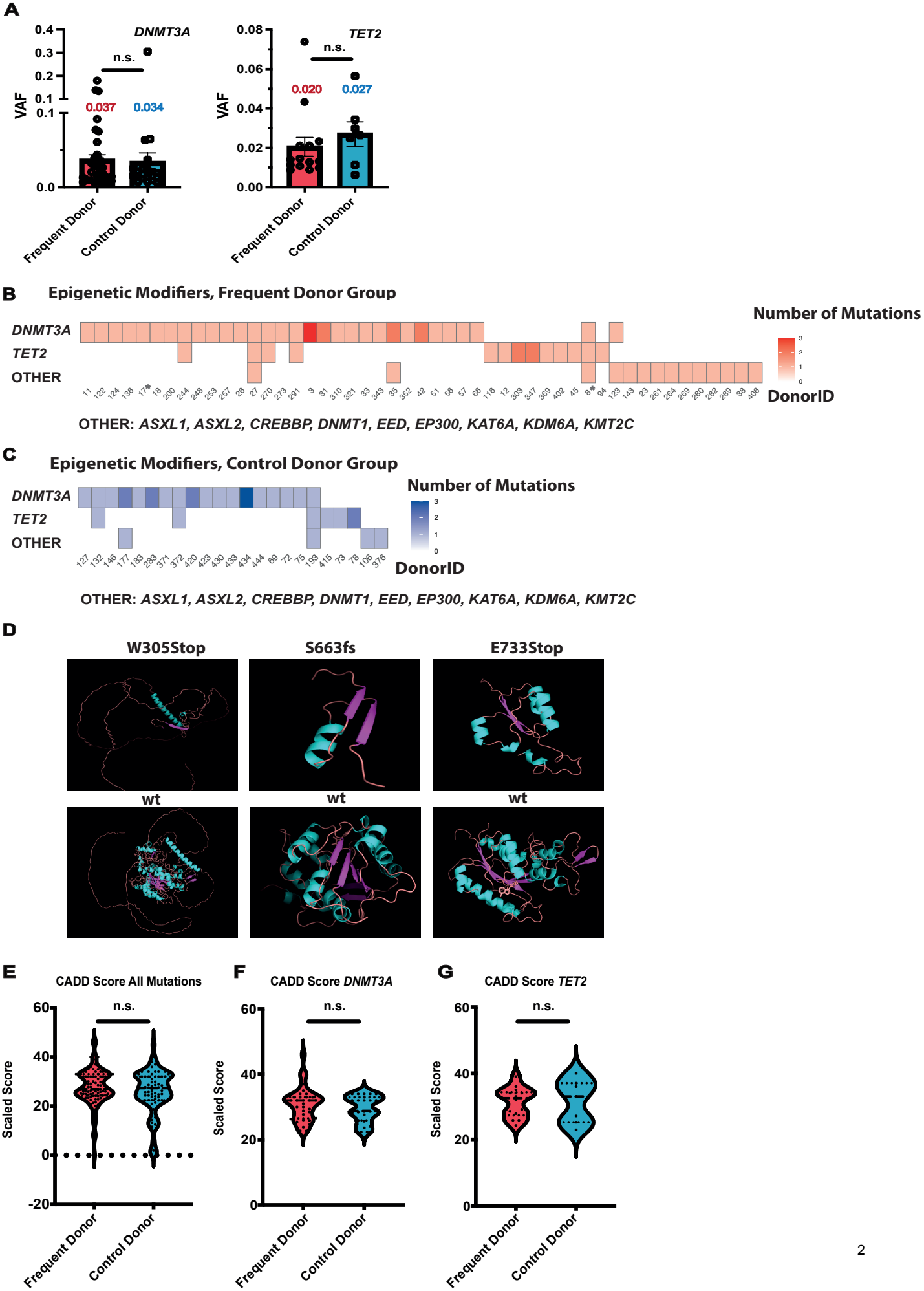
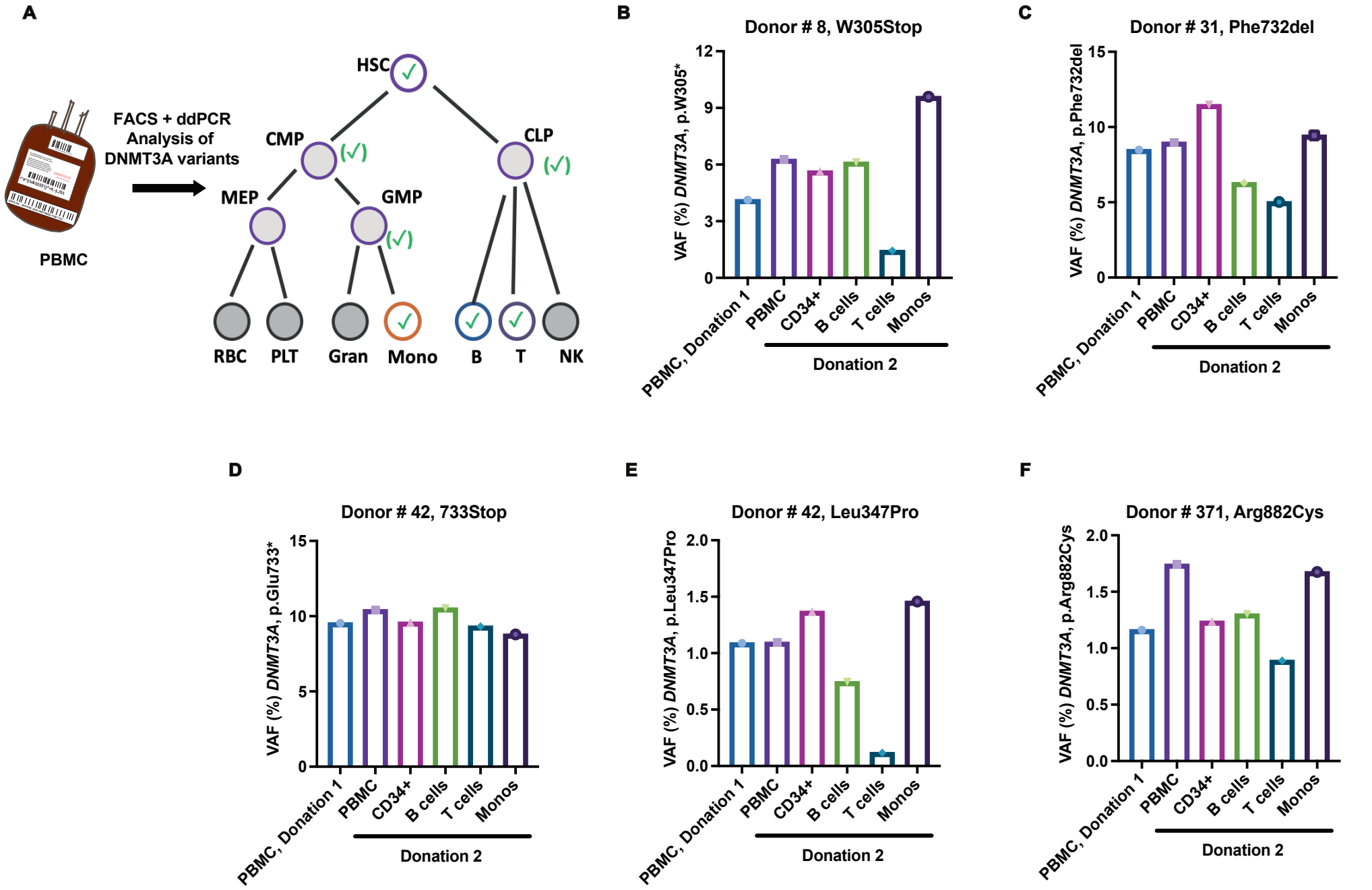
Supplementary Appendix

Supplement to: Karpova Darja, Huerga Encabo Hector, et al.: Frequent whole blood donations select for DNMT3A variants mediating enhanced response to erythropoietin.

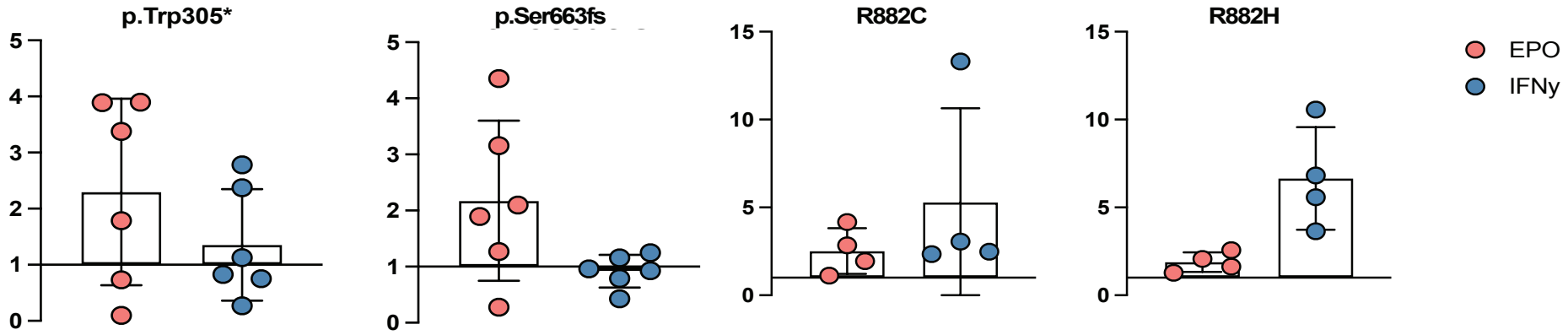
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure Legends

Supplemental Figure 1. Clonal hematopoiesis found in frequent blood donors is enriched in genes encoding for epigenetic regulators.
(A) VAF of all *DNMT3A* (left) and *TET2* (right) variants in the frequent (FD) vs. control donor cohort (CD) detected at a VAF $\geq$ 0.005. Mean values are specified above the bars. (B-C) Co-mutational analysis of the variants in epigenetic regulators detected in the (extended) FD (B) and CD (C) at a VAF $\geq$ 0.005. For each donor number and identity (*DNMT3A* vs. *TET2* vs. one of the following genes: *ASXL1, ASXL2, CREBBP, DNMT1, EED, EP300, KAT6A, KDM6A* or *KMT2C* (termed OTHER)) are depicted. Upon presence of one DNMT3A mutation a conditional odds ratio of 11/1 for having a second hit in *DNMT3A* or *TET2* as compared to OTHER was determined (p=0.006 based on McNemar exact test).
(D) Structural models of the DNMT3A variants W305*, S663fs (704*) and E733* in direct comparison to the wt protein were generated using homology modelling on SWISS-MODEL[83,84] based on the crystal structure of DNMT3A available on PDB under the alias 5YX2[37].
(E-G) Combined Annotation Dependent Depletion (CADD) based scoring[44,45] of the CH variants detected at a VAF $\geq$ 0.005 was performed. Scaled score for all (p=0.476), *DNMT3A* (p=0.358) and *TET2* (p=0.725) variants are shown in panel E, F and G, respectively.

Supplemental Figure 2. *DNMT3A* variants detected in blood donors are present in the myeloid and lymphoid lineage.
(A) Schematic presentation of analysis of mature and immature cell fractions isolated using FACS (green check mark) from PBMC of the frequent blood donors Donor 8 (B), 31, (C), 42 (D and E) and the control blood donor 371. VAF of each mutation was analyzed using digital droplet PCR (ddPCR) performed concurrently on the whole PBMC samples (timepoint 1 and 2) as well as the CD34+ cells, T- and B-cells and monocytes collected at the second donation timepoint. All five variants tested were detected in all 4 sorted cell populations, indicative of their presence in the myeloid (CMP, GMP, green mark in brackets) and lymphoid (CLP, green mark in brackets) lineage.

Supplemental Figure 3. Bone marrow HSPCs harboring R882 mutations expand in IFNγ-induced stress while non-preleukaemic *DNMT3A*-clones expand in EPO-induced stress.
Fold-change expansion (treated, with EPO or IFN***γ***, compared to untreated condition) of different mutations introduced in bone marrow HSPCs (4-6 biological donors tested). Each dot represents an independent biological donor. For each biological donor, a paired t-test was used to compare the percentage of the *DNMT3A*-mutant clones between different conditions

Supplemental Table 1. Blood Donor Cohorts Metadata

Group	DonorID	NumberOfDonations	SequencingDepth
FD	2	144	965.39
FD	3	115	1248.82
FD	4	120	958.07
FD	7	105	684.22
FD	9	133	664.41
FD	10	125	844.18
FD	11	112	805.95
FD	12	176	1016.42
FD	18	104	963.99
FD	19	101	2114.96
FD	20	136	853.85
FD	21	101	834.39
FD	22	110	1071.38
FD	23	126	633.68
FD	26	153	639.95
FD	27	107	809.51
FD	29	109	842.99
FD	30	110	715.83
FD	31	138	699.5
FD	33	105	431.14
FD	34	126	698.1
FD	35	104	873.91
FD	36	101	769.25
FD	38	151	1451.24
FD	40	154	1155.28
FD	41	167	626.71
FD	42	124	774.9
FD	44	146	855.66
FD	45	101	802.71
FD	46	125	804
FD	48	100	884.29
FD	50	127	536.82
FD	51	122	751.87
FD	53	101	692.95
FD	56	119	720.45
FD	57	122	1056.89
FD	66	102	1045.96
FD	94	161	1019.4
FD	116	102	1181.78
FD	122	107	1837.21
FD	123	115	1400.59
FD	124	128	1628.78
FD	126	130	1169.06
FD	136	111	1308.2
FD	143	134	1481.45
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FD	152	131	1473.09
FD	154	102	973
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FD	208	137	1369.68
FD	224	126	1440.35
FD	231	101	1470.76
FD	239	126	1385.89
FD	244	121	2061.18
FD	245	106	1501.18
FD	248	111	919.07
FD	253	103	1277.43
FD	256	103	1586.7
FD	257	125	1202.03
FD	261	149	1070.68
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FD	274	128	1061.47
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Supplemental Table 1. Blood Donor Cohorts Metadata

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FD	297	101	829.49
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FD	303	110	986.43
FD	305	144	849.68
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FD	373	111	1001
FD	389	144	1146.04
FD	391	111	1209.13
FD	401	139	1800.79
FD	402	100	2224.76
FD	406	178	633.25
FD	407	101	909.76
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CD	68	10	969.64
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CD	71	7	551.37
CD	72	2	1026.79
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CD	75	7	1029.25
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CD	104	5	567.11

Supplemental Table 1. Blood Donor Cohorts Metadata

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CD	157	7	1417.33
CD	166	2	865.39
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CD	184	3	1074.85
CD	193	6	1207.44
CD	196	3	1356.54
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CD	299	8	995.52
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CD	366	4	1114.64
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CD	420	3	1416.51
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CD	423	5	1456.79
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CD	442	9	1192.99
CD	444	2	1223.71
CD	445	8	1101.67
* extended FD			

Supplemental Table 2. List of all detected mutations

Group	DonorID	NumberOfDonations	SequencingDepth	Gene_Name	CHROM	TYPE	VariantAlleleFrequency (VAF)	EpigeneticModifier	CorrelationPlot
FD	2	144	965.39	NA	NA	NA	NA	NA	NA
FD	3	115	1248.82	DNMT3A	chr2	SNP	0.0065923	YES	DNMT3A
FD	3	115	1248.82	DNMT3A	chr2	SNP	0.0275362	YES	DNMT3A
FD	3	115	1248.82	DNMT3A	chr2	SNP	0.0321285	YES	DNMT3A
FD	4	120	958.07	NA	NA	NA	NA	NA	NA
FD	7	105	684.22	LRRC4	chr7	SNP	0.030853	NO	NA
FD*	8	102	825.06	CREBBP	chr16	SNP	0.0145278	YES	OTHER
FD*	8	102	825.06	DNMT3A	chr2	SNP	0.0210526	YES	DNMT3A
FD*	8	102	825.06	TET2	chr4	INDEL	0.0288684	YES	TET2
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FD	10	125	844.18	NA	NA	NA	NA	NA	NA
FD	11	112	805.95	DNMT3A	chr2	SNP	0.0276596	YES	DNMT3A
FD	12	176	1016.42	TET2	chr4	INDEL	0.0113636	YES	TET2
FD*	17	80	577.82	DNMT3A	chr2	INDEL	0.0225873	YES	DNMT3A
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FD	20	136	853.85	NA	NA	NA	NA	NA	NA
FD	21	101	834.39	NA	NA	NA	NA	NA	NA
FD	22	110	1071.38	NA	NA	NA	NA	NA	NA
FD	23	126	633.68	EP300	chr22	SNP	0.0122449	YES	OTHER
FD	26	153	639.95	DNMT3A	chr2	INDEL	0.0182927	YES	DNMT3A
FD	27	107	809.51	DNMT1	chr19	SNP	0.0094637	YES	OTHER
FD	27	107	809.51	TET2	chr4	SNP	0.0233645	YES	TET2
FD	27	107	809.51	DNMT3A	chr2	SNP	0.037225	YES	DNMT3A
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FD	30	110	715.83	NA	NA	NA	NA	NA	NA
FD	31	138	699.5	DNMT3A	chr2	SNP	0.0151515	YES	DNMT3A
FD	31	138	699.5	DNMT3A	chr2	INDEL	0.0751174	YES	DNMT3A
FD	33	105	431.14	DNMT3A	chr2	INDEL	0.0204082	YES	DNMT3A
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FD	35	104	873.91	DNMT3A	chr2	INDEL	0.0099256	YES	DNMT3A
FD	35	104	873.91	KMT2C	chr7	SNP	0.0192132	YES	OTHER
FD	36	101	769.25	NA	NA	NA	NA	NA	NA
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FD	38	151	1451.24	HNRNPK	chr9	SNP	0.0106222	NO	NA
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FD	42	124	774.9	DNMT3A	chr2	SNP	0.091858	YES	DNMT3A
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FD	45	101	802.71	CRLF2	chrX	SNP	0.3	NO	NA
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FD	51	122	751.87	DNMT3A	chr2	INDEL	0.060844	YES	DNMT3A
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FD	56	119	720.45	DNMT3A	chr2	INDEL	0.179669	YES	DNMT3A
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FD	94	161	1019.4	TET2	chr4	SNP	0.0131579	YES	TET2
FD	116	102	1181.78	TET2	chr4	SNP	0.0140625	YES	TET2
FD	122	107	1837.21	DNMT3A	chr2	SNP	0.0096043	YES	DNMT3A
FD	123	115	1400.59	DNMT3A	chr2	SNP	0.014549	YES	DNMT3A
FD	123	115	1400.59	ASXL1	chr20	INDEL	0.0460594	YES	OTHER
FD	124	128	1628.78	SF3B1	chr2	SNP	0.0102564	NO	NA
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FD	126	130	1169.06	NA	NA	NA	NA	NA	NA
FD	136	111	1308.2	DNMT3A	chr2	SNP	0.0082902	YES	DNMT3A
FD	143	134	1481.45	KMT2C	chr7	SNP	0.04323	YES	OTHER
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FD	152	131	1473.09	NA	NA	NA	NA	NA	NA
FD	154	102	973	BCR	chr22	INDEL	0.0397351	NO	NA
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FD	224	126	1440.35	NA	NA	NA	NA	NA	NA
FD	231	101	1470.76	NA	NA	NA	NA	NA	NA
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FD	244	121	2061.18	TET2	chr4	SNP	0.0212187	YES	TET2
FD	244	121	2061.18	DNMT3A	chr2	SNP	0.1382037	YES	DNMT3A
FD	245	106	1501.18	NA	NA	NA	NA	NA	NA
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FD	253	103	1277.43	DNMT3A	chr2	SNP	0.007014	YES	DNMT3A
FD	256	103	1586.7	CBL	chr11	SNP	0.0130152	NO	NA
FD	256	103	1586.7	JAK3	chr19	SNP	0.0635593	NO	NA
FD	257	125	1202.03	DNMT3A	chr2	SNP	0.0064	YES	DNMT3A
FD	261	149	1070.68	EED	chr11	SNP	0.0086207	YES	OTHER
FD	264	172	1048.1	WAS	chrX	SNP	0.01373	NO	NA
FD	264	172	1048.1	KMT2C	chr7	SNP	0.021164	YES	OTHER
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FD	269	109	1211.45	KMT2C	chr7	COMPLEX	0.1280559	YES	OTHER
FD	270	100	1088.81	TET2	chr4	SNP	0.0088409	YES	TET2
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FD	273	135	1548.4	DNMT3A	chr2	SNP	0.013459	YES	DNMT3A
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FD	278	111	2289.06	BRINP3	chr1	SNP	0.0078377	NO	NA
FD	280	133	1127.18	KMT2C	chr7	SNP	0.2608696	YES	OTHER
FD	282	104	1609.41	KAT6A	chr8	INDEL	0.0124052	YES	OTHER
FD	289	134	1526.98	KDM6A	chrX	SNP	0.0225806	YES	OTHER

Supplemental Table 2. List of all detected mutations

FD	291	116	1421.81	TET2	chr4	SNP	0.0212528	YES	TET2
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FD	303	110	986.43	TET2	chr4	SNP	0.0107095	YES	TET2
FD	303	110	986.43	TET2	chr4	SNP	0.0433071	YES	TET2
FD	305	144	849.68	NA	NA	NA	NA	NA	NA
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FD	342	177	1148.35	NA	NA	NA	NA	NA	NA
FD	343	103	939.79	DNMT3A	chr2	SNP	0.0067822	YES	DNMT3A
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CD	60	7	753.56	NA	NA	NA	NA	NA	NA
CD	61	6	1067.14	NA	NA	NA	NA	NA	NA
CD	63	4	1125.29	DDX41	chr5	SNP	0.0575658	NO	NA
CD	65	5	1176.42	NA	NA	NA	NA	NA	NA
CD	67	9	1034.84	NA	NA	NA	NA	NA	NA
CD	68	10	969.64	NA	NA	NA	NA	NA	NA
CD	69	10	1867.19	HNRNPK	chr9	INDEL	0.0063839	NO	NA
CD	69	10	1867.19	DNMT3A	chr2	INDEL	0.0077419	YES	DNMT3A
CD	70	9	1045.26	TERT	chr5	SNP	0.0102421	NO	NA
CD	70	9	1045.26	FIP1L1	chr4	SNP	0.0267062	NO	NA
CD	71	7	551.37	SMC1A	chrX	SNP	0.1501976	NO	NA
CD	72	2	1026.79	DNMT3A	chr2	SNP	0.0210526	YES	DNMT3A
CD	73	3	1117	TET2	chr4	INDEL	0.0113493	YES	TET2
CD	74	5	1079.51	NA	NA	NA	NA	NA	NA
CD	75	7	1029.25	DNMT3A	chr2	INDEL	0.0192593	YES	DNMT3A
CD	76	10	456.5	NA	NA	NA	NA	NA	NA
CD	77	3	822.63	NA	NA	NA	NA	NA	NA
CD	78	5	757.04	TET2	chr4	SNP	0.0343511	YES	TET2
CD	78	5	757.04	TET2	chr4	SNP	0.0564706	YES	TET2
CD	79	9	501.04	ANKRD26	chr10	SNP	0.0172414	NO	NA
CD	80	6	514.87	NA	NA	NA	NA	NA	NA
CD	81	7	542.05	NA	NA	NA	NA	NA	NA
CD	82	5	471.94	NA	NA	NA	NA	NA	NA
CD	84	7	1553.71	NA	NA	NA	NA	NA	NA
CD	85	8	1429.82	NOTCH1	chr9	SNP	0.0069174	NO	NA
CD	85	8	1429.82	FAM47A	chrX	SNP	0.0163551	NO	NA
CD	85	8	1429.82	JAK3	chr19	SNP	0.0318991	NO	NA
CD	86	2	1184.06	NA	NA	NA	NA	NA	NA
CD	87	7	1157.13	RUNX1	chr21	SNP	0.0106257	NO	NA
CD	88	7	675.78	NA	NA	NA	NA	NA	NA
CD	89	3	732.62	NA	NA	NA	NA	NA	NA
CD	90	6	1818.44	NA	NA	NA	NA	NA	NA
CD	91	10	784.15	NA	NA	NA	NA	NA	NA
CD	95	2	780.4	NA	NA	NA	NA	NA	NA
CD	97	4	765.02	NA	NA	NA	NA	NA	NA
CD	98	10	627.72	BCR	chr22	SNP	0.0296736	NO	NA
CD	99	10	501.82	NA	NA	NA	NA	NA	NA
CD	100	10	649.8	NA	NA	NA	NA	NA	NA
CD	101	5	463.59	NA	NA	NA	NA	NA	NA
CD	102	8	418.31	FAM47A	chrX	SNP	0.0431655	NO	NA
CD	103	10	486.56	NA	NA	NA	NA	NA	NA
CD	104	5	567.11	NA	NA	NA	NA	NA	NA
CD	105	4	600.85	NA	NA	NA	NA	NA	NA
CD	106	8	590.3	U2AF1	chr21	SNP	0.0263975	NO	NA
CD	106	8	590.3	ASXL1	chr20	INDEL	0.030445	YES	OTHER
CD	107	3	662.19	ATM	chr11	SNP	0.0116822	NO	NA
CD	127	5	1137.27	DNMT3A	chr2	SNP	0.0113924	YES	DNMT3A
CD	132	5	1146.5	TET2	chr4	SNP	0.0248619	YES	TET2
CD	132	5	1146.5	WAS	chrX	SNP	0.0677966	NO	NA
CD	132	5	1146.5	DNMT3A	chr2	SNP	0.3051085	YES	DNMT3A
CD	144	9	1237.83	NA	NA	NA	NA	NA	NA
CD	146	2	1336.53	DNMT3A	chr2	SNP	0.01375	YES	DNMT3A
CD	148	8	1028.87	NA	NA	NA	NA	NA	NA
CD	157	7	1417.33	NA	NA	NA	NA	NA	NA
CD	166	2	865.39	NA	NA	NA	NA	NA	NA

Supplemental Table 2. List of all detected mutations

CD	177	3	896.57	ASXL2	chr2	INDEL	0.0145228	YES	OTHER
CD	177	3	896.57	DNMT3A	chr2	SNP	0.0155521	YES	DNMT3A
CD	177	3	896.57	DNMT3A	chr2	INDEL	0.0636833	YES	DNMT3A
CD	183	8	1011.07	BRCA2	chr13	SNP	0.0162866	NO	NA
CD	183	8	1011.07	DNMT3A	chr2	SNP	0.0193322	YES	DNMT3A
CD	184	3	1074.85	NA	NA	NA	NA	NA	NA
CD	193	6	1207.44	SETBP1	chr18	SNP	0.0181818	YES	OTHER
CD	193	6	1207.44	DNMT3A	chr2	INDEL	0.0211679	YES	DNMT3A
CD	193	6	1207.44	TET2	chr4	SNP	0.0297806	YES	TET2
CD	196	3	1356.54	SF3B1	chr2	INDEL	0.0108254	NO	NA
CD	201	3	1267.44	DNM2	chr19	SNP	0.04209	NO	NA
CD	220	9	1425.11	NA	NA	NA	NA	NA	NA
CD	222	4	1584.5	CBL	chr11	SNP	0.0080175	NO	NA
CD	228	8	1532.17	NA	NA	NA	NA	NA	NA
CD	234	9	1606.52	NA	NA	NA	NA	NA	NA
CD	241	2	734.54	NA	NA	NA	NA	NA	NA
CD	243	6	1383.55	NA	NA	NA	NA	NA	NA
CD	250	5	1413.03	NA	NA	NA	NA	NA	NA
CD	251	4	874.87	SH2B3	chr12	SNP	0.0094439	NO	NA
CD	259	4	873.74	NA	NA	NA	NA	NA	NA
CD	283	2	2170.12	HNRNPK	chr9	INDEL	0.0059752	NO	NA
CD	283	2	2170.12	DNMT3A	chr2	SNP	0.0234443	YES	DNMT3A
CD	283	2	2170.12	DNMT3A	chr2	INDEL	0.0244845	YES	DNMT3A
CD	283	2	2170.12	NPAT	chr11	INDEL	0.352575	NO	NA
CD	287	3	2382.11	NA	NA	NA	NA	NA	NA
CD	296	8	729.72	NA	NA	NA	NA	NA	NA
CD	299	8	995.52	NA	NA	NA	NA	NA	NA
CD	304	2	979.31	NA	NA	NA	NA	NA	NA
CD	308	3	1217.92	NA	NA	NA	NA	NA	NA
CD	309	4	904.62	NA	NA	NA	NA	NA	NA
CD	315	4	1011.33	NA	NA	NA	NA	NA	NA
CD	323	4	1280.06	NA	NA	NA	NA	NA	NA
CD	356	4	1229.8	BRCA2	chr13	SNP	0.0465116	NO	NA
CD	366	4	1114.64	NA	NA	NA	NA	NA	NA
CD	371	3	1071.62	DNMT3A	chr2	SNP	0.0131805	YES	DNMT3A
CD	371	3	1071.62	DNM2	chr19	SNP	0.0136986	NO	NA
CD	372	6	2685.15	TET2	chr4	INDEL	0.0062684	YES	TET2
CD	372	6	2685.15	DNMT3A	chr2	SNP	0.0091463	YES	DNMT3A
CD	376	9	1041.21	KMT2C	chr7	SNP	0.048913	YES	OTHER
CD	378	2	922.1	NA	NA	NA	NA	NA	NA
CD	387	2	1290.16	CUX1	chr7	SNP	0.0168498	NO	NA
CD	398	8	1201.88	NA	NA	NA	NA	NA	NA
CD	408	8	1164.44	NA	NA	NA	NA	NA	NA
CD	410	3	910.63	IL7R	chr5	SNP	0.3724792	NO	NA
CD	413	2	1939.22	NA	NA	NA	NA	NA	NA
CD	415	4	2331.81	TET2	chr4	SNP	0.0263415	YES	TET2
CD	416	9	2257.19	GJB3	chr1	SNP	0.0051787	NO	NA
CD	416	9	2257.19	TAL1	chr1	SNP	0.0066815	NO	NA
CD	417	9	1059.48	NA	NA	NA	NA	NA	NA
CD	418	9	1000.44	NA	NA	NA	NA	NA	NA
CD	419	2	1105.3	NA	NA	NA	NA	NA	NA
CD	420	3	1416.51	DNMT3A	chr2	SNP	0.0151362	YES	DNMT3A
CD	420	3	1416.51	DNMT3A	chr2	SNP	0.037037	YES	DNMT3A
CD	422	7	1620.38	BRCA1	chr17	SNP	0.0082353	NO	NA
CD	422	7	1620.38	ABL1	chr9	SNP	0.0084122	NO	NA
CD	423	5	1456.79	DNMT3A	chr2	INDEL	0.064877	YES	DNMT3A
CD	426	2	1280.99	NA	NA	NA	NA	NA	NA
CD	430	8	2539.58	CHEK2	chr22	INDEL	0.0062406	NO	NA
CD	430	8	2539.58	DNMT3A	chr2	SNP	0.0069212	YES	DNMT3A
CD	433	7	1623.37	DNMT3A	chr2	SNP	0.0178069	YES	DNMT3A
CD	434	4	1624.36	DNMT3A	chr2	SNP	0.012973	YES	DNMT3A
CD	434	4	1624.36	DNMT3A	chr2	SNP	0.0143296	YES	DNMT3A
CD	434	4	1624.36	DNMT3A	chr2	INDEL	0.0257787	YES	DNMT3A
CD	434	4	1624.36	SMC1A	chrX	SNP	0.0388889	NO	NA
CD	436	2	2241.62	NA	NA	NA	NA	NA	NA
CD	437	3	2060.62	NA	NA	NA	NA	NA	NA
CD	438	4	1418.54	NA	NA	NA	NA	NA	NA
CD	439	5	1660.14	NA	NA	NA	NA	NA	NA
CD	440	8	969.64	NA	NA	NA	NA	NA	NA
CD	442	9	1192.99	NA	NA	NA	NA	NA	NA
CD	444	2	1223.71	DNMT3A	chr2	INDEL	0.0077419	YES	DNMT3A
CD	445	8	1101.67	NA	NA	NA	NA	NA	NA
* extended FD									

Supplemental Table 3. List of all DNMT3A mutations

Group	UMIDepth	DonorID	NumberOfDonations	CHROM	POS	ID	REF	ALT	QUAL	FILTER	TYPE	DP	UMT	VMT	VMF	Allele	Annotation	Annotation_Impact	Gene_Name	HGVS.c	Exchange	IDandPos	Stability Score	Premature STOP?
CD	1029.25	75	7	chr2	25469990		C	CA	12	PASS	INDEL	5861	675	13	0.0192593	CA	frameshift_variant	HIGH	DNMT3A	c.1051dupT	p.Cys351fs	DNMT3A c.1051dupT	NA	STOP
CD	1623.37	433	7	chr2	25469919		C	T	15	PASS	SNP	5423	1067	19	0.0178069	T	splice_donor_variant&intron_variant	HIGH	DNMT3A	c.1122+1G>A	NA	DNMT3A c.1122+1G>A	NA	NOT STOP
CD	1207.44	193	6	chr2	25469588		CA	C	24	PASS	INDEL	8936	1370	29	0.0211679	C	frameshift_variant	HIGH	DNMT3A	c.1179delT	p.Ser393fs	DNMT3A c.1179delT	NA	STOP
CD	1416.51	420	3	chr2	25467023		C	T	38	PASS	SNP	4067	1080	40	0.037037	T	splice_donor_variant&intron_variant	HIGH	DNMT3A	c.1851+1G>A	NA	DNMT3A c.1851+1G>A	NA	NOT STOP
CD	2170.12	283	2	chr2	25466784		AAG	A	67	PASS	INDEL	14465	3880	95	0.0244845	A	frameshift_variant	HIGH	DNMT3A	c.1917_1918delCT	p.Phe640fs	DNMT3A c.1917_1918delCT	NA	STOP
CD	1416.51	420	3	chr2	25464456		T	C	11	PASS	SNP	4131	991	15	0.0151362	C	missense_variant	MODERATE	DNMT3A	c.2057A>G	p.Asp686Gly	DNMT3A c.2057A>G	NA	NOT STOP
CD	1624.36	434	4	chr2	25463576		A	C	9	PASS	SNP	5430	925	12	0.012973	C	structural_interaction_variant	HIGH	DNMT3A	c.2106T>G	p.Asp702Glu	DNMT3A c.2106T>G	NA	NOT STOP
CD	1026.79	72	2	chr2	25463568	COSM1583102	A	G	11	PASS	SNP	3991	570	12	0.0210526	G	missense_variant	MODERATE	DNMT3A	c.2114T>C	p.Ile705Thr	DNMT3A c.2114T>C	0,3875	NOT STOP
CD	896.57	177	3	chr2	25463549		AT	A	46	PASS	INDEL	4942	581	37	0.0636833	A	frameshift_variant	HIGH	DNMT3A	c.2132delA	p.Asn711fs	DNMT3A c.2132delA	NA	STOP
CD	1867.19	69	10	chr2	25463291		GAACTCAAA	G	6	PASS	INDEL	5422	1550	12	0.0077419	G	frameshift_variant	HIGH	DNMT3A	c.2194_2201delTTTGAGTT	p.Phe732fs	DNMT3A c.2194_2201delTTTGAGTT	NA	STOP
CD	1137.27	127	5	chr2	25463289	COSM133126	T	C	7	PASS	SNP	5360	790	9	0.0113924	C	missense_variant	MODERATE	DNMT3A	c.2204A>G	p.Tyr735Cys	DNMT3A c.2204A>G	0,8631	NOT STOP
CD	1223.71	444	2	chr2	25463235	COSM133723	CAGA	C	7	PASS	INDEL	14479	1550	12	0.0077419	C	protein_protein_contact	HIGH	DNMT3A	c.2255_2257delTCT	p.Phe752fs	DNMT3A c.2255_2257delTCT	NA	
CD	1624.36	434	4	chr2	25463235	COSM133723	CAGA	C	36	PASS	INDEL	10019	1862	48	0.0257787	C	structural_interaction_variant	HIGH	DNMT3A	c.2255_2257delTCT	p.Phe752fs	DNMT3A c.2255_2257delTCT	NA	
CD	2170.12	283	2	chr2	25463232		A	T	56	PASS	SNP	13133	3455	81	0.0234443	T	missense_variant	MODERATE	DNMT3A	c.2261T>A	p.Leu754His	DNMT3A c.2261T>A	NA	NOT STOP
CD	2539.58	430	8	chr2	25463212		T	C	11	PASS	SNP	12828	4479	31	0.0069212	C	missense_variant	MODERATE	DNMT3A	c.2281A>G	p.Met761Val	DNMT3A c.2281A>G	NA	NOT STOP
CD	1336.53	146	2	chr2	25462068	COSM1583121	A	G	9	PASS	SNP	4647	800	11	0.01375	G	missense_variant	MODERATE	DNMT3A	c.2339T>C	p.Ile780Thr	DNMT3A c.2339T>C	0,4926	NOT STOP
CD	2685.15	372	6	chr2	25459806	COSM1583124	T	C	8	PASS	SNP	5301	1640	15	0.0091463	C	missense_variant&splice_region_variant	MODERATE	DNMT3A	c.2477A>G	p.Lys826Arg	DNMT3A c.2477A>G	1,0628	NOT STOP
CD	896.57	177	3	chr2	25458652		T	C	9	PASS	SNP	5143	643	10	0.0155521	C	missense_variant	MODERATE	DNMT3A	c.2521A>G	p.Lys841Glu	DNMT3A c.2521A>G	NA	NOT STOP
CD	1456.79	423	5	chr2	25458596		TA	T	69	PASS	INDEL	5259	894	58	0.064877	T	frameshift_variant	HIGH	DNMT3A	c.2576delT	p.Leu859fs	DNMT3A c.2576delT	NA	STOP
CD	1011.07	183	8	chr2	25458595	COSM231568	A	G	10	PASS	SNP	4259	569	11	0.0193322	G	missense_variant	MODERATE	DNMT3A	c.2578T>C	p.Trp860Arg	DNMT3A c.2578T>C	0,9209	NOT STOP
CD	1624.36	434	4	chr2	25458595	COSM231568	A	G	10	PASS	SNP	6675	977	14	0.0143296	G	missense_variant	MODERATE	DNMT3A	c.2578T>C	p.Trp860Arg	DNMT3A c.2578T>C	0,9209	NOT STOP
CD	1071.62	371	3	chr2	25457243	COSM1166704;COSM53042	G	A	14	PASS	SNP	8986	1745	23	0.0131805	A	missense_variant	MODERATE	DNMT3A	c.2644C>T	p.Arg882Cys	DNMT3A c.2644C>T	NA	NOT STOP
CD	1146.5	132	5	chr2	25457242	COSM442676;COSM52944	C	T	200	PASS	SNP	9591	1429	436	0.3051085	T	missense_variant	MODERATE	DNMT3A	c.2645G>A	p.Arg882His	DNMT3A c.2645G>A	0,9765	NOT STOP
FD	1088.81	270	100	chr2	25470472	COSM133727	GC	G	34	PASS	INDEL	7848	834	33	0.0395683	G	frameshift_variant	HIGH	DNMT3A	c.1001delG	p.Gly334fs	DNMT3A c.1001delG	NA	STOP
FD	774.9	42	124	chr2	25470002		A	G	7	PASS	SNP	6174	570	8	0.0140351	G	missense_variant	MODERATE	DNMT3A	c.1040T>C	p.Leu347Pro	DNMT3A c.1040T>C	NA	NOT STOP
FD	1421.81	291	116	chr2	25469945		C	T	190	PASS	SNP	7838	986	132	0.1338742	T	missense_variant	MODERATE	DNMT3A	c.1097G>A	p.Arg366His	DNMT3A c.1097G>A	0,7082	NOT STOP
FD	431.14	33	105	chr2	25469613	COSM1717669;COSM1717670	CG	C	6	PASS	INDEL	3562	245	5	0.0204082	C	frameshift_variant	HIGH	DNMT3A	c.1154delC	p.Pro385fs	DNMT3A c.1154delC	NA	STOP
FD	639.95	26	153	chr2	25469083		TC	T	11	PASS	INDEL	4805	656	12	0.0182927	T	frameshift_variant	HIGH	DNMT3A	c.1374delG	p.Lys459fs	DNMT3A c.1374delG	NA	STOP
FD	919.07	248	111	chr2	25468928		GC	G	16	PASS	INDEL	4400	523	15	0.0286807	G	frameshift_variant	HIGH	DNMT3A	c.1434delG	p.Leu479fs	DNMT3A c.1434delG	NA	STOP
FD	1202.03	257	125	chr2	25467467		A	T	6	PASS	SNP	11472	1875	12	0.0064	T	missense_variant	MODERATE	DNMT3A	c.1609T>A	p.Cys537Ser	DNMT3A c.1609T>A	0,4449	NOT STOP
FD	1432.01	200	111	chr2	25467408		C	T	16	PASS	SNP	3563	881	19	0.0215664	T	splice_donor_variant&intron_variant	HIGH	DNMT3A	c.1667+1G>A	NA	DNMT3A c.1667+1G>A	NA	NOT STOP
FD	873.91	35	104	chr2	25467104		T	TGC	6	PASS	INDEL	5687	806	8	0.0099256	TGC	frameshift_variant	HIGH	DNMT3A	c.1770_1771insGC	p.Thr591fs	DNMT3A c.1770_1771insGC	NA	STOP
FD	1248.82	3	115	chr2	25466800	COSM1407108;COSM87012	G	A	6	PASS	SNP	9796	1972	13	0.0065923	A	structural_interaction_variant	HIGH	DNMT3A	c.1903C>T	p.Arg635Trp	DNMT3A c.1903C>T	0,4060	NOT STOP
FD	1308.2	136	111	chr2	25466800	COSM1407108;COSM87012	G	A	9	PASS	SNP	16504	1930	16	0.0082902	A	structural_interaction_variant	HIGH	DNMT3A	c.1903C>T	p.Arg635Trp	DNMT3A c.1903C>T	0,4060	NOT STOP
FD	873.91	35	104	chr2	25464525		G	C	6	PASS	SNP	8606	1263	8	0.0063341	C	missense_variant	MODERATE	DNMT3A	c.1988C>G	p.Ser663Trp	DNMT3A c.1988C>G	0,4803	NOT STOP
FD	751.87	51	122	chr2	25464524		CG	C	73	PASS	INDEL	8683	1019	62	0.060844	C	frameshift_variant	HIGH	DNMT3A	c.1988delC	p.Ser663fs	DNMT3A c.1988delC	NA	STOP
FD	1628.78	124	128	chr2	25463600		C	T	8	PASS	SNP	4293	1069	12	0.0112254	T	splice_acceptor_variant&intron_variant	HIGH	DNMT3A	c.2083-1G>A	NA	DNMT3A c.2083-1G>A	NA	NOT STOP
FD	699.5	31	138	chr2	25463297		AAAG	A	42	PASS	INDEL	4982	426	32	0.0751174	A	disruptive_inframe_deletion	MODERATE	DNMT3A	c.2193_2195delCTT	p.Phe732del	DNMT3A c.2193_2195delCTT	NA	
FD	774.9	42	124	chr2	25463296		C	A	71	PASS	SNP	3927	479	44	0.091858	A	stop_gained	HIGH	DNMT3A	c.2197G>T	p.Glu733*	DNMT3A c.2197G>T	NA	STOP
FD	1387.65	352	147	chr2	25463292		A	G	48	PASS	SNP	5810	1044	46	0.0440613	G	missense_variant	MODERATE	DNMT3A	c.2201T>C	p.Phe734Ser	DNMT3A c.2201T>C	NA	NOT STOP
FD	963.99	18	104	chr2	25463287	COSM231560	G	A	15	PASS	SNP	8939	1152	19	0.0164931	A	protein_protein_contact	HIGH	DNMT3A	c.2206C>T	p.Arg736Cys	DNMT3A c.2206C>T	0,3159	NOT STOP
FD	1064.53	310	100	chr2	25463248	COSM219133	G	A	6	PASS	SNP	8828	1594	11	0.0069009	A	structural_interaction_variant	HIGH	DNMT3A	c.2245C>T	p.Arg749Cys	DNMT3A c.2245C>T	0,3393	NOT STOP
FD	1056.89	57	122	chr2	25463229		A	G	7	PASS	SNP	9377	1172	11	0.0093857	G	missense_variant	MODERATE	DNMT3A	c.2264T>C	p.Phe755Ser	DNMT3A c.2264T>C	NA	NOT STOP
FD	1125.23	321	143	chr2	25463184	COSM231549	G	A	75	PASS	SNP	4663	776	60	0.0773196	A	missense_variant	MODERATE	DNMT3A	c.2309C>T	p.Ser770Leu	DNMT3A c.2309C>T	0,4189	NOT STOP
FD	1248.82	3	115	chr2	25463182	COSM231563	G	A	18	PASS	SNP	4148	690	19	0.0275362	A	stop_gained	HIGH	DNMT3A	c.2311C>T	p.Arg771*	DNMT3A c.2311C>T	NA	STOP
FD	1548.4	273	135	chr2	25463182	COSM231563	G	A	8	PASS	SNP	5110	743	10	0.013459	A	stop_gained	HIGH	DNMT3A	c.2311C>T	p.Arg771*	DNMT3A c.2311C>T	NA	STOP
FD	805.95	11	112	chr2	25462085		C	A	17	PASS	SNP	3420	470	13	0.0276596	A	splice_acceptor_variant&intron_variant	HIGH	DNMT3A	c.2323-1G>T	NA	DNMT3A c.2323-1G>T	NA	NOT STOP
FD	720.45	56	119	chr2	25459847		CT	C	125	PASS	INDEL	3922	423	76	0.179669	C	frameshift_variant	HIGH	DNMT3A	c.2435delA	p.Lys812fs	DNMT3A c.2435delA	NA	STOP
FD	699.5	31	138	chr2	25458661	COSM231575	T	C	7	PASS	SNP	5729	528	8	0.0151515	C	missense_variant	MODERATE	DNMT3A	c.2512A>G	p.Asn838Asp	DNMT3A c.2512A>G	0,9860	NOT STOP
FD	1045.96	66	102	chr2	25457285		A	G	15	PASS	SNP	7376	564	15	0.0265957	G	protein_protein_contact	HIGH	DNMT3A	c.2602T>C	p.Phe868Leu	DNMT3A c.2602T>C	NA	NOT STOP
FD	1277.43	253	103	chr2	25457243	COSM1166704;COSM53042	G	A	7	PASS	SNP	11726	1996	14	0.007014	A	missense_variant	MODERATE	DNMT3A	c.2644C>T	p.Arg882Cys	DNMT3A c.2644C>T	NA	NOT STOP
FD	1837.21	122	107	chr2	25457243	COSM1166704;COSM53042	G	A	13	PASS	SNP	11184	2603	25	0.0096043	A	missense_variant	MODERATE	DNMT3A	c.2644C>T	p.Arg882Cys	DNMT3A c.2644C>T	NA	NOT STOP
FD	2061.18	244	121	chr2	25457242	COSM442676;COSM52944	C	T	200	PASS	SNP	13037	3162	437	0.1382037	T	missense_variant	MODERATE	DNMT3A	c.2645G>A	p.Arg882His	DNMT3A c.2645G>A	0,9765	NOT STOP
FD	939.79	343	103	chr2	25457204		C	G	7	PASS	SNP	10791	1327	9	0.0067822	G	structural_interaction_variant	HIGH	DNMT3A	c.2683G>C	p.Val895Leu	DNMT3A c.2683G>C	NA	NOT STOP
FD*	577.82	17	80	chr2	25470903		CA	C	11	PASS	INDEL	4478	487	11	0.0225873	C	splice_donor_variant&intron_variant	HIGH	DNMT3A	c.855+2delT	NA	DNMT3A c.855+2delT	NA	NOT STOP
FD*	825.06	8	102	chr2	25470560	COSM1169636	C	T	13	PASS	SNP	4890	665	14	0.0210526	T	stop_gained	HIGH	DNMT3A	c.914G>A	p.Trp305*	DNMT3A c.914G>A	NA	STOP
FD	1248.82	3	115	chr2	25470559		C	T	37	PASS	SNP	5874	1245	40	0.0321285	T	stop_gained	HIGH	DNMT3A	c.915G>A	p.Trp305*	DNMT3A c.915G>A	NA	STOP
FD	809.51	27	107	chr2	25470498		G	C	29	PASS	SNP	5058	591	22	0.037225	C	missense_variant	MODERATE	DNMT3A	c.976C>G	p.Arg326Gly	DNMT3A c.976C>G	NA	NOT STOP
FD	1400.59	123	115	chr2	25470481		G	T	14	PASS	SNP	7617	1031	15	0.014549	T	missense_variant	MODERATE	DNMT3A	c.993C>A	p.Phe331Leu	DNMT3A c.993C>A	NA	NOT STOP
* extended FD																								

Supplemental Table 4. List of all *TET2* mutations

Group	UMIDepth	VariantsTotal	DonorID	NumberOfDonations	CHROM	POS	ID	REF	ALT	QUAL	FILTER	TYPE	DP	UMT	VMT	VMF	Allele	Annotation	Annotation_Impact	Gene_Name	HGVS.c	Exchange	IDandPos
CD	757.04	149	78	5	chr4	106196309	COSM51209	C	T	19	PASS	SNP	6420	524	18	0.0343511	T	stop_gained	HIGH	TET2	c.4705C>T	p.Gln1569*	TET2 c.4705C>T
CD	757.04	149	78	5	chr4	106197285	COSM41741	T	C	30	PASS	SNP	3903	425	24	0.0564706	C	missense_variant	MODERATE	TET2	c.5681T>C	p.Ile1894Thr	TET2 c.5681T>C
FD	802.71	164	45	101	chr4	106157515	.	CT	C	8	PASS	INDEL	6188	785	10	0.0127389	C	frameshift_variant	HIGH	TET2	c.2480delT	p.Leu827fs	TET2 c.2480delT
FD	809.51	193	27	107	chr4	106162586	.	G	A	14	PASS	SNP	5627	642	15	0.0233645	A	missense_variant&splice_region_variant	MODERATE	TET2	c.3563G>A	p.Arg1188Lys	TET2 c.3563G>A
FD*	825.06	165	8	102	chr4	106193849	.	G	GA	24	PASS	INDEL	8010	866	25	0.0288684	GA	frameshift_variant	HIGH	TET2	c.4380dupA	p.Arg1461fs	TET2 c.4380dupA
FD	986.43	199	303	110	chr4	106164772	.	C	T	6	PASS	SNP	4671	747	8	0.0107095	T	protein_protein_contact	HIGH	TET2	c.3640C>T	p.Arg1214Trp	TET2 c.3640C>T
FD	986.43	199	303	110	chr4	106158075	.	T	A	30	PASS	SNP	2738	508	22	0.0433071	A	stop_gained	HIGH	TET2	c.3039T>A	p.Cys1013*	TET2 c.3039T>A
FD	1016.42	172	12	176	chr4	106196229	.	T	TCATGCAGCAGTCCCAGC	7	PASS	INDEL	6604	792	9	0.0113636	TCATGCAGCAGTCCCAGC	frameshift_variant	HIGH	TET2	c.4626_4642dupCATGCAGCAGTCCCAGC	p.Gln1548fs	TET2 c.4626_4642dupCATGCAGCAGTCCCAGC
FD	1019.4	172	94	161	chr4	106194066	.	C	T	7	PASS	SNP	4204	608	8	0.0131579	T	stop_gained	HIGH	TET2	c.4591C>T	p.Gln1531*	TET2 c.4591C>T
FD	1088.81	186	270	100	chr4	106157474	.	C	G	8	PASS	SNP	8129	1018	9	0.0088409	G	stop_gained	HIGH	TET2	c.2438C>G	p.Ser813*	TET2 c.2438C>G
CD	1117	164	73	3	chr4	106157069	.	C	CA	7	PASS	INDEL	5497	793	9	0.0113493	CA	frameshift_variant	HIGH	TET2	c.2034dupA	p.His679fs	TET2 c.2034dupA
CD	1146.5	165	132	5	chr4	106196374	.	C	A	16	PASS	SNP	4431	724	18	0.0248619	A	stop_gained	HIGH	TET2	c.4770C>A	p.Tyr1590*	TET2 c.4770C>A
FD	1181.78	154	116	102	chr4	106180790	COSM87135	G	C	7	PASS	SNP	5085	640	9	0.0140625	C	missense_variant	MODERATE	TET2	c.3881G>C	p.Cys1294Ser	TET2 c.3881G>C
CD	1207.44	172	193	6	chr4	106164061	.	C	T	19	PASS	SNP	4832	638	19	0.0297806	T	stop_gained	HIGH	TET2	c.3634C>T	p.Gln1212*	TET2 c.3634C>T
FD	1266.67	154	369	136	chr4	106180835	.	G	A	59	PASS	SNP	4293	622	46	0.073955	A	missense_variant	MODERATE	TET2	c.3926G>A	p.Gly1309Asp	TET2 c.3926G>A
FD	1305	172	347	100	chr4	106196551	.	T	G	6	PASS	SNP	7347	1152	10	0.0086806	G	stop_gained	HIGH	TET2	c.4947T>G	p.Tyr1649*	TET2 c.4947T>G
FD	1305	172	347	100	chr4	106196664	.	CT	C	6	PASS	INDEL	5298	819	8	0.009768	C	frameshift_variant	HIGH	TET2	c.5061delT	p.Leu1688fs	TET2 c.5061delT
FD	1421.81	172	291	116	chr4	106156825	.	G	T	15	PASS	SNP	7077	894	19	0.0212528	T	stop_gained	HIGH	TET2	c.1789G>T	p.Glu597*	TET2 c.1789G>T
FD	2061.18	155	244	121	chr4	106157212	.	C	T	28	PASS	SNP	7583	1838	39	0.0212187	T	stop_gained	HIGH	TET2	c.2176C>T	p.Gln726*	TET2 c.2176C>T
FD	2224.76	154	402	100	chr4	106158419	.	C	CA	15	PASS	INDEL	9707	1444	21	0.0145429	CA	frameshift_variant	HIGH	TET2	c.3384dupA	p.Pro1129fs	TET2 c.3384dupA
CD	2331.81	205	415	4	chr4	106164936	.	G	A	23	PASS	SNP	3346	1025	27	0.0263415	A	splice_donor_variant&intron_variant	HIGH	TET2	c.3866+1G>A	NA	TET2 c.3866+1G>A
CD	2685.15	166	372	6	chr4	106156744	.	ACACGAGATCTTGTG	A	7	PASS	INDEL	7350	2712	17	0.0062684	A	frameshift_variant	HIGH	TET2	c.1710_1723delACGAGATCTTGTGC	p.Arg571fs	TET2 c.1710_1723delACGAGATCTTGTGC
* extended FD																							

Supplemental Table 5. DNMT3A fitness scores and site-specific mutation rates

Group	DonorID	AminoAcidExchange	FitnessScore (% growth per year)	SiteSpecificMutationRate (μ x10*-9 per year)
FD	253	p.Arg882Cys	12,30	1,42E-03
FD	122	p.Arg882Cys	12,30	1,42E-03
FD	42	p.Glu733*	12,38	5,40E-05
FD	244	p.Arg882His	13,07	1,88E-03
FD	31	p.Asn838Asp	14,59	4,96E-05
FD	57	p.Phe755Ser	14,96	4,96E-05
FD	291	p.Arg366His	7,30	1,88E-03
FD	321	p.Ser770Leu	8,04	1,20E-03
FD	3	p.Arg635Trp	8,98	1,42E-03
FD	136	p.Arg635Trp	8,98	1,42E-03
FD	310	p.Arg749Cys	9,22	1,20E-03
FD	3	p.Arg771*	9,27	1,88E-03
FD	273	p.Arg771*	9,27	1,88E-03
FD	18	p.Arg736Cys	9,42	1,42E-03
FD	3	p.Trp305*	9,88	5,14E-04
CD	430	p.Met761Val	11,66	1,49E-04
CD	371	p.Arg882Cys	12,30	1,42E-03
CD	72	p.Ile705Thr	12,32	1,35E-04
CD	146	p.Ile780Thr	12,91	1,35E-04
CD	132	p.Arg882His	13,07	1,88E-03
CD	183	p.Trp860Arg	15,45	1,99E-03
CD	434	p.Trp860Arg	15,45	1,99E-03
CD	127	p.Tyr735Cys	19,93	8,81E-05

Supplemental Table 6. Longitudinal analysis

Group	UMIDepth	DonorID	DateOfDonation	NumberOfDonations	Donation	DaysBetweenDonations	CHROM	VMF	Gene_Name	HGVS.c	VMF(based on actual read counts)
FD	1048.53	3	12.01.21 00:00	121	2	399	chr2	0.0234043	DNMT3A	c.2311C>T	
FD	1048.53	3	12.01.21 00:00	121	2	399	chr2	0.0080808	DNMT3A	c.1903C>T	
FD	1048.53	3	12.01.21 00:00	121	2	399	chr2	0.0328228	DNMT3A	c.915G>A	
FD	1248.82	3	10.12.19 00:00	115	1	0	chr2	0.0275362	DNMT3A	c.2311C>T	
FD	1248.82	3	10.12.19 00:00	115	1	0	chr2	0.0065923	DNMT3A	c.1903C>T	
FD	1248.82	3	10.12.19 00:00	115	1	0	chr2	0.0321285	DNMT3A	c.915G>A	
FD*	616.36	8	05.05.21 00:00	106	2	512	chr2	0.0595483	DNMT3A	c.914G>A	
FD*	616.36	8	05.05.21 00:00	106	2	512	chr4	0.0495726	TET2	c.4380dupA	
FD*	825.06	8	10.12.19 00:00	102	1	0	chr2	0.0210526	DNMT3A	c.914G>A	
FD*	825.06	8	10.12.19 00:00	102	1	0	chr4	0.0288684	TET2	c.4380dupA	
FD	805.95	11	10.12.19 00:00	112	1	0	chr2	0.0276596	DNMT3A	c.2323-1G>T	
FD	2056.83	11	20.04.21 00:00	115	2	497	chr2	0.0448679	DNMT3A	c.2323-1G>T	
FD	699.5	31	11.12.19 00:00	138	1	0	chr2	0.0151515	DNMT3A	c.2512A>G	
FD	699.5	31	11.12.19 00:00	138	1	0	chr2	0.0751174	DNMT3A	c.2193_2195delCTT	
FD	3571.48	31	09.12.20 00:00	143	2	364	chr2	0.0310289	DNMT3A	c.2512A>G	
FD	3571.48	31	09.12.20 00:00	143	2	364	chr2	0.0788127	DNMT3A	c.2193_2195delCTT	
FD	774.9	42	11.12.19 00:00	124	1	0	chr2	0.0140351	DNMT3A	c.1040T>C	
FD	774.9	42	11.12.19 00:00	124	1	0	chr2	0.091858	DNMT3A	c.2197G>T	
FD	1315.09	42	10.02.21 00:00	128	2	427	chr2	0.0145985	DNMT3A	c.1040T>C	
FD	1315.09	42	10.02.21 00:00	128	2	427	chr2	0.11323	DNMT3A	c.2197G>T	
FD	802.71	45	11.12.19 00:00	101	1	0	chr4	0.0127389	TET2	c.2480delT	
FD	1079.14	45	10.02.21 00:00	105	2	427	chr4	0.0108803	TET2	c.2480delT	
FD	1056.89	57	11.12.19 00:00	122	1	0	chr2	0.0093857	DNMT3A	c.2264T>C	
FD	1130.58	57	08.12.20 00:00	126	2	363	chr2	0.0099458	DNMT3A	c.2264T>C	
FD	1019.4	94	15.01.20 00:00	161	1	0	chr4	0.0131579	TET2	c.4591C>T	
FD	1411.76	94	24.02.21 00:00	165	2	406	chr4	0.0214944	TET2	c.4591C>T	
FD	1308.2	136	18.05.20 00:00	111	1	0	chr2	0.0082902	DNMT3A	c.1903C>T	
FD	1481.27	136	17.05.21 00:00	116	2	364	chr2	0.0067797	DNMT3A	c.1903C>T	
FD	1116.78	200	08.03.21 00:00	115	2	287	chr2	0.0200445	DNMT3A	c.1667+1G>A	
FD	1432.01	200	25.05.20 00:00	111	1	0	chr2	0.0215664	DNMT3A	c.1667+1G>A	
FD	919.07	248	27.05.20 00:00	111	1	0	chr2	0.0286807	DNMT3A	c.1434delG	
FD	1094.42	248	19.01.21 00:00	115	2	237	chr2	0.0444785	DNMT3A	c.1434delG	
FD	1057.75	310	06.01.21 00:00	103	2	218	chr2	0.0074129	DNMT3A	c.2245C>T	
FD	1064.53	310	02.06.20 00:00	100	1	0	chr2	0.0069009	DNMT3A	c.2245C>T	
FD	1125.23	321	02.06.20 00:00	143	1	0	chr2	0.0773196	DNMT3A	c.2309C>T	
FD	1356.21	321	04.06.21 00:00	149	2	367	chr2	0.0569444	DNMT3A	c.2309C>T	
FD	1108.1	352	12.04.21 00:00	151	2	308	chr2	0.0514019	DNMT3A	c.2201T>C	
FD	1387.65	352	08.06.20 00:00	147	1	0	chr2	0.0440613	DNMT3A	c.2201T>C	
FD	802.55	369	02.03.21 00:00	140	2	267	chr4	0.1003788	TET2	c.3863G>A	
FD	1266.67	369	08.06.20 00:00	136	1	0	chr4	0.073955	TET2	c.3863G>A	
FD	2224.76	402	05.06.20 00:00	100	1	0	chr4	0.0145429	TET2	c.3384dupA	
FD	1775.54	402	04.06.21 00:00	105	2	364	chr4	0.0099010	TET2	c.3384dupA	
CD	1867.19	69	08.01.20 00:00	10	1	0	chr2	0.0077419	DNMT3A	c.2194_2201delTTTGAGTT	
CD	1677.33	69	16.06.21 00:00	14	2	520	chr2	0.0106007	DNMT3A	c.2194_2201delTTTGAGTT	
CD	1029.25	75	07.01.20 00:00	7	1	0	chr2	0.0192593	DNMT3A	c.1051dupT	
CD	1323.62	75	04.03.21 00:00	11	2	422	chr2	0.021645	DNMT3A	c.1051dupT	
CD	896.57	177	25.05.20 00:00	3	1	0	chr2	0.0155521	DNMT3A	c.2521A>G	
CD	896.57	177	25.05.20 00:00	3	1	0	chr2	0.0636833	DNMT3A	c.2132delA	
CD	1208.98	177	30.06.21 00:00	7	2	401	chr2	0.0168675	DNMT3A	c.2521A>G	
CD	1208.98	177	30.06.21 00:00	7	2	401	chr2	0.1228346	DNMT3A	c.2132delA	
CD	850.12	371	11.03.21 00:00	5	2	275	chr2	0.0204878	DNMT3A	c.2644C>T	
CD	1071.62	371	09.06.20 00:00	3	1	0	chr2	0.0131805	DNMT3A	c.2644C>T	
CD	1629.93	415	10.02.21 00:00	5	2	245	chr4	0.0291153	TET2	c.3866+1G>A	
CD	2331.81	415	10.06.20 00:00	4	1	0	chr4	0.0263415	TET2	c.3866+1G>A	
CD	1223.71	444	26.06.20 00:00	2	1	0	chr2	0.0077419	DNMT3A	c.2255_2257delTCT	0.006
CD	1125.80	444	10.09.21 00:00	7	2	441	chr2	0	DNMT3A	c.2255_2257delTCT	0.002
* extended FD											

Supplemental Table 7. Differential blood count analysis of Buffy Coats

Bzffy Coat ID		7,032E+10	7,032E+10	7,042E+10	7,042E+10	7,012E+10	7,042E+10	7,042E+10	7,032E+10	7,042E+10	7,042E+10	7,032E+10	7,032E+10	7,032E+10	7,032E+10	7,042E+10	7,042E+10	7,032E+10	7,032E+10	7,042E+10	7,032E+10	7,042E+10
Day 1	Hb(g/dl)	17,0	17,1	17,2	19,2	20,3	18,8	18,2	20,1	18,1	19,1	20,5	16,8	19,0	21,3	18,2	15,9	19,2	18,0	16,9	17,7	18,7
	Hct(%)	56,6	58,5	58,1	62,6	65,3	60,0	56,3	67,1	62,5	65,3	67,1	56,4	64,1	68,5	62,3	54,3	64,5	60,7	56,2	61,2	65,2
	MCV(fl)	103,5	100,2	106,8	104,2	108,3	97,7	99,8	104,0	104,0	105,7	98,8	105,0	106,3	103,9	100,0	100,9	107,5	103,9	109,6	103,7	97,3
	MCH(pg)	31,1	29,3	31,6	31,9	33,7	30,6	32,3	31,2	30,1	30,9	30,2	31,3	31,5	32,3	29,2	29,6	32,0	30,8	32,9	30,0	27,9
	MCHC(g/dl)	30,0	29,2	29,6	30,7	31,1	31,3	32,3	30,0	29,0	29,2	30,6	29,8	29,6	31,1	29,2	29,3	29,8	29,7	30,1	28,9	28,7
	RDW-CV(%)	13,9	18,7	13,3	14,5	14,3	14,8	14,4	14,2	14,2	14,5	14,4	13,1	14,1	15,4	14,1	12,9	13,7	14,1	13,4	13,4	16,5
	Plt(x10³/µl)	3049	2966	2491	3095	2643	2969	800	2599	2643	3099	3428	2989	3209	3724	3422	3855	3522	1993	1930	3614	3849
	MPV(fl)	10,0	9,3	8,8	10,7	9,7	9,8	8,8	10,1	10,3	9,2	8,5	9,4	10,2	8,8	9,6	9,0	8,9	10,3	11,4	9,7	8,4
	PDW(fl)	13,2	11,6	10,4	14,3	12,4	12,9	10,2	13,5	13,5	11,4	10,5	11,8	13,4	11,2	12,3	10,9	10,8	13,5	15,8	12,6	10,3
	WBC (x10³/µ	65,58	61,57	54,05	53,02	61,59	87,05	19,23	56,40	48,54	58,45	63,47	44,10	77,12	81,24	79,49	62,22	60,33	82,09	70,90	44,47	89,20
	Neut(%)	31,45	22,85	24,53	21,62	41,26	52,03	6,87	23,04	22,47	19,54	28,06	12,99	31,91	44,48	29,83	23,30	25,84	39,92	34,42	7,01	33,64
	Lymph(%)	27,01	32,12	20,38	24,21	15,28	26,63	9,42	25,48	18,96	27,29	24,88	24,95	36,44	29,11	34,89	28,74	29,36	33,44	25,44	31,62	45,00
	Mono(%)	4,92	5,05	7,27	5,83	3,61	6,97	1,89	6,65	6,15	10,96	9,54	5,68	7,43	5,53	12,36	8,77	4,41	7,25	7,06	4,99	9,19
	Eo(%)	1,67	0,98	1,28	0,41	1,03	0,40	0,90	0,11	0,35	0,23	0,59	0,11	0,44	1,40	1,22	0,96	0,30	0,57	0,45	0,61	0,77
	Baso(%)	0,53	0,57	0,59	0,95	0,41	1,02	0,15	1,12	0,61	0,43	0,40	0,37	0,90	0,72	1,19	0,45	0,42	0,91	0,53	0,24	0,60
	IG(%)	0,24	0,20	0,20	0,14	0,30	0,72	0,02	0,19	0,14	0,17	0,24	0,12	0,37	0,48	0,26	0,20	0,21	0,90	0,50	0,06	0,64
Day 2	Hb(g/dl)	16,6	17,0	17,3	18,7	19,3	18,7	18,0	19,7	17,9	19,1	20,6	16,9	19,0	21,2	18,1	15,9	19,5	17,8	16,9	17,9	18,9
	Hct(%)	59,3	62,0	61,2	67,2	66,1	65,1	57,4	68,7	65,4	68,2	71,1	61,5	69,8	73,4	67,5	60,8	68,4	63,5	60,3	66,7	70,4
	MCV(fl)	112,1	108,6	112,9	105,7	115,8	106,2	102,7	112,1	111,0	112,2	105,5	108,5	108,4	109,1	103,7	107,0	115,5	112,0	111,3	107,2	106,8
	MCH(pg)	31,4	29,8	31,9	29,4	33,8	30,5	32,2	32,1	30,4	31,4	30,6	29,8	29,5	31,5	27,8	28,0	32,9	31,4	31,2	28,8	28,7
	MCHC(g/dl)	28,0	27,4	28,3	27,8	29,2	28,7	31,4	28,7	27,4	28,0	29,0	27,5	27,2	28,9	26,8	26,2	28,5	28,0	28,0	26,8	26,8
	RDW-CV(%)	13,7	18,9	13,4	20,0	13,3	15,0	14,8	14,4	14,1	14,2	13,7	17,5	17,8	16,9	17,7	20,8	13,9	14,0	17,8	18,4	16,7
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	Plt(x10³/µl)	3417	4275	2860	33	3026	3372	902	2773	2750	3494	3966	3428	3525	4303	3920	4484	4153	2120	2033	4043	4598
	MPV(fl)	11,5	10,9	9,8	12,4	10,5	11,5	8,9	11,6	11,3	10,5	9,6	11,4	11,8	10,4	11,6	11,7	10,3	12,1	12,7	11,6	10,4
	PDW(fl)	16,7	15,4	12,6	18,4	14,3	16,6	10,3	17,2	16,5	14,4	12,6	16,1	17,4	14,6	17,0	16,8	13,5	18,2	19,9	16,9	14,2
	WBC (x10³/µ	65,37	60,94	51,00	53,15	61,22	88,96	18,49	56,22	45,54	58,45	64,34	44,12	75,77	77,61	78,72	61,66	60,31	83,72	69,18	45,67	88,73
	Neut(%)	31,60	22,11	22,78	21,91	40,21	51,24	6,66	22,46	20,18	19,88	28,69	12,55	30,77	41,27	29,19	23,18	24,83	39,77	35,90	7,07	32,80
	Lymph(%)	25,54	30,83	18,01	23,00	15,42	27,53	9,02	23,49	17,09	25,16	24,32	24,35	36,02	26,39	33,48	26,13	29,83	33,80	25,14	31,41	42,92
	Mono(%)	5,51	5,75	7,44	6,69	3,68	7,52	1,80	8,45	6,92	12,60	10,40	6,49	7,52	6,97	12,45	10,65	4,30	7,66	6,80	6,10	10,72
	Eo(%)	1,50	0,98	1,16	0,33	1,00	0,44	0,83	0,14	0,31	0,28	0,42	0,09	0,37	1,10	1,20	0,80	0,24	0,62	0,43	0,70	0,75
	Baso(%)	1,22	1,27	1,61	1,22	0,91	2,23	0,18	1,68	1,04	0,53	0,87	0,64	1,08	1,88	2,40	0,69	1,11	1,87	0,91	0,39	1,54
	IG(%)	0,44	0,68	0,58	0,52	0,65	0,81	0,05	0,61	0,35	0,78	0,54	0,35	0,57	0,61	0,66	0,61	0,27	1,14	0,62	0,12	1,02
Day 3	Hb(g/dl)	18,2	17,2	17,0	18,7	19,2	18,4	16,3	18,7	18,0	18,9	20,0	16,8	19,0	21,1	17,3	15,8	19,3	17,8	16,9	17,7	18,6
	Hct(%)	67,1	62,3	60,3	68,3	66,8	64,4	52,8	67,4	66,0	68,4	70,5	60,4	69,4	74,1	63,5	59,5	69,0	65,3	61,4	66,4	70,4
	MCV(fl)	107,5	109,1	114,2	105,9	112,6	107,5	104,6	107,5	107,8	112,7	106,5	112,9	107,9	109,6	108,9	112,9	111,7	107,8	112,5	106,4	107,3
	MCH(pg)	29,2	30,1	32,2	29,0	32,4	30,7	32,3	29,8	29,4	31,1	30,2	31,4	29,5	31,2	29,7	30,0	31,2	29,4	31,0	28,4	28,4
	MCHC(g/dl)	27,1	27,6	28,2	27,4	28,7	28,6	30,9	27,7	27,3	27,6	28,4	27,8	27,4	28,5	27,2	26,6	28,0	27,3	27,5	26,7	26,4
	RDW-CV(%)	19,4	18,6	13,8	20,9	16,8	15,7	12,0	17,6	16,9	14,8	14,6	13,2	18,8	17,8	13,7	13,2	16,5	17,2	19,3	19,4	16,7
	Plt(x10³/µl)	3320	3818	2959	3115	3079	3022	898	2706	2812	3288	3855	3279	3204	4240	3588	3878	4141	2020	1991	3801	3916
	MPV(fl)	11,9	11,1	10,2	12,9	11,1	12,0	9,9	12,0	11,6	11,2	9,9	11,5	12,3	10,7	11,5	11,7	10,5	12,5	13,2	12,0	11,1
	PDW(fl)	17,9	15,7	13,3	19,6	15,5	18,2	12,0	17,9	16,8	16,1	13,0	16,3	18,7	14,9	16,9	16,8	14,4	19,4	21,0	17,9	15,9
	WBC (x10³/µ	68,72	61,05	51,11	53,56	62,35	86,51	17,75	53,99	46,15	56,96	61,87	43,79	74,26	76,96	75,08	61,80	58,52	81,66	68,46	44,90	89,50
	Neut(%)	32,71	20,52	25,02	22,02	41,42	50,36	6,65	20,87	21,58	20,22	29,49	13,44	32,04	41,33	29,93	25,29	24,10	39,66	34,39	8,16	33,10
	Lymph(%)	25,82	30,17	16,55	22,03	14,59	25,11	8,87	20,67	16,75	23,04	21,34	23,57	33,88	23,38	29,85	25,87	27,00	31,03	22,76	29,46	41,08
	Mono(%)	5,47	6,62	5,67	6,96	3,12	7,09	1,19	9,48	5,91	12,35	8,63	5,39	6,19	8,06	10,69	8,82	4,99	7,43	7,62	5,89	11,91
	Eo(%)	1,54	0,86	1,12	0,36	0,87	0,42	0,75	0,12	0,30	0,22	0,44	0,09	0,37	1,01	1,07	0,76	0,22	0,57	0,38	0,61	0,75
	Baso(%)	3,18	2,88	2,75	2,19	2,35	3,53	0,29	2,85	1,61	1,13	1,97	1,30	1,78	3,18	3,54	1,06	2,21	2,97	3,31	0,78	2,66
	IG(%)	1,25	0,59	2,08	1,09	1,66	11,70	0,43	0,92	1,09	1,70	1,48	1,17	1,60	1,18	1,70	1,53	0,53	1,63	1,31	0,60	1,50

Supplemental Table 8. Digital droplet PCR Assays

AssayName	AssayID	AminoAcidExchange	NucleotideMutation	COSMICID
DNMT3A p.W305* c.914G>A	dHsaMDS311847198	p.W305*	c.914G>A	COSM1169636
DNMT3A p.F543delF / DNMT3A p.F731del c.2191_2193delTTC	dHsaMDS549125373 / dHsaMDS802387875	p.F732del (old: p.F543delF)	c.2191_2193delTTC	COSM99742
DNMT3A p.E733* c.2197C>T	dHsaMDS529169084	pE733*	c.2197C>T	
DNMT3A p.L347P c.1040T>C	dHsaMDS126094721	p.L347P	c.1040T>C	COSM5944978
DNMT3A p.R882H c.2645G>A	dHsaMDV2010089	p.R882H	c.2645G>A	COSM52944
DNMT3A p.R882C c.2644C>T	dHsaMDS475153762	p.R882C	c.2644C>T	COSM53042

Supplemental Table 9. CRISPR guides and HDR donor templates

CHROM	HGVS.c	HGVS.p	sgRNA	Donor template	MiSeq primers
chr2	c.2197G>T	p.Glu733*	TCCGACCTCTCAGAGGGCAC	GCGATCATCTCCCTCCTTGGGCCGCGCATCATGCAGGAGGCGGTAGAACT AAAAGAAGAGCCGGCCAGTGCCCTCTGAGAGGTCGGAAGAGAAAGCCATC	**FWD:** TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTTGCTGGCTATACCTCGAG. **REV:** GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGATATTTCTGCCCTGGGAC.
chr2	c.914G>A	p.Trp305*	GGAAACTGCGGGGCTTCTCC	AGCTGCTCGGCTCCGGCCCGTCATCCACCAAGACACAATGCGGCCTGGCC ACTAGGAGAAGCCCCGCAGTTTCCCCCACACCAGCTCCCCAATGCCAAAG	**FWD:** TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTACTGCCAAACCCCACAAC. **REV:** GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCGTGACCACTGTGTAATG.
chr2	c.1988delC	p.Ser663fs	GGACCGCTACATTGCCTCGG	TTCCCCTGGTGCCGCACCATGCCCACCGTGATGGAGTCCTCACACACCTC CAGGCAATGTAGCGGTCCACCTGAATGCCCAAGTCCTTCAGCACCAGGA	**FWD:** TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCACAGCATGGACATACATGC. **REV:** GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATGGGTCCTGTTGGCCAGCA.
chr2	c.2644C>T (R882C)	p.Arg882Cys	CCTCGCCAAGCGGCTCATGT	GATGACTGGCACGCTCCATGACCGGCCCAGCAGTCTCTGTCTCGCTAAGCAGC TCATGTTCGAGACGTCAGTATAGTGGACTGGGAAACCAAATACCCTG	**FWD:** TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGAGTTGGTGGGTGTGAGT. **REV:** GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCACGCAAAATACTCCTTCAGC.
chr2	c.2645G>A (R882H)	p.Arg882His	CCTCGCCAAGCGGCTCATGT	GATGACTGGCACGCTCCATGACCGGCCCAGCAGTCTCTGTCTCGCTAAGTGGC TCATGTTCGAGACGTCAGTATAGTGGACTGGGAAACCAAATACCCTG	**FWD:** TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGAGTTGGTGGGTGTGAGT. **REV:** GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCACGCAAAATACTCCTTCAGC.

Supplemental Methods

Healthy Blood Donors

Buffy coats, waste products of component preparation from whole blood donations, of selected healthy male volunteer blood donors donating between December 2019 and June 2020 as well as December 2020 and November 2021 (for consecutive samples) at the German Red Cross Blood Service Baden-Württemberg-Hessen were used for the study. All donors signed an informed consent allowing for anonymous processing of the samples. Database query parameter were set to select male individuals over the age of 60 with greater than 100 (frequent blood donors) or fewer than 10 (infrequent control donors) whole blood donations. Buffy coats produced in the course of processing of erythrocyte concentrates meeting these criteria were flagged by the IT system during blood processing. Subsequent verification of the donor characteristics showed that 212 out of 218 samples matched the set criteria. Further restriction with regard to inclusion of the donors was related to the quality of the sequencing (coverage depth and number of variants detected per sample) as described below under "Analysis of the variants". Based on sequencing metrics 4 additional donors (3x UMI Depth < 400; 1x 225 total variants per sample) were excluded. Thus 105 donors in the frequent donor (FD) cohort and 103 donors from the control donor (CD) cohort, were included as **main cohorts** (see Table 1 and Supplemental Table 1). These main cohorts were used for all statistical comparisons of FDs and CDs. All donor IDs are research IDs introduced in course of the study for the purpose of data analysis exclusively and do not allow an identification of the donors to anyone outside the research group.

In two of the "wrongly" processed and analyzed samples, from donor #8 (age 51-55 / 102 donations) and donor #17 (age 61-65 / 80 donations) mutations in CH drivers *DNMT3A* and *TET2* were identified. Both donors were therefore kept as part of the **extended** FD cohort despite being formally too young (donor #8) or having donated too few whole blood units for inclusion (donor #17) since they were of an age where CH can be detected at the set depth of the sequencing and met a general definition of frequent donor. Variants from these extended FD cohort donors were only considered in qualitative assessment of the variants, including longitudinal and functional analysis as well as lineage tracing.

Samples

Bulk / Whole PBMC Samples

Buffy coats (BC) were kept at RT for up to 3 days prior to shipment and processing at the German Cancer Research Center in Heidelberg. Before initiation of the study, we had verified that short term storage prior to freezing the cell pellet did not affect the leukocyte composition of the samples (see supplemental Table 7) and therefore did not introduce a bias in case of a different penetrance of certain mutations in different lineages such as have been reported e.g. for *DNMT3A* and *TET2* mutations[1].

5-10 ml BC suspension were washed with PBS and the cell pellet then spun down again to remove excessive plasma prior to freezing it at -80 °C. BC cell pellets were thawed on ice immediately prior to DNA isolation.

Selected fractions

For donors' consecutive sample analysis, BCs were obtained within 24 hours of whole blood donation. Whole PBMC sample was generated and processed as described above. The remaining sample (30-50 ml) was washed and subjected to Ficoll density centrifugation to isolate PBMC for subsequent freezing of live cells. On the days of sorting cryopreserved cells were gently thawed, washed and stained with anti-human CD3, CD14, CD19, CD34 and CD45 antibodies. Sorting of the cell fractions of interest was performed based on following immunophenotypes: T cells: $CD45^{high}CD34^{neg}CD3^{pos}CD14^{neg}CD19^{neg}$, B cells: $CD45^{high}CD34^{neg}CD3^{neg}CD14^{neg}CD19^{pos}$, Monocytes: $CD45^{high}CD34^{neg}CD3^{neg}CD14^{pos}CD19^{neg}$ and HSPC: $CD45^{dim}CD34^{pos}CD3^{neg}CD14^{neg}CD19^{neg}$. Cells were sorted into PBS/BSA, spun down and frozen as pellets at -80 °C until immediately prior to DNA isolation.

DNA isolation

DNA isolated was performed as per manufacturer's instructions using Qiagen DNA isolation kits: QiaAMP DNA Blood Maxi Kit (for up to 1 ml BC cell pellet), QiaAMP DNA Mini kit (for up to 200 µl BC cell pellet and more than 200K sorted cells) and QiaAMP DNA Micro kit (for fewer than 200K sorted cells).

Library preparation and Sequencing

Library preparation for targeted sequencing of PBMC samples was performed using the Human Myeloid Neoplasms Panel (Qiagen) that covers 141 genes and a total of 436 kilobase pairs. Per sample, 40 ng of genomic DNA were processed according to manufacturer's instructions to obtain dual indexed, molecularly barcoded (unique molecular barcodes, UMI) libraries. Library quality and size were assessed using Agilent 2100 Bioanalyzer. Quantitative verification was performed using qPCR (QIASeq Library Quant Assay Kit, Qiagen) and Qubit dsDNA HS Assay (Life Technologies). Sequencing was performed on an Illumina NextSeq 550 sequencer, with an average UMI based coverage of 1000x. Raw sequencing data along with the metadata of the analyzed cohorts will be submitted to the European Genome-Phenome Archive (EGA), hosted by the European Bioinformatics Institute and Centre for Genomic Regulation.

Analysis of the variants

Sequencing reads were mapped and annotated using the Qiagen web-based tool for QiaSeq Targeted DNA Enrichment Variant Calling[2]. Read processing pipeline along with the applied variant caller have been described previously[2] and are available at https://github.com/qiaseq/qiaseq-dna under GNU Affero General Public License v3.0. An average of 170 mutations were called per sample. Full lists of variant calls will be submitted to the GEO database. Following criteria were subsequently applied to account for sequencing artefacts as well as to reduce the variant lists to CHIP relevant mutations: Samples with an average UMI depth of less than 400x and an average number of variants per sample higher than 210 were excluded from the analysis. Only variants with a VAF of ≤ 0.4 were extracted to

exclude germline variants. VAF values determined by the QiaSeq pipeline were used for all analysis except for the longitudinal sample (donation #2) from donor #444, where the read count ratio extracted from the Integrative Genomic Viewer (IGV, https://software.broadinstitute.org/software/igv/) were used. Synonymous variants, variants predicted to have a low effect along with common SNPs were excluded. The pipeline specific quality parameters "Filter" and "RepRegion" were set to "PASS" and "NA", respectively. Furthermore, given the size of the cohort, variants (same gene and position) found to occur more than 10 times were considered to be panel artefacts and excluded from the final analysis. Variants included in the final list of mutations had a VAF between 0.005 and 0.37 % and variant allele coverage of an average of 32 UMI family-based reads. Analyses were run in R software, v 4.0.1. Overall pathogenicity scoring of mutations was performed using the Combined Annotation Dependent Depletion (CADD) tool[3,4]. **C**OSMIC database[5] was used for manual curation of the variants.

Lollipop plots for DNMT3A and TET2 were generated using the lolliplot function from the R package trackViewer. Spliceosome mutations were excluded. Protein domain annotations for the plots were downloaded from https://genome.ucsc.edu/. Heatmap plots of the epigenetic modifier mutation frequencies were generated using the R package ggplot2.

Digital Droplet PCR (ddPCR)

Digital droplet PCR was performed for validation of the targeted sequencing as well as when screening for presence of selected mutations in specific cell fractions of the sample. All assays were designed and purchased from Bio-Rad. ddPCR Supermix for Probes (No dUTP, Bio-Rad) was used for all reactions. Assay IDs are listed in the Supplemental Table 8. Reactions were set up according to manufacturer's instructions with 5-50 ng genomic DNA as input and annealing / extension temperatures of 53-55 °C for 40 cycles. Bio-Rad QX200 Droplet Digital PCR System was used for droplet generation and analysis of the samples.

Flow cytometry analysis and cell sorting

All experiments were analyzed at the Flow Cytometry core facility of The Francis Crick Institute using the LSR FORTESSA (BD Biosciences) equipped with a 488-nm laser, a 561-nm laser, a 633-nm laser, and a 405-nm laser. For sorting, cell suspensions were filtered through a 35-µm nylon mesh (Falcon, Cat# 352235) and sorted in a BD FACS FUSION cell sorter equipped with 488-nm, 561-nm, 633-nm, and 405-nm lasers. The antibodies used were: CD45-FITC (clone HI30, Biolegend), CD34- PerCP-Cy5.5 (clone 8G12, BD Pharmingen) and CD38-PECy7 (clone HIT2, BD Pharmingen) for sorting of HSPCs and CD33-PE (clone P67.6, Biolegend), CD19-APCCy7 (clone HIB19, Biolegend), CD71-APC (clone OKT9, eBioscience) and CD235a-FITC (clone HIR2, BD Pharmingen) for flow cytometry analysis. Exclusion of dead cells was done by staining with the fluorescent dye DAPI (1 µg/ml; BD Biosciences, Cat# 564907) and gating out the positive cells. All experiments were analyzed with FACSDiva 6.2 (BD Biosciences) and FCS Express 7 software.

Sample preparation for DNA sequencing

Sorted cells were pelleted and DNA was extracted using EZNA Tissue DNA kit (Omega Bio-tek). Targeted sequencing to the region of interest was performed after PCR amplification using the corresponding primers listed in the supplemental Table 10.

***In silico* structural analysis**

Models were generated using homology modelling on SWISS-MODEL[6,7] based on the crystal structure of DNMT3A available on PDB under the alias 5YX2[8]. UCSF ChimeraX[9] and PyMOL[10] were used for model visualization.

Statistical analysis

Comparisons between the frequent and control donor group with respect to the probability of observing at least one mutation from any gene at VAF threshold 0.5% or 2%, or of at least one *DNMT3A*, *TET2*, *DNMT3A* or *TET2*, Epigenetic or Non-epigenetic gene modifier mutation at VAF threshold 0.5%, were obtained as odds-ratios (OR) based on binomial generalized linear model fits. A quasipoisson generalized linear model accounting for potential over-dispersion was fitted instead to test for the difference in expected number of mutations between the two groups. VAF scores associated with each mutation were compared after log-transformation. Differences in expected log-VAF scores between the two donor groups were tested by fitting linear mixed models, including random intercept terms for donor and gene (where relevant) grouping. All group effects were estimated while controlling for donor age and sequencing depth.

The longitudinal analysis focused on expected VAF score log-fold changes between time-points 1 and 2, which was adopted as response variable. Robust linear modeling was used to account for potential outlier values. The donor group (taking as reference the control donor group), gene (taking as reference *DNMT3A*), donor age at time-point 1, number of days as well as the number of donations between the two donation time-points, and difference in sequencing depth between the two donation time-points, were included as explanatory variables and their effect was therefore estimated mutually adjusted for any other included variable. The VAF log-fold change of donor 444 was imputed from the actual read counts.

CADD scores, fitness scores, stability scores and site mutation rate values were also compared via robust linear modeling; stability scores and site mutation rate values were log-transformed to improve model fitting stability. The models controlled for donor age and included random intercept terms for donor and gene (where relevant) grouping.

For patients having at least one *DNMT3A* mutation, a McNemar exact test was performed to test for the difference in the proportions of presence of a second mutation from the *DNMT3A/TET2* group *vs*. the group of all other epigenetic modifier genes.

All continuous explanatory variables were included after standardization to enhance interpretability and model fitting stability. Analyses were run in R software, v 4.1.1. Linear mixed model fitting was performed via maximum likelihood with the lme4 R package[11] and p-values obtained via the lmerTest R package (Satterthwaite approximation)[12]. The McNemar

exact test was calculated with the exact2x2 R package[13]. Robust linear mixed models were fitted with the robustlmm R package[14], and p-values obtained again via the Satterthwaite approximation. Finally, robust modeling without random effects was performed with robustbase R package[15].

Statistical methods used for analysis of in vitro HSPC culture results are outlined in the figure legend. Sample size was not predetermined. Data are presented as means with standard deviation (SD) to indicate the variation within each experiment. For each biological donor a paired t-test was used to compare the percentage of the *DNMT3A*-mutant clones between different conditions.

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