Phospho-specific flow cytometry reveals signaling heterogeneity in T-cell acute lymphoblastic leukemia cell lines

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Supplementary Tables

Supplementary Table S1. Antibodies used for the detection of protein expression

Antibody	Clone	Fluorochrome	Manufacturer
CD1a	HI149	FITC	BD Biosciences
CD2	S5.2	V450	BD Biosciences
cCD3	SK7	PE-Cy7	BD Biosciences
mCD3	UCHT1	APC	BD Biosciences
CD5	L17F12	PerCp-Cy5.5	BD Biosciences
CD7	M-T701	APC	BD Biosciences
CD8	SK1	FITC	BD Biosciences
CD45	HI30	PerCp-Cy5.5	BD Biosciences
CD127	HIL-7R-M21	PE-Cy7	BD Biosciences
CD184	12G5	PE	BD Biosciences

cCD3: cytoplasmic CD3; mCD3: membrane CD3.

Supplementary Table S2. Modulators used in the study

Modulator	Concentration	Manufacturer
Unmodulated	-	-
H2O2	3,3 mM	Sigma-Aldrich
PMA	400 nM	Sigma-Aldrich
IL-7	10 ng/mL	Peprotech
CXCL12	100 ng/mL	Peprotech

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Supplementary Table S3. Antibodies used for the detection of phosphoproteins

Antibody	Clone	Fluorochrome	Manufacturer
pSTAT5 (Y694)	47/Stat5(pY694)	Alexa Fluor 488	BD Biosciences
pp38 (T180/Y182)	36/p38(pT180/pY182)	Alexa Fluor 488	BD Biosciences
pRb (S780)	J146-35	Alexa Fluor 488	BD Biosciences
pLck (Y505)	MOL171	Alexa Fluor 488	BD Biosciences
pNF-κB p65 (S529)	K10-895.12.50	PE	BD Biosciences
pErk1/2 (T202/Y204)	20A	PE	BD Biosciences
pZAP70 (Y292)	J34-602	PE	BD Biosciences
pZAP70 (Y319)	17A/P-ZAP70	PE	BD Biosciences
pAkt (S473)	M89-61	Alexa Fluor 647	Cell Signaling Technologies
pJNK (Thr183/Tyr185)	G9	Alexa Fluor 647	Cell Signaling Technologies
pSTAT3 (S727)	49/p-Stat3	Alexa Fluor 647	BD Biosciences
CD45 (for HDs)	HI30	PerCp-Cy5.5	BD Biosciences
cCD3*	UCHT1	PE-Cy7	BD Biosciences

*: cytoplasmic CD3

Supplementary Table S4. Staining panels for the detection of phosphoproteins

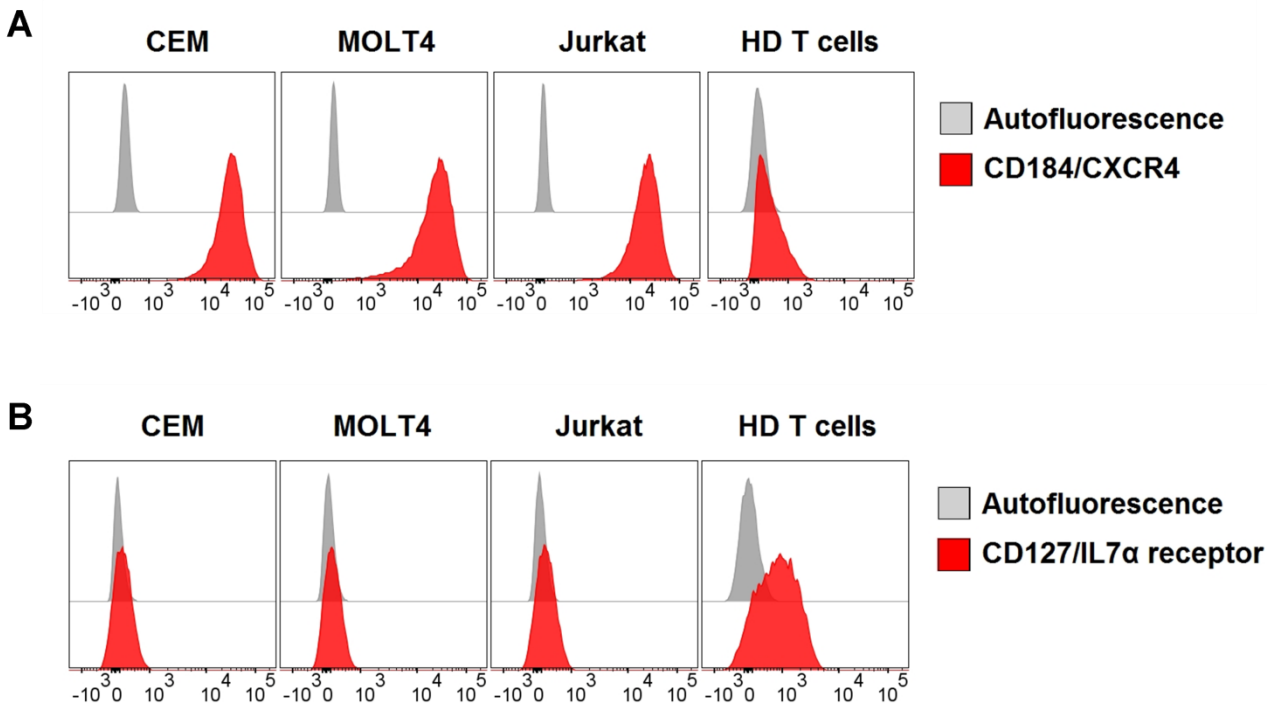
Alexa Fluor 488	PE	PerCp-Cy5.5	Pe-Cy7	Alexa Fluor 647	Near-IR
pSTAT5	pNF-kB p65	CD45	cCD3	pAkt	LIVE/DEAD
pp38	pErk1/2	CD45	cCD3	pJNK	LIVE/DEAD
pRb	pZAP70 (Y292)	CD45	cCD3	pSTAT3	LIVE/DEAD
pLck	pZAP70 (Y319)	CD45	cCD3	-	LIVE/DEAD

cCD3: cytoplasmic CD3.

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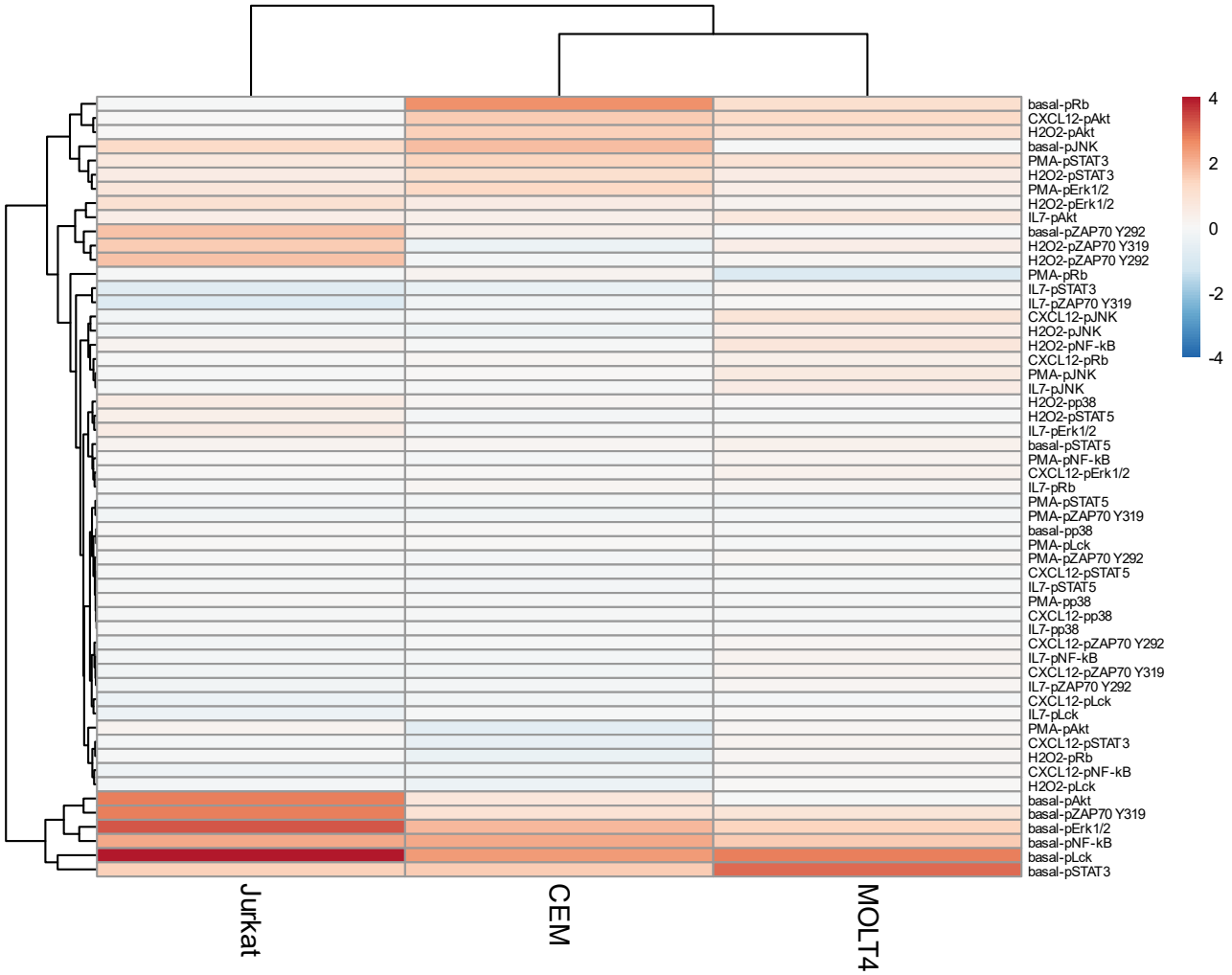
Supplementary Figures



Supplementary Figure S1. Comparison of expression of receptors for physiological stimuli. Surface expression of CD184/CXCR4 (**A**) and CD127/IL7α (**B**) on T-ALL cell lines and T cells from healthy donor (HD) was evaluated by flow cytometry, using autofluorescence as control. Data are representative of two independent experiments.

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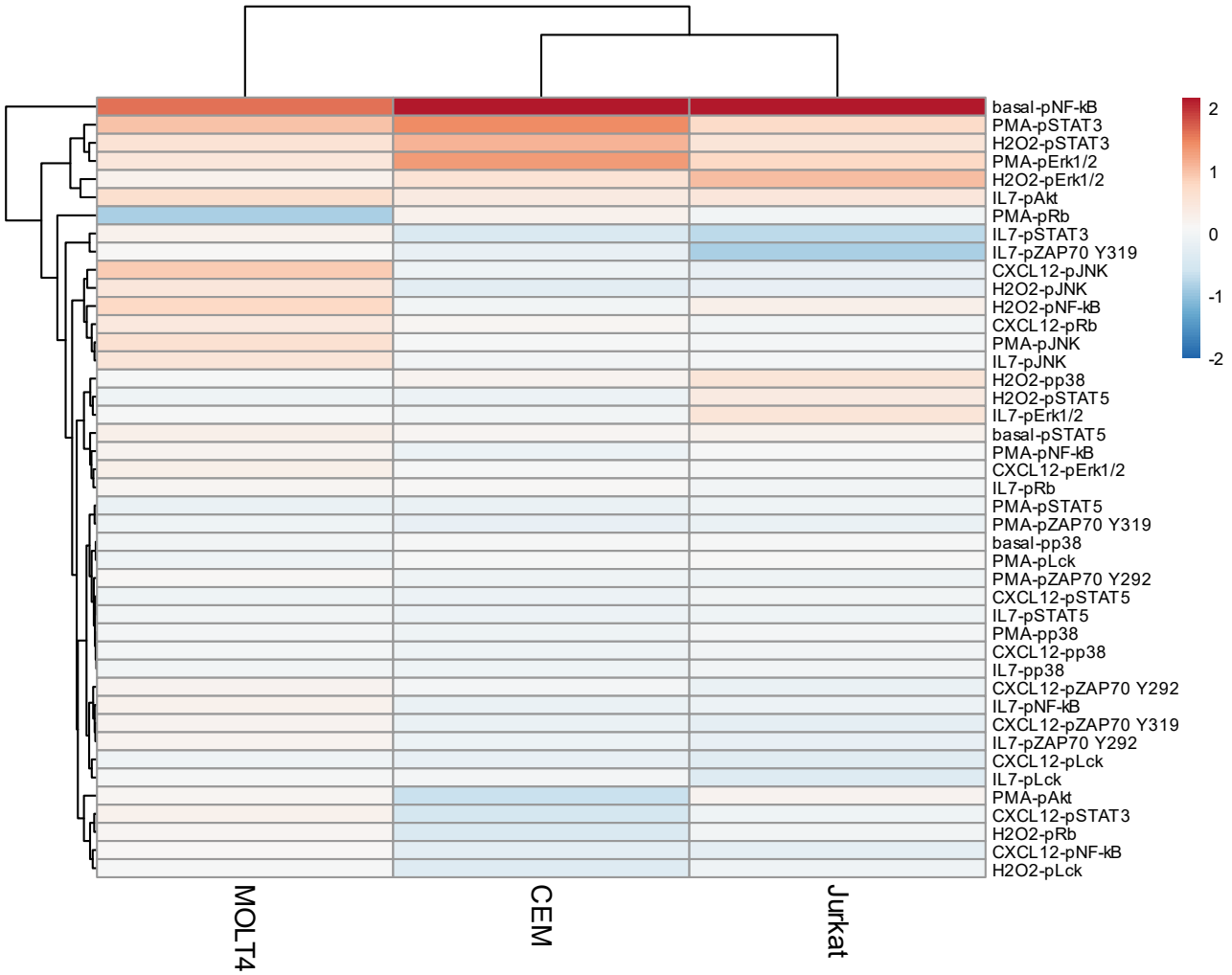
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Supplementary Figure S2. Unsupervised hierarchical clustering analysis of all the analyzed nodes. Unsupervised hierarchical clustering analysis of all the analyzed nodes (n=55) within the T-ALL cell lines CEM, MOLT4, and Jurkat.

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Supplementary Figure S3. Unsupervised hierarchical clustering analysis on selected nodes. Unsupervised hierarchical clustering analysis of nodes (n = 43) following exclusion of nodes showing higher variation (σ^2 of $\log_2$ values >0.5) across samples within the T-ALL cell lines CEM, MOLT4, and Jurkat.