E08-04-0373 Melancon

Supplementary Figure 1. Class I and II Arfs localize to the Golgi complex and peripheral puncta in HeLa cells. HeLa cells were transfected with plasmids encoding GFP-tagged forms of either Arfs 1, 3, 4 and 5 as described and imaged for GFP signal. Single frames of a movie acquired from live cells are shown. Bar represent 5 μm for images acquired with a 100X objective. Bar for the magnified images represent 1 μm.

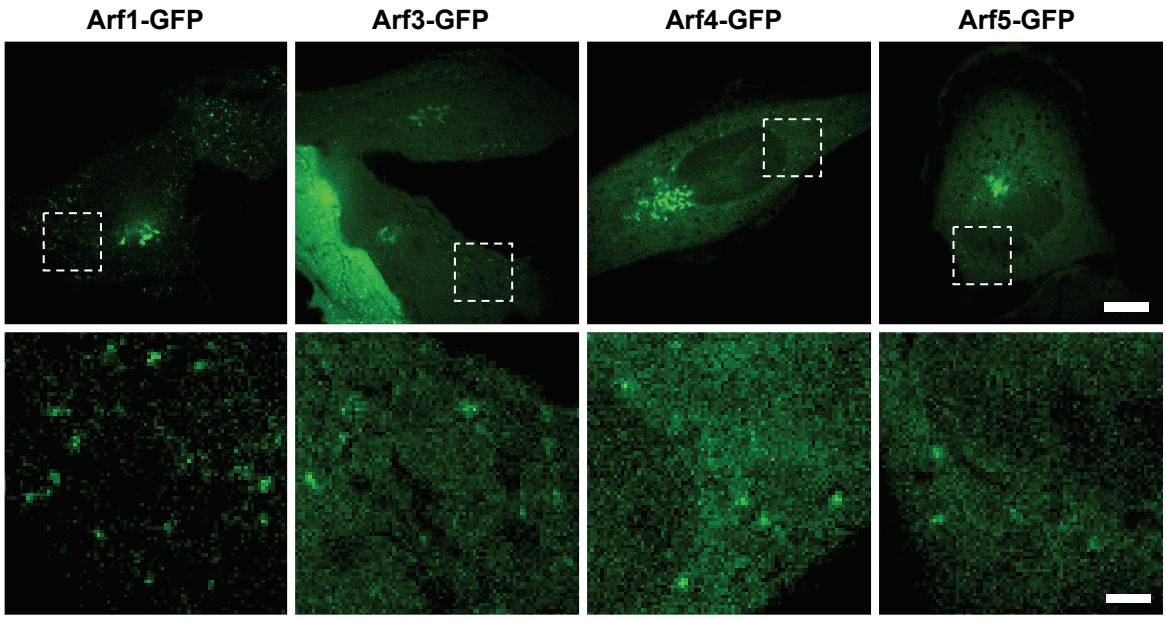
Supplementary Figure 2. Arf4, but not Arf1, remains associated with the ERGIC after treatment with nocodazole and BFA.
COS1 cells co-transfected with plasmids encoding Arf1-GFP and Arf4-mCherry were incubated on ice for 2 min followed by treatment with 20 μg/ml NOZ for 15 min on ice. Several minutes (5-10 min) after transfer to a heated stage, cells were treated with BFA and imaged every five seconds for 10 min. Images correspond to frames captured at the indicated time points. Bottom panels show single and merged images acquired in the boxed area from the mCherry and GFP channels. Bar, 10 μm.

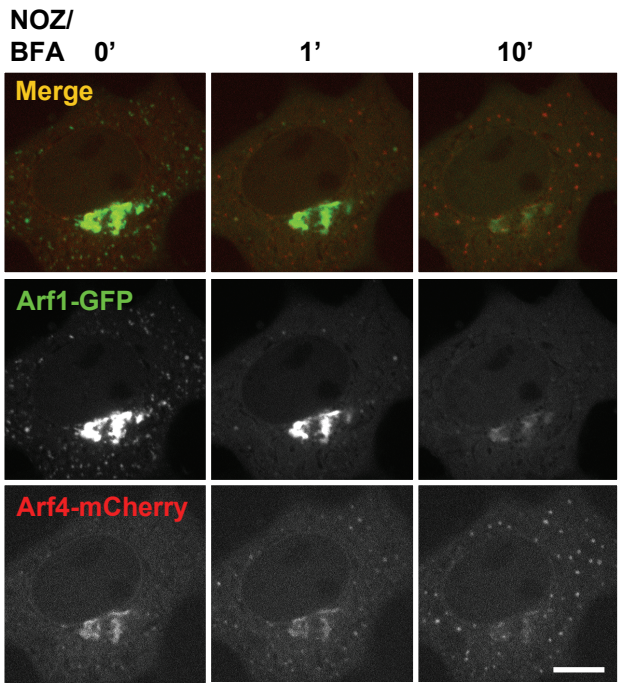
Supplementary Figure 3. Exo1 causes rapid accumulation of endogenous GBF1 but not BIG1 onto ERGIC membranes.
NRK, HeLa and COS1 cells were treated with carrier DMSO or 100 μM Exo1 for 90 sec, fixed and processed for IF using a mixture of anti-GBF1 monoclonal antibody and anti-BIG1 polyclonal antibody as described in Methods. All images were acquired and processed identically. Bar, 20 μm.

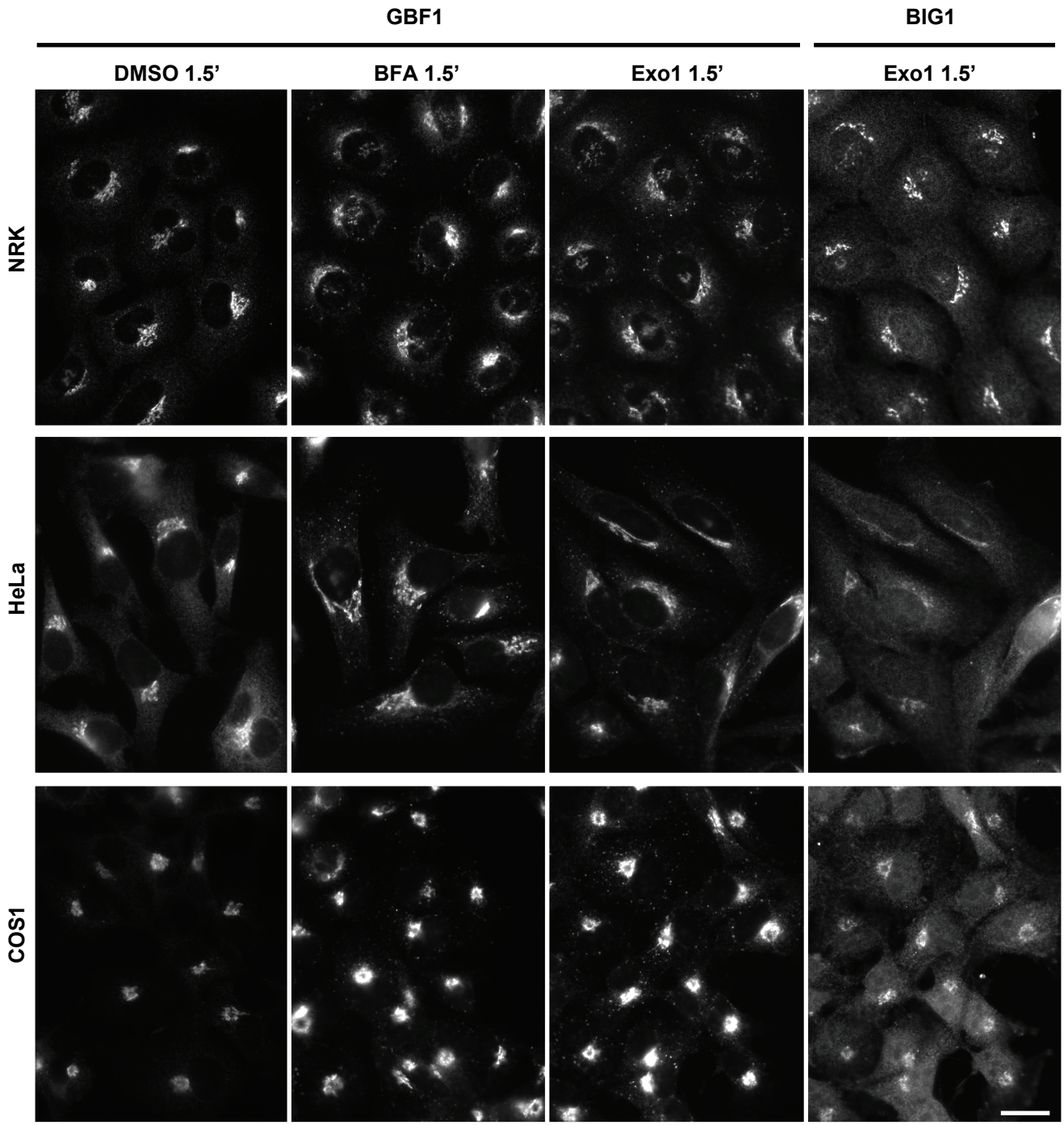
Supplementary Figure 4. Exo1 causes rapid loss of Arfs from Golgi membranes but leads to eventual concentration of GFP-GBF1 and class II Arfs at the ERGIC.
NRK-GFP-GBF1 cells transfected with plasmids encoding Arfs tagged with mCherry were treated with carrier DMSO or 100 μM Exo1 and imaged for 20 min as described in Methods. Images correspond to single frames captured at the indicated time points. Bar, 10 μm.

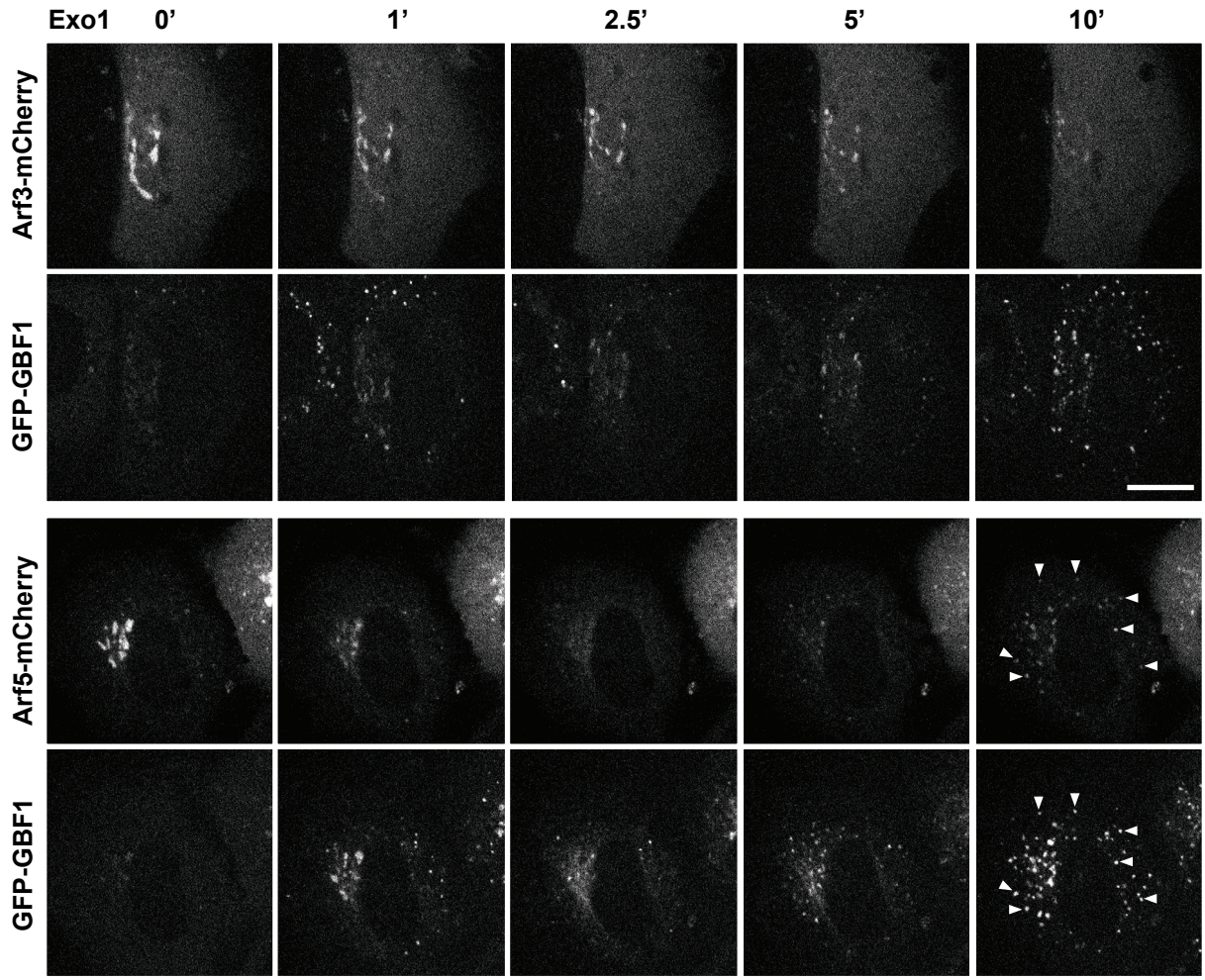
Supplementary Figure 5. Arf1(T31N) and Arf5(T31N), but not Arf4(T31N), disrupt the Golgi complex.
NRK cells co-transfected with plasmids encoding the indicated wild-type or mutant Arfs tagged with GFP (green label) or mCherry (red label) were fixed and processed for IF as described in Methods. The two rightmost panels display the green and red channels of the same field. Whereas Arf1(T31N)

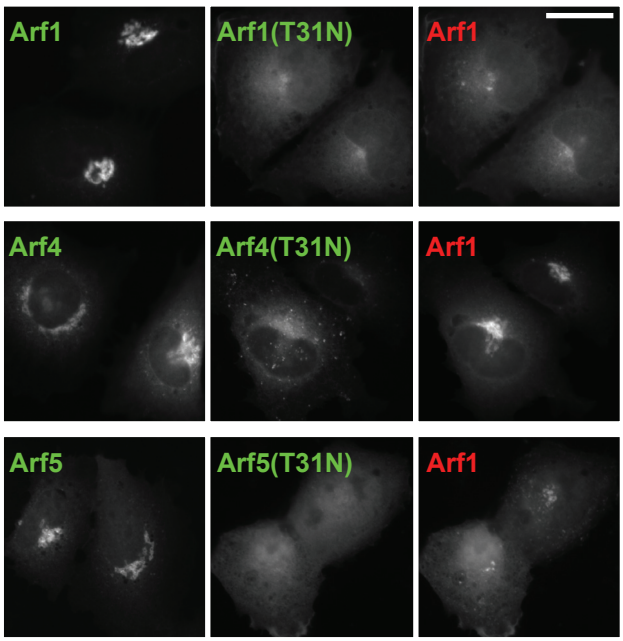
expression caused disappearance of a detectable Golgi complex in all transfectants examined, Arf4(T31N) expression had no detectable impact in any transfectant. Expression of Arf5(T31N) caused intermediate effect with about 50% of transfectants showing Golgi dispersal. Bar, 20 μm.


Arf1-GFP
Arf3-GFP
Arf4-GFP
Arf5-GFP


NOZ/
BFA 0’
1’
10’
Merge
Arf1-GFP
Arf4-mCherry


GBF1
BIG1
DMSO 1.5'
BFA 1.5'
Exo1 1.5'
Exo1 1.5'
NRK
HeLa
COS1


Exo1
0’
1’
2.5’
5’
10’
Arf3-mCherry
GFP-GBF1
Arf5-mCherry
GFP-GBF1


Arf1
Arf1(T31N)
Arf1
Arf4
Arf4(T31N)
Arf1
Arf5
Arf5(T31N)
Arf1