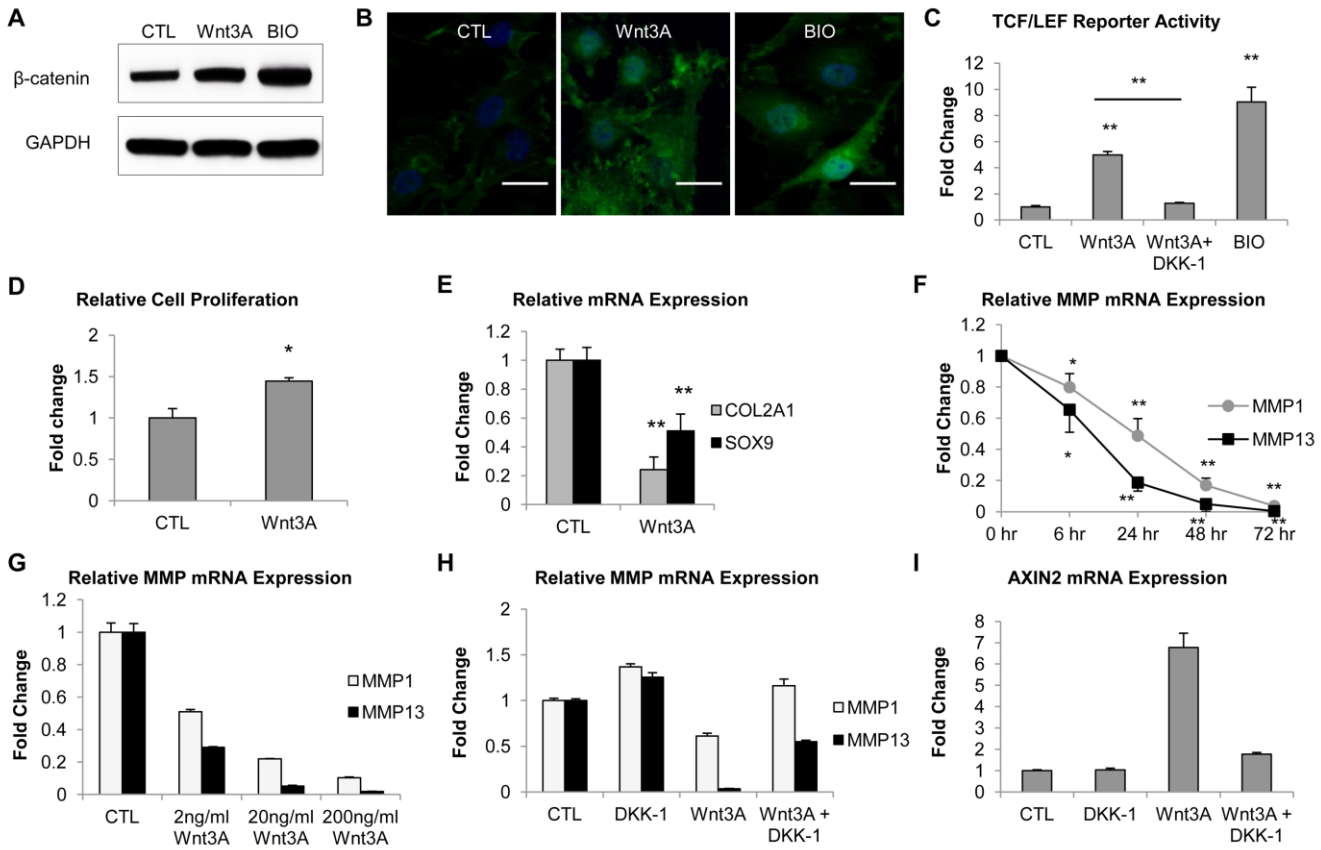
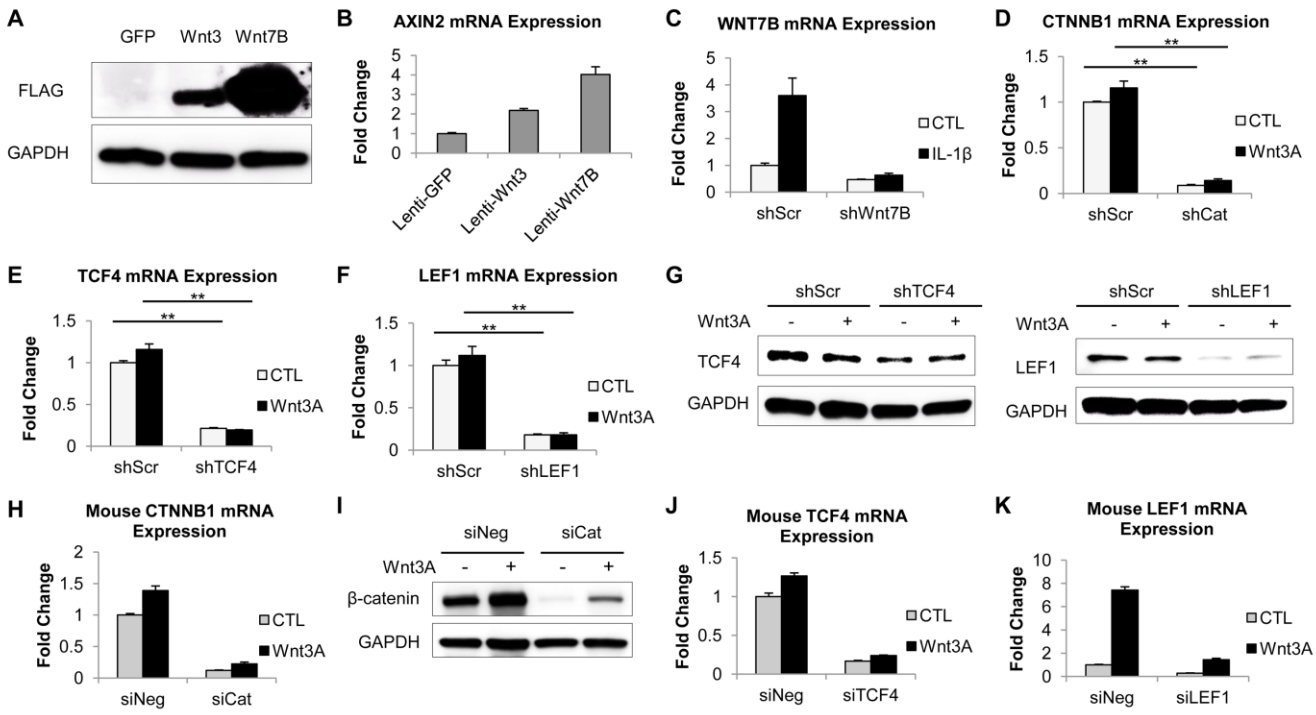
Supplemental Figure 1



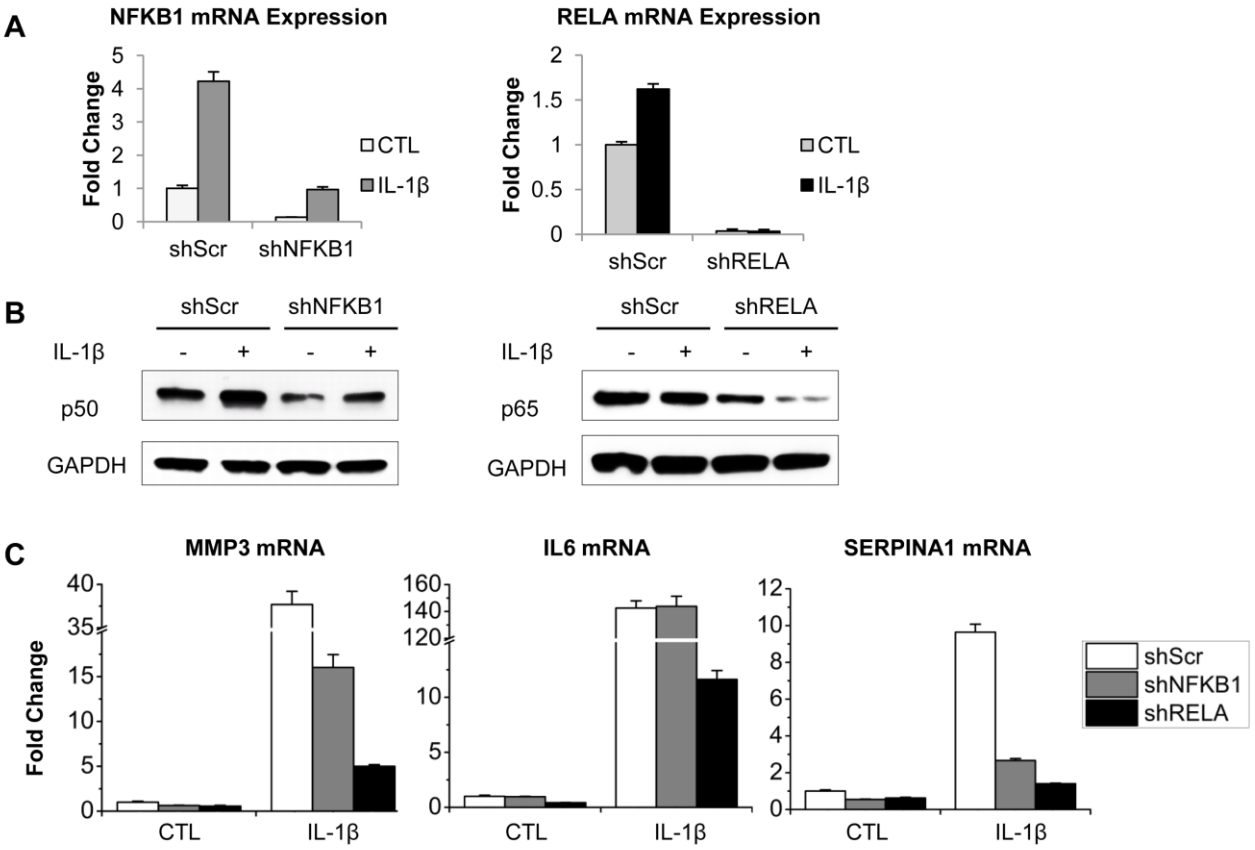
Supplemental Figure 1. Effects of Wnt/β-catenin activation in human chondrocytes. A&B, Human chondrocytes were treated with 200 ng/ml recombinant human (rh) Wnt-3A or 1 μM BIO, or left untreated (control [CTL]) for 48 hours and protein expression of β-catenin was detected by immnunoblotting (A) or immunofluorescence (green: β-catenin, blue: nuclei, scale bar = 5 μm) (B). Anti-β-catenin antibody and Alexa Fluor 546 goat anti-mouse antibody were used to stain β-catenin and cell nuclei were stained by DAPI (B). **C,** To measure TCF/LEF reporter activity, human chondrocytes were infected with a lentiviral TCF/LEF reporter construct and stimulated with 100 ng/ml rhWnt-3A, 1 μM BIO or a combination of 100 ng/ml rhWnt-3A and 300 ng/ml DKK-1 for 48 hours and luciferase activity was measured. Data are expressed as fold change compared to CTL. ** = $P < 0.01$, n = 3 donors each measured in triplicate. **D,** Proliferation of human chondrocytes was measured using a BrdU proliferation assay (Roche) according to the manufacturer's instructions in the presence or absence (CTL) of 200 ng/ml rhWnt-3A for 48 hours. Data are expressed as fold change relative to control. * = $P < 0.05$, n = 3 donors each measured in triplicate. **E,** Human chondrocytes were stimulated with 200 ng/ml rhWnt-3A for 48 hours. *COL2A1* and *SOX9* mRNA expression was measured by qPCR. ** = $P < 0.01$, n = 4 donors each measured in triplicate. **F,** Human chondrocytes were stimulated with 200 ng/ml rhWnt-3A for indicated time points and MMP mRNA expression was measured by qPCR. * = $P < 0.05$, ** = $P < 0.01$, n = 3 donors each measured in triplicate. **G,** Human chondrocytes were treated with increasing doses of Wnt-3A for 48 hours and MMP mRNA expression was measured by qPCR. n = triplicate cultures for 1 donor. **H&I,** Human chondrocytes were stimulated with 300 ng/ml DKK-1, 100 ng/ml rhWnt-3A or the combination of both for 24 hours and MMP mRNA (H) or Axin2 mRNA expression (I) was measured by qPCR. n = triplicate cultures for 1 donor.

Supplemental Figure 2



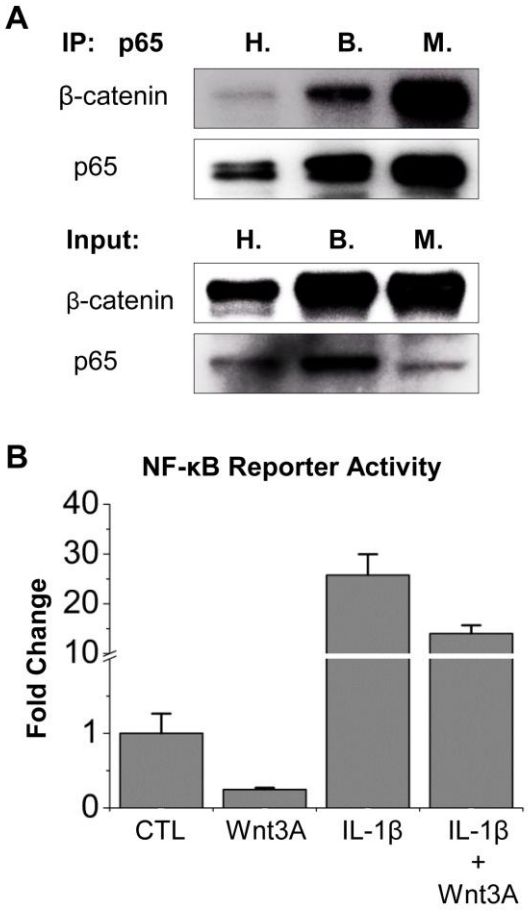
Supplemental Figure 2. Efficiency of gene overexpression and knockdown in human chondrocytes and MEFs. A&B, Lentivirus-mediated overexpression of FLAG-tagged Wnt-3 or Wnt-7B in human chondrocytes was validated by immunoblotting (A) and by upregulation of the established target gene of canonical Wnt signaling *AXIN2* meassured by qPCR, n = triplicate cultures for 1 donor (B). **C-G,** Efficiency of lentiviral shRNA-mediated knockdown was measured by qPCR after knockdown of Wnt-7B (shWnt7B) (C), β-catenin (shCat) (D), TCF-4 (shTCF4) (E) and LEF-1 (shLEF1) (F) in human chondrocytes treated with 10 ng/ml IL-1β or 50 ng/ml rhWnt-3A for 24 hours after lentiviral infection. Data are expressed as fold change compared to the scrambled (shScr) control. n = triplicate cultures from one donor (C). ** = $P < 0.01$, n = 3 donors each measured in triplicate (D-F). Proteins levels of TCF-4 and LEF-1 were measured after knockdown and treatment by immunoblotting (G). **H-K,** MEFs were transfected with siRNA and then stimulated with 200 ng/ml recombinant mouse Wnt-3A for 48 hours. β-Catenin mRNA (H) and protein expression (I) and TCF-4 mRNA (J) and LEF-1 mRNA (K) expression were detected by qPCR or immunoblotting. Data are expressed as fold change compared to the negative control (siNeg) (H, J, K). n = triplicate cultures.

Supplemental Figure 3



Supplemental Figure 3. Efficiency and effects of NF-κB knockdown in human chondrocytes. Human chondrocytes were infected with lentiviruses expressing shRNA against *NFKB1* (shNFKB1) and *RELA* (shRELA) and stimulated with 10 ng/ml IL-1β for 24 hours. Efficiency of lentiviral shRNA-mediated mRNA knockdown was measured at the mRNA level by qPCR for *NFKB1* and *RELA* or at the protein level by immunoblotting (B). (C) mRNA expression of MMP-3, IL-6 and SERPINA1 was measured by qPCR in human chondrocytes infected with lenviruses expressing shRNA against NFKB1 and RELA. Data are expressed as fold change compared to control. n = triplicate cultures for 1 donor.

Supplemental Figure 4



Supplemental Figure 4. Interaction of β-catenin with NF-κB. A, Proteins from human chondrocytes (H.), bovine chondrocytes (B.) and mouse embryonic fibroblasts (M.) were immunoprecitated with NF-κB p65 antibody and subjected to immunoblotting to detect co-precipitated proteins. **B,** MEFs were treated with 200 ng/ml recombinant mouse Wnt-3A, 10 ng/ml IL-1β or both for 24 hours after transduction of lentiviral NF-κB reporter and *Renilla* control. n = triplicate cultures.