SUPPLEMENTARY MATERIAL

Loss of Calreticulin Uncovers a Critical Role for Calcium in Regulating Cellular Lipid Homeostasis

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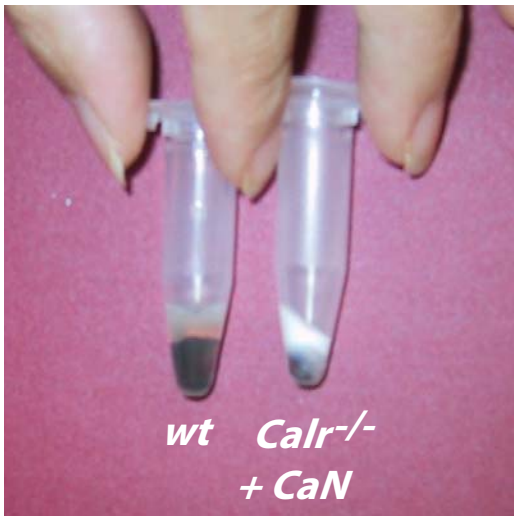
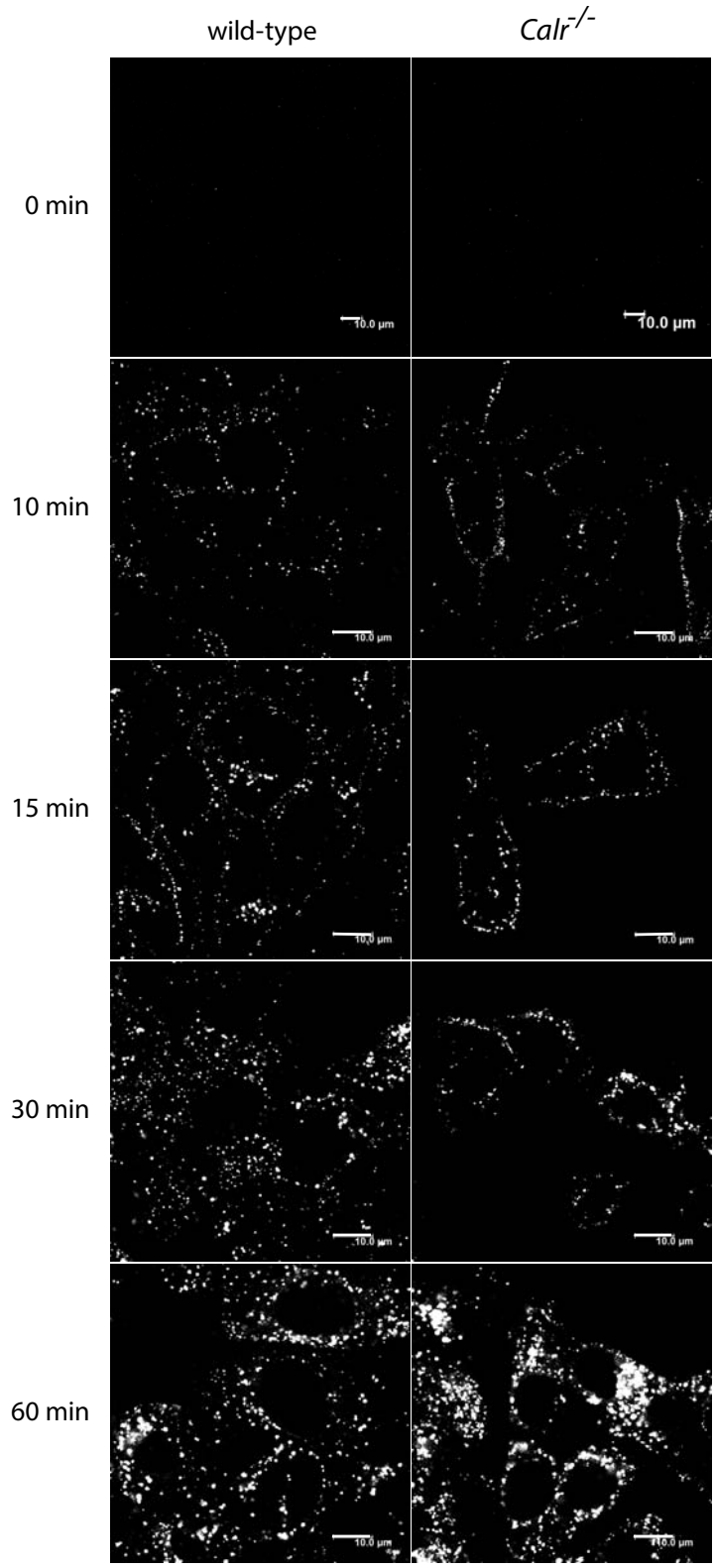
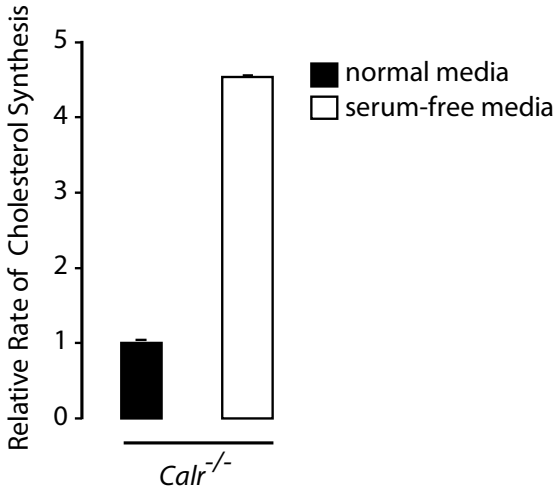


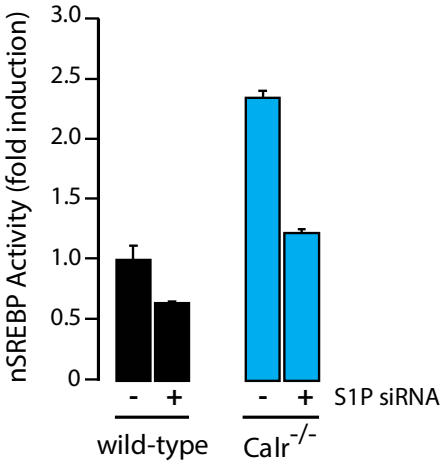
Supplementary Figure S1. Serum from wild-type (*wt*) and calreticulin-deficient (*Calr*$^{-/-}$) mouse rescued by cardiac-specific expression of the activated calcineurin (*Calr*$^{-/-}$+CaN)[12].



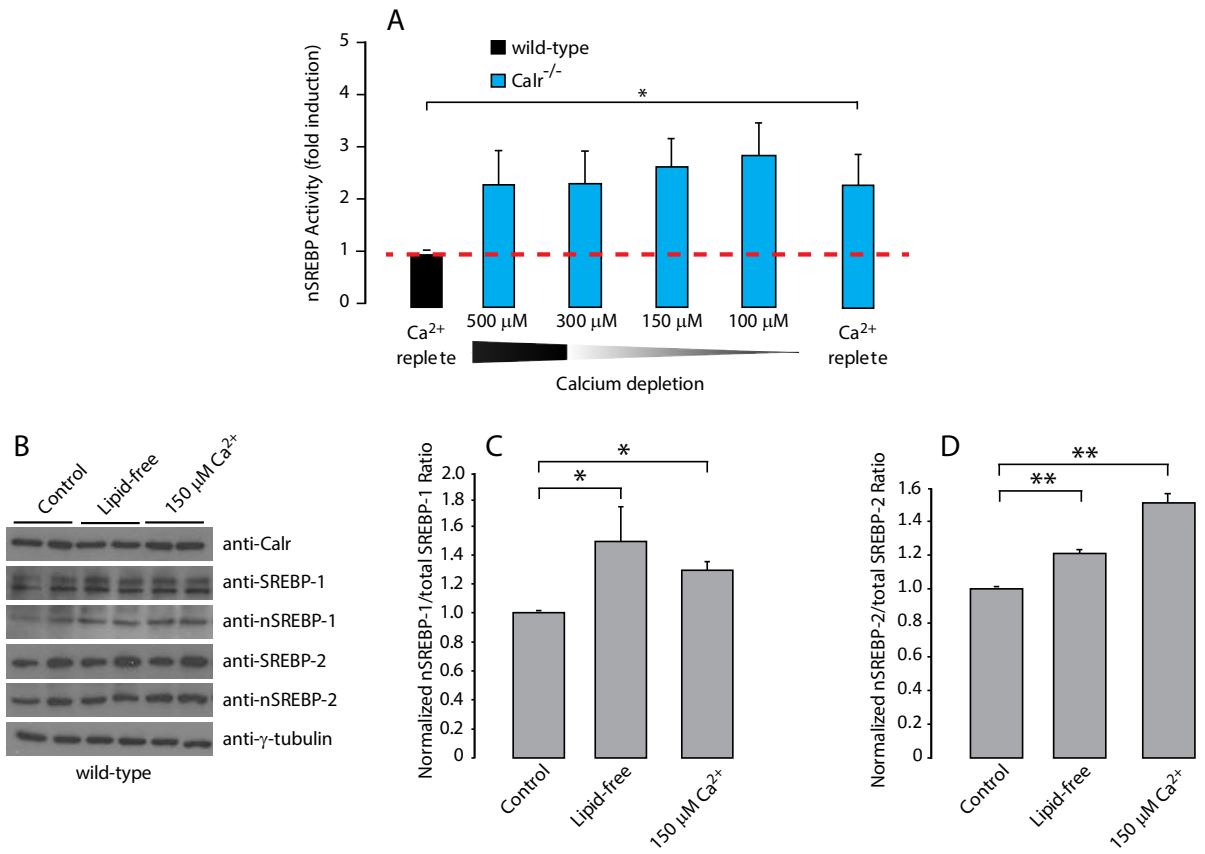
Supplementary Figure S2. Uptake of fluorescent LDL in wild-type and *Calr*$^{-/-}$ cells. The time of incubation with LDL fluorescent complex with BODIPY as indicate in the Figure.



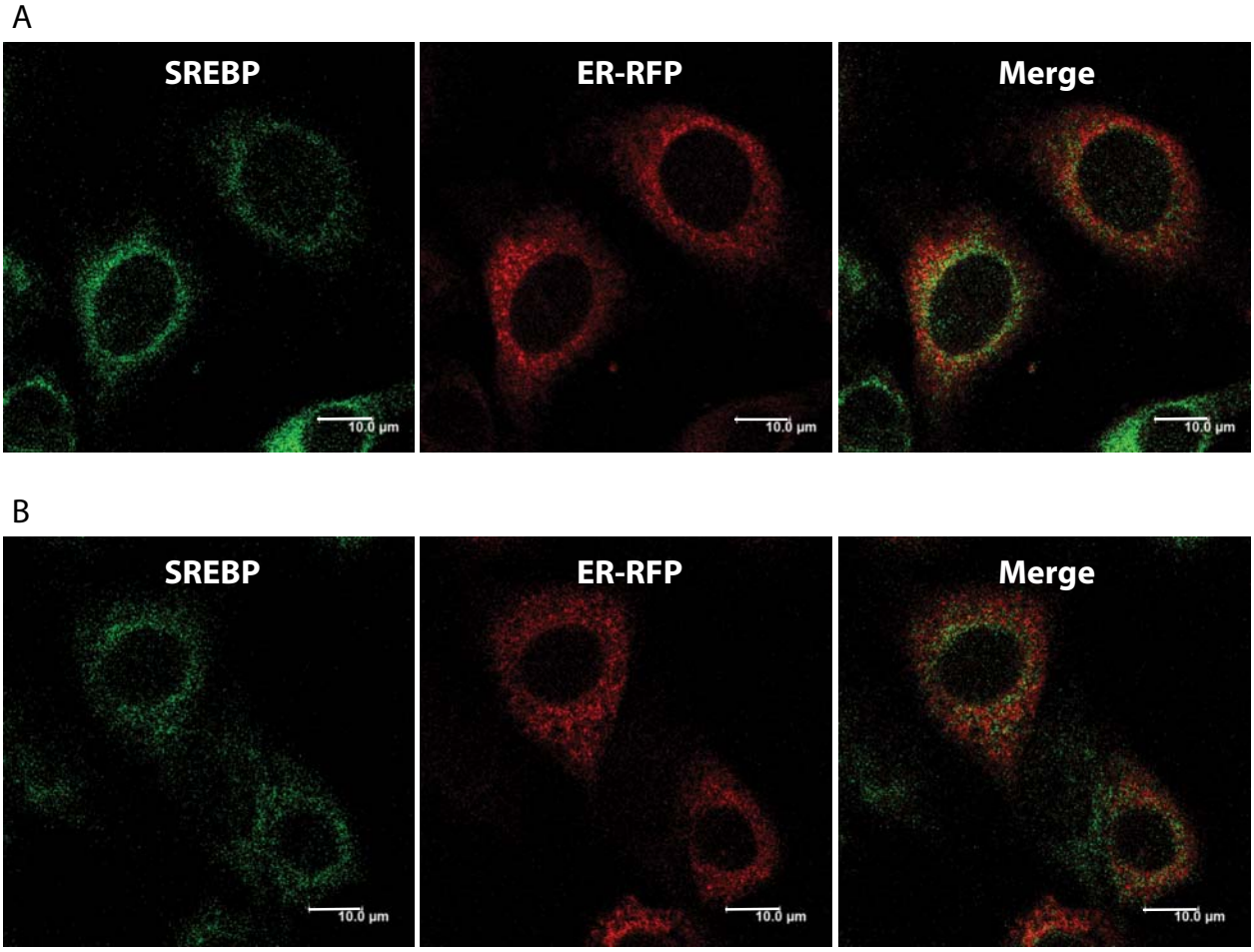
Supplementary Figure S3. Rate of cholesterol synthesis in *Calr*$^{-/-}$ cells cultured with normal and serum-free media.



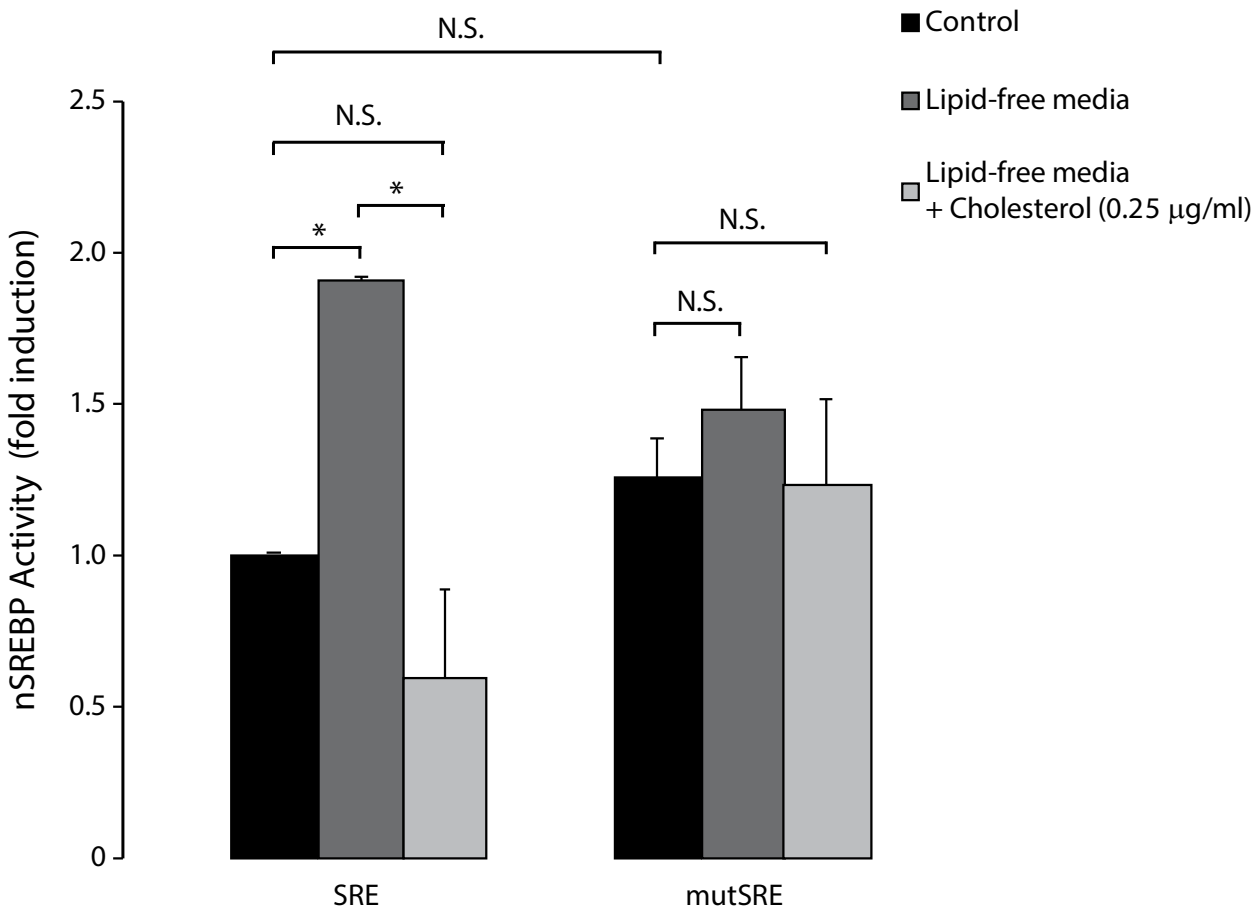
Supplementary Figure S4. Wild-type and *Calr*$^{-/-}$ cells were transfected with siRNA specific for S1P followed by nSREBP activity assay.



Supplementary Figure S5. Ca^{2+} effects on SREBP processing in *Calr*$^{-/-}$ cells. A. nSREBP activity in *Calr*$^{-/-}$ cells exposed to decreasing extracellular Ca^{2+} concentration as indicated in the Figure. Ca^{2+} replete represents 2.17 mM extracellular Ca^{2+} concentration. *Indicates statistically significant differences, wild-type vs. *Calr*$^{-/-}$ cells in control conditions *p*-value=0.0235. Representative of 7 biological replicates. **B.** Representative immunoblot analysis of SREBP-1, nSREBP-1, SREBP-2 and nSREBP-2 protein in wild-type cells grown in cholesterol-free media or treated with 150 µM extracellular Ca^{2+}. Anti-γ-tubulin antibodies were used as a loading control. Representative of 3 biological replicates. **C, D.** Quantitative analysis of Immunoblots, ratio of nuclear to total SREBP-1 (**C**) and total SREBP-2 (**D**). *Indicates statistically significant differences, in **C**, for SREBP-1, control vs. cholesterol-free *p*-value=0.0432 and control vs. 150 µM Ca^{2+}, *p*-value=0.0146. **Indicates statistically significant differences, in **D**, for SREBP-2, control vs. cholesterol free, *p*-value=0.0012; control vs. 150 µM Ca^{2+}, *p*-value=0.0013. Representative of 3 biological replicates. The images of (**B**) shown are cropped. The full-length gel/blots are shown in Fig. S17.

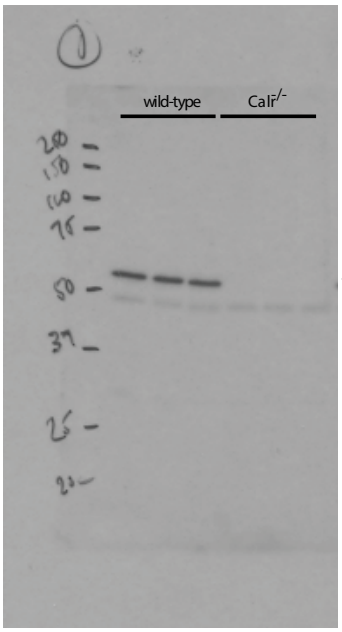


Supplementary Figure S6. Cellular distribution of SREBP-2 in wild-type and *Calr*$^{-/-}$ cells. Wild-type cells (**A**) and *Calr*$^{-/-}$ cells (**B**) expressing ER-targeted red fluorescent protein (ER-RFP) were stained with anti-SREBP antibodies. Wild-type cells Pearson's coefficient=0.231±0.035 (n=15); *Calr*$^{-/-}$ cells Pearson's coefficient=0.291±0.038 (n=15). Graphic representation of overlap SREBP and ER-RFP signals from representative cells is presented in the Figure. The arrows indicate the direction of the scan represented in the graph.

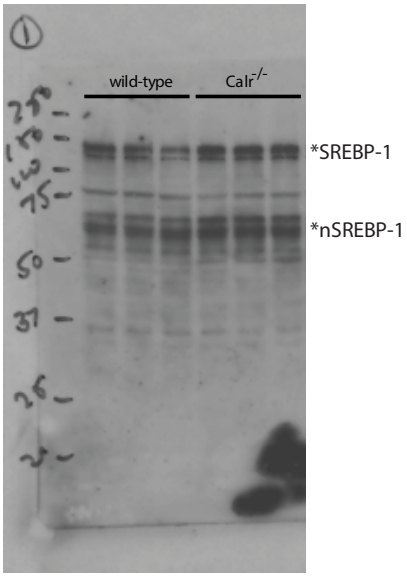


Supplementary Figure S7. Mutational analysis of the SRE element in HeLa cells. nSREBP activity in HeLa cells transfected with the SRE luciferase reporter plasmid or the mutated SRE (mutSRE) luciferase reporter plasmid treated with normal media, lipid-free media and lipid-free media plus cholesterol (0.25 μg/ml). SRE nucleotide sequence was mutated from ATCACCCCAC to ATTACCACGC. *Indicates statistically significant differences: HeLa cells with the SRE luciferase reporter in normal vs. lipid-free media, *p*-value<0.05 (ANOVA); HeLa cells with the SRE luciferase reporter in lipid-free media vs. lipid-free media plus cholesterol (0.25 μg/ml), *p*-value<0.05 (ANOVA). NS, not significant. Representative of 3 trials with 3 replicates.

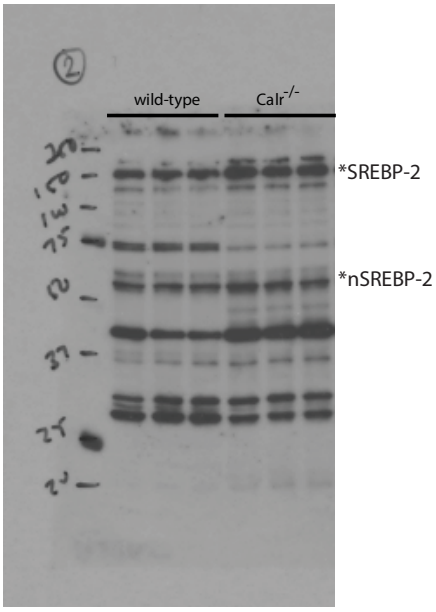
Anti-Calr



Anti-SREBP-1



Anti-SREBP-2



Anti-γ-tubulin

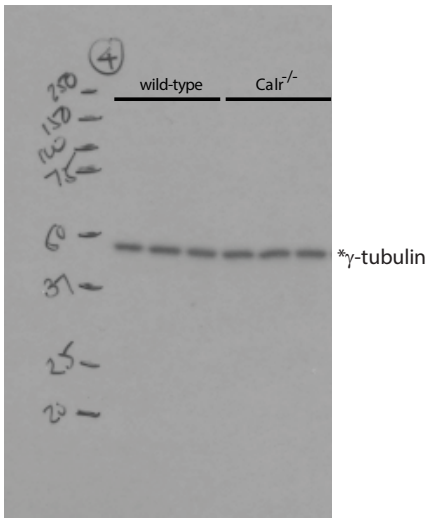


Figure S8. The full-length blots for Figure 2B

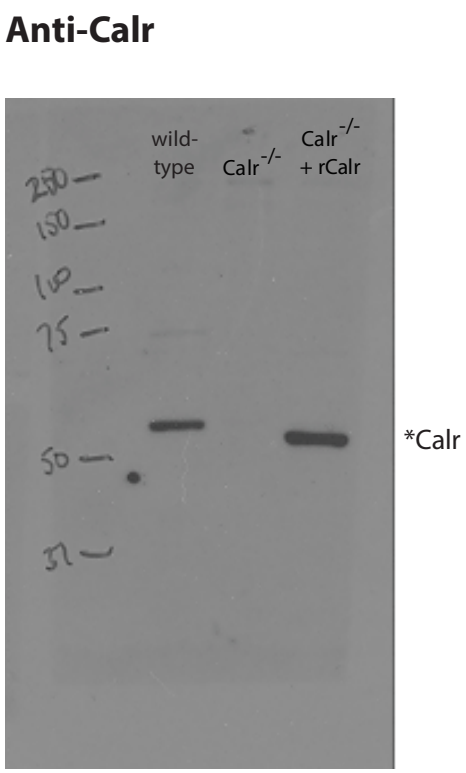
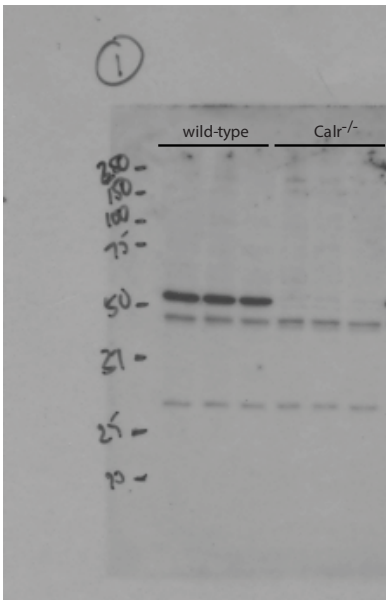
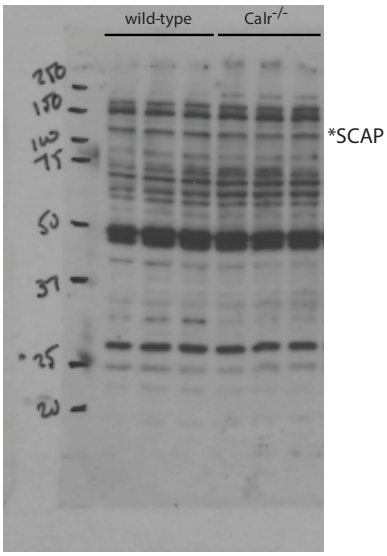


Figure S9. The full-length blots for Figure 2F

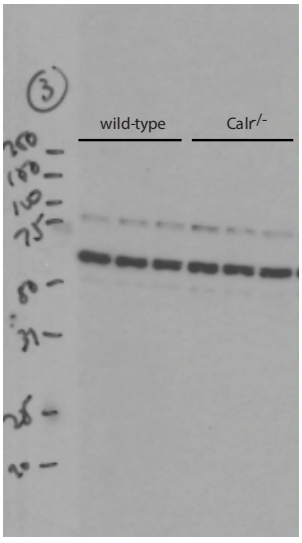
Anti-Calr



Anti-SCAP



Anti-INSIG



Anti-γ-tubulin

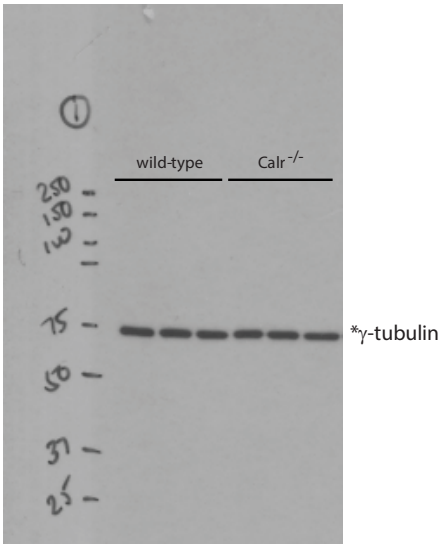


Figure S10. The full-length blots for Figure 3B

Anti-INSIG

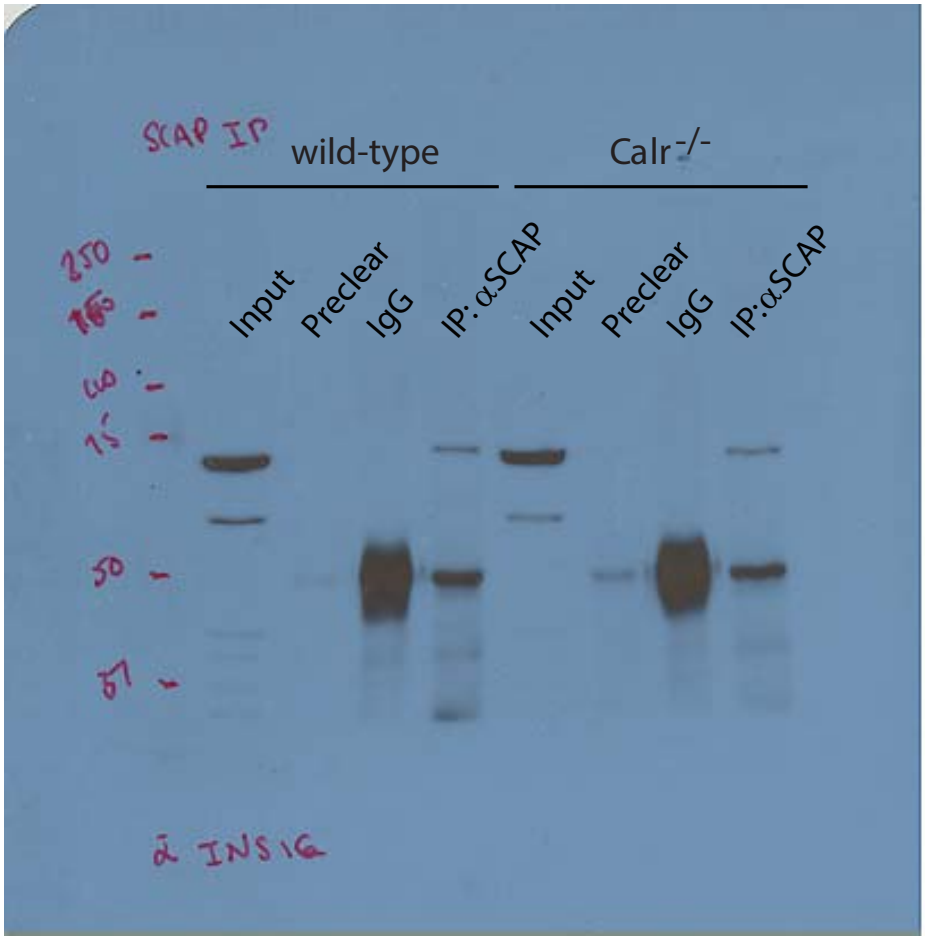


Figure S11. The full-length blot for Figure 3C

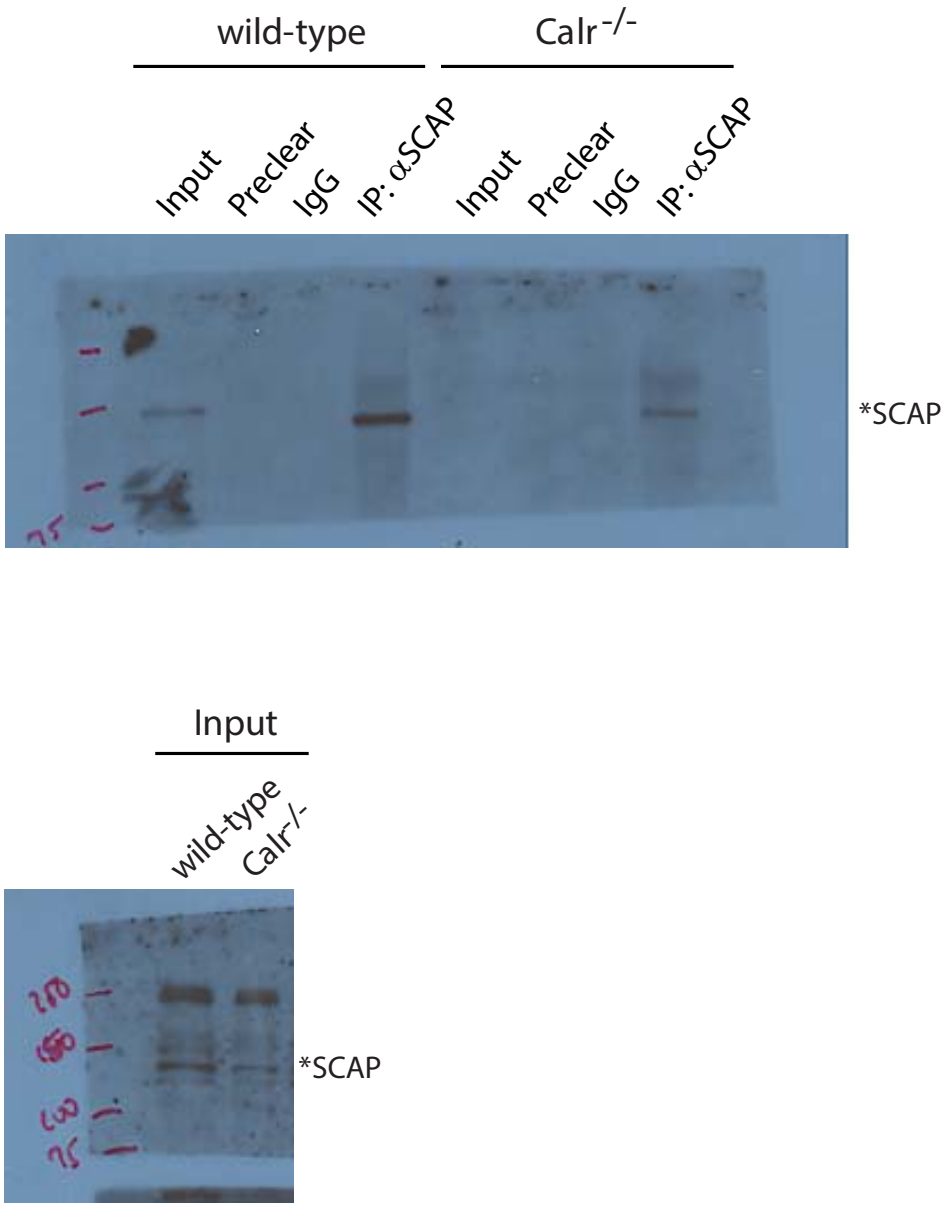
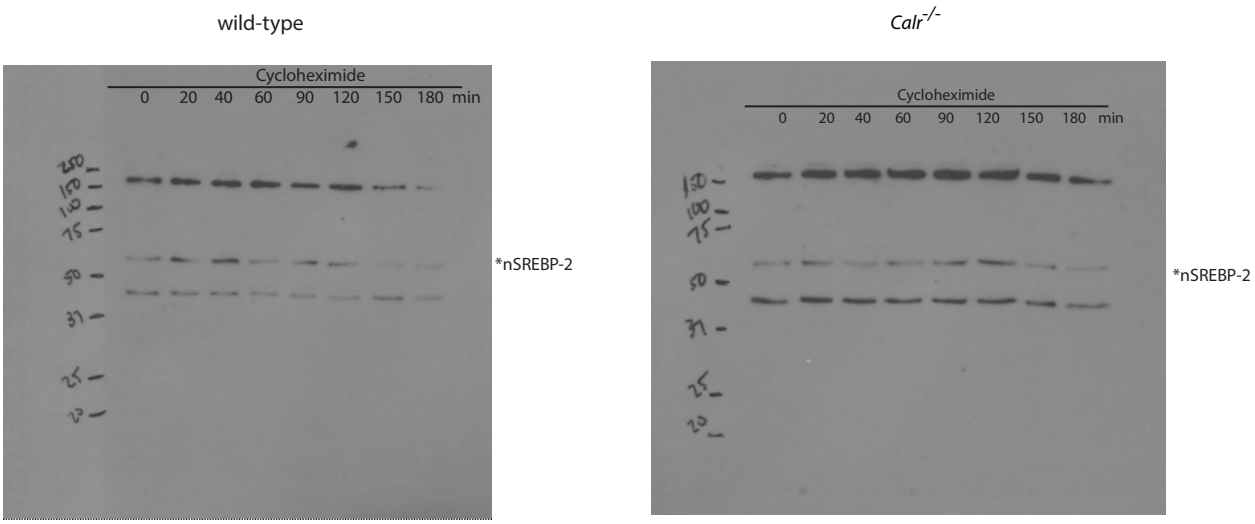


Figure S12. The full-length blots for Figure 3D

Anti-SREBP-2



Anti-γ-tubulin

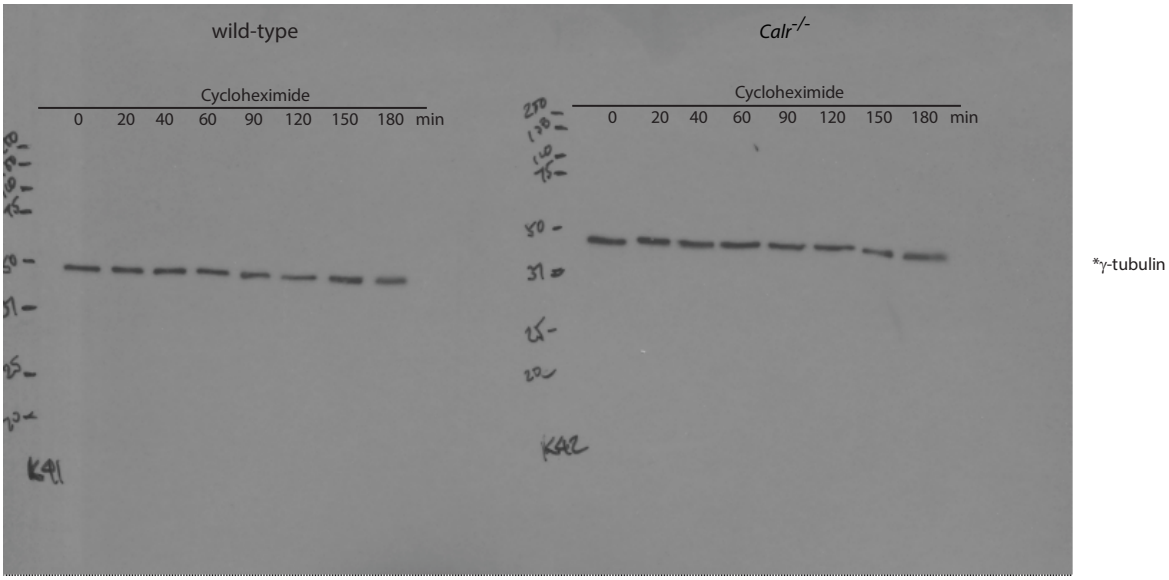


Figure S13. The full-length blots for Figure 3F

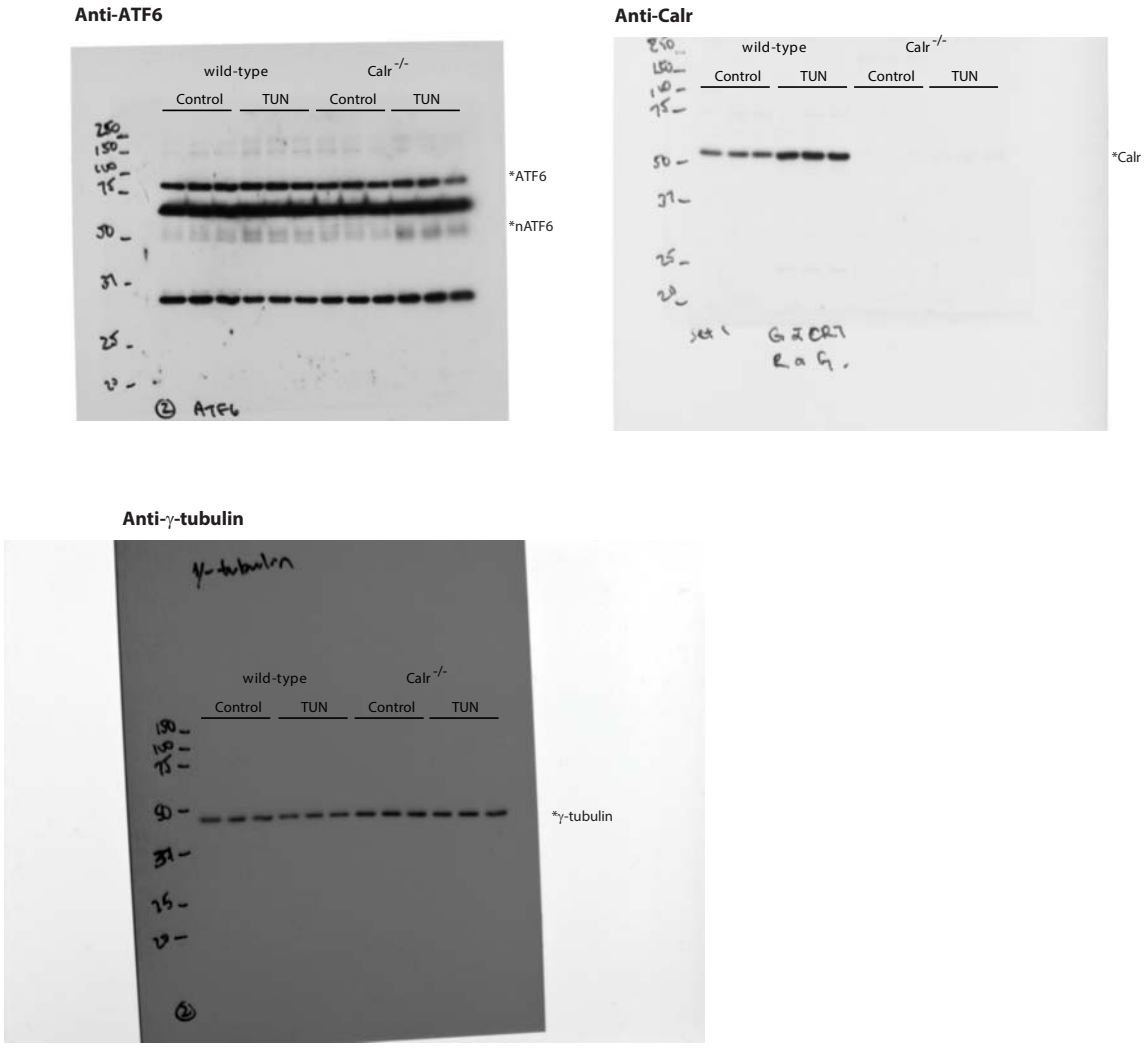
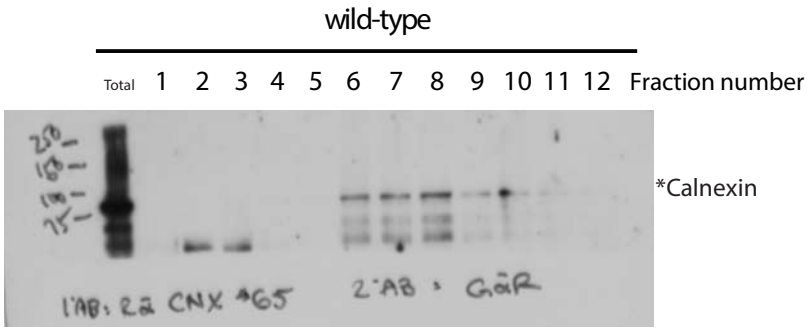


Figure S14. The full-length blots for Figure 4A

Anti-Calnexin



Anti-GAPDH

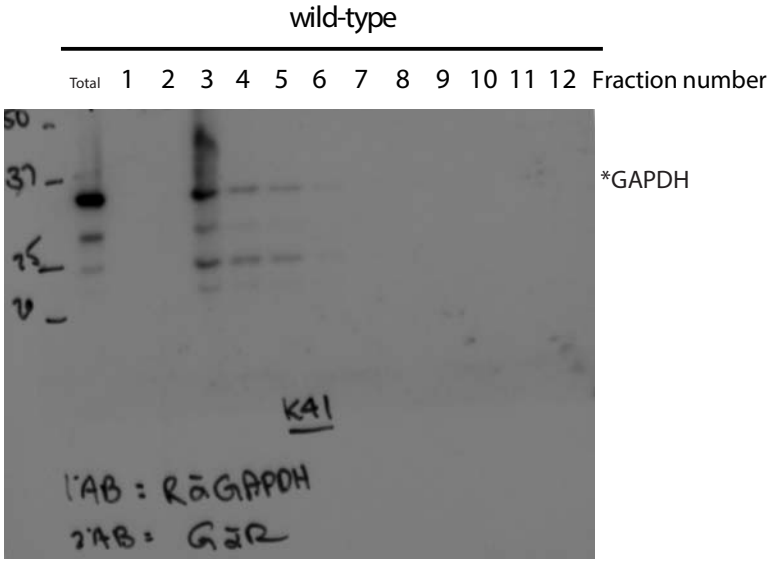


Figure S15. The full-length blots for Figure 7A

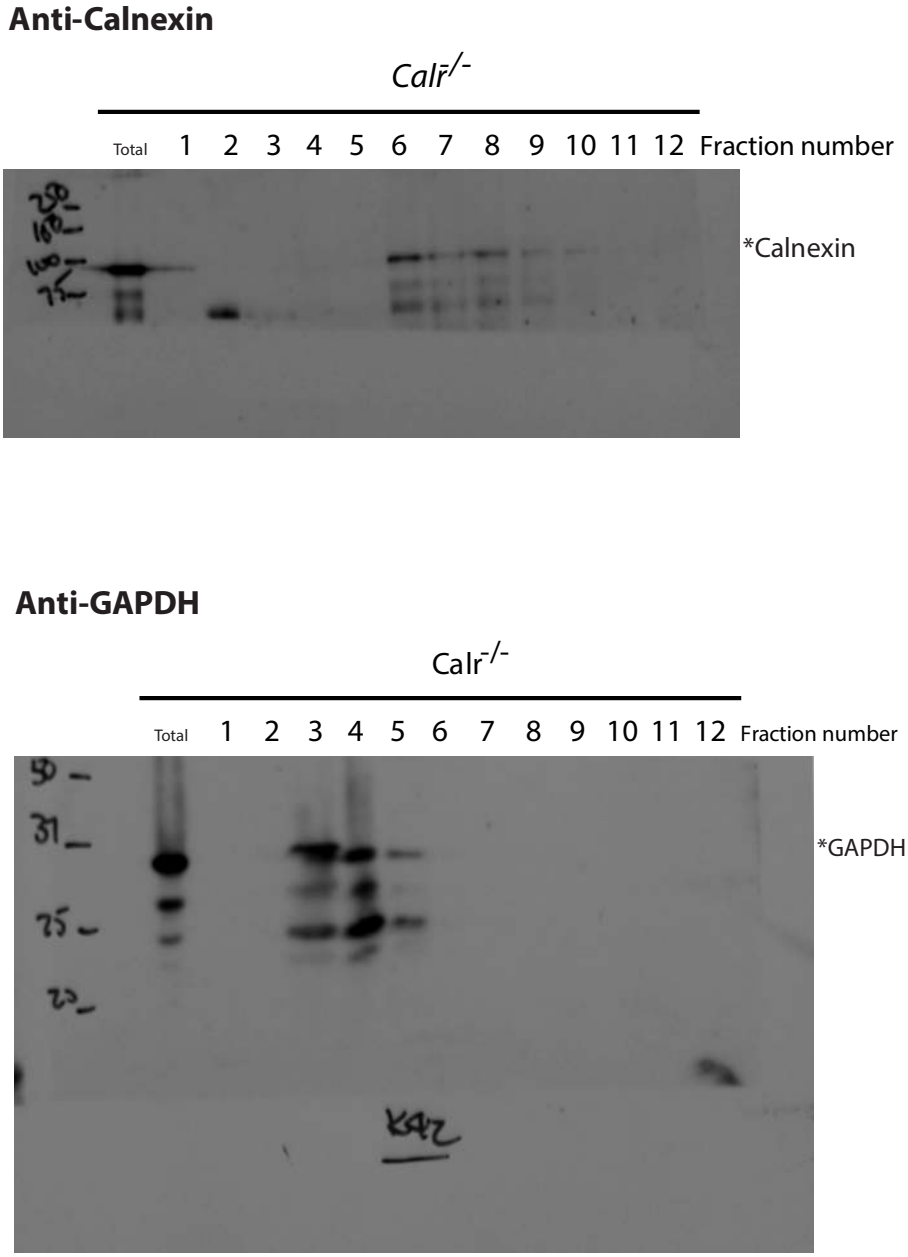


Figure S16. The full-length blots for Figure 7A

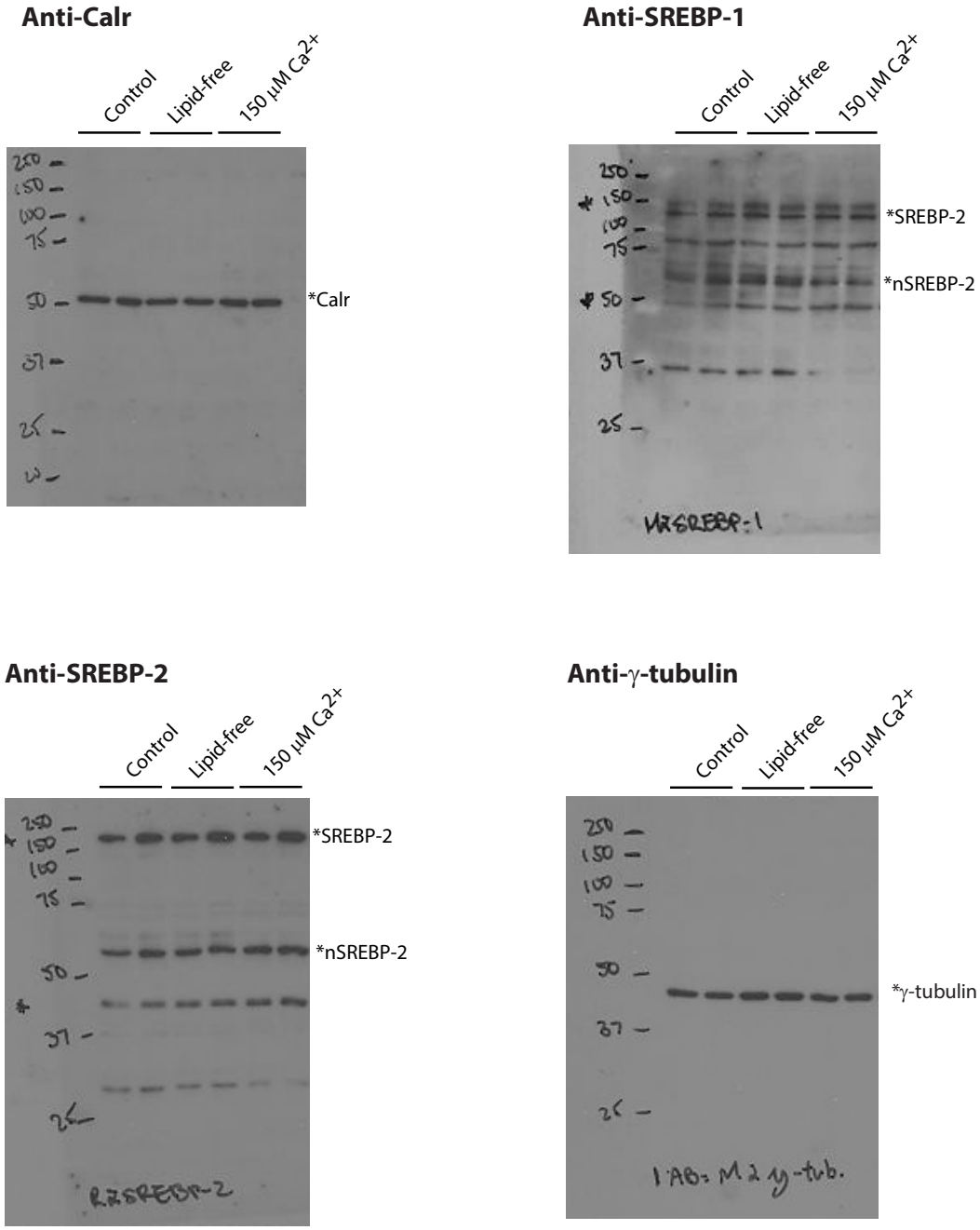


Figure S17. The full-length blots for Figure S5B