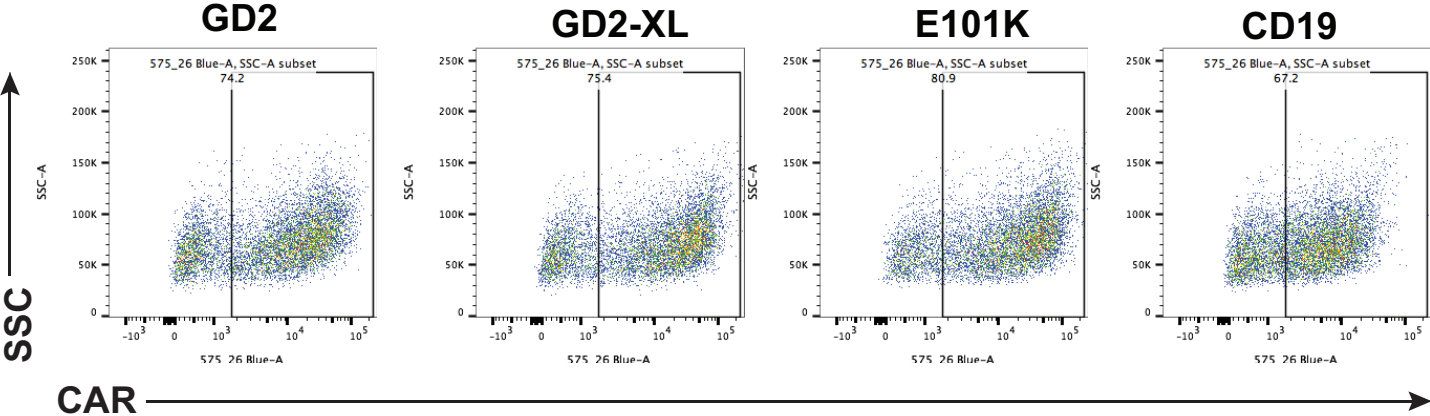
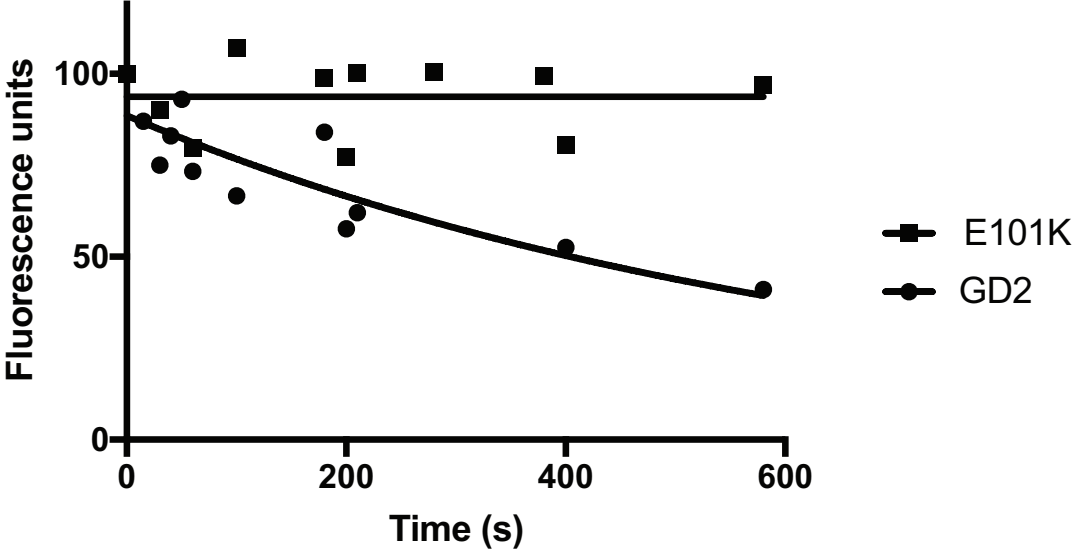


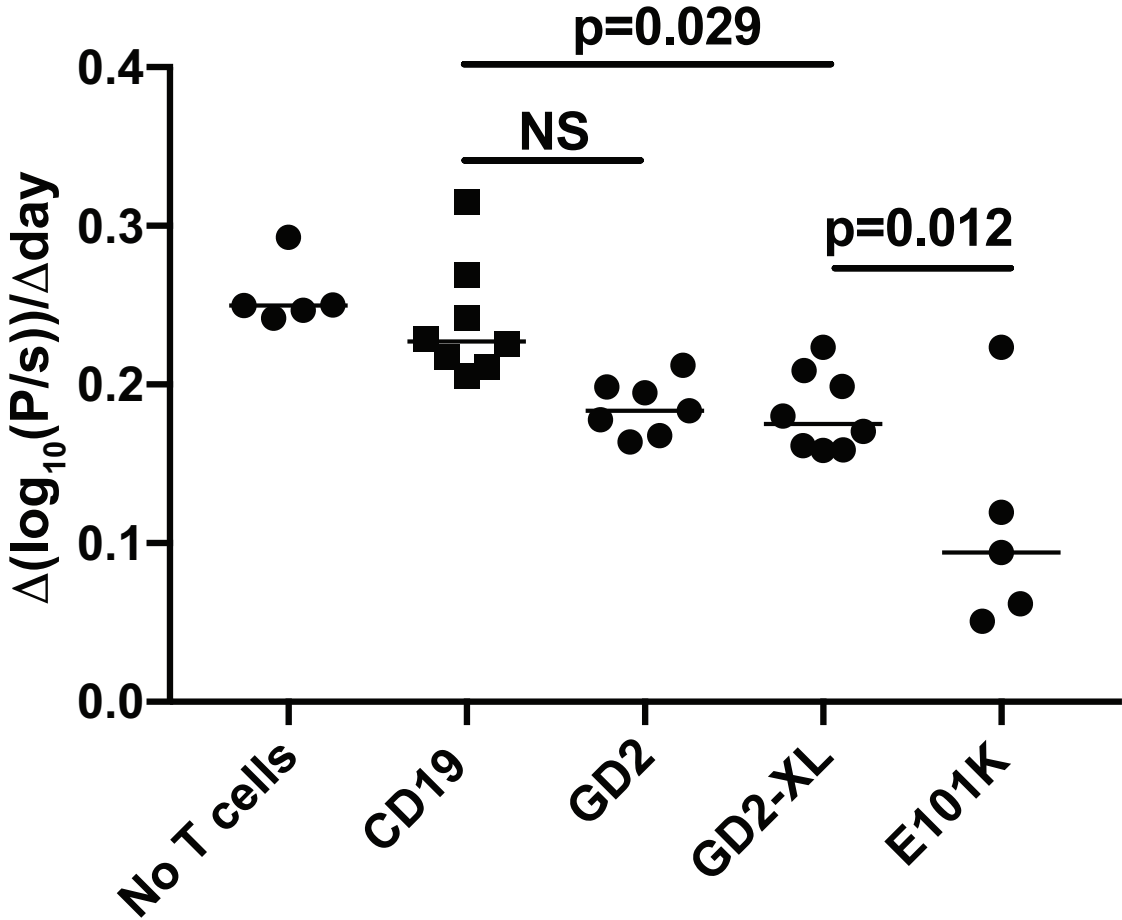
Supplementary Figure S1



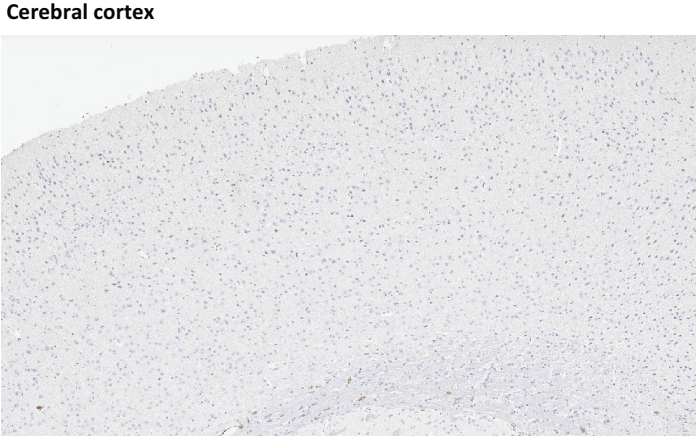
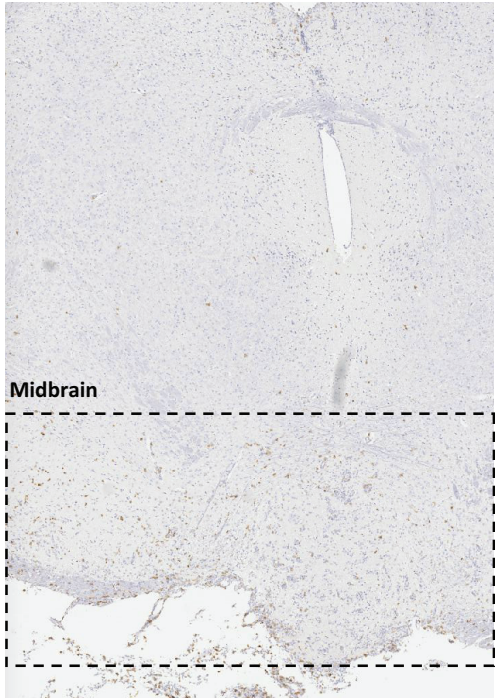
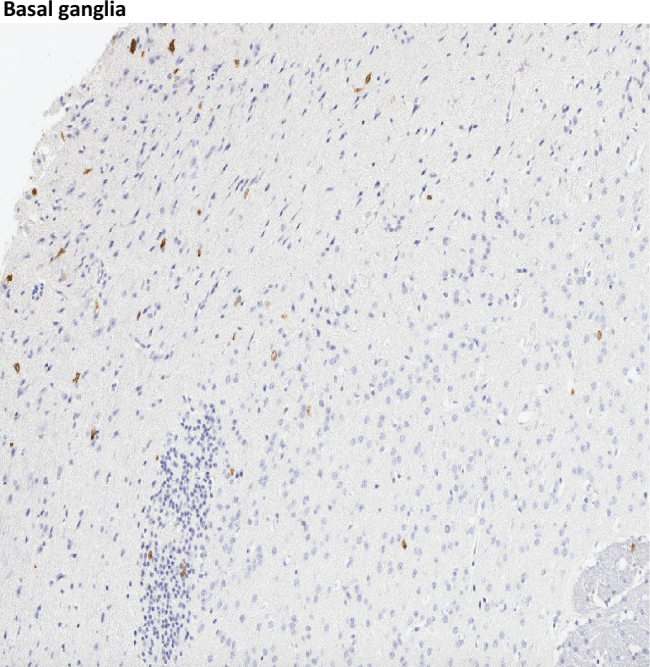
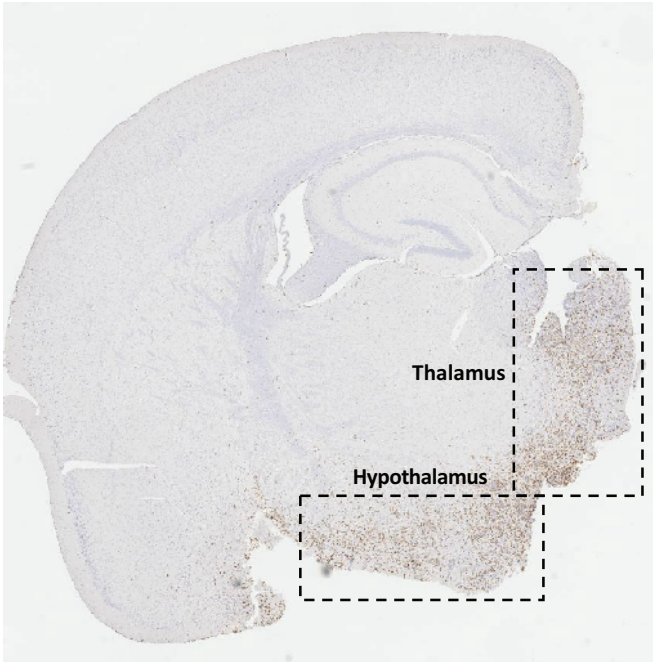
Supplementary Figure S2



Supplementary Figure S3

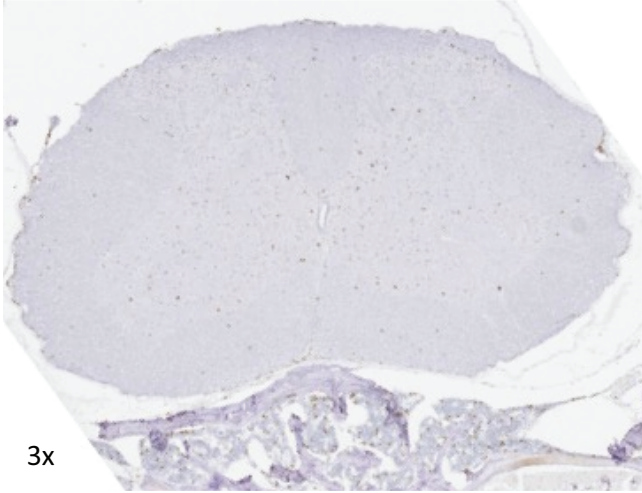


Supplementary Figure S4

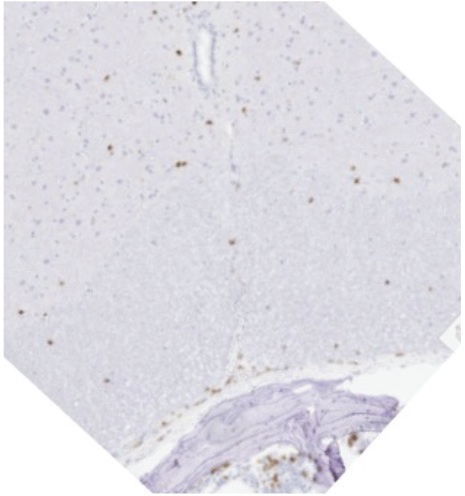


Supplementary Figure S5

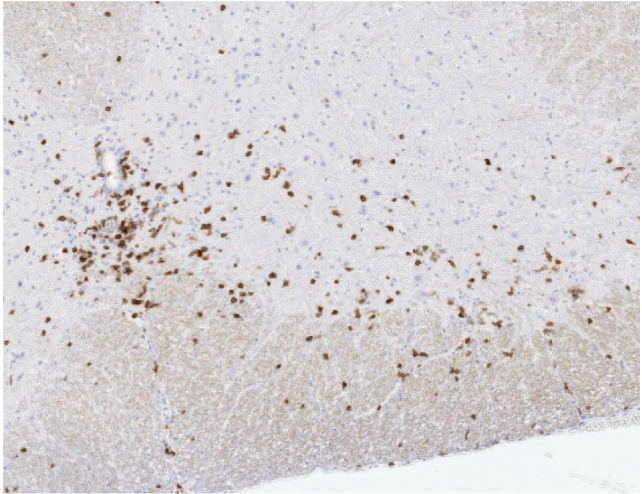
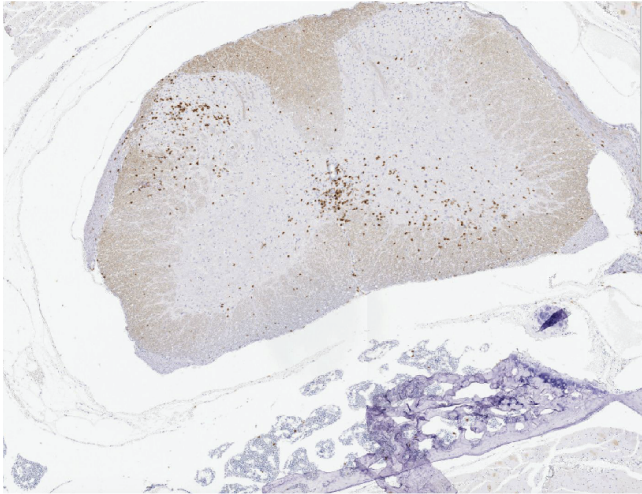
E101K



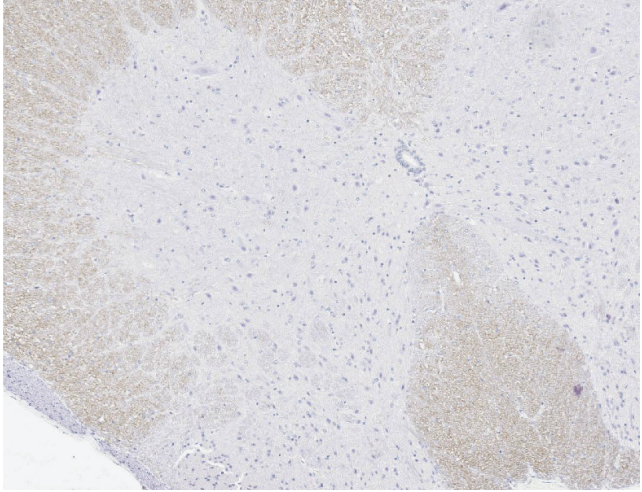
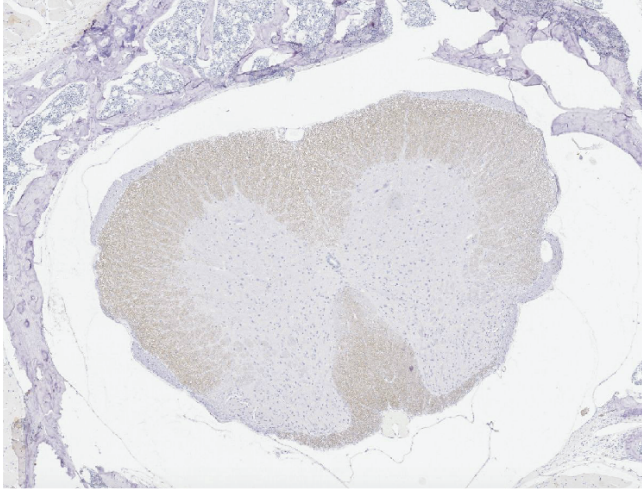
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m3F8

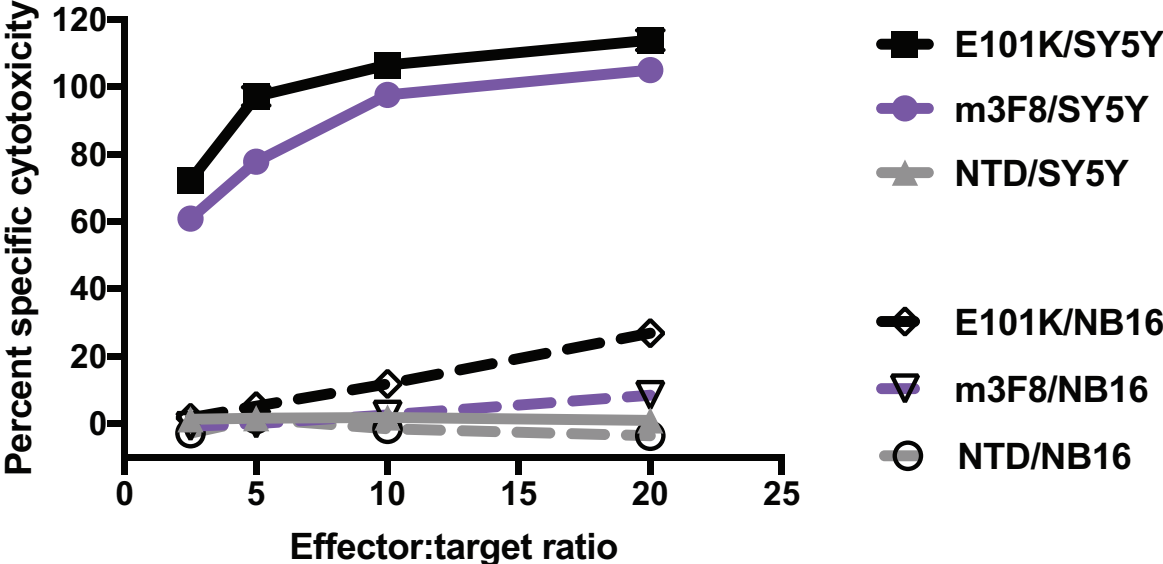


NTD

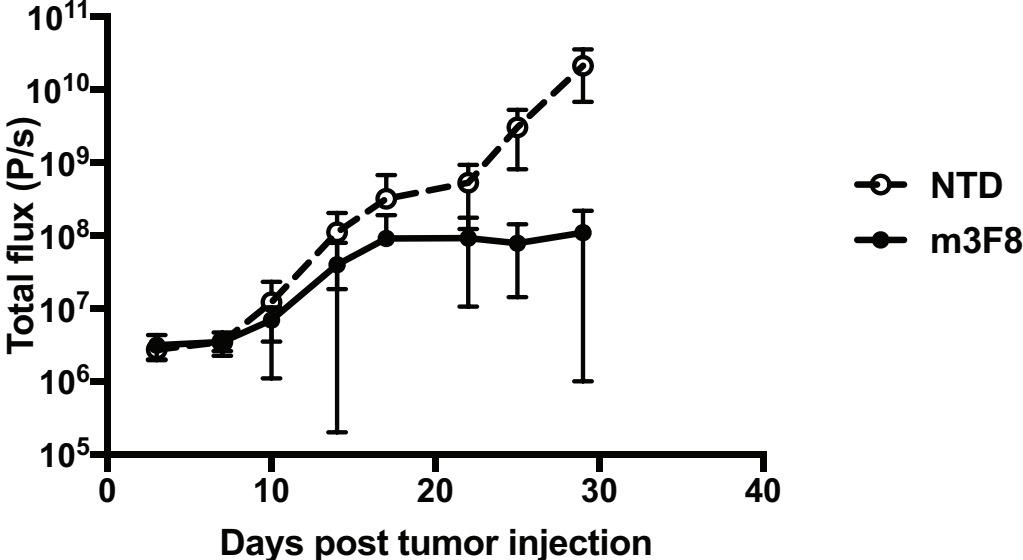


Supplementary Figure S6

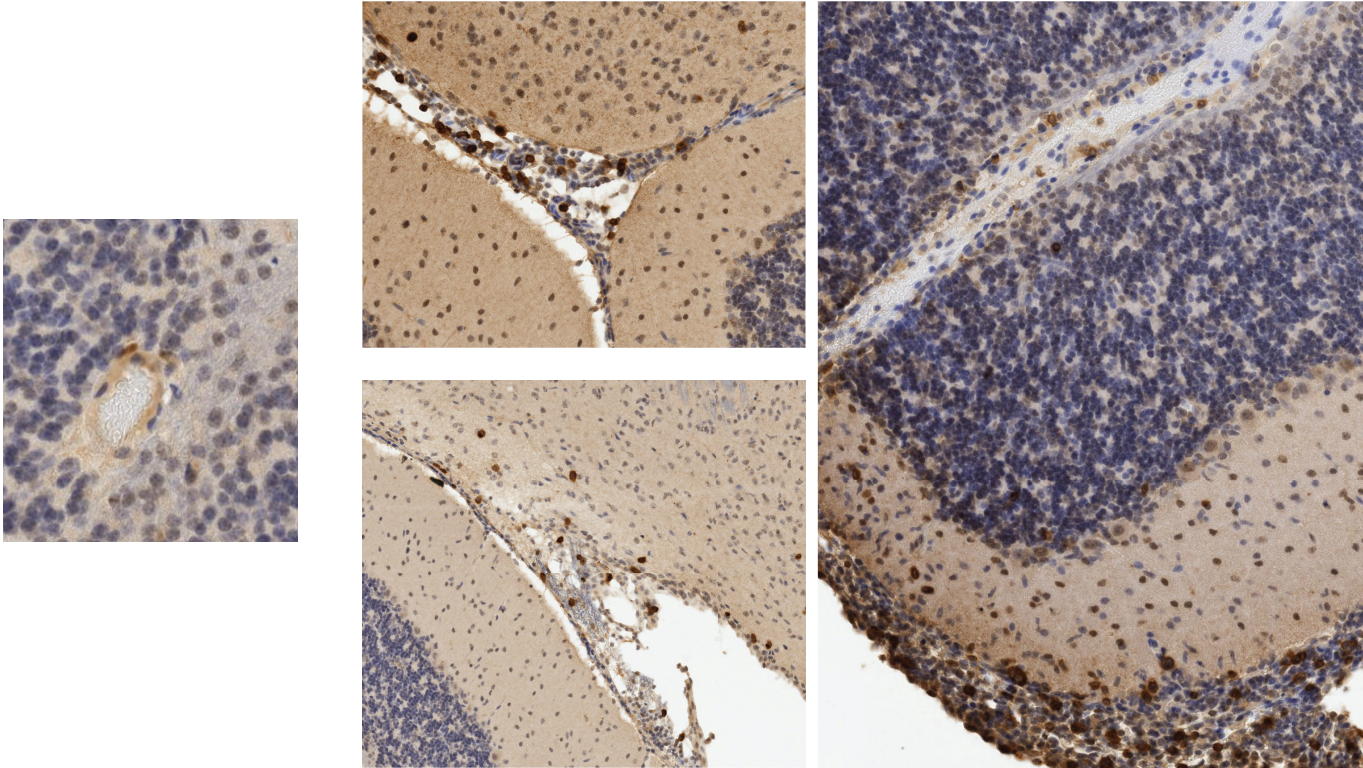
A



B



Supplementary Figure S7



1 **Supplemental Figure Legends**

2 **Supplemental Figure 1:** Comparison of surface expression of CAR constructs used in these
3 studies. T cells were transduced with indicated lentivirus at an MOI of 5. At day 8 of
4 expansion, T cells were stained with anti-mouse antibody that recognizes the mouse scFv.

5

6 **Supplemental Figure 2:** A, In vitro cytotoxic activity of m3F8-based anti-GD2 CAR, E101K
7 CAR T cells, and NTD T cells was measured in chromium release assay as described for
8 Figure 1, using the indicated target cells (either the GD2⁺ human neuroblastoma line SY5Y
9 or GD2^{low} human neuroblastoma line NB16.) Data represent one experiment using
10 triplicate wells and are shown as mean +/- standard error of the mean (SEM). B,
11 Neuroblastoma cell line SY5Y-CBG was injected via tail vein, followed by the injection of
12 indicated T cells as described for Figure 4. Tumor burden was monitored by
13 bioluminescence at the indicated time points. Groups contained 4-5 mice, and data
14 represent the mean total flux +/- standard deviation.

15

16 **Supplemental Figure 3:** Comparison of dissociation of GD2 and E101K IgG. SY5Y cells
17 were incubated with mouse-IgG containing the variable domains from either the GD2 or
18 E101K scFvs. Antibody was then allowed to dissociate in room temperature buffer for the
19 indicated number of seconds, and bound antibody was then detected by flow cytometry
20 using AF-647 labeled secondary antibody.

21

22

23 **Supplemental Figure 4:** Slides of brain tissue from 129X1/SvJ mice that received
24 syngeneic CAR T cells expressing the E101K-CD28-CD3 ζ CAR were stained with anti-mouse
25 CD3 by IHC. Left field, cerebellar parenchymal blood vessel, 20x. Right three panels,
26 cerebellar regions with associated meninges.

27

28 **Supplemental Figure 5:** CD8 IHC on indicated brain structures from mice receiving E101K
29 CAR T cells

30

31 **Supplemental Figure 6:** CD8 IHC on spinal cord from mice receiving indicated CAR T cells.

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