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Supplemental Information

Rebuilding Pluripotency from Primordial Germ Cells

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Inventory of Supplemental Information

File contains;

Figure S1 – related to Figure 1

Figure S2 – related to Figure 3

Figure S3 – related to Figure 4

Table S1 – related to Figures 1,2 and S1

and

Move S1 legend – related to Figure 2

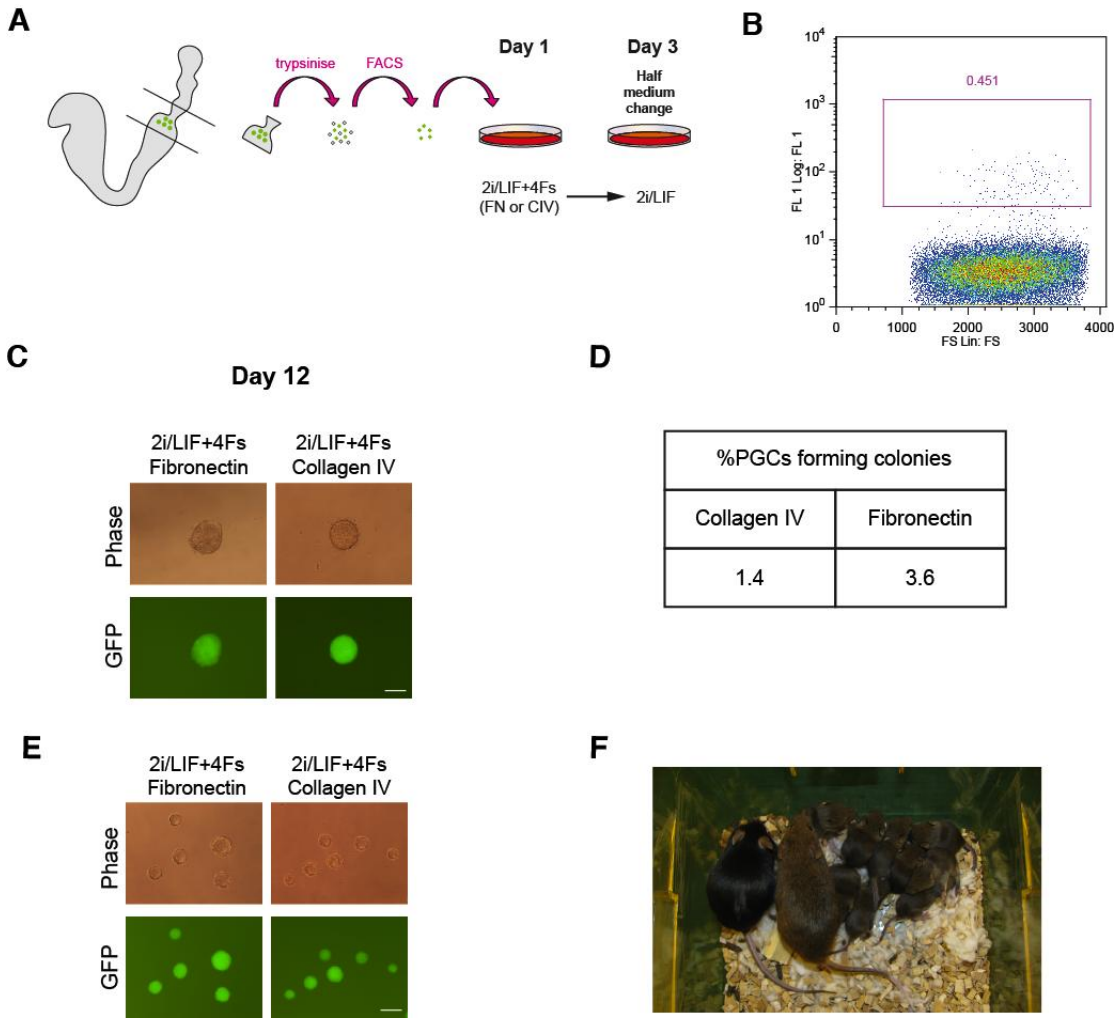

Figure S1. A defined system for EG cell derivation, related to Figure 1

(A) Schematic of derivation protocol incorporating flow cytometry to isolate PGCs prior to plating. FN=fibronectin, CIV=collagen IV. (B) FACS-plot showing gated GFP-positive E8.5 PGCs isolated from *Oct4*-ΔPE-GFP embryos. (C) Phase contrast and fluorescence images of EG cell colonies on day 12 (D) Table showing derivation efficiency on fibronectin and collagen IV. (E) Phase contrast and fluorescence images of EG cell lines derived from picked colonies. (F) An adult chimaera obtained by injecting EG cells (brown) into C57BL/6 blastocyst (black), with C57BL/6 mate and all brown litter indicating successful germline transmission. Scale bars, 100μM.

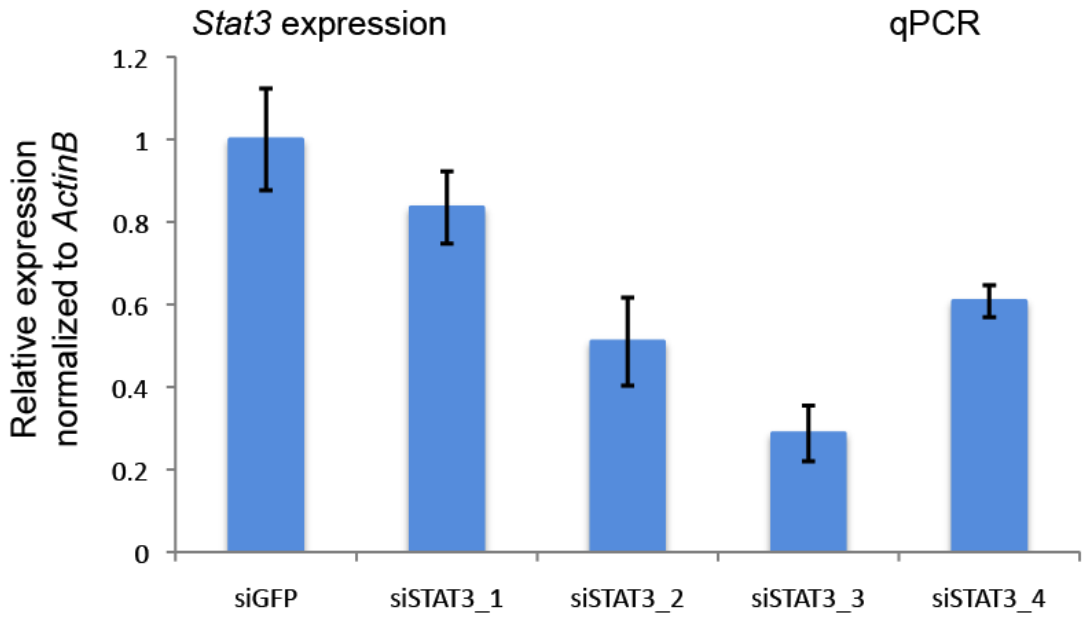

Figure S2. Validation of the STAT3 siRNAs, related to Figure 3

ES cells were transfected with the indicated siRNAs and analyzed after 24h by qPCR for *Stat3* expression. *ActinB* served as an endogenous control and data are normalized to siGFP transfected cells. Data in the figure are average and standard deviation of 2 independent experiments

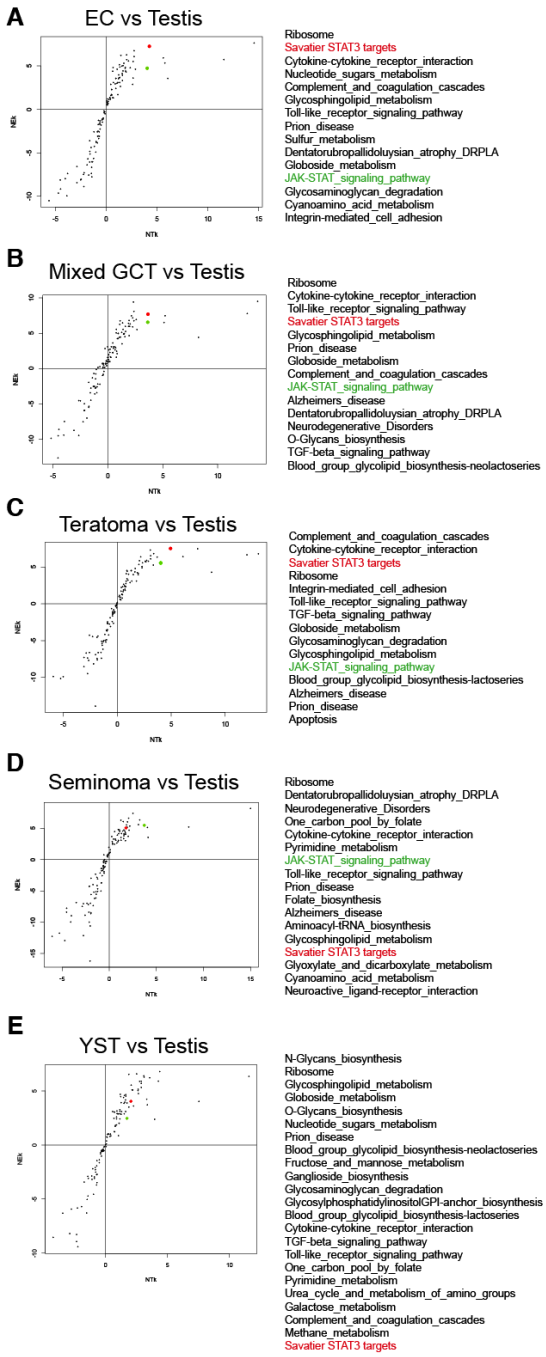

Figure S3. Expression of STAT3 target genes in GCT tumour subtypes, related to Figure 4

NTk and NEk values for KEGG pathways enriched in (A) EC, (B) mixed GCT, (C) Teratoma, (D) Seminoma and (E) YST over normal testis. The most enriched pathways in each comparison are shown below. Stat3 target genes were fed into the algorithm as a KEGG pathway ("Savatier STAT3 targets" – coloured red) for unbiased analysis of enrichment over other pathways. The standard JAK-STAT KEGG pathway is shown in green.

Table S1.

Summary of blastocyst injections and germline transmission of EG cells, related to Figures 1, 2 and S1.

EGC line	No. blastocysts injected	No. born	No. chimaeras	Germline transmission?
Figure 1	30	15	5	Yes
Figure 2	28	7	4	Yes
Figure S1	45	9	5	Yes

Movie S1.

Timelapse movie showing a single PGC giving rise to nine separate EG cell colonies, related to Figure 2.