Supplemental material

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

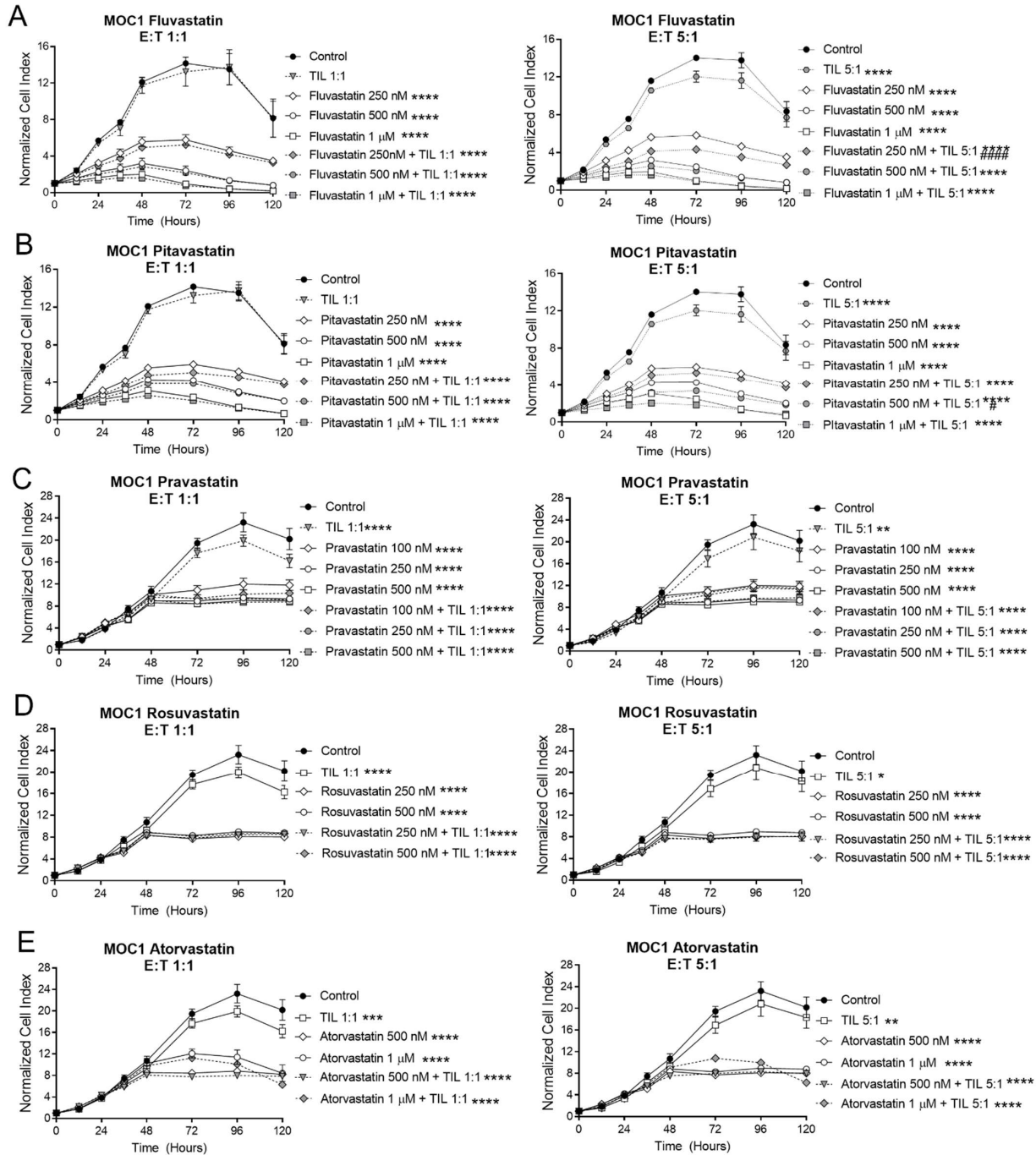*J Immunother Cancer*

Supplemental Figures

Figure S1: Different statins enhance direct and T-cell-induced inhibition of MOC1 tumor cell growth to variable degrees. MOC1 cells were plated in 96-well plates and allowed to adhere overnight. Some wells were then treated with statin drug and/or TIL at a 1:1 or 5:1 effector: target (E:T) ratio for 120 hours. Data represent mean ±SEM of 4 replicates, normalized to a cell index of 1.0 when statin and/or TIL were added (time 0 on graph). Graphs are representative of at least two independent experiments done in quadruplicate. **p<0.01, ***p<0.001, ****p<0.0001 versus control; #p<0.05, ####p<0.0001 versus statin alone. TIL, tumor infiltrating lymphocytes.
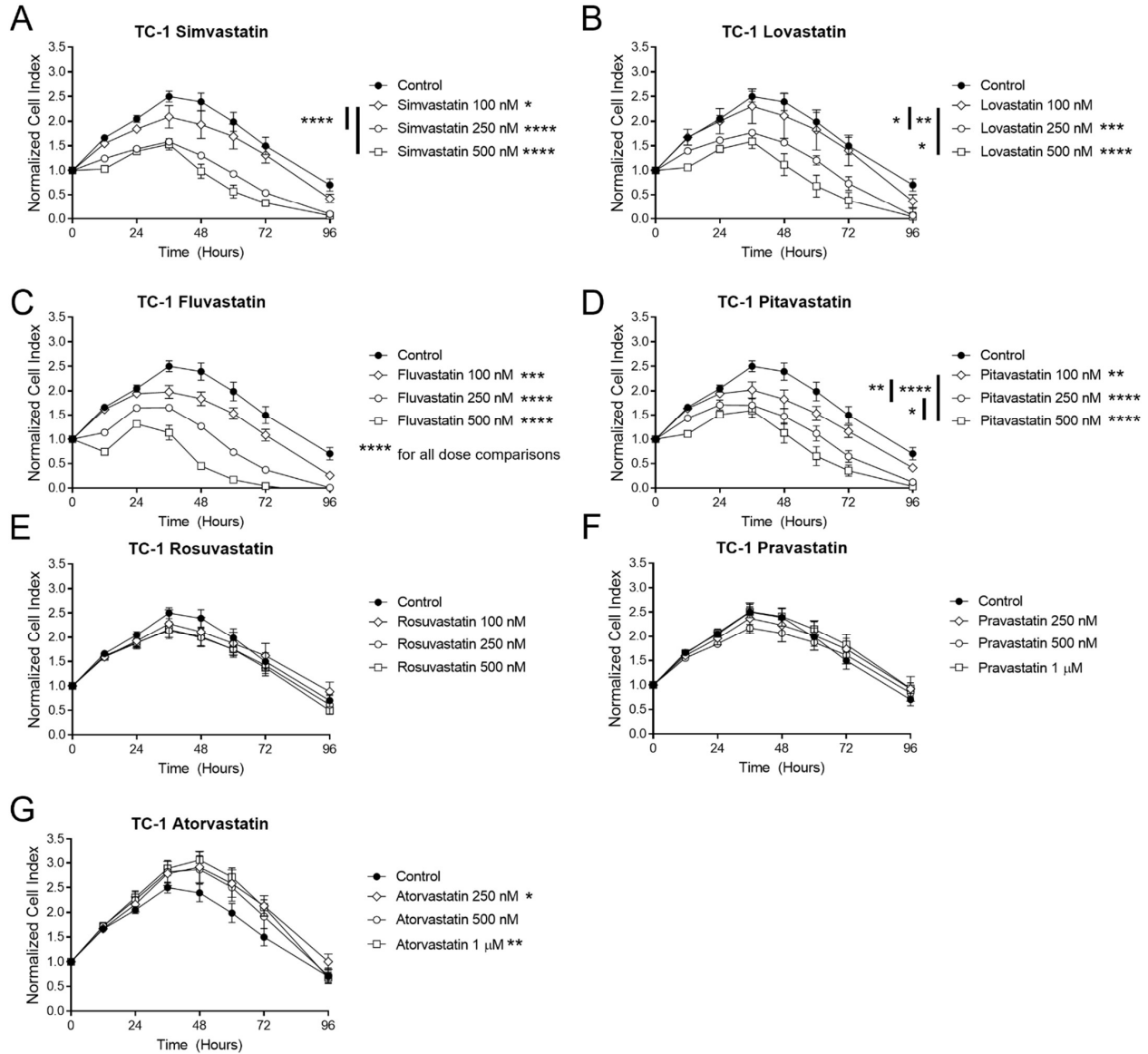
Figure S2: Different statins inhibit TC-1 tumor cell growth to variable degrees. TC-1 cells were plated in 96-well plates and allowed to adhere overnight. Some wells were then treated with statin drug for 96 hours. Data represent mean ±SEM of 4 replicates, normalized to a cell index of 1.0 when statin was added (time 0 on graph). Graphs are representative of at least two independent experiments done in quadruplicate. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 versus control or as indicated. TIL, tumor infiltrating lymphocytes.
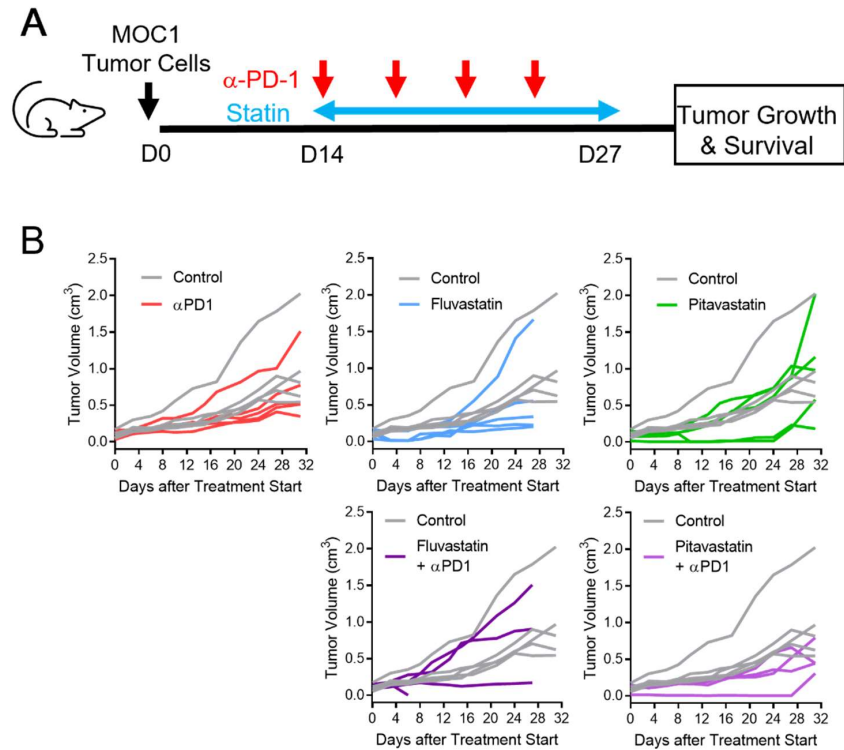
Supplemental Figure S3

Figure S3: Fluvastatin and pitavastatin have anti-tumor activity *in vivo*. MOC1 cells were injected into the right flank, and animals were randomized on day 14 to treatment with fluvastatin (60 mg/kg/day by oral gavage), pitavastatin (3 mg/kg/day by oral gavage), anti-PD-1 (200 mcg IP twice/week) or statin + anti-PD-1. **A,** schema of experiment. **B,** Tumor growth curves showing individual animals, compared with control.
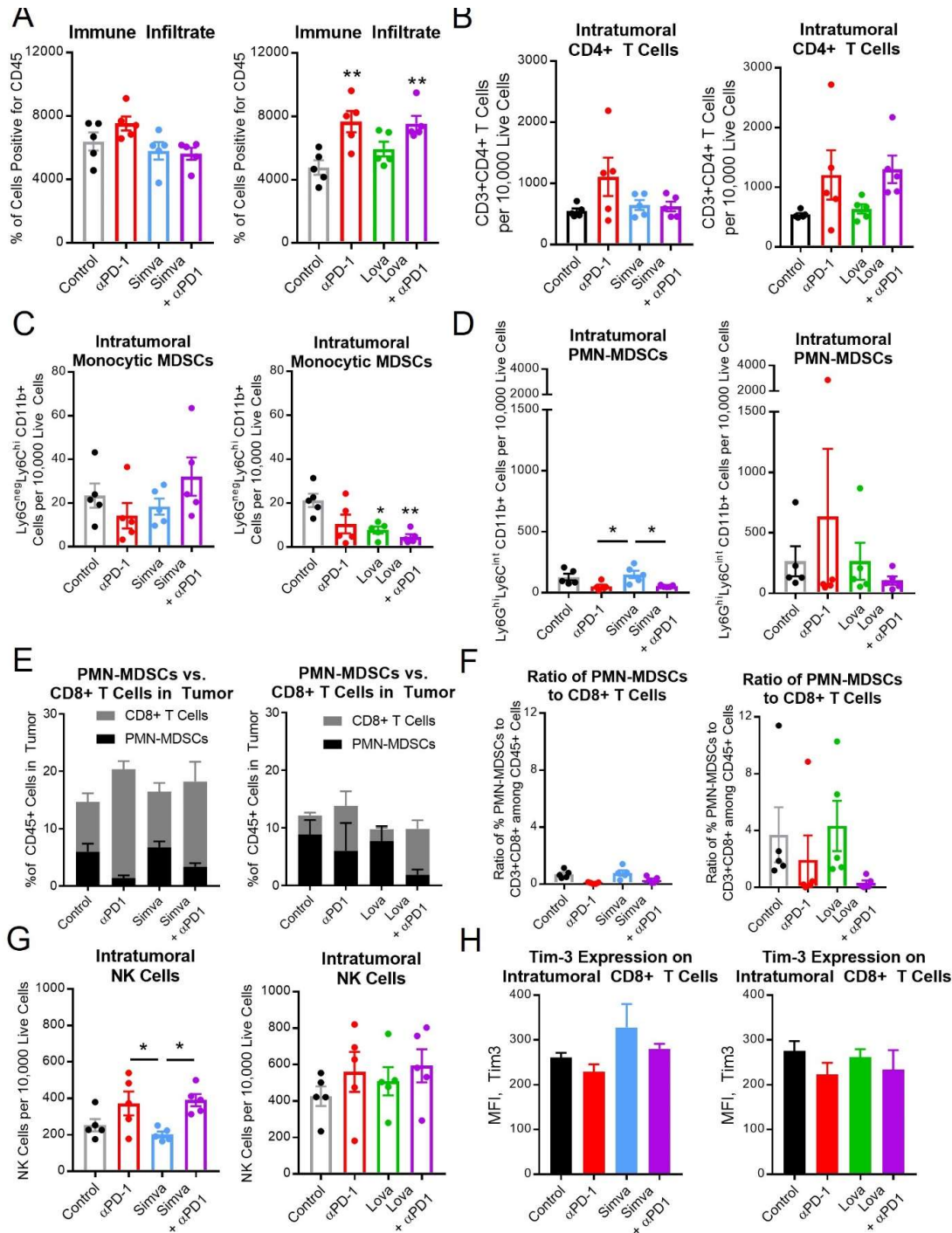
Figure S4: Simvastatin and lovastatin did not consistently alter the density of overall immune cells (A), CD4+ T cells (B), MDSCs (C-F), NK cells (G), or Tim3-expressing T cells (H). MOC1 tumor-bearing mice were treated with simvastatin/lovastatin and anti-PD-1 as in Figure 3. Mice were sacrificed during the second week of treatment, then tumors were harvested and analyzed by flow cytometry. D and E represent the relative percentages of PMN-MDSCs versus CD8+ T cells among live intratumoral CD45+ cells. Data are mean ± SEM, n= 5, * p <0.05, **p <0.01 (where indicated or versus control). MDSC, myeloid derived suppressor cell; NK, natural killer; PMN, polymorphonuclear.
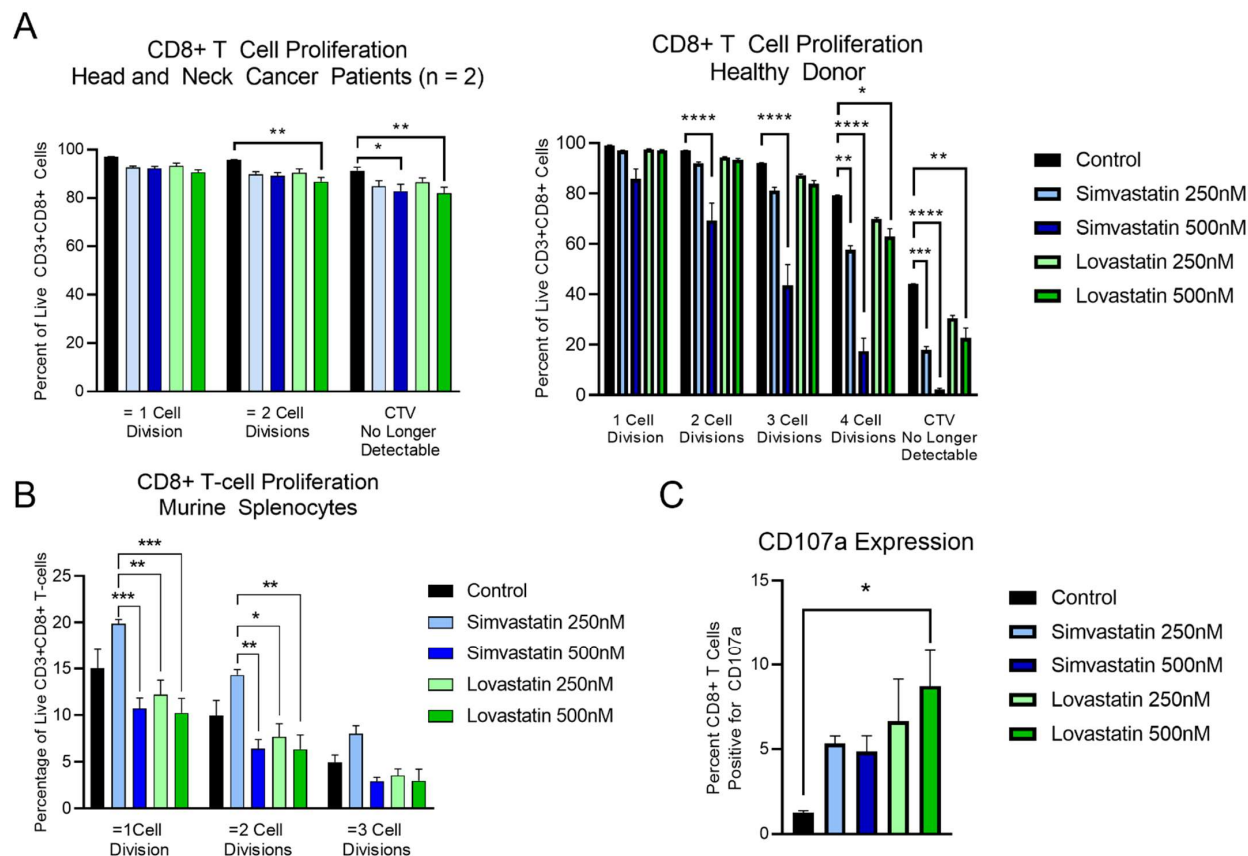
Figure S5: Statins may directly alter T cell function. **A,** T cells from the peripheral blood of two previously untreated head and neck cancer patients scheduled to undergo surgery (left) and a healthy donor (right) were stained with CellTrace Violet and stimulated with CD2/CD3/CD28 beads and IL-2 (50 IU/ml), with or without simvastatin or lovastatin, for 5 days (statin and IL-2 replenished on day 2). CellTrace Violet dilution was analyzed by flow cytometry. **B,** T cells sorted from mouse splenocytes were stained with CellTrace Violet and stimulated with IL-2 (50 IU/ml), with or without simvastatin or lovastatin, for 5 days (statin and IL-2 replenished on day 2). CellTrace Violet dilution was analyzed by flow cytometry. **C,** T cells from the same two head and neck cancer patients were treated as in **A**, then stained for surface CD107a and analyzed by flow cytometry. Data are mean + SEM from triplicate experiments. * $p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.
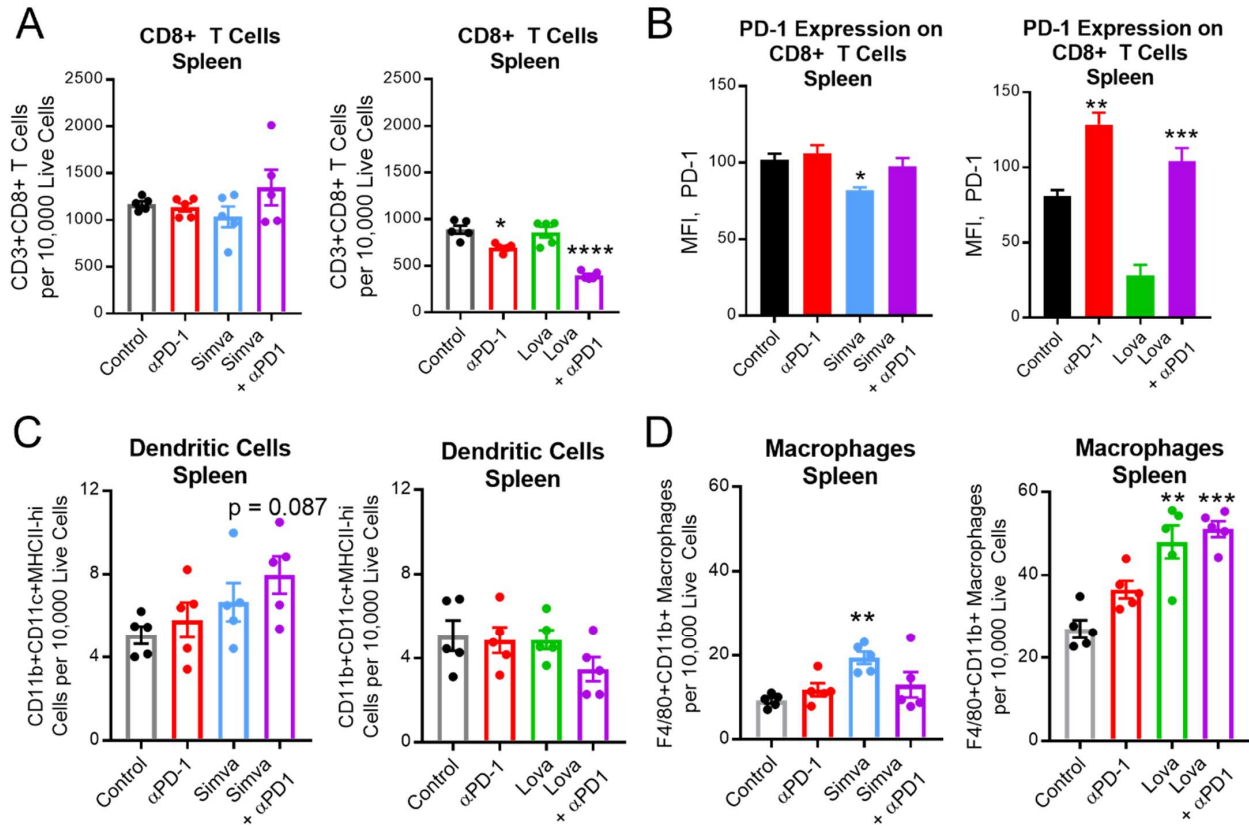
Figure S6: Statins did not have any consistent effect on immune cells in the spleen. MOC1 tumor-bearing mice were treated with simvastatin/lovastatin and anti-PD-1 as in Figure 3. Mice were sacrificed during the second week of treatment, then spleens were harvested and analyzed by flow cytometry. Data are mean ± SEM, n= 5, ** p <0.01, ***p <0.001 versus control.

Supplemental material

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)
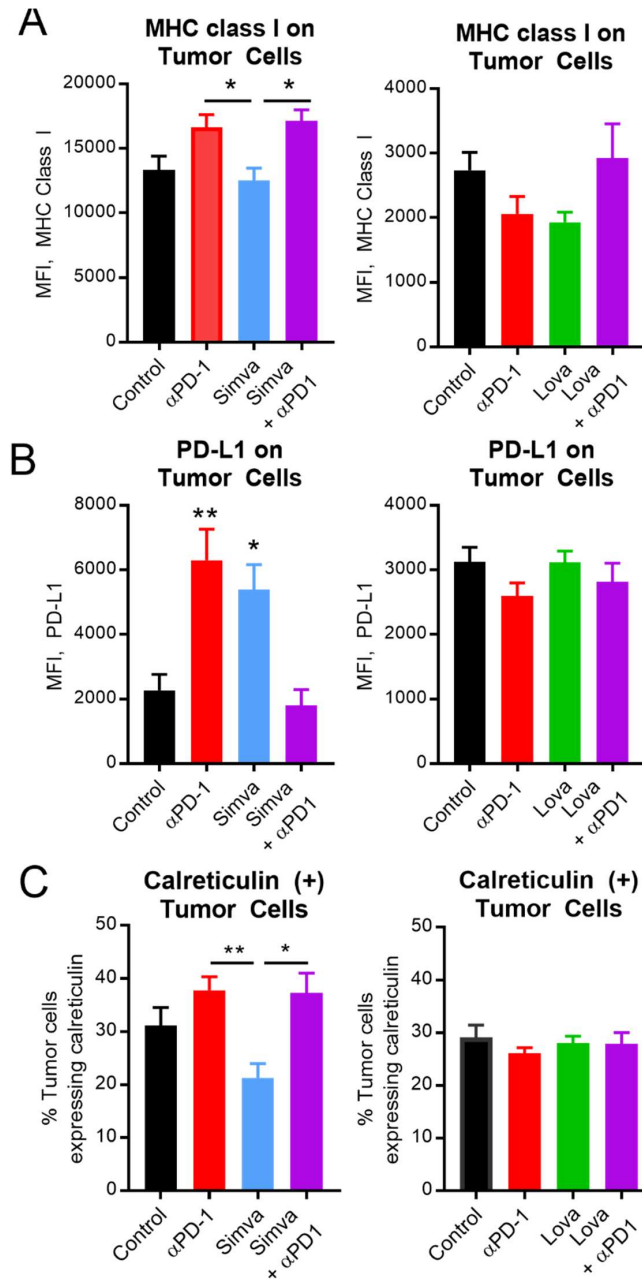
J Immunother Cancer

Figure S7: Statins did not have any consistent effect on MHC class I (A), PD-L1 (B), or calreticulin (C) surface expression on tumor cells. MOC1 tumor-bearing mice were treated with simvastatin/lovastatin and anti-PD-1 as in Figure 3. Mice were sacrificed during the second week of treatment, then tumors were harvested and analyzed by flow cytometry. Data are mean + SEM, n= 5, * p <0.05, **p <0.01 (where indicated or versus control).

Supplemental material

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

J Immunother Cancer

Supplemental Methods

Table S1: Antibodies used for flow cytometry.

Antibody	Vendor	Catalog#
Calreticulin	Abcam	Ab209577
CellTrace Violet	ThermoFisher	C34571
CD3	BD	563565
CD3	BD	741352
CD4	Biolegend	116020
CD4	BD	612936
CD8	Biolegend	100722
CD8	BD	563795
CD11b	Biolegend	101257
CD11c	Biolegend	117336
CD45	BD	566439
CD80	Biolegend	104706
CD107a	Biolegend	121614
CD107a	Biolegend	328607
CD206	Biolegend	141712
EpCAM	Biolegend	118245
F4/80	Biolegend	123112
H-2Kb-H-2Db (MHC class I)	Biolegend	114612
IFN-γ	Miltenyi	130-117-668
Ly6-C	Biolegend	128046
Ly6-G	Biolegend	127608
MHC class II (IA/IE)	Biolegend	107632
NK1.1	Biolegend	108714
PD-L1	Biolegend	124312
PD-1	Biolegend	135218
Tim-3	Biolegend	119727
Viability	BD	565694
Viability	BD	565388
ZUV (viability)	Biolegend	423108