Table S1. Hematological parameters of individual Hypoallelic mice, classified according to Bethesda criteria as MDS/MPN, at time of sacrifice

Genotype	Mouse #	WBC (x 10^3/μl)	RBC (x 10^6/μl)	HCT (%)	PLT (x 10^3/μl)	Neut (x 10^3/μl)	Lymph (x 10^3/μl)	Mono, Eosin, Baso (%)	Blasts (%)
WT	N=4	5.98 (± 0.9)	9.57 (± 0.5)	45.1 (± 1.2)	1164 (± 155)	0.60 (10.0%) (± 1.7%)	5.02 (83.9%) (± 1.6%)	1.5, 2.4, 0.1 (± 0.3, 0.4, 0.1)	0.97 (±0.8)
NR4A1$^{+/-}$	3197	4.52	9.42	47.6	1038	1.60 (35.4%)	2.66 (58.8%)	0.0, 0.0,0.0	6.1
NR4A3$^{-/-}$	3330	11.00	10.92	54.8	1260	5.67 (51.5%)	4.70 (42.7%)	0.4, 3.0, 0.1	2.3
	3526	5.60	7.40	38.2	486	1.20 (21.4%)	3.66 (65.4%)	4.3, 5.2, 0.4	3.8
	3509	7.10	9.20	44.8	1524	1.68 (23.7%)	5.12 (72.1%)	1.0, 0.4, 0.1	2.5
	3215	2.88	4.74	23.0	694	1.08 (37.5%)	1.42 (49.4%)	3.4, 6.1, 0.2	3.4
	3511	18.9	10.9	54.6	1264	12.3 (64.9%)	4.76 (25.2%)	2.1, 1.5, 0.0	6.3
	3460	12.88	8.66	44.8	1050	4.08 (31.7%)	6.00 (46.6%)	5.4, 4.2, 2.2	1.8
	3499	72.8	5.3	20.6	720	31.52 (43.3%)	18.8(25.8 %)	8.4, 9.2, 2.4	9.9
	3402	5.82	6.58	33.6	580	2.02 (34.7%)	2.69 (46.2%)	4.9, 7.8, 2.1	4.3
	3507	6.66	11.10	49.8	596	1.47 (22.0%)	4.32 (64.9%)	2.9, 6.2, 0.2	3.8
	3904	5.72	5.08	25.2	804	2.56 (44.8%)	3.03 (53.0%)	0.2, 0.3, 0.0	1.7
	3889	8.66	7.22	32.4	914	3.20 (37.0%)	5.20 (60.0%)	0.3, 0.3, 0.1	2.3
	3832	15.30	8.64	35.5	420	6.36 (41.6%)	6.00 (39.2%)	5.1, 7.1, 0.3	6.7
	3923	17.80	9.60	41.2	214	7.62 (42.8%)	4.68 (26.3%)	7.2, 9.3, 0.7	13.7
	3936	14.60	10.81	52.1	711	5.49 (37.6%)	6.04 (41.4%)	5.4, 4.1, 0.3	11.2
	3902	32.90	9.12	37.5	909	13.9 (42.3%)	13.6 (40.1%)	3.4, 9.4, 0.2	4.6
	3709	10.90	10.70	29.8	527	2.55 (23.4%)	6.02 (55.2%)	2.1, 1.4, 0.0	17.9
	3830	13.10	11.34	31.6	220	4.31 (32.9%)	7.52 (57.4%)	1.1, 2.7, 0.1	5.8
	3853	14.20	8.29	27.4	1240	5.20 (36.6%)	5.72 (40.3%)	7.1, 7.5, 0.4	8.1
	3462	10.66	1.96	12.0	102	1.76 (16.5%)	8.48 (79.5%)	2.2, 1.2, 0.1	0.8
	3329	11.18	9.10	39.6	702	1.90 (17%)	8.10 (68.6%)	3.6, 6.1, 0.2	1.2
	3447	3.76	9.02	43.4	400	0.60 (16%)	3.04 (80.8%)	0.2, 0.9, 0.0	2.1
	3340	0.94	3.72	18.8	500	0.11 (11.7%)	0.65 (69.1%)	2.7, 1.9, 0.3	12.5
	3527	4.79	5.60	46.7	564	0.685 (14.3%)	3.36 (70.1%)	0.8, 2.4, 0.2	12.2

Genotype	Mouse #	WBC (x 10^3/μl)	RBC (x 10^6/μl)	HCT (%)	PLT (x 10^3/μl)	Neut (x 10^3/μl)	Lymph (x 10^3/μl)	Mono, Eosin, Baso (%)	Blasts (%)
	3719	7.96	9.62	49.2	1174	1.58 (19.8%)	5.35 (67.2%)	3.6, 5.9, 0.1	3.4
NR4A1$^{-/-}$	3227	9.44	8.18	40.0	1444	2.26 (23.9%)	6.04 (64%)	1.9, 7.9, 0.4	1.7
NR4A3$^{+/-}$	3341	15.30	6.70	33.0	1646	7.30 (47.7%)	7.01 (46.4%)	0.8, 2.4, 0.3	1.9
	3467	0.64	9.12	46.0	1108	0.21 (33.0%)	0.09 (14%)	14.5, 15.2, 6.3	17.0
	3403	32.82	9.7	50.4	1594	24.9 (76.0%)	2.49 (7.6%)	5.0, 6.1, 1.1	3.2
	3424	12.87	9.71	42.0	240	5.43 (42.2%)	5.64 (43.8%)	2.4, 3.0, 0.1	8.5
	3468	11.32	9.40	46.2	1068	2.22 (19.6%)	8.30 (73.3%)	1.7, 2.1, 1.1	2.1
	3192	10.94	5.47	33.6	780	4.37 (39.9%)	3.61 (33.0%)	4.6, 5.0, 2.2	5.3
	3589	4.38	7.64	48.0	1358	1.45 (33.1%)	2.16 (49.3%)	4.0, 8.7, 0	4.9
	3461	15.50	12.06	54.6	1308	3.40 (21.9%)	10.85 (70%)	3.2, 3.4, 0.2	1.2
	3488	13.40	8.76	52.3	817	5.88 (43.9%)	5.37 (40.1%)	3.1, 5.0, 0.2	7.7
	3504	11.80	9.28	47.6	740	4.86 (41.2%)	5.46 (46.3%)	2.7, 5.8, 0.4	3.6
	3538	6.72	7.61	37.8	319	1.70 (25.3%)	4.49 (66.8%)	1.6, 3.4, 0.0	2.9
	3570	12.91	7.78	41.4	1101	3.74 (29.0%)	6.48 (50.2%)	6.1, 9.0, 0.1	5.6
	3615	14.50	6.40	40.2	510	5.42 (37.4%)	6.49 (44.8%)	4.2, 6.7, 2.0	4.9
	3836	14.20	6.73	44.7	212	6.04 (42.5%)	5.31 (37.4%)	1.3, 2.6, 0.6	15.6
	3411	6.36	7.32	34.4	324	0.76 (12%)	5.36 (84.3%)	0.2, 0.6, 0	2.8
	3338	7.08	7.74	42.8	390	1.02 (14.4%)	5.52 (78%)	2.1, 4.0, 0.2	1.1
	3515	7.60	10.12	32.9	422	1.33 (17.6%)	4.31 (56.7%)	7.2, 5.1, 0.7	12.7
	3720	4.77	8.51	29.6	54	0.77 (16.2%)	2.46 (51.6%)	7.1, 9.8, 0.5	14.8
	3339	4.12	8.60	48.0	1242	0.68 (16.5%)	2.69 (65.3%)	11.1, 4.2, 1.3	1.6

HCT, hematocrit; PLT, platlet; Neut, neutrophils; Lymph, lymphocytes; Mono, monocytes; Eosin, eosinophils; Baso, basophils. Normal Values: wbc, 3.2-12.7 x 10^3/μl; rbc, 7.0-10.1 x 10^6/μl; HCT, 36.7- 46.8%; PLT, 766-1657 x 10^3/μl; Neut, 0.5- 2.0 x 10^3/μl; Lymph, 3.80- 8.90 x 10^3/μl; Mono, 0-4.3%; Eosin, 0.2- 5.9%; Baso, 0- 0.3%; Blasts, 0 - 3.2%. Red color denotes mice displaying neutrophilia (>20% as defined in Bethesda guide); blue color denotes mice displaying thrombocytopenia (<800 x 10^3/μl).

Table S2. Hematological parameters of individual Hypoallelic mice, classified according to Bethesda criteria as AML, at time of sacrifice

Genotype	Mouse #	WBC (x $10^3/\mu l$)	RBC (x $10^6/\mu l$)	HCT (%)	PLT (x $10^3/\mu l$)	Neut (x $10^3/\mu l$)	Lymph (x $10^3/\mu l$)	Mono, Eosin, Baso (%)	Blasts (%)
WT	N=4	5.98 (± 0.9)	9.57 (± 0.5)	45.1 (± 1.2)	1164 (± 155)	0.60 (10.0%) (± 1.7%)	5.02 (83.9%) (± 1.6%)	1.5, 2.4, 0.1 (± 0.3, 0.4, 0.1)	0.97 (±0.8)
$NR4A1^{+/-}$	2996	47.3	1.84	8.7	544	12.7 (26.8%)	16.4 (34.6%)	1.7, 2.5, 0.2	34.2
$NR4A3^{-/-}$	1061	10.80	8.21	44.1	120	2.21 (20.5%)	4.03 (37.3%)	2.5, 3.0, 0.4	36.3
	3649	12.91	8.95	35.3	47	2.98 (23.1%)	6.33 (49.0%)	2.1, 3.2, 0.1	22.5
$NR4A1^{-/-}$	2140	9.31	7.70	25.7	132	1.51 (16.2%)	4.08 (43.8%)	1.4, 4.6, 0.1	33.9
$NR4A3^{+/-}$	3523	7.10	5.31	19.8	98	1.01 (14.2%)	3.83 (53.9%)	2.3, 2.7, 0.6	26.3

HCT, hematocrit; PLT, platlet; Neut, neutrophils; Lymph, lymphocytes; Mono, monocytes; Eosin, eosinophils; Baso, basophils. Red color denotes mice displaying neutrophilia (>20% as defined in Bethesda guide); blue color denotes mice displaying thrombocytopenia (<800 x $10^3/\mu l$).

Table S3. Mouse data

Mouse MPD (Kogan et al)	Hypoallelic Mice
1. By definition, this diagnosis is for lesions that are nonreactive, persistent, genetically determined and meet criteria 2-4 below.	The disease is nonreactive, persistent and genetically determined.
2. Mice exhibit increased nonlymphoid hematopoietic cells as evidenced by <u>any combination</u> of the following: (A) Erythrocytosis, leukocytosis of myeloid cells, and/or thrombocytosis/circulating micromegakaryocytes (B) Increased nonlymphoid hematopoietic cells in spleen and/or bone marrow	(A) leukocytosis of myeloid cells (neutrophils represent >20% of PB leukocytes) (B) Mice display increased myeloid cells in both the spleen and bone marrow as measured by cytospin, flow cytometry and immunohistochemistry.
3. Disorder is not a nonlymphoid leukemia	Peripheral blood and bone marrow and spleen cytospins show <20% blasts (except in rare, transformed mice).
4. Disorder is not a myeloid dysplasia (if a disorder satisfies criteria 1 and 2 but also fulfills criteria for myeloid dysplasia, the diagnosis of myeloid dysplasia should also be included)	Mice also exhibit features of myeloid dysplasia including dysgranulopoiesis Therefore the diagnosis of MDS was included.

Table S4. Blood analysis of individual mice used for qPCR analysis

Early Stage Mice used for NR4A1$^{+/-}$ NR4A3$^{-/-}$ mice used for qPCR

WBC	RBC	HCT	PLT	Neut (%)	Lymph (%)	Blasts
5.98	9.87	48	1219	28.53%	66.67%	0.96%
8.66	9.28	47.2	1170	6.90%	84.80%	1.20%
9.62	7.88	43	1424	11.10%	82.50%	3.9%

Late Disease stage NR4A1$^{+/-}$NR4A3$^{-/-}$ mice used for qPCR

Mouse	WBC	RBC	HCT	PLT	Neut (%)	% Ly Lymph (%)	PB Blasts	BM Blasts	Sp Blasts
*3426	12.26	7.88	37.6	760	16.6	61.40%	18%	25%	29%
3524	7.22	8.38	11.4	278	11.8	65.70%	13%	18%	14%
3527	3.57	7.94	37.7	215	24.1	59.20%	12.40%	15%	16%

* Myeloid blast counts in BM and spleen showed # 3426 classified as AML

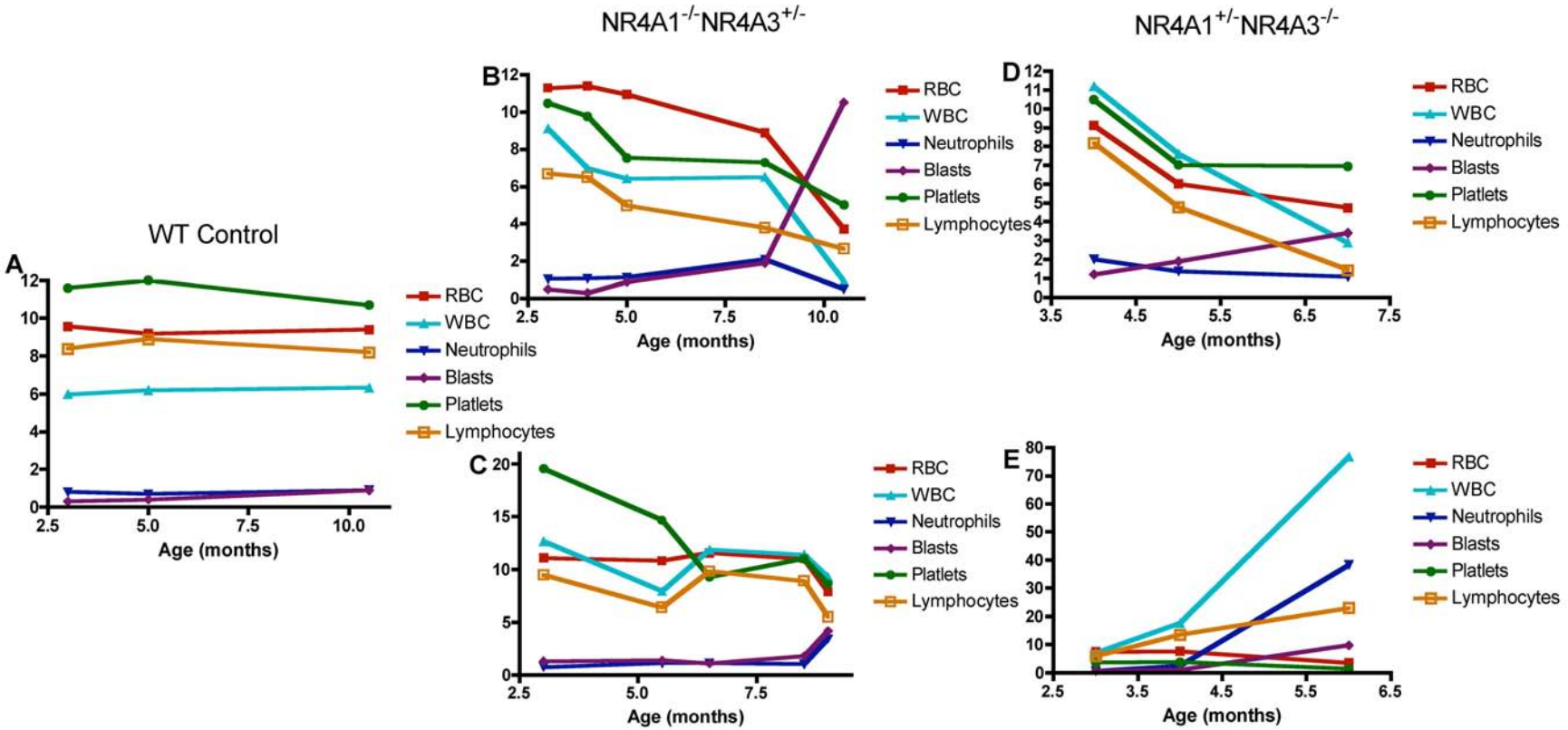


Figure S1. Complex disease progression evidenced by serial blood counts of hypoallelic mice.
(A–E) Serial peripheral blood counts of rbc (× 10^6/µl), wbc (× 10^3/µl), neutrophils (× 10^3/µl), blasts (%), platlets (× 10^5/µl) and lymphocytes (× 10^3/µl) taken by retrorbital bleeding of WT (A) or MDS/MPN *NR4A1*$^{-/-}$*NR4A3*$^{+/-}$ (B–C) and *NR4A1*$^{+/-}$*NR4A3*$^{-/-}$ (D–E) mice at indicated time points shows an increase in neutrophil number at disease onset (B and D) in some hypoallelic mice. With disease progression mature lineages (neutrophils, RBC, WBC, lymphocytes and platelets) decline with a concomitant increase in blast number (B–D) leading to pancytopenia in some hypoallelic mice, while others die from massive neutrophil expansion (E). N=2 for WT control and n=4 for hypoallelic mice (2 mice per indicated genotype, one mouse shown per graph).

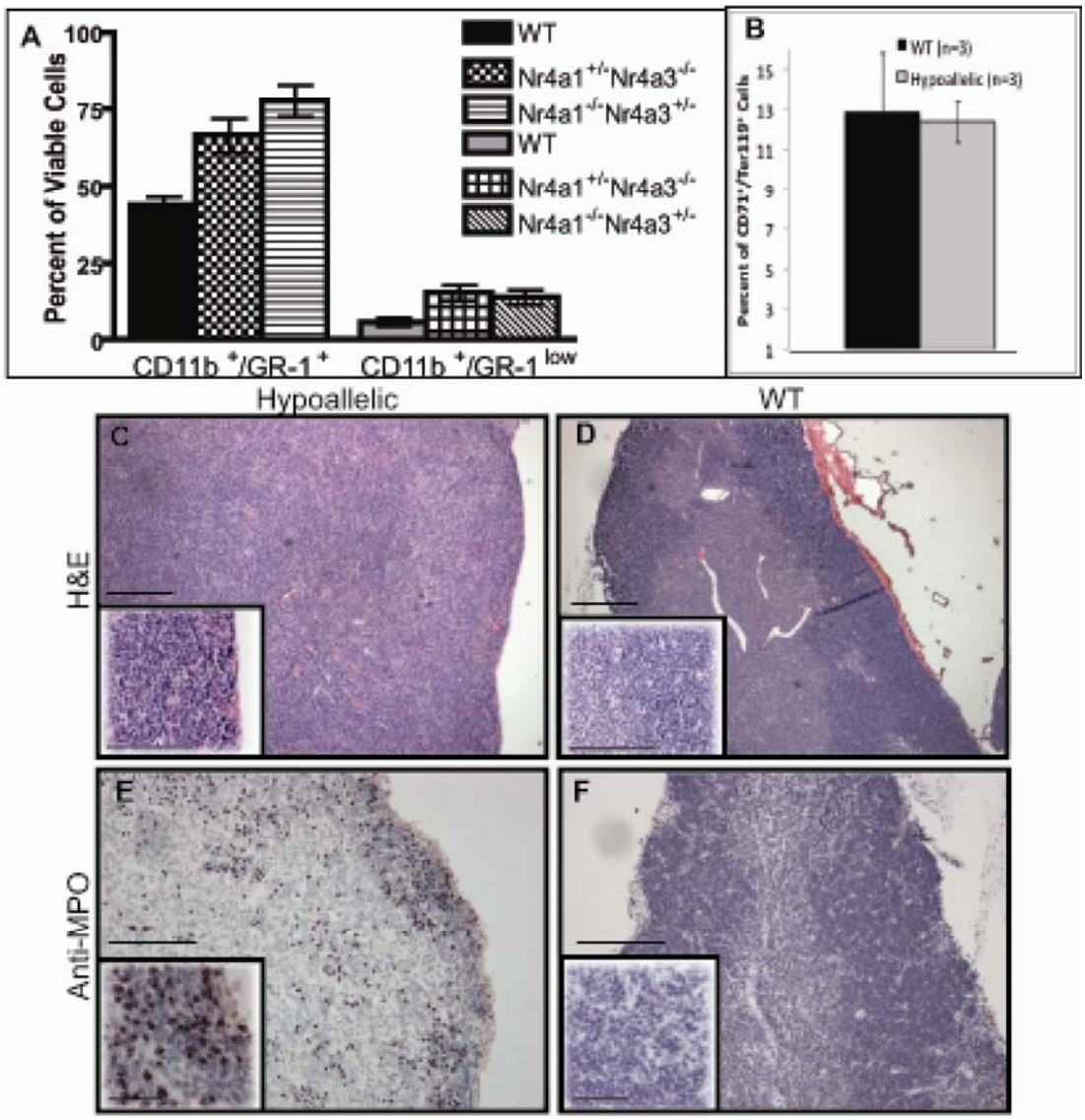


Figure S2. Expansion of myeloid elements in the bone marrow and perivascular infiltration of thymic cortex of hypoallelic mice.

(A) Flow cytometric analysis of bone marrow from moribund *NR4A1*$^{+/-}$*NR4A3*$^{-/-}$ mice (n=3, at 4–7 months) shows expansion of myeloid cells (CD11b^{+}/Gr-1^{low} and CD11b^{+}/Gr-1^{+}) compared to healthy littermates. Expansion of myeloid lineages was comparable to that seen in *NR4A1*$^{-/-}$*NR4A3*$^{+/-}$ mice (n=4, at 9–12 months). Numbers shown are the average percent of total cells for the associated region. (B) Flow cytometric analysis of bone marrow from moribund *NR4A1*$^{+/-}$*NR4A3*$^{-/-}$ mice (n=3, at 3–5 months) demonstrated no increase in CD71^{+}/Ter119^{+} cells, indicating erthryopoesis is normal in these mice. (C–F) Hematoxylin and eosin stained paraffin sections of thymus. Thymus from *NR4A1*$^{+/-}$*NR4A3*$^{-/-}$ hypoallelic mouse (at 5 months) (C) revealed loss of distinct thymic architecture, including well-defined cortex needed for T cell maturation, compared to control mice (D). Scale bar, 500μm for main image, 250μm for insets. (E–F) Antimyeloperoxidase immunohistochemistry revealed myeloid contribution to infiltrates in hypoallelic thymus (E), which were not present in control (F). Scale bar, 250μm for main image, 50μm for insets.

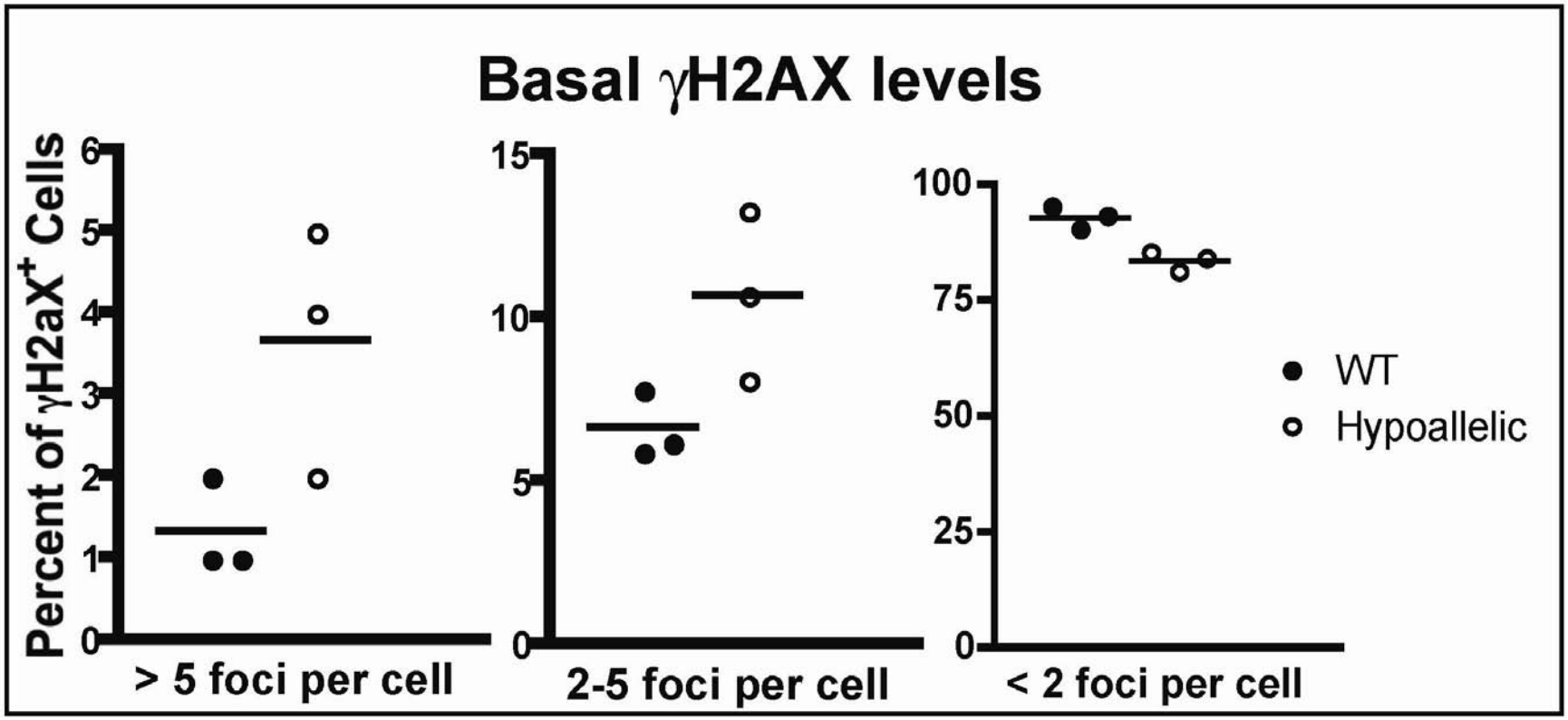
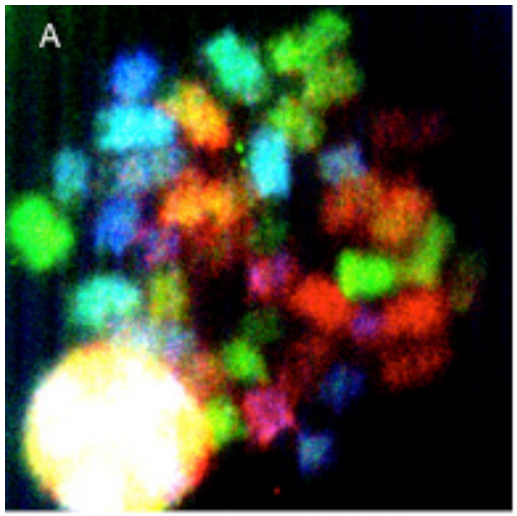
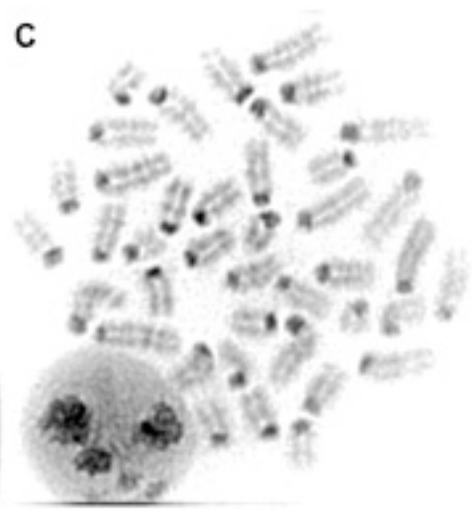


Figure S3. Basal DNA damage in myeloid progenitor cells.
(A) CD11b^{+}/Gr-1$^{low\text{-}neg}$ cells from MDS/MPN *NR4A1*$^{+/-}$*NR4A3*$^{-/-}$ mice (n=4) were analyzed for DNA damage. Scatter plot of percent γH2AX positive cells (200 cells counted per mouse) in un-irradiated samples shows 3 out of 4 hypoallelic mice analyzed have increased basal DNA damage.



Display Colors

Classification Colors



DAPI

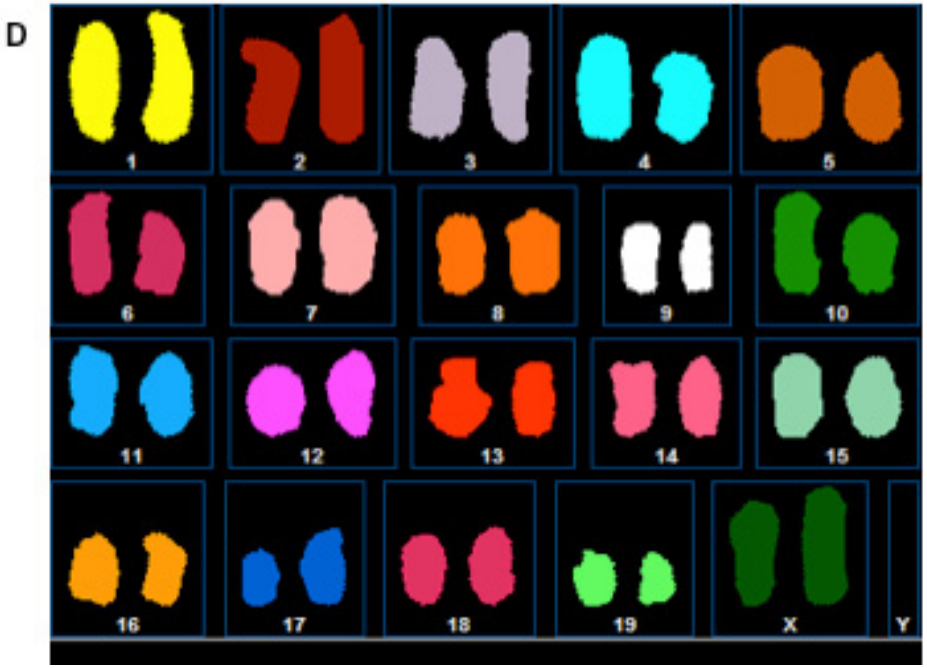


Figure S4. Blast crisis transformation does not require cooperation between *NR4A* silencing and gross genetic abnormalities. (A) Microscope display image. (B) Spectral karyotype (SKY) analysis DAPI banding pattern. (C) SKY analysis classification colors. (D) SKY analysis of whole bone marrow isolated from blast crisis phase (>20% blasts) *NR4A1*$^{-/-}$*NR4A3*$^{+/-}$ animals. Analysis shown is from a representative animal. 5–10 metaphase spreads were analyzed per animal. N=3 (age 9–12 months).

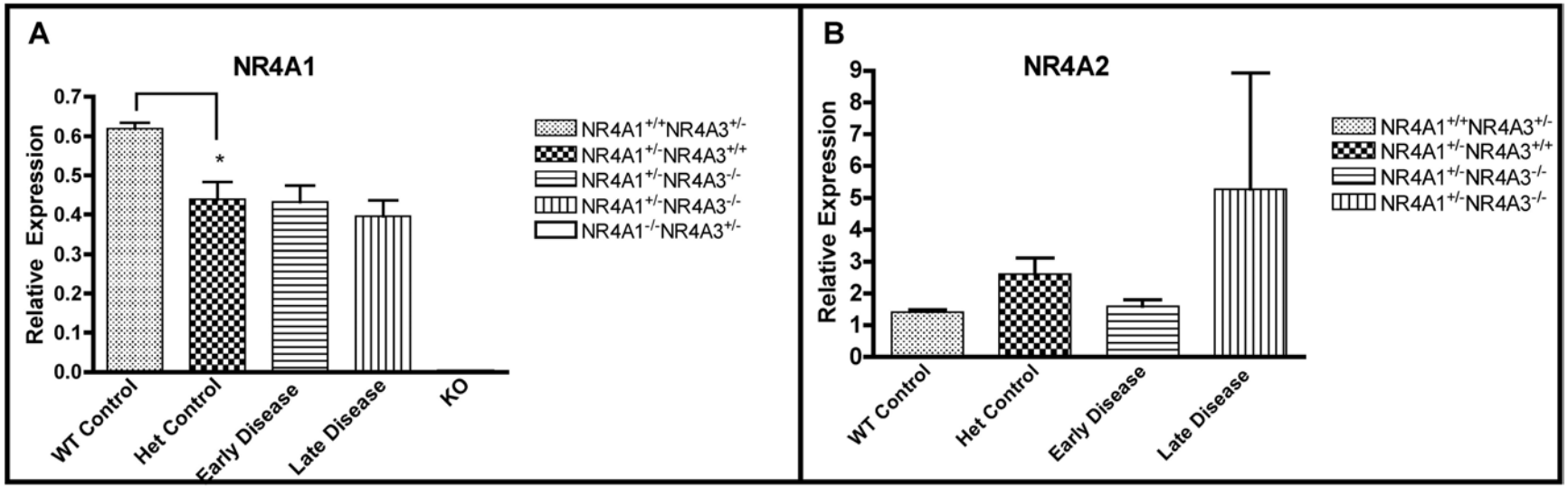


Figure S5. Silencing of the remaining NR4A alleles is not required for disease progression. (A) Real-time PCR analysis of *NR4A1* using RNA isolated from CD11b^{+}/Gr-1$^{low\text{-}neg}$ cells from healthy *NR4A1*$^{+/+}$*NR4A3*$^{+/-}$ mice (WT control for *NR4A1)*, healthy *NR4A1*$^{+/-}$*NR4A3*$^{+/+}$ (heterozygous (Het) control for *NR4A1*), early disease *NR4A1*$^{+/-}$*NR4A3*$^{-/-}$ hypoallelic mice (6–8 weeks of age, 1–4% blasts) and late disease MDS/MPN *NR4A1*$^{+/-}$*NR4A3*$^{-/-}$ hypoallelic mice (at 4–10 months of age, 12–18% blasts) shows the remaining *NR4A1* allele is not silenced with disease progression. Additionally, *NR4A1*$^{-/-}$*NR4A3*$^{+/-}$ mice (null control (KO) for *NR4A1*) were used as a control for qPCR. N=3 for each genotype and disease stage. Results were normalized to *β2m* expression. Error bars denote s.e.m. (B) Because MDS/MPN *NR4A1*$^{+/-}$*NR4A3*$^{-/-}$ mice are wild type for the third family member, *NR4A2*, we also conducted real-time PCR analysis of *NR4A2* using RNA isolated from CD11b^{+}/Gr-1$^{low\text{-}neg}$ cells, from the same cohorts of mice, to determine whether disease progression was associated with loss of *NR4A2* expression. We found that *NR4A2* is not silenced in MDS/MPN mice. N=3 for each genotype and disease stage. Results were normalized to *β2m* expression.