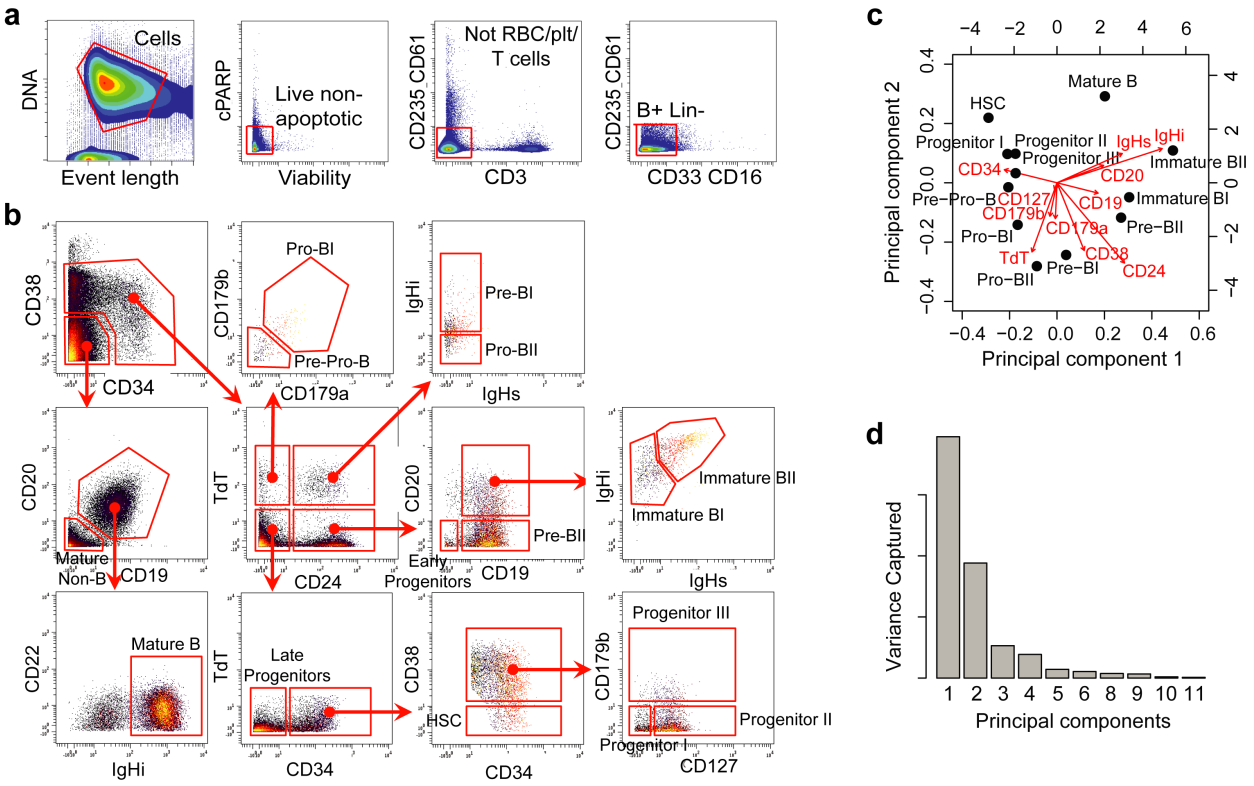
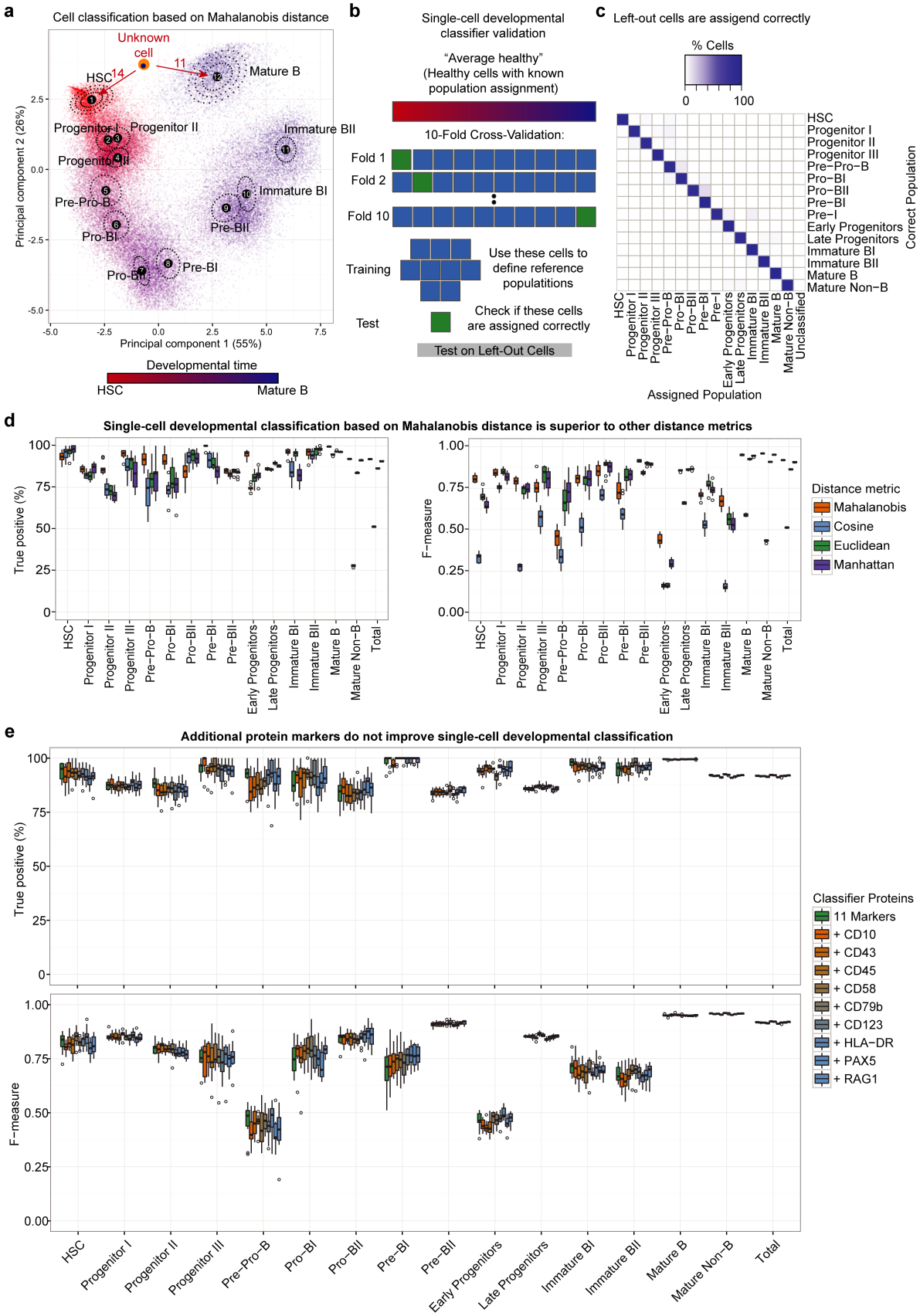
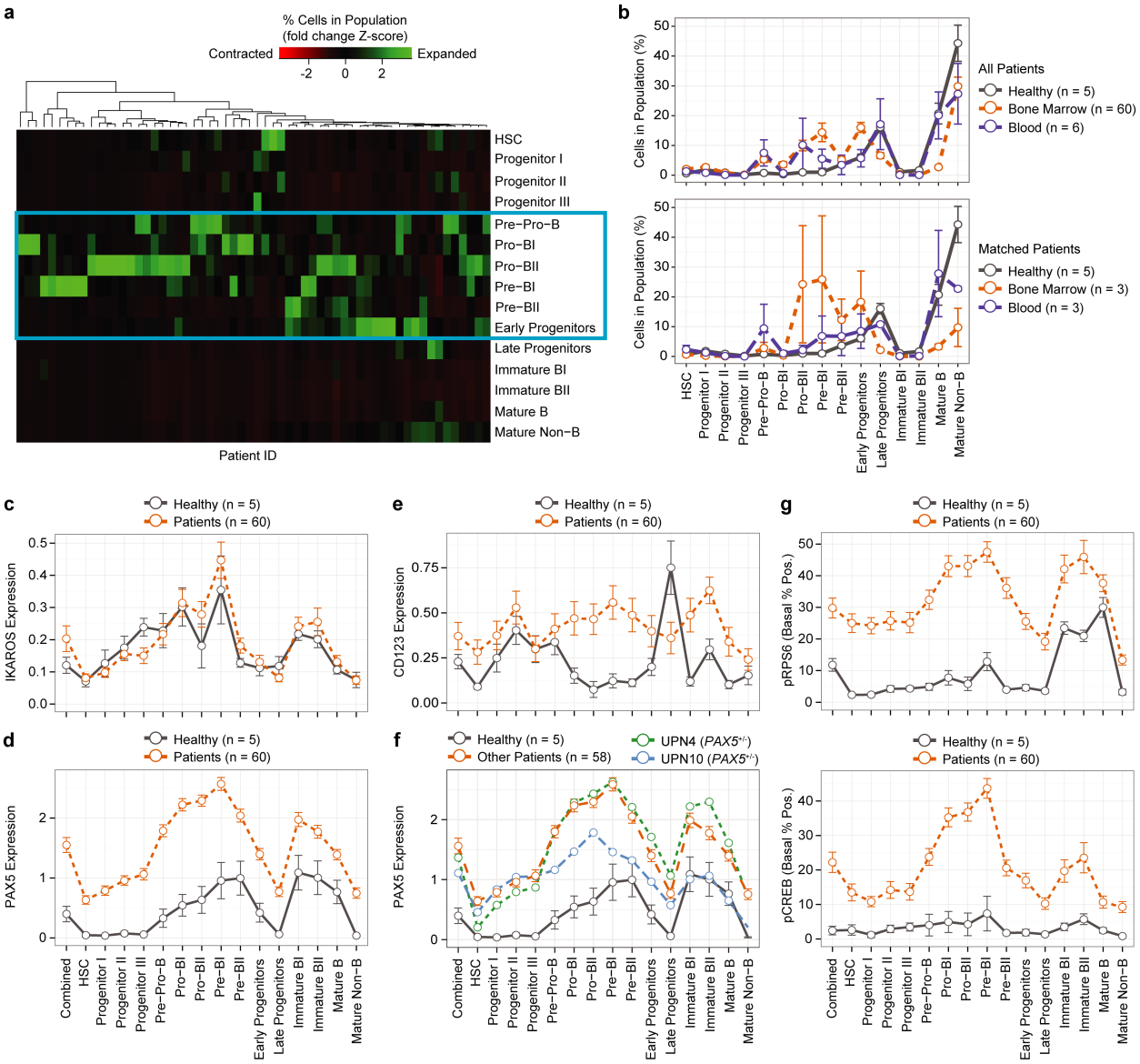
Supplementary Information



Supplementary Figure 1. Gating strategy for leukemic blasts and normal developing B cells. (a) Gating strategy for lineage-negative blasts (B^+ Lin^- cells), the starting population for all subsequent analyses of normal and leukemic samples. Mass cytometry results from a representative (of n = 5) lineage-depleted healthy donor bone marrow aspirate are shown. **(b)** Gating strategy to identify 12 subpopulations of B lymphopoiesis and 3 non-B populations among B^+ Lin^- cells from (a). **(c)** Principle component analysis (PCA) supplement for healthy populations from 5 healthy bone marrow donors shown in Figure 1c. Arrows indicate protein vectors defining the PCA space. **(d)** Bar plot demonstrating how much variance is captured by each principal component in (c).

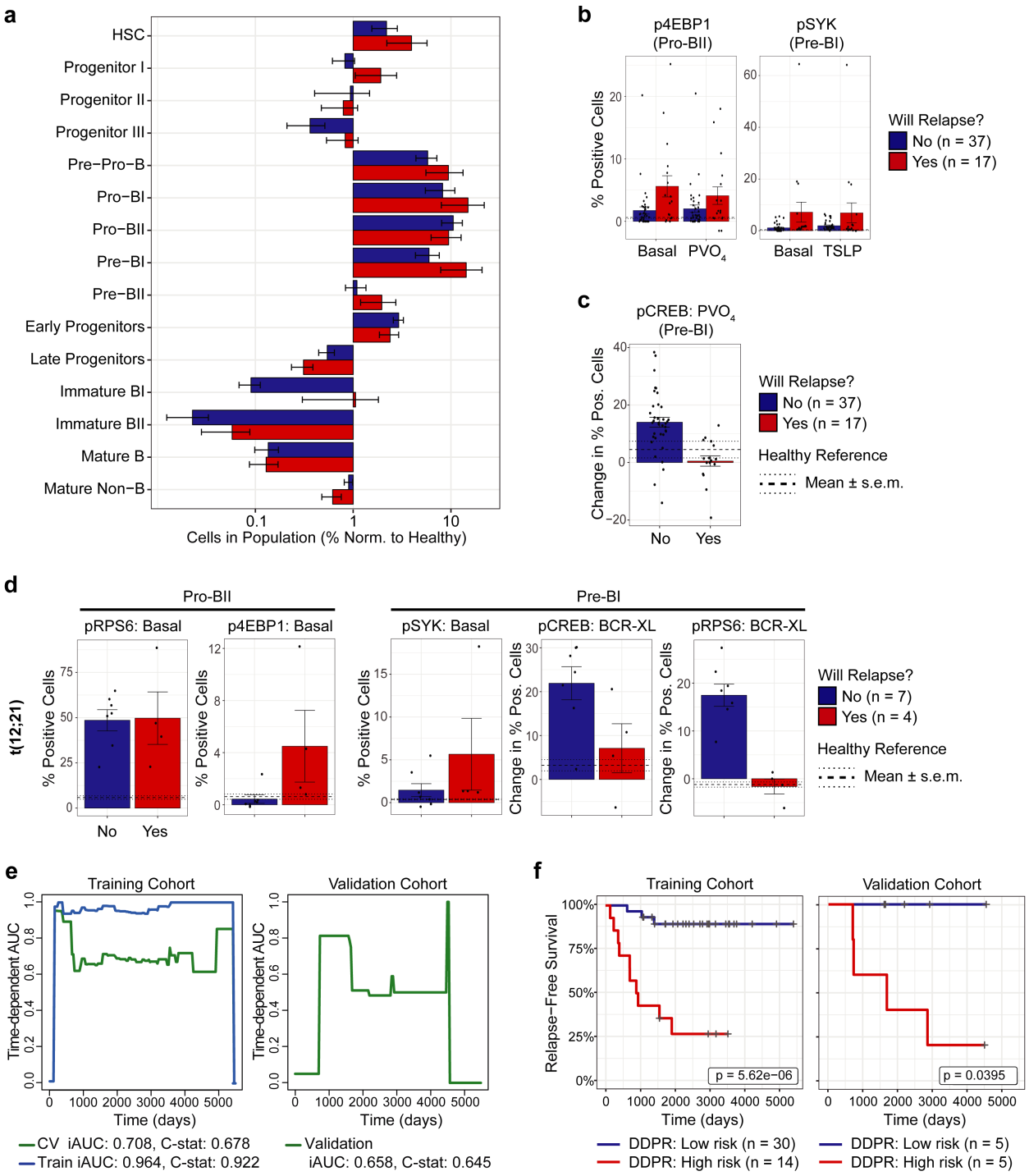

a
Cell classification based on Mahalanobis distance
Unknown cell
HSC
Mature B
Progenitor I
Progenitor II
Progenitor III
Immature BII
Immature BI
Pre-Pro-B
Pre-BII
Pro-BI
Pre-BI
Principal component 2 (26%)
Principal component 1 (55%)
Developmental time
HSC
Mature B
b
Single-cell developmental classifier validation
"Average healthy" (Healthy cells with known population assignment)
10-Fold Cross-Validation:
Fold 1
Fold 2
Fold 10
Training
Use these cells to define reference populatitions
Test
Check if these cells are assigned correctly
Test on Left-Out Cells
c
Left-out cells are assigend correctly
% Cells
0
100
Correct Population
Assigned Population
HSC
Progenitor I
Progenitor II
Progenitor III
Pre−Pro−B
Pro−BI
Pro−BII
Pre−BI
Pre−I
Early Progenitors
Late Progenitors
Immature BI
Immature BII
Mature B
Mature Non−B
Unclassified
d
Single-cell developmental classification based on Mahalanobis distance is superior to other distance metrics
True positive (%)
F−measure
Distance metric
Mahalanobis
Cosine
Euclidean
Manhattan
Total
e
Additional protein markers do not improve single-cell developmental classification
True positive (%)
F−measure
Classifier Proteins
11 Markers
+ CD10
+ CD43
+ CD45
+ CD58
+ CD79b
+ CD123
+ HLA−DR
+ PAX5
+ RAG1

Supplementary Figure 2. Optimization of single-cell developmental classifier. (a) Single-cell developmental classification assigns a given cell to its most similar healthy population based on the shortest Mahalanobis distance. Mahalanobis distance takes into account the center of a given population as well as its variation in high-dimensional space (covariance matrix). Thus, a cell with the same Euclidean distance to two populations (*e.g.*, HSC and mature B cells) is closer to that population with higher variation (in this example, mature B cells). **(b)** Schematic of single-cell developmental classifier validation. Cells from healthy samples were averaged into an "average healthy", and then 10-fold cross-validation was performed to assess developmental classifier performance in (c-e). **(c)** Heatmap demonstrating effect of misclassification in healthy controls. In rare cases when the developmental classifier misclassified a cell, it was most often assigned to a nearby population. **(d)** Optimization of distance metric for developmental classifier using true positive rate (left) or F-measure (right). **(e)** Optimization of proteins used for cell assignment. Addition of individual markers does not improve either true positive rate (top) or F-measure (bottom) over the original 11 developmental proteins. Box plots in (d) and (e) show 10 predicted (via cross-validation) values from 4 pooled healthy donors; box is defined by the first and third quartiles, band inside shows the median, whiskers show 1.5x interquartile range, and circles show outliers.



Supplementary Figure 3. BCP-ALL viewed through the 'lens' of normal B-cell development. (a) Heatmap demonstrates developmental classification of each of 60 primary BCP-ALL diagnostic samples (frequencies of cells in each developmental fraction normalized to an average of healthy bone marrow controls; Z-scores for each patient are shown; columns are hierarchically clustered data from individual patients). Blue box indicates area of shared expansion across most samples. **(b)** Comparison of developmental classification of bone marrow and peripheral blood samples obtained at diagnosis (all patients, top; matched samples

when available from the same patient, bottom). **(c-e)** Mean arsinh-transformed expression of IKAROS (c), PAX5 (d), and CD123 (e) on healthy bone marrow (grey) *vs.* classified leukemia (orange) populations. **(f)** Mean arsinh-transformed expression of PAX5 in two patients with known heterozygous deletions in *PAX5*: UPN4 (exons 2-6) and UPN10 (exons 1-10). The anti-PAX5 antibody used for mass cytometry was raised against the portion of the protein encoded by exons 5-7. PAX5 expression in bone marrow from 5 healthy donors (grey) and in other BCP-ALL patients (orange) is shown for reference. **(g)** Percentages of cells positive for pRPS6 (top) and pCREB (bottom) in healthy bone marrow *vs.* classified leukemia populations. Graphs in (b-g) show mean ± s.e.m. for all available diagnostic samples. “Combined” in (c-g) denotes expression in all cells without developmental classification.

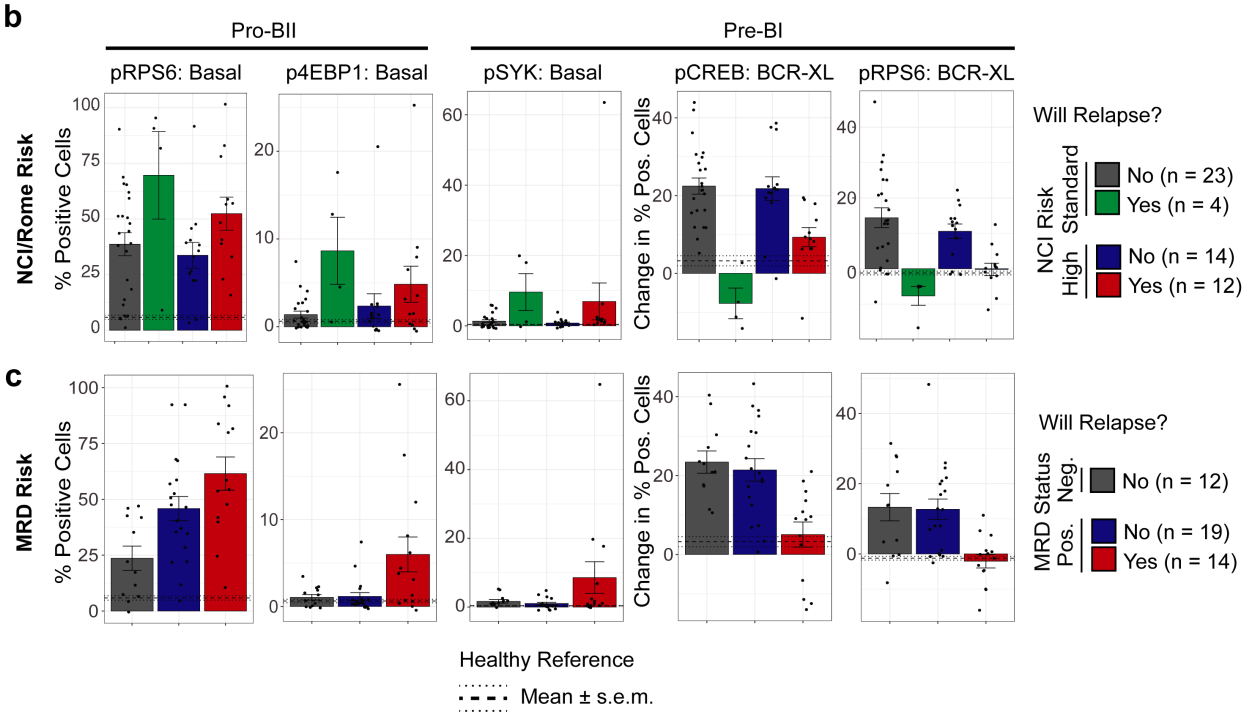


Supplementary Figure 4. Cellular features at diagnostic for patients who either would, or would not go on to relapse. (a) Developmental classification of diagnostic bone marrow samples binned by the last documented relapse status. Patients who would go on to relapse are shown in red (n = 17), patients who would remain in continuous remission are in blue (n = 37).

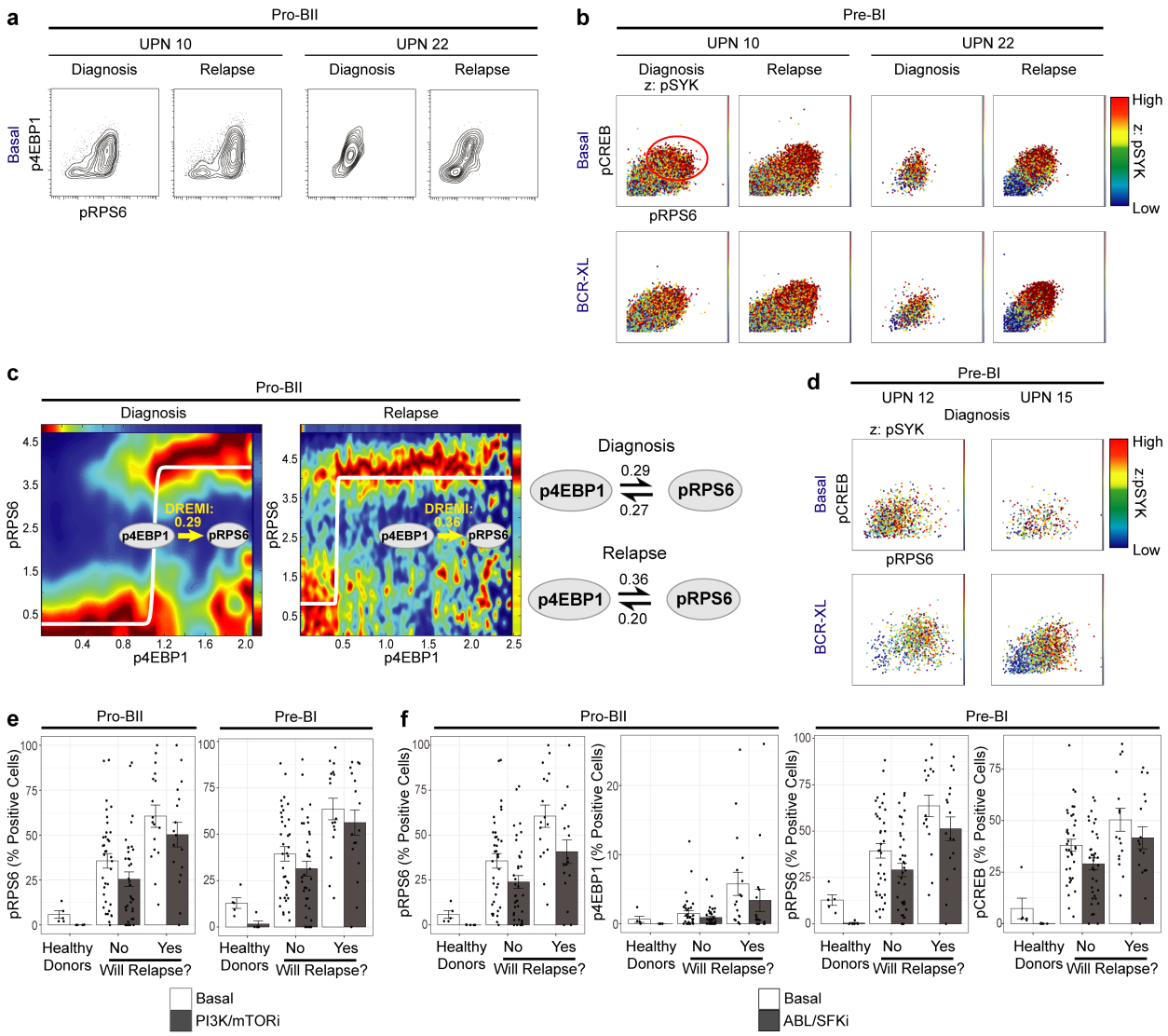
There are no significant differences in the frequencies of classified populations between groups (unpaired two-tailed Welch's *t* test with Bonferroni correction for multiple comparisons). **(b)** Bar plots of basal and stimulated levels of activated signaling proteins identified by DDPR: p4EBP1 in pro-BII in response to pervanadate (left) and pSYK in pre-BI in response to TSLP (right). **(c)** A bar plot of an additional cellular feature identified by DDPR: pCREB in pre-BI in response to pervanadate. Bar plots in (a-c) show mean ± s.e.m. for 54 patients with ≥3 years of follow-up; where indicated, dashed lines show mean levels in the corresponding developmental populations within healthy bone marrow aspirates of five healthy donors; dotted lines indicate one standard error. **(d)** Key DDPR features for all patients bearing the same single prognostic genetic translocation of chromosomes 12;21 (n = 11) are shown as bar plots (mean ± s.e.m). **(e)** Time-dependent AUC curves showing performance for relapse prediction in the training (left; CV test: green, overall training fit: blue) and validation (right) cohorts using bulk leukemic blast data without developmental classification. Integrated dynamic/cumulative AUC (iAUC) and C-statistic (C-stat) summary measures are shown for each curve. **(f)** Kaplan-Meier analysis of relapse-free survival in training (left; based on pre-validated relative risk) and validation (right; based on predicted relative risk) cohorts stratified by DDPR risk group (see Methods). RFS estimates with standard error, number of patients at risk, and p-values for both groups at 5 and 7 years are as follows: (left) training cohort: 5 years, low risk (n = 20) 89.4±5.8% *vs.* high risk (n = 4) 35.7±12.8%, $p = 6.7 \times 10^{-3}$; 7 years, low risk (n = 14) 89.4±5.8% *vs.* high risk (n = 3) 26.8±12.3%, $p = 7.4 \times 10^{-5}$; (right) validation cohort: 5 years, low risk (n = 3) 100.0±0.0% *vs.* high risk (n = 2) 40.0±21.9%, p = 0.20; 7 years, low risk (n = 2) 100.0±0.0% *vs.* high risk (n = 2) 40.0±21.9%, p = 0.063. P-values were calculated using the log-rank test. CV, cross-validation; TSLP, thymic stromal lymphopoietin; PVO_4, pervanadate; t(12;21), translocation t(12;21)(p13;q22) *ETV6/RUNX1*.

a

	NCI Risk + DDPR	MRD Risk + DDPR	Final Risk + DDPR
Integrated Discrimination Improvement Index	0.403 (0.097 - 0.618) p = 0.020	0.345 (0.003 - 0.593) p = 0.040	0.381 (0.028 - 0.640) p = 0.040
Net Reclassification Improvement	0.723 (0.089 - 0.873) p = 0.027	0.315 (0.022 - 0.579) p = 0.033	0.715 (0.030 - 0.900) p = 0.013
Median Improvement	0.375 (0.057 - 0.618) p = 0.013	0.313 (0.002 - 0.834) p = 0.020	0.697 (0.043 - 0.840) p = 0.020



Supplementary Figure 5. Comparison of DDPR performance to current clinical standards in patient risk stratification. (a) Improvement in relapse prediction at 5 years resulting from combining DDPR with current risk stratification methods, as assessed by integrated discrimination improvement index, continuous net reclassification improvement, and median improvement for censored time-to-event data. Shown: estimate (95% confidence interval) p-value in n = 53 (NCI risk + DDPR) and n = 45 (MRD risk + DDPR and Final Risk + DDPR) patients with ≥3 years of follow-up and available risk data. **(b-c)** Key DDPR features when stratified by the last documented relapse status and either NCI/Rome risk group (b, n = 53), or MRD risk (c, n = 45). Bar plots in (b-c) show mean ± s.e.m. for all patients with known risk status and ≥3 years of follow-up. BCR-XL, B-cell receptor crosslink; MRD, minimal residual disease.



Supplementary Figure 6. Single-cell analysis and targeting of DDPR functional features at diagnosis and relapse. **(a)** Single-cell data demonstrating two DDPR proteins in pro-BII cells in basal state: pRPS6 (X-axis) and p4EBP1 (Y-axis) in 2 representative (of n = 7) diagnosis-relapse pairs. **(b)** Single-cell data demonstrating three DDPR proteins in pre-BI cells in basal state (top) and in response to BCR crosslinking (BCR-XL; bottom): pRPS6 (X-axis), pCREB (Y-axis), and pSYK (Z-axis) in two representative diagnosis-relapse pairs. **(c)** Same as (b), but for two representative diagnostic samples from n = 37 patients who did not relapse. **(d)** DREMI analysis and DREVI visualization for DDPR features in pro-BII cells. Up to 5,000 cells from

matched diagnosis-relapse pairs (n = 7) were sampled and pooled prior to analysis. Left: Estimated conditional density functions for p4EBP1→pRPS6 at diagnosis and relapse; sigmoidal response functions were fitted to each plot. Right: Quantification for strengths of pairwise signaling relationships within the network formed by p4EBP1 and pRPS6 in pro-BII cells from paired diagnosis-relapse patient samples. **(e)** Response of DDPR features to short-term *ex vivo* treatment (as in Supplementary Table 3) in healthy donors (n = 5) or diagnostic samples (no relapse: n = 37, relapse: n = 17): effects of a dual PI3K and mTOR inhibitor (PI3K/mTORi) BEZ235 on the frequencies of pRPS6+ pro-BII and pre-BI cells. **(f)** Effects of a dual BCR-ABL and SRC family kinase inhibitor (ABL/SFKi) dasatinib on frequencies of pRPS6+ and p4EBP1+ pro-BII cells and on frequencies of pRPS6+ and pCREB+ pre-BI cells in healthy donors (n = 5) or diagnostic samples (no relapse: n = 37, relapse: n = 17).

Supplementary Items. Related to Methods.

Supplementary Table 1. Clinical, cytogenetic, and outcome data for the BCP-ALL patient cohort.

Patient ID*	Gender	Age at Diagnosis	WBC Count (Cells/μL)	Prognostic Translocations	Treatment Protocol	Final Risk	MRD Risk	NCI/Rome Risk	Date of Diagnosis	Relapse Status[§]	Time to Relapse (Days)	Type of Relapse[#]	CCR (Days)	Cohort	DDPR Risk
UPN1	Male	2	44100	Negative	AIEOP2000	Intermediate	Intermediate	Standard	10/23/00	Yes[M]	1043	Early	NA	Training	**Low**
UPN2	Male	2	36650	t(1;19)	AIEOP2000	Intermediate	Intermediate	Standard	7/31/01	No	NA	NA	5406	Training	Low
UPN3	Female	1	49250	Negative	AIEOP2000	Standard	Standard	Standard	12/2/02	No	NA	NA	4917	Training	Low
UPN4	Male	2	33000	t(12;21)	AIEOP2000	Standard	Standard	Standard	12/16/03	No	NA	NA	4538	Validation	Low
UPN5	Female	2	150000	Negative	AIEOP2000	Standard	Standard	High	2/2/04	No	NA	NA	4490	Validation	**High**
UPN6	Female	5	113000	t(12;21)	AIEOP2000	Intermediate	Intermediate	High	10/29/04	No	NA	NA	4220	Training	Low
UPN7	Male	14	230000	t(9;22)	AIEOP2000	High	Intermediate	High	2/25/05	Yes	136	Very early	NA	Training	High
UPN8	Female	3	120000	t(12;21)	AIEOP2000	Intermediate	Intermediate	High	4/1/05	Yes	364	Very early	NA	Training	High
UPN9	Male	5	88670	Negative	AIEOP2000	High	High	High	5/19/05	Yes	237	Very early	NA	Training	High
UPN10	Female	5	23520	t(12;21)	AIEOP2000	Intermediate	Intermediate	Standard	2/20/06	Yes[M]	886	Early	NA	Training	High
UPN11	Female	15	14100	t(12;21)	AIEOP2000	Intermediate	Intermediate	High	4/6/06	No	NA	NA	3696	Training	Low
UPN12	Male	6	48820	Negative	AIEOP2000	Standard	Standard	Standard	8/16/06	No	NA	NA	3564	Training	Low
UPN13	Female	2	21270	t(1;19)	AIEOP2000	Standard	Standard	Standard	9/4/06	No	NA	NA	3545	Training	Low
UPN14	Male	2	53580	t(12;21)	AIEOP2000	Intermediate	Intermediate	High	11/21/06	Yes	2848	Late	NA	Validation	High
UPN15	Male	4	44730	t(12;21)	AIEOP2000	Intermediate	Intermediate	Standard	11/29/06	No	NA	NA	3459	Training	Low
UPN16	Male	12	11500	Negative	AIEOP2000	Intermediate	Intermediate	High	1/21/06	No	NA	NA	3771	Training	Low
UPN17	Male	8	35830	Negative	AIEOP2000	Standard	Intermediate	Standard	9/6/07	No	NA	NA	3178	Training	**High**
UPN18	Male	5	2490	t(12;21)	AIEOP2000	Intermediate	Intermediate	Standard	4/9/08	No	NA	NA	2962	Training	Low
UPN19	Female	3	20300	Negative	AIEOP2000	Standard	Standard	Standard	4/18/08	No	NA	NA	2953	Training	**High**
UPN20	Male	3	34200	Negative	AIEOP2000	Intermediate	Standard	Standard	5/30/08	No	NA	NA	2911	Validation	Low
UPN21	Male	2	116000	t(12;21)	AIEOP2000	Standard	Intermediate	High	6/9/08	Yes	1907	Late	NA	Training	High
UPN22	Male	6	6220	Negative	AIEOP2000	Intermediate	Intermediate	Standard	8/7/08	Yes[M]	1552	Late	NA	Training	High
UPN23	Female	2	21700	Negative	AIEOP2000	Intermediate	Standard	Standard	5/22/08	No	NA	NA	2919	Training	Low
UPN24	Female	3	30680	Negative	AIEOP2000	Intermediate	Intermediate	Standard	12/11/08	No	NA	NA	2716	Training	Low
UPN25	Female	15	257000	Negative	AIEOP2000	Intermediate	Intermediate	High	12/17/08	Yes	698	Early	NA	Training	High
UPN26	Male	13	29200	Negative	AIEOP2000	Intermediate	Intermediate	High	1/15/09	Yes	699	Early	NA	Training	High
UPN27	Male	2	27970	Negative	AIEOP2000	Standard	Intermediate	Standard	6/8/09	No	NA	NA	2537	Training	Low
UPN28	Female	4	36700	t(1;19)	AIEOP2000	Intermediate	Standard	Standard	10/19/09	No	NA	NA	2404	Training	Low
UPN29	Male	10	11610	Negative	AIEOP2000	Intermediate	Intermediate	High	1/18/10	No	NA	NA	2313	Training	Low
UPN30	Male	10	21750	Negative	AIEOP2000	Intermediate	Intermediate	High	5/25/10	No	NA	NA	2186	Validation	Low
UPN31	Female	3	10100	t(1;19)	AIEOP2000	Standard	Standard	Standard	9/11/10	No	NA	NA	2077	Training	Low
UPN35	Male	13	41800	Negative	AIEOP2000	Intermediate	Intermediate	High	11/8/10	Yes[M]	624	Early	NA	Training	**Low**
UPN45	Female	6	59910	Negative	AIEOP2000	Intermediate	Intermediate	High	9/8/06	Yes[M]	700	Early	NA	Validation	High
UPN47	Male	15	1870	CRLF2r	AIEOP2009	High	High	High	12/5/11	Yes	395	Very early	NA	Training	High
UPN48	Male	12	193750	CRLF2r	AIEOP2009	Intermediate	Intermediate	High	10/18/10	No	NA	NA	2229	Training	Low
UPN49	Female	4	15910	CRLF2r	AIEOP2009	High	Intermediate	Standard	11/21/13	No	NA	NA	1099	Training	Low
UPN50	Female	17	5800	CRLF2r	AIEOP2009	Standard	Standard	High	1/13/13	No	NA	NA	1411	Training	Low
UPN51	Male	2	23940	CRLF2r	AIEOP2009	Standard	Standard	Standard	7/5/12	No	NA	NA	1603	Validation	Low
UPN52	Female	12	53640	CRLF2r	AIEOP2009	High	Intermediate	High	8/23/12	No	NA	NA	1554	Training	**High**
UPN53	Female	6	4200	t(12;21)	AIEOP2009	Intermediate	Intermediate	Standard	8/3/11	No	NA	NA	1940	Training	Low
UPN54	Male	4	12650	t(12;21)	AIEOP2009	High	High	Standard	2/29/12	No	NA	NA	1730	Training	Low
UPN55	Female	4	25030	Negative	AIEOP2009	High	High	Standard	3/15/12	No	NA	NA	1715	Training	Low
UPN56	Female	4	34800	Negative	AIEOP2009	Intermediate	Intermediate	Standard	6/5/12	Yes	938	Early	NA	Training	High
UPN57	Female	2	21950	Negative	AIEOP2009	Intermediate	Intermediate	Standard	5/29/12	No	NA	NA	1640	Validation	Low
UPN58	Female	16	6110	Negative	AIEOP2009	Intermediate	Intermediate	High	3/26/13	No	NA	NA	1339	Training	Low
UPN60	Female	1	34580	t(12;21)	AIEOP2009	Intermediate	Intermediate	Standard	6/17/13	NA	NA	NA	NA	NA	NA
UPN62	Male	1	20200	Negative	AIEOP2009	Intermediate	Intermediate	Standard	4/2/14	NA	NA	NA	NA	NA	NA
UPN63	Male	2	18130	t(12;21)	AIEOP2009	Intermediate	Intermediate	Standard	7/10/14	NA	NA	NA	NA	NA	NA
UPN67	Male	17	166370	CRLF2r	AIEOP2009	High	High	High	2/19/15	NA	NA	NA	NA	NA	NA
UPN68	Female	4	1660	CRLF2r	AIEOP2009	Intermediate	Intermediate	Standard	10/10/14	NA	NA	NA	NA	NA	NA
UPN69	Male	4	5890	CRLF2r	AIEOP2009	High	Intermediate	Standard	5/6/15	NA	NA	NA	NA	NA	NA
UPN90	Male	7	NA	t(9;22)	AALL0031	NA	NA	NA	10/1/04	Yes[M]	1400	Late	NA	Training	**Low**
UPN91	Male	18	250000	t(9;22)	AALL622	NA	NA	High	4/6/08	No	NA	NA	2991	Training	Low
UPN92	Male	4	49800	t(9;22)	AALL0031	NA	NA	Standard	4/1/08	No	NA	NA	3163	Training	Low
UPN93	Female	14	45000	t(9;22)	AALL1131	NA	NA	High	3/26/14	No	NA	NA	1073	Training	Low
UPN94	Male	8	143000	t(9;22)	AALL1131	NA	NA	High	4/11/14	Yes	725	Early	NA	Validation	High
UPN95	Male	15	4	t(9;22)	AALL0622	NA	NA	High	4/27/09	Yes[M]	1680	Late	NA	Validation	High
UPN96	Female	9	106000	t(9;22)	AALL0031	NA	NA	High	11/1/06	No	NA	NA	3521	Training	**High**
UPN97	Female	4	11000	t(9;22)	AALL1131	NA	NA	Standard	12/6/12	No	NA	NA	1336	Training	Low
UPN98	Male	15	100000	t(9;22)	AALL0622	NA	NA	High	3/23/09	No	NA	NA	2790	Training	Low

*Patients UPN1 - UPN69 are from Pediatric Clinic University of Milano-Bicocca (Monza, Italy). Patients UPN90 - UPN98 are from Lucile Packard Children's Hospital at Stanford (Stanford, CA, USA). No reported deaths without relapse occurred in this study.

[§]Based on the most recent follow-up data. Yes[M] denotes matched diagnosis-relapse bone marrow pairs.

[#]Clinical definitions for type of relapse:

- Very early: ≤18 months (1.5 years / 540 days for this study) from diagnosis.
- Early: >18m from diagnosis, but ≤6 months from end of therapy (1.5-3.0 years / 540-1100 days for this study).
- Late: >6 months from end of therapy (3.0 years / 1100 days for this study).

CCR, continuous complete remission; DDPR, developmentally dependent predictor of relapse; MRD, minimal residual disease; NCI, National Cancer Institute; WBC, white blood cell.

Supplementary Table 2. Mass cytometry antibody reagents. Surface and intracellular antibody staining panel clones, lot numbers, suppliers, isotope reporters, and final concentrations used for mass cytometry experiments.

Protein	Clone	Lot Number	Manufacturer	Metal Isotope	Final Concentration (µg/mL)	Surface or Intracellular Stain
			Phenotype			
CD10	HI10a	6155527	Biolegend	Gd156	1	S
CD123	6H6	B199259	Biolegend	Eu151	2	S
CD127	HCD127	B173990	Biolegend	Dy162	1	S
CD16	3G8	B175991	Biolegend	FITC*	20	S
CD179a	HSL96	B129864	Biolegend	Sm149	1.5	I
CD179b	HSL11	B179047	Biolegend	Gd158	1	I
CD19	H1B19	B157781	Biolegend	Nd142	2	S
CD20	2H7	B164952	Biolegend	Sm147	2	S
CD22	HIB22	B165323	Biolegend	Nd143	2	S
CD235	HIR2	B132247	Biolegend	In113	2	S
CD24	ML5	B167884	Biolegend	Gd160	2	S
CD3	HIT3a	B151232	Biolegend	Er170	0.5	S
CD33	HIM3-4	B183522	Biolegend	FITC*	20	S
CD34	8G12	B163230	Biolegend	Nd148	1	S
CD38	HIT2	B170151	Biolegend	Er168	2	S
CD43	CD43-10G7	B149905	Biolegend	Er167	2	S
CD45	HI30	B159992	Biolegend	In115	2	S
CD58	TS2-9	B145718	Biolegend	Tm169	2	S
CD61	VI-PL2	B176028	BD Biosciences	In113	1	S
CD79b	CB3-1	4203934	Biolegend	Nd146	6	S
HLA-DR	L243	B161762	Biolegend	Yb174	2	S
IgHi	polyclonal	10689	Invitrogen	Eu153	1	I
IgHs	polyclonal	10689	Invitrogen	Lu175	1	S
IKAROS	D10E5	2	Cell Signaling Technology	Nd145	4	I
IgL kappa	MHK-49	B162243	Biolegend	Sm154	2	I
IgL lambda	MHL-38	B171739	Biolegend	SM154	2	I
PAX5	1H9	B178991	eBioscience	Ho165	1	I
RAG1	D36B3	3968BF	Cell Signaling Technology	Dy163	2	I
TdT	E17-1519	21361	BD Biosciences	Dy164	2	I
CRLF2	1B4	E028811	eBioscience	Dy161	1	S
FITC	FIT-22	B174064	Biolegend	Yb171	2	I
			Functional			
AKT (pS473)	193H12	4060BF	Cell Signaling Technology	Tb159	1	I
4EBP1(pT37/T46)	236B4	18	Cell Signaling Technology	Nd144	1	I
cPARP	F21-852	5089576	BD Biosciences	La139	1.5	I
CREB (pS133)	87G3	9198BF	Cell Signaling Technology	Yb176	0.5	I
ERK1/2 (pT202/pY204)	D13	4370BF	Cell Signaling Technology	Yb173	0.5	I
Ki-67	B56	3305519	BD Biosciences	Sm152	2	I
IKAROS (pS63)	STA9	1	Epitomics (made for Nolan lab)	Gd155	2	I
PLCg2 (pY759)	K86-689.37	26057	BD Biosciences	Pr141	1	I
RPS6 (pS235/pS236)	N7-548	4044686	BD Biosciences	Yb172	2	I
STAT5 (pY694)	47	4044688	BD Biosciences	Nd150	1	I
ZAP70/SYK (pY319/pY352)	17a	85582	BD Biosciences	Er166	2	I

*These were commercial FITC-conjugated antibodies; an anti-FITC metal-conjugated (Yb171) antibody was used as secondary reporter.

Supplementary Table 3. Human marrow perturbations. Stimulation conditions for mass cytometry experiments. Final concentrations are for approximately 1 to 5 million cells in 1 mL volume for the indicated duration.

Perturbation	Manufacturer	Final Concentration	Duration
Basal	----	----	----
Pervanadate*	----	125 μM	15 minutes
IL-7	BD Biosciences	20 ng/mL	15 minutes
BCR Crosslink	Invitrogen MD Biomedicals	Anti-IgM 4 μg/mL H2O2 3.3 mM	5 minutes
TSLP	R&D systems	25 ng/mL	15 minutes
Dasatinib	LC Laboratories	100 nM	30 minutes
BEZ-235	Selleck Chemicals	1 μM	30 minutes
Tofacitinib	Selleck Chemicals	100 nM	30 minutes

*Made in-house from 200 mM sodium orthovanadate (EMD Millipore) dissolved in PBS containing 53 mM H_2O_2.

Supplementary Table 4. Targeted genetic analysis for selected patients.

Patient ID	Genetic Status*								
	IKZF1	*CRLF2r*	*CDK2A/B*	*PAX5*	*ETV6*	*BTG1*	*RB1*	*ERG*	*JAK2*
UPN4	WT	WT	deletion	del (ex 2-6)	deletion	WT	WT	WT	
UPN5	WT	WT	WT	WT	gain	gain	WT	WT	
UPN9	WT	WT	deletion	WT	deletion	WT	deletion	WT	
UPN10	WT	WT	del (homozygous)	del (ex 1-10, het.)	WT	WT	WT	WT	
UPN22	WT	WT	WT	WT	WT	WT	WT	WT	
UPN47	del (ex 2-7)	P2RY8-CRLF2	del (homozygous)	WT	wt	WT	WT		L681- I682 ins GL
UPN48		IGH@CRLF2							R683G
UPN49		P2RY8-CRLF2							WT
UPN50	del (ex 2-3)	P2RY8-CRLF2	deleted	del ex 1 and 5-6	WT	WT	WT		WT
UPN51	WT	P2RY8-CRLF2	WT	WT	WT	WT	WT		WT
UPN52	WT		WT	WT	WT	WT	WT		WT
UPN53	del (ex 2-3)	WT	deleted	WT	WT	WT	WT		WT
UPN54	WT	WT	WT	WT	deleted	del ex 2	WT		WT
UPN55	WT	WT	WT	WT	del (ex 5-6, hom.)	WT	WT		WT
UPN56	deleted	WT	deleted (all chr 9)	del (ex 7-10)	WT	WT	WT		WT
UPN57	WT	WT	WT	WT	WT	WT	WT		WT
UPN58	WT	WT	WT	del (ex 2-10)	del (ex 2)	WT	deleted		WT
UPN66	WT	P2RY8-CRLF2	deletion	WT	WT	del (ex5)	WT		WT
UPN67		P2RY8-CRLF2							WT
UPN68	WT	IGH@CRLF2	WT	del ex 5	WT	WT	WT		WT
UPN69		P2RY8-CRLF2							WT

*Copy number and mutation status for *IKZF1, CDKN2A/B, PAX5, ERG, BTG1, ETV6,* and *RB1,* as well as the presence of *P2RY8-CRLF2* gene fusion, were assessed by multiplex ligation-dependent probe amplification (MLPA); *JAK2* mutations were identified by high-resolution melt (HRM); *IGH@CRLF2* rearrangement was tested by fluorescent *in situ* hybridization (FISH); genetic analysis has not been performed for cells shown in grey. WT, wild-type.

Supplementary Table 5. Cellular features within each developmental fraction extracted from patient mass cytometry data.

This table is too large to display and is provided as a supplementary CSV file.

Supplementary Table 6. Lineage depletion antibodies. Non-B cell lineage depletion antibody staining panel clones, suppliers, and staining concentrations, as used for healthy controls.

Antibody	Clone	Manufacturer	Final Concentration
CD16	3G8	Biolegend	2 μg/mL
CD14	HCD-14	Biolegend	2 μg/mL
CD11c	3.9	Biolegend	2 μg/mL
CD56	HCD56	Biolegend	2 μg/mL
CD3	UCHT1	Biolegend	2 μg/mL
CD66	G10F5	Biolegend	2 μg/mL

Supplementary Table 7. Feature scaling parameters and final DDPR coefficients. These parameters are needed to apply the Developmentally Dependent Predictor of Relapse (DDPR) model to new patient data*.

Formula for the DDPR risk group assignment:

$$\textbf{Relative Risk (RR)} = e^{\beta 1*x1 + \beta 2*x2 + \beta 3*x3 + \beta 4*x4 + \beta 5*x5 + \beta 6*x6},$$

where features x1 – x6 have been scaled to mean (μ1 – μ6) and s.d. (σ1 – σ6) from the original training cohort:

Feature	Mean	S.D.	Coefficient
x1 = % pRPS6+ pro-BII cells in basal state	**μ1 =** 42.383637404	**σ1 =** 3.861958629	**β1 =** 0.001068559
x2 = Change in % p4EBP1+ pro-BII cells in response to pervanadate	**μ2 =** -0.200045619	**σ2 =** 0.244233419	**β2 =** -0.037058968
x3 = Change in % pSYK+ pre-BI cells in response to TSLP	**μ3 =** 0.366274681	**σ3 =** 0.130263455	**β3 =** -0.013482758
x4 = Change in % pRPS6+ pre-BI cells in response to pre-BCR crosslink	**μ4 =** 7.688111392	**σ4 =** 1.979755352	**β4 =** -0.024862815
x5 = Change in % pCREB+ pre-BI cells in response to pre-BCR crosslink	**μ5 =** 16.63420474	**σ5 =** 2.064346649	**β5 =** -0.011677451
x6 = Change in % pCREB+ pre-BI cells in response to pervanadate	**μ6 =** 10.12094851	**σ6 =** 1.652814498	**β6 =** -0.002623108

If **RR ≥ 0.9967361**, a patient is in **high** DDPR risk group, and in **low** DDPR risk group otherwise.

*Prior to constructing the DDPR model, all features from BCP-ALL patients with ≥3 years on follow-up (n = 54, see Methods and Supplementary Table 5) were scaled to mean = 0 and s.d. = 1 from the training cohort (n = 44). The scaling parameters were then applied to the validation cohort (n = 10). The same scaling parameters will need to be applied to other new patient samples.