Scale bar = 50 μm (D) PD0325901 can augment Nanog expression in STAT3$^{-/-}$ + DD-STAT3-S727A mES cells.

Figure 6. Proposed model for the role of differential STAT3 phosphorylation in regulating stem cell fates. In mESCs, activation of LIF/JAK signaling induces phosphorylation of STAT3 Y705 to maintain self-renewal. Upon withdrawal of LIF from the culture environment, mESCs differentiate into mEpiSCs concomitant with a switch from LIF/JAK-mediated phosphorylation of Y705 to FGF/Erk-mediated phosphorylation of S727. In the mEpiSC stage, STAT3 pS727 promotes neural commitment and secures this differentiation-primed state by inhibiting JAK/pY705-induced reprogramming

Supplementary figures

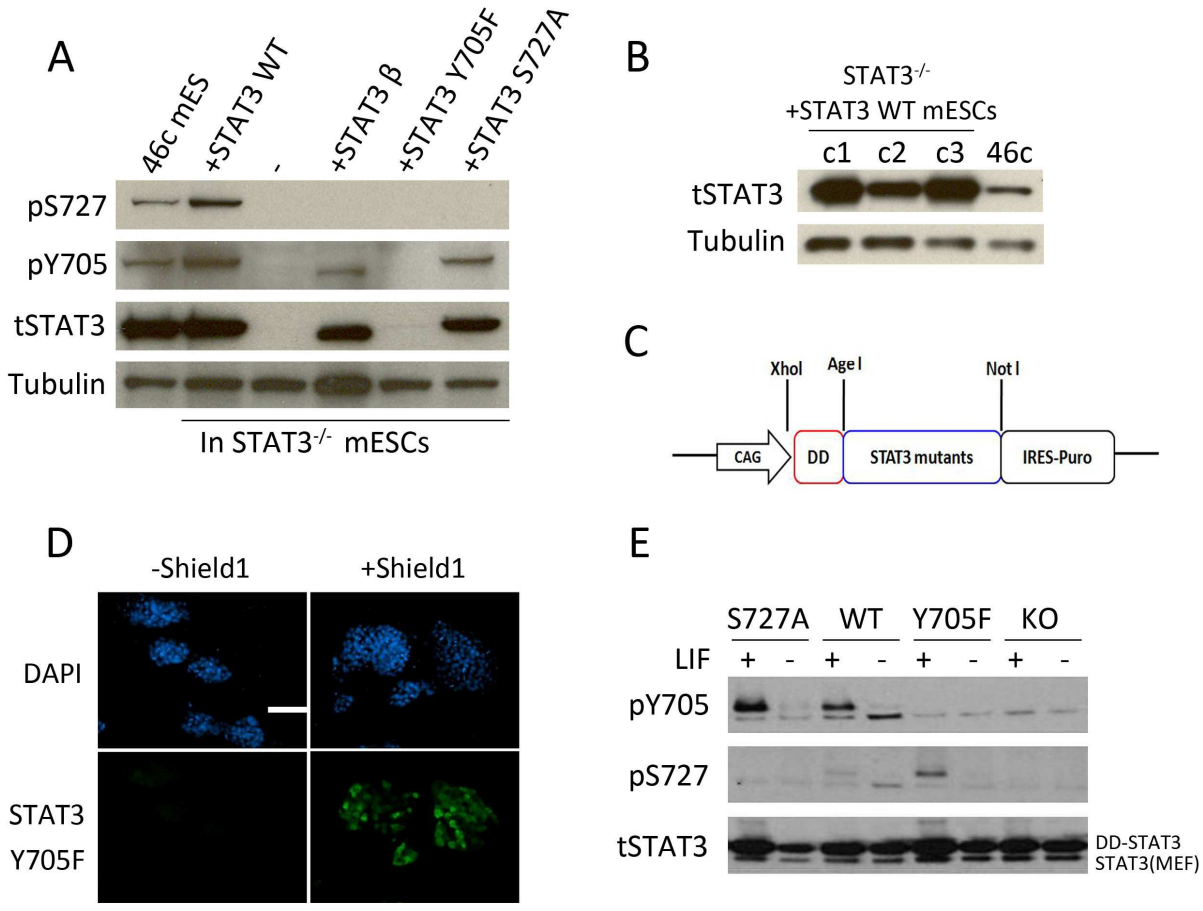

Figure S1. Stable transfection of STAT3 mutants into STAT3$^{-/-}$ mES cells led to aberrant expression of transgenes, so the DD-STAT3 system was used to fine-tune STAT3 quantity. (A) Cytotoxicity of STAT3-Y705F transgene in STAT3$^{-/-}$ mES cells. (B) Aberrant STAT3 expression level in stable STAT3$^{-/-}$ + STAT3-WT mES cells. (C) Diagram showing the DD-STAT3 expression vector used in the following experiments. (D) Immuno-fluorescence analysis of STAT3 (green) quantity modulated by S1. DAPI stains the nuclei (blue). STAT3$^{-/-}$ + DD-STAT3-Y705F mES cells were used as an example. Scale bar = 50 μm (E) Characterization of different STAT3$^{-/-}$ + DD-STAT3 mES cells. Cells were cultured in N2B27+2i+S1 medium and

stimulated with LIF for 1 hour before lysates were collected for immunoblotting with pSTAT3 antibodies.

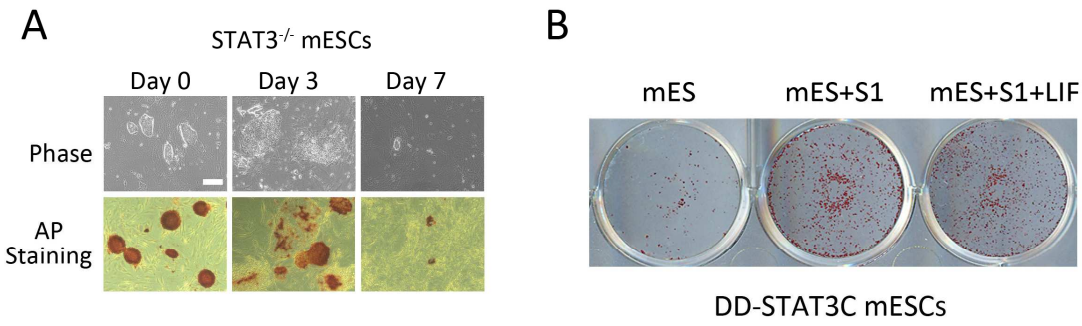

Figure S2. Self-renewal potential of STAT3$^{-/-}$ and STAT3$^{-/-}$+ DD-STAT3C mES cells. (A) Alkaline phosphatase staining and phase contrast images of STAT3$^{-/-}$ mES cells, indicating spontaneous differentiation and death after LIF withdrawal. Scale bar = 50 µm (B) Alkaline phosphatase staining of STAT3$^{-/-}$ + DD-STAT3C mES cells, indicating self-renewal in the absence of LIF.

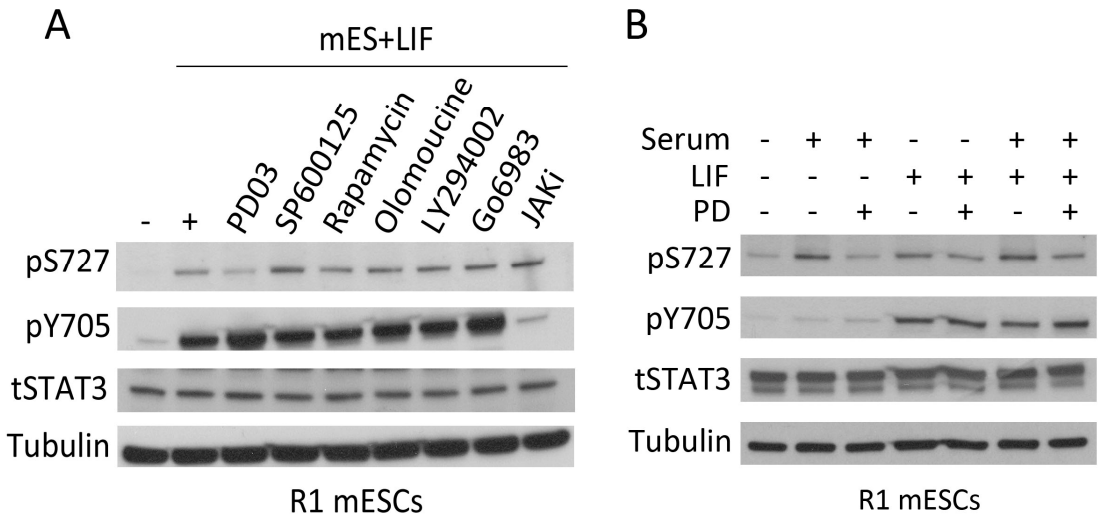

Figure S3. Serum-induced S727 phosphorylation of STAT3 was specifically inhibited by PD0325901 in R1 ES cells. (A) After overnight starvation with basal medium +S1, cells were pre-treated with various inhibitors for 2 hours, and then switched to mES+LIF for 1h before being lysed. (B) Serum and LIF stimulation was applied separately before immunoblot analysis.