Supplementary Materials

DnaJs are enriched in tau regulators

Abigail R. Esquivel[a,b], Shannon E. Hill[a,b], Laura J. Blair[a,b,c,*]

[a]*Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL 33612, USA*
[b]*USF Health Byrd Alzheimer's Institute, University of South Florida, Tampa, FL 33613, USA*
[c]*Research Service, James A Haley Veterans Hospital, Tampa, FL 33612, USA*

*Corresponding Author
Email:
laurablair@usf.edu

Address:
4001 E. Fletcher Ave.
MDC 36
Tampa, FL 33613

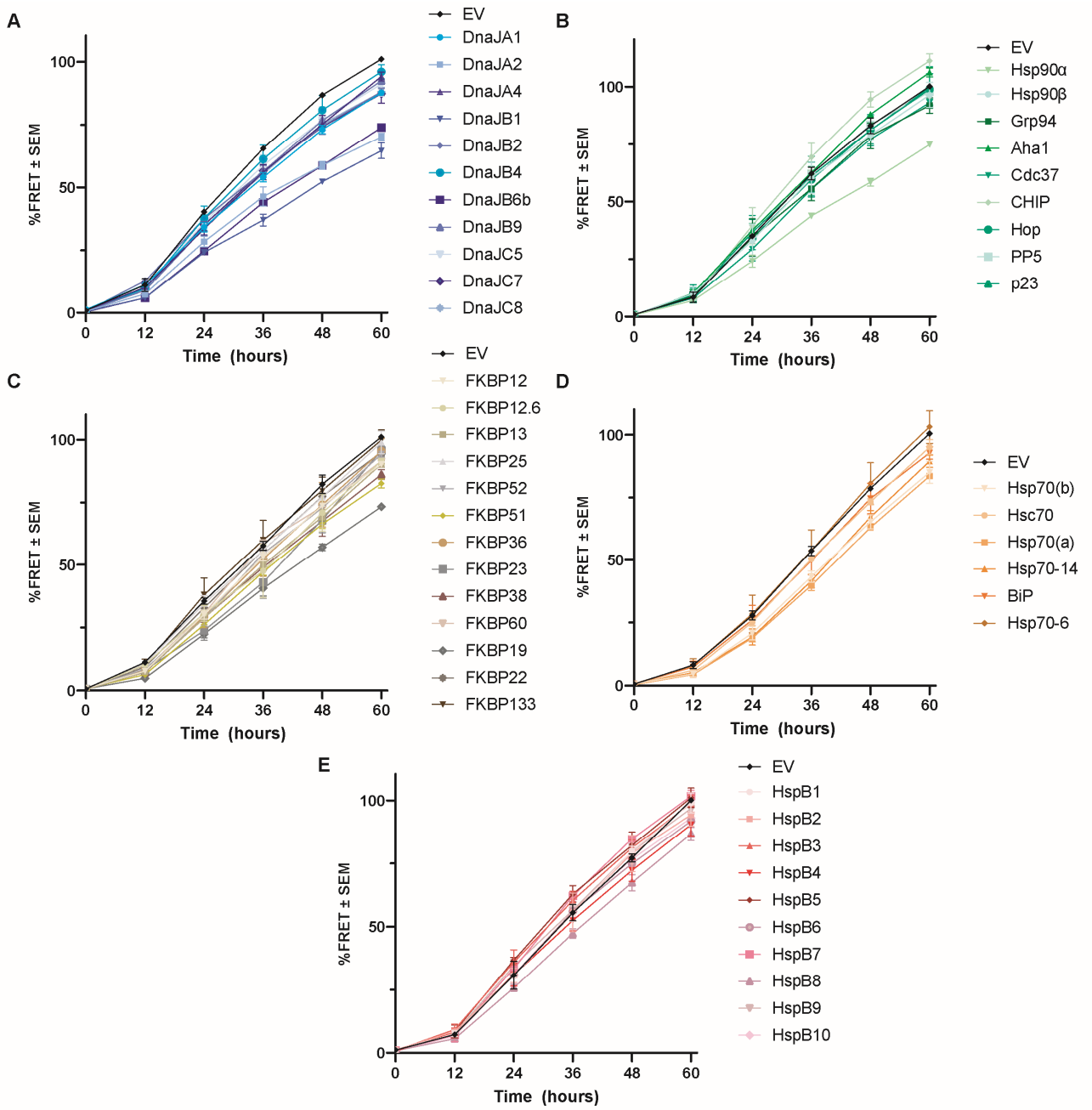

Figure S1. Kinetic data from Tau RD P301S FRET biosensor screen. A semi-high throughput Tau RD P301S FRET biosensor assay was used to screen 49 molecular chaperones from five chaperone families were screened for their effects on tau seeding. For each chaperone, FRET intensity within the total cell area at 60 hours was normalized to EV control to calculate the relative %FRET signal. Kinetic data show the average of 2 independent experiments as %FRET ± S.E.M. for chaperone members of the (A) DnaJ family, (B) Hsp90 and Hsp90 cochaperone families, (C) FKBP family, (D) Hsp70 family, and (E) sHsp family compared to their EV control, respectively. Data analysis was performed by repeated measures ANOVA with a Greenhouse-Geisser correction over the course of the experiment across each family of chaperones, except for Hsp90 and Hsp90 cochaperones, which were combined. Within subjects ANOVA showed the DnaJ group [$F(20.532,111.991)=3.415$], FKBP group [$F(24.705,133.028)=1.794$], Hsp90 and Hsp90 cochaperone group [$F(17.779,98.770)=6.090$] and Hsp70 group [$F(24.705,133.028)=1.794$] were all significant on tau seeding. Within each group, Dunnett's post hoc test revealed individual chaperone members with significant effects on tau seeding in DnaJ (DnaJA2, DnaJB1 and DnaJB6b), Hsp90 and Hsp90 cochaperone (Hsp90α) and FKBP (FKBP19) groups. The 60-hour timepoint from these data are displayed as bar graphs in Figure 2 to display significance.

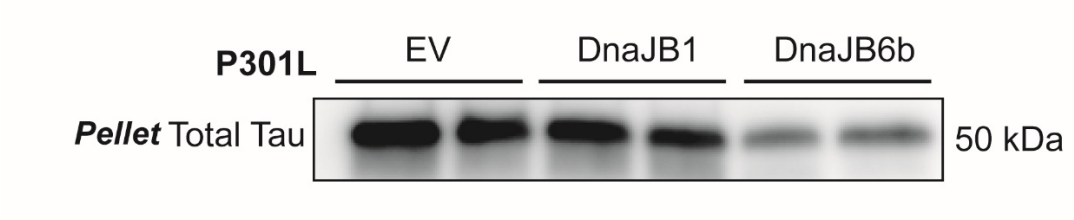

Figure S2. DnaJB6b decreases tau recovered from the pellet. Tau expression was induced by tetracycline for 48 hours in iHEK P301L, followed by transfection of DnaJB1, DnaJB6b, or EV for 48 hours prior to harvesting for analysis. Pellets were re-solublized and analyzed by western blot as shown.

Table S1. Summary of statistical analysis.

Figure	Type of Test	Factor	Group	Statistical Value	P value	Post Hoc p<0.05
2A-E /S1	Within Subjects Repeated measures ANOVA with Greenhouse-Geisser correction	Time Elapsed x Chaperone	DnaJ	F(20.532,111.991)=3.415	0.000	DnaJA2, DnaJB1, DnaJB6b
			FKBP	F(24.705,133.028)=1.794	0.019	FKBP19
			Hsp90 and Hsp90 cochaperone	F(17.779,98.770)=6.090	0.000	Hsp90a
			Hsp70	F(24.705,133.028)=1.794	0.033	None
			sHsp	F(14.108,77.591)=1.103	0.369	None
3B	One-way ANOVA	Chaperone	DnaJ	F(3,4)=240.9	<0.001	DnaJA2, DnaJB1, DnaJB6b
3B			Hsp90, Hsp90 cochaperone, and FKBP	F(2,3)=17.52	<0.05	None
4B		Chaperone	DnaJ	F(3,4)=20.07	<0.01	DnaJB1 and DnaJB6b
5B		Chaperone	iHEK P301L	F(2,9)=7.443	<0.05	DnaJB6b
5D		Chaperone	iHEK WT	F(2,9)=19.97	<0.001	DnaJB6b
5F		Chaperone	iHEK ΔK280	F(2,9)=12.05	<0.05	DnaJB6b
6B	Two-way ANOVA	Chaperone	Inhibitors	F (1, 8) = 32.46	<0.05	DnaJB6b DMSO and DnaJB6b Leup

Table S2. Summary of plasmids used in experiments.

Plasmid Name	Alternative Names	Vector Backbone	Tags	Source or Reference
Hsp90α	HSP90AA1	pCMV6	N-FLAG	Dr. Leonard Neckers
Hsp90β	HSP90AB1	pCMV6	N-FLAG	Dr. Leonard Neckers
Grp94	HSP90B1	pCMV6		Generated by our lab
Hsp70	HSPA1B/Hsp72	pCMV6		[30]
Hsc70	HSPA8/Hsp73	pCMV6		[30]
BIP	HSPA5/Grp78	pCMV KDEL	C-Myc	[100]
Hsp70 -14	HSPA14	pCDNA5 FRT/TO/V5		[101]
Hsp70 - 6	HSPA6/HSP70B	pCDNA5 FRT/TO/V5		[101]
Hsp70	HSPA1A/Hsp72	pCDNA5 FRT/TO/V5		[101]
p23	Prostaglandin E synthase 3	pCMV6		[102]
Aha1	AHSA1/p38	pCDNA 3.1		[40]
Cdc37		pCMV6		[102]
PP5	PPP5C	pCMV6-SPORT6		Generated by our lab
CHIP	STUB1	pCDNA3.1		[103]
HOP	HOPX	pCMV6		Generated by our lab
DnaJA1	HDJ2	pCMV6	N-FLAG	[64]
DnaJA2		pCMV6	N-FLAG	Subcloned into pCMV6 from cDNA [101]
DnaJA4		pCMV6	N-FLAG	Subcloned into pCMV6 from cDNA [101]
DnaJB1	HDJ1	pCMV6	N-FLAG	Subcloned into pCMV6 from cDNA [30]
DnaJB2A		pCMV6	N-FLAG	Subcloned into pCMV6 from cDNA [101]
DNAJB4		pCDNA5 FRT/TO/V5		[101]
DnaJB6B	MRJ	pCMV6	N-FLAG	Subcloned into pCMV6 from cDNA [101]
DnaJB9	MDG1/ERDj4	pCMV6	N-FLAG	Subcloned into pCMV6 from cDNA [101]
DnaJC5	CSPα	pCMV6	N-FLAG	[30]
DnaJC7	TPR2	pCMV6		[30]
DnaJC8	SPF31	pCMV6	N-FLAG	[30]
FKBP12	FKBP1A	pCMV6	N-FLAG	Generated by our lab
FKBP12.6	FKBP1B	pCMV6		Generated by our lab
FKBP13	FKBP2	pCMV6	C-FLAG	Generated by our lab
FKBP19	FKBP11	pCMV6	C-FLAG	Generated by our lab
FKBP22	FKBP14	pCMV6		Generated by our lab
FKBP23	FKBP7	pCMV6	C-FLAG	Generated by our lab
FKBP25	FKBP3	pCMV6	C-FLAG	Generated by our lab
FKBP36	FKBP6	pCMV6		Generated by our lab
FKBP38	FKBP8	pCMV6		Generated by our lab
FKBP51	FKBP5	pCMV6		[104]

FKBP52	FKBP4	pCMV6		[104]
FKBP60	FKBP9	pCMV6	C-FLAG	Generated by our lab
FKBP133	FKBP15	pCMV6	C-FLAG	Generated by our lab
HspB1	Hsp27	pCDNA5 FRT/TO		[105]
HspB2		pCDNA5 FRT/TO		[105]
HspB3		pCDNA5 FRT/TO		[105]
HspB4		pCDNA5 FRT/TO		[105]
HspB5	CRYAB/αB-crystallin	pCDNA5 FRT/TO		[105]
HspB6		pCDNA5 FRT/TO		[105]
HspB7		pCDNA5 FRT/TO		[105]
HspB8	Hsp22	pCDNA5 FRT/TO		[105]
HspB9		pCDNA5 FRT/TO		[105]
HspB10		pCDNA5 FRT/TO		[105]
P301L 4R0N Tau		pET-28a	6x His	Generated by our lab
P301L 4R0N Tau		pCMV6		Generated by our lab
empty vector		PCMV6		Origene, #RC223397
empty vector		PCMV6	N-FLAG	Origene, #PCMV6XL6
empty vector		pCDNA5 FRT/TO		Invitrogen #V6520-20

[100] J. Shen, X. Chen, L. Hendershot, R. Prywes, ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of golgi localization signals, Developmental Cell 3(1) (2002) 99-111.
[101] J. Hageman, H.H. Kampinga, Computational analysis of the human HSPH/HSPA/DNAJ family and cloning of a human HSPH/HSPA/DNAJ expression library, Cell Stress Chaperones 14(1) (2009) 1-21.
[102] U.K. Jinwal, J.H. Trotter, J.F. Abisambra, J. Koren, 3rd, L.Y. Lawson, G.D. Vestal, J.C. O'Leary, 3rd, A.G. Johnson, Y. Jin, J.R. Jones, Q. Li, E.J. Weeber, C.A. Dickey, The Hsp90 kinase co-chaperone Cdc37 regulates tau stability and phosphorylation dynamics, J. Biol. Chem. 286(19) (2011) 16976-83.
[103] C.A. Dickey, A. Kamal, K. Lundgren, N. Klosak, R.M. Bailey, J. Dunmore, P. Ash, S. Shoraka, J. Zlatkovic, C.B. Eckman, C. Patterson, D.W. Dickson, N.S. Nahman, Jr., M. Hutton, F. Burrows, L. Petrucelli, The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins, J. Clin. Invest. 117(3) (2007) 648-58.
[104] U.K. Jinwal, J. Koren, 3rd, S.I. Borysov, A.B. Schmid, J.F. Abisambra, L.J. Blair, A.G. Johnson, J.R. Jones, C.L. Shults, J.C. O'Leary, 3rd, Y. Jin, J. Buchner, M.B. Cox, C.A. Dickey, The Hsp90 cochaperone, FKBP51, increases Tau stability and polymerizes microtubules, J. Neurosci. 30(2) (2010) 591-9.
[105] M.J. Vos, M.P. Zijlstra, B. Kanon, M.A. van Waarde-Verhagen, E.R. Brunt, H.M. Oosterveld-Hut, S. Carra, O.C. Sibon, H.H. Kampinga, HSPB7 is the most potent polyQ aggregation suppressor within the HSPB family of molecular chaperones, Hum. Mol. Genet. 19(23) (2010) 4677-93.