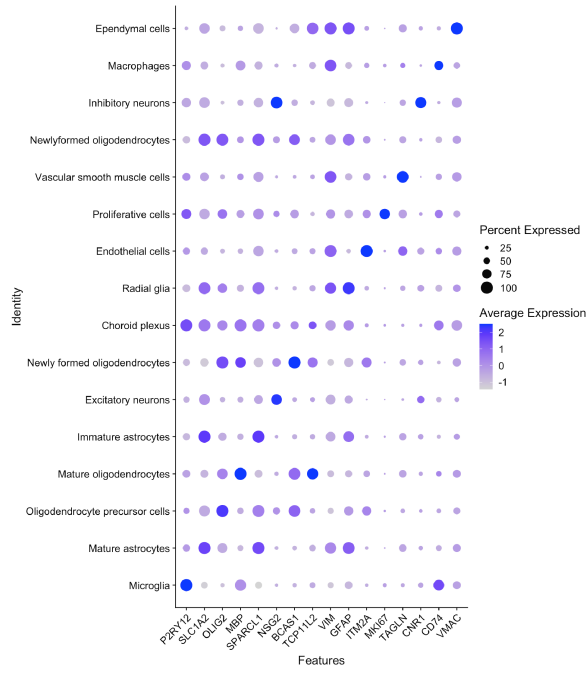
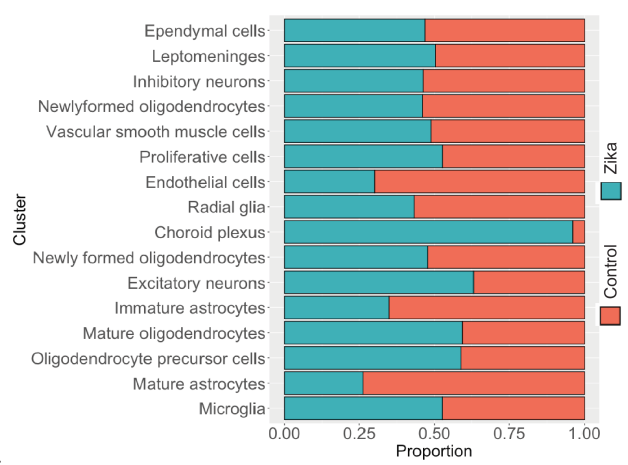


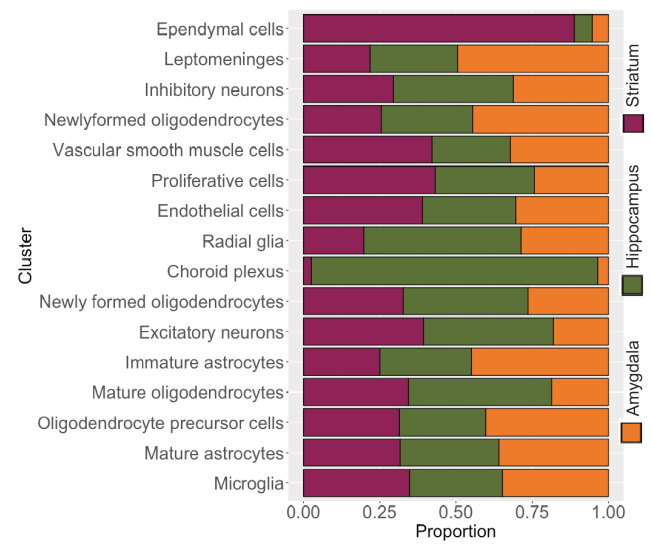
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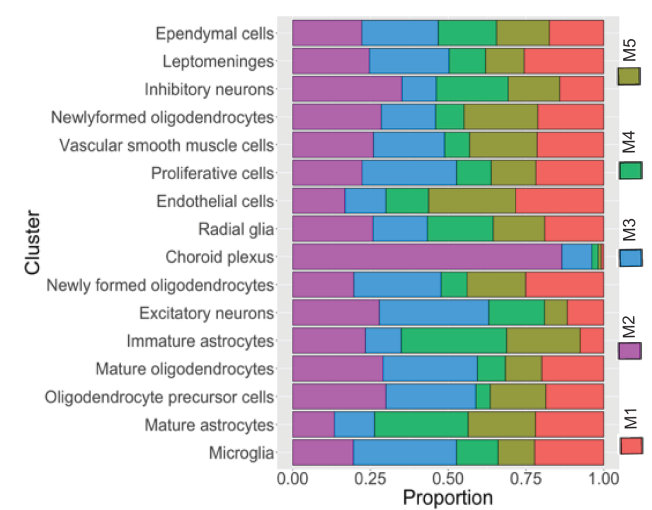
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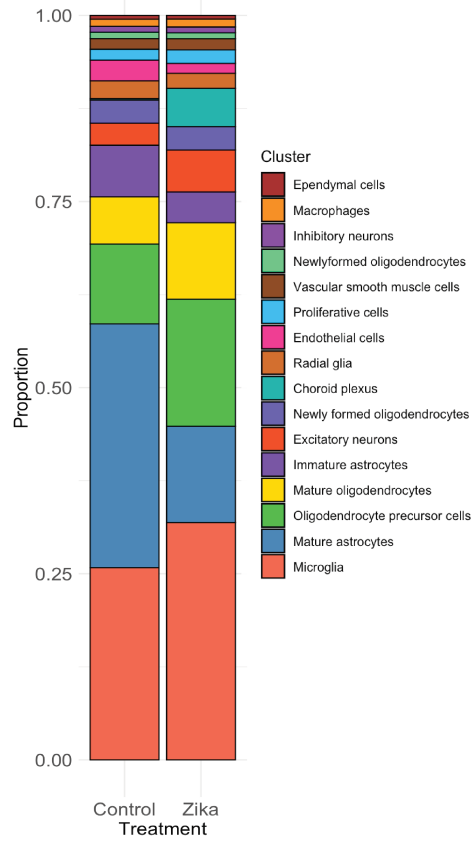
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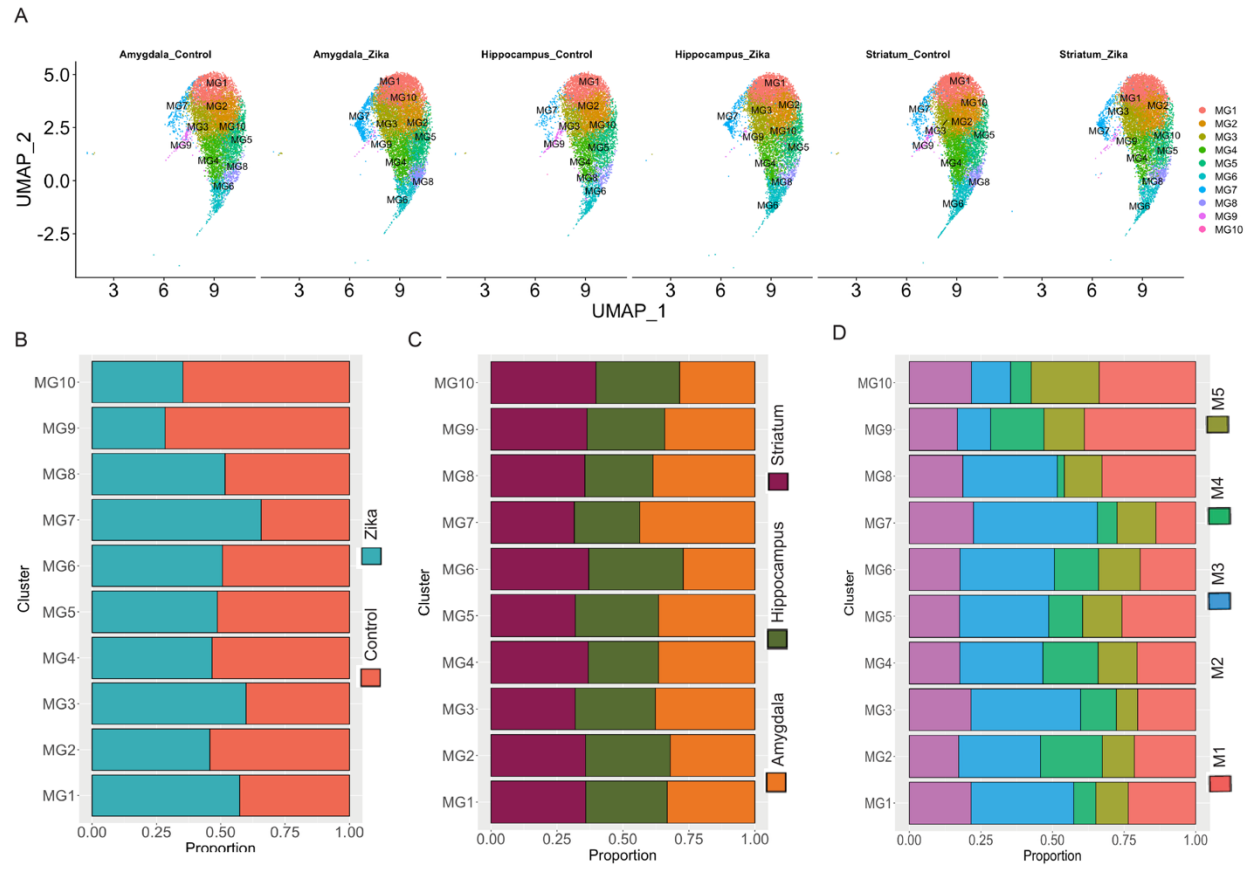
D



E



Supplemental Figure 1. Characterization and distribution of cell type clusters across treatment groups, brain regions and individual animal, related to Figure 1. (A) Cell type identities were assigned based on canonical marker gene expression. Dot plot showing scaled expression and percent of cells expressing representative marker genes for each identified cell type cluster. (B-D) Bar plots showing proportion of cells within each cluster, stratified by treatment group (B), brain region (C), and individual animal (D). Bars represent the relative abundance of each cluster within the specified category. (E) Bar plot showing the relative proportions of all identified cell type clusters across control and ZIKV-infected groups.



Supplemental Figure 2. UMAP visualization and distribution of microglial subclusters

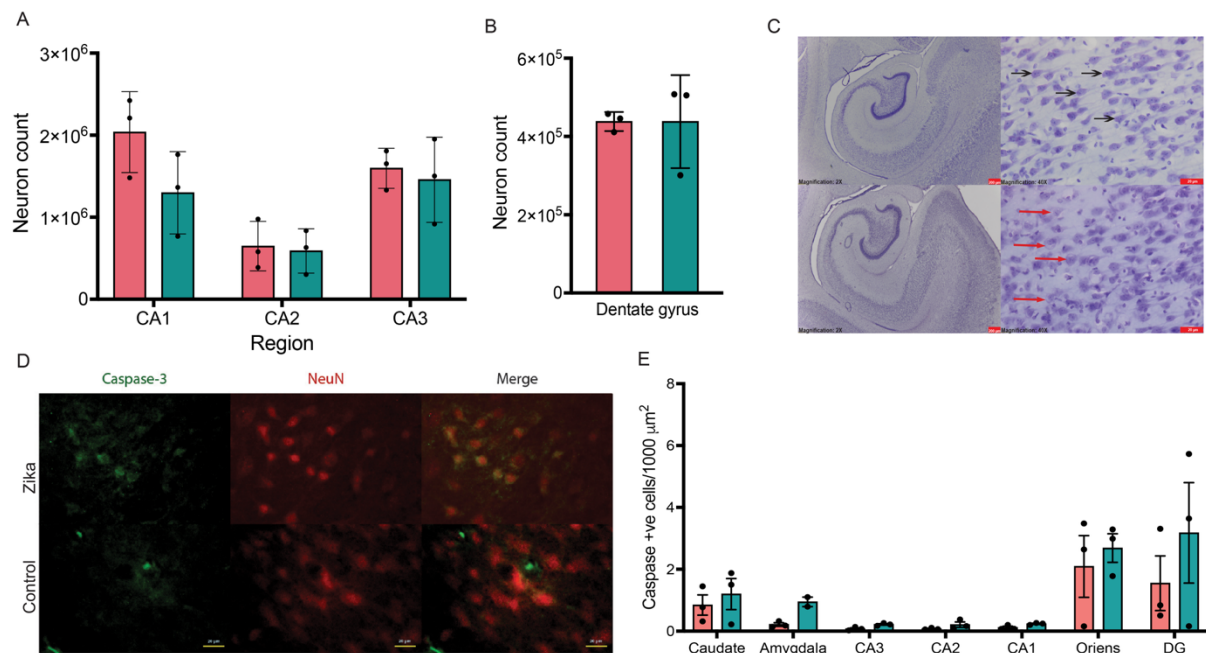
across treatment groups, brain regions and individual animal, related to Figure 2. (A)

UMAP projections of microglia subclusters from the amygdala, hippocampus, and striatum, with each region's cells split by treatment group (ZIKV-infected vs. control). Subclusters were

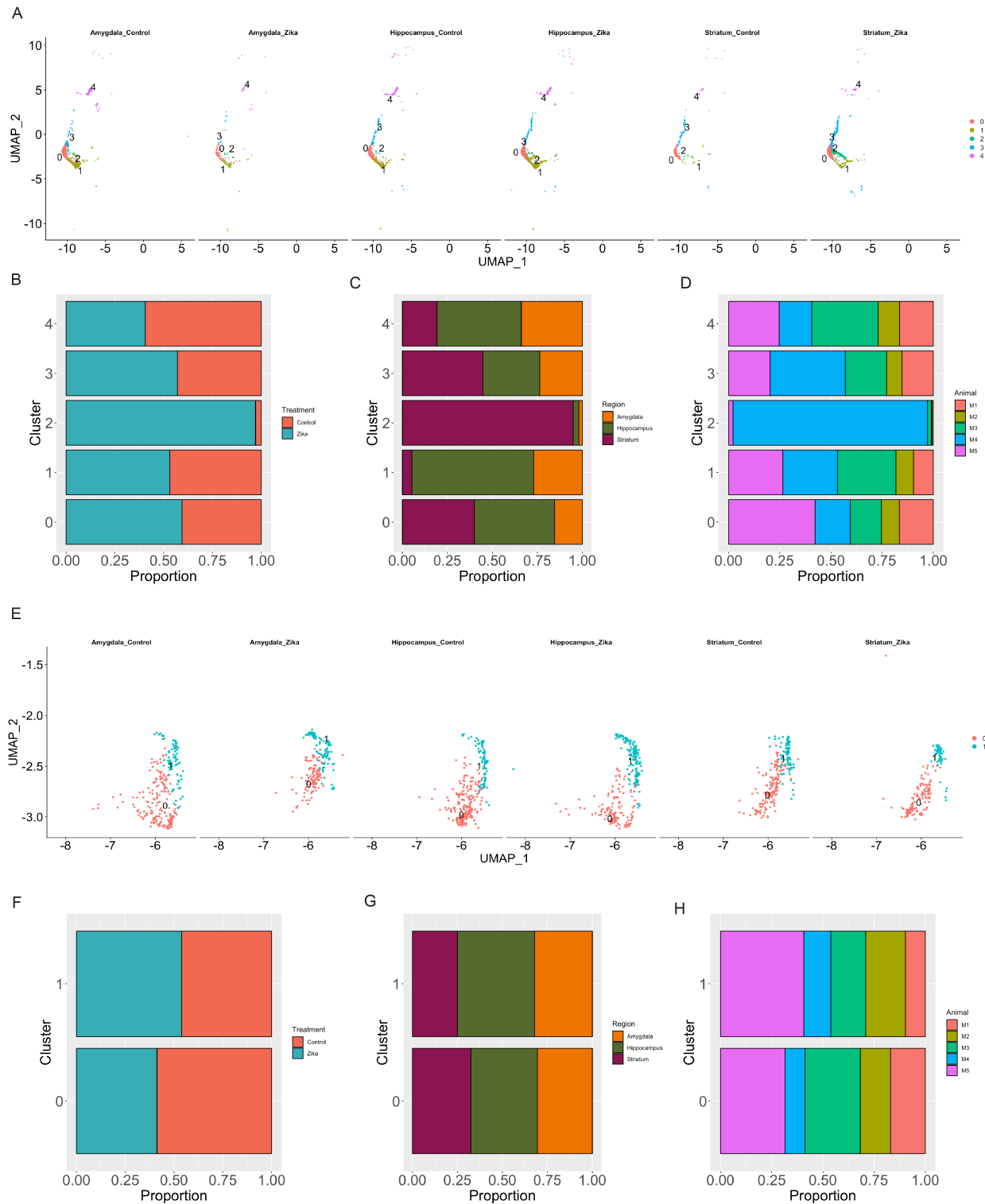
identified based on transcriptional profiles and are color-coded consistently across plots. **(B-D)**

Bar plots showing the proportion of each microglial subcluster across treatment groups **(B)**, brain regions **(C)**, and individual animal **(D)**.

Supplemental Figure 3. Gene expression in total microglia and MG6 subcluster across brain regions shows induction of interferon response genes, related to Figure 2. (A) Volcano plots showing differential expression of a curated panel of interferon-stimulated genes (ISGs) in the total microglial population, comparing ZIKV-infected and control infant rhesus macaques in the combined dataset and within individual brain regions (amygdala, hippocampus, and striatum). (B) Volcano plots of ISG expression within the MG6 subcluster, analyzed similarly across the combined dataset and individual brain regions. Each black point represents an individual gene, with interferon-stimulated genes (ISGs) highlighted in red. Plots display $\log_2$ fold change on the x-axis and $-\log_{10}(\text{p-value})$ on the y-axis.



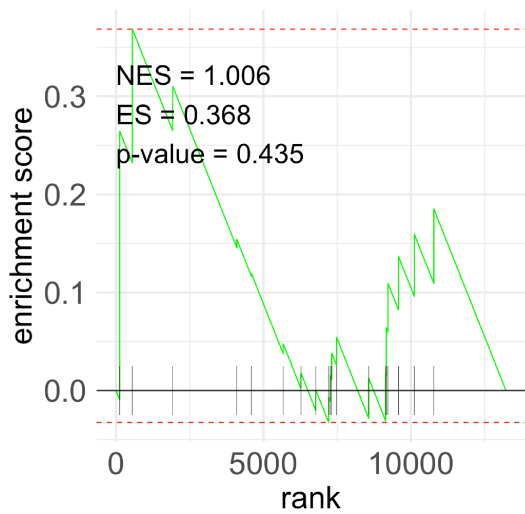
Supplemental Figure 4. Zika virus infection decreases neuron count in the hippocampal CA1 region, related to Figure 3. (A) Design-based stereologic quantification of hippocampal neuronal populations of the pyramidal layer in CA subfields 1-3 and **(B)** Design-based stereologic quantification of nestin positive immature neurons in the dentate gyrus. **(C)** Cresyl violet stained mature neurons in the hippocampal CA1 subfield showing frequent cytoplasmic inclusions (red arrows) in ZIKV-infected macaques and healthy neurons (black arrows) in control macaques. Top panels are representative images from control and bottom panels are from ZIKV-infected animals (scale bar left panels=200 μm; scale bar right panels=20 μm). **(D)** Matched sections from ZIKV-infected (top panels) and control (bottom panels) subjects were double-labelled for activated caspase-3 and the mature neuronal marker NeuN. ZIKV-infected subjects display double-labelling in the hippocampus (top right panel). Scale bar=20μm. **(E)** Quantification of caspase positive cells in different regions of hippocampus, caudate and amygdala.



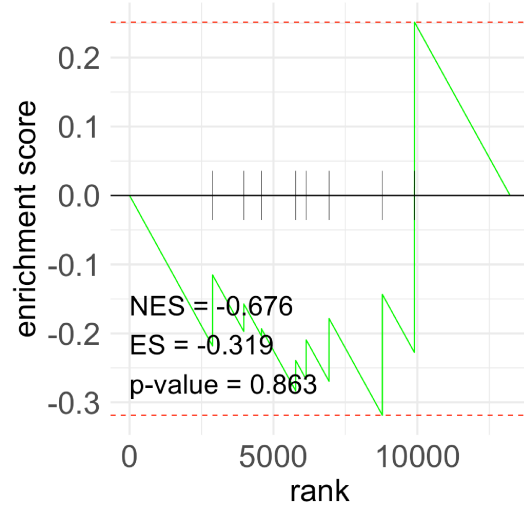
Supplemental Figure 5. UMAP visualization and distribution of excitatory and inhibitory neuron subclusters across treatment groups, brain regions and individual animal, related to Figure 3. (A) UMAP projections of excitatory neuron subclusters from the amygdala,

hippocampus, and striatum, with each region's cells split by treatment group (ZIKV-infected vs. control). Subclusters are color-coded consistently across plots. **(B-D)** Bar plots showing the proportion of each excitatory neuron subcluster across treatment groups **(B)**, brain regions **(C)**, and individual animal **(D)**. **(E)** UMAP projections of inhibitory neuron subclusters from the amygdala, hippocampus, and striatum, with each region's cells split by treatment group (ZIKV-infected vs. control). Subclusters are color-coded consistently across plots. **(F-H)** Bar plots showing the proportion of each inhibitory neuron subcluster across treatment groups **(F)**, brain regions **(G)**, and individual animal **(H)**.

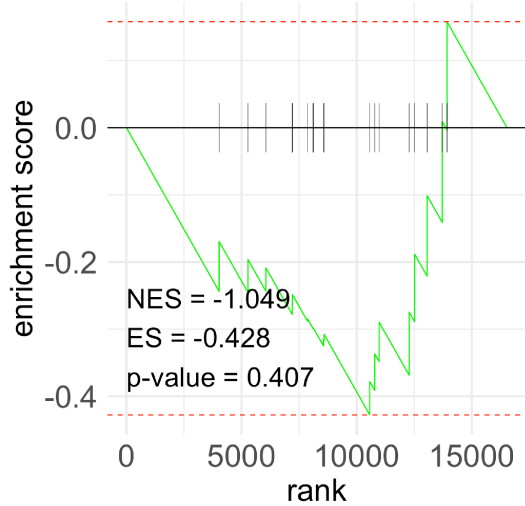
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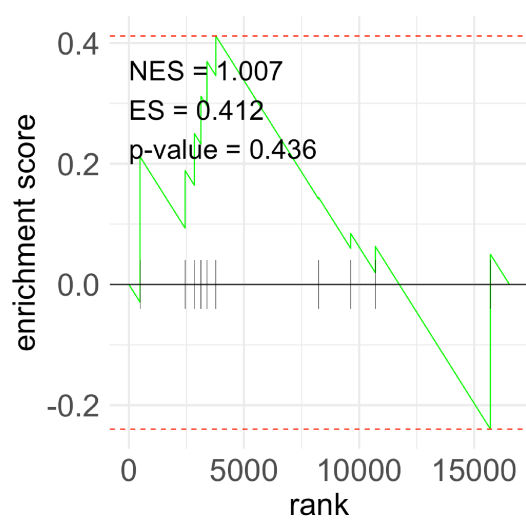
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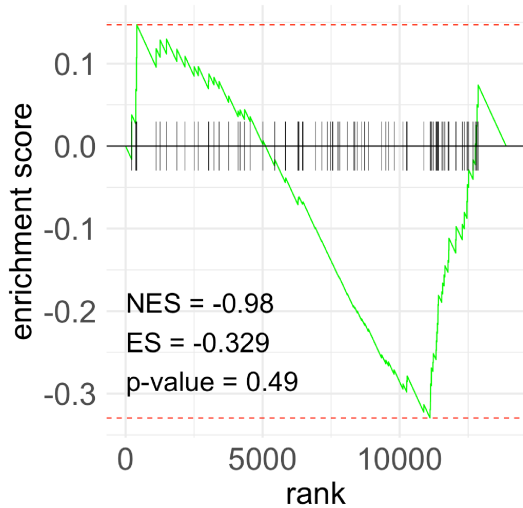
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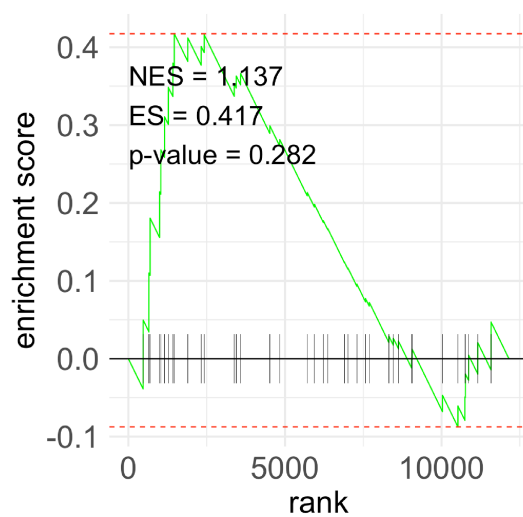
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E

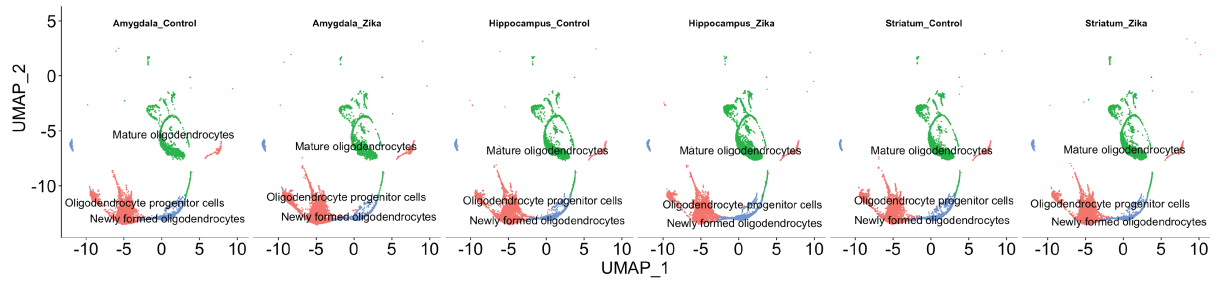


F

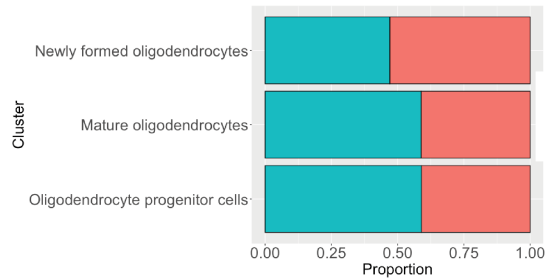


Supplemental Figure 6. Gene Set Enrichment Analysis (GSEA) of cerebral palsy and autism spectrum disorder (ASD) gene sets in neuronal and mature oligodendrocyte populations show selective enrichment of ASD genes in neuronal populations, related to Figure 4. (A & B) GSEA plots of cerebral palsy-associated gene sets in excitatory neurons, derived from the Clinical Genome Resources **(A)** and Harmonizome **(B)** databases. **(C & D)** GSEA plots of cerebral palsy-associated gene sets in inhibitory neurons, derived from the Clinical Genome Resources **(C)** and Harmonizome **(D)** databases. **(E & F)** GSEA plots of Epoch 1 **(E)** and Epoch 2 **(F)** ASD gene sets in mature oligodendrocytes. Enrichment scores and significance values reflect the degree of overrepresentation of disease-associated gene sets within the specified cell populations.

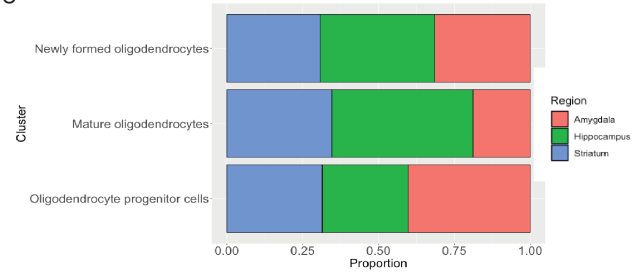
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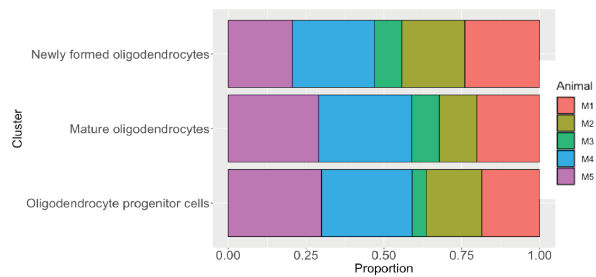
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C

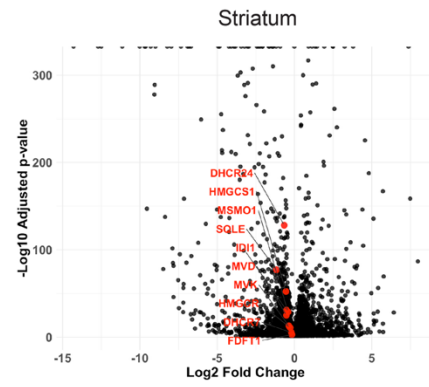
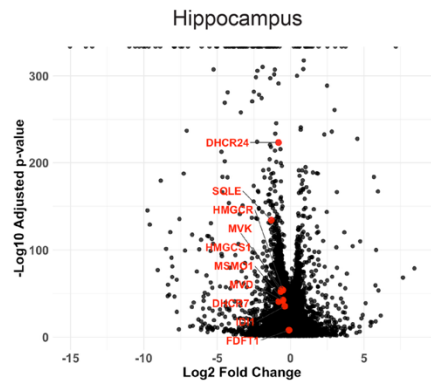
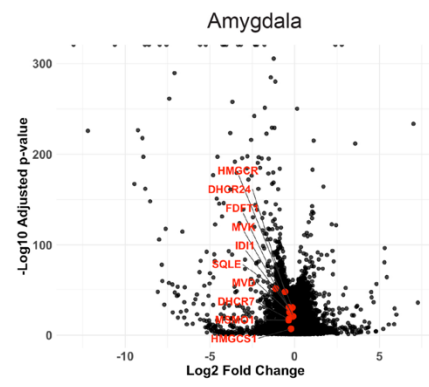
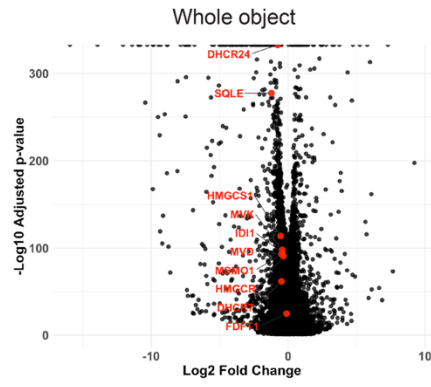


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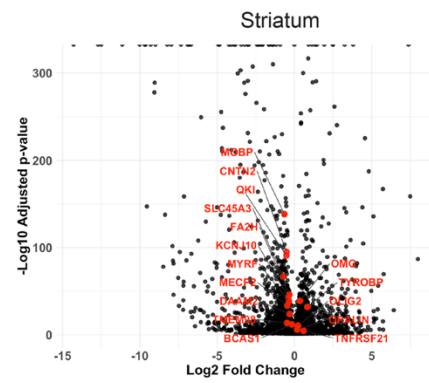
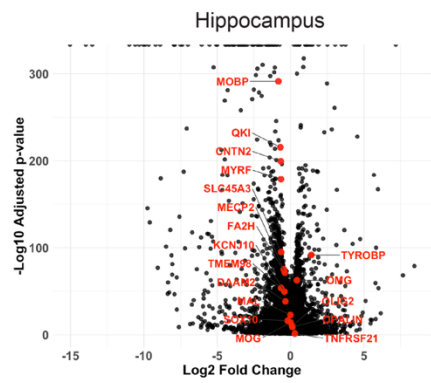
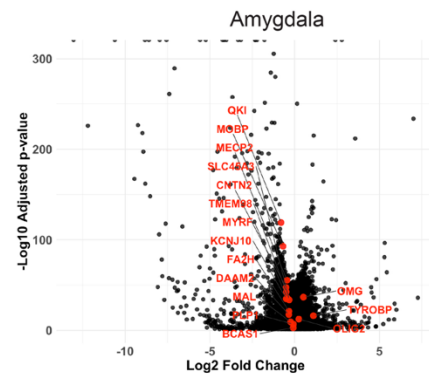
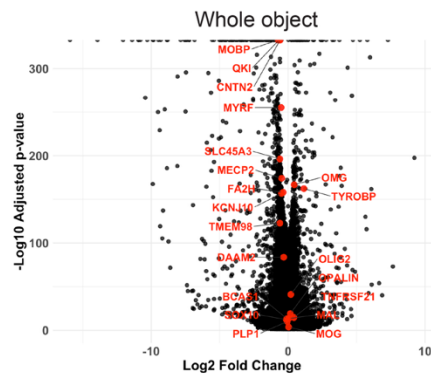


Supplemental Figure 7. UMAP visualization and distribution of oligodendrocyte subclusters related to Figure 5. (A) UMAP projections of oligodendrocyte subclusters from the amygdala, hippocampus, and striatum, with each region's cells split by treatment group (ZIKV-infected vs. control). Subclusters were identified based on transcriptional profiles and are color-coded consistently across plots. **(B-D)** Bar plots showing the proportion of each oligodendrocyte subcluster across treatment groups **(B)**, brain regions **(C)**, and individual animal **(D)**.

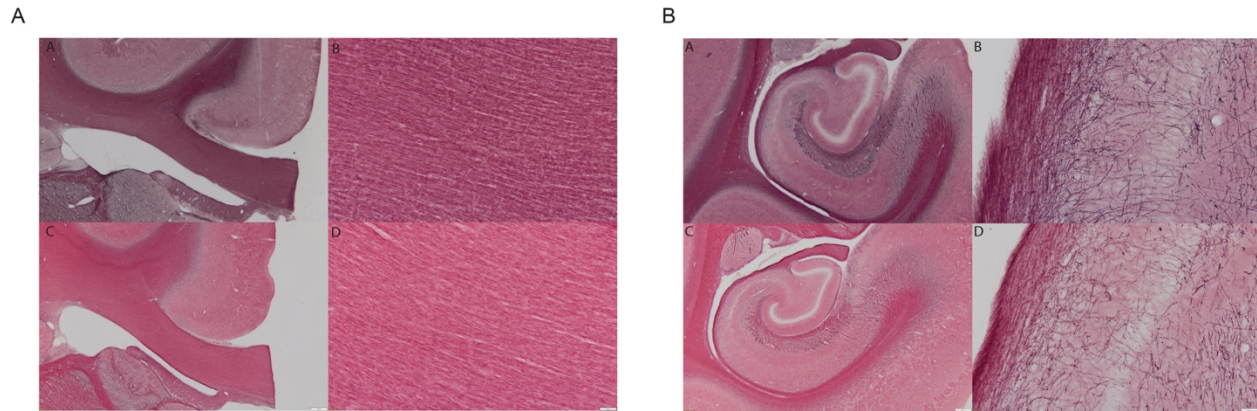
A



B



Supplemental Figure 8. Gene expression in mature oligodendrocytes across brain regions shows downregulation of representative genes from sterol biosynthesis and oligodendrocyte differentiation pathways, related to Figure 5. (A) Volcano plot illustrating differential expression of key genes involved in the sterol biosynthesis pathway between experimental groups in the combined dataset and within individual brain regions (amygdala, hippocampus, and striatum). (B) Volcano plot showing differential expression of genes associated with oligodendrocyte differentiation across the combined dataset and individual brain regions. Each point represents an individual gene, plotted by $\log_2$ fold change (x-axis) versus $-\log_{10}(\text{p-value})$ (y-axis). Genes highlighted in red color are representative members of the respective pathways.



Supplemental Figure 9. Zika virus infection did not alter myelin density in corpus callosum and limbic system white matter tracts, related to Figure 6. Matched sections from each subject were stained with a 0.2% gold chloride solution from **(A)** corpus callosum and **(B)** hippocampal region. Density analysis with Image J/Fuji did not reveal significant differences in myelin density between groups in either area. Top panels are representative images from control and bottom panels are from Zika-infected subjects (scale bar left panels=200 μm ; scale bars right panels=20 μm).

Supplemental Table 1. Cell numbers for all 16 clusters by experimental group

Cluster	Zika	Control
Microglia	30265	27217
Mature astrocytes	12303	34518
Oligodendrocyte precursor cells	16209	11313
Mature oligodendrocytes	9767	6680
Immature astrocytes	3917	7312
Excitatory neurons	5354	3137
Newly formed oligodendrocytes	2987	3271
Choroid plexus	4882	193
Radial glia	1917	2508
Endothelial cells	1264	2953
Proliferative cells	1711	1537
Vascular smooth muscle cells	1425	1495
Newly formed oligodendrocytes	768	904
Inhibitory neurons	732	854
Leptomeninges	996	985
Ependymal cells	478	544

Supplemental Table 2. Cell numbers for all 16 clusters by individual animal

Cluster	M5	M4	M3	M2	M1
Microglia	11175	19090	7701	6734	12782
Mature astrocytes	6267	6036	14117	10115	10286
Oligodendrocyte precursor cells	8243	7966	1275	4918	5120
Mature oligodendrocytes	4774	4993	1481	1919	3280
Immature astrocytes	2623	1294	3805	2660	847
Excitatory neurons	2363	2991	1525	616	996
Newly formed oligodendrocytes	1226	1761	521	1179	1571
Choroid plexus	4390	492	102	53	38
Radial glia	1141	776	934	736	838
Endothelial cells	707	557	578	1181	1194
Proliferative cells	724	987	362	466	709
Vascular smooth muscle cells	757	668	236	634	625
Newly formed oligodendrocytes	475	293	153	397	354
Inhibitory neurons	556	176	367	263	224
Leptomeninges	488	508	232	247	506
Ependymal cells	227	251	192	173	179

Supplemental Table 3. Cell numbers for all 16 clusters by brain region

Cluster	Striatum	Hippocampus	Amygdala
Microglia	19983	17511	19988
Mature astrocytes	14847	15163	16811
Oligodendrocyte precursor cells	8660	7768	11094
Mature oligodendrocytes	5672	7719	3056
Immature astrocytes	2806	3375	5048
Excitatory neurons	3341	3618	1532
Newly formed oligodendrocytes	2048	2557	1653
Choroid plexus	131	4766	178
Radial glia	876	2279	1270
Endothelial cells	1645	1289	1283
Proliferative cells	1401	1057	790
Vascular smooth muscle cells	1229	752	939
Newly formed oligodendrocytes	426	501	745
Inhibitory neurons	469	622	495
Leptomeninges	432	570	979
Ependymal cells	907	61	54

Supplemental Table 4. Cell numbers for all microglial subclusters by experimental group

Cluster	Zika	Control
MG1	8143	6050
MG2	4788	5669
MG3	5633	3786
MG4	3302	3778
MG5	3246	3428
MG6	2093	2040
MG7	1848	966
MG8	943	882
MG9	181	457
MG10	88	161

Supplemental Table 5. Cell numbers for all microglial subclusters by individual animal

Cluster	M5	M4	M3	M2	M1
MG1	3063	5080	1101	1608	3341
MG2	1804	2984	2255	1173	2241
MG3	2030	3603	1175	703	1908
MG4	1246	2056	1366	963	1449
MG5	1170	2076	792	916	1720
MG6	730	1363	637	601	802
MG7	630	1218	193	381	392
MG8	341	602	45	240	597
MG9	107	74	119	90	248
MG10	54	34	18	59	84

Supplemental Table 6. Cell numbers for all microglial subclusters by brain region

Cluster	Striatum	Hippocampus	Amygdala
MG1	5094	4382	4717
MG2	3745	3360	3352
MG3	3004	2863	3552
MG4	2609	1885	2586
MG5	2131	2107	2436
MG6	1532	1478	1123
MG7	888	698	1228
MG8	649	471	705
MG9	232	188	218
MG10	99	79	71

Supplemental Table 7. Cell numbers for all excitatory neuron subclusters by experimental group

Cluster	Zika	Control
0	2018	1379
1	1247	1101
2	1459	44
3	436	329
4	194	284

Supplemental Table 8. Cell numbers for all excitatory neuron subclusters by individual animal

Cluster	M5	M4	M3	M2	M1
0	1436	582	517	298	564
1	622	625	670	204	227
2	32	1427	29	6	9
3	155	281	154	58	117
4	118	76	155	50	79

Supplemental Table 9. Cell numbers for all excitatory neuron subclusters by brain region

Cluster	Striatum	Hippocampus	Amygdala
0	1356	1516	525
1	124	1588	636
2	1427	48	28
3	342	242	181
4	92	224	162

Supplemental Table 10. Cell numbers for all inhibitory neuron subclusters by experimental group

Cluster	Zika	Control
0	399	569
1	333	285

Supplemental Table 11. Cell numbers for all inhibitory neuron subclusters by individual animal

Cluster	M5	M4	M3	M2	M1
0	305	94	262	143	164
1	251	82	105	120	60

Supplemental Table 12. Cell numbers for all inhibitory neuron subclusters by brain region

Cluster	Striatum	Hippocampus	Amygdala
0	315	357	296
1	154	265	199

Supplemental Table 13. Cell numbers for all oligodendrocyte subclusters by experimental group

Cluster	Zika	Control
Oligodendrocyte progenitor cells	16174	11255
Mature oligodendrocytes	10189	7122
Newly formed oligodendrocytes	3368	3791

Supplemental Table 14. Cell numbers for all oligodendrocyte subclusters by individual animal

Cluster	M5	M4	M3	M2	M1
Oligodendrocyte progenitor cells	8218	7956	1267	4893	5095
Mature oligodendrocytes	5026	5163	1545	2072	3505
Newly formed oligodendrocytes	1474	1894	618	1448	1725

Supplemental Table 15. Cell numbers for all oligodendrocyte subclusters by brain region

Cluster	Striatum	Hippocampus	Amygdala
Oligodendrocyte progenitor cells	8624	7762	11043
Mature oligodendrocytes	5978	8076	3257
Newly formed oligodendrocytes	2204	2707	2248

Supplemental Table 16. Comparison of fractional anisotropy (FA) across 15 white matter tracts between ZIKV-infected and control infants, including sex-based subgroup

Fiber tract		Female		Male		Overall	
		Control	ZIKA	Control	ZIKA	Control	ZIKA
		(N=7)	(N=6)	(N=5)	(N=6)	(N=12)	(N=12)
ccg	Mean	0.361	0.363	0.352	0.364	0.357	0.364
	SD	0.0127	0.00571	0.0173	0.0139	0.0147	0.0102
cc1d	Mean	0.368	0.375	0.358	0.382	0.364	0.378
	SD	0.0119	0.00889	0.0192	0.0172	0.0154	0.0136
cc2d	Mean	0.44	0.428	0.414	0.44	0.429	0.435
	SD	0.0172	0.0164	0.0309	0.0168	0.0263	0.0171
	QC excl	0	1	0	0	0	1
cc3d	Mean	0.378	0.371	0.344	0.36	0.364	0.366
	SD	0.0279	0.0184	0.0172	0.0182	0.0288	0.0184
ccs	Mean	0.504	0.516	0.488	0.512	0.497	0.514
	SD	0.0164	0.0263	0.0402	0.0128	0.0283	0.0198
uf_l	Mean	0.356	0.353	0.35	0.366	0.354	0.359
	SD	0.0145	0.0229	0.0174	0.00812	0.0153	0.0177
uf_r	Mean	0.363	0.369	0.365	0.373	0.364	0.371
	SD	0.0184	0.0176	0.0182	0.00967	0.0175	0.0137
cg_l	Mean	0.315	0.324	0.313	0.334	0.315	0.328
	SD	0.0133	0.0172	0.0223	0.00526	0.017	0.0136
	QC excl	1	1	0	2	1	3
cg_r	Mean	0.327	0.334	0.315	0.344	0.322	0.339
	SD	0.0142	0.0144	0.0209	0.0116	0.0176	0.0134
	QC excl	0	1	0	1	0	2
cst_l	Mean	0.433	0.418	0.412	0.442	0.424	0.43
	SD	0.0162	0.0267	0.0135	0.0257	0.0178	0.028
cst_r	Mean	0.434	0.41	0.406	0.441	0.422	0.425
	SD	0.0172	0.0191	0.0104	0.0175	0.02	0.0237
ilf_l	Mean	0.331	0.338	0.321	0.336	0.327	0.337
	SD	0.0222	0.0262	0.0149	0.00528	0.0195	0.018
ilf_r	Mean	0.347	0.355	0.339	0.358	0.344	0.356
	SD	0.0153	0.0178	0.0159	0.0119	0.0153	0.0145
fornix_l	Mean	0.3	0.301	0.292	0.316	0.296	0.309
	SD	0.0269	0.0183	0.0198	0.0112	0.0236	0.0164
fornix_r	Mean	0.285	0.291	0.287	0.31	0.286	0.301
	SD	0.0202	0.011	0.016	0.0129	0.0178	0.015

Supplemental Table 17. Infant Neurobehavioral Assessment Scale

Category	Description
<u>Orientation</u>	
Visual orientation	Does the infant look at an object presented in their visual field? right, left, up and down
Visual following	Does the infant track the movement of an object? horizontal and vertical planes
Attention span	How attentive is the infant during visual assessments?
Auditory orientation	Does the infant turn toward a sound? right and left
<u>Motor Abilities</u>	
Locomotion	Does the infant exhibit coordinated limb movement?
Coordination	Is the infant's movement clumsy, adequate, or agile?
Activity	How much time is the infant engaged in movement?
<u>Neuromotor Abilities</u>	
Palmar Grasp	Grasping reflex examined in right and left hands
Plantar Grasp	Grasping reflex examined in right and left feet
Tactile Response	Reflex of retracting right and left limbs from a tactile stroke
Galant Reflex	Spinal reflex from a tactile stroke down the back
Rooting	Suckling reflex examined on the right and left side of the mouth
Head Righting	Realigning the head when the body is tilted to the right, left, prone, and supine
Body Righting	Does the infant turn over when placed supine?
Response Speed	How quickly does the infant respond?
Pull to Sit	Does the infants head lag when gently pulled from supine to seated position?
Active Power	Muscle resistance and infant's attempt to flex their limbs
<u>State Control</u>	
Predominant State	Is the infant agitated or just awake, alert, and not agitated during testing?
Vocalizations	How much did the infant vocalize during testing?
Vocal Intensity	Are the infant's vocalizations mild, moderate, or shrill?
Fearfulness	Does this infant fear grimace during testing?
Squirming	Does the infant struggle during testing?
Irritability	How distressed was the infant during testing?
Consolability	Was the infant easily or impossible to soothe during distress?
Cuddliness	Did the infant cuddle or flex toward the examiner?
Aggression	Did the infant attempt to bite the examiner?

All items are scored using a five-point Likert scale ranging from 0 to 2 and then averaged per category.

Supplemental Table 18. Individual Scores on the Infant Neurobehavioral Assessment by Age

Age (weeks)	Group	Orientation	Motor	Neuromotor	State Control
3	Uninfected Controls	2.000	1.667	1.476	0.278
		1.889	1.667	1.476	0.389
		1.778	0.833	1.429	0.167
		mean	1.889	1.389	1.460
	ZIKV-infected	1.778	2.000	1.476	0.444
		1.944	1.333	1.381	0.278
		2.000	1.333	1.429	0.111
		mean	1.907	1.555	1.429
	Uninfected Controls	2.000	1.667	1.500	0.389
		2.000	1.667	1.778	0.389
		1.667	1.333	1.476	0.278
		mean	1.889	1.556	1.585
4	ZIKV-infected	2.000	1.667	1.619	0.167
		2.000	1.667	1.476	0.111
		1.778	1.667	1.571	0.389
		mean	1.926	1.667	1.555
		2.000	1.833	1.405	0.167
	Uninfected Controls	2.000	2.000	1.550	0.222
		1.833	1.333	1.548	0.611
		mean	1.944	1.722	1.501
	ZIKV-infected	1.667	2.000	1.714	0.778
		1.778	2.000	1.476	0.333
		1.778	2.000	1.667	0.611
		mean	1.741	2.000	1.619
5	Uninfected Controls	1.944	1.667	1.810	0.111
		2.000	2.000	1.714	0.111
		1.833	1.667	1.381	0.111
		mean	1.926	1.778	1.635
	ZIKV-infected	1.667	2.000	1.524	1.000
		1.778	2.000	1.690	0.611
	Uninfected Controls	1.944	1.667	1.810	0.111
		2.000	2.000	1.714	0.111
		1.833	1.667	1.381	0.111
		mean	1.926	1.778	1.635
6	ZIKV-infected	1.667	2.000	1.524	1.000
		1.778	2.000	1.690	0.611
	Uninfected Controls	1.944	1.667	1.810	0.111
		2.000	2.000	1.714	0.111
		1.833	1.667	1.381	0.111
		mean	1.926	1.778	1.635
	ZIKV-infected	1.667	2.000	1.524	1.000
		1.778	2.000	1.690	0.611
	Uninfected Controls	1.944	1.667	1.810	0.111
		2.000	2.000	1.714	0.111
		1.833	1.667	1.381	0.111
		mean	1.926	1.778	1.635

	NA	NA	NA	NA
mean	1.723	2.000	1.607	0.806
