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Supplemental information

CRISPR interference provides increased cell type-specificity compared to the Cre-loxP system

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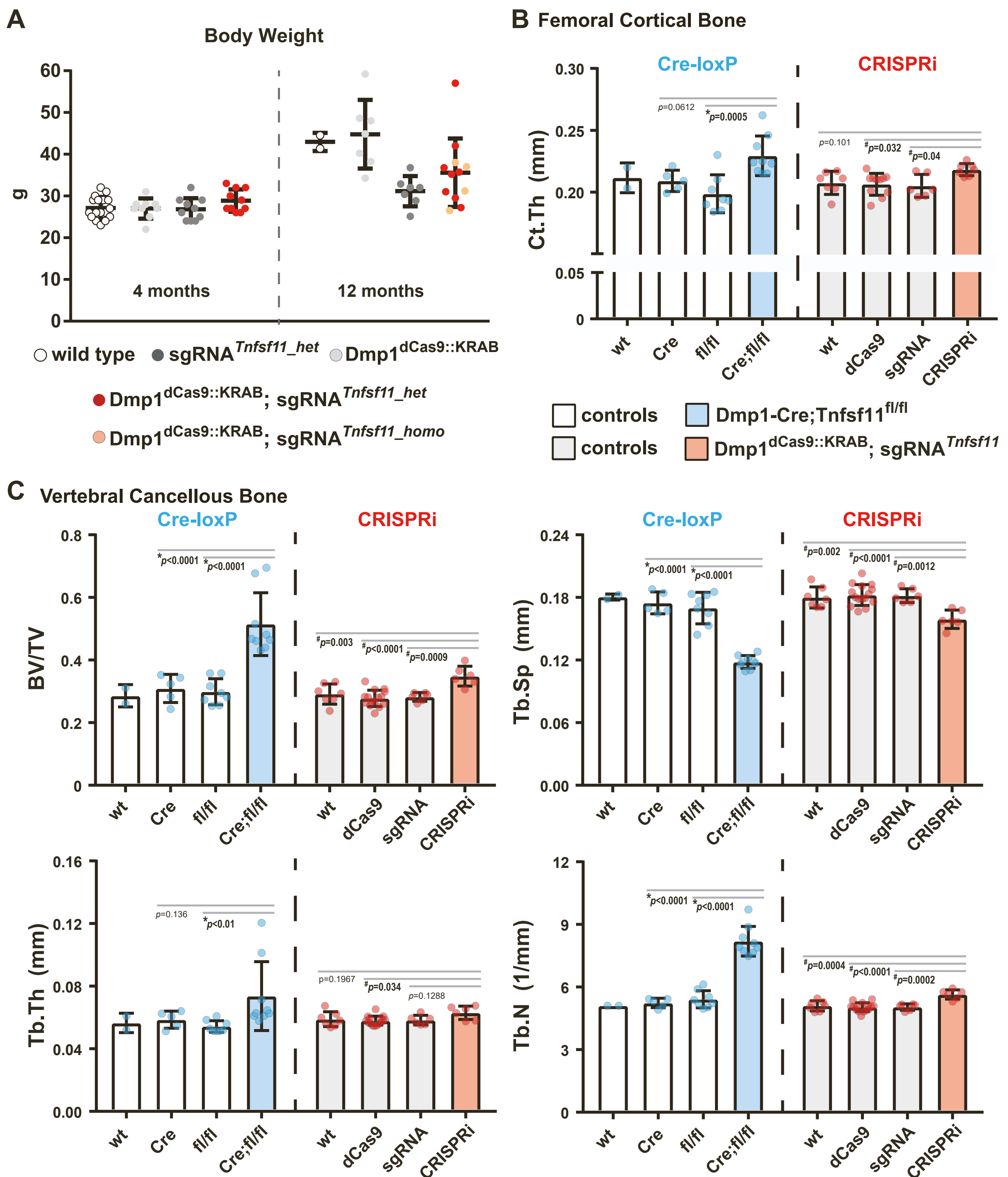
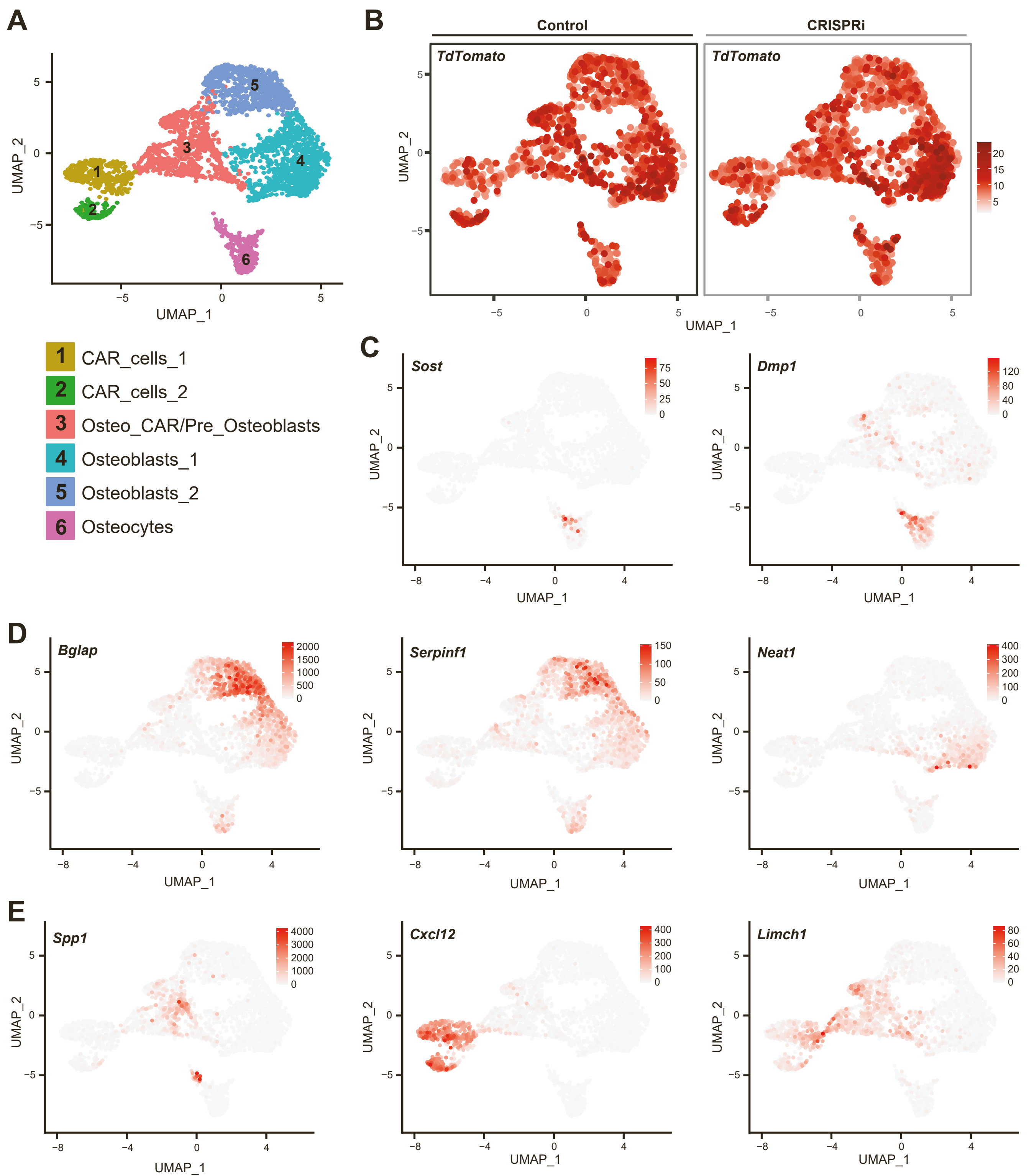
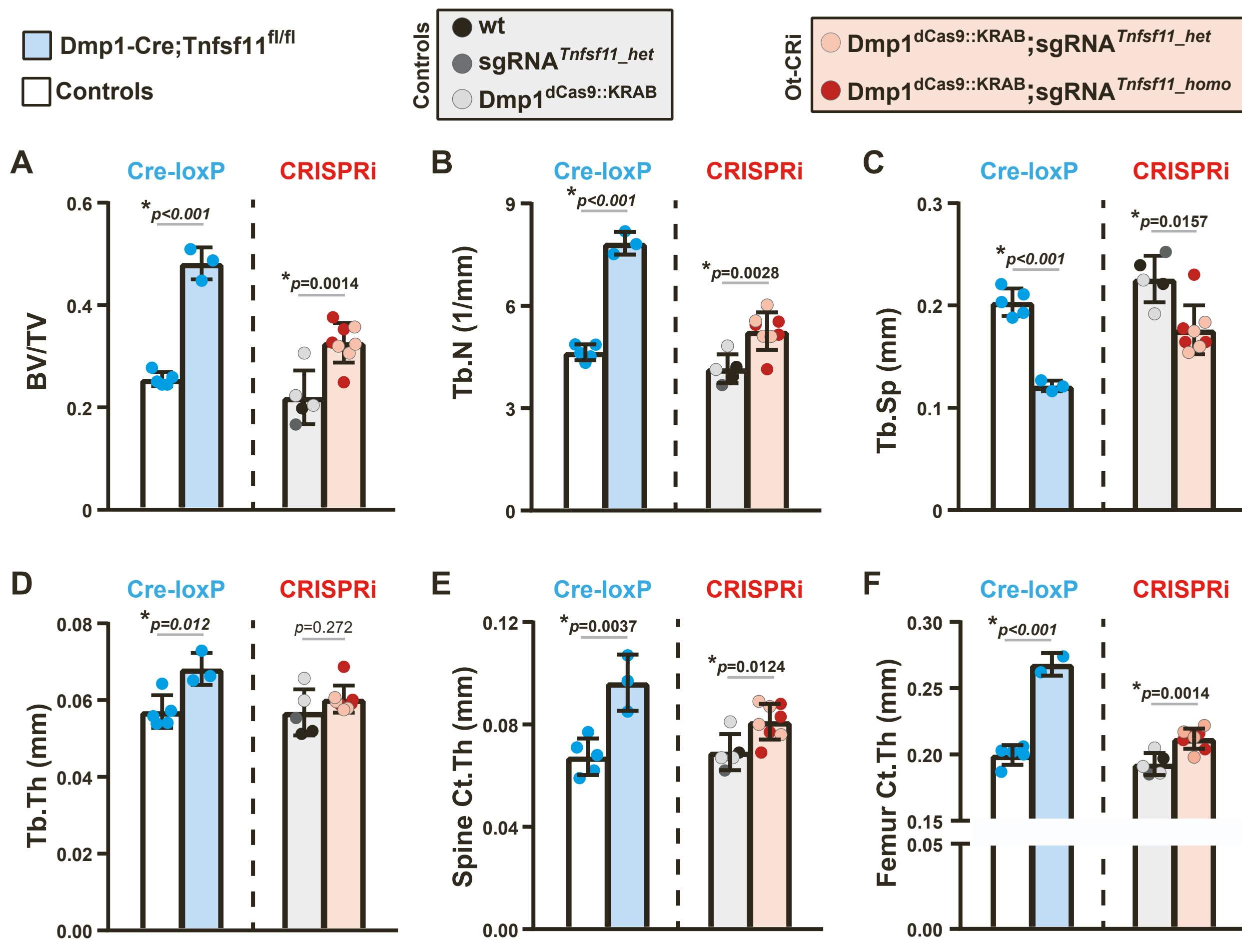


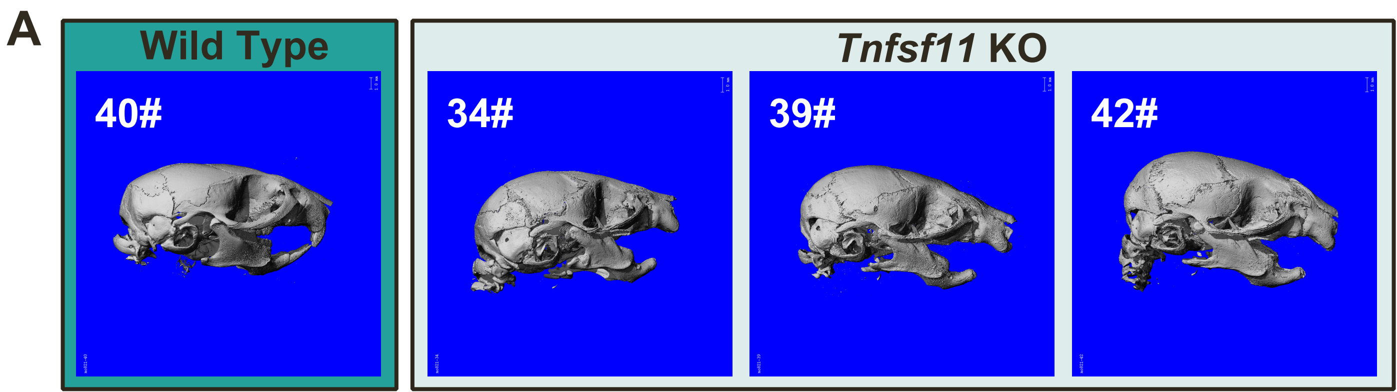
Supplementary Figure 1. *Tnfsf11* loss-of-function using *Dmp1*-driven Cre-loxP or *Dmp1*-driven CRISPRi, supplemental to Figure 2. A, Body weight of female Dmp1$^{dCas9::KRAB}$;sgRNA^{Tnfsf11} mice and their littermate controls (wild type, Dmp1$^{dCas9::KRAB,}$ and sgRNA^{Tnfsf11} mice) were measured at 4 and 12 months of age. n=2-17 mice/group. One-way ANOVA with Tukey adjustment was performed to compare *Dmp1*-driven CRISPRi to controls (all p>0.05). **B-C**, The skeletal phenotype of 6-month-old male mice was analyzed by μCT. **B**, Cortical thickness (Ct. Th) was measured at the femoral midshaft. **C**, Cancellous bone mass, and architecture were analyzed as bone volume over tissue volume (BV/TV), trabecular separation (Tb. Sp), trabecular thickness (Tb. Th), and trabecular number (Tb. N) in lumbar vertebrae 4. For deletion of *Tnfsf11* using Cre-loxP n=2-9 mice/group; *, p<0.05 comparing Dmp1-Cre; Tnfsf11$^{fl/fl}$ to its littermate controls using one-way ANOVA with Tukey adjustment. For suppression of *Tnfsf11* using *Dmp1*-driven CRISPRi n=6-15 mice/group; #, p<0.05 comparing *Dmp1*-driven CRISPRi to each littermate control using one-way ANOVA with Tukey adjustment. Individual Tukey *p* values for each comparison are provided in the graphs. All data presented as mean ± stdev.



Supplementary Figure 2. scRNA-seq clustering of TdTomato positive cells, supplemental to Figure 3. Femur and tibia shafts were subjected to serial digestions (see methods). Cells from fractions 2 to 8 were collected for flow cytometry. **A-E**, tdTomato-positive cells were sorted and used for scRNA-seq. **B**, TdTomato transcript in Control and CRISPRi samples. **C-E**, Cell-clustering was performed using cluster-specific markers: (**C**) *Sost* and *Dmp1* (cluster 6, osteocytes); (**D**) *Bglap, Serpinf1* and *Neat1* (cluster 4&5, osteoblasts); (**E**) *Cxcl12* (cluster 1&2, CAR cells), *Spp1* and *Limch1* (cluster 3, osteo-CAR cells).



Supplementary Figure 3. *Dmp1*-driven CRISPRi of *Tnfsf11* persists up to 12 months of age in male mice, supplemental to Figure 5. Skeletal phenotype of male Dmp1-Cre;Tnfsf11$^{fl/fl}$ mice (blue bars, n=3) and their littermate controls (white bars, n=5); Dmp1$^{dCas9::KRAB}$; sgRNA^{Tnfsf11} mice (Ot-CRi, pink bars, n=8) and their littermate controls (wild type, Dmp1$^{dCas9::KRAB,}$ and sgRNA^{Tnfsf11} mice, light grey bars, n=5) were assessed at 12 months of age by µCT analysis. **A-D** Cancellous bone mass and architecture were analyzed as bone volume over tissue volume (BV/TV) (**A**), trabecular number (Tb. N) (**B**), trabecular separation (Tb. Sp)(**C**), and trabecular thickness (Tb. Th) (**D**) in lumbar vertebrae 4 (L4). **E-F**, Vertebral cortical thickness (Spine Ct. Th) (**e**) was determined on the ventral cortical wall of L4 and the femoral cortical thickness (Femur Ct. Th) (**F**) was measured at femoral midshaft. *, p<0.05 comparing Dmp1$^{dCas9::KRAB}$; sgRNA^{Tnfsf11} mice (CRISPRi) or Dmp1-Cre; Tnfsf11$^{fl/fl}$ mice to their corresponding control mice using t-test or Rank sum test (in case of non-normality, specifically Tb.Sp and Tb.N of CRISPRi). Individual *p* values for each comparison are provided in the graphs. All data presented as mean $\pm$ stdev.



B

sgRNA^{Tnfsf11} (control)

82#

86#

91#

gCRi^{Tnfsf11}

80#

81#

83#

84#

88#

89#

90#

97#

98#

Supplementary Figure 4. Global suppression of *Tnfsf11* using CRISPRi prevents tooth eruption supplemental to Figure 1. A-B, µCT analysis of skulls of 5-week-old (**A**) wild type and their littermate *Tnfsf11* knockout (Tnfsf11 KO) mice, and (**B**) H11$^{dCas9\text{-}KRAB}$; sgRNA^{Tnfsf11} mice (gCRi^{Tnfsf11}) and their littermate controls (sgRNA^{Tnfsf11}_Control).