Supplementary Figure 1. Intracellular distribution of ER markers in cells treated with dispergo.

Cells were treated with DMSO (control) or dispergo for 3 hours and then immunostained with antibodies specific for various ER markers.

(A) BSC1 cells transiently expressing HA-DP1 immunostained for HA and endogenous TRAPα.

(B) Cells stably expressing GFP-Sec61β immunostained for endogenous calreticulin. The arrowhead highlights that calreticulin is not enriched in ER patches.

(C) BSC1 cell transiently expressing ssGFP-KDEL and mCherry-Sec61β imaged after fixation. The arrowheads highlight that ssGFP-KDEL is not enriched in ER patches labeled with mCherry-Sec61β.

(D) BSC1 cells transiently expressing GFP-Sec61β immunostained for endogenous TRAPα. Arrowheads indicate the formation of ER patches in a cell that didn't express GFP-Sec61β.

(E) Comparison of the effect of dispergo in BSC1 cells immunostained for Sec61β in the presence and absence of ectopic expression of GFP-Sec61β; the bottom panel shows that the proportion of cells with ER patches induced by dispergo was the same in the presence and absence of ectopic expression of GFP-Sec61β. (F) Comparison of untransfected NRK cells immunostained for endogenous TRAPα in DMSO (control) or dispergo treatment highlights the formation of ER patches induced by dispergo treatment in cells devoid of ectopic expression of proteins. Images in (C and E) were obtained using spinning disk confocal microscopy while the rest were acquired using wide-field fluorescence microscopy. Scale bars, 10 μm.

Supplementary Figure 2. Dispergo does not affect ERES.

(A) Dispergo does not affect the density of ERES. BSC1 cells stably expressing GFP-Sec61β were treated with DMSO (control) or dispergo for 1.5 hours and subsequently fixed and immunostained for Sec31a. Wide-field images acquired under the same imaging conditions for control and dispergo treated cells were then used to determine the location and frequency of ERES. The images were first background subtraction. A mask containing the peripheral region was selected by subtracting the total cellular area (traced manually) from the perinuclear region. The fluorescence intensity of Sec31a was segmented and the number of ERES objects was counted. The density of peripheral ERES was expressed as the number of the ERES spots contained within the selected area (number/μm^2). The peripheral ERES densities in control and

dispergo treated cells were 0.21 ± 0.07 (n=15 cells) and 0.25 ± 0.05 (n=20 cells) / μm^2, respectively. There was no statistically significant difference between the two groups (p=0.40). (B) Dispergo accelerates the exchange dynamics of COPII at ERES. BSC1 cells transiently expressing Sec31a-GFP were subjected to a FRAP experiment (boxed region) in control or 3 hours after dispergo treatment. The tracings correspond to the mean fluorescence intensity within the boxed region plotted against time. Time = 0 represents the start of FRAP experiment. The half life ($t_{1/2}$) values of the tracings were obtained by exponential fitting. The p value between control and dispergo was 0.06.

Supplementary Figure 3. Intracellular distribution of Golgi markers in cells treated with dispergo.

Cells were treated with DMSO (control) or dispergo for 3 hours, fixed and fluorescently labeled with antibodies specific for different ER or Golgi markers. (A) BSC1 cells transiently expressing GFP-ERGIC53 immunostained for the KDEL receptor. (B) BSC1 cells immunostained for Giantin and GPP130. (C) BSC1 cells immunostained for Vti1a and GPP130. (D) NRK cells immunostained for TGN38 and calreticulin. (E) BSC1 cells immunostained for Golgin97 and GPP130. (F) NRK cells immunostained for β-COP and GM130. The boxes represent enlarged regions indicated in the merge. All images were acquired using wide-field fluorescence microscopy. Scale bars, 10 μm.

Supplementary Figure 4. Dispergo does not affect the endocytic pathway, endosomes, actin and microtubule cytoskeletons but affects the distribution and morphology of mitochondria.

(A) Clathrin dependent endocytosis of transferrin. BSC1 cells were treated with DMSO (control) or dispergo for 2.5 hours and subsequently incubated with transferrin-Alexa Fluor 488 in the presence of DMSO (control) or dispergo for additional 30 min. GM130 was identified by immunostaining in images acquired using wide-field fluorescence microscopy. (B) Dynamics of clathrin coated pit formation. BSC1 cells stably expressing σ2-GFP (to label the endocytic clathrin adaptor AP2) were treated with DMSO (control) or dispergo for 1.5 hours. Images show a kymograph representation as a function of time obtained from a 2D time series acquired at the plasma membrane on bottom of the cell using live cell spinning disk confocal microscopy. (C)

BSC1 cells transiently expressing GFP-Sec61β and RFP-FYVEX3 (to label early endosomes) treated with DMSO (control) or dispergo for 3 hours imaged using live cell spinning disk confocal microscopy. (D, E) BSC1 cells stably expressing GFP-Sec61β treated with DMSO (control) or dispergo for 3 hours and immunostained with an antibody specific for CD63 (to label late endososomes/lysosomes) or with rhodamine-phalloidin (to label actin). (F) BSC1 cells transiently expressing mRFP1-Sec61β and GFP-tubulin were treated with DMSO (control) or dispergo for 3 hours. The images were acquired live by spinning disk confocal microscopy. (G) BSC1 cells stably expressing GFP-Sec61β stained with MitoTracker Red (to label mitochondria) and subsequently treated with DMSO (control) or dispergo for 3 hours. The images were acquired using live cell spinning disk confocal microscopy. Scale bars, 10 μm.

Supplementary Figure 5. Absence of detectable effect of DTT, tunicamycin and brefeldin A on the morphology of the ER.

(A-C) BSC1 cells stably expressing GFP-Sec61β were treated with DMSO (control), DTT or tunicamycin for 24 hours and subsequently fixed and immunostained for calreticulin.

(D) BSC1 cells stably expressing GFP-Sec61β were treated with brefeldin A for 24 hours and subsequently fixed and immunostained for calreticulin

MOVIE LEGENDS

Supplementary Movie 1

Time series corresponding to the upper row of Figure 3A. The display rate is 10 frames per second. The time stamp indicates hour:min:sec.

Supplementary Movie 2

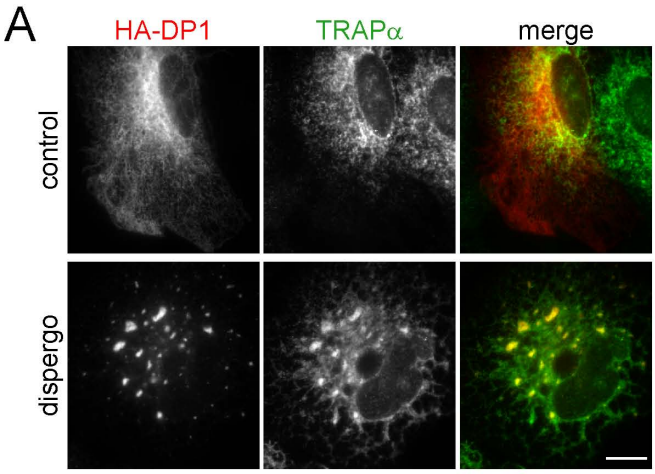
Time series corresponding to the lower row of Figure 3A. The display rate is 10 frames per second. The time stamp indicates hour:min:sec.

Supplementary Movie 3

Time series corresponding to Figure 3D. The ER patch that was photobleached is marked with a blue circle. The display rate is 10 frames per second. The time stamp indicates hour:min:sec.

Supplementary Movie 4

Time series corresponding to Figure 6A. Left panel, GFP-Sec61β; middle panel, GalT-tomato; right panel, merge image with GFP-Sec61β (green) and GalT-tomato (red). The display rate is 10 frames per second. The time stamp indicates hour:min:sec.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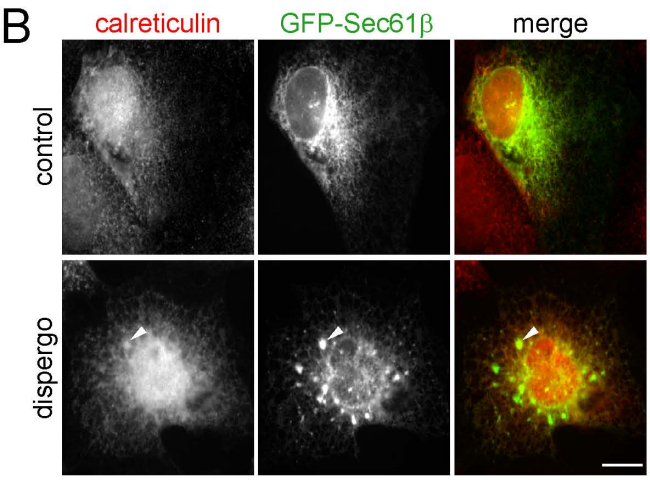
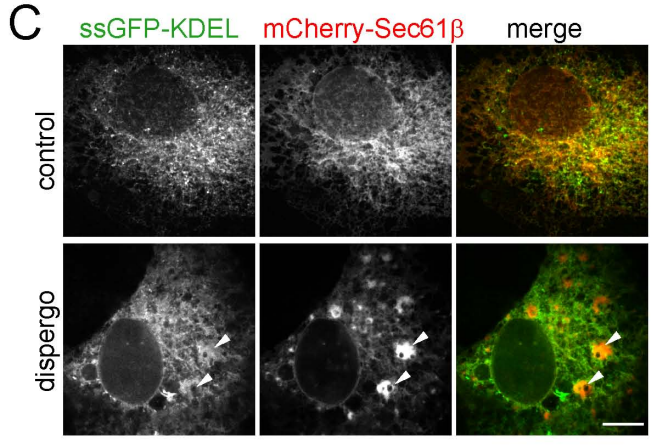
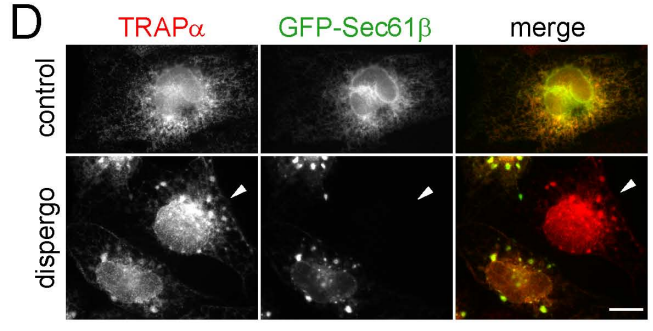
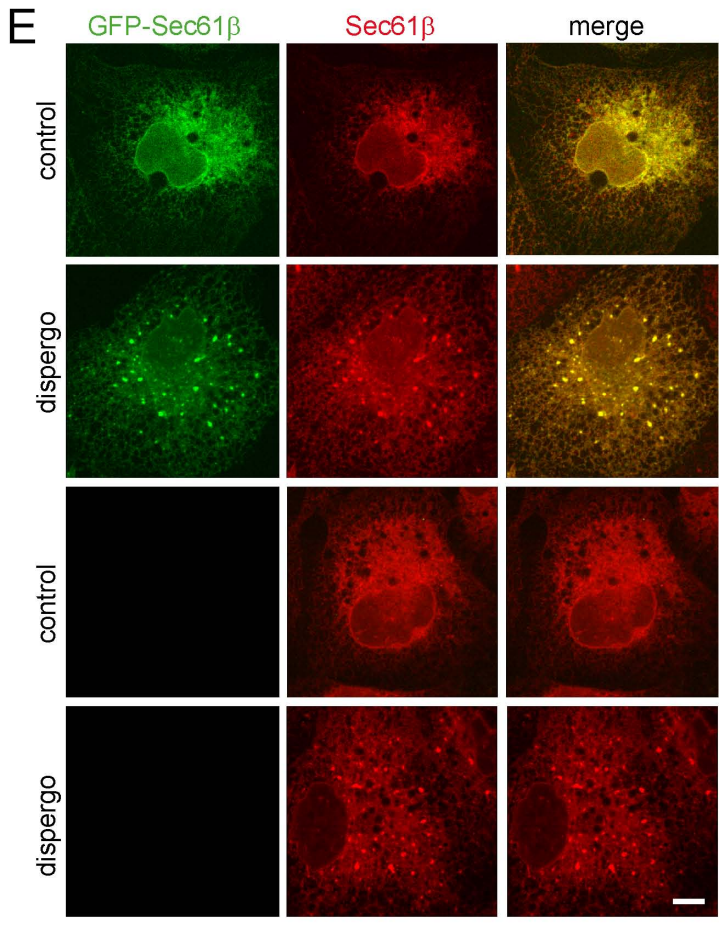
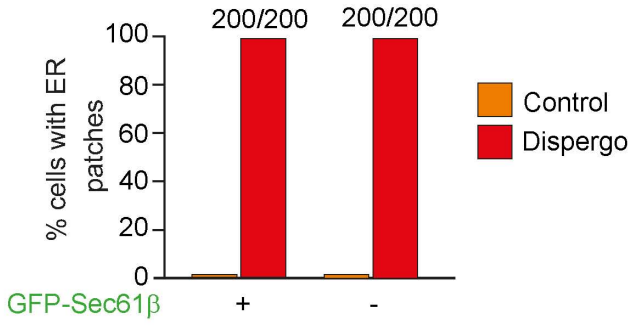
HA-DP1
TRAPα
merge
control
dispergo

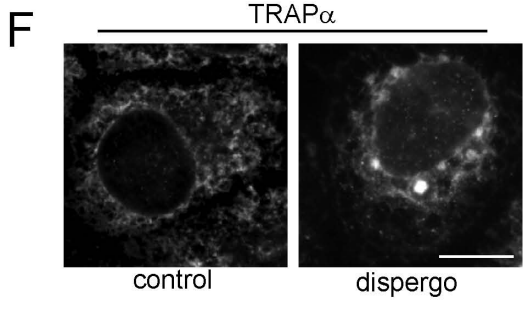

B
calreticulin
GFP-Sec61β
merge
control
dispergo


C
ssGFP-KDEL
mCherry-Sec61β
merge
control
dispergo

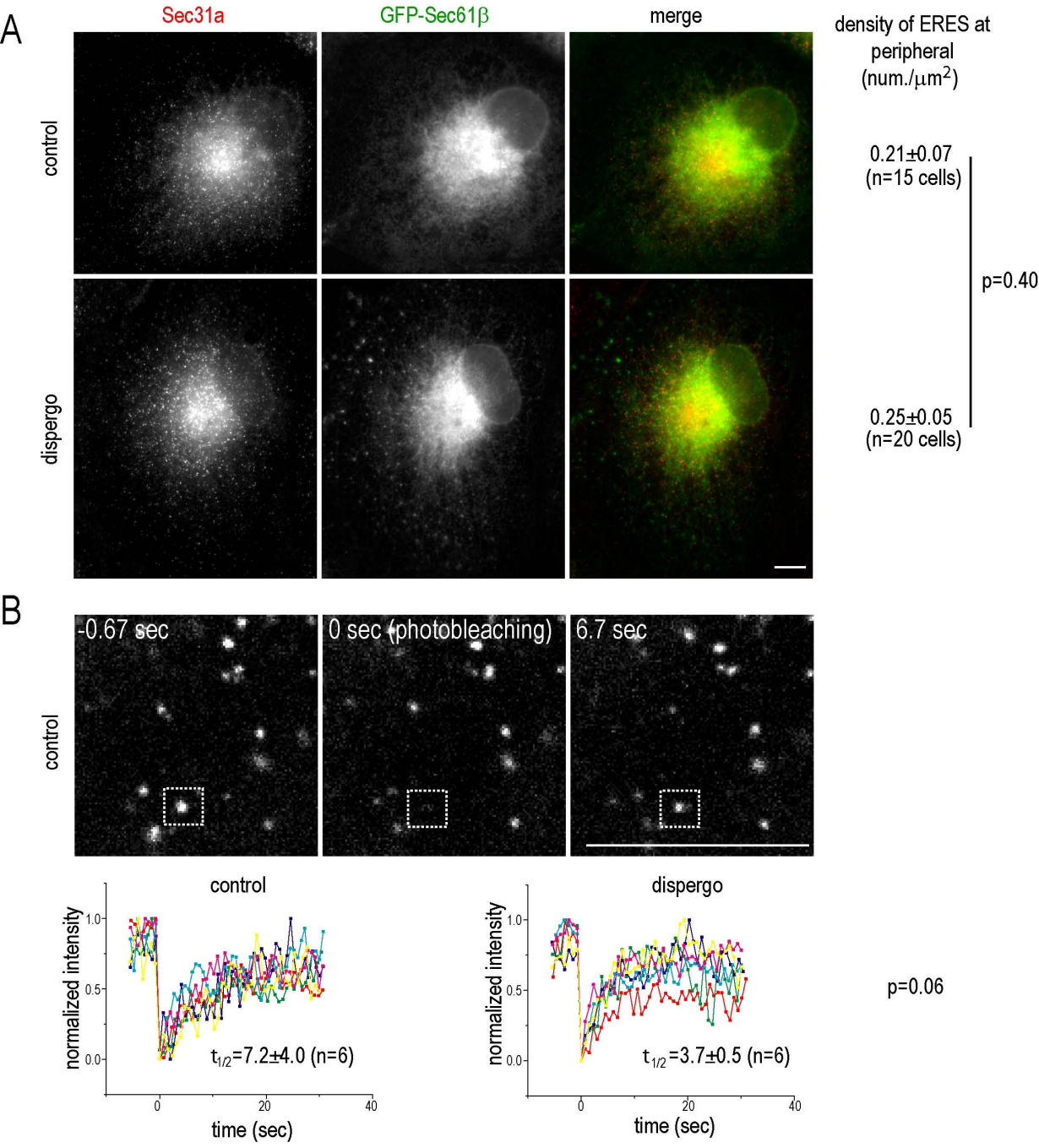

D
TRAPα
GFP-Sec61β
merge
control
dispergo


E
GFP-Sec61β
Sec61β
merge
control
dispergo
control
dispergo

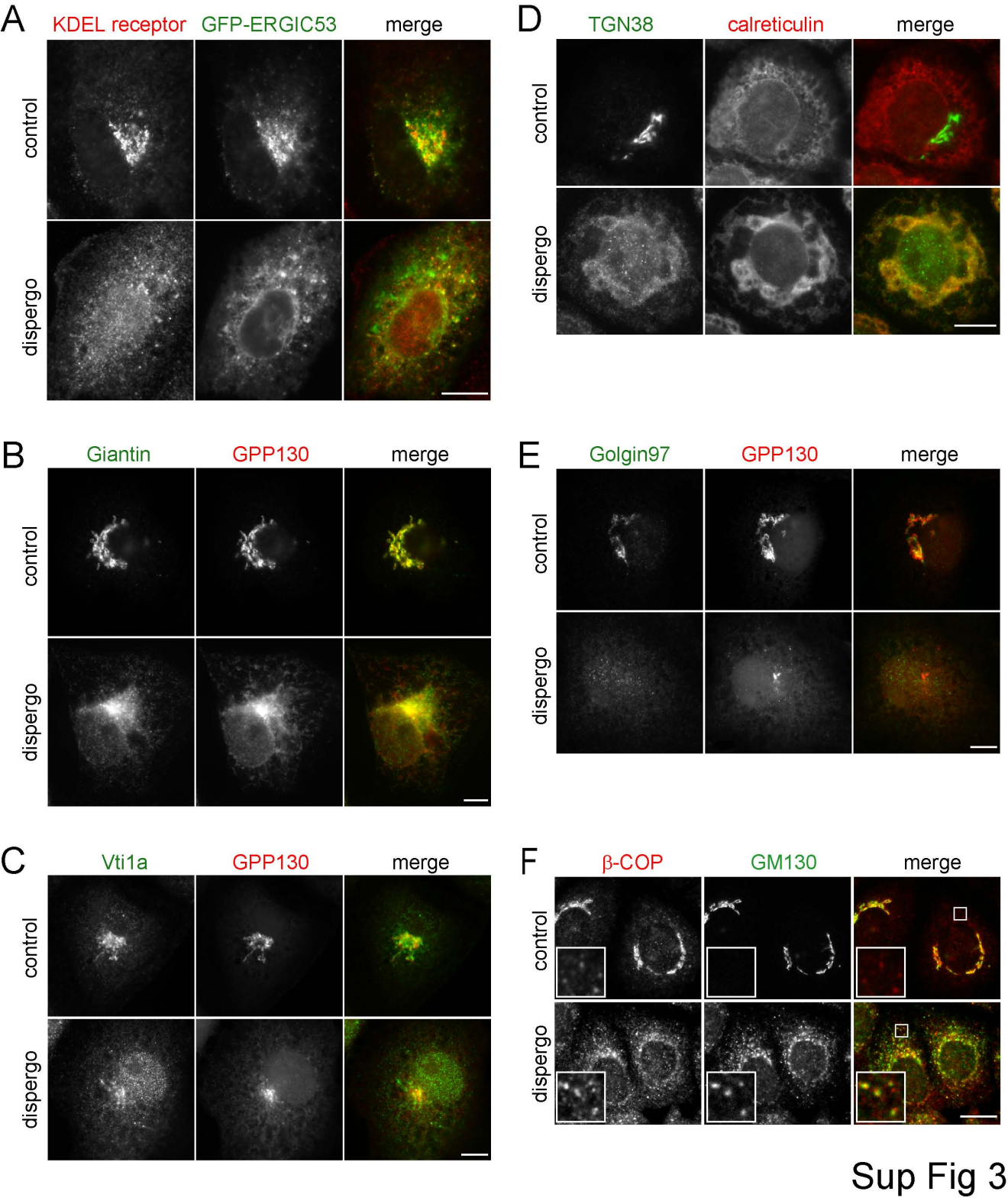

200/200
200/200
100
80
60
40
20
0
% cells with ER patches
Control
Dispergo
GFP-Sec61β
+
-


F
TRAPα
control
dispergo

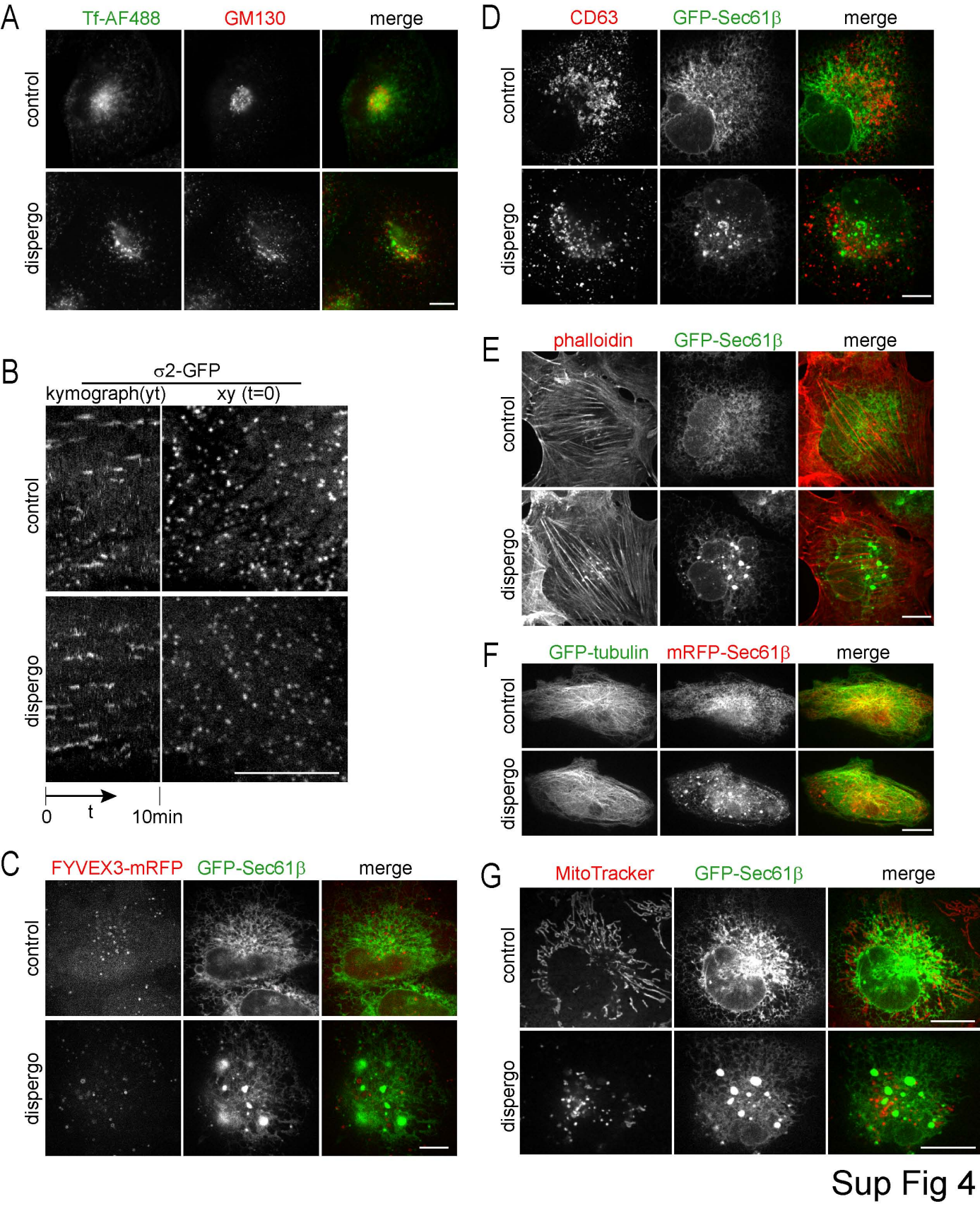
S Fig 1


A
Sec31a
GFP-Sec61β
merge
density of ERES at peripheral (num./μm²)
control
dispergo
0.21±0.07 (n=15 cells)
0.25±0.05 (n=20 cells)
p=0.40
B
-0.67 sec
0 sec (photobleaching)
6.7 sec
control
dispergo
normalized intensity
time (sec)
t1/2=7.2±4.0 (n=6)
t1/2=3.7±0.5 (n=6)
p=0.06

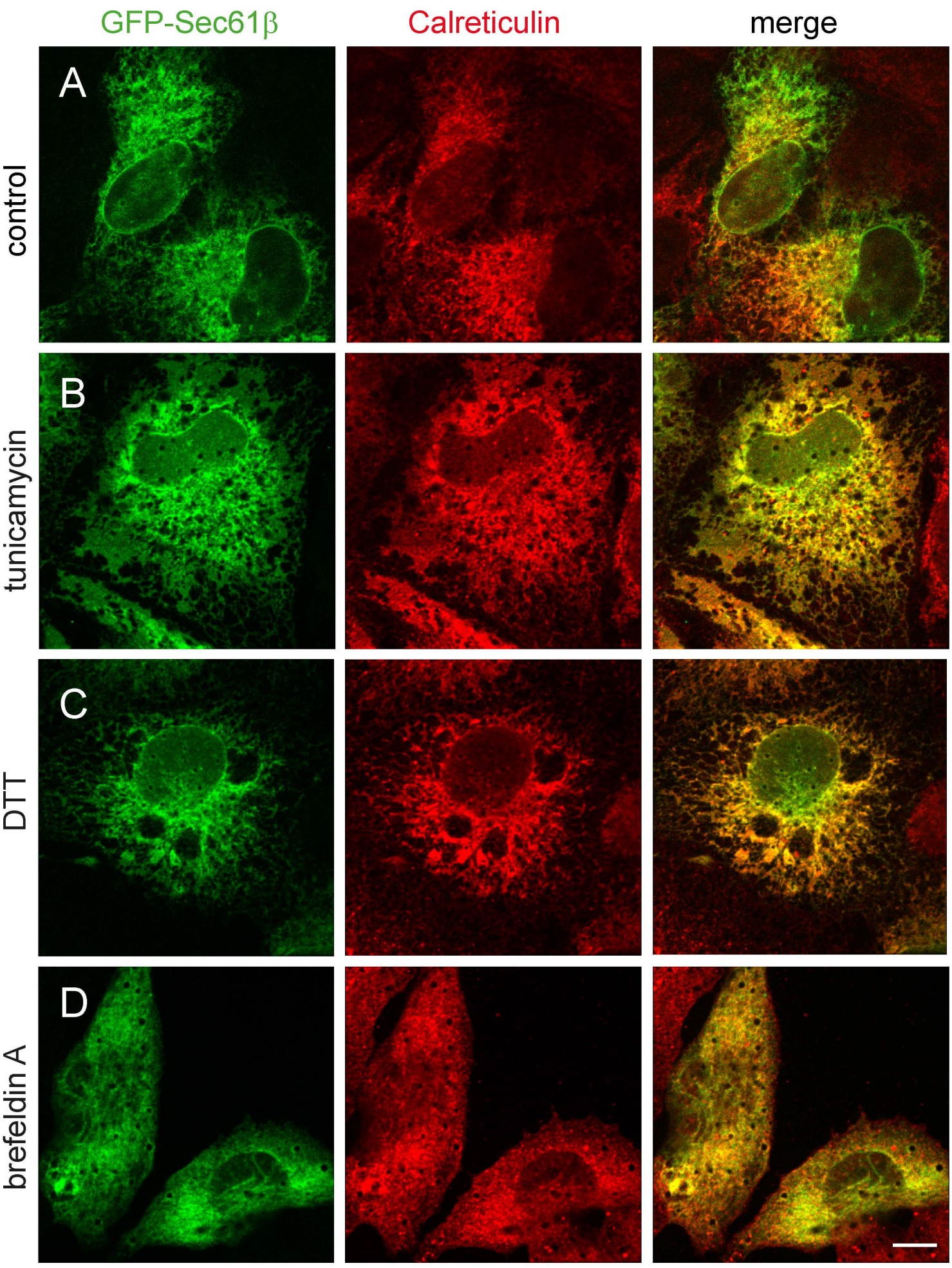
S Fig 2


A
KDEL receptor
GFP-ERGIC53
merge
control
dispergo
B
Giantin
GPP130
merge
control
dispergo
C
Vti1a
GPP130
merge
control
dispergo
D
TGN38
calreticulin
merge
control
dispergo
E
Golgin97
GPP130
merge
control
dispergo
F
β-COP
GM130
merge
control
dispergo

Sup Fig 3


A
Tf-AF488
GM130
merge
control
dispergo
B
σ2-GFP
kymograph(yt)
xy (t=0)
control
dispergo
0
t
10min
C
FYVEX3-mRFP
GFP-Sec61β
merge
control
dispergo
D
CD63
GFP-Sec61β
merge
control
dispergo
E
phalloidin
GFP-Sec61β
merge
control
dispergo
F
GFP-tubulin
mRFP-Sec61β
merge
control
dispergo
G
MitoTracker
GFP-Sec61β
merge
control
dispergo

Sup Fig 4


GFP-Sec61β
Calreticulin
merge
A
control
B
tunicamycin
C
DTT
D
brefeldin A

Sup Fig 5