

monitored by stimulating cells with the addition of 1 mM CaCl₂ to the external medium (indicated by arrow). Averaged data and the number of cells –‘n’ imaged are shown in *D*. *denotes values significantly different from control (P<0.05). (*E*) TIRFM images indicating YFP-STIM1^{D76A} punctae sensitive to raft disruption. (*F*) Immunoprecipitations indicating dependency of membrane rafts for TRPC1 and STIM1^{D76A} association. (*G*) Proposed model indicating raft recruitment of STIM1 as a step obligatory to SOCE.

Figure Legends (Supplementary figures)

Figure 1: Localization of STIM1 and TRPC1 in lipid rafts: Detergent free rafts were isolated from HSG cells as described in Experimental Procedures. Individual fractions were resolved on 4-12% NuPAGE gels and Western blots were performed using respective antibodies as described above at 1:000 dilutions. Both STIM1 and TRPC1 co-migrated with the raft markers as observed in Figure 1 (main text).

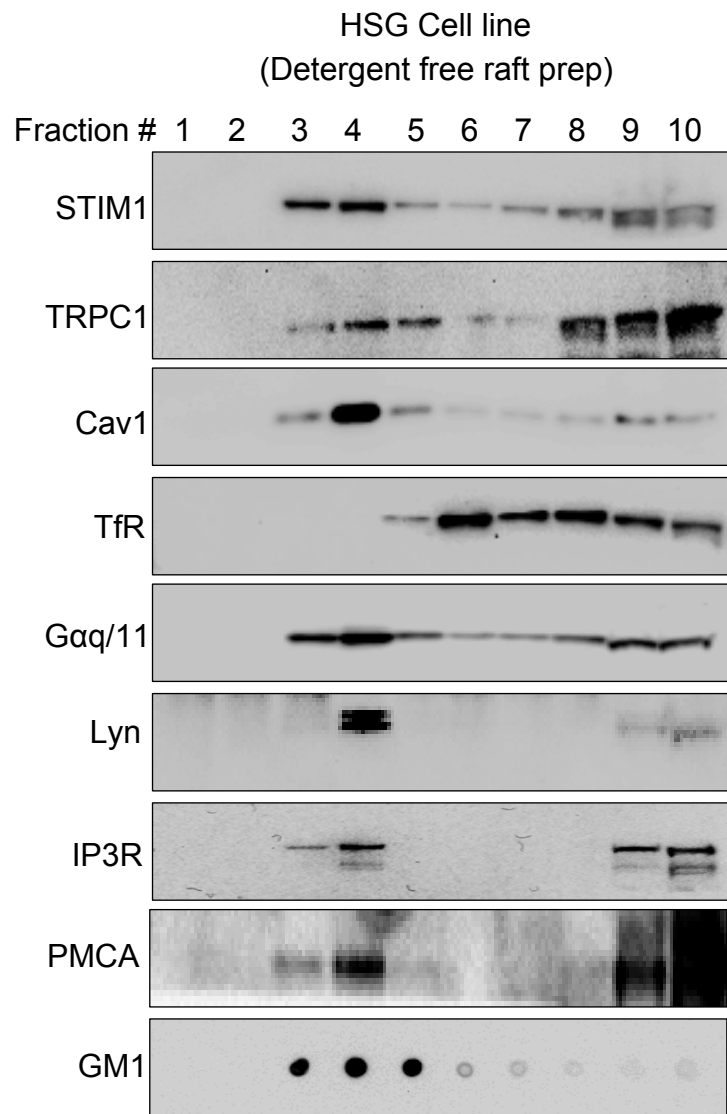
Figure 2: Lipid rafts dictates STIM1 movement and SOCE: (*A*) Composite confocal images of HSG cells expressing YFP-STIM1 (green) along with GM1 (red, a lipid raft marker) in control and treated cells (+ 2μM Tg). (*B*) Western blots showing Tg- mediated STIM1 movement in detergent resistant raft-‘R’ fractions from MβCD or cells treated with MβCD and replenished with cholesterol (as described in experimental procedures). (*C*) TIRF imaging on HEK-293 cells expressing either a control or TRPC1-Sh-RNA. (*D*) Tg-stimulated Ca²⁺ mobilization in control and MβCD treated HEK 293 cells.

Figure 3: Lipid raft integrity does not effect intracellular aggregation of STIM1 or STIM1^{D76A}: (*A*), indicates a representative epifluorescent image of YFP-STIM1 in control and MβCD (10 mM) treated HSG cells under both control (unstimulated) or store depleted (+Tg) conditions. (*B*), indicates epifluorescent image of YFP-STIM1^{D76A} mutant under control or MβCD treated HSG cells.

Table 1. List of Antibodies and Supplier

Antibody	Supplier
Anti-STIM1	BD-Transduction Laboratory and Abnova
Anti-TRPC1	Alemone, Sigma and Wang et al., *
Anti-Caveolin1	BD-Transduction Laboratories
Anti-PMCA, IP3R-III	Affinity BioReagents
Anti-Transferrin receptor	Zymed Laboratory
Anti-SERCA2	Calbiochem
Anti-Actin, Lyn, G _{αq/11} , Mouse IgG, Rabbit IgG	Santa Cruz Biotechnology
Anti-GFP	Clontech (JL8) and Torrey Pines Biolabs
Choleratoxin subunit B – HRP/Alexa Fluor 594 conjugated	Invitrogen
All secondary antibodies (HRP-conjugated)	Pierce

* Wang et al., (1999). Am. J. Physiol. 276:C969-79.



Pani et al., 2008 Supplement Fig.1

