

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	CGTTCTGGGTGCTTGCCGTCATAAAC	AACACCTTCAGACGAATTTTC
K130A	CGTCTGGCAGTGTTTCGTT	AATTTCTCGCCCAGTTCC
K140A	GCCGTCATGCACTGGTGT	AGCCACCCAGAACGAACA
C137A	CAGCAGTTCAATGCATTTCTC	AGTTTGTGACGCGCGCCAC
H139A	GCTGCCGTGCAAAACTGG	CACCCAGAACGAACACCT
K130M	CGTCTGATGGTGTTTCGTTTC	AATTTCTCGCCCAGTTCCC
V131I	TCGTCTGATGATCTTCGTTCTGGGTG	ATTTCTCGCCCAGTTCC
Δ 27	TAACTCGAGCACCACCACCAC	AATTTCTCGCCCAGTTCTC

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	0.023
L	34	N	0.031	N	0.039	N	0.046	N	0.012	N	0.011
G	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

G	57	B	0.135	N	0.048	N	0.055	N	0.054	N	0.03
L	58	N	0.066	N	0.06	N	0.055	N	0.015	N	0.021
P	59	N	0.054	N	0.035	N	0.038	N	0.022	N	0.024
V	60	N	0.06	N	0.031	N	0.044	N	0.025	N	0.022
C	61	N	0.045	N	0.029	N	0.031	N	0.037	N	0.027
A	62	N	0.04	N	0.016	N	0.035	N	0.043	N	0.026
F	63	N	0.043	N	0.037	N	0.025	N	0.026	N	0.03
S	64	N	0.041	N	0.035	N	0.041	N	0.032	N	0.041
S	65	N	0.034	N	0.036	N	0.039	N	0.053	N	0.029
A	66	N	0.087	N	0.048	N	0.06	N	0.04	N	0.026
G	67	N	0.057	N	0.041	B	0.236	N	0.025	N	0.027
P	68	N	0.028	N	0.031	N	0.044	N	0.03	N	0.032
C	69	N	0.09	N	0.024	N	0.071	N	0.072	N	0.032
A	70	N	0.037	N	0.027	N	0.034	N	0.06	N	0.027
L	71	N	0.038	N	0.022	N	0.034	N	0.024	N	0.015
R	72	N	0.04	N	0.031	N	0.041	N	0.028	N	0.019
F	73	N	0.03	N	0.039	N	0.043	N	0.011	N	0.034
T	74	N	0.025	N	0.02	N	0.052	N	0.009	N	0.038
S	75	N	0.035	N	0.029	N	0.058	N	0.015	N	0.032
A	76	N	0.067	N	0.038	N	0.05	N	0.02	N	0.022
R	77	N	0.058	N	0.051	N	0.083	N	0.029	N	0.034
C	78	N	0.08	N	0.04	N	0.037	N	0.023	N	0.027
M	79	N	0.058	N	0.035	N	0.035	N	0.022	N	0.023
E	80	N	0.044	N	0.017	N	0.03	N	0.017	N	0.021
T	81	N	0.027	N	0.022	N	0.045	N	0.012	N	0.018
T	82	N	0.024	N	0.035	N	0.023	N	0.038	N	0.022
V	83	N	0.035	N	0.019	N	0.028	N	0.008	N	0.019
N	84	N	0.028	N	0.022	N	0.054	N	0.014	N	0.018
A	85	N	0.023	N	0.025	N	0.032	N	0.019	N	0.022
H	86	N	0.022	N	0.036	N	0.031	N	0.014	N	0.025

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	B	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

P	147	N	0.033	N	0.034	N	0.04	N	0.03	N	0.041
C	148	N	0.073	N	0.024	N	0.041	N	0.051	N	0.028
N	149	N	0.042	N	0.036	N	0.035	N	0.03	N	0.032
F	150	N	0.065	N	0.054	N	0.03	N	0.042	N	0.038
F	151	N	0.044	N	0.032	N	0.051	N	0.031	N	0.033
T	152	N	0.038	N	0.025	N	0.032	N	0.007	N	0.024
S	153	N	0.038	N	0.029	N	0.031	N	0.022	N	0.021
A	154	N	0.03	N	0.035	N	0.033	N	0.027	N	0.016

Table S3. Purity of MBP-HBx variants as estimated by using the mass spectrometry emPAI values.

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
$\Delta 27$	85.2

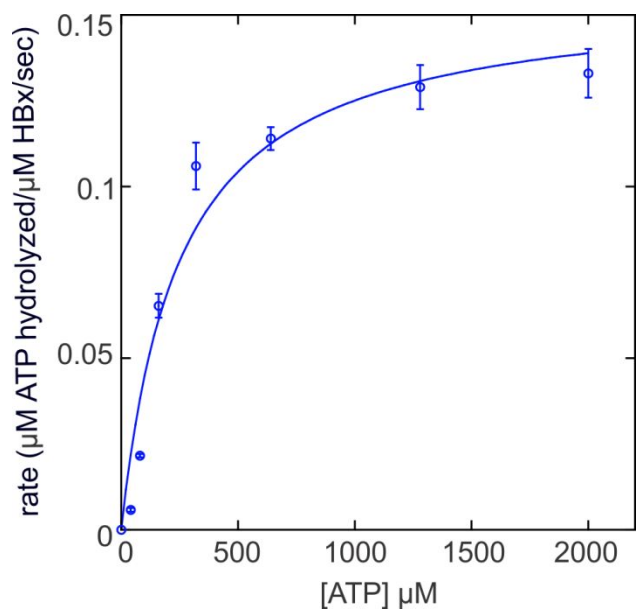


Figure S1. Michaelis-Menten kinetics of purified GroEL. From this fit, K_M and k_{cat} values were determined to be $255 \pm 14 \mu\text{M}$ and $0.15 \pm 0.007 \text{ s}^{-1}$ respectively. Error bars indicated standard error.

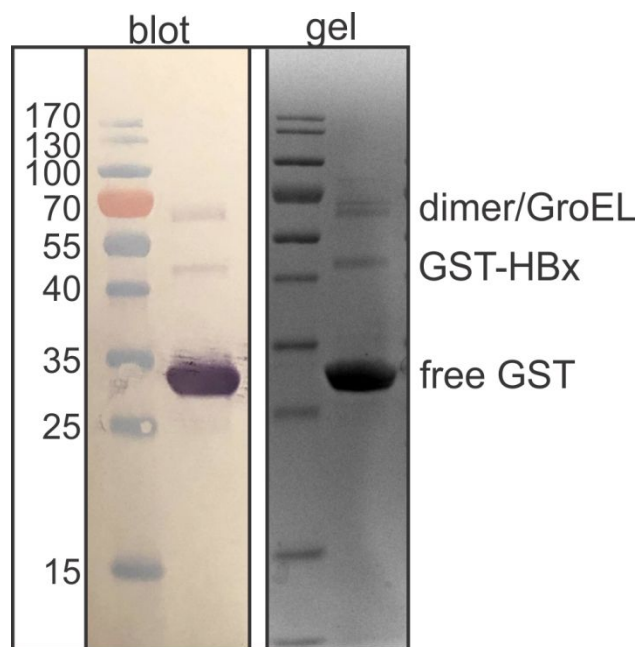


Figure S2. Western Blot and SDS-PAGE analysis of GST-HBx. Western blot was performed on a GST-HBx sample using His₆-antibody. Bands of approximate molecular weights of 60, 44 and 27 kDa efficiently bind the His-antibody (AbCam, ab49746) indicating that free GST in the sample is a result of proteolytic 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.

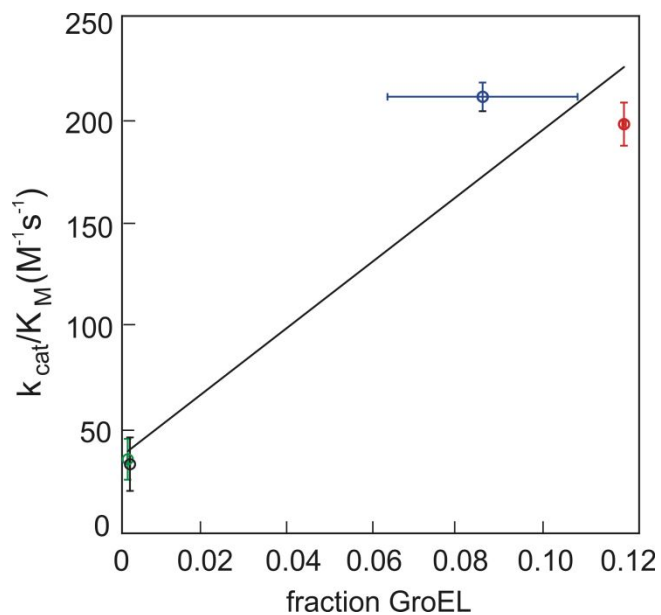


Figure S3. Correlation between the amount of GroEL and activity. MBP-HBx and GST-HBx samples containing GroEL are represented in red and blue, respectively. DsbC-HBx and NusA-HBx samples lacking the chaperone are shown in green and black, respectively. Vertical error bars indicate standard error in activity (k_{cat}/K_M values), while horizontal error bars indicate the variability of copurifying GroEL between individual samples (represented as standard error).