

Supplementary Fig. 1. a. Panel of representative immunohistochemical stainings for the myelin proteins MBP (top), PLP (middle) and CNP (bottom) and their quantification (right column) showing a significant reduction in wildtype CUP5W in the subcortical white matter (WM) and granule cell layer (GCL). **b.** Representative images and quantification of CD3-IHC showing a significant increase in T cells (arrowheads) in the lesion areas of the cerebellar nuclei (CN) in cuprizone-treated mice while no significant differences were observed in the cerebellar cortex in all conditions. Inset: Higher magnification image of the highlighted region. wt naive n = 4; wt CUP5W n = 5, MyD88^{-/-} naive n = 4, MyD88^{-/-} CUP5W n = 5. **c.** Representative images of the NAGM immunohistochemically stained for microglia (Iba1), TMEM119 (**d**) and S100A9 (**e**) in naive and cuprizone-fed animals. Morphologically, microglia appeared ramified in all conditions (insets, right column). Bottom panels: Quantification showing no differences in area coverage (Iba1, TMEM19) or cell density (S100A9). Each point represents a measurement from an individual animal (wt naive n = 4; wt CUP5W n = 6, MyD88^{-/-} naive n = 4, MyD88^{-/-} CUP5W n = 6 in a, c-e). Whiskers represent mean $\pm$ SEM. P values were obtained after two-way ANOVA followed by Tukey's multiple comparisons test (a-e). Asterisks represent significant p-values (*p < 0.05, **p < 0.01, ***p < 0.001).

Supplementary Fig. 2. a. S100 β -IHC showing nuclear staining of astroglia in the Purkinje cell layer (left panel). Quantification showing no significant differences in S100 β ⁺ cell density in any of the conditions studied (right panel). **b.** Representative images of Sox9 immunohistochemistry (left) and quantification (right) showing no significant differences between groups. **c.** EAAT1-IHC (top) showing a significant area reduction (bottom panel) in the molecular layer (ML) in wt CUP5W but not in cuprizone-fed MyD88^{-/-} mice. **d.** Representative images and quantification of area coverage for the neurite marker SMI31. **e.** MAP2 immunohistochemical staining (top) and quantification (bottom) showing a significant neurite reduction in the GCL in cuprizone-treated animals irrespective of genotype. The same dataset for MAP-IHC wt naive and CUP5W is also shown in Fig. 2b. Each point represents an individual animal (wt naive n = 4; wt CUP5W n = 6, MyD88^{-/-} naive n = 4, MyD88^{-/-} CUP5W n = 6). Whiskers represent mean $\pm$ SEM. P values were obtained after two-way ANOVA (a-e) followed by Tukey's multiple comparisons test. Asterisks represent significant p-values (*p < 0.05, **p < 0.01, ***p < 0.001).

Supplementary Fig. 3 a. Synaptophysin-IHC of the cerebellar nuclei (left) showing a tendency towards a reduction in synaptic density, which failed to reach statistical significance. Each point represents average values for a single animal (naive n = 4; CUP5W n = 5). **b.** Representative images of the cerebellar nuclei of naive (left) and cuprizone-fed (CUP5W, right) mice immunohistochemically stained against NF200. Quantification of NF200 + neuronal density (right) showing no significant differences between naive (n = 9) and CUP5W (n = 9) animals. Each point represents average values for a single animal. **c.** Representative images of the NAGM stained with Bielschowsky's silver impregnation. Insets (i,ii) show higher magnification images of highlighted regions. Quantification showing no differences in axonal crossings per field in the cerebellar nuclei (CN) but a significant reduction in the molecular layer (right). Each point represents average values for a single animal (naive n = 4; CUP5W n = 5). High axonal density in the subcortical lobar white matter (WM) is not suitable for quantification with this method and was excluded from the analysis. **d.** Golgi-Cox staining showing dendritic spines of Purkinje cells in the molecular layer. Right: Quantification showing no significant differences. Each point represents average density of spines per animal. A total length > 80 μ m per individual was quantified (naive n = 5; CUP5W n = 7). **e.** Representative images (top) and quantification (bottom) of synaptophysin-IHC of the granule cell layer showing no significant differences in the density of glomeruli between naive and CUP5W mice. **f.** Quantification of APP⁺ axonal spheroids showing no increase after cuprizone treatment (right). Each point represents average values for a single animal (naive n = 4; CUP5W n = 5). Whiskers represent mean $\pm$ SEM. P values were obtained after Mann Whitney test for a, b, d and e or two-way ANOVA with Sidak's multiple comparisons for c and d. Asterisks represent significant p-values (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). CN: cerebellar nuclei; WM: white matter; GCL: granule cell layer; ML: molecular layer.

Supplementary Fig. 4. Differential expression of proteins related to Bergmann glia and microglia. a Heatmap representation of proteome analysis revealing differential expression of

proteins related to Bergmann glia in all conditions studied. Note the rescue effect in MyD88^{-/-} CUP5W animals (first column) as compared to WT-CUP5W (third column). **b.** Similar representation of proteome data for microglial genes showing only small differences between conditions and no apparent rescue effect in MyD88^{-/-} CUP5W.

Supplementary Fig. 5. Frequency dependent reduction in facilitation in cuprizone-treated mice.

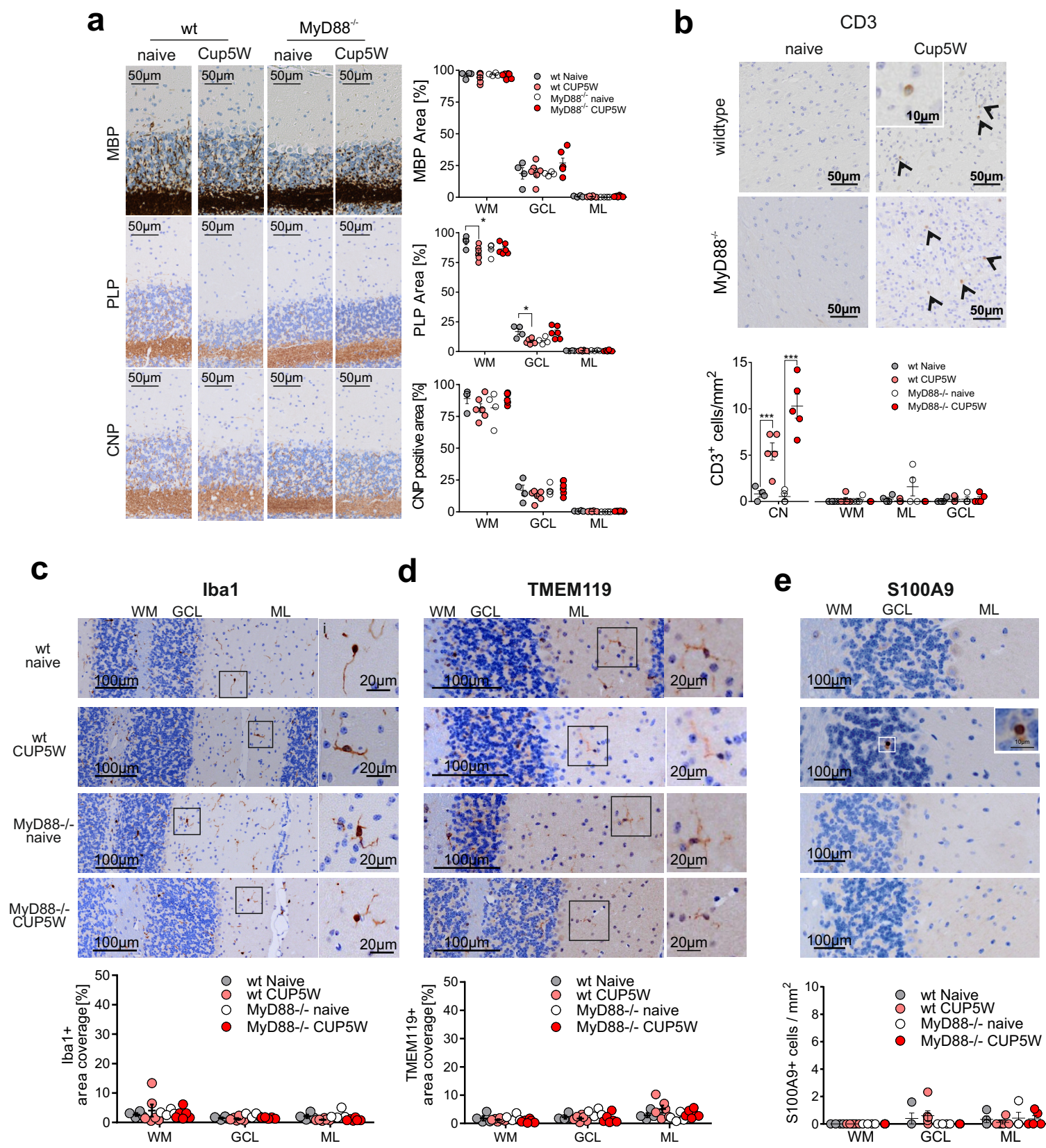
a Quantification of relative EPSC amplitude of current trains showing a frequency dependent reduction in facilitation in CUP5W animals. Each point represents mean normalized EPSC value $\pm$ SEM. **b** Relative facilitation as measured in EPSC₂ (50Hz) in animals fed with cuprizone for one (CUP1W) and 5 (CUP5W) weeks. Each point represents an individual cell. **c.** Decay time constant of Purkinje cell EPSCs in naive (gray) and 5-week cuprizone-fed wildtype mice (pink). Each point represents an individual cell. Whiskers represent mean $\pm$ SEM. P values were obtained after Mann Whitney test (b,c). Asterisks represent significant p-values (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

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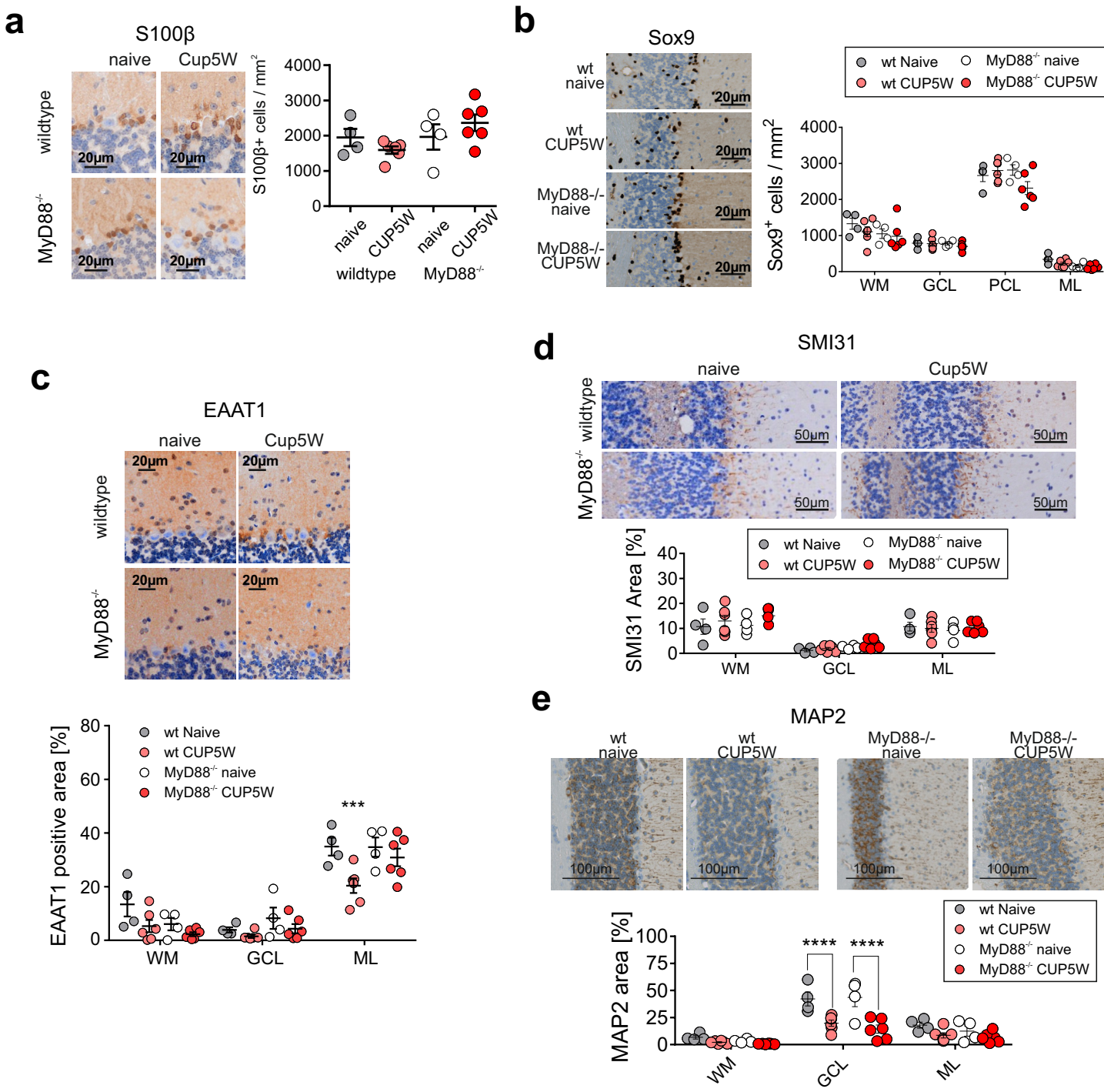
Supplementary Fig. 6 Proposed mechanism of synaptic pathology in the cerebellar NAGM in toxic demyelination.

a. Cuprizone feeding after 5 weeks (middle panel) leads to a prominent local activation of astrocytes/Bergmann glia (yellow) and discrete microglia activation, with increased expression of inflammatory mediators in demyelinated regions in the cerebellar nuclei e.g. TNF α and CCL3. Under these conditions, reactive astrocytes show a reduced expression of glutamate transport proteins such as GLAST and EAAT4 (green dots). Also, changes in glutamatergic synaptic transmission proteins as compared to physiological conditions (left panel) are observed, which is further accompanied by reduced facilitation of the PF-PC synapse. Cuprizone feeding in MyD88-deficient mice still leads to demyelinated lesion development, astrogliosis and discrete microglia activation, but is characterized by activation of NFkB signaling, a distinct composition of inflammatory mediators and is defined by more subtle changes in protein composition of glutamate transporters and synaptic proteins, without reduction in synaptic facilitation. **b.** Schematic overview of a glutamatergic synapse (modified from KEGG pathway mmu04724). Marked in yellow are proteins which are downregulated after cuprizone feeding in WT animals, but not, or significantly less, in MyD88^{-/-} animals. While some presynaptic proteins are identified, most are components of the postsynapse, including AMPA- and NMDA-receptors, structural proteins and voltage-gated ion channels. Further, the glutamate transports EAAT1 and 4 are among the most differentially expressed proteins between WT and MyD88^{-/-} animals after cuprizone administration. PC: Purkinje cell, ML: Molecular layer, GCL: Granule cell layer; WM: White matter.

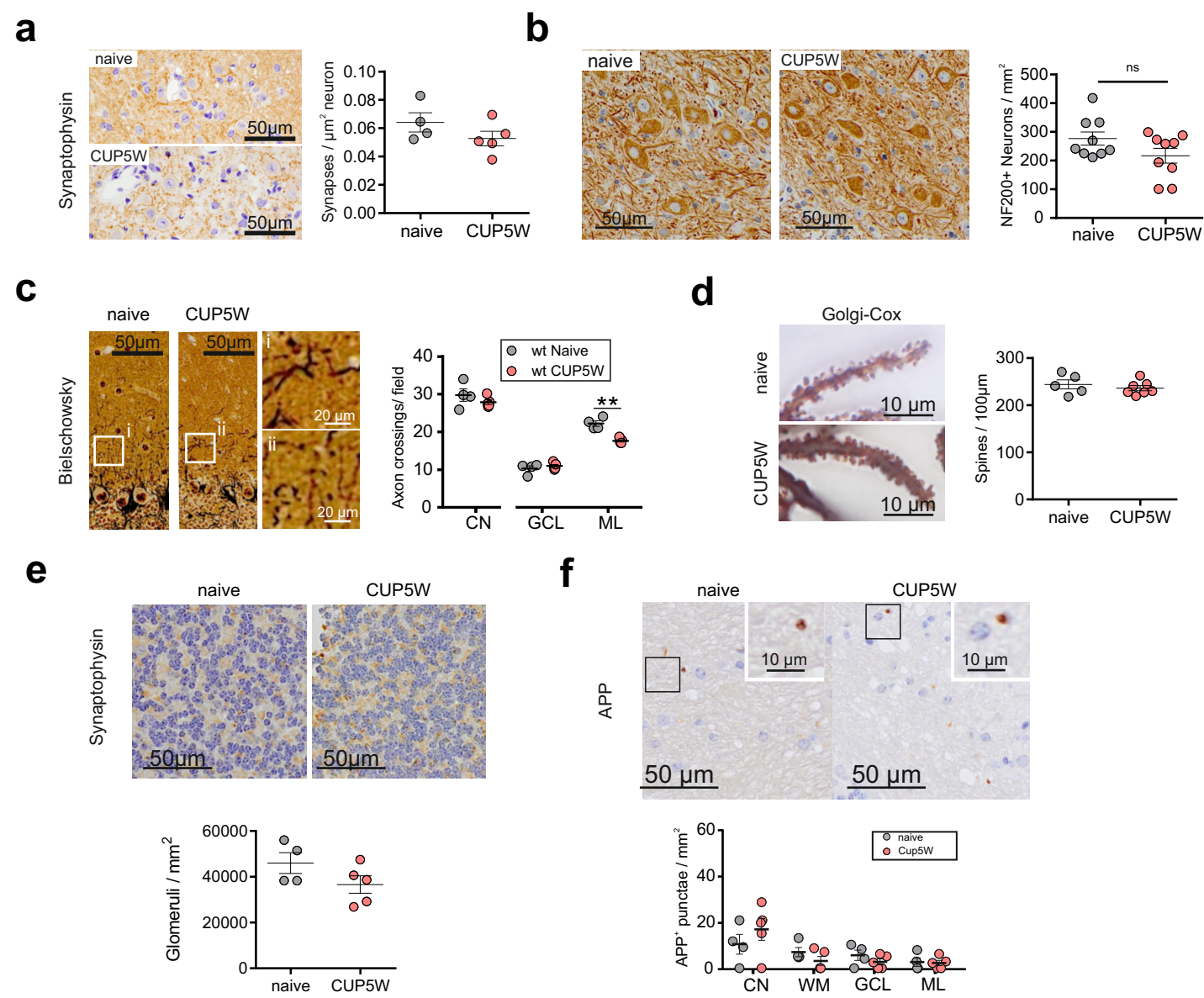
Supplementary Figure 1



Supplementary Figure 2

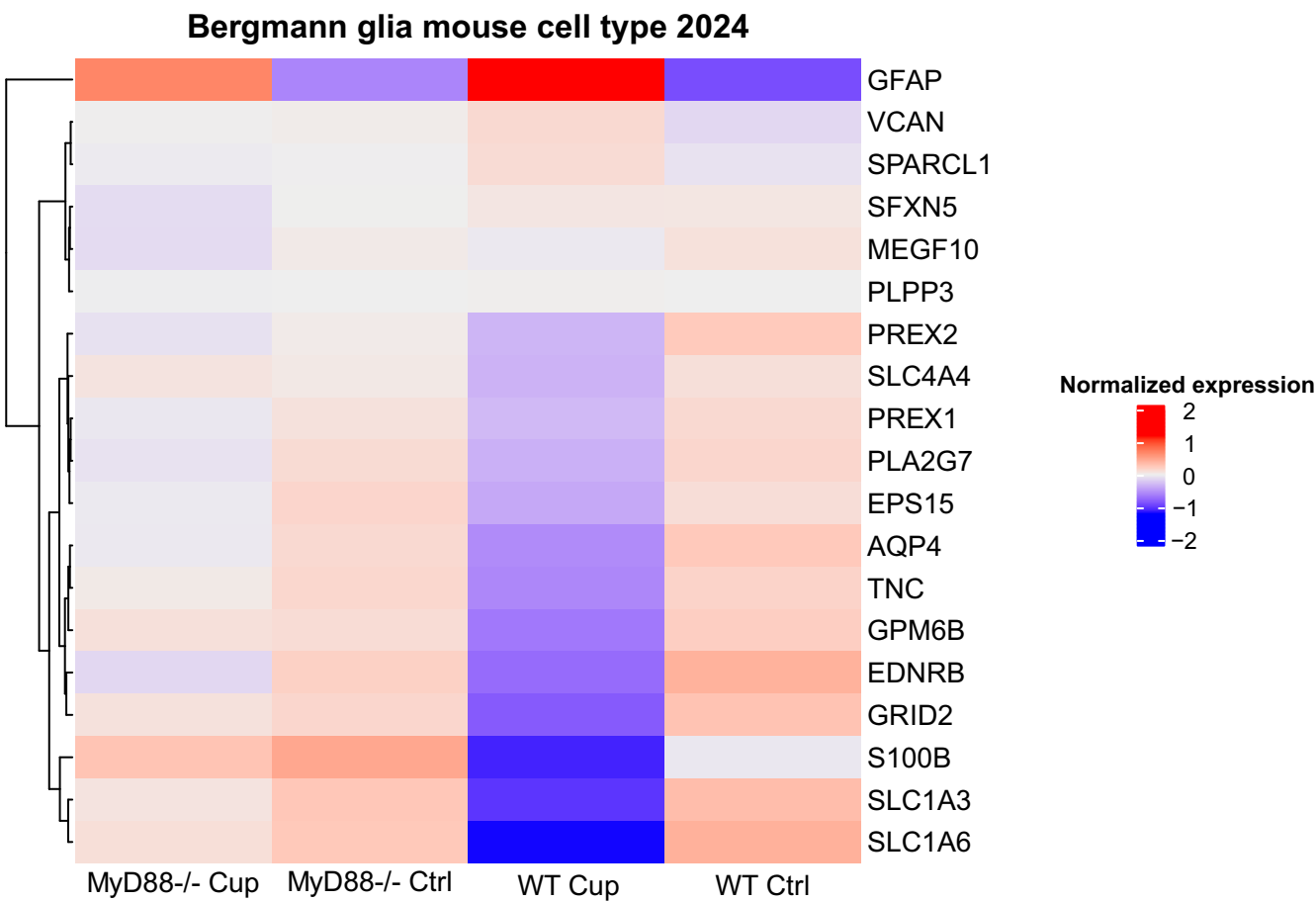


Supplementary Figure 3

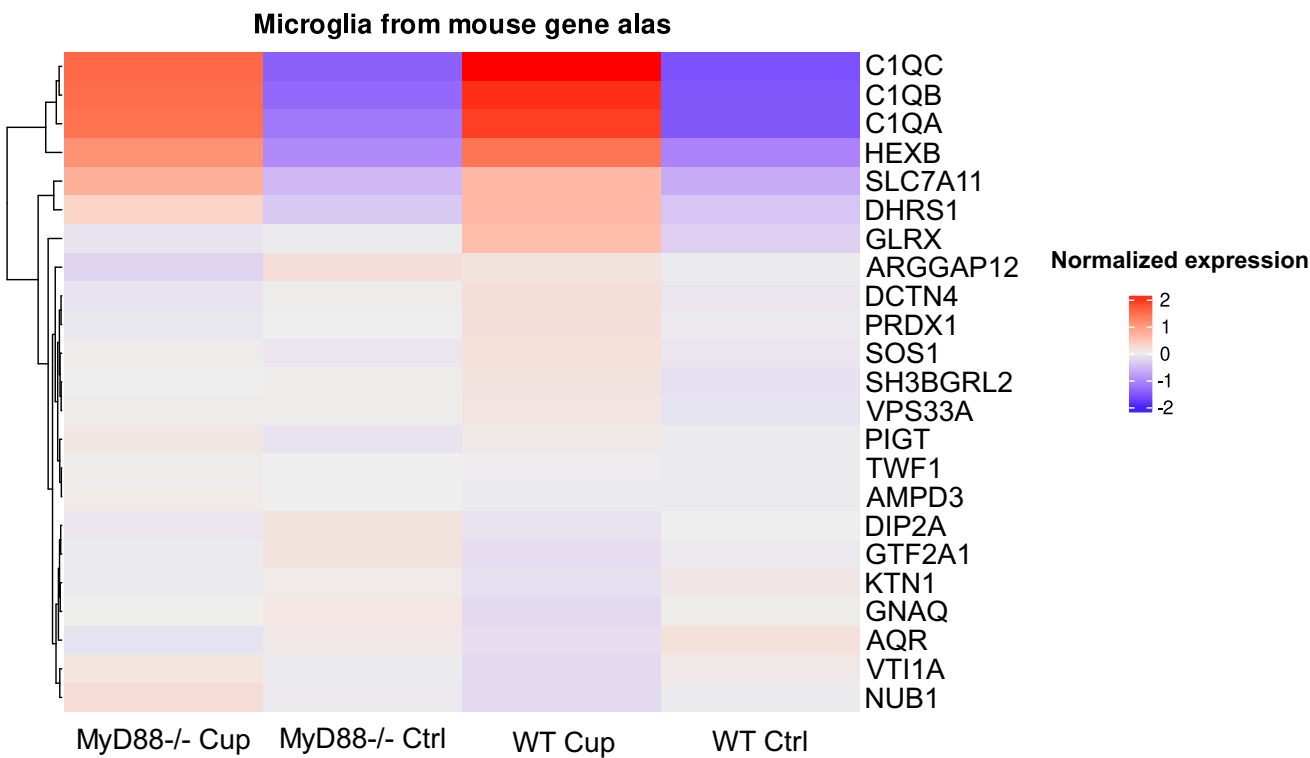


Supplementary Figure 4

a

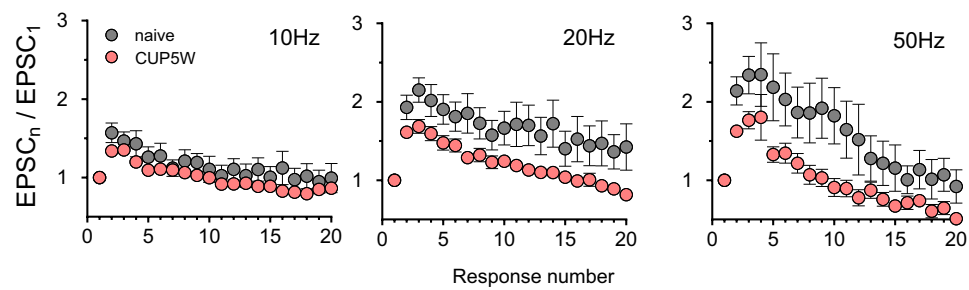


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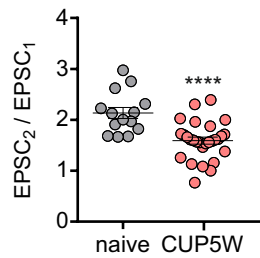


Supplementary Figure 5

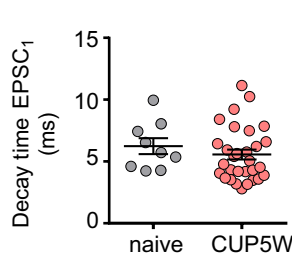
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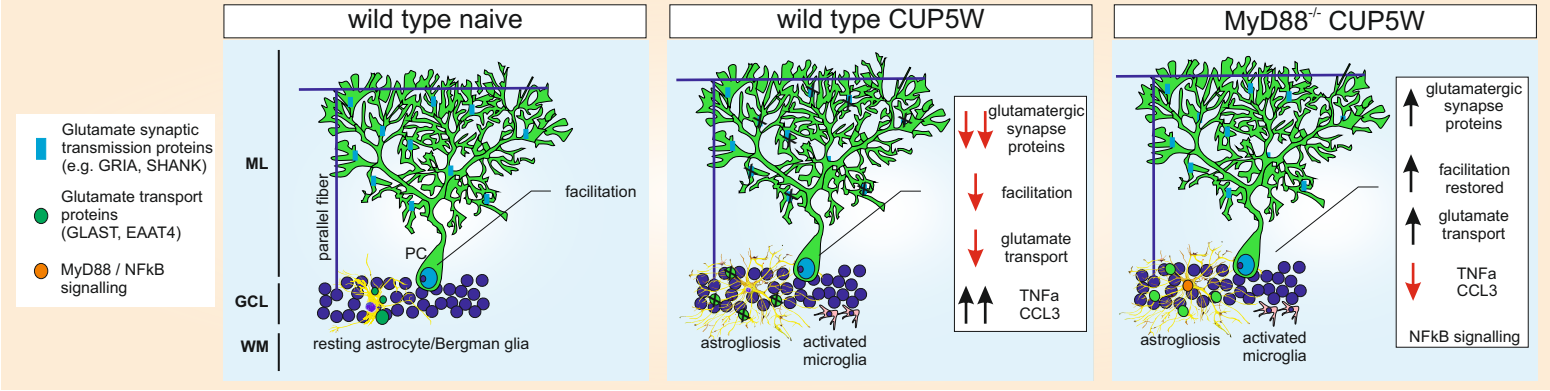


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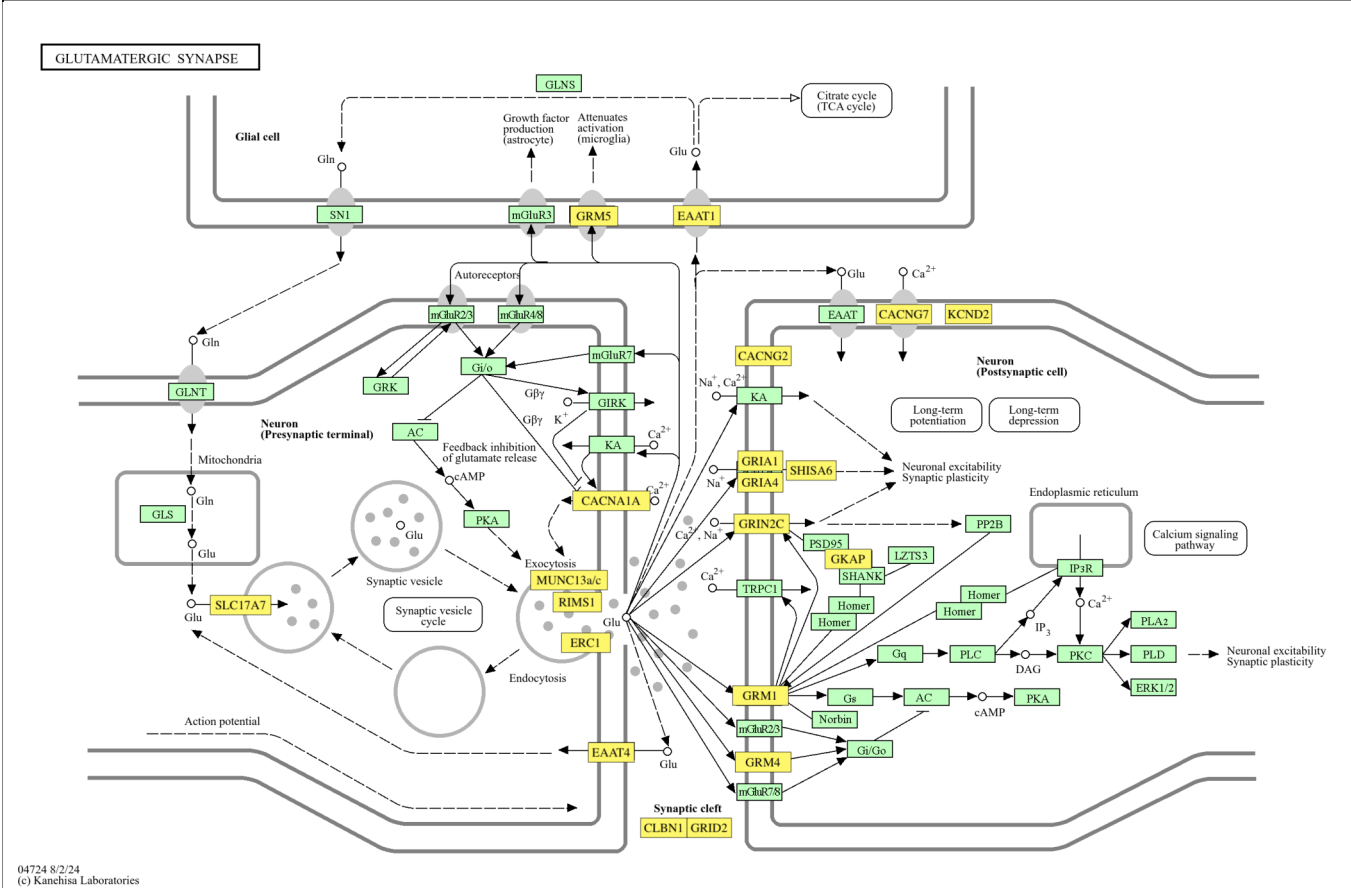


Supplementary Figure 6

a



b



Supplementary Table 1. Gene set enrichment analysis of differentially regulated gene sets in cuprizone-fed as compared to naive wildtype animals

Gene_set	Adjusted P-value	Odds Ratio	Combined Score
mRNA processing (GO:0006397)	2,31E-74	16,55	2938,48
RNA splicing, via transesterification reactions with bulged adenosine as nucleophile (GO:0000377)	6,50E-68	17,69	2866,72
mRNA splicing, via spliceosome (GO:0000398)	1,97E-67	16,20	2600,00
RNA processing (GO:0006396)	5,73E-37	13,45	1212,22
RNA splicing (GO:0008380)	2,63E-29	18,76	1355,03
mRNA metabolic process (GO:0016071)	1,31E-22	15,09	854,38
mRNA 3'-end processing (GO:0031124)	1,08E-19	15,74	783,15
spliceosomal complex assembly (GO:0000245)	1,31E-19	27,86	1377,30
RNA 3'-end processing (GO:0031123)	2,44E-19	17,51	852,94
mRNA transport (GO:0051028)	4,41E-19	11,98	575,14
mRNA export from nucleus (GO:0006406)	1,10E-18	11,49	540,15
RNA export from nucleus (GO:0006405)	8,33E-18	11,13	499,56
mRNA-containing ribonucleoprotein complex export from nucleus (GO:0071427)	8,93E-18	11,72	524,36
regulation of mRNA splicing, via spliceosome (GO:0048024)	6,69E-16	11,57	466,74
RNA transport (GO:0050658)	6,85E-16	13,29	535,08
nuclear export (GO:0051168)	1,23E-13	10,81	378,41
RNA metabolic process (GO:0016070)	3,29E-11	6,82	200,01
gene expression (GO:0010467)	3,40E-11	3,95	115,46
ribonucleoprotein complex assembly (GO:0022618)	5,30E-11	6,63	190,54
regulation of alternative mRNA splicing, via spliceosome (GO:0000381)	1,64E-09	11,61	293,55
mRNA splice site selection (GO:0006376)	3,93E-09	19,25	468,75
RNA splicing, via transesterification reactions (GO:0000375)	4,93E-09	23,21	558,85
chemical synaptic transmission (GO:0007268)	9,13E-09	3,76	87,98
spliceosomal snRNP assembly (GO:0000387)	1,17E-07	13,08	272,51
regulation of cardiac conduction (GO:1903779)	2,04E-07	9,45	191,10
mRNA cis splicing, via spliceosome (GO:0045292)	2,04E-07	32,21	650,38
nucleocytoplasmic transport (GO:0006913)	2,53E-06	10,77	189,88
regulation of neurotransmitter receptor activity (GO:0099601)	3,59E-06	8,01	138,12
termination of RNA polymerase II transcription (GO:0006369)	3,78E-06	12,00	205,97
nucleic acid metabolic process (GO:0090304)	7,42E-06	6,75	110,99
anterograde trans-synaptic signaling (GO:0098916)	8,93E-06	3,43	55,70
regulation of heart contraction (GO:0008016)	8,93E-06	5,64	91,33
glutamate receptor signaling pathway (GO:0007215)	8,93E-06	10,62	171,69
alternative mRNA splicing, via spliceosome (GO:0000380)	1,40E-05	20,01	314,03
import into nucleus (GO:0051170)	1,98E-05	6,09	93,31
cytosolic calcium ion transport (GO:0060401)	5,83E-05	11,86	168,50
regulation of mRNA processing (GO:0050684)	6,22E-05	9,64	136,06
regulation of gene silencing by RNA (GO:0060966)	8,04E-05	7,01	96,73
regulation of posttranscriptional gene silencing (GO:0060147)	8,04E-05	7,01	96,73
nuclear transport (GO:0051169)	8,42E-05	19,43	266,91
regulation of RNA splicing (GO:0043484)	8,69E-05	5,68	77,67
ncRNA export from nucleus (GO:0097064)	9,50E-05	8,95	121,40
regulation of gene silencing by miRNA (GO:0060964)	0,000107821	6,05	81,14
DNA-templated transcription, termination (GO:0006353)	0,00017708	5,73	73,88
regulation of cellular response to stress (GO:0080135)	0,000211332	4,24	53,84
ATP-dependent chromatin remodeling (GO:0043044)	0,000234623	7,83	98,42
positive regulation of synaptic transmission (GO:0050806)	0,000270679	5,44	67,51
spliceosomal tri-snRNP complex assembly (GO:0000244)	0,000294016	21,39	263,15
modulation of chemical synaptic transmission (GO:0050804)	0,000294057	4,33	53,18
calcium ion transmembrane transport (GO:0070588)	0,000378055	4,82	57,88
negative regulation of mRNA splicing, via spliceosome (GO:0048025)	0,000468355	18,72	220,40
regulation of cation channel activity (GO:2001257)	0,000477504	4,69	55,05
regulation of cellular response to heat (GO:1900034)	0,000592759	4,95	56,88
negative regulation of mRNA processing (GO:0050686)	0,000713027	16,64	187,95
positive regulation of RNA splicing (GO:0033120)	0,000997214	8,70	95,17
positive regulation of mRNA processing (GO:0050685)	0,001015893	10,93	119,19

cellular calcium ion homeostasis (GO:0006874)	0,001149759	3,60	38,71
transcription by RNA polymerase II (GO:0006366)	0,001170261	2,53	27,10
neurotransmitter transport (GO:0006836)	0,001174829	4,93	52,85
regulation of NMDA receptor activity (GO:2000310)	0,00117761	8,33	89,09
mRNA 3'-splice site recognition (GO:0000389)	0,001613103	49,80	515,88
endomembrane system organization (GO:0010256)	0,001643789	2,98	30,73
tRNA export from nucleus (GO:0006409)	0,001744252	7,69	78,56
tRNA-containing ribonucleoprotein complex export from nucleus (GO:0071431)	0,001744252	7,69	78,56
regulation of RNA metabolic process (GO:0051252)	0,001744252	5,10	52,10
chromatin organization (GO:0006325)	0,001744252	3,42	34,92
regulation of mRNA polyadenylation (GO:1900363)	0,001928725	12,48	125,84
chromatin remodeling (GO:0006338)	0,001971018	3,95	39,65
protein sumoylation (GO:0016925)	0,001971018	5,56	55,84
cellular metal ion homeostasis (GO:0006875)	0,002160757	3,91	38,79
calcium ion-regulated exocytosis of neurotransmitter (GO:0048791)	0,002361506	17,80	174,70
regulation of RNA export from nucleus (GO:0046831)	0,002361506	17,80	174,70
negative regulation of RNA splicing (GO:0033119)	0,00250253	11,52	112,17
tRNA transport (GO:0051031)	0,002951708	6,89	65,93
histone mRNA metabolic process (GO:0008334)	0,003092402	8,32	79,09
regulation of mRNA metabolic process (GO:1903311)	0,003519429	15,58	145,79
synaptic vesicle exocytosis (GO:0016079)	0,005098336	6,25	56,08
RNA secondary structure unwinding (GO:0010501)	0,005530301	24,90	221,12
signal release from synapse (GO:0099643)	0,008455482	5,71	48,23
positive regulation of histone modification (GO:0031058)	0,008833927	6,72	56,38
DNA topological change (GO:0006265)	0,009291366	19,92	165,80
neurotransmitter secretion (GO:0007269)	0,009621704	5,55	45,96
transmembrane receptor protein tyrosine kinase signaling pathway (GO:0007169)	0,00962231	2,10	17,38
ephrin receptor signaling pathway (GO:0048013)	0,011739512	3,88	31,24
regulation of synaptic transmission, glutamatergic (GO:0051966)	0,012763074	5,26	41,86
dosage compensation (GO:0007549)	0,013504252	16,60	130,20
L-glutamate transmembrane transport (GO:0015813)	0,013504252	16,60	130,20
lipid phosphorylation (GO:0046834)	0,013504252	16,60	130,20
nuclear pore complex assembly (GO:0051292)	0,013504252	16,60	130,20
lamellipodium assembly (GO:0030032)	0,013504252	7,48	58,62
positive regulation of histone methylation (GO:0031062)	0,013504252	7,48	58,62
transcription, DNA-templated (GO:0006351)	0,013586192	2,50	19,56
polyol metabolic process (GO:0019751)	0,013586192	5,12	40,00
inositol phosphate metabolic process (GO:0043647)	0,015522093	5,00	38,26
positive regulation of mRNA splicing, via spliceosome (GO:0048026)	0,015522093	9,58	73,08
synaptic transmission, glutamatergic (GO:0035249)	0,015522093	9,58	73,08
L-alpha-amino acid transmembrane transport (GO:1902475)	0,015522093	7,13	54,27
regulation of cardiac muscle cell contraction (GO:0086004)	0,015522093	7,13	54,27
endoplasmic reticulum organization (GO:0007029)	0,015522093	3,97	30,21