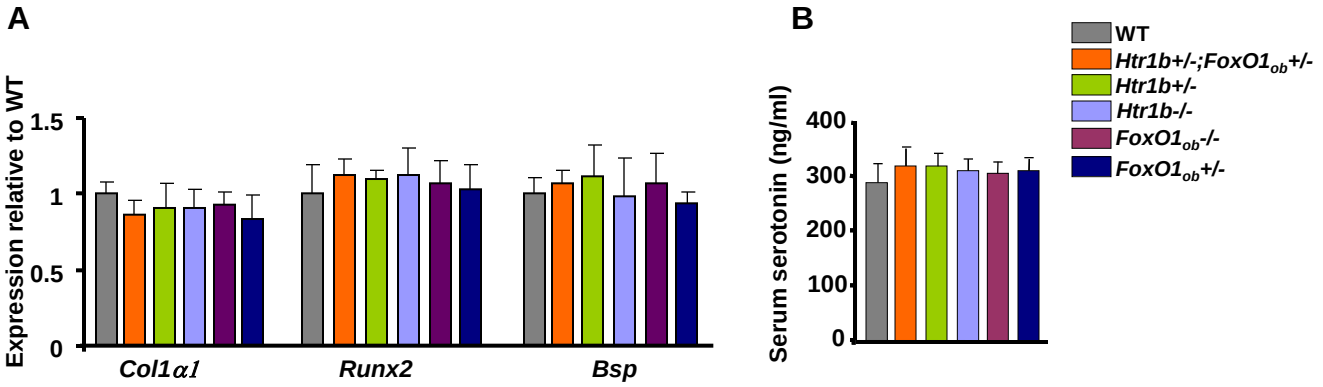
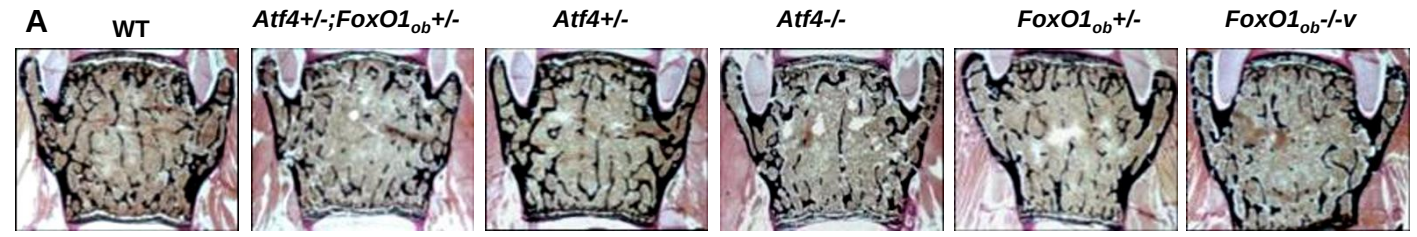
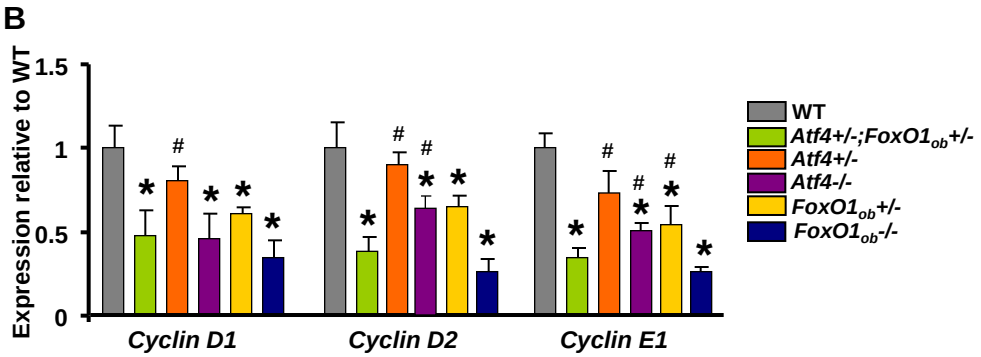
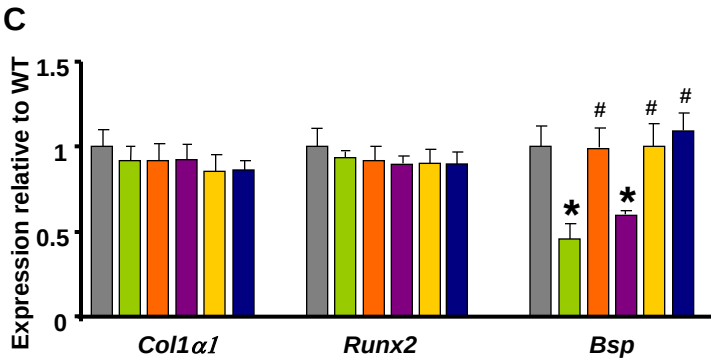


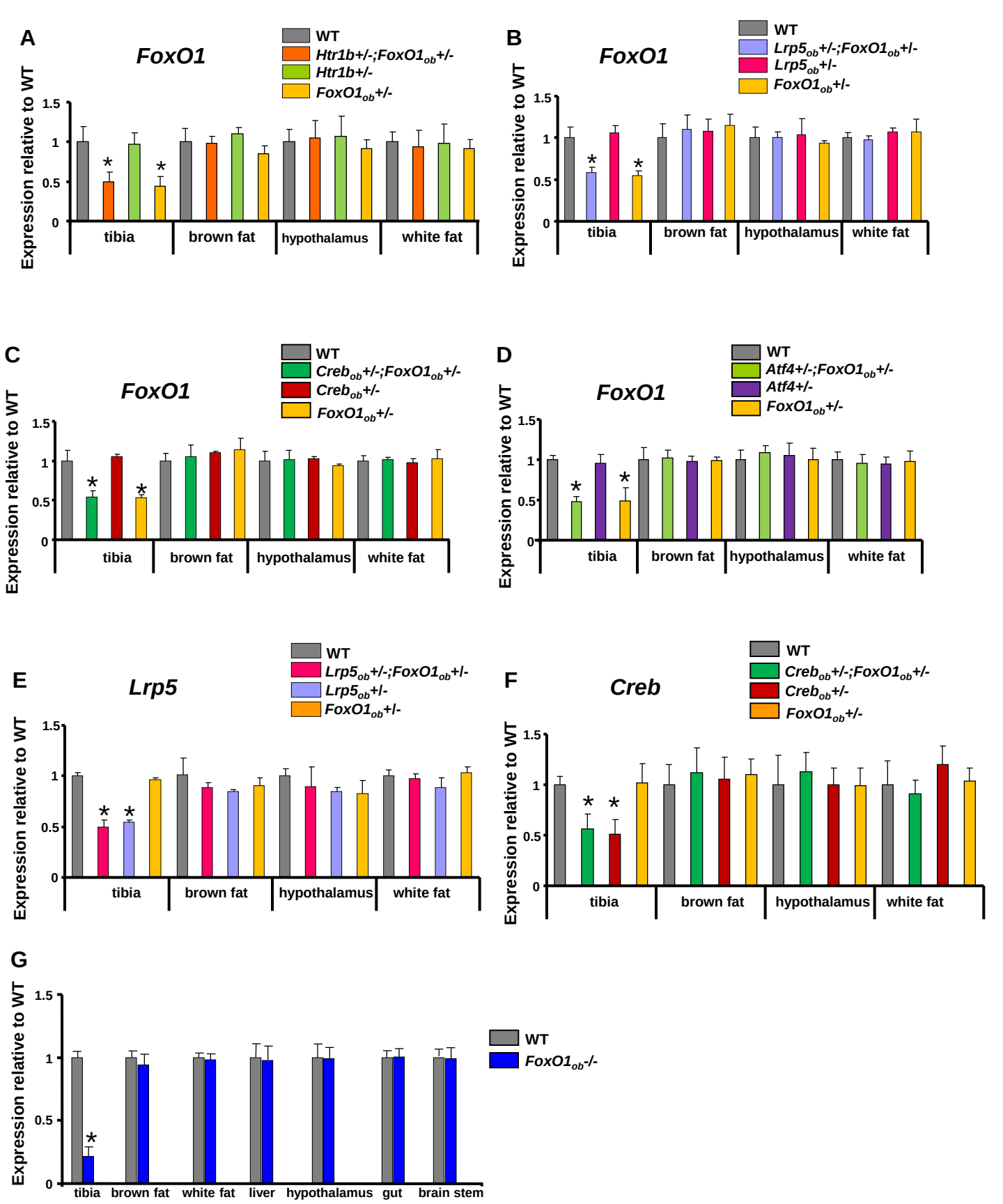
Supplemental Figure 1: Htr1b and FoxO1 interaction does not affect expression of osteoblast differentiation genes. (A) *Col1α1*, *Runx2* and *BSP* expression in bones of 2 month-old mice, n=4 mice/group. *p < 0.05 versus WT and #p < 0.05 versus *Htr1b+/-;FoxO1*$_{ob}$*+/-* group. **(B)** Serum serotonin levels. n=4 mice/group.



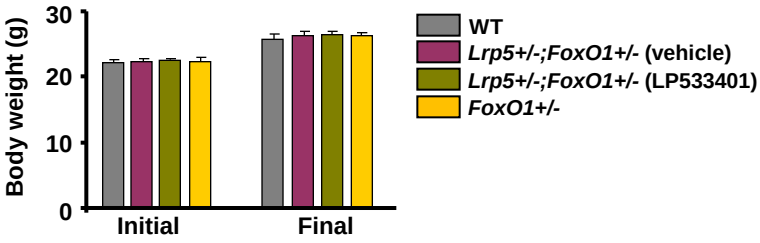




Supplemental Figure 2: FoxO1 and ATF4 synergize to maintain osteoblast proliferation. (A) Representative vertebral section images from 2 month-old mice. Mineralized bone matrix is stained in black by Von Kossa reagent. Images at 4x. **(B-C)** Real-time PCR analysis of indicated genes in bones, n=4 mice/group. *p < 0.05 versus WT and #p < 0.05 versus *Atf4*+/-;*FoxO1*$_{ob}$+/- group. Mice were 2 months old.



Supplemental Figure 3: Osteoblast-specific inactivation of *FoxO1*, *Lrp5*, and *Creb* in mice (A-D and G) *FoxO1*, **(E)** *Lrp5* and **(F)** *Creb* expression in tissues of WT and indicated mouse models, n=4 mice/group. *p < 0.05 versus WT.



Supplemental Figure 4: Normal body weight in mice treated with the gut serotonin synthesis inhibitor. The gut-derived serotonin synthesis inhibitor LP533401 was administered at 200mg/kg/day by oral gavage for 4 weeks. n=6 mice/group.. Mice were 2 months old.

Supplemental Table 1: FoxO1-ATF4 interaction is required for osteoblast proliferation.

	Wild type	*Atf4+/-; FoxO1$_{ob}$+/-*	*Atf4+/-*	*Atf4-/-*	*FoxO1$_{ob}$+/-*	*FoxO1$_{ob}$-/-*
BV/TV (%)	**15.09 0.9**	**9.85 1.2***	**13.31 0.5*#$**	**10.75 1.3*#$**	**12.95 0.6*#$**	**9.13 1.4***
N.Ob/T.Ar(/mm)	**95.12 10.3**	**41.26 7.6***	**87.31 6.5#$**	**51.56 8.3***	**71.32 11.6*#$**	**39.47 5.1***
BFR (μm^3/μm^2/yr)	**150.32 18.5**	**53.41 8.7***	**138.65 10.3#$**	**73.48 9.7*#$**	**83.49 9.8*#$**	**51.38 6.9***
OcS/BS	**9.56 1.4**	**10.24 1.2**	**9.36 1.5**	**9.11 0.1**	**10.57 0.9**	**18.16 2.3*#**

Histomorphometric analysis of vertebrae of WT, *Atf4*+/-; *FoxO1$_{ob}$*+/-, *Atf4*+/-, *Atf4*-/-, *FoxO1$_{ob}$*+/-, and *FoxO1$_{ob}$*-/- mice. BV/TV, bone volume over trabecular volume; N.Ob/T.Ar, number of osteoblasts per trabecular area; BFR, Bone Formation Rate, OcS/BS, osteoclast surface per bone surface. WT, *Atf4*+/-, *Atf4*-/-, *Atf4*+/-;*FoxO1ob*+/-, *FoxO1$_{ob}$*+/- , *FoxO1ob*-/-. (n=6-10 mice/group). *$p < 0.05$ versus wild type, $^{\#}p < 0.05$ versus *Atf4*+/-;*FoxO1$_{ob}$*+/- group and $^{\$}p < 0.001$ versus *ATF4*+/-; and *FoxO1$_{ob}$*+/- as determined by a test of multiplicative model in an One Way Anova. Mice were 2 months old.

Supplemental Table 2: Primer sequences used in real-Time PCR.

Primer	*Forward primer 5'->3'*	*Reverse primer 5'->3'*
β-Actin	*5'GACCTCTATGCCAACACAGT 3'*	*5' AGTACTTGCGCTCAGGAGGA3'*
BSP	*5'GGGAGGCAGTGACTCTTCAG 3'*	*5'CCCGAGAGTGTGGAAAGTGT3'*
Col1a1	*5'GAGAGCATGACCGATGGATT 3'*	*5' TGAGCTCGATCTCGTTGGAT3'*
Cyclin D1	*5' AGTGCGTGCAGAAGGAGATT3'*	*5'CACAACTTCTCGGCAGTCAA3'*
Cyclin D2	*5'TCCCGCAGTGTTCCTATTTC3'*	*5' CCAAGAAACGGTCCAGGTAA3'*
Cyclin E1	*5'CCTCCAAAGTTGCACCAGTT3'*	*5'CACCCGTGTCGTTGACATAG3'*
Runx2	*5' GCCGGGAATGATGAGAACTA3'*	*5' GGACCGTCCACTGTCACTTT3'*
Sod2	*5'CCGAGGAGAAGTACCACGAG3'*	*5'GCTTGATAGCCTCCAGCAAC3'*
$p19^{ARF}$	*5'CCACCCTTACCAGACCTGTG3'*	*5'AGGCGTCACACACATCCAG3'*
p16	*5'TCAACTACGGTGCAGATTCG3'*	*5'TCGCACGATGTCTTGATGTC3'*
Catalase	*5'CCTGACATGGTCTGGACTT3'*	*5'CAAGTTTTTGATGCCCTGGT3'*
Gadd45	*5'TGAGCTGCTGCTACTGGAGA3'*	*5'TCCCGGCAAAAACAAATAAG3'*