

1 **Supplementary Figure 1.**

2 (A) Tumour weight (in mg) measured on day 15 post B16F10 (2mm<sup>3</sup>) implantation; each dot  
3 represents an individual tumour. (B) Density of CD8<sup>+</sup> IFN- $\gamma$ <sup>+</sup> T cells in tumours as determined  
4 by flow cytometry following co-culture with B16F10 or MC57 cells in vitro. (C) Density of  
5 CD4<sup>+</sup> IFN- $\gamma$ <sup>+</sup> T cells in tumours as determined by flow cytometry following co-culture with  
6 B16F10 or MC57 cells in vitro. Statistical analysis by *t*-test (nonparametric). *N*=4.

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8 **Supplementary Figure 2.**

9 (A) Tumour weight (mg) measured on day 15 post B16F10 (2mm<sup>3</sup>) implantation; each dot  
10 represents an individual tumour. (B) Density of CD4<sup>+</sup> T cells in treated and untreated  
11 tumours in each group, as determined by flow cytometry. The densities were determined by  
12 dividing the total number of CD8<sup>+</sup> T cells in each tumour by the tumour weight (in mg), pre-  
13 gated on tumour infiltrating lymphocytes (TILs). (C) Representative FACS plots showing the  
14 total number of CD4<sup>+</sup> T cells in treated and untreated tumours in each group, pre-gated on  
15 TILs. (D and E) Percentage (%) of intratumoural and peritumoural CD4<sup>+</sup> T cells in both  
16 biologic groups. (F) Representative immunohistochemistry (IHC) stained for CD4<sup>+</sup> T cells to  
17 assess intratumoural and peritumoural cells, arrows point to CD4<sup>+</sup> T.

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19 **Supplementary Figure 3.**

20 (A) Percentage (%) of CD4<sup>+</sup> T cells in peripheral blood 48h post anti-CD4<sup>+</sup> mAb  
21 administration post tumour implantation; each dot represents an individual tumour. Depletion  
22 efficacy was ~99%. (B) Tumour weight (in mg) measured on day 15 post B16F10 (2mm<sup>3</sup>)  
23 implantation; each dot represents an individual tumour. (C) Density of CD8<sup>+</sup> T cells in  
24 tumours as determined by flow cytometry. The densities were determined by dividing the  
25 total number of CD8<sup>+</sup> T cells in each tumour (pre-gated on TILs) by the tumour weight (in  
26 mg). (D) Density of CD4<sup>+</sup> T cells in tumours as determined by flow cytometry. The densities  
27 were determined by dividing the total number of CD4<sup>+</sup> T cells in each tumour (pre-gated on  
28 TILs) by the tumour weight (in mg). (E) Representative FACS plots showing the percentage  
29 (%) of CD8<sup>+</sup> T cells in peripheral blood. Statistical analysis by *t*-test (nonparametric). *N*=4 or  
30 5.

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32 **Supplementary Figure 4.**

33 (A) A cartoon illustrating the intratumoural injection to study the depot effect using AF488-  
34 CuMV<sub>TT</sub> or AF488-CuMV<sub>TT</sub> formulated with MCT. (B) Percentage (%) CD11b<sup>+</sup> AF488-  
35 CuMV<sub>TT</sub> cells in tumour after 1 or 5 days of intratumoural injection. A mock group was  
36 included. *N*=3.

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40 **Supplementary Figure 5.**

41 (A and B) Go Gene Ontology and KEGG pathways enrichment were analysed to identify  
42 biological processes and pathways significantly enriched with upregulated and  
43 downregulated genes in tumours for each biological group. Enrichment scores showing gene  
44 count and statistical significance as determined with Fisher's exact test and presented for  
45 the top 30 Biological process-related GO terms and the top 30 KEGG pathways.

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