Supplementary Methods

Hodgkin lymphoma (HL) cell lines

Three HL cell lines KM-H2, L428 and HDLM2 (gift from Prof Volker Diehl, University of Cologne, Germany) were used. Control cell line HL-60 was from the Christchurch Haematology Research Group.

Flow Cytometry

The following antibodies were used: CD3-Alexa Fluor (AF)700 or CD3-Phycoerythrin (PE), CD4-PE-CF594, CD15-Violet (V)450, CD19-V450, CD20-V421, CD30-PE, CD40-PE-Cy7, CD279 (PD-1)- Brilliant Violet (BV)786, CD274 (PD-L1)-PE-Cy7 (all from BD Biosciences), CD25-BV421 and CD107-PE-Cy7 (Biolegend). Mouse anti-human CD83 monoclonal antibodies (mAbs), HB15a-Fluorescein Isothiocyanate (FITC) was obtained from Beckman and Coulter, and HB15e-FITC from BD Biosciences. 3C12C is a human IgG1 anti-human CD83 mAb made in house. Isotype control antibodies included mouse IgG1 Kappa- FITC, mouse IgG2b-FITC (BD Biosciences) and human IgG1 Kappa (Sigma Aldrich). For intracellular CD83 expression, cells were fixed and permeabilized with Foxp3 intracelluar staining kit (Ebioscience). Data were collected on a Fortessa X20 flow cytometer (BD Biosciences) and analyzed with FlowJoV9&10 software (TreeStar).

Immunofluorescence staining

KM-H2, L428 or HDLM2 cells (10^5 cells) were cytospun onto lysine coated microscope slides. Cells were fixed and permeabilized with acetone at -20°C overnight. This was followed by rehydration in PBS/1% BSA and blocking with 10% goat serum (Sigma Aldrich). Cells were stained with primary antibodies: HB15a (Beckman and Coulter), HB15e (STEMCELL Technologies) or 3C12C anti-CD83 antibodies, followed with goat anti-mouse IgG-AF647 (for HB15a, HB15e) or goat anti-human IgG-AF488 (for 3C12C) (Thermo Fisher Scientific). Nuclei

were stained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, Thermo Fisher Scientific). Cells were visualized using a laser scanning confocal microscope (Leica SP8) and composite images produced using Image J (National Institutes of Health).

In situ hybridization

Epstein-Barr virus encoding small RNA (EBER) in situ hybridization was performed using Bond EBER probe, Bond RNA negative control probe and Bond RNA positive control probe with Bond Anti-fluorescein antibody (Leica Biosystems). Staining was performed on 3µm sections of formalin fixed paraffin embedded tissue. Antigen retrieval, hybridisation and immunohistochemical staining was performed on an automated Leica Bond III (Leica Microsystems). Enzyme 1 (Leica Biosystems) was used for antigen retrieval for 15 minutes. A Bond Polymer Refine Detection system with 3,3'-diaminobenzidine (DAB) was used for chromogenic detection.

Trogocytosis analysis

Venous blood was collected from healthy donors (HD) under approval of SLHD HREC. Human PBMC were isolated by centrifugation on Ficoll-Paque-PLUS (GE Healthcare). T cells were isolated from PBMC using EasySep Human T cell Isolation Kit (STEMCELL Technologies) according to the supplier's instructions. KM-H2 cells were cultured with purified $CD3^+$ T cells from human PBMC for 4 hours at a ratio of 5:1. CD83 expression on T cells was analyzed by flow cytometry using HB15a mAb. For fluorescent imaging, KM-H2 cells were labelled with CellVue Claret Far Red Fluorescent Cell Linker Kits (Sigma-Aldrich) and co-cultured with $CD3^+$ T cells for 4 hours at ratio of 5:1. Cells were then stained with biotinylated mouse anti-human CD3 mAb (BD Bioscience) and Strepdavidin-AF488 (Thermo Fisher Scientific). In some experiments, 0.4µm transwell insert (Corning) were used to separate T cells from KM-H2 cells

during culture. CD83 expression on T cells was analyzed by flow cytometry after 4 hours of culture.

T cell proliferation analysis

Purified T cells of human PBMC were labelled with 5nM Carboxyfluorescein N-hydroxysuccinimidyl ester (CFSE ; Sigma-Aldrich) and stimulated with anti-CD2/CD3/CD28 T cell activation/expansion kit (Miltenyi) in the presence of supernatant from KM-H2 cells for 5 days. Cells were analysed by flow cytometry on a Fortessa (BD Bioscience). The proliferation Index (PI), that is defined as total number of divisions divided by the number of cells that went into division, and Division Index (DI), that is the average number of cell divisions that a cell in the original population has undergone, were analysed with FlowJo V9 (TreeStar).

PCR analysis

RNA was extracted with TRIzol (Life Technologies) and cDNA was transcribed from 100 ng RNA using SuperScript® III First-Strand Synthesis kit and random hexamers primer (Thermo Fisher Scientific) following the manufacturer's protocol. cDNA from the specified immune populations were amplified by PCR using human *CD83* exon 2 forward primer 5'-AGGTTCCCTACACGGTCTCC-3' and exon 5 reverse primer 5'-AAGATACTCTGTAGCCGTGCAAAC-3'. Primers to the *GAPDH* housekeeping gene 5'-ATGGGGAAGGTGAAGGTCGGA-3' (forward) and 5'-AGGGGCCATCCACAGTCTTCTG -3' (reverse) were used as an endogenous contol. Amplified fragments were separated on 2% agarose (Thermo Fisher Scientific) gel.

Stability of 3C12C antibody binding on the surface of HL lines.

KM-H2, L428 and HDLM2 cells were incubated with a saturated concentration of 3C12C (10µg/ml) for 30 minutes on ice. Cells were intensively washed, then cultured at 37°C 5% CO_2

from 0 minutes to 2 hours. Cells were stained with goat anti- human IgG (H+L)-AF488 (Invitrogen) and analyzed for the remaining surface 3C12C antibody level by flow cytometry. The geometric mean fluorescence (MFI) was analyzed using FlowJoV9&10 software (TreeStar). The remaining level of 3C12C on the cell surface was calculated as percentage of MFI at time 0.

Table S1: CD83 staining details in 35 HL patients:

Patient	Diagnosis	Stage	CD83 staining (Strong/Moderate/Weak)	% CD83+ HRS cells	CD30 staining	EBV (EBER)	Treatment response
1	NS	II	W	80	+	-	CR
2	NS	II	S	90	+	-	CR
3	MC	II	W	10	+	-	CR
4	MC	IV	W	70	+	-	CR
5	MC	II	S	80	+	+	CR
6	NS	II	M	70	+	-	CR
7	NS	II	M	70	+	-	NA
8	MC	II	S	20	+	-	CR
9	NS	II	S	<10	+	-	CR
10	NLP	II	S	70	+	-	CR
11	NLP	I	W	30	+	-	CR
12	CHL-U	III	S	90	+	+	CR
13	NS	II	S	80	+	-	CR
14	NS	IV	W	10	+	-	Relapsed -CR
15	MC	IV	W	<10	+	-	CR
16	MC	II	M	60	+	-	CR
17	NS	I	S	90	+	-	CR
18	NS	II	S	10	+	+	CR
19	LR	II	S	90	+	-	CR
20	NLP	I	S	>90	+	-	CR
21	NS	III	S	90	+	-	CR
22	NS	II	S	20	+	-	CR
23	NS	IV	W	<10	+	-	CR
24	NS	IV	W	80	+	+	CR
25	NS	II	W	10	+	-	CR
26	NS	II	M	<10	+	-	CR
27	MC	IV	S	20	+	+	Died
28	NS	III	M	<10	+	+	CR
29	NS	II	S	40	+	-	Died
30	NS	III	W	10	+	-	CR
31	NLP	IV	S	80	+	-	Relapsed
32	NS	II	S	>90	+	-	CR
33	NS	IIa	S	>90	+	-	Relapsed, CR after auto-HCT
34	cHL-U	IVb	M	40	+	+	CR
35	NS	IIIb	W	<10	+	-	CR

Table S2: Characteristics of 6 HL patients for analysis serum sCD83 level

Patient	Age	Diagnosis	Stage	Treatment	PET-CT
1	69	MC	IV	CHIVPP (cy1, 2, 3)	Post-cy2 (CR)
2	42	NS	III	ABVD (cy1, 2, 3, 4)	Post-cy2 (CR)
3	34	NS	II	ABVD (cy1, 2, 3, 4)	Post-cy2 (CR)
4	45	NS	III	ABVD (cy1, 2), BEACOPP (cy3, 4, 5, 6)	cy5 (PR), Post-cy2 (CR)
5	32	NS	II	ABVD (cy1, 2), BEACOPP (cy3, 4)	Post-cy2 (PD), Post-cy4 (PR)
6	36	NS	III	ABVD (cy1, 2, 3, 4, 5)	Post-cy2 (CR)

CR: complete response; PR: partial response; PD: progressive disease.

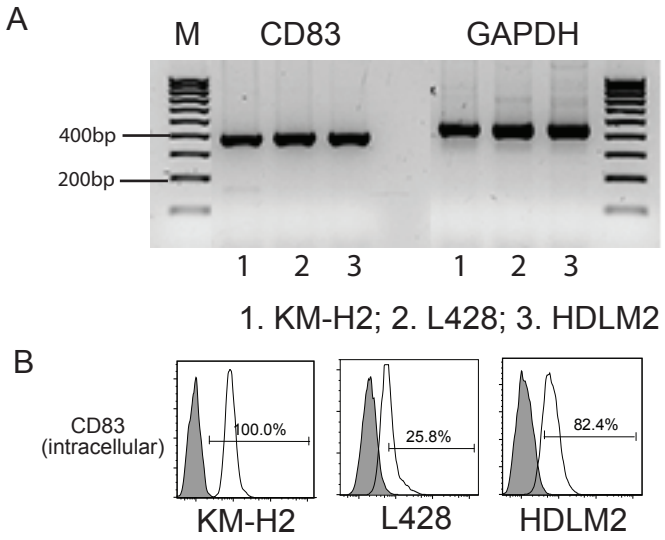

Fig. S1. CD83 transcript and intracellular expression in Hodgkin lymphoma cell lines.

(A) Total RNA was extracted from KM-H2, L428 and HDLM2 cells. cDNA was transcribed from 100 ng RNA. Expression of CD83mRNA and the house keeping gene GAPDH in KM-H2, L428 and HDLM2 were analysed by PCR. Amplified fragments were separated on 2% agarose gel. (B) KM-H2, L428 and HDLM2 cells were fixed and permeabilized, cells were stained with CD83 antibody (HB15e) and analysed for the intracellular CD83 expression by flow cytometry (n=3).

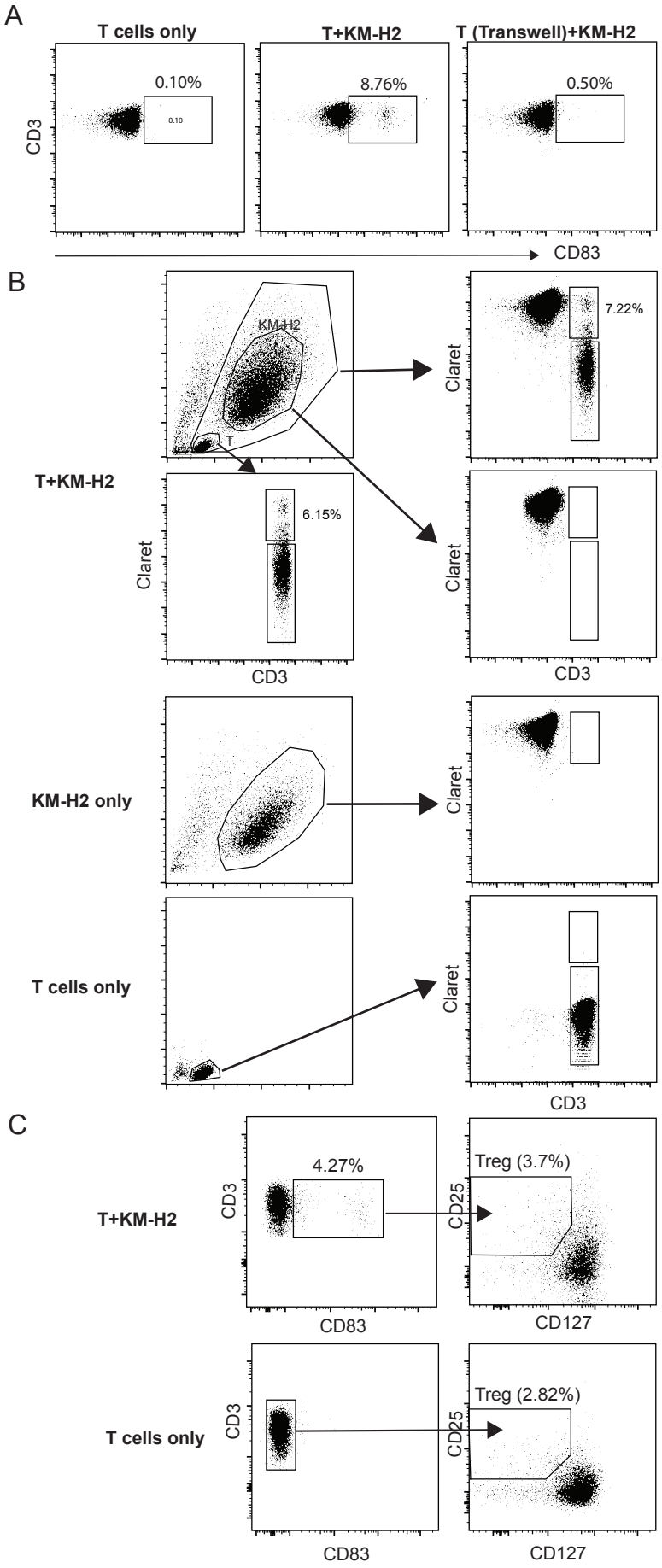

Fig. S2. Characterization the trogocytosis of Hodgkin and Reed-Sternberg (HRS) cells to T cells.

(A) T cells and KM-H2 cells were co-cultured with or without transwell, for 4 hours, CD83 expression on T cells was analyzed by flow cytometry, one of three reprsentative experiments shown. (B) KM-H2 cells were labelled with CellVue Claret and co-cultured with T cells at ratio of 5:1 for 4 hours. Claret expression were analysed on gated KM-H2, T cells or whole population. KM-H2 or T cells only used as control (n=3). (C) The proportion of $CD25^{high}CD127^{low}$ (Treg) in trogocytosed $CD83^{+}$ T cells were analysed, one of three representative experiments was shown.

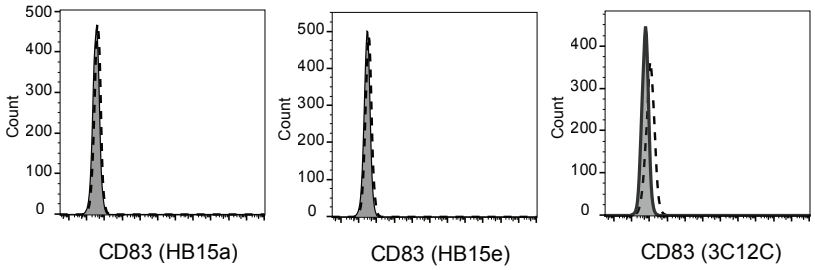

Fig. S3. CD83 expression on HL60 line.

CD83 expression on HL60 was analysed with mouse anti-human CD83 mAb HB15a, HB15e or human anti-human CD83 mAb 3C12C by flow cytometry. Grey filled histograms were isotype controls for CD83 antibodies.

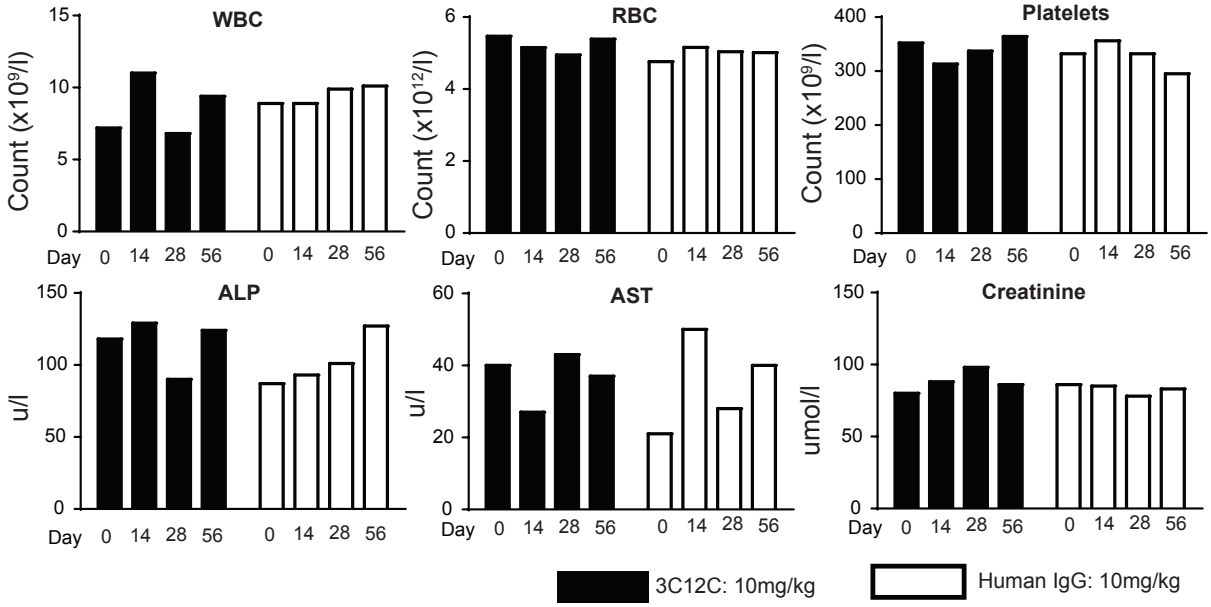

Fig. S4. 3C12C is safe in non-human primates.

Five non-human primates (Baboon) were injected with 3C12C (1, 5, 10, 10 mg/kg, n=4) or human IgG (10mg/kg, n=1) at day 0, 7, 14 and 21. Blood and serum samples were collected for blood cell counts (red cells, white cells and platelets), liver (ALP, AST level) and kidney function (Creatinine level) analysis. Data from the two animals receiving 10mg/kg of 3C12C or human IgG are shown.