Supplementary Information

Mitsugumin 53 regulates extracellular Ca^{2+} entry and intracellular Ca^{2+} release via Orai1 and RyR1 in skeletal muscle

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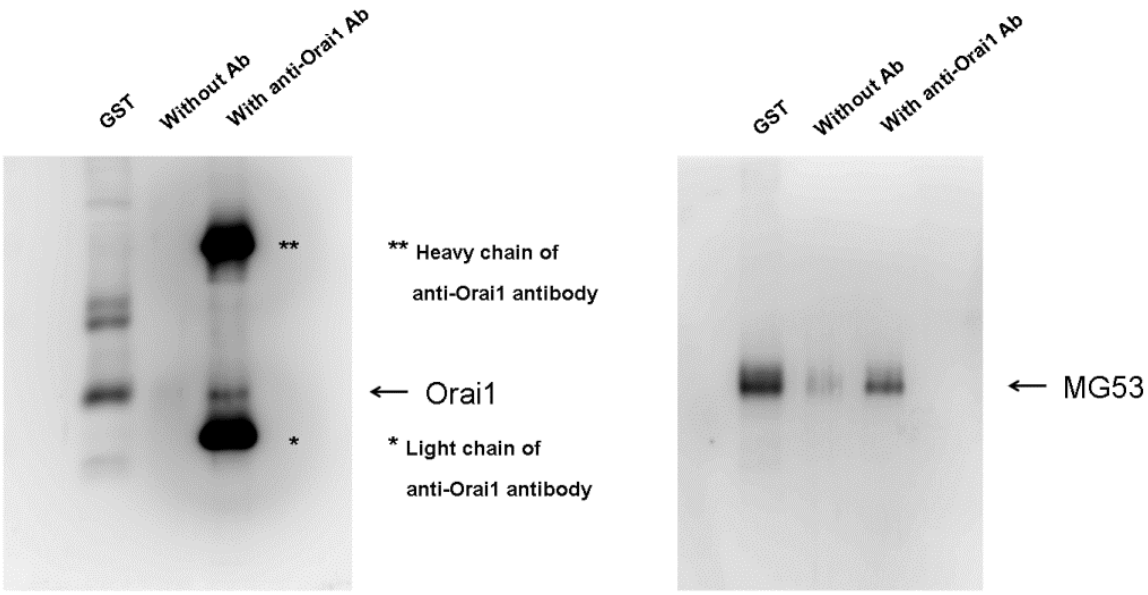
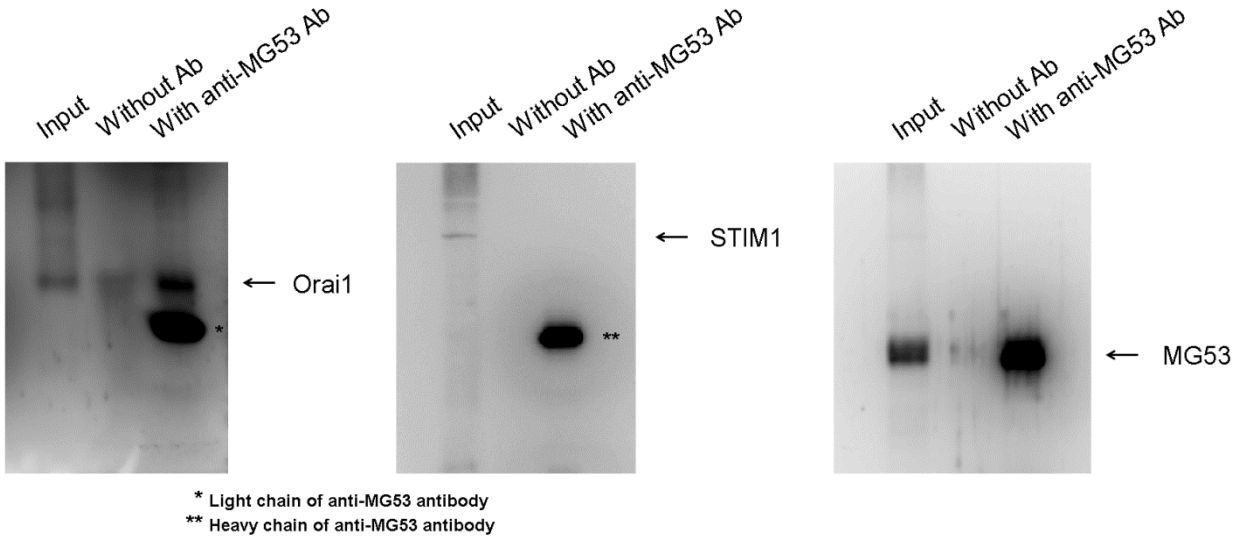
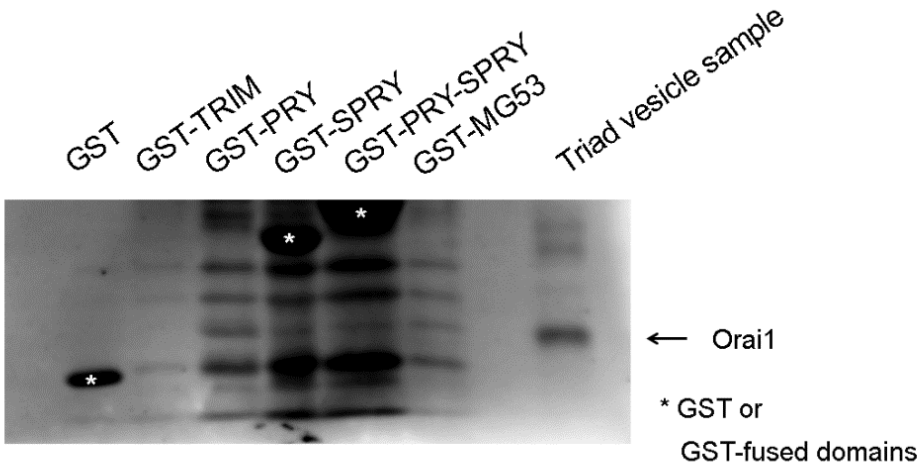
Supplemental Figure 1. The full-length blots for Orai1 and MG53 in Figure 1b (upper panel).



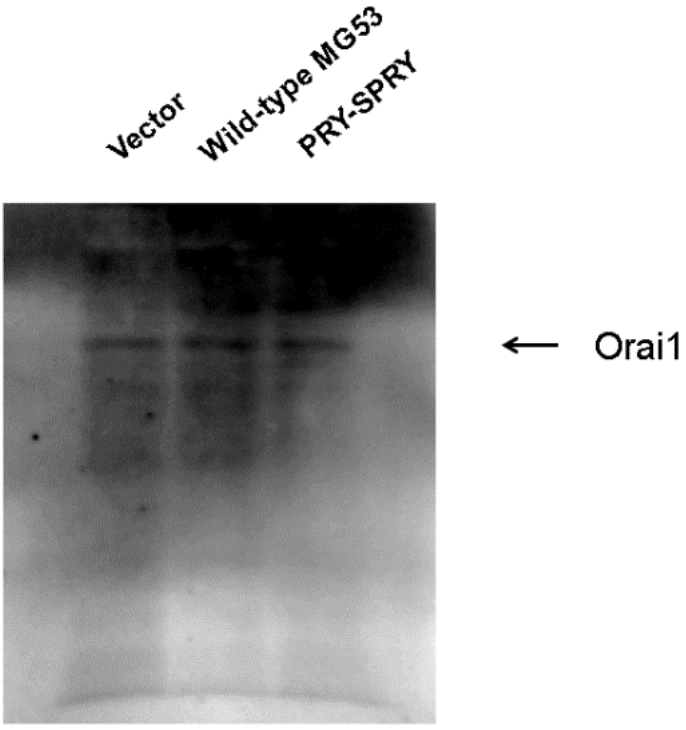
Supplemental Figure 2. The full-length blots for Orai1 and MG53 in Figure 1b (lower panel).



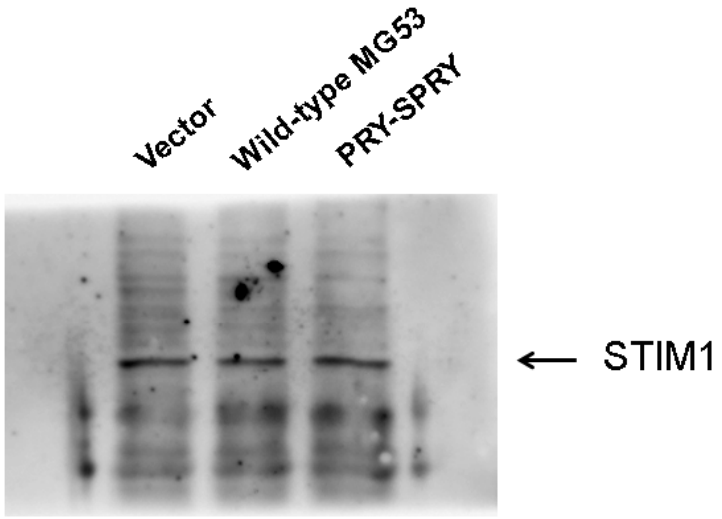
Supplemental Figure 3. The full-length blot for Orai1 in Figure 2c.



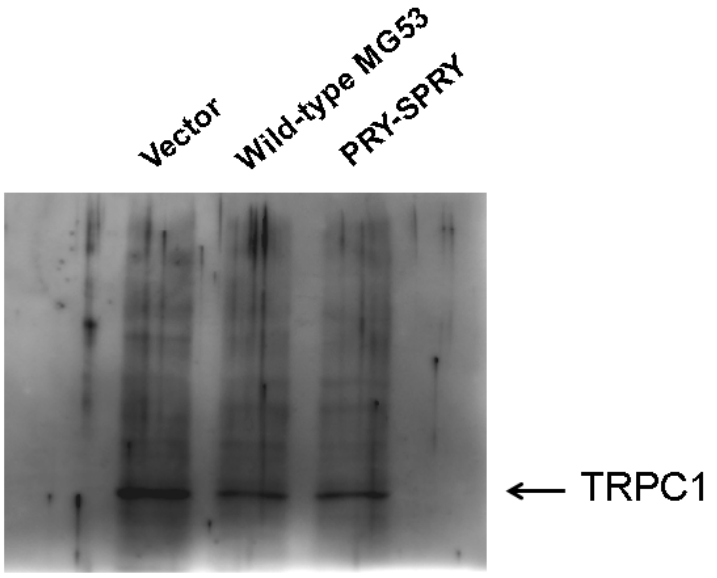
Supplemental Figure 4. The full-length blot for Orai1 in Figure 5a.



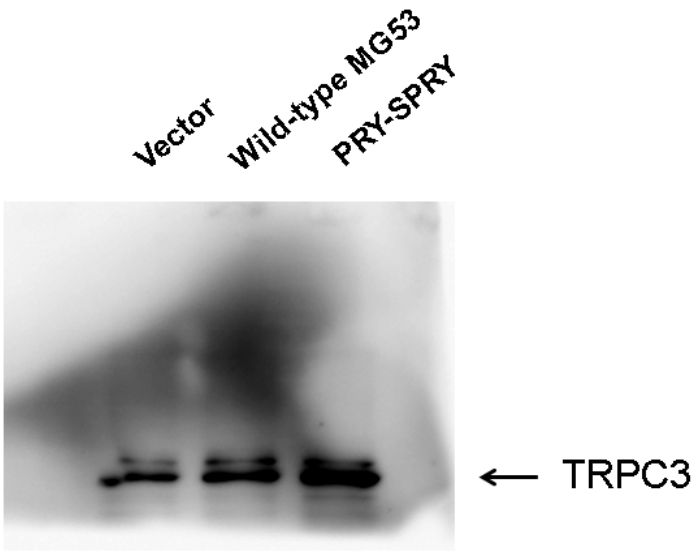
Supplemental Figure 5. The full-length blot for STIM1 in Figure 5a.



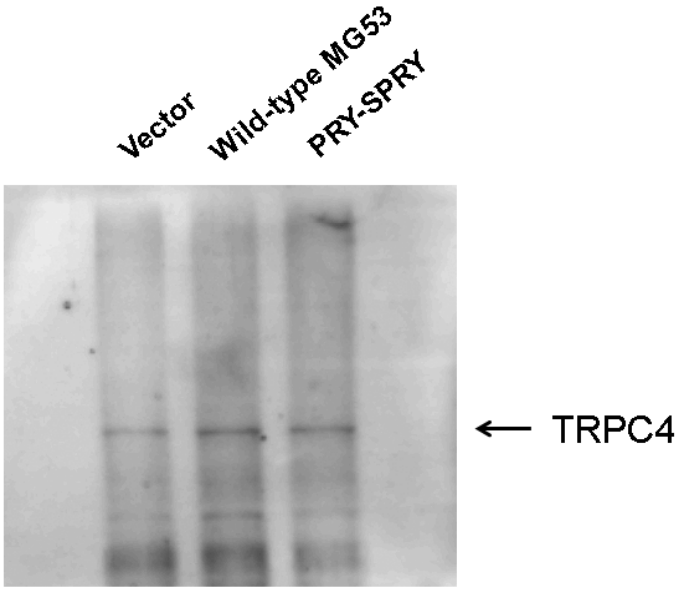
Supplemental Figure 6. The full-length blot for TRPC1 in Figure 5a.



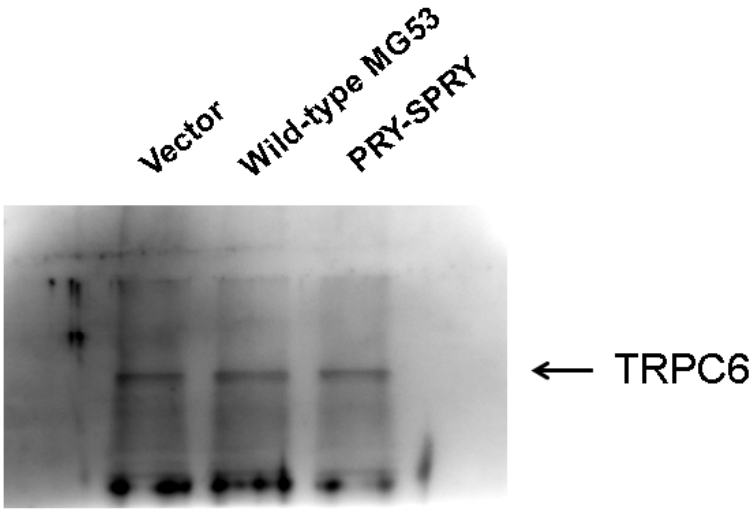
Supplemental Figure 7. The full-length blot for TRPC3 in Figure 5a.



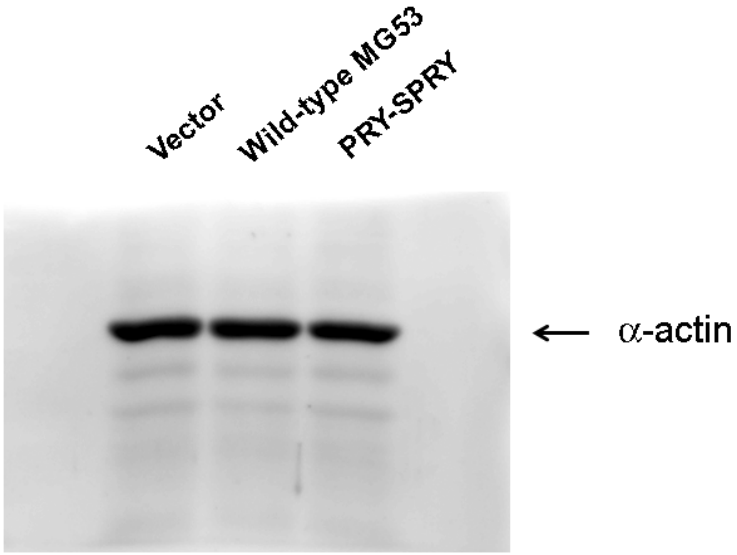
Supplemental Figure 8. The full-length blot for TRPC4 in Figure 5a.



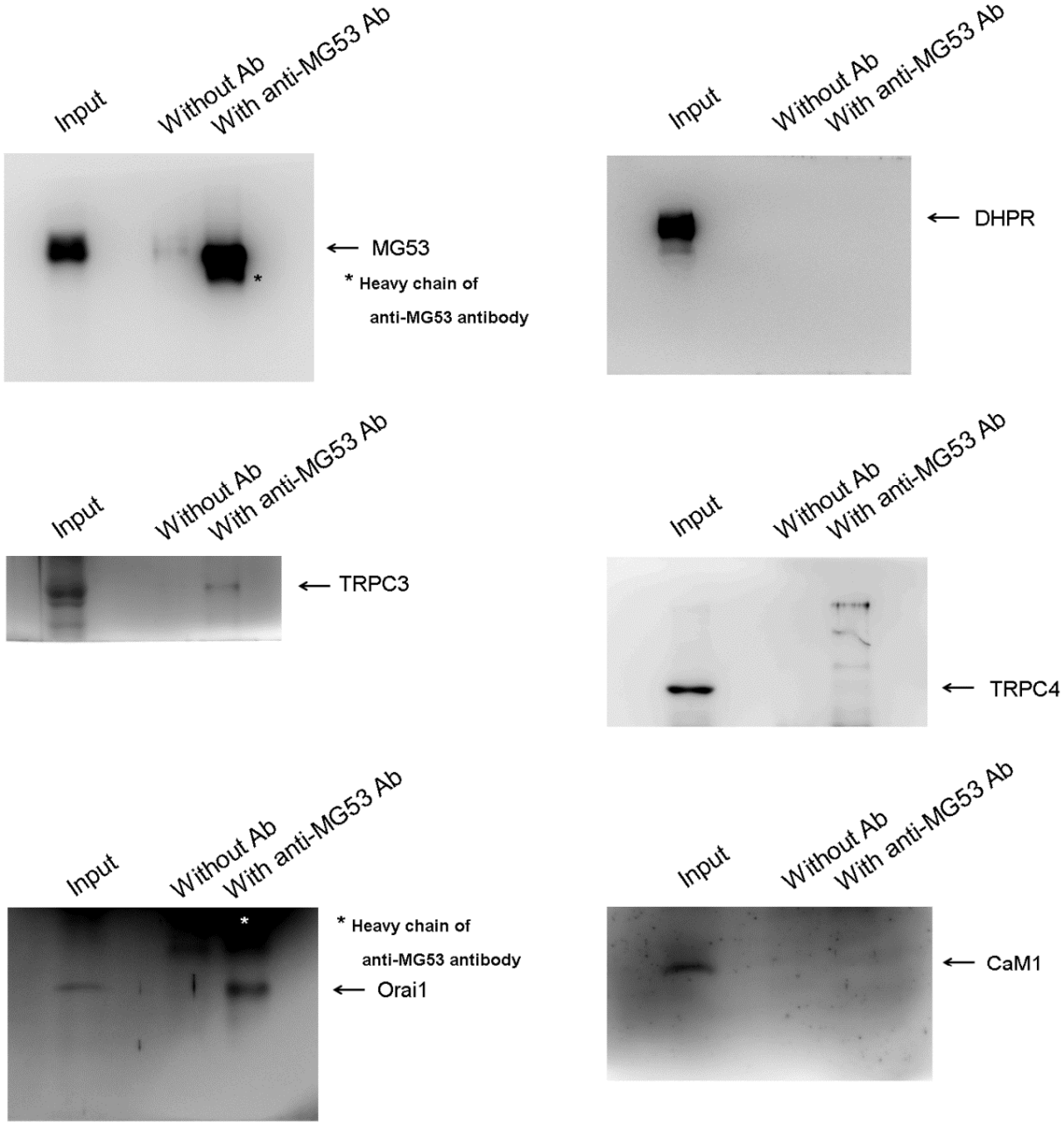
Supplemental Figure 9. The full-length blot for TRPC6 in Figure 5a.



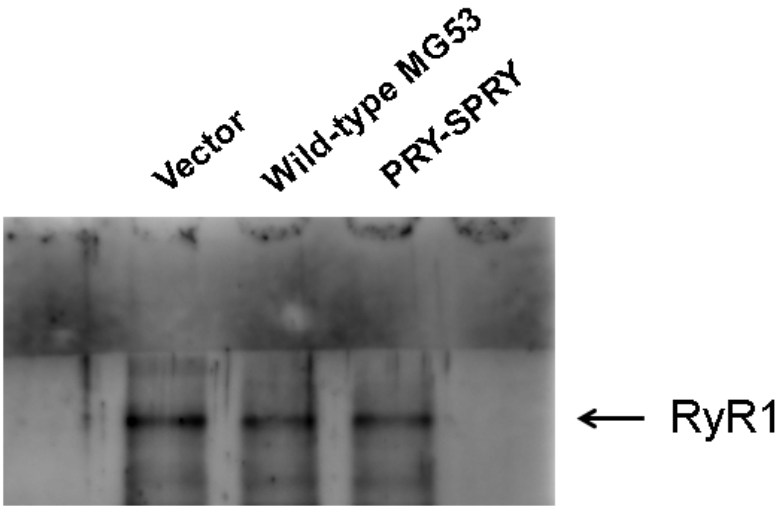
Supplemental Figure 10. The full-length blot for α-actin in Figure 5a.



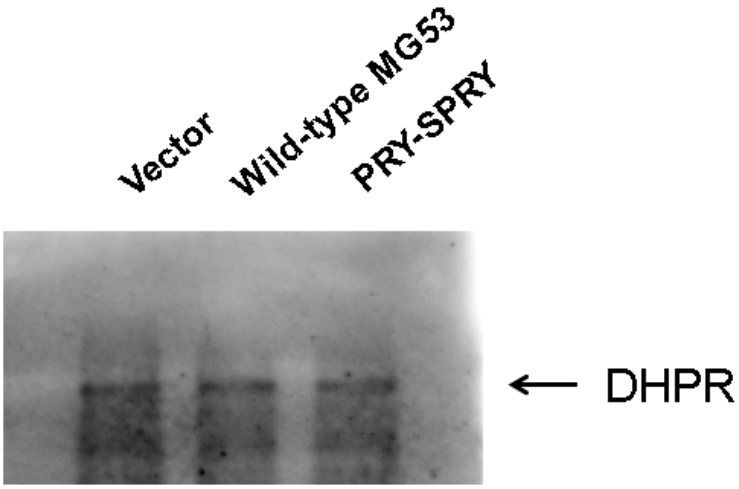
Supplemental Figure 11. The full-length blots in Figure 5c.



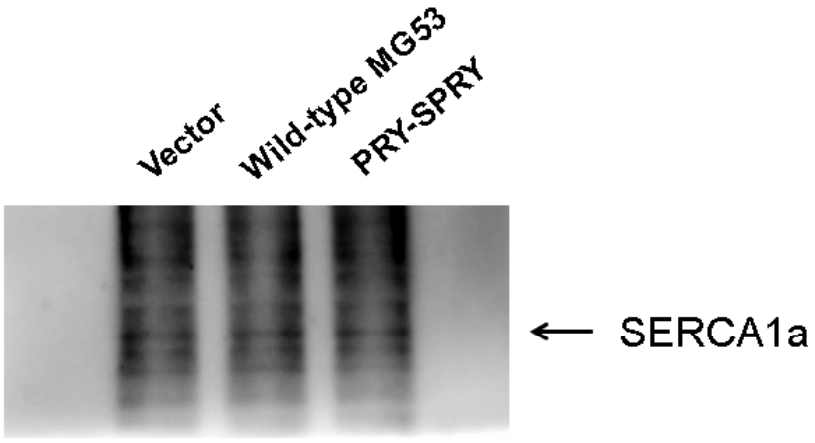
Supplemental Figure 12. The full-length blot for RyR1 in Figure 7a.



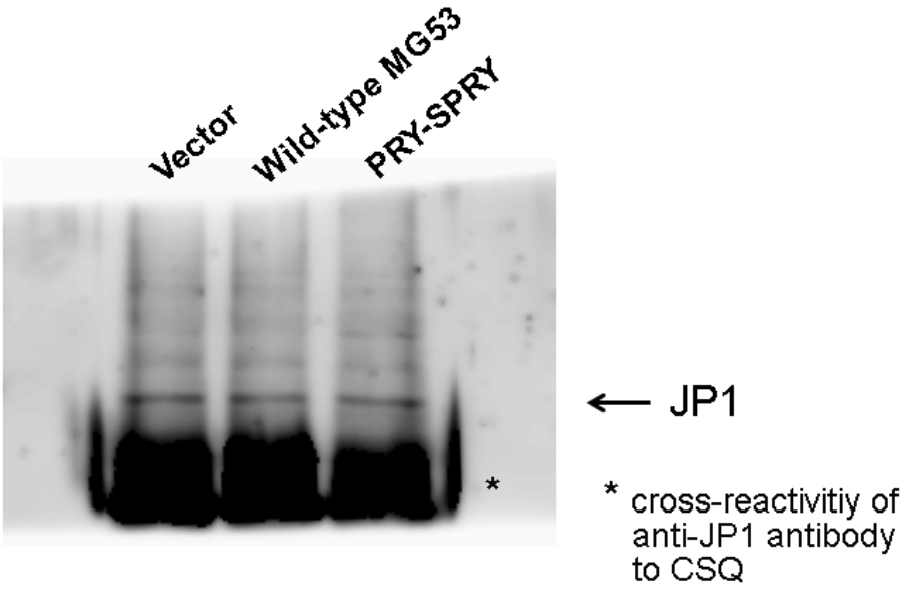
Supplemental Figure 13. The full-length blot for DHPR in Figure 7a.



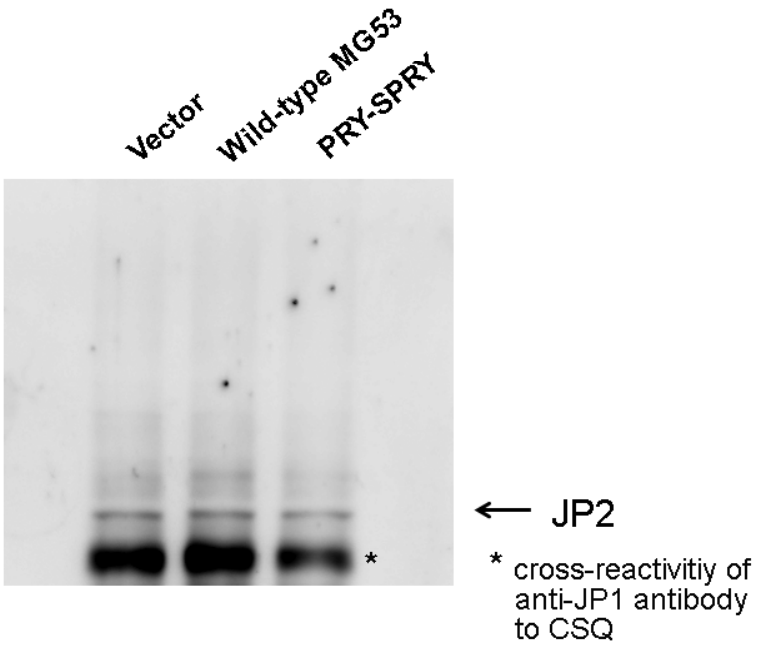
Supplemental Figure 14. The full-length blot for SERCA1a in Figure 7a.



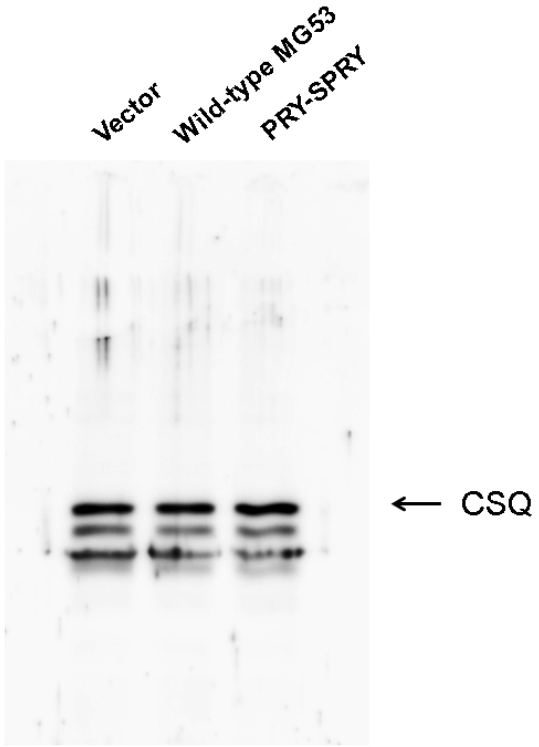
Supplemental Figure 15. The full-length blot for JP1 in Figure 7a.



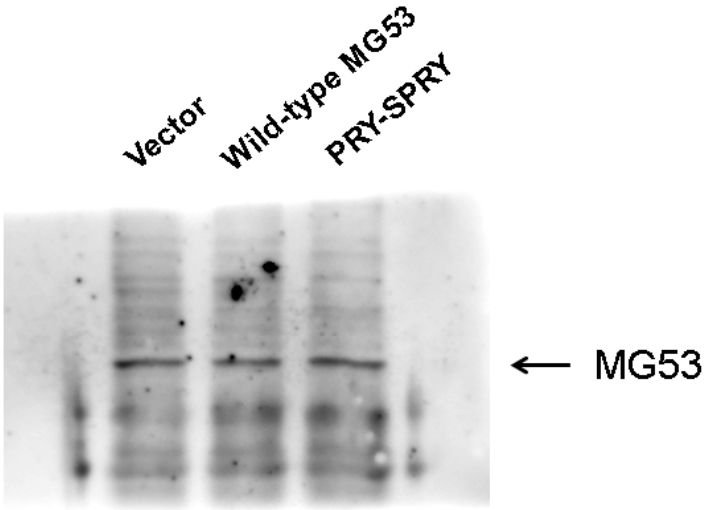
Supplemental Figure 16. The full-length blot for JP2 in Figure 7a.



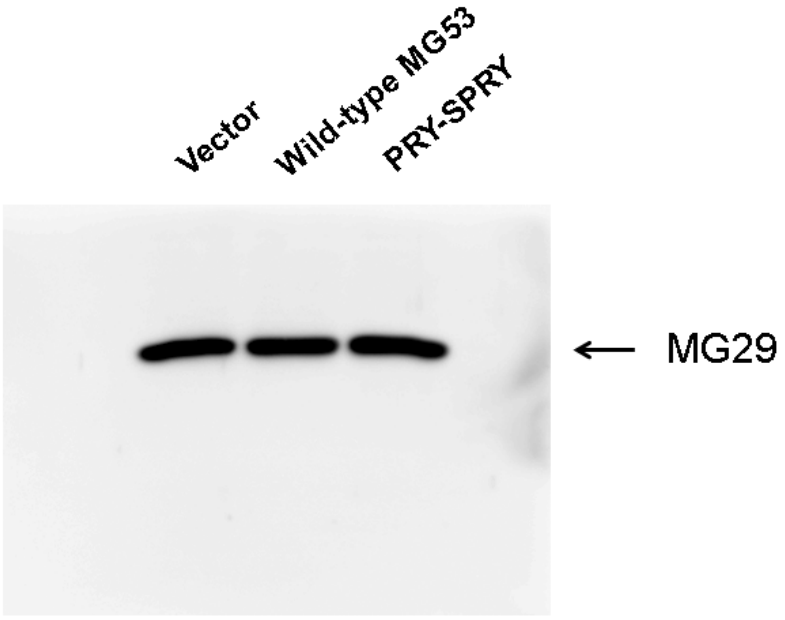
Supplemental Figure 17. The full-length blot for CSQ in Figure 7a.



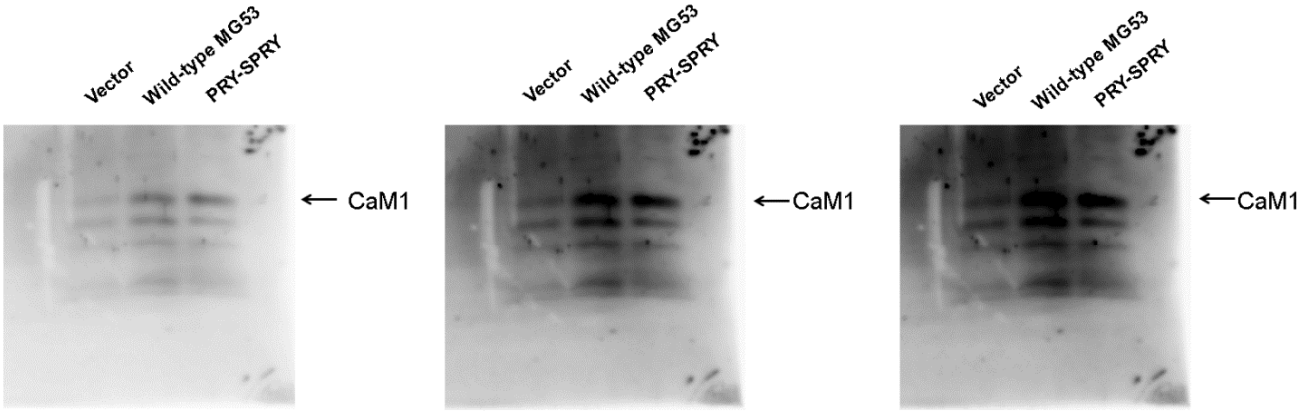
Supplemental Figure 18. The full-length blot for MG53 in Figure 7a.



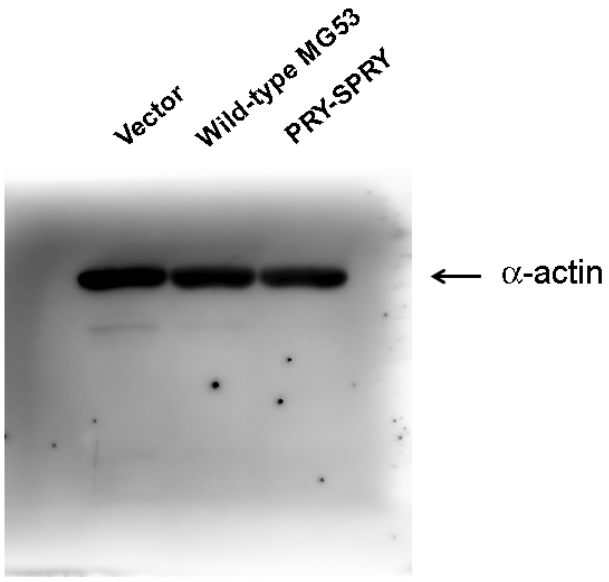
Supplemental Figure 19. The full-length blot for MG29 in Figure 7a.



Supplemental Figure 20. The full-length blots for CaM1 in Figure 7a. Blots were obtained from three different periods of exposure time.



Supplemental Figure 21. The full-length blot for α-actin in Figure 7a.



Supplemental Figure 22. Mn^{2+} quenching assay.

A Mn^{2+} quenching assay measured the unidirectional SOCE from intracellular Ca^{2+} release and Ca^{2+} extrusion[1-3]. Mn^{2+} permeates into cells via the SOCE mechanism while it is impervious to both the surface membrane extrusion processes and E/SR uptake by Ca^{2+} pumps due to its very high affinity with intracellular fura-2. Mn^{2+} with a Ca^{2+}-chelator such as BAPTA is added to the bath solutions (i.e., extracellular space) of cells loaded with fura-2. In this condition, extracellular Ca^{2+} is chelated by BAPTA, and the depletion of E/SR Ca^{2+} induces the entry of extracellular Mn^{2+} into the cells instead of Ca^{2+}. As a result, the quenching of intracellular fura-2 fluorescence occurs with the entry of Mn^{2+}. The quenching of intracellular fura-2 fluorescence by Mn^{2+} reflects the Ca^{2+} entry via the SOCE mechanism. The decreased rate of intracellular fura-2 fluorescence intensity by Mn^{2+}-quenching is measured. A steeper slope indicates a more active SOCE, while a shallower slope means a less active SOCE. The decrease rate (i.e., the rate of Mn^{2+} influx) is determined from the variable (i.e., slope) of a linear equation obtained from the linear fitting of the traces for the initial 10 seconds (i.e., the initial slope rather than the slower component from the late stages of the traces).

[1] Pan, Z., Zhao, X. & Brotto, M. Fluorescence-based measurement of store-operated calcium entry in live cells: from cultured cancer cell to skeletal muscle fiber. *Journal of visualized experiments : JoVE*, **60**, pii: 3415. doi:10.3791/3415 (2012).

[2] Shin, D. W. *et al.* A retrograde signal from calsequestrin for the regulation of store-operated Ca^{2+} entry in skeletal muscle. *J Biol Chem* **278**, 3286-3292, doi:10.1074/jbc.M209045200 (2003).

[3] Pan, Z. *et al.* Dysfunction of store-operated calcium channel in muscle cells lacking mg29. *Nat Cell Biol* **4**, 379-383, doi:10.1038/ncb788 (2002).