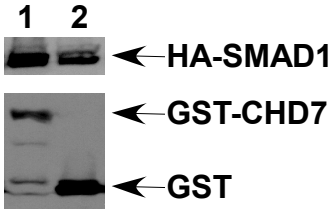
Supplementary figures.

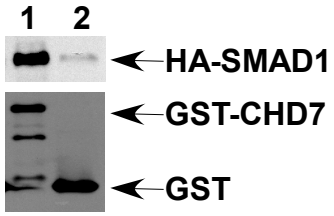
Sup. Fig. 1. Examination of SMAD1-CHD7 interaction by mammalian cell GST-pull down. **(A)** A plasmid expressing HA-SMAD1 was co-transfected with a plasmid expressing GST-CHD7 (a.a. 2301-2646) (lane 1) or GST alone (negative control, lane 2) into COS cells. Expression of HA-SMAD1 in COS cells was confirmed by Western analysis using an anti-HA antibody (top panel) and expression of GST-CHD7 or GST was confirmed by Western analysis using an anti-GST antibody (bottom panel). **(B)** The GST and GST-CHD7 fusion proteins were purified with GST-binding beads. After purification, samples were loaded onto an SDS-PAGE gel, and an HA antibody was applied to determine if HA-SMAD1 was co-purified with GST-CHD7 by Western analysis (top panel). Successful purification of GST and GST-CHD7 was confirmed with Western analysis using a GST antibody (bottom panel).

Sup. Figure-1, Jiao

A Cell lysate



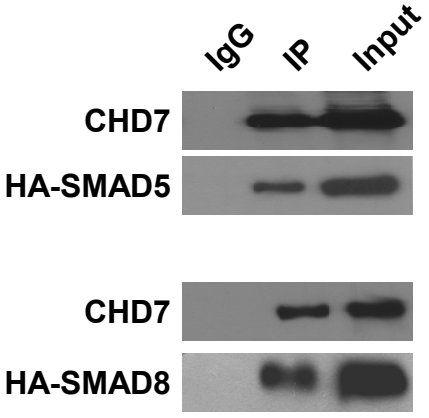
B Precipitation



Lane 1: COS cells with HA-*Smad1* + GST-*Chd7*
Lane 2: COS cells with HA-*Smad1* + GST

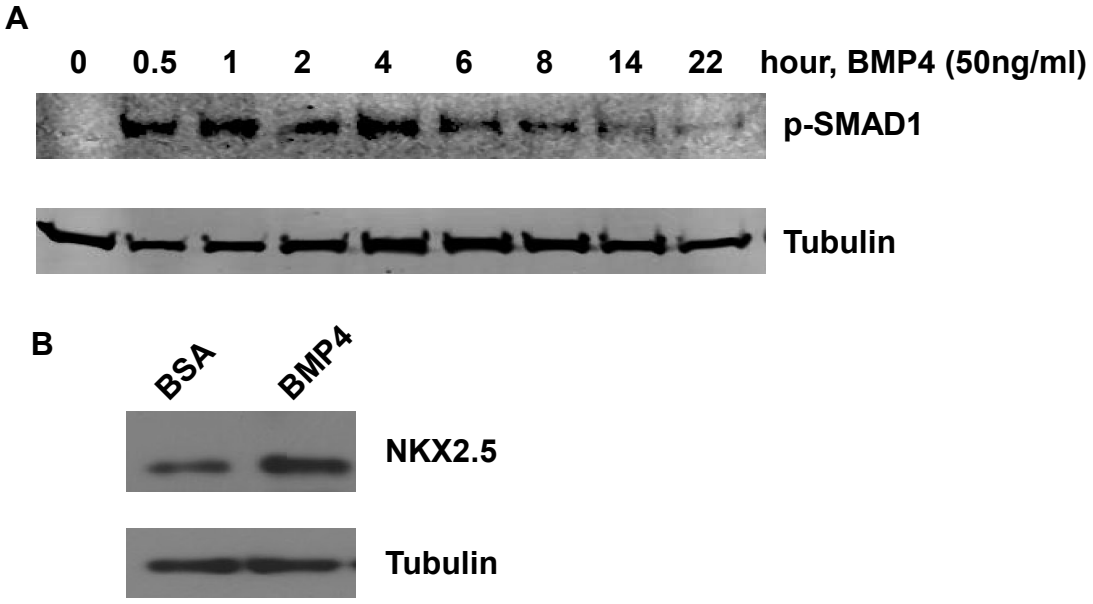
Sup. Fig. 2. CHD7 interacts with SMAD5 and SMAD8. A plasmid expressing HA-tagged hSMAD5 or hSMAD8 was co-transfected with a plasmid expressing constitutively active ALK6 into COS cells. 48-72 hours after transfection, IP analysis was performed using an anti-CHD7 antibody or pre-immune IgG (negative control) followed by Western analysis using antibodies against CHD7 and the HA tag. CHD7 interacts with both HA-SMAD5 and HA-SMAD8. In the absence of constitutively active ALK6, we could not detect the interaction between CHD7 and SMAD5 or SMAD8.

Sup. Figure-2, Jiao



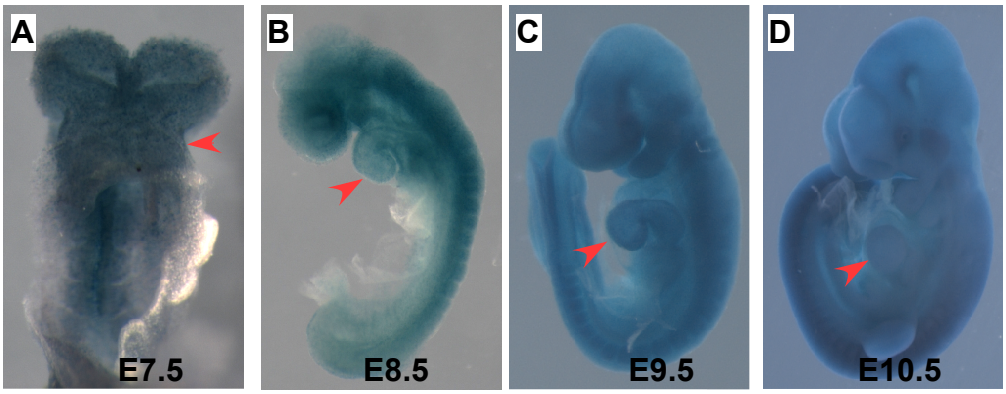
Sup. Fig. 3. Stimulation of NkL-TAg cells with BMP4. (A) NkL-TAg cells were treated with 50ng/ml BMP4 for the indicated times. Western analysis was performed using an anti-phosphorylated SMAD1 (p-SMAD1) antibody. Presence of p-SMAD1 indicates activation of BMP signaling. Tubulin was used as a loading control. This result shows that NkL-TAg cells can properly respond to BMP stimulation. **(B)** NkL-TAg cells were treated with 50ng/ml BMP4 for 48 hours followed by Western analysis using a NKX2.5 antibody. Tubulin was used as a loading control. Expression of NKX2.5 was upregulated by BMP4 stimulation.

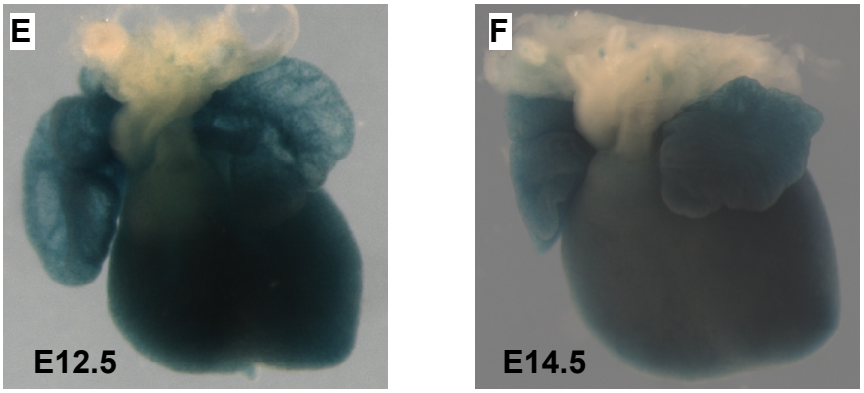
Sup. Figure-3, Jiao

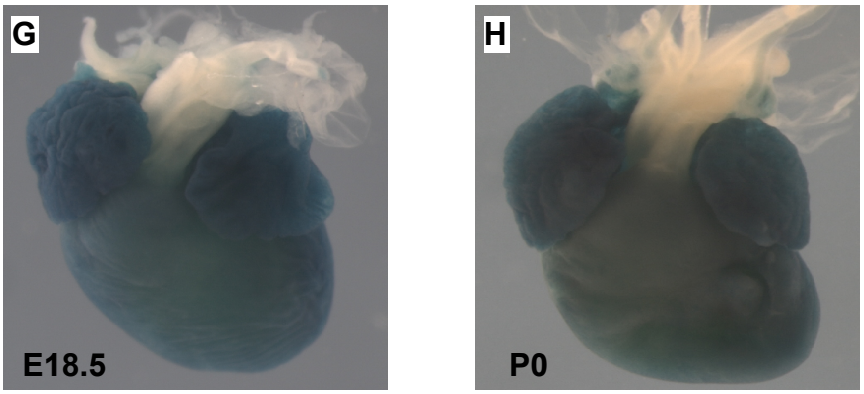


Sup. Fig. 4. Whole mount lacZ staining of *Chd7*$^{tm1a/+}$ embryos and embryonic hearts. (A-D) In the *Chd7*^{tm1a} allele, a lacZ reporter with an mRNA splicing acceptor was inserted between exons 2 and 3 of *Chd7*. Therefore, expression of lacZ is under the control of endogenous regulatory elements of *Chd7*. *Chd7*$^{tm1a/+}$ embryos at various stages were isolated and stained with lacZ. The arrow in panel A indicates the cardiogenic region (cardiac crescent), while the arrows in panels B-D indicate embryonic hearts. **(E-H)** *Chd7*$^{tm1a/+}$ hearts were isolated at the stages indicated and stained with X-gal. Expression of lacZ in blood vessels was reduced at later stages.

Sup. Figure-4, Jiao







Sup. Fig. 5. Sections of lacZ stained *Chd7*$^{tm1a/+}$ embryos or embryonic hearts. (A, A') An E9.5 *Chd7*$^{tm1a/+}$ embryo was stained with X-gal and sagittally sectioned. LacZ was ubiquitously expressed in the whole embryo. A' correspond to the boxed region of A. The red circle in A' shows the AV cushion area. Myocardial, endocardial and mesenchymal cells are all lacZ positive. **(B, B')** An E14.5 embryonic heart was lacZ stained and frontally sectioned. B' corresponds to the boxed region of B. The red arrows in B' indicate reduced expression of lacZ in AV valves. a: atrium; v: ventricle; la: left atrium; ra: right atrium; lv: left ventricle; rv: right ventricle.

Sup. Figure-5, Jiao