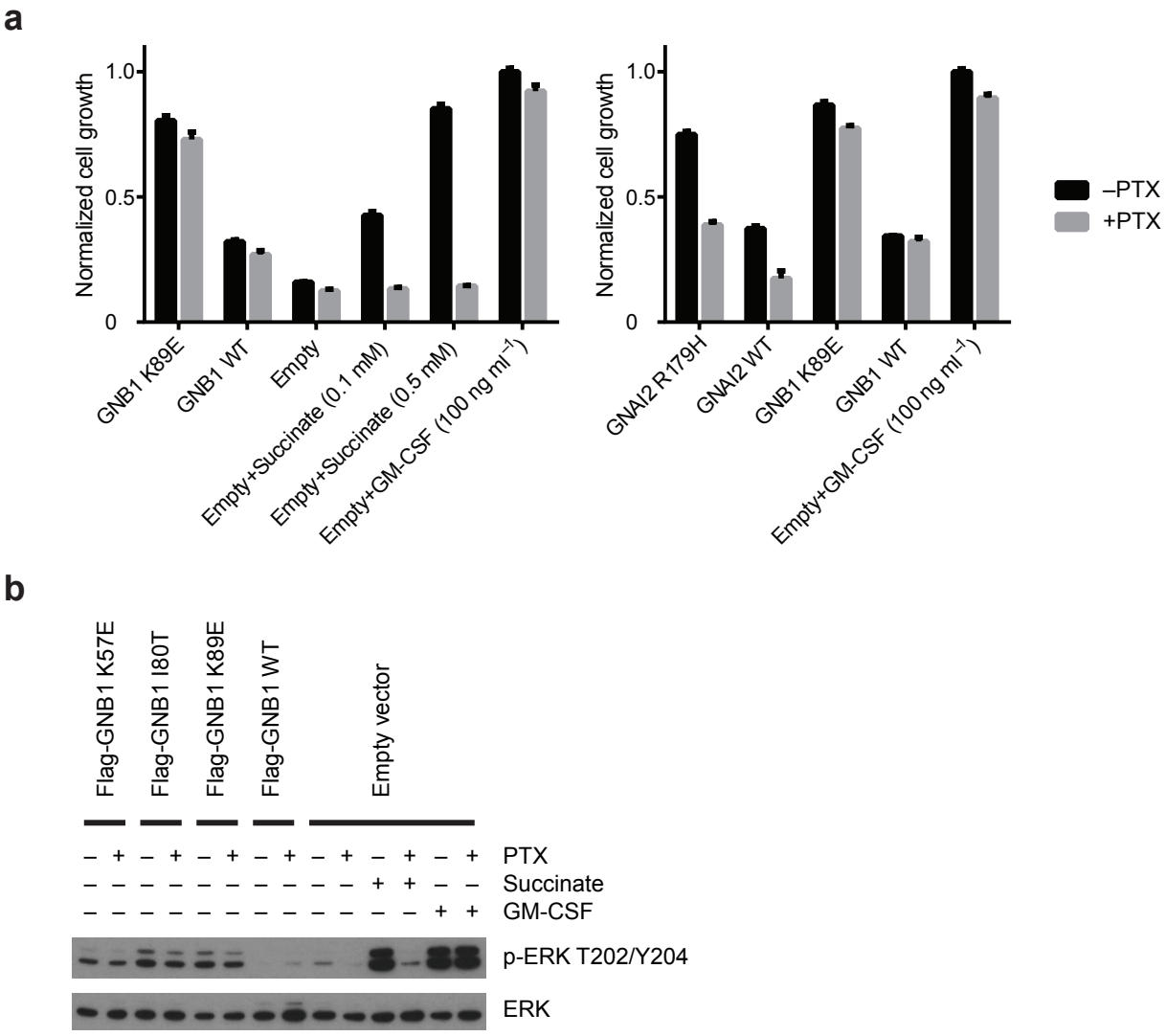
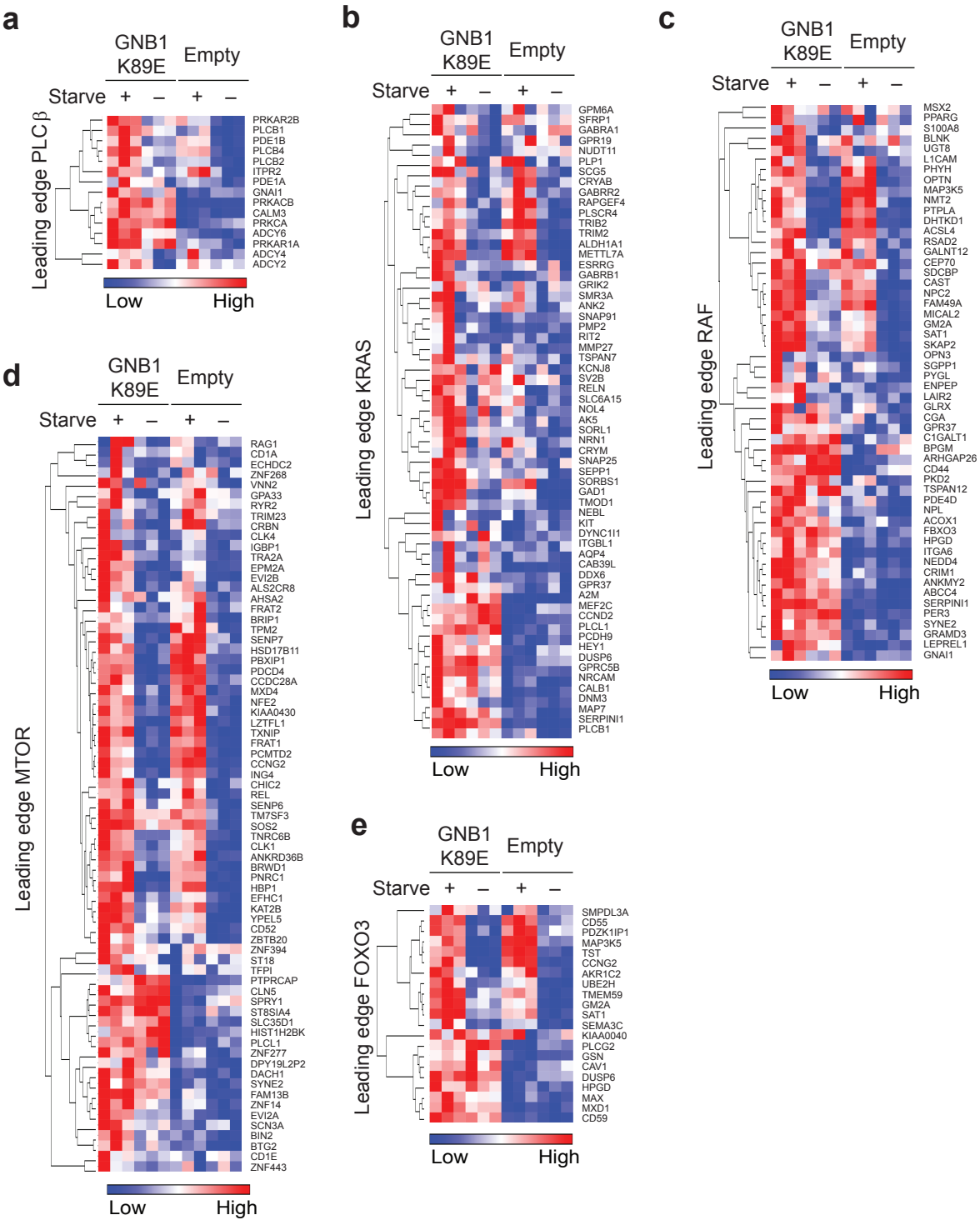
Supplementary Figure 1



Supplementary Figure 1. Pertussis toxin does not inhibit GNB1-mediated cytokine-independent cell growth or ERK activation.

(a) MTT assay on TF-1 cells expressing GNB1 alleles, GNAI2 alleles, or empty vector. Signaling via Gα subunits, such as with succinate stimulation[19] or oncogenic mutant GNA protein expression[20] is known to be inhibited by pertussis toxin (PTX). Cells were cultured with PTX (100 ng ml^{-1}) for 16 hrs and then stimulated by succinate, GM-CSF, or media alone for 2 days. pMSCV-puro (left) or pMSCV-ires-GFP (right) were used as backbone vectors, respectively. Graphs represent mean ± SD of three replicates (b) Western blotting of TF-1 cells expressing GNB1 alleles or empty vector. Cells were cultured with PTX (100 ng ml^{-1}) for 16 hrs and lysed after stimulation by succinate, GM-CSF or media alone for 5 min.

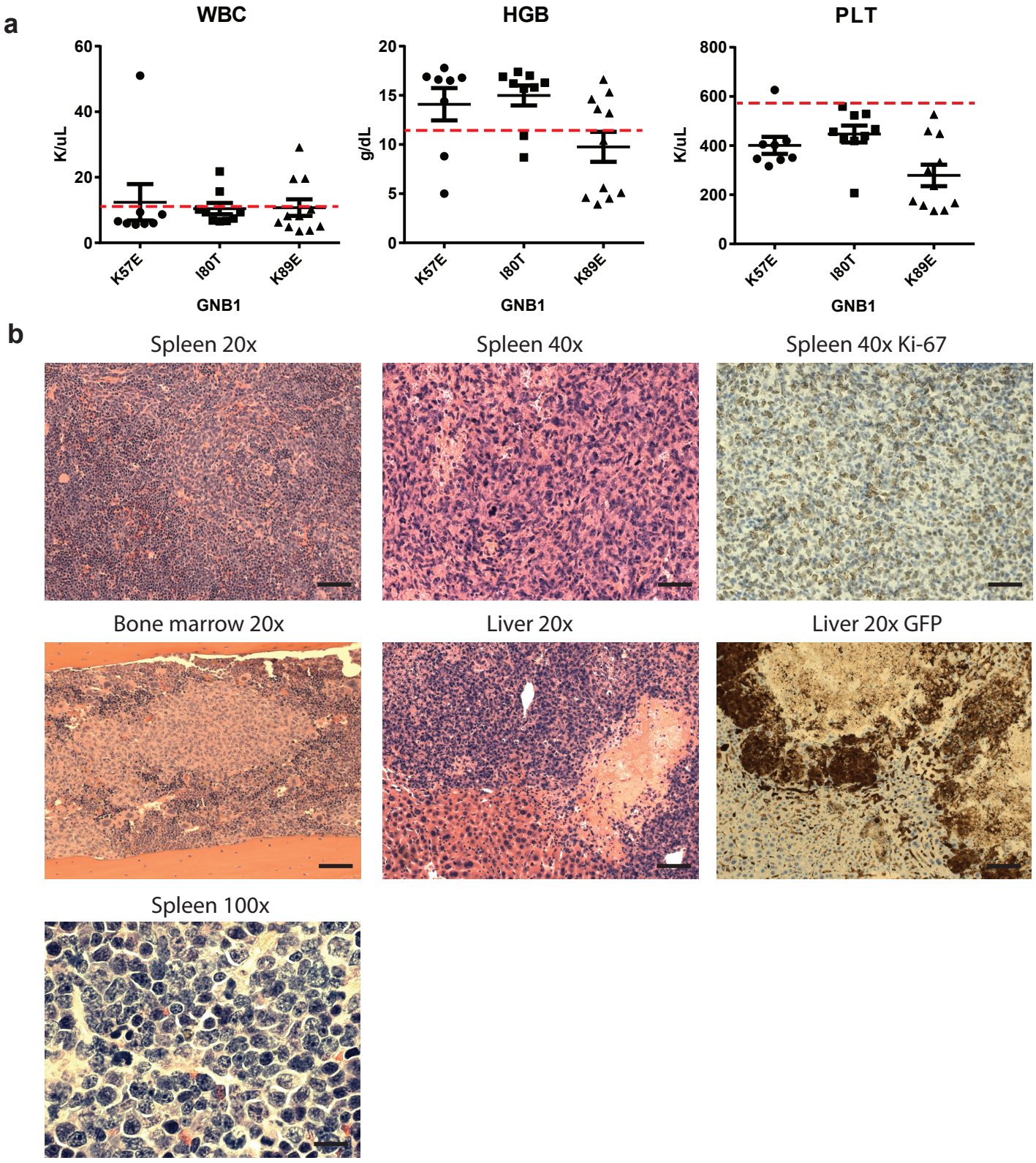
Supplementary Figure 2



Supplementary Figure 2. Leading edge analysis for selected pathways.

Heatmap of leading edge genes from GSEA plots shown in Figure 2 ((a) PLCβ, (b) KRAS, (c) RAF, (d) mTOR and (e) FOXO3 pathway components) in TF-1-GNB1 K89E or TF-1-empty vector cells, with (+) or without (–) 12 hour cytokine starvation.

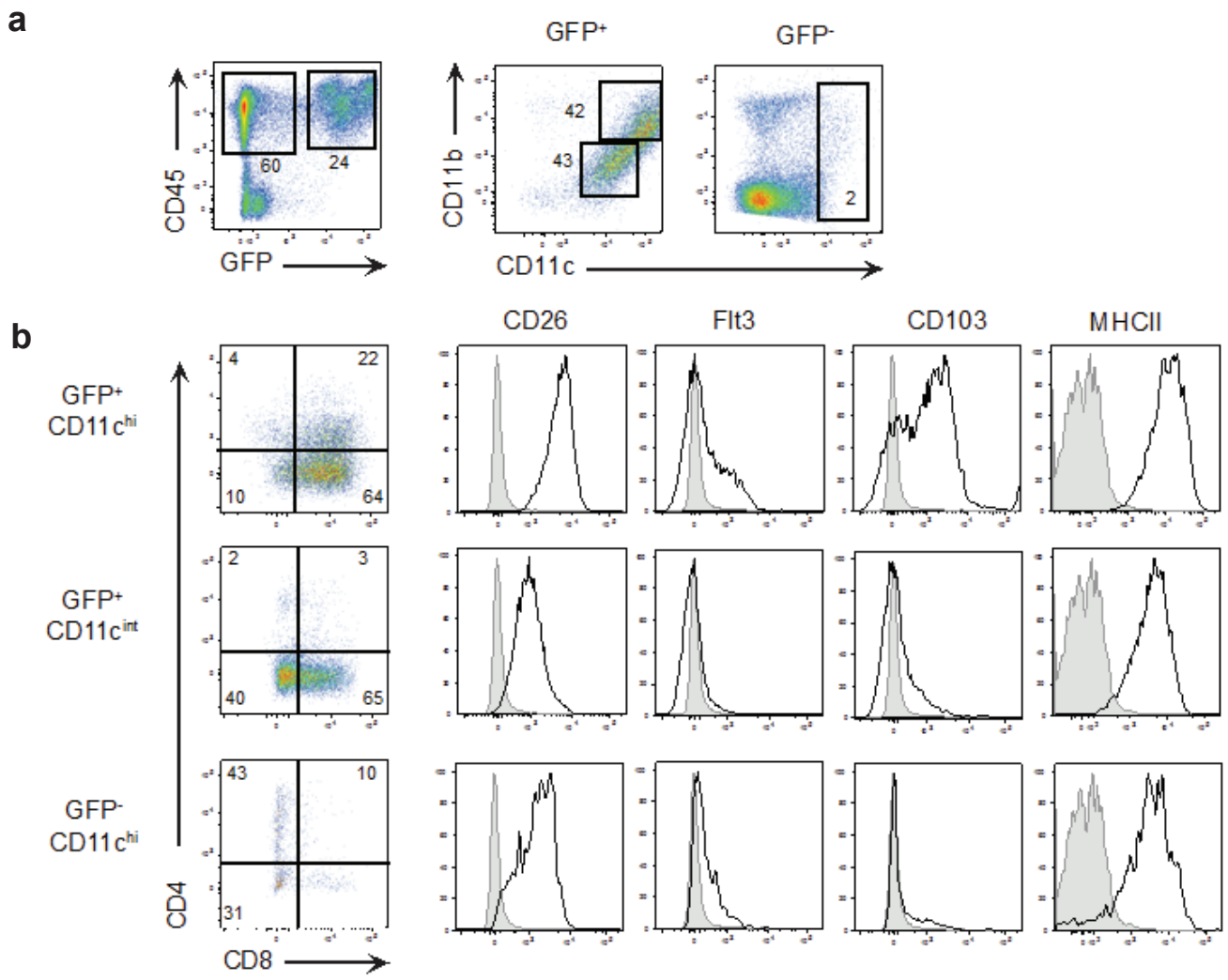
Supplementary Figure 3



Supplementary Figure 3. Complete blood counts and pathology from animals with GNB1-driven myeloid neoplasms.

(a) Peripheral blood white blood cell counts (WBC), hemoglobin (HGB), and platelet counts (PLT) from moribund mice with GNB1-associated myeloid neoplasms. Red dashed lines represent upper limit (WBC) or lower limit (HGB and PLT) of normal mouse blood counts. Horizontal bar represents mean, and error bars SEM. (b) Representative pathology of moribund mice with GNB1-associated neoplasms shows diffuse splenic involvement with high Ki-67 proliferation index, patchy tumor deposit in bone marrow, and infiltration of GFP-expressing tumor cells in liver (scale bar: 20x = 100 μm; 40x = 50 μm; 100x = 20 μm). Images show hematoxylin and eosin staining unless otherwise marked.

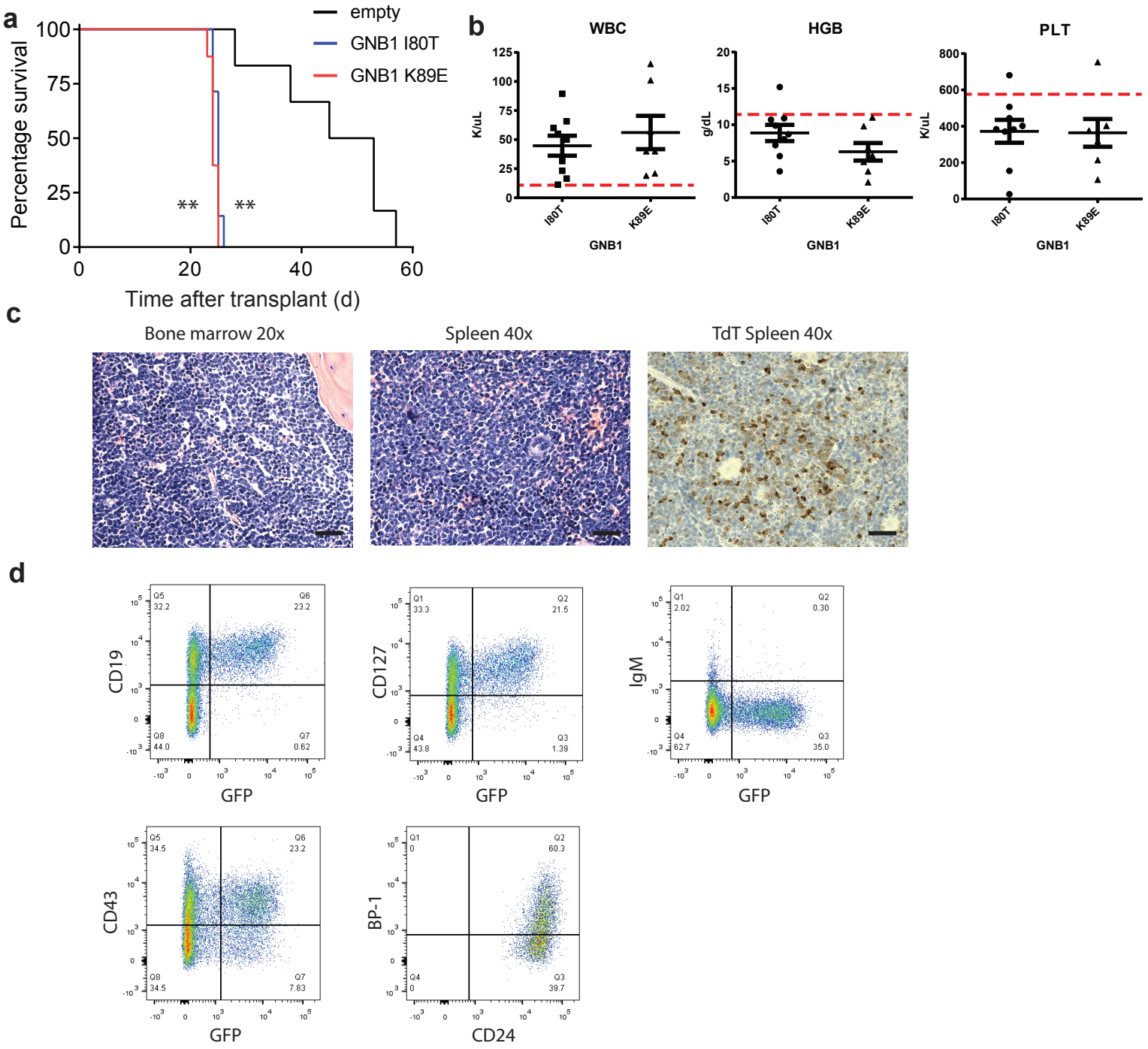
Supplementary Figure 4



Supplementary Figure 4. Flow cytometry analysis of myeloid dendritic cell neoplasms associated with GNB1 mutations.

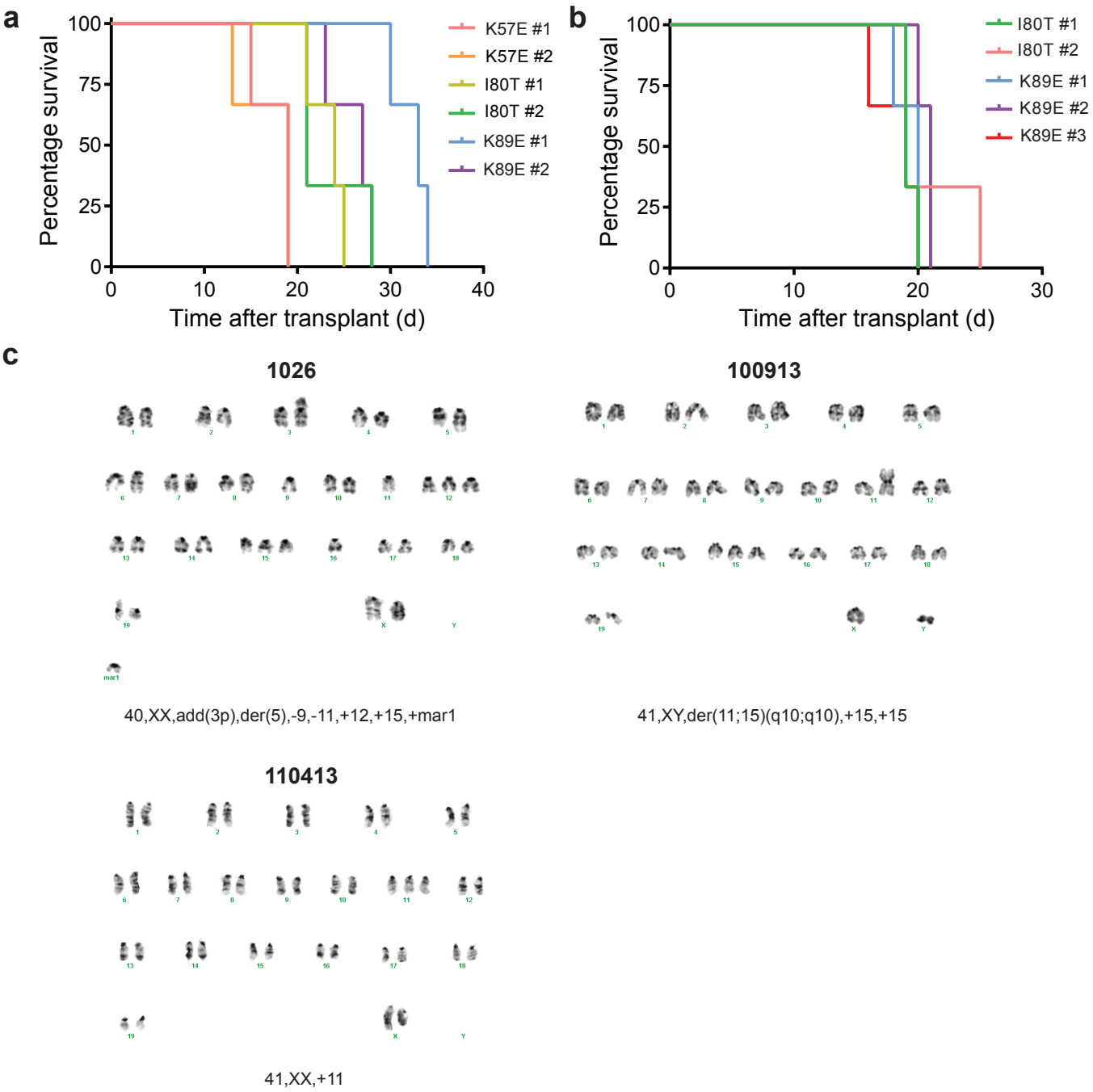
(a, b) Representative flow cytometry of splenocytes from recipients of 5-FU pre-treated *Cdkn2a*$^{-/-}$ bone marrow expressing GNB1 mutants. The tumor cells (CD45+GFP+) have a cell surface phenotype consistent with lymphoid organ resident conventional myeloid dendritic cells (CD11c^{hi}CD8a+CD103+, MHCII+).

Supplementary Figure 5



Supplementary Figure 5. Analysis of lymphoid leukemias associated with GNB1 mutations.
(a) Leukemia-free survival of recipient mice after transplantation of unmanipulated *Cdkn2a*$^{-/-}$ BM transduced with GNB1 mutants or empty vector (n = 8 mice/group; ** p < 0.001 vs empty, curves compared by log-rank test). (b) Peripheral blood white blood cell counts (WBC), hemoglobin (HGB), and platelet counts (PLT) from moribund mice with GNB1-associated lymphoid leukemias. Red dashed lines represent upper limit (WBC) or lower limit (HGB and PLT) of normal mouse blood counts. Horizontal bar represents mean, and error bars SEM. (c) Representative pathology of moribund mice with GNB1-associated lymphoid leukemia shows diffuse bone marrow and splenic involvement with terminal deoxynucleotidyl transferase (TdT) positivity by immunohistochemistry (scale bar: 20x = 100 μm; 40x = 50 μm). Images show hematoxylin and eosin staining unless otherwise marked. (d) Representative flow cytometry of leukemic splenocytes from recipients of non-5FU-pre-treated *Cdkn2a*–/– bone marrow expressing GNB1 mutants. The tumor cells (GFP+) are CD19+CD127+IgM– CD43+CD24+ (with TdT+ IHC) consistent with a pre-B cell acute lymphoblastic leukemia.

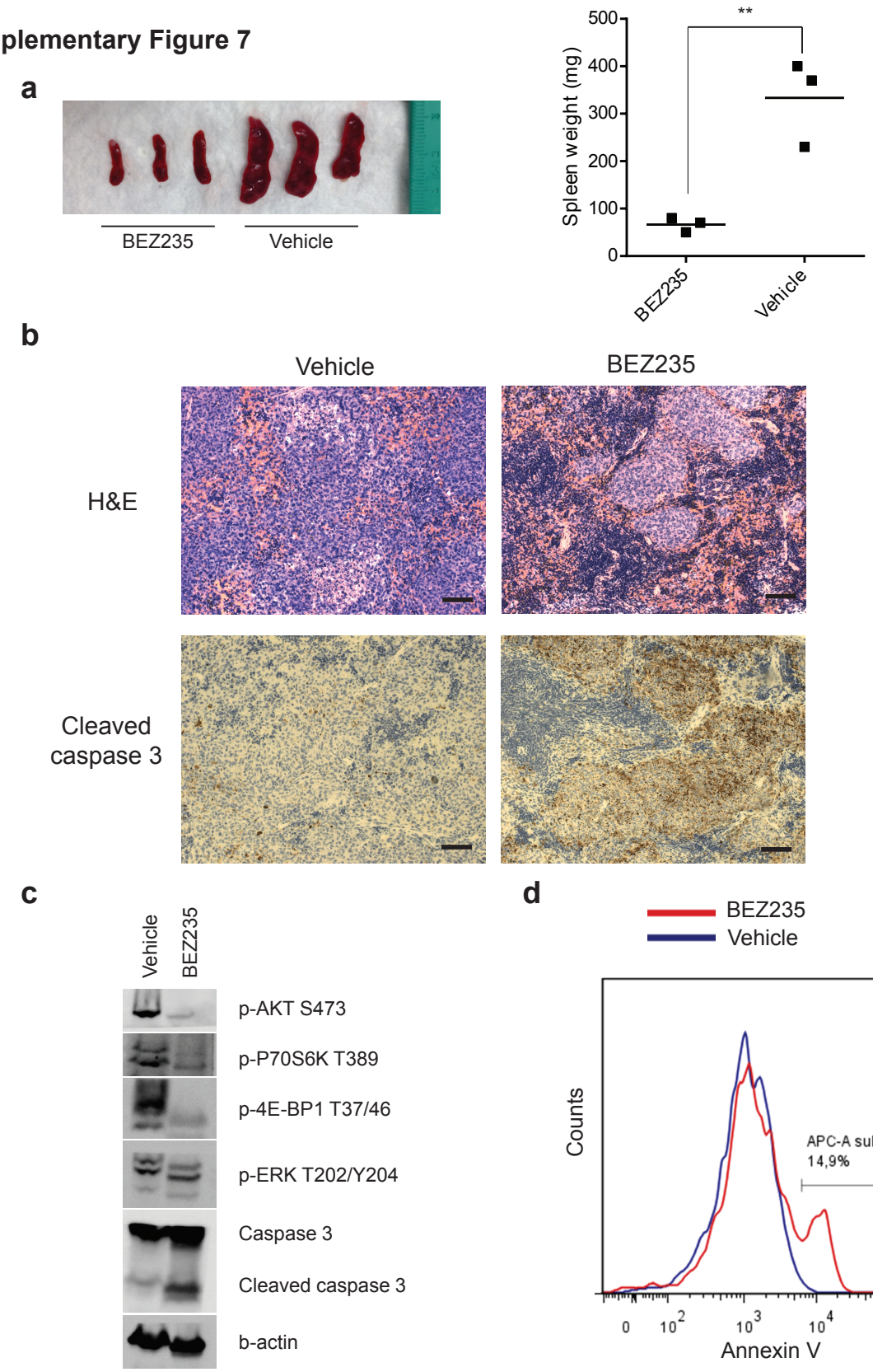
Supplementary Figure 6



Supplementary Figure 6. GNB1-driven myeloid and lymphoid neoplasms are secondarily transplantable and are clonally-derived.

(a) Survival of recipient mice after secondary transplantation of splenocytes from GNB1-associated myeloid neoplasms into sublethally irradiated syngeneic recipients. (b) Survival of recipient mice after secondary transplantation of splenocytes from GNB1-associated lymphoid leukemias into sublethally irradiated syngeneic recipients. (c) Karyotype of GNB1-associated malignancies demonstrates clonal recurrent abnormalities in chromosome number. 1026 and 100913 are myeloid neoplasms and 110413 is a lymphoid leukemia.

Supplementary Figure 7



Supplementary Figure 7. BEZ235 treatment results in decreased tumor burden *in vivo* associated with apoptotic cell death of GNB1-driven myeloid neoplasms.

(a) Spleen size and weight in secondary recipients of GNB1-associated myeloid neoplasms after 2 days of treatment with BEZ235 or vehicle. ** $p < 0.01$ by t-test. (b) Representative pathology of spleens from mice treated with BEZ235 or vehicle. Hematoxylin and eosin (H&E, top) shows shrinkage of infiltrating tumor nodules and reappearance of normal hematopoietic progenitors. Tumor deposits show induction of apoptosis evidenced by an increase in cleaved caspase 3 after BEZ235 treatment. (c) Western blotting of splenocytes after treatment with vehicle or BEZ235. (d) Flow cytometry showing induction of cell surface Annexin V positivity after treatment with BEZ235.