Supporting Information

“The Hepatitis B Virus oncoprotein HBx is not an ATPase”

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**Table S1.** Primers used for the generation of the MBP-HBx variants

| Mutation | Forward primer                  | Reverse Primer                  |
|----------|---------------------------------|---------------------------------|
| G136A    | CGTTCTGGGTGCTTGCCGTCAAAAAC      | AACACCTTCAGACGAATTTC            |
| K130A    | CGTCTGGCAGTGTTCGTT              | AATTTCCTCGCCCAGTTCC             |
| K140A    | GCCGTCATGCACCTGGGTGT            | AGCCACCCAGAAACGAACA             |
| C137A    | CAGCAGTTCAATGCAATTTC           | AGTTTGTGACGCGCGCCAC            |
| H139A    | GCTGCCGTGCAAAAACCTGG           | CACCCAGAACGAACACCT              |
| K130M    | CGTCTGATGGTTCTGGGTC            | AATTTCCTCGCCCAGTTCCC            |
| V131I    | TCGTCTGATGATCTTGCTGGGTG         | ATTTCCCTCGCCCAGTTCC            |
| Δ27      | TAACTCGAGCACCACCACCA          | AATTTCCTCGCCCAGTTCC           |
Table S2. NSitePred nucleotide binding prediction.

| AA | # | ATP binding res. | ATP prob. | ADP binding res. | ADP prob. | AMP binding res. | AMP prob. | GTP binding res. | GTP prob. | GDP binding res. | GDP prob. |
|----|---|------------------|-----------|------------------|-----------|------------------|-----------|------------------|-----------|------------------|-----------|
| M  | 1 | N                | 0.025     | N                | 0.025     | N                | 0.024     | N                | 0.021     | N                | 0.017     |
| A  | 2 | N                | 0.026     | N                | 0.022     | N                | 0.024     | N                | 0.025     | N                | 0.016     |
| A  | 3 | N                | 0.03      | N                | 0.022     | N                | 0.025     | N                | 0.024     | N                | 0.013     |
| R  | 4 | N                | 0.041     | N                | 0.027     | N                | 0.032     | N                | 0.013     | N                | 0.022     |
| M  | 5 | N                | 0.025     | N                | 0.029     | N                | 0.026     | N                | 0.016     | N                | 0.021     |
| Y  | 6 | N                | 0.028     | N                | 0.039     | N                | 0.04      | N                | 0.023     | N                | 0.02      |
| C  | 7 | N                | 0.024     | N                | 0.031     | N                | 0.029     | N                | 0.015     | N                | 0.022     |
| Q  | 8 | N                | 0.035     | N                | 0.036     | N                | 0.036     | N                | 0.062     | N                | 0.029     |
| L  | 9 | N                | 0.032     | N                | 0.023     | N                | 0.035     | N                | 0.025     | N                | 0.023     |
| D  | 10| N                | 0.048     | N                | 0.036     | N                | 0.035     | N                | 0.021     | N                | 0.025     |
| P  | 11| N                | 0.035     | N                | 0.032     | N                | 0.033     | N                | 0.044     | N                | 0.015     |
| S  | 12| N                | 0.038     | N                | 0.042     | N                | 0.032     | N                | 0.028     | N                | 0.017     |
| R  | 13| N                | 0.041     | N                | 0.033     | N                | 0.035     | N                | 0.047     | N                | 0.03      |
| D  | 14| N                | 0.043     | N                | 0.04      | N                | 0.025     | N                | 0.018     | N                | 0.029     |
| V  | 15| N                | 0.053     | N                | 0.033     | N                | 0.044     | N                | 0.024     | N                | 0.023     |
| L  | 16| N                | 0.037     | N                | 0.032     | N                | 0.028     | N                | 0.026     | N                | 0.015     |
| C  | 17| N                | 0.019     | N                | 0.03      | N                | 0.027     | N                | 0.034     | N                | 0.021     |
| L  | 18| N                | 0.03      | N                | 0.025     | N                | 0.036     | N                | 0.039     | N                | 0.027     |
| R  | 19| N                | 0.035     | N                | 0.028     | N                | 0.06      | N                | 0.023     | N                | 0.026     |
| P  | 20| N                | 0.028     | N                | 0.033     | N                | 0.034     | N                | 0.022     | N                | 0.089     |
| V  | 21| N                | 0.031     | N                | 0.051     | N                | 0.034     | N                | 0.033     | N                | 0.02      |
| G  | 22| N                | 0.032     | N                | 0.033     | N                | 0.04      | N                | 0.008     | N                | 0.018     |
| A  | 23| N                | 0.032     | N                | 0.062     | N                | 0.034     | N                | 0.017     | N                | 0.023     |
| E  | 24| N                | 0.041     | N                | 0.053     | N                | 0.029     | N                | 0.022     | N                | 0.04      |
| S  | 25| N                | 0.032     | N                | 0.04      | N                | 0.035     | N                | 0.021     | N                | 0.017     |
| R  | 26| N                | 0.038     | N                | 0.037     | N                | 0.027     | N                | 0.035     | N                | 0.023     |
|   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G | 27 | N | 0.05 | N | 0.029 | N | 0.03 | N | 0.027 | N | 0.02 |
| R | 28 | N | 0.027 | N | 0.035 | N | 0.027 | N | 0.012 | N | 0.012 |
| P | 29 | N | 0.023 | N | 0.019 | N | 0.024 | N | 0.026 | N | 0.009 |
| L | 30 | N | 0.031 | N | 0.018 | N | 0.031 | N | 0.026 | N | 0.014 |
| S | 31 | N | 0.043 | N | 0.028 | N | 0.033 | N | 0.029 | N | 0.015 |
| G | 32 | N | 0.029 | N | 0.019 | N | 0.041 | N | 0.013 | N | 0.017 |
| P | 33 | N | 0.027 | N | 0.03 | N | 0.047 | N | 0.013 | N | 0.02 |
| L | 34 | N | 0.031 | N | 0.039 | N | 0.046 | N | 0.012 | N | 0.011 |
| S | 35 | N | 0.046 | N | 0.018 | N | 0.022 | N | 0.042 | N | 0.006 |
| T | 36 | N | 0.051 | N | 0.065 | N | 0.026 | N | 0.017 | N | 0.012 |
| L | 37 | N | 0.027 | N | 0.018 | N | 0.026 | N | 0.016 | N | 0.024 |
| S | 38 | N | 0.052 | N | 0.017 | N | 0.039 | N | 0.008 | N | 0.018 |
| S | 39 | N | 0.048 | N | 0.038 | N | 0.043 | N | 0.024 | N | 0.011 |
| P | 40 | N | 0.035 | N | 0.016 | N | 0.028 | N | 0.022 | N | 0.013 |
| S | 41 | N | 0.031 | N | 0.045 | N | 0.063 | N | 0.021 | N | 0.017 |
| P | 42 | N | 0.046 | N | 0.06 | N | 0.031 | N | 0.018 | N | 0.012 |
| S | 43 | N | 0.025 | N | 0.044 | N | 0.027 | N | 0.013 | N | 0.026 |
| A | 44 | N | 0.037 | N | 0.04 | N | 0.027 | N | 0.013 | N | 0.02 |
| V | 45 | N | 0.028 | N | 0.047 | N | 0.03 | N | 0.018 | N | 0.018 |
| P | 46 | N | 0.028 | N | 0.019 | N | 0.032 | N | 0.035 | N | 0.02 |
| A | 47 | N | 0.019 | N | 0.026 | N | 0.024 | N | 0.023 | N | 0.017 |
| D | 48 | N | 0.02 | N | 0.037 | N | 0.025 | N | 0.018 | N | 0.013 |
| H | 49 | N | 0.034 | N | 0.042 | N | 0.033 | N | 0.023 | N | 0.025 |
| G | 50 | N | 0.03 | N | 0.034 | N | 0.028 | N | 0.028 | N | 0.023 |
| A | 51 | N | 0.022 | N | 0.051 | N | 0.026 | N | 0.041 | N | 0.014 |
| H | 52 | N | 0.029 | N | 0.029 | N | 0.026 | N | 0.019 | N | 0.029 |
| L | 53 | N | 0.021 | N | 0.016 | N | 0.027 | N | 0.015 | N | 0.019 |
| S | 54 | N | 0.025 | N | 0.02 | N | 0.035 | N | 0.026 | N | 0.013 |
| L | 55 | N | 0.023 | N | 0.032 | N | 0.028 | N | 0.027 | N | 0.028 |
| R | 56 | N | 0.05 | N | 0.025 | N | 0.082 | N | 0.025 | N | 0.017 |
| Column | Value | Column | Value | Column | Value | Column | Value |
|--------|-------|--------|-------|--------|-------|--------|-------|
| G      | 57    | B      | 0.135 | N      | 0.048 | N      | 0.055 |
| L      | 58    | N      | 0.066 | N      | 0.06  | N      | 0.055 |
| P      | 59    | N      | 0.054 | N      | 0.035 | N      | 0.038 |
| V      | 60    | N      | 0.06  | N      | 0.031 | N      | 0.044 |
| C      | 61    | N      | 0.045 | N      | 0.029 | N      | 0.031 |
| A      | 62    | N      | 0.04  | N      | 0.016 | N      | 0.035 |
| F      | 63    | N      | 0.043 | N      | 0.037 | N      | 0.025 |
| S      | 64    | N      | 0.041 | N      | 0.035 | N      | 0.041 |
| S      | 65    | N      | 0.034 | N      | 0.036 | N      | 0.039 |
| A      | 66    | N      | 0.087 | N      | 0.048 | N      | 0.06  |
| G      | 67    | N      | 0.057 | N      | 0.041 | B      | 0.236 |
| P      | 68    | N      | 0.028 | N      | 0.031 | N      | 0.044 |
| C      | 69    | N      | 0.09  | N      | 0.024 | N      | 0.071 |
| A      | 70    | N      | 0.037 | N      | 0.027 | N      | 0.034 |
| L      | 71    | N      | 0.038 | N      | 0.022 | N      | 0.034 |
| R      | 72    | N      | 0.04  | N      | 0.031 | N      | 0.041 |
| F      | 73    | N      | 0.03  | N      | 0.039 | N      | 0.043 |
| T      | 74    | N      | 0.025 | N      | 0.02  | N      | 0.052 |
| S      | 75    | N      | 0.035 | N      | 0.029 | N      | 0.058 |
| A      | 76    | N      | 0.067 | N      | 0.038 | N      | 0.05  |
| R      | 77    | N      | 0.058 | N      | 0.051 | N      | 0.083 |
| C      | 78    | N      | 0.08  | N      | 0.04  | N      | 0.037 |
| M      | 79    | N      | 0.058 | N      | 0.035 | N      | 0.035 |
| E      | 80    | N      | 0.044 | N      | 0.017 | N      | 0.03  |
| T      | 81    | N      | 0.027 | N      | 0.022 | N      | 0.045 |
| T      | 82    | N      | 0.024 | N      | 0.035 | N      | 0.023 |
| V      | 83    | N      | 0.035 | N      | 0.019 | N      | 0.028 |
| N      | 84    | N      | 0.028 | N      | 0.022 | N      | 0.054 |
| A      | 85    | N      | 0.023 | N      | 0.025 | N      | 0.032 |
| H      | 86    | N      | 0.022 | N      | 0.036 | N      | 0.031 |
|  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|
| Q | 87 | N | 0.025 | N | 0.03 | N | 0.038 | N | 0.018 | N | 0.021 |
| I | 88 | N | 0.029 | N | 0.02 | N | 0.029 | N | 0.017 | N | 0.017 |
| L | 89 | N | 0.027 | N | 0.024 | N | 0.032 | N | 0.018 | N | 0.022 |
| P | 90 | N | 0.031 | N | 0.026 | N | 0.03 | N | 0.023 | N | 0.016 |
| K | 91 | N | 0.032 | N | 0.025 | N | 0.031 | N | 0.015 | N | 0.022 |
| V | 92 | N | 0.015 | N | 0.017 | N | 0.021 | N | 0.008 | N | 0.02 |
| L | 93 | N | 0.027 | N | 0.022 | N | 0.03 | N | 0.015 | N | 0.02 |
| H | 94 | N | 0.048 | N | 0.06 | N | 0.059 | N | 0.038 | N | 0.02 |
| K | 95 | N | 0.027 | N | 0.034 | N | 0.034 | N | 0.015 | N | 0.018 |
| R | 96 | N | 0.019 | N | 0.018 | N | 0.101 | N | 0.032 | N | 0.015 |
| T | 97 | N | 0.027 | N | 0.046 | N | 0.024 | N | 0.013 | N | 0.011 |
| L | 98 | N | 0.025 | N | 0.019 | N | 0.026 | N | 0.014 | N | 0.014 |
| G | 99 | N | 0.037 | N | 0.035 | N | 0.032 | N | 0.033 | N | 0.014 |
| L | 100 | N | 0.028 | N | 0.029 | N | 0.04 | N | 0.017 | N | 0.021 |
| P | 101 | N | 0.027 | N | 0.022 | N | 0.034 | N | 0.017 | N | 0.022 |
| A | 102 | N | 0.027 | N | 0.022 | N | 0.037 | N | 0.009 | N | 0.024 |
| M | 103 | N | 0.023 | N | 0.032 | N | 0.036 | N | 0.008 | N | 0.026 |
| S | 104 | N | 0.046 | N | 0.03 | N | 0.025 | N | 0.011 | N | 0.055 |
| T | 105 | N | 0.087 | N | 0.028 | N | 0.031 | N | 0.008 | N | 0.041 |
| T | 106 | N | 0.03 | N | 0.018 | N | 0.029 | N | 0.01 | B | 0.117 |
| D | 107 | N | 0.02 | N | 0.014 | N | 0.025 | N | 0.009 | N | 0.054 |
| L | 108 | N | 0.03 | N | 0.015 | N | 0.018 | N | 0.027 | N | 0.02 |
| E | 109 | N | 0.018 | N | 0.022 | N | 0.019 | N | 0.021 | N | 0.021 |
| A | 110 | N | 0.023 | N | 0.03 | N | 0.024 | N | 0.021 | N | 0.015 |
| Y | 111 | N | 0.025 | N | 0.043 | N | 0.029 | N | 0.01 | N | 0.023 |
| F | 112 | N | 0.066 | N | 0.034 | N | 0.022 | N | 0.013 | N | 0.022 |
| K | 113 | N | 0.017 | N | 0.025 | N | 0.022 | N | 0.023 | N | 0.022 |
| D | 114 | N | 0.03 | N | 0.038 | N | 0.03 | N | 0.027 | N | 0.021 |
| C | 115 | N | 0.039 | N | 0.035 | N | 0.028 | N | 0.011 | N | 0.021 |
| V | 116 | N | 0.043 | N | 0.032 | N | 0.034 | N | 0.026 | N | 0.021 |
|   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|
|F | 117| N | 0.038| N | 0.051| N | 0.029| N | 0.023| N | 0.022|
|K | 118| N | 0.032| N | 0.038| N | 0.033| N | 0.029| N | 0.022|
|D | 119| N | 0.037| N | 0.032| N | 0.03 | N | 0.019| N | 0.016|
|W | 120| N | 0.033| N | 0.037| N | 0.03 | N | 0.024| N | 0.022|
|E | 121| N | 0.032| N | 0.018| N | 0.036| N | 0.024| N | 0.022|
|E | 122| N | 0.031| N | 0.023| N | 0.03 | N | 0.02 | N | 0.02 |
|L | 123| N | 0.035| N | 0.029| N | 0.031| N | 0.032| N | 0.02 |
|G | 124| N | 0.034| N | 0.043| N | 0.027| N | 0.024| N | 0.018|
|E | 125| N | 0.028| N | 0.031| N | 0.024| N | 0.021| N | 0.019|
|E | 126| N | 0.032| N | 0.022| N | 0.023| N | 0.022| N | 0.018|
|I | 127| N | 0.018| N | 0.021| N | 0.033| N | 0.018| N | 0.018|
|R | 128| N | 0.051| N | 0.099| N | 0.029| N | 0.019| N | 0.021|
|L | 129| N | 0.031| N | 0.025| N | 0.032| N | 0.02 | N | 0.019|
|K | 130| N | 0.025| N | 0.038| N | 0.03 | N | 0.021| N | 0.02 |
|V | 131| N | 0.019| N | 0.031| N | 0.031| N | 0.016| N | 0.018|
|F | 132| N | 0.031| N | 0.062| N | 0.035| N | 0.023| N | 0.021|
|V | 133| N | 0.024| N | 0.03 | N | 0.027| N | 0.016| N | 0.028|
|L | 134| N | 0.054| N | 0.033| N | 0.036| N | 0.012| N | 0.022|
|G | 135| B | 0.283| N | 0.041| N | 0.059| N | 0.024| N | 0.028|
|G | 136| B | 0.394| N | 0.03 | B | 0.151| N | 0.026| N | 0.033|
|C | 137| B | 0.179| N | 0.035| N | 0.046| N | 0.05 | N | 0.031|
|R | 138| N | 0.073| N | 0.026| N | 0.036| N | 0.031| N | 0.033|
|H | 139| N | 0.097| N | 0.048| N | 0.031| N | 0.023| N | 0.062|
|K | 140| N | 0.048| N | 0.052| N | 0.03 | N | 0.029| N | 0.029|
|L | 141| N | 0.033| N | 0.031| N | 0.026| N | 0.034| N | 0.02 |
|V | 142| N | 0.032| N | 0.034| N | 0.028| N | 0.037| N | 0.015|
|C | 143| N | 0.018| N | 0.028| N | 0.045| N | 0.029| N | 0.026|
|A | 144| N | 0.026| N | 0.013| N | 0.033| N | 0.024| N | 0.023|
|P | 145| N | 0.054| N | 0.072| N | 0.042| N | 0.043| N | 0.021|
|A | 146| N | 0.036| N | 0.042| N | 0.033| N | 0.033| N | 0.018|
| Variant       | Average Purity (% Fusion) |
|--------------|---------------------------|
| G136A        | 83.3                      |
| K130A        | 76.7                      |
| K130A/K140A  | 83.3                      |
| K140A        | 77.7                      |
| C137A        | 75.9                      |
| H139A        | 79.5                      |
| K130M        | 80.5                      |
| K130M/V131I  | 80.9                      |
| Δ27          | 85.2                      |

**Table S3.** Purity of MBP-HBx variants as estimated by using the mass spectrometry emPAI values.
Figure S1. Michaelis-Menten kinetics of purified GroEL. From this fit, $K_M$ and $k_{cat}$ values were determined to be $255 \pm 14 \, \mu\text{M}$ and $0.15 \pm 0.007 \, \text{s}^{-1}$ respectively. Error bars indicated standard error.
Figure S2. Western Blot and SDS-PAGE analysis of GST-HBx. Western blot was performed on a GST-HBx sample using His$_6$-antibody. Bands of approximate molecular weights of 60, 44 and 27 kDa efficiently bind the His-antibody (AbCam, ab49746) indicating that free GST in the sample is a result of proteolytic degradation of the GST-HBx fusion protein. This GST readily forms dimer species at a similar apparent molecular weight as GroEL (i.e. 60 kDa). Mass-spectrometric analysis indicate that the gel band at ~60 kDa is composed of this dimer as well as GroEL contaminant and perhaps minimal amounts of GST-HBx/HBx dimer.
Figure S3. Correlation between the amount of GroEL and activity. MBP-HBx and GST-HBx samples containing GroEL are represented in red and blue, respectively. DsbC-HBx and NusA-HBx samples lacking the chaperone are shown in green and black, respectively. Vertical error bars indicate standard error in activity ($k_{\text{cat}}/K_M$ values), while horizontal error bars indicate the variability of copurifying GroEL between individual samples (represented as standard error).