SUPPORTING INFORMATION

Inhibitors of Difficult Protein-Protein Interactions Identified by High Throughput Screening of Multi-Protein Complexes

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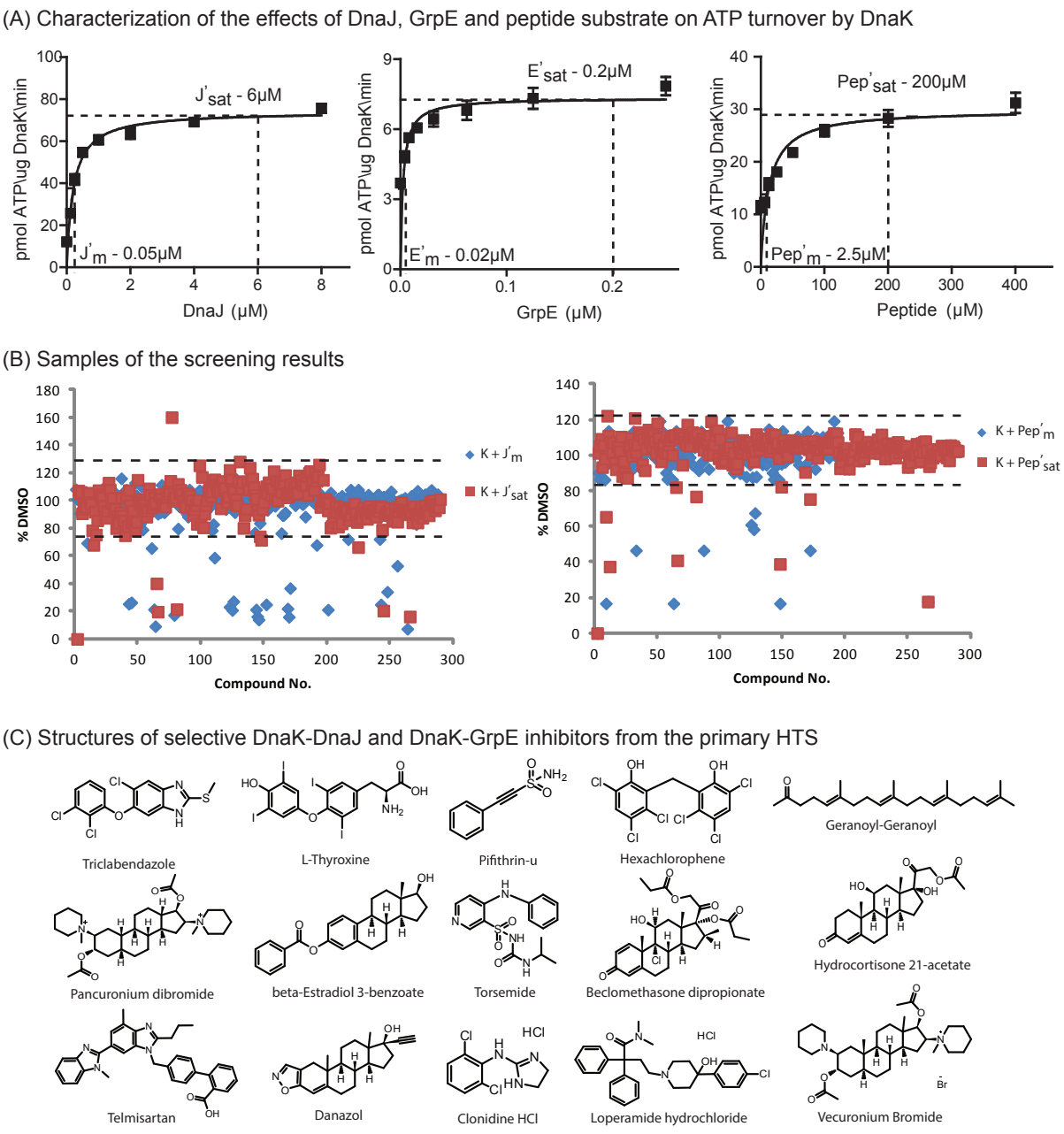
Supplemental Figure 1. Characterization of the DnaK, DnaJ and GrpE proteins and additional information on the high throughput screening results, including the chemical structures of the active molecules.

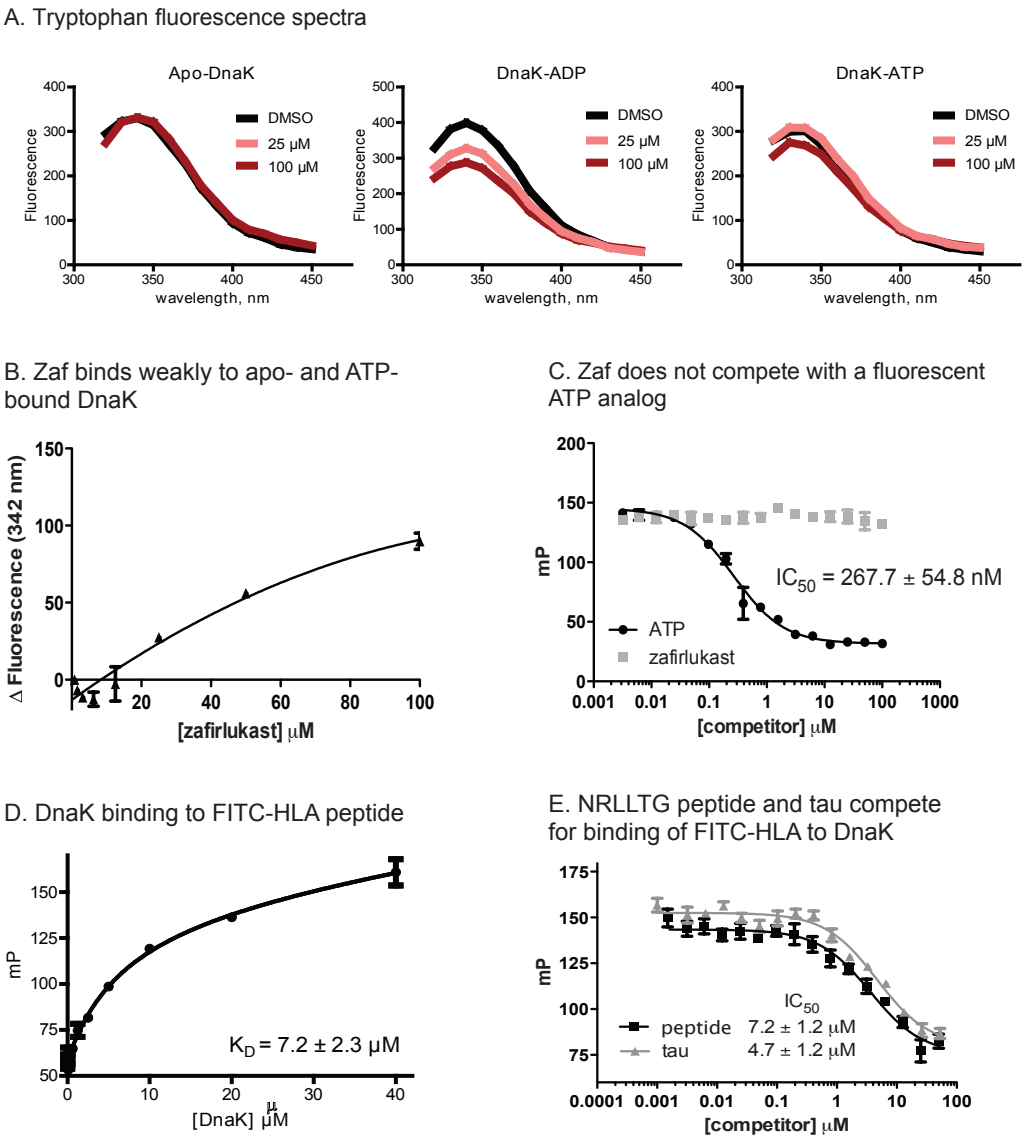
Supplemental Figure 2. Control and characterization of Zaf as an inhibitor of DnaK-DnaJ-GrpE, including raw tryptophan fluorescence, competition studies and peptide substrate binding.

Supplemental Figure 3. List of residues in DnaK's NBD and the chemical shift pertubations from Tel binding, as measured by NMR.

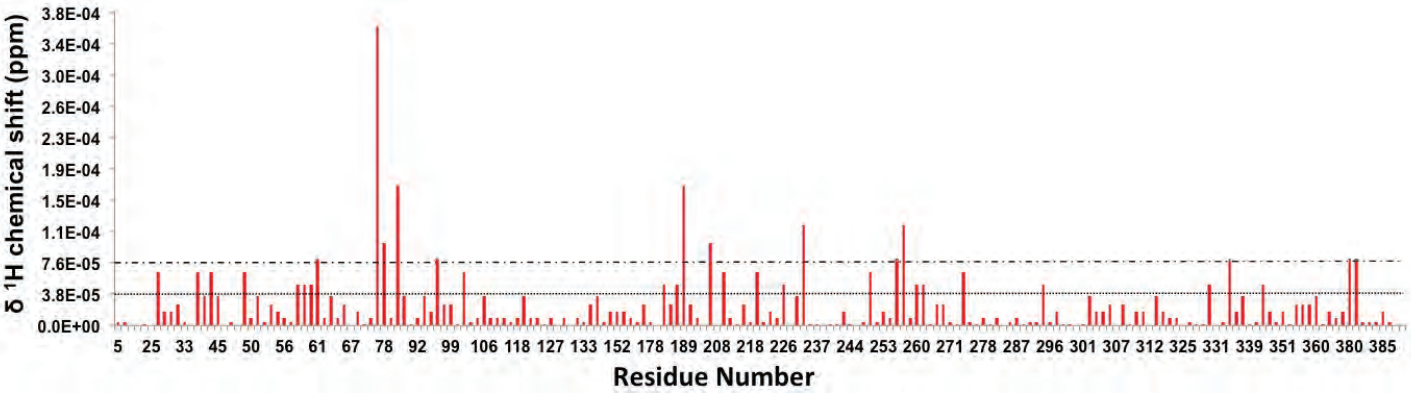
Supplemental Figure 4. Controls and characterization of Tel as an inhibitor of DnaK-DnaJ-GrpE, including ITC data, nucleotide binding and peptide substrate binding.



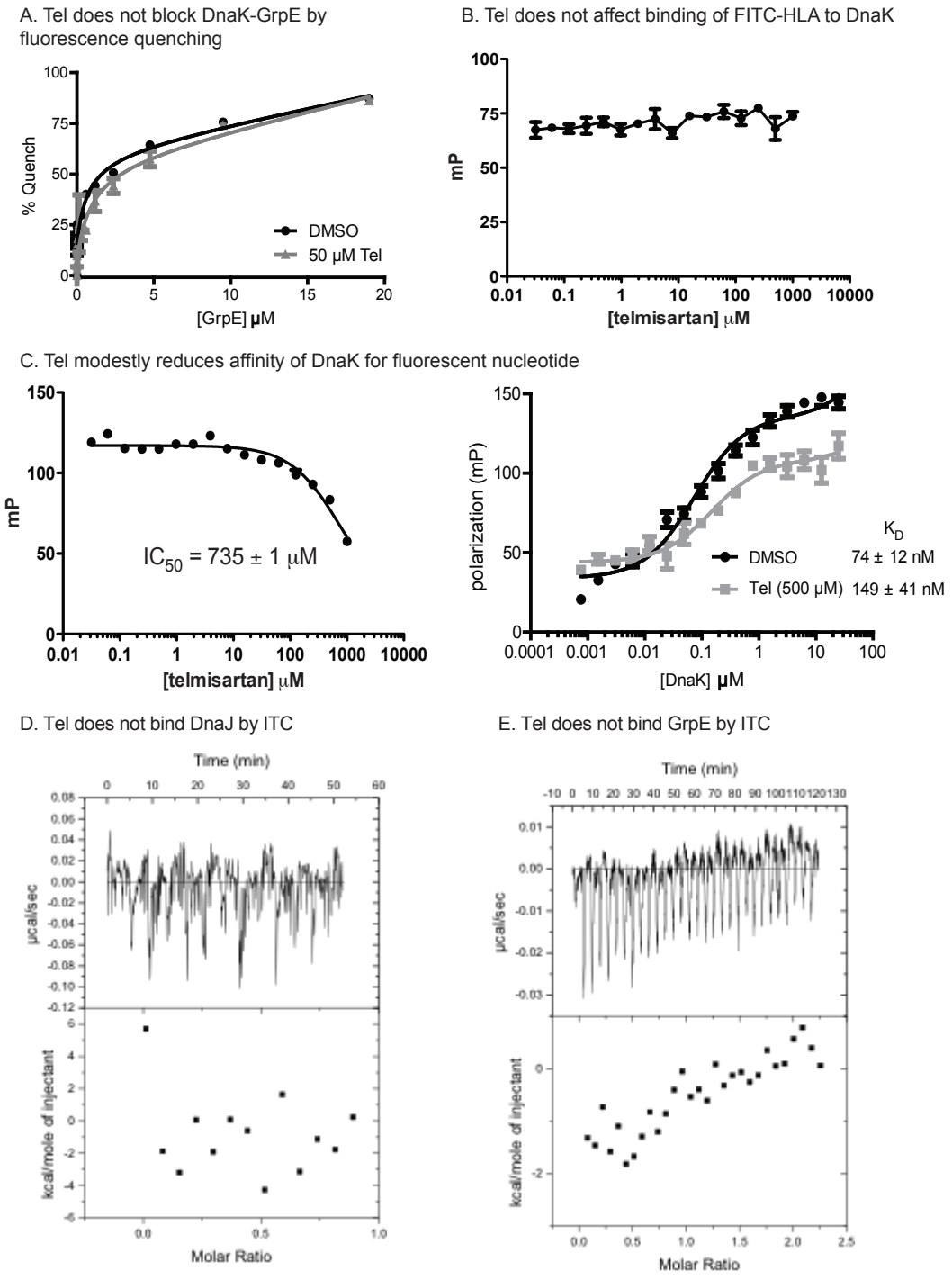
Suppl. Fig 1. Additional details and results from the parallel HTS campaigns. (A) Characterization of the stimulatory effects of DnaJ,GrpE and NRLLLTG peptide on ATP turnover, as measured by malachite green. Results are the representative averages of triplicates of three independent experiments, and error is SEM. Data were fit to the Michaelis-Menten equation. The saturation (sat) and half-maximal (m) values are shown. DnaK = 0.4 μM. (B) Representative examples of the primary, raw screening results, highlighting the effects of saturating non-enzyme partner. (C) Chemical structures of the molecules re-purchased and confirmed as inhibitors of DnaK-DnaJ or DnaK-GrpE.



Suppl. Fig 2. Controls and characterization of Zaf activity in the DnaK-DnaJ-GrpE systems. (A) Raw tryptophan fluorescence spectra of Zaf binding to DnaK in the apo ADP- and ATP-bound forms. Nucleotide was added at 1 mM. Signal for Zaf alone was subtracted. (B) Zaf binds very weakly to the ATP-bound form of DnaK, as measured by tryptophan fluorescence (see Fig 3A for the ADP results). (C) Zaf does not compete with fluorescent nucleotide for binding to DnaK. ATP is shown as a positive control. (D) DnaK binds to the FITC-HLA peptide, similar to what was previously reported for human Hsp72. (E) As positive controls, both NRLLLTG peptide and human tau compete for binding with the FITC-HLA, showing that binding occurs in the SBD. Results are the representative averages of triplicates of three independent experiments, and error bars represent SEM. Binding data were fit to the Langmuir binding equation; inhibition data were fit to the Hill equation.



Suppl. Figure 3. Tel binds DnaK$_{NBD}$ by NMR. Quantification of the changes in the proton NMR chemical shifts after Tel treatment. The dotted line represents the standard deviation of all the changes (s); the dashed line represents 2s.



Suppl. Fig 4. Characterization of Tel effects on binding to GrpE and peptide substrate. (A) Labeled DnaK binding to GrpE was measured as in Fig 3B. Tel did not have any effect on the apparent affinity. (p = 0.33) (B) Tel did not inhibit inhibit binding of DnaK to the FITC-HLA peptide. (C) Tel inhibits binding of fluorescent nucleotide (FAM-ATP) to DnaK, as measured by FP. All results are the representative averages of triplicates of three independent experiments, and error bars represent SEM. Binding data were fit to the Langmuir binding equation; inhibition data were fit to the Hill equation. Tel did not bind to either DnaJ (D) or GrpE (E), as measured by ITC.