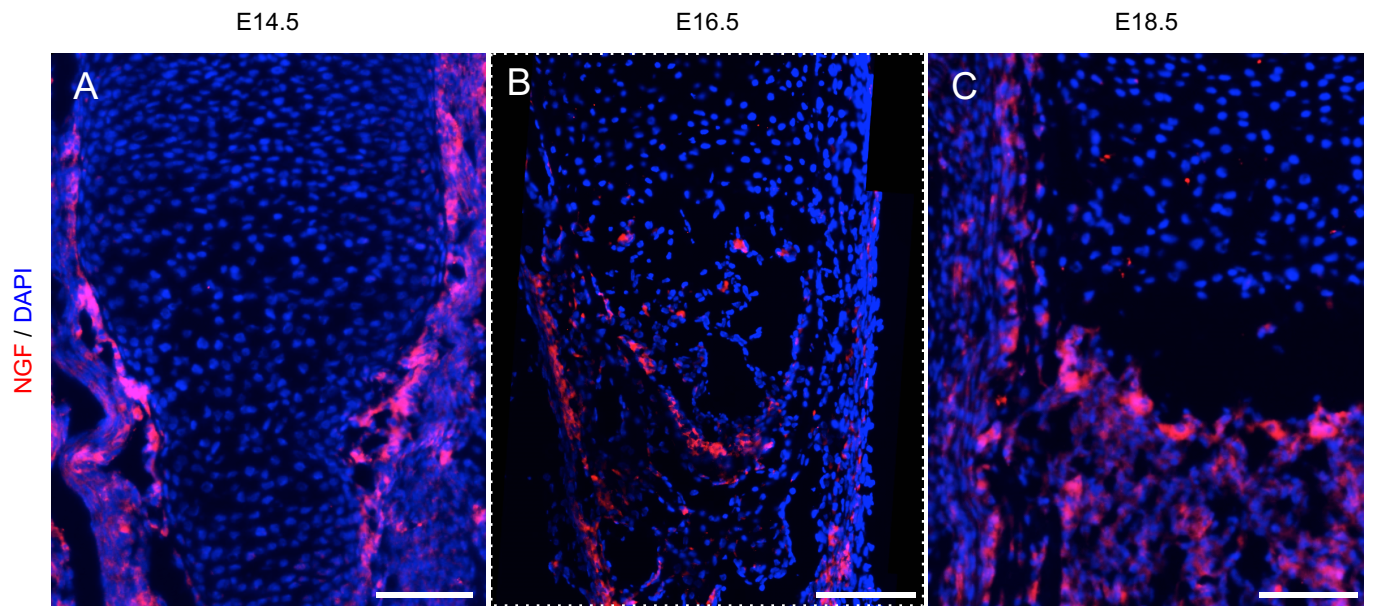


**Figure S1. Expression of NGF during embryogenesis**

Femurs harvested from NGF-eGFP mice were sectioned to illustrate NGF expression through the developing mouse bone at A) E16.5 and B) E18.5. Scale bars are 100 microns. **Related to Figure 2.**

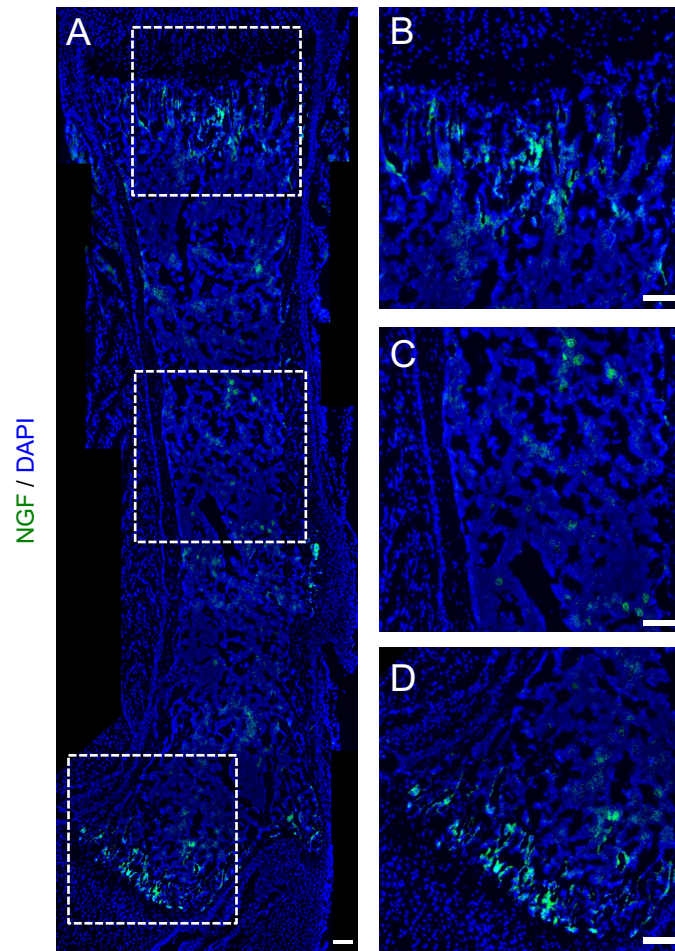


**Figure S2. NGF immunohistochemistry during embryogenesis**

Femurs harvested from wild type mice were sectioned and stained with antibodies against NGF and imaged at A) E14.5 B) E16.5 and C) E18.5 to confirm the expression pattern of NGF-eGFP mice during skeletal development. Scale bars are 100 microns. **Related to Figure 2.**

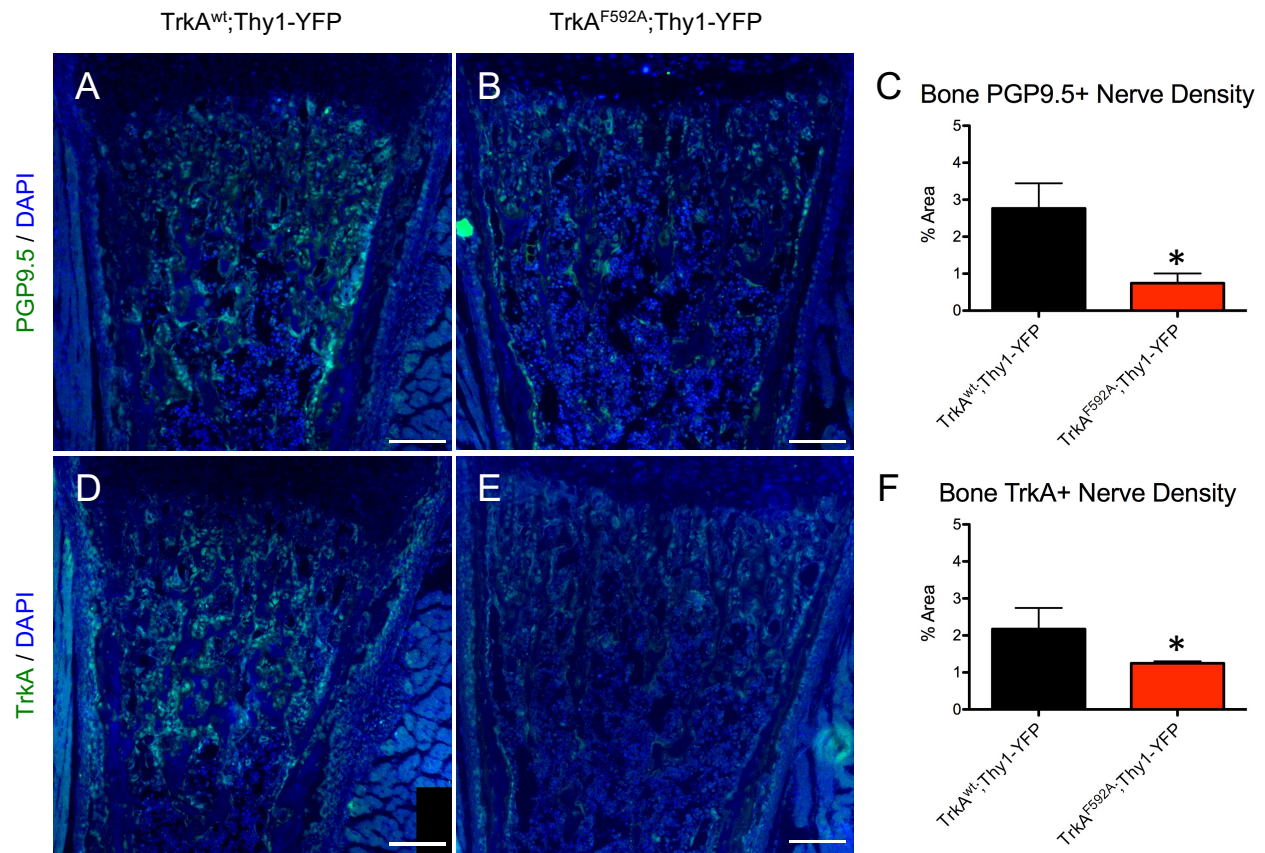


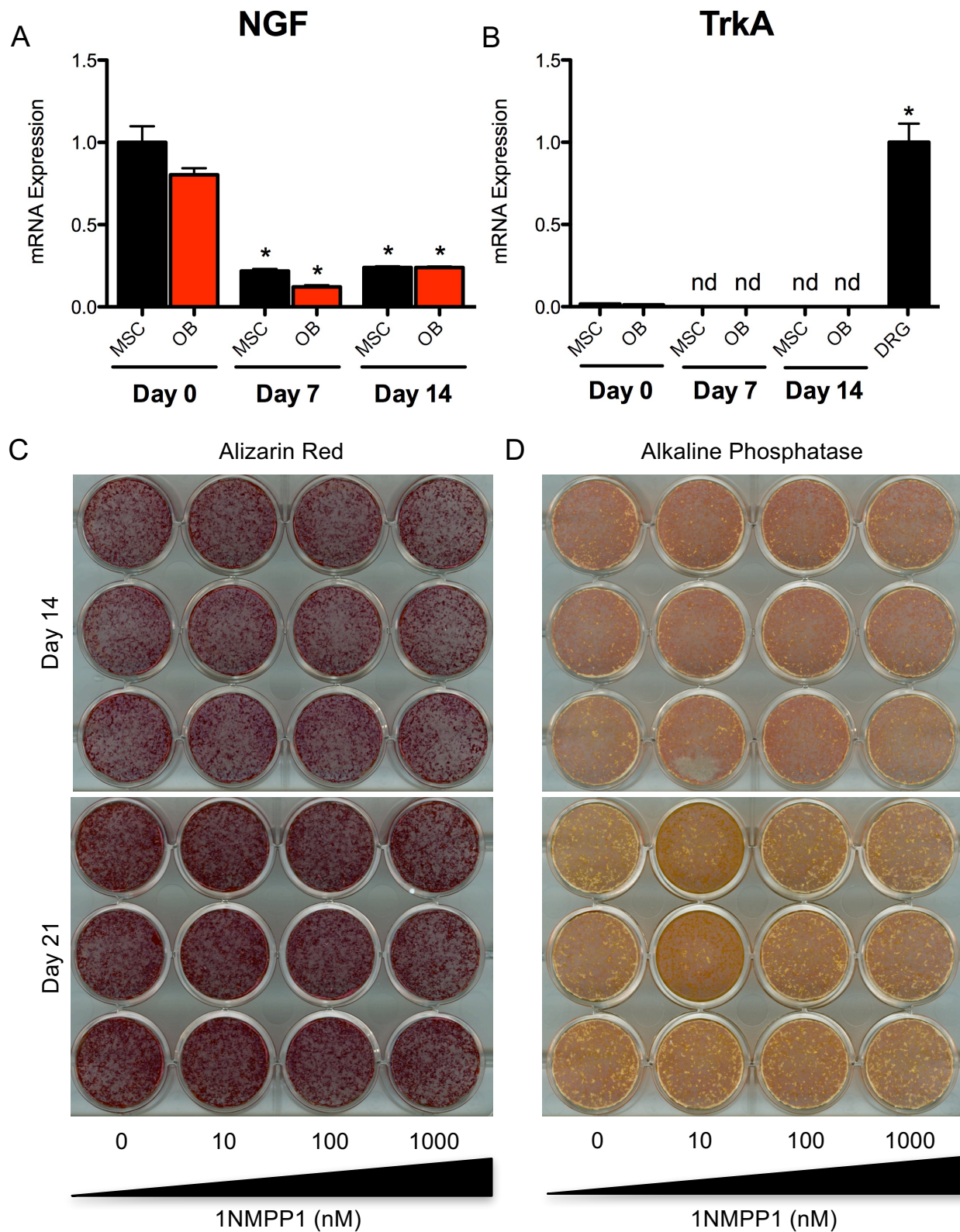
P7



**Figure S3. Postnatal expression of NGF in bone**

A) Femurs harvested from NGF-eGFP mice were sectioned at postnatal day 7 to illustrate the diminished NGF signal, with B-D) high powered insets. Scale bars are 100 microns. **Related to Figure 2.**

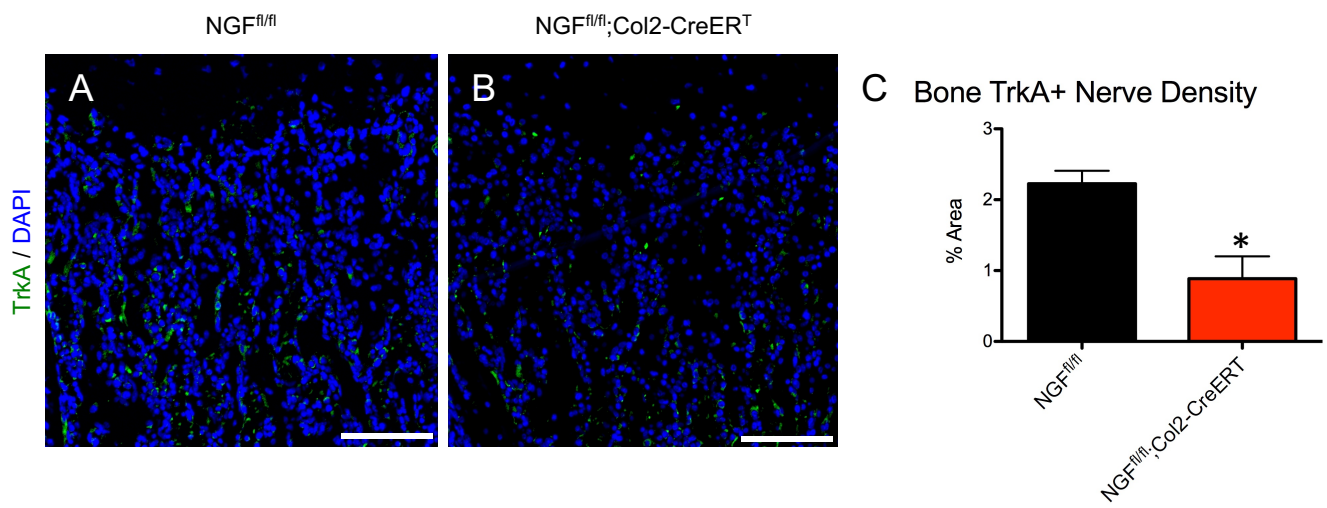




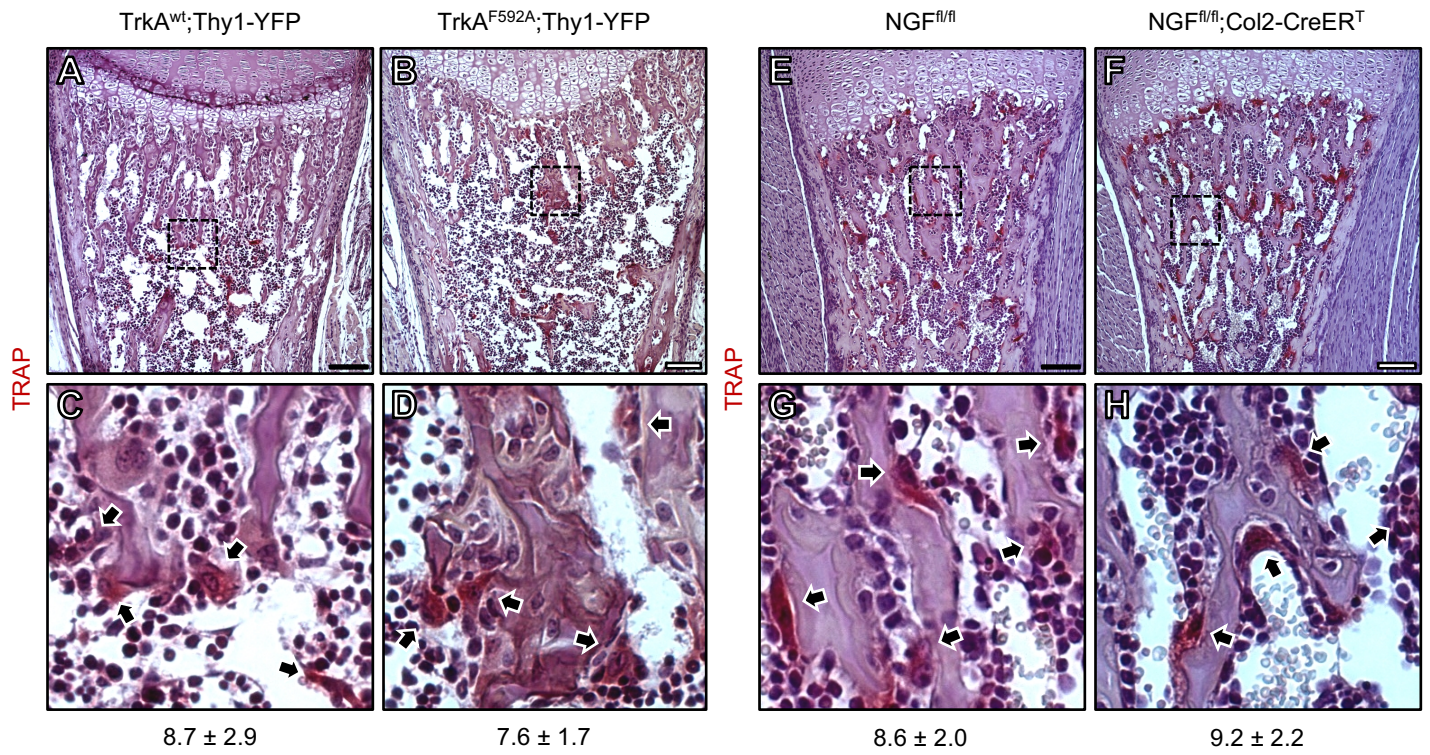
**Figure S5. TrkA inhibition does not affect primary osteoblasts or MSCs *in vitro***

A) mRNA expression of NGF was quantified for primary osteoblasts and MSCs after 0, 7, and 14 days of differentiation. B) mRNA expression of NGF was quantified for primary osteoblasts after 0, 7, and 14 days of differentiation and compared to freshly harvested DRG control. C) Alizarin red and D) alkaline phosphatase staining was performed on primary osteoblasts harvested from TrkA<sup>F592A</sup>;Thy1-YFP neonates and cultured for 14 or 21 days with increasing concentrations of 1NMPP1. nd = not detected. \* p < 0.05 vs. day 0 by Student's t-test. **Related to Figure 4.**





**Figure S6. Disruption of NGF in osteochondral progenitors impairs innervation of femoral metaphysis**  
Immunohistochemistry was performed with antibodies against TrkA on paraffin embedded sections from postnatal day 0 A) NGF<sup>fl/fl</sup> and B) NGF<sup>fl/fl</sup>; Col2-CreERT<sup>T</sup> mice with C) quantification. \* p < 0.05 by Student's t-test. Scale bars are 100 microns. **Related to Figure 6.**



**Figure S7. Inhibition of NGF-TrkA signaling does not affect osteoclast number at birth**  
 Osteoclasts were visualized at the femoral metaphysis by TRAP staining on paraffin sections from A) TrkA<sup>wt</sup>;Thy1-YFP and B) TrkA<sup>F592A</sup>;Thy1-YFP mice harvested at postnatal day 0, with high powered insets (C,D). Similarly, TRAP staining was performed on paraffin sections from E) NGF<sup>fl/fl</sup> and F) NGF<sup>fl/fl</sup>;Col2-CreERT mice dosed with tamoxifen at E11.5 and harvested at postnatal day 0, with high powered insets (G,H). Mean osteoclast number per millimeter of bone perimeter (N.Oc/BPm) ± standard deviation is noted below images. Arrow denotes positive TRAP staining. Scale bars are 100 microns. **Related to Figures 4 and 6.**

## Supplemental Tables

Target	Catalog	Manufacturer	Dilution
pTrkA	4619	Cell Signaling	1:500 (IHC), 1:1000 (WB)
NGF	sc-548	Santa Cruz	1:400
TrkA	06-574	Millipore	1:200
PGP9.5	sc-25800	Santa Cruz	1:200
CD31	553370	BD Biosciences	1:100
Osx	sc-133871	Santa Cruz	1:250

**Table S1.** Antibodies and dilutions used for immunohistochemistry (IHC) and western blot (WB).

Target	Forward (5'-3')	Reverse (5'-3')
NGF	TGATCGGCGTACAGGCAGA	GCTGAAGTTTAGTCCAGTGGG
TrkA	AGAGTGGCCTCCGCTTTGT	CGCATTGGAGGACAGATTCA

**Table S2.** Primers used for qRT-PCR.