

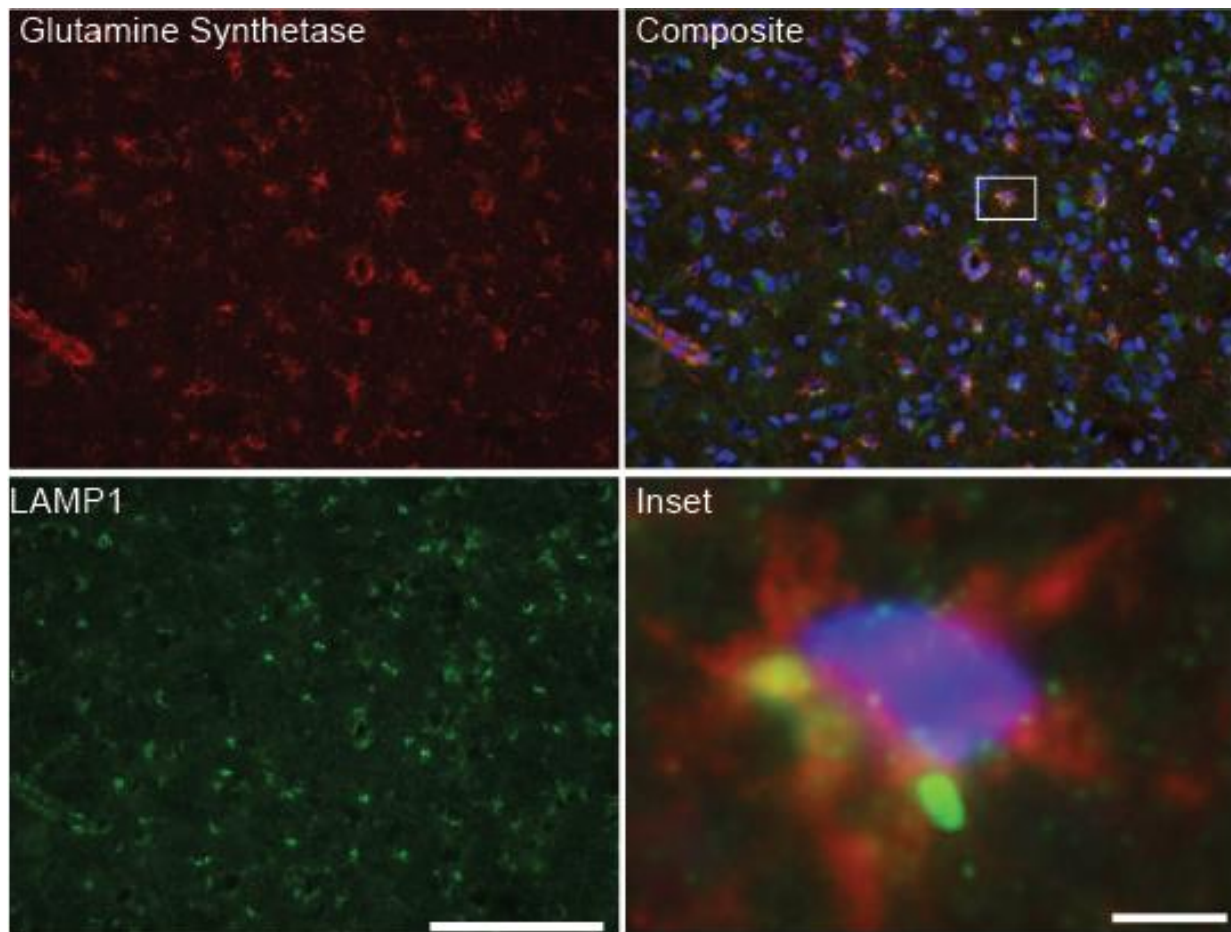


Figure S1. Images of lysosomal LAMP1 localization in glutamine synthetase-positive astrocytes. Representative images of glutamine synthetase (GS; red) LAMP1 (green) and Hoechst (blue) labeling in zebra finch brain tissue. GS immunoreactivity highlights astrocytic cell bodies and proximal processes (top left 40×) while LAMP1 labeling identifies lysosomal structures (bottom left 40×). The merged image (top right 40×) illustrates the spatial relationship between astrocytes and lysosomal compartments. The boxed region denotes the area shown at higher magnification (bottom right 263× digital zoom). The inset demonstrates the subcellular distribution of LAMP1 signal within GS-positive astrocytes with larger LAMP1-positive structures predominantly localized within GS-positive cells. Scale bars: 100 μ m (top left top right bottom left) 10 μ m (bottom right).

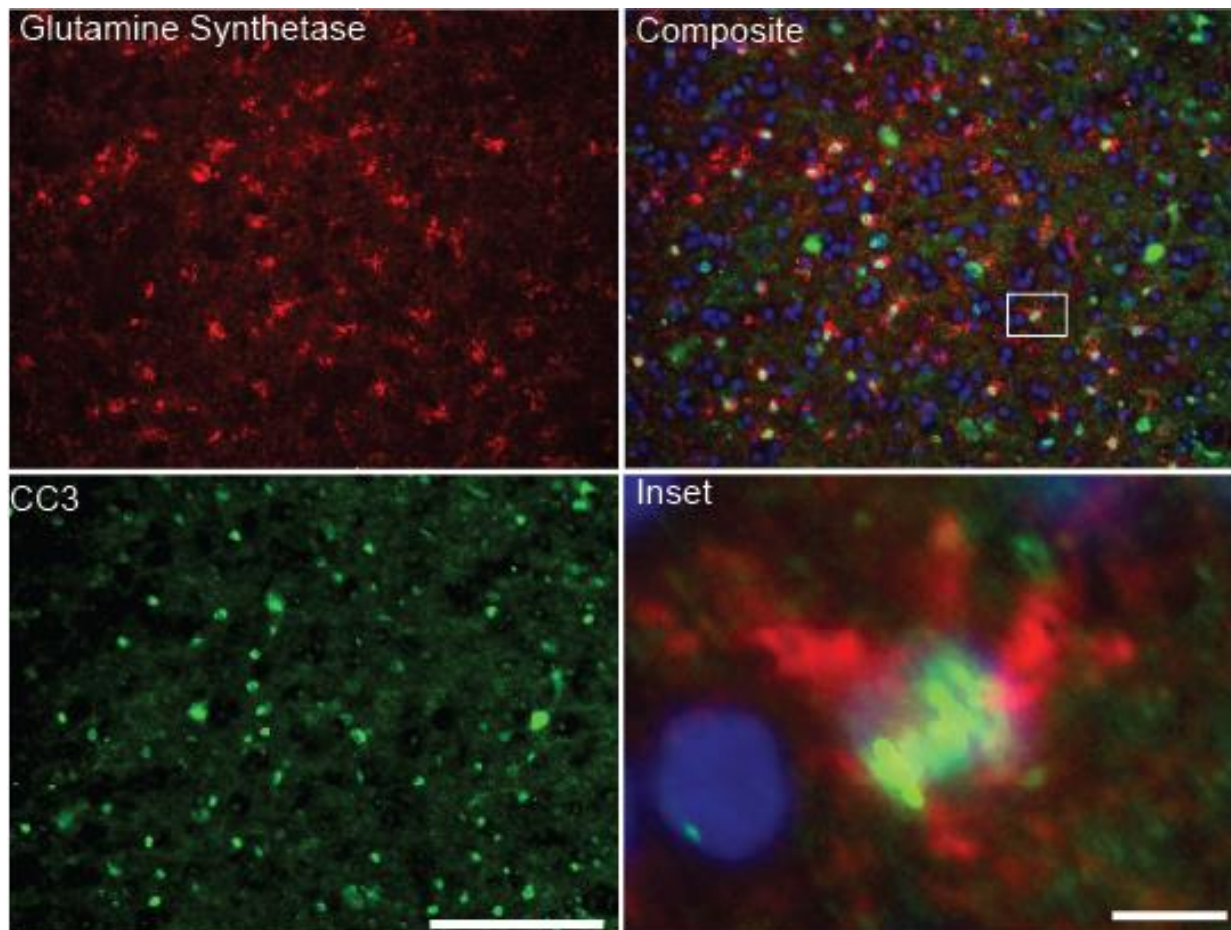


Figure S2. Apoptotic (cleaved caspase-3-positive) glutamine synthetase-positive astrocytes. Representative images of glutamine synthetase (GS; red) cleaved caspase-3 (CC3; green) and Hoechst (blue) labeling in zebra finch brain tissue. GS immunoreactivity highlights astrocytic cell bodies and proximal processes (top left 40 $\times$) while CC3 labeling identifies apoptotic cells (bottom left 40 $\times$). The merged image (top right 40 $\times$) illustrates the spatial relationship between CC3 expression and astrocytes. The boxed region denotes the area shown at higher magnification (bottom right 263 $\times$ digital zoom). Most CC3-positive cells are localized within GS-positive astrocytes. Scale bars: 100 μ m (top left top right bottom left) 10 μ m (bottom right).

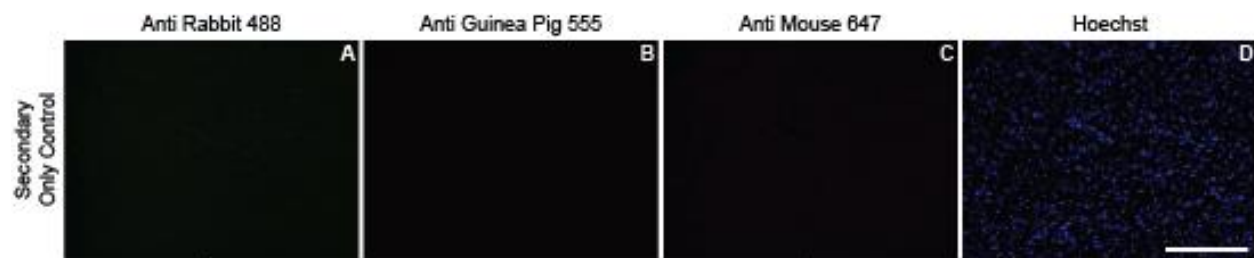


Figure S3. Representative images of secondary-only control sections (20X, scale bar = 200 μm). Representative images of secondary-only control sections (20 $\times$ scale bar = 200 μm). Sections were incubated with secondary antibodies only including goat anti-rabbit Alexa Fluor 488 (Thermo Fisher Scientific Cat. #A-11008 1:500) (A) goat anti-guinea pig Alexa Fluor 555 (Thermo Fisher Scientific Cat. #A-21435 1:500) (B) and goat anti-mouse Alexa Fluor 647 (Thermo Fisher Scientific Cat. #A-21235 1:500) (C) with nuclei counterstained using Hoechst (D). Lipofuscin autofluorescence was quenched using TrueBlack Lipofuscin Autofluorescence Quencher (Cell Signaling Technology Cat. #92401 1:20) which reduces lipofuscin-associated and general background fluorescence. Minimal background fluorescence was observed across all channels confirming specificity of primary antibody labeling.

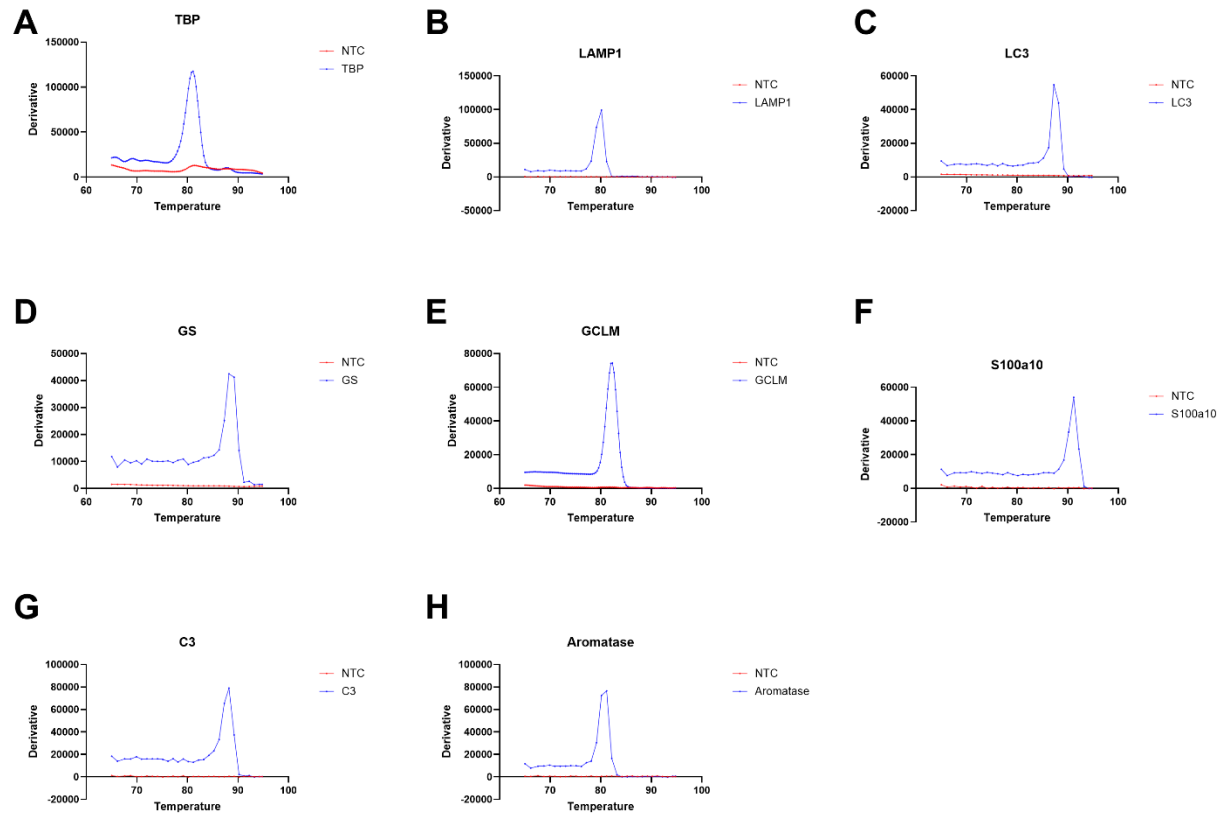


Figure S4. Melt curve analysis confirms amplification specificity of qPCR targets. Panels correspond to: (A) TATA binding protein (TBP), (B) LAMP1, (C) LC3, (D) GS, (E) GCLM, (F) S100a10, (G) C3, and (H) Aromatase. For each gene, a single, sharp melt peak was observed in reactions containing cDNA template, indicating amplification of a single specific product. In contrast, no distinct melt peak was observed in no-template controls (NTC), confirming the absence of non-specific amplification or primer-dimer formation

Table S1. Summary of main effects and interactions from mixed-effects models ANOVAs.

Figure	Effect	F (DFn, DFd)	p-value
4D	Region	F(2, 12) = 5.333	0.022
4D	Lesion condition	F(1, 6) = 11.36	0.015
4D	Treatment	F(1, 6) = 42.47	0.0006
4D	Region × Lesion condition	F(2, 12) = 0.3589	0.7057
4D	Region × Treatment	F(2, 12) = 0.8693	0.4441
4D	Lesion condition × Treatment	F(1, 6) = 8.026	0.0298
4D	Region × Lesion condition × Treatment	F(2, 12) = 5.793	0.0173
3D	Region	F(2, 12) = 30.69	<0.0001
3D	Lesion condition	F(1, 6) = 0.4152	0.5432
3D	Treatment	F(1, 6) = 48.33	0.0004
3D	Region × Lesion condition	F(2, 12) = 9.122	0.0039
3D	Region × Treatment	F(2, 12) = 8.061	0.006
3D	Lesion condition × Treatment	F(1, 6) = 1.994	0.2076
3D	Region × Lesion condition × Treatment	F(2, 12) = 2.347	0.1379
3F	Region	F(2, 12) = 252.8	<0.0001
3F	Lesion condition	F(1, 6) = 15.65	0.0075
3F	Treatment	F(1, 6) = 2.078	0.1995
3F	Region × Lesion condition	F(2, 12) = 6.610	0.0116
3F	Region × Treatment	F(2, 12) = 10.61	0.0022
3F	Lesion condition × Treatment	F(1, 6) = 0.1862	0.6811
3F	Region × Lesion condition × Treatment	F(2, 12) = 3.044	0.0853
3G	Region	F(2, 12) = 5.755	0.0177
3G	Lesion condition	F(1, 6) = 8.513	0.0267
3G	Treatment	F(1, 6) = 1.853	0.2223
3G	Region × Lesion condition	F(2, 12) = 9.148	0.0039
3G	Region × Treatment	F(2, 12) = 4.351	0.0379
3G	Lesion condition × Treatment	F(1, 6) = 3.154	0.1261
3G	Region × Lesion condition × Treatment	F(2, 12) = 5.408	0.0212
3E	Region	F(2, 12) = 15.22	0.0005
3E	Lesion condition	F(1, 6) = 120.7	<0.0001
3E	Treatment	F(1, 6) = 39.35	0.0008
3E	Region × Lesion condition	F(2, 12) = 14.03	0.0007
3E	Region × Treatment	F(2, 12) = 0.1776	0.8395
3E	Lesion condition × Treatment	F(1, 6) = 24.17	0.0027
3E	Region × Lesion condition × Treatment	F(2, 12) = 1.176	0.3417
6A	Region	F(2, 18) = 11.23	0.0007
6A	Treatment	F(1, 18) = 51.09	<0.0001
6A	Region × Treatment	F(2, 18) = 19.29	<0.0001
6B	Region	F(2, 18) = 15.92	0.0001
6B	Treatment	F(1, 18) = 34.23	<0.0001
6B	Region × Treatment	F(2, 18) = 16.51	<0.0001
5A	Region	F(2, 18) = 162.9	<0.0001
5A	Treatment	F(1, 18) = 30.12	<0.0001
5A	Region × Treatment	F(2, 18) = 19.13	<0.0001
5B	Region	F(2, 18) = 117.9	<0.0001
5B	Treatment	F(1, 18) = 10.40	0.0047
5B	Region × Treatment	F(2, 18) = 27.50	<0.0001
7C	Region	F(2, 12) = 70.95	<0.0001
7C	Treatment	F(1, 6) = 24.82	0.0025
7C	Region × Treatment	F(2, 12) = 47.72	<0.0001
7A	Region	F(2, 12) = 37.39	<0.0001
7A	Treatment	F(1, 6) = 5.312	0.0607
7A	Region × Treatment	F(2, 12) = 9.643	0.0032
7B	Region	F(2, 12) = 84.37	<0.0001
7B	Treatment	F(1, 6) = 14.71	0.0086
7B	Region × Treatment	F(2, 12) = 0.8347	0.4577

Table S2. Region-specific post hoc comparisons across treatment and lesion conditions

Figure	Region	Comparison	Mean Difference	95% CI	Adjusted p-value
3D	HVC	VEH Un-lesioned vs. VEH Lesioned	-1.217	-3.735 to 1.301	0.4237
3D	HVC	CBD Un-lesioned vs. CBD Lesioned	-4.131	-6.649 to -1.612	0.0025
3D	HVC	VEH Un-lesioned vs. CBD Un-lesioned	-2.414	-4.932 to 0.1047	0.0606
3D	HVC	VEH Lesioned vs. CBD Lesioned	-5.327	-7.845 to -2.809	0.0003
3D	RA	VEH Un-lesioned vs. VEH Lesioned	-0.3385	-5.167 to 4.490	0.9752
3D	RA	CBD Un-lesioned vs. CBD Lesioned	1.487	-3.341 to 6.315	0.6366
3D	RA	VEH Un-lesioned vs. CBD Un-lesioned	-9.619	-14.60 to -4.635	0.0007
3D	RA	VEH Lesioned vs. CBD Lesioned	-7.793	-12.78 to -2.809	0.0036
3D	Area X	VEH Un-lesioned vs. VEH Lesioned	5.016	1.275 to 8.757	0.0147
3D	Area X	CBD Un-lesioned vs. CBD Lesioned	1.351	-2.390 to 5.092	0.5462
3D	Area X	VEH Un-lesioned vs. CBD Un-lesioned	-0.6725	-4.503 to 3.158	0.8858
3D	Area X	VEH Lesioned vs. CBD Lesioned	-4.338	-8.169 to -0.5068	0.0269
3E	HVC	VEH Un-lesioned vs. VEH Lesioned	-32.81	-43.26 to -22.37	0.0002
3E	HVC	CBD Un-lesioned vs. CBD Lesioned	-9.774	-20.22 to 0.6739	0.0640
3E	HVC	VEH Un-lesioned vs. CBD Un-lesioned	6.407	-4.772 to 17.58	0.3096
3E	HVC	VEH Lesioned vs. CBD Lesioned	29.45	18.27 to 40.63	<0.0001
3E	RA	VEH Un-lesioned vs. VEH Lesioned	-21.25	-42.57 to 0.0655	0.0498
3E	RA	CBD Un-lesioned vs. CBD Lesioned	-6.914	-28.23 to 14.41	0.6085
3E	RA	VEH Un-lesioned vs. CBD Un-lesioned	7.94	-10.54 to 26.42	0.5018
3E	RA	VEH Lesioned vs. CBD Lesioned	22.28	3.802 to 40.76	0.019
3E	Area X	VEH Un-lesioned vs. VEH Lesioned	-53.11	-63.75 to -42.47	<0.0001
3E	Area X	CBD Un-lesioned vs. CBD Lesioned	-24.23	-34.87 to -13.59	0.0010
3E	Area X	VEH Un-lesioned vs. CBD Un-lesioned	0.515	-10.05 to 11.08	0.9906
3E	Area X	VEH Lesioned vs. CBD Lesioned	29.4	18.83 to 39.96	<0.0001
3F	HVC	VEH Un-lesioned vs. VEH Lesioned	-12.69	-26.24 to 0.8679	0.0638
3F	HVC	CBD Un-lesioned vs. CBD Lesioned	-13.45	-27.01 to 0.1033	0.0515
3F	HVC	VEH Un-lesioned vs. CBD Un-lesioned	-14.02	-27.98 to -0.0695	0.0489
3F	HVC	VEH Lesioned vs. CBD Lesioned	-14.79	-28.74 to -0.8342	0.0378
3F	RA	VEH Un-lesioned vs. VEH Lesioned	6.07	-4.046 to 16.19	0.2364
3F	RA	CBD Un-lesioned vs. CBD Lesioned	-5.677	-15.79 to 4.439	0.2739
3F	RA	VEH Un-lesioned vs. CBD Un-lesioned	4.293	-6.305 to 14.89	0.5396
3F	RA	VEH Lesioned vs. CBD Lesioned	-7.455	-18.05 to 3.143	0.1859
3F	Area X	VEH Un-lesioned vs. VEH Lesioned	-14.36	-28.13 to -0.6018	0.0624
3F	Area X	CBD Un-lesioned vs. CBD Lesioned	-6.989	-20.75 to 6.773	0.3335
3F	Area X	VEH Un-lesioned vs. CBD Un-lesioned	-0.8486	-13.03 to 11.34	0.9809
3F	Area X	VEH Lesioned vs. CBD Lesioned	6.526	-5.658 to 18.71	0.3544
3G	HVC	VEH Un-lesioned vs. VEH Lesioned	-14.51	-25.04 to -3.984	0.0130
3G	HVC	CBD Un-lesioned vs. CBD Lesioned	-2.587	-13.12 to 7.942	0.7446
3G	HVC	VEH Un-lesioned vs. CBD Un-lesioned	0.1046	-9.089 to 9.298	0.9995
3G	HVC	VEH Lesioned vs. CBD Lesioned	12.03	2.836 to 21.22	0.0117
3G	RA	VEH Un-lesioned vs. VEH Lesioned	-1.634	-8.937 to 5.670	0.7816
3G	RA	CBD Un-lesioned vs. CBD Lesioned	-1.063	-8.366 to 6.241	0.8988
3G	RA	VEH Un-lesioned vs. CBD Un-lesioned	-0.1096	-6.634 to 6.415	0.9989
3G	RA	VEH Lesioned vs. CBD Lesioned	0.4611	-6.063 to 6.985	0.9803
3G	Area X	VEH Un-lesioned vs. VEH Lesioned	-2.082	-4.921 to 0.7570	0.1408
3G	Area X	CBD Un-lesioned vs. CBD Lesioned	-0.7842	-3.623 to 2.055	0.6919
3G	Area X	VEH Un-lesioned vs. CBD Un-lesioned	-1.063	-3.541 to 1.415	0.5030
3G	Area X	VEH Lesioned vs. CBD Lesioned	0.2352	-2.243 to 2.713	0.9649

Figure	Region	Comparison	Mean Difference	95% CI	Adjusted p-value
4D	HVC	VEH Un-lesioned vs. VEH Lesioned	-0.3163	-2.096 to 1.463	0.8540
4D	HVC	CBD Un-lesioned vs. CBD Lesioned	-1.815	-3.595 to -0.0359	0.0463
4D	HVC	VEH Un-lesioned vs. CBD Un-lesioned	4.86	2.407 to 7.313	0.0006
4D	HVC	VEH Lesioned vs. CBD Lesioned	3.361	0.9073 to 5.814	0.0088
4D	RA	VEH Un-lesioned vs. VEH Lesioned	1.347	-1.050 to 3.745	0.2729
4D	RA	CBD Un-lesioned vs. CBD Lesioned	-2.677	-5.074 to -0.2797	0.0324
4D	RA	VEH Un-lesioned vs. CBD Un-lesioned	4.973	2.649 to 7.297	0.0003
4D	RA	VEH Lesioned vs. CBD Lesioned	0.9488	-1.375 to 3.273	0.5347
4D	Area X	VEH Un-lesioned vs. VEH Lesioned	-1.501	-3.383 to 0.3807	0.1094
4D	Area X	CBD Un-lesioned vs. CBD Lesioned	-0.9359	-2.818 to 0.9458	0.3464
4D	Area X	VEH Un-lesioned vs. CBD Un-lesioned	3.051	1.073 to 5.028	0.0039
4D	Area X	VEH Lesioned vs. CBD Lesioned	3.616	1.638 to 5.594	0.0011
5A	HVC	VEH Lesioned vs. CBD Lesioned	0.7235	0.4153 to 1.0320	<0.0001
5A	RA	VEH Lesioned vs. CBD Lesioned	0.6066	0.2984 to 0.9148	0.0002
5A	Area X	VEH Lesioned vs. CBD Lesioned	-0.2166	-0.5248 to 0.0916	0.2238
5B	HVC	VEH Lesioned vs. CBD Lesioned	0.6258	0.3482 to 0.9034	<0.0001
5B	RA	VEH Lesioned vs. CBD Lesioned	0.3913	0.1137 to 0.6689	0.0048
5B	Area X	VEH Lesioned vs. CBD Lesioned	-0.4278	-0.7054 to -0.1503	0.0022
6A	HVC	VEH Lesioned vs. CBD Lesioned	-1.035	-1.505 to -0.5655	<0.0001
6A	RA	VEH Lesioned vs. CBD Lesioned	-1.329	-1.798 to -0.8586	<0.0001
6A	Area X	VEH Lesioned vs. CBD Lesioned	0.1529	-0.3170 to 0.6228	0.7874
6B	HVC	VEH Lesioned vs. CBD Lesioned	-0.9411	-1.268 to -0.6139	<0.0001
6B	RA	VEH Lesioned vs. CBD Lesioned	-0.3868	-0.7140 to -0.0596	0.0180
6B	Area X	VEH Lesioned vs. CBD Lesioned	0.0677	-0.2595 to 0.3949	0.9324
7A	HVC	VEH Lesioned vs. CBD Lesioned	1.219	0.5593 to 1.8790	0.0004
7A	RA	VEH Lesioned vs. CBD Lesioned	0.0095	-0.6505 to 0.6694	>0.9999
7A	Area X	VEH Lesioned vs. CBD Lesioned	-0.1713	-0.8312 to 0.4886	0.8774
7B	HVC	VEH Lesioned vs. CBD Lesioned	1.549	0.2904 to 2.8070	0.0136
7B	RA	VEH Lesioned vs. CBD Lesioned	1.457	0.1988 to 2.7160	0.0207
7B	Area X	VEH Lesioned vs. CBD Lesioned	0.8456	-0.4129 to 2.1040	0.2564
7C	HVC	VEH Lesioned vs. CBD Lesioned	2.268	1.698 to 2.838	<0.0001
7C	RA	VEH Lesioned vs. CBD Lesioned	-0.2136	-0.7835 to 0.3563	0.7087
7C	Area X	VEH Lesioned vs. CBD Lesioned	0.1575	-0.4124 to 0.7274	0.8565

Table S3. Primers used for quantitative RT-PCR

Gene Symbol	Accession Number	Forward Primer	Reverse Primer
TBP	XM_030268321.4	TCACACACCAGCAGTTCAGC	CTGCTCGTACTTTAGCACCCAGTTA
GS	XM_030279644.4	AATCTGCTCCCCAGCTTCCC	TCAGCCTTTCAGAGCAGATCA
GCLM	XM_030279191.4	GACTGAGTGGAGCTCAAAGGT	CCAGGTCAACTGCGTCTCTG
LAMP1	XM_002191571.5	TGCATCAACTCCAAGCACATC	AGAGACCCTATCTTCAGCACATTC
LC3B	NM_001245293.2	AGCTTCCAGTTTTGGATAAGACC	TTCTCGCTCTCGTACACCTC
CYP19A1	AH008871.2	CTCCACCGACAAAAATCCAC	GGGTTTCCAGGACCATCTTT
S100a10	XM_030291396.4	TCATGGACAAGGAGTTCCCG	TGCTGCACGAAGTAGTCGTT
C3	XM_072920066.1	GGCTCTCCCAGCAGAACTAC	AGAACCGACACGTCCAATC