Table S1. Data collection and refinement

Data collection	
Space group	*C*2
Cell dimensions	*a*=206.95, *b*=49.77, *c*=147.36 Å *α*=*γ*=90.0, *β*=115.0
Mol. in ASU	4
Resolution range (Å)	50.0-2.1 (2.15-2.11)*
Redundancy	3.6 (2.5)
Completeness (%)	96.3 (51.9)
$I/\sigma I$	19.5 (1.7)
R_{sym} (%)	7.0 (45.6)
Refinement	
Resolution range (Å)	41.2-2.1
No. Reflections	77,439
R_{free} (%)	22.1
R_{cryst} (%)	17.3
Protein atoms (solvent)	9,469 (444)
Average B-factor (Å^2)	49.7
RMSD bond lengths (Å)	0.009
RMSD bond angles (°)	1.2
Ramachandran analysis	
Favored (%)	97.7
Allowed (%)	2.0
Outliers (%)	0.3

Data were obtained from a single crystal.
*Values in parentheses refer to the high-resolution shell.

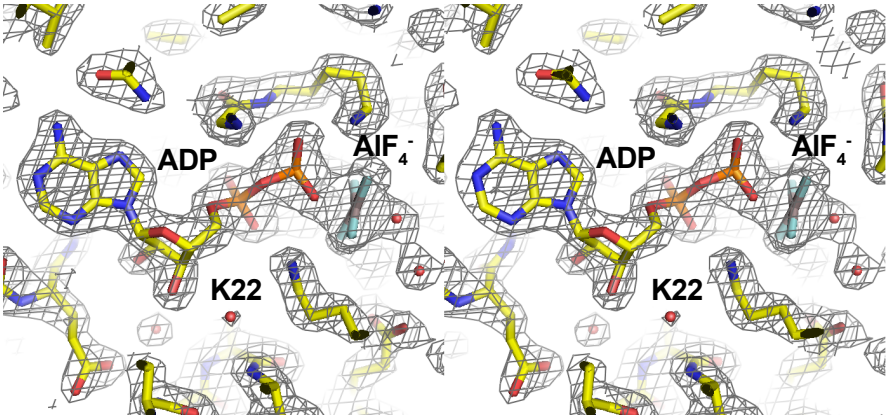

Figure S1. Cross-eyed stereo view of electron density for the Mg^{2+}ADP•AlF_4^- complex of *M. thermautotrophicus* TRC40. The refined model is superimposed onto a σ_A-weighted $2F_o$-F_c map calculated at 2.1 Å resolution and contoured at 2σ.

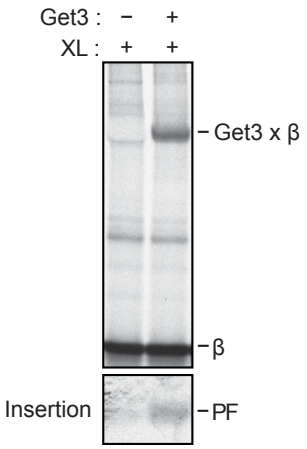

Figure S2. Characterization of the depleted translation extract (PD-RRL). Sec61β was translated in PD-RRL in the presence or absence of recombinant yeast Get3. An aliquot was subjected to chemical crosslinking with DSS to evaluate substrate interactions with 5 ng/µl Get3 (top panel). The remainder was incubated with yRMs and analyzed for insertion by the protease protection assay (bottom panel). PD-RRL lacks all known chaperones that interact with ER-directed TA proteins, and is deficient for insertion; activity was restored by supplementing with physiological levels of TRC40/ Get3.