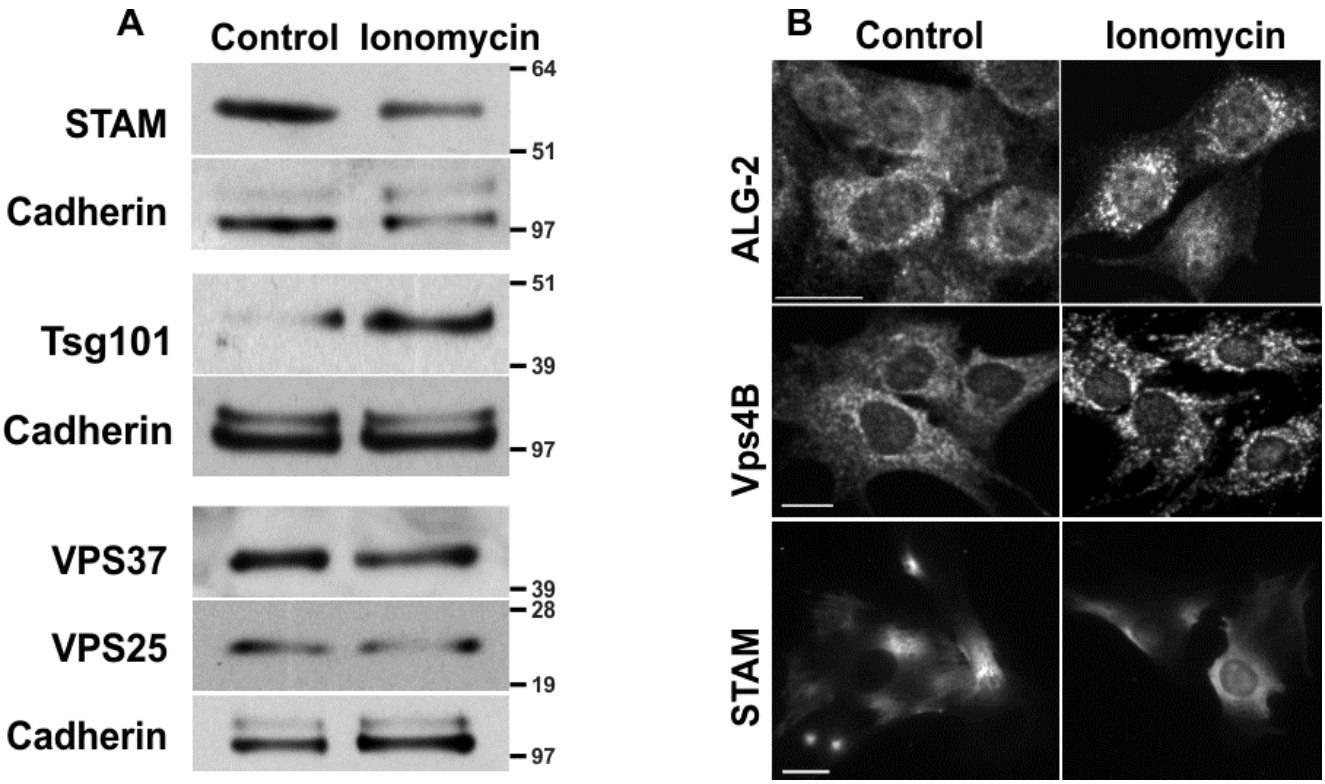
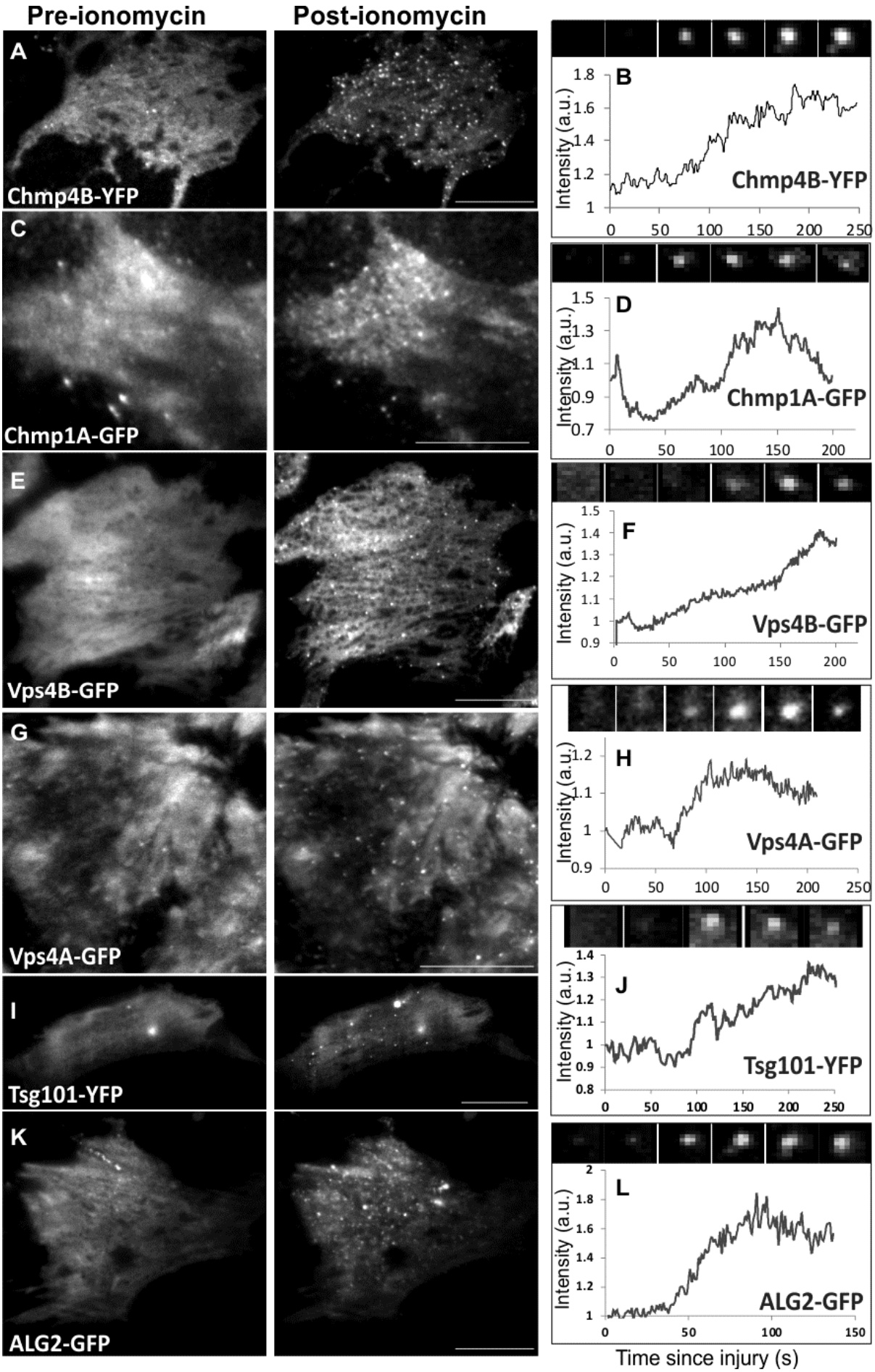
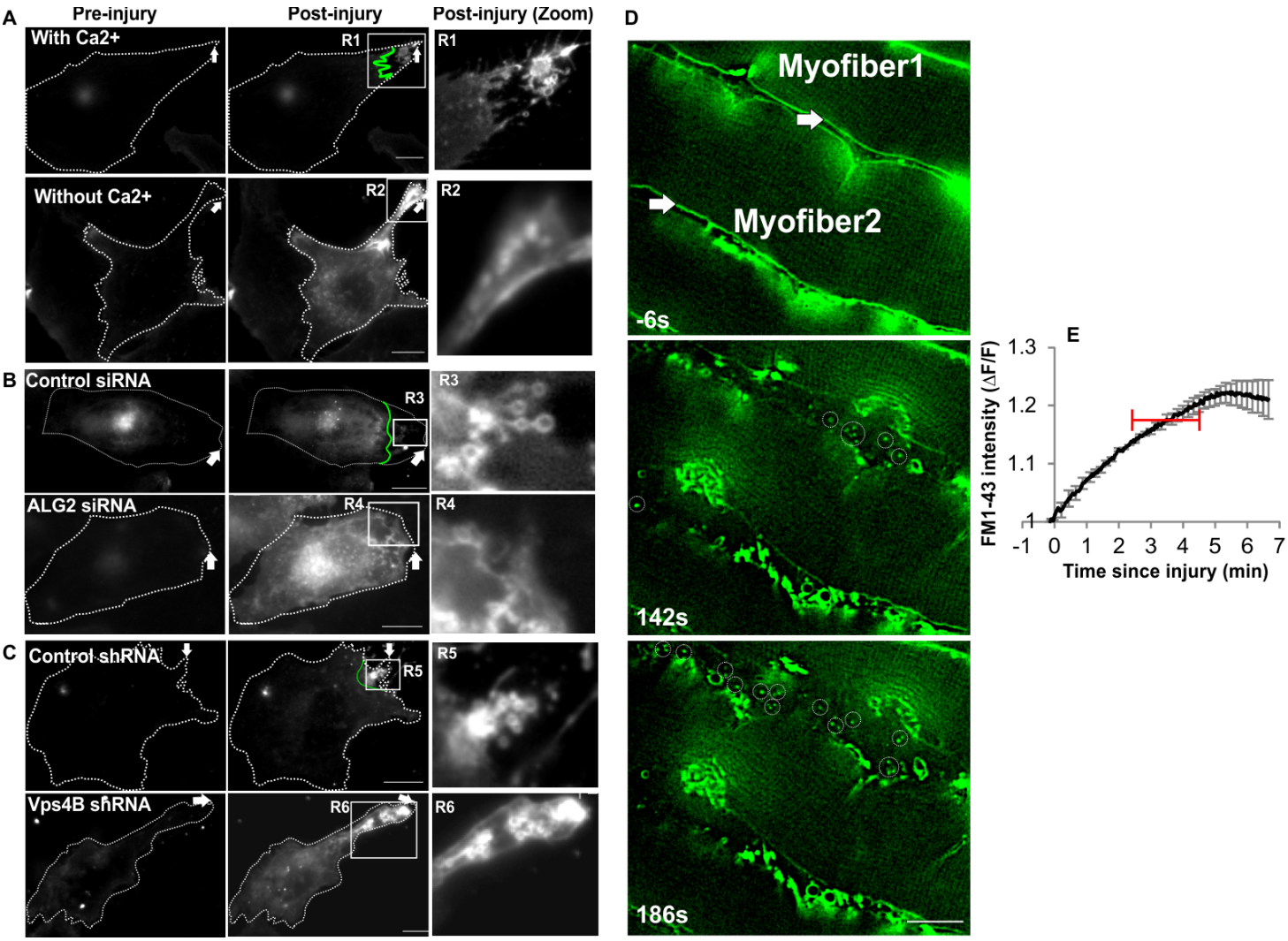


Supplementary Figure 1: Effect of Ca2+ increase on cell surface level and distribution of ESCRT0-II and accessory proteins. (A) Representative Western blots (n≥3) showing ionomycin triggered change in cell surface level of ESCRT0-II complexes. Protein molecular weight markers (in KDa) are listed for each blot. Data from these and two other similar blots were used to calculate the averaged fold change presented in figure 1F). (B) Images of C2C12 myoblasts immunostained for indicated proteins following 5 minute treatment DMSO (1%) (Control) or with 10μM ionomycin (Ionomycin), scale bar represents 10μm.


Pre-ionomycin
Post-ionomycin
A
Chmp4B-YFP
B
Intensity (a.u.)
Chmp4B-YFP
C
Chmp1A-GFP
D
Chmp1A-GFP
E
Vps4B-GFP
F
Vps4B-GFP
G
Vps4A-GFP
H
Vps4A-GFP
I
Tsg101-YFP
J
Tsg101-YFP
K
ALG2-GFP
L
ALG2-GFP
Time since injury (s)

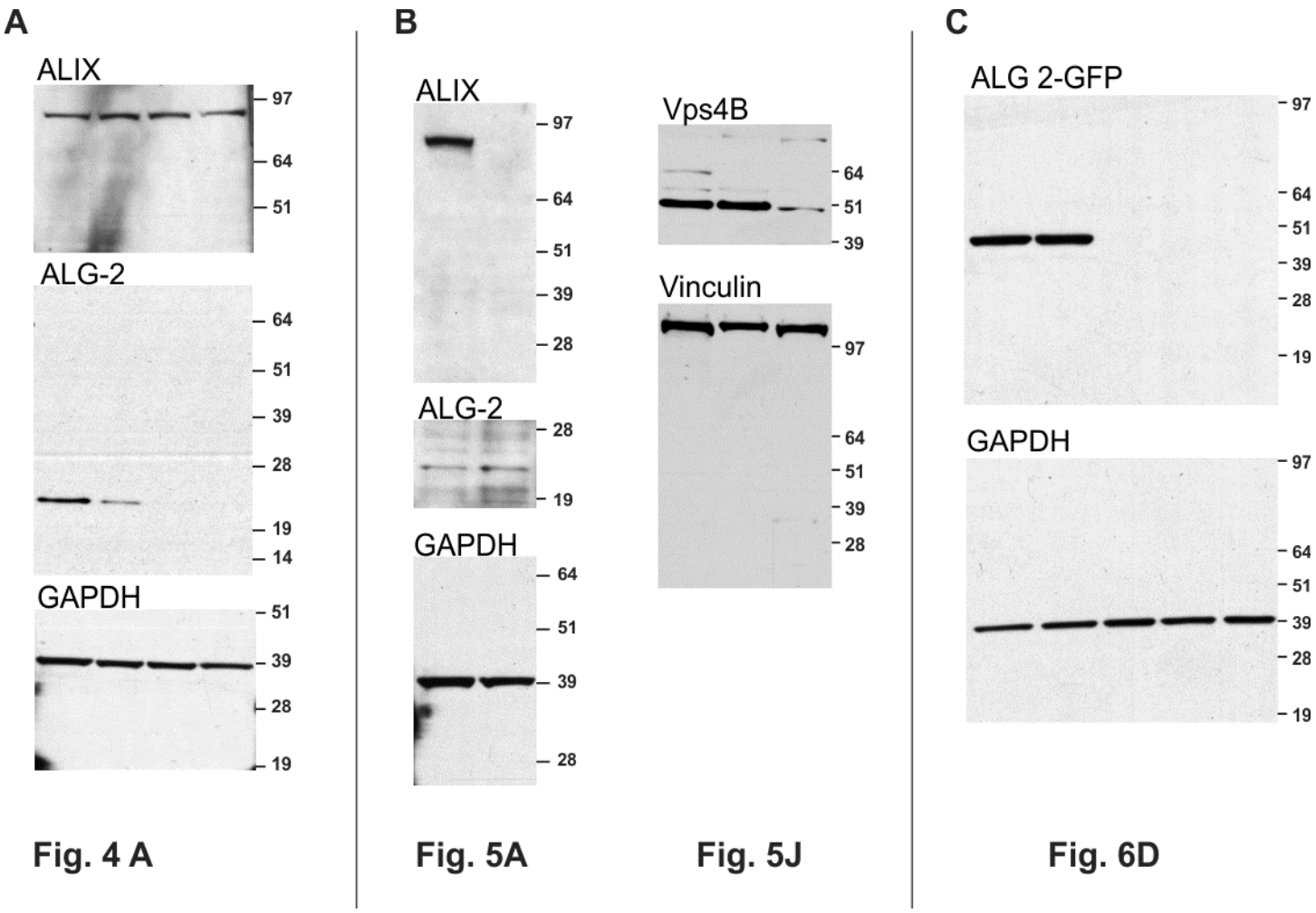
Supplementary Figure 2: Ca2+-triggers punctate recruitment of ESCRTIII and associated protein. C2C12 myoblasts expressing fluorescently tagged (A) Chmp4B-YFP, (C) Chmp1A-GFP (E) Vps4B-GFP, (G) Vps4A-GFP, (I) Tsg101-YFP and (K) ALG2-GFP proteins were treated with 10μM ionomycin while being imaged by TIRF microscopy to monitor Ca^{2+}-triggered cell surface accumulation of these proteins. Images show each cell 5s prior to ionomycin addition (Pre-ionomycin) and at the time point following ionomycin addition when most punctae have accumulated at the cell membrane (Post-ionomycin). The kinetics of ionomycin-induced formation of individual puncta is shown in corresponding plots (B, D, F, H, J, and L). Insets show the buildup of the proteins into a single puncta. The time points for image in the insets are roughly aligned with the time on the X-axis in the corresponding plot below. Scale bars represent 10μm.



Supplementary Figure 3: ALG-2 dependent ESCRT accumulation facilitates cell membrane repair by shedding of injured cell membrane. Fate of the injured cells monitored by widefield imaging of cellular membranes labeled by FM1-43 dye after (A) myoblasts were injured in presence or absence of Ca^{2+} (B) HeLa expressing or lacking ALG-2, and (C) expressing or lacking Vps4B were injured by a laser pulse. These cells were used in part to generate the plots for FM dye entry in figures 4A-C. These images show cellular FM-dye intensity 5 seconds prior to (Pre-injury) and 150-175 s post injury (Post-injury) at the site marked by the arrows and scale bar represents 5μm. The insets are the zoomed up image of the region around the site of injury showing the membrane scission and vesicles formation during the course of repair. The region where the damaged cell membrane is excised was identified using a combination of FM dye fluorescence and transmitted light image of the cell and is marked by green line. Only cells injured in presence of Ca^{2+} and not knocked down for ALG-2 or Vps4B manage to prevent dye entry and also exhibit membrane scission and vesicle formation. (D) Image of myofibers in an intact mouse biceps muscle were incubated in Tyrode's buffer with FM dye and injured by pulsed laser at sites marked by the arrows. The fibers were imaged by spinning disc confocal microscopy using a 40x objective. Images were subtracted for the background and thresholded to show the vesicles marked by dotted circles. The time stamp show time before (-6.0s) and after (142s, 186s) laser injury and scale bar represents 10μm. (E) Plot showing the kinetics of biceps myofiber cell membrane repair as assessed by FM1-43 dye influx. Red bar represents time during repair when maximal vesicle release occurred, which precedes block in FM dye entry indicating repair of injured muscle cell membrane.

Process	ESCRT-0	ESCRT-I	Bro1-domain protein	ESCRT-III and accessory proteins	Vps4 complex
Cytokinesis	Cep55	Tsg101	ALIX	Chpm1, Chmp2, Chmp3, Chmp4, Chmp5, Chmp6, Ist1	Vps4, Lip5
Retrovirus budding	Gag	Tsg101	ALIX	Chmp2, Chmp4	Vps4, Lips5
Cell membrane repair	ALG-2 (+Ca2+)	Tsg101	ALIX	Chmp1, Chmp4, Chmp6	Vps4

Supplementary Figure 4: Comparison of the sequential ESCRT assembly involved in cell membrane processes mediated by ESCRT complex. The ESCRT and associated proteins reported in literature to regulate cytokinesis and retrovirus budding are tabulated and equivalent proteins are listed that we have identified to remodel cell membrane during its repair from a focal injury.



Supplementary Figure 5: Uncropped Western blots. Western blots corresponding to (A) figure 4, (B) figure 5, and (C) figure 6 are shown. The molecular weight markers (in KDa) for individual blot are marked and labeled.