Stimulation of GMP formation in hGBP1 is mediated by W79 and its effect on the antiviral activity

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Supporting Information

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Table S1. List of primers used for the preparation of point mutants.

Mutant	Primer	Sequence
W79A	Sense	5' CACACTAAAGGAATCGCGATGTGGTGTGTGCC 3'
	Anti-Sense	5' GGCACACACCACATCGCGATTCCTTTAGTGTG 3'
W81A	Sense	5' CACTAAAGGAATCTGGATGGCGTGTGTGCCCCACCCAAG 3'
	Anti-Sense	5' CTTGGGGTGGGGCACACACGCCATCCAGATTCCTTTAGTG 3'
W114A	Sense	5' CAGAATGACTCCGCGATCTTCGCCCTG 3'
	Anti-Sense	5' CAGGGCGAAGATCGCGGAGTCATTCTG 3'
W180A	Sense	5' CTTCCCAGACTTTGTGGCGACACTGAGAGATTTC 3'
	Anti-Sense	5' GAAATCTCTCAGTGTCGCCACAAAGTCTGGGAAG 3'
W79F	Sense	5' CTAAAGGAATCTTCATGTGGTGTGTGC 3'
	Anti-Sense	5' GCACACACCACATGAAGATTCCTTTAG 3'
W114F	Sense	5' GAATGACTCCTTCATCTTCGCCC 3'
	Anti-Sense	5' GGGCGAAGATGAAGGAGTCATTC 3'

Table S2: The n value derived from the steady-state kinetic analysis.

Protein	n^{GDP}	n^{GMP}
WT	1.0	1.4
W79A	1.0	0.95
W81A	0.9	1.05
W114A	1.0	1.7
W180A	1.1	1.3
W79F	1.0	0.97
W114F	1.0	1.5
W79F/W114F	0.95	1.1

Figure S1A-C. Intrinsic tryptophan fluorescence spectra of the mutants in the absence and presence of GDP.AIF₄: A) W81A, B) W114A and C) W180A mutants.

Figure S2. Dynamic tryptophan fluorescence quenching of wild type and mutant proteins in the absence and presence of GDP.AIF₄. The fluorescence intensity at the emission maximum was measured and the Stern-volmer plot (F_0/F vs concentration of CsCl) was generated. The slope of the curve gave the value of K_{sv} .

Figure S3A-H. Kinetic analysis of GMP and GDP formation in the mutants and wild-type: (A) W79A, (B) W81A, (C) W114A, (D) W180A, (E) W79F, (F) W79F/W114F, (G) W114F and (H) WT. The initial rate versus the concentrations of GTP was fitted to a sigmoidal equation, $\text{Rate} = k_{\text{cat}} \times [\text{E}_0] \times [\text{GTP}]^n / (K_{0.5}^n + [\text{GTP}]^n)$ using Sigmaplot, yielding the steady-state kinetic parameters, apparent $K_{0.5}$, k_{cat} and n (degree of cooperativity). The quality of the fit was judged by a theoretical line drawn through the experimental data points and highest confidence limit.