Supplementary Table 1

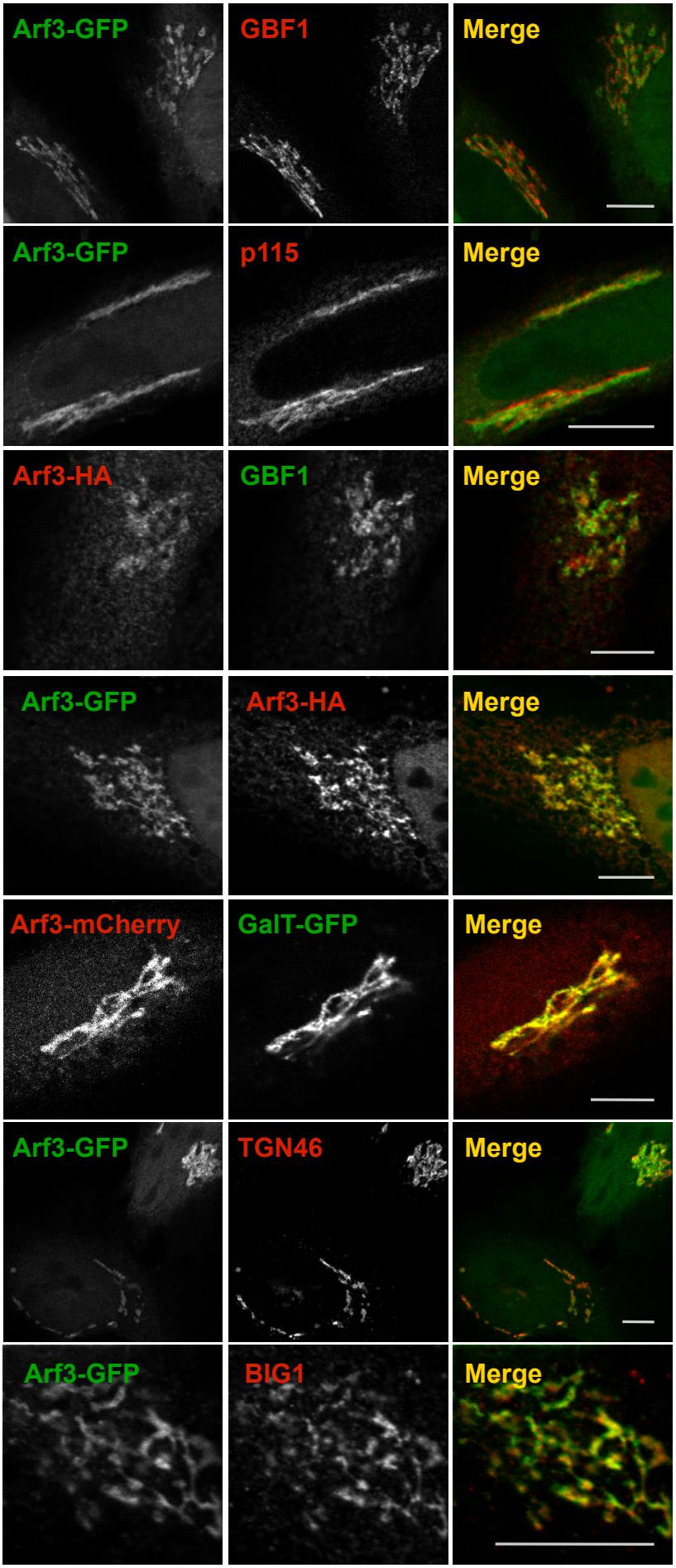
Organism	Classification	Accession number	Abbreviation
Bos taurus	Arf1	NP_788826	BosArf1
Bos taurus	Arf2	NP_777114	BosArf2
Bos taurus	Arf3	NP_001015571	BostArf3
Caenorhabditis elegans	ArfI	NP_498235	CeArfI
Caenorhabditis elegans	ArfII	NP_501336	CeArfII
Caenorhabditis elegans	ArfIII	NP_503011	CeArfIII
Canis lupus	Arf1	XP_531820	ClArf1
Canis lupus	Arf2	XP_537606	ClArf2
Canis lupus	Arf3	XP_543688	ClArf3
Ciona Intestinalis	ArfI-a	XP_002119700	Ci19700
Ciona Intestinalis	ArfI-b	XP_002125592	Ci25592
Ciona Intestinalis	ArfII	XP_002130554	Ci30554
Ciona Intestinalis	ArfIII-a	XP_002129954	Ci29954
Ciona Intestinalis	ArfIII-b	XP_002129929	Ci29929
Danio rerio	ArfI-a	NP_958888	Dr8888
Danio rerio	ArfI-b	NP_958860	Dr8860
Danio rerio	ArfI-c	AAH66632	Dr6632
Danio rerio	ArfI-d	NP_958912	Dr8912
Danio rerio	Arf3-a	NP_001012248	Dr2248
Danio rerio	Arf3-b	NP_001003441	Dr3441
Drosophila melanogaster	ArfI	NP_476955	DmArf79F
Drosophila melanogaster	ArfII	NP_524631	DmArf102F
Drosophila melanogaster	ArfIII	NP_523751	DmArf51F
Gallus gallus	Arf1	NP_001006352	GgArf1
Gallus gallus	Arf4	XP_001232784	GgArf4
Gallus gallus	Arf5	NP_990656	GgArf5
Gallus gallus	Arf6	NP_001075174	GgArf6
Homo sapiens	Arf1	NP_001649	HsArf1
Homo sapiens	Arf3	NP_001650	HsArf3
Homo sapiens	Arf4	NP_001651	HsArf4
Homo sapiens	Arf5	NP_001653	HsArf5
Homo sapiens	Arf6	NP_001654	HsArf6
Maccaca mulatta	Arf1-a	XP_001106772	MmuArf1
Maccaca mulatta	Arf3	XP_001104727	MmuArf3

Maccaca mulatta	Arf1-b	XP_001105536	MmuArf3x
Monosiga brevicolis	ArfI	XP_001748945	MonArfI
Monosiga brevicolis	ArfII	XP_001749883	MonArfII
Monosiga brevicolis	ArfIII	XP_001743406	MonArfIII
Mus musculus	Arf1	NP_031502	MusmArf1
Mus musculus	Arf2	NP_031503	MusmArf2
Mus musculus	Arf3	NP_031504	MusmArf3
Mus musculus	Arf4	NP_031505	MusmArf4
Mus musculus	Arf5	NP_031506	MusmArf5
Mus musculus	Arf6	NP_031507	MusmArf6
Nematostella vectensis	ArfI/II-a	XP_001634219	Nem2285
Nematostella vectensis	ArfI/II-b	XP_001640423	Nem8733
Nematostella vectensis	ArfIII-a	XP_001632921	Nem6955
Nematostella vectensis	ArfI/II-c	XP_001634746	Nem6810
Nematostella vectensis	ArfI/II-d	XP_001640422	Nem8132
Nematostella vectensis	ArfI/II-e	XP_001640455	Nem8803
Nematostella vectensis	ArfI/II-f	XP_001628436	Nem1050
Nematostella vectensis	ArfIII-b	XP_001632965	Nem6872
Ornithorhynchus anatinus	Arf3	XP_001516517	Orn16517
Ornithorhynchus anatinus	Arf4	XP_001511094	Orn11094
Ornithorhynchus anatinus	ArfIII-a	XP_001514159	Orn14159
Ornithorhynchus anatinus	ArfIII-b	XP_001512800	Orn12800
Pan troglodytes	Arf1	XP_513698	PantArf1
Pan troglodytes	Arf3	XP_509036	PantArf3
Pan troglodytes	Arf4	XP_001173782	PantArf4
Pan troglodytes	Arf6	XP_509935	PantArf6
Ratus norvegicus	Arf1	NP_071963	RnArf1
Ratus norvegicus	Arf2	NP_077064	RnArf2
Ratus norvegicus	Arf3	NP_543180	RnArf3
Saccharomyces cerevisiae	Arf I/II-1	NP_010089	ScArf 1
Saccharomyces cerevisiae	ArfI/II-2	NP_010144	ScArf 2
Saccharomyces cerevisiae	ArfIII	NP_014737	ScArf3
Salmo salar	ArfI-a	ACI33932	SsArf1
Salmo salar	ArfI-b	ACH70742	SsArf2
Salmo salar	Arf3	NP_001133306	SsArf3
Salmo salar	ArfII-a	ACI33187	SsArf4
Salmo salar	ArfII-b	ACM09449	SsArf4b
Salmo salar	Arf6	ACI33167	SsArf6

Schizosaccharomyces pombe	ArfI/II-1	NP_596118	Sp596118
Schizosaccharomyces pombe	ArfI/II-2	NP_596822	Sp596822
Schizosaccharomyces pombe	ArfIII	NP_593036	Sp593036
Strongylocentrotus purpuratus	Arf I/II-a	XP_791037	Str91037
Strongylocentrotus purpuratus	Arf I/II-b	XP_783520	Str83520
Strongylocentrotus purpuratus	Arf I/II-c	XP_787374	Str87374
Strongylocentrotus purpuratus	ArfIII-a	XP_001191776	Str191776
Strongylocentrotus purpuratus	ArfIII-b	XP_796400	Str96400
Taeniopygia guttata	Arf1	XP_002194825	Tae4825
Taeniopygia guttata	Arf4	XP_002193649	Tae3649
Taeniopygia guttata	Arf6	XP_002200661	Tae0061
Trichoplax adhaerens	ArfI	XP_002110783	Tric63238
Trichoplax adhaerens	ArfII	XP_002108209	Tric19616
Trichoplax adhaerens	ArfIII	XP_002107960	Tric49674
Xenopus laevis	ArfI	NP_001080474	XlArf1
Xenopus laevis	Arf3	NP_001086694	XlArf3
Xenopus laevis	ArfII	NP_001082540	XlArf4
Xenopus laevis	Arf6	NP_001086677	XlArf6

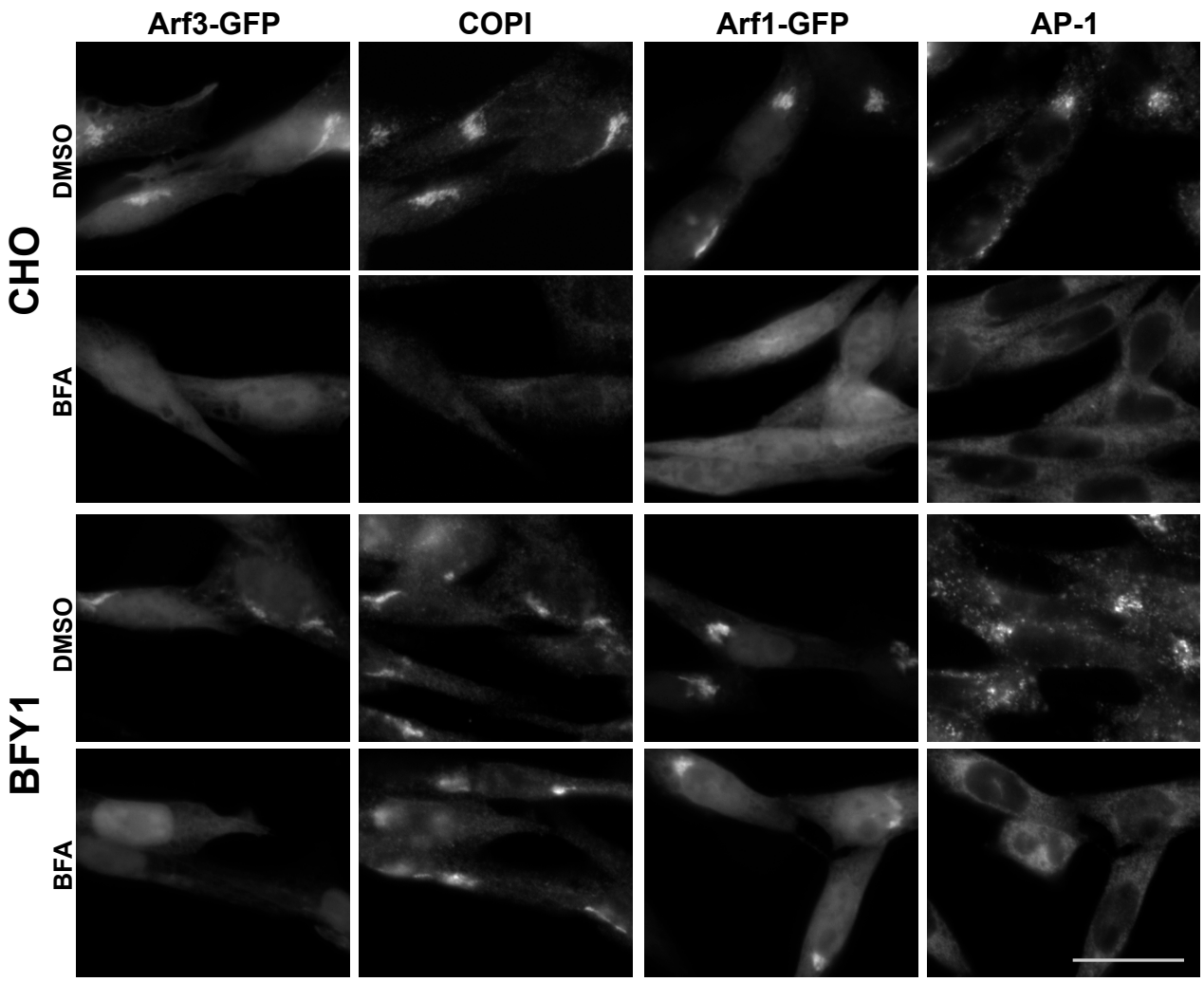
Information for sequences used in phylogenetic analyses.

Information for all sequences used in all phylogenetic analyses (including the initial analyses not shown) is provided by organism name, our assessment of the classification of Arf homology, accession number and abbreviation used in the dataset used for analysis.



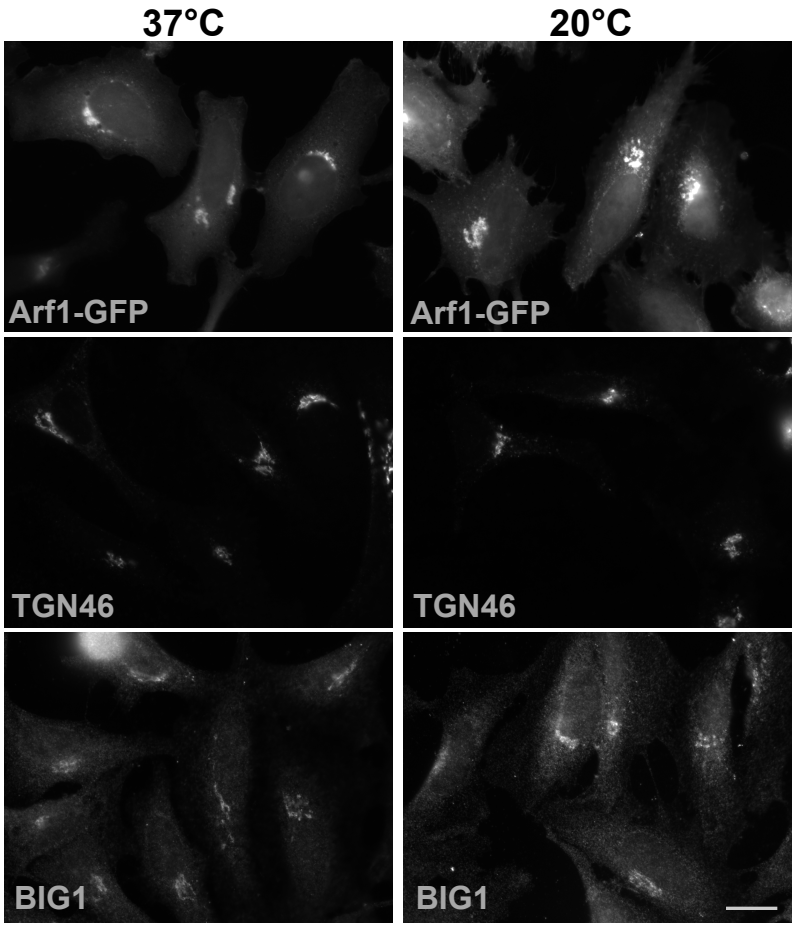
Supplementary Figure 1. Arf3 also localizes to the *trans*-side of the Golgi complex in HeLa cells, regardless of the tag.

HeLa cells were transfected with plasmids encoding Arf3-GFP, Arf3-mCherry, Arf3-HA and/or GalT-GFP as indicated. Fixed cells were stained for the specified markers and images were acquired using a confocal microscope. Representative images selected from at least 3 separate experiments are shown. Although red and green signal vary in intensity, individual channels reveal different (first 3 set of panels) or similar (last 4 set of panels) patterns. Bar, 10μm.

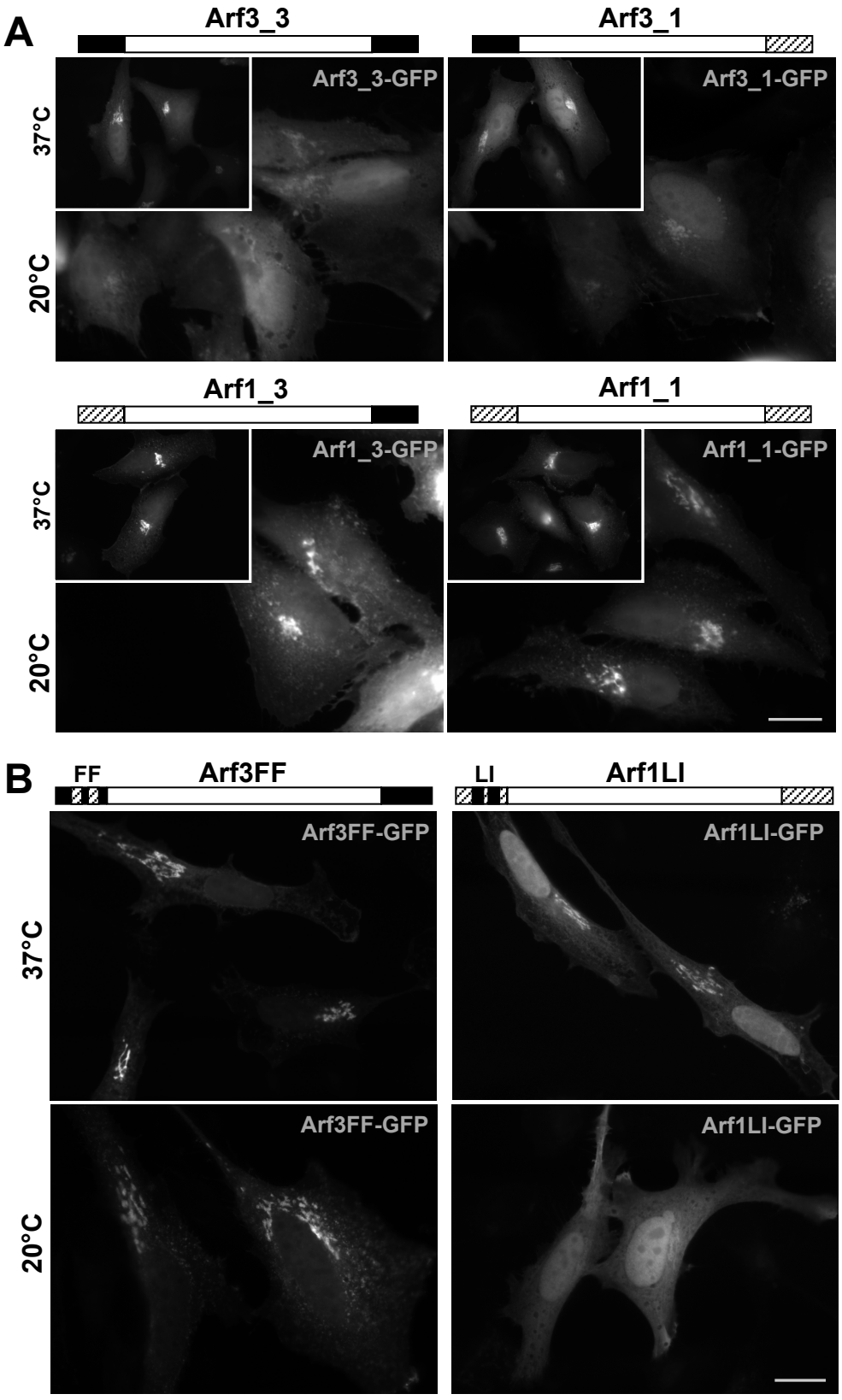


Supplementary Figure 2. Membrane recruitment of Arf3 remains BFA sensitive in BFY1 cells.

CHO and BFY1 cells were transfected with a plasmid encoding Arf3-GFP or Arf1-GFP. After 24 hours, cells were treated with 5μg/ml BFA for 2 min and then fixed and stained for COPI or AP-1. Representative epifluorescence images are shown selected from at least 2 separate experiments. Bar, 20μm.

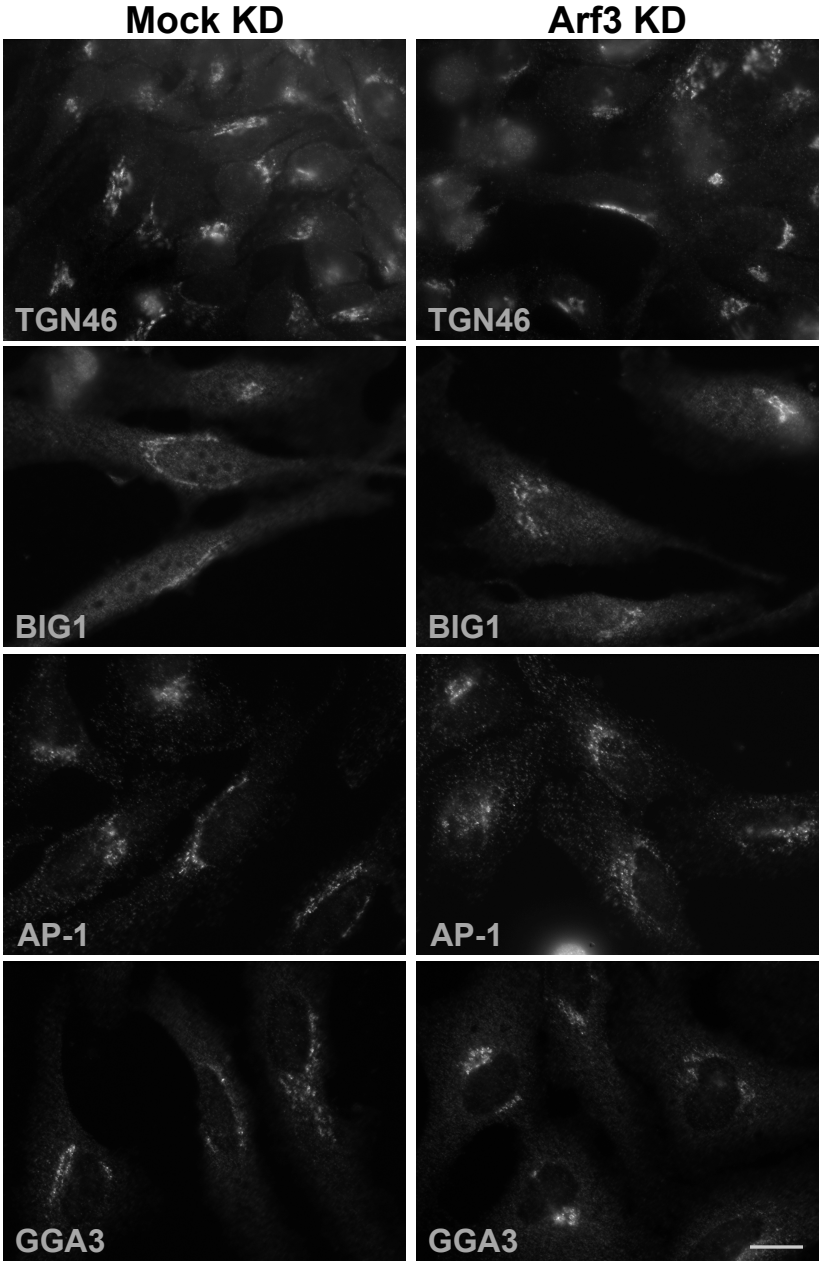


Supplementary Figure 3. Temperature shift to 20°C has no impact on Golgi membrane localization of Arf1-GFP, TGN46 or BIG1.
HeLa cells were transfected with a plasmid encoding Arf1-GFP or transfection reagent only for 24 hours. Cells were either fixed directly from 37°C (left panels) or shifted from 37°C to 20°C for 2 hours and then fixed. Cells were stained for the specified markers and images were acquired using identical settings for the 37°C and the 20°C samples. Representative epifluorescence images selected from at least 2 separate experiments are shown. Bar, 20μm.

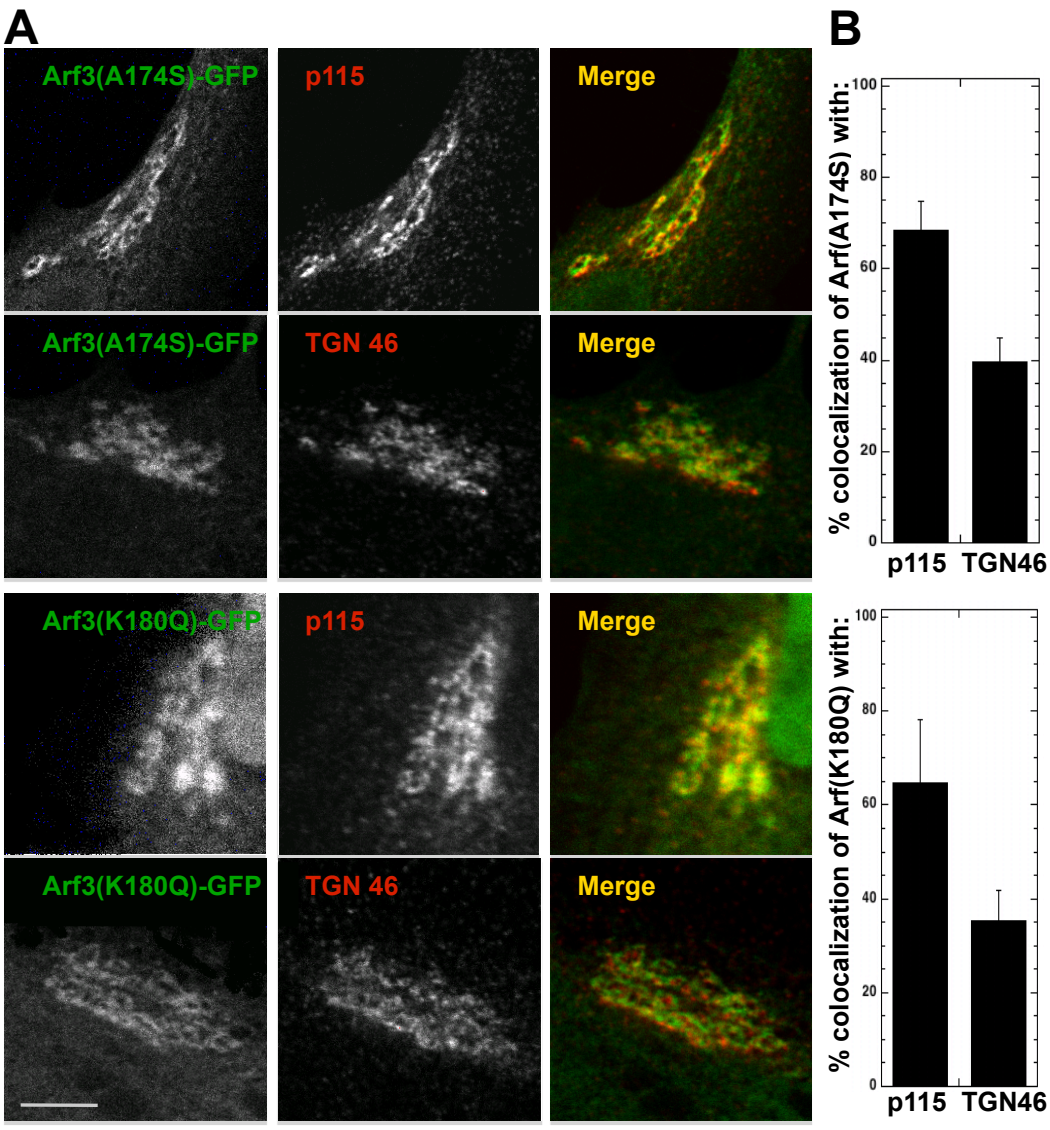


Supplementary Figure 4. Two residues in the N-terminal helix dictate the temperature sensitivity for membrane recruitment of Arf3 and Arf1.

HeLa cells were transfected with Arf3/Arf1 chimeras (**A**) or double mutants (**B**) tagged with GFP, as indicated. After 24 hours, cells were either kept 37°C or shifted to 20°C for 30 minutes and then fixed. Epifluorescence images are shown. Schematic representation of chimeras shown above each panel. Bar, 20μm.

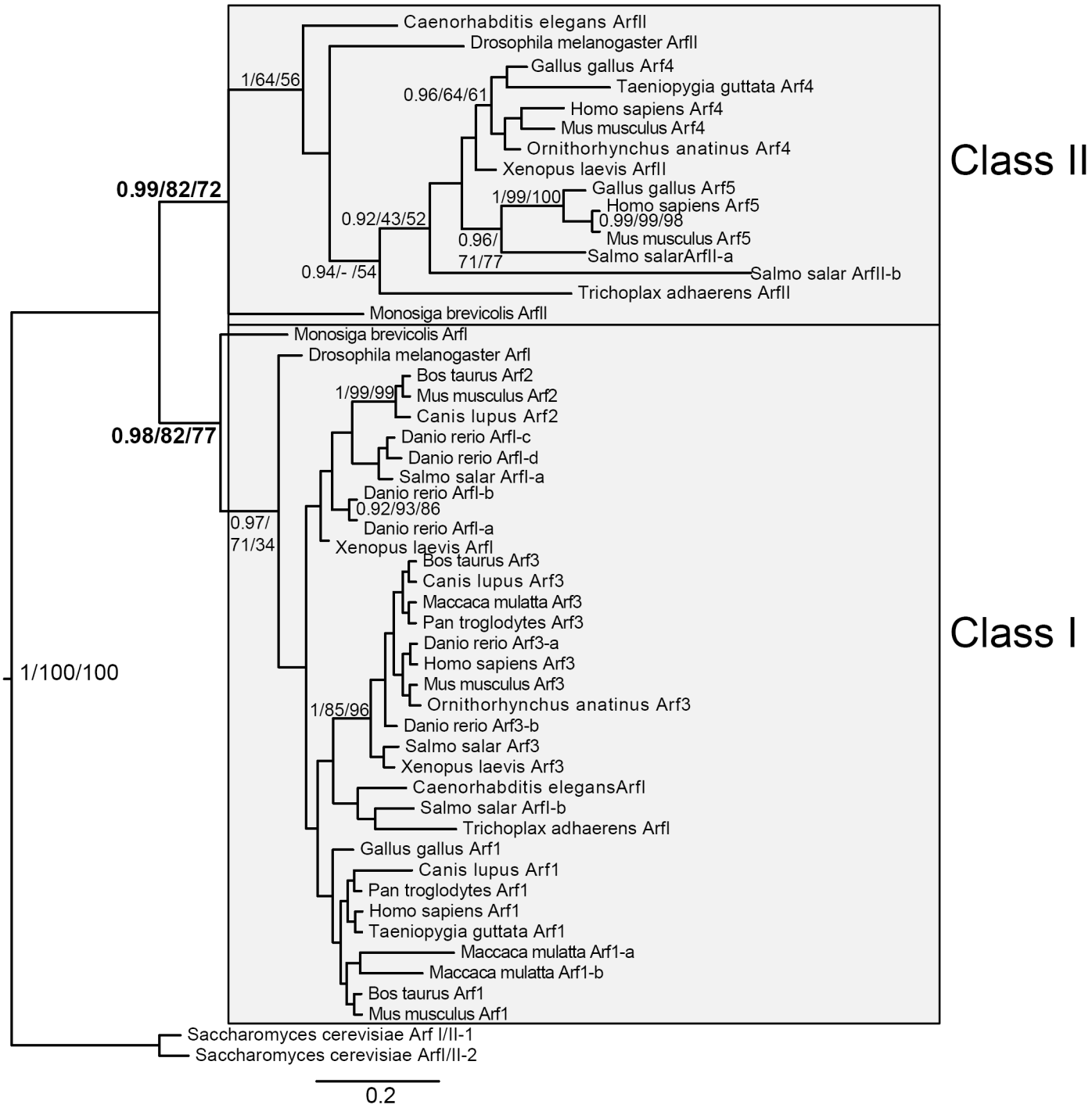


Supplementary Figure 5. Arf3 knockdown has no effect on either TGN structure or membrane recruitment of clathrin adaptors AP1 and GGA3.
HeLa cells were transfected with either irrelevant siRNA (Mock KD, left panels) or a pool of two validated Arf3 siRNA duplexes (Arf3 KD, right panels). 72 hours post transfection cells were fixed and stained for the specified markers. Representative epifluorescence images selected from at least 2 separate experiments are shown. Bar, 20μm.



Supplementary Figure 6. Two residues in the C-terminal helix of Arf3 are required for concentration on the TGN in HeLa cells.

A. HeLa cells were transfected with the indicated Arf3A174S-GFP, Arf3K174R-GFP, Arf3A174S-GFP or Arf3K180Q-GFP chimeras. After 24 hours, cells were fixed and stained for p115 or TGN46. Images were acquired using a confocal microscope. Bar, 5μm. **B.** Quantitative analysis of experiments similar to **A** showing signal overlap between the Arf3/ Arf1 swap single mutants and the specified markers. Error bars correspond to the mean ± SD (n ≥ 10 cells from 2 separate experiments).



Supplementary Figure 7. Class I and II Arfs evolved prior to the origin of multicellular animals.

Phylogenetic analysis of Class I/II Arf homologues rooted by the *Saccharomyces cerevisiae* ArfI and II sequences. This tree shows the best Bayesian topology and support values for nodes with greater support than 0.85 posterior probability and 50% in one of the two ML methods. Node values are given in the order of posterior probability values/PhyML bootstraps and RAxML bootstraps. Classification of Arfs with Roman numerals may reflect either the lack of resolution into an Arabic numbered Arf clade or that they diverged prior to the duplications giving rise to that clade. The nodes delimiting the Class I vs Class II Arfs are bolded and the clades are enclosed in shaded boxes.