SUPPLEMENTAL METHODS

Cell lines and cell culture

The Hodgkin lymphoma (HL) cell lines L428 (nodular sclerosis), L1236 (mixed cellularity), L540 (nodular sclerosis, T cell derived), HDLM2 (nodular sclerosis; T cell derived), KMH2 (mixed cellularity), L591 (nodular sclerosis, EBV+), DEV (nodular lymphocyte predominant (NLPHL)) and the diffuse large B cell lymphoma (DLBCL) cell line SUDHL6 were cultured as previously described.[1,2] Cell line ID was checked using DNA-based STR analysis with the powerplex 16 HS kit (Promega, Madison, WI, USA). Routinely performed mycoplasma tests were consistently negative. Human leukocyte antigen (HLA) typing of the HL cell lines was determined by SSOP (Immucor, Peachtree Corners, GA, USA) as described previously (Supplemental Table 1).[3] The high resolution typing results of the cell lines for HLA-DRB1 and HLA-DQB1 were confirmed using next generation sequencing.

Flow cytometry

Flow cytometry was performed by incubating cells with antibodies against HLA-A,B,C, HLA-DP,DQ,DR, APC labeled antibody for HLA-DR and PE labeled antibodies for CD54 and CD58 for 30 minutes on ice. Unlabeled antibodies were followed by a goat anti-mouse IgG FITC. As a negative control the first antibody was omitted or isotype control antibodies were used. All antibodies used in this study are summarized in Supplemental Table 3. Samples were analyzed on a BD FACS Caliber flow cytometer (BD Biosciences) and analyzed using FlowJo version 10.6.1 software.

Healthy controls

Six unmatched and nineteen HLA-II matched healthy donors were selected from a pool of HLA typed blood donors (Supplemental Table 2). The matching was based on HLA-II type of both PBMC donors and HL cell lines and was perfect at second field resolution, referring to matching based on the precise and unique HLA amino acid sequence according to the HLA nomenclature.[4] The only exception was KMH2, carrying the HLA-DRB1*04:65 allele that is uncommon in Caucasians.

Peripheral blood mononuclear cells (PBMCs) were isolated from full blood of healthy donors using Ficoll-Paque (GE Healthcare, Chicago, IL, USA) following standard procedures. PBMCs were resuspended in FCS + 10% dimethyl sulfoxide (Sigma-Aldrich, Saint Louis, MO, USA) and frozen in liquid nitrogen. This study was approved by the Institutional Review Board of the UMCG (METc UMCG 2018/150) and all healthy donors gave informed consent. Each experiment was done with at least three different HLA-II unmatched or matched PBMC donors per cell line or condition.

Rosetting assay

HL cell lines were incubated with PBMCs in a HL cell:PBMC ratio of 1:20 with $1x10^6$ PBMCs/mL RPMI 1640 medium supplemented with 10% FCS. A 1:50 ratio was only used for visualization purposes, because we observed some clustering of rosettes at a 1:20 ratio at longer co-culture times (e.g. 2-4h). Cells were incubated at 37^{o}C for five minutes to four hours to allow the formation of rosettes. After incubation rosettes were gently resuspended, diluted and cytospins were made using a CytoSpin 4 cytocentrifuge (ThermoFischer Scientific). For accurate counting of T cells within tumor cell rosettes both the shape of the nucleus and the size of the cells were considered to reliably discriminate lymphocytes from monocytes. In addition, in double staining experiments (see below) monocytes were excluded from the counts also based on their CD4dim staining pattern.[5] A rosette was defined as a cluster containing one tumor cell and three or more adherent lymphocytes. The number of rosettes was counted for at least 50 tumor cells by two individual observers. Infrequent tumor cell clumps of three or more tumor cells were excluded, as this influenced the formation of rosettes. To block the interaction between CD2 and CD58, cells were incubated with 5µg anti-human CD2 as described previously.[6] Enzyme-linked immunosorbent assay (ELISA) was used to measure interleukin-2 (IL-2) in supernatant of 18 hour co-culture experiments. Experiments to determine IL-2 production were performed in duplicate and the mean of duplicates was used. If samples were measured on different ELISA plates at least three control samples were taken along

and values were normalized to the control samples. ELISA was performed according to manufacturer's protocol (IL-2 kits: HS200 and DY202, R&D systems).

Immunofluorescence

Cytospin slides were fixed with 4% paraformaldehyde (PFA) for 10 minutes, incubated with primary antibodies against CD2, TCR or CD11a and a secondary anti-mouse IgG Alexa Fluor 555 antibody. F-actin was stained with Phalloidin-iFluor 555 reagent. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (1:1000, Invitrogen). Fluorescent signals were visualized using the Leica DM4000B (Wetzlar, Germany) microscope equipped with a Leica DFC345FX Camera using the LAS V4.8 imaging software. Overlays were made and brightness and contrast were adjusted using Fiji software.

Immunohistochemistry

Immunohistochemistry was performed on cytospins and snap-frozen tissues of classical HL patients (cHL) using standard procedures. For CD8/CD4 double staining, cytospins were fixed in acetone and incubated with anti-CD8, followed by incubations with rabbit anti-mouse immunoglobulins/HRP, goat anti-rabbit immunoglobulins/HRP and visualized with diaminobenzidine (DAB). Subsequent staining for CD4 was done by incubating with anti-CD4, followed by incubations with goat anti-mouse IgG2a-biotin, streptavidin alkaline phosphatase and visualized using an alkaline phosphatase staining reaction. Appropriate positive and negative control slides were included. Single staining for CD20 and CD56 on cytospins was visualized using 3-amino-9-ethylcarbazole (AEC) after incubation with appropriate secondary and tertiary antibodies. This procedure was also used for CD2 and TCR staining on tissue sections that were cut from snap-frozen cHL tissue. Single stained slides were counterstained with hematoxylin.

Generation of CRISPR-Cas9 knockout cell lines

Guide RNAs used to target CIITA, CD58 and CD54 were selected from the study of Abrahimi et al.[7] or designed using the online optimization software tool GuideScan (http://www.guidescan.com/) and the GPP sgRNA designer of the Broad Institute (https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design) (Supplemental Table 4). The single guide RNAs (sgRNAs) were cloned into the BsmBI restriction site in a modified lentiCRISPR v2 vector.[8] Lentiviral particles were produced after which L428 cells were infected as described previously.[9-11] Knockout (KO) efficiency was determined by flow cytometry of HLA-DR (to monitor efficiency of CIITA-KO), CD58 and CD54 using a FACS Caliber flow cytometer. HLA-DR, CD58 and CD54 negative cells were sorted by fluorescent activated cell sorting (FACS) using a SH800S sorter (Sony Biotechnology, San Jose, CA, USA) approximately 2-3 weeks after infection for further studies. The HL cell line L428 was selected to generate KOs as the lack of HLA-I expression in this cell line prevents bias that would likely be caused by activation of CD8+ T cells.

Proximity ligation assay

A duolink in situ proximity ligation assay (PLA) kit was used to detect close proximity of molecules of interest according to manufacturer's instructions (DUO92102 and DUO92104, Sigma-Aldrich). The duolink PLA signal is generated only if the proteins of interest are within 40 nm and is therefore considered as a marker for a direct interaction. Antibodies used for PLA were mouse anti-human CD2, goat anti-human CD58/LFA-3, rabbit anti-human CD4 and mouse anti-human HLA-DP,DQ,DR. To study the interaction of the TCR with HLA-II we used a CD4 antibody in combination with a pan-HLA-II antibody as there were no appropriate antibodies for the TCR-HLA-II combination. CD4 is a good alternative as it binds together with the TCR to HLA-II. Slides were imaged using a Leica DM4000B microscope. PLA signals were quantified at the tumor cell T cell interface by determining the average number of signals per attached cell for every visualized rosette. As controls for noise, slides were included in which one of the two primary antibodies was omitted. These controls were consistently negative.

Set-up of the *in vitro* model to study rosette formation

A short-term *in vitro* model system was developed and optimized using HLA-II unmatched PBMCs from healthy donors and HL cell lines. Read-out parameters included the percentage of tumor cells with rosettes (see representative example in Methods Figure 1A), the CD4 and CD8 T cell subsets present in the rosettes (see representative example in Methods Figure 1B), activation of T cells and relocalization of IS components.

The most optimal co-culture time point was determined, aiming for a percentage of tumor cells with rosettes that did not already reach a maximum to allow identification of potential differences between cell lines and conditions. For L428 we observed rosetting of PBMCs for 65, 77 and 85% of tumor cells after 15, 30 and 60 min respectively (Methods Figure 1C). A co-culture time of 30 minutes was chosen as the most optimal time point for subsequent experiments. At this time point the percentage of rosettes ranged from 47-80% for the different cHL cell lines. The percentage of rosetting around the NLPHL cell line DEV, the only cell line that lacked HLA-I, HLA-II and CD58 expression was lower with only 36% of tumor cells with rosettes (Methods Figure 1D). A much lower number of tumor cell rosettes was observed for the DLBCL cell line SUDHL6, corroborating the notion that massive rosetting is unique for HL. Double staining for CD4 and CD8 showed that approximately 60% of the rosetting cells were CD4+, 20% were CD8+ and 20% were CD4-CD8- cells. No clear differences were observed between the HL cell lines (Methods Figure 1E). The CD4-CD8- cell population is a mixture of NK cells (CD56+) and B cells (CD20+). TCR mediated activation of T cells was assessed by measuring IL-2 levels in the culture supernatant. PBMCs of all five donors responded to L428, L1236 and L591 by producing IL-2 (Methods Figure 1F). Three out of five donors produced IL-2 in response to L540, while hardly any responses were observed upon co-culture with HDLM2, SUPHD1, KMH2 and DEV.

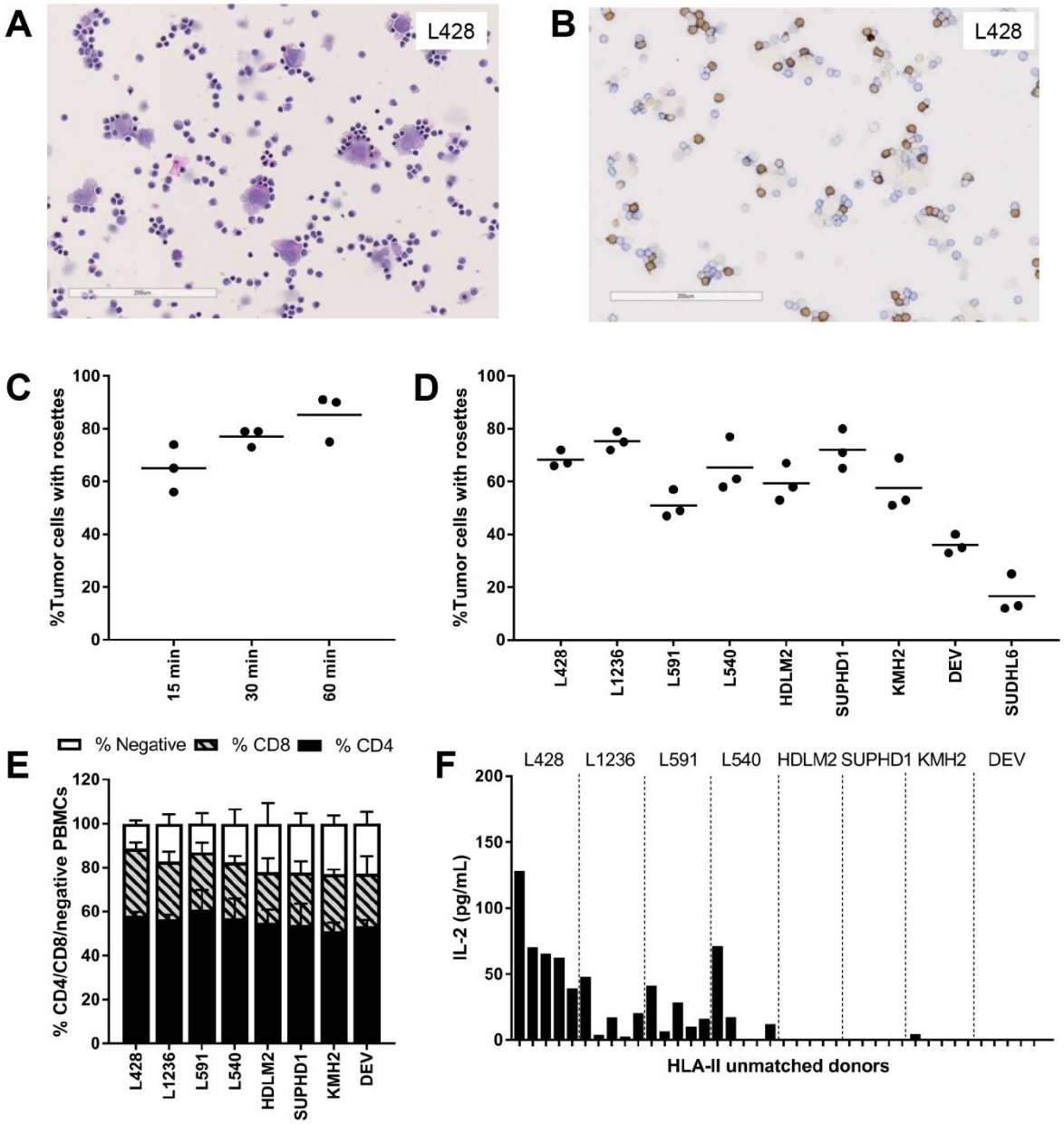
Immunofluorescence was used to study F-actin polymerization in tumor cells and relocalization of IS components in T cells upon rosetting. For this analysis we used co-culture times

varying between 5 min and 4h. F-actin polymerization was prominent in tumor cells and increased over time. It presented as spider-web like structures stretching out towards the T cells (Methods Figure 2A). For CD2 on T cells, a clear relocalization to the physical interface with tumor cells was observed already at five minutes and lasting up to four hours. This relocalization occurred in response to the HL cell lines that are CD58 positive, but not in the context of CD58 negative cell lines (Methods Figure 2B). No relocalization of CD11a nor TCR was observed.

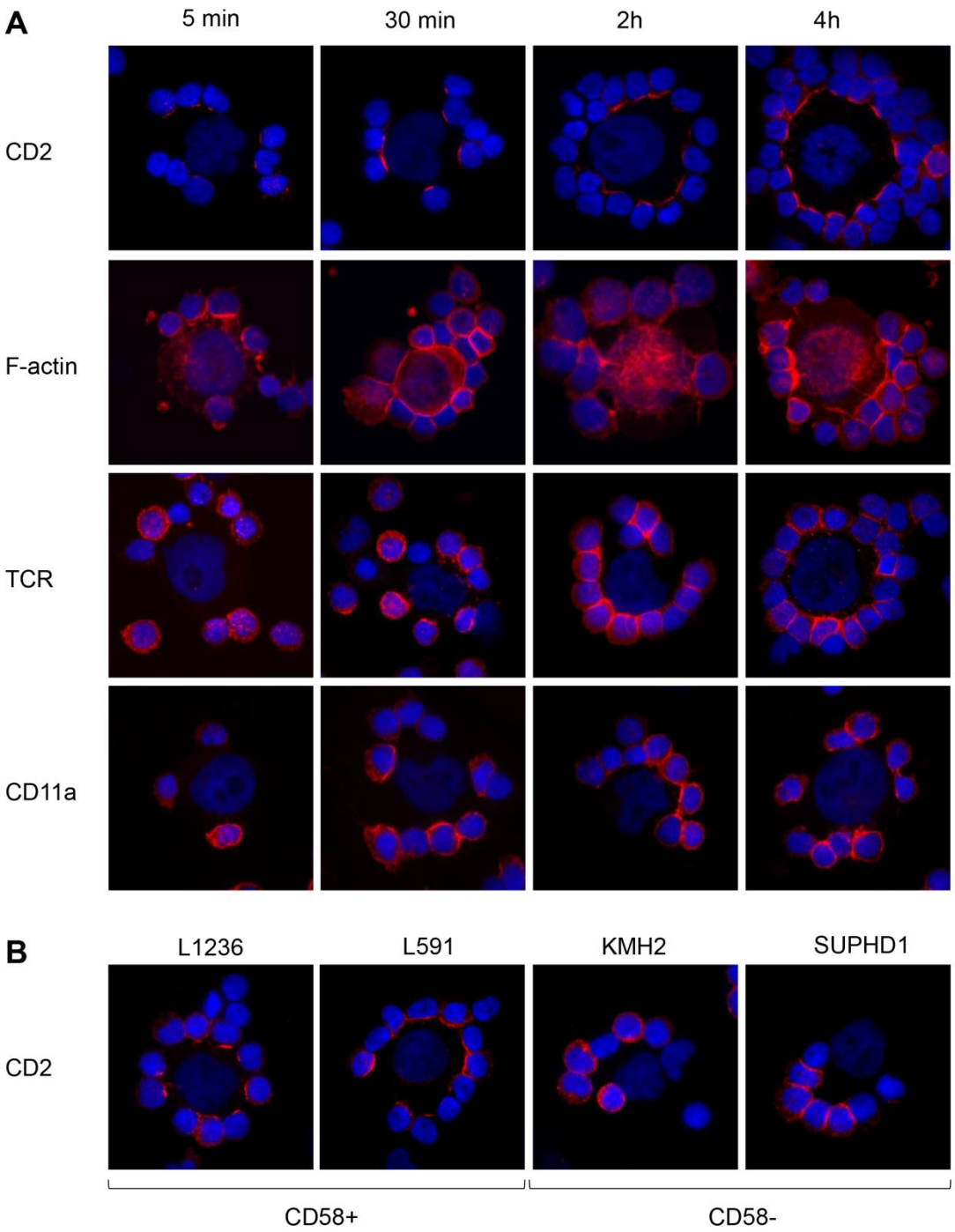
The model and read-out parameters described above were also used to study the effect of IS component KOs with HLA-II unmatched PBMCs (Methods Figure 3). In general effects observed with unmatched PBMCs were similar to matched PBMCs.

Statistical analysis

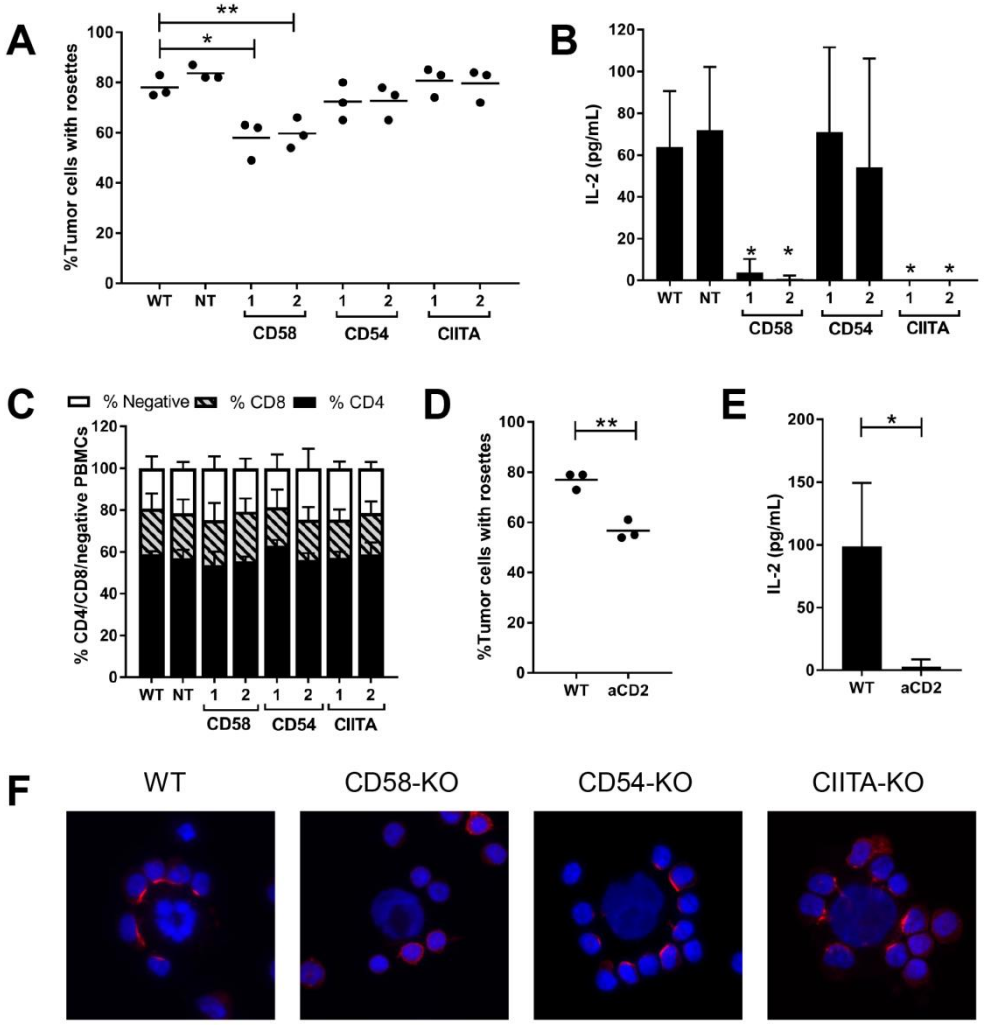
Data are represented for all individual PBMC donors and either displayed separately or as mean of PBMC donors plus standard deviation. Statistical significance was calculated with GraphPad Prism 7.02 using a one-tailed paired t-test if different conditions employed on the same cell line were compared or a one-tailed Mann-Whitney U test if different cell lines are compared. The one-tailed test was done with the hypothesis that the interventions would decrease rosette formation and T cell activation. Values $P < 0.05$ were considered statistically significant.



Methods Figure 1. An *in vitro* model to study T cell rosetting of HLA-II unmatched PBMCs with HL cell lines. (A) Hematoxylin and eosin staining of rosettes after a 30 minute co-culture of L428. (B) CD4 (blue) and CD8 (brown) double staining after a 30 minute co-culture of L428. (C) The percentage of L428 cells with rosettes quantified after 15, 30 or 60 minutes of co-culture. (D) The percentage of tumor cells with rosettes for different HL cell lines and as a control the DLBCL cell line SUDHL6 upon co-culture for 30 minutes. SUDHL6 cells expressed all components of the immunological synapse. (E) The percentage of CD4+, CD8+ and CD4-CD8- cells present in rosettes of HL cell lines co-cultured for 30 minutes. Data is presented as mean of different donors plus standard deviation. In panels C-E the experiments were performed with three different unmatched donors, which were the same for all cell lines. (F) IL-2 production of unmatched PBMCs in response to different HL cell lines. Each bar represents a single donor. The same five donors were used for all HL cell lines and presented in the same order.



Methods Figure 2. Relocalization of immunological synapse components in response to HL cell lines using HLA-II unmatched PBMCs. (A) Immunological synapse components CD2, F-actin, TCR and CD11a were stained (red signal) upon co-culture of PBMCs with L428 for four different incubation times. (B) CD2 staining (red signal) on PBMCs co-cultured with CD58 positive L1236 and L591 or CD58 negative KMH2 and SUPHD1 cell lines. Clear relocalization of CD2 can be observed as discrete red signals at the interface between T cells and tumor cells using CD58 positive cell lines in contrast to the homogenous circumferential staining of entire T cells using CD58 negative cell lines. Relocalization of F-actin in L428 tumor cells presented as spider-web like structures stretching out towards the T cells. Representative images are shown at a 400x magnification.



Methods Figure 3. Relevance of HLA-II, CD58 and CD54 in adhesion and activation of rosetting T cells using HLA-II unmatched PBMCs. (A) The percentage of tumor cells with rosettes upon co-culture of L428 wild-type (WT), non-targeting control (NT), CD58-KO, CD54-KO or CIITA-KO cell lines with PBMCs for 30 minutes. (B) IL-2 production by PBMCs in response to L428 WT, NT, CD58-KO, CD54-KO and CIITA-KO cell lines. (C) The percentage CD4+, CD8+ and CD4-CD8- cells present in rosettes upon co-culture of L428 WT, NT, CD58-KO, CD54-KO or CIITA-KO cell lines with PBMCs for 30 minutes. (D) The percentage of tumor cells with rosettes upon a 30 minute co-culture of L428 with untreated or anti-CD2 (aCD2) blocking antibody treated PBMCs. (E) IL-2 production of PBMCs co-cultured with L428 cells in the absence or presence of an aCD2 blocking antibody. (F) CD2 staining (red signal) of WT, CD58-KO, CD54-KO or CIITA-KO L428 cells co-cultured with PBMCs. Nuclei were counterstained with DAPI (blue signal). Clear relocalization of CD2 can be observed as discrete red signals at the interface between T cells and WT, CD54-KO and CIITA-KO tumor cells in contrast to the homogenous circumferential staining of entire T cells in CD58-KO cells. Representative images are shown at a 400x magnification. All experiments were performed with at least three different unmatched PBMC donors. Statistics were calculated using a one-tailed paired t-test compared to the WT. Values $P<0.05$ were considered statistically significant. *$P<0.05$; **$P<0.01$.

SUPPLEMENTAL REFERENCES

1. Liu Y, Abdul Razak FR, Terpstra M, et al. The mutational landscape of hodgkin lymphoma cell lines determined by whole-exome sequencing. *Leukemia*. 2014;28(11):2248-2251.

2. de Jong MRW, Langendonk M, Reitsma B, et al. WEE1 inhibition synergizes with CHOP chemotherapy and radiation therapy through induction of premature mitotic entry and DNA damage in diffuse large B-cell lymphoma. *Ther Adv Hematol*. 2020;11:2040620719898373.

3. Huang X, Kushekhar K, Nolte I, et al. HLA associations in classical hodgkin lymphoma: EBV status matters. *PLoS One*. 2012;7(7):e39986.

4. The IPD-IMGT/HLA database. HLA nomenclature. https://www.ebi.ac.uk/ipd/imgt/hla/nomenclature/index.html. Accessed on: 20 April 2020.

5. Filion LG, Izaguirre CA, Garber GE, Huebsh L, Aye MT. Detection of surface and cytoplasmic CD4 on blood monocytes from normal and HIV-1 infected individuals. *J Immunol Methods*. 1990;135(1-2):59-69.

6. Fromm JR, Kussick SJ, Wood BL. Identification and purification of classical hodgkin cells from lymph nodes by flow cytometry and flow cytometric cell sorting. *Am J Clin Pathol*. 2006;126(5):764-780.

7. Abrahimi P, Chang WG, Kluger MS, et al. Efficient gene disruption in cultured primary human endothelial cells by CRISPR/Cas9. *Circ Res*. 2015;117(2):121-128.

8. Wallace J, Hu R, Mosbruger TL, et al. Genome-wide CRISPR-Cas9 screen identifies MicroRNAs that regulate myeloid leukemia cell growth. *PLoS One*. 2016;11(4):e0153689.

9. Dzikiewicz-Krawczyk A, Kok K, Slezak-Prochazka I, et al. ZDHHC11 and ZDHHC11B are critical novel components of the oncogenic MYC-miR-150-MYB network in burkitt lymphoma. *Leukemia*. 2017;31(6):1470-1473.

10. Yuan Y, Kluiver J, Koerts J, et al. miR-24-3p is overexpressed in hodgkin lymphoma and protects hodgkin and reed-sternberg cells from apoptosis. *Am J Pathol*. 2017;187(6):1343-1355.

11. Yuan Y, Niu F, Nolte IM, et al. MicroRNA high throughput loss-of-function screening reveals an oncogenic role for miR-21-5p in hodgkin lymphoma. *Cell Physiol Biochem*. 2018;49(1):144-159.

SUPPLEMENTAL TABLES

Supplemental Table 1. HLA typing results of Hodgkin lymphoma cell lines

Cell line	HLA-A		HLA-B		HLA-C		HLA-DRB1		HLA-DQB1	
L1236	02		51		02		14:54		05:03	
L428	03		35		04		12:01		03:01	
L540	03	11	51		02	15	04:04	11:01	03:01	03:02
HDLM2	01	02	08	44	05	07	13:01	15:01	06:02	06:03
L591	01	33	08	35	04	07	03:01		02:01	
SUPHD1	01		38		12		13:01		06:03	
KMH2	11	24	52	62	04	12	04:65	11:01	03:01	03:02
DEV	02		44	60	10	05	04:01	15:01	06:02	03:01

Missing alleles either represent homozygosity for the indicated allele or loss of heterozygosity.

Supplemental Table 2. HLA genotype of healthy PBMC donors

Donor	HLA-A		HLA-B		HLA-C		HLA-DRB1		HLA-DQB1		Matched to cell line
UC01	02	68	53	61	02	04	13:01	13:02	06:03	06:04	*Unmatched*
UC02	02	31	27	57	02	06	04:01	07:01	03:02	03:03	*Unmatched*
UC03	02	24	62		03		04:01		03:02		*Unmatched*
UC04	31		51	60	03		04:04	11:04	03:01	03:02	*Unmatched*
UC05	24	30	13	51	06	14	07:01	09:01	02:02	03:03	*Unmatched*
UC06	01	25	08	18	07		11:04	15:01	03:01	06:02	*Unmatched*
MC01	02	11	51	55	05	09	13:01	14:54	05:03	06:03	L1236
MC02	26	68	35	51	04	15	01:01	14:54	05:01	05:03	L1236
MC03	02	03	44	55	04	05	13:01	14:54	05:03	06:03	L1236
MC04	24	32	44	51	05	15	01:01	12:01	03:01	05:01	L428
MC05	01	02	07	44	05	07	12:01	15:01	03:01	06:02	L428
MC06	32	68	07	44	05	07	12:01	15:01	03:01	06:02	L428
MC07	01	23	08	49	07		04:04[+]	11:01	03:01	03:02	L540 and KMH2
MC08	02		37	62	10	06	04:04[+]	11:01	03:01	03:02	L540 and KMH2
MC09	02	29	51	60	02	10	04:04[+]	11:01	03:01	03:02	L540 and KMH2
MC10	11	68	35	71	04	07	04:01[+]	11:01	03:01	03:02	KMH2
MC11	02	68	07	08	07	07	13:01	15:01	06:02	06:03	HDLM2
MC12	03	68	07	61	02	07	13:01	15:01	06:02	06:03	HDLM2
MC13	02	26	27	62	02	09	13:01	15:01	06:02	06:03	HDLM2
MC14	01	03	08		07		03:01		02:01		L591
MC15	01	11	08	35	04	07	03:01		02:01		L591
MC16	01	03	08	35	04	07	01:01	03:01	02:01	05:01	L591
MC17	24	30	13	38	06	12	11:01	13:01	06:03	03:01	SUPHD1
MC18	01	31	08	38	07	12	03:01	13:01	02:01	06:03	SUPHD1
MC19	01	02	08	62	09	07	03:01	13:01	02:01	06:03	SUPHD1

[+]KMH2 has an uncommon HLA-DRB1*04:65 and was therefore matched with HLA-DRB1*04:01 or HLA-DRB1*04:04 PBMC donors. HLA-DRB1*04:04 has three and HLA-DRB1*04:01 has four amino acids difference in exon-2 with HLA-DRB1*04:65. Green indicates a perfect second field match.

Supplemental Table 3. Antibodies used during this study

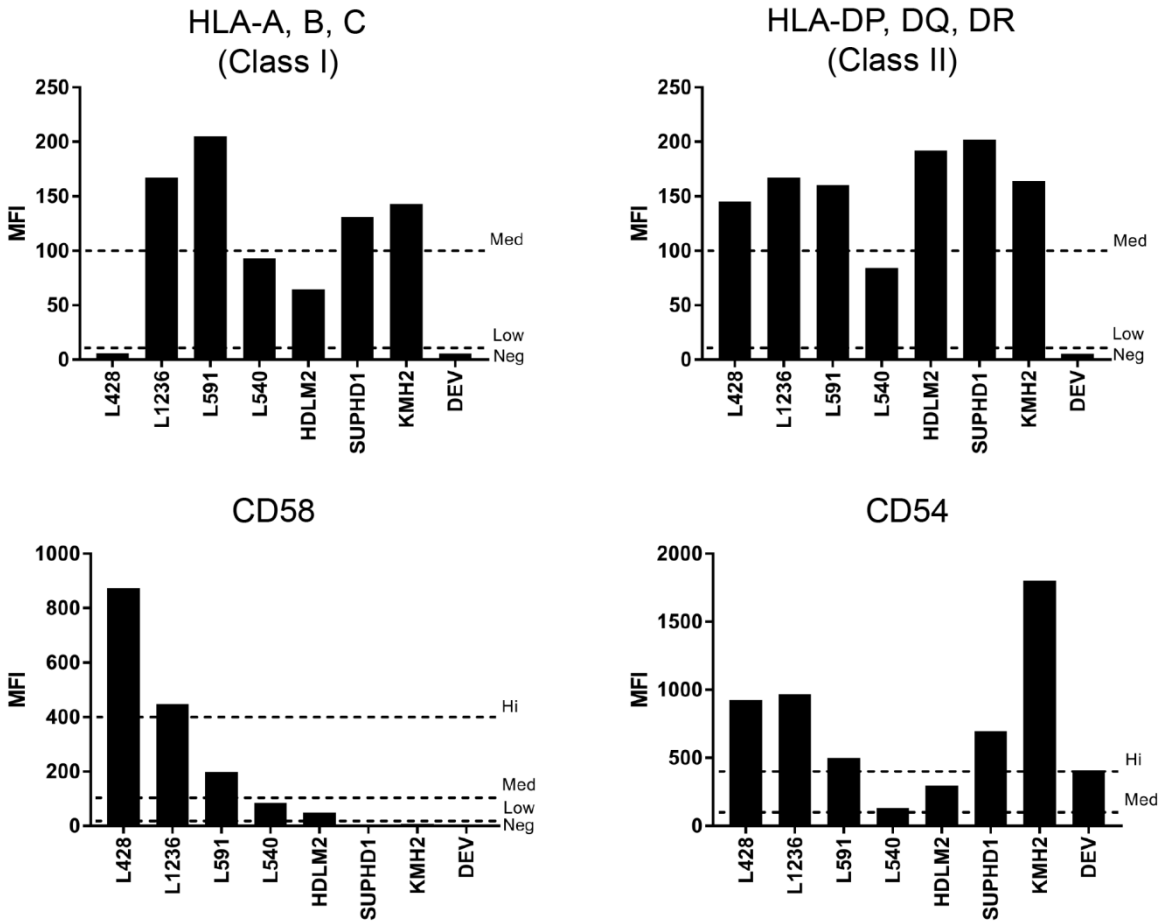
Antibody name	Clone	Dilution	Application	Company
Purified anti-human HLA-A,B,C	W6/32	1:500	FC	Biolegend (San Diego, CA, USA)
Mouse anti-human HLA-DP,DQ,DR	CR3/43	1:200	FC PLA	Dako (Santa Clara, CA, USA)
APC anti-human HLA-DR	L243	1:20	FC	Biolegend
CD54 (ICAM-1) monoclonal antibody PE	HA58	1:40	FC	ThermoFisher Scientific (Waltham, MA, USA)
PE mouse anti-human CD58	1C3	1:20	FC	BD Biosciences (San Jose, CA, USA)
Goat anti-mouse IgG, Human ads-FITC	Polyclonal	1:100	FC	Southern Biotech (Birmingham, AL, USA)
APC mouse IgG2a,κ isotype ctrl antibody	MPOC-173	1:160	FC	Biolegend
Mouse IgG1 kappa isotype control PE	P3.6.2.8.1	1:160	FC	ThermoFisher Scientific
PE mouse IgG2a, κ isotype control	G155-178	1:20	FC	BD Biosciences
Purified mouse anti-human CD2	RPA-2.10	5 µg 1:50	*In vitro* PLA, IHC	BD Biosciences
Human CD58/LFA-3 antibody	Polyclonal	1:50	PLA	R&D Systems (Minneapolis, MN, USA)
Anti-CD4 antibody	EPR6855	1:50	PLA	Abcam (Cambridge, UK)
Mouse anti-human CD2	OKT11	Undiluted	IF	Cell culture supernatant ATCC® CRL-8027™
Mouse anti-human CD11a	LFA1/2	1:100	IF	Sanquin (Amsterdam, NL)
Mouse anti-human TCR	W4F.5B	Undiluted	IF	Cell culture supernatant ATCC® HB-9282™
Donkey anti-mouse IgG (H+L) highly cross-adsorbed Secondary antibody Alexa 555	Polyclonal	1:100	IF	ThermoFisher Scientific
Phalloidin-iFluor 555 reagent – cytopainter	-	1:1000	IF	Abcam
Purified mouse anti-human CD8	MCD8	1:10	IHC	IQ Products (Groningen, NL)
Purified mouse anti-human CD4	Edu-2	1:10	IHC	IQ Products
Purified mouse anti-human CD56	MOC-1	1:5	IHC	IQ Products
Purified mouse anti-human CD20	B-ly 1	1:5	IHC	IQ Products
Rabbit anti-mouse IgG/HRP	Polyclonal	1:100	IHC	Dako
Goat anti-rabbit IgG/HRP	Polyclonal	1:100	IHC	Dako
Goat anti-mouse IgG2a biotin	Polyclonal	1:100	IHC	Southern Biotech
Streptavidin alkaline phosphatase	Polyclonal	1:300	IHC	Dako

Supplemental Table 4. Sequences of single guide RNAs used in this study

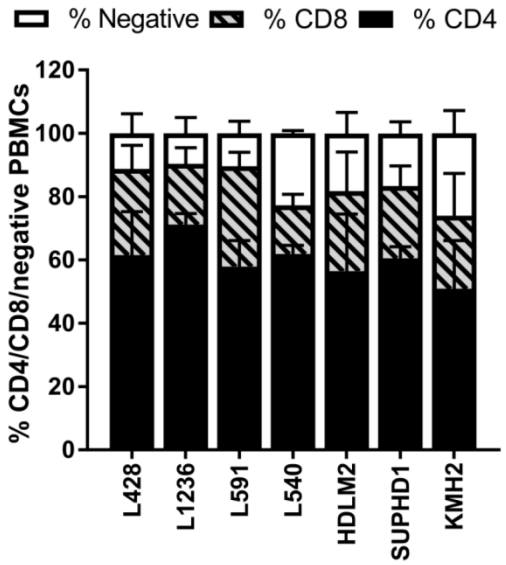
Guide RNA	Exon	Strand	Sequence 5'→3' *
CIITA sgRNA1	3	Sense	**CACCG**TCAACTGCGACCAGTTCAGC
		Antisense	**AAAC**GCTGAACTGGTCGCAGTTGA**C**
CIITA sgRNA2	3	Sense	**CACCG**GATATTGGCATAAGCCTCCC
		Antisense	**AAAC**GGGAGGCTTATGCCAATATC**C**
CD58 sgRNA1	1	Sense	**CACCG**TGGTTGCTGGGAGCGACGC
		Antisense	**AAAC**GCGTCGCTCCCAGCAACCA**C**
CD58 sgRNA2	1	Sense	**CACCG**GACCACGCTGAGGACCCCCA
		Antisense	**AAAC**TGGGGGTCCTCAGCGTGGTC**C**
CD54 sgRNA1	1	Sense	**CACCG**CGCACTCCTGGTCCTGCTCG
		Antisense	**AAAC**CGAGCAGGACCAGGAGTGCG**C**
CD54 sgRNA2	1	Sense	**CACCG**CCGAGCAGGACCAGGAGTGC
		Antisense	**AAAC**GCACTCCTGGTCCTGCTCGG**C**
NT	-	Sense	**CACCG**ATCGTTTCCGCTTAACGGCG
		Antisense	**AAAC**CGCCGTTAAGCGGAAACGAT**C**

*Bold indicates the parts within the sgRNAs that create overhangs after annealing to fit at the BsmBI cut site in the lentiCRISPR v2 vector.

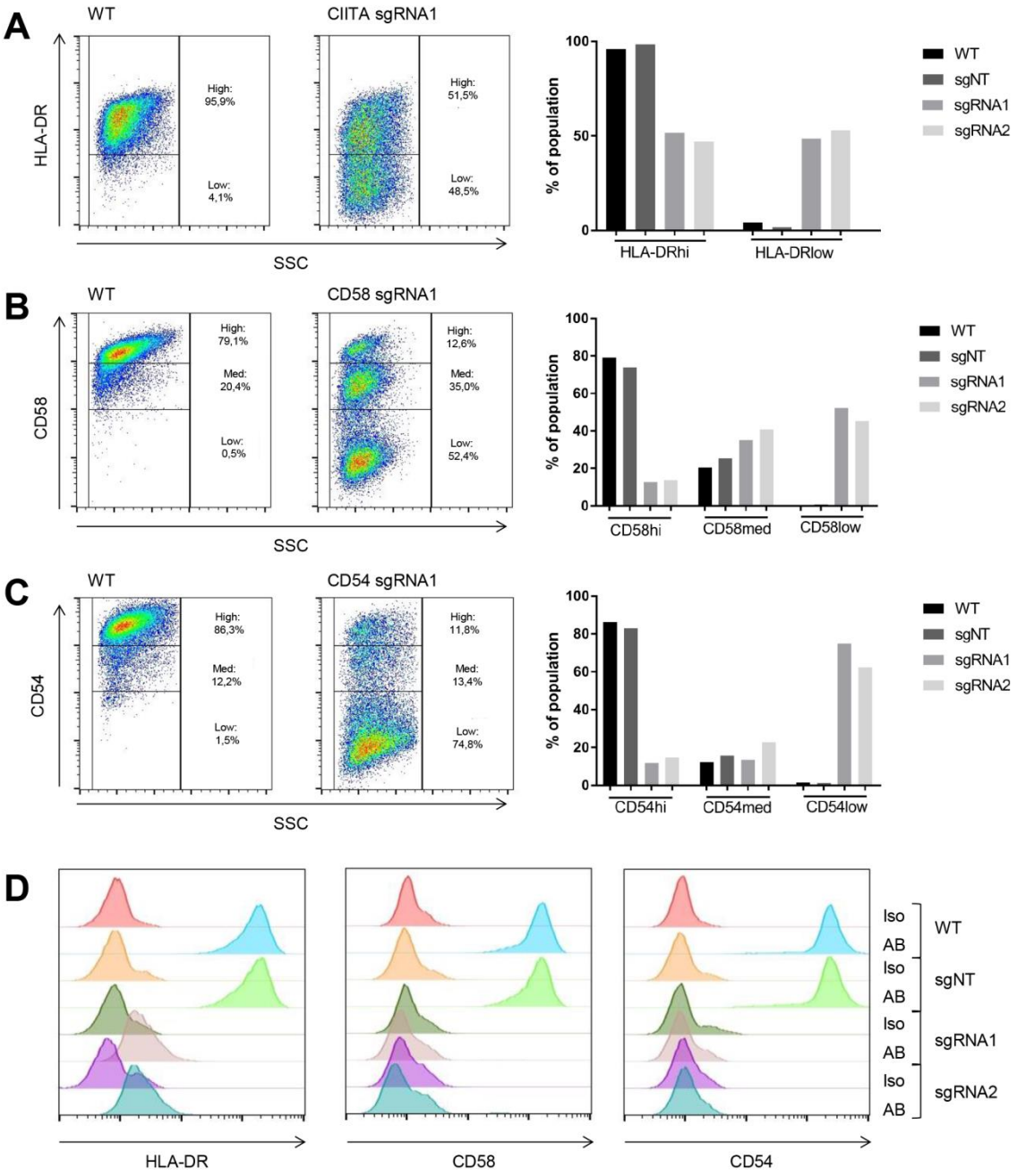
SUPPLEMENTAL FIGURES



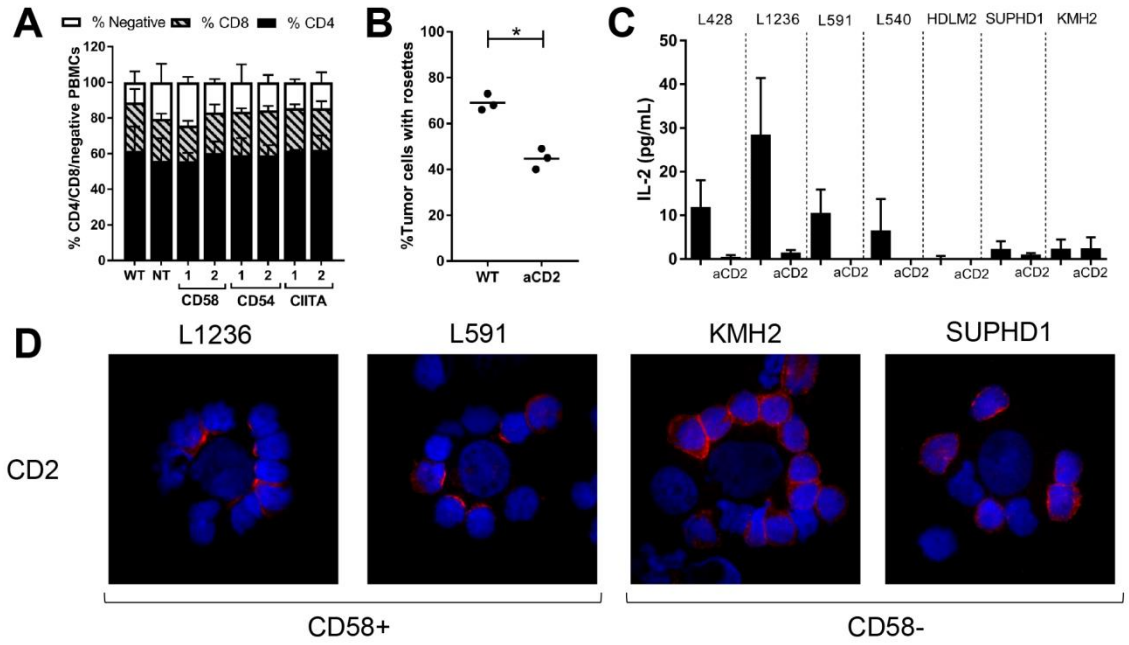
Supplemental Figure 1. Expression of immunological synapse components on HL cell lines. Flow cytometry analysis of cell-surface HLA class I, HLA class II, CD58 and CD54 expression in eight HL cell lines visualized by median fluorescent intensity values (MFI). Classification of negative (neg), low, medium (med) and high (hi) cell lines is based on MFI. Range: 0-10=neg; 10-100=low; 100-400=med; >400=hi.



Supplemental Figure 2. T cell composition in rosettes around different Hodgkin lymphoma cell lines using HLA-II matched PBMCs. HL cell lines were co-cultured with HLA-II matched PBMCs for 30 minutes and the percentage of CD4+, CD8+ or CD4-CD8- cells present in rosettes was quantified using a double staining. Data are represented as mean plus standard deviation of at least three different HLA-II matched PBMC donors.



Supplemental Figure 3. Generation of CIITA, CD58, CD54 CRISPR-Cas9 knockout L428 cell lines. Cell surface expression of HLA-DR, CD58 and CD54 in L428 cells infected with lentiviral vectors containing two independent sgRNAs for (A) CIITA, (B) CD58 or (C) CD54. After 2-3 weeks of culture a significant proportion of the cells has reduced expression of HLA-DR, CD58 or CD54. The cells with no or low expression of the respective proteins were sorted and expanded for further studies. (D) Flow cytometry staining of HLA-DR, CD58 or CD54 (indicated by AB) of L428 wild-type (WT), NT and the knockout (KO) cell lines and controls after sorting and expansion. The sorted KO populations showed a complete KO of CD58 or CD54, while HLA-DR was strongly decreased after CIITA KO. NT cells showed no change in cell surface expression levels.



Supplemental Figure 4. Involvement of CD58 in adhesion and activation of rosetting T cells using HLA-II matched PBMCs.

(A) The percentages of CD4+, CD8+ and CD4-CD8- cells within the rosettes upon co-culture of L428 wild-type (WT), non-targeting (NT) control, CD58-KO, CD54-KO and CIITA-KO with HLA-II matched PBMCs for 30 minutes. Data are represented as the mean plus standard deviation of three independent donors. (B) The percentage of tumor cells with rosettes upon co-culture of L428 with untreated or anti-CD2 (aCD2) blocking antibody treated PBMCs for 30 minutes. Each dot indicates one independent HLA-II matched donor. (C) IL-2 production of matched PBMCs in response to different HL cell lines in the absence or presence of an aCD2 blocking antibody. Data are represented as the mean plus standard deviation of at least three independent donors. (D) CD2 staining (red signal) of matched PBMCs co-cultured with the CD58 positive L1236 and L591 or CD58 negative KMH2 and SUPHD1 cell lines. Nuclei were counterstained with DAPI (blue signal). Relocalization of CD2 can be observed as discrete red signals at the interface between T cells and CD58 positive tumor cells in contrast to homogenous circumferential staining of entire T cells in CD58 negative tumor cells. Representative images are shown at a 400x magnification. Statistics were calculated using a one-tailed paired t-test. Values $P<0.05$ were considered statistically significant. *$P<0.05$.