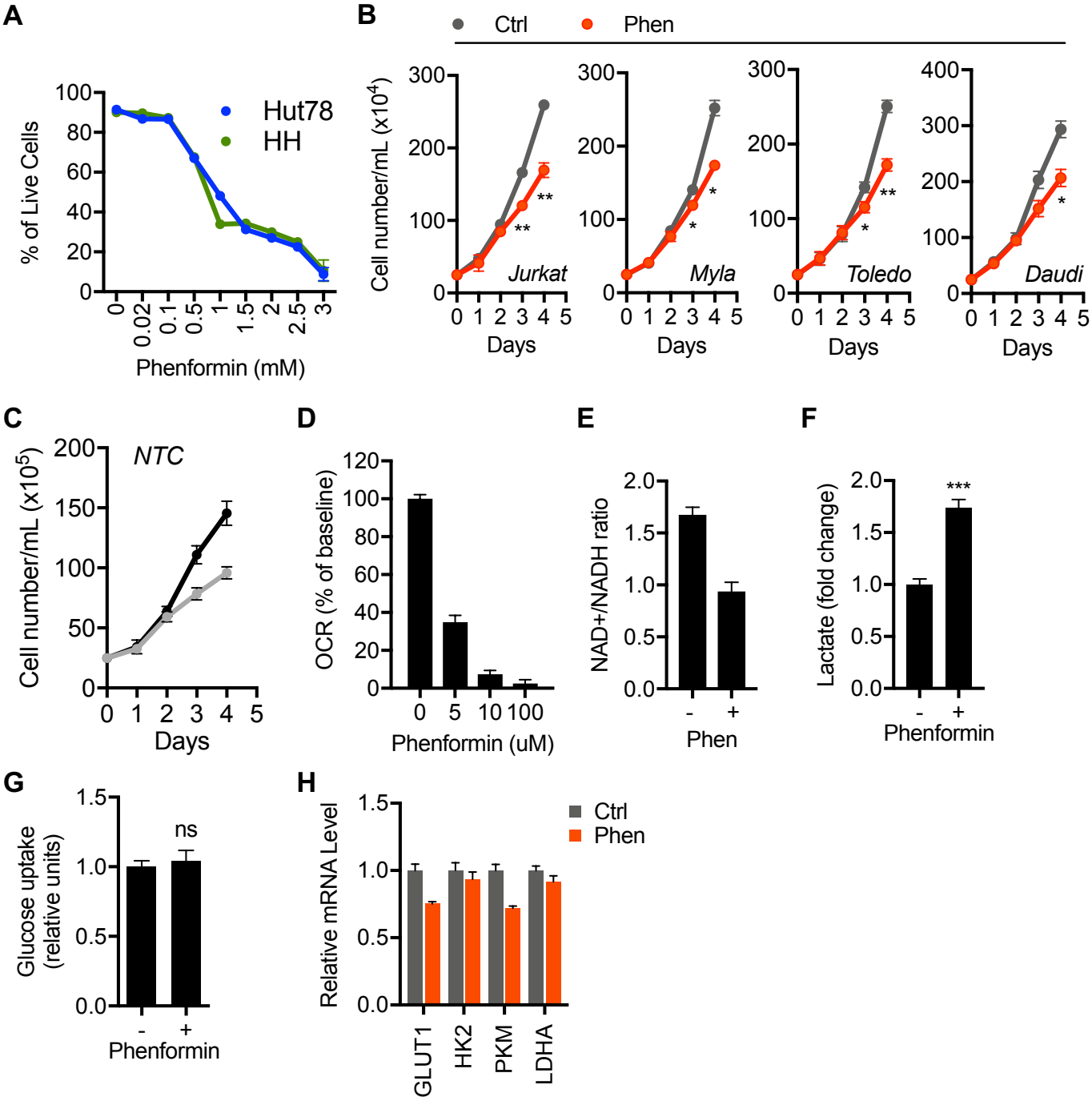

Figure S1. Phenformin reduces growth rate and mitochondrial respiration at non-toxic concentrations. Related to Figure 1.

(A) Viability of indicated cell lines after exposure to indicated concentrations of phenformin for 72 hours. (B) Live cell number over four days of indicated cell lines during treatment with 10 μM phenformin. (C-G) Effect of phenformin (10 μM) on normal CD4+ T-cells (NTCs) from healthy donors with live cell number during treatment with phenformin (C), mitochondrial oxygen consumption rate (D), NAD+/NADH ratio (E), extracellular lactate levels (F), and glucose uptake (G) after treatment with phenformin (10 μM) for 24 hours prior to assay (D-G). (H) Levels of mRNA of glucose transporter 1 (GLUT1) and key glycolytic enzymes, hexokinase 2 (HK2), pyruvate kinase M (PKM), phosphofructokinase (PFK), and lactate dehydrogenase A (LDHA) in NTC after treatment with 10 mM phenformin for 24 hrs. Data presented as mean ± SD of triplicate measurements representative of at least three independent experiments.

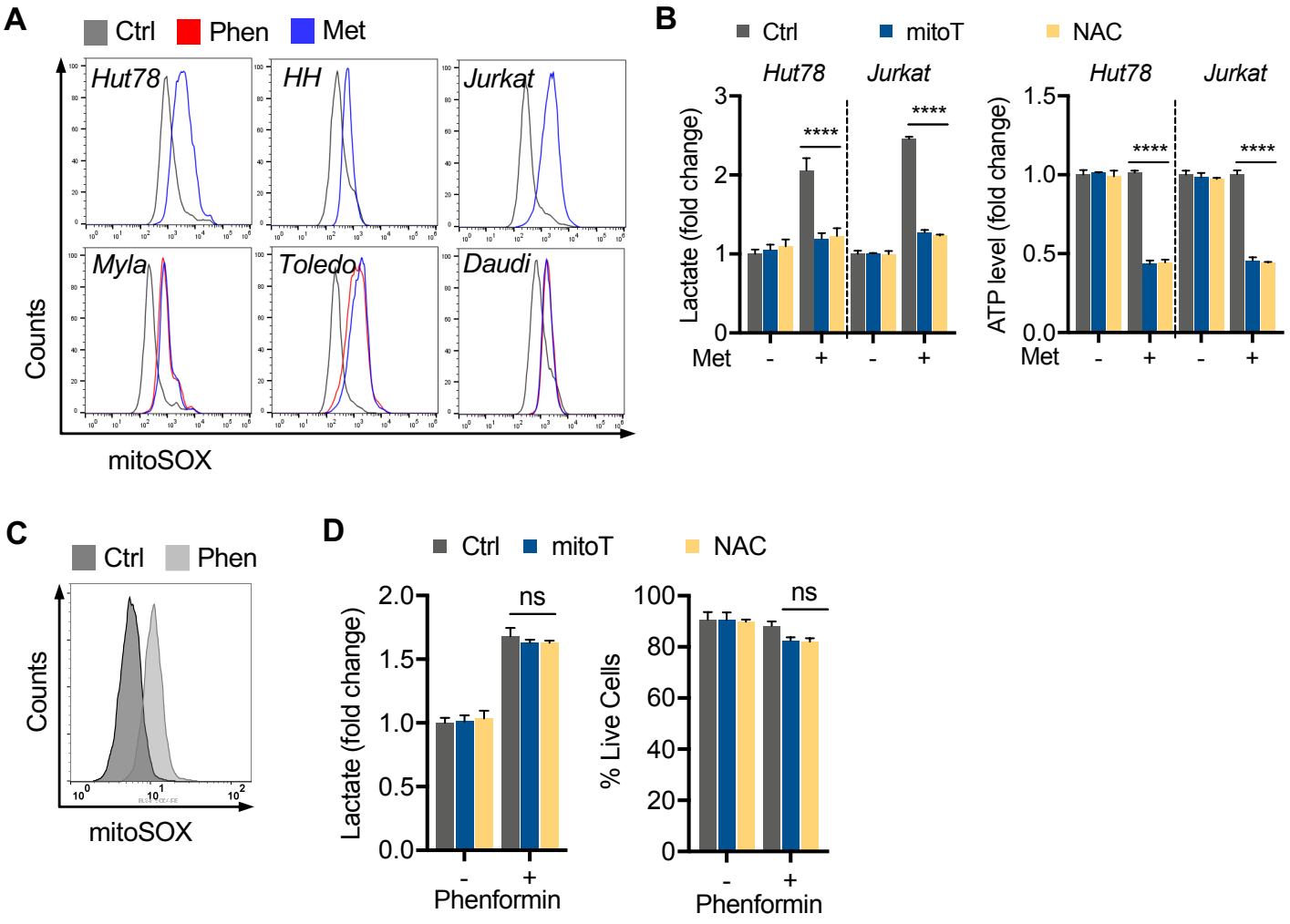

Figure S2. Mitochondrial ROS are required for metabolic adaption during phenformin and metformin treatment. Related to Figure 2.

(A) Mitochondrial ROS levels detected by mitoSOX Red fluorescence using flow cytometry after treatment with phenformin (10 mM) or metformin (5 mM) for 24 hrs (representative histograms). (B) Effect of antioxidants, n-acetylcysteine (NAC; 4 mM) and mitoTEMPO (mitoT; 30 mM), on lactate production and intracellular ATP levels after exposure to metformin for 24 hrs. (C) Mitochondrial ROS levels detected by mitoSOX Red fluorescence using flow cytometry after 24 hours of treatment with phenformin (10 mM) in NTC (representative histograms). (D) Effect of NAC and mitoTEMPO on lactate production and survival of NTC during treatment with phenformin. (B and D) All data points presented as mean ± SD of triplicate measurements.

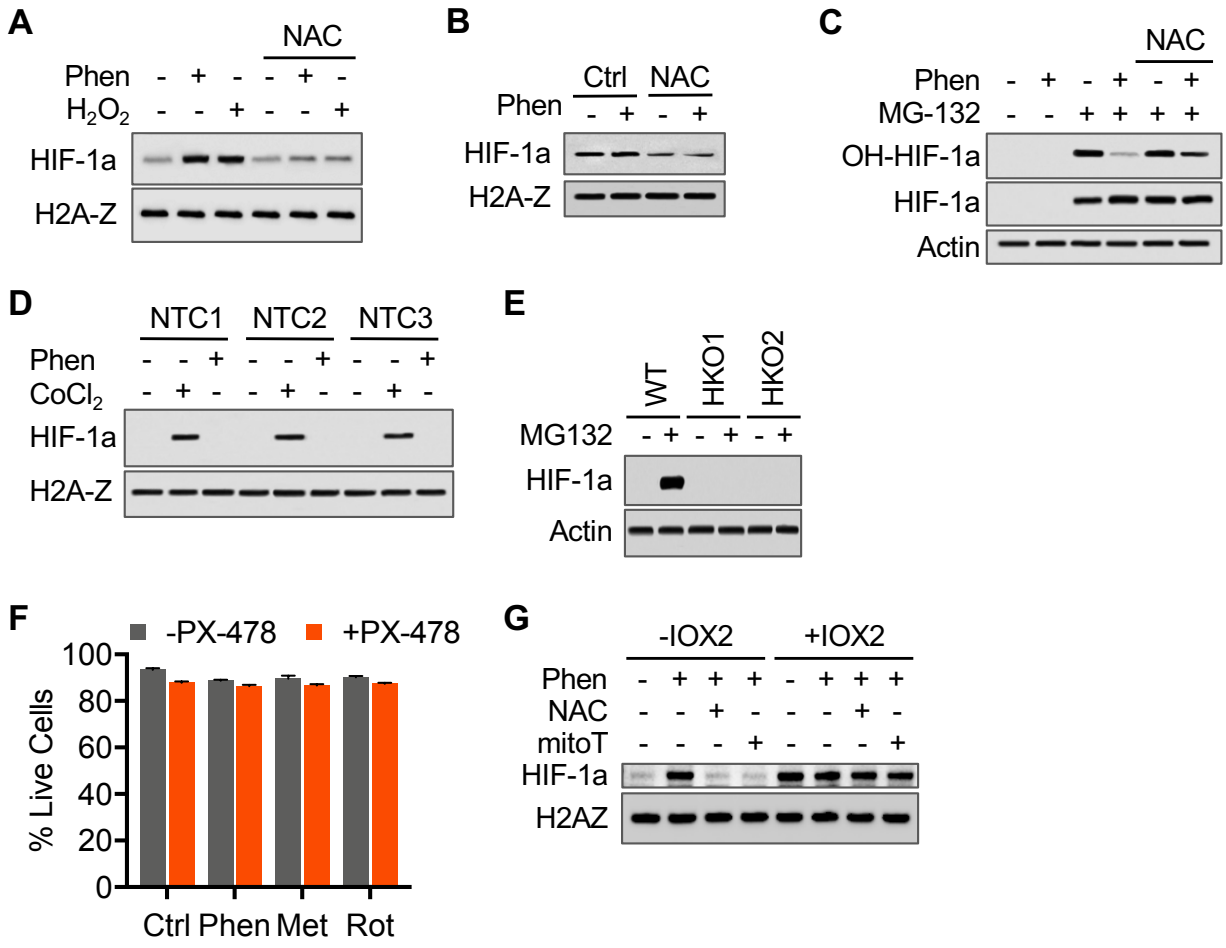

Figure S3. Phenformin modulates HIF-1a levels. Related to Figures 3, 4, and 5.
(A) Immunoblot of nuclear extracts of Hut78 cells showing upregulation of HIF-1a levels in response to treatment with 10 mM phenformin for 24 hrs and 500 mM hydrogen peroxide (H_2O_2) for 1 hr. Antibody against histone H2A-Z was used as a loading control. (B) Immunoblots of tumor lysates showing reduced HIF-1a levels in NAC-treated tumors. (C) Immunoblots of whole cell lysates of Hut78 cells treated as indicated were immunoblotted with antibodies specific to hydroxylated HIF-1a (OH-HIF-1a), total HIF-1a. Proteasome activity was inhibited with MG-132 (25 μM).
(D) Immunoblots of nuclear extracts of isolated normal CD4+ T cells from 3 different donors (NTC1-3) show lack of HIF-1a upregulation after exposure to 10 μM phenformin. $CoCl_2$ treatment was used as a positive control (100 mM for 4 hrs). (E) Immunoblots from two separate CRISPR/Cas9-generated knockout clones (HKO1 and HKO2) show loss of HIF-1a expression. Proteasome activity was inhibited with MG-132. β-Actin was used as a loading control. (F) Cell viability of isolated normal $CD4^+$ T cells treated with 10 μM phenformin, 5 mM metformin, 1 μM rotenone for 72 hrs. Data presented as mean ± SD of triplicate measurements. (G) Immunoblot of nuclear extracts of Hut78 cells treated as indicated showing constitutive activation of HIF-1a by PHD inhibitor, IOX2 (50 μM).

Diagnosis	Age/Sex	WBC (1Kcells/uL)	Neutrophils	Lymphocytes	Monocyte	Eosinophils	Basophils	Leukemic burden
B-Lymphoblastic Leukemia (ALL1)	63/F	5.7	2%	28%	0%	1%	0%	69%
B-Lymphoblastic Leukemia (ALL2)	52/M	23.9	8%	18%	1%	0%	0%	71%
T-Lymphoblastic Leukemia (ALL3)	17/M	933.9	1%	3%	1%	0%	0%	95%
T-Lymphoblastic Leukemia (ALL4)	24/M	103.3	13%	5%	4%	0%	0%	76%
Chronic Lymphocytic Leukemia (CLL1)	49/M	256.6	3%	96%	1%	0%	0%	95%
Chronic Lymphocytic Leukemia (CLL2)	62/F	11.6	25%	72%	3%	0%	0%	60%
Chronic Lymphocytic Leukemia (CLL3)	64/M	18.2	31%	62%	4%	3%	0%	86%
Chronic Lymphocytic Leukemia (CLL4)	72/M	10.8	23%	74%	2%	0%	1%	97%

Supplemental Table S1. Characteristics of leukemic samples. Related to Figure 6.
All acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL) samples were collected from different patients with age and sex as indicated. Leukemic burden indicated as blasts as % of white blood cells (WBC) for ALL and as leukemic cells as % of lymphocytes for CLL. For standard immunophenotypic definition see STAR methods.