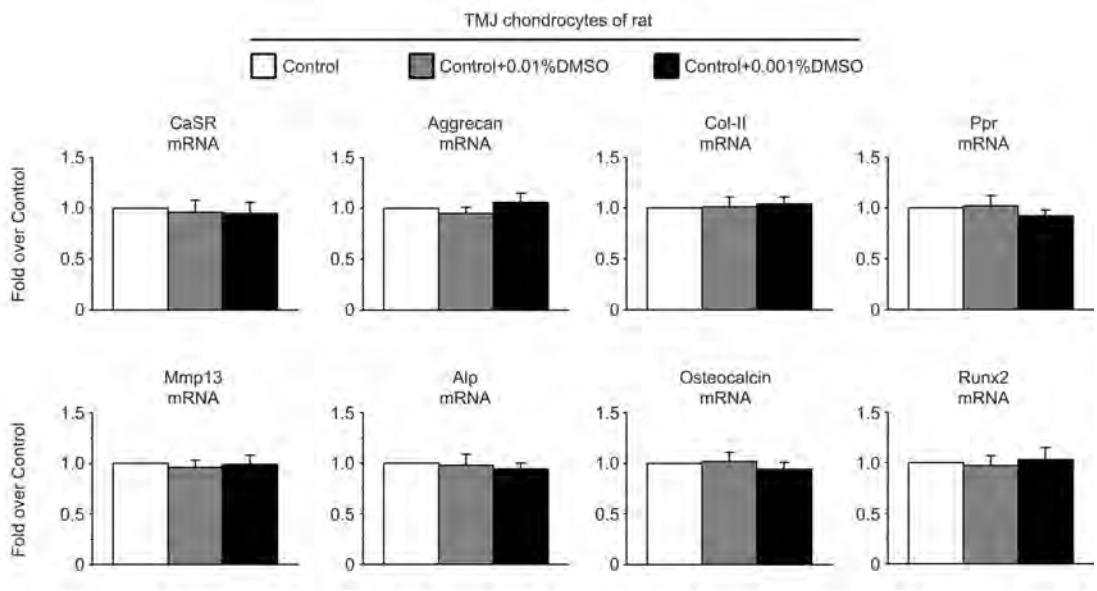
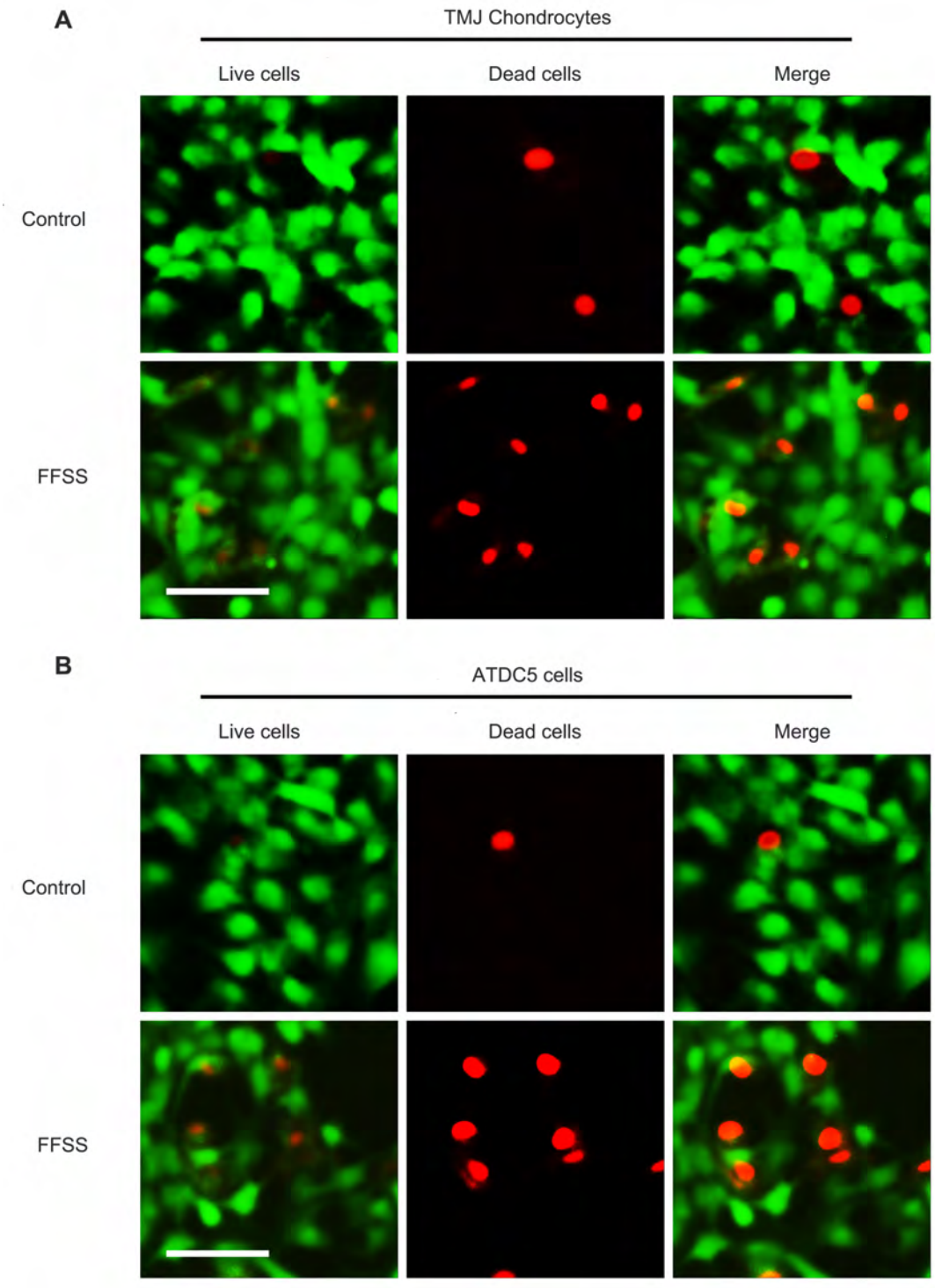
## Supplemental figure legends

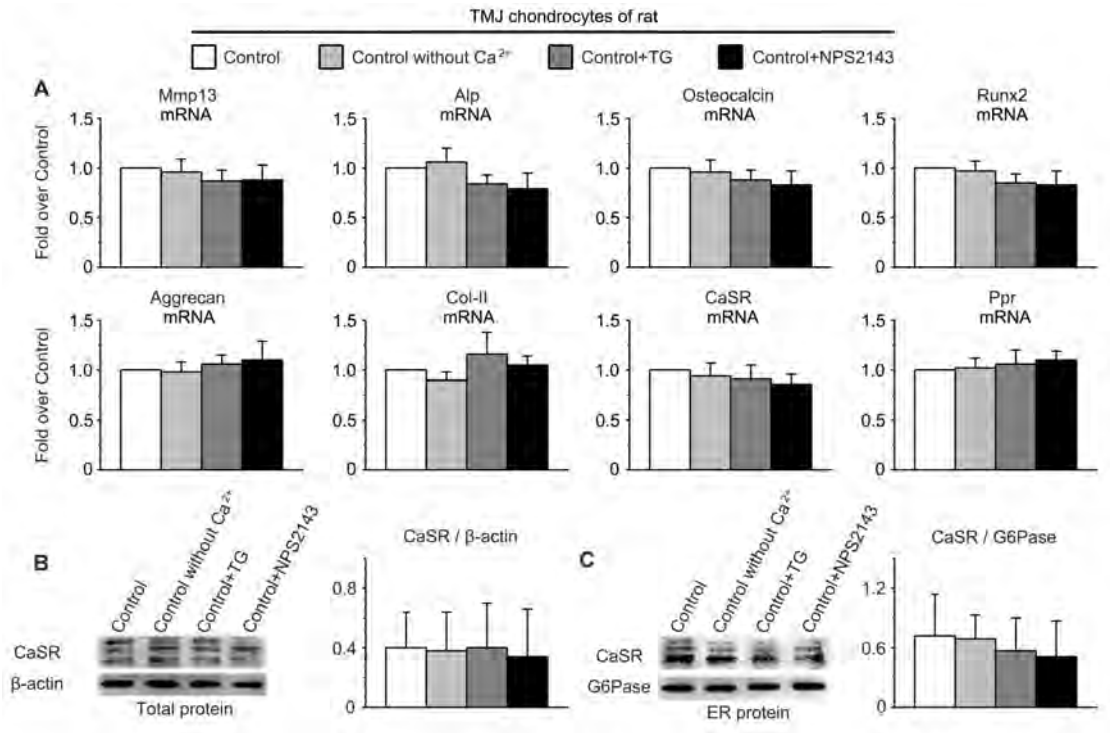



**Supplemental Fig. 1.** RNA Expression of CaSR and differentiation marker is indifferent in rat TMJ-chondrocytes cultured with 0.01% or 0.001% DMSO from no treatment controls. qPCR was performed with probe sets for CaSR, early (Aggrecan, Col-II and PPR) and late (Mmp13, ALP, Osteocalcin, Runx2) differentiation markers. mRNA levels were normalized to housekeeping GAPDH and presented as fold-increase over control treatment. n=4 separate cultures. Control, cultured cells without DMSO or other treatment. Values are presented as the mean±SD.

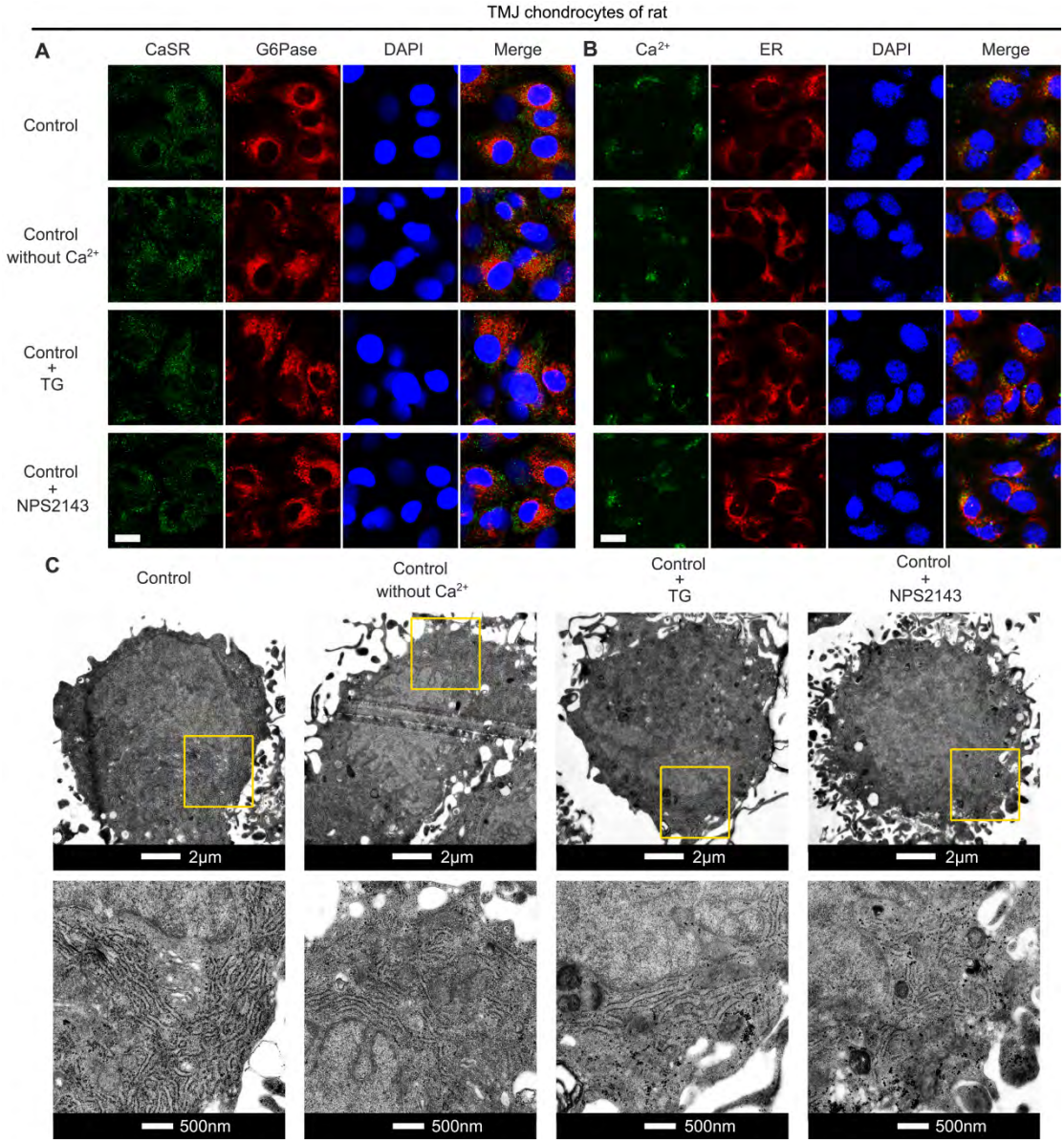



**Supplemental Fig. 2.** Acute FFSS (16dyn/cm$^2$ for 1 hr) induced modest cell death of (**A**) primary chondrocytes cultured from TMJs of SD rats or (**B**) chondrogenic ATDC5 cells. Apoptotic cells were stained by ethidium homodimer-1 (red) while living cells

were stained by calcein-AM (green) using a Live/Dead commercial kit (R37601, Invitrogen) according to the manufacturer's protocol. n=5 separate cultures.

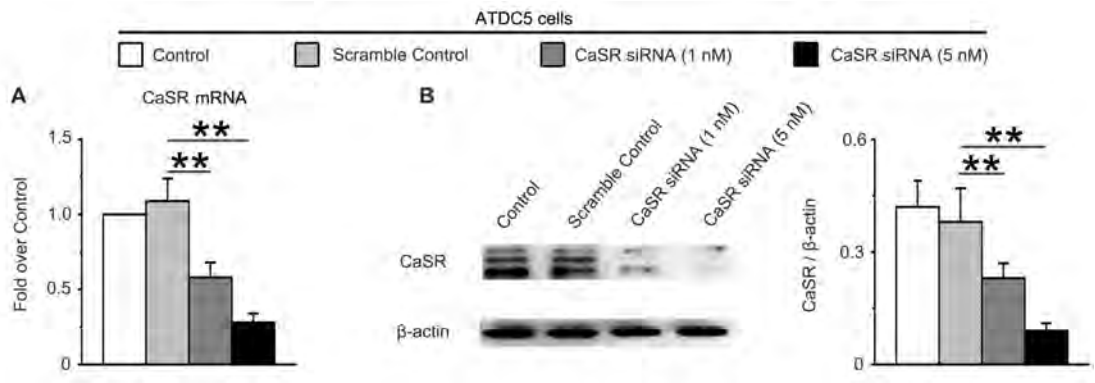



**Supplemental Fig. 3.** Expression of terminal differentiation marker and localization of CaSR in ERs in rat TMJ-chondrocytes cultured with or without $Ca^{2+}$, TG ($10^{-6}$M), or NPS2143 ($10^{-5}$M). (**A**) mRNA expression by qPCR showed no obvious difference on the expression of terminal differentiation markers -- Mmp13, ALP, Osteocalcin, Runx2 and CaSR and the expression of early differentiation makers -- Aggrecan, Col-II and PPR in all groups without FFSS treatment. mRNA levels were normalized to housekeeping GAPDH and presented as fold-increase over control treatment. (**B, C**) Immunoblotting of (**B**) total and (**C**) ER proteins with anti-CaSR antibody demonstrated that no change in the expression of CaSR protein and its localization in ERs in cells without FFSS treatmen. β-actin and G6Pase were used as loading controls for total and ER protein, respectively, and for normalization to calculate the expression ratios in the histograms. n=4 separate cultures. Control, cultured cells without FFSS or other treatment. Values are presented as the mean±SD.

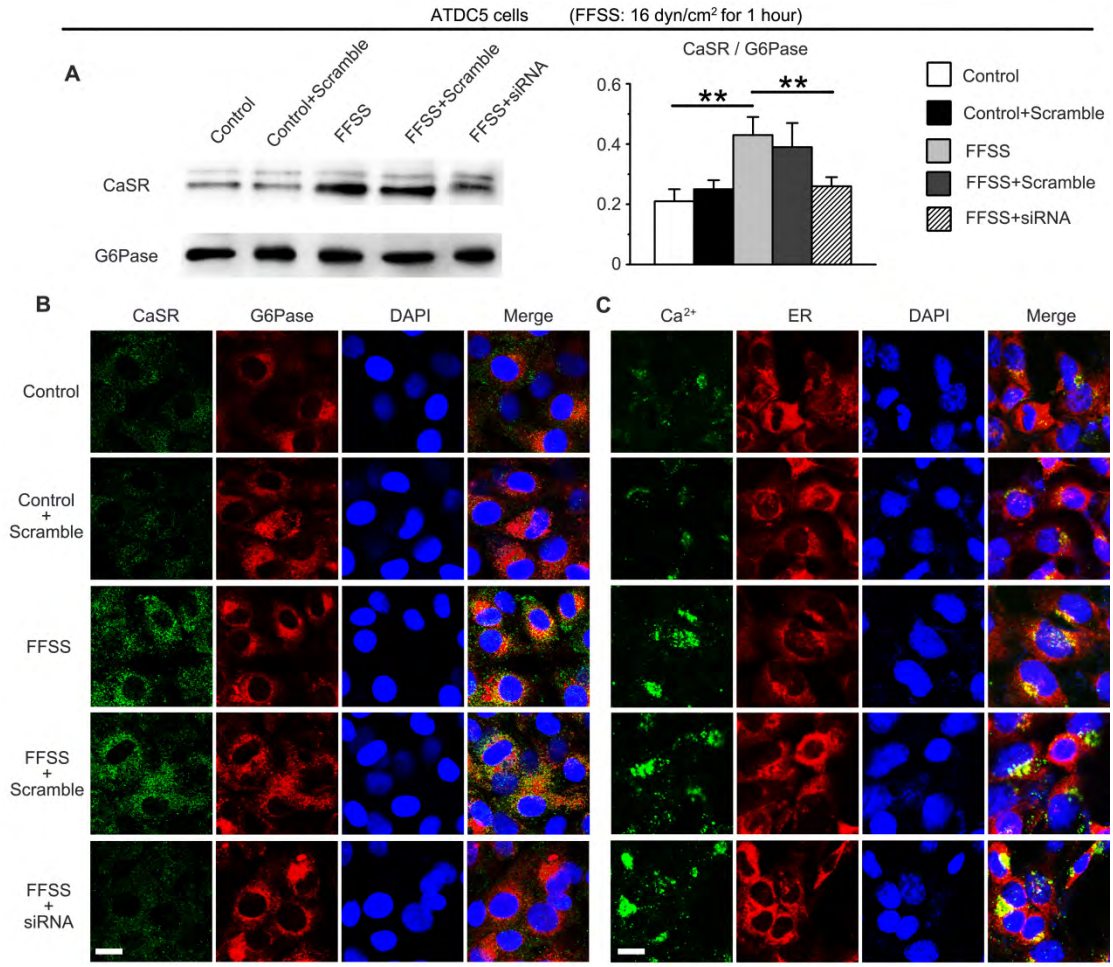



**Supplemental Fig. 4.** (**A**) CaSR localization, (**B**) $Ca^{2+}$ loading, and (**C**) organelle swelling in ERs of rat TMJ-chondrocytes cultured with or without $Ca^{2+}$, TG ($10^{-6}$M), or NPS2143 ($10^{-5}$M). (**A**) Dual-fluorescence staining with anti-CaSR (in green) and anti-glucose-6-phosphatase (anti-G6Pase, in red) antibodies showed similar CaSR localization in ERs. (**B**) Dual-fluorescence staining with Fluo-8 (in green) and ER-tracker (in red) showed similar [$Ca^{2+}$] in ERs. n=4 separate cultures. Bar=5μm. (**C**) Representative transmission electron microscopy (TEM) images showed no obvious

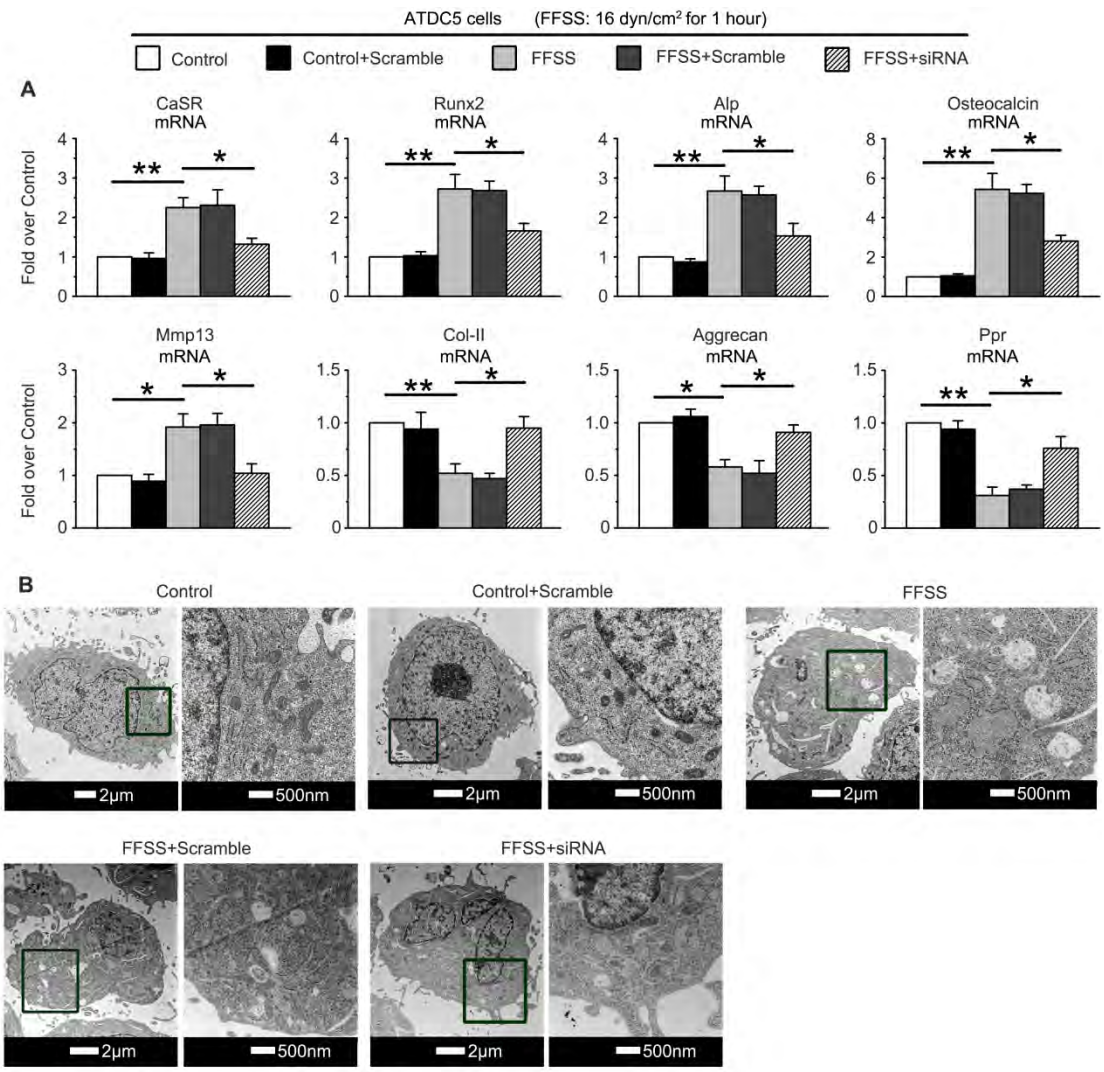
swelling of mitochondria and ERs in all groups. Control: cultured cells without FFSS or other treatment.




**Supplemental Fig. 5.** The impact of CaSR RNA knockdown on the (**A**) mRNA and (**B**) protein expression of CaSR in ATDC5 chondrogenic cell cultures. ATDC5 cells, cultured at a density of $5\times10^3/cm^3$, were transfected with CaSR and scramble siRNA with a final concentration of 1nM and 5 nM for 6 hrs, before their RNA and protein were extracted for qPCR and immunoblotting analyses for CaSR and/or β-actin expression. n=5 separate cultures. Values are presented as the mean ± SD. **p< 0.01 vs non-transfected (Control) and scramble siRNA cultures.

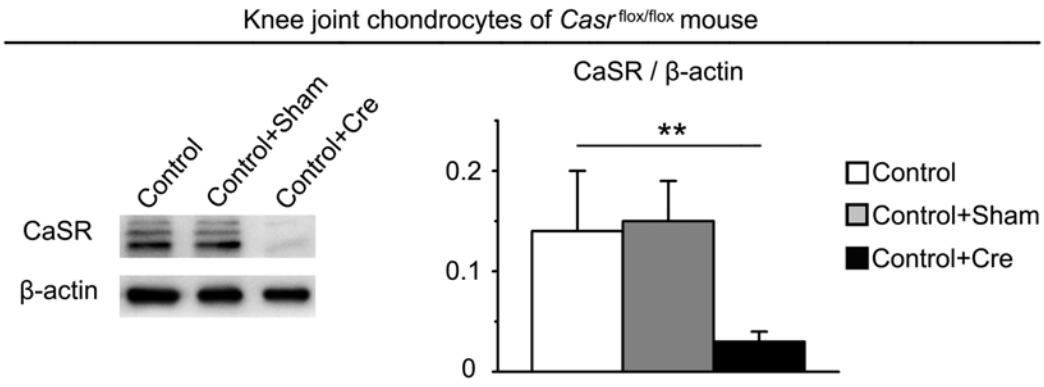



**Supplemental Fig. 6.** The impact of CaSR mRNA knockdown (siRNA concentration: 5 nM) on FFSS-induced changes in CaSR localization and $Ca^{2+}$ loading in ERs. (**A**) Immunoblotting and (**B**) dual-fluorescence staining of CaSR (in green) and G6Pase (in red) showed the ability of CaSR siRNA to abrogate the FFSS-induced CaSR localization in ERs. Bar=5μm. (**C**) Dual-fluorescence Fluo-8 (in green) and ER-tracker (in red) staining showed the inability of CaSR siRNA to block the FFSS-induced $Ca^{2+}$loading in ERs. Bar=5μm. n=5 separate cultures. Values are presented as the mean ± SD. ** $p<0.01$ between groups as specified by top horizontal bars in each panel.

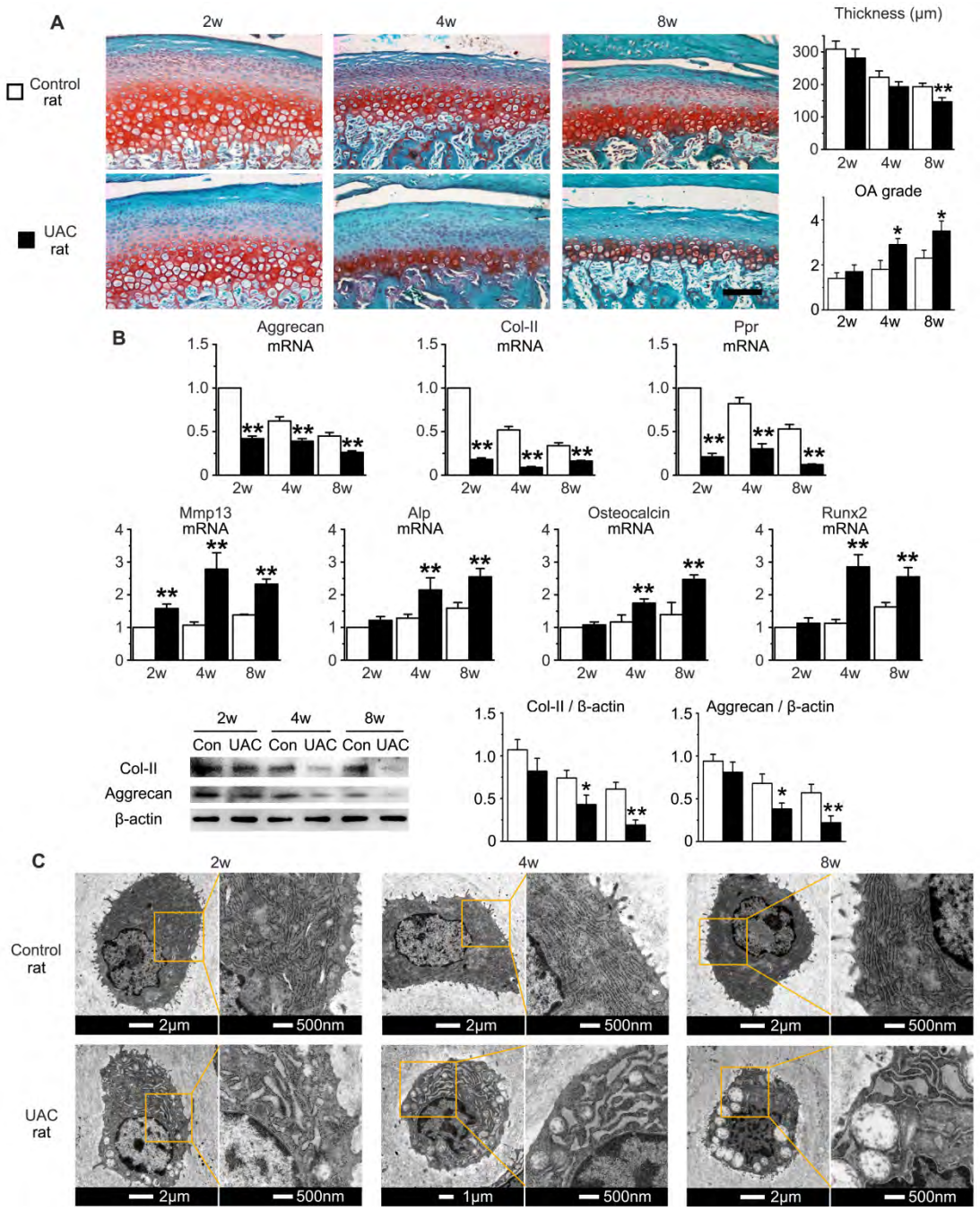



**Supplemental Fig. 7.** The impact of CaSR mRNA knockdown (siRNA concentration: 5 nM) on FFSS-induced changes in expression of cell differentiation markers, and organelle swelling in ATDC5 chondrogenic cell cultures. (**A**) mRNA expression profiles by qPCR showed the ability of FFSS to upregulate the expression of Mmp13, ALP, Osteocalcin, Runx2 and CaSR and downregulate the expression of Aggrecan, Col-II and PPR as well as the ability of CaSR knockdown to prevent these changes. n=5 separate cultures. Values are presented as the mean ± SD. $^{**}p<0.01$, $^{*}p<0.05$ between groups as specified by top horizontal bars in each panel. (**B**) TEM images

showed the inability of CaSR knockdown to affect the FFSS-induced swelling of mitochondria and ERs.

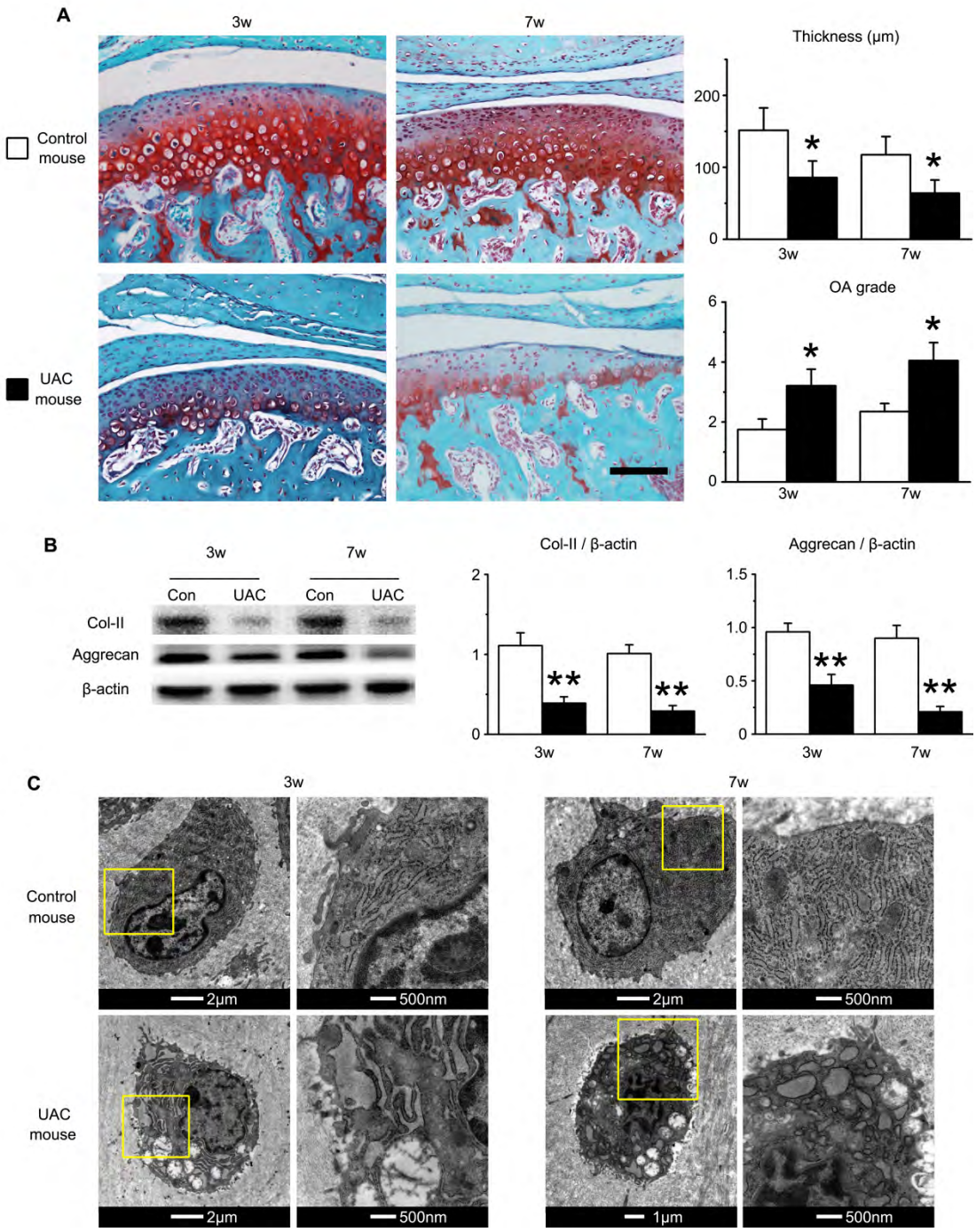



**Supplemental Fig. 8.** The impact of Cre recombinase on CaSR protein expression in cultured chondrocytes from knee joint cartilage of *Casr*$^{flox/flox}$ mice. Chondrocytes cultured at a density of $5\times10^3/cm^3$, were pretreated with Cre recombinase (2μM) for 5 hrs before their protein were extracted for immunoblotting analyses for CaSR and/or β-actin expression. n=5 separate cultures. Values are presented as the mean ± SD. **p< 0.01 vs non-pretreated (Control) cultures.



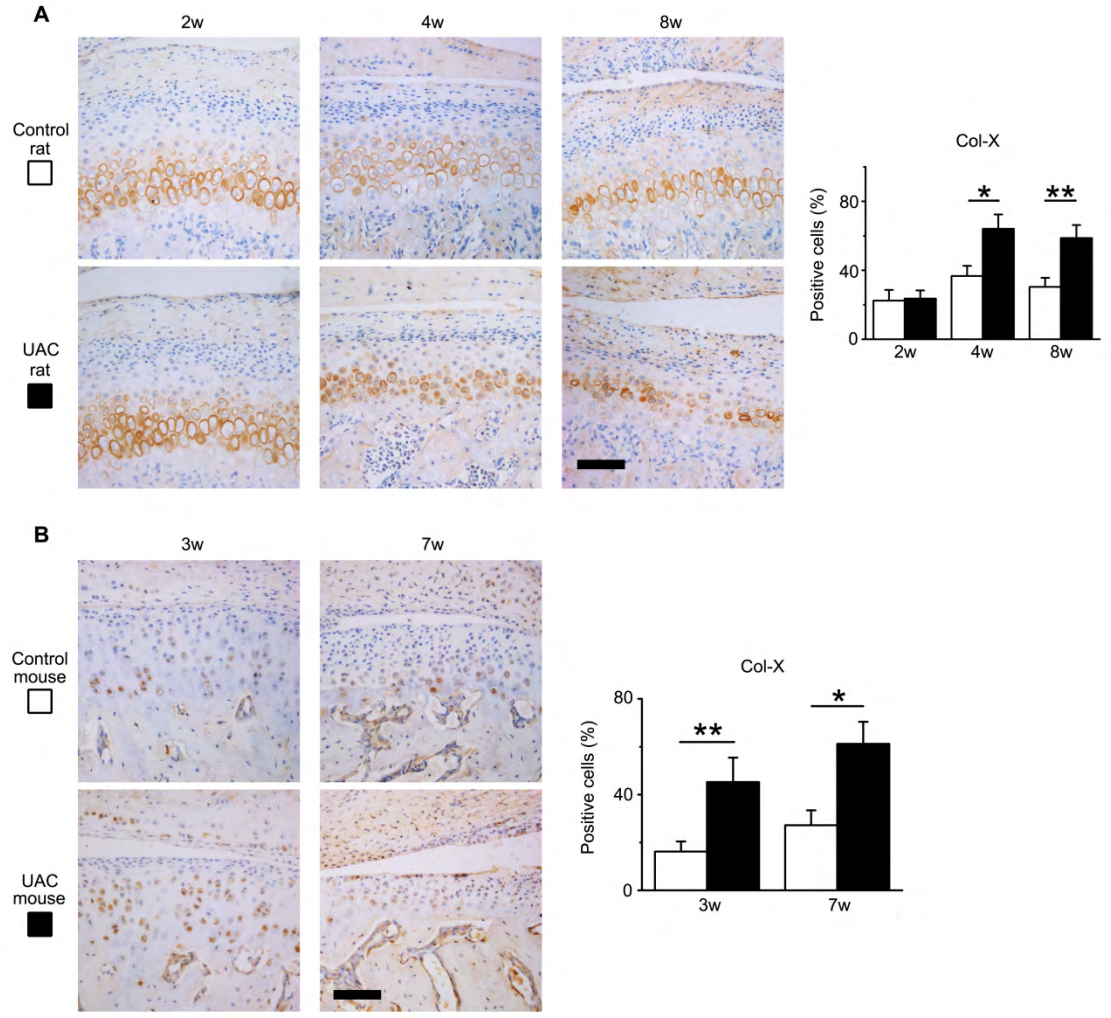

**Supplemental Fig. 9.** Effects of UAC on (**A**) tissue morphology, (**B**) gene and protein expression, and (**C**) organelle morphology in condylar cartilage in rat TMJs subjected to UAC for 2, 4 and 8 weeks. n=12 rats per group. Six right TMJs from rats were used for safranin O staining and the other six right TMJs were used for TEM observation. Twelve left TMJ cartilages were pooled into 6 samples (2 cartilages per sample) for RNA and protein extraction and used for qPCR and immunoblotting analyses. (**A**)

Safranin O staining showed age-dependent decreases in proteoglycan content, thinning of cartilage, and increased OA grade in non-UAC control rats and these effects were further exacerbated in UAC-treated rats. Bar=100μm. (**B**) qPCR analyses of mRNA and immunoblotting analysis of protein extracted from the condylar cartilage of the above rats showed age-dependent decreases in the expression of early differentiation markers (Aggrecan, Col-II and PPR) and increases in markers of terminal differentiation (ALP, Runx2, Mmp13and Osteocalcin) in non-UAC control rats, and these effects were greatly amplified in UAC-treated rats. mRNA levels are expressed as fold-change over 2 weeks non-UAC control. Values are presented as the mean ± SD. $^{**}p<0.01$, $^{*}p<0.05$ vs age-matched non-UAC control. (**C**) TEM images showed swollen mitochondria and ERs in mandibular condylar cartilage subjected to UAC vs control and these changes intensified with the length of UAC treatment.

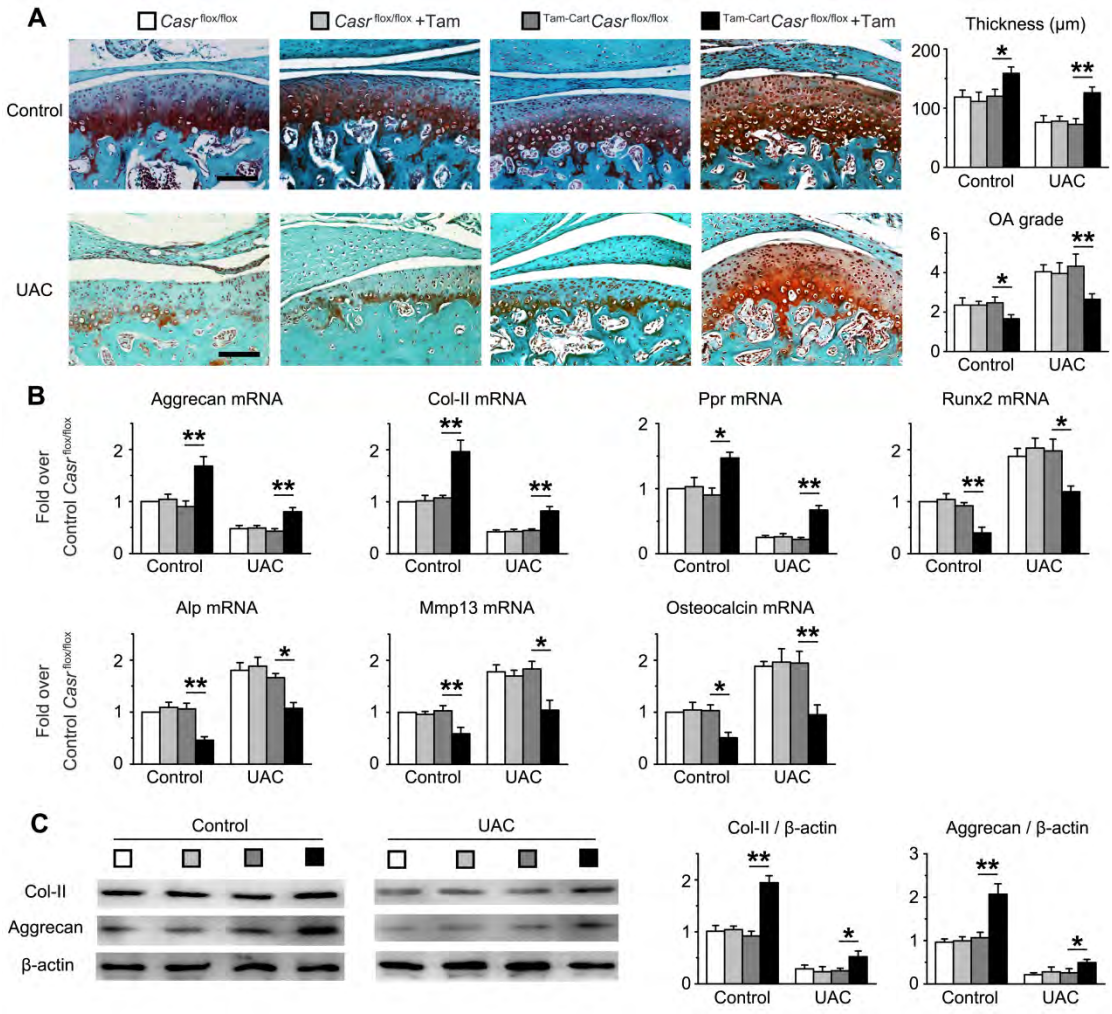



**Supplemental Fig. 10.** Effects of UAC on (**A**) tissue morphology, (**B**) gene and protein expression, and (**C**) organelle morphology in condylar cartilage in mice TMJs subjected to UAC for 3 and 7 weeks. n=18 mice per group. Six right TMJs from mice were used for safranin O staining and another six right TMJs were used for TEM studies. Six right TMJ cartilage and eighteen left TMJ cartilages were pooled into 4 samples (6 cartilages

per sample) for protein extraction and used for immunoblotting analyses. (**A**) Safranin O staining showed age-dependent decreases in proteoglycan content, thinning of cartilage and increased OA grade in non-UAC control mice and these effects were further exacerbated in UAC-treated mice. Bar=100μm. (**B**) Immunoblotting analysis of protein extracted from the condylar cartilage of the above mice showed decreases in the expression of Aggrecan and Col-II in UAC-treated mice. Values are presented as the mean ± SD. $^{**}p<0.01$, $^{*}p<0.05$ vs age-matched non-UAC control. (**C**) TEM images showed swollen mitochondria and ERs in mandibular condylar cartilage subjected to UAC vs control and these changes intensified with the length of UAC treatment.

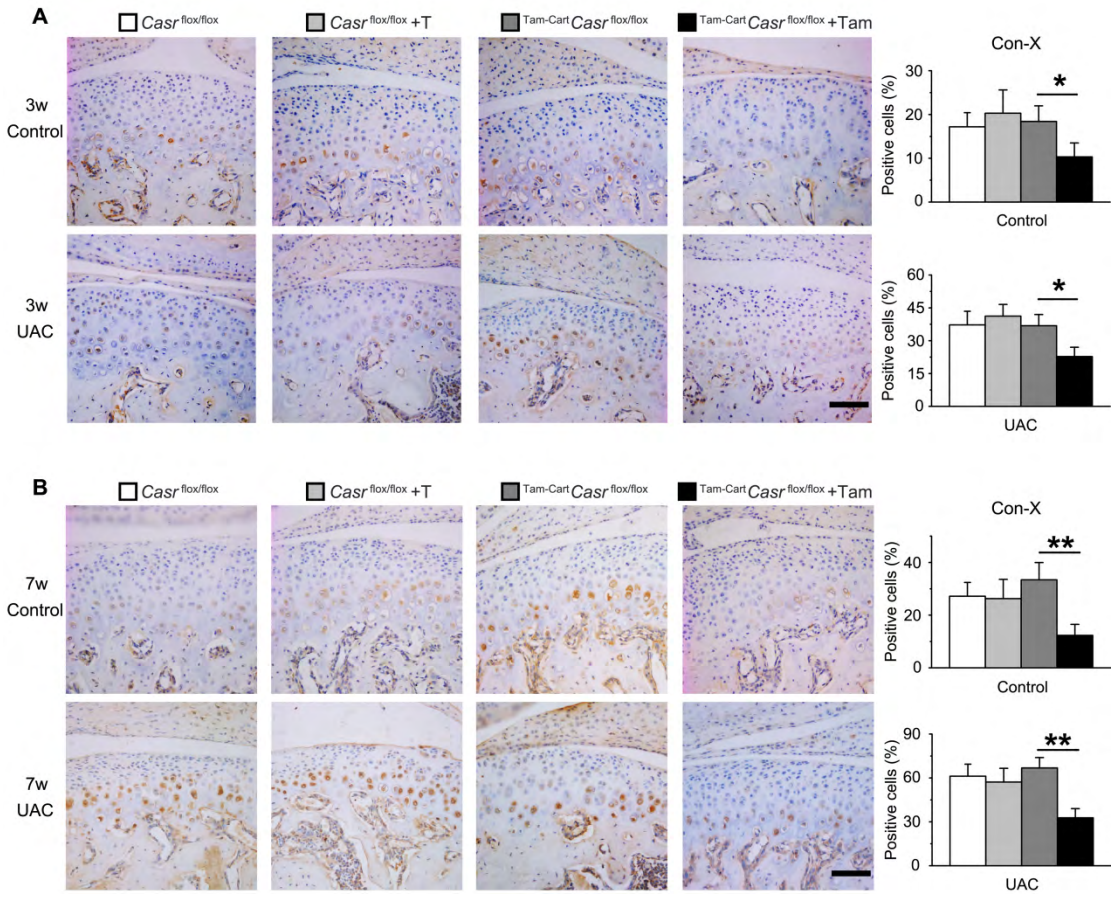



**Supplemental Fig. 11.** Col-X expression in chondrocytes in (**A**) rat and (**B**) mouse TMJ cartilage without (Control) or with UAC treatment. (**A**) n=6 rats per group. TMJs from six rats were used for immunohistochemical staining. Immunohistochemical staining showed increased % of Col-X positive cells in cartilage of UAC rats. (**B**) n=6 mice per group. TMJs from six mice were used for immunohistochemical staining. Immunohistochemical staining showed increased % of Col-X positive cells in cartilage of UAC mice. Bar=100μm. Values are presented as the mean ± SD. **p<0.01, *p<0.05 vs age-matched non-UAC control.



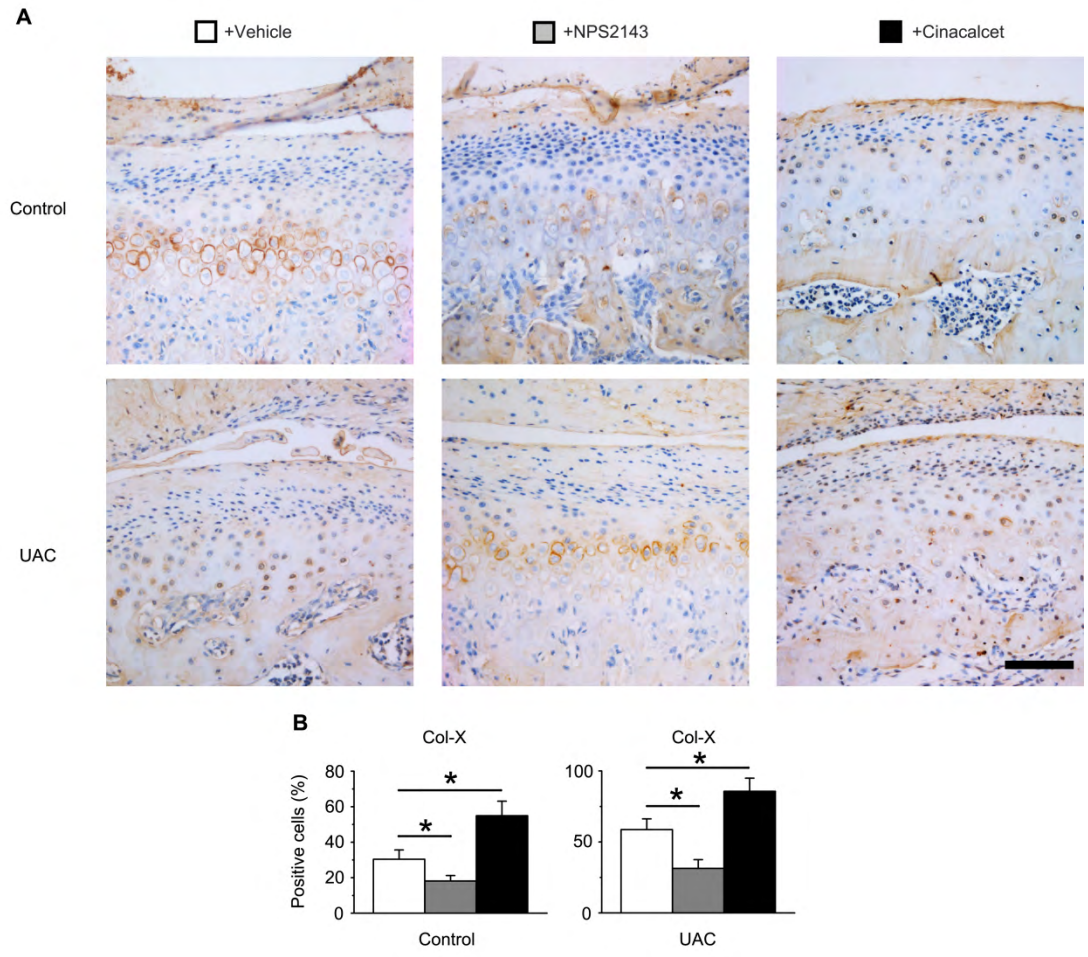

**Supplemental Fig. 12.** Effects of Tamoxifen (Tam)-induced *Casr* gene KO on chondrocytic functions in TMJ-cartilage of mice without (Control) or with UAC. The *Casr* gene KO was induced by 5daily intraperitoneal (IP) Tam injections in $^{Tam\text{-}Cart}Casr^{flox/flox}$ mice at 8 weeks of age, followed by 7 weeks of UAC. Three control groups -- $Casr^{flox/flox}$, $Casr^{flox/flox}$ injected with Tamoxifen ($Casr^{flox/flox}$+Tam), or $^{Tam\text{-}Cart}Casr^{flox/flox}$ without Tamoxifen injections --, which did not have their *Casr* genes ablated, were subjected to the same UAC regimen. (**A**) Safranin O staining (in red) showed profoundly increased proteoglycan content and thickness of TMJ-cartilage and decreased OA grade in $^{Tam\text{-}Cart}Casr^{flox/flox}$ mice injected with Tamoxifen vs the other 3 control groups with or without UAC treatment. Bar=100μm. n=6 mice per group. (**B**)

qPCR analyses of mRNA and (**C**) immunoblotting analysis of protein extracted from the TMJ-cartilage of the above mice showed the inability of UAC to reduce the expression of early differentiation markers and to increase terminal differentiation makers in $^{\text{Tam-Cart}}Casr^{\text{flox/flox}}$ mice injected with Tamoxifen vs other 3 controls with or without UAC. Eighteen TMJ-cartilages were pooled into 3 samples (6 cartilage per sample) for qPCR and immunoblotting analysis. All values in (**B**) were normalized to the $Casr^{\text{flox/flox}}$ Control. Values are presented as the mean±SD. $^{**}p<0.01$, $^{*}p<0.05$ between groups as specified by top horizontal bars in each panel.

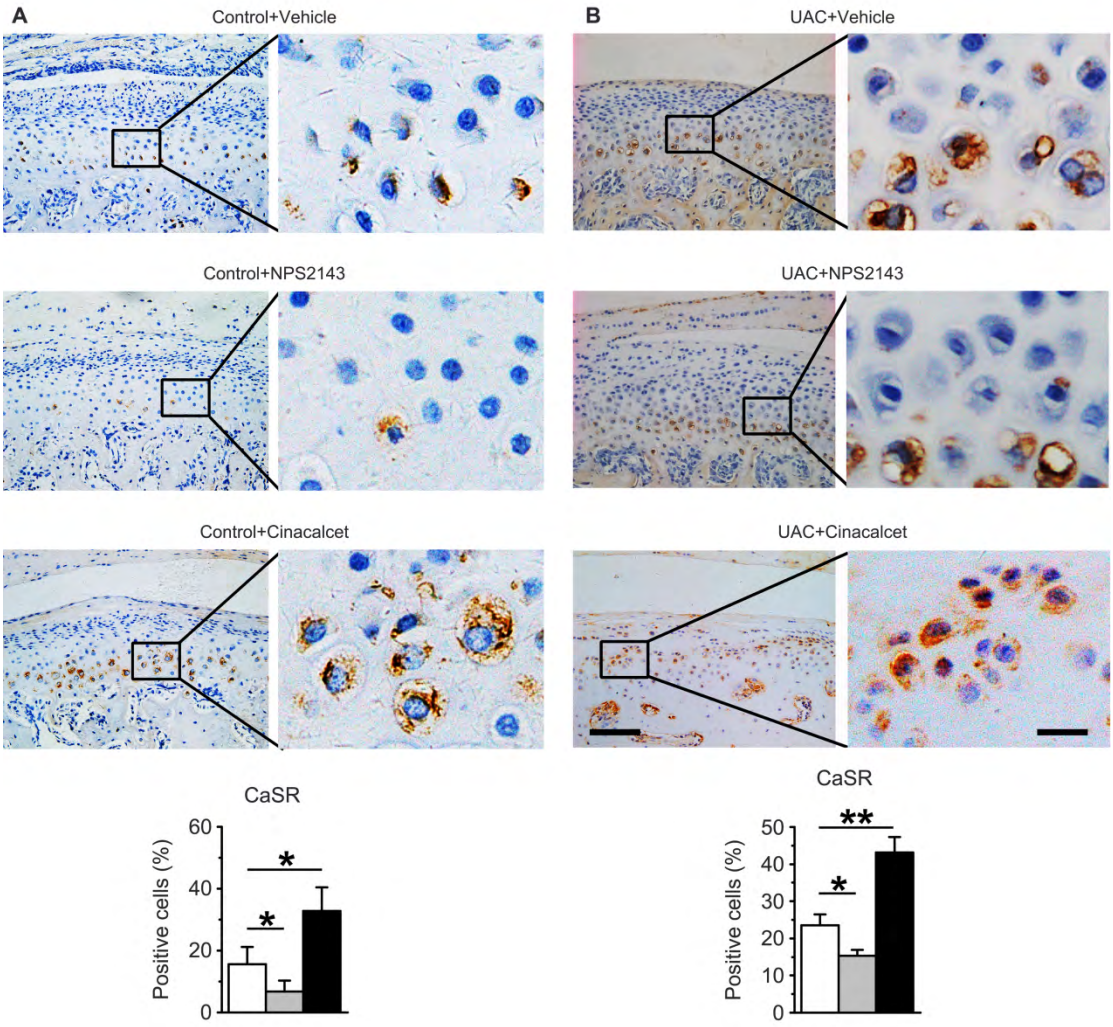



**Supplemental Fig. 13.** Effects of Tamoxifen (Tam)-induced *Casr* gene KO on Col-X expression in TMJ-cartilage of mice without or with UAC for 3 (**A**) or 7 weeks (**B**). The *Casr* gene KO was induced by 5daily intraperitoneal (IP) Tam injections in $^{Tam\text{-}Cart}Casr^{flox/flox}$ mice at 8 weeks of age, followed by 3 or 7 weeks of UAC. Three control groups -- $Casr^{flox/flox}$, $Casr^{flox/flox}$ injected with Tamoxifen ($Casr^{flox/flox}$+Tam), or $^{Tam\text{-}Cart}Casr^{flox/flox}$ without Tamoxifen injections --, which did not have their *Casr* genes ablated, were subjected to the same UAC regimen. n=6 mice per group. TMJs from six mice were used for immunohistochemical staining. Immunohistochemical staining showed profoundly decreased % of Col-X positive cells in $^{Tam\text{-}Cart}Casr^{flox/flox}$ mice injected with Tamoxifen vs the other 3 control groups with or without UAC treatment.

Bar=100μm. Values are presented as the mean ± SD. **$p<0.01$, *$p<0.05$ between groups as specified by top horizontal bars in each panel.

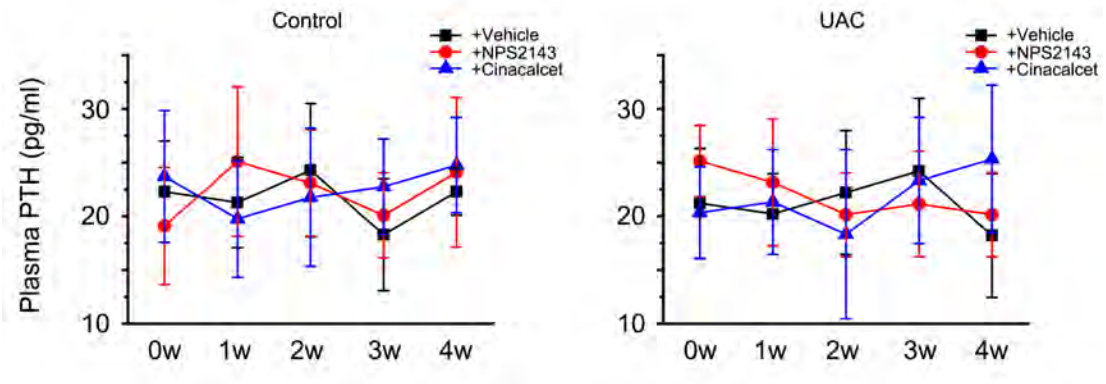



**Supplemental Fig. 14.** Effects of local injections of NPS2143 or Cinacalcet on Col-X expression in chondrocytes in rat TMJ cartilage without (Control) or with UAC treatment. Drug and vehicle injections were performed on control and UAC rats as described in Fig. 6. TMJs from six rats were used for immunohistochemical staining (n=6). Immunohistochemical staining (**A**) showed decreased % of Col-X positive cells in cartilage of either control or UAC rats injected with NPS2143, and vice versa for the injection of Cinacalcet (**B**). Bar=100μm or 20μm in enlarged pictures. Values are presented as the mean ± SD. *$p<0.05$ vs the corresponding vehicle-injected groups.




**Supplemental Fig. 15.** Effects of local injections of NPS2143 or Cinacalcet on CaSR expression in chondrocytes in rat TMJ-cartilage (**A**) without (Control) or (**B**) with UAC treatment. Drug and vehicle injections were performed on control and UAC rats as described in Fig. 6. Immunohistochemical staining showed decreased % of CaSR-positive cells in cartilage of either control or UAC rats injected with NPS2143, and vice versa for the injection of Cinacalcet. Interestingly, Cinacalcet injections also increased localization of CaSR in the intracellular compartments of chondrocytes in both control and UAC groups. n=6 rats per group. Bar=100μm or 20μm in enlarged pictures. Values

are presented as the mean ± SD. **p<0.01, *p<0.05 vs the corresponding vehicle-injected groups.




**Supplemental Fig. 16.** Effects of local injections of NPS2143 and Cinacalcet on plasma PTH level in rat (A) without (Control) or (B) with UAC treatment. Plasma PTH level was first measured the day before injection and marked as "0w" and then measured weekly at the end of each week (1w, 2w, 3w and 4w). No obvious difference was observed. n=6 rats per group. Values are presented as the mean ± SD.