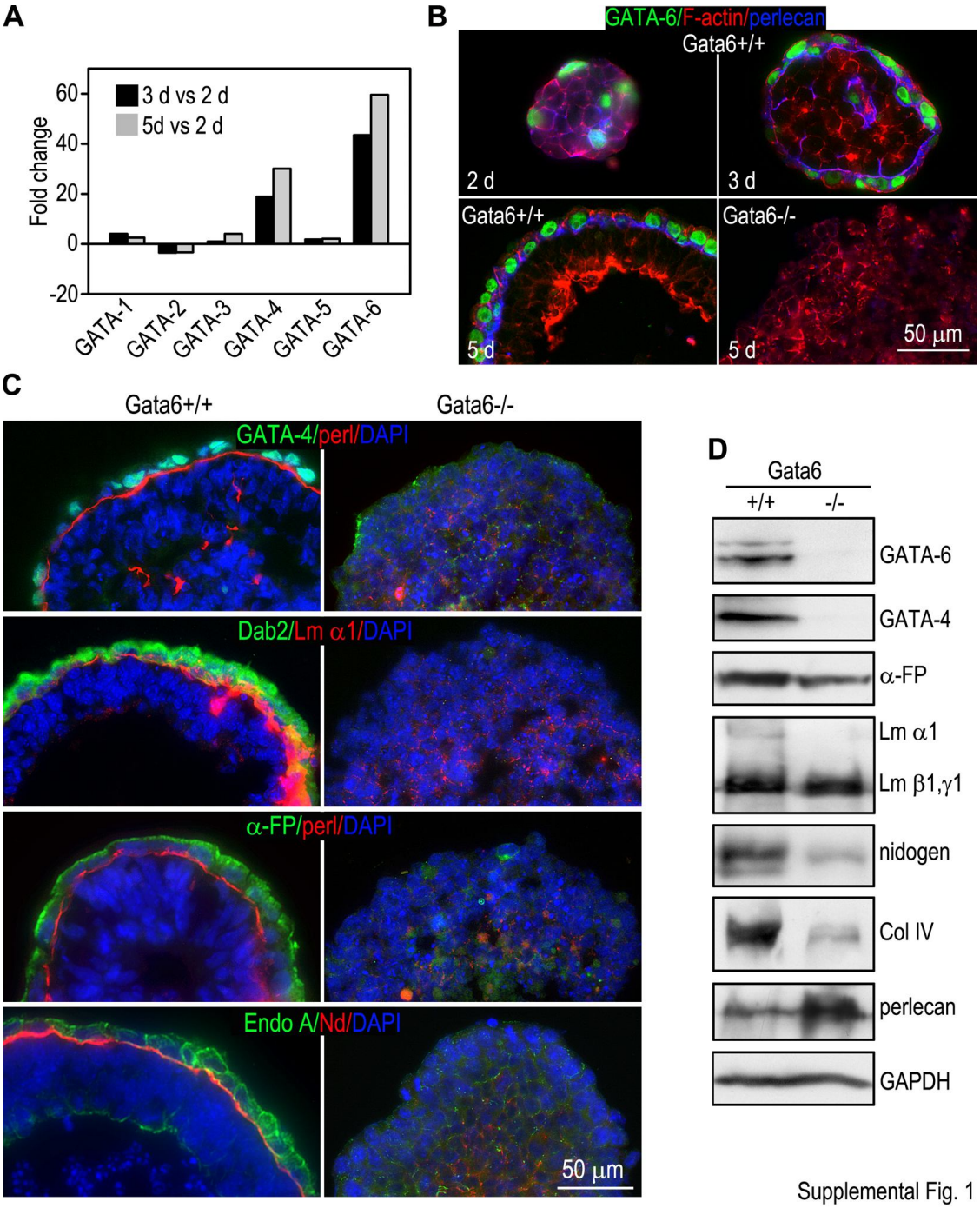
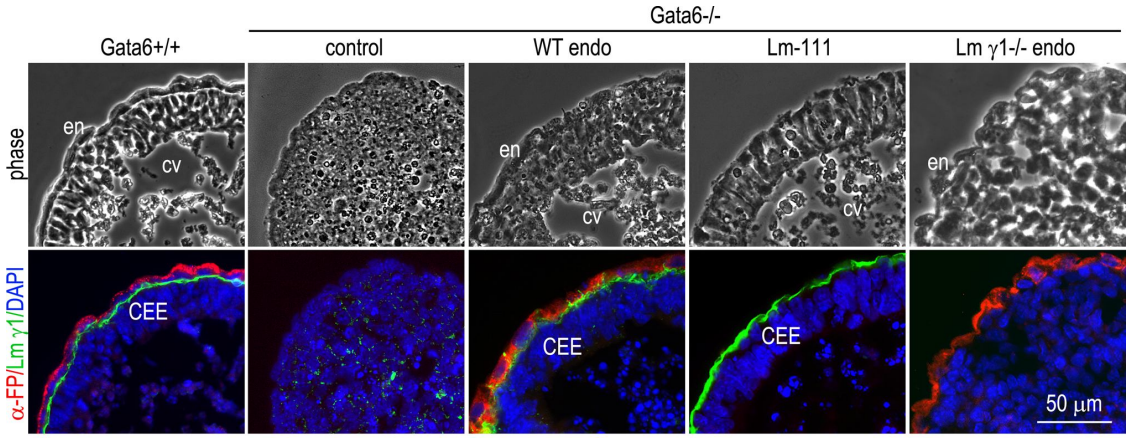


Supplemental Fig. 1

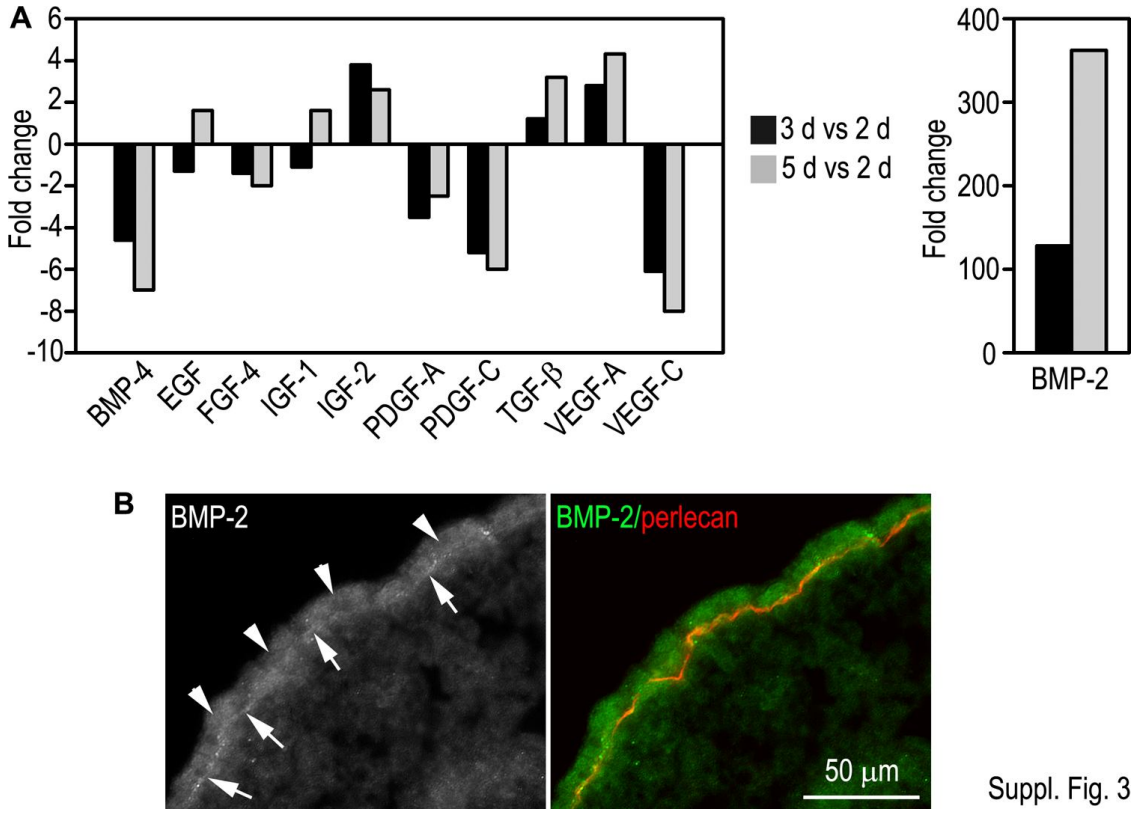
Supplemental Figure 1. Ablation of GATA-6 blocks endoderm differentiation and BM assembly. (A) Microarray analysis of mRNAs for GATA transcription factors revealed significant upregulation of GATA-4 and 6 during normal EB differentiation. The data shown are the average of two biological replicates. (B) Immunofluorescence microscopy showed that GATA-6 was expressed in the nucleus of normal endodermal cells. Gata6$^{-/-}$ EBs were used as negative controls. Rhodamine-phalloidin was used to stain F-actin to show the formation of an actin belt in 5-day normal EBs but not *Gata6*$^{-/-}$ EBs. (C) Immunostaining of 4-day EBs for the endoderm markers GATA-4, disabled-2 (Dab2), α-

fetoprotein (α-FP) and cytokeratin Endo A showed no endoderm formation in *Gata6*$^{-/-}$ EBs. BM was also absent from *Gata6*$^{-/-}$ EBs as evidenced by immunofluorescence of laminin α1 (Lm α1), perlecan (perl) or nidogen (Nd). (D) Immunoblots show that GATA-6 was not expressed in *Gata6*$^{-/-}$ EBs. The expression of the laminin α1 chain (Lm α1), nidogen, collagen IV (Col IV) and the endodermal markers GATA-4 and α-fetoprotein (α-FP) was reduced in *Gata6*$^{-/-}$ EBs.



Supplemental Fig. 2

Supplemental Figure 2. Contributions of endoderm-derived factors to epiblast polarization and cavitation. GATA-6 -/- EBs were treated with 100 μg/ml laminin (Lm)-111 or grafted with wild-type (WT) or Lm γ1-null endoderm (endo) cells and cultured for 5 days. *Gata6*$^{+/+}$ and untreated -/- EBs were used as controls. EBs were immunostained for α-fetoprotein and Lm γ1. Nuclei were counterstained with DAPI. Grafting normal but not Lm γ1-null endoderm cells onto GATA-6-null EBs induced basement membrane assembly, the formation of a columnar epiblast epithelium (CEE) and cavitation. Treatment of the null EBs with Lm-111 had a similar effect.



Suppl. Fig. 3

Supplemental Figure 3. BMP-2 is upregulated in endoderm and enriched in the basement membrane during EB differentiation. (A) Microarray analysis of mRNAs for growth factors showed significant upregulation of BMP-2 during normal EB differentiation. The data shown are the average of two detections. (B) Immunostaining of 4-day EBs showed that BMP-2 was mainly expressed in endoderm (arrowheads) of normal EBs and was enriched in the underlying BM (arrows).