Supporting Information

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SI Materials and Methods

DNA Constructs. ***Tgn38-FRB-CFP.*** The human TGN38 (accession number BC008461, obtained as a full-length clone: 4272702 from Open Biosystems) was fused to the N terminus of the human FRB protein through a short linker (DPTRSANSGAGAGAGAILSR). This fusion protein was tagged with CFP using the pEGFP-N1 plasmid backbone. To obtain the **Tgn38-FRB** recruiter construct without the fluorescent tag, the Tgn38-FRB part of the construct was amplified with the primer pairs:

Fwd: 5′-ATATGCTAGCACCATGCGGTTCGTAGTTG-3′,
Rev: 5′-ATATGGTACCACCGGTGCGTAGTCTGGTACG-TCG-3′

and the PCR product was subcloned into the HA-pcDNA3.1 vector after cutting with NheI and KpnI restriction enzymes. The mRFP-FKBP-only plasmid has been described earlier (1).

The **mRFP-FKBP12-hSac1** was constructed by amplification of the enzyme devoid of the C-terminal localization sequence (2-516) from a full-length EST clone (Invitrogen, Clone ID: 3049075) using the following primer pairs:

5′-TATACGATCGGCGACGGCGGCCTACGAGC-3′,
5′-tataggtaccTCATGGAACACTTAAAGGACTATGA-GATTCTAATTCATCC-3′.

After cutting the product with PvuI and KpnI, this insert was used to replace the 5-phosphatase domain in the mRFP-FKBP12-5PTase construct described previously. To increase the distance between the Sac1 phosphatase and FKBP12, an additional linker sequence was inserted between the two proteins (SAGG-SAGGSAGGSAGGSAGGPRAQASR).

GFP-OSH1-PH was a gift from Mark Lemmon (University of Pennsylvania, Philadelphia, PA) and the three **GFP-GGA** constructs were kindly provided by Juan Bonifacino (NICHD, NIH, Bethesda, MD). These were then also subcloned into a pEGFP-C1 vector driven by timidine-kinase (TK) promoter. **Arf1-GFP** was a gift from Julie Donaldson (National Heart, Lung, and Blood Institute (NHLBI), NIH, Bethesda, MD), and the **VSVg-GFP** construct was from Jennifer Lippincott-Schwartz (NICHD, NIH). The photoactivable (PA) version of this construct was generated by replacing EGFP with PA-GFP also kindly provided by the Lippincott-Schwartz laboratory. The light chain of clathrin tagged with EGFP (**Clathrin LC-GFP**) was a kind gift from Lois Greene (NHLBI, NIH). The YFP-tagged μ1 subunit of AP-1 was generously provided by Alexander Sorkin (University of Colorado, Denver). The TK-driven FAPP1-PH-GFP and CERT-PH-GFP were derived from the respective constructs described previously (2) by replacing the CMV promoter with the TK promoter in the respective plasmids as described previously for the STIM1 protein (3).

NES-GFP-OSBP-PH. A pEGFP-C1 plasmid containing an N-terminal nuclear export signal (NES-GFP-C1) was initially generated using the NES sequence of the MAPKK (ALQKKLEELELDE) with two oligonucleotides:

Fwd: 5′-CTAGCCACCATGGCTCTGCAGAAAAAGTTG-GAAGAGCTTGAGCTGGATGAGGCA-3′ and
Rev: 5′-CCGGTGCCTCATCCAGCTCAAGCTCTTCCAA-CTTTTTCTGCAGAGCCATGGTGG-3′,

which after annealing yielded Nhe and AgeI sites at the 5′ and 3′ ends, respectively. This piece was inserted in front of the GFP between the NheI and AgeI sites. The OSBP-PH was then cut out from the previously described OSBP-PH-GFP plasmid (2) and subcloned between the BglII and EcoRI sites of this NES-EGFP-C1 vector.

The **pEGFPN3-CI-mannose-6-P** receptor was a gift from Jeffry Pessin (University of Iowa, Iowa City, IA). To obtain the photoactivable-GFP version of the construct PA-GFP was amplified out from the PA-GFP-N1 vector with the following primer pairs:

Fwd: 5′-ATATGTCGACCTGGTACCGCGGGCC-3′ (containing SalI site) and
Rev: 5′-ATATGCGGCCGCTTTACTTGTACAGCTCGTC-3′ (containing a NotI site).

The product was then subcloned between the SalI–NotI sites of the pEGFPN3-mannose-6-P receptor construct after digestion with the same enzymes.

1. Varnai P, Thyagarajan B, Rohacs T, Balla T (2006) Rapidly inducible changes in phosphatidylinositol 4,5-bisphosphate levels influence multiple regulatory functions of the lipid in intact living cells. *J Cell Biol* 175:377–382.
2. Balla A, Tuymetova G, Tsiomenko A, Várnai P, Balla T (2005) A plasma membrane pool of phosphatidylinositol 4-phosphate is generated by phosphatidylinositol 4-kinase type-III alpha: Studies with the PH domains of the oxysterol binding protein and FAPP1. *Mol Biol Cell* 16:1282–1295.
3. Várnai P, Tóth B, Tóth DJ, Hunyady L, Balla T (2007) Visualization and manipulation of plasma membrane-endoplasmic reticulum contact sites indicates the presence of additional molecular components within the STIM1-Orai1 Complex. *J Biol Chem* 282:29678–29690.

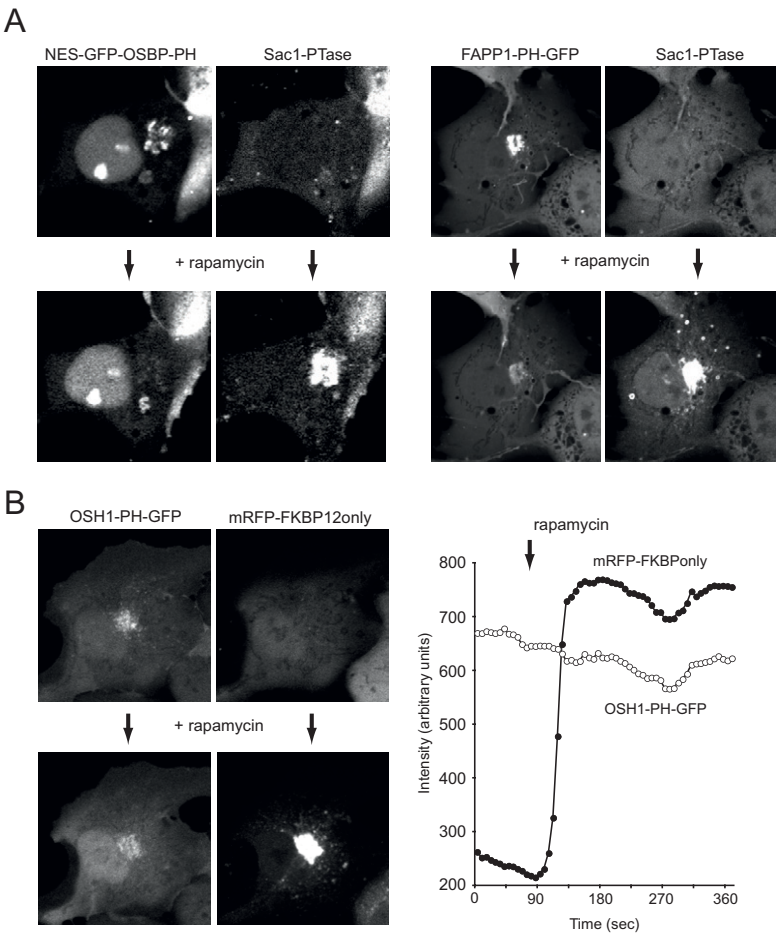

Fig. S1. (*A*) Rapamycin-induced recruitment of the Sac1 phosphatase releases the OSBP and FAPP1 PH domains from the Golgi. (*B*) Recruitment of the mRFP-FKBP-only construct does not release OSH1-PH domain from the Golgi.