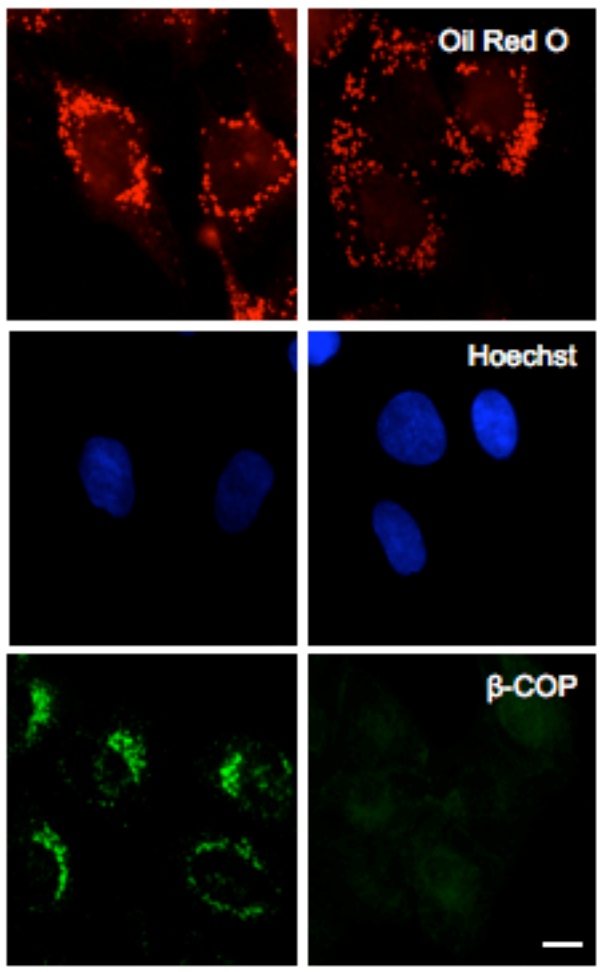
Figure 1

A.


Oil Red O
Hoechst
β-COP
DMSO
Exo2

B.

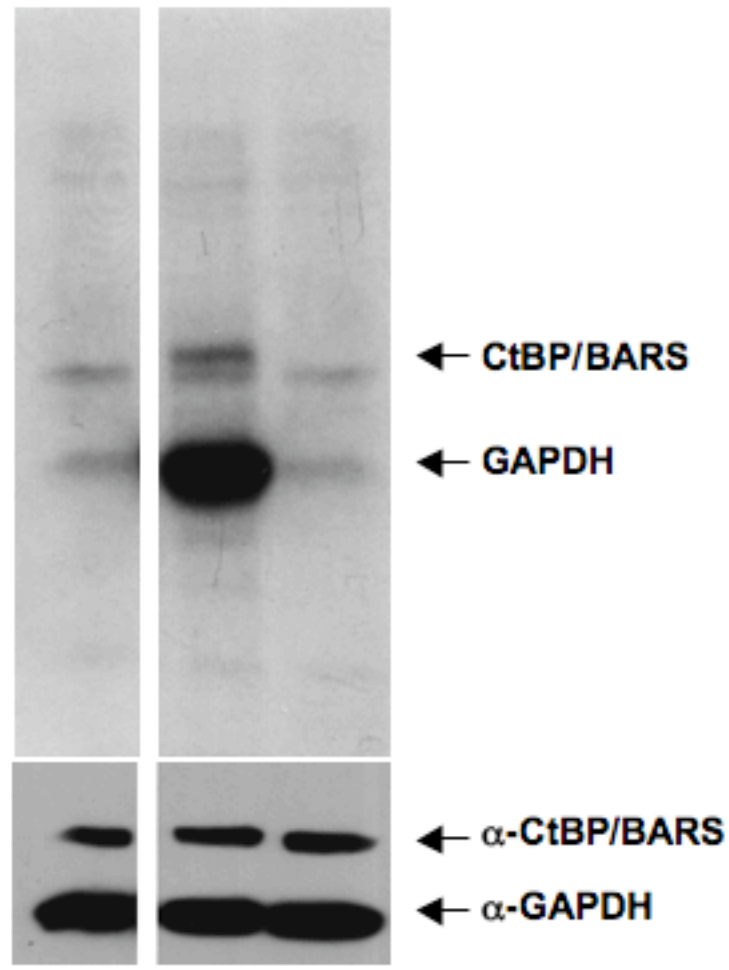

CtBP/BARS
GAPDH
α-CtBP/BARS
α-GAPDH
1
2
3

Figure 2

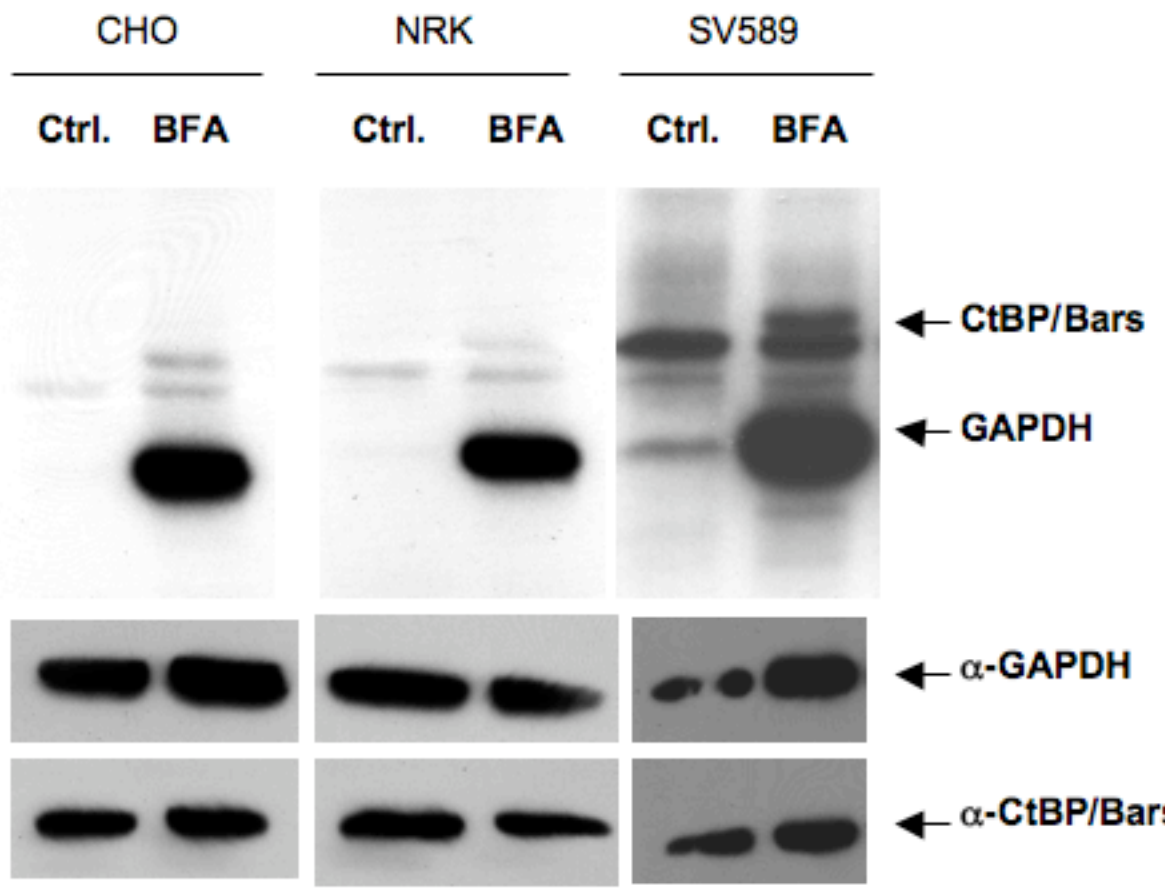
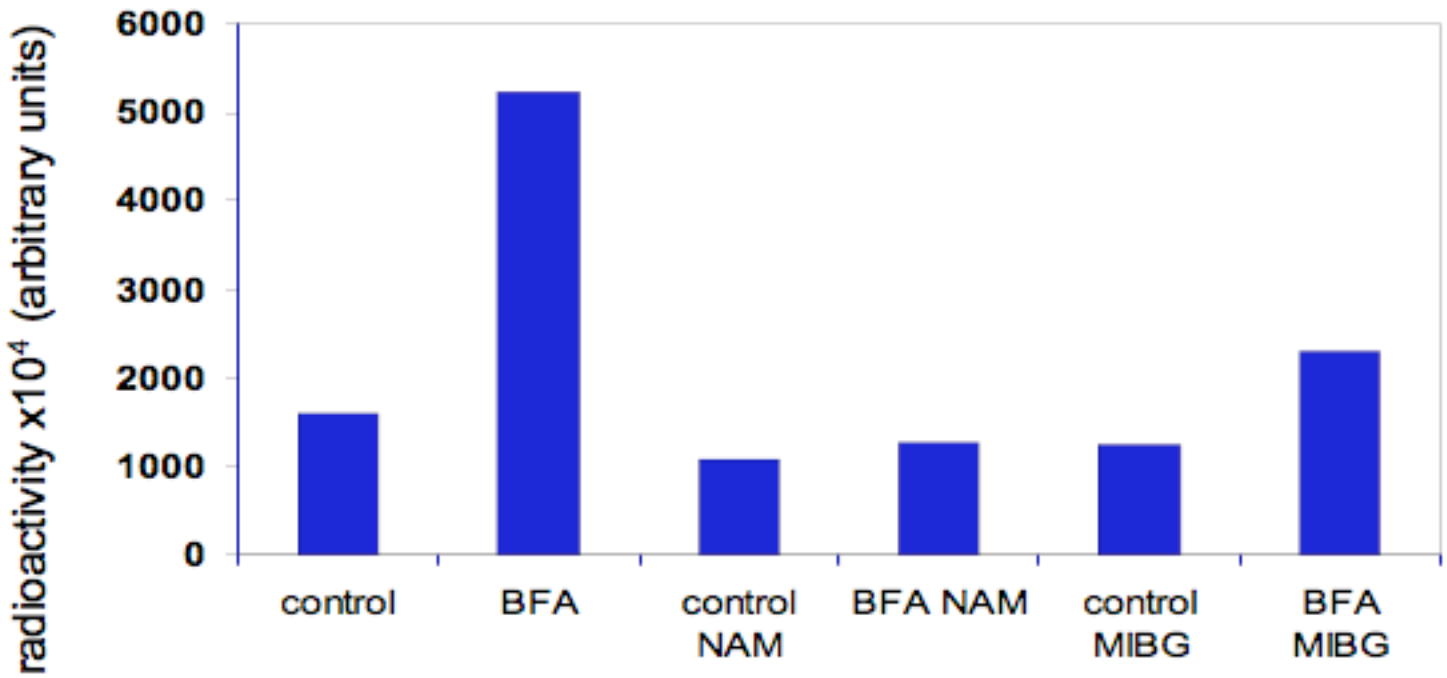

CHO
NRK
SV589
Ctrl.
BFA
Ctrl.
BFA
Ctrl.
BFA
CtBP/Bars
GAPDH
α-GAPDH
α-CtBP/Bars

Figure 3


radioactivity x10⁴ (arbitrary units)
6000
5000
4000
3000
2000
1000
0
control
BFA
control NAM
BFA NAM
control MIBG
BFA MIBG

Supplemental Movie BFA does not inhibit the uptake of free fatty acids. NRK cells were incubated in the presence of 100 μM oleate plus 2 μg/ml BFA in CO_2 independent media for up to 18 h at 37°C. The original DIC images were taken at 1 frame per minute for 12 h after the start of incubation.

Supplemental Figure 1 Lipid loss does not occur in response to Exo2 induced Golgi fragmentation. A) NRK cells were treated with 100 μM oleate for 24 hrs, washed and then incubated in the presence of 2 μg/ml Exo2 or DMSO carrier for 12 h at 37°C. Cells were fixed and stained for neutral lipids with oil red O or DNA with Hoechst dye (upper panel). To confirm that Exo2 caused Golgi fragmentation, another coverslip from the same experiment was fixed, permeabilized and stained with antibodies against βCOP (lower panel). Scale bar =10 μm. B) cytosol from CHO cells containing [^{32}P-NAD] was either treated with DMSO (control, lane 1), incubated in the presence of 60 μg/ml BFA (lane 2) or 60 μg/ml Exo2 (lane 3) for 2 hr at 37°C. Membranes and cytosol were separated by centrifugation. The supernatant fraction was separated by SDS-PAGE, transferred to PVDF-membrane and processed for autoradiography. The PVDF-membrane was then processed for detection of GAPDH and CtBP1/BARS by immunoblotting.

Supplemental Figure 2 BFA stimulates ribosylation of CtBP/BARS and GAPDH in various cell lines. Cytosol from cells as indicated (0.3 mg/ml for CHO and NRK, 0.5 mg/ml for SV589) containing [^{32}P-NAD] were either treated with DMSO (Ctrl.) or 60 μg/ml BFA for 2 hr at 37°C. Membranes and cytosol were separated by centrifugation. The supernatant fraction was separated by SDS-PAGE, transferred to PVDF-membrane and processed for autoradiography. The PVDF-membrane was then processed for detection of GAPDH and CtBP1/BARS by immunoblotting.

Supplemental Figure 3 Quantification of BARS ribosylation. Samples from Fig 6A were processed for SDS-PAGE and transferred onto PVDF-membrane as described earlier. Membranes were exposed to a storage phosphor screen for 24 hr. Radioactivity was then visualized using a Phospho imager (Typhoon 9410 Image analysis; GE Healthcare, Piscataway, NJ) and quantified using ImageQuant TL software (GE Healthcare).