

Supplemental Table S1. List of reagents

REAGENT or RESOURCE	SOURCE	IDENTIFIER	Clone/variant	Dilution/conc.
<b>Cell Lines</b>				
NCI-H1650 bronchoalveolar adenocarcinoma	ATCC	CRL-5883		
NCI-H1703 squamous cell lung carcinoma	ATCC	CRL-5889		
A-431 Skin Epidermoid carcinoma	ATCC			
A549 lung adenocarcinoma	ATCC			
FaDu Pharynx Squamous cell carcinoma	ATCC			
MCF7 Mammary Gland-Breast Adenocarcinoma	ATCC			
MDA-MB-231 Mammary Gland-Breast Adenocarcinoma	ATCC			
MRC-5 Lung Fibroblast	ATCC			
NCI-H1355 lung adenocarcinoma	ATCC			
NCI-H1792 lung adenocarcinoma	ATCC			
NCI-H1975 lung adenocarcinoma	ATCC			
NCI-H292 pulmonary mucoepidermoid carcinoma	ATCC			
NCI-H441 papillary lung adenocarcinoma	ATCC			
NCI-H460 large cell lung carcinoma	ATCC			
NCI-H522 lung adenocarcinoma	ATCC			
NCI-H661 large cell lung carcinoma	ATCC			
SK-OV-3 Ovary Adenocarcinoma	ATCC			
U-87 MG Brain Glioblastoma	ATCC			
<b>Cell culture</b>				
RPMI 1640 (with 2mM L-Glutamine)	Sartorius	01-100-1A		
Fetal Bovine Serum, USA origin, Heat Inactivated	Sigma-Aldrich	F4135		10%
Sodium pyruvate solution, 11mg/ml (100mM)	Sartorius	03-042-1B		1mM
Penicillin-Streptomycin (10000 U/ml, 10mg/ml)	Sigma-Aldrich	P4333-100ML		100U/ml, 0.1mg/ml
PBS (Dulbecco's Phosphate Buffered Saline Without Calcium and Magnesium)	Sartorius	02-023-1A		
Trypsin-EDTA	Sartorius	03-050-1B		For culture
Tryple Express Enzyme 1x w/ Phenol Red	Thermo Fisher	12605010		For functional assays
Mycoalert detection kit	Lonza	LNV-LT07-418		
<b>Cell line engineering</b>				
Firefly Luciferase Lentivirus (EF1 $\alpha$ , G418)	BPS Bioscience	79692-G		MOI = 1
NuLight Green Lentivirus (EF1 $\alpha$ , Puro)	Sartorius	4624	Nuc-GFP	MOI = 3

NuLight Red Lentivirus (EF1 $\alpha$ , Puro)	Sartorius	4625	Nuc-mKate2	MOI = 3
hCD19 lentivirus (EF1 $\alpha$ , Blasticidin)	Vector Builder	LVM(VB900133-6392gkb)-K1		MOI = 10
G418	Sigma-Aldrich	G8168-10ml		200-800 $\mu$ g/ml
Puromycin	Sigma-Aldrich	P9620-10ML		0.5-2 $\mu$ g/ml
Blasticidin S ready made solution 10mg	Sigma-Aldrich	SBR00022-1ml 10		10 $\mu$ g/ml
Lipofectamine CRISPRMAX Reagent	Thermo Fisher	CMAX00003		
TrueCut Cas9 Protein v2 (100 $\mu$ g)	Thermo Fisher	A36498		0.1 $\mu$ g/ $\mu$ l
TrueGuide custom sgRNA HLA2 ACCCTCGTCCTGCTACTCTC FAM SEQ.GGG	Thermo Fisher	A35514		0.4 $\mu$ g/ $\mu$ l
TrueGuide Synthetic sgRNA (ID# CRISPR1097726_SGM; HLA-A)	Thermo Fisher	A35533		0.4 $\mu$ g/ $\mu$ l
HLA-A seq primer FW: GAGAAGCAAGGGGCCCTC	Metabion			
HLA-A seq primer Rev: CTGCTCTATCCACGGCGC	Metabion			
Modified sgRNA Length 17-23 ERBB2 TCATCGCTCACAACCAAGTG	Syntego	003-1C0-000-000		0.4 $\mu$ g/ $\mu$ l
ECM 830 Electroporation System, with Safety Dome	BTX	45-2052		
NEPA Electroporation Cuvettes 1mm gap, 50 pcs/pack	NepaGene	EC-002		
NCI-H1650-Luc	This manuscript (ffLuc lentiviral transduction)	H1650 (A2+HER2+) Luc		
NCI-H1650-Luc A2KO (Clone 2) HLA-A*02 neg	This manuscript (CRISPR KO)	H1650 (A2-HER2+) Luc		
NCI-H1650-Luc-nGFP	This manuscript (NuLight Green lentiviral transduction)	H1650 (A2+HER2+) Luc-GFP "T2g"		
NCI-H1650-Luc-nRFP	This manuscript (NuLight Red lentiviral transduction)	H1650 (A2+HER2+) Luc-RFP "T2"		
NCI-H1650-Luc A2KO-nGFP	This manuscript (NuLight Green lentiviral transduction)	H1650 (A2-HER2+) Luc-GFP "T1"		
NCI-H1650-Luc A2KO-nRFP	This manuscript (NuLight Red lentiviral transduction)	H1650 (A2-HER2+) Luc-RFP "T1r"		
NCI-H1650-Luc-nGFP HER2 KO	This manuscript (CRISPR KO)	H1650 (A2+HER2-) Luc-GFP "T3g"		
NCI-H1650-Luc-nRFP HER2 KO	This manuscript (CRISPR KO)	H1650 (A2+HER2-) Luc-RFP "T3"		
NCI-H1650-Luc-nGFP A2 KO +HER2 KO	This manuscript (CRISPR KO)	H1650 (A2-HER2-) Luc-GFP "T4"		
NCI-H1650-Luc-nGFP-CD19	This manuscript (CD19 lentiviral transduction)	H1650 (A2+HER2+CD19+) Luc-GFP "T7"		
NCI-H1703-Luc	This manuscript (ffLuc lentiviral transduction)	H1703 (A2+) Luc		
NCI-H1703-Luc A2KO (Clone 1) HLA-A*02 neg	This manuscript (CRISPR KO)			
NCI-H1703-Luc A2KO (Clone 2) HLA-A*02 neg	This manuscript (CRISPR KO)	H1703 (A2-) Luc		

NCI-H1703-Luc-nRFP	This manuscript (Nuclight Red lentiviral transduction)	H1703 (A2+) Luc-RFP "T6"		
NCI-H1703-Luc A2KO (Clone 2)-nGFP	This manuscript (Nuclight Green lentiviral transduction)	H1703 (A2-) Luc-GFP "T5"		
<b>Flow cytometry (where given two concentrations, the higher one colored blue was used for quantification)</b>				
Anti-DYKDDDDK (Flag) APC	Miltenyi Biotec	130-119-584	REA216	1:50, 1:25
Anti-cMyc FITC	Miltenyi Biotec	130-116-485	SH1-26E7.1.3	1:50, 1:25
HLA-A2 Tetramer PE (NY-ESO-1 peptide)	MBL International	TB-M105-1	SLLMWITQV	1:10, 1:5
Anti-Trastuzumab (naked IgG1)	Absolute antibodies	Ab00618-10.0	scFv69	1:25
Anti-human-IgG1 APC	BioLegend	406609	QA19A42	1:20
Anti-Trastuzumab APC	Absolute antibodies	Ab00618-10.0	scFv69	1:10
Anti-TNG3.2 (naked IgG1)	This manuscript (manufactured by Accurus bioscience)	NA	R2 S2	10ug/ml
Anti-Her2 PE	Miltenyi Biotec	130-124-466	REA1232	1:5
Anti-EGFR PE	R&D Systems	FAB9577P-100	Cetuximab	1:5
Anti-HLA-A2 APC	Thermo Fisher	17-9876-42	BB7.2	1:20, 1:10
Anti-Trastuzumab APC	Absolute antibodies	Ab00618-10.0	scFv69	1:10
Anti-HLA-A2 APC	Thermo Fisher	17-9876-42	BB7.2	1:20
Quantum FITC-5 MESF (Premix)	Bangs laboratories	555p		
Quantum R-PE MESF	Bangs laboratories	827		
Quantum APC MESF	Bangs laboratories	823		
Quantum Simply Cellular anti-Mouse IgG	Bangs laboratories	815		
Quantum Simply Cellular anti-Human IgG	Bangs laboratories	816		
PBS (Dulbecco's Phosphate Buffered Saline Without Calcium and Magnesium)	Sartorius	02-023-1A		
Fetal Bovine Serum, USA origin, Heat Inactivated	Sigma-Aldrich	F4135		2%
DAPI ready made solution	Sigma-Aldrich	MBD0015-1		1:2000
Attune® NxT Acoustic Focusing Cytometer, blue/red/violet/yellow	Thermo Fisher	A24858		
BD Celesta Flow Cytometer, blue/red/violet	BD Biosciences	BD FACSCelesta		
FlowJo v10.8.1	FlowJo, LLC	N/A		
QUICKCAL V. 3.0	Bangs Laboratories	N/A		
<b>CAR-T</b>				
Whole Blood Samples of Healthy donors	Sheba Blood Bank, Ramat gan, Israel	Leukocytes for research		D26, 28, 30
Human Leukopak	Hemacare corporation, Northridge, CA	PB001F-1		D144, 466, 9587

PBMC	Hemacare corporation, Northridge, CA	PB009C-3		
Ficoll Paque Plus	Sigma-Aldrich	GE17-1440-02		
LymphoOne T-cell expansion Xeno-Free medium	Takara Bio	WK552S		
TransAct	Miltenyi Biotec	130-111-160		1:100
Human IL-2 IS, premium grade	Miltenyi Biotec	130-097-746		100 U/ml
Custom made lentivirus (EF1 $\alpha$ )	Alstem	CS-LV02X		
Custom made lentivirus (EF1 $\alpha$ )	Vector Builder	Lentivirus packaging, custom made, medium scale		
Male human AB serum heat inactivated	Access Biologicals	AC-001-1B		1%
CryoStor CS10	Sigma-Aldrich	C2874		
MACS BSA Stock solution	Miltenyi Biotec	130-091-376		0.5%
HLA-A2 Tetramer PE (NY-ESO-1 peptide)	MBL International	TB-M105-1	SLLMWITQV	1:10
Anti-PE MicroBeads	Miltenyi Biotec	130-105-639		
G-Rex 24 well plate	Wilson Wolf	80192M		
G-Rex 6 well plate	Wilson Wolf	80240M		
LS columns	Miltenyi Biotec	130-042-401		
MS columns	Miltenyi Biotec	130-042-201		
TrueCut Cas9 Protein v2 (100 $\mu$ g)	Thermo Fisher	A36498		0.1 $\mu$ g/ $\mu$ l
TrueGuide custom sgRNA HLA-A2 probe#150 TACCACCAGTACGCCTACGA	Thermo Fisher	A35514 "sgRNA #150"		0.4 $\mu$ g/ $\mu$ l
Modified sgRNA Length 17-23 TCR Control AGAGTCTCTCAGCTGGTACA	Syntego	003-1C0-000-000		0.4 $\mu$ g/ $\mu$ l
Anti-HLA-A2 APC	Thermo Fisher	17-9876-42	BB7.2	1:20
Anti-APC MicroBeads	Miltenyi Biotec	130-090-855		
Anti-cMyc FITC	Miltenyi Biotec	130-116-485	SH1-26E7.1.3	1:25
Anti-FITC MicroBeads	Miltenyi Biotec	130-048-701		
Neon Transfection System	Thermo Fisher	MPK5000		
Neon Transfection System 100 $\mu$ L Kit	Thermo Fisher	MPK10025		
<b>CAR constructs</b>				
VR33	This manuscript (synthesized by Alstem)	HER2 aCAR (Trastuzumab scFv)		MOI = 10
VR49	This manuscript (synthesized by Vector Builder)	BB7.2 KIR2DL1 dual CAR		MOI = 20
VR51	This manuscript (synthesized by Vector Builder)	BB7.2 LIR1 dual CAR	PD1 HTM, T2A	MOI = 20
VR54	This manuscript (synthesized by Vector Builder)	BB7.2 CD33 dual CAR		MOI = 20
VR354	This manuscript (synthesized by Vector Builder)	BB7.2 LIR1 dual CAR	LIR1 HTM (52), T2A	MOI = 20
VR362	This manuscript (synthesized by Vector Builder)	BB7.2 PD1 dual CAR		MOI = 20
VR363	This manuscript (synthesized by Vector Builder)	BB7.2 PD1x2 dual CAR		MOI = 20

VR428	This manuscript (synthesized by Vector Builder)	HzBB7.2.1 LIR1 dual CAR	LIR1 HTM (52), IRES	MOI = 20
VR447	This manuscript (synthesized by Vector Builder)	TNF3.2 LIR1 dual CAR	LIR1 HTM (30), IRES	MOI = 20
VR492	This manuscript (synthesized by Vector Builder)	HzBB7.2.2 iMut dual CAR	LIR1 HTM (52), T2A	MOI = 20
VR495	This manuscript (synthesized by Vector Builder)	Non-binder LIR1 dual CAR	LIR1 HTM (52), T2A	MOI = 20
Dual CAR HTM variants	This manuscript (synthesized by Vector Builder)	BB7.2 LIR1 dual CAR (several HTM)	LIR1 D3-4, D4, CD8, CD28	MOI = 20
Dual CAR short LIR1 hinge length variants	This manuscript (synthesized by Vector Builder)	HzBB7.2.1 LIR1 dual CARs (LIR1 HTM)	64, 52, 44, 36, 30, 26, 20aa	MOI = 20
Dual CAR iCAR scFv variant HzBB7.2.2	This manuscript (synthesized by Vector Builder)	HzBB7.2.2 LIR1 dual CARs	LIR1 HTM (52), T2A or IRES	MOI = 20
Dual CAR iCAR scFv variant TNF3.3	This manuscript (synthesized by Vector Builder)	TNF3.3 LIR1 dual CAR	LIR1 HTM (26), T2A or IRES	MOI = 20
CD19 CAR	This manuscript (synthesized by Vector Builder)	CD19 aCAR (FMC63 scFv)		MOI = 10
<b>Luciferase killing assay</b>				
Bio-Glo Luciferase Assay system	Promega	G7940		
Corning 96 well solid white polystyrene plates	Corning	CLS3917		
Varioskan LUX multimode microplate reader	Thermo Fisher	VL0L00D0		
GraphPad Prism v9	GraphPad Software Inc.	N/A		
<b>IFN<math>\gamma</math> secretion assay</b>				
Human IFN $\gamma$ CBA Flex Set E7,100T	BD Biosciences	BD558269		
96 Well Plate, V bottom, Non-Binding	Greiner Bio-One	651901		
<b>IncuCyte 2D and 3D assays</b>				
IncuCyte <sup>®</sup> S3 Live-Cell Analysis Instrument	Sartorius	4647		
Nunc <sup>™</sup> MicroWell <sup>™</sup> 96-Well, Nunclon Delta-Treated, Flat-Bottom Microplate	Thermo Fisher	167008		For 2D
Corning Costar Ultra-Low Attachment 96 well plate, Round bottom, Clear	Corning	CLS7007		For 3D
IncuCyte Annexin-V Red Reagent	Sartorius	4641		
IncuCyte 2019B rev2	Essen Bioscience	N/A		
<b>Reversibility and Repeated Antigen Challenge</b>				
EasySep Human PE Position Selection Kit II	StemCell	17664		
Anti-hCD324 (E-Cadherin) PE	Miltenyi Biotec	130-111-839	8C11	1:50
Anti-human P-Cadherin PE	R&D Systems	FAB861P	104805	1:5

PBS (Dulbecco's Phosphate Buffered Saline Without Calcium and Magnesium)	Sartorius	02-023-1A		
MACS BSA Stock solution	Miltenyi Biotec	130-091-376		0.5%
EDTA Disodium Solution, 0.5M, pH=8	Biological Industries	01-862-1B		10 mM
<b>Mouse xenograft studies</b>				
Mice: NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ)	The Jackson Laboratory	Cat# JAX:005557; RRID:IMSR_JAX:005557		
IFNg was IFN gamma Human ELISA Kit, High Sensitivity (in vivo)	Thermo Fisher	BMS228HS		
Human AB Serum	MP Biomedical	2938249		1:20
Mouse Serum	Thermo Fisher	24-5544-94		1:20
Rabbit Serum	MilliporeSigma	NS01L1ML		1:20
Dulbecco's Phosphate-Buffered Saline, no Ca2+ or Mg2+	Thermo Fisher	14287080		
TruStain Monocyte Blocker	BioLegend	426103		1:20
Cell Stain Buffer (with BSA) (CSB)	BD Biosciences	554657		
ACK Lysing Buffer	Lonza	10548E		
Anti-hCD3 APC-Cy7	BioLegend	344818	SK7	1:80
TruStain FcX PLUS (anti-mouse CD16/32) Antibody	BioLegend	156604		1:200
CountBright Absolute Counting Beads	Thermo Fisher	C36950		

**Supplemental Table S2.** Determination of A2 and HER2 expression in cancer cell lines

Histology	Cell Line	HLA-A Alleles <sup>1</sup>		Receptors per cell (ABC) <sup>2</sup>			CCLE RNAseq (TPM)		
		I	II	Her2	HLA-A2	Ratio	Her2	HLA-A	Ratio
Skin Epidermoid carcinoma	A-431	03:01	03:01	2,818	ND		NA	NA	NA
lung adenocarcinoma	A549	25:01	30:01	ND	ND		17	57	3.4
Pharynx Squamous cell carcinoma	FaDu	01:01	01:01	39,783	ND		56	193	3.4
Pharynx Squamous cell carcinoma	FaDu-A2+ (Jet Prime, 1B10 clone)	01:01	01:01	ND	1,349,780		56	193	3.4
Pharynx Squamous cell carcinoma	FaDu-A2+ (Lipofectamine FAL clone)	01:01	01:01	ND	2,177,934		56	193	3.4
Mammary Gland-Breast Adenocarcinoma	MCF7	02:01	02:01	56,182	126,085	2.2	46	39	0.8
Mammary Gland-Breast Adenocarcinoma	MDA-MB-231	02:17	02:01	16,944	2,468,107	145.7	24	840	35.0
Lung Fibroblast	MRC-5	2:01	29:02	10,569	385,253	36.5	NA	NA	NA
lung adenocarcinoma	NCI-H1355	02:01	02:01	21,817	210,501	9.6	41	135	3.3
lung adenocarcinoma	NCI-H1355-Luc	02:01	02:01	10,608	405,293	38.2	41	135	3.3
lung adenocarcinoma	NCI-H1355-Luc A2KO (Clone C9)	02:01	02:01	10,139	ND		41	135	3.3
bronchoalveolar adenocarcinoma	NCI-H1650	02:01	02:01	55,207	813,485	14.7	79	128	1.6
bronchoalveolar adenocarcinoma	NCI-H1650-Luc	02:01	02:01	20,475	1,247,812	60.9	79	128	1.6
bronchoalveolar adenocarcinoma	NCI-H1650-Luc A2KO (Clone 2)	02:01	02:01	21,122	ND		79	128	1.6
bronchoalveolar adenocarcinoma	NCI-H1650-Luc A2KO (Clone 7)	02:01	02:01	35,472	ND		79	128	1.6
squamous cell lung carcinoma	NCI-H1703	01:01	02:01	21,322	878,876	41.2	33	551	16.7
squamous cell lung carcinoma	NCI-H1703-Luc	01:01	02:01	24,739	1,684,284	68.1	33	551	16.7
squamous cell lung carcinoma	NCI-H1703-Luc A2KO (Clone 2)	01:01	02:01	27,286	ND		33	551	16.7
lung adenocarcinoma	NCI-H1792	26:08'	02:01	18,606	483,418	26.0	22	283	12.9
lung adenocarcinoma	NCI-H1792-Luc	26:08'	02:01	9,264	726,720	78.4	22	283	12.9
lung adenocarcinoma	NCI-H1792-Luc A2KO (Clone F6)	26:08'	02:01	7,925	ND		22	283	12.9
lung adenocarcinoma	NCI-H1975	01:01	01:01	49,165	ND		36	483	13.4
lung adenocarcinoma	NCI-H1975-Luc	01:01	01:01	26,792	ND		36	483	13.4
pulmonary mucopidermoid carcinoma	NCI-H292-Luc	68:02	74:01	14,623	ND		30	54	1.8
papillary lung adenocarcinoma	NCI-H441	03:01	02:01'	39,368	121,410	3.1	74	846	11.4
papillary lung adenocarcinoma	NCI-H441-Luc A2KO (Clone C7)	03:01	02:01'	53,857	ND		74	846	11.4
large cell lung carcinoma	NCI-H460	68:01'	24:02	19,642	ND		13	170	13.1
lung adenocarcinoma	NCI-H522	02:01	24:18'	68,995	90,674	1.3	150	74	0.5
large cell lung carcinoma	NCI-H661	02:01'	24:02'	67,437	25,376	0.4	62	2	0.0
Ovary Adenocarcinoma	SK-OV-3	68:01	03:01	ND	ND		2,123	1,350	0.6
Brain Glioblastoma	U-87 MG	02:01	02:01	0	602,864		3	589	196.3
<b>Column Statistics</b>									
Median				22,844	494,059	31.2	39	182	4.7
25% Quartile				14,514	124,916	4.7	24	70	3.0
75% Quartile				41,490	1,093,381	56.0	65	561	8.6
Min				0	25,376	0.4	3	2	0.7
Max				68,995	2,468,107	145.7	2,123	1,350	0.6
Mean				29,658	734,068	37.6	176	362	2.1
Std Dev				21,508	765,947	40.6	520	385	NA

**Supplemental Table S2 Notes**

1. The HLA-A alleles in each cell line were obtained using the Tron Cell Lines Portal (<http://celllines.tron-mainz.de/>)[7]
2. The cell surface expression of HER2 and A2 were measured by qFACS in 2D cultures using anti HER2 and A2 allele specific monoclonal antibody BB7.2 as described in **Methods**.