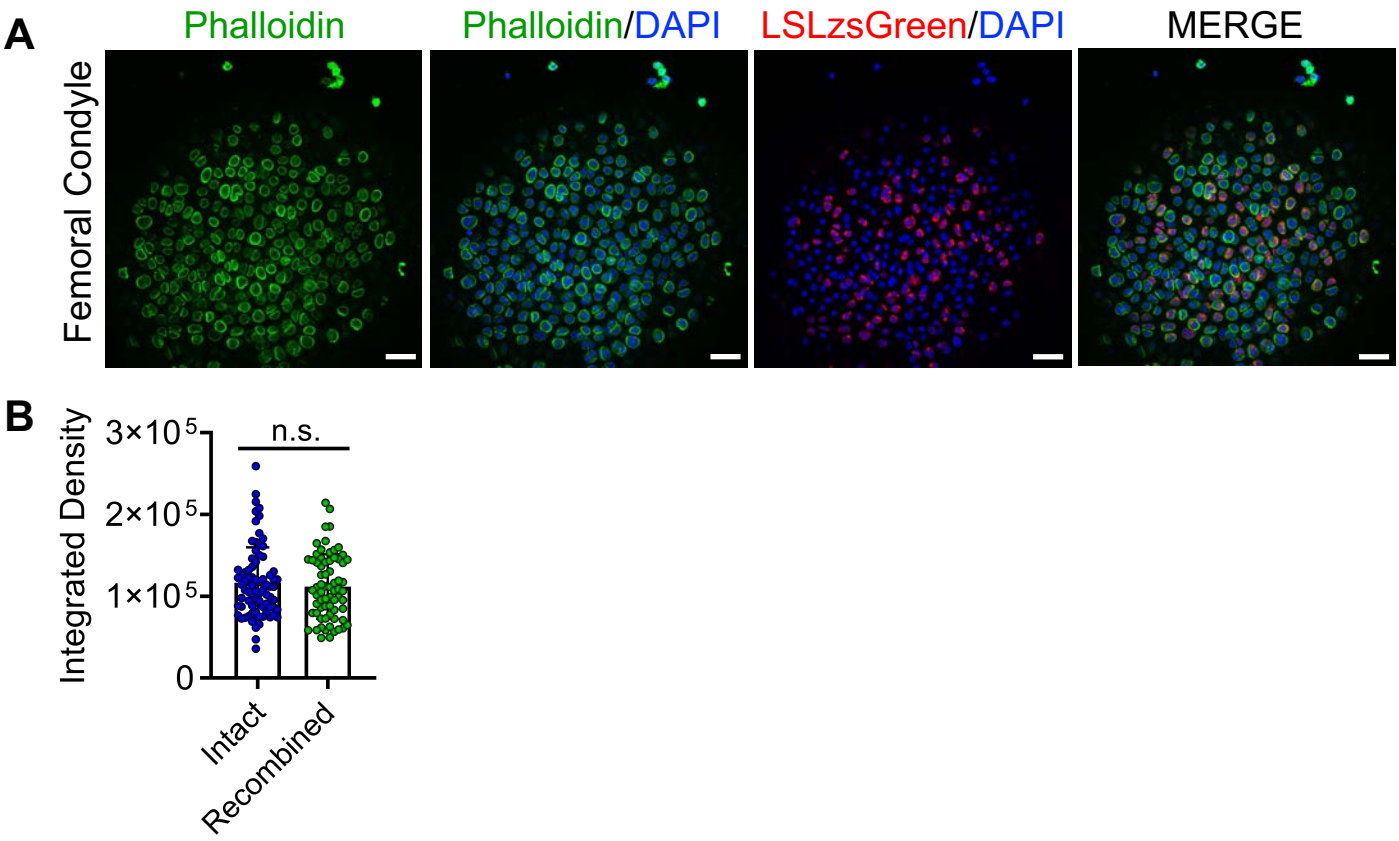
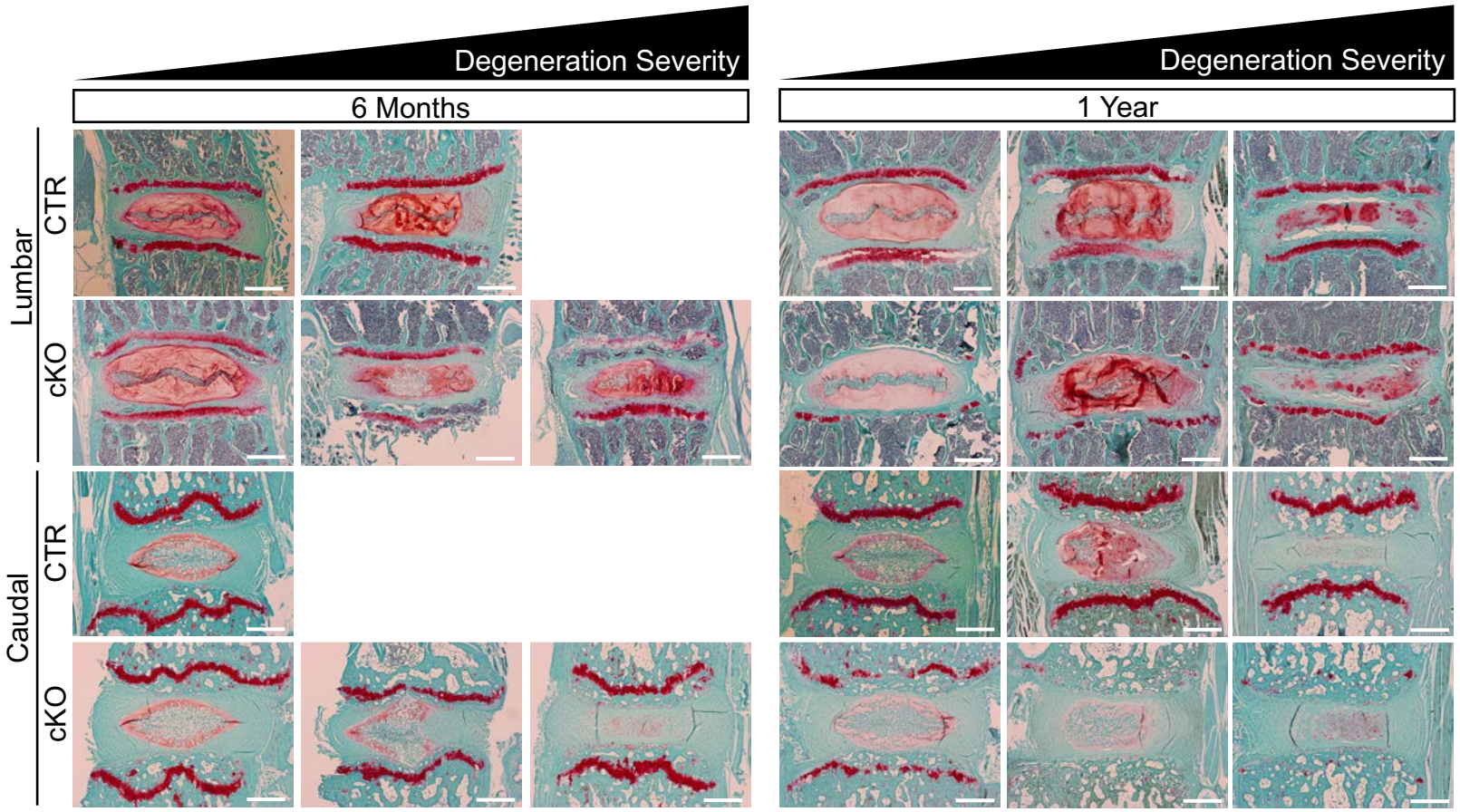
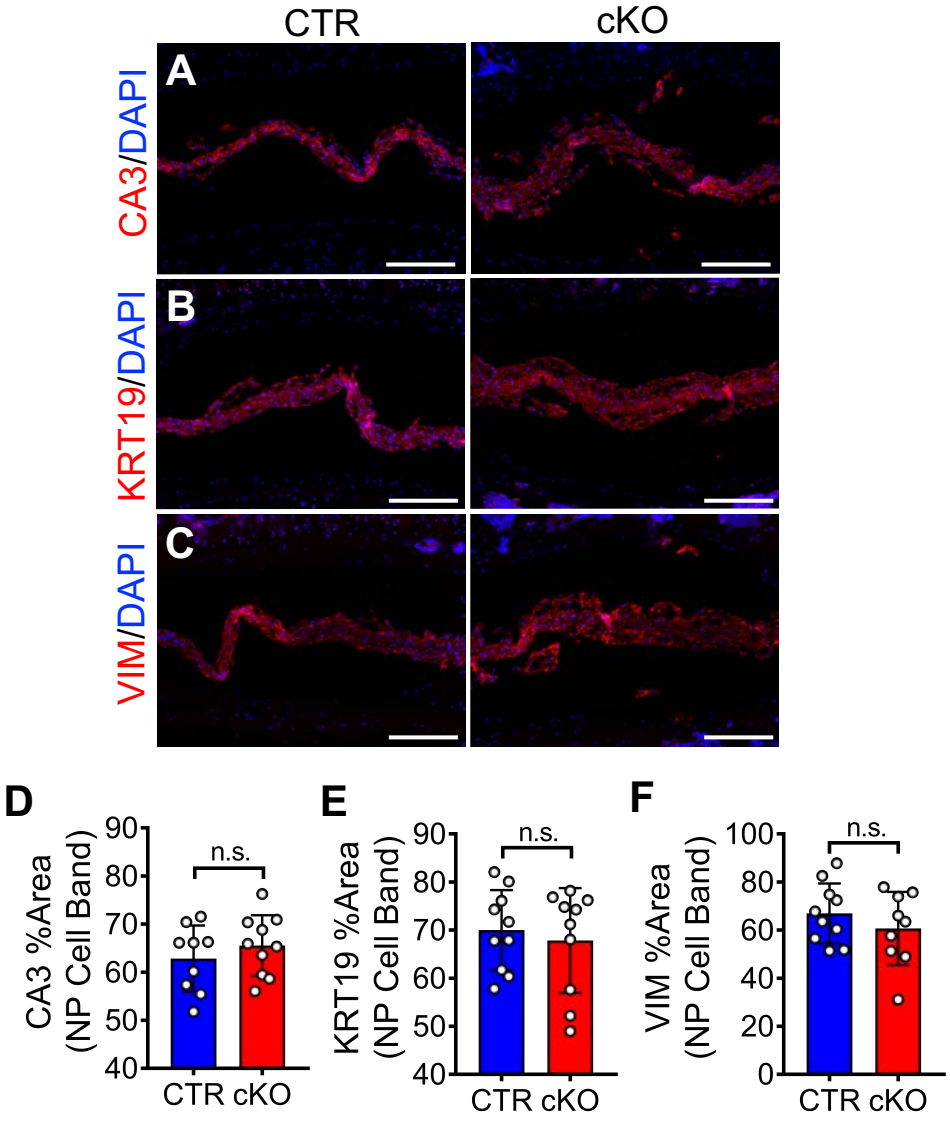


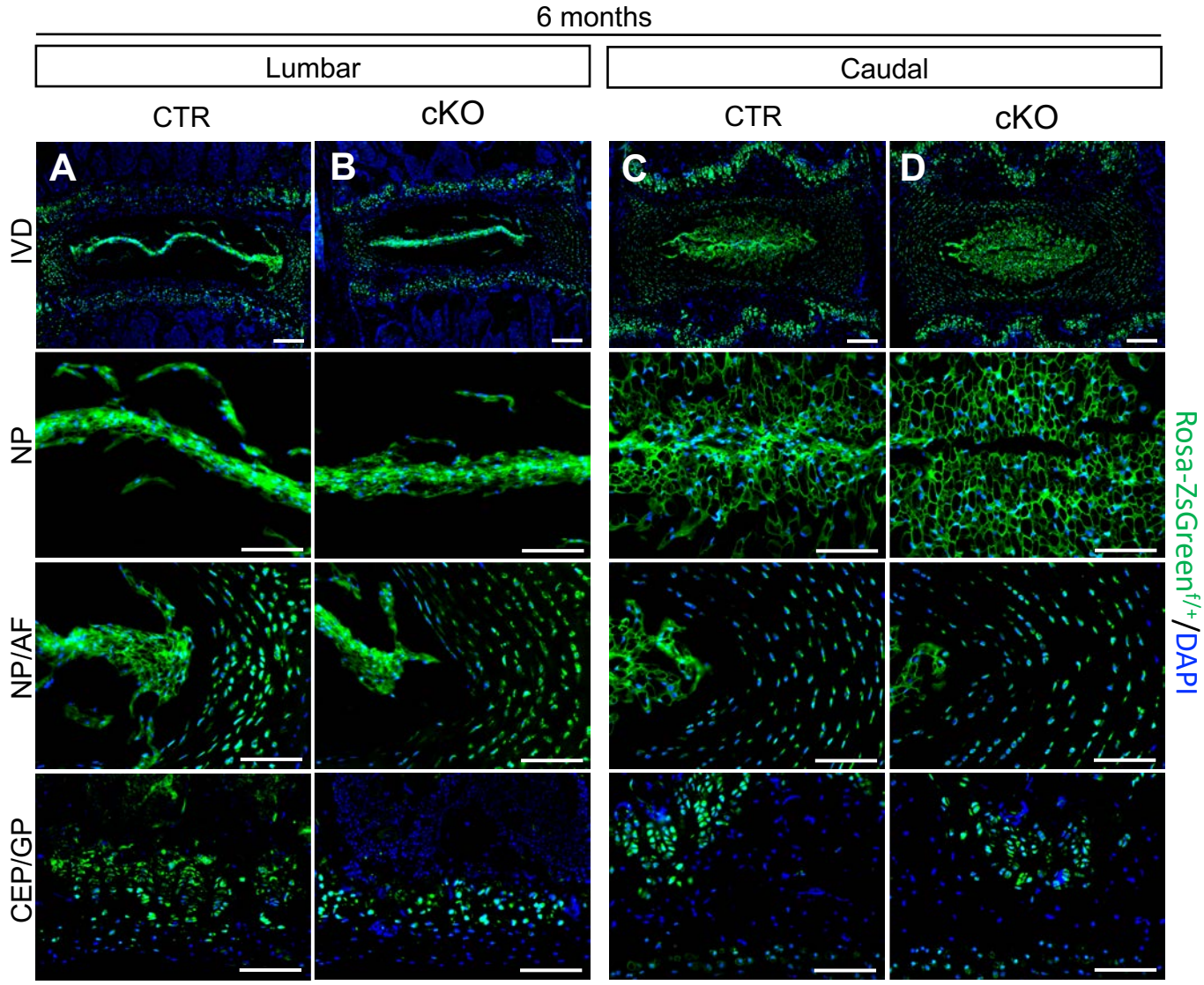
Supplemental Figure 1: Arp2/3 inactivation does not affect actin abundance in chondrocytes. (A) Confocal microscopy analyzing actin abundance in chondrocytes with and without loss of *Arpc2* from femoral condyles of Acan-CreER^{T2};Arpc2$^{f/f}$;ROSA-lox-stop-lox-ZsGreen mice. (B) Quantification of the integrated pixel intensity of 78 *Arpc2*-intact cells (no recombination as denoted by lack of a ZsGreen signal) and 69 *Arpc2*-loss cells (recombination as denoted by a positive ZsGreen signal) by ImageJ. Note that for ease of viewing, the ZsGreen signal is pseudo-colored red and the phalloidin signal is pseudo-colored green. (Scale, 20 μm) (quantification was done from 3 mice/group). Quantitative measurements represent mean ± SD. Significance was determined using Mann-Whitney *U*. n.s. = not significant.



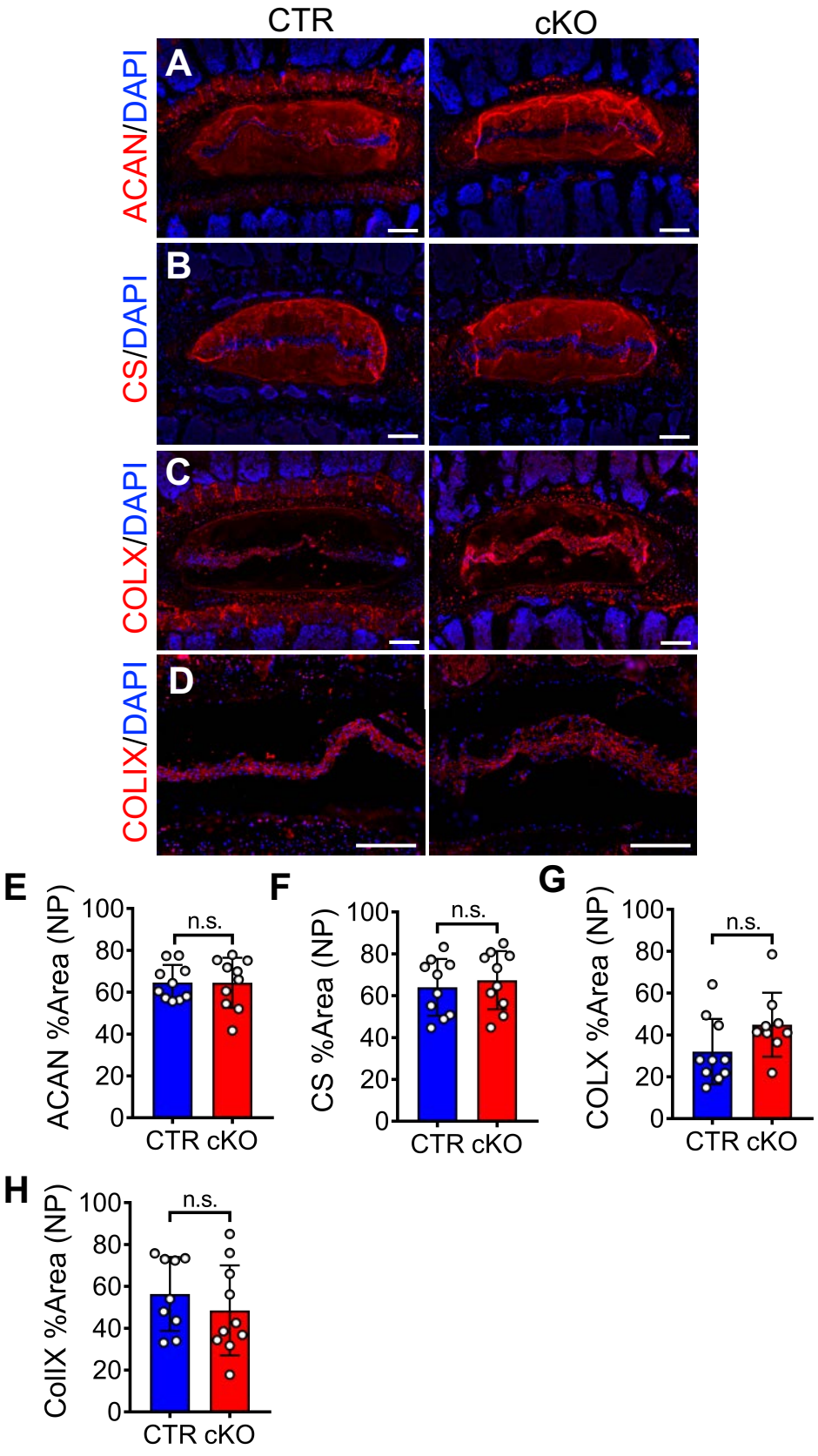
Supplemental Figure 2: Phenotypic variability in lumbar and caudal discs of wild-type and *Arpc2* mutants. Safranin-O/Fast Green/Hematoxylin staining of lumbar and caudal discs from 6-month and 1-year-old Acan-CreER^{T2}; Arpc2$^{f/f}$ mice injected with tamoxifen at 4 months (Scale, 200 μm).



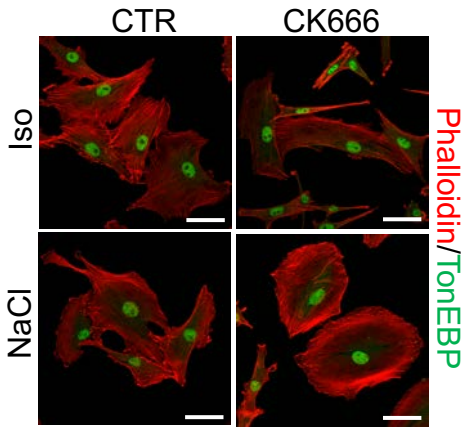
Supplemental Figure 3: Inactivation of Arp2/3 in postnatal mice does not affect NP cell phenotypic markers. Quantitative immunohistochemistry performed on 1-year-old lumbar discs from Acan-CreER^{T2}; Arpc2$^{f/f}$ mice stained by the following: (A, D) carbonic anhydrase 3 (CA3); (B, E) keratin-19 (KRT19); (C, F) vimentin (VIM) (Scale, 100 μm) (n=10 discs; 5 mice). Quantitative measurements represent mean ± SD. Significance was determined using unpaired Student's *t* test or Mann-Whitney *U*. n.s. = not significant.



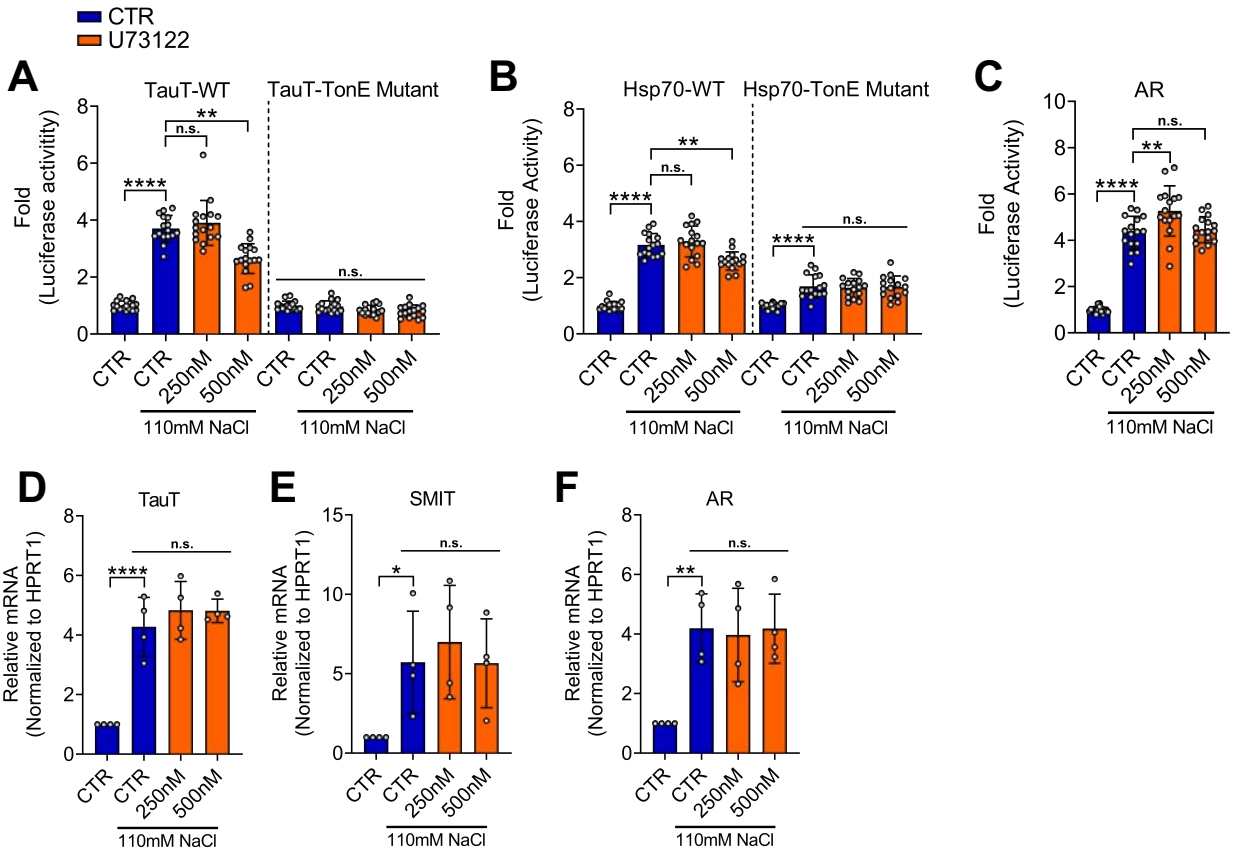
Supplemental Figure 4: Fate mapping confirms tamoxifen-induced Cre recombination driven by Acan-CreER^{T2}. A lox-stop-lox ZsGreen reporter (green) was used to verify the tamoxifen-induced Cre recombination of floxed alleles. (A) 6-month-old lumbar and (C) caudal discs of control animals showing ZsGreen signal confined to cells of the NP, AF, and growth-plate. (B) 6-month-old lumbar and (D) caudal discs of mutant animals showing ZsGreen signal confined to cells of the NP, AF, and growth-plate. (Top row: Scale, 200 μm; High magnification view: Scale, 100 μm).



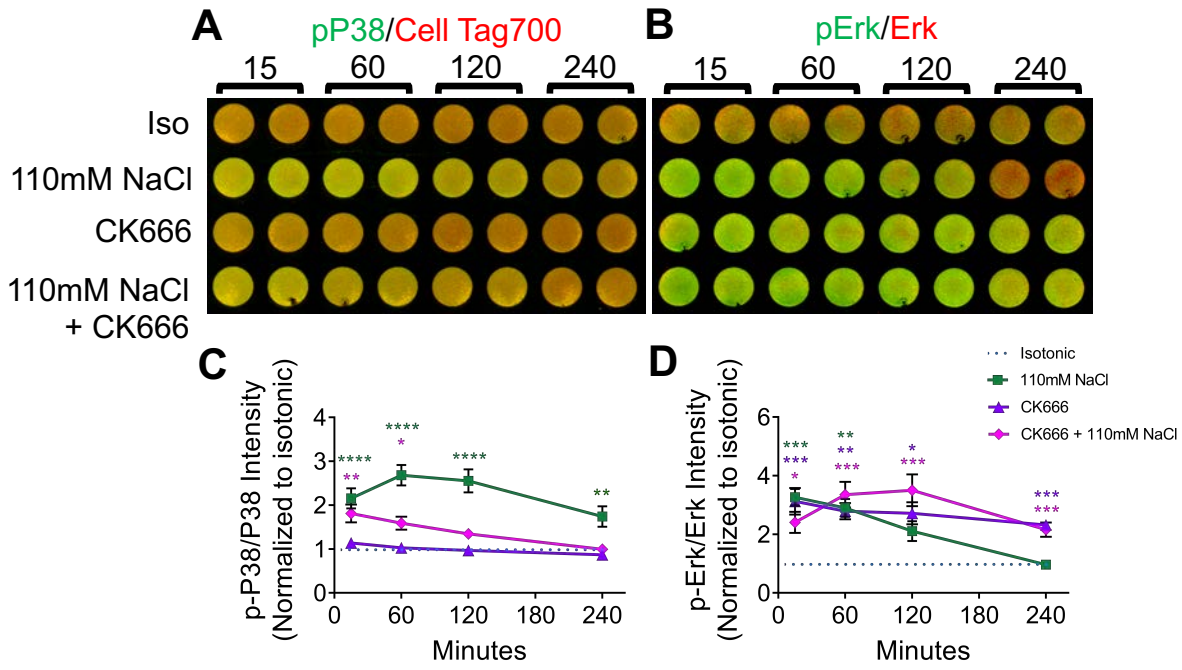
Supplemental Figure 5: Loss of Arp2/3 in postnatal mice does not affect NP matrix composition. Quantitative immunohistochemistry against 1-year-old lumbar discs from Acan-CreER^{T2}; Arpc2$^{f/f}$ mice stained by the following: (A, E) aggrecan (ACAN); (B, F) chondroitin sulfate (CS); (C, G) collagen X (COLX) (Scale, 200 μm); (D, H) collagen IX (COLIX) (Scale, 100 μm) (n=10 discs; 5 mice). Quantitative measurements represent mean ± SD. Significance was determined using unpaired Student's *t* test or Mann-Whitney *U*. n.s. = not significant.



Supplemental Figure 6: NaCl-induced TonEBP nuclear translocation is not impaired by Arp2/3 inhibition. High magnification images of cultured NP cells stained for actin (red) and TonEBP (green) (Scale, 50 μm) (n=3 independent biological experiments).



Supplemental Figure 7: Phospholipase C is dispensable for hypertonic induction of TonEBP activity in NP cells. Luciferase assays measuring (A) TauT-WT/TauT-TonE mutant and (B) Hsp70-WT/Hsp70-TonE mutant promoter activities with NaCl treatment and PLC (U73122) inhibition in primary NP cells. (C) NaCl-induced activation of AR-reporter with inhibition of PLC (n=4, 4 replicates/experiment). qRT-PCR measuring mRNA levels of (D) TauT, (E) SMIT, and (F) AR from primary NP cells treated with NaCl and PLC inhibitor (n=4 independent experiments). Quantitative measurements represent mean ± SD. Significance was determined using one-way ANOVA. n.s. = not significant; **, $p \leq 0.01$; ****, $p \leq 0.0001$.



Supplemental Figure 8: Arp2/3 is required for phosphorylation of p38 MAPK upon hypertonic stimulation in NP cells. In cell Western assay treated with NaCl and/or CK666 showing (A, B) scanned imaging results and (C, D) intensity ratios of pP38/Cell Tag700 and pErk/Erk, respectively (n=5 independent biological experiments). Quantitative measurements represent mean ± SD. Significance was determined using Kruskal-Wallis. *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$.