Qi et al., http://www.jcb.org/cgi/content/full/jcb.201111063/DC1

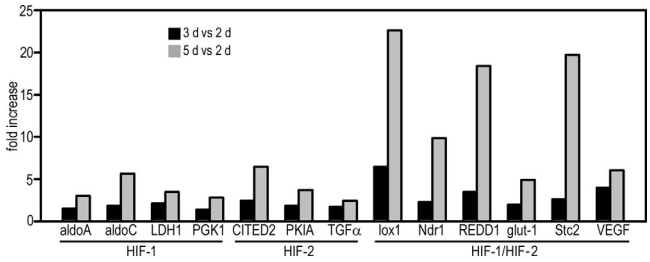

Figure S1. **Microarray analysis of hypoxia-induced genes during EB differentiation.** Normal EBs were cultured for 2, 3, and 5 d and analyzed by mRNA profiling. The fold increase of mRNA levels in 3- or 5-d EBs versus those in 2-d EBs was plotted. The data shown are the mean of two detections. aldoA, aldolase A; aldoC, aldolase C; LDH1, lactate dehydrogenase 1; PGK1, phosphoglycerate kinase 1; CITED2, Cbp/p300-interacting transactivator with Glu/Asp-rich C-terminal domain 2; PKIA, protein kinase inhibitor α; lox1, lysyl oxidase 1; Ndr1, N-myc downstream regulated 1; REDD1, regulated in development and DNA damage response 1; glut-1, glucose transporter–1; Stc2, stanniocalcin 2. The expression of aldoA, aldoC, LDH1, and PGK1 has been reported to respond only to HIF-1, whereas CITED2, PKIA, and TGFα respond only to HIF-2. Lox1, Ndr1, REDD1, glut-1, Stc2, and VEGF are regulated by both HIF-1 and HIF-2.

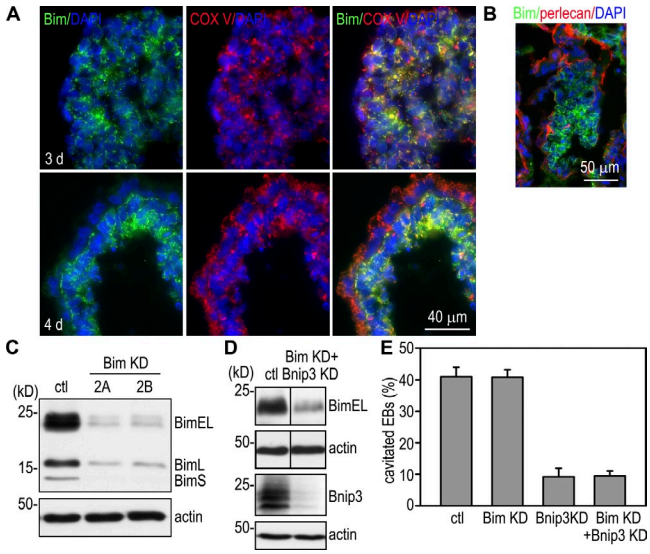

Figure S2. **Bim is not involved in EB cavitation.** (A) Normal EBs were cultured for 3 and 4 d and immunostained for Bim and mitochondrial complex V (COX V). Bim was mainly expressed in epiblast cells and colocalized with complex V. (B) E5.0 mouse embryo was immunostained for Bim and basement membrane perlecan. Bim was expressed in the epiblast cells in a punctate pattern. (C) Normal ES cells were stably transfected with Bim shRNAs (Bim knockdown [KD]) or the scrambled control (ctl). 3-d EBs were analyzed for Bim expression by immunoblotting. In clone 2A, Bim was reduced by 88% at the protein level. (D) Bnip3 knockdown ES cells were transfected with the Bim shRNA, and stable clones were selected based on RFP expression. Immunoblots show silencing of both Bim and Bnip3 in 3-d EBs. Black lines indicate that intervening lanes have been spliced out. (E) Cavitation of 4-d EBs was quantitated by phase microscopy. Knockdown of Bnip3 but not Bim markedly reduced the cavitation efficiency. However, knockdown of both Bnip3 and Bim did not further inhibit EB cavitation. $n = 5$ independent experiments with a total of 432–501 EBs for each group. Error bars represent the mean ± SD.

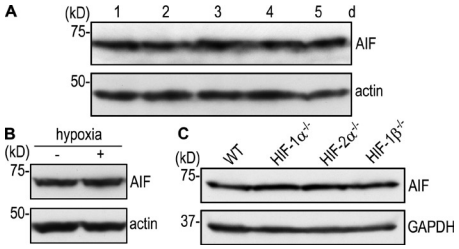

Figure S3. **AIF expression is unchanged during EB differentiation and in response to hypoxia.** (A) Normal EBs were cultured for 1–5 d and analyzed by immunoblotting for AIF. AIF expression was unchanged during EB differentiation. (B) 1-d EBs were cultured in hypoxia pouches for 16 h and subjected to immunoblot analysis. AIF expression was not altered in hypoxia. (C) 4-d EBs were analyzed by immunoblotting for AIF. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) serves as a loading control. Ablation of HIF-1α, HIF-2α, or HIF-1β had no influence on AIF expression. WT, wild type.

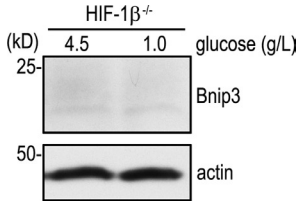

Figure S4. **Low glucose induces Bnip3 expression through HIFs.** 1-d HIF-1$\beta^{-/-}$ EBs were cultured for 24 h in the medium containing 4.5 or 1.0 g/L. EBs were then analyzed by immunoblotting for Bnip3. Actin serves as a loading control. Low glucose failed to induce the elevation of Bnip3 in HIF-1$\beta^{-/-}$ EBs.