SUPPORTING INFORMATION

Affinity Labeling of Hepatitis C Virus Replicase with a Nucleotide Analog: Identification of binding site

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Chart S1.

1. **37 mer self-annealing RNA template primer:**

2. **$rU_{15}.rA_{10\text{-}3'deoxy}$: Oligo rU_{15} annealed with 3'dexoy terminated rA_{10}**

3. **Primer oligo for in-vitro mutagenesis:**

Y38A: Up: 5'-CGTCACCACAACATGGTCGCTGCTACAACATCTCGCAG-3'

Down: 5'-CTGCGAGATGTTGTAGCAGCGACCATGTTGTGGTGACG-3'

Y382A: Up: 5'-CATCTGGCAAAAGGGTGGCCTATCTCACCCGTGACC-3'

Down: 5'-GGTCACGGGTGAGATAGGCCACCCTTTTGCCAGATG-3'

K491A: Up: 5'-GCTTCATGCCTCAGGGCACTTGGGGTACCGCC-3'

Down: 5'-GGCGGTACCCCAAGTGCCCTGAGGCATGAAGC-3'

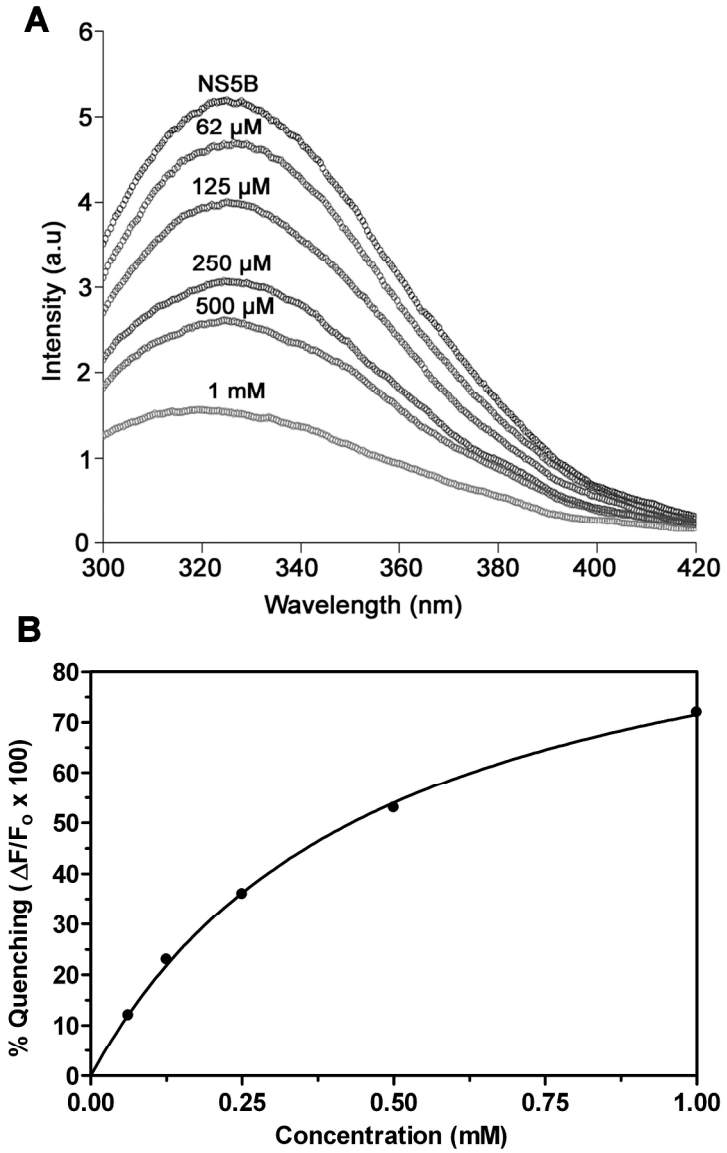

Figure S1. Fluorescence quenching of NS5B by FSBA. HCV NS5B (1µM) was incubated with increasing concentrations of FSBA. The change of intrinsic protein fluorescence was monitored at the emission spectrum of 300 to 420 nm, with excitation at 280 nm. (A) Emission fluorescence spectrum of NS5B in the presence of increasing concentrations of FSBA is shown. (B) Fluorescence quenching of NS5B in the presence of increasing concentrations of FSBA at emission fluorescence intensity of 330 nm, with excitation at 280 nm. The percentage of fluorescence quenching ($\Delta F/F_o$ x 100) was calculated relative to the fluorescence of NS5B.

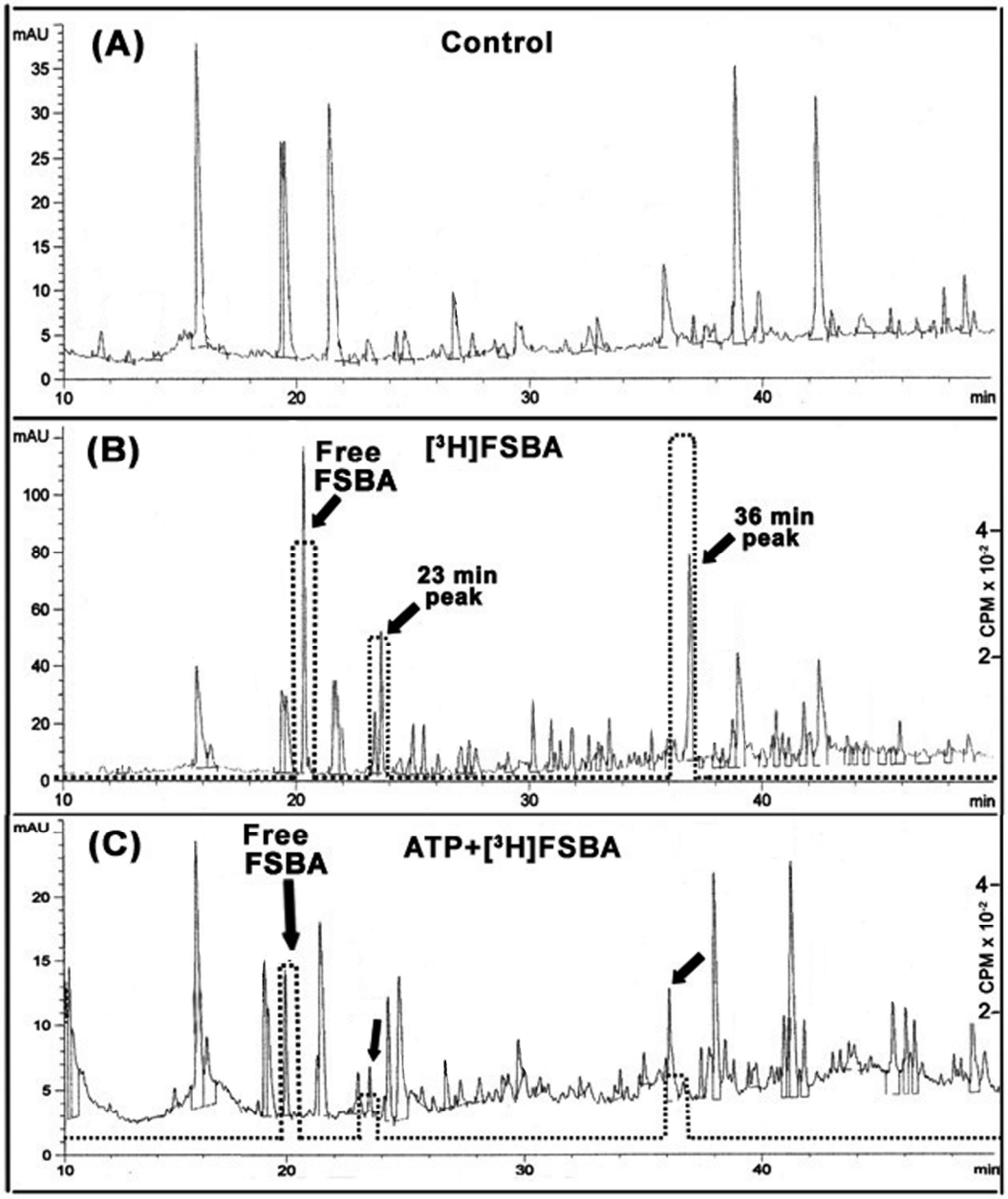

Figure S2. Comparison of tryptic peptides of NS5B in the absence (A) and presence (B) of [^{3}H]-FSBA and ATP (C). The labeling reaction containing 15 μM HCV NS5B was incubated in the presence or absence of 1 mM [^{3}H]-FSBA for 1 h at 37^{O}C. The protein was TCA-precipitated and trypsinized. The tryptic peptides were resolved using a Phenomenex Kinetex XB-C18 reverse-phase column using a linear gradient of solvent B (CH_3CN) into solvent A (H_2O, 0.1% TFA), 0-10 min (0-1% B), 11-50 min (1-50% B), and 51-80 min (51-100% B 1.5 ml of eluants were collected; aliquots were withdrawn and assayed for ^{3}H radioactivity by liquid scintillation counting. Dotted line represents the amount of radioactivity detected. Tryptic peptide profile is shown in the full scale of each experiment at 254 nm absorbance.