Liu et al., http://www.jcb.org/cgi/content/full/jcb.200810137/DC1

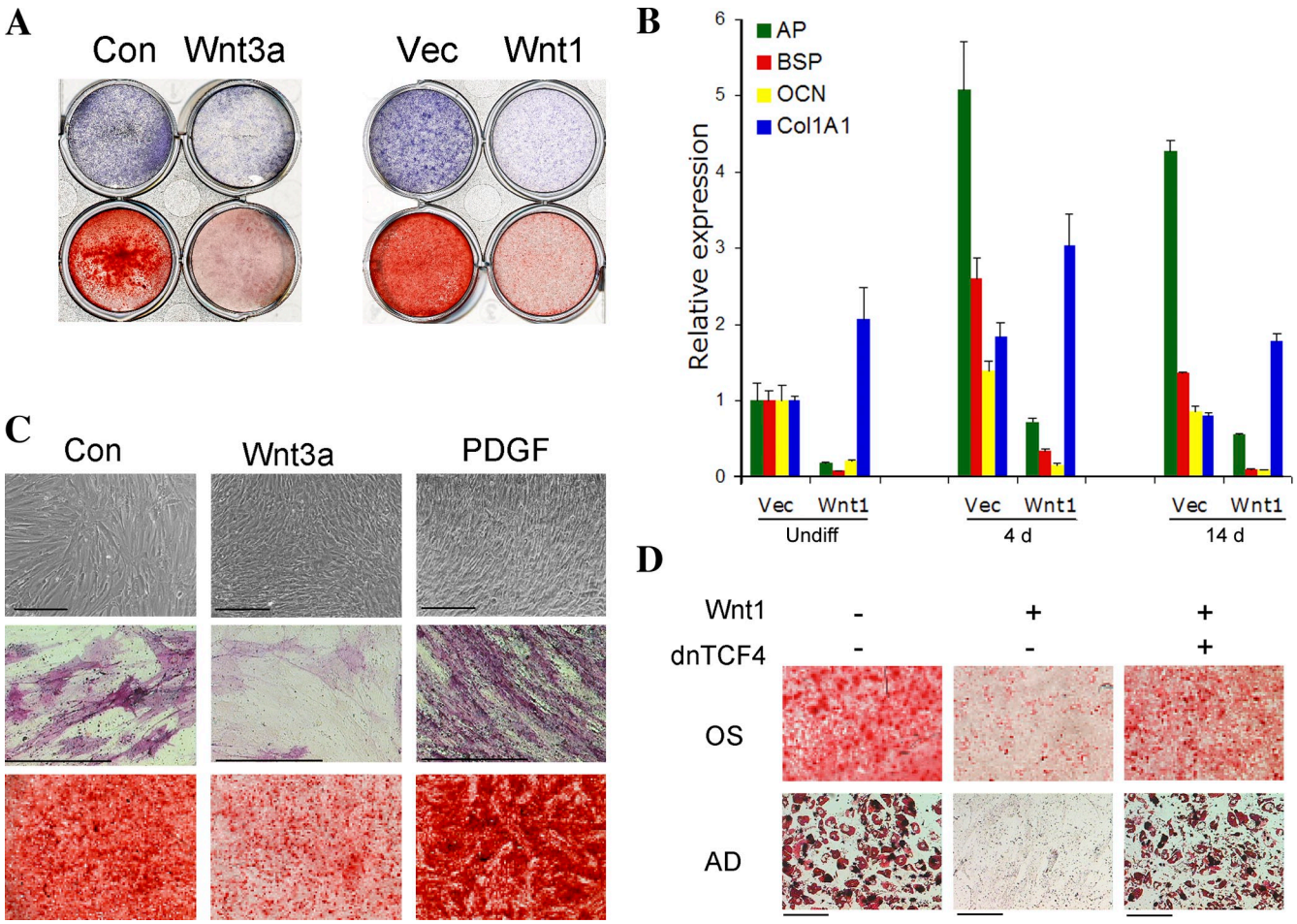

Figure S1. **Effects of canonical Wnts on hMSC differentiation and proliferation.** (A) Canonical Wnts inhibit osteogenic differentiation by hMSCs. hMSCs in 12-well plates were stimulated with osteogenic medium in the presence of either control (Con) or Wnt3a CM or after stable lentiviral transduction of vector (Vec) or Wnt1. Staining for AP activity (top) or mineralization by Alizarin red (bottom) was performed after 2 and 3 wk of differentiation, respectively. (B) Real-time PCR analysis of expression of genes associated with osteoblastic differentiation in vector- or Wnt1-expressing hMSCs after induction with osteogenic medium for 0 (Undiff), 4, or 14 d. Genes include AP, bone sialoprotein (BSP), osteocalcin (OCN), and collagen 1A1 (Col1A1). Results represent mean values ± SD from experiments performed in triplicate. (C) Comparison of effects of Wnt3a and PDGF on hMSC proliferation and differentiation. The top panel shows phase-contrast images of hMSCs grown in 50% control CM, 50% Wnt3a CM, or 50 ng/ml PDGF-containing media. The middle and bottom panels show AP and Alizarin red staining, respectively, of hMSCs induced with osteogenic medium containing 50% control CM, 50% Wnt3a CM, or 50 ng/ml PDGF for 3 wk. (D) Inhibition of β-catenin signaling antagonizes Wnt1 inhibition of osteogenic (OS) and adipogenic (AD) differentiation. hMSCs were transduced with lentivirus-expressing vector, Wnt1, or dnTCF4 in addition to Wnt1 and were then subjected to osteogenic or adipogenic differentiation for 3 wk. Cultures were stained at 3 wk with Alizarin red (top) or Oil red O (bottom). Bars, 20 μm.

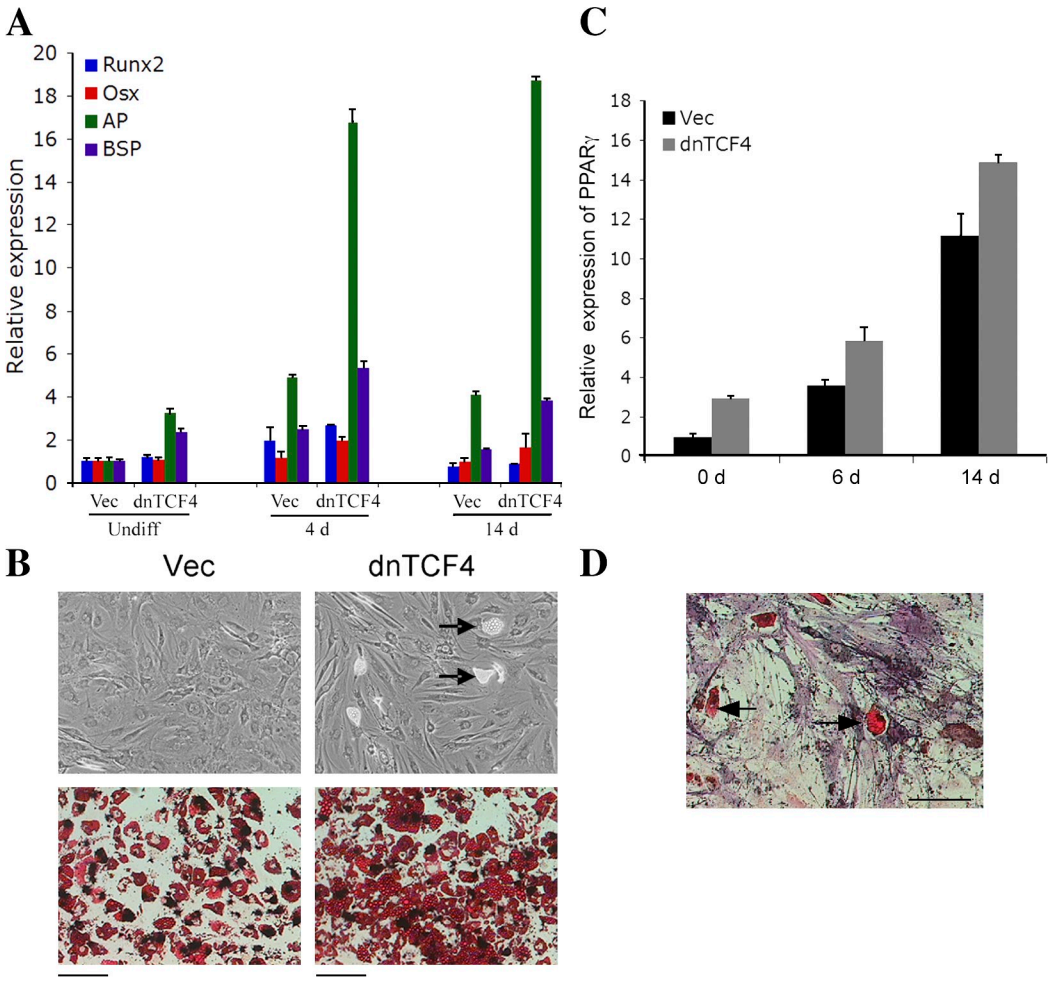

Figure S2. **Inhibition of endogenous Wnt/β-catenin signaling on hMSC differentiation in vitro.** (A) dnTCF4 enhances gene expression associated with osteogenic differentiation as determined by real-time PCR in vector (Vec)- or dnTCF4-expressing hMSCs grown in basal or osteogenic medium for 4 and 14 d. BSP, bone sialoprotein. (B) Spontaneous hMSC adipogenic differentiation in basal medium stimulated by dnTCF4 expression (top). dnTCF4 expression enhanced adipocyte differentiation after 3 wk in adipogenic medium (bottom; stained with Oil red O). (C) Real-time PCR analysis of PPAR-γ expression in vector- or dnTCF4-expressing hMSCs grown in basal (0 d) or adipogenic medium for 6 and 14 d. (D) dnTCF4-expressing hMSCs were subjected to osteogenic differentiation as in Fig. 3 D and stained for AP and Oil red O. (B and D) Arrows indicate adipocytes. (A and C) Results represent mean values ± SD from two independent experiments performed in triplicate. Bars, 20 µm.

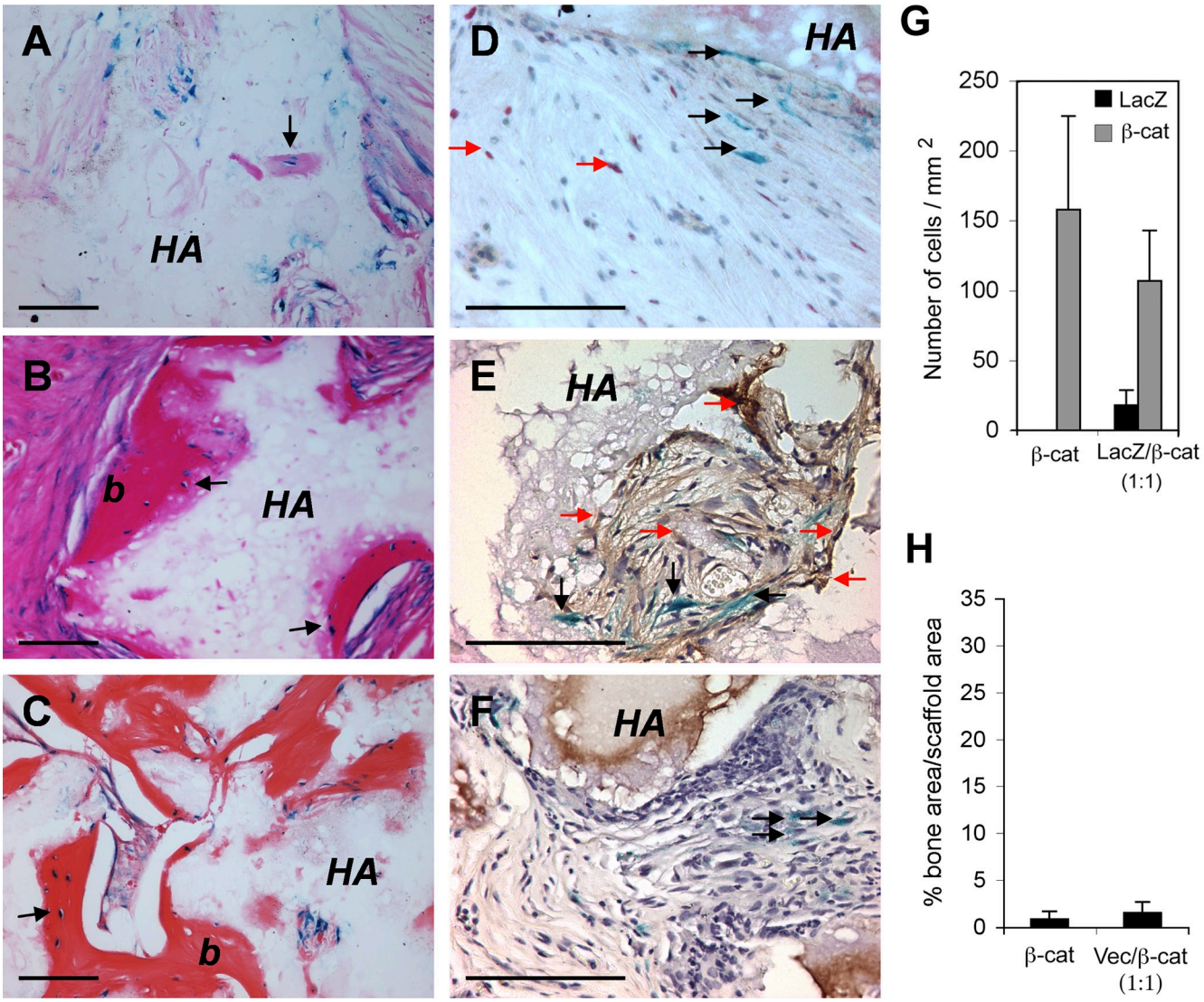

Figure S3. **Paracrine Wnt signaling stimulates ectopic bone formation by hMSCs in vivo.** (A–C) hMSC-LacZ cells alone (A) or hMSC-LacZ mixed with hMSC-Wnt1 cells (B and C) at a ratio of 1:1 (B) or 3:1 (C). Sections were stained for LacZ (blue) and eosin (red). Arrows indicate osteocytes with positive LacZ staining. (D) PCNA staining of a section as in A. Black arrows indicate hMSC-LacZ cells, and red arrows indicate mouse cells stained positive for PCNA. (E and F) β-Catenin staining in implants with 1:1 mixture of hMSCs expressing stabilized β-catenin (S33Y) and LacZ (E) or with hMSC-LacZ cells alone (F). Black arrows indicate LacZ staining (blue). Red arrows indicate β-catenin staining (brown). (D–F) Sections were counterstained with hematoxylin. (G) Quantification of cell numbers in implants with hMSCs expressing β-catenin (S33Y) alone or mixed with hMSC-LacZ cells at a ratio of 1:1. (H) Quantitative analysis of bone formation in recovered implants with hMSCs expressing stabilized β-catenin (S33Y) alone or mixed with vector (Vec) hMSCs at a ratio of 1:1. (G and H) Results reflect mean values ± SD of experiments performed in triplicate. b, bone; HA, HA/TCP. Bars, 100 μm.