Supplemental Information

The Germ Cell Determinant Blimp1 Is Not Required for Derivation of Pluripotent Stem Cells

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INVENTORY OF SUPPLEMENTAL INFORMATION

Figure S1 (related to Figure 2)
Figure S2 (related to Figure 3)
Table S1 (related to Figure 1)
Table S2 (related to Figure 1)
Table S3 (related to Figure 2)
A

| Epiblast | EpiSC | Genotype | rESC |
|----------|-------|----------|------|
| BL2      | √     | -/-      | √    |
| BL3      | √     | +/-      | √    |
| BL4      | √     | +/-      | ND   |
| BL5      | √     | +/-      | ND   |
| 2BL1     | √     | +/-      | ND   |
| 2BL2     | x     | x        | x    |
| 2BL3     | x     | x        | x    |
| 2BL4     | √     | +/-      | ND   |
| 2BL5     | √     | +/-      | ND   |
| 2BL6     | √     | +/-      | √    |
| 2BL7     | √     | +/-      | √    |
| 2BL8     | √     | +/-      | ND   |

B

C

D

E

Correlation = 0.98
31 genes (>2-fold up)
104 genes (>2-fold down)

Correlation = 0.88
2 genes (>2-fold up)
81 genes (>2-fold down)

Correlation = 0.98
21 genes (>2-fold up)
86 genes (>2-fold down)
Figure S1. Characterisation of Blimp1 -/- EpiSC and their reversion to rESC.
(A) Table of EpiSC derivation from E6.5 epiblasts obtained from Blimp1 heterozygous
intercrosses. 2 null, 2 heterozygous and 1 wildtype EpiSC were successfully reverted to
rESC. ND = not done. (B) Representative genotyping results for 4 of the lines shown in
(A). WT = wildtype band. (C) Representative images of emerging rESC on day 19 and 21
of reversion. (D) Immunostaining of Blimp1 -/- EpiSC and rESC for Oct4 and H3K27me3.
H3K27me3 nuclear foci can be observed in Oct4-positive cells in EpiSC cultures
(examples denoted by arrowheads) but not rESC derived from them. DNA
counterstained with DAPI. Oct4-negative cells are feeders. Scale bars: 20 µm. (E)
Scatter plot analysis of microarray data comparing Blimp1 null and heterozygous epiSC
lines.
Figure S2. Blimp1 null rESC can generate overtly normal E8.5 embryos in tetraploid rescue experiments. (A) E8.5 embryos obtained following injection of Blimp1 null rESC into tetraploid blastocysts. Stained with alkaline phosphatase.

(B) Summary of a tetraploid injection experiment comparing Blimp1 null rESC with wildtype control rESC.

|                  | No. of injected 4N blastocysts | No. of implantation sites | No. of E8.0 - 8.5 embryos |
|------------------|-------------------------------|---------------------------|---------------------------|
| Blimp1 -/- rESC  | 47                            | 29 (62%)                  | 7 (24%)                   |
| Wild type rESC   | 35                            | 23 (66%)                  | 6 (26%)                   |
**Table S1.** Summary of an independent ES cell derivation experiment from a Blimp1+/- mating.

| Embryo | ESC line (Yes/No)? | Blimp1 genotype |
|--------|--------------------|-----------------|
| 1      | Yes                | +/+             |
| 2      | Yes                | +/+             |
| 3      | Yes                | +/+             |
| 4      | Yes                | +/-             |
| 5      | Yes                | +/-             |
| 6      | Yes                | +/+             |
| 7      | Yes                | +/-             |
| 8      | Yes                | +/+             |
| 9      | Yes                | +/-             |
| 10     | Yes                | +/+             |

**Table S2.** Chimera generation from Blimp1-/- ESC

| Blimp1 -/- ESC clone | No. blastocysts injected | No. born | No. chimaeras | Germline transmission? |
|----------------------|--------------------------|----------|---------------|------------------------|
| 1                    | 25                       | 8        | 6             | No                     |
| 2                    | 23                       | 5        | 4             | No                     |

**Table S3.** Sequences of qPCR primers

| qPCR Primers | Primer Sequences |
|--------------|------------------|
| Gapdh        | CATGGCCTTCCTGGTTCCT | GCGGCACGTGACATCCA |
| Oct4 (Pou5f1)| TGGATCCTCGAACTGGCTA | CCTCCGGCGAAGCTGATG |
| Nanog        | AATGCTGTACGACCTCATAA | TAAAATGCGCATGGCTTTCC |
| Klf2         | TAAAGGCGCATCTCGTACA | CGACAAGTGGCAGTGAAAG |
| Fgf5         | AAACCTCATGCAAGTGCAAAAT | TCTCGGCTGTCTTTTCAGTTC |
| Klf4         | GCACACCTGCAGTGGCACTACAC | GTTTGCCTGTGGCTGGTC |
| Foxa2        | TCTCCGTGTACGAGGCAAA | AGGCAGGTGCTCCCTTAGC |