Stefanovic et al., http://www.jcb.org/cgi/content/full/jcb.200901040/DC1

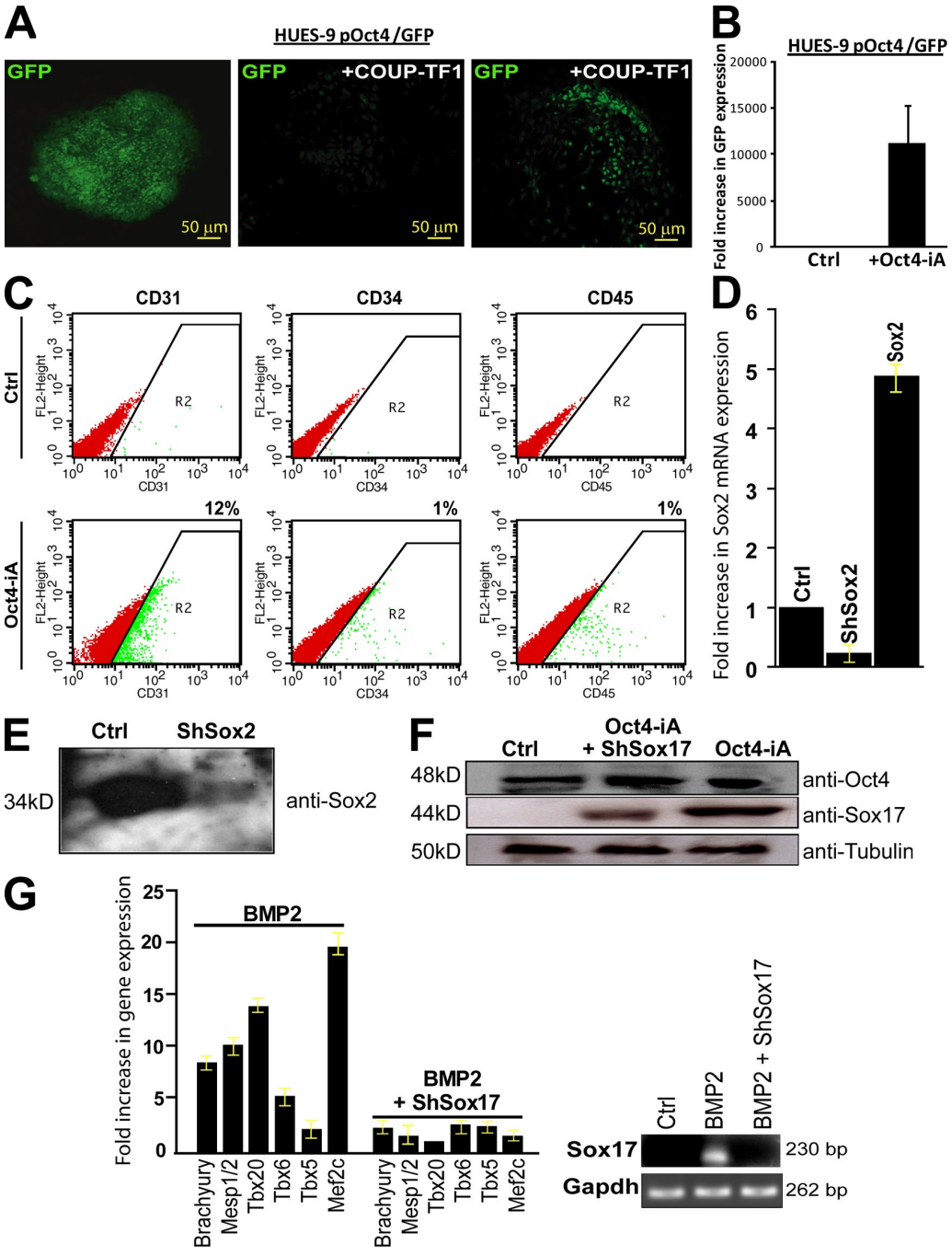

Figure S1. **Characterization of HUES-9 pOct4/GFP clone.** Non-cardiac potential of differentiation of Oct4-OEC. Knock-out of Sox2/Sox17 and consequences on BMP2-induced gene expression. (A) HUES-9 pOct4/GFP cell line. Left image: mock cell clone; middle and right images: HES cells expressing COUP-TFI. Raw images were acquired using a laser-scanning microscope (LSM-510 Meta; Carl Zeiss, Inc.) at 20x (NA 0.75) and visualized using the LSM software. (B) HUES-9 pOct4/GFP cell line was nucleofected or not (Ctrl) with Oct4-iA, and RNA was extracted 2 d later to carry out real-time PCR of GFP. (C) FACS analysis of mock (Ctrl) or Oct4-iA–overexpressing HUES cells (Oct4-iA) Cells were stained with anti-CD31, -CD34, or -CD45 antibodies. (D) Real-time PCR of *Sox2* cDNA reverse transcribed from RNA extracted from control (mock GFP-nucleofected cells), Sox2 ShRNA-nucleofected cells (ShSox2), and Sox2-nucleofected cells ($n = 2$). (E) Anti-Sox2 Western blot of 50 µg proteins extracted from control or shRNA Sox2-nucleofected HUES cells using an anti-Sox2 antibody. (F) Expression of Oct4-iA and Sox17 was monitored by Western blot. Anti–Oct4-iA and -Sox17 Western blot of 50 µg proteins extracted from control or Oct4-iA–nucleofected HUES cells with or without Sox17 ShRNA, using an anti–C-terminal Oct-3/4 or anti-Sox17 (R&D Systems) antibody. Blots are normalized with an anti–tubulin antibody. (G) Mock-nucleofected cells or cells nucleofected with *Sox17* ShRNA were stimulated with BMP2 and SU5402 for 4 d, RNA extracted, and mesodermal and cardiac gene monitored by RT-Q-PCR ($n = 2$). Right panel: *Sox17* expression was monitored by Q-PCR and the amplicon run on gel.

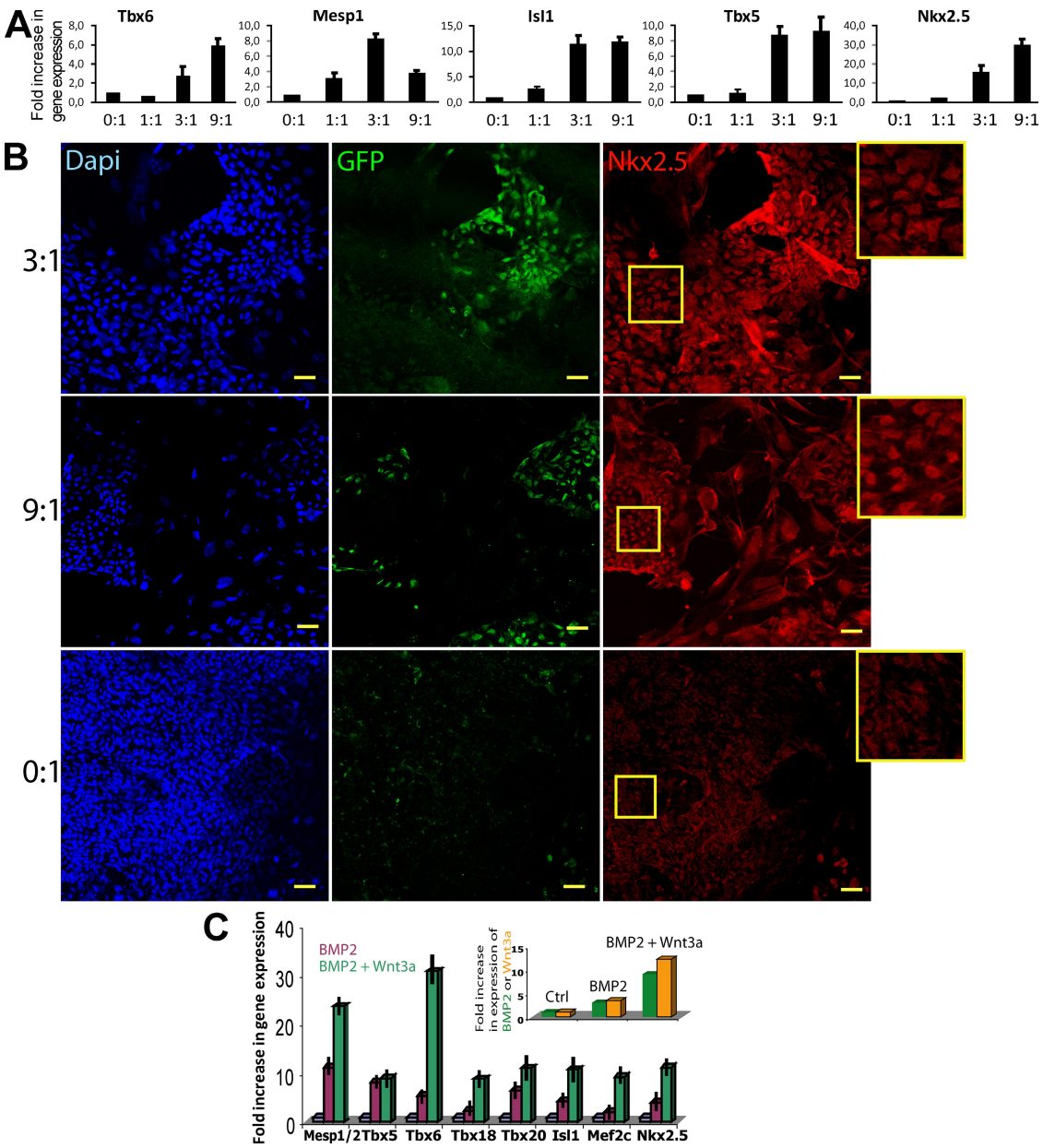

Figure S2. **Paracrine action of Oct4-overexpressing cells.** HUES-9 pOct4/GFP cells nucleofected with Oct4-iA cDNA and wild-type mock cells were mixed together in the same wells at different ratios (1:1; 3:1; 9:1). After 4 d of co-culture, wild-type mock cell colonies were dissected out and used in real-time PCR (A), or cells were fixed and stained with an anti-Nkx2.5 antibody (B). (A) PCR data are normalized to expression of the housekeeping gene GAPDH and expressed as fold increased compared with wild-type HESC (ratio 0:1). (B) The left panels show DAPI; the middle, GFP; and the right, Nkx2.5. Insets: magnifications of a ROI within non-GFP cells show the nuclear staining or just nonspecific cytosolic background in control wild-type cells. The top panels illustrate a well with cells mixed at a 3:1 ratio, the middle one, a well with cells mixed at a 9:1 ratio, and the bottom only wild-type cells. The experiment was performed in duplicate with similar results. Images were acquired at room temperature using a laser-scanning microscope (LSM-510 Meta; Carl Zeiss, Inc.) at 40x (oil Fluor objective, NA 1.3); images were visualized using the LSM software. Bar, 20 µm. (C) Real-time PCR of RNA extracted from control, BMP2- treated, or Wnt3a- and BMP2-treated HESC. Early cardiac marker genes as well as BMP2 and Wnt3a mRNA (inset) were amplified in real-time PCR. Data are normalized to human GAPDH expression and expressed as means ± SEM (n = 3–5). *, Significantly different from control ($P \leq 0.001$).

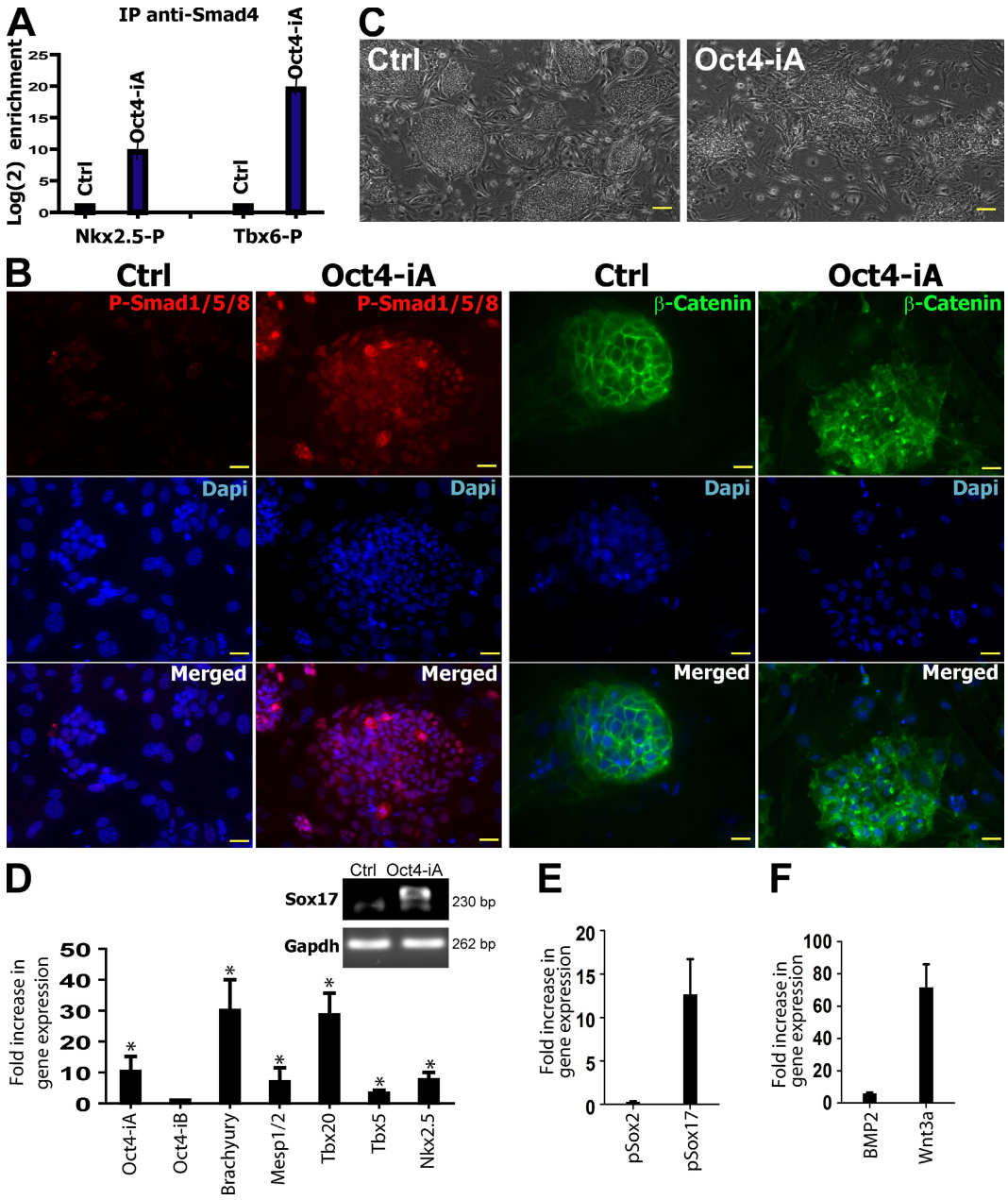

Figure S3. **Oct4-overexpressing cells activate Smad and β-catenin pathways.** The Oct4/Sox17 network is conserved in iPSCs. (A) HESC (control [Ctrl] or overexpressing Oct4-iA [Oct4-iA]) were subjected to ChIP assay using an anti-Smad4 antibody. Real-time PCR was used to amplify the Nkx2.5 and Tbx6 promoter regions. Results are expressed as log(2) enrichment, assuming that 1% of nucleosomes were immunoprecipitated. (B) HESC (control [Ctrl] or overexpressing Oct4-iA [Oct4-iA]) were fixed and stained 2 d after nucleofection using an anti–phospho-Smad1/5/8 or anti–β-catenin antibody and with DAPI. Raw images were acquired at room temperature using an epifluorescence microscope (AxioImager; Carl Zeiss, Inc.) at 10x Achroplan (NA 0.25) and an AxioCam CCD, and visualized using AxioVision software. Bar, 20 µm. (C) Colonies of wild-type (Ctrl, left) and Oct4-iA–overexpressing (+Oct4-iA, right) iPSCs cultured in the presence of FGF2 visualized at room temperature using an Axiovert microscope (Carl Zeiss, Inc.) at 10x Achroplan (NA 0.25) and an AxioCam CCD; Images were visualized without any processing with the AxioVision software. Bar, 20 µm. (D) iPSCs were transfected with Oct4-iA or GFP as a control and gene expression was monitored 6 d later by RT-Q-PCR. Data are normalized to GAPDH expression and expressed as means ± SEM (n = 3–5). *, Significantly different from control ($P \leq 0.001$). The inset shows the gel of the Sox17 and GAPDH amplicons following real-time PCR. (E) ChIP analysis of Sox2 and Sox17 DNA promoter region (pSox) coimmunoprecipitated with an anti-Oct4 antibody in iPSCs overexpressing Oct4-iA. Data are normalized to GAPDH DNA (input) and fold changes compared with mock cells (GFP-nucleofected) and expressed as means ± SEM (n = 3–5). (F) RT-Q-PCR of BMP2 and Wnt3a mRNA in control and Oct4-iA–expressing HUES cells. Data are normalized to GAPDH expression and expressed as means ± SEM (n = 3–5). *, Significantly different from control ($P \leq 0.001$).