

# Supplementary Figure 1

			1	→	
hBiP	1	-----MKLSLVAAMLLLLSAARAEEDKK	EDVGT	VVGIDL	35
dBiP	1	-----MKLCILLAVVAFVGLSLGEEKKEKDKELGTVIGIDL			36
gBiP	1	-----MARSWRASGSLVALAIVLSCFFAISIAKEEAAKLGTVIGIDL			43
Kar2	1	---MFFNRLSAGKLLVPLSVVLYALFVVILPLQNSFHSSNVLVRGADDVENYGTVIGIDL			57
hHsp70	1	-----MAKAAAIGIDL			11
dHsp70	1	-----MPAIGIDL			8
gHsp70	1	-----MAGKSEGSAIGIDL			14
bHsc70	1	-----MSKGPVAVGIDL			11
Ssa1	1	-----MSKGPVAVGIDL			11
hmtHsp70	1	MISASRAAAARLVGAAASRGPTAARHQDSWNGLSHEAFRLVSRDYASEAIKGVAVGIDL			60
Ssc1	1	-----MLAAKNIILNRSSLSSSFRIATRLQSTKVQGSVIGIDL			37
eDnaK	1	-----MGKIIIGIDL			9
dsDnaK	1	-----MGKIIIGIDL			9
abDnaK	1	-----MAKIIIGIDL			9
laDnaK	1	-----MSKIIIGIDL			9
vhDnaK	1	-----MSKIIIGIDL			9

		2	3	4	5	A	C		
		→	→	→	→	▭	▭		
hBiP	36	GTTTSCVGVFKNGRVEIIANDQGNRITPSYVAFTPEGERLIGDAAKNQLTNPENTVFDA							95
dBiP	37	GTTTSCVGVYKNGRVEIIANDQGNRITPSYVAFTADGERLIGDAAKNQLTNPENTVFDA							96
gBiP	44	GTTTSCVGVYKNGHVEIIANDQGNRITPSVVAFT-DSERLIGEAAKNQAAVNAERTIFDV							102
Kar2	58	GTTTSCVAVMKNKTEILANEQGNRITPSYVAFT-DDERLIGDAAKNQVAANPQNTIFDI							116
hHsp70	12	GTTTSCVGVFQHGKVEIIANDQGNRTTPSYVAFT-DTERLIGDAAKNQVALNPQNTVFDA							70
dHsp70	9	GTTTSCVGVYQHGKVEIIANDQGNRTTPSYVAFT-DSERLIGDPAKNQVAMNPRNTVFDA							67
gHsp70	15	GTTTSCVGVWQHDRVEIIANDQGNRTTPSYVAFT-DSERLIGDAAKNQVAMNPTNTVFDA							73
bHsc70	12	GTTTSCVGVFQHGKVEIIANDQGNRTTPSYVAFT-DTERLIGDAAKNQVAMNPTNTVFDA							70
Ssa1	12	GTTTSCVGVFQHGKVEIIANDQGNRTTPSYVAFT-DTERLIGDAAKNQVAMNPTNTVFDA							70
hmtHsp70	61	GTTNSCVAVMEGKRAKVLNAEGARTTPSVVAFTADGERLVGMPAKRQAVTNPNTFYAT							120
Ssc1	38	GTTNSAVAIMEGKVPKIIENAEGSRTTPSVVAFTKEGERLVGIPAKRQAVVNPENTLFAT							97
eDnaK	10	GTTNSCVAIMDGTTPRVLNAEGDRTPSIIAYTQDGETLVGQPAKRQAVTNPQNTLFAI							69
dsDnaK	10	GTTNSCVSVMEGNEPVVIPNSEGKRTTPSIVAFVNGERKVGDPAKRQAITNPKTIFSI							69
abDnaK	10	GTTNSCVAVLEGDKVKVIEAEGARTTPSIIAY-KDGEILVQSAKRQAVTNPKNLFAI							68
laDnaK	10	GTTNSAVAVLEGGEAKIIPNPEGARTTPSVVGF-KNGERQVGEVAKRAAITNP-NTVISI							68
vhDnaK	10	GTTNSCVSVMEGGEAVVIPNPEGNRTTPSVVAF-KNGERQVGEVAKRQAITNP-NTIQSV							68

		D	7	8	9	E		
		▭	▭	→	→	→	▭	
hBiP	96	KRLIGRTWNDPSVQODIKFLPFKVVVEKKT-KPYIQVDIGGGQTKTFAPEEISAMVLTRMK						154
dBiP	97	KRLIGREWSDTNVQHDIKFFPFKVVVEKNS-KPHISVDTSQG-AKVFAPPEEISAMVLGKMK						154
gBiP	103	KRLIGRKFEDKEVQRDMKLVPIKIVNKDG-KPYIQVKIKDGETKVFSPPEEISAMVLTRMK						161
Kar2	117	KRLIGLKYNDRSVQDKIKHLPFNVNKDG-KPAVEVSVK-GEKKVFTPEEISGMILGKMK						174
hHsp70	71	KRLIGRKFDPVQSDMKHWPQVINDGD-KPKVQVSYK-GETKAFYPEEISSMVLTRMK						128
dHsp70	68	KRLIGRKYDDPKIAEDMKHWPQVVSDDG-KPKIGVEYK-GESKRFAPPEEISSMVLTRMK						125
gHsp70	74	KRLIGRRFSDASVQSDMKLWPFVTPGAGDKPMITVAYK-GEDKMFAEEEISSMVLTRMK						132
bHsc70	71	KRLIGRRFDDAVVQSDMKHWPFMVNDAG-RPKVQVEYK-GETKSFYPEEVSSMVLTRMK						128
Ssa1	71	KRLIGRRFDDAVVQSDMKHWPFMVNDAG-RPKVQVEYK-GETKSFYPEEVSSMVLTRMK						128
hmtHsp70	121	KRLIGRRYDPEVQDKIKNVPFKIVRASN-GDAWVEAHG----KLYSPQIGAFVLMKMK						175
Ssc1	98	KRLIGRRFEDAQVQDKIKVPIKIVKHSN-GDAWVEARG----QTYSPAQIGGFVLMKMK						152
eDnaK	70	KRLIGRRFQDEEVQRDVSIMPFKIIAADN-GDAWVEVKG----QKMAPPQISAEVLKMK						124
dsDnaK	70	KRFMGETFD--QVSKEVNRVPIKVVVRGDN-NTPRVEIDD----RKYSPEEISAMTLQKMK						124
abDnaK	69	KRLIGRRYEDQAVQDKIGLVPYKIIKADN-GDAWVEVND----KKLAPQISAEILKMK						123
laDnaK	69	KRHMGTDYK-----ETIEG----KDYSPEEISAILQYLK						99
vhDnaK	69	KRHMGTDYK-----VKIED----KEFTPEQVSAIILQHIK						99

# Supplementary Figure 1

hBiP	155	<b>ETAEAYLGKKVTHAVVTVPAYFNDAQRQATKDAGTIAGLNVMRIINEPTAAAIAYGLDKR</b>	214
dBiP	155	ETAEAYLGKKVTHAVVTVPAYFNDAQRQATKDAGVIAGLQVMRIINEPTAAAIAYGLDKK	214
gBiP	162	ETAEAYLGKKIKDAVTVPAYFNDAQRQATKDAGIIAGLNVARIINEPTAAAIAYGLDKK	221
Kar2	175	QIAEDYLGTKVTHAVVTVPAYFNDAQRQATKDAGTIAGLNVLRIVNEPTAAAIAYGLDKS	234
hHsp70	129	EIAEAYLGYPVTVNAVITVPAYFNDSQRQATKDAGVIAGLNVLRINEPTAAAIAYGLDRT	188
dHsp70	126	ETAEAYLGESITDAVITVPAYFNDSQRQATKDAGHIAGLNVLRINEPTAAALAYGLDKN	185
gHsp70	133	ETAEAYLGSSVKNGVVTVPAYFNDSQRQATKDAGVIAGLNVMRIINEPTAAAIAYGLDKK	192
bHsc70	129	EIAEAYLGKTVTNAVTVPAYFNDSQRQATKDAGTIAGLNVLRINEPTAATIAYGLDKK	188
Ssa1	129	EIAEAYLGKTVTNAVTVPAYFNDSQRQATKDAGTIAGLNVLRINEPTAATIAYGLDKK	188
hmtHsp70	176	ETAENYLGRYAKNAVITVPAYFNDSQRQATKDAGQISGLNVLRVINEPTAAALAYGLDKS	235
Ssc1	153	ETAEAYLGKPVKNVAVTVPAYFNDSQRQATKDAGQIVGLNVLRVINEPTAAALAYGLEKS	212
eDnaK	125	KTAEDYLGEVTEAVITVPAYFNDAQRQATKDAGRIGLEVKRIINEPTAAALAYGLDKG	184
dsDnaK	125	KTAEDYLGQEVTEAVTVPAYFSDSQRQATKEAGEIAGLKVRRIINEPTAASLAYGLDKM	184
abDnaK	124	KTAEDYLGETVTEAVITVPAYFNDAQRQATKDAGKIAGLDVKRIINEPTAALAFGMDKK	183
laDnaK	100	GYAEDYLGETVDKAVITVPAYFNDAQRQATKDAGKIAGLEVERINEPTAAALAYGMDKT	159
vhDnaK	100	SYAEDYIGETVEKAVITVPAYFNDAERQATKDAGKIAGLEVERINEPTAAALAYGIDKD	159

hBiP	215	<b>E---GEKNILVFDLGGGTFDVSLLTI---DNG-VFEVVATNGDTHLGGEDFDQRMVMEHFI</b>	267
dBiP	215	E---GEKNVLVFDLGGGTFDVSLLTI---DNG-VFEVVATNGDTHLGGEDFDQRMVDHFI	267
gBiP	222	G---GEKNILVFDLGGGTFDVSILTI---DNG-VFEVLSTNGDTHLGGEDFDQRI MEYFI	274
Kar2	235	D---KEHQIIVYDLGGGTFDVSLLSI---ENG-VFEVQATSGDTHLGGEDFDYKIVRQLI	287
hHsp70	189	GK--GERNVLIFDLGGGTFDVSILTI---DDG-IFEVKATAGDTHLGGEDFDNRLVNHFV	242
dHsp70	186	LK--GERNVLIFDLGGGTFDVSILTI---DEGSLFEVVRSTAGDTHLGGEDFDNRLVTHLA	240
gHsp70	193	ASSVGEKNVLIFDLGGGTFDVSLLTI---EEG-IFEVKATAGDTHLGGEDFDNRMVNHFV	248
bHsc70	189	VG--AERNVLIFDLGGGTFDVSILTI---EDG-IFEVKSTAGDTHLGGEDFDNRMVNHFI	242
Ssa1	189	VG--AERNVLIFDLGGGTFDVSILTI---EDG-IFEVKSTAGDTHLGGEDFDNRMVNHFI	242
hmtHsp70	236	E---DKVIAVYDLGGGTFDISILEI---QKG-VFEVKSTNGDTHLGGEDFDQALLRHIV	287
Ssc1	213	D---SKVAVFDLGGGTFDISILDI---DNG-VFEVKSTNGDTHLGGEDFDIYLLREIV	264
eDnaK	185	T---GNRTIAVYDLGGGTFDISIIEIDEVDGEKTFEVLATNGDTHLGGEDFDSRLINYLIV	241
dsDnaK	185	D---RDMKIAVFDLGGGTFDISILEL---GDG-VFEVKSTGDTHLGGDDFDQVIIDWLA	237
abDnaK	184	E---GDRKVAVYDLGGGTFDVSIIIEIADLDGDDQIEVLSSTNGDTHLGGEDFDNALIEYLV	240
laDnaK	160	D---KDQTIIVFDLGGGTFDVSILEL---GDG-VFEVHSTAGDNHLGGDDFDQKIIDYLV	212
vhDnaK	160	D---QDQTIIVYDLGGGTFDVSILDI---GDG-TFEVVSTAGDNRLGGDDFDEVIINHMV	212

hBiP	268	<b>KLYKKKTGKDVKDNRAVQKLRREVEKAKRALSSQHQAARIEIESFYE---GEDFSETLT</b>	323
dBiP	268	KLYKKKKGKDIRKDNRAVQKLRREVEKAKRALSGSHQVRVRIEIESFFE---GDDFSETLT	323
gBiP	275	KLIKKKHGKDISKDNRALGKLRREAERAKRALSSQHQRVEIESLFD---GVDFSEPLT	330
Kar2	288	KAFKKKHGIDVSDNNKALAKLKREAERAKRALSSQMSSTRIEIDSFVD---GIDLSETLT	343
hHsp70	243	EEFKRKHKKDISQNKRAVRLRTACERAKRTLSSSTQASLEIDSLFE---GIDFYTSIT	298
dHsp70	241	DEFKRYKLDLRNPRALRRLRTAERAKRTLSSSTEATIEIDALFE---GQDFYTKVS	296
gHsp70	249	QEFKRKHKKDISGNPRALRRLRTSCERAKRTLSSSTAQTIEIDSLYE---GIDFYTSIT	304
bHsc70	243	AEFKRKHKKDISENKRAVRLRTACERAKRTLSSSTQASIEIDSLYE---GIDFYTSIT	298
Ssa1	243	AEFKRKHKKDISENKRAVRLRTACERAKRTLSSSTQASIEIDSLYE---GIDFYTSIT	298
hmtHsp70	288	KEFKRETGVDLTKDNMALQVRVREAAEKAKCELSSSVQTDINLPYLTMDSSGPKHLNMKLT	347
Ssc1	265	SRFKTETGIDLENDRAIQVIREAAEKAKIELSSSTVSTEINLPFITADASGPKHINMKFS	324
eDnaK	242	EEFKDQGDILRNDPLAMQRLKEAAEKAKIELSSAQQTDVNLPIYTADATGPKHMNIKVT	301
dsDnaK	238	DEFNSEEGIDLRKDPMALQRLKEAAEKAKIELSSSSQTEINLPYIMPVNGIPKHLVKTLS	297
abDnaK	241	EEFKKEQNVNLKNDPLALQRLKEAAEKAKIELSSSNATEINLPYITADATGPKHLVINVT	300
laDnaK	213	SEFKKENGIDLSQDKMALQRLKDAAEKAKKDLSGVMSTQISLPFITAGEAGPLHLEVNLT	272
vhDnaK	213	QEFKKENGIDLSKDKMATQRLKDAAEKAKKDLSGVSTQISLPFITAGEAGPLHLEMNLT	272

# Supplementary Figure 1

Protein	Residue Range	Sequence	Residue Range
hBiP	324	RAKFEELNMDLFRSTMKPVQKVLKSDIDEIVLVGGSTRIPKIQQLVKEFFNGKE	383
dBiP	324	RAKFEELNLDLFRSTLKPVQKVLKEDADMNKKDVHEIVLVGGSTRIPKVQQLVKDFFGGKE	383
gBiP	331	RARFEELNNDLFRKTMGPVKKAMEDAGLQKSQIDEIVLVGGSTRIPKVQQLLKDYFDGKE	390
Kar2	344	RAKFEELNLDLFRKTLKPVKVLQDSGLEKKDVDDIVLVGGSTRIPKVQQLLESYFDGK	403
hHsp70	299	RARFEELCSDLFRSTLEPVEKALRDAKLKDAQIHDIVLVGGSTRIPKVQQLQDFFNGRD	358
dHsp70	297	RARFEELCADLFRNTLQPVKALNDAKMDKGQIHDIVLVGGSTRIPKVQSLQDFFHGKN	356
gHsp70	305	RARFEELNMDLFRKCMPEVEKCLRDAKMDKSTVHDVVLVGGSTRIPKVQQLQDFFNGKE	364
bHsc70	299	RARFEELNADLFRGTLDPVEKALRDAKLKDSQIHDIVLVGGSTRIPKIQQLQDFFNGKE	358
Ssa1	299	RARFEELNADLFRGTLDPVEKALRDAKLKDSQIHDIVLVGGSTRIPKIQQLQDFFNGKE	358
hmtHsp70	348	RAQFEGIVTDLIRRTIAPCQKAMQDAEVSKSDIGEVILVGGMTRMPKVQQTVDLFGRA	406
Ssc1	225	RAQFETLTAPLVKRTVDPVKALKDAGLSTSDISEVLLVGGMSRMPKVVEVTKSLF-GKD	383
eDnaK	302	RAKLESLVEDLVNRSIEPLKVALQDAGLSVSDIDVILVGGQTRMPMVQKKVAEFF-GKE	360
dsDnaK	298	RAKFEQLADSLINATIEPCRKALKNAGMTASDIDEVILVGGSTRIPAIQDKVKEFF-GKE	356
abDnaK	301	RAKLEGLVGDIVARTIEPCKIALKDALGSLTSDISDVILVGGQSRMPLVQKQVQEFF-GRE	359
laDnaK	273	RAKFDELTSDLVERTVGPTRQALKDAGLSASDIDQVILVGGSTRIPAVQEAITKEL-GKE	331
vhDnaK	273	RAKFEELSDDLVERTMVPTRKALSASLSANDIHKVILVGGSTRIPAVQEAIKREI-GQE	331

Protein	Residue Range	Sequence	Residue Range
hBiP	384	PSRGINPDEAVAYGAAVQAGVLSGD--QDTGDLVLLHVCPLTLGIEITVGGVMTKLI PSNT	441
dBiP	384	PSRGINPDEAVAYGAAVQAGVLSGE--QDTDAIVLLDNPLTMGIEITVGGVMTKLI PRNT	441
gBiP	391	PNKGVNPDEAVAYGAAVQGGILSGEGGDETKDILLLDVAPLTLGIEITVGGVMTKLI PRNT	450
Kar2	404	ASKGINPDEAVAYGAAVQAGVLSGE--EGVEDIVLLDVNALTGLGIEITGGVMTPLIKRNT	461
hHsp70	358	LNKSINPDEAVAYGAAVQAAIILMGDKSENVQDLLLLDVAPLSLGLLETAGGVMTALIKRNS	418
dHsp70	357	LNLSINPDEAVAYGAAVQAAIILSGDQSGKIQDVLLVDVAPLSLGLLETAGGVMTKLIERN	416
gHsp70	365	LCKSINPDEAVAYGAAVQAAIILSGEGNEKVDLDDVTPLSLGLLETAGGVMTVLI PRNT	424
bHsc70	359	LNKSINPDEAVAYGAAVQAAIILSGDKSENVQDLLLLDVTPLSLGLLETAGGVMTVLIKRN	418
Ssa1	359	LNKSINPDEAVAYGAAVQAAIILSGDKSENVQDLLLLDVTPLSLGLLETAGGVMTVLIKRN	418
hmtHsp70	407	PSKAVNPDEAVAIGAAIQGGVLAG---DVTDLVLLDVTPLSLGLLETGGVFTKLIERN	462
Ssc1	384	PSKAVNPDEAVAIGAAVQAGVLSG---EVTDLVLLDVTPLSLGLLETGGVFTRLI PRNT	439
eDnaK	361	PRKDVNPDEAVAIGAAVQGGVLTG---DVKDVLLDVTPLSLGLLETGGVMTTLIAKNT	416
dsDnaK	357	ASKGVNPDEVVAIGAAIQGGVLTG---EVKDVLLDVTPLSLGLLETGGVMTKLIERN	412
abDnaK	360	PRKDVNPDEAVAIGAAIQGAVLSG---DKNDVLLDVTPPLTLGIEITMGGVLTPIIEKNT	415
laDnaK	332	PHRGVNPDEVVAMGAAIQGGVITG---DVKDVLLDVTPLSLGLLETGGVMTSLIERNT	387
vhDnaK	332	PSKGVNPDEVVALGAAIQGGVLTG---DVKDVLLDVTPLSLGLLETGGVFTKLIERN	387

Protein	Residue Range	Sequence	Residue Range
hBiP	442	VVPTKKSQIFSTASDNQPTVTIKVYEGERPLTKDNHLLGTFDLTGIPAPRGVPOIEVTF	501
dBiP	442	VIPTKKSQVFSTASDNQHTVTIQVYEGERPMTKDNHLLGKFDLTGIPAPRGIPQIEVFS	501
gBiP	451	VIPTKKSQVFSTYQDQQTTVSIQVFEGERSLTKDCRLLGKFDLTGIPAPRGTPQIEVTF	510
Kar2	462	AIPTKKSQIFSTAVDNQPTVMIKVYEGERAMSKDNLLGKFFELTGIPAPRGVPOIEVTF	521
hHsp70	419	TIPTKQTQIFSTYSDNQPGVLIQVYEGERAMTKDNLLGRFELSGIPAPRGVPOIEVTF	478
dHsp70	417	RIPCKQTKTFSTYADNQPGVSIQVYEGERAMTKDNLLGTFDLGIPAPRGVPOIEVTF	476
gHsp70	425	TIPTKKEQVFSTYSDNQPGVLIQVYEGERTTRDNLLGKFFELSGIPAPRGVPOITVCF	484
bHsc70	419	TIPTKQTQIFSTYSDNQPGVLIQVYEGERAMTKDNLLGKFFELTGIPAPRGVPOIEVTF	478
Ssa1	419	TIPTKQTQIFSTYSDNQPGVLIQVYEGERAMTKDNLLGKFFELTGIPAPRGVPOIEVTF	478
hmtHsp70	463	TIPTKKSQVFSTAAAGQTVSIVKQGEREMAGDNKLLGQFTLIGIPAPRGVPOIEVTF	522
Ssc1	440	TIPTKKSQIFSTAAAGQTSVEIRVQGERELVRDNKLLGNFTLAGIPAPRGVPOIEVTF	499
eDnaK	417	TIPTKKSQVFSTAEQNSAVTIHVLQGERKRAADNKSLGQFNLDGINPAPRGMPQIEVTF	476
dsDnaK	413	TIPTKKSQVFSTAEQNSAVTIHVLQGERPIASGNKTIGRFHLDGIPAPRGIPQIEVTF	472
abDnaK	416	TIPTKKSQVFSTAAADNPVAVISVYQGERKMAQQNKLGNFQLDGIPAPRGVPOIEVFS	475
laDnaK	388	TIPTKKSQVFSTAAADNPVAVIHLVQGERPMAKDNKTLGRFQLADIPAPRGVPOIEVFS	447
vhDnaK	388	TIPTKKSQVFSTAAADNPVAVIHLVQGEREMADNKTLGRFQLTDIPAPRGVPOIEVFS	447

# Supplementary Figure 1

hBiP	502	<b>EIDVNGILRVTAEDKGTGNKNKIIITINDQNRLTPEEIERMVNDAEKFAEDDKLKERIDT</b>	561
dBIP	502	EIDANGILQVSAEDKGTGNKEKIVITINDQNRLTPEDIDRMIRDAEKFADEDKLLKERVES	561
gBiP	511	EVDANGILNVKAEDKGTGKSEKIIITINDKGRLSQEEIERMVREAEEFAEDDKKVKERIDA	570
Kar2	522	ALDANGILKVSATDKGTGKSESIIITINDKGRLLTQEEIDRMVVEAEKFASEDASIKAKVES	581
hHsp70	479	DIDANGILNVTATDKSTGKANKIITITNDKGRLSKEEIERMVQEAKEYKAEDVQREVRSA	538
dHsp70	477	DLDANGILNVSAREMSTGKAKNIIITINDKGRLSQAEIDRMVNEAEKYADEDEKHRQRITS	536
gHsp70	485	DIDANGILNVSAREMSTGKAKNIIITINDKGRLSKEEIEKMQEAKEYKSEDEEHKKKVEA	544
bHsc70	479	DIDANGILNVSAREMSTGKAKNIIITINDKGRLSKEDIERMVQEAKEYKAEDKQDRKVSS	538
Ssa1	479	DIDANGILNVSAREMSTGKAKNIIITINDKGRLSKEDIERMVQEAKEYKAEDKQDRKVSS	538
hmtHsp70	523	DIDANGIVHVSAREMSTGKAKNIIITINDKGRLSKEDIERMVQEAKEYKAEDRRKKERVEA	581
Ssc1	500	DIDADGIINVSARDKATNKDSSITVAGSSG-LSENEIEQMVNDAEKFKSQDEARKQAIET	558
eDnaK	477	DIDADGILHVSAREMSTGKAKNIIITINDKGRLSKEDIERMVQEAKEYKAEDRRKKERVEA	535
dsDnaK	473	DIDANGILHVHAKDKATGKSQSIRIEASSG-LSDEEINRMKSEAEANAEDRKKAKETADK	531
abDnaK	476	DINADGILKVSAREMSTGKAKNIIITINDKGRLSKEDIERMVQEAKEYKAEDRKKAKETADK	534
laDnaK	448	DIDKNGIVTVRAKDLGTGKEQNIVIKSSSG-LTDEEIDRMVKDAEANAEDRKKAKETADK	506
vhDnaK	448	DIDANGIVNVRADMGTNKEQSITIKSSSG-LSDEEVDKRVKAEANAEDRKKAKETADK	506

hBiP	562	<b>RNELESYAYSLKNQIGDKKLGKLSSEDKETMEKAVEEKIEWLES---HQDADIEDFKA</b>	618
dBIP	562	RNELESYAYSLKNQIGDKKLGKLSSEDKKLESAIDESIKWLEQ---NPDADPEEYKK	618
gBiP	571	RNSLETYIYNMKNQINDKDKLADKLESEDEKEKVVETAVKEALEWLDD---NQSAEKEDYEE	627
Kar2	582	RNKLENYAHSLSKNQVNGDLGE--KLEEDKETLLDAANDVLEWLDD---NFETAIAEDFDE	637
hHsp70	539	KNALESYAFNMKSAVEDEGLKG-KISEADKKKVLKDCQEVISWLDA---NTLAEKDEFEH	594
dHsp70	537	RNALESYVFNKQAVEQAPAG--KLDEADKNSVLDKCNDRIRWLDS---NTTAEKEEFDH	591
gHsp70	545	KNALESYAFNMKSAVEDEGLKG-KISEADKKKVLKDCQEVISWLDA---NTLAEKDEFEH	600
bHsc70	539	KNSLESYAFNMKATVEDEKLQK-KINDEKQKILDKCNEIINWLDK---NQTAKEEFEH	594
Ssa1	539	KNSLESYAFNMKATVEDEKLQK-KINDEKQKILDKCNEIINWLDK---NQTAKEEFEH	594
hmtHsp70	582	VNMAEGIIHDTETKMEEFK---QLPADECNKLKEEISKMRELLAR---KDSETGENIRQ	635
Ssc1	559	ANKADQLANDTENSLEFEG---KVDKAEAQKVRDQITSLKELVARVQGGEEVNAEELKT	615
eDnaK	536	RNQGDLHLHSTRKQVEEAGD---KLPADDKTAIESALTALETALKG-----EDKAAIEA	586
dsDnaK	532	INQADSLIFQTEKQLKEYGD---KLPADKKGPIEDALKKLEAHAS-----KDLAAIEA	582
abDnaK	535	RNEADALISSSNKAVKDLGD---KVTEDEKTAVNTAVSELEAATKE-----NDVEAIEA	585
laDnaK	507	RNNADQLIFTVDKTLKELEG---KVDADDEVKKAETARDELQEAALKG-----EDFDAIKE	557
vhDnaK	507	RNEADQLIFTTDKTIKDLGE---KVSDEEKEKAKEAKEELKSALES-----DDQEIQIE	557

hBiP	619	<b>KKKELEEIVQPIISK</b> LYGSAGPPPTGEEDTAE-----KDEL	654
dBIP	619	QKLDLEAIVQPVIAKLYQAGGAPPPEGDDADL-----KDEL	656
gBiP	628	KLKEVEAVCNPIITAVYQRSGGAPGGGSTEEDDDS-----HDEL	666
Kar2	638	KFESLSKVAYPIITSKLYGGADGSGAADYDEDEDGDDYFEHDEL	682
hHsp70	595	KRKELEQVCNPIISGLYQAGGP-GPGGFQAQGPKGSGSGSPTIEEVD	641
dHsp70	592	KLEELTRHCSPIIMTKMHQQGAGAGAGGPGANCGQQAGGFGGYSGPTVEEVD	642
gHsp70	601	KMKELENMCNPIIAKMYQAGGEPAGKAGEAPPAGGSGAGPKIEEVD	648
bHsc70	595	QQKELEKVCNPIITKLYQSAGGMPGGMPGGMPGGFPGGGAPPSSGASSGPTIEEVD	650
Ssa1	595	QQKELEKVCNPIITKLYQSAGGMPGGMPGGMPGGFPGGGAPPSSGASSGPTIEEVD	650
hmtHsp70	636	AASSLQOASLKLFEFEMAYKMASEREGSGSSGTGEQKEDQKEEKQ	679
Ssc1	616	KTEELQTSMSKLFQLYKNSNNNNNNNGNNAESGETKQ	654
eDnaK	587	KMQELAQVSQKLMEIAQQQHAQQOTAGADASANNAKDDVVDVDAEFEEVKDKK	638
dsDnaK	583	AMNELNTVFAASQEMYNASQAQGGAGPDPDAGQQAGPQDQPKDDGEVTDVDFEEVK	637
abDnaK	586	KTEALQNIILMPITQRAYEQAQAGGAEQFDPNFAFQGGDAGQQKADDGVVDAEFTEVKDDK	646
laDnaK	558	KSDALNEIVQSLSVKLYEQAAAAQQAQGENPEANAADGSEDVVDADFEEINDDDKKEDK	615
vhDnaK	558	KKEALEEQVQQLSVKMYEQMQQEAQAQQQEDQGSDDVVDADYQEVDEEKDEKDNK	613

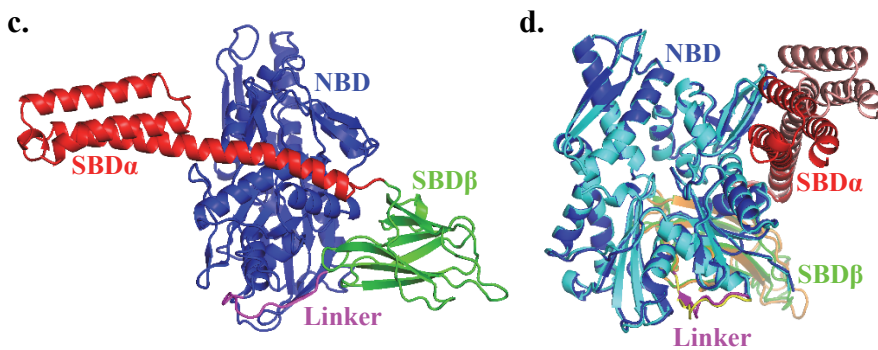
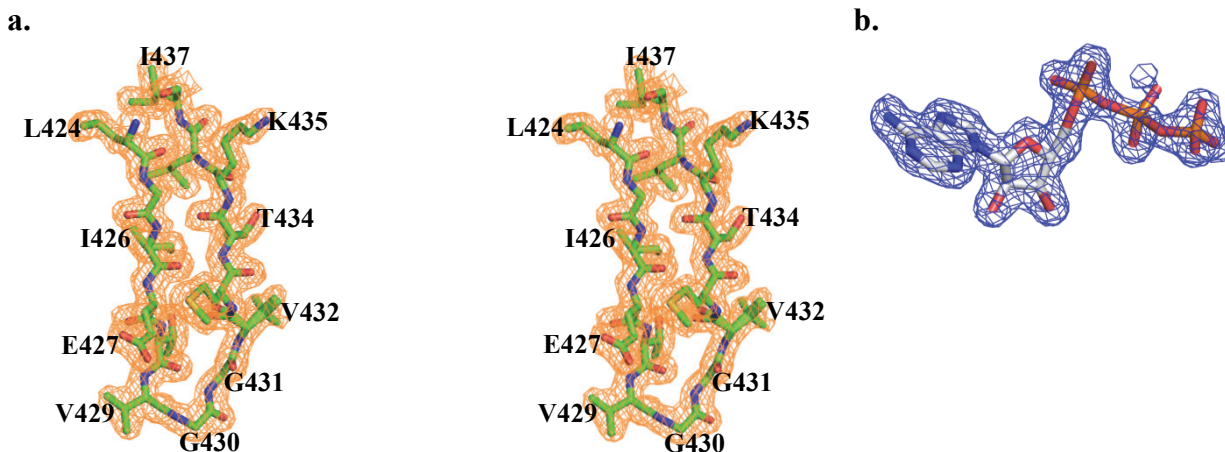
## Supplementary Figure 1

### Supplementary Figure 1. Structure-based sequence alignment of representative Hsp70s.

The amino acid sequence of human BiP (hBiP) is aligned with the sequences of a number of selected Hsp70s from both eukaryotes (different cellular compartments) and prokaryotes. The BiP construct used in crystallization is from residue 25 to 633, and all the residues in the construct (highlighted in red) are ordered in both protomers. L<sub>3,4</sub>, which is mutated to VGG (L<sub>3,4</sub>'), is highlighted in blue. Elements of secondary structure in the BiP-ATP2 structure are specified by cylinders for helices and arrows for strands and labeled. The coloring of structural elements is: NBD (blue), Linker (purple), SBD $\beta$  (green) and SBD $\alpha$  (red). The highly conserved Gly425, Gly430 and Gly431 are highlighted in purple. The first four sequences are Hsp70s from endoplasmic reticulum (ER); the next 5 sequences (names in red) are Hsp70s from eukaryotic cytosol; the following two sequences (names in green) are Hsp70s from mitochondria; and the last five sequences are Hsp70s from prokaryotes (names in blue). hBiP: human ER Hsp70; dBiP: *Drosophila melanogaster* ER Hsp70; gBiP: *Gossypium hirsutum* ER Hsp70; Kar2: *S. cerevisiae* ER Hsp70; hHsp70: human Hsp70; dHsp70: *Drosophila melanogaster* Hsp70; gHsp70: *Gossypium hirsutum* Hsp70; bHsc70: bovine Hsc70; Ssa1: *S. cerevisiae* Hsp70; hmtHsp70: human mitochondrial Hsp70; bmtHsp70: bovine mitochondrial Hsp70; Ssc1: *S. cerevisiae* mitochondrial Hsp70; eDnaK: *Escherichia coli* Hsp70 (gram-negative); dsDnaK: *Draconibacterium* sp. Hsp70 (gram-negative); abDnaK: *Acinetobacter baumannii* Hsp70 (gram-negative); laDnaK: *Listeria aquatica* Hsp70 (gram-positive); vhDnaK: *Virgibacillus halodenitrificans* Hsp70 (gram-positive).



## Supplementary Figure 2



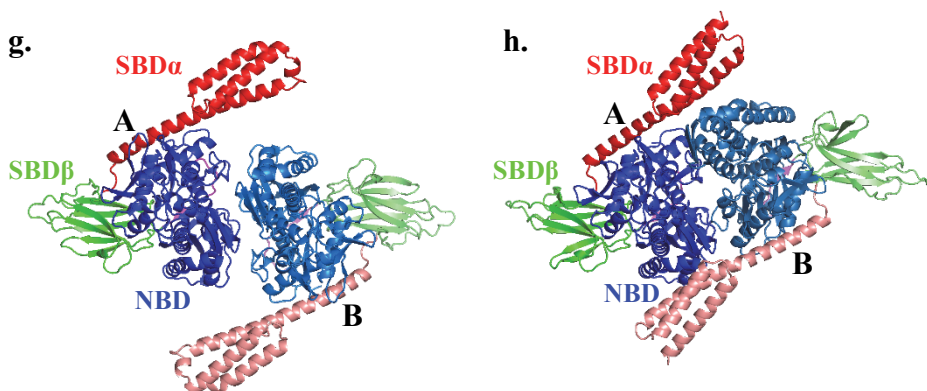
**e.**

rmsd (Å)	BiP-ATP2/BiP-ATP	BiP-ATP2/DnaK-ATP
NBD	0.513	0.920
SBDβ	2.337	2.618
SBDβ-core	0.591	0.615
SBDα	1.076	2.174

SBDβ-core: SBDβ excluding L<sub>1,2</sub>, L<sub>3,4</sub>, L<sub>4,5</sub>, L<sub>5,6</sub>, and β8.

**f.**

	$k_{cat}$ ( $\times 10^{-3}$ min <sup>-1</sup> )
- Pi	12.92±0.92
+ Pi	2.55±0.36



## Supplementary Figure 2

### Supplementary Figure 2. The BiP-ATP2 structure and structural comparison.

**a-b**, Electron-density maps for  $L_{1,2}$  (**a**, stereo image) and ATP (**b**) of the BiP-ATP2 structure. 2Fo-Fc maps (contoured at  $1\sigma$ ) were shown as meshes.  $L_{1,2}$  and ATP were drawn as sticks.

**c**, The BiP-ATP2 structure. Orthogonal view of Fig. 1**b**.

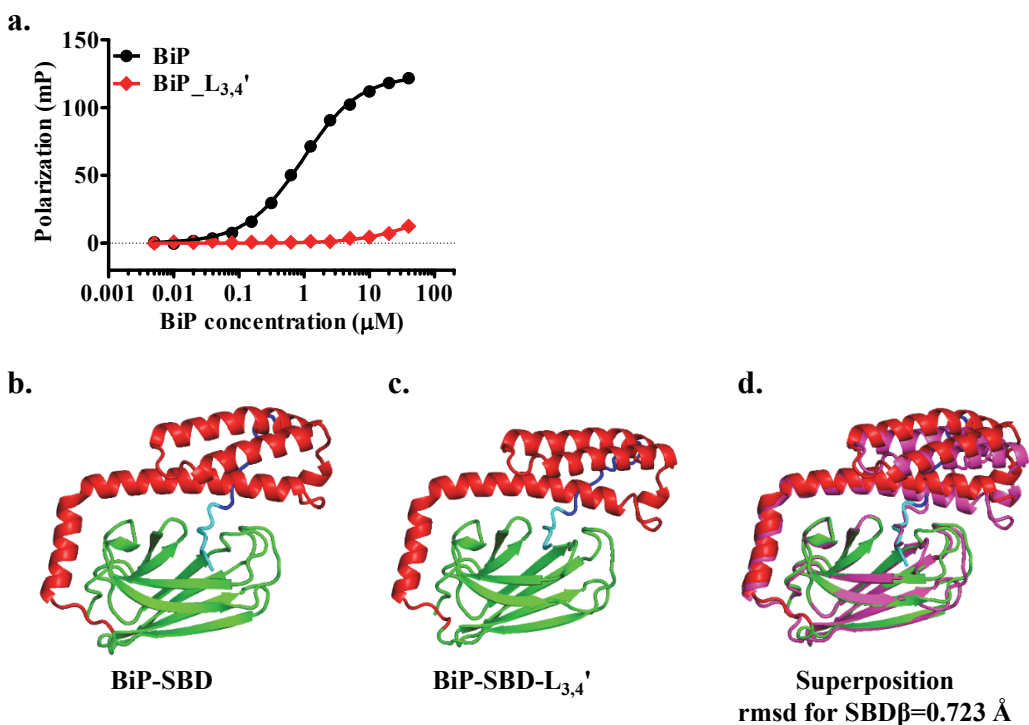
**d**, Structural comparison of BiP-ATP2 with BiP-ATP (PDB code: 5E84). Orthogonal view of Fig. 1**c**.

**e**,  $C\alpha$  root-mean-square deviation (rmsd) between the Hsp70-ATP structures. NBD and SBD $\beta$ -core have the smallest rmsd.

**f**, Phosphate reduces the ATPase activity of BiP. Single-turnover ATPase assay was carried with BiP protein in the presence of 100 mM phosphate (+ Pi), and the catalytic constant ( $k_{cat}$ ) was calculated after fitting the data using Prism. The ATPase activity in the absence of phosphate (- Pi) was used as a control.

**g-h**, Ribbon diagrams of BiP-ATP2 dimer (**g**) and DnaK-ATP-dimer (**h**, PDB code: 4JNE). Protomer As from BiP-ATP2 and DnaK-ATP are superimposed. The domain coloring for protomer A is the same as Fig. 1**b**, and the domain coloring for protomer B is: NBD (light blue), SBD $\beta$  (light green), SBD $\alpha$  (pink), and inter-domain linker (magenta).

## Supplementary Figure 3



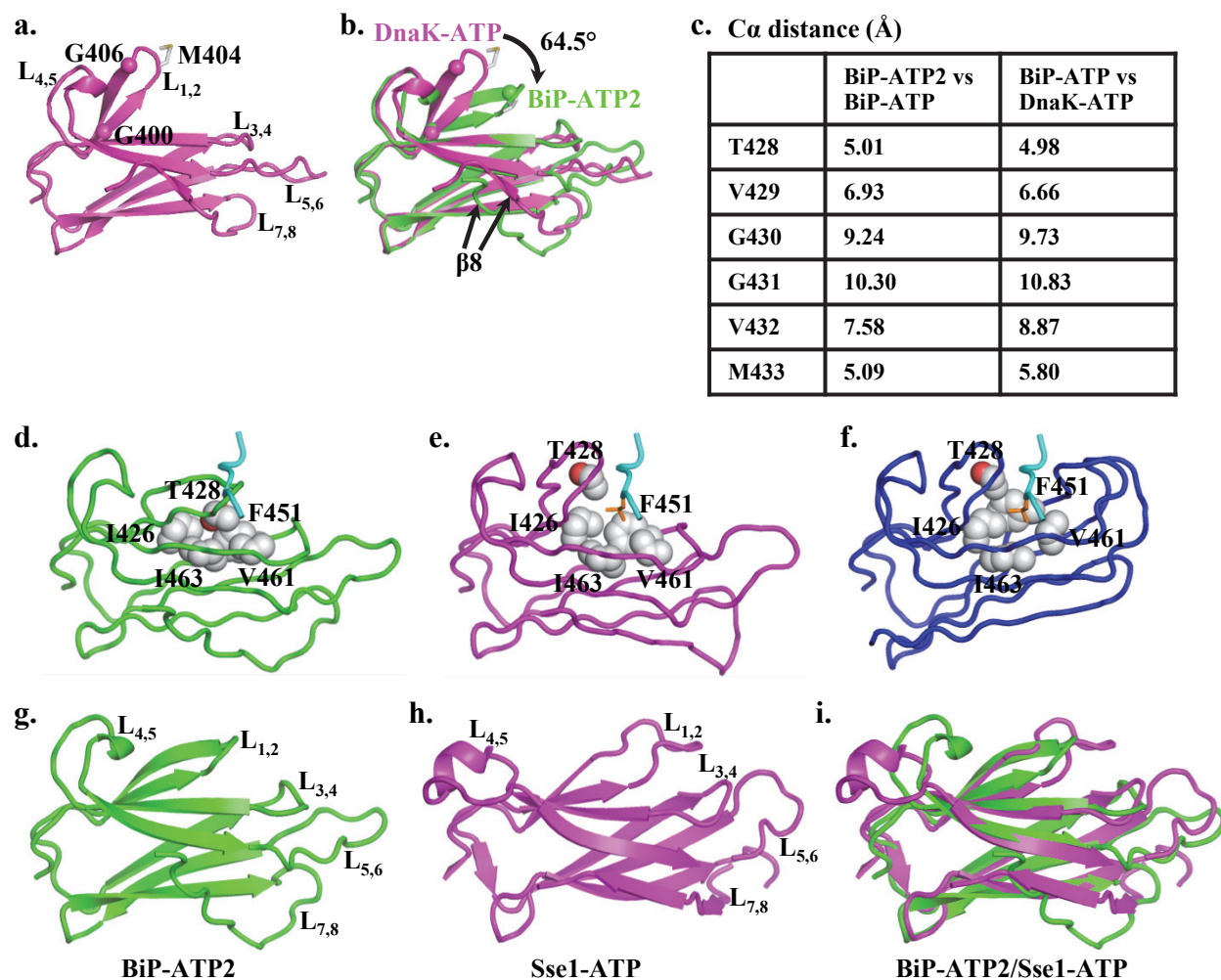
### Supplementary Figure 3. The $L_{3,4}'$ modification in BiP significantly compromises peptide substrate binding but has little structural impact in the isolated domain structure.

**a**, Peptide substrate binding to BiP proteins using fluorescence polarization. The NR peptide was labeled with fluorescein at the N-terminus. This F-NR peptide was incubated with serial dilutions of BiP proteins. After binding reached equilibrium, fluorescence polarization was measured and fitted to one-site binding equation. The dissociation constant ( $K_d$ ) for the WT BiP is 0.95  $\mu\text{M}$ , consistent with published results. The binding for BiP- $L_{3,4}'$  protein was drastically reduced.

**b-d**, Structural comparison of the isolated SBD domain of BiP. The structures of isolated SBD from WT BiP (**b**, PDB code: 5E85) and BiP- $L_{3,4}'$  (**c**, PDB code: 5E86) were superimposed based on the C $\alpha$ s of SBD $\beta$ . The coloring for both structures is: SBD $\beta$  (green), SBD $\alpha$  (red), linked NR (cyan), and the Tev linker between SBD and NR peptide (blue). **d** is the superposition of the two structures. The WT SBD structure has the same color code as in **b** and the SBD- $L_{3,4}'$  is colored in purple. The rmsd for the SBD $\beta$  is 0.723 Å.



## Supplementary Figure 4



### Supplementary Figure 4. The conformations of the polypeptide-binding pocket.

**a,** Ribbon diagram of the SBD $\beta$  from the DnaK-ATP structure (PDB code: 4JNE). The C $\alpha$  atoms of Gly400 and Gly406 are shown as balls.

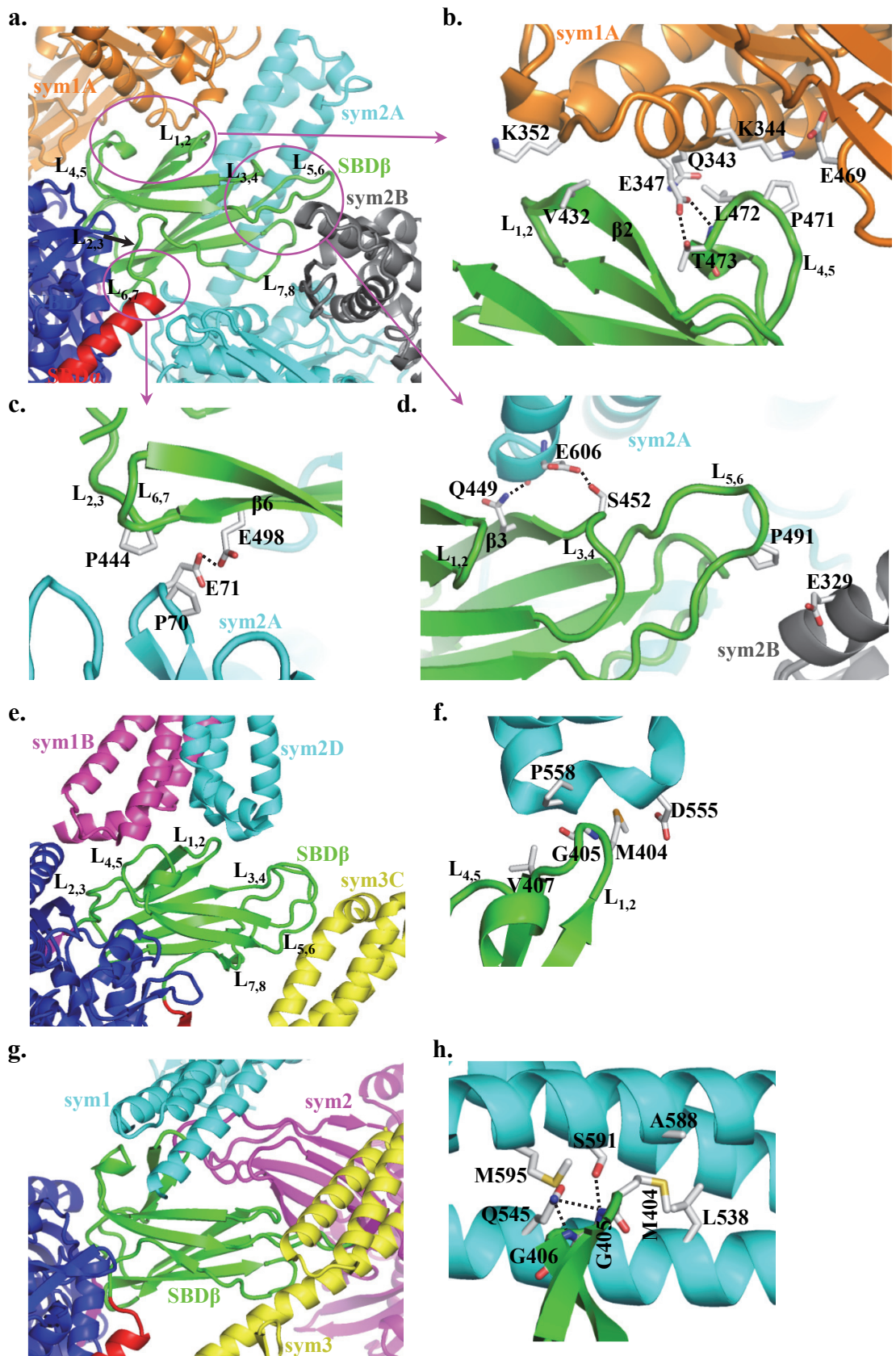
**b,** Superposition of the SBD $\beta$ s from BiP-ATP2 (green) and DnaK-ATP (purple). The structures were superimposed based on the C $\alpha$  atoms of the SBD $\beta$ -core. The C $\alpha$  atoms of Gly400 and Gly406 are shown as balls. The rotation of L<sub>1,2</sub> between BiP-ATP2 and DnaK-ATP is labeled.

**c,** Distances between the C $\alpha$  atoms in the  $\beta$ 1-L<sub>1,2</sub>- $\beta$ 2 segment from the BiP-ATP2, BiP-ATP and DnaK-ATP structures.

**d-f,** The polypeptide-binding pockets in BiP-ATP2 (**d**), BiP-ATP (**e**, PDB code: 5E84), and isolated BiP SBD (**f**, PDB code: 5E85). SBD $\beta$ s are shown as worm representation. The NR peptide bound to the isolated SBD is shown in cyan with the side chain of the central leucine, Leu4, highlighted in yellow. SBD $\beta$  side chains that make van der Waals contacts with Leu4 in the isolated BiP SBD structure are shown as sphere drawings. Since the polypeptide-binding pocket is fully closed, these residues pack against each other to exclude the binding of the NR peptide.

**g-i,** Structural comparison of the SBD $\beta$ s from BiP-ATP2 (**g**) and Sse1-ATP (**h**, PDB code: 2QXL). **i** is the superposition of **g** and **h**. The SBD $\beta$ s are superimposed based on C $\alpha$  atoms. Except L<sub>4,5</sub> and  $\beta$ 8, the two structures aligned well (rmsd: 1.511 Å), especially the peptide-binding loops. The disordered region in L<sub>7,8</sub> of Sse1-ATP is not shown.

# Supplementary Figure 5



## Supplementary Figure 5

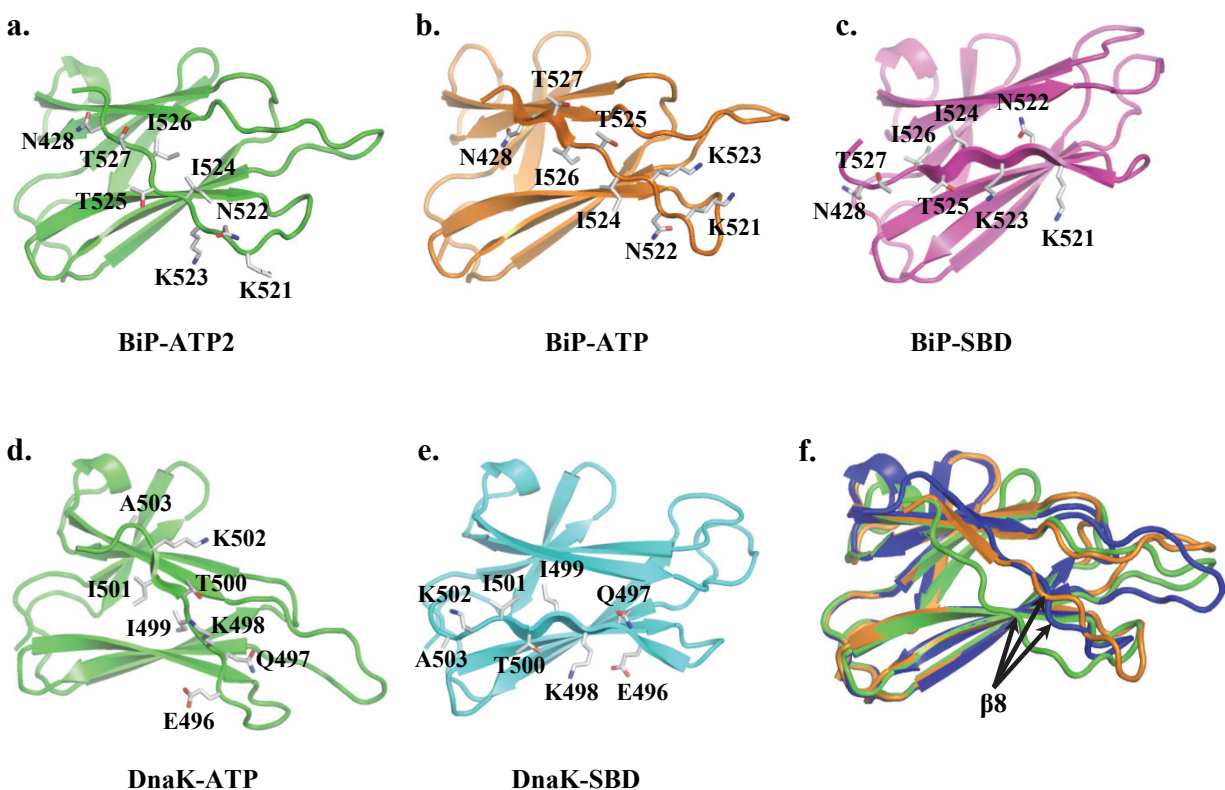
### Supplementary Figure 5. Crystal contacts surrounding the SBD $\beta$ region.

**a-d**, Crystal contacts in the BiP-ATP2 structure. **a**, Overall crystal contacts surrounding the SBD $\beta$ . Only protomer A was shown (NBD: blue; SBD $\beta$ : green; and SBD $\alpha$ : red). Three molecules from two symmetry mates form crystal contacts with SBD $\beta$ : protomer A from symmetry mate 1 (sym1A, orange), and both protomers from symmetry mate 2 (sym2A and sym2B, cyan and grey, respectively). **b**, Details of the crystal contacts formed between SBD $\beta$  and sym1A. L<sub>1,2</sub> and L<sub>4,5</sub> of SBD $\beta$  are involved in these crystal contacts. A hydrophobic contact was formed between Val432 (in L<sub>1,2</sub>) and Lys352 (sym1A). Two hydrogen bonds were formed between the side-chain of Glu347 (sym1A) and Thr473 in L<sub>4,5</sub> (both side-chain and main-chain). There are a number of hydrophobic contacts formed between two residues on L<sub>4,5</sub> (Lys472 and Pro471) and three residues on sym1A (Gln343, Lys344 and Glu469). **c**, Details of the crystal contacts formed between SBD $\beta$  (the L<sub>2,3</sub> and L<sub>6,7</sub> region) and sym2A. Hydrophobic contacts were formed between the side-chains of Pro444 (in L<sub>2,3</sub>) and Pro70 and Glu71 from sym2A. Moreover, a hydrogen bond was formed between Glu498 (in  $\beta$ 6) and Glu71 in sym2A. **d**, Details of the crystal contacts formed between SBD $\beta$  and sym2A and sym2B around the L<sub>3,4</sub> and L<sub>5,6</sub> region. There are two hydrogen bonds: between Ser452 in L<sub>3,4</sub> and Glu606 of Sym2A, and between Gln449 in  $\beta$ 3 and Glu606 of Sym2A. Hydrophobic contacts were formed between Pro491 in L<sub>5,6</sub> and side-chain of Glu329 from sym2B.

**e-f**, Crystal contacts around the peptide-binding loops in the DnaK-ATP structure solved by the Mayer's group (PDB code: 4B9Q). Only protomer A was shown (NBD: blue; SBD $\beta$ : green; and SBD $\alpha$ : red). **e**, Overall crystal contacts around the peptide-binding loops. Three symmetry mates form crystal contacts around the peptide-binding loops: sym1B (purple), sym2D (cyan), and sym3C (yellow). **f**, L<sub>1,2</sub> forms crystal contacts with symmetry mate sym2D. This crystal contact features a number of hydrophobic interactions between the side-chain of Met404 in L<sub>1,2</sub> and side-chains of both Asp555 and Pro558 (sym2D), between the main-chain of Gly405 in L<sub>1,2</sub> and side-chain of Pro558 (sym2D), and between the side-chains of Val407 in L<sub>1,2</sub>- $\beta$ 2 junction and Pro558 (sym2D).

**g-h**, Crystal contacts around the peptide-binding loops in the DnaK-ATP structure solved by our group (PDB code: 4JNE). Only protomer A was shown (NBD: blue; SBD $\beta$ : green; and SBD $\alpha$ : red). **g**, Overall crystal contacts around the peptide-binding loops. Three symmetry mates form crystal contacts around the peptide-binding loops: sym1 (purple), sym2 (cyan), and sym3 (yellow). **h**, The crystal contacts formed between L<sub>1,2</sub> and symmetry mate sym1. A number of hydrophobic interactions and hydrogen bonds are formed on these crystal contacts. The hydrogen bonds are labeled as dotted lines. The side-chain of Met404 in L<sub>1,2</sub> forms hydrophobic contacts with the side-chains of Leu538, Ala588 and Ser591 on sym1. The main-chain C $\alpha$  of Gly406 in L<sub>1,2</sub> forms hydrophobic contact with Met595 on sym1.

## Supplementary Figure 6



### Supplementary Figure 6. The conformation of $\beta$ 8.

**a-e,** The SBD $\beta$ s from BiP-ATP2 (**a**), BiP-ATP (**b**, PDB code: 5E84), BiP-SBD (**c**, PDB code: 5E85), DnaK-ATP (**d**, PDB code: 4JNE), and DnaK-SBD (**e**, PDB code: 1DKX) are drawn in ribbon diagram. Side chains of  $\beta$ 8 are highlighted in stick representation. The  $\beta$ 8 in BiP-ATP2 has similar side-chain orientations as those in the isolated BiP-SBD; whereas the side-chain orientations of  $\beta$ 8 in BiP-ATP are flipped. The side-chain orientations of  $\beta$ 8 in the isolated DnaK SBD are similar to those of the isolated BiP SBD. Interestingly, the first two residues at the N-terminal end of  $\beta$ 8 in DnaK-ATP have similar side-chain orientations as the isolated SBD structures, whereas for the rest of  $\beta$ 8 starting from the third residue, the side-chain orientations are flipped as those in BiP-ATP. Thus, the side-chain orientations of  $\beta$ 8 in DnaK-ATP seem to be a hybrid between BiP-ATP and BiP-ATP2.

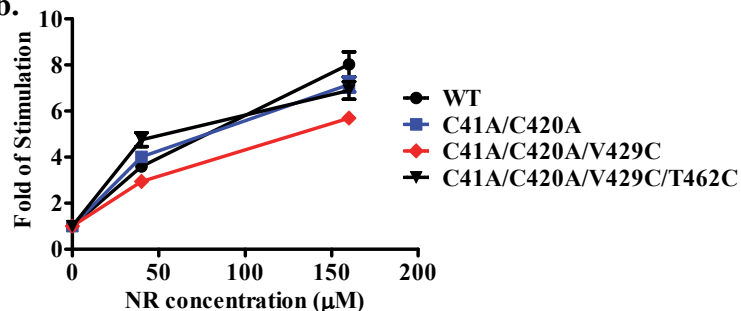
**f,** Superposition of SBD $\beta$ s from BiP-ATP2 (green), BiP-ATP (orange) and DnaK-ATP (blue). The SBD $\beta$ s were superimposed based on the C $\alpha$  atoms of SBD $\beta$ -core.  $\beta$ 8 is indicated by arrows.

## Supplementary Figure 7

a.

BiP proteins	$k_{\text{cat}}$ ( $\times 10^{-3} \text{ min}^{-1}$ )	$K_d$ ( $\mu\text{M}$ )
WT	12.04 $\pm$ 0.80	0.91 $\pm$ 0.042
C41A/C420A	12.46 $\pm$ 0.43	0.85 $\pm$ 0.012
C41A/C420A/V429C	10.47 $\pm$ 0.74	0.82 $\pm$ 0.026
C41A/C420A/V429C/T462C	9.11 $\pm$ 1.38	0.61 $\pm$ 0.023

b.



**Supplementary Figure 7. The cysteine modifications used in the FRET and EPR analysis have little influence on BiP's biochemical activities.**

**a.** The intrinsic ATPase and peptide-binding activities of the site-directed cysteine mutations in BiP. The single-turnover ATPase assay was carried out at 20°C, and the catalytic constants ( $k_{\text{cat}}$ ) were calculated after fitting the data using Prism. Peptide substrate binding affinities were assayed using fluorescence polarization with the NR peptide, and the dissociation constants ( $K_d$ ) were calculated by fitting one-site binding equation using Prism.

**b.** The allosteric coupling in BiP proteins was analyzed using the NR peptide stimulation on the ATPase activity. The intrinsic ATPase activities were set as 1. Fold of stimulation was plotted.



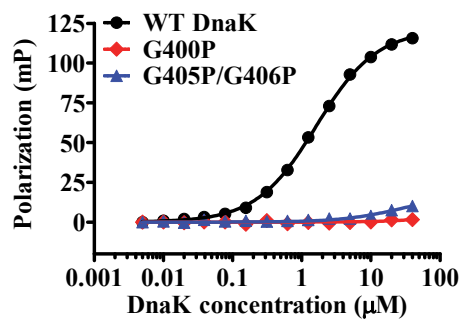
## Supplementary Figure 8

BiP	BiP-ATP2		BiP-ATP		BiP-SBD		DnaK	DnaK-ATP		DnaK-SBD		Sse1	Sse1-ATP	
	$\varphi$ (°)	$\psi$ (°)	$\varphi$ (°)	$\psi$ (°)	$\varphi$ (°)	$\psi$ (°)		$\varphi$ (°)	$\psi$ (°)	$\varphi$ (°)	$\psi$ (°)		$\varphi$ (°)	$\psi$ (°)
T423	-63	130	-58	133	-66	132	S398	-68	136	-71	147	S401	-74	133
L424	-101	134	-100	148	-107	143	L399	-99	125	-121	136	V402	-123	124
<b>G425</b>	<b>-136</b>	<b>164</b>	<b>-154</b>	<b>-176</b>	<b>-170</b>	<b>-166</b>	<b>G400</b>	<b>-142</b>	<b>-178</b>	<b>-168</b>	<b>-161</b>	<b>S403</b>	<b>-106</b>	<b>153</b>
I426	-126	151	-128	155	-128	162	I401	-134	154	-134	163	Y404	-117	141
E427	-120	112	-77	137	-86	127	E402	-83	116	-83	129	S405	-124	162
T428	-92	120	-134	159	-108	177	T403	-108	166	-116	173	W406	-155	167
V429	-62	125	-26	130	-55	126	M404	-43	134	-50	124	D407	-62	130
G430	72	14	87	-137	104	11	G405	102	-4	110	-9			
<b>G431</b>	<b>-134</b>	<b>-158</b>	<b>-140</b>	<b>66</b>	<b>64</b>	<b>43</b>	<b>G406</b>	<b>75</b>	<b>20</b>	<b>76</b>	<b>34</b>			
V432	-83	153	-96	130	-99	124	V407	-86	154	-106	147	D414	-122	31
M433	-109	131	-77	88	-73	117	M408	-90	172	-89	131	H415	-170	137
T434	-154	146	-81	105	-112	117	T409	-109	118	-112	122	M416	-172	132
K435	-64	143	-58	146	-65	130	T410	-76	132	-83	130	E417	-74	134
L436	-120	-55	-132	-53	-111	-56	L411	-104	-41	-101	-41	V418	-119	-51
I437	-134	112	-128	113	-114	112	I412	-133	116	-121	118	F419	-117	100

**Supplementary Figure 8. Backbone conformation of the  $\beta$ 1-L<sub>1,2</sub>- $\beta$ 2 segment in Hsp70 structures.** The  $\psi$  and  $\varphi$  angles of Gly425 and Gly431 are highlighted in red.

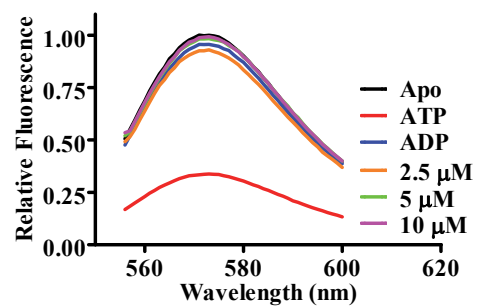


## Supplementary Figure 9



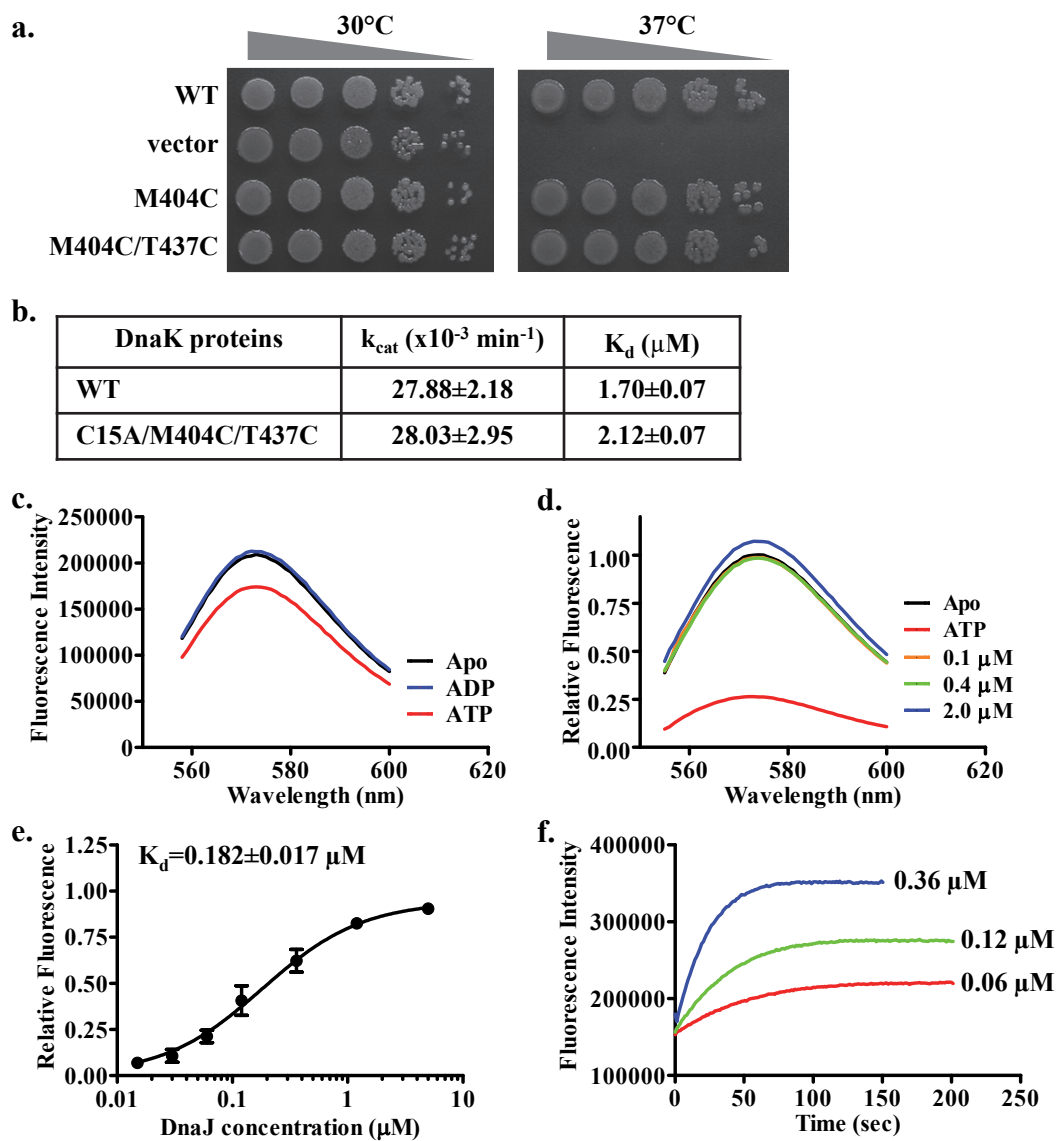
**Supplementary Figure 9. The G400P and G405P/G406P mutations drastically reduced the peptide-binding activity of DnaK.** The fluorescence polarization assay for peptide binding activity was carried out on the DnaK proteins in the same way as in Fig. 5b.

## Supplementary Figure 10



**Supplementary Figure 10. ERdj3 has little effect on the fluorescence intensity of the TMR-labeled BiP in the presence of ADP.** Assay was carried out essentially the same way as in Fig. 6a except that ADP was added in place of ATP.

# Supplementary Figure 11



## Supplementary Figure 11

### Supplementary Figure 11. Conserved dynamics of $L_{1,2}$ in DnaK.

**a,** Neither M404C nor T437C modification has appreciable impact on the *in vivo* function of DnaK. Growth test of DnaK mutants in Hsp70-deficient *E.coli* was carried out. Serial dilutions of fresh cultures from fresh transformants were spotted onto plates and incubated at 37 °C with a 30 °C duplicate plate as growth control. WT DnaK and empty vector were used as positive and negative controls, respectively.

**b,** Both the intrinsic ATPase and peptide-binding activities of DnaK are largely unaffected by the M404C and T437C modifications. Assays were performed at room-temperature. For the peptide binding activity, fluorescence polarization assay with the model peptide NR was used and dissociation constants ( $K_d$ ) were calculated. Single-turnover ATPase assay was used to determine the catalytic constants ( $k_{cat}$ ).

**c,** The G400P mutation almost abolished the ATP-induced quenching of the TMR-labeled DnaK's fluorescence. Fluorescence emission spectra were collected in the same way as in Fig. 7a.

**d,** DnaJ has little influence on the fluorescence of the TMR-labeled DnaK in the absence of ATP. Assay was carried out essentially the same way as in Fig. 7c except that ATP was not included.

**e,** Dissociate constant ( $K_d$ ) of DnaJ for DnaK in the FRET assay. Relative fluorescence was calculated by setting the fluorescence intensities of Apo and ATP alone at 573 nm (the peak emission) as 1 and 0, respectively.

**f,** The kinetics of DnaJ-induced opening of  $L_{1,2}$ . Assays were performed on DnaJ and the TMR-labeled DnaK in essentially the same way as in Fig. 6c. The concentrations of DnaJ were labeled on the right.