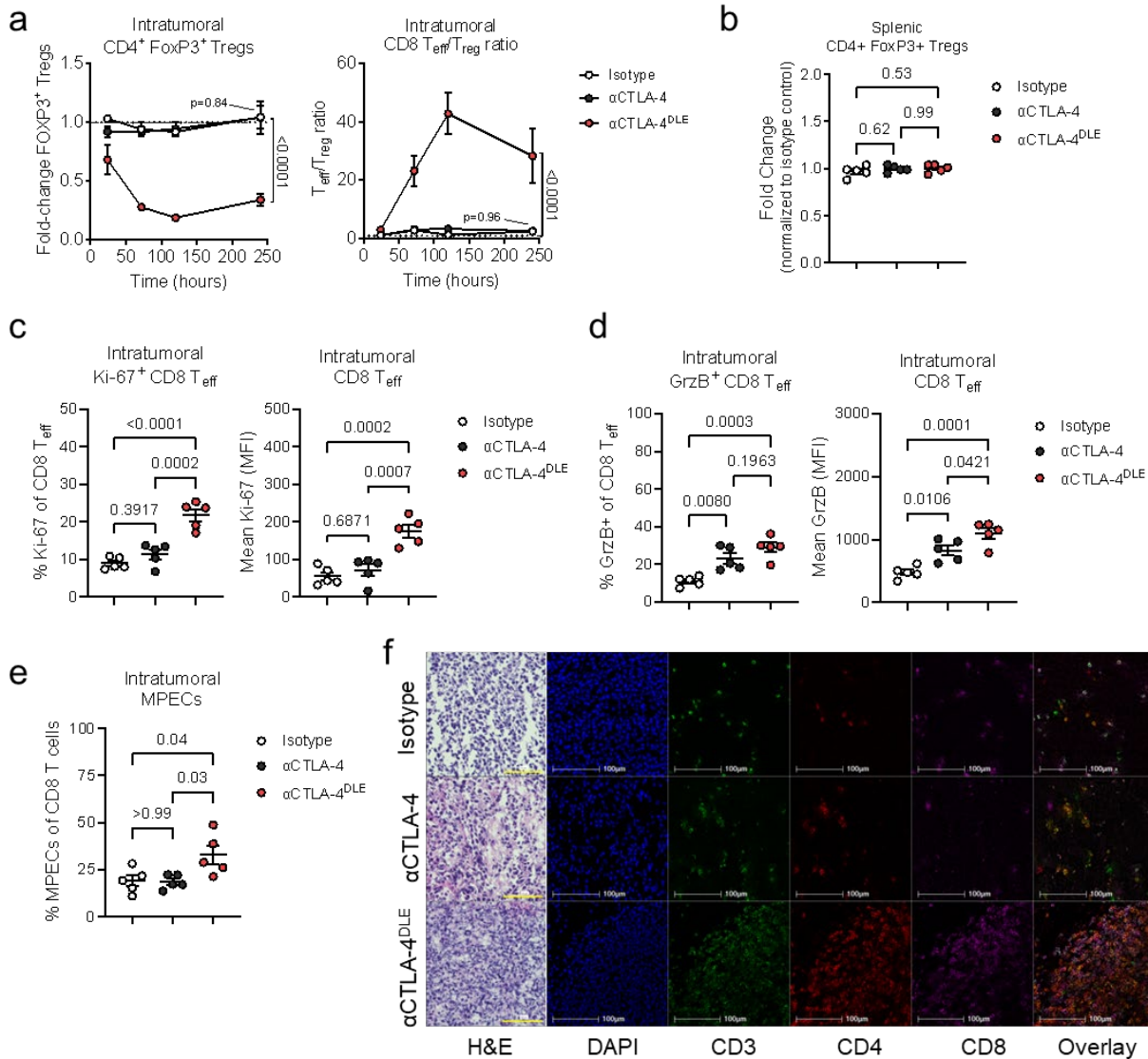


1 **Supplementary Figure S3**



2

3 **Supplementary Figure S3. αCTLA-4<sup>DLE</sup> promotes superior T cell infiltration in the tumor**

4 **microenvironment versus parental αCTLA-4 in MC38 and CT26 tumor-bearing mouse**

5 **models. (a-e) C57BL/6 mice bearing MC38 subcutaneous tumors (~120 mm<sup>3</sup>) treated once**

6 **intraperitoneally with 100 µg of indicated antibodies. (a) Fold-change of intratumoral FoxP3<sup>+</sup> Tregs**

7 **relative to vehicle control (dashed line) and ratio of intratumoral CD8<sup>+</sup> effector T cells to FoxP3<sup>+</sup>**

8 **Tregs (n=5 mice/treatment time point), and (b) fold-change of splenic FoxP3<sup>+</sup> Tregs relative to**

9 **isotype at 72 hours (n=5 mice/group). (c) Percentage of intratumoral Ki-67<sup>+</sup> and Ki-67 mean**

10 fluorescent intensity (MFI) on CD8<sup>+</sup> T effector cells (Teff; CD44<sup>+</sup>CD62L<sup>-</sup>), **(d)** Percentage of  
11 intratumoral granzyme B (GrzB<sup>+</sup>) and GrzB<sup>+</sup> MFI on CD8 Teff cells and **(e)** memory precursor  
12 effector cells (MPECs) subsets (CD62L<sup>-</sup>PD-1<sup>-</sup> Slamf7<sup>+</sup>CX3CR1<sup>-</sup>) evaluated by flow cytometry 10  
13 days following treatment (n=5 mice/group). Data were analyzed using a two-way ANOVA **(a)** or  
14 one-way ANOVA **(b-e)** followed by a Tukey's multiple comparisons test. **(f)** Representative  
15 hematoxylin & eosin (H&E) and multiplex immunofluorescence images from isotype (top panel)  
16 or αCTLA-4 (middle panel) and αCTLA-4<sup>DLE</sup> (bottom panel) treated CT26 tumor-bearing (~50-75  
17 mm<sup>3</sup>) treated once intraperitoneally with 100 μg of indicated antibodies are shown. H&E staining  
18 and immunofluorescent labelling of CD3, CD4 and CD8 from fresh frozen tumor sections collected  
19 9 days post-treatment. Imaging was performed with a Keyence BZ-X800 inverted fluorescence  
20 microscope equipped with a 20X Plan Apo λ NA 0.75 objective (Nikon), an Akoya CODEX  
21 microfluidics instrument.