

**Fig S1.** Representative images showing immunostaining of differentiated EPS cells by EB assay. EB was immunostained by anti-FOXA2, anti- $\beta$ -III TUBULIN, anti-GATA3, anti-EOMES and anti-CDX2 antibodies.

**Fig S2.** The generation of *IL6* knock-in EPS cells. Diagrams of the generation human *IL6* gene knock-in EPS cells. Primers for knock-in detection are indicated as pairs of arrows.

**Fig S3. (A)** Off-target effects of human *IL3* knock-in EPS cells. Left, T7E1 assay. Right, sequencing (20 clones per sample). **(B)** Off-target effects of human *IL6* knock-in EPS cells. Left, T7E1 assay. Right, sequencing (20 clones per sample).

**Fig S4.** Representative images showing immunostaining of EPS and EPS-*IL3* with lineage-specific markers. Error bar, 25  $\mu$ m.

**Fig S5.** ES-IL3 cells cultured in 2i condition were generated using the same gene targeting strategy with EPS-IL3 cells. Two ES-IL3 sub-lines were selected for tetraploid complementation experiments at passage 20. 46 blastocysts were injected with ES-IL3 cells and surrogate mothers were sacrificed on day E10.5. (A) Placenta obtained from tetraploid assay using ES-IL3 cells, E10.5. (B) The development of EPS-IL3 and ES-IL3 cells in tetraploid blastocysts.

Fig. S1

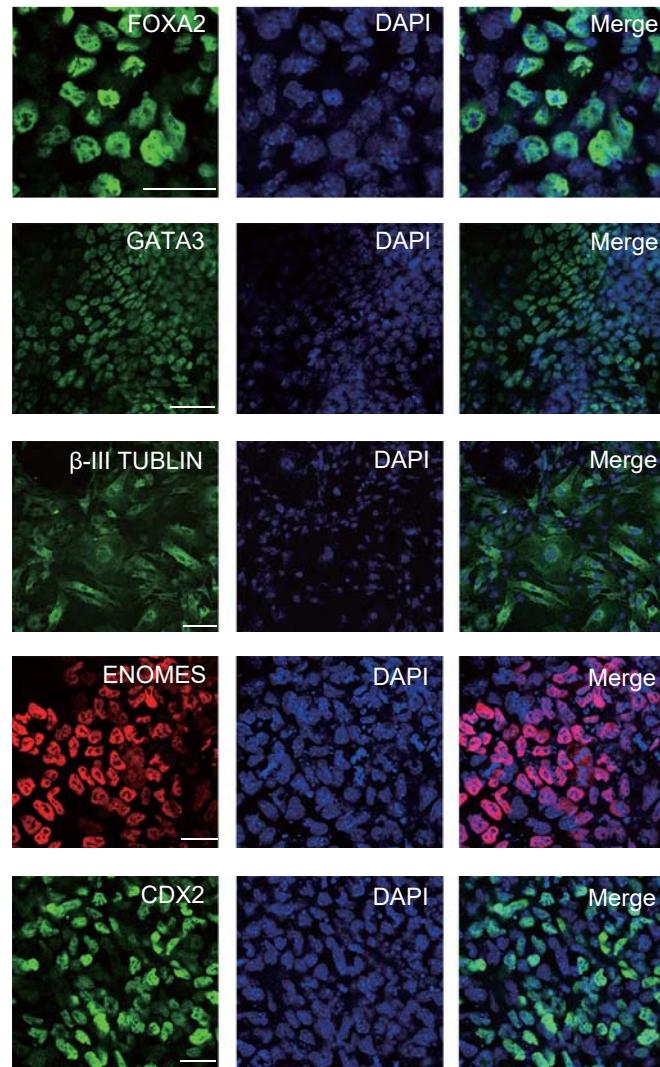


Fig. S2

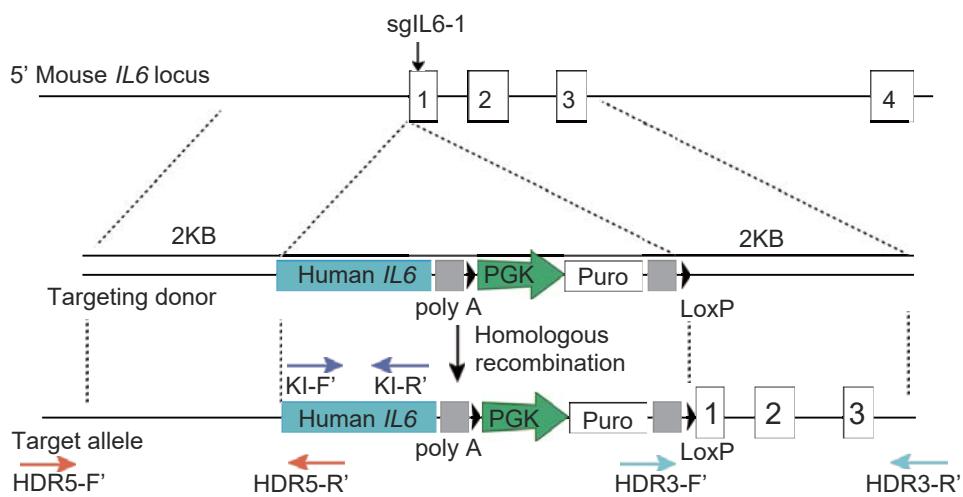


Fig. S3

A Mouse *IL3* locus

5' GATGCTGGTGGTAGAGCTGGCAAGAACCATTTGTCGTTCTGGTCCCTCCAAGGGGTTCTGAAGAAG 3'  
 target PAM

offtarget 1 GTGGATACATTGTCGTTCAGG

offtarget 2 TTTTGGATATTGTCGTTCAGG

off-target 1

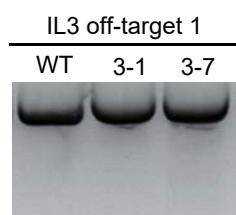
WT CTGGGGGAGGTGGGATACATTGTCGTTCAGGGCTAGACACTCACA

3-1 OFF 1-1 CTGGGGGAGGTGGGATACATTGTCGTTCAGGGCTAGACACTCACA

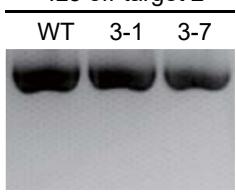
3-1 OFF 1-2 CTGGGGGAGGTGGGATACATTGTCGTTCAGGGCTAGACACTCACA

3-7 OFF 1-1 CTGGGGGAGGTGGGATACATTGTCGTTCAGGGCTAGACACTCACA

3-7 OFF 1-2 CTGGGGGAGGTGGGATACATTGTCGTTCAGGGCTAGACACTCACA



IL3 off-target 2



B Mouse *IL6* locus

5' TGCCTCACACTCCTCTCTCACGTCTCAATAGCTCCGCCAGAGGG CAAGGAAC TGCTTCACTTACT 3'  
 target PAM

offtarget 1 GTGATCCTAGCTCCGCCAGTGGG

offtarget 2 GTAAGCAGGGCTCCGCCAGAGGG

off-target 1

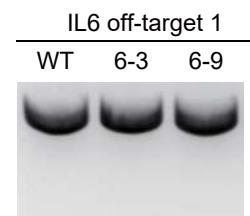
WT CAACTCTTGTGATCCTAGCTCCGCCAGTGGG TGTGATGCAGTATC

6-3 OFF 1-1 CAACTCTTGTGATCCTAGCTCCGCCAGTGGG TGTGATGCAGTATC

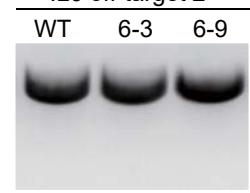
6-3 OFF 1-2 CAACTCTTGTGATCCTAGCTCCGCCAGTGGG TGTGATGCAGTATC

6-9 OFF 1-1 CAACTCTTGTGATCCTAGCTCCGCCAGTGGG TGTGATGCAGTATC

6-9 OFF 1-2 CAACTCTTGTGATCCTAGCTCCGCCAGTGGG TGTGATGCAGTATC



IL6 off-target 2



off-target 2

WT GAGCCAGTAAGCAGGGCTCCGCCAGAGGG GAGTGCTCAAAGGACA

6-3 OFF 2-1 GAGCCAGTAAGCAGGGCTCCGCCAGAGGG GAGTGCTCAAAGGACA

6-3 OFF 2-2 GAGCCAGTAAGCAGGGCTCCGCCAGAGGG GAGTGCTCAAAGGACA

6-9 OFF 2-1 GAGCCAGTAAGCAGGGCTCCGCCAGAGGG GAGTGCTCAAAGGACA

6-9 OFF 2-2 GAGCCAGTAAGCAGGGCTCCGCCAGAGGG GAGTGCTCAAAGGACA

Fig. S4

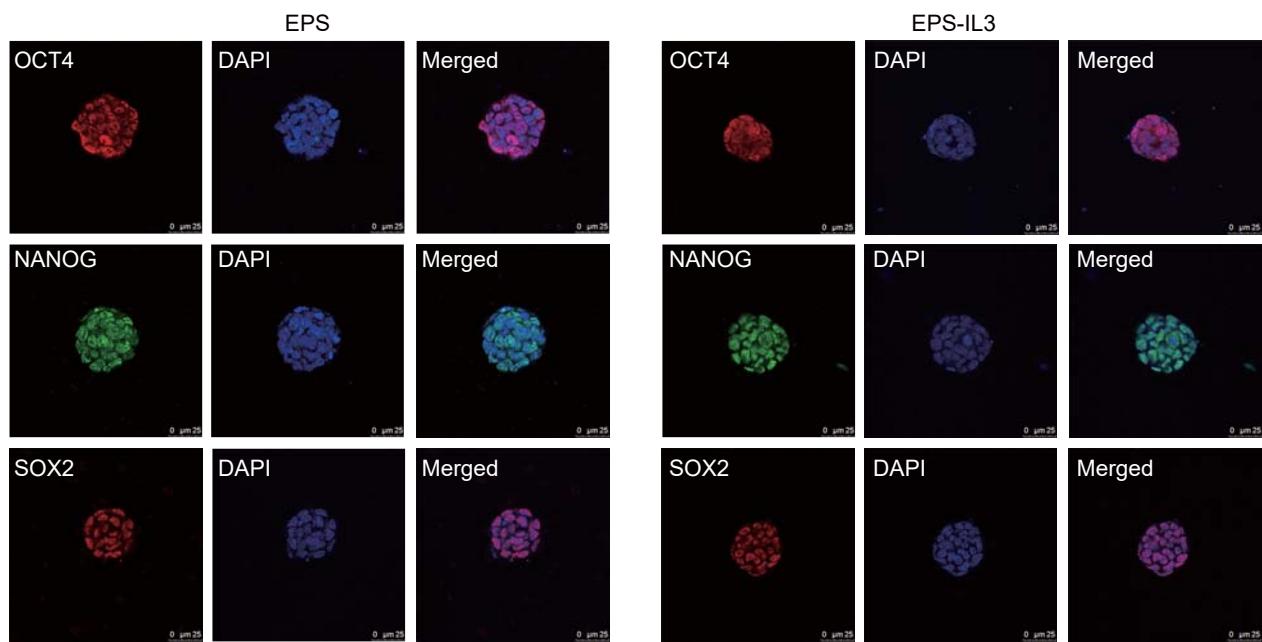


Fig. S5

