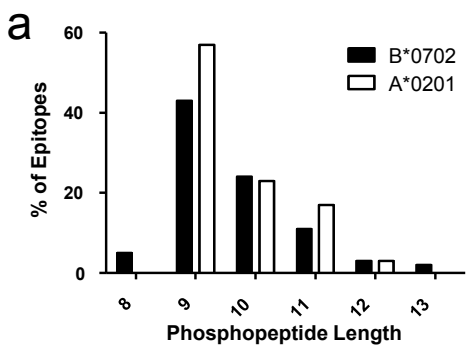
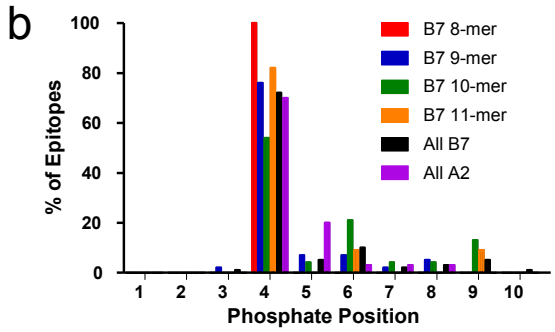
Supplemental Material





c

9mer HLA-B7 Phosphopeptides

	P1	P2	P3	P4	P5	P6	P7	P8	P9
A	2	2	15	0	2	12	20	12	7
C	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	5	2	5	0
E	5	0	2	0	0	7	5	7	0
F	0	0	5	0	0	2	0	0	10
G	0	0	2	0	0	20	2	5	0
H	0	0	2	2	0	0	7	0	0
I	0	0	0	0	0	0	2	2	5
K	20	0	12	2	2	2	10	10	0
L	2	0	2	2	5	5	10	0	61
M	0	0	0	0	0	2	0	2	7
N	0	0	0	0	0	0	2	0	0
P	0	85	12	2	63	5	10	5	2
Q	0	0	2	0	2	0	2	0	0
R	61	2	24	7	10	7	10	15	0
S	7	7	2	2	5	15	7	20	2
T	2	0	2	2	0	2	0	2	0
V	0	2	7	0	0	7	7	7	5
W	0	0	2	0	0	0	0	0	0
Y	0	0	2	2	2	0	0	2	0
pSer (X)	0	0	2	71	7	7	2	2	0
pThr (Z)	0	0	0	5	0	0	0	2	0

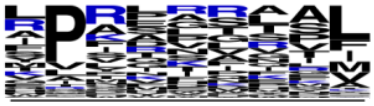
d

9mer Predicted HLA-B7 Phosphopeptides with P4=pSer(X)

	P1	P2	P3	P4	P5	P6	P7	P8	P9
A	11	0	12	0	2	7	8	7	25
C	1	0	0	0	0	1	0	1	0
D	1	0	4	0	1	6	4	5	0
E	9	0	9	0	1	9	9	9	0
F	0	0	1	0	2	1	2	2	3
G	6	0	8	0	0	11	6	5	1
H	2	0	1	0	0	1	0	2	0
I	3	0	2	0	1	4	1	2	8
K	9	0	5	0	0	6	7	2	0
L	4	0	11	0	1	4	5	9	30
M	2	0	1	0	0	1	1	1	2
N	3	0	2	0	0	2	1	1	0
P	13	100	9	0	87	12	22	20	1
Q	4	0	4	0	1	1	3	5	0
R	10	0	8	0	1	11	5	7	0
S	10	0	13	0	1	7	13	10	0
T	7	0	6	0	0	9	7	4	17
V	4	0	5	0	2	5	7	6	13
W	1	0	0	0	0	1	0	0	0
Y	1	0	0	0	1	1	0	2	0
pSer (X)	0	0	0	100	0	0	0	0	0
pThr (Z)	0	0	0	0	0	0	0	0	0

e

9mer HLA-B7 non-phosphopeptides



	P1	P2	P3	P4	P5	P6	P7	P8	P9
A	8	5	11	7	8	6	10	19	6
C	1	0	1	1	1	1	1	1	1
D	1	0	2	6	3	3	2	2	0
E	3	1	2	8	3	2	4	3	1
F	7	1	7	4	5	5	4	3	12
G	4	2	3	5	8	8	5	4	1
H	3	0	2	3	4	2	3	1	1
I	7	3	6	4	5	5	4	6	12
K	5	0	7	4	6	3	4	3	2
L	15	7	5	8	8	7	10	4	36
M	5	2	3	3	2	3	3	3	11
N	3	1	3	4	7	4	5	3	0
P	0	60	4	8	7	5	4	10	1
Q	3	2	3	6	2	5	5	3	0
R	12	2	20	6	9	13	7	4	0
S	6	2	6	8	6	10	9	12	1
T	5	3	4	6	6	8	10	8	2
V	4	8	5	4	7	6	6	8	11
W	3	0	2	2	2	2	2	1	1
Y	6	1	4	3	2	2	3	3	1

f

8mer HLA-B7 phosphopeptide ligands

	P1	P2	P3	P4	P5	P6	P7	P8
A	0	0	0	0	20	0	0	0
C	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	20	0
E	0	0	0	0	0	0	0	0
F	20	0	0	0	0	0	0	20
G	0	0	0	0	0	0	20	0
H	0	0	0	0	0	0	0	0
I	0	0	20	0	0	0	0	0
K	0	0	0	0	0	0	0	0
L	0	0	20	0	20	20	40	60
M	0	0	0	0	0	0	0	0
N	0	0	0	0	0	0	0	0
P	0	60	0	0	60	20	0	20
Q	0	0	0	0	0	0	0	0
R	80	0	40	0	0	0	20	0
S	0	40	20	0	0	0	0	0
T	0	0	0	0	0	20	0	0
V	0	0	0	0	0	40	0	0
W	0	0	0	0	0	0	0	0
Y	0	0	0	0	0	0	0	0
pSer (X)	0	0	0	100	0	0	0	0
pThr (Z)	0	0	0	0	0	0	0	0

g

10mer HLA-B7 phosphopeptide ligands

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
A	0	0	8	0	21	0	4	0	4	8
C	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	4	0	4	0	4	0
E	0	0	4	0	0	8	0	0	4	0
F	4	0	0	0	0	0	8	4	0	8
G	8	0	4	0	4	4	4	8	0	0
H	0	0	0	0	4	0	4	0	0	0
I	0	0	4	0	0	0	0	8	0	8
K	8	0	0	0	0	0	8	0	4	4
L	0	0	0	4	0	0	13	25	4	63
M	0	0	0	0	0	0	0	0	0	4
N	0	0	4	0	0	0	0	17	0	0
P	0	92	17	13	54	17	25	4	4	4
Q	8	0	8	4	0	4	4	4	0	0
R	50	4	46	13	0	13	8	8	0	0
S	13	0	0	8	4	25	8	8	33	0
T	8	4	0	0	0	8	4	8	0	0
V	0	0	0	0	4	0	0	0	21	0
W	0	0	0	0	0	0	0	0	0	0
Y	0	0	4	0	0	0	0	0	4	0
pSer (X)	0	0	0	42	4	17	4	0	17	0
pThr (Z)	0	0	0	17	0	4	0	4	0	0

h

11mer HLA-B7 phosphopeptide ligands

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
A	0	0	18	9	18	0	9	9	0	0	0
C	0	0	0	0	0	0	0	0	0	0	0
D	0	0	9	0	0	9	0	9	0	9	0
E	0	0	9	0	0	0	9	0	9	9	0
F	0	0	0	0	9	0	9	0	0	0	9
G	0	0	0	0	0	36	27	9	0	27	0
H	0	0	0	0	0	0	0	0	0	0	0
I	0	0	9	0	0	0	0	9	0	0	0
K	9	0	0	9	0	9	0	9	0	9	0
L	0	0	0	0	0	9	0	0	0	0	64
M	0	0	0	0	0	0	0	0	0	0	9
N	0	0	0	0	9	0	0	9	9	0	0
P	0	91	0	0	55	0	9	0	9	0	0
Q	9	9	0	0	0	0	0	9	0	9	0
R	45	0	45	0	9	9	18	18	9	0	0
S	18	0	9	0	0	9	9	0	18	27	0
T	9	0	0	0	0	9	0	0	9	9	0
V	0	0	0	0	0	0	9	9	18	0	18
W	0	0	0	0	0	0	0	0	0	0	0
Y	9	0	0	0	0	0	0	0	9	0	0
pSer (X)	0	0	0	82	0	9	0	9	9	0	0
pThr (Z)	0	0	0	0	0	0	0	0	0	0	0

Fig. S1. Detailed HLA binding motif analysis. **(a)** proportion of HLA-A2 and -B7 phosphopeptides of given residue length. **(b)** Phosphate position in B7 and A2 phosphopeptides. Residue frequency (%) depicted in combined logoplots and heatmaps for: 41 9-mer HLA-B7 phosphopeptides in this study **(c)**; 164 9-mer phosphopeptides predicted to bind to HLA-B7 where pSer was at P4 (d); 1038 regular non-phosphorylated 9-mer HLA-B7 peptides from the Immune Epitope database (e); five 8-mer (f), twenty-one 10-mer (g) and eleven 11-mer (h) HLA-B7 phosphopeptides identified in this study. Heatmaps denote percentage frequency of residues at each position.

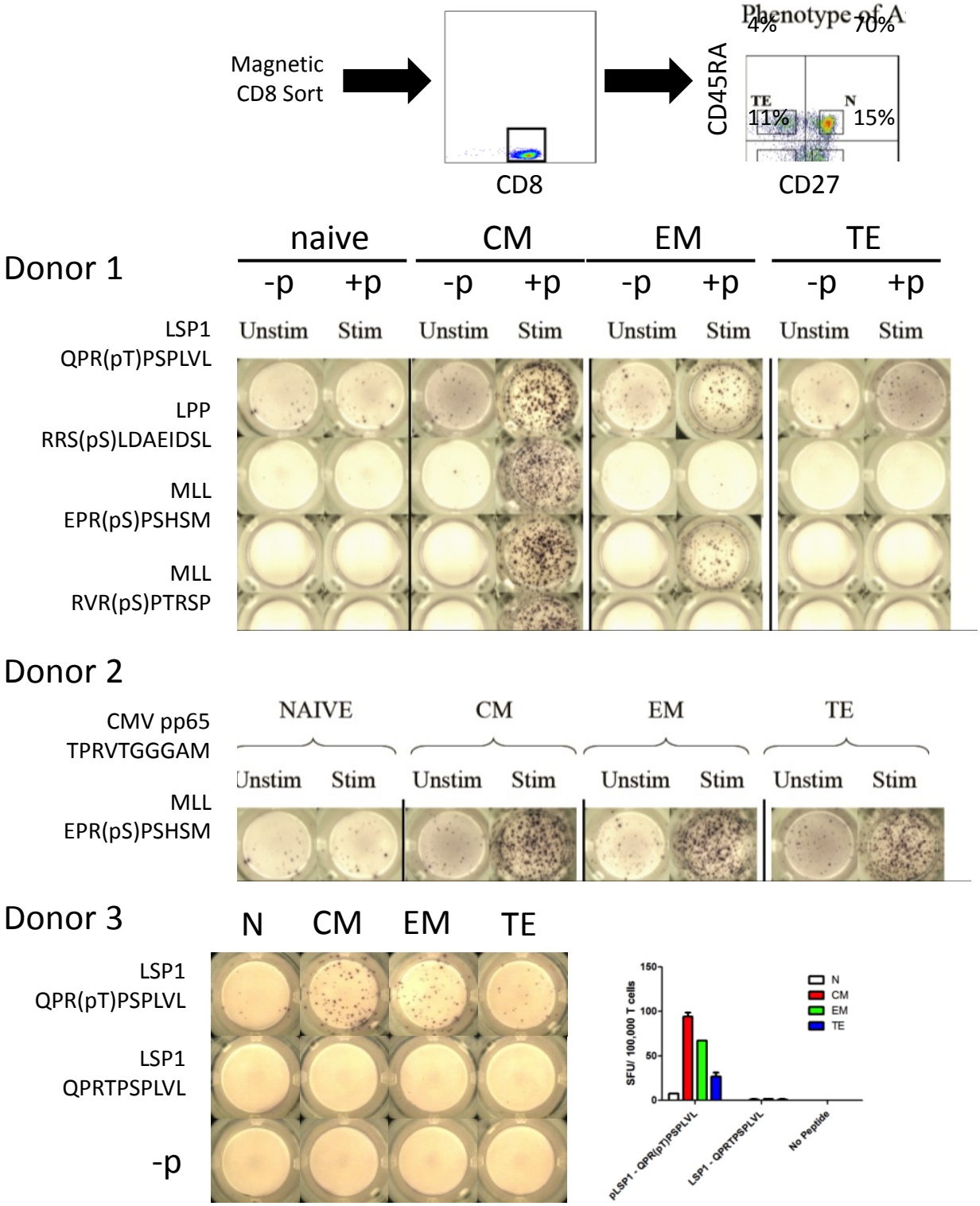


Fig. S2. T-cell memory subset mapping of anti-phosphopeptide responses in healthy donors. Representative FACS-sorted T-cell subset profile of freshly isolated, magnetically enriched CD8 T-cells from peripheral blood of healthy donors (upper panel). 100,000 T-cells from each compartment were cultured with phosphopeptides for 7-days. 7-day ELISpot analysis of each T-cell memory subset reveals level of immunity against each respective phosphopeptide in 3 donors. For Donor 3 we tested reactivity against the non-phosphopeptide.

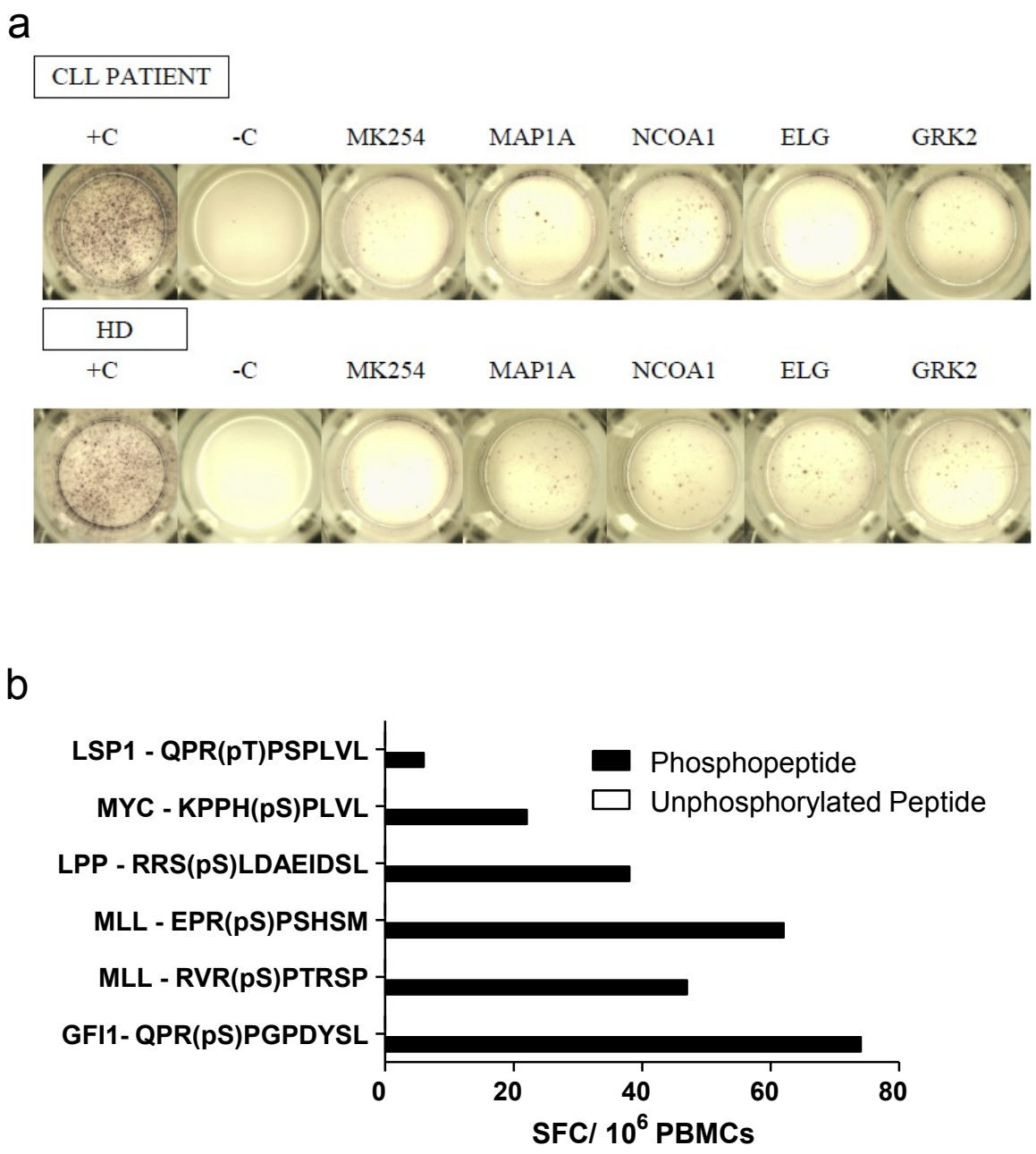


Fig. S3. Leukemia-associated phosphopeptide-specific immunity is lacking in CLL patients.
(a) Example of ELISpot of enriched CD8+ T-cells from a patient with CLL and a healthy donor against selected CLL-associated antigens. **(b)** Example of responses observed for phosphopeptide antigens (filled) and counterpart non-phosphorylated peptide in a healthy donor.

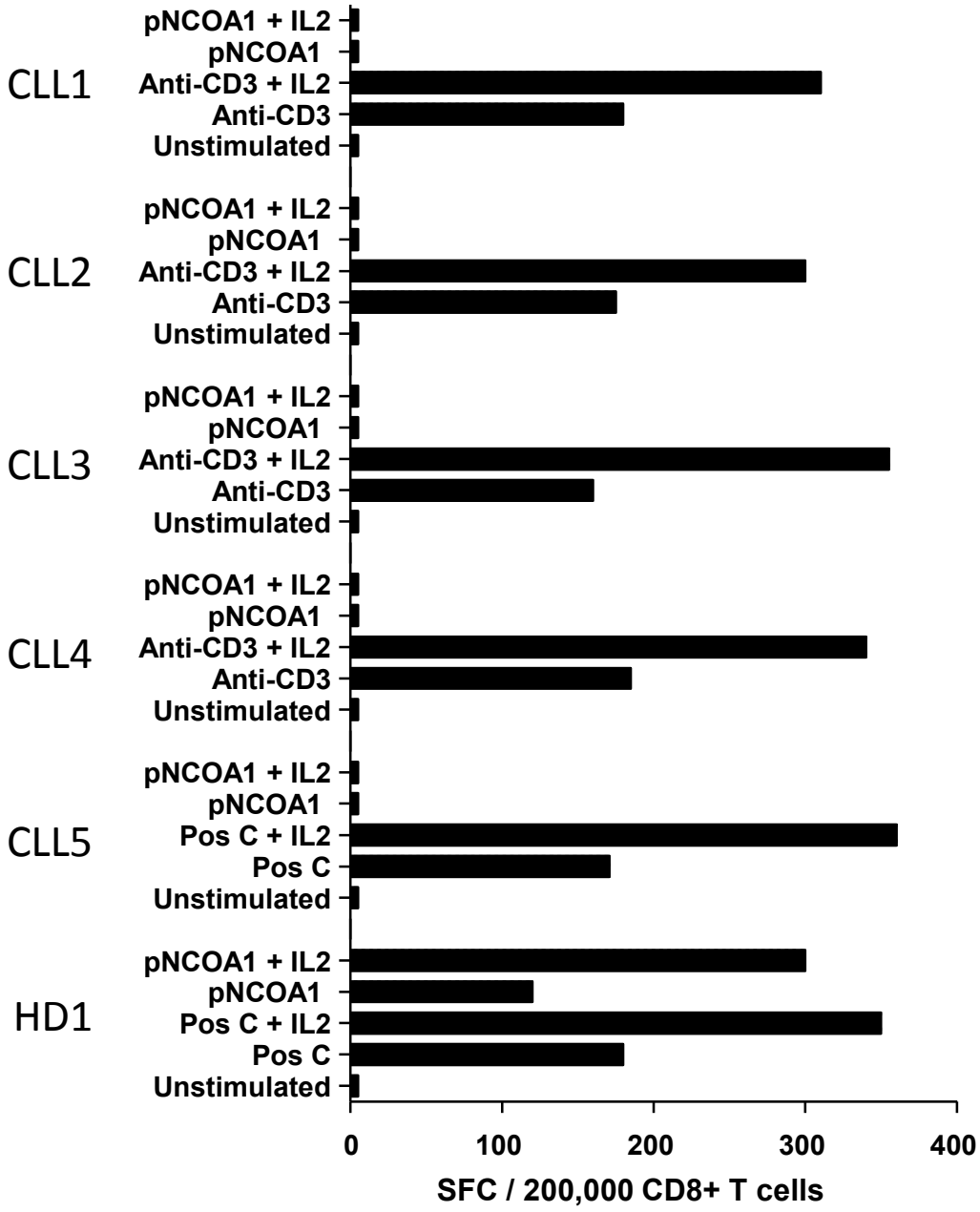


Fig. S4. Deletion (rather than anergy) of anti-phosphopeptide immunity in five patients with CLL. Representative 24-hour ELISpot from enriched CD8 T-cells from a healthy donor (HD) and five patients with CLL who lacked anti-phosphopeptide immunity stimulated in presence or absence of IL-2. Responses to anti CD3 (positive control) and the HLA-B7 restricted phosphopeptide (pNCOA1) was assessed.

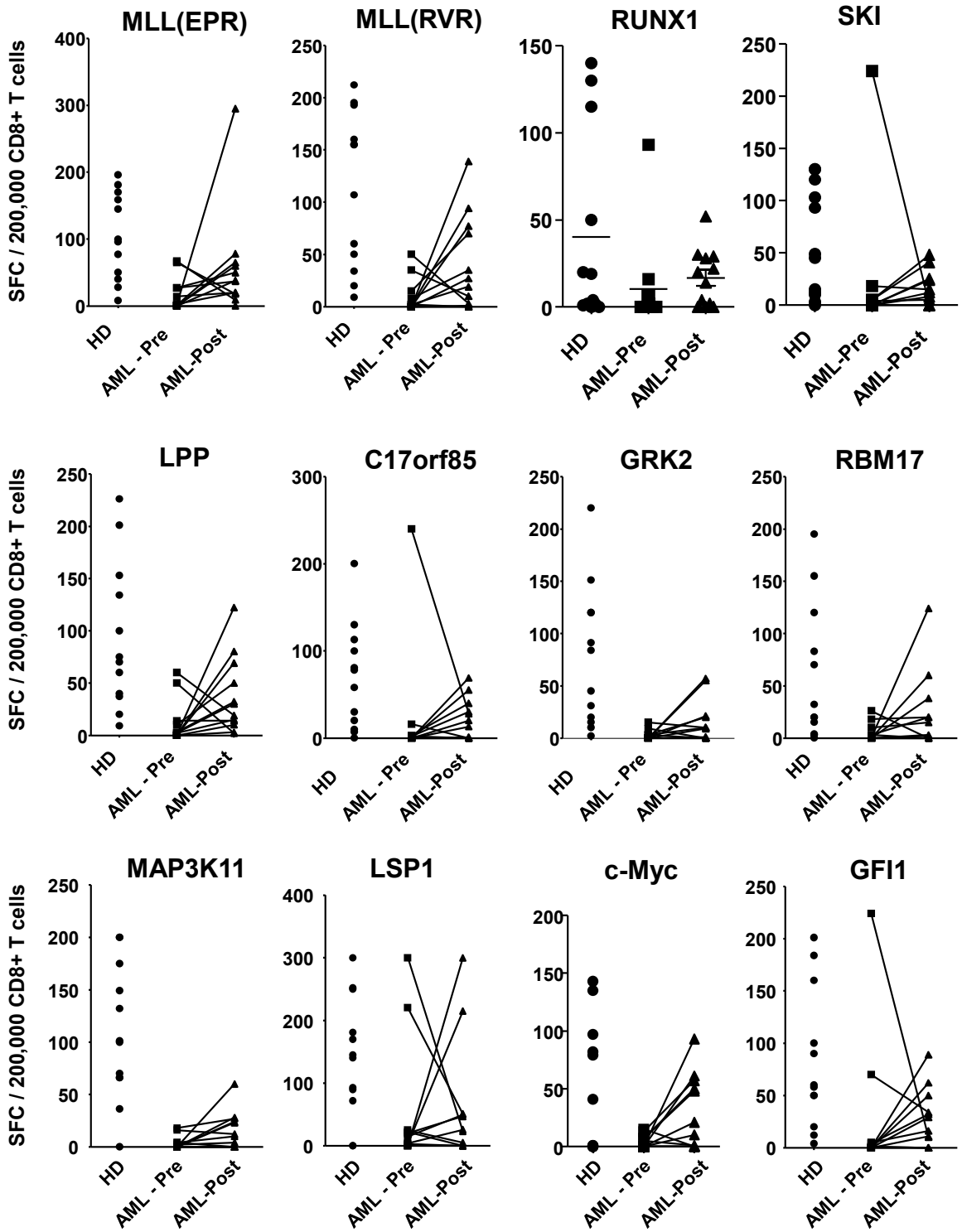


Fig. S5. Leukemia-associated phosphopeptide-specific immunity is lacking in AML patients but restored following SCT. ELISpot data for each phosphopeptide between AML patients pre- and post-transplant compared with healthy donors.

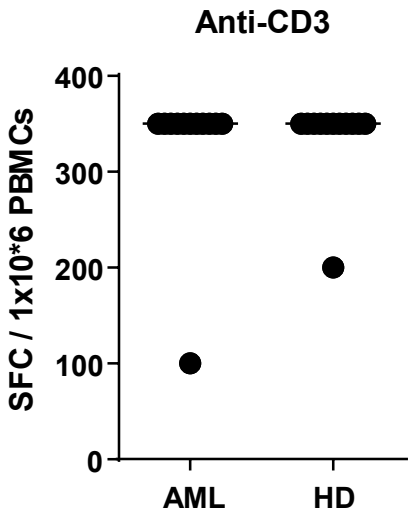


Fig. S6. Immunocompetence of patients with AML in complete remission prior to transplantation. Anti-CD3 responses measured by ELISpot among healthy donors (HD) and patients with AML whilst in complete remission pre-transplant

Patient ID	Time Since Sample	Age	Sex	Stage at presentation (Binet stage)	CD38	Zap-70	Genetic aberration	IgVH	Time to first treatment (days)	Progression free survival after first treatment	Timing of sample(s)	Treatment, status
CLL1	890	79		A	POS	POS	Normal	UM	2387	Progressed through chlorambucil	Previously treated, slow progressive disease	Died, Chl, FC, methylpred, died of disease +4695
CLL2	420	71		A	NEG	NEG	NA	NA	Untreated	Untreated	Untreated	Alive day +506
CLL3	1048	64		A	NEG	NA	NA	NA	685	Progressed 301 days post first tx	Untreated	Alive, FCR, transformed to Hodgkins ABVD, day +1142
CLL4	945	75		A	NEG	NEG	Normal	M	Untreated	Untreated	Untreated	Alive day +5341
CLL5	688	63		A	NEG	NA	NA	NA	Untreated	Untreated	Untreated	Alive day +1749
CLL6	586	86		A	NEG	NEG	Normal	M	3334	Died on tx	Prior to first treatment for progressive disease	Died, Chl, died of disease day +3467
CLL7	359	70		A	NEG	NEG	NA	NA	Untreated	Untreated	untreated	Alive, day +1201
CLL8	359	67		A	NEG	NA	13q	NA	1141	Not progressed	Prior to first treatment for progressive disease	Alive, FCR, day +1460
CLL9	897	78		A	NEG	NEG	Normal	M	Untreated	Untreated	Untreated	Alive day +6133
CLL10	1091	89		C	NA	NA	NA	NA	0	Partial response	All taken on treatment (chlorambucil)	Died, Chl, ?died of disease day +362
CLL11	540	65		B	NEG	POS	NA	NA	30	Progressed 631 days post first tx	Previously treated, progressive disease	Alive, Chl, FCO, day +1399
CLL12	1064	84		C	NEG	NEG	NA	NA	68	Not progressed	18/2/09 – Prior to first treatment for progressive disease 6/1/10 – 4 months post treatment	Alive, Chl/R, day +1129
CLL13	523	63		A	NEG	NEG	NA	NA	Untreated	Untreated	6/1/10 – 4 months post treatment	Alive, day +993
CLL14	579	63		A	POS	NEG	NA	M	Untreated	Untreated	Untreated	Alive day +2708

IgVH, Immunoglobulin Variable Region Mutation status:- M indicates mutated; UM, unmutated; NA, not assessed;
NA, Not available; FC, fludarabine+cyclophosphamide; FCR, fludarabine+cyclophopshamide+rituximab; FCO, fludarabine+cyclophopshamide+ofatumumab; Chl, chlorambucil; Chl/R, chlorambucil+rituximab; Methylpred, methylprednisolone

Table S1. CLL patient characteristics.

AML Patient ID	Age	Gender	Diagnosis and Treatment	Cytogenetics	Transplant History	Samples Analyzed	GvHD	ALC	Clinical Condition at the end of study (12/2012)
AML1	65	M	AML, ADE x2	monosomy 7	MUD	21 months post SCT	No	1.2	23 months post transplant: Death due to Relapse †
AML2	67	F	AML, DAx3, CR1	normal	MUD	11 months post SCT	No	1.3	26 months post transplant: In Remission and well
AML3	64	F	AML DA x2, MIDAC, CR1	FLT3pos	2 Cord Bloods	9 months post SCT	No	1.3	26 months post transplant: In Remission and well
AML4	67	M	AML MIDAC x3 CR1	normal	Sibling	10 months post SCT	Skin	2.3	10 months post transplant: Relapsed. Patient died 13 months post trasnplant
AML5	62	F	AML, DAx2, MACE	normal	MUD	21 months post SCT	No	1.3	39 months post transplant: In Remission and well
AML6	65	M	AML DA/Myelotarg, DA CR1	normal	MUD	3 months post SCT	No	1.5	3 months post transplant: Death †
AML7	54	F	AML, AML 17 (ADE X2) CR1	monosomy 7	MUD	7 months post SCT	Gut	0.9	25 months post transplant: In Remission and well
AML8	56	M	MDS transformed to AML, [DAx2, MACE, MIDAC] [ARA-C], CR2	normal	MUD	6 months post SCT	Skin now resolved	1	25 months post transplant: In Remission and well
AML9	51	F	MDS no treatment	trisomy 6	Sibling	113 months post SCT	Eyes, mouth, skin and liver	2.2	131 months post transplant: In Remission and well
AML10	66	F	AML, [AML 16] [FLAG x2] CR2	normal	MUD	34 months post SCT	Skin and gut	2.7	46 months post transplant: In Remission and well
AML11	67	F	AML, [DA x2, MIDAC] [FLAG/Myelotarg, FLAG] [FLAG x 2] CR3	normal	2 Cord Bloods	9 months post SCT	Gut	5.4	27 months post transplant: In Remission and well
AML12	41	M	AML, DA x 2, MIDAC, CR1	normal	Sibling	15 months post SCT	Skin	1.8	31 months post transplant: In Remission and well

MUD, matched unrelated donor; ADE, Ara-C, daunorubicin, etoposide; FLAG, fludarabine, Ara-C, idarubicin; DA, daunorubicin, Ara-c; MIDAC; amsacrine, Ara-C, etoposide, mitozantrone; CR = complete remission. (CR1,2,3 = 1st 2nd or 3rd CR). ALC = absolute lymphocyte count ($x10^9$/L)

Table S2. AML patient characteristics.

Supplementary Methods