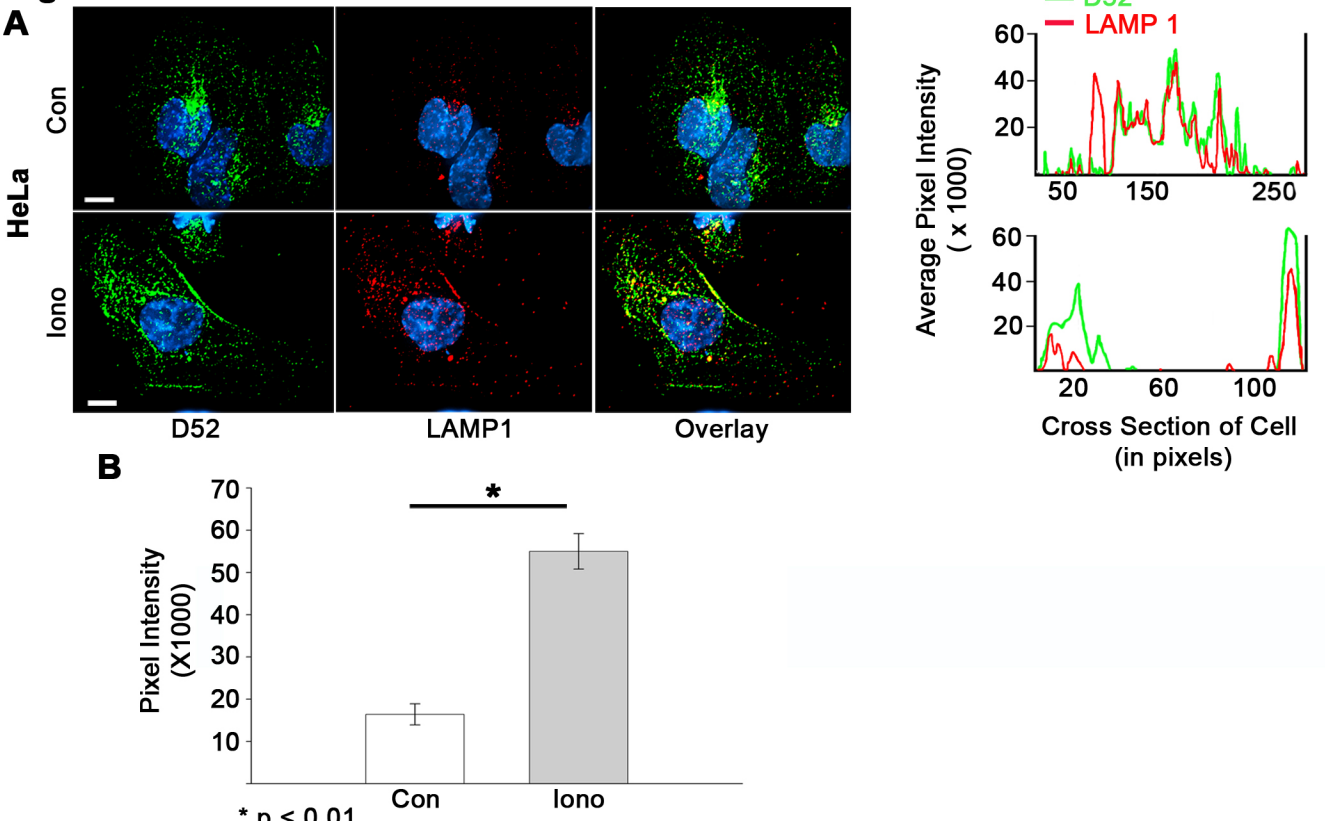
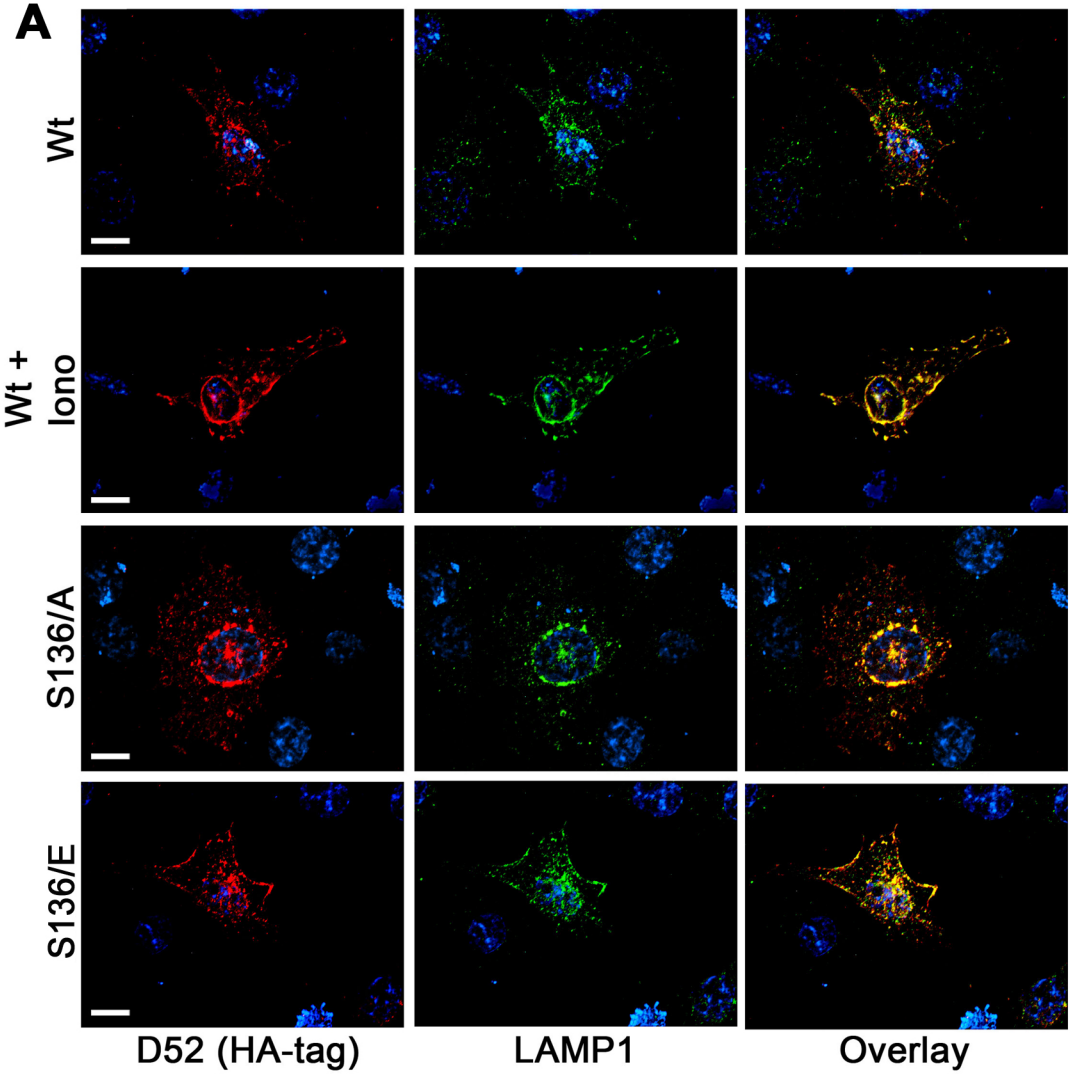
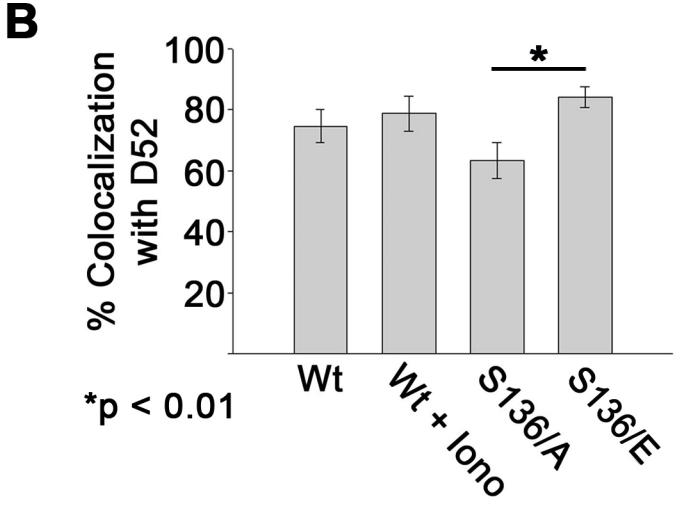
**Figure S1**

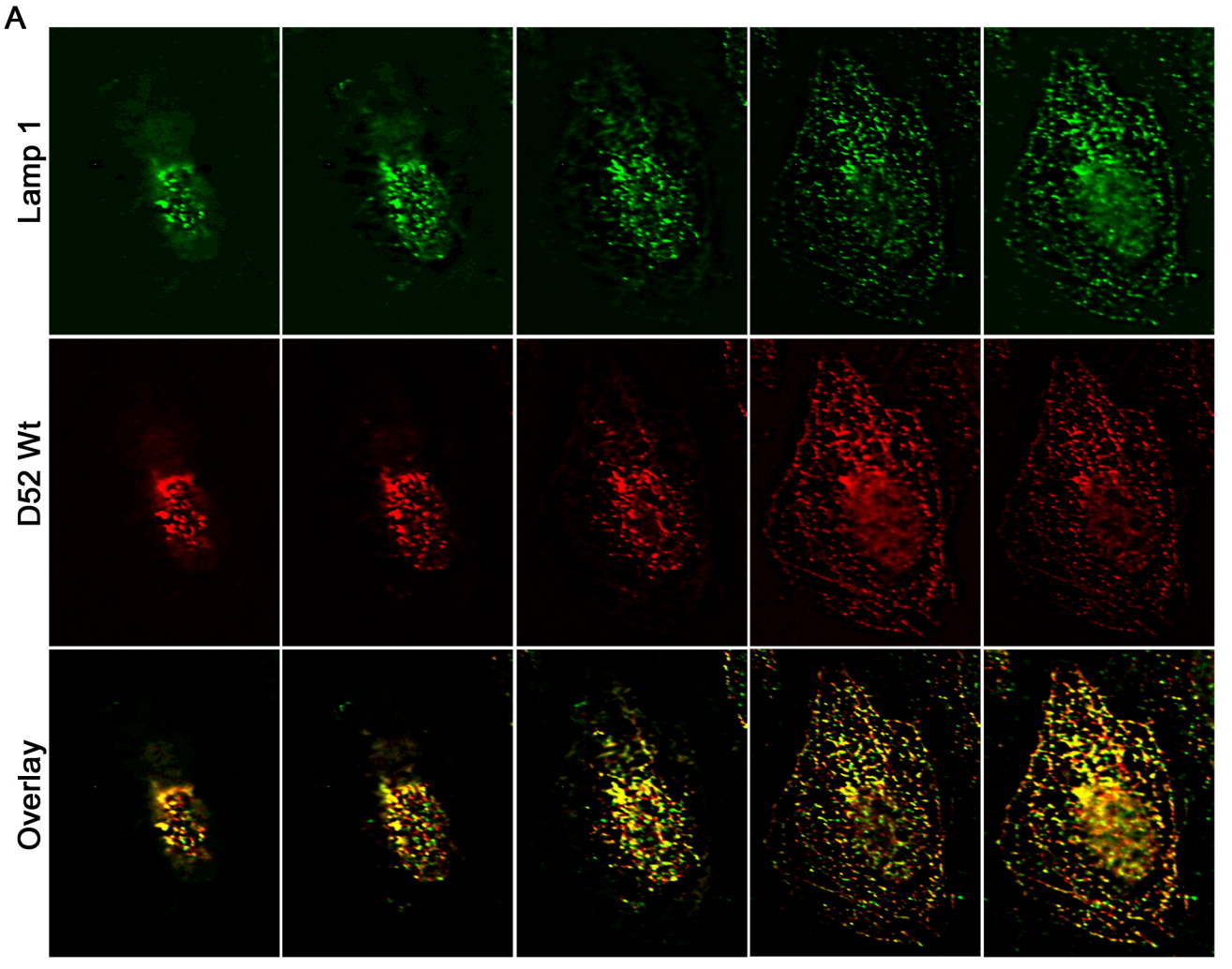

A
HeLa
Con
Iono
D52
LAMP1
Overlay
D52
LAMP 1
Average Pixel Intensity
( x 1000)
60
40
20
50
150
250
60
40
20
20
60
100
Cross Section of Cell
(in pixels)
B
Pixel Intensity
(X1000)
70
60
50
40
30
20
10
*
Con
Iono
* p < 0.01

# Figure S2


A
Wt
Wt + Iono
S136/A
S136/E
D52 (HA-tag)
LAMP1
Overlay


B
% Colocalization with D52
100
80
60
40
20
*
Wt
Wt + Iono
S136/A
S136/E
*p < 0.01

# Figure S3



## Supplemental Figures

**Supplemental Figure 1. Endogenous D52 translocates to plasma membrane with LAMP1 during Ca2+ -stimulated exocytosis in HeLa cells.** D52 and LAMP1 were analyzed in HeLa cells treated with 0.01% DMSO as control (Con) or 2 μM ionomycin (Iono) for 5 minutes and then incubated at 4$^{o}$C with anti-LAMP1 to label externalized LAMP1 as described in Fig 5. Each image is a reconstructed z-series obtained by brightfield microscopy. All images are a single representative experiment performed in at least three separate tissue preparations. Bars, 13 μm. *Panel A right side*: Line density analysis of D52 (green) and LAMP1 (red) staining across a single cell. *Panel B*: Quantitative analysis of LAMP1 localization at the plasma membrane designated as the first 20 pixels of signal acquired at the cell periphery from multiple line density plots. Data are mean and S.E (n = 10 for each experimental condition) performed in at least three separate tissue preparations.

**Supplemental Figure 2. Effects of D52 overexpression in NRK cells.** NRK cells were transfected with wt-D52 or indicated phospho-mutants for 18 hours. Cells were treated with 0.01% DMSO as control or with 2 μM ionomycin for 5 minutes prior to fixation and permeabilization. LAMP1 was detected as described in Fig 3. Transfected D52 was detected using an anti-HA antibody (1:100) in combination with Alexa Fluor 546-conjugated anti-rat IgG (1:100). All images are reconstructive z-series images representative of multiple determinations performed on at least three separate tissue preparations. Bars, 13 μm. Panel B: Quantification of cellular LAMP1 and overexpressed wt-D52 of D52 phsophomutants. Note the extensive colocalization of D52 and LAMP1 in cells overexpressing D52 and D52 mutants.

**Supplemental Figure 3. Sequential series of z-plane images of external LAMP1 and intracellular D52 in CHO-K1 cells transfected with wt-D52.** Each image is a sequential 500 nm z-plane section depicting external LAMP1 and intracellular D52. Images shown can be seen as a reconstructed 3-D image in Fig 5A. D52 and LAMP1 (both at 1:100) were analyzed in CHO-K1 cells transfected with wt-D52 and fixed in 2% formaldehyde. Immunoreactivity was determined using Alexa Fluor 546-conjugated anti-rabbit IgG (1:500) and Alexa Fluor 488-conjugated anti-mouse IgG (1:250), respectively. Images were captured at 5 nm intervals. Post-collection, D52 and LAMP1 were applied green and red pseudo-colors, respectively. Images were obtained by brightfield microscopy. Bars, 13 μm.