

List of Supplementary Materials

Supplementary figure 1. *Notum* mRNA expression in primary cultures of bone cells from calvaria and long bones. (A) mRNA expression analyses of *Notum* in calvarial periosteal cells from *Runx2-cre Notum^{flox/flox}* and *Notum^{flox/flox}* mice cultured for 4 (D4) and 7 (D7) days in osteogenic media. (B) mRNA expression analyses of *Notum* in long bone osteoblasts from *WT* C57BL/6N mice cultured in osteogenic media for 1 (D1), 3 (D3) and 6 (D6) days.

Supplementary figure 2. *Notum* mRNA expression in the MC3T3-E1 osteoblastic cell line mRNA expression analyses of *Notum* in MC3T3-E1 cells cultured for 2, 4 and 11 days.

Supplementary figure 3. Enzymatic activity of recombinant NOTUM protein. Lipase activity of recombinant human NOTUM protein and wheat germ lipase analyzed by cleavage of the fluorogenic substrate OPTS and fluorescence at 535 nm after 10, 20 and 30 min.

Supplementary figure 4. Regional association plots for the two independent signals in the *NOTUM* locus \pm 25Kb on chromosome 17 with either (A) rs 35344256 or (B) rs 147901986 indicated by purple diamond to evaluate linkage with other single-nucleotide polymorphisms in the region. The r^2 is based on the hg19-1000 Genomes Nov 2014 EUR samples. The blue line and right hand Y axis represent EUR 1000 Genomes based recombination rates.

Supplementary table 1. Bone characteristics in male *Notum* heterozygote mice.

Supplementary table 2: Cortical bone analysis of vertebral body L5 in *Runx2-creNotum^{flox/flox}* mice.

Supplementary table 3: Histomorphometric parameters in the diaphyseal region of femur in *Runx2-creNotum^{flox/flox}* mice.

Supplementary table 4: Body weight and organ weights in mice with inducible *Notum* inactivation.

Supplementary Table 1. Bone characteristics in male *Notum*^{+/-} mice

	WT	<i>Notum</i> ^{+/-}
Cortical bone area (mm ²)	1.1 ± 0.03	1.2 ± 0.03
Cortical thickness (µm)	211.7 ± 5.17	218.3 ± 4.64
Cortical density (mg/mm ³)	1151.8 ± 8.43	1162.4 ± 5.85
Trabecular Bone Mineral Density (mg/cm ³)	428.8 ± 26.3	436.0 ± 22.5
Total body Bone Mineral Density (mg/cm ³)	546.0 ± 19.9	556.4 ± 16.7

Bone parameters measured by pQCT analyses in femur and total body bone mineral density as measured by DXA. Values are given as means ± SEM (WT, n=7; *Notum*^{+/-}, n=8, 13-week-old male mice).

Supplementary Table 2: Cortical bone analysis of vertebral body L5 in *Runx2-creNotum*^{flox/flox} mice

	Female		Male	
	<i>Notum</i> ^{flox/flox}	<i>Runx2-creNotum</i> ^{flox/flox}	<i>Notum</i> ^{flox/flox}	<i>Runx2-creNotum</i> ^{flox/flox}
Cortical area (mm ²)	366 ± 11	430 ± 18**	334 ± 14	357 ± 5(*)
Cortical thickness (µm)	84.6 ± 2.7	101.6 ± 5.0**	74.3 ± 3.7	76.8 ± 1.4

Cortical bone analysis of vertebral body L5 as measured by µCT. Values are given as means ± SEM. 17-week-old *Runx2-creNotum*^{flox/flox} mice (females, n=11; males, n=15) and *Notum*^{flox/flox} mice (females, n=13; males, n=11);). ***P*<0.01, (*)*P*=0.09 vs. *Notum*^{flox/flox} using Student's *t* test.

Supplementary Table 3: Histomorphometric parameters in the diaphyseal region of femur in *Runx2-creNotum^{flox/flox}* mice

	<i>Notum^{flox/flox}</i>	<i>Runx2-creNotum^{flox/flox}</i>
Static parameters		
<i>Periosteal</i>		
Number of osteoblasts/perimeter (N.Ob/B.Pm; mm ⁻¹)	7.41 ± 2.15	3.86 ± 1.11
Number of osteoclasts/perimeter (N.Oc/B.Pm; mm ⁻¹)	1.17 ± 0.25	1.09 ± 0.26
<i>Endocortical</i>		
Number of osteoblasts/perimeter (N.Ob/B.Pm; mm ⁻¹)	6.97 ± 1.71	5.64 ± 1.69
Number of osteoclasts/perimeter (N.Oc/B.Pm; mm ⁻¹)	0.96 ± 0.48	0.67 ± 0.15
Dynamic parameters		
<i>Periosteal</i>		
Mineralizing surface/bone surface (MS/BS; %)	46.0 ± 4.10	47.1 ± 2.02
Mineral apposition rate (MAR; μm/d)	1.23 ± 0.12	1.27 ± 0.05
Bone formation rate (BFR; μm ³ /μm ² /d)	221 ± 36	220 ± 16
<i>Endocortical</i>		
Mineralizing surface/bone surface (MS/BS; %)	35.4 ± 2.76	35.0 ± 3.90
Mineral apposition rate (MAR; μm/d)	1.22 ± 0.13	1.15 ± 0.08
Bone formation rate (BFR; μm ³ /μm ² /d)	167 ± 22	155 ± 23

Values are given as means ± SEM. (*Notum^{flox/flox}*, n=11; *Runx2-creNotum^{flox/flox}*, n=15; 17-week-old male mice). ****P*<0.001, ***P*<0.01, vs. *Notum^{flox/flox}* using Student's *t* test.

Supplementary Table 4: Body weight and organ weights in mice with inducible *Notum* inactivation

	<i>Notum</i> ^{flx/flx}	<i>CAGG-Cre-ER-Notum</i> ^{flx/flx}
Body weight (g)	23.2 ± 0.5	23.9 ± 0.7
Femur length (mm)	16.3 ± 0.1	16.6 ± 0.1
Tibia length (mm)	18.7 ± 0.1	18.7 ± 0.1
<i>Organ weights (mg/g body weight)</i>		
Liver	43.2 ± 1.4	43.1 ± 2.2
Gonadal fat	19.8 ± 2.6	19.2 ± 1.2
Kidney	8.9 ± 0.5	9.7 ± 0.2
Spleen	3.0 ± 0.2	3.2 ± 0.2
Uterus	2.4 ± 0.5	2.6 ± 0.4

Values are given as means ± SEM. (*Notum*^{flx/flx}, n=10; *CAGG-Cre-ER-Notum*^{flx/flx}, n=12; 15-week-old female mice).