Supplemental Figures

Figure S1. Reverse transcription polymerase chain reaction using wild-type blastocysts recovered from the uterus *in vivo*.

Abundant expression of the *Fthl17* gene in female embryos was confirmed using wild-type blastocysts recovered from the uterus (see the Materials and Methods for details).

Figure S2. Alignment of the *Fthl17* family of genes and position of specific PCR primers for each gene.

Dots indicate identical nucleotides among the seven sequences. The sequence of each primer is listed in Supplemental Table S1. The RT–PCR primers for *Fthl17-L2* and *Fthl17-L4* could not be designed to distinguish between these genes.

Figure S3. Southern genomic hybridization analysis of the Fthl17 family of genes.

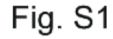
Southern hybridization was carried out using the full length Fthl17 sequence as a probe.

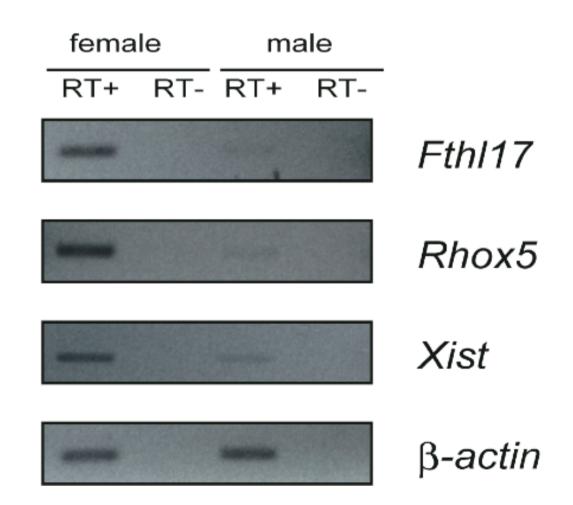
Figure S4. Analysis of X inactivation status at the blastocyst stage.

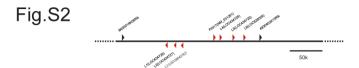
Comparison of our microarray data (fold change in female/male gene expression) with *in situ* hybridization data (relative Xp expression level at the blastocyst stage) reported by Patrat et al. (Proc Natl Acad Sci U S A 106: 5198–5203). The gray box indicates our group 1 genes (see Results; fold change -1.2 to +1.2) considered to be inactivated at this stage. The results from the *in situ* hybridization experiments were consistent with our microarray data. Although some probes were redundant, the results from all probes are listed. The relative positions of X-linked genes are indicated.

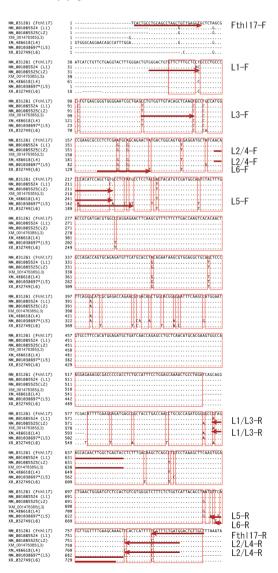
Figure S5. Methylation analysis of the H19 locus at the blastocyst stage.

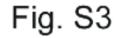
The differentially methylated region of the H19 locus was also analyzed by bisulfite sequencing as a control.

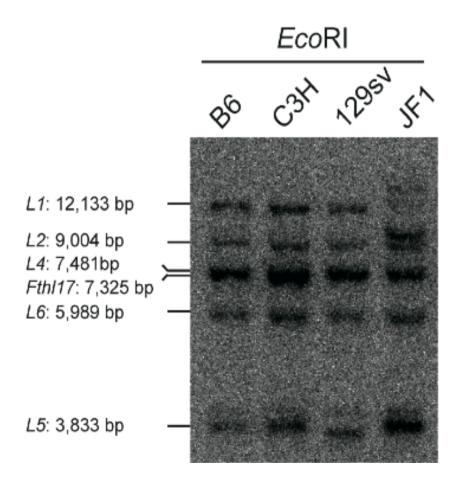












N.D.L3: 529 bp

Fig. S4

| | no | gene name | primary sequence name | fold change | relative Xp expression (%) |
|--------|-----|-----------|-----------------------|-------------|----------------------------|
| | I 1 | Atp6ap2 | TF11785 | 1.79 | 70-99 |
| | 2 | Utx | TB5892 | 1.27 | 50-69 |
| | 2 | Utx | 1D121-5 A02 | 1.25 | 50-69 |
| | 3 | SIc25a5 | TB759 | 1.40 | 14-29 |
| | 4 | Lamp2 | TB3265 | 1.16 | 14-29 |
| | 1 5 | Fmr1 | TB2174 | 1.29 | 14-29 |
| | 5 | Fmr1 | 1L014 E01 | 1.22 | 14-29 |
| | 6 | Mecp2 | TB3649 | 1.10 | 50-69 |
| | 6 | Mecp2 | TF32714 | 1.06 | 50-69 |
| | 7 | G6pdx | TB2240 | 1.12 | 30-49 |
| | 8 | Kif4 | TB3151 | -1.02 | 14-29 |
| | 9 | Rps4x | TB4924 | 1.56 | 14-29 |
| | 10 | Chic1 | Y11896.1 | 1.02 | <15 |
| | 11 | Rnf12 | TB6737 | 1.12 | <15 |
| | 11 | Rnf12 | 1D118-5 F05 | 1.05 | <15 |
| | 11 | Rnf12 | TB4862 | 1.02 | <15 |
| Xist | 12 | Atrx | TB6003 | 1.11 | <15 |
| | 13 | Atp7a | TB919 | 1.20 | <15 |
| | 13 | Atp7a | 1K012 B12 | 1.03 | <15 |
| | 13 | Atp7a | TF23690 | -1.05 | <15 |
| | 13 | Atp7a | TF27254 | -1.09 | <15 |
| | 14 | Gla | TB650 | -1.20 | 30-49 |
| | 15 | Fgd1 | TB2102 | 1.04 | <15 |
| | 16 | Huwe1 | TF31367 | 1.30 | 100 |
| | | Huwe1 | TB7858 | 1.26 | 100 |
| | 17 | Jarid1c | TB5217 | 1.03 | <15 |
| \cup | | | | | |

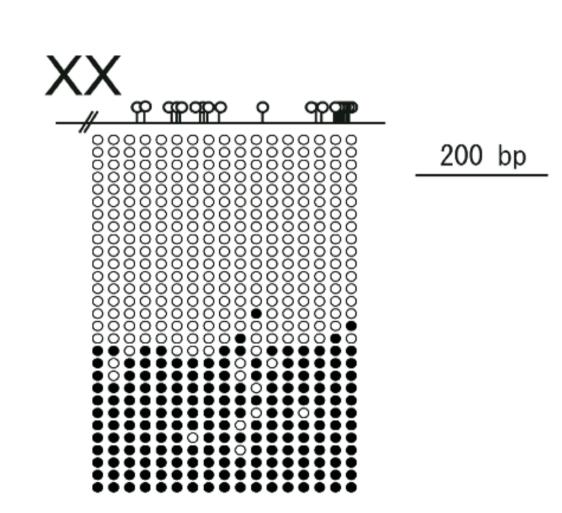


Fig. S5

Table S1 Specific PCR primers used for amplifying the *Fthl17* gene family members.

| Gene | | | | | |
|-----------|-----------------------------|--------------|---------------------|------------------------|---------------------------|
| name | Official symbol | Protein | mRNA | PCR primer: forward | PCR primer: reverse |
| Fthl17 | Fthl17 | NP_112551 | NM_031261 | cactgcctgcagcctagct | gcaacagtccatcagaaacca |
| Fthl17-L1 | EG434726 | NP_001078993 | NM_001085524 | gggactgtgttctttgctca | gtactcagccaagttgtgctctat |
| Fthl17-L2 | EG434727/OTTMUSG00000016933 | NP_001078994 | NM_001085525 | aacacccacatccagctgc | tcagaaaccgaaaatgagtgg |
| Fthl17-L3 | OTTMUSG0000016694 | XP_001476435 | XM_001476385 | cctgtggttgtacagctcaagt | gtactcagccaagttgtgctctat |
| Fthl17-L4 | EG434728/OTTMUSG00000016862 | XP_486618 | XM_486618.4 | aacacccacatccagctgc | tcagaaaccgaaaatgagtgg |
| Fthl17-L5 | EG434729 | NP_001033786 | NM_001038697.1** | acatccagctgtacctccaat | actttgcttcaaaaccaacataaat |
| Fthl17-L6 | EG628008 | pseudo | XR_031510/XR_032749 | tctcgagtgcggcagaactt | ttgcttcaaaaccaacatgc |

**Differences found between NM_001038697 and the mouse C57BL/6J genome (NT_039700) are described in 'misc_difference features of BC104362'.

Table S2 PCR primers used for searching DMR of Fthl17 loci

| primer name | sequence | mer | amplified fragment size(bp) | amplified loci |
|--|---|-----|-----------------------------|-------------------|
| Fthl17 CpG1-F | actcaactttctccaaacttctaaacc | | 776 | CpG1 |
| Fthl17 CpG1-R | ıl17 CpG1-R ggtagtttttggaatagttgta | | | |
| Fthl17 CpG2-9-F | 7 CpG2-9-F gaggtattttgggattgtgggat | | 314 | CpG2,3,4,6,7,8,9* |
| Fthl17 CpG2-9-R aaacatctctacactaatctaacaattatataacttaatc | | 40 | | |
| Fthl17 CpG5-F tgaaggtgaaggtttaaaataggatt | | 26 | 635 | CpG5 |
| Fthl17 CpG5-R | ataccaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa | 27 | | |

*This primer set amplified all seven *Fthl17* family loci (Fthl17, L1, L2, L3, L4, L5, L6), and each locus was distinguished with its SNPs inside *Fthl17* genes.