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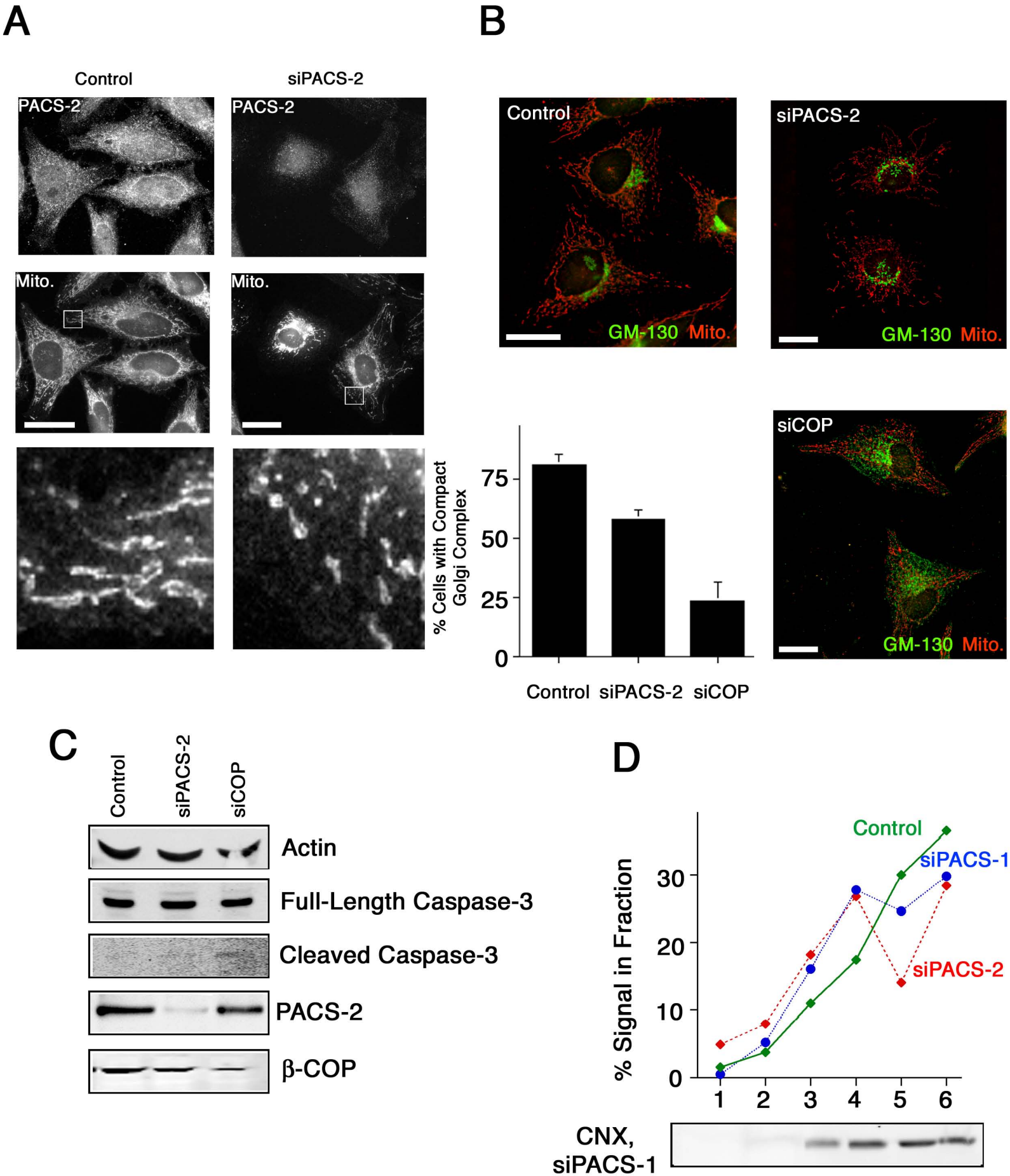
Supplemental Figure 1. A. Mitochondria morphology and PACS-2 signal in control and PACS-2 depleted cells. HeLa cells were transiently depleted of PACS-2 and imaged two days after transfection. Cells were loaded with Mitotracker and immunostained with anti-PACS-2. Scale bar = 25 μm. B. Golgi morphology upon PACS-2 and β-COP knockdown. HeLa cells were transiently depleted of PACS-2 or β-COP and imaged two days after transfection. Cells were loaded with Mitotracker and immunostained with anti-GM-130. Scale bar = 25 μm. Large fields (n = 3) containing about 100 cells each were assayed for compact Golgi morphology, as seen under control conditions. C. Toxicity of PACS-2 and β-COP knockdown. HeLa cells were depleted of PACS-2 or β-COP as in A and probed by Western blot for cleaved caspase-3 (p19), full-length caspase-3, PACS-2 and β-COP. D. OPTIPREP calnexin fractionation upon PACS-1 knockdown. Cells were depleted of PACS-1 as in Figure 4A and assayed for calnexin. The diagram shows the summary of the fractionation of calnexin control cells (green), PACS-1 depleted cells (blue) and PACS-2 depleted cells (red), without error bars to increase clarity.

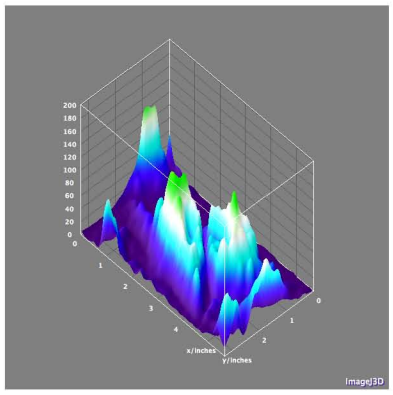
Supplemental Figure 2. Quantification of immunofluorescence images used in Figure 3. A. Images used for Figure 3, as indicated, were transformed into 3D surface heat maps using ImageJ, which allows the correlation of exposure to colors. B. Quantification of the gradient of staining of 8 individual cells each for every condition indicated. HeLa cells were treated as indicated in Figure 3 and the gradient of staining was assayed as indicated in Materials and Methods. $P<0.0001$ between CNX control and CNX siPACS-2.

Supplemental Figure 3. A. Binding of calnexin constructs to PACS-2 FBR. PACS-2 (FBR) was incubated with GST-tagged calnexin tail constructs (wt, SSAA, SSDD) and bound GST-tagged molecules were detected by Western blot using anti-GST antibodies. Equal loading was verified by Ponceau. B. Cell surface calnexin upon 2h incubation with 10 mM β-mercaptoethanol. A7 cells were assayed for cell surface calnexin. Irrelevant intervening bands were cut out.

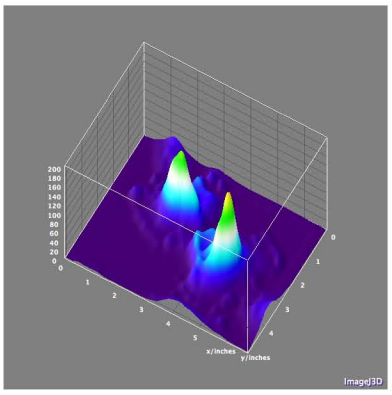


Myhill et al.,
Supplemental Figure 1

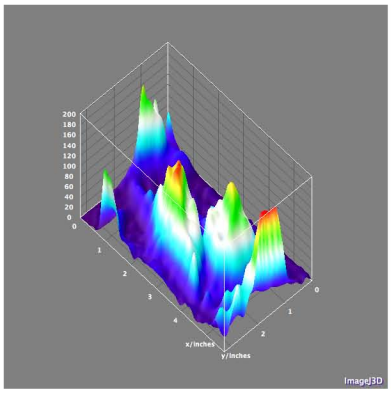
A



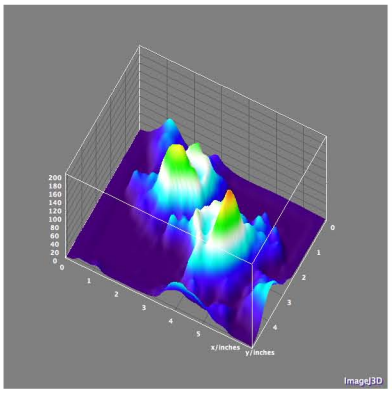
CNX, Figure 3A



CNX, Figure 3E

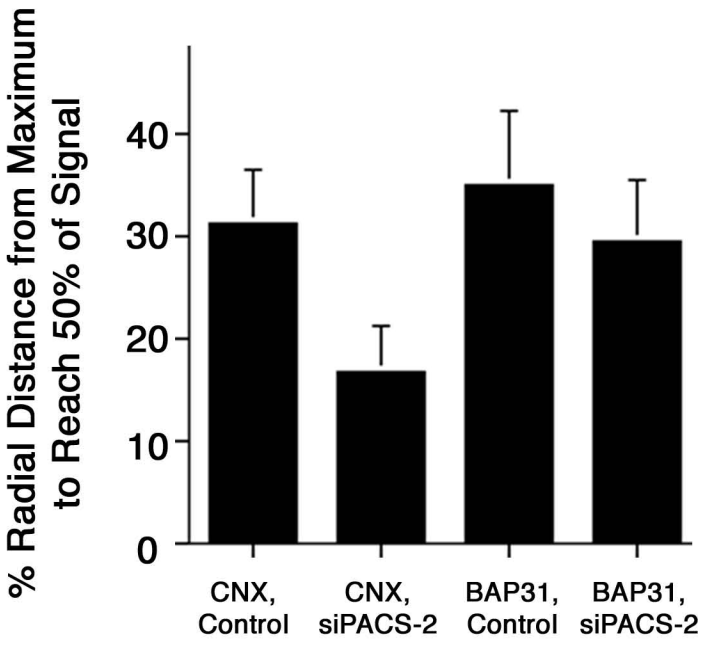


BAP31, Figure 3C

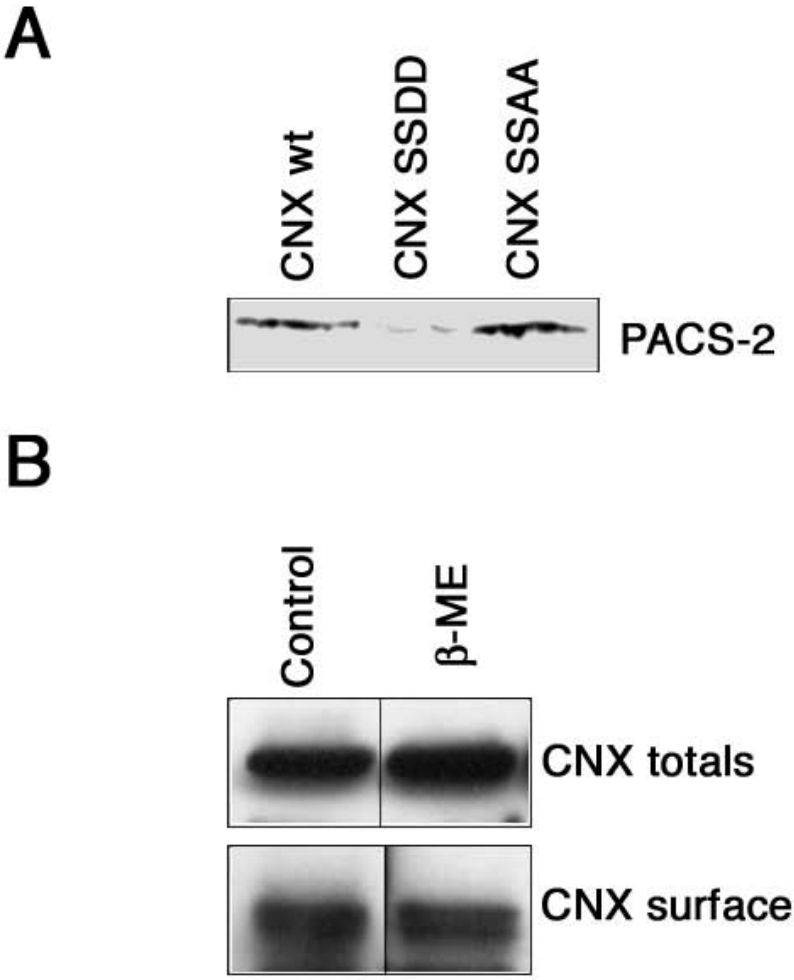


BAP31, Figure 3G

B



Myhill et al.,
Supplemental Figure 2



Myhill et al., Supplemental Figure 3