

## **Supplementary Data**

**Effect of rapamycin on bone mass and strength in the  $\alpha 2(I)$ -G610C mouse model of osteogenesis imperfecta**

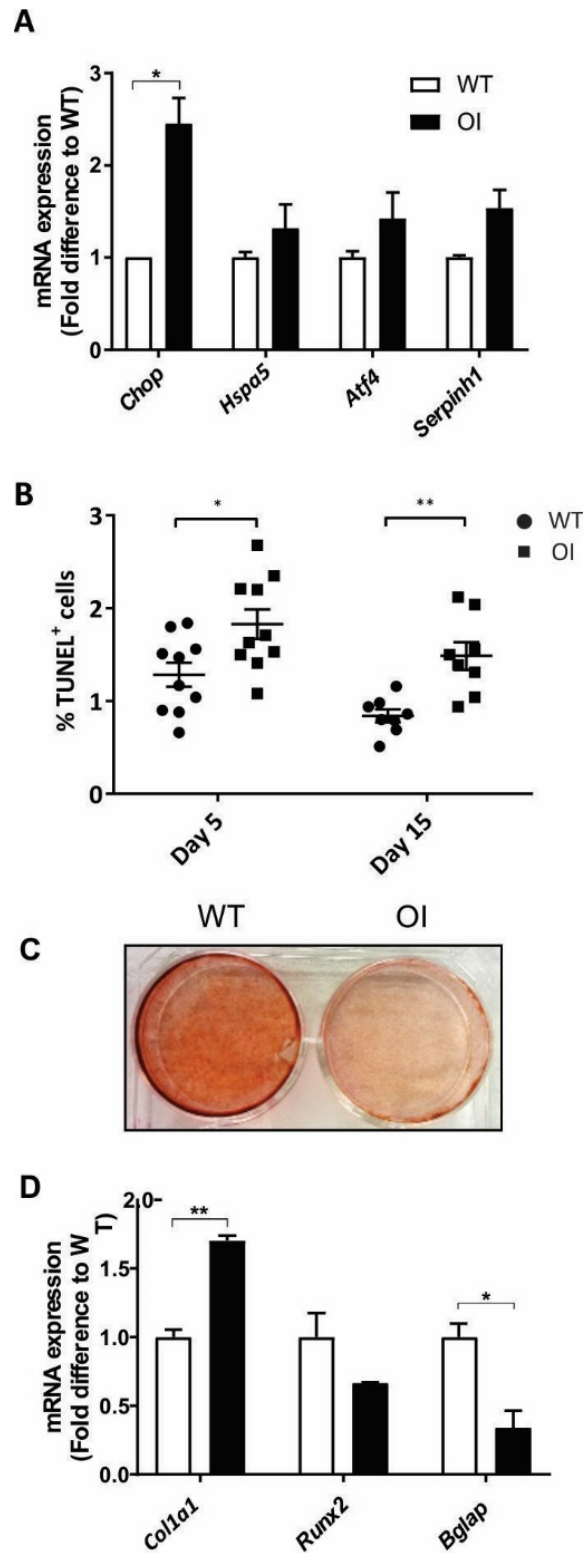
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## Supplementary Table 1

### RT-qPCR primer list

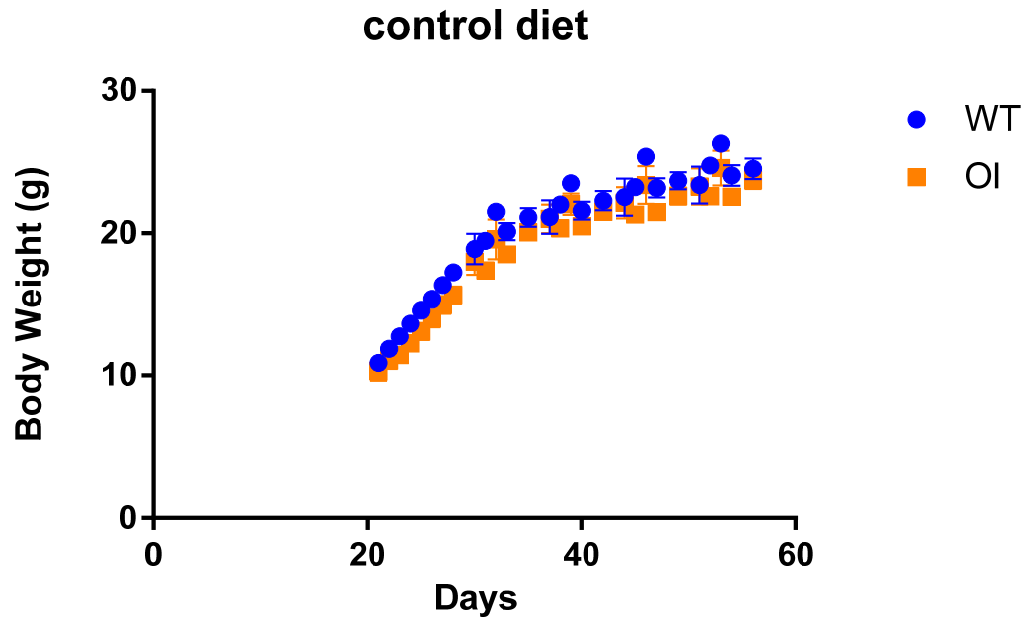
<b>Gene</b>	<b>Forward primer</b>	<b>Reverse Primer</b>
<i>Gapdh</i>	AATGTGTCCGTCGTGGATCT	GCTTCACCACCTTCTTGATGT
<i>Hspa5 (BiP)</i>	TGAAACTGTGGGAGGAGTCA	TTCAGCTGTCACTCGGAGAA
<i>Ddit3 (Chop)</i>	GCGACAGAGCCAGAATAACA	GATGCACTTCCTTCTGGAACA
<i>Serpinh1 (Hsp47)</i>	ATGCCTGCCCTAGAATTGTTAT	AAAAGGGAGAAAAACAAAAGTTCA
<i>Atf4</i>	AGAAGCTGAAAAAGATGGAGCA	GCTTCTTCTGGCGGTACCTA
<i>Col1a1</i>	GACTGGCAACCTCAAGAAGG	CAAGTTCGGGTGTGACTCG
<i>Runx2</i>	TCTCCACACCATTAGAGGTTGA	GGGTGCTTCTGCTACCACTC
<i>Bglap</i>	AGACTCCGGCGCTACCTT	CTCGTCACAAGCAGGGTTAAG

Supplementary Figure 1. Cell stress, apoptosis and differentiation in cultured parietal bone osteoblasts



**A)** Primary osteoblasts from 10 day old wild type (WT) and OI mice were cultured for 21 days in osteogenic medium, mRNA extracted and RT-qPCR performed in triplicate using primer pairs for the indicated genes normalised to the housekeeping gene *Gapdh*. Plots depict mean fold difference of OI to WT control with standard deviation from the mean, N = 3 independent experiments, statistical significance was determined assuming a normal distribution among the groups using unpaired one-tail Student's *t* test, \*  $p < 0.05$ ; **B)** Apoptosis in calvarial osteoblasts. Calvarial cells were cultured *in vitro* in osteogenic medium, for 5 and 15 days, fixed and apoptosis was assayed by TUNEL staining. TUNEL-positive nuclei were counted and expressed related to the total number of DAPI-positive nuclei. □ WT, ■ OI. All values are mean  $\pm$  S.E.M. Student's t-test, \*  $p < 0.05$ , \*\* $p < 0.01$ ; **C)** Cultures stained with Alizarin Red to visualize calcification after 21 days in osteogenic medium; **D)** RT-qPCR of osteoblast differentiation markers after 21 days culture as described in A, \*  $p < 0.05$ , \*\* $p < 0.01$ .

Supplementary Figure 2. OI and WT mouse weight



Wild type mice (N=8) and OI mice (N=8) were weighed daily from 3 weeks to 8 weeks of age.

All values are mean  $\pm$  S.E.M.