An Evolutionarily Conserved piRNA-producing Locus Required for Male Mouse Fertility

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SUMMARY (≤150 words; now 150)

Pachytene piRNAs, which comprise >80% of all small RNAs in the adult mouse testis, have been proposed to bind and regulate target RNAs like miRNAs, to cleave targets like siRNAs, or to lack biological function altogether. Although mutants lacking proteins that make pachytene piRNAs are male sterile, no biological function has been identified for any mammalian piRNA-producing locus. Here, we report that loss of piRNA precursor transcription from a conserved pachytene piRNA locus on mouse chromosome 6 (*pi6*) perturbs male fertility. Loss of *pi6* piRNAs has no measurable effect on sperm quantity or transposon repression, yet *pi6*$^{-/-}$ mice produce sperm with defects in motility, egg fertilization, and embryo development, severely reducing pup production even at the peak of male reproduction. Our data establish a direct role for pachytene piRNAs in spermiogenesis and embryo viability and enable new strategies to identify the RNA targets of individual piRNA species.

Keywords: PIWI-interacting RNA; piRNA; MIWI; A-MYB; MYBL1, spermatogenesis; acrosome; zona pellucida; sperm; pachytene piRNA; meiosis

Highlights

- Normal male mouse fertility and spermiogenesis require piRNAs from the *pi6* locus
- Normal sperm motility and binding to zona pellucida require *pi6* piRNAs
- Sperm from *pi6* males fail to support embryo development
- Defects in *pi6* sperm reflect changes in the abundance of specific mRNAs

INTRODUCTION

Only animals produce PIWI-interacting RNAs (piRNAs), 21–35-nt small RNAs that form the most abundant class of small RNA in the germline. In most animals, piRNAs protect the germline genome from transposons and repetitive sequences, and, in many arthropods, piRNAs fight viruses and transposons in somatic tissues (Houwing et al., 2007; Aravin et al., 2008; Batista et al., 2008; Das et al., 2008; Lewis et al., 2018). The mammalian male germline makes three classes of piRNAs: (1) 26–28 nt transposon-silencing piRNAs predominate in the fetal testis (Aravin et al., 2008); (2) shortly after birth 26–27 nt piRNAs derived from mRNA 3′ untranslated regions (UTRs) emerge (Robine et al., 2009); and (3) at the pachytene stage of meiosis, ~30 nt, non-repetitive pachytene piRNAs appear. Pachytene piRNAs accumulate to comprise >80% of all small RNAs in the adult mouse testis, and they continue to be made throughout the male mouse reproductive lifespan. These piRNAs contain fewer transposon sequences than the genome as a whole, and most pachytene piRNAs map only to the loci from which they are produced. The diversity of pachytene piRNAs is unparalleled in development, with >1 million distinct species routinely detected in spermatocytes or spermatids. Intriguingly, the sequences of pachytene piRNAs are not themselves conserved, but piRNA-producing loci have been maintained at the syntenic regions across eutherian mammals (Girard et al., 2006; Chirn et al., 2015), suggesting that the vast sequence diversity of pachytene piRNAs is itself biologically meaningful.

In mice, 100 pachytene piRNA-producing loci have been annotated (Girard et al., 2006; Grivna et al., 2006; Lau et al., 2006; Ro et al., 2007; Li et al., 2013). All are coordinately regulated by the transcription factor A-MYB (MYBL1), which also promotes expression of proteins that convert piRNA precursor transcripts into mature piRNAs, as well as proteins required for cell cycle progression and meiosis (Bolcun-Filas et al., 2011). Of the 100 piRNA-producing loci, 15 pairs of pachytene piRNA-producing genes

are divergently transcribed from bidirectional, A-MYB-binding promoters (Li et al., 2013). The contribution of pachytene piRNAs from each piRNA-producing locus is unequal, with just five loci—*pi2*, *pi6*, *pi7*, *pi9*, and *pi17*—contributing to >50% of all pachytene piRNA production at 17 days postpartum (dpp).

Loss of proteins required to make pachytene piRNAs, including the pachytene piRNA-binding protein, MIWI (PIWIL1), invariably arrests spermatogenesis and renders males sterile (Deng and Lin, 2002; Reuter et al., 2011; Zheng and Wang, 2012; Li et al., 2013; Castañeda et al., 2014; Wasik et al., 2015). Yet, loss of the chromosome 17 pachytene piRNA-producing locus, *17-qA3.3-27363(−),26735(+)* (henceforth, *pi17*), has no detectable phenotype or impact on male fertility (Homolka et al., 2015), even though *pi17* produces ~30% of all pachytene piRNAs. Similarly, mice disrupted in expression of a piRNA cluster on chromosome 2 are viable and fertile (P.-H.W., K.C., and PDZ, unpublished; Xu et al., 2008). Consequently, the function of pachytene piRNAs in mice is actively debated. One model proposes that pachytene piRNAs regulate meiotic progression of spermatocytes by cleaving mRNAs during meiosis (Goh et al., 2015; Zhang et al., 2015). Another model posits that pachytene piRNAs direct degradation of specific mRNAs via a miRNA-like mechanism involving mRNA deadenylation (Gou et al., 2014). A third model proposes that MIWI functions without piRNAs, and that piRNAs are byproducts without a critical function (Vourekas et al., 2012). Compelling evidence exists to support each model.

In fact, direct demonstration of piRNA function in any animal has proven elusive. Only two piRNA-producing loci have been directly shown to have a biological function—both are in flies and were identified genetically before the discovery of piRNAs (Livak, 1984; Livak, 1990; Palumbo et al., 1994; Pélisson et al., 1994; Bozzetti et al., 1995; Prud'homme et al., 1995; Robert et al., 2001; Robert et al., 2001; Mével-Ninio et al., 2007). In male flies, piRNAs from *Suppressor of Stellate*, a multi-copy gene on the Y chromosome, silence the selfish gene *Stellate*, and deletion of *Suppressor of Stellate*

leads to Stellate protein crystals in spermatocytes (Aravin et al., 2001; Aravin et al., 2003). In female flies, deletion of the piRNA-producing *flamenco* gene, which is expressed in somatic follicle cells that support oogenesis, leads to *gypsy* family transposon expression and infertility (Brennecke et al., 2007; Saito et al., 2009).

Here, we report that a small promoter deletion in the chromosome 6 pachytene piRNA cluster *6-qF3-28913(−),8009(+)* (henceforth, *pi6*) that eliminates *pi6* piRNA production disrupts male fertility. The *pi6* locus, one of the five most productive piRNA-producing loci in mice, generates 5.8% of pachytene piRNAs in the adult testis and is conserved among eutherian mammals. Mice lacking *pi6*-derived piRNAs produce normal numbers of sperm and continue to repress transposons. However, *pi6* mutant sperm fertilize eggs poorly due to defective sperm motility and zona pellucida penetration. Consistent with these phenotypes, the steady-state abundance of mRNAs encoding proteins crucial for cilial function, zona pellucida proteolysis, and egg binding was significantly decreased in sperm progenitor cells from *pi6* males. Our findings provide direct evidence for a biological function for pachytene piRNAs in male mouse fertility, and *pi6* promoter deletions provide a new model for the future identification of piRNA targets in vivo.

RESULTS

pi6 Promoter Deletion Eliminates *pi6* pachytene piRNAs

To eliminate production of *pi6* pachytene piRNAs while minimizing the impact on adjacent genes, we used a pair of single-guide RNAs to delete 227 bp, including the A-MYB-binding promoter sequences, from *pi6* (Figure 1, S1A, and S1B, and Table S1; Li et al., 2013). For comparison, we created an analogous promoter deletion in *pi17*. We established stable mutant lines (*pi6*^{em1}-1, -2, and -3 in Figure S1B) from three founders whose *pi6* promoter deletion sizes range from 219 to 230 bp and differ at their deletion boundaries, reflecting imprecise DNA repair after Cas9 cleavage. All three deletions

eliminated *pi6* primary transcripts and mature pachytene piRNAs from both arms of the locus (Figure 1). Because these lines were created using the same pair of sgRNA guides, we refer to all as the *pi6*^{em1} allele.

pi6 is Required for Male Mouse Fertility

When paired with C57BL/6 females, *pi6*$^{em1/em1}$ males between 2 and 8 months old produced fewer pups compared to their littermates, even at peak reproductive age (Figure 2A and S2A). In six months, C57BL/6 males produced 7 ± 1 (n = 5) litters, while *pi6*$^{em1/em1}$ males produced 2 ± 2 (n = 6) litters. The significantly smaller number of progeny produced by *pi6*$^{em1/em1}$ males over their reproductive lifetime does not reflect fewer pups produced in each litter: *pi6*$^{em1/em1}$ males sired 5 ± 2 (n = 4) pups per litter compared to 6 ± 2 (n = 27) pups per litter for C57BL/6 control males (Figure 2A). Moreover, *pi6*$^{em1/em1}$ males regularly produced mating plugs, a sign of mating, in cohabiting females. Instead, the reduced progeny from *pi6*$^{em1/em1}$ males reflects two abnormal aspects of their fertility (Figure 2B). First, 29% of *pi6*$^{em1/em1}$ males never produced pups. Second, the mutants that did sire pups did so less frequently. These defects are specific for the loss of *pi6* piRNAs in males, because *pi6*$^{+/em1}$ heterozygous males and *pi6*$^{em1/em1}$ homozygous mutant females showed no discernable phenotype. As observed previously for a partial-loss-of-function *pi17* promoter deletion (Homolka et al., 2015), males and females carrying a ~583-bp promoter deletion in *pi17* were fully fertile, despite loss of primary transcripts and mature piRNAs from both arms of the *pi17* locus (Figure 1).

To test that the reduced fertility of *pi6*$^{em1/em1}$ male mice reflects loss of the *pi6* promoter—and not an undetected Cas9-induced off-target mutation elsewhere in the genome—we used Cas9 and a second pair of sgRNAs to generate a 117 bp *pi6* promoter deletion, *pi6*^{em2} (Figures 1, S1A, and S1C, and Table S1). Like *pi6*$^{em1/em1}$ male mice, *pi6*$^{em2/em2}$ males produced neither primary *pi6* transcripts nor mature *pi6* piRNAs

and showed reduced fertility (Figure S2A). We conclude that *pi6* piRNAs are required for C57BL/6 male fertility in mice.

pi6$^{em1/em1}$ Males Produce Fewer Embryos

pi6 mutant male matings were less likely to produce fully developed embryos. We examined the embryos produced by natural mating of C57BL/6 females housed with C57BL/6, *pi6*$^{+/em1}$, or *pi6*$^{em1/em1}$ males at 8.5, 14.5, or 16.5 days after occurrence of a mating plug. At 8.5 days after mating, C57BL/6 females housed with *pi6*$^{em1/em1}$ males carried fewer embryos (2 ± 2, n = 3) compared to the females paired with *pi6*$^{+/em1}$ (6 ± 5, n = 2) or C57BL/6 control (7 ± 4, n = 1) males (Figure 2C). At 14.5 and 16.5 days post-mating, female mice paired with *pi6*$^{em1/em1}$ males had even fewer embryos. Consistent with the observation that naturally-born pups sired by *pi6*$^{em1/em1}$ males were rare but healthy, the surviving embryos resulting from natural mating showed no obvious abnormalities.

Moreover, *pi6* piRNAs appear to play little if any role in the soma of the developing embryo. *pi6*$^{+/em1}$ heterozygous males mated to *pi6*$^{+/em1}$ heterozygous females yielded progeny at the expected Mendelian and sex ratios. Moreover, the weight of *pi6*$^{em1/em1}$ homozygous pups (28.3 ± 0.6 g, n = 8) that developed to adulthood was indistinguishable from their C57BL/6 (26.9 ± 0.3 g, n = 8) or heterozygous littermates (28.6 ± 0.3 g, n = 8) (Figure S2B). We detected no difference in the gross appearance or obvious changes in behavior among these pups.

pi6$^{em1/em1}$ Males Produce Mature Spermatozoa

Two-to-four months after birth, both *pi6*$^{+/em1}$ and *pi6*$^{em1/em1}$ testes weighed slightly less than C57BL/6 testes (Figure S2B). Nonetheless, *pi6*$^{em1/em1}$ testis gross histology was normal, with all expected germ cell types present in seminiferous tubules and sperm clearly visible in the lumen (Figure 2D). The quantity of caudal epididymal sperm

produced by *pi6*$^{em1/em1}$ mice (19 ± 10 million sperm per ml; *n* = 6) was also comparable to that of their *pi6*$^{+/em1}$ (23 ± 7 million sperm/ml; *n* = 4) or C57BL/6 (20 ± 10 million sperm per ml; *n* = 13) littermates (Figure 2E).

Although *pi6*$^{em1/em1}$ mice produce normal numbers of sperm, the sperm showed signs of agglutination compared to C57BL/6 sperm after 90 min of incubation in vitro, and ~10% of *pi6*$^{em1/em1}$ caudal epidydimal sperm had abnormal head morphology (Figure S2C). Defects in germ cell chromosomal synapsis, triggering errors in gene expression, have been linked to abnormal sperm head shape (Wong et al., 2008; de Boer et al., 2015). In fact, 22 ± 7 percent of *pi6*$^{em1/em1}$ pachytene spermatocytes had unsynapsed sex chromosomes or incompletely synapsed autosomal chromosomes, compared to 7 ± 3 percent for C57BL/6 (*n* = 4) (Figure S2E).

pi6$^{em1/em1}$ Sperm Fail to Fertilize

pi6 mutant males produce ordinary numbers of normally shaped sperm (~90%), yet are ineffectual at siring offspring. We used in vitro fertilization (IVF) to distinguish between defects in mating behavior and sperm function, incubating sperm from C57BL/6, *pi6*$^{+/em1}$, or *pi6*$^{em1/em1}$ males with wild-type oocytes and scoring for the presence of both male and female pronuclei and the subsequent development of the resulting bi-pronuclear zygotes into two-cell embryos 24 h later (Figure 3A). The majority of oocytes incubated with C57BL/6 (91 ± 5%; *n* = 5) or *pi6*$^{+/em1}$ (60 ± 35%; *n* = 3) sperm developed into two-cell embryos. By contrast, only 7 ± 5% (*n* = 7) of oocytes incubated with *pi6*$^{em1/em1}$ sperm reached the two-cell stage. The majority of these oocytes remained one-cell embryos, and few contained a male pronucleus, suggesting that *pi6*$^{em1/em1}$ sperm are defective in fertilization.

pi6$^{em1/em1}$ Sperm Nuclei Support Fertilization

The best studied piRNA function is transposon silencing, and mouse *pi2* has been proposed to be involved in LINE1 element silencing, although *pi2* mutant males are fertile (Xu et al., 2008). Moreover, LINE1 transcript abundance increases in mice bearing inactivating mutations in the catalytic site of MIWI (Reuter et al., 2011). Transposon activation can produce DNA damage, and genomic integrity is critical for fertilization (Ahmadi and Ng, 1999; Morris et al., 2002; Bourc'his and Bestor, 2004; Lewis and Aitken, 2005). However, pachytene piRNAs are depleted of repetitive sequences in contrast to other types of piRNA-producing genomic loci (Figure S3A; Aravin et al., 2006; Girard et al., 2006; Gainetdinov et al., 2018).

We asked whether the defect in fertilization by *pi6*$^{em1/em1}$ might reflect DNA damage or epigenetic dysregulation of the *pi6*$^{em1/em1}$ sperm genome. *pi6*$^{+/em1}$ *or* *pi6*$^{em1/em1}$ sperm heads were individually injected into the cytoplasm of wild-type oocytes (intracytoplasmic sperm injection, or ICSI) (Figure 3B), bypassing the requirement for sperm motility, acrosome reaction, egg binding, or sperm-egg membrane fusion (Kuretake et al., 1996). *pi6*$^{em1/em1}$ sperm heads delivered by ICSI fertilized the oocyte at a rate similar to that of *pi6*$^{+/em1}$ sperm: 66% of oocytes injected with homozygous mutant *pi6*$^{em1/em1}$ sperm heads reached the two-cell stage, compared to 79% for *pi6*$^{+/em1}$. Thus, most *pi6*$^{em1/em1}$ nuclei are capable of fertilization.

The steady-state abundance of transposon RNA in *pi6*$^{em1/em1}$ testicular germ cells further supports the view that the fertilization defect caused by loss of *pi6* piRNAs does not reflect a failure to silence transposons. We used RNA-seq to measure the abundance of RNA from 1,007 transposons in four distinct germ cell types, purified by fluorescence-activated cell sorting: pachytene spermatocytes (4C), diplotene spermatocytes (4C), secondary spermatocytes (2C), and spermatids (1C). *pi6* piRNAs are plentiful in pachytene spermatocytes onwards (Figure S3B), yet when *pi6* piRNAs

were eliminated, we found no significant changes in steady-state RNA abundance (i.e., an increase or decrease ≥ 2-fold and FDR ≤ 0.05) for any transposon family compared to C57BL/6 cells (Figure S3C). We also note that, similar to C57BL/6 testis, γH2AX expression is confined to meiotic spermatocytes in *pi6*$^{em1/em1}$ testis, indicating absence of DNA damage (data not shown). Together with the rescue of the fertilization defects of *pi6*$^{em1/em1}$ sperm by ICSI, these data suggest that transposon silencing is unlikely to be the biological function of *pi6* piRNAs.

Impaired Motility in *pi6* Mutant Sperm

To assess whether abnormal sperm motility might contribute to *pi6*$^{em1/em1}$ male subfertility, we observed freshly extracted caudal epididymal sperm from *pi6*$^{em1/em1}$ or C57BL/6 mice for 5 h. Ten minutes after sperm extraction, most *pi6*$^{em1/em1}$ sperm moved more slowly than C57BL/6 control sperm (Movies S1 and S2). With time, *pi6*$^{em1/em1}$ sperm motility declined more rapidly than C57BL/6 sperm (Movies S3–S10). At 4 and 5 h, most *pi6*$^{em1/em1}$ sperm only moved in place and showed signs of agglutination (Movies S8 and S10).

To quantify the differences between *pi6* mutant and control sperm, we used computer-assisted sperm analysis (CASA) to measure *pi6*$^{em2/em2}$ sperm motility 10 min after isolation (Mortimer, 2000). While control sperm swam at a path velocity comparable to previously reported (110 ± 50 μm/sec for 221 ± 75 cells measured; n = 3; Ren et al., 2001), *pi6*$^{em2/em2}$ sperm moved at a lower average path velocity (80 ± 60 μm/sec for 232 ± 57 cells measured; n = 3) (Table 1). Similarly, The *pi6*$^{em2/em2}$ sperm also showed less forward, progressive movement (progressive velocity = 50 ± 60 μm/sec for 232 ± 57 cells measured; n = 3) compared to control sperm (progressive velocity = 70 ± 50 μm/sec for 221 ± 75 cells measured; n = 3). For comparison, knockout of *CatSper1* leads to ~65% reduction in path velocity and ~62% reduction in progressive velocity (Ren et al., 2001). As a population, the speed and

progressivity of *pi6* mutant sperm motility patterns varied more widely than control sperm (Movies S1–S10 and Table 1). Lower average path and progressive velocity in sperm populations is linked to worse outcomes in fertilization and pregnancy in IVF (Donnelly et al., 1998). Thus, the slower and less progressive movement in *pi6*$^{em1/em1}$ sperm likely contributes to the subfertility of *pi6*$^{em1/em1}$ males.

pi6 Mutant Sperm Struggle to Penetrate the Zona Pellucida

Mammalian spermatozoa stored in the epididymis are dormant. Sperm "capacitate," i.e., resume maturation, only upon entering the female reproductive tract (de Lamirande et al., 1997). Upon capacitation, sperm become capable of undergoing the acrosome reaction, which is required to bind and penetrate the outer oocyte glycoprotein layer, the zona pellucida (Florman and Storey, 1982; de Lamirande et al., 1997; Jin et al., 2011). To test whether the defect in fertilization by *pi6* mutant sperm was due to impaired binding to or penetration of zona pellucida, we compared IVF using wild-type oocytes with their zona pellucida either intact or removed (Figure 4A). As before, 10 ± 6% (n = 3) of intact oocytes incubated with *pi6*$^{em1/em1}$ sperm reached the two-cell stage, compared to 94 ± 5% (n = 3) for C57BL/6 sperm (Figure 4B). Strikingly, removing the zona pellucida from the wild-type oocytes fully rescued the fertilization rate of *pi6* mutant sperm: 92 ± 7% (n = 3) of zona pellucida-free oocytes incubated with *pi6*$^{em1/em1}$ sperm reached the two-cell stage, compared to those with intact zona pellucida (10 ± 6%; n = 3)

Ex vivo, the acrosome reaction occurs spontaneously in some sperm and can be further triggered by inducing Ca^{2+} influx using the ionophore A23187 (Talbot et al., 1976), which results in an acrosome reaction visually indistinguishable from that triggered by natural ligands such as progesterone (Osman et al., 1989) or ZP3 (Arnoult et al., 1996), while bypassing signaling pathways essential for acrosome reaction in vivo (Tateno et al., 2013) (Figure 4C and 4D). The spontaneous acrosome reaction rates for

C57BL/6 (19 ± 3%; *n* = 3) and *pi6* mutant sperm were similar (17 ± 8%; *n* = 3). Acrosome reaction triggered by ionophore-induced Ca^{2+} influx differed between the two genotypes: 45 ± 14% of *pi6* mutant sperm (*n* = 3) underwent partial or complete reaction, compared to 66 ± 6% (*n* = 3) for C57BL/6 (Figure 4C). Our data suggest that *pi6* mutant sperm less effectively undergo an acrosome reaction triggered by ionophore-induced Ca^{2+} influx, a defect expected to impair binding and penetrating the zona pellucida.

Potential Role of Paternal *pi6* piRNAs in Embryo Development

Even when *pi6* sperm successfully fertilize the oocyte, the resulting heterozygous embryos are less likely to complete gestation. Two-cell embryos generated by IVF using heterozygous or homozygous *pi6* mutant or C57BL/6 control sperm were transferred to C57BL/6 surrogate mothers (Figure 5A). At least half of embryos from $pi6^{+/em1}$ (50 ± 10%; *n* = 3) or C57BL6 control (70 ± 10%; *n* = 3) sperm developed to term (Figure 5B), a rate typical for the C57BL/6 background (González-Jara et al., 2017).

The low number of fertilized two-cell embryos produced in IVF using $pi6^{em1/em1}$ sperm precluded transferring the standard number of embryos to surrogate mothers. For example, in two IVF experiments using $pi6^{em1/em1}$ sperm, only 5 or 7 embryos could be transferred; the surrogate females failed to become pregnant (Figure 5B and S4A, Trials 1 and 2). In theory, this result might suggest a paternal role for *pi6*. A more mundane explanation is that the low number of embryos transferred reduced the yield of live fetuses, as reported previously (McLaren , 1955; Johnson et al., 1996; González-Jara et al., 2017). We conducted additional experiments to distinguish between these two possibilities. Oocytes were again fertilized by IVF with $pi6^{em1/em1}$ or C57BL/6 control sperm, and two-cell embryos transferred to surrogate females, but matching the number of embryos transferred to each surrogate for the two sperm genotypes. We used two strategies. First, similar numbers of embryos derived from $pi6^{em1/em1}$ sperm and filler

embryos derived from control sperm were transferred to separate oviducts (Figure 5B, Trials 3 and 4). Again, fewer embryos developed to term for *pi6*$^{em1/em1}$ (17%) compared to control sperm (37%). Second, embryos were mixed before transfer and then equal numbers of embryos, selected randomly, were implanted in each oviduct (Figure 5B, Trial 5). Pups isolated by cesarean section 18.5 days after transfer were genotyped by PCR. In this experiment, only 40% of embryos derived from *pi6*$^{em1/em1}$ sperm developed to term, compared to 80% of filler embryos. Finally, in one experiment (Trial 6) where we obtained sufficient numbers of embryos derived from *pi6*$^{em1/em1}$ sperm, 10 *pi6*$^{em1/em1}$-derived two-cell embryos were transferred to each oviduct of the surrogate female. Nevertheless, only 15% of the *pi6*$^{em1/em1}$-derived embryos developed to term, compared to 85% of the control.

We also monitored pre-implantation development ex vivo for up to 96 h, a period during which the one-cell embryo develops into a blastocyst. Of all the oocytes incubated with *pi6*$^{em1/em1}$ sperm, 40% remained one cell without evidence of a male pronucleus, presumably because they were not fertilized by *pi6*$^{em1/em1}$ mutant sperm. Among the remaining 60% oocytes that progressed to at least two-cell stage, which indicated successfully fertilization by *pi6*$^{em1/em1}$ sperm, 82% showed delayed development, requiring 48 h to reach the two-cell stage. None of these developed further. Only 3% of fertilized oocytes progressed to the blastocyst stage by 96 h, compared to 98% of oocytes fertilized by C57BL/6 sperm (Figure 5C).

Further support for this idea comes from transfer of embryos generated by ICSI (Figure 5D). ICSI with *pi6*$^{em1/em1}$ or *pi6*$^{+/em1}$ sperm yielded comparable normal numbers of fertilized oocytes (Figure 3B), so no filler embryos were used; all embryos were transferred into a single oviduct of the surrogate female. In two independent experiments in which embryos generated by ICSI were transferred to surrogate mothers, only 19% of two-cell embryos derived from *pi6*$^{em1/em1}$ sperm heads developed to term, compared to 34% for embryos fertilized with *pi6*$^{+/em1}$ (Figure 5C). Only four of

seven (57%) surrogate mothers carrying embryos derived from *pi6*$^{em1/em1}$ sperm became pregnant. All three surrogate mothers receiving embryos derived from *pi6*$^{+/em1}$ sperm became pregnant (Figure S4B).

We note that the live fetuses generated using *pi6*$^{em1/em1}$ sperm in IVF or sperm heads in ICSI, like those produced by natural mating using *pi6*$^{em1/em1}$ males, showed no obvious morphological abnormalities and grew to adulthood normally when fostered by host mothers. This suggests a direct or indirect requirement for paternal *pi6* piRNAs in early embryogenesis.

Changes in Spermatocyte mRNA Abundance Accompany Loss of *pi6* piRNAs

To characterize the molecular phenotypes of *pi6* and *pi17* mutants, we used RNA-seq to measure steady-state RNA abundance in pachytene spermatocytes, diplotene spermatocytes, secondary spermatocytes, and spermatids purified from *pi6*$^{em1/em1}$, *pi17*$^{-/-}$, and C57BL/6 adult testis (Figure 6A). *pi6* and *pi17* precursor transcripts are abundant in meiotic pachytene spermatocytes (tetraploid), decrease in diplotene spermatocytes, and fall to low levels in post-meiotic spermatids (haploid) (Figure S5B). Compared with C57BL/6 controls, *pi6*$^{em1/em1}$ mutants had widespread changes in mRNA abundance in pachytene spermatocytes—481 mRNAs more than doubled, while 394 fell by more than half (FDR ≤ 0.05; Figure 6B and S5A, and Table S2)—but caused little alteration in mRNA abundance in diplotene spermatocytes, secondary spermatocytes, or spermatids. In contrast, *pi17*$^{-/-}$ mutants showed significant changes in mRNA abundance in diplotene (10 mRNAs increased, 267 decreased) and secondary spermatocytes (103 mRNA increased, 400 decreased) but not in pachytene spermatocytes or spermatids (Figure S5A). Among the mRNAs that changed in the diplotene spermatocytes of *pi17*$^{-/-}$ mutants, 56% remained different from controls in secondary spermatocytes in these mutants. These data suggest that, despite similar temporal expression, *pi6* piRNAs function primarily in pachytene spermatocytes, while

pi17 piRNAs may be more important at a later stage of spermatogenesis. Furthermore, 734 (84%) of mRNAs with altered abundance in *pi6*$^{em1/em1}$ pachytene spermatocytes were unchanged in any *pi17*$^{-/-}$ sorted germ cell type we examined, suggesting that distinct sets of genes are dysregulated in *pi6*$^{em1/em1}$ and *pi17*$^{-/-}$ mutants.

The abundance of piRNAs from the other four major pachytene piRNA clusters, including *pi17*, was unaffected by loss of *pi6* piRNAs, and loss of neither *pi6* nor *pi17* piRNAs had any significant effect on the abundance of mRNAs encoding piRNA pathway proteins (Table S3), suggesting that the changes in mRNA abundance in *pi6*$^{em1/em1}$ or *pi17*$^{-/-}$ cells reflect direct regulation of target genes by *pi6* or *pi17* piRNAs or the downstream regulation through the direct targets of these piRNAs.

Gene Ontology (GO) analysis of the 481 up-genes found over 354 significantly enriched GO biological processes (FDR ≤ 0.01 and enrichment ≥ 2). Curiously, 106 of these GO terms correspond to developmental processes that do not normally occur in testis, suggesting a failure to suppress inappropriate programs without *pi6* piRNAs. Similarly, *pi6*$^{em1/em1}$ mutants show increased mRNA abundance for 20 transcription factors that normally act in undifferentiated spermatogonia or spermatogonial stem cells or the stem cells of other tissues (Table S4).

The mRNA abundance of several miRNA pathway genes also increased in *pi6*$^{em1/em1}$ pachytene spermatocytes, including *Lin28a* (5.6-fold), *Zc3h7b* (5-fold), and *Ajuba* (5.3-fold; Figure S5C) (Dresios et al., 2005; James et al., 2010; Pilotte et al., 2011; Piskounova et al., 2011). LIN28A inhibits *let-7* biogenesis by binding to the loop of pre-*let-7*, blocking its processing by DICER (Piskounova et al., 2008; Hagan et al., 2009; Heo et al., 2009), and *let-7* promotes *Lin28a* degradation by binding two conserved sites in the *Lin28a* 3′ untranslated region (Reinhart et al., 2000; Agarwal et al., 2015) predicting that *let-7* levels should fall and *let-7* targets should rise in *pi6*$^{em1/em1}$. Indeed, in *pi6*$^{em1/em1}$ adult testis, the aggregate abundance of *let-7a, let-7b, let-7c, let-7e, let-7f, let-7g, and let-7i*, the seven most abundant *let-7* family members (≥ 10 ppm in

wild-type testis) fell to less than half of wild-type, suggesting *pi6* regulation of downstream target genes via *let-7*. Moreover, 48 predicted *let-7* targets (Agarwal et al., 2015) increased in the absence of *pi6*$^{em1/em1}$, including *Lin28a* and the mRNAs encoding three transcription factors: *Sall4* (increased 8.7-fold), *Elf4* (increased 7-fold), and *Pbx2* (increased 6.7-fold). SALL4 is normally expressed in undifferentiated spermatogonia where it represses genes that specify somatic gene expression programs (Gassei and Orwig, 2013; Yamaguchi et al., 2015; Chan et al., 2017). ELF4 has been implicated in regulation of quiescence in hematopoietic stem cells (Lacorazza et al., 2006). Our data suggest that piRNAs, miRNAs, and transcription factors collaborate to ensure precise regulation of gene expression in spermatogenesis.

Genes that Function in the Cilium Assembly, Cilium Motility, and Fertilization Pathways Decrease in mRNA Abundance upon Loss of *pi6* piRNAs

GO analysis of the 394 down-genes revealed only 36 significantly enriched GO biological processes (FDR ≤ 0.01 and fold enrichment ≥ 2), of which 34 are related to the production and function of sperm and can be organized into four sets (Table S5). One set encompasses broad spermatogenesis terms (e.g., *male gamete generation*, 4.6-fold enriched, FDR = 5.8×10^{-11}; *sperm capacitation*, 12-fold enriched, FDR = 7.4×10^{-3}) while three sets are highly specific and match the in vivo phenotypes of *pi6* mutant males. The first specific set includes *cilium assembly* (6.2-fold enriched, FDR = 4.1×10^{-9}) and *axonemal dynein complex assembly* (18-fold enriched, FDR = 1.1×10^{-5}). The second set contains *sperm motility* (13-fold enriched, FDR = 6.0×10^{-10}) and *cilium movement involved in cell motility* (27-fold enriched, FDR = 2.0×10^{-3}). The third set involves *fertilization* (6.2-fold enriched, FDR = 1.7×10^{-5}) and *binding of sperm to zona pellucida* (12-fold enriched, FDR = 2.3×10^{-3}). None of these three sets of GO terms is enriched in the 481 genes whose mRNA levels increased in *pi6*$^{em1/em1}$ pachytene spermatocytes. The three sets of specific GO terms contain 28, 36, and 22 genes

whose mRNAs decreased (63 total and 23 shared between sets; Figure 6C and Table S6). The last two general GO terms—*microtubule-based process* (GO:0007017; with 27 genes whose mRNA abundance declined) and *organelle assembly* (GO:0070925; with 28 genes whose mRNA abundance decreased)—likely gained their enrichment from the large number of genes they share with Cilium assembly and Sperm motility processes (23 and 25 genes for the two GO terms, respectively).

Master Regulators of Cilium Assembly and Sperm Motility

The 63 Cilium Assembly, Sperm Motility, or Fertility genes with reduced mRNA abundance in *pi6* mutants include two transcription factors, *Rfx2* and *Foxj1*, that act as master regulators of ciliogenesis (Figure 6C). Like *pi6* itself, *Rfx2* transcription is activated by A-MYB, and RFX2 also binds its own promoter (Horvath et al., 2009). Of the genes with decreased mRNA abundance in *pi6*$^{em1/em1}$ pachytene spermatocytes, 31 both bind RFX2 and have reduced mRNA abundance in *Rfx2*$^{-/-}$ testis, suggesting they are direct targets of RFX2 (Figure 6C and Table S7) (Kistler et al., 2015). Intriguingly, 23 of these 31 RFX2-regulated genes also bind A-MYB (Table S7). *A-Myb* mRNA levels are normal in *pi6*$^{em1/em1}$, which may account for the relatively modest decreases in the mRNA abundance of these 23 genes. Unlike RFX2, the role of FOXJ1 in sperm flagellar assembly has not been extensively studied but its role in general ciliogenesis is well established: *FoxJ1*$^{-/-}$ mouse died at or soon after birth due to absence of cilia in multiple organs (Chen et al., 1998; Blatt et al., 1999; Brody et al., 2000; Yu et al., 2008). Six genes—*Tekt4, Spa17, Drc1, Rsph1, Meig1,* and *Tsnaxip1*—out of the 394 genes with reduced mRNA abundance in *pi6*$^{em1/em1}$ pachytene spermatocytes are regulated by FOXJ1 in ciliogenesis in other tissues (Yu et al., 2008; Stauber et al., 2017). Fourteen genes whose mRNA abundances decrease in *pi6*$^{em1/em1}$ are uniquely annotated with the GO term Fertilization (Figure 6C and Table S6). Several are required for sperm to bind the zona pellucida or for acrosome function, including *Acrosin* (halved in *pi6*$^{em1/em1}$

pachytene spermatocytes), *Adam3* (decreased 2.5-fold), *Zpbp2* (decreased 3.3-fold), and the FOXJ1-regulated gene *Spa17* (decreased 5-fold). Among the genes with decreased or increased mRNA abundance in *pi6*$^{em1/em1}$ cells, 28 have been reported to disrupt mouse or human male fertility or to play a role in spermatogenesis, spermiogenesis, or sperm function (Table S8).

DISCUSSION

Deletion of the mouse pachytene piRNA *pi6* locus results in specific, quantifiable defects in male fertility. These include impaired sperm mobility and failure in sperm to bind and penetrate the zona pellucida. The male fertility defects accompanying loss of *pi6* piRNAs are specific to this locus, as deletion of the promoter of *pi17*, which eliminates *pi17* piRNAs, had no detectable effect on male or female fertility or viability, as reported previously (Homolka et al., 2015). The phenotypic defects of *pi6* mutants reflect the molecular changes—decreased steady-state abundance of mRNAs encoding proteins that function in cilial motility and fertilization. Mutations in four of these genes also cause infertility in men. The molecular changes were detected only in pachytene spermatocytes but not in diplotene spermatocytes, secondary spermatocytes, or spermatids. By contrast, RNA-seq for 17.5 dpp or adult *pi6*$^{em1/em1}$ testes revealed no changes in mRNA abundance compared to controls. These results underscore the power of analyzing sorted germ cells.

Pachytene piRNAs have been proposed to act collectively in meiotic spermatocytes or post-meiotic spermatids to target mRNAs for destruction (Gou et al., 2014; Goh et al., 2015), but the extent to which piRNAs from different pachytene piRNA loci regulate overlapping sets of targets is unknown. Transcriptome analysis of sorted germ cells from *pi6*$^{em1/em1}$ and *pi17*$^{-/-}$ mutant mice revealed distinct changes in mRNA abundance, suggesting that, despite the coordinate temporal expression of pachytene piRNAs, individual pachytene piRNA loci regulate distinct sets of genes. Given that *pi6*

produces 95,677 distinct piRNA sequences, the phenotypic specificity of the *pi6* mutant is extraordinary. For both miRNAs and siRNAs, the seed sequence plays a central role in determining a small RNA's regulatory target. Assuming that pachytene piRNAs find their target RNAs by a similar mechanism, the sequence diversity of the small RNAs produced by individual loci is enormous: *pi6* piRNAs encompass 9,880 distinct seed (g2–g8 or 7mer-m8; Bartel, 2009) and 17,304 distinct extended seed sequences (g2–g9) in adult mouse testis, while *pi17* generates 134,358 distinct piRNA sequences, encompassing 11,324 distinct g2–g8 seed and 21,972 distinct g2–g9 seed sequences. Yet, the g2–g9 seed sequences of the 100 most abundant *pi6* piRNAs are not found among the 100 most abundant *pi17* piRNAs. Furthermore, 97 of these *pi6* g2–g9 seed sequences are not found among any of the 100 most-abundant piRNAs produced by *pi2*, *pi7*, *pi9*, or *pi17*. Together with *pi6*, these loci produce more than half of all pachytene piRNAs. The unique seed sequences of the most abundant *pi6* piRNAs are consistent with the lack of compensation of loss of *pi6* piRNAs by other piRNA-producing loci.

We envision that piRNAs from distinct loci target overlapping sets of genes, ensuring robust control of mRNA abundance across spermatogenesis. Our data show that *pi6* piRNAs regulate—directly or by regulating upstream factors—a specific set of mRNAs whose protein products must be eliminated for successful spermiogenesis. In this view, *pi6* piRNAs target mRNAs whose expression must decline at the onset of the pachynema in order to allow new sets of mRNAs to accumulate, such as the RFX2-regulated genes required for ciliogenesis. While we cannot exclude a direct role for piRNAs in activating gene expression or increasing mRNA stability, we note that the overwhelming majority of siRNAs and miRNAs in plants and animals act as repressors not activators.

The phenotypic and molecular specificity of *pi6* may reflect a lower degree of redundancy with other piRNA clusters. Nonetheless other piRNA clusters may partially

rescue the *pi6* phenotype, accounting for the incomplete penetrance of the *pi6* sterility phenotype. Conversely, the lack of a phenotype for other pachytene piRNA clusters may simply reflect greater redundancy with their piRNA-producing peers. Loss of regulation of the targets of *pi17* piRNAs may be compensated by piRNAs from other loci. Testing this hypothesis is clearly a prerequisite for explaining why loss of *pi6* and not *pi17* piRNAs has a measurable biological consequence.

Beyond the requirement for *pi6* piRNAs to produce fully functional sperm, *pi6* piRNAs appear to play an additional role in embryo development. Our data suggest that the arrested development and reduced viability of embryos derived from *pi6* mutant sperm reflects a paternal defect and not the embryonic genotype. Damaged sperm DNA, abnormal sperm chromatin structure, and failure to form a male pronucleus in fertilized embryos have been reported to be linked to retarded embryo development (Sakkas et al., 1998; Borini et al., 2006). Our analysis of transposon RNA abundance in *pi6* mutant germ cells argues against a role for *pi6* piRNAs in transposon silencing during spermatogenesis, but we cannot currently exclude a direct or indirect role for *pi6* piRNAs in silencing transposons in the early embryo (Peaston et al., 2004). Of course, DNA damage might reflect incomplete repair of the double-stranded DNA breaks required for recombination, rather than transposition or transposon-induced illegitimate recombination.

How piRNAs identify their targets remains poorly understood, in part because suitable biochemical or genetic model systems are not available. The availability of a mouse mutant missing a specific set of piRNAs whose absence causes a readily detectable phenotype should provide an additional tool for understanding the base-pairing rules that govern the binding of piRNAs to their RNA targets and for unraveling the regulatory network created by pachytene piRNAs.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, Figures S1–5, Tables S1–S7, and Movies S1–S10.

AUTHOR CONTRIBUTIONS

P.H.W., K.C., Y.F., Z.W., and P.D.Z. conceived and designed the experiments. P.H.W. and K.C. performed the experiments. Y.F. analyzed the sequencing data. D.M.Ö generated A-MYB ChIP-seq datasets. P.H.W., Y.F., and P.D.Z. wrote the manuscript.

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FIGURE LEGENDS

Figure 1. *pi6*$^{em1/em1}$, *pi6*$^{em2/em2}$, and pi17$^{-/-}$ promoter deletion in mice

Scissors indicate sites targeted by sgRNAs used to guide the Cas9-catalyzed promoter deletions. RNA-seq was used to measure the steady-state abundance of piRNA primary transcripts, and sequencing of $NaIO_4$ oxidation-resistant small RNA was used to measure the abundance of mature piRNAs in 17.5 dpp testes.

See also Figure S1 and Table S1.

Figure 2. Reduced fertility in *pi6*$^{em1/em1}$ males by natural mating

(A) Number of litters and pups per litter produced by male mice between 2–8 months of age. **(B)** Frequency and periodicity of litter production. Each bar represents a litter. **(C)** Number of embryos produced by males mated with C57BL/6 females. **(D)** Testis morphology analyzed by hematoxylin and eosin staining. **(E)** Concentration of sperm from the caudal epididymis.

See also Figure S2.

Figure 3. Fertilization defects of *pi6*$^{em1/em1}$ sperm revealed by IVF and ICSI

(A) Sperm function analyzed by in vitro fertilization (IVF). **(B)** Sperm function analyzed by intracytoplasmic sperm injection (ICSI).

Thick lines denote the median, and whiskers report the 75th and 25th percentiles.

See also Figure S3

Figure 4. Impaired motility and zona pellucida-binding in *pi6*$^{em1/em1}$ sperm

(A) Strategy for zona-free IVF. **(B)** Comparison of sperm function in standard and zona-free IVF. **(C)** Acrosome reaction triggered with the Ca^{2+} ionophore A23187 in vitro. The results using *pi6*$^{em1/em1}$ and *pi6*$^{em2/em2}$ sperm are combined and indicated. **(D)** Representative caudal epididymal spermatozoa with distinct acrosome reaction status. Green, peanut agglutinin to detect the acrosome; blue, DAPI to detect DNA.

See also Movies S1–S10.

Figure 5. Embryos derived from *pi6*$^{em1/em1}$ sperm fail to develop

(A) Strategy for surgical transfer of fertilized two-cell embryos to surrogate mothers. **(B)** Rates of IVF-derived two-cell embryos that developed to term. Each uterine cartoon represents one surrogate mother, and the colored circles represent embryos. The number of embryos transferred to each side of the oviduct is also indicated. **(C)** Development of IVF-derived embryos. Red, the number of embryos that developed to the stage expected for the time after fertilization. **(D)** Rates of ICSI-derived two-cell embryos that developed to term.

See also Figure S4

Figure 6. The abundance of mRNAs encoding proteins required for sperm motility and zona pellucida-binding is decreased in *pi6*$^{em1/em1}$ germ cells

(A) Strategy for purifying specific male germ cell types. **(B)** Volcano plots of steady-state transcript abundance in sorted testicular germ cells. Control cells were sorted from C57BL/6 testis. Each dot represents the mean abundance of an mRNA measured using three biologically independent samples. Differentially expressed transcripts (≥ 2 fold-change and ≤ 0.05 FDR) are indicated. **(C)** Major GO categories containing enriched

GO terms associated with genes with decreased expression in *pi6*$^{em1/em1}$ pachytene spermatocytes (FDR ≤ 0.01 and fold enrichment ≥ 2). Genes annotated for a single category that are discussed in the main text are listed in respective categories. **(D)** RFX2 and A-MYB target genes with significantly decreased mRNA abundance in *pi6*$^{em1/em1}$ pachytene spermatocytes and established functions in sperm motility and zona pellucida-binding. ChIP-seq peaks around respective transcription start sites (TSS) are shown. . RFX-2 or A-MYB occupancy is reported as fold enrichment of ChIP-seq reads relative to input.

See also Table S7 for the complete list of genes regulated by *pi6*, RFX2, and A-MYB.

Table 1. Sperm motility measured by computer-assisted sperm analysis (CASA)

	C57BL/6		*pi6*$^{+/em2}$		*pi6*$^{em2/em2}$			
	Exp. 1	**Exp. 2**	**Exp. 1**	**Mean ± SD**	**Exp.1**	**Exp. 2**	**Exp. 3**	**Mean ± SD**
Cells counted	271	135	257	n/a	273	167	257	n/a
Motile cells	256	106	227	n/a	247	111	208	n/a
Progressive cells	217	87	187	n/a	146	81	166	n/a
Percent motile	94	79	83	87 ± 8	90	66	81	80 ± 10
Percent progressive	80	64	73	70 ± 8	53	49	65	56 ± 8
Path Velocity (μm/s)	110 ± 50	110 ± 60	110 ± 50	110 ± 50	70 ± 80	80 ± 40	90 ± 60	80 ± 60
Progressive Velocity (μm/s)	60 ± 50	50 ± 60	70 ± 40	70 ± 50	50 ± 70	40 ± 30	50 ± 60	50 ± 60
Track speed (μm/s)	210 ± 90	220 ± 80	200 ± 100	210 ± 90	200 ± 100	210 ± 100	210 ± 100	200 ± 100
Lateral Amplitude (μm)	13 ± 8	13 ± 7	13 ± 8	13 ± 8	12 ± 8	13 ± 7	13 ± 7	13 ± 7
Beat Frequency (%)	30 ± 10	30 ± 20	30 ± 20	30 ± 20	30 ± 20	40 ± 20	30 ± 20	40 ± 10
Straightness (%)	60 ± 30	50 ± 30	60 ± 30	60 ± 30	60 ± 20	50 ± 20	50 ± 30	50 ± 20

Linearity (%)	30 ± 20	30 ± 20	40 ± 20	30 ± 20	30 ± 20	20 ± 10	20 ± 20	20 ± 20
Elongation	40 ± 20	40 ± 10	40 ± 10	40 ± 10	40 ± 20	40 ± 20	40 ± 10	40 ± 20
Area (μm^2)	90 ± 80	80 ± 50	80 ± 60	80 ± 70	60 ± 40	80 ± 60	80 ± 60	70 ± 50
Rapid cells (> 50 μm/s)	217	87	187	n/a	146	81	166	n/a
Medium cells (25–50 μm/s)	4	1	3	n/a	9	0	3	n/a
Slow cells (< 25 μm/s)	35	18	37	n/a	92	30	39	n/a
Static cells (< 10 μm/s)	15	29	30	n/a	26	56	49	n/a
Percent rapid cells	80	64	73	74 ± 8	53	49	65	57 ± 8
Percent medium cells	1	1	1	1 ± 0	3	0	1	2 ± 2
Percent slow cells	13	13	14	13.4 ± 0.6	34	18	15	20 ± 10
Percent static cells	6	21	12	13 ± 8	10	34	19	20 ± 10

STAR METHODS

Mouse mutants

Mice were maintained and sacrificed according to guidelines approved by the Institutional Animal Care and Use Committee of the University of Massachusetts Medical School (A-2222-17).

Small guide RNAs (sgRNAs) flanking piRNA promoters were designed using CRISPR design tools (crispr.mit.edu/). DNA oligos containing guide sequences were cloned into pX330 vectors (Cong et al., 2013), and their cleavage activity tested in NIH3T3 cells by co-transfecting pX330 constructs containing sgRNA sequences and puromycin-resistant plasmid (pPUR) using TransIT-X2 (Mirus Bio, Madison, WI). Puromycin (3 μg/μl) was added 24 h after transfection and DNA extracted 48 h afterwards. Promoter deletions were detected by PCR using primers flanking the predicted Cas9 cleavage sites.

For mice, sgRNAs were generated by in vitro transcription and purified by electrophoresis on 8% (w/v) polyacrylamide gels. To generate the *pi6*$^{em1/em1}$ and *pi17*$^{-/-}$ lines used in this study, in vitro transcribed sgRNAs (10 ng/μl each) targeting *pi6* and *pi17* were mixed with Cas9 mRNA (40 ng/μl) and injected together into the cytoplasm of one-cell C57BL/6 zygotes (RNA only). For some founders, the sgRNA and Cas9 mRNA mixture was combined with pX330 plasmids expressing the same four sgRNAs and Cas9 and injected into both the cytoplasm and pronuclei of one-cell C57BL/6 zygotes (RNA + DNA). For *pi6*$^{em2/em2}$, in vitro transcribed sgRNAs and Cas9 mRNA were injected into the cytoplasm of one-cell C57BL/6 embryos. Embryos were transferred to pseudopregnant females using standard methods. To screen for mutant founders, DNA was extracted from small pieces of tail clipped from three-week-old pups (Truett et al., 2000). Deletions were detected by PCR, and PCR products purified and cloned into TOPO blunt vectors. Mutant sequences were determined by Sanger sequencing.

Mouse fertility test

Each 2–8 month-old male mouse was housed with one 2–4 month-old C57BL/6 female, who was examined for the presence of a vaginal plug the following morning. When a plug was observed, the female was housed separately. For male mice who did not produce pups after 3 months (~3 cycles), the original female was replaced with a new female and the fertility test continued.

Testis histology, sperm count, and sperm morphology

Mouse testes were fixed in Bouin's solution overnight, washed with 70% ethanol, embedded in paraffin, and sectioned at 5 μm thickness. Sections were stained with hematoxylin solution, countered stained with eosin solution, and imaged using Leica DMi8 brightfield microscope equipped with an 20× 0.4 N.A. objective (HC PL FL L 20×/0.40 CORR PH1, Leica Microbiosystems, Buffalo Grove, IL). To quantify sperm abundance, the cauda epididymides were collected from mice and placed in phosphate-buffered saline (PBS) containing 4% (w/v) bovine serum albumin. A few incisions were made in the epididymides with scissors to release the sperm, followed by incubation at 37°C and 5% CO_2 for 20 min. A 20 μl aliquot of sperm suspension was diluted in 480 μl of 1% (w/v) paraformaldehyde (PFA), and sperm cells counted at 10× by brightfield microscopy. To assess sperm morphology, caudal epididymal sperm were fixed in 1% (w/v) PFA, stained with trypan blue, and a Leica DMi8 brightfield microscope equipped with an 63× 1.4 N.A. oil immersion objective (HC PL APO; Leica Microbiosystems, Buffalo Grove, IL). Sperm stained with Alexa 488-conjugated PNA (see below) were also used to assess sperm morphology.

Meiotic chromosome spreads

Meiotic chromosome spreads were prepared as described (Holloway et al., 2014). Mouse testes were incubated in hypotonic buffer (30 mM Tris-Cl, pH 8.2, 50 mM

sucrose, 17 mM sodium citrate, 5 mM EDTA, 0.5 mM DTT) for 30 min on ice, then small fragments of seminiferous tubules were moved to 100 mM sucrose solution and pulled apart with forceps to release germ cells. A drop of sucrose solution containing germ cells was pipetted onto a glass slide with a thin layer of 1× PBS containing 1% PFA and 0.15% (v/v) Triton-X100 (pH 9.2) and spread by swirling. Slides were placed in a humidifying chamber for 2.5 h, air-dried, and washed twice with 1× PBS with 0.4% Photo-Flo 200 (Kodak, Rochester, NY) and once with water with 0.4% Photo-Flo 200, and air-dried. For immunostaining of meiotic chromosomes, slides were sequentially washed with (1) 1× PBS with 0.4% Photo-Flo 200, (2) 1× PBS containing 0.1% (v/v) Triton-X, and (3) blocked with PBS containing 3% (w/v) BSA, 0.05% (v/v) Triton X-100, and 10% (v/v) goat serum in 1× PBS at room temperature. The slides were then incubated with primary antibodies, anti-SCP1 (1:1000 dilution) and anti-SCP3 (1:1000 dilution), in a humidifying chamber overnight at room temperature. Washing and blocking steps were repeated the next day, and the slides were incubated with Alexa 488- or Alexa 594-conjugated secondary antibodies (1:10,000 dilution) for 1 h at room temperature. Slides were washed thrice with 1× PBS containing 0.4% (v/v) Photo-Flo 200, once with water containing 0.4% Photo-Flo 200 mixture, air-dried in the dark, mounted by incubation in ProLong Gold Antifade Mountant with DAPI (4',6'-diamidino-2-phenylindole; Thermo Fisher Scientific, Waltham, MA) overnight in the dark, and imaged using a Leica DMi8 fluorescence microscope equipped with an 63× 1.4 N.A. oil immersion objective (HC PL APO; Leica Microbiosystems, Buffalo Grove, IL).

Cell sorting by FACS

Testicular cell sorting was performed as described (Cole et al., 2014). Testes were collected, decapsulated, and incubated in 0.4 mg/ml collagenase type IV (Worthington LS004188) in 1× Grey′s Balanced Salt Solution (GBSS, Sigma, G9779) at 33°C rotating at 150 rpm for 15 min. Separated seminiferous tubules were washed with 1× GBSS and

incubated in 0.5 mg/ml Trypsin and 1 µg/ml DNase I in 1× GBSS at 33°C rotated at 150 rpm for 15 min. Tubules were dissociated on ice by gentle pipetting, and then 7.5% (v/v) fetal bovine serum (f.c.) was added to inactivate trypsin. The cell suspension was filtered through a pre-wetted 70 µm cell strainer, and cells pelleted at 300 × *g* for 10 min at 4ºC. Cells were resuspended in 1× GBSS containing 5% (v/v) FBS, 1 µg/ml DNase I, and 5 µg/ml Hoechst 33342 (Thermo Fisher Scientific, Waltham, MA) and rotated at 150 rpm at 33ºC for 45 min. Propidium iodide (0.2 µg/ml, f.c.; Thermo Fisher Scientific, Waltham, MA) was added, and cells strained through a pre-wetted 40 µm cell strainer. Cell sorting was performed on a FACSAria II (BD Biosciences, Franklin Lakes, NJ). The purity of sorted fractions was assessed by immunostaining. Secondary spermatocyte and spermatid populations were >90% pure, and the pachytene spermatocytes and diplotene spermatocytes were >80% pure.

In vitro fertilization (IVF) and embryo transfer

In vitro fertilization was performed as previously described (Nagy et al., 2003) using spermatozoa from caudal epididymis of either C57BL/6, *pi6*$^{+/em1}$, or *pi6*$^{em1/em1}$ mice. Spermatozoa were incubated in human tubal fluid (HTF; 101.6 mM NaCl, 4.69 mM KCl, 0.37mM KH_2PO_4, 0.2 mM $MgSO_4{\cdot}7H_2O$, 21.4 mM Na-lactate, 0.33 mM Na-pyruvate, 2.78 mM glucose, 25 mM $NaHCO_3$, 2.04 mM $CaCl_2{\cdot}2H_2O$, 0.075 mg/ml Penicillin-G, 0.05 mg/ml streptomycin sulfate, 0.02% (v/v) phenol red, 4 mg/ml BSA) with oocytes (98–146 for control sperm and 120–293 for *pi6*$^{em1/em1}$ sperm) from B6SJLF1/J mice for 3–4 h at 37ºC with constant 5% O_2, 90% N_2, and 5% CO_2 concentration. Oocyte viability and the presence of pronuclei were assessed under a Nikon SMZ-2B (Nikon, Tokyo, Japan) dissecting microscope. To observe embryo development, embryos were moved into potassium-supplemented simplex optimized media (KSOM; 95 mM NaCl, 2.5 mM KCl, 0.35 mM KH_2PO_4, 0.2 mM $MgSO_4{\cdot}7H_2O$, 10 mM Na-lactate, 0.2 mM Na-pyruvate, 0.2 mM glucose, 25 mM $NaHCO_3$, 1.71 mM $CaCl_2{\cdot}2H_2O$, 1 mM L-glutamine, 0.01 mM

EDTA, 0.075 mg/ml Penicillin-G, 0.05 mg/ml streptomycin sulfate, 0.02% (v/v) phenol red, 1 mg/ml BSA; Millipore Sigma, Burlington, MA) after IVF and assessed every 24 h. To measure birth rates, two-cell embryos were transferred to Swiss Webster pseudopregnant females, and fetuses isolated by cesarean section 18.5 d after embryo transfer.

For zona-free IVF, the zona pellucida of oocytes was removed with acid Tyrode's solution as described (Yanagimachi et al., 1976; Johnson et al., 1991).

Intracytoplasmic sperm injection (ICSI)

Frozen caudal epididymal spermatozoa were thawed, the sperm tails detached (Nagy et al., 2003), and individual *pi6*$^{+/em1}$ or *pi6*$^{em1/em1}$ sperm heads injected into B6D2F1/J oocytes in Chatot-Ziomek-Bavister media (CZB; 81.62 mM NaCl, 4.83 mM KCl, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4 \cdot 7H_2O$, 25 mM Na_2HCO_3, 1.70 mM $CaCl_2 \cdot 2H_2O$, 0.11 mM Na_2-ETDA$\cdot 2H_2O$, 1 mM L-glutamine, 28 mM Na-lactate, 0.27 mM Na-pyruvate, 5.55 mM glucose, Penicillin-G 0.05 mg/ml, 0.07 mg/ml streptomycin sulfate, 4 mg/ml BSA) (Millipore Sigma, Burlington, MA) using the PiezoXpert (Eppendorf, Hamburg, Germany; Cat#5194000024). Surviving oocytes were counted, collected, and cultured in KSOM (Millipore Sigma, Burlington, MA) at 37ºC and 5% CO_2 for 24 h. Two-cell embryos were surgically transferred unilaterally into the oviducts of pseudopregnant Swiss Webster females. At 16.5 days after the surgery, live fetus isolated by cesarean section.

Sperm motility

Cauda epidydimal sperm were collected from mice and placed in 37ºC HTF media in an incubator with 5% CO_2. A drop of sperm was removed from the suspension and pipetted into a sperm counting glass chamber, then assayed by CASA or video acquisition. CASA was conducted using an IVOS II instrument (Hamilton Thorne, Beverly, MA) with the following settings: 100 frames acquired at 60 Hz; minimal

contrast = 50; 4 pixel minimal cell size; minimal static contrast = 5; 0%straightness (STR) threshold; 10 µm/s VAP Cutoff; prog. min VAP, 20 µm/s; 10 µm/s VSL Cutoff; 5 pixel cell size; cell intensity = 90; static head size = 0.30–2.69; static head intensity = 0.10–1.75; static elongation = 10–94; slow cells motile = yes; 0.68 magnification; LED illumination intensity = 3000; IDENT illumination intensity = 3603; 37°C. Agglutination of *pi6*$^{em1/em1}$ sperm prevented CASA measurements at later times. A Nikon Diaphot 200 microscope (Nikon, Tokyo, Japan) with darkfield optics equipped with Nikon E Plan 10×/0.25 160/- Ph1 DL objective (Nikon, Tokyo, Japan), ZWO ASI 174mm Monochrome CMOS Imaging camera (ZWO, SuZhou, China), and the SharpCap software (https://docs.sharpcap.co.uk/2.9/) using darkfield at 10× magnification were used to record sperm movement at 37ºC.

In vitro acrosome reaction assay

Acrosome reaction was assessed as described (Talbot et al., 1976). Cauda epididymides were collected from mice, placed in HTF media pre-warmed for at least 2 h in a 37ºC incubator at 5% CO_2. A few incisions were made in the epididymides with scissors to release the sperm, followed by incubation at 37°C in 5% CO_2 for 90 min. Calcium ionophore A23187 (10 µm f.c. in DMSO) was added, and incubation continued for 30 min. Sperm were fixed at room temperature for 10 min by adding two volumes of 4% (w/v) PFA, pelleting at 1,000 × *g* for 5 min, washed with 1× PBS, resuspended in fresh 1× PBS, spotted on a glass slide, and air-dried. Methanol was pipetted onto the sperm to permeabilize the cells, followed by washing with 1× PBS. Slides were incubated overnight in 10 µg/ml Alexa Fluor 488-conjugated peanut agglutinin (PNA) in 1× PBS (Mortimer D., 1987), washed with 1× PBS, air-dried, and mounted with ProLong Gold Antifade Mountant with DAPI (Thermo Fisher Scientific, Waltham, MA). Sperm were imaged using a Leica DMi8 fluorescence microscope equipped with a 63× 1.4 N.A.

oil immersion objective (HC PL APO; Leica Microbiosystems, Buffalo Grove, IL) and analyzed using ImageJ (version 2.0.0-rc-68/1.52e; https://fiji.sc/).

Chromatin Immunoprecipitation (ChIP) and sequencing

Frozen testes were cross-linked with 2% (w/v) formaldehyde at room temperature for 30 min using an end-over-end tumbler. Fixed tissues were homogenized in buffer containing 1% (w/v) sodium lauryl sulfate (SDS), 10mM EDTA, and 50mM Tris-HCl (pH 8.1) by 40 strokes in a Dounce tissue grinder with Pestle B (Kimble-Chase, Rockwood, TN). Lysed samples were sonicated using the E220 Covaris ultrasonicator (Covaris, Woburn, MA) to shear the chromatin to 150–200 bp fragments and diluted 1:10 with a buffer containing 0.01% (w/v) SDS, 1.1% (v/v) Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl (pH 8.1), 167 mM NaCl. Immunoprecipitation was performed using 5.5 µg of rabbit anti-A-MYB antibody (Sigma, St. Louis, MO), DNA was extracted with phenol:chloroform:isoamyl alcohol (25:24:1) (pH 8), and ChIP-seq libraries were prepared as previously described (Li et al., 2013). Libraries were sequenced using paired-end reading on NextSeq500 (Illumina, San Diego, CA), and reads were mapped to mouse genome assembly mm10 using Bowtie2 (v2.2.5). ChIP-seq peaks were determined using MACS2 (v2.1.1) and unique mapping reads were reported in this study as fold enrichment over input.

RNA-seq and small RNA-seq

Small RNA-seq and RNA-seq libraries were constructed and sequenced using NextSeq 500 (Illumina, San Diego, CA) as described (Fu et al., 2018). To sequence mature piRNAs, small RNA was oxidized with 25 mM $NaIO_4$ in 30 mM sodium borate, 30 mM boric acid (pH 8.6; Sigma Aldrich, St. Louis, MO) at 25ºC for 30 min. RNA was precipitated with ethanol before adapter ligation. Small RNA-seq and RNA-seq reads were mapped to mouse genome assembly mm10 using piPipes (Han et al., 2015).

Transcript abundance between *pi6*$^{+/em1}$ and C57BL/6 testes were indistinguishable (< 2-fold change and FDR > 0.05). Transcripts with low abundance (< 1 fpkm) in both C57BL/6 and *pi6*$^{em1/em1}$ cells were excluded.

Transposon mapping

RNA-seq reads were intersected using BEDtools (Quinlan and Hall, 2010) with Repeat Masker annotation from UCSC (downloaded from https://genome.ucsc.edu/cgi-bin/hgTables). Reads mapping to multiple genomic locations were apportioned. Reads for individual repeats were aggregated to obtain reads counts for repeat families.

Statistics

All statistics were performed using R (https://www.rstudio.com/) and graphs were generated using Igor Pro v7.08 (WaveMetrics) or ggplot2 v3.0.0 (https://ggplot2.tidyverse.org/). Unless otherwise stated, Mann-Whitney-Wilcoxon test was used to calculate *p* values.

ACCESSION NUMBERS

All sequencing data are available through the NCBI Sequence Read Archive using accession number PRJNA480354.

SUPPLEMENTAL FIGURE, TABLE, AND MOVIES

Supplemental Figure Legends

Figure S1. Confirmation of mutant founder genotypes. Related to Figure 1 and Table S1.

(A) Genotyping of mutant founders by PCR. Genomic sequences of *pi6* promoter region in *pi6*$^{em1/em1}$ **(B)** and *pi6*$^{em2/em2}$ **(C)** mouse lines. **(D)** Genomic sequences of *pi17* promoter region in *pi17*$^{-/-}$ mouse lines. Dashes, genomic sequences deleted by CRISPR; dots, unaltered sequence omitted for clarity.

Figure S2. *pi6*$^{em1/em1}$ adult male phenotype. Related to Figure 2.

(A) Number of litters produced in 6 months by 2–8 month-old males. **(B)** Body and testis weight of 2–4 month-old *pi6*$^{em1/em1}$ and *pi6*$^{em2/em2}$ males. Each dot represents an individual mouse. The thick lines denote median values, and whiskers indicate the 75th and 25th percentiles. **(C)** Representative spermatozoon. **(D)** Representative patterns of meiotic chromosome synapsis in $^{pi6em1/em1}$ pachytene spermatocytes. SYCP1, Synaptonemal complex protein 1; SYCP3, Synaptonemal complex protein 3. **(E)** Quantification of patterns of meiotic chromosome synapsis depicted in (D).

Figure S3. Abundance of transposons in *pi6*$^{em1/em1}$ germ cells. Related to Figure 3.

(A) Proportions of the whole genome or piRNA sequences composed of repetitive sequences. **(B)** Abundance of repetitive sequences in mouse germ cells. A pseudocount of 1 was added to each value. Each dot represents the mean value of three biologically independent RNA-seq experiments.

Figure S4. Pregnancy rate of surrogate mothers in IVF and ICSI experiments. Related to Figure 5.

Percent of pregnant surrogate mothers in IVF **(A)** and ICSI **(B)**.

Figure S5. Transcriptome changes in *pi6*$^{em1/em1}$ cells. Related to Figure 6.

(A) Number of altered genes with mRNA abundance altered by ≥ 2-fold with FDR ≤ 0.05 in indicated cell types. **(B)** Abundance of pachytene piRNAs and their precursors in C75BL/6 purified germ cells. For piRNA precursor levels, each dot represents the mean value of triplicate datasets and each error bar indicates the standard deviation. For mature piRNAs, each dot represents the mean abundance of unique-mapping reads of two duplicate datasets. **(C)** mRNAs with altered abundance in *pi6*$^{em1/em1}$ cells and encoding protein with functions in meiotic chromosome organization and miRNA-mediated regulation.

Supplemental Table Legends

Table S1. Statistics of CRISPR injection for *pi6* mutant generation. Related to Figure 1 and S1.

Table S2. Differentially expressed genes in *pi6*$^{em1/em1}$ germ cells. Related to Figure 6 and S5.

Mean abundance (fpkm) of significantly altered mRNAs (≥ 2-fold change ∩ FDR 0.05) in C57BL/6 versus *pi6*$^{em1/em1}$ cells of RNA-seq triplicate datasets. A pseudocount of 0.5 was added to each value to calculate the differences. Transcripts with < 1 rpkm in both C57BL/6 and *pi6*$^{em1/em1}$ cells prior to adding pseudocount were excluded.

Table S3. Expression of piRNA pathway genes in *pi6*$^{em1/em1}$ cells. Related to Figure 6 and S5

Mean expression (fpkm) of piRNA genes in C57BL/6 versus *pi6*$^{em1/em1}$ cells of RNA-seq triplicate datasets. A pseudocount of 0.5 was added to each value to calculate the differences. Significant changes were ≥ 2-fold increase or decrease and FDR ≤ 0.05.

Table S4. Transcription factors with altered mRNA abundance in *pi6*$^{em1/em1}$ pachytene spermatocytes. Related to Figure 6 and S5.

Table S5. Gene Ontology of genes with decreased expression in *pi6*$^{em1/em1}$ pachytene spermatocytes. Related to Figure 6 and S5.

Table S6. Genes with reduced expression in *pi6*$^{em1/em1}$ pachytene spermatocytes that are mapped to major Gene Ontology categories.

Related to Figure 6 and S5.

Table S7. RFX2 and A-MYB target genes with decreased abundance in *pi6*$^{em1/em1}$ pachytene spermatocytes.

Related to Figure 6 and S5.

Table S8. Published male fertility genes with altered expression in *pi6*$^{em1/em1}$ cells. Related to Figure 6 and S5.

Legends to Movies

Movies S1-10. *pi6*$^{em1/em1}$ sperm motility.

Movie S1. C57BL/6 sperm motility at 10 minute time point.

Movie S2. *pi6*$^{em1/em1}$ sperm motility at 10 minute time point.

Movie S3. C57BL/6 sperm motility at 90 minute time point.

Movie S4. *pi6*$^{em1/em1}$ sperm motility at 90 minute time point.

Movie S5. C57BL/6 sperm motility at 3 hour time point.

Movie S6. *pi6*$^{em1/em1}$ sperm motility at 3 hour time point

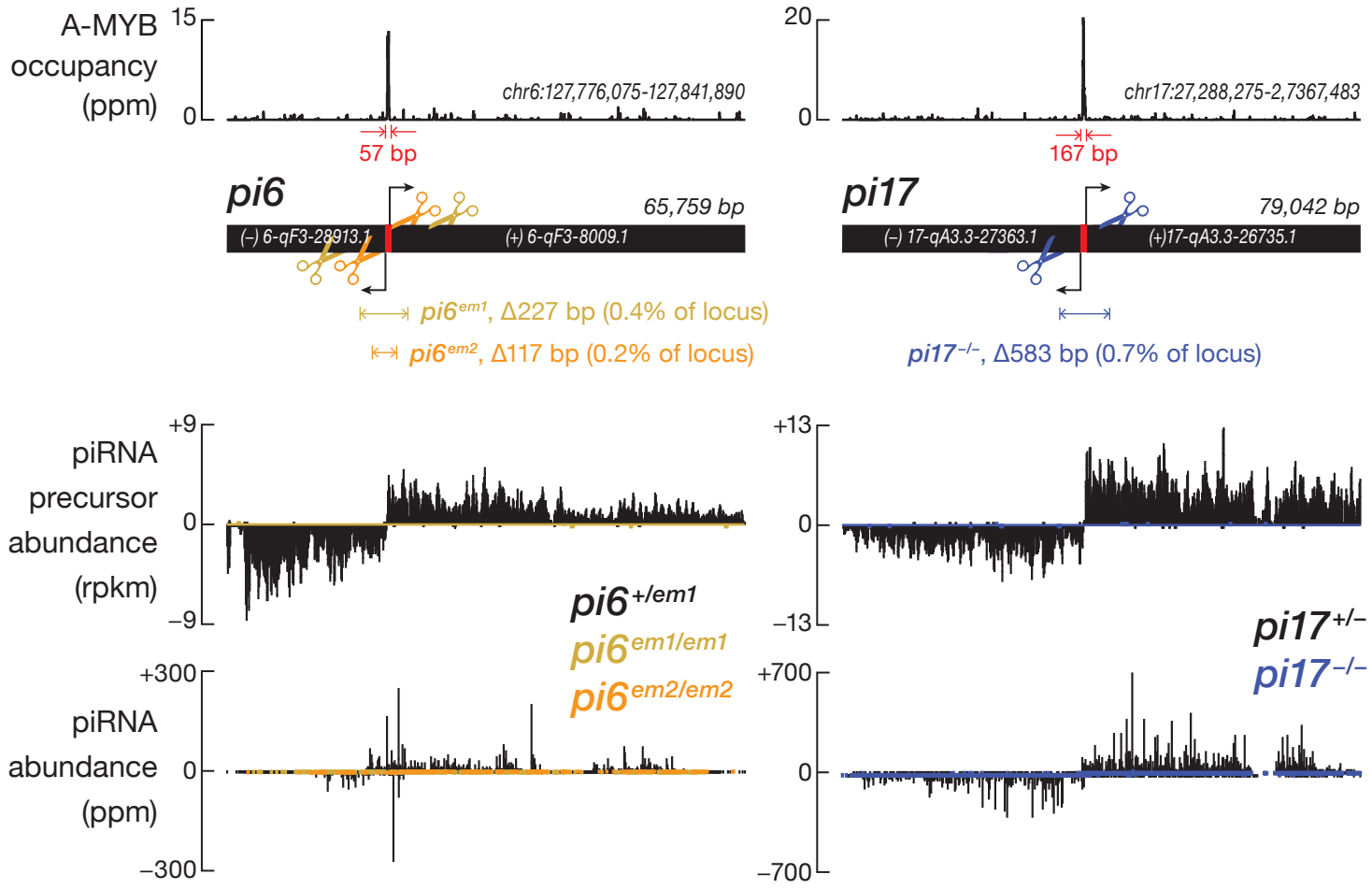
Movie S7. C57BL/6 sperm motility at 4 hour time point.

Movie S8. *pi6*$^{em1/em1}$ sperm motility at 4 hour time point.

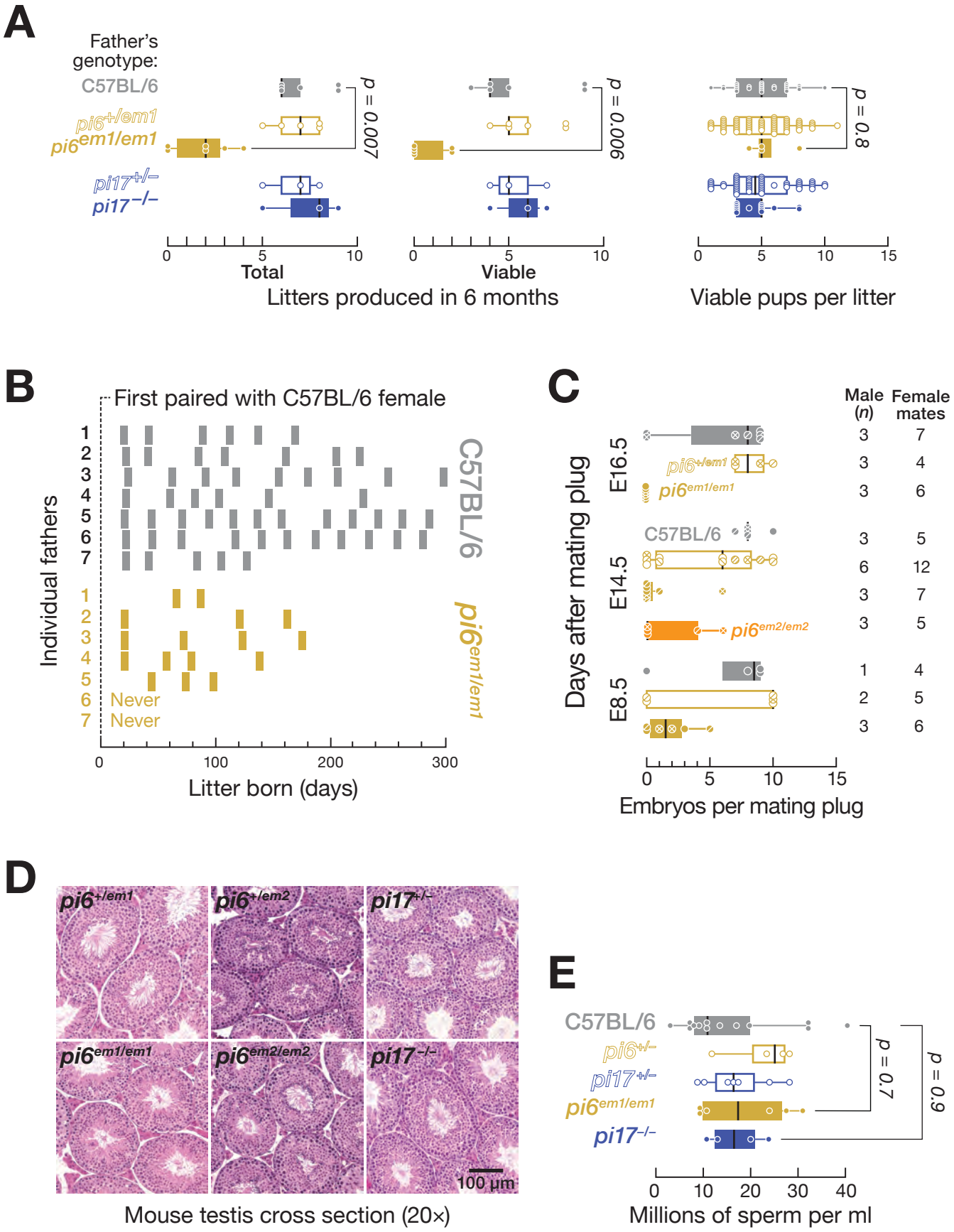
Movie S9. C57BL/6 sperm motility at 5 hour time point.

Movie S10. *pi6*$^{em1/em1}$ sperm motility at 5 hour time point.

Wu et al. Figure 1



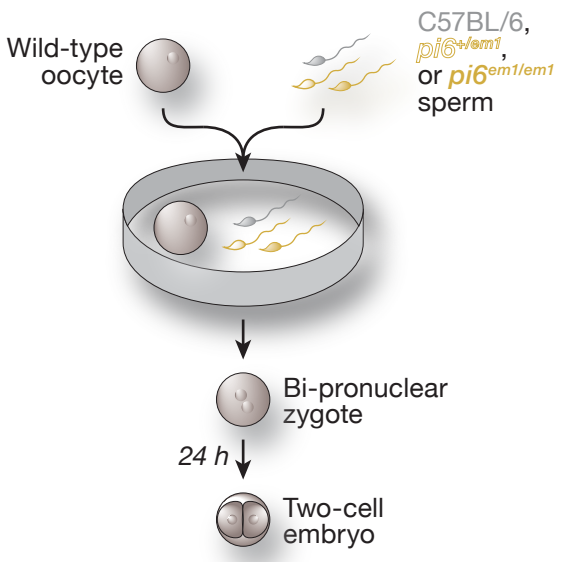
Wu et al. Figure 2

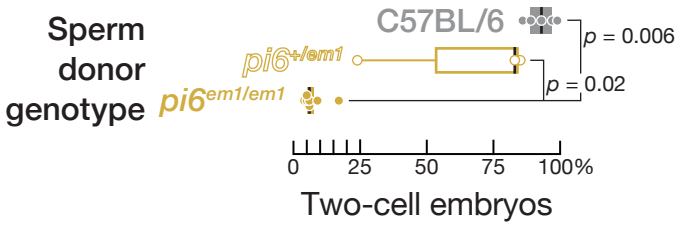


Wu et al. Figure 3

A

In vitro fertilization (IVF)

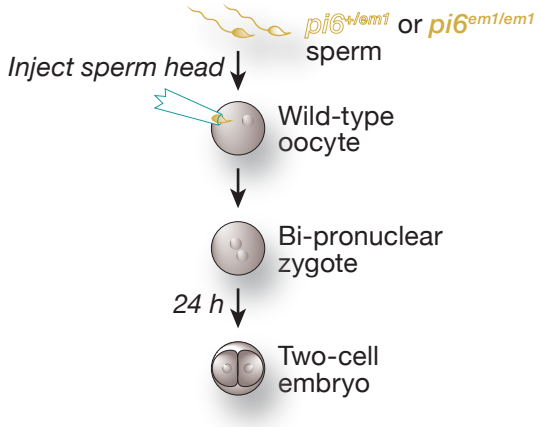




Sperm donor genotype	Trial	Oocytes	Two-cell embryos
C57BL/6	1	98	84 (86%)
	2	128	124 (97%)
	3	117	109 (93%)
	4	134	119 (89%)
	5	144	143 (99%)
		mean ± SD = *91 ± 5*	
pi6+/em1	1	118	28 (24%)
	2	148	125 (85%)
	3	146	121 (83%)
		mean ± SD = *60 ± 35*	
pi6em1/em1	1	120	5 (4%)
	2	150	7 (5%)
	3	125	8 (6%)
	4	293	16 (6%)
	5	94	5 (5%)
	6	115	20 (17%)
	7	129	12 (9%)
		mean ± SD = *7 ± 5*	

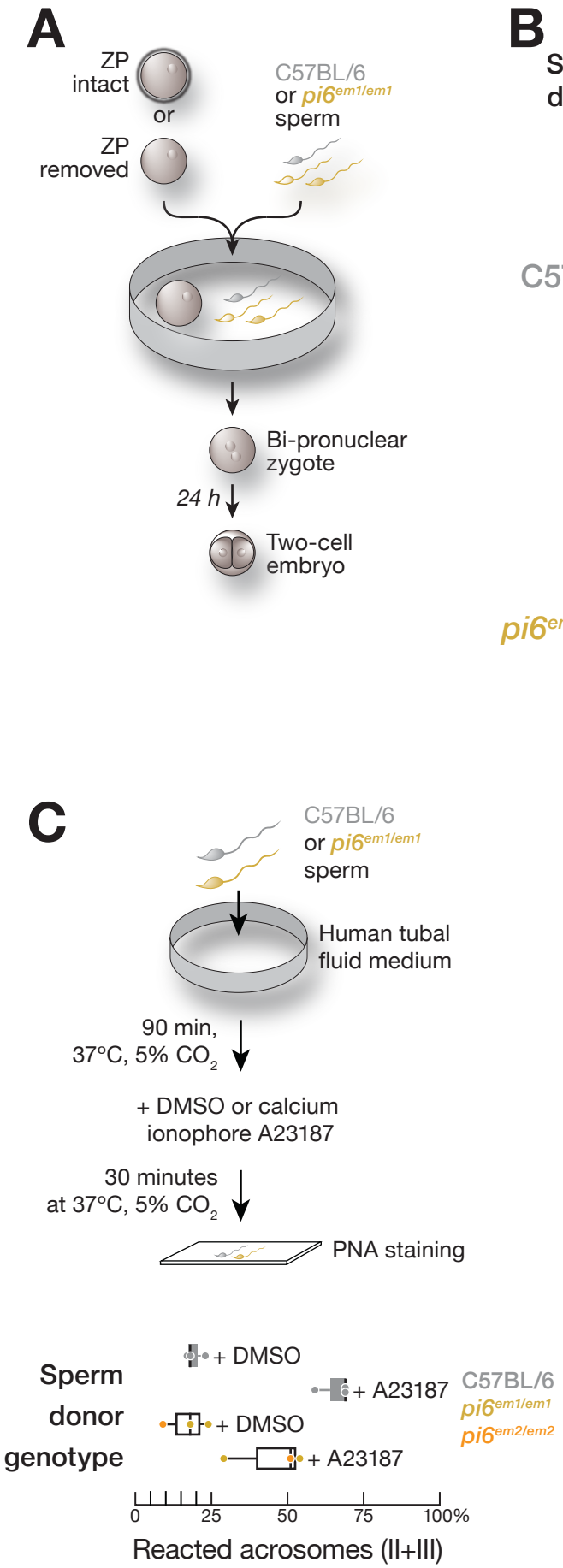
B

Intracytoplasmic injection (ICSI)



Sperm donor genotype	Trial	Viable injected oocytes	Two-cell embryos
pi6+/em1	1	37	29 (78%)
	2	24	19 (79%)
pi6em1/em1	1	63	40 (64%)
	2	98	66 (67%)

Wu et al. Figure 4



Sperm donor	Zona pellucida	Trial	Oocytes	Two-cell embryos
C57BL/6		1	117	109 (93%)
		2	134	119 (89%)
		3	144	143 (99%)
		mean ± SD = *94 ± 5*		
		1	46	33 (72%)
		2	76	39 (51%)
		3	69	35 (51%)
		mean ± SD = *58 ± 12*		
pi6$^{em1/em1}$		1	94	5 (5%)
		2	115	20 (17%)
		3	129	12 (9%)
		mean ± SD = *10 ± 6*		
		1	112	102 (91%)
		2	105	90 (86%)
		3	99	99 (100%)
		mean ± SD = *92 ± 7*		

Wu et al. Figure 5

A

Isolate Sperm

↓ IVF or ICSI

Paternal genotype

- C57BL/6
- *pi6*$^{+/em1}$
- *pi6*$^{em1/em1}$
- *pi6*$^{em1/em1}$, mixed with C57BL/6 filler embryos

↓ Implant in left or right horn of surrogate mother

↓ C-section before birth

Genotype embryos

C

Incubation	C57BL/6 sperm 24	48	72	96	*pi6*$^{em1/em1}$ sperm 24	48	72	96 h
One-cell	0	0	0	0	117	52	52	52
Two-cell	143	0	0	0	12	63	63	63
Four-cell		143	0	0		14	14	12
Morula			143	3			0	0
Blastocyst				140				2

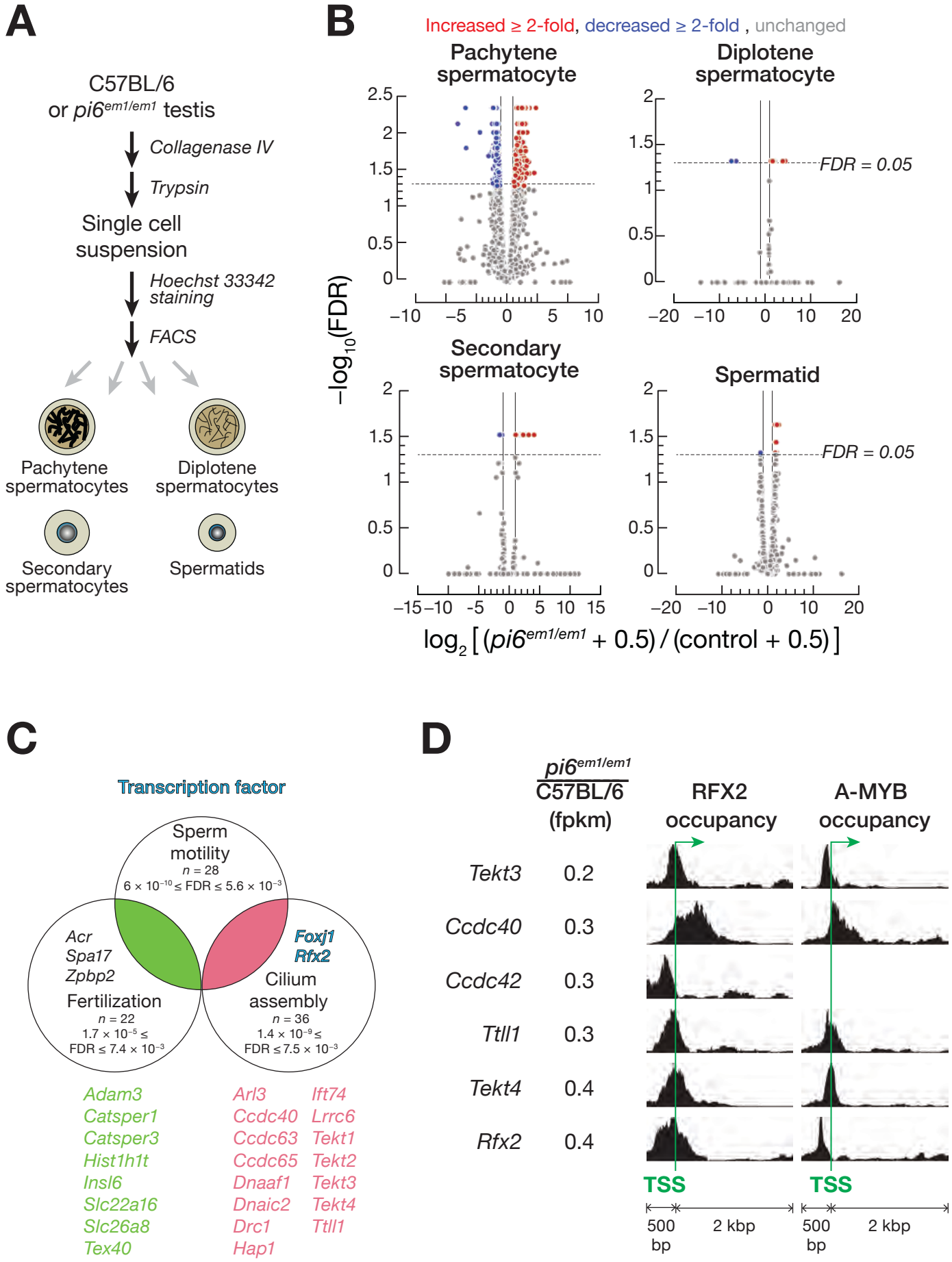
D

Sperm donor genotype	Trial	Number and placement of two-cell embryos in surrogate mother	Percent developed to live fetus
pi6$^{+/em1}$	1	0 15 0 15	21
	2	19	47
			mean 34
pi6$^{em1/em1}$	1	0 13 0 13 0 14	20
	2	0 12 0 13 0 13 0 13	18
			mean 19

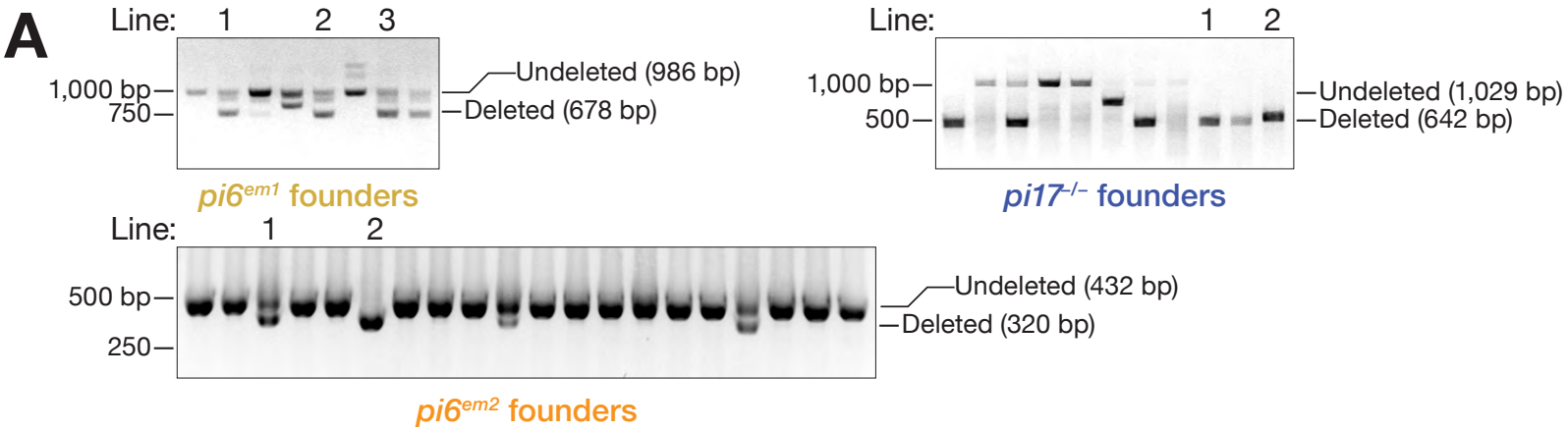
B

Sperm donor genotype	Trial	Number and placement of two-cell embryos in surrogate mother	Embryos developed to live fetus (%)	Filler embryos developed to live fetus (%)
C57BL/6	1	12 12 12 12 12 12	60	n/a
	2	9 9 9 9	72	n/a
	3	10 10	85	n/a
			mean ± SD 70 ± 10	n/a
pi6$^{+/em1}$	1	12 12 12 12 12 12 12 12 12 12	58	n/a
	2	12 13 12 13 12 13 12 13 12 12	39	n/a
	3	7 7 7 7	54	n/a
			mean ± SD 50 ± 10	n/a
pi6$^{em1/em1}$	1	0 5	0	n/a
	2	0 7	0	n/a
	3	8 12	50	33
	4	8 9 8 9	0	39
	5	10 10	40	80
	6	10 10	15	n/a
			mean ± SD 20 ± 20	50 ± 20

Wu et al. Figure 6



Wu et al., Figure S1, related to Figure 1



B

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C57BL/6     AGAAGACTGCCTACTCCAAGATAGTGGG......CACACAAGTGCCCAACGAAATGGAAAACA
sgRNA1      GACTGCCTACTCCAAGATAG    sgRNA2 CACACAAGTGCCCAACGAAA
pi6em1  1   AGAAGACTGCCTACTCCAAG-------(Δ219 bp)--------TGCCCAACGAAATGGAAAACA
pi6em1  2   AGAAGACTGCCTACTCCAAG-------(Δ230 bp)------------------ATGGAAAACA
pi6em1  3   AGAAGACTGCCTACTCCAA--------(Δ228 bp)---------------GAAATGGAAAACA
pi6em1  4   AGAAGACTGCCTACTCCAA--------(Δ233 bp)--------------------GGAAAACA
pi6em1  5   AGAAGACTGCCTACTCCAAGA------(Δ227 bp)----------------AAATGGAAAACA
pi6em1  6   AGAAGACTGCCTACTCCAA--------(Δ231 bp)------------------ATGGAAAACA
```

C

```
C57BL/6     ACGGTGGGTTCTATCCAATGAGGTC......GGGATAGAGTAAGTGAGAAGCTGGCCCTTACATCAT
sgRNA1      ACGGTGGGTTCTATCCAATG      sgRNA2 GGATAGAGTAAGTGAGAAGC
pi6em2  1   ACGGTGGGTTCTATCCAA-----(Δ116 bp)--------------------GCTGGCCCTTACATCAT
pi6em2  2   ACGGTGGG---------------(Δ125 bp)------------------AGCTGGCCCTTACATCAT
```

D

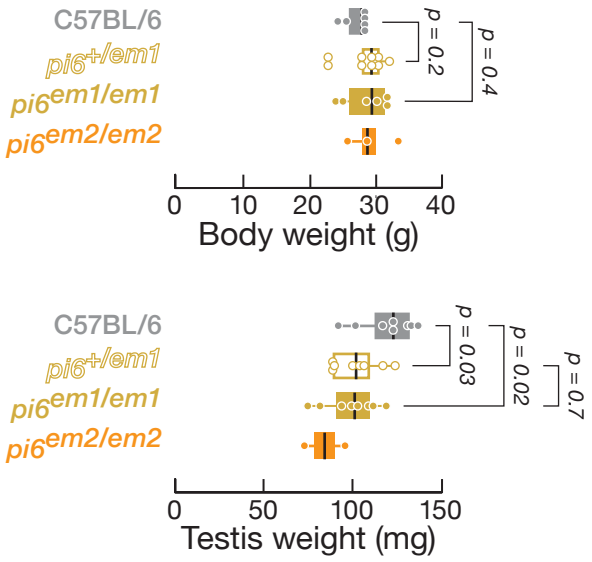
```
C57BL/6     GGGCTGCTCTGTCTGACAACGGGAC...TCACATCTCTGTGCAG...TCCCTTCACACGGCCGTTTA...CCGTCCCTGATAGTGG
sgRNA2      GCTCTGTCTGACAACGGGAC                  sgRNA1 TCCCTTCACACGGCCGTTTA
pi17-/-  1  GGGCTGCT---------------(Δ606 bp)-------------------------------------CCGTCCCTGATAGTGG
pi17-/-  2  GGGCTGCTCTGTCTGACAACG---(Δ583 bp)--------------------------------TTTA...CCGTCCCTGATAGTGG
pi17-/-  3  GGGCT------------------(Δ543 bp)----TCTGTGCAG...TCCCTTCACACGGCCGTTTA...CCGTCCCTGATAGTGG
```

Wu et al. Figure S2, related to Figure 2

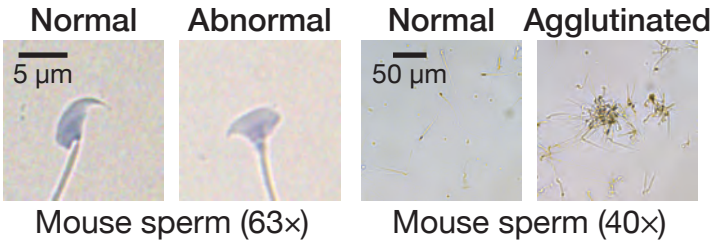
A

Father's genotype	Trial	Total litters	Viable litters
C57BL/6	1	6	5
	2	6	4
	3	7	3
	4	9	9
	5	6	4
pi6$^{+/em1}$	1	6	4
	2	5	5
	3	8	8
	4	7	5
	5	8	6
pi6$^{em1/em1}$	1	4	2
	2	0	0
	3	0	0
	4	2	0
	5	3	2
	6	2	0
pi6$^{em2/em2}$	1	4	3
	2	4	4

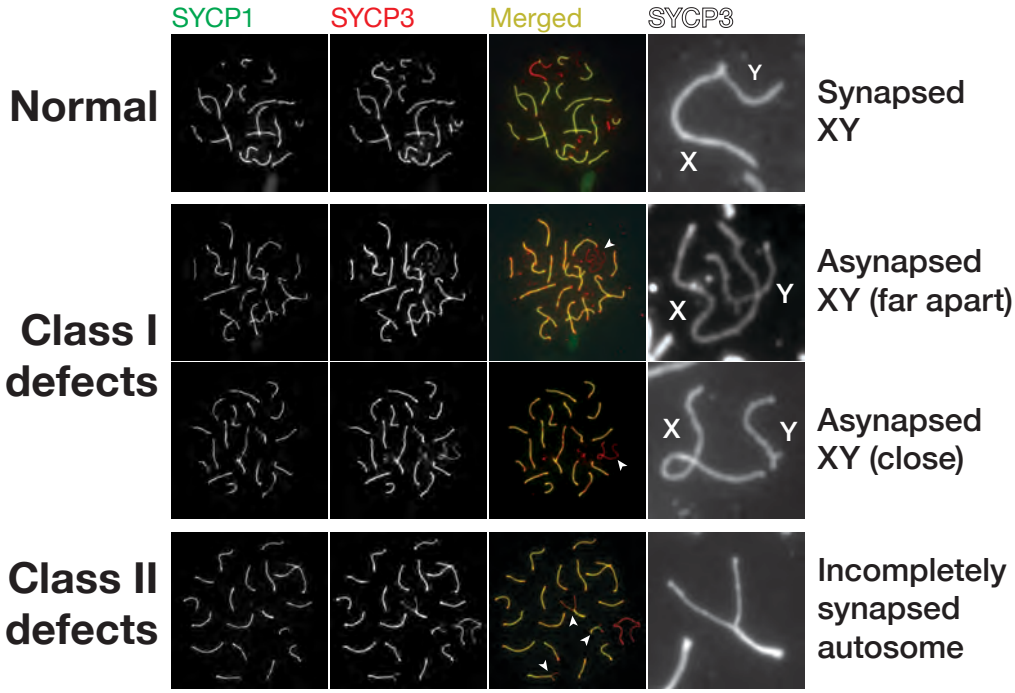
B



C



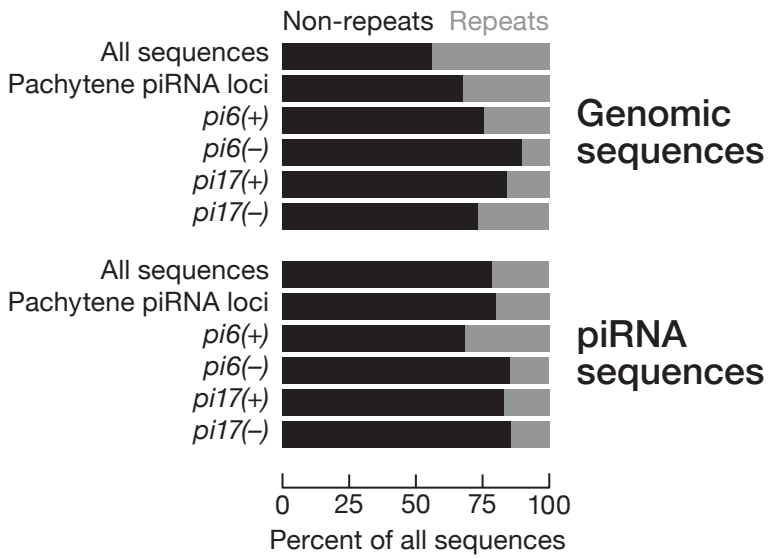
D



E

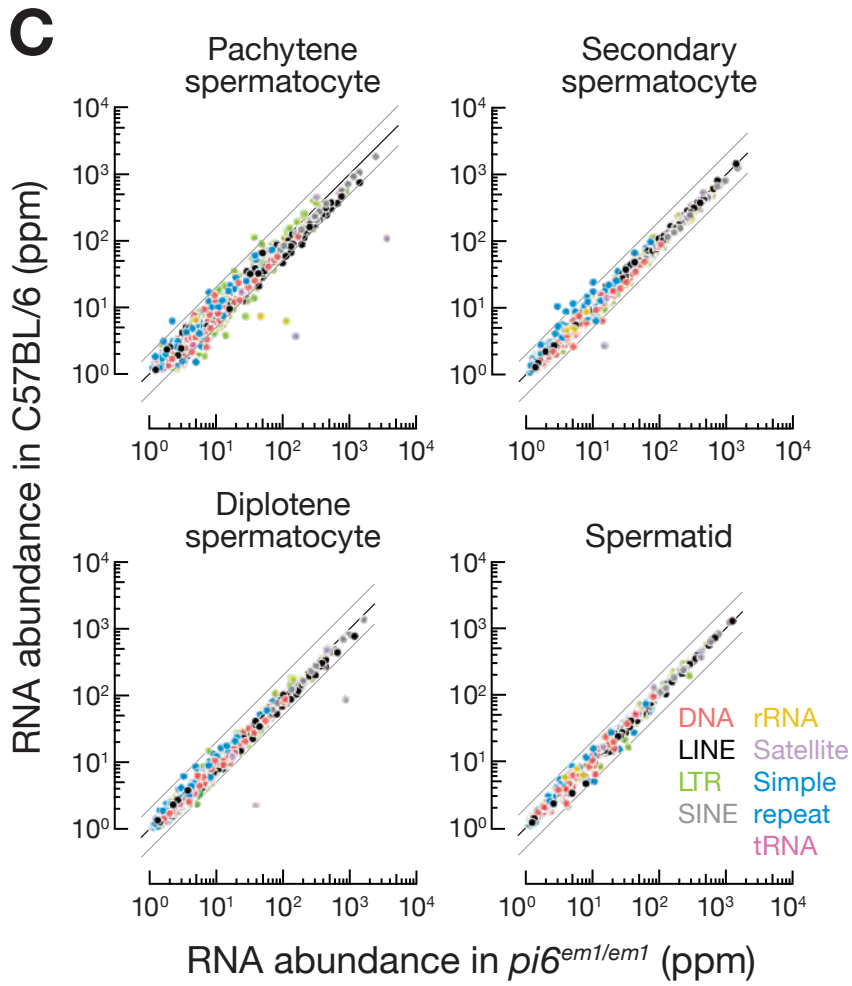
	C57BL/6					*pi6*$^{em1/em1}$					
Trial	**1**	**2**	**3**	**4**		**1**	**2**	**3**	**4**		
Cells counted	77	88	60	87	mean ± SD	129	74	82	88	mean ± SD	*p*-value
Class I (%)	6	8	2	8	6 ± 3	12	20	22	17	18 ± 5	0.03
Class II (%)	3	0	0	1	1 ± 1	3	18	11	2	8 ± 7	0.06
Class I and II (%)	1	0	0	0	0 ± 1	1	8	7	2	5 ± 4	0.05
Class I or II (%)	8	8	2	9	7 ± 3	14	30	26	17	22 ± 7	0.03

Wu et al. Figure S3, related to Figure 3



B

	Cell type	Exp.	piRNA abundance (ppm)
pi6	Pachytene spermatocyte	1	49,544
		2	45,166
	Diplotene spermatocyte	1	51,002
		2	50,994
	Secondary spermatocyte	1	53,028
		2	52,954
	Spermatid	1	53,532
		2	53,177
pi17	Pachytene spermatocyte	1	92,101
		2	147,559
	Diplotene spermatocyte	1	78,450
		2	82,417
	Secondary spermatocyte	1	59,860
		2	61,722
	Spermatid	1	62,025
		2	59,683
pi2	Pachytene spermatocyte	1	49,005
		2	43,379
	Diplotene spermatocyte	1	50,951
		2	50,434
	Secondary spermatocyte	1	53,282
		2	52,813
	Spermatid	1	53,985
		2	52,984



Wu et al. Figure S4, related to Figure 5.

A

Sperm donor genotype	Trial	Number and placement of two-cell embryos in surrogate mother	Surrogate mothers	Pregnant surrogate mothers
C57BL/6	1	12 12 12 12 12 12	3	3
	2	9 9 9 9	2	2
	3	10 10	1	1
				100%
pi6$^{+/em1}$	1	7 7 7 7	2	2
	2	12 13 12 13 12 13 12 13 12 12	5	5
	3	12 12 12 12 12 12 12 12 12 12	5	5
				100%
pi6$^{em1/em1}$	1	0 5	1	0
	2	0 5	1	0
	3	8 12	1	1
	4	8 9 8 9	2	1
	5	10 10	1	1
	6	10 10	1	1
				67%

B

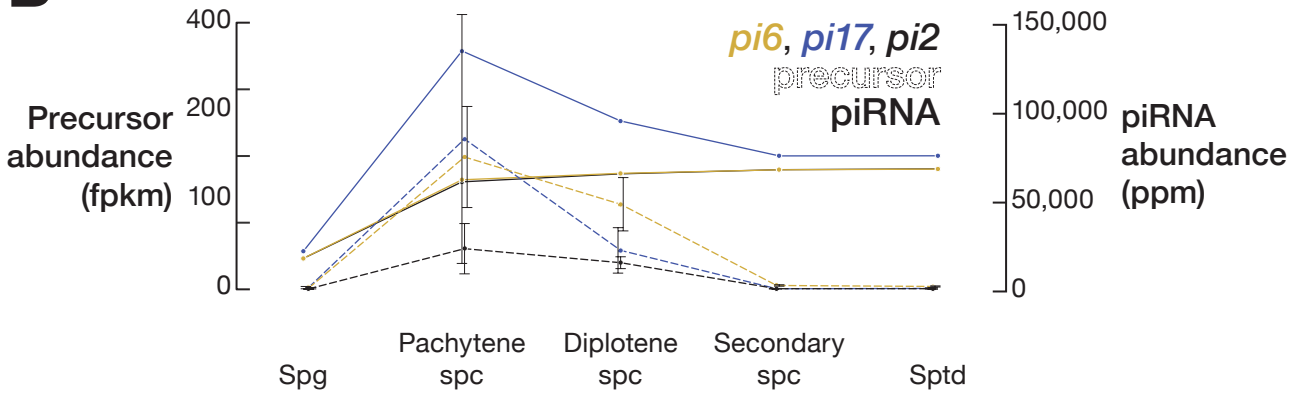
Sperm donor genotype	Trial	Number and placement of two-cell embryos in surrogate mother	Surrogate mothers	Pregnant surrogate mothers
pi6$^{+/em1}$	1	0 15 0 15	2	2
	2	19	1	1
				100%
pi6$^{em1/em1}$	1	0 13 0 13 0 14	3	2
	2	0 12 0 13 0 13 0 13	4	2
				57%

Wu et al. Figure S5, Related to Figure 6

A

Cell type	Number of genes with altered RNA abundance: *pi6*$^{em1/em1}$	*pi17*$^{-/-}$
Pachytene spermatocytes (4C)	875	0
Diplotene spermatocytes (4C)	9	277
Secondary spermatocytes (2C)	20	503
Spermatids (1C)	45	0
Total altered unique genes	*928*	*625*

B



C

	Genes	C57BL/6 (fpkm)	*pi6*$^{em1/em1}$ (fpkm)	*pi6*$^{em1/em1}$ / C57BL/6	FDR
Meiotic	*Atm*	3.5	12.3	3.2	2.2×10^{-2}
chromosome	*Dmc1*	1.6	9.2	4.6	2.6×10^{-2}
organization	*Syce1*	215.2	71.6	0.3	4.3×10^{-3}
miRNA	*Lin28a*	0.9	7.6	5.6	1.6×10^{-2}
pathway	*Zc3h7b*	1.6	10.1	5.0	4.3×10^{-3}
genes	*Ajuba*	0.6	5.4	5.3	7.0×10^{-3}

Table S1. Statistics of CRISPR injection for *pi6* mutant generation.

Allele	*pi6*^{em1}				*pi6*^{em2}
Nucleic acid injected	sgRNA + Cas9 mRNA	pX330 construct	sgRNA + Cas9 mRNA + pX330 construct	Total	sgRNA + Cas9 mRNA
Number of pups screened	55	45	42	142	23
Number of founders	5 (9%)	1 (2%)	2 (5%)	8 (6%)	5 (22%)
Number of female founders	3 (60%)	1 (100%)	1 (50%)	5 (63%)	2 (40%)
Number of male founders	2 (40%)	0 (0%)	1 (50%)	3 (38%)	3 (60%)
Number of surviving founders	5 (100%)	0 (0%)	2 (100%)	7 (88%)	5 (100%)

Table S2. Differentially expressed genes in *pi6*$^{em1/em1}$ germ cells.

Cell type	Ensembl ID	Gene	Genomic Location (mm10)	C57BL/6 (fpkm)	*pi6*$^{em1/em1}$ (fpkm)	*pi6*$^{em1/em1}$ / C57BL/6	FDR
Pac spc	ENSMUSG00000075014.1	*Gm10800*	chr2:98666546-98667301	132.8	3644.7	27.3	4.3×10^{-3}
Pac spc	ENSMUSG00000075015.3	*Gm10801*	chr2:98662236-98664083	12.7	299.3	22.6	3.3×10^{-2}
Pac spc	ENSMUSG00000021451.11	*Sema4d*	chr13:51701245-51793747	0.4	12.6	14.1	4.3×10^{-3}
Pac spc	ENSMUSG00000031584.12	*Gsr*	chr8:33652522-33698163	1.8	29.0	13.0	4.3×10^{-3}
Pac spc	ENSMUSG00000031229.12	*Atrx*	chrX:105797614-105929397	0.9	18.2	13.0	4.3×10^{-3}
Pac spc	ENSMUSG00000003949.12	*Hlf*	chr11:90336535-90390895	0.3	10.3	12.8	3.3×10^{-2}
Pac spc	ENSMUSG00000032841.11	*Prr5l*	chr2:101714284-101883256	0.3	9.1	12.8	2.0×10^{-2}
Pac spc	ENSMUSG00000042105.14	*Inpp5f*	chr7:128611327-128696425	0.7	14.2	12.2	2.3×10^{-2}
Pac spc	ENSMUSG00000081327.1	*Gm11819*	chr4:13444769-13445141	0.0	5.4	11.9	4.3×10^{-3}
Pac spc	ENSMUSG00000016386.11	*Mpped2*	chr2:106693268-106868356	0.4	9.4	11.0	1.8×10^{-2}
Pac spc	ENSMUSG00000083546.1	*Tpt1-ps1*	chr3:101233459-101233895	0.0	4.8	10.6	4.3×10^{-3}
Pac spc	ENSMUSG00000031161.11	*Hdac6*	chrX:7930119-7947889	2.5	30.7	10.4	4.3×10^{-3}
Pac spc	ENSMUSG00000039428.6	*Tmem135*	chr7:89139722-89404222	4.1	46.9	10.3	2.2×10^{-2}
Pac spc	ENSMUSG00000038080.12	*Kdm1b*	chr13:47025169-47084613	0.6	10.6	10.2	9.2×10^{-3}
Pac spc	ENSMUSG00000008318.5	*Relt*	chr7:100845847-100863446	0.3	7.9	10.2	3.1×10^{-2}
Pac spc	ENSMUSG00000005078.12	*Jkamp*	chr12:72085588-72185029	0.8	12.2	9.9	3.3×10^{-2}
Pac spc	ENSMUSG00000059625.6	*Sohlh1*	chr2:25842994-25847248	0.6	10.4	9.9	2.2×10^{-2}
Pac spc	ENSMUSG00000032135.10	*Mcam*	chr9:44123767-44142727	1.5	18.9	9.9	4.3×10^{-3}
Pac spc	ENSMUSG00000042453.10	*Reln*	chr5:21884453-22344702	1.6	19.4	9.6	4.3×10^{-3}
Pac spc	ENSMUSG00000016262.10	*Sertad4*	chr1:192844487-192856246	0.5	9.5	9.6	4.5×10^{-2}
Pac spc	ENSMUSG00000039323.14	*Igfbp2*	chr1:72824502-72852474	1.3	16.9	9.5	2.6×10^{-2}
Pac spc	ENSMUSG00000028487.14	*Bnc2*	chr4:84275094-84675275	1.6	19.5	9.5	4.3×10^{-3}
Pac spc	ENSMUSG00000068270.11	*Shroom4*	chrX:6399853-6637448	0.3	6.5	9.2	4.9×10^{-2}
Pac spc	ENSMUSG00000032598.8	*Nckipsd*	chr9:108808367-108818844	2.0	22.5	9.2	4.3×10^{-3}
Pac spc	ENSMUSG00000017760.11	*Ctsa*	chr2:164830731-164857711	0.6	9.9	9.1	4.3×10^{-3}

Pac spc	ENSMUSG00000019849.10	*Prep*	chr10:45067205-45158997	1.2	14.6	9.1	4.3×10^{-3}
Pac spc	ENSMUSG00000026923.11	*Notch1*	chr2:26445695-26516663	0.1	5.0	8.9	4.3×10^{-3}
Pac spc	ENSMUSG00000070371.7	*Prss36*	chr7:127932637-127946725	0.3	6.7	8.9	3.8×10^{-2}
Pac spc	ENSMUSG00000000247.7	*Lhx2*	chr2:38339280-38369733	0.1	5.3	8.9	4.3×10^{-3}
Pac spc	ENSMUSG00000027200.13	*Sema6d*	chr2:124089968-124667770	0.2	5.3	8.8	4.3×10^{-3}
Pac spc	ENSMUSG00000018417.10	*Myo1b*	chr1:51749764-51916071	0.9	12.1	8.8	7.0×10^{-3}
Pac spc	ENSMUSG00000050708.10	*Ftl1*	chr7:45457943-45459884	6.6	61.9	8.8	4.3×10^{-3}
Pac spc	ENSMUSG00000027547.13	*Sall4*	chr2:168748331-168768108	0.6	9.3	8.7	4.3×10^{-3}
Pac spc	ENSMUSG00000031431.9	*Tsc22d3*	chrX:140539527-140600659	3.8	36.4	8.6	4.3×10^{-3}
Pac spc	ENSMUSG00000071369.6	*Map3k5*	chr10:19934471-20142753	1.0	12.2	8.5	1.7×10^{-2}
Pac spc	ENSMUSG00000030796.11	*Tead2*	chr7:45215752-45239115	0.5	8.0	8.4	4.3×10^{-3}
Pac spc	ENSMUSG00000022763.12	*Aifm3*	chr16:17489610-17507485	0.6	8.5	8.4	1.1×10^{-2}
Pac spc	ENSMUSG00000058454.10	*Dhcr7*	chr7:143823144-143848410	2.5	24.8	8.4	4.3×10^{-3}
Pac spc	ENSMUSG00000086481.1	*Gm11707*	chr11:106972057-106973090	0.0	3.7	8.4	4.3×10^{-3}
Pac spc	ENSMUSG00000007891.11	*Ctsd*	chr7:142325836-142388038	3.7	34.1	8.3	4.3×10^{-3}
Pac spc	ENSMUSG00000025261.13	*Huwe1*	chrX:151800806-151935417	8.8	76.5	8.3	4.3×10^{-3}
Pac spc	ENSMUSG00000017386.6	*Traf4*	chr11:78158498-78165589	0.8	10.0	8.2	9.2×10^{-3}
Pac spc	ENSMUSG00000053398.7	*Phgdh*	chr3:98313169-98339990	0.7	9.3	8.2	1.8×10^{-2}
Pac spc	ENSMUSG00000005672.8	*Kit*	chr5:75574915-75656722	2.0	20.2	8.1	4.3×10^{-3}
Pac spc	ENSMUSG00000009376.11	*Met*	chr6:17463799-17573980	0.3	6.0	8.1	2.1×10^{-2}
Pac spc	ENSMUSG00000028293.10	*Slc35a1*	chr4:34663256-34687438	0.9	11.2	8.1	1.5×10^{-2}
Pac spc	ENSMUSG00000061462.11	*Obscn*	chr11:58994255-59136402	0.5	7.8	8.0	1.9×10^{-2}
Pac spc	ENSMUSG00000031353.9	*Rbbp7*	chrX:162760401-162829454	2.5	23.6	8.0	2.8×10^{-2}
Pac spc	ENSMUSG00000039382.7	*Wdr45*	chrX:7714332-7728201	0.7	9.0	8.0	4.3×10^{-3}
Pac spc	ENSMUSG00000032291.8	*Crabp1*	chr9:54764747-54773110	2.9	26.6	7.9	7.0×10^{-3}
Pac spc	ENSMUSG00000027669.10	*Gnb4*	chr3:32580331-32616585	0.3	5.7	7.9	1.6×10^{-2}
Pac spc	ENSMUSG00000039683.12	*Sdk1*	chr5:141241489-142215586	1.7	16.5	7.9	1.5×10^{-2}
Pac spc	ENSMUSG00000025577.7	*Cbx2*	chr11:119022961-119031270	0.9	10.7	7.8	4.3×10^{-3}
Pac spc	ENSMUSG00000030199.12	*Etv6*	chr6:134035699-134270158	1.4	14.2	7.7	3.8×10^{-2}

Pac spc	ENSMUSG00000022433.14	*Csnk1e*	chr15:79417855-79443919	0.6	8.1	7.6	7.0×10^{-3}
Pac spc	ENSMUSG00000001525.10	*Tubb5*	chr17:35833920-35838306	2.2	19.8	7.6	4.3×10^{-3}
Pac spc	ENSMUSG00000036893.12	*Ehmt1*	chr2:24790768-24919609	2.4	21.3	7.6	4.3×10^{-3}
Pac spc	ENSMUSG00000031403.10	*Dkc1*	chrX:75095853-75131016	1.2	12.7	7.6	2.2×10^{-2}
Pac spc	ENSMUSG00000006494.7	*Pdk1*	chr2:71873223-71903858	0.3	5.7	7.5	2.4×10^{-2}
Pac spc	ENSMUSG00000029177.5	*Cenpa*	chr5:30666776-30674827	1.2	11.7	7.4	4.8×10^{-2}
Pac spc	ENSMUSG00000035202.7	*Lars2*	chr9:123366939-123462664	2.7	22.9	7.3	4.3×10^{-3}
Pac spc	ENSMUSG00000002227.11	*Mov10*	chr3:104794835-104818563	1.4	13.3	7.2	4.3×10^{-3}
Pac spc	ENSMUSG00000042439.8	*Zfp532*	chr18:65580229-65689443	1.2	11.6	7.2	4.3×10^{-3}
Pac spc	ENSMUSG00000001082.8	*Mfsd10*	chr5:34633641-34637212	1.1	10.7	7.2	4.3×10^{-3}
Pac spc	ENSMUSG00000041417.11	*Pik3r1*	chr13:101680562-101768217	0.2	4.5	7.2	1.8×10^{-2}
Pac spc	ENSMUSG00000025272.12	*Tro*	chrX:150645303-150657583	0.5	6.5	7.2	4.3×10^{-3}
Pac spc	ENSMUSG00000004317.10	*Clcn5*	chrX:7153809-7319358	0.6	7.8	7.2	1.8×10^{-2}
Pac spc	ENSMUSG00000012123.11	*Aim1l*	chr4:134065911-134095082	1.5	13.5	7.2	2.7×10^{-2}
Pac spc	ENSMUSG00000024968.9	*Rcor2*	chr19:7267324-7275225	1.0	10.4	7.1	2.0×10^{-2}
Pac spc	ENSMUSG00000036564.12	*Ndrg4*	chr8:95676979-95715119	1.6	14.5	7.1	4.3×10^{-3}
Pac spc	ENSMUSG00000004328.11	*Hif3a*	chr7:17031506-17062427	0.1	3.6	7.1	3.0×10^{-2}
Pac spc	ENSMUSG00000016239.7	*Lonrf3*	chrX:36328352-36362341	2.1	18.2	7.1	4.3×10^{-3}
Pac spc	ENSMUSG00000002870.7	*Mcm2*	chr6:88883474-88898780	2.5	20.7	7.1	4.3×10^{-3}
Pac spc	ENSMUSG00000040749.7	*Siah1b*	chrX:164070704-164076493	1.5	13.6	7.1	4.9×10^{-2}
Pac spc	ENSMUSG00000026956.11	*Uap1l1*	chr2:25359888-25365682	0.4	6.1	7.1	2.3×10^{-2}
Pac spc	ENSMUSG00000025815.9	*Dhtkd1*	chr2:5895509-5942792	0.5	6.2	7.0	4.4×10^{-2}
Pac spc	ENSMUSG00000000787.8	*Ddx3x*	chrX:13280969-13294052	1.0	9.9	7.0	1.5×10^{-2}
Pac spc	ENSMUSG00000030091.13	*Nup210*	chr6:91013067-91116829	1.7	14.7	7.0	4.3×10^{-3}
Pac spc	ENSMUSG00000000325.11	*Arvcf*	chr16:18348181-18479073	1.1	10.8	7.0	4.3×10^{-3}
Pac spc	ENSMUSG00000073294.4	*AU022751*	chrX:6027055-6092269	0.3	5.3	7.0	3.7×10^{-2}
Pac spc	ENSMUSG00000031103.8	*Elf4*	chrX:48411045-48463132	0.3	5.3	7.0	1.6×10^{-2}
Pac spc	ENSMUSG00000024837.11	*Dmrt1*	chr19:25505617-25604329	3.8	29.4	7.0	4.3×10^{-3}
Pac spc	ENSMUSG00000000037.12	*Scml2*	chrX:161117192-161258213	3.2	24.6	6.8	7.0×10^{-3}

Pac spc	ENSMUSG00000009670.7	*Tex11*	chrX:100838647-101059667	1.5	12.9	6.8	1.3×10^{-2}
Pac spc	ENSMUSG00000056004.12	*9330182L06Rik*	chr5:9266117-9481825	0.1	3.8	6.8	4.3×10^{-3}
Pac spc	ENSMUSG00000034168.6	*Irf2bpl*	chr12:86880702-86884814	0.6	6.6	6.8	4.3×10^{-3}
Pac spc	ENSMUSG00000000420.11	*Galnt1*	chr18:24205343-24286818	1.9	15.5	6.7	4.3×10^{-3}
Pac spc	ENSMUSG00000034673.10	*Pbx2*	chr17:34589805-34597400	2.1	16.8	6.7	1.8×10^{-2}
Pac spc	ENSMUSG00000034926.3	*Dhcr24*	chr4:106561037-106589113	1.4	11.8	6.6	4.3×10^{-3}
Pac spc	ENSMUSG00000045374.14	*Wdr81*	chr11:75440943-75454717	0.6	7.0	6.6	4.3×10^{-3}
Pac spc	ENSMUSG00000043993.6	*2900052L18Rik*	chr11:120229801-120231585	0.3	5.0	6.6	3.9×10^{-2}
Pac spc	ENSMUSG00000037138.12	*Aff3*	chr1:38177325-38664955	0.4	5.8	6.6	3.2×10^{-2}
Pac spc	ENSMUSG00000030123.11	*Plxnd1*	chr6:115954810-115995005	0.8	8.4	6.6	4.3×10^{-3}
Pac spc	ENSMUSG00000038764.10	*Ptpn3*	chr4:57190840-57307305	0.2	4.1	6.6	1.7×10^{-2}
Pac spc	ENSMUSG00000006378.9	*Gcat*	chr15:79030873-79043558	0.8	8.0	6.5	1.8×10^{-2}
Pac spc	ENSMUSG00000039316.10	*Rftn1*	chr17:49992256-50190674	0.8	8.2	6.5	4.9×10^{-2}
Pac spc	ENSMUSG00000020387.11	*Jade2*	chr11:51813454-51857653	0.6	6.4	6.5	4.3×10^{-3}
Pac spc	ENSMUSG00000051817.8	*Sox12*	chr2:152393610-152398063	0.6	6.3	6.5	7.0×10^{-3}
Pac spc	ENSMUSG00000069053.7	*Uba1y*	chrY:818648-847750	2.0	15.3	6.4	4.3×10^{-3}
Pac spc	ENSMUSG00000030530.11	*Furin*	chr7:80388584-80405436	1.0	9.3	6.4	9.2×10^{-3}
Pac spc	ENSMUSG00000019822.8	*Smpd2*	chr10:41476313-41490369	1.6	13.1	6.4	1.7×10^{-2}
Pac spc	ENSMUSG00000026944.14	*Abca2*	chr2:25428702-25448540	2.4	18.3	6.4	4.3×10^{-3}
Pac spc	ENSMUSG00000028980.10	*H6pd*	chr4:149979474-150009023	0.2	3.7	6.4	3.1×10^{-2}
Pac spc	ENSMUSG00000042506.11	*Usp22*	chr11:61151784-61175055	1.9	15.0	6.3	9.2×10^{-3}
Pac spc	ENSMUSG00000039741.11	*Bahcc1*	chr11:120232946-120292296	1.1	9.4	6.3	4.3×10^{-3}
Pac spc	ENSMUSG00000015291.6	*Gdi1*	chrX:74304997-74311862	0.7	7.3	6.3	1.7×10^{-2}
Pac spc	ENSMUSG00000006369.10	*Fbln1*	chr15:85205948-85286535	1.0	8.9	6.3	2.0×10^{-2}
Pac spc	ENSMUSG00000046774.12	*8030474K03Rik*	chrX:101794655-101798642	1.2	10.1	6.2	1.7×10^{-2}
Pac spc	ENSMUSG00000025764.10	*Jade1*	chr3:41555730-41616864	2.0	15.0	6.2	4.3×10^{-3}
Pac spc	ENSMUSG00000044349.11	*Snhg11*	chr2:158375637-158386145	0.2	4.1	6.2	1.7×10^{-2}
Pac spc	ENSMUSG00000074480.4	*Mex3a*	chr3:88532394-88541396	0.5	5.7	6.2	4.3×10^{-3}
Pac spc	ENSMUSG00000034714.9	*Ttyh2*	chr11:114675430-114720977	0.7	7.0	6.2	9.2×10^{-3}

Pac spc	ENSMUSG00000022216.12	*Psme1*	chr14:55578122-55585302	1.8	13.7	6.2	4.0×10^{-2}
Pac spc	ENSMUSG00000026074.10	*Map4k4*	chr1:39900912-40026310	1.9	14.4	6.2	1.3×10^{-2}
Pac spc	ENSMUSG00000021466.7	*Ptch1*	chr13:63508327-63573598	0.2	3.6	6.1	2.3×10^{-2}
Pac spc	ENSMUSG00000013033.12	*Lphn1*	chr8:83900104-83955205	2.4	17.1	6.1	4.1×10^{-2}
Pac spc	ENSMUSG00000032312.6	*Csk*	chr9:57626646-57645653	0.7	7.0	6.1	1.6×10^{-2}
Pac spc	ENSMUSG00000099502.1	*Gm28640*	chr2:74130180-74130730	0.0	2.5	6.1	4.9×10^{-2}
Pac spc	ENSMUSG00000025558.11	*Dock9*	chr14:121542038-121797734	1.1	9.5	6.1	4.3×10^{-3}
Pac spc	ENSMUSG00000023262.8	*Acy1*	chr9:106432980-106438319	0.4	5.0	6.0	3.0×10^{-2}
Pac spc	ENSMUSG00000082670.1	*Gm14050*	chr2:122207919-122208265	0.0	2.5	6.0	1.7×10^{-2}
Pac spc	ENSMUSG00000055780.6	*Usp26*	chrX:51753958-51801233	1.6	12.3	6.0	4.3×10^{-3}
Pac spc	ENSMUSG00000034690.8	*Nlrp4c*	chr7:6045160-6105149	0.8	7.3	6.0	1.3×10^{-2}
Pac spc	ENSMUSG00000019087.9	*Atp6ap1*	chrX:74297096-74304721	1.6	11.7	5.9	7.0×10^{-3}
Pac spc	ENSMUSG00000047945.6	*Marcksl1*	chr4:129513580-129515985	7.6	47.5	5.9	4.3×10^{-3}
Pac spc	ENSMUSG00000020806.11	*Rhbdf2*	chr11:116598164-116627019	0.7	6.6	5.9	1.8×10^{-2}
Pac spc	ENSMUSG00000032812.12	*Arap1*	chr7:101348066-101412586	0.5	5.1	5.9	4.3×10^{-3}
Pac spc	ENSMUSG00000020661.11	*Dnmt3a*	chr12:3806006-3914443	1.6	11.6	5.9	4.3×10^{-3}
Pac spc	ENSMUSG00000046574.7	*Prr12*	chr7:45027706-45052881	0.9	7.5	5.8	4.3×10^{-3}
Pac spc	ENSMUSG00000005533.9	*Igf1r*	chr7:67952858-68226780	2.5	16.7	5.8	4.3×10^{-3}
Pac spc	ENSMUSG00000042410.11	*Agps*	chr2:75832176-75931350	1.7	12.2	5.8	4.3×10^{-3}
Pac spc	ENSMUSG00000072944.7	*Nup62cl*	chrX:140006804-140062712	0.8	7.3	5.8	3.4×10^{-2}
Pac spc	ENSMUSG00000004221.12	*Ikbkg*	chrX:74393289-74453854	0.2	3.7	5.8	7.0×10^{-3}
Pac spc	ENSMUSG00000033792.8	*Atp7a*	chrX:106027275-106124926	0.4	4.5	5.8	3.1×10^{-2}
Pac spc	ENSMUSG00000071773.4	*Rhox1*	chrX:37213803-37222258	0.0	2.4	5.8	4.3×10^{-3}
Pac spc	ENSMUSG00000027359.12	*Slc27a2*	chr2:126521201-126588243	0.3	4.0	5.7	2.5×10^{-2}
Pac spc	ENSMUSG00000025503.4	*Taldo1*	chr7:141392198-141402968	2.6	17.1	5.7	1.6×10^{-2}
Pac spc	ENSMUSG00000019055.11	*Plod1*	chr4:147909752-147936767	1.8	12.4	5.7	4.3×10^{-3}
Pac spc	ENSMUSG00000029223.9	*Uchl1*	chr5:66626494-66687231	16.5	96.1	5.7	4.3×10^{-3}
Pac spc	ENSMUSG00000028782.10	*Bai2*	chr4:129984869-130022633	0.4	4.8	5.7	4.3×10^{-3}
Pac spc	ENSMUSG00000055612.11	*Cdca7*	chr2:72476158-72486893	1.8	12.7	5.7	7.0×10^{-3}

Pac spc	ENSMUSG00000050966.5	*Lin28a*	chr4:134003329-134019869	0.9	7.6	5.6	1.6×10^{-2}
Pac spc	ENSMUSG00000031157.6	*Pqbp1*	chrX:7894518-7899269	0.4	4.7	5.6	2.2×10^{-2}
Pac spc	ENSMUSG00000079487.7	*Med12*	chrX:101274029-101325963	3.0	18.9	5.6	4.3×10^{-3}
Pac spc	ENSMUSG00000028434.8	*Epb4.1l4b*	chr4:56991971-57143437	0.8	6.8	5.6	2.1×10^{-2}
Pac spc	ENSMUSG00000037344.9	*Slc12a9*	chr5:137314557-137333597	1.2	8.8	5.6	1.3×10^{-2}
Pac spc	ENSMUSG00000028078.10	*Dclk2*	chr3:86786150-86920852	0.3	4.1	5.6	1.7×10^{-2}
Pac spc	ENSMUSG00000016534.11	*Lamp2*	chrX:38401356-38456454	2.2	14.3	5.6	4.2×10^{-2}
Pac spc	ENSMUSG00000057897.10	*Camk2b*	chr11:5969643-6066362	0.3	4.0	5.5	2.1×10^{-2}
Pac spc	ENSMUSG00000002028.8	*Kmt2a*	chr9:44803354-44881296	1.2	9.0	5.5	4.3×10^{-3}
Pac spc	ENSMUSG00000020097.10	*Sgpl1*	chr10:61098641-61147703	2.4	15.4	5.5	4.3×10^{-3}
Pac spc	ENSMUSG00000037824.5	*Tspan14*	chr14:40906444-40966807	1.0	7.6	5.5	1.3×10^{-2}
Pac spc	ENSMUSG00000030084.7	*Plxna1*	chr6:89304629-89362613	3.2	20.0	5.5	4.3×10^{-3}
Pac spc	ENSMUSG00000039262.12	*Prrc2b*	chr2:32151081-32236382	1.8	12.0	5.5	1.7×10^{-2}
Pac spc	ENSMUSG00000066687.4	*Zbtb16*	chr9:48654310-48835945	0.9	7.0	5.5	4.3×10^{-3}
Pac spc	ENSMUSG00000029804.12	*Herc3*	chr6:58831464-58920398	0.8	6.5	5.5	4.3×10^{-3}
Pac spc	ENSMUSG00000020653.7	*Klf11*	chr12:24651370-24662774	1.2	8.7	5.5	1.4×10^{-2}
Pac spc	ENSMUSG00000005413.7	*Hmox1*	chr8:75093590-75100596	0.6	5.3	5.4	4.2×10^{-2}
Pac spc	ENSMUSG00000028032.9	*Papss1*	chr3:131564767-131643670	0.8	6.5	5.4	1.3×10^{-2}
Pac spc	ENSMUSG00000041936.14	*Agrn*	chr4:156165289-156197488	0.9	7.2	5.4	2.6×10^{-2}
Pac spc	ENSMUSG00000045237.5	*1110012L19Rik*	chrX:70385876-70389417	0.0	2.2	5.4	4.3×10^{-3}
Pac spc	ENSMUSG00000031167.12	*Rbm3*	chrX:8138974-8147964	2.4	15.1	5.4	3.3×10^{-2}
Pac spc	ENSMUSG00000044150.8	*A830080D01Rik*	chrX:159526687-159593081	1.2	8.4	5.4	1.8×10^{-2}
Pac spc	ENSMUSG00000017561.12	*Crlf3*	chr11:80046492-80080991	1.2	8.5	5.4	3.2×10^{-2}
Pac spc	ENSMUSG00000045294.10	*Insig1*	chr5:28071362-28078662	3.1	19.0	5.4	7.0×10^{-3}
Pac spc	ENSMUSG00000001506.10	*Col1a1*	chr11:94936223-94953042	0.1	3.0	5.4	1.8×10^{-2}
Pac spc	ENSMUSG00000045659.13	*Plekha7*	chr7:116123492-116308376	0.6	5.2	5.4	4.3×10^{-3}
Pac spc	ENSMUSG00000007379.11	*Dennd2c*	chr3:103102603-103169769	0.8	6.5	5.4	3.8×10^{-2}
Pac spc	ENSMUSG00000033434.11	*Gtpbp6*	chr5:110099968-110108197	3.5	20.7	5.4	3.8×10^{-2}
Pac spc	ENSMUSG00000021996.12	*Esd*	chr14:74732296-74750765	4.1	24.0	5.3	3.2×10^{-2}

Pac spc	ENSMUSG00000057541.10	*Pus7*	chr5:23740647-23783711	1.3	9.2	5.3	2.1×10^{-2}
Pac spc	ENSMUSG00000013089.11	*Etv5*	chr16:22381308-22439719	0.4	4.3	5.3	1.9×10^{-2}
Pac spc	ENSMUSG00000008489.14	*Elavl2*	chr4:91250762-91400785	1.2	8.5	5.3	2.5×10^{-2}
Pac spc	ENSMUSG00000022178.10	*Ajuba*	chr14:54567468-54577661	0.6	5.4	5.3	7.0×10^{-3}
Pac spc	ENSMUSG00000038437.7	*Mllt6*	chr11:97663216-97685463	1.3	8.7	5.3	4.3×10^{-3}
Pac spc	ENSMUSG00000000838.13	*Fmr1*	chrX:68678484-68717963	3.1	18.2	5.3	4.3×10^{-3}
Pac spc	ENSMUSG00000028654.9	*Mycl*	chr4:122995651-123002485	0.1	2.7	5.3	1.9×10^{-2}
Pac spc	ENSMUSG00000034771.11	*Tle2*	chr10:81572611-81590845	0.2	2.9	5.2	4.3×10^{-3}
Pac spc	ENSMUSG00000025795.7	*Rassf3*	chr10:121410349-121476250	0.2	3.2	5.2	4.1×10^{-2}
Pac spc	ENSMUSG00000028405.9	*Aco1*	chr4:40143080-40198338	0.6	5.3	5.2	1.7×10^{-2}
Pac spc	ENSMUSG00000048240.10	*Gng7*	chr10:80948623-81014945	0.1	2.8	5.2	3.5×10^{-2}
Pac spc	ENSMUSG00000052373.10	*Mpp3*	chr11:101999651-102028461	0.2	3.2	5.2	4.0×10^{-2}
Pac spc	ENSMUSG00000018899.12	*Irf1*	chr11:53770013-53778374	0.4	4.1	5.2	1.1×10^{-2}
Pac spc	ENSMUSG00000097119.1	*B230354K17Rik*	chr17:45433851-45442544	1.5	9.6	5.2	4.3×10^{-3}
Pac spc	ENSMUSG00000034160.9	*Ogt*	chrX:101640059-101684351	1.7	10.9	5.1	1.6×10^{-2}
Pac spc	ENSMUSG00000037315.10	*Jade3*	chrX:20425687-20519939	1.2	8.5	5.1	4.0×10^{-2}
Pac spc	ENSMUSG00000036591.11	*Arhgap21*	chr2:20847918-20968881	1.4	9.4	5.1	1.8×10^{-2}
Pac spc	ENSMUSG00000002059.13	*Rab34*	chr11:78188429-78192193	0.7	5.8	5.1	2.8×10^{-2}
Pac spc	ENSMUSG00000017009.3	*Sdc4*	chr2:164424246-164443887	1.4	9.0	5.1	1.9×10^{-2}
Pac spc	ENSMUSG00000029822.11	*Osbpl3*	chr6:50293329-50456201	0.1	2.7	5.1	1.1×10^{-2}
Pac spc	ENSMUSG00000045411.12	*2410002F23Rik*	chr7:44246721-44262720	4.7	25.7	5.1	1.8×10^{-2}
Pac spc	ENSMUSG00000102859.1	*RP23-20B1.1*	chr3:73933045-73934122	0.0	2.0	5.1	4.3×10^{-3}
Pac spc	ENSMUSG00000003070.6	*Efna2*	chr10:80179481-80190010	0.3	3.3	5.0	4.0×10^{-2}
Pac spc	ENSMUSG00000038072.10	*Galnt11*	chr5:25222847-25265918	0.9	6.8	5.0	2.2×10^{-2}
Pac spc	ENSMUSG00000022390.10	*Zc3h7b*	chr15:81744847-81796269	1.6	10.1	5.0	4.3×10^{-3}
Pac spc	ENSMUSG00000031012.13	*Cask*	chrX:13517079-13851367	0.4	4.0	5.0	2.9×10^{-2}
Pac spc	ENSMUSG00000037706.12	*Cd81*	chr7:143021783-143067934	2.1	12.3	4.9	3.9×10^{-2}
Pac spc	ENSMUSG00000040732.14	*Erg*	chr16:95359168-95586593	0.2	3.1	4.9	1.5×10^{-2}
Pac spc	ENSMUSG00000060216.11	*Arrb2*	chr11:70432634-70440828	0.9	6.5	4.9	4.9×10^{-2}

Pac spc	ENSMUSG00000031150.8	*Ccdc120*	chrX:7731713-7750905	0.1	2.4	4.9	1.1×10^{-2}
Pac spc	ENSMUSG00000038677.9	*Scube3*	chr17:28142315-28174852	0.7	5.2	4.9	1.8×10^{-2}
Pac spc	ENSMUSG00000020923.13	*Ubtf*	chr11:102304559-102319742	2.7	15.0	4.9	1.3×10^{-2}
Pac spc	ENSMUSG00000024640.5	*Psat1*	chr19:15904677-15947337	2.6	14.7	4.9	1.9×10^{-2}
Pac spc	ENSMUSG00000103643.1	*RP24-271K21.1*	chr3:32260332-32261104	0.0	1.9	4.9	4.9×10^{-2}
Pac spc	ENSMUSG00000021318.11	*Gli3*	chr13:15440301-15730026	1.1	7.2	4.8	3.9×10^{-2}
Pac spc	ENSMUSG00000031558.11	*Slit2*	chr5:47983154-48306282	0.7	5.2	4.8	4.9×10^{-2}
Pac spc	ENSMUSG00000079584.2	*Gm364*	chrX:57409153-57488767	4.3	22.3	4.8	1.7×10^{-2}
Pac spc	ENSMUSG00000005125.8	*Ndrg1*	chr15:66929320-67013039	0.3	3.2	4.8	1.4×10^{-2}
Pac spc	ENSMUSG00000042515.9	*Mum1l1*	chrX:139210041-139238335	0.8	5.7	4.7	1.3×10^{-2}
Pac spc	ENSMUSG00000067873.7	*Htatsf1*	chrX:57053582-57067183	0.8	5.6	4.7	1.4×10^{-2}
Pac spc	ENSMUSG00000090673.1	*Gm340*	chr19:41582369-41586536	0.9	6.3	4.7	2.9×10^{-2}
Pac spc	ENSMUSG00000037712.11	*Fermt2*	chr14:45458791-45530118	0.8	5.8	4.7	2.6×10^{-2}
Pac spc	ENSMUSG00000008435.11	*Rdh13*	chr7:4424769-4445649	0.3	3.2	4.7	1.7×10^{-2}
Pac spc	ENSMUSG00000031397.7	*Tktl1*	chrX:74177258-74208500	1.6	9.5	4.7	3.1×10^{-2}
Pac spc	ENSMUSG00000061731.5	*Ext1*	chr15:53064037-53346159	0.5	4.1	4.7	2.4×10^{-2}
Pac spc	ENSMUSG00000017724.10	*Etv4*	chr11:101769741-101785371	0.3	3.3	4.7	2.9×10^{-2}
Pac spc	ENSMUSG00000024070.11	*Prkd3*	chr17:78949404-79020816	2.7	14.6	4.7	1.4×10^{-2}
Pac spc	ENSMUSG00000031314.13	*Taf1*	chrX:101532733-101601789	2.1	11.7	4.7	4.1×10^{-2}
Pac spc	ENSMUSG00000031214.9	*Ophn1*	chrX:98554276-98891025	1.0	6.5	4.7	1.7×10^{-2}
Pac spc	ENSMUSG00000032511.13	*Scn5a*	chr9:119483407-119579016	0.3	3.4	4.7	4.3×10^{-3}
Pac spc	ENSMUSG00000025246.9	*Tbl1x*	chrX:77511012-77662983	0.7	4.9	4.7	1.9×10^{-2}
Pac spc	ENSMUSG00000067768.8	*Xlr4b*	chrX:73107634-73292976	0.0	2.0	4.6	3.8×10^{-2}
Pac spc	ENSMUSG00000022429.10	*Dmc1*	chr15:79561499-79605084	1.6	9.2	4.6	2.6×10^{-2}
Pac spc	ENSMUSG00000002900.11	*Lamb1*	chr12:31265233-31329644	0.5	4.2	4.6	1.6×10^{-2}
Pac spc	ENSMUSG00000032936.9	*Camkv*	chr9:107935076-107949691	0.4	3.7	4.6	2.6×10^{-2}
Pac spc	ENSMUSG00000029366.9	*Dck*	chr5:88764995-88783281	0.8	5.3	4.6	1.3×10^{-2}
Pac spc	ENSMUSG00000026860.12	*Sh3glb2*	chr2:30344808-30359337	2.4	13.0	4.6	3.0×10^{-2}
Pac spc	ENSMUSG00000029998.10	*Pcyox1*	chr6:86386005-86397150	0.3	3.3	4.6	2.5×10^{-2}

Pac spc	ENSMUSG00000013936.8	*Myl2*	chr5:122100950-122138957	0.0	1.8	4.6	4.3×10^{-3}
Pac spc	ENSMUSG00000009941.6	*Nxf2*	chrX:134944525-134964754	2.0	10.9	4.6	2.9×10^{-2}
Pac spc	ENSMUSG00000028080.11	*Lrba*	chr3:86224679-86782692	3.0	15.5	4.5	4.3×10^{-3}
Pac spc	ENSMUSG00000036989.11	*Trim3*	chr7:105604462-105633571	1.0	6.1	4.5	4.0×10^{-2}
Pac spc	ENSMUSG00000027395.11	*Polr1b*	chr2:129100994-129126594	3.4	17.1	4.5	4.3×10^{-3}
Pac spc	ENSMUSG00000026643.12	*Nmt2*	chr2:3284211-3328877	6.5	31.2	4.5	7.0×10^{-3}
Pac spc	ENSMUSG00000024811.7	*Tnks2*	chr19:36834231-36893477	5.6	26.9	4.5	4.3×10^{-3}
Pac spc	ENSMUSG00000020432.8	*Tcn2*	chr11:3917191-3932159	0.3	3.0	4.5	3.8×10^{-2}
Pac spc	ENSMUSG00000063239.12	*Grm4*	chr17:27422386-27513341	0.1	2.1	4.5	3.6×10^{-2}
Pac spc	ENSMUSG00000089989.5	*Flt3l*	chr7:45125557-45136432	0.3	3.0	4.5	4.8×10^{-2}
Pac spc	ENSMUSG00000026192.9	*Atic*	chr1:71557149-71579631	2.2	11.6	4.5	9.2×10^{-3}
Pac spc	ENSMUSG00000037553.10	*Zdhhc18*	chr4:133605298-133650154	0.7	4.8	4.5	3.9×10^{-2}
Pac spc	ENSMUSG00000042644.8	*Itpr3*	chr17:27057303-27122223	0.7	4.9	4.5	1.1×10^{-2}
Pac spc	ENSMUSG00000020715.5	*Ern1*	chr11:106394649-106487852	2.1	11.1	4.5	1.1×10^{-2}
Pac spc	ENSMUSG00000021069.12	*Pygl*	chr12:70190810-70234165	0.5	3.8	4.4	4.5×10^{-2}
Pac spc	ENSMUSG00000047098.13	*Rnf31*	chr14:55591707-55610030	2.2	11.5	4.4	2.7×10^{-2}
Pac spc	ENSMUSG00000030110.9	*Ret*	chr6:118151747-118197744	0.1	2.1	4.4	2.8×10^{-2}
Pac spc	ENSMUSG00000045071.9	*E130308A19Rik*	chr4:59626210-59761439	0.7	4.6	4.4	3.2×10^{-2}
Pac spc	ENSMUSG00000016757.6	*Ttll12*	chr15:83575118-83595157	1.4	8.1	4.4	4.3×10^{-3}
Pac spc	ENSMUSG00000021109.9	*Hif1a*	chr12:73901374-73949785	2.3	11.9	4.4	4.3×10^{-2}
Pac spc	ENSMUSG00000034311.3	*Kif4*	chrX:100622882-100727214	0.6	4.5	4.4	1.7×10^{-2}
Pac spc	ENSMUSG00000002058.9	*Unc119*	chr11:78343481-78349164	1.6	9.0	4.4	1.5×10^{-2}
Pac spc	ENSMUSG00000103155.1	*RP23-234G15.1*	chr3:54021163-54021909	0.0	1.7	4.4	4.3×10^{-3}
Pac spc	ENSMUSG00000062949.9	*Atp11c*	chrX:60223289-60807993	1.4	7.6	4.4	1.9×10^{-2}
Pac spc	ENSMUSG00000049672.10	*Zbtb14*	chr17:69383049-69390750	0.3	2.9	4.4	4.6×10^{-2}
Pac spc	ENSMUSG00000028527.14	*Ak4*	chr4:101419276-101466995	1.6	8.9	4.4	9.2×10^{-3}
Pac spc	ENSMUSG00000015243.4	*Abca1*	chr4:53030786-53159895	0.6	4.5	4.4	4.3×10^{-3}
Pac spc	ENSMUSG00000025105.8	*Bnc1*	chr7:81966671-81992618	0.6	4.2	4.4	2.8×10^{-2}
Pac spc	ENSMUSG00000033295.9	*Ptprf*	chr4:118208212-118291405	6.7	31.0	4.4	4.3×10^{-3}

Pac spc	ENSMUSG00000040363.10	*Bcor*	chrX:12036739-12160355	0.2	2.4	4.4	3.7×10^{-2}
Pac spc	ENSMUSG00000026193.11	*Fn1*	chr1:71585519-71662843	3.5	16.9	4.3	9.2×10^{-3}
Pac spc	ENSMUSG00000040856.13	*Dlk1*	chr12:109452822-109463336	0.3	3.2	4.3	3.3×10^{-2}
Pac spc	ENSMUSG00000025854.11	*Fam20c*	chr5:138754513-138810077	0.0	1.9	4.3	4.6×10^{-2}
Pac spc	ENSMUSG00000024909.10	*Efemp2*	chr19:5473972-5481853	2.1	10.7	4.3	2.9×10^{-2}
Pac spc	ENSMUSG00000050947.8	*Amigo1*	chr3:108186334-108192286	0.6	4.3	4.3	1.4×10^{-2}
Pac spc	ENSMUSG00000029096.11	*Htra3*	chr5:35652040-35679782	0.1	2.0	4.3	1.8×10^{-2}
Pac spc	ENSMUSG00000057530.10	*Ece1*	chr4:137862236-137965229	0.9	5.5	4.3	4.6×10^{-2}
Pac spc	ENSMUSG00000033170.10	*Card10*	chr15:78775137-78803042	0.3	3.0	4.3	2.0×10^{-2}
Pac spc	ENSMUSG00000069044.6	*Usp9y*	chrY:1298960-1459782	0.7	4.5	4.3	4.0×10^{-2}
Pac spc	ENSMUSG00000026641.9	*Usf1*	chr1:171411312-171420352	1.8	9.1	4.3	4.9×10^{-2}
Pac spc	ENSMUSG00000032366.11	*Tpm1*	chr9:67022589-67049406	0.7	4.4	4.2	4.3×10^{-3}
Pac spc	ENSMUSG00000022175.8	*Lrp10*	chr14:54464163-54471497	0.5	3.7	4.2	2.3×10^{-2}
Pac spc	ENSMUSG00000037552.13	*Plekhg2*	chr7:28359603-28372599	4.5	20.4	4.2	1.1×10^{-2}
Pac spc	ENSMUSG00000020167.10	*Tcf3*	chr10:80409513-80433647	4.5	20.3	4.2	1.1×10^{-2}
Pac spc	ENSMUSG00000030872.10	*Gga2*	chr7:121986721-122021222	1.9	9.7	4.2	2.9×10^{-2}
Pac spc	ENSMUSG00000038576.11	*Susd4*	chr1:182763859-182896591	0.1	2.1	4.2	3.7×10^{-2}
Pac spc	ENSMUSG00000055067.11	*Smyd3*	chr1:178951959-179518041	5.0	22.4	4.2	3.1×10^{-2}
Pac spc	ENSMUSG00000059895.8	*Ptp4a3*	chr15:73723144-73758766	2.0	10.0	4.2	1.5×10^{-2}
Pac spc	ENSMUSG00000008682.9	*Rpl10*	chrX:74270811-74273135	4.7	21.1	4.1	2.6×10^{-2}
Pac spc	ENSMUSG00000039713.12	*Plekhg5*	chr4:152072497-152115400	0.7	4.6	4.1	2.0×10^{-2}
Pac spc	ENSMUSG00000032311.13	*Nrg4*	chr9:55208924-55326844	0.3	2.6	4.1	1.3×10^{-2}
Pac spc	ENSMUSG00000053436.10	*Mapk14*	chr17:28691341-28748404	3.1	14.3	4.1	2.5×10^{-2}
Pac spc	ENSMUSG00000031523.12	*Dlc1*	chr8:36567750-36953143	0.3	2.9	4.1	4.3×10^{-3}
Pac spc	ENSMUSG00000035284.9	*Vps13c*	chr9:67840395-67995634	2.3	10.9	4.0	4.3×10^{-3}
Pac spc	ENSMUSG00000063382.5	*Bcl9l*	chr9:44499135-44510388	0.9	5.0	4.0	1.4×10^{-2}
Pac spc	ENSMUSG00000034708.7	*Grn*	chr11:102430314-102447682	1.3	6.6	4.0	4.0×10^{-2}
Pac spc	ENSMUSG00000035621.9	*Midn*	chr10:80148271-80158368	2.6	11.8	4.0	9.2×10^{-3}
Pac spc	ENSMUSG00000056153.10	*Socs6*	chr18:88665223-88927481	1.5	7.6	4.0	2.1×10^{-2}

Pac spc	ENSMUSG00000059991.6	*Nptx2*	chr5:144545886-144557478	1.7	8.5	4.0	1.1×10^{-2}
Pac spc	ENSMUSG00000025269.12	*Apex2*	chrX:150519518-150643878	0.3	2.8	4.0	1.9×10^{-2}
Pac spc	ENSMUSG00000013629.12	*Cad*	chr5:31054779-31078479	10.2	42.1	4.0	3.1×10^{-2}
Pac spc	ENSMUSG00000025019.11	*Lcor*	chr19:41482644-41562246	1.3	6.8	4.0	2.5×10^{-2}
Pac spc	ENSMUSG00000028633.7	*Ctps*	chr4:120539867-120570276	3.3	14.8	4.0	2.2×10^{-2}
Pac spc	ENSMUSG00000070462.4	*Mesdc1*	chr7:83879872-83884305	0.1	2.1	4.0	3.9×10^{-2}
Pac spc	ENSMUSG00000003545.2	*Fosb*	chr7:19302720-19310045	0.6	3.9	4.0	3.2×10^{-2}
Pac spc	ENSMUSG00000032280.12	*Tle3*	chr9:61372365-61418497	8.4	34.8	4.0	1.3×10^{-2}
Pac spc	ENSMUSG00000025612.5	*Bach1*	chr16:87698944-87733346	0.5	3.7	4.0	2.7×10^{-2}
Pac spc	ENSMUSG00000042035.7	*Igsf3*	chr3:101377124-101463059	1.6	7.9	4.0	7.0×10^{-3}
Pac spc	ENSMUSG00000059316.2	*Slc27a4*	chr2:29802633-29817522	1.6	7.9	3.9	1.5×10^{-2}
Pac spc	ENSMUSG00000031386.10	*Hcfc1*	chrX:73942791-73966357	2.4	11.0	3.9	1.8×10^{-2}
Pac spc	ENSMUSG00000001034.13	*Mapk7*	chr11:61485430-61494406	7.0	29.1	3.9	7.0×10^{-3}
Pac spc	ENSMUSG00000027087.7	*Itgav*	chr2:83724396-83806916	0.2	2.3	3.9	4.3×10^{-3}
Pac spc	ENSMUSG00000029767.12	*Calu*	chr6:29348068-29388468	4.8	20.4	3.9	4.3×10^{-3}
Pac spc	ENSMUSG00000041329.9	*Atp1b2*	chr11:69599735-69605942	1.0	5.6	3.9	3.0×10^{-2}
Pac spc	ENSMUSG00000034472.9	*Rasd2*	chr8:75213943-75224113	1.8	8.4	3.9	2.7×10^{-2}
Pac spc	ENSMUSG00000051592.10	*Ccnb3*	chrX:6979651-7041619	0.8	4.4	3.9	3.5×10^{-2}
Pac spc	ENSMUSG00000018651.10	*Tada2a*	chr11:84078919-84129600	1.8	8.5	3.9	3.0×10^{-2}
Pac spc	ENSMUSG00000060671.8	*Atp8b2*	chr3:89939480-89963508	3.9	16.5	3.9	1.7×10^{-2}
Pac spc	ENSMUSG00000028444.13	*Cntfr*	chr4:41657497-41697089	0.8	4.4	3.9	2.9×10^{-2}
Pac spc	ENSMUSG00000042686.5	*Jph1*	chr1:16898184-17097889	0.4	3.0	3.9	3.0×10^{-2}
Pac spc	ENSMUSG00000041263.10	*Rusc1*	chr3:89083980-89093363	0.1	1.9	3.9	3.3×10^{-2}
Pac spc	ENSMUSG00000048752.3	*Prss50*	chr9:110857966-110864628	6.6	27.1	3.9	1.1×10^{-2}
Pac spc	ENSMUSG00000025809.11	*Itgb1*	chr8:128685653-128733200	4.5	18.8	3.8	1.6×10^{-2}
Pac spc	ENSMUSG00000026837.11	*Col5a1*	chr2:27882924-28039514	0.9	4.8	3.8	4.3×10^{-3}
Pac spc	ENSMUSG00000020821.13	*Kif1c*	chr11:70700547-70731964	2.2	9.7	3.8	1.6×10^{-2}
Pac spc	ENSMUSG00000019943.9	*Atp2b1*	chr10:98915151-99026143	0.9	4.7	3.8	1.4×10^{-2}
Pac spc	ENSMUSG00000015501.6	*Hivep2*	chr10:13966074-14154446	0.2	2.2	3.8	4.3×10^{-3}

Pac spc	ENSMUSG00000029676.11	*Pot1a*	chr6:25743736-25809246	2.2	9.7	3.8	4.8×10^{-2}
Pac spc	ENSMUSG00000030201.11	*Lrp6*	chr6:134446475-134566965	2.1	9.3	3.8	4.3×10^{-3}
Pac spc	ENSMUSG00000032228.12	*Tcf12*	chr9:71842687-72111871	9.5	36.8	3.8	1.8×10^{-2}
Pac spc	ENSMUSG00000015944.8	*Gatsl2*	chr5:134099710-134144343	0.5	3.4	3.8	3.5×10^{-2}
Pac spc	ENSMUSG00000048277.11	*Syngr2*	chr11:117809667-117839908	3.3	13.8	3.7	3.8×10^{-2}
Pac spc	ENSMUSG00000045348.11	*Nyap1*	chr5:137730882-137741607	0.2	2.1	3.7	3.7×10^{-2}
Pac spc	ENSMUSG00000029207.12	*Apbb2*	chr5:66298860-66618828	1.1	5.6	3.7	3.3×10^{-2}
Pac spc	ENSMUSG00000025323.9	*Sp4*	chr12:118234932-118301440	1.2	5.8	3.7	1.7×10^{-2}
Pac spc	ENSMUSG00000027794.4	*Sohlh2*	chr3:55182027-55209957	4.1	16.6	3.7	4.8×10^{-2}
Pac spc	ENSMUSG00000038212.11	*Hiatl1*	chr13:65064662-65112982	3.2	13.2	3.7	3.1×10^{-2}
Pac spc	ENSMUSG00000028906.11	*Epb4.1*	chr4:131923412-132076992	0.9	4.9	3.7	2.9×10^{-2}
Pac spc	ENSMUSG00000052911.5	*Lamb2*	chr9:108479735-108490530	2.0	8.7	3.7	4.4×10^{-2}
Pac spc	ENSMUSG00000048170.10	*Mcmbp*	chr7:128696440-128740495	3.9	15.7	3.7	1.3×10^{-2}
Pac spc	ENSMUSG00000060685.4	*Gm14511*	chrX:8975709-8976559	0.0	1.3	3.7	4.3×10^{-3}
Pac spc	ENSMUSG00000042364.10	*Snx18*	chr13:113592179-113618564	1.3	6.0	3.7	1.6×10^{-2}
Pac spc	ENSMUSG00000022436.11	*Sh3bp1*	chr15:78899666-78919517	1.0	5.1	3.7	1.7×10^{-2}
Pac spc	ENSMUSG00000027353.10	*Mcm8*	chr2:132816140-132844197	2.6	10.8	3.7	4.2×10^{-2}
Pac spc	ENSMUSG00000032875.7	*Arhgef17*	chr7:100869745-100932161	0.3	2.5	3.6	1.8×10^{-2}
Pac spc	ENSMUSG00000041415.9	*Dicer1*	chr12:104687741-104751952	3.0	12.1	3.6	4.3×10^{-3}
Pac spc	ENSMUSG00000003500.9	*Impdh1*	chr6:29200433-29216364	1.7	7.5	3.6	4.0×10^{-2}
Pac spc	ENSMUSG00000034413.10	*Neurl1b*	chr17:26414828-26446349	0.1	1.8	3.6	4.0×10^{-2}
Pac spc	ENSMUSG00000037606.13	*Osbpl5*	chr7:143688761-143756985	0.1	1.6	3.6	3.9×10^{-2}
Pac spc	ENSMUSG00000024130.11	*Abca3*	chr17:24351949-24414542	1.0	4.8	3.6	4.0×10^{-2}
Pac spc	ENSMUSG00000095078.1	*Gm5866*	chr5:52582319-52583227	0.0	1.3	3.6	4.9×10^{-2}
Pac spc	ENSMUSG00000071553.6	*Cpa2*	chr6:30541581-30564476	0.5	3.0	3.6	4.5×10^{-2}
Pac spc	ENSMUSG00000027333.14	*Smox*	chr2:131491495-131525922	0.5	3.2	3.5	2.9×10^{-2}
Pac spc	ENSMUSG00000018547.8	*Pip4k2b*	chr11:97715156-97744704	0.6	3.5	3.5	4.0×10^{-2}
Pac spc	ENSMUSG00000032485.10	*Scap*	chr9:110333292-110384935	3.9	15.2	3.5	4.3×10^{-3}
Pac spc	ENSMUSG00000031295.9	*Phka2*	chrX:160502165-160598878	3.2	12.7	3.5	4.3×10^{-2}

Pac spc	ENSMUSG00000039967.10	*Zfp292*	chr4:34803112-34882960	2.2	8.9	3.5	7.0×10^{-3}
Pac spc	ENSMUSG00000025586.12	*Cpeb1*	chr7:81347025-81455465	5.6	21.2	3.5	1.4×10^{-2}
Pac spc	ENSMUSG00000031310.12	*Zmym3*	chrX:101404383-101420849	3.2	12.5	3.5	1.4×10^{-2}
Pac spc	ENSMUSG00000045817.8	*Zfp36l2*	chr17:84183930-84187947	2.3	9.5	3.5	1.1×10^{-2}
Pac spc	ENSMUSG00000033763.10	*Mtss1l*	chr8:110721475-110741400	1.1	5.3	3.5	4.5×10^{-2}
Pac spc	ENSMUSG00000003410.7	*Elavl3*	chr9:22015004-22052023	0.3	2.3	3.5	1.5×10^{-2}
Pac spc	ENSMUSG00000001924.11	*Uba1*	chrX:20658325-20683179	8.6	31.3	3.5	7.0×10^{-3}
Pac spc	ENSMUSG00000050846.8	*Zfp623*	chr15:75940951-75949377	0.6	3.2	3.5	3.8×10^{-2}
Pac spc	ENSMUSG00000031987.5	*Egln1*	chr8:124908595-124949254	0.5	2.8	3.5	3.1×10^{-2}
Pac spc	ENSMUSG00000009596.5	*Taf7l*	chrX:134460117-134476490	8.1	29.5	3.5	4.3×10^{-3}
Pac spc	ENSMUSG00000029478.12	*Ncor2*	chr5:125017152-125179219	4.5	16.8	3.5	4.3×10^{-3}
Pac spc	ENSMUSG00000054520.11	*Sh3bp2*	chr5:34525837-34563638	0.4	2.8	3.5	4.0×10^{-2}
Pac spc	ENSMUSG00000022350.6	*E430025E21Rik*	chr15:59331997-59374167	2.0	8.1	3.5	4.0×10^{-2}
Pac spc	ENSMUSG00000030757.9	*Zkscan2*	chr7:123479515-123500449	3.4	13.0	3.5	3.1×10^{-2}
Pac spc	ENSMUSG00000063410.7	*Stk24*	chr14:121286342-121379334	0.6	3.2	3.5	4.8×10^{-2}
Pac spc	ENSMUSG00000024074.7	*Crim1*	chr17:78200247-78376592	0.5	2.9	3.5	2.6×10^{-2}
Pac spc	ENSMUSG00000051586.10	*Mical3*	chr6:120931706-121003153	1.6	6.6	3.5	3.6×10^{-2}
Pac spc	ENSMUSG00000022443.12	*Myh9*	chr15:77760586-77842175	1.1	5.2	3.4	3.1×10^{-2}
Pac spc	ENSMUSG00000026979.12	*Psd4*	chr2:24367579-24414954	0.1	1.4	3.4	9.2×10^{-3}
Pac spc	ENSMUSG00000044167.6	*Foxo1*	chr3:52268335-52353221	1.5	6.3	3.4	4.3×10^{-3}
Pac spc	ENSMUSG00000023927.11	*Satb1*	chr17:51736186-51834723	0.2	1.9	3.4	2.3×10^{-2}
Pac spc	ENSMUSG00000062542.7	*Syt9*	chr7:107370727-107548656	1.0	4.8	3.4	3.5×10^{-2}
Pac spc	ENSMUSG00000020422.9	*Tns3*	chr11:8431651-8664535	0.3	2.3	3.4	1.4×10^{-2}
Pac spc	ENSMUSG00000046139.7	*Patl1*	chr19:11912398-11945096	2.6	10.0	3.4	1.9×10^{-2}
Pac spc	ENSMUSG00000035778.13	*Ggta1*	chr2:35400178-35463231	0.1	1.7	3.4	3.1×10^{-2}
Pac spc	ENSMUSG00000001507.12	*Itga3*	chr11:95044473-95076801	0.5	2.9	3.4	2.3×10^{-2}
Pac spc	ENSMUSG00000022673.4	*Mcm4*	chr16:15623896-15637400	2.6	9.9	3.4	1.6×10^{-2}
Pac spc	ENSMUSG00000037679.8	*Inf2*	chr12:112588783-112615556	0.4	2.5	3.4	3.8×10^{-2}
Pac spc	ENSMUSG00000028530.10	*Jak1*	chr4:101068982-101265282	5.7	20.3	3.4	1.6×10^{-2}

Pac spc	ENSMUSG00000019256.13	*Ahr*	chr12:35497973-35535038	0.2	1.7	3.4	4.8×10^{-2}
Pac spc	ENSMUSG00000074796.6	*Slc4a11*	chr2:130684112-130697519	0.2	1.7	3.3	5.0×10^{-2}
Pac spc	ENSMUSG00000040007.8	*Bahd1*	chr2:118900376-118924528	4.3	15.4	3.3	4.8×10^{-2}
Pac spc	ENSMUSG00000041225.12	*Arhgap12*	chr18:6024426-6136098	2.8	10.5	3.3	1.6×10^{-2}
Pac spc	ENSMUSG00000038160.6	*Atg5*	chr10:44268357-44364291	1.7	6.7	3.3	4.0×10^{-2}
Pac spc	ENSMUSG00000036902.9	*Neto2*	chr8:85636587-85690973	0.6	3.1	3.3	4.3×10^{-2}
Pac spc	ENSMUSG00000053716.9	*Dusp7*	chr9:106368631-106375724	1.4	5.9	3.3	3.8×10^{-2}
Pac spc	ENSMUSG00000025151.12	*Maged1*	chrX:94535473-94542143	4.9	17.5	3.3	7.0×10^{-3}
Pac spc	ENSMUSG00000027070.10	*Lrp2*	chr2:69424339-69586065	0.1	1.6	3.3	2.4×10^{-2}
Pac spc	ENSMUSG00000032392.10	*Parp16*	chr9:65214689-65239219	0.5	2.8	3.3	3.7×10^{-2}
Pac spc	ENSMUSG00000031328.11	*Flna*	chrX:74223460-74249820	1.5	6.1	3.3	1.3×10^{-2}
Pac spc	ENSMUSG00000036523.12	*Greb1*	chr12:16670614-16800886	0.2	1.8	3.3	2.9×10^{-2}
Pac spc	ENSMUSG00000098195.1	*Gm7693*	chr7:72712633-72713621	0.0	1.1	3.2	4.3×10^{-3}
Pac spc	ENSMUSG00000051790.11	*Nlgn2*	chr11:69823121-69837784	1.2	4.9	3.2	3.0×10^{-2}
Pac spc	ENSMUSG00000027340.11	*Slc23a2*	chr2:132052495-132220250	1.5	6.0	3.2	3.8×10^{-2}
Pac spc	ENSMUSG00000054321.6	*Taf4b*	chr18:14783244-14900359	3.5	12.4	3.2	1.4×10^{-2}
Pac spc	ENSMUSG00000027932.10	*Slc27a3*	chr3:90385238-90389938	0.6	3.0	3.2	4.1×10^{-2}
Pac spc	ENSMUSG00000034902.13	*Pip5k1c*	chr10:81292962-81319973	5.5	18.8	3.2	1.8×10^{-2}
Pac spc	ENSMUSG00000028661.8	*Epha8*	chr4:136929418-136956816	0.3	2.1	3.2	2.9×10^{-2}
Pac spc	ENSMUSG00000005373.9	*Mlxipl*	chr5:135106890-135138382	0.3	2.1	3.2	3.3×10^{-2}
Pac spc	ENSMUSG00000048897.11	*Zfp710*	chr7:80024813-80092751	0.4	2.5	3.2	5.0×10^{-2}
Pac spc	ENSMUSG00000024457.12	*Trim26*	chr17:36837133-36859398	1.7	6.4	3.2	3.7×10^{-2}
Pac spc	ENSMUSG00000068876.10	*Cgn*	chr3:94760068-94786492	0.9	3.9	3.2	4.8×10^{-2}
Pac spc	ENSMUSG00000030309.12	*Caprin2*	chr6:148842491-148896237	12.0	39.5	3.2	4.1×10^{-2}
Pac spc	ENSMUSG00000020092.8	*Pald1*	chr10:61319656-61383523	0.1	1.5	3.2	4.7×10^{-2}
Pac spc	ENSMUSG00000010592.8	*Dazl*	chr17:50279393-50293599	50.8	162.9	3.2	4.3×10^{-3}
Pac spc	ENSMUSG00000032902.1	*Slc16a1*	chr3:104638667-104658462	4.3	14.7	3.2	1.6×10^{-2}
Pac spc	ENSMUSG00000040249.11	*Lrp1*	chr10:127538160-127621148	0.2	1.8	3.2	1.1×10^{-2}
Pac spc	ENSMUSG00000021294.7	*Kif26a*	chr12:112146207-112181747	0.4	2.2	3.2	3.1×10^{-2}

Pac spc	ENSMUSG00000049699.3	*Ucn2*	chr9:108986162-108987164	0.0	1.1	3.2	4.3×10^{-3}
Pac spc	ENSMUSG00000034218.11	*Atm*	chr9:53439148-53536740	3.5	12.3	3.2	2.2×10^{-2}
Pac spc	ENSMUSG00000055491.9	*Pprc1*	chr19:46032592-46072915	9.5	31.1	3.2	3.3×10^{-2}
Pac spc	ENSMUSG00000005034.11	*Prkacb*	chr3:146729578-146812960	3.0	10.5	3.1	1.9×10^{-2}
Pac spc	ENSMUSG00000017550.10	*Atad5*	chr11:80089399-80135794	1.3	5.1	3.1	4.8×10^{-2}
Pac spc	ENSMUSG00000019877.6	*Serinc1*	chr10:57515773-57532530	10.2	32.9	3.1	4.8×10^{-2}
Pac spc	ENSMUSG00000026478.10	*Lamc1*	chr1:153218921-153332786	0.2	1.7	3.1	1.1×10^{-2}
Pac spc	ENSMUSG00000095123.1	*Gm21781*	chr10:4391586-4396424	1.6	5.9	3.1	4.3×10^{-2}
Pac spc	ENSMUSG00000041351.12	*Rap1gap*	chr4:137664725-137729861	2.7	9.4	3.1	4.0×10^{-2}
Pac spc	ENSMUSG00000019179.6	*Mdh2*	chr5:135778479-135790398	16.4	52.0	3.1	1.9×10^{-2}
Pac spc	ENSMUSG00000071076.5	*Jund*	chr8:70697738-70700616	4.3	14.4	3.1	2.6×10^{-2}
Pac spc	ENSMUSG00000033352.7	*Map2k4*	chr11:65688242-65788297	0.9	4.0	3.1	2.8×10^{-2}
Pac spc	ENSMUSG00000000184.9	*Ccnd2*	chr6:127125778-127212411	0.3	2.0	3.1	3.0×10^{-2}
Pac spc	ENSMUSG00000034903.14	*Cobll1*	chr2:65088338-65239675	0.2	1.7	3.1	1.1×10^{-2}
Pac spc	ENSMUSG00000034762.5	*Glis1*	chr4:107434571-107635061	0.5	2.6	3.1	4.1×10^{-2}
Pac spc	ENSMUSG00000024151.9	*Msh2*	chr17:87672329-87723713	3.4	11.6	3.1	3.3×10^{-2}
Pac spc	ENSMUSG00000033059.7	*Pygb*	chr2:150786734-150831758	1.6	6.0	3.1	3.8×10^{-2}
Pac spc	ENSMUSG00000032898.6	*Fbxo21*	chr5:117976769-118010191	4.5	14.7	3.0	1.6×10^{-2}
Pac spc	ENSMUSG00000028030.8	*Tbck*	chr3:132684143-132838506	1.7	6.1	3.0	5.0×10^{-2}
Pac spc	ENSMUSG00000020782.14	*Llgl2*	chr11:115824048-115855780	1.6	5.8	3.0	4.5×10^{-2}
Pac spc	ENSMUSG00000004113.14	*Cacna1b*	chr2:24603886-24763152	0.9	3.6	3.0	3.6×10^{-2}
Pac spc	ENSMUSG00000057672.11	*Pkn1*	chr8:83666832-83699179	1.9	6.8	3.0	4.0×10^{-2}
Pac spc	ENSMUSG00000026238.10	*Ptma*	chr1:86526725-86530712	18.8	57.0	3.0	4.0×10^{-2}
Pac spc	ENSMUSG00000022791.12	*Tnk2*	chr16:32643873-32683493	3.0	9.9	3.0	2.9×10^{-2}
Pac spc	ENSMUSG00000015647.9	*Lama5*	chr2:180176372-180225859	1.6	5.7	3.0	2.4×10^{-2}
Pac spc	ENSMUSG00000031657.12	*Heatr3*	chr8:88137854-88172027	5.4	17.1	3.0	4.4×10^{-2}
Pac spc	ENSMUSG00000034282.3	*Evpl*	chr11:116220558-116238077	0.3	1.9	3.0	4.8×10^{-2}
Pac spc	ENSMUSG00000070570.4	*Slc17a7*	chr7:45163920-45176138	1.0	3.9	3.0	3.8×10^{-2}
Pac spc	ENSMUSG00000052298.8	*Cdc42se2*	chr11:54717455-54787675	11.9	36.2	3.0	1.1×10^{-2}

Pac spc	ENSMUSG00000072825.6	*Cep170b*	chr12:112722173-112746591	1.1	4.1	2.9	2.3×10^{-2}
Pac spc	ENSMUSG00000032547.8	*Ryk*	chr9:102834916-102908305	4.4	13.8	2.9	4.5×10^{-2}
Pac spc	ENSMUSG00000024098.5	*Twsg1*	chr17:65923065-65951187	3.3	10.7	2.9	4.7×10^{-2}
Pac spc	ENSMUSG00000021910.11	*Nisch*	chr14:31170929-31216946	14.0	41.8	2.9	4.3×10^{-3}
Pac spc	ENSMUSG00000009035.9	*Tmem184b*	chr15:79360683-79403303	1.6	5.6	2.9	3.6×10^{-2}
Pac spc	ENSMUSG00000023977.10	*Ubr2*	chr17:46928291-47010532	3.3	10.7	2.9	2.1×10^{-2}
Pac spc	ENSMUSG00000021611.8	*Tert*	chr13:73627000-73649041	0.4	2.0	2.9	4.4×10^{-2}
Pac spc	ENSMUSG00000067336.6	*Bmpr2*	chr1:59763399-59879014	1.0	3.9	2.9	1.3×10^{-2}
Pac spc	ENSMUSG00000007817.10	*Zmiz1*	chr14:25455736-25666743	0.3	1.9	2.9	3.3×10^{-2}
Pac spc	ENSMUSG00000036046.10	*5031439G07Rik*	chr15:84943935-84988551	1.4	5.0	2.9	4.8×10^{-2}
Pac spc	ENSMUSG00000032849.9	*Abcc4*	chr14:118482691-118706219	0.3	1.8	2.8	3.7×10^{-2}
Pac spc	ENSMUSG00000007564.10	*Ppp2r1a*	chr17:20945310-20965916	9.4	27.3	2.8	2.7×10^{-2}
Pac spc	ENSMUSG00000056724.10	*Nbeal2*	chr9:110624788-110654161	1.2	4.2	2.8	4.2×10^{-2}
Pac spc	ENSMUSG00000029863.9	*Casp2*	chr6:42264984-42282508	7.9	23.0	2.8	2.9×10^{-2}
Pac spc	ENSMUSG00000027646.11	*Src*	chr2:157418443-157471862	1.0	3.8	2.8	4.8×10^{-2}
Pac spc	ENSMUSG00000042978.9	*Sbk1*	chr7:126272618-126294999	3.4	10.3	2.8	2.6×10^{-2}
Pac spc	ENSMUSG00000053617.7	*Sh3pxd2a*	chr19:47260173-47464411	0.6	2.5	2.8	1.5×10^{-2}
Pac spc	ENSMUSG00000033624.6	*Pdpr*	chr8:111094629-111145480	5.8	17.0	2.8	1.7×10^{-2}
Pac spc	ENSMUSG00000035898.9	*Uba6*	chr5:86110719-86172803	9.6	27.3	2.7	3.8×10^{-2}
Pac spc	ENSMUSG00000042700.11	*Sipa1l1*	chr12:82170015-82451782	5.1	15.0	2.7	1.8×10^{-2}
Pac spc	ENSMUSG00000063455.12	*D630045J12Rik*	chr6:38048482-38254009	2.0	6.2	2.7	4.5×10^{-2}
Pac spc	ENSMUSG00000033228.7	*Scaf11*	chr15:96411697-96460843	3.0	8.9	2.7	4.0×10^{-2}
Pac spc	ENSMUSG00000003812.9	*Dnase2a*	chr8:84908559-84937359	0.1	1.1	2.7	4.0×10^{-2}
Pac spc	ENSMUSG00000037410.9	*Tbc1d2b*	chr9:90163068-90270804	0.8	2.9	2.7	4.5×10^{-2}
Pac spc	ENSMUSG00000005802.8	*Slc30a4*	chr2:122681232-122721456	4.2	12.3	2.7	4.2×10^{-2}
Pac spc	ENSMUSG00000061313.7	*Ddhd2*	chr8:25725323-25754280	4.4	12.7	2.7	3.6×10^{-2}
Pac spc	ENSMUSG00000028961.11	*Pgd*	chr4:149149990-149166771	10.9	29.7	2.7	3.2×10^{-2}
Pac spc	ENSMUSG00000005410.5	*Mcm5*	chr8:75109527-75128439	9.7	26.4	2.6	3.8×10^{-2}
Pac spc	ENSMUSG00000053198.9	*Prx*	chr7:27499323-27520214	0.1	1.1	2.6	4.9×10^{-2}

Pac spc	ENSMUSG00000056938.12	*Acbd4*	chr11:103101681-103112200	0.1	1.0	2.6	4.3×10^{-3}
Pac spc	ENSMUSG00000037815.6	*Ctnna1*	chr18:35118887-35254773	4.2	11.8	2.6	4.5×10^{-2}
Pac spc	ENSMUSG00000052085.6	*Dock8*	chr19:24999528-25202432	0.2	1.2	2.6	1.7×10^{-2}
Pac spc	ENSMUSG00000018846.8	*Pank3*	chr11:35769483-35791285	4.6	12.8	2.6	3.1×10^{-2}
Pac spc	ENSMUSG00000014602.11	*Kif1a*	chr1:93015463-93101951	0.3	1.4	2.6	3.6×10^{-2}
Pac spc	ENSMUSG00000027312.10	*Atrn*	chr2:130906494-131030333	6.9	18.4	2.5	4.1×10^{-2}
Pac spc	ENSMUSG00000009995.13	*Taz*	chrX:74273216-74290151	0.5	1.9	2.5	2.8×10^{-2}
Pac spc	ENSMUSG00000075470.1	*Alg10b*	chr15:90224310-90230554	6.6	17.6	2.5	2.9×10^{-2}
Pac spc	ENSMUSG00000033948.3	*Zswim5*	chr4:116877375-116989264	3.8	10.4	2.5	1.8×10^{-2}
Pac spc	ENSMUSG00000032340.7	*Neo1*	chr9:58874678-59036441	4.1	11.0	2.5	3.5×10^{-2}
Pac spc	ENSMUSG00000003316.10	*Glg1*	chr8:111154420-111259216	11.4	29.1	2.5	3.1×10^{-2}
Pac spc	ENSMUSG00000033767.10	*D930015E06Rik*	chr3:83897654-84040175	7.9	20.0	2.4	4.7×10^{-2}
Pac spc	ENSMUSG00000041859.10	*Mcm3*	chr1:20802967-20820312	11.1	27.4	2.4	5.0×10^{-2}
Pac spc	ENSMUSG00000062296.4	*Trank1*	chr9:111311738-111395774	3.1	8.2	2.4	4.0×10^{-2}
Pac spc	ENSMUSG00000033253.14	*Szt2*	chr4:118359989-118409273	8.7	20.9	2.3	4.0×10^{-2}
Pac spc	ENSMUSG00000038644.10	*Pold1*	chr7:44532745-44548849	22.1	51.8	2.3	4.3×10^{-2}
Pac spc	ENSMUSG00000063146.7	*Clip2*	chr5:134489385-134552434	0.2	1.2	2.3	4.2×10^{-2}
Pac spc	ENSMUSG00000032267.7	*Usp28*	chr9:48985384-49042517	6.9	16.3	2.3	4.2×10^{-2}
Pac spc	ENSMUSG00000050310.8	*Rictor*	chr15:6708380-6800398	8.2	19.2	2.3	4.3×10^{-2}
Pac spc	ENSMUSG00000027878.10	*Notch2*	chr3:98013537-98150367	3.4	8.3	2.2	3.6×10^{-2}
Pac spc	ENSMUSG00000029512.7	*Ulk1*	chr5:110784487-110810097	7.0	15.9	2.2	4.3×10^{-2}
Pac spc	ENSMUSG00000005469.9	*Prkaca*	chr8:83972977-83996445	55.2	25.9	0.5	4.4×10^{-2}
Pac spc	ENSMUSG00000030086.12	*Chchd6*	chr6:89383145-89595652	157.2	74.1	0.5	4.8×10^{-2}
Pac spc	ENSMUSG00000032396.13	*Dis3l*	chr9:64306755-64341288	162.8	76.5	0.5	4.8×10^{-2}
Pac spc	ENSMUSG00000028937.10	*Acot7*	chr4:152178133-152271855	332.0	154.5	0.5	4.9×10^{-2}
Pac spc	ENSMUSG00000021771.9	*Vdac2*	chr14:21831268-21856926	249.6	115.8	0.5	4.7×10^{-2}
Pac spc	ENSMUSG00000024897.8	*Apba1*	chr19:23758875-23949597	58.8	27.0	0.5	3.3×10^{-2}
Pac spc	ENSMUSG00000036211.3	*Hist1h1t*	chr13:23695813-23696542	1424.2	658.2	0.5	3.8×10^{-2}
Pac spc	ENSMUSG00000040548.11	*Tex2*	chr11:106502146-106613423	125.0	56.6	0.5	3.8×10^{-2}

Pac spc	ENSMUSG00000000552.9	*Zfp385a*	chr15:103313894-103340086	77.0	34.7	0.5	3.8×10^{-2}
Pac spc	ENSMUSG00000074734.2	*4933416C03Rik*	chr10:116018212-116274932	129.5	58.4	0.5	4.8×10^{-2}
Pac spc	ENSMUSG00000022622.4	*Acr*	chr15:89568325-89574585	125.8	56.7	0.5	4.0×10^{-2}
Pac spc	ENSMUSG00000030357.6	*Fkbp4*	chr6:128429734-128438677	229.3	102.8	0.4	3.6×10^{-2}
Pac spc	ENSMUSG00000058297.12	*Spock2*	chr10:60106218-60135198	29.4	12.9	0.4	4.0×10^{-2}
Pac spc	ENSMUSG00000020078.11	*Vps26a*	chr10:62454842-62486805	97.4	43.4	0.4	4.2×10^{-2}
Pac spc	ENSMUSG00000022092.10	*Ppp3cc*	chr14:70217897-70289449	89.0	39.5	0.4	3.5×10^{-2}
Pac spc	ENSMUSG00000023456.10	*Tpi1*	chr6:124808660-124814296	100.4	44.4	0.4	3.9×10^{-2}
Pac spc	ENSMUSG00000034274.7	*Thoc5*	chr11:4895319-4928867	62.8	27.5	0.4	4.0×10^{-2}
Pac spc	ENSMUSG00000033213.12	*AA467197*	chr2:122636985-122641191	367.2	162.0	0.4	4.0×10^{-2}
Pac spc	ENSMUSG00000031554.13	*Adam5*	chr8:24727092-24824369	289.5	126.8	0.4	3.5×10^{-2}
Pac spc	ENSMUSG00000026163.13	*Sphkap*	chr1:83254138-83408200	27.9	12.0	0.4	4.0×10^{-2}
Pac spc	ENSMUSG00000063229.10	*Ldha*	chr7:46841474-46855627	571.8	250.6	0.4	4.0×10^{-2}
Pac spc	ENSMUSG00000036196.11	*Slc26a8*	chr17:28637782-28689987	55.6	24.0	0.4	3.8×10^{-2}
Pac spc	ENSMUSG00000025171.1	*Ubtd1*	chr19:41981762-42034641	75.8	32.8	0.4	4.4×10^{-2}
Pac spc	ENSMUSG00000040734.10	*Ppp1r13l*	chr7:19359748-19378533	64.3	27.7	0.4	4.3×10^{-2}
Pac spc	ENSMUSG00000072295.5	*Als2cr11*	chr1:59014223-59094900	151.0	65.0	0.4	4.4×10^{-2}
Pac spc	ENSMUSG00000025509.11	*Pnpla2*	chr7:141455197-141460743	164.1	70.6	0.4	4.0×10^{-2}
Pac spc	ENSMUSG00000022246.9	*Rai14*	chr15:10568978-10714631	87.2	37.3	0.4	3.1×10^{-2}
Pac spc	ENSMUSG00000045466.14	*Zfp956*	chr6:47943170-47965300	78.6	33.5	0.4	4.2×10^{-2}
Pac spc	ENSMUSG00000051768.8	*Xrcc1*	chr7:24547149-24573438	54.9	23.1	0.4	4.2×10^{-2}
Pac spc	ENSMUSG00000024206.10	*Rfx2*	chr17:56775896-56831008	182.8	77.6	0.4	3.8×10^{-2}
Pac spc	ENSMUSG00000039183.5	*Nubp2*	chr17:24882610-24886350	126.2	53.4	0.4	3.9×10^{-2}
Pac spc	ENSMUSG00000075706.6	*Gpx4*	chr10:80047165-80056439	509.6	216.5	0.4	3.5×10^{-2}
Pac spc	ENSMUSG00000029131.10	*Dnajb6*	chr5:29735636-29786478	150.3	63.3	0.4	3.9×10^{-2}
Pac spc	ENSMUSG00000017843.9	*Ppp2r5c*	chr12:110485738-110583061	117.1	49.2	0.4	2.5×10^{-2}
Pac spc	ENSMUSG00000028878.7	*Fam76a*	chr4:132899212-132922558	33.4	13.7	0.4	4.8×10^{-2}
Pac spc	ENSMUSG00000049792.6	*Bag5*	chr12:111709487-111713257	114.8	47.9	0.4	2.6×10^{-2}
Pac spc	ENSMUSG00000039936.14	*Pik3cd*	chr4:149649167-149702571	16.8	6.8	0.4	4.0×10^{-2}

Pac spc	ENSMUSG00000035211.8	*Xrra1*	chr7:99859217-99917824	82.9	34.4	0.4	4.0×10^{-2}
Pac spc	ENSMUSG00000042246.4	*Tmc7*	chr7:118535842-118584736	16.6	6.6	0.4	4.9×10^{-2}
Pac spc	ENSMUSG00000002102.11	*Psmc3*	chr2:91054008-91070417	344.2	142.9	0.4	3.5×10^{-2}
Pac spc	ENSMUSG00000027550.10	*Lrrcc1*	chr3:14533787-14572658	76.1	31.3	0.4	2.5×10^{-2}
Pac spc	ENSMUSG00000024154.6	*Gtf2a1l*	chr17:88668659-88715152	119.6	49.4	0.4	3.3×10^{-2}
Pac spc	ENSMUSG00000056665.2	*Them6*	chr15:74721203-74728034	52.6	21.5	0.4	3.4×10^{-2}
Pac spc	ENSMUSG00000033210.12	*Slc9c1*	chr16:45535308-45607001	40.4	16.4	0.4	3.6×10^{-2}
Pac spc	ENSMUSG00000045107.4	*Saysd1*	chr14:20075645-20083172	49.4	20.1	0.4	3.8×10^{-2}
Pac spc	ENSMUSG00000013822.6	*Elof1*	chr9:22112988-22117148	314.9	129.8	0.4	3.8×10^{-2}
Pac spc	ENSMUSG00000020520.10	*Galnt10*	chr11:57623697-57787514	25.1	10.1	0.4	2.3×10^{-2}
Pac spc	ENSMUSG00000027363.11	*Usp8*	chr2:126707327-126783458	111.1	45.5	0.4	2.6×10^{-2}
Pac spc	ENSMUSG00000024474.5	*Ik*	chr18:36744655-36757639	120.9	49.4	0.4	1.8×10^{-2}
Pac spc	ENSMUSG00000025035.8	*Arl3*	chr19:46531108-46573085	184.1	75.4	0.4	3.1×10^{-2}
Pac spc	ENSMUSG00000035890.8	*Rnf126*	chr10:79758514-79766952	266.5	109.1	0.4	2.3×10^{-2}
Pac spc	ENSMUSG00000020472.10	*Zkscan17*	chr11:59485519-59526751	62.4	25.3	0.4	4.9×10^{-2}
Pac spc	ENSMUSG00000053624.3	*Gykl1*	chr18:52693678-52695668	118.9	48.2	0.4	2.6×10^{-2}
Pac spc	ENSMUSG00000027378.12	*Nphp1*	chr2:127740731-127788897	348.5	141.4	0.4	2.5×10^{-2}
Pac spc	ENSMUSG00000025793.11	*Hgs*	chr11:120467634-120483984	99.1	40.0	0.4	4.9×10^{-2}
Pac spc	ENSMUSG00000022013.3	*Dnajc15*	chr14:77826216-77874917	151.1	61.0	0.4	3.9×10^{-2}
Pac spc	ENSMUSG00000062732.6	*Lypd4*	chr7:24864619-24869941	210.4	85.0	0.4	2.2×10^{-2}
Pac spc	ENSMUSG00000058741.3	*Prr19*	chr7:25301358-25304133	55.7	22.3	0.4	2.5×10^{-2}
Pac spc	ENSMUSG00000039886.4	*Tmem120a*	chr5:135735484-135744271	92.6	37.2	0.4	2.9×10^{-2}
Pac spc	ENSMUSG00000040097.11	*Flywch1*	chr17:23755422-23771591	119.6	48.1	0.4	2.4×10^{-2}
Pac spc	ENSMUSG00000025218.6	*Poll*	chr19:45552274-45560531	44.5	17.7	0.4	2.9×10^{-2}
Pac spc	ENSMUSG00000035560.4	*Wdr20rt*	chr12:65225516-65228454	60.9	24.3	0.4	2.0×10^{-2}
Pac spc	ENSMUSG00000084883.1	*Ccdc85c*	chr12:108206344-108275417	19.4	7.5	0.4	3.2×10^{-2}
Pac spc	ENSMUSG00000042156.11	*Dzip1*	chr14:118875519-118925314	37.5	14.8	0.4	1.7×10^{-2}
Pac spc	ENSMUSG00000019834.11	*Slc22a16*	chr10:40570335-40604132	96.9	38.7	0.4	2.6×10^{-2}
Pac spc	ENSMUSG00000030096.7	*Slc6a6*	chr6:91684066-91759063	6.0	2.1	0.4	4.8×10^{-2}

Pac spc	ENSMUSG00000045211.4	*Nudt18*	chr14:70577846-70582571	54.4	21.6	0.4	1.8×10^{-2}
Pac spc	ENSMUSG00000025337.6	*Sbds*	chr5:130245730-130255530	66.6	26.4	0.4	4.8×10^{-2}
Pac spc	ENSMUSG00000029310.9	*Nudt9*	chr5:104046305-104065379	42.8	16.9	0.4	2.9×10^{-2}
Pac spc	ENSMUSG00000016982.6	*Pom121l2*	chr13:21981193-21988734	150.6	60.0	0.4	2.6×10^{-2}
Pac spc	ENSMUSG00000026331.9	*Slco6c1*	chr1:97059037-97128301	91.6	36.3	0.4	3.2×10^{-2}
Pac spc	ENSMUSG00000027702.7	*Lrrc34*	chr3:30624266-30672431	77.7	30.8	0.4	4.8×10^{-2}
Pac spc	ENSMUSG00000030216.10	*Wbp11*	chr6:136813653-136828233	303.5	121.1	0.4	2.5×10^{-2}
Pac spc	ENSMUSG00000058709.7	*Egln2*	chr7:27153713-27166802	263.5	105.0	0.4	3.3×10^{-2}
Pac spc	ENSMUSG00000031553.11	*Adam3*	chr8:24677224-24725852	182.1	72.4	0.4	3.1×10^{-2}
Pac spc	ENSMUSG00000069805.6	*Fbp1*	chr13:62864752-62888282	387.3	153.8	0.4	1.9×10^{-2}
Pac spc	ENSMUSG00000050035.6	*Fhl4*	chr10:85097018-85102495	573.4	226.2	0.4	1.5×10^{-2}
Pac spc	ENSMUSG00000074749.6	*Kiz*	chr2:146855863-146970097	100.3	39.3	0.4	1.4×10^{-2}
Pac spc	ENSMUSG00000070953.9	*Rabepk*	chr2:34777555-34799912	66.4	25.9	0.4	4.9×10^{-2}
Pac spc	ENSMUSG00000025762.10	*Larp1b*	chr3:40950630-41040234	194.3	76.4	0.4	1.9×10^{-2}
Pac spc	ENSMUSG00000024304.10	*Cdh2*	chr18:16588876-16809246	25.9	9.9	0.4	3.5×10^{-2}
Pac spc	ENSMUSG00000029147.7	*Ppm1g*	chr5:31202667-31220545	243.8	95.8	0.4	1.8×10^{-2}
Pac spc	ENSMUSG00000024158.13	*Hagh*	chr17:24840142-24864450	183.5	71.7	0.4	3.1×10^{-2}
Pac spc	ENSMUSG00000024654.8	*Asrgl1*	chr19:9109867-9279175	282.2	110.2	0.4	1.6×10^{-2}
Pac spc	ENSMUSG00000030792.7	*Dkkl1*	chr7:45207524-45211883	833.7	326.1	0.4	3.3×10^{-2}
Pac spc	ENSMUSG00000031631.11	*Cfap97*	chr8:46033260-46195590	165.1	64.2	0.4	2.2×10^{-2}
Pac spc	ENSMUSG00000034932.4	*Mrpl54*	chr10:81264712-81266934	103.2	39.9	0.4	3.9×10^{-2}
Pac spc	ENSMUSG00000019906.10	*Lin7a*	chr10:107271842-107425143	30.4	11.6	0.4	2.9×10^{-2}
Pac spc	ENSMUSG00000020462.10	*Cfap36*	chr11:29221531-29247409	195.0	75.6	0.4	4.4×10^{-2}
Pac spc	ENSMUSG00000022671.8	*Mzt2*	chr16:15848440-15863369	74.4	28.6	0.4	4.1×10^{-2}
Pac spc	ENSMUSG00000042797.8	*Aqp11*	chr7:97726378-97738247	123.6	47.6	0.4	4.5×10^{-2}
Pac spc	ENSMUSG00000073730.2	*4933415F23Rik*	chr1:23048294-23235673	149.2	57.4	0.4	1.4×10^{-2}
Pac spc	ENSMUSG00000038026.8	*Kcnj9*	chr1:172320500-172329318	29.9	11.2	0.4	3.9×10^{-2}
Pac spc	ENSMUSG00000032239.9	*Rp9*	chr9:22448310-22468356	99.1	37.7	0.4	4.5×10^{-2}
Pac spc	ENSMUSG00000022085.3	*Pebp4*	chr14:69840419-70059886	190.7	72.7	0.4	1.8×10^{-2}

Pac spc	ENSMUSG00000032497.11	*Lrrfip2*	chr9:111118110-111225668	30.9	11.5	0.4	2.9×10^{-2}
Pac spc	ENSMUSG00000055720.9	*Ubl7*	chr9:57910985-57929968	171.4	65.2	0.4	1.1×10^{-2}
Pac spc	ENSMUSG00000033128.8	*Gga1*	chr15:78877189-78894585	49.8	18.7	0.4	2.0×10^{-2}
Pac spc	ENSMUSG00000016626.8	*Nlrp14*	chr7:107166989-107198102	73.3	27.6	0.4	3.9×10^{-2}
Pac spc	ENSMUSG00000019944.10	*Rhobtb1*	chr10:69151433-69291791	15.7	5.7	0.4	4.4×10^{-2}
Pac spc	ENSMUSG00000020434.4	*4921536K21Rik*	chr11:3886087-3895098	72.1	26.9	0.4	1.1×10^{-2}
Pac spc	ENSMUSG00000027088.6	*Phospho2*	chr2:69789622-69800005	177.7	66.6	0.4	2.6×10^{-2}
Pac spc	ENSMUSG00000053030.7	*Spink2*	chr5:77205106-77211471	391.6	146.7	0.4	7.0×10^{-3}
Pac spc	ENSMUSG00000050623.4	*Tex40*	chr19:6922425-6925380	236.1	88.2	0.4	1.7×10^{-2}
Pac spc	ENSMUSG00000001794.8	*Capns1*	chr7:30186941-30195164	128.2	47.7	0.4	2.7×10^{-2}
Pac spc	ENSMUSG00000016526.8	*Dyrk3*	chr1:131127454-131138340	36.5	13.3	0.4	1.8×10^{-2}
Pac spc	ENSMUSG00000031839.6	*Hsbp1*	chr8:119344537-119348927	279.4	104.1	0.4	9.2×10^{-3}
Pac spc	ENSMUSG00000037617.7	*Spag1*	chr15:36179367-36235610	29.3	10.6	0.4	2.4×10^{-2}
Pac spc	ENSMUSG00000039168.11	*Dap*	chr15:31224313-31274341	36.2	13.2	0.4	2.5×10^{-2}
Pac spc	ENSMUSG00000050553.2	*Gk2*	chr5:97392439-97588125	204.0	75.6	0.4	1.4×10^{-2}
Pac spc	ENSMUSG00000009115.5	*Spatc1l*	chr10:76562271-76570532	25.2	9.0	0.4	4.9×10^{-2}
Pac spc	ENSMUSG00000030030.4	*1700003E16Rik*	chr6:83156403-83162975	214.5	79.3	0.4	1.3×10^{-2}
Pac spc	ENSMUSG00000075227.6	*Znhit2*	chr19:6061191-6062472	140.2	51.7	0.4	2.0×10^{-2}
Pac spc	ENSMUSG00000050996.6	*Cetn1*	chr18:9615523-9619478	198.1	73.1	0.4	1.3×10^{-2}
Pac spc	ENSMUSG00000052566.7	*Hook2*	chr8:84990594-85003364	20.6	7.3	0.4	4.2×10^{-2}
Pac spc	ENSMUSG00000028294.11	*1700003M02Rik*	chr4:34688558-34730206	279.3	103.2	0.4	1.4×10^{-2}
Pac spc	ENSMUSG00000033368.8	*Trim69*	chr2:122120107-122186189	58.7	21.4	0.4	2.9×10^{-2}
Pac spc	ENSMUSG00000030801.9	*Kat8*	chr7:127912516-127930113	68.5	25.0	0.4	2.6×10^{-2}
Pac spc	ENSMUSG00000040794.5	*C1qtnf4*	chr2:90885859-90890525	106.5	38.9	0.4	9.2×10^{-3}
Pac spc	ENSMUSG00000028392.11	*Bspry*	chr4:62480052-62497298	98.6	36.0	0.4	1.8×10^{-2}
Pac spc	ENSMUSG00000025324.7	*Atp10a*	chr7:58658201-58829426	8.9	3.0	0.4	1.7×10^{-2}
Pac spc	ENSMUSG00000047383.7	*Als2cr11*	chr1:58997314-59006218	62.1	22.5	0.4	1.1×10^{-2}
Pac spc	ENSMUSG00000022972.5	*1110004E09Rik*	chr16:90925808-90934927	138.7	50.6	0.4	1.1×10^{-2}
Pac spc	ENSMUSG00000027793.2	*Ccna1*	chr3:55045468-55055055	33.7	12.0	0.4	3.4×10^{-2}

Pac spc	ENSMUSG00000029073.5	*Cptp*	chr4:155864722-155869440	70.9	25.7	0.4	1.4×10^{-2}
Pac spc	ENSMUSG00000052075.6	*1700029F12Rik*	chr13:97021863-97034362	291.8	106.2	0.4	4.3×10^{-3}
Pac spc	ENSMUSG00000024937.10	*Ehbp1l1*	chr19:5707375-5726317	31.2	11.1	0.4	1.4×10^{-2}
Pac spc	ENSMUSG00000022525.9	*Hrasls*	chr16:29209694-29230531	84.8	30.5	0.4	4.7×10^{-2}
Pac spc	ENSMUSG00000026807.8	*Ak8*	chr2:28700163-28813165	88.2	31.7	0.4	7.0×10^{-3}
Pac spc	ENSMUSG00000061032.8	*Rrp1*	chr10:78400361-78413043	245.9	88.9	0.4	1.1×10^{-2}
Pac spc	ENSMUSG00000037979.9	*Ccdc92*	chr5:124834417-124862424	269.0	96.9	0.4	2.5×10^{-2}
Pac spc	ENSMUSG00000042404.12	*Dennd4b*	chr3:90265184-90280669	16.5	5.6	0.4	1.5×10^{-2}
Pac spc	ENSMUSG00000038587.8	*Akap12*	chr10:4266328-4359468	86.7	31.0	0.4	1.9×10^{-2}
Pac spc	ENSMUSG00000026790.15	*Odf2*	chr2:29889220-29931746	609.6	217.8	0.4	1.6×10^{-2}
Pac spc	ENSMUSG00000023170.10	*Gps2*	chr11:69913887-69916591	120.5	42.8	0.4	1.9×10^{-2}
Pac spc	ENSMUSG00000050957.4	*Insl6*	chr19:29321343-29325356	137.4	48.8	0.4	1.1×10^{-2}
Pac spc	ENSMUSG00000028576.8	*Ift74*	chr4:94614490-94693229	68.2	24.0	0.4	2.3×10^{-2}
Pac spc	ENSMUSG00000031786.6	*Drc7*	chr8:95055102-95078141	36.5	12.7	0.4	7.0×10^{-3}
Pac spc	ENSMUSG00000047104.4	*Pbp2*	chr6:135309783-135310347	119.8	42.3	0.4	2.6×10^{-2}
Pac spc	ENSMUSG00000022375.6	*Lrrc6*	chr15:66379857-66500910	44.1	15.4	0.4	2.6×10^{-2}
Pac spc	ENSMUSG00000045246.7	*Kcng4*	chr8:119623853-119635680	8.0	2.5	0.4	2.2×10^{-2}
Pac spc	ENSMUSG00000035314.8	*Gdpd5*	chr7:99381548-99460983	3.2	0.8	0.4	4.5×10^{-2}
Pac spc	ENSMUSG00000024387.9	*Csnk2b*	chr17:35116195-35128855	719.3	254.7	0.4	1.6×10^{-2}
Pac spc	ENSMUSG00000068854.7	*Hist2h2be*	chr3:96221118-96223738	17.1	5.7	0.4	2.9×10^{-2}
Pac spc	ENSMUSG00000046447.3	*Camk2n1*	chr4:138454313-138460123	7.8	2.4	0.4	2.5×10^{-2}
Pac spc	ENSMUSG00000074384.3	*AI429214*	chr8:36993574-36995531	35.6	12.2	0.4	4.0×10^{-2}
Pac spc	ENSMUSG00000048707.9	*Tprn*	chr2:25262617-25269885	104.4	36.4	0.4	2.2×10^{-2}
Pac spc	ENSMUSG00000029798.9	*Herc6*	chr6:57580991-57665136	4.5	1.2	0.4	4.4×10^{-2}
Pac spc	ENSMUSG00000062270.9	*Morf4l1*	chr9:90091664-90114774	622.7	218.1	0.4	1.5×10^{-2}
Pac spc	ENSMUSG00000037001.10	*Zfp39*	chr11:58888152-58904225	43.3	14.9	0.4	1.6×10^{-2}
Pac spc	ENSMUSG00000031027.11	*Stk33*	chr7:109279222-109444893	180.4	62.6	0.3	1.5×10^{-2}
Pac spc	ENSMUSG00000054428.8	*Atpif1*	chr4:132530554-132533659	159.8	55.1	0.3	3.3×10^{-2}
Pac spc	ENSMUSG00000026650.11	*Meig1*	chr2:3409042-3422648	953.3	329.8	0.3	9.2×10^{-3}

Pac spc	ENSMUSG00000042293.7	*Gm5617*	chr9:48495344-48495964	406.6	140.4	0.3	1.6×10^{-2}
Pac spc	ENSMUSG00000038782.4	*1700028J19Rik*	chr7:44229932-44236122	318.6	109.7	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000073758.6	*Sh3d21*	chr4:126150601-126163491	37.7	12.7	0.3	3.1×10^{-2}
Pac spc	ENSMUSG00000041399.3	*1700013G24Rik*	chr4:137453283-137455461	53.7	18.1	0.3	3.4×10^{-2}
Pac spc	ENSMUSG00000045835.4	*Hdgfl1*	chr13:26768172-26770119	180.7	61.6	0.3	1.6×10^{-2}
Pac spc	ENSMUSG00000038729.16	*Akap2*	chr4:57434246-57896984	1.3	0.1	0.3	4.3×10^{-2}
Pac spc	ENSMUSG00000034227.7	*Foxj1*	chr11:116330703-116335399	15.4	4.9	0.3	1.6×10^{-2}
Pac spc	ENSMUSG00000038949.8	*Cnst*	chr1:179546369-179627478	12.8	4.0	0.3	1.4×10^{-2}
Pac spc	ENSMUSG00000027517.9	*Ankrd60*	chr2:173568665-173578365	44.8	15.0	0.3	3.7×10^{-2}
Pac spc	ENSMUSG00000042249.7	*Adrbk2*	chr5:112910477-113015514	8.4	2.5	0.3	2.4×10^{-2}
Pac spc	ENSMUSG00000073471.2	*Rsph3a*	chr17:7881105-7979824	89.2	30.0	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000046487.6	*Mospd4*	chr18:46465214-46465790	85.8	28.8	0.3	2.9×10^{-2}
Pac spc	ENSMUSG00000037418.5	*Best1*	chr19:9985173-10001633	19.9	6.4	0.3	2.1×10^{-2}
Pac spc	ENSMUSG00000050107.2	*Gsg2*	chr11:73090582-73147446	161.2	54.2	0.3	2.5×10^{-2}
Pac spc	ENSMUSG00000029151.10	*Slc30a3*	chr5:31086105-31112526	91.4	30.5	0.3	4.2×10^{-2}
Pac spc	ENSMUSG00000034552.4	*Zswim2*	chr2:83915078-83941228	26.7	8.7	0.3	2.8×10^{-2}
Pac spc	ENSMUSG00000026255.11	*Efhd1*	chr1:87264362-87310839	280.4	94.2	0.3	1.1×10^{-2}
Pac spc	ENSMUSG00000078627.5	*43169*	chr11:105360797-105456735	296.4	99.5	0.3	1.5×10^{-2}
Pac spc	ENSMUSG00000031849.8	*Comp*	chr8:70373547-70382065	42.8	14.1	0.3	1.9×10^{-2}
Pac spc	ENSMUSG00000024565.8	*Sall3*	chr18:80966375-80988575	3.6	0.9	0.3	4.3×10^{-2}
Pac spc	ENSMUSG00000099958.1	*1700010B13Rik*	chr15:73645851-73652347	19.3	6.2	0.3	4.9×10^{-2}
Pac spc	ENSMUSG00000039963.14	*Ccdc40*	chr11:119228571-119265236	68.8	22.8	0.3	3.1×10^{-2}
Pac spc	ENSMUSG00000021997.4	*Lrrc63*	chr14:75084302-75130881	37.7	12.3	0.3	1.8×10^{-2}
Pac spc	ENSMUSG00000047841.8	*BC051628*	chr2:181220012-181222854	37.4	12.2	0.3	3.0×10^{-2}
Pac spc	ENSMUSG00000068860.5	*Gm128*	chr3:95236919-95251193	229.1	76.2	0.3	1.7×10^{-2}
Pac spc	ENSMUSG00000025480.4	*Syce1*	chr7:140777228-140787854	215.2	71.6	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000070424.7	*Art5*	chr7:102096878-102111148	26.8	8.6	0.3	3.0×10^{-2}
Pac spc	ENSMUSG00000028555.11	*Ttc39a*	chr4:109406622-109444745	16.1	5.1	0.3	3.5×10^{-2}
Pac spc	ENSMUSG00000037910.2	*1700018B24Rik*	chr3:48605731-48609102	192.6	63.8	0.3	1.4×10^{-2}

Pac spc	ENSMUSG00000020878.6	*Lrrc46*	chr11:97034601-97041407	383.9	127.5	0.3	9.2×10^{-3}
Pac spc	ENSMUSG00000022442.11	*Ttll1*	chr15:83483771-83510893	21.5	6.8	0.3	2.9×10^{-2}
Pac spc	ENSMUSG00000024033.9	*Rsph1*	chr17:31255018-31277356	743.0	246.0	0.3	9.2×10^{-3}
Pac spc	ENSMUSG00000064280.9	*Ccdc146*	chr5:21292960-21424677	27.4	8.7	0.3	4.7×10^{-2}
Pac spc	ENSMUSG00000035420.6	*Fam170a*	chr18:50278368-50283019	32.3	10.4	0.3	4.8×10^{-2}
Pac spc	ENSMUSG00000036398.9	*Ppp1r11*	chr17:36948355-36951741	298.8	98.6	0.3	3.3×10^{-2}
Pac spc	ENSMUSG00000040424.11	*Hipk4*	chr7:27523266-27531175	16.4	5.1	0.3	1.6×10^{-2}
Pac spc	ENSMUSG00000032334.9	*Loxl1*	chr9:58287722-58313212	4.1	1.0	0.3	4.3×10^{-2}
Pac spc	ENSMUSG00000044566.11	*Cage1*	chr13:38006051-38061433	128.1	42.0	0.3	4.3×10^{-2}
Pac spc	ENSMUSG00000043621.9	*Ubxn10*	chr4:138709836-138746132	89.4	29.1	0.3	1.8×10^{-2}
Pac spc	ENSMUSG00000006930.11	*Hap1*	chr11:100347326-100356128	27.3	8.6	0.3	1.4×10^{-2}
Pac spc	ENSMUSG00000028976.6	*Slc2a5*	chr4:150119282-150144169	223.2	72.7	0.3	1.6×10^{-2}
Pac spc	ENSMUSG00000021660.10	*Btf3*	chr13:98309895-98324415	173.5	56.4	0.3	1.1×10^{-2}
Pac spc	ENSMUSG00000031518.6	*Spata4*	chr8:54550330-54610098	555.1	180.8	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000017195.11	*Zpbp2*	chr11:98551096-98558665	133.8	43.2	0.3	1.3×10^{-2}
Pac spc	ENSMUSG00000031893.6	*Tsnaxip1*	chr8:105827743-105844676	60.8	19.4	0.3	1.3×10^{-2}
Pac spc	ENSMUSG00000024430.9	*Cabyr*	chr18:12741323-12755146	56.3	17.9	0.3	1.3×10^{-2}
Pac spc	ENSMUSG00000035785.5	*Cmtm2b*	chr8:104322236-104330756	245.4	79.1	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000003354.5	*Ccdc65*	chr15:98708206-98723326	121.7	39.0	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000074575.4	*Kcng1*	chr2:168260116-168281736	6.3	1.7	0.3	2.0×10^{-2}
Pac spc	ENSMUSG00000050677.2	*Ccdc96*	chr5:36484587-36488172	46.2	14.5	0.3	1.1×10^{-2}
Pac spc	ENSMUSG00000026578.6	*Ccdc181*	chr1:164275584-164287847	149.5	47.6	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000027528.8	*Fabp9*	chr3:10179850-10197283	1302.0	415.4	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000080268.3	*Brms1*	chr19:5041403-5049917	65.1	20.4	0.3	7.0×10^{-3}
Pac spc	ENSMUSG00000041566.3	*Tssk1*	chr16:17894222-17897922	14.4	4.2	0.3	1.8×10^{-2}
Pac spc	ENSMUSG00000024973.12	*Hrasls5*	chr19:7612540-7639642	480.9	152.5	0.3	1.6×10^{-2}
Pac spc	ENSMUSG00000087122.1	*4930403D09Rik*	chr11:34226814-34783892	111.3	34.9	0.3	4.3×10^{-2}
Pac spc	ENSMUSG00000073380.1	*Arrdc5*	chr17:56294112-56300286	23.4	7.1	0.3	3.8×10^{-2}
Pac spc	ENSMUSG00000044581.7	*4932415D10Rik*	chr10:82282115-82285278	16.0	4.7	0.3	2.4×10^{-2}

Pac spc	ENSMUSG00000000942.10	*Hoxa4*	chr6:52162510-52221854	30.8	9.4	0.3	2.3×10^{-2}
Pac spc	ENSMUSG00000045915.11	*Ccdc42*	chr11:68587020-68597966	58.3	18.0	0.3	2.5×10^{-2}
Pac spc	ENSMUSG00000033739.8	*Fkbpl*	chr17:34644763-34646324	62.3	19.2	0.3	1.6×10^{-2}
Pac spc	ENSMUSG00000052273.2	*Dnah3*	chr7:119922716-120095177	8.4	2.3	0.3	1.3×10^{-2}
Pac spc	ENSMUSG00000022445.6	*Cyp2d26*	chr15:82790106-82794245	7.7	2.1	0.3	4.6×10^{-2}
Pac spc	ENSMUSG00000084135.3	*Pom121l12*	chr11:14599313-14599862	79.2	24.4	0.3	1.8×10^{-2}
Pac spc	ENSMUSG00000030672.8	*Mylpf*	chr7:127211607-127214298	1.1	0.0	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000035085.5	*1700020L24Rik*	chr11:83437676-83463071	25.5	7.6	0.3	4.0×10^{-2}
Pac spc	ENSMUSG00000024175.1	*Tekt4*	chr17:25471589-25476594	51.7	15.7	0.3	7.0×10^{-3}
Pac spc	ENSMUSG00000029188.10	*Slc34a2*	chr5:53038081-53071664	28.2	8.4	0.3	1.3×10^{-2}
Pac spc	ENSMUSG00000026546.12	*Cfap45*	chr1:172520800-172563717	62.6	19.1	0.3	2.0×10^{-2}
Pac spc	ENSMUSG00000021977.7	*1700129C05Rik*	chr14:59133039-59142893	104.2	31.9	0.3	1.4×10^{-2}
Pac spc	ENSMUSG00000042190.8	*Cmklr1*	chr5:113612353-113650426	10.4	2.9	0.3	3.6×10^{-2}
Pac spc	ENSMUSG00000021258.9	*Ccnk*	chr12:108179737-108203359	174.0	53.4	0.3	7.0×10^{-3}
Pac spc	ENSMUSG00000027030.11	*Stk39*	chr2:68210444-68472268	207.1	63.5	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000056223.7	*Spata31*	chr13:64917405-64923184	14.8	4.2	0.3	3.8×10^{-2}
Pac spc	ENSMUSG00000049985.10	*Ankrd55*	chr13:112288450-112384002	6.6	1.7	0.3	3.3×10^{-2}
Pac spc	ENSMUSG00000100075.1	*1700018L02Rik*	chr19:29020832-29048729	53.7	16.1	0.3	9.2×10^{-3}
Pac spc	ENSMUSG00000030189.11	*Ybx3*	chr6:131364857-131388450	619.0	189.5	0.3	2.0×10^{-2}
Pac spc	ENSMUSG00000029517.9	*Ankrd7*	chr6:18866317-18879586	56.9	17.0	0.3	2.0×10^{-2}
Pac spc	ENSMUSG00000030590.10	*Fam98c*	chr7:29134853-29156920	142.6	43.2	0.3	1.6×10^{-2}
Pac spc	ENSMUSG00000022439.5	*Parvg*	chr15:84324025-84342978	4.5	1.0	0.3	3.6×10^{-2}
Pac spc	ENSMUSG00000090843.2	*Gm17673*	chr12:83954498-83984852	6.5	1.6	0.3	4.2×10^{-2}
Pac spc	ENSMUSG00000102758.1	*RP23-349M18.1*	chr3:23804334-23939477	1.1	0.0	0.3	1.3×10^{-2}
Pac spc	ENSMUSG00000074127.5	*Cmtm2a*	chr8:104281041-104310145	397.0	120.0	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000078907.1	*Fam186b*	chr15:99271017-99287180	50.9	15.1	0.3	1.1×10^{-2}
Pac spc	ENSMUSG00000043986.5	*Spata31d1d*	chr13:59725924-59731752	6.3	1.6	0.3	2.1×10^{-2}
Pac spc	ENSMUSG00000046173.2	*Pabpc6*	chr17:9666496-9669704	224.3	67.5	0.3	9.2×10^{-3}
Pac spc	ENSMUSG00000045573.9	*Penk*	chr4:4133530-4188703	39.2	11.5	0.3	2.2×10^{-2}

Pac spc	ENSMUSG00000020679.7	*Hnf1b*	chr11:83850062-83905819	4.6	1.0	0.3	4.8×10^{-2}
Pac spc	ENSMUSG00000049115.10	*Agtr1a*	chr13:30336440-30382867	7.7	2.0	0.3	3.8×10^{-2}
Pac spc	ENSMUSG00000008482.8	*Rnf151*	chr17:24715838-24718057	31.6	9.1	0.3	3.3×10^{-2}
Pac spc	ENSMUSG00000028560.7	*Usp1*	chr4:98923809-98935543	138.2	41.0	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000053783.5	*1700016K19Rik*	chr11:75999911-76003569	103.3	30.5	0.3	2.1×10^{-2}
Pac spc	ENSMUSG00000046755.5	*Kif2b*	chr11:91575314-91577558	42.6	12.3	0.3	7.0×10^{-3}
Pac spc	ENSMUSG00000026125.5	*Prss39*	chr1:34498409-34503063	68.8	20.1	0.3	1.4×10^{-2}
Pac spc	ENSMUSG00000031841.14	*Cdh13*	chr8:118283732-119324921	1.9	0.2	0.3	2.5×10^{-2}
Pac spc	ENSMUSG00000021643.10	*Serf1*	chr13:100106794-100114571	403.1	119.2	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000028813.2	*CK137956*	chr4:127927591-127970951	71.5	20.8	0.3	1.1×10^{-2}
Pac spc	ENSMUSG00000057816.3	*1700007G11Rik*	chr5:98329353-98801910	96.1	28.1	0.3	3.3×10^{-2}
Pac spc	ENSMUSG00000073102.3	*Drc1*	chr5:30281387-30366708	39.1	11.2	0.3	1.1×10^{-2}
Pac spc	ENSMUSG00000043913.10	*Ccdc60*	chr5:116123613-116288985	185.4	54.5	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000046750.12	*BC089491*	chr7:28284651-28291186	35.3	10.1	0.3	7.0×10^{-3}
Pac spc	ENSMUSG00000029679.7	*Hyal6*	chr6:24733244-24745452	39.7	11.4	0.3	7.0×10^{-3}
Pac spc	ENSMUSG00000027968.7	*Larp7*	chr3:127536953-127553348	118.1	34.5	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000032204.9	*Aqp9*	chr9:71110658-71168682	77.4	22.4	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000038555.7	*Reep2*	chr18:34840588-34847463	31.4	8.8	0.3	9.2×10^{-3}
Pac spc	ENSMUSG00000071234.2	*Syndig1l*	chr12:84677277-84698807	5.5	1.3	0.3	3.6×10^{-2}
Pac spc	ENSMUSG00000020475.3	*Pgam2*	chr11:5801639-5803733	601.0	175.4	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000022269.9	*43170*	chr15:26309047-26409576	256.1	74.3	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000070980.4	*Actl7b*	chr4:56740004-56741443	157.5	45.6	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000020799.12	*Tekt1*	chr11:72344721-72362442	128.5	36.9	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000078442.2	*Ccdc105*	chr10:78746923-78753067	27.7	7.6	0.3	1.3×10^{-2}
Pac spc	ENSMUSG00000028314.6	*Toporsl*	chr4:52596273-52612430	36.7	10.2	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000036557.4	*1700011E24Rik*	chr17:87389570-87427741	297.1	84.8	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000037568.8	*Vash2*	chr1:190947645-190979296	16.8	4.4	0.3	2.9×10^{-2}
Pac spc	ENSMUSG00000038398.7	*Upf3a*	chr8:13785614-13798538	108.0	30.3	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000021585.8	*Cast*	chr13:74694285-74807921	12.7	3.2	0.3	3.8×10^{-2}

Pac spc	ENSMUSG00000050114.7	*Prdx6b*	chr2:80292471-80295356	131.5	36.9	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000021552.6	*Gkap1*	chr13:58233350-58274188	202.5	56.9	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000038025.7	*Phf2*	chr13:48801749-48870885	46.5	12.7	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000040829.10	*Zmynd15*	chr11:70453982-70466202	38.5	10.5	0.3	7.0×10^{-3}
Pac spc	ENSMUSG00000074764.7	*Sel1l2*	chr2:140229854-140389706	21.0	5.5	0.3	2.2×10^{-2}
Pac spc	ENSMUSG00000063971.6	*1700011A15Rik*	chr15:101447744-101453909	30.5	8.2	0.3	4.9×10^{-2}
Pac spc	ENSMUSG00000029624.10	*Ptcd1*	chr5:145140361-145167108	40.5	11.0	0.3	3.8×10^{-2}
Pac spc	ENSMUSG00000030847.7	*Bag3*	chr7:128523582-128546977	10.6	2.6	0.3	7.0×10^{-3}
Pac spc	ENSMUSG00000029235.10	*Pdcl2*	chr5:76312114-76331156	291.3	81.0	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000036463.7	*4930544G11Rik*	chr6:65952570-65954012	146.2	40.4	0.3	7.0×10^{-3}
Pac spc	ENSMUSG00000037638.5	*Zbtb42*	chr12:112678827-112682747	22.5	5.9	0.3	1.3×10^{-2}
Pac spc	ENSMUSG00000039540.8	*4921524L21Rik*	chr18:6603632-6638966	45.6	12.3	0.3	1.9×10^{-2}
Pac spc	ENSMUSG00000021545.4	*1700067P10Rik*	chr17:48089631-48090920	28.2	7.5	0.3	4.7×10^{-2}
Pac spc	ENSMUSG00000034675.13	*Dbn1*	chr13:55473428-55488111	9.3	2.2	0.3	3.9×10^{-2}
Pac spc	ENSMUSG00000028845.11	*Tekt2*	chr4:126322120-126325688	85.5	23.3	0.3	1.6×10^{-2}
Pac spc	ENSMUSG00000039335.7	*Spata16*	chr3:26637619-26983212	159.5	43.6	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000021846.8	*Peli2*	chr14:48120868-48260883	14.6	3.7	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000028310.2	*Ppp3r2*	chr4:49661610-49845744	132.7	35.9	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000036214.9	*Znrd1as*	chr17:36958591-36965622	57.3	15.3	0.3	4.3×10^{-2}
Pac spc	ENSMUSG00000024116.5	*Prss21*	chr17:23868055-23873114	30.1	7.9	0.3	1.6×10^{-2}
Pac spc	ENSMUSG00000053868.3	*Gm5142*	chr14:59158502-59178749	88.4	23.8	0.3	1.4×10^{-2}
Pac spc	ENSMUSG00000042581.10	*Thsd7b*	chr1:129273301-130219278	11.5	2.8	0.3	1.8×10^{-2}
Pac spc	ENSMUSG00000021499.8	*Catsper3*	chr13:55784567-55808998	12.6	3.1	0.3	4.9×10^{-2}
Pac spc	ENSMUSG00000070331.9	*Qrich2*	chr11:116441324-116455237	207.4	56.2	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000059810.14	*Rgs3*	chr4:62559846-62704001	15.4	3.8	0.3	1.6×10^{-2}
Pac spc	ENSMUSG00000028637.11	*Ccdc30*	chr4:119322892-119415521	166.0	44.3	0.3	2.6×10^{-2}
Pac spc	ENSMUSG00000033579.12	*Fa2h*	chr8:111345134-111393824	21.1	5.3	0.3	9.2×10^{-3}
Pac spc	ENSMUSG00000032680.7	*6820408C15Rik*	chr2:152415586-152444330	25.1	6.3	0.3	1.5×10^{-2}
Pac spc	ENSMUSG00000023873.8	*1700010I14Rik*	chr17:8988332-9008319	313.4	83.5	0.3	4.3×10^{-3}

Pac spc	ENSMUSG00000044362.7	*Ccdc89*	chr7:90426311-90428660	47.0	12.2	0.3	1.1×10^{-2}
Pac spc	ENSMUSG00000067367.5	*Lyar*	chr5:38220469-38234306	529.1	140.6	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000031831.6	*Dnaaf1*	chr8:119575234-119605222	308.3	81.6	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000097863.1	*1010001B22Rik*	chr5:109995510-109996398	1.4	0.0	0.3	1.8×10^{-2}
Pac spc	ENSMUSG00000051732.2	*Pabpc2*	chr18:39773496-39776082	280.5	73.2	0.3	7.0×10^{-3}
Pac spc	ENSMUSG00000029999.10	*Tgfa*	chr6:86195250-86275639	8.3	1.8	0.3	9.2×10^{-3}
Pac spc	ENSMUSG00000031493.9	*Ggn*	chr7:29170219-29173933	150.0	38.6	0.3	9.2×10^{-3}
Pac spc	ENSMUSG00000035522.3	*Tsga8*	chrX:82948869-85206141	40.9	10.2	0.3	3.8×10^{-2}
Pac spc	ENSMUSG00000049476.8	*1700104B16Rik*	chr8:33730533-33731819	64.0	16.2	0.3	2.6×10^{-2}
Pac spc	ENSMUSG00000037621.7	*Atoh8*	chr6:72206176-72235577	18.2	4.3	0.3	9.2×10^{-3}
Pac spc	ENSMUSG00000059395.4	*Nkapl*	chr13:21467046-21468509	87.3	22.1	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000030549.5	*Rhcg*	chr7:79593362-79617657	14.8	3.4	0.3	2.6×10^{-2}
Pac spc	ENSMUSG00000000632.9	*Sez6*	chr11:77930799-77979048	6.5	1.3	0.3	1.5×10^{-2}
Pac spc	ENSMUSG00000017417.10	*Plxdc1*	chr11:97923237-97986444	14.5	3.4	0.3	7.0×10^{-3}
Pac spc	ENSMUSG00000071104.5	*Ccdc110*	chr8:45934618-45944145	20.5	4.9	0.3	1.5×10^{-2}
Pac spc	ENSMUSG00000023949.6	*Tcte1*	chr17:45523433-45549677	71.1	17.8	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000071636.6	*Rimbp3*	chr16:17208134-17213921	123.3	31.1	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000062075.9	*Lmnb2*	chr10:80901202-80918245	12.1	2.7	0.3	3.9×10^{-2}
Pac spc	ENSMUSG00000022620.10	*Arsa*	chr15:89472475-89484847	99.8	24.9	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000040866.9	*Rsph6a*	chr7:19054689-19074447	92.8	23.1	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000030292.7	*Smco2*	chr6:146850103-146871406	62.5	15.4	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000024209.9	*1700061G19Rik*	chr17:56875476-56888904	17.2	3.9	0.3	7.0×10^{-3}
Pac spc	ENSMUSG00000022915.3	*1700093J21Rik*	chr16:96082675-96089070	1.5	0.0	0.3	4.3×10^{-3}
Pac spc	ENSMUSG00000079334.4	*Nat6*	chr9:107575819-107587425	15.5	3.5	0.3	4.4×10^{-2}
Pac spc	ENSMUSG00000048988.7	*Elfn1*	chr5:139907942-139974711	7.0	1.4	0.2	2.5×10^{-2}
Pac spc	ENSMUSG00000017832.2	*Hspb9*	chr11:100713849-100714575	352.7	87.7	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000022441.13	*Efcab6*	chr15:83866711-84065379	70.3	17.1	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000038246.6	*Fam50b*	chr13:34734849-34747613	67.2	16.3	0.2	1.8×10^{-2}
Pac spc	ENSMUSG00000034683.8	*Ppp1r1c*	chr2:79707779-79818496	22.3	5.1	0.2	3.8×10^{-2}

Pac spc	ENSMUSG00000034706.12	*Dnaic2*	chr11:114727407-114757889	18.4	4.2	0.2	7.0×10^{-3}
Pac spc	ENSMUSG00000078127.2	*Fam170b*	chr14:32833961-32836789	18.9	4.3	0.2	9.2×10^{-3}
Pac spc	ENSMUSG00000044117.8	*2900011O08Rik*	chr16:13981701-14101500	10.6	2.2	0.2	4.8×10^{-2}
Pac spc	ENSMUSG00000012042.4	*4930579F01Rik*	chr3:138164134-138186713	12.1	2.6	0.2	4.8×10^{-2}
Pac spc	ENSMUSG00000026649.10	*1700009P17Rik*	chr1:171113917-171126967	42.3	9.8	0.2	1.5×10^{-2}
Pac spc	ENSMUSG00000079523.4	*Tmsb10*	chr6:72957346-72958748	655.0	156.7	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000100937.1	*1700020D05Rik*	chr19:5495277-5510489	122.8	29.0	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000085464.1	*Gm16208*	chr8:107029674-107031188	1.6	0.0	0.2	2.6×10^{-2}
Pac spc	ENSMUSG00000043859.4	*1700049L16Rik*	chr10:71979889-71980694	22.5	5.0	0.2	1.5×10^{-2}
Pac spc	ENSMUSG00000027518.3	*1700021F07Rik*	chr2:173522585-173528501	54.5	12.6	0.2	2.9×10^{-2}
Pac spc	ENSMUSG00000035179.3	*Ppp1r32*	chr19:10474256-10482897	48.4	11.1	0.2	7.0×10^{-3}
Pac spc	ENSMUSG00000047025.4	*Ccer1*	chr10:97693058-97694926	46.2	10.5	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000028610.12	*Dmrtb1*	chr4:107676289-107684230	370.7	86.9	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000039330.4	*Tsga10ip*	chr19:5390048-5394401	78.3	18.0	0.2	1.8×10^{-2}
Pac spc	ENSMUSG00000036168.11	*Ccdc38*	chr10:93540631-93605245	123.1	28.5	0.2	1.7×10^{-2}
Pac spc	ENSMUSG00000021056.7	*Tex21*	chr12:76198691-76246746	19.1	4.1	0.2	1.8×10^{-2}
Pac spc	ENSMUSG00000055602.12	*Tcp10b*	chr17:13061103-13082481	47.8	10.7	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000083649.5	*Rasl2-9*	chr7:5124937-5125950	72.2	16.3	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000022602.10	*Arc*	chr15:74669082-74672570	69.8	15.7	0.2	7.0×10^{-3}
Pac spc	ENSMUSG00000039391.7	*Ccdc81*	chr7:89866147-89903629	16.9	3.5	0.2	1.9×10^{-2}
Pac spc	ENSMUSG00000030544.5	*Mesp1*	chr7:79792240-79793788	44.4	9.7	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000020023.13	*Tmcc3*	chr10:94311948-94612084	9.5	1.8	0.2	2.6×10^{-2}
Pac spc	ENSMUSG00000001948.9	*Spa17*	chr9:37603294-37613720	164.8	36.8	0.2	9.2×10^{-3}
Pac spc	ENSMUSG00000018776.9	*Slc35g3*	chr11:69759889-69761968	16.5	3.3	0.2	1.6×10^{-2}
Pac spc	ENSMUSG00000038498.3	*Catsper1*	chr19:5335740-5344153	15.1	3.0	0.2	9.2×10^{-3}
Pac spc	ENSMUSG00000081360.1	*Gm11718*	chr11:107191093-107191630	1.8	0.0	0.2	2.4×10^{-2}
Pac spc	ENSMUSG00000020268.9	*Lyrm7*	chr11:54826865-54860916	15.3	3.0	0.2	4.0×10^{-2}
Pac spc	ENSMUSG00000090273.3	*Prr22*	chr17:56770249-56772208	27.7	5.7	0.2	9.2×10^{-3}
Pac spc	ENSMUSG00000084938.1	*BB557941*	chr2:57127478-57181754	1.8	0.0	0.2	1.3×10^{-2}

Pac spc	ENSMUSG00000071322.8	*Tcp10a*	chr17:7324645-7345974	80.2	17.2	0.2	9.2×10^{-3}
Pac spc	ENSMUSG00000011263.11	*Exoc3l2*	chr7:19489055-19496760	10.0	1.8	0.2	1.4×10^{-2}
Pac spc	ENSMUSG00000023165.9	*Ssxb2*	chrX:8454344-8461726	1.8	0.0	0.2	1.4×10^{-2}
Pac spc	ENSMUSG00000021534.7	*1700001L19Rik*	chr13:68597438-68614231	14.8	2.8	0.2	4.6×10^{-2}
Pac spc	ENSMUSG00000052469.8	*Tcp10c*	chr17:13354571-13377223	65.1	13.6	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000043036.9	*Ccdc63*	chr5:122100950-122138957	22.1	4.3	0.2	1.8×10^{-2}
Pac spc	ENSMUSG00000101963.1	*1700001J11Rik*	chr9:40050364-40053028	188.7	39.7	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000042189.5	*Tekt3*	chr11:63061653-63094964	63.2	13.0	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000104111.1	*RP23-71J17.3*	chr1:160041700-160044331	1.9	0.0	0.2	3.6×10^{-2}
Pac spc	ENSMUSG00000036598.3	*Ccdc113*	chr8:95534099-95558888	106.7	22.1	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000032023.7	*4931429I11Rik*	chr9:40894848-40964118	24.5	4.7	0.2	2.9×10^{-2}
Pac spc	ENSMUSG00000027505.2	*Fam209*	chr2:172472519-172474331	39.6	7.9	0.2	1.5×10^{-2}
Pac spc	ENSMUSG00000084837.1	*1700108N11Rik*	chr2:144305174-144332639	43.2	8.5	0.2	4.5×10^{-2}
Pac spc	ENSMUSG00000046585.8	*Cfap58*	chr19:47937711-48035379	11.3	1.9	0.2	1.9×10^{-2}
Pac spc	ENSMUSG00000062154.9	*Tex33*	chr15:78378399-78395912	40.9	7.9	0.2	9.2×10^{-3}
Pac spc	ENSMUSG00000024306.8	*Ccdc178*	chr18:21810896-22171396	18.1	3.3	0.2	4.0×10^{-2}
Pac spc	ENSMUSG00000097562.1	*Gm26639*	chr13:65590292-65591561	2.0	0.0	0.2	2.4×10^{-2}
Pac spc	ENSMUSG00000091955.2	*Gm9844*	chr7:24862212-24862697	2.0	0.0	0.2	2.0×10^{-2}
Pac spc	ENSMUSG00000087510.1	*1700112K13Rik*	chr4:127810637-127812173	2.0	0.0	0.2	1.4×10^{-2}
Pac spc	ENSMUSG00000072878.4	*1700123L14Rik*	chr6:96113153-96657198	254.1	49.8	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000012211.9	*Tex22*	chr12:113074501-113088917	102.9	19.8	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000080059.4	*Rps19-ps3*	chr4:147821776-147822202	2.1	0.0	0.2	2.7×10^{-2}
Pac spc	ENSMUSG00000021338.13	*Lrrc16a*	chr13:24012343-24280795	11.2	1.7	0.2	1.7×10^{-2}
Pac spc	ENSMUSG00000100585.1	*1700108J01Rik*	chr14:122181693-122402232	181.0	33.8	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000049526.7	*Tmem202*	chr9:59518685-59525501	28.7	5.0	0.2	7.0×10^{-3}
Pac spc	ENSMUSG00000029784.9	*Ssmem1*	chr6:30509848-30520254	90.5	16.0	0.2	4.3×10^{-3}
Pac spc	ENSMUSG00000084475.1	*Gm25782*	chr16:8449497-8449786	2.5	0.0	0.2	3.8×10^{-2}
Pac spc	ENSMUSG00000087335.2	*4930526F13Rik*	chr13:54926762-54930256	6.0	0.6	0.2	4.7×10^{-2}
Pac spc	ENSMUSG00000097066.1	*Gm26758*	chr13:65780904-65867305	2.9	0.0	0.1	2.1×10^{-2}

Pac spc	ENSMUSG00000086443.1	*4933421A08Rik*	chr4:122961308-122963475	6.6	0.5	0.1	5.0×10^{-2}
Pac spc	ENSMUSG00000087332.1	*Gm12690*	chr4:99569499-99573011	2.9	0.0	0.1	2.3×10^{-2}
Pac spc	ENSMUSG00000030617.8	*Ccdc83*	chr7:90223877-90265432	38.7	5.1	0.1	1.8×10^{-2}
Pac spc	ENSMUSG00000094338.1	*Hist1h2bl*	chr13:21715762-21716143	3.4	0.0	0.1	4.3×10^{-3}
Pac spc	ENSMUSG00000053896.9	*4933409G03Rik*	chr2:68582412-68616387	74.9	8.9	0.1	1.9×10^{-2}
Pac spc	ENSMUSG00000103011.1	*RP23-241J7.2*	chr3:9072766-9073211	4.3	0.0	0.1	1.4×10^{-2}
Pac spc	ENSMUSG00000095331.3	*Ptma-ps1*	chr7:24063831-24064140	4.6	0.0	0.1	2.6×10^{-2}
Pac spc	ENSMUSG00000084372.1	*Gm13988*	chr2:123273923-123274211	10.1	0.0	0.0	9.2×10^{-3}
Pac spc	ENSMUSG00000048559.4	*4930555K19Rik*	chr15:41173700-41173871	50.6	0.0	0.0	1.5×10^{-2}
Dip spc	ENSMUSG00000075014.1	*Gm10800*	chr2:98666546-98667301	52.6	1072.4	20.2	4.9×10^{-2}
Dip spc	ENSMUSG00000075015.3	*Gm10801*	chr2:98662236-98664083	5.6	84.6	14.0	4.9×10^{-2}
Dip spc	ENSMUSG00000000278.10	*Scpep1*	chr11:88905927-88955465	13.6	61.1	4.4	4.9×10^{-2}
Dip spc	ENSMUSG00000023572.12	*Ccndbp1*	chr2:121008402-121016904	4.0	17.7	4.1	4.9×10^{-2}
Dip spc	ENSMUSG00000058569.7	*Tmed9*	chr13:55593134-55597663	8.2	29.1	3.4	4.9×10^{-2}
Dip spc	ENSMUSG00000097164.1	*Cep83os*	chr10:94673492-94688613	38.4	125.2	3.2	4.9×10^{-2}
Dip spc	ENSMUSG00000022136.7	*Dnajc3*	chr14:118937931-118981702	21.8	61.6	2.8	4.9×10^{-2}
Dip spc	ENSMUSG00000022501.5	*Prm1*	chr16:10796325-10796886	126.4	308.2	2.4	4.9×10^{-2}
Dip spc	ENSMUSG00000038015.6	*Prm2*	chr16:10791379-10796134	107.0	248.8	2.3	4.9×10^{-2}
Secondary spc	ENSMUSG00000023572.12	*Ccndbp1*	chr2:121008402-121016904	0.2	12.0	17.3	3.1×10^{-2}
Secondary spc	ENSMUSG00000033713.7	*Foxn3*	chr12:99194979-99450111	1.2	15.3	9.4	3.1×10^{-2}
Secondary spc	ENSMUSG00000000278.10	*Scpep1*	chr11:88905927-88955465	4.9	33.9	6.3	3.1×10^{-2}
Secondary spc	ENSMUSG00000022136.7	*Dnajc3*	chr14:118937931-118981702	4.2	29.2	6.3	3.1×10^{-2}
Secondary spc	ENSMUSG00000022300.9	*Dcaf13*	chr15:39112864-39146856	2.0	12.6	5.2	3.1×10^{-2}
Secondary spc	ENSMUSG00000048310.8	*Pskh1*	chr8:105900440-105931778	2.2	10.2	4.0	3.1×10^{-2}
Secondary spc	ENSMUSG00000028684.10	*Urod*	chr4:116989964-116994413	2.4	10.0	3.7	3.1×10^{-2}
Secondary spc	ENSMUSG00000058569.7	*Tmed9*	chr13:55593134-55597663	0.9	4.3	3.5	3.1×10^{-2}
Secondary spc	ENSMUSG00000025134.2	*Alyref*	chr11:120592120-120598365	9.0	31.6	3.4	3.1×10^{-2}
Secondary spc	ENSMUSG00000074997.3	*Pin1rt1*	chr2:104713925-104716379	3.7	10.9	2.7	3.1×10^{-2}
Secondary spc	ENSMUSG00000083282.2	*Ctsf*	chr19:4855128-4860912	0.4	1.9	2.7	3.1×10^{-2}

Secondary spc	ENSMUSG00000053453.8	*Thoc7*	chr14:13918443-13961225	37.5	100.8	2.7	3.1×10^{-2}
Secondary spc	ENSMUSG00000079606.1	*Gm595*	chrX:48841465-48877713	18.5	45.3	2.4	3.1×10^{-2}
Secondary spc	ENSMUSG00000019210.8	*Atp6v1e1*	chr6:120795244-120822685	7.9	18.7	2.3	3.1×10^{-2}
Secondary spc	ENSMUSG00000102483.1	*RP23-474A1.1*	chr1:177808549-177962233	14.7	31.9	2.1	3.1×10^{-2}
Secondary spc	ENSMUSG00000021534.7	*1700001L19Rik*	chr13:68597438-68614231	108.1	48.8	0.5	3.1×10^{-2}
Secondary spc	ENSMUSG00000001948.9	*Spa17*	chr9:37603294-37613720	529.1	239.1	0.5	3.1×10^{-2}
Secondary spc	ENSMUSG00000099863.1	*1700031L13Rik*	chr5:82122407-82124713	103.6	36.1	0.4	3.1×10^{-2}
Secondary spc	ENSMUSG00000036249.12	*Rbm43*	chr2:51924447-51935163	22.0	6.9	0.3	3.1×10^{-2}
Secondary spc	ENSMUSG00000064288.4	*Hist1h4k*	chr13:21750193-21750505	1.5	0.0	0.3	3.1×10^{-2}
Sptd	ENSMUSG00000023572.12	*Ccndbp1*	chr2:121008402-121016904	0.3	9.5	12.5	2.4×10^{-2}
Sptd	ENSMUSG00000022136.7	*Dnajc3*	chr14:118937931-118981702	4.0	24.1	5.5	2.4×10^{-2}
Sptd	ENSMUSG00000002985.11	*Apoe*	chr7:19696108-19699166	3.0	18.6	5.4	2.4×10^{-2}
Sptd	ENSMUSG00000058252.6	*1700008I05Rik*	chrX:135654697-135693790	16.2	87.7	5.3	2.4×10^{-2}
Sptd	ENSMUSG00000022300.9	*Dcaf13*	chr15:39112864-39146856	1.5	9.5	5.0	2.4×10^{-2}
Sptd	ENSMUSG00000000278.10	*Scpep1*	chr11:88905927-88955465	2.9	15.3	4.6	2.4×10^{-2}
Sptd	ENSMUSG00000047654.6	*Tssk6*	chr8:69887787-69903518	82.3	378.9	4.6	2.4×10^{-2}
Sptd	ENSMUSG00000048310.8	*Pskh1*	chr8:105900440-105931778	2.0	11.1	4.6	2.4×10^{-2}
Sptd	ENSMUSG00000053453.8	*Thoc7*	chr14:13918443-13961225	18.4	83.6	4.4	2.4×10^{-2}
Sptd	ENSMUSG00000019210.8	*Atp6v1e1*	chr6:120795244-120822685	2.8	14.2	4.4	2.4×10^{-2}
Sptd	ENSMUSG00000045217.5	*Ppp1r2-ps9*	chrX:15110584-15111466	18.7	82.6	4.3	2.4×10^{-2}
Sptd	ENSMUSG00000029766.4	*1700012A03Rik*	chr6:32050245-32058921	32.5	136.3	4.1	2.4×10^{-2}
Sptd	ENSMUSG00000036002.8	*Fam214b*	chr4:43027689-43053253	10.2	43.7	4.1	2.4×10^{-2}
Sptd	ENSMUSG00000051896.4	*Tex37*	chr6:70913086-70918927	55.7	232.4	4.1	2.4×10^{-2}
Sptd	ENSMUSG00000036918.11	*Ttc7*	chr17:87282885-87381769	14.1	58.6	4.1	2.4×10^{-2}
Sptd	ENSMUSG00000031085.11	*Gm498*	chr7:143866870-143897506	31.3	127.5	4.0	3.7×10^{-2}
Sptd	ENSMUSG00000026473.11	*Glul*	chr1:153849541-153909723	63.6	255.3	4.0	4.8×10^{-2}
Sptd	ENSMUSG00000076438.5	*Oxct2b*	chr4:123105164-123139951	47.5	190.9	4.0	2.4×10^{-2}
Sptd	ENSMUSG00000050087.3	*Cby3*	chr11:50354461-50359699	14.3	57.6	3.9	4.8×10^{-2}
Sptd	ENSMUSG00000076436.1	*Oxct2a*	chr4:123312644-123343252	44.3	173.4	3.9	2.4×10^{-2}

Sptd	ENSMUSG00000027562.8	*Car2*	chr3:14886272-14900770	29.2	112.3	3.8	2.4×10^{-2}
Sptd	ENSMUSG00000047394.7	*Odf3b*	chr15:89377449-89379254	10.0	38.4	3.7	2.4×10^{-2}
Sptd	ENSMUSG00000049653.4	*Spatc1*	chr15:76268088-76292572	38.1	139.6	3.6	3.7×10^{-2}
Sptd	ENSMUSG00000078346.3	*Gm5132*	chrX:14211147-14211661	13.0	47.7	3.6	3.7×10^{-2}
Sptd	ENSMUSG00000003178.7	*Mical3*	chr6:121007240-121081609	41.8	148.6	3.5	2.4×10^{-2}
Sptd	ENSMUSG00000000125.5	*Wnt3*	chr11:103774149-103817957	5.2	18.8	3.4	3.7×10^{-2}
Sptd	ENSMUSG00000074259.6	*Gramd2*	chr9:59680143-59718874	2.3	8.9	3.4	2.4×10^{-2}
Sptd	ENSMUSG00000021791.6	*Dydc2*	chr14:41049208-41069074	13.9	47.5	3.3	2.4×10^{-2}
Sptd	ENSMUSG00000027482.8	*Bpifa3*	chr2:154130335-154138356	29.1	98.1	3.3	2.4×10^{-2}
Sptd	ENSMUSG00000036046.10	*5031439G07Rik*	chr15:84943935-84988551	8.1	27.7	3.3	2.4×10^{-2}
Sptd	ENSMUSG00000021194.5	*Chga*	chr12:102554968-102565027	0.6	2.9	3.2	2.4×10^{-2}
Sptd	ENSMUSG00000020307.10	*Cdc34*	chr10:79682194-79688394	102.2	325.7	3.2	3.7×10^{-2}
Sptd	ENSMUSG00000031770.11	*Herpud1*	chr8:94377920-94395377	49.9	157.5	3.1	2.4×10^{-2}
Sptd	ENSMUSG00000056508.5	*1700001K19Rik*	chr12:110667688-110682619	49.4	153.8	3.1	4.8×10^{-2}
Sptd	ENSMUSG00000050721.8	*Plekho2*	chr9:65554385-65580040	5.3	16.9	3.0	3.7×10^{-2}
Sptd	ENSMUSG00000058794.8	*Nfe2*	chr15:103248211-103258403	0.5	2.6	3.0	3.7×10^{-2}
Sptd	ENSMUSG00000031930.10	*Wwp2*	chr8:107436397-107558594	13.0	39.9	3.0	4.8×10^{-2}
Sptd	ENSMUSG00000071076.5	*Jund*	chr8:70697738-70700616	13.6	41.3	3.0	2.4×10^{-2}
Sptd	ENSMUSG00000048038.6	*4932418E24Rik*	chr2:26271645-26294557	31.4	91.7	2.9	4.8×10^{-2}
Sptd	ENSMUSG00000024197.9	*Plin3*	chr17:56278961-56290511	1.9	5.9	2.6	3.7×10^{-2}
Sptd	ENSMUSG00000083282.2	*Ctsf*	chr19:4855128-4860912	0.2	1.4	2.6	3.7×10^{-2}
Sptd	ENSMUSG00000014791.9	*Elmo3*	chr8:105305600-105310623	0.3	1.3	2.3	2.4×10^{-2}
Sptd	ENSMUSG00000036949.12	*Slc39a12*	chr2:14388315-14494977	4.7	1.4	0.4	2.4×10^{-2}
Sptd	ENSMUSG00000099508.1	*1700030L20Rik*	chr3:136435269-136449349	16.5	5.3	0.3	4.8×10^{-2}
Sptd	ENSMUSG00000102758.1	*RP23-349M18.1*	chr3:23804334-23939477	8.4	2.4	0.3	2.4×10^{-2}

Table S3. Expression of piRNA pathway genes in *pi6*$^{em1/em1}$ cells.

Gene	Ensembl ID	C57BL/6 (fpkm)	*pi6*$^{em1/em1}$ (fpkm)	*pi6*$^{em1/em1}$ / C57BL/6	FDR
Pachytene Spermatocyte					
Piwil1	ENSMUSG00000029423.6	491.0	377.3	0.8	0.7
Piwil2	ENSMUSG00000033644.4	154.5	237.4	1.5	0.4
Mov10l1	ENSMUSG00000015365.11	100.0	164.5	1.6	0.5
A-Myb	ENSMUSG00000025912.12	51.6	49.8	1.0	1.0
Tdrd1	ENSMUSG00000025081.9	188.6	194.1	1.0	1.0
Tdrd6	ENSMUSG00000040140.10	272.3	117.1	0.4	0.1
UAP56/Ddx39b	ENSMUSG00000019432.11	90.5	115.8	1.3	0.6
PLD6	ENSMUSG00000043648.7	121.0	85.9	0.7	0.5
Papi/Tdrkh	ENSMUSG00000041912.8	29.4	36.4	1.2	0.7
Tdrd12	ENSMUSG00000030491.12	109.4	119.1	1.1	0.9
Ddx4	ENSMUSG00000021758.9	259.6	220.5	0.8	0.8
Piwil4	ENSMUSG00000036912.13	0.0	1.8	4.4	0.5
Mael	ENSMUSG00000040629.4	600.4	340.9	0.6	0.2
Rnf17	ENSMUSG00000000365.8	85.1	99.4	1.2	0.8
Henmt1	ENSMUSG00000045662.12	37.1	30.7	0.8	0.8
PNLDC1	ENSMUSG00000073460.4	8.0	11.3	1.4	0.6
Diplotene Spermatocyte					
Piwil1	ENSMUSG00000029423.6	270.5	344.8	1.3	1.0
Piwil2	ENSMUSG00000033644.4	54.6	75.7	1.4	1.0
Mov10l1	ENSMUSG00000015365.11	33.9	43.2	1.3	1.0
A-Myb	ENSMUSG00000025912.12	43.2	47.4	1.1	1.0
Tdrd1	ENSMUSG00000025081.9	79.7	109.9	1.4	1.0
Tdrd6	ENSMUSG00000040140.10	473.1	473.7	1.0	1.0
UAP56/Ddx39b	ENSMUSG00000019432.11	42.4	54.3	1.3	1.0
PLD6	ENSMUSG00000043648.7	90.7	110.3	1.2	1.0
Papi/Tdrkh	ENSMUSG00000041912.8	14.8	17.6	1.2	1.0
Tdrd12	ENSMUSG00000030491.12	62.5	78.4	1.3	1.0
Ddx4	ENSMUSG00000021758.9	216.5	190.6	0.9	1.0
Piwil4	ENSMUSG00000036912.13	0.0	0.0	1.0	1.0
Mael	ENSMUSG00000040629.4	673.1	637.9	0.9	1.0
Rnf17	ENSMUSG00000000365.8	36.4	48.7	1.3	1.0
Henmt1	ENSMUSG00000045662.12	40.7	45.4	1.1	1.0
PNLDC1	ENSMUSG00000073460.4	4.6	4.7	1.0	1.0

Secondary Spermatocyte

Piwil1	ENSMUSG00000029423.6	33.3	40.7	1.2	1.0
Piwil2	ENSMUSG00000033644.4	12.7	21.6	1.7	0.6
Mov10l1	ENSMUSG00000015365.11	10.2	15.0	1.5	1.0
A-Myb	ENSMUSG00000025912.12	30.6	34.5	1.1	1.0
Tdrd1	ENSMUSG00000025081.9	9.7	13.8	1.4	1.0
Tdrd6	ENSMUSG00000040140.10	444.0	489.6	1.1	1.0
UAP56/Ddx39b	ENSMUSG00000019432.11	14.3	17.0	1.2	1.0
PLD6	ENSMUSG00000043648.7	20.5	32.0	1.5	1.0
Papi/Tdrkh	ENSMUSG00000041912.8	5.5	5.1	0.9	1.0
Tdrd12	ENSMUSG00000030491.12	20.7	22.9	1.1	1.0
Ddx4	ENSMUSG00000021758.9	294.1	223.8	0.8	1.0
Piwil4	ENSMUSG00000036912.13	0.0	0.0	0.9	1.0
Mael	ENSMUSG00000040629.4	797.2	797.4	1.0	1.0
Rnf17	ENSMUSG00000000365.8	38.0	30.3	0.8	1.0
Henmt1	ENSMUSG00000045662.12	22.7	28.5	1.3	1.0
PNLDC1	ENSMUSG00000073460.4	1.9	1.6	0.9	1.0

Spermatid

Piwil1	ENSMUSG00000029423.6	14.2	21.0	1.5	0.7
Piwil2	ENSMUSG00000033644.4	7.8	12.2	1.5	0.7
Mov10l1	ENSMUSG00000015365.11	8.0	6.6	0.8	0.9
A-Myb	ENSMUSG00000025912.12	12.9	18.3	1.4	0.8
Tdrd1	ENSMUSG00000025081.9	14.8	16.7	1.1	1.0
Tdrd6	ENSMUSG00000040140.10	283.6	389.5	1.4	0.9
UAP56/Ddx39b	ENSMUSG00000019432.11	21.1	15.2	0.7	0.8
PLD6	ENSMUSG00000043648.7	24.0	15.8	0.7	0.7
Papi/Tdrkh	ENSMUSG00000041912.8	4.5	5.6	1.2	0.9
Tdrd12	ENSMUSG00000030491.12	14.8	16.7	1.1	1.0
Ddx4	ENSMUSG00000021758.9	34.1	60.6	1.8	0.6
Piwil4	ENSMUSG00000036912.13	0.0	0.0	1.0	1.0
Mael	ENSMUSG00000040629.4	997.0	728.6	0.7	0.8
Rnf17	ENSMUSG00000000365.8	35.1	26.7	0.8	0.8
Henmt1	ENSMUSG00000045662.12	28.1	17.9	0.6	0.7
PNLDC1	ENSMUSG00000073460.4	2.2	1.1	0.6	0.6

Table S4. Transcription factors with altered mRNA abundance in *pi6*$^{em1/em1}$ pachytene spermatocytes.

Genes	Ensembl ID	C57BL/6 (fpkm)	*pi6*$^{em1/em1}$ (fpkm)	*pi6*$^{em1/em1}$ / C57BL/6	FDR
Sohlh1	ENSMUSG00000059625.6	0.6	10.4	9.9	2.2×10^{-2}
Sall4	ENSMUSG00000027547.13	0.6	9.3	8.7	4.3×10^{-3}
Etv6	ENSMUSG00000030199.12	1.4	14.2	7.7	3.8×10^{-2}
Elf4	ENSMUSG00000031103.8	0.3	5.3	7.0	1.6×10^{-2}
Dmrt1	ENSMUSG00000024837.11	3.8	29.4	7.0	4.3×10^{-3}
Pbx2	ENSMUSG00000034673.10	2.1	16.8	6.7	1.8×10^{-2}
Lin28a	ENSMUSG00000050966.5	0.9	7.6	5.6	1.6×10^{-2}
Erg	ENSMUSG00000040732.14	0.2	3.1	4.9	1.5×10^{-2}
Ubtf	ENSMUSG00000020923.13	2.7	15.0	4.9	1.3×10^{-2}
Gli3	ENSMUSG00000021318.11	1.1	7.2	4.8	3.9×10^{-2}
Hif1a	ENSMUSG00000021109.9	2.3	11.9	4.4	4.3×10^{-2}
Usf1	ENSMUSG00000026641.9	1.8	9.1	4.3	4.9×10^{-2}
Tcf3	ENSMUSG00000020167.10	4.5	20.3	4.2	1.1×10^{-2}
Tcf12	ENSMUSG00000032228.12	9.5	36.8	3.8	1.8×10^{-2}
Sohlh2	ENSMUSG00000027794.4	4.1	16.6	3.7	4.8×10^{-2}
Zfp292	ENSMUSG00000039967.10	2.2	8.9	3.5	7.0×10^{-3}
Foxo1	ENSMUSG00000044167.6	1.5	6.3	3.4	4.3×10^{-3}
Mlxipl	ENSMUSG00000005373.9	0.3	2.1	3.2	3.3×10^{-2}
Jund	ENSMUSG00000071076.5	4.3	14.4	3.1	2.6×10^{-2}
Notch2	ENSMUSG00000027878.10	3.4	8.3	2.2	3.6×10^{-2}
Rfx2	ENSMUSG00000024206.10	182.8	77.6	0.4	3.8×10^{-2}
Hoxa4	ENSMUSG00000000942.10	30.8	9.4	0.3	2.3×10^{-2}
Foxj1	ENSMUSG00000034227.7	15.4	4.9	0.3	1.6×10^{-2}

References

Goertz et al., 2011; Howard et al., 2014; Hough et al., 2014; Kistler et al., 2015; Lacorazza et al., 2006; McIntyre et al., 2013; Saleh et al., 2000; Sakashita et al., 2018; Selleri et al., 2004; Stauber et al., 2017; Suzuki et al., 2012; Thépot et al., 2000; Wang et al., 20115; Yamaguchi et al., 2015; Yu et al., 2008; Zhang et al., 2016; Zheng et al., 2009; Zhou et al., 2017

Table S5. Gene Ontology of genes with decreased expression in *pi6*$^{em1/em1}$ pachytene spermatocytes.

GO Biological process	Mus musculus reference list (22,262 genes)	Number of genes	Expected enrichment	Observed enrichment	*p*-value	FDR
Cilium organization (GO:0044782)	292	27	4.54	5.95	7.46×10^{-13}	1.44×10^{-9}
Cilium assembly (GO:0060271)	261	25	4.06	6.16	2.66×10^{-12}	4.13×10^{-9}
Cell projection assembly (GO:0030031)	363	27	5.64	4.79	7.85×10^{-11}	8.68×10^{-8}
Plasma membrane bounded cell projection assembly (GO:0120031)	350	26	5.44	4.78	1.83×10^{-10}	1.89×10^{-7}
Axonemal dynein complex assembly (GO:0070286)	33	9	0.51	17.55	1.19×10^{-8}	1.08×10^{-5}
Axoneme assembly (GO:0035082)	65	11	1.01	10.89	2.17×10^{-8}	1.68×10^{-5}
Microtubule bundle formation (GO:0001578)	95	11	1.48	7.45	7.01×10^{-7}	4.17×10^{-4}
Cell projection organization (GO:0030030)	1059	36	16.46	2.19	1.97×10^{-5}	7.45×10^{-3}
Sperm motility (GO:0097722)	84	17	1.31	13.02	2.34×10^{-13}	6.03×10^{-10}
Flagellated sperm motility (GO:0030317)	80	15	1.24	12.06	1.60×10^{-11}	1.90×10^{-8}
Cilium movement (GO:0003341)	55	11	0.85	12.87	4.67×10^{-9}	4.52×10^{-6}
Cilium or flagellum-dependent cell motility (GO:0001539)	24	8	0.37	21.45	2.12×10^{-8}	1.73×10^{-5}
Cilium-dependent cell motility (GO:0060285)	24	8	0.37	21.45	2.12×10^{-8}	1.82×10^{-5}
Cilium movement involved in cell motility (GO:0060294)	12	5	0.19	26.81	4.34×10^{-6}	2.04×10^{-3}
Microtubule-based movement (GO:0007018)	240	15	3.73	4.02	1.00×10^{-5}	4.45×10^{-3}
Regulation of cilium movement (GO:0003352)	15	5	0.23	21.45	1.05×10^{-5}	4.50×10^{-3}
Regulation of microtubule-based movement (GO:0060632)	29	6	0.45	13.31	1.37×10^{-5}	5.60×10^{-3}
Fertilization (GO:0009566)	166	16	2.58	6.2	2.26×10^{-8}	1.67×10^{-5}
Single fertilization (GO:0007338)	123	12	1.91	6.28	1.17×10^{-6}	6.48×10^{-4}
Binding of sperm to zona pellucida (GO:0007339)	38	7	0.59	11.85	5.09×10^{-6}	2.32×10^{-3}
Sperm-egg recognition (GO:0035036)	43	7	0.67	10.47	1.05×10^{-5}	4.40×10^{-3}
Sperm capacitation (GO:0048240)	31	6	0.48	12.45	1.92×10^{-5}	7.42×10^{-3}
Sexual reproduction (GO:0019953)	806	49	12.53	3.91	1.53×10^{-15}	2.38×10^{-11}
Spermatogenesis (GO:0007283)	529	39	8.22	4.74	4.90×10^{-15}	2.53×10^{-11}

Multi-organism reproductive process (GO:0044703)	929	52	14.44	3.6	4.55×10^{-15}	3.52×10^{-11}
Male gamete generation (GO:0048232)	549	39	8.53	4.57	1.51×10^{-14}	5.84×10^{-11}
Multicellular organismal reproductive process (GO:0048609)	786	45	12.22	3.68	1.84×10^{-13}	5.70×10^{-10}
Multicellular organism reproduction (GO:0032504)	798	45	12.4	3.63	3.03×10^{-13}	6.71×10^{-10}
Gamete generation (GO:0007276)	664	40	10.32	3.88	1.01×10^{-12}	1.74×10^{-9}
Reproduction (GO:0000003)	1334	57	20.73	2.75	9.11×10^{-12}	1.18×10^{-8}
Reproductive process (GO:0022414)	1333	57	20.72	2.75	8.86×10^{-12}	1.25×10^{-8}
Spermatid differentiation (GO:0048515)	217	18	3.37	5.34	2.58×10^{-8}	1.82×10^{-5}
Spermatid development (GO:0007286)	209	17	3.25	5.23	8.23×10^{-8}	5.31×10^{-5}
Germ cell development (GO:0007281)	313	17	4.86	3.49	1.49×10^{-5}	5.91×10^{-3}
Organelle assembly (GO:0070925)	620	28	9.64	2.91	9.23×10^{-7}	5.30×10^{-4}
Microtubule-based process (GO:0007017)	628	27	9.76	2.77	3.51×10^{-6}	1.70×10^{-3}

Table S6. Genes with reduced expression in *pi6*$^{em1/em1}$ pachytene spermatocytes that are mapped to major Gene Ontology categories.

Gene	Cilium assembly	Sperm motility	Fertilization
Acr			+
Adam3		+	+
Arl3	+	+	
Arsa			+
Cabyr			+
Cast			+
Catsper1		+	+
Catsper3		+	+
Ccdc40	+	+	
Ccdc63	+	+	
Ccdc65	+	+	
Ccdc113	+		
Cdh13	+		
Dkkl1			+
Dnaaf1	+	+	
Dnah3		+	
Dnaic2	+	+	
Drc1	+	+	
Dzip1	+		
Efhd1	+		
Fbp1	+		
Foxj1	+		
Gdpd5	+		
Hap1	+	+	
Hist1h1t		+	+
Ift74	+	+	
Insl6		+	+
Kif2b		+	
Lrrc6	+	+	
Lrrc46	+		

Lrrcc1	+		
Nphp1	+		
Nubp2	+		
Odf2	+		
Parvg	+		
Pbp2			+
Prkaca			+
Rfx2	+		
Rimbp3			+
Rsph1	+		
Slc9c1		+	
Slc22a16		+	+
Slc26a8		+	+
Spa17			+
Spata4		+	
Spag1			+
Spink2			+
Tcp10a	+		
Tcp10b	+		
Tcp10c	+		
Tcte1		+	
Tekt1	+	+	
Tekt2	+	+	
Tekt3	+	+	
Tekt4	+	+	
Tex40		+	+
Tprn	+		
Tsga10ip	+		
Ttll1	+	+	
Ubxn10	+		
Vdac2			+
Ybx3			+
Zpbp2			+

Table S8. Published male fertility genes with altered expression in *pi6*$^{em1/em1}$ cells.

Gene	ENSEMBL ID	Reference	C57BL/6 (fpkm)	*pi6*$^{em1/em1}$ (fpkm)	$\frac{pi6^{em1/em1}}{C57BL/6}$	FDR
Adam3	ENSMUSG00000031553.11	Yamaguchi et al., 2009	182.1	72.4	0.4	3×10^{-2}
Catsper1	ENSMUSG00000038498.3	Ren et al., 2009; Avenarius et al., 2009; Qi et al., 2007	15.1	3.0	0.2	9×10^{-3}
Catsper3	ENSMUSG00000021499.8	Qi et al., 2007	12.6	3.1	0.3	5×10^{-2}
Ccdc40	ENSMUSG00000039963.14	Antony et al., 2013; Becker-Heck et al., 2011	68.8	22.8	0.3	3×10^{-2}
Ccdc42	ENSMUSG00000045915.11	Pasek et al., 2016	58.3	18.0	0.3	3×10^{-2}
Ccdc65	ENSMUSG00000003354.5	Horani et al., 2013	121.7	39.0	0.3	4×10^{-3}
Ccna1	ENSMUSG00000027793.2	Liu et al., 1998	33.7	12.0	0.4	3×10^{-2}
Dnaic2	ENSMUSG00000034706.12	Guichard et al., 2001	18.4	4.2	0.2	7×10^{-3}
Drc1	ENSMUSG00000073102.3	Wirschell et al., 2013	39.1	11.2	0.3	1×10^{-2}
Gga1	ENSMUSG00000033128.8	International Mouse Phenotyping Consortium	49.8	18.7	0.4	2×10^{-2}
Hnf1b	ENSMUSG00000020679.7	Mieusset et al., 2017	4.6	1.0	0.3	5×10^{-2}
Ift74	ENSMUSG00000028576.8	San Agustin et al., 2015	68.2	24.0	0.4	2×10^{-2}
Lrrcc1	ENSMUSG00000027550.10	International Mouse Phenotyping Consortium	76.1	31.3	0.4	2×10^{-2}
Meig1	ENSMUSG00000026650.11	Zhang et al., 2009; Salzberg et al., 2010	953.3	329.8	0.3	9×10^{-3}
Ppp3cc	ENSMUSG00000022092.10	Miyata et al., 2015	89.0	39.5	0.4	3×10^{-2}
Ppp3r2	ENSMUSG00000028310.2	International Mouse Phenotyping Consortium	132.7	35.9	0.3	4×10^{-3}
Prm1	ENSMUSG00000022501.5	Haueter et al., 2010	126.4	308.2	2.4	5×10^{-2}
Rfx2	ENSMUSG00000024206.10	Kistler et al., 2015; Shawlot et al., 2015	182.8	77.6	0.4	4×10^{-2}
Spink2	ENSMUSG00000053030.7	International Mouse Phenotyping Consortium	391.6	146.7	0.4	7×10^{-3}
Stk33	ENSMUSG00000031027.11	Martins et al., 2018	180.4	62.6	0.3	2×10^{-2}
Syce1	ENSMUSG00000025480.4	Bolcun-Filas et al., 2009; Maor-Sagie et al., 2015	215.2	71.6	0.3	4×10^{-3}
Tekt2	ENSMUSG00000028845.11	Iguchi et al., 1999; Tanaka et al., 2004	85.5	23.3	0.3	2×10^{-2}
Tekt3	ENSMUSG00000042189.5	Roy et al., 2009	63.2	13.0	0.2	4×10^{-3}
Tekt4	ENSMUSG00000024175.1	Roy et al, 2007	51.7	15.7	0.3	7×10^{-3}
Ttll1	ENSMUSG00000022442.11	Vogel et al., 2010	22.0	7.3	0.3	3×10^{-2}

Zpbp2	ENSMUSG000 00017195.11	Lin et al., 2007	133.8	43.2	0.3	1×10^{-2}