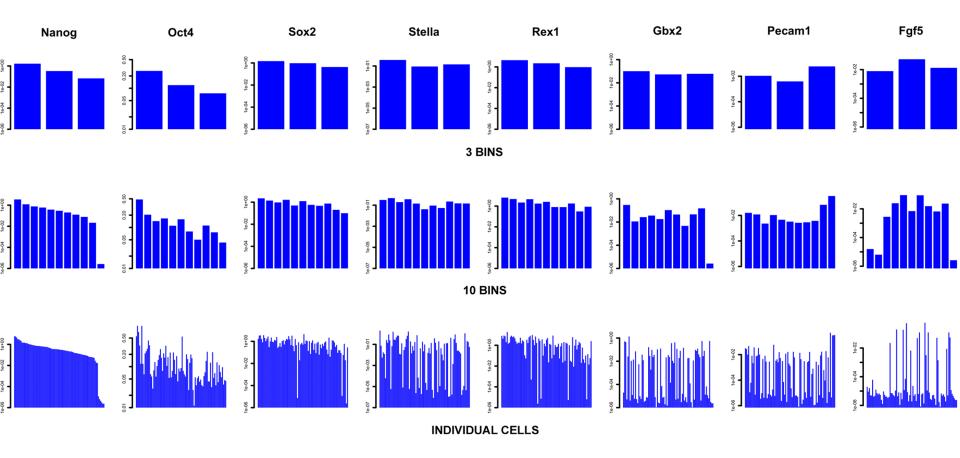
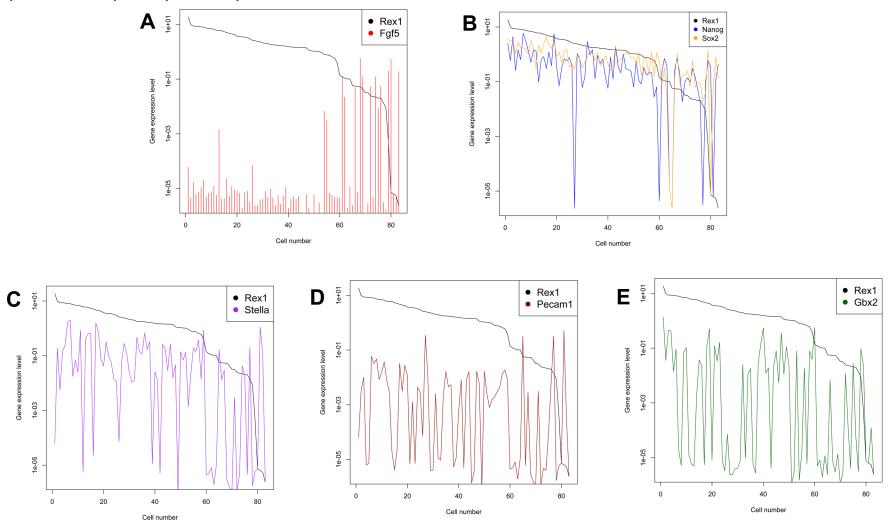


Supplementary figure 1: Expression level distribution of mRNAs in single mES cells. The expression level distribution of all the genes, with the exception of Oct4, appear to be bimodal, suggesting two distinct states: on and off. Recorded mRNA levels for each gene were normalised to the level of Gapdh mRNA in each cell before natural logarithms of the normalised values were calculated. The histograms above show the distributions of the natural logarithms of recorded mRNA levels in single mES cells cultured under conditions that promote pluripotency. Of the eight genes one is a marker of epiblast differentiation (Fgf5), whilst the other genes are associated with pluripotency.



Supplementary figure 2: Bar plots of Fgf5 and pluripotency gene expression in mES cells. The 83 mES cells were ordered according to decreasing Nanog expression (bottom row) then split into three (top row) or ten (middle row) equal groups. The average expression level of each gene in each group was calculated and plotted on one of the bar plots above. The bar plots in the bottom row contain gene expression levels in individual cells, ordered according to decreasing Nanog expression. The simple correlations between average expression levels observable in the top row of bar plots break down as the bins used to average similar expression levels become narrower and as one tends towards looking at single cells.



Supplementary figure 3: Expression levels of Fgf5 and pluripotency genes in single mES cells ordered by decreasing Rex1 expression. Cells were ordered according to decreasing Rex1 expression (black lines in all plots). Plotted in each panel are the mRNA levels of Fgf5 (A, red bars), Nanog (B, blue line), Sox2 (B, orange line), Stella (C, purple line), Pecam1 (D, dark red line) and Gbx2 (E, green line) mRNA in each cell were plotted. Fgf5 expression appears to correlate strongly with low levels of Rex1 expression (A), whilst expression of Nanog and Sox2 broadly, if inconsistently, correlate with Rex1 expression (B). Note that, although the levels of Pecam1, Stella and Gbx2 correlate with Rex1 at the population level, there is considerable variation at the single cell level (C-E).