Cytomegalovirus-specific T cells restricted for shared and donor human leukocyte antigens differentially impact on cytomegalovirus reactivation risk after allogeneic hematopoietic stem cell transplantation

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Supplementary Material for

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This file includes:

- additional information regarding materials and methods

- supplementary figures

- supplementary figure legends

- supplementary tables

SUPPLEMENTARY MATERIALS AND METHODS

HSCT procedures

The majority of patients received a conditioning regimen based on Treosulfan (14 g/m^2/day) on days -6 to -4 and Fludarabine (30 mg/m^2/day) on days -6 to -2, considered a reduced-toxicity conditioning regimen (RTC)[1-3]. The majority of patients received a myeloablative conditioning (MAC) regimen, intensified with the addition of a second alkylating agent or total body irradiation (TBI). Graft source was predominantly (91%) unmanipulated PBSCs. The majority of patients received PT-Cy (50 mg/kg/day), on days three and four, and Sirolimus. PT-Cy was omitted only in patients receiving graft from cord blood (CB), according to Institutional guidelines. Mycophenolate mofetil (MMF) was added if the donor was a matched unrelated donor (MUD) or a mis-matched related donor (MMRD).

Definitions

Patients were stratified by disease and status at the time of transplantation according to the refined disease risk index (DRI)[4] validated by Armand et al. Comorbidities were evaluated according to the age-adjusted Hematopoietic cell transplantation-specific comorbidity index (HCT-CI)[5]. HLA compatibility among donor-recipient pairs was assessed by five loci molecular typing (HLA-A, -B, -C, -DRB1, -DQB1) at the allelic level. For MUD, 10/10 and 9/10 HLA-matched donors were included. Acute GvHD was defined and scored following the Center for International Blood and Marrow Transplant Research *(*CIBMTR) Severity Index and the Glucksberg criteria[6-8]. Chronic GvHD was defined and scored according to the NIH consensus criteria[9]. Neutrophil engraftment was defined as achievement of an absolute neutrophil count >500 cells/mm^3 for three consecutive days, and platelet engraftment was defined as platelet counts ≥ 20×10^9/L in the absence of transfusions during the preceding seven days.

Absolute counts of immune cell subsets

IR was evaluated by flow cytometry at -7, +30, +60, +90, +120, +180 and +365 days from HSCT on whole blood samples, using a lyse-no-wash technique and a panel of directly conjugated antibodies

specific for CD45, CD3, CD4, CD8, CD19, CD16, CD56, CD25 and CD127, according to the International Society for Cell Therapy (ISCT) immunological gating protocol[10]. The single platform method was used to determine absolute counts, employing fluorospheres (Flow-Count™, Beckman Coulter). Cells were acquired with a Navios cytometer (Beckman Coulter).

Detailed procedure for Dextramer staining on fresh blood

Within 24 hours from bleeding, 200 μl of whole blood were incubated separately with 10 μl of each Dextramer PE matching the HLA type of the patient or donor. A separate tube with a negative Dextramer control PE was always performed. For HLA-A*01:01 and HLA-A*03:01 Dextramer reagents, the incubation was performed together with CD8 antibody to avoid background signal. After 10 minutes at room temperature, 10 μl each of anti-CD8 FITC and anti-CD3 PerCP were added and incubated at 4°C for 30 minutes. A separate Trucount tube (Becton Dickinson, BD) with 100 μl of whole blood and 10 μl each of anti-CD4 PE, anti-CD8 FITC and anti-CD3 PerCP (BD) was prepared. For apheresis product the same procedure was used with 50 μl (Dextramer and negative control tubes) and 25 μl (Trucount tube) of fresh sample. After incubation, red blood cells were lysed with FACS Lysing Solution (BD) for 10 minutes at room temperature, then cells were washed with PBS and fixed with 300 μl of Fixing solution (Biolegend). Acquisition was performed within 6 hours from sampling with a Navios (Beckman Coulter) cytometer. At least 15'000 events in the $CD3^+CD8^+$ region and 10'000 count beads were acquired. Data were analyzed with Kaluza v2 software (Beckman Coulter).

Absolute counts of Dextramer-positive (Dex^+) cells were calculated with the following formulas:

$$\text{Absolute count } CD3^+CD8^+ \text{ cells} = \frac{CD3^+CD8^+ \text{events}}{\text{bead events}} \times \frac{\text{bead events per test}}{100\ \mu l}$$

$$\text{Absolute count } CMV^+ \text{ T cells} = \frac{(\text{absolute count } CD3^+CD8^+ \text{cells}) \times (\%\ Dex^+ \text{ T cells})}{100}$$

The manufacturer reported LOD values for Dextramer CMV kit that range from 0.53 cells/μl for HLA-B*0702 to 2.41 cells/μl for HLA-B*0801. The analytical specificity was also calculated on 16 CMV-seronegative subjects for the 7 alleles covered by the kit and results are within 0.00-0.03 cells/μl. Given the low number of subjects (n=5) and alleles (n=4) tested to define the LOD and, most importantly, the low numbers of $CD3^+$ T cells/μl normally observed in the first months after HSCT, we considered any CMV-specific quantification informative, in samples with at least 15'000 $CD3^+CD8^+$ cells acquired by the cytometer.

Flow cytometry analysis on frozen PBMC

Patients' PBMC were thawed and kept in colture overnight in IMDM (Lonza) supplemented with Glutamine 1%, Penicillin/Streptomycin 1% and 10% human serum (HS) in presence of low doses of recombinant human IL-2 (rhIL-2, 20 UI/ml; Novartis). Next, were stained with Fixable Viability Dye 620 (BD) 1:1'000 for 10 minutes at room temperature, washed and incubated with the specific Dextramer reagents matching the HLA type of patients and donors, and with the antibodies included in Supplementary Table 1. A separate tube with FITC, PE and APC negative Dextramer controls was always performed. Cells were incubated first with FITC, PE and APC Dextramer reagents for 10 minutes at room temperature and washed after each Dextramer staining to avoid unspecific signal, then with antibodies for 20 minutes at room temperature. All the washing steps were performed with autoMacs® Rinsing Solution (Miltenyi) supplemented with 0.5% Bovine Serum Albumin (Miltenyi). Cells were acquired within 6 hours with a FACSymphony cytometer (BD). Data were analyzed by FlowJo software version 10 (BD). Absolute counts of CMV-specific $CD8^+$ T cells in frozen samples were calculated based on percentage of $CD3^+CD8^+Dextramer^+$ lymphocytes and on the absolute counts of $CD8^+$ T cells in corresponding whole fresh blood.

High-dimensional analysis of flow cytometry data

After compensation optimization and doublets and dead cells exclusion, $CD3^+CD8^+Dextramer^+$ events were exported to a new .fcs file. CytoChain[11] was used for subsequent data handling. Data

were then optimized for acquisition stability and all the channels' fluorescence intensities transformed by the arcSin function (cofactor 150). The optimized flowset was concatenated and the total expression matrix with all the amount of events was processed using the Flow-Self Organized Map (FlowSOM) package, identifying 100 clusters. These were then collapsed into 25 metaclusters by the ConsensusClusterPlus package and distributed on a two-dimension map by the dimensionality reduction algorithm t-distributed Stochastic Neighbor Embedding (t-SNE, perplexity 30). The concatenated flowset was also analysed by FlowJo version 10 (BD) to validate cytoChain results. After a first t-SNE mapping, LAG-3 and CD25 markers showed a low and almost uniform expression, with very little differences among the clusters. Therefore, these markers were removed from mapping and clustering analysis to exclude a potential confounding effect.

IFN-γ ELISpot

Patients' PBMC were thawed and kept in colture as for flow cytometry analysis. Next, PBMC were washed to eliminate rhIL-2, plated at the final concentration of 10^5 cells per well and stimulated overnight at 37°C with three different CMV antigen sources: a glycine extract of a human fibroblast-cell line infected with the CMV strain AD169.2 (CMV-antigen)[12] and two libraries of overlapping peptides covering pp65 (0.25 μg/ml) and IE-1 (1 μg/ml) viral proteins (JPT). Polyclonal stimulation was provided by both Phytohemagglutinin (PHA, Lophius) and a peptide pool of immunodominant epitopes (CEF pool, JPT). Next, the substrate was added according to manufacturer instructions to detects specific spot forming cells. Medium alone and unstimulated PBMC used as negative controls were subtracted. Given the high variability of $CD3^+$ T-cell counts after transplantation, ELISpot results were normalized for the CD3-absolute content of each sample.

CMV QuantiFERON

QuantiFERON-CMV (Qiagen) specialised collection tubes include a tube coated with peptides simulating $CD8^+$ T cells with specific epitopes of CMV proteins, along with negative and positive control tubes. No more than 2 hours after blood collection, tubes were re-mixed and blood was

incubated at 37°C for 16-24 hours. Tubes were then centrifuged and plasma samples were collected and frozen to -20 °C for maximum 7 days before analysis. A fully automated plate enzyme-linked immunosorbent assay (ELISA) processing system was then used (Automatic ELISA workstation DSX® - Dynex Technologies, Inc.) to measure the amount of IFN-γ present in plasma from each of the three tubes (CMV-antigen, positive and negative controls), according to manufacturer's instructions. To discriminate between reactive and non-reactive samples we used the recommended clinical test threshold in the CMV antigen tube (0.2 IU/ml).

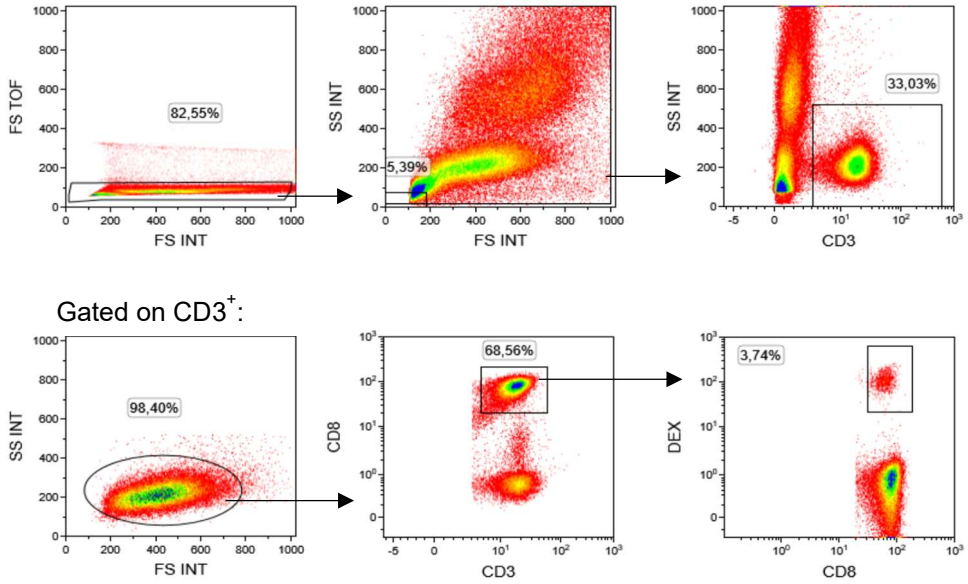
Selection criteria for patients analyzed by QuantiFERON-CMV and IFN-γ ELISpot

QuantiFERON-CMV was performed on 47 whole blood samples from 16 patients based on the availability of the diagnostic kit. Frozen PBMC collected at the same timepoints were also analysed by IFN-γ ELISpot for CMV. In addition, IFN-γ ELISpot for CMV was performed on frozen PBMC harvested from HLA-mismatched allo-HSCT, resulting in a total of 113 samples analysed from 34 patients.

SUPPLEMENTARY FIGURES

Supplementary Figure 1

A



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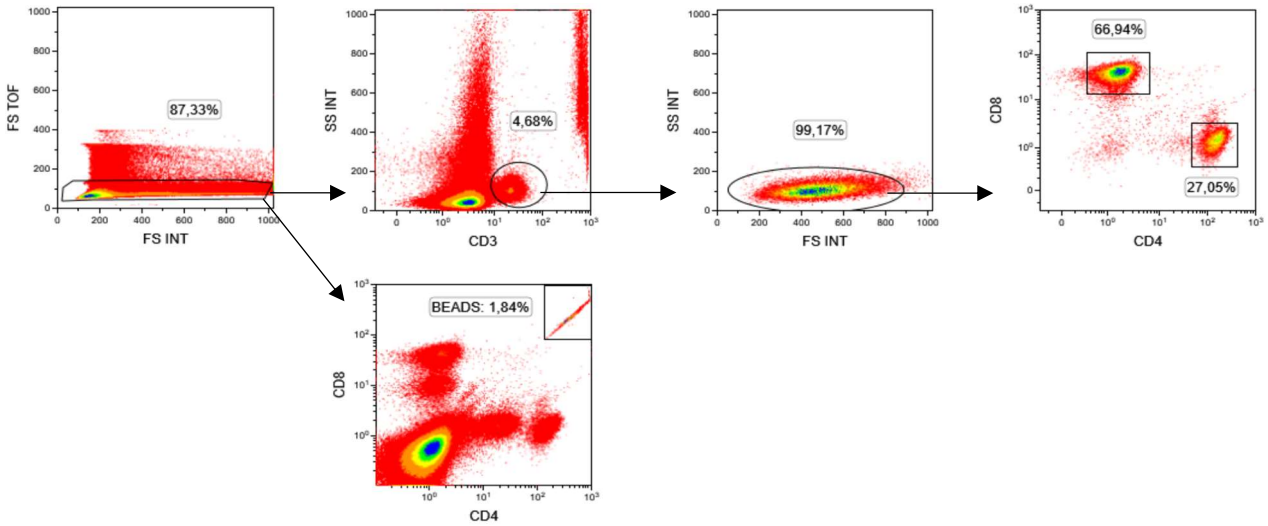
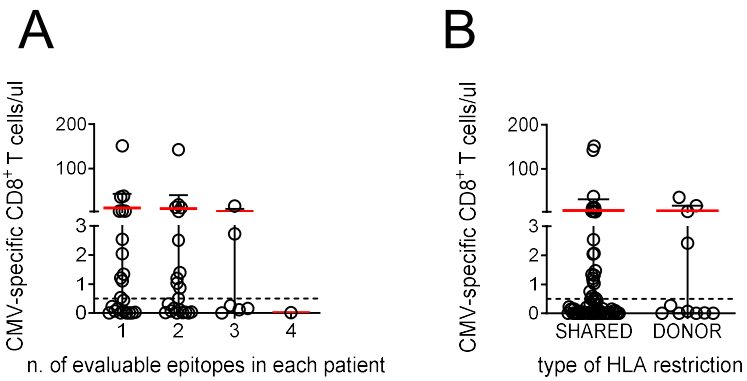
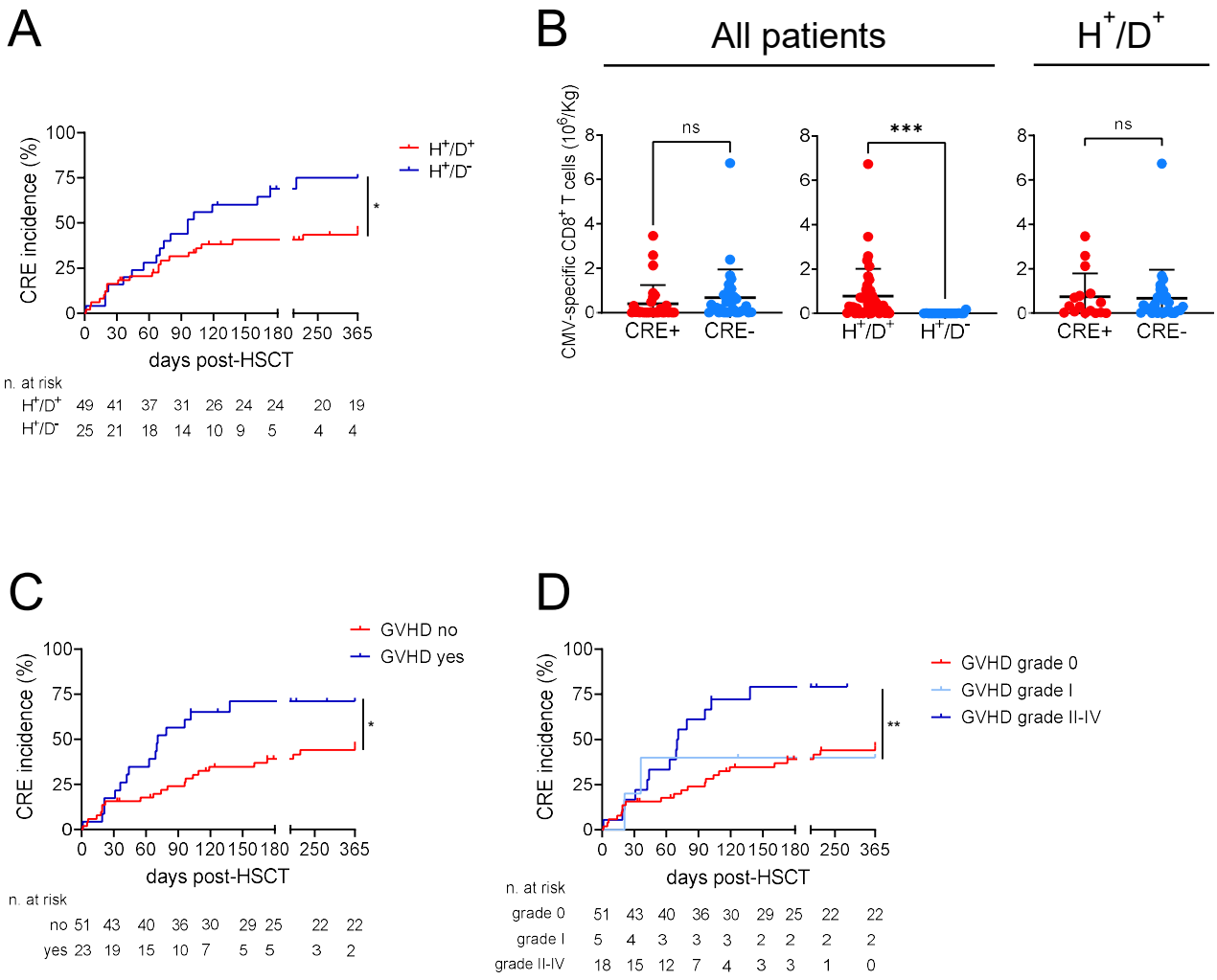


Supplementary Figure 2



Supplementary Figure 3



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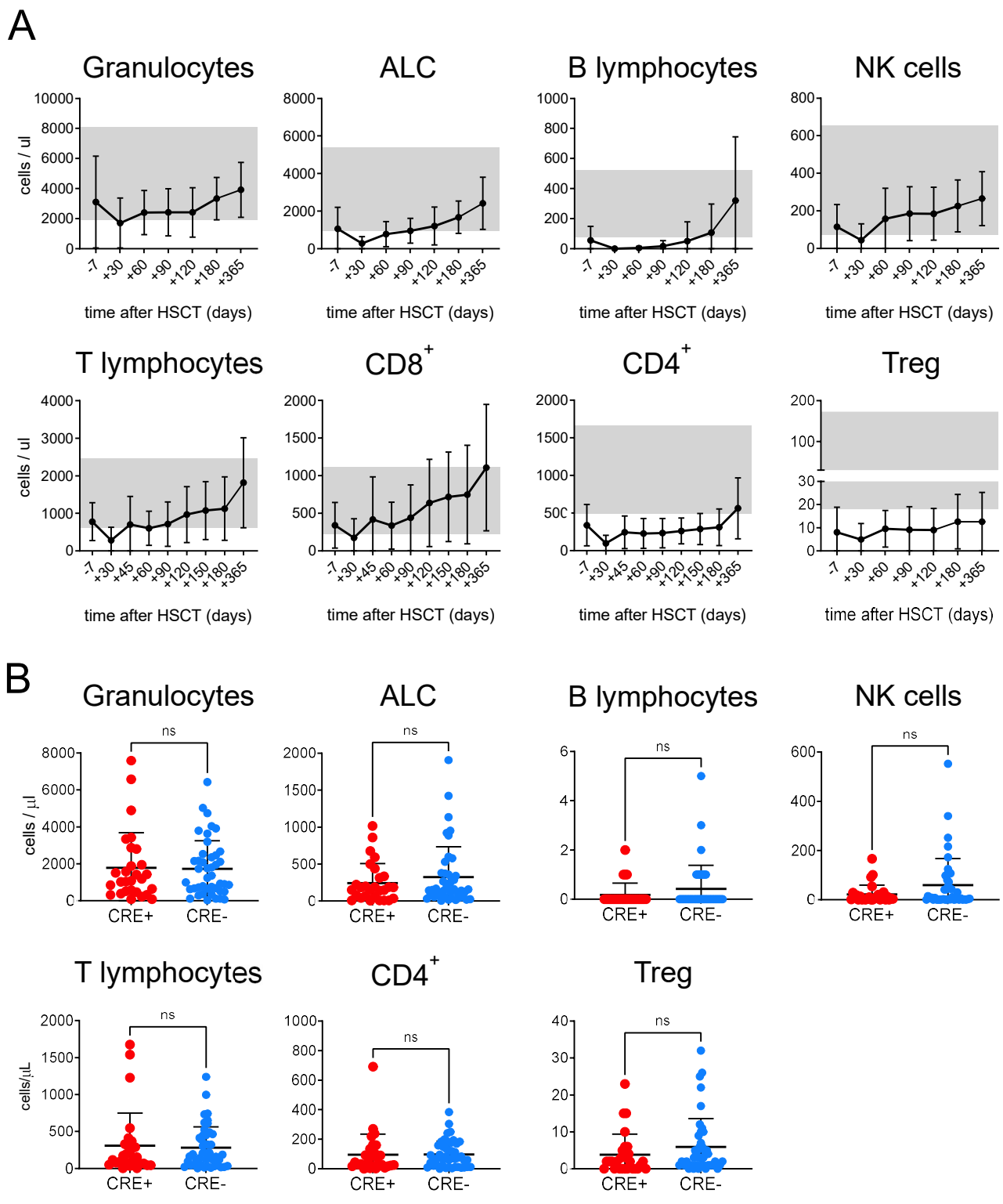

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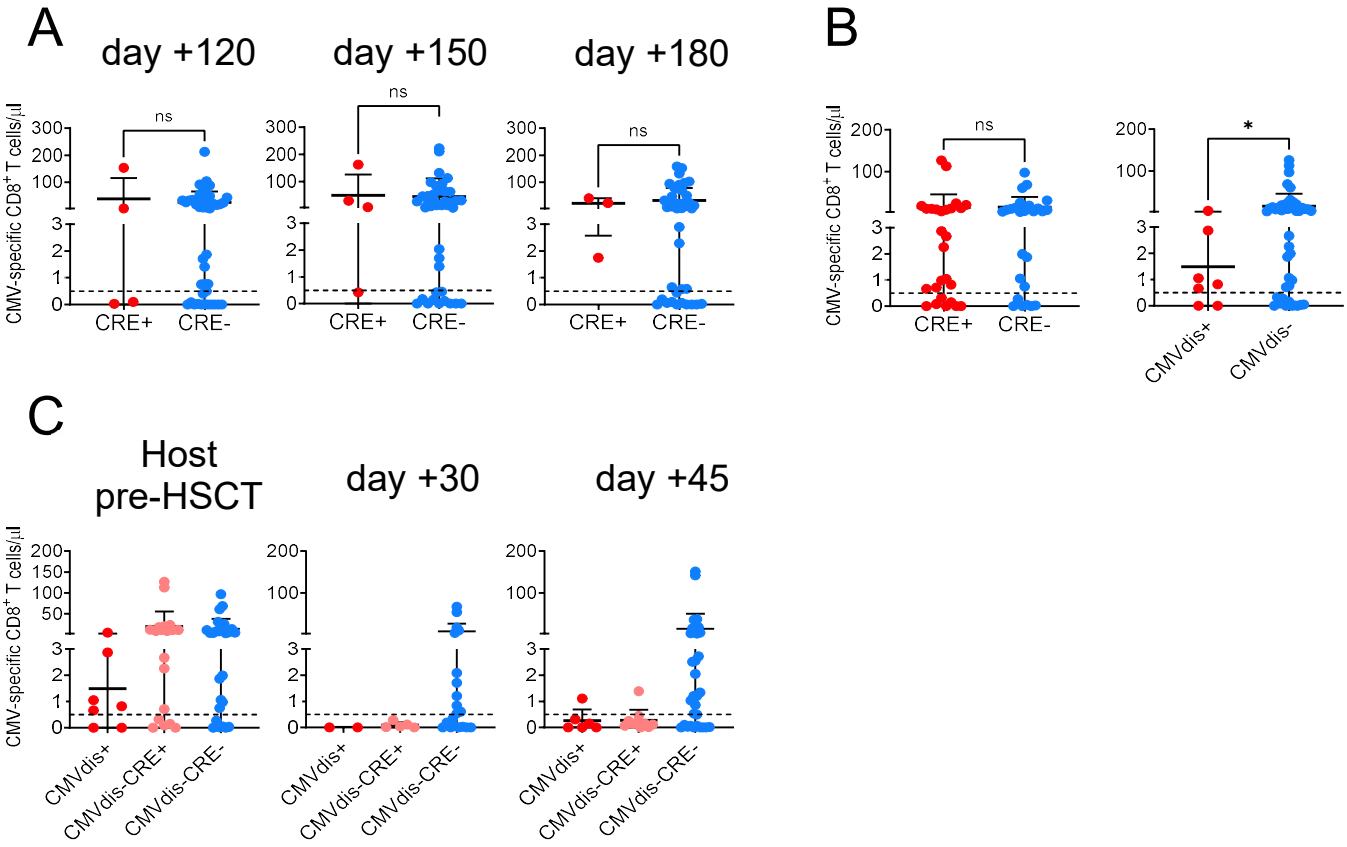
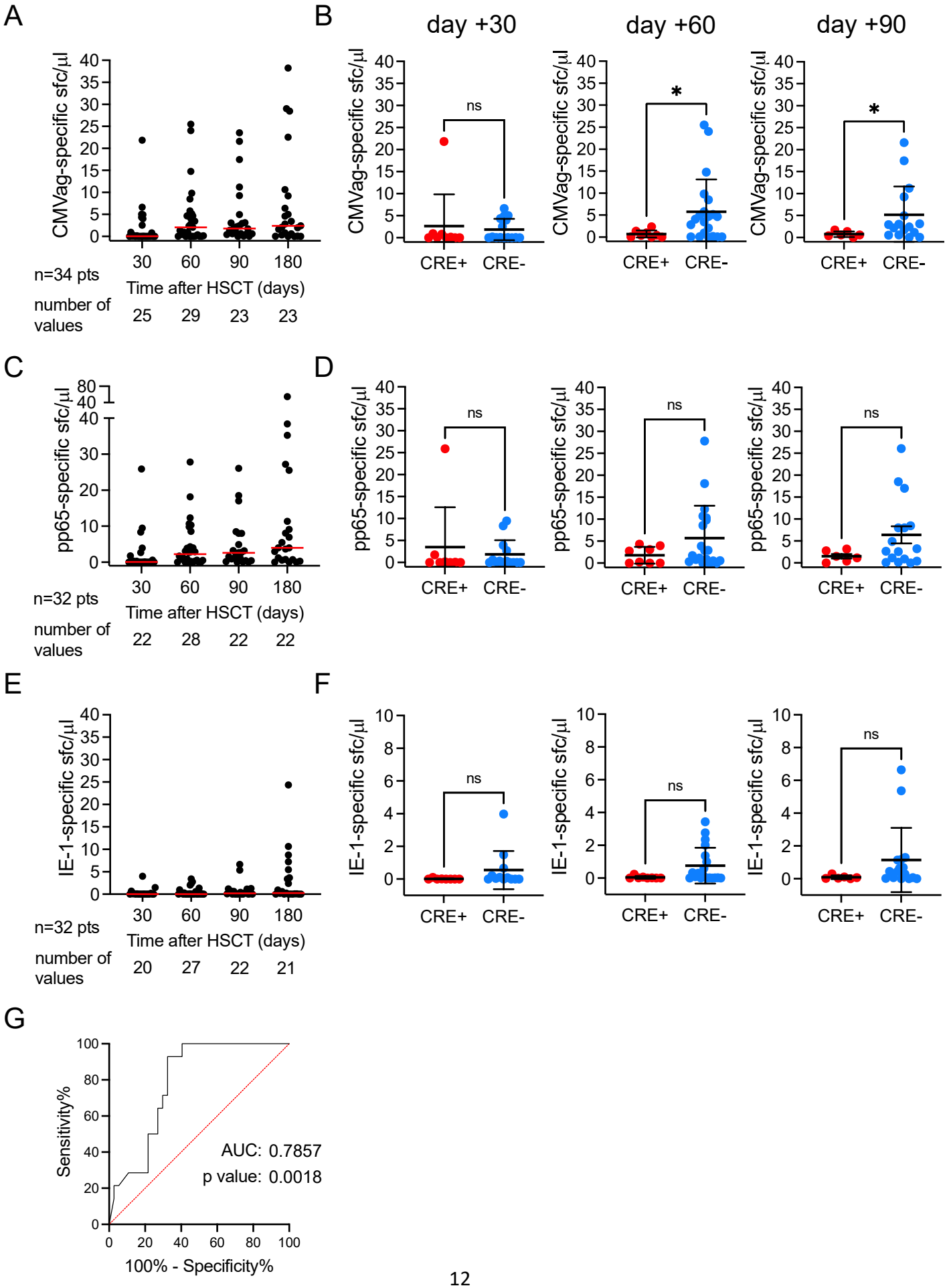
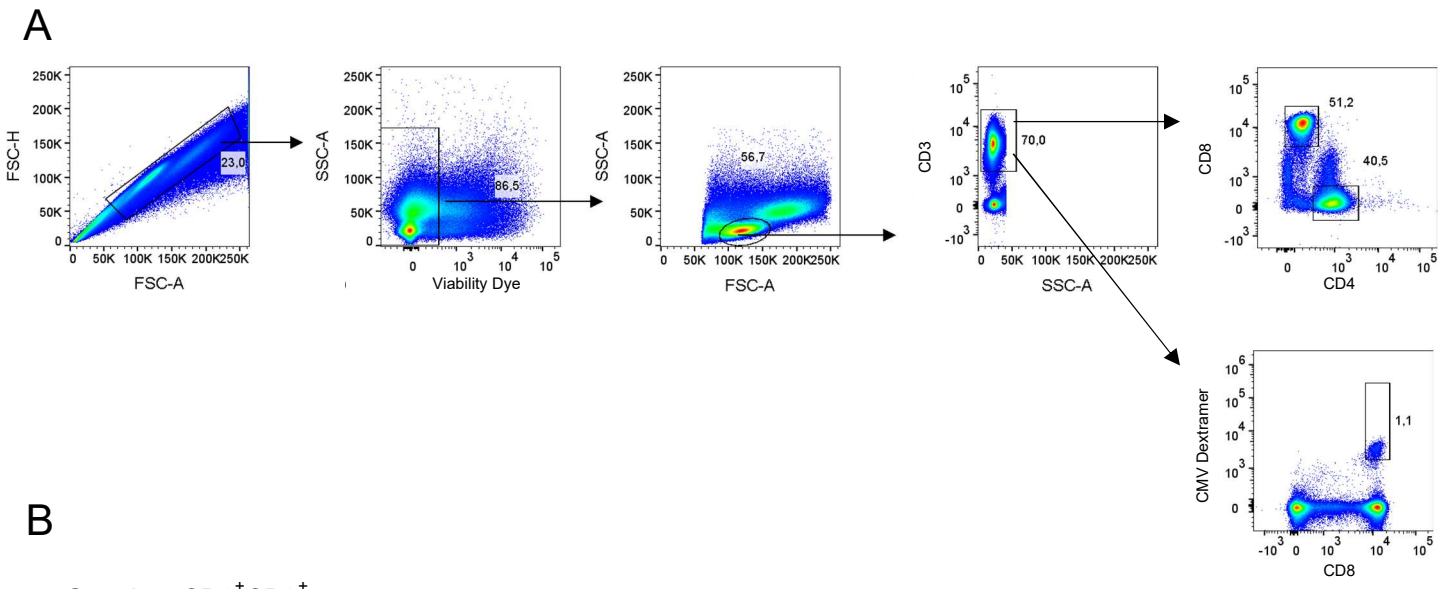
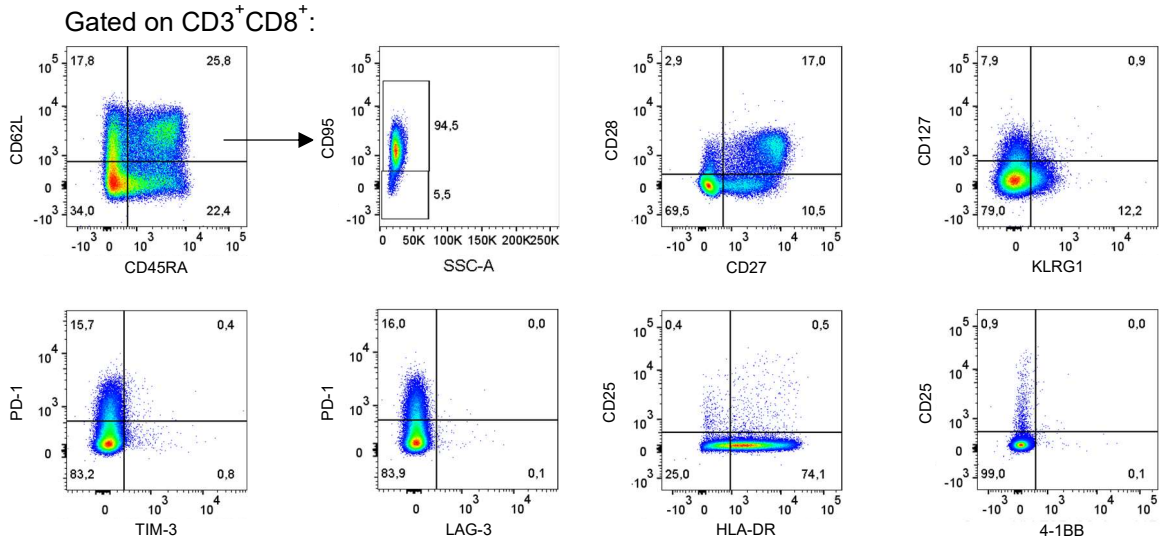


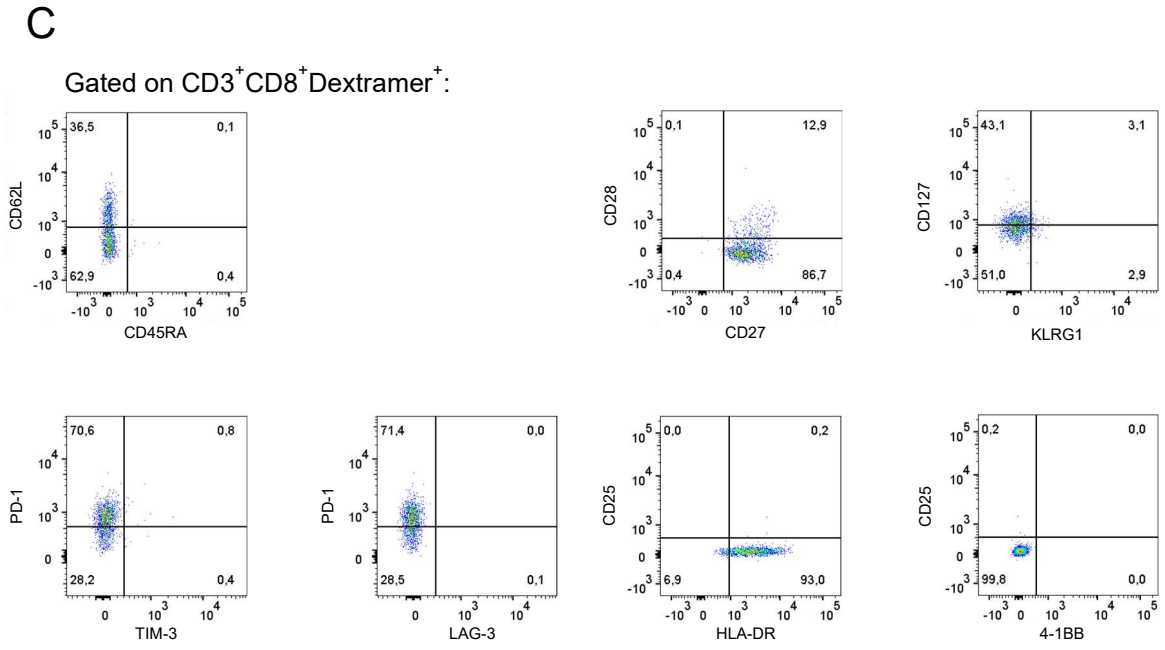
Supplementary Figure 6



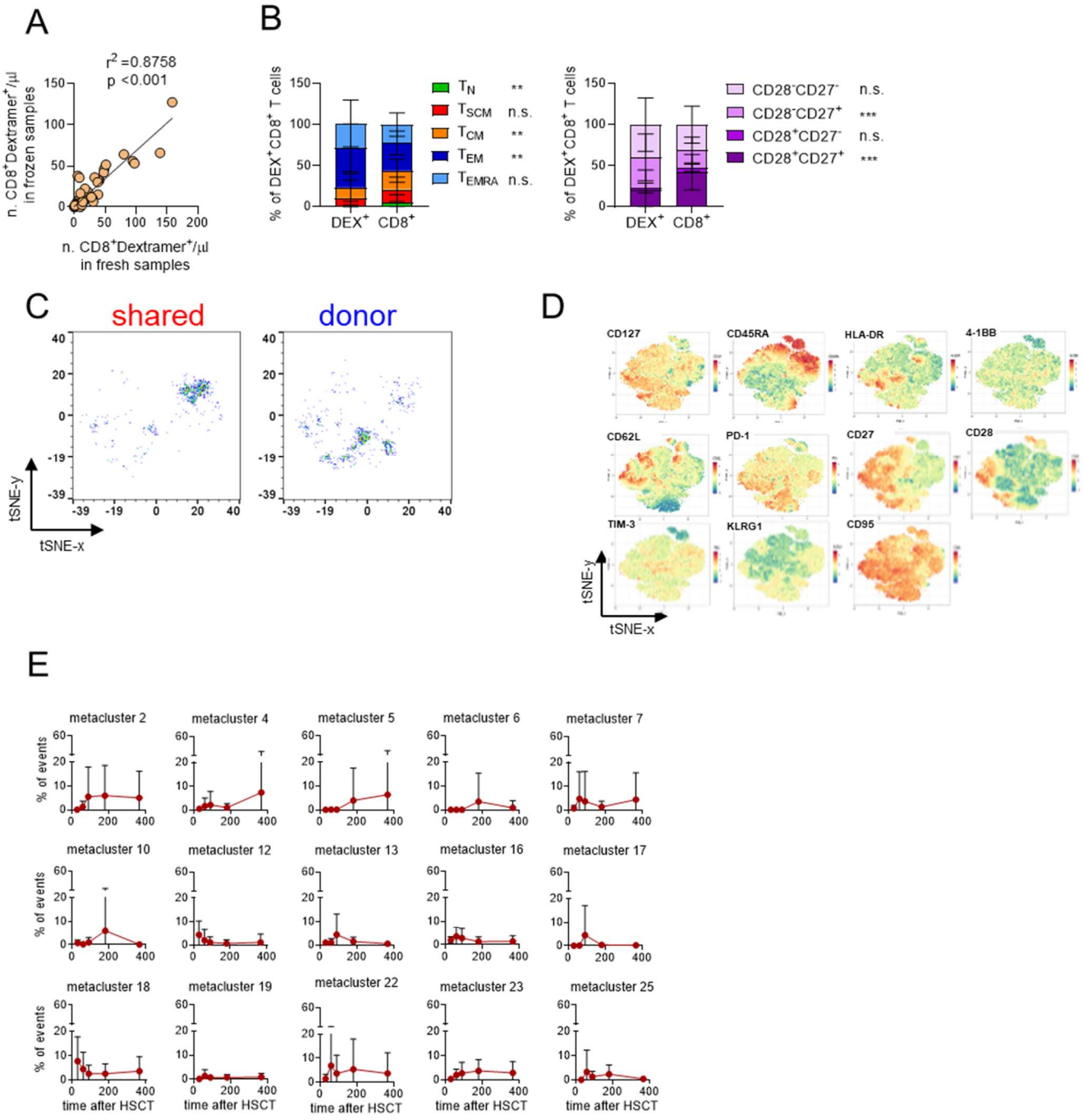
Supplementary Figure 7







Supplementary Figure 8



SUPPLEMENTARY FIGURES LEGENDS

Supplementary Figure 1. Representative gating strategy for Dextramer CMV Kit. The gating strategies used to identify CMV-specific $CD8^+$ T cells (**A**) and to quantify $CD4^+$ and $CD8^+$ T lymphocytes by Trucount beads (**B**) in fresh blood samples are shown.

Supplementary Figure 2. CMV-specific $CD8^+$ T cells counts in patients evaluated with different numbers and types of HLA alleles. A, Absolute counts of CMV-specific $CD8^+$ T cells at +45 days after HSCT according to the number of epitopes evaluable in each patient. Each point indicates the sum of all the counts for each patient. **B,** Absolute counts of CMV-specific $CD8^+$ T cells at +45 days after HSCT according to the type of restriction. Analyses were performed by Kruskal-Wallis (A) or Mann-Whitney (B) test. No significant differences were found. Lines, mean±SD. Dotted line indicates the threshold of 0.5 CMV-specific T cells/μl.

Supplementary Figure 3. Clinical variables associated with CMV CRE and impact of CMV-specific $CD8^+$ T cell counts in the donors' apheresis. A, incidence of CRE according to donor's serostatus. **B,** absolute numbers of CMV-specific $CD8^+$ T lymphocytes in the donors' apheresis in patients experiencing or not subsequent CRE (left panel), according to host/donor serostatus (middle panel) or only in patients receiving seropositive grafts experiencing or not subsequent CRE (right panel). The number of CMV-specific $CD8^+$ T cells infused within the graft was calculated based on the counts of total T cells infused (absolute counts/kg). Lines, mean±SD. Analysis by Mann-Whitney test: ns, not significant; *** $p<0.001$. **C** and **D,** the incidence of CMV CRE is shown according to the occurrence of acute GvHD of any grade (C) or requiring (grade II-IV) or not (grade I) steroids (D). Analysis in A, C and D by Log-rank test: * $p<0.05$; ** $p<0.01$.

Supplementary Figure 4. Reconstitution of several immune subsets do not predict CRE. A, kinetics of reconstitution after HSCT of the indicated immune populations expressed as absolute

numbers/μl. Grey areas indicate the normal values. **B,** absolute numbers of Granulocytes, Absolute Lymphocyte Counts (ALC), B lymphocytes, NK cells, T lymphocytes, $CD8^+$, $CD4^+$, regulatory T lymphocytes (Treg) at 30 days after HSCT in patients experiencing or not subsequent CRE. Lines, mean±SD. Analyses in B were performed by Mann-Whitney test: ns, not significant.

Supplementary Figure 5. Association of CMV-specific T lymphocytes at different timepoints with subsequent CRE or CMV-disease. A, absolute numbers of CMV-specific $CD8^+$ T lymphocytes at the indicated timepoints after HSCT in patients experiencing or not subsequent CRE. **B**, absolute counts of CMV-specific $CD8^+$ T lymphocytes in recipient's pre-transplant blood compared between patients experiencing or not CMV clinically relevant events (CRE+ or CRE-, left panel) or CMV-disease (CMVdis+ or CMVdis-, right panel) after HSCT. **C,** absolute numbers of CMV-specific $CD8^+$ T lymphocytes at the indicated timepoints in patients segregated for experiencing subsequent CMV-disease (CMVdis+), no CMV-disease with CRE (CMVdis-CRE+) or no CMV-disease without CRE (CMVdis-CRE-). Analyses in **A, B** were performed by Mann-Whitney test. Analyses in **C** were performed by Kruskal-Wallis test. *, $p<0.05$; ns, not significant. Lines, mean±SD. Dotted line indicates the level of 0.5 CMV-specific T cells/μl.

Supplementary Figure 6. Kinetics of CMV-specific T-cell reconstitution following allo-HSCT measured by IFN-γ ELISpot. Functional response of CMV-specific T cells measured at the indicated timepoints by IFN-γ ELISpot after stimulation with CMV antigen (CMVag; **A, B**) and pp65 (**C, D**) or IE-1 (**E, F**) peptide pools. Results are expressed as spot forming cells (sfc)/μl. Panels A, C and E show the kinetics of CMV-specific responses; red lines indicate mean values. Panels B, D and F show the absolute numbers of CMV-specific T lymphocytes at the indicated timepoints after HSCT in patients experiencing (CRE+) or not (CRE-) subsequent CRE. **G,** ROC analysis for the identification of a protective threshold of 1.75 sfc/μl at 60 and 90 days after HSCT (AUC, area under

the curve). Analysis in B, D and F were performed by Mann-Whitney test: *, $p<0.05$; ns, not significant. Lines, mean±SD.

Supplementary Figure 7. Representative gating strategy for $CD8^+Dextramer^+$ T lymphocytes in frozen samples. A, representative gating strategy for the identification of total $CD4^+$, total $CD8^+$ and $CD8^+Dextramer^+$ T cells. **B** and **C**, representative dot plots showing memory, differentiation, activation and exhaustion phenotype of total (B) and CMV-specific (C) $CD8^+$ T lymphocytes.

Supplementary Figure 8. CMV-specific T cells quantification and extensive characterization in frozen samples. A, linear regression analysis showing the correlation between the number of $CD8^+Dextramer^+$ T lymphocytes in fresh and in frozen samples. **B,** manual gating analysis for the distribution of differentiation T-cell subsets in $Dextramer^+$ or bulk $CD8^+$ T cells. Differentiation T-cell subsets were defined according to the expression profile of CD45RA, CD62L and CD95 markers (left panel) or to the combination of CD28 and CD27 (right panel). T_N, $CD45RA^+CD62L^+CD95^-$; T_{SCM}, $CD45RA^+CD62L^+CD95^+$; T_{CM}, $CD45RA^-CD62L^+$; T_{EM}, $CD45RA^-CD62L^-$; T_{EMRA}, $CD45RA^+CD62L^-$. Analysis by two-way Anova: **, $p<0.01$; ***, $p<0.001$; n.s., not significant. **C,** tSNE maps of CMV-specific T cells restricted for a S or D-specific HLA analysed in the same patient at +60 days after HSCT. **D,** scaled fluorescence distribution of the indicated markers in the tSNE map (markers map). **E,** kinetics of common metaclusters of CMV-specific T cells expressed as the percentage of events in each metacluster among total $CD8^+Dextramer^+$ T lymphocytes. Among the CMV-specific T cell populations evaluated, three were obtained at 30 days after HSCT, 11 at +60, 16 at +90, 12 at +180 and 13 at +365.

SUPPLEMENTARY TABLES

Supplementary Table 1. Flow cytometry Dextramer reagents and antibodies.

Specificity	Fluorochrome	Clone	Company	Catalogue number	Allele frequency (%)[13]	Average frequency of Caucasian individuals having the allele (%)[14]
Dextramer HLA-A*01:01 VTEHDTLLY	PE	n.a.	Immudex	Dextramer CMV Kit	11.528	27.7
Dextramer HLA-A*02:01 NLVPMVATV	PE	n.a.	Immudex	Dextramer CMV Kit	22.820	42.7
Dextramer HLA-A*03:01 KLGGALQAK	PE	n.a.	Immudex	Dextramer CMV Kit	10.628	23.0
Dextramer HLA-A*24:02 QYDPVAALF	PE	n.a.	Immudex	Dextramer CMV Kit	12.286	18.6
Dextramer HLA-B*07:02 TPRVTGGGAM	PE	n.a.	Immudex	Dextramer CMV Kit	5.239	19.0
Dextramer HLA-B*07:02 RPHERNGFTVL	PE	n.a.	Immudex	Dextramer CMV Kit	5.239	19.0
Dextramer HLA-B*08:01 ELRRKMMYM	PE	n.a.	Immudex	Dextramer CMV Kit	5.760	18.4
Dextramer HLA-B*35:01 IPSINVHHY	PE	n.a.	Immudex	Dextramer CMV Kit	8.004	12.3
Dextramer HLA-A*01:01 VTEHDTLLY	FITC/APC	n.a.	Immudex	CA2131	11.528	27.7
Dextramer HLA-A*02:01 NLVPMVATV	APC	n.a.	Immudex	CB2132	22.820	42.7
Dextramer HLA-A*02:01 VLEETSVML	FITC	n.a.	Immudex	WB2658	22.820	42.7
Dextramer HLA-A*03:01 KLGGALQAK	FITC/APC	n.a.	Immudex	CC2197	10.628	23.0
Dextramer HLA-A*24:02 AYAQKIFKI	FITC/APC	n.a.	Immudex	WF2196	12.286	18.6
Dextramer HLA-A*24:02 QYDPVAALF	FITC	n.a.	Immudex	CF2133	12.286	18.6
Dextramer HLA-B*07:02 TPRVTGGGAM	APC	n.a.	Immudex	CH2136	5.239	19.0
Dextramer HLA-B*08:01 QIKVRVDMV	APC	n.a.	Immudex	WI2659	5.760	18.4
Dextramer HLA-B*35:01 LPLNVGLPIIGVM	FITC	n.a.	Immudex	WK3479	8.004	12.3
CD3	BUV661	UCHT1	BD	612964		
CD4	BUV496	SK3	BD	564651		
CD8	APC-H7	SK1	BD	560179		
CD25	BV421	M-A251	BD	562442		
CD27	BV605	L128	BD	562655		
CD28	BV650	CD28.2	BD	740593		
CD45RA	AlexaFluor700	HI100	BD	560673		
CD62L	BUV805	DREG-56	BD	742024		
CD95	PE-Cy7	DX2	BD	561636		
CD127	BB700	HIL-7RM21	BD	566398		
CD137 (4-1BB)	BUV737	4B4-1	BD	741861		
CD223 (LAG-3)	BUV395	T47-530	BD	745640		
CD279 (PD-1)	BV480	EH12.1	BD	566112		
CD366 (TIM-3)	BV711	344823	BD	747959		
HLA-DR	BUV563	G46-6	BD	748340		
KLRG1	BV786	2F1/KLRG1	Biolegend	138429		

Abbreviations: n.a., not applicable; BD, Becton Dickinson.

Supplementary Table 2. CRE timepoint and CMV-specific T cells at +30 and +45 days after HSCT.

	days from HSCT				absolute counts of CMV+ T cells	
Patient#	1st CRE	2nd CRE	3rd CRE	CMV disease	+30 days post-HSCT	+45 days post-HSCT
2	14	NO			0,03	0,00
3	NO				n.e	0,07
5	70	NO			0,11	0,16
6	NO				67,15	142,32
8	69	NO			n.e	0,00
9	19	21	NO		0,00	n.e
12	5	NO			1,71	3,64
14	0	NO			53,99	151,26
18	45	54	73	69	0,00	0,00
19	119	NO		119	n.e	0,31
20	80	NO			n.e	0,25
22	NO				n.e	37,40
23	71	NO			0,02	0,07
25	161	236	NO		n.e	0,15
26	NO				0,00	0,00
27	21	NO			0,84	1,20
28	79	NO			0,28	0,11
29	NO				0,35	1,21
32	6	NO			17,46	35,87
34	NO				0,07	2,51
35	NO				n.e	4,80
36	NO				n.e	1,34
37	67	NO			n.e	0,14
38	NO				n.e	2,05
39	22	NO		22	0,00	n.e
41	NO				0,54	2,55
42	NO				n.e	2,72
43	NO				0,01	n.e
45	102	NO			0,00	1,39
46	97	286	NO		0,04	0,02
47	NO				0,61	0,53
49	69	NO		69	n.e	0,00
50	NO				n.e	0,10
51	19	NO		19	n.e	0,01
53	NO				n.e	0,03
54	NO				n.e	0,86
58	104	NO		104	n.e	1,11
59	NO				n.e	0,02
63	NO				0,19	1,03
65	NO				n.e	0,00
66	NO				n.e	3,25
71	NO				10,19	14,93
72	NO				1,20	17,98
75	208	NO		210	n.e	0,01
77	52	NO			0,00	0,50
78	NO				n.e	0,00
80	NO				2,09	15,32
81	NO				n.e	12,60
82	45	NO		58	0,00	0,15
83	NO				n.e	0,07
84	138	191	NO		n.e	0,44

Red and blue cells indicate, respectively, patients experiencing (CRE+, red) or not (CRE-, blue) CMV clinically relevant events after the evaluated time-point post-HSCT. n.e., not evaluable.

Supplementary Table 3. Putative protective thresholds of CMV-specific $CD8^+$ T cells/μl

	Sensitivity %	95% CI	Specificity %	95% CI	Likelihood ratio
< 0.2050	64,71	41,30% to 82,69%	67,74	50,14% to 81,43%	2,006
< 0.2800	70,59	46,87% to 86,72%	67,74	50,14% to 81,43%	2,188
< 0.3750	76,47	52,74% to 90,44%	67,74	50,14% to 81,43%	2,371
< 0.4700	82,35	58,97% to 93,81%	67,74	50,14% to 81,43%	2,553
< 0.5150	**88,24**	**65,66% to 97,91%**	**67,74**	**50,14% to 81,43%**	**2,735**
< 0.6950	88,24	65,66% to 97,91%	64,52	46,95% to 78,88%	2,487
< 0.9450	88,24	65,66% to 97,91%	61,29	43,82% to 76,27%	2,279
< 1.070	88,24	65,66% to 97,91%	58,06	40,77% to 73,58%	2,104
< 1.155	94,12	73,02% to 99,70%	58,06	40,77% to 73,58%	2,244

ROC analysis for the absolute amount of $CD8^+Dextramer^+$ T cells at +45 days after HSCT correlated with protection against subsequent CRE. Values between 0.2 and 1.1 are shown.

Supplementary Table 4. Correlation between Dextramer CMV kit, IFN-γ ELISpot and QuantiFERON-CMV.

		IFN-γ ELISpot		
		Reactive	**Non-Reactive**	**Total**
Dextramer CMV kit	**Positive**	42	13	55 (76%)
	Negative	0	17	17 (24%)
	Total	42 (58%)	30 (42%)	72 (100%)
		QuantiFERON-CMV		
		Reactive	**Non-Reactive**	**Total**
Dextramer CMV kit	**Positive**	20	2	22 (81%)
	Negative	1	4	5 (19%)
	Total	21 (78%)	6 (22%)	27 (100%)
		QuantiFERON-CMV		
		Reactive	**Non-Reactive**	**Total**
IFN-γ ELISpot	**Reactive**	20	1	21 (64%)
	Non-Reactive	5	7	12 (36%)
	Total	25 (76%)	8 (24%)	33 (100%)

Positivity thresholds: 0.5 $CD8^+Dextramer^+$ T cells/μl for Dextramer CMV kit, 1.75 sfc/μl after CMV antigen stimulation for IFN-γ ELISpot and 0.2 IU/ml in the CMV tube for QuantiFERON.

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