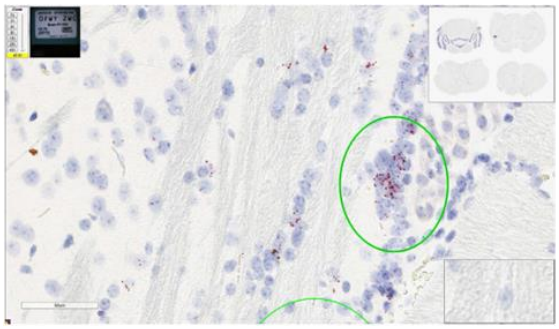
Fig. S6

A

Sample	Section	DLL3 RNA score	% Positive	Comments
Animal 1	1	1	<10%	
	2	1	<10%	Subset of cells score 2
	3	1	<10%	Subset of cells score 2
	4	2	<10%	Localized in ventricular system
Animal 2	1	1	<10%	
	2	1	<10%	
	3	1	<10%	Subset of cells score 2
	4	2	<10%	Localized in ventricular system
Animal 3	1	1	<10%	
	2	1	<10%	
	3	2	<10%	
	4	2	<10%	

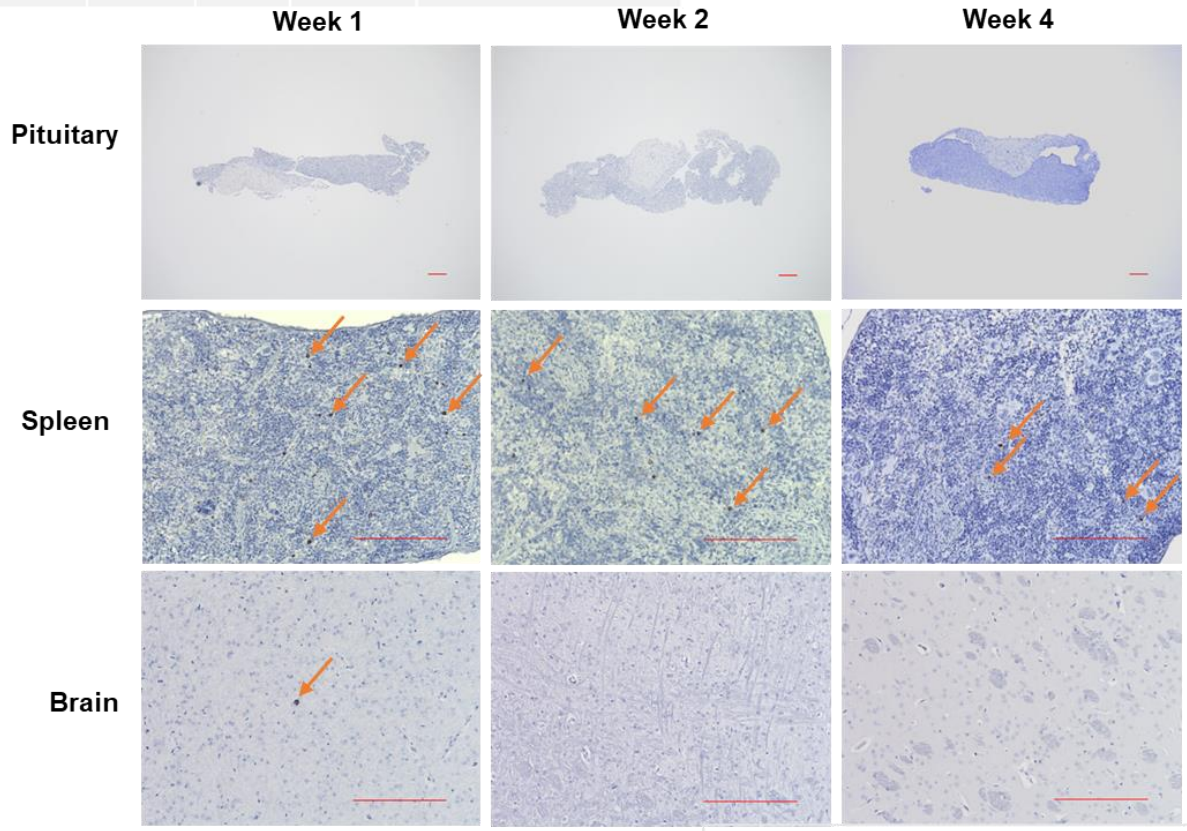
Animal#1 Section 2



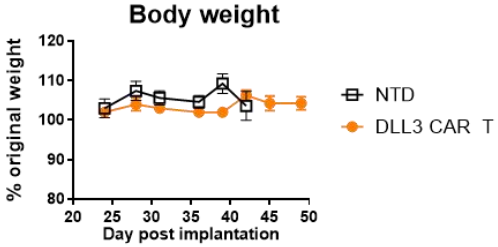
Green circle: subset of cells score 2

RNA score 0 = No staining or <1 RNA molecule / 10 cells, 1 = 1-3 RNA molecules/cell , 2 = 4-9 RNA molecules/cell, no or very few dot clusters, 3 = 10-15 RNA molecules/cell and/or <10% dots are in clusters, 4 = >15 RNA molecules/cell and/or >10% dots are in clusters. Percentage of cells positive is scored visually based on number of cells with >1 dot/cell and binned into categories (i.e., 0%, 1-25%, 26-50%, 51-75%, 76-99%, 100%)

B



C



D

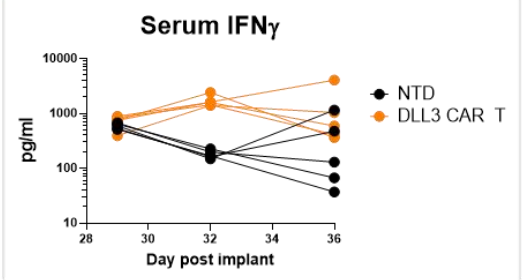


Fig. S6 continued

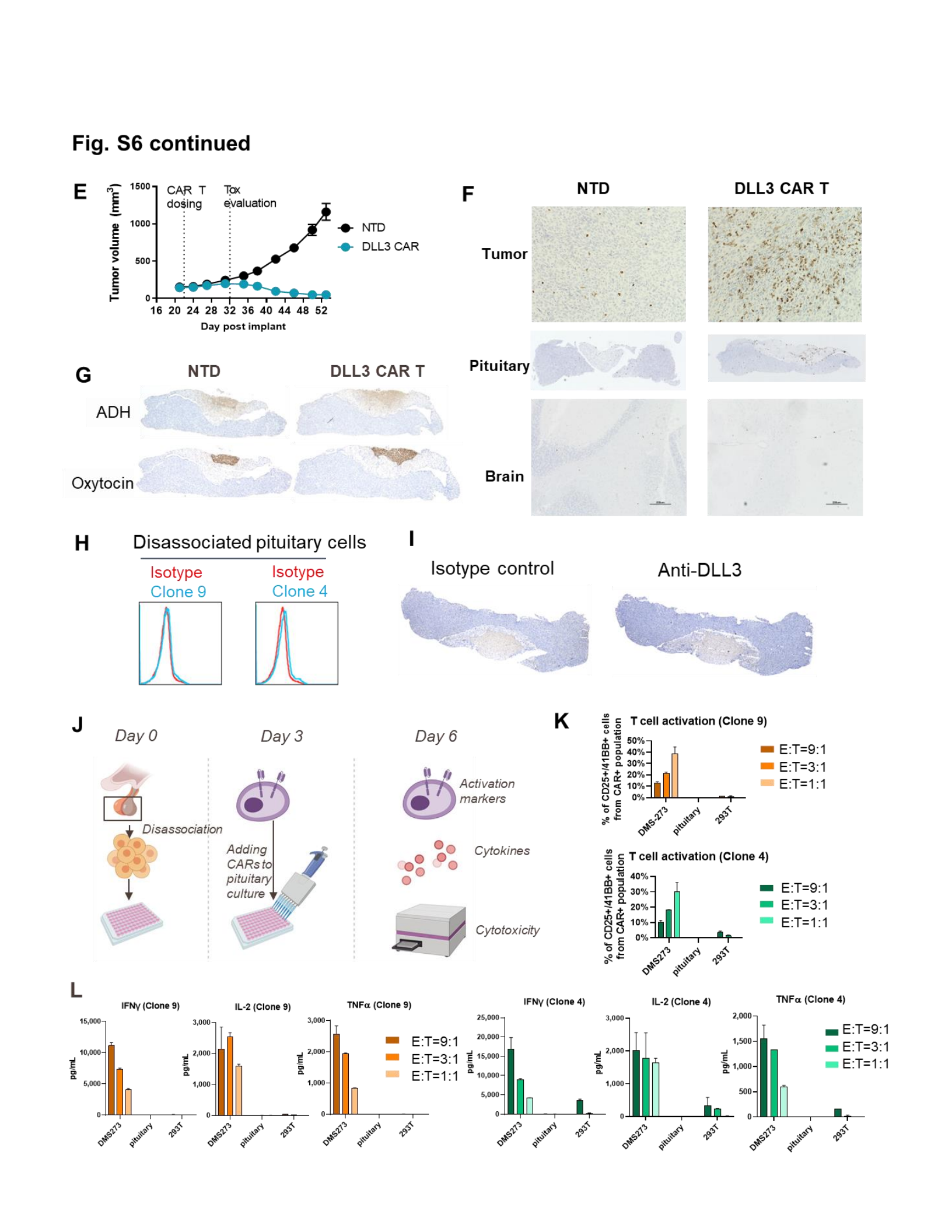


Fig. S6. Infusion of DLL3 CAR T cells did not induce toxicity in non-tumor-bearing model or subcutaneous tumor model. (**A**) Mouse brain tissues express DLL3 RNA. Brain tissues from three tumor-free and treatment-free NSG mice were harvested and used for single-color chromogenic RNAscope assay with mouse DLL3 specific probe. Discrete red dots corresponded to individual RNA targets. Visual scoring is performed by a qualified scientist to assign a single score to a sample based on the predominant staining pattern throughout the entire sample. (**B**) T cells were not detected in brain or pituitary samples from non-tumor bearing animals injected with a high dose ($8X10^6$) of NTD or DLL3 CAR T cells. Tissues were collected 1, 2, or 4 weeks post T cell injection. Scale bars represent 200 μm. Orange arrows point to T cells in the tissues. (**C**) DLL3 CAR T cells did not induce obvious changes of body weight throughout the study. (**D**) Serum was collected from animals treated with NTD or DLL3 CAR T cells on day 29, 32 and 36 post T cell infusion and analyzed using meso-scale discovery (MSD) to measure cytokines. Serum collected on day 32 from DLL3 CAR T treated animals has the highest levels of IFNγ. (**E**) NTD or DLL3 CAR T cells were injected into animals bearing LN229-mDLL3 subcutaneous tumors. A subset of animals were euthanized on day 32 (time of peak of CAR T activity) post CAR T infusion to analyze T cell infiltration into tumor, pituitary and brain. Error bars represent SEM, n=5. (**F**) IHC study using anti-human CD45 antibody revealed dense infiltration into subcutaneous tumor and sparse infiltration into pituitary tissues. (**G**) Despite low levels of T cell infiltration into pituitary tissues, pituitary hormones (ADH and oxytocin) can still be detected using IHC, suggesting hormone-secreting function of pituitary was not affected. (**H**) Surface DLL3 protein was not detected on disassociated mouse pituitary cells using flow cytometry. Disassociated mouse pituitary cells were stained with 2ug/ml of clone 4, clone 9 or isotype control antibody followed by anti-human IgG secondary antibody conjugated with PE. (**I**) DLL3 protein was not detected in mouse pituitary samples using IHC. (**J**) Design of in vitro cytotoxicity study (**K**) Surface staining for CD25 and 41BB of DLL3 CAR T cells co-cultured with targets for 3 days, demonstrating that mouse pituitary cells do not activate DLL3 CAR Ts. Error bars represent standard deviation, n=3. (**L**) DLL3 CAR Ts do not secret cytokines when co-cultured with mouse pituitary cells. Error bars represent standard deviation, n=3.