

Title: GTP-binding of ARL-3 is activated by ARL-13 as a GEF and stabilized by UNC-119.

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Supplementary Materials:

Supplementary Figure legends

Figure S1. UNC-119 localizes to the InV-like compartment of cilia. (a). UNC-119 is highly conserved across ciliated species. *Ce*, *Caenorhabditis elegans*; *Cr*, *Chlamydomonas reinhardtii*; *Dr*: *Danio rerio*; *Mm*, *Mus musculus*; *Hs*, *Homo sapiens*. (b). *unc-119 (ed3)* mutants are dye-filling defective in phasmid cilia. The wild-type copy of GFP-tagged UNC-119 used in localization studies can fully rescue ciliogenesis defect of *unc-119* mutants. *: $p < 0.01$. (c). GFP-tagged UNC-119 only localizes along the middle segment. mCherry-tagged β -tubulin TBB-4 was used to label the whole cilium. (d). The N-terminal sequence but not the PDE-delta domain is required for UNC-119 ciliary targeting. (e) Negative controls for BiFC studies. UNC-119, ARL-13, and ARL-3 do not show fluorescence complementation with IFT components (CHE-11 and IFT-20) in BiFC studies. Scale bar: 5 μ m. Arrowheads indicate the ciliary tip, and stars indicate the ciliary base.

Figure S2. Characterization of UNC-119-ARL-13-ARL-3 association. (a). The Palmitoylation or SUMOylation modification of ARL-13 is not required for its association with UNC-119 or ARL-3 in BiFC study. (b). Dominant negative ARL-3 or Dominant negative ARL-13 still show normal mutual interactions with other components in the UNC-119-ARL-13-ARL-3 module in BiFC study. (c). Deletion of UNC-119, or ARL-13, or ARL-3 does not affect the ciliary entry of other proteins in the UNC-119-ARL-13-ARL-3 module. mCherry-tagged MKS-5 was used to label the transition zone at the ciliary base. Arrows point to the abnormal bulge formed in residual cilia in *arl-13* mutants. (d). ARL-13 exclusively localizes to the middle segment of cilia in wide-type worms and mislocalizes to the dendrite and the cell body in *unc-119* mutants. E. *arl-3* mutants possess normal axonemal doublets. Scale bars: (a-d), 5 μm ; E, 100 nm. Arrowheads indicate the ciliary tip, and stars indicate the ciliary base.

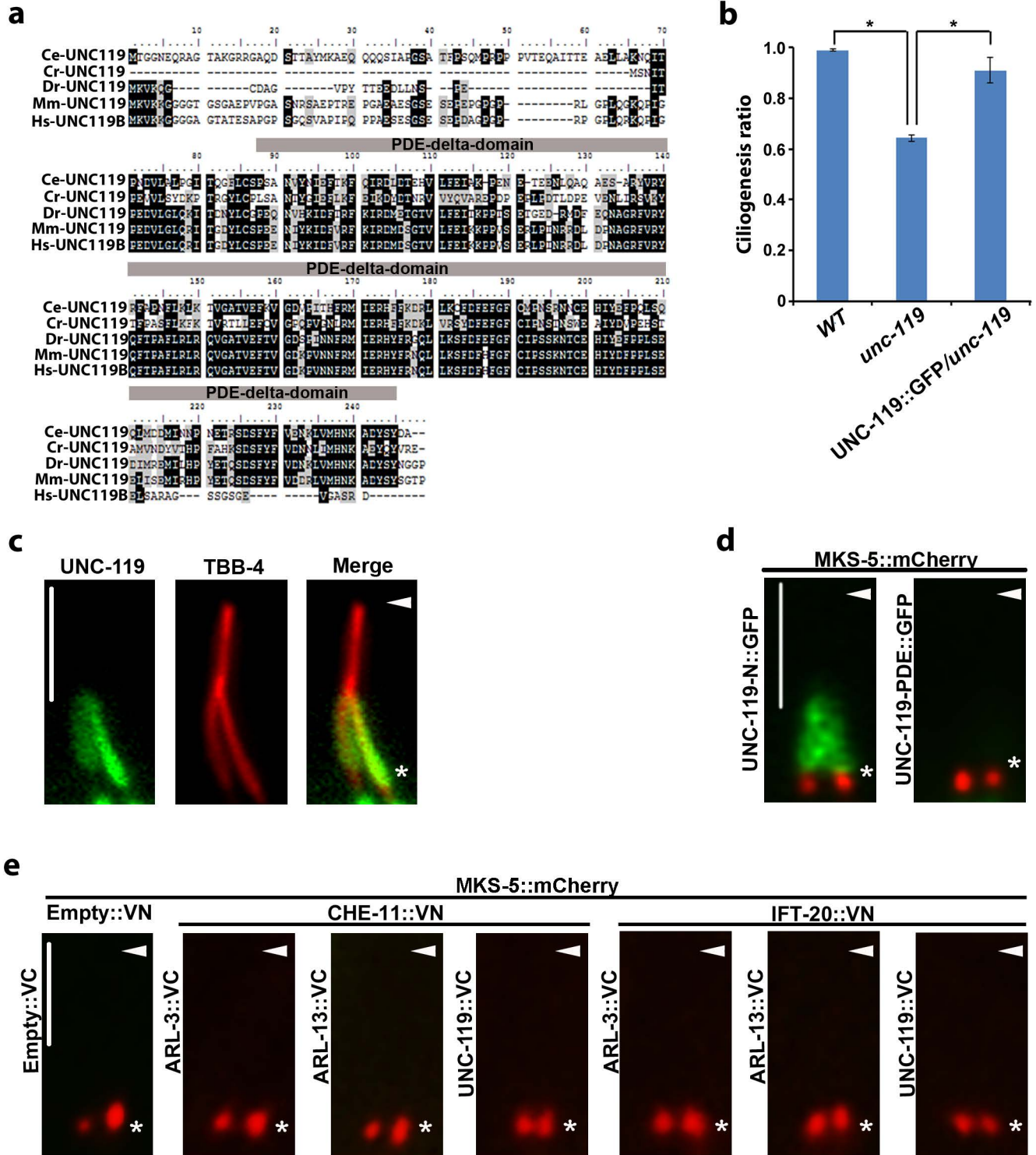
Figure S3. ARL-13 promotes GDP release from ARL-3. (a). All purified proteins used in this manuscript were showed here by SDS-PAGE and stained by Coomassie blue. 5 μg protein was loaded in each lane. BSA served as indication for the protein amount. (b). ARL-13 but not UNC-119 accelerates GDP release from His-tagged ARL-3.

Figure S4. The N terminal but not the C terminal domain of ARL-13 can accelerates GDP release from GST-ARL-3. (a). GEF activity of the indicated concentrations of ARL-13 for 0.05 μM ARL-3 \cdot mantGDP. K_{obs} ($\text{s}^{-1}\times 10^{-3}$) and fold change are shown in the table below the graph. (b). ARL-3 \cdot GTP cannot promote nucleotide dissociation of ARL-

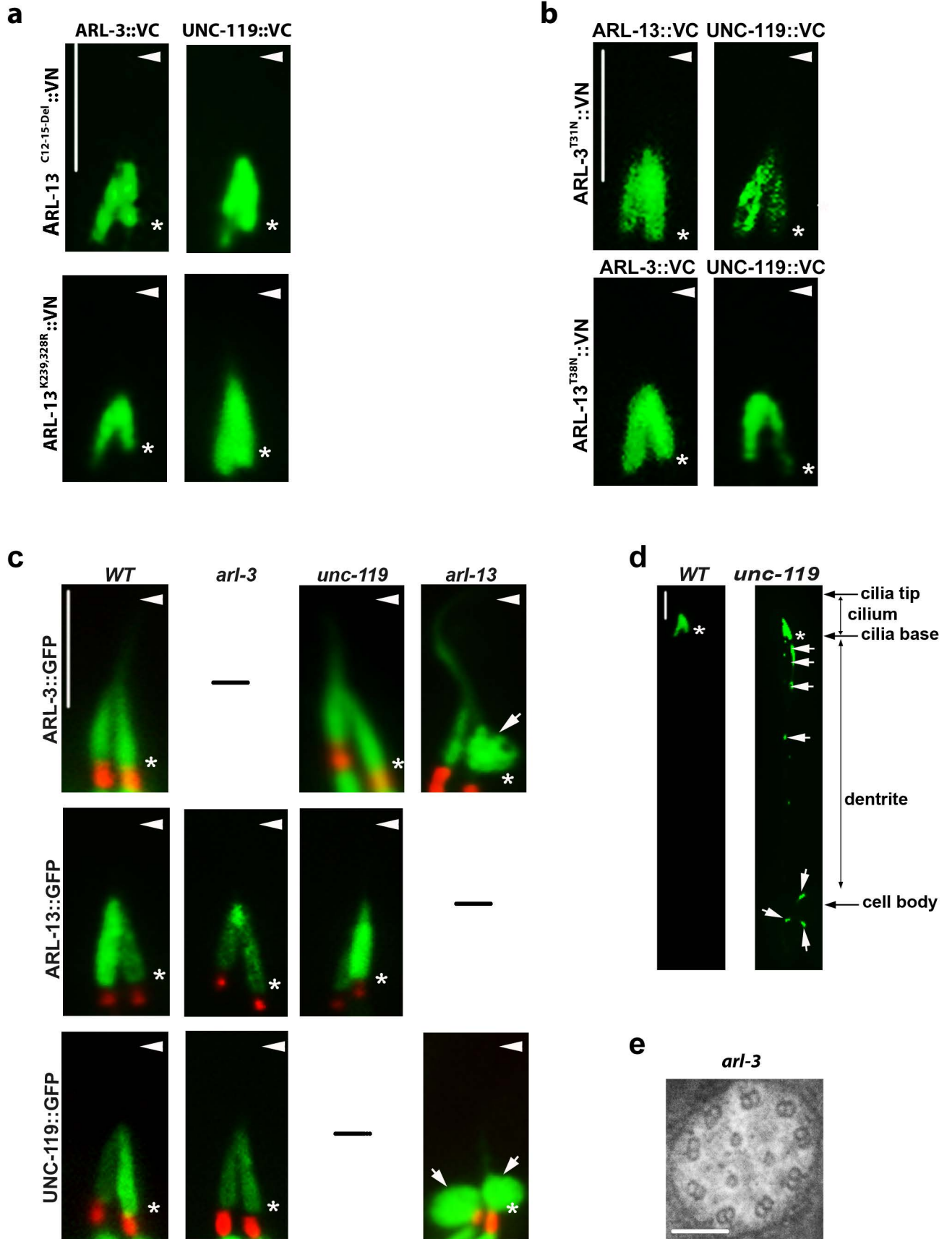
13·mantGppNHp. (c). The N-terminal domain of ARL-13 containing the GTPase domain (1-176aa) and the coil-coil domain (177-250aa) is able to accelerate the GDP release from ARL-3·mantGDP. (d). Adding UNC-119 together with FL or truncated ARL-13 does not change the nucleotide exchange rate of ARL-3.

Figure S5. ARL-3 and ARL-13 show no intrinsic GTP hydrolysis activity. No GTP hydrolysis was detected when mixing Mant-GTP with either ARL-3 or ARL-3 +ARL-13 at room temperature (a) or 37°C (b) for 60 min. RhoA and RhoA GAP (P50) served as positive control. (c). GFP-tagged ARL-3(T31N) normally localizes in cilia. mCherry-tagged MKS-5 was used to label the transition zone at the ciliary base. Scale bars: 5 µm; The arrowhead indicates the ciliary tip, and the star indicates the ciliary base.

Supplementary Fig. 1

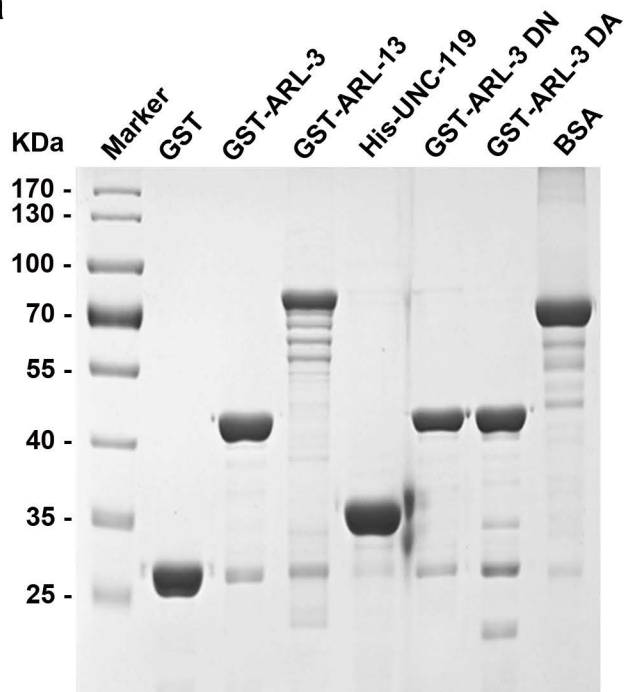


Supplementary Fig. 2

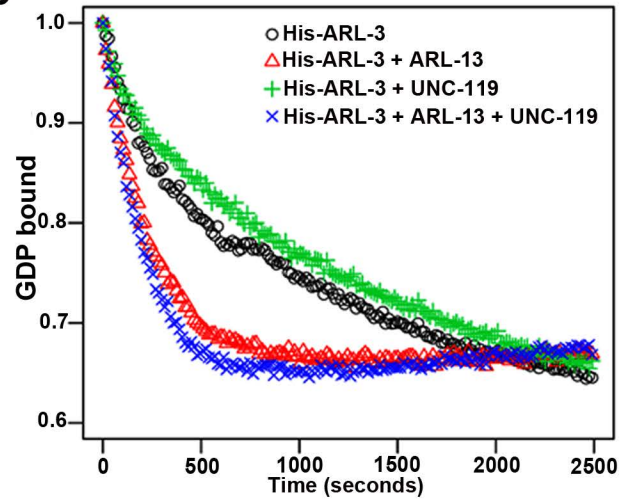


Supplementary Fig. 3

a

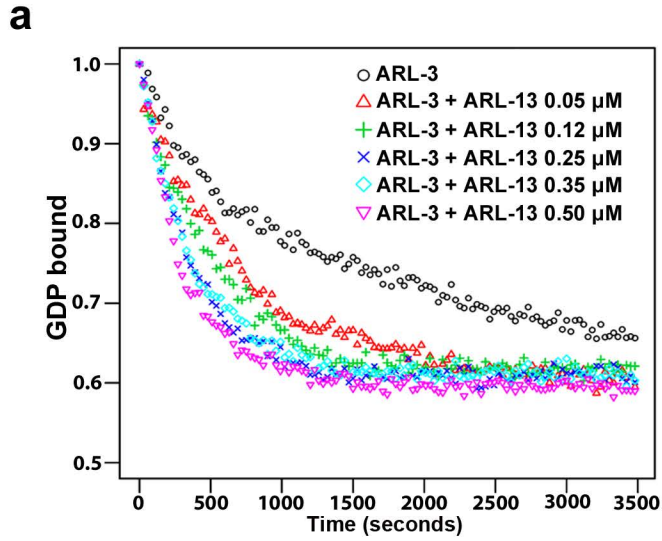


b

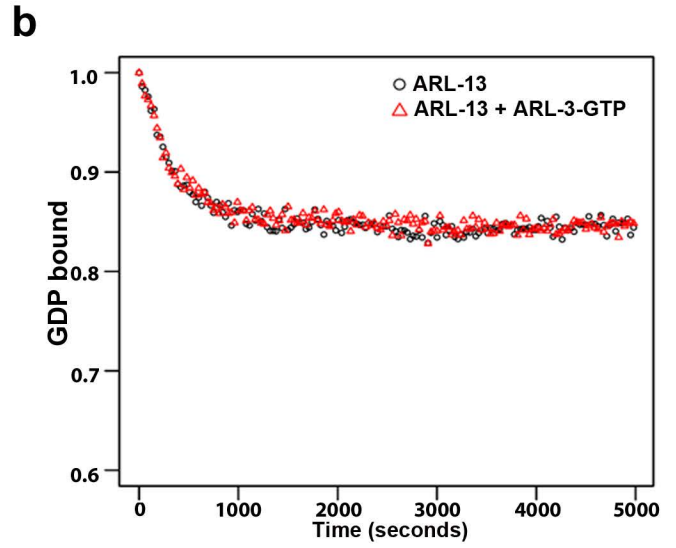


	k_{obs} ($s^{-1} \times 10^{-3}$)	Fold stimulation
His-ARL-3	1.07 ± 0.03	-
His-ARL-3 + ARL-13	4.74 ± 1.13	4.45
His-ARL-3 + UNC-119	0.83 ± 0.04	0.78
His-ARL-3 + ARL-13 + UNC-119	7.66 ± 0.43	7.19

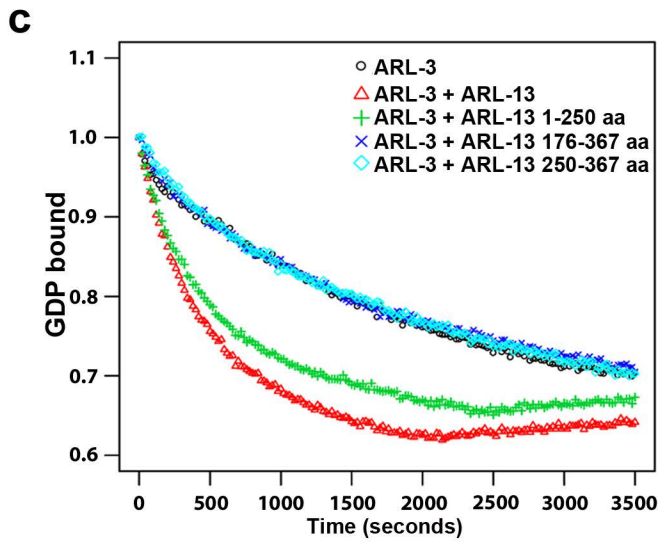
Supplementary Fig. 4



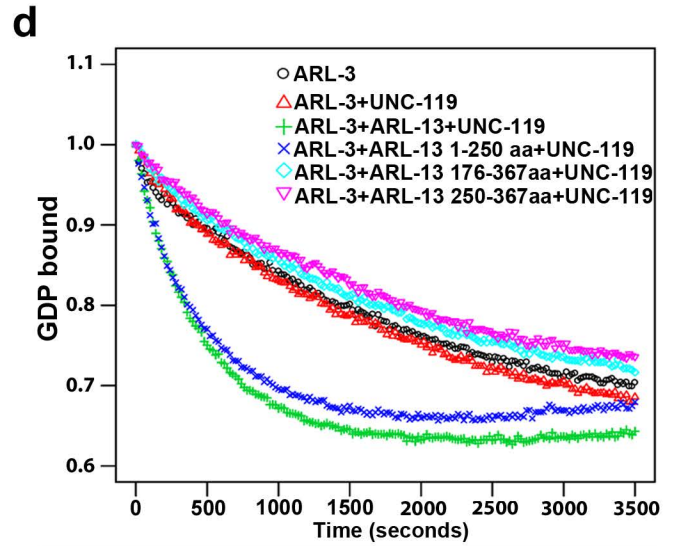
mantGDP-GST-ARL-3		
	K_{obs} ($S^{-1} \times 10^{-3}$)	Fold stimulation
None	0.59 ± 0.03	-
ARL-13 0.05 μ M	1.45 ± 0.06	2.44
ARL-13 0.12 μ M	2.53 ± 0.36	4.26
ARL-13 0.25 μ M	2.78 ± 0.16	4.69
ARL-13 0.35 μ M	2.83 ± 0.08	4.76
ARL-13 0.5 μ M	2.71 ± 0.06	4.57



mantGppNHp-GST-ARL-13		
	K_{obs} ($S^{-1} \times 10^{-3}$)	Fold stimulation
None	2.87 ± 0.13	-
ARL-3-GTP	2.66 ± 0.22	0.93



mantGDP-GST-ARL-3		
	K_{obs} ($S^{-1} \times 10^{-3}$)	Fold stimulation
None	0.50 ± 0.02	-
ARL-13	1.79 ± 0.03	3.57
ARL-13 1-250aa	1.38 ± 0.04	2.74
ARL-13 176-367aa	0.51 ± 0.03	1.01
ARL-13 250-367aa	0.58 ± 0.04	1.16



mantGDP-GST-ARL-3		
	K_{obs} ($S^{-1} \times 10^{-3}$)	Fold stimulation
None	0.50 ± 0.02	-
ARL-3 + UNC-119	0.50 ± 0.01	0.98
ARL-13 + UNC-119	2.23 ± 0.10	4.44
ARL-13 1-250aa + UNC-119	2.06 ± 0.03	4.11
ARL-13 176-367aa + UNC-119	0.50 ± 0.03	1.00
ARL-13 250-367aa + UNC-119	0.50 ± 0.01	0.99

Supplementary Fig. 5

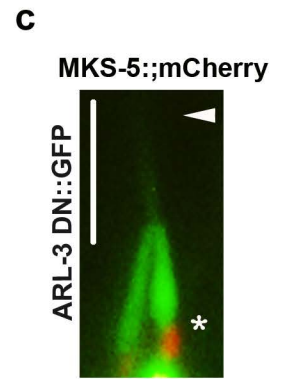
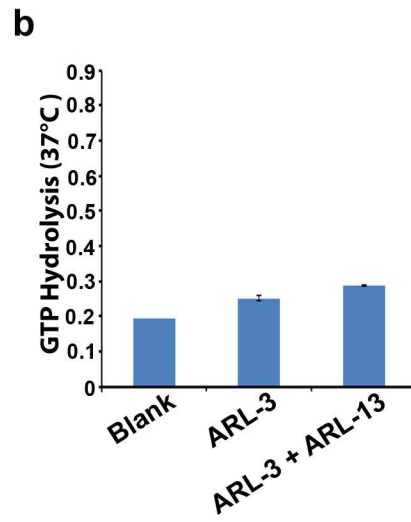
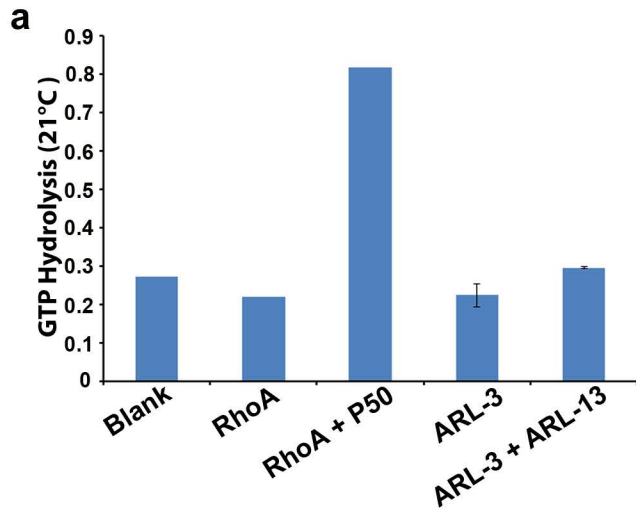


Table S1. Transgenic strains used in this study

Strain name	Genotype
ZP1863	<i>arl-3 (tm1703); arl-13 (gk513)</i>
ZP1865	<i>arl-3 (tm1703); arl-13 (gk513); unc-119 (ed3)</i>
ZP1866	<i>arl-13 (gk513); [Parl-13::UNC-119::GFP; Parl-13::TBB-4::mCherry; PR4]</i>
ZP1867	<i>arl-3 (tm1703); [UNC-119::VN; ARL-13::VC; PR4]</i>
ZP1870	<i>N₂ [ARL-13T38N::VN; ARL-3::VC; PR4]</i>
ZP1871	<i>N₂ [ARL-13T38N::VN; UNC-119::VC; PR4]</i>
ZP1886	<i>arl-3 (tm1703); unc-119 (ed3)</i>
ZP1887	<i>unc-119 (ed3); [Parl-13::ARL-13::GFP]</i>
ZP1889	<i>arl-13 (gk513); [ARL-3::VN; UNC-119::VC; RP4]</i>
ZP1892	<i>arl-13 (gk513); him8</i>
ZP1893	<i>N₂ [ARL-13::VN; ARL-3::VC; ccGFP]</i>
ZP1895	<i>N₂ [ARL-3::VN; UNC-119::VC; ccGFP]</i>
ZP1900	<i>N₂ [ARL-13::VN; UNC-119::VC; MKS-5::mCherry; ccGFP]</i>
ZP1901	<i>N₂ [ARL-13::VN; ARL-3::VC; MKS-5::mCherry; ccGFP]</i>
ZP1905	<i>N₂ [ARL-3::VN; UNC-119::VC; MKS-5::mCherry; ccGFP]</i>
ZP1910	<i>arl-3 (tm1703); OSM-6::GFP</i>
ZP1911	<i>unc-119 (ed3); OSM-6::GFP</i>
ZP1912	<i>arl-3 (tm1703); arl-13 (gk513); OSM-6::GFP</i>
ZP1913	<i>arl-3 (tm1703); unc-119 (ed3); OSM-6::GFP</i>
ZP1914	<i>unc-119 (ed3); OSM-6::GFP</i>
ZP1916	<i>N₂ [Parl-13::ARL-3::GFP; MKS-5::mCherry; PR4]</i>
ZP1917	<i>unc-119 (ed3) [Parl-13::ARL-3::GFP; MKS-5::mCherry; PR4]</i>
ZP1919	<i>N₂ [Parl-13::UNC-119::GFP; MKS-5::mCherry; PR4]</i>
ZP1920	<i>arl-3 (tm1703); [Parl-13::UNC-119::GFP; MKS-5::mCherry; PR4]</i>
ZP1921	<i>arl-13 (gk513); [Parl-13::UNC-119::GFP; MKS-5::mCherry; PR4]</i>
ZP1926	<i>unc-119 (ed3); [Parl::ARL-13::GFP]</i>
ZP1927	<i>arl-3 (tm1703); [Parl::ARL-13::GFP]</i>
ZP1953	<i>arl-13 (gk513); unc-119 (ed3)</i>
ZP1959	<i>N₂ [Parl-13::ARL-13::GFP; MKS-5::mCherry; PR4]</i>