Supplementary Table 1. Gene Trap Mutations in Rat Strains Screened for Reproduction Phenotypes

Gene symbol	Intron	Native Protein	Trapped Protein	Product (blue font = translated epitope from gene trap construct)
Abca13	49 of 62	5041 aa	4416 aa	exon 49 to bgeo splice (aa4377)VWYNQKGFHSLPSYLNHLNNLILWQNLPAHAADWRQYARG
Alk3	1 of 10	532 aa	25 aa	exon 1 to bgeo splice MTQLYTYIRLLGACLFIISHVQARG
Atg13	17 of 17	516 aa	515 aa	exons 1-17 to bgeo splice METDLSSQDRKDLDKFIKFFALKTVQVIVQARLGEKICTRSSSSPTGSDWFNLAIKDIPEVTHEAKKALSGQLPAVGRSM CVEISLKTSEGDSMELEIWCLEMNEKCDKEIKVSYTVYNRLSLLLKSLLAITRVTPAYRLSRKQGHEYVILYRIYFGEVQLNG LGEGFQTVRVGTVGTPVGTLTLSCAYRINLAFMSTRQFERTPPIMGIIIDHFVDRPYPSSSPMHPCNYRTAEDAGVAYPS VEDSQEVCTTSFSTSPPSQLSSSRLSYQPAVLGLGSADLAYPVVFTAGLNTTHAHQLMVPGKEGGVPLAPNHPAHGAQ ADQERLVVHMPSDGTHCAATPSSSEDTETVSNSSEGRASPHDILETIFVRKVGAFVNKPINQVTLTSLDIPFAMFAPKNL ELEDADPMVNPPDSPETTSPLHGSLHSEGSSGGSSGNAHDDFVMIDFKPAFSKDDILPMDLGTFYREFQNPPQLSSLSI DFGAQSMAEDLLAVEDKLFAVFPVGIDGIDKLDDL
Btrc	1 of 14	619 aa	40 aa	exon 1 to bgeo splice MDPAEAVLQEKALKFMLAVEDKLFAVFPVGIDGIDKLDDL
Dlg1	6 of 28	911 aa	218 aa	exons 1-6 to bgeo splice

				MPVRKQDTQRALHLLEEYRSKLSQTEDRQLRSSIERVISIFQSNLFQALIDIQEFYEVTLLDNPKCVDHSKQCEPVQPGNP WESGSLSSAAVTSESLPGGLSPPVEKYRYQDEEVLPSERISPQVPLAVEDKLFAVFPVGIDGIDKLDDL
Exoc6b	1 of 21	810 aa	1355 aa	exon 1 to bgeo splice MERVKMAEESLETAAEHERILREIESTDTACIGPTLRSVYDGEEHGRFMEKLETRIRNHDREIEKMCNFHY QGFVDSITELLKVRGEAQKLKNQVTDTNRKLQHEGKELVIAMEELKQCRLQQRNISATVDKLMLCLPVLE MYSKLRDQMKTKRHYPALKTLEHLEHTYLPQVSHYRFCKVMVDNIPKLREEIKDVSMSDLKDFLESIRKH SDKIGETAMKQAQQQRNLDNIVLQQPRLGSKRKSKKDVYTIFDTEVESTSPKSEQDSGILDVEDEEDDEE VPGAQDLVDFSPVYRCLHIYSVLGARETFENYYRKQRRKQARLVLQPPSNMHETLDGYRKYFNQIVGFF VVEDHILHTTQGLVNRAYIDELWEMALSKTIAALRTHSSYCSDPNLVLDLKNLIVLFADTLQVYGFPVNQLF DMLLEIRDQYSETLLKKWAGVFRNILDSDNYSPIPVTSEETYKKVVGQFPFQDTELEKQPFPKKFPFSEFV PKVYNQIKEFIYACLKFSEDLHLSSTEVDDMIRKSTNLLLTRTLSNSLQNVIKRKNIGLTELVQIIINTTHLEKS CKYLEEFITNITNVLPETVHTTKLYGTTTFKDARHAAEEEIYTNLNQKIDQFLQLADYDWMTGELDNKASDY LVDLIAFLRSTFAVFTHLPGKVAQTACMSACKHLATSLMQLLLEAEVRQLTLGALQQFNLDVRECEQFAR SGPVPGFQEDTLQLAFIDLRQLLDLFIQWDWSTYLADYGQPNCKYLRVNPVTALTLLEKMKDTSRKNNMF AQFRKNERDKQKLIDTVAKQLRGLISSHHS
Gsg1l	3 of 6	230 aa	84 aa	exons 1-3 to bgeo splice MDKGQQEAQPGQGGGEKCRSFIDLAPASEKGVLWLSVVSEVLYILLLVVGFSLMCLELLHSSSVIDGLKLNAFAAVFTVL SARG
Grik3	In exon 11 of 16	910 aa	557 aa	Exons 1- truncated 11 to bgeo genomic

				MTAPWRRLRSLVWEYWAGFLVCAFWIPDSRGMPHVIRIGGIFEYADGPNAQVMNAEEHAFRFSANIINRNRTLLPNT TLTYDIQRIHFHDSFEATKKACDQLALGVVAIFGPSQGSCTNAVQSICNALEVPHIQLRWKHHPLDNKDTFYVNLYPDYA SLSHAILDLVQSLKWRSATVVYDDSTGLIRLQELIMAPSRYNIRLKIRQLPIDSDDSRPLLKEMKRGREFRIIFDCSHTMAA QILKQAMAMGMMTEYYHFIFTTLDLYALDLEPYRYSGVNLTGFRILNVDNPHVSAIVEKWSMERLQAAPRAESGLLDG VMMTDAALLYDAVHIVSVCYQRAPQMTVNSLQCHRHKAWRFGGRFMNFIKEAQWEGLTGRIVFNKTSGLRTDFDLD IISLKEDGLEKVGVWSPADGLNITEVAKGRGPNVTDSLTNRSLIVTTLLEEPFVMFRKSDRTLYGNDRFEGYCIDLLKELAH ILGFSYEIRLVEDGKYGAQDDKGQWNGMVKELIDHKADLAVAPLTITHVREKAIDFSKPFMTLGVSILQLKSEVYIHLSW SH
Fstl5	3 of 15	847 aa	56 aa	1-3 to bgeo splice MFKCWSAVLILGFIFLASEGRPTKESGYGLKYYQPLTRLRHKQEKSQESSRIKARG
Pan3	5 of 18	833 aa	278 aa	exons 1-5 to bgeo splic MNSGGGLPPPSAAASPSSSSLAAAVAVAVAASSGVGGVPGGPAAGVKLKYCRYYAKDKTCFYGEECQFLHEDPASGA APGLGLHSNSVPLALAAASGAGFPPGALPGGGAGPPAGPKKPELGVPGAATAGGGLDGPRVAIPGMDGGALTDTSLT DSYFSTSFIGVNGFGSPVETKYPLMQRMTSSSSSPSLLNDSAKPYTGHDPLTSSASSLFNDFGALNISQRRKTPNPTASEFI PKGGSTSRLSNVSQSNMSAFSQVFSHPSMGSPATAGLAPARG
Pclo	3 of 24	4880-5085 aa	1075 aa	exons 1-3 to bgeo splice

				MGNEASLEGEGLPEGLAAAAGAGGSGSALHPGIPAGMEADLSQLSEEERRQIAAVMSRAQGLPKGSVPPAAAESPSM HRKQELDSSQAPQQPGKPPDPGRPTQPGLSKSRTTDTFRSEQKLPGRSPSTISLKESKSRTDFKEEYKSSMMPGFFSDV NPLSAVSSVVNKFNPFDLISDSEASQEETTKKQKVVQKEQGKSEGMAKPPLQQPSPKPIPKQQGQVKEVIQQDSSPKSV SSQQAEKVKPQAPGTGKPSQQSPAQTPAQQASPGKPVAQQPGSAKATVQQPGPAKSPAQPAGTGKSPAQPPAKTP GQQAGLEKTSSSQQPGPKSLAQTPGHGKFPLGPVKSPAQQPGTAKHPAQQPGPQTAAKVPGPTKTPAQQSGPGKTP AQQPGPTKPSPQQPIPAKPQPQQPVATKTQPQQSAPAKPQPQQPAPAKPQPQQPTPAKPQPQPPTPAKPQPQPPT ATKPQPQPPTATKPHHQQPGLAKPSAQQPTKSISQTVTGRPLQPPPTSAAQTPAQGLSKTICPLCNTTELLLHIPEKANF NTCTECQSTVCSLCGFNPNPHLTEIKEWLCLNCQMQRALGGDLAAAIPSSPQPTPKAATAPTATASKSPVPSQQASPKK EPPSKQDSPKALESKKPPEPKKPPEPKKPPEPKKPPPLVKQPTLHGPTPATAPQLPVAEALPEPAPPKEPSGPLPEQAKAP VGDVEPKQPKMTETRADIQSSSTTKPDILSSQVQSQAQVKTASPLKTDSAKPSQSFPPTGEKTTPLDSKAMPRPASDSKI ISQPGPGSESKDPKHIDPIQKKDEPKKAQPKGSPKPETKPVPKGSPTPSGTRPTAGQAAPPSQQPPKPQEQSRRFSLNLG GITDAPKSQPTTPQETVTGKLFGFGASIFSQASNLISTAGQQGPHPQTGPAAPSKQAPTPSQSPAAQGPAKSTGQLPPA PAKATAVKKEAKAAAAENLESKPEQAPTAKKTEKDKKPPPAKVGKPPPSEPEKAVPAHKPDKTTKPKPACPLCRTELNLG SQEPPNFNTCTECKNQVCNLCGFNPTPHLTELAVEDKLFAVFPVGIDGIDKLDDL
Slc1a3	2 of 9	543 aa	63 aa	exons 1-2 to bgeo splice MTKSNGEEPRMGSRMERFQQGVRKRTLLAKKKVQNITKEDVKSYLFRNAFVLLTVSAVIVARG
Spaca6	2 of 8	324 aa	100 aa	exons 1-2 to bgeo splice MGLVALVGSFVLLLLLIFRASTWACLFCFTTHEERLSLCRMFVGSEDSKIRKCRDALTDAFEGFSDMEINYDERSHLHDEF TQMTVFLQEVAAVQGESRG
Tmx4	5 of 7	336 aa	193 aa	exons 1-5 to bgeo splice MTGGFCVPVFLAAWLAAAAAEAEGLEQAALPAEESRVQPMTASNWTLVMEGEWMLKFYAPWCPSCQQTDSEWET FAKNGETLQISVGKVDVIQEPGLSGRFFVTTLPAFFHAKDGIFRRYRGPGIYEDLQNYILEKKWQSVEPLTGWKSPASLT MSGMAGLFSISGKIWLAVEDKLFAVFPVGIDGIDKLDDL
Ube2K	1 of 6	200 aa	45 aa	exon 1 to bgeo splice

				MANIAVQRIKREFKEVLKSEELAVEDKLFAVFPVGIDGIDKLDDL
Ube2q2	1 of 14	345 aa	87 aa	exon 1 to bgeo splice MSVSGLKAELKFLASIFDKNHELFRIVSWKLDELHCQFLVPPPPPPGSSHSPPPPLTLHCNITLAVEDKLFAVFPVGIDGIDKLDDL
Zmynd8	3 of 21	1208 aa	82 aa	exons 1-3 to bgeo splice MDISTRSKDPGSTERTAQKRKVPSPPHSSNGHSPQDSSTSPIKKKKKPGLLNSSSKDQLAVEDKLFAVFPVGIDGIDKLDDL

Supplementary Table 2. Reproduction Phenotypes of Rats with Gene Trap Mutations

Mutated Gene	Intronic Insertion	Breeders	Breeding Pairs	Litter Number	Mean Litter Size (±S.E.M.)		Genotypes Ratio (% WT:Het:Homo)	Sex Ratio (Female:Male)	Reproduction Phenotype
Wildtype	None	WT x WT	3	10	11.8±1.27		100:0:0	53:47	Normal
Abca13	Intron 49 of 62	Het x Het	2	6	7.33±0.80	p=0.01*	25:61:14	55:45	Subfertile
		F Homo x WT	2	6	9.50±1.19		0:100:0	52:48	Normal
		M Homo x WT	2	6	12.8±1.08		0:100:0	39:61	Normal
Alk3	Intron 1 of 10	Het x Het	2	6	5.83±0.60	p=0.001*	49:51:0	43:57	Embryonic Lethal
		F Homo x WT	0	0	-		-	-	-
		M Homo x WT	0	0	-		-	-	-
Atg13	Intron 16 of 16	Het x Het	2	6	8.33±1.08	p=0.06*	30:42:28	42:58	Subfertile
		F Homo x WT	2	0	0		-	-	Adult Lethal
		M Homo x WT	2	0	0		-	-	Adult Lethal
Btrc	Intron 1 of 14	Het x Het	2	6	11.6±1.50		28:43:29	47:53	Normal
		F Homo x WT	2	6	9.00±1.88		0:100:0	54:46	Normal
		M Homo x WT	2	0	0		-	-	Gametogenesis Defect
Dlg1†	Intron 6 of 28	Het x Het	-	-	-		-	-	Unknown†
		F Homo x WT	-	-	-		-	-	-
		M Homo x WT	-	-	-		-	-	-
Exco6b	Intron 1 of 21	Het x Het	2	6	11.6±0.40		31:69:0	41:59	Embryonic Lethal
		F Homo x WT	0	0	-		-	-	-
		M Homo x WT	0	0	-		-	-	-
Gsgl1	Intron 3 of 6	Het x Het	2	5	5.80±1.02	p=0.003*	3:69:28	52:48	Subfertile
		F Homo x WT	2	6	9.33±2.58		0:100:0	46:54	Normal
		M Homo x WT	2	5	12.8±1.16		0:100:0	48:52	Normal
Grik3	Exon 11 of 16	Het x Het	2	6	9.50±0.84		19:51:30	44:56	Normal
		F Homo x WT	2	5	5.80±1.07	p=0.003*	0:100:0	48:52	Subfertile
		M Homo x WT	2	6	11.3±2.07		0:100:0	54:46	Normal
Fstl5	Intron 3 of 15	Het x Het	2	5	12.2±0.97		27:39:34	52:48	Normal
		F Homo x WT	2	5	11.2±1.88		0:100:0	55:45	Normal
		M Homo x WT	2	3	14.0±1.00		0:100:0	50:50	Normal
Pan3	Intron 5 of 18	Het x Het	2	6	9.67±1.58		27:48:25	36:64	Normal
		F Homo x WT	2	6	6.00±1.55	p=0.014*	0:100:0	44:56	Subfertile
		M Homo x WT	2	0	0		-	-	Gametogenesis Defect
Pclo¶	Intron 3 of 25	Het x Het	2	6	11.3±1.26		26:62:12	53:47	Normal <8 mo
		F Homo x WT	2	0	0		-	-	Behavioral
		M Homo x WT	2	0	0		-	-	Behavioral
Rgs22	Intron 2 of 24	Het x Het	2	4	14.3±1.93		24:53:23	52:48	Normal
		F Homo x WT	2	4	13.8±0.63		0:100:0	55:45	Normal

		M Homo x WT	2	4	11.8±1.77		0:100:0	53:47	Normal
Slc1a3	Intron 2 of 9	Het x Het	2	12	6.92±1.33	p=0.01*	22:77:1	51:49	Embryonic Lethal
		F Homo x M Het	0	0	-		-	-	-
		M Homo x WT	0	0	-		-	-	-
Spaca6	Intron 2 of 8	Het x Het	2	5	10.0±0.89		24:42:34	50:50	Normal
		F Homo x WT	2	5	9.40±2.20		0:100:0	51:49	Normal
		M Homo x WT	2	0	0		-	-	Fertilization
Tmx4	Intron 5 of 7	Het x Het	2	8	9.63±1.25		39:61:0	51:49	Embryonic Lethal
		F Homo x WT	0	0	-		-	-	-
		M Homo x WT	0	0	-		-	-	-
Ube2K	Intron 1 of 6	Het x Het	2	6	9.40±1.14	p=0.036*	21:53:26	55:45	Subfertile
		F Homo x WT	2	0	0		-	-	Gametogenesis Defect
		M Homo x WT	2	0	0		-	-	Gametogenesis Defect
Ube2q2	Intron 1 of 14	Het x Het	2	6	9.50±1.32		19:50:31	42:58	Normal
		F Homo x WT	2	5	12.2±1.93		0:100:0	47:53	Normal
		M Homo x WT	2	6	12.0±1.06		0:100:0	44:56	Normal
Zmynd8	Intron 3 of 21	Het x Het	2	7	7.80±1.22	p=0.003*	75:25:00	44:56	Embryonic Lethal
		F Homo x WT	0	0	-		-	-	-
		M Homo x WT	0	0	-		-	-	-

*Potentially Subfertile due to smaller mean letter sizes ($p<0.1$ shown versus wildtype).
†Heterozygous *Dlg1*-wt/gt mutant females produced from wildtype recipient males displayed problems breeding for undefined reasons.
¶Heterozygous *Pclo*-wt/gt females 8-14 months of age did not reproduce when paired with wildtype males of similar age (n=4 breeder pairs). Additional analyses confirmed reproductive failure in *Spaca6*$^{gt/gt}$ males (n=4), *Pclo*$^{gt/gt}$ females (n=6) and *Pclo*$^{gt/gt}$ males (n=6).

Supplementary Table 3. Body, Testis and Epididymal Weights in Mutant Rats

Mutant*	Rats (n)	Body wt (g) ±S.E.M.	Testis wt (g) ±S.E.M.		Epididymus wt (g) ±S.E.M.		Testis:Body wt† ±S.E.M.	Epid:Body wt† ±S.E.M.
Atg13	3	354±14	1.51±0.07	p=0.0054	0.49±0.04	p=0.025	4.27±0.08	1.38±0.01
Btrc	3	428±40	0.67±0.04	P<0.0001	0.30±0.03	P<0.0001	1.55±0.10	0.70±0.03
Grik3	4	511±63	1.86±0.13	p>0.10	0.61±0.05	p>0.10	3.64±0.24	1.19±0.11
Pan3	4	436±19	1.10±0.04	P<0.0001	0.45±0.02	p=0.001	2.53±0.15	1.03±0.09
Pclo	4	536±58	1.95±0.05	P=0.1	0.64±0.01	p>0.10	3.64±0.39	1.19±0.13
Spaca6	6	447±20	2.01±0.06	p>0.10	0.57±0.02	p>0.10	4.50±0.18	1.28±0.09
Ube2k	4	302±38¶	0.35±0.01	P<0.0001	0.24±0.03	P<0.0001	1.16±0.25	0.79±0.38
Ube2q2	3	486±22	2.17±0.04	p>0.10	0.65±0.03	p>0.10	4.47±0.20	1.33±0.09
Wildtype	6	506±28	1.98±0.10	n.a.	0.64±0.02	n.a.	3.91±0.26	1.26±0.07

* Male rats were analyzed between 90-250 days of age; significant differences in mutant testis and epididymal weights are compared to wildtype littermates at the respective age of analysis.

¶ Homozygous mutant *Ube2k* rats displayed significantly reduced body weights ($p<0.001$).

† Organ:body weight (wt) ratios x 1000.

Supplementary Table 4. Mutant Rat Phenotypes in Current Study* Compared Across Species.

Mutated Gene	Insertion Site	Rat*	Mouse[n]	Human[n]	Other Organisms[n]
Abca13	Intron 49 of 62	None observed	Unknown	Schizophrenia, bipolar disorder, depression[1]	Unknown
Atg13	Intron 16 of 16	Shortened lifespan; renal failure; inflammation; autophagy defect	Embryonic lethal, cardiovascular development defects[2]	Autoimmune Disease[3]	Premature senescence in plants[4]; reduced lifespan in yeast[5] and worms[6]; embryonic lethal in flies[7].
Alk3	Intron 1 of 10	Stunted growth in heterozygotes; embryonic lethal in homozygotes	Normal growth in heterozygotes; embryonic lethal in homozygotes; epiblast defects[8]	Juvenile and hamartomatous polyposis syndromes[9-11]	Anterior-posterior wing patterning and oogenesis in flies[12-14];
Btrc	Intron 1 of 14	Spermatogonial development block	Males sub-fertile[15], neural Stem cell fate[16,17], longer circadian period[18], DNA stability[16]	10q Medulloblastoma tumor suppressor locus[19], 10q split hand/foot disease[20,21]; promotes HIV infection[22]	Xenopus dorsal axis duplication[23]
Dlg1	Intron 6 of 28	Dominant¶	Recessive effects on aging, renal function, craniofacial, vision, and urogenital defects[24-27].	Schizophrenia[28]; 3q29 microdeletion syndrome[29]	Tumor suppressor[30]; alcohol tolerance in flies[31]
Exoc6b	Intron 1 of 21	Embryonic lethal	Unknown	Behavioral defects, epilepsy[32,33]	Unknown
Gsg1l	Intron 3 of 6	None observed	Unknown	Candidate risk factor for colon cancer[34]	Unknown
Grik3	Exon 11 of 16	None observed	Synaptic transmission[35]	Behavioral disorders[36]; schizophrenia[37]	Unknown
Fstl5	Intron 3 of 15	None observed	Unknown	Adiposity-Related Anthropometric Traits[38]	Unknown
Pan3	Intron 5 of 18	Post-meiotic spermatogenesis	Unknown	Unknown	Non-lethal, polyadenylation defects in yeast[39]
Pclo	Intron 3 of 25	Female & male social behavior, mating behaviors, seizures	Adults reproduce and behave normally, postnatal growth and lifespan stunted[40]	Major depressive, bipolar disorders, seizures, pontocerebellar hypoplasia, emotional processing[41-46]	Unknown
Rgs22†	Intron 2 of 24	None observed	Unknown	Unknown	Unknown
Slc1a3	Intron 2 of 9	Embryonic lethal	Adult fertile, aging, behavior disorders[47-49]	Behavior; addiction, seizure[50,51]	Unknown
Spaca6	Intron 2 of 8	Sperm function	Sperm function[52]	Unknown	Unknown
Tmx4	Intron 5 of 7	Embryonic lethal	Unknown	Unknown	Unknown
Ube2K	Intron 1 of 6	Female & male meiosis	Adults fertile; resistant to amyloid-β toxicity[53]	Unknown	Unknown
Ube2q2	Intron 1 of 14	None observed	Unknown	Renal failure[54,55]; arthritis[56]	Unknown
Zmynd8	Intron 3 of 21	Embryonic lethal	P14 viable[21]	Unknown	Unknown

* Reproduction phenotypes observed in current study; all phenotypes recessive unless stated as dominant or occurring in heterozygotes. † Gene trap is in Reverse Orientation to the *Rgs22* gene, gene trap is predicted to be non-functional. ¶ Strain was not propagated from F1 *Dlg1* heterozygote mutant females for unknown reasons but displayed solitary behavior in cage (potential mating behavior or other reproduction defect). [n] PubMed citation referenced below; only spontaneous or non-conditional experimental mutations referenced.

References to Supplementary Table 4

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Supplementary Material

Supplementary Results

A majority of protein coding genes are essential for rat reproduction

Rats harboring *Sleeping Beauty* genetrap transposons within 18 distinct protein coding genes were crossed with wildtype rats to generate heterozygous breeding pairs (Supplementary Table 1). Heterozygous breeding pairs were successfully generated for 17 of 18 mutant alleles (Supplementary Table 2). Only female rats heterozygous for a genetrap (gt) mutation within intron 5 of *Dlg1* (i.e. *Dlg1*$^{wt/gt}$) failed to transmit their mutation more than one generation (Supplementary Table 2). Male and female heterozygotes for the remaining 17 strains were paired to generate homozygous mutant strains. Mean litter sizes produced by crossing heterozygotes for 12 of 18 mutant strains were not significantly different from wildtype breeder pairs (Supplementary Table 2). Heterozygous breeding pairs from each *Alk3*$^{wt/gt}$, *Abca13*$^{wt/gt}$, *Gsg1l*$^{wt/gt}$, *Slc1a3*$^{wt/gt}$, *Ube2k*$^{wt/gt}$ and *Zmynd8*$^{wt/gt}$ strains consistently reproduced, but their litter sizes were significantly smaller compared to wildtype (Supplementary Table 2).

Mean litter sizes generated by crossing homozygous mutant *Abca13*$^{gt/gt}$, *Fstl5*$^{gt/gt}$, *Grik3*$^{gt/gt}$ (males only), *Gsg1l*$^{gt/gt}$, *Rg22*$^{gt/gt}$ and *Ube2q2*$^{gt/gt}$ rats to wildtype rats appeared normal (Supplementary Table 2). *Grik3*$^{gt/gt}$ and *Pan3*$^{gt/gt}$ females were fertile, but produced smaller litters after crossing with wildtype males (p=0.003). In contrast, homozygous genetrap mutations in *Btrc, Ube2k*, *Pan3*, *Atg13*, *Spaca6* and *Pclo* blocked reproduction by males during peak reproductive age (Fig. 2a and Supplementary Table 2); in this group, *Ube2k*$^{gt/gt}$, *ATG13*$^{gt/gt}$ and *Pclo*$^{gt/gt}$ mutant females also did not reproduce (Supplementary Table 2). *Btrc*$^{gt/gt}$ and *Pan3*$^{gt/gt}$ female rats were fertile (Supplementary Table 2). Despite their inability to reproduce, *Ube2k*$^{gt/gt}$ and *Pclo*$^{gt/gt}$ female rats developed oocytes (Supplementary Fig. 2c).

Spermatogenic cells in *Ube2k*$^{gt/gt}$ rats arrested early during meiosis I postnatally and in young adults (Fig. 2a, b), and progressively regressed to arrest at pre-meiotic steps during adulthood (Supplementary Fig. 2a). *Ube2k*$^{gt/gt}$ mutant rats were also significantly smaller in body size compared to wildtype littermates ($p<0.001$) (Supplementary Table 3). Spermatogenic cells in *Pan3*$^{gt/gt}$ mutant rats arrested during spermatid elongation (Fig. 2a, b and Supplementary Fig. 2). Rats with heterozygous genetrap mutations predicted to disrupt expression of *Alk3*, *Exoc6b*, *Slc1a3*, *Tmx4 and Zmynd8* reproduced, but demonstrated reduced Mendelian rates towards generation of homozygous mutant progeny (Supplementary Table 2). Homozygous genetrap mutations in these later genes were classified as embryonic lethal. Heterozygous *Alk3*$^{wt/gt}$ mutants also displayed a reduced growth phenotype compared to wildtype littermates (D24 mean body weight, *Alk3*$^{wt/wt}$ 69.4±1.7g, *Alk3*$^{wt/gt}$ 58.6±2.7g, n=36 total pups, 3 litters; ±SD $p<0.001$).

Comparative Analysis of Rat Genetrap Mutations

Phenotypes transmitted by rat genetrap mutations were compared across different species, as follows:

Alk3$^{gt/gt}$: Distinct from reduced growth in *Alk3*$^{wt/gt}$ rats, heterozygous mutant *Alk3* mice display normal growth[1]. Consistent with current Mendelian rates of germline transmission in *Alk3*$^{gt/gt}$ rats, homozygous mutations are embryonic lethal in mice, which caused defects stemming from epiblast development[1]. In flies, homozygous *Alk3* mutations disrupt wing patterning[2,3], as well as germline stem cell development[4]. In humans, recessive *Alk3* mutations are linked to Juvenile and Hamartomatous Polyposis syndromes[5-7].

Atg13$^{gt/gt}$: Analogous to reduced postnatal lifespan in *Atg13*$^{gt/gt}$ rats, disrupting *Atg13* expression induces nutrient dependent senescence in plants[8] and reduces lifespan in *C. Elegans*[9]. However, *Atg13* loss-of-function mutations are embryonic lethal in flies[10]. Spermatozoa from the cauda

epididymus of *Atg13*$^{gt/gt}$ mutants appeared immotile when analyzed between 60 and 120 days of age. In addition to the reduced sperm count (Supplementary Fig. 3), flagella from *Atg13*$^{gt/gt}$ cauda epididymal spermatozoa were largely immotile, and often displayed detached heads and tails (not shown). Notably, the health decline of *Atg13*$^{gt/gt}$ rats was observed ~90-100 days of age, which is only 2-3 weeks after rats reach reproductive age (*also see* Discussion section in manuscript). Livers and kidneys in *Atg13*$^{gt/gt}$ rats appeared abnormal (Fig. 3c). In the liver, occasional vacuolated cells were scattered throughout histological sections. These cells contained small spherical vacuoles consistent with accumulation of triglycerides (Supplementary Fig. 4a).

All kidneys examined displayed marked glomerulonephritis and moderate tubulointerstitial disease (Fig. 3d). Glomeruli were diffusely altered by membranous change; glomerular tufts were thickened by expansion of mesangium and thickening of basements membranes (Figs. 3d and Supplementary 4a). Bowman's capsule was also thickened, and in some glomeruli, Bowman's space was filled with crescentic proliferations (Fig. 3d). Numerous tubules were dilated and contained protein-rich casts (Fig. 3d and Supplementary 4a). PAS and Trichrome stains indicated that hyaline deposits consisted of both PAS positive material and collagen. There was scattered interstitial inflammation consisting of lymphocytes and plasma cells; and, small numbers of tubules contained neutrophils (Supplementary Fig. 4a).

Btrc$^{gt/gt}$: Distinct from *Btrc*$^{gt/gt}$ rats, *Btrc*-deficient mice develop sufficient numbers of functional sperm to reproduce by natural mating, but their litter sizes are significantly smaller when compared to wildtype[11]. Reduced litter size in *Btrc*-deficient mice is thought to be caused by abnormal meiotic divisions in some segments of seminiferous tubules, which would reduce total sperm counts[11]. In contrast, normal elongating spermatids were not observed in *Btrc*$^{gt/gt}$ rats (Supplementary Figs. 2a, b). And, most seminiferous tubule cross sections from *Btrc*$^{gt/gt}$ rats

contained only Sertoli cells and type A spermatogonia (~85%, n=2 rats; Supplementary Figs. 2a, b). A lower percent tubules (~15%) uniquely contained spermatocytes, round spermatids and abnormal elongating spermatids (Supplementary Fig. 2b). *Btrc* encodes an E3 ubiquitin ligase, and analogous to effects observed here on spermatogonial development, *Btrc* is essential for proper differentiation of neural progenitor cells[12,13]. In *Xenopus*, dominant negative *Btrc* constructs missing the N-terminal F-box domain, but retaining its C-terminal WD40 repeat domains induce duplication of the dorsal tadpole axis[14]. Interestingly, absolute testis weights in *Btrc*$^{gt/gt}$ rats are greater than in *Ube2k*$^{gt/gt}$ rats (Supplementary Table 3). This is despite the fact that spermatogenesis arrested at later developmental steps in *Ube2k*$^{gt/gt}$ compared to a vast majority of tubules in *Btrc*$^{gt/gt}$ rats (Supplementary Fig. 2a, b). However, it should be noted that *Ube2k*$^{gt/gt}$ male rats are between 30-40% smaller in body size compared to *Btrc*$^{gt/gt}$ or wildtype rats (Supplementary Table 3).

Dlg1$^{gt/gt}$: Female *Dlg1*$^{wt/gt}$ rats were consistently observed in back cage corners, remained socially isolated following pairing with respective wildtype males, did not produce offspring, and appeared otherwise healthy. In contrast to the reproduction deficiency of heterozygous female *Dlg1*$^{wt/gt}$ rats, heterozygous *Dlg1* mutant female mice breed normally[15-18]. However, homozygous *Dlg1* mouse mutants display overt urogenital, craniofacial and lymphocyte developmental defects linked to shorter postnatal lifespan[15-18]. Here, a similar *Dlg1*$^{wt/gt}$ mutation with respect to deleted domains was transmitted through the rat male germline by spermatogonial stem cells transplanted into the somatic background of wildtype founder rats. This, and the abnormal breeding by female *Dlg1*$^{wt/gt}$ rats, is consistent with species-dependent dominant and recessive somatic defects in rats and mice, respectively.

Pan3$^{gt/gt}$: Like in *Pan3*$^{gt/gt}$ rats, yeast harboring *Pan3* deletion mutations are viable; however, effects on yeast gametogenesis were not reported. Here, spermatogenic cells in *Pan3*$^{gt/gt}$ mutant rats arrested during spermatid elongation (Fig. 2a, b and Supplementary Fig. 2a). One hypothesis is that the *Pan3* mutation in rats disrupts control over mRNA stability and/or translation during steps critical for spermatid elongation. This is because PAN3 functions as a co-activator of the PAN2 polyA specific ribonuclease subunit homolog[19].

Pclo$^{gt/gt}$: Unlike effects of *Pclo*$^{gt/gt}$ in rats, current strains of mutant *Pclo* mice reproduce normally and do not display neurological and/or behavioral phenotypes (*also see* Discussion section in manuscript)[20].

Slc1a3$^{gt/gt}$: Unlike embryonic lethality in *Slc1a3*$^{gt/gt}$ rats, adult homozygous mutant *Slc1a3* mice are viable and reproduce normally, but display measurable behavioral phenotypes[21-23].

Spaca6$^{gt/gt}$: Here, based on normal testis histology, epididymal sperm counts and mating behavior, the rat *Spaca6*$^{gt/gt}$ mutation is predicted to disrupt spermatozoan function at some point within the female reproductive tract. In mutant mice lacking a 11kb region of chromosome 17, *Spaca6* was initially implicated in gamete membrane fusion[24].

Ube2k$^{gt/gt}$: Unlike in rats, male and female mice harboring a very similar *Ube2k*$^{gt/gt}$ mutation reproduced normally, had normal body weights and did not show age related myogenic/motor neuron-like phenotypes[25]. *Ube2k* encodes the ubiquitin conjugating enzyme E2-25k[26].

Zmynd8$^{gt/gt}$: Distinct from embryonic lethal effects of *Zmynd8*$^{gt/gt}$ rats, *Zmynd8*-deficient mice are reported as postnatal lethal, surviving to postnatal day 14 (IMPC database. http://mousephenotype.org/)[27].

Supplementary Figure Legends

Supplementary Fig. 1. Rat gene-trap mutations screened for reproduction effects.

Schematic of mutant proteins predicted to be produced in rat strains harboring intronic insertions of *Sleeping Beauty β-geo* genetrap transposons[28]. Exon sequences predicted to be excluded (Δ) from mRNAs encoding truncated polypeptides (aa) generated by imposed splicing to the genetrap transposon are shown below respective wildtype proteins for 17 of the 18 mutant rat strains screened for effects on reproduction. A transposon insertion within intron 2 of *Rgs22* is not shown, and is not predicted to truncate the RGS22 open reading frame due to its genetrap cassette inserting in the 3' to 5' orientation to *Rgs22*. *See* Supplementary Table S1 for full amino acid sequences of the predicted mutant proteins, which contain additional epitopes of either 3, 24 or 1319 (β-GEO) amino acids derived from the genetrap construct.

TM, Transmembrane domain; AAA, ATPase Associated with a variety of cellular activities; GS, GS Motif; L27, domain in receptor targeting proteins Lin-2 and Lin-7; MG-PEST, Polyubiquitination (PEST) N-terminal domain of MAGUK; PDZ, Domain present in PSD-95; β-TrCP, D domain of beta-TrCP; FBOX, A Receptor for Ubiquitination Targets; Dlg, and ZO-1/2; SH3, Src homology 3 domain; GuKc, Guanylate kinase homologue; CC, coil coil region; KAZAL, Kazal type serine protease inhibitors; IGc2, Immunoglobulin C-2 Type; Lg-Ch-Bd, Ligated ion channel L-glutamate- and glycine-binding site; ZnF_C3H1, Zinc Finger Domain; STYKc, Protein kinase; unclassified specificity; C2, Protein kinase C conserved region 2 (CalB); UBCc, Ubiquitin-conjugating enzyme E2, catalytic domain homologue; UBA, Ubiquitin associated domain; RWD, domain in RING finger and WD repeat containing proteins and DEXDc-like helicases subfamily related to the UBCc domain; PHD, PHD zinc finger; BROMO, bromo domain; PWWP, domain with conserved PWWP motif.

Supplementary Fig. 2. Gametogenesis defects in mutant rats

a) H&E stained testis sections from wildtype and respective homozygous mutant rats. Scale bar, 100 μm.

b) Immunofluorescence labeling of cells in wildtype and mutant *Btrc*$^{gt/gt}$ rat testis sections using an antibody to γH2AX and Hoechst 43332 dye. Scale bar, 100 μm.

c) H&E stained ovarian sections from wildtype and respective homozygous mutant rats. Scale bar, 100 μm.

Supplementary Fig. 3. Sperm counts correlate with infertility in mutant rats

Mean testis weight in Grams (tan bars; left y-axis) and epididymal sperm counts plotted as Millions/Rat (blue bars; right y-axis) from respective homozygous mutant rat strains (±SEM, n=4-6 rats/strain). Measurements taken between postnatal days 120-180. Caudal epididymal spermatozoa from *Spaca6*$^{gt/gt}$ (n=6) and *Pclo*$^{gt/gt}$ (n=4) rats displayed similar basal activity compared to wildtype. *Atg13*$^{gt/gt}$ (n=3) and Btrc$^{gt/gt}$ (n=2) rat caudal epididymal sperm were immotile and morphologically abnormal compared to wildtype.

Supplementary Fig. 4. Pathology in *Atg13* mutant rats

a) Hematoxylin and Eosin stained sections illustrating fatty liver (Left) and pyelonephritis (Right) in *Atg13*$^{gt/gt}$ rats. Note: Renal tubules (Right) are dilated and filled with protein-rich filtrate (asterisks) and numerous neutrophils (arrows). Scale bar, 50 μm.

b) Western blot analysis of autophagy marker proteins (ATG13, p62) and TUBA1a in wildtype and *Atg13*$^{gt/gt}$ primary rat embryonic fibroblast cultures shown in Fig. 3f. Cultures were pretreatment with rapamycin (100 nM), ammonium chloride (3 mM) and/or Bafilomycin A1 (3 nM). The loading control blot shown for TUBA1a is the same blot shown in Fig. 3f.

Supplementary Fig 5. Synaptic transmission is downregulated in the brain of mutant *Piccolo* rats.

Scatter plots of Log2-fold change in transcript abundance versus mean relative expression levels (Log2 FPKM values) in *Pclo*$^{wt/gt}$ and *Pclo*$^{gt/gt}$ brain (left panels) and testis (right panel) compared to wildtype. Differentially expressed genes (DEGS) are shown in red (increased abundance) and blue (decreased abundance), respectively (log2-fold change >1 or <-1; FDR < 0.05). Note that the *Pclo* mutation caused more DEGS in the brain vs testis transcriptome. *Gabra6* and *Gabrg3*, the most significantly affected genes in brain and testis, respectively, have similar expression pattern in the homozygous and heterozygous mutant rats.

Supplementary Fig. 6. Gonadotropin Releasing Hormone (GnRH) pathway genes are downregulated in the brain of mutant *Piccolo* rats.

a) Panther Pathway finder analysis on down regulated Hormonal Secretion GO: gene sets identify GnRH signaling pathway as a prominent cluster of down-regulated genes in *Pclo*$^{gt/gt}$ rat brain versus *Pclo*$^{wt/wt}$ rat brains.

b) Coronal brain sections from Pclo$^{wt/wt}$ or Pclo$^{gt/gt}$ postnatal day 100 rat brains immuno-stained with GnRH antibodies. Lower panel, 5x magnification of the preoptic area (POA) located in the boxed area reveal presence of somata and GnRH positive neuron processes flanking the third ventricle. Scale bars = upper panel 0.3 cm, lower panel 1000 μm.

c) Relative abundance of GnRH signaling pathway GO: gene set components in *Pclo*$^{gt/gt}$ rat brains versus *Pclo*$^{wt/wt}$ rat brains.

d) KEGG pathway analysis predicts downregulation of GnRH signaling pathway in *Pclo*$^{gt/gt}$ rat brains versus *Pclo*$^{wt/wt}$ rat brains.

Supplementary Fig. 7. Misregulated gene networks downstream of GnRH Signaling.

a) Relative abundance of G-Protein Coupled Receptors (GPCRs) involved in conducting GnRH signaling on gonadotropes GO: gene set components in *Pclo*$^{gt/gt}$ (KO) and *Pclo*$^{wt/gt}$ (Het) rat brains versus *Pclo*$^{wt/wt}$ (WT) rat brains.

b) Relative abundance of Calcium Signaling GO: gene set components in *Pclo*$^{gt/gt}$ (KO) rat brains versus *Pclo*$^{wt/wt}$ (WT) rat brains.

c) Relative abundance of Gonadotropin Regulated Genes in *Pclo*$^{gt/gt}$ (KO) rat testes versus *Pclo*$^{wt/wt}$ (WT) rat testes. FSH, Follicle Stimulating Hormone; LH, Luteinizing Hormone, T, Testosterone.

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