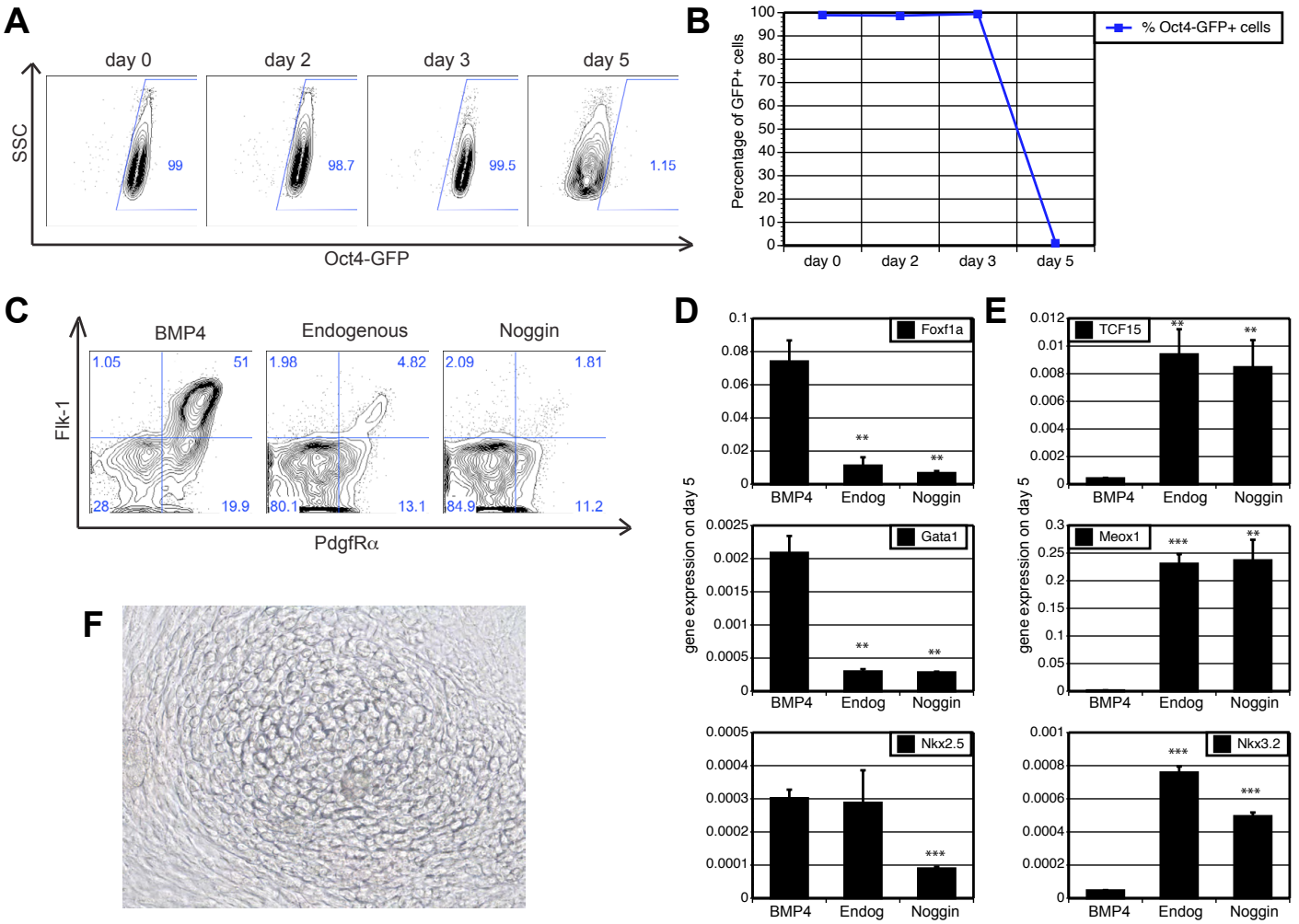

Fig. S1. BMP signaling influences mesoderm development from mouse iPSCs. Oct4-GFP miPSCs were differentiated according to the serum-free protocol shown in Fig. 1A. miPSCs were induced at day 2 for 24 hours with activin, Wnt3a, VEGF, and one of: BMP4, no BMP4 added (Endogenous, Endog), or noggin. On day 3 of culture, EBs were re-aggregated in the presence of bFGF for 2 days and then plated in monolayer culture in SFD containing BMP4 and bFGF for up to 12 days. (**A**) Flow cytometric analysis showing downregulation of Oct4-GFP over the first 5 days of differentiation (Act, Wnt3a and noggin-induced). (**B**) Quantification of GFP$^+$ cells over the course of differentiation by flow cytometry (n=6). (**C**) Flow cytometric analysis showing expression of Flk-1 and Pdgfrα after 1 day induction in indicated conditions. (**D**,**E**) Q-PCR analysis of expression of (D) the lateral plate mesoderm gene (*Foxf1a*), hematopoietic (*Gata1*) and cardiac (*Nkx2.5*) transcription factors, and (E) paraxial mesoderm (*Tcf15*) and somite (*Meox1, Nkx3.2*) genes in day 5 aggregates. Significance relative to BMP4-induced cultures. Values represent copy number relative to β-actin. *P<0.05, **P<0.01, ***P<0.001. (**F**) Micrograph of chondrocytes in monolayer culture derived from Activin, Wnt3a and noggin-induced miPSCs. Magnification 20×.

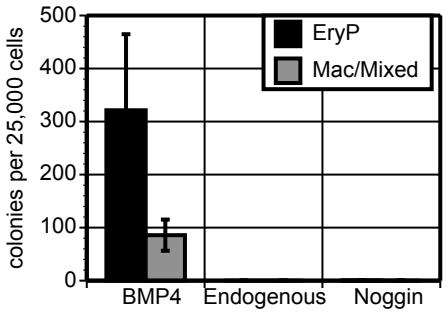

Fig. S2. Hematopoietic potential is restricted to mESC-derived cells induced with BMP4 (in combination with activin, Wnt and VEGF). Total number of primitive erythroid colonies (EryP) and macrophage/mixed colonies (Mac/Mixed) formed from day 5 populations induced as in Fig. 1. Colonies were scored following 7 days of culture in methylcellulose-based media containing hematopoietic cytokines. Error bars indicate s.e.m. (*n*=3). EB-derived cells were plated in 1% (w/v) methylcellulose containing 10% (v/v) plasma-derived serum, 5% (v/v) protein-free hybridoma medium (PFHM-II) and the following cytokines: 1% (v/v) kit ligand conditioned medium, 1% (v/v) thrombopoietin conditioned medium, erythropoietin (2 U/ml), IL11 (25 ng/ml), 1% (v/v) IL3 conditioned medium, 1% (v/v) GM-CSF conditioned medium and IL6 (5 ng/ml).

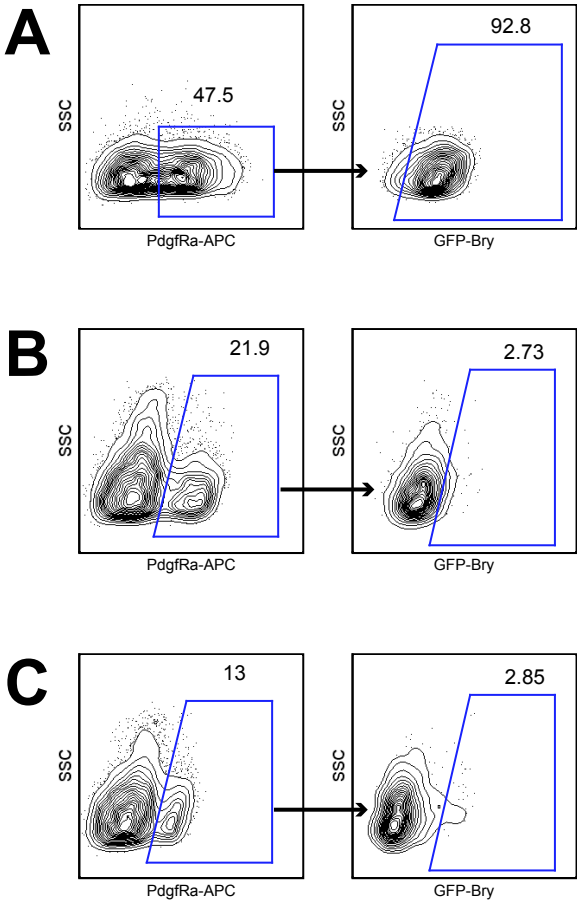

Fig. S3. Pdgfrα populations at day 3 and day 5 are distinguished by the expression of Brachyury. Populations were first gated on P^+ cells, expression of GFP-Bry was observed in P^+ cells. (**A**) The majority (>92%) of day 3 P^+ cells in noggin-induced EBs express GFP-Bry. (**B**,**C**) Less than 3% of day 5 P^+ cells derived from either day 3 P^- aggregates (B) or bulk (unsorted on day 3) aggregates (C) express GFP-Bry.

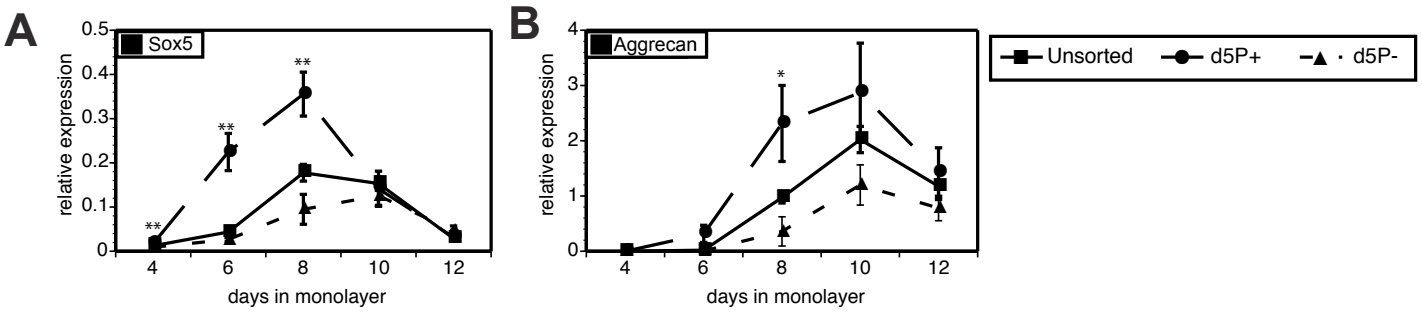

Fig. S4. Chondrogenic potential is detected in the Bry^{-}P^{+} cell fraction isolated from bulk differentiation cultures on day 5. (**A**,**B**) P^{+} and P^{-} populations were isolated by FACS from day 5 aggregates and cultured in monolayers in CH media for an additional 12 days. *Sox5* (A) and aggrecan (B) expression were significantly higher in d5P^{+}-derived monolayers compared with d5P^{-}-derived cultures. Significance of gene expression values in d5P^{+} monolayer cultures was compared with d5P^{-}-derived cultures. Error bars indicate s.e.m. (n=3). Values represent copy number relative to β-actin. *P<0.05, **P<0.01, ***P<0.001.

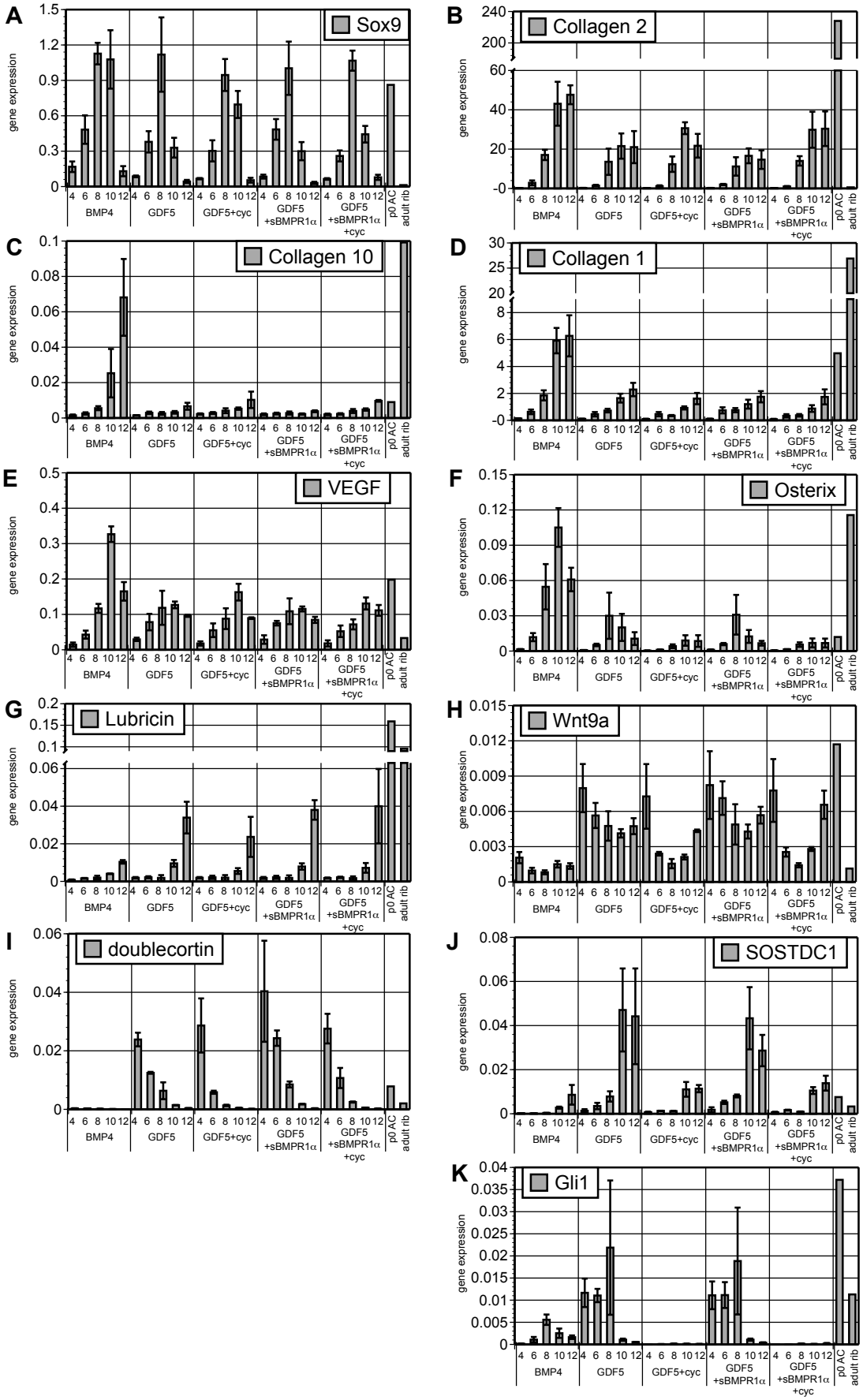

Fig. S5. Gene expression profiles of BMP4- and Gdf5-treated mESC-derived mesoderm in the presence of the BMP4 inhibitor sBmpr1α and the hedgehog inhibitor cyclopamine (cyc) during monolayer culture. (**A-K**) Gene expression analyses of the indicated genes following 4, 6, 8, 10 and 12 days of monolayer culture under different culture conditions. Values represent copy number relative to β-actin.

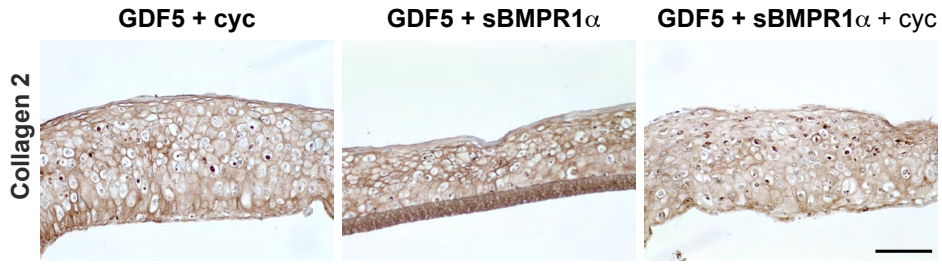

Fig. S6. Chondrocytes that develop in cultures with Gdf5, the hedgehog inhibitor (cyc) and/or the BMP4 inhibitor (sBmpr1α) generated cartilage-like tissues that express collagen 2. Filter cultures were analyzed after 5 weeks and immunohistochemistry was used to visualize collagen 2 expression. Scale bar: 100 μm.

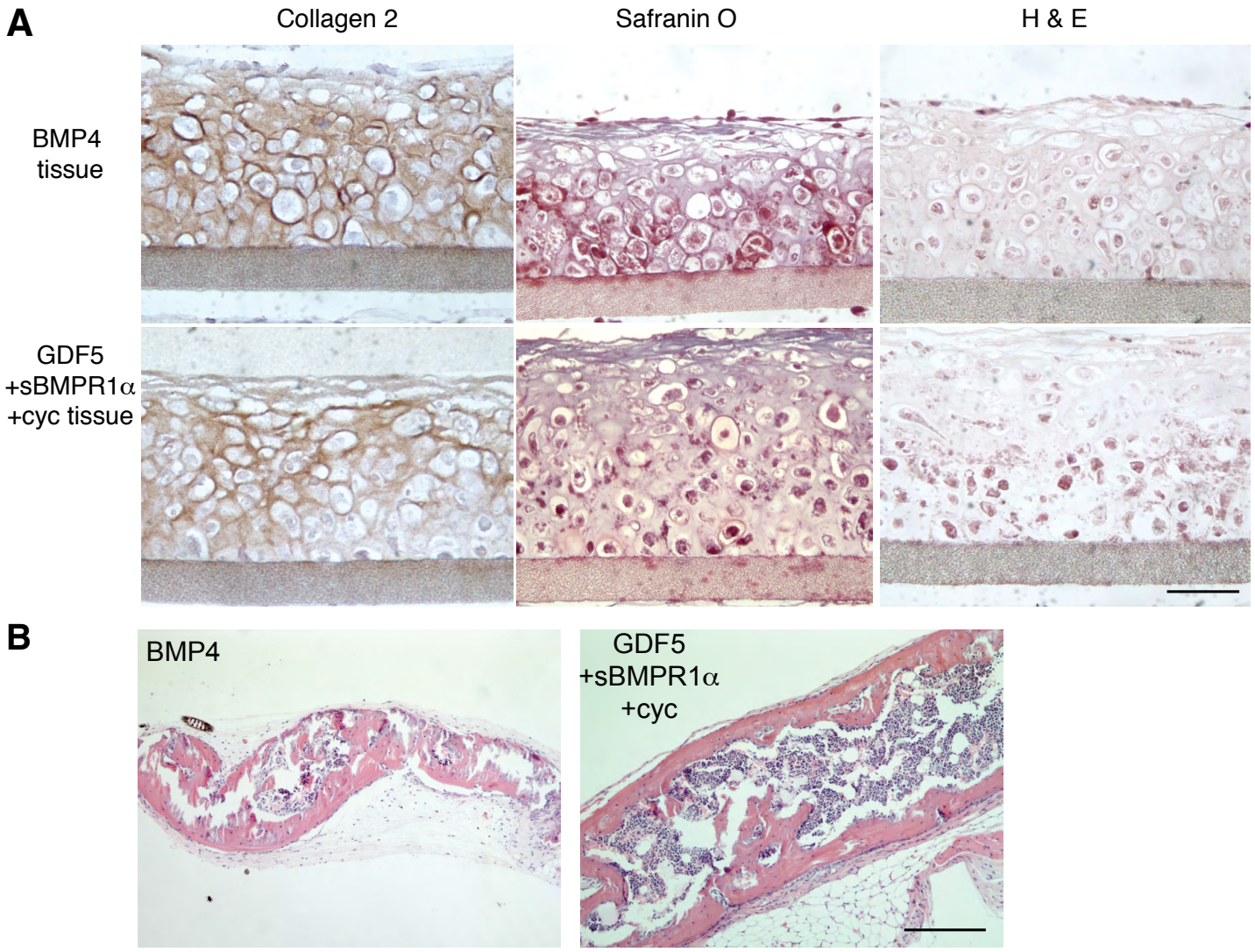

Fig. S7. Mouse ESC-derived cartilage tissue transplanted within diffusion chambers for 4 weeks shows no evidence of ossification, whereas comparable tissue transplanted without the chambers for the same period of time is completely ossified. Cartilage-like tissue was generated from BMP4-treated or Gdf5+sBmpr1α+cyc-treated chondrocytes for 4 weeks *in vitro* and then placed within diffusion chambers prior to subcutaneous implantation into immunodeficient mice. Mice were sacrificed at 4 weeks post transplantation, the diffusion chambers were isolated and the contents analyzed histologically for the presence of stable cartilage and/or bone-like tissue. (**A**) Collagen 2 immunohistochemistry, safranin O and Hematoxylin and Eosin (H&E) staining of cartilage-like tissue that was harvested after 4 weeks *in vivo*. (**B**) Three-dimensional cartilage tissue derived from mESCs were transplanted subcutaneously for 4 weeks. H&E staining of the grafts after 4 weeks shows bone-like tissue and hematopoietic cells contained within the graft. Scale bars: 50 in A; 100 μm in B.

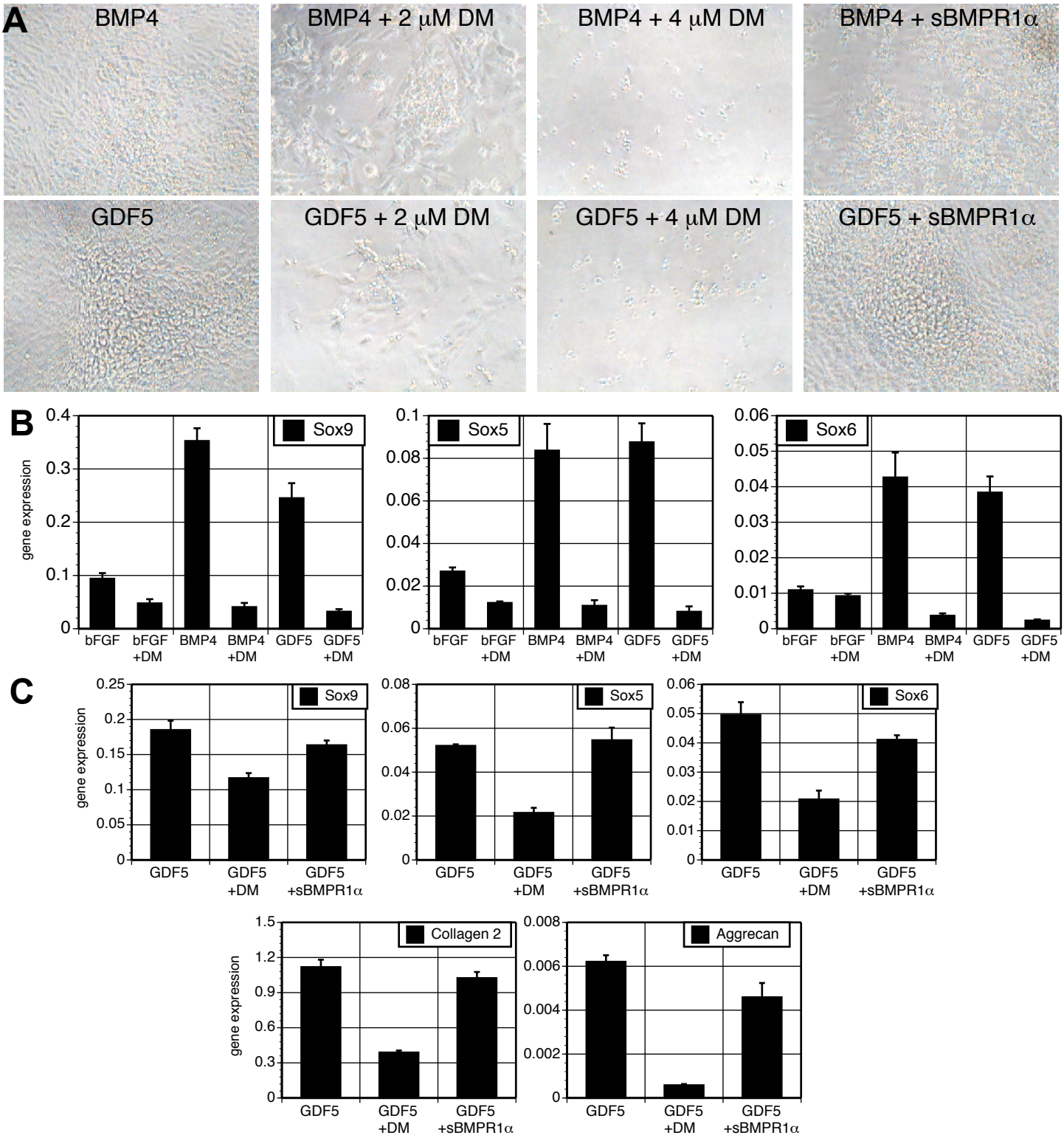

Fig. S8. BMP4 and Gdf5-mediated chondrogenic effects appear to be dependent on Bmpr1-mediated phosphorylation events. mESC-derived chondrogenic mesoderm was cultured in the presence of BMP4 or Gdf5 in combination with effective doses of dorsomorphin (DM), the small molecule inhibitor of the type I receptors Alk-2, -3, -6, or with the soluble Bmpr1α (sBmpr1α). (**A**) Micrographs of monolayer cultures in indicated conditions after 7 days. DM treatment at 4 μM resulted in cell death, whereas a lower concentration (2 μM) inhibited chondrogenesis in both BMP4- and Gdf5-treated cells. Gdf5-mediated chondrogenesis was not inhibited by sBmpr1α, whereas BMP4-mediated chondrogenesis was inhibited by sBmpr1α. Magnification 20×. (**B**) DM treatment (2 μM) prevented the upregulation of chondrocyte transcription factors *Sox9, Sox5* and *Sox6* in day 6 monolayers treated with BMP4 or Gdf5. (**C**) sBmpr1α did not prevent the expression of chondrogenic transcription factors or collagen 2 and aggrecan expression in Gdf5-treated cultures on day 7. Gene expression is copy number relative to β-actin.

Table S1. Primers

Gene	Forward	Reverse
β-actin	TGA GCG CAA GTA CTC TGT GTG GAT	ACT CAT CGT ACT CCT GCT TGC TGA
Tcf15	AAG GAC TCC AGA GAA AGA GGC CAT	TCC TTA CAC AAC GCA GGA GTG GTT
Meox1	AGC GTC TTG TGT TCT CCA AGG	ATG TGT GTG AAC CTG GGA GGT
Nkx3.2	TCA GAA CCG TCG CTA CAA GAC CAA	CAG CAC CTT TAC GGC CAC TTT CTT
Tbx18	CGC CCA GCC ATT CCT GTT TAT TCT	AAG GCT GTC ATC CAC CTT CCT TCA
Mesp1	TTT CCT TTG GTC TTG GCA CCT TCG	TCC AAG GAG GGT TGG AAT GGT ACA
Foxf1a	ATG CCA TGG CCT CTT CTT CTA TGC	ACA CGG CTT GAT GTC TTG GTA GGT
Gata1	ATG GAA TCC AGA CGA GGA	CTC CCC ACA ATT CCC ACT
Nkx2.5	ACC TTT AGG AGA AGG GCG ATG ACT	AAG TGG GAT GGA TCG GAG AAA GGT
Sox9	AGG TTT CAG ATG CAG TGA GGA GCA	CAC AAC ACA CGC ACA CAT CCA CAT
Sox5	ATA AGG GCC AAC AGA CTG TGG TGA	GTT AAT GTG CTT GGC CAC TGG GAA
Sox6	ATT CCT CCT CCA AGG CTG ACA ACA	GGC CAG CAT TTG ACA AGT GGA ACA
Col2a1	CCA AAC ACT TTC CAA CCG CAG TCA	AGT CTG CCC AGT TCA GGT CTC TTA
aggrecan	ATC CCA CCC ACA TGG TGT CTT CTT	TTA GAT GCA GTT TGG GTG ATG CGG
Col10a1	TGG AGA TGC ATT TGG AGG TAG GCT	AGG GCT TTA GGA TTG CTG AGT GCT
Col1a1	TGG CGG TTA TGA CTT CAG CTT CCT	GGT CAC GAA CCA CGT TAG CAT CAT’
0sterix	TTG CCA GTG CCT AGT TCC TAT GCT	AGG CCA GAT GGA AGC TGT GAA GAA
Vegf	GTG TGT GTG TGT GTG TGT GTG TGT	TCA CCG ATC TGG GAG AGA GAG ATT
lubricin	AGC CAA TGA AGA AGT GCA CAG GGA	AGG TGT GTG TCT GGA AAG GTC CAA
Wnt9a	AGG CCA GAG CCT CTT TGA TCT TCT	AAA GAC AGC TCC CTT GTG AGT CCA
doublecortin	GGA ATG TTT GGC AAG GCC CAT GTA	AGT CCT CAA ACC AAA CAG CCC TCA
Sostdc1	AGT CCA GCC ACA ACT TTG AAA GCG	AGA CTG TGC TTG CTG GAT TTG CTG
Gli1	ACA CTC AGC TGG ACT TTG TGG CTA	AGA CAC TCA TGT TAC CCA CTG CCA