Transforming activities of the *NUP98-KMT2A* fusion gene associated with myelodysplasia and acute myeloid leukemia

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Supplementary materials for

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This supplementary file includes:

supplementary methods, 3 supplementary figures, 5 supplementary tables.

Supplementary Methods

Establishment of rtTA;NUP98-KMT2A transgenic mice

A human full-length *NUP98-KMT2A* cDNA was cloned and fully sequenced before cloning it into the *p2Lox* targeting vector and electroporation into A2Lox-Cre ES cells. The transgene is targeted into a region upstream of the *Hprt* locus on the X chromosome. *rtTA;NUP98-KMT2A* double transgenic mice were established (here referred as "iNUP98-KMT2A") and backcrossed for over 10 generations to C57BL/6 mice.

Bone marrow reconstitution experiments

Prior to BM transplantation (BMT), recipient mice (6-10 week-old C57BL/6) were irradiated: 1 dose of 600 cGy (sublethal) or 2 doses of 600 cGy 4 hours apart (lethal) (Gammacell 40 Exactor, Best Theratronics, Canada). Donor mice were sacrificed by asphyxiation with CO_2 and total BM was isolated from the long bones, the hips and the spine. Where indicated, C57BL/6 wild type (WT) support BM cells were isolated in a similar manner and mixed with donor cells prior to injection. Recipient mice were provided with DOX-impregnated chow pellets or normal chow from the day of transplant.

Blood Analysis

Peripheral blood (PB) was isolated by tail vein bleeding or from cardiac puncture after sacrifice, diluted in 0.9% saline solution and analyzed using an Advia 2120 hematology analyzer (Siemens).

Histology and PB smear staining

Mouse organs were fixed in 4% buffered formaldehyde, cut in two halves along long respective axes, dehydrated, embedded in paraffin blocks, sectioned (4µm), automatically H&E-stained and covered analogously to the accredited standard operating procedures at the Institute of Pathology of the University Hospital Basel. PB smears were stained with Wright Giemsa solution.

qPCR of iNUP98-KMT2A hematopoietic stem and progenitor cell mRNA

Total RNA was extracted from fresh and frozen cells using the NucleoSpin® RNA XS kit (Macherey-Nagel, Germany). 0.5-1 µg of total RNA was reverse transcribed with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) using random hexamer primers. cDNA was analyzed by quantitative real-time PCR (ABI prism 7700, Applied Biosystems) using power SYBR PCR (Applied Biosystems, USA) using the primers listed in **Supplementary Table S1**. Ct values for each sample were normalized to *Gapdh* mRNA levels.

Bioinformatic Analysis

From raw Ct values on qPCR arrays, deltaCt values was calculated by subtraction of the average values of housekeeping genes: *Actb, B2m, Gapdh, Gusb*, and *Hsp90ab*. A linear model was set up using limma[22] for R, and the model formular was "~ 0 + Group", where Group is a factor reflecting passage (late or early) and mutation status (iNUP98-KMT2A or Control). In order to account for the batch effect of the experiments having been performed at different dates, and by different experimentalists, the linear model was fitted while regressing out this effect. Briefly, the function *duplicateCorrelation* was used to identify the inter subject correlation, and the *lmFit* was called providing this trimmed average estimated inter-duplicate correlation

as input. Furthermore, information about batch was provided as a blocking factor. This batch correction method fits the model, while simultaneously removing the effect of batch. In order to calculate moderated t-statistics for each gene between Groups eBayes was applied for the desired comparisons (contrasts).

Supplementary Figure 1

A. Peripheral blood values for control (WT), iNUP98-KMT2A mice on DOX, and iNUP98-KMT2A mice kept off DOX (mean age: 56 weeks).

B. Breakdown of number of total BM cells immunophenotyped as either myeloid (Mac-1$^+$, Gr-1$^+$, n=4), B-cells (B220$^+$, n=2), or T-cells (CD3$^+$, n=2).

C. Percentages of apoptotic total BM cells immunophenotyped as either myeloid (Mac-1$^+$, Gr-1$^+$), erythroid (CD71$^+$/Ter119$^+$), B-cells (B220$^+$), or T-cells (CD3$^+$) (n=4).

D. Absolute number of LSK collected from control (WT) and iNUP98-KMT2A mice. The mean +/-SD of 3 biological replicates is shown.

E. The percentage of iNUP98-KMT2A total BM cells immunophenotyped as either myeloid (Mac-1$^+$, Gr-1$^+$), erythroid (CD71$^+$/Ter119$^+$), B-cells (B220$^+$), or T-cells (CD3$^+$) in G_0, G_1, or G_2/M phase of the cell cycle. The mean +/-SD of 3 biological replicates is shown. *<0.05, paired t-test, n=3.

Supplementary Figure 2

A. Histological sections and peripheral blood smears from 6 leukemic iNUP98-KMT2A mice. Images for M1-4 are repeated from Fig. 3B and are included for completeness. Scale bars: BM: 100µm; liver: 100µm; PB: 10µm; lung: 100µm; spleen: 100µm.

B. Percentages of total BM cells stained with Mac-1 and Gr-1-binding antibodies. Data for M1-4 is repeated from Fig. 3C and is included for completeness.

C. Percentages of total BM cells stained with CD3 and B220-binding antibodies. N.A.: data not available for these mice.

D. Percentages of total BM cells stained with FcyRII/III and c-Kit-binding antibodies.

Supplementary Figure 3

A. Kaplan-Meier curves indicating the disease-free survival times for iNUP98-KMT2A mice given DOX food and exposed or not to sublethal irradiation. **<0.01, log-rank test, n=5.

B. The growth of Lin-negative BM cells from control (WT) and iNUP98-KMT2A mice grown *in vitro* was assessed over a period of seven days in the presence of DOX (1µg/mL). The mean values +/-SD of triplicate cultures are shown, *<0.05, **<0.01, unpaired t-test, n=5.

C. *NUP98-KMT2A* expression was analyzed by qPCR after 6 days *in vitro*. *<0.05, **<0.01, unpaired t-test, n=3.

D. Numbers of colonies formed after 5000 control (WT) and iNUP98-KMT2A Lin^- negative BM cells were grown in methylcellulose (MC) containing cytokines and DOX (1µg/mL). The mean +/-SD is reported. No significant differences were observed.

E. The percentage of iNUP98-KMT2A Lin^- cells in G_0, G_1, or G_2/M phase of the cell cycle. The mean +/-SD of 3 biological replicates is shown. *<0.05, paired t-test, n=3.

F. The growth of control (WT) and iNUP98-KMT2A MEF in liquid medium at early passage is shown. The mean +/-SD of two biological replicates is shown.

G. The growth of control (WT) and iNUP98-KMT2A MEF in liquid medium at late passage is shown. The mean +/-SD of two biological replicates is shown. *<0.05, **<0.01, 2-way ANOVA.

Supplementary Table 1

Primers used in the current study for qPCR.

Supplementary Table 2

Values derived from analysis of NUP98-KMT2A MEF by RT^2 qPCR array. Values represent late passage MEF and show the expression levels of 79 genes in NUP98-KMT2A positive MEF relative to control MEF cultures.

Supplementary Table 3

Mouse characteristics and PB data for primary-induced NUP98-KMT2A and BMT mice.

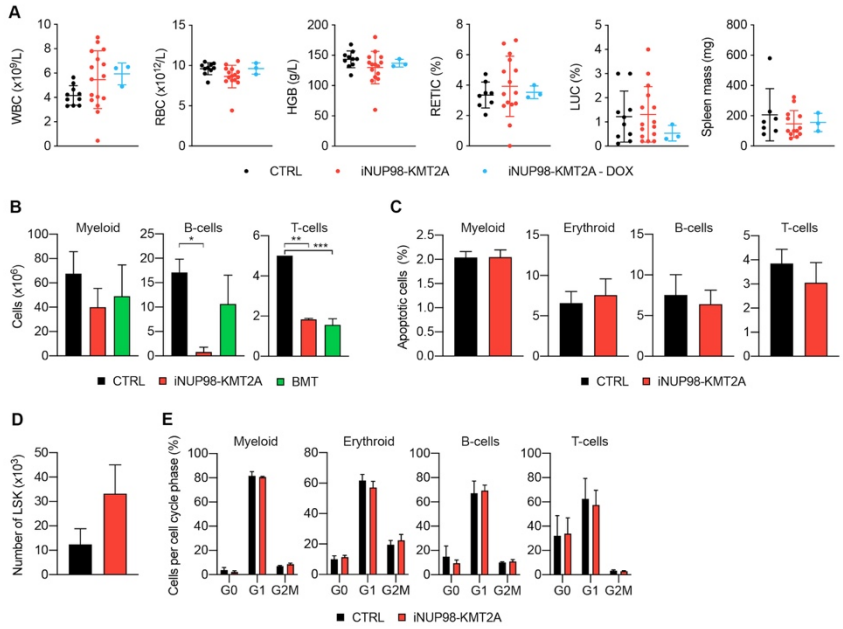
Supplementary Table 4

Mouse characteristics and PB data for primary-induced NUP98-KMT2A mice which developed AML.

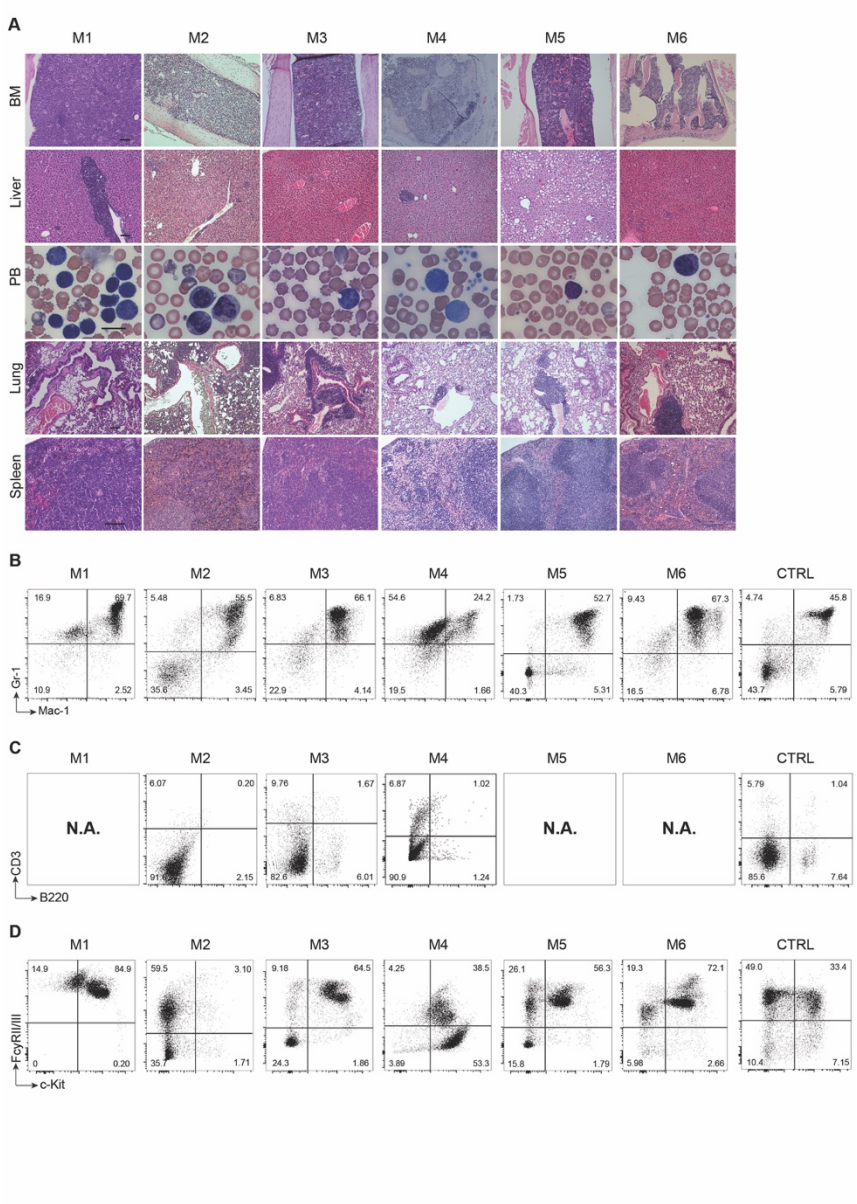
Supplementary Table 5

Mouse characteristics and PB data for sublethally-irradiated NUP98-KMT2A mice.

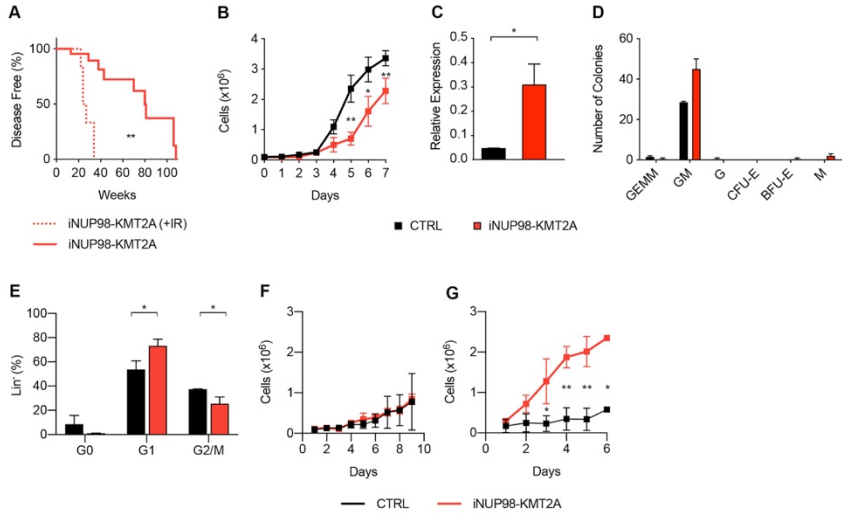
Fisher et al. Sup. Figure 1



Fisher et al. Sup. Figure 2



Fisher et al. Sup. Figure 3



Fisher et al. Sup. Table 1. Primers used in the current study for qPCR analysis.

Target	FWD (5'-3')	REV (5'-3')
Gapdh	ATG ACA TCA AGA AGG TGG TG	CAT ACC AGG AAA TGA GCT TG
HoxA5	CTC ATT TTG CGG TCG CTA TCC	ATC CAT CGG ATT GTA GCC GTA
HoxA9	AGACCGAGCAAAAGACGAG	CTGAGGTTAGAGCCGCTTT
HoxA10	CAC CAC CCA CTC TGG TTT G	TGC ATT TTC GCC TTT GGA ACT
HoxB3	GCA CCT GGA GGG TGA CTA C	CCC CCG TTA TTG CTG TTG C
HoxB4	CAG AGC GAT TAC CTA CCC AGC	CGT AGC GCT GCA CAG TGC AC
HoxB6	GCT CTA CTC GTC TGG CTA TGC	GTG GGT AAT AGG AGG ACG CC
HoxB8	ACA GCT CTT TCC CTG GAT GC	CGT GCG ATA CCT CGA TCC TC
HoxC6	AAT TCC ACC GCC TAT GAT CCA	ACA TTC TCC TGT GGC GAA TAA AA
NUP98-KMT2A	CCAGCAGCACATCAATAGTC	AGCTGAATTTCGGTCAGAGC
NUP98	CCAGGAGCCAGTTCCAGATT	GCAATGATGCTTTCATGATCTGT
Sirt1	GGAGCAGATTAGTAAGCGGCTTG	GTTACTGCCACAGGAACTAGAGG
Prkcd	ACA TTC TGC GGC ACT CCT GAC T	CCG ATG AGC ATT TCG TAC AGG AG
Twist1	GATTCAGACCCTCAAACTGGCG	AGACGGAGAAGGCGTAGCTGAG
Vim	CGGAAAGTGGAATCCTTGCAGG	AGCAGTGAGGTCAGGCTTGGAA
Tert	GAAAGTAGAGGATTGCCACTGGC	CGTATGTGTCCATCAGCCAGAAC
Rbl2	TCT CGG TGT CTA AGT GCT GCC T	GTT CTC CTG AAC ATA CCT CAC GC

Fisher et al. Sup. Table 2. NUP98-KMT2A MEF RT^2 qPCR array data.

	logFC	AveExpr	P.Value
Prkcd	-2.6644001	5.48562532	0.00069013
Twist1	-2.6574001	3.98449998	0.00259186
Rbl2	-2.0243998	6.73387532	0.02600805
Col1a1	-1.7269001	-0.7655003	0.03363591
Tert	-2.3943996	9.6901248	0.03741225
Sirt1	-1.4443989	6.00712495	0.04294075
Igf1r	-0.9944	3.68249945	0.04409883
Vim	-1.270401	-1.242625	0.04509946
Ccna2	1.11509991	2.66387491	0.05176579
Ets1	1.44460011	6.00012546	0.05717406
Morc3	-1.1644001	6.27487474	0.05946814
Ing1	-1.3023996	5.02162485	0.06098824
Akt1	-1.2409	2.92337494	0.06176317
Cdkn2d	-1.4778995	8.15124946	0.06821009
Cdk6	-2.6798992	6.0486248	0.0692436
Map2k6	-4.6549005	8.59662514	0.07330706
E2f1	-1.0313997	6.51449995	0.08244486
Cdk2	1.01660061	4.39487486	0.08267551
Terf2	-0.9274006	6.04237514	0.08372268
Col3a1	-3.1904001	-1.4281249	0.08851775
Ccnd1	1.27610016	0.52149992	0.09286648
Creg1	1.15209961	4.03825025	0.09821737
Cdkn1c	-4.0764007	4.74350004	0.1082637
Cdkn2c	-1.2614002	4.75650005	0.11512015
Ccnb1	1.3621006	4.15812497	0.1152938
Id1	-1.6243992	2.09275012	0.14494891
Mdm2	0.88159943	2.05237537	0.14717842
Pten	-1.0524006	1.75724964	0.16026999
Cdkn2b	1.27909947	2.51612477	0.16990731
Sod2	0.79610062	2.98262505	0.18763854
Igfbp7	0.7571001	1.37037497	0.23163661
Irf3	0.95410061	4.17887526	0.24069374
Trp53bp1	-0.9478989	6.11200004	0.24214803
Serpine1	2.72659969	0.95425015	0.25481097
Nbn	0.57159996	5.56637506	0.28216403
Ccne1	1.16660023	6.05687528	0.29694269
Tbx2	-1.2838993	9.21412496	0.30803044
Ets2	-0.6939011	6.13900023	0.32067927
Igfbp3	-3.8539	2.76312475	0.32640552
Pik3ca	-0.5634003	3.77525024	0.33372849

Map2k1	-0.7059002	4.17312532	0.34318979
Rb1	0.53760052	4.98587518	0.34793639
Abl1	-0.7349014	6.21287518	0.34842797
Plau	0.86959935	5.15500026	0.35944757
Sparc	-0.6244001	-1.8087499	0.36002515
Egr1	-1.1279001	0.98024993	0.3721072
Calr	0.44609928	-0.9050002	0.38371221
Pcna	-0.5474005	2.98700027	0.38981351
Atm	-0.482399	6.59862523	0.3918157
Cdc25c	0.52109909	6.47999983	0.40403863
Nfkb1	-0.6004	4.01587491	0.41140048
Cdkn1b	-0.6373997	3.27300005	0.41251743
Aldh1a3	-0.9548988	9.26649957	0.42027465
Bmi1	-0.3383999	3.7048748	0.42443822
Nox4	-0.4988995	6.57824974	0.44734398
Cdk4	-0.3873997	1.96049981	0.47344011
Chek2	-0.8658991	7.74075012	0.48662207
Hras	0.36209965	3.17775016	0.4962286
Tgfb1i1	0.33759975	2.22287516	0.49645811
Sod1	0.38910008	1.14075022	0.50411162
Rbl1	0.42410088	4.88637524	0.50594409
Glb1	-0.4798994	5.11462503	0.55873858
Gadd45a	0.68710041	6.4563747	0.56363706
Thbs1	0.55659962	-2.1503746	0.57609433
Myc	0.29109955	4.80524998	0.58141749
Igf1	-0.7289009	4.26425033	0.66266563
Trp53	0.16359997	4.76512508	0.70395286
Chek1	0.22510147	7.13312512	0.7226313
Cdkn1a	-0.1769009	2.2937501	0.74135841
Tgfb1	0.11359978	3.51762466	0.79719852
Cd44	0.28759956	2.20762496	0.80264652
E2f3	0.08060074	5.38312511	0.8513705
Mapk14	0.10560036	4.754	0.85772016
Irf5	0.21859932	8.65649991	0.8760064
Fn1	-0.0864	-0.803375	0.88341639
Map2k3	0.08759976	4.22662477	0.90376734
Cdkn2a	0.13610077	3.67325001	0.9273292
Gsk3b	0.03209877	2.79812508	0.95737138
Cited2	-0.0049	1.86862497	0.99228674

Fisher et al. Sup. Table 3. Data for primary-induced NUP98-KMT2A and BMT mice.

Mouse ID	Experimental setting	Sex	Latency (weeks)	WBC (x10^9 cells/L)	RBC (x 10^12 cells/L)	HGB (g/L)	RDW (%)	PLT (x 10^9 cells/L)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)	LUC (x 10^9cells/L)	LUC (%)	RETIC (x 10^9cells/L)	RETIC (%)	Spleen (mg)	Liver (mg)
82	1° NUP98-KMT2A	F	25	3.33	9.54	147	15.9	573	6.2	88.7	1.7	0.00	0.00	0.06	1.60	128.4	1.35	50	1760
167	1° NUP98-KMT2A	F	30	3.78	7.89	135	15.6	399	3.6	91.3	1.3	3.20	0.10	0.03	0.50	331.2	4.21	181	1874
212	1° NUP98-KMT2A	F	29	2.82	8.07	120	16.3	756	4.2	90.2	1.3	3.10	0.20	0.03	1.00	396.0	4.90	210	1780
213	1° NUP98-KMT2A	F	29	8.55	8.52	138	15.6	798	15.9	75.2	2.9	3.30	0.30	0.21	2.50	573.3	6.74	324	1723
264	1° NUP98-KMT2A	F	21	3.93	9.21	138	12.4	1155	42.7	48.6	3.9	4.40	0.20	0.00	0.20	251.1	2.73	119	1350
293	1° NUP98-KMT2A	M	72	1.65	4.53	87	14.6	222	7.3	77.8	6.5	5.20	0.40	0.06	2.80	362.1	8.00	159	2351
294	1° NUP98-KMT2A	M	72	5.55	6.96	108	15.5	1134	17.9	72.2	3.7	4.60	0.40	0.06	1.30	214.5	3.08	126	1694
304	1° NUP98-KMT2A	F	79	4.20	9.15	132	12.9	1509	12.0	69.0	4.9	11.70	0.80	0.06	1.60	323.7	3.50	297	1630
437	1° NUP98-KMT2A	M	106	0.45	4.41	60	18.5	108	15.9	73.0	5.5	2.80	0.80	0.00	2.10	307.8	6.96	197	4600
534	1° NUP98-KMT2A	F	65	6.72	8.16	108	14.1	1500	34.7	56.1	2.5	2.50	0.10	0.27	4.00	495.0	6.07	100	1700
733	1° NUP98-KMT2A	F	33	5.76	9.60	135	12.5	1593	6.2	89.7	1.1	2.00	0.30	0.03	0.70	275.1	2.87	NA	NA
755	1° NUP98-KMT2A	F	21	7.95	8.55	114	13.8	1719	6.4	79.6	12.3	0.60	0.30	0.06	0.80	484.8	5.67	100	1300
756	1° NUP98-KMT2A	F	39	4.29	10.56	153	13.4	861	9.9	82.4	2.3	4.00	0.50	2.70	0.01	333.9	3.16	80	1230
942	1° NUP98-KMT2A	F	43	7.80	10.14	147	12.7	1548	6.7	87.8	3.2	1.70	0.20	0.03	0.40	283.8	2.80	113	2000
950	1° NUP98-KMT2A	F	13	7.14	10.56	156	12.7	1254	8.8	81.8	2.4	3.70	0.30	0.21	3.00	362.1	3.42	80	1000
951	1° NUP98-KMT2A	F	41	4.74	8.85	129	13.1	1629	43.0	53.8	0.6	1.40	0.10	0.06	1.10	157.5	1.78	130	1000
1107	BMT + DOX	F	30	7.95	4.92	135	17.5	5685	10.1	84.4	1.2	2.1	0.3	0.15	1.9	248.1	5.04	75	1000
1108	BMT + DOX	F	32	5.46	9.09	129	13.5	1380	23.9	66.7	0.4	3.5	1.1	0.24	4.4	261	2.87	80	1000
1109	BMT + DOX	F	32	9.75	9.84	135	12.8	1104	3.3	92.2	1.6	2	0.2	0.06	0.6	278.7	2.84	80	1000
1110	BMT + DOX	F	32	5.13	9	123	14	1263	11.5	81	1.43	2.2	0.4	0.18	3.6	360.3	4	80	1000
1111	BMT + DOX	F	34	8.22	8.82	111	16	822	18.1	73.6	2.9	3.3	0.3	0.15	1.8	521.1	5.9	150	1000
1136	BMT DOX rem	F	34	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	60	NA
1137	BMT DOX rem	F	46	8.37	8.49	129	14	237	39.7	51	3.1	5.1	0.5	0.06	0.6	729.9	8.59	130	1360
1138	BMT DOX rem	F	48	7.74	7.74	108	16.6	1377	28.2	65.1	2	2.6	0.7	0.12	1.4	479.1	6.18	140	1390
1141	BMT DOX rem	F	50	5.55	8.25	120	14.3	39	27.7	59.7	4.3	7.2	0.2	0.06	1	378	4.59	70	780
1140	BMT DOX rem	F	81	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	107	1410
1139	BMT DOX rem	F	81	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	70	934

Fisher et al. Sup. Table 4. Data for primary-induced NUP98-KMT2A mice which developed AML.

Mouse ID	Experimental setting	Sex	Latency (weeks)	WBC (x10^9 cells/L)	RBC (x 10^12 cells/L)	HGB (g/L)	RDW (%)	PLT (x 10^9 cells/L)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)	LUC (x 10^9cells/L)	LUC (%)	RETIC (x 10^9cells/L)	RETIC (%)	Spleen (mg)	Liver (mg)
49	1° NUP98-KMT2A	F	108	22.32	9.33	135	14.1	1245	8.3	88.0	0.8	0.30	0.20	0.51	2.30	345.0	3.69	374	1556
253	1° NUP98-KMT2A	F	38	20.52	6.99	84	22.0	237	11.1	67.7	1.9	0.30	13.00	3.90	19.10	1788.9	25.54	1400	2900
436	1° NUP98-KMT2A	M	81	13.38	9.60	129	14.8	678	26.0	64.2	2.5	2.20	1.80	0.42	3.20	365.1	3.80	200	1800
532	1° NUP98-KMT2A	F	43	12.42	10.62	165	15.3	843	23.6	70.0	1.3	4.30	0.30	0.06	0.50	218.4	2.06	190	1300
533	1° NUP98-KMT2A	F	70																
438	1° NUP98-KMT2A	M	106	8.94	8.76	126	13.0	1935	8.9	66.0	1.5	2.20	0.80	1.83	20.60	314.4	3.59	100	1100

Fisher et al. Sup. Table 5. Data for primary-induced sublethally-irradiated NUP98-KMT2A mice.

Mouse ID	Experimental setting	Sex	Latency (weeks)	WBC (x10^9 cells/L)	RBC (x 10^12 cells/L)	HGB (g/L)	RDW (%)	PLT (x 10^9 cells/L)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)	LUC (x 10^9cells/L)	LUC (%)	RETIC (x 10^9cells/L)	RETIC (%)	Spleen (mg)	Liver (mg)
374	1° NUP98-KMT2A + IR	F	24	**2.16**	7.23	138	27.6	294	14.6	67.9	0.6	1.9	7.9	0.33	15	429.9		234	2055
978	1° NUP98-KMT2A + IR	F	27	**9.33**	10.44	147	13.3	1590	6.8	87.7	1.9	1.2	0.3	0.18	2.1	439.8		80	1350
375	1° NUP98-KMT2A + IR	F	24	**5.01**	8.31	123	14.1	1113	3.3	91.7	0.3	2.6	0.7	0.06	1.4	339		84	1045
979	1° NUP98-KMT2A + IR	F	34	**3.81**	6.93	117	16.9	666	2	90.8	0.3	0.6	2	0.15	4.2	182.4		200	1740
980	1° NUP98-KMT2A + IR	F	34	**4.92**	6.78	111	15.9	204	1.4	95.2	0.2	0.5	1.3	0.09	1.5	182.4		523	1850
981	1° NUP98-KMT2A + IR	F	22	**33.33**	9.48	138	13.3	486	6.6	81.6	8.2	0.6	1.5	0.51	1.5	124.5		490	1400