Supplementary Table S1 List of the primary antibodies used in the present study

In-house produced monoclonal antibodies

Target protein	*Immunogen*	*Host*	*Clone*	*Subclass*	*WB dilution*[a]	*IHC concentration*[b]	*Reference*
Syntaxin-1	Crude human brain immunoprecipitate	Mouse	SP7	IgG2a	1:100	3 µg/ml	Honer *et al*., 1993
Syntaxin-1	Crude human brain immunoprecipitate	Mouse	SP6	IgG1	1:100	–	Honer *et al*., 1993
SNAP25	Crude human brain immunoprecipitate	Mouse	SP12	IgG1	1:100	3 µg/ml	Honer *et al*., 1993
VAMP	Crude human brain immunoprecipitate	Mouse	SP10	IgM	1:10	6 µg/ml	Honer *et al*., 1993
VAMP	Crude human brain immunoprecipitate	Mouse	SP11	IgG1	1:10	6 µg/ml	Honer *et al*., 1993
Complexin-I	Purified synthetic complexin-I	Mouse	SP33	IgG1	1:10	6 µg/ml	Takahashi *et al*., 1995
Complexin-II	Purified synthetic complexin-II	Mouse	LP27	IgG1	1:10	10 µg/ml	Takahashi *et al*., 1995
Synaptophysin	Schizophrenia brain homogenate	Mouse	EP10	IgG1	1:100	–	Honer *et al*., 1989
Synaptotagmin[c]	Rat brain synaptic membranes	Mouse	MAB30	IgG1	1:100	–	Matthew *et al*., 1981
Tau (misfolded)	Crude homogenate of human AD brain	Mouse	Alz-50	IgM	–	1 µg/ml	Wolozin *et al*., 1986

Commercially available antibodies

Target protein	*Immunogen*	*Host*	*Clone*	*Subclass*	*WB dilution*	*IHC dilution*	*Cat. no.*	*Vendor*
SNAP25	Human brain synaptosomal extract	Mouse	SMI 81	IgG1	1:2,000		SMI-81R	Covance
SNAP25B	Rat SNAP25B, residues 58-72	Rabbit	Polyclonal	–	1:1,000		111 113	Synaptic Systems
HLA-DP/DQ/DR	Human	Mouse	CR3/43	IgG1	–	1:100	M0775	Dako
β-amyloid	Synthetic $A\beta_{17-24}$ peptide	Mouse	4G8	IgG2b	–	1:500	800709	BioLegend
pS^{202}/pT^{205}-Tau	Partially purified human PHF-tau	Mouse	AT8	IgG1	–	1:1,000	MN1020	ThermoFisher

Abbreviations: AD, Alzheimer's disease; IHC, immunohistochemistry; PHF, paired helical filament; pS, phosphoserine; pT, phosphothreonine; SNAP25, synaptosome-associated protein of 25 kDa; STXBP1, syntaxin-binding protein-1; VAMP, vesicle-associated membrane protein; WB, Western blotting

[a] Dilutions correspond to hybridoma cell culture supernatants, and were equal to the dilutions used in WB assays

[b] Concentrations correspond to affinity column-purified antibodies from hybridoma cell culture supernatant

[c] Hybridomas were obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biology, Iowa City, IA 52242

References

Honer WG, Kaufmann CA, Kleinman JE, Casanova MF, Davies P. Monoclonal antibodies to study the brain in schizophrenia. Brain Res. 1989;500:379–83.

Honer WG, Hu L, Davies P. Human synaptic proteins with a heterogeneous distribution in cerebellum and visual cortex. Brain Res. 1993;609:9–20.

Matthew WD, Tsavaler L, Reichardt LF. Identification of a synaptic vesicle-specific membrane protein with a wide distribution in neuronal and neurosecretory tissue. J. Cell Biol. 1981;91:257–69.

Takahashi S, Yamamoto H, Matsuda Z, Ogawa M, Yagyu K, Taniguchi T, et al. Identification of two highly homologous presynaptic proteins distinctly localized at the dendritic and somatic synapses. FEBS Lett. 1995;368:455–60.

Wolozin BL, Pruchnicki A, Dickson DW, Davies P. A neuronal antigen in the brains of Alzheimer patients. Science. 1986;232:648–50.

Supplementary Table S2 Characteristics of the recombinant proteins used in reconstitution assays

Protein	***Origin***	***Sequence***	***Tag (position)***	***Theoretical MW***	***Preparation***	***Catalogue no.***	***Vendor***
Syntaxin-1A	Human	aa 1-265 (full)	Untagged	30.7 kDa	unknown	NBP2-52366	Novus Biologicals
SNAP25 isoform B	Human	aa 1-206 (full)	Untagged	23.0 kDa	*E. coli*	NBC1-18344	Novus Biologicals
SNAP25 isoform B	Human	aa 1-206 (full)	Myc-DDK (C-t)	23.1 kDa	HEK293	TP312596	OriGene
VAMP2	Human	aa 1-89 (full)	His (N-t)	13.8 kDa	*E. coli*	NBP1-18335	Novus Biologicals
M18L	Human	aa 1-603 (full)	GST (N-t)	95.1 kDa	Wheat germ	H00006812-P01	Abnova
M18S	Human	aa 1-594 (full)	GST-His (N-t)	95.4 kDa	Insect cells	11751-H20B-100	Sino Biologicals
Complexin-1	Human	aa 1-134 (full)	His (N-t)	17.1 kDa	*E. coli*	NBC1-18349	Novus Biologicals
Complexin-2	Human	aa 1-134 (full)	His (N-t)	16.8 kDa	*E. coli*	11927H07E50	ThermoFisher
Synaptotagmin-1	Human	aa 1-422 (full)	GST (N-t)	72.0 kDa	Wheat germ	ab132655	Abcam

Abbreviations: C-t, carboxyl-terminus; GST, glutathione-S-transferase; HEK, human embryonic kidney cells; His, poly-histidine tag; M18L, munc18-1 long variant (a.k.a. isoform 1); M18S, munc18-1 short variant (a.k.a. isoform 2); MW, molecular weight; N-t, amino-terminus; SNAP25, synaptosome-associated protein of 25 kDa; VAMP, vesicle-associated membrane protein; WB, Western blotting

Supplementary Table S3 Effects of IT and MF immunodensities of the identified presynaptic complexes on MAP participants’ cognitive function nearest death assessed in independent linear regression models [a]

Protein complex	IT (*n* = 154)			MF (*n* = 174)		
	Std. β	% cont. [b]	FDR *p*-value	Std. β	% cont. [b]	FDR *p*-value
30k STX1	0.1676	1.61	0.0801	0.1054	0.44	0.3023
150k STX1	0.3457	8.61	<0.0001*	0.0819	0.13	0.3845
450k STX1	0.0930	0.35	0.3040	0.0697	0.01	0.4897
70k SNAP25	0.1931	2.43	0.0364*	0.2377	4.74	0.0081*
150k SNAP25	0.2079	3.29	0.0139*	0.0937	0.32	0.3559
450k SNAP25	0.1780	2.09	0.0479*	0.1552	1.83	0.0579
50k CPLX1	0.0433	-0.30	0.6625	0.1045	0.58	0.2487
200k CPLX1	0.1691	1.92	0.0545	0.1871	2.67	0.0256*
500k CPLX1	0.0708	-0.05	0.4612	0.2191	4.28	0.0071*
50k CPLX2	0.0198	-0.44	0.9057	-0.0044	-0.47	0.9519
200k CPLX2	0.1208	0.75	0.2414	0.0490	-0.24	0.9071
500k CPLX2	0.1152	0.63	0.2846	-0.0179	-044	0.7979
50k CPLX1/2 ratio [c]	0.0971	0.37	0.3394	0.2134	3.88	0.0077*
200k CPLX1/2 ratio [c]	0.1467	1.63	0.0786	0.1988	3.26	0.0165*
500k CPLX1/2 ratio [c]	0.0335	-0.38	0.7896	0.3127	9.56	<0.0001*

Abbreviations: % cont., percent contribution value; CPLX1/2, complexin-I/II; FDR, false discovery rate; S.E., standard error; SNAP25, synaptosome-associated protein of 25 kDa; Std. β, standardized beta estimate; STX1, syntaxin-1

[a] All models were adjusted for age, sex, education, postmortem interval, local (i.e. IT or MF) β-amyloid and phosphotau load, macroinfarcts, Lewy bodies, hippocampal sclerosis, and synaptic density (mean of total syntaxin-1, SNAP25 and VAMP immunodensities). Group sizes, individual standardized β-coefficients, and FDR-adjusted *p*-values are also reported.

[b] Percent contribution values were calculated as the difference between the whole model adjusted R^2 values obtained before and after adding each of the indicated neurochemical measures as terms into the reference model containing all covariates.

[c] Ratios of CPLX1 to CPLX2 for each of the indicated complex sizes.

Supplementary Table S4 Linear regression models[a] showing the associations between most relevant presynaptic complexes identified in the IT and MF of MAP participants (*n* = 140), as predictors, and the different cognitive domains or cognitive decline as outcomes

Model terms	**Episodic memory**		**Semantic memory**		**Working memory**		**Perceptual speed**		**Visuospatial skills**		**Global cognitive decline slope**	
	Std. β	FDR *p*-value	Std. β	FDR *p*-value	Std. β	FDR *p*-value	Std. β	FDR *p*-value	Std. β	FDR *p*-value	Std. β	FDR *p*-value
Age at death	-0.1249	0.1176	-0.0705	0.6405	-0.1517	0.1644	-0.2216	0.0479*	-0.2516	0.0088*	-0.0730	0.4531
Sex	0.1272	0.1176	0.0082	0.9173	0.0028	0.9724	-0.0069	0.9914	-0.1851	0.0669	0.0135	0.8740
Education	-0.0713	0.4148	-0.0599	0.6747	-0.1260	0.2264	-0.0946	0.4562	-0.1758	0.0709	-0.0767	0.4531
PMI	-0.0115	0.8717	-0.0219	0.8600	-0.0960	0.3439	-0.0159	0.9914	-0.0600	0.5104	-0.1238	0.2754
Macroinfarcts	0.0624	0.4182	0.0677	0.6405	0.1226	0.2264	-0.0420	0.9030	-0.0034	0.9652	-0.1093	0.2754
Lewy bodies	0.0959	0.2482	0.1514	0.1870	0.1993	0.0925	0.1110	0.4416	0.2330	0.0176*	0.1977	0.0533
Hipp sclerosis	0.1536	0.1095	0.0553	0.6747	-0.1119	0.2945	0.1055	0.4562	-0.1063	0.2931	-0.1940	0.0699
β-amyloid[b]	-0.2304	0.0132*	-0.2468	0.0465*	-0.2010	0.0925	-0.2082	0.0843	-0.1830	0.0709	-0.2801	0.0177*
Phosphotau[b]	-0.3043	0.0012*	-0.1401	0.2592	0.0196	0.9022	0.0010	0.9914	0.0938	0.3429	0.3521	0.0034*
Synapse density[c]	-0.0143	0.8717	-0.0405	0.7298	-0.0323	0.8326	0.0234	0.9914	-0.0983	0.2931	-0.0185	0.8740
IT 150k STX1	0.1497	0.1146	0.2324	0.0465*	0.2472	0.0743	0.1426	0.3519	0.2965	0.0084*	0.2593	0.0181*
MF 500k ratio[d]	0.1966	0.0208*	0.1493	0.1870	0.1506	0.1644	0.2257	0.0479*	0.1530	0.0977	0.2379	0.0181*

Abbreviations: CPLX, complexin; FDR, false discovery rate; Hipp, hippocampal; IT, inferior temporal gyrus; MF, middle-frontal gyrus; PMI, postmortem interval; S.E., standard error; STX1, syntaxin-1.

[a] Each model was constructed independently with the above indicated terms. Individual standardized coefficients (Std. β) and FDR-adjusted *p*-values are reported.

[b] Overall brain load.

[c] Synapse density was estimated as the overall brain levels of the three SNARE proteins (syntaxin-1, SNAP-25 and VAMP) averaged.

[d] Ratio between complexin-I (GABAergic) to complexin-II (glutamatergic) 500-kDa complexes.

* Statistically significant.

Supplementary Table S5 Linear mixed-effect models for longitudinal data showing the associations between IT 150-kDa STX1 levels and MF 500-kDa CPLX1/2 ratio and global cognitive decline (n = 125)

Model terms	**Model 1**[a]			**Model 2**[b]			**Model 3**[c]		
	β	SD	p-value	β	SD	p-value	β	SD	p-value
Intercept	-0.702	0.120	–	-0.681	0.099	–	-0.653	0.101	–
Age at death	-0.020	0.008	0.012*	-0.020	0.008	0.010*	-0.018	0.008	0.020*
Sex	-0.171	0.122	0.161	–	–	–	–	–	–
Education	0.025	0.018	0.165	–	–	–	–	–	–
PMI	-0.006	0.014	0.652	–	–	–	–	–	–
Macroinfarcts	-0.068	0.108	0.529	–	–	–	–	–	–
LBD	-0.368	0.139	0.008*	-0.381	0.131	0.004*	-0.244	0.134	0.068
Hippocampal sclerosis	0.003	0.197	0.987	–	–	–	–	–	–
β-amyloid[d]	-0.106	0.047	0.024*	-0.115	0.045	0.011*	-0.288	0.065	<0.001*
Phosphotau[d]	-0.026	0.009	0.005*	-0.023	0.009	0.010*	-0.020	0.009	0.026*
Synapse density[e]	0.055	0.093	0.555	–	–	–	–	–	–
IT 150k STX1	0.005	0.003	0.072	0.006	0.002	0.022*	0.015	0.004	<0.001*
MF 500k CPLX1/2	0.075	0.054	0.161	0.081	0.053	0.129	0.190	0.078	0.015*
Time	-0.106	0.015	<0.001*	-0.105	0.015	<0.001*	-0.092	0.015	<0.001*
Post-3yr DOD	0.093	0.028	<0.001*	0.093	0.028	<0.001*	0.053	0.029	0.068
LBD × Post-3yr DOD	–	–	–	–	–	–	0.296	0.059	<0.001*
β-amyloid × Time	–	–	–	–	–	–	-0.033	0.009	<0.001*
IT 150k STX1 × Time	–	–	–	–	–	–	0.002	0.001	<0.001*
MF 500k CPLX1/2 × Time	–	–	–	–	–	–	0.025	0.012	0.032*
Random Effects									
Subject	0.835 (88.3%)			0.850 (88.4%)			0.847 (88.6%)		
Time	0.014 (1.5%)			0.014 (1.5%)			0.012 (1.3%)		
Post-3yr DOD	0.043 (4.5%)			0.043 (4.5%)			0.044 (4.6%)		
Residual	0.054 (5.7%)			0.054 (5.6%)			0.053 (5.5%)		
Model Fit									
Deviance	637.6			641.7			582.7		
AIC	681.6			673.7			622.7		
R-squared	0.327			0.318			0.427		

Abbreviations: AIC, Akaike information criterion; CPLX1/2, complexin I/II ratio; IT, inferior temporal gyrus; LBD, Lewy body disease; MF, middle-frontal gyrus; Post-3yr DOD, 3-year period before date of death; SD, standard deviation; STX1, syntaxin-1.

[a] Additive model with IT 150k STX1 and MF 500k CPLX1/2 ratio, and other covariates.

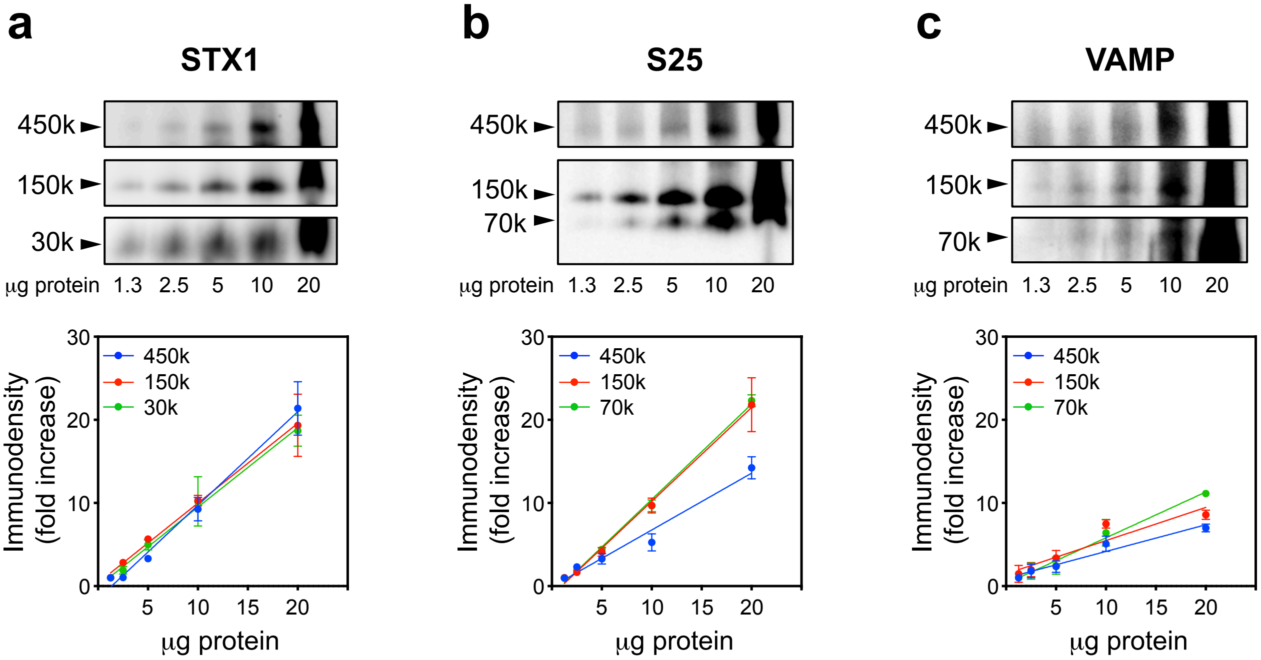
[b] Inclusion of only significant covariates that improve model fit.

[c] Inclusion of significant interactions with time.

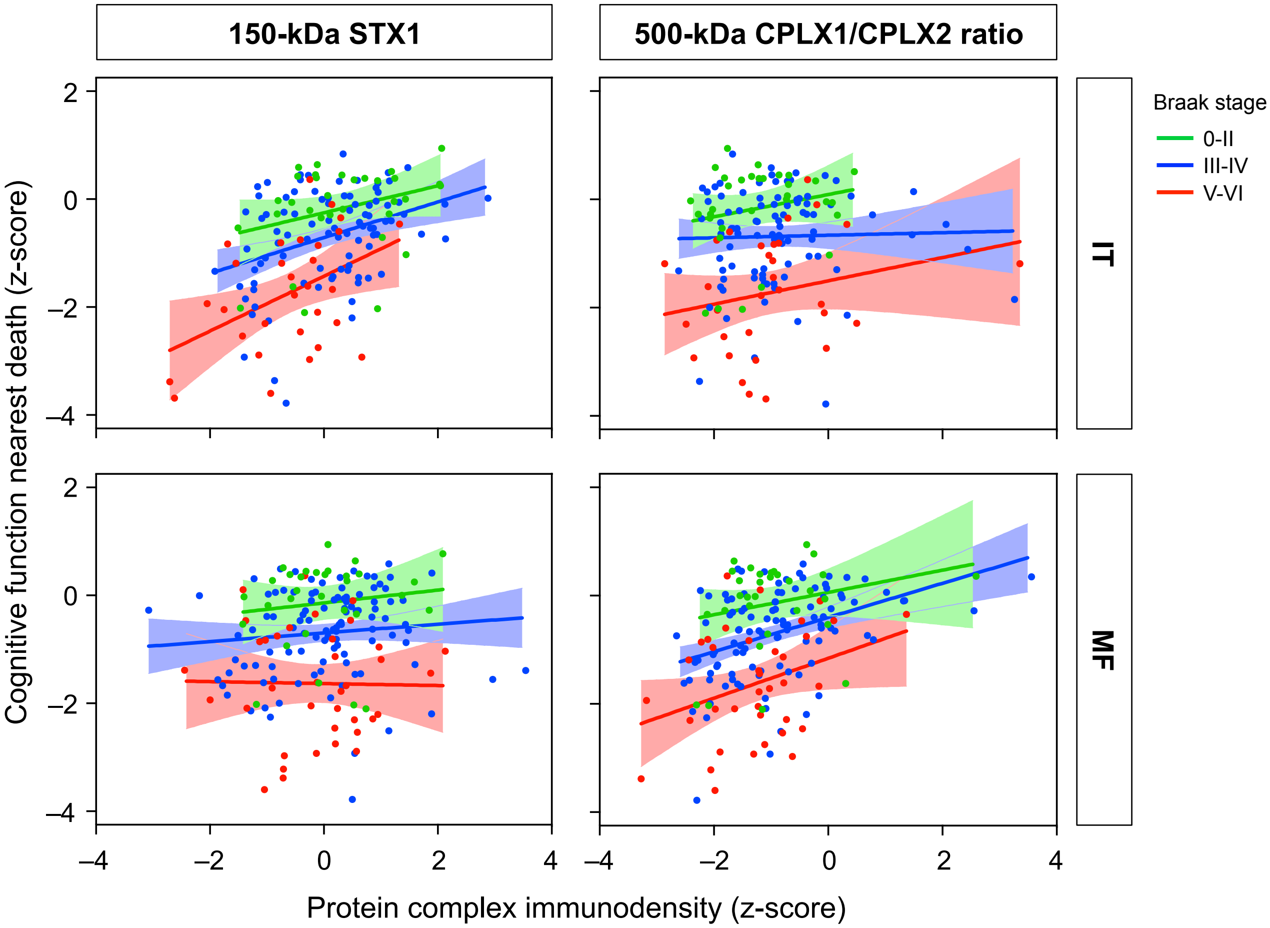
[d] Overall brain load

[e] Estimated as the overall cortical levels of the three SNARE proteins averaged.

* Statistically significant.



Supplementary Fig. S1. Linearity range of the main immunoquantitated complexes. Solubilized brain protein complexes from human inferior temporal cortex (IT) were serially diluted (final total protein contents ranging as indicated), resolved by blue-native (BN)-PAGE and immunoblotted (IB) with specific antibodies against (**a**) syntaxin-1 (STX1), (**b**) SNAP25 (S25), and (**c**) VAMP (SP10) (see Supplementary Table S1). (**a–c**) Representative immnoblots are shown on top. Points in the scatterplots below are the mean ± standard error of 3 independent experiments. Lines represent the best fit for the associations between the loading amounts and the obtained immunodensities. For STX1 and S25, all R-squared values were above 0.90 ($P < 0.001$). Molecular masses were estimated (in kDa) from native standards loaded aside.



Supplementary Fig. S2. Scatterplots representing standardized global cognitive function nearest death against the levels of 150-kDa syntaxin-1 (STX1; left panels) or the ratio of complexin-I (CPLX1) to complexin-II (CPLX2; right panel) in samples from the temporal (upper panels) and frontal (MF; bottom panels) lobes of MAP participants, grouped into Braak 0–II (blue), III–IV (green), or V–VI (red). Best fit (solid lines) and the 95% confidence intervals (shaded areas) calculated for each group are overlaid.