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## Supplemental information

# Partial prevention of glucocorticoid-induced osteocyte deterioration in young male mice with osteocrin gene therapy

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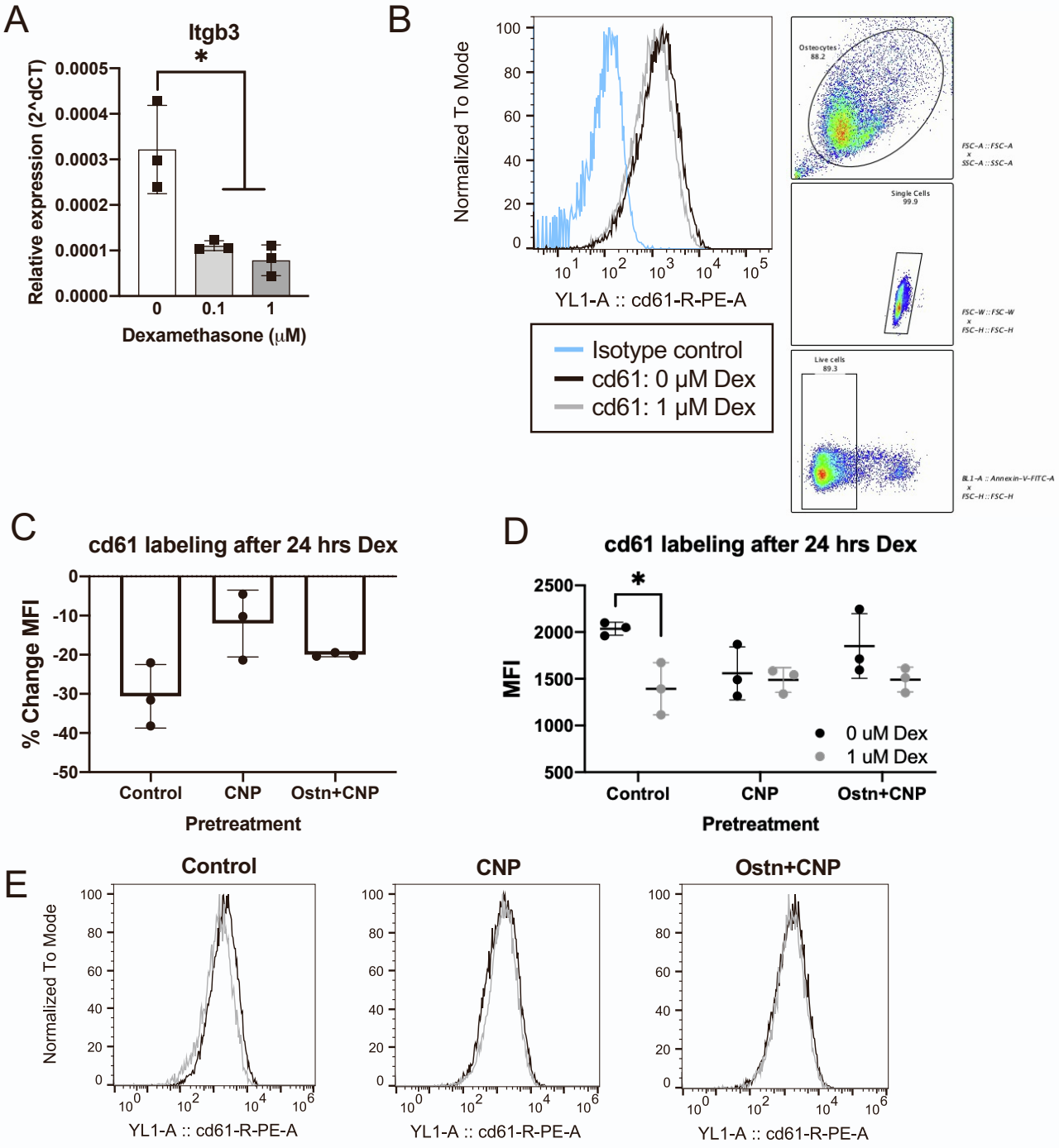


**Figure S1:** Effects of glucocorticoids and CNP/Ostn on integrin β3 expression *in vitro,* related to Figure 1.

(A) Ocy454 cells were treated with dexamethasone at the indicated concentrations for 24 hours. Expression of *Itgb3* was measured by RT-qPCR and presented relative to *Actb*. n=3. *p<0.05 by Tukey's multiple comparisons tests.

(B) Integrin β3 (CD61) surface labeling in Ocy454 cells is reduced following 24 hours of dexamethasone (Dex) treatment. Plots show representative histograms and gating strategy used for flow cytometry.

(C-E) Ocy454 cells were pretreated with CNP (100 nM), CNP+Ostn (100 nM + 500 nM), or vehicle for one hour, followed by 24 hours dexamethasone (1 μM) treatment. Dexamethasone-induced reduction in median fluorescence intensity (MFI) of CD61 labeling is shown for three independent experiments in (C), for one representative experiment with three biologic replicates in (D), and for one sample per condition in (E). *p<0.05 by Sidak's multiple comparisons tests.

All error bars show +/- SD.

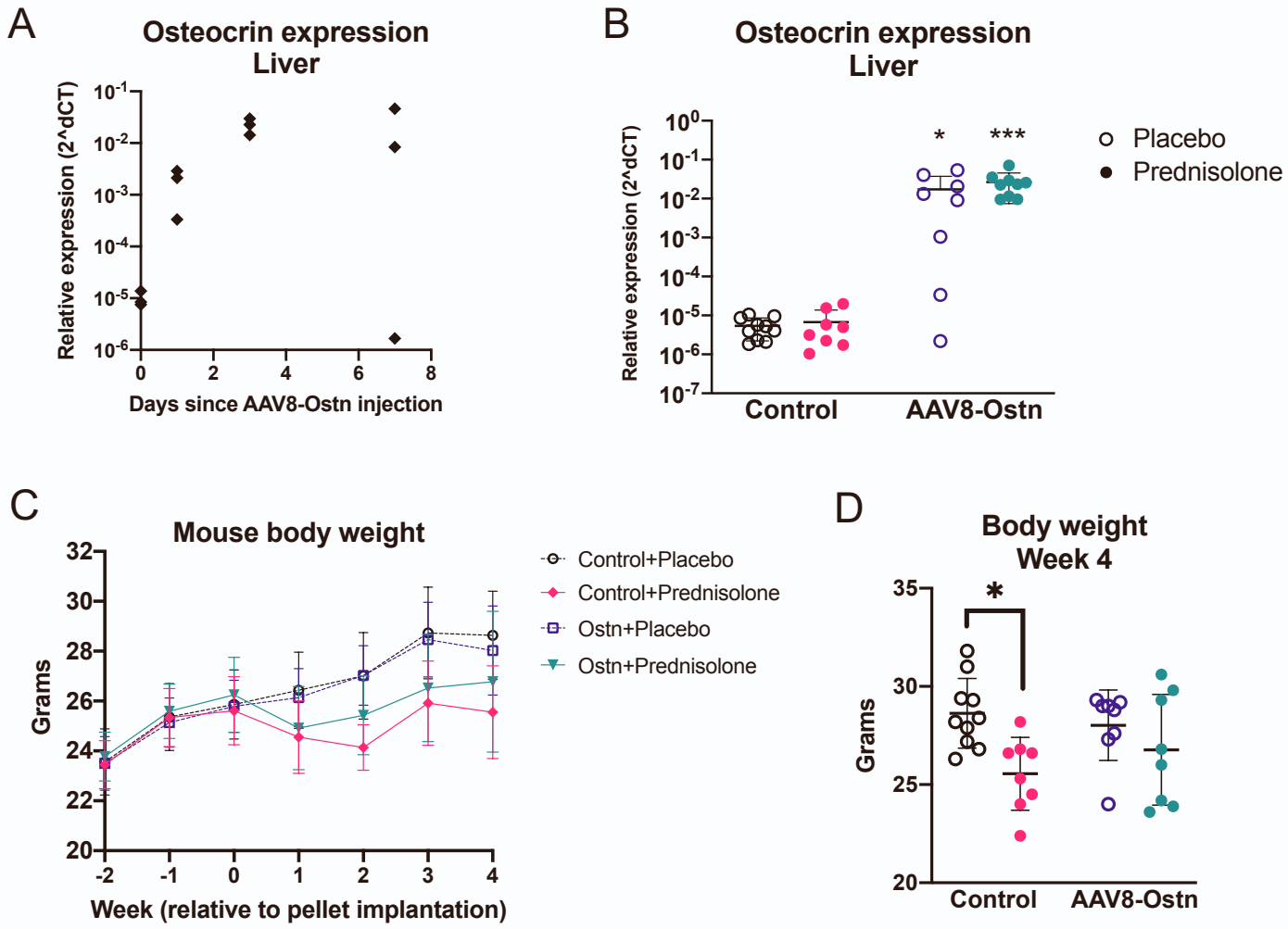


**Figure S2:** Systemic effects of AAV8-Ostn and prednisolone in mice, related to Figures 2-5.
(A) Hepatic expression of *Ostn* mRNA was measured relative to *Actb* 1, 3, and 7 days after intraperitoneal injection of AAV8-Ostn in C57BL/6J mice (n=3).
(B) Hepatic expression of *Ostn* mRNA was measured relative to *Actb* 35 days after intraperitoneal injection of AAV8-Ostn or AAV8-Control and 28 days after placebo or prednisolone pellet implantation in FVB mice. n=8-10. *p<0.05, ***p<0.001 relative to corresponding AAV8-control group by Sidak's multiple comparisons tests.
(C-D) Mice were weighed weekly starting 2 weeks prior to GC pellet implantation, and statistical comparisons were performed at the end of the experiment. n=8-10. *p<0.05 compared to corresponding placebo group by Sidak's multiple comparisons tests.
All error bars show +/- SD.

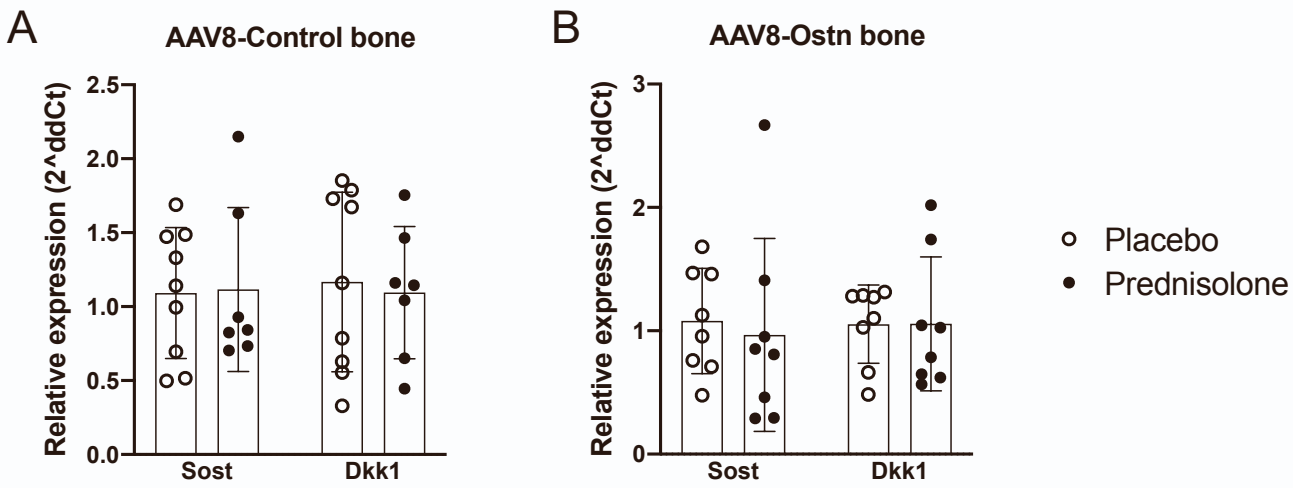


**Figure S3:** Prednisolone-induced changes in bone gene expression, related to Figure 2. Gene expression was measured relative to *Actb* in humeri after 28 days of prednisolone or placebo treatment in (A) AAV8-Control (n=7-9) and (B) AAV8-Ostn-treated (n=8) mice. Error bars show +/- SD.

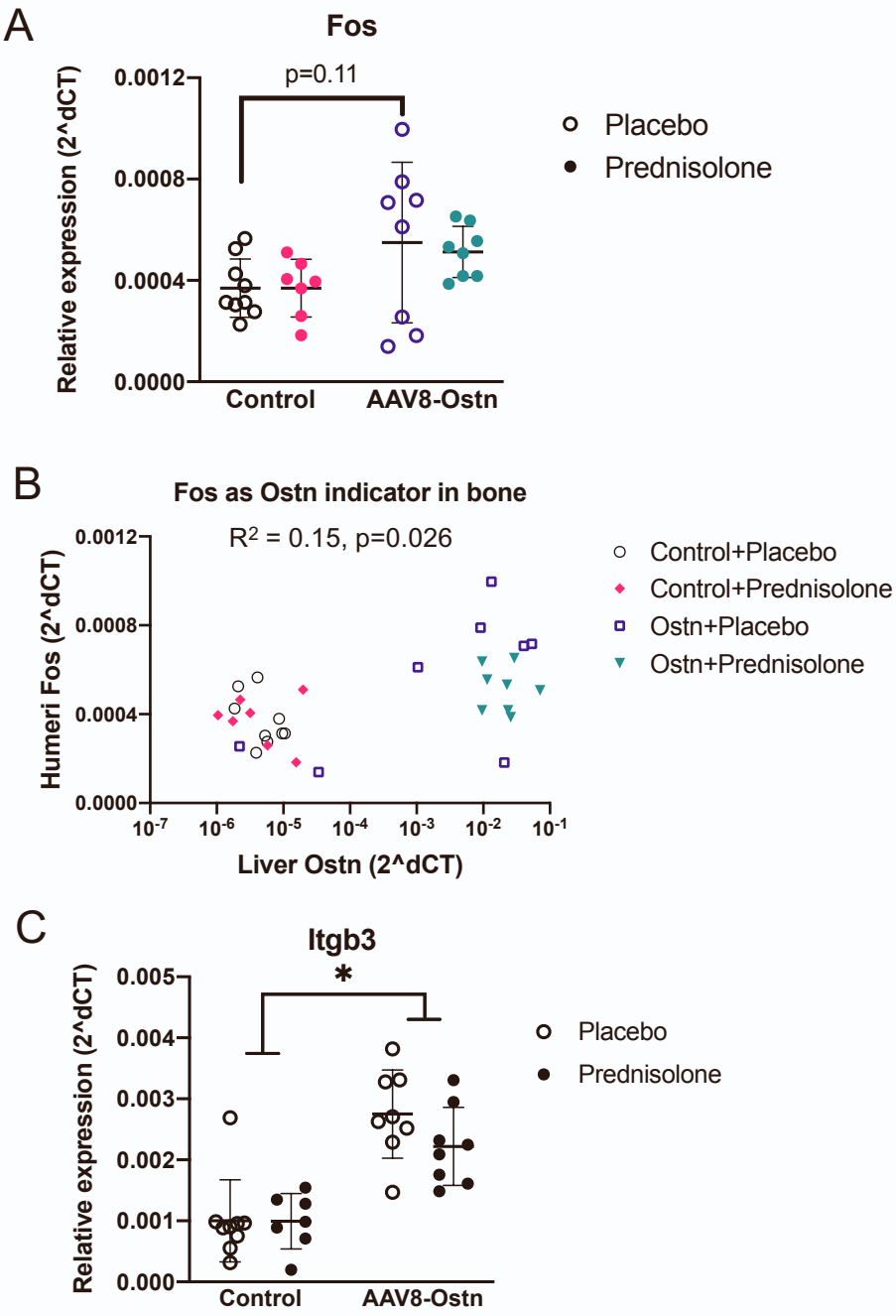


**Figure S4:** Osteocrin-induced changes in gene expression, related to Figure 2, Figure S1, Figure S2.
(A) Expression of Erk1/2-responsive gene *Fos* was measured in marrow-depleted humeri and normalized to *Actb* 35 days after intraperitoneal injection of AAV8-Ostn or AAV8-Control. n=7-10. p value shown compared to corresponding AAV8-Control group by Sidak's multiple comparisons test. Error bars show +/- SD.
(B) *Fos* expression in marrow-depleted humeri (normalized to *Actb*) is plotted relative to hepatic *Ostn* expression (normalized to *Actb*) for each mouse ($R^2$ = 0.15, p=0.026, n=32).
(C) Expression of *Itgb3* was measured in marrow-depleted humeri and normalized to *Actb* 35 days after intraperitoneal injection of AAV8-Ostn or AAV8-Control. n=7-10. *p<0.05 compared to corresponding AAV8-Control group by Sidak's multiple comparisons tests. Error bars show +/- SD.

**Table S1**: Oligonucleotide primer sequences, related to STAR Methods.

| | |
|---|---|
| Actb Fwd | CCT CTA TGC CAA CAC AGT GC |
| Actb Rev | ACA TCT GCT GGA AGG TGG AC |
| Ostn Fwd | AGT TTG GGA TAA GCT GCA GG |
| Ostn Rev | AGT CCA CAG AGA ATG CCT TTC |
| Sp7 Fwd | CCT CTC CCT TCT CCC TCT C |
| Sp7 Rev | CTG GAG CCA TAG TGA GCT TC |
| Gilz Fwd | TGA CTG CAA CGC CAA AGC |
| Gilz Rev | CTG ATA CAT TTC GGT GTT CAT GGT T |
| Acp5 Fwd | CCA TTG TTA GCC ACA TAC GG |
| Acp5 Rev | ACT CAG CAC ATA GCC CAC AC |
| Ctsk Fwd | CAT GGT GAG CTT TGC TCT GT |
| Ctsk Rev | CCG AGA GAT TTC ATC CAC CT |
| Mmp13 Fwd | TGT GTT TGC AGA GCA CTA CT |
| Mmp13 Rev | CTA AGC CAA AGA AAG ATT GCA TTT C |
| Fos Fwd | TGC GTT GCA GAC TGA GAT TG |
| Fos Rev | ATC TCC TCT GGG AAG CCA AG |
| Bglap Fwd | CGC TCT GTC TCT CTG ACC TC |
| Bglap Rev | GAC TGA GGC TCC AAG GTA GC |
| Sost Fwd | GCC TCA TCT GCC TAC TTG TG |
| Sost Rev | CTG TGG CAT CAT TCC TGA AG |
| Dkk1 Fwd | GAG GGG AAA TTG AGG AAA GC |
| Dkk1 Rev | AGC CTT CTT GTC CTT TGG TG |
| Itgb3 Fwd | AGG ATG CGA GCG CAG TG |
| Itgb3 Rev | TGG TAC AGA TGT TGG ACT CTC C |