Supporting Information for:

Development of a Capillary Electrophoresis Platform for Identifying Inhibitors of Protein-Protein Interactions

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Supporting Information:

Figure S-1. ATPase activity of Hsp70-488 compared to Hsp70

Figure S-2. Isothermal calorimetry measurement of binding affinity between Hsp70 and Bag3

Figure S-3. Dose response curves for Hsp70-Bag3 inhibitors identified by CE and FCPIA and cross-testing.

Figure S-4. Correlation of IC50s by CE and FCPIA for confirmed inhibitors

Figure S1. ATPase activity of Hsp70 is unaffected by labeling as shown by the lack of difference between Hsp70 and labeled Hsp70 (Hsp70-488) activity. (A) ATPase stimulation by the Hsp70 co-chaperone J protein. (B) Bag3 effect on Hsp70/J protein ATPase stimulation.

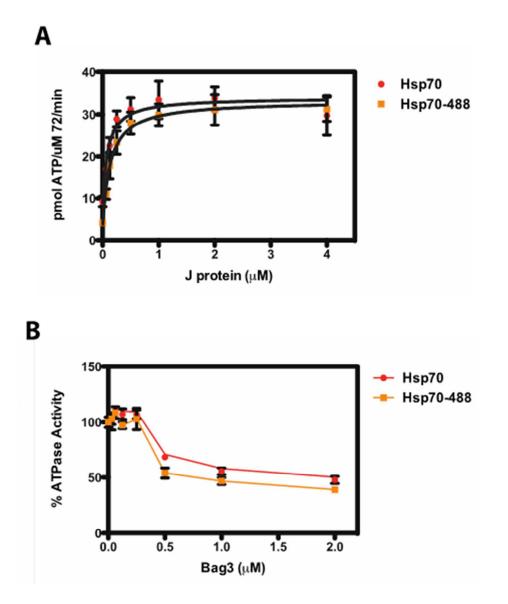


Figure S2. Calorimetric isothermal-titration measurement of Bag3 interaction Hsp70. Both proteins are unlabeled so this measurement is native proteins yet yields a similar K_D to the CE method using labeled Hsp70.

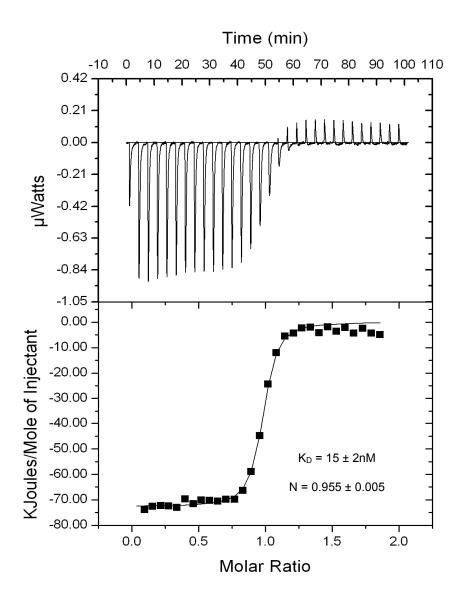
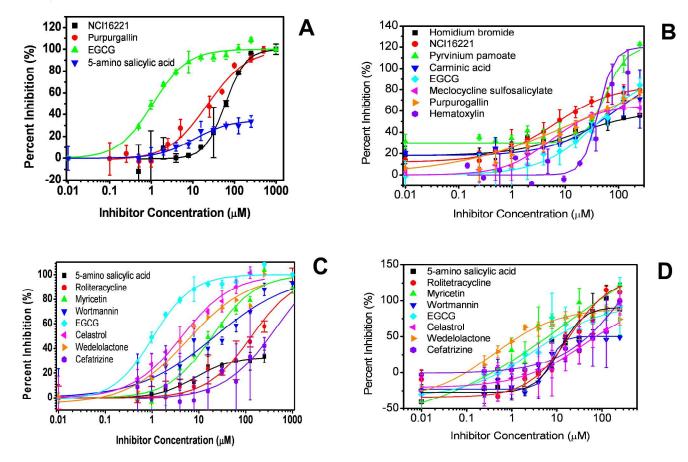


Figure S3. DRCs of confirmed FCPIA hits using (A) CE, (B) FCPIA and CE hits using (C) CE, (D) FCPIA. Only 4 compounds are shown (A) because other FCIPIA hits showed no activity at 100 μ M in the CE assay. Error bars are the range for two replicates.



Inhibitor Concentration (µM)

Percent Inhibition

Figure S4. Comparison of IC50s found by CE and FCPIA. Only compounds that gave IC50 < 400 μ M by both methods were used. Error bars are the range for two replicates.

