Supplemental Data

Tumor-conditional anti-CTLA-4 uncouples anti-tumor efficacy from immunotherapy-related toxicity

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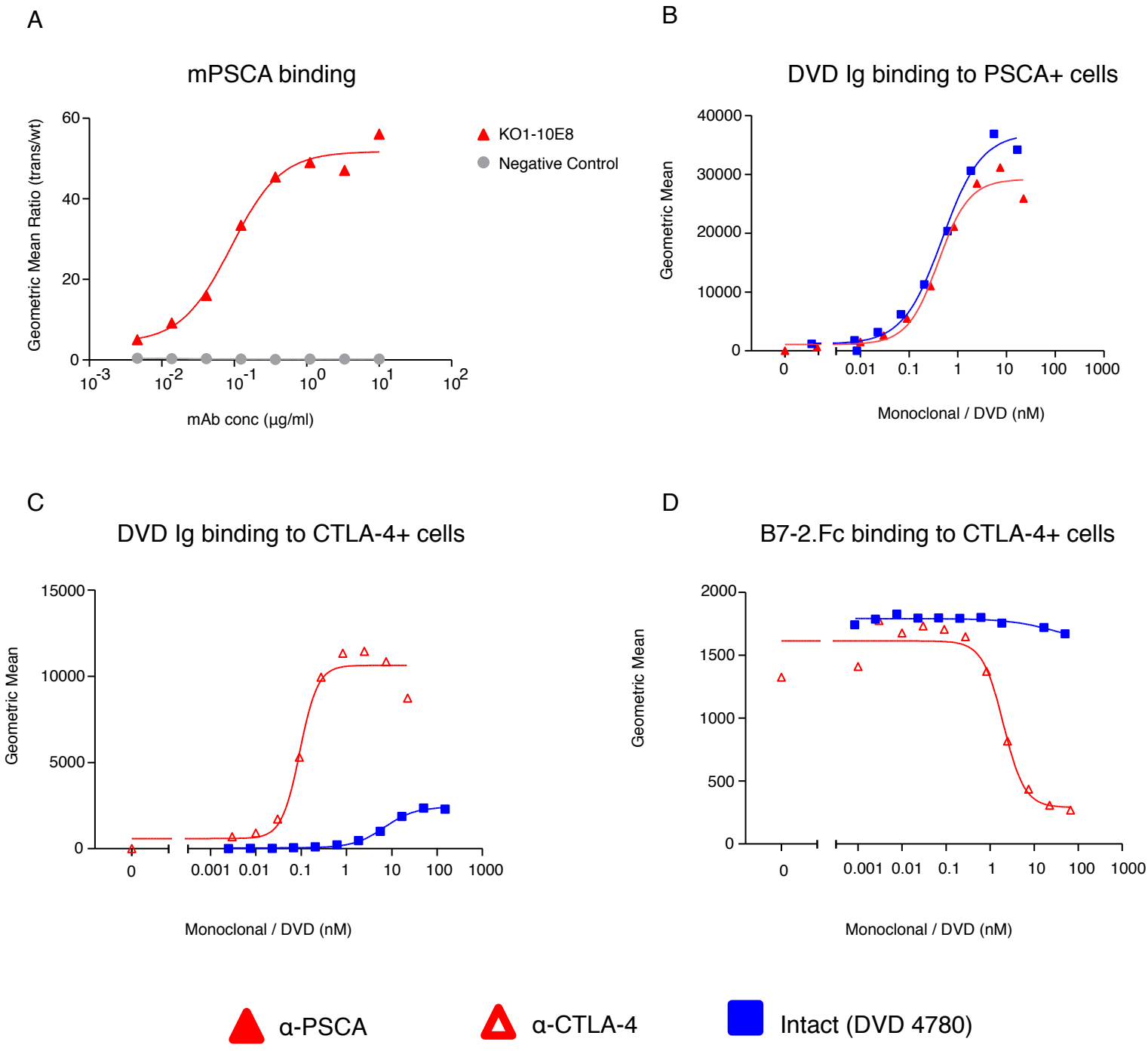
Table 1. Key reagents

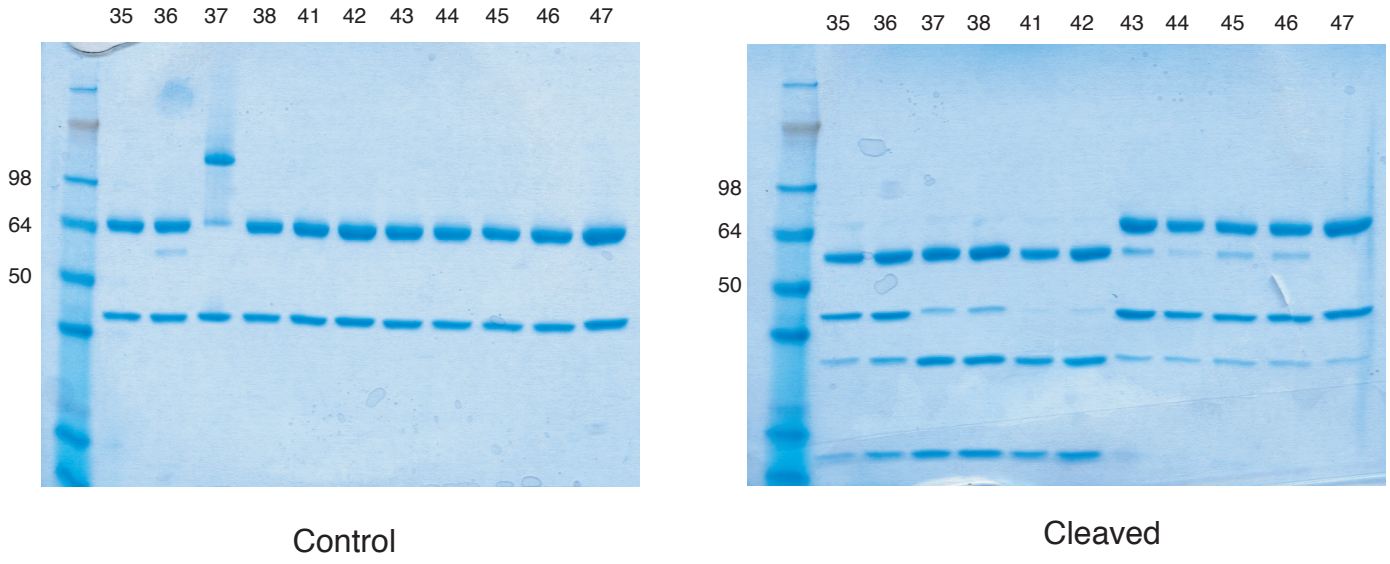
In vivo antibodies		
Reagents	**Source**	**Note**
Anti-CTLA-4	Abbvie	24H2 Clone/ Rat anti-mouse CTLA-4 with mIgG2a/k constant regions/ In vivo injection
Anti-CTLA-4 DVD	Abbvie	24H2 Clone/ Rat anti-mouse CTLA-4 with mIgG2a/k constant regions/ In vivo injection/ With PSCA targeting outer domain
Anti-PSCA	Abbvie	10E8 clone/ Rat anti-mouse PSCA with mIgG2a/k constant regions
mIgG2a/k	Abbvie	Isotype control
^{89}Zr-anti-CTLA-4	Abbvie/UCSF	24H2 clone with radio isotope labeling/ Image studies
^{89}Zr-anti-CTLA-4 DVD	Abbvie/UCSF	24H2 clone with radio isotope labeling/ Image studies
Cell lines		
Cell lines	**Source**	**Note**
TRAMP-C2	ATCC	Murine prostate adenocarcinoma
HEK293 (PSCA overexpressing)	ATCC/Abbvie	PSCA overexpressing cell line/ Semi-adherent HEK293 engineered by Abbvie.
HEK293 (CTLA-4 overexpressing)	ATCC/ Abbvie	CTLA-4 overexpressing cell line/ Semi-adherent HEK293 engineered by Abbvie.
Mouse		
Strain	**Source**	**Note**
C57BL/6j	Jackson	Cat# 000664
Rag 1-/-	Jackson	Cat# 002216
BALB/c	Taconic	Model# BALB
C.B17 SCID	Taconic	Model# CB17SC
Flow Antibodies		
Antibodies	**Source**	**Note**
CD4	Biolegend	Clone: GK1.5 / Cat#100447
CD8	BD Bioscience	Clone: 53-6.7/ Cat#552877
CD8	Biolegend	Clone: 53-6.7/ Cat#100743
CD45	Biolegend	Clone: 30-F11/ Cat#103138
CD3	BD Bioscience	Clone: 145-2C11/ Cat#563565
Foxp3	eBioscience	Clone: FJK-16s/ Cat# 11-5773-82
CD44	BD Bioscience	Clone: IM7/ Cat#563736
Spas-1	NIH tetramer core	Sequence: STHVNHLHC, H-2D^{b}
CD25	Biolegend	Clone: PC61/ Cat#102026
CD45RB	BD Bioscience	Clone: 16A/Cat#553100
TNF-alpha	eBioscience	Clone: MP6-XT22/ Cat# 12-7321-81
ICOS	BD Bioscience	Clone: 7E.17G9/Cat#564070
ICOS	eBioscience	Clone: C398.4A/ Cat# 25-9949-82
Ki-67	BD Bioscience	Clone: B56/ Cat# 566109.
Zombie dye	Biolegend	Live/dead staining

Supplemental Figure 1



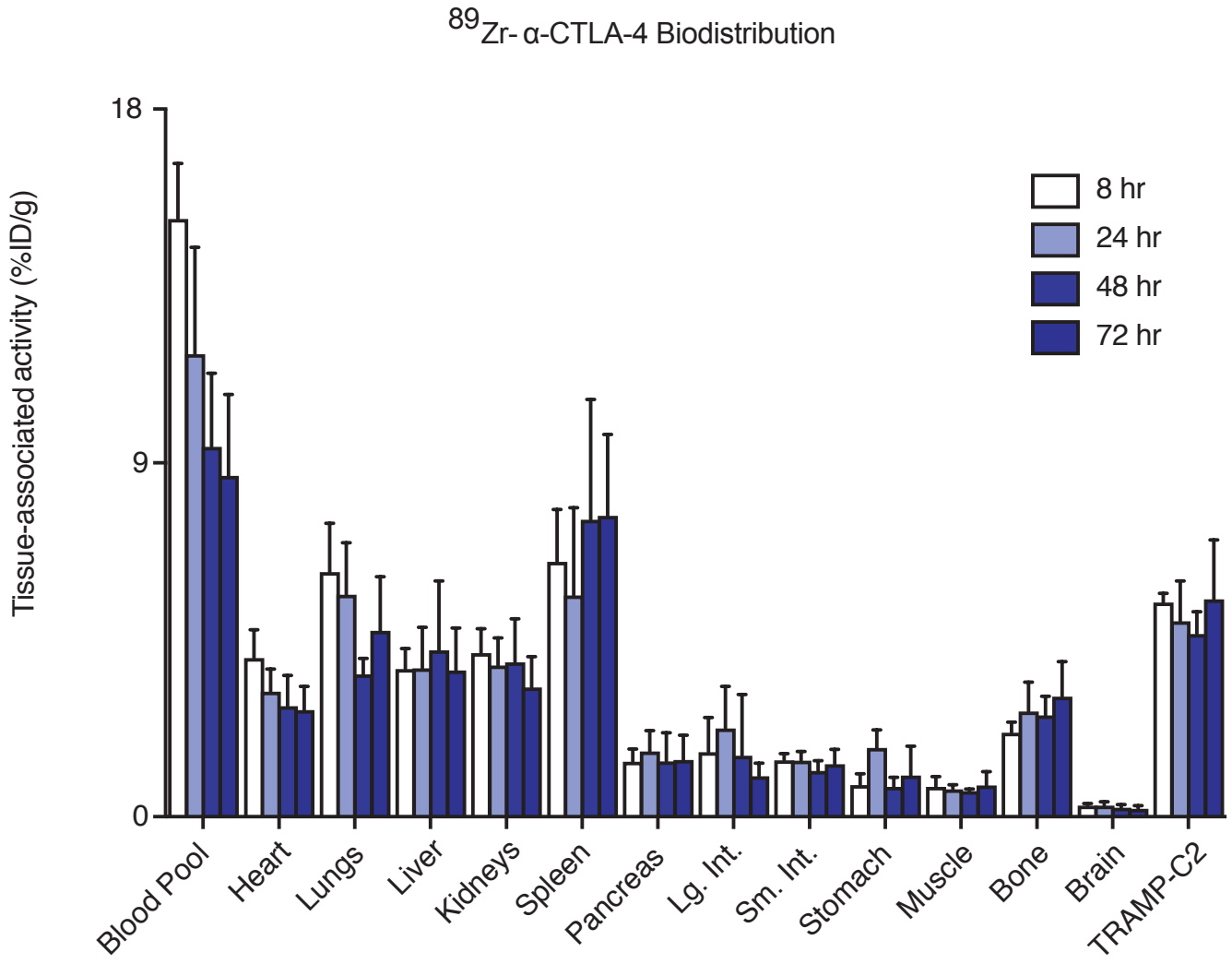
Supplemental Figure 1. Characterization of PSCA mAb 10E8 and generation of PSCA/CTLA-4 DVD Ig molecules.
(A) A chimeric anti-mouse PSCA antibody derived from the variable domains of the KO1-10E8 hybridoma was expressed as mouse isotype IgG2a and tested for binding to mouse PSCA overexpressing HEK293 cells. (B and C) A candidate PSCA/CTLA-4 DVD Ig was tested for binding to HEK293 cells overexpressing PSCA or CTLA-4. (D) The DVD Ig from C was evaluated for neutralization of B7-2.Fc binding to CTLA-4 overexpressing HEK293 cells by FACS. Representative figure from each in vitro experiment was shown. Each experiment was conducted three times independently.

Supplemental Figure 2



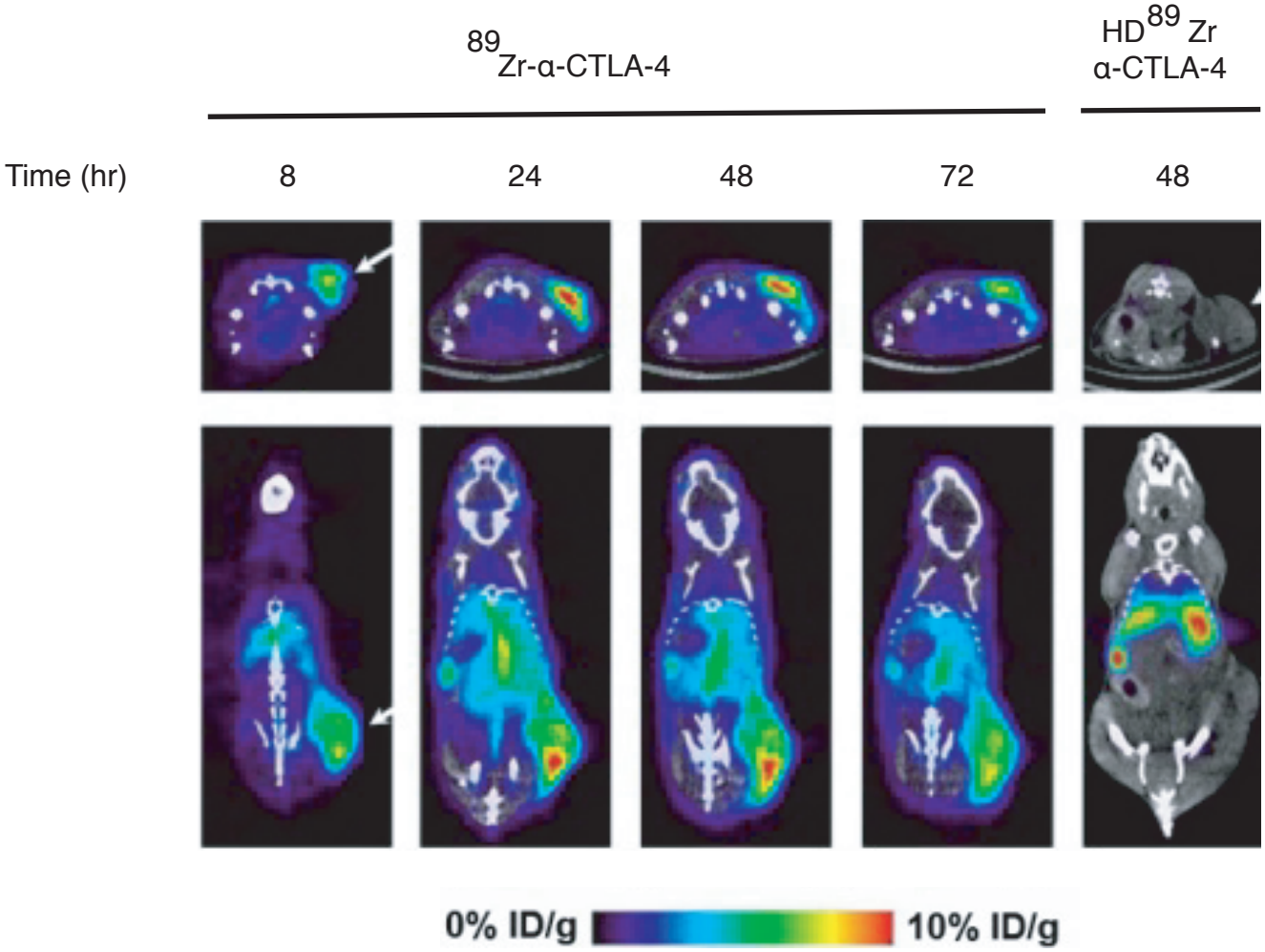
Supplemental Figure 2. Original raw gel files of Figure 3B.
This figure shows the raw gel files. The antibodies are DVD5035 (35), DVD 5036 (36), DVD5037 (37), DVD5038 (38), DVD5041 (41), DVD5042 (42) respectively. DVD 5047 (47) is the linker control (non-cleavable). The remaining lanes are DVD Ig with optimized linkers that are cleaved less efficiently.

Supplemental Figure 3



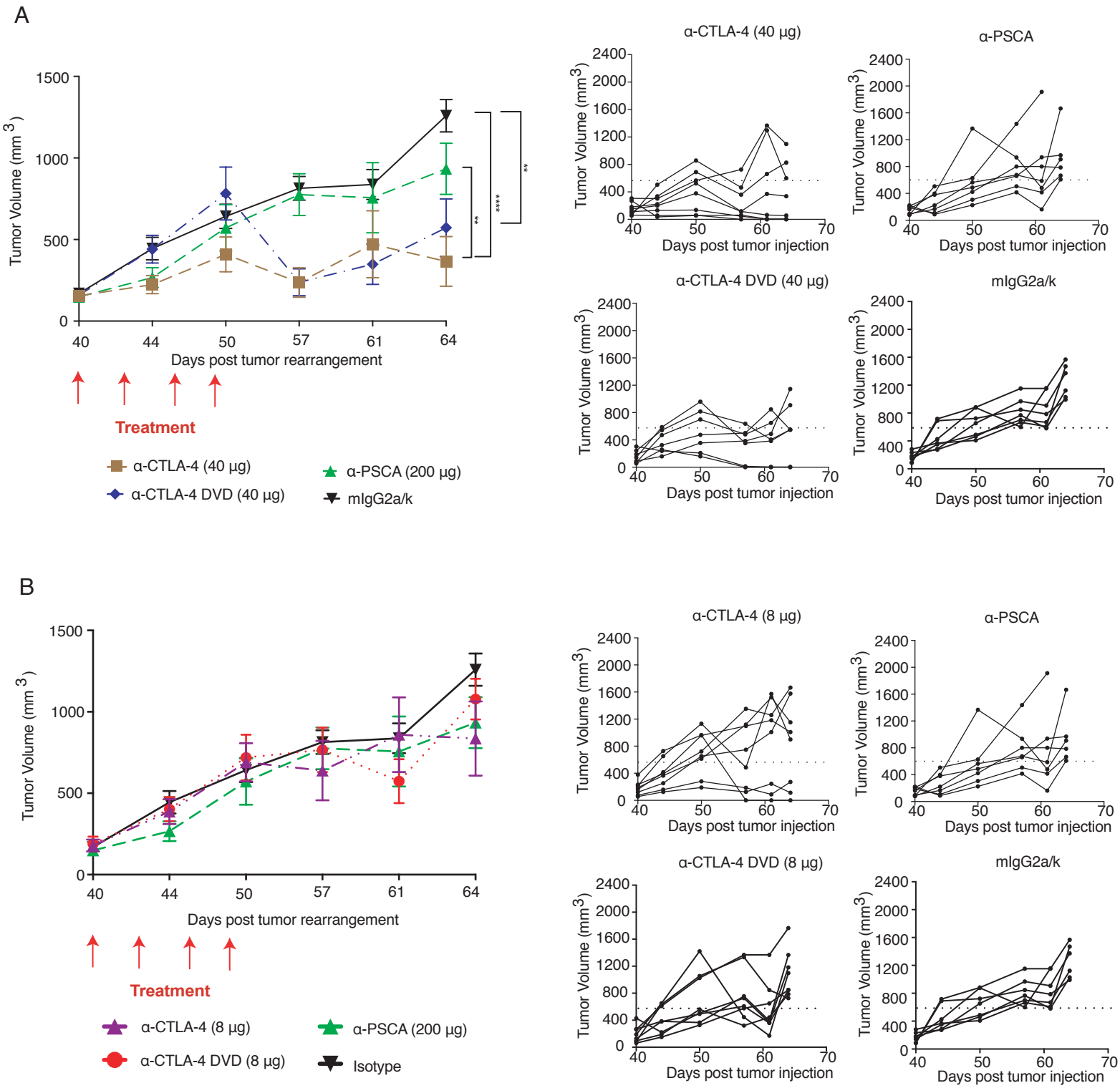
Supplemental Figure 3. Tissue distribution of CTLA-4 blockades over time.
A time course study showing the biodistribution of ^{89}Zr-α-CTLA-4 in intact male C57BL6/j mice with subcutaneous TRAMP-C2 xenografts. Tumor uptake was consistent between 8 – 72 hours, while retention of the radiotracer in normal tissues generally declined from 8 – 72 hours. Bone uptake of the radiotracer was low, underscoring that the ^{89}Zr-α-CTLA-4 construct is stable in vivo. These data were used to identify that 48 hours post injection was a suitable time point for comparing the intact and cleavable antibodies. Data were collected from 4 mice per time point for a total of 16 mice from one experiment. Bars represent mean ± SE

Supplemental Figure 4



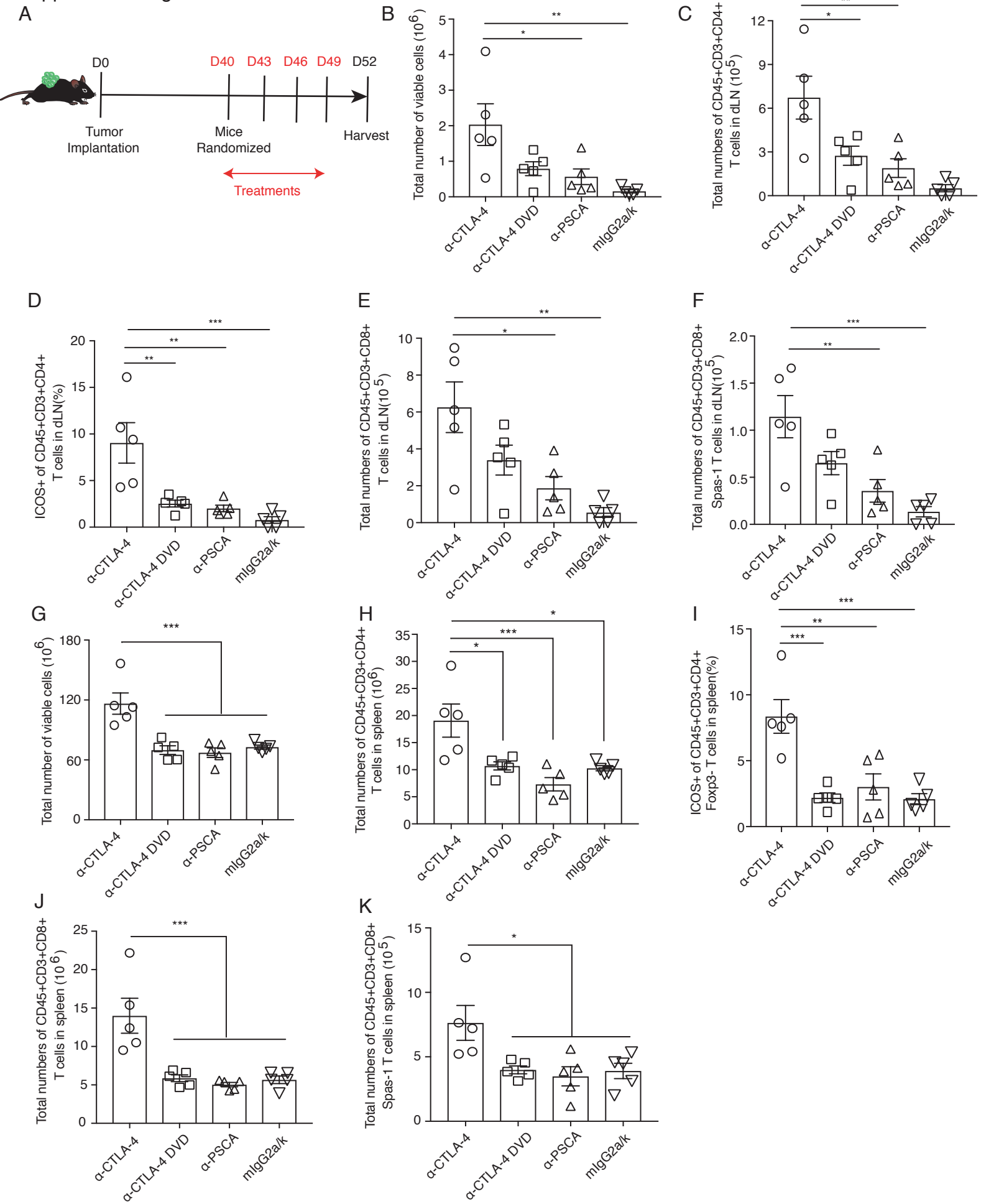
Supplemental Figure 4. Tissue distribution of CTLA-4 and heat-denatured CTLA-4 blockades. Representative transverse and coronal PET images from mice treated with ^{89}Zr-α-CTLA-4 from Supplemental Figure 3. Tumor uptake was visible from 8 – 72 hours post injection (white arrow), as well as uptake in the liver. Heat-denatured ^{89}Zr-α-CTLA-4 (HD) was not detected in the tumor, as expected.

Supplemental Figure 5



Supplemental Figure 5. Dose responses of α-CTLA-4 DVD in anti-tumor activities.
C57BL/6j male mice were implanted with TRAMP-C2 tumors at Day 0, and tumors were allowed to grow for 40 days. Mice were randomized into different treatment groups before antibody injection and then received different treatments at day 43, day 46, and day 49. (A) Tumor growth kinetics from mice treated with medium doses of α-CTLA-4 or α-CTLA-4 DVD. (B) Tumor growth kinetics from mice treated with low doses of α-CTLA-4 or α-CTLA-4 DVD. Data were conducted with two independent experiments. Each treatment arm was collected from 7 mice per group. Bars represent mean ± SE. Statistical significance was calculated using two-way ANOVA with post-hoc Tukey test (**P<0.01, ****P<0.0001).

Supplemental Figure 6



Supplemental Figure 6. Systemic immune-profiling post checkpoint inhibitor treatments. C57BL/6j male mice were implanted with TRAMP-C2 tumors at day 0, and tumors were allowed to grow for 40 days. Mice were randomized into different treatment groups before antibody injection and then received different treatments at day 43, day 46, and day 49. Draining lymph nodes (dLN) and spleens were harvested at day 52. (A) Treatment schema. (B-F) Data from dLN. (G-K) Data from spleens. Data were shown as 5 mice per group from one representative experiment. Bars represent mean ± SE. Statistical significance was calculated using one-way ANOVA with post-hoc Tukey test (*P<0.05, **P<0.01, ***P<0.001).