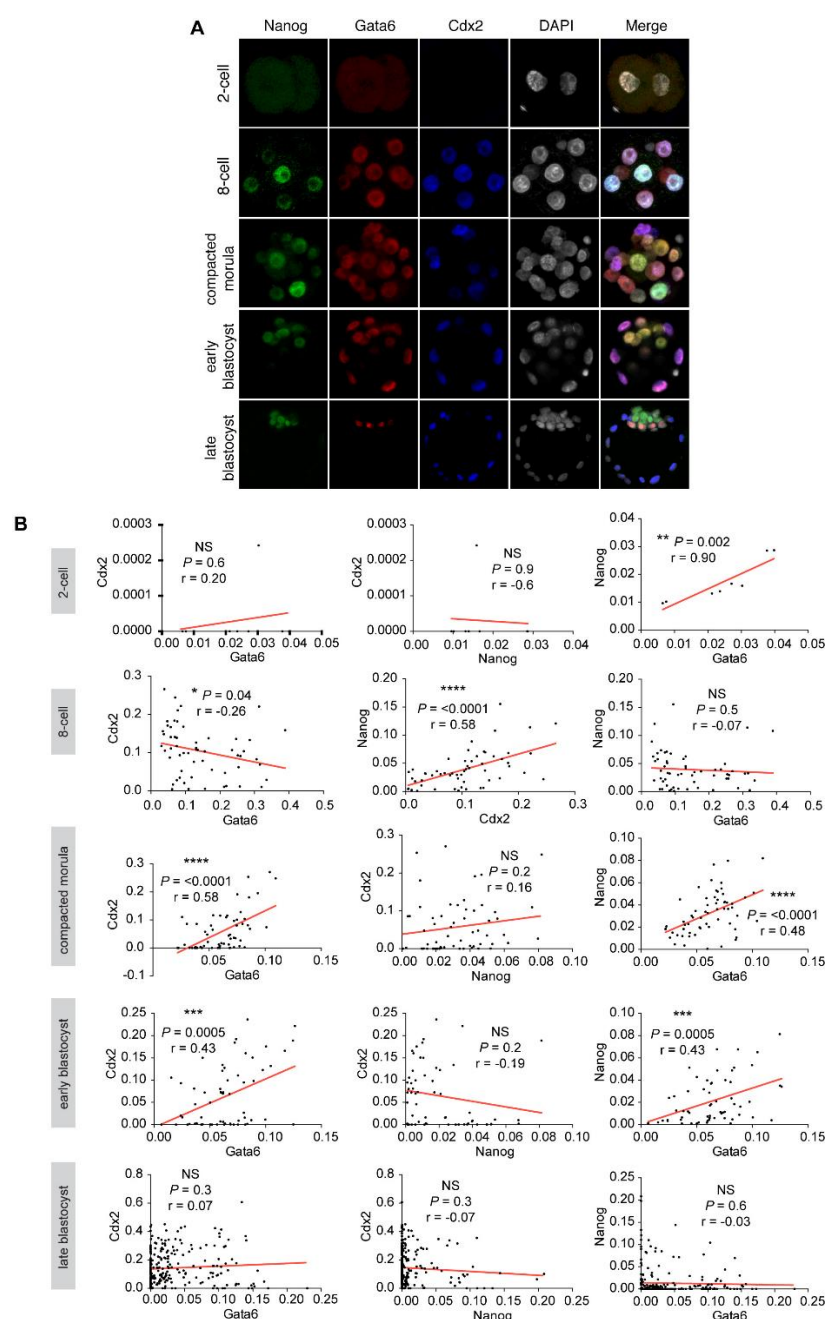
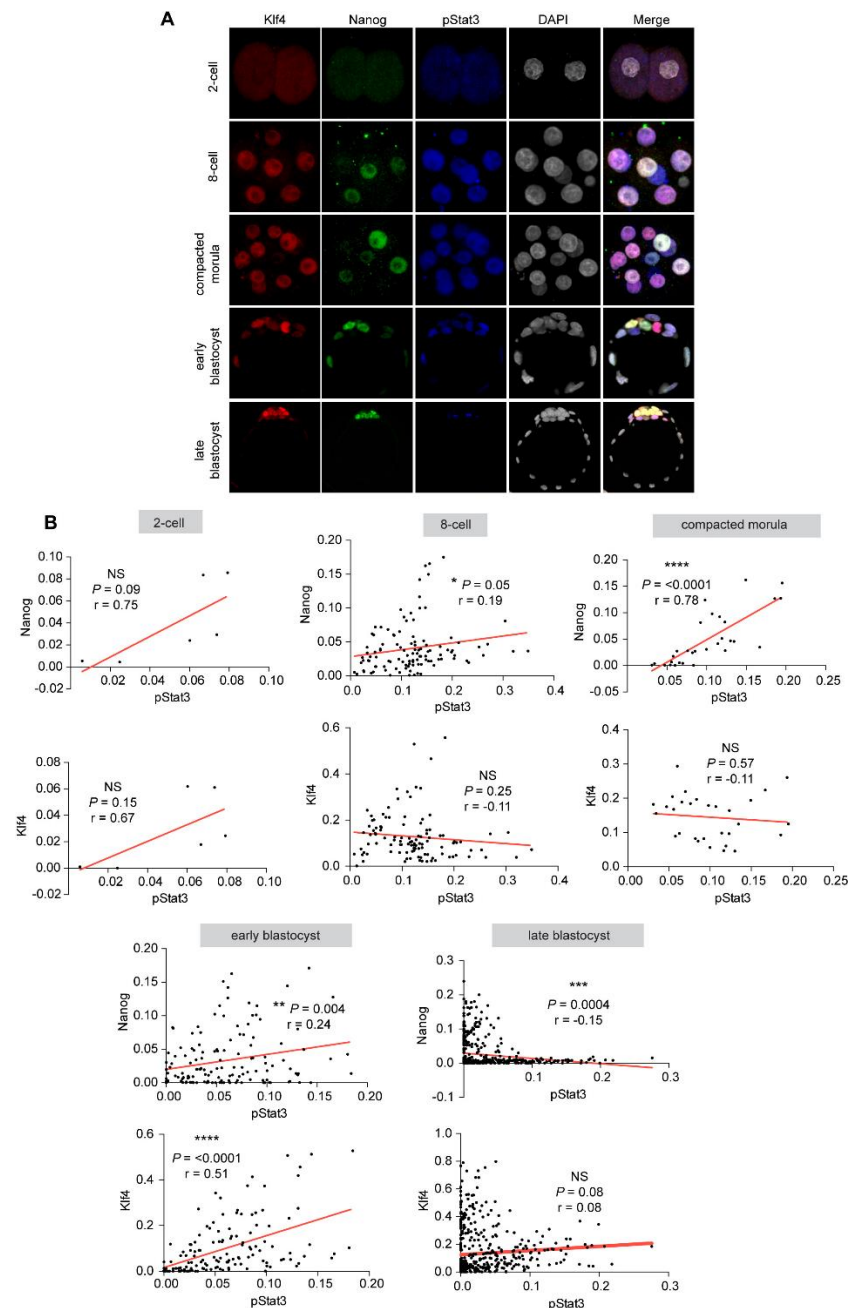


**Supplementary Figure 1. A.** HV ESCs were cultured in 2i or 2i/LIF for 3 passages. ESCs from 2i were then switched to 2i/LIF and the change in HV fluorescence was measured by flow cytometry each day. Data is shown relative to 2i/LIF mean fluorescence. Error bars represent the s.d. of 3 biological replicates. **B.** qRT-PCR time course of ESCs cultured in 2i for 3 passages before adding LIF for up to 3 passages. Data is shown relative to the geometric mean of the housekeeping genes, TBP and PGK1. Values represent the mean  $\pm$  the s.d. of 3 biological replicates of independent ESC lines. **C.** Histograms showing flow cytometry analysis of unstained ESCs or ESCs stained with an isotype control for the LIFR antibody as a control for LIFR flow cytometry in Fig. 1D,E. Only cells expressing the marker of undifferentiated ESCs, SSEA-1, were analysed for the expression of the LIFR. The top (HV<sup>+</sup>) and bottom (HV<sup>-</sup>) 10% of HV-expressing cells were selected and analysed for the expression of LIFR. Any difference in signal would correspond to autofluorescence.

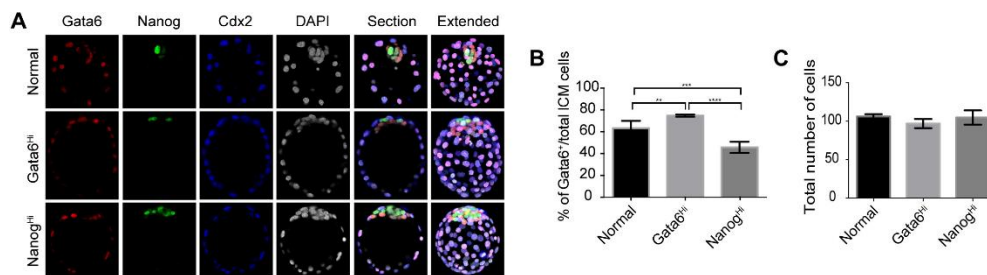


## Supplementary Figure 2. Validation of coexpression quantification methodology.

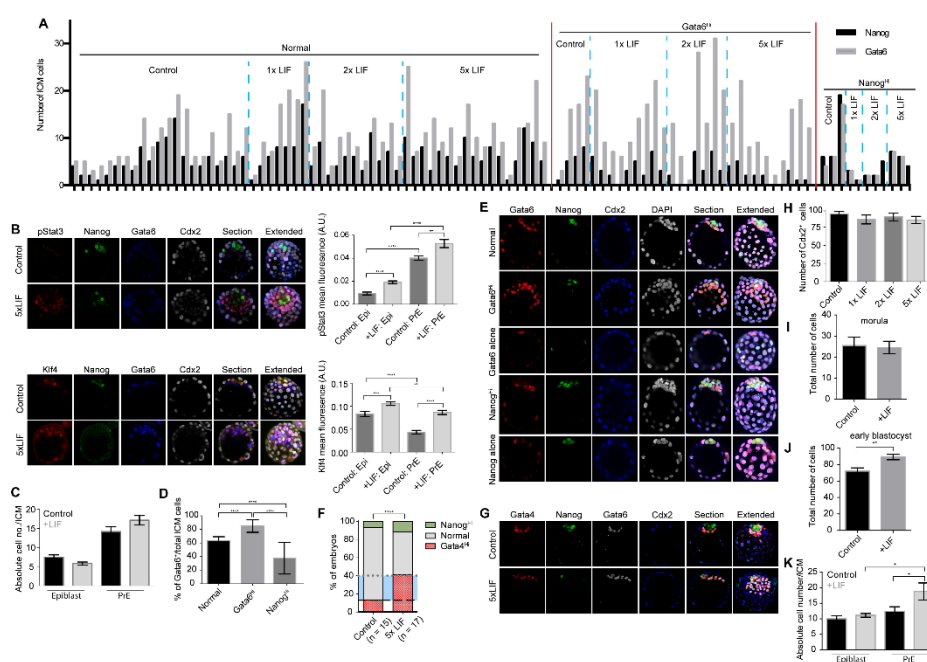
**A.** Immunostaining of embryos at different stages of pre-implantation development. Images from blastocyst-stage embryos represent confocal optical sections through the ICM while images of earlier stages are extended focus showing the entire embryo. **B.** Quantification of colocalisation of NANOG, GATA6 and CDX2 during pre-implantation development. CellProfiler was used to quantify immunostaining in individual cells. Cell nuclei were identified by manual selection and the mean pixel intensity measured in arbitrary units of intensity (a.u.). Each point represents the intensity of the noted markers within a single nucleus. Linear regression line is shown in red.  $P$  values indicate correlation.



**Supplementary Figure 3. Quantification of pSTAT3 and KLF4 expression during pre-implantation development.** **A.** Immunostaining of embryos at different stages of pre-implantation development. Images from blastocyst-stage embryos represent confocal optical sections through the ICM while images of earlier stages are extended focus showing the entire embryo. **B.** Quantification of colocalisation of NANOG, pSTAT3 and KLF4 during pre-implantation development. CellProfiler was used to quantify immunostaining in individual cells. Cell nuclei were identified by manual selection and the mean pixel intensity measured in arbitrary units of intensity (a.u.). Each point represents the intensity of the noted markers within a single nucleus. Linear regression line is shown in red. *P* values indicate correlation.

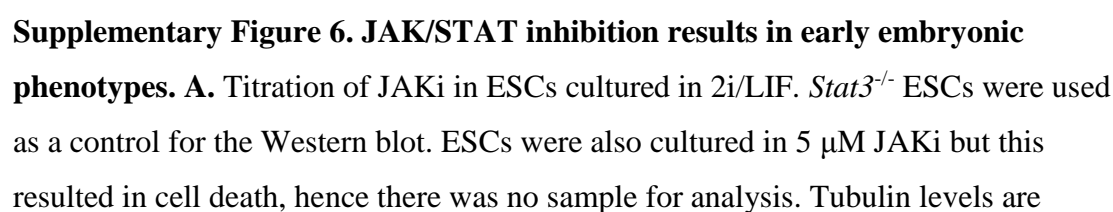


**Supplementary Figure 4. Variation in PrE vs. Epi normally observed within embryo culture.** Embryos were flushed from oviducts at E2.5 and cultured for 3 days in KSOM. **A.** Confocal optical sections through the ICM of late blastocysts immunostained for the 3 lineage markers, NANOG (Epi), GATA6 (PrE) and CDX2 (trophoblast) are shown for phenotype categories in control embryos. **B.** Graph displaying the % of GATA6<sup>+</sup> ICM cells in each category in control (KSOM-cultured) embryos. ‘GATA6<sup>Hi</sup>’/‘GATA6 alone’ and ‘NANOG<sup>Hi</sup>’/‘NANOG alone’ embryos were pooled. \*\* $P = 0.0025$ , \*\*\*  $P = 0.0003$ , \*\*\*\* $P < 0.0001$ , Student’s unpaired t-test. **C.** Graphs displaying the total number of cells within embryos of each category. Error bars indicate average  $\pm$  s.e.m.



**Supplementary Figure 5. LIF supports PrE cells *in vivo*.** Embryos were flushed from oviducts at E2.5 and cultured for 3 days in KSOM with 5000 U (5x) LIF. **A.** Graphs displaying absolute GATA6<sup>+</sup> and NANOG<sup>+</sup> cell numbers in the ICM of individual embryos in ‘normal’, ‘GATA6<sup>Hi</sup>’ and ‘NANOG<sup>Hi</sup>’ embryos. Red lines divide categories and blue dotted lines divide treatments. **B.** Confocal optical sections through the ICM of late blastocysts immunostained for the 3 lineage markers, NANOG (Epi), GATA4 (PrE) and CDX2 (trophoblast) and the LIF targets pSTAT3 or KLF4. An extended focus image shows the whole embryo. Graphs show the level of pSTAT3 and KLF4 upon LIF treatment, quantified using CellProfiler (see methods). Error bars represent average  $\pm$  s.e.m.  $**P = <0.01$ ,  $***P = <0.001$ ,  $****P = <0.0001$ , Student’s unpaired t-test **C.** Graph showing the absolute number of NANOG<sup>+</sup> Epi and GATA6<sup>+</sup> PrE ICM cells in embryos cultured in control conditions (KSOM) or with LIF E2.5 for 3 days. Error bars indicate average  $\pm$  s.e.m. **D.** Graph displaying the % of GATA6<sup>+</sup> ICM cells in each category in LIF-treated embryos. ‘GATA6<sup>Hi</sup>’/‘GATA6 alone’ and ‘NANOG<sup>Hi</sup>’/‘NANOG alone’ embryos were pooled.  $**P = 0.0025$ ,  $***P = 0.0003$ ,  $****P < 0.0001$ , Student’s unpaired t-test. **E.** Confocal optical sections through the ICM of late blastocysts immunostained for the lineage markers, NANOG (Epi), GATA6 (PrE) and CDX2 (trophoblast) are shown for phenotype categories in LIF-treated embryos. **F-G.** Embryos were flushed from

oviducts at E2.5 and cultured for 3 days in KSOM with 5000 U (5x) LIF. **F.** Graph showing categorisation of embryos immunostained for GATA4 as a marker of PrE.. The ICM of embryos was analysed based on the proportion of GATA4<sup>+</sup> and NANOG<sup>+</sup> cells. Normal embryos are those with the same proportions of PrE and Epi cells as the proportions +/- the s.d. in control (KSOM-cultured) embryos. GATA4<sup>Hi</sup> or NANOG<sup>Hi</sup> categories correspond to embryos that fell outside of the average control proportions +/- the s.d. due to having more GATA4<sup>+</sup> or NANOG<sup>+</sup> cells. The number of embryos analysed is shown below each bar. The dashed black line indicates the proportion of GATA4<sup>Hi</sup> embryos in control conditions. The dotted line indicates the number of embryos with high levels of GATA4. \*\*\*\* $P < 0.0001$ , two-tailed chi-square test. **G.** Confocal optical sections through the ICM of late blastocysts immunostained for the 3 lineage markers, NANOG (Epi), GATA4 (PrE) and CDX2 (trophoblast). An extended focus image also shows the whole embryo. **H.** Graph displaying number of CDX2<sup>+</sup> trophoblast cells within each condition. Error bars indicate average +/- s.e.m. **I-J.** Graph showing the total number of cells in embryos cultured in KSOM or KSOM + 5x LIF for 1 day (**I**) or 2 days (**J**). \*\* $P = < 0.01$ , Student's unpaired t-test. Error bars indicate average +/- s.e.m. **K.** Graph showing the absolute number of NANOG<sup>+</sup> Epi and GATA4<sup>+</sup> PrE ICM cells in embryos cultured in control conditions (KSOM) or with LIF E2.5 for 2 days. Error bars indicate average +/- s.e.m. \* $P = < 0.05$ , Student's unpaired t-test.



shown as a loading control. **B.** Confocal optical sections through ESC colonies immunostained for pSTAT3, KLF4 and NANOG after treatment for 4 days in JAKi. *Stat3*<sup>-/-</sup> ESCs were used as a control for antibody specificity. At 5  $\mu$ M JAKi, the majority of the ESC culture underwent cell death. A representative cluster of ESCs is shown where nuclei are enlarged and beginning to fragment. **C-E.** Embryos were flushed from oviducts at E2.5 and cultured for 3 days in KSOM alone or KSOM with 500 nM JAKi. Confocal optical sections through the ICM of late blastocysts immunostained for the 3 lineage markers, NANOG (Epi), GATA6 (PrE), CDX2 (trophoblast) and pSTAT3 (**C**) or KLF4 (**D**). **E.** Representative embryos for the JAKi phenotype categories quantitated in Fig. 5B. ‘? cells’ refers to embryos where cells were present within the ICM that expressed neither NANOG, GATA6 nor CDX2. **F.** Graphs displaying absolute GATA6<sup>+</sup> and NANOG<sup>+</sup> cell numbers in the ICM of individual embryos in ‘normal’, ‘GATA6<sup>Hi</sup>’, ‘NANOG<sup>Hi</sup>’, ‘Cavity’ and ‘? cells’ embryos. Red lines divide categories and blue dotted lines divide treatments. **G.** Embryos were flushed from oviducts at E2.5 and cultured for 3 days in KSOM alone or KSOM with 500 nM JAKi. Confocal optical sections through the ICM of late blastocysts immunostained for OCT4, NANOG (Epi) and GATA6 (PrE). **H.** Graph displaying number of CDX2<sup>+</sup> trophoblast cells within each condition. Error bars indicate average  $\pm$  s.e.m. **I.** Graphs displaying the total number of cells within embryos of each category. Error bars indicate average  $\pm$  s.e.m. **J-K.** Graph showing the total number of cells in embryos cultured in KSOM or KSOM + 500 nM JAKi for 1 day (**J**) or 2 days (**K**). Error bars indicate average  $\pm$  s.e.m. **L.** Graph showing categorisation of immunostained embryos. Embryos were cultured in KSOM or KSOM + 500 nM JAKi for 2 days. The ICM of embryos was analysed based on the proportion of GATA6<sup>+</sup> and NANOG<sup>+</sup> cells. Normal embryos are those with the same proportions of PrE and Epi cells as the proportions  $\pm$  the s.d. in control (KSOM-cultured) embryos. GATA6<sup>Hi</sup> or NANOG<sup>Hi</sup> categories correspond to embryos that fell outside of the average control proportions  $\pm$  the s.d. due to having more GATA6<sup>+</sup> or NANOG<sup>+</sup> cells. Cells that coexpressed NANOG and GATA6 were quantified in the same manner. The number of embryos analysed is shown below each bar. The dashed black line indicates the proportion of GATA6<sup>Hi</sup> embryos in control conditions. The dotted line indicates the average number of GATA6<sup>Hi</sup> embryos. \*\*\*\**P* < 0.0001, two-tailed chi-square test.