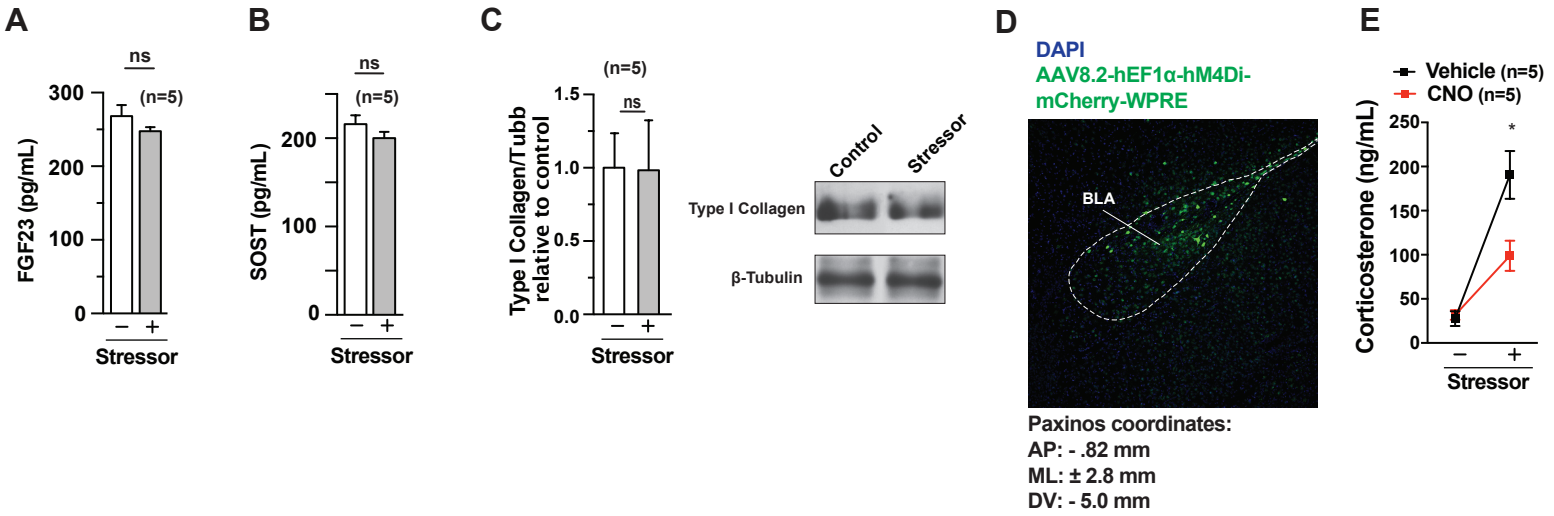

Fig. S1. Related to Figure 1. Stressors trigger a rapid surge of circulating bioactive osteocalcin (Ocn) in rodents and humans.

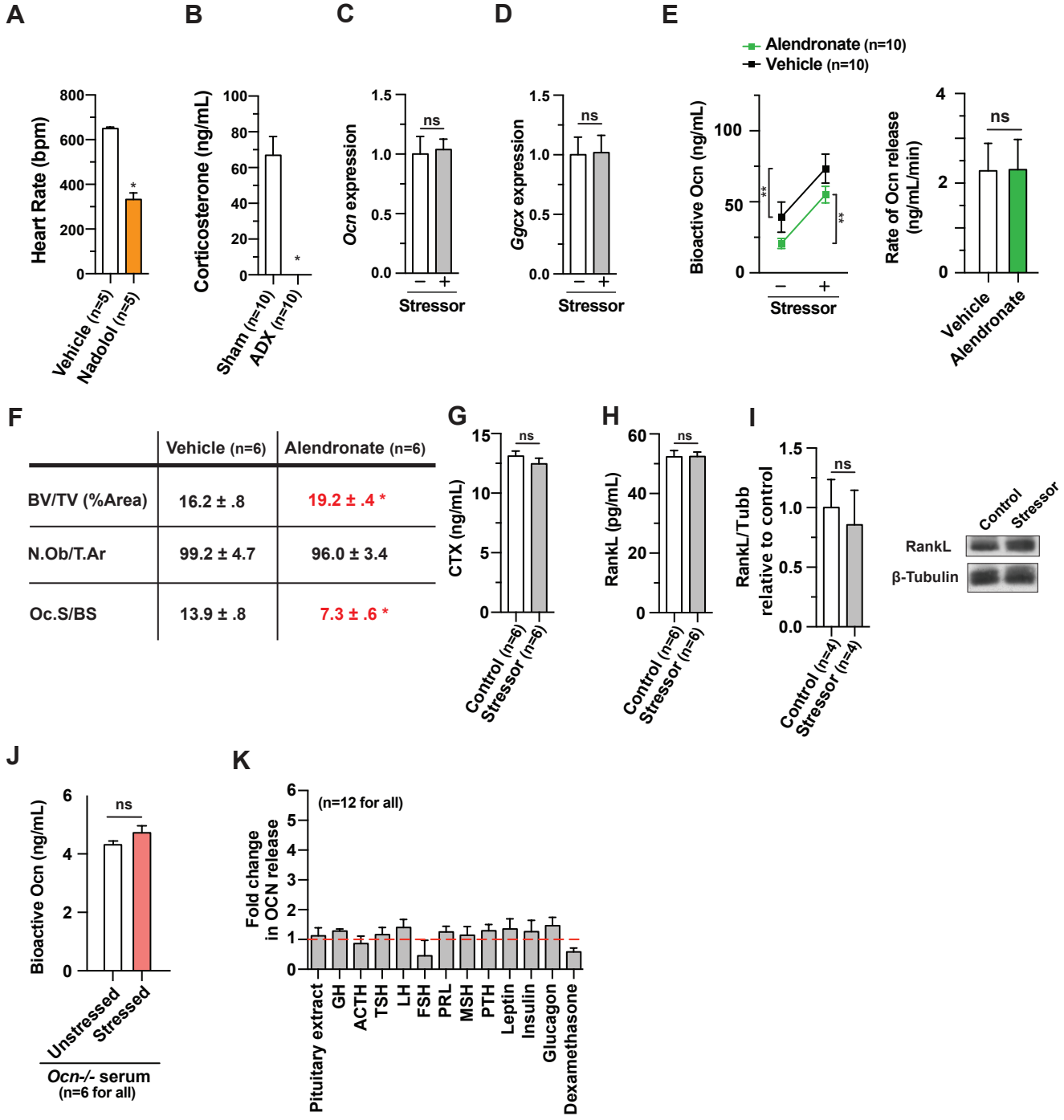

Fig. S2. Related to Figure 2. Bioactive osteocalcin is released from cells of the osteoblast lineage during an acute stress response.

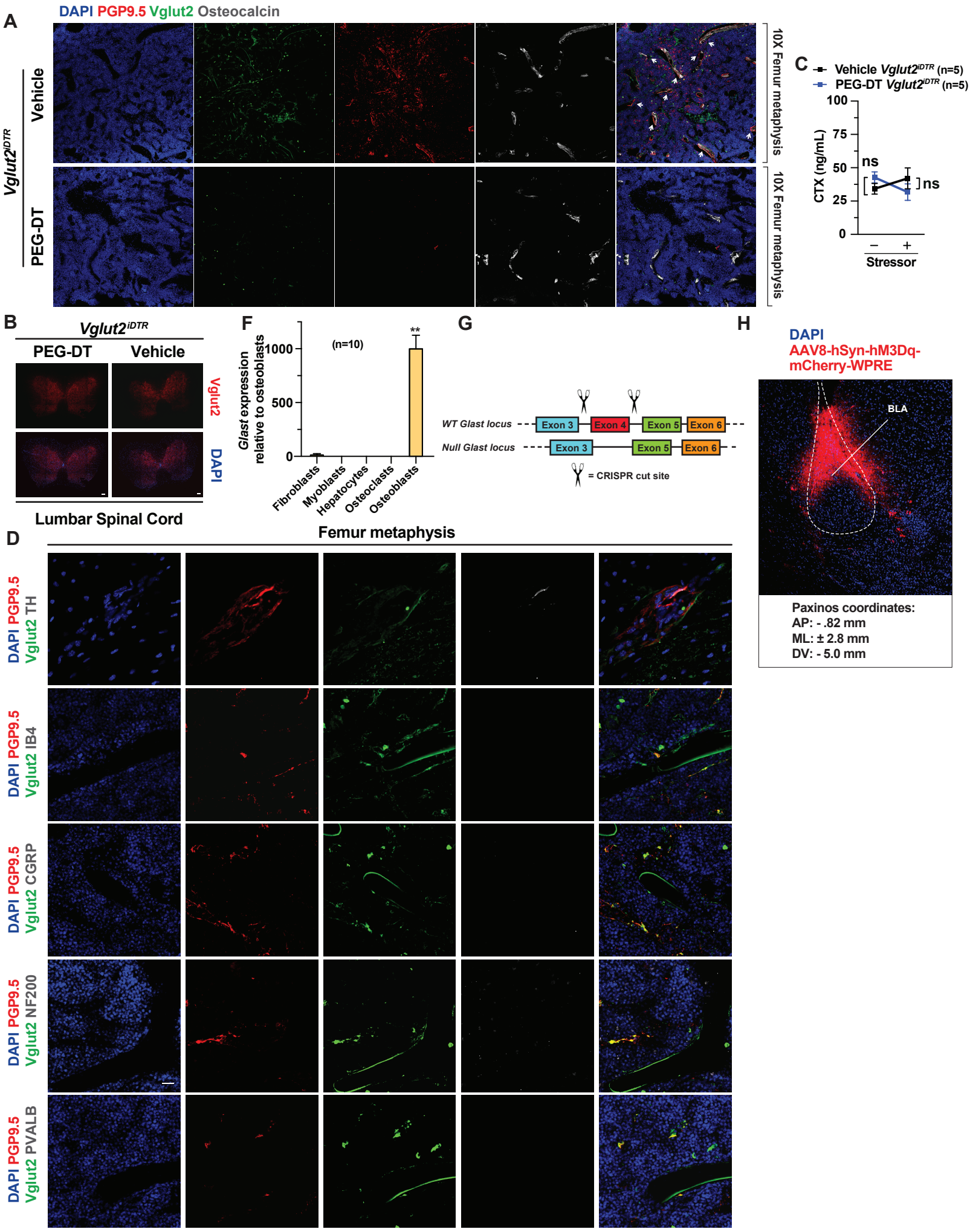

Fig. S3. Related to Figure 3. Glutamate mediates the stressor-induced release of bioactive osteocalcin from osteoblasts.

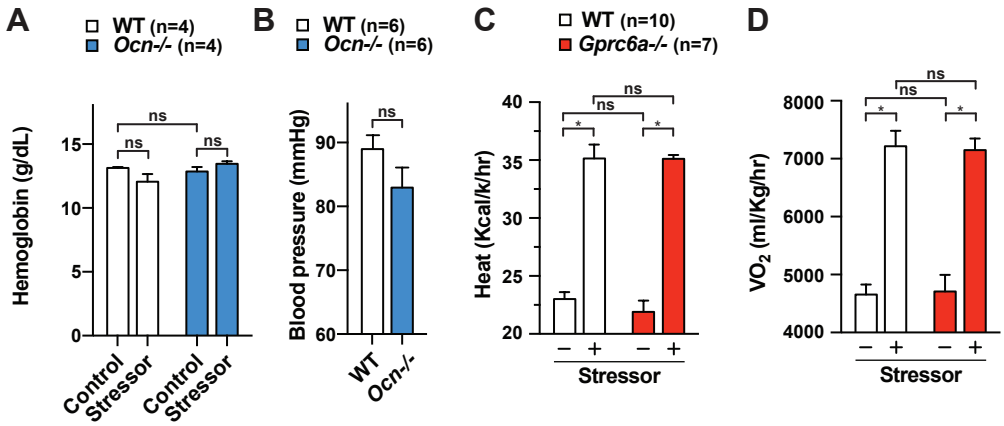

Fig. S4. Related to Figure 4. Osteocalcin signaling in peripheral organs is necessary to mount an ASR.

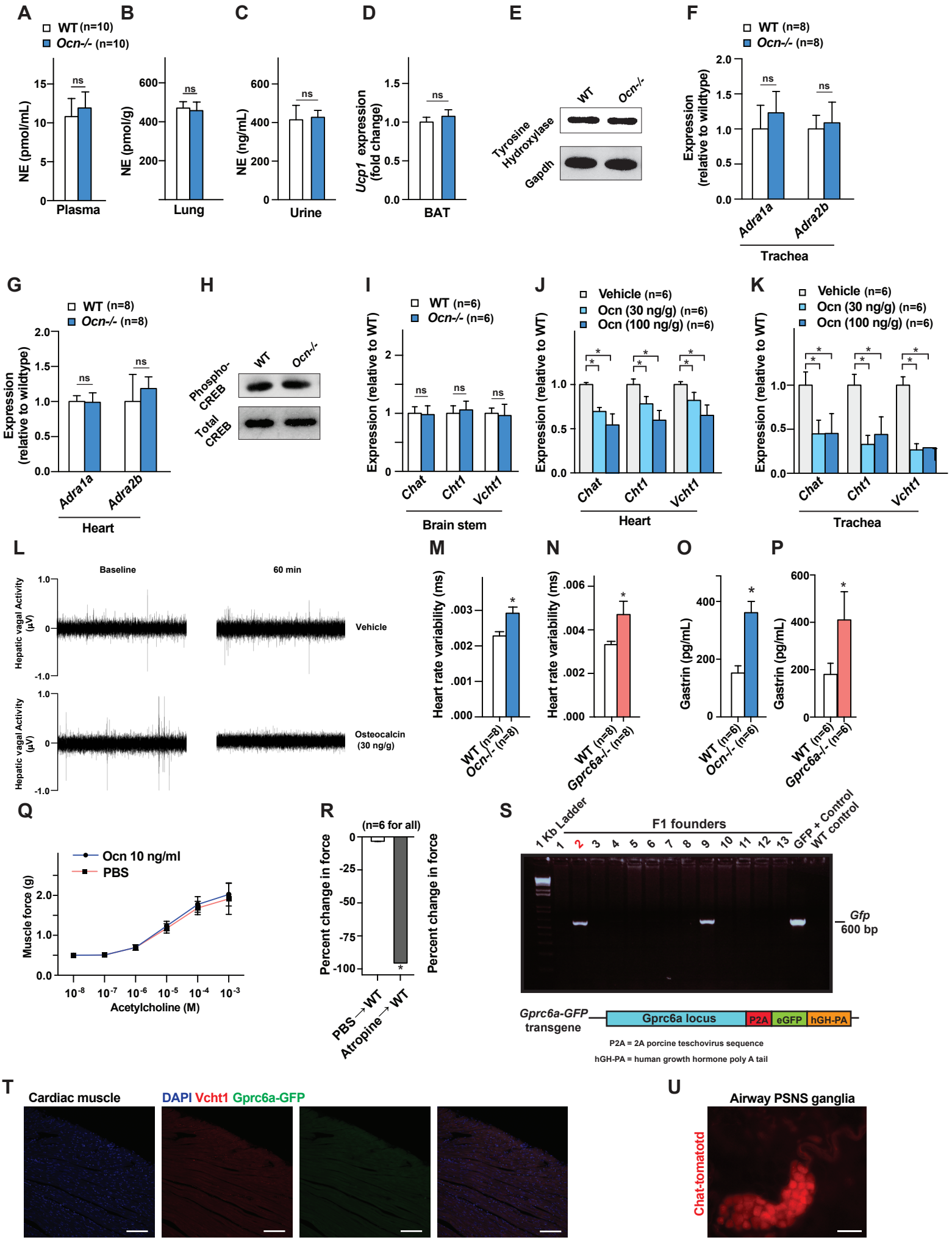

Fig. S5. Related to Figure 5. Osteocalcin inhibits the parasympathetic tone to trigger an ASR.

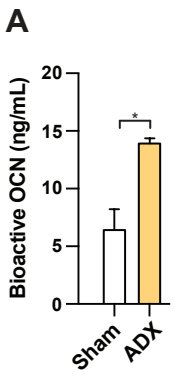

Fig. S6. Related to Figure 6. High circulating osteocalcin levels account for the ability of adrenalectomized mice to develop an ASR.

SUPPLEMENTAL INFORMATION

Figure S1. Related to Figure 1. Stressors trigger a rapid surge of circulating bioactive osteocalcin (Ocn) in rodents and humans. (A-B) FGF23 and sclerostin (SOST) circulating levels in TMT-exposed WT mice. **(C)** Bone Type (I) Collagen content in WT mice before and after TMT. **(D)** Immunofluorescence of basolateral amygdala (BLA) 3 weeks post injection of *AAV8.2-hEF1α-hM4Di-mCherry-WPRE.* **(E)** Serum corticosterone levels before and after TMT exposure and after i.p. injection of CNO or vehicle into WT mice expressing *hM4Di* in the BLA. Mice are 3-month-old females. Values are mean ±SEM. ns, not significant; *, p<.05; **, p<.01; by Student's t-test or one-way ANOVA with bonferroni post hoc test.

Figure S2. Related to Figure 2. Bioactive osteocalcin is released from cells of the osteoblast lineage during an acute stress response. (A) Heart rate in nadolol- and vehicle-treated WT mice. **(B)** Serum corticosterone levels in ADX and sham-operated WT mice. **(C-D)** Expression of *Ocn* or *Ggcx* in WT tibia before and after TMT. **(E)** Serum Ocn levels and rate of Ocn release in alendronate- and vehicle-treated WT mice exposed to TMT. **(F)** Histomorphometric analysis of L4 vertebrae of alendronate- and vehicle-treated mice. **(G)** Serum CTX levels in WT mice before and after TMT. **(H)** Serum RankL levels in WT mice before and after TMT. **(I)** Bone RankL content in WT mice before and after TMT. **(J)** Supernatant Ocn levels after 1-hour treatment of osteoblasts with serum from unstressed and stressed *Ocn-/-* mice. **(K)**

Supernatant Ocn levels after 1-hour treatment of osteoblasts with indicated hormones (n=12). Mice are 3-month-old females. Values are mean ±SEM. ns, not significant; *, p<.05; **, p<.01; by Student's t-test or one-way ANOVA with bonferroni post hoc test.

Figure S3. Related to Figure 3. Glutamate mediates the stressor-induced release of bioactive osteocalcin from osteoblasts. (A) Immunofluorescence in femur metaphysis of pegylated diphtheria toxin (PEG-DT)- or vehicle-injected *Vglut2*^{iDTR} mice (scale: 50 µm). **(B)** Immunofluorescence of lumbar spinal cord (scale: 80 µm) for indicated markers in PEG-DT- or vehicle-injected *Vglut2*^{iDTR} mice**. (C)** Serum CTX levels in PEG-DT- or vehicle injected *Vglut2*^{iDTR} mice. **(D-E)** Immunofluorescence of WT femoral metaphysis **(D)** or dorsal root ganglia (DRG) **(E)** for Th (scale: 5 µm), IB4, CGRP, NF200, PAVLB (scale: 20 µm). **(F)** Expression of *Glast* in indicated cell types**. (G)** Design of *Glast-/-* mice. **(H)** Immunofluorescence of basolateral amygdala (BLA) 3 weeks post injection of *AAV8-hSyn-hM3Dq-mCherry-WPRE.* Mice are 3-month-old females. Values are mean ±SEM. ns, not significant; *, p<.05; **, p<.01; by Student's t-test or one-way ANOVA with bonferroni post hoc test.

Figure S4. Related to Figure 4. Osteocalcin signaling in peripheral organs is necessary to mount an ASR. (A) Serum hemoglobin levels in *Ocn-/-* and WT mice before and after foot shock. **(B)** Blood pressure in *Ocn-/-* and WT mice (n=6). **(C-D)** Energy expenditure and oxygen consumption in *Gprc6a-/-* and WT

mice before and after foot shock. Mice are 3-month-old females. Values are mean ±SEM. ns, not significant; *, p<.05; **, p<.01; by Student's t-test or one-way ANOVA with bonferroni post hoc test.

Figure S5. Related to Figure 5. Osteocalcin inhibits the parasympathetic tone to trigger an ASR. (A-B) Plasma norepinephrine (NE) levels and lung NE content in *Ocn-/-* and WT mice.

(C-D) Urine norepinephrine (NE) and *Ucp1* expression in brown adipose tissue in *Ocn-/-* and WT mice (n=10). **(E)** Tyrosine hydroxylase (Th) content in heart of *Ocn-/-* and WT littermates. **(F-G)** *Adra1a* and *Adra2b* expression in trachea and heart of *Ocn-/-* and WT mice. **(H)** Phospho-CREB content in heart of *Ocn-/-* and WT littermates. **(I)** *Chat*, *Cht1* and *Vacht1* expression in brain stem of *Ocn-/-* and WT mice. **(J-K)** *Chat*, *Cht1* and *Vacht1* expression in trachea and heart two hours after injection of Ocn or vehicle into WT mice. **(L)** Representative raw tracing of sympathetic nerve activity in WT mice before and after treatment with Ocn or Vehicle. **(M-N)** Serum gastrin levels in *Ocn-/-*, *Gprc6a-/-* and WT littermates. **(O-P)** Heart rate variability in *Ocn-/-*, *Gprc6a-/-* and WT littermates. **(Q)** Contraction of mouse tracheal rings treated with increasing doses of acetylecholine without electrical stimulation and treated with either Ocn or vehicle. **(R)** Contraction of electrically stimulated *Gprc6a-/-* or WT mouse tracheal rings treated with atropine (10^{-6} M) or vehicle. **(S)** Construct of *Gprc6a-Gfp* mice and *Gfp* status of founding litter (line founder in red). **(T)** Immunofluorescence of cardiac muscle (scale bar is 100 μm). **(U)** Endogenous *tdTomato* fluorescence of

airway parasympathetic ganglia from *Chat-tdTomato* mice used for single cell electrophysiological recordings (scale bar is 8 μm). Mice are 3-month-old females. Values are mean ±SEM. ns, not significant; *, p<.05; **, p<.01; by Student's t-test or one-way ANOVA with bonferroni post hoc test.

Figure S6. Related to Figure 6. High circulating osteocalcin levels account for the ability of adrenalectomized mice to develop an ASR. (A) Serum Ocn levels in adrenalectomized (ADX) and sham-operated WT rats. Values are mean ±SEM. ns, not significant; *, p<.05; **, p<.01; by Student's t-test or one-way ANOVA with bonferroni post hoc test.