

Expanded View Figures

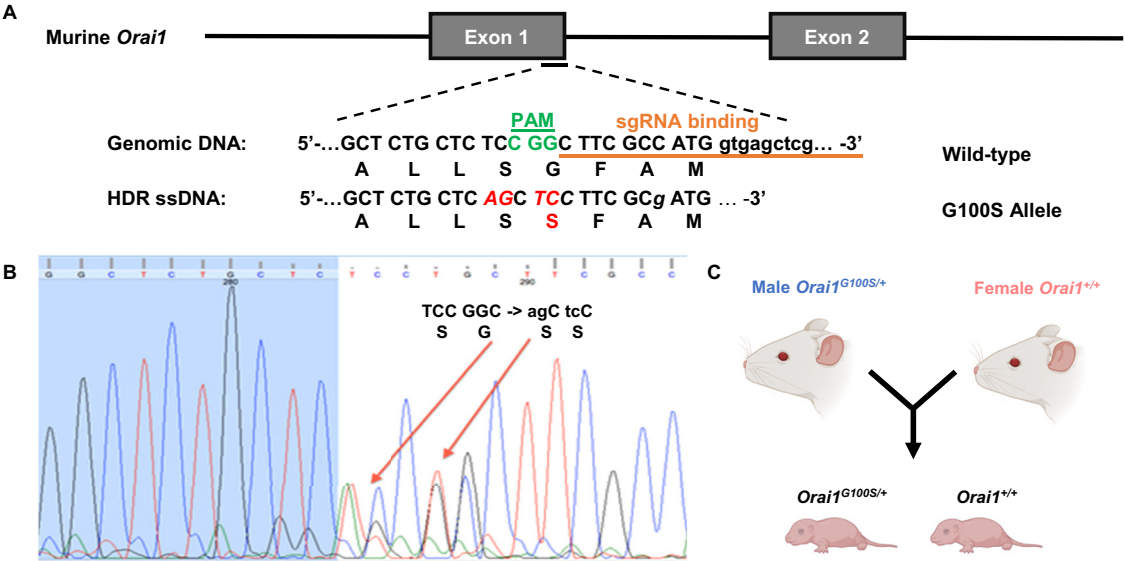


Figure EV1. Generation of GS mouse model.

(A) CRISPR-Cas9 strategy used to generate heterozygous GS knock-in mice. The G100S mutation is located at the end of exon 1. A donor template with desired glycine-to-serine mutation and altered PAM region was introduced to mouse embryos. (B) Sanger sequencing confirming heterozygous expression of the glycine to serine mutation at ORAI1 amino acid position 100. (C) Cartoon depicting the breeding strategy used in this study to generate WT and GS mice.

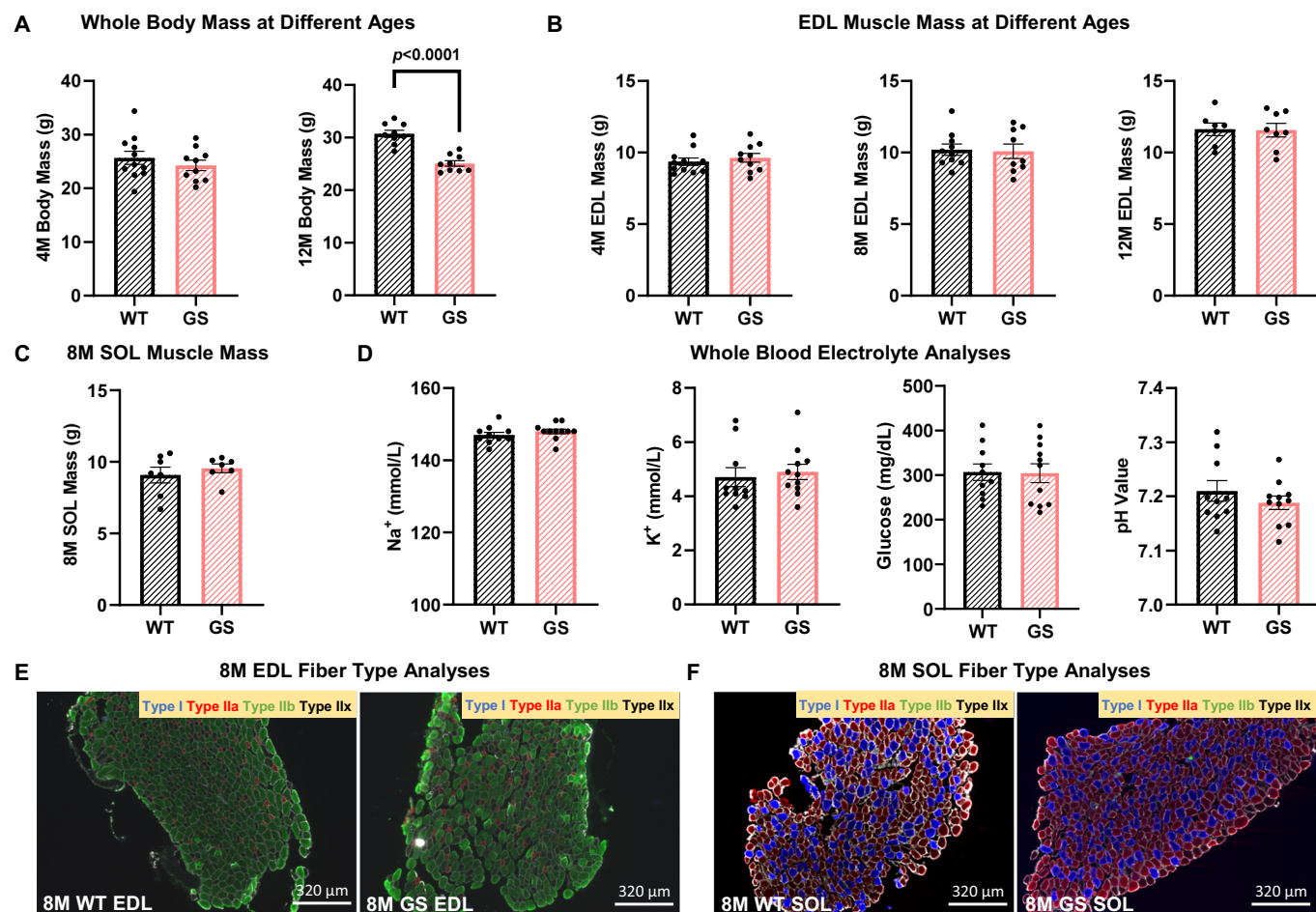


Figure EV2. Additional phenotypic characterization of GS mice.

(A) Compared to age-matched WT mice, GS mice showed no significant differences in average body mass at 4 M of age, but significantly reduced body mass at 12 M of age. For 4 M old mice, $n = 11$ and 10 for WT and GS mice, respectively. For 12 M old mice, $n = 9$ for WT mice and $n = 9$ for GS mice. Significance was calculated using Student's t test. The exact P value is labeled in the figure when $P < 0.05$. (B) GS mice showed similar EDL muscle mass at 4, 8 and 12 M of age as WT mice. For 4 M old mice, $n = 11$ and 10 for WT and GS mice, respectively. For 8 M old mice, $n = 10$ and 9 for WT and GS mice, respectively. For 12 M old mice, $n = 7$ and 8 for WT and GS mice, respectively. (C) WT ($n = 7$) and GS ($n = 7$) mice exhibited similar SOL muscle mass at 8 M of age. (D) Whole blood analysis of Na^+ , K^+ , glucose, and pH from 8 M old WT ($n = 10$) and GS ($n = 11$) mice. (E, F) Representative fiber type images of EDL (E) and SOL (F) muscles from 8 M old WT and GS mice.

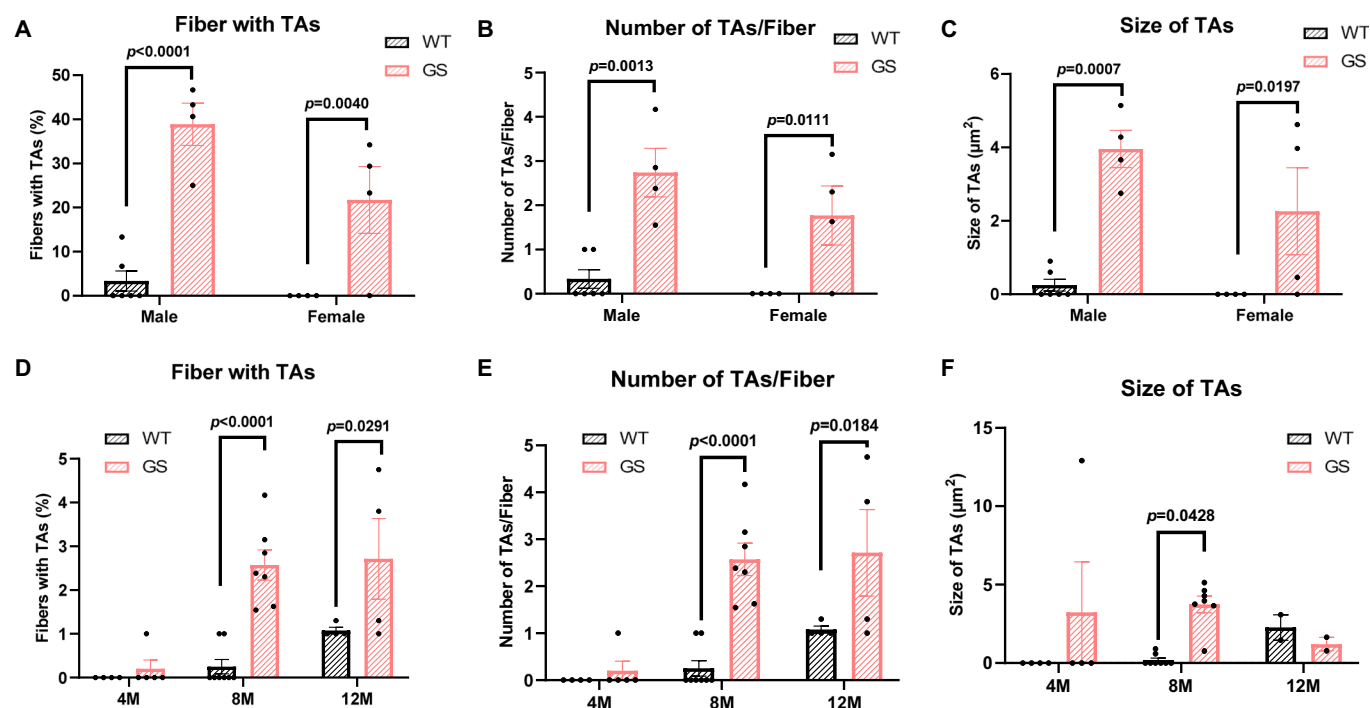


Figure EV3. Sex- and age-dependent analyses of tubular aggregates in EDL muscle from 8 M WT and GS mice.

(A–C) Average ($\pm$ SEM) percentage of fibers with TAs (A), number of TAs/fiber (B), and TA size (C) in EDL muscles from 8 M old male and female WT and GS mice. For male group, $n = 6$ and 4 for WT and GS group, respectively. For female mice, $n = 4$ for WT mice and $n = 4$ GS mice. Significance was calculated using 2-way ANOVA followed by Holm–Sidak’s multiple comparisons post hoc test. The exact P value is labeled in the figure when adjusted $P < 0.05$. (D–F) Average ($\pm$ SEM) percentage of fibers with TAs (D), number of TAs/fiber (E), and TA size (F) in EDL muscles from 4 M, 8 M, and 12 M old WT and GS mice. For 4 M and 12 M mice, $n = 4$ for WT mice and $n = 4$ GS mice. For 8 M old mice, $n = 8$ and 7 for WT and GS mice, respectively. Significance was calculated using 2-way ANOVA followed by Holm–Sidak’s multiple comparisons post hoc test. The exact P value is labeled in the figure when adjusted $P < 0.05$.

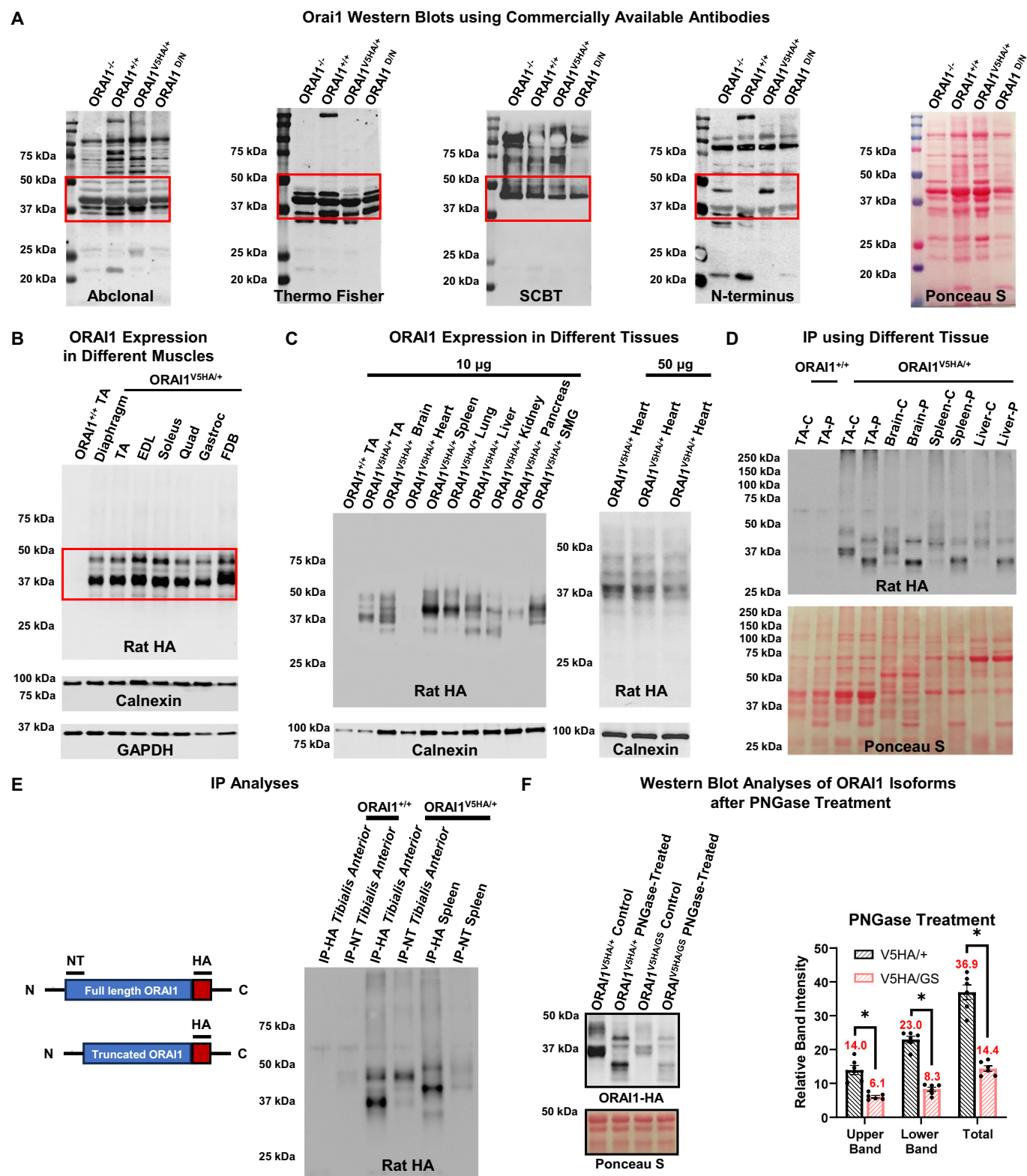


Figure EV4. Western blots analyses using commercially available ORAI1 and HA antibodies in various tissues.

(A) Representative western blots of muscle lysates from WT mice ($ORAI1^{+/+}$), constitutive muscle-specific ORAI1 KO mice ($ORAI1^{-/-}$), heterozygous V5HA/+ mice ($ORAI1^{V5HA/+}$), and muscle-specific dominant negative ORAI1 transgenic mice ($ORAI1^{DN}$) probed with commercially available ORAI1 antibodies from Abclonal, ThermoFisher, Santa Cruz Biotechnology (SCBT), and a rabbit polyclonal antibody raised against the first 20 amino acids of the ORAI1 N-terminus. The final gel (right) is the Ponceau S stained gel used for protein loading. No specific ORAI1 band at the expected molecular weight (and absent in the ORAI1 KO lane) was observed for any of these antibodies. (B) Representative western blot of endogenous ORAI1 expression probed with rat HA primary antibody across multiple different skeletal muscles (diaphragm, *tibialis anterior* (TA), EDL, SOL, *quadriceps* (Quad), *gastrocnemius* (Gastroc), and FDB) obtained from V5HA/+ mice. The first lane is a *tibialis anterior* (TA) muscle lysate from a WT ($Orai1^{+/+}$) mouse used as a negative control. Calnexin and GAPDH loading control blots are shown below the rat HA blot. Two different Orail-V5HA-tagged isoforms are observed in each muscle. (C) Representative western blot of endogenous ORAI1 expression probed with rat HA primary antibody across multiple different organs (*tibialis anterior* (TA), brain, heart, spleen, lung, liver, kidney, pancreas, and submandibular gland (SMG)) obtained from V5HA/+ mice. 10 μ g (left) or 50 μ g (right) lysate was loaded in each lane. Calnexin loading control blots are shown below the rat HA blots. A *tibialis anterior* muscle lysate from a control WT mouse ($Orai1^{+/+}$) was used as a negative control. Note: Low level ORAI1 protein expression in the heart is best observed when loading 50 μ g of lysate. Two distinct Orail-V5HA-tagged isoforms are observed, though at somewhat different levels of glycosylation. (D) Representative rat HA western blot (upper) of skeletal muscle (*tibialis anterior* (TA)), brain, spleen, and liver lysates from $Orai1^{V5HA/+}$ mice without (C) and after treatment with PNGase (P). A skeletal muscle (*tibialis anterior* (TA)) lysate from a control WT mouse ($ORAI1^{+/+}$) was used as a negative control. The lower blot shows protein loading using Ponceau S stain. (E) Schematic of full-length and N-terminal truncated V5-3xHA-tagged ORAI1 isoforms (left) and a representative western blot of *tibialis anterior* (TA) immunoprecipitation (IP) using either a rat HA antibody (left lane pairs) or the rabbit polyclonal antibody raised against the first 20 amino acids of the mouse ORAI1 N-terminus (right lane pairs) and then blotted with rat HA primary antibody. While both ORAI1 isoforms are readily pulled-down with the rat HA antibody, the larger full-length ORAI1 isoform is preferentially pulled-down by the N-terminal antibody that recognizes the full-length isoform. These results are consistent with the smaller isoform reflecting an alternative N-terminal translation initiation as reported previously (Desai et al, 2015; Fukushima et al, 2012). (F) Representative HA western blot (left) of *tibialis anterior* muscle in the absence (control) and after treatment with PNGase (PNGase-treated) of skeletal muscle (*tibialis anterior*) from either a control V5HA/+ mouse or a V5HA/GS mouse. The lower blot shows total protein loading using Ponceau S stain. Average ($\pm$ SEM) bar plot of relative HA-reactive upper, lower, and total (upper+lower) band intensity after PNGase treatment for muscle lysates from V5HA/+ (black, $n = 6$) and V5HA/GS mice (pink, $n = 6$) (right). Protein expression levels were normalized to Ponceau S. Mean relative band intensity for each condition is noted in red above each bar. Significance was calculated using 2-way ANOVA followed by Holm-Sidak's multiple comparisons post hoc test. * indicates adjusted $P < 0.05$ with all $P < 0.0001$.

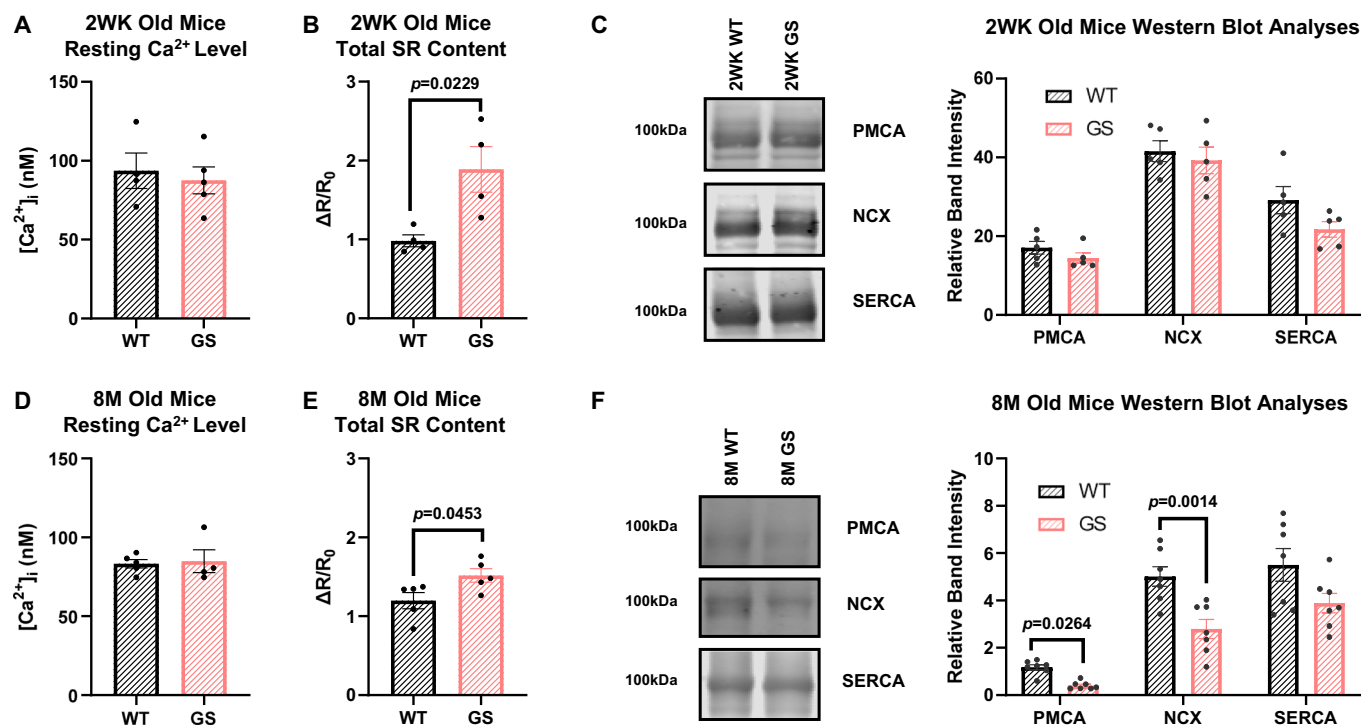


Figure EV5. GS mice show altered Ca^{2+} handling ability at both 2 WK and 8 M of age.

(A, D) No significant alterations in resting Ca^{2+} level were observed in FDB fibers from GS mice at both 2 WK and 8 M of age. For 2 WK old mice, $n = 4$ and 5 for WT and GS mice, respectively. For 8 M old mice, $n = 5$ and 4 for WT and GS mice, respectively. (B, E) SR Ca^{2+} storage was significantly increased in FDB fibers from GS mice at both 2 WK and 8 M of age. For 2 WK old mice, $n = 4$ WT mice and $n = 4$ GS mice. For 8 M old mice, $n = 5$ WT mice and $n = 5$ GS mice. Significance was calculated using Student's t test. The exact P value is labeled in the figure when $P < 0.05$. (C) Expression of PMCA, NCX and SERCA were not significantly altered in *tibialis anterior* muscle lysates from 2 WK old GS mice. Protein expression levels were normalized to Ponceau S. $n = 5$ for all experiment groups. (F) Expression of PMCA and NCX, but not SERCA, were significantly decreased in *tibialis anterior* muscle lysates from 8 M old GS mice. Protein expression levels were normalized to Ponceau S. $n = 5$ for all experiment groups. Significance was calculated using 2-way ANOVA followed by Holm-Sidak's multiple comparisons post hoc test. The exact P value is labeled in the figure when adjusted $P < 0.05$.