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Supplemental material

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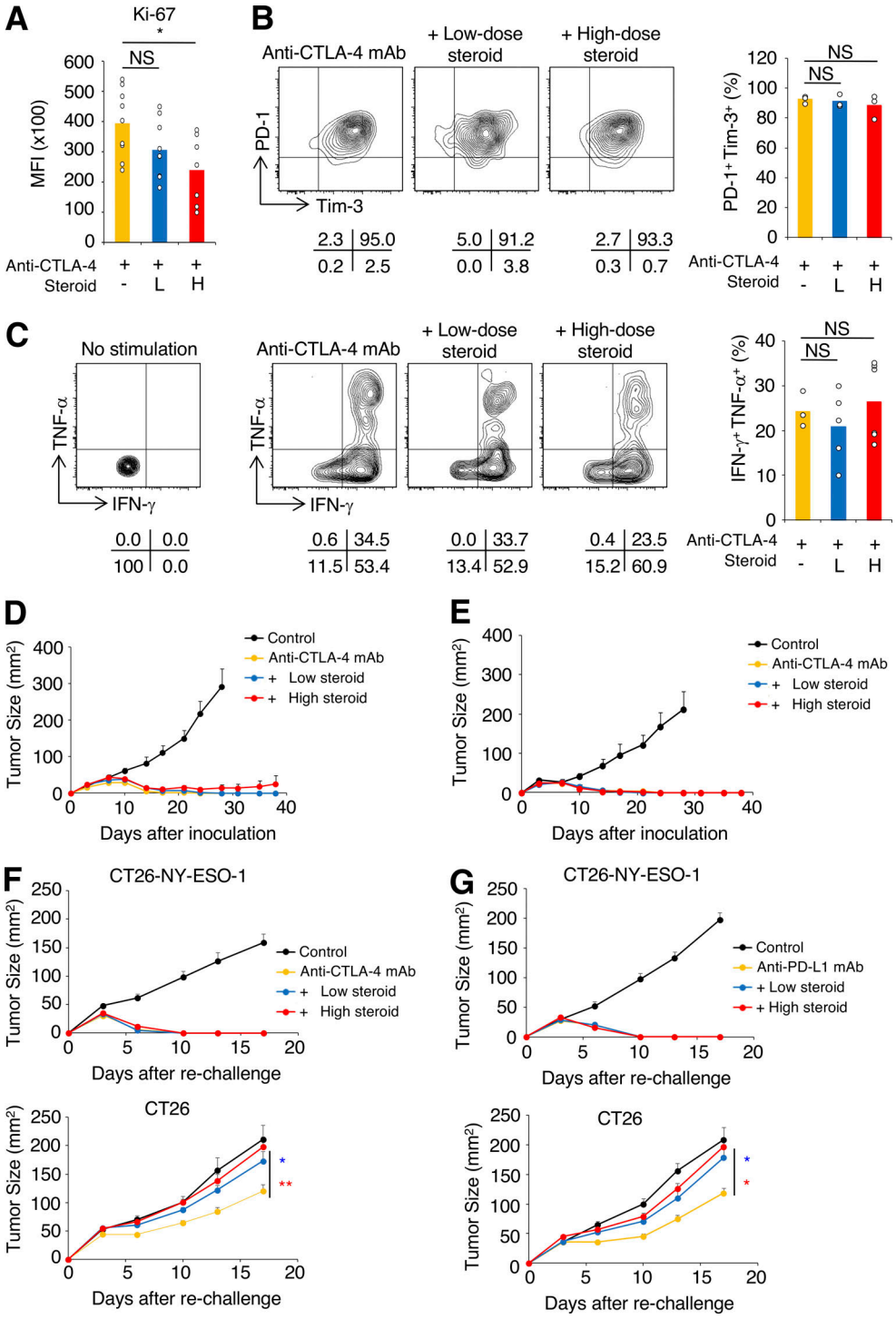

Figure S1. **Early corticosteroid treatment reduces antitumor activity and impairs low-affinity memory T cell differentiation. (A)** Ki-67 expression by NY-ESO-1 tetramer⁺CD8⁺ T cells in CMS5a-NY-ESO-1 tumors at 10 d after tumor inoculation (n = 7–9). MFI, mean fluorescence intensity. L, low dose; H, high dose. **(B)** Representative flow-cytometric analysis (left) and summary (right) of PD-1 and Tim-3 expression on NY-ESO-1-tetramer⁺CD8⁺ T cells in CMS5a-NY-ESO-1 tumors at 10 d after tumor inoculation (n = 3). **(C)** Representative flow cytometry (left) and summary (right) of IFN-γ and TNF-α production by NY-ESO-1-tetramer⁺CD8⁺ T cells in CMS5a-NY-ESO-1 tumors at 10 d after tumor inoculation (n = 4–6). **(D and E)** Tumor growth curves of early (D) and late (E) corticosteroid treatment. BALB/c mice were inoculated with CT26-NY-ESO-1 and injected with anti–CTLA-4 mAb on days 3, 6, and 9 after tumor inoculation. Corticosteroid administration was started on the same day (D) or on day 17 (E) of anti–CTLA-4 mAb (n = 4–10). **(F)** Tumor growth curves (upper: CT26-NY-ESO-1; lower: CT26) of rechallenge to early corticosteroid-treated mice. CT26-NY-ESO-1–bearing mice were treated as in (D). Mice that had completely rejected the initial tumors were secondarily inoculated with CT26-NY-ESO-1 and parental CT26 (day 39; n = 5–10). **(G)** Tumor growth curves (upper: CT26-NY-ESO-1; lower: CT26) of rechallenge to early corticosteroid-treated mice. CT26-NY-ESO-1–bearing mice were treated as in Fig. 2 A. Mice that had completely rejected the initial tumors were inoculated with CT26-NY-ESO-1 and parental CT26 (day 39; n = 5–9). Data in D–G are mean + SE. Statistical analysis by Dunnett's test; *, $P < 0.05$; **, $P < 0.01$. These experiments were performed independently three times with similar results.

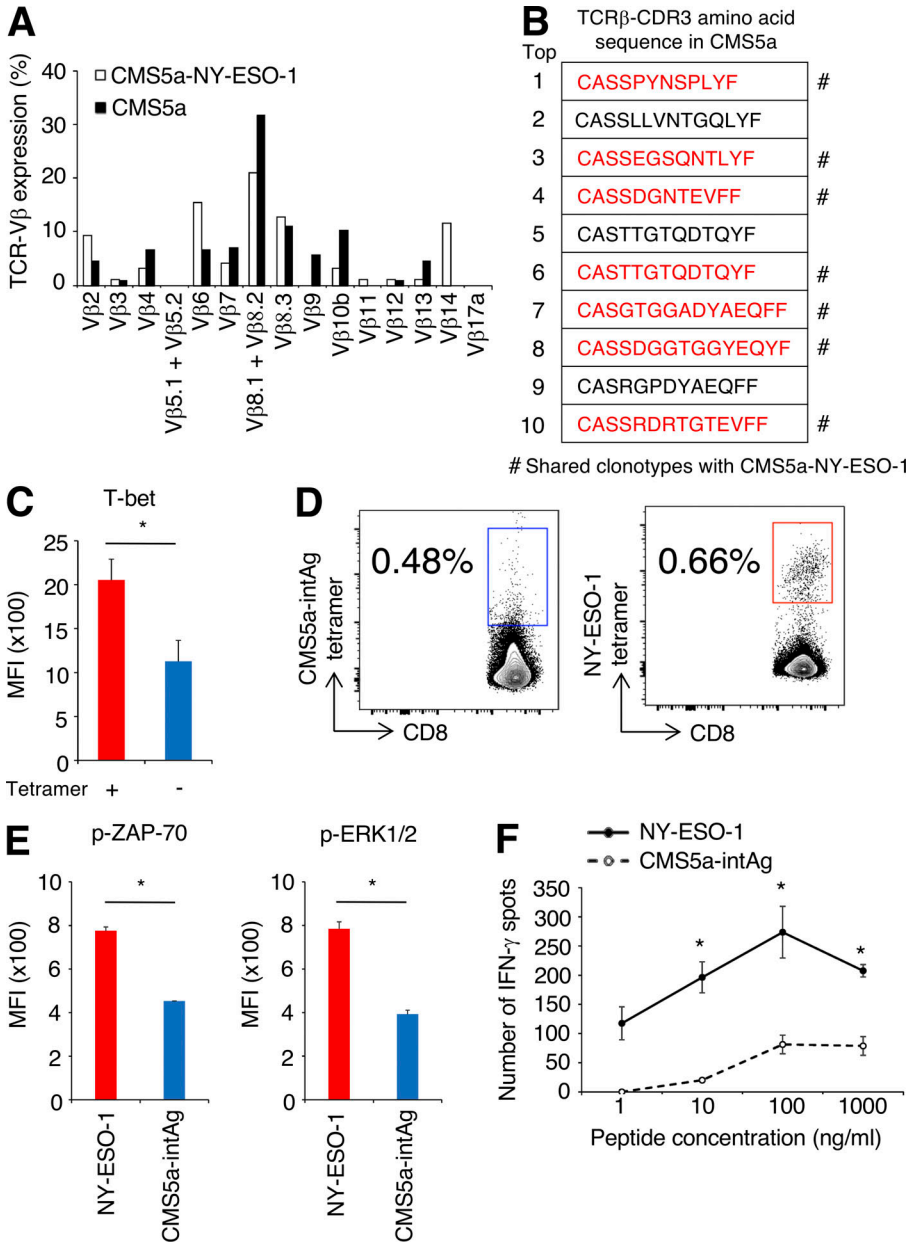

Figure S2. **CMS5a-intAg–specific CD8⁺ T cells have low-affinity TCR. (A)** Comparison of TCR repertoires expressed by NY-ESO-1-tetramer⁻CD69⁺CD8⁺ T cells. **(B)** TCR vβ-repertoire analysis of NY-ESO-1-tetramer⁻CD69⁺CD8⁺ T cells. Shared top 10 clonotypes of CD69⁺CD8⁺ T cells in CMS5a tumors and all clonotypes of NY-ESO-1-tetramer⁻CD69⁺CD8⁺ T cells in CMS5a-NY-ESO-1 tumors were analyzed. BALB/c mice were inoculated with CMS5a-NY-ESO-1 or CMS5a, and administered with anti–CTLA-4 mAb on days 3, 6, and 9 after tumor inoculation. TCR repertoires were examined at 10 d after tumor inoculation (n = 4). **(C)** T-bet expression in NY-ESO-1-tetramer⁺ or NY-ESO-1-tetramer⁻ CD69⁺CD8⁺ T cells in CMS5a-NY-ESO-1 tumors at 10 d after tumor inoculation (n = 3). MFI, mean fluorescence intensity. **(D)** NY-ESO-1– or CMS5a-intAg–specific CD8⁺ T cell induction. Mice were immunized with NY-ESO-1 peptide (RGPESRLL) or CMS5a-intAg peptide (QYIHSANVL) 100 µg/mouse by CASAC-vaccination. Cells from draining lymph nodes in the immunized mice on day 16 after two vaccinations (day 0 and day 10) were stained with MHC/peptide dextramers and analyzed with flow cytometry. **(E)** Phosphorylation of TCR signaling molecules in NY-ESO-1– and CMS5a-intAg–specific CD8⁺ T cells. NY-ESO-1–specific and CMS5a-intAg–specific CD8⁺ T cells were cultured with P1.HTR pulsed with cognate peptides with/without corticosteroids (25 ng/ml). Phosphorylation of ZAP-70 (left) and ERK1/2 (right) was determined by flow cytometry (n = 3). **(F)** IFN-γ secretion by NY-ESO-1– and CMS5a-intAg–specific CD8⁺ T cells. NY-ESO-1–specific and CMS5a-intAg–specific CD8⁺ T cells were cultured with antigen-presenting cells (CD90-negative splenocytes) pulsed with cognate peptides. The number of IFN-γ–secreting Ag-specific CD8⁺ T cells was assessed by ELISPOT assays (n = 3). Data in C, E, and F are mean ± SD. Statistical analysis by Student's t test; *, $P < 0.05$. These experiments were performed independently three times with similar results.

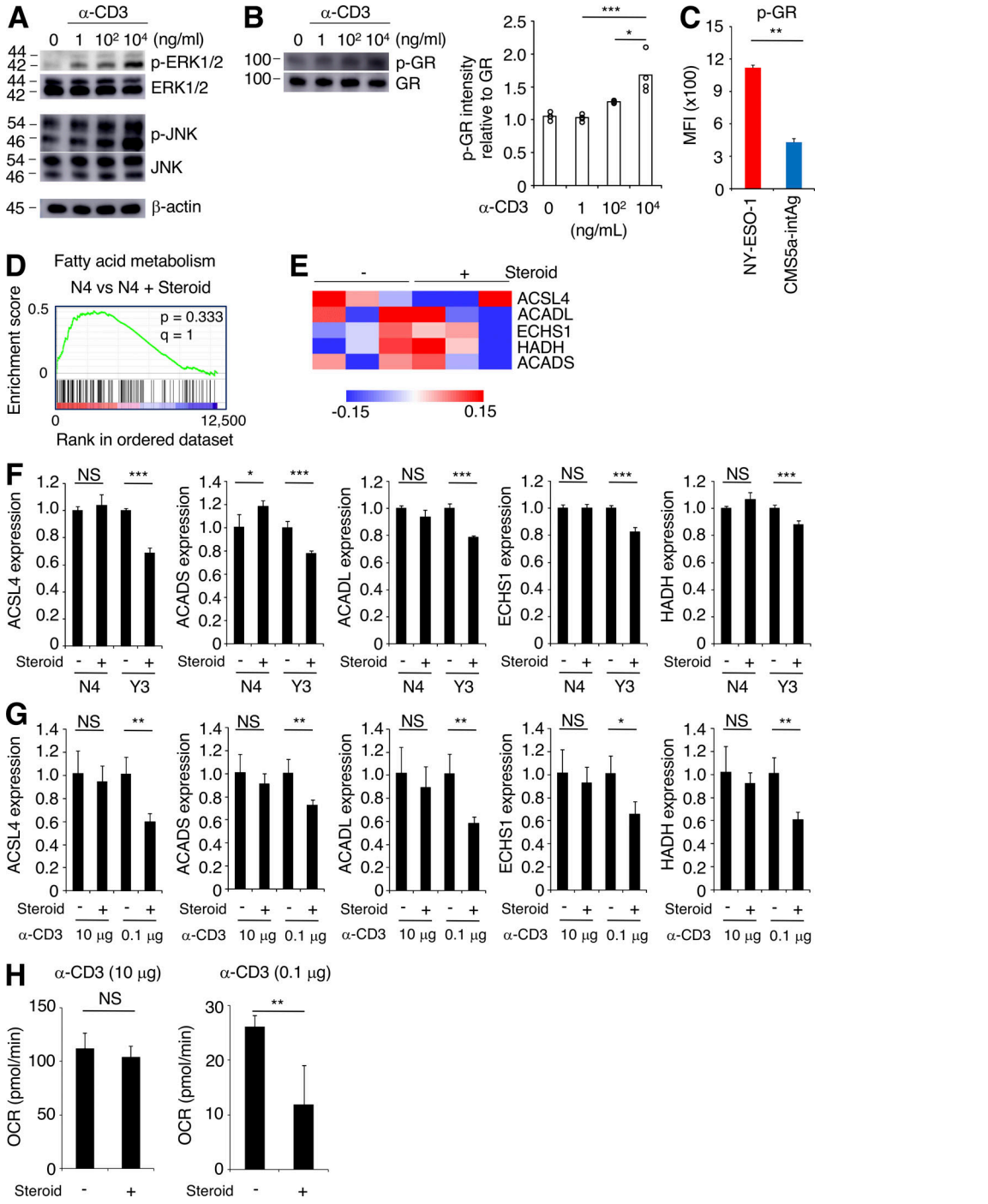

Figure S3. **Corticosteroids compromise FAO in low-affinity but not high-affinity memory T cells. (A)** Phosphorylation of TCR signaling molecules by immunoblot. CD8⁺ T cells were stimulated with titrated doses of anti-CD3 mAb. Phosphorylation of ERK1/2 and JNK was determined by immunoblot. **(B)** Phosphorylation of GR. CD8⁺ T cells were stimulated with titrated doses of anti-CD3 mAb. Phosphorylation of GR was determined by immunoblot (right: relative levels of phosphorylated GR protein). Protein levels were normalized with total GR signal (n = 4). **(C)** Phosphorylation of GR in NY-ESO-1– and CMS5a-intAg antigen–specific CD8⁺ T cells. NY-ESO-1–specific and CMS5a-intAg–specific CD8⁺ T cells were cultured with P1.HTR pulsed with cognate peptides with/without corticosteroids (25 ng/ml). Phosphorylation of GR was determined by flow cytometry (n = 3). **(D)** GSEA of fatty acid metabolism-related genes in N4-stimulated OT-I cells relative to those treated with corticosteroids. **(E)** Heat map of expression of FAO-related genes. OT-I cells were stimulated with N4 peptide, and treated with corticosteroids in vitro for 4 d. mRNA expression was examined with microarray. **(F)** FAO-related mRNA expression in OT-I cells. OT-I cells were stimulated with N4 or Y3 peptide, and treated with corticosteroids in vitro for 4 d. mRNA expression was determined by quantitative PCR (n = 3). **(G)** FAO-related mRNA expression in CD8⁺ T cells activated by anti-CD3 mAb. CD8⁺ T cells were stimulated with anti-CD3 mAb, and treated with corticosteroids in vitro for 4 d. mRNA expression was determined by quantitative PCR (n = 3). **(H)** OCR of anti-CD3 mAb-stimulated CD8⁺ T cells with etomoxir. CD8⁺ T cells were stimulated as described. FAO was determined with etomoxir by measuring OCR using extracellular flux analyzer (n = 4, 5). Data in C, F, G, and H are mean + SD. Statistical analysis by Dunnett's test (B) or Student's t test (C, F, G, and H); *, P < 0.05; **, P < 0.01; ***, P < 0.001. These experiments were performed independently three times with similar results.