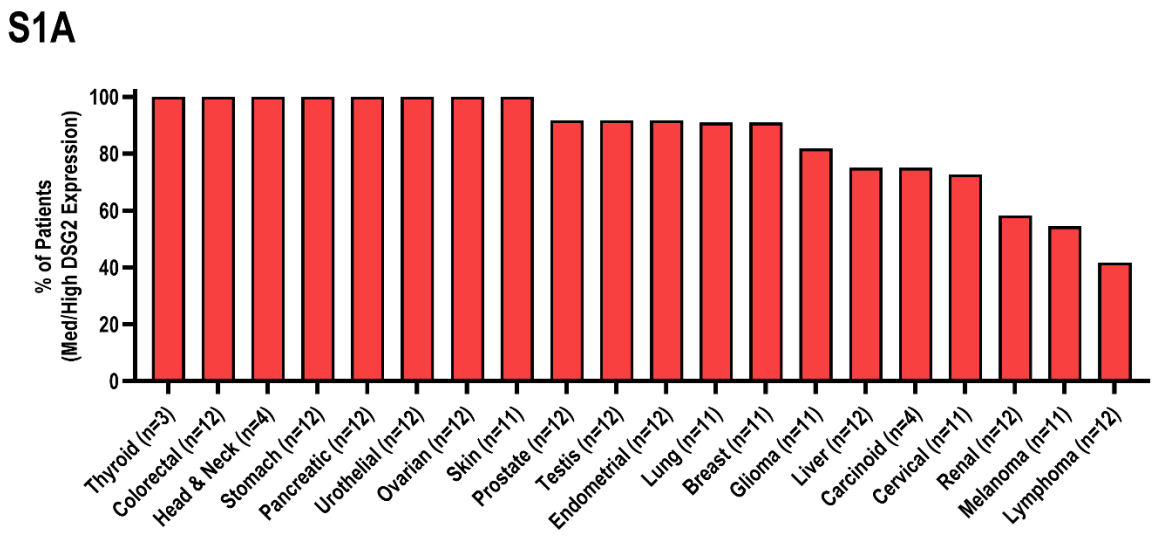
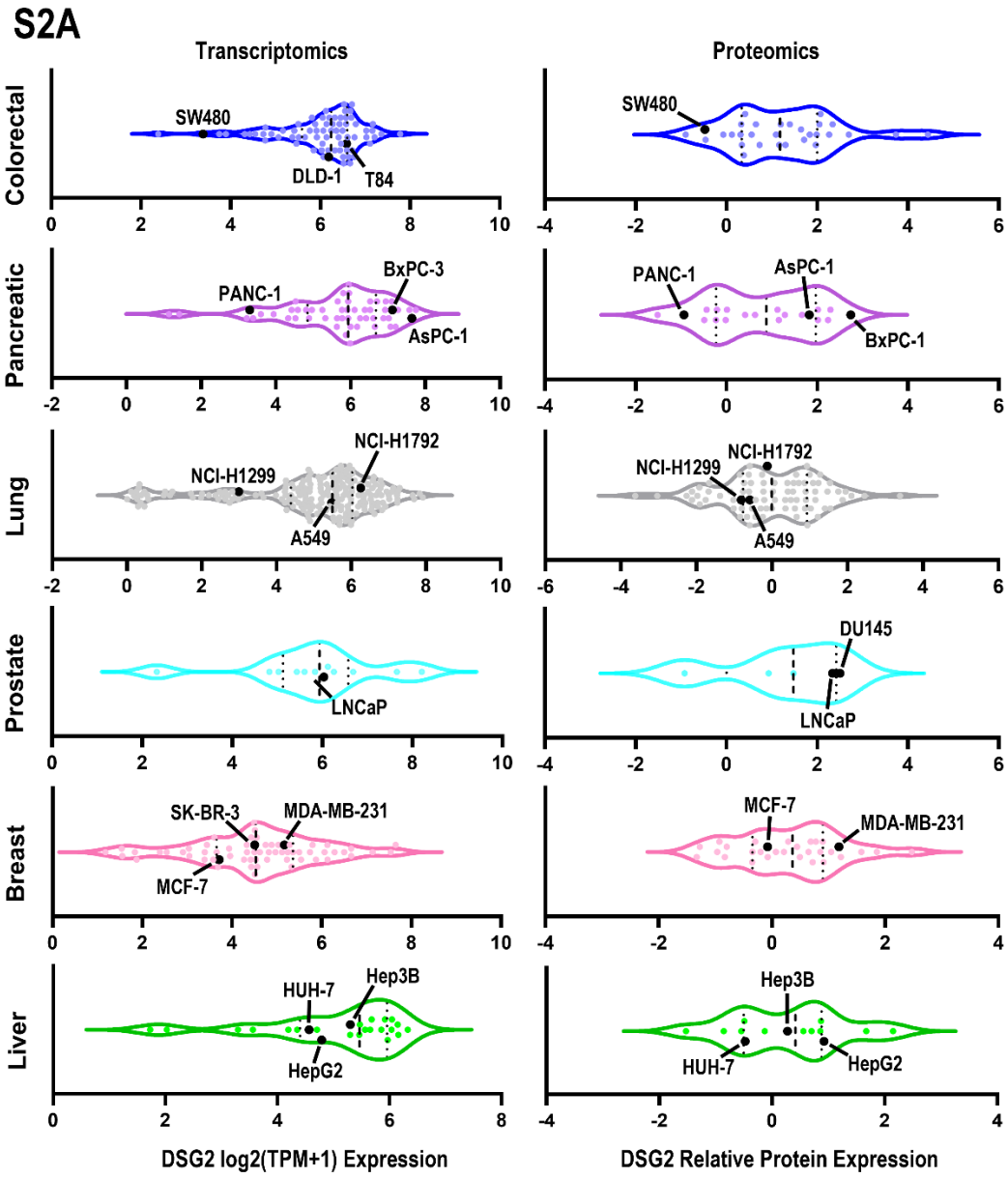
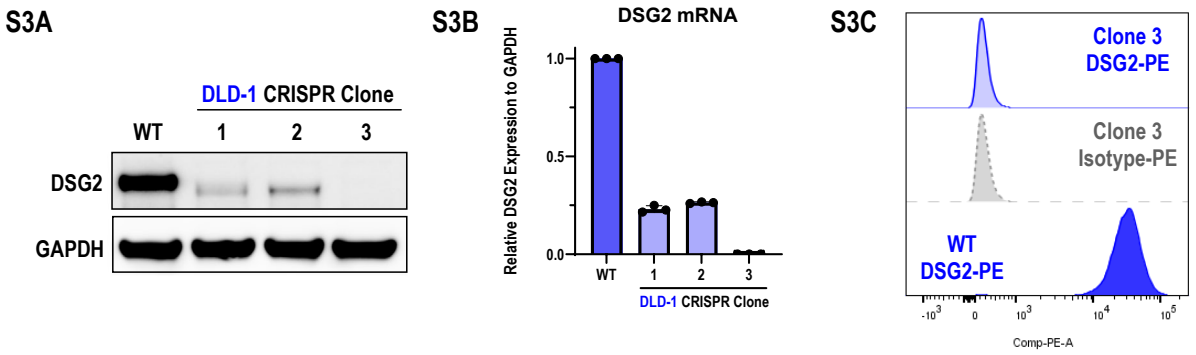


Supplementary Figure S1: DSG2 protein is abundantly expressed in patient solid tumor specimens. (**A**) Percentage of patient tumors scoring medium-high for DSG2 protein expression via HPA IHC-scoring criteria. Percentage based on number of medium/high IHC-scored tissues divided by total number of available patient specimens per tumor type using the HPA004896 dataset.



Supplementary Figure S2: DSG2 mRNA and protein expression stratification in CCLE-indexed cell lines. (**A**) Select cancer cell lines stratified by DSG2 transcriptional (left; Expression Public 24Q2) and proteomic (right; UniProt identifier: Q14126) expression data among all available CCLE-indexed cell lines per cohort, representing the six most lethal solid tumor types. Black dotted lines represent expression quartiles. Cohort inclusion is dependent on cell line availability in CCLE data sets. Expression data was retrieved and normalized via the Broad Institute's Cancer Dependency Map (DepMap) Portal (06/2024). TPM; transcripts per million.



Supplementary Figure S3: Validation of DSG2 CRISPR-knockout in DLD-1 colorectal cancer cells. (**A**) Whole cell lysate (WCL) from three separate DLD-1 CRC cell monoclonal colonies following bulk cell transfection with DSG2 CRISPR synthetic guide RNA (sgRNA) pool analyzed by western blot for DSG2 full length (DSG2; 160 kDa) protein. Wildtype (WT) DLD-1 cells served as positive control for DSG2. GAPDH (37 kDa) was used as the loading control. (**B**) DSG2 mRNA expression in three separate DLD-1 DSG2-KO cell clones relative to GAPDH. Relative DSG2 mRNA expression from each clone was further normalized to WT DLD-1 control cells. DSG2 transcript expression was undetectable in DLD-1 DSG2-KO clone 3. n=3 technical replicates/clone. (**C**) DSG2 surface expression in DLD-1 DSG2-KO cell clone 3 (light blue) and WT DLD-1 cells (dark blue) measured via flow cytometry with a DSG2-PE monoclonal antibody. IgG2b-PE mouse isotype antibody (grey) served as a negative stain control.

S4A

Nb of 5' trimmed-n:3. Nb of 3' trimmed-n:14. Analysed sequence length:439.

Sequence analysis category: 1 (no indel search)

Sequence compared with the *Mus musculus* (mouse) IG set from the IMGT reference directory

IMGT HC Query

>Additional_Sequence_HC_480_-_Rev

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gtggccaagcacagatccagttggtgcagtctggacctgagctgaagaagcctggagaga
cagtcaagatctcctgcaagggcttctgggtatacctt cacaaactatggaatgaactggg
tgaagcaggctccaggaagggtttaaagtggatgggctggataaacacctacactggaa
acccaacatatgctgatgacttcaagggacggtttgacttctctttggaaacctntgcca
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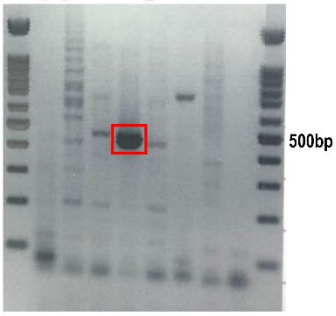
caagagatcgtggtaattcctttgactactggggccaaggcaccactctcacantntnnt
cannca aacgncnnnccc

Result summary:	Productive IGH rearranged sequence: (no stop codon and in-frame junction)		
V-GENE and allele	Musmus IGHV9-1*02 F	score = 1367	identity = 97.22% (280/288 nt)
J-GENE and allele	Musmus IGHJ2*01 F	score = 197	identity = 85.42% (41/48 nt)
D-GENE and allele by IMGT/JunctionAnalysis	Musmus IGHD1-1*02 F	D-REGION is in reading frame 3	
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[25.17.38.11]	[8.8.10]	CARDRGNSFDYW

V-REGION uncertain nt nb: 1



Nb of 5' trimmed-n:6. Nb of 3' trimmed-n:17. Analysed sequence length:398.

Sequence analysis category: 1 (no indel search)

Sequence compared with the *Mus musculus* (mouse) IG set from the IMGT reference directory

IMGT κLC Query

>Original_KLC_(No_Aberrant_Primers)_-_Rev

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cagaaacagggaaaatctcctcagctcctggtctatattgcaataaacttagcagatggt
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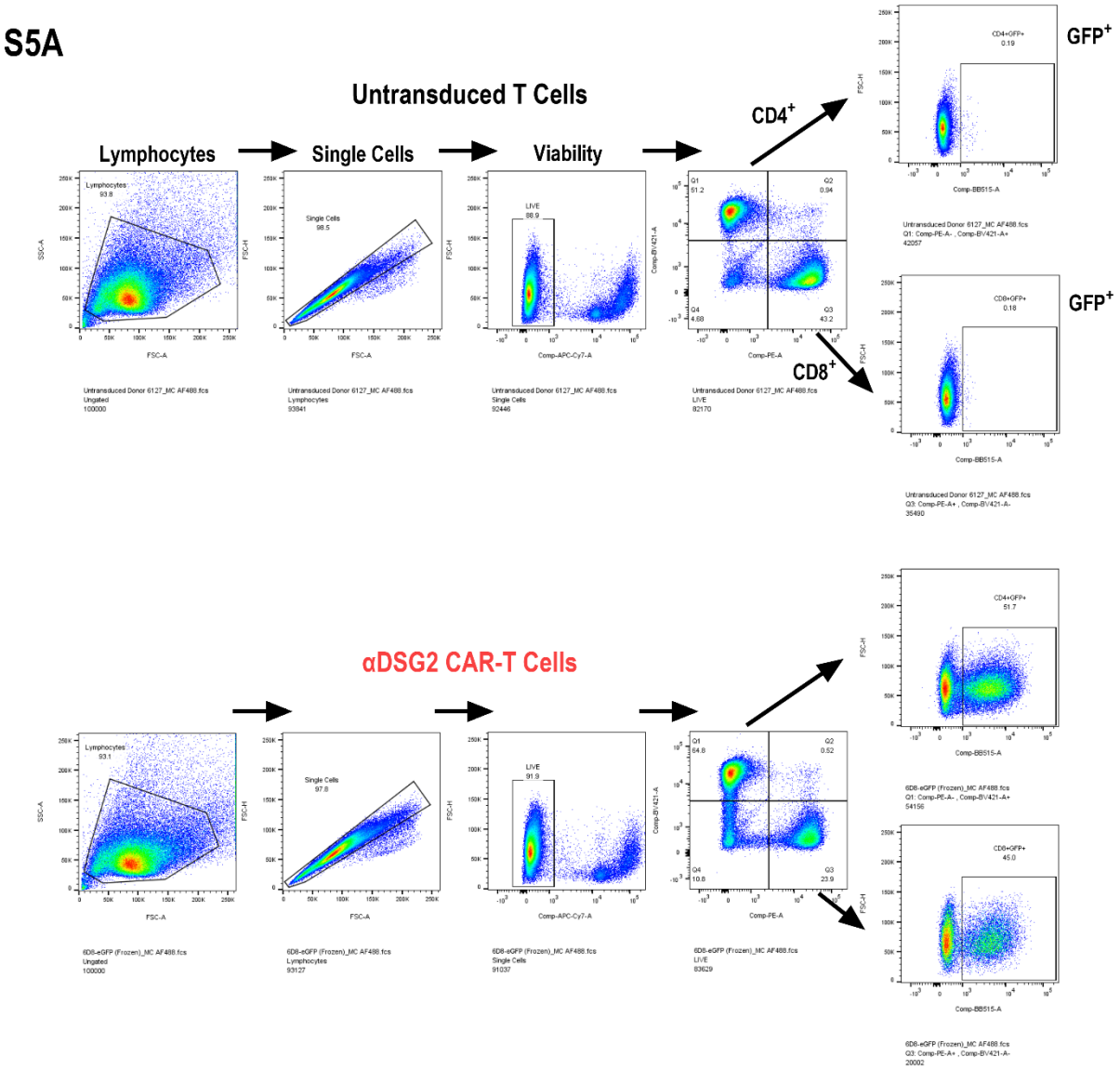
Result summary:	Productive IGK rearranged sequence: (no stop codon and in-frame junction)		
V-GENE and allele	Musmus IGKV12-46*01 F	score = 1340	identity = 97.85% (273/279 nt)
J-GENE and allele	Musmus IGKJ1*01 F	score = 165	identity = 91.67% (33/36 nt)
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[26.17.36.10]	[6.3.9]	CQHFWGTPRTF

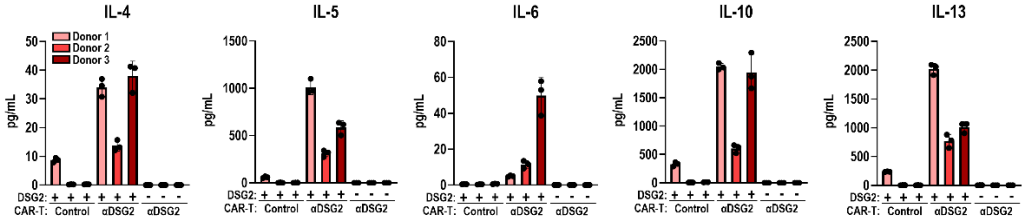
V-REGION uncertain nt nb: 1

Supplementary Figure S4: Sequence identification of αDSG2 monoclonal antibody (6D8) from hybridoma genomic DNA. (**A**) Hybridoma 6D8 genomic DNA isolated and analyzed via polymerase chain reaction (PCR) with degenerate primers (Heavy Chain, HC: MHALT1.RV, MgC.CH1AS; Kappa Light Chain, κLC: MLALT4.RVG, MCkAS.XBA). Amplicon (left, red box) extracted, sequenced, and queried in ImMunoGeneTics (IMGT) V-Quest tool to determine antibody variable region identity and associated complementarity-determining regions (CDRs) for αDSG2 CAR scFv construction.

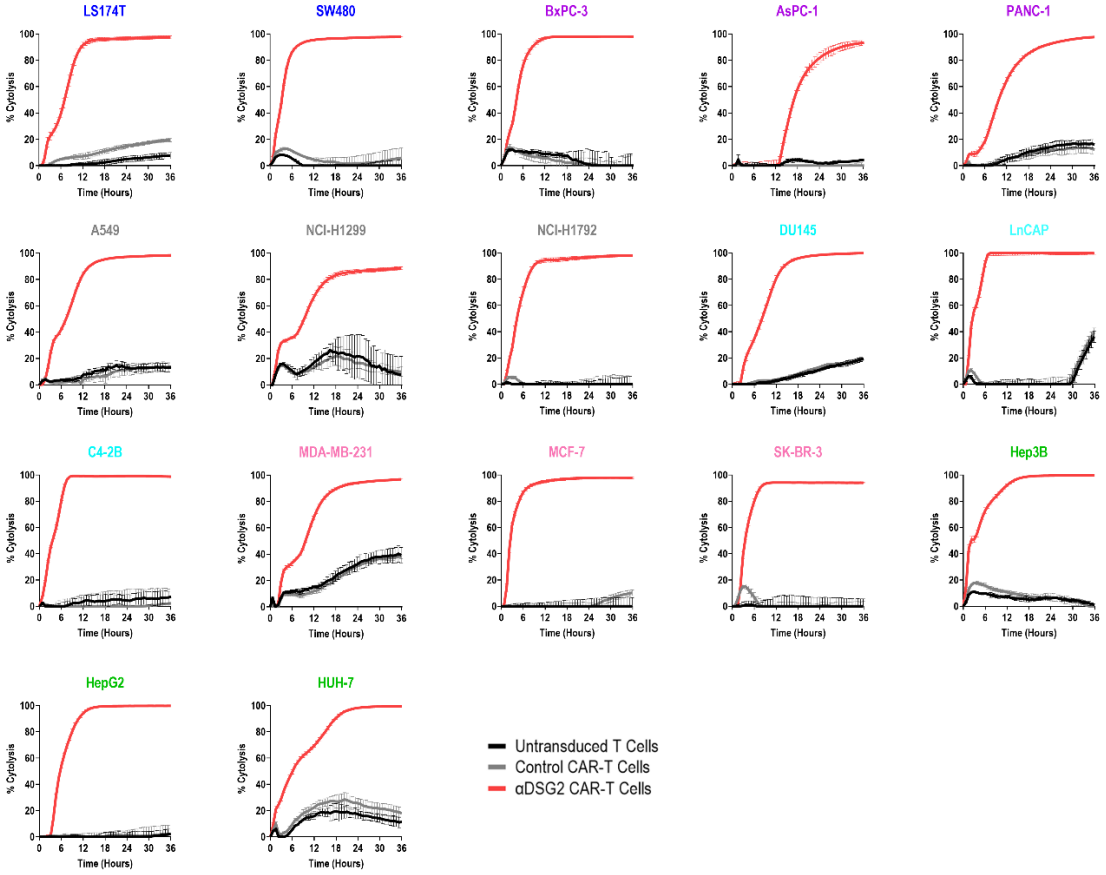


Supplementary Figure S5: Flow cytometry gating strategy for determining CAR transduction efficiency. (**A**) Representative flow cytometry gating strategy for analyzing CAR-T cell transduction efficiency via bicistronic GFP-expression after 14 days in culture. Following bulk lymphocyte gating on size (FSC) and granularity (SSC), doublets were excluded, followed by dead cell exclusion in cells containing the viability dye. $CD4^+$ and $CD8^+$ T cells were split, and relative transduction efficiency (via % GFP^+) was compared to human donor-matched untransduced T cells.

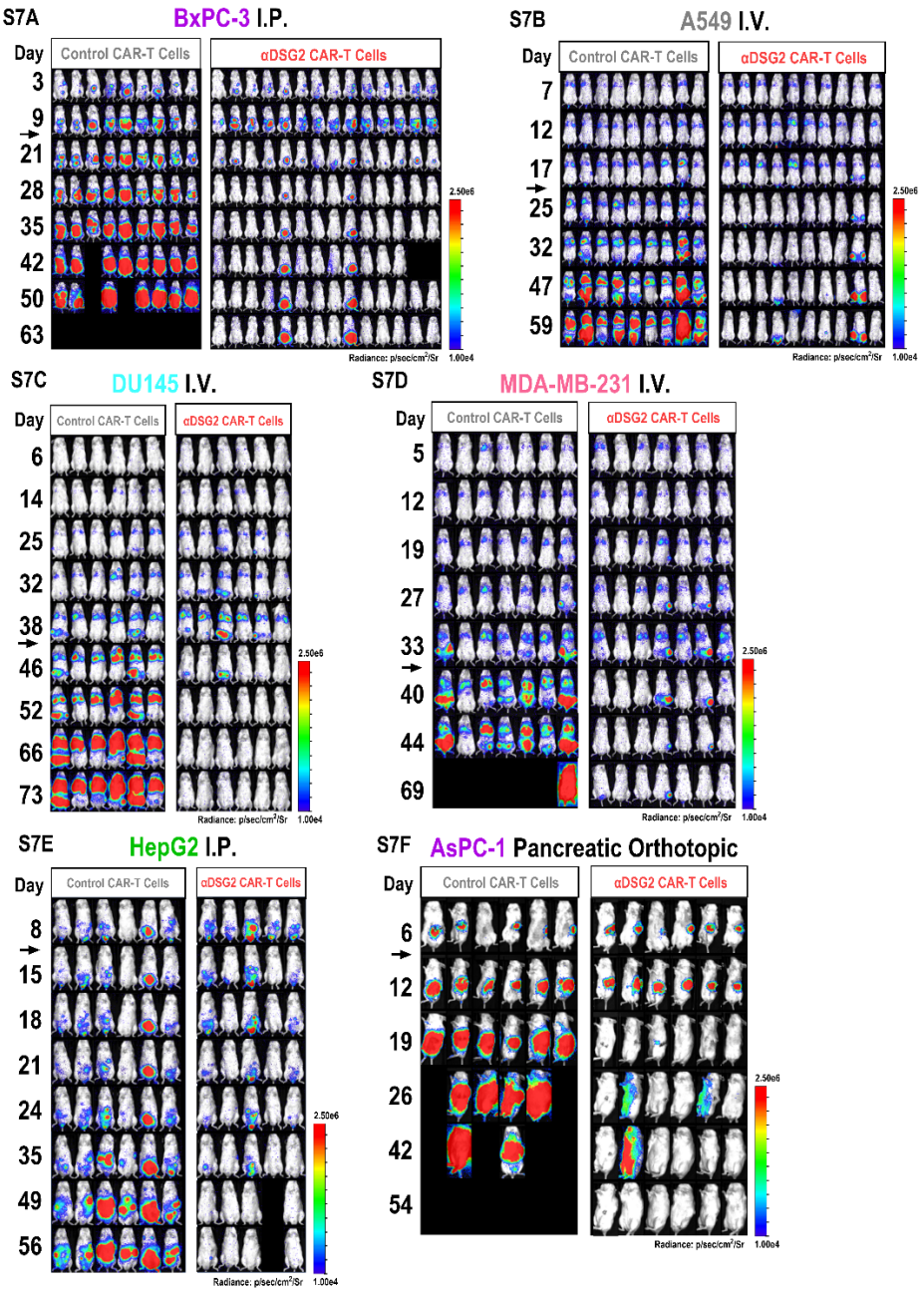
S6A



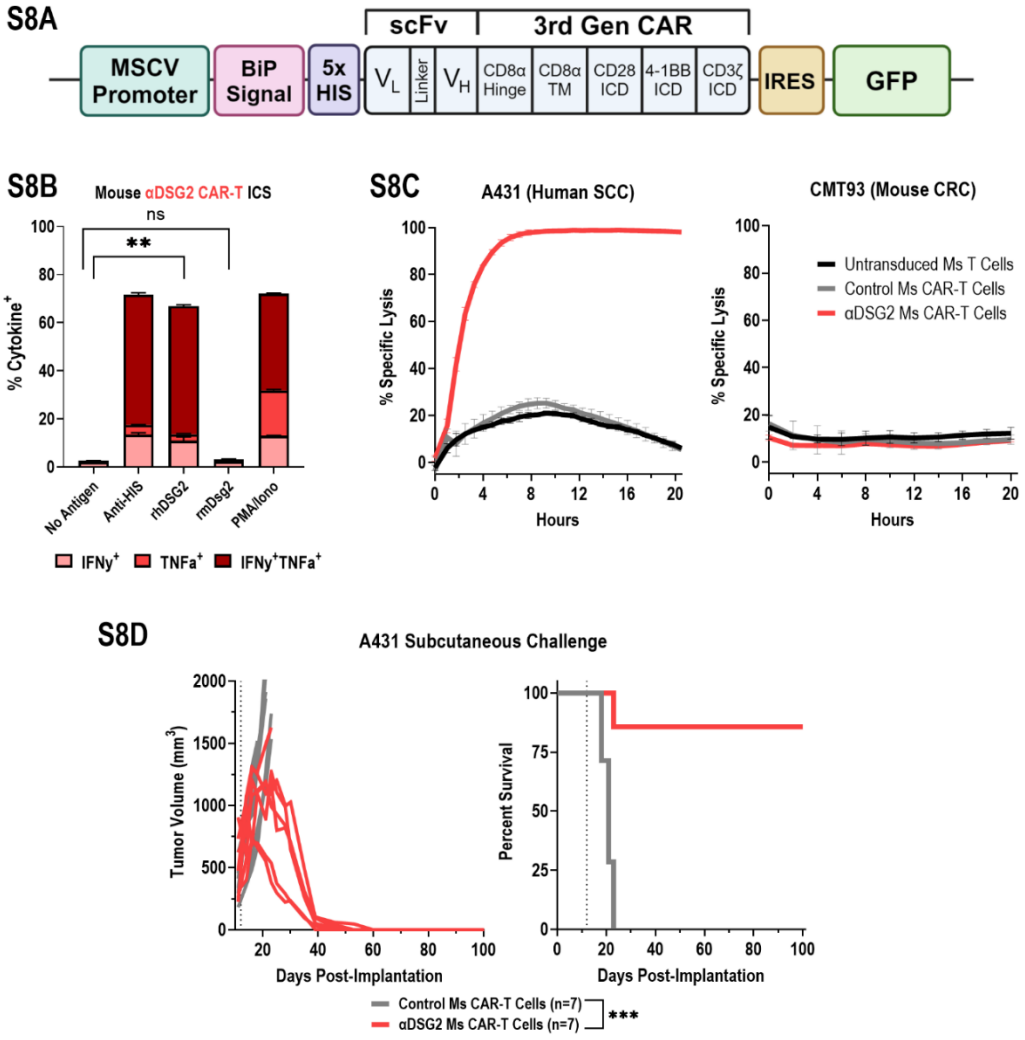
S6B



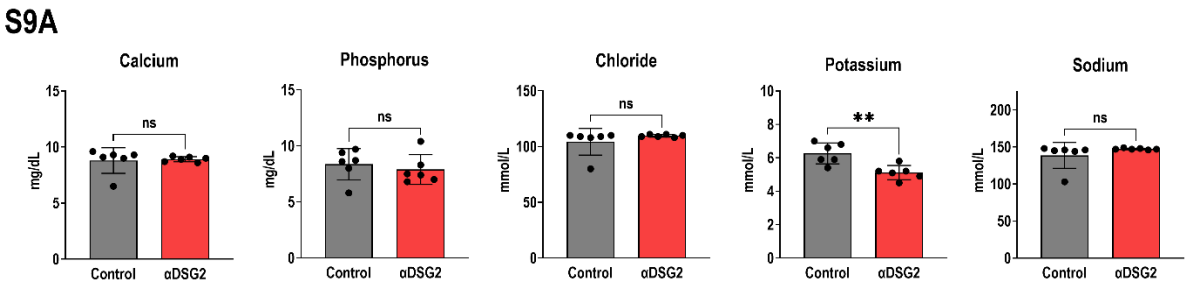
Supplementary Figure S6: Cytokine secretion and aggregate cancer cell cytolysis from αDSG2 CAR-T cell co-cultures. (**A**) Secreted cytokine content (in addition to **Fig. 2C**) evaluated in supernatants collected from control (αCD19) or αDSG2 CAR-T cells co-cultured with either wildtype (DSG2+) or DSG2-deficient (DSG2-) DLD-1 colorectal cells after 24 hours. Data presented as mean ± SD. n=3 technical replicates/donor. (**B**) Individual xCELLigence cytolysis data of colorectal (LS174T, SW480; dark blue), pancreatic (BxPC-3, AsPC-1, PANC-1; purple), lung (A549, NCI-H1299, NCI-H1792; grey), prostate (DU145, LnCAP, C4-2B; cyan), breast (MDA-MB-231, MCF7, SK-BR-3; pink), and liver (Hep3B, HepG2, HUH-7; green) cancer cell lines following 36-hour co-culture with untransduced, control, or αDSG2 CAR-T cells at a 5:1 E:T ratio (composite data are shown in **Fig. 2H**).



Supplementary Figure 7: *In vivo* antitumor activity of αDSG2 CAR-T cells. Bioluminescence imaging (BLI) of mouse tumor burden following luciferase-expressing (**A**) BxPC-3 pancreatic i.p. challenge, (**B**) A549 lung i.v. challenge, (**C**) DU145 prostate i.v challenge, (**D**) MDA-MB-231 breast i.v. challenge, (**E**) HepG2 liver i.p. challenge, and (**F**) AsPC-1 pancreatic orthotopic challenge. Black arrows signify day of CAR-T cell treatment.



Supplementary Figure 8: Mouse αDSG2 CAR construct specificity and validation. (**A**) Schematic diagram of the αDSG2 murine CAR construct containing a murine stem cell virus (MSCV)-driven promoter followed by a BiP signal sequence, 5xHis, DSG2-specific (6D8) scFv, CD8α hinge and transmembrane (TM) regions, and CD28, 4-1BB, and CD3ζ intracellular signaling domains (ICD) flanked by an internal ribosome entry site (IRES) upstream of a GFP reporter. (**B**) Intracellular cytokine staining of IFNγ and TNFα in αDSG2 mouse CAR-T cells following 6-hour incubation with plate-coated anti-5xHIS antibody, recombinant human DSG2 protein (rhDSG2), or recombinant mouse Dsg2 protein (rmDsg2). PMA/Ionomycin and no antigen served as positive and negative controls, respectively. Stacked bars represent the percentage of GFP^+ CAR-T cells staining positive for individual IFNγ, TNFα, or combined cytokine. Data presented as mean ± SD. n=2 technical replicates/antigen type and analyzed via unpaired t test with Welch's correction. **, $P < 0.01$; ns, not significant. (**C**) Cytolysis of human A431 SCC cells (left) by αDSG2 mouse CAR-T cells compared to untransduced and control (αCD19, 1D3) mouse CAR-T cells at 5:1 E:T ratio. Absence of cytolysis observed in mouse CMT93 colorectal cancer cell line (right) under same conditions. Cytolysis curves depict group mean cytolysis ± SD. n=3 technical replicates/CAR or T cell type. (**D**) Tumor growth curves (left) and overall mouse survival (right) of A431 SCC cells implanted subcutaneously in flanks of NSG mice treated with control or αDSG2 mouse CAR-T cells. Vertical dotted line denotes treatment on day 12. Survival was analyzed using the log-rank (Mantel-Cox) test. ***, $P < 0.001$.



Supplementary Figure 9: Serum blood chemistry and ion concentrations in hDSG2^{Tg} safety model. (**A**) Circulating ion concentrations in hDSG2^{Tg} mice following syngeneic adoptive transfer of either control or αDSG2 mouse CAR-T cells (n=6/group; 3 male, 3 female). Serum collected at study end (day=14). Data presented as means ± SD. Analysis was performed by unpaired t test with Welch's correction. **, $P < 0.01$; ns, not significant.

Supplementary Table S1

Cancer Type	Cell Line	ATCC Designation	Media	Note
Colon	DLD-1	CCL-221	RPMI + 10% FBS	ATCC
	DLD-1 (DSG2-KO)	CCL-221	RPMI + 10% FBS	Wildtype from ATCC, see methods.
	LS174T	CL-188	MEM + 10% FBS	ATCC
	SW480	CL-228	DMEM + 10% FBS	ATCC
Pancreas	BxPC-3	CRL-1687	RPMI + 10% FBS	Courtesy of Dr. Elda Grabocka, PhD (Thomas Jefferson University)
	AsPC-1	CRL-1682	RPMI + 10% FBS	Courtesy of Dr. Jonathan Brody, PhD (Oregon Health & Science University)
	PANC-1	CRL-1469	DMEM + 10% FBS	Courtesy of Dr. Jonathan Brody, PhD (Oregon Health & Science University)
Lung	A549	CCL-185	F-12K + 10% FBS	ATCC
	NCI-H1299	CRL-5803	RPMI + 10% FBS	ATCC
	NCI-H1792	CRL-5895	RPMI + 10% FBS	ATCC
Breast	MDA-MB-231	HTB-26	DMEM + 10% FBS	ATCC
	SK-BR-3	HTB-30	McCoy's 5A + 10% FBS	ATCC
	MCF-7	HTB-22	MEM + 10% FBS	ATCC
Prostate	DU145	HTB-81	DMEM + 10% FBS	Courtesy of Dr. Matthew Schiewer, PhD (Thomas Jefferson University)
	LnCAP	CRL-1740	RPMI + 10% FBS	Courtesy of Dr. Matthew Schiewer, PhD (Thomas Jefferson University)
	C4-2B	CRL-3315	RPMI + 10% FBS	ATCC
Liver	Hep3B	HB-8064	DMEM + 10% FBS	Courtesy of Dr. Ashesh Shah, MD & Dr. Hien Dang, PhD (Thomas Jefferson University)
	HepG2	HB-8065	DMEM + 10% FBS	ATCC
	HUH-7	N/A	DMEM + 10% FBS	Courtesy of Dr. Ashesh Shah, MD & Dr. Hien Dang, PhD (Thomas Jefferson University)