Yamamoto et al., http://www.jcb.org/cgi/content/full/jcb.200808044/DC1

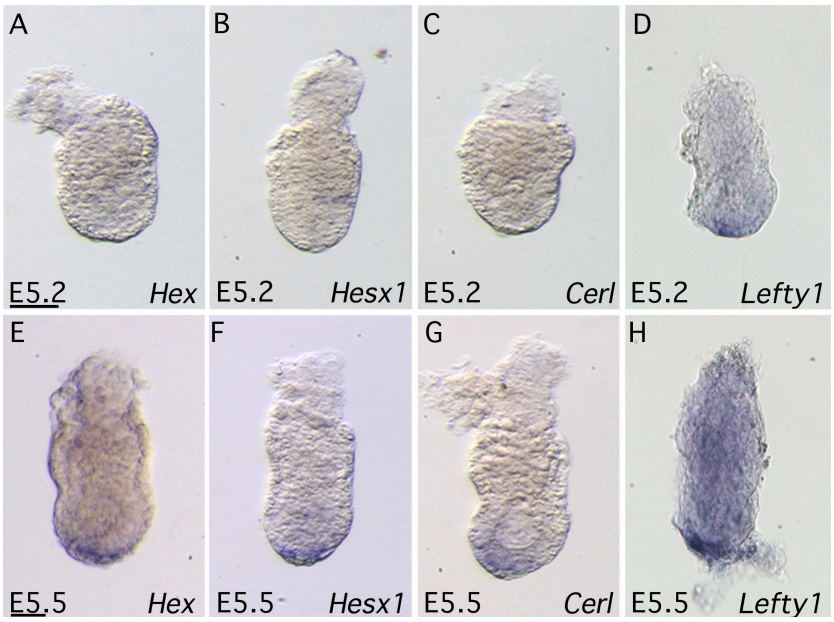

Figure S1. **Expression of DVE markers at E5.2 and E5.5.** Expression of *Hex* (A and E), *Hesx1* (B and F), *Cerl* (C and G), and *Lefty1* (D and H) was examined by in situ hybridization in wild-type mouse embryos at E5.2 or E5.5. Expression of *Hex*, *Hesx1*, and *Cerl* is absent at E5.2 but is apparent at E5.5. Bars, 50 µm.

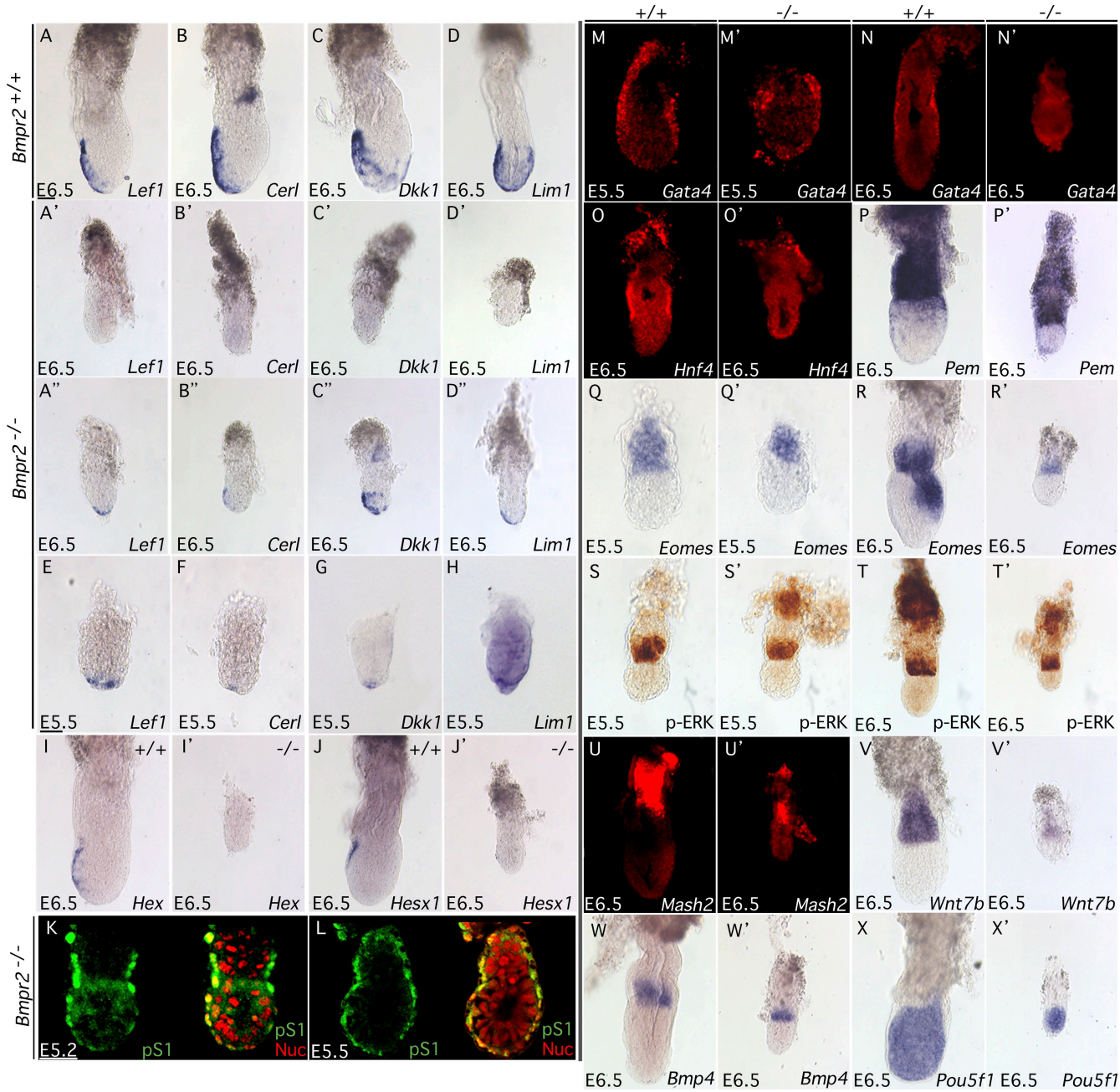

Figure S2. **Phenotype of AVE, VE, and ExE in *Bmpr2*$^{-/-}$ embryos.** Expression of *Lefty1* (A–A" and E), *Cerl* (B–B" and F), *Dkk1* (C–C" and G), *Lim1* (D–D" and H), *Hex* (I and I'), *Hesx1* (J and J'), *Gata4* (M, M', N, and N'), *Hnf4* (O, O'), *Pem* (P and P'), *Eomes* (Q, Q', R, and R'), *Mash2* (U and U'), *Wnt7b* (V and V'), *Bmp4* (W and W'), and *Pou5f1* (X and X') was examined by in situ hybridization in wild-type (*Bmpr2*$^{+/+}$) and *Bmpr2*$^{-/-}$ mouse embryos at the indicated stages. Expression of p-Smad1 (pS1; green) as determined by immunohistofluorescence staining was impaired in *Bmpr2*$^{-/-}$ embryos at the indicated stages (K and L); merged images with nuclear staining (Nuc; red) are also shown. Expression of phosphorylated extracellular signal–regulated kinase (p-ERK) was also examined by immunohistochemical staining with specific antibodies (S, S', T, and T'; Corson, L.B., Y. Yamanaka, K.M. Lai, and J. Rossant. 2003. *Development.* 130:4527–4537). The AVE was absent (A'–D', I', and J') or was present but remained in the distal region or had migrated only slightly toward the anterior side (A"–D") or the DVE was present but abnormal (E–H) in the mutant embryos. *Dkk1* expression was absent (C') or remained relatively normal (C''). Expression of the VE marker gene *Gata4* was normal at E5.5 but was reduced at E6.5 in *Bmpr2*$^{-/-}$ embryos, which is indicative of a partial defect in VE formation. Expression of the ExE marker genes *Eomes*, *Bmp4*, and *Mash2* was normal, whereas that of *Wnt7b* was slightly decreased, in the mutant embryos. Staining for phosphorylated ERK and *Pou5f1* expression were normal in the mutant. VE, ExE, and epiblast of *Bmpr2*$^{-/-}$ embryos thus appear normal at E5.5. Lateral views are shown for each embryo, with the anterior side on the left (A–D, I, J, N–P, T–X, and A"–D"). Bars, 50 µm.

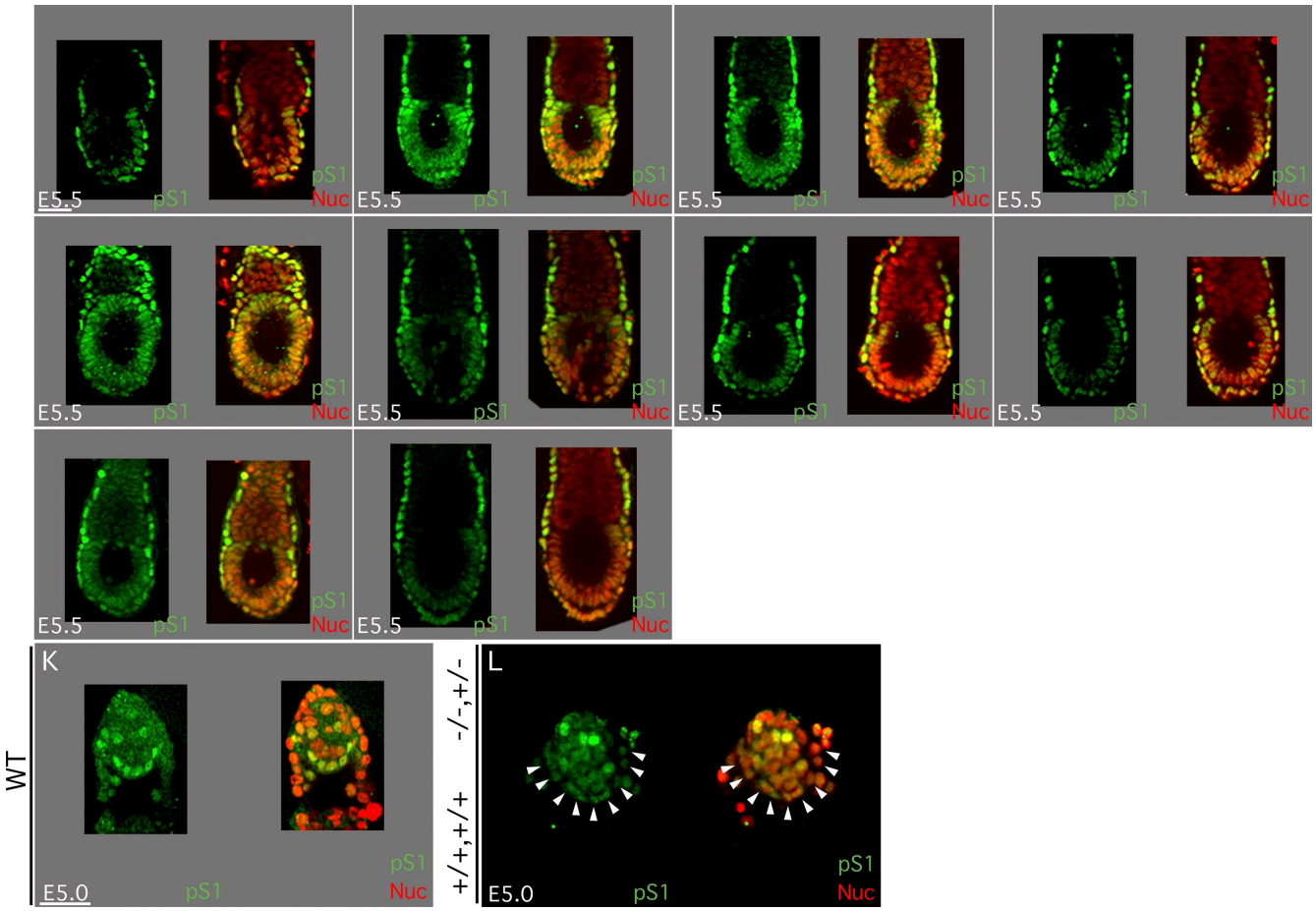

Figure S3. **p-Smad1 staining in wild-type E5.5 embryos and in the chimeric embryo at E5.0.** (A–J) p-Smad1 staining (pS1; green) and merged images with nuclear staining (Nuc; red) are shown for wild-type embryos at E5.5 (n = 10) obtained from a single litter. In all cases, p-Smad1 staining in the DVE region was either absent or greatly reduced. (K and L) Expression of p-Smad1 (pS1; green) and merged images with nuclear staining (Nuc; red) are shown for wild-type (WT) embryos (K) and green ES FM260 cell (+/+,+/+)←→*Bmpr2*$^{-/-}$,*Actr2b*$^{+/-}$ (−/−,+/−) tetraploid chimeric embryos (L) at E5.0. Staining for p-Smad1 in the chimeric embryo is similar to that in the embryonic portion of the wild-type embryo and to that in the extraembryonic portion (VE) of *Bmpr2*$^{-/-}$,*Actr2b*$^{+/-}$ embryos. Staining for p-Smad1 in VE was not detected in the chimeric embryo (arrowheads). Bars, 50 μm.

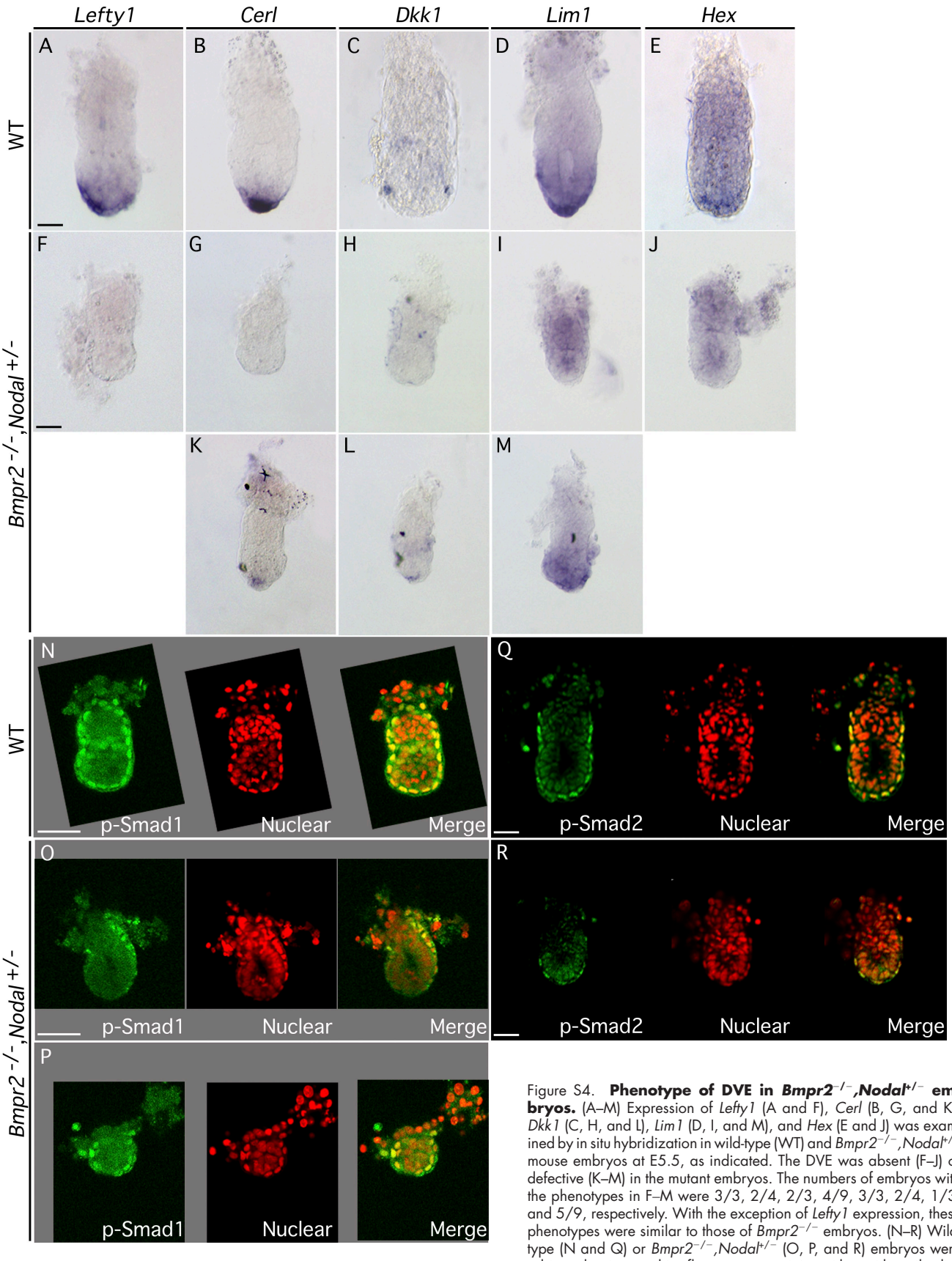

Figure S4. **Phenotype of DVE in *Bmpr2*$^{-/-}$,*Nodal*$^{+/-}$ embryos.** (A–M) Expression of *Lefty1* (A and F), *Cerl* (B, G, and K), *Dkk1* (C, H, and L), *Lim1* (D, I, and M), and *Hex* (E and J) was examined by in situ hybridization in wild-type (WT) and *Bmpr2*$^{-/-}$,*Nodal*$^{+/-}$ mouse embryos at E5.5, as indicated. The DVE was absent (F–J) or defective (K–M) in the mutant embryos. The numbers of embryos with the phenotypes in F–M were 3/3, 2/4, 2/3, 4/9, 3/3, 2/4, 1/3, and 5/9, respectively. With the exception of *Lefty1* expression, these phenotypes were similar to those of *Bmpr2*$^{-/-}$ embryos. (N–R) Wild-type (N and Q) or *Bmpr2*$^{-/-}$,*Nodal*$^{+/-}$ (O, P, and R) embryos were subjected to immunohistofluorescence staining either with antibodies to p-Smad1 (green) at E5.2 (N–P) or with antibodies to p-Smad2 (green) at E5.5 (Q and R). Nuclei were stained with YOYO-1 (red). Merged images are also shown. Staining for p-Smad1 in *Bmpr2*$^{-/-}$,*Nodal*$^{+/-}$ embryos is similar to that in *Bmpr2*$^{-/-}$ embryos. Staining for p-Smad2 is down-regulated in *Bmpr2*$^{-/-}$,*Nodal*$^{+/-}$ embryos. Bars, 50 µm.

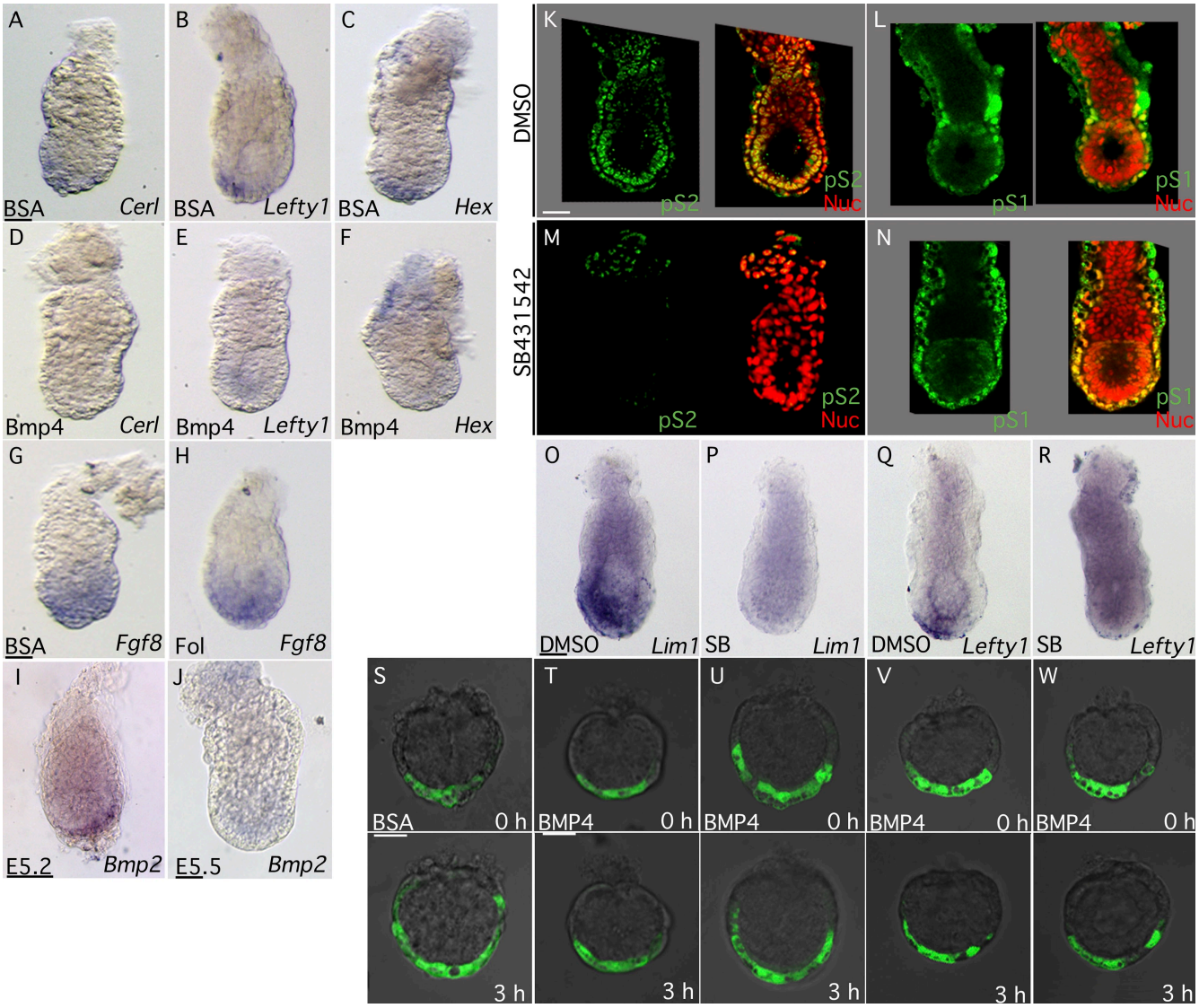

Figure S5. **Effects of SB431542, follistatin, and BMP4 on DVE formation.** (A–J and O–R) Expression of *Cerl* (A and D), *Lefty1* (B, E, Q, and R), *Hex* (C and F), *Fgf8* (G and H), *Bmp2* (I and J), and *Lim1* (O and P) was examined by in situ hybridization in E5.2 (A–H and I) or E5.5 (O–R and J) wild-type embryos treated with DMSO, SB431542 (SB), BSA, follistatin (Fol), or Bmp4 for 7 h, as indicated. (H) *Fgf8* expression is maintained in follistatin-treated embryos. (D–F) Expression of *Cerl*, *Lefty1*, and *Hex* was absent in BMP4-treated embryos. (I and J) *Bmp2* expression is detectable at E5.2 but is absent at E5.5. (K–N) Staining of p-Smad2 (pS2; green) or p-Smad1 (pS1; green), and merged images with nuclear staining (Nuc; red), for E5.5 wild-type embryos treated with DMSO (vehicle) or SB431542 for 7 h, as indicated. Treatment with SB431542 leads to not only reduction of p-Smad2 (M) but also enhancement of p-Smad1 (N). Inhibition of ALK*4/5/7* type I receptor by SB431542 may decrease the interaction between ALK*4/5/7* and their type II receptors, which would lead to more type II receptor available for BMP signaling. (S–W) The embryo explants lacking ExE were prepared from E5.5 embryos and were cultured with BSA or BMP4. The DVE region was monitored by a *Hex-Venus* transgene (green) at 0 or 3 h after removal of ExE. Five independent explants (S–W) are shown here. In all BMP4-treated explants, expansion of the DVE region was inhibited by BMP4 (T–W). Bars, 50 μm.