

Supplementary information for

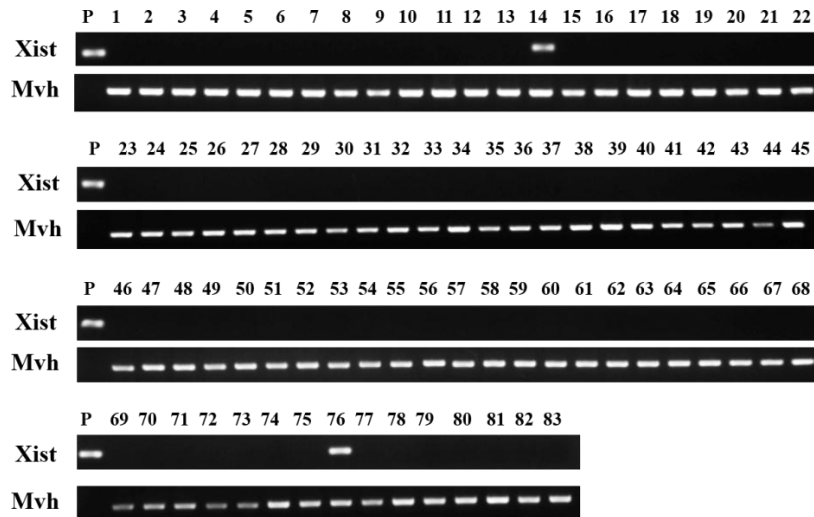
Dosage compensation in the process of inactivation/reactivation during both germ cell development and early embryogenesis in mouse

Xiaoyong Li¹¶, Zhiqiang Hu^{2,3}¶, Xuelin Yu^{2,3}¶, Chen Zhang¹, Binbin Ma¹, Lin He¹, Chaochun Wei^{2,3*} and Ji Wu^{1,4*}

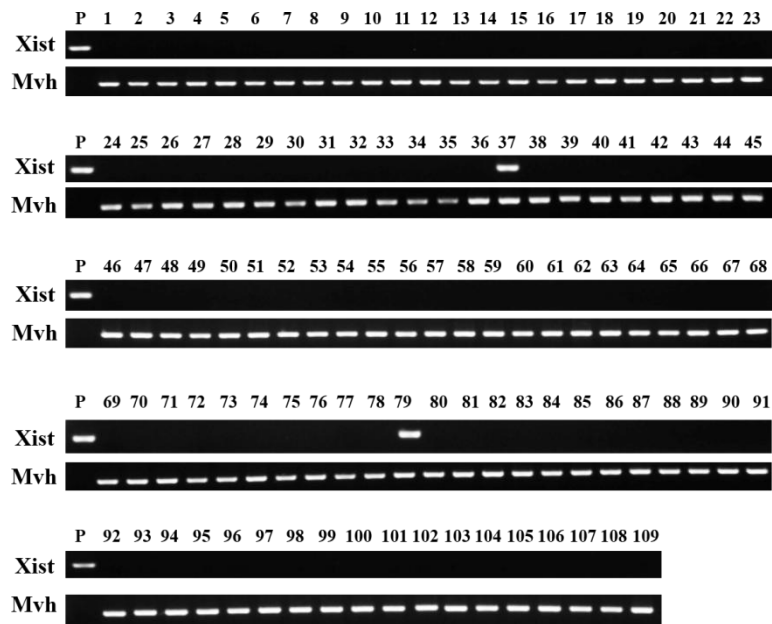
Supplementary information includes

Supplementary Figure S1- S15.

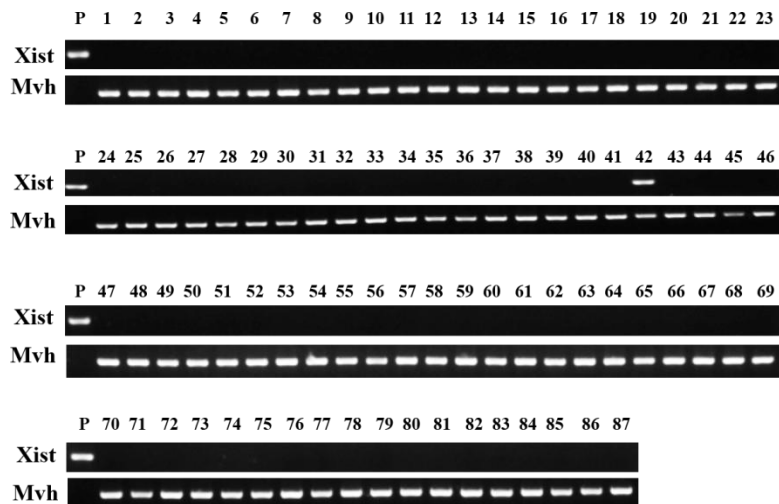
Supplementary Table S1- S8.



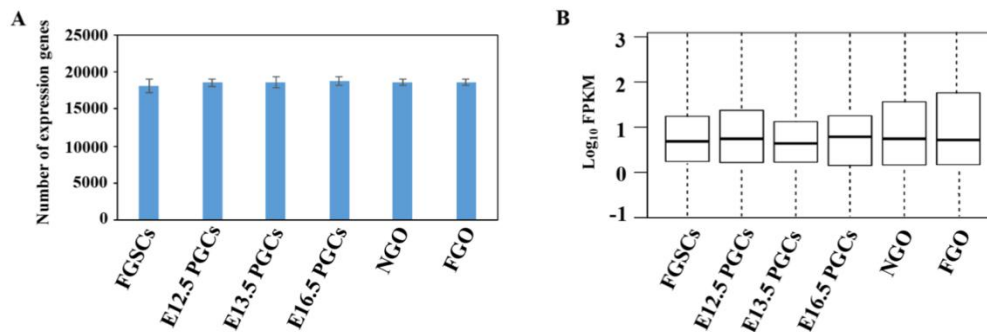
Supplementary Figure S1. Single cell RT-PCR analysis of Xist, Mvh for E12.5 PGCs. Lane P, female tail fibroblasts (positive control); lane 1-83, single cell E12.5 PGCs.



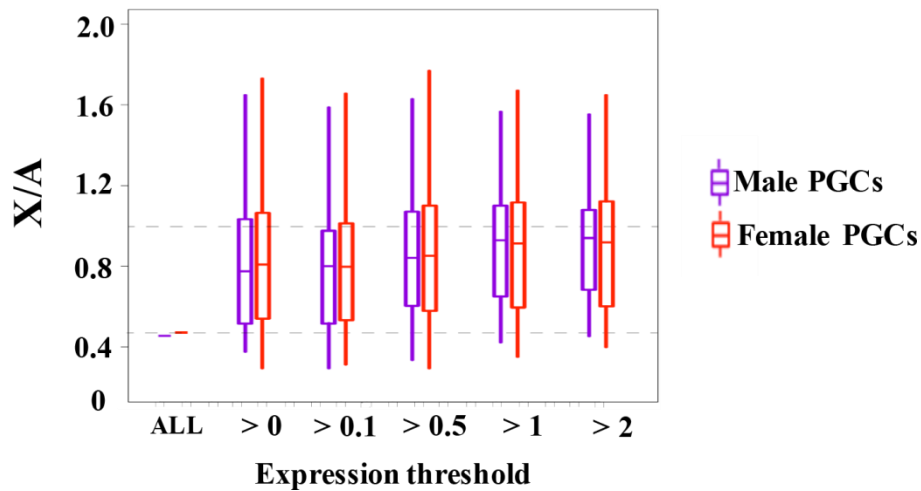
Supplementary Figure S2. Single cell RT-PCR analysis of Xist, Mvh for E13.5 PGCs. Lane P, female tail fibroblasts (positive control); lane 1-109, single cell E13.5 PGCs.



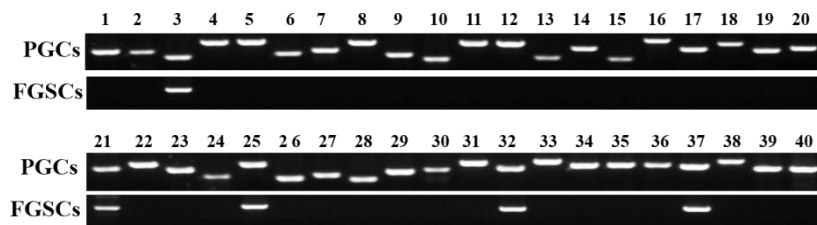
Supplementary Figure S3. Single cell RT-PCR analysis of Xist, Mvh for E16.5 PGCs. Lane P, female tail fibroblasts (positive control); lane 1-87, single cell E16.5 PGCs.



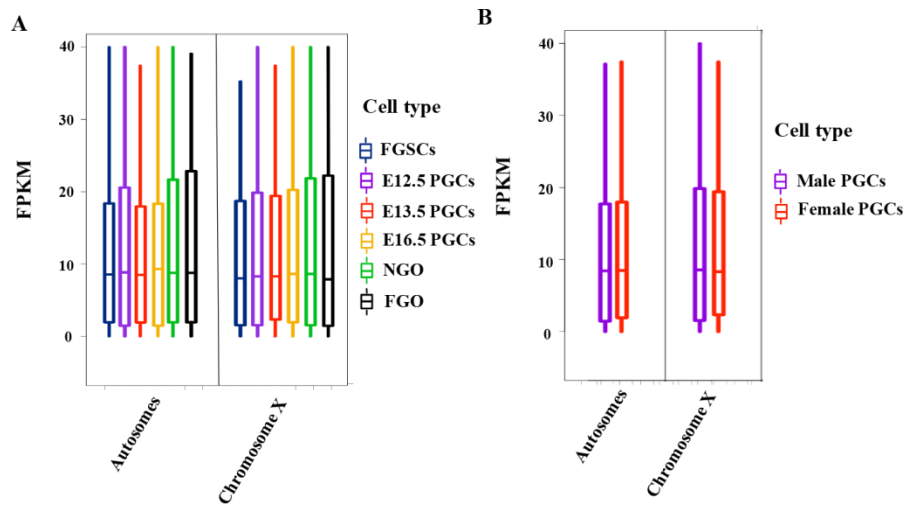
Supplementary Figure S4. There are no statistically significant differences between the RNA-seq data of each types of germ cells. (A) Similar expression gene number between each types of germ cells. (B) Similar gene expression level between each types of germ cells.



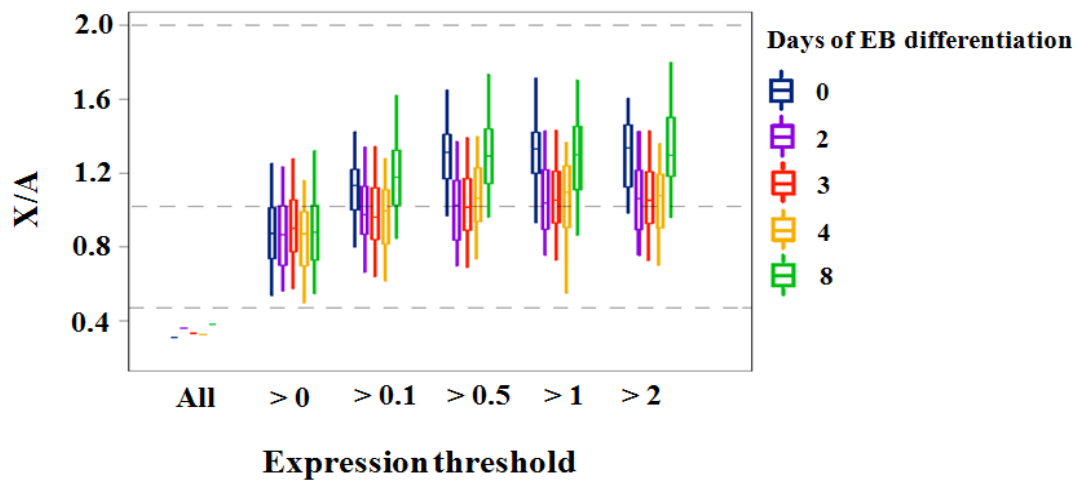
Supplementary Figure S5. Comparisons of X: A ratio between male and female E13.5 PGCs. For both male and female E13.5 PGCs, the X: A median expression ratios increased to close to 1 as no-/low-expression genes were gradually removed and remained stable after genes with $FPKM \leq 1$ were removed. There were no differences between each types of germ cells. ($P > 0.05$, by student's t-test).



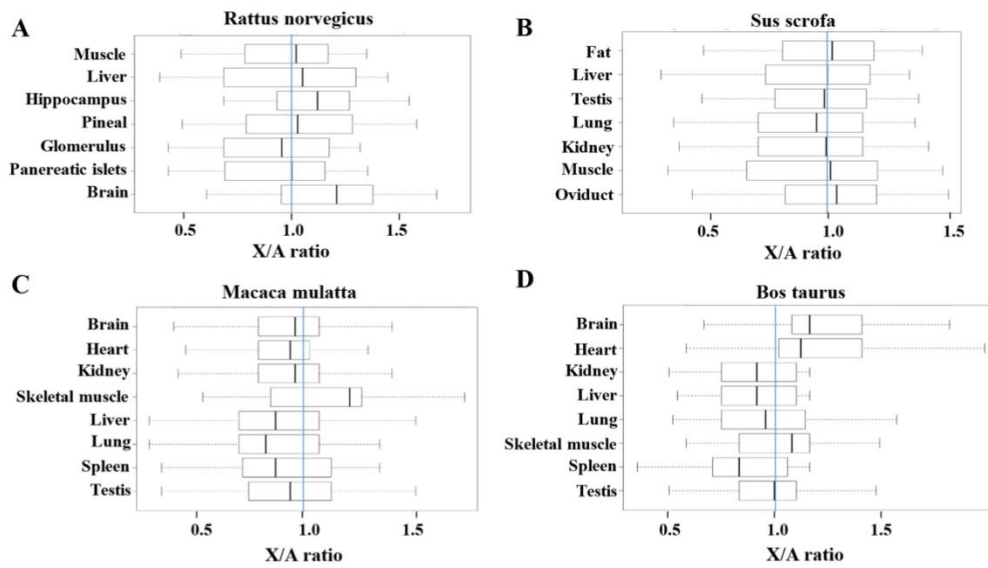
Supplementary Figure S6. Low-abundance transcripts result from technical or biological noise rather than active transcripts. Forty genes with low-expression ($FPKM < 1$) in FGSCs while high-expression ($FPKM > 1$) in PGCs were randomly selected from our RNA-seq data. lane 1-40: Pfn4, Cxcl14, Fam3b, Eno2, Fam131c, Rab25, Cd48, Cacnb4, Mmp9, Lama2, Tnfaip6, Xylt1, Omd, Lrriq1, Scd3, Lctl, Nek5, Cldn18, Fank1, Cela1, Gad2, Nppb, Lsamp, Grid1, Exd1, Rab25, Kcnc3, Ica11, Filip1, Pde4d, Wdfy4, Fzd4, Thsd7b, Ang, Atg9b, Yod1, Slc5a5, Cabp1, Sbp, Spink2.



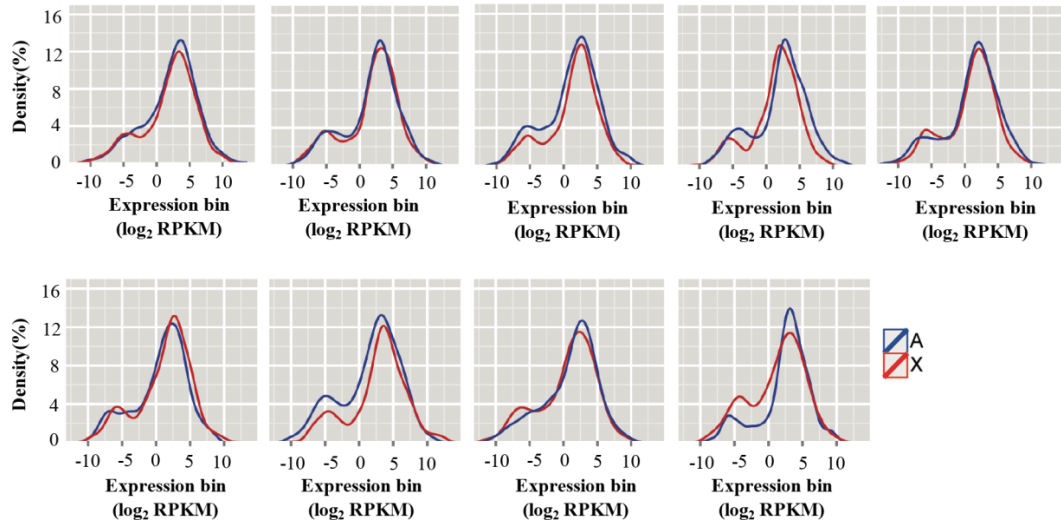
Supplementary Figure S7. Distribution of gene expression across all different samples. A. Distribution of gene expression in germ cell development. B. Distribution of gene expression in male and female E13.5 PGCs. All genes with an expression level of FPKM > 1 are included.



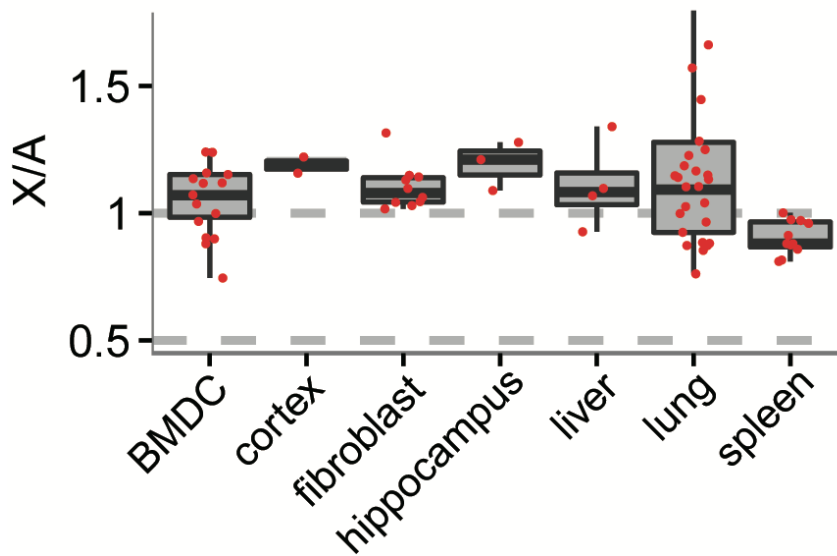
Supplementary Figure S8. X: A median expression ratios during the early stages of XCI by differentiation of female ESCs to EBs were strongly dependent on FPKM cutoffs (>0, ≥0.1, ≥0.5, ≥1, ≥2).



Supplementary Figure S9. Comparisons of RNA-Seq gene expression levels between the X chromosome and autosomes from mammalian tissues (seven *Rattus norvegicus* tissues, seven *Sus scrofa* tissues, eight *Bos taurus* tissues and eight *Macaca mulatta* tissues) by excluding no-/low-expression genes. (A) X: A ratios of median expressions from seven *Rattus norvegicus* tissues. The X: A ratio is significant closer to 1 compared to 0.5 (student's t-test, $p < 0.001$). (B) X: A ratios of median expressions from seven *Sus scrofa* tissues. The X: A ratio is significant closer to 1 compared to 0.5 (student's t-test, $p < 0.001$). (C) X: A ratios of median expressions from eight *Macaca mulatta* tissues. The X: A ratio is significant closer to 1 compared to 0.5 (student's t-test, $p < 0.001$). (D) X: A ratios of median expressions from eight *Bos taurus* tissues. The X: A ratio is significant closer to 1 compared to 0.5 (student's t-test, $p < 0.001$). The percentiles in all boxplots are 0.05, 0.25, 0.5, 0.75 and 0.95.

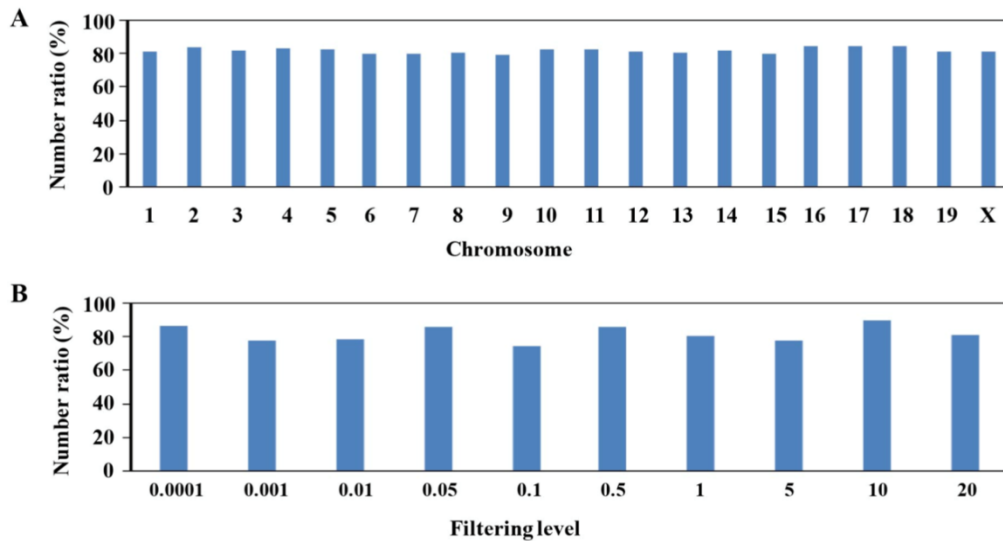


Supplementary Figure S10. Histograms of X-linked and autosomal expression distributions ($P > 0.05$, by Kolmogorov-Smirnov test).

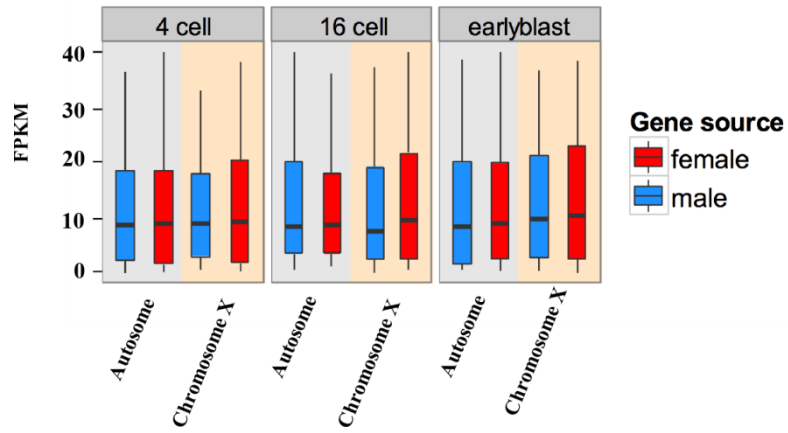


Supplementary Figure S11. X: A ratios of median expressions based on single cell RNA-Seq from bone marrow-derived dendritic cells (BMDC) (male, $N=15$), cortex cells ($N=2$), fibroblast cells (female, $N=10$), hippocampus cells ($N=3$), liver cells (female, $N=4$), lung cells ($N=25$) and spleen cells (male, $N=10$) when excluding no-/low-expression genes. Sex information is listed in parentheses after the cell names,

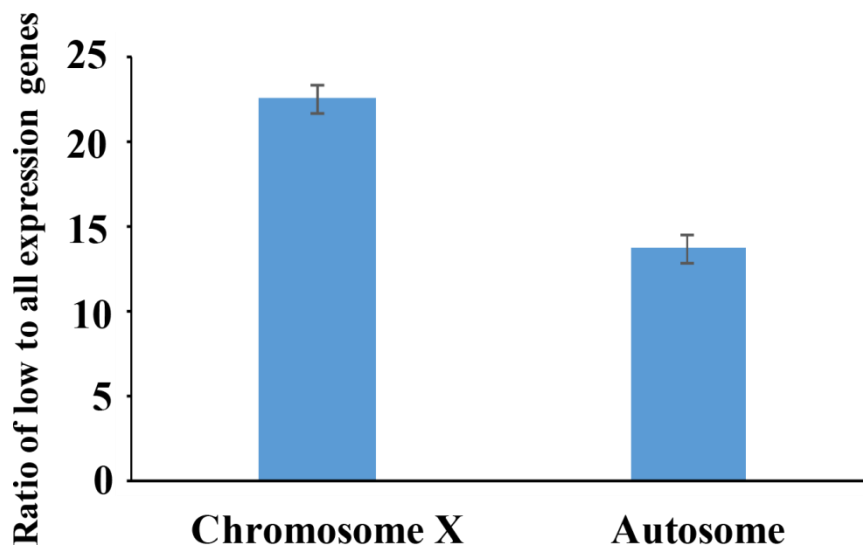
and N represents the number of single cell RNA-seq data with this type of cells in the parentheses after the cell names. There were no differences between each types of single cells. ($P > 0.05$, by student's t-test) except cortex and hippocampus ($P < 0.05$, by student's t-test). And the X: A ratio is significant closer to 1 compared to 0.5 (student's t-test, $p < 0.001$). The percentiles in all boxplots are 0.05, 0.25, 0.5, 0.75 and 0.95.



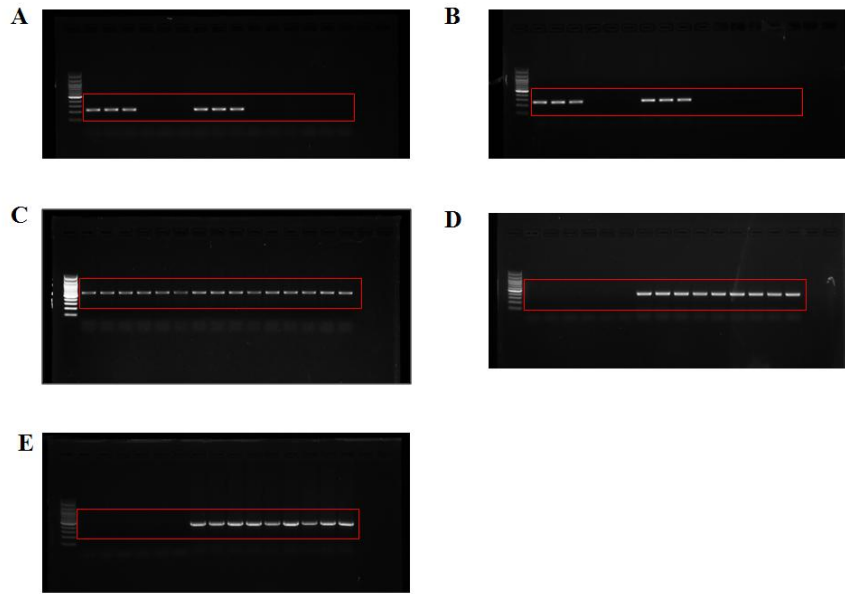
Supplementary Figure S12. The SNPs containing genes were widely scattered on all chromosomes and each level of filtering. (A) Chromosomes are on the X axis, and the distribution ratio is on the Y axis. Vertical bands show the ratio (SNPs containing genes/ total genes) of SNPs containing genes derived from each chromosome. (B) Filtering level are on the X axis, and the distribution ratio is on the Y axis. Vertical bands show the ratio (SNPs containing expression genes/ total expression genes in this filtering level) of SNPs containing genes derived from each level of filtering. In each filter level, our SNP containing genes indicated the remaining number of SNP containing genes after filtering while total genes indicated the remaining number of all of the genes (including SNP containing genes and no SNP containing genes).



Supplementary Figure S13. Distribution of gene expression across all different samples in mouse early embryogenesis. All genes with an expression level of $\text{FPKM} > 0.1$ are included.



Supplementary Figure S14. The low expressed genes are much more distributed on the X chromosome. We used female E12.5 PGCs as an example.



Supplementary Figure S15. Uncropped gels for Figure 1J. (A) Uncropped gels for single cell RT-PCR analysis of Xist ex1. (B) Uncropped gels for single cell RT-PCR analysis of Xist ex2. (C) Uncropped gels for single cell RT-PCR analysis of Gapdh. (D) Uncropped gels for single cell RT-PCR analysis of Mvh. (E) Uncropped gels for single cell RT-PCR analysis of Oct4. Red boxes correspond to those shown in the cropped images within the manuscript.

Supplementary Table S1. The information of RNA-seq data produced in our laboratory

| Stages | No. of libraries | No. of cells in each libraries | Total raw reads |
|--------|------------------|--------------------------------|-----------------|
| FGSCs | 2 | 8 | 30,333,613 |
| PGCs | 2 | 8 | 23,107,536 |
| FGO | 2 | 5 | 29,323,069 |

Supplementary Table S2. Percentage of expressed genes left after each level of filtering in germ cell development

| Cell type | Percentage of genes left after each level of filtering (%) | | |
|------------|--|------|------|
| | 0.1 | 0.5 | 1 |
| FGSCs | 98.2 | 92.9 | 82.5 |
| E12.5 PGCs | 98.6 | 90.1 | 81.6 |
| E13.5 PGCs | 97.3 | 89.3 | 80.7 |
| E16.5 PGCs | 92.6 | 88.4 | 79.8 |
| NGO | 96.9 | 88.1 | 79.8 |
| FGO | 97.2 | 88.5 | 80.4 |

Supplementary Table S3. The median X: A ratio with expression threshold for single cells

| Single cells | Access numbers | Expression threshold | | | | | | | | | | |
|--------------|----------------|----------------------|------------|-------|-------|------|------|------|------|------|------|------|
| | | 0.00 0001 | 0.00 01 | 0.001 | 0.005 | 0.01 | 0.05 | 0.1 | 0.5 | 1 | 5 | 10 |
| BMDC | SRR578548 | 1.76 | 1.76 | 1.76 | 1.83 | 1.76 | 1.73 | 1.68 | 1.33 | 1.3 | 1.21 | 1.04 |
| | SRR578549 | 1.09 | 1.09 | 1.09 | 1.09 | 1.04 | 1.19 | 1.03 | 1.13 | 1.12 | 1.15 | 1.09 |
| | SRR578550 | 1.07 | 1.07 | 1.07 | 1.07 | 1.13 | 1.17 | 1.18 | 1.07 | 1.18 | 1.12 | 1.1 |
| | SRR578551 | 0.61 | 0.61 | 0.61 | 0.61 | 0.55 | 1.07 | 1.08 | 1.08 | 1.24 | 1.19 | 1.1 |
| | SRR578552 | 0.56 | 0.56 | 0.55 | 0.59 | 0.83 | 1.28 | 1.17 | 1.2 | 1.2 | 1.17 | 1.22 |
| | SRR578553 | 0.83 | 0.83 | 0.83 | 0.82 | 0.85 | 0.77 | 0.86 | 1.46 | 1.3 | 1.56 | 1.36 |
| | SRR578554 | 0.52 | 0.52 | 0.52 | 0.47 | 0.92 | 1.72 | 1.47 | 1.11 | 1.26 | 1.05 | 0.95 |
| | SRR578555 | 0.79 | 0.79 | 0.77 | 0.93 | 0.83 | 1.07 | 1.21 | 1.14 | 1.28 | 1 | 0.94 |
| | SRR578556 | 1.05 | 1.05 | 1.04 | 0.9 | 0.76 | 0.8 | 1.31 | 1.11 | 1.24 | 1.07 | 0.91 |
| | SRR578557 | 1.52 | 1.52 | 1.73 | 1.57 | 2.05 | 2.1 | 1.64 | 1.43 | 1.36 | 1.2 | 1.16 |
| | SRR578558 | 0.92 | 0.92 | 0.95 | 0.9 | 0.9 | 1.14 | 1.27 | 1.14 | 1.18 | 1.25 | 1.15 |
| | SRR578559 | 0.86 | 0.86 | 0.85 | 1.04 | 1.16 | 2.14 | 1.38 | 1.39 | 1.21 | 1.23 | 1.23 |
| | SRR578560 | 1.2 | 1.2 | 1.19 | 1.91 | 1.72 | 1.27 | 0.92 | 1.01 | 1.09 | 1.15 | 1.26 |
| | SRR578561 | 2.06 | 2.06 | 2.03 | 2.1 | 1.95 | 1.6 | 1.16 | 1.13 | 1.05 | 1.28 | 1.23 |
| | SRR578562 | 1.69 | 1.69 | 1.64 | 1.82 | 1.76 | 1.26 | 1.07 | 1.12 | 1.04 | 1.19 | 1.24 |
| | SRR578563 | 0.95 | 0.95 | 0.95 | 0.94 | 0.93 | 0.95 | 0.99 | 0.79 | 0.76 | 1.01 | 0.97 |
| | SRR578564 | 0.99 | 0.99 | 0.99 | 0.99 | 0.98 | 1 | 1 | 1.07 | 0.95 | 1.04 | 0.92 |
| | SRR578565 | 0.96 | 0.96 | 0.96 | 0.96 | 0.95 | 0.96 | 0.98 | 1 | 0.97 | 1.05 | 0.92 |
| | SRR578566 | 0.8 | 0.8 | 0.8 | 0.9 | 0.93 | 1.03 | 1 | 0.96 | 1.02 | 1.11 | 0.89 |
| | SRR578567 | 0.96 | 0.96 | 0.96 | 0.96 | 0.95 | 1.02 | 0.99 | 0.99 | 1 | 1.08 | 0.93 |
| | SRR578568 | 0.75 | 0.75 | 0.75 | 0.98 | 0.98 | 0.99 | 1.01 | 0.99 | 1 | 1.1 | 0.91 |
| SRR578569 | 0.83 | 0.83 | 0.83 | 0.84 | 0.83 | 0.88 | 0.89 | 0.95 | 0.95 | 1.08 | 0.9 | |
| SRR578570 | 0.81 | 0.81 | 0.81 | 0.8 | 0.8 | 0.88 | 0.98 | 0.99 | 0.94 | 1.05 | 0.93 | |
| SRR578571 | 0.77 | 0.77 | 0.77 | 0.81 | 0.8 | 0.89 | 0.97 | 0.96 | 0.93 | 1.07 | 0.92 | |
| Cortex | SRR1033341 | 1.16 | 1.16 | 1.17 | 1.12 | 1.19 | 1.28 | 1.35 | 1.2 | 1.24 | 1.21 | 1.13 |
| | SRR1033342 | 1.17 | 1.17 | 1.17 | 1.25 | 1.34 | 1.29 | 1.34 | 1.35 | 1.33 | 1.27 | 1.6 |
| Fibroblast | SRR1041755 | 0.62 | 0.62 | 0.67 | 0.67 | 0.75 | 0.95 | 0.88 | 0.97 | 1.04 | 1.15 | 1.37 |
| | SRR1041756 | 0.68 | 0.68 | 0.7 | 0.73 | 0.77 | 1.06 | 1.12 | 1.18 | 1.15 | 1.27 | 1.2 |
| | SRR1041757 | 0.64 | 0.64 | 0.63 | 0.69 | 0.72 | 0.92 | 0.98 | 1.1 | 1.13 | 1.28 | 1.13 |
| | SRR1041758 | 0.76 | 0.76 | 0.76 | 0.76 | 0.84 | 0.9 | 0.95 | 1.01 | 1.14 | 1.08 | 1.06 |
| | SRR1041759 | 0.81 | 0.81 | 0.82 | 0.88 | 0.93 | 1.11 | 1.13 | 1.28 | 1.32 | 1.21 | 1.19 |
| | SRR1041760 | 1.11 | 1.11 | 1.1 | 1.17 | 1.19 | 1.12 | 1.13 | 1.07 | 1.02 | 0.91 | 0.92 |
| | SRR1041761 | 0.75 | 0.75 | 0.75 | 0.85 | 0.91 | 1.03 | 1.07 | 1.08 | 1.06 | 0.99 | 1 |

| | | | | | | | | | | | | |
|-------------|------------|------|------|------|------|------|------|------|------|------|------|------|
| | SRR1041762 | 0.96 | 0.96 | 0.98 | 1.05 | 1.06 | 1.12 | 1.13 | 1.13 | 1.1 | 1.03 | 1.05 |
| | SRR1041763 | 0.98 | 0.98 | 0.97 | 0.96 | 0.97 | 0.94 | 1.05 | 1.04 | 1.03 | 0.93 | 1.09 |
| | SRR1041764 | 0.8 | 0.8 | 0.8 | 0.78 | 0.81 | 0.88 | 0.96 | 1 | 1.05 | 1.16 | 0.99 |
| Hippocampus | SRR1033343 | 1.2 | 1.2 | 1.29 | 1.29 | 1.34 | 1.36 | 1.33 | 1.34 | 1.3 | 1.31 | 1.35 |
| | SRR1033344 | 1.29 | 1.29 | 1.29 | 1.38 | 1.35 | 1.37 | 1.31 | 1.32 | 1.32 | 1.14 | 1.24 |
| | SRR1033345 | 1.28 | 1.28 | 1.28 | 1.34 | 1.31 | 1.39 | 1.39 | 1.29 | 1.34 | 1.32 | 1.27 |
| Liver | SRR805273 | 0.69 | 0.69 | 0.69 | 0.68 | 0.8 | 0.78 | 0.89 | 0.97 | 1.17 | 1.12 | 0.91 |
| | SRR805274 | 0.58 | 0.58 | 0.58 | 0.63 | 0.85 | 0.86 | 1.1 | 1.09 | 0.93 | 0.61 | 1.21 |
| | SRR805275 | 0.38 | 0.38 | 0.42 | 0.47 | 0.6 | 0.52 | 0.61 | 1.63 | 1.34 | 1.82 | 1.26 |
| | SRR805276 | 0.82 | 0.82 | 1.04 | 1.04 | 1.04 | 0.99 | 0.98 | 1.27 | 1.1 | 0.62 | 0.74 |
| | SRR805277 | 0.69 | 0.69 | 0.69 | 0.72 | 0.75 | 0.85 | 0.87 | 0.97 | 1.35 | 1.37 | 1.34 |
| Lung | SRR1033981 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 1.01 | 1.28 | 1.07 | 1.12 |
| | SRR1033982 | 1.13 | 1.13 | 1.13 | 1.13 | 1.13 | 1.18 | 1.18 | 1.14 | 1.44 | 1.54 | 1.31 |
| | SRR1033984 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 1.07 | 1.04 | 1.03 | 0.96 |
| | SRR1033985 | 1.21 | 1.21 | 1.21 | 1.21 | 1.21 | 1.21 | 1.21 | 1.19 | 1.2 | 1.04 | 1.03 |
| | SRR1033986 | 0.95 | 0.95 | 0.95 | 0.95 | 0.95 | 0.95 | 0.95 | 0.89 | 0.85 | 0.89 | 0.8 |
| | SRR1033987 | 0.91 | 0.91 | 0.91 | 0.91 | 0.91 | 0.91 | 0.91 | 0.91 | 0.87 | 1.04 | 0.98 |
| | SRR1033988 | 1.23 | 1.23 | 1.23 | 1.23 | 1.23 | 1.22 | 1.39 | 1.39 | 1.3 | 1.3 | 1.33 |
| | SRR1033989 | 1.43 | 1.43 | 1.43 | 1.43 | 1.43 | 1.43 | 1.53 | 1.53 | 1.53 | 1.49 | 1.53 |
| | SRR1033990 | 1.48 | 1.48 | 1.48 | 1.48 | 1.48 | 1.48 | 1.48 | 1.43 | 1.48 | 1.25 | 1.26 |
| | SRR1033991 | 1.32 | 1.32 | 1.32 | 1.32 | 1.32 | 1.32 | 1.32 | 1.3 | 1.28 | 1.18 | 1.17 |
| | SRR1033992 | 1.18 | 1.18 | 1.18 | 1.18 | 1.18 | 1.18 | 1.19 | 1.17 | 1.15 | 1.14 | 1.22 |
| | SRR1033993 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.19 | 1.42 | 1.49 | 1.24 | 1.27 |
| | SRR1033994 | 1.23 | 1.23 | 1.23 | 1.23 | 1.23 | 1.22 | 1.24 | 1.19 | 1.23 | 1.22 | 1.32 |
| | SRR1033995 | 1.13 | 1.13 | 1.13 | 1.13 | 1.13 | 1.13 | 1.13 | 1.12 | 1.1 | 1.11 | 1.45 |
| | SRR1033996 | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 | 0.95 | 1.06 | 1.19 | 1.26 | 1.85 | 1.56 |
| | SRR1033997 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 | 1.19 | 1.27 | 1.16 | 1.17 |
| | SRR1033998 | 1.09 | 1.09 | 1.09 | 1.09 | 1.09 | 1.08 | 1.06 | 1.09 | 1.31 | 1.26 | 1.25 |
| | SRR1033999 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.78 | 0.76 | 0.91 | 0.82 |
| | SRR1034000 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 | 1.17 | 1.49 |
| | SRR1034001 | 1.18 | 1.18 | 1.18 | 1.18 | 1.18 | 1.18 | 1.17 | 1.15 | 1.13 | 1.13 | 1.14 |
| | SRR1034002 | 1.84 | 1.84 | 1.84 | 1.84 | 1.84 | 1.84 | 1.83 | 1.81 | 1.79 | 1.68 | 1.52 |
| | SRR1034003 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.95 | 1 |
| | SRR1034004 | 1.56 | 1.56 | 1.56 | 1.56 | 1.56 | 1.56 | 1.56 | 1.64 | 1.49 | 2.37 | 2 |
| | SRR1034006 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.98 | 0.92 | 0.78 | 0.77 |
| | SRR1034007 | 1.77 | 1.77 | 1.77 | 1.77 | 1.77 | 1.77 | 1.77 | 1.74 | 1.64 | 1.38 | 1.58 |
| SRR1034008 | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 | 1 | 0.99 | 0.94 | 0.87 | 1.2 | 1.29 | |
| SRR1034009 | 1.29 | 1.29 | 1.29 | 1.29 | 1.29 | 1.28 | 1.28 | 1.16 | 1.58 | 1.76 | 1.78 | |
| SRR1034011 | 1.51 | 1.51 | 1.51 | 1.51 | 1.51 | 1.51 | 1.51 | 1.5 | 1.5 | 1.34 | 1.12 | |
| SRR1034012 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.15 | 1.07 | 1.18 | |
| SRR1034013 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 | 1.11 | 1.22 | 1.13 | 1.16 | |

| | | | | | | | | | | | | |
|-------|------------|------|------|------|------|------|------|------|------|------|------|------|
| | SRR1034014 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 | 1.13 | 1.14 | 1.16 | 1.39 |
| | SRR1034015 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.07 | 1.02 | 1.06 |
| | SRR1034016 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 | 1.13 | 1.08 |
| | SRR1034017 | 1.27 | 1.27 | 1.27 | 1.27 | 1.27 | 1.33 | 1.31 | 1.34 | 1.25 | 1.25 | 1.32 |
| | SRR1034018 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.88 | 0.8 | 0.74 |
| | SRR1034019 | 1.17 | 1.17 | 1.17 | 1.17 | 1.17 | 1.17 | 1.17 | 1.14 | 1.11 | 1.15 | 1.27 |
| | SRR1034020 | 1.47 | 1.47 | 1.47 | 1.47 | 1.47 | 1.47 | 1.45 | 1.26 | 1.18 | 1.31 | 1.35 |
| | SRR1034021 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.17 | 1.19 | 1.12 | 1.17 | 1.18 | 1.16 |
| | SRR1034022 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.09 | 1.01 | 1.19 | 1.24 | 1.16 |
| | SRR1034023 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.8 | 0.86 | 0.92 | 0.86 |
| | SRR1034024 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.19 | 1.14 | 1.46 | 1.3 | 1.27 | 1.34 |
| | SRR1034025 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.83 |
| | SRR1034026 | 1.02 | 1.02 | 1.02 | 1.02 | 1.02 | 1.02 | 1.02 | 1.07 | 1 | 1.09 | 0.87 |
| Speen | SRR1106612 | 0.35 | 0.35 | 0.37 | 0.62 | 0.71 | 0.83 | 0.85 | 0.93 | 0.88 | 0.92 | 0.92 |
| | SRR1106613 | 0.47 | 0.47 | 0.48 | 0.64 | 0.75 | 0.73 | 0.72 | 0.74 | 0.81 | 0.96 | 0.92 |
| | SRR1106614 | 0.62 | 0.62 | 0.65 | 0.71 | 0.76 | 0.8 | 0.81 | 0.94 | 1 | 0.97 | 0.95 |
| | SRR1106615 | 0.61 | 0.61 | 0.61 | 0.68 | 0.68 | 0.72 | 0.73 | 0.78 | 0.82 | 1.23 | 0.99 |
| | SRR1106616 | 0.4 | 0.4 | 0.4 | 0.46 | 0.54 | 0.71 | 0.73 | 0.78 | 0.76 | 0.95 | 1.1 |
| | SRR1106618 | 0.7 | 0.7 | 0.72 | 0.73 | 0.73 | 0.81 | 0.95 | 0.93 | 0.97 | 1.16 | 0.98 |
| | SRR1106619 | 0.73 | 0.73 | 0.72 | 0.76 | 0.75 | 0.81 | 0.79 | 0.77 | 0.82 | 0.93 | 1.12 |
| | SRR1106620 | 0.79 | 0.79 | 0.78 | 0.87 | 0.84 | 0.85 | 0.87 | 0.92 | 0.88 | 0.87 | 0.97 |
| | SRR1106617 | 0.73 | 0.73 | 0.73 | 0.73 | 0.72 | 0.78 | 0.81 | 0.87 | 0.91 | 1.08 | 1 |
| | SRR1106622 | 0.33 | 0.33 | 0.37 | 0.39 | 0.44 | 0.58 | 0.62 | 0.68 | 0.78 | 1.12 | 1.04 |
| | SRR1106623 | 0.84 | 0.84 | 0.83 | 0.82 | 0.81 | 0.83 | 0.92 | 0.96 | 0.97 | 0.93 | 0.95 |

Supplementary Table S4. Percentage of expressed genes left after each level of filtering in single fibroblast cells

| Sample | Percentage of genes left after each level of filtering (%) | | |
|------------|--|------|------|
| | 0.1 | 0.5 | 1 |
| SRR1041755 | 93.1 | 84.7 | 78.7 |
| SRR1041756 | 92.1 | 82.7 | 77 |
| SRR1041757 | 93.9 | 82.6 | 78.2 |
| SRR1041758 | 92.9 | 82.8 | 78.9 |
| SRR1041759 | 93.6 | 83.2 | 76.3 |
| SRR1041760 | 92.2 | 84.1 | 79.7 |
| SRR1041761 | 93.5 | 84.5 | 78.6 |
| SRR1041762 | 93.9 | 83.1 | 78.1 |
| SRR1041763 | 92 | 82.6 | 78.7 |
| SRR1041764 | 92.3 | 82.6 | 77.1 |

Supplementary Table S5. The median X: A ratio of paternal X chromosome (Xp) and maternal X chromosome (Xm) in early embryogenesis

| Single cells | Access numbers | Xp/A | Xm/A | X/A | number | Expression level |
|--------------------|----------------|------|------|-------|--------|------------------|
| earlyblas (male) | SRR805294 | 0 | 0.89 | 1.06 | 14774 | 0.88 |
| | SRR805295 | 0 | 1.07 | 1.3 | 14477 | 0.94 |
| | SRR805296 | 0 | 0.89 | 0.95 | 14133 | 0.88 |
| | SRR805297 | 0 | 1.14 | 1.14 | 14854 | 0.93 |
| | SRR805298 | 0 | 1.07 | 1.22 | 14004 | 0.93 |
| | SRR805299 | 0 | 0.85 | 0.91 | 15013 | 0.9 |
| | SRR805300 | 0 | 1.07 | 1.47 | 15245 | 0.97 |
| | SRR805301 | 0 | 1 | 1.11 | 14693 | 0.94 |
| | SRR805302 | 0 | 0.86 | 0.91 | 14972 | 0.94 |
| | SRR805303 | 0 | 0.83 | 0.89 | 14127 | 0.95 |
| | SRR805304 | 0 | 0.93 | 0.98 | 15068 | 0.89 |
| | SRR805305 | 0 | 0.77 | 0.87 | 14066 | 0.91 |
| | SRR805306 | 0 | 0.93 | 1.1 | 15027 | 0.92 |
| | SRR805307 | 0 | 0.93 | 1.01 | 14866 | 0.9 |
| | SRR805308 | 0 | 0.82 | 1.1 | 14347 | 0.88 |
| | SRR805324 | 0 | 1.07 | 1.07 | 15280 | 0.91 |
| | SRR805325 | 0 | 1.1 | 1.38 | 14485 | 0.88 |
| | SRR805326 | 0 | 0.91 | 1.23 | 14389 | 0.89 |
| | SRR805327 | 0 | 1.06 | 1.31 | 14981 | 0.96 |
| | SRR805328 | 0 | 1.07 | 1.15 | 14024 | 0.96 |
| | SRR805329 | 0 | 1.07 | 1.38 | 15143 | 0.91 |
| | SRR805330 | 0 | 0.87 | 0.98 | 14178 | 0.89 |
| | SRR805331 | 0 | 1.11 | 1.31 | 14259 | 0.89 |
| | SRR805332 | 0 | 1 | 1.34 | 14826 | 0.92 |
| SRR805333 | 0 | 1.05 | 1.26 | 14601 | 0.91 | |
| SRR805334 | 0 | 1.21 | 1.33 | 14987 | 0.98 | |
| SRR805335 | 0 | 0.94 | 0.99 | 14065 | 0.92 | |
| SRR805336 | 0 | 1.03 | 1.16 | 14064 | 0.96 | |
| earlyblas (female) | SRR805309 | 0.26 | 0.83 | 1.09 | 15190 | 0.96 |
| | SRR805310 | 0.1 | 0.86 | 1.1 | 14952 | 0.98 |
| | SRR805311 | 0.02 | 0.87 | 1.11 | 14190 | 0.9 |
| | SRR805312 | 0.08 | 0.8 | 1.09 | 14987 | 0.95 |
| | SRR805313 | 0.05 | 0.89 | 0.97 | 14679 | 0.96 |
| | SRR805314 | 0.3 | 0.85 | 1.11 | 14679 | 0.88 |
| | SRR805315 | 0.2 | 0.75 | 1.15 | 15049 | 0.94 |
| | SRR805316 | 0.07 | 0.94 | 1.26 | 15358 | 0.98 |
| SRR805317 | 0.2 | 0.83 | 1.08 | 15233 | 0.95 | |

| | | | | | | |
|-----------------|-----------|------|------|-------|-------|------|
| | SRR805318 | 0.02 | 0.89 | 1.12 | 14767 | 0.93 |
| | SRR805319 | 0.09 | 0.87 | 1.18 | 14957 | 0.98 |
| | SRR805320 | 0.14 | 0.81 | 1.2 | 14812 | 0.89 |
| | SRR805321 | 0.1 | 1.02 | 1.2 | 14416 | 0.88 |
| | SRR805322 | 0.01 | 1.08 | 1.29 | 14015 | 0.9 |
| | SRR805323 | 0.19 | 0.79 | 1.33 | 14349 | 0.95 |
| 16cell (male) | SRR805173 | 0 | 1.17 | 1.33 | 14314 | 0.9 |
| | SRR805174 | 0 | 0.86 | 0.91 | 15037 | 0.95 |
| | SRR805175 | 0 | 0.97 | 1.17 | 15406 | 0.89 |
| | SRR805176 | 0 | 0.91 | 0.72 | 14028 | 0.95 |
| | SRR805177 | 0 | 0.87 | 0.94 | 15132 | 0.89 |
| | SRR805178 | 0 | 0.97 | 1.04 | 15192 | 0.91 |
| | SRR805179 | 0 | 0.91 | 0.96 | 14956 | 0.96 |
| | SRR805180 | 0 | 0.96 | 0.93 | 14047 | 0.88 |
| | SRR805181 | 0 | 1.02 | 1.1 | 15133 | 0.98 |
| | SRR805182 | 0 | 0.98 | 1.08 | 14634 | 0.97 |
| | SRR805183 | 0 | 0.93 | 0.88 | 14173 | 0.93 |
| | SRR805184 | 0 | 1 | 1.18 | 14947 | 0.95 |
| | SRR805185 | 0 | 0.96 | 0.85 | 14557 | 0.9 |
| | SRR805186 | 0 | 0.93 | 1.1 | 15434 | 0.92 |
| | SRR805198 | 0 | 1.07 | 1.22 | 14496 | 0.98 |
| | SRR805199 | 0 | 0.8 | 0.81 | 14768 | 0.88 |
| | SRR805200 | 0 | 0.88 | 0.98 | 14424 | 0.94 |
| | SRR805201 | 0 | 0.83 | 0.86 | 14835 | 0.9 |
| | SRR805202 | 0 | 0.9 | 0.98 | 15226 | 0.88 |
| | SRR805203 | 0 | 0.85 | 0.95 | 14785 | 0.92 |
| SRR805204 | 0 | 0.96 | 1.06 | 14505 | 0.88 | |
| SRR805205 | 0 | 0.87 | 0.97 | 15261 | 0.89 | |
| SRR805206 | 0 | 0.93 | 0.53 | 14288 | 0.89 | |
| SRR805207 | 0 | 0.88 | 0.85 | 14949 | 0.96 | |
| SRR805208 | 0 | 0.89 | 0.84 | 15027 | 0.88 | |
| SRR805209 | 0 | 1.01 | 1.05 | 15006 | 0.96 | |
| SRR805210 | 0 | 0.99 | 1 | 15384 | 0.96 | |
| 16cell (female) | SRR805189 | 0.21 | 0.74 | 1.18 | 14886 | 0.97 |
| | SRR805190 | 0.1 | 0.9 | 1.01 | 14508 | 0.98 |
| | SRR805191 | 0.35 | 0.54 | 1.06 | 14630 | 0.96 |
| | SRR805192 | 0.31 | 0.7 | 1.15 | 14430 | 0.96 |
| | SRR805193 | 0.28 | 0.6 | 1.14 | 14450 | 0.98 |
| | SRR805194 | 0.26 | 0.63 | 0.99 | 15209 | 0.91 |
| | SRR805195 | 0.34 | 0.68 | 1.19 | 15043 | 0.88 |
| | SRR805196 | 0.27 | 0.63 | 1.38 | 14619 | 0.89 |
| | SRR805197 | 0.15 | 0.81 | 1.17 | 14843 | 0.94 |
| | SRR805211 | 0.15 | 0.71 | 1.01 | 14509 | 0.9 |

| | | | | | | |
|----------------|-----------|------|------|-------|-------|------|
| | SRR805212 | 0.16 | 0.69 | 1.38 | 14639 | 0.92 |
| | SRR805213 | 0.21 | 0.64 | 1.02 | 14045 | 0.94 |
| | SRR805214 | 0.33 | 0.65 | 1.04 | 14015 | 0.9 |
| | SRR805215 | 0.19 | 0.72 | 1.02 | 14653 | 0.96 |
| | SRR805216 | 0.46 | 0.69 | 1.1 | 14913 | 0.9 |
| | SRR805217 | 0.27 | 0.6 | 1.04 | 14223 | 0.92 |
| | SRR805218 | 0.27 | 0.79 | 1.3 | 15278 | 0.92 |
| | SRR805219 | 0.25 | 0.68 | 1.19 | 14344 | 0.92 |
| | SRR805220 | 0.17 | 0.72 | 1.24 | 14991 | 0.93 |
| | SRR805221 | 0.31 | 0.78 | 1.19 | 14977 | 0.95 |
| | SRR805222 | 0.19 | 0.8 | 1.33 | 14476 | 0.96 |
| 4cell (male) | SRR805223 | 0 | 0.85 | 0.97 | 15336 | 0.89 |
| | SRR805224 | 0 | 0.95 | 1.06 | 14767 | 0.9 |
| | SRR805225 | 0.03 | 0.91 | 0.94 | 14624 | 0.89 |
| 4cell (female) | SRR805226 | 0.49 | 0.29 | 1.02 | 14707 | 0.91 |
| | SRR805227 | 0.36 | 0.49 | 1.03 | 14890 | 0.94 |
| | SRR805228 | 0.49 | 0.7 | 1.01 | 14887 | 0.97 |
| | SRR805229 | 0.47 | 0.42 | 1.06 | 14261 | 0.96 |
| | SRR805230 | 0.49 | 0.47 | 1.14 | 14688 | 0.95 |
| | SRR805231 | 0.44 | 0.51 | 1.18 | 14137 | 0.97 |
| | SRR805232 | 0.63 | 0.51 | 0.92 | 15096 | 0.94 |
| | SRR805233 | 0.56 | 0.35 | 1.01 | 14391 | 0.88 |
| | SRR805234 | 0.4 | 0.41 | 1.02 | 14890 | 0.95 |
| | SRR805235 | 0.48 | 0.46 | 0.83 | 14688 | 0.88 |
| SRR805236 | 0.33 | 0.49 | 1.04 | 15158 | 0.92 | |

Supplementary Table S6. List of primer pairs used in single cell RT-PCR analyses

| Gene | Forward | Reverse | PCR product size (bp) |
|------------|-----------------------|----------------------|-----------------------|
| Xist exon1 | CTAAAACTCAGCCC GTTCCA | GCAACCCAGCAATAGTCAT | 217 |
| Xist exon7 | GCCCAGGTCACATTATGGTT | CTCCAATTTCTGGGCTCAAG | 232 |
| GAPDH | TGGCCTTCCGTGTTCTTAC | GAGTTGCTGTTGAAGTCGCA | 568 |
| Oct4 | GTCAGCCAGACCACCATCT | TGGGAAAGGTGTCCCTGTAG | 385 |
| Mvh | GCCAGAGGGCTTGATATTGA | CAACTGGATTGGGAGCTTGT | 370 |

Supplementary Table S7. List of primer of 40 genes with low-expression in FGSC while high-expression in PGC

| Gene name | Forward | Reverse | PCR product size |
|-----------|---------------------------|------------------------|------------------|
| Pfn4 | CTCACCTTCATAAACCATTCTGTGA | ACAGGGTCTTTTCCTGGAGTTT | 311 |
| Cxcl14 | ACCAAGAGCATGTCCAGGTA | GGAAGCCTTTCACACACAGC | 301 |
| Fam3b | TCAGATCAAGCTGGGTGTTTGT | TCCTTCACAGCTGGACTTCAA | 249 |
| Eno2 | AGCCCTCATCAGCTCAGGTA | CCCACGTTAGTGGCATCCTT | 399 |
| Fam131c | TCTCCTAGCCTGCTCTCCTG | TGCCCCTGGTGTCTGCTTAG | 403 |
| Rab25 | CCGGACCAGTGCCATCAC | GCCTGTATAGGGTCCAGGTG | 262 |
| Cd48 | CTGCCCTTGGGAAGTGGATT | TTCACGCAGCACTCTCATGT | 291 |
| Cacnb4 | GTACCTGGAGGCATACTGGC | GCCTATGTCGGGAGTCATGG | 380 |
| Mmp9 | GAGACTCTACACGGAGCACG | GGAAGACGAAGGGGAAGACG | 239 |
| Lama2 | AGAGCCATCTGAAGAACACATTGA | ACAACCTCTGGGAGGTGGAGTT | 200 |
| Tnfaip6 | TGCAACCGAAGAGATGGTCG | CCTGTTGAGCCGGATTCCAT | 370 |
| Xylt1 | TGGGACTTCTTCATCAACCTCA | CCCTGGGGCTTTCCTAGAAC | 366 |
| Omd | GTGAAAATGGCTTTACTTCATAAGC | AGTCGTCAGGCTCGGTAGTA | 211 |
| Lrriq1 | TTGGTGAGTGAGGAGGGTGT | CTCTGAGCTTTGCCTCGCT | 300 |
| Scd3 | AAGTATGGGCGAGGCTTCCA | CCACTCTTGACTCCCGTC | 203 |
| Lctl | TGAAGAGCAACATCACCCCC | GGTTGTCGATGTCCACAGGT | 390 |
| Nek5 | GGTTCTGAAGATTTGTCAAGGACG | CCACCCACGCATGCTTTTAAAT | 301 |
| Cldn18 | GCTGTACGAGCCCTGATGAT | ATCACTCCCCAATCAGGGT | 350 |
| Fank1 | CAACCCGAGCGACGCC | CCCGTGTAATGACACCGTAG | 280 |

| | | | |
|--------|-------------------------|--------------------------|-----|
| Cela1 | GGACGTTCCGGAAACTGACG | CCCGCAAATGTCAACCAGAC | 302 |
| Gad2 | CCGGGAAGGTTCGCCTG | CCTTTATTATTGCTGGTTGCACAG | 345 |
| Nppb | GAGGCGAGACAAGGGAGAAC | TACAGCCCAAACGACTGACG | 400 |
| Lsamp | AGGGCAGCAATGTAACCCTG | TGTCATCCCGGTACCACTCA | 333 |
| Grid1 | CATGCACATCCCACACCTCT | CATCCAGACCCAGCCTTGAG | 251 |
| Exd1 | TTTGGTGTTCGCTTCTCTCC | TGGGTCAGCTACAGAGACGA | 380 |
| Rab25 | CCCTGGGGAAAAGAGGGC | GCCTGTATAGGGTCCAGGTG | 211 |
| Kcnc3 | ACAAGAGCCCAATCACTCCC | AACACAGGCTCACTGTTGGG | 240 |
| Ica11 | GATGAGGGTGTGACGCCTCG | TTGCTCGTGAGAAGTGACGG | 202 |
| Filip1 | GGAAGGCGTGAGTCCAGTTAT | GAGCTCACTCGCAACTGGT | 279 |
| Pde4d | TGACATGCCTGGAGAATTGGAG | AAGCCACAAAGAGAAAAGTCTT | 299 |
| Wdfy4 | CCAGATGTGCTAAGCCCACG | ACCAGGGTTAGGAAGCGCAG | 380 |
| Fzd4 | CCCACAAGACTCCCATCCAG | GCCTACAGTCAGCCGAACAA | 320 |
| Thsd7b | CAATGGATGCGCAGAGGTGT | CCGGTTTTCCAGAGGAACTGA | 399 |
| Ang | GGGACGAGATTCCAAAGGA | TTGATGTTGCTCTTGTTGCCA | 350 |
| Atg9b | CTGCTGGTTTTGGACGTCAG | AGATGCCCAACCCAAACCTT | 350 |
| Yod1 | ACCTCTCGCGATGTTTGGAG | CAGCATGTCACCTGACTGGA | 349 |
| Slc5a5 | CCAGTACCTAGAAGTGCCT | CAGGTTGATCCGGGAATGGT | 340 |
| Cabp1 | GCTCCCCGCCACAGC | CGGAGCTCTTCAATCTCCTCT | 401 |
| Sbp | TCTAAGTGCCAGATTGTACCACA | GCACTGCCCTCTACCTTTATCA | 312 |
| Spink2 | TGCCGTTACTGGCGGTTCT | AGCACCGGAGGAGAGGAGTT | 313 |

Supplementary Table S8. The accession numbers of the datasets

| No. | Cell line or tissue | Database | Access numbers | data source |
|-----|-------------------------|----------|----------------|------------------|
| 1 | mouse FGSCs | GEO | GSE75738 | Our lab |
| 2 | mouse E12.5 PGCs | GEO | GSE75738 | Our lab |
| 3 | mouse E13.5 PGCs | GEO | GSE41908 | ^{64,65} |
| 4 | mouse E16.5 PGCs | ENA | ERP001953 | ⁶⁶ |
| 5 | mouse NGO | DDBJ | DRP002743 | ⁶⁷ |
| 6 | mouse FGO | GEO | GSE75738 | Our lab |
| 8 | Single BMDC | GEO | GSE41265 | ⁶⁸ |
| 9 | Single fibroblast cell | GEO | GSE45719 | ³³ |
| 10 | Single liver cell | GEO | GSE45719 | ³³ |
| 11 | Single lung cell | GEO | GSE52583 | ⁶⁹ |
| 12 | Single cortex cell | GEO | GSE52525 | ⁷⁰ |
| 13 | Single hippocampus cell | GEO | GSE52525 | ⁷⁰ |
| 14 | Single spleen cell | GEO | GSE54006 | ⁷¹ |
| 15 | Single embryonic cell | GEO | GSE45719 | ³³ |