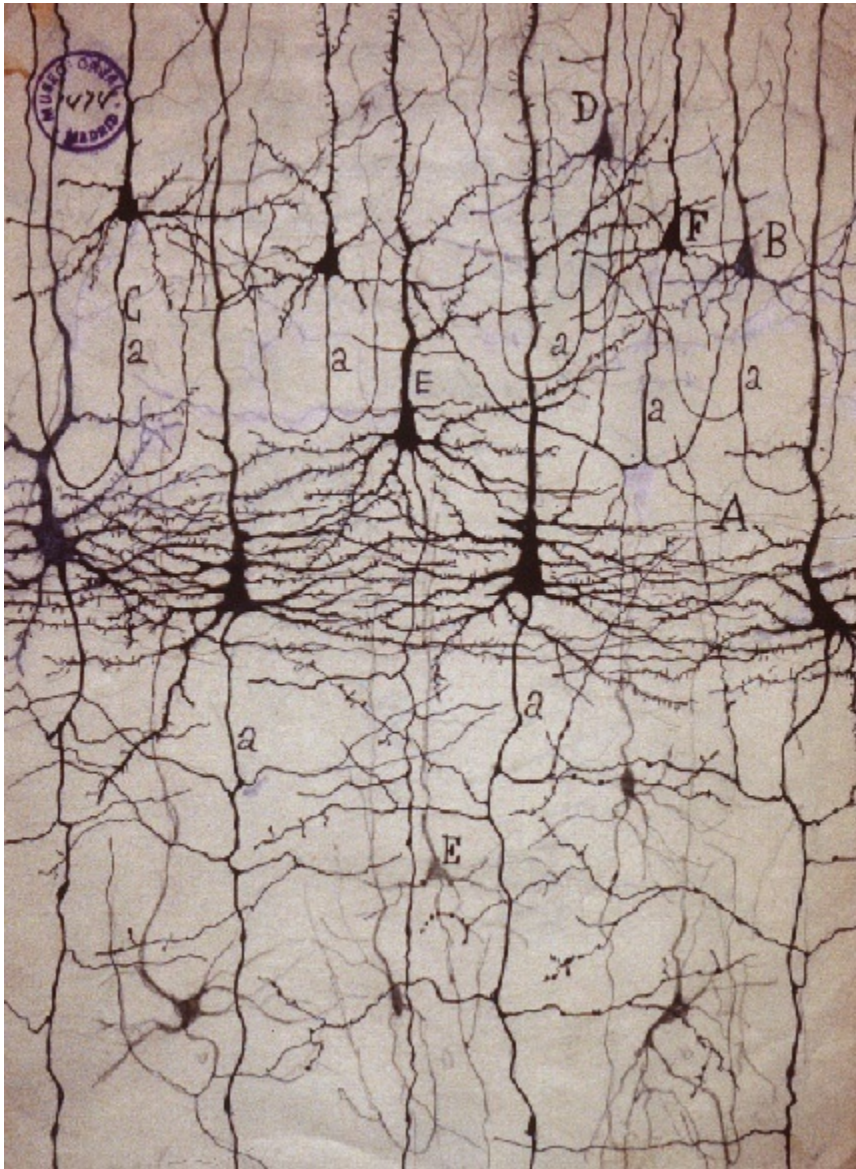


Neuronal Substrates Required for Synapse Maintenance



Sreeganga S. Chandra

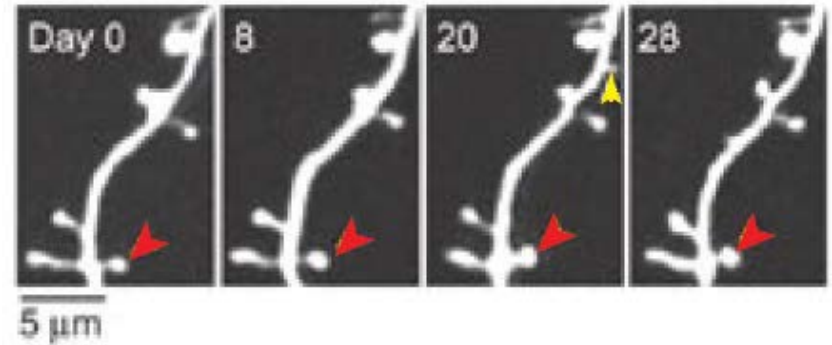
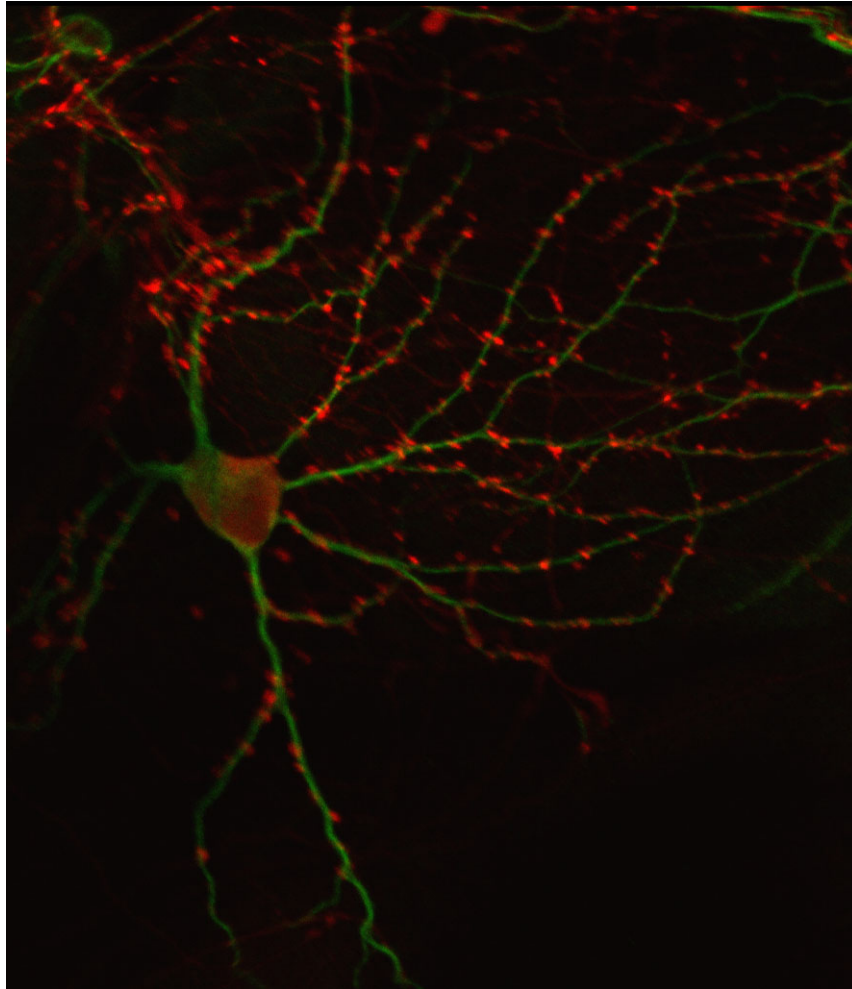
CNNR Program

Dept. of Neurology

Dept. of Mol. Cell & Dev. Biol.

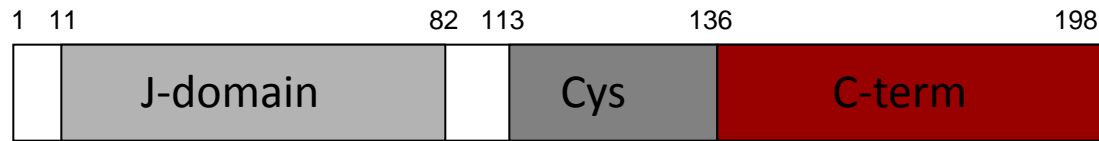
Yale University

The Majority of Synapses are Stable in the Healthy Brain

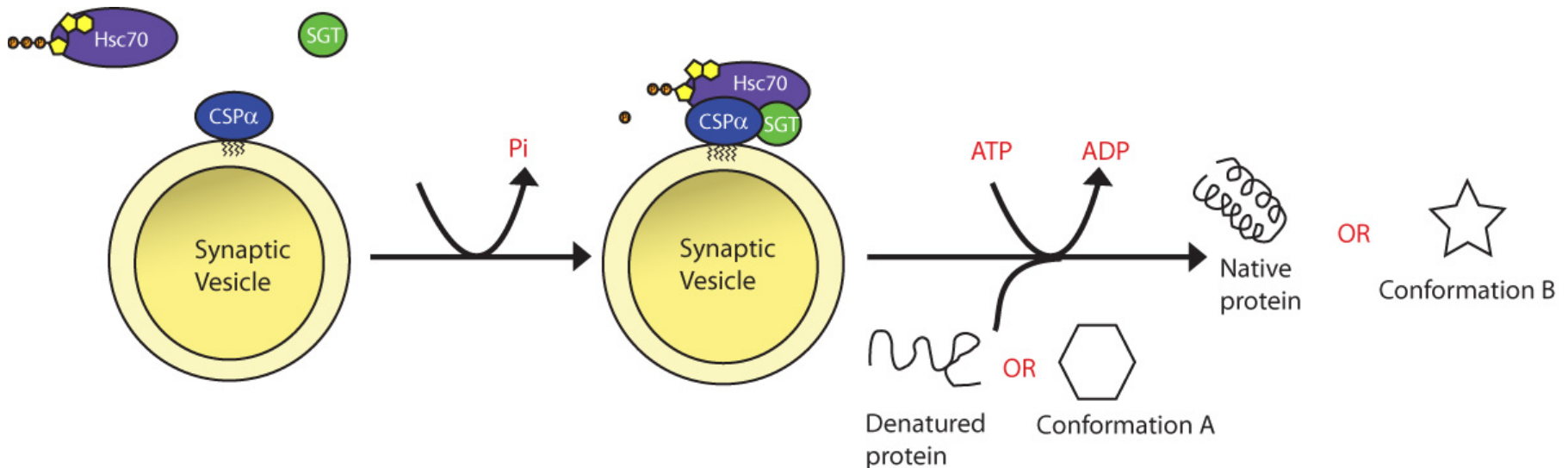


- *Two-photon imaging studies have shown that > 70% synapses are stable for 15 months*

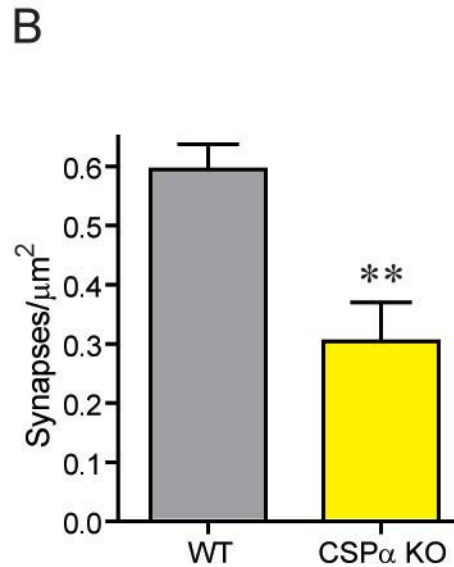
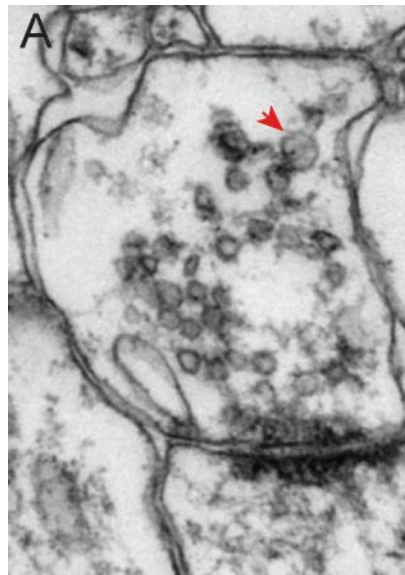
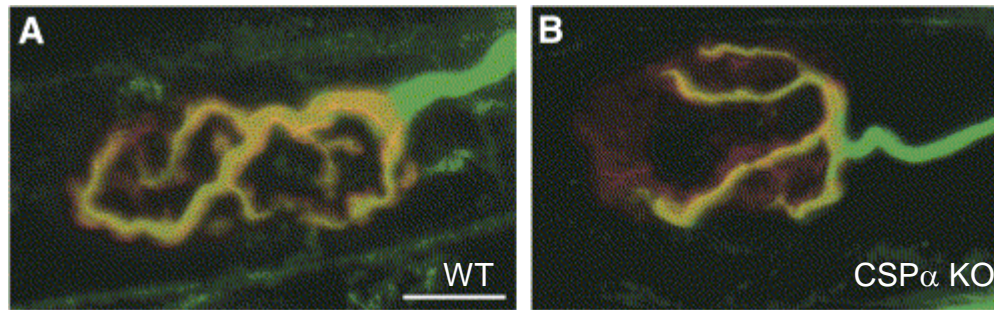
Synapse Maintenance--Cysteine String Protein α



- *CSP α is brain specific; synaptic vesicle protein*
- *Co-chaperone, has a N-terminal J-domain*
- *Binds Hsc70 and SGT (small glutamine-rich tetratricopeptide repeat-containing protein) to form a functional chaperone*

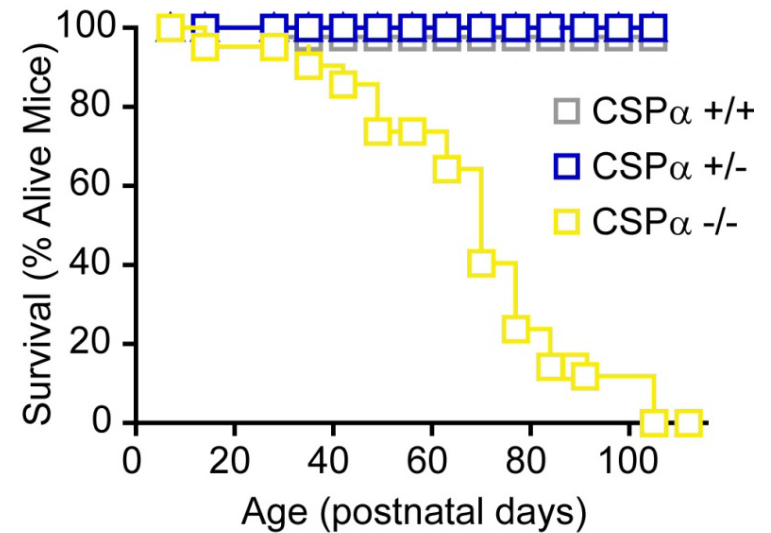
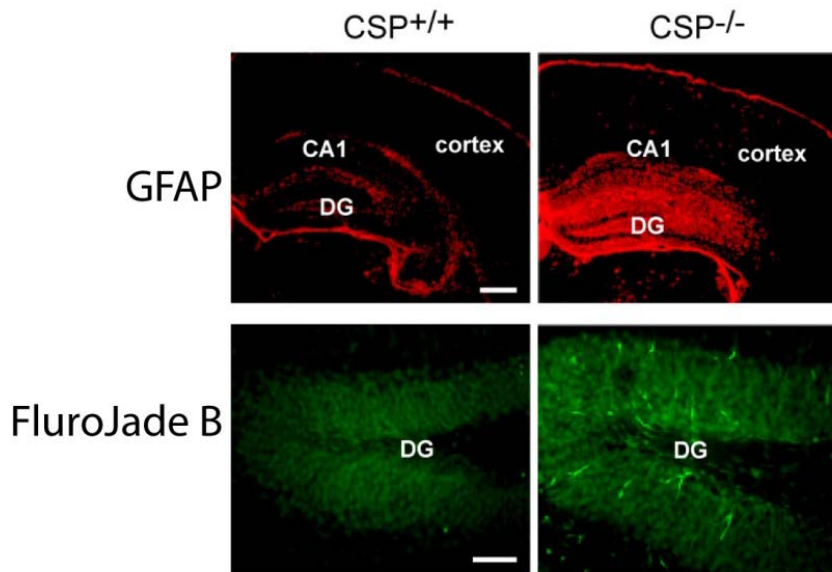


CSP α Is Critical For Synapse Maintenance

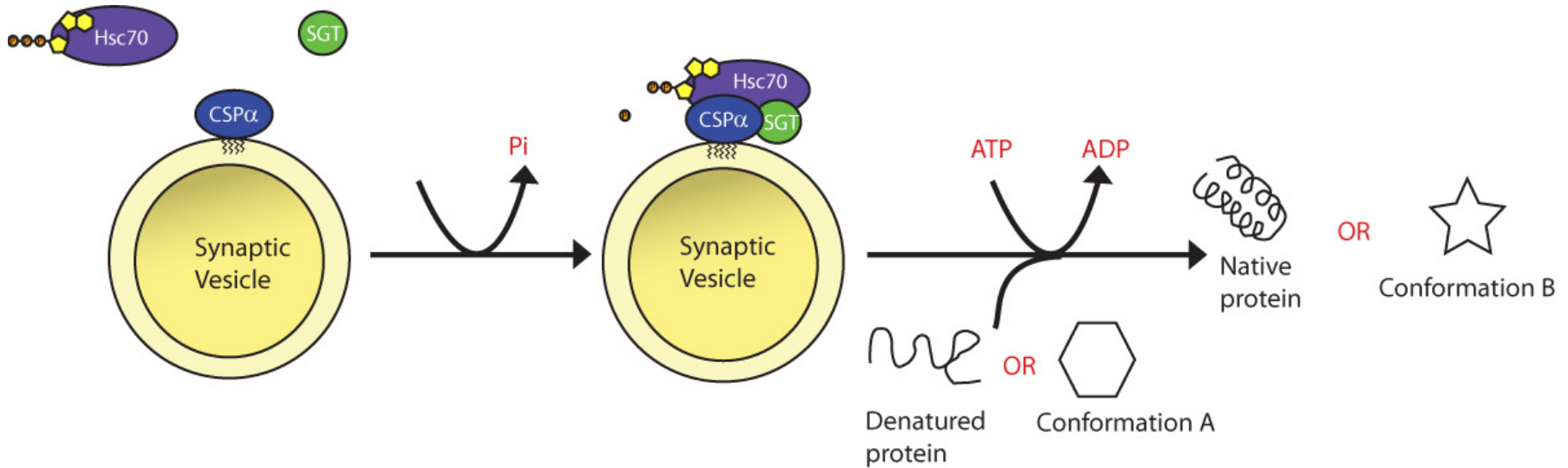


- *CSP α KO have normal neurodevelopment (P20), but subsequently exhibit progressive synapse loss*

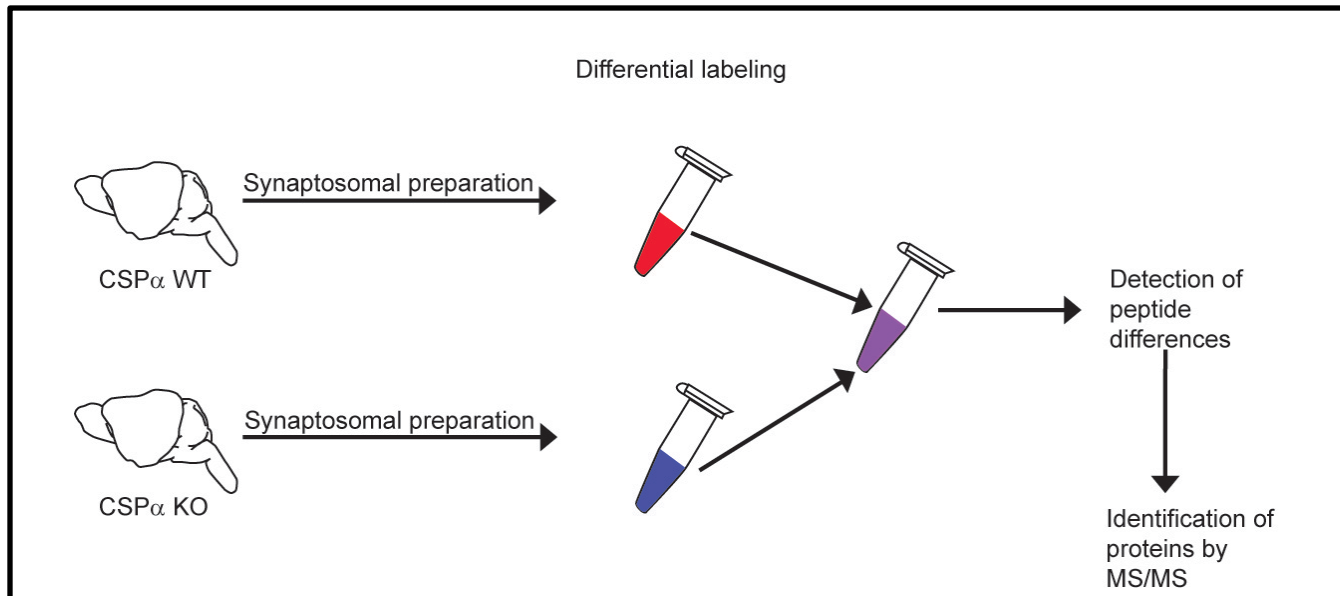
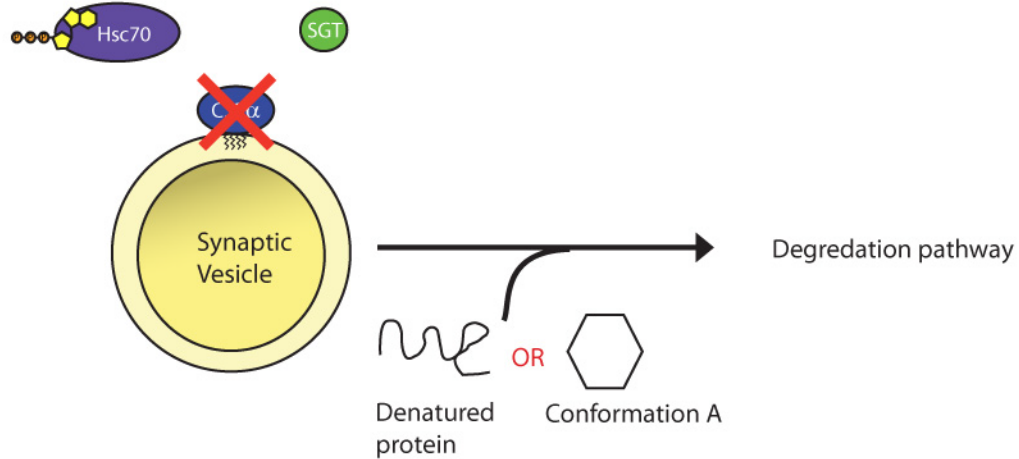
CSP α KO Exhibits Neurodegeneration & Early Lethality



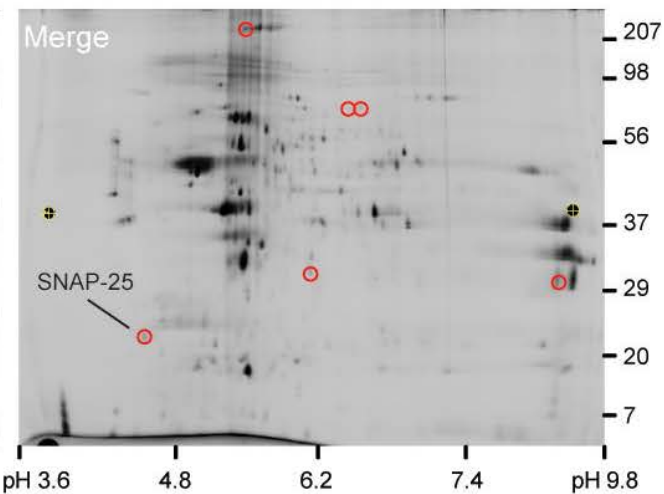
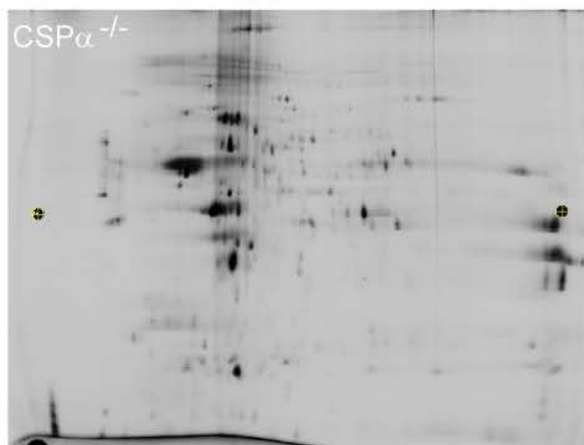
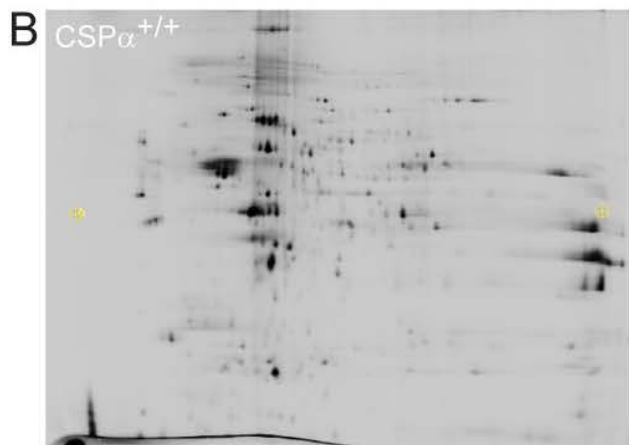
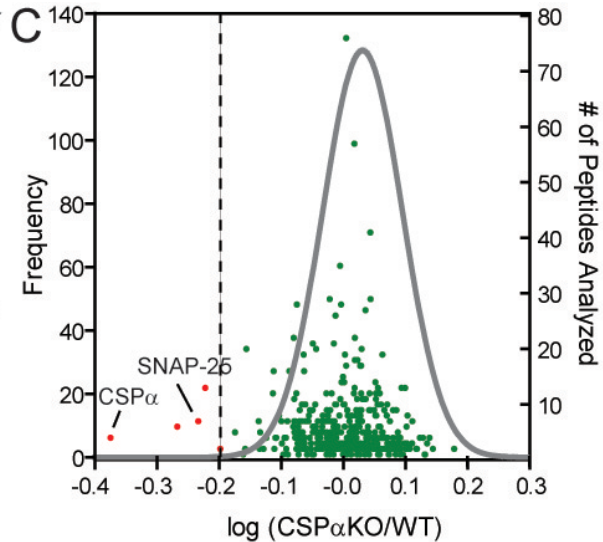
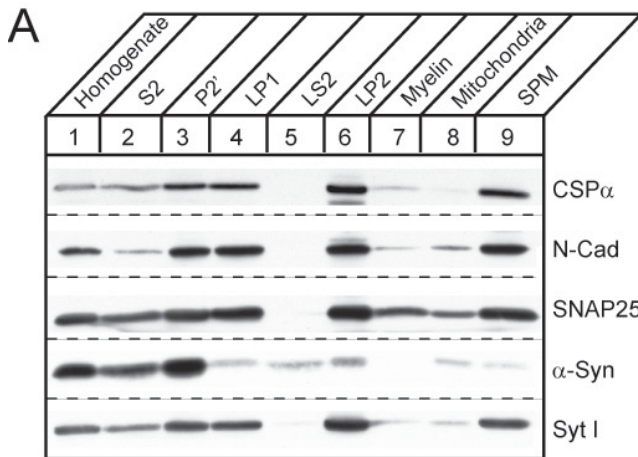
How Does CSP α Maintain Synapse Stability?



Rationale for Proteomic Screen for CSP α clients



DIGE & iTRAQ Analysis of CSP α KO Synaptic Fractions



SNAP-25 is a known client of the CSP α chaperone complex

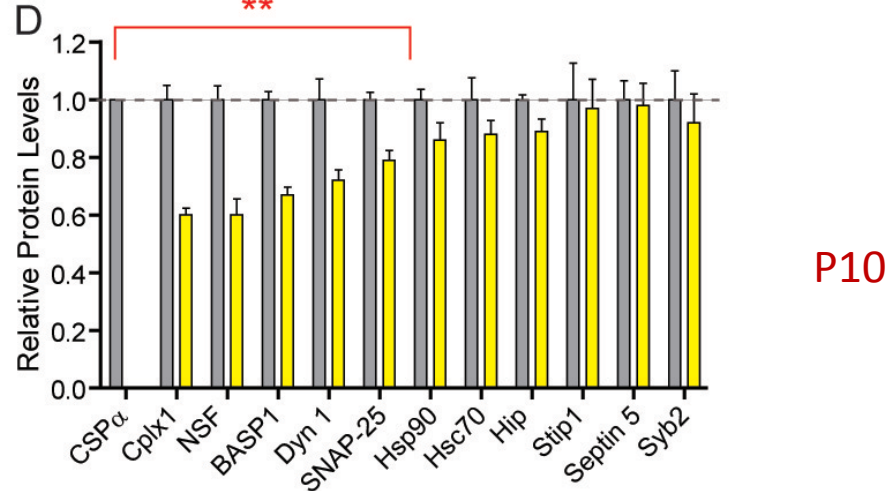
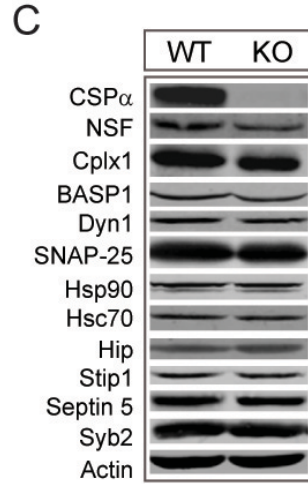
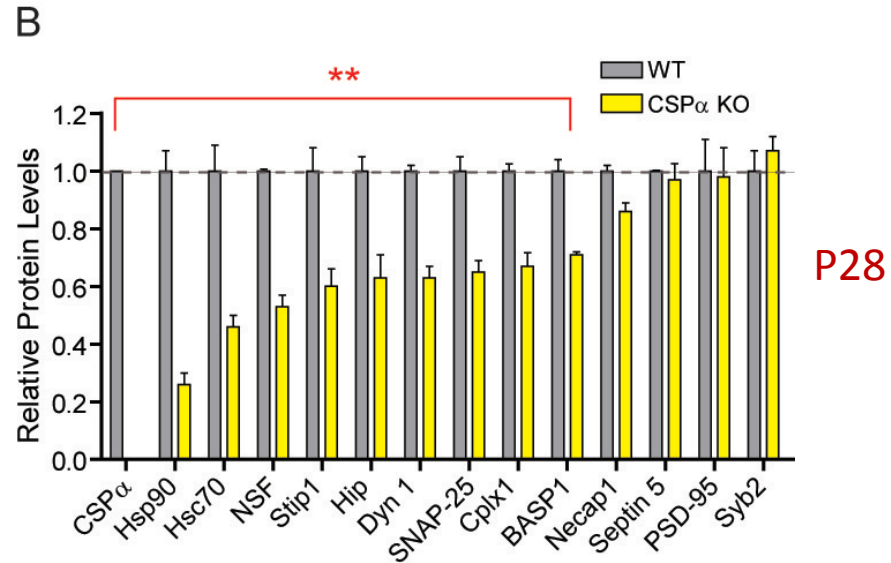
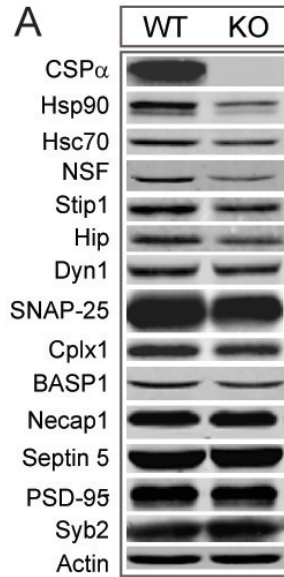
DIGE and iTRAQ Identify Novel CSP α Client Proteins

<i>Protein ID</i>	<i>Gene</i>	<i>Protein Name</i>	<i>Fold Change</i>	<i>Method</i>	<i>Peptides</i>	<i>Fraction</i>	<i>Validated</i>	<i>Method</i>
CHAPERONES								
IPI00875866	Dnajc5	CSP α	-4.18	iTRAQ	3	Vesicle/Memb	+	WB
IPI00330804	Hsp90aa1	Inducible Hsp90	-1.80	DIGE	34, 29	Cytosol	+	WB
IPI00229080	Hsp90ab1	Constitutive Hsp90	-1.80	DIGE	31-38	Cytosol	NT	
IPI00319992	Hspa5	Hsp70-5/GRP78	-1.78	DIGE	21	Membrane/Cytosol	+	MRM
IPI00123802	Hsph1	Hsp 105/110	-1.71	iTRAQ	5	Cytosol	NT	
IPI00323357	Hspa8	Hsc70	-1.67	DIGE/iTRAQ	21-33	Vesicle/Memb/Cytosol	+	MRM/WB
IPI00121514	Stip1	HOP	-1.54	DIGE	42, 33	Cytosol	+	MRM/WB
IPI00317711	Hspa4l	Hsp70-4 like	-1.47	iTRAQ	7	Cytosol	NT	
IPI00116308	St13	Hip	-1.40	DIGE/iTRAQ	4	Cytosol	+	WB
IPI00116279	Cct5	Chaperonin TriC	-1.44	DIGE	20	Cytosol	+	MRM
EXOCYTOSIS								
IPI00125635	Snap25	SNAP-25b	-3.26	DIGE/iTRAQ	21-22	Vesicle/Memb/Cytsol	+	WB
IPI00626660	Cacnb4	Calcium channel β 4	-1.64	iTRAQ	2	Membrane	NT	
IPI00656325	Nsf	NSF	-2.32	DIGE	7	Membrane	+	MRM/WB
IPI00132278	Cplx1	Complexin 1	-1.46	DIGE/iTRAQ	3, 7	Cytosol	+	WB
ENDOCYTOSIS								
IPI00845595	Dnm1	Dynamin 1	-2.44	DIGE	9-41	Vesicle/Memb	+	MRM/WB
IPI00225533	Necap1	Necap 1	-1.61	iTRAQ	4	Vesicle	+	MRM/WB

DIGE and iTRAQ Identify Novel CSP α Client Proteins

<i>CYTOSKELETAL</i>								
IPI00845581	Dpysl4	Crmp3	-2.34	DIGE	14	Vesicle	NT	
IPI00808312	Sept3	Septin 3	-2.20	DIGE	7	Vesicle	+	MRM
IPI00850740	Sept5	Septin 5	-1.97	DIGE	15-22	Vesicle/Memb	-	WB
IPI00224626	Sept7	Septin 7	-1.90	DIGE	8-16	Vesicle	+	MRM
IPI00473707	Sept6	Septin 6	-1.64	DIGE	13	Vesicle	NT	
IPI00129519	Basp1	BASP1	-1.50	DIGE	3	Vesicle	+	WB
IPI00114375	Dpysl2	Crmp2	-1.41	DIGE	13	Cytosol	+	MRM
<i>SIGNALING</i>								
IPI00222430	Dbi	Diazepam binding inhib.	-2.00	iTRAQ	2	Vesicle	NT	
IPI00750256	Ak1	Adenylate kinase 1	-1.94	DIGE	6	Vesicle	+	MRM
IPI00227773	Ppp1cc	Protein phosphatase 1c	-1.68	iTRAQ	2	Vesicle	NT	
IPI00626797	Dlg4	PSD-95	-1.67	iTRAQ	2	Vesicle	-	MRM/WB
IPI00309207	Pafah1b1	PAFacetylhydrolase IB α	-1.60	iTRAQ	3	Vesicle	NT	
IPI00230418	Vsnl1	Visinin-like	-1.47	DIGE	16	Cytosol	+	MRM
IPI00653237	Wdr7	Rabconnectin 3 β	-1.46	iTRAQ	1	Cytosol	-	MRM
IPI00761443	Iqsec1	ARF-GEP	-1.42	iTRAQ	1	Cytosol	+	MRM
<i>OTHER</i>								
IPI00331016	Sec24b	Sec24b	-2.44	iTRAQ	2	Vesicle	NT	
IPI00263013	Plp1	Proteolipid protein 1	-1.91	iTRAQ	6	Membrane	NT	
IPI00223377	Mbp	Myelin basic protein	-1.68	iTRAQ	13	Membrane	NT	
IPI00132734	Dynl12	Dynein light chain 2	-1.57	iTRAQ	5	Vesicle	NT	
IPI00845803	Snca	α -Synuclein	-1.49	DIGE/iTRAQ	3	Vesicle	NT	
IPI00131614	Snca	β -Synuclein	-1.43	DIGE	8	Cytosol	NT	

Confirmation of CSP α Client Protein Levels-Quantitative Immunoblotting

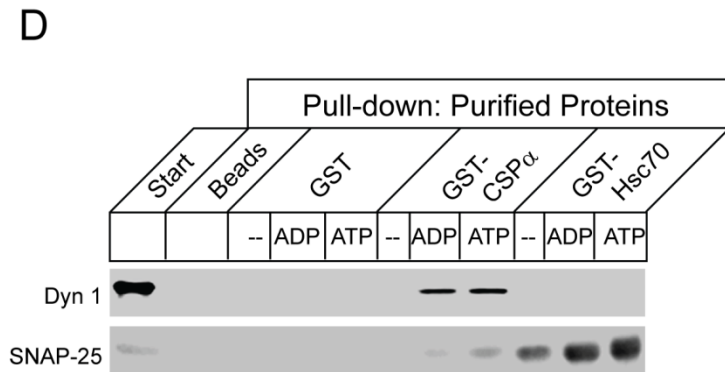
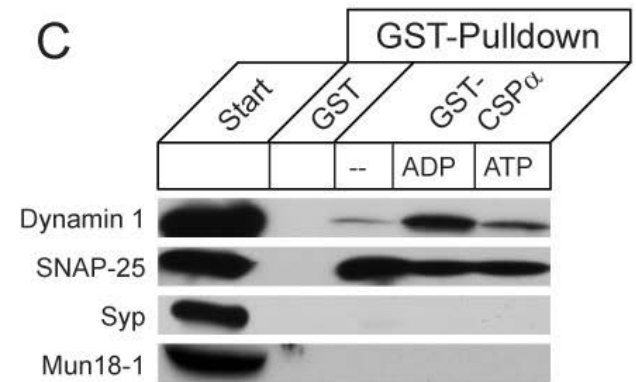
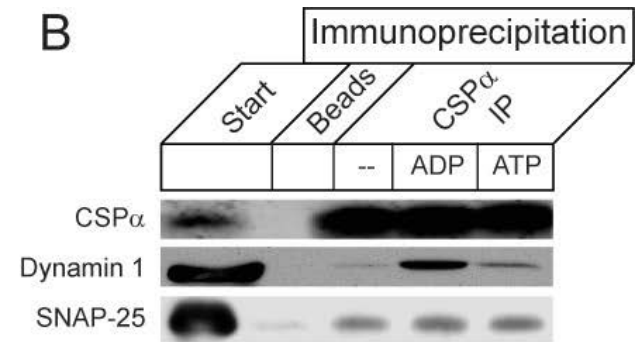
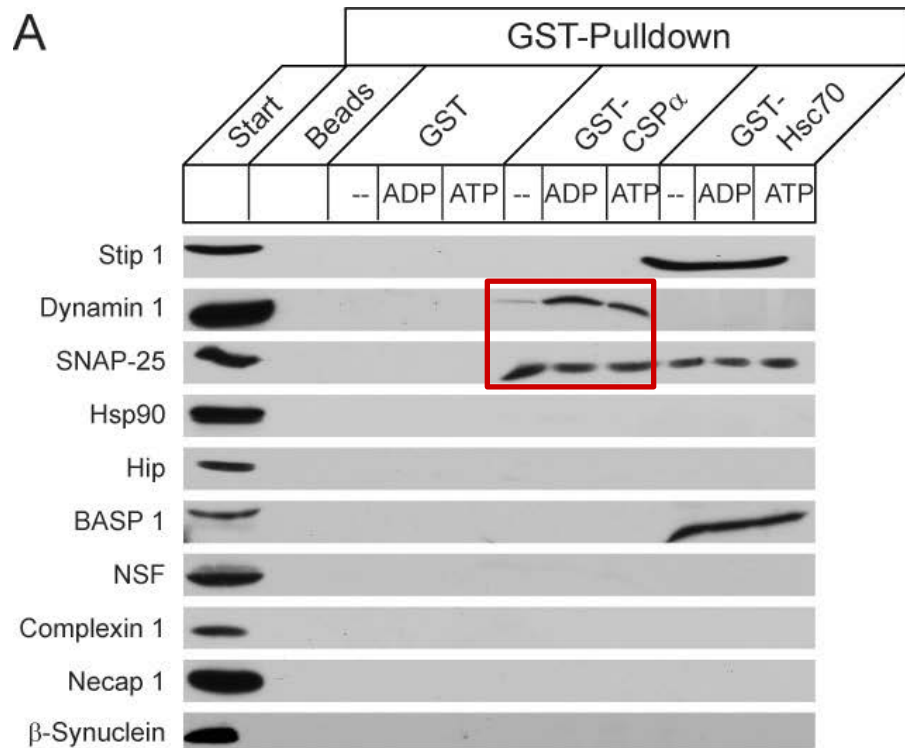


Confirmation of CSP α Client Protein Levels- MRM Analysis

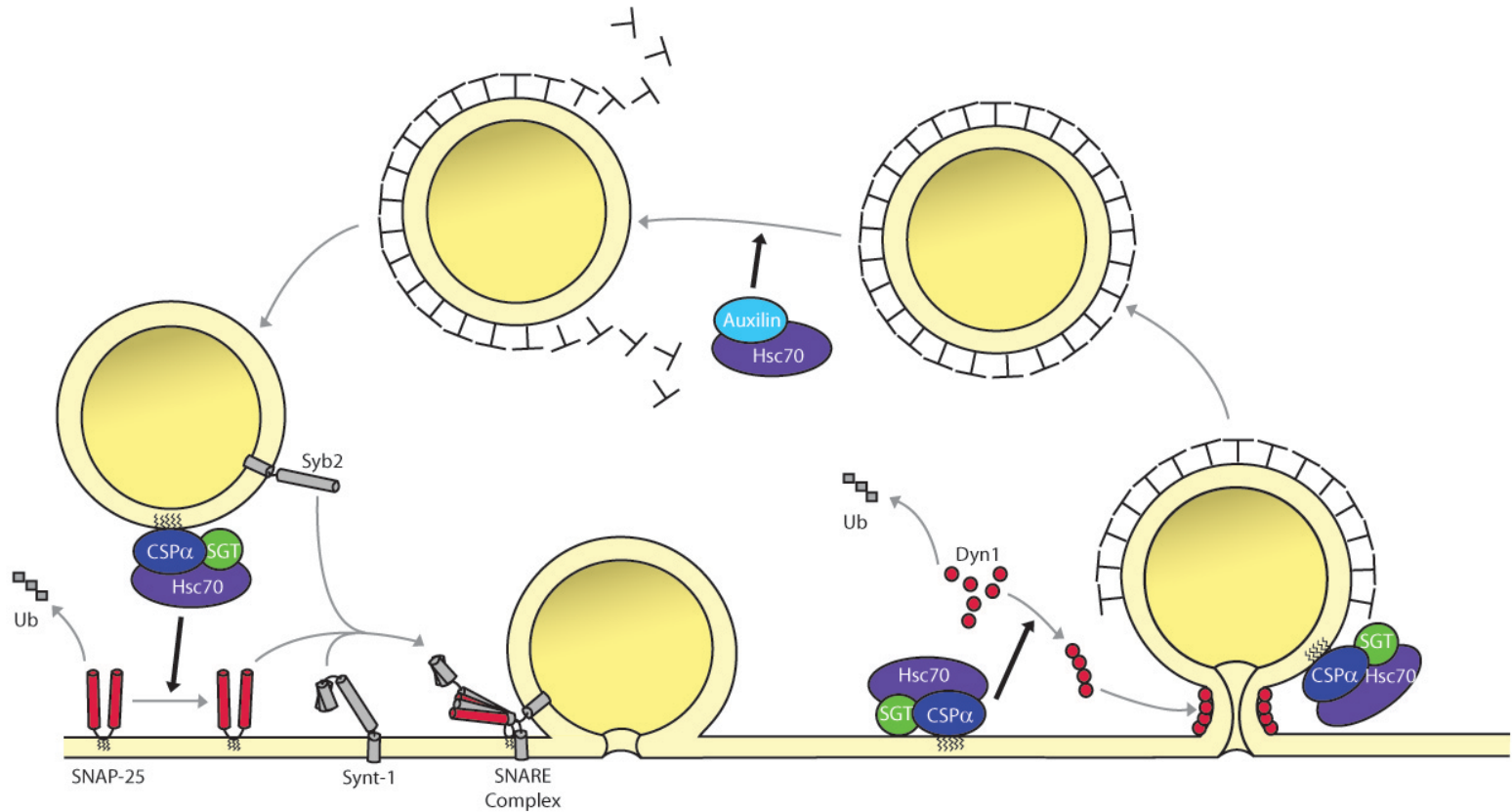
Gene	Protein	Peptide	Vesicle (CSP α /WT)			Membrane (CSP α /WT)			Cytosol (CSP α /WT)		
			Avg	SEM	p-value	Avg	SEM	p-value	Avg	SEM	p-value
Ak1	Adenylate kinase	IIFVVGPGSGK	0.598	0.0128	3.51E-16	0.721	0.0234	5.26E-07	0.809	0.00620	1.49E-12
Cct5	Chaperonin Tric	AVTIFIR	0.603	0.0346	4.25E-08	0.555	0.0476	1.46E-07	0.906	0.0101	6.53E-06
Dlg4	PSD-95	DYHFVSSR				0.946	0.0757	0.507362			
		IIPGAAAQDGR				0.789	0.0269	9.18E-07	0.918	0.0490	0.246505
Dnm1	Dynamin 1	NTYDVVYLK	0.403	0.0200	2.91E-18	0.936	0.0124	0.003323	0.934	0.0735	0.457958
		SSVLENFVGR	0.636	0.00909	6.95E-13	0.765	0.00830	5.1E-11	0.858	0.00801	3.35E-09
		DITAAALAAER	0.672	0.00475	9.91E-13	0.788	0.0138	2.56E-08	1.019	0.0127	0.315132
		FPFELVK	0.568	0.00981	9.01E-10	0.781	0.0157	7.66E-11	0.837	0.00431	3.36E-14
		GYIGVNR	0.602	0.0150	1.63E-17	0.706	0.0137	1.81E-10	0.795	0.0109	4.7E-10
Dpyl2	Crmp2	LQSQLLSIEK	0.621	0.0235	4.09E-11	0.866	0.0285	0.001559	0.822	0.0111	3.04E-08
		SPTSSPTPQR	0.541	0.119	0.001847	0.846	0.0998	0.17452	1.025	0.0234	0.366095
		GSPLVVISQGK	0.627	0.0105	9.11E-13	0.682	0.00876	1.29E-13	0.812	0.00328	2.23E-11
		SAAEVIAQAR	0.534	0.00782	1.15E-25	0.720	0.0142	2.09E-10	0.785	0.00657	5.39E-12
Hspa5	Hsp70	VFNLVPR	0.555	0.0173	2.03E-14	0.718	0.00831	1.15E-13	0.779	0.00916	1.29E-11
		ALSSQHQAR	0.631	0.00602	3.35E-14	0.737	0.0155	1.16E-06	0.857	0.0215	6.1E-05
Hspa8	Hsc70	VYEGERPLTK	0.350	0.0718	1.79E-07	0.528	0.0209	3.68E-08	0.560	0.0359	6.73E-05
		EIAEAYLGK	0.474	0.0129	2.01E-20	0.593	0.00855	1.52E-11	0.639	0.00510	3.35E-14
Iqsec1	ARF-GEP	DAGTIAGNLVLR	0.476	0.00290	6.07E-12	0.605	0.0108	2.94E-20	0.603	0.00668	9.31E-15
		VEIANDQGGR	0.466	0.00387	6.66E-15	0.624	0.0116	2.05E-18	0.587	0.00574	3.12E-08
		IGLNLFNK	0.707	0.0150	8.92E-10	0.795	0.0125	5.88E-09	0.792	0.0259	6.15E-05
Necap	Necap	LIEAFSQR	0.696	0.0121	3.97E-16	0.759	0.00790	6.9E-14	0.874	0.00915	7.35E-06
		LDQPDWTGR	0.559	0.0162	3.19E-18	0.808	0.0109	1.25E-06	0.964	0.0145	0.032693
Nsf	NSF	AENSSLNLIGK	0.813	0.0170	6.05E-08	0.749	0.0146	1.69E-08	0.968	0.0307	0.607495
		LFADAEQQR	0.567	0.0110	1.93E-21	0.780	0.00878	4.06E-08	0.848	0.0158	3.5E-06
		LLDYVPIGPR	0.788	0.00406	3.07E-07	0.673	0.00735	7.74E-14	1.222	0.00934	3.51E-11
		LLIIGTTSR	0.716	0.0212	2.08E-09	0.636	0.00667	6.59E-11	0.889	0.00585	4.86E-10
		TTIAQQVK				0.630	0.0377	1.74E-05	1.014	0.0542	0.822594
		VVNGPEILNK	0.926	0.0273	0.09396	0.762	0.0145	4E-12	0.950	0.0363	0.268993
		YIFTLR	0.732	0.00710	3.05E-11	0.649	0.00938	6.11E-11	0.945	0.00837	0.000118
Sept3	Septin 3	YVGESEANIR	0.691	0.0153	6.06E-15	0.808	0.0201	0.000127	1.389	0.0236	0.004282
		STLVNTLFK	0.537	0.00406	1.12E-11	0.658	0.00941	4.27E-17	0.899	0.0124	2.5E-05
		VNIIPVIK	0.490	0.00283	2E-12	0.656	0.00964	2.5E-15	0.885	0.00817	3.74E-08
Sept7	Septin 7	VNIIPVIK	0.491	0.00628	5.04E-21	0.679	0.00870	4.85E-11	0.897	0.0112	3.28E-06
		FEDYLNESR	0.436	0.00935	5.79E-23	0.695	0.0125	4.14E-11	1.275	0.0140	2.18E-10
Stip1	HOP	VNIPLIK	0.448	0.00919	1.77E-14	0.665	0.00974	3.04E-20	0.834	0.00508	1.73E-12
		LLEFQLALK	0.118	0.0586	1.96E-10	0.864	0.0523	0.26738	0.665	0.0970	0.020414
Vsnl	Visinin like protein	FAQHAFR						0.652	0.00636	2.54E-11	
Wdr7	Rabconnectin 3 β	LLIQDSSGR						1.806	0.0160	8.99E-08	
		AAVLFQQVK	1.152	0.0275	0.000108	0.888	0.0455	0.080795	1.050	0.0621	0.630733
		TATDLIGR	0.9832	0.00799	0.295298	0.723	0.0304	4.96E-06	0.791	0.0545	0.018092

Validation Rate= 86%

Identification of CSP α Client Proteins

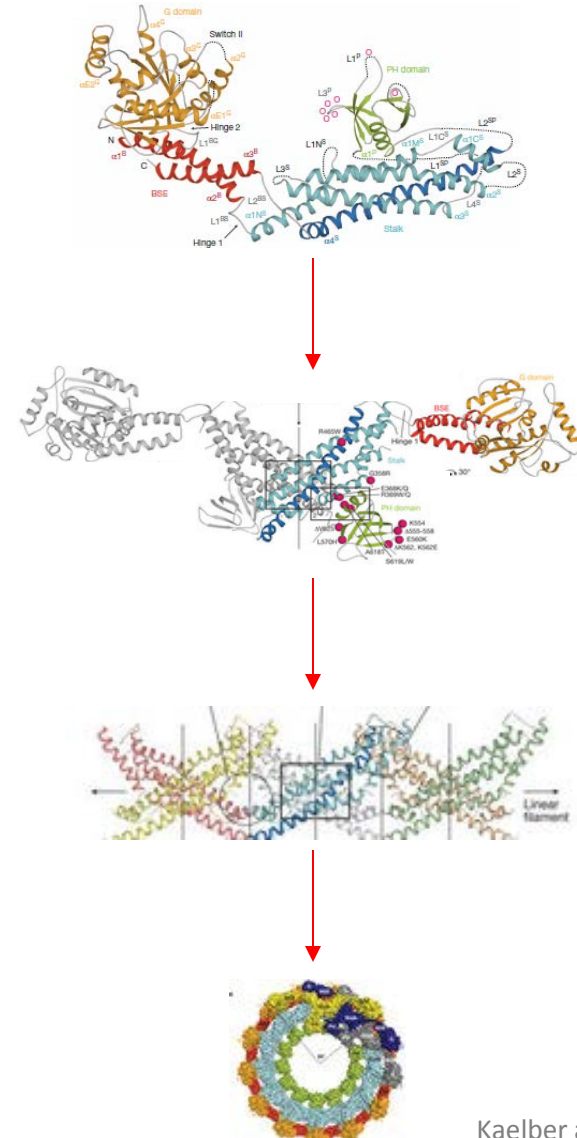
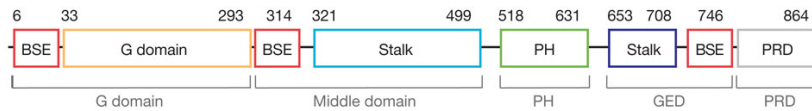
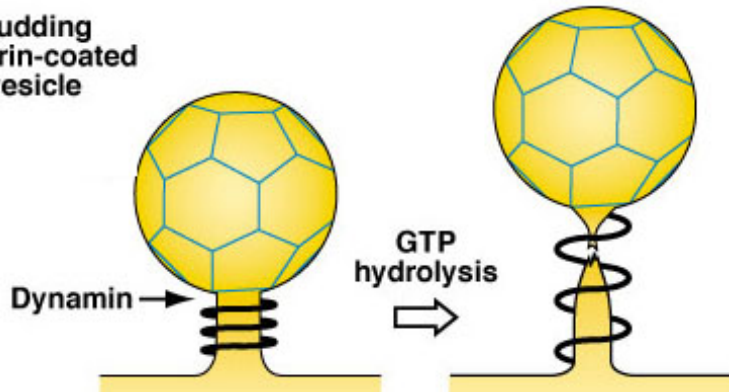


CSP α Functions at Two Steps in the Synaptic Vesicle Cycle

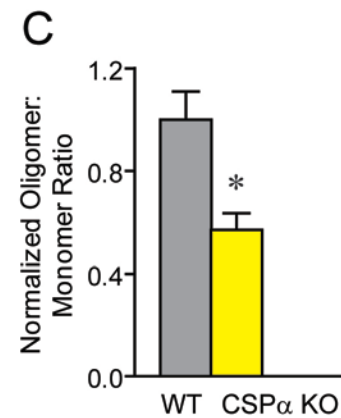
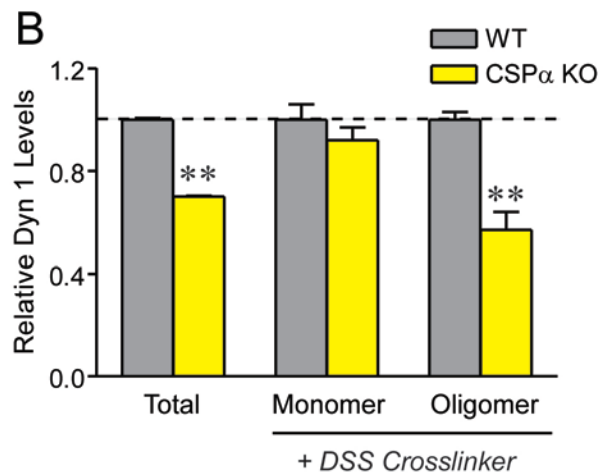
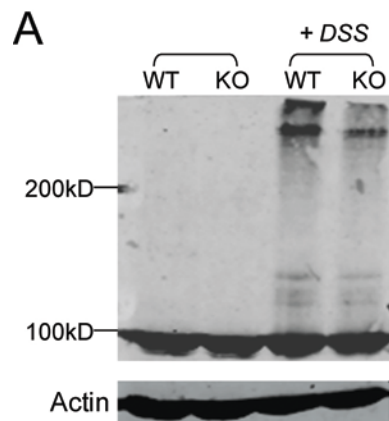


Dynamin 1 Oligomerizes During Synaptic Vesicle Endocytosis

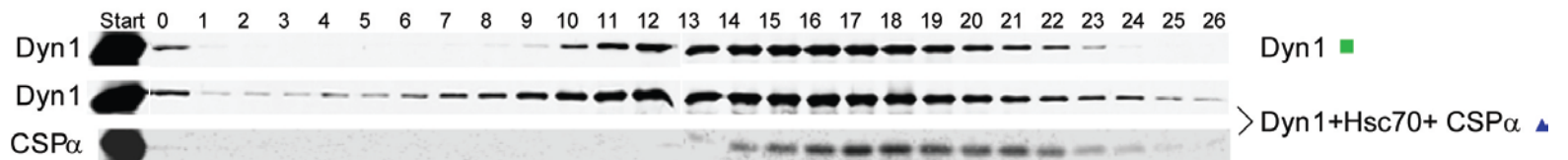
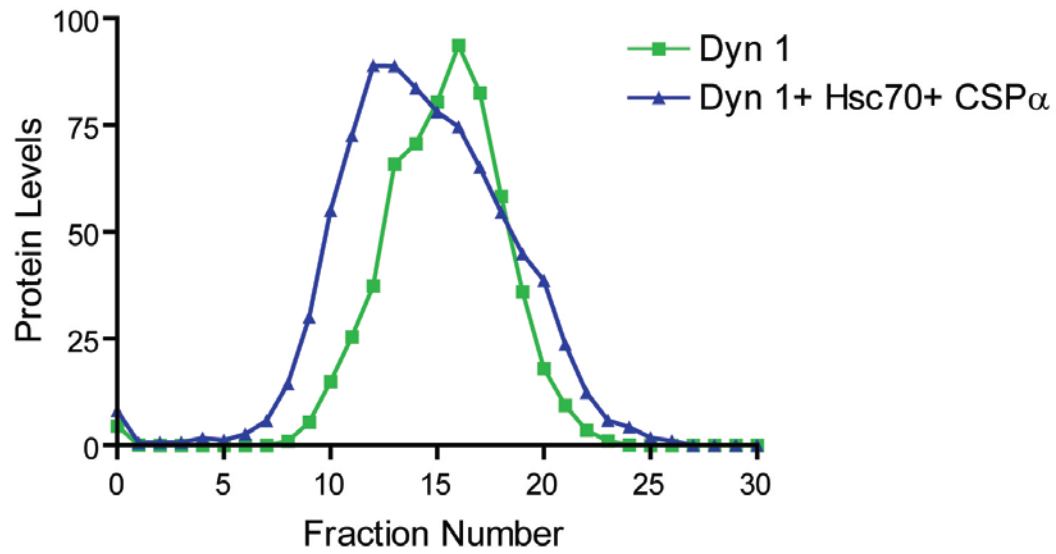
Budding clathrin-coated vesicle



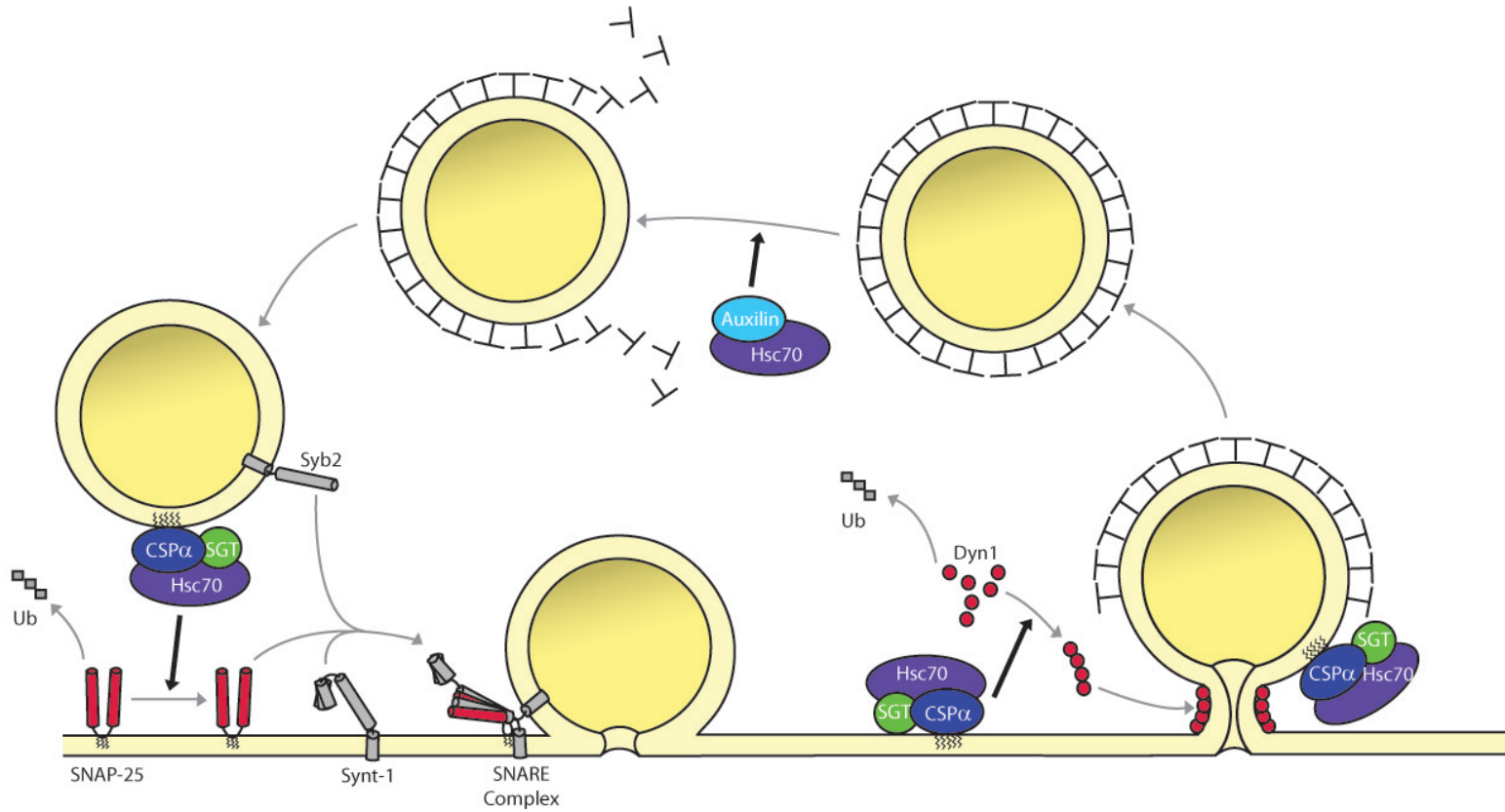
Deficits in Dynamin 1 Oligomerization in CSP α KO mice *in vivo*



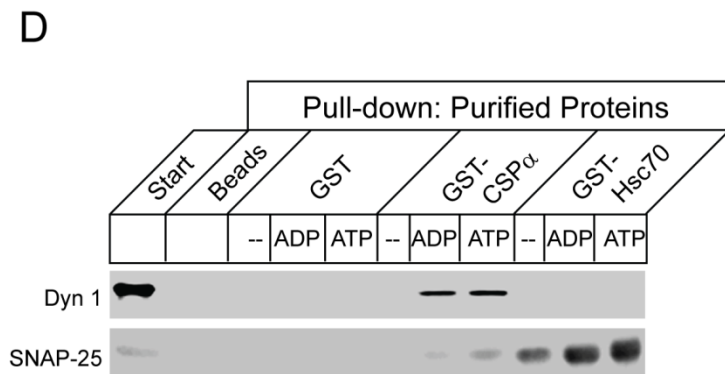
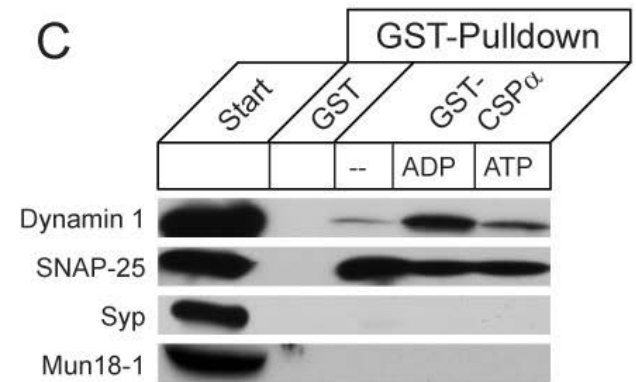
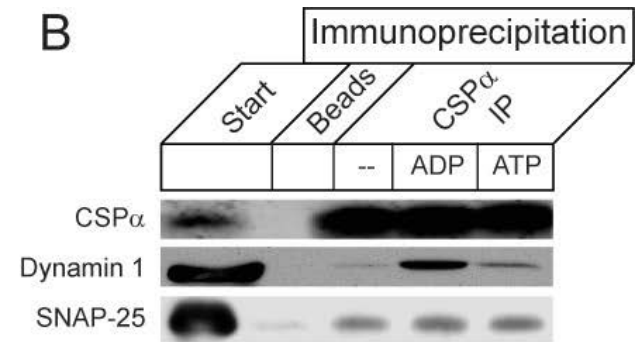
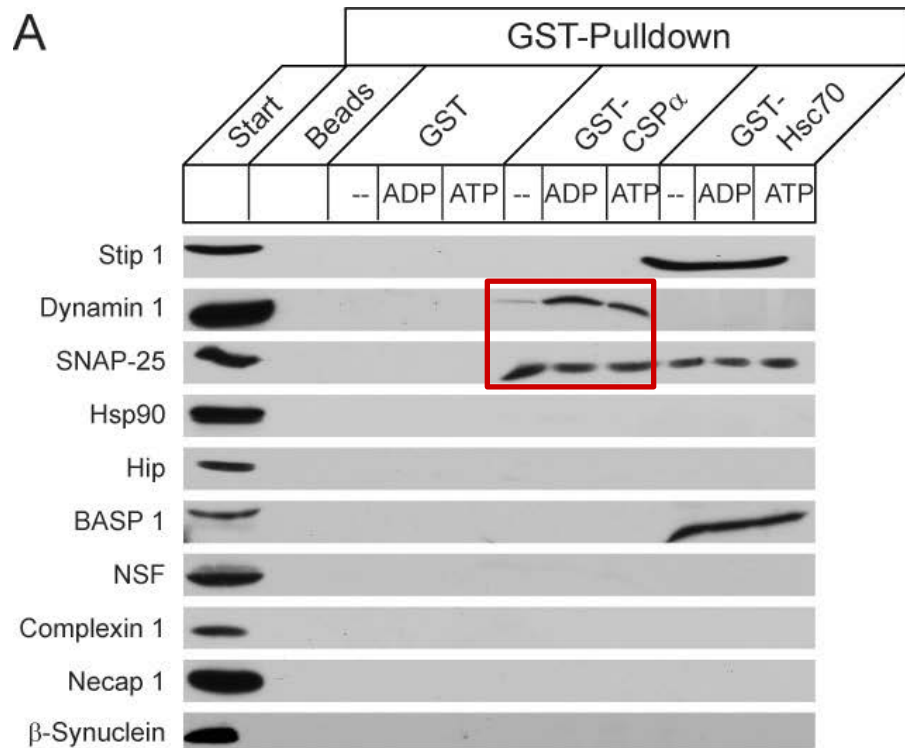
CSP α Promotes Polymerization of Dynamin 1



CSP α Deletion Perturbs Exo-Endocytosis Coupling



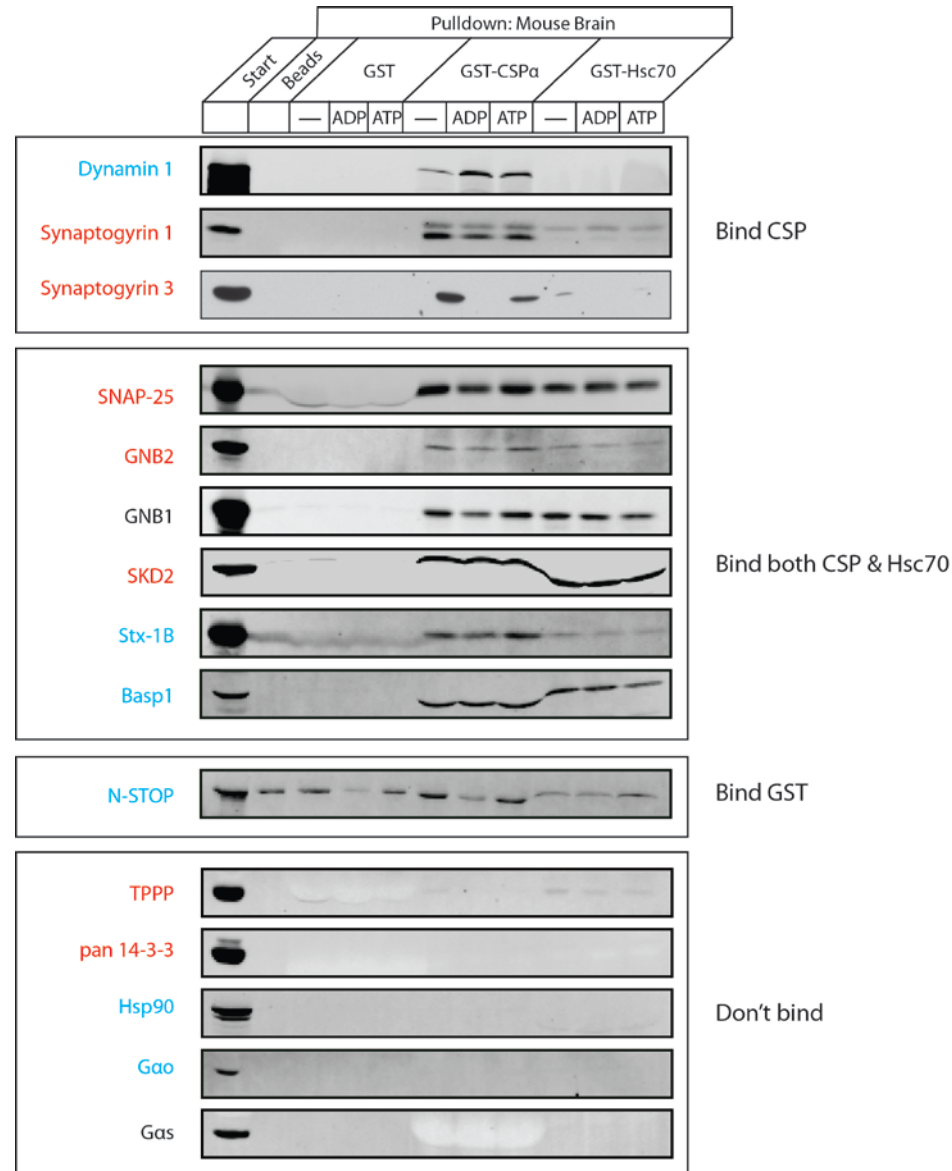
Identification of CSP α Client Proteins



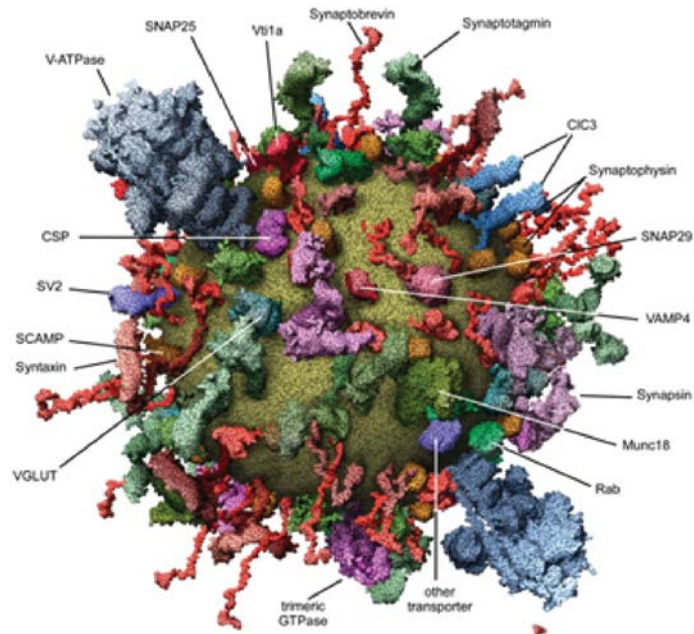
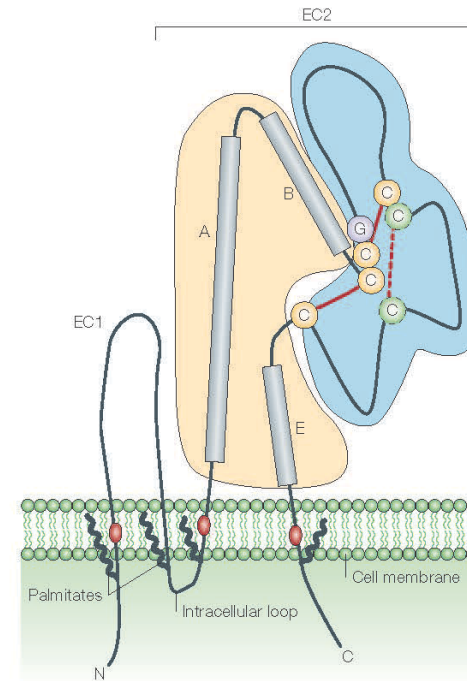
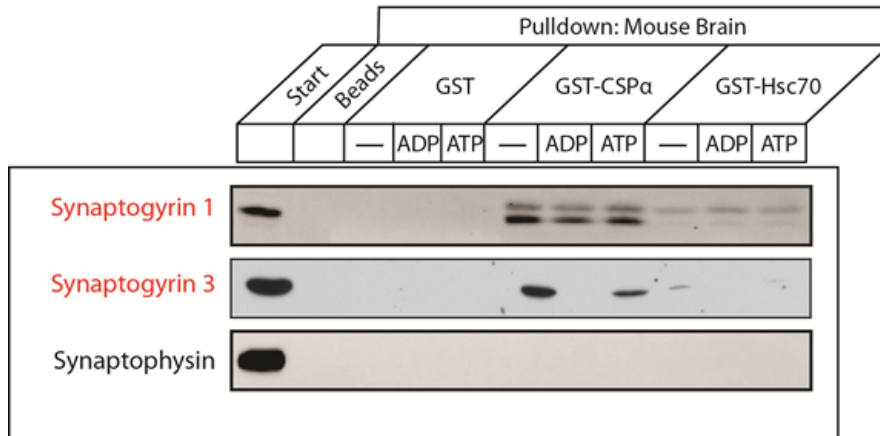
MS Screen for CSP α Client Proteins

<i>Score</i>	<i>Expectation</i>	<i>Protein Name</i>	<i># Samples</i>	<i>Experiments</i>	<i>MW</i>
<i>All 3 Experiments</i>					
143	7.10e-10	Synaptogyrin 3	9	1,2,3	24511
73	0.0077	SNAP-25	9	1,2,3	23300
81	0.0011	G protein beta 2 subunit	6	1,2,3	37380
136	3.30e-9	TPPP	6	1,2,3	23560
259	1.80e-21	SKD2	5	1,2,3	82513
75	0.0049	Synaptogyrin-1	4	1,2,3	21281
426	3.60e-38	14-3-3 zeta	4	1,2,3	27708
<i>At Least 2 Experiments</i>					
92	0.000084	G protein, alpha o	6	1,2	51447
189	1.90e-14	14-3-3 protein gamma	5	1,2	35075
515	4.40e-47	Neuronal-STOP protein	4	2,3	96346
156	3.90e-11	PHGP	4	2,3	19510
131	1.20e-8	Lasp-1	3	1,2	23087
108	0.0000024	Reticulon-1	3	2,3	23543
366	3.80e-32	14-3-3 beta	2	2,3	28079
222	8.50e-18	Dynamamin 1	2	1,2	84987
141	1.10e-9	14-3-3 epsilon	2	2,3	29155
130	1.50e-8	Hsp84	2	1,3	83296
68	0.021	Syntaxin-1B	2	1,2	33224
202	9.30e-16	Cofilin-1	2	2,3	18548

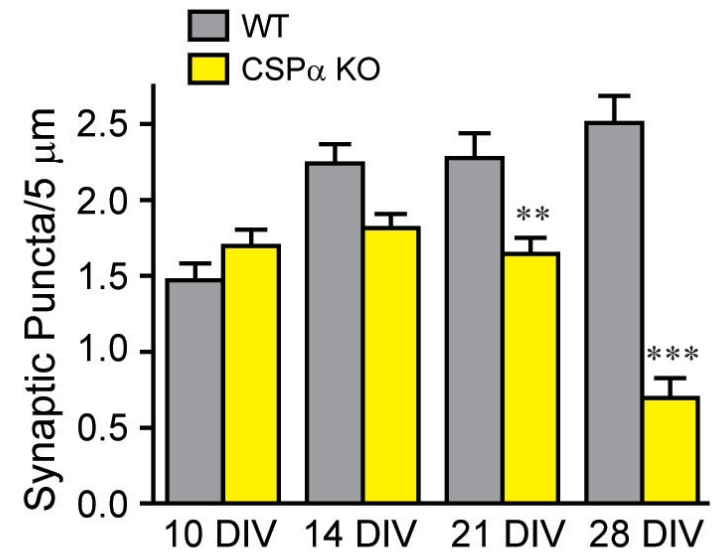
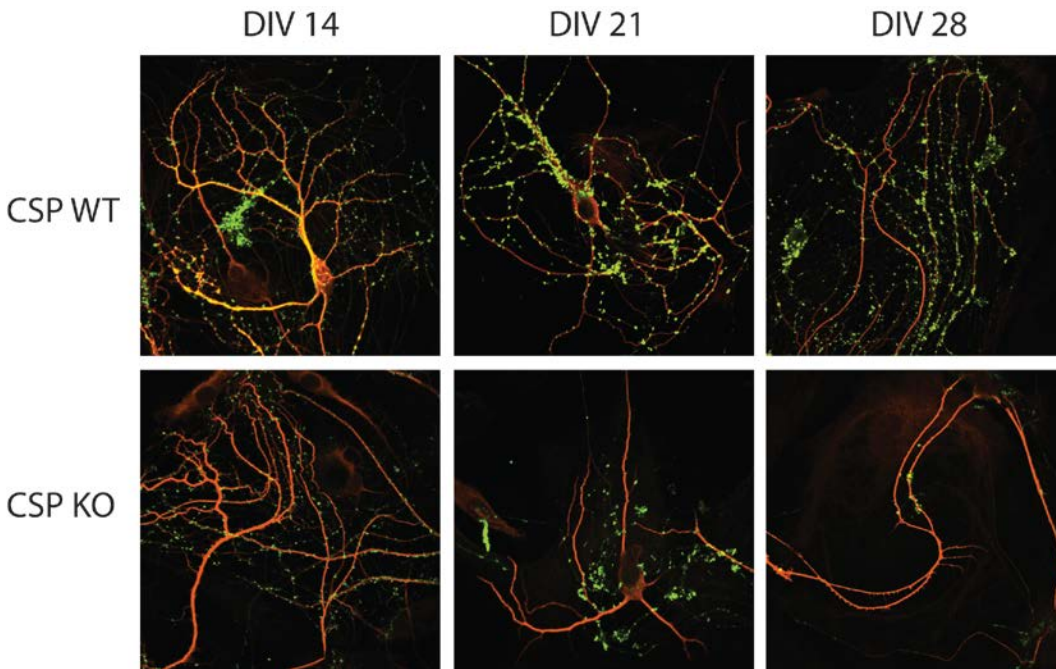
We Have Identified Additional Novel CSP α Client Proteins



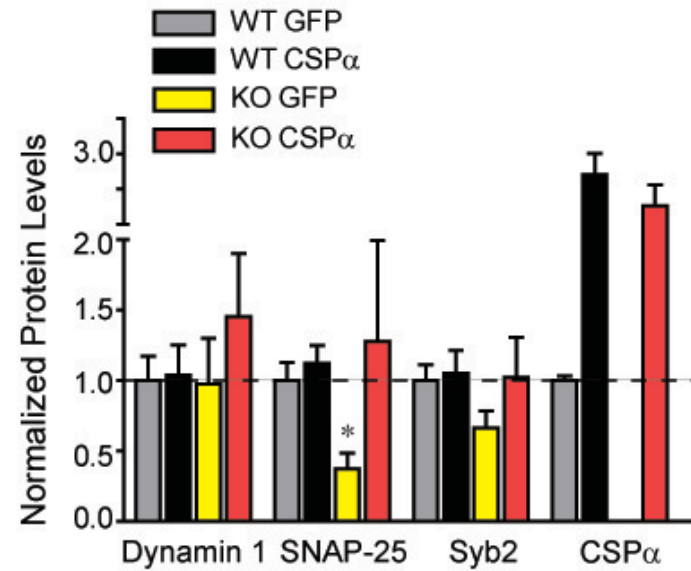
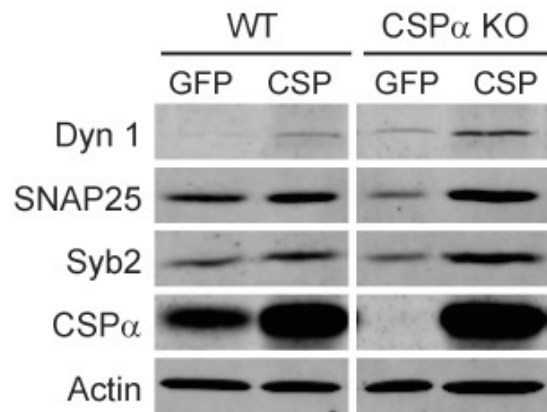
Synaptogyrins: Enigmatic CSP α Clients



CSP α KO: Cell Culture Model For Synapse Loss



CSP α KO Shows Decreased Levels of CSP α Clients



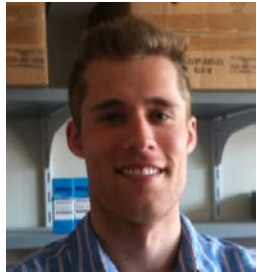
Future Directions

- *How to CSP α clients link to the actin cytoskeleton to confer synaptic stability?*
- *Are the CSP α clients degraded in via the lysosome?*
- *To evaluate the role of CSP α in ameliorating synapse loss in neurodegeneration*
- *What is the relationship between synapse stability and structural plasticity?*

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