

Supplemental Figures

Suppression of Glut1 and Glucose Metabolism by Decreased Akt/mTORC1 Signaling Drives T Cell Impairment in B Cell Leukemia

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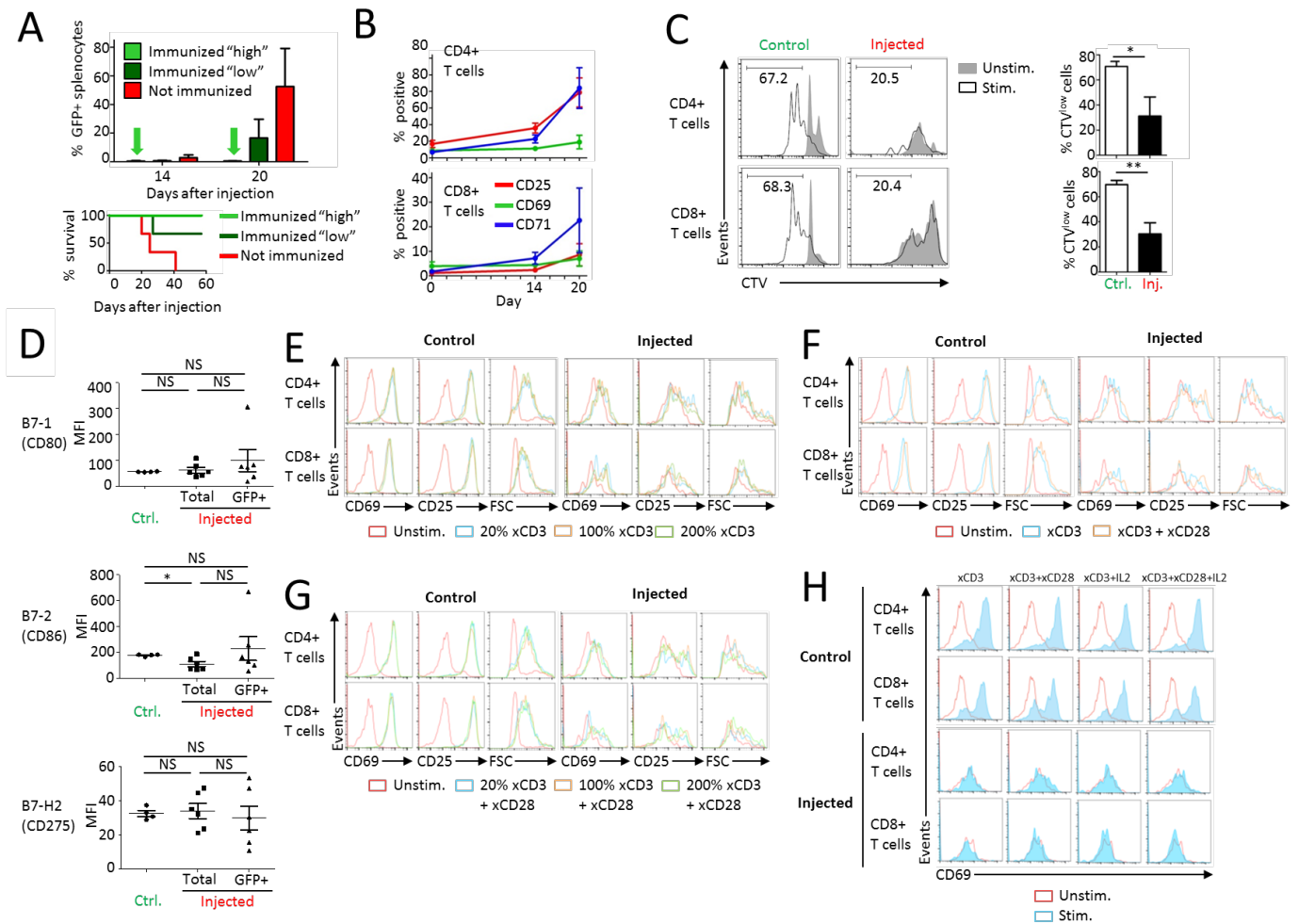
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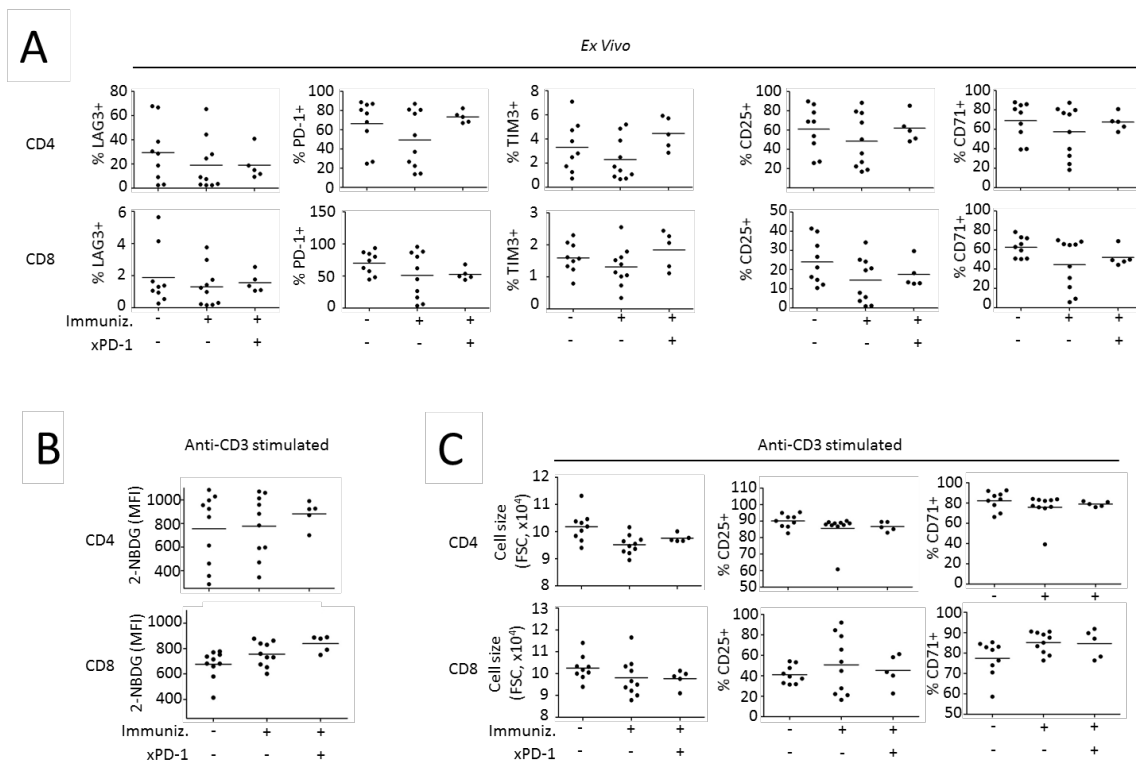
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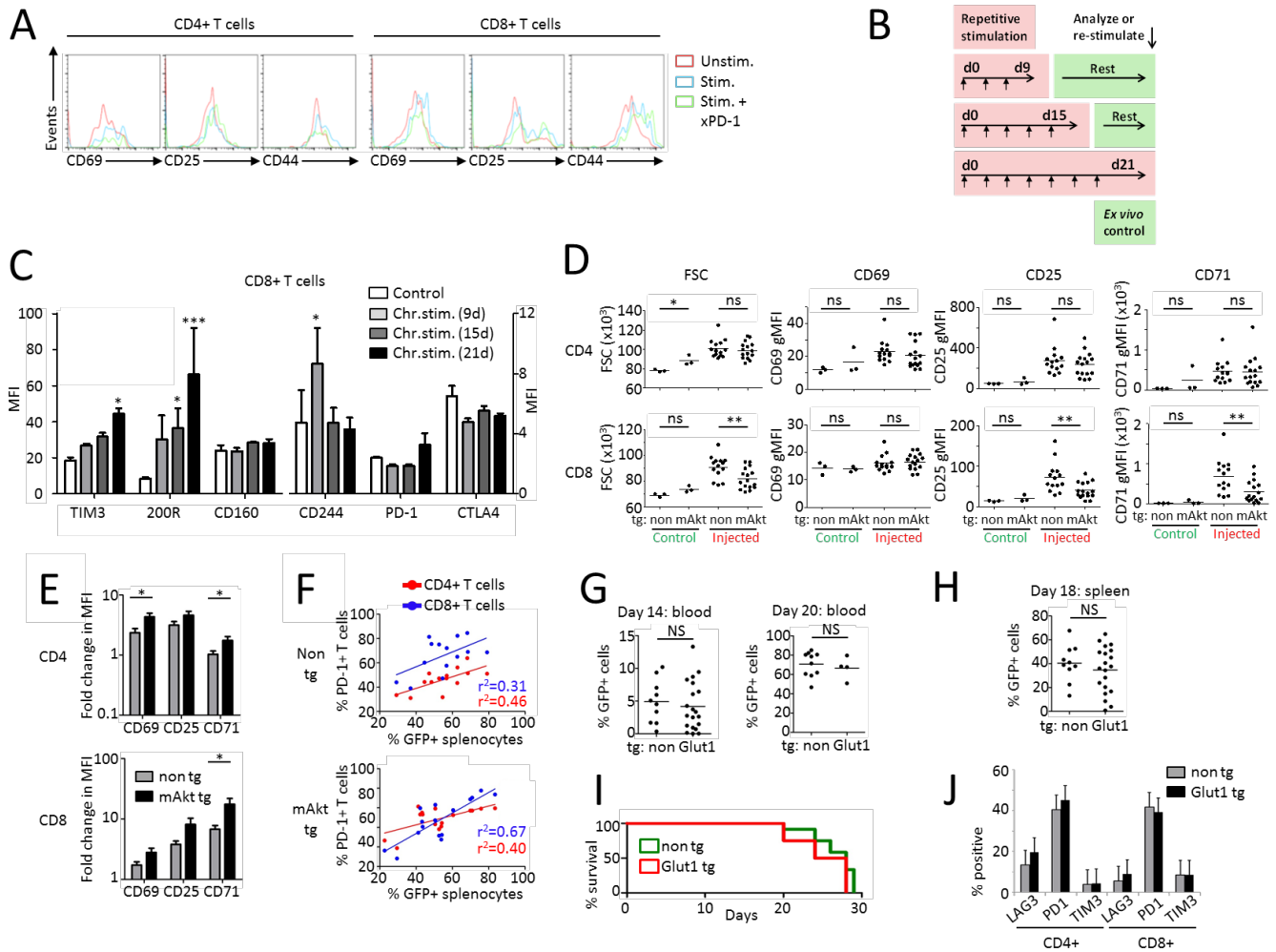
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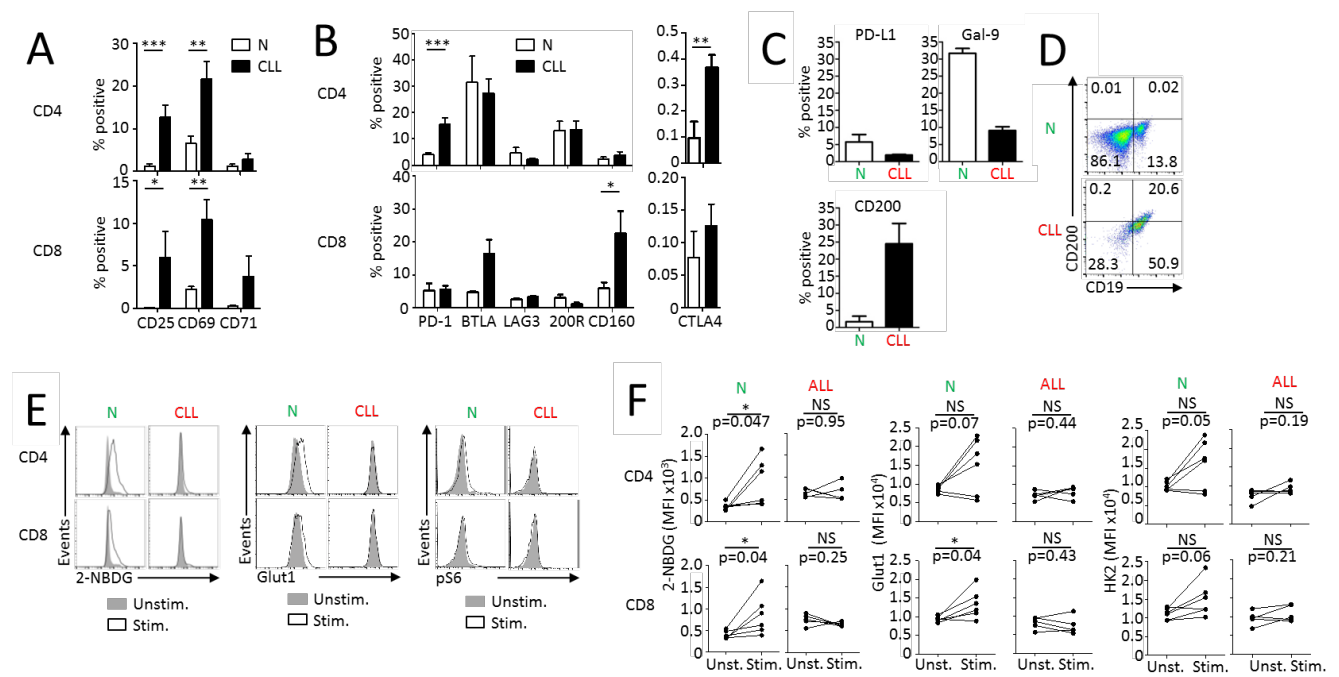
Supplemental Figure 1. Murine B cell lymphoblastic leukemia model to assess leukemia associated T cell dysfunction. BALB/c mice were injected with FL5.12 pro-B-cells harboring BCR/Abl. Leukemia associated T cells in spleens or leukemic cells in blood were analyzed at specified time points. **(A)** Survival and percentage of GFP⁺ cells in blood 14 and 20 after injection of FL5.12 cells with or without immunization with low (0.1×10^6) or high (1×10^6) concentration of irradiated FL5.12 cells ($n=3/\text{group}$). **(B)** Expression of activation markers CD25, CD69 and CD71 assessed by flow cytometry ($n=3-4$ mice/group). **(C)** Splenic T cells were stimulated with anti-CD3 and proliferation of T cells from control animals ($n=3$) or FL5.12 injected animals ($n=2-3$) was measured using Cell Trace Violet dye. **(D)** Expression of B7-1, B7-2 and B7-H2 on total or GFP⁺ splenocytes from injected or control animals. **(E-H)** Control mice ($n=1-2$) or mice injected with FL5.12 cells ($n=1-3$) were stimulated in indicated conditions and expression of activation markers was measured by flow cytometry.



Supplemental Figure 2. In vivo PD-1 blockade is not sufficient to revert B cell leukemia induced T cell dysfunction. BALB/c mice were immunized with 0.02×10^6 irradiated FL5.12 cells seven days prior to leukemia transfer. Immunized mice were injected with live FL5.12 cells and were subject to i.p. anti-PD-1 therapy ($250 \mu\text{g}/\text{mouse}$) every three days for total of four doses. **(A)** Expression of activation and exhaustion markers *ex vivo*. Glucose uptake **(B)**, cell size and expression of CD25 and CD71 **(C)** on T cells from leukemia bearing animals with or without anti-PD-1 therapy.



Supplemental Figure 3. The role of inhibitory pathways and chronic stimulation in the induction of metabolic T cell dysfunction. (A) T cells from FL5.12 leukemia-bearing mice were *in vitro* stimulated with or without anti-PD-1 treatment and expression of specified markers was assessed by flow cytometry. (B-C) Purified healthy human blood T cells were repeatedly stimulated with low-dose anti-CD3, anti-CD28, anti-CD2 coated beads for indicated times with or without rest until restimulation on day 21. (B) Model. (C) Expression of specified markers on CD8+ T cells. (D-F) Splenic T cells from FL5.12 injected mice with (n=16) or without (n=14) T cell specific mAkt tg were stimulated over-night with anti-CD3 and expression of CD69, CD25 and CD71 was measured with flow cytometry. Fold change in MFI was calculated as the MFI ratio of stimulated/unstimulated cells. (G,H) Splenocytes and blood samples from FL5.12 injected mice with or without T cell specific Glut1 tg were analyzed 14 or 20 days after injection and percentage of GFP+ cells was assessed with flow cytometry. (I) Survival of FL5.12 injected mice with (n=4) or without (n=12) T cell specific Glut1 tg. (J) *Ex vivo* expression of indicated markers on T cells from mice described in G,H. Data are mean \pm s.e.m. * P < 0.05, ** P < 0.01 and *** P < 0.001.



Supplemental Figure 4. T cells from human B cell leukemia patients show signs of chronic stimulation and exhaustion that correlate with metabolic defects after *in vitro* stimulation. (A,B) Peripheral blood mononuclear cells (PBMCs) from 5-8 healthy donors (N) and CLL patients (n=6) were analyzed for the specified markers using flow cytometry. The percentage of positive cells was established using not stained controls. (C,D) Expression of PD-L1, Gal-9 and CD200 measured on total PBMCs (C) or CD19+ B cells (D) from healthy donors (n=2) and CLL patients (n=6). (E) CLL cohort 2 was analyzed as CLL cohort 1 (Fig. 5E). PBMCs from CLL patients (n=5) or healthy controls (n=4) were stimulated for three days with anti-CD3 and indicated measurements were performed using flow cytometry. (F) Samples from healthy controls (n=5) or BCR/Ab1+ B cell ALL patients (n=5) were stimulated and analyzed as in E.

A

Nr.	Sex	Age (yr.)	WBC	Ever treated	Rai stage	FISH	Zap70	IgH mutated
		At draw date			(at diagnosis)			
CLL Cohort 1 Patient Data								
3	m	47	105.4	no	0	13q+17p	pos	no
4	f	71	43.2	no	0	13q	pos	yes
5	f	68	95.9	no	0	tri12	pos	no
7	m	67	37.9	yes	0	11q+13q	pos	ND
8	m	67	33.7	no	0	17p	neg	no
9	m	61	105.5	yes	2	13q	pos	yes
19	f	67	27.5	no	0	13q+tri12	neg	yes
20	m	63	283.2	no	0	13q	pos	no
21	f	86	53.7	yes	0	13q+17p	neg	no
26	f	55	54.3	no	0	13q	neg	yes
27	m	78	37.1	yes	0	17p+tri12	neg	yes
28	f	87	61	yes	1	17p	neg	yes
29	f	68	25.9	no	0	normal	neg	yes
30	m	72	33.9	yes	0	tri12	neg	yes
31	f	73	103.2	no	0	ND	pos	yes
32	f	62	32.5	no	0	ND	neg	yes
33	m	48	269	no	1	ND	neg	no
34	m	73	150.3	no	0	tri12	neg	no
36	m	66	88.1	no	2	ND	pos	no
39	m	79	45.8	yes	0	17p+tri12	neg	yes
40	m	61	126.6	yes	2	13q	pos	yes
41	m	73	43.3	no	0	13q	pos	yes
42	m	63	36.6	no	ND	ND	pos	ND
43	m	79	12	yes	0	13q	pos	yes
44	m	71	62.1	no	0	normal	neg	yes
45	m	69	33.5	no	0	13q	pos	no
46	m	75	37	no	0	13q	pos	yes
47	m	60	17.1	no	0	13q	neg	ND
14	m	64	58.2	no	0	tri12	pos	no
38	f	74	49.2	no	0	13q	neg	yes
37	ND	58	70.8	no	ND	ND	neg	yes
30	ND	72	33.9	yes	0	tri12	pos	yes
CLL Cohort 2 Patient Data								
1292	m	55	85.6	no	0	ND	ND	yes
1706	m	66	74.6	no	0	ND	ND	ND
1664	m	66	32.07	no	2	ND	ND	ND
1418	v	64	115	no	0	ND	ND	polyclonal
1754	m	46	180	no	ND	ND	ND	ND

B

ALL Patient Data						
Nr.	Sex	Age (yr.)	Karyotype	Phenotype	Blast %	
15-08-020	m	63	46,XY,t(9;22)(q34;q11.2)[1]/47~48,idem, +2,+5,+8[cp5]/46,XY[1]	93% of cells = B-Lymphoblasts (CD19+, CD20-)	87.5	
15-11-009	f	59	46,XX,t(9;22)(q34;q11.2)[1]/46,idem,del(12)(p13),der(19)t(17;19)(q21;p13)[16]/ 46,XX[3]	Abnormal B-Lymphoblasts. CD10+, CD19+, CD34(+), CD58+, CD20-	80	
16-01-019	m	67	39~43,XY,add(1)(p36.3),-3,-7-9,t(9;22)(q34;q11.2),-13,-14,+mar[cp17]/46,XY[3]	B Lymphoblasts; CD9+, CD19+, CD34(+), CD58+, CD20-	95	
110304A	m	43	46,XY,t(9;22)(q34;q11.2)[2]/46,XY[5]	CD19+, CD33-, CD45dim lymphoblasts	100	
110615B	m	70	46,XY,t(9;22)(q34;q11.2)[20]	Atypical blasts CD19+, CD10+, CD34+, CD20-	51.5	

Supplemental Table 1. Patient Characteristics.