ORAI1 inhibition as an efficient preclinical therapy for tubular aggregate myopathy and Stormorken syndrome

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SUPPLEMENTAL MATERIAL

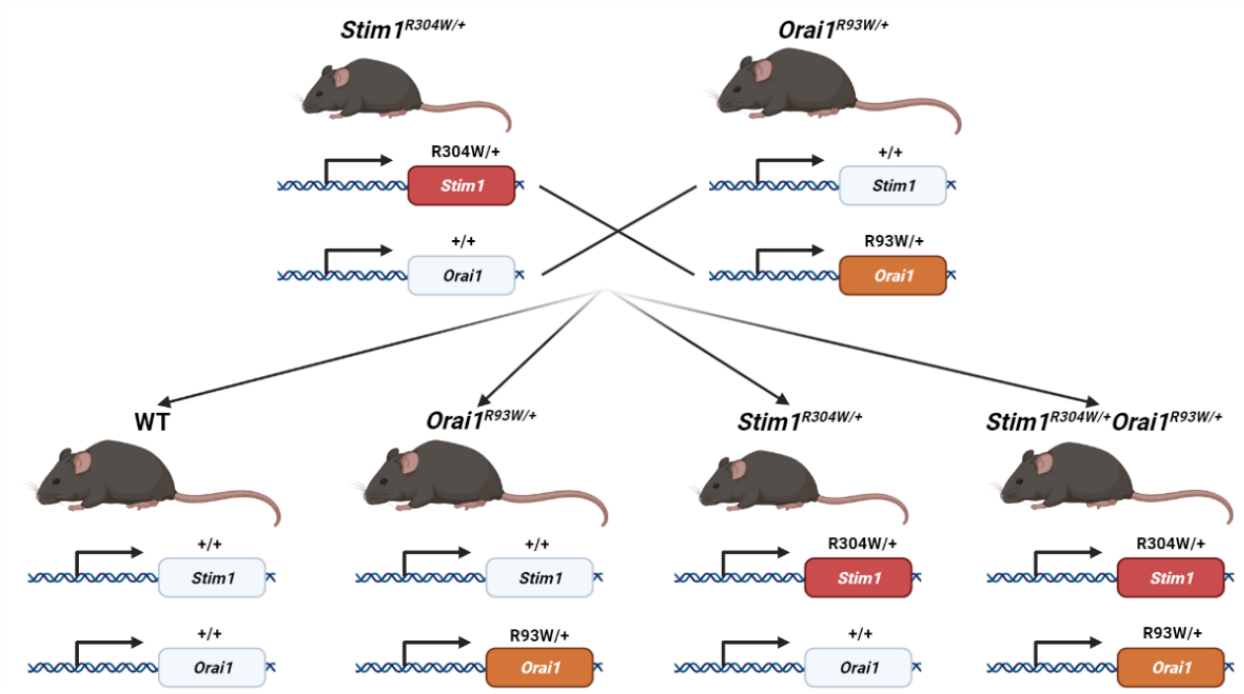

Figure S1. Crossing scheme. (A) Crossing of *Stim1*$^{R304W/+}$ and *Orai1*$^{R93W/+}$ mice resulted in offspring with either of the four equiprobable genotypes: WT, *Orai1*$^{R93W/+}$, *Stim1*$^{R304W/+}$, and *Stim1*$^{R304W/+}$*Orai1*$^{R93/W+}$.

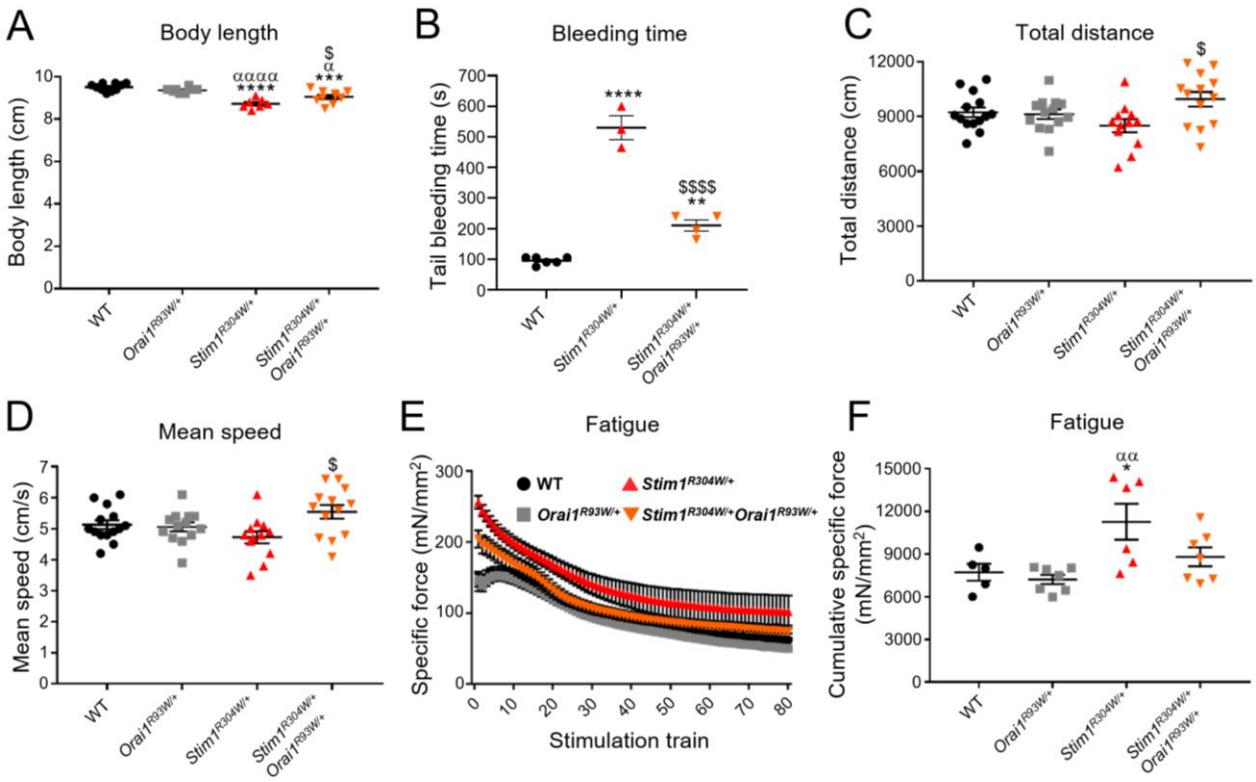

Figure S2. Improved body length, bleeding times, activity, and fatigue profiles of *Stim1$^{R304W/+}$Orai1$^{R93W/+}$* mice. (A) At 4 months, *Stim1$^{R304W/+}$* mice were smaller than WT and *Orai1$^{R93W/+}$* littermates, and body length was rescued in *Stim1$^{R304W/+}$Orai1$^{R93W/+}$* mice (n=7-10, one-way ANOVA and Tukey's post hoc test). (B) Tail bleeding assays revealed excessive bleeding times in *Stim1$^{R304W/+}$* mice and significantly improved coagulation in *Stim1$^{R304W/+}$Orai1$^{R93W/+}$* littermates at 4 months (n=3-6, one-way ANOVA and Tukey's post hoc test). (C-D) Open field activity tended to be reduced in *Stim1$^{R304W/+}$* mice as illustrated by the non-significant decrease of covered distance and mean speed at 10 weeks of age. Both parameters were improved in *Stim1$^{R304W/+}$Orai1$^{R93W/+}$* mice (n=11-14, one-way ANOVA and Tukey's post hoc test). (E-F) At 4 months, *Stim1$^{R304W/+}$* mice displayed abnormal fatigue curves associated with higher levels of accumulative force compared with WT and *Orai1$^{R93W/+}$* controls, and both shifted towards normal values in *Stim1$^{R304W/+}$Orai1$^{R93W/+}$* mice (n=5-7, one-way ANOVA and Tukey's post hoc test). Graphs represent mean ± SEM. Significant differences are indicated as */α/$ P<0.05, **/αα/$$ P<0.01, ***/ααα/$$$ P<0.001, and

****/αααα/$$$$ P<0.0001 with * reflecting the comparison of *Stim1*$^{R304W/+}$ with the WT group, α the comparison with the *Orai1*$^{R93W/+}$ group, and $ the comparison with the *Stim1*$^{R304W/+}$*Orai1*$^{R93W/+}$ group.

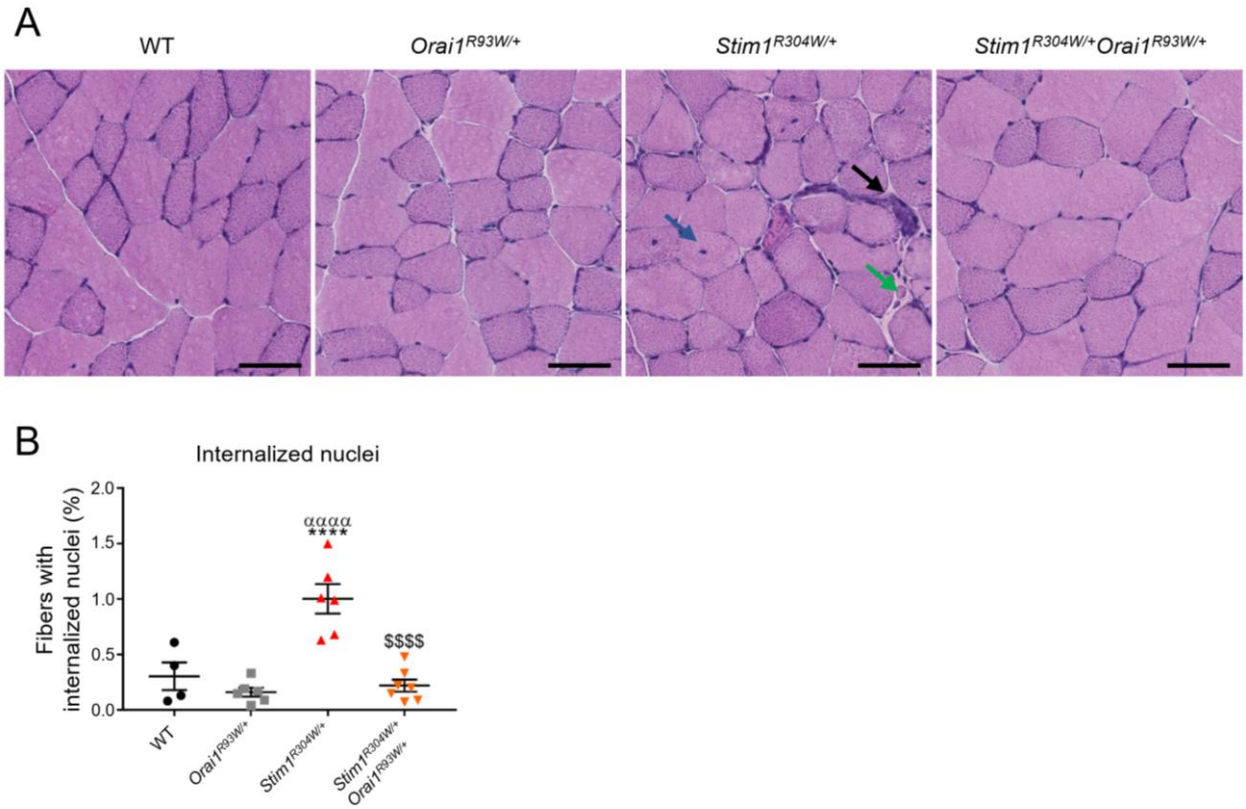

Figure S3. Resolved myofiber degeneration in *Stim1*$^{R304W/+}$*Orai1*$^{R93W/+}$ gastrocnemius. (A) Representative H&E pictures of gastrocnemius sections at 4 months showing internalized nuclei (blue arrow), regenerating fibers (green arrow) and infiltration of immune cells (black arrow) in *Stim1*$^{R304W/+}$ mice, and a complete absence of the histopathological hallmarks of muscle fiber degeneration in *Stim1*$^{R304W/+}$*Orai1*$^{R93W/+}$ sections. Scale bar = 50 µm (n=5-7). (B) Fibers with internalized nuclei are increased in *Stim1*$^{R304W/+}$ gastrocnemius and normalized in *Stim1*$^{R304W/+}$*Orai1*$^{R93W/+}$ muscle (n=4-6, one-way ANOVA and Tukey's post hoc test). The graph represents mean ± SEM. Significant differences are indicated as ****/αααα/$$$$ P<0.0001 with * reflecting the comparison of *Stim1*$^{R304W/+}$ with the WT group, α the comparison with the *Orai1*$^{R93W/+}$ group, and $ the comparison with the *Stim1*$^{R304W/+}$*Orai1*$^{R93W/+}$ group.

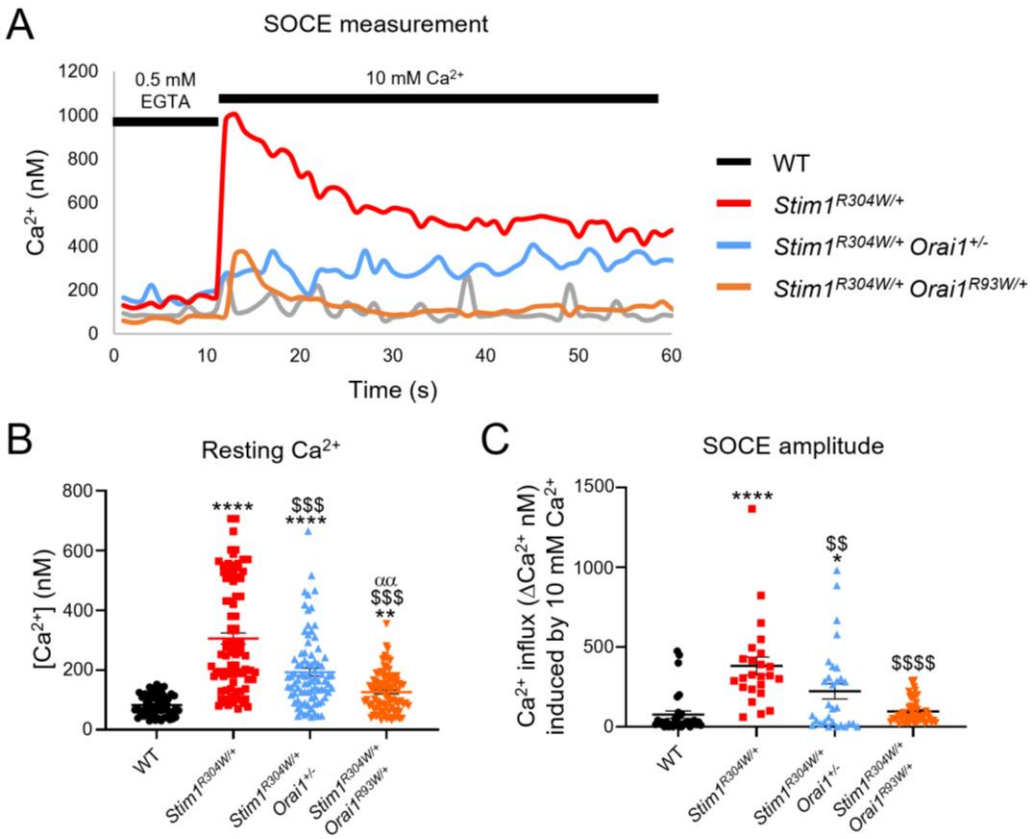

Figure S4. Comparison of resting Ca^{2+} levels and SOCE amplitude in WT, *Stim1*$^{R304W/+}$, *Stim1*$^{R304W/+}$*Orai1*$^{+/-}$, and *Stim1*$^{R304W/+}$*Orai1*$^{R93W/+}$ myotubes. (A) Representative traces showing major extracellular Ca^{2+} entry in *Stim1*$^{R304W/+}$ myotubes upon addition of 10 mM Ca^{2+} to the medium compared with the WT, and significantly less Ca^{2+} influx in *Stim1*$^{R304W/+}$*Orai1*$^{+/-}$ and *Stim1*$^{R304W/+}$*Orai1*$^{R93W/+}$ myotubes (n=24-55 cells). (B-C) Quantification revealed increased resting Ca^{2+} levels and SOCE amplitude in *Stim1*$^{R304W/+}$ myotubes compared with the WT. Both parameters were moderately improved in *Stim1*$^{R304W/+}$*Orai1*$^{+/-}$ myotubes and strongly improved in *Stim1*$^{R304W/+}$*Orai1*$^{R93W/+}$ myotubes (resting Ca^{2+} n=58-89 and SOCE amplitude n=24-55, Kruskal-Wallis and Dunn's multiple comparison test). Graphs represent mean ± SEM. Significant differences are indicated as **/αα/$$ P<0.01, ***/ααα/$$$ P<0.001, and ****/αααα/$$$$ P<0.0001 with * reflecting the comparison with the WT group, α the comparison with the *Stim1*$^{R304W/+}$*Orai1*$^{+/-}$ group, and $ the comparison with the *Stim1*$^{R304W/+}$ group.

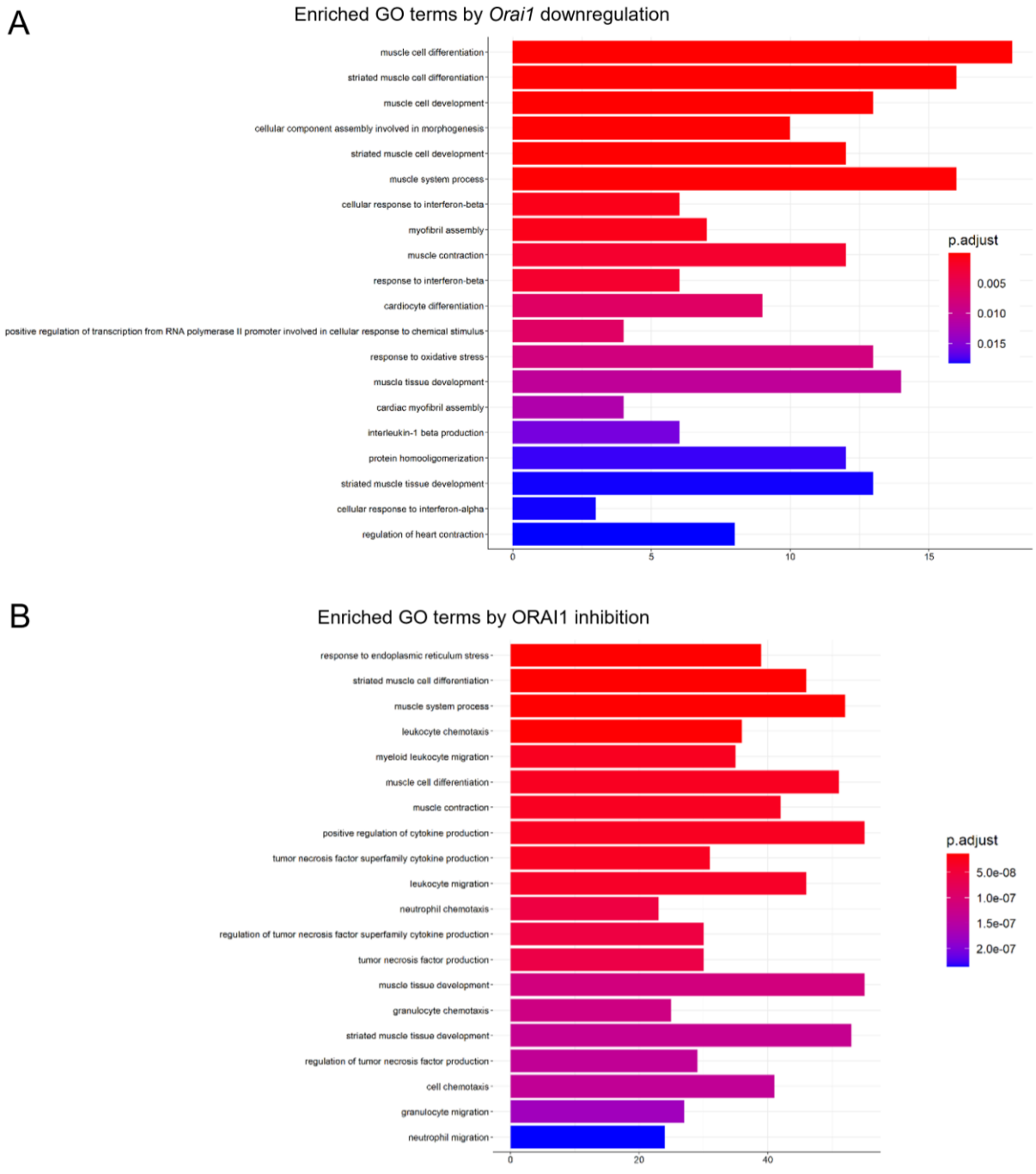

Figure S5. Comparison of RNA-seq-derived enriched GO terms between ORAI1 inhibition and *Orai1* downregulation. (A-B) GO term enrichment analysis of genes with partially or completely normalized expression revealed terms associated with muscle contraction and differentiation in both *Stim1*$^{R304W/+}$*Orai1*$^{+/-}$ and *Stim1*$^{R304W/+}$*Orai1*$^{R93W/+}$ mice. Only *Stim1*$^{R304W/+}$*Orai1*$^{R93W/+}$ muscle samples showed an improved expression of genes implicated in immune systems processes and in the response to ER/SR stress (n=4).

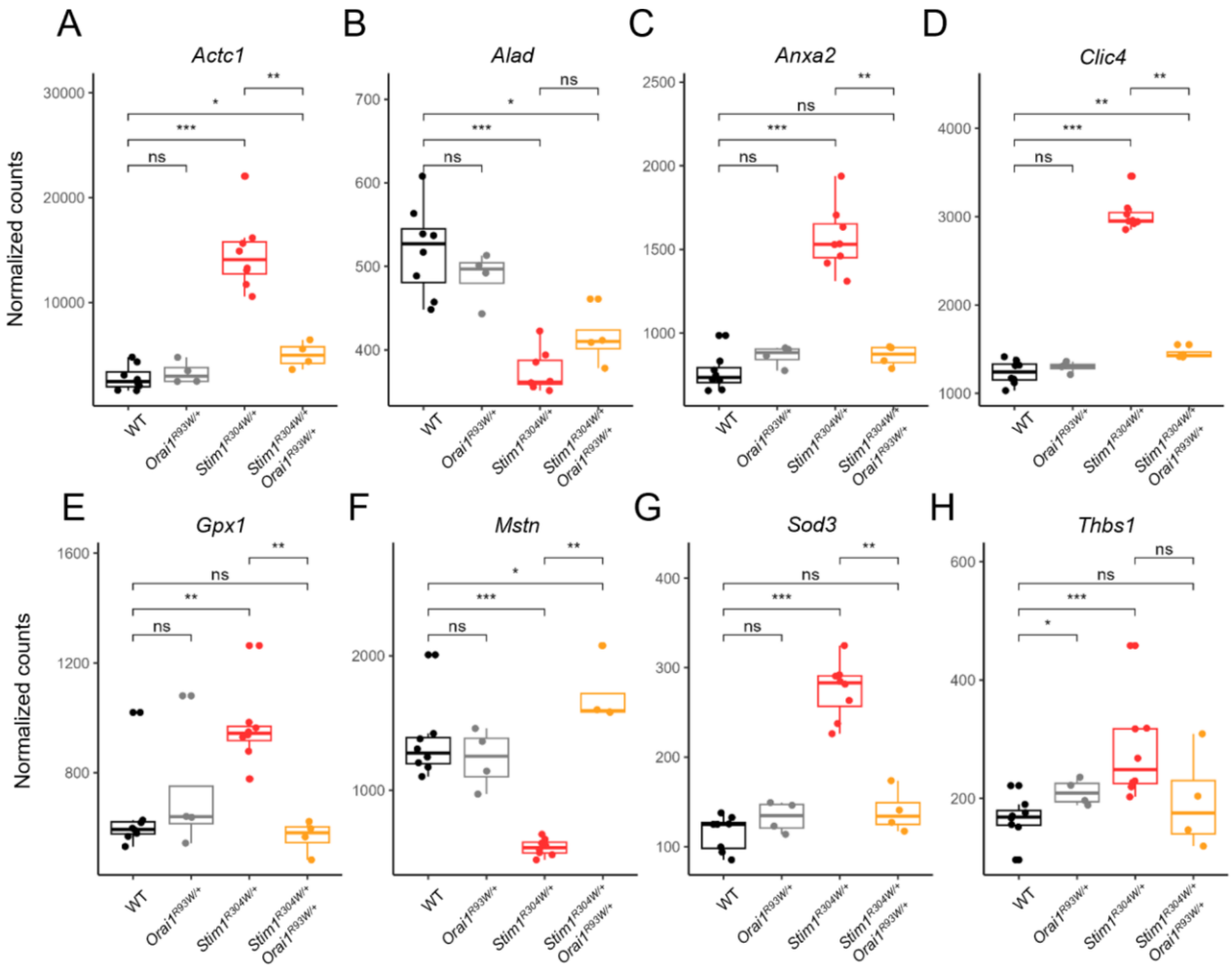

Figure S6. Selection of *Mstn* as potential biomarker for TAM/STRMK. (A-H) The known circulating biomarkers *Actc1*, *Alad*, *Anxa2*, *Clic4*, *Gpx1*, *Mstn*, *Sod3*, and *Thbs1* were differentially expressed in *Stim1*$^{R304W/+}$ mice compared with the WT, and all were partially or fully normalized in *Stim1*$^{R304W/+}$*Orai1*$^{R93W/+}$ muscle (n=3-5, one-way ANOVA and Tukey's post hoc test). Graphs represent mean ± SEM. Significant differences are indicated as */α/$ P<0.05, **/αα/$$ P<0.01, and ***/ααα/$$$ P<0.001.

Gene	Forward primer	Reverse primer
Rpl27	AAGCCGTCATCGTGAAGAACA	CTTGATCTTGGATCGCTTGGC
Hspa5	CTATTCCTGCGTCGGTGTGT	ATTCCAAGTGCGTCCGATGA
Hspb90b1	CCACTCAAATCGAACACGGC	AGATTCCGCCTCCTTTCTGC
Spliced Xbp1	GCTGAGTCCGCAGCAGGT	CAGGCTCCAACTTGTCCAGAAT
Unspliced Xbp1	CAGACTATGTGCACCTCTGC	CAGGCTCCAACTTGTCCAGAAT

Supplemental Table S1. List of RT-qPCR primers

	BV/TV (%)	Tb.Th (μm)	Tb.N (1/mm)	Tb.Sp (μm)
WT	5.48 ± 1.03	66.08 ± 3.70	0.80 ± 0.13	455.13 ± 29.63
$Orai1^{R93W/+}$	3.44 ± 0.44	63.39 ± 2.20	0.54 ± 0.06	538.69 ± 37.32
$Stim1^{R304W/+}$	0.40 ± 0.10	49.66 ± 2.25	0.08 ± 0.02	770.95 ± 11.96
$Stim1^{R304W/+}Orai1^{R93W/+}$	3.55 ± 0.78	66.95 ± 2.99	0.51 ± 0.10	555.80 ± 43.69
p value $Stim1^{R304W/+}Orai1^{R93W/+}$ vs $Stim1^{R304W/+}$	0.0257	0.0012	0.0564	0.0393
p value WT control vs $Stim1^{R304W/+}Orai1^{R93W/+}$	>0.999	0.9962	0.6968	0.9156
p value $Orai1^{R93W/+}$ control vs $Stim1^{R304W/+}Orai1^{R93W/+}$	>0.999	0.8089	>0.999	>0.999

Supplemental Table S2. Trabecular bone parameters of the femur

BV/TV, bone volume fraction; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation. P values refer to the comparison of $Stim1^{R304W/+}$ and $Stim1^{R304W/+}Orai1^{+/-}$ by Tukey's post hoc test one-way ANOVA of all groups (n=6-7).