Supplementary materials

Immunosuppressive regimen of patients

For all patients, induction therapy was anti-IL2RA (20mg on day 0 and day 4) (SIMULECT®, Novartis Pharma SAS) and Methylprednisolone: 500 mg at day 0, 120 mg at day 1, then prednisone or equivalent, 20 mg/day from day 3. Maintenance immunosuppressive regimen was either based on everolimus 0.75mg bid, targeted to 3-8 ng/ml and Cyclosporin A (CsA) with target ranges 100-200 ng/ml from day 3 to month 2, 75-150 ng/mL from month 2 to month 4 and 25-50 ng/mL from months 6 to 12, either Csa with target ranges of 150–220 ng/mL from day 3 to month 2, 100–150 ng/mL from month 2 to month 12 and mycophenolic acid 1080 mg bid for one month, then 720 mg bid. Ciclosporin and everolimus whole blood concentrations were performed at day 7, day 14 and month 6 post transplantation.

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Figure S10 No effect of mTORi on Akt phosphorylation after TCR stimulation of Vδ2^{neg} γδ T cells

Supplemental table 1. References of flow cytometry material

	MANUFACTURER	CLONE	N°CATALOG	LAST BATCH	DILUTION
PER-CP CD3	BD biosciences	SP34-2	9091596	552851	0.4
FITC CD16	BD biosciences	3g8	8255938	560996	
BV421 CD154	BD biosciences	TRAP-1	9171764	566268	0.1
fixable viability stain 575V	BD biosciences		7020921	565694	0.0002
BV510 CD8	BD biosciences	SK-1	8003887	561617	0.1
BV786 CD27	BD biosciences	L128	8236716	563328	0.02
PE pan-δ	Miltenyi	REA 591	5190321373	130-113-512	0.02
PC-7 Vδ2	Miltenyi	REA 711	5190131222	130-111-012	0.1
BV650 CD161	BD biosciences	DX12	7235832	563864	0.1
BV786 CD16	BD biosciences	3G8	7139586	563690	0.1
FITC CD85J	BD biosciences	GHI/75	6214793	555942	0.4
PE VIO-615 KLRG1	Miltenyi	REA 261	5171115662	130-108-395	0.06
BV650 PD1	BD biosciences	EH12	7258845	564104	0.1
BV711 TIM3	BD biosciences	7D3	8310921	565566	0.1
BV786 DNAM	BD biosciences	DX11	8318701	742497	0.04
FITC Granulysin	BD biosciences	RB1	7089964	558254	0.04
APC pan-δ	Miltenyi	REA591	5190314461	130-113-508	0.06
PE-CF594 CD8	BD biosciences	RPA-T8	7150677	562282	0.04
V450 CD3	BD biosciences	UCHT1	8164556	560365	0.06
BV786 Interferon-γ	BD biosciences	4S-B3	7187947	563731	0.04
PE Granzym B	Molecular probes	GB12	1735130	MHB04	0.04
APC-H7 CD45RA	BD biosciences	HI 100	7226562	560674	0,1
APC Perforin	BD biosciences	dG9	7124573	563576	0,1
BV421 Granzym	BD biosciences	GB11		563389	0,1
APC CY7 CD16	BD biosciences	3G8	7075615	560195	0,1
FITC CD16	BD biosciences	3g8	8255938	560996	0.1
PC7 P-S6	Cell signalling	D57.2.2E	2	#34411	0,005
Alexa fluor 647 P-38 MAPK	Cell signalling	3D7	7	#14594	0,02
PE PAKT T308	BD biosciences	J1-223.371	9192801	558275	0,1
FITC PERK 1/2	BD biosciences	20A	720836	612592	0,1
FITC PAKT S473	BD biosciences	M89-61	9282654	560404	0.1
PE AKT	BD biosciences	55/PKBa/Akt	8310873	560049	0,1
PE S6	Cell signalling	54D2	1	55594S	0,005
Alexa fluor 647 HOBIT (ZNF683)	BD biosciences	sanquin-hobit/1	8201604	566250	0.06
PC5.5 Tbet	ebioscience	P3.6.2.8.1	4289808	45-4714-80	0.06
PC7 EOMES	ebioscience	WD1928	192396	25-4877-42	0.06
PE BLIMP1	BD biosciences	6D3	7290934	564702	0.02
Foxp3 transcription factor staining buffer	fisher scientific		2075534	115-000-597	
BV510 CD3	BD biosciences	UCHT1	8297756	563109	0.06
Pacific blue TCR Vδ2	Beckman coulter	IMMU389	5	B49310	0.04
FITC TCR Vδ2	Beckman coulter	IMMU389	2000040	I1464	0.04

FITC KLRG1	ebioscience	13F13F2	4345831	53948842	0.4
PE CD69	Beckman coulter	TP1.55.3	41	IM19930	0.1
Alexa fluor 700 CD69	BD biosciences	FN50	7258876	560739	0.1
PE-CY5.5 Panδ	Beckman coulter	IMMU510	30	A99021	01:50
live viability e fluor 780	ebioscience		4302692	65-0865-13	0.0002
BD cytofix/cytoperm^{tm}	BD biosciences			555028	
Protein transport inhibitor	BD biosciences		9011506	554724	
Phospho buffer perm III	BD biosciences		5260671	558050	
Lyse/fix buffer 5X	BD biosciences		7180900	558049	
Purified anti-CD3	Beckman coulter	UCHT1	200036	IM1304	
Purified anti-Vδ1	produced in the lab				

Supplemental Figures

Figure S1. Flow chart of the ancillary study

Samples came from patients initially includes in a French multicenter study (n=186) from which 83 were included at Bordeaux University Hospital. Among those 83 patients, 77 could be followed for more than 1 month, 44 were treated with mycophenolic acid (MPA-treated patients) and 33 were treated with everolimus (EVR-treated patients). Among EVR-treated patients, 6 have been switched to MPA and were excluded of analyses. Consequently, the analyses of the study included 44 MPA-treated patients and 27 EVR-treated patients.

Figure S2 Flow chart of MPA-treated patients of the ancillary study

Among the 44 MPA-treated patients of the ancillary study, 33 had available frozen PBMC at day 0 of transplantation. 21 were used to constitute the first set of patients with total CD8 and γδ T cell phenotype, and 12 were used for the second set of patients with CD8, CMV-specific CD8 and γδ T cell phenotype.

Figure S3 $V\delta2^{neg}$ γδ T cells and CD8+ αβ T cells phenotype at baseline in CMV seropositive patients

A. $V\delta2^{neg}$ γδ T cell and CD8+ αβ T cell expression of CD27 and CD45RA analyzed by flow cytometry separating patients with no CMV DNAemia or CMV DNAemia requiring no treatment (well-controlled CMV, n=12) versus patients requiring CMV antiviral treatment (severe CMV, n=9).

B. $V\delta2^{neg}$ γδ T cells andCD8+ T cells phenotypes for inhibitory receptors and KLRG1 expression were validated in an internal cohort of patients with well-controlled CMV (n=5) versus patients with severe CMV (n=7).

Each symbol represents an individual donor; large horizontal lines indicate the mean and small horizontal lines indicate the standard deviation, 0.05>p>0.01*; **0.01>p>0.001; ***p<0.001; as determined by the Mann-Withney U test.

Figure S4 Phenotype of Vδ2^{neg} γδ T cells and CMV-specific αβ T cells during in vitro culture

A. One representative phenotype at day 0 of CMV positive patient's PBMC used for *in vitro* IL2-IL15 culture of Vδ2^{neg} γδ T cells, analyzed by flow cytometry.

B. One representative proliferation assay of TEMRA Vδ2^{neg} γδ T cells analyzed by CFSE staining of CD45+ CD27- cells by flow cytometry.

C. One representative phenotype at day 7, 14 and 21 of CMV positive patient's PBMC during *in vitro* culture of Vδ2^{neg} γδ T cells analyzed by flow cytometry.

D. Evolution of Vδ2^{neg} γδ T cell number during 21 days of PBMC cultures with IL2 (n=4) and IL2-IL15 (n=4) ,obtained by Neubauer counting associated with analysis of Vδ2^{neg} γδ T cells percentage by flow cytometry. Results are the mean $\pm$ SD of the cell number fold increase from day 0, of PBMC cultures from those 4 different donors.

E. One representative phenotype at day 0 of CMV positive patient's PBMC used for *in vitro* culture of CMV-specific αβ T cells analyzed by flow cytometry.

Figure S5 Low dose of mTORi decrease the number of Vδ2^{neg} γδ T cells and CMV-specific αβ T cell expressing CD85j.

Expression of CD85j was analyzed in Vδ2^{neg} γδ T cells and CMV specific αβ T cells by flow cytometry after culturing PBMC of CMV seropositive donors (one representative, Figure A; cumulative results for 5 donors, Figure B) *in vitro* with or without a low dose (0.5nM) of everolimus during 21 days for the culture of Vδ2^{neg} γδ T cells and during 16 days for the culture

of CMV specific αβ T cells (stimulated overnight by pp65 peptides and gated on non-γδ T cells positive for CD69 and IFNγ). Each symbol represents an individual donor; 0.05>p>0.01*; **0.01>p>0.001; as determined by Wilcoxon test.

Figure S6 mTORi does not affect CMV-specific αβ T cell frequencies during in vitro culture

One representative phenotype of CMV-specific αβ T cells after 7 days of PBMC culture with IL2 with or without 0.5nM everolimus. After overnight stimulation with CMV peptides, CMV-specific αβ T cells were gated by flow cytometry through their expression of CD69 and IFNg among non γδ T cells (left). Cumulative results for 5 donors(right).

Figure S7 mTORi improvement of the T cell dysfunctional profile is maintained when combined with ciclosporin

(A) $V\delta2^{neg}$ γδ T cells (after 7, 14 and 21 days for expansion and 21 days for PD1 and CD85j expression) and (B) CMV-specific αβ T cells (after 16 days) with 0/25/75/200mg/mL^{-1} with either medium, 0.5nM everolimus or mycophenolate mofetil (MMF) were analyzed after *in vitro* culture of PBMC from CMV-seropositive KTR. Expansion are expressed as fold increase from day 0 of culture. PD-1 and CD85j are expressed as proportion among total $V\delta2^{neg}$ γδ T cells or CMV-specific αβ T cells.

Each symbol represents an individual donor. ns, not significant, 0.05>p>0.01*; **0.01>p>0.001; as determined by Wilcoxon test.

Figure S8 Blocking anti-CD3 mAb had no effect on $V\delta2^{neg}$ γδ T cell viability.

Vδ2^{neg} γδ T cells were negatively sorted after 21 days of culture with 0 or 0.5 nM everolimus and were cultured in the same medium alone (either with 0 or 0.5 nM everolimus), with non-infected (NI), or with CMV-infected (CMV) fibroblasts with or without blocking anti-CD3 mAb (10µg/ml) during 24 hours and cells were stained with 1µM DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride) to assess their viability by flow cytometry.

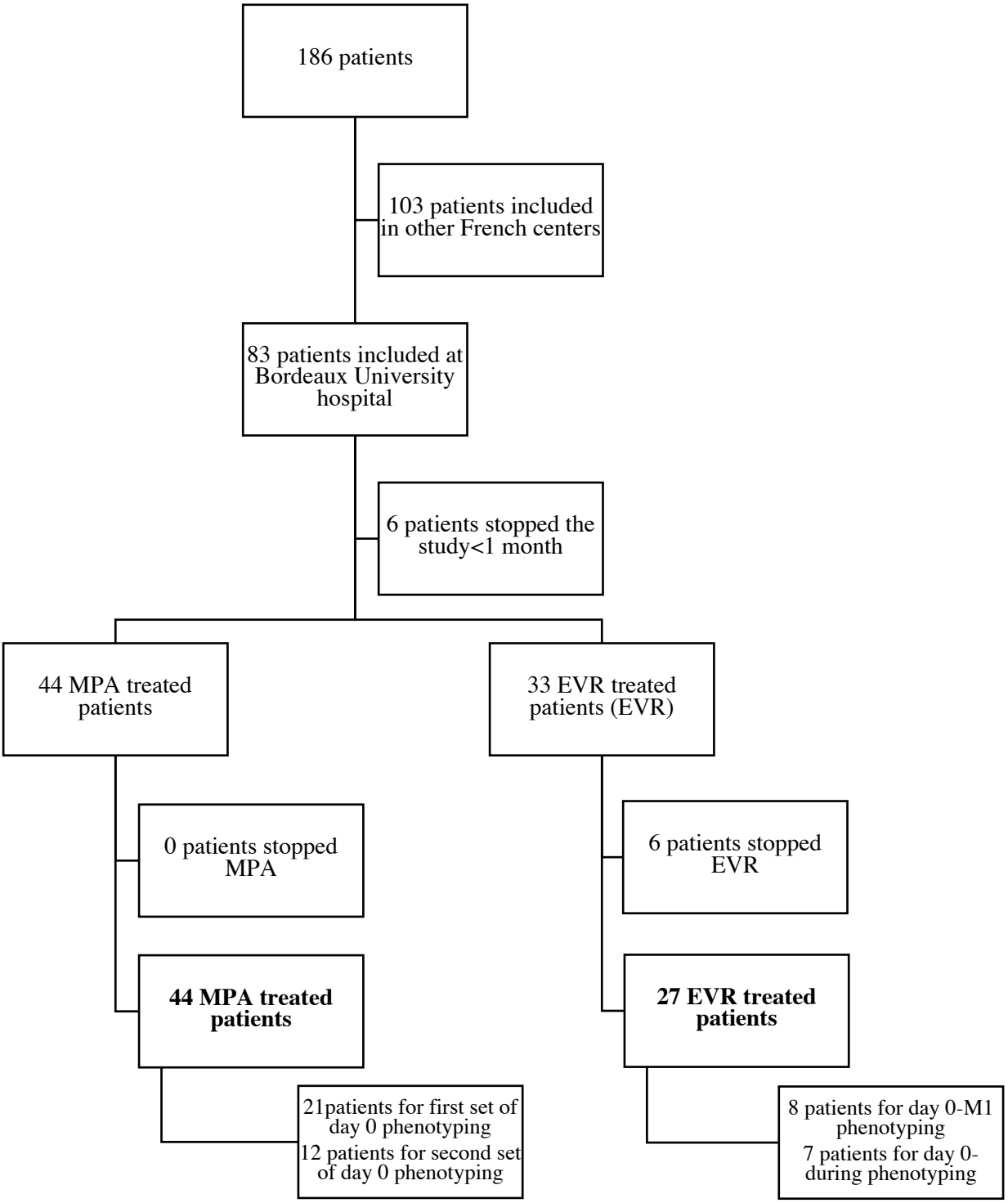
Figure S9 mTORi does not affect SLP-76 and MAP kinase 38 signaling after TCR stimulation of Vδ2^{neg} γδ T cells

Vδ2^{neg} γδ T cells were negatively sorted after 21 days of culture with 0 or 0.5 nM of everolimus , and stimulated with an agonist anti-CD3 mAb (UCHT1, 10µg/ml) for the indicated durations. SLP-76 phosphorylation (A) and MAP kinase 38 phosphorylation (B) were measured by flow cytometry in 4 donors (right, mean ± ranges) and in a representative donor (left).

Figure S10 No effect of mTORi on Akt phosphorylation after TCR stimulation of Vδ2^{neg} γδ T cells

After 21 days of culture with 0 or 0.5 nM of everolimus, PBMC were stimulated using an agonist anti-Vδ1 mAb (10µg/ml) for 30 minutes, 2 and 4 hours and Akt phosphorylation was measured and analyzed by flow cytometry (T308 phosphorylation site, left; S473 phosphorylation site, right) for 4 donors (mean ± ranges).

Figure S1



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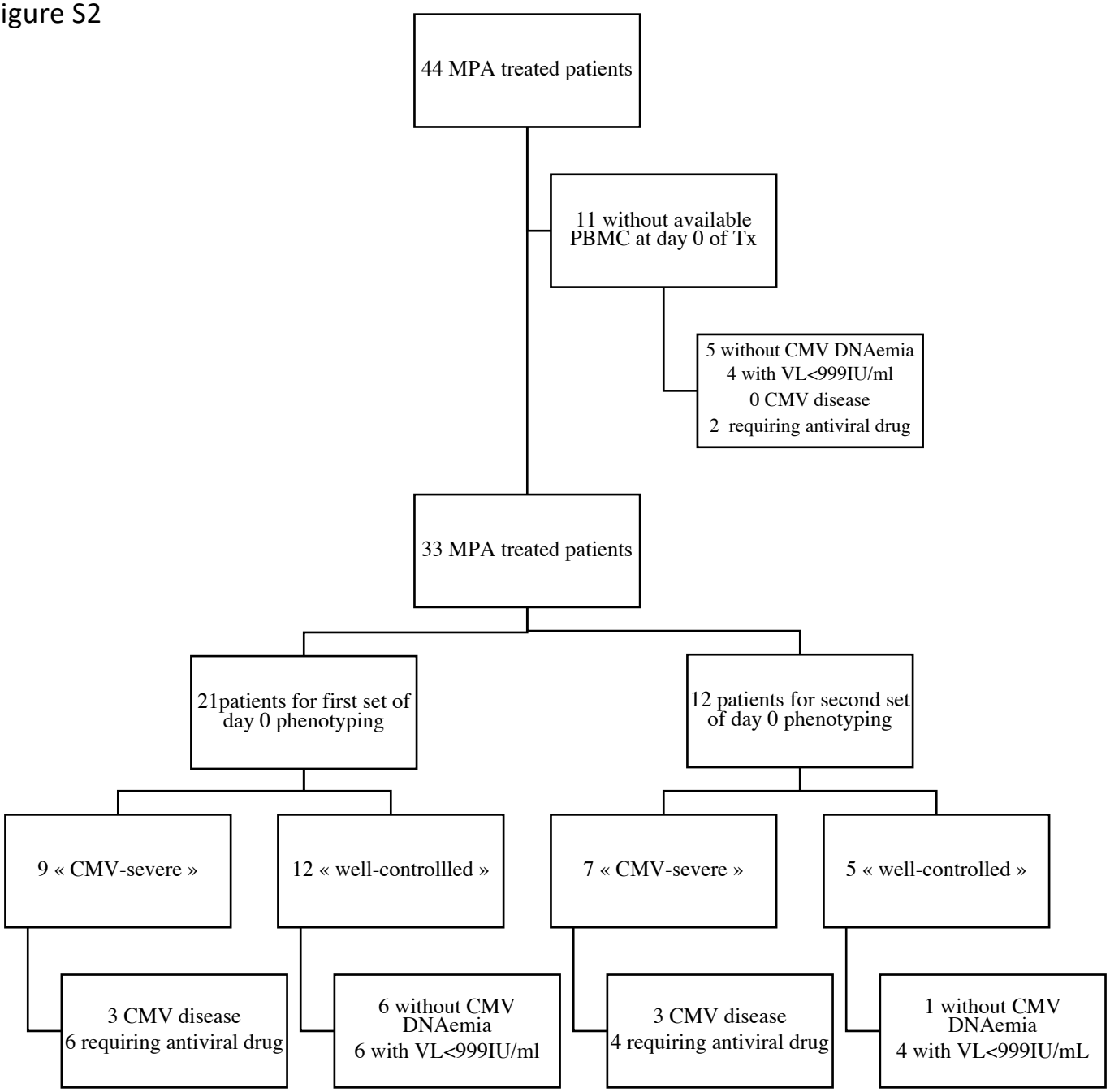

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Figure S3

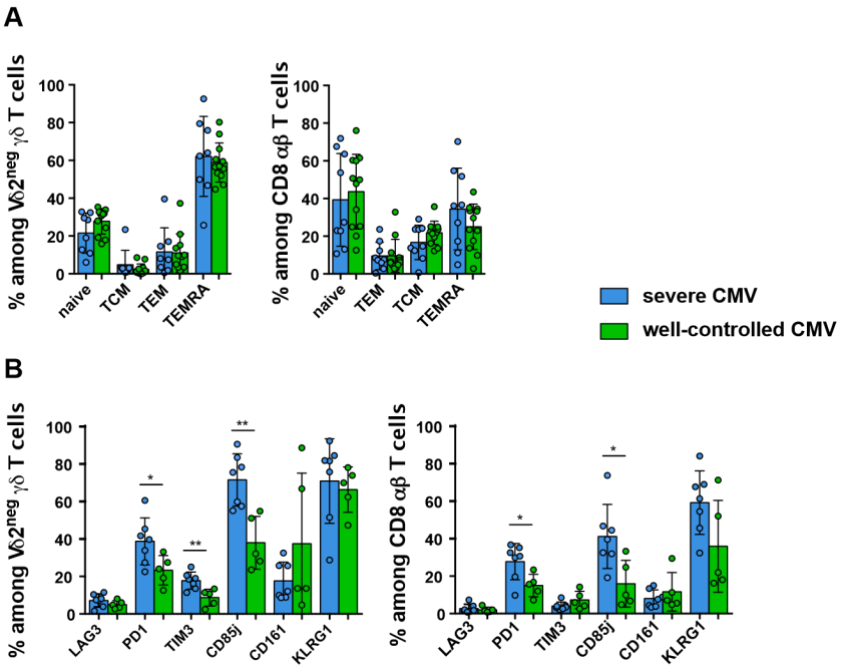

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Figure S4

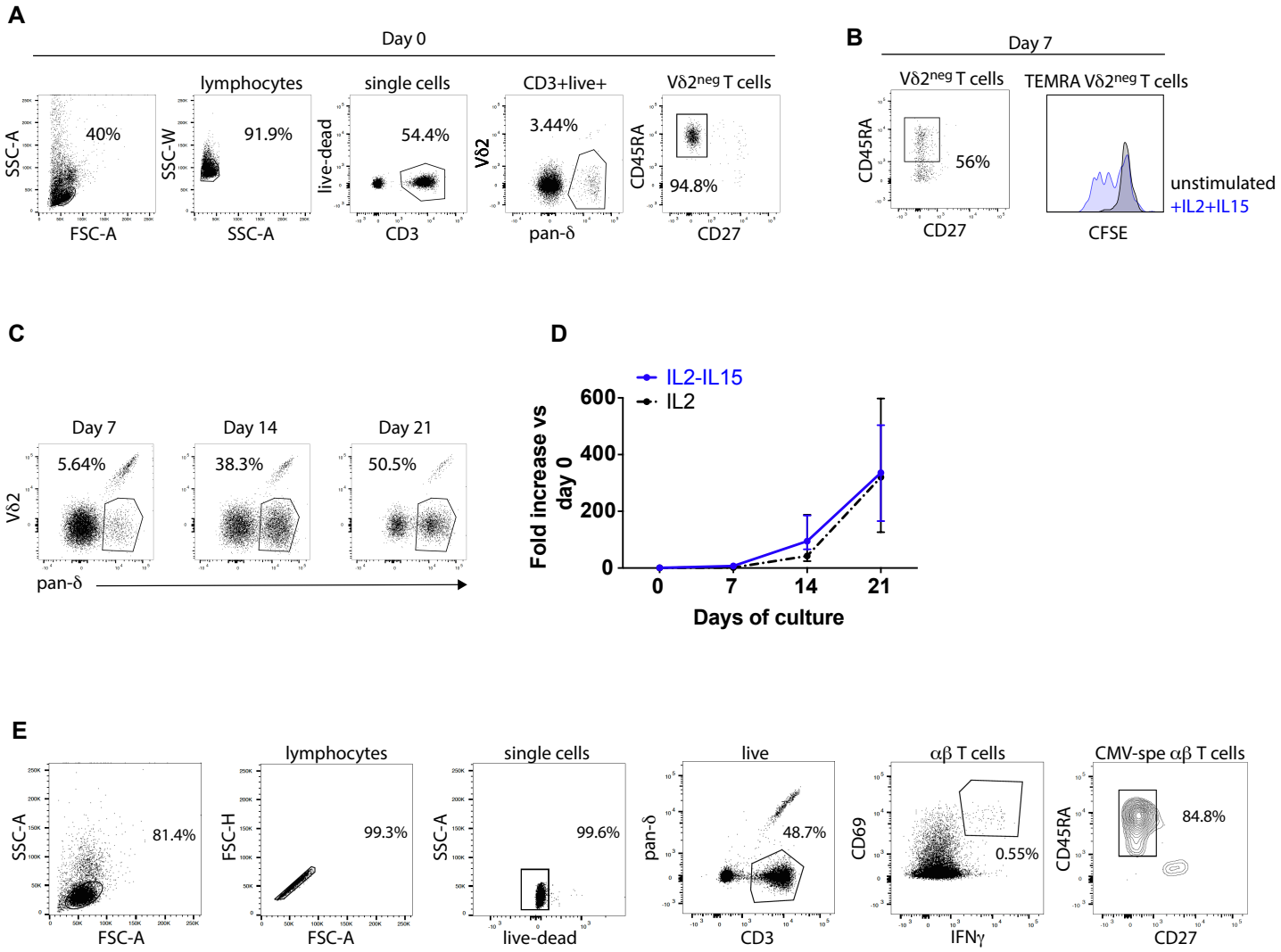

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Figure S5

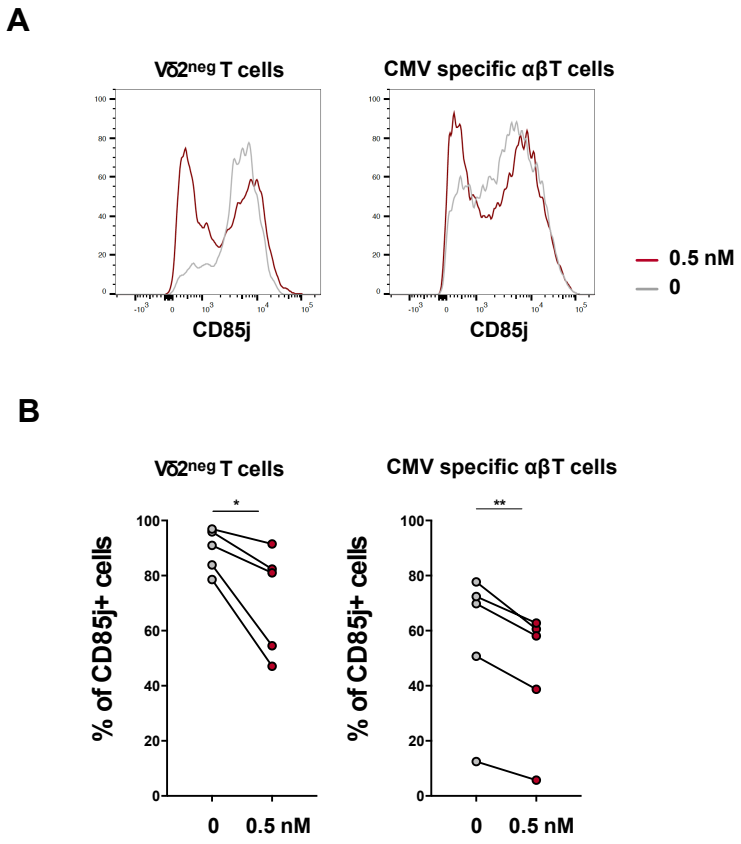

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Figure S6

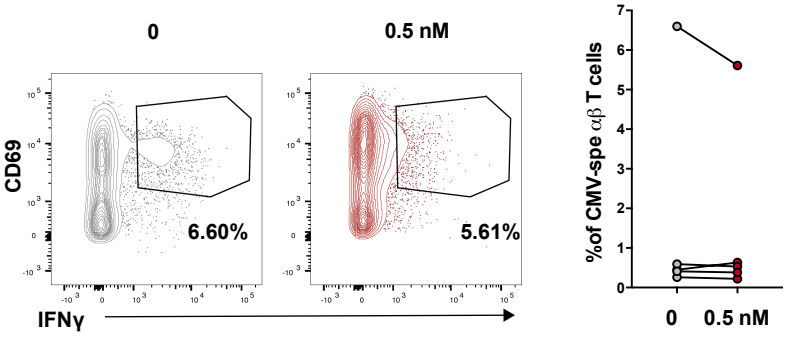

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Figure S7

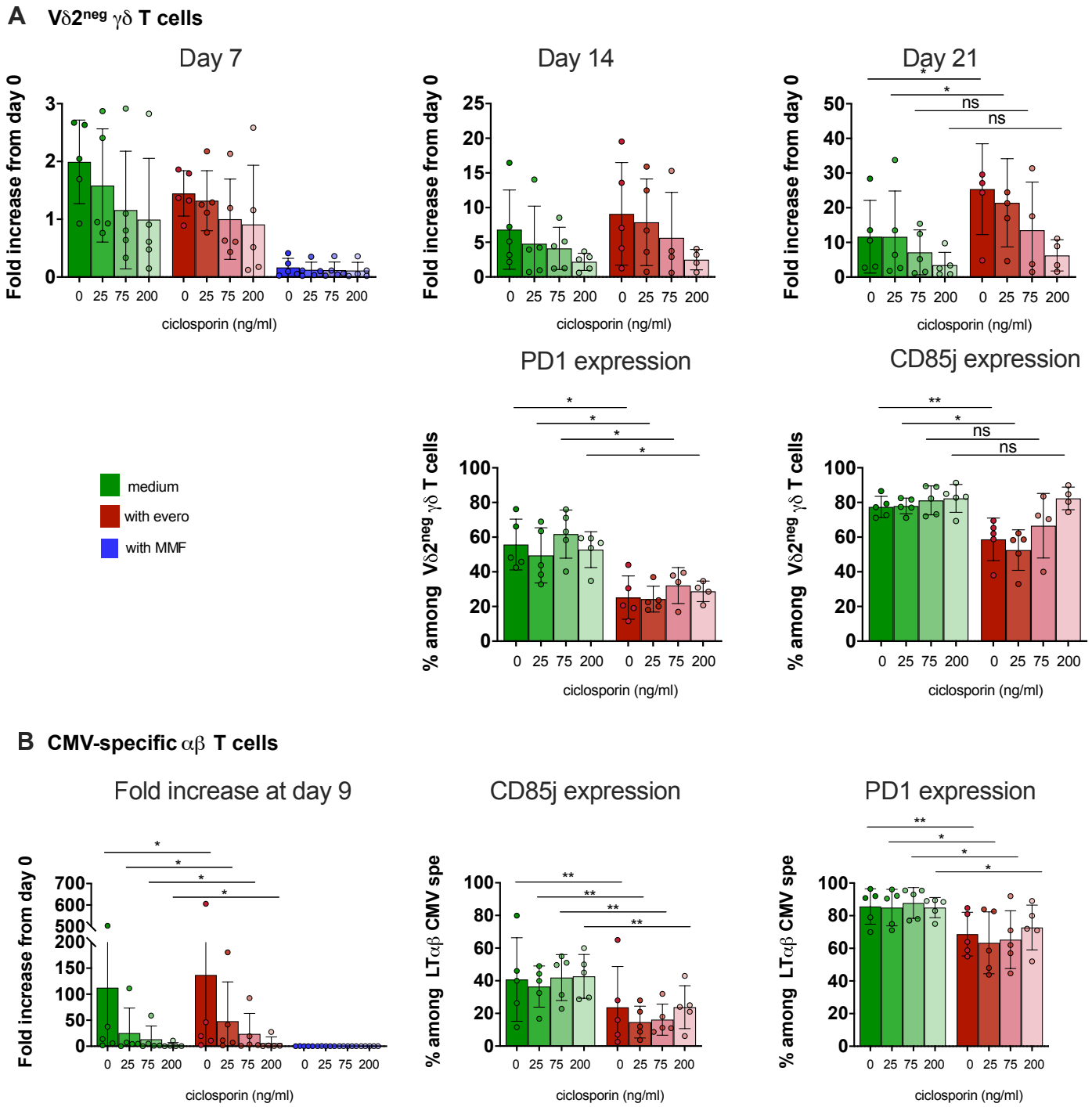

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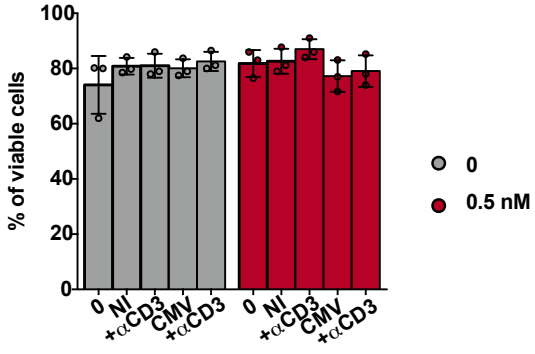
Figure S8



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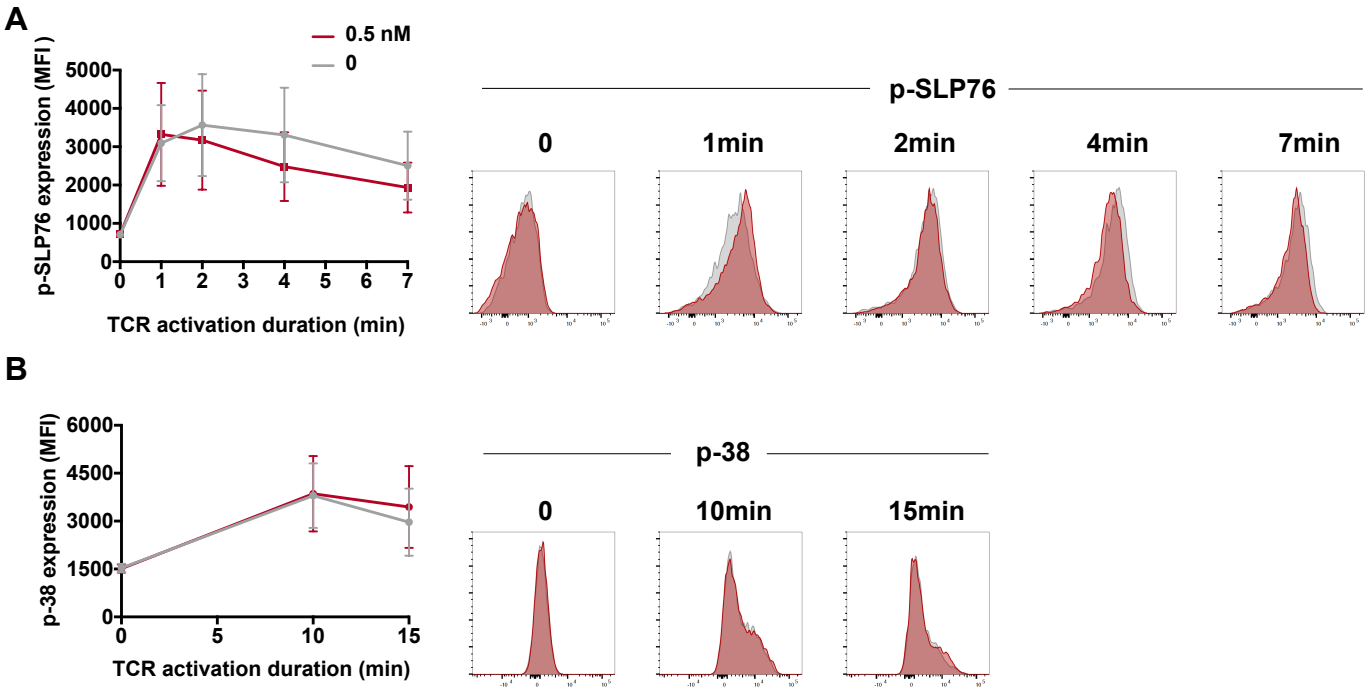
Supplemental figure S9



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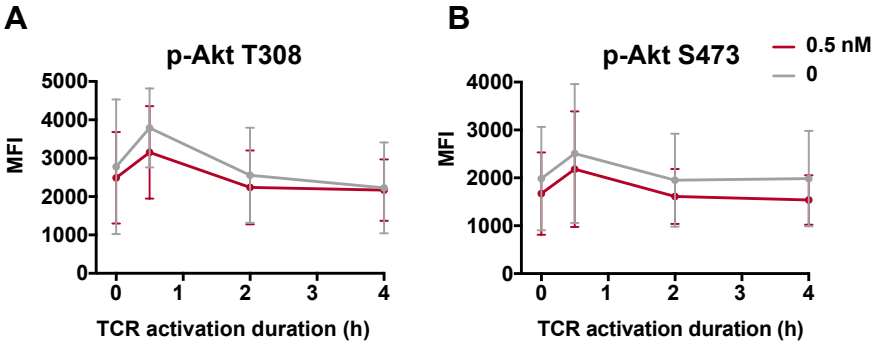
Supplemental figure S10



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