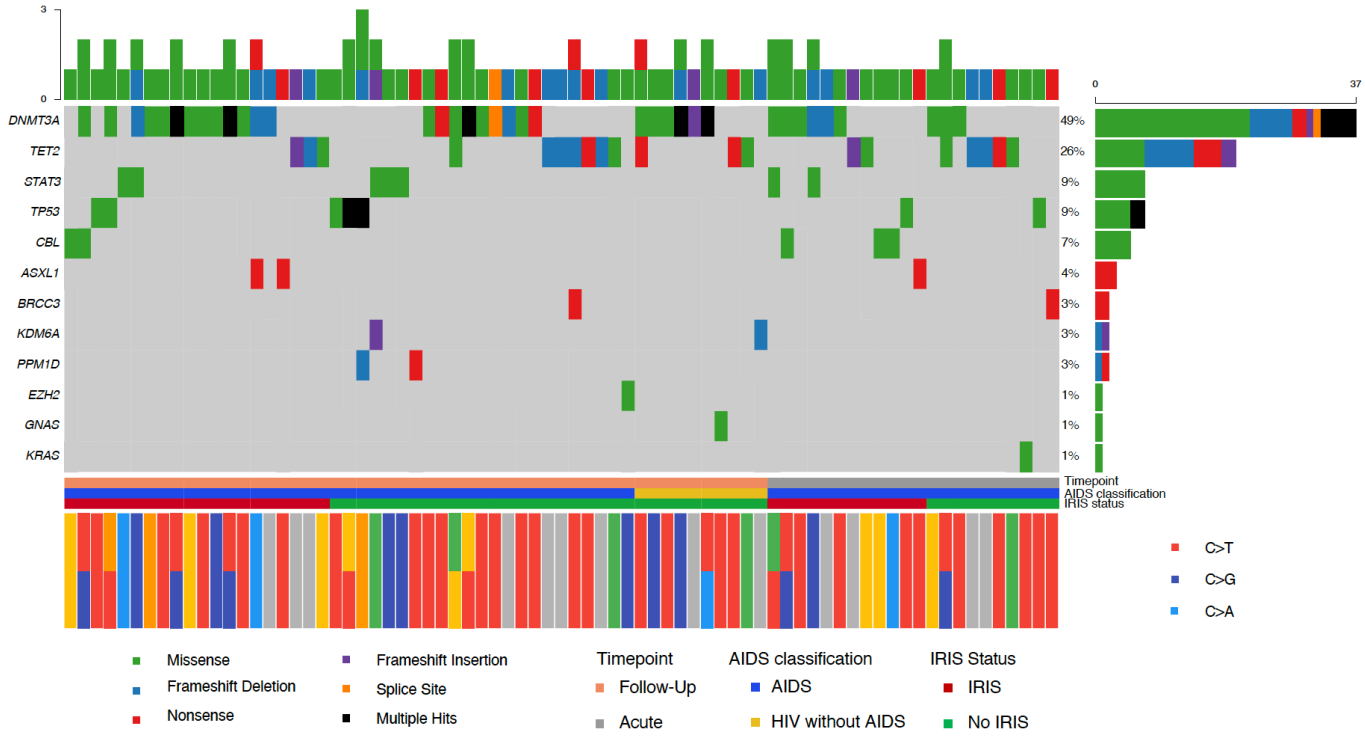
Supplemental Methods

Clinical Protocols

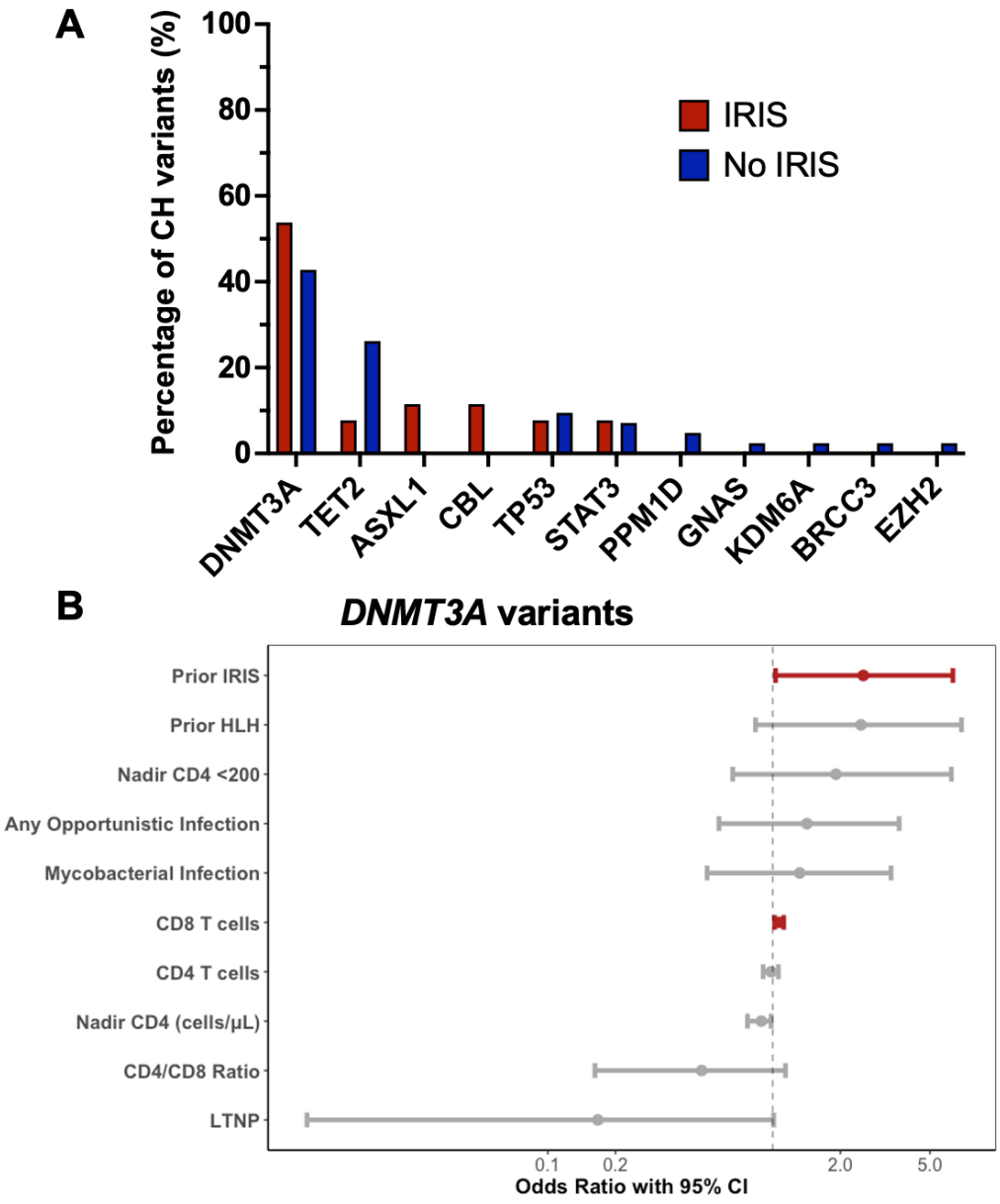
All 197-participants were previously enrolled in one of three prior National Institute of Allergy and Infectious Diseases institutional review board approved observational, prospective studies of individuals with HIV (NCT00286767, NCT02147405, NCT02081638). These included the "Immune Reconstitution Inflammatory Syndrome in HIV-Infected Patients Taking Antiretroviral Therapy" study (IRIS - NCT00286767) and the "PET Imaging and Lymph Node Assessment of IRIS in Persons with AIDS" study (PANDORA - NCT02147405) which were both observational longitudinal studies evaluating the immunopathogenesis of IRIS in HIV-1 infected patients with CD4 count <100 cells/μL. All patients were seen and evaluated at the National Institutes of Health in Maryland, USA. In both studies, IRIS events were defined using the AIDS Clinical Trials Group IRIS definition criteria which include evidence of ART initiation with resultant increase in CD4 count (≥50 cells/μL or a ≥2-fold rise) and/or virologic suppression (>0.5 log10 decrease in plasma HIV viremia), clinical presentation consistent with an infectious or inflammatory condition, and the absence of an alternative etiology such as the expected course of a previously recognized infection or side-effects of medications. Peripheral blood mononuclear cells (PBMCs) were collected prior to ART and at weeks 2, 4, 8, 12, 24, 36, 48, 64, 80, 96 and during episodes of IRIS. The third study (NCT02081638) was the "Elite Controller and ART-treated HIV+ Statin Versus ASA Treatment Intervention Study" (ECSTATIN) which was an open label randomized controlled trial of either aspirin or atorvastatin given for 9-months to study their potential effect in immune activation and inflammation. All participants were 18 years or older with plasma viremia <40 copies/ml. Women who were lactating or were pregnant, or actively trying to become pregnant were excluded from participation. Other exclusion criteria

were known hypersensitivity or allergy or other contraindication to study medications, diagnosis of cardiovascular disease or hypercholesterolemia (LDL cholesterol ≥ 190 mg/dL), any chronic inflammatory condition requiring treatment with anti-inflammatory medications, active drug or alcohol use disorder. All participants signed informed consent and all procedures were in accordance with the Helsinki Declaration of the World Medical Association.

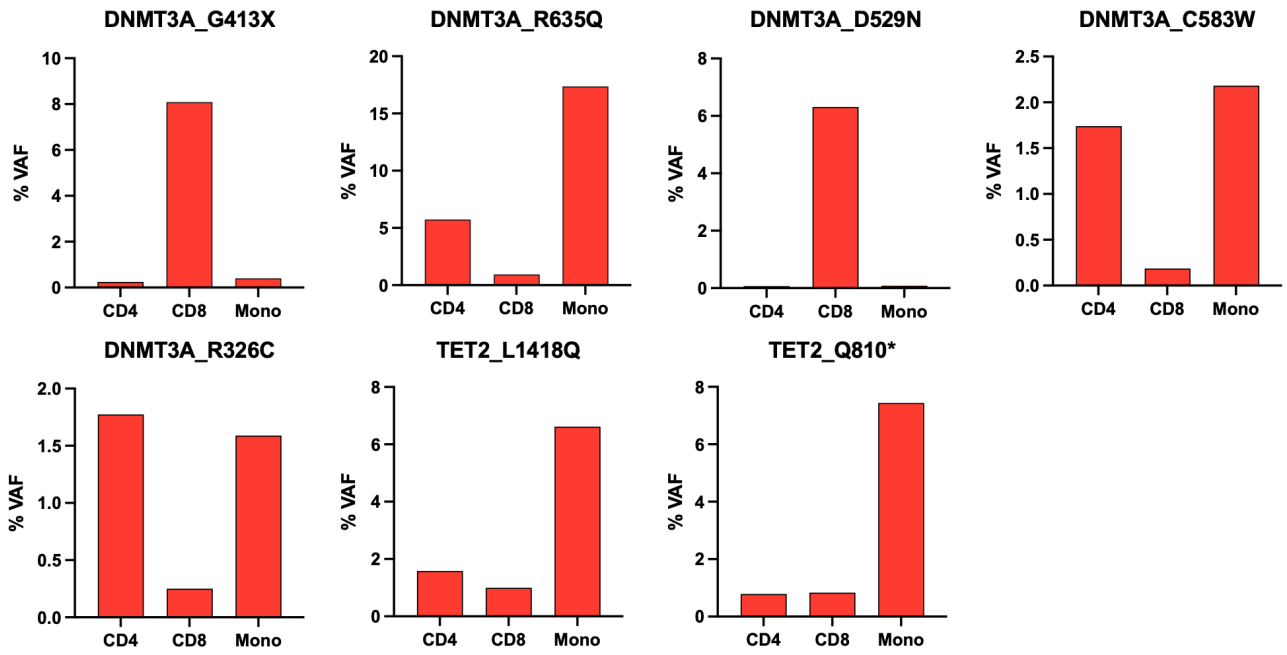
Supplemental Figures



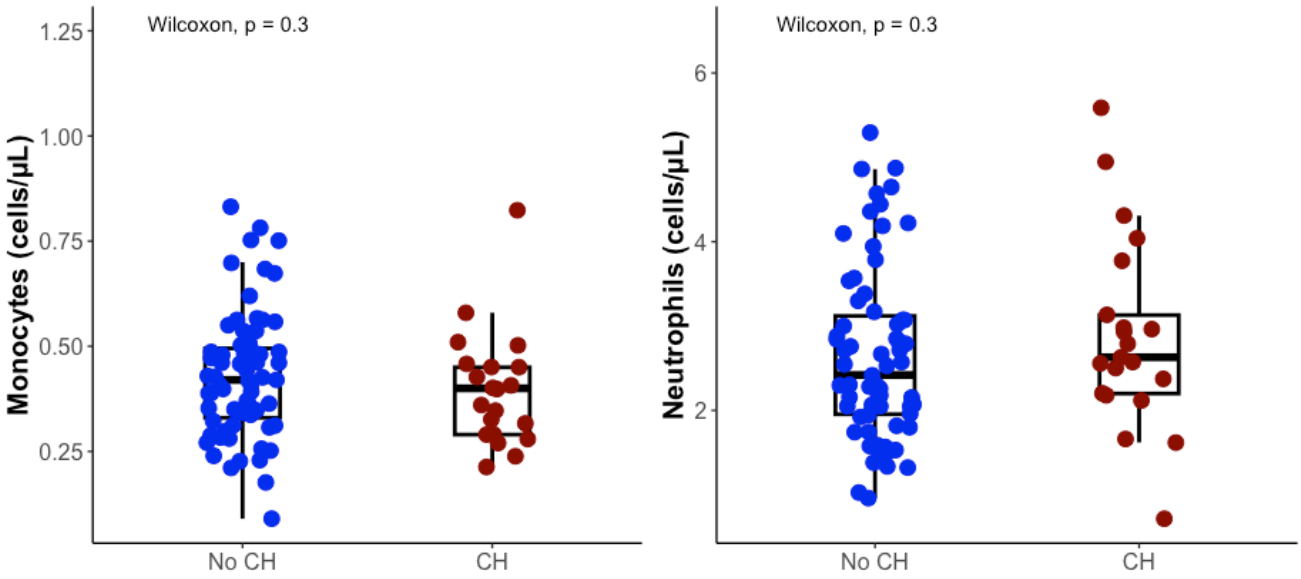
Supplemental Figure 1: Waterfall plot demonstrating specific clonal hematopoiesis variants in each gene for individual patients at the acute and follow-up timepoints. All gene mutations (N=100) in 54-people with HIV are depicted, and the proportion of each specific mutation identified is depicted on the right. Subgroups at the bottom represent the Acute and Follow-up Timepoints, history of CD4 nadir <200 cells/μL (AIDS classification), and history of immune reconstitution inflammatory syndrome (IRIS status). Specific nucleotide changes are also color-coded and summarized at the bottom of the figure.



Supplemental Figure 2: (A) Proportion of clonal hematopoiesis variants identified in each gene at the follow-up timepoint stratified by history of IRIS. (B) Adjusted logistic regression with 95% CIs of key variables and their impact on risk of clonal hematopoiesis in *DNMT3A* in people with HIV. CD4 and CD8 T-cell counts are presented as an OR per 50 cells/µL change. CD4 T-cell nadir was evaluated as a categorical variable (CD4 T-cells nadir <200 cells/µL) and a continuous variable (per 50 cells/µL change).

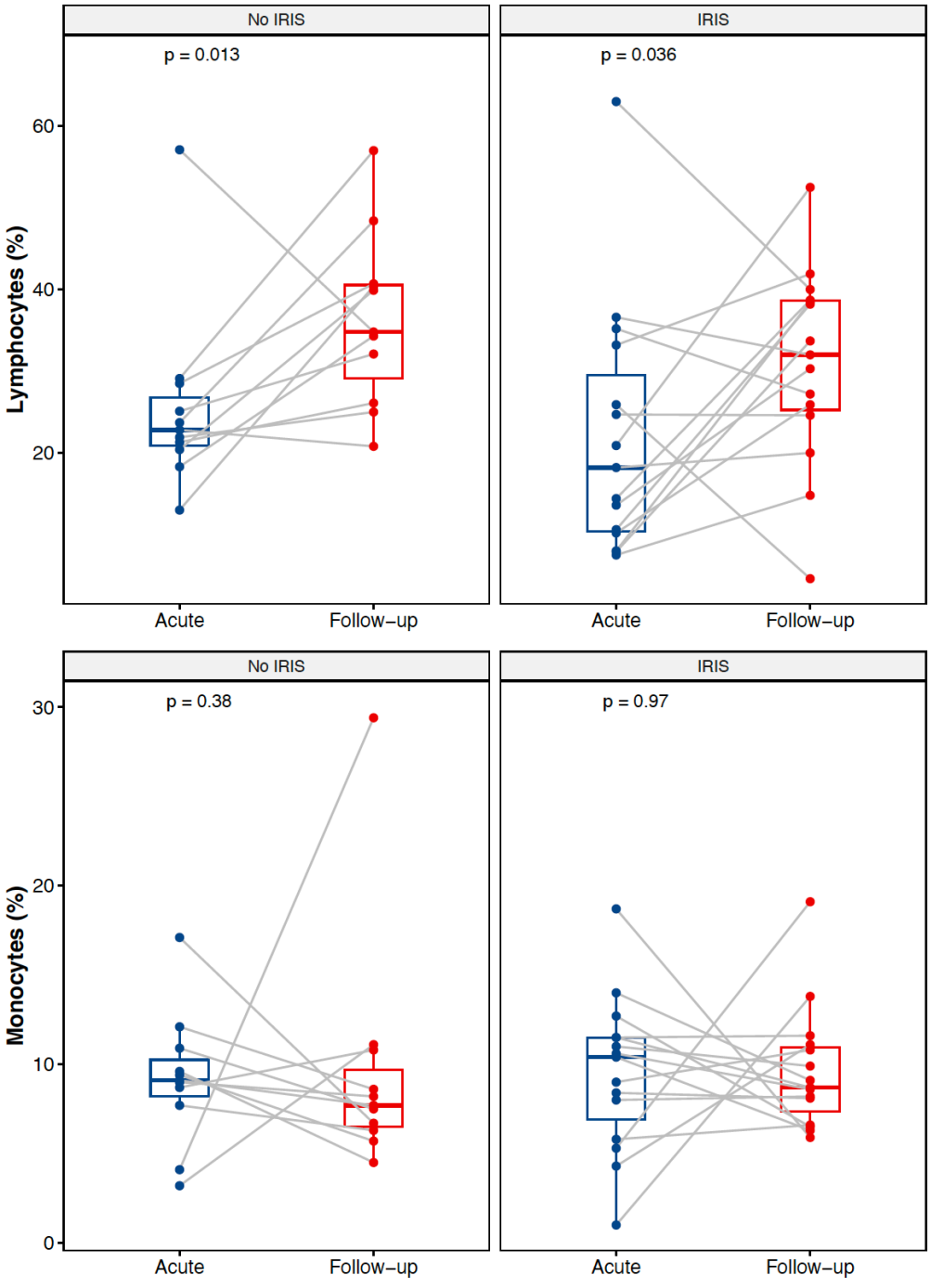


Supplemental Figure 3: Variant allele frequencies in purified peripheral blood lineages of 7-participants with HIV and clonal hematopoiesis in *DNMT3A* or *TET2*. Abbreviations: VAF – variant allele frequency; Mono – monocytes; CD4 – CD4 T-cells; CD8 – CD8 T-cells.



Supplemental Figure 4: Comparison of peripheral blood absolute monocyte and neutrophil counts between the people with HIV without clonal hematopoiesis and with clonal hematopoiesis. Data are presented as boxplots with medians and interquartile ranges. Groups were compared using Wilcoxon rank sum test and resultant p-values are shown.

Abbreviations: CH - clonal hematopoiesis.



Supplemental Figure 5: Comparison of peripheral blood monocyte and lymphocyte frequencies between the acute and follow-up sample in people with HIV and CH. Data are presented as boxplots with medians and interquartile ranges. Groups were compared using Wilcoxon rank sum test and resultant p-values are shown.

Supplemental Tables

Supplemental Table 1: Demographic and clinical characteristics of people with HIV with or without clonal hematopoiesis.

Characteristic	**CH** (n = 54)	**No CH** (n = 143)
Age (yr)	48 (41-55)	40 (35-50)
Female – no. (%)	14 (26)	38 (27)
Race/Ethnicity – no. (%)		
Hispanic	5 (9.3)	38 (26.6)
African/African-American	34 (63)	78 (54.5)
White	9 (16.6)	13 (9.1)
Other[A]	6 (11.1)	14 (9.8)
Nadir CD4 T-cells (cells/μL)	36 (11-103)	32 (11-107)
HIV viral load (IU/mL)	<40	<40
Smoking – no. (%)	25 (46)	58 (41)
Hematologic malignancy – no. (%)		
Lymphoma	5 (9.3)	3 (2.1)

KSHV	2 (3.7)	18 (12.6)
Prior Chemotherapy	3 (5.6)	7 (4.9)
Opportunistic Infection – no. (%)		
Mycobacteria	22(40.7)	53 (37.1)
Histoplasmosis	3 (5.6)	9 (6.3)
Cryptococcus	2 (3.7)	8 (5.6)
Toxoplasmosis	2 (3.7)	5 (3.5)
IRIS – no. (%)	21 (38.9)	47 (32.9)
HLH – no. (%)	11 (20.4)	22 (15.3)
LTNP – no. (%)	4 (7.4)	14 (9.8)

Data represent medians with interquartile ranges for continuous variables and frequencies with percentages for categorical variables. Abbreviations: Yr – Years; IRIS – immune reconstitution inflammatory syndrome; HIV – human immunodeficiency virus; KSHV – Kaposi sarcoma herpesvirus; HLH – hemophagocytic lymphohistiocytosis; LTNP – long-term nonprogressor. [A]Other race/ethnicity includes: Asian (n=3), Multiple (n=1), and Unknown (n=15).

Supplemental Table 2: Primer and adapter sequences used for mutation validation

DNMT3A_G413X_ 129bp Forward (adapter)	5' ACACTCTTTCCCTACACGACGCTCTTCCGATCTACAGCGATG AGAGTGACACT 3'
DNMT3A_G413X_ 129bp Reverse (adapter)	5' GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTGCCCTCA TTTACCTTCTG 3'
DNMT3A_R635Q_ 145bp Forward (adapter)	5' ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCCCAGCTG AGAAGAGGAA 3'
DNMT3A_R635Q_ 145bp Reverse (adapter)	5' GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTCGTTTTGCC AGAGTTGCC 3'
DNMT3A_D529N_ 150bp Forward (adapter)	5' ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGCTTTCTG GAGTGTGCG 3'
DNMT3A_D529N_ 150bp Reverse (adapter)	5' GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCAGCTAAG GAGACCACTG 3'

DNMT3A_C583W_ 149bp Forward (adapter)	5' ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGGAGTGT GTGGACCTCTT3'
DNMT3A_C583W_ 149bp Reverse (adapter)	5' GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGAACATCT GGAGCCGGG3'
DNMT3A_R326C_ 150bp Forward (adapter)	5' ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTTGGCATT GGGGAGCTG 3'
DNMT3A_R326C_ 150bp Reverse (adapter)	5' GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCACTGAGA ATTTGCCGTC 3'
TET2_Q810X_143b p Forward (adapter)	5' ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGCCAGACT AAAGTGGAAGA 3'
TET2_Q810X_143b p Reverse (adapter)	5' GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCATGGTCTG ACTATAAGGGGA 3'

TET2_L1418Q_114 bp Forward (adapter)	5' ACACTCTTTCCCTACACGACGCTCTTCCGATCTACCAGGTAT GCACTCTCACT 3'
TET2_L1418Q_114 bp Reverse (adapter)	5' GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCCAAACTC ATCCACGTCA 3'
TET2_I1873T_150bp Forward (adapter)	5' ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGAGGACA ACGATGAGGTC 3'
TET2_I1873T_150bp Forward (adapter)	5' GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGGATTCTTT AAAGGGGTTGTG 3'

Supplemental Table 3: Target genes analyzed via panel targeted error-corrected sequencing

ASXL1	*ETV6*	*MPL*	*SF1*
BCOR	*EZH2*	*MYD88*	*SF3A1*
BCORL1	*FLT3*	*NOTCH1*	*SF3B1*
BRAF	*GATA2*	*NPM1*	*SMC1A*
BRCC3	*GNAS*	*NRAS*	*SMC3*
CALR	*GNB1*	*PHF6*	*SRSF2*
CBL	*IDH1*	*PIGA*	*STAG2*
CEBPA	*IDH2*	*PPM1D*	*STAT3*
CREBBP	*JAK2*	*PRPF40B*	*TET2*
CSF1R	*JAK3*	*PTEN*	*TP53*
CSF3R	*KDM6A*	*PTPN11*	*U2AF1*
CTCF	*KIT*	*RAD21*	*U2AF2*
CUX1	*KMT2A*	*RUNX1*	*WT1*
DNMT3A	*KRAS*	*SETBP1*	*ZRSR2*

Supplemental Table 4: Clonal Hematopoiesis Driver classification criteria

Gene name	Criteria for classification as a driver mutation	Transcript
ASXL1	Frameshift/nonsense/splice-site in exon 11-12	NM_015338
BCOR	Frameshift/nonsense/splice-site	NM_001123385
BCORL1	Frameshift/nonsense/splice-site	NM_021946
BRAF	Missense in aa range p.590-615; Missense at G469	NM_004333
BRCC3	Frameshift/nonsense/splice-site	NM_024332
CALR	Frameshift in exon 9	NM_004343
CBL	Missense in Linker/RING finger domains (p.345-434)	NM_005188
CEBPA	Frameshift/nonsense/splice-site	NM_004364

CREBBP	Frameshift/nonsense/splice-site	NM_004380
CSF1R	Missense at L301 / Y969	NM_005211
CSF3R	T615A, T618I, truncating c.741-791	NM_000760
CTCF	Frameshift/nonsense/splice-site, R377C, R377H, P378A, P378L	NM_006565
CUX1	Frameshift/nonsense/splice-site	NM_181552
DNMT3A	Frameshift/nonsense/splice-site; Missense in PWWP (p.292-350) / ADD (p.482-614) / MTase (p.634-912) domains	NM_022552
ETV6	Frameshift/nonsense/splice-site	NM_001987
EZH2	Frameshift/nonsense/splice-site; Missense in SET domain (p.617-732)	NM_001203247
FLT3	V579A, V592A, V592I, F594L, FY590-591GD, D835Y, D835H, D835E, del835	NM_004119
GATA2	Frameshift/nonsense/splice-site, R293Q, N317H, A318T, A318V, A318G, G320D, L321P, L321F, L321V, Q328P, R330Q, R361L, L359V, A372T, R384G, R384K	NM_001145661
GNAS	Missense at R201 (844)	NM_016592
GNB1	Missense at K57 / I80	NM_002074
IDH1	Missense at R132	NM_005896
IDH2	Missense at R140 / R172	NM_002168
JAK2	V617F; Missense/indel in aa range p.536-547	NM_004972
JAK3	M511T, M511I, A572V, A572T, A573V, R657Q, V715I, V715A	NM_000215

KDM6A	Frameshift/nonsense/splice-site	NM_021140
KIT	ins503, V559A, V559D, V559G, V559I, V560D, V560A, V560G, V560E, del560, E561K, del579, P627L, P627T, R634W, K642E, K642Q, V654A, V654E, H697Y, H697D, E761D, K807R, D816H, D816Y, D816F, D816I, D816V, D816H, del551-559	NM_000222
KMT2A	Frameshift/nonsense/splice-site	NM_005933
KRAS	Missense at G12 / G13 / Q61 / A146	NM_033360
MPL	S505G, S505N, S505C, L510P, del513, W515A, W515R, W515K, W515S, W515L, A519T, A519V, Y591D, W515-518KT	NM_005373
MYD88	L265P	NM_002468
NOTCH1	Frameshift/nonsense/splice-site/missense in exon 26-34	NM_017617
NPM1	Frameshift in exon 12	NM_002520
NRAS	Missense at G12 / G13 / Q61	NM_002524
PHF6	Frameshift/nonsense/splice-site	NM_001015877
PIGA	Frameshift/nonsense/splice-site	NM_002641
PPM1D	Frameshift/nonsense/splice-site in exon 5/6	NM_003620
PRPF40B	Frameshift/nonsense/splice-site	NM_001031698
PTEN	Frameshift/nonsense/splice-site	NM_000314
PTPN11	Missense in aa range p.58-76 and p.491-510	NM_002834
RAD21	Frameshift/nonsense/splice-site	NM_006265
RUNX1	Frameshift/nonsense/splice-site, S73F, H78Q, H78L, R80C, R80P, R80H, L85Q, P86L, P86H, S114L, D133Y, L134P, R135G, R135K,	NM_001001890

	R135S, R139Q, R142S, A165V, R174Q, R177L, R177Q, A224T, D171G, D171V, D171N, R205W, R223C	
SETBP1	D868N, D868T, S869N, G870S, I871T, D880N, D880Q	NM_015559
SF1	Frameshift/nonsense/splice-site	NM_004630
SF3A1	Frameshift/nonsense/splice-site	NM_005877
SF3B1	Missense in terminal HEAT domains (p.529-1201)	NM_012433
SMC1A	Missense at R96 / R586	NM_006306
SMC3	Frameshift/nonsense/splice-site	NM_005445
SRSF2	Missense/deletion involving p.P95	NM_003016
STAG2	Frameshift/nonsense/splice-site	NM_006603
STAT3	Missense in SH2 domain (p.580-670)	NM_139276
TET2	Frameshift/nonsense/splice-site; Missense in conserved domains (p.1104-1481 and p.1843-2002)	NM_001127208
TP53	Frameshift/nonsense/splice-site; Missense in DNA-binding domain (p.95-288); Missense at P72 / R337	NM_001126112
U2AF1	Missense at S34 / R156 / Q157	NM_006758
U2AF2	Missense in RNA recognition motifs domains (p.149-231, p.259-337, p.381-462)	NM_007279
WT1	Frameshift/nonsense/splice-site	NM_024426
ZRSR2	Frameshift/nonsense/splice-site	NM_005089

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