Supplemental Tables and Figures

Table S1. Summary of mouse leukemias analyzed in this study

Mouse ID	Sex	Age at euthanasia (weeks)	CD19 cells in thymus (per cent)	Health score at euthanasia[a]	Thymus weight (g)
406	F	13.4	96.5	10	0.63
853	M	20.6	92.6	5	0.07
854	M	20.6	97.3	10	0.61
857	M	20.6	96.9	5	0.21
968	F	18.4	97.7	10	0.60
973	F	18.1	99.3	10	0.64
856	M	20.6	86.6	10	0.48
932	M	14.3	96.7	5	0.24

[a]Health score of 10 indicates lethargy, piloerection and dyspnea. 5 indicates lethargy and/or piloerection.

Table S2. Summary of whole exome sequencing analysis.

Mouse ID	Leukemia # Reads	Control # Reads	>0.1 SNVs (Strelka)	>0.1 SNVs (VarScan)	>0.1 SNVs (FreeBayes)	Gene Count >0.1 VAF MSSS (Strelka)	Gene Count >0.1 VAF MSSS (VarScan)	Gene Count >0.1 VAF MSSS (FreeBayes)	Gene Count (3 Callers)
406	60,500,407	59,438,806	412	7140	47301	73	248	675	19
853	67,958,871	67,817,215	399	18389	258161	94	277	1261	21
854	68,410,460	63,035,473	578	19918	274870	109	353	1434	24
857	54,677,683	51,126,996	444	19034	93623	91	365	1173	10
968	57,551,810	59,980,031	335	6999	50200	64	236	642	16
973	75,511,794	58,495,688	273	8885	28877	40	208	608	23
856	65,397,737	64,757,981	343	6742	53732	57	248	584	20
932	53,180,631	63,388,629	440	7171	24075	73	311	736	24

SNV, single nucleotide variants. VAF, variant allele frequency. MSSS, missense, stop, start, and splice variants.

Table S3. Summary of mutations identified in *Jak3*, *Jak1*, and *Ikzf3* genes.

Gene	Mouse	CCF (Strelka)	CCF (VarScan)	CCF (FreeBayes)	Location		Base Change	Amino Acid Change	Effect
Jak3									
Jak3	853	0.509	0.508	0.515	chr8	71684008	C>T	R653H	Missense variant
Jak3	857	0.332	0.321	0.327	chr8	71685437	C>T	T844M	Missense variant
Jak3	406	0.401	0.379	0.396	chr8	71682913	C>T	A568V	Missense variant
Jak3	856	0.540	0.496	0.534	chr8	71684059	T>C	V670A	Missense variant
Jak3	968	0.483	0.447	0.478	chr8	71684008	C>T	R653H	Missense variant
Jak1									
Jak1	854	0.255	0.223	0.257	chr4	101163673	C>A	V657F	Missense variant
Jak1	973	0.509	0.606	0.507	chr4	101159057	T>G	F837V	Missense variant
Ikzf3									
Ikzf3	853	0.453	0.456	0.449	chr11	98488858	C>T	H195Y	Missense variant
Ikzf3	857	0.552	0.541	0.547	chr11	98490344	C>T	R137*	Stop gained
Ikzf3	932	0.278	0.240	0.275	chr11	98490344	C>T	R137*	Stop gained

CCF, cancer cell frequency

Table S4. Identities of Mice Included in Ruxolitinib Chow Experiment

Mouse ID	Ruxolitinib Chow	Sex	Age at euthanasia (weeks)	B-ALL	RNA-seq	WES
229	None	F	14.3	✓	✓	✓
258	None	M	17.4	✓	✓	✓
262	None	M	17.4	✓	✓	✓
484	None	F	15.3	✓	✓	✓
485	None	F	21.9	x[a]	✓	✓
224	4-8 wk	F	58.1	✓	x	✓
226	4-8 wk	F	27.3	✓	✓	✓
261	4-8 wk	F	22.7	✓	✓	✓
307	4-8 wk	F	27.7	✓	✓	✓
310	4-8 wk	F	27.7	✓	✓	✓
339	4-8 wk	F	51.9	✓	x	✓
486	4-8 wk	M	40.0	✓	✓	✓

[a] determined to be other than B-ALL as indicated by low frequency $CD19^+$ cells and low frequency of pro-B cell specific gene expression.

Table S5. Primer Sequences.

Gene	Primer orientation	Oligonucleotide sequence
Tpb1	FORWARD	5'- ACCGTGAATCTTGGCTGTAAAC -3'
Tpb1	REVERSE	5'- GCAGCAAATCGCTTGGGATTA -3'
Gpx1	FORWARD	5'- GTGTGGCCGATGTGTCTATT -3'
Gpx1	REVERSE	5'- GCGTTTCCTGTCTTTGTACTTTC -3'
Cat	FORWARD	5'- GATGGTAACTGGGATCTTGTGG -3'
Cat	REVERSE	5'- GTGGGTTTCTCTTCTGGCTATG -3'
Neil1	FORWARD	5'- CAGCCATTCCTCCTTCAGAAT -3'
Neil1	REVERSE	5'- TCCACACACCCACCAAATAC -3'
Sod2	FORWARD	5'- CAGATTGCTGCCTGCTCTAA -3'
Sod2	REVERSE	5'- CTGAAGGTAGTAAGCGTGCTC -3'
Rad51	FORWARD	5' - GGTTAGAGCAGTGTGGCATAA -3'
Rad51	REVERSE	5' - TAGTTCCTTCTTCGGTGCATAAG -3'

Figure S1. Mutational signature analysis. (A-H) Bar graphs indicate frequencies of variant types (in color, see legend) as well as trinucleotide context (x-axis) plotted against fraction (y-axis). Signatures identified by deconstructSigs are indicated above each graph.

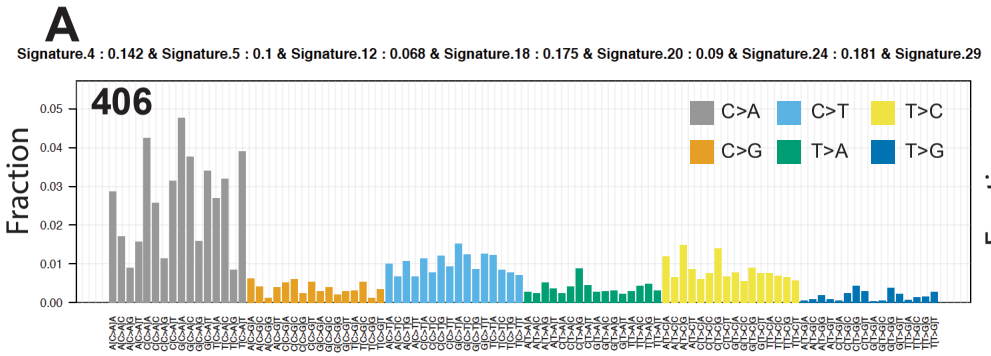
Figure S2. Increased ROS in bone marrow cells in the absence of PU.1. Bone marrow cells from Spi-B-deficient (DB) or PU.1 and Spi-B-deficient (DPB) mice were placed in culture with IL-7 for one week followed by staining with H_2DCFDA and flow cytometric analysis. A) Shows one representative experiment. B) Shows mean and standard deviation of mean fluorescence intensity (MFI) from three independent experiments (unpaired Student's *t*-test).

Figure S3. Effect of Necrox-5 and ML334 on cell proliferation and gene expression of cultured leukemia cells. (A) Necrox-5 treatment reduces cell counts of cultured 973 cells in a dose-dependent manner. MTT assay was performed, and y-axis indicates absorbance at the indicated wavelength. P value was determined by ordinary one-way ANOVA of n=3 biological replicate experiments. (B) Gene expression analysis. RT-qPCR was performed to determine relative fold changes in the indicated mRNA transcripts after 24 hours of culture with Necrox-5. Statistical analysis was performed by one sample t and Wilcoxon test (n=5 biological replicate experiments). Error bars indicate standard error of the mean. (C) Reduced ROS in 973 cells treated for 24 hours with Necrox-5, as determined by H_2DCFDA staining. Numbers indicate mean fluorescence intensity (MFI). (D) ML334 treatment reduces cell counts of cultured 973 cells in a dose-dependent manner. Cell counting assays were performed, and y-axis indicates cell numbers. P value was determined by ordinary one-way ANOVA of n=3 biological replicate experiments. (B) Gene expression analysis. RT-qPCR was performed to

determine relative fold changes in the indicated mRNA transcripts after 24 hours of culture with ML334. Statistical analysis was performed by one sample t and Wilcoxon test (*n*=5 biological replicate experiments). Error bars indicate standard error of the mean.

Figure S4. Mutational signatures of variants called by Varscan separated into VAF <0.3 or VAF>0.3. (A) Weights of mutational signatures called using Strelka for leukemias from Mb1-CreΔPB mice fed with Control or Ruxolitinib (Ruxo) chow between 4-8 weeks of age. (B, C) Heat maps of mutational signatures in leukemias from Mb1-CreΔPB mice fed with Control or Ruxolitinib (Ruxo) chow between 4-8 weeks of age. Colors indicate weights of mutational signatures called by Varscan for WES results of leukemias separated into VAF <0.3 (B) or VAF > 0.3 (C).

Figure S1


A
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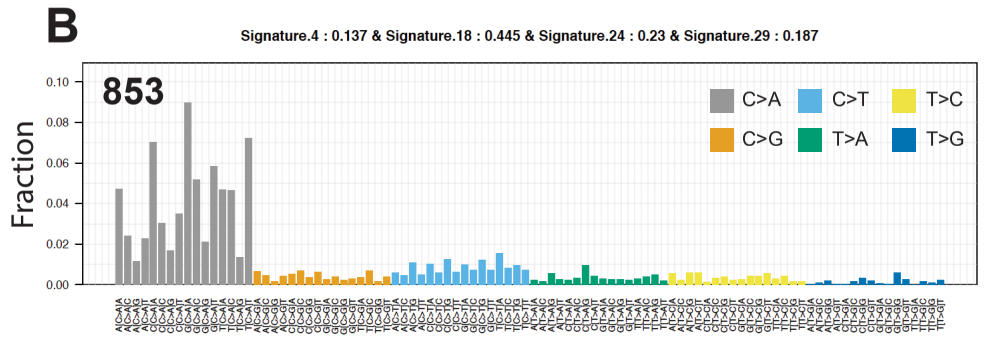
406
C>A
C>T
T>C
C>G
T>A
T>G
Fraction


B
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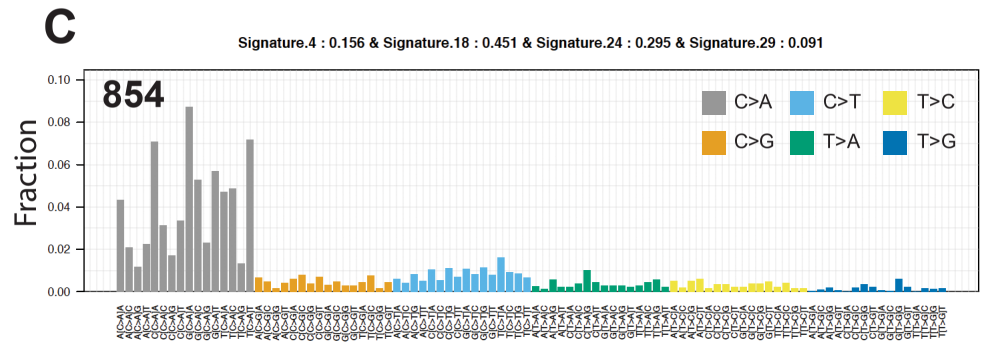
853
C>A
C>T
T>C
C>G
T>A
T>G
Fraction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854
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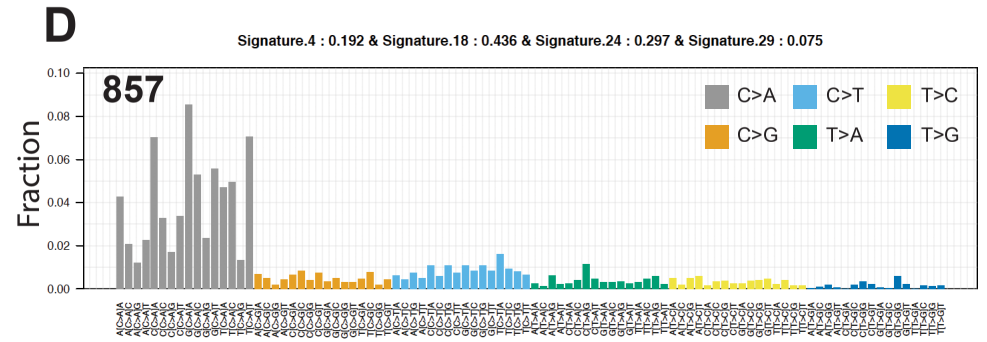
C>T
T>C
C>G
T>A
T>G
Fraction


D
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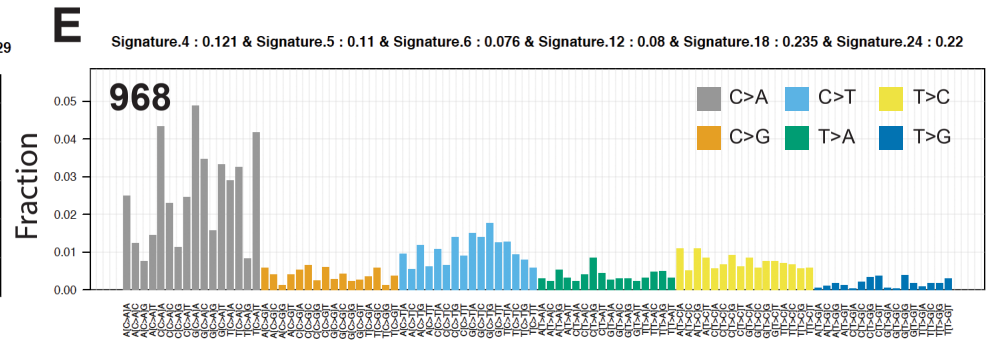
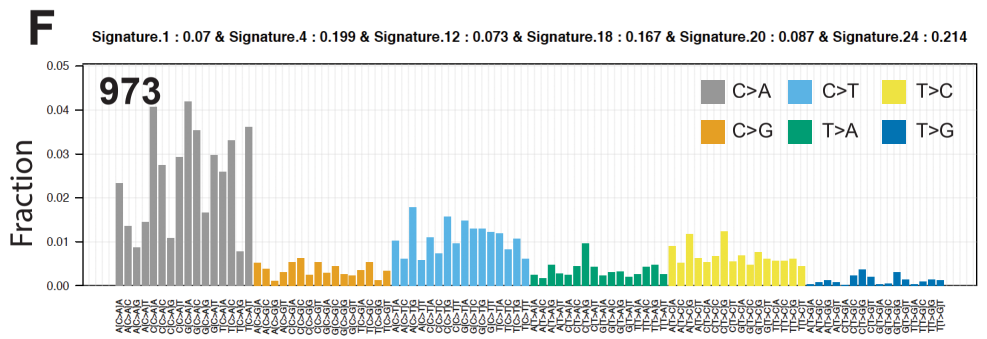
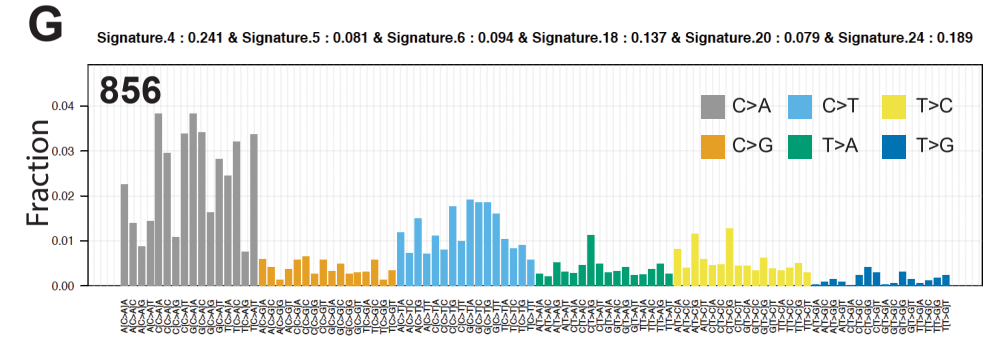
857
C>A
C>T
T>C
C>G
T>A
T>G
Fraction


E
Signature.4 : 0.121 & Signature.5 : 0.11 & Signature.6 : 0.076 & Signature.12 : 0.08 & Signature.18 : 0.235 & Signature.24 : 0.22
968
C>A
C>T
T>C
C>G
T>A
T>G
Fraction


F
Signature.1 : 0.07 & Signature.4 : 0.199 & Signature.12 : 0.073 & Signature.18 : 0.167 & Signature.20 : 0.087 & Signature.24 : 0.214
973
C>A
C>T
T>C
C>G
T>A
T>G
Fraction


G
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856
C>A
C>T
T>C
C>G
T>A
T>G
Fraction

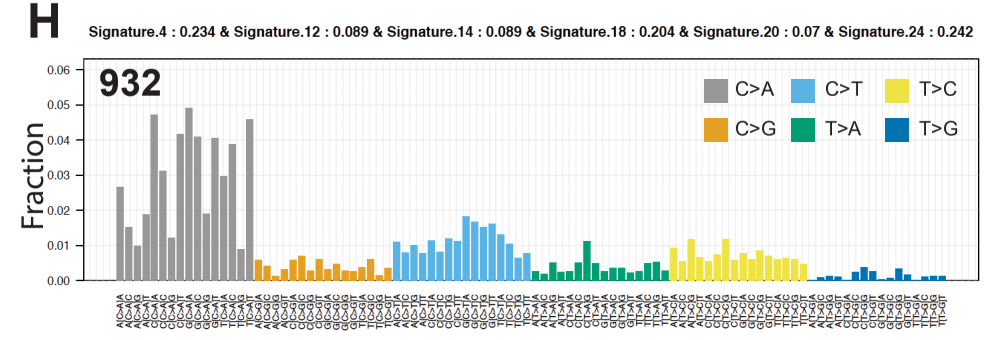

H
Signature.4 : 0.234 & Signature.12 : 0.089 & Signature.14 : 0.089 & Signature.18 : 0.204 & Signature.20 : 0.07 & Signature.24 : 0.242
932
C>A
C>T
T>C
C>G
T>A
T>G
Fraction

Figure S2

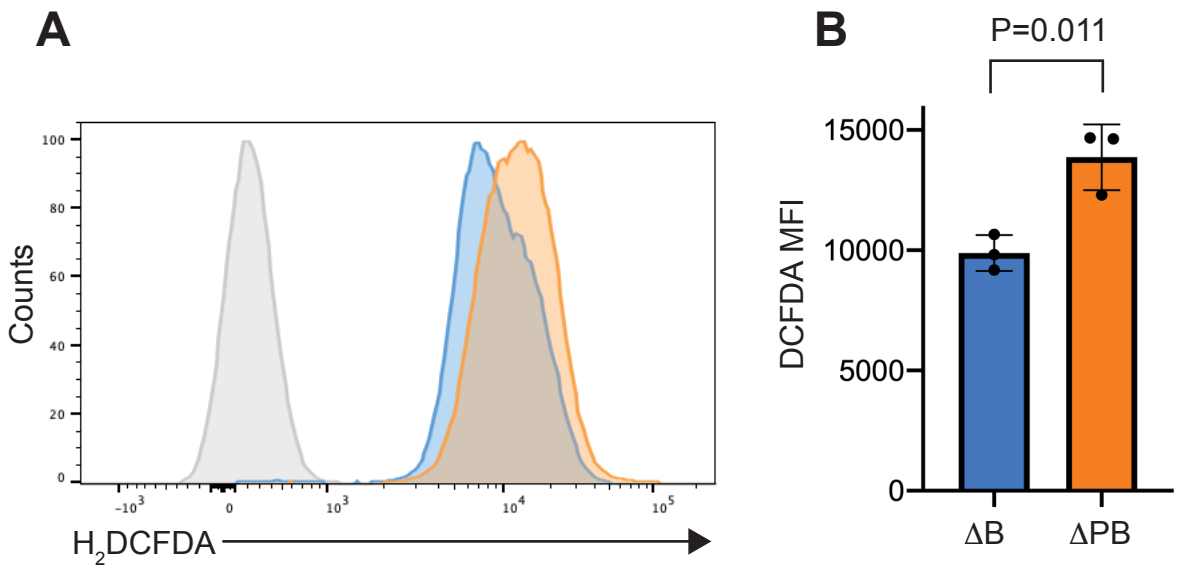

A
Counts
100
80
60
40
20
0
−10^3
0
10^3
10^4
10^5
H2DCFDA
B
P=0.011
DCFDA MFI
15000
10000
5000
0
ΔB
ΔPB

Figure S3

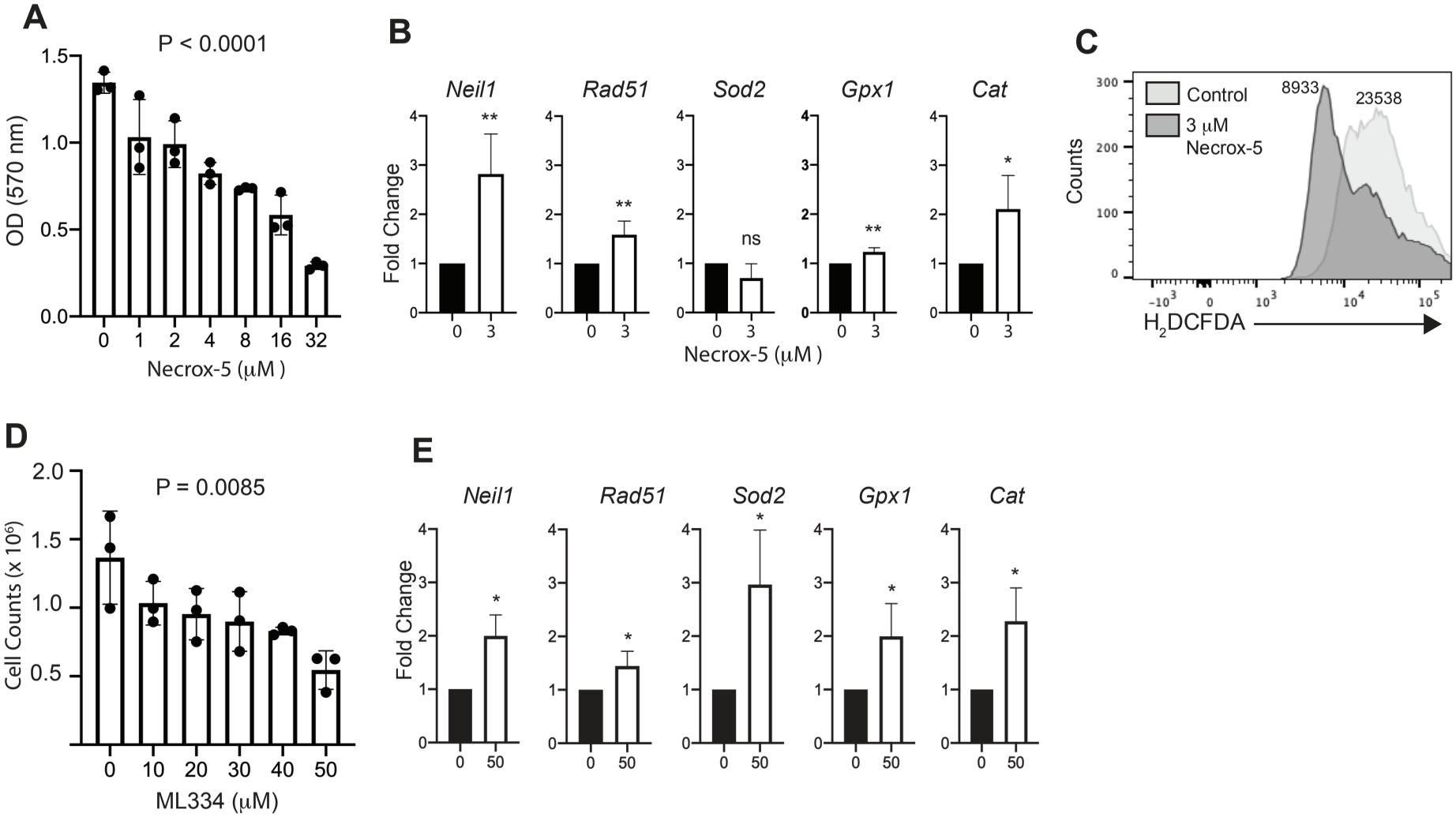
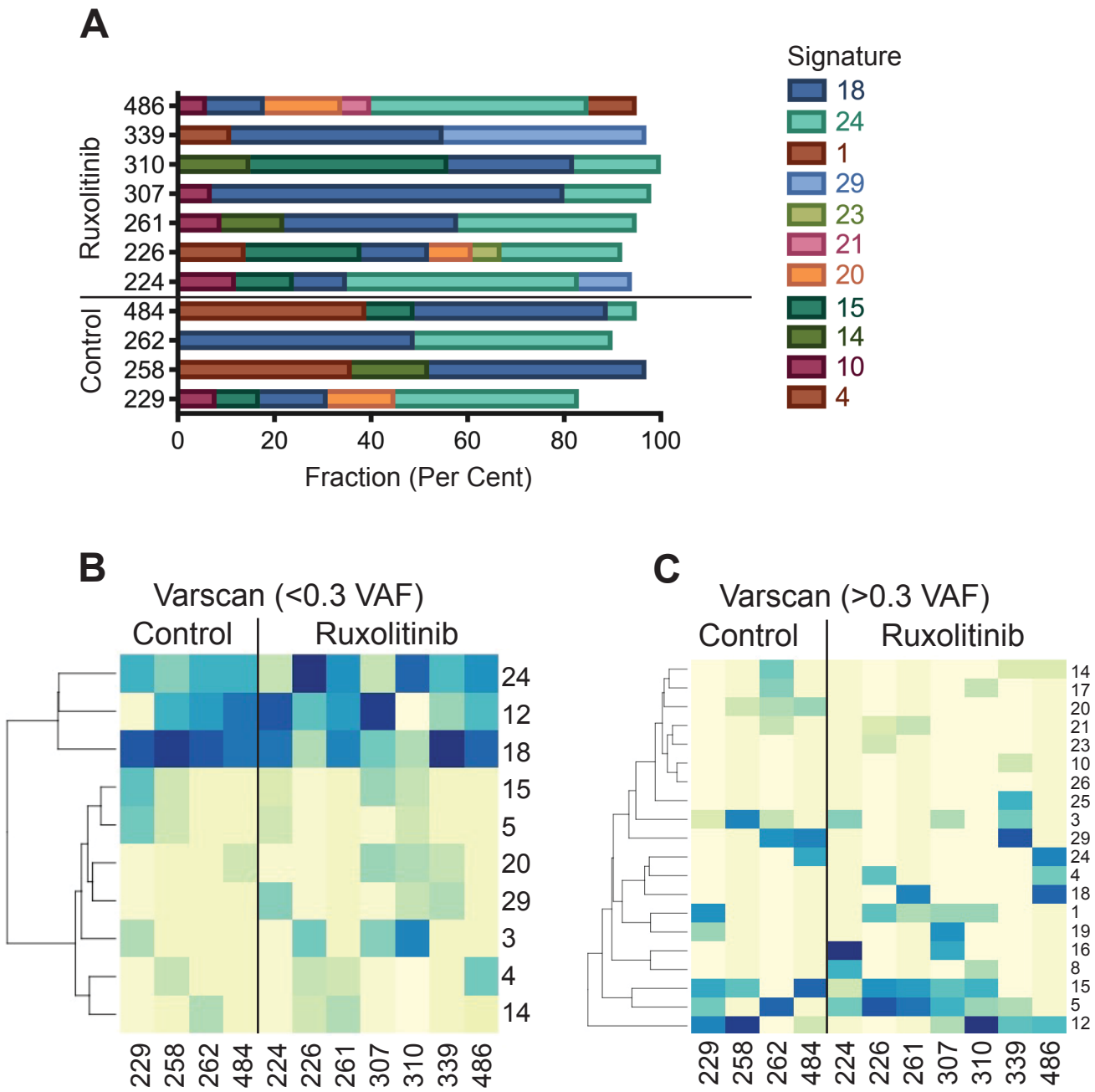

A
P < 0.0001
OD (570 nm)
1.5
1.0
0.5
0.0
0 1 2 4 8 16 32
Necrox-5 (μM)
B
Neil1
Rad51
Sod2
Gpx1
Cat
Fold Change
**
**
ns
**
*
0 3
Necrox-5 (μM)
C
Control
3 μM
Necrox-5
8933
23538
Counts
300
200
100
0
H_2DCFDA
D
P = 0.0085
Cell Counts (x 10^6)
2.0
1.5
1.0
0.5
0 10 20 30 40 50
ML334 (μM)
E
Neil1
Rad51
Sod2
Gpx1
Cat
Fold Change
*
*
*
*
*
0 50

Figure S4


A
Signature
18
24
1
29
23
21
20
15
14
10
4
Ruxolitinib
Control
486
339
310
307
261
226
224
484
262
258
229
0
20
40
60
80
100
Fraction (Per Cent)
B
Varscan (<0.3 VAF)
Control
Ruxolitinib
24
12
18
15
5
20
29
3
4
14
229
258
262
484
224
226
261
307
310
339
486
C
Varscan (>0.3 VAF)
Control
Ruxolitinib
14
17
20
21
23
10
26
25
3
29
24
4
18
1
19
16
8
15
5
12
229
258
262
484
224
226
261
307
310
339
486