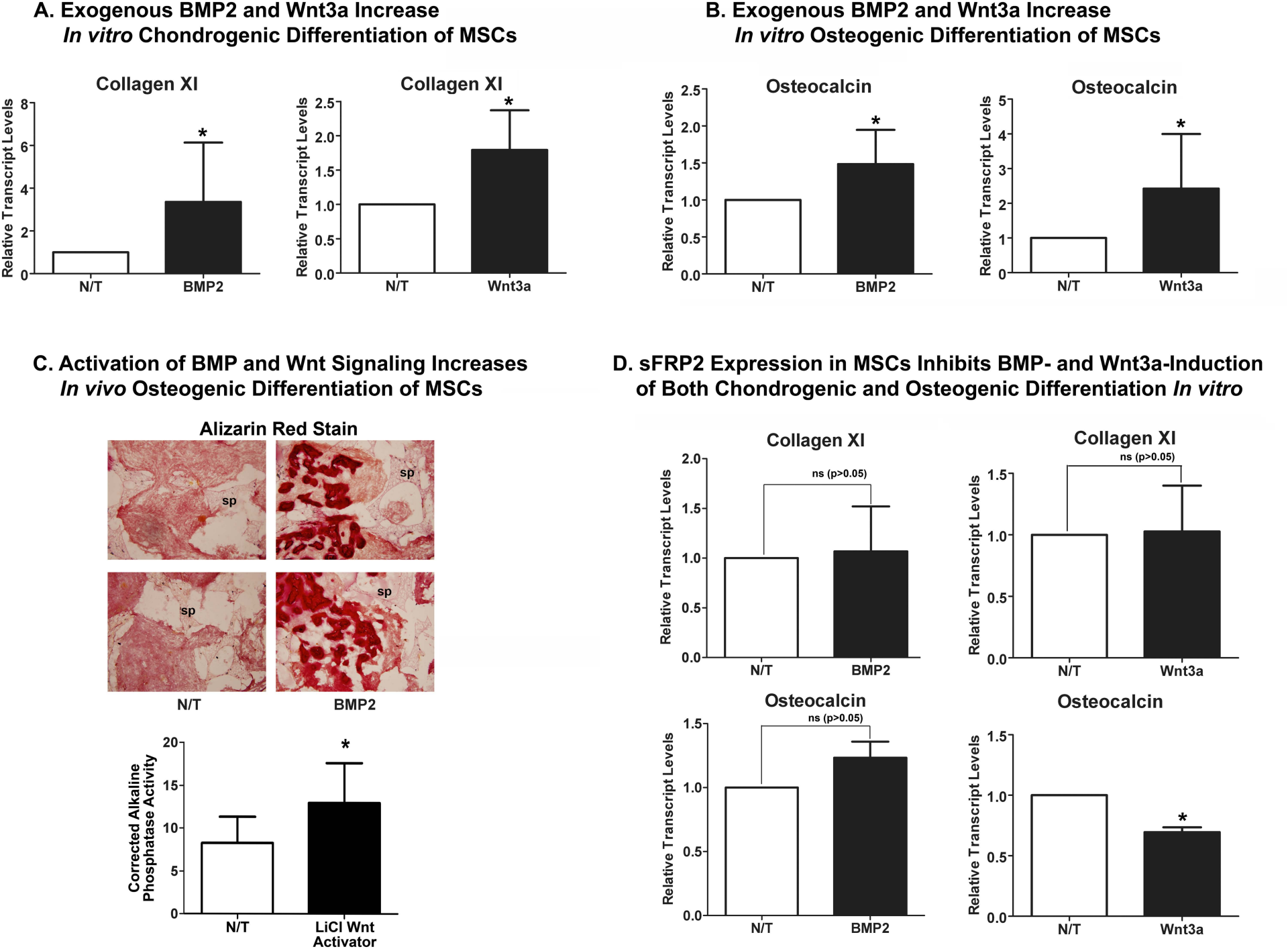
Supplemental Fig.1. BMP and Wnt signaling are involved in MSC lineage commitment *in vitro* and *in vivo*; sFRP2 inhibits this effect A. Exogenous recombinant BMP2 (100 ng/ml) and Wnt3a (50 ng/ml) increase the levels of collagen XI transcripts in MSCs *in vitro*. B. Addition of BMP2 (100 ng/ml) and Wnt3a (50 ng/ml) increase the osteocalcin transcript levels of MSCs *in vitro*. n=6 cohorts per treatment group per lineage. C. Representative images of Alizarin Red stained PVA sponges that were treated with 30 μg/kg/day of BMP2 admixed with growth factor reduced matrigel or matrigel alone. BMP2 treatment increased extracellular calcification as observed by the bright red nodules. sp=sponge (20X); n=4. Wnt activation by LiCl increases osteogenic differentiation of MSCs within PVA sponges. The levels of alkaline phosphatase activity (normalized to DNA content) in LiCl-treated sponges is higher compared to PBS-treated controls. D. The increase in collagen XI and osteocalcin transcript levels is not observed in the sFRP2-MSC cohort after addition of BMP2 (100 ng/ml) or Wnt3a (50 ng/ml) to the differentiating conditions. *$p \leq 0.05$, Two-tailed, paired student's T-test; ns=not significant; n=3; N/T=no treatment.

Supplemental Fig 2. BMP signaling is decreased by sFRP2 even in the presence of a Wnt inhibitor. BMP2 treatment (50 ng/ml) increases pSMAD 1/5/8 levels dramatically in GFP-MSCs. This increase is not as dramatic in sFRP2-MSCs and is not rescued by the Wnt inhibitor, pyrvinium. N/T=no treatment.


A. Exogenous BMP2 and Wnt3a Increase
In vitro Chondrogenic Differentiation of MSCs
Collagen XI
Relative Transcript Levels
N/T
BMP2
Collagen XI
Relative Transcript Levels
N/T
Wnt3a
B. Exogenous BMP2 and Wnt3a Increase
In vitro Osteogenic Differentiation of MSCs
Osteocalcin
Relative Transcript Levels
N/T
BMP2
Osteocalcin
Relative Transcript Levels
N/T
Wnt3a
C. Activation of BMP and Wnt Signaling Increases
In vivo Osteogenic Differentiation of MSCs
Alizarin Red Stain
sp
N/T
BMP2
Corrected Alkaline Phosphatase Activity
N/T
LiCl Wnt Activator
D. sFRP2 Expression in MSCs Inhibits BMP- and Wnt3a-Induction
of Both Chondrogenic and Osteogenic Differentiation In vitro
Collagen XI
ns (p>0.05)
Relative Transcript Levels
N/T
BMP2
Collagen XI
ns (p>0.05)
Relative Transcript Levels
N/T
Wnt3a
Osteocalcin
ns (p>0.05)
Relative Transcript Levels
N/T
BMP2
Osteocalcin
Relative Transcript Levels
N/T
Wnt3a

Supplemental Figure 2