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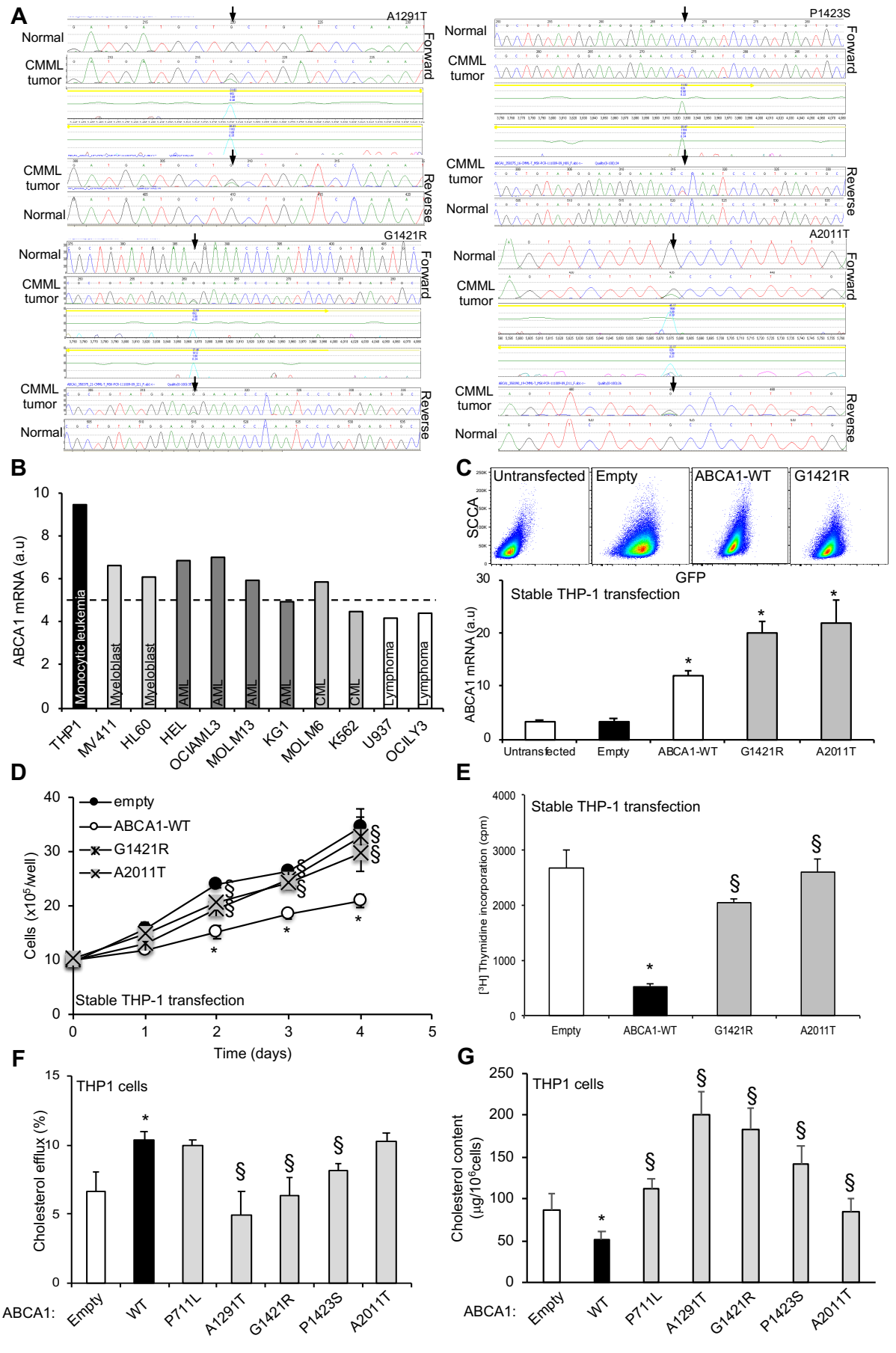
Supplemental Information

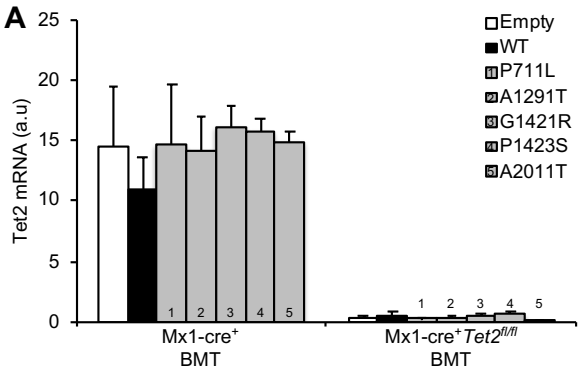
ABCA1 Exerts Tumor-Suppressor Function in Myeloproliferative Neoplasms

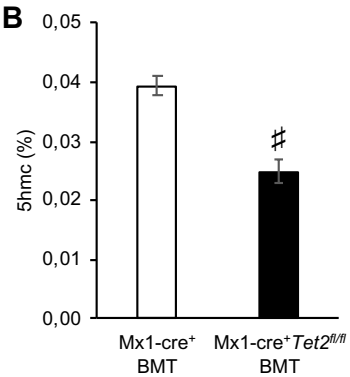
Manon Viaud, Omar Abdel-Wahab, Julie Gall, Stoyan Ivanov, Rodolphe Guinamard, Sophie Sore, Johanna Merlin, Marion Ayrault, Emma Guilbaud, Arnaud Jacquel, Patrick Auberger, Nan Wang, Ross L. Levine, Alan R. Tall, and Laurent Yvan-Charvet

Supplemental Table 1: Primer sequences used to sequence all coding exons of ABCA1, ABCG1, NR1H2, and NR1H3 **(**related to Figure 1)


A
A1291T
Normal
CMML tumor
Forward
CMML tumor
Reverse
Normal
G1421R
Normal
CMML tumor
Forward
CMML tumor
Reverse
Normal
P1423S
Normal
CMML tumor
Forward
CMML tumor
Reverse
Normal
A2011T
Normal
CMML tumor
Forward
CMML tumor
Reverse
Normal
B
ABCA1 mRNA (a.u)
Monocytic leukemia
Myeloblast
Myeloblast
AML
AML
AML
AML
CML
CML
Lymphoma
Lymphoma
THP1
MV411
HL60
HEL
OCIAML3
MOLM13
KG1
MOLM6
K562
U937
OCILY3
C
Untransfected
Empty
ABCA1-WT
G1421R
SCCA
GFP
Stable THP-1 transfection
ABCA1 mRNA (a.u)
Untransfected
Empty
ABCA1-WT
G1421R
A2011T
D
empty
ABCA1-WT
G1421R
A2011T
Cells (x10^5/well)
Stable THP-1 transfection
Time (days)
E
Stable THP-1 transfection
[3H] Thymidine incorporation (cpm)
Empty
ABCA1-WT
G1421R
A2011T
F
THP1 cells
Cholesterol efflux (%)
ABCA1:
Empty
WT
P711L
A1291T
G1421R
P1423S
A2011T
G
THP1 cells
Cholesterol content (µg/10^6cells)
ABCA1:
Empty
WT
P711L
A1291T
G1421R
P1423S
A2011T

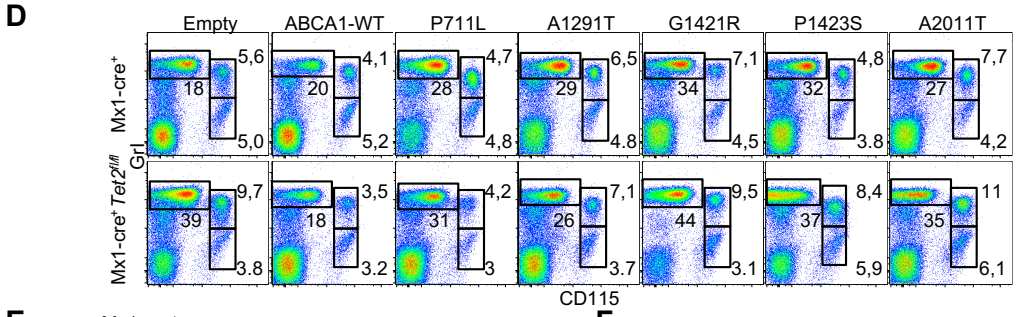
Supplemental Figure 1. *ABCA1* mutants confer a growth advantage to THP-1 monocytic leukemia cells (related to Figure 1). *ABCA1* mutants are co-expressed with common oncogenes (JAK2-V617F, Flt3-ITD, N- or K-Ras-G12D) in myeloid cell DNA from patients with chronic myelomonocytic leukemia (CMML) **(A)**. *Abca1* transcripts expressed as arbitrary unit (a.u.) in several leukemic cell lines. THP-1 cells are monocytic leukemia cells that express relatively high amount of *Abca1* transcripts compared to myeloblastic MV411 and HL60 cells, acute myeloid leukemia (AML) HEL, OCIAML3, MOLM13 and KG1 cells, chronic myeloid leukemia (CML) MOLM6 and K562 cells or lymphoma U237 and OCILY3 cells **(B)**. Monocytic leukemia THP-1 cells stably transfected with lentiviral particles at MOI of 5 were validated by flow cytometry analysis of GFP expression **(C, upper panel)** and *Abca1* mRNA expression **(C, lower panel)**. Stable THP-1 cells transduced with *ABCA1* mutants (G1421R or A2011T) exhibit growth advantage over a 4-day period compared with *ABCA1-WT* **(D)** and higher proliferation rate using [3H]-thymidine proliferation assay **(E)**. Cholesterol efflux from the aforementioned THP-1 macrophages was quantified after overnight [^{3}H]-cholesterol loading and determined 6 hours after incubation with apoA-I (15μg/mL) **(F)**. Cellular cholesterol content was determined in these cells **(G)**. Values are mean $\pm$ SEM of at least three experiments performed in triplicate. * $P < 0.05$ versus empty control. § $P < 0.05$ versus *ABCA1-WT*.

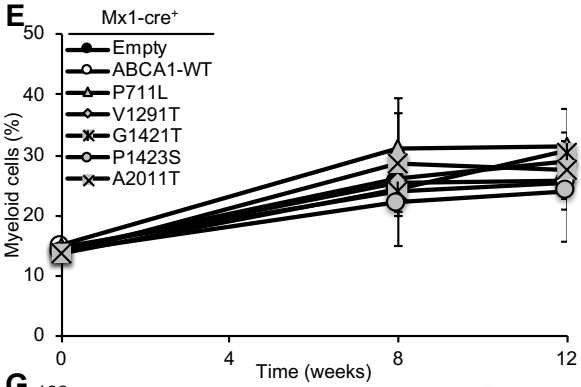


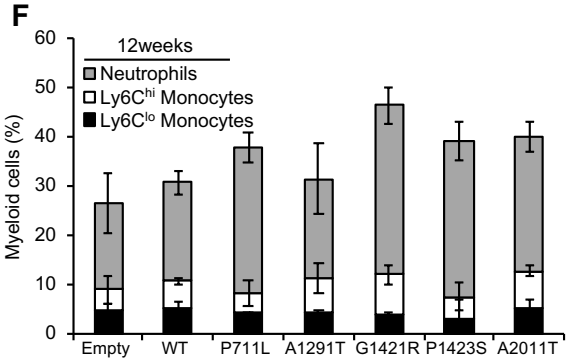


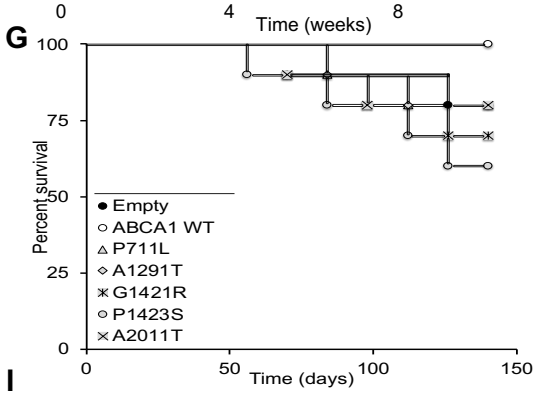
C

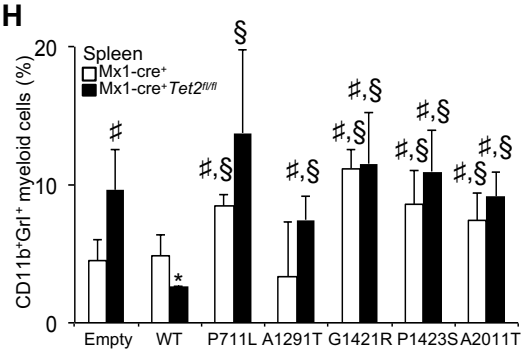
WBC (K/uL)	Empty	ABCA1-WT	P711L	A129T	G1421R	P1423S	A2011T	Abca1+/+	Abca1fl/fl
Mx1-cre+	4.61±0.45	3.62±0.27	4.10±1.72	5.76±0.92	5.54±0.34	4.39±0.38	4.21±0.36	8.85±0,47	7.86±0,88
Mx1-cre+Tet2fl/fl	5.78±0.55	4.69±0.33	5.30±1.75	6.13±0.59	6.83±1.26	5.45±0.49	6.23±1.08	8.06±0,74	8.08±0,40

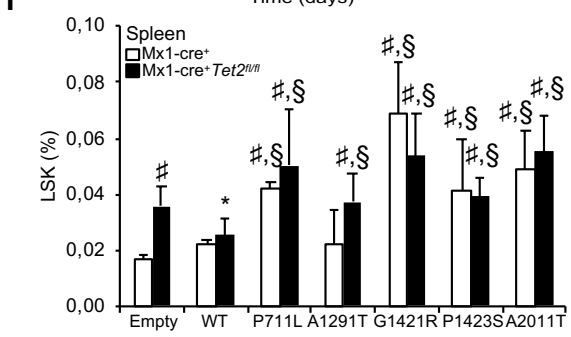




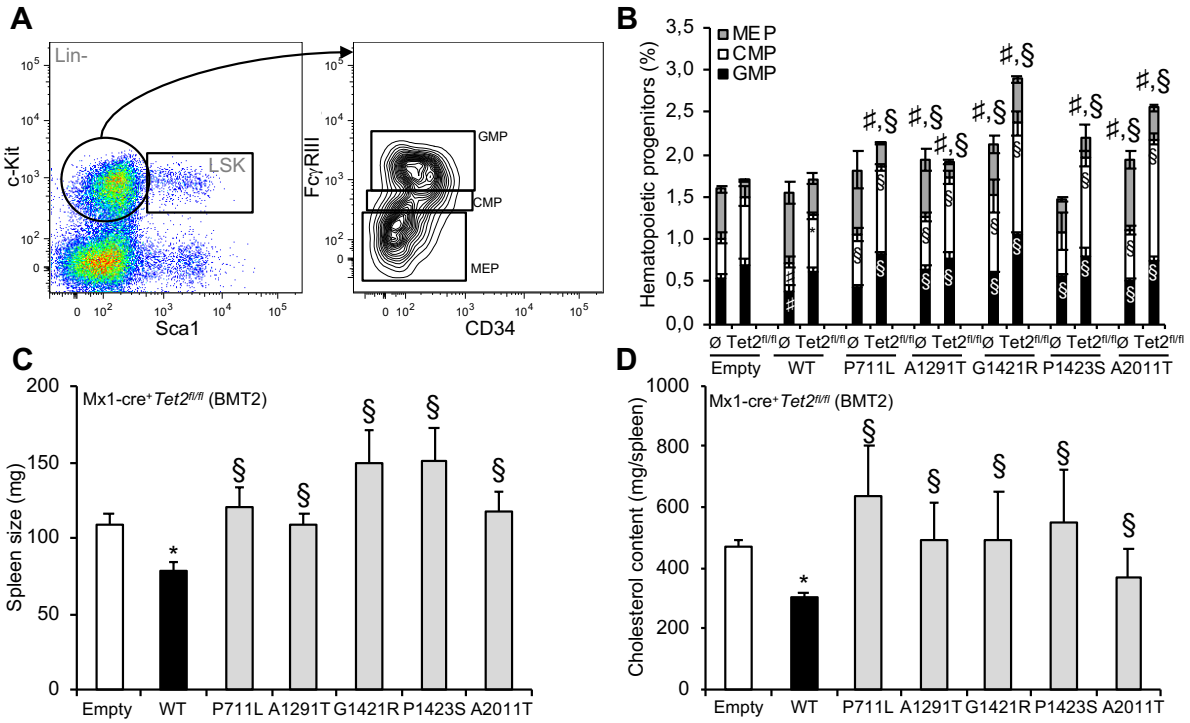




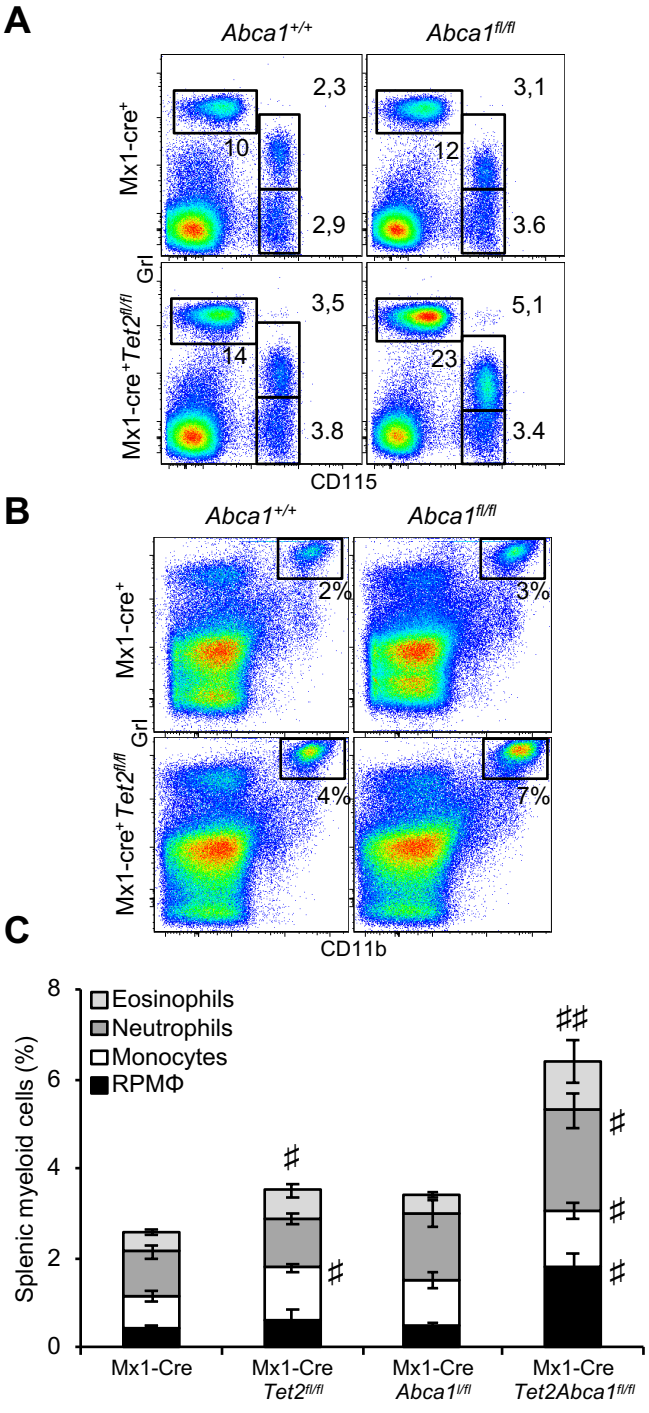




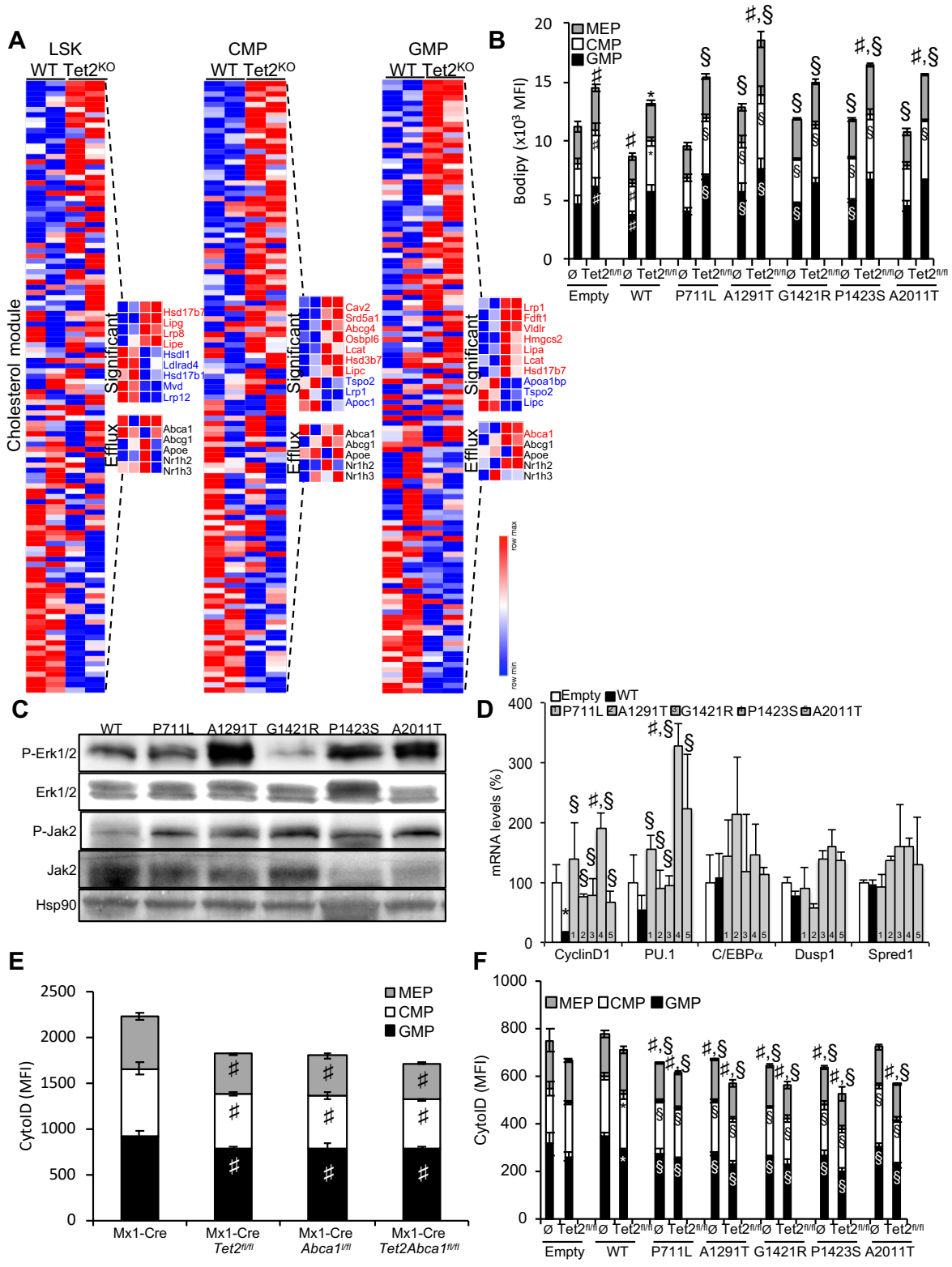
Supplemental Figure 2. *ABCA1* mutants are not sufficient to promote myelopoiesis on a WT background but accelerates extramedullary myelopoiesis on a Tet2 deficient background (related to Figure 2). Quantification of Tet2 mRNA expression levels in the BM of lethally irradiated WT recipient mice that were transplanted with the BM of Mx1-Cre^{+} or Mx1-Cre^{+}Tet2$^{fl/fl}$ mice after transduction with lentiviral particles expressing *ABCA1-WT*, *ABCA*1 mutants or empty vector and induced 5 weeks later with Poly:IC injection **(A)**. Percentage of 5hmC in genomic DNA isolated from a pool of blood cells of these mice and quantified by ELISA **(B)**. Complete blood counts show similar white blood cell (WBC) numbers in *ABCA1* mutant BMT model compared with empty or *ABCA1-WT* at the end of the study period. Standard deviation is indicated **(C)**. Analysis of peripheral blood myeloid cells by flow cytometry. Representative dot plot of CD115^{+}Ly6C^{hi} and CD115^{+}Ly6C^{lo} monocytes and CD115^{-}Ly6C^{hi} neutrophils in peripheral blood of these mice at end of the study period **(D)**. Quantification of peripheral blood myeloid cells is depicted over the course of 12 weeks after Poly:IC injection in recipients mice transplanted with control BM expressing empty, *ABCA1-WT* or ABCA1 mutants **(E)**. Quantification of myeloid subsets at the end of the study period in these mice **(F)**. Kaplan-Meier survival curve of these animals (n=10) **(G)**. Percentage of CD11b^{+} GrI^{+} myeloid cells determined by flow cytometry in the spleen of recipient mice transplanted with control or Mx1-Cre^{+}Tet2$^{fl/fl}$ BM expressing empty, *ABCA1-WT* or *ABCA1* mutants **(H)** Quantification of Lin^{-}Sca1^{+}c-Kit^{+} (LSK) hematopoietic stem and progenitor cells was also determined by flow cytometry in the spleen of *ABCA1* mutants-transduced animals **(I)**. The results are means ± SEM of 5-9 animals per group. * $P < 0.05$ versus empty control transduced animals on a Tet2 deficient background. § $P < 0.05$ versus *ABCA1-WT*. $^{\#}$ $P < 0.05$ versus Mx1-Cre^{+} controls.



Supplemental Figure 3. *ABCA1* invalidation disengages the tumor suppressive effect of *ABCA1* on HSPC expansion in Tet2-deficient mice and fails to repress splenomegaly in serial BM transplantation (related to Figure 3). Gating strategy of hematopoietic stem and progenitor cells **(A)**. Hematopoietic stem cells were identified as Lin$^-$ Sca1$^+$ c-Kit$^+$ (LSK cells) and Lin$^-$ Sca1$^-$ c-Kit$^+$ progenitor cells including MEPs (Megakaryocyte-Erythrocyte Progenitors), CMPs (Common Myeloid Progenitors) and GMPs (Granulocyte-Monocyte Progenitors) were identified based on the cell surface markers CD34 and FcγRII **(A)**. Quantification of hematopoietic progenitor cells in the BM of recipient mice transplanted with control or Mx1-Cre$^+$Tet2$^{fl/fl}$ BM expressing empty, *ABCA1-WT* or *ABCA1* mutants **(B)**. Spleen weight **(C)** or cholesterol content **(D)** of recipient mice serially transplanted with control or Mx1-Cre$^+$Tet2$^{fl/fl}$ BM expressing empty, *ABCA1-WT* or *ABCA1* mutants**.** Results are means ± SEM of 5-9 animals per group. * $P < 0.05$ versus empty control on a Tet2 deficient background. § $P < 0.05$ versus *ABCA1-WT*. $^{\#}$ $P < 0.05$ versus Mx1-Cre$^+$ controls.



Supplemental Figure 4. *ABCA1* deficiency exacerbates extramedullary myelopoiesis on a Tet2 deficient background (related to Figure 4). Representative dot plot of $CD11b^+$ $CD115^+$ or $CD11b^+$ Grl^+ myeloid cells determined by flow cytometry in the blood **(A)** and spleen **(B)** of recipient mice transplanted with $Mx1\text{-}Cre^+$, $Mx1\text{-}Cre^+Abca1^{fl/fl}$, $Mx1\text{-}Cre^+Tet2^{fl/fl}$ and $Mx1\text{-}Cre^+Tet2^{fl/fl}Abca1^{fl/fl}$ BM. Percentage of $CD11b^+$ Grl^+ myeloid cells determined in the spleen of these mice **(C)**. Results are means ± SEM of 5-7 animals per group. $^{\#}P < 0.05$ versus $Mx1\text{-}Cre^+$ controls.



Supplemental Figure 5. Metabolic and signaling rewiring in BM cells after *ABCA1* invalidation and Tet2 loss (related to Figure 5). Heatmap of genes encoding molecules involved in cholesterol metabolism in Tet2 deficient LSK, CMP and GMP cells **(A)**. Genes labeled in blue or red are statistically down- or up-regulated, respectively. Quantification of BODIPY staining by flow cytometry as a surrogate of cellular cholesterol neutral lipid in BM hematopoietic progenitor cells (i.e, MEPs, CMPs and GMPs) of recipient mice transplanted with control and Mx1-Cre^{+}Tet2$^{fl/fl}$ BM expressing empty, *ABCA1-WT* or *ABCA1* mutants **(B)**.

Results are means ± SEM of 5-9 animals per group. Western blot analysis for pErk1/2, Erk1/2, pJak2, Jak2, and Hsp90 in BM cells obtained from *ABCA1-WT* and *ABCA1* mutants-transduced animals on a Tet2 deficient background at the end of the study period **(C)**. mRNA expression of MAPK target genes (Cyclin D1 and PU.1), C/EBPα and negative regulators of RAS signaling (Dusp1 and Spred1) from empty, WT and *ABCA1* mutants-transduced BM on a Tet2 deficient background isolated at the end of the study period **(D)**. Expression of mRNA was normalized to m36B4. mRNA levels were expressed as percentage over WT cells. Autophagic flux was quantified using the Cyto-ID probe by flow cytometry and expressed as the mean fluorescence intensity (MFI) in BM hematopoietic progenitor cells (i.e, MEP, CMP and GMP) of recipient mice transplanted with Mx1-Cre^{+}, Mx1-Cre^{+}Abca1$^{fl/fl}$, Mx1-Cre^{+}Tet2$^{fl/fl}$ and Mx1-Cre^{+}Tet2$^{fl/fl}$Abca1$^{fl/fl}$ BM **(E)** or control and Mx1-Cre^{+}Tet2$^{fl/fl}$ BM expressing empty, *ABCA1-WT* or *ABCA1* mutants **(F)**. Results are means ± SEM of 5-9 animals per group. * $P <$ 0.05 versus empty control transduced animals on a Tet2 deficient background. § $P < 0.05$ versus *ABCA1-WT*. $^{\#}$ $P < 0.05$ versus Mx1-Cre^{+} controls.