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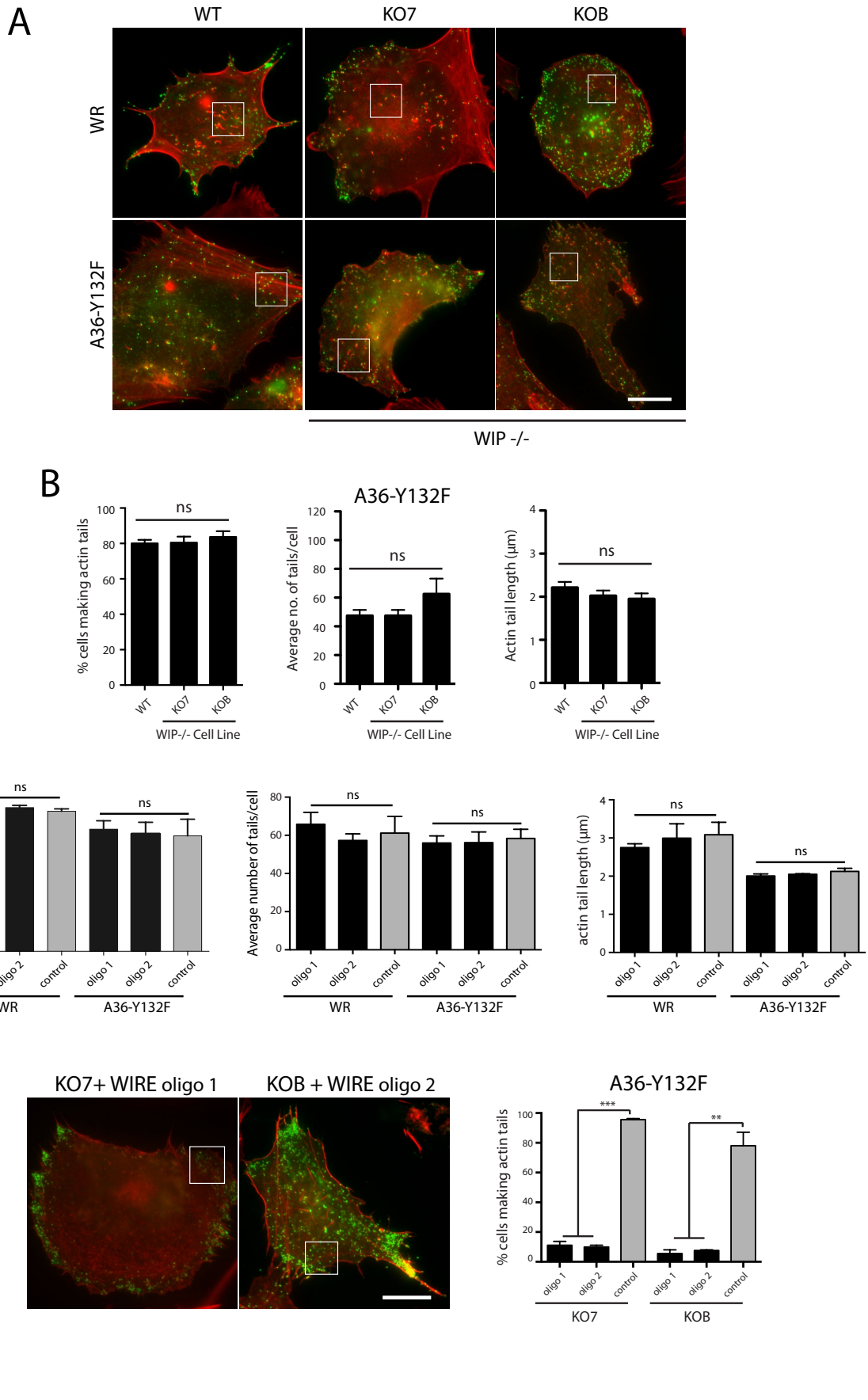
Supplemental Information

WIP Provides an Essential Link between Nck and N-WASP during Arp2/3-Dependent Actin Polymerization

Sara K. Donnelly, Ina Weisswange, Markus Zettl, and Michael Way


A
WT
KO7
KOB
WR
A36-Y132F
WIP -/-
B
A36-Y132F
% cells making actin tails
Average no. of tails/cell
Actin tail length (µm)
ns
WT
KO7
KOB
WIP-/- Cell Line
C
% cells making actin tails
Average number of tails/cell
actin tail length (µm)
ns
oligo 1
oligo 2
control
WR
A36-Y132F
D
KO7+ WIRE oligo 1
KOB + WIRE oligo 2
A36-Y132F
% cells making actin tails
***
**
KO7
KOB

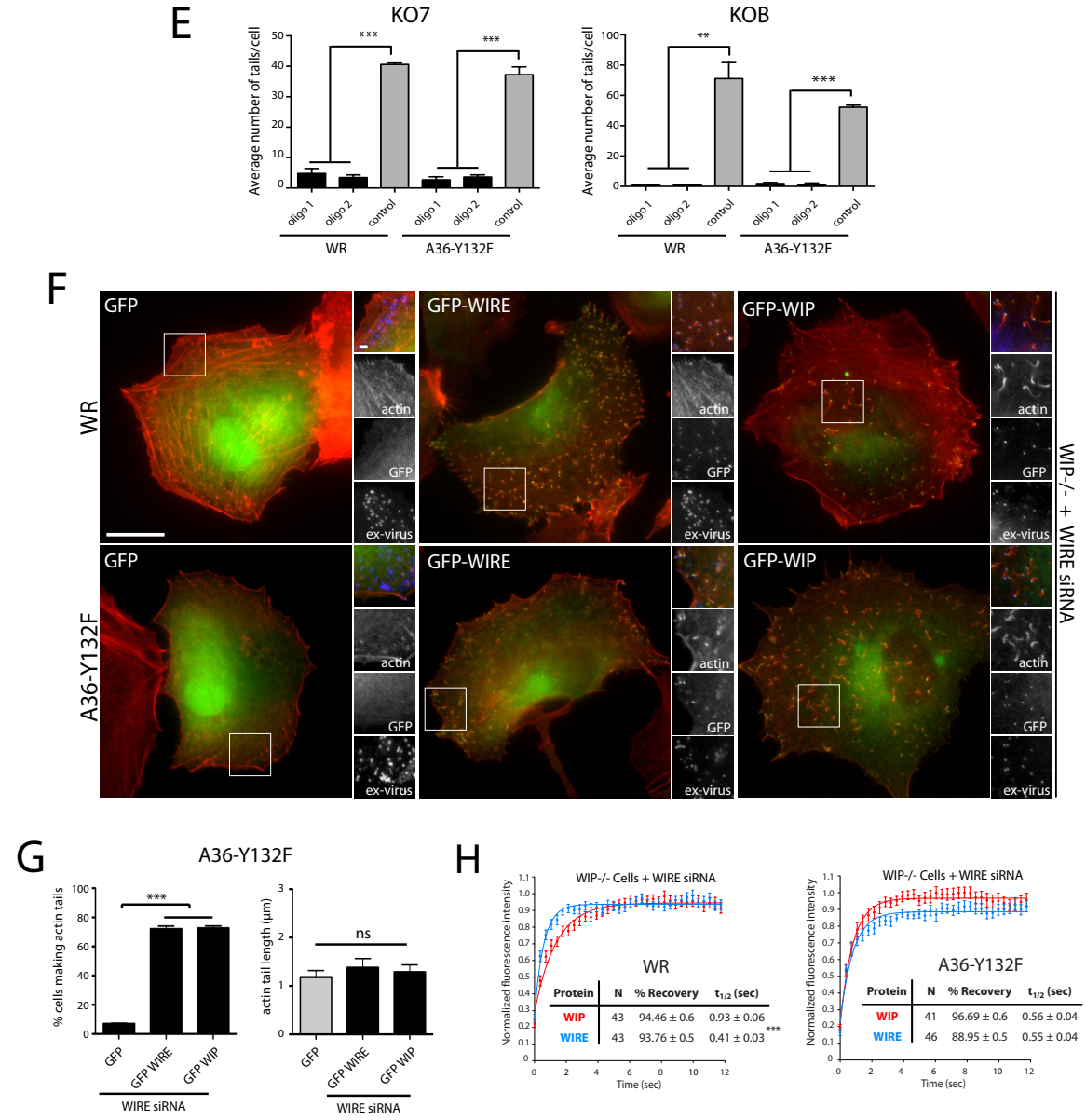


Figure S1., related to Figure 1

(A) Immunofluorescence images showing actin tails (red) induced by Western Reserve (WR) and A36-Y132F viruses (Ex-virus) in wild type (WT) or WIP-/- (KO7 and KOB) MEFs. White boxes represent images shown in Fig. 1B of main paper. **(B)** Quantification of the % cells with actin tails, the average number actin tails and their length in WT or WIP-/- MEFs infected with the A36-Y132F virus. **(C)** Quantification of the % cells with actin tails, the average number actin tails and their length in WT MEFs treated with WIRE siRNA and infected with WR or A36-Y132F virus. **(D)** Immunofluorescence images of WIP-/- MEFs (KO7 and KOB) treated with indicated WIRE siRNA and infected with WR. White boxes correspond to images shown in Fig. 1F of main paper. The graph shows the quantification of the % of A36-Y132F infected cells with at least one actin tail in WIRE siRNA (black bars) or control (grey bar) treated WIP-/- cell lines. **(E)** The graphs show the average number of actin tails induced by WR or A36-Y132F in WIP-/- MEFs (KO7 and KOB) treated with WIRE (black bars) or control (grey bar) siRNA. **(F)** GFP-tagged WIP and WIRE but not GFP are recruited to WR and A36-Y132F viruses (ex-virus) and rescue actin tail formation in WIP-/- MEFs (KO7) subjected to WIRE siRNA. **(G)** Quantification of the % of A36-Y132F infected cells expressing the indicated GFP-tagged protein with at least one actin tail and their average length in WIP-/- cells treated with control (grey bars) or WIRE (black bars) siRNA. **(H)** Comparison of the recovery kinetics of GFP-tagged WIP or WIRE on WR or A36-Y132F after photobleaching in WIRE siRNA treated WIP-/- cells (KO7). All error bars in the graphs represent SEM from 3 independent experiments.
ns = not significant ** p<0.01, *** = p<0.001. All scale bars = 2 or 20μm.

A

WIP Peptide List

Nr.	Sequence	Nr.	Sequence
1	M-P-V-P-P-P-P-A-P-P-P-P-P-T-F	91	V-G-N-R-P-S-I-H-R-E-A-V-P-P-P
2	P-P-P-P-A-P-P-P-P-P-P-T-F-A-L-A	92	R-P-S-I-H-R-E-A-V-P-P-P-P-P-Q
3	P-A-P-P-P-P-P-T-F-A-L-A-N-T-E	93	I-H-R-E-A-V-P-P-P-P-P-Q-N-N-K
4	P-P-P-P-T-F-A-L-A-N-T-E-K-P-T	94	E-A-V-P-P-P-P-P-Q-N-N-K-P-P-V
5	P-T-F-A-L-A-N-T-E-K-P-T-L-N-K	95	P-P-P-P-P-Q-N-N-K-P-P-V-P-S-T
6	A-L-A-N-T-E-K-P-T-L-N-K-T-E-Q	96	P-P-Q-N-N-K-P-P-V-P-S-T-P-R-P
7	N-T-E-K-P-T-L-N-K-T-E-Q-A-G-R	97	N-N-K-P-P-V-P-S-T-P-R-P-S-A-P
8	K-P-T-L-N-K-T-E-Q-A-G-R-N-A-L	98	P-P-V-P-S-T-P-R-P-S-A-P-H-R-P
9	L-N-K-T-E-Q-A-G-R-N-A-L-L-S-D	99	P-S-T-P-R-P-S-A-P-H-R-P-H-L-R
#	T-E-Q-A-G-R-N-A-L-L-S-D-I-S-K	100	P-R-P-S-A-P-H-R-P-H-L-R-P-P-P
#	A-G-R-N-A-L-L-S-D-I-S-K-G-K-K	101	S-A-P-H-R-P-H-L-R-P-P-P-P-S-R
#	N-A-L-L-S-D-I-S-K-G-K-K-L-K-K	102	H-R-P-H-L-R-P-P-P-P-S-R-P-G-P
#	L-S-D-I-S-K-G-K-K-L-K-K-T-V-T	103	H-L-R-P-P-P-P-S-R-P-G-P-P-P-L
#	I-S-K-G-K-K-L-K-K-T-V-T-N-D-R	104	P-P-P-P-S-R-P-G-P-P-P-L-P-P-S
#	G-K-K-L-K-K-T-V-T-N-D-R-S-A-P	105	P-S-R-P-G-P-P-P-L-P-P-S-S-S-G
#	L-K-K-T-V-T-N-D-R-S-A-P-I-L-D	106	P-G-P-P-P-L-P-P-S-S-S-G-N-D-E
#	T-V-T-N-D-R-S-A-P-I-L-D-K-P-K	107	P-P-L-P-P-S-S-S-G-N-D-E-T-P-R
#	N-D-R-S-A-P-I-L-D-K-P-K-G-A-G	108	P-P-S-S-S-G-N-D-E-T-P-R-L-P-Q
#	S-A-P-I-L-D-K-P-K-G-A-G-A-G-G	109	S-S-G-N-D-E-T-P-R-L-P-Q-R-N-L
#	I-L-D-K-P-K-G-A-G-A-G-G-G-G-G	110	N-D-E-T-P-R-L-P-Q-R-N-L-S-L-S
#	K-P-K-G-A-G-A-G-G-G-G-G-F-G-G	111	T-P-R-L-P-Q-R-N-L-S-L-S-S-S-T
#	G-A-G-A-G-G-G-G-G-G-F-G-G-G-G	112	L-P-Q-R-N-L-S-L-S-S-S-T-P-P-L
#	A-G-G-G-G-G-G-F-G-G-G-G-G-F-G	113	R-N-L-S-L-S-S-S-T-P-P-L-P-S-P
#	G-G-G-G-F-G-G-G-G-G-F-G-G-G-G	114	S-L-S-S-S-T-P-P-L-P-S-P-G-R-S
#	G-F-G-G-G-G-G-F-G-G-G-G-G-G-G	115	S-S-T-P-P-L-P-S-P-G-R-S-G-P-L
#	G-G-G-G-F-G-G-G-G-G-G-G-G-G-G	116	P-P-L-P-S-P-G-R-S-G-P-L-P-P-P
#	G-F-G-G-G-G-G-G-G-G-G-S-F-G	117	P-S-P-G-R-S-G-P-L-P-P-P-P-S-E
#	G-G-G-G-G-G-G-G-S-F-G-G-G-G	118	G-R-S-G-P-L-P-P-P-P-S-E-R-P-P
#	G-G-G-G-G-G-S-F-G-G-G-G-P-P-G	119	G-P-L-P-P-P-P-S-E-R-P-P-P-P-V
#	G-G-G-S-F-G-G-G-G-P-P-G-L-G-G	120	P-P-P-P-S-E-R-P-P-P-P-V-R-D-P
#	S-F-G-G-G-G-P-P-G-L-G-G-L-F-Q	121	P-S-E-R-P-P-P-P-V-R-D-P-P-G-R
#	G-G-G-P-P-G-L-G-G-L-F-Q-A-G-M	122	R-P-P-P-P-V-R-D-P-P-G-R-S-G-P
#	P-P-G-L-G-G-L-F-Q-A-G-M-P-K-L	123	P-P-V-R-D-P-P-G-R-S-G-P-L-P-P
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#	D-N-D-S-G-G-S-R-P-P-L-L-P-P-G	130	N-G-S-T-S-R-A-L-P-A-T-P-Q-L-P
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#	R-N-R-M-P-P-P-R-P-D-V-G-S-K-P	146	S-I-R-N-G-F-Q-D-S-P-C-E-D-E-W
#	M-P-P-P-R-P-D-V-G-S-K-P-D-S-I	147	N-G-F-Q-D-S-P-C-E-D-E-W-E-S-R
#	P-R-P-D-V-G-S-K-P-D-S-I-P-P-P	148	Q-D-S-P-C-E-D-E-W-E-S-R-F-Y-F
#	D-V-G-S-K-P-D-S-I-P-P-P-V-P-S	149	P-C-E-D-E-W-E-S-R-F-Y-F-H-P-I
#	S-K-P-D-S-I-P-P-P-V-P-S-T-P-R	150	D-E-W-E-S-R-F-Y-F-H-P-I-S-D-L
#	D-S-I-P-P-P-V-P-S-T-P-R-P-I-Q	151	E-S-R-F-Y-F-H-P-I-S-D-L-P-P-P
#	P-P-P-V-P-S-T-P-R-P-I-Q-S-S-L	152	F-Y-F-H-P-I-S-D-L-P-P-P-E-P-Y
#	V-P-S-T-P-R-P-I-Q-S-S-L-H-N-R	153	H-P-I-S-D-L-P-P-P-E-P-Y-V-Q-T
#	T-P-R-P-I-Q-S-S-L-H-N-R-G-S-P	154	S-D-L-P-P-P-E-P-Y-V-Q-T-T-K-S
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#	G-G-P-R-Q-P-S-P-G-P-T-P-P-P-F	160	K-L-A-R-N-E-S-R-S-G-S-N-R-R-E
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#	R-G-T-A-L-G-G-G-S-I-R-Q-S-P-L		
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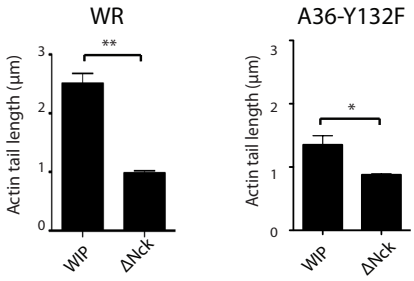
B



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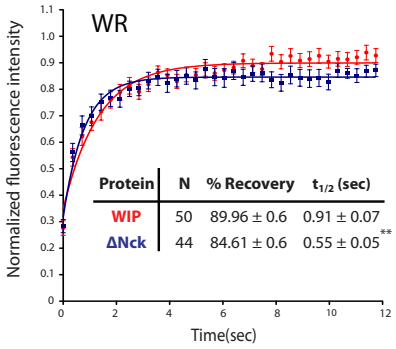

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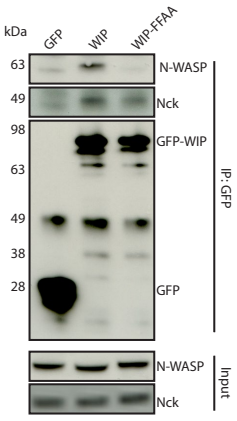

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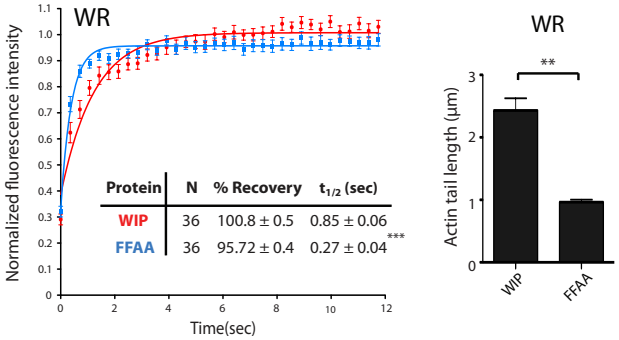

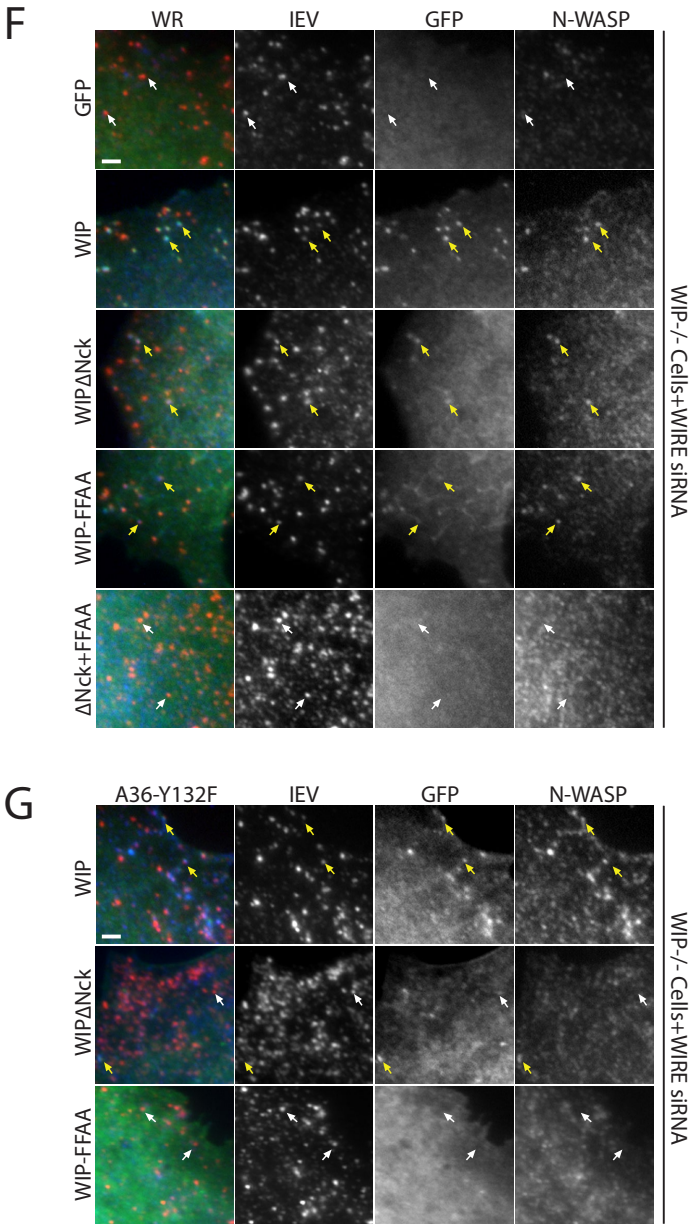

Figure S2, related to Figure 2.
(A) List of individual overlapping 15mer WIP peptides spotted on the arrays. Adjacent peptides are shifted by 3 amino acids and His-Nck binding peptides are shown in red. **(B)** Quantification of the length of actin tails induced by WR and A36-Y132F viruses in WIP-/- MEFs treated with WIRE siRNA and expressing GFP-tagged WIP or WIPΔNck. **(C)** Comparison of the recovery kinetics of GFP-tagged WIP and WIPΔNck on WR after photobleaching in WIRE siRNA treated WIP-/- cells. **(D)** Immunoblot analysis demonstrates that endogenous N-WASP co-immunoprecipitates with GFP-tagged WIP but not WIP-FFAA in HeLa cells. Nck is pulled down by GFP-tagged WIP and WIP-FFAA. The N-WASP and Nck inputs and the immunoprecipitated GFP-tagged proteins are indicated. **(E)** Comparison of the recovery kinetics of GFP-tagged WIP and WIP-FFAA on WR after photobleaching in WIRE siRNA treated WIP-/- cells. The graph shows the length of actin tails induced by WR in WIP-/- MEFs treated with WIRE siRNA and expressing GFP-tagged WIP or WIP-FFAA. **(F)** Immunofluorescence images showing the recruitment of endogenous N-WASP (yellow arrows) to WR in WIP-/- cells treated with WIRE siRNA and expressing the indicated GFP-tagged protein. White arrows indicate the absence of N-WASP recruitment to virus particles. **(G)** Images showing the recruitment of endogenous N-WASP (yellow arrows) to the A36-Y132F virus in WIP-/- cells treated with WIRE siRNA and expressing the indicated GFP-tagged protein. White arrows indicate the absence of N-WASP recruitment to virus particles and scale bars = 2 μm. All error bars represent SEM from three independent experiments. * = $p< 0.05$, ** = $p< 0.01$, *** = $p<0.001$, Scale bars = 2 μm.

A

N-WASP Peptide List

Nr.	Sequence	Nr.	Sequence
1	M-S-S-G-Q-Q-P-P-R-R-V-T-N-V-G	91	R-Q-A-P-P-P-P-P-P-S-R-G-G-P-P
2	G-Q-Q-P-P-R-R-V-T-N-V-G-S-L-L	92	P-P-P-P-P-P-S-R-G-G-P-P-P-P-P
3	P-P-R-R-V-T-N-V-G-S-L-L-L-T-P	93	P-P-P-S-R-G-G-P-P-P-P-P-P-P-P
4	R-V-T-N-V-G-S-L-L-L-T-P-Q-E-N	94	S-R-G-G-P-P-P-P-P-P-P-P-H-S-S
5	N-V-G-S-L-L-L-T-P-Q-E-N-E-S-L	95	G-P-P-P-P-P-P-P-P-P-H-S-S-G-P-P
6	S-L-L-L-T-P-Q-E-N-E-S-L-F-S-F	96	P-P-P-P-P-P-H-S-S-G-P-P-P-P-P
7	L-T-P-Q-E-N-E-S-L-F-S-F-L-G-K	97	P-P-P-H-S-S-G-P-P-P-P-P-A-R-G
8	Q-E-N-E-S-L-F-S-F-L-G-K-K-C-V	98	H-S-S-G-P-P-P-P-P-A-R-G-R-G-A
9	E-S-L-F-S-F-L-G-K-K-C-V-T-M-S	99	G-P-P-P-P-P-P-A-R-G-R-G-A-P-P-P
10	F-S-F-L-G-K-K-C-V-T-M-S-S-A-V	100	P-P-P-A-R-G-R-G-A-P-P-P-P-P-S
11	L-G-K-K-C-V-T-M-S-S-A-V-V-Q-L	101	A-R-G-R-G-A-P-P-P-P-P-S-S-R-A-P
12	K-C-V-T-M-S-S-A-V-V-Q-L-Y-A-A	102	R-G-A-P-P-P-P-P-S-R-A-P-T-A-A
13	T-M-S-S-A-V-V-Q-L-Y-A-A-D-R-N	103	P-P-P-P-P-S-R-A-P-T-A-A-P-P-P
14	S-A-V-V-Q-L-Y-A-A-D-R-N-C-M-W	104	P-P-S-R-A-P-T-A-A-P-P-P-P-P-P
15	V-Q-L-Y-A-A-D-R-N-C-M-W-S-K-K	105	R-A-P-T-A-A-P-P-P-P-P-P-S-R-P
16	Y-A-A-D-R-N-C-M-W-S-K-K-C-S-G	106	T-A-A-P-P-P-P-P-P-S-R-P-G-V-V
17	D-R-N-C-M-W-S-K-K-C-S-G-V-A-C	107	P-P-P-P-P-P-S-R-P-G-V-V-V-P-P
18	C-M-W-S-K-K-C-S-G-V-A-C-L-V-K	108	P-P-P-S-R-P-G-V-V-V-P-P-P-P-P
19	S-K-K-C-S-G-V-A-C-L-V-K-D-N-P	109	S-R-P-G-V-V-V-P-P-P-P-P-N-R-M
20	C-S-G-V-A-C-L-V-K-D-N-P-Q-R-S	110	G-V-V-V-P-P-P-P-P-N-R-M-Y-P-P
21	V-A-C-L-V-K-D-N-P-Q-R-S-Y-F-L	111	V-P-P-P-P-P-N-R-M-Y-P-P-P-P-P
22	L-V-K-D-N-P-Q-R-S-Y-F-L-R-I-F	112	P-P-P-N-R-M-Y-P-P-P-P-P-A-L-P
23	D-N-P-Q-R-S-Y-F-L-R-I-F-D-I-K	113	N-R-M-Y-P-P-P-P-P-A-L-P-S-S-A
24	Q-R-S-Y-F-L-R-I-F-D-I-K-D-G-K	114	Y-P-P-P-P-P-A-L-P-S-S-A-P-S-G
25	Y-F-L-R-I-F-D-I-K-D-G-K-L-L-W	115	P-P-P-A-L-P-S-S-A-P-S-G-P-P-P
26	R-I-F-D-I-K-D-G-K-L-L-W-E-Q-E	116	A-L-P-S-S-A-P-S-G-P-P-P-P-P-P
27	D-I-K-D-G-K-L-L-W-E-Q-E-L-Y-N	117	S-S-A-P-S-G-P-P-P-P-P-P-L-S-M
28	D-G-K-L-L-W-E-Q-E-L-Y-N-N-F-V	118	P-S-G-P-P-P-P-P-P-L-S-M-A-G-S
29	L-L-W-E-Q-E-L-Y-N-N-F-V-Y-N-S	119	P-P-P-P-P-P-L-S-M-A-G-S-T-A-P
30	E-Q-E-L-Y-N-N-F-V-Y-N-S-P-R-G	120	P-P-P-L-S-M-A-G-S-T-A-P-P-P-P
31	L-Y-N-N-F-V-Y-N-S-P-R-G-Y-F-H	121	L-S-M-A-G-S-T-A-P-P-P-P-P-P-P
32	N-F-V-Y-N-S-P-R-G-Y-F-H-T-F-A	122	A-G-S-T-A-P-P-P-P-P-P-P-P-P-P
33	Y-N-S-P-R-G-Y-F-H-T-F-A-G-D-T	123	T-A-P-P-P-P-P-P-P-P-P-P-P-G-P
34	P-R-G-Y-F-H-T-F-A-G-D-T-C-Q-V	124	P-P-P-P-P-P-P-P-P-P-G-P-P-P-P
35	Y-F-H-T-F-A-G-D-T-C-Q-V-A-L-N	125	P-P-P-P-P-P-G-P-P-P-P-P-G-L
36	T-F-A-G-D-T-C-Q-V-A-L-N-F-A-N	126	P-P-P-P-G-P-P-P-P-P-G-L-P-S-D
37	G-D-T-C-Q-V-A-L-N-F-A-N-E-E-E	127	P-G-P-P-P-P-P-G-L-P-S-D-G-D-H
38	C-Q-V-A-L-N-F-A-N-E-E-E-A-K-K	128	P-P-P-P-G-L-P-S-D-G-D-H-Q-V-P
39	A-L-N-F-A-N-E-E-E-A-K-K-F-R-K	129	P-G-L-P-S-D-G-D-H-Q-V-P-A-S-S
40	F-A-N-E-E-E-A-K-K-F-R-K-A-V-T	130	P-S-D-G-D-H-Q-V-P-A-S-S-G-N-K
41	E-E-E-A-K-K-F-R-K-A-V-T-D-L-L	131	G-D-H-Q-V-P-A-S-S-G-N-K-A-A-L
42	A-K-K-F-R-K-A-V-T-D-L-L-G-R-R	132	Q-V-P-A-S-S-G-N-K-A-A-L-L-D-Q
43	F-R-K-A-V-T-D-L-L-G-R-R-Q-R-K	133	A-S-S-G-N-K-A-A-L-L-D-Q-I-R-E
44	A-V-T-D-L-L-G-R-R-Q-R-K-S-E-K	134	G-N-K-A-A-L-L-D-Q-I-R-E-G-A-Q
45	D-L-L-G-R-R-Q-R-K-S-E-K-R-R-D	135	A-A-L-L-D-Q-I-R-E-G-A-Q-L-K-K
46	G-R-R-Q-R-K-S-E-K-R-R-D-A-P-N	136	L-D-Q-I-R-E-G-A-Q-L-K-K-V-E-Q
47	Q-R-K-S-E-K-R-R-D-A-P-N-G-P-N	137	I-R-E-G-A-Q-L-K-K-V-E-Q-N-S-R
48	S-E-K-R-R-D-A-P-N-G-P-N-L-P-M	138	G-A-Q-L-K-K-V-E-Q-N-S-R-P-V-S
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50	A-P-N-G-P-N-L-P-M-A-T-V-D-I-K	140	V-E-Q-N-S-R-P-V-S-C-S-G-R-D-A
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52	L-P-M-A-T-V-D-I-K-N-P-E-I-T-T	142	P-V-S-C-S-G-R-D-A-L-L-D-Q-I-R
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54	D-I-K-N-P-E-I-T-T-N-R-F-Y-S-S	144	R-D-A-L-L-D-Q-I-R-Q-G-I-Q-L-K
55	N-P-E-I-T-T-N-R-F-Y-S-S-Q-V-N	145	L-L-D-Q-I-R-Q-G-I-Q-L-K-S-V-S
56	I-T-T-N-R-F-Y-S-S-Q-V-N-N-I-S	146	Q-I-R-Q-G-I-Q-L-K-S-V-S-D-G-Q
57	N-R-F-Y-S-S-Q-V-N-N-I-S-H-T-K	147	Q-G-I-Q-L-K-S-V-S-D-G-Q-E-S-T
58	Y-S-S-Q-V-N-N-I-S-H-T-K-E-K-K	148	Q-L-K-S-V-S-D-G-Q-E-S-T-P-P-T
59	Q-V-N-N-I-S-H-T-K-E-K-K-K-G-K	149	S-V-S-D-G-Q-E-S-T-P-P-T-P-A-P
60	N-I-S-H-T-K-E-K-K-K-G-K-A-K-K	150	D-G-Q-E-S-T-P-P-T-P-A-P-T-S-G
61	H-T-K-E-K-K-K-G-K-A-K-K-K-R-L	151	E-S-T-P-P-T-P-A-P-T-S-G-I-V-G
62	E-K-K-K-G-K-A-K-K-K-R-L-T-K-A	152	P-P-T-P-A-P-T-S-G-I-V-G-A-L-M
63	K-G-K-A-K-K-K-R-L-T-K-A-D-I-G	153	P-A-P-T-S-G-I-V-G-A-L-M-E-V-M
64	A-K-K-K-R-L-T-K-A-D-I-G-T-P-S	154	T-S-G-I-V-G-A-L-M-E-V-M-Q-K-R
65	K-R-L-T-K-A-D-I-G-T-P-S-N-F-Q	155	I-V-G-A-L-M-E-V-M-Q-K-R-S-K-A
66	T-K-A-D-I-G-T-P-S-N-F-Q-H-I-G	156	A-L-M-E-V-M-Q-K-R-S-K-A-I-H-S
67	D-I-G-T-P-S-N-F-Q-H-I-G-H-V-G	157	E-V-M-Q-K-R-S-K-A-I-H-S-S-D-E
68	T-P-S-N-F-Q-H-I-G-H-V-G-W-D-P	158	Q-K-R-S-K-A-I-H-S-S-D-E-D-E-D
69	N-F-Q-H-I-G-H-V-G-W-D-P-N-T-G	159	S-K-A-I-H-S-S-D-E-D-E-D-D-D-D
70	H-I-G-H-V-G-W-D-P-N-T-G-F-D-L	160	I-H-S-S-D-E-D-E-D-D-D-E-E-D
71	H-V-G-W-D-P-N-T-G-F-D-L-N-N-L	161	S-D-E-D-E-D-D-D-D-E-E-D-F-Q-D
72	W-D-P-N-T-G-F-D-L-N-N-L-D-P-E	162	D-E-D-D-D-D-E-E-D-F-Q-D-D-D-E
73	N-T-G-F-D-L-N-N-L-D-P-E-L-K-N	163	D-D-D-E-E-D-F-Q-D-D-D-E-W-E-D
74	F-D-L-N-N-L-D-P-E-L-K-N-L-F-D		
75	N-N-L-D-P-E-L-K-N-L-F-D-M-C-G		
76	D-P-E-L-K-N-L-F-D-M-C-G-I-S-E		
77	L-K-N-L-F-D-M-C-G-I-S-E-A-Q-L		
78	L-F-D-M-C-G-I-S-E-A-Q-L-K-D-R		
79	M-C-G-I-S-E-A-Q-L-K-D-R-E-T-S		
80	I-S-E-A-Q-L-K-D-R-E-T-S-K-V-I		
81	A-Q-L-K-D-R-E-T-S-K-V-I-Y-D-F		
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84	K-V-I-Y-D-F-I-E-K-T-G-G-V-E-A		
85	Y-D-F-I-E-K-T-G-G-V-E-A-V-K-N		
86	I-E-K-T-G-G-V-E-A-V-K-N-E-L-R		
87	T-G-G-V-E-A-V-K-N-E-L-R-R-Q-A		
88	V-E-A-V-K-N-E-L-R-R-Q-A-P-P-P		
89	V-K-N-E-L-R-R-Q-A-P-P-P-P-P-P		
90	E-L-R-R-Q-A-P-P-P-P-P-P-S-R-G		

Figure S3 related to Figure 3.
(A) List of individual overlapping 15mer N-WASP peptides spotted on the arrays.
Adjacent peptides are shifted by 3 amino acids and His-Nck binding peptides are shown in red.

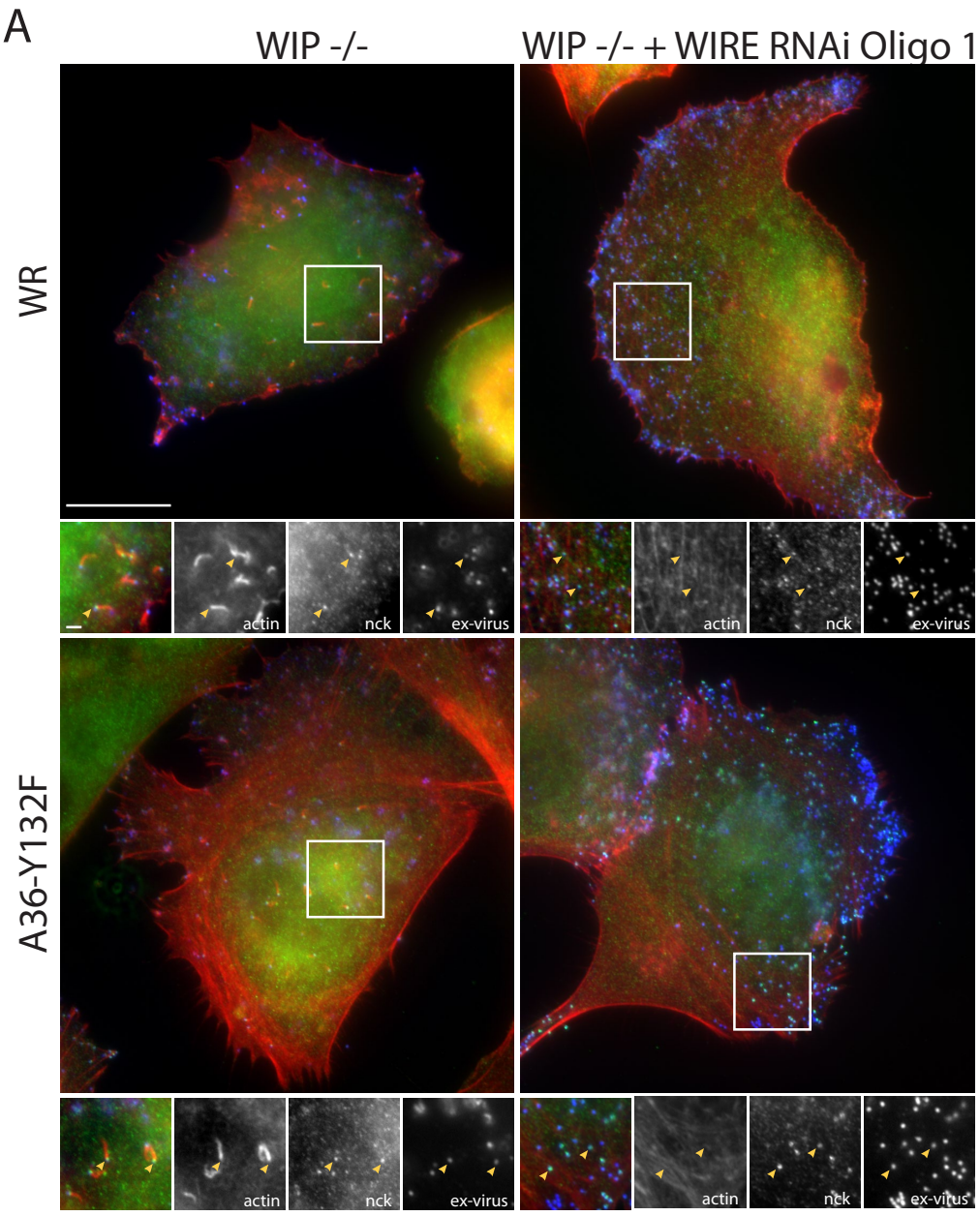

Figure S4 related to Figure 4.
(A) Immunofluorescence images showing the recruitment of endogenous Nck (yellow arrows) to WR and A36-Y132F viruses in WIP-/- cells treated with control or WIRE siRNA.
Scale bars = 20 or 2 μm.

Supplemental Experimental Procedures

Cell Lines, Infection, and Immunofluorescence

Wild type and WIP-/- MEFs (KO7 and KOB) [1, 2] were kindly provided by Raif Geha (Harvard Medical School, Boston, USA). Nck-/- and N-WASP-/- MEFS [3, 4] were provided by Tony Pawson (Samuel Lunenfeld Research Institute, Canada) and Scott Snapper (Dept. of Medicine and Immunology, MGH, Boston, USA) respectively. Wild type and WIP-/- MEFs were infected with the indicated viruses for 9 hours, while N-WASP-/- and Nck-/- cell lines were infected with the indicated virus for 15 hours before being processed for immunofluorescence as described previously [5]. Extracellular virus was labeled with anti-B5 19C2 antibody as previously described [6]. Intracellular enveloped virus (IEV) were detected using anti-F13 antibody [7]. Actin was detected with texas red phalloidin (Invitrogen, USA). WIRE polyclonal antibody against a peptide corresponding to residues 19-39 of human WIRE (FHQANTEQPKLSRDEQRGRGA) was produced in rabbits and purified as described previously [6]. The anti-N-WASP antibody has been described previously [8]. Immunofluorescence images were acquired and figures prepared as before [6]. The percentage of cells making actin tails was determined in 100 cells in 3 independent experiments. The average number of actin tails and their lengths were determined for 10 randomly selected cells and 50 actin tails, respectively in three independent experiments.

RNAi Transfections, Immunoprecipitation, and Immunoblot Analysis

WIP-/- cells were transfected with 20nM of siRNA oligo: All-Star control (Qiagen, Germany), WIRE oligo 1 (D-041519-01) and oligo 2 (D-041519-02) (Dharmacon, USA) using the HiPerFect fast-forward protocol (Qiagen). After two days the cells were infected and then processed for immunofluorescence, live cell imaging or immunoblot analysis 9 hours later. Immunoprecipitations of GFP-WIP were performed by lysing cells in 20mM Tris pH 7.5, 150 mM NaCl, 1mM EDTA, 1% NP-40, 1.5mM $MgCl_2$ and 10% glycerol with protease inhibitors. Lysates were pre-cleared for 1 hour with washed Protein G beads (Sigma), before incubation over night at 4°C with anti-GFP (4E12 Cancer Research UK) antibody. Lysates and antibody were subsequently incubated with washed Protein G beads for 1 hour at 4°C then washed 4X with lysis buffer before being processed for SDS-PAGE. In addition, GFP-WIP/WIRE (Fig. 1I), GFP-N-WASP and GFP-Nck pulldowns were performed using the GFP-trap (Chromotek) as described [6]. Immunoblot analysis was performed using antibodies against WIRE (HPA024467, Sigma Aldrich, UK); N-WASP [8]; Grb2 (610112, BD biosciences, USA); Nck (06-288 Millipore, USA), GFP (3E1 Cancer Research UK); and RFP (to detect mCherry) (Chemicon).

Vector Construction and Generation of Stable Cell Lines

The GFP-WIRE expression vector was generated by cloning human WIRE into the NotI-EcoRI sites of the pEL-GFP vaccinia expression vector [9]. GFP-tagged Nck, WIP, N-WASP pE/L expression vectors and Lentivirus pLL 3.7 GFP-Nck have been described [8-10]. N-WASPΔPolyPro in the pEL-GFP vaccinia expression vector was generated by deleting the base pairs corresponding to amino acid residues 273-386. Proline to alanine substitutions in WIP and N-WASP as well as the Nck mutants W38K, W143K, and W229K that disrupt individual SH3 domains [11] were generated using the Stratagene site directed mutagenesis kit. The resulting mutants were cloned into the Not1/EcoRI sites of pE/L GFP or the Lentivirus vectors pLL 3.7 GFP and a modified pLVX-puro-GFP (Clontech). Lentiviruses were generated with the pL/L 3.7 GFP Nck mutants, pLVX-puro-GFP N-WASP or N-WASPΔNck vectors and subsequently used to establish stable Nck-/- or N-WASP-/- cell as described previously [10]. GFP-Nck mutant cell lines were selected using FACS, while N-WASP cell lines were selected

using 1µg/ml of puromycin. Nck-/- cells expressing GFP-Nck have been described [10].

Live Imaging, FRAP, WIRE Intensity, and Statistical Analyses

At 4 hours post infection, WIP-/- cells were transfected with pEL expression vectors using effectene (Qiagen, Germany). Images were captured from 5 hrs using a Plan-Achromat 63x lens (Carl Zeiss, Germany) and an Evolve 512 camera (Photometrics, AZ) on an Axio Observer Microscope controlled by Slidebook (3i intelligent imaging innovations, USA). FRAP experiments and analysis of GFP-recovery rates were performed as previously described [10, 12]. To determine the level of endogenous WIRE associated with virus particles we used MetaMorph (Molecular Devices Corporation) to measure the intensity of anti-WIRE staining on virions inducing actin tails in cells infected with WR expressing RFP-A3 [10]. RFP-A3, a core viral protein, provides a constant internal fluorescence reference and anti-Cortactin antibody (05-180, Millipore, USA) was used to label actin tails. The ratio of WIRE intensity to RFP-A3 intensity is shown. Fifty particles in each of 3 independent experiments were analyzed. All data are presented as mean ± standard error of the mean and were analyzed by ANOVA or Student's t test using Prism 5.0 (GraphPad Software, CA). A P value of <0.05 was considered statistically significant.

Peptide Arrays and Pull-Downs

His-Nck1 was expressed and purified as described [13]. Overlapping 15mer WIP and N-WASP peptide arrays in which adjacent peptides are shifted by 3 amino acids were provided by the Peptide Synthesis Laboratory (LRI). Peptide arrays were blocked and incubated for 1 hour in 5% Milk/PBS/0.1% Tween-20 containing 2.0 µg/ml His-Nck1 at 4°C. Probed membranes were washed 5 times for 20 mins with 5% Milk/PBS/0.1% Tween-20 and probed with anti-His antibody (Qiagen). The peptides WIP1: FSNRPPLPPTPSRALDDKPP; WIP Mut1: FSNRPPLAPTASRALDDKPP; WIP2: SSGNDETPRLPQRNLSLSSS; WIP Mut2: SSGNDETARLAQRNLSLSSS; N-WASP1: APPPPPPSRGGPPPPPPPPH; N-WASP1Mut: APPAPPASRGGPPPPPPPPH; N-WASP2: PPPPPPPPHSSGPPPPPARG; N-WASP2Mut: PPPPPPPPHSSGPAPPAARG were coupled via an additional N-terminal CGG to SulfoLink resin (Pierce Biotechnology) and His-Nck1 pulldowns performed as previously described [13].

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