

The Role of Tenascin-C on the Structural Plasticity of Perineuronal Nets and Synaptic Expression in the Hippocampus of Male Mice

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Supplementary Table S1. Acquisition settings used for confocal fluorescence imaging.

Experimental setup of confocal fluorescence microscopy								
	Objective	Laser Unit				Channel settings		
		Laser source	Wavelength	Power	Excitation transmission	Pinhole	Detector gain	Amplifier Offset
WFA	Plan-Neofluar	Argon	488 nm	30 mW	35%	4 µm	672	-0.3
PV	40x/1.30 Oil	HeNe1	543 nm	1 mW	30%		691	-0.3
WFA	Plan-Apochromat	Argon	488 nm	30 mW	35%	2.2 µm	790	-0.3
VGAT	63x/1.4 Oil	HeNe1	543 nm	1 mW	30%		763	-0.3
VGLUT1		HeNe2	633 nm	5 mW	30%		866	-0.3

Supplementary Table S2. Number of investigated PNNs and PV cells in the four subfields of the hippocampus

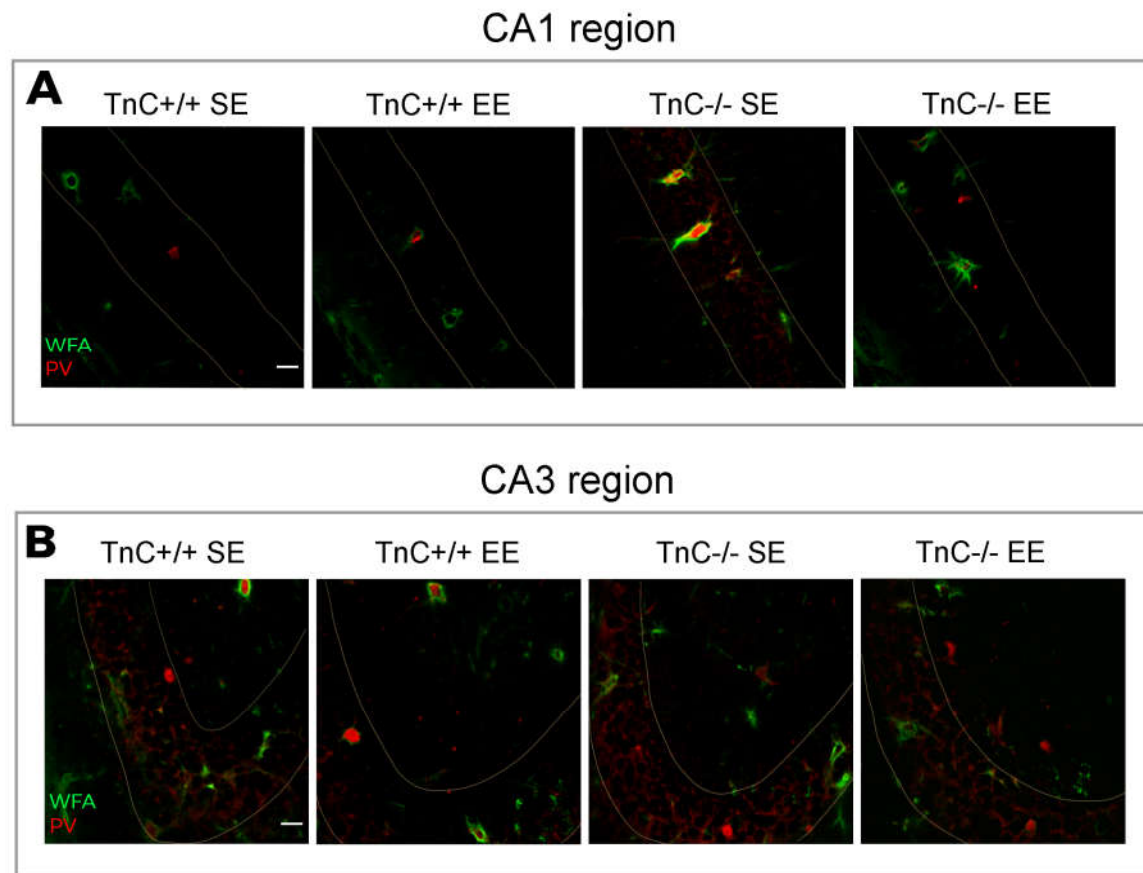
Group	Number of investigated PNNs	Number of investigated PV+ cells	Number of investigated PNNs for ultrastructure analysis	Number of investigated PNNs with VGAT and VGLUT1 puncta
<i>DG</i>				
TnC +/+ SE	64	58	23	14
TnC +/+ EE	70	55	23	15
TnC -/- SE	77	49	23	17
TnC -/- EE	58	57	23	16
<i>CA1</i>				
TnC +/+ SE	81	86	36	20
TnC +/+ EE	101	98	36	16
TnC -/- SE	90	68	36	17
TnC -/- EE	95	99	36	16
<i>CA2</i>				
TnC +/+ SE	48	44	23	17
TnC +/+ EE	75	59	23	17
TnC -/- SE	90	65	23	14
TnC -/- EE	78	57	23	16
<i>CA3</i>				
TnC +/+ SE	88	84	14	18
TnC +/+ EE	91	91	14	18
TnC -/- SE	101	104	14	17
TnC -/- EE	92	91	14	18

Supplementary Table S3. Results of statistical tests for every parameter in the four hippocampal subregions DG, CA1, CA2 and CA3.

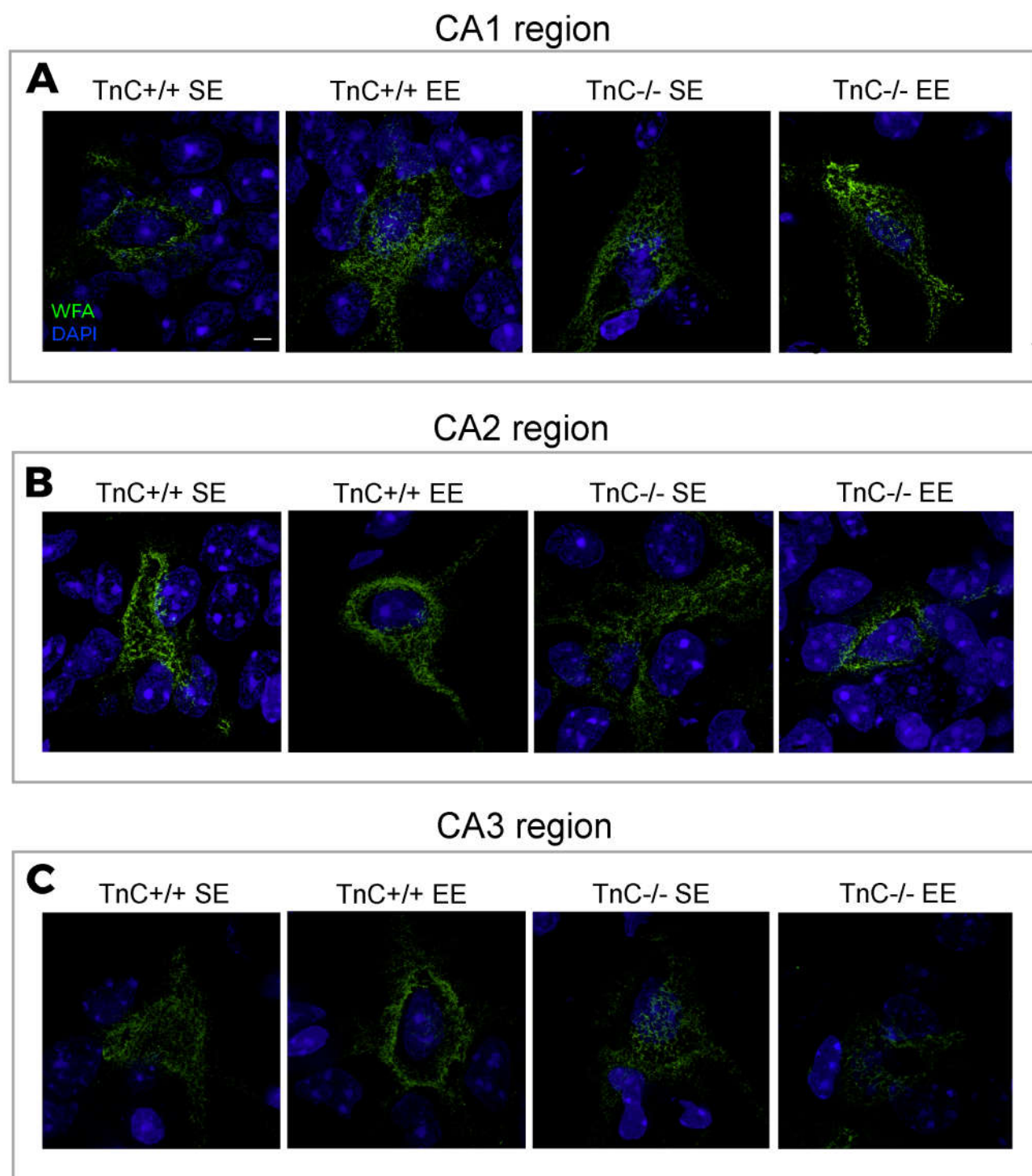
Measured parameter	DG	CA1	CA2	CA3
Statistical test:				
Two-way ANOVA, F value, degrees of freedom (df), p value				
PNN intensity	Genotype	Genotype	Genotype	Genotype
	F (1,46) = 4.638	F (1,47) = 1.017	F (1,45) = 0.731	F (1,45) = 0.367
	p = 0.037	p = 0.319	p = 0.397	p = 0.548
	No <i>post hoc</i> difference	Environment	Environment	Environment
	Environment	F (1,47) = 1.505	F (1,45) = 0.501	F (1,45) = 0.425
	F (1,46) = 2.177	p = 0.226	p = 0.483	p = 0.518
	p = 0.147	Genotype x Environment	Genotype x Environment	Genotype x Environment
PNN number	Genotype	Genotype	Genotype	Genotype
	F (1,45) = 0.031	F (1,46) = 0.139	F (1,45) = 8.694	F (1,45) = 0.021
	p = 0.861	p = 0.711	p = 0.005	p = 0.885
	Environment	Environment	Tukey <i>post hoc</i> test TnC+/+ SE vs. TnC-/-SE p = 0.002	Environment
	F (1,45) = 0.456	F (1,46) = 0.459		F (1,45) = 0.133
	p = 0.503	p = 0.502		p = 0.717
	Genotype x Environment	Genotype x Environment		Genotype x Environment
	F (1,45) = 3.102	F (1,46) = 0.291		F (1,45) = 0.613
	p = 0.085	p = 0.593		p = 0.438
PNN+/PV+ intensity	Genotype	Genotype	Genotype	Genotype
	F (1,42) = 5.482	F (1,45) = 4.910	F (1,43) = 0.728	F (1,45) = 0.212
	p = 0.024	p = 0.032	p = 0.399	p = 0.648
	No <i>post hoc</i> difference	No <i>post hoc</i> difference	Environment	Environment
	Environment	Environment	F (1,43) = 0.236	F (1,45) = 0.538
	F (1,42) = 0.499	F (1,45) = 0.301	p = 0.630	p = 0.468
	p = 0.484	p = 0.586	Genotype x Environment	Genotype x Environment
PNN+/PV+ number	Genotype	Genotype	Genotype	Genotype
	F (1,42) = 0.584	F (1,45) = 0.022	F (1,43) = 0.158	F (1,45) = 0.601
	p = 0.449	p = 0.883	p = 0.693	p = 0.443
	Environment	Environment	Environment	Environment
	F (1,42) = 0.499	F (1,45) = 0.301	F (1,43) = 0.236	F (1,45) = 0.538
	p = 0.484	p = 0.586	p = 0.630	p = 0.468
	Genotype x Environment	Genotype x Environment	Genotype x Environment	Genotype x Environment
PNN+/PV+ number	Genotype	Genotype	Genotype	Genotype
	F (1,46) = 0.006	F (1,46) = 0.011	F (1,45) = 1.435	F (1,45) = 0.113
	p = 0.939	p = 0.917	p = 0.238	p = 0.739
	Environment	Environment	Environment	Environment
	F (1,46) = 0.315	F (1,46) = 0.011	F (1,45) = 0.000	F (1,45) = 0.245
	p = 0.577	p = 0.917	p = 1.000	p = 0.623
	Genotype x Environment	Genotype x Environment	Genotype x Environment	Genotype x Environment
PNN+/PV+ number	Genotype	Genotype	Genotype	Genotype
	F (1,46) = 0.594	F (1,46) = 0.044	F (1,45) = 0.283	F (1,45) = 0.400
	p = 0.445	p = 0.834	p = 0.597	p = 0.531
	Environment	Environment	Environment	Environment
	F (1,46) = 0.315	F (1,46) = 0.011	F (1,45) = 0.000	F (1,45) = 0.245
	p = 0.577	p = 0.917	p = 1.000	p = 0.623
	Genotype x Environment	Genotype x Environment	Genotype x Environment	Genotype x Environment

PNN+/PV-intensity	Genotype F (1,40) = 1.240 p = 0.273 Environment F (1,40) = 6.408 p = 0.016 Tukey post hoc test TnC+/+ EE vs. TnC+/+ SE p=0.017 Genotype x Environment F (1,40) = 3.737, p = 0.061	Genotype F (1,44) = 0.008 p = 0.929 Environment F (1,44) = 0.064 p = 0.902 Genotype x Environment F (1,44) = 0.160 p = 0.691	Genotype F (1,41) = 0.640 p = 0.429 Environment F (1,41) = 0.797 p = 0.377 Genotype x Environment F (1,41) = 0.003 p = 0.954	Genotype F (1,42) = 1.830 p = 0.184 Environment F (1,42) = 0.004 p = 0.953 Genotype x Environment F (1,42) = 1.449 p = 0.236
PNN+/PV- number	Genotype F (1,46) = 0.243 p = 0.625 Environment F (1,46) = 0.243 p = 0.625 Genotype x Environment F (1,46) = 1.727 p = 0.196	Genotype F (1,47) = 0.249 p = 0.620 Environment F (1,47) = 0.390 p = 0.536 Genotype x Environment F (1,47) = 0.249 p = 0.620	Genotype F (1,45) = 9.612 p = 0.003 Tukey post hoc test TnC+/+ SE vs. TnC- /-SE p<0.001 Environment F (1,45) = 0.736 p = 0.396 Genotype x Environment F (1,45) = 11.356 p = 0.002 Tukey post hoc test TnC-/ EE vs. TnC-/ SE p=0.023	Genotype F (1,45) = 0.285 p = 0.596 Environment F (1,45) = 0.023 p = 0.879 Genotype x Environment F (1,45) = 0.023 p = 0.879
PV+/PNN- number	Genotype F (1,46) = 0.591 p = 0.446 Environment F (1,46) = 0.322 p = 0.573 Genotype x Environment F (1,46) = 2.092 p = 0.155	Genotype F (1,47) = 2.279 p = 0.138 Environment F (1,47) = 2.693 p = 0.108 Genotype x Environment F (1,47) = 2.279 p = 0.138	Genotype F (1,45) = 0.035 p = 0.852 Environment F (1,45) = 0.162 p = 0.689 Genotype x Environment F (1,45) = 0.950 p = 0.335	Genotype F (1,45) = 0.285 p = 0.596 Environment F (1,45) = 0.022 p = 0.882 Genotype x Environment F (1,45) = 0.384 p = 0.539
PV+ number	Genotype F (1,46) = 0.021 p = 0.886 Environment F (1,46) = 0.086 p = 0.771 Genotype x Environment F (1,46) = 0.339 p = 0.564	Genotype F (1,46) = 0.488 p = 0.489 Environment F (1,46) = 0.934 p = 0.339 Genotype x Environment F (1,46) = 2.090 p = 0.156	Genotype F (1,46) = 0.758 p = 0.389 Environment F (1,46) = 0.030 p = 0.863 Genotype x Environment F (1,46) = 1.216 p = 0.276	Genotype F (1,46) = 0.758 p = 0.389 Environment F (1,46) = 0.030 p = 0.863 Genotype x Environment F (1,46) = 1.216 p = 0.276
Statistical test:				
Kruskal Wallis, H value, degrees of freedom (df), p value				
Nodes number in	H(3) = 15.761	H(3) = 1.637	H(3) = 5.769	H(3) = 0.826
PNN mesh	p = 0.001	p = 0.651	p = 0.123	p = 0.843
Dunn post hoc test				

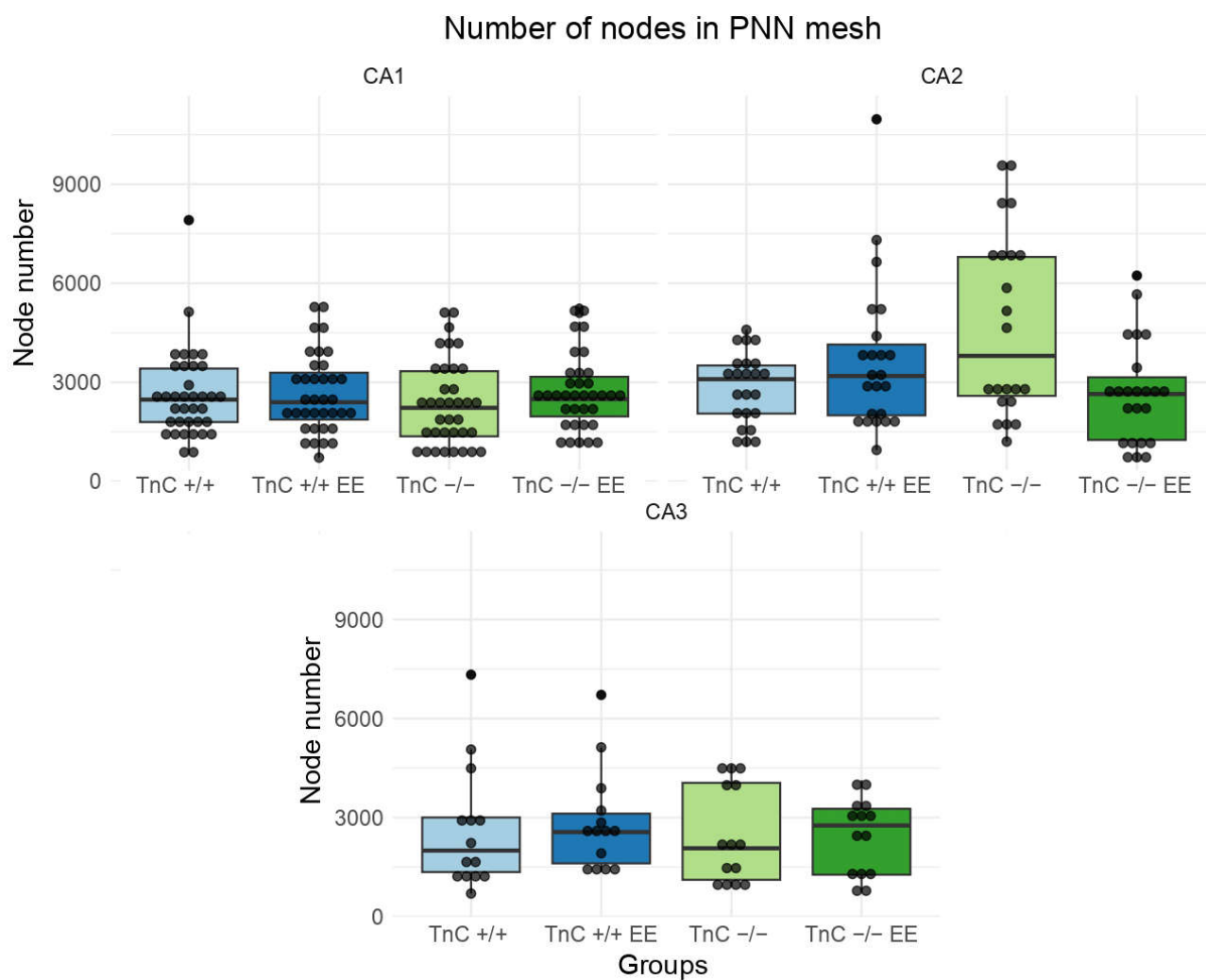
	TnC+/+ SE vs. TnC-/- SE $p = 0.001$ TnC-/- SE vs. TnC-/- EE $p = 0.026$			
Nodes percentage in PNN mesh	H(3) = 6.934 $p = 0.074$	H(3) = 4.373 $p = 0.224$	H(3) = 3.646 $p = 0.302$	H(3) = 4.668 $p = 0.198$
Nodes connectivity degree	H(3) = 4.862 $p = 0.182$	H(3) = 2.551 $p = 0.466$	H(3) = 4.918 $p = 0.178$	H(3) = 6.878 $p = 0.076$
Average distance between nodes	H(3) = 4.576 $p = 0.206$	H(3) = 4.410 $p = 0.221$	H(3) = 3.064 $p = 0.382$	H(3) = 4.497 $p = 0.213$
Maximal distance between nodes - R95	H(3) = 7.136 $p = 0.068$	H(3) = 4.322 $p = 0.229$	H(3) = 3.559 $p = 0.313$	H(3) = 2.013 $p = 0.570$
VGAT density	H(3) = 9.436 $p = 0.024$ Dunn post hoc test TnC+/+ SE vs. TnC-/- SE $p = 0.014$ TnC+/+ SE vs. TnC+/+ EE $p = 0.037$	H(3) = 1.588 $p = 0.662$	H(3) = 9.597 $p = 0.022$ Dunn post hoc test TnC+/+ SE vs. TnC+/+ EE $p = 0.004$ TnC+/+ EE vs. TnC-/- EE $p = 0.017$	H(3) = 8.012 $p = 0.046$ Dunn post hoc test TnC+/+ SE vs. TnC+/+ EE $p = 0.016$ TnC+/+ EE vs. TnC-/- EE $p = 0.016$
VGAT size	H(3) = 5.183 $p = 0.159$	H(3) = 5.376 $p = 0.146$	H(3) = 5.521 $p = 0.137$	H(3) = 4.730 $p = 0.193$
VGAT intensity	H(3) = 0.218 $p = 0.975$	H(3) = 4.668 $p = 0.198$	H(3) = 10.605 $p = 0.014$ Dunn post hoc test TnC+/+ SE vs. TnC-/- SE $p = 0.015$ TnC+/+ EE vs. TnC-/- EE $p = 0.044$	H(3) = 2.453 $p = 0.484$
VGLUT1 density	H(3) = 7.569 $p = 0.056$ Dunn post hoc test TnC-/- SE vs. TnC-/- EE $p = 0.011$	H(3) = 2.065 $p = 0.559$	H(3) = 3.423 $p = 0.331$	H(3) = 6.035 $p = 0.110$
VGLUT1 size	H(3) = 1.868 $p = 0.600$	H(3) = 2.039 $p = 0.564$	H(3) = 4.342 $p = 0.227$	H(3) = 2.658 $p = 0.447$
VGLUT1 intensity	H(3) = 11.646 $p = 0.009$ Dunn post hoc test TnC-/- SE vs. TnC-/- EE $p = 0.005$ TnC+/+ SE vs. TnC-/- SE $p = 0.005$	H(3) = 0.103 $p = 0.991$	H(3) = 6.465 $p = 0.091$	H(3) = 2.056 $p = 0.561$



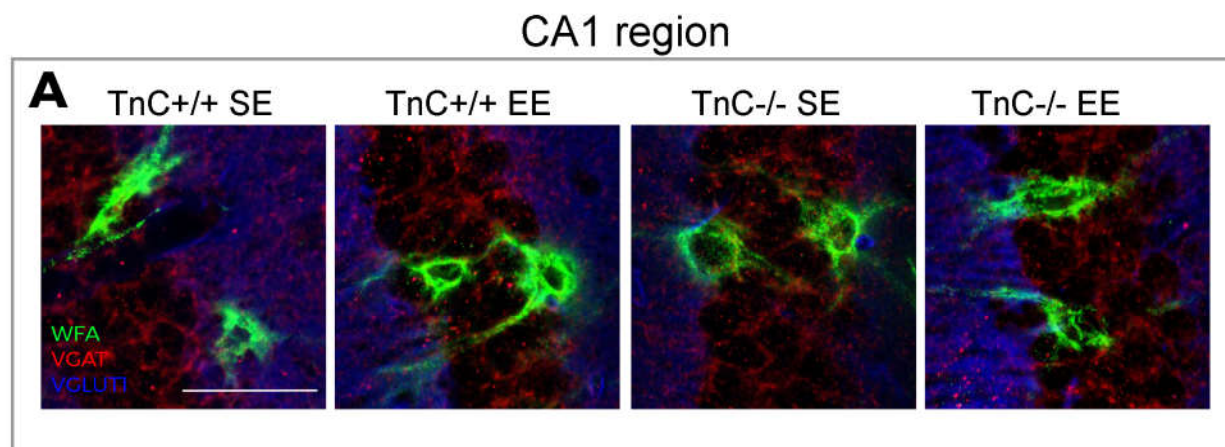
Supplementary Figure S1. Expression of PNNs and PV+ neurons in the region of CA1 and CA3 in TnC+/+ and TnC-/- mice after exposure to SE and EE. (**a, b**) Representative confocal images shows double immunofluorescent staining for PNN marker WFA (green). Pyramidal layer of CA regions is delineated with light yellow line. Scale bar (**a**): 50 μ m.



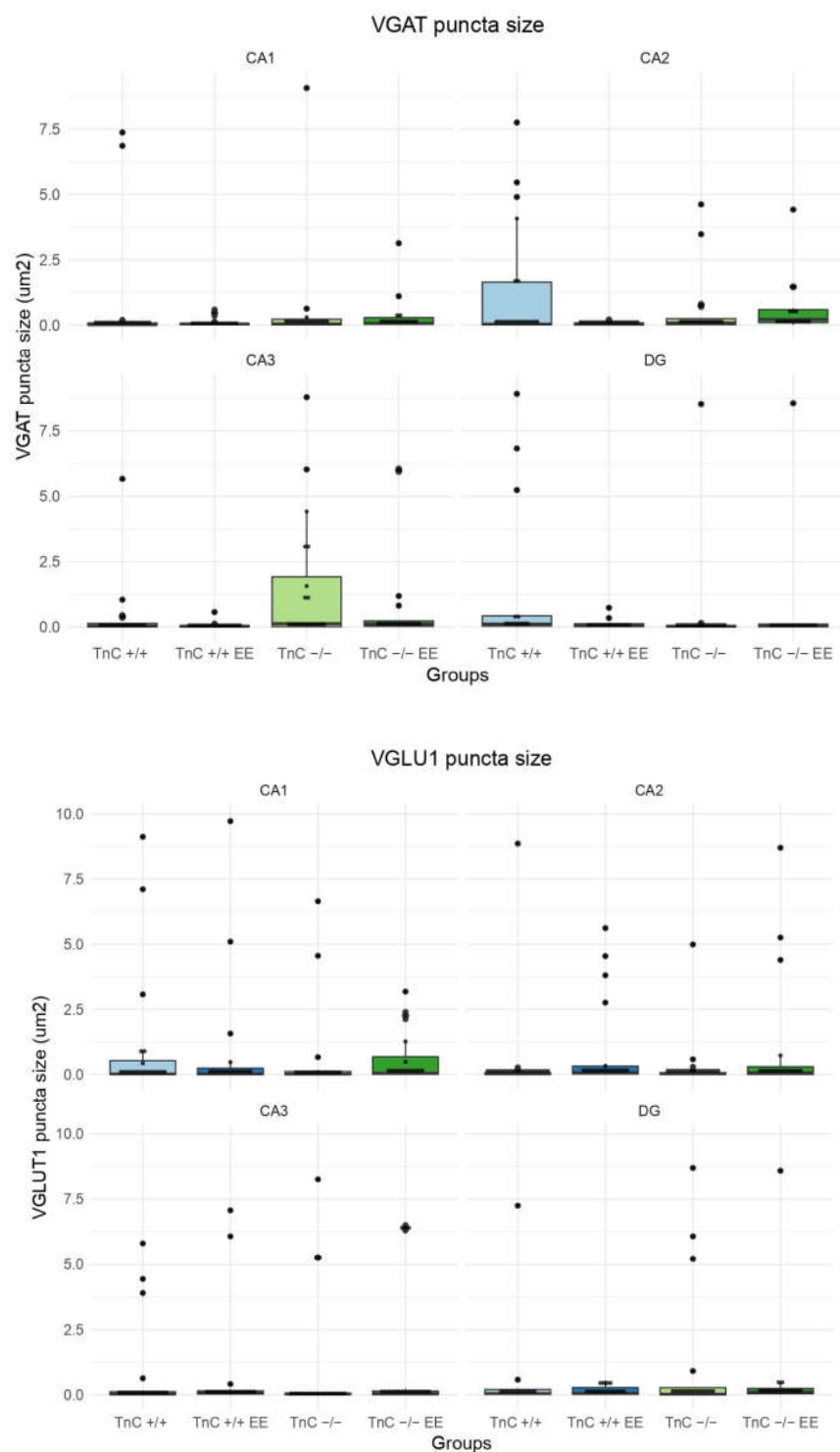
Supplementary Figure S2. Ultrastructure of PNN in the CA1 (a), CA2 (b) and CA3 (c) in the hippocampus of TnC+/+ and TnC-/- mice after exposure to SE and EE. Representative 3D super-resolution images from SIM shows double immunofluorescent staining for PNN marker WFA (green) and DAPI (blue). Scale bar (a): 3 μ m.



Supplementary Figure S3. Quantification of PNNs ultrastructure in the four subfields of the hippocampus of TnC+/+ and TnC-/- mice after exposure to SE and EE. Box plots represents median number of nodes in PNN mesh (4 animals/experimental group, 3 brain section/animal), whiskers extending from the box are indicating range of data within 1.5 times the interquartile range. No significant difference between groups is found.



Supplementary Figure S4. Expression of inhibitory and excitatory synapses in the CA1 region in the hippocampus of TnC+/+ and TnC-/- mice after exposure to SE and EE. **(a)** Representative 3D confocal images from shows triple immunofluorescent staining for PNN marker WFA (green), inhibitory marker VGAT (red) and excitatory marker VGLUT1 (blue). Scale bar **(a)**: 50 μ m.



Supplementary Figure S5. Quantification of inhibitory and excitatory synapses size in the four subfields of the hippocampus of TnC+/+ and TnC-/- mice after exposure to SE and EE. Box plots represents size of inhibitory and excitatory puncta that are penetrating PNN (3-4 animals/experimental group, 3 brain section/animal), whiskers extending from the box are indicating range of data within 1.5 times the interquartile range. No significant difference between groups is found.