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Effects of a male meiotic driver on male and female transcriptomes in the house mouse

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Abstract

Not all genetic loci follow Mendel's rules, and the evolutionary consequences of this are not yet fully known. Genomic conflict involving multiple loci is a likely outcome, as restoration of Mendelian inheritance patterns will be selected for, and sexual conflict may also arise when sexes are differentially affected. Here we investigate effects of the *t* haplotype, an autosomal male meiotic driver in house mice, on genome-wide gene expression patterns in males and females. We analysed gonads, liver and brain in adult same-sex sibling pairs differing in genotype, allowing us to identify *t*-associated differences in gene regulation. In testes, only 40% of differentially expressed genes mapped to the approximately 708 annotated genes comprising the *t* haplotype. Thus, much of the activity of the *t* haplotype occurs in *trans*, and as up-regulation. Sperm maturation functions were enriched among both *cis* and *trans* acting *t* haplotype genes. Within the *t* haplotype, we observed more down-regulation and differential exon usage. In ovaries, liver, and brain, the majority of expression differences mapped to the *t* haplotype, and were largely independent of the differences seen in the testis. Overall, we found widespread transcriptional effects of this male meiotic driver in the house mouse genome.

Introduction

As a rule, nuclear genes in diploids have an equal 50% chance of being transmitted from the germline into gametes. This has important implications, as the route to increasing fitness lies in cooperating with other genes to increase organismal relative fitness [1]. Some loci, called meiotic drivers, adopt a different, selfish strategy, by distorting segregation in their favour in heterozygotes, so that the genomic region bearing the driver is over-represented among gametes. As a consequence, meiotic drivers increase in frequency through segregation distortion if they have positive, neutral, or even detrimental fitness effects on the organism, as long as the fitness costs are not too high. Negative fitness effects of drivers slow fixation or prevent it altogether, and select for counter-adaptations of the genome against the driver in the form of drive resistance loci. This in turn selects for evolution in the driver to overcome such defenses in the genome, potentially leading to an arms race between driver and the genome that affects multiple genes.

Detailed studies of several systems where drive occurs in males, including the *SD* drive system in *Drosophila melanogaster*, *SR* in *D. simulans, D. neotestacea* and the stalk-eyed fly *Teleopsis dalmanni,* and the *t* haplotype in house mice, have shown that these systems have in common that one or more driver loci act in *trans* on a target locus on the homologous autosome or on the other sex chromosome, which causes the developmental failure of wildtype sperm [2-6]. Loci in linkage disequilibrium with a driver, for example within an inversion, may also be divergent from wildtype variants and increase in frequency by hitchhiking. With reduced recombination, Muller's ratchet predicts an accumulation of deleterious mutations [7]. Selection could favour the evolution of linked enhancers of a driver, to improve drive, or linked loci that otherwise improve the fitness of drive carriers. Sexually antagonistic selection could also play a role, as meiotic drive favours transmission either in males or in females. In male meiotic drive, for example, drive loci are transmitted more often through males than females, so trait expression of driver-linked genes could be pulled away from the average optimum for both sexes to one closer to the optimum for males, the sex in which the driver is active [8, 9]. This could potentially explain shared patterns of expression of genes in ovaries and testes of driving X chromosome carriers in stalk-eyed flies, *T. dalmanni* [10]. Drive also selects for the evolution of unlinked suppressor loci. Thus, meiotic drive might lead not only to differences in expression of genes associated with the drive phenotype, but also in linked genes, sexually antagonistic genes, and unlinked loci throughout the genome.

Here, we use the *t* haplotype in house mice to investigate genome-wide patterns of gene expression related to a male meiotic driver. We already have a relatively rich understanding of specific effects of the *t* haplotype. It is transmitted to about 90% of offspring when inherited from the father. Females transmit the *t* gametes in the usual 50% ratio [11, 12]. The mechanism of segregation distortion is like a poison-antidote system. Males heterozygous for the *t* haplotype (+/*t*) produce equal proportions of +

and *t* sperm [13], but maturing *t* sperm produce gene products from the transmission distortion loci (*Tagap, Fgd2*, *Nme3,* and *Tiam2)* that ultimately hyperactivate a target gene (Sperm motility kinase *Smok*) in + sperm [14-19], leaving + sperm impaired. *t* sperm themselves have a hypoactive version of the target *Smok* gene ($Smok^{Tcr}$) to compensate [17]. The balance of these effects is that *t* sperm are successful when competing against damaged + sperm within the ejaculate, but are very poor in competition with + sperm from other males [20, 21] when females mate multiply. Sperm from +/*t* males have been found to be slower, to hyperactivate more quickly, and to be less able to enter the oocyte [22-26].

The distorter loci are situated within four large adjacent inversions on chromosome 17 that greatly reduce recombination and make it more likely that the complex is inherited as one intact entity [27]. A recent study has shown a more heterogeneous picture – some regions of the *t* haplotype show signs of recombination, while others do not [28]. However, as predicted under the mutation accumulation hypothesis, the *t* haplotype often carries a recessive lethal allele, so that *t* homozygotes die prenatally [21, 29]. Litter sizes are reduced by 40% in crossings of +/*t* individuals [11]. There are however, several different *t* variants, each with different recessive lethal effects, or that do not have lethal effects, but male-sterile effects when homozygous [12]. Detailed studies of a natural population of *t* bearing house mice have led to the discovery of other traits associated with heterozygosity at the *t* haplotype: increased longevity in females [30], and decreased activity levels in females [31], an increased propensity to emigrate [32], and decreased fitness in males [20, 21, 33].

In a previous study, Kelemen and Vicoso [28] made use of the finding of +/*t* individuals among a set of wild-caught house mice from a genome sequencing and gene expression study [34]. They analysed the pooled transcriptomes of 4 male +/*t* mice with 12 male +/+ mice from two different populations in France and Germany, [34]. Their main findings were that gene regulation divergence was highly variable across the *t* haplotype, with increased divergence in regions in which no recombination events with the wildtype chromosome could be detected, parallel with an increase in the ratio of nonsynonymous to synonymous SNPs in this region. They also found very few genes that were differentially expressed that were not within the *t* haplotype.

In this study we compare the transcriptome of pairs of full sibling male and female adult house mice reared under standardised conditions in which one same-sex sibling is heterozygous for the *t* haplotype, while the other carries two wildtype alleles. The same laboratory population has been used in numerous studies of the *t* haplotype [11, 21, 25, 35-38] and descends from a well-studied field population [32, 39]. We analyse gene expression divergence in gonads, liver and brain attributable to the *t* haplotype in both males and females, making this the first study of effects of a male meiotic driver on female gene expression. By incorporating analysis of differential exon usage, we also advance on previous studies of gene regulation associated with meiotic drivers.

Understanding the effects of the *t* haplotype may be useful in contexts other than evolutionary biology, as it has been proposed as a conservation tool to control house mouse populations on islands [40]. The *t* haplotype could be transformed into a sex ratio driver by genetically engineering the incorporation of the male *Sry* gene, a Y-linked gene that initiates male sexual development, onto the *t* [40, 41]. Offspring inheriting the modified *t* would develop a male phenotype regardless of their sex chromosomes, thereby reducing the proportion of fertile females to all offspring produced. Strongly biased sex ratios can lead to population crashes [42-44]. An improved understanding of the effects of the *t* haplotype on gene expression may be useful in better predicting the fate of a *t-Sry* construct.

Methods

Animal breeding

We maintain a laboratory population of wild house mice descended from animals wild-caught from an intensively studied free-living population in Illnau, Switzerland [see 11, 38, 45 for details of the source population, and the *t* haplotype]. All mice were maintained at standard conditions (22–25°C, 40–50% humidity, at a 14h:10h light:dark cycle starting at 5:30 CET) in the same room. Further details are available in the ESM.

We created 10 breeding pairs from unrelated +/*t* females and +/+ males. Each pair contributed their first litter to the study, over 21 days. These 10 litters generated 7 male and 8 female sibling pairs, with each pair consisting of one +/+ and one +/*t*. Five litters provided both male and female sibling pairs. At 23 days of age, offspring were moved to single sex cages and an ear punch taken for *t* haplotype diagnosis by PCR [46]. At five weeks of age, males were placed into single cages (Macrolon type II), and at 8 weeks of age were euthanised with CO_2. Sister pairs were put into two single cages connected by a tube, to equalise per capita space available to males and females. For females, we prioritized standardization between paired siblings of the female oestrus cycle over age. From 8 weeks of age, we examined females visually for oestrus [47], checking for colour and swelling of the external genitalia, backed up by a vaginal smear [48] when in doubt. Females in a sibling pair were euthanised as soon as both were not in oestrus and appeared to be in dioestrus. Mice within pairs were processed sequentially during daylight hours, with one researcher extracting the brain and another gonads and liver. The organs were put into an RNA stabilisation buffer [49] at 10X volume of the sample, and stored at 4°C. After 4 hours the RNA buffer was replaced. After 24 hours the buffer was removed and samples frozen at -80°C. All experimental procedures were conducted blind with respect to genotype.

RNA isolation, sequencing and processing

Samples were shipped on dry ice to the MPI for Evolutionary Biology in Germany for RNA isolation and sequencing. RNA from testis, ovaries and liver was extracted using the Ambion PureLink RNA

Mini Kit with Trizol and a DNase step, while RNA from whole brain was isolated using a phenol-chloroform protocol. RNA libraries were prepared using Set A and B of the TruSeq Stranded mRNA Library Kit (Illumina). All samples, in total 90, were measured with the Fluoreszenz NanoDrop (NanoDrop 3300 Fluorospectrometer) and pooled at equimolar concentrations. The final pools were measured with the Agilent 2100 Bioanalyzer using the Agilent DNA 7500 Kit. The library was clustered to a density of approximately 180 K/mm^2. Sequencing was performed on an Illumina NextSeq 500 using five HighOutput 300 cycle kits, corresponding to 5 lanes. PhiX Control library (Illumina) was combined with the library at 1%.

The RNA-seq data processing consisted of the following steps. Adapter trimming of the reads was performed with Trimmomatic [50]. We filtered reads with an average quality below 20. Poor quality reads led to the omission of one testis, one male brain and one female liver sample. Read-alignment was performed with STAR 2.5.3a [51]. As reference we used the Ensembl genome build GRCm38, with the gene annotations downloaded on 2017-05-31. Gene expression values were computed with the function featureCounts from *Rsubread* [52]. The Supplementary Material details the STAR alignment and featureCounts options. Genes were considered as expressed in a sample if they had at least 10 reads assigned. All figures were generated using more general R/Bioconductor functionality and UpsetR [53].

Differential Gene Expression and Differential Exon Usage

For the tissues we computed differential expression as gene-level differences and differential exon usage. In the gene-level differences we analysed the total number of reads assigned to a gene locus, using the generalised linear model implemented in the Bioconductor package *DESeq2* [54]. Specifically, the design parameter we used as input for DESeq2 was *Genotype+Gender+Sibling*. These factors are available as sample descriptions in GEO under the accession GSE138839. We computed the contrast between +/*t* and +/+. The model used for *DEXSeq* [55] was extended to include exons and the genotype-exon interaction. Differential exon usage (DEU) will be significant if there is alternative splicing, or usage of alternative transcript start sites. If differential exon usage is detected for a gene, the increase or decrease of the gene's total read counts must be interpreted with caution. For those genes it cannot be assumed that an increase in the gene counts is associated with an overexpression. Thus, we report genes only as DEU if they show next to the DEU also an increased or decreased global read count. The thresholds we used were the following: up-regulation: log2 ratio>0.1, fdr<0.1; down-regulation: log2 ratio<-0.1, fdr <0.1; differential exon usage: splicing q-value <0.1.

We used the software VLAD [56] via the Mouse Genomic Informatics website (http://proto.informatics.jax.org/prototypes/vlad/) accessed 25.7.2019 to test for functional themes among differentially expressed gene sets. To test for enrichment of ontology terms within the *t* haplotype we had to define the set of genes associated with the *t* haplotype. The precise boundaries of

the *t* haplotype have not been identified [57]. Kelemen and Vicoso [28] gave approximate beginning and end points of the *t* haplotype as 5 – 40 Mb, based on increased SNP heterozygosity in a heterogeneous sample of +/+ and +/*t* mice. However, a recently discovered *t* haplotype distorter, *Tiam2* [18], is located at 3.2 Mb, suggesting that this window is too small. We therefore expanded the window by 2 Mb in each direction (including 20 additional genes), at the risk of considering genes beyond the start and end points of the *t* haplotype as within it. We set $q<0.01$ to limit results to the top terms.

Results

We found in all tissues a total of 434 genes with a differential expression status between +/*t* and +/+ siblings. 198 of these genes mapped to chromosome 17, with 195 falling within our defined limit of the *t* haplotype (3–42 Mb, making up 1.4% of the mouse genome [58] and containing 708 annotated genes). These 195 genes were located from 5.9 – 40.9 Mb, the remaining three at 43.9, 80.5 and 86.9 Mb, the latter two clearly outside of the *t* haplotype.

We found that the *t* haplotype region is highly enriched for antigen processing and presentation genes, as the *t* contains 78% of all MHC protein complex genes. The *t* haplotype also includes 82% of all pheromone activity genes (via exocrine gland secreted peptide genes), and is enriched for plasma membrane part genes, and response to pheromone and G-protein coupled receptor activity genes, due to numerous vomeronasal receptor genes. It does not show depletion of any biological or molecular process, but in terms of cellular processes, it is deficient in intracellular parts.

We visualized gene regulation differences between +/*t* and +/+ siblings as heatmaps (Figure 1, see also ESM Figure S1). Expression differences were observed in all tissues. In testes, expression differences, measured as absolute log2 ratios, were greater in the *t* haplotype region (mean±sd of 0.83±1.09) than in the rest of the genome (0.35±0.65; Wilcoxon test, $Z=2883$, $p<0.00001$; ESM Figure S2). Remarkably, brain tissue also showed a strong upregulation of a few *t* haplotype genes not differentially expressed in testes. This contrasts with non-*t* haplotype genes in the brain, which showed only very small changes.

We also observed differential exon usage in all tissues, and mostly in genes mapping to the *t* haplotype (Figure 2). Examples include the *t* haplotype distorter genes *Tagap* (but notably not *Tiam2*), the *responder* $Smok^{Tcr}$ locus element *Rps6ka2,* and spermatogenesis genes such as *Dynlt1c, Dynlt1f, Tcp1, Tcp10a, Tcp10b, Tcp11, Tcte2, Tcte3,* as well as the embryonic lethal gene *Vps52*. As an example of differential exon usage, we show in Figure S3 differences in the distribution of reads across exons between +/*t* and +/+ individuals in the chromosome 5 gene *Ppp1cb*, identified as showing the strongest differential expression between +/*t* and +/+ in Kelemen & Vicoso [28]. Thus, our final dataset scores three categories: significant differential exon usage, or significant up- or down-

regulation. ESM Table S2 provides the differential expression status of all genes, and ESM Figure S4 illustrates differential expression status of genes along chromosome 17.

The testis dominated other tissues regarding the number of differentially expressed genes (Figure 2), but only 40% (105/263) of all differentially expressed testis genes mapped to the *t* haplotype. This was significantly less than in other tissues: in ovaries 83% (65/78, χ^2=43.6, p>0.001), in male and female liver combined 64% (74/116 ,χ^2=17.5, p<0.001), and in male and female brain combined, 77.0% (99/135, χ^2=38.5, p<0.001) of differentially expressed genes were within the *t* haplotype. The *t* haplotype also significantly influenced the pattern of gene expression differences. In the testis, up-regulation was observed in 30.5% (32/105) of differentially expressed *t* genes, compared to 69.0% (108/158) of non-*t* haplotype genes (χ^2=34.8, p<0.001).

We next asked whether the direction or type of expression difference in the ovary, liver and brain mirrored those of the testis. Usually they did not (Figure 3, see also ESM Figure S4). Many genes were up or down-regulated only in the testis, but in every tissue (male and female gonad, liver and brain) we found genes with tissue-specific changes. We also identified other genes with similar expression changes in multiple tissues. Remarkably, those genes were nearly always located on the *t* haplotype (Figure 3). Males and females showed independent, but also some shared regulation differences within tissues. A small number of genes showed up-regulation in all tissues (*Rsph3a, Zrnd1*), down-regulation in all tissues (*Jmjd8*) and differential exon usage in all tissues (*H2-D1, Ppp1r11, Rnpepl1, Tcp1, Tmem181*), with all these genes but *Rnpepl1* located within the *t* haplotype.

We also compared females and males more generally, asking how often a gene is significantly differentially expressed in females but not in males between *+/t* and +/+. In the gonads, 51 genes were differentially expressed in ovaries but not in testes, 26 genes in female compared to male brains, and 36 genes in female vs male livers. One gene *Zfp994* showed up-regulation in all female, but not in any male tissue, and one gene, *Tagap,* showed differential expression in all male but not in any female tissues.

We then tested if differentially expressed (DE) genes from the testis showed enrichment for particular functions (Figure S5, ESM GO_analysis). Spermatogenesis genes were significantly enriched, with 11% of all differentially expressed genes having this function, representing 4% of all known spermatogenesis genes. Some functions were up-regulated, such as syncytium formation, with 15% of syncytium formation genes affected. Down-regulated functions included regulation of protein catabolic process (5% of DE genes, 3% of genes of this function) and chylomicron remnant clearance (1% of DE genes, 50% of genes attached to this function). Differentially expressed genes were involved in endocytic recycling (2% of DE genes, 10% of endocytic recycling genes). The top category of enrichment of cellular process was up-regulation of genes contributing to the sperm flagellum (7% of DE genes, 14% of all sperm flagellum genes), followed by differential exon usage of

dynein complex genes (4% of DE genes, 14% of genes of this function) and protein phosphatase type 1 complex genes, including the gene Ppp1cb (1.5% of DE genes, 23% of genes of this function). No molecular functions were significantly enriched.

We next asked whether the differentially expressed genes from the *t* haplotype were significantly associated with biological functions, also using a Gene Ontology enrichment analysis. In *t* genes of the testis, we found significant enrichment and upregulation of the cytoplasmic dynein complex and enrichment of sperm flagellum genes (ESM Table S1, ESM GO_analysis). With the analogous analysis for non-*t* genes, we found enrichment of sperm flagellum genes, spermatogenesis, but also syncytium formation, GTP biosynthetic process and alcohol and steroid metabolism.

Some functions carried over to other tissues. In the ovary, there was enrichment of gene function only within the *t* region. The dynein complex was enriched but down-regulated. Sperm flagellum genes and the function of membrane bound organelles were also enriched. Unlike in testis, there was enrichment of the MHC protein complex. In male liver, no functions were enriched, but in females some metabolic processes were enriched, in the non-*t* part of the genome. In male brain, differential expression of cytoplasmic dynein genes was enriched, but mainly due to differential exon usage, unlike in the ovary. In both male and female brain intracellular organelle was enriched.

Discussion

In this study of gene expression associated with the *t* haplotype, including analysis of differential exon usage, we show that most expression differences are associated with the testis, where the loci causing drive of the *t* haplotype are known to be active. Expression effects were larger within the *t* haplotype than in chromosomal regions outside of the *t* haplotype, in line with previous studies comparing the magnitude of *cis* and *trans* expression [59]. We found that 60% of genes showing expression differences in testes map outside of the *t* haplotype, suggesting a high degree of activity in *trans* of *t* haplotype genes. In ovaries, liver and brain, most expression changes were associated with genes of the *t* haplotype itself. Both *cis* and *trans* changes are important in adaptive evolution, but it appears that *trans* changes feature more prominently in adaptation within species, whereas *cis* changes play a larger role in divergence between species [60-62]. This is interesting in the context of the *t* haplotype because it is thought to have evolved 1-3 million years ago [63, 64] and then recently introgressed into *Mus musculus* populations, either from an isolated *M. musculus* lineage [12] or from another species [65]. This suggests that the gene expression differences outside of the testis may simply reflect a neutral evolution of *cis*-acting elements. The *trans* effects in the testis, on the other hand, indicate the direct interaction of genes from the *t* haplotype with other parts of the genome to regulate sperm maturation.

Activity by transcription factors *in trans* may explain why the vast majority of expression differences off the *t* haplotype were in activation or repression of transcription, rather than in alternative splicing, which also requires *cis*-elements [66]. Testis genes affected in *trans* included those conferring functions that seem beneficial to the driving *t* haplotype genes. For example, *t* distorter products encounter developing + sperm in the syncytium, thus up-regulation of syncytium formation genes may enhance dissemination of *t* distorter proteins, potentially increasing drive. The *t* haplotype influences flagellar function, decreasing sperm motility and altering movement patterns [24, 25], and it is thought that + sperm of the +/*t* male are primarily affected, with the *t* sperm retaining normal function [17, 67]. As dynein genes affect flagellar function, and spermatogenesis genes can affect many aspects of sperm function, activating such genes off the *t* haplotype could enhance drive. Protein phosphatase type 1 complex genes were enriched in the testis. These function in signaling, in an opposite way to protein kinases, such as *Smok*, and thus could conceivably also play a role in drive. Differentially expressed genes involved in protein catabolism, endocytic recycling and chylomicron (small lipoprotein particle) clearance may contribute to general maturation pathways of the sperm that respond to the signaling changes induced by the *t* haplotype genes.

Expression of *t* genes with function in the testis was generally tissue-specific, according with organ-specific gene expression in mammals [68]. Some *t* genes were differentially expressed in the testis and also in other tissues, but most of these are likely due to the differences in *cis*-elements that have accumulated on the *t* haplotype. One unusual example is that of dynein complex genes, which showed differential exon usage in testis and male brain, but were down-regulated in ovaries, were they are unlikely to be useful, and were not differentially expressed in female brain. Sex- and tissue-specific expression of many *t* genes suggests that selection has finely tuned expression patterns of most *t*-beneficial genes, and that sexually antagonistic selection for male beneficial gene regulation has not generally shaped expression profiles in females. Differential expression of *t* genes outside of the testis is not associated with an enrichment of gene ontology functions that link to phenotypic changes that have been observed in female +/*t*, including increased viability [39], decreased activity in the home cage [35], or altered mate choice [69], although we measured gene expression in dioestrus, rather than oestrus, when it is more relevant for mate choice [70]. Carboxylic acid metabolism in liver has been linked to sexual dimorphism in mice [71] and this function was enriched outside of the *t* haplotype in +/*t* female liver. In all +/*t* female tissues, there was up-regulation of one gene, a zinc finger protein gene with KRAB domain, associated with retrotransposon defense [72].

Our success in identifying expression differences in nearly all genes in which we expected to find differences validates our experimental and analytical approach. In testis, many *t* haplotype loci previously investigated in the search for genes directly involved in drive showed expression differences in this study. Nearly half of all *t* haplotype genes that showed differential exon usage are known to be associated with differences between +/*t* and +/+: *Tagap* [14], *Rps6ka2 [17], Tcte2* [73],

Tcte3 [74], *Tcp1* [75], *Tcp10a* and *Tcp10b* [76, 77], *Tcp11* [78], *Dynlt1c* and *Dynlt1f* [79], *Vps52* [80], *Fgfr1op* [17], *Ppp1r11* [81], *Synj2* [82], and *Tulp4* [83]. 19 genes showing alternative transcripts are left which have as yet no known function in the *t* haplotype, but are now new candidates: the protein kinase *Map3k4*, sperm genes *Ift140, Hmga1, Slc26a8*, as well as a mixed collection: *Tmem181, Ube2i, Rsph3b, 1700010I14Rik, Rab11b, Rab11fip3, Fkbpl, Afdn, Znrd1as, Gm3448, Tbp, Rps2*, and *H2-D1*. Copy number variation in the *t* haplotype [28] could contribute to the prevalence of alternative splicing among differentially expressed genes . The driving gene *Tagap*, for example, is present in four copies [14]. We found however, that differential exon usage was largely tissue-specific, and not across tissues. Down-regulation was seen in five well-studied *t* haplotype genes: *Dynlt1b [84], Tagap1 [17], Fgd2 [15], DNAh8 [85, 86].* Up-regulation of known *t*-haplotype active genes was rare, with only two cases: the maternally imprinted gene *Slc22a3* [87, 88] and the sperm glycolysis gene *Pgk2 [89, 90]*. We found no expression differences in the *t* loci *Tiam2 [18], Nme3 [16], Tcp10c [91], Slc22a2 [87], Acat2* and *Acat3 [92], Serac1 [82]*, and *Prdm9 [93]*, or the classic markers *T*, *qk*, *Itpr3* (previously *tf)* [57]. Lack of detecting differences in *Tiam2* was unexpected, given previously reported differential exon usage [18]. Expression differences might be due to differences between wildtype strains or between *t* variants, as shown for *Fgd2 [15]*, possibly indicating variation in which transmission distortion loci function in different *t* variants or different genetic backgrounds. *Nme3*, in contrast, was not expected to show expression differences [16]. These genes are not typical of the content of the *t* haplotype region, as it is enriched for immune function and pheromone activity and pheromone response, yet none of these functions were enriched in differentially expressed genes of the testis, liver or brain.

Our finding that most differentially expressed genes in the testis were not within the *t* haplotype region differs from stalk-eyed flies *Teleopsis dalmanni* and *Drosophila neotestacea*, and in a previous study of the *t* haplotype, in which most expression differences in testis were linked to the driving chromosome [5, 10, 28]. The differences with the latter study are likely to have been influenced by sampling scheme, as distant populations were pooled for that analysis [28]. Strikingly, functional themes among enriched transcripts in *T. dalmanni* and *D. neotestacea* show little overlap with each other or with this study, even though all three systems damage + sperm development. This highlights the varied evolutionary routes leading to male meiotic drive, but more work is needed to better understand the differences and commonalities between systems.

Ethics

This research was approved by the Veterinaeramt of Kanton Zurich, under permit 110/2013.

Data accessibility

Sequence reads can be accessed at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE138839. Other data supporting this study are available in the electronic supplementary material.

Authors' contributions

A.L. and D.T. designed the study. A.L. set up the live animal study and A.L. and A.S. collected samples. S.K. performed RNA isolation and RNA sequencing. H.R. analysed the RNA data. A.L. wrote the manuscript with input from H.R., A.S. and D.T. All authors contributed to editing the manuscript.

Competing interests

We declare we have no competing interests.

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References

[1] Frank, S.A. 2003 Repression of competition and the evolution of cooperation. *Evolution* **57**, 693-705.

[2] Larracuente, A.M. & Presgraves, D.C. 2012 The selfish *Segregation Distorter* gene complex of *Drosophila melanogaster*. *Genetics* **192**, 33-53.

[3] Tao, Y., Araripe, L., Kingan, S.B., Ke, Y., Xiao, H. & Hartl, D.L. 2007 A *sex-ratio* meiotic drive system in *Drosophila simulans*. II: An X-linked distorter. *PLoS Biol* **5**, e293.

[4] Herrmann, B.G. & Bauer, H. 2012 The mouse *t*-haplotype: a selfish chromosome – genetics, molecular mechanism, and evolution. In *Evolution of the house mouse* (eds. M. Macholán, S.J.E. Baird, P. Munclinger & J. Piálek). Cambridge, Cambridge University Press.

[5] Pieper, K.E., Unckless, R.L. & Dyer, K.A. 2018 A fast-evolving X-linked duplicate of *importin-α2* is overexpressed in sex-ratio drive in *Drosophila neotestacea*. *Molecular Ecology* **27**, 5165-5179.

[6] Burt, A. & Trivers, R. 2006 *Genes in conflict: the biology of selfish genetic elements*. Cambridge, Harvard University Press.

[7] Muller, H.J. 1964 The relation of recombination to mutational advance. *Mutation Research* **1**, 2-9.

[8] Manser, A., Lindholm, A.K., König, B. & Bagheri, H.C. 2012 The effect of polyandry on a distorter system with differential viabilities in the sexes. *Communicative & Integrative Biology* **5**, 550-552.

[9] Patten, M.M. 2014 Meiotic drive influences the outcome of sexually antagonistic selection at a linked locus. *Journal of Evolutionary Biology* **27**, 2360-2370.

[10] Reinhardt, J.A., Brand, C.L., Paczolt, K.A., Johns, P.M., Baker, R.H. & Wilkinson, G.S. 2014 Meiotic drive impacts expression and evolution of X-linked genes in stalk-eyed flies. *PLOS Genetics* **10**, e1004362.

[11] Lindholm, A.K., Musolf, K., Weidt, A. & König, B. 2013 Mate choice for genetic compatibility in the house mouse. *Ecology and Evolution* **3**, 1231-1247.

[12] Silver, L.M. 1993 The peculiar journey of a selfish chromosome: mouse *t* haplotypes and meiotic drive. *Trends in Genetics* **9**, 250-254.

[13] Hammerberg, C. & Klein, J.A.N. 1975 Evidence for postmeiotic effect of *t* factors causing segregation distortion in mouse. *Nature* **253**, 137-138.

[14] Bauer, H., Willert, J., Koschorz, B. & Herrmann, B.G. 2005 The *t*-complex-encoded GTPase-activating protein Tagap1 acts as a transmission ratio distorter in mice. *Nature Genetics* **37**, 969-973.

[15] Bauer, H., Véron, N., Willert, J. & Herrmann, B.G. 2007 The *t*-complex-encoded guanine nucleotide exchange factor *Fgd2* reveals that two opposing signaling pathways promote transmission ratio distortion in the mouse. *Genes & Development* **21**, 143-147.

[16] Bauer, H., Schindler, S., Charron, Y., Willert, J., Kusecek, B. & Herrmann, B.G. 2012 The nucleoside diphosphate kinase gene *Nme3* acts as quantitative trait locus promoting non-Mendelian inheritance. *PLOS Genetics* **8**, e1002567.

[17] Herrmann, B.G., Koschorz, B., Wertz, K., McLaughlin, J. & Kispert, A. 1999 A protein kinase encoded by the *t complex responder* gene causes non-mendelian inheritance. *Nature* **402**, 141-146.

[18] Charron, Y., Willert, J., Lipkowitz, B., Kusecek, B., Herrmann, B.G. & Bauer, H. 2019 Two isoforms of the RAC-specific guanine nucleotide exchange factor TIAM2 act oppositely on transmission ratio distortion by the mouse *t*-haplotype. *PLOS Genetics* **15**, e1007964.

[19] Veron, N., Bauer, H., Weisse, A.Y., Luder, G., Werber, M. & Herrmann, B.G. 2009 Retention of gene products in syncytial spermatids promotes non-Mendelian inheritance as revealed by the *t* complex responder. *Genes & Development* **23**, 2705-2710.

[20] Manser, A., Lindholm, A.K., Simmons, L.W. & Firman, R.C. 2017 Sperm competition suppresses gene drive among experimentally evolving populations of house mice. *Molecular Ecology* **26**, 5784-5792.

[21] Sutter, A. & Lindholm, A.K. 2015 Detrimental effects of an autosomal selfish genetic element on sperm competitiveness in house mice. *Proceedings of the Royal Society B: Biological Sciences* **282**, 20150974.

[22] Johnson, L.R., Pilder, S.H., Bailey, J.L. & Olds-Clarke, P. 1995 Sperm from mice carrying one or two *t* haplotypes are deficient in investment and oocyte penetration. *Developmental Biology* **168**, 138-149.

[23] Olds-Clarke, P. 1989 Sperm from t^{w32}/+ mice: capacitation is normal, but hyperactivation is premature and nonhyperactivated sperm are slow. *Developmental Biology* **131**, 475-482.

[24] Olds-Clarke, P. & Johnson, L.R. 1993 *t* haplotypes in the mouse compromise sperm flagellar function. *Developmental Biology* **155**, 14-25.

[25] Sutter, A. & Lindholm, A.K. 2016 Meiotic drive changes sperm precedence patterns in house mice: potential for male alternative mating tactics? *BMC Evolutionary Biology* **16**, 133.

[26] Redkar, A.A., Si, Y., Twine, S.N., Pilder, S.H. & Olds-Clarke, P. 2000 Genes in the first and fourth inversions of the mouse *t* complex synergistically mediate sperm capacitation and interactions with the oocyte. *Developmental Biology* **226**, 267-280.

[27] Artzt, K., McCormick, P. & Bennett, D. 1982 Gene mapping within the *T/t* complex of the mouse. I: *t*-lethal genes are nonallelic. *Cell* **28**, 463-470.

[28] Kelemen, R.K. & Vicoso, B. 2018 Complex history and differentiation patterns of the *t*-haplotype, a mouse meiotic driver. *Genetics* **208**, 365-375.

[29] Safronova, L.D. 2009 Embryonal effects of *t*-haplotypes in mice. *Russian Journal of Developmental Biology* **40**, 23-30.

[30] Manser, A., Lindholm, A.K., König, B. & Bagheri, H.C. 2011 Polyandry and the decrease of a selfish genetic element in a wild house mouse population. *Evolution* **65**, 2435-2447.

[31] Auclair, Y., König, B. & Lindholm, A.K. 2013 A selfish genetic element Influencing longevity correlates with reactive behavioural traits in female house mice (*Mus domesticus)*. *PLoS ONE* **8**, e67130.

[32] Runge, J.-N. & Lindholm, A.K. 2018 Carrying a selfish genetic element predicts increased migration propensity in free-living wild house mice. *Proceedings of the Royal Society B: Biological Sciences* **285**, 20181333.

[33] Carroll, L.S., Meagher, S., Morrison, L., Penn, D.J. & Potts, W.K. 2004 Fitness effects of a selfish gene (the *Mus t* complex) are revealed in an ecological context. *Evolution* **58**, 1318 - 1328.

[34] Harr, B., Karakoc, E., Neme, R., Teschke, M., Pfeifle, C., Pezer, Ž., Babiker, H., Linnenbrink, M., Montero, I., Scavetta, R., et al. 2016 Genomic resources for wild populations of the house mouse, *Mus musculus* and its close relative *Mus spretus*. *Scientific Data* **3**, 160075.

[35] Auclair, Y., König, B. & Lindholm, A.K. 2013 A selfish genetic element influencing longevity correlates with reactive behavioural traits in female house mice (*Mus domesticus*). *PLoS One* **8**.

[36] Manser, A., König, B. & Lindholm, A.K. 2015 Female house mice avoid fertilization by *t* haplotype incompatible males in a mate choice experiment. *J Evol Biol* **28**, 54-64.

[37] Sutter, A. & Lindholm, A.K. 2016 No evidence for female discrimination against male house mice carrying a selfish genetic element. *Current Zoology* **62**, 675-586.

[38] Ferrari, M., Lindholm, A.K. & König, B. 2014 A genetic tool to manipulate litter size. *Frontiers in Zoology* **11**, 18.

[39] Manser, A., Lindholm, A.K., König, B. & Bagheri, H.C. 2011 Polyandry and the decrease of a selfish genetic element in a wild house mouse population. *Evolution* **65**, 2436-2447.

[40] Leitschuh, C.M., Kanavy, D., Backus, G.A., Valdez, R.X., Serr, M., Pitts, E.A., Threadgill, D. & Godwin, J. 2018 Developing gene drive technologies to eradicate invasive rodents from islands. *Journal of Responsible Innovation* **5**, S121-S138.

[41] Backus, G. & Gross, K. 2016 Genetic engineering to eradicate invasive mice on islands: Modeling the efficiency and ecological impacts. *Ecosphere* **7**, e01589.

[42] Hamilton, W.D. 1967 Extraordinary sex ratios. *Science* **156**, 477-488.

[43] Lyttle, T.W. 1977 Experimental population genetics of meiotic drive systems. 1. Pseudo-*Y* chromosomal drive as a means of eliminating cage populations of *Drosophila melanogaster*. *Genetics* **86**, 413-445.

[44] Kyrou, K., Hammond, A.M., Galizi, R., Kranjc, N., Burt, A., Beaghton, A.K., Nolan, T. & Crisanti, A. 2018 A CRISPR–Cas9 gene drive targeting *doublesex* causes complete population suppression in caged *Anopheles gambiae* mosquitoes. *Nature Biotechnology* **36**, 1062.

[45] König, B. & Lindholm, A.K. 2012 The complex social environment of female house mice (*Mus domesticus*). In *Evolution of the House Mouse* (eds. M. Macholán, S.J.E. Baird, P. Munclinger & J. Piálek). Cambridge, Cambridge University Press.

[46] Schimenti, J. & Hammer, M. 1990 Rapid identification of mouse *t* haplotypes by PCR polymorphism (PCRP). *Mouse Genome* **87**, 108 - 108.

[47] Champlin, A.K., Dorr, D.L. & Gates, A.H. 1973 Determining the stage of the estrous cycle in the mouse by the appearance of the vagina. *Biology of Reproduction* **8**, 491-494.

[48] Byers, S.L., Wiles, M.V., Dunn, S.L. & Taft, R.A. 2012 Mouse estrous cycle identification tool and images. *PLoS One* **7**.

[49] De Wit, P., Pespeni, M.H., Ladner, J.T., Barshis, D.J., Seneca, F., Jaris, H., Therkildsen, N.O., Morikawa, M. & Palumbi, S.R. 2012 The simple fool's guide to population genomics via RNA-Seq: an introduction to high-throughput sequencing data analysis. *Molecular Ecology Resources* **12**, 1058-1067.

[50] Bolger, A.M., Lohse, M. & Usadel, B. 2014 Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114-2120.

[51] Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M. & Gingeras, T.R. 2013 STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15-21.

[52] Liao, Y., Smyth, G.K. & Shi, W. 2013 The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. *Nucleic Acids Research* **41**, e108.

[53] Conway, J.R., Lex, A. & Gehlenborg, N. 2019 UpSetR: An R package for the visualization ofiIntersecting sets and their properties *Bioinformatics* **33**, 2938-2940.

[54] Love, M.I., Huber, W. & Anders, S. 2014 Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**, 550.

[55] Anders, S., Reyes, A. & Huber, W. 2012 Detecting differential usage of exons from RNA-seq data. *Genome Research* **22**, 2008.

[56] Richardson, J.E. & Bult, C.J. 2015 Visual annotation display (VLAD): a tool for finding functional themes in lists of genes. *Mamm Genome* **26**, 567-573.

[57] Sugimoto, M. 2014 Developmental genetics of the mouse *t* complex. *Genes & Genetic Systems* **89**, 109-120.

[58] EMBL-EBI. 2019 Mouse assembly and gene annotation https://www.ensembl.org/Mus_musculus/Info/Annotation. (

[59] Meiklejohn, C.D., Coolon, J.D., Hartl, D.L. & Wittkopp, P.J. 2014 The roles of *cis*- and *trans*-regulation in the evolution of regulatory incompatibilities and sexually dimorphic gene expression. *Genome Research* **24**, 84-95.

[60] Metzger, B.P.H., Duveau, F., Yuan, D.C., Tryban, S., Yang, B. & Wittkopp, P.J. 2016 Contrasting frequencies and effects of *cis*- and *trans*-regulatory mutations affecting gene expression. *Molecular Biology and Evolution* **33**, 1131-1146.

[61] Rhoné, B., Mariac, C., Couderc, M., Berthouly-Salazar, C., Ousseini, I.S. & Vigouroux, Y. 2017 No excess of *cis*-regulatory variation associated with intraspecific selection in wild pearl millet (*Cenchrus americanus*). *Genome Biology and Evolution* **9**, 388-397.

[62] Signor, S.A. & Nuzhdin, S.V. 2018 The evolution of gene expression in *cis* and *trans*. *Trends in Genetics* **34**, 532-544.

[63] Morita, T., Kubota, H., Murata, K., Nozaki, M., Delarbre, C., Willison, K., Satta, Y., Sakaizumi, M., Takahata, N. & Gachelin, G. 1992 Evolution of the mouse *t* haplotype: recent and worldwide introgression to *Mus musculus*. *Proceedings of the National Academy of Sciences of the United States of America* **89**, 6851-6855.

[64] Hammer, M.F. & Silver, L.M. 1993 Phylogenetic analysis of the alpha-globin pseudogene-4 *(Hba-ps4)* locus in the house mouse-species complex reveals a stepwise evolution of *t*-haplotypes. *Molecular Biology and Evolution* **10**, 971-1001.

[65] Silver, L.M. 1982 Genomic analysis of the *H-2*-complex region associated with mouse *t*-haplotypes. *Cell* **29**, 961-968.

[66] Wang, Y., Liu, J., Huang, B.O., Xu, Y.-M., Li, J., Huang, L.-F., Lin, J., Zhang, J., Min, Q.-H., Yang, W.-M., et al. 2015 Mechanism of alternative splicing and its regulation. *Biomed Rep* **3**, 152-158.

[67] Olds-Clarke, P. & Peitz, B. 1985 Fertility of sperm from *t*/+ mice: evidence that + -bearing sperm are dysfunctional. *Genetic Research* **47**, 49-52.

[68] Brawand, D., Soumillon, M., Necsulea, A., Julien, P., Csárdi, G., Harrigan, P., Weier, M., Liechti, A., Aximu-Petri, A., Kircher, M., et al. 2011 The evolution of gene expression levels in mammalian organs. *Nature* **478**, 343.

[69] Lenington, S. & Egid, K. 1985 Female discrimination of male odors correlated with male genotype at the *T* locus: a response to *T*-locus or *H-2*-locus variability? *Behavior Genetics* **15**, 53-67.

[70] Williams, J.R. & Lenington, S. 1993 Factors modulating preferences of female house mice for males differing in *t*-complex genotype: Role of *t*-complex genotype, genetic background, and estrous condition of females. *Behavior Genetics* **23**, 51-58.

[71] Yang, X., Schadt, E.E., Wang, S., Wang, H., Arnold, A.P., Ingram-Drake, L., Drake, T.A. & Lusis, A.J. 2006 Tissue-specific expression and regulation of sexually dimorphic genes in mice. *Genome Research* **16**, 995-1004.

[72] Jacobs, F.M.J., Greenberg, D., Nguyen, N., Haeussler, M., Ewing, A.D., Katzman, S., Paten, B., Salama, S.R. & Haussler, D. 2014 An evolutionary arms race between KRAB zinc-finger genes *ZNF91/93* and SVA/L1 retrotransposons. *Nature* **516**, 242.

[73] Braidotti, G. & Barlow, D.P. 1997 Identification of a male meiosis-specific gene, *Tcte2*, which is differentially spliced in species that form sterile hybrids with laboratory mice and deleted in *t* chromosomes showing meiotic drive. *Developmental Biology* **186**, 85-99.

[74] Huw, L.Y., Goldsborough, A.S., Willison, K. & Artzt, K. 1995 TCTEX2-a sperm tail surface protein mapping to the *t*-complex. *Developmental Biology* **170**, 183-194.

[75] Silver, L.M., Kleene, K.C., Distel, R.J. & Hecht, N.B. 1987 Synthesis of mouse *t* complex proteins during haploid stages of spermatogenesis. *Developmental Biology* **119**, 605-608.

[76] Schimenti, J., Cebra-Thomas, J.A., Decker, C.L., Islam, S.D., Pilder, S.H. & Silver, L.M. 1988 A candidate gene family for the mouse *t complex responder (Tcr)* locus responsible for haploid effects on sperm function. *Cell* **55**, 71-78.

[77] Cebrathomas, J.A., Decker, C.L., Snyder, L.C., Pilder, S.H. & Silver, L.M. 1991 Allele-specific and haploid-specific product generated by alternative splicing from a mouse *t*-complex-responder locus candidate. *Nature* **349**, 239-241.

[78] Fraser, L.R., Hosseini, R., Hanyalogou, A., Talmor, A. & Dudley, R.K. 1997 TCP-11, the product of a mouse *t*-complex gene, plays a role in stimulation of capacitation and inhibition of the spontaneous acrosome reaction. *Molecular Reproduction and Development* **48**, 375-382.

[79] Ha, H., Howard, C.A., Yeom, Y.I., Abe, K., Uehara, H., Artzt, K. & Bennett, D. 1991 Several testis-expressed genes in the mouse *t*-complex have expression differences between wild-type and *t*-mutant mice. *Developmental Genetics* **12**, 318-332.

[80] Sugimoto, M., Kondo, M., Hirose, M., Suzuki, M., Mekada, K., Abe, T., Kiyonari, H., Ogura, A., Takagi, N., Artzt, K., et al. 2012 Molecular identification of t^{w5}: *Vps52* promotes pluripotential cell differentiation through cell–cell interactions. *Cell Reports* **2**, 1363-1374.

[81] Han, Y.B., Feng, H.L., Cheung, C.K., Lam, P.M., Wang, C.C. & Haines, C.J. 2007 Expression of a novel *T-complex testis expressed 5 (Tctex5)* in mouse testis, epididymis, and spermatozoa. *Molecular Reproduction and Development* **74**, 1132-1140.

[82] Schimenti, J.C., Reynolds, J.L. & Planchart, A. 2005 Mutations in *Serac1* or *Synj2* cause proximal *t* haplotype-mediated male mouse sterility but not transmission ratio distortion. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 3342-3347.

[83] Chao, H.H.J., Mentzer, S.E., Schimenti, J.C. & You, Y. 2003 Overlapping deletions define novel embryonic lethal loci in the mouse *t* complex. *Genesis* **35**, 133-142.

[84] Duguay, D., Bélanger-Nelson, E., Mongrain, V., Beben, A., Khatchadourian, A. & Cermakian, N. 2011 Dynein light chain Tctex-type 1 modulates orexin signaling through its interaction with orexin 1 receptor. *PLoS One* **6**, e26430-e26430.

[85] Fossella, J., Samant, S.A., Silver, L.M., King, S.M., Vaughan, K.T., Olds-Clarke, P., Johnson, K.A., Mikami, A., Vallee, R.B. & Pilder, S.H. 2000 An axonemal dynein at the *Hybrid Sterility 6* locus: implications for *t* haplotype-specific male sterility and the evolution of species barriers. *Mammalian Genome* **11**, 8-15.

[86] Samant, S.A., Ogunkua, O.O., Hui, L., Lu, J., Han, Y., Orth, J.M. & Pilder, S.H. 2005 The mouse *t* complex distorter/sterility candidate, *Dnahc8*, expresses a γ-type axonemal dynein heavy chain isoform confined to the principal piece of the sperm tail. *Developmental Biology* **285**, 57-69.

[87] Zwart, R., Verhaagh, S., de Jong, J., Lyon, M. & Barlow, D.P. 2001 Genetic analysis of the organic cation transporter genes *Orct2/Slc22a2* and *Orct3/Slc22a3* reduces the critical region for the *t* haplotype mutant t^{w73} to 200 kb. *Mammalian Genome* **12**, 734-740.

[88] Zwart, R., Sleutels, F., Wutz, A., Schinkel, A.H. & Barlow, D.P. 2001 Bidirectional action of the *Igf2r* imprint control element on upstream and downstream imprinted genes. *Genes & Development* **15**, 2361-2366.

[89] Fraser, L.R. & Dudley, K. 1999 New insights into the *t*-complex and control of sperm function. *Bioessays* **21**, 304-312.

[90] Danshina, P.V., Geyer, C.B., Dai, Q., Goulding, E.H., Willis, W.D., Kitto, G.B., McCarrey, J.R., Eddy, E.M. & O'Brien, D.A. 2010 Phosphoglycerate kinase 2 (PGK2) is essential for sperm function and male fertility in mice. *Biology of Reproduction* **82**, 136-145.

[91] Bullard, D.C. & Schimenti, J.C. 1991 Molecular structure of *Tcp-10* genes from the *t* complex responder locus. *Mammalian Genome* **1**, 228-234.

[92] Ashworth, A. 1993 Two acetyl-CoA acetyltransferase genes located in the *t*-complex region of mouse chromosome 17 partially overlap the *Tcp-1* and *Tcp-1 x* genes. *Genomics* **18**, 195-198.

[93] Kono, H., Tamura, M., Osada, N., Suzuki, H., Abe, K., Moriwaki, K., Ohta, K. & Shiroishi, T. 2014 *Prdm9* polymorphism unveils mouse evolutionary tracks. *DNA Research* **21**, 315-326.

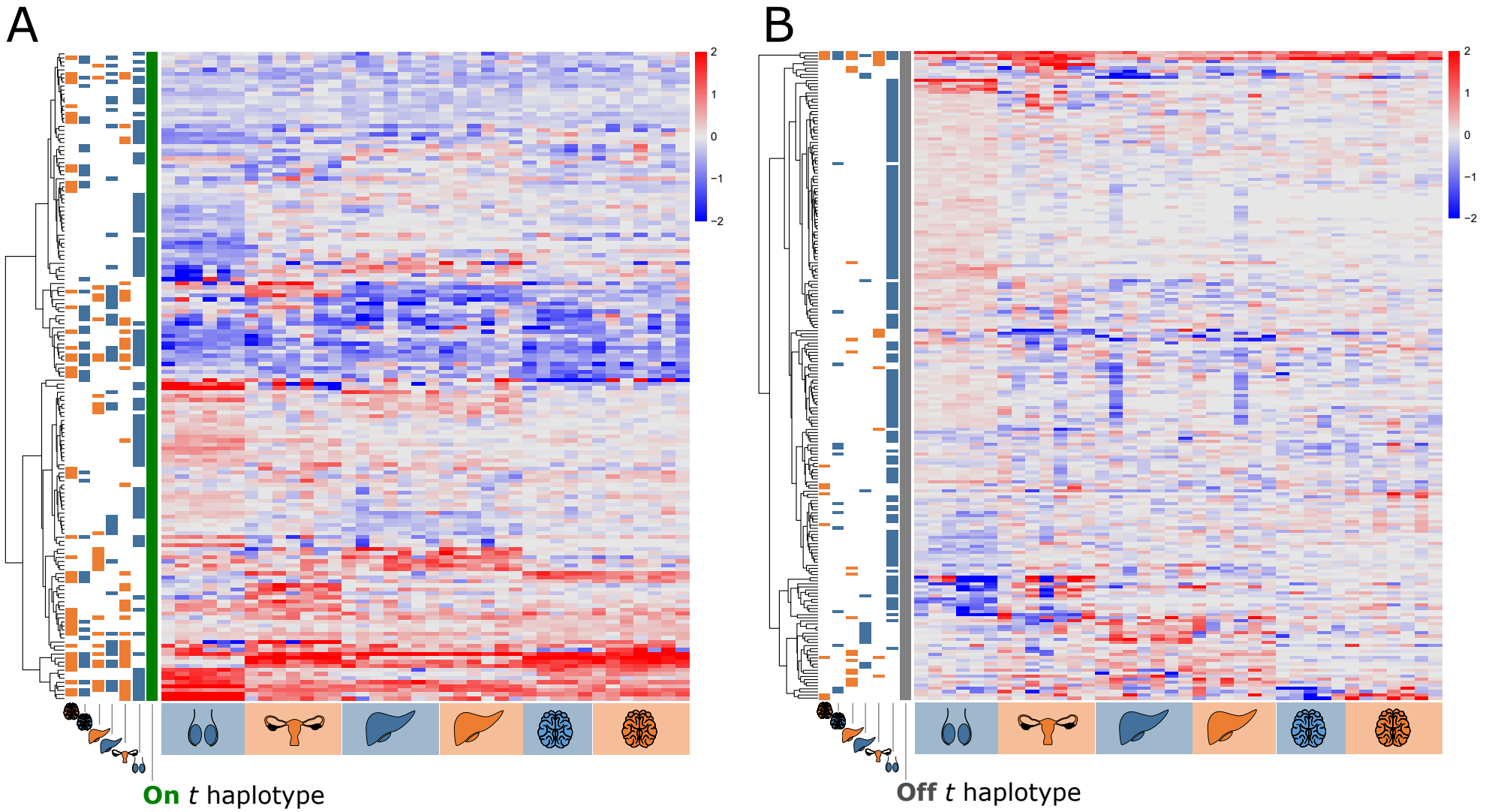
Figure legends

Figure 1: Heatmaps indicating expression differences between +/*t* and +/+ males and females for all genes DE in at least one tissue, with red indicating increased expression and blue decreased expression in +/*t*. Each column indicates the comparison between a +/*t* mouse and its +/+ sibling, for gonads, liver and brain for males (blue) and females (orange). On the left of each panel, blue and orange bars indicate in which comparison a specific gene showed a significant gene-level expression difference: (*a*) with the green bar shows the genes located on the *t* haplotype, while (*b*) with the grey bar shows genes located in other genomic regions.

Figure 2: The number of significantly DE genes per tissue and sex (with blue representing male tissue, and orange female tissue), grouped by up- or downregulation (indicated by up or down facing arrows) or DEU. Darker bars represent genes in the *t* haplotype region; lighter bars represent genes elsewhere in the genome.

Figure 3: Shared expression patterns according to whether genes are located within the *t* haplotype or in the rest of the genome, for (*a*) upregulated genes, (*b*) downregulated genes or (*c*) genes with differential exon usage. The *y*-axis indicates the number of genes of each category, with the darker part of each bar representing genes in the *t* haplotype region, and the lighter part representing genes elsewhere in the genome. The *x*-axis indicates categories of shared expression. Single dots indicate expression differences within a category of sex and tissue, and vertical lines connect dots whenever

the same gene is DE in more than one category. Blue and orange organs represent male and female tissues, respectively.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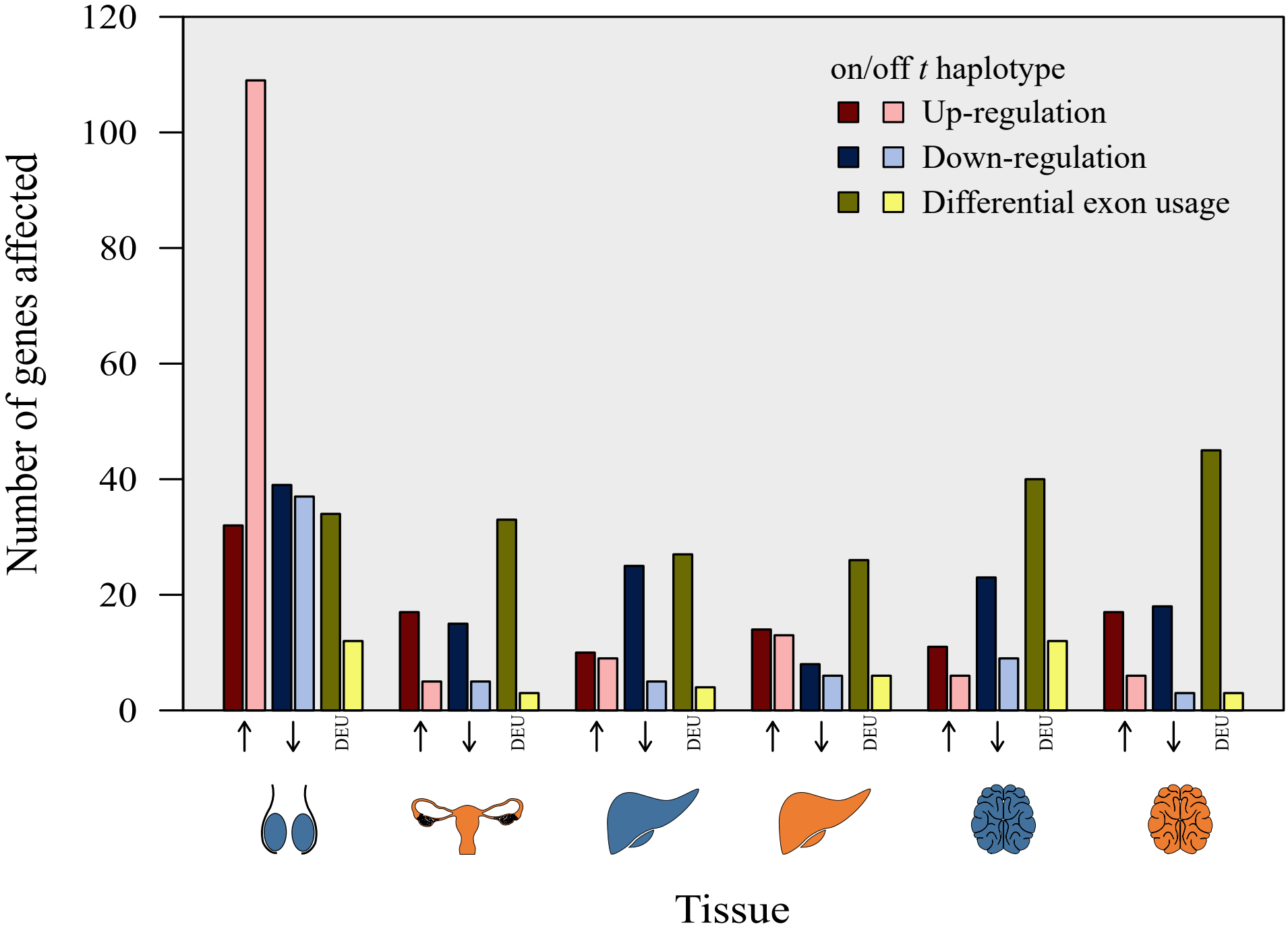
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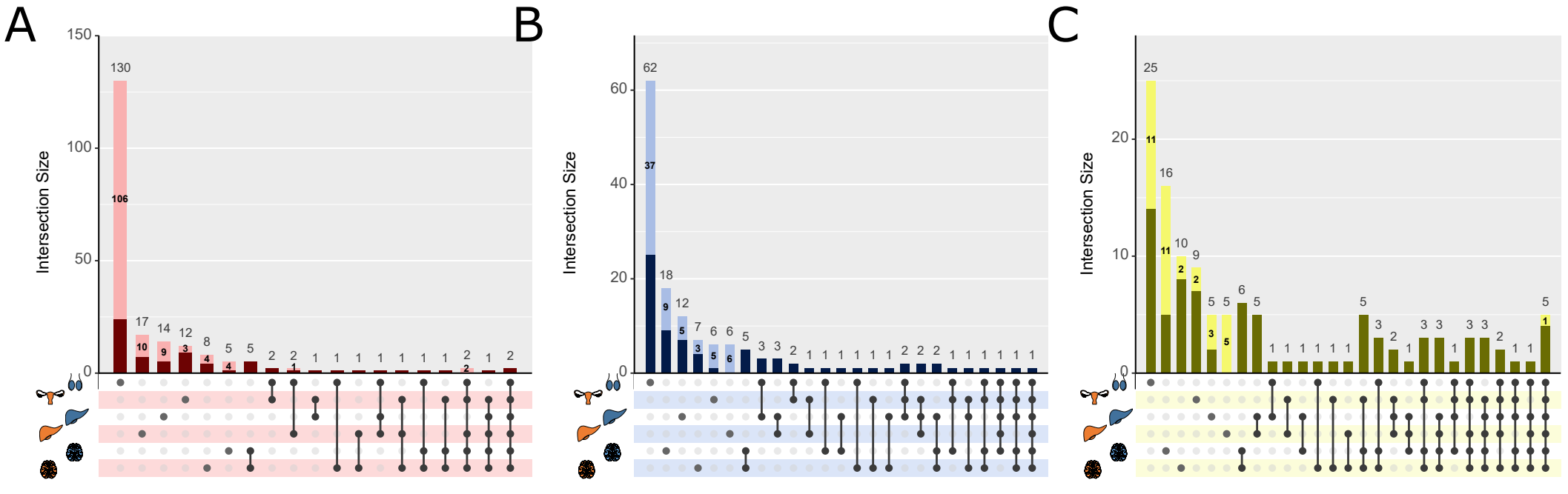
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Supplementary Methods, Figures and Table

Housing conditions

Mice were housed in Makrolon type III cages (425 mm × 266 mm × 155 mm) with animal bedding (Lignocel Hygienic Animal Bedding, JRS), cardboard rolls and pieces, and paper towels as nesting material, and provided with Kliba mouse chow (laboratory animal diet for mice and rats, no. 3430).

At 5 weeks of age, just prior to the typical onset of male-male aggression and sexual maturity, brother pairs were isolated into single cages (Macrolon type II) and placed in a cage rack so that one brother was just above the other (in half of the pairs, the +/*t* male was above the +/+, otherwise reversed), with no other immediate neighbour. Sister pairs (differing in genotype) were put into two single cages connected by a tube, so that per capita space available to males and females was the same. Males and females were all uninjured and healthy. Each cage had bedding, two toilet paper rolls, one piece of cardboard and one piece of paper towel, and food and water were provided *ad libitum*.

Five days per week each cage of paired mice was given mixed soiled bedding to provide standardized odorant input to stimulate reproductive development. 50 ml of soiled bedding was taken from three cages each of group-housed adult females and adult males (from mice outside the experiment), with both +/+ and +/*t* genotypes represented. The 300 ml of soiled bedding was mixed and 10 ml put into each cage containing focal individuals.

RNAseq data processing additional information

The STAR alignment options used were "--outFilterType BySJout --outFilterMatchNmin 30 --outFilterMismatchNmax 10 --outFilterMismatchNoverLmax 0.05 --alignSJDBoverhangMin 1 --alignSJoverhangMin 8 --alignIntronMax 100000 --alignMatesGapMax 100000 --outFilterMultimapNmax 50 --twopassMode Basic".

The options for featureCounts were the following: min mapping quality of 10; min feature overlap of 10bp; count multi-mapping reads yes; count only primary alignments yes; count reads if they overlap multiple genes yes.

Supplementary Figures

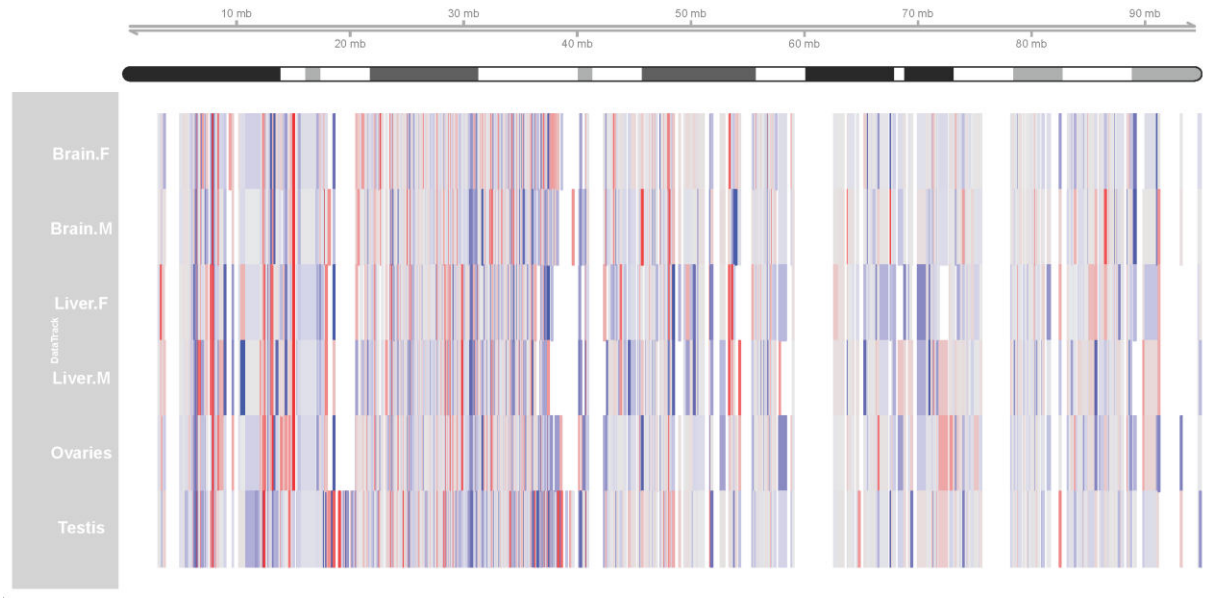

Figure S1. Plot along chromosome 17 of differential transcript abundance between +/*t* and +/+ for each gene and tissue (male and female brain, male and female liver and ovaries and testis), with red indicating increased expression and blue decreased expression in +/*t*. Genes that are expressed without significant change are in dark grey, while genes with below analysis threshold reads are depicted in light gray. White indicates regions with no mapped reads. For this study we defined 3-42 Mb as the *t* haplotype region.

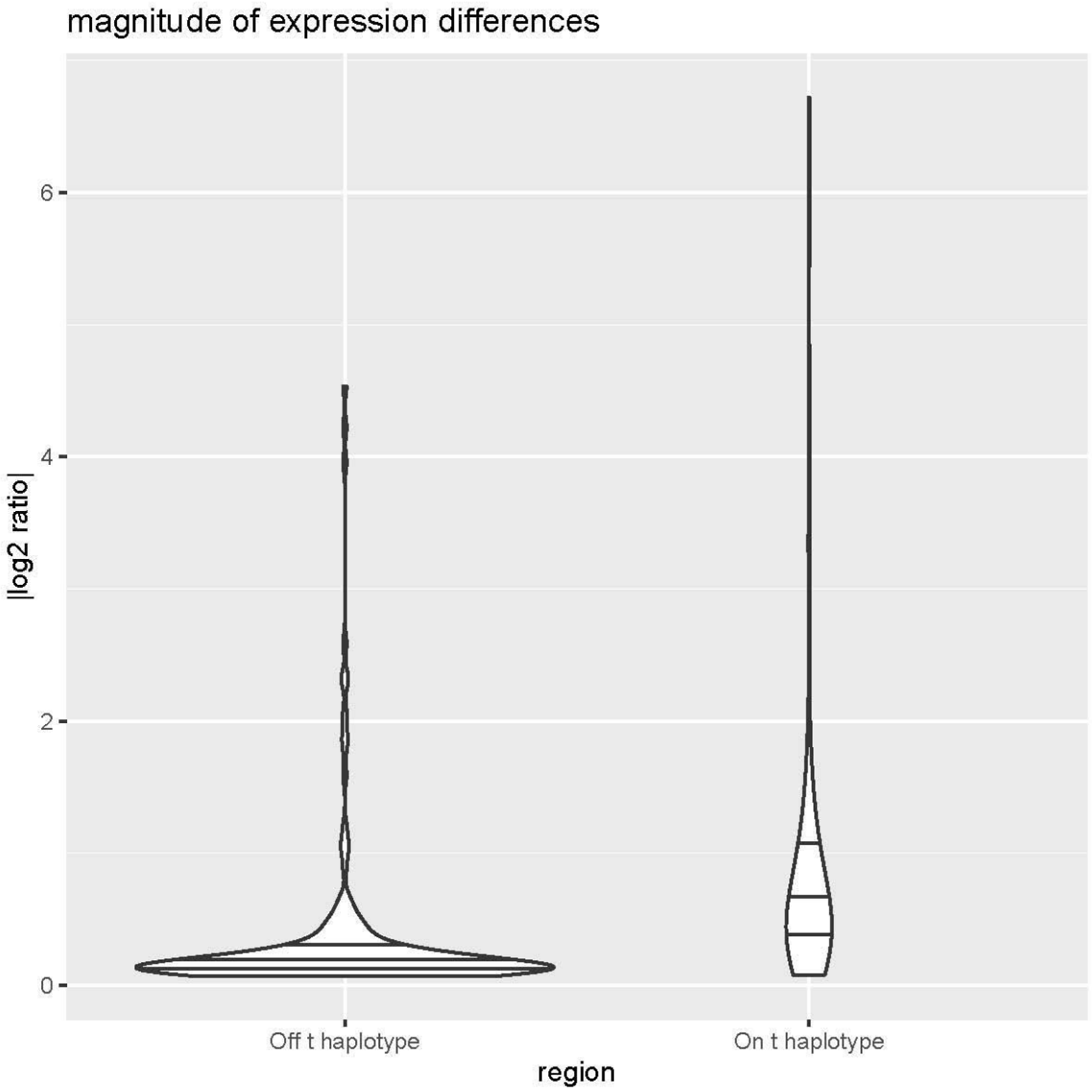

Figure S2. Violin plot of expression differences between +/t and +/+ males, in absolute values of log2 ratios, from testis for genes within the t haplotype (on t haplotype) and in the rest of the genome (off t haplotype).

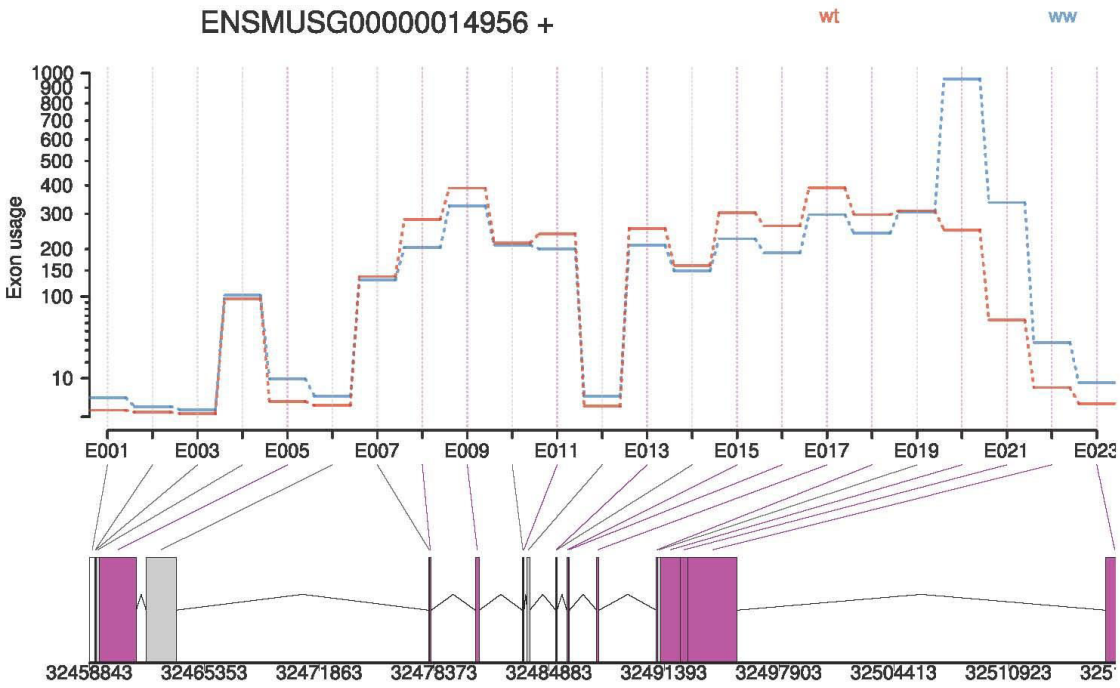

Figure S3: An example of differential exon usage in the chr 5 gene Ppp1cb from testis. The x-axis indicates position on the chromosome and the y-axis indicates the number of reads (shown in red for +/t and blue for +/+). Coloured vertical lines indicate exons.

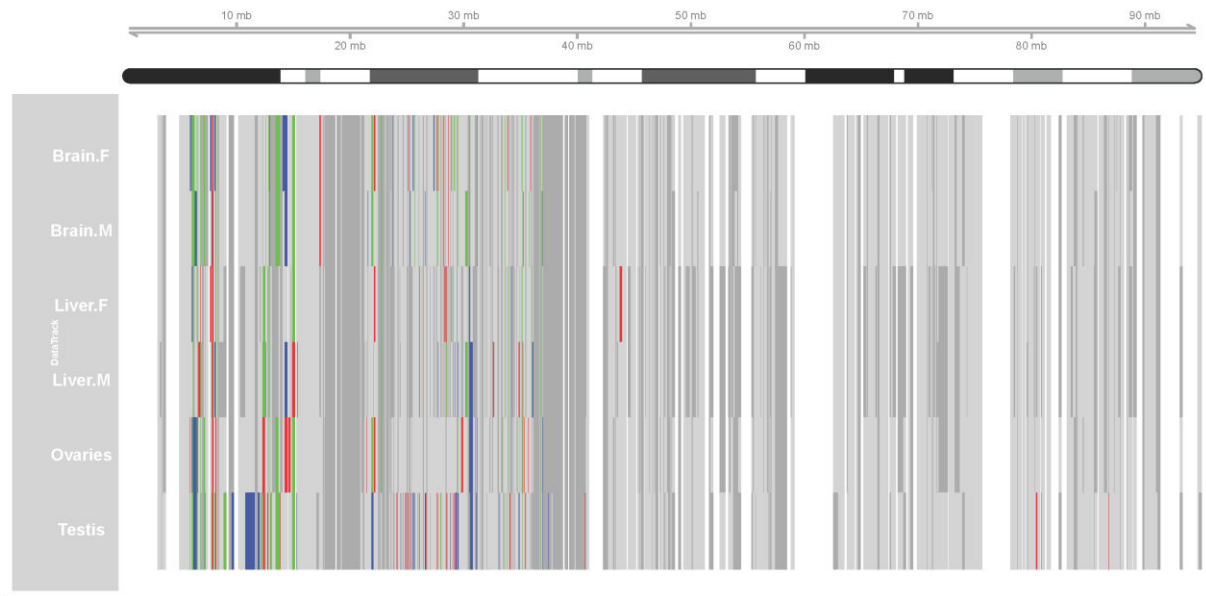

Figure S4. Plot along chromosome 17 of differential expression between +/t and +/+ for each gene and tissue (male and female brain, male and female liver and ovaries and testis). Red denotes up-regulation, blue down-regulation and green differential exon usage. Genes that are expressed without significant change are in dark grey, while genes with below analysis threshold reads are depicted in light gray. White indicates regions with no mapped reads. Above is a cytogenetic map of chromosome 17 showing cytobands. For this study we defined 3-42 Mb as the t haplotype region.

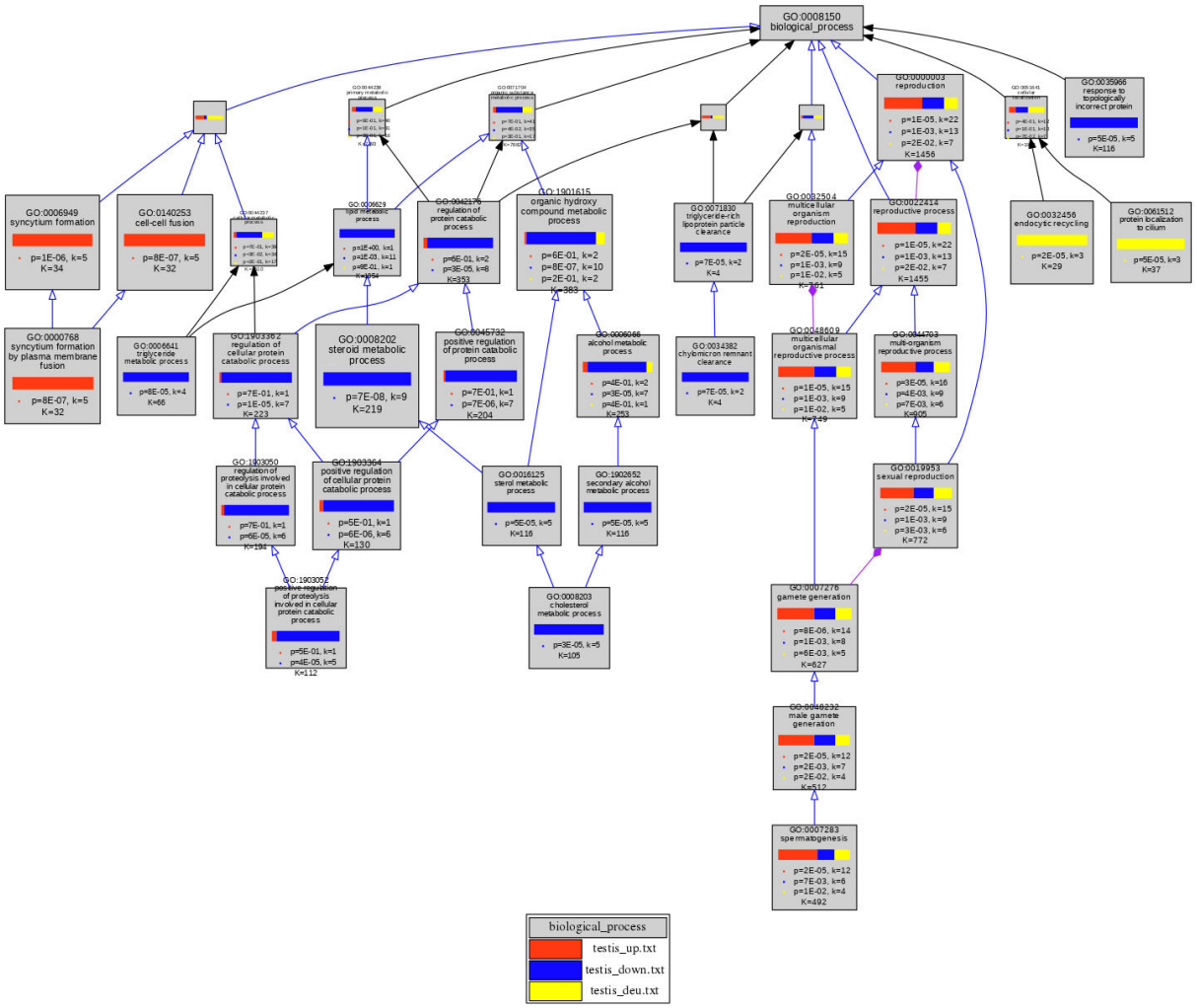

Figure S5. Top 25 enriched gene ontology terms for biological processes, with ancestral nodes, for testis genes differing in expression between +/t and +/+ brothers. Red indicates the contribution of up-regulated genes (testis_up), blue down-regulated genes (testis_down), and yellow genes with differential exon usage (testis_deu). Image generated by VLAD (Richardson and Bult 2015b). Black arrows indicate multiple connections between B and A, blue arrows that B is part of A. p is the p-value for enrichment given k, the number of differentially expressed genes annotated to the term, and K, the number of genes annotated to the term .

Table S1. Gene function analysis of genes differentially expressed between +/t and +/+ siblings, according to gene location.

Tissue	Sex	Within *t*	Rest of genome
Gonad	M	Cytoplasmic dynein complex Sperm flagellum	Spermatogenesis, Sperm flagellum Syncytium formation GTP biosynthetic process Alcohol metabolic process Steroid metabolic process
	F	Dynein complex Sperm flagellum Membrane bound organelle MHC protein complex	None
Liver	M	None	None
	F	None	Saturated and unsaturated monocarboxylic acid metabolic process Myristol-coA and Palmitoyl-coA hydrolase activity,
Brain	M	Cytoplasmic dynein complex Intracellular organelle	None
	F	Membrane bound organelle	None