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3 # Placental effects on maternal brain revealed by disrupted placental gene

4 # expression in mouse hybrids

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13 Paternal effects, *Mus musculus domesticus*, *Mus spretus*, Genomic imprinting

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15 Running title: Placental effects on maternal brain

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23 **Abstract**

24 The mammalian placenta is both the physical interface between mother and fetus, and the source
25 of endocrine signals that target the maternal hypothalamus, priming females for parturition,
26 lactation and motherhood. Despite the importance of this connection, the effects of altered
27 placental signaling on the maternal brain are unstudied. Here, we show that placental dysfunction
28 alters gene expression in the maternal brain, with the potential to affect maternal behavior. Using
29 a cross between the house mouse and the Algerian mouse in which hybrid placental development
30 is abnormal, we sequenced late gestation placental and maternal medial preoptic area
31 transcriptomes and quantified differential expression and placenta-maternal brain co-expression
32 between normal and hybrid pregnancies. The expression of *Fmn1, Drd3, Caln1* and *Ctsr* was
33 significantly altered in the brains of females exposed to hybrid placentas. Most strikingly,
34 expression patterns of placenta-specific gene families and *Drd3* in the brains of house mouse
35 females carrying hybrid litters matched those of female Algerian mice, the paternal species in the
36 cross. Our results indicate that the paternally-derived placental genome can influence the
37 expression of maternal-fetal communication genes, including placental hormones, revealing a
38 previously unrecognized effect of the offspring's father on the mother's brain.

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39 **Introduction**

40 The placenta is a unique, transient organ shared by two organisms. Placental morphology is
41 surprisingly diverse across vertebrates and is subject to rapid evolutionary change and
42 convergent evolution (Blackburn 1993, Reznick et al. 2002, Roberts et al. 2016, Armstrong et al.
43 2017). In most eutherian mammals, including mice and humans, successful blastocyst
44 implantation relies on endometrial invasion by the embryonic trophoblast cells that give rise to
45 the mature placenta (Cross et al. 1994). As such, the placenta provides the closest physical and
46 molecular link between mother and offspring seen in any animal (Wagner et al. 2014). This
47 intimate connection promotes an array of maternal-fetal interactions, including bidirectional
48 hormonal regulation and even the exchange of entire cells. These interactions are not spatially
49 limited, but extend to both the fetal and the maternal brain (Bridges et al. 1996, Ladyman et al.
50 2010, Boddy et al.2015).

51 Throughout pregnancy the placenta mediates the regulation of resource allocation,
52 immune tolerance, fetal development and, importantly, hormonal priming of the maternal brain.
53 A key subset of placenta-secreted molecules reaches the maternal brain, priming maternal
54 physiology for parturition and lactation, and promoting the onset of maternal behaviors in late
55 gestation. In rodents, these placental molecules mainly target the medial preoptic area (MPoA) in
56 the hypothalamus (Bridges et al. 1996, Mann and Bridges 2001, Larsen and Grattan 2012),
57 which has been characterized as the central hub of parenting behavior (Kohl and Dulac 2018).
58 Receptors for key pregnancy hormones and neurotransmitters, including estrogen, prolactin and
59 dopamine, are highly expressed in this area and interact with ligands of both maternal and
60 placental origin (Numan and Insel 2003).

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61 Two classes of placental genes are of particular importance to the interaction between
62 placenta and maternal brain: imprinted genes (IGs) and placenta-specific gene families (PSFs).
63 IGs and PSF genes have overlapping expression patterns, especially in the placental endocrine
64 compartment (Tunster et al. 2013). IGs are exclusively or predominantly expressed from one
65 allele, and are highly enriched in placenta and brain. The silencing or repression of the second
66 allele is determined by opposing, heritable epigenetic marks ("imprints") in maternal and
67 paternal germ cells, such that some IGs are maternally imprinted and paternally expressed,
68 whereas others are paternally imprinted and maternally expressed (Reik and Walter 2001,
69 Ferguson-Smith 2011). During pregnancy, IGs are critical to placental development and function,
70 maintaining the balance between maternal supply and embryonic demand, and regulating
71 maternal-fetal exchange (Constancia et al. 2005, Lefebvre 2012, Tunster et al. 2013).
72 PSFs arose through lineage-specific gene duplication events during placental evolution
73 (Rawn and Cross 2008). In rodents, these are the prolactin gene family (placental lactogens
74 (Prls)), placental cathepsin proteases and their inhibitors (PECs) and pregnancy specific
75 glycoproteins (PSGs) (Zebhauser et al. 2005, Soares et al. 2007, Mason 2008). PSF gene
76 products are mainly expressed from the placental endocrine compartment and many are secreted
77 into the maternal bloodstream; key functions include placental development, immunoregulation,
78 and physiological and neurological priming of the maternal organism (Rawn and Cross 2008).
79 Most notably, a subset of PRLs binds prolactin receptors in the maternal MPoA and affects
80 maternal endocrine state and behaviour (Larsen and Grattan 2012). IGs are implicated in
81 regulating PSF secretion via their effects on the structure and function of the placental endocrine
82 compartment (John 2017). However, our current understanding of the role of IGs in PSF

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83 signaling is rudimentary, and the relationship between gene expression in placenta and the
84 maternal MPoA is uncharted.

85 The majority of the placenta, including the endocrine compartment, is derived from
86 embryonic tissue. Placental representation of both parental genomes sets the stage for conflict
87 (maternal-paternal and parent-offspring), and for coadaptation (mother-offspring), with
88 imprinted genes uniquely positioned to mediate both types of interactions (Moore and Haig 1991,
89 Wolf and Hager 2006, Keverne and Curly 2008, Haig 2014). However, while evolutionary
90 models for imprinted gene expression abound (reviewed in Patten et al. 2014), few have
91 considered the interaction between paternally-derived placental signals and signal reception in
92 the maternal brain (Haig 1996, Creeth et al. 2018).

93 Here, we use a natural hybrid system to explore the effects of placental dysregulation on
94 gene expression in the maternal brain. Over- or under-growth that depends on the direction of the
95 cross is a signature of disrupted imprinting in mammalian hybrids (Vrana 2007). This pattern is
96 documented in several orders (Gray 1972), with the best-studied examples in rodents (multiple
97 species in the genera *Peromyscus, Mus* and *Phodopus* (Zechner et al. 1996, Vrana et al. 1998,
98 Brekke and Good 2014)). Parent-of-origin growth effects in the cross between the house mouse,
99 *Mus m. domesticus* (*Dom*) and the Algerian mouse, *M. spretus* (*Spret*), were first described over
100 20 years ago: placentas are undersized when the mother is *Dom* and the father is *Spret*, and
101 severely oversized in the reciprocal cross (Zechner et al. 1996). Subsequent studies confirmed
102 altered expression and methylation of candidate IGs, and disrupted placental organization
103 (Hemberger et al. 1999, Zechner et al. 2002, 2004, Shi et al. 2005). Specifically, the placental
104 endocrine compartment (or junctional zone) was shown to be reduced and disorganized (Kurz et
105 al. 1999). However, the extent of placental misexpression has not been measured on a genome

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106 scale, and this system's potential to uncover the maternal consequences of altered placental
107 signaling has not been considered.
108 The characteristics of this classic system, together with the availability of high quality
109 genomes for both parental species (Keane et al. 2011), make the cross between *Dom* and *Spret* an
110 excellent model in which to explore the effects of placental disruptions on the maternal brain. By
111 comparing MPoA expression between females of the same species that differ only in the type of
112 pregnancy/placenta they experience (hybrid vs. conspecific), we specifically isolate the effect of
113 placental gene expression differences on the maternal brain (Fig. 1). Characterization of altered
114 gene expression at the maternal-fetal interface provides insight into the mechanisms of maternal-
115 fetal communication, the contribution of the paternal genome to this interaction, and identifies
116 promising candidate genes for future evolutionary and biomedical work.
117

118 **Results**

119 To study the relationship between placenta and maternal MPoA on a molecular level we
120 produced three crosses resulting in three types of pregnancy: *Dom* x *Dom* (*Dom* pregnancy, n=5),
121 *Dom* x *Spret* (hybrid pregnancy, n=5) and *Spret* x *Spret* (*Spret* pregnancy, n=5) (in all crosses,
122 female is first). For each type of cross we produced 5 biological replicates and extracted the
123 maternal MPoA and placentas from each pregnant female in late gestation at embryonic day (e)
124 17.5. During late gestation the effect of placental signaling on the maternal MPoA is specifically
125 important for the onset of maternal care at parturition (Bridges et al. 1996, Mann and Bridges
126 2001, Larsen and Grattan 2012). We sequenced the maternal MPoA transcriptome and the
127 placental transcriptomes of 1 male and 1 female per mother (n=9-10/type of pregnancy, hybrid
128 pregnancy female placentas n=4), and evaluated differential expression between all pregnancy

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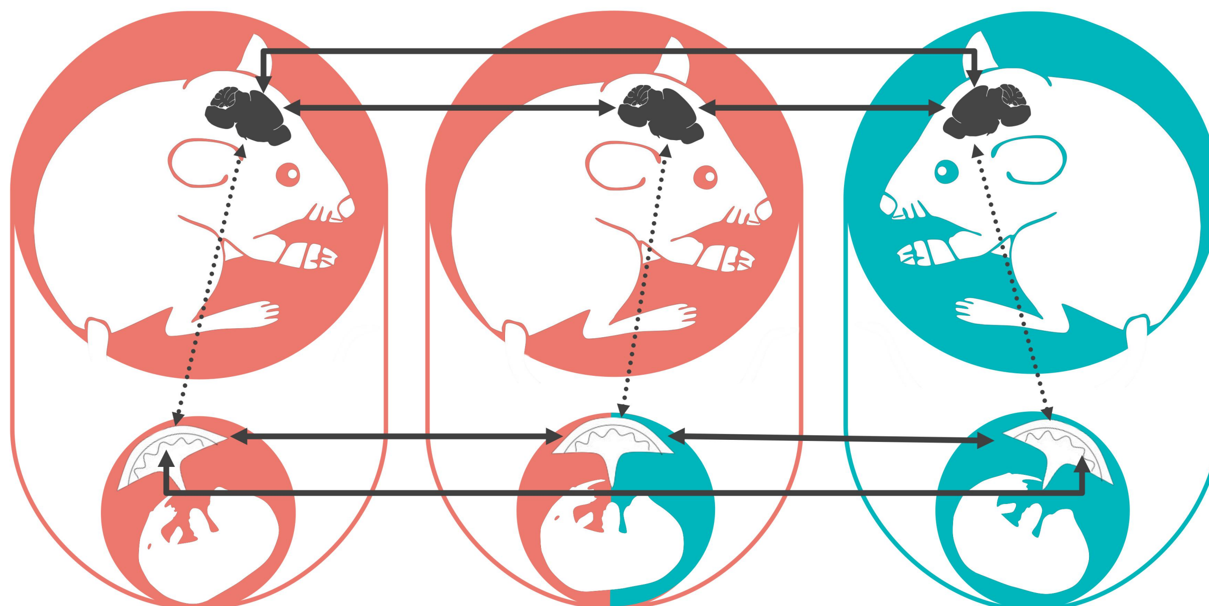
129 types (Fig. 1). Because the maternal brain is exposed to the placental signals of both sexes
130 simultaneously, male and female placental expression was analyzed jointly. Additionally, we
131 assessed co-expression between the two tissues for each type of pregnancy and determined the
132 differences in co-expression between pregnancy types (Fig. 1).




133

134 **Figure 1**. Experimental design. Schematic representation of the comparisons performed to test for differential gene
135 expression in the medial preoptic area of the maternal brain and the placenta during late gestation. *Mus m.*
136 *domesticus* is depicted in red and *Mus spretus* in blue. Hybrid tissue is indicated by a combination of red and blue.
137 Solid arrows indicate differential gene expression analysis. Dashed arrows indicate co-expression analysis.
138

139 **Differential expression in the placenta**

140 We tested for differential expression in three pairwise comparisons: hybrid vs. *Dom*, hybrid vs.
141 *Spret,* and *Dom* vs. *Spret* placentas. For placental comparisons, only genes with log2 fold change
142 (LFC) in expression $\geq 0.5$ (1.5 times higher or lower expression), and Benjamini-Hochberg-
143 corrected $p \leq 0.05$, were considered significantly differentially expressed (DE). We define
144 transgressive expression in hybrids as expression that is significantly higher or lower compared

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145 to both parental species. Hybrid genes that were DE compared to *Dom* but not to *Spret* are
146 defined as having *Spret*-like expression patterns, and vice versa.
147 In hybrid placentas 9.73% of all tested genes were expressed higher and 7.79% lower
148 compared to *Dom* placentas (up: 1,781/18,298, including 11 IGs; down: 1,426/18,298, including
149 3 IGs) (Fig. 2*,* Supplemental Fig. S1 and Supplemental Dataset S1). Compared to *Spret* placentas,
150 16.32% of genes were expressed higher and 9.6% lower in hybrids (up: 3,036/18,529, including
151 16 IGs; down: 1,801/18,529, including 7 IGs) (Fig. 2*,* Supplemental Fig. S2, Supplemental
152 Dataset S1). Thirty-two percent of all tested genes were DE between *Dom* and *Spret* (up:
153 4,014/19,079, including 19 IGs; down: 3,278/19,079, including 13 IGs) (Supplemental Fig. S3,
154 Supplemental Dataset S1).
155 To explain hybrid placental phenotypes that are not intermediate to both parents*,* genes
156 with transgressive expression are of specific interest. We found 275 genes that were expressed at
157 higher, and 167 at lower, levels in hybrids compared to both parental species (Fig. 2*A*).
158 Transgressively upregulated genes were significantly enriched for B-cell receptor activation and
159 integrin cell surface interaction pathways (Fig. 2*S,* Supplemental Dataset S1). Interestingly,
160 transgressively down-regulated genes were enriched for prolactin and growth hormone receptor
161 signaling, ERBB signaling, and cytokine signaling in immune system, among others (Fig. 2*A,*
162 Supplemental Dataset S1). Many Prls are involved in these pathways, along with other genes.
163 PSF genes were highly overrepresented among DE genes in hybrids compared to both *Dom*
164 (Fisher's exact test: $p<0.001$, odds ratio = 5.65) and *Spret* ($p<0.001$, odds ratio = 3.41). Multiple
165 members of these gene families were misexpressed in the hybrid placenta, with the majority
166 being expressed lower compared to both parental species (14 transgressively lower, 7 DE
167 intermediate) (Table 1).

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168 Notably, approximately twice as many genes in hybrid placentas were uniquely DE
169 relative to *Spret* (3,271) as opposed to *Dom* (1,659) (Fig. 2*A*). Thus, the general expression
170 pattern in hybrid placentas was more similar to the maternal species. *Dom*-like expressed genes
171 were enriched for multiple immune related pathways, together with angiogenesis, vascular
172 development and hemostasis related terms, among others (Supplemental Dataset S1).

173 Genes with a *Spret*-like expression pattern in hybrid placentas are of particular interest,
174 since these have the potential to alter communication with the *Dom* maternal brain. Of the 1,659
175 genes with *Spret*-like expression (Fig. 2*A*), 12 were PSF genes and 6 were IGs (Tables 1 and 2).
176 *Spret*-like expressed genes in the hybrid were enriched for WNT signaling and extracellular
177 matrix organization pathways, among others (Fig. 2*A,* Supplemental Dataset S1).

178 IGs were significantly overrepresented among hybrid DE genes compared to both *Spret*
179 (Fisher's exact test: p=0.02, odds ratio=0.59) and *Dom* (p=0.03, odds ratio=0.55). Three IGs
180 (*Tnfrsf23, Phlda2* and *Klf14*) were transgressively upregulated and two, (*Ascl2* and *Sfmbt2*) were
181 transgressively down-regulated. Two additional IGs, *Tspan32* and *Th*, were significantly DE
182 compared to both parental species but intermediate between the two. Five of these misexpressed
183 IGs belong to the same imprinting cluster (IC2) on the distal part of mouse chromosome 7 (dist7),
184 and are normally maternally expressed (Table 2*,* Supplemental Dataset S1).

185

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Table 1. Differential expression of PSF genes in hybrid placenta compared to both parental species

| | Chr. | Hybrid vs. *Dom* placenta | | | Hybrid vs. *Spret* placenta | | | Hybrid vs. both parental species |
|---|---|---|---|---|---|---|---|---|
| | | LFC | Stat | padj | LFC | Stat | padj | |
| *Prl3c1* | 13 | -1.60 | -7.81 | <0.001 | -2.31 | -8.78 | <0.001 | Transgressive lower |
| *Prl7d1* | 13 | -0.95 | -7.80 | <0.001 | -0.93 | -4.36 | <0.001 | Transgressive lower |
| *Prl2c5* | 13 | -0.89 | -7.04 | <0.001 | -1.26 | -5.75 | <0.001 | Transgressive lower |
| *Ceacam12* | 7 | -0.87 | -4.72 | <0.001 | -0.88 | -3.73 | <0.001 | Transgressive lower |
| *Ctsm* | 13 | -0.82 | -3.19 | 0.01 | -1.22 | -4.24 | <0.001 | Transgressive lower |
| *Prl7a1* | 13 | -0.82 | -3.21 | 0.01 | -1.72 | -5.57 | <0.001 | Transgressive lower |
| *Psg25* | 7 | -0.76 | -3.45 | <0.001 | -1.44 | -5.54 | <0.001 | Transgressive lower |
| *Cts3* | 13 | -0.76 | -3.01 | 0.01 | -1.55 | -5.94 | <0.001 | Transgressive lower |
| *Prl2c3* | 13 | -0.75 | -4.64 | <0.001 | -1.36 | -3.58 | <0.001 | Transgressive lower |
| *Psg28* | 7 | -0.73 | -3.26 | 0.01 | -0.62 | -2.38 | 0.04 | Transgressive lower |
| *Prl4a1* | 13 | -0.72 | -2.80 | 0.02 | -1.46 | -5.20 | <0.001 | Transgressive lower |
| *Prl7a2* | 13 | -0.71 | -2.91 | 0.01 | -1.69 | -6.09 | <0.001 | Transgressive lower |
| *Prl2b1* | 13 | -0.64 | -3.64 | <0.001 | -1.26 | -7.05 | <0.001 | Transgressive lower |
| *Prl2a1* | 13 | -0.59 | -3.45 | <0.001 | -1.41 | -5.30 | <0.001 | Transgressive lower |
| *Cts7* | 13 | -3.68 | -13.40 | <0.001 | 3.34 | 7.92 | <0.001 | Intermediate |
| *Prl3d1* | 13 | -3.39 | -21.70 | <0.001 | 2.41 | 5.89 | <0.001 | Intermediate |
| *Ceacam5* | 7 | -1.47 | -8.01 | <0.001 | 2.25 | 10.25 | <0.001 | Intermediate |
| *Ctsr* | 13 | -0.68 | -6.29 | <0.001 | 0.83 | 6.43 | <0.001 | Intermediate |
| *Psg20* | 7 | 1.43 | 6.58 | <0.001 | -2.13 | -7.59 | <0.001 | Intermediate |
| *Psg22* | 7 | 2.26 | 11.63 | <0.001 | -2.17 | -9.72 | <0.001 | Intermediate |
| *Ceacam3* | 7 | 2.73 | 11.03 | <0.001 | -1.67 | -6.30 | <0.001 | Intermediate |
| *Cts6* | 13 | -0.97 | -7.65 | <0.001 | -0.12 | -0.97 | 0.46 | *Spret-like* expression |
| *Prl2c1* | 13 | -1.49 | -7.78 | <0.001 | 0.43 | 1.39 | 0.26 | *Spret-like* expression |
| *Psg26* | 7 | -0.92 | -3.80 | <0.001 | 0.46 | 1.97 | 0.10 | *Spret-like* expression |
| *Psg27* | 7 | -0.90 | -4.08 | <0.001 | -0.36 | -1.50 | 0.22 | *Spret-like* expression |
| *Prl7c1* | 13 | -0.84 | -2.54 | 0.03 | 0.05 | 0.11 | 0.95 | *Spret-like* expression |
| *Psg19* | 7 | -0.79 | -3.39 | <0.001 | -0.08 | -0.34 | 0.82 | *Spret-like* expression |
| *Prl3d2* | 13 | -0.74 | -2.54 | 0.03 | -0.48 | -1.11 | 0.39 | *Spret-like* expression |
| *Ceacam11* | 7 | -0.73 | -3.91 | <0.001 | -0.43 | -1.88 | 0.12 | *Spret-like* expression |
| *Psg29* | 7 | -0.67 | -2.85 | 0.02 | -0.30 | -0.96 | 0.46 | *Spret-like* expression |
| *Ceacam15* | 7 | 1.24 | 4.17 | <0.001 | -0.50 | -1.79 | 0.14 | *Spret-like* expression |
| *Prl3b1* | 13 | -1.10 | -10.71 | <0.001 | 0.41 | 3.05 | 0.01 | *Spret-like* expression |
| *Tpbpa* | 13 | -0.58 | -3.64 | <0.001 | -0.49 | -2.38 | 0.04 | *Spret-like* expression |

Chr.=Chromosome, DE=differential expression, LFC=log2 Fold Change of expression, Padj=adjsted p-value according to Benjamini-Hochberg method, Stat=Wald test (DESeq2).

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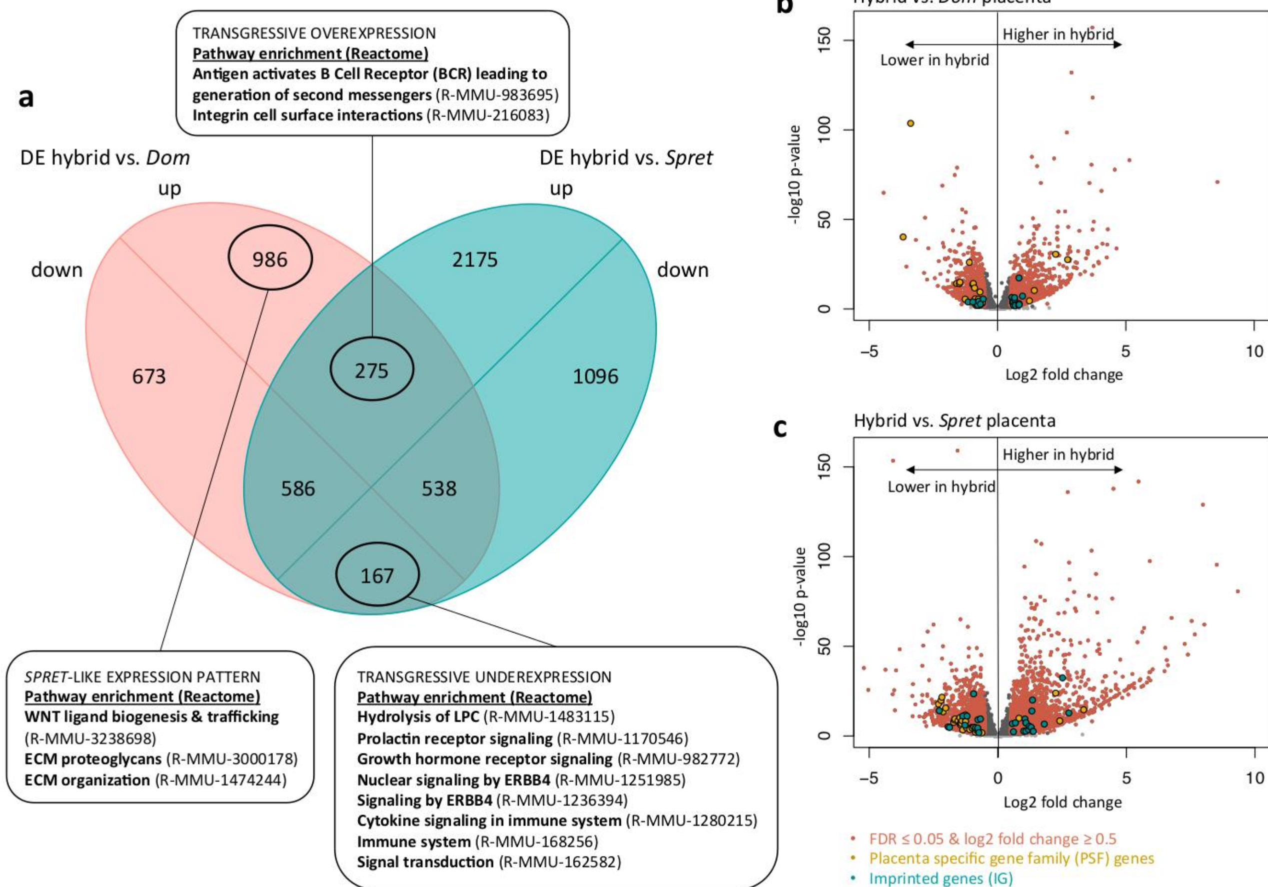


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188 **Figure 2**. Placental gene expression. Summary of results of differential gene expression (DE) analysis between *Mus*
189 *m. domesticus* (*Dom*), *Mus spretus* (*Spret*) and hybrid placentas. (A) Venn diagram indicating the overlap of
190 differentially expressed genes between the comparisons hybrid vs. *Dom* and hybrid vs. *Spret*; up = genes expressed
191 higher in hybrids compared to parental species, down = genes expressed lower in hybrids compared to parental
192 species. Genes expressed higher or lower compared to both parental species (transgressive expression) and genes
193 with *Spret*-like expression in the hybrid are marked in the diagram. Results of pathway overrepresentation
194 (Reactome, version 58,Mi et al. 2017) are provided in connected text boxes. Volcano plot of DE analysis results of
195 (B) hybrid vs. *Dom* and (C) hybrid vs. *Spret* placentas. Significantly differentially expressed genes with FDR $\leq$ 0.05
196 and log2 fold change $\geq$ 0.5 are depicted in red. Imprinted genes are indicated in blue and placenta-specific gene
197 family genes in yellow.

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Table 2. Differential expression of imprinted genes in hybrid placenta compared to both parental species

| | IC/Chr. | Hybrid vs. *Dom* placenta | | | Hybrid vs. *Spret* placenta | | | Hybrid vs. both parental species | expressed allele |
|---|---|---|---|---|---|---|---|---|---|
| | | LFC | Stat | padj | LFC | Stat | padj | | |
| *Phlda2* | dist7-IC2 | 0.97 | 5.39 | <0.001 | 1.09 | 5.44 | <0.001 | Transgressive higher | Maternal |
| *Klf14* | prox6 | 0.67 | 5.01 | <0.001 | 1.06 | 6.29 | <0.001 | Transgressive higher | Maternal |
| *Tnfrsf23* | dist7-IC2 | 0.59 | 3.57 | <0.001 | 1.97 | 9.02 | <0.001 | Transgressive higher | Maternal |
| *Tspan32* | dist7-IC2 | -0.66 | -3.11 | 0.01 | 2.01 | 6.43 | <0.001 | Intermediate | Maternal |
| *Th* | dist7-IC2 | 0.69 | 2.48 | 0.04 | -2.28 | -7.76 | <0.001 | Intermediate | Maternal |
| *Ascl2* | dist7-IC2 | -0.96 | -3.85 | <0.001 | -1.28 | -4.90 | <0.001 | Transgressive lower | Maternal |
| *Sfmbt2* | 2 | -0.56 | -4.69 | <0.001 | -0.67 | -6.30 | <0.001 | Transgressive lower | Paternal |
| *Magel2* | cent7 | 0.61 | 3.81 | <0.001 | 0.48 | 2.25 | 0.06 | *Spret-like* expression | Paternal |
| *Dcn* | 10 | 0.63 | 2.45 | 0.04 | -0.06 | -0.16 | 0.92 | *Spret-like* expression | Maternal |
| *Nap1l5* | prox6 | 0.82 | 2.63 | 0.03 | 0.31 | 0.91 | 0.49 | *Spret-like* expression | Paternal |
| *Grb10* | prox11 | 0.83 | 8.65 | <0.001 | -0.06 | -0.47 | 0.74 | *Spret-like* expression | Maternal |
| *Nnat* | dist2 | 0.84 | 3.29 | <0.001 | -0.07 | -0.27 | 0.86 | *Spret-like* expression | Paternal |
| *Igf2* | dist7-IC1 | 0.84 | 2.89 | 0.01 | 0.02 | 0.08 | 0.96 | *Spret-like* expression | Paternal |

IC=imprinting cluster (https://www.mousebook.org (03.22.18)), Chr.=Chromosome, DE=differential expression, LFC=log2 Fold Change of expression, Padj=adjsted p-value according to Benjamini-Hochberg method, Stat=Wald test (DESeq2), expressed allele according to https://www.mousebook.org (03.22.18).

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204 **Overlap of placental DE genes with genes involved in preeclampsia**

205 Among the misexpressed genes in the hybrid placenta we noticed several that are also
206 misexpressed in the human pregnancy pathology preeclampsia. Although mice do not develop
207 preeclampsia, preeclampsia-like phenotypes can occur and several rodent models have been
208 developed to study key symptoms such as hypertension, proteinura, and altered inflammatory
209 response (Podjarny et al. 2004, Dokras et al. 2006). To test if DE genes in the hybrid overlap
210 with genes related to preeclampsia we extracted genes associated with preeclampsia from the
211 database for preeclampsia (http://ptbdb.cs.brown.edu/dbpec/, (Uzun et al. 2016)) and obtained
212 the mouse orthologs for these from biomart (R-package biomaRt, (Durinck et al. 2005)). Of the
213 490 mouse orthologs we obtained, 26 were transgressively misexpressed in the hybrid placenta.
214 Preeclampsia related genes were significantly overrepresented among transgressively
215 misexpressed genes (Fisher's exact test: p=0.003, odds ratio=1.91). An altered inflammatory
216 response at the fetal-maternal interface is involved in preeclampsia in humans (Harmon et al.
217 2016); in the hybrid placenta, both up- and down-regulated transgressively expressed genes were
218 enriched for immune-related and cytokine signaling pathways (Supplemental Dataset S1).

219

220 **Differential expression in the MPoA**

221 To explore maternal gene expression in response to placental genotype we compared late
222 gestation MPoA of *Dom* females during *Dom* pregnancies (MPoA-*dom*), *Dom* females during
223 hybrid pregnancies (MPoA-*hy*) and *Spret* females during *Spret* pregnancies (MPoA-*spret*).
224 Neural and placental tissues were collected from the same females. Gene expression in MPoA-*hy*
225 vs. MPoA-*dom* is expected to be highly similar, with any DE attributable to carrying a hybrid
226 litter, while the other comparisons should result in a large number of DE genes attributable to

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227 interspecific differences. For the intraspecific comparison we report significantly DE genes with
228 log2 fold change (LFC) ≥ 0.2, since differences are expected to be subtle. For all other
229 comparisons we report significantly DE genes with LFC ≥ 0.5.
230 Four genes were DE between MPoA-*hy* and MPoA-*dom*: Cathepsin-R (*Ctsr*) was
231 expressed higher in MPoA-*hy* compared to MPoA-*dom*, and Dopamine receptor 3 (*Drd3*),
232 Calneuron 1 (*Caln1*) and Formin 1 (*Fmn1*) were expressed lower (up: 1/18,779; down: 3/18,779)
233 (Fig. 3, Table 3, Supplemental Fig. S4 and Supplemental Dataset S2). In MPoA-*hy* compared to
234 MPoA-*spret* 10.61% of all tested genes were expressed higher and 10.32% lower (up:
235 1,608/15,154; down: 1,565/15,154) (Fig. 3, Supplemental Fig. S5, Supplemental Dataset S2). In
236 the interspecific comparison of conspecific pregnancies, 11.32% of all tested gene were
237 expressed higher and 10.76% lower in MPoA-*dom* compared to MPoA-*spret* (up: 1,757/15,515;
238 down: 1,670/15,515) (Fig. 3, Supplemental Fig. S6, Supplemental Dataset S2).
239 Hybrid placentas express both *Spret* and *Dom* alleles and 1,659 genes had *Spret*-like
240 expression. Therefore, the MPoA in *Dom* females carrying hybrid litters might exhibit
241 expression patterns more similar to *Spret* female MPoA for some maternal-fetal communication
242 genes. We extracted a list of 959 genes that were DE in MPoA-*dom* vs. MPoA-*spret* but not in
243 MPoA-*hy* vs. MPoA-*spret.* This list includes *Drd3,* which was expressed lower in MPoA-*hy*
244 compared to MPoA-*dom* (Fig. 3, Supplemental Dataset S2). Thus, *Drd3* could be defined as
245 having *Spret*-like expression in MPoA-*hy*. *Fmn1* and *Ctsr* were expressed lower and higher,
246 respectively, in MPoA-*hy* compared to both MPoA-*dom* and MPoA-*spret*, and were not DE
247 between *Dom* and *Spret* MPoA during regular pregnancies (Fig. 3, Supplemental Dataset S2).
248

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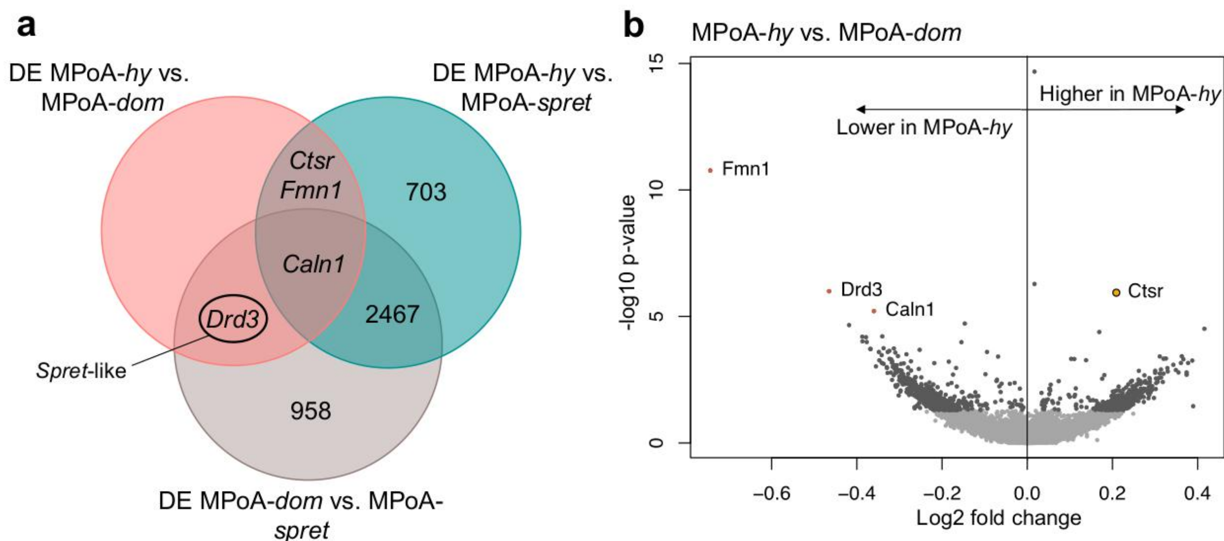


249

250 **Figure 3**. Maternal medial preoptic area (MPoA) gene expression. Summary of results of differential gene
251 expression (DE) analysis between *Mus m. domesticus* MPoA during normal pregnancy (MPoA-*dom*), *Mus m.*
252 *domesticus* MPoA during hybrid pregnancy (MPoA-*hy*) and *Mus spretus* MPoA during normal pregnacy (MPoA-
253 *spret*). (A) Venn diagram indicating the overlap of differentially expressed genes between the comparisons MPoA-
254 *hy* vs. MPoA-*dom*, MPoA-*hy* vs. MPoA-*spret* and MPoA-*dom* vs. MPoA-*spret*. Genes with *Spret*-like expression in
255 MPoA-*hy* are marked in the diagram. (B) Volcano plot of DE analysis results of MPoA-*hy* vs. MPoA-*dom*.
256 Significantly differentially expressed genes with FDR ≤ 0.05 and log2 fold change ≥ 0.2 are depicted in red,
257 placenta-specific gene family genes in yellow.

258

Table 3. Differential expression in MpoA-*hy* compared to MPoA-*dom*.

| | Chr. | LFC | Stat | padj |
|---|---|---|---|---|
| *Fmn1* | 2 | -0.74 | -6.73 | <0.001 |
| *Drd3* | 16 | -0.47 | -4.89 | <0.001 |
| *Caln1* | 5 | -0.36 | -4.52 | 0.02 |
| *Ctsr* | 13 | 0.21 | 4.86 | <0.001 |

Chr.=Chromosome, LFC=log2 Fold Change of expression, Padj=adjsted p-value according to Benjamini-Hochberg method, Stat=Wald test (DESeq2).

259

## 260 Co-expression between the placenta and MPoA

261 The MPoA is an important target of placenta-secreted molecules and we found the placenta-
262 specific gene, *Ctsr*, to be expressed in the maternal MPoA during hybrid pregnancies. Therefore,
263 co-expression between placenta and MPoA is of particular interest. We determined the level of

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264 placenta-MPoA co-expression for the three pregnancy types (hybrid, *Dom*, *Spret*), and assessed
265 differences between them.
266 10,839 genes were co-expressed between placenta and MPoA in all 3 comparisons. 172
267 genes were only co-expressed in *Dom* and 494 only in *Spret* pregnancies (Fig. 4, Supplemental
268 Dataset S3). 176 genes were uniquely co-expressed in hybrid pregnancies. This gene set is of
269 specific interest since these genes are indicators of MPoA response to abnormal placental
270 expression. Uniquely co-expressed genes in hybrid pregnancies included 45 genes that were DE
271 between hybrid and *Dom* placentas and 16 that were DE compared to both parental species'
272 placentas. *Ctsr*, which was significantly upregulated in MPoA-*hy* compared to MPoA-*dom*, was
273 uniquely co-expressed in hybrid pregnancies (Fig. 4, Supplemental Dataset S3).
274 228 genes were co-expressed in *Spret* and hybrid pregnancies but not in *Dom* pregnancies.
275 Thus, these genes exhibit *Spret*-like co-expression in the MPoA of *Dom* females carrying a
276 hybrid litter. There was pathway overrepresentation overlap between this gene set and genes with
277 transgressive misexpression in hybrid placenta (Fig. 2*A*), including prolactin and growth
278 hormone receptor signaling, and ERBB signaling (Fig. 4, Supplemental Dataset S3). Moreover,
279 PSFs were significantly overrepresented among these co-expressed genes (Fisher's exact test:
280 $p<0.001$, odds ratio=12.57). Although expression levels were far lower in the MPoA (range=10-
281 589, mean=100 normalized counts) than in the placenta (range=20-1,127,603, mean=97,988
282 normalized counts), these results are striking. To explore this relationship further, we tested for
283 correlated expression of PSF genes between placenta and MPoA. We found very strong, positive
284 correlations for hybrid ($R^2$adj=0.95, $p<0.001$) and *Spret* ($R^2$adj=0.9, $p<0.001$) and a significant
285 but, surprisingly, weaker positive correlation for *Dom* ($R^2$adj=0.38, $p=0.01$) (Fig. 4*B*, Table 3).
286 Additionally, we found that MPoA-*hy* express significantly more PSF genes (sum of PSF read

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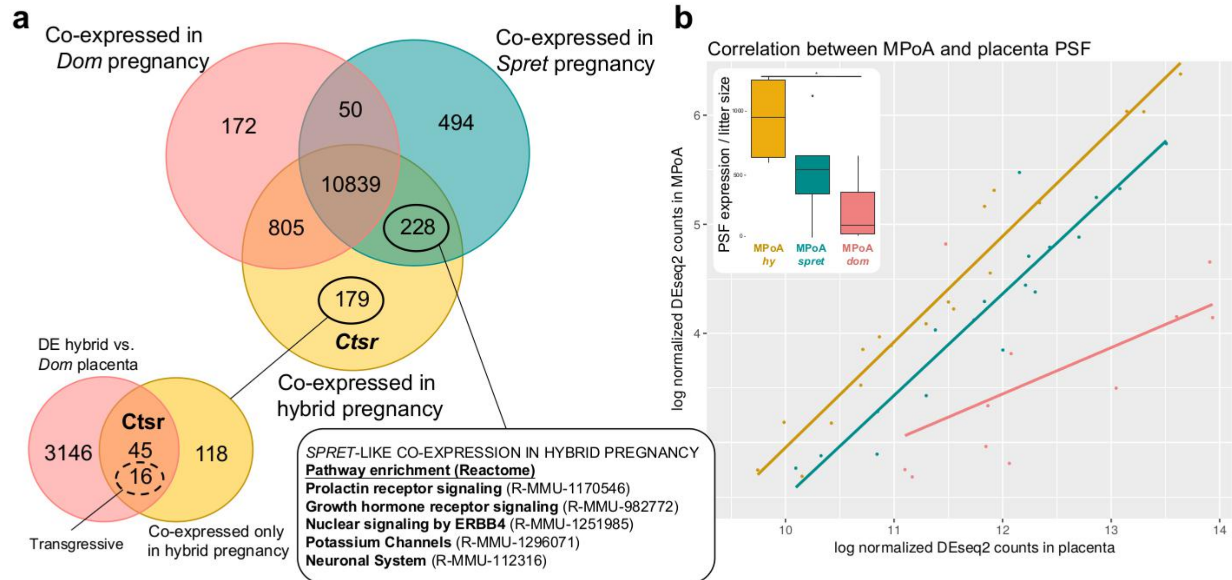
287 counts divided by litter size) than MPoA-*dom* but not than MPoA-*spret* (One-way ANOVA:

288 $F_{2,12}$=5.44, p=0.02, Tukey HSD: MPoA-*hy* vs. *MPoA-dom*: p=0.016, MPoA-*hy* vs. MPoA-*spret*:

289 p=0.18, MPoA-*dom* vs. MPoA-*spret*: p=0.36) (Fig. 4*B*).

290




291

292 **Figure 4**. Co-expression between maternal medial preoptic area (MPoA) and placenta during late gestation.
293 Summary of results of co-expression analysis for *Mus m. domesticus* during normal pregnancy (*Dom*-pregnancy),
294 *Mus m. domesticus* during hybrid pregnancy (hybrid pregnancy) and *Mus spretus* during normal pregnancy (*Spret*
295 pregnancy). (A) Venn diagram indicating the overlap of co-expressed genes between the three pregnancy types.
296 Genes that are only co-expressed in hybrid pregnancy are marked in the diagram. A secondary Venn diagram for
297 this gene set shows its overlap with differentially expressed genes in hybrid vs. *Dom* placentas. Transgressively
298 expressed genes contained in this overlap are marked. Genes that are co-expressed in *Spret* and hybrid pregnancies
299 but not in *Dom* pregnancies (*Spret*-like co-expression) are marked in the primary Venn diagram. Results of pathway
300 overrepresentation (Reactome, version 58, Mi et al. 2017) for this gene set are provided in the connected text box.
301 (B) Scatterplot showing the correlation between placenta specific gene family (PSF) gene expression in placenta and
302 MPoA for the three pregnancy types. Red = *Dom* pregnancy ($R^2_{adj}$=0.38, p=0.01), Blue = *Spret* pregnancy ($R^2_{adj}$=0.9,
303 p<0.001), Yellow = *hybrid* pregnancy ($R^2_{adj}$=0.95, p<0.001). Inset boxplot shows total PSF gene expression in
304 MPoA (sum of normalized PSF counts/litter size), asterix indicates significant difference between MPoA-*hy* and
305 MPoA-*dom*.

306

## 307 Pairwise evolutionary rates of selected PSF genes

308 PSF genes were previously shown to exhibit accelerated evolutionary rates, potentially driven by

309 maternal-fetal conflict (Chuong et al. 2010). We selected the top 10 co-expressed PSF genes with

310 the highest expression in MPoA and extracted pairwise evolutionary rates (dN/dS) from Biomart

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311 (R-package biomaRt, (Durinck et al. 2005)) to test for evidence of positive selection. We also
312 included the PSF gene *Ctsr*, which was significantly differentially expressed between MPoA-*hy*
313 and MPoA-*dom*.

314 dN/dS, the per site ratio of nucleotide substitutions that change amino acid identity to
315 those that do not, is an indicator of selective pressure, with dN/dS = 1 indicating neutral
316 evolution, dN/dS < 1 purifying selection, and dN/dS > 1 diversifying positive selection
317 (Goldman and Yang 1994). Of the 11 genes, three (*Prl8a6*, *Tpbpb* and *Ctsr*) had pairwise dN/dS
318 >1 between *Dom* and *Spret* (Fig. 5, Supplemental Table S1). To infer which lineage experienced
319 selection, we analyzed these three genes using the application CodeML (implemented in PAML
320 4; (Yang and Rannala 1997, Yang 2007)), and including sequences from additional *Mus*
321 subspecies and species (*Mus m. musculus* (*Musc*), *Mus m. castaneus* (*Cast*), *Mus caroli* (*Car*)
322 and *Mus pahari* (*Pah*)). CodeML fits alternative models to the data; the best-fit model is chosen
323 based on likelihood ratio tests (LTRs). The two main models tested were M0, one evolutionary
324 rate for the whole tree, and MC, selected branches (foreground) evolve at a different rate than the
325 rest of the tree (background).

326 For all three genes, we found evidence for positive selection on *Dom* (*Prl8a6*) or *Spret*
327 branches (*Ctsr*), or both (*Tpbpb*) (Fig. 5, Supplemental Table S1). Specifically, there was
328 evidence for positive selection on *Prl8a6* in the *Mus musculus* subspecies clade ($LRT_{(M0-}$
329 $_{MC)}$=7.50, p=0.01, foreground $dN/dS_{(MC)}$=3.78, background $dN/dS_{(MC)}$=0.25). Evolutionary rates
330 for *Ctsr* were elevated on branches leading to *Pah, Spret* and *Musc* relative to the rest of the tree
331 ($LRT_{(M0-MC)}$=6.93, p=0.01, foreground $dN/dS_{(MC)}$>10, background $dN/dS_{(MC)}$=0.26). Results for
332 *Tpbpb* suggest high evolutionary rates across the whole tree ($LRT_{(M0-MC)}$=3.56, p=0.1,
333 $dN/dS_{(M0)}$=1.43) (Fig. 5, Supplemental Table S1).

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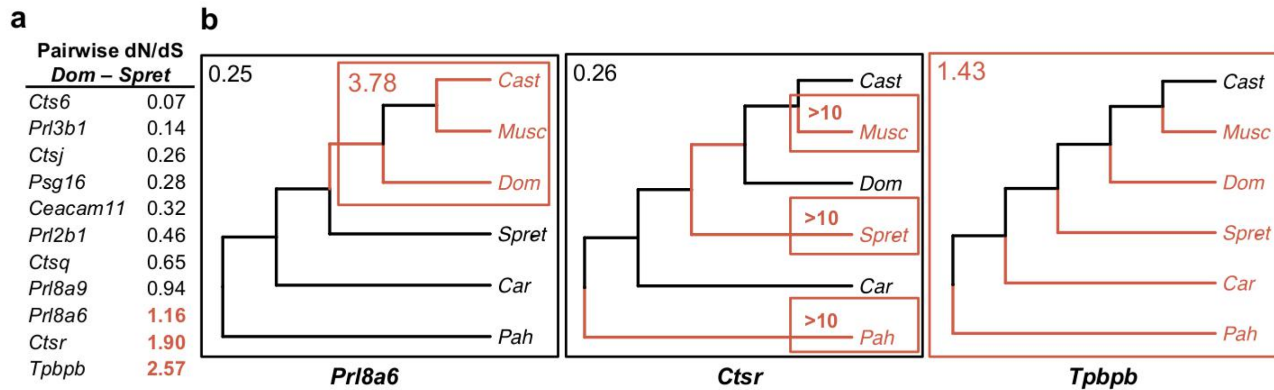
336 


337 **Figure 5**. Evolutionary rates of placenta-specific gene family (PSF) genes expressed in the maternal medial preoptic
338 area (MPoA). (A) Pairwise evolutionary rate between *Mus m. domesticus (Dom)* and *Mus spretus (Spret)* for *Ctsr*
339 and the 10 co-expressed PSF genes with the highest expression in MPoA. Evolutionary rate (dN/dS) > 1 is marked
340 in red throughout the figure and is indicative of positive selection. (B) PAML4 CodeML analysis results for the
341 three genes with pairwise dN/dS>1. Species are *Mus m. castaneus (Cast), Mus m. musculus (Musc), Mus m.*
342 *domesticus (Dom), Mus spretus (Spret), Mus caroli (Car), Mus pahari (Pah).* dN/dS values are indicated for groups
343 of branches depending on which CodeML model provided the best fit for the data (M0: one evolutionary rate for the
344 whole tree, MC: selected branches evolve at a different rate than the rest of the tree). dN/dS values depicted in black
345 for *Prl8a6* and *Ctsr* indicate background evolutionary rates.

346

347

## 348 Discussion

349 Molecular communication between the placenta and the maternal brain is crucial for the
350 expression of maternal behavior in rodents (Bridges et al. 1996, Larsen and Grattan 2012).
351 Disruption of this interaction in humans is detrimental to both mother and offspring (Redline
352 2008). In this study, we used a hybrid mouse model to characterize the extent to which placental
353 disruption influences gene expression in the maternal brain. Several maternally expressed
354 imprinted genes were transgressively misexpressed in the hybrid placenta. In *Mus m. domesticus*
355 females carrying hybrid litters we found altered placenta-specific gene family expression in the
356 placenta, and in the maternal MPoA. Surprisingly, the expression of these genes was highly
357 correlated between the two tissues, and was *Mus spretus*-like in the MPoA. This suggests that

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358 paternally inherited alleles in the placenta exert substantial influence on expression in the
359 maternal brain. Collectively, our results reveal reciprocal effects of mothers on offspring and
360 offspring on mothers, mediated in both cases by the placenta. We discuss these findings in light
361 of maternal-fetal coevolution and parental conflict, and identify potential implications for
362 placental pathologies.
363

364 **Maternal effects on placental expression**

365 Global patterns of expression in the placenta were strongly associated with maternal genotype. In
366 hybrids, half as many genes were DE relative to normal *Dom* placentas as opposed to *Spret*
367 placentas. Notably, the >1,600 genes with *Dom*-like expression in hybrid placenta were highly
368 enriched for terms associated with immunity and regulation of blood flow, both of which are
369 essential to placental mediation between mother and embryo (Cross et al. 1994). Because
370 maternal vasculature is incorporated into the placenta, whole placenta transcriptomes necessarily
371 include some transcripts of maternal origin. However, maternal blood flow within the placenta is
372 under the direct control of placental cell lineages; trophoblast giant cells invade and replace
373 maternal vascular endothelium, limiting maternally-derived tissue to blood (Rai and Cross 2014).
374 Therefore, while contamination from maternal transcripts may contribute to this pattern it is
375 unlikely to bias the expression of such a large number of genes. The regulatory effects of
376 maternal hormones, and of maternally inherited genes in the placenta, are non-mutually
377 exclusive alternative explanations. For example, because paternal X chromosome inactivation is
378 maintained in mouse placenta (Tagaki and Sasaki 1975), maternally inherited X-linked genes are
379 strong candidates for modulating autosomal expression in both sexes. While disentangling
380 maternal effects (*sensu* Wolf and Wade 2009) from the effects of maternally inherited genes is a

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381 challenge for future studies, we note that the match between maternal genotype and placental
382 expression of genes that modulate maternal immune tolerance and angiogenesis is consistent
383 with the expectation of molecular coadaptation between mother and offspring (Wolf and Brodie
384 1998, Keverne and Curly 2008), and the well-established effect of maternal environment on
385 placental function (Cottrell and Seckl 2009, Monk et al. 2012).
386

387 **Altered PSF and IG expression in the placenta**

388 Maternal adaptation to pregnancy relies to a great extent on placental signaling (Bridges et al.
389 1996). Thus, altered expression of genes encoding or influencing placental signaling molecules
390 can ultimately affect maternal physiological and behavioral response to pregnancy.
391 Misexpression in the hybrid placenta was substantial. However, the most striking pattern we
392 found was the reduced expression of a large number of PSFs. In mice, most of these genes are
393 expressed from the placental endocrine compartment and many are found in maternal plasma
394 during pregnancy (Rawn and Cross 2008). In this hybrid mouse model, the endocrine
395 compartment is markedly reduced when the mother is *Dom* (Zechner et al. 1996, Kurz et al.
396 1999). Thus, reduced abundance of PSF producing cell types likely contributes to overall
397 reduction in PSF expression.

398 IGs are thought to modulate PSF expression, primarily through effects on placental
399 endocrine cell abundance, with maternally expressed genes (MEGs) repressing and paternally
400 expressed genes (PEGs) promoting cell proliferation (John 2013, 2017). Two such MEGs,
401 *Phlda2* and *Ascl2*, were transgressively misexpressed in hybrid placentas. Misexpression of
402 either of these genes in lab mouse models results in an undersized endocrine compartment,
403 altered glycogen energy stores and reduced PSF gene expression (Tunster et al. 2010, 2016a,b).

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404 Indeed, *Phlda2* and *Ascl2* seem to be critical co-regulators of placental endocrine compartment
405 development (John 2017). Two other MEGs, *Dcn* and *Grb10*, were overexpressed when
406 compared to *Dom* placentas. While neither is specifically implicated in placental endocrine
407 function, both are key modulators of placental growth, and *Dcn* overexpression represses cellular
408 proliferation (Yamaguchi and Ruoslahti 1988, Kresse and Schönherr 2001, Garfield et al. 2011).
409 Collectively, our results are consistent with the proposed role of IGs in placental signaling (Haig
410 1996, Tunster et al. 2013, John 2013,2017), and identify MEG misexpression as a candidate
411 mechanism for the undersized endocrine compartment and consequent global reduction in PSF
412 expression in hybrid placenta.
413

414 **The effects of hybrid placental dysfunction on the maternal brain**

415 Altered signaling in hybrid placentas has the potential to affect the maternal brain. We found
416 subtle but significant differences in the expression of four genes in the MPoA of *Dom* females
417 exposed to hybrid relative to conspecific placentas. Both *Fmn1* (Formin1) and *Caln1*
418 (Calneuron1) were underexpressed. In the brain, *Fmn1* is involved in the formation of adherens
419 junctions and in linear actin cable polymerization (Kobielak et al. 2004). The formation of
420 adherens junctions is important in the maintenance of the blood brain barrier (BBB), a highly
421 specialized structure that regulates the influx of molecules into the brain (Stamatovic et al. 2008).
422 During pregnancy, the permeability of the BBB is increased by placenta-derived factors to which
423 the maternal brain must respond in order to maintain this barrier (Cipolla 2007, Schreurs et al.
424 2012). Reduced expression of *Fmn1* therefore suggests alterations in BBB adaptation during
425 hybrid pregnancies. *Caln1* encodes a neuron-specific protein with sequence similarities to
426 calcium-binding calmodulins. While the function of *Caln1* is uncharacterized, homology to

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427 calmodulin suggests a role in neuronal calcium signaling (Wu et al. 2001). Decreased expression
428 of a calcium-binding protein could indicate alterations in neuronal activity in the MPoA exposed
429 to hybrid placentas.

430 *Drd3* (Dopamine receptor D3) was also underexpressed compared to *Dom* mothers, but
431 not to *Spret* mothers, in the hybrid pregnancy MPoA. DRD3, a D2-like receptor with a generally
432 inhibitory role, is implicated in treatment-resistant major depression (Lattanzi et al. 2002) and
433 *Drd3* knock-out mice exhibit a suite of anxiety- and depressive-like behaviors (Moraga-Amaro et
434 al. 2014). Given that the action of dopamine in the MPoA is critical for the expression of
435 maternal behavior in rats (Numan and Stolzenberg 2009), and hypothalamic dopamine is altered
436 in a mouse model for post-partum depression (Avraham et al. 2017), reduced *Drd3* expression in
437 the MPoA might cause deficits in maternal behavior. However, *M. spretus* mothers are more
438 responsive to pups than *M. m. domesticus* mothers (Cassaing et al. 2010) and *Drd3* expression in
439 the hybrid pregnancy was statistically indistinguishable from the normal *Spret* pregnancy.
440 Whether placental expression of paternally inherited alleles promotes maternal behaviors is an
441 intriguing question for future study.

442 *Ctsr* (Cathepsin R), a placenta-specific cathepsin, was the only gene that was
443 overexpressed in the MPoA exposed to hybrid placentas. Unlike other PSF genes, expression of
444 *Ctsr* in the maternal brain was unique to the hybrid pregnancy. Interestingly, loss of the IG *Peg3*
445 leads to de-repression of several PSF members, including *Ctsr*, in the fetal and adult brain (Kim
446 et al. 2013). While *Peg3* was not misexpressed in the hybrid placenta, the transgressively
447 overexpressed MEG, *Phlda2*, was recently shown to perturb maternal behaviour and neural gene
448 expression when misexpressed in mouse placenta (Creeth et al. 2018). Specifically,
449 overexpression of placental *Phlda2* reduced postpartum nurturing behaviour, while

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450 underexpression increased maternal behavior (Creeth et al. 2018). Since *Phlda2* and *Ascl2* jointly
451 regulate development of the endocrine compartment (John 2017), it is likely that transgressive
452 misexpression of both genes in hybrid placenta impacts the maternal brain via effects on
453 placental hormone expression.
454

455 **Paternal effects on the maternal brain**

456 The hybrid placenta expresses both maternally derived (*Dom*) and paternally derived (*Spret*)
457 alleles. Thus, females pregnant with hybrids are exposed to gene products from a foreign
458 paternal genome. In *Dom* females exposed to hybrid placentas we found a substantial subset of
459 genes, including PSF genes and *Drd3*, with expression patterns that differed from *Dom* mothers
460 with conspecific litters, but closely matched those of *Spret* mothers.

461 A surprisingly large number of genes were co-expressed between placenta and MPoA in
462 hybrid and *Spret* pregnancies but not in *Dom* pregnancies. In particular, placental and MPoA
463 PSF gene expression was highly correlated in hybrid pregnancies and in *Spret* pregnancies, while
464 *Dom* pregnancies showed a weaker correlation. Likewise, total MPoA PSF gene expression was
465 *Spret*-like in hybrid pregnancies. The positive correlation between placental and MPoA
466 expression, and the striking similarity to *Spret*, preclude maternal compensation for hybrid
467 placental PSF misexpression as an explanation for these patterns. Instead, these results provide
468 two novel insights into placenta-maternal brain interactions. (1) Expression levels of PSFs in the
469 maternal brain are driven by placental expression levels of the same genes. (2) PSF and *Drd3*
470 expression in the maternal MPoA is strongly influenced by paternally inherited alleles in the
471 placenta. Thus, natural differences between the parental species used in this study uncover a
472 significant and previously unrecognized effect of the paternal genome on the maternal brain.

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473

474 **A signature of conflict in PSF evolution and expression in the maternal brain**

475 Pregnancy requires substantial investment from the mother, which is offset by costs to her
476 capacity to invest in future offspring (Trivers 1972). However, when offspring are sired by
477 multiple males, selection favors fathers who extract maximal maternal resources for their own
478 offspring (Trivers 1972). Haig and colleagues proposed that these asymmetries in the
479 reproductive interests of males and females, and the coefficients of relatedness between mothers
480 and offspring (always 0.5) vs. fathers and offspring (0.5 or 0), should promote parental
481 antagonism, played out at the molecular level between maternally and paternally expressed IGs
482 in the placenta (Moore and Haig 1991, Haig and Graham 1991, Haig 2000). Because placental
483 endocrine signals promote maternal investment in current offspring, placental hormones are also
484 proposed players in both parental and mother-offspring conflicts (Trivers 1985, Haig 1996).
485 Consistent with a history of antagonistic coevolution, PSFs in general are the fastest evolving
486 genes in the rodent placenta (Chuong et al. 2010). We report a similar signature of selection on
487 three PSF genes that are co-expressed in the hybrid placenta and the maternal MPoA.

488 Trivers (1985) described placental hormones as the molecular equivalent of begging calls.
489 Here we show for the first time that the expression of Prls and other PSFs is highly correlated
490 between placenta and maternal brain. While the function of PSFs in the brain is undefined,
491 placental genotype-dependent differences between *Dom* females in the strength of the correlation
492 and the number of co-expressed genes indicate that the relationship is driven by the placenta not
493 the mother. Moreover, the *Spret*-like co-expression patterns of PSF genes in mothers of litters
494 sired by *Spret* males implicate the paternally inherited genome as the driver of these placental
495 begging calls, which are echoed in the maternal brain. Given that these patterns of expression are

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496 consistent with parental conflict, it is noteworthy that the opportunity for sperm competition
497 (inferred from testis-body mass ratios) is higher in *M. spretus* than in *M. m. domesticus* (Gomez
498 Montoto et al. 2011).
499

500 **Preeclampsia related gene expression in hybrid pregnancies**

501 Preeclampsia is a serious pregnancy complication and the lead cause of maternal and fetal
502 morbidity and mortality (Burton and Jauniaux 2004, Redman and Sargent 2009). We found
503 significant overlap between transgressively misexpressed genes in hybrid placentas and
504 preeclampsia related genes and pathways. Haig (1993) interpreted preeclampsia as a
505 consequence of conflicts between maternal and paternal genomes, played out in the placenta and
506 potentially involving IGs. While the genetic basis of preeclampsia is complex (Uzun et al. 2016),
507 involvement of IGs is supported in both humans and mouse models. For example, human
508 chromosome 10 regions containing imprinting clusters are associated with preeclampsia
509 (Oudejans et al. 2004), and loss of the MEG *Cdkn1c* causes preeclampsia-like symptoms in mice
510 (Kanayama et al. 2002). Interestingly, *Cdkn1c* is in the same imprinting cluster as *Th, Phlda2*
511 and *Ascl2* (dist7, IC2), all of which were misexpressed in hybrid placentas. It is possible that
512 misexpression in this imprinting cluster is a general contributor to preeclampsia-like placental
513 phenotypes. Because preeclampsia can significantly alter permeability of the BBB (Cipolla
514 2007), it is also notable that *Fmn1*, a gene implicated in BBB maintenance, was underexpressed
515 in brains of mothers exposed to hybrid placentas.
516

517 **Conclusions**

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518 Evolutionary theoreticians have modeled mammalian pregnancy as both intimate cooperation
519 and antagonistic struggle between two genetically distinct organisms (Trivers 1974, Haig 1993,
520 Wolf and Hager 2006). Whether driven by conflict or coadaptation, it is clear that the placenta is
521 the mediator of these complex interactions between mother and offspring. Here we concentrated
522 on placental effects on the maternal brain during the final stages of pregnancy, when it is a
523 critical source of signal molecules that prime female physiology and behavior for motherhood.
524 We found both hybrid placental misexpression with the potential to disrupt maternal-fetal
525 communication, and altered expression in the brains of mothers exposed to hybrid placentas.
526 Expression in the hybrid placenta seems to be dominated by the maternally derived genome
527 and/or driven by maternal effects. Maternal-placental communication genes co-expressed in
528 maternal brain and placenta show elevated evolutionary rates, consistent with antagonistic
529 coevolutionary processes. The expression of a proportion of transcripts of these genes from a
530 foreign paternal genome in the placenta has the potential to affect the maternal brain and alter
531 maternal behavior. In addition to the effects of placental disruption on the maternal brain, natural
532 differences between the parental species in this hybrid system reveal a previously described
533 influence of the paternal placental genome on the maternal brain. These paternal effects on the
534 maternal brain could play a major role in the expression of maternal behavior and the quality of
535 maternal care, and open novel avenues of research in both evolutionary and biomedical fields.
536

537 **Methods**

538 **Animals and tissue collection**

539 Mice used in this study were maintained on a 12:12 light:dark cycle with lights on at 9:00 AM,
540 and were provided with 5001 Rodent Diet (LabDiet, Brentwood, MO, U.S.A.) and water ad lib.

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541 All animal procedures were approved by the Oklahoma State University IACUC under protocol
542 #141-AS. *Mus m. domesticus* (*Dom*) was represented by the wild-derived inbred strain WSB/EiJ
543 (Jackson Laboratory) and *Mus spretus* (*Spret*) was represented by the wild-derived inbred strain
544 SFM/Pas (Montpellier Wild Mice Genetic Repository). We conducted three crosses (female
545 shown first): *Dom* X *Dom* (n=5), *Dom* X *Spret* (n=5), *Spret* X *Spret* (n=5). Prior to pairing,
546 females were placed in a cage with soiled conspecific male bedding for ~48 hrs to induce
547 receptivity to mating (Whitten 1956). Mice were paired between 5:00 and 6:00 PM, left
548 undisturbed for two nights, and split on the morning of the second day. The second night was
549 counted as embryonic day 0 (e0). Females were weighed after two weeks to confirm pregnancy
550 but were otherwise left undisturbed. Pregnant females (n=5/type of pregnancy) were euthanized
551 by cervical dislocation between 10:00 and 11:00 AM on embryonic day 17-18 (e17.5) and the
552 maternal brain was extracted. Embryos were separated from placentas, and the maternally-
553 derived decidual layer was removed as previously described (Qu et al. 2014). All tissues were
554 transferred immediately to RNAlater (Thermo Fisher, USA), kept at 4°C overnight to allow
555 RNAlater perfusion, and stored at -20°C until microdissection and RNA extraction.
556
557 **Brain microdissection and RNA extraction**
558 The maternal MPoA was localized using the Mouse Brain atlas (Figs. 26-33, (Paxinos and
559 Franklin 2013)), and microdissected by sectioning the RNAlater perfused brain at 100µm on a
560 Leica CM 1950 cryostat, followed by dissection under a dissecting microscope in chilled PBS
561 droplets for improved visibility of brain microstructure. DNA was extracted from embryonic
562 tissue using the DNeasy Blood & Tissue Kit (Qiagen, USA) followed by PCR for the Y-linked
563 gene, *Zfy1*, to determine sex. Placentas from one male and one female per litter were used for

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564 RNA extraction (n=5 males/cross, n=4 females/hybrid cross, n=5 females/conspecific cross).
565 RNA was extracted from all tissues immediately after microdissection using the RNeasy Plus
566 Universal Mini Kit (Qiagen) for MPoA, and the AllPrep RNA/DNA Mini Kit (Qiagen) for
567 placenta. RNA was stored at -80°C until sequencing.
568

569 **RNAseq pipeline**

570 Sequencing (RNAseq): RNA integrity (RIN) was determined by the sequencing facility
571 (Novogene, Sacramento, CA) using the RNA Nano 6000 Assay Kit with the Agilent Bioanalyzer
572 2100 system (Agilent Technologies, Santa Clara, CA, USA). RIN values ranged from 8.2-10 for
573 all samples. Library preparation was performed by the sequencing facility, using the NEB Next
574 Ultra RNA library prep kit for Illumina. RNAseq was performed on the Illumina HiSeq 4000
575 platform, producing >30 million, 150bp paired-end reads per sample.
576 Mapping: QC of raw sequencing reads and trimming were performed in Trim Galore! 0.4.5
577 (Brabraham Bioinformatics, http://www.bioinformatics.babraham.ac.uk/projects/trim_galore),
578 using a phred score cutoff of 30 and minimum sequence length of 100 after trimming. In order to
579 map hybrid placenta reads we generated a pseudo-hybrid genome using the genome preparation
580 tool of the program SNPsplit (Brabraham Bioinformatics, (Krueger and Andrews 2016)). Briefly,
581 SNPs from both *Dom* (WSB/EiJ) and *Spret* (SPRET/EiJ) relative to the mouse genome
582 (GRCm38.89) available from the Ensembl FTP server (ftp://ftp.ensembl.org) were introduced
583 into the mouse genome (GRCm38.89). SNPs between *Dom* and *Spret* were then N-masked to
584 allow mapping of both *Dom*- and *Spret*-derived reads. To improve comparability, all placenta
585 samples (*Dom*, *Spret* and hybrid) were mapped to the pseudo-hybrid genome. MPoA samples
586 were mapped to their corresponding genomes (WSB/EiJ_v1 for *Dom* MPoA, SPRET/EiJ_v1 for

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587 *Spret* MPoA, (Keane et al. 2011)). Mapping was done using HISAT2 2.1 (Kim et al. 2015). After
588 mapping we filtered the resulting alignment files using SAMtools 0.1.19 (Li et al. 2009),
589 retaining only high quality (HISAT2 MAPQ score 40), uniquely mapped, paired reads for
590 analysis.
591 Post-processing of alignments: Before filtering, average alignment rate for MPoA samples was
592 87% for *Spret* MPoA samples and 88% for *Dom* MPoA samples. For placenta samples the
593 alignment rate was slightly lower for *Spret* samples (88%) compared to *Dom* (91%) and *hybrid*
594 (91%). We therefore randomly downsampled all alignment files to ~40 million reads using
595 SAMtools 0.1.19 (Li et al. 2009) to account for a possible mapping bias and to improve
596 comparability.
597 Quantification: Transcript quantification and annotation was done using StringTie 1.3.3 (Pertea
598 et al. 2015). Gene annotation information was retrieved from the Ensembl FTP server
599 (ftp://ftp.ensembl.org) for *Spret* (SPRET/EiJ_v1.86) and *Dom* (WSB/EiJ_v1.86). Mouse genome
600 annotation was used for samples mapped to the pseudo-hybrid genome (GRCm38.89). We used
601 the python script (preDE.py) included in the StringTie package to prepare gene-level count
602 matrices for analysis of differential gene expression.
603 Differential expression (DE) analysis: Differential expression was tested with DESeq2 1.16.1
604 (Love et al. 2014). Pseudogenes were removed from the count matrices based on "biotype"
605 annotation information extracted from Biomart (R-package biomaRt, (Durinck et al. 2005)). Low
606 counts were removed by the independent filtering process implemented in DESeq2 (Bourgon et
607 al. 2010). The adjusted p-value (Benjamini-Hochberg method) cutoff for DE was set at 0.05. Due
608 to variation in litter size, especially in females carrying hybrid litters (range=3-6), and its
609 potential effect of on MPoA expression, we corrected for litter size in all MPoA sample

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610 comparisons. To analyze DE between *Spret* and *Dom* MPoA, which were mapped to their
611 respective genomes, we extracted homologous gene names from the mouse genome database
612 using Ensembl Biomart (R-package biomaRt, (Durinck et al. 2005)) and merged the dataset
613 based on the genes that had a clear mouse homolog in both. Normalized read count tables
614 produced by DESeq2 were used in subsequent co-expression analyses.
615 Co-expression: To determine co-expression between placenta and MPoA we set a cutoff of 10
616 normalized counts for at least 4 out of 5 observations each tissue type (MPoA, male placenta and
617 female placenta). Based on this cutoff we report genes expressed in both placenta and MPoA for
618 each type of pregnancy. We then determined differences in co-expression between the three
619 pregnancy types.
620 Gene ontology (GO) term and pathway overrepresentation analysis: We performed GO term and
621 pathway overrepresentation analyses on relevant lists of genes from DE and co-expression
622 analyses using the PANTHER gene list analysis tool with Fisher's exact test and FDR correction
623 (Mi et al. 2017). We tested for overrepresentation based on the GO annotation database
624 (Biological Processes) (released 07-Jan-2017, (Ashburner et al. 2000, The Gene Ontology
625 Consortium 2017)) and the Reactome pathway database (version 58,(Fabregat et al. 2017)).
626

627 **Evolutionary rates for selected genes**

628 We extracted the pairwise evolutionary rate (dN/dS = nonsynonymous to synonymous
629 substitution rate ratio) between *Dom* and *Spret* from Biomart (R-package biomaRt, (Durinck et al.
630 2005)). dN/dS is an index of selective pressure on coding sequence, with dN/dS = 1 indicating
631 neutral evolution, dN/dS < 1 purifying selection, and dN/dS > 1 diversifying positive selection
632 (Goldman and Yang 1994). Further analysis of genes with dN/dS > 1 (*Prl8a6*, *Ctsr*, *Tpbpb*) was

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633 performed with CodeML implemented in PAML 4.8 (Yang and Rannala 1997, Yang 2007),
634 including sequences from related *Mus* subspecies and species (*Mus m. musculus (Musc)*, *Mus m.*
635 *castaneus (Cast)*, *Mus caroli (Car)* and *Mus pahari (Pah)*). Coding sequences for all three genes
636 for *Musc*, *Cast* and *Car* were available from Ensembl. For *Pah*, coding sequences for *Prl8a6* and
637 *Ctsr* were downloaded from NCBI Genbank. For *Tpbpb*, we ran blastn on NCBI with the *Dom*
638 *Tpbpb* coding sequence against the nr/nt database and found two matches for *Pah*, of which one
639 showed higher similarity to *Dom Tpbpa* and the other to *Dom Tpbpb*. The latter was included in
640 the CodeML analysis (Supplemental Table S5). CodeML calculates evolutionary rates by
641 applying different models to an alignment and a phylogeny. To prepare the alignments,
642 sequences were visualized with Geneious 9.1.8 (Biomatters, http://www.geneious.com/) and
643 trimmed to coding sequence. Translation alignments were performed using the MUSCLE
644 alignment algorithm, implemented in Geneious. The phylogeny was built based on recent
645 phylogenomic analyses of house mice and related species (White et al. 2009, Sarver et al. 2017).
646 For the CodeML codon frequency setting we used the setting with the best fit for each analysis
647 according to the preliminary likelihood ratio analysis.
648 To calculate individual evolutionary rates for each branch in the tree we used CodeML's
649 "free-ratio" model. This served as an initial indication as to which branches might show higher
650 evolutionary rates. After this, two models were computed: Model M0 "one ratio" in which all
651 branches were constrained to evolve at the same rate and MC "two-ratio" in which selected
652 branches are allowed to evolve at a different rate than the rest of the tree. Branches with
653 potentially higher evolutionary rate based on the "free-ratio" model result were marked as
654 foreground branches and were allowed to evolve differently from the background. To test if MC
655 provides a better fit for the data than M0 we performed Likelihood Ratio Tests. When MC

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656 provided the better fit, and dN/dS calculated for the foreground branches was > 1 and dN/dS
657 calculated for the background branches was < 1, we inferred positive selection on the foreground
658 branches. When M0 provided the better fit and dN/dS for the whole tree was > 1, we inferred
659 positive selection for the whole tree (Yang 1998).
660

661 **Data Access**

662 RNA-seq data from this study have been submitted to the NCBI Gene Expression Omnibus
663 (GEO; https://www.ncbi.nlm.nih.gov/geo/) under accession number XXXX.
664

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