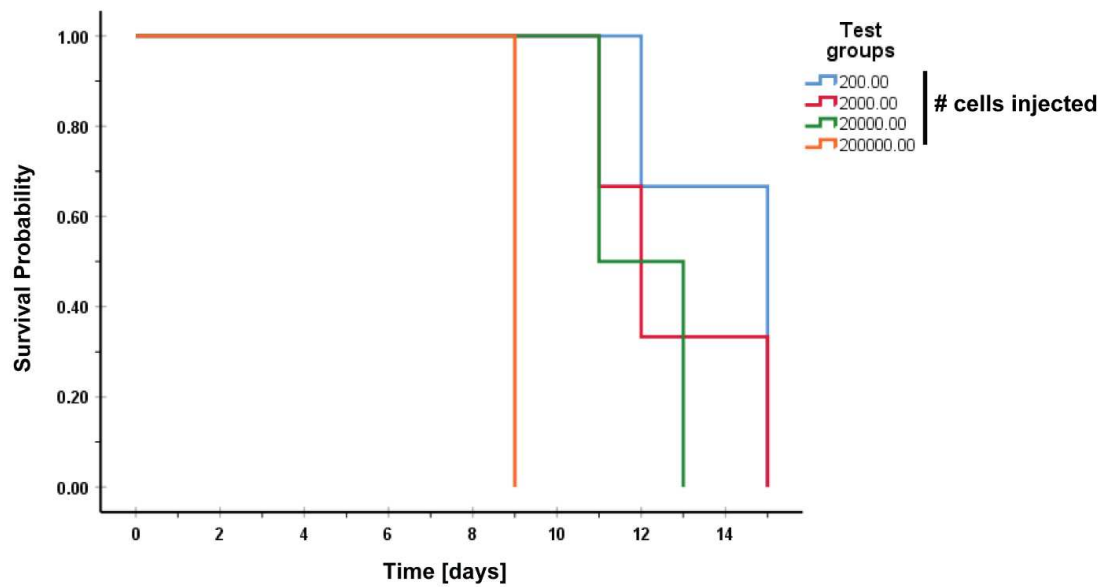
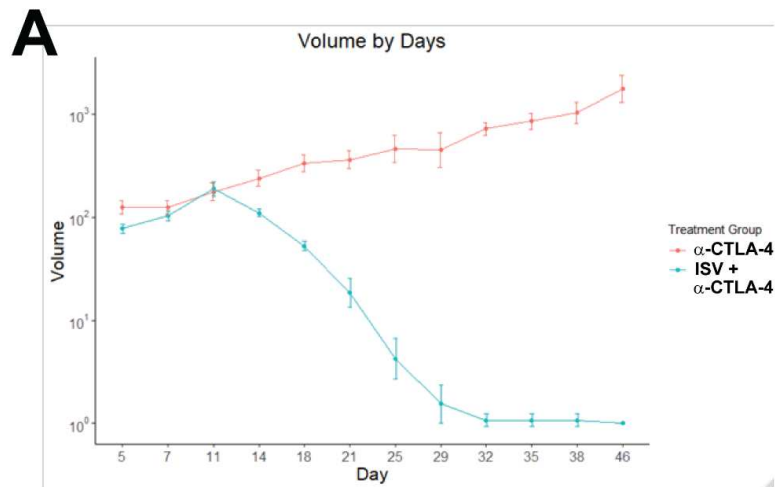


B16 melanoma - intracranial



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2 **Supplementary Figure 1:** Survival time for mice intracranially injected with B16 (GD2⁻)
3 melanoma cells ranging from 200 – 200,000 cells.
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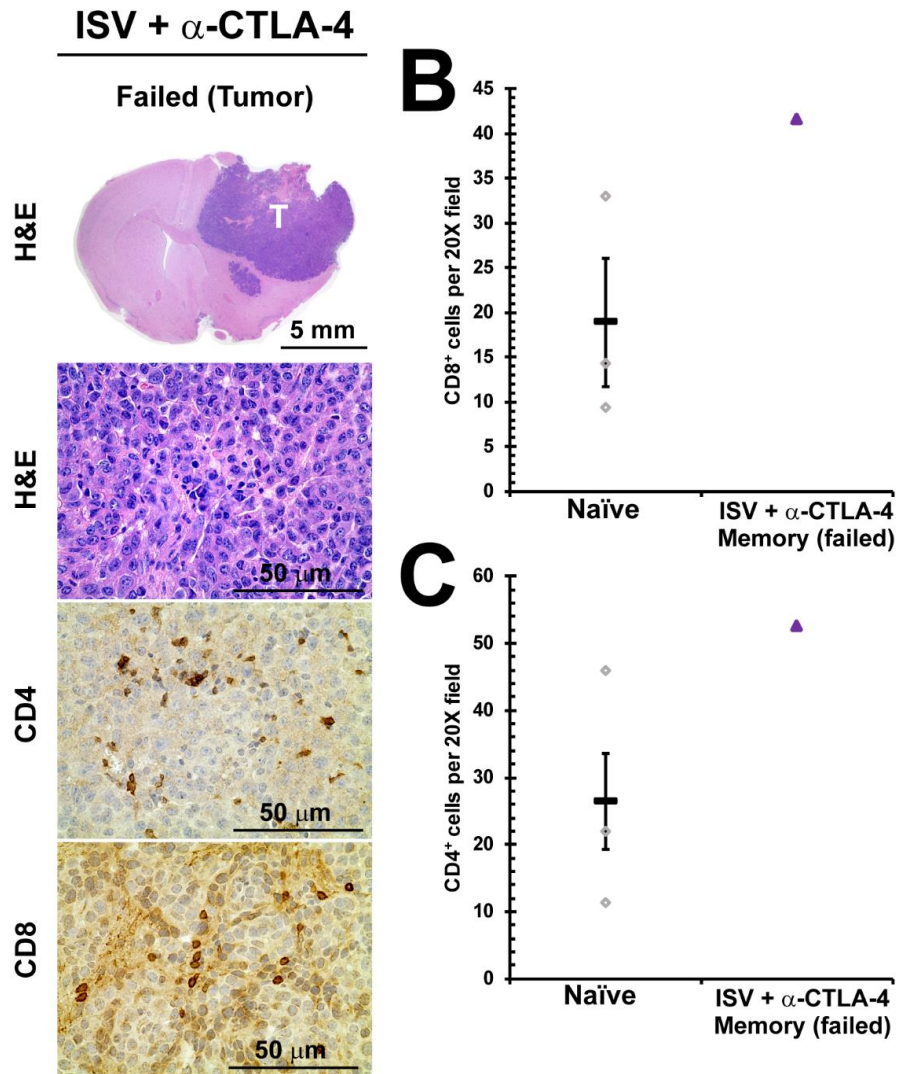
B

Day	Treatment Group	n	mean_vol	sd_vol	p_value
5	A	8	123.99	11.92	<0.001
5	B	22	76.08	8.29	
7	A	8	123.75	11.49	0.07
7	B	22	102.71	12.53	
11	A	8	174.38	21.82	0.61
11	B	22	186.77	30.45	
14	A	8	237.90	25.58	<0.001
14	B	22	109.18	10.54	
18	A	8	332.56	39.67	<0.001
18	B	22	51.70	5.60	
21	A	8	360.41	45.10	<0.001
21	B	22	17.36	5.80	
25	A	8	456.20	85.36	<0.001
25	B	22	3.24	1.52	
29	A	8	445.62	107.36	<0.001
29	B	22	0.55	0.24	
32	A	8	715.28	62.96	<0.001
32	B	22	0.08	0.01	
35	A	8	848.70	92.72	<0.001
35	B	22	0.08	0.01	
38	A	8	1022.44	152.44	<0.001
38	B	22	0.08	0.01	
46	A	8	1752.89	334.04	<0.001
46	B	22	0.00	0.00	

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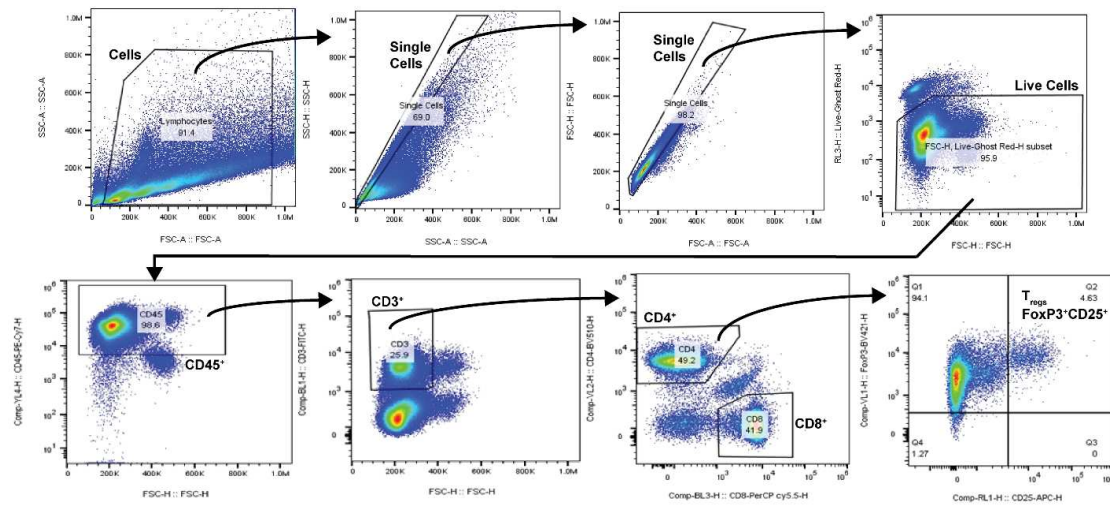
Supplementary Figure 2: (A) Log₁₀-transformed tumor growth curve of Fig. 1B. **(B)** Log₁₀ transformed data was used to determine significantly different tumor growth rates at multiple time points. Treatment groups, A: α-CTLA-4 only, B: ISV + α-CTLA-4.

A B78 melanoma - intracranial (memory)



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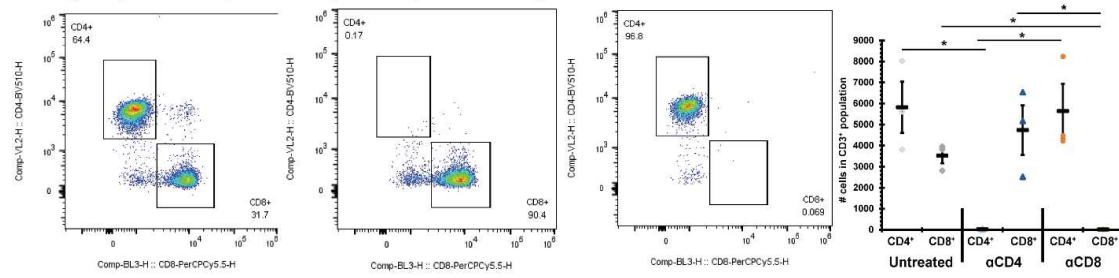
Supplementary Figure 3: Immune cell analysis of 1 (of 12) mouse that failed to establish immune memory in CNS. (A) Immunohistochemistry representative images of CD4⁺ and CD8⁺ within developed brain tumor (T: tumor, brown: positive immunolabeling, H&E: hematoxylin and eosin). Quantification of CD8⁺ (B) and CD4⁺ (C) T-cells demonstrated increases in brain tumor of 1 (of 12) mouse that failed to establish immune memory compared to naïve mice (euthanized due to brain tumor burden, not time-matched).



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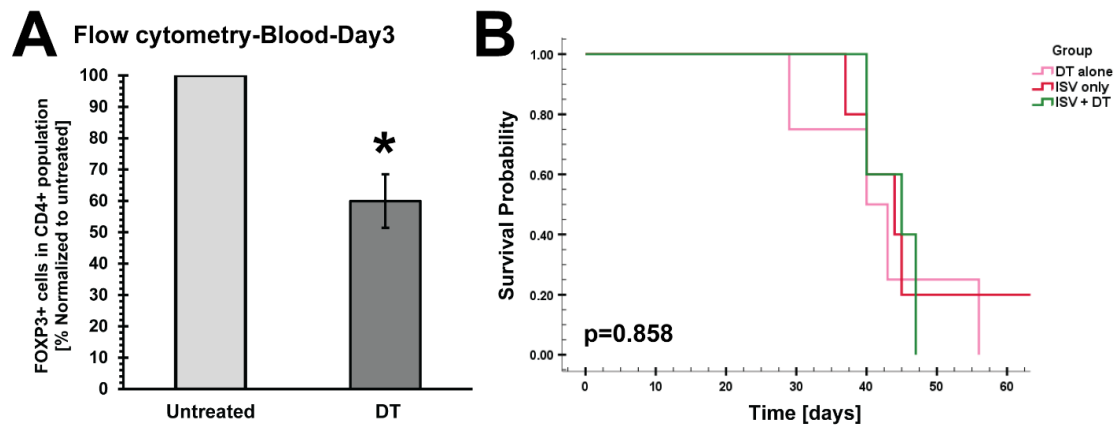
Supplementary Figure 4: Flow cytometry gating strategy.

Blood @ 7 days after 1st injection of respective depletion antibody



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Supplementary Figure 5: Depletion efficiency for α -CD4 and α -CD8 antibodies. Injection (i.p.) of α -CD4 and α -CD8 antibodies resulted in significant $>97\%$ depletion of CD4⁺ and CD8⁺ cells, respectively, in mouse blood 7 days after first administration ($*p < 0.05$, mean \pm S.E., $n = 3$ mice in a single animal experiment, ANOVA with post-hoc Bonferroni).



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30 **Supplementary Figure 6: FOXP3⁺ cell depletion in DEREK mouse model. (A)** Flow cytometric31 **analysis at day 3 post diphtheria toxin (DT) injection (* $p < 0.05$; mean \pm S.E., $n = 3$ mice total in two**32 **separate animal experiments, Student's t -test). (B) Survival curve for mice harboring intracranial**33 **and extracranial B78 tumors, and receiving ISV and/or DT treatment ($p = 0.858$, Kaplan-Meier,** **$n \geq 4$ in a single animal experiment).**