Supporting Information

Hale et al. 10.1073/pnas.0910715107

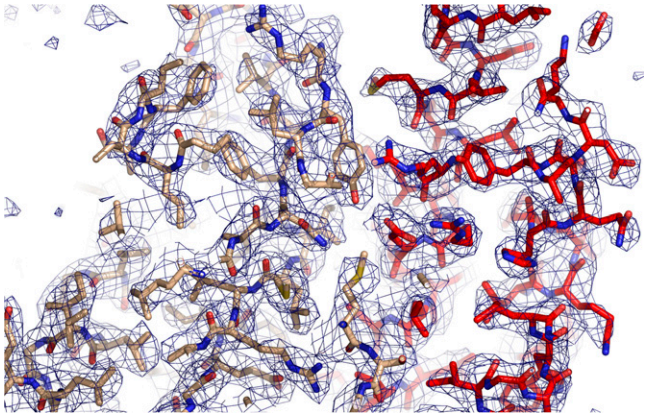

Fig. S1. Representative example of $2F_o$-F_c electron density (contoured at 1 σ) for the ED:β-iSH2 complex.

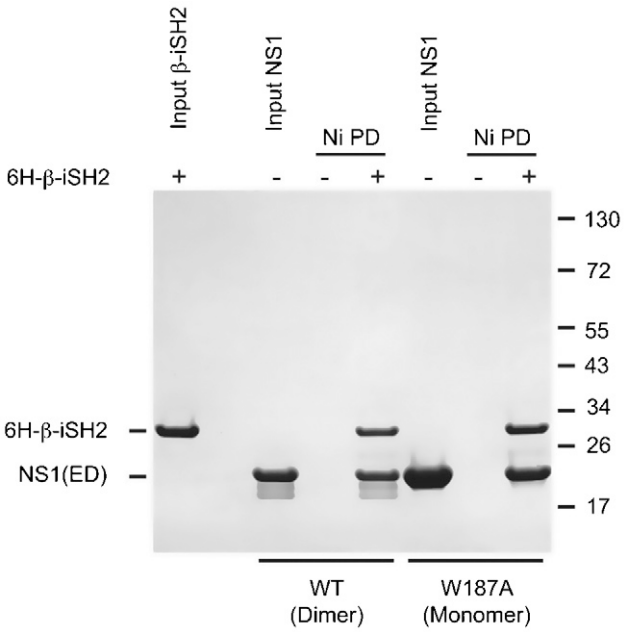

Fig. S2. The NS1 ED-W187A (monomeric) mutant binds β-iSH2. 6H-tagged β-iSH2 was expressed and purified as described previously (1). Untagged PR8/NS1 EDs (WT and W187A) were purified as for the Alb/NS1 ED (2), and confirmed to be dimeric (WT) or monomeric (W187A) by analytical gel filtration (2). Ni-NTA pull-downs (PD) were performed after individually mixing the purified ED proteins with 6H-β-iSH2, and were analyzed by SDS-PAGE followed by Coomassie Blue staining. As shown in the figure, both WT and W187A ED proteins were efficiently precipitated with β-iSH2. Neither ED protein was precipitated in the absence of β-iSH2. Molecular weight markers (kDa) are shown to the right.

1. Hale BG, Batty IH, Downes CP, Randall RE (2008) Binding of influenza A virus NS1 protein to the inter-SH2 domain of p85 suggests a novel mechanism for phosphoinositide 3-kinase activation. *J Biol Chem* 283(3):1372–1380.
2. Hale BG, Barclay WS, Randall RE, Russell RJ (2008) Structure of an avian influenza A virus NS1 protein effector domain. *Virology* 378(1):1–5.

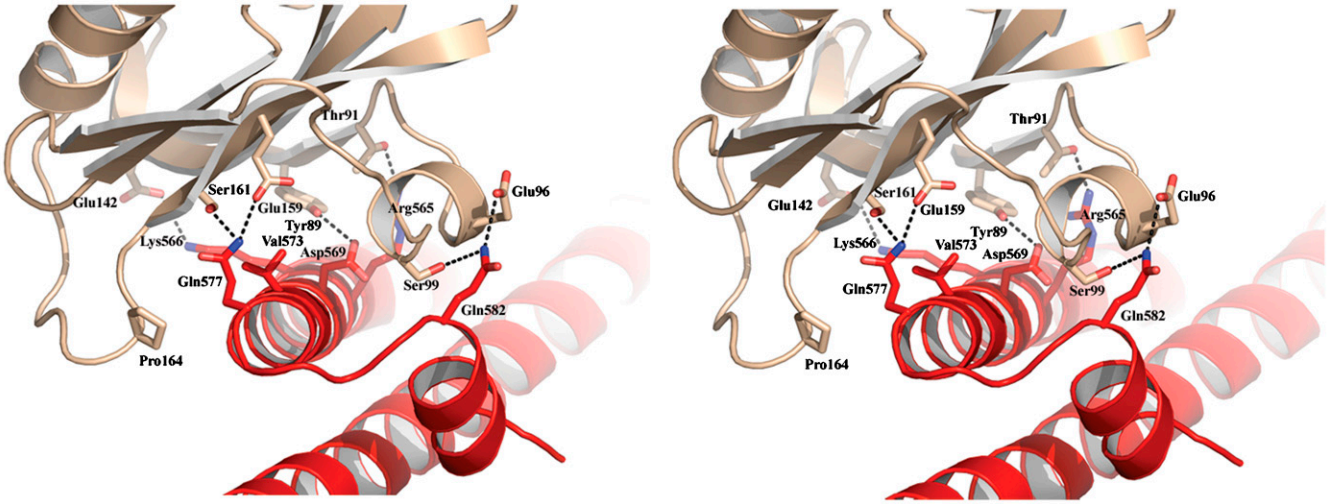

Fig. S3. Residues at the ED:β-iSH2 interface (stereo version of Fig. 2*C*). Individual residues are shown in stick representation. NS1 ED is colored gold, whilst β-iSH2 is colored red.

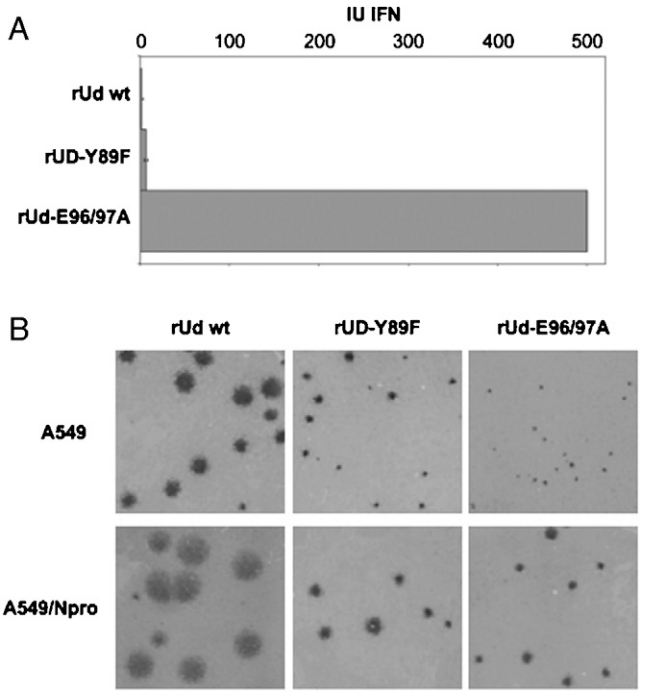

Fig. S4. rUd-E96/97A induces greater amounts of IFN than rUd wt and rUd-Y89F. A549 monolayers were infected with rUd wt, rUd-Y89F or rUd-E96/97A in serum-free DMEM at an MOI of 5 PFU/cell. 24 h later, supernatants were harvested, clarified by centrifugation, and infectious virus inactivated by UV treatment. The amount of IFNα/β secreted by infected cells was estimated by a biological EMCV-inhibition assay. A549/BVDV-NPro cells (which are unable to synthesize IFN, but can respond to exogenous IFN (3, 4) were incubated with dilutions of culture supernatant for 24 h before cells were infected with EMCV. The endpoint for each sample was taken to be the dilution at which cells exhibited 50% cytopathic effect. The amount of IFNα/β in each sample was calculated in International Units (IU) per 10^6 cells by comparison of the endpoint with an IFNα standard. Data represent the mean values of three independent experiments (±S.D.). (*B*) Plaque size of recombinant rUd wt, rUd-Y89F and rUd-E96/97A viruses in A549 cells (*Upper*), or A549/BVDV-NPro cells (*Lower*).

3. Hilton L, et al. (2006) The NPro product of bovine viral diarrhea virus inhibits DNA binding by interferon regulatory factor 3 and targets it for proteasomal degradation. *J Virol* 80 (23):11723–11732.
4. Carlos TS, Young DF, Schneider M, Simas JP, Randall RE (2009) Parainfluenza virus 5 genomes are located in viral cytoplasmic bodies whilst the virus dismantles the interferon-induced antiviral state of cells. *J Gen Virol* 90(Pt 9):2147–2156.

Table S1. Residues of NS1 ED present at the ED:β-iSH2 interface

	ASA	BSA
ARG 88	176.74	19.87
TYR 89	106.51	92.87
THR 91	72.91	27.93
LEU 95	141.62	88.52
GLU 96	94.87	14.41
MET 98	49.85	46.21
SER 99	80.46	79.30
ARG 100	84.21	15.11
ASP 101	131.19	61.74
CYS 116	10.60	0.67
ARG 118	68.69	13.47
SER 135	15.68	13.51
GLU 142	63.82	8.69
THR 143	42.89	18.82
LEU 144	3.17	0.49
ILE 145	67.64	65.07
LEU 146	21.25	21.25
ARG 148	16.89	12.09
GLU 159	14.86	10.93
SER 161	18.76	18.76
PRO 162	34.39	15.65
LEU 163	55.50	2.31
PRO 164	135.78	96.14

ASA is the solvent accessible surface area of the residue in Å^2. BSA is solvent-accessible surface area of the corresponding residue that is buried upon interface formation in Å^2.

Table S2. Residues of β-iSH2 present at the ED:β-iSH2 interface

	ASA	BSA
HIS 444	26.62	6.84
MET 562	117.34	57.21
ARG 565	116.37	55.86
LYS 566	108.12	75.79
ARG 568	40.80	21.22
ASP 569	53.54	52.68
GLN 570	99.95	30.73
LEU 572	44.49	44.33
VAL 573	80.39	74.20
TRP 574	103.10	41.33
THR 576	70.34	43.38
GLN 577	130.87	94.37
LYS 578	110.57	14.11
ARG 581	173.41	25.33
GLN 582	140.51	96.44
LYS 583	106.78	1.18
ILE 585	9.71	9.71
ASN 586	79.30	4.95
LEU 589	34.73	17.40

ASA is the solvent accessible surface area of the residue in Å^2. BSA is solvent-accessible surface area of the corresponding residue that is buried upon interface formation in Å^2.