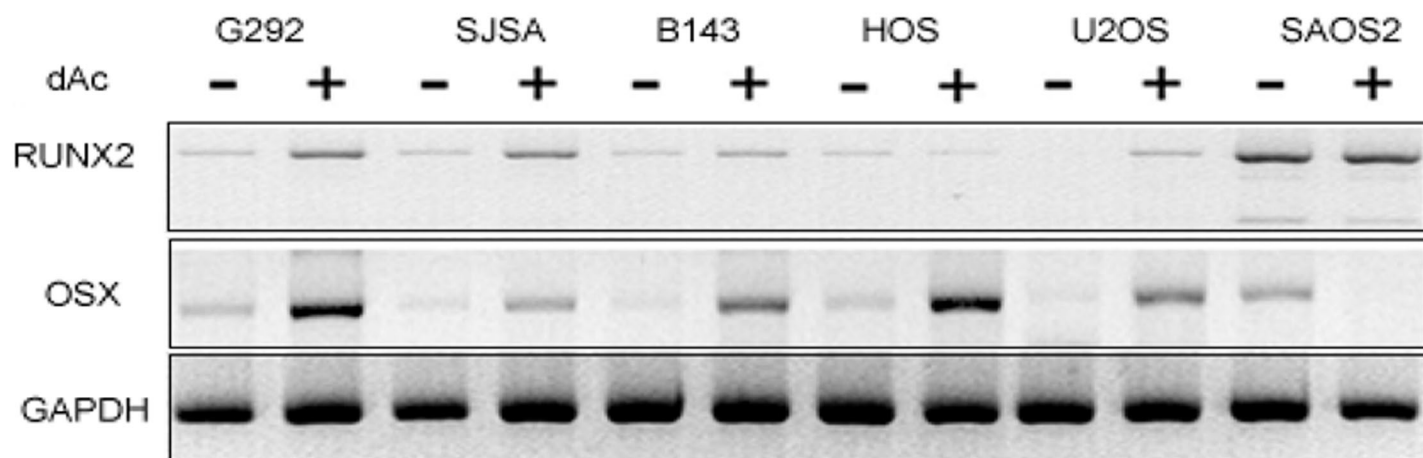


SUPPLEMENTAL FIGURE LEGENDS

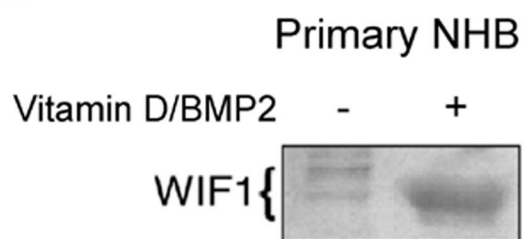
Supplemental Figure 1. dAc and Wif1 regulate osteoblast differentiation in vitro. . A: RT-PCR confirmation of changes in Runx2 and OSX expression in a panel of osteosarcoma cell lines following treatment with dAc. B: Normal human bone cells were treated with BMP2 and Vitamin D for 21 days, and 20 µg of protein subject to Western blotting for analysis of Wif1 protein expression. C: Primary human bone-derived osteoblasts were flow sorted using antibodies STRO1 and ALP and semi quantitative RT-PCR analysis carried out to assess transcript levels of Wif1, OC, COL1A1. D: NHB cells and osteosarcoma cell lines were treated in 24 well plates with 2µg/ml of Wif1-GST recombinant protein or equimolar amounts of control GST protein for 16 hr. Cells were lysed, and equal amounts of protein lysates were assayed for ALP activity using a colorimetric kit. Data represent the mean and SEM. Each value represents the mean ± of three separate determinations, expressed as nmole of p-nitrophenyl produced/mg protein/minute.

Supplemental Figure 2. A: Right hindlimbs were obtained from 4-7 *Wif1^{LacZ/LacZ}* and wildtype male and female mice at 16 ± 1 weeks of age. Data is expressed as means ± S.E.M. Statistically significant differences are depicted by * P<0.05 (2 way ANOVA analysis of variance). Femoral length and width of femurs determined by X-ray. Histomorphometric analysis of tibial bone structure. Mineralised surface/bone surface (MS/BS), mineral appositional rates (MAR), bone formation rate/bone surface (BFR/BS), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular space (Tb.Sp), bone volume/tissue volume (BV/TV), osteoblast number per mm of bone perimeter (Nob/Bpm), and osteoid thickness (O.Th). B: Effect of genotype on expression of osteoblast marker genes. Quantitative RT-PCR examining changes in gene expression in femurs from 8-week-old *Wif1^{LacZ/LacZ}* and wildtype mice and MC3T3E1 Wif1 knockdown cells compared to control cells.

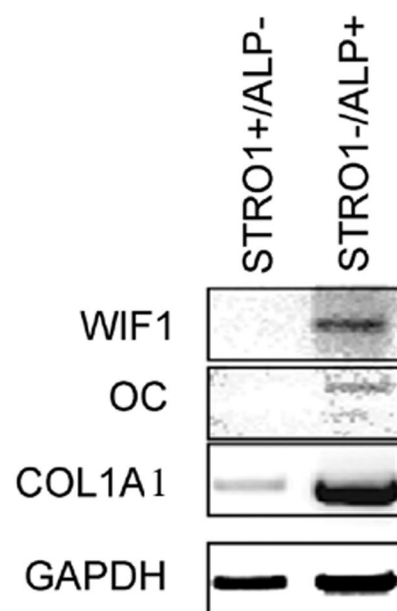
A



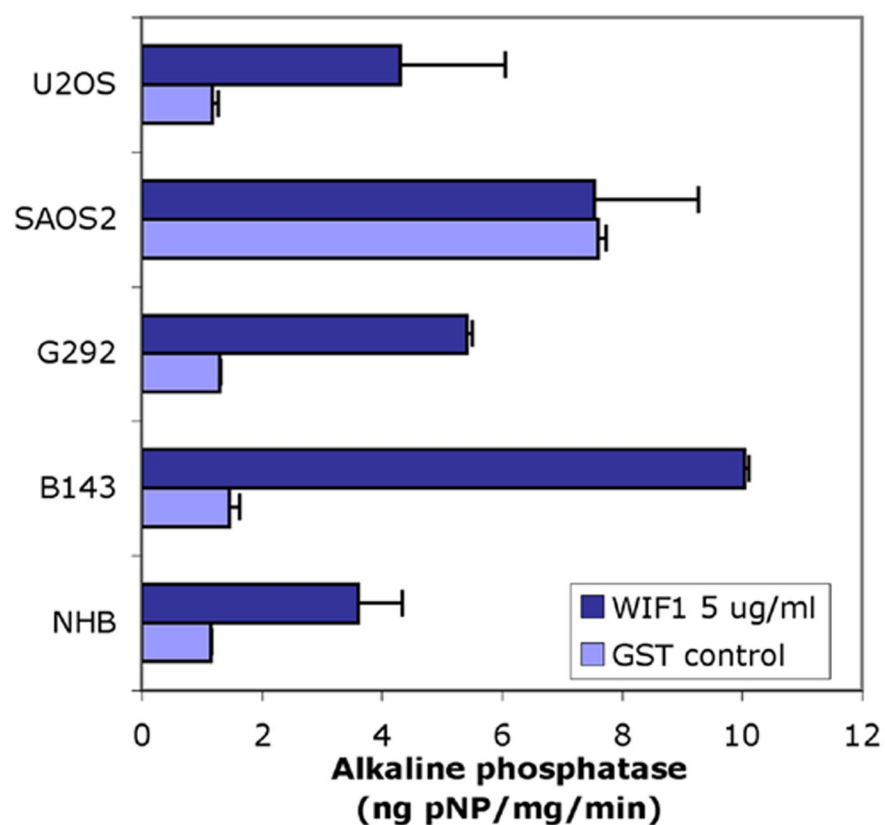
B



C



D



A

	Male		Female		2-way ANOVA (p-values)		
	Wif1 +/+	Wif1 -/-	Wif1 +/+	Wif1 -/-	Genotype	Gender	Interaction
Femoral length (mm)	15.4±0.4	15.0±0.2	14.1±0.3	14.7±0.4	0.7541	0.0221*	0.1315
Femoral width (mm)	1.4±0.04	1.3±0.1	1.1±0.1	1.2±0.1	1.000	0.0652	0.3357
MS/BS (%)	9.1±1.7	11.8±4.4	11.5±2.6	11±0.7	0.7721	0.8331	0.6740
MAR (µm/day)	1.1±0.17	1.4±0.15	1.9±0.2	1.42±0.2	0.6301	0.0407*	0.0501
BFR/BS (%)	37.5±6.8	74.4±35	75.4±14	55.7±7	0.7690	0.7404	0.1315
Tb.N (/mm)	2.8±0.4	2.0±0.4	2.0±0.4	1.7±0.3	0.2061	0.2061	0.5570
Tb.Th (µm)	40.9±5	35.5±4	33.1±5.2	29.3±3	0.3303	0.1465	0.8634
Tb.Sp (µm)	319±42	774±231	466±63	620±146	0.1400	0.9859	0.4531
BV/TV (%)	11.4±2.8	8.4±2.5	6.2±3.2	4.2±1.5	0.3769	0.1075	0.8579
Nob/BPm	3.6±1.6	7.4±2.1	10.8±3.3	16.1±7.5	0.8500	0.2614	0.8500
O.Th (µm)	1.2±0.3	1.3±0.3	2.0±0.4	2.1±0.9	0.8387	0.1182	1.0000

B

