Supplementary Information

Title:

27-Hydroxycholesterol acts on myeloid immune cells to induce T cell dysfunction, promoting breast cancer progression.

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Running Head: cholesterol metabolite works on myeloid cells to impair T cells**.**

Supplemental Table 1a. Cytokine secretion of macrophages under two time points after Veh or 27HC treatment only.

		24hrs post treatment				72hrs post co-culture		
Cytokine	Treatment	Average Concentration*	SEM	FDR	Treatment	Average Concentration	SEM	FDR
ENA-78	Veh	out of range	NA	NA	Veh	3.46	0.85	NA
ENA-78	27HC	out of range	NA		27HC	out of range	NA	
Eotaxin	Veh	out of range	NA	NA	Veh	out of range	NA	NA
Eotaxin	27HC	out of range	NA		27HC	out of range	NA	
G-CSF	Veh	out of range	NA	NA	Veh	0.79	0.2	0.7898
G-CSF	27HC	0.58	0.1		27HC	0.97	0.05	
GM-CSF	Veh	out of range	NA	NA	Veh	out of range	NA	NA
GM-CSF	27HC	out of range	NA		27HC	out of range	NA	
GRO-alpha	Veh	99.19	1.81	0.9820	Veh	109.46	3.48	0.0169
GRO-alpha	27HC	99.49	7.93		27HC	69.8	2.89	
IFN-a	Veh	12.48	2.04	0.5984	Veh	15.43	1.8	0.8713
IFN-a	27HC	15.64	1.59		27HC	14.17	2.49	
IFN-g	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IFN-g	27HC	out of range	NA		27HC	out of range	NA	
IL-10	Veh	2.65	0.58	0.4249	Veh	1.76	0.54	0.9053
IL-10	27HC	4.89	1.07		27HC	1.63	0.15	
IL-12p70	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IL-12p70	27HC	out of range	NA		27HC	out of range	NA	
IL-13	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IL-13	27HC	out of range	NA		27HC	out of range	NA	
IL-15	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IL-15	27HC	out of range	NA		27HC	out of range	NA	
IL-17A	Veh	0.65	0.05	0.3276	Veh	1.08	0.25	0.4097
IL-17A	27HC	1.84	0.42		27HC	1.65	0.09	
IL-18	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IL-18	27HC	out of range	NA		27HC	out of range	NA	
IL-1a	Veh	out of range	NA	NA	Veh	out of range	NA	NA

		24hrs post treatment			72hrs post co-culture			
Cytokine	Treatment	Average Concentration*	SEM	FDR	Treatment	Average Concentration	SEM	FDR
IL-1a	27HC	out of range	NA		27HC	out of range	NA	
IL-1b	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IL-1b	27HC	out of range	NA		27HC	out of range	NA	
IL-2	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IL-2	27HC	out of range	NA		27HC	out of range	NA	
IL-22	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IL-22	27HC	out of range	NA		27HC	out of range	NA	
IL-23	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IL-23	27HC	out of range	NA		27HC	out of range	NA	
IL-27	Veh	0.49	0.17	NA	Veh	0.45	0.08	0.5474
IL-27	27HC	0.76	0.14		27HC	0.61	0.08	
IL-28	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IL-28	27HC	out of range	NA		27HC	out of range	NA	
IL-3	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IL-3	27HC	out of range	NA		27HC	out of range	NA	
IL-31	Veh	out of range	NA	NA	Veh	6.07	1.06	NA
IL-31	27HC	out of range	NA		27HC	6.39	0.74	
IL-4	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IL-4	27HC	out of range	NA		27HC	out of range	NA	
IL-5	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IL-5	27HC	out of range	NA		27HC	out of range	NA	
IL-6	Veh	out of range	NA	NA	Veh	1.29	0.57	NA
IL-6	27HC	7.76	2.81		27HC	1.03	0.42	
IL-9	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IL-9	27HC	out of range	NA		27HC	out of range	NA	
IP-10	Veh	192.42	13.49	0.5474	Veh	104.84	15.2	0.4011
IP-10	27HC	147.53	26.31		27HC	69.28	4.98	

		24hrs post treatment			72hrs post co-culture			
Cytokine	Treatment	Average Concentration*	SEM	FDR	Treatment	Average Concentration	SEM	FDR
LIF	Veh	2.13	0.55	0.7898	Veh	5.37	0.05	NA
LIF	27HC	2.6	0.16		27HC	5.29	0.27	
M-CSF	Veh	out of range	NA	NA	Veh	out of range	NA	NA
M-CSF	27HC	out of range	NA		27HC	out of range	NA	
MCP-1	Veh	91.16	10.89	0.1965	Veh	33.07	3.49	0.1306
MCP-1	27HC	196.22	28.23		27HC	16.51	3.21	
MCP-3	Veh	77.84	7.56	0.2824	Veh	152.97	8.23	0.1335
MCP-3	27HC	114.25	12.78		27HC	91.99	13.8	
MIP-1a	Veh	4.39	0.12	0.0156	Veh	3.16	0.05	0.8595
MIP-1a	27HC	6.49	0.09		27HC	3.22	0.1	
MIP-1b	Veh	38.27	1.77	0.0279	Veh	27.17	0.86	0.8392
MIP-1b	27HC	62.35	2.55		27HC	27.76	0.24	
MIP-2	Veh	519.17	23.68	0.3009	Veh	284.96	7.8	NA
MIP-2	27HC	604.1	29.96		27HC	191.19	6.02	
RANTES	Veh	23.3	2.25	0.2409	Veh	24.32	1.39	0.0634
RANTES	27HC	15.47	2.25		27HC	10.81	0.45	
TNF-a	Veh	32.95	2.88	0.6104	Veh	15.76	0.23	NA
TNF-a	27HC	39.88	5.14		27HC	15.13	0.73	

*concentration as pg/ml

Supplemental Table 1B. Cytokine secretion of macrophages under two time points after LPS and Veh or 27HC treatment.

		24hrs post treatment			72hrs post co-culture			
Cytokine	Treatment	Average Concentration*	SEM	FDR	Treatment	Average Concentration	SEM	FDR
ENA-78	Veh	174.47	12.86	0.8598	Veh	69.14	4.59	0.7990
ENA-78	27HC	182.74	11.45		27HC	84.88	19.94	
Eotaxin	Veh	out of range	NA	NA	Veh	out of range	NA	NA
Eotaxin	27HC	out of range	NA		27HC	out of range	NA	
G-CSF	Veh	5.34	0.61	0.9053	Veh	3.92	0.43	0.5474
G-CSF	27HC	5.71	1.08		27HC	3.06	0.39	
GM-CSF	Veh	7.92	0.28	NA	Veh	out of range	NA	NA
GM-CSF	27HC	8.18	0.44		27HC	out of range	NA	
GRO-alpha	Veh	1993.92	119.05	0.2409	Veh	566.87	31.96	0.2409
GRO-alpha	27HC	1614.83	55.72		27HC	847.87	86.06	
IFN-a	Veh	15.38	4.05	0.5984	Veh	15.98	2.48	0.9105
IFN-a	27HC	21.24	0.03		27HC	16.62	3.16	
IFN-g	Veh	4.29	0.45	0.1095	Veh	0.84	0.47	0.6104
IFN-g	27HC	1.61	0.17		27HC	0.22	0.05	
IL-10	Veh	1058.73	18.36	NA	Veh	21.79	1.53	0.5984
IL-10	27HC	1052.2	13.84		27HC	18.65	2.08	
IL-12p70	Veh	6.83	0.25	NA	Veh	0.7	0.18	0.8713
IL-12p70	27HC	6.98	0.15		27HC	0.92	0.54	
IL-13	Veh	2.95	0.03	0.6104	Veh	0.92	0.09	0.6769
IL-13	27HC	3.04	0.06		27HC	1.16	0.22	
IL-15	Veh	26.45	0.9	0.5474	Veh	13.43	0.37	0.1095
IL-15	27HC	24.73	0.89		27HC	10.78	0.5	
IL-17A	Veh	14.08	0.95	0.9820	Veh	2.89	0.51	NA
IL-17A	27HC	14.12	1.19		27HC	3.33	0.93	
IL-18	Veh	879.91	46.17	0.1260	Veh	212.34	8.59	0.0674
IL-18	27HC	1143.92	12.38		27HC	144.8	10.31	
IL-1a	Veh	111.48	7.24	0.0279	Veh	85.53	3.38	0.1260
IL-1a	27HC	238.05	1.44		27HC	105.1	4.1	

		24hrs post treatment			72hrs post co-culture			
Cytokine	Treatment	Average Concentration*	SEM	FDR	Treatment	Average Concentration	SEM	FDR
IL-1b	Veh	274.45	6.14	0.0156	Veh	30.6	0.7	0.0156
IL-1b	27HC	378.89	7.54		27HC	11.18	1.24	
IL-2	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IL-2	27HC	out of range	NA		27HC	out of range	NA	
IL-22	Veh	176.97	20.57	0.4249	Veh	46.84	2.37	NA
IL-22	27HC	226.03	19.66		27HC	33.48	2.27	
IL-23	Veh	26.03	3.01	0.8303	Veh	5.04	2.2	NA
IL-23	27HC	23.87	0.94		27HC	1.16	0.28	
IL-27	Veh	141.61	4.34	NA	Veh	9.7	0	0.1335
IL-27	27HC	119.44	4.31		27HC	6.95	0.48	
IL-28	Veh	308.37	34.4	0.9105	Veh	127.24	16.28	0.8595
IL-28	27HC	300.85	31.42		27HC	99.97	42.92	
IL-3	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IL-3	27HC	out of range	NA		27HC	out of range	NA	
IL-31	Veh	17.72	0.99	NA	Veh	10.01	1.12	0.8598
IL-31	27HC	19.49	0.84		27HC	8.96	1.6	
IL-4	Veh	out of range	NA	NA	Veh	out of range	NA	NA
IL-4	27HC	out of range	NA		27HC	out of range	NA	
IL-5	Veh	7.69	0.56	0.6769	Veh	4.16	0.49	NA
IL-5	27HC	8.43	0.51		27HC	3.26	0.43	
IL-6	Veh	7600.34	164.28	0.0674	Veh	347.71	41.42	NA
IL-6	27HC	5766.51	13.33		27HC	368.53	32.73	
IL-9	Veh	28.88	2.27	NA	Veh	out of range	NA	NA
IL-9	27HC	29.87	3.82		27HC	out of range	NA	
IP-10	Veh	714.98	31.69	0.8871	Veh	817.83	11.48	NA
IP-10	27HC	703.04	13.19		27HC	777.67	72.42	
LIF	Veh	17.01	0.65	0.6464	Veh	8.88	0.6	0.7898
LIF	27HC	17.97	0.63		27HC	9.67	0.88	
M-CSF	Veh	1.08	0.02	0.8713	Veh	0.83	0.11	0.4249
M-CSF	27HC	1.07	0.02		27HC	0.6	0.05	

		24hrs post treatment			72hrs post co-culture			
Cytokine	Treatment	Average Concentration*	SEM	FDR	Treatment	Average Concentration	SEM	FDR
MCP-1	Veh	10177.69	1525.37	NA	Veh	1508.6	21.21	0.1570
MCP-1	27HC	9294.78	919.08		27HC	1266.96	57.22	
MCP-3	Veh	472	23.77	NA	Veh	312.62	7.3	0.9053
MCP-3	27HC	483.91	49.4		27HC	315.95	14.05	
MIP-1a	Veh	39.22	0.51	NA	Veh	4.54	0.21	0.7508
MIP-1a	27HC	31.39	0.42		27HC	5.17	0.65	
MIP-1b	Veh	684.83	34.65	0.1350	Veh	16.94	0.82	0.1350
MIP-1b	27HC	538.14	25.19		27HC	30.42	2.79	
MIP-2	Veh	7852.33	241.26	0.6670	Veh	4145.43	330.06	NA
MIP-2	27HC	7462.6	294.32		27HC	3787.83	353.77	
RANTES	Veh	679.87	20.08	0.8084	Veh	620.1	6.97	0.9187
RANTES	27HC	661.36	17.96		27HC	625.15	34.76	
TNF-a	Veh	1493.98	41.81	NA	Veh	194.18	0.21	NA
TNF-a	27HC	1472.32	38.99		27HC	196.34	10.98	

*concentration as pg/ml

Supplemental Table 2. LXR and/or ER ligands

Ligand	Class	Documented Activity	Identified Activity	Literature
4βHC	oxysterol	LXR ligand	putative ER ligand	[124]
20(S)HC	oxysterol	LXR ligand		[124, 125]
22(R)HC	oxysterol	LXR ligand		[124, 126, 133]
24(S)HC	oxysterol	LXR ligand		[124, 126]
25HC	oxysterol	LXR ligand, ER ligand		[124, 126, 128]
27HC	oxysterol	LXR ligand, ER ligand		[22, 108, 124, 129, 130]
24, 25EC	oxysterol	LXR ligand		[126]
7KC	oxysterol	LXR ligand, ER ligand		[127, 131]
Dendrogenin A	conjugation of 5,6α-epoxy-cholesterol with histamine	LXR ligand		[132, 133]
Desmosterol	cholesterol precursor	LXR ligand	putative ER ligand	[134, 135]
GW3965	synthetic LXR agonist	LXR ligand		[133]
T091317	synthetic LXR agonist	LXR ligand		[125, 133]

Supplemental Figure Legends:

Supplemental Figure 1. 27HC-treated macrophages suppress T cell expansion. (A) Representative histograms from flow cytometry. CD3+ T cells were isolated from the spleen of OT-1 mice, stained with CFSE, were co-cultured with BMDMs treated with $OVA_{257-264}$ and either vehicle- or 27HC. 72hrs after co-culture, T cells were harvested for flow cytometry analysis. This data is from Fig. 3E, and is also representative of **Figs. 2D&E**. **(B)** Naïve T cells were isolated from the spleen of wildtype C57BL/6 mouse, chemically activated and cultured in the presence of conditioned media harvested from vehicle- or 27HC-treated BMDMs. 72hrs after co-culture, T cells were harvested for flow cytometry analysis. Data are presented as mean ± SEM and asterisks (*) indicate statistical significance from the vehicle control (N=3/group for T cell only control, N=6/group for conditioned media two-tailed t-test, $p < 0.05$). **(C)**. CD3+ T cells were isolated from the spleen of wildtype C57BL/6 mice, labeled with CFSE, chemically activated and cultured with conditioned media harvested from vehicle- or 27HC-treated BMDMs. 72hrs after co-culture, T cells were harvested for flow cytometry analysis. Data are presented as mean ± SEM and asterisks (*) indicate statistical significance from the respective vehicle control (N=3/group for T cell only controls, for conditioned-media conditions, N=5-6/group). (two-tailed t-test, $p < 0.05$). This sub-figure is an independent repeat of **Fig. 1I.**

Supplemental Figure 2. Blockade of MHCII does not attenuate pro-colonizing effects of 27HC. Mice were pretreated with placebo or 27HC (20mg/kg/d) for 5d, as in [23]. 36h and 12h prior to cell graft, mice were injected (i.p) with an isotype control antibody (IgG) or an antibody against murine I-A/I-E. Mice were then grafted with Met1 cells expressing iRFP. Resulting cancer colonies within the lung were quantified by *ex vivo* imaging for iRFP fluorescence. Different letters denote statistical significance, N=8/group. (one-way ANOVA followed by Neuman-Kuels multiple comparison test, $p < 0.05$).

Supplemental Figure 3. Expression of granzyme B (GZMB) and perforin (PRF1) normalized to CD8A expression was modestly but significantly correlated with CYP7B1 expression. mRNA expressions from Human RNA sequencing data from TCGA Pan Cancer for invasive breast carcinoma were log2 transformed and Pearson's correlation was assessed (N=1077 in (A) and 1081 in (B)). These data partner with **Fig. 3L and M**.

Supplemental Figure 4. Cholesterol efflux from macrophages unlikely to mediate the suppressive effect of 27HC on T cells. CD3+ T cells were isolated from wildtype C57BL/6 or $LDLR^{-/-}$ mice, labeled with CFSE and chemically activated. They were then co-cultured with vehicle- or 27HC-treated BMDMs. 72hrs after co-culture, T cells were harvested for flow cytometry analysis. Data are presented as mean ± SEM and asterisks (*) indicate statistical significance from the respective vehicle control (N=3/group for all T cell only controls, for co-culture conditions, N=5/group). (two-tailed t-test, $p < 0.05$).

Supplemental Figure 5. ELISA analysis of TGFβ after macrophages were treated with LPS and either vehicle or 27HC. BMDMs were treated with LPS and either vehicle or 27HC for 24hrs. Conditioned media was harvested and TGFβ was quantified by ELISA (R&D, DY1679-05).

Supplemental Figure 6. Previously reported pathways involved in myeloid cell suppression of T cells (PI3K, AKT, NOTCH and CaMKK2) were ruled out from the suppressive mechanism of 27HC-treated macrophages. BMDMs were treated with vehicle ± indicated inhibitors or 27HC ± indicated inhibitors for 24hrs. CD3+ T cells were then isolated from wildtype C57BL/6 mouse, labeled with CFSE and chemically activated at the time of co-culture with pre-treated BMDMs. 72hrs after co-culture, T cells were harvested for flow cytometry analysis. Data are presented as mean ± SEM and asterisks (*) indicate statistical significance from the respective vehicle control (N=3/group for all T cell only controls, for co-culture conditions, N=3-4/group). (two-tailed t-test, $p < 0.05$).

Supplemental Figure 7. Estrogenic activity of 13 ligands. T47D cells stably expressing a ERE luciferase reporter were treated with indicated compounds and the activation of ER was measured by luciferase activity. Data are presented as mean ± SEM (N=4/treatment).

Supplemental Figure 8. LXR-activating activity of 12 cholesterol metabolites, synthetic LXR ligands and E2. BMDMs were treated with indicated compounds and mRNA expression of **(top)** ABCA1 (N=3/group for all

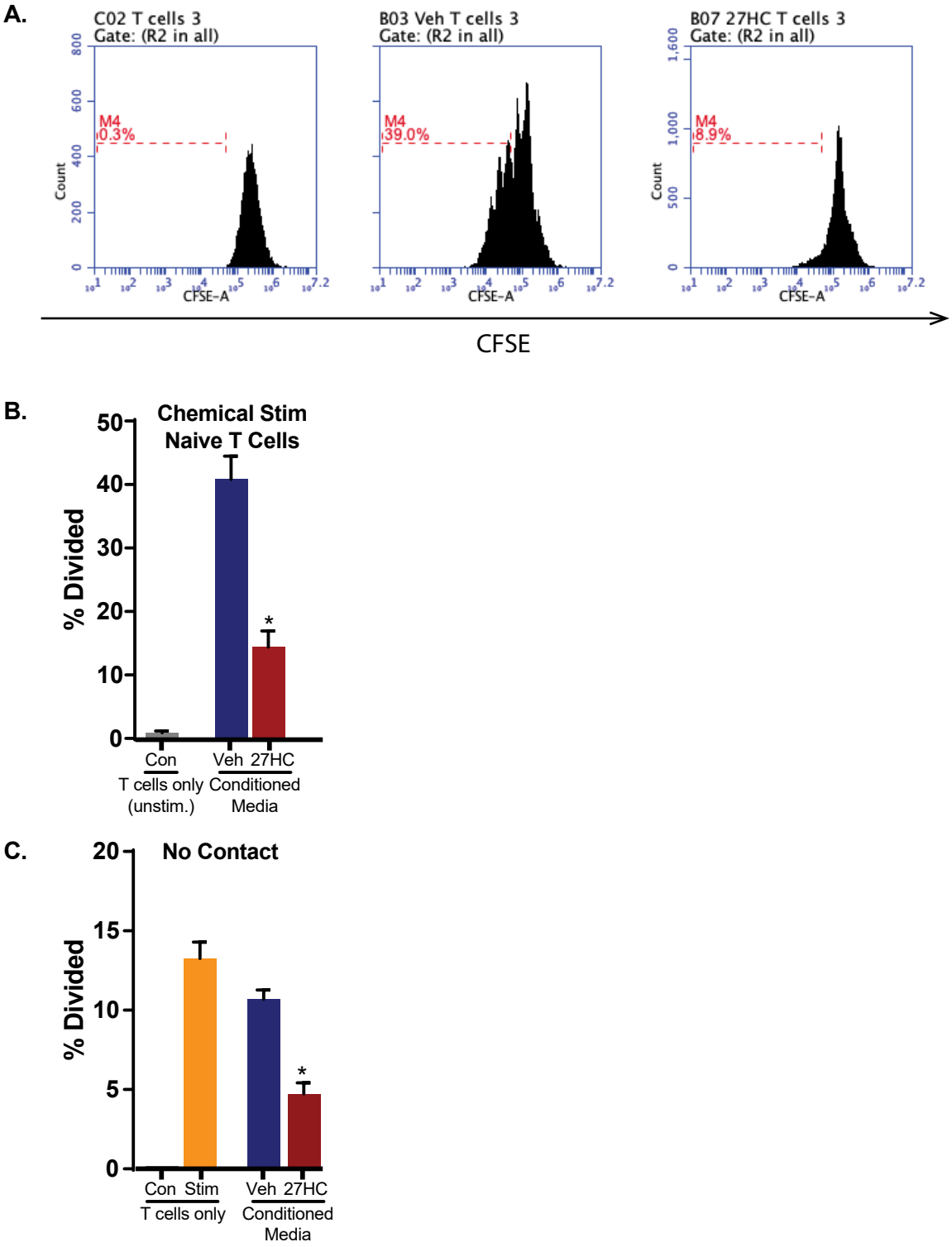
cholesterol metabolites and synthetic LXR ligands, N=5 or 6/group for E2) and **(bottom)** ABCG1 (N=3/group), were quantified by qPCR. Data are presented as mean ± SEM relative to the respective vehicle control.

Supplemental Figure 9. Knockdown or inhibition of LXR isoforms attenuates immunosuppressive effects of 27HC and the synthetic LXR agonist, GW3965 (A) Representative LXRα and β expression after siRNA knockdown for 48hrs as measured by qPCR (N=3/group; This corresponds with **Fig. 5G**). **(B)** LXRα and β expression after lentivirus-delivered shRNA knockdown for 96hrs as measured by qPCR (N=2/group; this corresponds with **Fig. 5H**). **(C)** T cell proliferation was restored with the LXR antagonist, GSK2033. After 24hrs, the treatments were washed off and treated BMDMs were co-cultured with chemically activated wildtype T cells for 72hrs. T cells were labeled with CFSE and proliferation measured by flow cytometry (N=3/group for T cell only controls, for co-culture conditions, N=4/group). Data are presented as mean ± SEM and asterisks (*) indicate statistical significance from the respective vehicle control. (two-tailed t-test, $p < 0.05$). This corresponds with **Fig. 5I**.

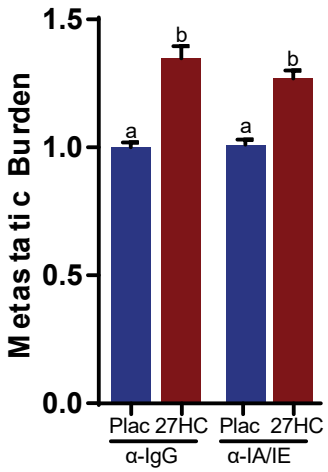
Supplemental Figure 10. 27HC-treated macrophages induce T cell apoptosis. (A) Representative flow cytometry dot plots of **Fig. 6B** (48hrs), where T cells were harvested and labeled with Annexin V and PI, after co-culture with vehicle- (top) or 27HC-treated BMDMs (bottom) for 48hrs. **(B)** Representative flow cytometry dot plots of **Fig. 6C** (48hrs), where T cells were harvested and labeled with FLICA-FAM and PI, after co-culture with vehicle- (top) or 27HC-treated BMDMs (bottom) for 48hrs.

Supplemental Figure 11. CYP27A1$^{fl/fl}$;LysMCre+ mice do not express CYP27A1 in bone marrow-derived macrophages. Tissues were harvested from 3 separate CYP27A1$^{fl/fl}$;LysMCre+ and 3 CYP27A1$^{+/+}$;LysMCre^{+} (control) male mice. Tissue was harvested from a traditional (global) CYP27A1$^{-/-}$ mouse as a control. Bone marrow was harvested and used to derive macrophages. **(A)** Genotyping analysis indicating presence of floxed alleles in the CYP27A1 gene (smaller PCR amplicon). **(B)** Protein was extracted in RIPA buffer and run on SDS-page for Western analysis using anti-CYP27A1 (Abcam 126785) or cyclophilin B (Cyp B; Santa Cruz Biotechnology sc-517566) as an internal control. As can be noted, CYP27A1 was not expressed in macrophages, but continued to be expressed in the liver, spleen and lungs of CYP27A1$^{fl/fl}$;LysMcre^{+} mice.

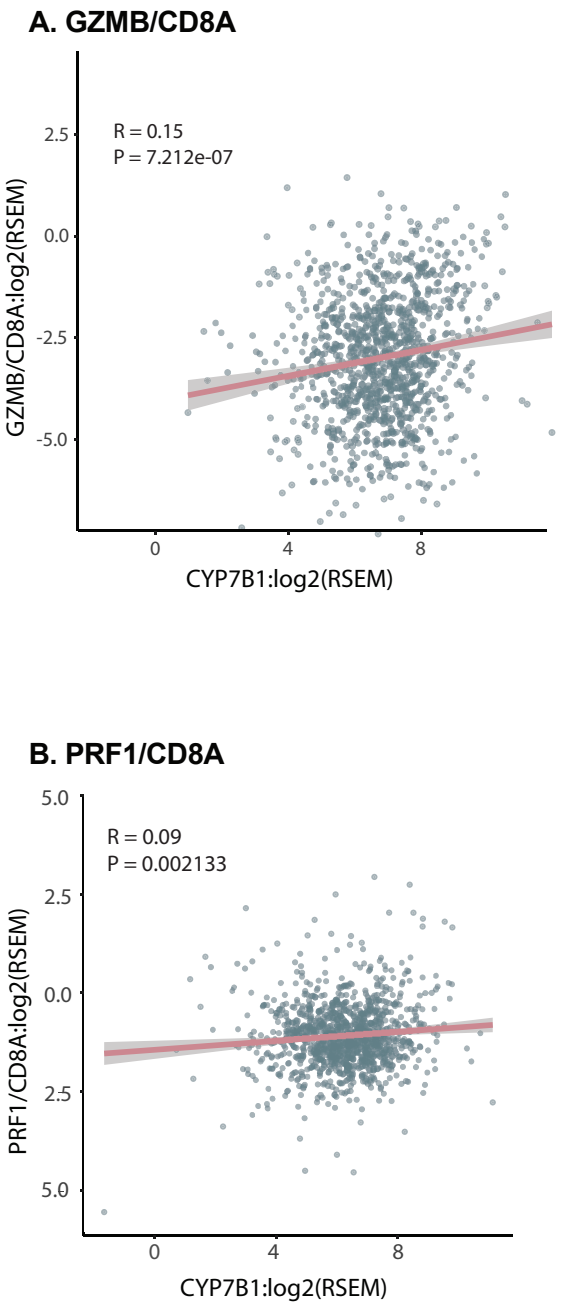
Supplemental Figure 12. Absence of myeloid CYP27A1 decreased tumor growth post T-cell graft. Mice with myeloid cell knockout of CYP27A1 (CYP27A1$^{fl/fl}$;LysMCre^{+}) exhibited lower level of antigen-specific primary tumor growth. CYP27A1$^{fl/fl}$;LysMCre^{+} mice (N=8) and their replete controls (N=7) were grafted orthotopically with E0771-OVA cells and tumors allowed to grow to ~200 mm^3. Control (CYP27A1$^{+/+}$;LysMCre^{+}) mice received an adoptive transfer of CD3+ T cells isolated from OT-I mice 12 days post-cancer graft (first dashed line), and CYP27A1$^{fl/fl}$;LysMCre^{+} mice received an adoptive transfer of CD3+ T cells isolated from OT-I mice 13 days post-cancer graft. Control and CYP27A1$^{fl/fl}$;LysMCre^{+} mice were euthanized 25 days after initial cancer cell graft respectively. **(A)** Tumor volumes were measured through time by digital calipers and plotted as mean ± SEM. Two way ANOVA followed by Sidak's multiple comparison test on data up to and including day 25 found a statistically significant difference between the groups on day 25 ($p < 0.05$, denoted by asterisk). **(B)** Growth of tumors post T cell transfer. This is the same data from **(A), but** adjusted for time post T cell transfer. Data were then fit to a curve with non-linear regression (four parameter variable slope). This corresponds with **Fig. 7A**.



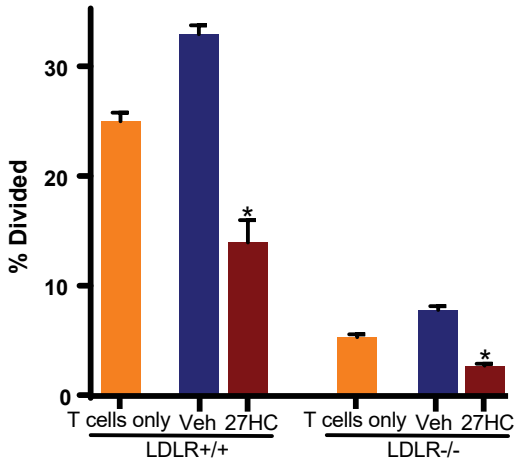
Supplemental Figure 1. 27HC-treated macrophages suppress T cell expansion. (A) Representative histograms from flow cytometry. CD3+ T cells were isolated from the spleen of OT-1 mice, stained with CFSE, were co-cultured with BMDMs treated with $OVA_{257-264}$ and either vehicle- or 27HC. 72hrs after co-culture, T cells were harvested for flow cytometry analysis. This data is from Fig. 3E, and is also representative of **Figs. 2D&E**. **(B)** Naïve T cells were isolated from the spleen of wildtype C57BL/6 mouse, chemically activated and cultured in the presence of conditioned media harvested from vehicle- or 27HC-treated BMDMs. 72hrs after co-culture, T cells were harvested for flow cytometry analysis. Data are presented as mean ± SEM and asterisks (*) indicate statistical significance from the vehicle control (N=3/group for T cell only control, N=6/group for conditioned media two-tailed t-test, $p < 0.05$). **(C)**. CD3+ T cells were isolated from the spleen of wildtype C57BL/6 mice, labeled with CFSE, chemically activated and cultured with conditioned media harvested from vehicle- or 27HC-treated BMDMs. 72hrs after co-culture, T cells were harvested for flow cytometry analysis. Data are presented as mean ± SEM and asterisks (*) indicate statistical significance from the respective vehicle control (N=3/group for T cell only controls, for conditioned-media conditions, N=5-6/group). (two-tailed t-test, $p < 0.05$). This sub-figure is an independent repeat of **Fig. 1I.**



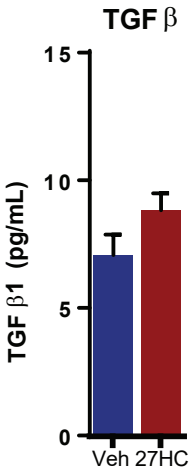
Supplemental Figure 2. Blockade of MHCII does not attenuate pro-colonizing effects of 27HC. Mice were pretreated with placebo or 27HC (20mg/kg/d) for 5d, as in [23]. 36h and 12h prior to cell graft, mice were injected (i.p) with an isotype control antibody (IgG) or an antibody against murine I-A/I-E. Mice were then grafted with Met1 cells expressing iRFP. Resulting cancer colonies within the lung were quantified by *ex vivo* imaging for iRFP fluorescence. Different letters denote statistical significance, N=8/group. (one-way ANOVA followed by Neuman-Kuels multiple comparison test, $p < 0.05$).



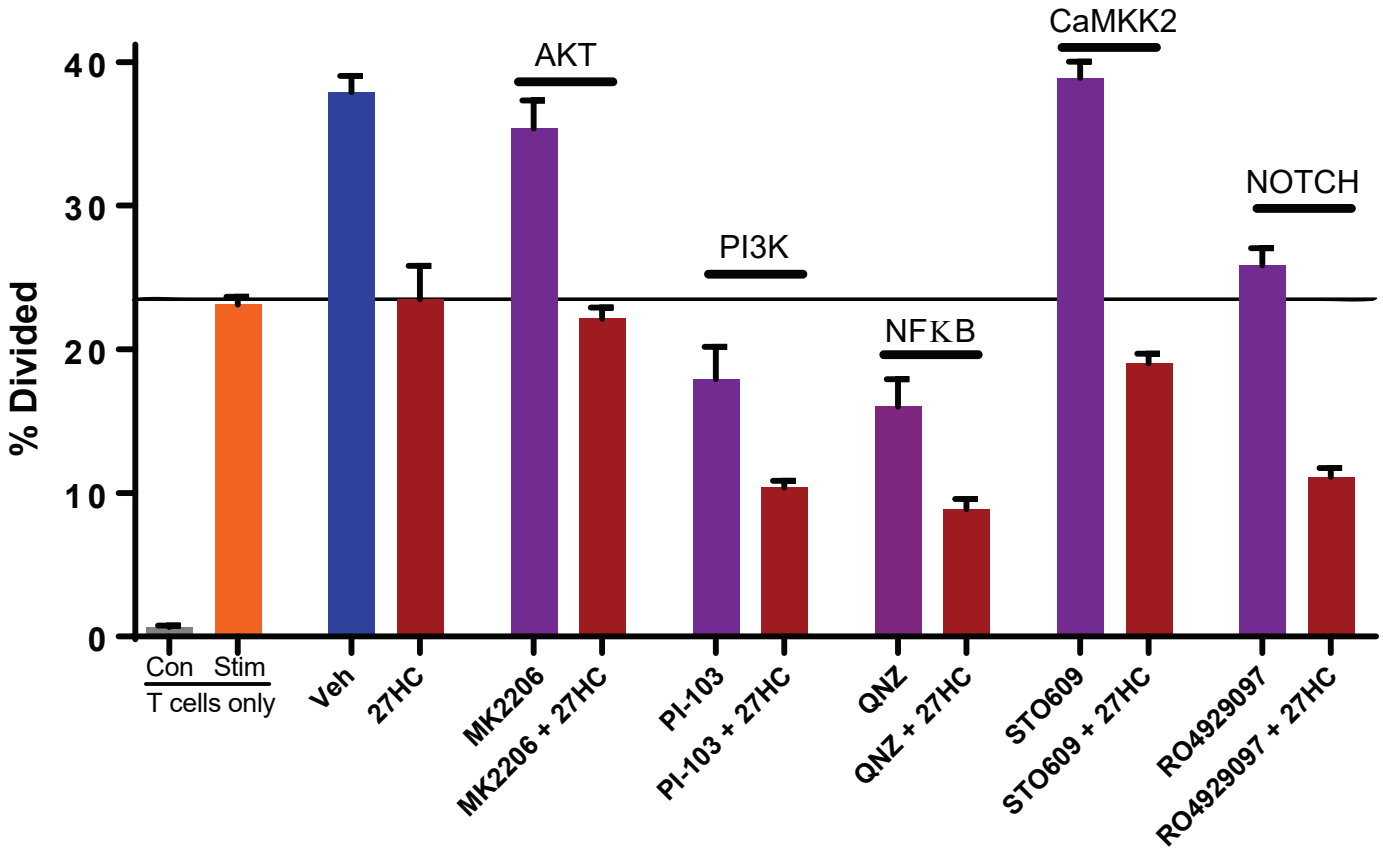
Supplemental Figure 3. Expression of granzyme B (GZMB) and perforin (PRF1) normalized to CD8A expression was modestly but significantly correlated with CYP7B1 expression. mRNA expressions from Human RNA sequencing data from TCGA Pan Cancer for invasive breast carcinoma were log2 transformed and Pearson's correlation was assessed (N=1077 in (A) and 1081 in (B)). These data partner with **Fig. 3L and M**.



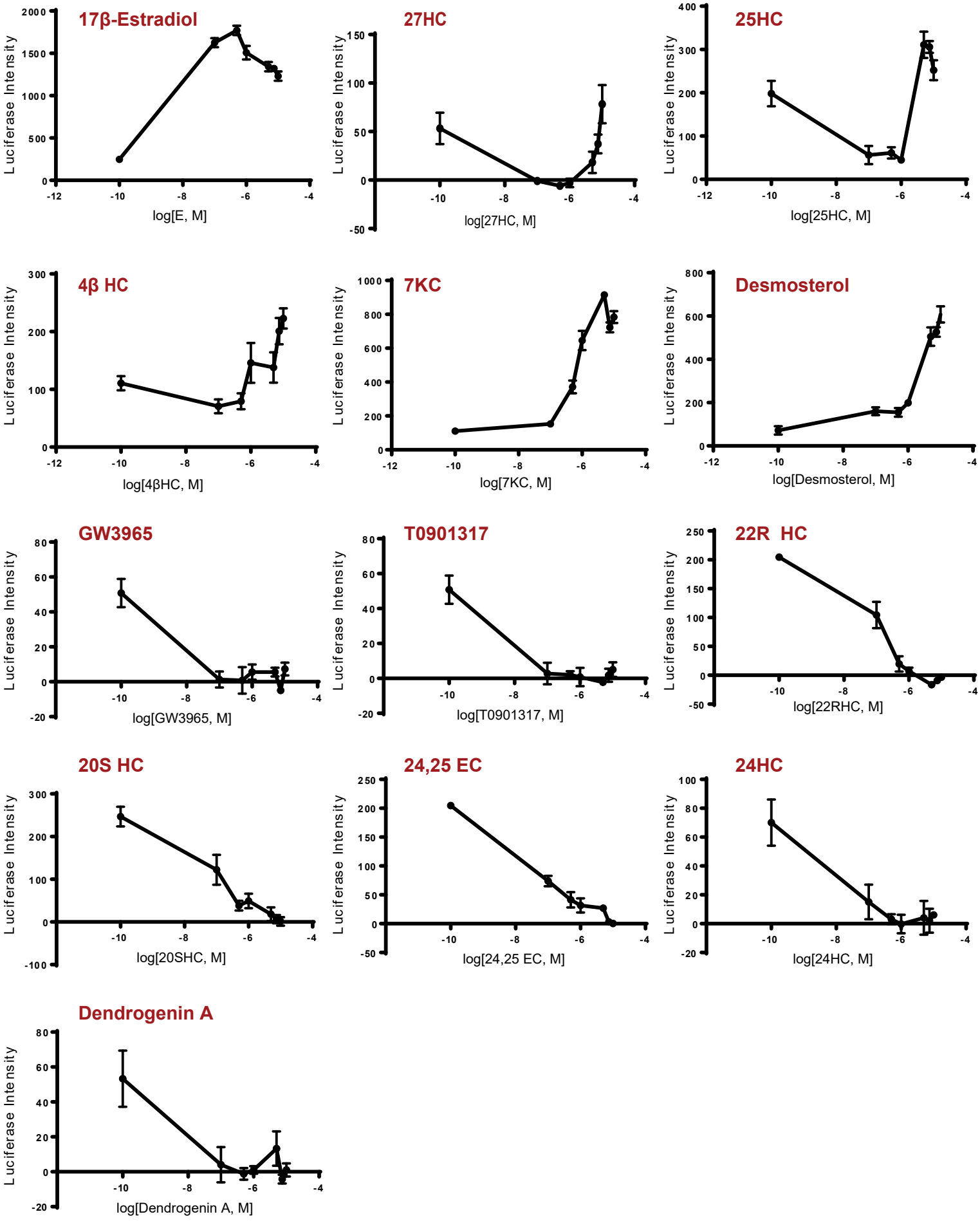
Supplemental Figure 4. Cholesterol efflux from macrophages unlikely to mediate the suppressive effect of 27HC on T cells. CD3+ T cells were isolated from wildtype C57BL/6 or LDLR$^{-/-}$ mice, labeled with CFSE and chemically activated. They were then co-cultured with vehicle- or 27HC-treated BMDMs. 72hrs after co-culture, T cells were harvested for flow cytometry analysis. Data are presented as mean ± SEM and asterisks (*) indicate statistical significance from the respective vehicle control (N=3/group for all T cell only controls, for co-culture conditions, N=5/group). (two-tailed t-test, $p < 0.05$).



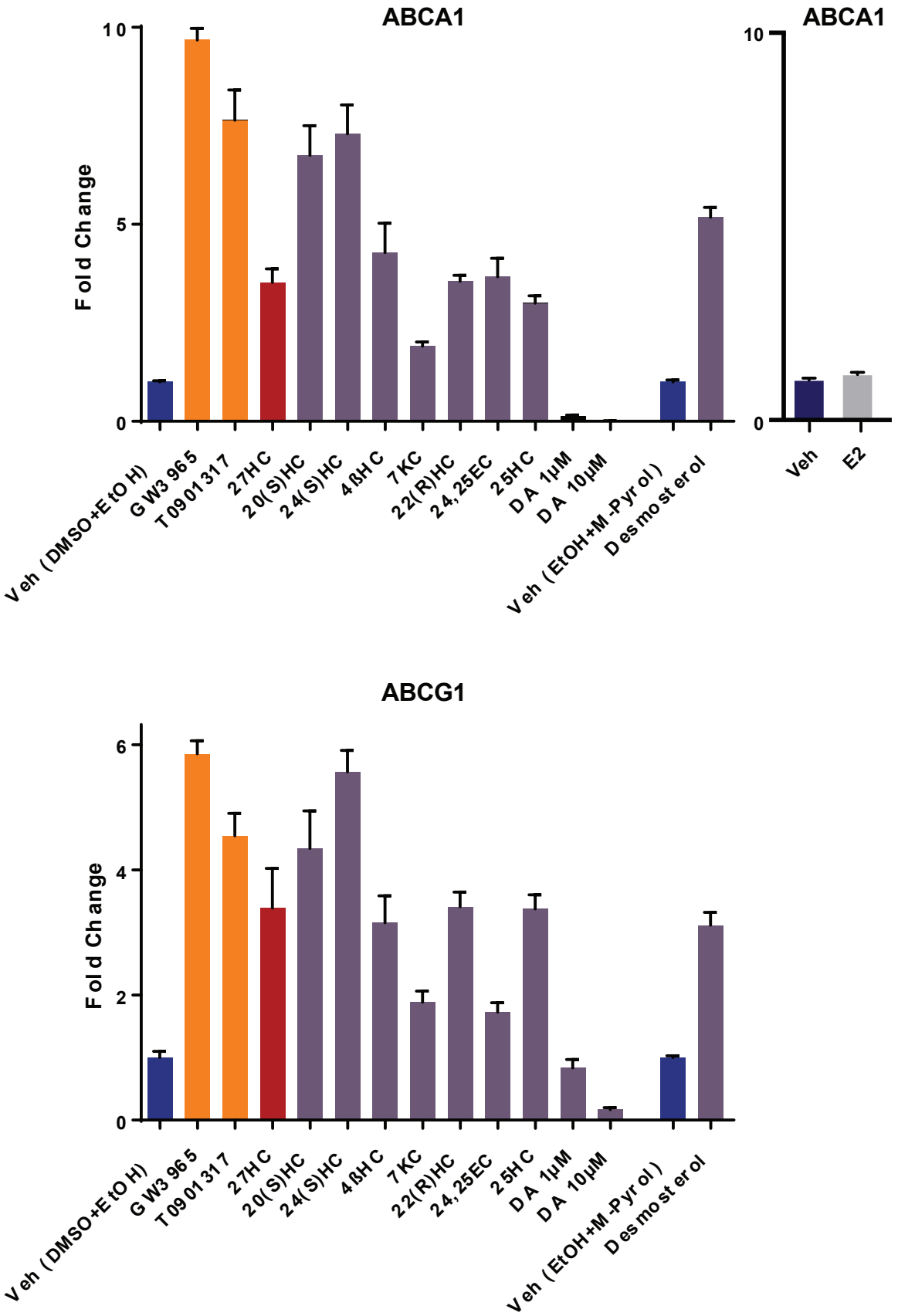
Supplemental Figure 5. ELISA analysis of TGFβ after macrophages were treated with LPS and either vehicle or 27HC. BMDMs were treated with LPS and either vehicle or 27HC for 24hrs. Conditioned media was harvested and TGFβ was quantified by ELISA (R&D, DY1679-05).



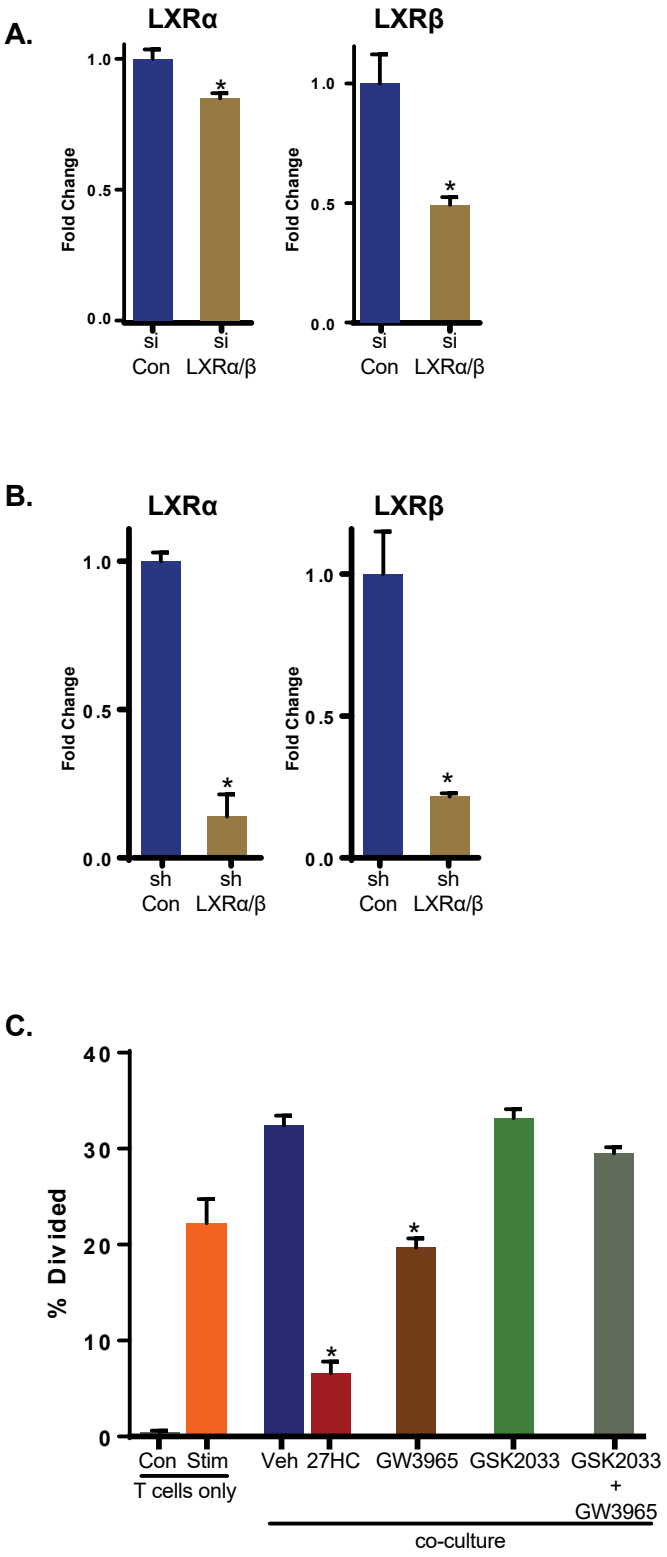
Supplemental Figure 6. Previously reported pathways involved in myeloid cell suppression of T cells (PI3K, AKT, NOTCH and CaMKK2) were ruled out from the suppressive mechanism of 27HC-treated macrophages. BMDMs were treated with vehicle ± indicated inhibitors or 27HC ± indicated inhibitors for 24hrs. CD3+ T cells were then isolated from wildtype C57BL/6 mouse, labeled with CFSE and chemically activated at the time of co-culture with pre-treated BMDMs. 72hrs after co-culture, T cells were harvested for flow cytometry analysis. Data are presented as mean ± SEM and asterisks (*) indicate statistical significance from the respective vehicle control (N=3/group for all T cell only controls, for co-culture conditions, N=3-4/group). (two-tailed t-test, $p < 0.05$).



Supplemental Figure 7. Estrogenic activity of 13 ligands. T47D cells stably expressing a ERE luciferase reporter were treated with indicated compounds and the activation of ER was measured by luciferase activity. Data are presented as mean ± SEM (N=4/treatment).

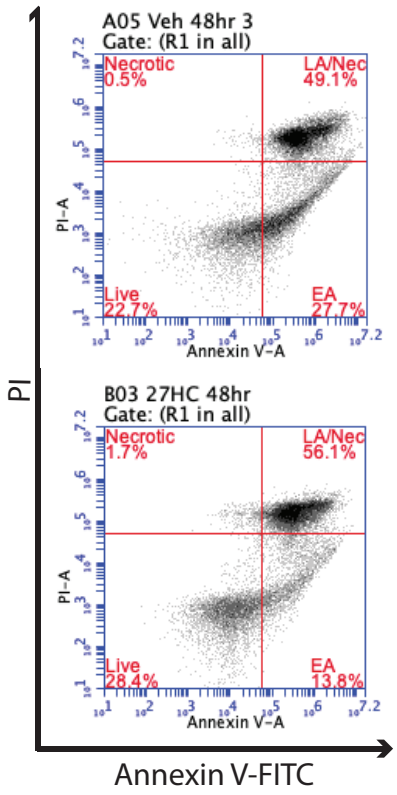


Supplemental Figure 8. LXR-activating activity of 12 cholesterol metabolites, synthetic LXR ligands and E2. BMDMs were treated with indicated compounds and mRNA expression of **(top)** ABCA1 (N=3/group for all cholesterol metabolites and synthetic LXR ligands, N=5 or 6/group for E2) and **(bottom)** ABCG1 (N=3/group), were quantified by qPCR. Data are presented as mean ± SEM relative to the respective vehicle control.



Supplemental Figure 9. Knockdown or inhibition of LXR isoforms attenuates immunosuppressive effects of 27HC and the synthetic LXR agonist, GW3965 (A) Representative LXRα and β expression after siRNA knockdown for 48hrs as measured by qPCR (N=3/group; This corresponds with **Fig. 5G**). **(B)** LXRα and β expression after lentivirus-delivered shRNA knockdown for 96hrs as measured by qPCR (N=2/group; this corresponds with **Fig. 5H**). **(C)** T cell proliferation was restored with the LXR antagonist, GSK2033. After 24hrs, the treatments were washed off and treated BMDMs were co-cultured with chemically activated wildtype T cells for 72hrs. T cells were labeled with CFSE and proliferation measured by flow cytometry (N=3/group for T cell only controls, for co-culture conditions, N=4/group). Data are presented as mean ± SEM and asterisks (*) indicate statistical significance from the respective vehicle control. (two-tailed t-test, $p < 0.05$). This corresponds with **Fig. 5I**.

A.



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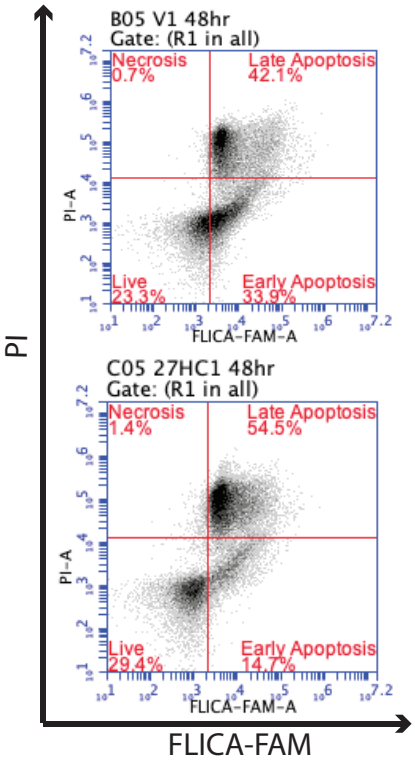
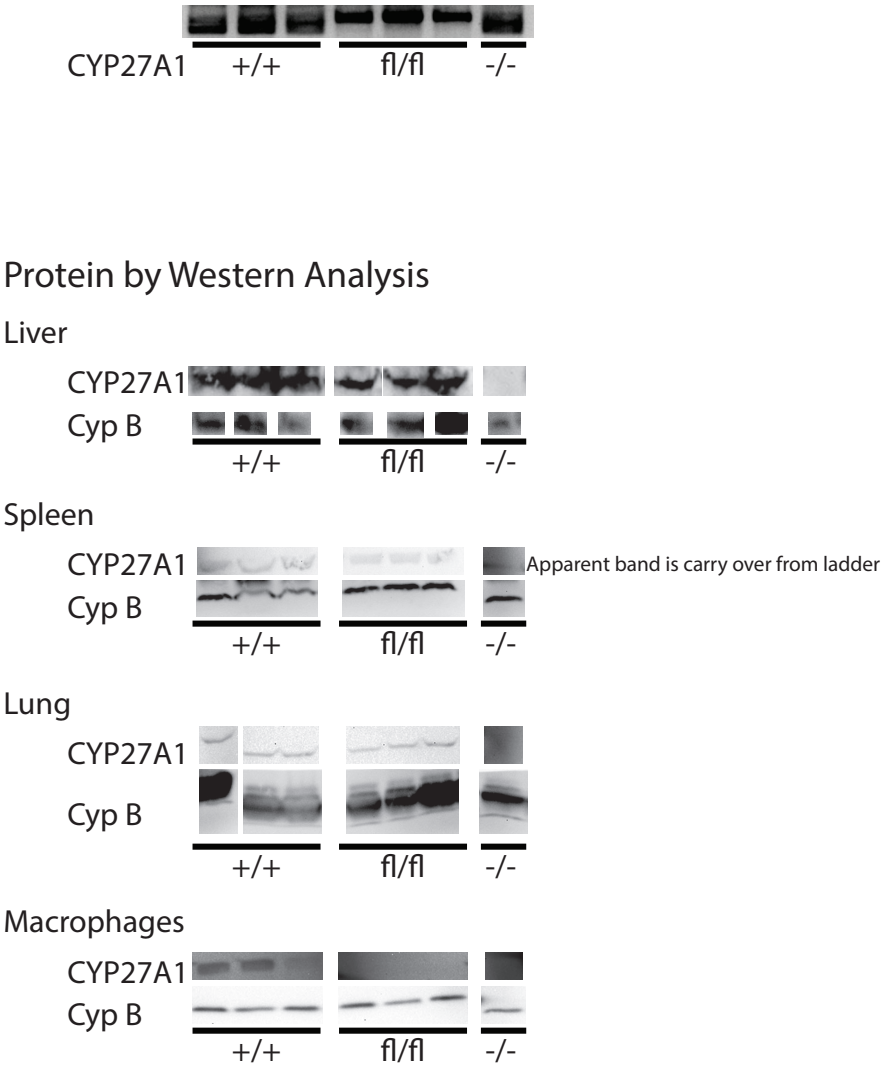
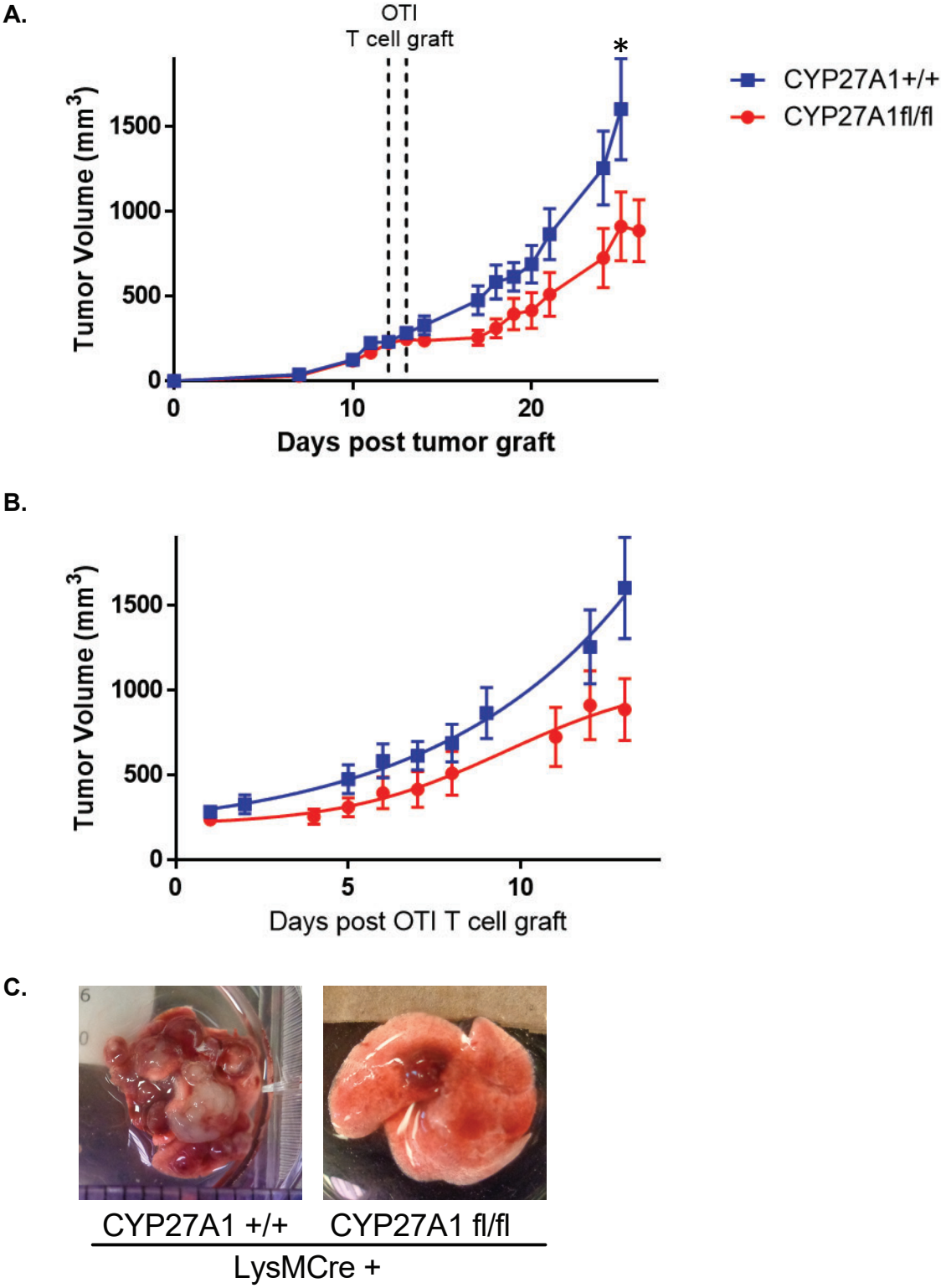


Supplemental Figure 10. 27HC-treated macrophages induce T cell apoptosis. (A) Representative flow cytometry dot plots of **Fig. 6B** (48hrs), where T cells were harvested and labeled with Annexin V and PI, after co-culture with vehicle- (top) or 27HC-treated BMDMs (bottom) for 48hrs. **(B)** Representative flow cytometry dot plots of **Fig. 6C** (48hrs), where T cells were harvested and labeled with FLICA-FAM and PI, after co-culture with vehicle- (top) or 27HC-treated BMDMs (bottom) for 48hrs.



Supplemental Figure 11. CYP27A1$^{fl/fl}$;LysMCre+ mice do not express CYP27A1 in bone marrow-derived macrophages. Tissues were harvested from 3 separate CYP27A1$^{fl/fl}$;LysMCre+ and 3 CYP27A1$^{+/+}$;LysMCre^{+} (control) male mice. Tissue was harvested from a traditional (global) CYP27A1$^{-/-}$ mouse as a control. Bone marrow was harvested and used to derive macrophages. **(A)** Genotyping analysis indicating presence of floxed alleles in the CYP27A1 gene (smaller PCR amplicon). **(B)** Protein was extracted in RIPA buffer and run on SDS-page for Western analysis using anti-CYP27A1 (Abcam 126785) or cyclophilin B (Cyp B; Santa Cruz Biotechnology sc-517566) as an internal control. As can be noted, CYP27A1 was not expressed in macrophages, but continued to be expressed in the liver, spleen and lungs of CYP27A1$^{fl/fl}$;LysMcre^{+} mice.



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