**Supplementary Information: Analysis of *Mater* duplication**

*Mater* is a maternal effect gene that is essential for early embryonic development in mice [25]. A detailed analysis of *Mater* also has clinical relevance, as it is the predominant autoantigen in mouse autoimmune oophoritis, a model for autoimmune premature ovarian failure in women [24]. *Mater* is expressed exclusively in oocytes and accumulates in the egg during oogenesis. Eggs from mice lacking *Mater* are fertilized but are unable to progress past the 2-cell stage [25].

A 42 kb segmental duplication involving two duplicons (DUP1 – where *Mater* is located; and DUP2 – where a novel *Mater2* is located) reside about 5 Mb apart and are situated in an inverted orientation. Furthermore, an intron-less *Mater* pseudogene (*MaterP*), sharing 87 percent DNA sequence identity to *Mater*, is located 10 Mb proximal to *Mater*. DUP1 and DUP2 are on average 91.1 percent identical over the entire 57 kb genomic region with a 96.6 percent average in the exonic regions. DUP2 contains similarity to all *Mater* exons except for exon 1 and a deletion corresponding to *Mater* exon 8, which encodes the leucine-rich domain (Fig. A). One full length cDNA sequence (AK016782) and three separate EST sequences (BB645510, BB624558, and AV367637) align unambiguously to DUP2 supporting a putative *Mater2* transcript sequence that is 1878 bp in length, with an open reading frame of 269 amino acids. This open reading frame shares 95 percent amino acid identity with the C-terminal end of *Mater*. These *Mater2* transcripts are derived from testis, adipose, and lung tissue libraries suggesting it may have a different expression pattern than the oocyte-specific *Mater* [40]. It is still unclear whether *Mater2* represents a pseudogene or a functional gene that has evolved a different expression pattern and function.

The characterization of the regulatory elements surrounding duplicated gene loci are important for sub-functionalization hypotheses, as it is believed that mutations in these regions may alter expression patterns that can allow for gene specialization (reviewed by [3]). While examining conserved non-coding sequences (CNS) between *Mater* and the related loci, we found two EST sequences (BB608289 and BB666495) suggesting the presence of an additional exon 18 kb upstream of previously labeled exon 1 [41]. This new exon, labeled as exon 1 in Fig. A, is found conserved in human but not in the *Mater2* locus. While no expression data supports the existence of this exon 1 in human, its conservation further supports *MATER* and *Mater*'s proposed orthologous relationship [5]. Using rVISTA [42] we looked for conserved regulatory elements and identified a conserved CCAAT motif 59 bp upstream of exon 1. This conserved non-coding segment (CNS) may contain the proximal promoter responsible for the oocyte specific expression observed in both species. We utilized Laj (Local Alignments with Java; [43]) to interactively display the annotation, genomic alignments and coordinates, and can be found at http://marcie.ceh.uvic.ca/~mdwilson/mater.html. Similar analyses and tools will be useful for preliminary analyses of other duplicated loci in the mouse genome.

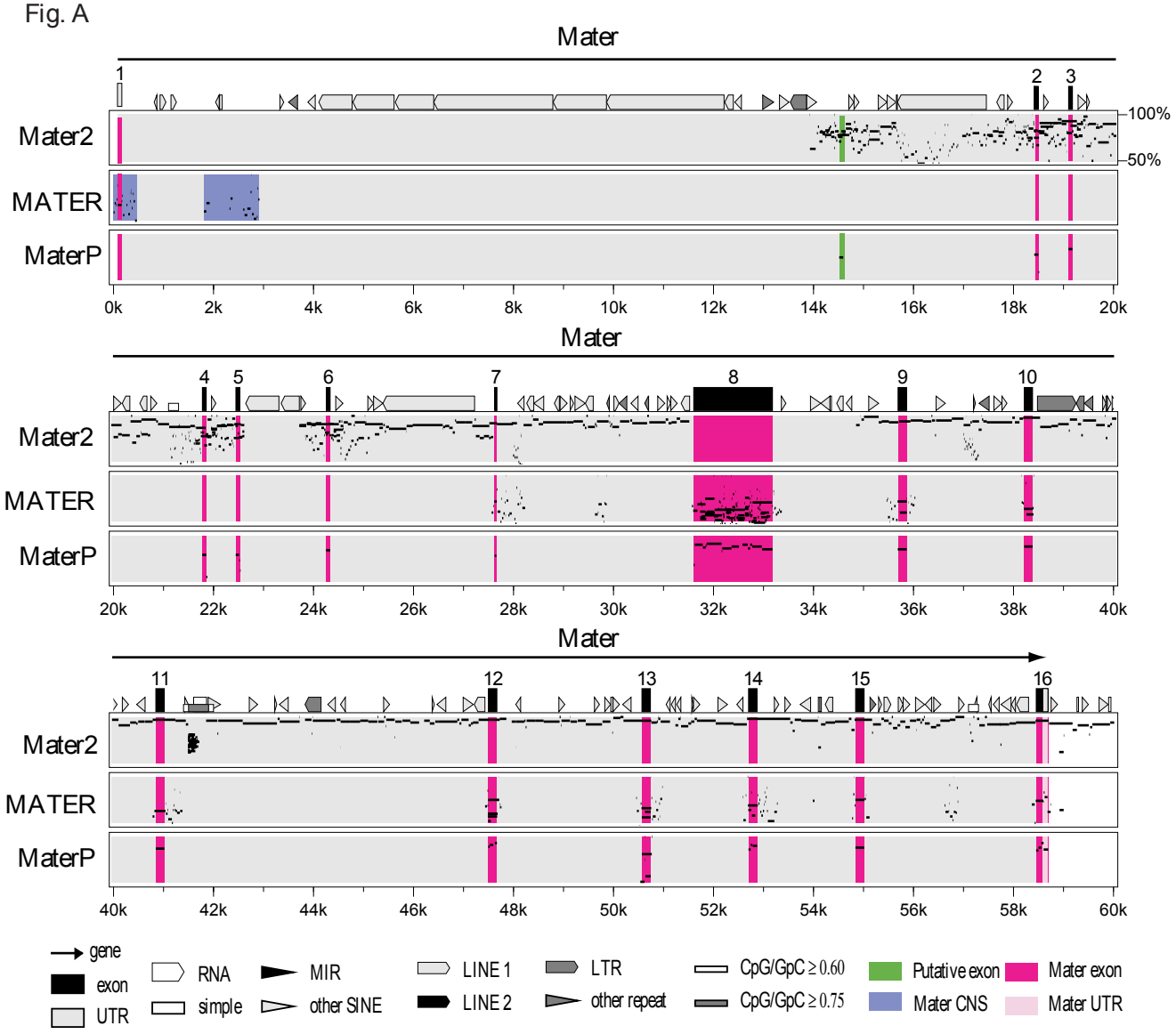
**Methods:**

**Comparative sequence analysis of *Mater*.** Repeat elements were characterized using RepeatMasker (Smit and Green unpublished). Comparative sequence alignments were generated using PipMaker [44] and VISTA [45]. Conserved regulatory elements were examined using regulatory VISTA [42]. DNA and protein sequences were compared using BLAST-2-Seq [46].

**Figure Legend:**

**Fig. A.** Multiple percent identity plot of *Mater* versus: *Mater2*, *MATER* (human), and *MaterP*. 60 kb of sequence from the mouse *Mater* genomic locus (found at chromosome 7, position 15617364-15677363 on the February 2002 mouse genome assembly) is compared to 500 kb surrounding mouse *Mater2*; 509 kb surrounding human *MATER*; and 42 kb surrounding the intron-less pseudogene *MaterP*. The percent identity, which ranges from 50 percent to 100 percent is indicated on the y-axis and is drawn as a series of horizontal lines indicating similarity between the 60 kb *Mater* genomic region and each target sequence. Discontinuity in the horizontal lines and dots along the x-axis corresponds to gaps in the alignment. Stacking of these lines indicates more than one section of the target sequence aligns to the same position on *Mater*. Purple shading indicates regions corresponds to *Mater* exons, blue shading corresponds to conserved non-coding sequence (CNS) between *Mater* and *MATER*, and green shading corresponds to a putative exon containing splice acceptor/donor sites that are highly similar to *Mater* exons 2 through 6 (all of which appear to have arisen through duplication events).

Fig. A


Mater
1
2
3
100%
50%
Mater2
MATER
MaterP
0k
2k
4k
6k
8k
10k
12k
14k
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16
Mater2
MATER
MaterP
40k
42k
44k
46k
48k
50k
52k
54k
56k
58k
60k
gene
exon
UTR
RNA
simple
MIR
other SINE
LINE 1
LINE 2
LTR
other repeat
CpG/GpC ≥ 0.60
CpG/GpC ≥ 0.75
Putative exon
Mater CNS
Mater exon
Mater UTR