Supplementary Table S1: Patient demographics for MPM PDOTs and TILs

MPM Sample ID	ADU-S100 Response	TAK-676 Response	Age	Gender	Histology	Neoadjuvant	Figures
1	NR	NT	77	M	E	N	2C, 2D, S5B, S5C
2	NR	NT	79	F	E	N	2C, 2D, S5B, S5C
3	NR	NT	70	M	B	N	2C, 2D, S5C
4	NR	NT	69	M	E	Y	2C, 2D, S5B
5	NR	NT	U	U	U	U	2C, 2D, S5B, S5C
6	NR	NT	68	M	B	Y	2C, 2D, S5C
7	NR	NT	38	F	E	N	2C, 2D, S5B, S5C
8	NR	NR	66	M	E	Y	2C, 2D, 2F, S5C, S5B
9	NR	NR	68	M	E	N	2C, 2D, 2F, S5C
10	NR	NR	79	M	E	N	2C, 2D, 2F, S5C
11	NR	NT	52	M	B	N	2C, 2D, S5B, S5C
12	NR	NR	74	M	E	N	2C, 2D, 2F, 4A, S5C, S9A
13	NR	NT	71	M	E	Y	2C, 2D, S5B, S5C
14	NR	NT	75	F	B	N	2C, 2D, S5B, S5C
15	NR	NT	71	F	B	N	2C, 2D, S5C
16	NR	NT	77	M	E	N	2C, 2D, S5C
17	NR	NR	53	M	E	N	2C, 2D, 2F, S5C
18	NR	NT	80	M	B	N	2C, 2D, S5C
19	NR	NT	76	M	E	N	2C, 2D, S5B, S5C
20	NR	NT	72	M	B	N	2C, 2D, S5B, S5C
21	NR	NT	79	M	B	N	2C, 2D, S5B, S5C
22	NR	NR	77	M	E	N	2C, 2D, 2F, S5C

23	NR	NR	73	M	E	N	2C, 2D, 2F, S5C
24	NR	R	69	M	E	Y	2C, 2D, 2F, S5C
25	NR	NT	79	F	B	N	2C, 2D, S5C
26	NR	NR	78	F	E	N	2C, 2D, 2F, 2G, 3, S5C, S6B, S6C, S7, S8
27	NR	NT	82	F	E	N	2C, 2D, S5B, S5C
28	NR	NT	68	F	E	Y	2C, 2D, S5B, S5C
29	R	NT	67	M	E	N	2C, 2D, S5C
30	R	NT	79	M	E	Y	2C, 2D, S5B, S5C
31	R	NT	58	F	E	N	2C, 2D, S5B, S5C
32	R	NT	75	M	E	N	2C, 2D, 5C, S5C, S10C
33	R	R	60	M	E	N	2C, 2D, 2F, S5A, S5C
34	R	R	66	M	E	N	2C-E, 2F, S5C, S6A, S6E
35	R	R	80	M	B	Y	2A-D, 2F, S5A, S5C
36	NR	NT	83	M	E	U	5A, S10A
37	NR	NT	30	F	B	U	5B

R = response to treatment with cell death >20% above control, T-test $p<0.05$; NR = non-responder; NT = Not tested. F = female, M = Male gender. E = epithelioid MPM, B = biphasic MPM, Y/N = yes/no neoadjuvant treatment. U = unknown.

TIL Batch Number	Age	Gender	Diagnosis	Figures
1	69	F	Stage II NSCLC	S9D
2	77	F	Stage II NSCLC	S9E, S9F
3	66	M	Stage I NSCLC	4, S9C

F = female, M = Male gender. NSCLC = non-small cell lung cancer.

Supplementary Table S2: Summary of patient demographics for e*x vivo* STING agonist treatment of MPM tumors

	ADU-S100 Responder	Non-responder
Total Patients	7	30
Median Age (years)	67	73
Gender		
Male	6 (86%)	20 (67%)
Female	1 (14%)	9 (30%)
Not recorded	0	1 (3%)
Histology		
Epithelioid	6 (86%)	19 (63%)
Biphasic	1 (14%)	10 (33%)
Sarcomatoid	0	0
Not recorded	0	1 (4%)
Neoadjuvant Therapy		
Yes	2 (29%)	6 (20%)
No	5 (71%)	21 (70%)
Not recorded	0	3 (10%)

Supplementary Table S3: Antibodies used in flow cytometry and western blot experiments

Target	Clone	Manufacturer	Cat. No.
CD69	FN50	BioLegend	310904
CD16	3G8	BioLegend	302006
CD8	RPA-T8	Thermo Fisher	BDB560662
CCR2	K036C2	Biolegend	357203
CD38	HIT2	BioLegend	303506
CD11c	3.9	BioLegend	301605
CCR7	150503	Thermo Fisher	BDB62381
CD56	GDC56	BioLegend	318348
LAG-3	11C3C65	BioLegend	369309
CD103	B-Ly7	Thermo Fisher	25-1038-41
TIM-3	F38-2E2	BioLegend	345012
PD-L1	29E.2A3	BioLegend	329708
CD3	UCHT1	BioLegend	300424
PD-1	EH12.2H7	BioLegend	329920
HLA-DR	G46-6	Thermo Fisher	BDB562804
CD45RA	HL100	BioLegend	304142
CD15	SSEA-1	BioLegend	323028
CTLA-4	BNI3	BioLegend	369609
CD19	HIB19	BioLegend	302243
CD45	H130	BioLegend	304050
CD4	PRA-T4	BioLegend	300554
CD14	M5E2	BioLegend	301840
Mesothelin	REA1057	Miltenyi	130-118-168
STING	D2P2F	Cell Signaling	13647
pTBK1	D52C2	Cell Signaling	5483
TBK1	Polyclonal	Cell Signaling	3013
pIRF3	4D4G	Cell Signaling	4947
IRF3	D6I4C	Cell Signaling	11904
pSTAT1	58D6	Cell Signaling	9167
STAT1	Polyclonal	Cell Signaling	9172
IFNAR-1	Polyclonal	Thermal Fischer	PA5-79441
β-Actin	C4	Santa Cruz	sc-47778

Supplementary Table S4: Gene signatures for scRNAseq

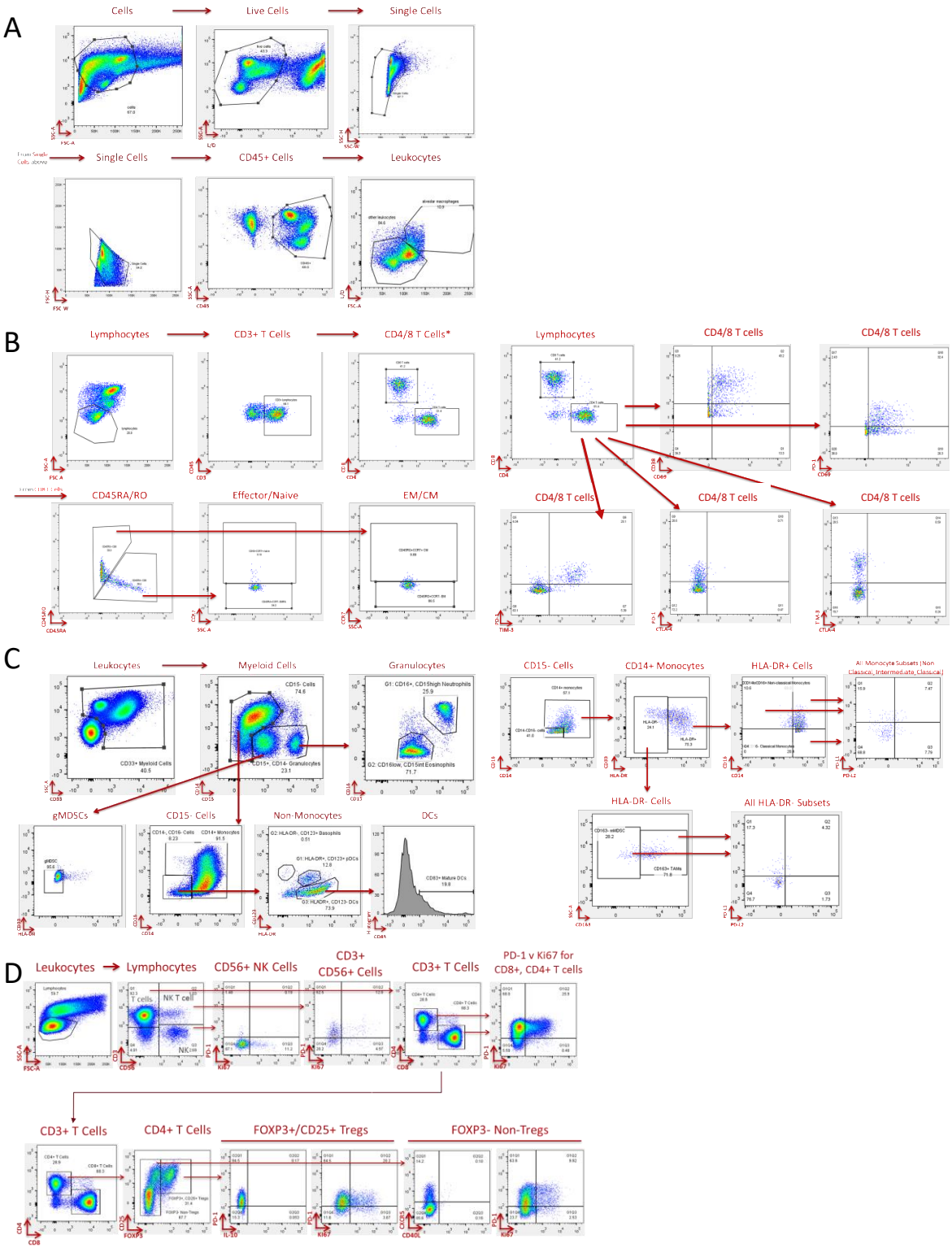
B-cell	Macrophage	Fibroblast	T-Cell	Tumor	NK Cell	NK Active	NK T-cell	Other T-cell	V-gene		
CD79A	*CD14*	*COL3A1*	*TCF7*	*MSLN*	*SH2D1B*	*PRF1*	*CD3D*	*SELL*	*TRBV2*	*TRBV12-4*	*TRAV8-3*
IGHM	*VSIG4*	*COL4A1*	*CD3G*	*ANXA8*	*TRDC*	*KLRB1*	*CD3E*	*CCR7*	*TRBV3-1*	*TRBV14*	*TRAV13-1*
CD79B	*C1QB*	*COL4A2*	*CD3D*	*CALB2*	*TYROBP*	*TNFSF10*	*CD2*	*S1PR1*	*TRBV4-1*	*TRBV18*	*TRAV12-2*
MS4A1	*C1QA*	*COL4A5*	*CD4*	*INHBA*	*KLRD1*	*CCL5*	*CCL5*	*SPOCK2*	*TRBV5-1*	*TRBV19*	*TRAV8-4*
	APOE	*COL4A6*	*CD28*	*ITLN1*	*TNFRSF18*	*NKG7*	*CST7*	*GIMAP5*	*TRBV6-1*	*TRBV20-1*	*TRAV13-2*
		COL5A1	*BCL11B*	*MGARP*	*NKG7*	*KLRD1*	*TRBC2*	*GIMAP7*	*TRBV4-2*	*TRBV21-1*	*TRAV14DV4*
		COL5A2	*CD8A*	*HEG1*	*KLRB1*	*NCAM1*	*CXCR4*	*LTB*	*TRBV6-2*	*TRBV24-1*	*TRAV9-2*
		COL6A1	*CD8B*		*GNLY*	*NCR1*	*CD8A*	*CXCR4*	*TRBV7-2*	*TRBV27*	*TRAV12-3*
		COL6A2			*IL2RB*	*NCR2*	*GZMK*	*IL7R*	*TRBV6-4*	*TRBV28*	*TRAV8-6*
		COL8A1			*CTSW*	*NCR3*	*IL32*	*YNE2*	*TRBV7-3*	*TRBV29-1*	*TRAV17*
		COL12A1			*ALOX5AP*	*FCER1G*	*PLAAT4*	*SARAF*	*TRBV9*	*TRBV30*	*TRAV19*
		COL17A1			*GZMB*	*KIR2DL1*	*GZMA*	*IFITM1*	*TRBV11-2*	*TRAV1-2*	*TRAV21*
		S100A16				*KIR2DL3*			*TRBV6-5*	*TRAV2*	*TRAV22*
		UGDH				*KIR2DL4*			*TRBV7-4*	*TRAV4*	*TRAV23DV6*
		LOXL1				*KLRC1*			*TRBV5-4*	*TRAV5*	*TRAV24*
		PCOLCE2				*KLRC4*			*TRBV7-6*	*TRAV6*	*TRAV25*
		ADAMTS2				*FCGR3A*			*TRBV7-9*	*TRAV8-1*	*TRAV26-1*
						KIR3DL1			*TRBV13*	*TRAV10*	*TRAV29DV5*
						KIR3DL2			*TRBV10-3*	*TRAV12-1*	*TRAV26-2*
									TRBV12-3	*TRAV8-2*	*TRAV35*
											TRAV36DV7
											TRAV38-1
											TRAV38-2DV8
											TRAV39
											TRAV41

Supplementary Table S5: Expression changes with STING agonist treatment

40 Most Increased Genes with Treatment (10μM ADU-S100)					
Gene	**p value**	**avg log2FC**	**pct.1**	**pct.2**	**p val adj**
ISG15	3.23E-39	5.19	1.00	0.71	7.54E-35
IFI27	4.01E-29	4.89	0.87	0.12	9.35E-25
IFI6	1.01E-38	4.76	1.00	0.57	2.35E-34
RSAD2	2.73E-37	4.43	0.97	0.02	6.37E-33
IFIT3	1.04E-36	4.01	0.98	0.56	2.43E-32
IFIT2	1.94E-30	3.99	0.92	0.39	4.53E-26
IFIT1	1.83E-36	3.89	0.97	0.26	4.28E-32
ISG20	4.04E-36	3.89	0.98	0.72	9.43E-32
IL32	1.86E-23	3.43	0.82	0.23	4.34E-19
IFITM1	3.04E-23	3.42	0.85	0.33	7.10E-19
OAS1	4.07E-38	3.40	0.99	0.13	9.51E-34
WARS	1.33E-27	3.24	0.94	0.80	3.11E-23
LY6E	1.61E-38	3.24	1.00	0.95	3.77E-34
CXCL10	2.50E-10	3.10	0.43	0.00	5.83E-06
C15orf48	1.18E-25	3.02	0.91	0.54	2.76E-21
OASL	9.30E-35	3.01	0.94	0.05	2.17E-30
TFPI2	5.10E-12	2.88	0.60	0.16	1.19E-07
BST2	1.38E-16	2.86	0.61	0.02	3.22E-12
MX1	5.77E-36	2.84	0.96	0.10	1.35E-31
LAP3	1.26E-33	2.78	0.97	0.90	2.94E-29
IFI35	6.12E-34	2.69	0.97	0.84	1.43E-29
PLSCR1	1.58E-35	2.64	0.97	0.75	3.69E-31
SAA1	7.21E-12	2.46	0.63	0.15	1.68E-07
CXCL11	4.41E-12	2.45	0.48	0.00	1.03E-07
IRF7	5.88E-36	2.44	0.98	0.82	1.37E-31
PLAAT4	2.08E-25	2.38	0.90	0.49	4.86E-21
IFI30	3.79E-21	2.24	0.75	0.15	8.85E-17
IL1B	2.62E-07	2.23	0.55	0.30	0.0061082
IL1RN	1.12E-15	2.21	0.74	0.28	2.62E-11
CXCL1	3.53E-08	2.20	0.61	0.30	0.0008241
CMPK2	1.25E-31	2.19	0.89	0.03	2.91E-27
CCL5	1.26E-06	2.10	0.29	0.00	0.0294404
HLA-B	1.84E-36	2.09	1.00	1.00	4.29E-32
STAT1	8.11E-32	2.09	0.94	0.57	1.89E-27
HLA-C	1.34E-36	2.08	1.00	1.00	3.12E-32
HES4	2.74E-25	2.07	0.91	0.56	6.40E-21
WFDC2	3.52E-07	2.06	0.73	0.62	0.0082103
TNFSF13B	5.30E-23	2.03	0.77	0.08	1.24E-18
OAS3	9.16E-33	2.02	0.93	0.28	2.14E-28
MDK	3.49E-16	2.01	0.72	0.28	8.15E-12
20 Most Decreased Genes with Treatment					
Gene	**p value**	**avg log2FC**	**pct.1**	**pct.2**	**p val adj**
TGFBI	4.65E-28	-2.38	0.57	0.98	1.09E-23
SERPINB3	2.15E-28	-2.15	0.52	0.98	5.02E-24
KRT19	1.57E-17	-1.81	0.83	1.00	3.66E-13
SERPINB7	1.88E-19	-1.73	0.58	0.95	4.40E-15
CCDC80	3.66E-23	-1.57	0.79	1.00	8.56E-19
ANGPTL4	9.13E-13	-1.48	0.33	0.72	2.13E-08
IGFBP4	5.58E-13	-1.42	0.75	0.97	1.30E-08

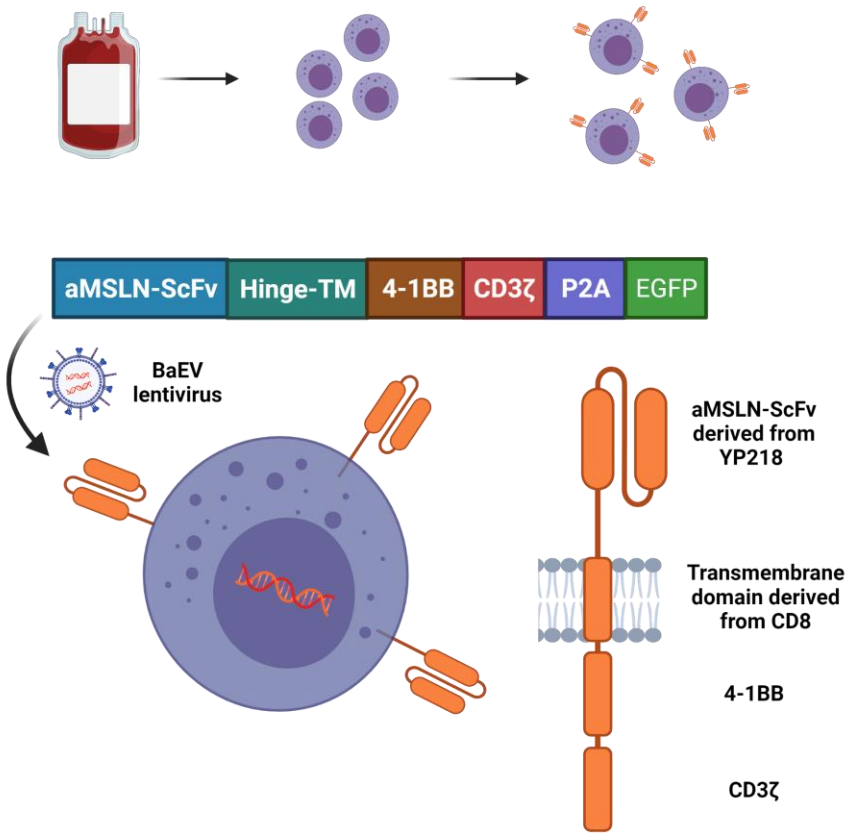
GPI	4.12E-23	-1.27	0.71	0.98	9.62E-19
SERPINB4	2.22E-17	-1.25	0.46	0.90	5.19E-13
CEMIP	1.11E-33	-1.20	0.14	0.71	2.59E-29
LDHA	5.51E-25	-1.16	0.98	1.00	1.29E-20
PABPC1	8.78E-22	-1.11	0.95	1.00	2.05E-17
TPI1	5.70E-21	-1.11	0.96	1.00	1.33E-16
RPSA	1.71E-27	-1.10	0.97	1.00	4.00E-23
RPS29	1.97E-31	-1.10	0.89	1.00	4.61E-27
RPL41	1.57E-31	-1.09	0.95	1.00	3.66E-27
NDRG1	6.92E-13	-1.07	0.48	0.90	1.62E-08
ID1	1.11E-13	-1.07	0.29	0.72	2.60E-09
PGK1	2.74E-22	-1.04	0.92	1.00	6.39E-18
TUBA1A	6.65E-22	-1.02	0.23	0.75	1.55E-17

Supplementary Figure S1: Flow cytometry dot plots and gating strategy



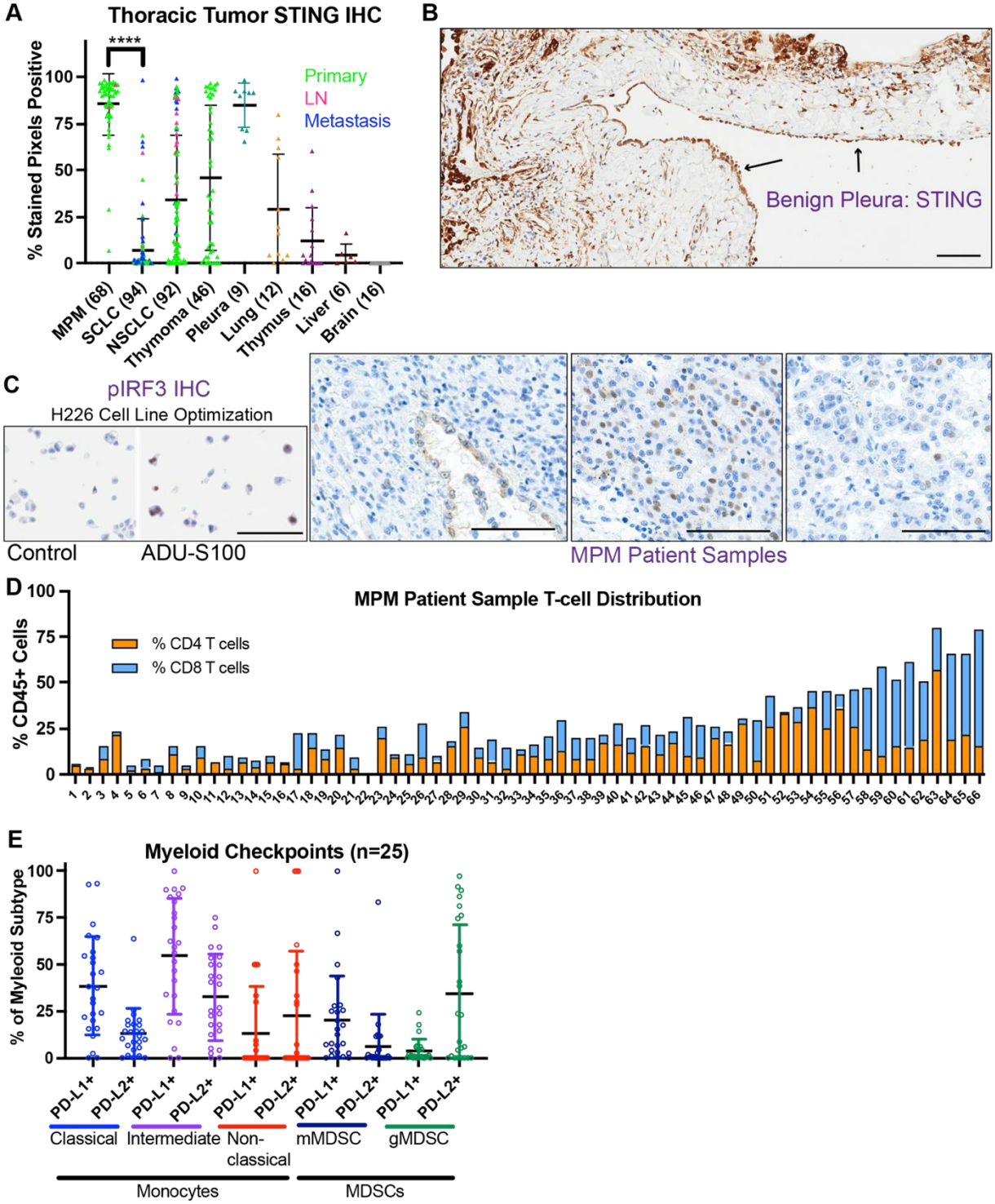
A, Gating strategy and example for viable CD45+ cells. **B,** Gating hierarchy for T cell lineage and phenotypic marker**. C,** Myeloid cell gating hierarchy**. D,** Gating for NK lineage and Tregs.

Supplementary Figure S2: MSLN CAR Construct Design and Map



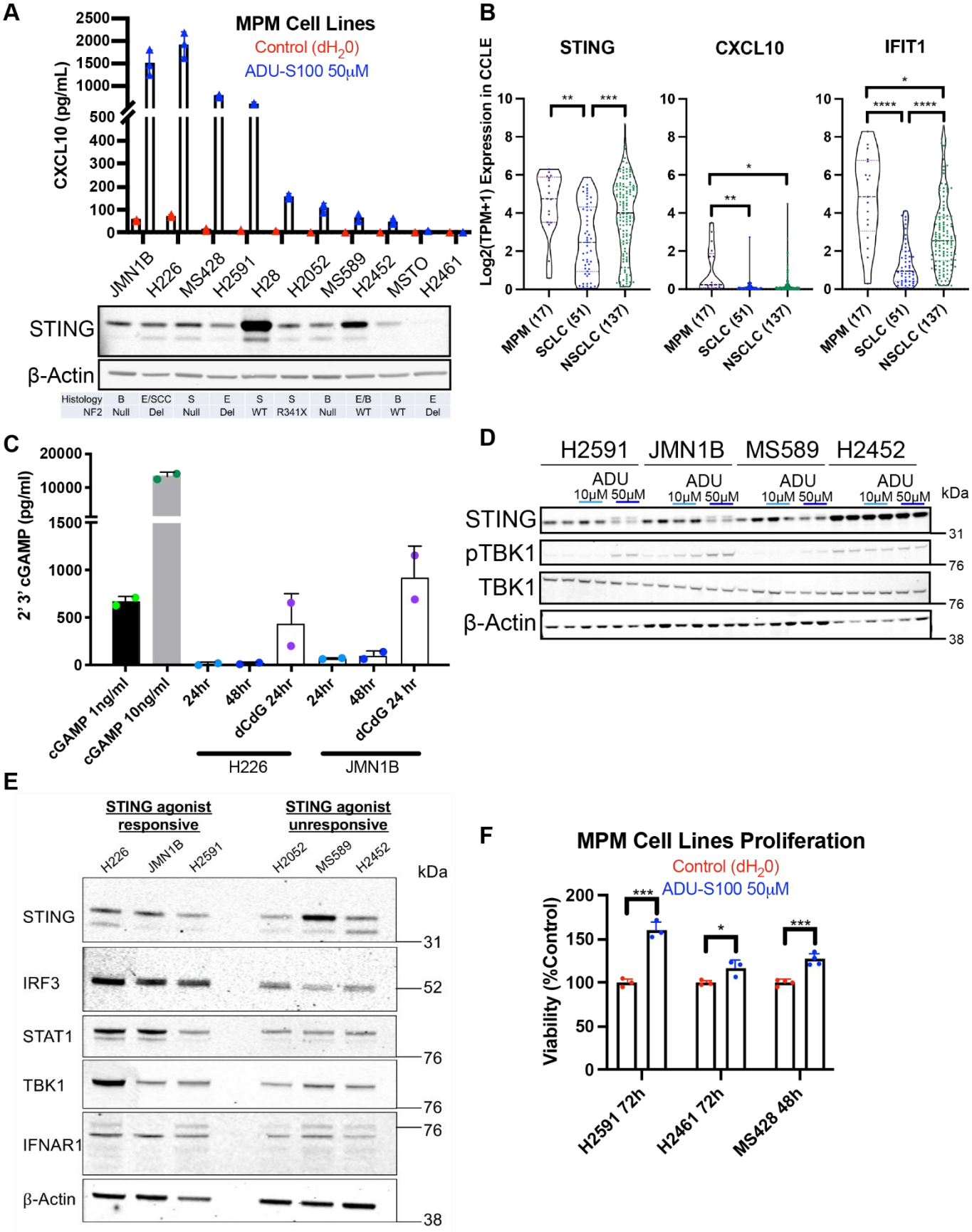
NK-cell isolation and transduction with anti-mesothelin (MSLN) CAR construct (see methods for details).

Supplementary Figure S3: MPM STING and immune characterization



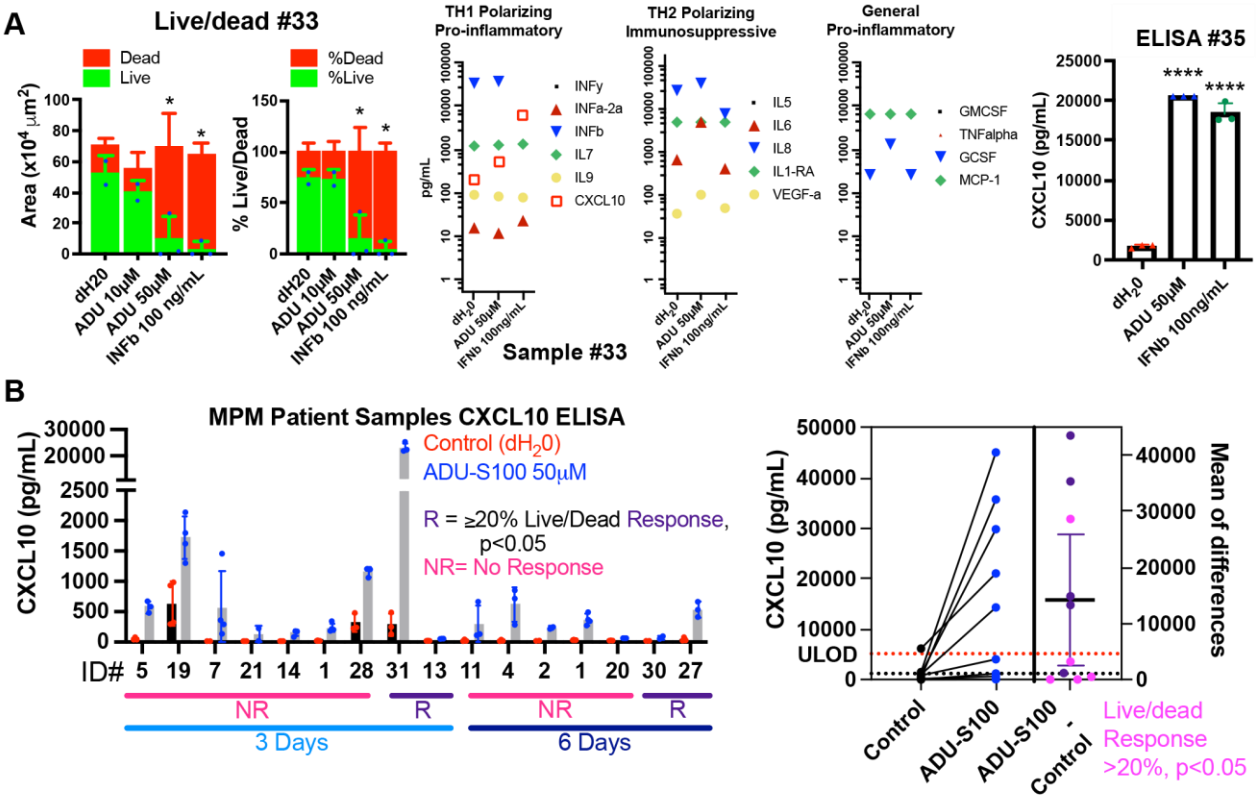
A, STING IHC in thoracic cancers and adjacent normal tissues quantified using QuPath software. Source of tissue indicated by color of dot (primary tumor, LN=lymph node, distant metastasis). MPM = malignant pleural mesothelioma; SCLC = small-cell lung carcinoma; NSCLC = non-small cell lung carcinoma. Kruskal Wallis $p<0.0001$ with Dunn's multiple comparisons test: ****$p<0.0001$. **B,** STING IHC in normal pleura (n=9 total samples with similar appearance). Scale bar = 100 μm. **C,** Phospho-IRF3 (pIRF3) IHC optimization in H226 MPM cells after 24-hour treatment with 50 μM ADU-S100. pIRF3 IHC in MPM tumor specimens (n=31 total samples stained). Scale bars = 100 μm. **D** and **E,** Flow cytometry from freshly resected MPM specimens with number of samples specified either in the graph title or in parentheses on the x axis label. Flow cytometry antibody details provided in **Supplementary Table S3**. mMDSC = monocytic myeloid-derived suppressor cells; gMDSC = granulocytic myeloid-derived suppressor cells.

Supplementary Figure S4: STING expression and activation in MPM cell lines



A, ELISA for CXCL10 after 24-hour treatment with 50 μM ADU-S100 or dH20 control in MPM cell lines. Western blot for STING and beta-actin load control using untreated lysates from each cell line. Publicly available characteristics for each cell line shown in the table below. **B,** mRNA expression data from the Cancer Cell Line Encyclopedia (CCLE) database comparing expression in malignant pleural mesothelioma (MPM), small-cell lung carcinoma (SCLC) and non-small cell lung carcinoma cell lines. Kruskal Wallis $p<0.0001$ for STING and IFIT1, $p<0.01$ for CXCL10 with Dunn's multiple comparisons tests as shown: *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$. **C,** 2'3' cGAMP ELISA from MPM cell lines at 24 and 48-hours of culture with poly (dG:dC) treatment used as positive control (1 μg). **D,** Western blot for STING, phospho-TBK1 (pTBK1), TBK1, and beta-actin load control in MPM cell lines treated for 6 hours with the indicated doses of ADU-S100 in duplicate. **E,** Western blot for STING pathway components and beta-actin load control in MPM cell lines. **F,** CellTiter-Glo viability assay in MPM cell lines treated with 50 μM ADU-S100 or dH20 control for the indicated number of hours. T-test: *$p<0.05$, ***$p<0.001$.

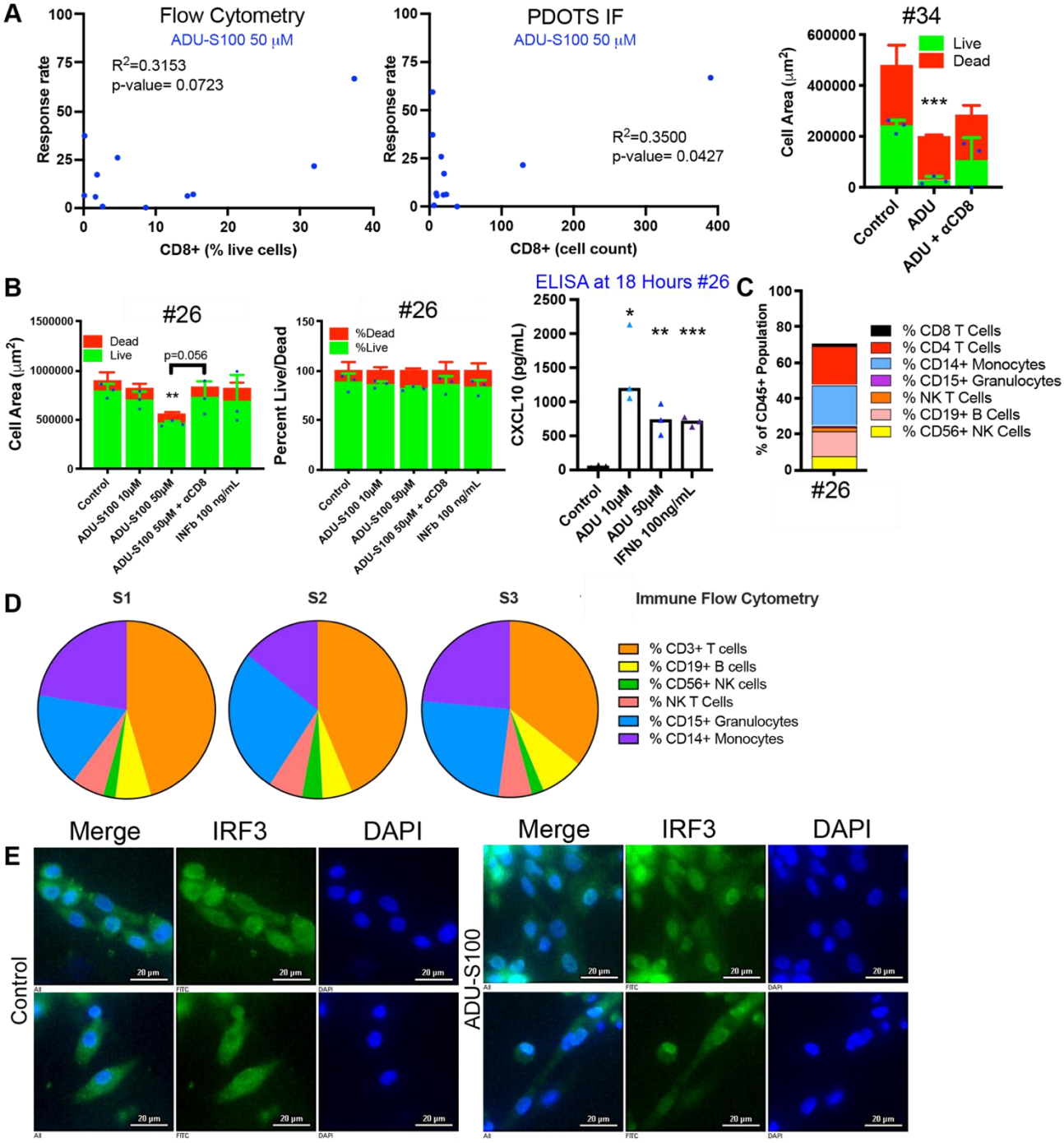
Supplementary Figure S5: *Ex vivo* STING agonist treatment of MPM tumors in PDOTS



Sample ID	31	25	8	5	4	26	29	12	32	35	22	33	27	15	9	20	28	14	3	10	30	1	23	6	2	13	24	17	7	34	18	21	16	19	11
Response	R	R					R		R	R		R	R			R	R				R	#			#		R			R						
Histology	E	B	E	U	E	E	E	E	E	B	E	E	E	B	E	B	E	B	B	E	E	E	E	B	E	E	E	E	E	E	B	B	E	E	B
Neoadjuvant	N	N	Y	U	Y	N	N	N	N	Y	N	N	N	N	N	N	Y	N	N	N	Y	N	N	Y	N	Y	Y	N	N	N	N	N	N	N	N
Adjuvant	Y	U	U	U	Y	U	Y	U	U	N	N	U	Y	N	N	U	Y	Y	Y	N	Y	Y	U	Y	Y	N	Y	Y	U	N	N	Y	Y	Y	Y
Age	58	79	66	U	69	78	67	74	75	80	77	60	82	71	68	72	68	75	70	79	79	77	73	68	79	71	69	53	38	66	80	79	77	76	52
Gender	F	F	M	U	M	F	M	M	M	M	M	M	F	F	M	M	F	F	M	M	M	M	M	M	F	M	M	M	F	M	M	M	M	M	M
Race	W	W	W	U	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	O	W	W	W	W	W	W	W

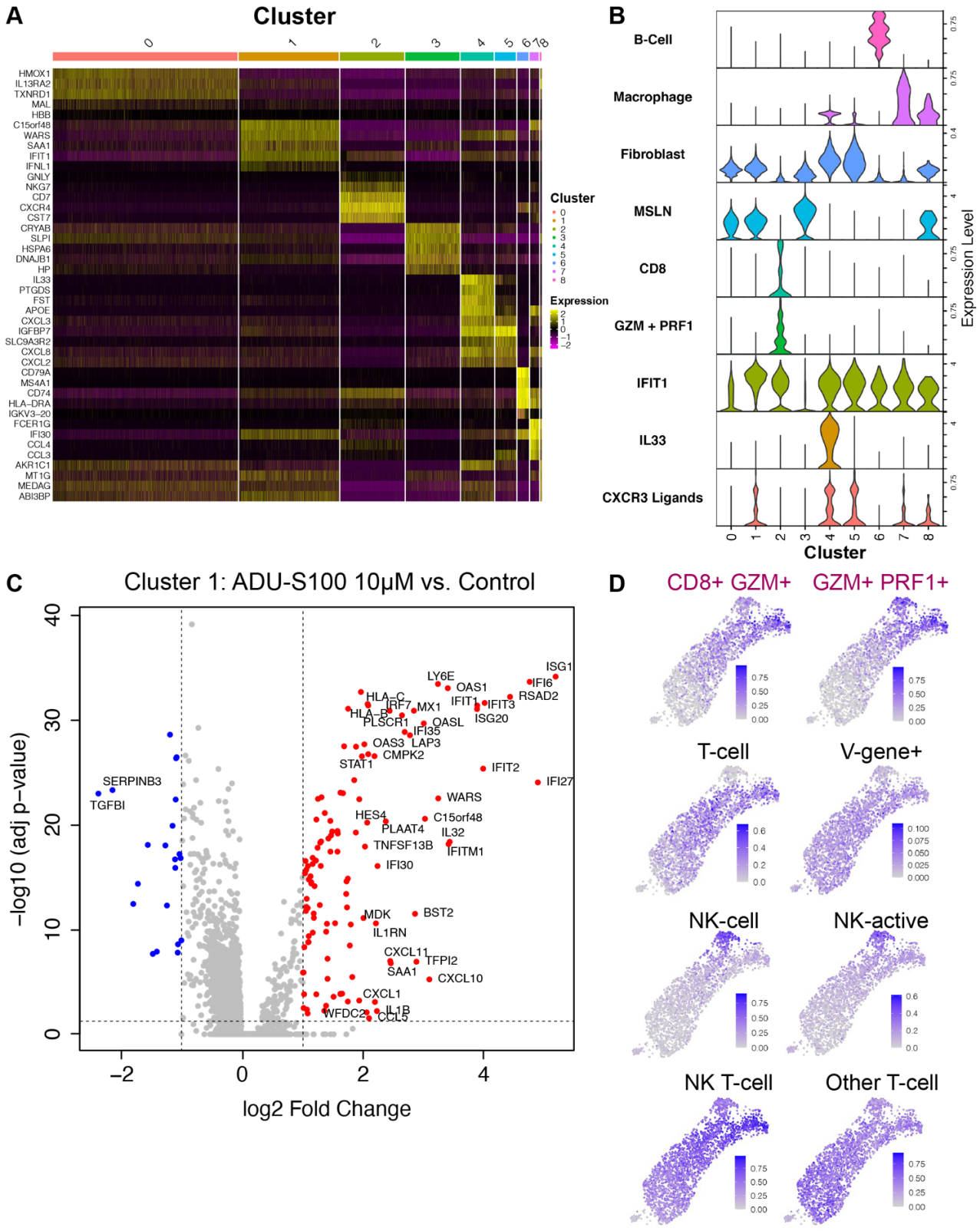
A, Hoechst/propidium iodide cell area and percent live/dead quantification from case #33 after 6-day treatment with 50 μM ADU-S100 (ADU) or dH20 and 100ng/mL IFNb controls. T-test vs. dH20 control: *p<0.05. MSD cytokine array performed on conditioned media from samples in the previous panel. ELISA for CXCL10 performed on conditioned media from tumor explants (S1 fragment) of case #35 with exceptional live/dead response. Three-day treatment with ADU-S100 or IFNb positive control. T-test vs. dH20 control: ****p<0.0001. **B,** CXCL10 ELISA from MPM explants treated for 3 days (S1) or PDOTS treated for 6 days (S2) with 50 μM ADU-S100 (ADU) or dH20 control. Estimation plot for CXCL10 from MSD cytokine panel performed on 10 MPM PDOTS after 3 days of explant (S1) treatment with 50 μM ADU-S100 or dH20 control. Live/dead response from IF stain designated by pink dot color. **C,** Summary of MPM PDOTS cell death after 6-day treatment with 50 μM ADU-S100 or dH20 control, plotted from left to right with increasing cell death in the control condition. R = response to treatment with cell death >20% above control, T-test p<0.05; # = less cell death with ADU-S100 treatment, p<0.05. E = epithelioid MPM, B = biphasic MPM, Y/N = yes/no neoadjuvant treatment**.** F = female, M = Male gender. W = white/Caucasian, O = other race. U = unknown demographic.

Supplementary Figure S6: STING agonists activate tumor cell STING causing CD8-cell killing



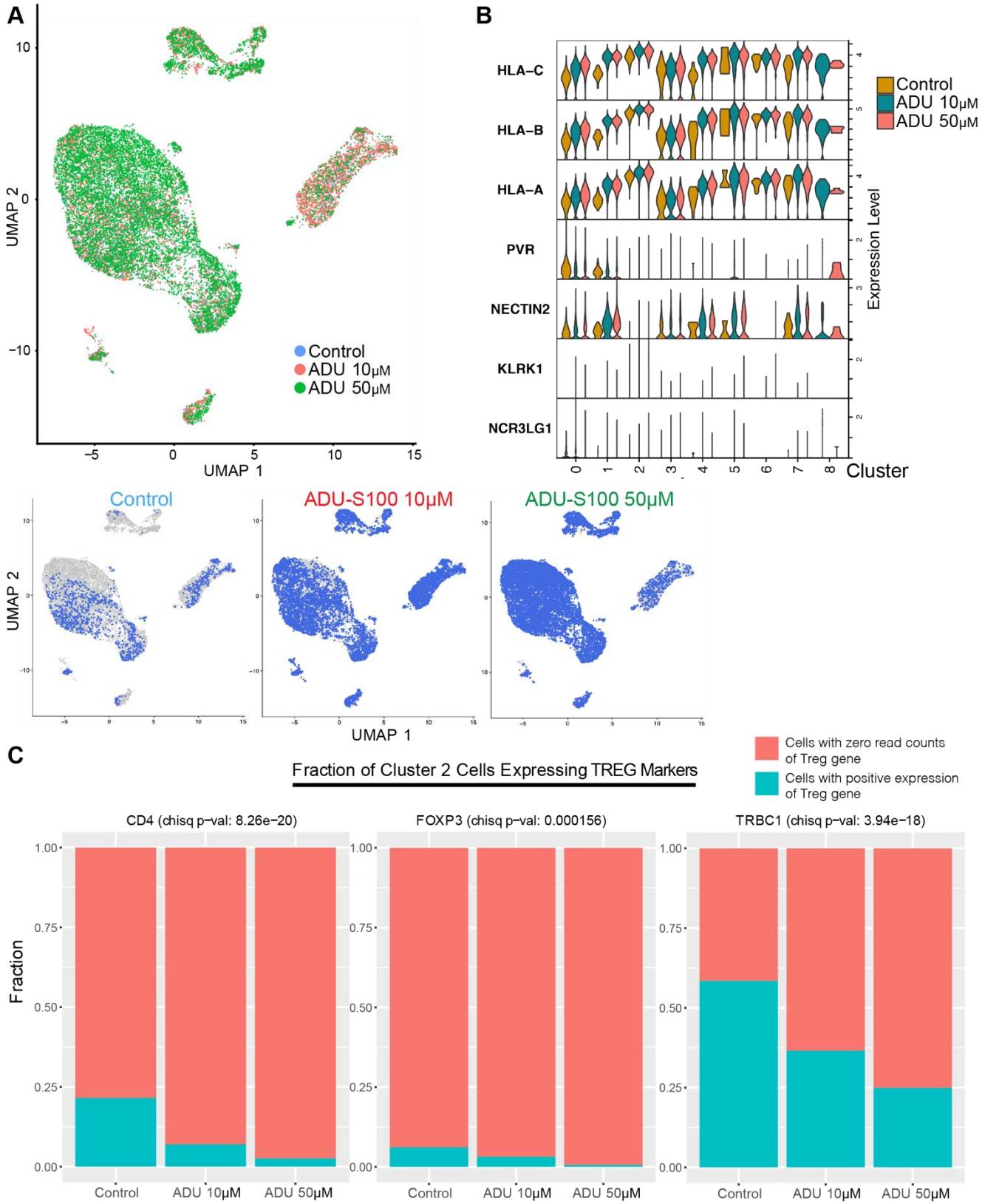
A, Correlation between CD8 flow cytometry (S3, n = 11) or CD8 immunofluorescence (S2, n = 12) at the time of sample collection for MPM PDOTS and subsequent live/dead response (6 days 50 μM ADU-S100 treatment cell death minus control cell death). CD8 cell count per PDOTS well/chamber. Linear regression analysis shown. Hoechst/propidium iodide cell area for sample #34 after 6 days of treatment with 50 μM ADU-S100 or dH20 control and CD8 neutralizing antibody (αCD8). T-test vs. dH20 control: ***p<0.001. **B,** Characterization of sample #26 where scRNA sequencing was performed**.** Hoechst/propidium iodide cell area and percent live/dead quantification after 6-day treatment with ADU-S100 +/- anti-CD8 neutralizing antibody, dH20 control or 100ng/mL IFNb. T-test vs. dH20 control. CXCL10 ELISA from S1 explant conditioned media after 18 hours of treatment with 10 or 50 μM ADU-S100 or 100ng/mL IFNb. T-test vs. dH20 control: *p<0.05, **p<0.01, ***p<0.001. **C,** Flow cytometry from S3 of sample #26. **D,** MPM immune flow cytometry profiling example comparing different sample fractions. **E,** IRF3 immunofluorescence in PDOTS #34 after 3-hours of treatment with 50 μM ADU-S100 or dH20 control. Scale bar = 20 μm.

Supplementary Figure S7: scRNA seq demonstrates STING activation in tumor cells and fibroblasts



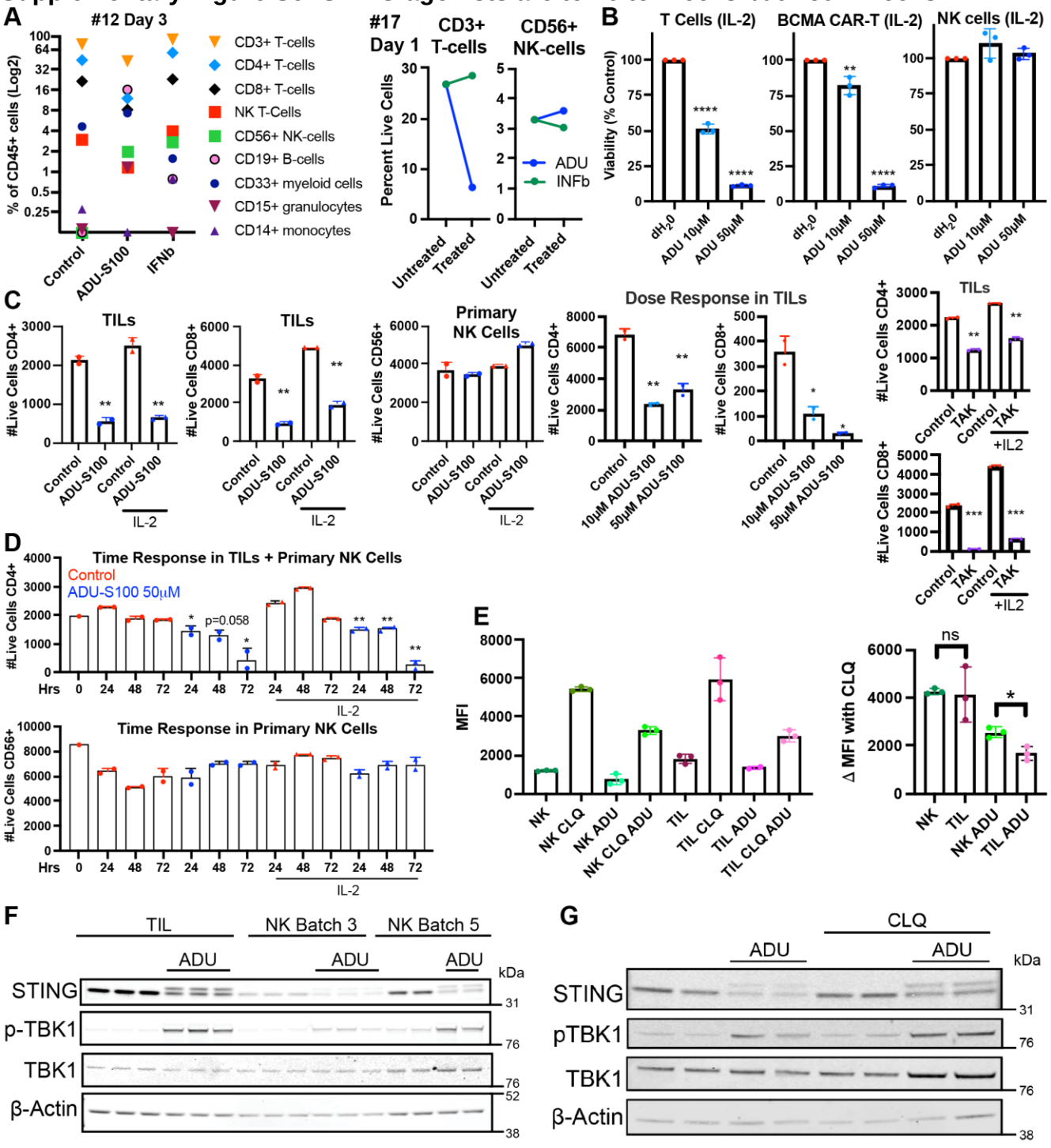
A, Heat maps for cluster-defining genes. **B,** Violin plots for immune cell and fibroblast signatures in addition to mesothelin (MSLN), CD8, combined granzyme + perforin (GZM + PRF1), IFIT1, IL33 and CXCR3 ligands (CXCL9/10/11) shown by cluster. **C,** Volcano plot of differentially expressed genes after 24 hours of treatment with 10 μM ADU-S100 or dH20 control. **D,** UMAP plots for specified transcripts and signatures focused on effector cell cluster 2.

Supplementary Figure S8: scRNAseq suggests STING agonist toxicity in T-cells



A, Combined UMAP plot from broad clustering of scRNA sequencing of MPM specimen #26 after 24 hours of treatment with dH20 control, 10 μM and 50 μM ADU-S100, with the contribution of each treatment shown together and separately. **B,** Violin plots for select NK cell activating/inhibitory transcripts from combined broad clustering split by treatment condition/dose of ADU-S100. **C,** Fraction bar graph showing expression of Treg transcripts in cluster 2 for each treatment, normalized to number of cells per sample.

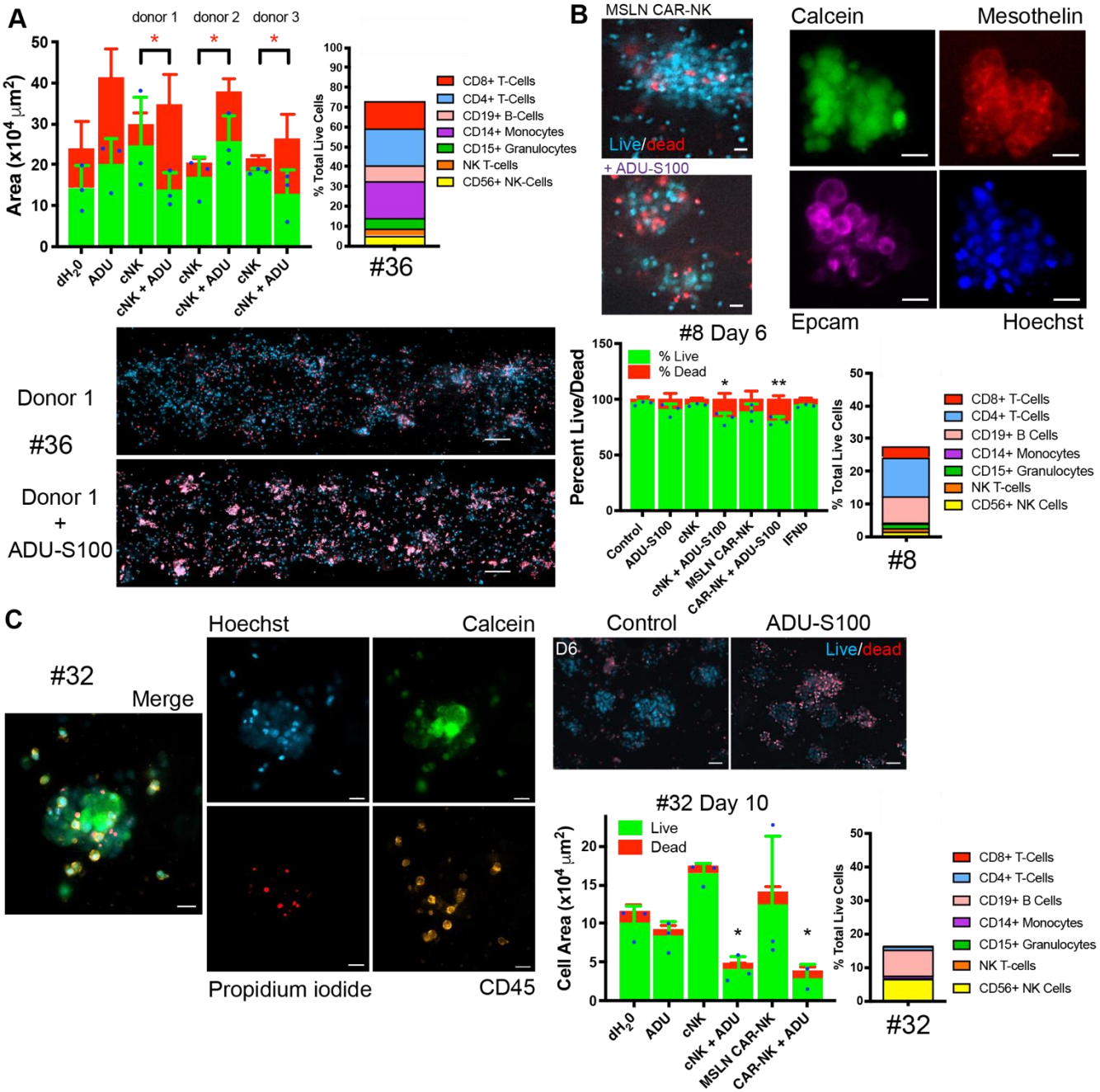
Supplementary Figure S9: STING agonists are toxic to T-cells but not NK cells



A, CD3/CD56 flow cytometry after 3- or 1-day treatment with 50 μM ADU-S100, 100ng/mL IFNb, or dH20 control S1 explants from samples #12 and #17. **B,** CellTiter-Glo viability in primary T-cells, BCMA CAR T-cells, and batch 2 primary NK cells treated for 24-hours with 10 or 50 μM ADU-S100 or dH20 control in the presence of 200 U/mL IL-2. T-test vs. dH20 control: **p<0.01, ****p<0.0001. **C,** CD4/CD8 flow cytometry after 72-hour treatment with 50 μM ADU-S100, 10 μM TAK-676, or dH20 control in batch 3 TILs compared with flow for CD56 in batch 2 NK cells expanded from PBMCs +/- 200 U/mL IL-2. T-test vs. control: *p<0.05, **p<0.01, ***p<0.001. **D,** Time course of toxicity for CD4+ T-cells from batch 1 TILs co-cultured with batch 2 NK cells. T-test vs. control at that timepoint: *p<0.05, **p<0.01. Dose-dependent toxicity for CD4/CD8 T-cells from TILs. One-way ANOVA p<0.01 with corrected pairwise comparisons: *p<0.05, **p<0.01. **E,** Mean fluorescence intensity (MFI) plots from flow cytometry for autophagolysosome vacuoles after 24-hour treatment with 10 μM chloroquine (CLQ) followed by 24-hour treatment with CLQ +/- 50 μM ADU-S100 (ADU) in batch 6 NK cells or

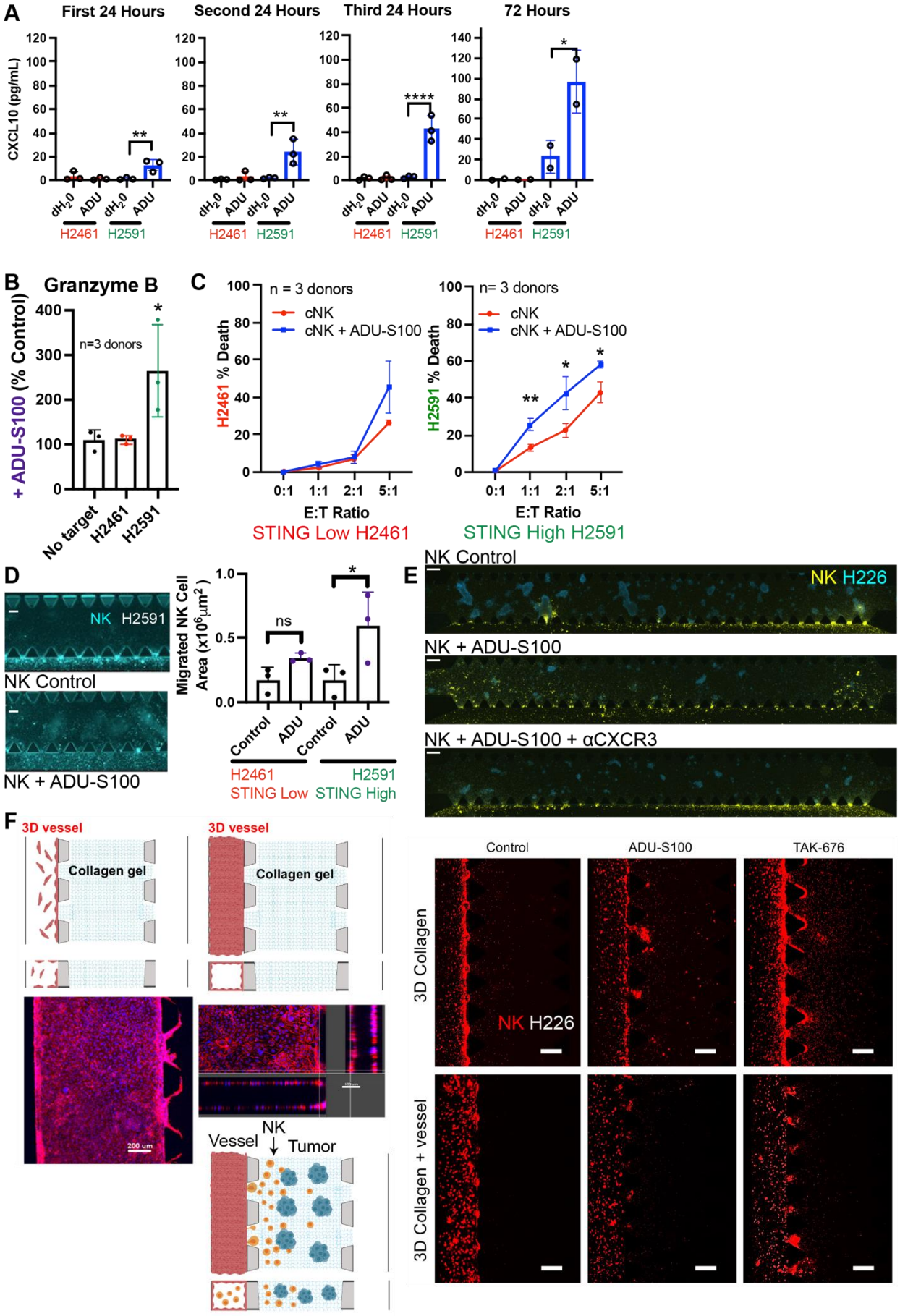
batch 2 TILs. T-test: *p<0.05. **F,** Western blot for STING, phospho-TBK1 (pTBK1), TBK1 and beta-actin load control after 6-hour treatment with 50 μM ADU-S100 or dH20 control in batch 2 TILs compared with batch 3 NK cells in triplicate and batch 5 NK cells in duplicate. **G,** Western blot from batch 6 NK cells treated for 6 hours with 50 μM ADU-S100 or dH20 and 10 μM chloroquine or DMSO control in duplicate.

Supplementary Figure S10: STING agonists enhance NK cell killing in MPM PDOTs



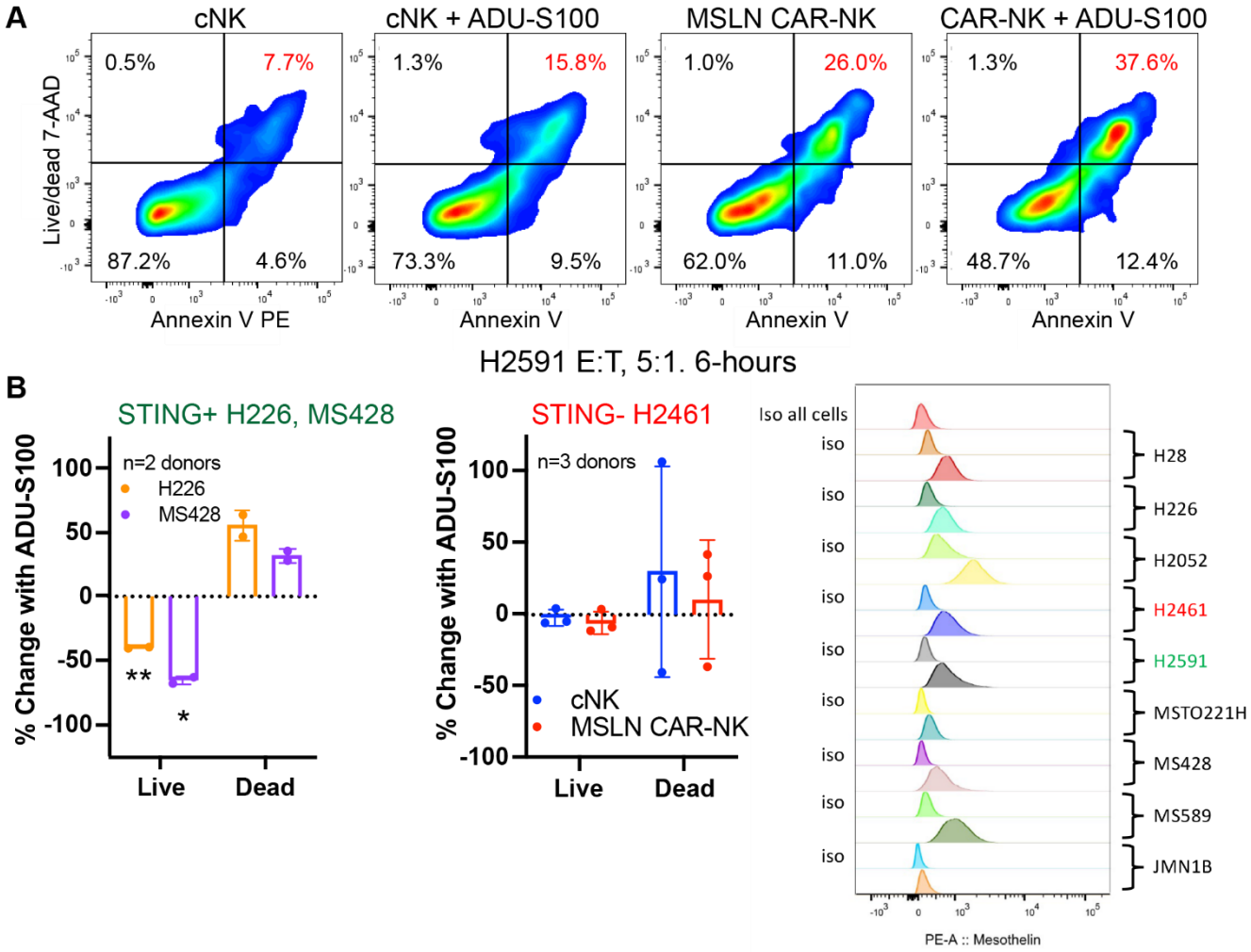
A, Hoechst/propidium iodide cell area quantification and live/dead immunofluorescence from sample #36 after 6-day treatment with 50 μM ADU-S100 (ADU) or dH20 +/- untransduced primary NK cells (cNK) from three donors. T-test: *p<0.05. Baseline immune flow cytometry from S3. Scale bars = 50 μm. **B,** IF and Hoechst/propidium iodide live/dead quantification from S2 for sample #8 after 6-day treatment with 50 μM ADU-S100 (ADU) or dH20 +/- untransduced primary NK cells (cNK) or anti-mesothelin (MSLN) CAR-NK cells, 100ng/mL IFNb control. Scale bars = 20 μm. T-test vs. dH20 control: *p<0.05, **p<0.01. Baseline immune flow cytometry from S3. **C,** IF and live/dead cell area quantification from S2 for sample #32 after 6- or 10-day treatment with 50 μM ADU-S100 (ADU) or dH20 +/- untransduced primary NK cells (cNK) or anti-mesothelin (MSLN) CAR-NK cells. Scale bars for Hoechst, Calcein, propidium iodide, CD45 = 20 μm. Live/dead scale bars = 50 μm. T-test vs. dH20 control: *p<0.05. Baseline immune flow cytometry from S3.

Supplementary Figure S11: STING agonists enhance NK cell migration and killing



A, CXCL10 ELISA on conditioned media from STING high and low MPM cell lines seeded in collagen in AIM chips and treated for 24-72 hours with 50 μM ADU-S100 or dH20 control in the media side channels. T-test: *p<0.05, **p<0.01, ****p<0.0001. **B,** Granzyme B ELISA after 24 hours of treatment with 50 μM ADU-S100 or dH20 control in primary NK cells -/+ target cell lines. One-sample t-test with expected percent control of 100: *p<0.05. **C,** Flow cytometry for annexin V and live/dead with H2591 MPM cells in co-culture with NK cells. Quantification for three NK cell donors across E:T ratios, graphed as percent target cell death. T-test: *p<0.05, **p<0.01. **D,** Representative IF images of primary NK cells labeled with cell tracker migrating toward H2591 MPM cells after 4-day treatment with 50 μM ADU-S100 or dH20 control. Scale bar = 200 μm. Quantification of NK cell migration from experimental triplicate. One-way ANOVA p<0.05 with corrected pairwise comparisons: *p<0.05. **E,** Representative IF images of NK cell migration towards H226 MPM tumor cells after 4-day treatment with 50 μM ADU-S100 +/- 5 μg/mL CXCR3 neutralizing antibody (αCXCR3). Scale bar = 150 μm. **F,** Schematic and IF for 3D migration assay with vessel co-culture and representative IF images of NK cell migration towards H226 MPM tumor cells after 24-hour treatment with 50 μM ADU-S100, 1 μM TAK-676, or dH20 control. Scale bar = 150 μm.

Supplementary Figure S12: STING agonists enhance CAR-NK cell killing in MPM



A, Flow cytometry for annexin V and live/dead of H2591 MPM cells in co-culture with control NK (cNK) or MSLN CAR-NK cells (E:T 5:1) following 6-hour treatment with 50 μM ADU-S100 or dH20 control. **B,** Quantification of two NK cell donors targeting two additional STING-expressing MPM cell lines (H226, MS428), graphed as percent change with ADU-S100 vs. dH20 control treatment. One-sample t-test with expected difference of zero: *p<0.05, **p<0.01. Quantification of three NK cell donors with cNK and MSLN CAR NK targeting STING-negative H2461, graphed as percent change with ADU-S100 vs. dH20 control. MSLN flow cytometry in MPM cell lines.