

Supplementary Materials for

Arf GTPase activates the WAVE regulatory complex through a distinct binding site

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Sci. Adv. **8**, eadd1412 (2022)
DOI: 10.1126/sciadv.add1412

The PDF file includes:

Figs. S1 to S6
Tables S1 and S2
Legends for movies S1 and S2

Other Supplementary Material for this manuscript includes the following:

Movies S1 and S2

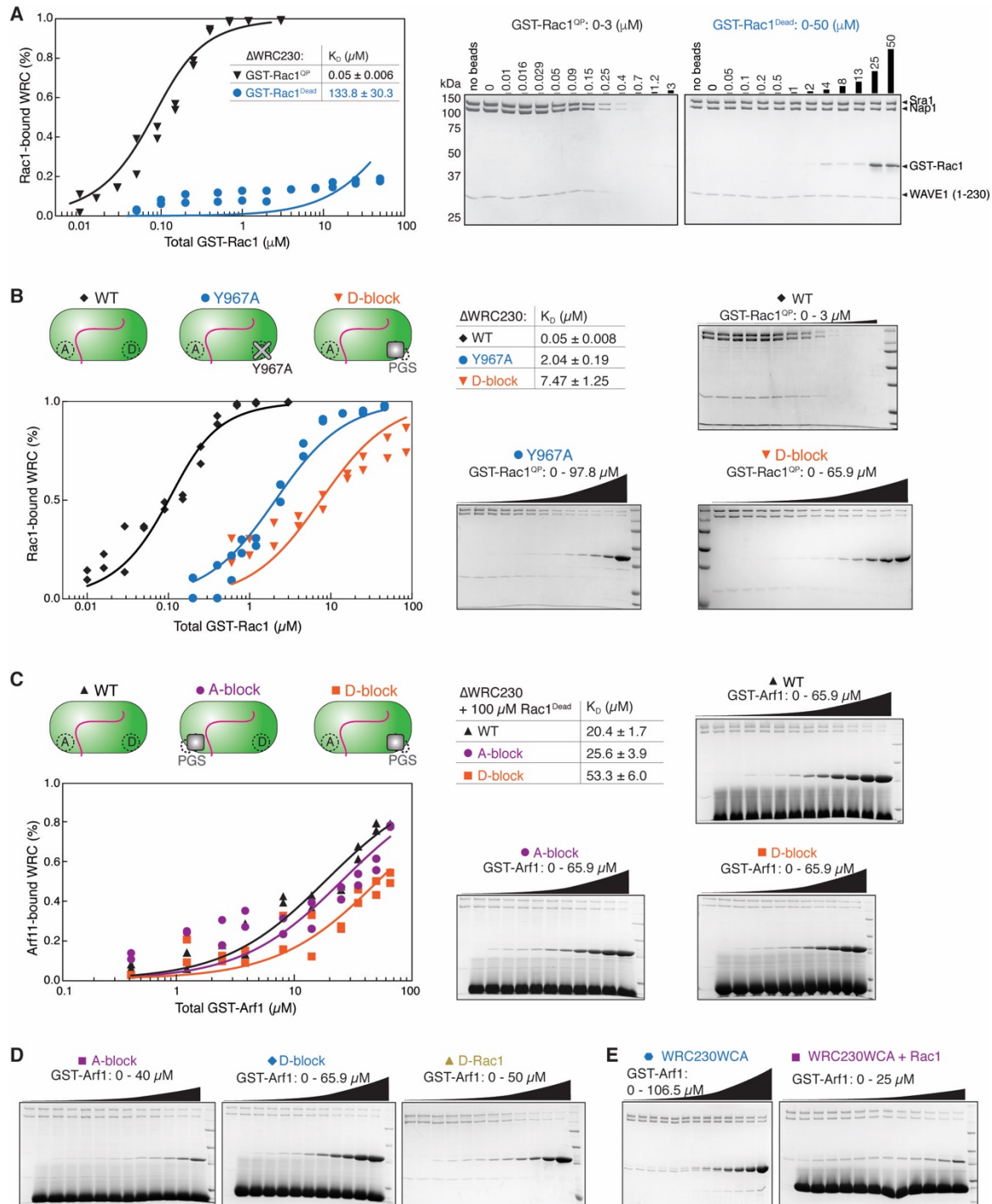


Fig. S1. Supporting EPD data for Fig. 1, 2, and 3. (A) EPD measurement, including quantification and representative Coomassie blue-stained SDS PAGE gels, showing GST-Rac1^{Dead} does not bind to WRC. **(B)** EPD measurement showing that, compared to the Y967A mutation, D-block further reduces potential leaky binding of Rac1 to the D site. **(C)** EPD measurement showing

that blocking the A site or the D site does not significantly affect the basal level binding of Arf1 to WRC. **(D-E)** Example EPD gels for Fig. 2B and 3B, respectively.

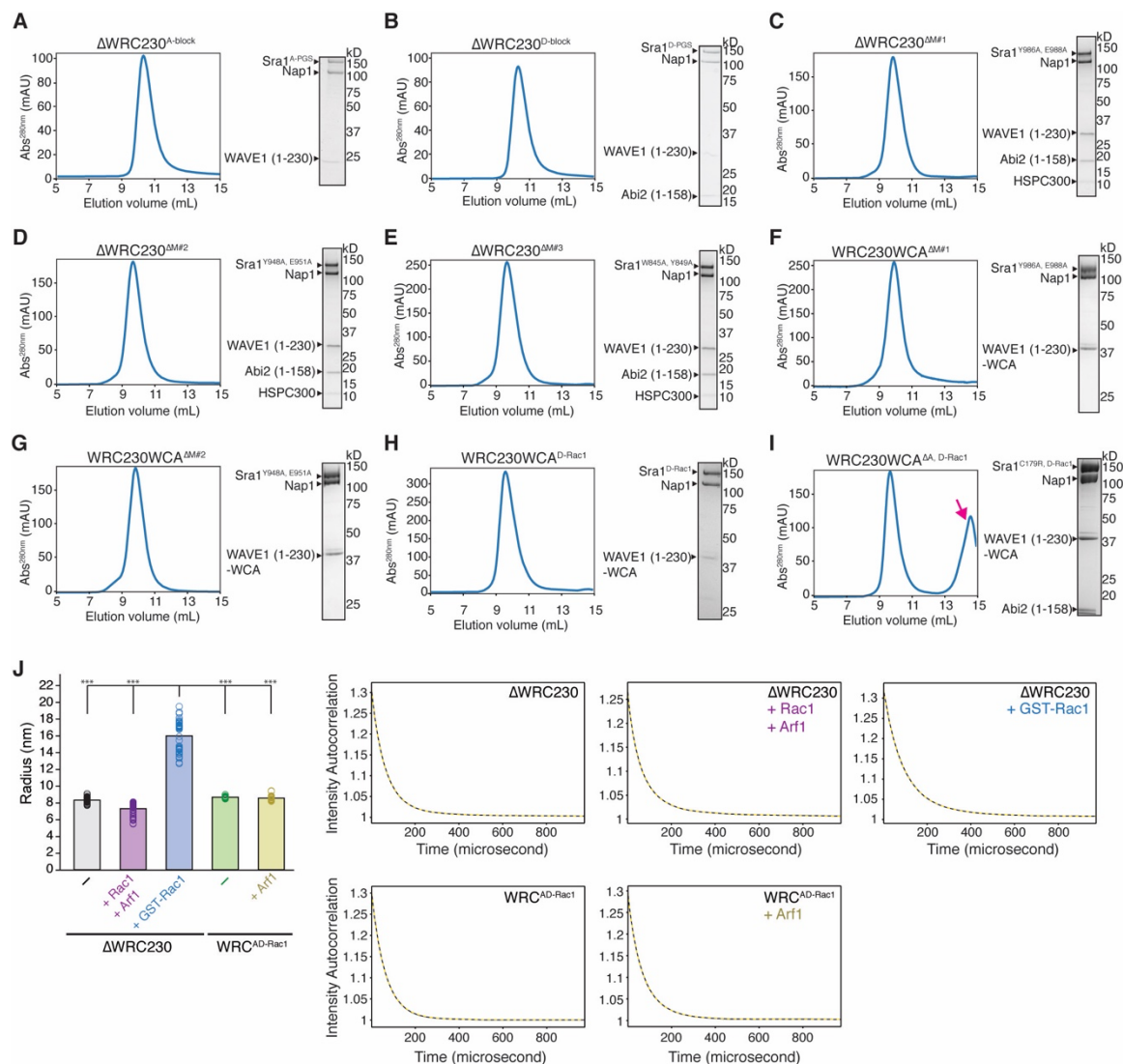


Fig. S2. Quality control of the WRCs newly developed in this study. (A-I) Shown are the final steps or analytical steps of WRC purifications using 24-ml Superdex 200 gel filtration columns, with Coomassie blue-stained SDS-PAGE gels showing the peak or pooled fractions. Depending on whether the preceding purification step included a Source Q15 ion exchange column, different amounts of Tev and cleaved MBP tag may show as clearly separated peaks (indicated by magenta arrow) following the WRC peak. (J) Dynamic Light Scattering (DLS) measurements of indicated WRC mixed with GTPases. GST-Rac1 is used as a positive control to show radius change when WRC is dimerized by GST-Rac1. On the left is radius values for 3.3 μ M Δ WRC230 alone ($n = 54$), 3.5 μ M Δ WRC230 + 30 μ M Rac1^{QP} + 125 μ M Arf1 ($n = 44$), 1 μ M Δ WRC230 + 30 μ M GST-Rac1^{QP} ($n = 51$), 5 μ M WRC^{AD-Rac1} alone ($n = 16$), and 5 μ M WRC^{AD-Rac1} + 125 μ M Rac1^{QP} ($n = 24$). n equals total number of acquisitions, *** indicates $p < 0.001$, ANOVA test. Radius

values are reported as the average values from all requisitions. All experiments were collected in 50-100 mM NaCl and 5% (w/v) glycerol and at room temperature. The slightly reduced radius for Δ WRC230 + Rac1 + Arf1 sample was likely due to the addition of large amounts of Rac1 and Arf1, which have lower molecular weight. On the right is a representative plot of intensity autocorrelation (black solid curve) and the regularization fit (yellow dashed curve) for each experiment.

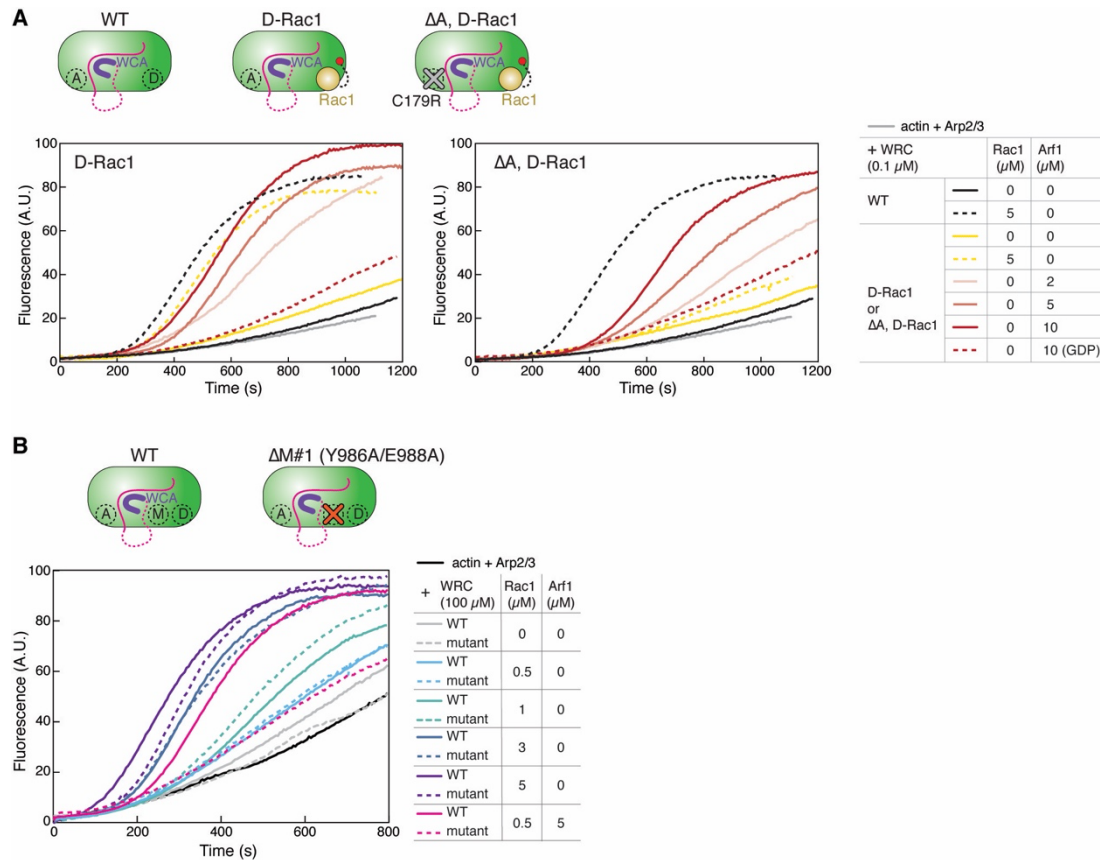


Fig. S3. Additional actin polymerization assays comparing Arf1- vs. Rac1-mediated WRC activation. (A-B) Pyrene-actin polymerization assays comparing indicated WRC variants in response to the addition of free Rac1^{QP} or Arf1. WT WRC230WCA is used as a reference point. Reactions contained 3.5 μM actin (5% pyrene labeled), 10 nM Arp2/3 complex, 100 nM indicated WRC, and indicated amounts of Rac1^{QP} and/or Arf1. In all assays, Arf1 is loaded with GNPPNP, unless it is designated with GDP.

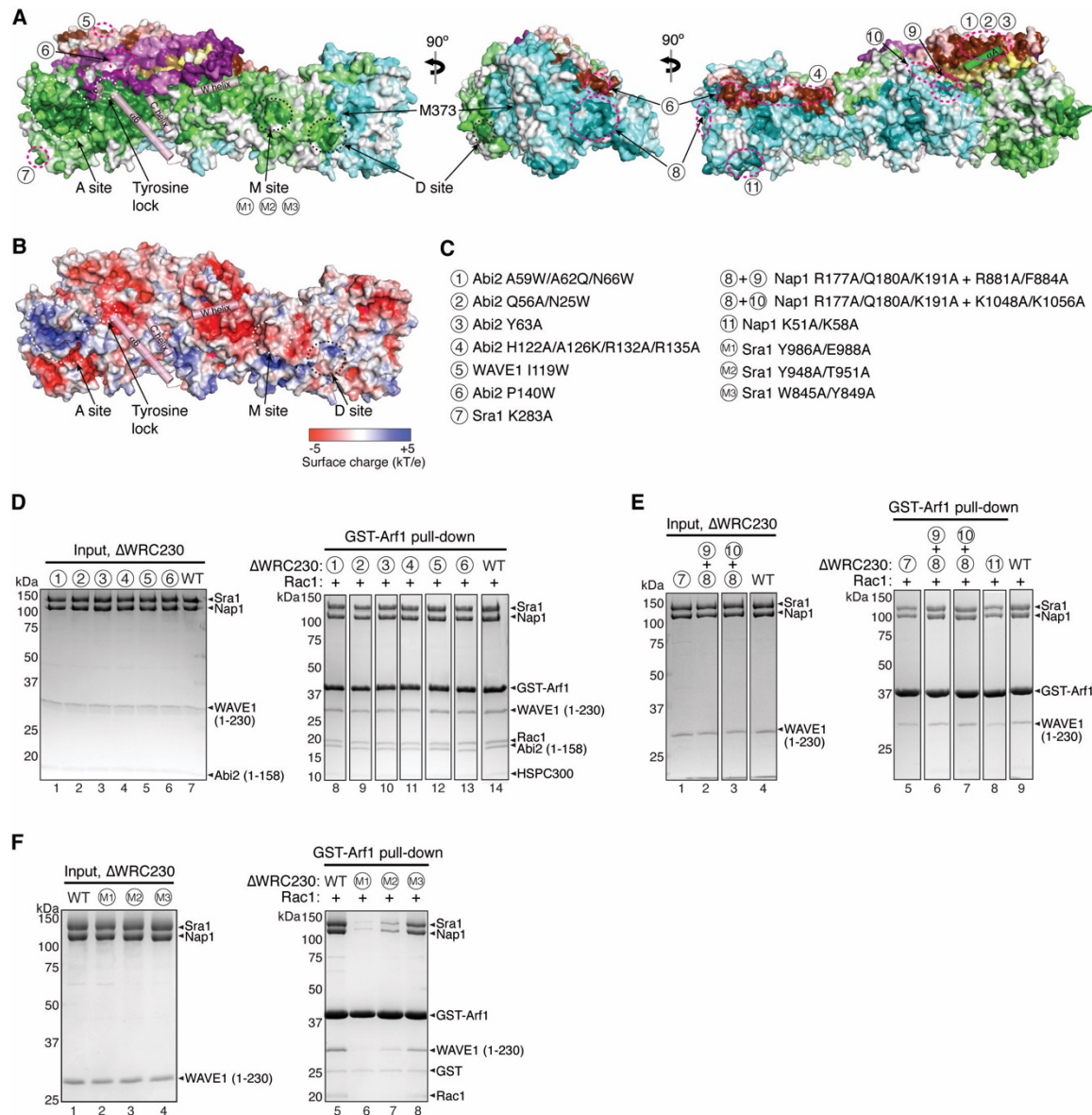
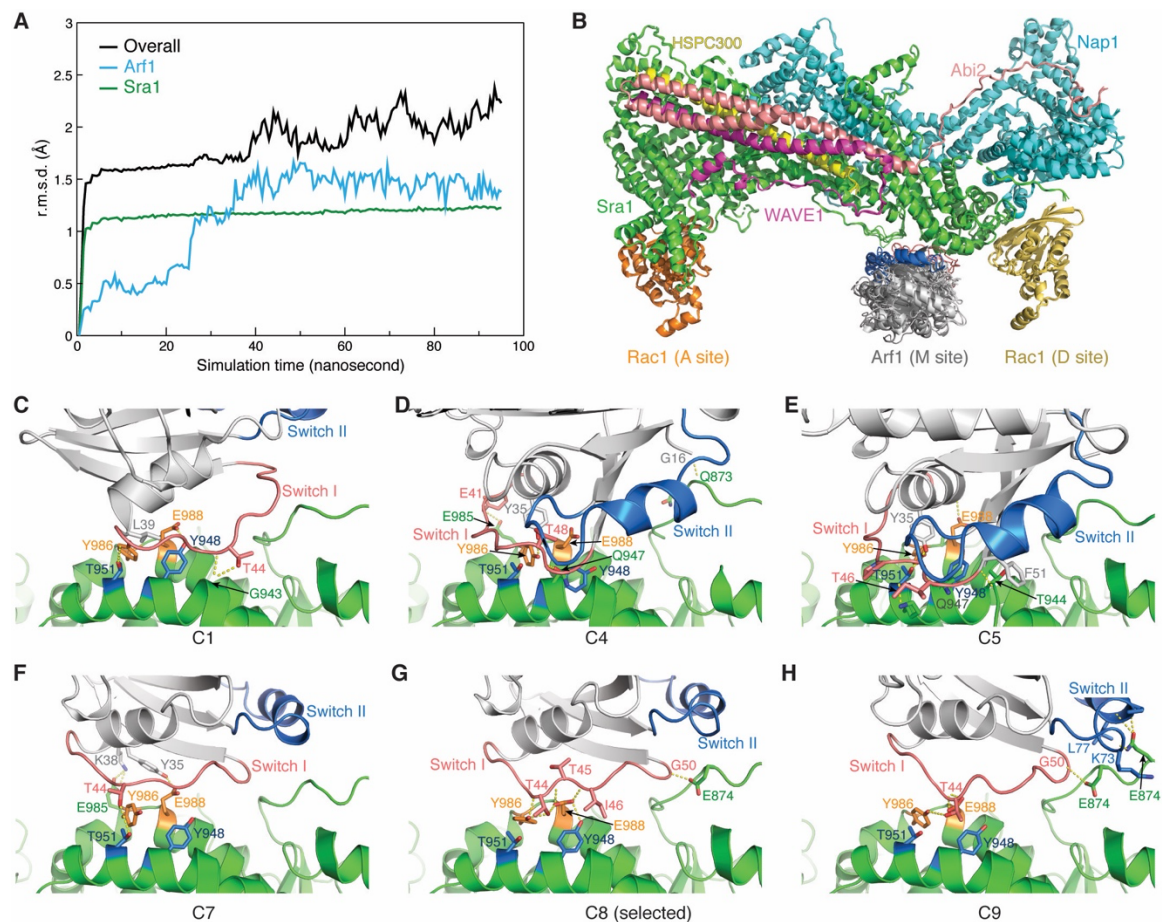


Fig. S4. Surveying various conserved surfaces on WRC to identify the Arf1-binding site. (A-B) Surface conservation (A) and electrostatic charge (B) representation of the WRC. (A) was calculated by ConSurf (61), and (B) by APBS in Pymol (85). In (A), color to white gradients represent the most conserved surface residues (ConSurf score = 9 for darkest colors) to the least conserved residues (ConSurf score = 1 for white color). Important sites on Sra1, including the identified Arf1 binding site (M site), are indicated with white or black dotted circles. Positions of mutations examined in (D-F) are indicated with magenta dotted circles. Semitransparent pink cylinders refer to the sequences in WAVE1 that are destabilized upon WRC activation by Rac1. The α A helix in Sra1 is also shown as a semitransparent green cylinder for clarity. (C) Information

of the mutations indicated in (A) and examined in (D-F). **(D-F)** Coomassie blue-stained SDS PAGE gels showing GST-Arf1 pull-down of Δ WRC230 carrying indicated surface mutations.



System		Free energies of the whole Arf1-WRC ^{AD-Ract} complex						Free energies of Arf1 binding to WRC ^{AD-Ract}					
model	mutation	E _{vdw}	E _{elec}	G ^{vdw} _{total}	G ^{vdw} _{nonpolar}	TS	G _{MM-PBSA-WSAS}	E _{vdw}	E _{elec}	G ^{vdw} _{total}	G ^{vdw} _{nonpolar}	TS	G _{MM-PBSA-WSAS}
C1	WT	-15569.59±6.45	-25689.81±4.53	-9969.32±0.86	694.41±0.64	35791.12±0.89	-9539.18±11.25	-43.36±0.49	331.84±1.27	-304.51±1.59	-4.25±0.02	-19.57±0.25	-0.71±0.67
	ΔM#1	-15559.76±7.33	-25771.60±0.43	-9873.65±0.13	694.98±0.45	35779.34±1.93	-9531.29±8.63	-39.64±0.92	304.68±1.30	-282.04±1.11	-3.81±0.05	-18.68±0.33	-2.13±0.57
	ΔM#2	-15560.81±7.28	-25674.19±1.04	-9972.81±0.66	694.92±0.45	35782.28±1.89	-9536.06±9.36	-37.64±0.71	332.23±1.20	-306.10±1.05	-3.89±0.04	-18.03±0.30	2.64±0.72
C4	WT	-15532.98±21.22	-25690.78±15.42	-9976.65±13.12	692.84±0.16	35780.95±0.48	-9501.03±24.31	-36.08±0.87	291.85±1.99	-270.47±1.57	-4.13±0.09	-19.03±0.13	0.20±0.51
	ΔM#1	-15519.39±20.84	-25756.19±15.41	-9893.34±13.27	693.32±0.17	35772.16±0.47	-9481.03±24.51	-30.78±0.56	280.67±1.78	-260.83±1.45	-3.72±0.08	-16.30±0.13	1.65±0.27
	ΔM#2	-15521.08±21.51	-25676.08±15.32	-9977.85±13.03	693.18±0.16	35773.25±0.45	-9487.85±24.49	-33.34±0.87	291.96±1.92	-271.27±1.52	-4.05±0.09	-18.35±0.10	1.65±0.54
C5	WT	-15530.30±16.40	-25745.00±6.63	-9918.23±5.24	689.54±0.10	35776.63±1.53	-9503.97±21.48	-65.27±0.77	302.36±0.69	-275.42±0.58	-6.74±0.08	-26.86±0.17	-18.21±0.59
	ΔM#1	-15517.55±16.21	-25816.75±6.39	-9828.87±4.95	690.12±0.08	35766.63±1.44	-9483.19±21.90	-61.05±1.00	287.15±1.01	-261.53±0.84	-6.22±0.07	-25.09±0.19	-16.56±0.69
	ΔM#2	-15519.42±15.86	-25727.59±6.72	-9922.05±5.33	689.94±0.09	35769.63±1.49	-9492.22±22.26	-61.88±0.93	306.56±0.57	-279.82±0.46	-6.56±0.08	-25.19±0.20	-16.52±0.71
C7	WT	-15515.32±16.65	-25740.41±6.45	-9917.68±5.14	689.59±0.09	35762.44±1.54	-9494.00±21.19	-64.96±0.73	302.46±0.69	-275.53±0.59	-6.73±0.07	-26.86±0.17	-17.89±0.56
	ΔM#1	-15542.18±12.06	-25776.00±12.13	-9899.55±11.90	686.19±0.21	35771.98±1.90	-9505.71±26.51	-71.40±0.87	274.46±3.24	-248.44±2.58	-9.08±0.07	-28.28±0.06	-26.19±0.38
	ΔM#2	-15529.34±12.05	-25840.27±12.56	-9815.78±12.19	686.61±0.22	35763.39±2.00	-9487.10±26.59	-66.61±0.74	260.04±3.13	-235.37±2.75	-8.78±0.08	-26.10±0.10	-24.62±0.23
C8	WT	-15528.76±12.19	-25771.69±12.19	-9899.73±12.03	686.28±0.22	35757.17±1.90	-9498.29±26.56	-71.31±0.87	274.56±3.23	-248.51±2.57	-9.08±0.07	-28.28±0.06	-26.06±0.39
	ΔM#1	-15572.61±26.33	-25722.41±14.16	-9942.87±9.72	690.31±0.43	35784.32±1.15	-9524.61±44.36	-76.97±1.61	301.80±0.32	-272.67±0.36	-8.93±0.08	-29.31±0.36	-27.46±1.12
	ΔM#2	-15557.84±25.88	-25785.51±13.42	-9858.63±9.28	690.65±0.44	35776.05±1.09	-9502.55±44.63	-70.33±1.49	292.08±0.42	-261.90±0.26	-8.76±0.10	-26.48±0.41	-22.43±0.75
C9	WT	-15559.40±26.38	-25707.60±13.89	-9944.34±9.77	690.59±0.44	35777.61±1.10	-9510.56±43.58	-72.47±1.28	301.15±0.31	-272.96±0.51	-8.91±0.07	-27.81±0.35	-25.38±0.67
	ΔM#1	-15559.55±26.48	-25719.08±14.21	-9942.22±9.81	690.38±0.41	35769.74±1.11	-9516.65±43.91	-76.70±1.61	301.83±0.28	-272.80±0.35	-8.89±0.08	-29.30±0.36	-27.26±1.12
	ΔM#3	-15538.55±4.49	-25794.92±11.65	-9876.27±6.87	693.73±0.23	35784.20±0.72	-9438.42±43.10	-50.51±0.68	310.70±1.03	-287.05±1.04	-5.39±0.10	-21.77±0.16	-10.47±0.52
C9	WT	-15528.50±4.59	-25864.25±11.53	-9787.16±6.74	694.10±0.21	35774.96±0.66	-9421.61±43.86	-48.91±0.46	293.71±1.18	-270.60±1.19	-5.15±0.10	-20.17±0.21	-10.78±0.27
	ΔM#2	-15526.45±4.44	-25777.97±11.56	-9878.46±6.74	693.97±0.23	35777.26±0.71	-9424.98±43.14	-45.89±0.64	310.47±1.01	-287.38±1.00	-5.39±0.10	-20.69±0.14	-7.49±0.56
	ΔM#3	-15522.34±4.68	-25790.76±11.87	-9876.08±7.08	693.97±0.23	35769.97±0.77	-9426.93±42.75	-49.34±0.60	310.94±1.17	-287.52±1.13	-5.13±0.06	-21.50±0.16	-9.55±0.47

Fig. S5. Molecular dynamics simulation and energy minimization of the top M-site Arf1 docking models. (A) Root-mean-square deviation (r.m.s.d.) time course of the MD simulation of the C8 model, showing the system reached equilibrium after 30 nanoseconds. Other models showed similar r.m.s.d. time courses. (B) Overlay of all six MD energy minimized models. (C-H) Detailed view of the Arf1-M site interactions in indicated models. Sra1 is kept in the same

orientation to demonstrate different orientations of Arf1 in various MD models. Yellow dotted lines indicate polar or π - π interactions. **(I)** List of MM-PBSA-WSAS energy terms of whole complex free energies (left) and Arf1-binding free energies (right) of six Arf1-WRC^{AD-Rac1} complexes, each including the WT WRC and M-site mutants, with $\Delta M\#1$ for Sra1^{Y986A/E988A}, $\Delta M\#2$ for Sra1^{948A/T951A}, and $\Delta M\#3$ for Sra1^{W845A/Y849A} (as a negative control). All energy terms, including E_{vdw} and E_{eel} for the van der Waals and electrostatic interactions, respectively; G_{polar}^{sol} and $G_{nonpolar}^{sol}$ for the polar and nonpolar parts of the solvation free energy, respectively, and TS for the entropic term, are in kcal/mol. Model C8 agreed best with the experimental results, in that $\Delta M\#1$ and $\Delta M\#2$, but not $\Delta M\#3$ mutations, increased by free energies for Arf1 binding.

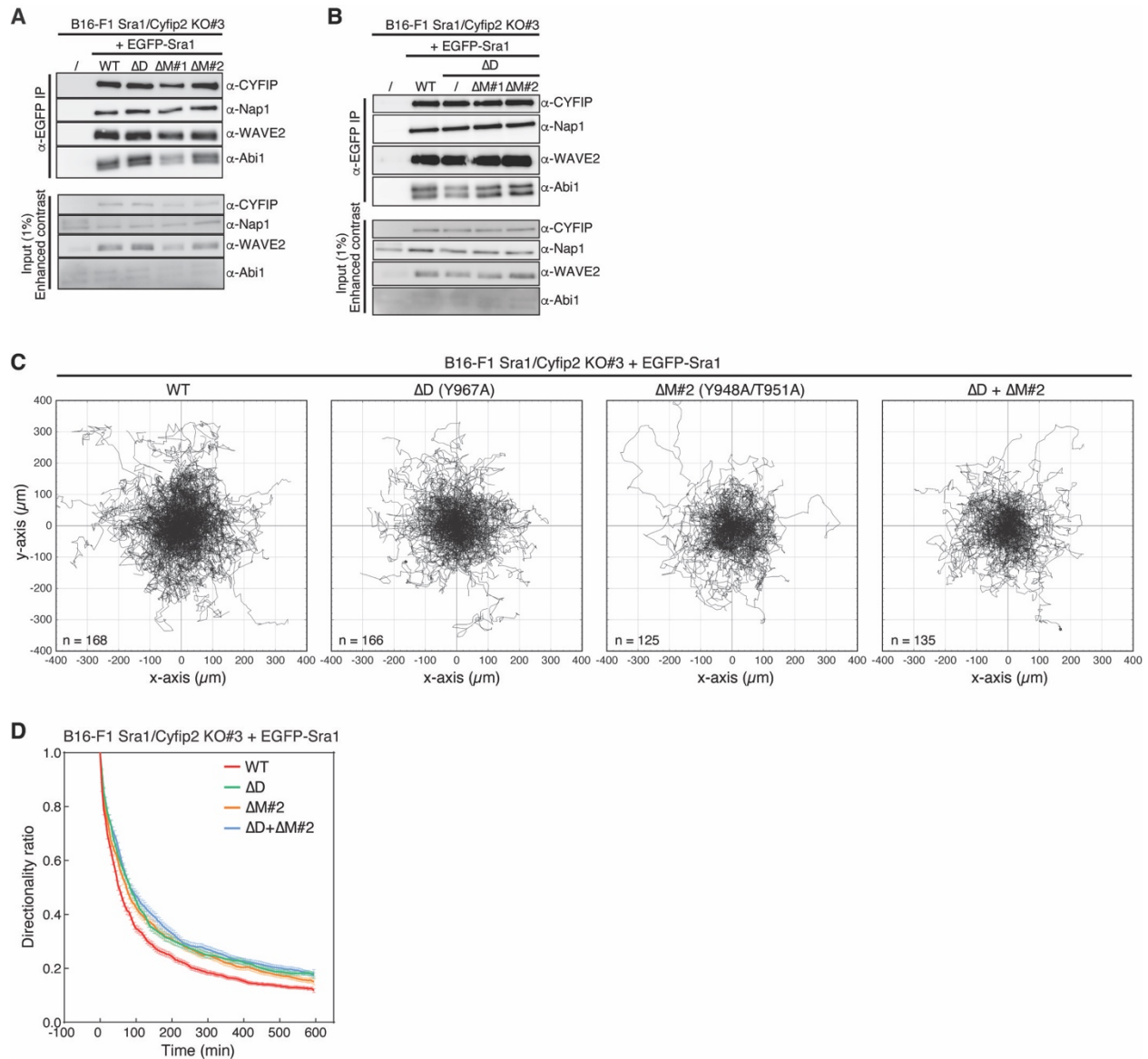


Fig. S6. Supporting data for the cellular studies in Fig.6. (A-B) Immunoprecipitation (IP) and Western blot of the same B16-F1 *Sra1/CYFIP2* double KO#3 cells used in Fig. 6. The cells were transfected with indicated EGFP-tagged Sra1 variants, lysed, and probed for the expression and assembly of WRC, as exemplified by CYFIP (for both Sra1 and Cyfp2), Nap1, WAVE2, and Abi1. (C) Trajectory plots of *Sra1/Cyfp2* KO#3 cells rescued with EGFP-Sra1 WT and indicated variants and subjected to random cell migration assays. “n” equals to number of cell trajectories analyzed. Migration rates derived from trajectory analyses are shown in Fig. 6L. (D) Analysis of migration directionality of indicated Sra1-rescued cells by showing directionality ratios over elapsed time. Data are from the same cells as used in Fig. 6M. Compared to the directionality ratio at the last point of trajectory shown in Fig. 6M, this figure displays the directionality ratios of

migration trajectories at all measured time points throughout the entire migration process. Solid curves are arithmetic means of directionality ratios at given time points of all measured cells, while error bars are standard error of means.

Table S1. DNA constructs and WRC assemblies used in this study

Name	Description	Source/reference	Identifier
Individual proteins/subunits			
Sra1	His6-Tev-hSra1 (1-1253, full length) in pAV5a vector, His6-Tev finally removed	(35)	pYS1
Sra1 ^{D-Rac1}	His6-Tev-Sra1-(GGG) ₄ -Rac1 ^{Q61L/P29S} (1-188) in pAV5a vector, His6-Tev finally removed	(46)	pYS11
Sra1 ^{A-Rac1}	His6-Tev-Sra1 Y423-[(GGG) ₆ -Rac1 ^{Q61L/P29S} (1-188)-(GS) ₆]-S424 (Rac1 is inserted in a loop of Sra1 between Y423/S424), in pAV5a vector, His6-Tev finally removed	This study	pYS88
Sra1 ^{Y986A, E988A}	986A, E988A in Sra1 (Δ M#1)	This study	pYS197
Sra1 ^{Y948A, T951A}	Y948A, T951A in Sra1 (Δ M#2)	This study	pYS198
Sra1 ^{W845A, Y849A}	W845A, Y849A in Sra1 (Δ M#3)	This study	pYS199
Sra1 ^{D-PGS}	His6-Tev-Sra1 H965-(G)-PGS-(G)-G968 (PGS is inserted between H965 and G968, with E966 and Y967 replaced by Gly), in pAV5a vector, His6-Tev finally removed (D-block)	This study	pYS110
Sra1 ^{A-PGS}	His6-Tev-Sra1 K178-(G)-PGS-(G)-S180 (PGS is inserted between K178 and S180, with C179 replaced by Gly. An extra Gly is tethered to C terminus of PGS), in pAV5a vector, His6-Tev finally removed (A-block)	This study	pYS112
Sra1 ^{Y967A}	Y967A in Sra1 (Δ D)	(41)	cbyd-150807-3 (AT3-1)
Sra1 ^{C179R}	C179R in Sra1 (Δ A)	(36)	cbyd-150807-8
Sra1 ^{C179R, D-Rac1}	His6-Tev-Sra1 ^{C179R} -(GGG) ₄ -Rac1 ^{Q61L/P29S} (1-188) in pAV5a vector, His6-Tev finally removed	This study	pYS196
Nap1	His6-Tev-hNap1 (1-1128, full length), in pAV5a vector, His6-Tev finally removed	(35)	pYS2
WAVE1 (1-230)	MBP-Tev-hWAVE1 (1-230) in pMalC2Tev vector, MBP-Tev finally removed	(41)	pYS8
WAVE1 (1-230)-WCA	MBP-Tev-hWAVE1 (1-230)-(GGG) ₆ -WCA(485-559) in pMalC2Tev vector, MBP-Tev finally removed	(41)	pYS9
WAVE1(1-230)-Rac1	MBP-Tev-WAVE1 (1-230)-(GGG) ₆ -Rac1 ^{Q61L/P29S} (1-188) in pMalC2Tev vector, MBP-Tev finally removed	(41)	cbyd-131103-2 (AE9-2)
Abi2 (1-158)	MBP-Tev-hAbi2 (1-158) in pMalC2Tev vector, MBP-Tev finally removed	(35)	pYS3

HSPC300	MBP-Tev-hHSPC300 (1-79, full length) in pMalC2Tev vector, MBP-Tev finally removed	(35)	pYS4
WCA	hWAVE1(485-559) in pET11a vector	(35)	cbyd-090413-13 (pBC6)
GST-Arf1	GST-Thrombin-Arf1 Δ^{17} (18-181) in pGEX Thrombin vector	This study	pYS30
His8-Arf1	His ₈ -(GGS) ₂ -Arf1 Δ^{17} (18-181) in pET11a vector	This study	pYS186
GST-Arf5	GST- Thrombin-Arf5 Δ^{17} (18-180) in pGEX Thrombin vector	From Neal Alto	pYS29
GST-Arf6	GST- Thrombin-Arf6 Δ^{13} (14-175) in pGEX Thrombin vector	From Neal Alto	pYS28
GST-Arl1	GST- Thrombin-Arl1 Δ^{17} (18-181) in pGEX Thrombin vector	From Neal Alto	pYS27
GST-Arl2	GST- Thrombin-Arl2 Δ^{13} (14-184) in pGEX Thrombin vector	From Neal Alto	pYS31
His6-EspG	His ₆ -EspG ^{A41} (42-398) in pPROEX HTb vector	From Neal Alto	pYS70
GST-Rac1 ^{QP}	GST-Tev-Rac1 ^{Q61L/P29S} (1-188) in pGEXTev vector	(41)	pYS7
GST-Rac1 ^{dead}	Switch I (a.a. 25-39) replaced by (GGS) ₅ in GST-Rac1	This study	pYS107
Untagged Rac1 ^{QP}	Rac1 ^{Q61L/P29S} (1-188) in pET11a vector	(41)	pYS108
EGFP-mCyfip1	EGFP-mCyfip1 in pEGFP vector	(62)	pMS1
EGFP-mCyfip1 ^{Y967A}	EGFP-mCyfip1 ^{Y967A} in pEGFP vector (Δ D)	(62)	pMS5
EGFP-mCyfip1 ^{Y986A, E988A}	EGFP-mCyfip1 ^{Y986A, E988A} in pEGFP vector (Δ M#1)	This study	pMS140
EGFP-mCyfip1 ^{Y948A, T951A}	EGFP-mCyfip1 ^{Y948A, T951A} in pEGFP vector (Δ M#2)	This study	pMS141
EGFP-mCyfip1 ^{Y967A, Y986A, E988A}	EGFP-mCyfip1 ^{Y967A, Y986A, E988A} in pEGFP vector (Δ D, Δ M#1)	This study	pMS156
EGFP-mCyfip1 ^{Y967A, Y948A, T951A}	EGFP-mCyfip1 ^{Y967A, Y948A, T951A} in pEGFP vector (Δ D, Δ M#2)	This study	pMS157
Assembled WRC (refer to the above table for subunit information)			
WRC ^{230WCA} , or WRC ^{apo}	Sra1, Nap1, WAVE1 (1-230)-WCA, Abi2 (1-158), and HSPC300	(41)	WRC ^{230WCA} , or WRC ^{apo}
WRC ^{D-Rac1}	Sra1 ^{D-Rac1} , Nap1, WAVE1 (1-230)-WCA, Abi2 (1-158), and HSPC300	(46)	WRC ^{D-Rac1}
WRC ^{$\Delta\Delta$, D-Rac1}	Sra1 ^{C179R, D-Rac1} , Nap1, WAVE1 (1-230)-WCA, Abi2 (1-158), and HSPC300	This study	WRC ^{$\Delta\Delta$, D-Rac1}
WRC ^{ΔM#1}	Sra1 ^{Y986A, E988A} , Nap1, WAVE1 (1-230)-WCA, Abi2 (1-158), and HSPC300	This study	WRC ^{ΔM#1}

WRC ^{ΔM#2}	Sra1 ^{Y948A, T951A} , Nap1, WAVE1 (1-230)-WCA, Abi2 (1-158), and HSPC300	This study	WRC ^{ΔM#2}
ΔWRC230	Sra1, Nap1, WAVE1 (1-230), Abi2 (1-158), and HSPC300	(41)	ΔWRC230
ΔWRC230 ^{D-Rac1}	Sra1 ^{D-Rac1} , Nap1, WAVE1 (1-230), Abi2 (1-158), and HSPC300	(46)	ΔWRC230 ^{D-Rac1}
ΔWRC230 ^{A-Rac1}	Sra1 ^{A-Rac1} , Nap1, WAVE1 (1-230), Abi2 (1-158), and HSPC300	This study	ΔWRC230 ^{A-Rac1}
ΔWRC230 ^{AWCA-Rac1}	Sra1, Nap1, WAVE1(1-230)-Rac1, Abi2 (1-158), and HSPC300	(41)	ΔWRC230 ^{AWCA-Rac1}
ΔWRC230 ^{D-block}	Sra1 ^{D-PGS} , Nap1, WAVE1 (1-230), Abi2 (1-158), and HSPC300	This study	ΔWRC230 ^{D-block}
ΔWRC230 ^{A-block}	Sra1 ^{A-PGS} , Nap1, WAVE1 (1-230), Abi2 (1-158), and HSPC300	This study	ΔWRC230 ^{A-block}
ΔWRC230 ^{ΔM#1}	Sra1 ^{Y986A, E988A} , Nap1, WAVE1 (1-230)-WCA, Abi2 (1-158), and HSPC300	This study	ΔWRC230 ^{ΔM#1}
ΔWRC230 ^{ΔM#2}	Sra1 ^{Y948A, T951A} , Nap1, WAVE1 (1-230)-WCA, Abi2 (1-158), and HSPC300	This study	ΔWRC230 ^{ΔM#2}
ΔWRC230 ^{ΔM#3}	Sra1 ^{W845A, Y849A} , Nap1, WAVE1 (1-230)-WCA, Abi2 (1-158), and HSPC300	This study	ΔWRC230 ^{ΔM#3}

Table S2. Sequences of recombinant proteins used in this study

<p>Only sequences in the final product (i.e., after protease cleavage to remove the affinity tag) are shown.</p>
<p>>Sra1 GAMAAQVTLLEDALSNDLLEELPLPDQQPCIEPPSSLLYQPNFNTNFEDRNAFVTGIARYIEQATVHSSMNEMLEEGQEYAVMLYT WRSCSRAIPQVKCNEQPNRVEIYEKTEVELEPEVTKLMNFMFYQRNAIERFCGEVRRLLCHAERRKDFVSEAYLITLGGKFINMFAVLD ELKNMKCSVKNDHSA YKRAAQFLRKMADPQSIQESQNL SMFLANHNKITQSLQQQLEVISGYEELLADIVNLCVDYYENRMYLTPS EKHMMLKVMGFGLYLMDGSVSNYKLD AKKRINLSKIDKYFKQLQVVPLFGDMQIELARYIKTSAHYEENKSRWCTCTSSGSSPQYNI CEQMIQIREDHMRFISELARYSNSEVVTGSGRQEAQKTD AEYRKLFDLALQGLQLLSQWSAHVMEVYVSWKLVHPTDKYSNKDCPD SAEYERATRYNYTSEEKFA LVEVIAMIKGLQVLMGRMESVFNHAI RHTVY AALQDFSQVTLREPLRQA IKKKKNVIQSVLQAI RKT VCDWETGHEPFNDPALRGEKDPKSGFDIKVPRRAVGPSTQLY MVRTMLES LIADKSGSKTLRSSLEGPTILDIEKFHRESFFYTHLI NFSETLQCCDLSQLWFRFFLELTMGRRIQFPIEMSMPWILTDHILETKEASMM EYVLYSLDLYNDSAHYALTRFNKQFLYDEIEAE VNLCFDQFVYKLADQIFAYYKVMAGSLLDKRLRSECKNQGATHLPPSNRYETLLKQRHVQLGRSIDLNRLITQRVSAAMYKSE LAIGRFES EDTSIVELDGLLEINRMTHKLLSRYLTLDGFDAMFREANHNVSAPYGRITLHVFWELNYDFLPNYCYNGSTNRFVRTV LPFSQEFQRDKQPNAQPQYLHGSKALNLA YSSYGSYRNFVGP PHFQVICRLLGYQGIAV VMEELLKVVKSLLOGTILQYVKTLM EV MPKICRLPRHEYGSPGILEFFHHQLKDIVEY AELKTVCFQNLREVGNAILFCLLIEQSLSLEEVCDLLHAAPFQNLPRVHVKEGERLD AKMKRLESKYAPLHLVPLIERLGT PQQIAIAREGDLLTKERLCCGLSMFEVILTRIRSFDDPIWRG PLPSNGVMHVDECVEFHRLWS AMQFVY CIPVGTHEFTVEQCFDGLHWAGCMII VLLGQRRFAVLD FCYHLLKVQKHDGKDEIKNVPLKKMVERIRK FQILNDEI I TILDKYLSKSGDGEGTPVEHVRCFQPIHQSLASS</p>
<p>>Sra1^{D-Rac1}, or Sra1-(GGG)₄-Rac1^{Q61L/P29S}(1-188) GAMAAQVTLLEDALSNDLLEELPLPDQQPCIEPPSSLLYQPNFNTNFEDRNAFVTGIARYIEQATVHSSMNEMLEEGQEYAVMLYT WRSCSRAIPQVKCNEQPNRVEIYEKTEVELEPEVTKLMNFMFYQRNAIERFCGEVRRLLCHAERRKDFVSEAYLITLGGKFINMFAVLD ELKNMKCSVKNDHSA YKRAAQFLRKMADPQSIQESQNL SMFLANHNKITQSLQQQLEVISGYEELLADIVNLCVDYYENRMYLTPS EKHMMLKVMGFGLYLMDGSVSNYKLD AKKRINLSKIDKYFKQLQVVPLFGDMQIELARYIKTSAHYEENKSRWCTCTSSGSSPQYNI CEQMIQIREDHMRFISELARYSNSEVVTGSGRQEAQKTD AEYRKLFDLALQGLQLLSQWSAHVMEVYVSWKLVHPTDKYSNKDCPD SAEYERATRYNYTSEEKFA LVEVIAMIKGLQVLMGRMESVFNHAI RHTVY AALQDFSQVTLREPLRQA IKKKKNVIQSVLQAI RKT VCDWETGHEPFNDPALRGEKDPKSGFDIKVPRRAVGPSTQLY MVRTMLES LIADKSGSKTLRSSLEGPTILDIEKFHRESFFYTHLI NFSETLQCCDLSQLWFRFFLELTMGRRIQFPIEMSMPWILTDHILETKEASMM EYVLYSLDLYNDSAHYALTRFNKQFLYDEIEAE VNLCFDQFVYKLADQIFAYYKVMAGSLLDKRLRSECKNQGATHLPPSNRYETLLKQRHVQLGRSIDLNRLITQRVSAAMYKSE LAIGRFES EDTSIVELDGLLEINRMTHKLLSRYLTLDGFDAMFREANHNVSAPYGRITLHVFWELNYDFLPNYCYNGSTNRFVRTV LPFSQEFQRDKQPNAQPQYLHGSKALNLA YSSYGSYRNFVGP PHFQVICRLLGYQGIAV VMEELLKVVKSLLOGTILQYVKTLM EV MPKICRLPRHEYGSPGILEFFHHQLKDIVEY AELKTVCFQNLREVGNAILFCLLIEQSLSLEEVCDLLHAAPFQNLPRVHVKEGERLD AKMKRLESKYAPLHLVPLIERLGT PQQIAIAREGDLLTKERLCCGLSMFEVILTRIRSFDDPIWRG PLPSNGVMHVDECVEFHRLWS AMQFVY CIPVGTHEFTVEQCFDGLHWAGCMII VLLGQRRFAVLD FCYHLLKVQKHDGKDEIKNVPLKKMVERIRK FQILNDEI I TILDKYLSKSGDGEGTPVEHVRCFQPIHQSLASSGGSGSGSGSGSMAIKCVVVG DGA VGKTCLLISYTTNAFSGEYIPTVFDNYS ANVMVDGKPVNLGLWDTAGLEDYDRLRPLSY PQTDFVFLICFSLVSPASFENVR AKWYPEVRHHCNTPHILVGT KLDLDRDDKDTIE KLKEKKTLPITYPQGLAMAKEIGAVKYLECSALTQRGLKTVFDEAIRAVLCP PPVKKRKRK</p>
<p>>Sra1^{A-Rac1} or Sra1 Y423-[(GGG)₆-Rac1^{Q61L/P29S}(1-188)-HRV_3C-(GG)₆]-S424 GAMAAQVTLLEDALSNDLLEELPLPDQQPCIEPPSSLLYQPNFNTNFEDRNAFVTGIARYIEQATVHSSMNEMLEEGQEYAVMLYT WRSCSRAIPQVKCNEQPNRVEIYEKTEVELEPEVTKLMNFMFYQRNAIERFCGEVRRLLCHAERRKDFVSEAYLITLGGKFINMFAVLD ELKNMKCSVKNDHSA YKRAAQFLRKMADPQSIQESQNL SMFLANHNKITQSLQQQLEVISGYEELLADIVNLCVDYYENRMYLTPS EKHMMLKVMGFGLYLMDGSVSNYKLD AKKRINLSKIDKYFKQLQVVPLFGDMQIELARYIKTSAHYEENKSRWCTCTSSGSSPQYNI CEQMIQIREDHMRFISELARYSNSEVVTGSGRQEAQKTD AEYRKLFDLALQGLQLLSQWSAHVMEVYVSWKLVHPTDKYGGSGGS GGSGSGSGSGSMQAICV VVG DGA VGKTCLLISYTTNAFSGEYIPTVFDNYSANVMVDGKPVNLGLWDTAGLEDYDRLRPLSY PQTDVFLICFSLVSPASFENVR AKWYPEVRHHCNTPHILVGT KLDLDRDDKDTIEKLEKKTLPITYPQGLAMAKEIGAVKYLECSAL TQRGLKTVFDEAIRAVLCP PPVKKRKRKGSLEVL FQGP GSGSGSGSSNKDCPD SAEYERATRYNYTSEEKFA LVEVIAMIKGL QVLMGRMESVFNHAI RHTVY AALQDFSQVTLREPLRQA IKKKKNVIQSVLQAI RKT VCDWETGHEPFNDPALRGEKDPKSGFDIKV PRRAVGPSTQLY MVRTMLES LIADKSGSKTLRSSLEGPTILDIEKFHRESFFYTHLI NFSETLQCCDLSQLWFRFFLELTMGRRIQ FPIEMSMPWILTDHILETKEASMM EYVLYSLDLYNDSAHYALTRFNKQFLYDEIEAEVNLCFDQFVYKLADQIFAYYKVMAGSLL DKRLRSECKNQGATHLPPSNRYETLLKQRHVQLGRSIDLNRLITQRVSAAMYKSELAIGRFES EDTSIVELDGLLEINRMTHKLL SRYLTLDGFDAMFREANHNVSAPYGRITLHVFWELNYDFLPNYCYNGSTNRFVRTVLPFSQEFQRDKQPNAQPQYLHGSKALNLA YSSYGSYRNFVGP PHFQVICRLLGYQGIAV VMEELLKVVKSLLOGTILQYVKTLM EVMPKICRLPRHEYGSPGILEFFHHQLKDIVE Y AELKTVCFQNLREVGNAILFCLLIEQSLSLEEVCDLLHAAPFQNLPRVHVKEGERLDAKMKRLESKYAPLHLVPLIERLGT PQQIAI AREGDLLTKERLCCGLSMFEVILTRIRSFDDPIWRG PLPSNGVMHVDECVEFHRLWSAMQFVY CIPVGTHEFTVEQCFDGLHWA GCMII VLLGQRRFAVLD FCYHLLKVQKHDGKDEIKNVPLKKMVERIRK FQILNDEI I TILDKYLSKSGDGEGTPVEHVRCFQPIHQ S LASS</p>
<p>> Sra1^{Y986A, E988A} GAMAAQVTLLEDALSNDLLEELPLPDQQPCIEPPSSLLYQPNFNTNFEDRNAFVTGIARYIEQATVHSSMNEMLEEGQEYAVMLYT WRSCSRAIPQVKCNEQPNRVEIYEKTEVELEPEVTKLMNFMFYQRNAIERFCGEVRRLLCHAERRKDFVSEAYLITLGGKFINMFAVLD ELKNMKCSVKNDHSA YKRAAQFLRKMADPQSIQESQNL SMFLANHNKITQSLQQQLEVISGYEELLADIVNLCVDYYENRMYLTPS EKHMMLKVMGFGLYLMDGSVSNYKLD AKKRINLSKIDKYFKQLQVVPLFGDMQIELARYIKTSAHYEENKSRWCTCTSSGSSPQYNI CEQMIQIREDHMRFISELARYSNSEVVTGSGRQEAQKTD AEYRKLFDLALQGLQLLSQWSAHVMEVYVSWKLVHPTDKYSNKDCPD SAEYERATRYNYTSEEKFA LVEVIAMIKGLQVLMGRMESVFNHAI RHTVY AALQDFSQVTLREPLRQA IKKKKNVIQSVLQAI RKT VCDWETGHEPFNDPALRGEKDPKSGFDIKVPRRAVGPSTQLY MVRTMLES LIADKSGSKTLRSSLEGPTILDIEKFHRESFFYTHLI</p>

NFSETLQQCCDLSQLWFREFFLELTMGRRIQFPIEMSPWPILTDHILETKEASMMMEYVLYSLDLYNDSAHYALTRFNKQFLYDEIEAE
VNLCFDQFVYKLDQIFAYYKVMAGSLLLDKRLRSECKNQGATHLPPSNRYETLLKQRHVQLLGRSIDLNRLITQRVSAAMYKSL
LAIGRFESDLTSIVELDGLLEINRMTHKLLSRYLTDGDFDAMFREANHNVSAPYGRITLHVFWELNYDFLPNYCYNGSTNRFVRTV
LPFSQEFQRDKQPNAQPQYLHGSKALNLA YSSYGSYRNFVGPFFHQVICRLLGYQGIAVVMEEELLKVVKSLLQGTLQYVKTLMVEV
MPKICRLPRHEYGSPGILEFFHHQLKDIVEAAALKTVCFQNLREVGNAIFCLLIEQSLSEEVCDLLHAAPFQNLPRVHVKEGERLD
AKMKRLESKYAPLHLVPLIERLGTQQIAIAREGDLLTKERLCCGLSMFEVILTRIRSFLDDPIWRGPLPSNGVMHVDECVEFHRLWS
AMQFVYVIPVGTHEFTVEQCFDGLHWAGCMIIVLLGQRRFAVLDFCYHLLKVQKHDGKDEIKNVPLKKMVERIRKQFQILNDEII
TILDKYLKSGDGEPTVEHVRCFQPIHQSLASS

> **Sra1**^{Y948A, T951A}
GAMAAQVTLLEDALSNDLLEELPLPDQPCIEPPSSLLYQPNFNTNFEDRNAFVTGIARYIEQATVHSSMNEMLEEGQEYAVMLYT
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EKHMLLKVMGFLYLMDGSVSNYKLDACKRINLSKIDKYFKQLQVVPFLGDMQIELARYIKTSAHYEENKSRWCTSSGSSPQYNI
CEQMIQIREDHMRFISELARYSNSEVVTGSGRQEAQKTDAYRKLFDLALQGLQLLSQWSAHVMEVYVSWKLVHPTDKYSNKDCPD
SAEYERATRYNYTSEEKFALEVIAMIKGLQVLMGRMESVFNHAIHRTVY AALQDFSQVTLREPLRQAIAKKNVQSVLQAIKRT
VCDWETGHEPFNDPALRGEKDPKSGFDIKVPRRAVGPSTQLY MVRTMLESLIADKSGSKTLRSSLEGPTILDIEKFHRESFFYTHLI
NFSETLQQCCDLSQLWFREFFLELTMGRRIQFPIEMSPWPILTDHILETKEASMMMEYVLYSLDLYNDSAHYALTRFNKQFLYDEIEAE
VNLCFDQFVYKLDQIFAYYKVMAGSLLLDKRLRSECKNQGATHLPPSNRYETLLKQRHVQLLGRSIDLNRLITQRVSAAMYKSL
LAIGRFESDLTSIVELDGLLEINRMTHKLLSRYLTDGDFDAMFREANHNVSAPYGRITLHVFWELNYDFLPNYCYNGSTNRFVRTV
LPFSQEFQRDKQPNAQPQYLHGSKALNLA YSSYGSYRNFVGPFFHQVICRLLGYQGIAVVMEEELLKVVKSLLQGTLQAVKALMEV
MPKICRLPRHEYGSPGILEFFHHQLKDIVEYAEKTVCFQNLREVGNAIFCLLIEQSLSEEVCDLLHAAPFQNLPRVHVKEGERLD
AKMKRLESKYAPLHLVPLIERLGTQQIAIAREGDLLTKERLCCGLSMFEVILTRIRSFLDDPIWRGPLPSNGVMHVDECVEFHRLWS
AMQFVYVIPVGTHEFTVEQCFDGLHWAGCMIIVLLGQRRFAVLDFCYHLLKVQKHDGKDEIKNVPLKKMVERIRKQFQILNDEII
TILDKYLKSGDGEPTVEHVRCFQPIHQSLASS

> **Sra1**^{W845A, Y849A}
GAMAAQVTLLEDALSNDLLEELPLPDQPCIEPPSSLLYQPNFNTNFEDRNAFVTGIARYIEQATVHSSMNEMLEEGQEYAVMLYT
WRSCSRAIPQVKCNEQPNRVEIYEKTEVELEPEVTKLMNMFYQFQNAIERFCGEVRRLCHAERRKDFVSEAYLITLTKFINMFAVLD
ELKNMKCSVKNDHSAYKRAAQFLRKMADPQSIQESQNLMSFLANHNKITQSLQQQLEVISGYEELLADIVNLCVDYENRMYLTPS
EKHMLLKVMGFLYLMDGSVSNYKLDACKRINLSKIDKYFKQLQVVPFLGDMQIELARYIKTSAHYEENKSRWCTSSGSSPQYNI
CEQMIQIREDHMRFISELARYSNSEVVTGSGRQEAQKTDAYRKLFDLALQGLQLLSQWSAHVMEVYVSWKLVHPTDKYSNKDCPD
SAEYERATRYNYTSEEKFALEVIAMIKGLQVLMGRMESVFNHAIHRTVY AALQDFSQVTLREPLRQAIAKKNVQSVLQAIKRT
VCDWETGHEPFNDPALRGEKDPKSGFDIKVPRRAVGPSTQLY MVRTMLESLIADKSGSKTLRSSLEGPTILDIEKFHRESFFYTHLI
NFSETLQQCCDLSQLWFREFFLELTMGRRIQFPIEMSPWPILTDHILETKEASMMMEYVLYSLDLYNDSAHYALTRFNKQFLYDEIEAE
VNLCFDQFVYKLDQIFAYYKVMAGSLLLDKRLRSECKNQGATHLPPSNRYETLLKQRHVQLLGRSIDLNRLITQRVSAAMYKSL
LAIGRFESDLTSIVELDGLLEINRMTHKLLSRYLTDGDFDAMFREANHNVSAPYGRITLHVFAELNADFLPNYCYNGSTNRFVRTV
PFSQEFQRDKQPNAQPQYLHGSKALNLA YSSYGSYRNFVGPFFHQVICRLLGYQGIAVVMEEELLKVVKSLLQGTLQYVKTLMVEV
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AMQFVYVIPVGTHEFTVEQCFDGLHWAGCMIIVLLGQRRFAVLDFCYHLLKVQKHDGKDEIKNVPLKKMVERIRKQFQILNDEII
TILDKYLKSGDGEPTVEHVRCFQPIHQSLASS

>**Sra1**^{D-PGS} or **Sra1 H965-(G)-PGS-(G)-G968**
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ELKNMKCSVKNDHSAYKRAAQFLRKMADPQSIQESQNLMSFLANHNKITQSLQQQLEVISGYEELLADIVNLCVDYENRMYLTPS
EKHMLLKVMGFLYLMDGSVSNYKLDACKRINLSKIDKYFKQLQVVPFLGDMQIELARYIKTSAHYEENKSRWCTSSGSSPQYNI
CEQMIQIREDHMRFISELARYSNSEVVTGSGRQEAQKTDAYRKLFDLALQGLQLLSQWSAHVMEVYVSWKLVHPTDKYSNKDCPD
SAEYERATRYNYTSEEKFALEVIAMIKGLQVLMGRMESVFNHAIHRTVY AALQDFSQVTLREPLRQAIAKKNVQSVLQAIKRT
VCDWETGHEPFNDPALRGEKDPKSGFDIKVPRRAVGPSTQLY MVRTMLESLIADKSGSKTLRSSLEGPTILDIEKFHRESFFYTHLI
NFSETLQQCCDLSQLWFREFFLELTMGRRIQFPIEMSPWPILTDHILETKEASMMMEYVLYSLDLYNDSAHYALTRFNKQFLYDEIEAE
VNLCFDQFVYKLDQIFAYYKVMAGSLLLDKRLRSECKNQGATHLPPSNRYETLLKQRHVQLLGRSIDLNRLITQRVSAAMYKSL
LAIGRFESDLTSIVELDGLLEINRMTHKLLSRYLTDGDFDAMFREANHNVSAPYGRITLHVFWELNYDFLPNYCYNGSTNRFVRTV
LPFSQEFQRDKQPNAQPQYLHGSKALNLA YSSYGSYRNFVGPFFHQVICRLLGYQGIAVVMEEELLKVVKSLLQGTLQYVKTLMVEV
MPKICRLPRHEYGSPGILEFFHHQLKDIVEYAEKTVCFQNLREVGNAIFCLLIEQSLSEEVCDLLHAAPFQNLPRVHVKEGERLD
AKMKRLESKYAPLHLVPLIERLGTQQIAIAREGDLLTKERLCCGLSMFEVILTRIRSFLDDPIWRGPLPSNGVMHVDECVEFHRLWS
AMQFVYVIPVGTHEFTVEQCFDGLHWAGCMIIVLLGQRRFAVLDFCYHLLKVQKHDGKDEIKNVPLKKMVERIRKQFQILNDEII
TILDKYLKSGDGEPTVEHVRCFQPIHQSLASS

>**Sra1**^{A-PGS} or **K178-(G)-PGS-(G)-S180**
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WRSCSRAIPQVKCNEQPNRVEIYEKTEVELEPEVTKLMNMFYQFQNAIERFCGEVRRLCHAERRKDFVSEAYLITLTKFINMFAVLD
ELKNMKGGIDCSFVNESYLTGSRDERKKSLLSKFGMDEGVTFMFIGRFRDQKGVVDVLLKAIIEILSSKKEFQEMRFIIIGKGDPELEG
WARSLEEKHGNVAVITMELSRFVRELYGSVDFVIIPSYFEPFGLVALEAMCLGAIPIASAVGGLRDIITNETGILVKAGDPGELANAI
LKALELSRDLKSFRENCKKRAMSFSGSGPILGFFHHQLKDIVEYAEKTVCFQNLREVGNAIFCLLIEQSLSEEVCDLLHAA
PFQNLPRVHVKEGERLDAKMKRLESKYAPLHLVPLIERLGTQQIAIAREGDLLTKERLCCGLSMFEVILTRIRSFLDDPIWRGPLPSN
GVMHVDECVEFHRLWSAMQFVYVIPVGTHEFTVEQCFDGLHWAGCMIIVLLGQRRFAVLDFCYHLLKVQKHDGKDEIKNVPL
KKMVERIRKQFQILNDEIITILDKYLKSGDGEPTVEHVRCFQPIHQSLASS

YSWKL VHPD KYSNKDCPDSAEYERATRYNYTSEEK FALVEVIAMIKGLQVLMGRMESVFNHAI RHTVYAALQDFSQVTLREPLR
QAIKKKKNVIQSVLQAI RKT VCDWETGHEPFNDPALRGEKDPKSGFDIKVPRRAVGPSS TQLYMVRTMLES LIADKSGSKTLRSSL
EGPTIL DIEKFHRESFFYTHLINFSETLQCCDLSQLWFR EFFLELTMGRRIQFPIEMSPWILTDHILETKEASMM EYVLYSLDLYND
SAHYALTRFNKQFLYDEIEAEVNLCFDQFVYK LADQIFAYYKVMAGSLLLDKRLRSECKNQGATHLPPSNRYETLLKQRHVQLLG
RSIDLNRLITQRVSAAMYKSLELAIGRFESEDLTSIVELDGLLEINRMTHKLLSRYLTLDGFDAMFREANHNVSAPYGRITLHVFWEL
NYDFLPNYCYNGSTNRFRVTVLPFSQEFQRDKQPNAQPQYLHGSKALNLA YSSYGSYRN FVGPFFHQVICRLLGYQGIAVVM EELL
KVVKSLLOGTILQYVKTLM EVMPIKICRLPRHEYGSPGILEFFHHQLKDIVEY AELKTVCFQNLREVGNAILFCLLIEQSL SLEEVCDLL
HAAPFQNILPRVHVKEGERLDAKMKRLESKYAPLHLVPLIERLGT PQQIAIAREGDLLTKERLCCGLSMFEVILTRIRSFLDDPIWRGP
LPSNGVMHVDECEVFEHRLWSAMQFVY CIPVGTHEFTVEQCFGDGLHWAGCMIIVLLGQRRFAVLDFCYHLLKVQKHDGKDEI IK
NVPLKMMVERIRK FQILNDEIITILD KYLKS GGDGEGTPVEHVRFCFPPIHQSLASS

>Sra1^{Y967A}

GAMAAQVTL EDALSNDLLEELPLPDQPCIEPPSSLLYQPNFNTNFEDRNAFVTGIARYIEQATVHSSMNEMLEEGQEYAVMLYT
WRSCSRAIPQVKCNEQPNRVEIYEKTVEVLEPEVTKLMNFMFYQRNAIERFCGEVRR LCHAERRKDFVSEAYLITLGKFINMFVAVLD
ELKNMKCSVKNDHSAYKRAAQFLRKMADPQSIQESQNSMFLANHNKITQSLQQQLEVISGYEELLADIVNLCVDY YENRMYLTPS
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CEQMIQIREDHMRFISELARYSNSEVVTGSGRQEAQKTD AEYRKLFDLALQGLQLLSQWSAHVMEVY SWKLVHPTDKYSNKDCPD
SAEYERATRYNYTSEEK FALVEVIAMIKGLQVLMGRMESVFNHAI RHTVYAALQDFSQVTLREPLRQA IKKKNVIQSVLQAI RKT
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VNLCFDQFVYK LADQIFAYYKVMAGSLLLDKRLRSECKNQGATHLPPSNRYETLLKQRHVQLLGRSIDLNRLITQRVSAAMYKSLE
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LPFSQEFQRDKQPNAQPQYLHGSKALNLA YSSYGSYRN FVGPFFHQVICRLLGYQGIAVVM EELLKVVKSLLOGTILQYVKTLM E
VMPKICRLPRHEAGSPGILEFFHHQLKDIVEY AELKTVCFQNLREVGNAILFCLLIEQSL SLEEVCDLLHAAPFQNILPRVHVKEGERLD
AKMKRLESKYAPLHLVPLIERLGT PQQIAIAREGDLLTKERLCCGLSMFEVILTRIRSFLDDPIWRGPLPSNGVMHVDECEVFEHRLWS
AMQFVY CIPVGTHEFTVEQCFGDGLHWAGCMIIVLLGQRRFAVLDFCYHLLKVQKHDGKDEI IKNVPLKMMVERIRK FQILNDEI
TILD KYLKS GGDGEGTPVEHVRFCFPPIHQSLASS

>Sra1^{C179R}

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WRSCSRAIPQVKCNEQPNRVEIYEKTVEVLEPEVTKLMNFMFYQRNAIERFCGEVRR LCHAERRKDFVSEAYLITLGKFINMFVAVLD
ELKNMKRSVKNDHSAYKRAAQFLRKMADPQSIQESQNSMFLANHNKITQSLQQQLEVISGYEELLADIVNLCVDY YENRMYLTPS
EKHMLLKVMGFGLYLM DGSVSNYKLD AKKRINLSKIDKYFKQLQVVP LFGDMQIELARYIKTSAHYEENKSRWCTSSGSSPQYNI
CEQMIQIREDHMRFISELARYSNSEVVTGSGRQEAQKTD AEYRKLFDLALQGLQLLSQWSAHVMEVY SWKLVHPTDKYSNKDCPD
SAEYERATRYNYTSEEK FALVEVIAMIKGLQVLMGRMESVFNHAI RHTVYAALQDFSQVTLREPLRQA IKKKNVIQSVLQAI RKT
VCDWETGHEPFNDPALRGEKDPKSGFDIKVPRRAVGPSS TQLYMVRTMLES LIADKSGSKTLRSSLEGP TILDIEKFHRESFFYTHL
NFSETLQCCDLSQLWFR EFFLELTMGRRIQFPIEMSPWILTDHILETKEASMM EYVLYSLDLYNDSAHYALTRFNKQFLYDEIEAE
VNLCFDQFVYK LADQIFAYYKVMAGSLLLDKRLRSECKNQGATHLPPSNRYETLLKQRHVQLLGRSIDLNRLITQRVSAAMYKSLE
LAIGRFESEDLTSIVELDGLLEINRMTHKLLSRYLTLDGFDAMFREANHNVSAPYGRITLHVFWELNYDFLPNYCYNGSTNRFRVTV
LPFSQEFQRDKQPNAQPQYLHGSKALNLA YSSYGSYRN FVGPFFHQVICRLLGYQGIAVVM EELLKVVKSLLOGTILQYVKTLM E
VMPKICRLPRHEYGSPGILEFFHHQLKDIVEY AELKTVCFQNLREVGNAILFCLLIEQSL SLEEVCDLLHAAPFQNILPRVHVKEGERLD
AKMKRLESKYAPLHLVPLIERLGT PQQIAIAREGDLLTKERLCCGLSMFEVILTRIRSFLDDPIWRGPLPSNGVMHVDECEVFEHRLWS
AMQFVY CIPVGTHEFTVEQCFGDGLHWAGCMIIVLLGQRRFAVLDFCYHLLKVQKHDGKDEI IKNVPLKMMVERIRK FQILNDEI
TILD KYLKS GGDGEGTPVEHVRFCFPPIHQSLASS

>Sra1^{C179R, D-Rac1}, or Sra1^{C179R}-(GGS)₄-Rac1^{O61L/P29S}(1-188)

GAMAAQVTL EDALSNDLLEELPLPDQPCIEPPSSLLYQPNFNTNFEDRNAFVTGIARYIEQATVHSSMNEMLEEGQEYAVMLYT
WRSCSRAIPQVKCNEQPNRVEIYEKTVEVLEPEVTKLMNFMFYQRNAIERFCGEVRR LCHAERRKDFVSEAYLITLGKFINMFVAVLD
ELKNMKRSVKNDHSAYKRAAQFLRKMADPQSIQESQNSMFLANHNKITQSLQQQLEVISGYEELLADIVNLCVDY YENRMYLTPS
EKHMLLKVMGFGLYLM DGSVSNYKLD AKKRINLSKIDKYFKQLQVVP LFGDMQIELARYIKTSAHYEENKSRWCTSSGSSPQYNI
CEQMIQIREDHMRFISELARYSNSEVVTGSGRQEAQKTD AEYRKLFDLALQGLQLLSQWSAHVMEVY SWKLVHPTDKYSNKDCPD
SAEYERATRYNYTSEEK FALVEVIAMIKGLQVLMGRMESVFNHAI RHTVYAALQDFSQVTLREPLRQA IKKKNVIQSVLQAI RKT
VCDWETGHEPFNDPALRGEKDPKSGFDIKVPRRAVGPSS TQLYMVRTMLES LIADKSGSKTLRSSLEGP TILDIEKFHRESFFYTHL
NFSETLQCCDLSQLWFR EFFLELTMGRRIQFPIEMSPWILTDHILETKEASMM EYVLYSLDLYNDSAHYALTRFNKQFLYDEIEAE
VNLCFDQFVYK LADQIFAYYKVMAGSLLLDKRLRSECKNQGATHLPPSNRYETLLKQRHVQLLGRSIDLNRLITQRVSAAMYKSLE
LAIGRFESEDLTSIVELDGLLEINRMTHKLLSRYLTLDGFDAMFREANHNVSAPYGRITLHVFWELNYDFLPNYCYNGSTNRFRVTV
LPFSQEFQRDKQPNAQPQYLHGSKALNLA YSSYGSYRN FVGPFFHQVICRLLGYQGIAVVM EELLKVVKSLLOGTILQYVKTLM E
VMPKICRLPRHEYGSPGILEFFHHQLKDIVEY AELKTVCFQNLREVGNAILFCLLIEQSL SLEEVCDLLHAAPFQNILPRVHVKEGERLD
AKMKRLESKYAPLHLVPLIERLGT PQQIAIAREGDLLTKERLCCGLSMFEVILTRIRSFLDDPIWRGPLPSNGVMHVDECEVFEHRLWS
AMQFVY CIPVGTHEFTVEQCFGDGLHWAGCMIIVLLGQRRFAVLDFCYHLLKVQKHDGKDEI IKNVPLKMMVERIRK FQILNDEI
TILD KYLKS GGDGEGTPVEHVRFCFPPIHQSLASSGGSGGSGGSGSMQAICV VVGDAVGKTCLLISYTTNAFSGEYIPTVFDNYS
ANVMVDGKPVNGLWDTAGLEDYDRLRPLSYPTDVF LICFSLVSPASFENVR AKWYPEVRHHCNTPHILVGT KLDLRDDKDTIE
KLKEKLTPTIPYQGLAMAKEIGAVKYLECSALTQRGLKTVFDEAIRAVLCP PPKRKRK

>Nap1

GAMSRSVLQPSQQLAEKLTILNDRGVGMLTRLYNIKKACGDPKAKPSYLIDKNLES AVK FIVRKFPAVETRNNQQLAQLQKEKS
EILKNLALYYFTFDVDMFEKDHVCELLNTIDVCCVFFDITVNF DLTKNYLDLIITYTLMILLSRIEERKAIIGLYNYAHEMTHGASDR
EYRGLGQMIVDYENPLKMMEEFVPHSKSLSDALISLQMVYPRNLSADQWRNAQLLSLISAPSTMLNPAQSDTMPCEYLSLDAME
KWIIFGILCHGILNTDATALNWKLALQSSSCLSLFRDEVFHIHKAEDLQVFNIRGYNKRINDIRECKEAAVSHAGSMHRRERKFLR
SALKELATVLS DQPGLLGPKALFVFMALSFARDEIHWLLRHADNMPKKSADDFIDKHIAELIFYMEELRAHV RKYGPVMQRYVYQY

<p>LSGFDVAVLNLVQNLVCPEDESIIMSSFVNTMTSLSVKQVEDGEVDFRGMRLDWFRLQAYTSVSKASLGLADHRELGKMMNTI IFHTKMVDSLVEMLVETSLSIFCFYSRAFEKMFQQCLELPSQSRYSIAFPLLC THFMSC THELCPEERHHIGDRSLSLCNMFLDEMA KQARNLITDICTEQCTLSDQLPKHCAKTISQAVNKKSKKQTGKKGEPEREKPGVESMRKNRLVVTNLDKLHTALSELCSFINSYVPN MVVWEHTFTPREYLTSHLEIRFTKSIVGMTMYNQATQEIAPSELLTSVRAYMTVLQSIENYVQIDITRVFNNVLLQQTQHLDSHGE PTITSLYTNWYLETLLRQVSNGHIAFYFPAMKAFVNLPTENELTFNAEEYSDISEMRSLSELLGPYGMKFLSESLMWHISSQVAELKKL VVENVDLTQMRTSFDKPDQMAALFKRLSSVDSVLKRMITIGVILSFRSLAQEALRDVLSYHIPFLVSSIEDFKDHIPRETDKMKVAMN VYELSSAAGLPCEIDPALVVALSSQKSENISPEEYKIACLLMVFVAVSLPTLASNVMSQYSPAIEGHCCNIHCLAKAINQIAAALFTIH KGSIEDRLKEFLALASSLLKIGQETDKTTRNRRESVYLLDMIVQESPFLLTMDLLESCFPYVLLRNAYHA VYKQSVTSSA</p>
<p>>WAVE1 (1-230) GHMPLVKNRNDPRHLCHTALPRGIKNELECVTNISLANIIRQLSSLSKYAEDIFGELFNEAHSFSFRVNSLQERVDRLSVSVTQLDPKEE ELSLQDITMRKAFRSTIQDQQLFDRKTLPIPLQETYDVCEQPPLNLTYPYRDDGKEGLKFYTNPSYFFDLWKEKMLQDTEDKRKEK RKQKQKNLDRPHEPEKVPRAPHDRRREWQKLAQGPELAEDDANLLHKHIEVANG</p>
<p>>WAVE1 (1-230)-WCA, or WAVE1 (1-230)-(GGS)₆-WCA(485-559) GHMPLVKNRNDPRHLCHTALPRGIKNELECVTNISLANIIRQLSSLSKYAEDIFGELFNEAHSFSFRVNSLQERVDRLSVSVTQLDPKEE ELSLQDITMRKAFRSTIQDQQLFDRKTLPIPLQETYDVCEQPPLNLTYPYRDDGKEGLKFYTNPSYFFDLWKEKMLQDTEDKRKEK RKQKQKNLDRPHEPEKVPRAPHDRRREWQKLAQGPELAEDDANLLHKHIEVANGGGSGSGSGSGSGSGSGSKRHPSTLPVISD ARSVLEAIRKGIQLRKVEEQREAEKHERIENDVATILSRRIA VEYSDEDDSEFDEVDWLE</p>
<p>>WAVE1 (1-230)-Rac1, or WAVE1 (1-230)-(GGS)₆-Rac1^{Q61L/P29S} (1-188) GHMPLVKNRNDPRHLCHTALPRGIKNELECVTNISLANIIRQLSSLSKYAEDIFGELFNEAHSFSFRVNSLQERVDRLSVSVTQLDPKEE ELSLQDITMRKAFRSTIQDQQLFDRKTLPIPLQETYDVCEQPPLNLTYPYRDDGKEGLKFYTNPSYFFDLWKEKMLQDTEDKRKEK RKQKQKNLDRPHEPEKVPRAPHDRRREWQKLAQGPELAEDDANLLHKHIEVANGGGSGSGSGSGSGSGSGSMQAIKCVVVG GAVGKTCLLISYTTNAFSGEYIPTVFDNYSANVMVDGKPVNLGLWDTAGLEDYDRLRPLSYPTDVFLLICFSLVSPASFENVRAKW YPEVRRHCPNTPILVGTGLDRDDKDTIEKLEKELKTPITYPQLAMAKEIGAVKYLECSALTQRGLKTVFDEAIRAVLCPVPVKR KRK</p>
<p>>Abi2 (1-158) GHMAELQMLLEEIPGGRRALFDSYTNLERVADYCENNYIQSADKQRALEETKAYTTQSLASVAYLINTLANNVLQMLDIQASQLR RMESINHISQTVDIHKEKVARREIGILTNNKNTSRTHKIAPANLERPVRYIRKPIDYTILDDIGHGVKYSTQ</p>
<p>>HSPC300 GHMGAAMAGQEDPVQREIHQDWANREYIEIITSSIKKIADFLNSFDMSCRSRLATLNEKLTALERRIEYIEARVTKGETLT</p>
<p>>WCA KRHPSTLPVISDARSVLEAIRKGIQLRKVEEQREAEKHERIENDVATILSRRIA VEYSDEDDSEFDEVDWLE</p>
<p>> GST-Arf1 MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVVKLTQSMAIIRYIADKHNMLGGCP KERAESMLEGAVLDIRYGVSRIAYSKDFETLKVDVFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLD AFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSMRILMVGLDAAAGKTTILYKLLKGEIVTTIPTIGF NVETVEYKNISFTVWVGGQDKIRPLWRHYFQNTQGLIFVVDSDNRERVNEAREELMRMLAEDELDAVLLVFANKQDLPNAMN AAEITDKLGLHSLRHRNWIYQATCATSGDGLYEGLDWLSNQLRNQK</p>
<p>> His8-Arf1 HHHHHHHHGGSGGSMRILMVGLDAAAGKTTILYKLLKGEIVTTIPTIGFNVETVEYKNISFTVWVGGQDKIRPLWRHYFQNTQGLIF VVDSDNRERVNEAREELMRMLAEDELDAVLLVFANKQDLPNAMNAAEITDKLGLHSLRHRNWIYQATCATSGDGLYEGLDWLS NQLRNQK</p>
<p>> His6-EspG MSYHHHHHHHDYDIPTTENLYFQGAMGSKKSWDEMCAEKLKFKVLSFGLWNPTYSRERQSFQELLTVLEPVYPLPNELGRVSARF SDGSSLRISVTNSELVEAEIRTANNEKITVLESNEQNRLQLPIDRHMPYIQVHRALSEMDLTDTTSMRNLLGFTSKLSTTLIPHNA QTDPLSGPTPFSSIFMDTCRGLGNAKLSLNGVDIPANAQKLLRDALGLKDTHTSSPTRNVIDHGISRHDAEQIARESSGSDKQKAEVVE FLCHPEAATAICSAFYQSFNPALTLTHERISKASEYNAERSLDTPNACINISISQSSDGNIVYVTSHTGVLIMAPEDRPNEMGMLTNRTS YEVPOGVKCIIDEMVSALQPRYAASETYLQNT</p>
<p>> GST-Arf5 MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVVKLTQSMAIIRYIADKHNMLGGCP KERAESMLEGAVLDIRYGVSRIAYSKDFETLKVDVFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLD AFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSMRILMVGLDAAAGKTTILYKLLKGEIVTTIPTIGF NVETVEYKNICFTVWVGGQDKIRPLWRHYFQNTQGLIFVVDSDNRERVQESADELQKMLQEDELDAVLLVFANKQDMPNAMP VSELTDKLGLQHLRSRTWYVQATCATQGTGLYDGLDWLSHELKSR</p>
<p>> GST-Arf6 MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVVKLTQSMAIIRYIADKHNMLGGCP KERAESMLEGAVLDIRYGVSRIAYSKDFETLKVDVFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLD AFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSMRILMLGLDAAAGKTTILYKLLKQSVTTIPTVG FNVETVYKKNVFNWVWVGGQDKIRPLWRHYTGTQGLIFVVDCAADRDRIDEARQELHRIINDREMRDAIILIFANKQDLPDAMKP HEIQEKLGLTRIRDRNWIYVQSCATSGDGLYEGLTWLSNYKS</p>
<p>> GST-Arf11 MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVVKLTQSMAIIRYIADKHNMLGGCP KERAESMLEGAVLDIRYGVSRIAYSKDFETLKVDVFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLD</p>

AFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSMRILILGLDGAGKTILYRLQVGEVVTIPTIGF
NVETVYKLNKQVWDLGGQTSIRPYWRCYYSNTDAVIYVVDSCDRDRIGISKSELVAMLEEEELRKAILVVFANKQDMEQAMTSS
EMANSLGLPALKDRKWQIFKTSATKGTGLDEAMEWL VETLKS RQ

> **GST-Arl2**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHL YERDEGDKWRNKKFELGLEFPNLPYYIDGDV KLTQSM AIIRYIADKH NMLGGCP
KERA EISMLEGAVLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDHVTHPDFM LYDALDVVLYMDPMCLD
AFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSE RELRLLMLGLDNAGKTILKKFNGEDIDTISP
TLGFNIKLEHRGFKLNIWDVGGQKSLRSYWRNYFESTDGLI WVVD SADRQRMQDCQRELQSL LVEERLAGATLLIFANKQDLPGA
LSSNAIREALELDSIRSHHCWCIQGCSAVTGENLLPGIDWLLDDISSRIFTAD

> **GST-Rac1^{OP} or GST-Tev-Rac1^{Q61L/P29S}(1-188)**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHL YERDEGDKWRNKKFELGLEFPNLPYYIDGDV KLTQSM AIIRYIADKH NMLGGCP
KERA EISMLEGAVLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDHVTHPDFM LYDALDVVLYMDPMCLD
AFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSE NLYFQGHMQAIKCVVVG DGAVGKTCLLISYT
TNAFSGEYIPTVFDNYSANVMVDGKPVNLGLWDTAGLEDYDRLRPLSYPTDVF LICFSLVSPASFENVRAKWYPEVRHHCNP TPII
LVGTKL DLRDDKDTIEKLEK KLTPIY PQGLAMAKEIGAVKYLECSALTQRGLKTVFDEAIRAVLCPPPVKKRKRK

> **GST-Rac1^{dead} or GST-Tev-Rac1(1-188)**, with Switch I (a.a. 25-39) replaced by (GGS)s

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHL YERDEGDKWRNKKFELGLEFPNLPYYIDGDV KLTQSM AIIRYIADKH NMLGGCP
KERA EISMLEGAVLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDHVTHPDFM LYDALDVVLYMDPMCLD
AFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSE NLYFQGHMQAIKCVVVG DGAVGKTCLLISYT
GSGSGSGSGSGSYSANVMVDGKPVNLGLWDTAGLEDYDRLRPLSYPTDVF LICFSLVSPASFENVRAKWYPEVRHHCNP TPII
LVGTKL DLRDDKDTIEKLEK KLTPIY PQGLAMAKEIGAVKYLECSALTQRGLKTVFDEAIRAVLCPPPVKKRKRK

> **Rac1^{OP} or Rac1^{Q61L/P29S}(1-188)**

MQAIKCVVVG DGAVGKTCLLISYTTNAFSGEYIPTVFDNYSANVMVDGKPVNLGLWDTAGLEDYDRLRPLSYPTDVF LICFSLVS
PASFENVRAKWYPEVRHHCNP TPII LVGTKL DLRDDKDTIEKLEK KLTPIY PQGLAMAKEIGAVKYLECSALTQRGLKTVFDEAIR
AVLCPPPVKKRKRK

Movie S1. Lamellipodia protrusion efficiency in cells harboring wildtype WRC *versus* WRC deficient in Arf-binding or D site Rac binding or both. Phase-contrast time-lapse microscopy of B16-F1 Sra1/Cyfp2-KO#3 cells expressing constructs as indicated. Movie was accelerated 100 times.

Movie S2. Random cell migration in cells harboring wildtype WRC *versus* WRC deficient in Arf-binding or D site Rac binding or both. Low-mag phase contrast video microscopy of B16-F1 Sra1/Cyfp2-KO#3 cells expressing constructs as indicated. Movie was accelerated 100 times.