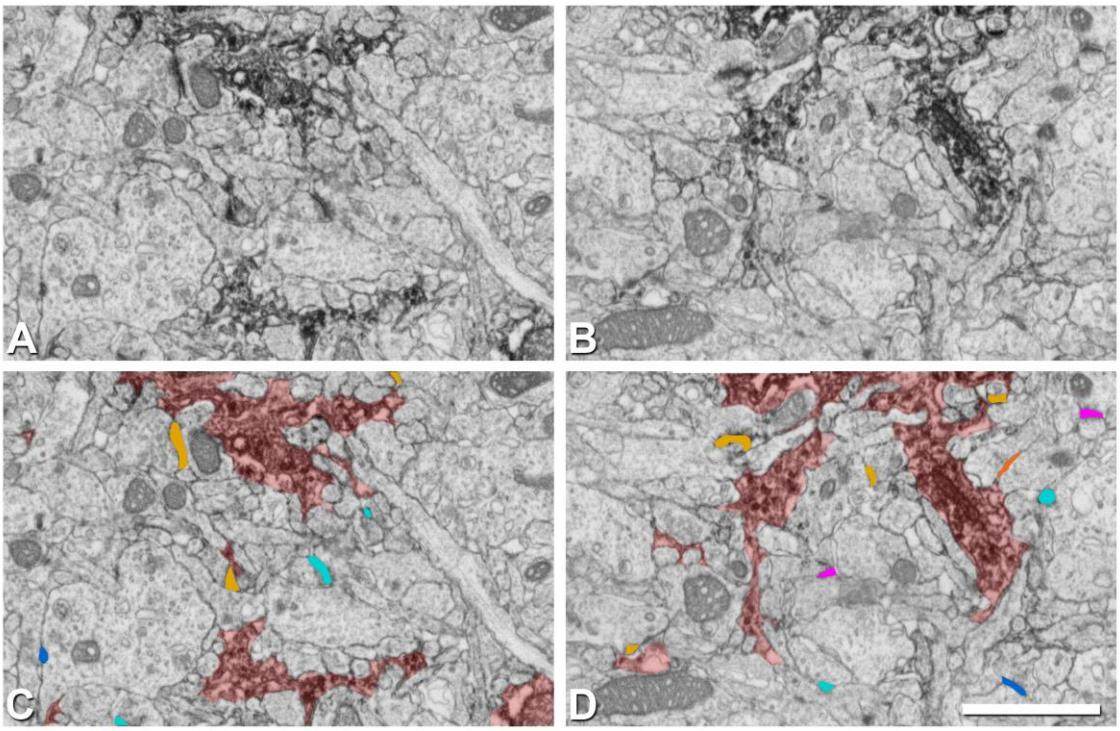
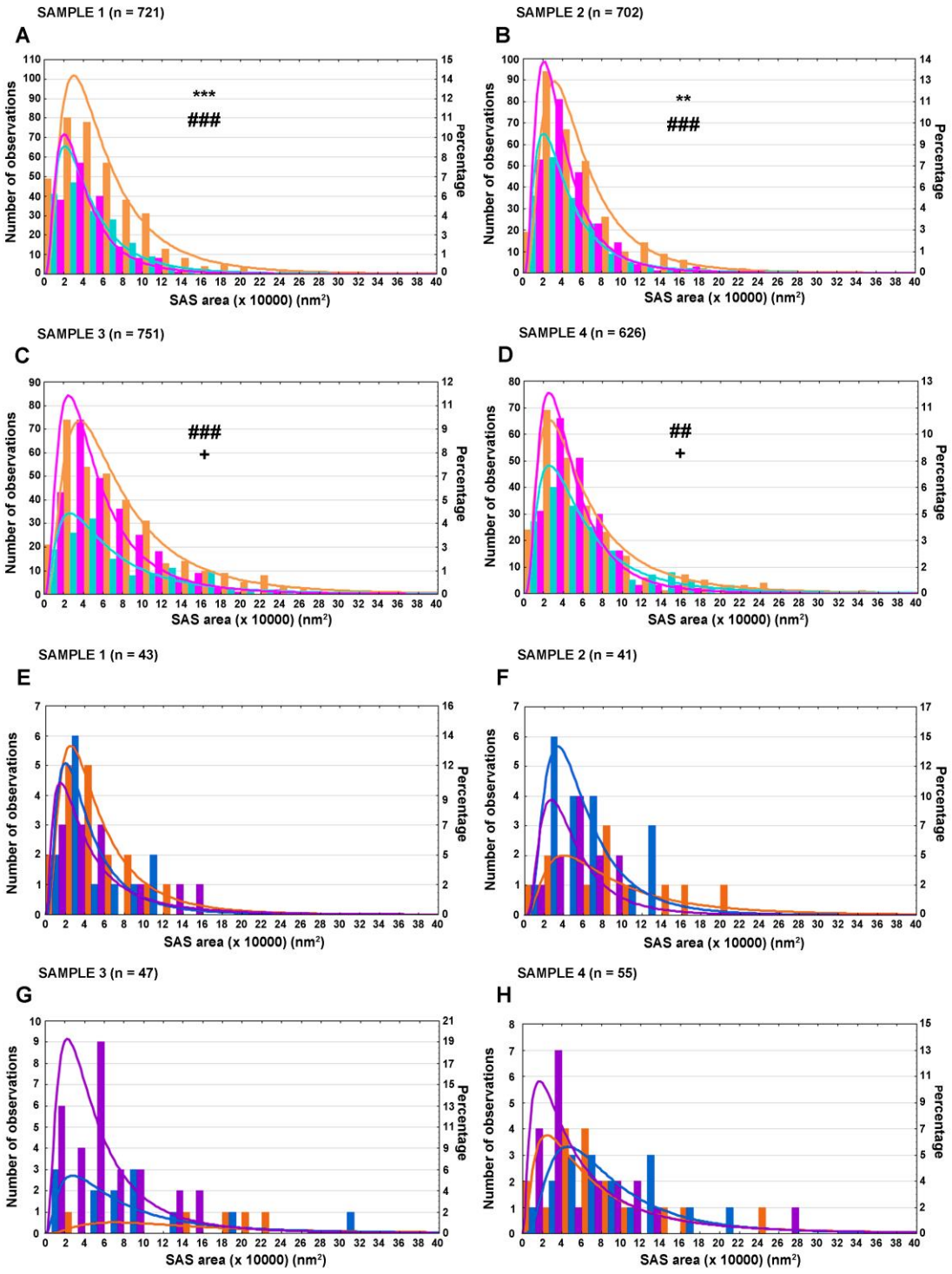
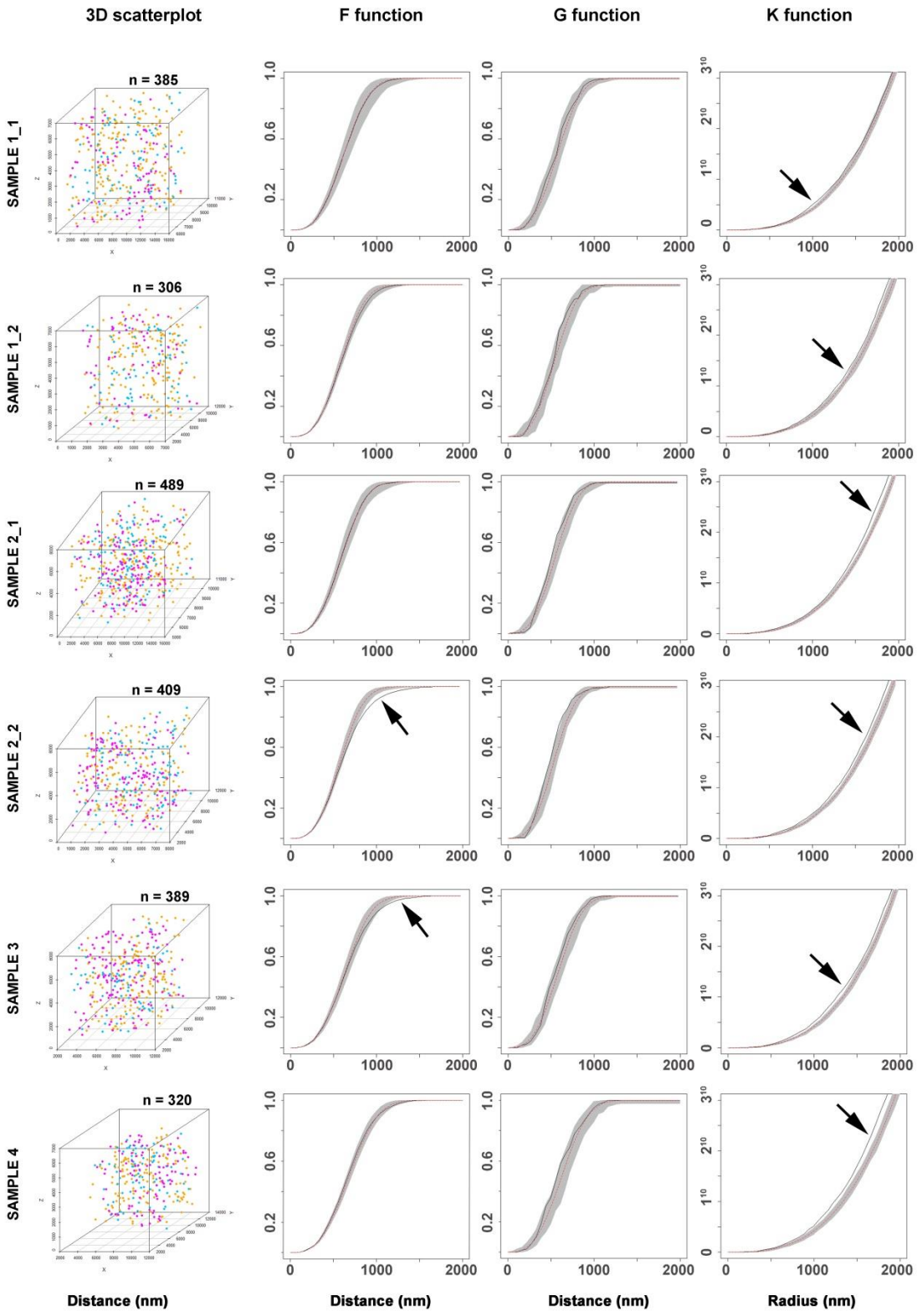
SUPPLEMENTARY FIGURES



Supplementary Figure 1. Example of FIB/SEM imaging and segmentation. A, B, Crop from images 88 and 147 in **A** and **B** in **Figure 3**. Electron dense deposits of DAB are visible within astrocytic processes in the FIB/SEM images. **C, D,** Same FIB/SEM images as in **A** and **B**, respectively, with segmented structures (astrocytic processes in red; synapses: asymmetric “Cleft Ast” in light orange, asymmetric “Pre/Post Ast” in light blue, asymmetric “Free Ast” in light purple, symmetric “Cleft Ast” in dark orange, symmetric “Pre/Post Ast” in dark blue). Scale bar shown in **D** indicates 1.78 µm in **A**–**D.**

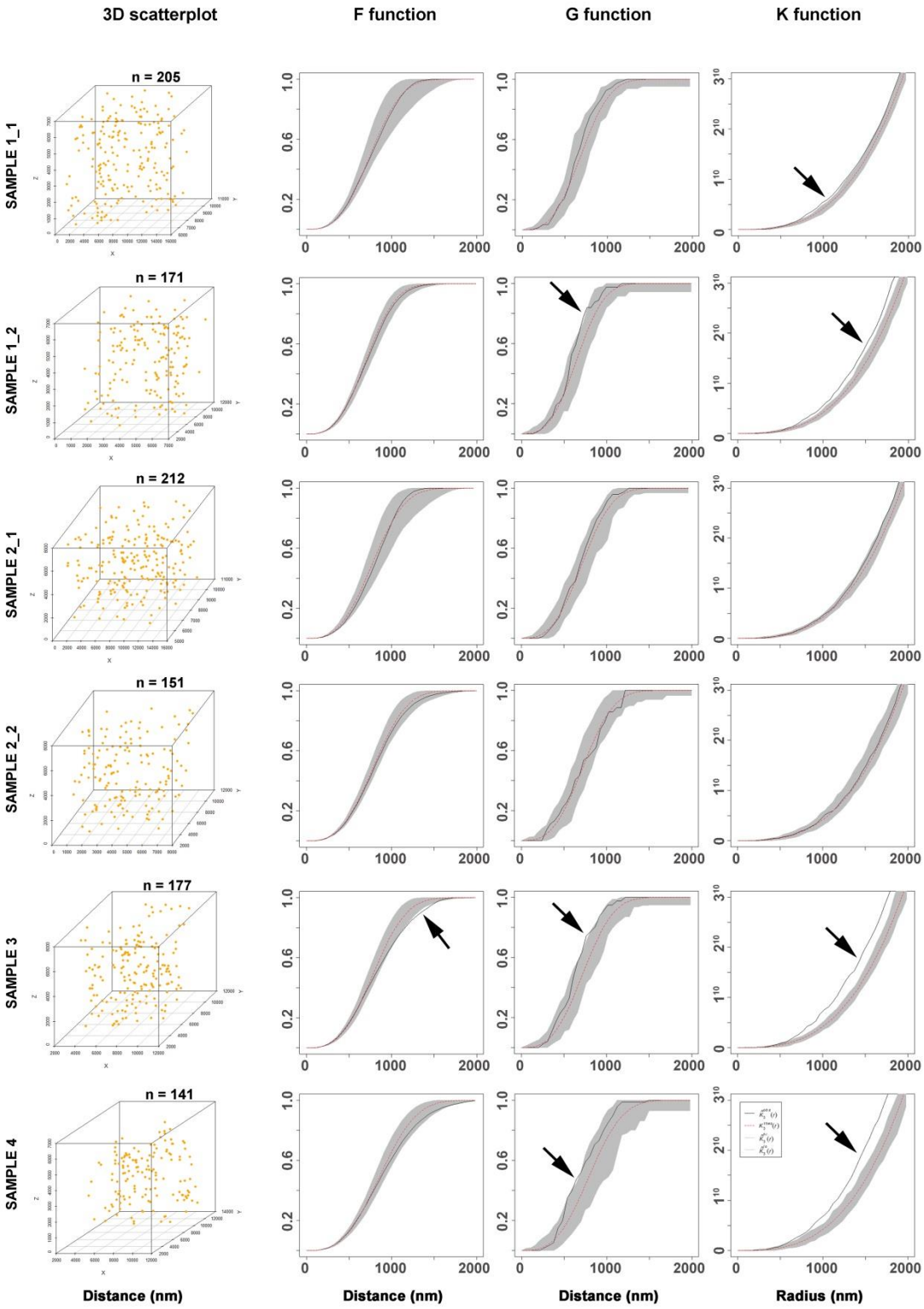


Supplementary Figure 2. Frequency distribution of asymmetric and symmetric synapse sizes classified according to their contacts with labeled astrocytic compartments. A, B, C, D, Frequency distribution histogram of SAS areas of asymmetric synapses classified according to their 3D contact with labeled astrocytic compartments in sample 1, sample 2, sample 3 and sample 4, respectively. **E, F, G, H,** Frequency distribution histogram of SAS areas of symmetric synapses classified according to their 3D contact with labeled astrocytic compartments in sample 1, sample 2, sample 3 and sample 4, respectively.
The log-normal function for each category has been represented. The x-axis bin = 2 (x10000) nm^2. See text and **Supplementary Table 6** for further details.



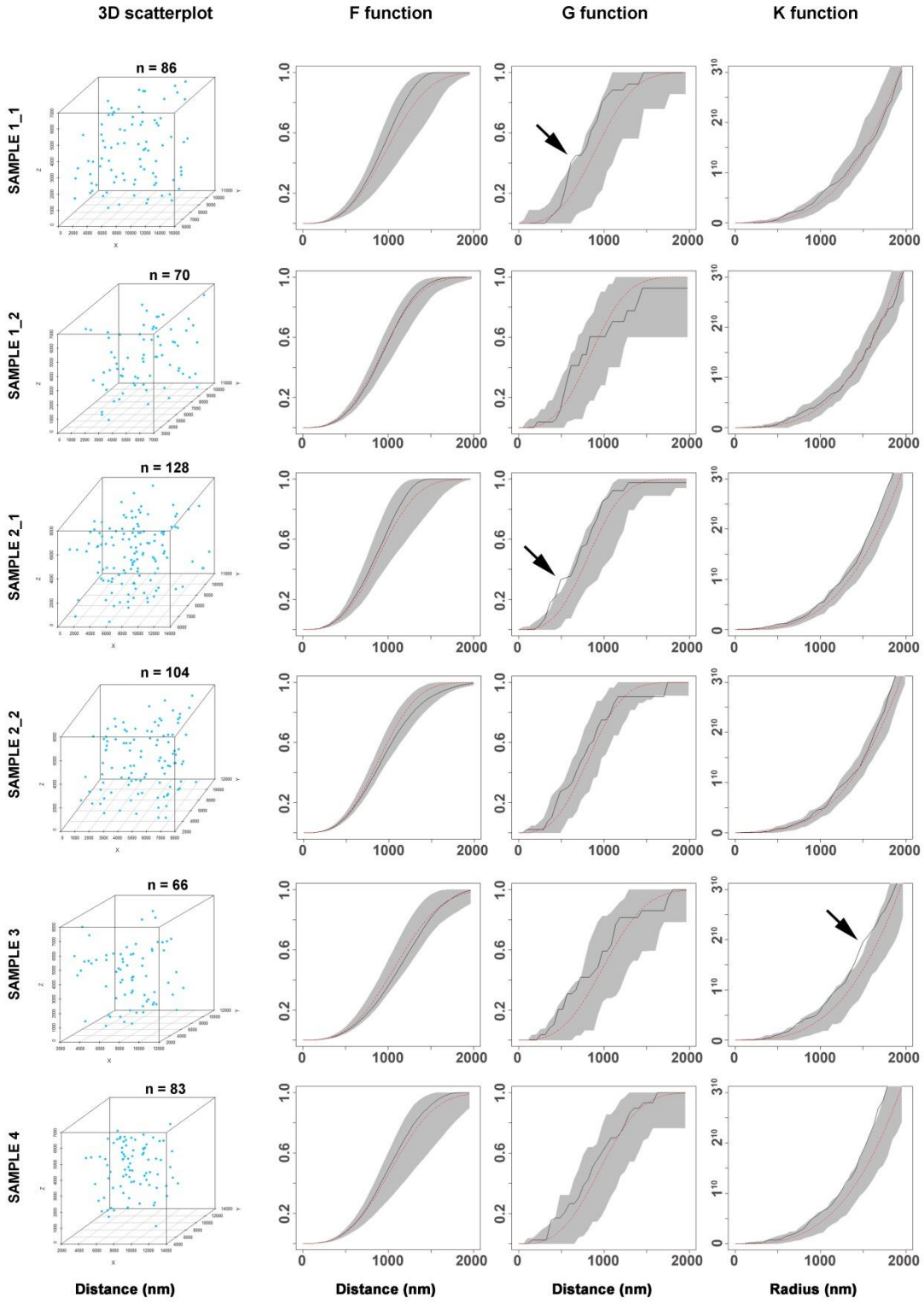
Supplementary Figure 3. 3D spatial distribution of synapses (All Synapses). The spatial distribution analysis of All Synapses together in the different samples: sample 1.1 (n = 385 synapses; top row), sample 1.2 (n = 306 synapses; second row), sample 2.1 (n = 489 synapses; third row), sample 2.2 (n = 409 synapses; fourth row), sample 3 (n = 389 synapses; fifth row) and sample 4 (n = 320 synapses; bottom row). For each synapse, we recorded the spatial positions of the center of gravity or centroid of the synaptic junction, as represented in the 3D scatterplots of the rightmost column. Three spatial statistical

functions (F, G and K) were calculated for each group of synapses in each sample. In each graph, the function corresponding to the actual sample is represented by a black line. The theoretical homogeneous Poisson distribution or complete spatial randomness (CSR) is represented as a red discontinuous trace, and the grey envelope is generated by 99 simulations of the CSR model. The spatial distributions of All Synapses were nearly random, since all three functions lay within the simulated envelopes or deviated only slightly (arrows).



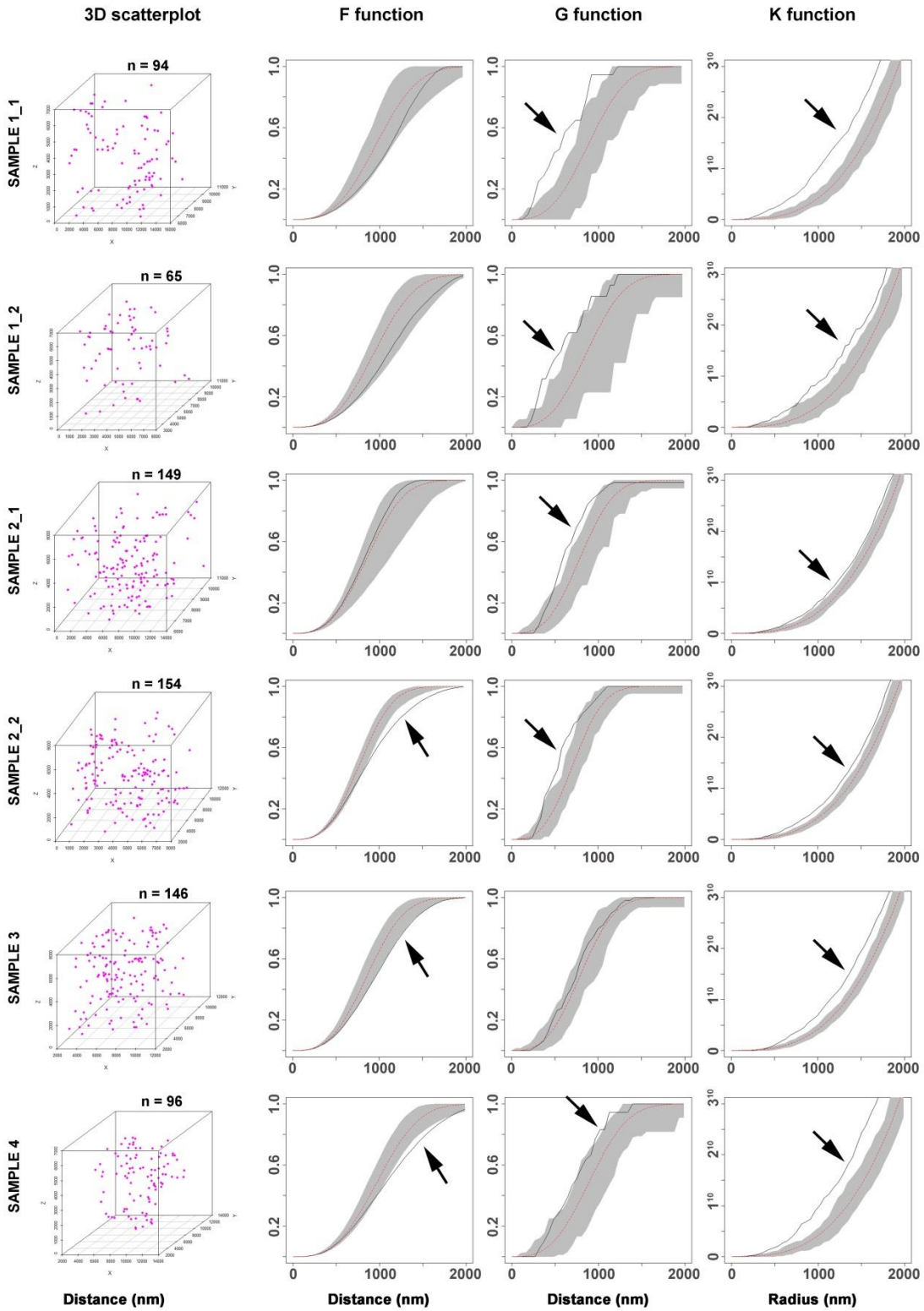
Supplementary Figure 4. 3D spatial distribution of synapses (Cleft Ast Synapses). The spatial distribution analysis of synapses whose synaptic cleft were in contact with astrocytic processes (Cleft Ast synapses) in the different samples: sample 1.1 (n = 205 synapses; top row), sample 1.2 (n = 171 synapses; second row), sample 2.1 (n = 212 synapses; third row), sample 2.2 (n = 151 synapses: fourth row), sample 3 (n = 177 synapses; fifth row) and sample 4 (n = 141 synapses; bottom row). For each synapse, we recorded the spatial positions of the center of gravity or centroid of the synaptic junction, as represented in the 3D scatterplots of the rightmost column. Three spatial statistical

functions (F, G and K) were calculated for each group of synapses in each sample. In each graph, the function corresponding to the actual sample is represented by a black line. The theoretical homogeneous Poisson distribution or complete spatial randomness (CSR) is represented as a red discontinuous trace, and the grey envelope is generated by 99 simulations of the CSR model. The spatial distributions of Cleft Ast Synapses were nearly random, since all three functions lay within the simulated envelopes or deviated only slightly (arrows).



Supplementary Figure 5. 3D spatial distribution of synapses (Pre/Post Ast Synapses). The spatial distribution analysis of synapses whose synaptic cleft were in contact with astrocytic processes (Pre/Post Ast synapses) in the different samples: sample 1.1 (n = 86 synapses; top row), sample 1.2 (n = 70 synapses; second row), sample 2.1 (n = 128 synapses; third row), sample 2.2 (n = 104 synapses; fourth row), sample 3 (n = 66 synapses; fifth row) and sample 4 (n = 83 synapses; bottom row). For each synapse, we recorded the spatial positions of the center of gravity or centroid of the synaptic junction, as

represented in the 3D scatterplots of the rightmost column. Three spatial statistical functions (F, G and K) were calculated for each group of synapses in each sample. In each graph, the function corresponding to the actual sample is represented by a black line. The theoretical homogeneous Poisson distribution or complete spatial randomness (CSR) is represented as a red discontinuous trace, and the grey envelope is generated by 99 simulations of the CSR model. The spatial distributions of Pre/Post Ast Synapses were nearly random, since all three functions lay within the simulated envelopes or deviated only slightly (arrows).



Supplementary Figure 6. 3D spatial distribution of synapses (Free Ast Synapses). The spatial distribution analysis of synapses whose synaptic cleft were in contact with astrocytic processes (Free Ast synapses) in the different samples: sample 1.1 (n = 94 synapses; top row), sample 1.2 (n = 65 synapses; second row), sample 2.1 (n = 149 synapses; third row), sample 2.2 (n = 154 synapses; fourth row), sample 3 (n = 146 synapses; fifth row) and sample 4 (n = 96 synapses; bottom row). For each synapse, we recorded the spatial positions of the center of gravity or centroid of the synaptic junction, as

represented in the 3D scatterplots of the rightmost column. Three spatial statistical functions (F, G and K) were calculated for each group of synapses in each sample. In each graph, the function corresponding to the actual sample is represented by a black line. The theoretical homogeneous Poisson distribution or complete spatial randomness (CSR) is represented as a red discontinuous trace, and the grey envelope is generated by 99 simulations of the CSR model. The spatial distributions of Free Ast Synapses were clearly incompatible with the CSR model, since —for the most part— both the G and K functions lay clearly outside the envelopes. In this case, it is a clustered distribution, since the G function indicates that the nearest neighbors are closer than expected by chance, and the K function shows higher than expected local densities of points.

SUPPLEMENTARY TABLES

Rat ID	Astrocyte ID	Brain cut orientation	Area of the Astrocyte cell arbor (μm^2)	Feret diameter of the Astrocyte cell arbor (μm)
1	1	Coronal	1935.42	57.14
1	2	Coronal	1607.35	52.98
2	3	Coronal	2529.91	68.08
2	4	Coronal	1882.65	63.74
2	5	Coronal	1356.79	55.59
2	6	Coronal	1806.68	57.86
2	7	Coronal	1937.00	57.68
2	8	Coronal	2169.18	63.38
2	9	Coronal	2154.90	63.86
2	10	Coronal	1718.11	57.19
2	11	Coronal	2175.58	60.82
2	12	Coronal	1574.90	51.12
3	13	Coronal	3126.02	84.91
3	14	Coronal	1796.15	65.57
3	15	Coronal	1704.73	54.32
3	16	Coronal	1255.61	45.05
3	17	Coronal	1644.34	61.24
3	18	Coronal	1224.07	47.96
3	19	Coronal	2806.00	75.51
4	20	Coronal	2445.86	70.57
4	21	Coronal	1840.29	60.47
4	22	Coronal	1125.07	46.94
4	23	Coronal	2216.44	64.27
MEAN (n = 23 cells)		**CORONAL**	**1915 ± 498**	**60 ± 9**
1	24	Horizontal	1756.93	59.06
1	25	Horizontal	2870.10	80.23
1	26	Horizontal	2224.52	59.43
1	27	Horizontal	3919.68	91.85
1	28	Horizontal	2255.47	69.96
1	29	Horizontal	2281.66	69.13
1	30	Horizontal	2274.46	72.97
1	31	Horizontal	1858.81	60.51
1	32	Horizontal	1258.90	50.06
2	33	Horizontal	2347.48	66.14
2	34	Horizontal	2112.94	66.21
2	35	Horizontal	1903.51	56.88
4	36	Horizontal	1918.36	69.68
4	37	Horizontal	1944.92	68.95
4	38	Horizontal	2355.07	62.87
4	39	Horizontal	2121.05	63.18
4	40	Horizontal	1369.36	50.11
MEAN (n = 17 cells)		**HORIZONTAL**	**2163 ± 591**	**66 ± 10**
MEAN (n = 40 cells)			**2010 ± 131**	**63 ± 2**

Supplementary Table 1. Area and feret diameter of the cell arbor of the 40 LY-injected astrocytes of layer IV. Rat ID and brain cut orientation of the injected slice has been indicated. Mean ± SD has been provided. The following statistical comparisons were performed:

- coronal arbor area *versus* horizontal arbor area: not significant, U-Mann Whitney = 258, p = 0.09.
- coronal feret diameter *versus* horizontal feret diameter: not significant, $t_{1,38}$= -1.758, p = 0.087.
- arbor area from different rats: not significant, $F_{3,36}$ = 0.569, p = 0.639, η^2 = 0.045
- feret diameter from different rats: not significant, $F_{3,36}$ = 0.536, p = 0.660, η^2 = 0.043

			Number of synapses	Density (syn/μm^3)	% within sample	% within AS	% within SS
Sample 1	**AS**	ALL	721	0.701	94.4	100	
		Cleft Ast	372	0.362	48.7	51.6	
		Pre/Post Ast	181	0.176	23.7	25.1	
		Free Ast	168	0.163	22.0	23.3	
	SS	ALL	43	0.042	5.6		100
		Cleft Ast	18	0.018	2.4		41.9
		Pre/Post Ast	13	0.013	1.7		30.2
		Free Ast	12	0.012	1.5		27.9
	TOTAL		**764**	**0.743**	**100**		
Sample 2	**AS**	ALL	709	0.881	94.5	100	
		Cleft Ast	305	0.379	40.7	43.0	
		Pre/Post Ast	175	0.215	23.3	24.7	
		Free Ast	229	0.284	30.5	32.3	
	SS	ALL	41	0.051	5.5		100
		Cleft Ast	11	0.013	1.5		26.8
		Pre/Post Ast	19	0.024	2.5		46.3
		Free Ast	11	0.014	1.5		26.8
	TOTAL		**750**	**0.932**	**100**		

			Number of synapses	Density (syn/μm^3)	% within sample	% within AS	% within SS
Sample 3	**AS**	ALL	751	0.684	94.1	100	
		Cleft Ast	341	0.311	42.7	45.4	
		Pre/Post Ast	142	0.130	17.8	18.9	
		Free Ast	268	0.245	33.6	35.7	
	SS	ALL	47	0.043	5.9		100
		Cleft Ast	5	0.005	0.6		10.6
		Pre/Post Ast	13	0.012	1.6		27.7
		Free Ast	29	0.026	3.6		61.7
	TOTAL		**798**	**0.729**	**100**		
Sample 4	**AS**	ALL	626	0.621	91.9	100	
		Cleft Ast	246	0.244	36.4	39.3	
		Pre/Post Ast	173	0.172	25.4	27.6	
		Free Ast	207	0.205	30.4	33.1	
	SS	ALL	55	0.055	8.1		100
		Cleft Ast	17	0.017	2.5		30.9
		Pre/Post Ast	17	0.017	2.5		30.9
		Free Ast	21	0.021	3.1		38.1
	TOTAL		**681**	**0.676**	**100**		

			Number of synapses	Density (syn/μm^3)	% within sample	% within AS	% within SS
All samples	**AS**	All	2807	0.722 ± 0.111	93.8 ± 1.2	100	
		Cleft Ast	1264	0.324 ± 0.061	42.1 ± 5.1	45 ± 5	
		Pre/Post Ast	671	0.173 ± 0.035	22.6 ± 3.3	24 ± 4	
		Free Ast	872	0.225 ± 0.052	29.1 ± 5.0	31 ± 5	
	SS	ALL	186	0.048 ± 0.006	6.2 ± 1.2		100
		Cleft Ast	51	0.013 ± 0.006	1.7 ± 0.9		27 ± 13
		Pre/Post Ast	62	0.017 ± 0.005	2.1 ± 0.5		34 ± 8
		Free Ast	73	0.018 ± 0.006	2.4 ± 1.1		39 ± 16
TOTAL			**2993**	**0.769 ± 0.111**	**100**		

Supplementary Table 2. Synapse information —numbers, density and percentage— per sample and the average of all samples. Once the counting frame was applied, the final total volume analyzed in sample 1 was 1028 μm^3, in sample 2: 805 μm^3, in sample 3: 1096 μm^3 and in sample 4: 1007 μm^3 (values corrected for tissue shrinkage). Average data correspond to mean ± SD. The following statistical comparisons were performed, applying one-way ANOVA together with Bonferroni multiple comparisons post hoc:

- Density of asymmetric synapses (AS) according to their contacts with the labeled astrocytic compartments: the density of synapses with the synaptic cleft in contact with astrocytic processes was significantly higher than the density of synapses with the Pre/Post in contact with astrocytic processes (t_9 = 4.249, p = 0.006). In addition, the density of synapses with the synaptic cleft in contact with astrocytic processes was nearly one third higher than the density of asymmetric synapses that were free of astrocytic processes (0.22 ± 0.05 asymmetric synapses / μm^3) — although this difference was not statistically significant (t_9 = 2.802, p = 0.062). $F_{2,9}$ = 9.334, p = 0.006, η^2 = 0.675

- Density of symmetric synapses (SS) according to their contacts with the labeled astrocytic compartments: not significant. $F_{2,9}$ = 0.694, p = 0.525, η^2 = 0.134

χ^2 test was applied:

- Percentage of AS according to their contacts with the labeled astrocytic compartments: significantly different (χ^2 = 196.340, p = 0.000)

- Percentage of SS according to their contacts with the labeled astrocytic compartments: not significant (χ^2 = 4.292, p = 0.117)

	Estimated Sample Volume (μm^3)	Estimated Astrocyte Volume (μm^3) (total)	Estimated Astrocyte Volume (μm^3) (soma)	Estimated Astrocyte (processes) Volume (μm^3) (processes)	Estimated Neuropil Volume (μm^3) (excluding somas)	% of sample volume occupied by astrocyte (total)	% of sample volume occupied by astrocyte (soma)	% of sample volume occupied by astrocyte (processes)	% of neuropil occupied by astrocyte (processes)
Sample 1	1091	264	142	122	866	24	13	11	14
Sample 2	1167	242	110	132	1003	21	9	11	13
Sample 3	1164	221	109	112	1038	19	9	10	11
Sample 4	1535	198	55	142	1324	13	4	9	11
MEAN	**1239 ± 200**	**231 ± 28**	**104 ± 36**	**127 ± 13**	**1057 ± 192**	**19 ± 5**	**9 ± 4**	**10 ± 1**	**12 ± 2**

Supplementary Table 3. Estimation of the volume fraction of photoconverted astrocytic compartments (soma and processes) and neuropil. Average data correspond to mean ± SD.

Asymmetric synapses	Pre (nº of synapses)	Post (nº of synapses)	Pre and Post (nº of synapses)	Pre (%)	Post (%)	Pre and Post (%)
Sample 1	70	81	30	38.7	44.7	16.6
Sample 2	76	67	30	43.9	38.7	17.4
Sample 3	62	52	28	43.7	36.6	19.7
Sample 4	97	57	19	56	33	11
MEAN				**38.3 ± 4.9**	**45.6 ± 7.4**	**16.1 ± 3.7**
Symmetric synapses						
Sample 1	1	8	4	7.7	61.5	30.8
Sample 2	10	5	4	52.6	26.3	21.1
Sample 3	5	5	3	38.5	38.5	23.0
Sample 4	8	6	3	47.1	35.3	17.6
MEAN				**40.4 ± 15.0**	**36.5 ± 20.0**	**23.1 ± 5.6**

Supplementary Table 4. Number and percentage of synapses (both asymmetric and symmetric) that had only the presynaptic (Pre), only the postsynaptic (Post) or both the presynaptic and the postsynaptic terminals (Pre and Post) in contact with the labeled astrocytic compartments. Average data correspond to mean ± SD.

χ^2 test was applied:

- Percentage of AS —within the population of Pre/Post Ast synapses— according to the contact of the presynaptic (only), the postsynaptic (only) or both the pre- and the postsynaptic element with the labeled astrocytic compartment: significantly different (χ^2 = 95.677, p = 0.000)

- Percentage of SS —within the population of Pre/Post Ast synapses— according to the contact of the presynaptic (only), the postsynaptic (only) or both the pre- and the postsynaptic element with the labeled astrocytic compartment: not significantly different (χ^2 = 3.226, p = 0.199)

	Asymmetric synapses (AS)	Number of synapses in contact with astrocyte (soma and processes)	% of synapses in contact with astrocyte (soma)	% of synapses in contact with astrocyte (processes)	% of the photoconverted astrocyte in the sample corresponding to the soma*	% of the photoconverted astrocyte in the sample corresponding to the processes**
Sample 1	Cleft Ast	**372**	**2.2 (8/372)**	**98.8 (364/372)**	**54**	**46**
	Pre/Post Ast	**181**	**4.4 (8/181)**	**95.6 (173/181)**		
Sample 2	Cleft Ast	**305**	**0.7 (2/305)**	**99.3 (303/305)**	**45**	**55**
	Pre/Post Ast	**175**	**3.4 (6/175)**	**96.6 (169/175)**		
Sample 3	Cleft Ast	**341**	**1.8 (6/341)**	**98.2 (335/341)**	**49**	**51**
	Pre/Post Ast	**142**	**1.4 (2/142)**	**98.6 (140/142)**		
Sample 4	Cleft Ast	**246**	**2.8 (7/246)**	**97.2 (239/246)**	**28**	**71**
	Pre/Post Ast	**173**	**1.7 (3/173)**	**98.3 (170/173)**		
MEAN	Cleft Ast		**1.9 ± 0.9**	**98.4 ± 0.9**	**44 ± 11**	**56 ± 11**
	Pre/Post Ast		**3.1 ± 1.1**	**97.3 ± 1.4**		
	Symmetric synapses (SS)					
Sample 1	Cleft Ast	**18**	**5.6 (1/18)**	**94.4 (17/18)**	**54**	**46**
	Pre/Post Ast	**13**	**0**	**100 (13/13)**		
Sample 2	Cleft Ast	**11**	**0**	**100 (11/11)**	**45**	**55**
	Pre/Post Ast	**19**	**0**	**100 (19/19)**		
Sample 3	Cleft Ast	**5**	**0**	**100 (5/5)**	**49**	**51**
	Pre/Post Ast	**13**	**0**	**100 (13/13)**		
Sample 4	Cleft Ast	**17**	**0**	**100 (17/17)**	**28**	**71**
	Pre/Post Ast	**17**	**0**	**100 (17/17)**		
MEAN	Cleft Ast		**1.4 ± 2.8**	**98.6 ± 2.8**	**44 ± 11**	**56 ± 11**
	Pre/Post Ast		**0**	**100**		

Supplementary Table 5. Number and percentage of synapses (asymmetric and

symmetric) that were in contact with the soma of the astrocyte or with their processes. * Data calculated from **Supplementary table 3** = % of sample volume occupied by astrocyte (soma) * 100 / % of sample volume occupied by astrocyte (total). ** Data calculated from **Supplementary table 3** = % of sample volume occupied by astrocyte (processes) * 100 / % of sample volume occupied by astrocyte (total). Average data correspond to mean ± SD.

		Type of synapse	No. synapses	SAS area (x 10000) (nm^2; mean ± SD)	μ (location) of the log-normal distribution	σ (scale) of the log-normal distribution
AS	Sample 1	Cleft Ast	372	6.666 ± 4.934	1.643	0.7431
		Pre/Post Ast	181	5.052 ± 3.889	1.3421	0.7758
		Free Ast	168	4.886 ± 3.013	1.2202	0.7105
	Sample 2	Cleft Ast	303	6.210 ± 4.334	1.6079	0.6845
		Pre/Post Ast	170	4.755 ± 3.537	1.2988	0.7609
		Free Ast	229	4.307± 3.236	1.2244	0.6918
	Sample 3	Cleft Ast	341	8.586 ± 6.835	1.8681	0.7678
		Pre/Post Ast	142	7.696 ± 6.074	1.7146	0.8656
		Free Ast	268	5.819 ± 4.327	1.4935	0.7617
	Sample 4	Cleft Ast	246	7.038 ± 6.337	1.6308	0.8267
		Pre/Post Ast	173	6.687 ± 5.672	1.5927	0.8019
		Free Ast	207	5.050 ± 3.702	1.3965	0.6814
	TOTAL	Cleft Ast	1264	7.148 ± 5.736	1.693	0.7602
		Pre/Post Ast	671	5.965 ± 4.979	1.4756	0.8148
		Free Ast	872	4.944 ± 3.727	1.3471	0.7239
SS	Sample 1	Cleft Ast	18	5.517 ± 3.190	1.4965	0.7553
		Pre/Post Ast	13	4.639 ± 3.457	1.2777	0.7574
		Free Ast	12	5.172 ± 4.506	1.2754	0.9356
	Sample 2	Cleft Ast	11	9.612 ± 6.242	2.0188	0.8033
		Pre/Post Ast	19	6.123 ± 3.770	1.6244	0.649
		Free Ast	11	4.948 ± 2.474	1.4377	0.6722
	Sample 3	Cleft Ast	5	15.953 ± 7.604	2.5864	0.8075
		Pre/Post Ast	13	8.997 ± 8.018	1.847	0.9072
		Free Ast	29	5.729 ± 4.101	1.4656	0.8122
	Sample 4	Cleft Ast	17	8.191 ± 6.125	1.7874	0.946
		Pre/Post Ast	17	9.075 ± 5.370	2.0103	0.7003
		Free Ast	21	8.144 ± 10.669	1.5584	1.0165
	TOTAL	Cleft Ast	51	8.314 ± 6.075	1.8129	0.8758
		Pre/Post Ast	62	7.223 ± 5.494	1.7042	0.7739
		Free Ast	73	6.214 ± 6.612	1.4568	0.8653

Supplementary Table 6. Synapse size information per sample and as a total for all samples together. The distribution of the size of synapses fits a log-normal distribution. Two

parameters of this function [μ (location) and σ (scale)] are showed. Average data correspond to mean ± SD. The nonparametric two samples Kolmogorov-Smirnov test was used to compare the cumulative distributions of two data sets (two categories of the classification of synapses regarding their 3D contact with labeled astrocytic compartments). Thus, all possible comparisons were made by pairs. Statistically significant differences were as follows:

Asymmetric synapses (AS)
All samples:
Synaptic cleft contact *versus* Pre-/Post contact: $D_{1264,671} = 2.419$, p = 0.000 (***)
Synaptic cleft contact *versus* NO contact: $D_{1264,872} = 4.303$, p = 0.000 (###)
Pre-/Post contact *versus* NO contact: $D_{872,671} = 1.828$, p = 0.003 (++)

Sample 1:
Synaptic cleft contact *versus* Pre-/Post contact: $D_{372,181} = 1.932$, p = 0.001 (***)
Synaptic cleft contact *versus* NO contact: $D_{372,168} = 2.896$, p = 0.000 (###)
Pre-/Post contact *versus* NO contact: not significant

Sample 2:
Synaptic cleft contact *versus* Pre-/Post contact: $D_{303,170} = 1.799$, p = 0.003 (**)
Synaptic cleft contact *versus* NO contact: $D_{303,229} = 2.918$, p = 0.000 (###)
Pre-/Post contact *versus* NO contact: not significant

Sample 3:
Synaptic cleft contact *versus* Pre-/Post contact: not significant
Synaptic cleft contact *versus* NO contact: $D_{341,268} = 2.536$, p = 0.000 (###)
Pre-/Post contact *versus* NO contact: $D_{142,268} = 1.607$, p = 0.011 (+)

Sample 4:
Synaptic cleft contact *versus* Pre-/Post contact: not significant
Synaptic cleft contact *versus* NO contact: $D_{246,207} = 1.699$, p = 0.006 (##)
Pre-/Post contact *versus* NO contact: $D_{173,207} = 1.444$, p = 0.031 (+)

Symmetric synapses (SS)
All samples:
Synaptic cleft contact *versus* Pre-/Post contact: not significant
Synaptic cleft contact *versus* NO contact: $D_{51,73} = 1.551$, p = 0.016 (#)
Pre-/Post contact *versus* NO contact: not significant

Sample 1:
Synaptic cleft contact *versus* Pre-/Post contact: not significant
Synaptic cleft contact *versus* NO contact: not significant
Pre-/Post contact *versus* NO contact: not significant

Sample 2:
Synaptic cleft contact *versus* Pre-/Post contact: not significant
Synaptic cleft contact *versus* NO contact: not significant
Pre-/Post contact *versus* NO contact: not significant

Sample 3:
Synaptic cleft contact *versus* Pre-/Post contact: not significant
Synaptic cleft contact *versus* NO contact: $D_{5,29} = 1.581$, p = 0.013 (#)
Pre-/Post contact *versus* NO contact: not significant

Sample 4:
Synaptic cleft contact *versus* Pre-/Post contact: not significant
Synaptic cleft contact *versus* NO contact: not significant

Pre-/Post contact *versus* NO contact: not significant

Synaptic cleft contact *versus* Pre-/Post contact: ***
Synaptic cleft contact *versus* NO contact: ###
Pre-/Post contact *versus* NO contact: +++

		SYNAPTIC SHAPE		
		MACULAR	HORSESHOE PERFORATED FRAGMENTED	Total
TYPE OF SYNAPSE	Cleft Ast	**1148** (1178.0)	**117** (87.0)	1265
	Pre/Post Ast	**618** (604.4)	**31** (44.6)	649
	Free Ast	**833** (816.7)	**44** (60.3)	877
	Total	2599	192	2791

Supplementary Table 7. Contingency table showing the type of synapse classified according to their 3D contact with labeled astrocytic compartments against the synaptic shape (macular vs. more complex shapes – horseshoe, perforated, fragmented) for asymmetric synapses. See text and **Figure 8**.

χ^2 test was applied:

- "Cleft Ast synapses" *versus* "Pre/Post Ast": significantly different (χ^2, p = 0.002, **)
- "Cleft Ast synapses" *versus* "Free Ast synapses": significantly different (χ^2, p = 0.001, ###).

		SYNAPTIC SHAPE		
		MACULAR	HORSESHOE PERFORATED FRAGMENTED	Total
TYPE OF SYNAPSE	Cleft Ast	**25** (34.6)	**26** (16.4)	51
	Pre/Post Ast	**46** (42.1)	**16** (19.9)	62
	Free Ast	**56** (50.3)	**18** (23.7)	74
	Total	127	60	187

Supplementary Table 8. Contingency table showing the type of synapse classified according to their 3D contact with labeled astrocytic compartments against the synaptic shape (macular vs. more complex shapes – horseshoe, perforated, fragmented) for symmetric synapses. See text and **Figure 8**.

χ^2 test was applied:

- "Cleft Ast synapses" *versus* "Pre/Post Ast": significantly different (χ^2, p = 0.018, *)
- "Cleft Ast synapses" *versus* "Free Ast synapses": significantly different (χ^2, p = 0.006, ##).

		No. of AS	DENSITY (No. AS / μm^3)	No. of synapses (AS + SS)	DENSITY (No. synapses / μm^3)
SAMPLE 1.1	**Cleft Ast**	205	0.46	217	0.49
	Pre/Post Ast	86	0.19	96	0.22
	Free Ast	94	0.21	101	0.23
	TOTAL	385	0.87	414	0.94
SAMPLE 1.2	**Cleft Ast**	171	0.56	178	0.58
	Pre/Post Ast	70	0.23	76	0.25
	Free Ast	65	0.21	67	0.22
	TOTAL	306	1.00	321	1.05
SAMPLE 2.1	**Cleft Ast**	212	0.42	220	0.44
	Pre/Post Ast	128	0.26	136	0.27
	Free Ast	149	0.30	155	0.31
	TOTAL	489	0.98	512	1.02
SAMPLE 2.2	**Cleft Ast**	151	0.40	156	0.41
	Pre/Post Ast	104	0.27	115	0.30
	Free Ast	154	0.40	156	0.41
	TOTAL	409	1.07	427	1.12
SAMPLE 3	**Cleft Ast**	177	0.36	178	0.36
	Pre/Post Ast	66	0.13	74	0.15
	Free Ast	146	0.30	165	0.34
	TOTAL	389	0.79	417	0.85
SAMPLE 4	**Cleft Ast**	141	0.33	151	0.36
	Pre/Post Ast	83	0.20	90	0.21
	Free Ast	96	0.23	105	0.25
	TOTAL	320	0.75	346	0.82
MEAN OF ALL SAMPLES	**TOTAL**		**0.91 ± 0.12**		**0.97 ± 0.12**

Supplementary Table 9. Sub-samples for sample 1 (sample 1.1 and 1.2), sample 2 (sample 2.1 and 2.2), sample 3 (sample 3) and sample 4 (sample 4) establishing orthogonal counting frames and avoiding cell somata to perform the analysis of the spatial distribution of all (asymmetric and symmetric) synapses and asymmetric synapses in the neuropil. Once the counting frame had been applied, the final total volume analyzed in sample 1.1 was 442 μm^3, in sample 1.2: 307 μm^3, in sample 2.1: 500 μm^3, in sample 2.2: 382 μm^3, in sample 3: 490 μm^3 and in sample 4: 424 μm^3 (values corrected for tissue shrinkage). Average data correspond to mean ± SD.

Reference and Specie	Type of tissue	Astrocyte detection method	% of total area occupied by astrocytic processes	Nº of synapses analyzed	% of synapses…						PSD area (μm^2)			
					…with the synaptic cleft in contact with astrocyte			…with the postsynaptic terminal in contact with astrocyte	…with the presynaptic terminal in contact with astrocyte	…free of astrocytes	Synapse with the synaptic cleft in contact with astrocyte	Synapse with the postsynaptic terminal in contact with astrocyte	Synapse with the presynaptic terminal in contact with astrocyte	Synapses free of astrocytes
					TOTAL	Macular (Simple)	Perforated (Complex)							
[1] Human (10y)	Acute slice from biopsy Hippocampus (CA1, str. Rad)	By their irregular, stellate shape and the presence of glycogen granules and bundles of intermediate filaments	4.8 ± 0.6 %	191***	40						0.065		0.050	
[2] Rat (7d)	Organotypic hippocampal slice cultures (CA1, str. Rad)	By their morphological characteristics: relatively lucid cytoplasm, irregular shape, presence of glycogen granules, and typical bundles of intermediate filaments in thicker processes	4.6 ± 0.4 %	149	85	77.8	96.6							
[3] Rat (40-77d)	Perfused-Fixed brain Hippocampus (CA1, str. Rad)	*Idem* to [1]	4 ± 1 %	229	57	52	88				-	-	-	-
[4] Rat (8w)	Dentate Gyrus (medial molecular layer)	*Idem* to [1]	9.18 ± 0.65 %	189	RESULT: Astroglia approached PSDs on thin dendritic spines (macular simple synapses) more closely than PSDs on mushroom spines (complex synapses).									
[5] Rat (65-75d)	Perfused-Fixed brain Hippocampus (CA1, str. Rad)	*Idem* to [1]	-	201	61.9 ± 3.7	60*	90 – 99**	6.6 ± 2.9	8.1 ± 2.7	23.9 ± 3.4	0.055 ± 0.003	0.047 ± 0.006	0.036 ± 0.004	0.036 ± 0.004
	Acute slice Hippocampus (CA1, str. Rad)	*Idem* to [1]	-	210	43 ± 3.5	40*	60**	10.6 ± 2.8	6.3 ± 2.7	39.6 ± 3.3				
[6] Rat (adult)	Somatosensory cortex, barrel cortex (Layer IV)	Glutamine synthetase pre-embedding immunohistochemistry (dark DAB reaction product)			>90									
[7] Rat (adult)	Somatosensory cortex, barrel cortex (Layer Vb)	Glutamine synthetase pre-embedding immunohistochemistry (dark DAB reaction product)		33	>90									
[8] Mouse (adult)	Somatosensory cortex (Layer IV)	-	-	-	68					10				

Supplementary Table 10. Data of the relationship of synapses and astrocytes obtained from EM studies in normal cerebral cortex of different species (d = day-old; w = week-old; y = year-old).

[1] Witcher et al., 2010

[2] Lushnikova et al., 2009

[3] Ventura and Harris, 1999

[4] Medvedev et al., 2014

[5] Witcher et al., 2007

[6] Rollenhagen, et al., 2015

[7] Rollenhagen, et al., 2018

[8] Bernardinelli et al., 2014a

*Only small macular synapses from thin spines

**Includes larger macular synapses from mushroom spines and perforated synapses

***Only data from patients with mild degree of epilepsy have been extracted from the paper (those closer to a “normal” situation)

SUPPLEMENTARY VIDEOS LEGENDS

Supplementary video 1. **Video of the EspINA software user interface.** FIB/SEM sections are viewed through the *xy*-plane (as obtained by FIB/SEM microscopy) as well as the *yz*- and *xz*-planes. 3D segmentation of a perforated synapse is shown. The 3D reconstruction of this perforated synapse (a "Pre/Post Ast synapse") is shown in the 3D viewer in light blue at the end of the video. Together with the 3D reconstruction, the synaptic apposition surface (SAS) (yellow) extracted from this same synapse is shown. The 3D reconstruction of the synapse allows us to identify the morphology of the synapse as perforated. This same "Pre/Post Ast synapse" is shown in **Figure 4H–N** where an arrow points out the perforation in the synaptic junction.

Supplementary video 2. **Video of the FIB/SEM serial sections and the synaptic classification according to the morphology of the PSD and their contacts with the labeled astrocytic processes**. This video shows FIB/SEM serial sections (labeled astrocytic processes are electron dense diaminobenzidine (DAB)-deposits), illustrating (i) how AS (excitatory) and SS (inhibitory) are identified; (ii) how synapses are classified according to their contacts with the labeled astrocytic processes *[with examples of an asymmetric synapse with the labeled astrocytic process in contact with the synaptic cleft (segmented in light orange), an asymmetric synapse with the labeled astrocytic process in contact with the postsynaptic element (synapse segmented in light blue), and an asymmetric synapse with no labeled astrocytic process in contact with it (synapse segmented in light purple)]*; and (iii) the 3D view of the orthogonal planes of the tissue together with all synapses segmented and classified according to their contacts with astrocytic processes in a stack of images.

Supplementary video 3. **Video of the 3D reconstruction to illustrate synapses classified according to their contacts with the labeled astrocytic processes.** The following elements are 3D reconstructed: an asymmetric "Pre/Post Ast synapse" (light blue) and two asymmetric "Cleft Ast synapses" (light orange), in addition to their respective postsynaptic (green) and presynaptic (blue) elements. Also, the surrounding astrocytic processes (red) are shown. See **Figures 5** and **6** to visualize the segmented structures in the FIB/SEM images (**Figure 5**) and images of the 3D reconstructions **(Figure 6**).