F) Supplemental Text and Figures

Supplemental Data

Table S1: Data collection and structure determination statistics.

Dataset	NBD-linker/Apo	NBD-linker/ADP	NBD-linker/ADP*Vi
A. Data processing			
Space group	$P2_1$	$P2_1$	$P2_1$
Unit cell parameters			
a (Å)	73.336	73.306	73.336
b (Å)	78.236	77.962	77.841
c (Å)	75.462	75.320	75.793
α (deg.)	90	90	90
β (deg.)	100.806	101.458	101.533
γ (deg.)	90	90	90
Resolution (Å)	50.0-1.85 (1.92-1.85)	50.0-1.75 (1.81-1.75)	50.0-1.86 (1.93-1.86)
No. of reflections	203449	303138	256805
No. of unique reflections	70516 (7093)	83895 (8294)	70267 (6981)
Wavelength (Å)	1.54	1.54	1.54
Completeness (%)	98.7 (99.9)	100 (100)	100 (99.8)
Multiplicity	2.9 (2.8)	3.6 (3.5)	3.7 (3.5)
Mosaicity (deg.)	0.739	0.500	0.399
R_{sym} (%)	4.8 (47.2)	3.3 (23.4)	6.1 (41.5)
$< I/\sigma>$	19.8 (2.1)	29.1 (3.9)	17.9 (2.9)
B. Refinement statistics			
Resolution range (Å)	35.09-1.85	35.16-1.75	36.06-1.86
R_{cryst} (%)	20.2	17.7	18.8
R_{free} (%)	23.6	20.9	22.4
Average B factor (Å^2)	30.121	12.886	12.398
RMSD bonds (Å)	0.012	0.011	0.010
RMSD angles (deg.)	1.232	1.233	1.173
Ramachandran analysis			
Most favored (%)	92.2	93.1	93.8
Additionally allowed (%)	7.8	6.8	6.2
Generously allowed (%)	---	0.1	---

Dataset	NBD: J/ADP*Pi	NBD-linker: J/ADP*Pi (#1)	NBD-linker: J/ADP*Pi (#2)
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a (Å)	38.627	38.698	38.654
b (Å)	58.262	58.543	58.641
c (Å)	225.929	226.236	225.558
α (deg.)	90	90	90
β (deg.)	90	90	90
γ (deg.)	90	90	90
Resolution (Å)	50.0-2.40 (2.49-2.40)	50.0-1.70 (1.76-1.70)	50.0-1.75 (1.81-1.75)
No. of reflections	75243	370038	289951
No. of unique reflections	20512 (2044)	56529 (4806)	48554 (3092)
Wavelength (Å)	1.54	1.00	1.00
Completeness (%)	97.3 (99.9)	98.2 (84.7)	91.2 (59.4)
Multiplicity	3.7 (3.7)	6.5 (4.0)	6.0 (4.8)
Mosaicity (deg.)	0.634	0.42	0.34
R_{sym} (%)	9.4 (58.2)	6.4 (49.6)	8.0 (51.8)
< I/σ>	13.1 (2.5)	26.8 (3.1)	19.4 (2.4)
B. Refinement statistics			
Resolution range (Å)	113.23-2.40	40.69-1.70	40.66-1.75
R_{cryst} (%)	20.8	19.3	20.2
R_{free} (%)	27.9	22.2	24.2
Average B factor (Å^2)	41.045	28.203	27.892
RMSD bonds (Å)	0.011	0.011	0.011
RMSD angles (deg.)	1.334	1.217	1.235
Ramachandran analysis			
Most favored (%)	93.2	93.2	93.7
Additionally allowed (%)	6.8	6.1	5.9
Generously allowed (%)	---	0.5	---
Overall coordinate error (from free Rfactor)	---	0.133 Å	0.108 Å

Dataset	NBD-linker: J/AMPPNP	
Space group	$P2_12_12_1$	
a (Å)	38.797	
b (Å)	56.494	
c (Å)	225.878	
α (deg.)	90	
β (deg.)	90	
γ (deg.)	90	
Resolution (Å)	50.0-2.20 (2.28-2.20)	
No. of reflections	86413	
No. of unique reflections	25924 (2561)	
Wavelength (Å)	1.54	
Completeness (%)	99.8 (100)	
Multiplicity	3.3 (3.3)	
Mosaicity (deg.)	0.968	
R_{sym} (%)	7.2	
< I/σ>	14.3 (2.4)	
B. Refinement statistics	hydrolyzed form	intact from
Resolution range (Å)	31.67-2.21	31.67-2.21
R_{cryst} (%)	24.0	23.0
R_{free} (%)	29.5	29.4
Average B factor (Å^2)	40.861	45.339
RMSD bonds (Å)	0.014	0.014
RMSD angles (deg.)	1.395	1.527
Ramachandran analysis		
Most favored (%)	92.2	92.2
Additionally allowed (%)	7.8	7.8
Overall coordinate error (from free Rfactor)	0.263 Å	0.261 Å

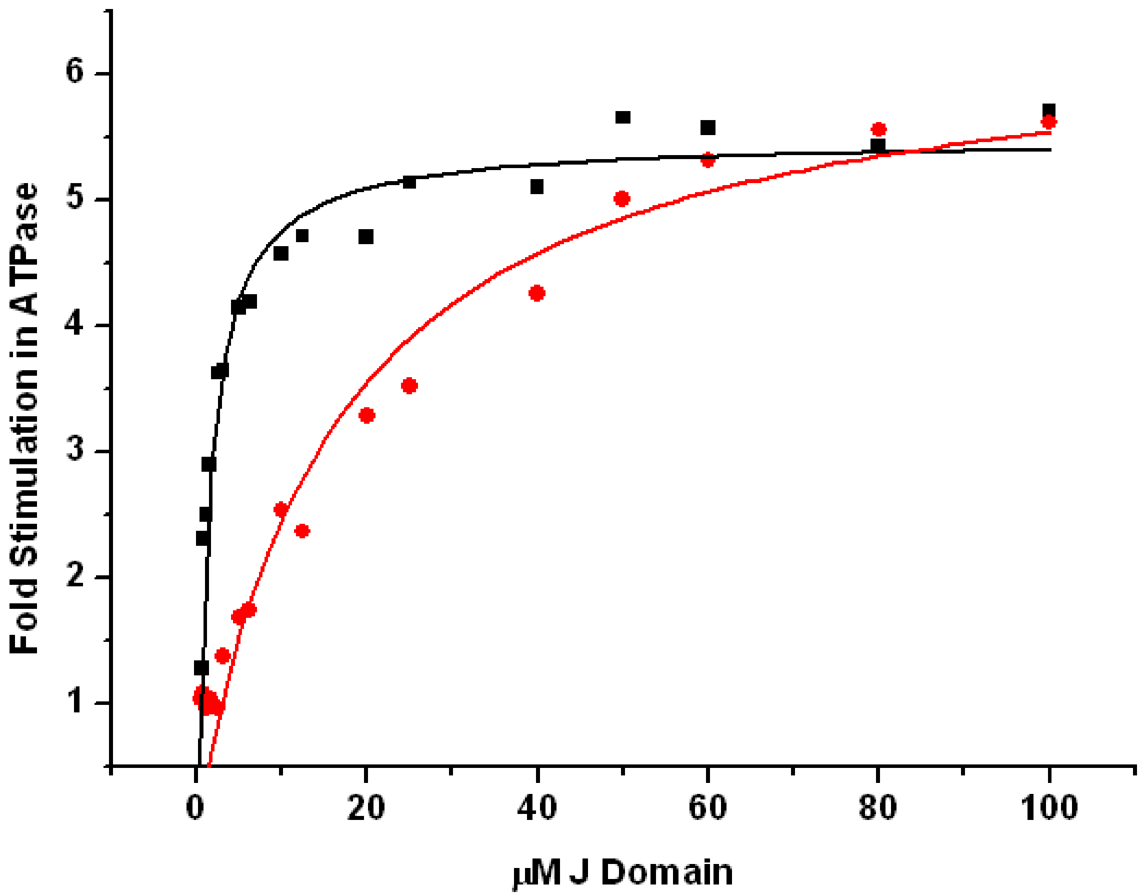

Fig. S1. Fold stimulation of 2-domain (aa 1-554: black curve) or NBD_linker (aa 1-394:red curve) Hsc70 ATPase activity as a function of auxilin J domain concentration. Hyperbolic fits are shown. Maximal stimulation of 2-domain Hsc70 is 5.4±0.1 fold with half-maximal stimulation occurring at 1.5±0.14 µM J domain (chi-square=0.059). For the NBD_Linker the corresponding values were: 6.4±0.4 and 16.2±2.96 µM (chi-square=0.17).

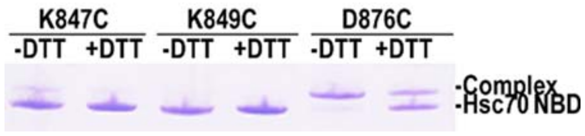

Fig. S2. Preparation of auxilin J domain:NBD complexes. Hsc70 NBD (aa 1-386) with cysteine substituted for R171 was mixed with 1 mM DTT and auxilin J domains (aa 803-910) bearing the indicated cysteine substitutions and dialyzed overnight against buffer with no reducing agent. Samples were then resolved on SDS PAGE in either the absence, or presence, of 2 mM DTT, as indicated.

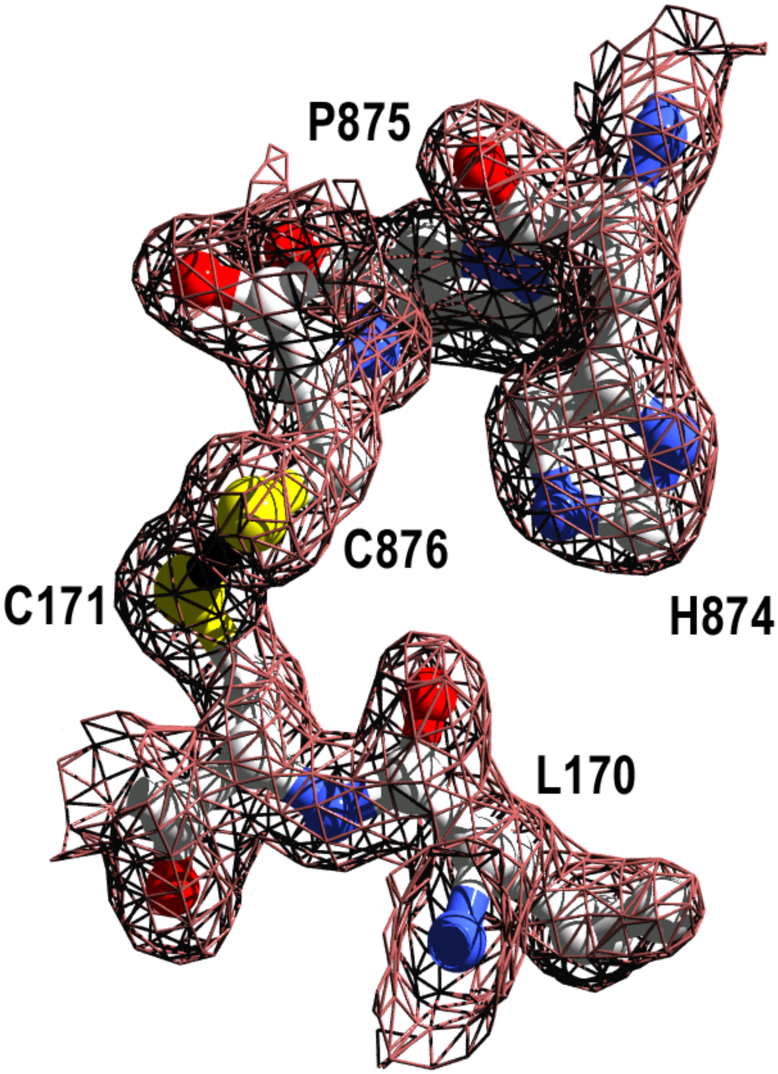

Fig. s3: Representative electron density (contoured at 1.0 σ) around the J domain $H_{874}P_{875}D_{876}$ motif (HPC in this mutant) and disulfide cross-link from the ADP*Pi*J*NBD_Linker structure with carbons in white, oxygens in red, nitrogens in blue, and sulfurs in yellow.