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Supplemental Information

CAR-T Cells Targeting Epstein-Barr Virus gp350 Validated in a Humanized Mouse Model of EBV Infection and Lymphoproliferative Disease

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Supplemental data
Figure S1

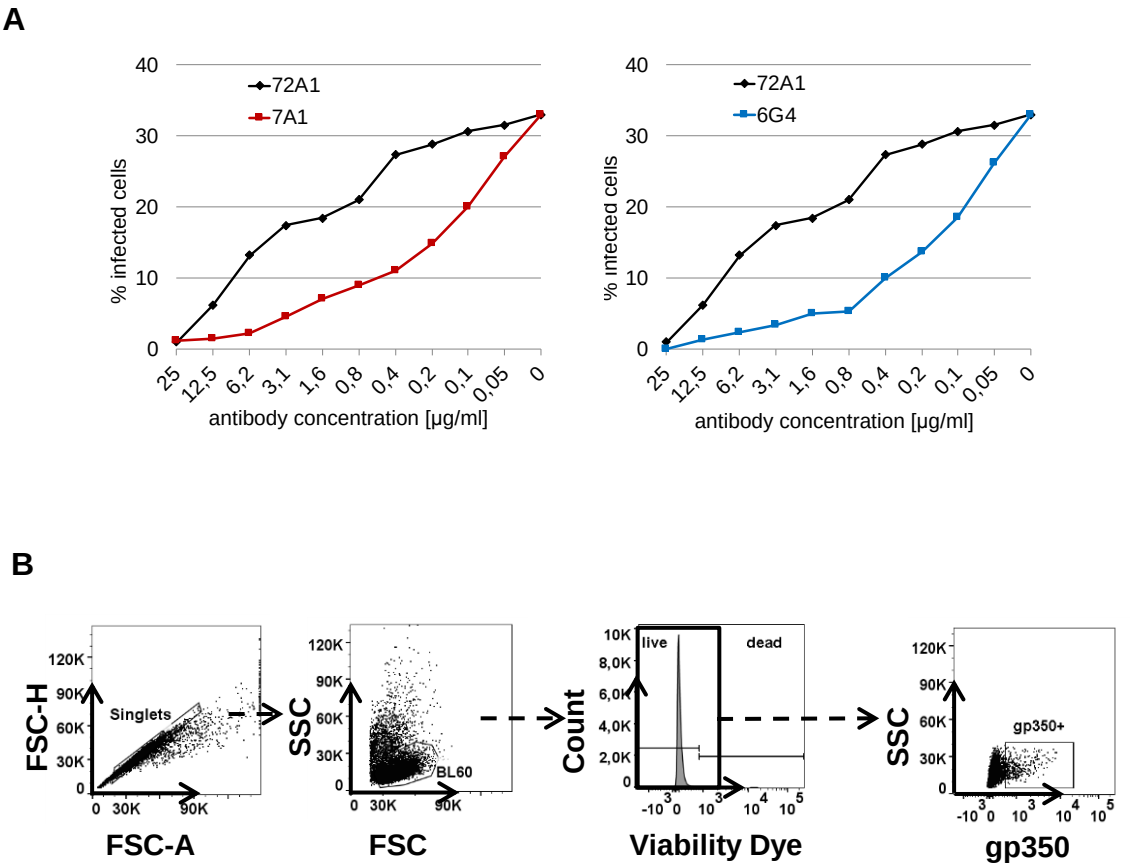

Figure S1 - *In vitro* neutralization assay for gp350-binding monoclonal antibodies and flow cytometry analyses for detection of gp350 on the cell surface (see Fig. 1A).

(A) Aliquots of EBV/B95-8/GFP (MOI of 0.2 for $2x10^5$ cells) were pre-incubated with serial dilutions of the monoclonal antibodies (72A1: black, 7A1: red and 6G4: blue) at the indicated concentrations and at room temperature for 30 min. Primary B cells were infected with the viral aliquotes in a final volume of 400µl. The frequencies of GFP^+ infected B cells were quantified by flow cytometry 2 days later. **(B)** Flow cytometry gating strategy for detection of gp350. Viable cells were gated showing representative example of BL-60 EBV^+ cell line.

Figure S2

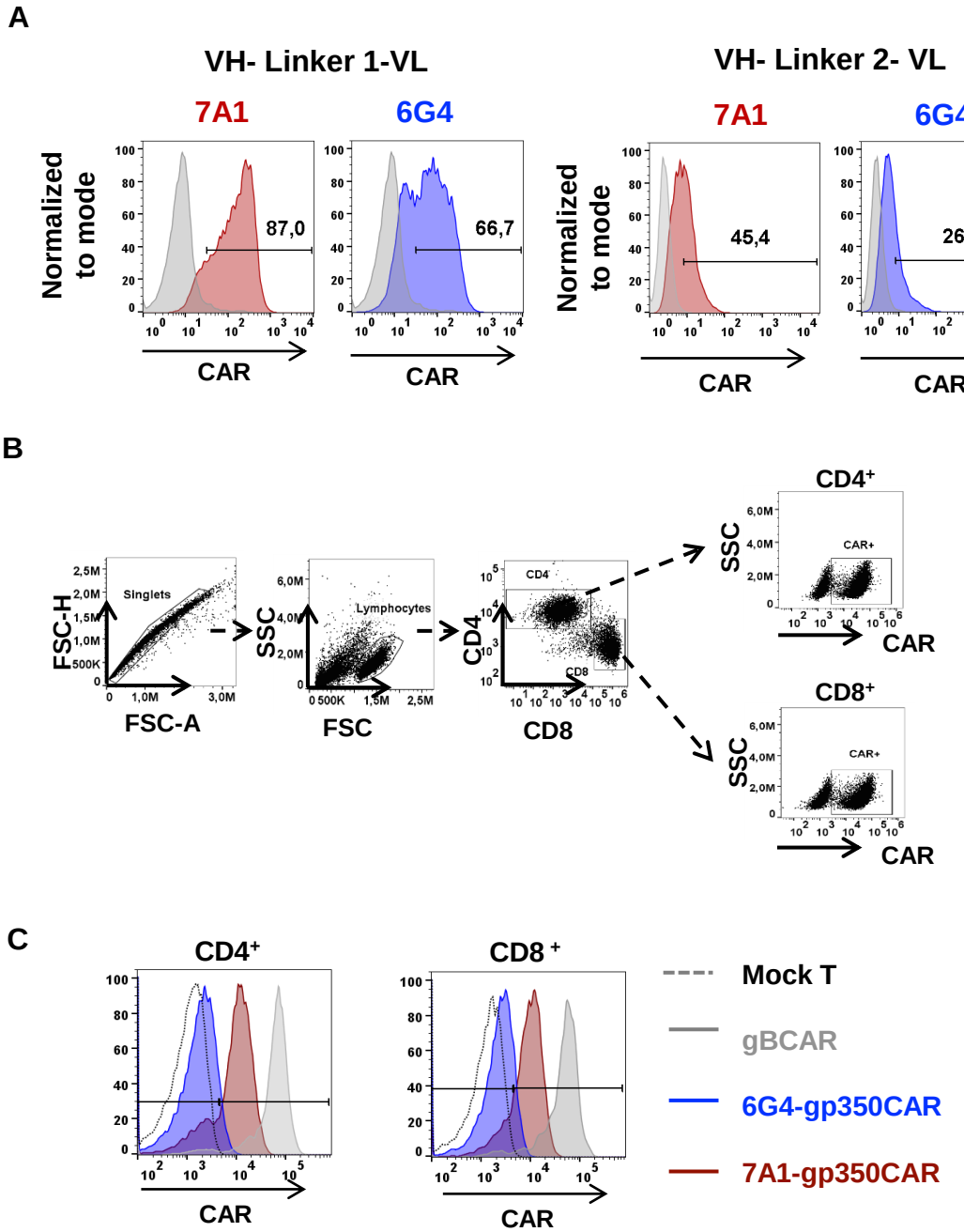

Figure S2 – Detection and quantification of gp350CAR$^+$ cells (see Fig. 1C, D).

(A) Effects of different linkers in scFvs. CARs with 7A1 or 6G4 scFvs and different linkers were pre-tested after transfection of 293T cells and CAR expression was analyzed by flow cytometry**. (B)** Flow cytometry gating strategy for analyses of CARs (see Fig. 1C). Representative example shows 7A1-gp350CAR expression on CD4$^+$ or CD8$^+$ T cells **(C)** Calculation of mean fluorescence intensity (MFI) of CAR$^+$ cells. MFI of non-transduced Mock T cells of the same donor was used as a reference.

Figure S3

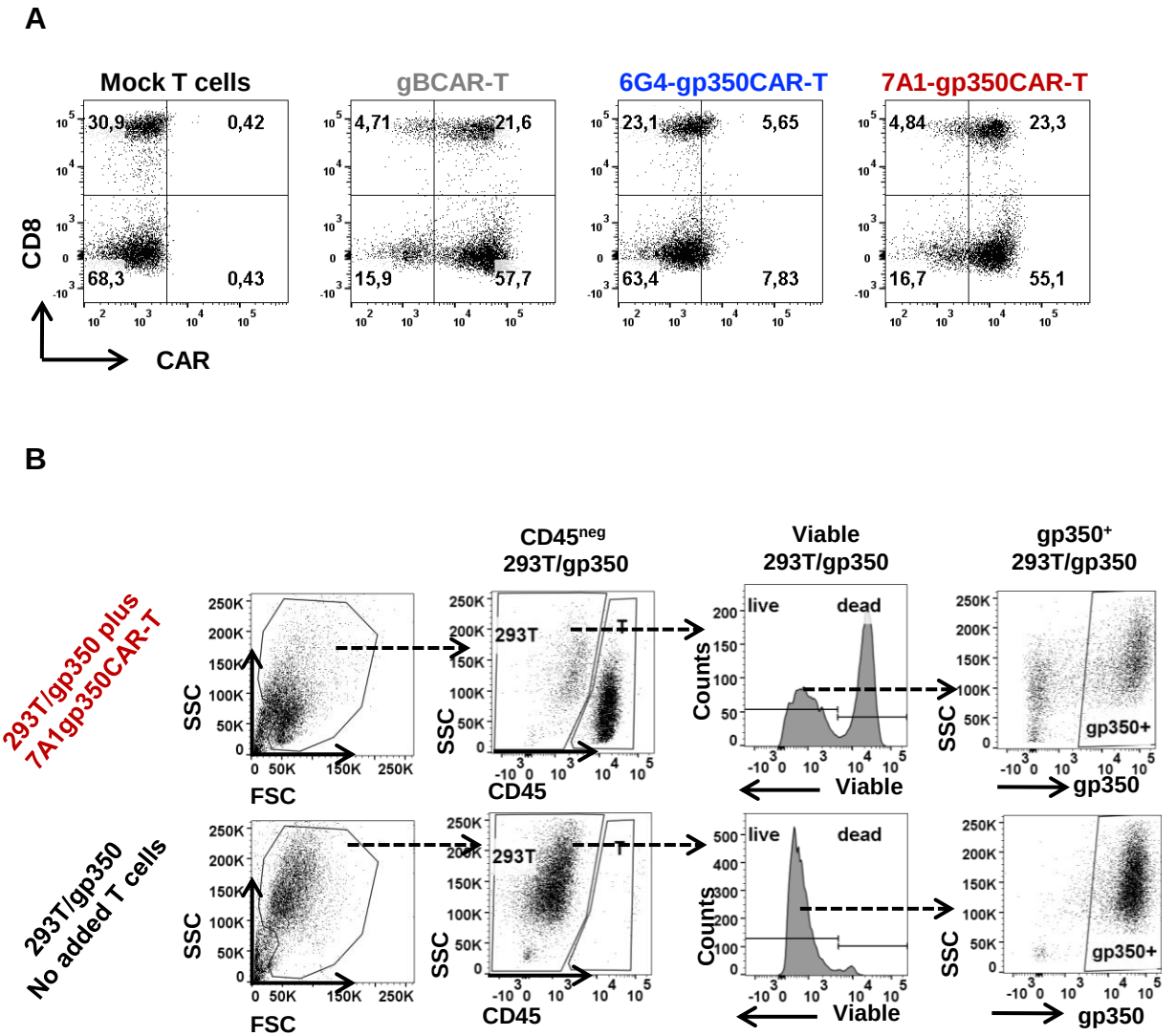

Figure S3 – Flow cytometry analyses of gp350CAR-T cells in co-culture with 293T targets (see Fig. 1G, H).

(A) Flow cytometry analyses of Mock T or CAR-T cells used for co-cultures with 293T/gp350. **(B)** Flow cytometry gating strategy for analyses of remaining viable gp350^{+} 293T/gp350 targets. Upper panels show 7A1-gp350CAR-T and 293T/gp350 targets at 3:1 effector to target ratio after 24 hours of co-culture. Lower panels show as 293Tgp350 targets with no added T cells. % viable targets were calculated as: % viable targets in CAR-T co-cultures/ % viable targets with no added T cells. % viable gp350^{+} targets were calculated as % viable gp350^{+} targets in co-cultures / % viable gp350^{+} viable targets with no added T cells.

Figure S4

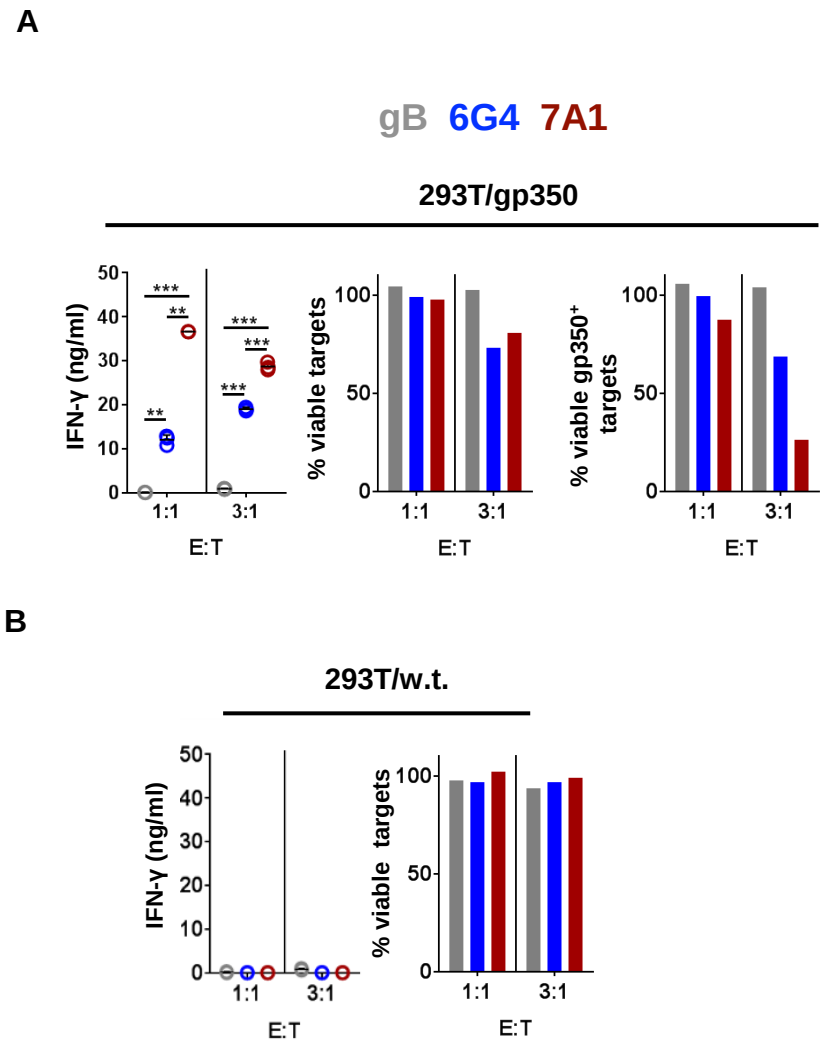

Figure S4 - 48 h co-cultures of gp350CAR-T cells with 293T targets (see Fig. 1G, H).

(A) 293T/gp350 cells were cultured with CAR-T cells (gB: grey; 6G4-gp350: blue; 7A1-gp350: red) for 48 h at 1:1 or 3:1 effector : target (E:T) ratios. Left panel: Concentrations of secreted IFN-γ (ng/ml) measured in the cell supernatants (n=3) ** $p<0.01$, *** $p<0.001$. Middle panel: Percentages of viable 293T/gp350 cells analyzed by flow cytometry shown for one experiment. Right panel: Percentages of viable $gp350^+$ 293T/gp350 cells analyzed by flow cytometry shown for one experiment. **(B)** Control co-culture of 293T/w.t. cells with CAR-T cells. Left panel: No detectable secreted IFN-γ . Right panel: No cell killing.

Figure S5

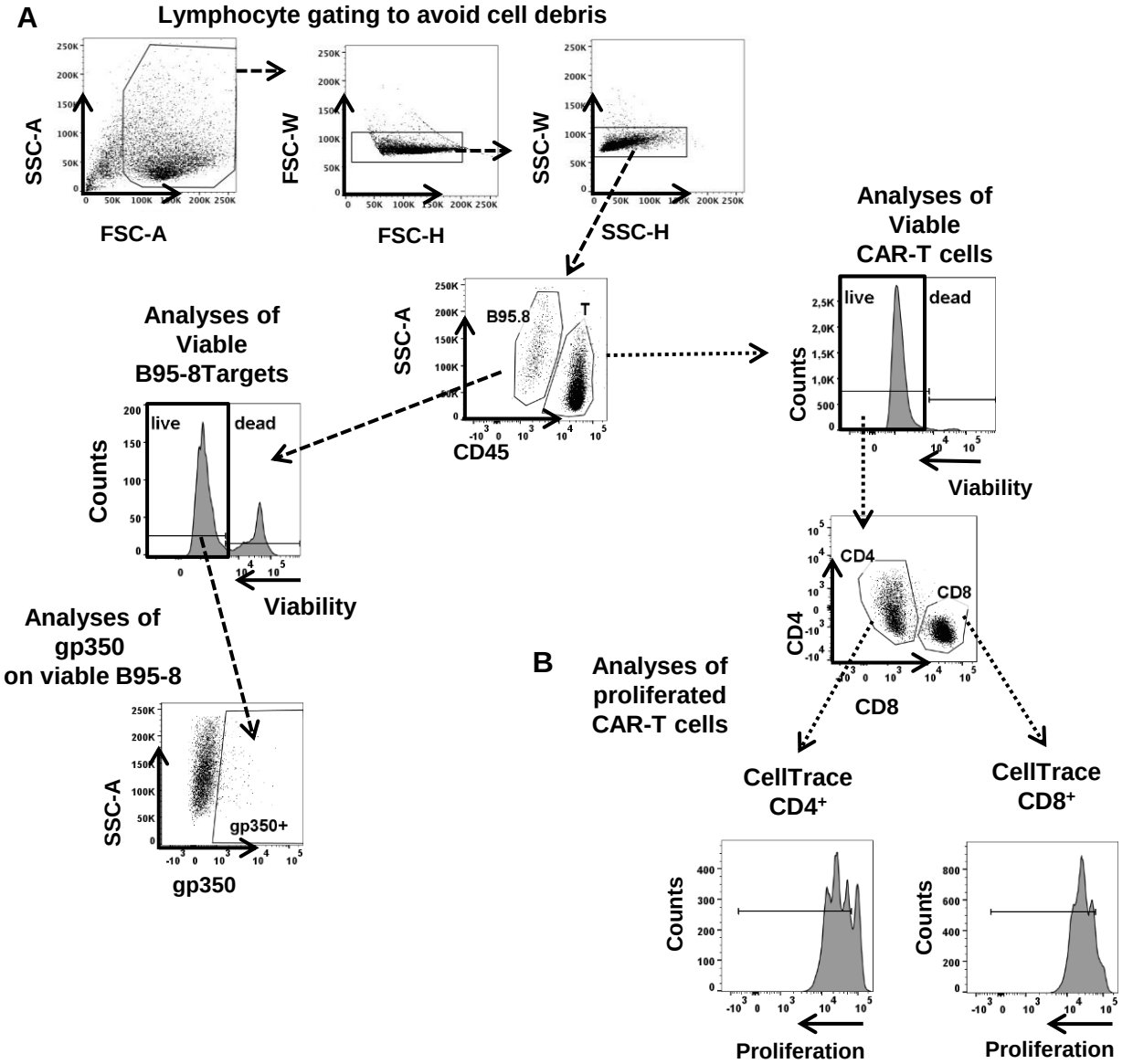

Figure S5 - Flow cytometry gating strategy for analyses of B95-8 co-cultures with CAR-T cells co-cultures (see Fig. 2 C-E).

(A) Representative gating strategy for analyses of B95-8 targets cells co-cultured with 7A1-gp350CAR-T cells for 38 hours at an effector : target ratio of 10:1. **(B)** Representative gating strategy for analyses of 7A1-gp350CAR-T cells for proliferation of $CD4^+$ and $CD8^+$ T cells after 86 hours of co-culture.at an effector : target ratio of 10:1.

Figure S6

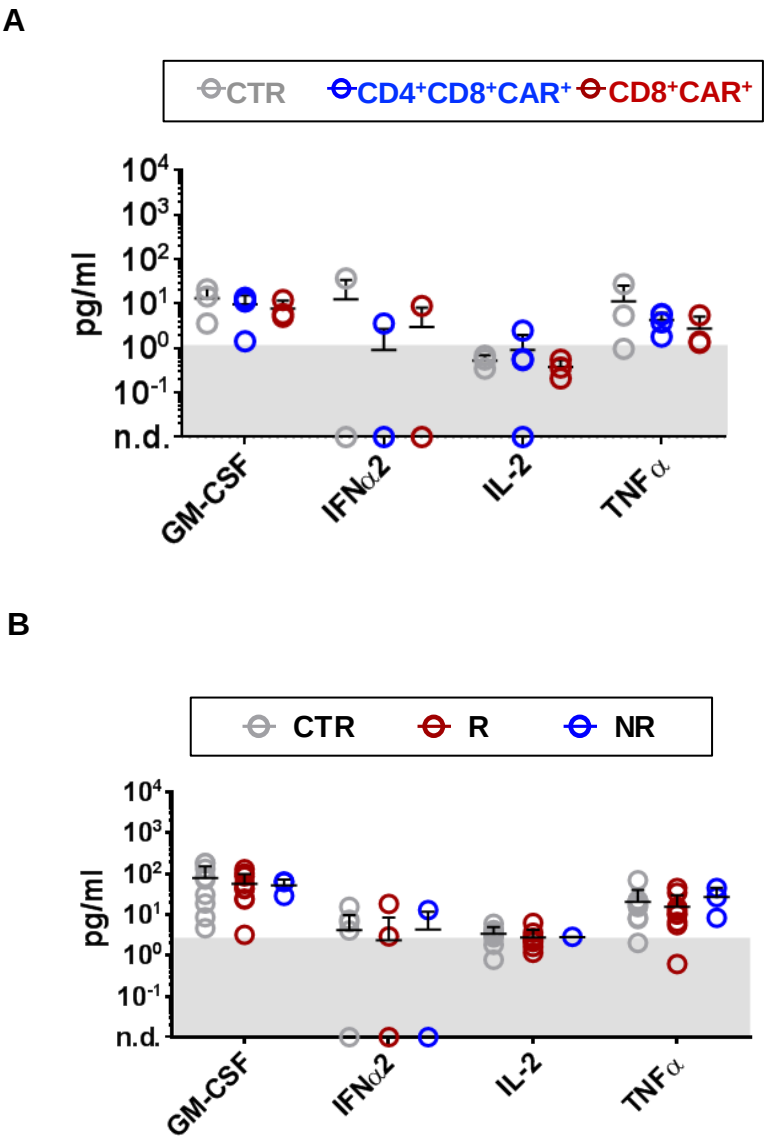

Figure S6 - Human cytokines detected in plasma of mice at endpoint analysis not varying among cohorts (see Fig. 4G and Fig. 7F).

Concentration (pg/ml) of GM-CSF, IFN-α2, IL-2, TNF-α measured for EBV-infection control and CAR-T cell treated cohorts. **(A)** Data for the protective model at 5 wpi. **(B)** Data for the therapeutic model at 8 wpi. No significant differences were observed.

Figure S7

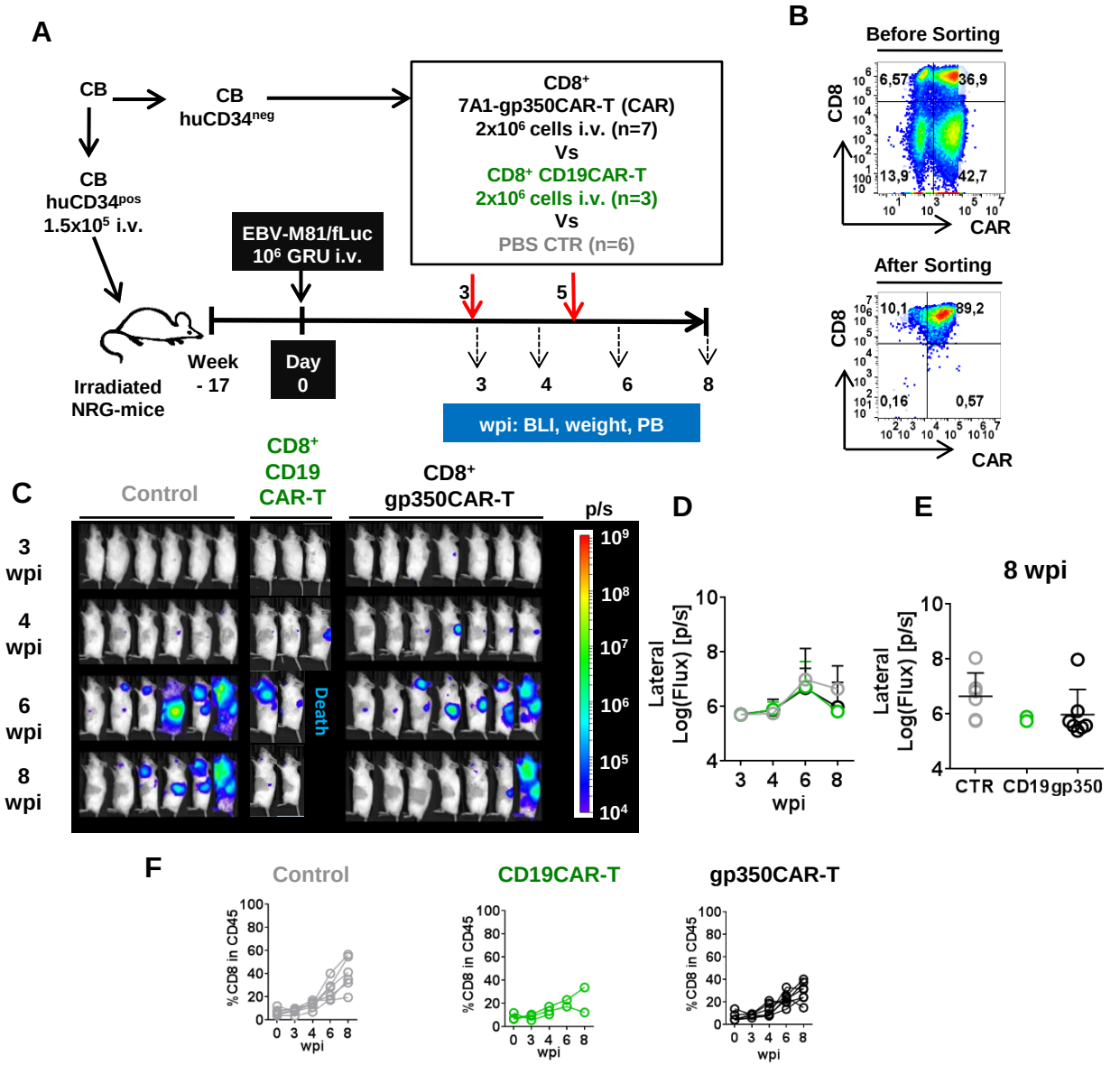

Figure S7 - Comparisons between Control, CD19CAR-T and gp350CAR-T for the therapeutic model (see Fig. 5).

(A) Schematic representation. **(B)** Flow cytometry dot-plot graphs showing CD8$^+$ CD19CAR$^+$ T cells analyzed before (upper panel) and after (lower panel) sorting for enrichment of CD8$^+$/CAR$^+$ T cells. **(C)** Sequential BLI analyses showing pictures of the left lateral view of individual mice performed at 3, 4, 6 and 8 wpi. Mice were transplanted with CD34^{pos} derived from CB1. The control cohort received PBS i.v. (CTR: n=7, grey) and the test groups received 2x10^6 CD8$^+$ CAR-T cells i.v. (CD19CAR-T: n=3, green; gp350CAR-T: n=7, black). One mouse of the CD19-CAR-T succumbed at 5 wpi. Signal intensity was measured with the same settings for all mice and depicted in logarithmically scale as Log (Flux) (photons/sec, p/s, see color-coded bar). **(D)** Serial quantification of the BLI analyses of lateral left body view showing each cohort. **(E)** Quantification of the BLI analyses of lateral left body view showing each mouse at 8wpi. **(F)** Sequential analyses of the frequencies of human CD8$^+$ T cells within huCD45$^+$ cells in blood for each cohort.

Figure S8

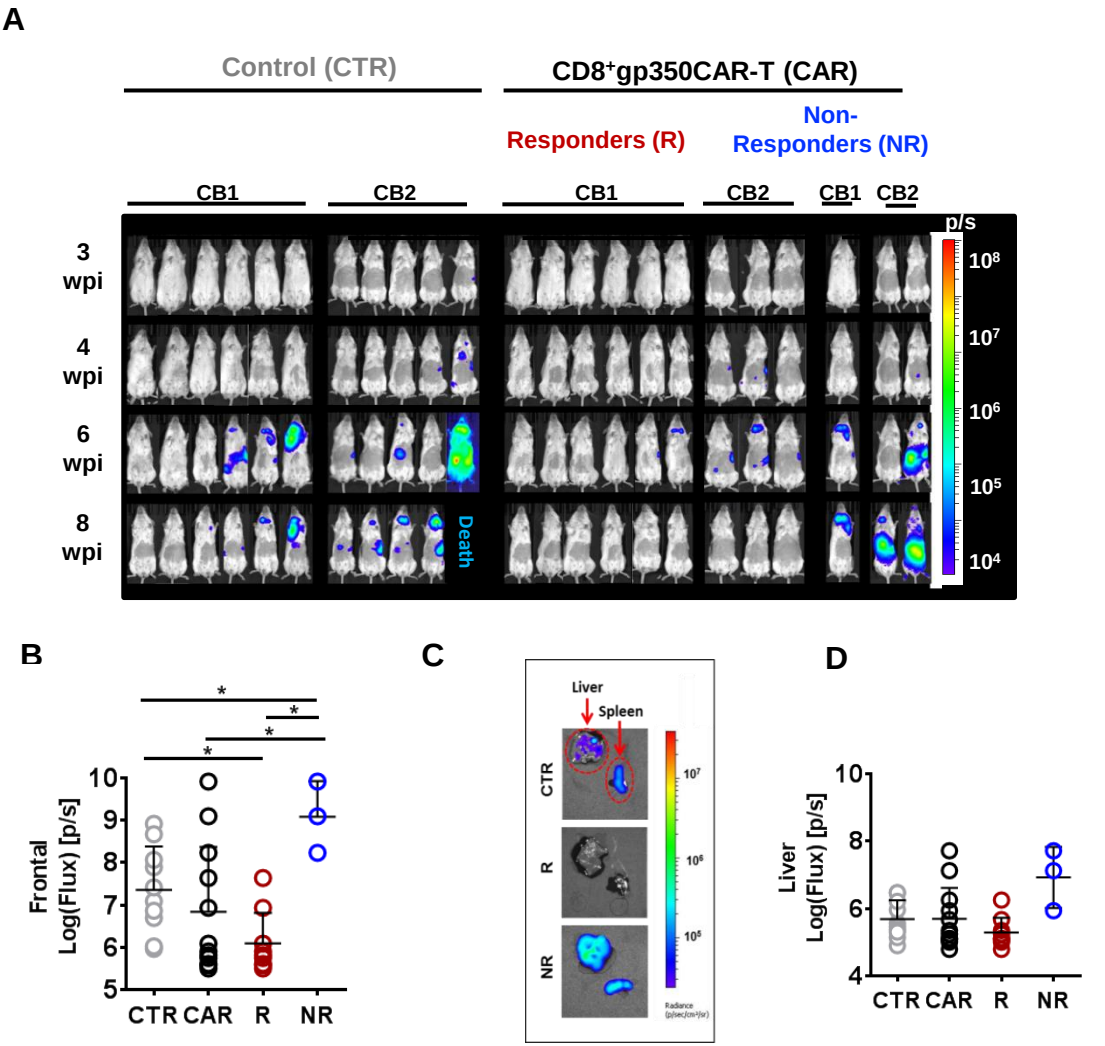

Figure S8 - Additional BLI analyses of therapeutic experiments (see Fig. 5).

(A) Sequential BLI analyses showing pictures of the frontal view of individual mice performed at 3, 4, 6 and 8 wpi. Mice transplanted with $CD34^{pos}$ derived from CB1 and CB2 are indicated. The control cohort received PBS i.v. (CTR: n=11, grey) and the test group received $2x10^6$ $CD8^+$gp350CAR-T cells i.v. (CAR: n=12, black; Responders: n=9, red; non-responders: n=3, blue). One mouse of the control group succumbed at 7 wpi. Signal intensity was measured with the same settings for all mice and depicted in logarithmically scale as Log (Flux) (photons/sec, p/s, see color-coded bar). **(B)** Quantification of the BLI analyses of frontal body view at endpoint analysis (Log (Flux) p/s). * $p<0.05$. **(C)** Representative examples of BLI of explanted organs showing lower signals for organs of responder mice. **(D)** Quantified BLI analyses of explanted livers.

Figure S9

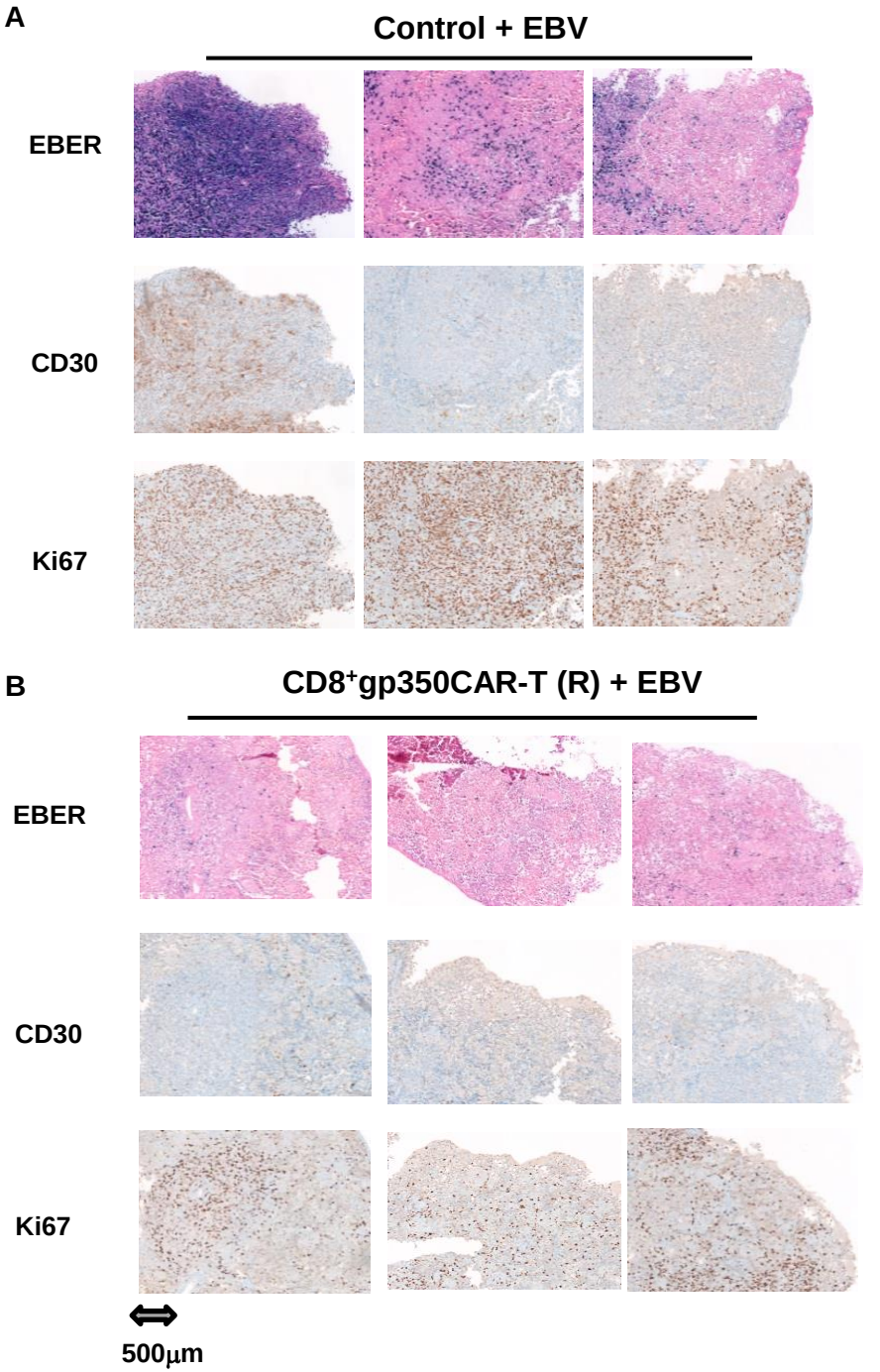

Figure S9 – Histopathology analyses of spleens .

(A) Control infected mice (n=3) and **(B)** Responder mice treated with gp350CAR-T cells (n=3). Upper panels: Detection of EBER by *in situ* hybridization. Middle panels: Immunohistochemistry for detection of $CD30^+$ cells. Lower panels: Immunohistochemistry for detection of $Ki67^+$ cells.

Figure S10

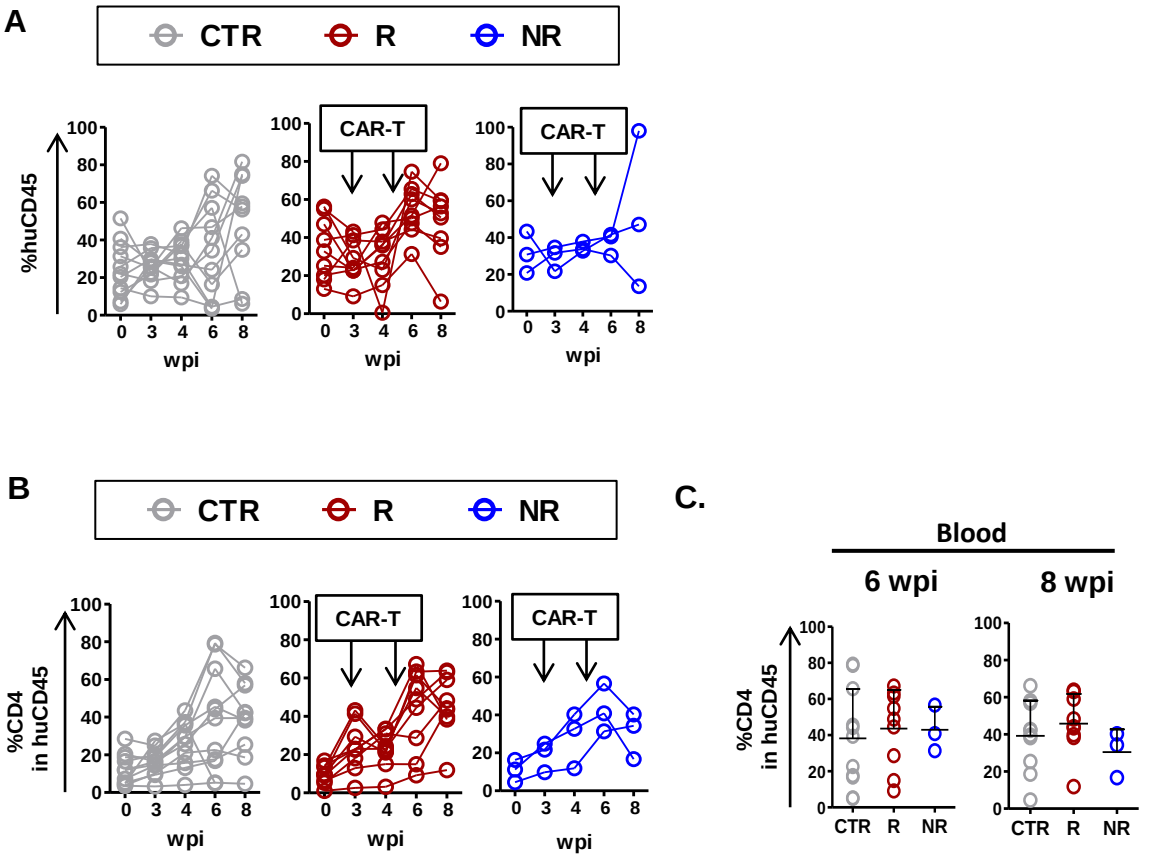

Figure S10 - Additional immune monitoring data for therapeutic gp350CAR-T cell administration (see Fig. 7).

(A) Mice were grouped in control (CTR, grey), Responders (R, red) and Non-responders (NR, blue). Analyses performed at baseline prior to (week 0) and after EBV infection (at 3, 4, 6 and 8 wpi). The percentages of human $CD45^+$ cells within the total blood lymphocytes are shown. Time points of CAR-T cell administrations are indicated. **(B)** Comparisons between the frequencies of human $CD4^+$ in $CD45^+$ cells in blood over the course of the experiment are displayed. Time points of CAR-T cell administrations are indicated. **(C)** $CD4^+$ in $CD45^+$ cells in blood analyzed on weeks 6 (left) and 8 (right) after EBV infection are shown with mean and standard deviation for each group.

Figure S11

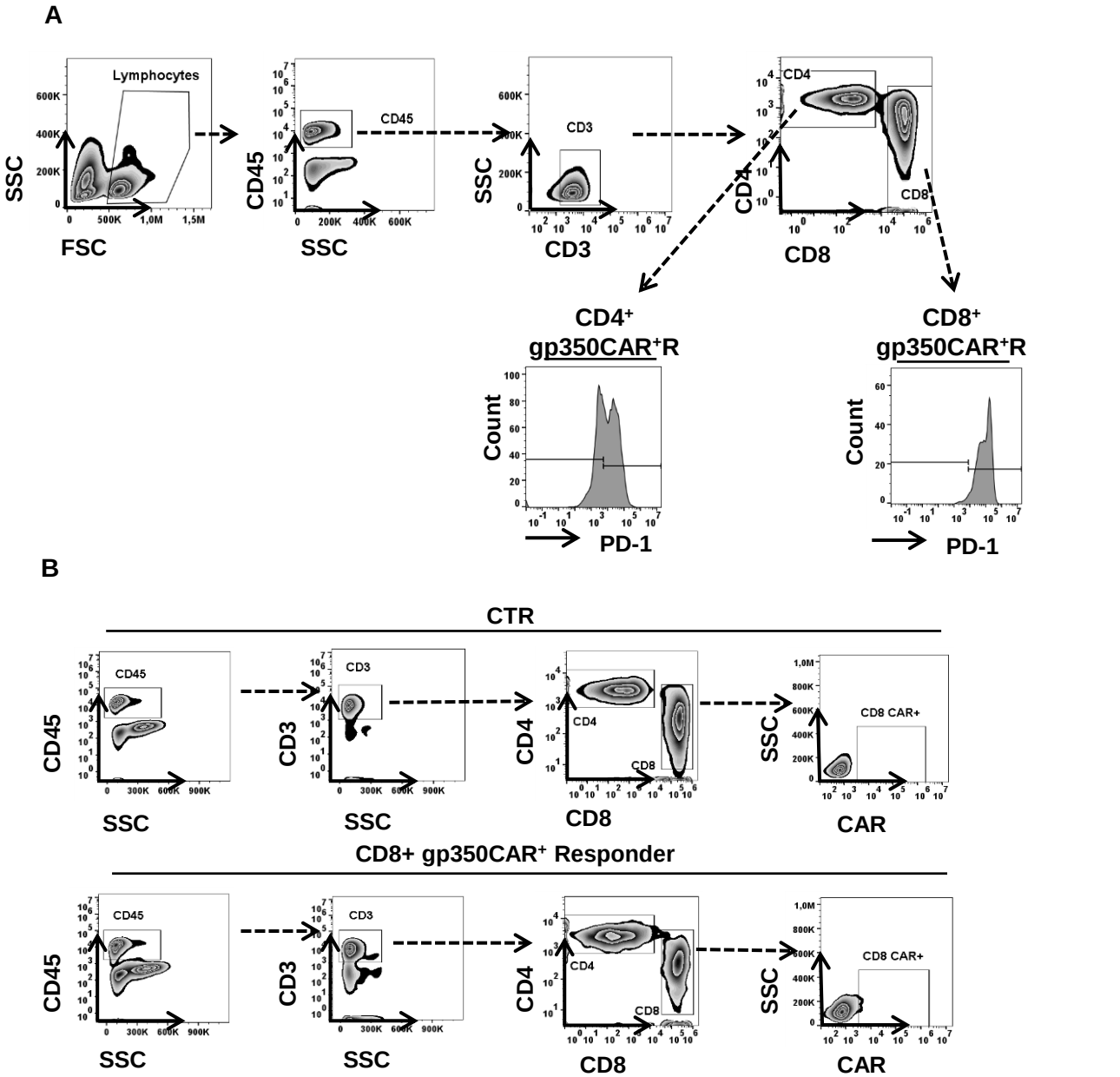

Figure S11 - Effects on PD-1 expression and CAR detection for therapeutic gp350CAR-T cell administration experiment (see Fig. 7).

(A) Representative example shows analyses of blood of a mouse treated with $CD8^+$-gp350CAR-T cells to quantify the MFI of PD-1 expression on $CD4^+$ and $CD8^+$ T cells . **(B)** Flow cytometry gating strategy for quantification of the frequencies of CAR^+ $CD8^+$ T cells. Representative example for detection of CAR^+ T cells in peripheral blood of a control mouse (upper panels) and mouse treated with $CD8^+$-gp350CAR-T cells (lower panels).

Table S1: Descriptive statistics for comparisons between gBCAR-T, 6G4-gp350CAR-T and 7A1-gp350CAR-T cells regarding the CAR expression levels and reactivity against 293T/gp350.

		gB		6G4		7A1		p		
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**gBx6G4**	**gBx7A1**	**6G4x7A1**
Fig.1D (gB: n=6; 6G4 and 7A1: n=7)	MFI of $CD4^+$ relative to MOCK $CD4^+$-T	95.68	72.59	1.91	0.36	11.11	3.50	0.00034	0.00312	0.00001
	MFI of $CD8^+$ relative to MOCK $CD8^+$-T	53.08	36.83	1.46	0.36	6.13	2.16	0.00035	0.00245	0.00002
Fig. 1E (n=5)	CD4 % CAR+					52.02	21.70			
	CD8 % CAR+					68.00	20.25			
Fig. 1G (n=3)	3:1 (E:T) IFN-γ (ng/ml)	0.18	0.04	15.51	0.46	21.08	0.48	0.00070	0.00070	0.00070
	1:1 (E:T) IFN-γ (ng/ml)	0.01	0.01	6.55	0.24	15.85	1.42	0.00200	0.00800	0.00980
Fig. S4 A (n=3)	3:1 (E:T) IFN-γ (ng/ml)	0.85	0.10	18.96	0.33	28.69	0.72	0.0002	0.00055	0.0006
	1:1 (E:T) IFN-γ (ng/ml)	0.03	0.04	12.00	0.91	36.59	0.02	0.0028	0.00000001	0.0013

Table S2: Descriptive statistics for comparisons between gBCAR-T and 7A1-gp350CAR-T cells regarding reactivity against B95-8.

			(E:T)	gB		7A1		*p*
				Mean	**SD**	**Mean**	**SD**	**gBx7A1**
Fig. 2B (n=9)	**IFN-γ (ng/ml)**	**38h**	**0.1:1**	0.07	0.09	0.26	0.19	0.02
			1:1	0.12	0.09	2.31	0.86	<0,0001
			10:1	0.82	0.90	3.96	2.36	0.01
Fig. 2C (n=3)	**% proliferating cells in $CD4^+$**	**38h**	**0.1:1**	5.63	2.81	5.78	3.44	0.96
			1:1	3.93	2.26	6.76	4.63	0.50
			10:1	5.01	3.08	8.36	6.24	0.55
	% proliferating cells in $CD4^+$	**86h**	**0.1:1**	32.37	6.13	75.60	1.16	0.02
			1:1	72.30	19.82	74.23	17.00	0.92
			10:1	18.18	18.87	64.10	5.98	0.13
Fig. 2D (n=3)	**% proliferating cells in $CD8^+$**	**38h**	**0.1:1**	4.03	0.75	4.08	1.78	0.98
			1:1	5.02	2.25	7.00	4.05	0.59
			10:1	7.09	3.82	11.05	6.24	0.50
	% proliferating cells in $CD8^+$	**86h**	**0.1:1**	30.80	3.80	78.87	9.72	0.03
			1:1	65.93	16.31	84.07	5.11	0.27
			10:1	25.03	24.00	64.17	14.99	0.27
Fig. 2E (n=3)	**% viable targets**	**38h**	**0.1:1**	95.33	5.79	95.33	4.99	1.00
			1:1	94.67	7.04	94.00	3.74	1.00
			10:1	98.67	4.11	87.33	8.73	0.60
Fig. 2F (n=3)	**% viable $gp350^+$ targets**	**38h**	**0.1:1**	135.36	27.77	111.35	19.27	0.38
			1:1	121.56	29.32	75.62	13.75	0.28
			10:1	102.42	14.07	24.78	7.21	0.02

Table S3: Descriptive statistics for comparisons between the cohorts PBS control, $CD4^+CD8^+$7A1-gp350CAR-T cells and $CD8^+$7A1-gp350CAR-T cells regarding protective effects against M81/fLuc infection.

			CTR (n=3)		$CD4^+/CD8^+$ CAR (n=4)		$CD8^+$ CAR (n=3)		*p*			statistical test applied
			Mean	SD	Mean	SD	Mean	SD	CTR x $CD4^+/CD8^+$ CAR	CTR x $CD8^+$ CAR	$CD4^+/CD8^+$ CAR x $CD8^+$ CAR	
Fig.3D	Lateral Log (Flux) (p/s)	week 2	6.16	0.36	5.93	0.03	5.78	0.13	<0.0165	<0.0165	ns	ANCOVA
		week 3	7.07	0.98	6.08	0.26	6.17	0.24				
		week 4	8.15	1.93	6.67	0.48	6.76	0.67				
		week 5	8.62	2.17	6.87	0.35	7.17	1.13				
		week 5	8.62	2.17	6.87	0.35	7.17	1.13	>0.1	>0.1	>0.1	Welch's t test with Bonferroni-Holm correction
Fig.3E	Relative weight change	week 1	105.33	1.25	98.00	0.71	107.00	2.83	<0.0001	ns	<0.0001	ANCOVA
		week 3	105.33	3.68	99.50	3.20	109.33	2.05				
		week 4	103.33	4.71	100.50	3.50	109.33	1.70				
		week 5	101.67	3.77	94.75	3.96	108.00	1.63				
		week 5	101.67	3.77	94.75	3.96	108.00	1.63	0.2497	0.2497	0.018	Welch's t test with Bonferroni-Holm correction
Fig.3F*	#EBER$^+$/mm^2 in spleen		3526.87	4572.53	34.14	18.86	354.43	491.17	>0.1	>0.1	>0.1	Welch's t test with Bonferroni-Holm correction
Fig. 3G*	Viral load in spleen (IU/ug DNA)		49876.63	45470.29	16262.98	20004.22	60972.52	84716.92	>0.1	>0.1	>0.1	Welch's t test with Bonferroni-Holm correction
Fig. 3H*	Viral load in bone marrow (IU/ug DNA)		15382.76	21646.46	16.28	7.40	346.02	312.19	>0.1	>0.1	>0.1	Welch's t test with Bonferroni-Holm correction

Table S4: Descriptive statistics for comparisons between the cohorts PBS control, $CD4^+CD8^+$ 7A-gp350CAR-T cells and $CD8^+$ 7A-gp350CAR-T cells regarding immune responses observed in the protective model.

			CTR (n=3)		$CD4^+/CD8^+$ CAR (n=4)		$CD8^+$ CAR (n=3)		*p*		
			Mean	SD	Mean	SD	Mean	SD	CTR x $CD4^+/CD8^+$ CAR	CTR x $CD8^+$ CAR	$CD4^+/CD8^+$ CAR x $CD8^+$ CAR
Fig. 4B	%huCD45	week 0	11.84	3.55	13.88	2.73	14.37	7.77	ns	ns	ns
		week 2	5.52	1.66	11.49	3.47	5.09	0.77			
		week 4	4.10	1.51	7.39	3.38	4.12	2.11			
		week 5	9.68	3.98	16.69	6.89	18.73	5.13			
Fig. 4C	%CD8 in huCD45	week 0	7.31	2.54	6.04	1.46	11.47	1.75	ns	ns	ns
		week 2	11.58	4.71	15.37	6.50	17.83	1.20			
		week 4	21.30	13.60	22.63	4.77	31.63	6.62			
		week 5	21.87	13.06	18.23	5.21	11.80	4.93			
Fig. 4D	%CD4 in huCD45	week 0	12.31	5.88	8.57	1.18	15.33	2.21	ns	ns	ns
		week 2	24.57	10.47	14.00	3.74	24.47	3.31			
		week 4	24.25	20.52	27.10	6.45	27.83	3.39			
		week 5	40.90	21.27	55.65	6.02	69.53	8.67			
Fig. 4E*	#CD8 in spleen		2.45E+06	2.32E+06	3.79E+05	1.77E+05	6.65E+05	3.59E+05	ns	ns	ns
Fig. 4F*	#CD4 in spleen		1.65E+06	1.08E+06	6.72E+05	3.89E+05	1.20E+06	9.97E+05	ns	ns	ns
Fig. 4G	Cytokines (pg/ml)	IFN-γ	231.54	302.70	11.98	11.74	1.21	0.09	0.79	0.79	0.63
		IL-10	2045.08	2770.27	3.55	3.25	10.82	14.09	1.00	1.00	1.00
		IL-12	2.15	3.05	n.d.	n.d.	n.d.	n.d.			
		IL-6	1.63	2.30	n.d.	n.d.	n.d.	n.d.			
		IL-8	7.47	10.57	0.63	0.63	n.d.	n.d.			
		MCP-1	62.23	88.01	n.d.	n.d.	1.30	1.84			
Fig. S6 A	Cytokines (pg/ml)	GM-CSF	13.19	7.27	9.76	4.89	7.79	3.21	1.00	1.00	1.00
		IFNα2	12.47	17.64	0.91	1.57	2.99	4.23			
		IL-2	0.53	0.14	1.20	0.91	0.38	0.14	0.43	0.43	0.43
		TNFα	11.38	11.68	4.32	1.65	2.76	1.97	1.00	1.00	1.00

Table S5: Descriptive statistics for comparisons between the cohorts PBS control, CD8$^+$ CD19CAR-T cells and CD8$^+$ 7A1-gp350CAR-T cells (all mice, responders or non-responders) cregarding therapeutic effects against M81/fLuc infection.

			CTR (n=6)		CD19CAR-T (n=3)		gp350CAR-T (n=7)		*p*		
			Mean	SD	Mean	SD	Mean	SD	CTRxCD19 CAR-T	CTRxgp350CAR-T	CD19CAR-Txgp350CAR-T
Fig. S7D	Optical imaging lateral log(flux)	week 3	5.71	0.05	5.69	0.06	5.72	0.03	1.00	1.00	1.00
		week 4	5.75	0.04	5.90	0.23	5.87	0.37	1.00	1.00	1.00
		week 6	6.99	1.04	only 2 mice left		6.68	0.68	1.00	1 (uncorrected 0.57)	1.00
		week 8	6.65	0.78	only 2 mice left		5.98	0.84	0.19	0.40 (uncorrected 0.20)	0.66
Fig. S7E	%CD8 in huCD45	week 0	6.97	2.65	8.49	2.41	6.40	3.42			
		week 3	7.80	2.14	8.48	2.07	7.65	1.45			
		week 4	12.96	3.20	13.83	2.79	12.47	5.16			
		week 6	25.67	8.00	only 2 mice left		22.81	5.52			
		week 8	39.90	12.92	only 2 mice left		29.24	8.08			

			CTR (n=11)		CAR total (n=12)		CAR R (n=8)		CAR NR (n=3)		*p*								
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	CTRxCAR	CARx CAR R	CAR x CAR NR	CTRxCAR R	CTRx CAR NR	CAR R x CAR NR	w6xw8 CTR	w6xw8 CAR R	w6xw8 CAR NR
Fig. 5D-F	Optical imaging lateral log(flux)	week 3	5.88	0.25	5.88	0.25	5.83	0.23	6.01	0.25									
		week 4	6.23	0.77	6.19	0.64	6.19	0.72	6.19	0.24									
		week 6	7.03	1.31	6.92	0.96	6.87	0.69	7.09	1.49							0.39	0.0007	0.25
		week 8	7.14	0.96	6.41	1.24	5.72	0.23	8.47	0.55	0.15	0.10	0.01	0.005	0.05	0.03			
Fig. 5G*	Viral load in spleen (IU/ug DNA)		4947.53	5320.47	1221.06	1604.84	1221.06	1604.84	4013.53	2396.57				ns	ns	ns			
Fig. 5 H*	Viral load in bone marrow (IU/ug DNA)		286.29	294.21	144.10	203.17	144.10	203.17	5884.33	7838.05				ns	ns	ns			
Fig. S8B*	Optical imaging frontal log(flux)	week 8	7.36	0.97	6.84	1.47	6.09	0.68	9.08	0.69	0.35	0.16	0.01	0.02	0.04	0.02			
Fig. S8D	Optical imaging Liver log(flux)		5.69	0.53	5.70	0.88	5.29	0.41	6.92	0.74	0.973	0.192	0.123	0.1	0.129	0.079			

Table S6: Descriptive statistics for comparisons between the cohorts PBS control and CD8+ 7A-gp350CAR-T cells (all mice, responders or non-responders) regarding weight and therapeutic effects against LPD.

			CTR (n=11)		CAR total (n=12)		CAR R (n=8)		CAR NR (n=3)		*p*					
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	CTRxCAR	CARx CAR R	CAR x CAR NR	CTRxCAR R	CTRx CAR NR	CAR R x CAR NR
Fig.6A	Relative weight change	week 1	102.56%	3.40%	103.02%	2.91%	102.47%	2.40%	104.68%	3.62%	0.74	0.66	0.59	1.00	1.00	1.00
		week 2	104.18%	2.37%	104.94%	1.94%	104.95%	2.17%	104.92%	0.96%	0.44	0.99	0.98	1.00	1.00	1.00
		week 3	105.68%	2.60%	105.47%	1.73%	105.63%	1.96%	105.00%	0.34%	0.84	0.86	0.42	1.00	1.00	1.00
		week 4	105.99%	4.26%	105.17%	3.87%	105.36%	3.58%	104.59%	4.59%	0.65	0.91	0.88	1.00	1.00	1.00
		week 5	102.87%	3.57%	104.00%	3.59%	104.29%	3.47%	103.14%	3.80%	0.48	0.86	0.79	1.00	1.00	1.00
		week 6	100.79%	3.18%	103.41%	3.69%	103.92%	3.79%	101.88%	2.85%	0.10	0.77	0.55	0.24	0.89	0.89
		week 7	104.01%	6.76%	106.42%	4.38%	107.50%	4.39%	103.20%	2.22%	0.35	0.61	0.17	0.41	0.77	0.29
		week 8	100.99%	9.17%	102.95%	7.64%	105.43%	4.42%	95.50%	10.03%	0.60	0.38	0.41	0.59	0.59	0.59
Fig. 6C*	#EBER$^+$/mm^2 in spleen		2035.73	3594.60	98.27	90.95	55.38	58.32	212.67	57.16	0.11	0.47	0.02	0.08	0.98	0.02
Fig. 6D	% EBER$^+$ in spleen		11.72	19.40	0.63	0.61	0.32	0.37	1.47	0.23	0.10	0.21	0.01	0.19	0.19	0.01
Fig. 6G	Spleen	% CD3$^+$/Ki67$^+$	29.32	8.14			24.30	10.73						0.3242		
		% CD20$^+$/Ki67$^+$	7.27	14.31			3.93	1.10						0.2827		

Table S7: Descriptive statistics for comparisons between the cohorts PBS control and CD8$^+$ 7A-gp350CAR-T cells (all mice, responders or non-responders) regarding the immunologic effects in the therapeutic model.

			CTR (n=11)		CAR R (n=8)		CAR NR (n=3)		*p*			statistical test applied
			Mean	SD	Mean	SD	Mean	SD	CTRxCAR R	CTRx CAR NR	CAR R x CAR NR	
Fig.7A+B	%CD8$^+$ in huCD45 in blood	week 0	5.62	2.54	5.08	2.77	7.61	4.56	0.94			ANCOVA
		week 3	7.28	2.42	7.47	2.06	9.36	4.13				
		week 4	11.61	4.92	15.37	9.03	14.27	5.57				
		week 6	27.22	12.28	32.62	15.43	34.33	6.20				
		week 8	49.71	16.56	34.00	11.25	57.90	14.93				
		week 6	27.22	12.28	32.62	15.43	34.33	6.20	0.86	0.82	0.86	Welch's t test with Bonferroni-Holm correction
		week 8	49.71	16.56	34.00	11.25	57.90	14.93	0.10	0.54	0.28	
Fig.7C	# CD8$^+$ in spleen		8.55E+06	5.76E+06	7.56E+06	5.42E+06	6.15E+06	1.30E+06	1.00	1.00	1.00	Welch's t test with Bonferroni-Holm correction
	# CD4$^+$ in spleen		5.12E+06	3.17E+06	1.02E+07	1.05E+07	3.31E+06	1.28E+06	0.65	0.75	0.53	
Fig.7D*	MFI PD-1 R1	in CD8	73993.17	30234.36	59176.50	10452.24	n=1		0.50			Welch's t test with Bonferroni-Holm correction
		in CD4	50503.17	15402.12	53875.50	13231.48			0.66			
	MFI PD-1 R2	in CD8	292.25	25.79	241.67	3.30	n=2		0.03			
		in CD4	556.25	79.32	442.67	41.77			0.08			
Fig. 7E	% CAR$^+$ in huCD45/CD8 at week 8		0.16	0.29	0.65	0.63	0.48	0.67	0.44	1.00	1.00	Wilcoxon test with correction for ties
Fig. 7F	Cytokines (pg/ml)	IFN-γ *	147.24	120.48	69.77	54.72	1745.88	1214.62	0.19	0.19	0.19	Welch's t test with Bonferroni-Holm correction
		IL-10*	39.25	37.27	16.57	9.56	631.27	521.94	0.25	0.25	0.12	
		IL-12	2.37	2.01	2.91	2.86	10.62	5.96	0.79	0.29	0.29	Wilcoxon test with correction for ties
		IL-6	4.59	8.29	0.01	0.00	30.48	24.69	0.41	0.41	0.16	
		IL-8*	16.02	29.65	7.07	13.01	34.42	35.47	0.80	0.65	0.65	Welch's t test with Bonferroni-Holm correction
		MCP-1*	38.71	31.14	25.78	14.40	71.24	61.45	1.00	1.00	1.00	

For Figures marked with a * in the tables, p-values were calculated with log values without log display in the figures or the tables.

Table S8: List of used antibodies

EBNA2 staining				
Antibody	**Flourochrome conjugate**	**Clone**	**Company**	**Order number**
Rat anti-EBNA2	-	R3	Merck	Q69022
Mouse anti-rat IgG AF 647	AF 647	Polyclonal	Jackson ImmunoResearch Laboratories	212-605-082
Blocking				
			Company	**Order number**
PBS with 10% human serum			Capricorn Scientific, Ebsdorfergrund, Germany	HUM-3B
PBS with 10 µg/ml mouse-IgG			Sigma-Aldrich, St. Louis, MO	MFCD00212351
gp350 Staining				
Antibody	**Flourochrome conjugate**	**Clone**	**Company**	**Order number**
gp350 primary antibodies	-	6G4, 7A1, 72A1	kindly provided by GeneVector Laboratory, Munich, Germany	-
Mouse anti-rat IgG AF 647	AF 647	Polyclonal	Jackson ImmunoResearch Laboratories	212-605-082
Goat anti-mouse IgG	AF 647	Polyclonal	Jackson ImmunoResearch Laboratories	115-605-003
Detection of hematopoetic cells				
Antibody	**Flourochrome conjugate**	**Clone**	**Company**	**Order number**
Anti-human CD45	Pacific Blue	HI30	Biolegend	304022
Anti-human CD19	AF700	HIB19	Biolegend	302225
Anti-human CD3	BV510	UGHT1	Biolegend	300448
Anti-human CD4	PerCP	Okt 04	Biolegend	317432
Anti-human CD8	PECy7	HIT8a	Biolegend	300914
Anti-human CD62-L	PECy5	DREG56	Biolegend	304808
Anti-human CD45RA	FITC	HI100	Biolegend	304106
Anti-human PD-1	PE	EH12.2H7	Biolegend	329906
CAR detection				
Antibody	**Flourochrome conjugate**	**Clone**	**Company**	**Order number**
Goat Anti-Human IgG, Fcγ fragment specific	AF 488	Polyclonal	Jackson ImmunoResearch Laboratories	109-545-008
Goat Anti-Human IgG, Fcγ fragment specific	AF 647	Polyclonal	Jackson ImmunoResearch Laboratories	109-605-008
Immunohistochemistry				
Antibody	**Flourochrome conjugate**	**Clone**	**Company**	**Order number**
Anti-human CD3	Opal 650	polyclonal	DAKO	A0452
Anti-human CD20	Opal 690	L26	DAKO	M0755
Anti-human Ki67	Opal 620	SP6	Thermofisher Scientific	RM9106-S1