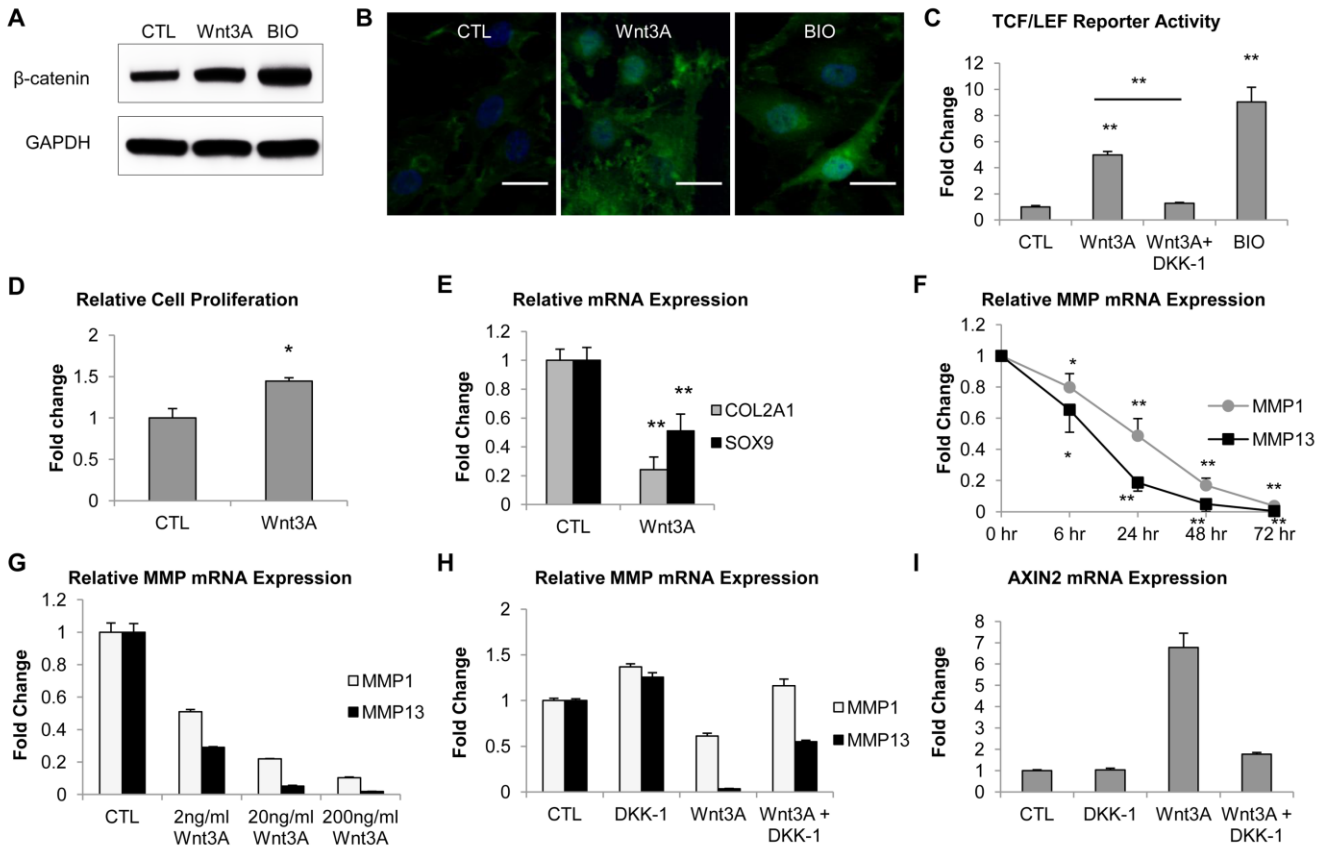
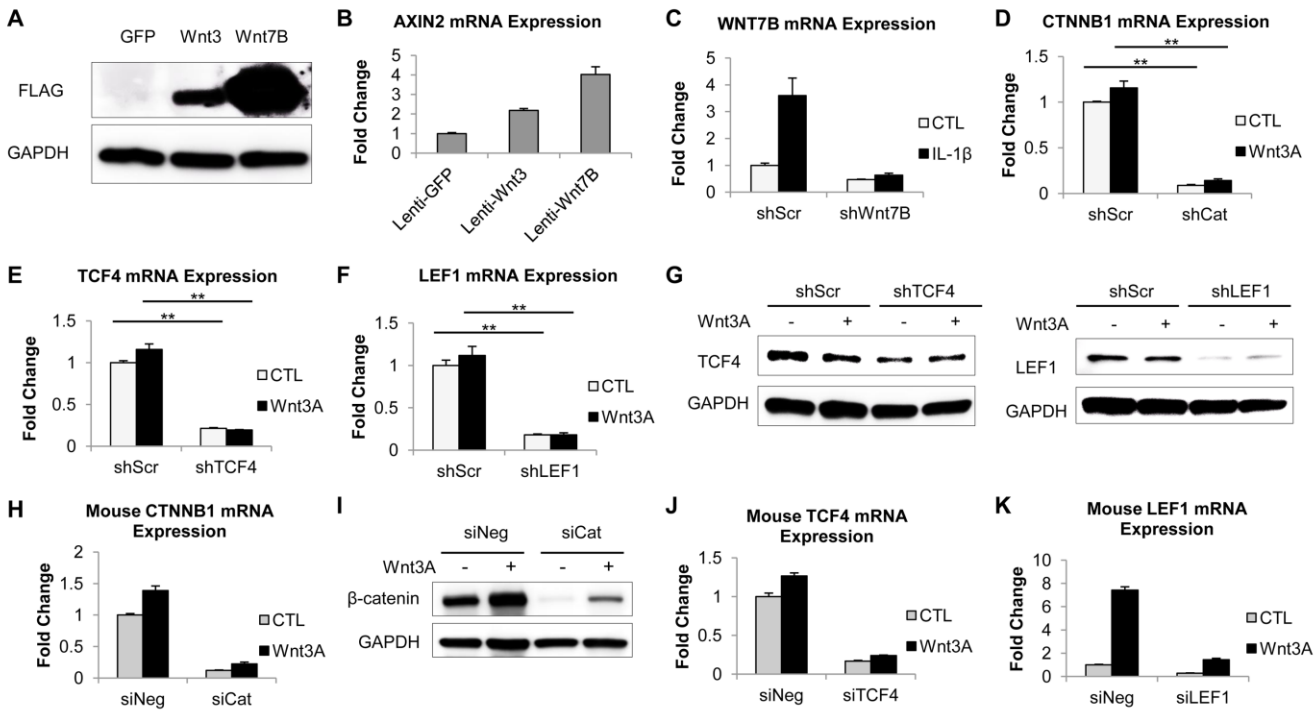
Supplemental Figure 1



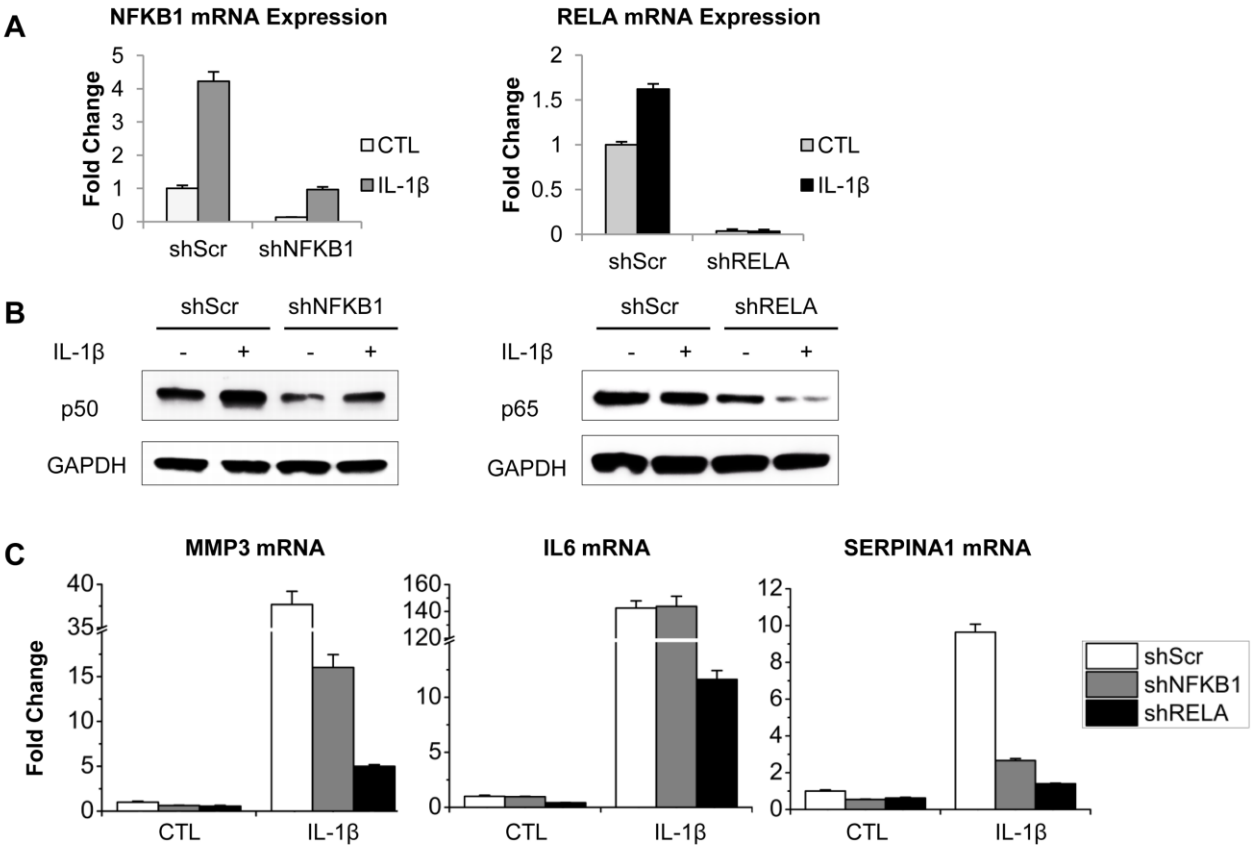
Supplemental Figure 1. Effects of Wnt/β-catenin activation in human chondrocytes. A&B, Human chondrocytes were treated with 200 ng/ml rhWnt3A or 1 μM BIO, or left untreated (control, CTL) for 48 hours and protein expression of β-catenin was detected by immnunoblot (A) or immunofluorescence (green: β-catenin, blue: nuclei, scale bar: 5 μm) (B). Anti-β-catenin antibody (BD Biosciences) and Alexa Fluor 546 goat anti-mouse antibody (Invitrogen) were used to stain β-catenin and cell nuclei were stained by DAPI (Invitrogen) (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 rhWnt3A, 1 μM BIO or a combination of 100 ng/ml rhWnt3A 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 rhWnt3A 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 rhWnt3A 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 rhWnt3A 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 Wnt3A for 48 hours and MMP mRNA expression was measured by qPCR. n = triplicate cultures from one donor. **H&I,** Human chondrocytes were stimulated with 300 ng/ml DKK-1, 100 ng/ml rhWnt3A or the combination of both for 24 hours and MMP mRNA (H) or Axin2 mRNA expression (I) was measured by qPCR. n = triplicate cultures from one donor.

Supplemental Figure 2



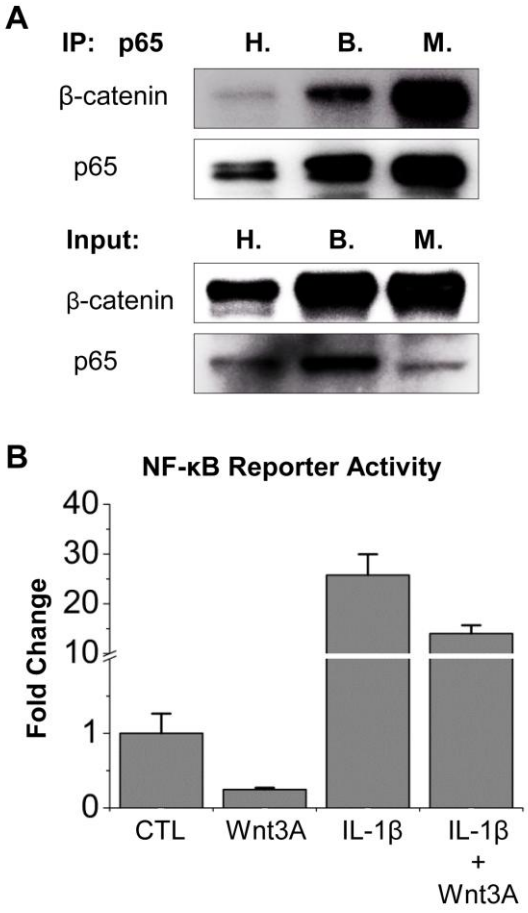
Supplemental Figure 2. Efficiency of gene overexpression and knockdown in human chondrocytes and MEFs. A&B, Lentivirus-mediated overexpression of FLAG-tagged Wnt3 or Wnt7B in human chondrocytes was validated by immunoblot (A) and by upregulation of the established target gene of canonical Wnt signaling Axin2 meassured by qPCR, n = triplicate cultures from one donor (B). **C-G,** Efficiency of lentiviral shRNA-mediated knockdown was measured by qPCR after knockdown of Wnt7B (C), CTNNB1/β-catenin (shCat) (D), TCF4 (E) and LEF1 (F) in human chondrocytes treated with 10 ng/ml IL-1β or 50 ng/ml rhWnt3A 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 TCF4 and LEF1 were measured after knockdown and treatment by immunoblot (G). **H-K,** MEFs were transfected with siRNA and then stimulated with 200 ng/ml rmWnt3A for 48 hours. β-catenin mRNA (H) and protein expression (I) and TCF4 mRNA (J) and LEF1 mRNA (K) expression were detected by qPCR or immunoblot. 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 and RELA 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 immunoblot (B). (C) mRNA expression of MMP3, IL6 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 from one 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 immunoblot to detect co-precipitated proteins. **B,** MEFs were treated with 200 ng/ml rmWnt3A, 10 ng/ml IL-1β or both for 24 hours after transduction of lentiviral NF-κB reporter and Renilla control. n = triplicate cultures.