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Supplemental information

Lipid nanoparticle delivery limits antisense oligonucleotide activity and cellular distribution in the brain after intracerebroventricular injection

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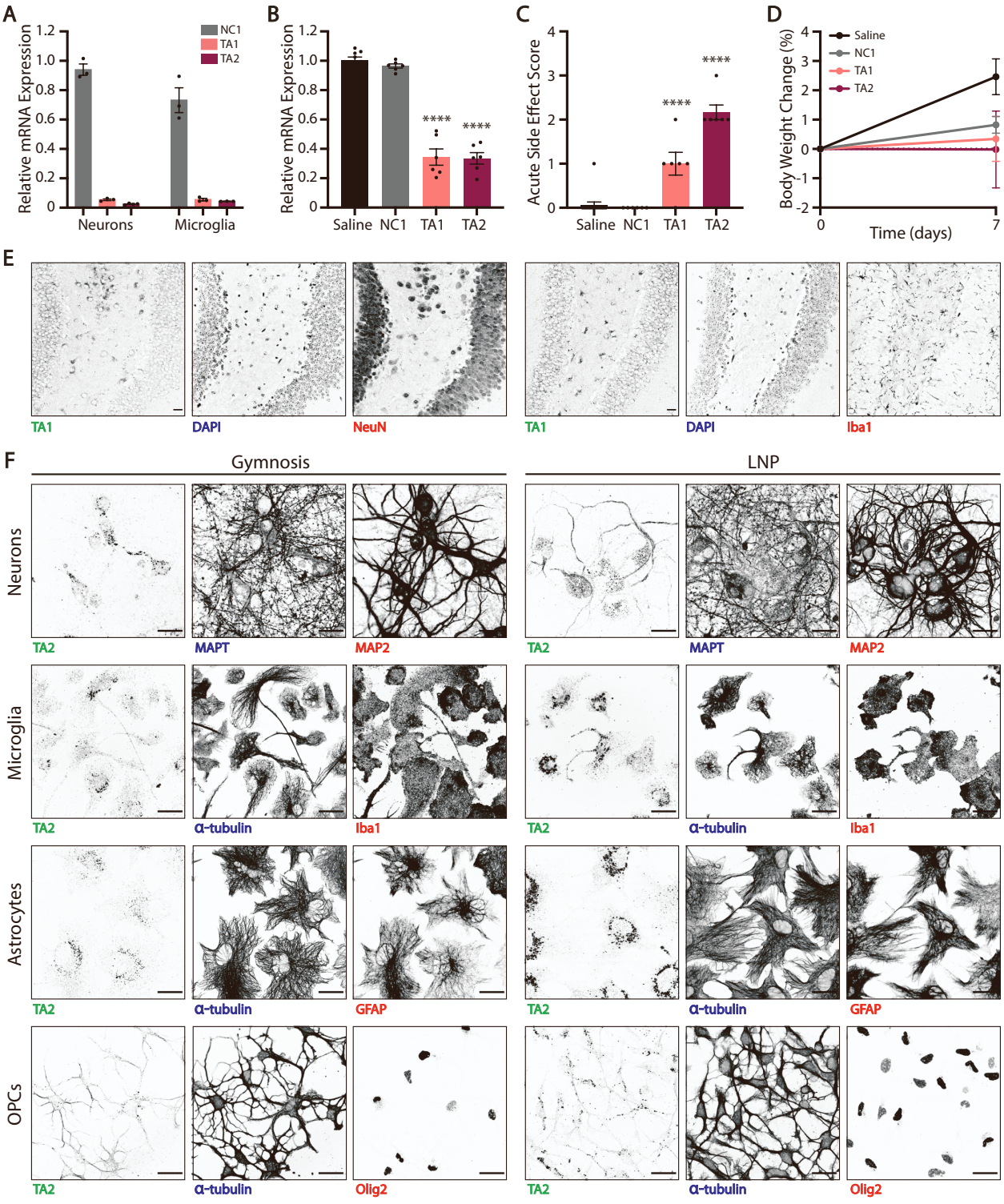


Figure S1. TA1 and TA2 show similar efficacy and localization in cells, but TA2 is slightly toxic when ICV injected in mice. (**A**) *TMEM106b* mRNA expression in cortical neurons and microglia as determined by RT-qPCR. Cells were treated with 10 μM NC1, TA1, or TA2 for 3 days. Data were normalized to GAPDH and PBS-treated control cells. Three internal replicates were averaged and the experiments were completed in triplicate. (**B-D**) C57BL/6N mice were ICV

injected with saline (n = 6/experiment), NC1 (100 µg, n = 6), TA1 (100 µg, n = 6), or TA2 (100 µg, n = 6) and analyzed over 7 days. (**B**) *TMEM106b* mRNA expression levels in the brain 7 days post ICV injection as assessed by RT-qPCR. Data were normalized to GAPDH and saline-treated controls. (**C**) Acute toxicity 1 hour post injection. (**D**) Percent body weight change normalized to day 0. (**E-F**) Greyscale single channel IF images showing ASO localization in the brain and primary CNS cells. (**E**) TA1 localization in the brain shown with DAPI, and NeuN (left) or Iba1 (right) co-staining, as shown in Figure 1E, 14 days post injection. Scale bars, 100 µm. (**F**) TA2 localization in primary CNS cells shown with cell type marker co-staining, as shown in Figure 2D (gymnosis) and 2L (LNP delivery). Cells were fixed 3 days following gymnosis or LNP delivery. Scale bars, 25 µm. For all *in vivo* statistical analyses, one-way ANOVA with multiple comparisons Dunnett's test (saline control) was used, ****$p < 0.0001$. Mean ± SEM are shown.

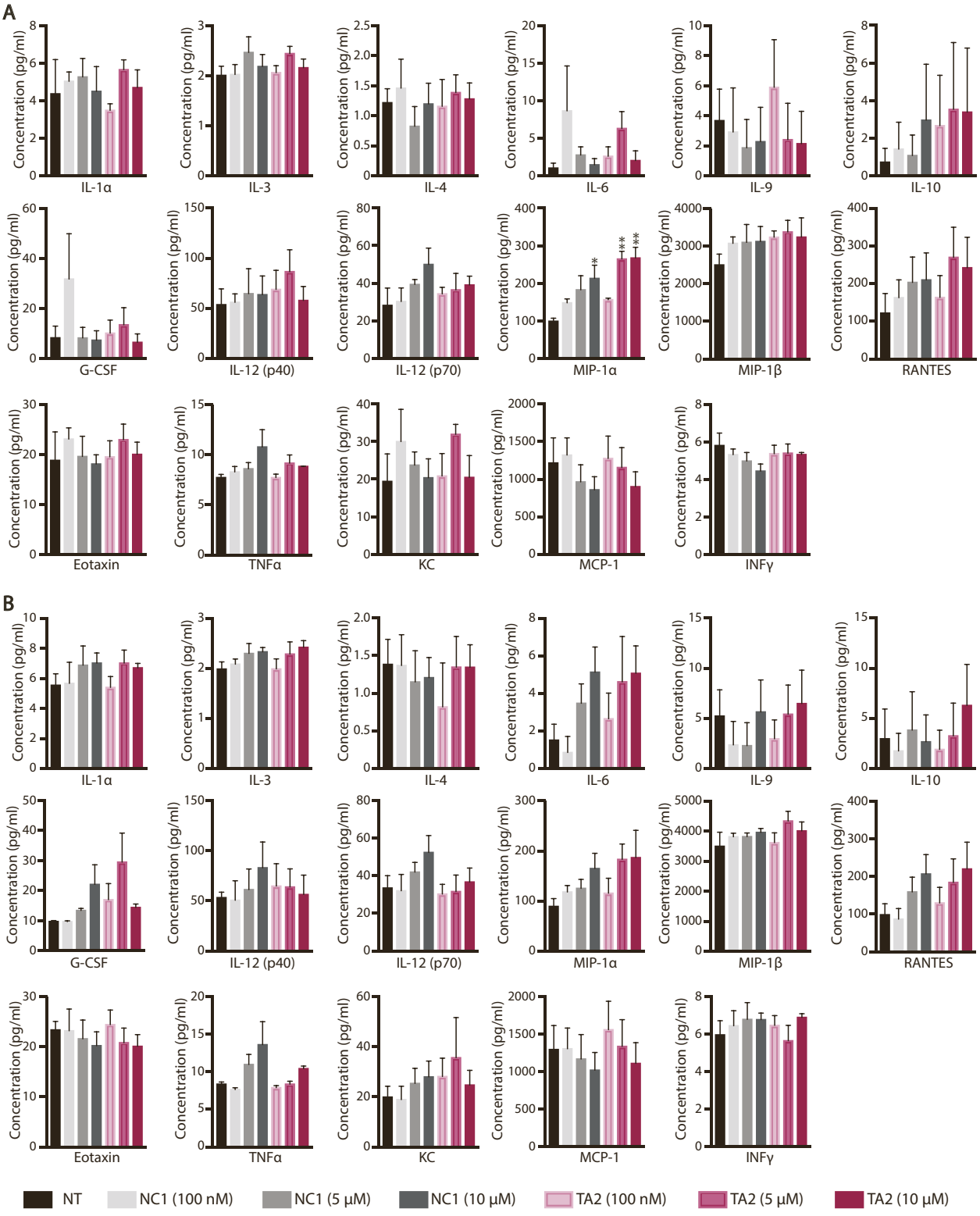
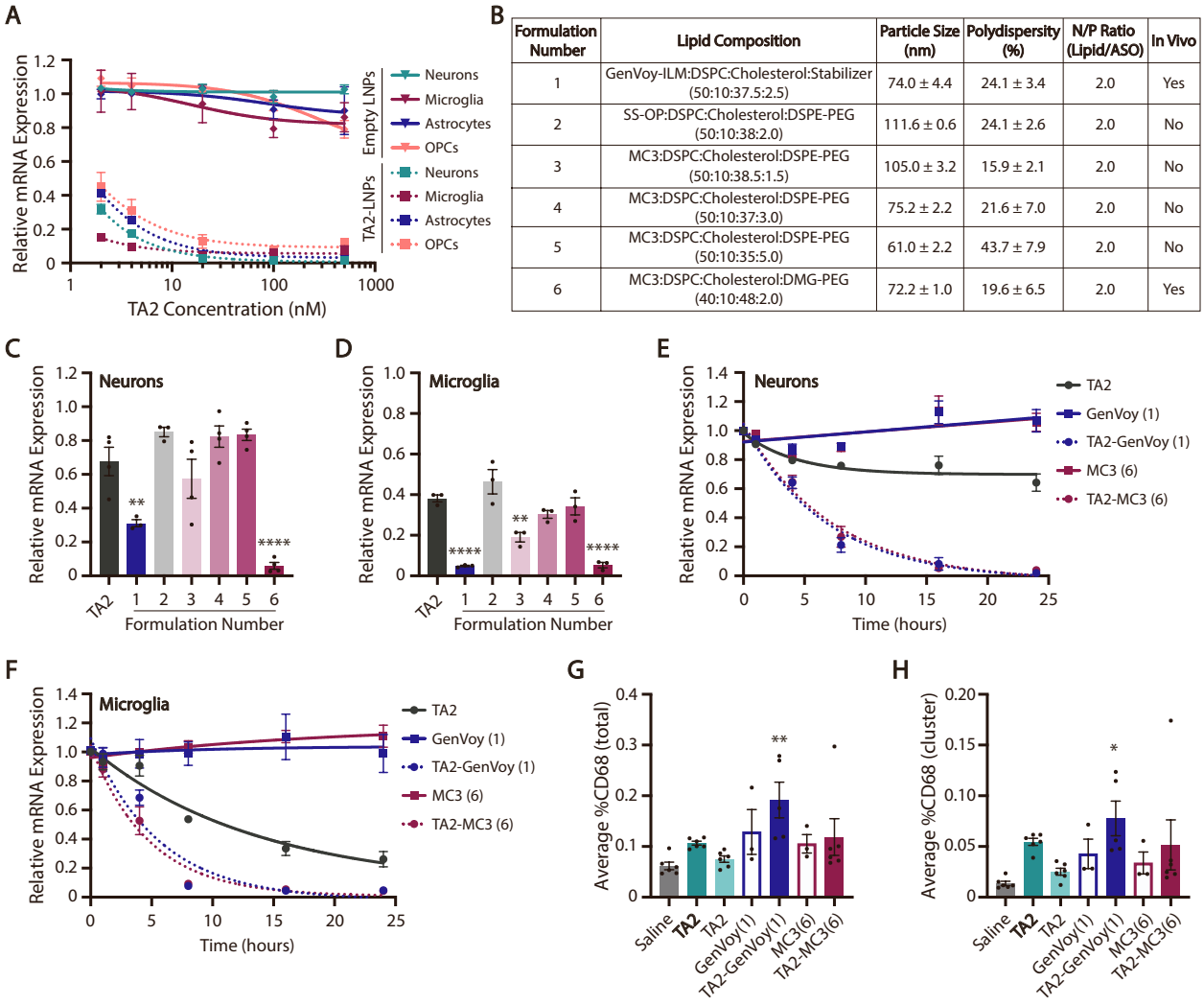


Figure S2. ASO treatment does not induce cytokine release from microglia *in vitro*. (**A-B**) Luminex multiplex analysis of cytokine release from primary microglia. Microglia were treated

with 100 nM, 5 μM, and 10 μM non-targeting ASO NC1 or *TMEM106b*-targeting ASO TA2, and media was collected after 1 day (**A**) or 3 days (**B**). Three internal replicates were averaged and experiments were completed in triplicate, and mean ± SEM are shown. One-way ANOVA with multiple comparisons Dunnett's test was used (not-treated (NT) control), *$p < 0.03$, **$p < 0.002$.



Formulation Number	Lipid Composition	Particle Size (nm)	Polydispersity (%)	N/P Ratio (Lipid/ASO)	In Vivo
1	GenVoy-ILM:DSPC:Cholesterol:Stabilizer (50:10:37.5:2.5)	74.0 ± 4.4	24.1 ± 3.4	2.0	Yes
2	SS-OP:DSPC:Cholesterol:DSPE-PEG (50:10:38:2.0)	111.6 ± 0.6	24.1 ± 2.6	2.0	No
3	MC3:DSPC:Cholesterol:DSPE-PEG (50:10:38.5:1.5)	105.0 ± 3.2	15.9 ± 2.1	2.0	No
4	MC3:DSPC:Cholesterol:DSPE-PEG (50:10:37:3.0)	75.2 ± 2.2	21.6 ± 7.0	2.0	No
5	MC3:DSPC:Cholesterol:DSPE-PEG (50:10:35:5.0)	61.0 ± 2.2	43.7 ± 7.9	2.0	No
6	MC3:DSPC:Cholesterol:DMG-PEG (40:10:48:2.0)	72.2 ± 1.0	19.6 ± 6.5	2.0	Yes

Figure S3. ASO-LNP screening and gliosis characterization. (**A**) Cells were treated with 2, 4, 20, 100, and 500 nM TA2-LNPs (dashed) or unloaded LNP controls (solid) for 3 days and *TMEM106b* mRNA expression was assess using RT-qPCR. (**B**) ASO-LNP formulation characteristics including particle size and polydispersity indices. (**C-D**) *TMEM106b* mRNA expression in cortical neurons (**C**) and microglia (**D**) as determined by RT-qPCR. Cells were treated with 200 nM TA2 (leftmost bar) or TA2-LNP formulations (1-6) for 24 hours. (**E-F**) *TMEM106b* mRNA expression over time in cortical neurons (**E**) and microglia (**F**) treated with 200 nM TA2 or TA2-LNP formulations (1 and 6), as determined by RT-qPCR. (**G-H**) IHC analysis of total (**G**) and clustered (**H**) CD68 staining in the brain 14 days post ICV injection. For all RT-qPCR data, three internal replicates were averaged and the experiments were completed in triplicate, and data were normalized to GAPDH and PBS-treated control cells. For all panels, mean ± SEM are shown. One-way ANOVA with multiple comparisons Dunnett's test (TA2 or saline controls) was used, *p < 0.03, **p < 0.002, ****p < 0.0001.

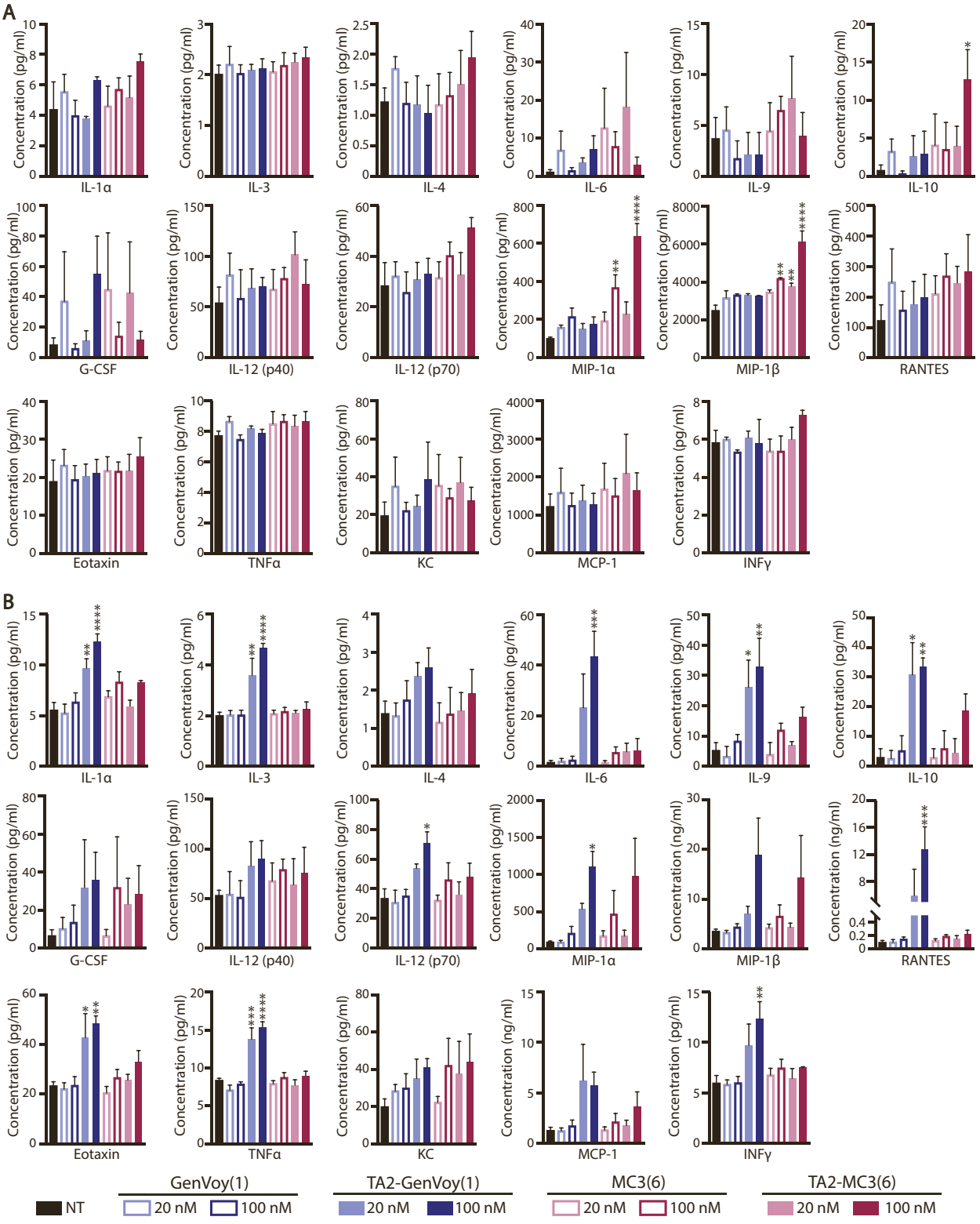


Figure S4. ASO-LNP treatment differentially regulates cytokine release from microglia *in vitro*. (**A-B**) Luminex multiplex analysis of cytokine release from primary microglia. Microglia were treated with 20 nM and 100 nM unloaded (GenVoy and MC3) or TA2-loaded (TA2-

GenVoy(1) and TA2-MC3(6)) LNPs, and media was collected after 1 day (**A**) or 3 days (**B**). Three internal replicates were averaged and experiments were completed in triplicate, and mean ± SEM are shown. One-way ANOVA with multiple comparisons Dunnett's test (NT control) was used, *$p < 0.03$, **$p < 0.002$, ***$p < 0.0002$, ****$p < 0.0001$.

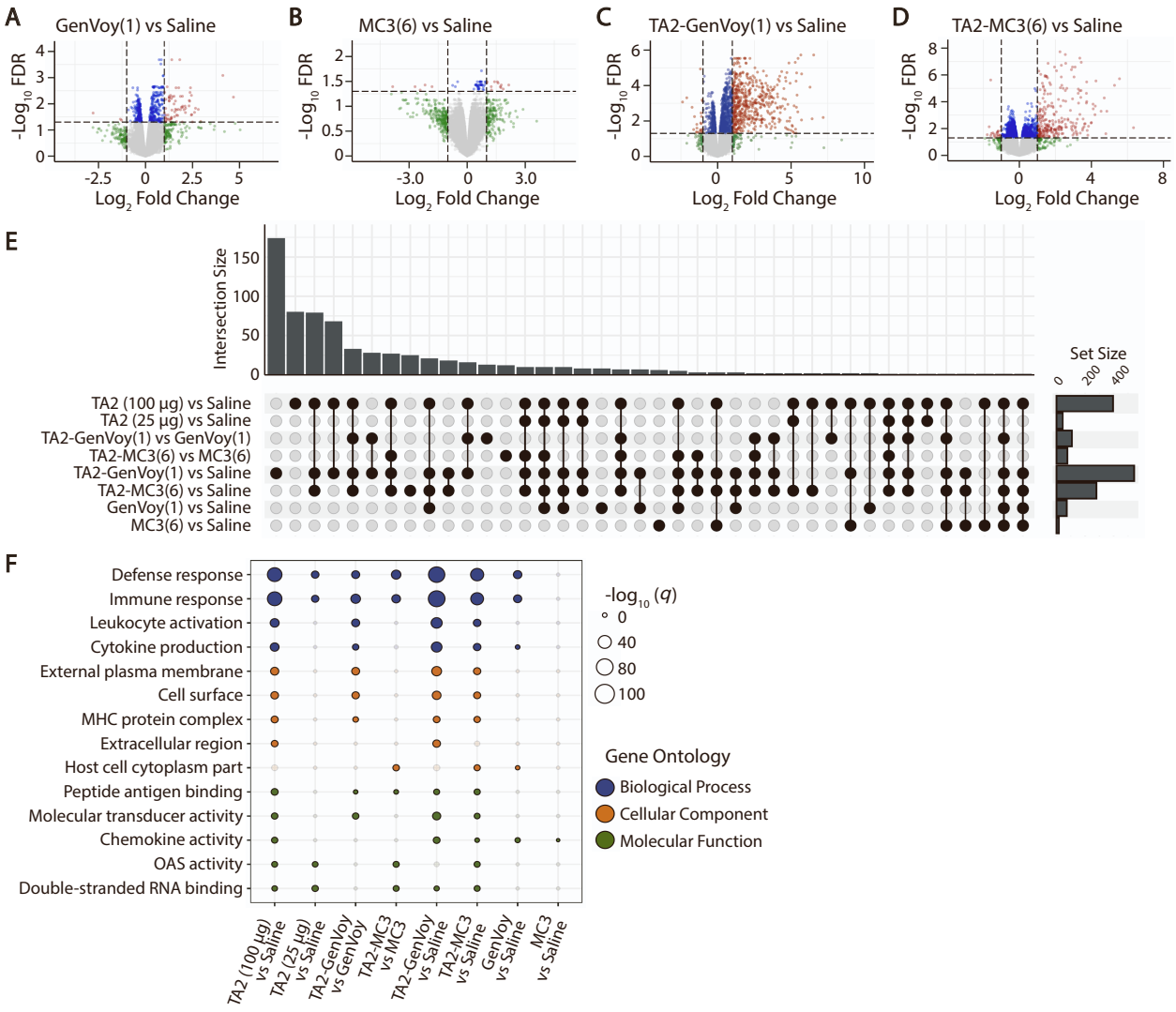


Figure S5. TA2 and TA2-LNP transcriptomic analysis. (**A-D**) Volcano plots of differential gene expression profiles from pairwise comparisons of GenVoy(1) vs Saline (**A**), MC3(6) vs Saline (**B**), TA2-GenVoy(1) vs Saline (**C**), and TA2-MC3(6) vs Saline (**D**). Red dots represent FDR < 0.05 and absolute log fold-change (logFC) > 1, blue dots represent FDR < 0.05 and absolute logFC < 1, green dots represent FDR > 0.05 and absolute logFC > 1, and grey dots represent FDR > 0.05 and absolute logFC < 1. (**E**) UpSet plot of overlaps between significant (FDR < 0.05 and absolute logFC > 1) differentially expressed genes (DEGs). (**F**) Gene ontology (GO) terms from analyses of significant (FDR < 0.05 and absolute logFC > 1) DEGs from all pairwise comparisons. Significant (FWER < 0.1) terms for a comparison are indicated by dots that are not transparent.

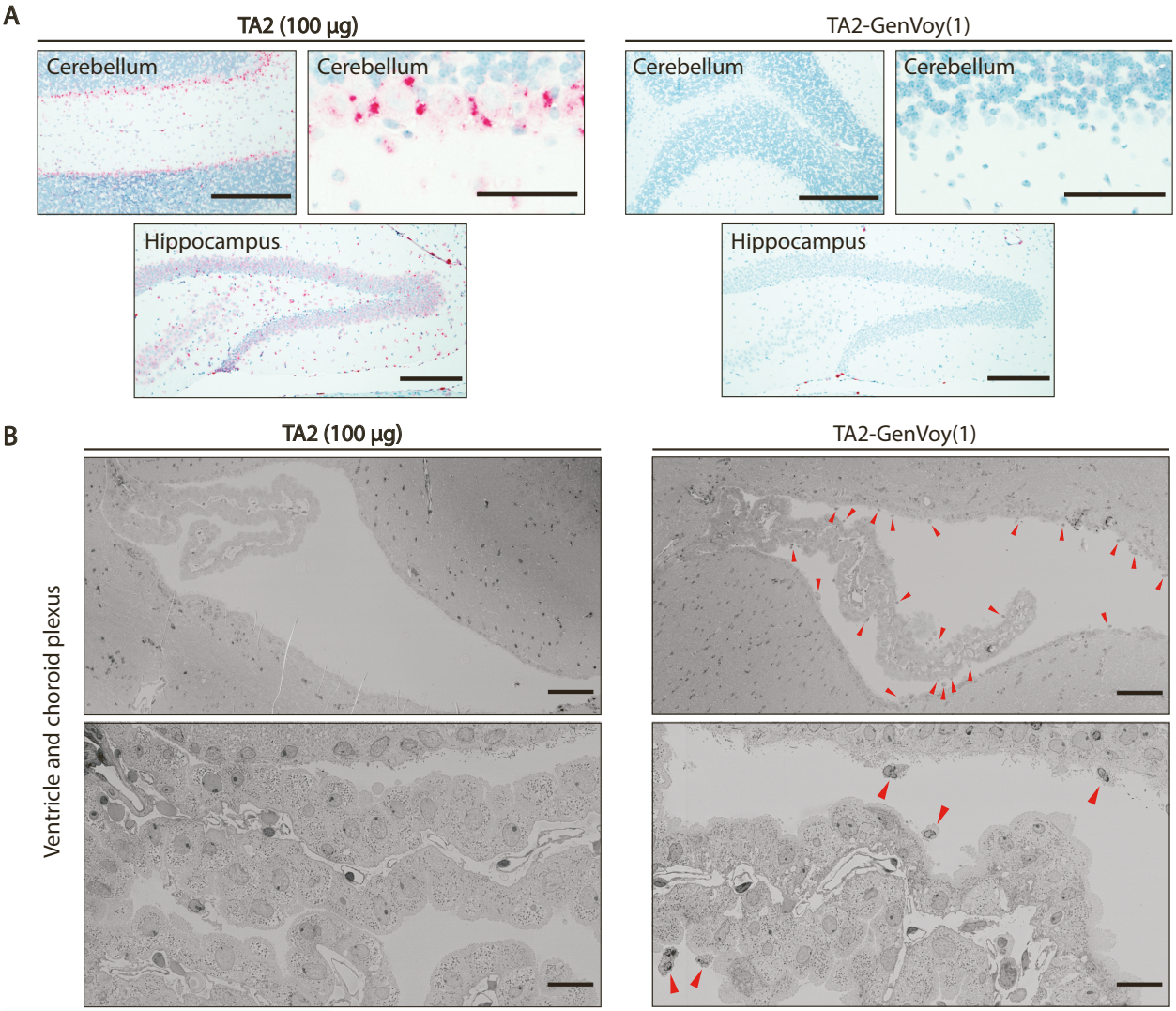


Figure S6. Differential ASO distribution in the brain following TA2-LNP delivery. (**A**) Representative anti-ASO IHC brain sections of free TA2 (100 µg) or TA2-GenVoy(1) (25 µg) treated animals 4 days following ICV injection, showing TA2 localization in the cerebellum and the hippocampus (red staining). Scale bars, 200 µm (left, cerebellum and hippocampus) and 50 µm (right, cerebellum). (**B**) Representative cryo-TEM images of macrophages, red arrows, lining the ventricle 4 days following TA2-GenVoy(1) administration. Scale bars, 100 µm (top) and 20 µm (bottom).