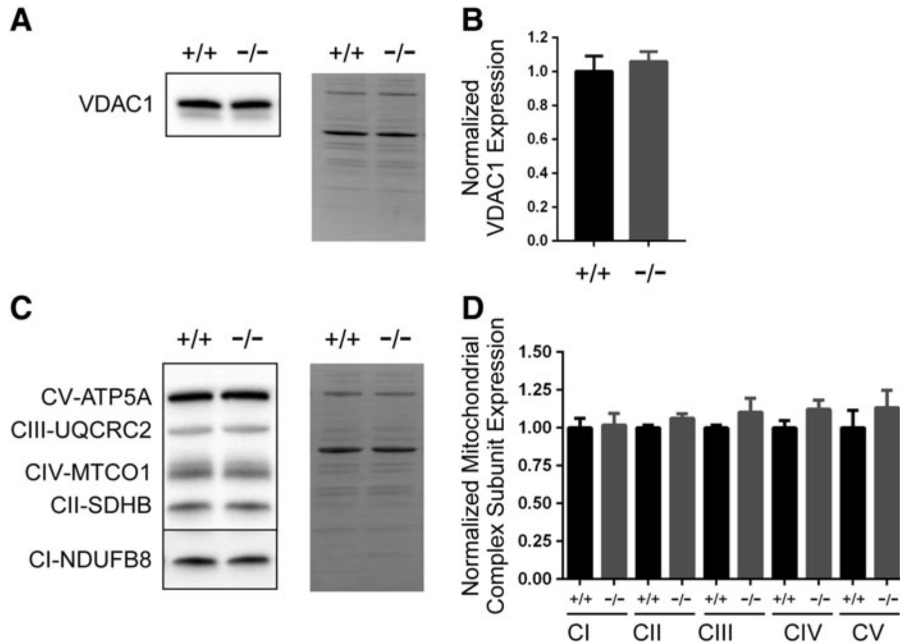

SUPPLEMENTARY FIG. S4. Expression of SERCA1, iNOS, eNOS, and phosho-eNOS in GSNOR null skeletal muscles. Thirty micrograms of skeletal muscle protein was loaded per lane, with proteins visualized on PVDF membrane using the Stain-Free™ system (Biorad). **(A)** The *top panel* is a representative Western blot showing SERCA1 expression in wild-type (+/+) and GSNOR null (–/–) tibialis anterior muscles. The *bottom panel* shows total protein loading for the Western blot. Densitometry of Western blots reveals that SERCA1 protein expression is unaffected by the absence of GSNOR ($n = 5$). These data suggest that the enhanced contractility of GSNOR null TA muscles is independent of changes in SERCA1 Ca^{2+} pump expression. **(B)** The *top panel* is a representative Western blot showing that iNOS protein expression is beyond the limits of detection in wild-type (+/+) and GSNOR null (–/–) tibialis anterior muscles. Note that mdx muscles were used as a positive control for the iNOS antibody (not shown). The *bottom panel* shows total protein loading for the Western blot. **(C)** The *top panel* is a representative Western blot showing eNOS expression in wild-type (+/+) and GSNOR null (–/–) tibialis anterior muscles. Lung tissue is used as a positive control for the eNOS antibody. The *bottom panel* shows total protein loading for the Western blot. Densitometry of Western blots reveals that eNOS protein expression is similar between wild-type and GSNOR null muscles ($n = 5$). **(D)** The *top panel* is a representative Western blot showing that serine 1177 phosphorylated eNOS is beyond the limits of detection in wild-type (+/+) and GSNOR null (–/–) tibialis anterior muscles. phospho-eNOS^{ser1177} is a marker of active eNOS. The *bottom panel* shows total protein loading for the Western blot. The data in **(B–D)** suggest that RyR1 hypernitrosylation is not driven by iNOS or eNOS in GSNOR null TA muscles.