SUPPLEMENTAL DATA

Kinetic studies of the Arf activator Arno on Model Membranes in the Presence of Arf Effectors Suggest Control by a Positive Feedback Loop

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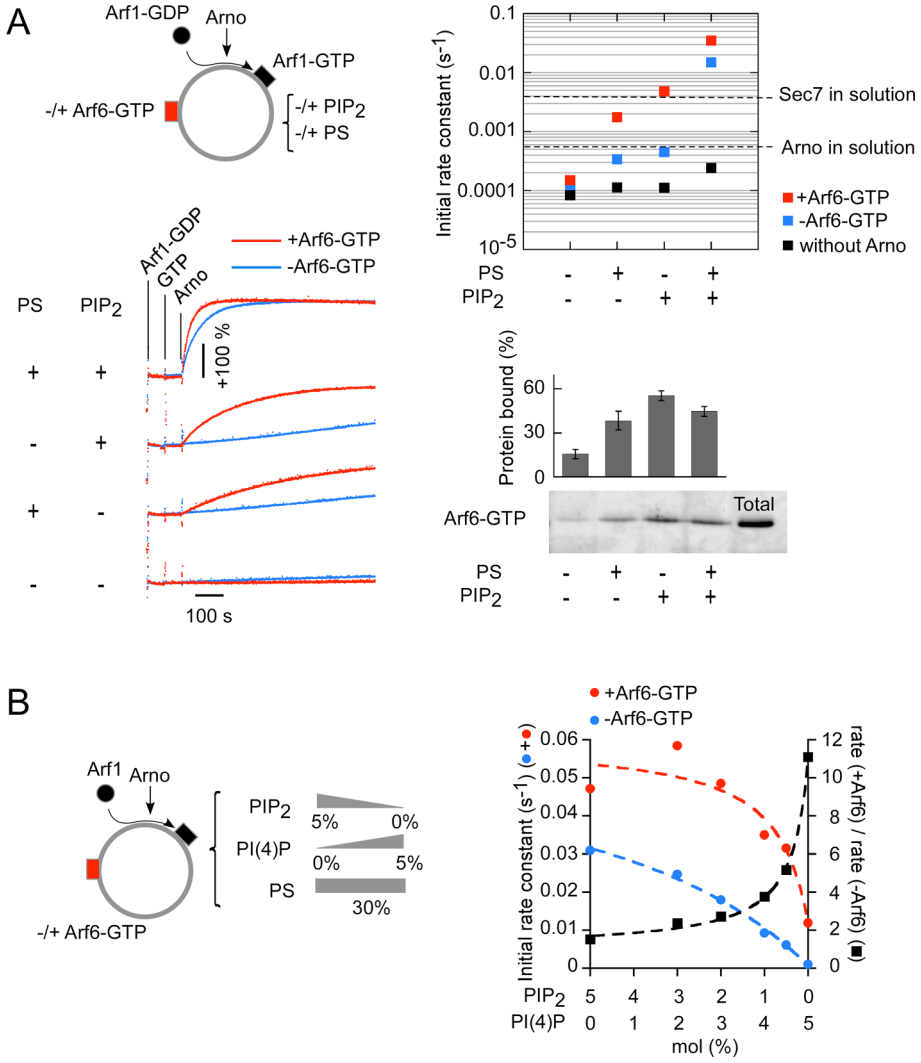
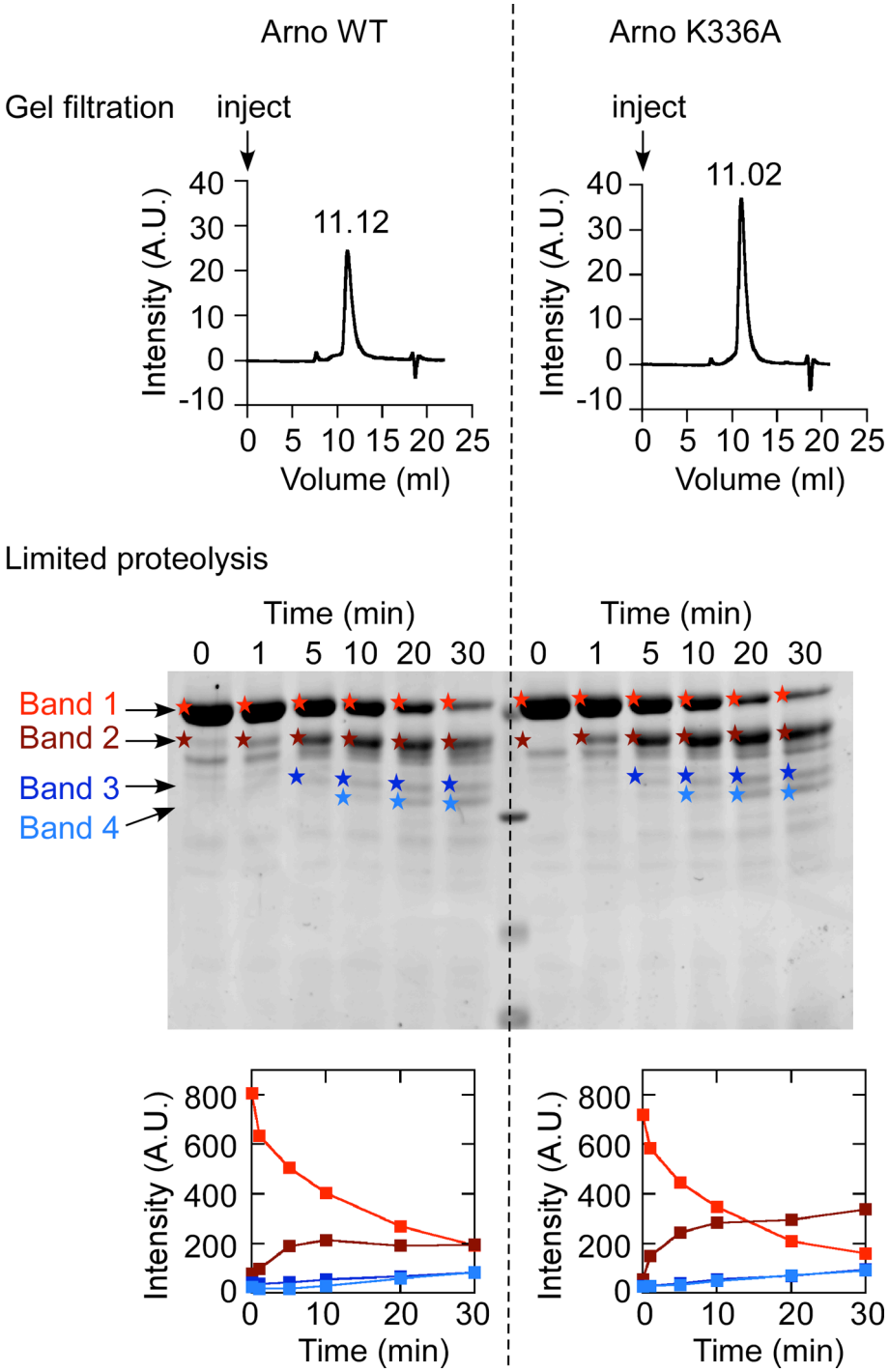
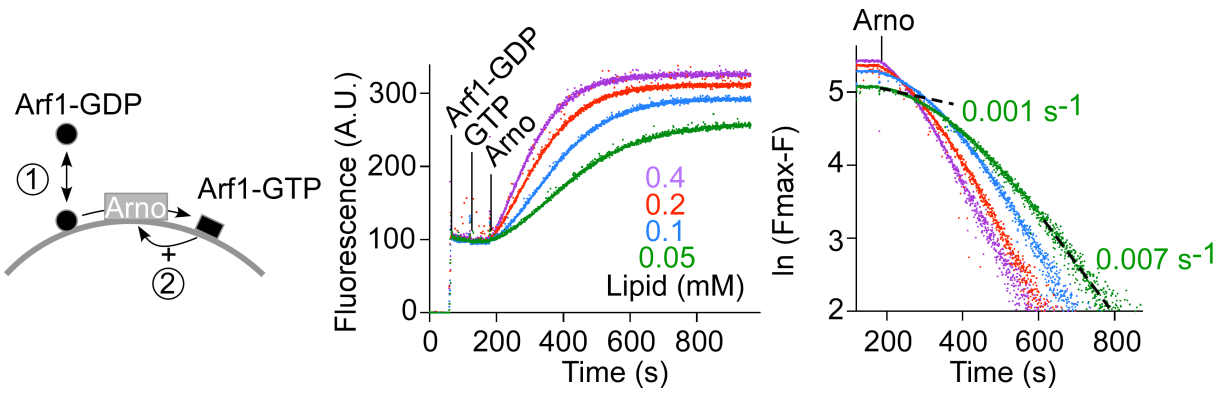


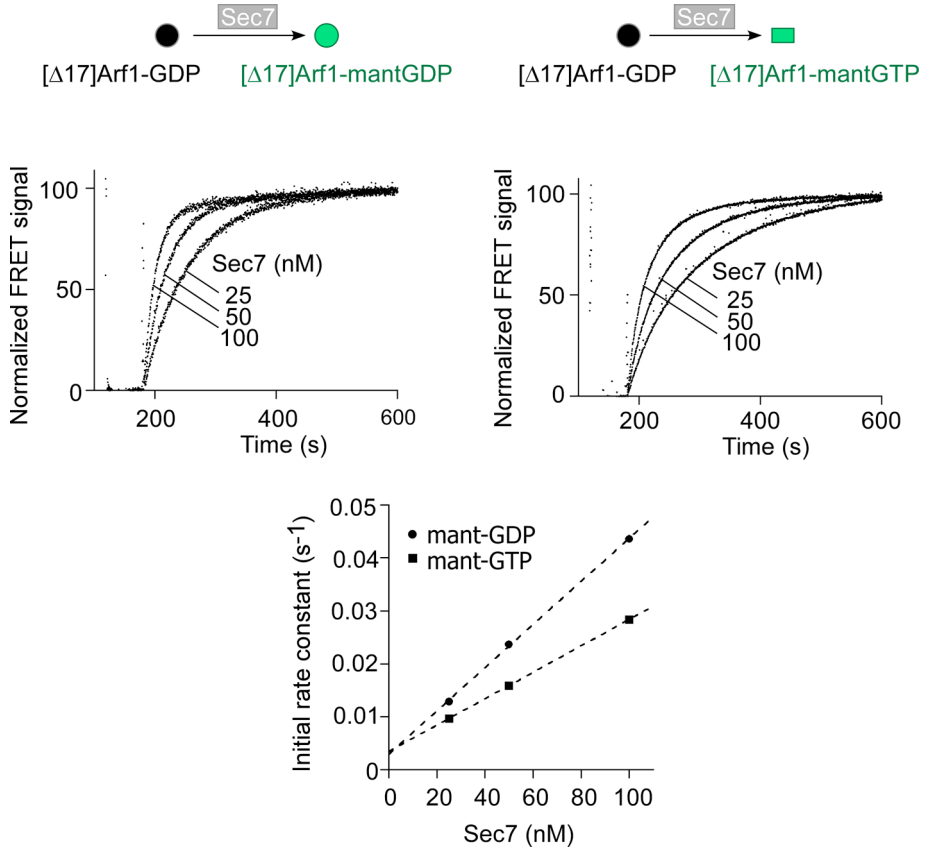
Supplemental Fig. S1. Activation of Arno by Arf6-GTP on liposomes. (**A**) Time course of GDP to GTP exchange on myristoylated Arf1 catalyzed by Arno on liposomes of defined composition. The liposomes (0.2 mM) were supplemented (red traces) or not (blue traces) with 300 nM myristoylated Arf6-GTP. At the times indicated, Arf1-GDP (400 nM), GTP (40 µM) and Arno (7.5 nM) were sequentially added. The fluorescence level before the addition of Arf1-GDP was arbitrary set at zero. The right panel compiles the apparent initial rate constant (k_{obs}) from the fluorescence recordings. The gel shows the % of membrane bound Arf6-GTP on the various liposomes as assessed by a flotation assay (mean ± SEM, n=3). (**B**) Apparent rate constant for the activation of Arf1 by Arno on liposomes containing increasing amounts of PIP at the expense of PIP_2 and with or without Arf6-GTP (300 nM).



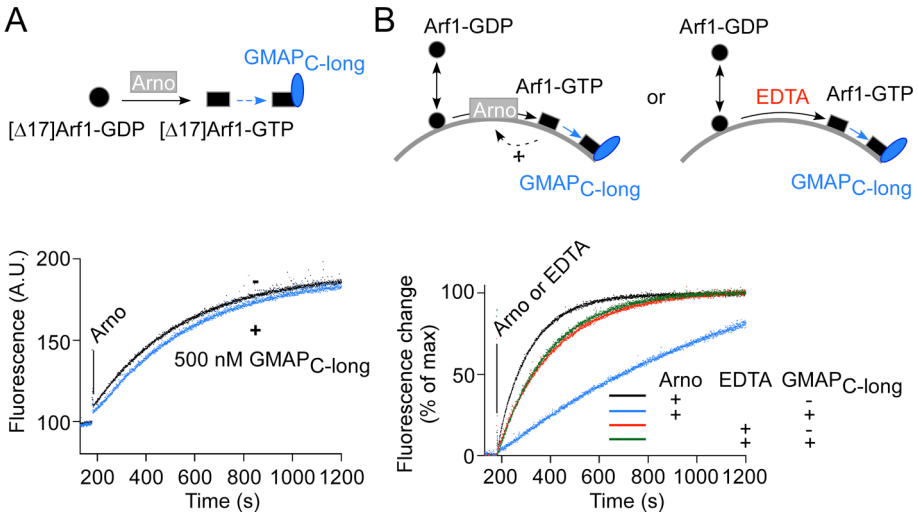
Supplemental Fig. S2. Biochemical analysis of wild-type and K336A Arno. Upper panels: elution profile of wild-type Arno and of the K336A mutant on a Superose 12 column. Lower panels: limited proteolysis. Arno or the K336A mutant (4 µM) was incubated with subtilisin (1 µg/ml) at 25°C in solution. At the indicated times an aliquot was withdrawn and supplemented with 0.2 mM PMSF to block the reaction before SDS page analysis.



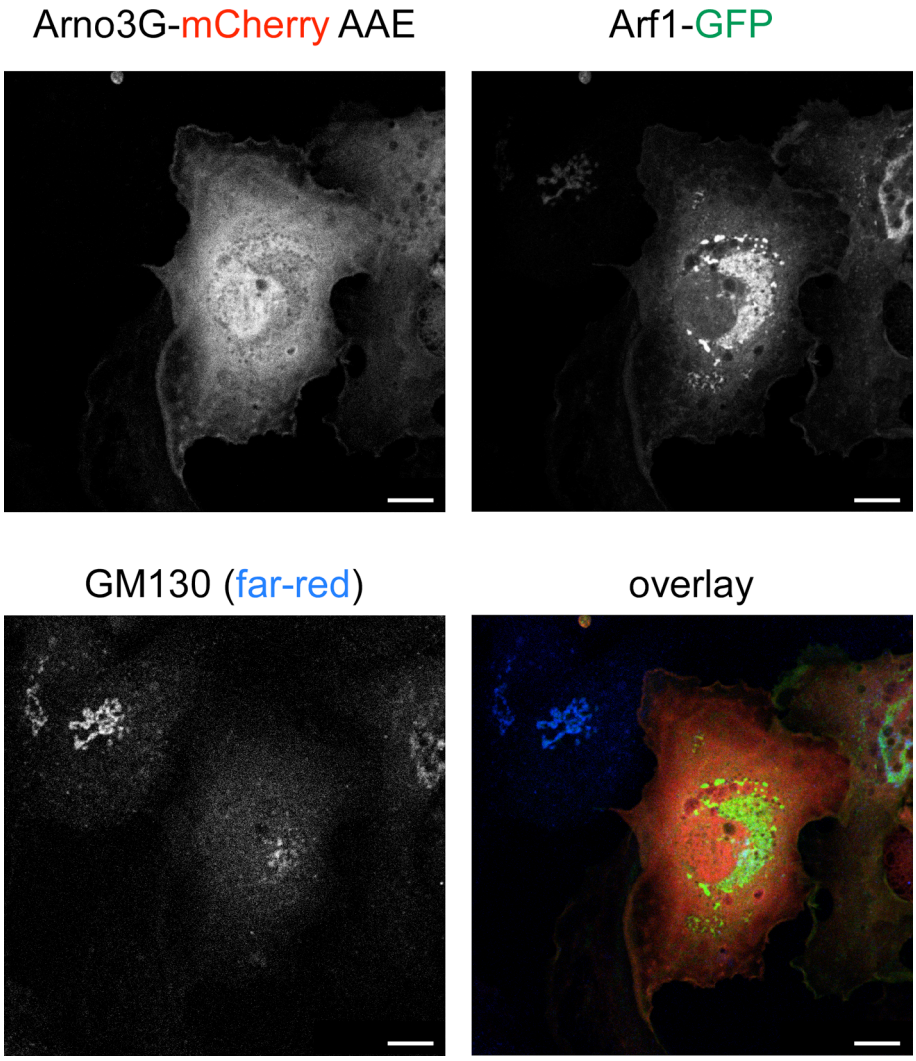
Supplemental Fig. S3. Arf1 activation by Arno is sensitive to the surface concentration of Arf1-GTP. On liposomes containing 1 % PIP_2 and 30% PS, the time course of Arno-catalyzed Arf1 activation displays a sigmoidal shape, which accentuates at lower lipid concentration. A log plot shows that the kinetics accelerates up to 7-fold as the reaction proceeds at the lowest lipid concentration used here. Protein concentration : Arno, 7.5 nM ; Arf1, 400 nM.



Supplemental Fig. S4. Binding kinetics of mant-GDP or mant-GTP (6.6 μM) to [Δ17]Arf1-GDP (1 μM) upon the addition of the Sec7 domain of Arno. Nucleotide binding was measured by FRET between the tryptophan groups of Arf1 and the mant moiety. In contrast to the experiments performed on liposomes with full-length Arno and Arf1 where mant-GTP binds much faster than mant-GDP (Fig. 4A), here the binding kinetics of mant-GTP is slightly slower than that of mant-GDP.



Supplemental Fig. S5. Specific inhibitory effect of $GMAP_{C\text{-}long}$ on the kinetics of Arno-mediated GDP to GTP exchange on Arf1 at the surface of liposomes. Tryptophan fluorescence was used to follow the activation of Arf1. (**A**) In solution. Protein concentration: [[Δ17]Arf1] = 1 μM, [Arno] = 100 nM, [$GMAP_{C\text{-}long}$] = 0 or 500 nM. (**B**) On liposomes. The liposomes (200 μM) contained 30 mol% PS and 1 mol% PIP_2. Protein concentration : [Arf1] = 400 nM ; [Arno] = 7.5 nM. [$GMAP_{C\text{-}long}$] = 0 or 500 nM. When indicated EDTA (2 mM) was used to chelate magnesium and to artificially stimulate nucleotide exchange. The blue and black traces are the same recordings as in Fig. 4B.



Supplemental Fig. S6. Effect of a 'loose' interdomain mutant of Arno on the structure of the Golgi apparatus. In RPE1 cells, expression of the Arno triple inter domain mutant (F380A-L384A-F257E) promotes the formation of intense Arf1-GFP labeled structures. This formation occurs at the expense of the normal Golgi structure as assessed here by an anti GM-130 antibody. Scale bar: 10 µm.