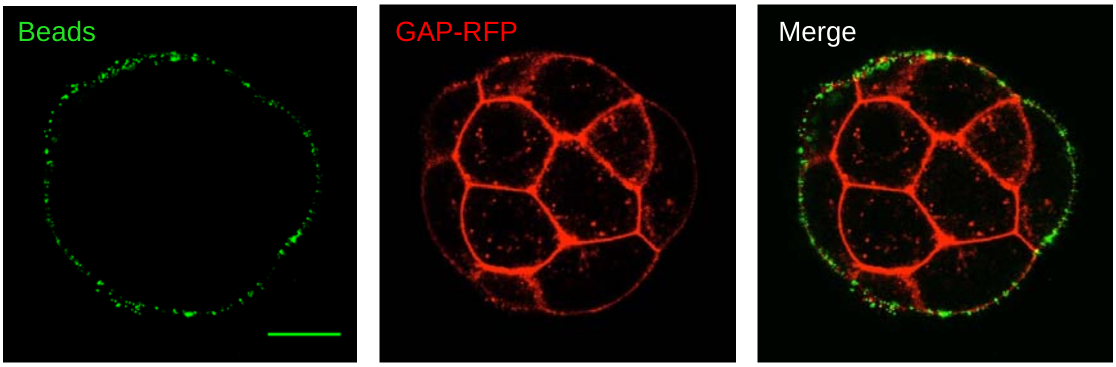
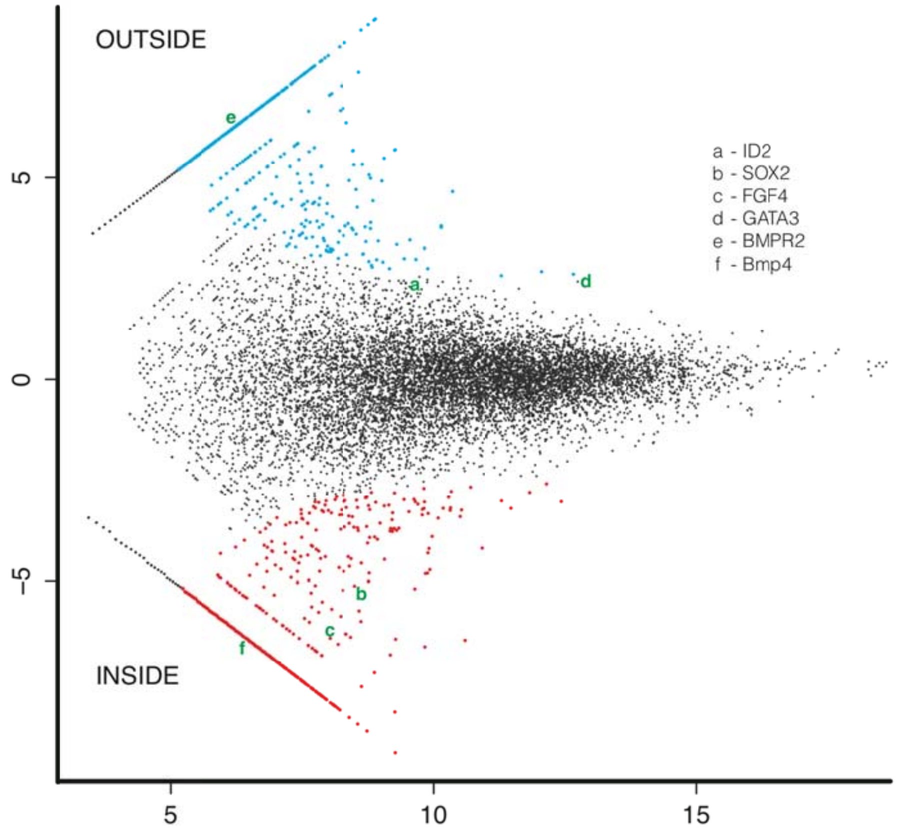
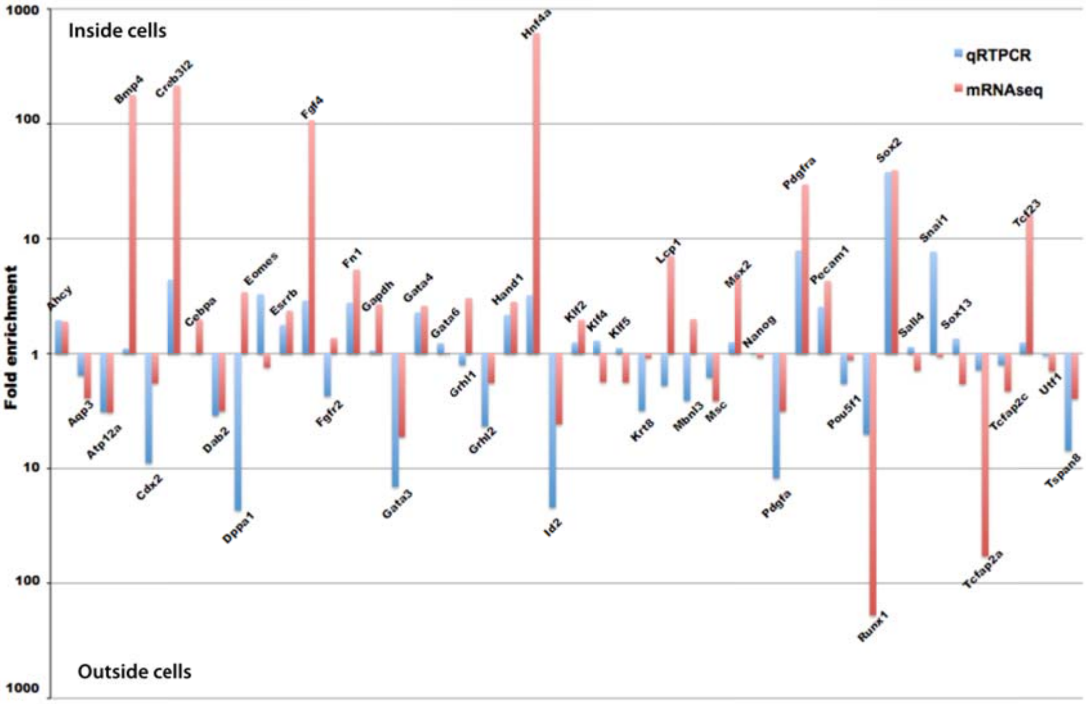


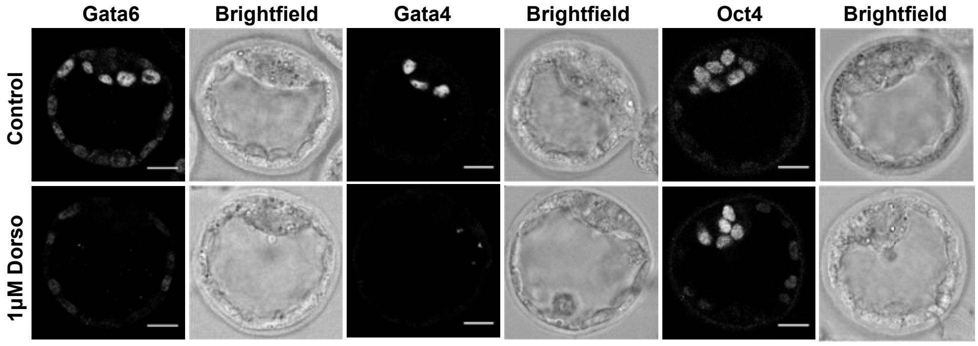
Supplementary Figure 1. Labelling of outside cells at the 16-cell stage. Example of 16-cell embryo that has been incubated in a suspension of 0.2 µm fluorescent beads. Scale bar = 20 µm. The embryo has been injected with *Gap43-RFP* mRNA so that cell boundaries can be clearly seen. The beads are only bound to outside cells that have an exposed surface.



Supplementary Figure 2. Differential gene expression in inside and outside cells. MA plot for hits of all identified genes in either of the cell types. Genes that had less than 5-fold differential expression between inside and outside cells are in black. Genes enriched by more than 5-fold in inside or outside cells are in red and blue, respectively.



Supplementary Figure 3. Confirmation of differential gene expression by qRT-PCR. qRT-PCR (blue bars) validation of the results obtained from mRNA-seq (red bars). Expression values given as the relative fold enrichment in cell type with higher levels over the level in other cell type. 74% of genes (34/46) show enrichment in the same cell type using both methods.



Supplementary Figure 4. Effect of Dorsomorphin treatment on expression of PE and ICM markers. Examples of expression of markers of the PE (Gata6 and Gata4) and ICM (Oct4) in embryos treated with 1.0 µM Dorsomorphin from E2.5 to E4.5. Scale bars = 20 µm**.**

Supplementary Table 1. Differential gene expression at the 16-cell stage. Numbers of genes with statistically significant (GFOLD p-value ≤ 0.01) value of particular fold enrichment in inside or outside cells. * For genes with hit count of 0, a value of 0.5 was used in the calculation.

Fold enrichment threshold*	Genes in inside cells	Genes in outside cells	Remaining genes
2	1,574	1,251	8,334
5	632	473	10,054
10	309	239	10,611
25	71	37	11,051
50	8	5	11,146