Supplementary Figure Legends:

Supp Figure 1: CD70 expression on in-house primary and recurrent GBM samples.

A. Manhattan plot of the top upregulated RNAseq genes in patient-derived GBM brain tumor initiating cells (GBM CSCs) BT698 (rGBM), BT956 (pGBM), BT618 (rGBM), compared to the publicly available TCGA database, identified CD70 (circled in red) as a target candidate upregulated in the CSC subpopulation, compared to tumor bulk. **B:** CD70 cell surface protein expression, assessed by flow cytometry cell surface staining on multiple in-house primary and recurrent cell lines, as well as normal human cell lines.

Supp Figure 2: **In silico analyses of CD70 expression in publicly-available glioma transcriptomic datasets.**

A:CD70 mRNA expression in the Chinese Glioma Genome Atlas (CGGA), stratified by primary, recurrent or secondary tumor. Boxes indicate 25th, 50th and 75th percentile while the whiskers extend to 1.4 times the IQR; P values <0.05 from unpaired T tests are as follows: primary-recurrent P = 1.3e-05. B-C, Kaplan-Meier survival analysis of glioma patients stratified by median CD70 expression from B: CGGA and C. the Cancer Genome Atlas (TCGA). P value from log-rank (Mantel-Cox) test. D: CD70 mRNA expression in the Ivy GAP, stratified by tumor cell location. Boxes indicate 25th, 50th and 75th percentile while the whiskers extend to 1.4 times the IQR; P values <0.05 from unpaired T tests are as follows: pseudopalisading cells compared to cellular tumor (P = 2.1e-05), infiltrating tumor (P = 8.3e-05), leading edge (P = 7.5e-05), and microvascular proliferation (P = 2.4e-03). E: CD70 mRNA expression in the CGGA (left) and TCGA (right), stratified by gender. Boxes indicate 25th, 50th and 75th percentile while the whiskers extend to 1.4 times the IQR; P values from unpaired T tests on plot. F: CD70 mRNA expression in the CGGA, stratified by WHO grade and age status. Boxes indicate 25th, 50th and 75th percentile while the whiskers extend to 1.4 times the IQR; P values from unpaired T tests on plot.

Seyfrid M, *et al. J Immunother Cancer* 2022; 10:e003289. doi: 10.1136/jitc-2021-003289

Supp Figure 3: Knockdown of CD70 improves survival *in vivo*.

A: Proliferation of sorted CD70-positive and -negative cells from BT698, BT428, BT458 GBM cultures was assessed using a PrestoBlue assay. **B:** Proliferation of GBM8 cultures transduced with shCD70-1, shCD70-2 or shGFP was assessed using a PrestoBlue assay. **C:** Limiting Dilution Analysis of BT241that was knocked out for CD70 (cr-CD70A and cr-CD70B) or control crAAVS-1.

D. Tumor area from animal-engrafted tumors of shCD70 GBM4 cells compared to control. E**:** *Kaplan-Meier* curve displaying survival of mice engrafted with shCD70 GBM4 cells compared to shGFP GBM4 controls.

Supplementary Figure 4: CD70 influence on the MES subtype and associated machinery.

A, B: RNA sequencing of CD70 silenced GBM CSC cells BT241, GBM8 and GBM4 permitted the gene set enrichment analysis (GSEA) (A) and Cytoscape Node map (B) depicting circuitries under CD70 dependence.

Supp Figure 5: Generation and *in vitro* Characterization of CD70-Specific CAR-T Cells.

A: Internalization studies showing Fab presence on the surface of BT241 cells after a 2 hour incubation. **B, C:** IFN-gamma and TNF-alpha release during coculture of CD70 CAR-T or ConCAR with GBM CSCs lines GBM4, GBM8, or BT935 CD70 CAR-T, at effector to target (E:T) ratios of 1:1, as analyzed by ELISA (n=3). **D:** Cytotoxicity assay assessing the killing capacity of CD70CAR-T cells on GBM8 CSCs compared to control, after co-culturing for 24 hours, as tested at various E:T ratios (n=3).

Supp Figure 6: CD70 CAR-Ts are efficacious against recurrent GBM8 cells *in vivo*.

Seyfrid M, *et al. J Immunother Cancer* 2022; 10:e003289. doi: 10.1136/jitc-2021-003289

NSG mice (at least n=6 per group) were intracranially implanted with 100,000 human GBM8 ffLuc GBM cells. Upon successful engraftment, mice were treated with $1x10^6$ CD70CAR-T or ConCAR-T cells, delivered intracranially once a week for two weeks. **A:** CAR-T treated mice a lower tumor burden in the CD70 CAR-T group compared to Control group as by IVIS imaging. **B:** CD70CAR-T treated mice also showed higher survival rate compared to that of the ConCAR-T cohort. **C:** Tumor burden was assessed in the CD70 CAR-T group compared to Control group, as measured using formalin-fixed, H&E stained mouse brain slices (representative picture on the right).

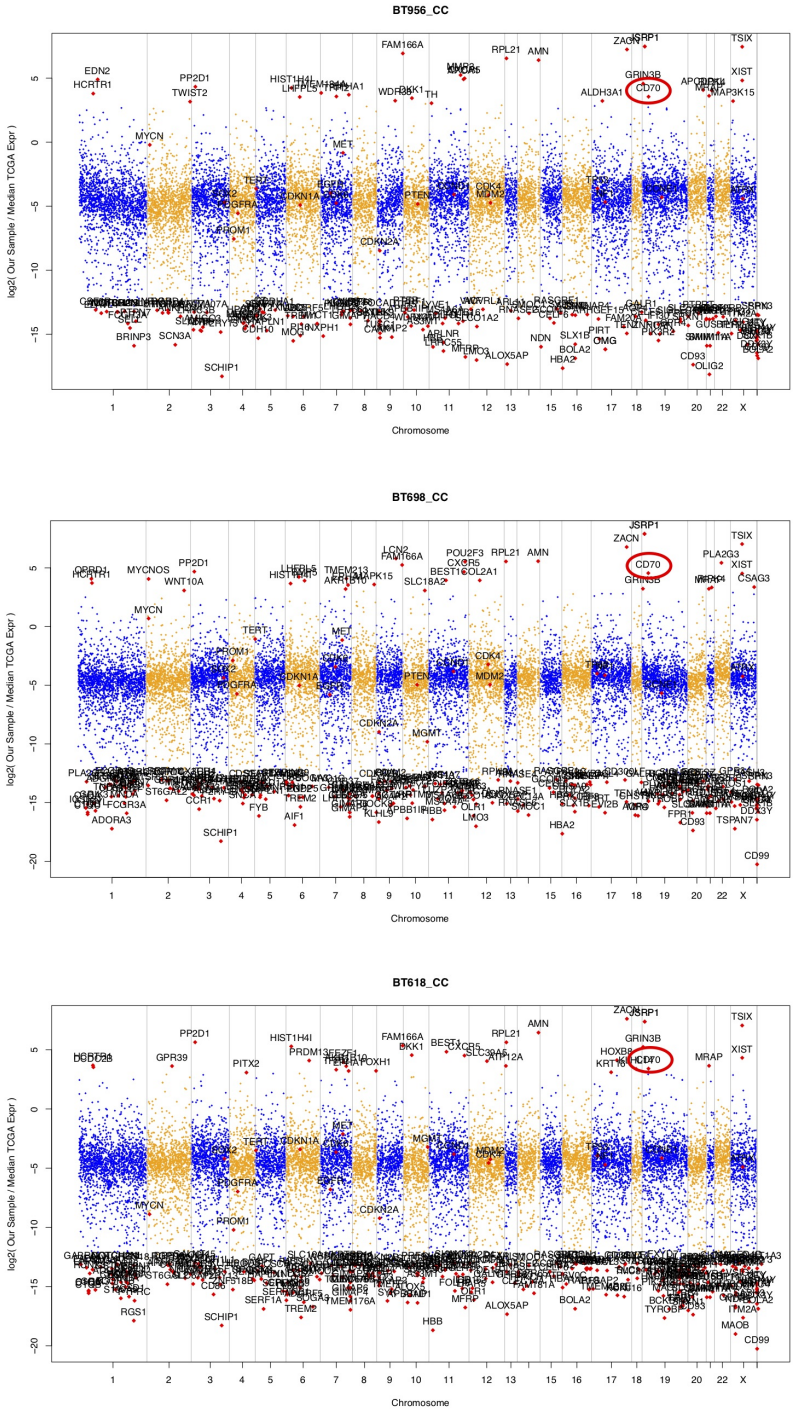
Supp Figure 7: Modelling CD70s influence on the GBM TIME.

A. Viability of CD3+ or CD3+CD27+ cells were assessed after co-culture with cells only or supernatant only from i) control knockdown BT241 cells; ii) one of two constructs that produce CD70KD BT241 cells. **B.** Tumor immune microenvironment (TIME) cells extracted from patient tumor samples were analyzed by flow cytometry, evaluating the pattern of expression of CD27 in non-lymphoid cells (CD45+CD3+) as well as cytotoxic T cell infiltration (CD8+ in CD45+CD3+).

Supp Figure 8: CD70 and CD27 expression in non-malignant tissue. **A.** Cell-type annotated UMAP of Cao 2020 scRNAseq data (top) and expression of CD70 (middle) and CD27 (bottom) visualized on UMAP and stratified by cell type. **B**. Cell-type annotated UMAP of Zeisel 2018 scRNAseq data (left) and expression of CD70 (top right) and CD27 (bottom right) visualized on UMAP and stratified by cell type. Expr; Expression.

Seyfrid M, *et al. J Immunother Cancer* 2022; 10:e003289. doi: 10.1136/jitc-2021-003289

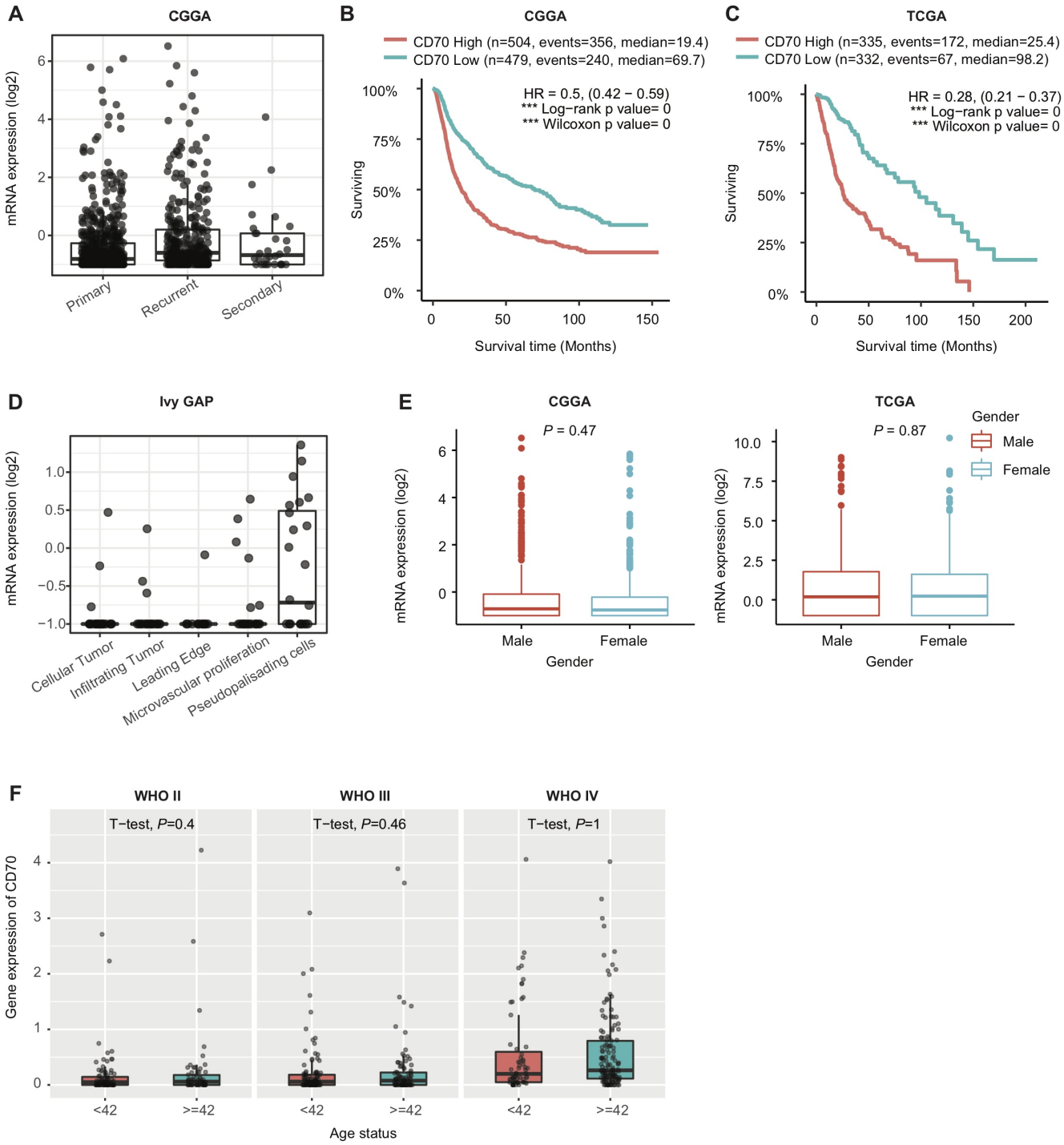
A



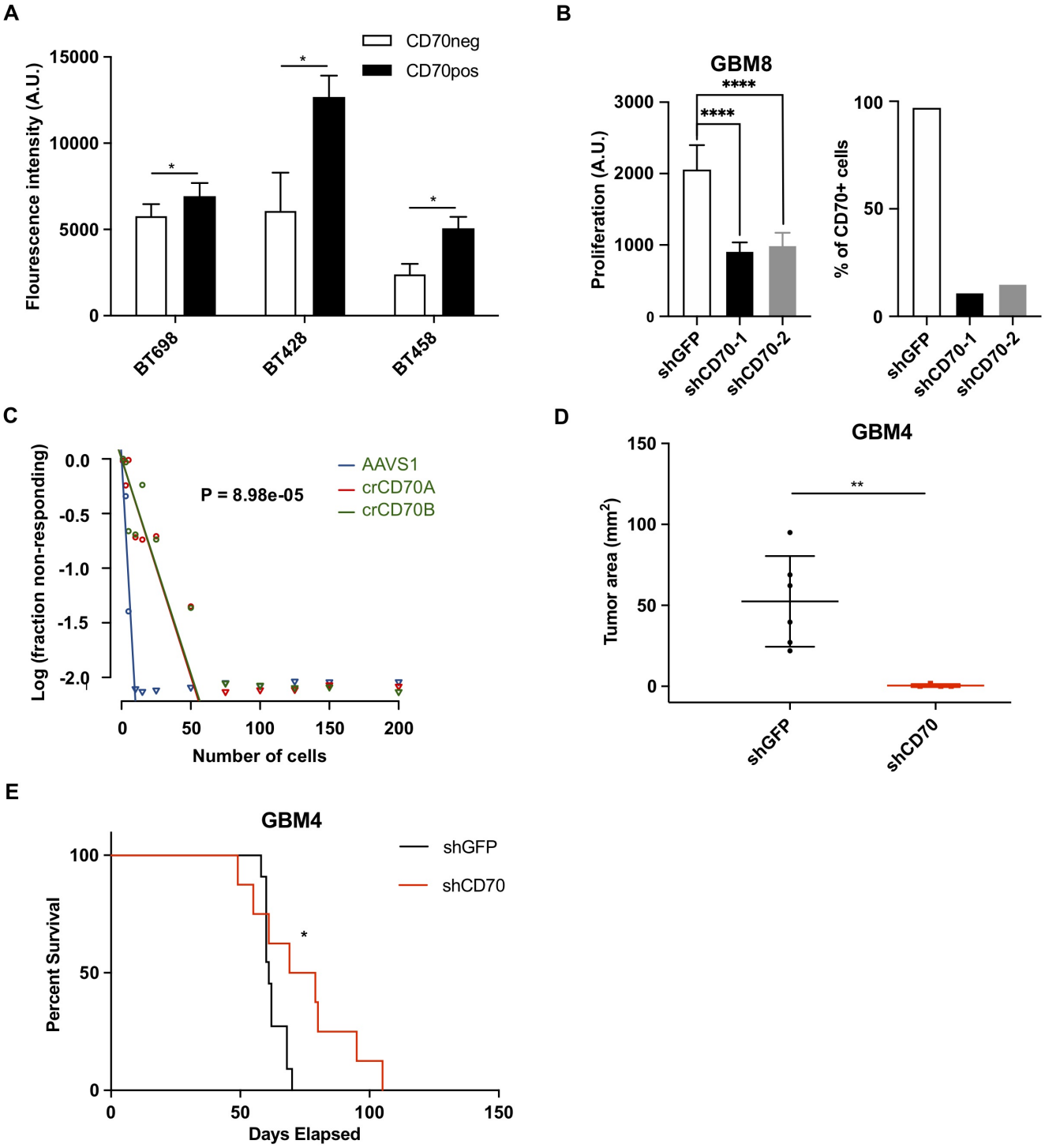
B

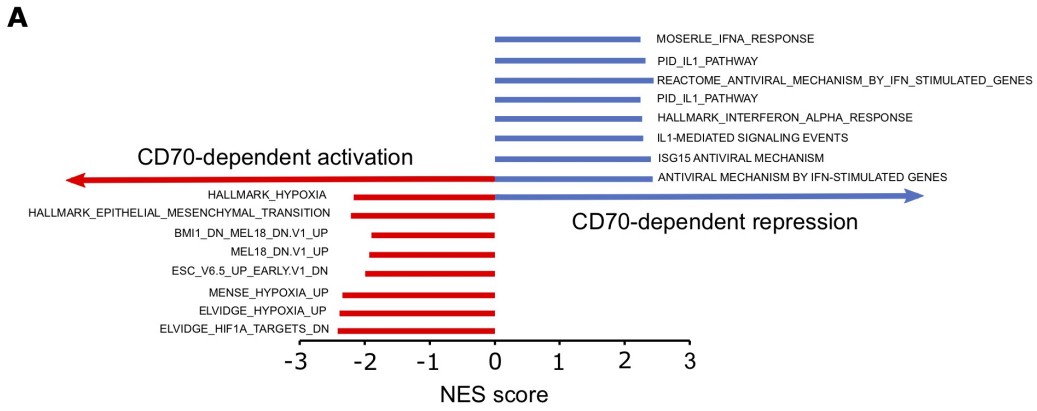
	Patient derived BTIC lines	%CD70+ cells
Recurrent	BT241	94
	MBT190	69
	MBT225	68
	BT618	46
	MBT173	41
	BT972	11.3
	SB2b	4
	BT566	3
Primary	GBM4	72
	GBM8	70
	BT698	65
	RN1	35
	MBT103	35
	MBT121	12
	BT458	9
	BT428	5
	BT954	4
	WK1	3
	BT799	2.2
	GBM123	1.5
	MBT63	1.3
	MBT27	1
	BT935	<1
	BT594	0.5
	BT459	<1
	BT778	<1
Normal brain cells	NSC195	1.8
	NSC201FT	8.5
	NSC198	0.12
	Astrocytes	1.2

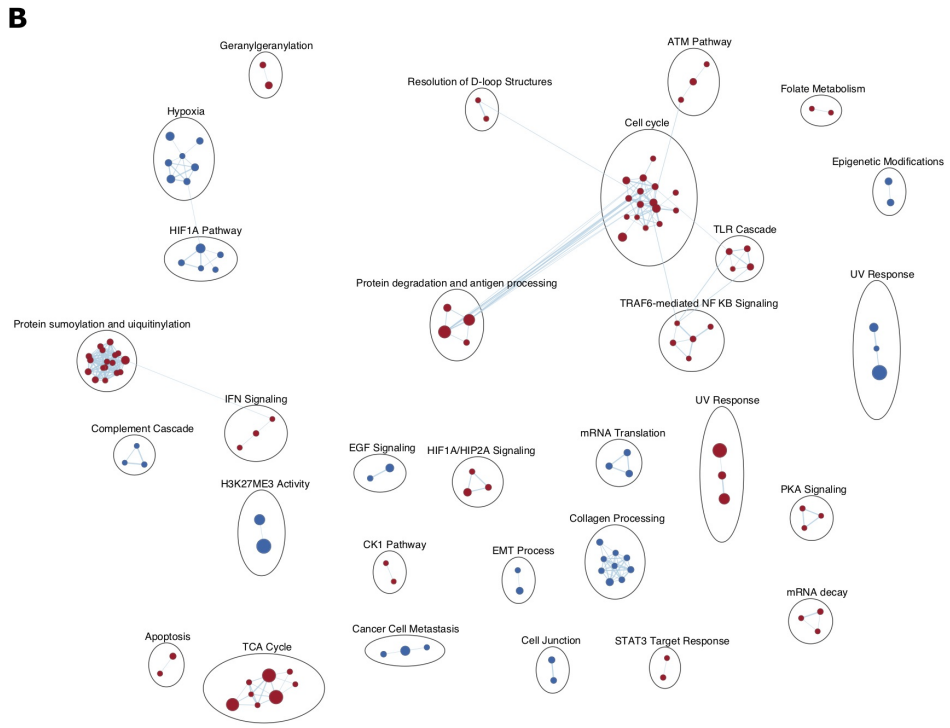
Sup Figure 1


A
CGGA
mRNA expression (log2)
Primary
Recurrent
Secondary
B
CGGA
CD70 High (n=504, events=356, median=19.4)
CD70 Low (n=479, events=240, median=69.7)
HR = 0.5, (0.42 − 0.59)
*** Log−rank p value= 0
*** Wilcoxon p value= 0
Surviving
Survival time (Months)
C
TCGA
CD70 High (n=335, events=172, median=25.4)
CD70 Low (n=332, events=67, median=98.2)
HR = 0.28, (0.21 − 0.37)
*** Log−rank p value= 0
*** Wilcoxon p value= 0
Surviving
Survival time (Months)
D
Ivy GAP
mRNA expression (log2)
Cellular Tumor
Infiltrating Tumor
Leading Edge
Microvascular proliferation
Pseudopalisading cells
E
CGGA
P = 0.47
mRNA expression (log2)
Male
Female
Gender
TCGA
P = 0.87
Gender
Male
Female
mRNA expression (log2)
Male
Female
Gender
F
WHO II
T−test, P=0.4
WHO III
T−test, P=0.46
WHO IV
T−test, P=1
Gene expression of CD70
<42
>=42
Age status

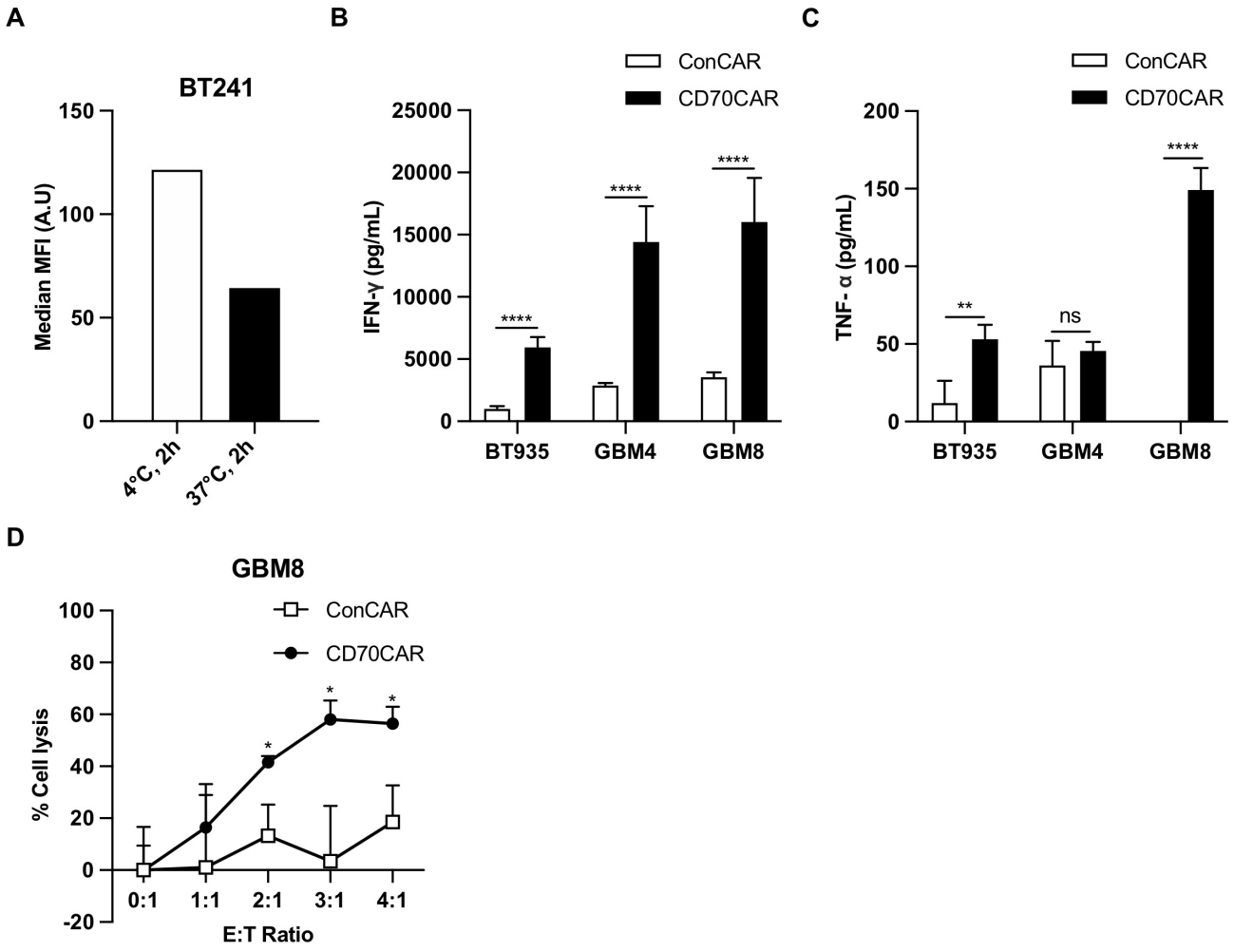
Sup Figure 2


A
CD70neg
CD70pos
Flourescence intensity (A.U.)
15000
10000
5000
0
*
*
*
BT698
BT428
BT458
B
GBM8
****
****
Proliferation (A.U.)
3000
2000
1000
0
shGFP
shCD70-1
shCD70-2
% of CD70+ cells
100
50
0
shGFP
shCD70-1
shCD70-2
C
Log (fraction non-responding)
0.0
-0.5
-1.0
-1.5
-2.0
P = 8.98e-05
AAVS1
crCD70A
crCD70B
0
50
100
150
200
Number of cells
D
GBM4
**
Tumor area (mm2)
150
100
50
0
shGFP
shCD70
E
GBM4
shGFP
shCD70
Percent Survival
100
50
0
*
0
50
100
150
Days Elapsed

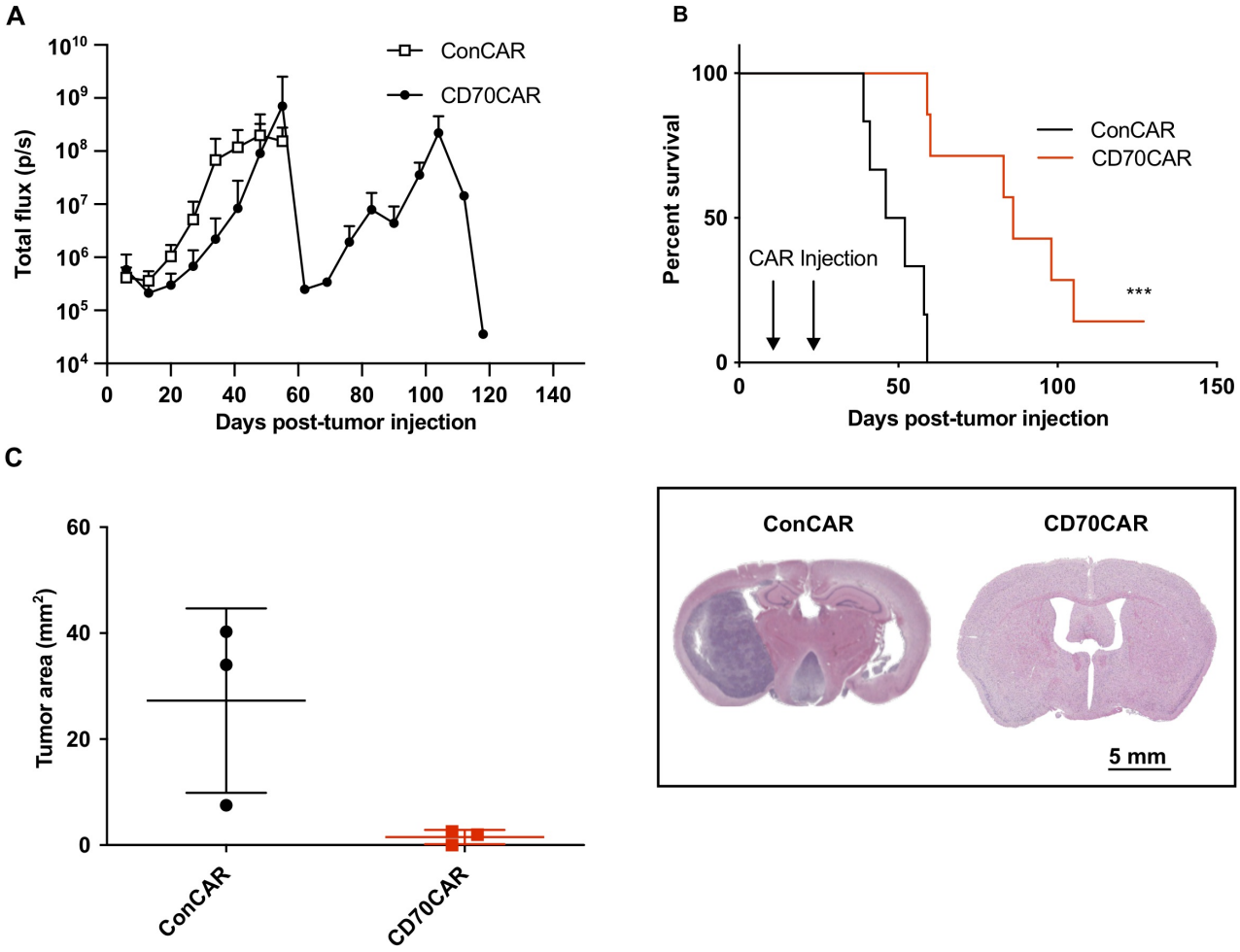

A
MOSERLE_IFNA_RESPONSE
PID_IL1_PATHWAY
REACTOME_ANTIVIRAL_MECHANISM_BY_IFN_STIMULATED_GENES
PID_IL1_PATHWAY
HALLMARK_INTERFERON_ALPHA_RESPONSE
IL1-MEDIATED SIGNALING EVENTS
ISG15 ANTIVIRAL MECHANISM
ANTIVIRAL MECHANISM BY IFN-STIMULATED GENES
CD70-dependent activation
CD70-dependent repression
HALLMARK_HYPOXIA
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
BMI1_DN_MEL18_DN.V1_UP
MEL18_DN.V1_UP
ESC_V6.5_UP_EARLY.V1_DN
MENSE_HYPOXIA_UP
ELVIDGE_HYPOXIA_UP
ELVIDGE_HIF1A_TARGETS_DN
-3
-2
-1
0
1
2
3
NES score


B
Geranylgeranylation
Hypoxia
HIF1A Pathway
Protein sumoylation and uiquitinylation
IFN Signaling
Complement Cascade
H3K27ME3 Activity
Apoptosis
TCA Cycle
Resolution of D-loop Structures
ATM Pathway
Folate Metabolism
Cell cycle
Epigenetic Modifications
TLR Cascade
Protein degradation and antigen processing
TRAF6-mediated NF KB Signaling
UV Response
EGF Signaling
HIF1A/HIP2A Signaling
mRNA Translation
UV Response
PKA Signaling
CK1 Pathway
EMT Process
Collagen Processing
mRNA decay
Cancer Cell Metastasis
Cell Junction
STAT3 Target Response

Sup Figure 4


A
BT241
Median MFI (A.U)
150
100
50
0
4°C, 2h
37°C, 2h
B
ConCAR
CD70CAR
IFN-γ (pg/mL)
25000
20000
15000
10000
5000
0
****
****
****
BT935
GBM4
GBM8
C
ConCAR
CD70CAR
TNF- α (pg/mL)
200
150
100
50
0
**
ns
****
BT935
GBM4
GBM8
D
GBM8
ConCAR
CD70CAR
% Cell lysis
100
80
60
40
20
0
-20
*
*
*
0:1
1:1
2:1
3:1
4:1
E:T Ratio

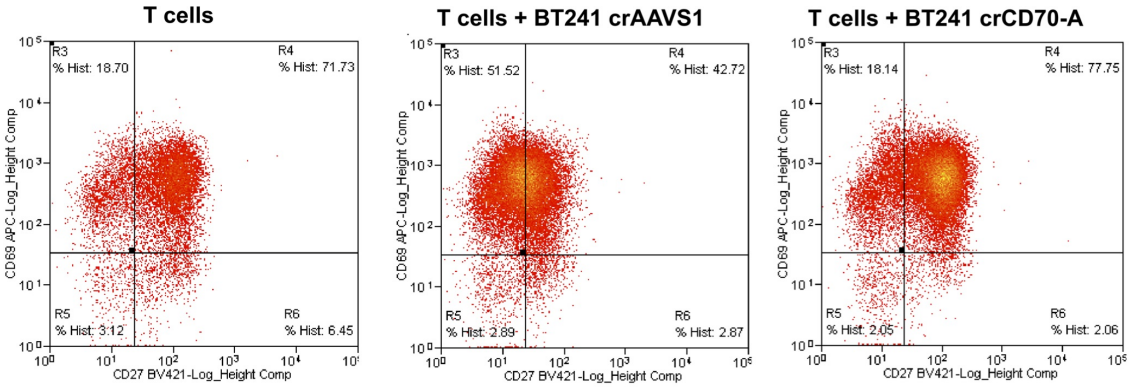
Sup Figure 5


A
Total flux (p/s)
ConCAR
CD70CAR
Days post-tumor injection
B
Percent survival
ConCAR
CD70CAR
CAR Injection
***
Days post-tumor injection
C
Tumor area (mm²)
ConCAR
CD70CAR
ConCAR
CD70CAR
5 mm

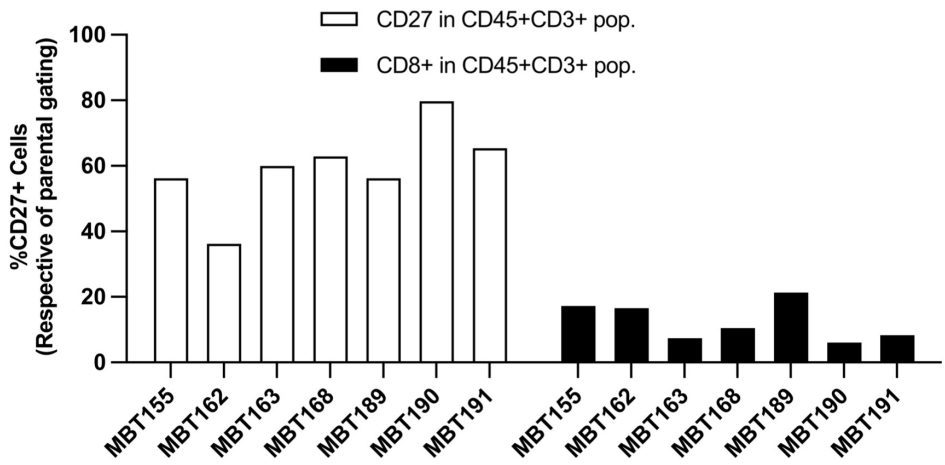
Sup Figure 6

A

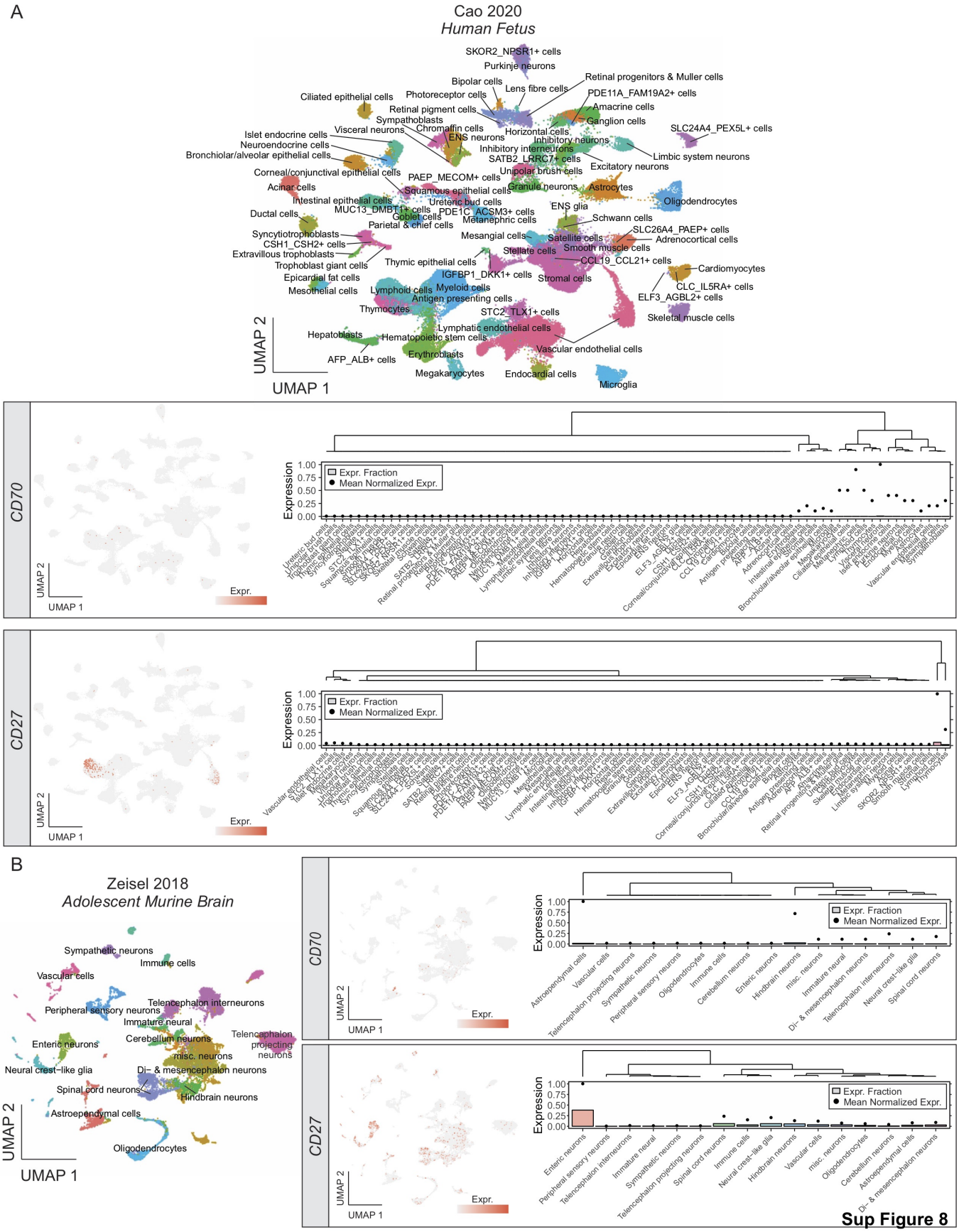
Experimental setup	%CD3+	%CD3+/CD27+
T cells alone	84	78
T cells + BT241 crAAVS1 (supernatant)	76	78
T cells + BT241 crCD70-A (supernatant)	76	78
T cells + BT241 crCD70-B (supernatant)	78	78
T cells + BT241 crAAVS1 (cells)	77	43
T cells + BT241 crCD70-A (cells)	77	79
T cells + BT241 crCD70-B (cells)	80	76



B



Sup Figure 7


A
Cao 2020
Human Fetus
SKOR2_NPSR1+ cells
Purkinje neurons
Retinal progenitors & Muller cells
Bipolar cells
Lens fibre cells
PDE11A_FAM19A2+ cells
Ciliated epithelial cells
Photoreceptor cells
Amacrine cells
Retinal pigment cells
Ganglion cells
Sympathoblasts
Chromaffin cells
Horizontal cells
SLC24A4_PEX5L+ cells
Visceral neurons
ENS neurons
Inhibitory neurons
Islet endocrine cells
Neuroendocrine cells
Inhibitory interneurons
Limbic system neurons
Bronchiolar/alveolar epithelial cells
SATB2_LRRC7+ cells
Excitatory neurons
Corneal/conjunctival epithelial cells
Unipolar brush cells
PAEP_MECOM+ cells
Acinar cells
Granule neurons
Astrocytes
Squamous epithelial cells
Intestinal epithelial cells
Ureteric bud cells
Oligodendrocytes
MUC13_DMBT1+ cells
PDE1C_ACSM3+ cells
ENS glia
Ductal cells
Goblet cells
Metanephric cells
Schwann cells
Parietal & chief cells
SLC26A4_PAEP+ cells
Syncytiotrophoblasts
Mesangial cells
Satellite cells
Adrenocortical cells
CSH1_CSH2+ cells
Smooth muscle cells
Extravillous trophoblasts
Stellate cells
Trophoblast giant cells
Thymic epithelial cells
CCL19_CCL21+ cells
Cardiomyocytes
Epicardial fat cells
IGFBP1_DKK1+ cells
Stromal cells
Mesothelial cells
CLC_IL5RA+ cells
Lymphoid cells
Myeloid cells
ELF3_AGBL2+ cells
Antigen presenting cells
Thymocytes
STC2_TLX1+ cells
Skeletal muscle cells
Lymphatic endothelial cells
Hepatoblasts
Hematopoietic stem cells
Vascular endothelial cells
AFP_ALB+ cells
Erythroblasts
Megakaryocytes
Endocardial cells
Microglia
UMAP 2
UMAP 1
CD70
CD27
Expr.
Expression
Expr. Fraction
Mean Normalized Expr.
1.00
0.75
0.50
0.25
0.00
B
Zeisel 2018
Adolescent Murine Brain
Sympathetic neurons
Immune cells
Vascular cells
Telencephalon interneurons
Peripheral sensory neurons
Immature neural
Cerebellum neurons
Telencephalon projecting neurons
Enteric neurons
misc. neurons
Neural crest-like glia
Di- & mesencephalon neurons
Spinal cord neurons
Hindbrain neurons
Astroependymal cells
Oligodendrocytes
Sup Figure 8