Supplemental material

Osteocyte- and late Osteoblast-derived NOTUM Reduces Cortical Bone Mass in Mice

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Supplemental figure 1: Total body bone mineral density measured by DXA in $Dmp1$-cre$Notum^{flox/flox}$ (females, n=7; males, n=5) mice and $Notum^{flox/flox}$ (females, n=10; males, n=8) mice. The results refer to 12-week-old mice. All values are given as mean ± SEM. A mixed model two-way ANOVA was used to assess the effects of genotype ($Dmp1$-cre$Notum^{flox/flox}$ and $Notum^{flox/flox}$), gender, as well as their interaction. A difference was considered significant when $P < 0.05$. 
Supplemental Table 1. mRNA expression analyses in diaphyseal cortical bone

| Genes          | Females Notum^{flox/flox} | Females Dmp1-creNotum^{flox/flox} | Males Notum^{flox/flox} | Males Dmp1-creNotum^{flox/flox} | P Genotype | P Gender | P Interaction |
|----------------|---------------------------|----------------------------------|-------------------------|---------------------------------|------------|----------|--------------|
| Alpl           | 9.42 ± 0.99               | 10.2 ± 1.44                      | 6.2 ± 0.29              | 3.25 ± 0.87                     | 0.5        | < 0.0001 | 1            |
| Col1a1         | 5.00 ± 0.61               | 4.71 ± 0.43                      | 4.25 ± 0.83             | 2.10 ± 0.45                     | 0.3        | 0.03     | 0.1          |
| Tnfrsf11b (OPG)| 4.84 ± 0.46               | 3.12 ± 0.36                      | 1.71 ± 0.24             | 1.0 ± 0.14                      | 0.8        | 0.009    | 0.3          |
| Tnfs11 (RANKL)| 3.63 ± 0.63               | 3.02 ± 0.29                      | 1.12 ± 0.36             | 0.2 ± 0.07                      | 0.2        | 0.7      | 0.9          |
| Ratio Rankl/OPG| 3.44 ± 0.43               | 2.87 ± 0.32                      | 2.07 ± 0.36             | 1.0 ± 0.14                      | 0.7        | 0.3      | 0.2          |
| Ctsk           | 2.68 ± 0.41               | 2.44 ± 0.70                      | 2.65 ± 0.30             | 3.66 ± 0.69                     | 0.3        | 0.2      | 0.1          |
| Tcf7           | 1.80 ± 0.16               | 1.84 ± 0.12                      | 1.47 ± 0.17             | 1.57 ± 0.22                     | 0.7        | 0.1      | 0.9          |
| Lrp5           | 3.62 ± 0.45               | 3.43 ± 0.21                      | 2.60 ± 0.36             | 2.72 ± 1.67                     | 0.9        | 0.004    | 0.7          |
| Lrp6           | 1.47 ± 0.10               | 1.37 ± 0.06                      | 1.25 ± 0.10             | 1.26 ± 0.01                     | 0.6        | 0.1      | 0.5          |
| Fzd1           | 2.70 ± 0.43               | 0.32 ± 0.06                      | 0.34 ± 0.07             | 2.57 ± 0.48                     | 0.7        | 0.9      | 0.4          |
| Dmp1           | 3.00 ± 0.33               | 2.74 ± 0.22                      | 1.89 ± 0.27             | 2.30 ± 0.51                     | 0.6        | 0.9      | 0.4          |
| Sost           | 3.95 ± 0.50               | 3.57 ± 0.44                      | 1.86 ± 0.23             | 2.13 ± 0.46                     | 0.9        | < 0.001  | 0.5          |
| Wnt10b         | 3.03 ± 0.35               | 2.45 ± 0.25                      | 1.65 ± 0.36             | 2.41 ± 0.63                     | 0.8        | 0.09     | 0.1          |
| Axin2          | 2.82 ± 0.38               | 2.92 ± 0.31                      | 1.82 ± 0.20             | 2.25 ± 0.29                     | 0.4        | 0.01     | 0.7          |
| cMyc           | 1.44 ± 0.10               | 1.52 ± 0.13                      | 1.80 ± 0.06             | 1.64 ± 0.15                     | 0.7        | 0.04     | 0.3          |

The expression of each genes is normalized to 18S ribosomal subunit, presented as arbitrary units.

Values are given as mean ± standard error of the mean. (Notum^{flox/flox}: females n=10, males n=8; Dmp1-creNotum^{flox/flox}: females n=7, males n=5, 20-week-old mice). A mixed model two-way ANOVA were used to evaluate the effect of genotype, gender, and their interaction. A difference was considered significant when $P < 0.05$. 
Supplemental Table 2. Serum levels of bone resorption and bone formation markers

|                  | Females                  |                  | Males                  |                  | 2-way-ANOVA       |
|------------------|--------------------------|------------------|------------------------|------------------|------------------|
|                  | *Notum*^flox/flox*       | *Dmp1-creNotum*^flox/flox* | *Notum*^flox/flox* | *Dmp1-creNotum*^flox/flox* | P Genotype | P Gender | P Interaction |
| P1NP ng/µL       | 73.5 ± 8.76              | 67.1 ± 12.0      | 72.8 ± 10.5            | 61.0 ± 11.7      | 0.4          | 0.8      | 0.8          |
| CTX ng/µL        | 17.2 ± 1.50              | 18.0 ± 1.34      | 26.0 ± 2.15            | 21.2 ± 3.42      | 0.3          | 0.007    | 0.2          |

P1NP: Procollagen type I N-terminal propeptide. CTX: C-terminal type I collagen. Values are given as mean ± standard error of the mean. (*Notum*^flox/flox*: females n=10, males n=8; *Dmp1-creNotum*^flox/flox*: females n=7, males n=5, 20-week-old mice). A mixed model two-way ANOVA were used to evaluate the effect of genotype, gender, and their interaction. A difference was considered significant when $P < 0.05$. 