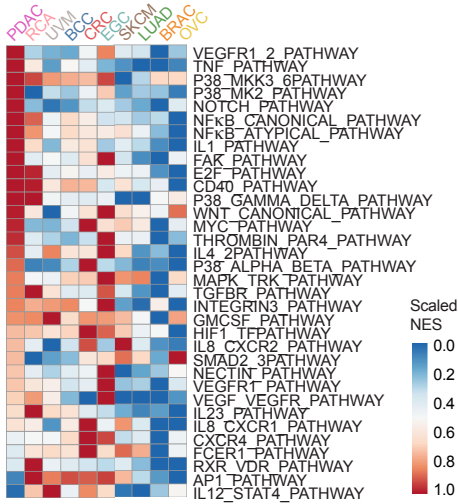
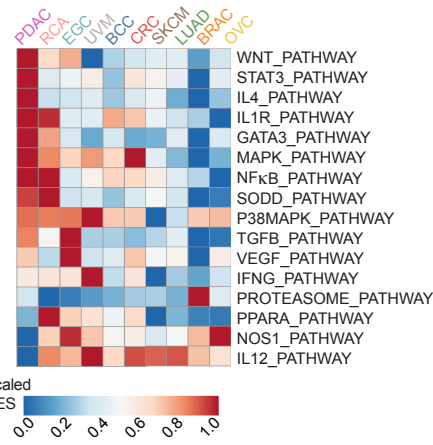


**A**

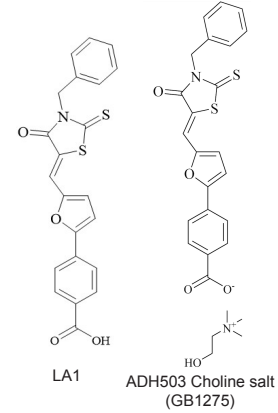
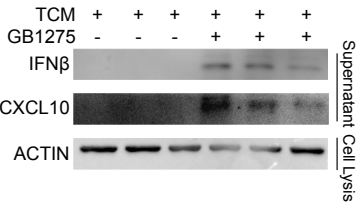
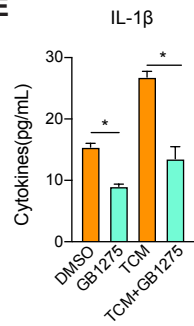
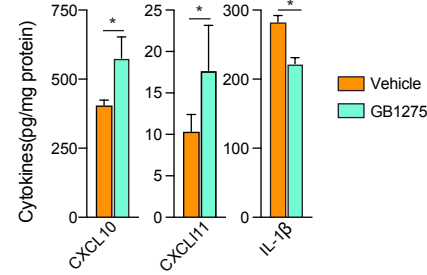
## GSEA Heatmap PID

**B**

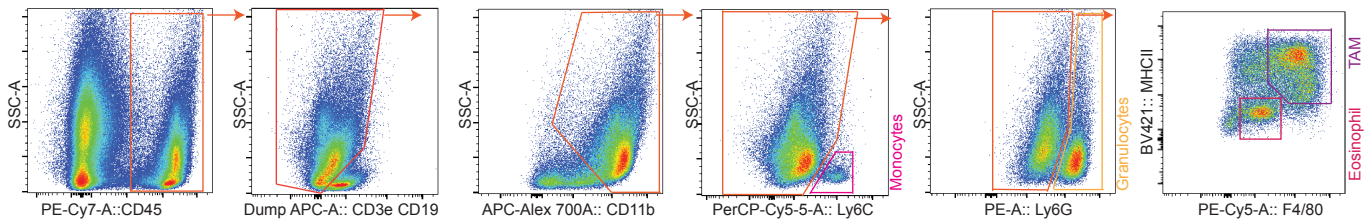
## GSEA Heatmap BIOCARTA

**C**

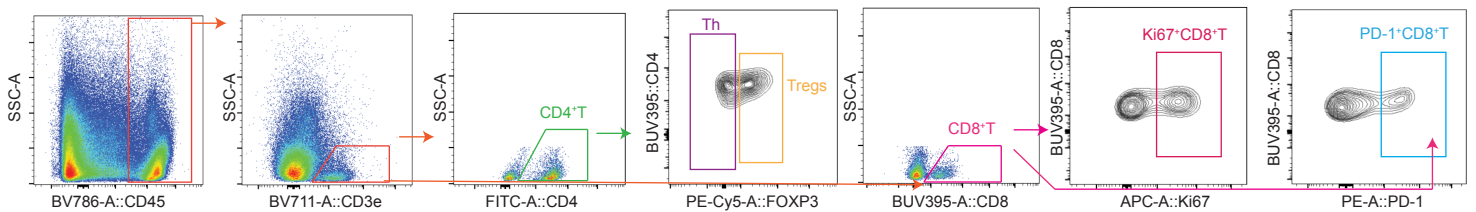
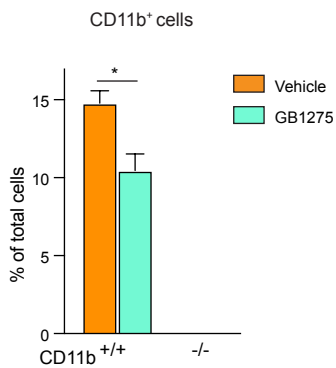
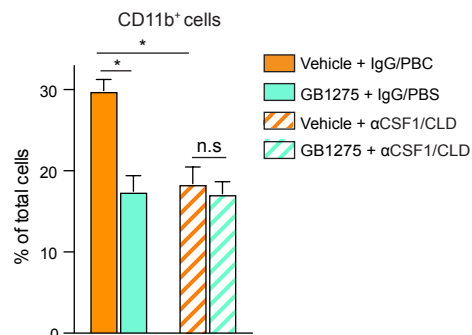
## CD11b agonists

**D****E****F****G**

## Gate strategy (Myeloid cells)

**H**

## Gate strategy (T cells)

**I****J**

**Figure S1. GB1275 exhibits anti-tumor function via CD11b, Related to Figure 1.**

(A) Gene set enrichment analysis (GSEA) identified pathway enrichment in tumor-associated macrophages in the above cancer types analyzed from curated data sources (Figure 1A), including PID (A) and BIOCARTA (B).

(C) Chemical structures of LA1 and ADH-503 (GB1275).

(D) Representative immunoblot for CXCL10 and IFN $\beta$  in supernatant from bone marrow-derived macrophages (BMDMs) treated with tumor cell-conditioned media (TCM)  $\pm$  GB1275 for 12 hours. The amount of loading protein was normalized by its cell lysis protein ( $\beta$ -ACTIN).

(E) IL-1 $\beta$  in supernatant from BMDMs treated with GB1275 for 12 hours in the presence or absence of TCM was measured by ELISA.

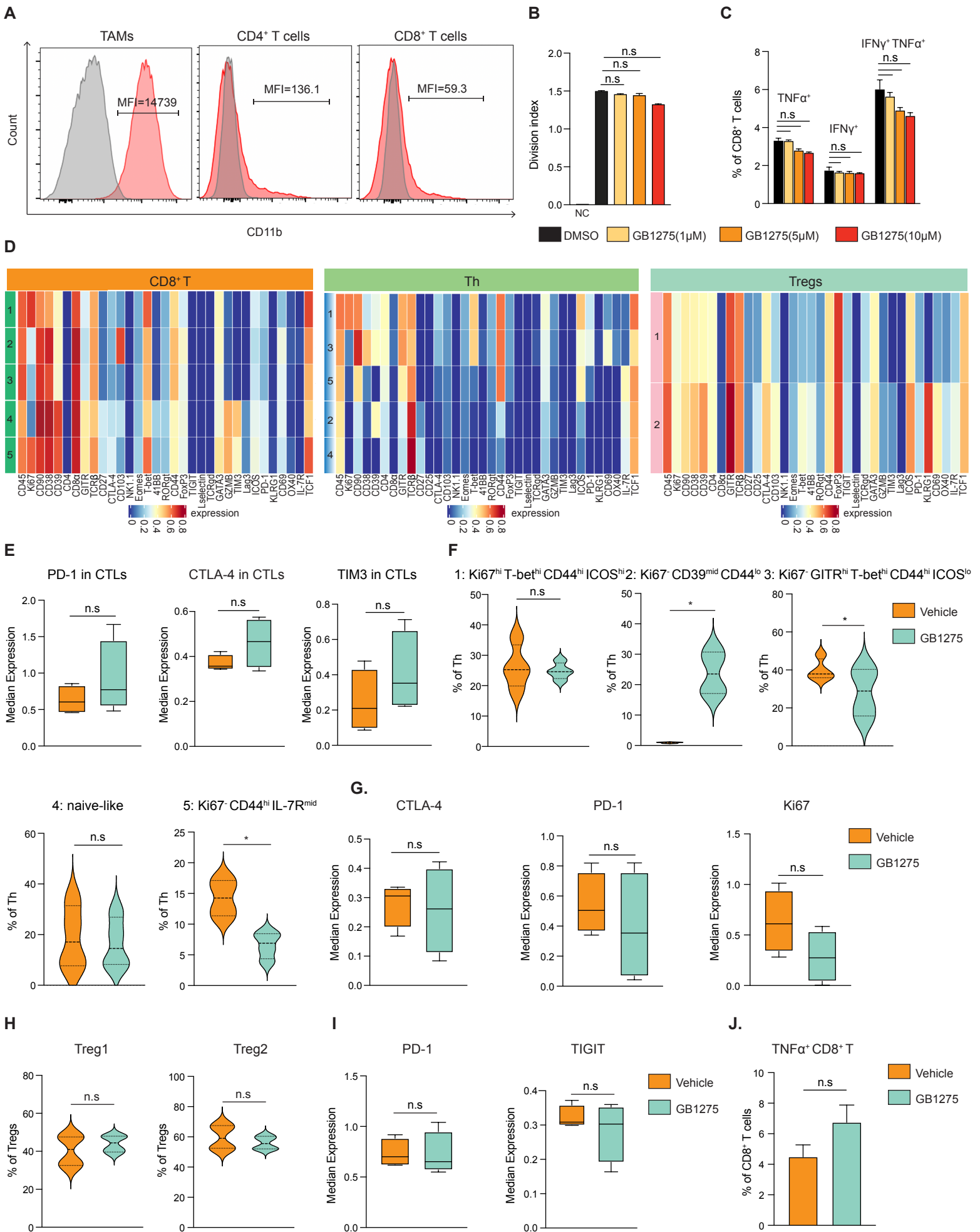
(F) CXCL10, 11, and IL-1 $\beta$  in tumor tissues from mice bearing syngeneic orthotopic KP2-OVA tumors treated with vehicle or GB1275 (120 mg/kg) for 12 days measured by ELISA (n =8/group).

(G) Representative flow cytometry gating strategies for myeloid cells.

(H) Representative flow cytometry gating strategies for T cells.

(I) Frequencies of tumor-infiltrating CD11b<sup>+</sup> cells from wild-type or CD11b<sup>-</sup> null mice bearing syngeneic orthotopic KP2 tumors treated with vehicle or GB1275 (120 mg/kg) for 14 days (n = 6/group).

(J) Frequencies of tumor-infiltrating CD11b<sup>+</sup> cells from mice bearing syngeneic orthotopic KI tumors with three doses of IgG+ phosphate-buffered saline/ aCSF-1 + Clodronate in the presence or absence of GB1275 (120 mg/kg) for 12 days (n = 6/group). Graphs show the mean  $\pm$  SEM; \*denotes p < 0.05 by a two-sided t-test for comparisons between two groups. In vitro data are representative of three independent experiments.



**Figure S2. GB1275 affects T cells phenotype, Related to Figure 2.**

- (A) Expression analysis of CD11b in tumor-associated macrophages, CD4<sup>+</sup> T, and CD8<sup>+</sup> T cells from KPC tumor tissues.
- (B) CFSE-labeled T cells were treated with GB1275 at different doses for 2 days. The proliferation of CD8<sup>+</sup> T cells was measured by flow cytometry.
- (C) Percentage of functional CD8<sup>+</sup> T cells from T cells treated with GB1275 at different doses.
- (D) Heat map of markers stained in CD8<sup>+</sup> T, CD4<sup>+</sup> Th and Tregs.
- (E) Median expressions of PD-1, CTLA-4, and TIM3 in CD8<sup>+</sup> T cells.
- (F) Percentage of individual subclusters in CD4<sup>+</sup> Th cells.
- (G) Median expressions of CTLA-4, PD-1, and Ki67 in CD4<sup>+</sup> Th cells.
- (H) Percentage of individual subclusters in Tregs.
- (I) Median expressions of PD-1 and TIGIT in Tregs.
- (J) Percentage of functional CD8<sup>+</sup> T cells from (Fig. 2H) (n = 7/group). Graphs show the mean ± SEM; \* denotes p < 0.05 by a two-sided *t*-test.

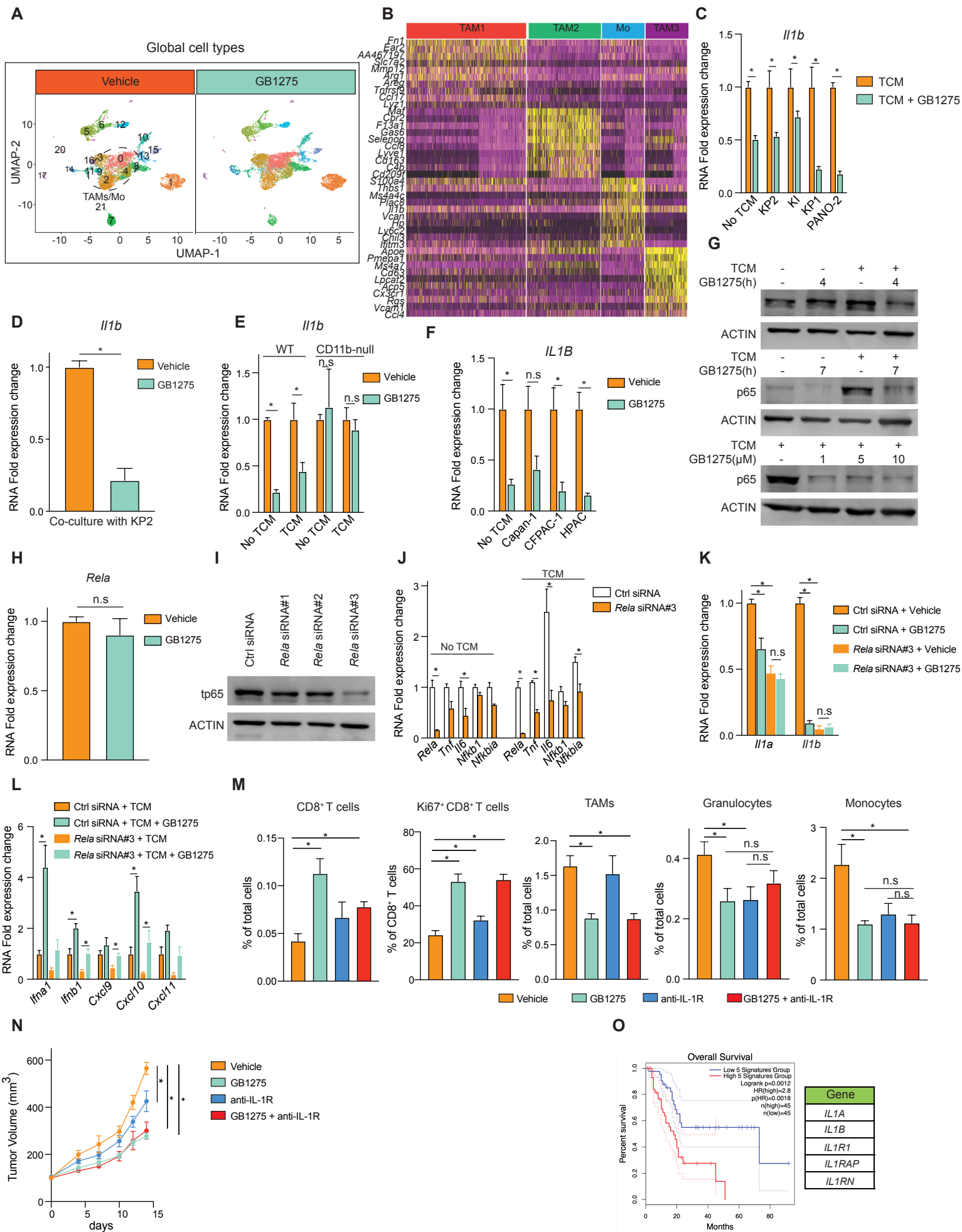


**Table S2. Gene List according to Genes regulated by NF- $\kappa$ B in response to TNF [GeneID=7124]. Related to Fig. 3E**

Gene name	avg_log2FC	-log10.p_val.	Gene name	avg_log2FC	-log10.p_val.	Gene name	avg_log2FC	-log10.p_val.
<i>Abca1</i>	-0.8383755	194.641539	<i>Fosb</i>	-0.202632	7.01914749	<i>Map3k14</i>	-0.2437754	42.7620249
<i>Arid2</i>	-0.1056034	10.5518372	<i>Fosl2</i>	-0.5211441	88.7405204	<i>Map3k7</i>	-0.0725858	5.08145945
<i>Atp2b1</i>	-0.277889	52.7892244	<i>G0s2</i>	0.0909763	9.397519	<i>Map3k8</i>	-0.1169387	8.19996336
<i>B4gal1</i>	0.25511586	35.2558242	<i>Gadd4a</i>	0.02435033	2.45935448	<i>Mapk14</i>	-0.4799102	159.617099
<i>Batf3</i>	-0.2656504	36.5353332	<i>Gadd4b</i>	-0.4182548	69.0592649	<i>Marcks1</i>	-1.1772583	300
<i>Bcl2ab</i>	0.5746906	215.595166	<i>Gch1</i>	-0.1673179	15.2564902	<i>Mxd1</i>	-0.2501254	13.1518109
<i>Bcl3</i>	-0.4243896	118.753212	<i>Gpr183</i>	0.27864034	27.4203131	<i>Myd88</i>	-0.4186959	119.287941
<i>Bhlhe0</i>	-0.1256872	26.100816	<i>Hbegf</i>	-0.4602758	41.7788174	<i>Nampt</i>	0.05739416	3.2268331
<i>Birc2</i>	-0.1013859	10.2169962	<i>Hes1</i>	0.0586757	2.01700954	<i>Nfat5</i>	-0.2539833	41.3278989
<i>Birc3</i>	-0.5085888	148.459671	<i>Ier2</i>	-0.7252434	134.694996	<i>Nfil3</i>	0.01349859	3.86279838
<i>Bmp2</i>	-0.5215322	115.219683	<i>Ier3ip1</i>	0.25164809	56.6004156	<i>Nfkb1</i>	-0.5965276	159.817982
<i>Btg1</i>	-0.1347422	1.56446766	<i>Ier5l</i>	-0.0232871	2.32407138	<i>Nfkb2</i>	-0.5042596	166.549262
<i>Btg2</i>	-0.374529	49.6089708	<i>Ilih1</i>	-0.245251	37.2019824	<i>Nfkbia</i>	-0.8376275	199.710505
<i>Btg3</i>	0.0576778	7.42663239	<i>Il15ra</i>	0.05885296	6.83765029	<i>Nfkbie</i>	-0.398704	87.2022177
<i>Ccl22</i>	-0.5318135	67.7737159	<i>Il18bp</i>	-0.0627294	5.34231582	<i>Ninj1</i>	-0.2537139	12.1275102
<i>Ccl4</i>	-0.6670718	120.555163	<i>Il1a</i>	-0.1371674	0.79336647	<i>Nr4a1</i>	-0.622014	82.9648383
<i>Ccl5</i>	0.5261759	5.30276626	<i>Il1b</i>	-0.1680165	1.94076115	<i>Nr4a2</i>	-0.1688019	3.7133669
<i>Ccnd1</i>	-0.2852683	27.7905845	<i>Il1rap</i>	0.02267265	3.46575393	<i>Nr4a3</i>	-0.4120524	26.108985
<i>Ccnl1</i>	-0.603519	182.562668	<i>Il1m</i>	-0.5297018	46.9136632	<i>Pde4b</i>	-0.3368215	34.2798252
<i>Ccr2</i>	0.1811769	10.8562748	<i>Il6st</i>	-0.2052886	28.9874399	<i>Per1</i>	-0.1452606	13.3448616
<i>Cd44</i>	-0.1638629	5.41305164	<i>Il7r</i>	0.17177735	13.2841795	<i>Pfkfb3</i>	-0.0838627	3.88766896
<i>Cd80</i>	0.0015904	4.56604789	<i>Inhba</i>	0.04681066	2.16530929	<i>Phlda1</i>	-0.7003989	86.3857097
<i>Cd83</i>	-0.6493465	112.058986	<i>Irak1</i>	-0.1952726	32.2444393	<i>Plaur</i>	0.71457546	232.975405
<i>Cdkn1a</i>	-0.151114	3.92659263	<i>Irak2</i>	0.0935217	8.66363756	<i>Plk2</i>	-0.461719	77.422352
<i>Cebpb</i>	0.0340785	10.5123304	<i>Irak3</i>	-0.0736737	6.66960922	<i>Pnrc1</i>	-0.2339431	30.7453046
<i>Cebpd</i>	-0.3673683	58.0710908	<i>Irf1</i>	-0.6709618	152.619834	<i>Ppp1r1a</i>	-0.8806561	249.311784
<i>Cflar</i>	-0.6177732	191.906655	<i>Irs2</i>	0.14212271	10.9795509	<i>Ptger4</i>	-0.4421047	81.2589541
<i>Chuk</i>	-0.1569163	22.4910651	<i>Jag1</i>	0.05841019	11.3135612	<i>Ptgs2</i>	-0.7943106	48.6363227
<i>Clcf1</i>	-0.0698502	5.84465787	<i>Kdm6b</i>	-0.9011994	253.957183	<i>Ptpre</i>	-0.2266869	38.6528049
<i>Csf1r</i>	-0.4957009	152.360514	<i>Klf2</i>	-0.3699222	24.2816673	<i>Rcan1</i>	-0.3096883	27.0428691
<i>Csf2rb</i>	0.0826627	18.7915023	<i>Klf4</i>	0.11648849	6.01472949	<i>Rela</i>	-0.3206074	71.6420038
<i>Cxcl10</i>	-0.3034904	22.5445749	<i>Klf6</i>	-0.4364123	53.1540872	<i>Relb</i>	-0.2030357	37.1081675
<i>Ddx58</i>	-0.265879	57.8482147	<i>Klf9</i>	0.44405784	118.174284	<i>Rhob</i>	-0.649193	89.6285495
<i>Marcks1</i>	-1.4373567	235.336835	<i>Ldlrad4</i>	0.03497074	3.28116677	<i>Rnf19b</i>	-0.5516989	115.161508
<i>Egr2</i>	-0.648375	126.89279	<i>Lifr</i>	-0.9273745	261.876185	<i>Sat1</i>	-0.3207461	39.4381805
<i>Egr3</i>	-0.3714816	37.1671868	<i>Litaf</i>	-0.2718494	57.6441994	<i>Sdc4</i>	-0.7312944	99.5091609
<i>Ehd1</i>	-0.3867548	76.0936176	<i>Maff</i>	-0.2469427	30.4228429	<i>Serpine1</i>	0.16445604	7.04704712
<i>Eif1ad</i>	0.1510942	20.3619067	<i>Map2k3</i>	-0.2744135	50.7957257	<i>Tgfb1</i>	-0.0874015	3.32061022
<i>Ets2</i>	-0.1064745	5.24629946	<i>Map3k0</i>	0.02903878	2.36697793	<i>Ticam1</i>	-0.0740738	13.1665631
<i>Sgk1</i>	0.3345507	38.8309289	<i>Socs3</i>	-0.3904057	45.9580777	<i>Trib1</i>	-0.4803535	78.6340325
<i>Smad3</i>	-0.0943288	24.6259832	<i>Stab1</i>	-0.8336481	113.019328	<i>Trip10</i>	-0.0450348	3.94538003
<i>Tnfaip1</i>	-0.1218112	14.3960346	<i>Traf1</i>	-0.2700676	23.0398546			
<i>Trnp1</i>	-0.0798396	6.54821356	<i>Traf6</i>	-0.0459017	2.88420338			
<i>Tsc221</i>	-0.1482281	17.4310284	<i>Zc3h12a</i>	-0.3023344	40.7936029			
<i>Tubba</i>	-0.1983393	21.5893251	<i>Zfp361</i>	-0.7423456	146.676965			

**Table S3. GB1275- downregulated top 80 genes from Genes regulated by NF- $\kappa$ B in response to TNF [GeneID=7124]. Related to Fig. 3M**

<b>Gene name</b>	<b>avg_log2FC</b>	<b>-log10.p_val.</b>	<b>Gene name</b>	<b>avg_log2FC</b>	<b>-log10.p_val.</b>
<i>Egr1</i>	-1.4373567	235.337242	<i>Klf2</i>	-0.3699222	24.2814983
<i>Marcks1</i>	-1.1772583	400	<i>Cebpd</i>	-0.3673683	58.0710923
<i>Lifr</i>	-0.9273745	261.876148	<i>Pde4b</i>	-0.3368215	34.2798407
<i>Kdm6b</i>	-0.9011994	253.958607	<i>Sat1</i>	-0.3207461	39.4377071
<i>Ppp1r15a</i>	-0.8806561	249.31158	<i>Rela</i>	-0.3206074	71.6420652
<i>Abca1</i>	-0.8383755	194.642065	<i>Rcan1</i>	-0.3096883	27.0428718
<i>Nfkbia</i>	-0.8376275	199.709965	<i>Cxcl10</i>	-0.3034904	22.5451551
<i>Ptgs2</i>	-0.7943106	48.636388	<i>Zc3h12a</i>	-0.3023344	40.7931741
<i>Zfp36l1</i>	-0.7423456	146.677781	<i>Ccnd1</i>	-0.2852683	27.790485
<i>Sdc4</i>	-0.7312944	99.5086383	<i>Atp2b1</i>	-0.277889	52.790485
<i>Ier2</i>	-0.7252434	134.694649	<i>Map2k3</i>	-0.2744135	50.79588
<i>Phlda1</i>	-0.7003989	86.3861582	<i>Litaf</i>	-0.2718494	57.6439741
<i>Irf1</i>	-0.6709618	152.619789	<i>Traf1</i>	-0.2700676	23.0400052
<i>Ccl4</i>	-0.6670718	120.554396	<i>Ddx58</i>	-0.265879	57.8477117
<i>Cd83</i>	-0.6493465	112.058986	<i>Nfat5</i>	-0.2539833	41.3279021
<i>Rhob</i>	-0.649193	89.6289321	<i>Ninj1</i>	-0.2537139	12.1272612
<i>Egr2</i>	-0.648375	126.89279	<i>Mxd1</i>	-0.2501254	13.1518109
<i>Nr4a1</i>	-0.622014	82.9665762	<i>Maff</i>	-0.2469427	30.4225082
<i>Cflar</i>	-0.6177732	191.906578	<i>Ifih1</i>	-0.245251	37.2020404
<i>Ccn1</i>	-0.603519	182.562249	<i>Pnrc1</i>	-0.2339431	30.7447275
<i>Nfkb1</i>	-0.5965276	159.818156	<i>Ptpre</i>	-0.2266869	38.653647
<i>Rnf19b</i>	-0.5516989	115.161781	<i>Il6st</i>	-0.2052886	28.9871628
<i>Ccl22</i>	-0.5318135	67.7746907	<i>Relb</i>	-0.2030357	37.1079054
<i>Bmp2</i>	-0.5215322	115.219683	<i>Fosb</i>	-0.202632	7.01908806
<i>Fosl2</i>	-0.5211441	88.7399286	<i>Tubb2a</i>	-0.1983393	21.5900669
<i>Birc3</i>	-0.5085888	148.459671	<i>Nr4a2</i>	-0.1688019	3.71336617
<i>Nfkb2</i>	-0.5042596	166.549751	<i>Il1b</i>	-0.1680165	1.94076119
<i>Trib1</i>	-0.4803535	78.634512	<i>Gch1</i>	-0.1673179	15.2564902
<i>Plk2</i>	-0.461719	77.4225082	<i>Cd44</i>	-0.1638629	5.4134127
<i>Hbegf</i>	-0.4602758	41.7798919	<i>Cdkn1a</i>	-0.151114	3.92659328
<i>Ptger4</i>	-0.4421047	81.2588484	<i>Tsc22d1</i>	-0.1482281	17.4306261
<i>Klf6</i>	-0.4364123	53.154282	<i>Per1</i>	-0.1452606	13.3448616
<i>Bcl3</i>	-0.4243896	118.752027	<i>Btg1</i>	-0.1347422	1.56446766
<i>Gadd45b</i>	-0.4182548	69.0594835	<i>Bhlhe40</i>	-0.1256872	26.1007268
<i>Nr4a3</i>	-0.4120524	26.1090204	<i>Map3k8</i>	-0.1169387	8.19997064
<i>Nfkbie</i>	-0.398704	87.2020404	<i>Ets2</i>	-0.1064745	5.24641694
<i>Socs3</i>	-0.3904057	45.9586073	<i>Birc2</i>	-0.1013859	10.2168113
<i>Ehd1</i>	-0.3867548	76.093665	<i>Smad3</i>	-0.0943288	24.6252517
<i>Btg2</i>	-0.374529	49.6090649	<i>Pfkfb3</i>	-0.0838627	3.88766987
<i>Egr3</i>	-0.3714816	37.1674911	<i>Tnip1</i>	-0.0798396	6.54821356



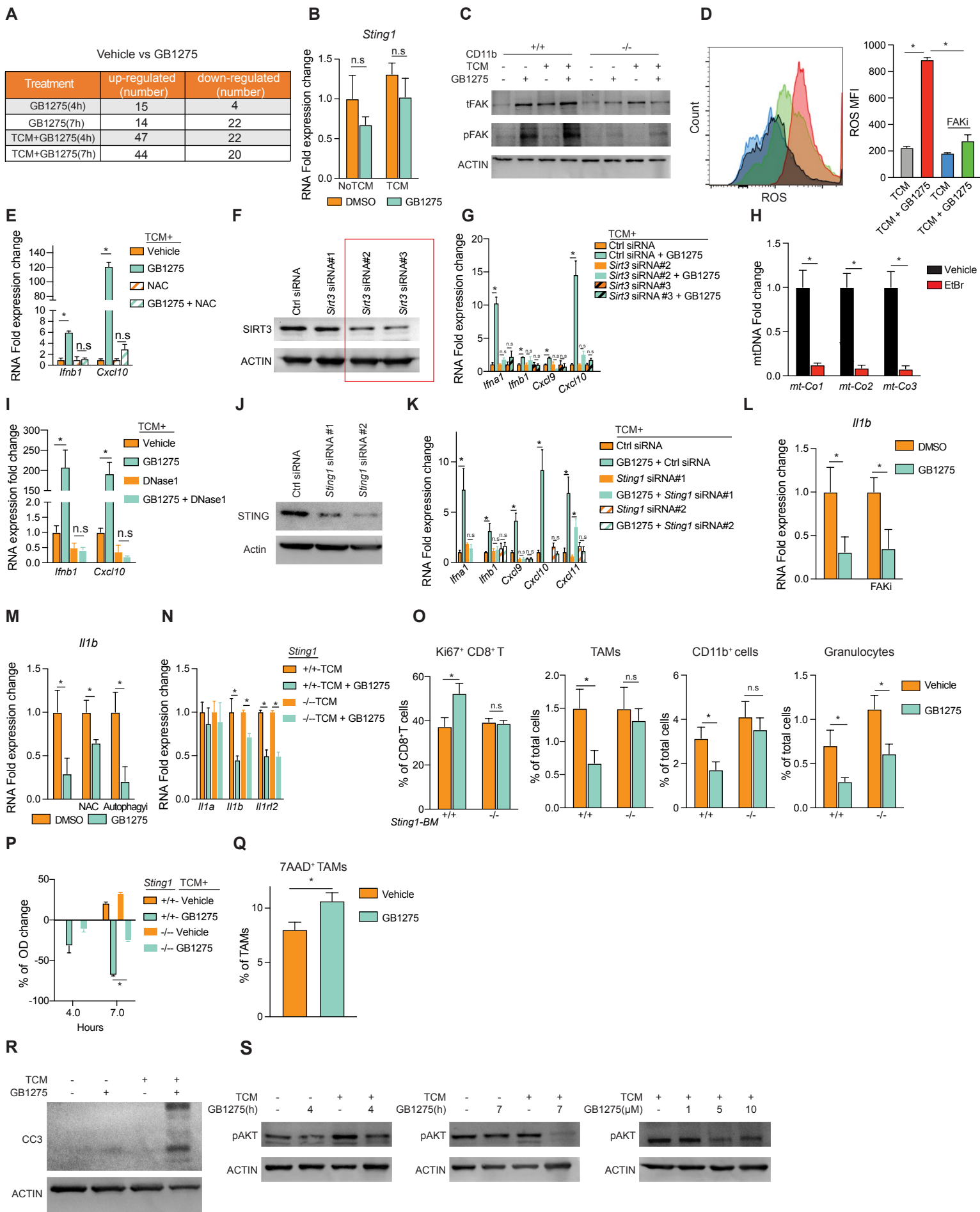
**Figure S3. GB1275 inhibits NF $\kappa$ b, Related to Figure 3.**

- (A) UMAP scRNAseq plots show CD45<sup>+</sup> cells in vehicle- and GB1275-treated groups.
- (B) Heat map showing differentially expressed genes in subclusters from tumor-associated macrophage/monocyte populations.
- (C) Quantitative PCR mRNA expression analysis of BMDMs treated with Vehicle or GB1275 for 7 hours in the presence or absence of different TCM. Changes in gene expression are depicted as the fold change from the vehicle baseline.
- (D) Quantitative PCR mRNA expression analysis of BMDMs co-cultured with KP2 cells  $\pm$  vehicle or GB1275 for 48 hours. Changes in gene expression are depicted as the fold change from the vehicle baseline.
- (E) Quantitative PCR mRNA expression analysis of BMDMs isolated from either wild-type or CD11b<sup>-</sup> null mice treated with TCM  $\pm$  vehicle or GB1275 for 7 hours. Changes in gene expression are depicted as the fold change from the vehicle baseline.
- (F) Quantitative PCR mRNA expression analysis of human monocyte-derived macrophages treated with different TCM  $\pm$  vehicle or GB1275 for 7 hours. Changes in gene expression are depicted as the fold change from the vehicle baseline.
- (G) Representative immunoblot for total p65 and  $\beta$ -ACTIN (loading control) in BMDMs treated with GB1275 $\pm$  TCM at different doses for indicated time points.
- (H) Quantitative PCR mRNA expression analysis of BMDMs treated with vehicle or GB1275 for 7 hours. Changes in gene expression are depicted as the fold change from the vehicle baseline.
- (I) Representative immunoblot for total p65 and  $\beta$ -ACTIN (loading control) in BMDMs transfected with siRNA targeting *Rela* and ctrl siRNA for 24 hours.
- (J) Quantitative PCR mRNA expression analysis of BMDMs transfected with *Rela* siRNA or ctrl siRNA for 24 hours, then incubated with TCM or 1% fetal bovine serum media for 6 hours. Changes in gene expression are depicted as the fold change from the vehicle baseline.
- (K) Quantitative PCR mRNA expression analysis of BMDMs transfected with *Rela* siRNA or ctrl siRNA for 24 hours prior to 7-hour vehicle or GB1275 treatment. Changes in gene expression are depicted as the fold change from the vehicle baseline.
- (L) Quantitative PCR mRNA expression analysis of BMDMs transfected with *Rela* siRNA or ctrl siRNA for 24 hours prior to 7-hour TCM or TCM + GB1275 treatment. Changes in gene expression are depicted as the fold change from the vehicle baseline.
- (M) Syngeneic model of KP2 treated with vehicle, GB1275 (120 mg/kg), anti-IL-1R (200  $\mu$ g/mouse), or the combination for 14 days. Relative frequencies of tumor-infiltrating CD8<sup>+</sup> T cells, Ki67<sup>+</sup>CD8<sup>+</sup> T cells, TAMs, granulocytes, and monocytes (n = 6/group).

(N) Tumor growth curve from the abovementioned four groups expressed by tumor volume (n = 8/group).

(O) Kaplan–Meier survival curves for genes shown in the right table in TCGA patient dataset for pancreatic adenocarcinoma. Graphs show the mean  $\pm$  SEM; \* denotes  $p < 0.05$  by the two-sided *t*-test for comparisons between any two groups or log-rank test for Kaplan–Meier survival curves.

*In vitro* data are representative of three independent experiments.



**Figure S4. GB1275 triggers STING signaling, Related to Figure 4.**

(A) The number of changed proteins following GB1275 ± TCM at 4 and 7 hours by RPPA as shown in the table.

(B) Quantitative PCR mRNA expression analysis of BMDMs treated with Vehicle or GB1275 for 7 hours in the presence or absence of TCM. Changes in gene expression are depicted as the fold change from the vehicle baseline.

(C) Representative immunoblots for total FAK, pFAK, and β-ACTIN (loading control) in BMDMs from wild-type or CD11b-null mice treated with vehicle or GB1275 ± TCM for 4 hours.

(D) FAKi (0.5 μM, VS4718)-pretreated BMDMs were stimulated by TCM + GB1275 for 7 hours, and the intracellular level of total reactive oxygen species (ROS) was measured by flow cytometry.

(E) Quantitative PCR mRNA expression analysis of 1 mM N-acetylcysteine (NAC)-pretreated BMDMs treated with TCM ± vehicle or GB1275 for 7 hours. Changes in gene expression are depicted as the fold change from the vehicle baseline.

(F) Representative immunoblot for SIRT3 and β-ACTIN (loading control) in BMDMs transfected with siRNA targeting *Sirt3* and ctrl siRNA for 24 hours.

(G) Quantitative PCR mRNA expression analysis of BMDMs transfected with *Sirt3* siRNA or ctrl siRNA for 24 hours prior to 7-hour vehicle or GB1275 + TCM treatment. Changes in gene expression are depicted as the fold change from the vehicle baseline.

(H) Mitochondrial genes released in cytoplasm were detected by quantitative PCR analysis from BMDMs treated with ethidium bromide (1.5 μg/mL) for 8 hours. Changes in gene expressions are depicted as the fold change from the vehicle baseline.

(I) BMDMs were pretreated with DNase1 (10 μg/mL) 1 hour ahead of TCM ± vehicle or GB1275 for 7 hours. Quantitative PCR mRNA expression analysis of the abovementioned cells with different stimulations. Changes in gene expressions are depicted as the fold change from the vehicle baseline.

(J) Representative immunoblot for STING and β-ACTIN (loading control) in BMDMs transfected with siRNA targeting *Sting1* and ctrl siRNA.

(K) Quantitative PCR mRNA expression analysis of BMDMs transfected with *Sting1* siRNA or ctrl siRNA for 24 hours prior to 7-hour vehicle or GB1275 + TCM treatment. Changes in gene expression are depicted as the fold change from the vehicle baseline.

(L) Quantitative PCR mRNA expression analysis of FAKi (0.5 μM, VS4718)-pretreated BMDMs treated with vehicle or GB1275 for 7 hours. Changes in gene expression are depicted as the fold change from the vehicle baseline.

(M) Quantitative PCR mRNA expression analysis of NAC (1 mM) or autophagy inhibitor (10  $\mu$ M)-pretreated BMDMs treated with vehicle or GB1275 for 7 hours. Changes in gene expression are depicted as the fold change from the vehicle baseline.

(N) Quantitative PCR mRNA expression analysis of BMDMs isolated from wild-type (WT) and STING-null mice treated with TCM  $\pm$  GB1275 for 7 hours. Changes in gene expression are depicted as the fold change from the vehicle baseline.

(O) Relative frequencies of tumor-infiltrating Ki67<sup>+</sup> CD8<sup>+</sup> T cells, TAMs, CD11b<sup>+</sup> cells and granulocytes (n = 6/group) in the bone marrow transplant model (4 M) treated with vehicle or GB1275 for 14 days.

(P) MTT proliferation assay using BMDMs from WT or STING-null mice treated with TCM  $\pm$  GB1275 for 4 or 7 hours. Average percentage of OD change after treatment.

(Q) Relative frequencies of 7AAD<sup>+</sup> TAMs in KP2-OVA orthotopic model treated with vehicle or GB1275 (120 mg/kg) for 12 days (n = 6/group).

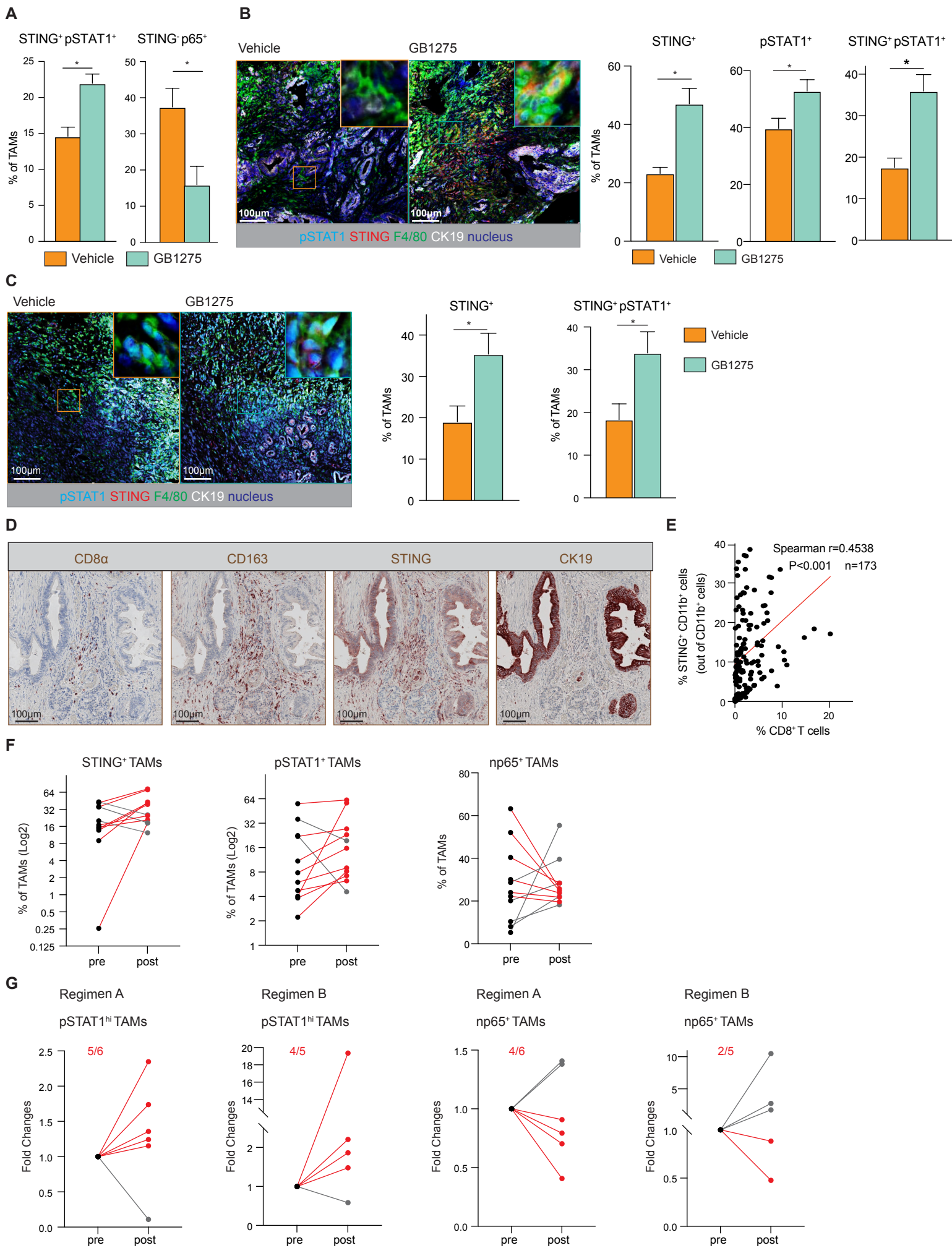
(R) Representative immunoblot for cleaved caspase-3 (CC3) and  $\beta$ -ACTIN (loading control) in BMDMs treated with TCM  $\pm$  GB1275 for 7 hours.

(S) Representative immunoblot for pAKT and  $\beta$ -ACTIN (loading control) in BMDMs treated with different doses of GB1275 for indicated time points in the presence or absence of TCM (same protein and experiment as shown in Figure S3G, sharing the same  $\beta$ -ACTIN data). Graphs show the mean  $\pm$  SEM; \*denotes p < 0.05 by the two-sided t-test for comparisons between two groups. In vitro data are representative of three independent experiments.



**Table S5. Biopsy information, Related to Fig. 5F**

<b>Sample ID</b>	<b>Sex</b>	<b>Age</b>	<b>Treatment Regimen</b>	<b>GB1275 Dose</b>	<b>Cancer type</b>	<b>Stage</b>
1	Female	70	GB1275+ pembrolizumab	100 mg	Pancreas	IV
2	Female	57	GB1275	1200 mg	Colon/Rectum	IV
3	Female	78	GB1275	100 mg	Colon/Rectum	IV
4	Female	73	GB1275	200 mg	Colon/Rectum	IV
5	Male	75	GB1275+ pembrolizumab	400 mg	Esophagus	IV
6	Male	73	GB1275	1200 mg	Esophagus	IV
7	Male	39	GB1275+ pembrolizumab	800 mg	Colon/Rectum	IV
8	Male	57	GB1275+ pembrolizumab	1200 mg	Colon/Rectum	IV
9	Female	43	GB1275+ pembrolizumab	100 mg	Gastric	IV
10	Male	59	GB1275	400 mg	Colon/Rectum	IV
11	Female	73	GB1275	400 mg	Pancreas	IV



**Figure S5. STING activation in humans, Related to Figure 5.**

(A) Proportion of STING<sup>+</sup> pSTAT1<sup>+</sup> and STING<sup>-</sup> p65<sup>+</sup> TAMs from KPC tumor tissue treated with vehicle or GB1275 for 14 days (n = 7–8 mice per group).

(B) Representative mplHC staining for pSTAT1, STING, F4/80, and CK19 in tumors from the 14-day vehicle and GB1275 (120 mg/kg)-treated KP2 orthotopic model. Scale bar, 100  $\mu$ m. Right, percentages of STING<sup>+</sup> TAMs, pSTAT1<sup>+</sup>, and STING<sup>+</sup>pSTAT1<sup>+</sup>TAMs from each treatment group (n = 7 mice per group).

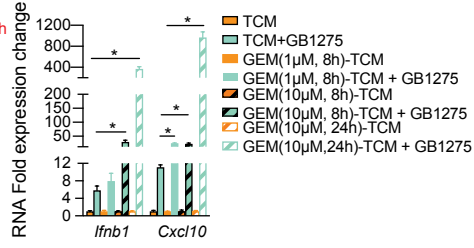
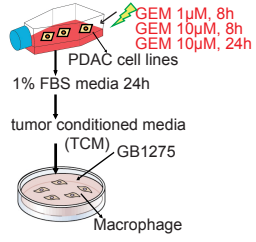
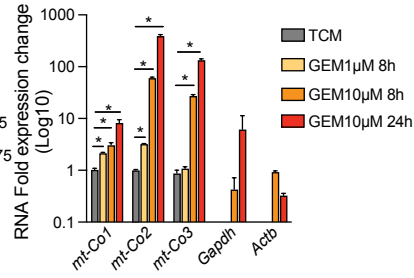
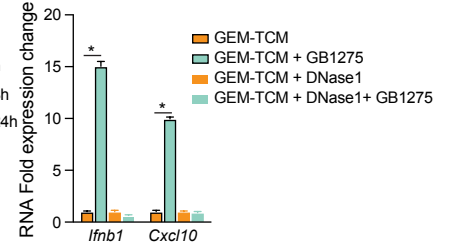
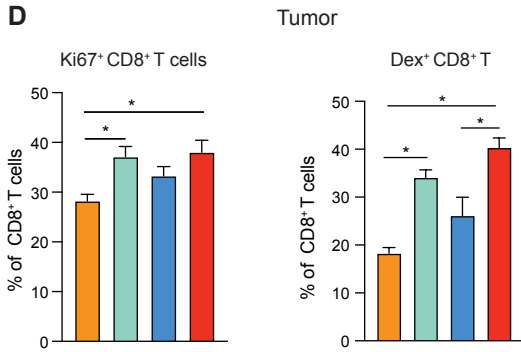
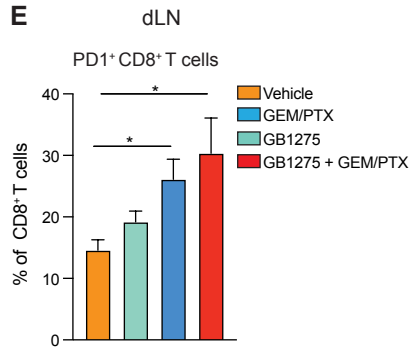
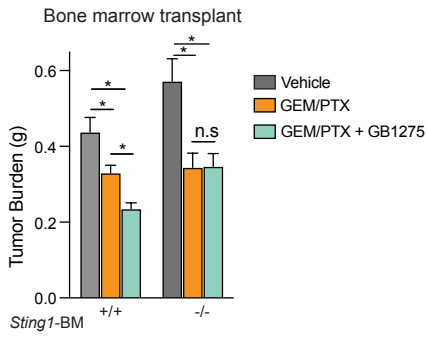
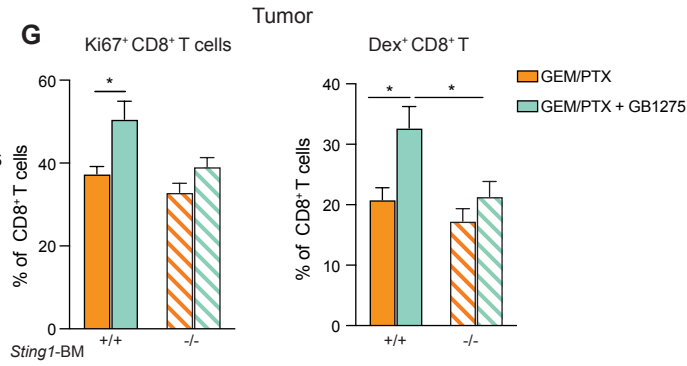
(C) Representative mplHC staining for pSTAT1, STING, F4/80, and CK19 in tumors from the 14-day vehicle and GB1275 (120 mg/kg)-treated KI orthotopic model. Scale bar, 100  $\mu$ m. Right, percentages of STING<sup>+</sup> TAMs and STING<sup>+</sup> pSTAT1<sup>+</sup> TAMs from each treatment group (n = 7 mice per group).

(D) Single staining of CD8 $\alpha$ , CD163, STING, and CK19 in human tumor microarrays (TMAs) by mplHC. Scale bar, 100  $\mu$ m.

(E) Scatter plot showing Spearman's correlation between the percentage of STING<sup>+</sup> CD11b<sup>+</sup> cells and CD8<sup>+</sup> T cells in human TMAs.

(F) Proportion of STING<sup>+</sup>, pSTAT1<sup>+</sup> and nuclear p65<sup>+</sup> macrophages in paired pre- and post-groups.

(G) Relative fold changes pSTAT1<sup>hi</sup> macrophages and nuclear p65<sup>+</sup> macrophages in paired pre- and post-groups from Regimen A and B. Graphs show the mean  $\pm$  SEM; \*denotes p < 0.05 by the two-sided *t*-test for comparisons between two groups.

**A****B****C****D****E****F****G**

**Figure S6. Chemotherapy combined with GB1275 amplifies the anti-tumor response, Related to Figure 6.**

(A) KP2 cells were treated with gemcitabine at different doses for different time periods and then TCM was made from the abovementioned cells. Quantitative PCR mRNA expression analysis of BMDMs treated with the abovementioned TCM  $\pm$  vehicle or GB1275 for 7 hours. Changes in gene expression are depicted as the fold changes from the vehicle baseline.

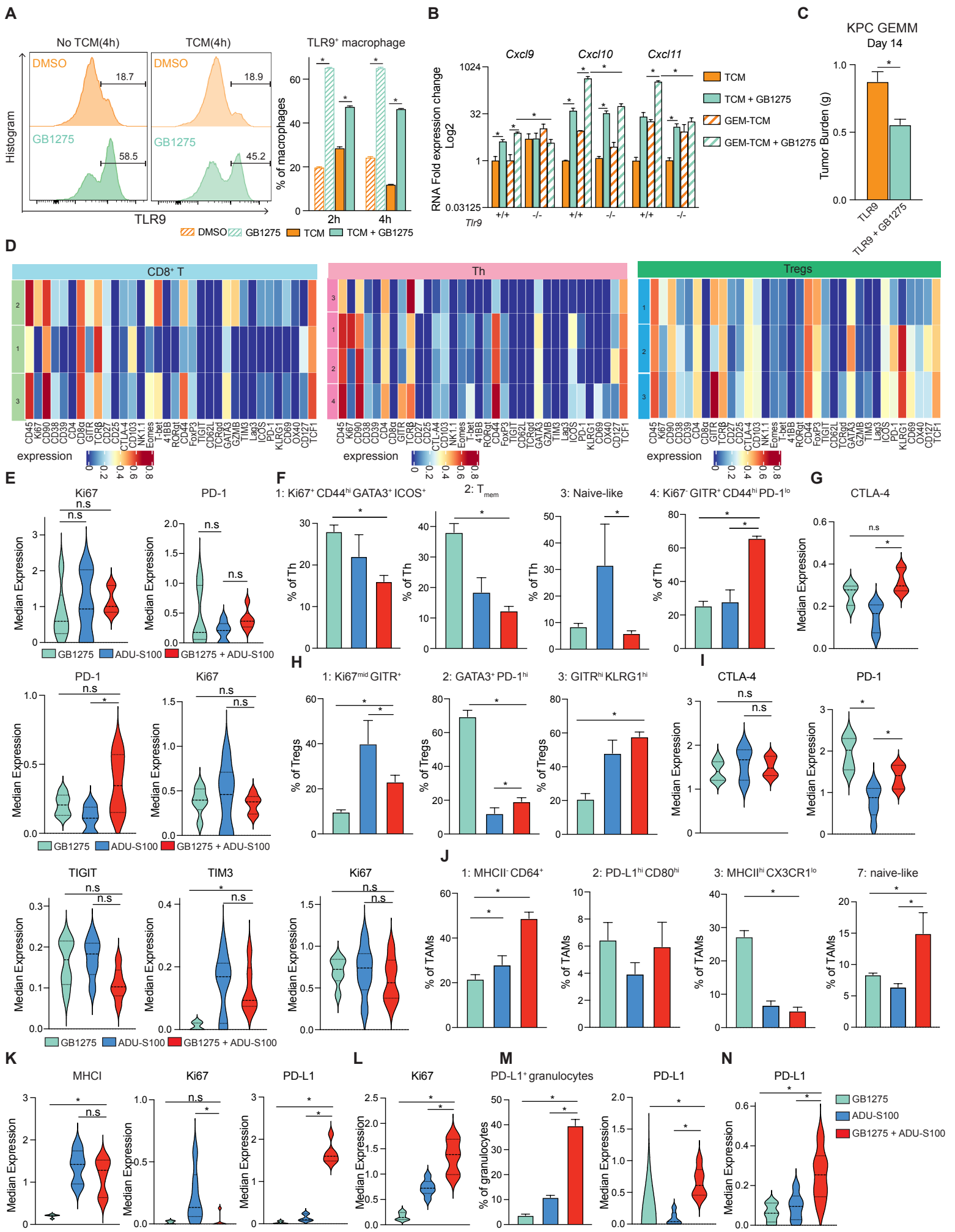
(B) DNA released in metabolites ( $< 3,000$  Da) from different TCM in A was detected by quantitative PCR analysis. Changes in gene expression are depicted as the fold change from the TCM group baseline.

(C) Quantitative PCR mRNA expression analysis of BMDMs treated with TCM made of GEM (10  $\mu$ M, 8 hours) in the presence or absence of DNase1 (10  $\mu$ g/mL) or GB1275 for 7 hours. Changes in gene expressions are depicted as the fold change from the vehicle baseline.

(D) Syngeneic orthotopic model of KP2-OVA cells treated with vehicle, GB1275 (120 mg/kg) chemotherapy (50 mg/kg gemcitabine + 10 mg/kg paclitaxel), or combination for 12 days. Relative frequencies of tumor-infiltrating Ki67<sup>+</sup> CD8<sup>+</sup> T and Dex<sup>+</sup> CD8<sup>+</sup> T cells from tumor tissue (D) and PD1<sup>+</sup> CD8<sup>+</sup> T cells in tumor-draining lymph node (E) from the abovementioned mice (n = 6/group).

(F) C57/BL-6 mice were lethally irradiated and adoptively transferred with bone marrow cells from either wild-type mice or STING-null mice. After 4 weeks, these mice were adopted to establish a KP2-OVA orthotopic model and after 7 days mice were treated with vehicle, chemotherapy (50 mg/kg gemcitabine + 10 mg/kg paclitaxel) or the combination of chemotherapy with GB1275 (120 mg/kg) for 12 days. Tumor burden in all groups is shown (n = 5–9/group).

(G) Relative frequencies of tumor-infiltrating Ki67<sup>+</sup> CD8<sup>+</sup> T and Dex<sup>+</sup> CD8<sup>+</sup> T cells from the abovementioned mice (n = 6/group). Graphs show the mean  $\pm$  SEM; \* denotes  $p < 0.05$  by the two-sided *t*-test for comparisons between two groups. *In vitro* data are representative of three independent experiments.



**Figure S7. Amplified IFN signaling regulates T cells and tumor-associated macrophages, Related to Figure 7.**

(A) Measurement of TLR9 expression on BMDMs treated with vehicle or GB1275 in the presence or absence of TCM for 2 and 4 hours. Percentage of TLR9<sup>+</sup> macrophages are shown (right).

(B) Quantitative PCR mRNA expression analysis of BMDMs isolated from both wild-type and *Tlr9*-null mice treated with different TCM ± vehicle or GB1275 for 7 hours. Changes in gene expressions are depicted as the fold change from the vehicle baseline.

(C) Tumor burden of KPC mice treated with TLR9 agonist (ODN) or GB1275(120 mg/kg) + TLR9 agonist (ODN) for 14 days.

(D) Heat map of markers stained in CD8<sup>+</sup> T cells, Th and Tregs.

(E) Median expression of Ki67 and PD-1 in CD8<sup>+</sup> T cells.

(F) Percentage of individual subclusters in Th from PDAC tissue treated with GB1275, ADU-S100, or the combination group.

(G) Median expressions of CTLA-4, PD-1, and Ki67 in Th.

(H) Percentage of individual subclusters in Tregs.

(I) Median expressions of CTLA-4, PD-1, TIGIT, TIM3, and Ki67 in Tregs.

(J) Percentage of individual subclusters in TAMs from PDAC tissue treated with GB1275, ADU-S100 or in the combination group.

(K) Median expressions of MHC-I, Ki67, and PD-L1 in TAMs.

(L) Median expression of Ki67 in monocytes.

(M) Percentage of PD-L1<sup>+</sup> granulocytes and median expression of PD-L1 in granulocytes.

(N) Median expressions of Ki67 and PD-L1 in DCs. Graphs show the mean ± SEM; \* denotes  $p < 0.05$  by analysis of variance.