Structure of the Dom34–Hbs1 complex and implications for its role in No-Go decay

Liming Chen, Denise Muhlrad, Vasili Hauryliuk, Zhihong Cheng , Meng Kiat Lim, Viktoriya Shyp, Roy Parker and Haiwei Song

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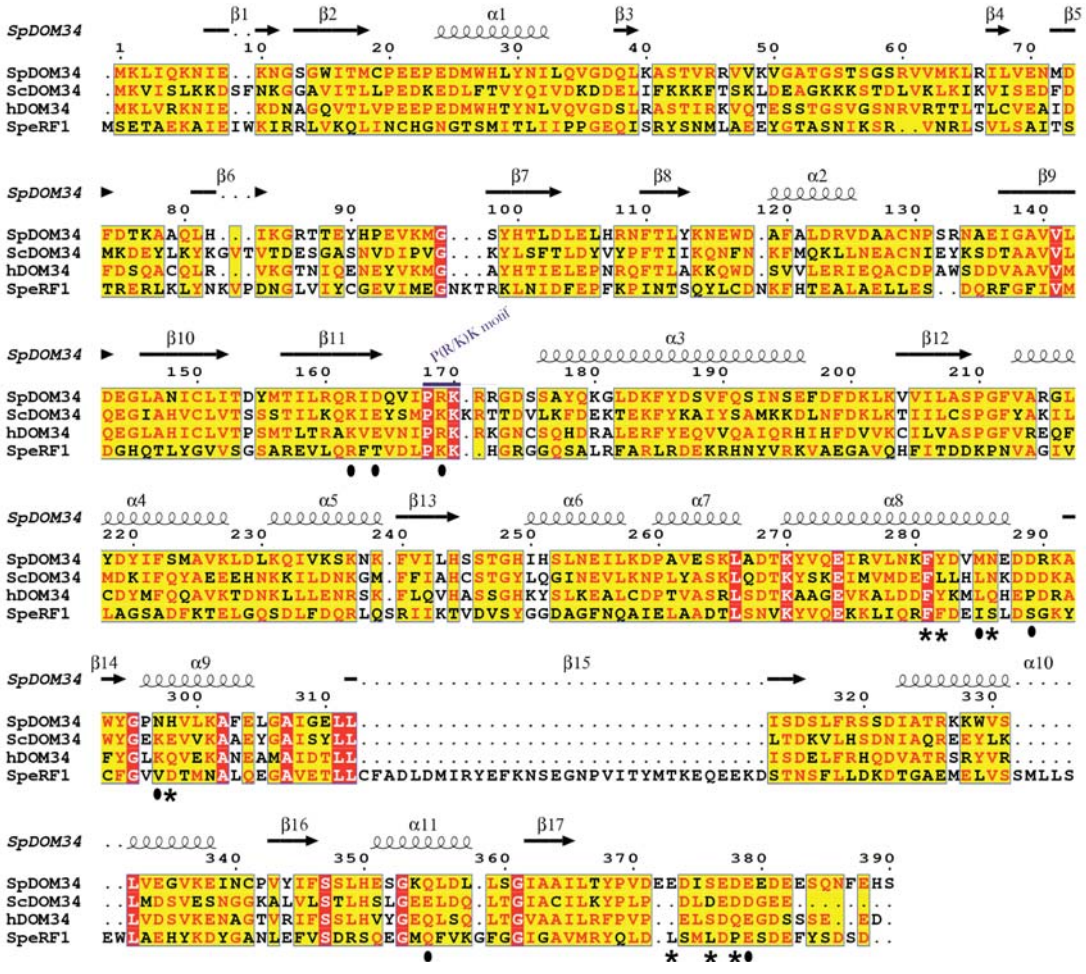
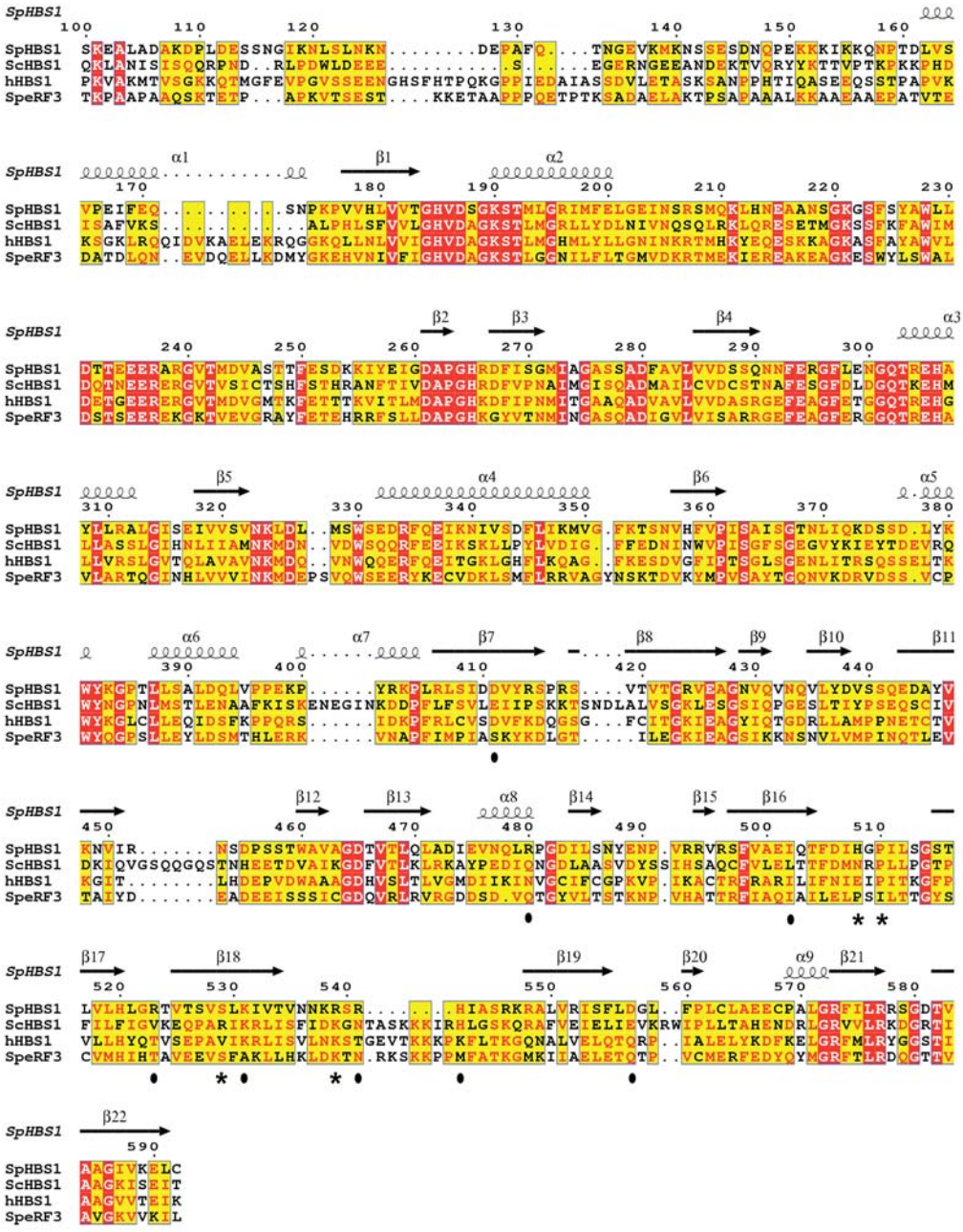
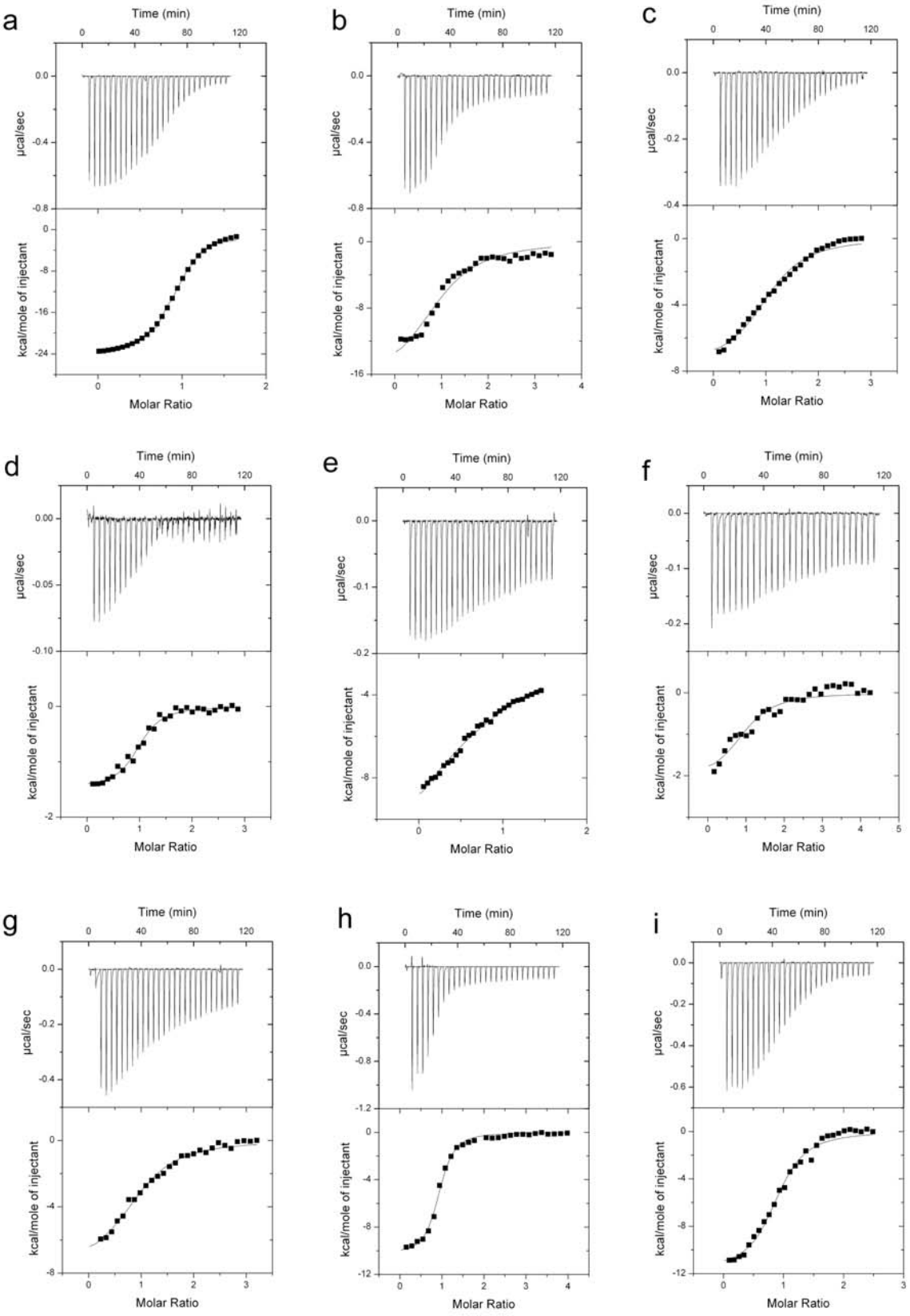


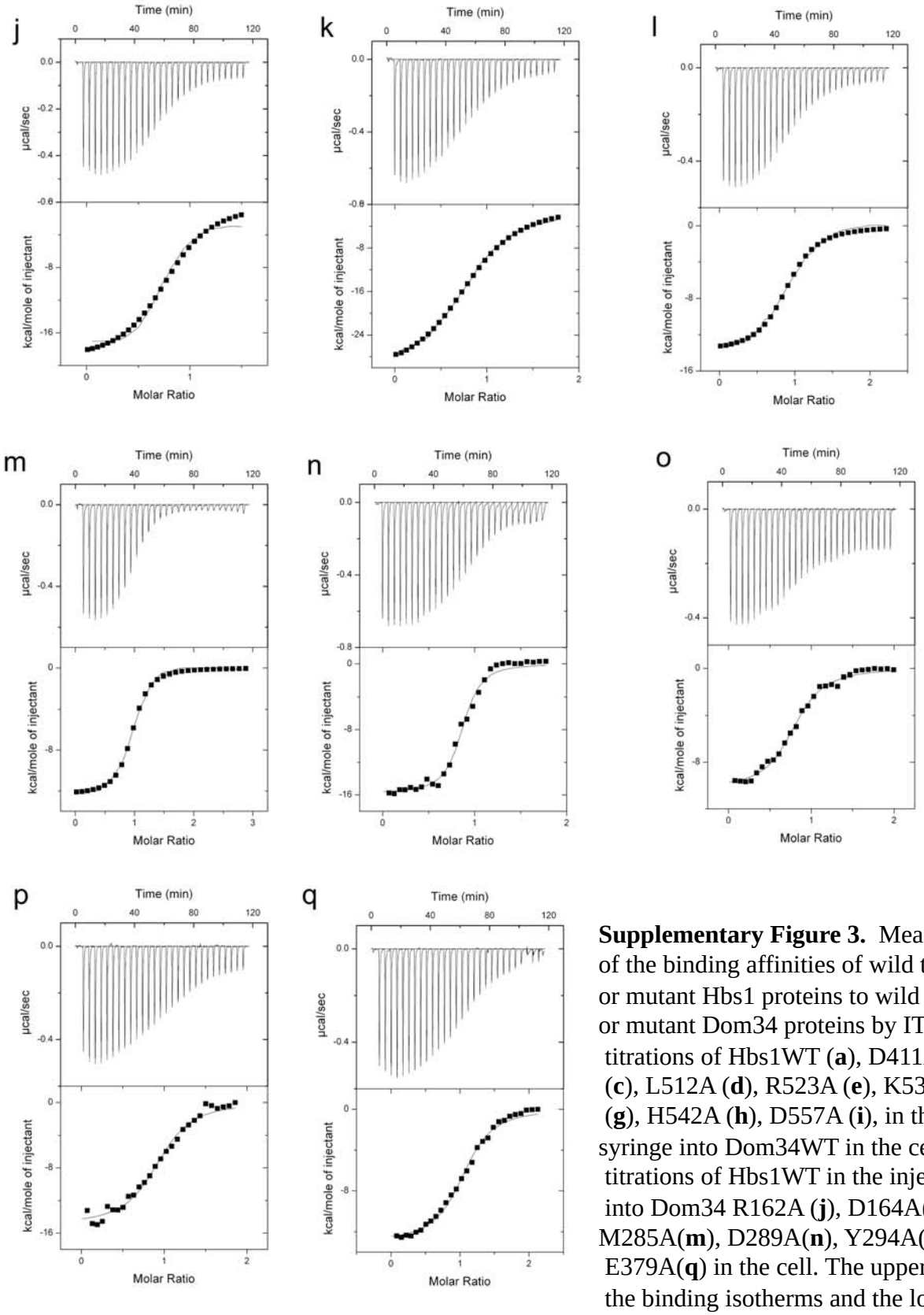
Supplementary Figure 1. Sequence alignment of the Dom34 proteins. Secondary structures of Dom34 are indicated at the top of the alignment. Residues involved in the Dom34/Hbs1 interface are marked with "*" with those subjected to mutational analysis marked with "•".



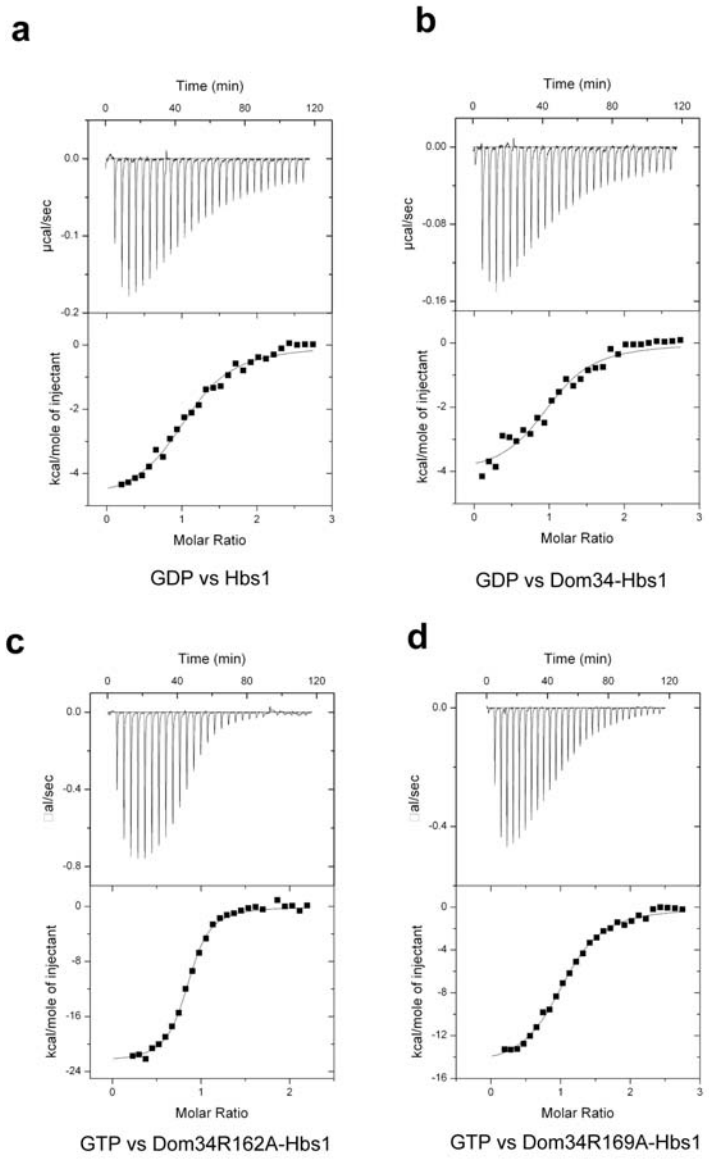
Supplementary Figure 2. Sequence alignment of the Hbs1 proteins. Secondary structures of Hbs1 are indicated at the top of the alignment. Residues involved in the Dom34/Hbs1 interface are marked with "*" with those subjected to mutational analysis marked with "•".



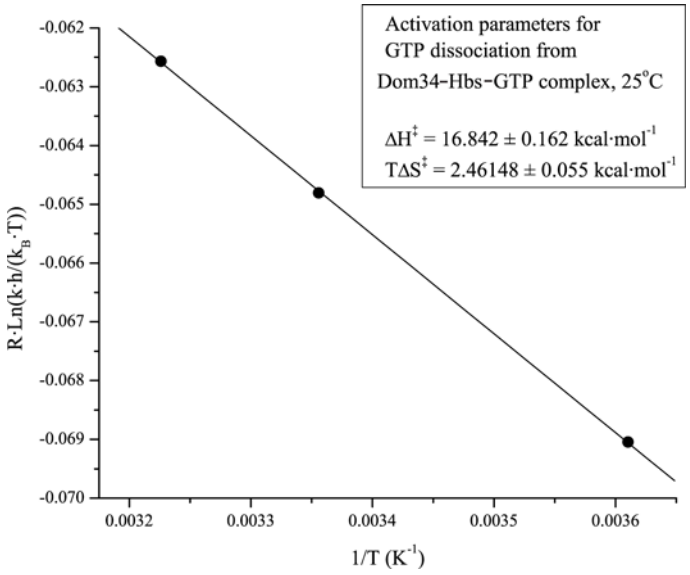
Supplementary Figure 3 continued in next page



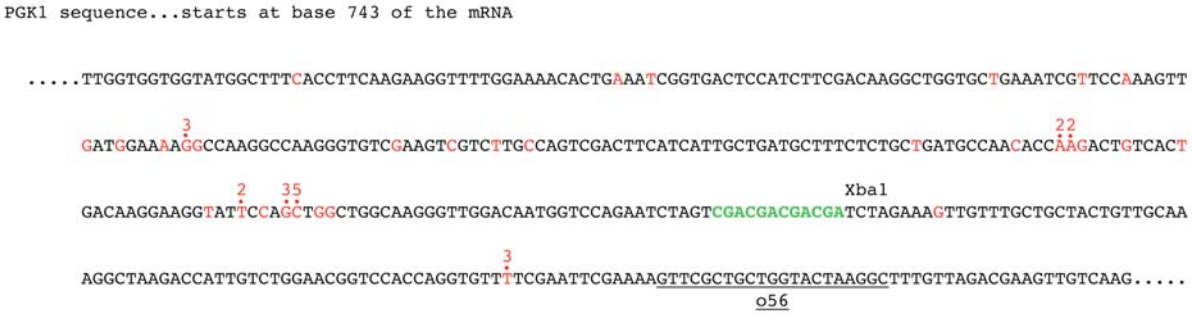
Supplementary Figure 3. Measurements of the binding affinities of wild type (WT) or mutant Hbs1 proteins to wild type (WT) or mutant Dom34 proteins by ITC. ITC titrations of Hbs1WT (**a**), D411A (**b**), R480A (**c**), L512A (**d**), R523A (**e**), K531A (**f**), R541A (**g**), H542A (**h**), D557A (**i**), in the injection syringe into Dom34WT in the cell. ITC titrations of Hbs1WT in the injection syringe into Dom34 R162A (**j**), D164A(**k**), R169A(**l**), M285A(**m**), D289A(**n**), Y294A(**o**), Q355A(**p**), E379A(**q**) in the cell. The upper panels show the binding isotherms and the lower panels show the integrated heat for each injection fitted to a single-site model.



Supplementary Figure 4. The effects of WT and mutant Dom34 proteins on the GDP/GTP binding to Hbs1. ITC titrations of GDP to HBS1 (**a**), GDP to Dom34-Hbs1 (**b**), GTP to Dom34R162A–Hbs1 (**c**), GTP to Dom34R169A–Hbs1 (**d**). The upper panels show the binding isotherms and the lower panels show the integrated heat for each injection fitted to a single-site model.



Supplementary Figure 5. Arrhenius analysis of the kinetics of GTP from Dom34–Hbs–GTP complex at different temperatures. Data are *linearized* using the following equation from the transition state theory: $R \cdot \ln(h \cdot k / k_B \cdot T) = \Delta S^{\ddagger} - \Delta H^{\ddagger} / T$, where R is the gas constant, T in the absolute temperature, k_B is Boltzmann's constant, h is Planck's constant, k is rate constant, $\Delta S^{\ddagger}$ is activation entropy and $\Delta H^{\ddagger}$ is activation enthalpy. Activation enthalpy is determined from the slope, and the activation entropy is determined from the ordinate intercepts of the linear fit.



Supplementary Figure 6. This figure shows the results of 5'-RACE experiments performed on total mRNA in an xrn1Δ strain. 5'-RACE was done by ligating an RNA oligo oRP1522(GCUGAUGGCGAUGAAUGAACACUGCGUUUGCUGGCCUUUGAUGAAA) to the mRNA 5' end and reverse transcribing from one of four internal PGK1 primers oRP56(GCCTTAGTACCAGCAGCGAAC), oRP70(CGGATAAGAAAGCAACACCTGG), oRP1113(CCAAAGAAGCACCACCACCAGT), or oRP154(GCCTTAGTACCAGCAGCGAAC). Subsequent PCR was done using oRP1523(CTGATGGCGATGAATGAACACT) homologous to the RNA oligo and one of the three internal primers from PGK1 depending on the position of the RT reaction oligo. PCR products were TA cloned and sequenced. The DNA sequence of PGK1CGApG is given starting from base 743 of the PGK1 mRNA. The 5'-RACE ends cloned are shown in red with the number of hits (if >1) given above for each base. The inserted CGAs are highlighted in green and the position of the 5'-most oligo used for PCR and cloning is underlined. Forty two mapped clones are shown.

Supplementary Table 1. Summary of ITC data

Cell ligand	Injectant	K_d, μM	ΔH, kcal/mol	ΔS,	N
Dom34 WT	Hbs1 WT	0.39 ± 0.02	-24.42 ± 0.18	-52.6	0.94 ± 0.005
	Hbs1 D411A	3.56 ± 0.90	-17.32 ± 1.77	-33.0	1.02 ± 0.076
	Hbs1 R480A	1.73 ± 0.26	-7.63 ± 0.29	0.8	1.10± 0.030
	Hbs1 L512A	0.58 ±0.11	-1.47 ± 0.04	23.7	0.99 ± 0.023
	Hbs1 R523A	27.29 ± 9.75	-28.7 ± 12.30	75.1	0.97 ± 0.026
	Hbs1 K531A	2.79 ± 1.21	-2.14 ± 0.32	18.6	1.01 ± 0.110
	Hbs1 R541A	2.27 ± 0.34	-7.74 ± 0.42	-0.1	0.99 ± 0.038
	Hbs1 H542A	0.39 ± 0.05	-10.34 ± 0.17	-5.3	0.90 ± 0.011
	Hbs1 D557A	1.02 ± 0.16	-11.77 ± 0.34	-12.0	0.91 ± 0.019
Dom34 R162A	Hbs1 WT	0.78 ± 0.11	-19.50 ± 0.48	-37.3	0.82 ± 0.015
Dom34 D164A		1.15 ± 0.05	-31.30 ± 0.31	-77.7	0.86 ± 0.006
Dom34 R169A		0.46 ± 0.04	-13.90 ± 0.19	-17.7	0.91 ± 0.009
Dom34 M285A		0.21 ± 0.02	-12.40 ± 0.11	-10.9	0.94 ± 0.006
Dom34 D289A		0.12 ± 0.02	-15.80 ± 0.25	-21.3	0.85 ± 0.009
Dom34 Y294A		0.43 ± 0.05	-10.20 ± 0.18	-5.2	0.80± 0.010
Dom34 Q355A		0.48 ± 0.08	-17.90 ± 0.36	-21.1	0.95 ± 0.017
Dom34 E379A		0.56 ± 0.06	-12.90 ± 0.18	-14.9	1.06 ± 0.011
Hbs1	GDP	0.90 ± 0.13	-4.91 ± 0.17	-52.6	1.09 ± 0.027
Dom34–Hbs1	GDP	0.89 ± 0.25	-4.12 ± 0.24	-14.0	1.04 ± 0.045
Hbs1	GTP	1.41 ± 0.20	-1.00 ± 0.03	23.5	1. 05± 0.022
Dom34–Hbs1	GTP	0.11 ± 0.01	-8.80 ± 0.05	2.3	1.08 ± 0.005
Dom34	Hbs1 GTP	0.04 ± 0.01	-29.01 ± 0.19	-63.6	1.06 ± 0.004
Dom34R162A–Hbs1	GTP	0.18 ± 0.02	-22.60 ± 0.29	-45.0	0.84 ± 0.007
Dom34R169A–Hbs1	GTP	0.69 ± 0.06	-15.04 ± 0.29	-22.2	1.06 ± 0.014

Supplementary Table 2. Kinetics of GTP interactions with the Dom34–Hbs1 complex

Sample	Temperature, (°C)	$1/k_{-1}$, sec	k_{-1}, sec^{-1}	K_d[a], μM	k_{+1}[b], $\mu M^{-1} \cdot sec^{-1}$
Dom34–Hbs1	4	218.26 ± 84.50	0.0046 ± 0.0018		
Dom34–Hbs1	25	24.07 ± 4.39	0.042 ± 0.0075	0.11	0.38 ± 0.07
Dom34–Hbs1	37	7.44 ± 0.96	0.134 ± 0.017		
eRF1–eRF3[c]	25	7.14 ± 1.26	0.14 ± 0.03	0.3 ± 0.1	0.5 ± 0.03
eRF1–eRF3[d]	37	19.8 ± 0.40	0.05 ± 0.01	0.7 ± 0.2	0.07 ± 0.02

[a] From ITC experiments

[b] k_{+1} is calculated from using the K_d and k_{-1} values and expression $k_{+1} = k_{-1}/K_d$

[c] from ref. 1

[d] from ref. 2

Supplementary references

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2. Hauryliuk V, Zavialov A, Kisselev L & Ehrenberg M. Class-1 release factor eRF1 promotes GTP binding by class-2 release factor eRF3. *Biochimie.* **88**, 747-757 (2006).