

## Supporting Information for

### Helical domain changes between hGBP3 and hGBP3 $\Delta$ C result in distinct oligomers and anti-HCV activity

Sowmiya Gupta<sup>1</sup>, Aunji Pradhan<sup>2,†</sup>, Divya Rashmi<sup>1,†</sup>, Monika Mittal<sup>1,†</sup>, Saumitra Das<sup>2</sup> and Apurba Kumar Sau<sup>\*,1</sup>

<sup>1</sup> National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi 110067, India;

<sup>2</sup> Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560012, India

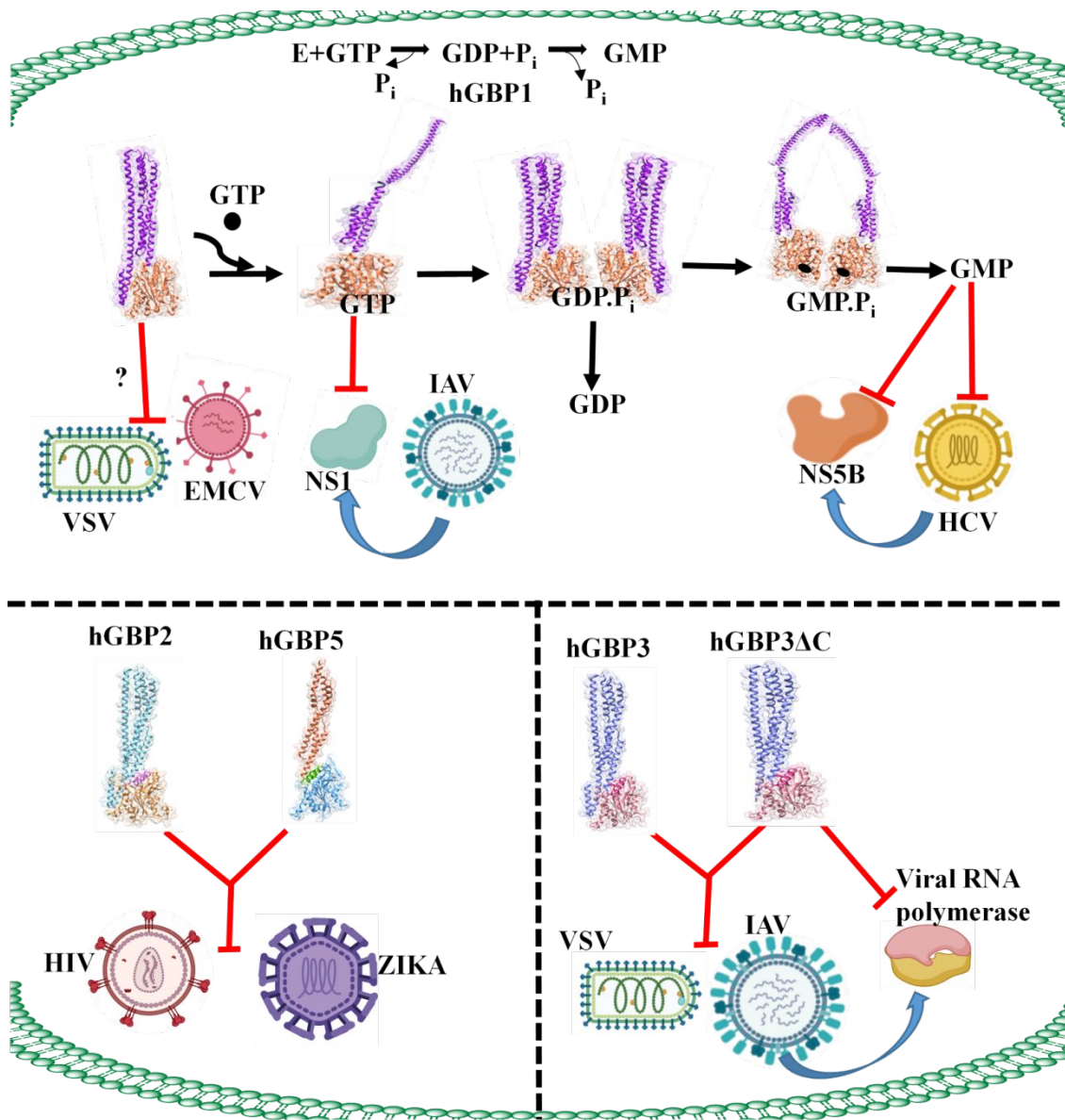
<sup>†</sup> These authors contributed equally to this work

\* To whom correspondence should be addressed: Dr. Apurba Kumar Sau, National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi, Delhi 110067, India; apurba@nii.ac.in, apurbaksau@gmail.com; Tel. +91-11-26703768; Fax. +91-11-26742125/26742626

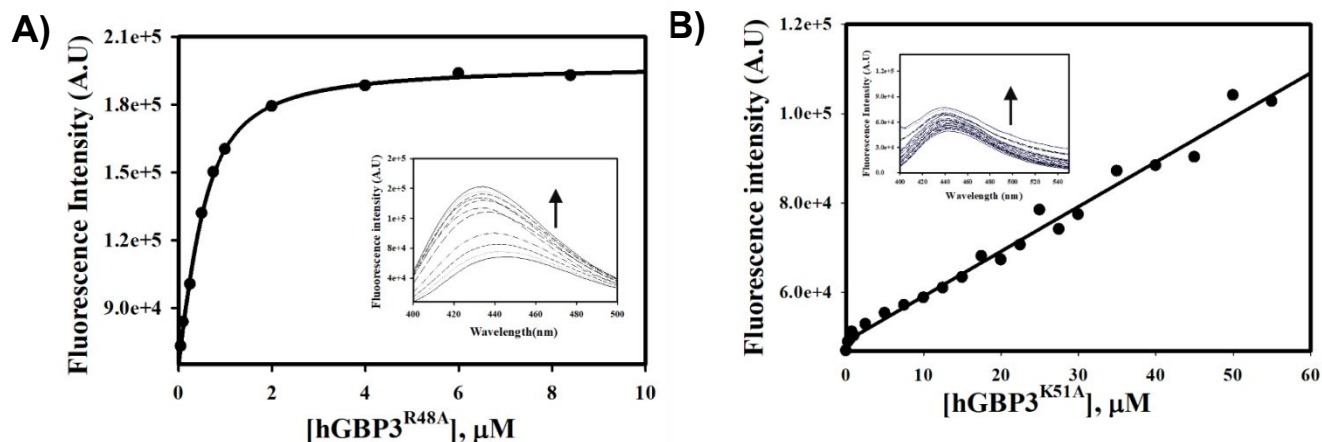
Running title: *hGBP3 helical domain and oligomer*

Table S1. List of primers used for the preparation of wild-type, splice variant, truncated and mutant hGBP3

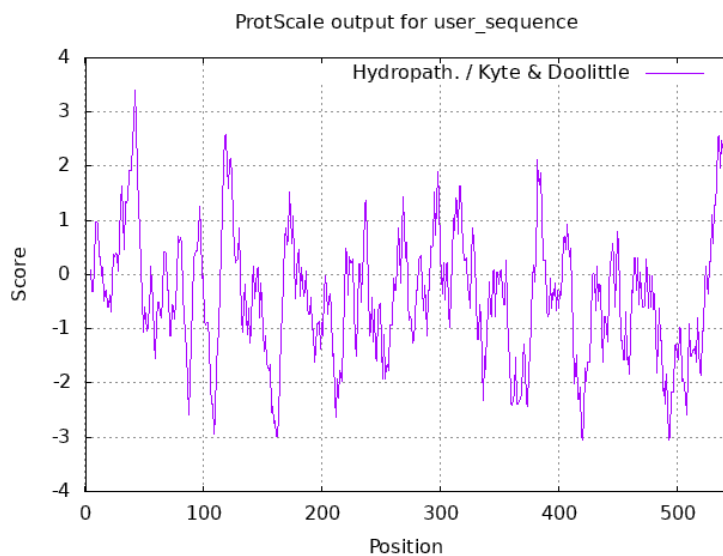
Vector	Construct	Primer	Sequence
<b>pET22b</b>	hGBP3	Sense	5'GTGCAAAGGCTATTGCCCACTATGACCAGC AGATGGGC 3'
		Antisense	5'GCGCTCGAGGATCTTTAGCTTATG 3'
<b>pET28a</b>	hGBP3 $\Delta$ C	Sense	5'GTGCAAAGGCTATTGCCCACTATGACCAGC AGATGGGC3'
		Antisense	5'GCACTCGAGGGAAGAACAGAGAG3'
<b>pET22b and pcDNA3.1</b>	hGBP3 <sup>K51A</sup>	Sense	5'GGCCTCTACCGCACAGGAGCATCCTACCTGA TGAACAAG3'
		Antisense	5'CTTGTTTCATCAGGTAGGATGCTCCTGTGCGGT AGAGGCC3'
<b>pET22b and pcDNA3.1</b>	hGBP3 <sup>R48A</sup>	Sense	5'GCAATTGTGGGCTCTACGCCACAGGAAAAT CCTACCTG3'
		Antisense	5'CAGGTAGGATTTTCCTGTGGCGTAGAGGCC ACAATTGC3'
<b>pcDNA3.1</b>	hGBP3	Sense	5'GAAGCGGCCGCTATGGCTCCAGAG3'
		Antisense	5'GCACTCGAGTTAGATCTTTAGCTT3'
<b>pcDNA3.1</b>	hGBP3 $\Delta$ C	Sense	5'GAAGCGGCCGCTATGGCTCCAGAG3'
		Antisense	5'GCACTCGAGCTAGGAAGAACAGAG3'
<b>pcDNA3.1</b>	hGBP3 <sup>1-309</sup>	Sense	5'GAAGCGGCCGCTATGGCTCCAGAG3'
		Antisense	5'GCGCTCGAGGCAGGGCAGATCCCCTCT 3'
<b>pcDNA3.1</b>	hGBP3 <sup>1-278</sup>	Sense	5'GAAGCGGCCGCTATGGCTCCAGAG3
		Antisense	5'GCGCTCGAGTTTAGTTTTGGA3'



**Figure S1. Schematic diagram of the antiviral effect of hGBP homologs against different viruses.** hGBP1 inhibits proliferation of both VSV and EMCV in hela cells but the mechanism of action is not yet known. In case of influenza virus, hGBP1 interacts with the viral NS1 protein which leads to decrease in viral titers. hGBP1 is also known to interact with NS5B subunit of hepatitis C virus RNA polymerase and decreases the viral propagation. Additionally, the enhanced GMP formation by hGBP1 is crucial for exhibiting anti-hepatitis C activity. hGBP2 and hGBP5 lead to inhibition of HIV and Zika virus proliferation by inhibiting the furin mediated viral envelope protein processing. hGBP3 and its splice variant inhibit the Influenza virus propagation by targeting its RNA polymerase activity and reducing the viral replication.



**Figure S2. Substrate binding measurements of hGBP3 mutants.** The binding experiments of A) hGBP3<sup>R48A</sup> and B) hGBP3<sup>K51A</sup> were performed using 0.5 μM MANT-GppNHp. Fluorescence titration with increasing concentrations of the protein. The data were fitted to a quadratic equation to determine the  $K_d$  value.



**Figure S3. Hydropathy index of hGBP3.** The hydropathy index was analyzed using ProScale online server. Position in X-axis represents position of amino acid residues. Y-axis represents hydropathy score. In this graph, more hydrophobic amino acids have the highest positive values while the more hydrophilic ones have the highest negative values.