Supplemental Figure Legend

Table S1 related to Table 1. Treatment information for relapsed AML patients that received venetoclax with azacitidine treatment.

Trial Patient	Therapy Prior to Ven/aza
Patient 1	Aza, cord blood BMT
Patient 2	7+3, decitabine, sorafenib
Patient 3	Aza, cord blood BMT
Patient 4	7+3, GCLAC
Patient 5	enasidenib, decitabine, AG881
Patient 6	7+3, 5+2
Patient 7	Aza + sorafenib
Patient 8	7+3, GLCAC, matched unrelated donor BMT, CLAG-M, Aza, decitabine
Patient 9	7+3, matched sibling BMT, 7+3 + midostaurin
Patient 10	7+3
Patient 11	7+3, GCLAC, haplo cord BMT
Patient 12	Aza + entospletinib

Treatment received prior to ven/aza listed in the order therapies were administered. 7+3 is cytarabine 7 days and idarubicin 3 days, 5+2 is cytarabine 5 days and idarubicin 2 days, enasidenib is a IDH2 inhibitor, Sorafenib is a protein kinase inhibitor, decitabine is a hypomethylating agent similar to azacitidine, BMT is bone marrow transplant, AG881 is a pan IDH inhibitor, entospletinib is a Syk inhibitor, midostaurin is a FLT3 inhibitor, GCLAC is granulocyte colony-stimulating factor [filgrastim], clofarabine, high-dose cytarabine, CLAG-M is cladribine, cytarabine, filgrastim and mitoxantrone.

Table S2 related to Table 1. Univariate logistic regression results comparing untreated to relapsed/refractory patients

Predictor	Odds Ratio (95% CI)	p-value
Age	0.90 (0.83, 0.97)	0.0075
Antecedent hematologic disorder	2.20 (0.59, 8.24)	0.2409
Treatment-related AML	1.76 (0.30, 10.41)	0.5331
Cytogenetic risk group	0.45 (0.11, 1.82)	0.0747
TP53 mutation	1.10 (0.11, 10.93)	0.6286

FLT3 ITD mutation	4.10 (0.92, 18.29)	0.9756
ASXL1 mutation presence (ref = no mutation)	1.43 (0.32, 6.38)	0.6663
European Leukemia Net Risk Group	4.71 (0.55, 40.71)	0.9458

Supplemental Figure 1 related to Figure 1: R/R and de novo LSCs are metabolically distinct

A. Graphs of metabolites that are significantly increased in R/R LSCs compared to de novo LSCs. AML specimens used in this analysis include AML1, 2, 3, 4, 5, 6, 7, 8, 9, 14, 15 and 16. Significance was determined using a student's t-test. **B.** Western blot for NAMPT and GAPDH in LSCs isolated from de novo and R/R AML specimens treated with vehicle control or 5μM AraC (cytarabine) for 4 hours. Cells were untreated or treated with AraC to determine if NAMPT expression was altered by chemotherapy treatment. **C.** Levels of NAMPT cofactor Phosphoribosyl diphosphate (PRPP) in de novo and relapsed AML specimens. AML specimens used in this analysis were AML 1, 2, 3, 5, 8, and 9. Significance determined by student's t-test. **D.** NAD+/H levels upon in vitro or in vivo chemotherapy treatment. **E.** Levels of stable isotope labelled tryptophan in de novo and R/R LSCs 12 hours post [$^{13}C_{11}$,$^{15}N_2$]tryptophan incubation. Statistical significance was determined using an unpaired student's t-test. **F.** Levels of stable isotope labelled formyl-kynuerine in de novo and R/R LSCs 12 hours post [$^{13}C_{11}$,$^{15}N_2$] tryptophan incubation. Statistical significance was determined using an unpaired student's t-test. **G.** Levels of parental NAD+ (^{13}C labelled not detected) in de novo and R/R LSCs 12 hours post [$^{13}C_{11}$,$^{15}N_2$] tryptophan incubation. **H.** Levels of NAD+ one-hour post treatment with increasing concentrations of tryptophan. Statistical significance was determined using an unpaired student's t-test. *p<0.05, **p<0.01, ****p<0.001

Supplemental Figure 2 related to Figure 2: Differences in metabolism between de novo and R/R LSCs.

A. Metabolites from $^{13}C^{15}N$ amino acids. Statistical significance determined by a two-way anova analysis. **B.** Metabolites from [$^{13}C_{16}$]palmitic acid. Statistical significance determined by a two-way anova analysis. *p<0.05, **p<0.01, ****p<0.001

Supplemental Figure 3 related to Figure 3: Other metabolic changes upon nicotinamide pretreatment.

Metabolites from $^{13}C_{16}$ palmitic acid (A), or $^{13}C_6$ glucose (B). Statistical significance was determined using an unpair student's t-test. **C.** Viability of LSCs upon ven, aza, or ven/aza treatment with or without a preincubation with nicotinamide. Statistical significance determined by two-way Anova analysis. **D.** Viability of LSCs upon cytarabine treatment with or without a preincubation with nicotinamide. Statistical significance determined by two-way Anova analysis. **E.** Viability of LSCs upon doxorubicin treatment with or without a preincubation with nicotinamide. Statistical significance determined by two-way Anova analysis. *p<0.05, **p<0.01, ***p<0.005, ****p<0.001.

Supplemental Figure 4 related to Figure 4: Inhibition of nicotinamide metabolism in LSCs. **A.** NAD+/H levels in de novo and R/R LSCs 4 hours post 10nM APO866 treatment. Each dot represents an individual patient specimen normalized to vehicle control. Statistical significance was determined by a two-way anova analysis. **B.** Viability of de novo and R/R LSCs treated with increasing concentration of APO866 or KPT-9472 for 24 hours. **C.** Colony-forming ability of normal hematopoietic cells upon treatment with increasing concentration of APO866 or KPT-9472. **D.** Colony-forming ability of normal bone marrow upon transfection with control scrambled siRNA or NAMPT targeting siRNA. **E.** Viability of ROS-low cells and ROS-high AML blasts upon APO866 or KPT-9274 treatment. Statistical significance determined by Anova analysis. **F.** RT-PCR for NAMPT expression upon NAMPT knockdown. AML specimens 5, 8, and 9 were used for this analysis. **G.** Viability of ROS-high AML blasts isolated from paired de novo relapse AML specimens upon ven/aza, APO866, KPT-9274, or cytarabine treatment for 24 hours. Statistical significance determined by two-way Anova analysis. *p<0.05, **p<0.01, ***p<0.005, ****p<0.001.

Supplemental Figure 5 related to Figure 5: Combination of ven/aza with NAMPT inhibition **A.** Leukemic burden in de novo AML specimen treated with APO866 (20mg/kg) or chemotherapy (50mg/kg cytarabine for 5 days with 1.5mg/kg doxorubicin for 2 days). **B.** Human monocytes (CD45+/CD33+) in mouse femur after 2 weeks of APO866 or KPT-9274 treatment. Each dot represents an individual mouse. Statistical significance was determined using an unpair student's t-test. **C.** Human lymphocytes (CD45+/CD19+) in mouse femur after 2 weeks of APO866 or KPT-9274 treatment. Each dot represents an individual mouse. Statistical significance was determined using an unpair student's t-test. **D.** Ratio of CD33+/CD19+ cells in mouse femur after 2 weeks of APO866 or KPT-9274 treatment. Each dot represents an individual mouse. Statistical significance was determined using an unpair student's t-test. **E.** Viability of LSCs upon treatment with ven/aza, APO866, KPT-9274 or the combination for 24 hours. ***p<0.005, ****p<0.001.

Supplemental Figure 6 related to Figure 6: Metabolic changes upon nicotinamide metabolism inhibition.

A. Glycolysis levels determined by seahorse assay in de novo and R/R LSCs after a 4 hour treatment with 10nM APO866. Each dot represents an individual patient specimen normalized to vehicle control. Statistical significance was determined using an unpair student's t-test. **B.** Glycolysis levels determined by seahorse assay in de novo and R/R LSCs after a 4 hour treatment with 100nM KPT-9274. Each dot represents an individual patient specimen normalized to vehicle control. Statistical significance was determined using an unpair student's t-test. **C.** Glycolysis levels determined by seahorse assay in leukemic cell isolated from mice treated with APO866 or KPT-9274 for 24 hours. Statistical significance was determined by Anova analysis. **D.** OXPHOS and glycolysis levels upon APO866 or KPT-9274 treatment in ROS-high AML blasts isolated from primary AML specimens. AML specimens used in this analysis include AML 5, 8, and 9. Statistical significance was determined using an Anova analysis. **E.** OXPHOS and glycolysis levels upon NAMPT knockdown in AML specimens. Statistical significance was determined using student's t-test. **F.** Abundance of metabolites in de novo(red/pink) and R/R (blue/light blue) LSCs treated with vehicle control or APO866 for 4 hours determined by mass spectrometry. Each dot represents an individual patient. Statistical significance was determined by a two-way anova analysis. **G.** Activity of 2-oxoglutarate dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase, or hexokinase in de novo LSCs upon treatment with vehicle control or 10nM APO866 for 4 hours. Each dot represents an individual patient specimen. Statistical significance was determined using an unpair student's t-test. *p<0.05, **p<0.01, ***p<0.005,****p<0.001

Supplemental Figure 7 related to Figure 7: TCA Cycle and glycolytic intermediates upon APO866 treatment

A. Metabolites from $^{13}C^{15}N$ amino acids upon vehicle control or 10nM APO866 treatment for 4 hours. Statistical significance determined by an unpaired student's t-test. **B.** Metabolites from $^{13}C_{16}$ palmitic acid upon vehicle control or 10nM APO866 treatment for 4 hours. Statistical significance determined by an unpaired student's t-test. **C.** $^{13}C_6$ glucose upon vehicle control or 10nM APO866 treatment for 4 hours. Statistical significance determined by an unpaired student's t-test. **D.** Metabolites from $^{13}C_6$ glucose upon vehicle control or 10nM APO866 treatment for 4 hours. Statistical significance determined by an unpaired student's t-test. **E.** RT-PCR for HADH upon HADH knockdown. AML specimens 5, 8, and 9 were used for this analysis.

Supplemental Table 3 related to methods section patient specimens: Patient specimen characteristics

Patient Sample	Diagnosis	Age/Sex	Cytogenetics	Mutations
AML1	De Novo	52/M	45,XY,-7[3]/46,sl,+r(7)(p11q21)[11]/46,sdl1,der(5)t(1;5)(q31;p14)[5]/46,XY[1]	ASXL1, DNMT3a, NOTCH, NRAS
AML2	De Novo	49/F	Normal karyotype (46, XX)	FLT3 ITD+; WT for CEBPA, NPM1, IDH1, IDH2, JAK2
AML3	De Novo	51/M	46,XY,add(1)(p11),del(5)(q15q33),del(7)(q22q36),der(11)t(1;11)(p31;p12-14)[20] , Loss of 5q31 and 7q31	FLT3 ITD, BCOR, NOTCH1
AML4	Relapse	21/M	inv(16) (p13.1q22)	NA
AML5	Refractory	49/F	46,XX[21]	FLT3 ITD+
AML6	Refractory	47/M	46,XY,del(7)(q21)[8]/46,sl,del(5)(q31q35),add(12)(p13)[7]/46,sl,add(12)(p13),del(17)(q21)[3]/46,XY,del(9)(q22q32)[2]	IDH1 R132; CKIT D816V; WT for IDH2, FLT3 and NPM1
AML7	Relapse	NA	NA	FLT3 ITD, NPM1
AML8	Relapse	79/M	46,XY,t(6;9) (p21;q34)	FLT3 ITD, FLT3 TKD, IDH2
AML9	Relapse	69/F	46,XX,add(14)(q22)[4]	FLT3, IDH1, NPM1
AML10	De Novo	77/F	NA	NPM1, RUNX1, TET2
AML11	De Novo	NA	Karyotype 45,X,-Y; t(9;11)(p22;q23)	Mutant for FLT3
AML12	De Novo	NA	Normal karyotype (46, XX)	NPM1 mutant

AML13	Relapse	23/F	Normal karyotype	NA
AML14	De novo AML	66/F	46,XX,1~26dmin[11]/46,sl,add(3)(q2?6),add(8)(q24),4~70dmin[4]/46,sdl1,add(5)(q31),del(14)(q32)[2]/46,sdl2,-del(14)(q32),+14,add(15)(q11.2)[2]/92,slx2[2]	TET2, ASXL1
AML15	De novo AML	56/F	46,XX[22] Normal	FLT3 ITD,DNMT3A, WT1, GATA2
AML16	De novo AML	63/F	46,XX[20] Normal	RUNX1, SF3B1, U2AF1
AML17	Paired	35/M	46,XY,inv(16)(p13.1q22)[20]	TET2 V218M
AML18	Paired	58/M	46,XY[20] Normal Male	IDH2 R140
AML19	Paired	59/M	46,XY[20] Normal Male	KIT D816V
AML20	Paired	70/M	46,XY,del(3)(q21q25)[2]/46,XY[19]	NA
AML21	Paired	63/M	46,XY[20] Normal Male	NPM1

Table S4 related to methods section patient specimens. Treatment information for relapsed AML patients.

Patient Sample	Treatment prior to specimen collection	Response
AML4	7+3	CR
AML5	7+3	refractory
AML6	7+3	refractory
AML7	na	na
AML8	Aza, Ven	CR, refractory
AML9	7+3	CR
AML13	na	na
AML17	7+3	CR
AML18	7+3 and then 5+2	CR-i

AML19	7+3	CR
AML20	7+3	CR
AML21	7+3	CR

7+3 is cytarabine 7 days and idarubicin 3 days, 5+2 is cytarabine 5 days and idarubicin 2 days

Figure S1

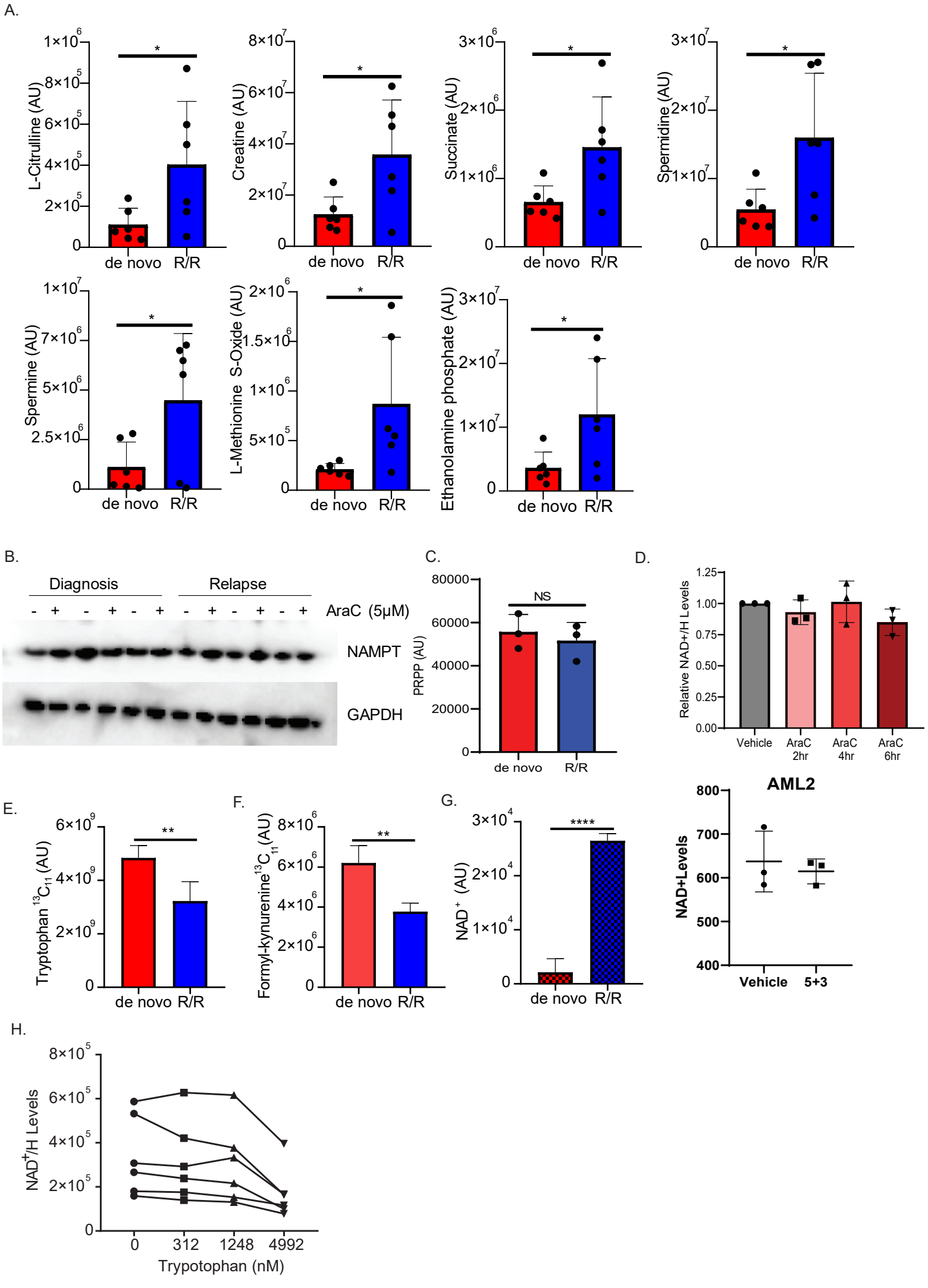

A.
L-Citrulline (AU)
Creatine (AU)
Succinate (AU)
Spermidine (AU)
Spermine (AU)
L-Methionine S-Oxide (AU)
Ethanolamine phosphate (AU)
de novo
R/R
B.
Diagnosis
Relapse
AraC (5μM)
NAMPT
GAPDH
C.
PRPP (AU)
NS
D.
Relative NAD+/H Levels
Vehicle
AraC 2hr
AraC 4hr
AraC 6hr
E.
Tryptophan $^{13}C_{11}$ (AU)
F.
Formyl-kynurenine $^{13}C_{11}$ (AU)
G.
NAD$^+$ (AU)
AML2
NAD+Levels
Vehicle
5+3
H.
NAD$^+$/H Levels
Trypotophan (nM)

Figure S2

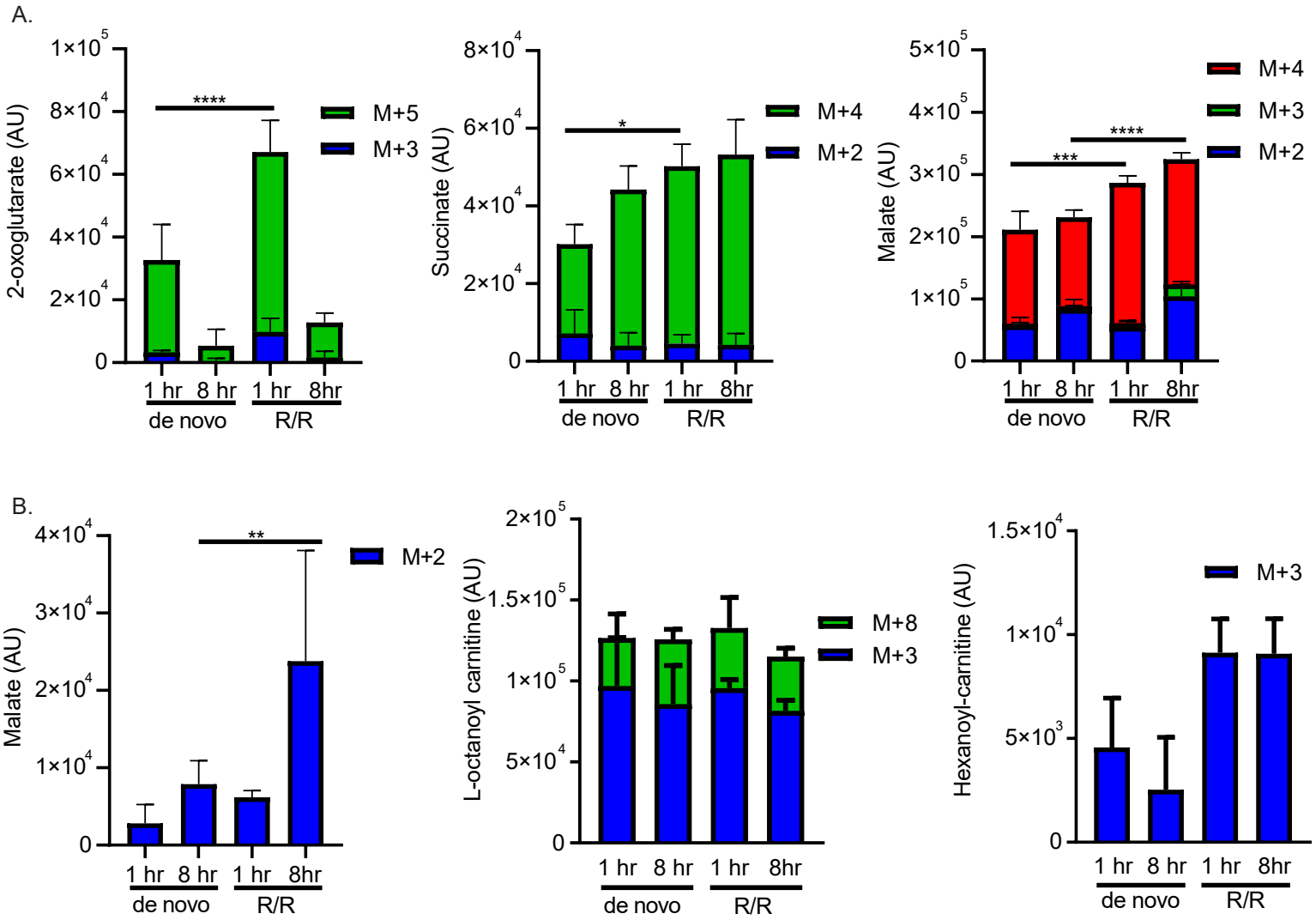

A.
2-oxoglutarate (AU)
1×10^5
8×10^4
6×10^4
4×10^4
2×10^4
0
****
M+5
M+3
1 hr 8 hr 1 hr 8hr
de novo
R/R
Succinate (AU)
8×10^4
6×10^4
4×10^4
2×10^4
0
*
M+4
M+2
1 hr 8 hr 1 hr 8hr
de novo
R/R
Malate (AU)
5×10^5
4×10^5
3×10^5
2×10^5
1×10^5
0
***
****
M+4
M+3
M+2
1 hr 8 hr 1 hr 8hr
de novo
R/R
B.
Malate (AU)
4×10^4
3×10^4
2×10^4
1×10^4
0
**
M+2
1 hr 8 hr 1 hr 8hr
de novo
R/R
L-octanoyl carnitine (AU)
2×10^5
1.5×10^5
1×10^5
5×10^4
0
M+8
M+3
1 hr 8 hr 1 hr 8hr
de novo
R/R
Hexanoyl-carnitine (AU)
1.5×10^4
1×10^4
5×10^3
0
M+3
1 hr 8 hr 1 hr 8hr
de novo
R/R

Figure S3

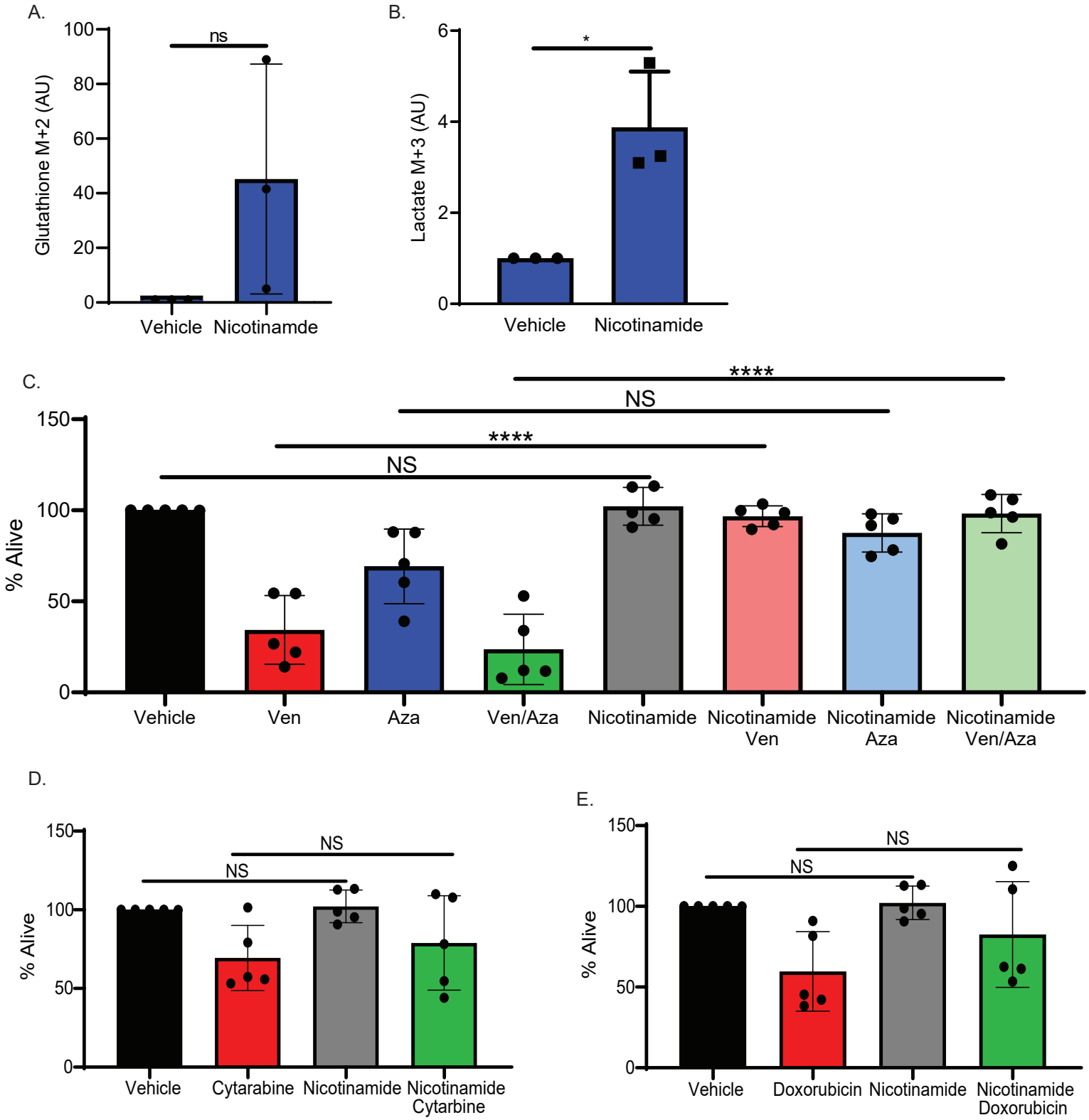

A.
Glutathione M+2 (AU)
100
80
60
40
20
0
ns
Vehicle
Nicotinamde
B.
Lactate M+3 (AU)
6
4
2
0
*
Vehicle
Nicotinamide
C.
% Alive
150
100
50
0
****
NS
****
NS
Vehicle
Ven
Aza
Ven/Aza
Nicotinamide
Nicotinamide Ven
Nicotinamide Aza
Nicotinamide Ven/Aza
D.
% Alive
150
100
50
0
NS
NS
Vehicle
Cytarabine
Nicotinamide
Nicotinamide Cytarbine
E.
% Alive
150
100
50
0
NS
NS
Vehicle
Doxorubicin
Nicotinamide
Nicotinamide Doxorubicin

Figure S4

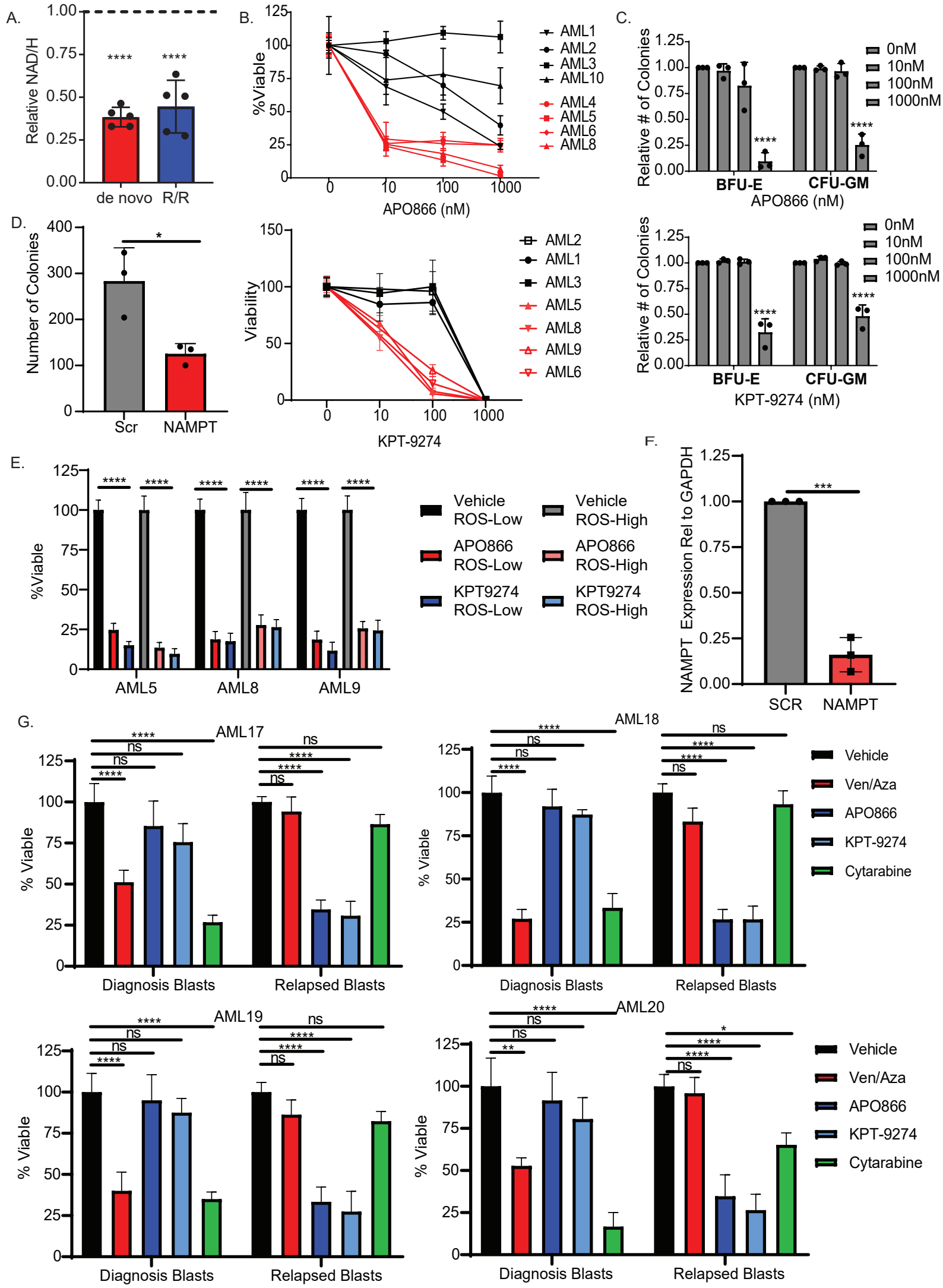

A.
Relative NAD/H
de novo
R/R
B.
%Viable
APO866 (nM)
AML1
AML2
AML3
AML10
AML4
AML5
AML6
AML8
C.
Relative # of Colonies
BFU-E
CFU-GM
APO866 (nM)
0nM
10nM
100nM
1000nM
D.
Number of Colonies
Scr
NAMPT
Viability
KPT-9274
AML2
AML1
AML3
AML5
AML8
AML9
AML6
Relative # of Colonies
KPT-9274 (nM)
E.
%Viable
AML5
AML8
AML9
Vehicle ROS-Low
Vehicle ROS-High
APO866 ROS-Low
APO866 ROS-High
KPT9274 ROS-Low
KPT9274 ROS-High
F.
NAMPT Expression Rel to GAPDH
SCR
NAMPT
G.
AML17
AML18
AML19
AML20
% Viable
Diagnosis Blasts
Relapsed Blasts
Vehicle
Ven/Aza
APO866
KPT-9274
Cytarabine

Figure S5

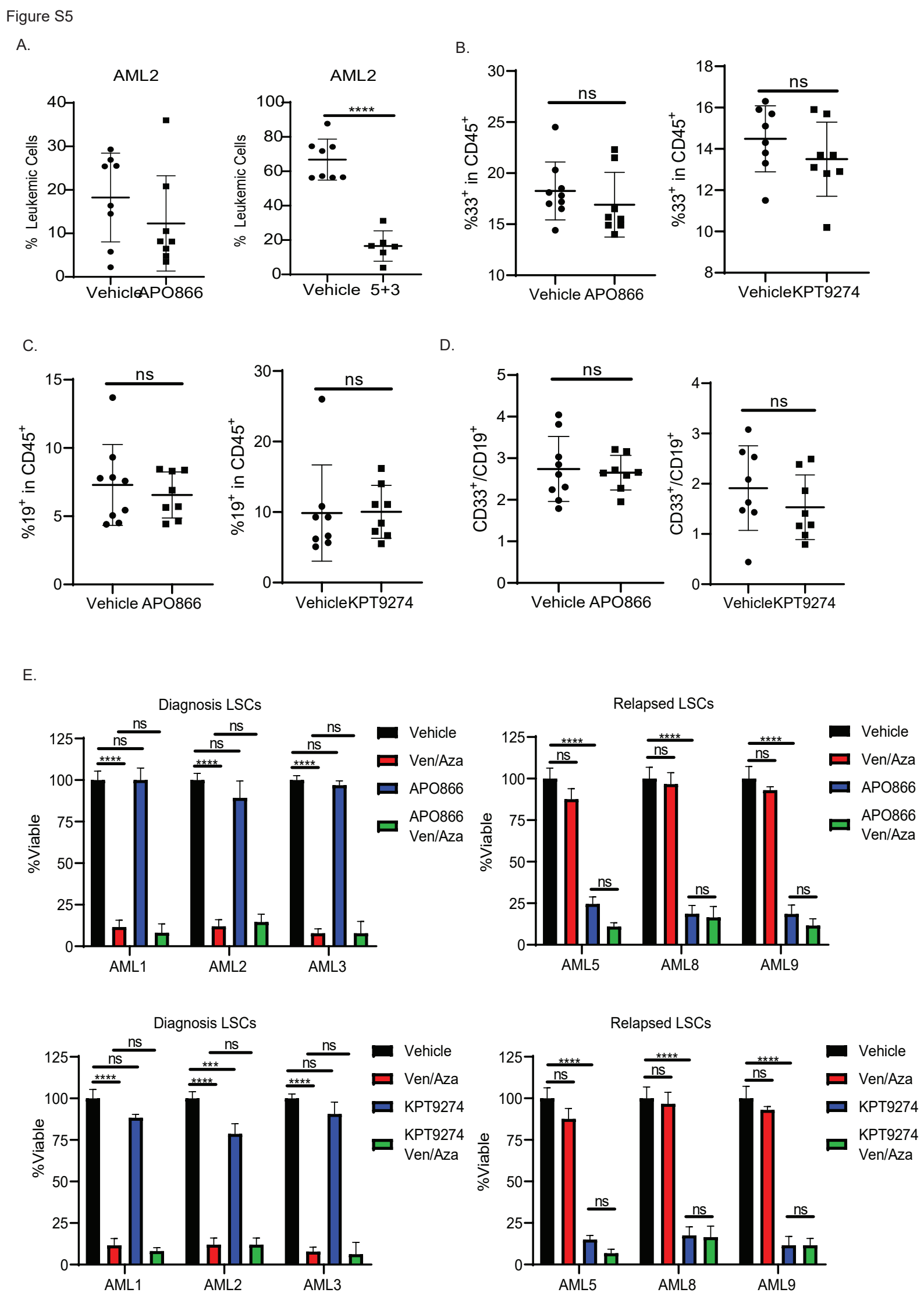

A.
AML2
% Leukemic Cells
Vehicle
APO866
AML2
****
% Leukemic Cells
Vehicle
5+3
B.
ns
%33+ in CD45+
Vehicle
APO866
ns
%33+ in CD45+
Vehicle
KPT9274
C.
ns
%19+ in CD45+
Vehicle
APO866
ns
%19+ in CD45+
Vehicle
KPT9274
D.
ns
CD33+/CD19+
Vehicle
APO866
ns
CD33+/CD19+
Vehicle
KPT9274
E.
Diagnosis LSCs
%Viable
AML1
AML2
AML3
Vehicle
Ven/Aza
APO866
APO866 Ven/Aza
Relapsed LSCs
%Viable
AML5
AML8
AML9
Vehicle
Ven/Aza
APO866
APO866 Ven/Aza
Diagnosis LSCs
%Viable
AML1
AML2
AML3
Vehicle
Ven/Aza
KPT9274
KPT9274 Ven/Aza
Relapsed LSCs
%Viable
AML5
AML8
AML9
Vehicle
Ven/Aza
KPT9274
KPT9274 Ven/Aza

Figure S6

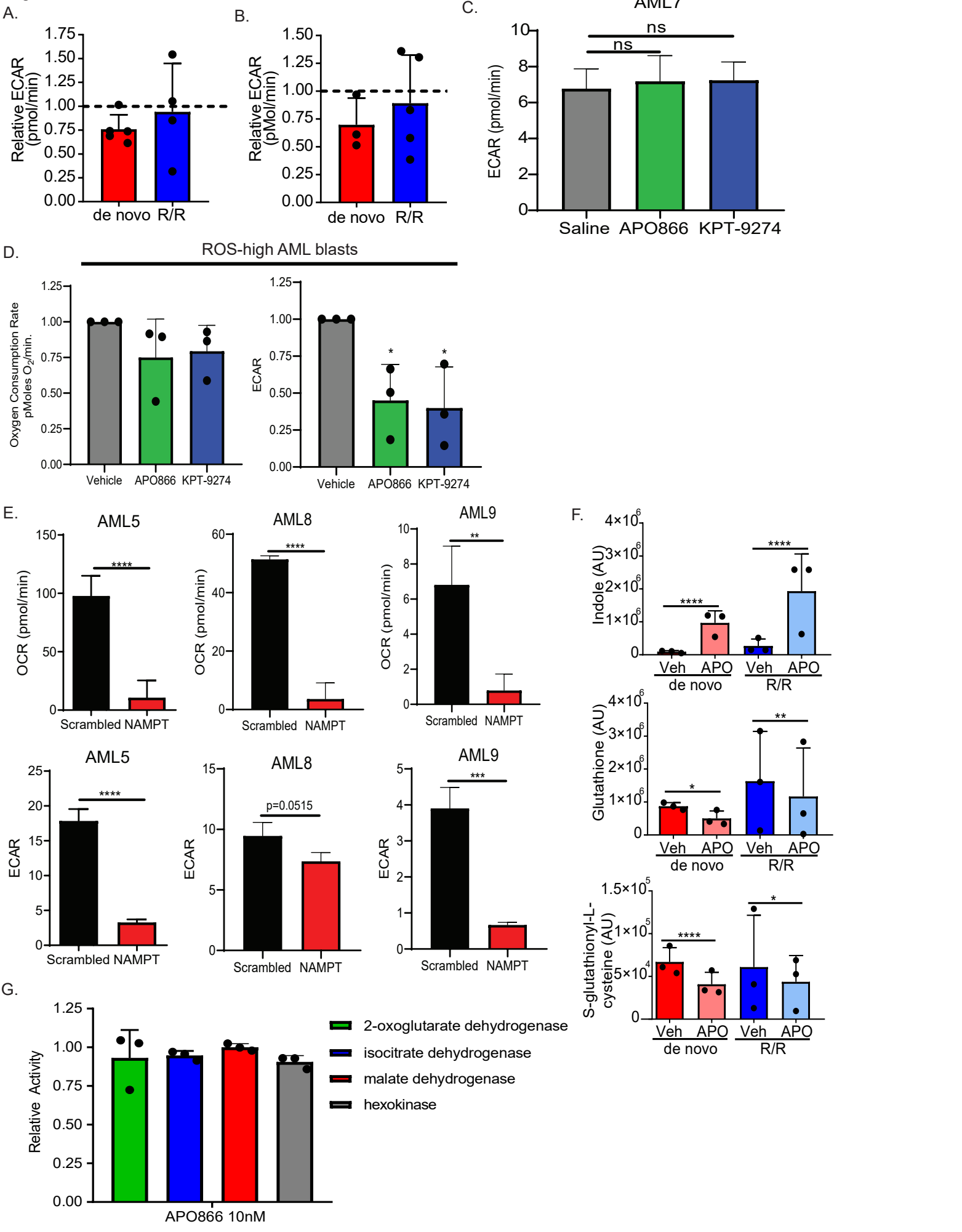
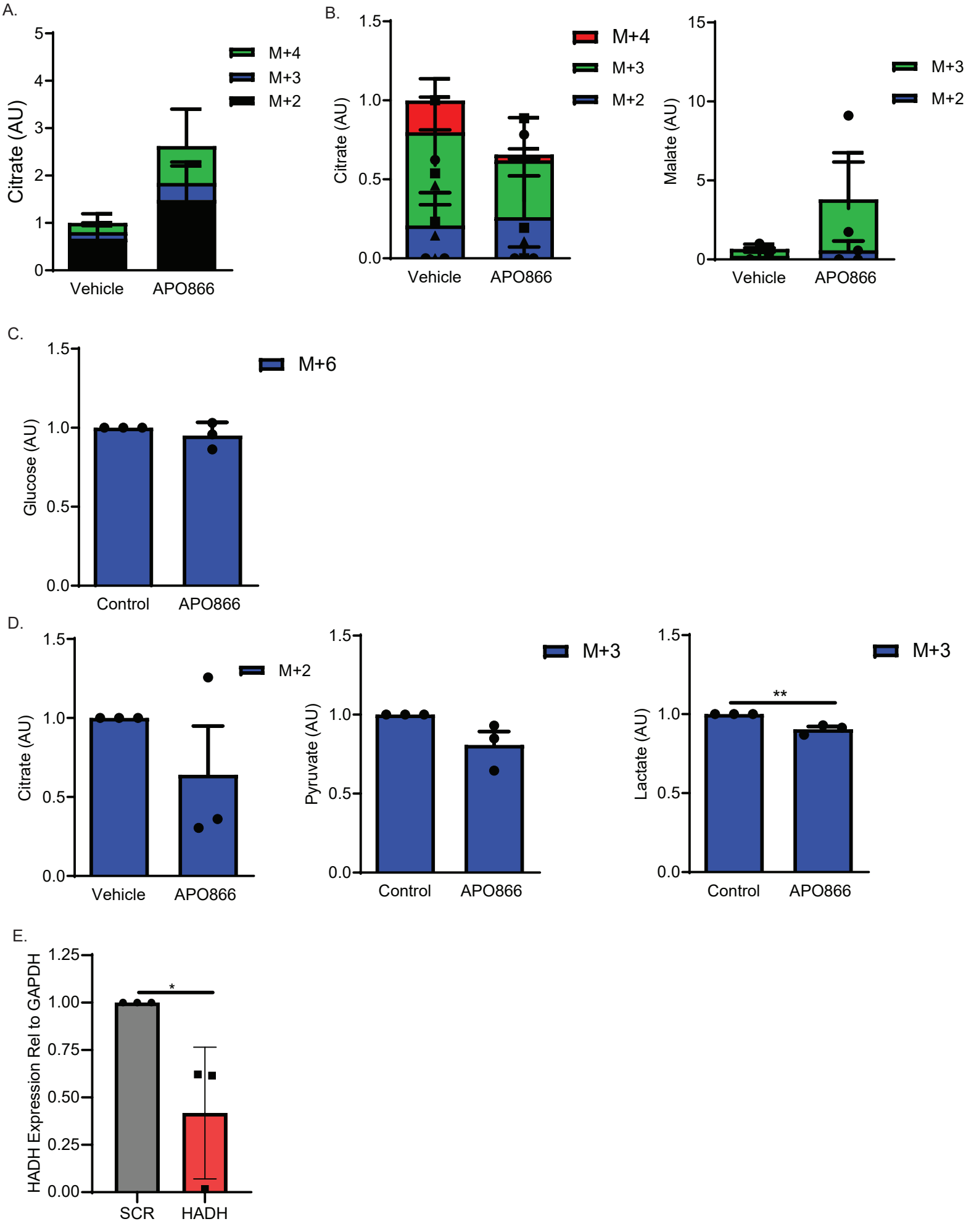

A.
Relative ECAR (pmol/min)
de novo R/R
B.
Relative ECAR (pMol/min)
de novo R/R
C.
AML7
ns
ns
ECAR (pmol/min)
Saline APO866 KPT-9274
D.
ROS-high AML blasts
Oxygen Consumption Rate pMoles O2/min.
Vehicle APO866 KPT-9274
ECAR
Vehicle APO866 KPT-9274
E.
AML5
OCR (pmol/min)
Scrambled NAMPT
AML8
OCR (pmol/min)
Scrambled NAMPT
AML9
OCR (pmol/min)
Scrambled NAMPT
AML5
ECAR
Scrambled NAMPT
AML8
p=0.0515
ECAR
Scrambled NAMPT
AML9
ECAR
Scrambled NAMPT
F.
Indole (AU)
Veh APO Veh APO
de novo R/R
Glutathione (AU)
Veh APO Veh APO
de novo R/R
S-glutathionyl-L-cysteine (AU)
Veh APO Veh APO
de novo R/R
G.
Relative Activity
APO866 10nM
2-oxoglutarate dehydrogenase
isocitrate dehydrogenase
malate dehydrogenase
hexokinase

Figure S7


A.
Citrate (AU)
M+4
M+3
M+2
Vehicle
APO866
B.
Citrate (AU)
M+4
M+3
M+2
Vehicle
APO866
Malate (AU)
M+3
M+2
Vehicle
APO866
C.
Glucose (AU)
M+6
Control
APO866
D.
Citrate (AU)
M+2
Vehicle
APO866
Pyruvate (AU)
M+3
Control
APO866
Lactate (AU)
M+3
**
Control
APO866
E.
HADH Expression Rel to GAPDH
*
SCR
HADH