Supplemental figures legends

Figure S1. Antiproliferative activity of ibrutinib in aggressive B-cell lymphoma cell lines. **(A)** Scatter dot plot showing the efficacy of treatment with increasing concentration of ibrutinib for 24 hours in B-cell lymphoma cell line panel (ABC n=6 and GCB n=3). Error bars represent standard error of the mean (S.E.M) of triplicate experiments. **(B)** Four ABC [n=3 MyD88 mutant (TMD-8, HBL-1 and OCI-LY-10) and n=1 MyD88 wild type (Ri1)] and one GCB (SUDHL-4) cells were transfected with 1 μM scramble siRNA or MyD88 siRNA, and MyD88 levels assessed by western blot assay after 24 hours to confirm efficient MyD88 knockdown. **(C)** Representative western blot demonstrating the efficacy of MyD88 knockdown. HBL-1, SUDHL-4 and Ri-1 cells were infected with either Ctrl (RENILLA.173) or two different hairpins shRNAs for MyD88, and MyD88 level assessed by western blot assay after selection and induction with doxycycline for 1 day.

Figure S2. MyD88 L265P mutation is a gain of function. **(A)** Scatter dot plot showing increased MyD88 mRNA level in Ri-1 cells that stably expressed MyD88 mutation compared to the parental. Differences between groups were calculated with the Student T test. *** p=0.003. **(B)** Representative western blot demonstrating the GFP expression in the transduced cells with MyD88 L265P. Enhanced levels of the NF-kB activation [pNF-kB (Ser 536), IRF4, pSTAT3 (T705)] are also shown in the constitutively activated MyD88 mutant L265P in Ri-1 cells compared to Ri-1 parent cell line. **(C)** Growth curves of Ri-1 DLBCL cells transduced with MyD88 L265P (red) or empty vector (blue) at the indicated times, normalized to the day 0 values. **(D)** Flow cytometric analysis of co-coltures of Ri-1 DLBCL cells transduced with MyD88 L265P (red) or empty vector (blue). Shown are the percentages of MyD88 L265P/GFP+ and empty vector/GFP- cells over time. Data are representative of three independent experiments.

Figure S3. Multiple HDACs control MyD88 expression. (A) Representative western blot analysis demonstrating the efficacy of HDAC knockdown and concomitant MyD88 decrease. HBL-1 and SUDHL-4 cells were infected with either Ctrl (RENILLA.173) or four different hairpins shRNAs for each HDAC, and HDAC and MyD88 levels assessed by western blot assay after selection and induction for 2 days.

Figure S4. HDAC inhibitors decrease STAT3 activation, resulting in MyD88 downregulation. **(A)** Representative western blot demonstrating decrease of STAT3 among all different transcription factors involved in MyD88 regulation [p-cJun (Ser 243), p-cFos (Ser 32), p-cATF2 (Thr 71)] after treatment with two different HDAC inhibitors (panobinostat and romidespin) for 6, 12 and 24 hours. **(B)** Box and whiskers plot showing no change in pSTAT1 (T701) levels in B-cell lymphoma cell line panel (GCB n=2 and ABC n=5) after treatment with either 0.05 μM panobinostat or romidepsin 0.01 μM for 12 hours. Error bars represent S.E.M of triplicates. **(C)** Schematic representation of MyD88 promoter structure: HDAC inhibitors decrease STAT3 activation, ultimately decreasing the activation of MyD88 transcription. **(D)** Western blot showing the effects of STAT3 depletion by RNA interference on MyD88 levels in TMD-8 and SUDHL-4 cells. Both cells were treated with 2μM scramble or STAT3 siRNAs for 48 hours, and the effects on the expression levels of STAT3 and MyD88 assessed by western blotting. **(E)** Box and whiskers plot showing the effects of STAT3 depletion by RNA interference on MyD88 protein levels in B-cell lymphoma cell line panel (ABC n=3 and GCB n=1). Error bars represent S.E.M of triplicate experiments. Differences between groups were calculated with the Student T test. ** $p<0.005$; ns, not significant. **(F)** Box and whiskers plot showing the effects of STAT3 depletion by RNA interference on relative mRNA levels of MyD88 in B-cell lymphoma cell line panel (ABC n=3 and GCB n=1) after 24 (black) and 48 (gray) hours. **(G)** TMD-8 and

SUDHL-4 cells transfected with 2 μM scramble and STAT3 siRNA were incubated with increasing concentrations of ibrutinib (0.1, 0.25, 0.5 μM) and cell viability assessed by MTS assay after 48 hours. STAT3 siRNA + ibrutinib viability data were normalized to the effect of STAT3 siRNA alone. Error bars represent S.E.M of triplicate experiments. Differences between groups were calculated with the Student T test. * p=0.01. **(H)** Western blot confirming the effects of STAT3 depletion by second RNA interference on MyD88 levels in HBL-1, TMD-8, Ri-1 and SUDHL-4 cells. Both cells were treated with 2μM scramble or STAT3 siRNAs (s744) for 48 hours, and the effects on the expression levels of STAT3 and MyD88 assessed by western blotting. **(I)** HBL-1, TMD-8, Ri-1 and SUDHL-4 cells transfected with 2 μM scramble and STAT3 siRNA (s744) were incubated with increasing concentrations of ibrutinib (0.1, 0.25, 0.5 μM) and cell viability assessed by MTS assay after 48 hours. STAT3 siRNA + ibrutinib viability data were normalized to the effect of STAT3 siRNA alone. Error bars represent S.E.M of triplicate experiments. Differences between groups were calculated with the Student T test. * p=0.01; *** p< 0.0005 **(F)** TMD-8 and HBL-1 cells were treated with either panobinostat (0.05 μM) or DMSO, and ChIP was performed with H3K4me antibody or control immunoglobulin G (IgG). Primers to amplify the STAT3-binding regions of the MyD88 promoter were used in qPCR to determine fold enrichment relative to a noncoding region. Error bars represent SEM of three independent experiments. ANOVA with Dunnett's test was performed to compare STAT3 wild type binding site versus the other conditions. * p< 0.05; ** p< 0.005; **** p< 0.0001.

Figure S5. Panobinostat and ibrutinib combination in DLBCL. **(A)** MTS assay confirming absence of synergistic effects of panobinostat in combination with ibrutinib in GCB cells (SUDHL-4, SUDHL-6, SUDHL-8). Cells were incubated with increasing concentrations of panobinostat (0.01, 0.025, 0.05, 0.075 μM) and ibrutinib (0.01, 0.025, 0.05, 0.075 μM), and viability assessed after 24 hours. Error bars represent SEM of three independent experiments. **(B)** Western blot showing enhanced downregulation on pNF-kB, IKK beta and IRF4 combining

panobinostat and ibrutinib in three ABC MyD88 mutant (HBL-1, TMD-8 and OCI-LY10), but not in two ABC MyD88 wild type (U-2932 and Ri-1). Cells were treated for 24 hours with indicated concentration of either panobinostat, ibrutinib, the combination of the two or DMSO. **(C)** Representative western blot demonstrating correlation between MyD88 mutational status and NF-kB and STAT3 activation. **(D)** Box and whiskers graph showing correlation between MyD88 mutational status and pSTAT3 (Tyr 705) levels. **(E)** IL-6 level change in three ABC DLBCL cell lines harboring MyD88 mutation (HBL-1, TMD-8 and OCI-LY-10). Cells were treated for 12 hours with indicated concentration of either DMSO, panobinostat, ibrutinib or the combination of the two drugs. Error bars represent S.E.M of triplicates. Differences between groups were calculated with the ANOVA with Dunnett's test. * p=0.05; **p<0.005. **(F)** Scatter dot plot showing the effects of rIL-6 and rIL-10 on relative mRNA levels of MyD88 in HBL-1 cells after 30 minutes, 3 and 6 hours. Error bars represent SEM of three independent experiments. **(G)** Box and whiskers plot showing increase in luciferase activity in two representative ABC (HBL-1 and OCI-LY-10) and one GCB (SUDHL-4) cell lines. Cells were treated for 6 hours with indicated concentration of either rIL-6 or rIL-10. Cells were incubated with 500 ng/ml of INF-γ as positive control for TLR activation. Error bars represent S.E.M. of triplicates. Differences between groups were calculated with the ANOVA with Dunnett's test. *p<0.05; ** p<0.005**.**

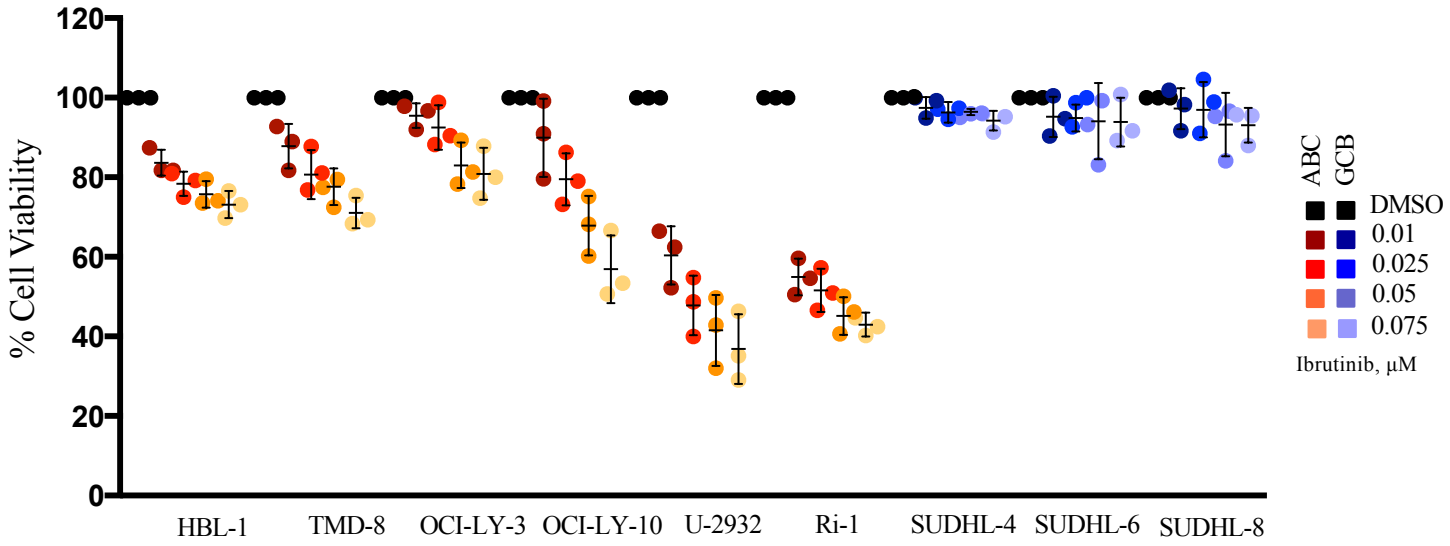
Figure S6. Safety and feasibility of panobinostat and ibrutinib combination in DLBCL mouse model. **(A)** Variations of body weight over time in TMD-8 and Ri-1 xenografts treated with vehicle, panobinostat (5 mg/kg), ibrutinib (2mg/kg) or the two drugs together.

Figure S7. Panobinostat synergizes with ibrutinib in xenograft model of Ri-1 ABC DLBCL cells stably expressing the MyD88 mutant L265P. (A) NSG mice (n=8 per treatment group) bearing Ri-1 stably expressing the MyD88 mutant L265P and Ri-1 with MyD88 wild type tumors

subQ were treated i.p. daily with either vehicle, panobinostat (5 mg/kg), ibrutinib (2mg/kg) or the two drugs together, 5 times weekly for three weeks. Tumor volumes were measured 3 times per week. Differences between groups were calculated with the ANOVA with Dunnett's test. **** $p<0.0001$; ns, not significant. (**B**) NSG mice (n=8 per treatment group, in Ri-1 stably expressing MyD88 mutant L265P or MyD88 wild type experiment respectively) were treated i.p. daily with either vehicle, panobinostat (5 mg/kg), ibrutinib (2mg/kg) or the two drugs together, 5 times weekly for three weeks and observed until death after the end of the treatment. Differences between groups were calculated with the ANOVA with Dunnett's test. **** $p<0.0001$; ns, not significant. (**C**) Kaplan-Meier plot of the percent survival as a function of time from last drug administration. Data are from n= 11 and 8 for all groups in TMD8 and Ri-1 tumors, respectively. *** $p<0.0005$; ns, not significant.

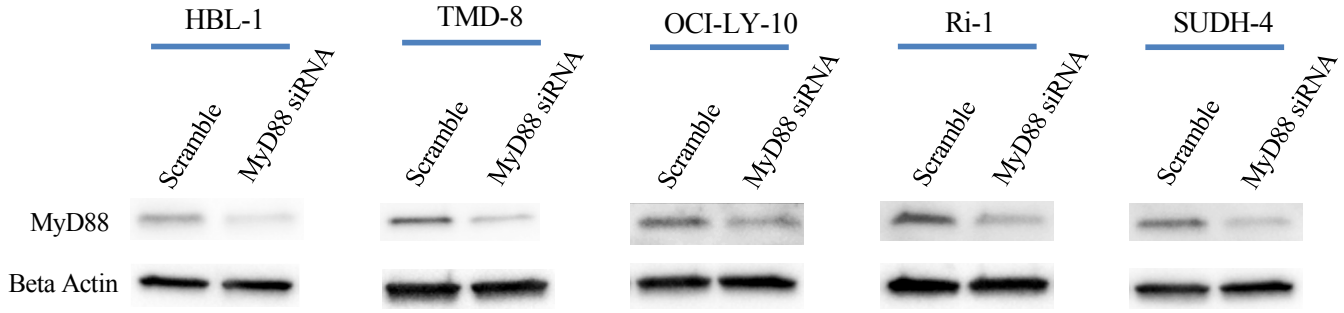
Figure S1

A



MyD88 mutant	L265P	L265P	L265P	L265P	-	-	-	S219C	-
CD79 mutant	Y197F	Y197H	X184	-	-	-	-	-	-

B



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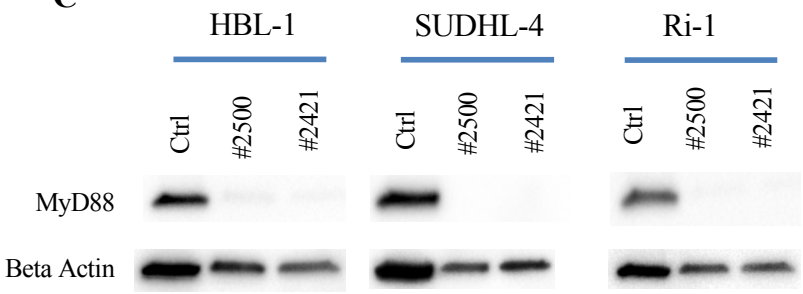


Figure S2

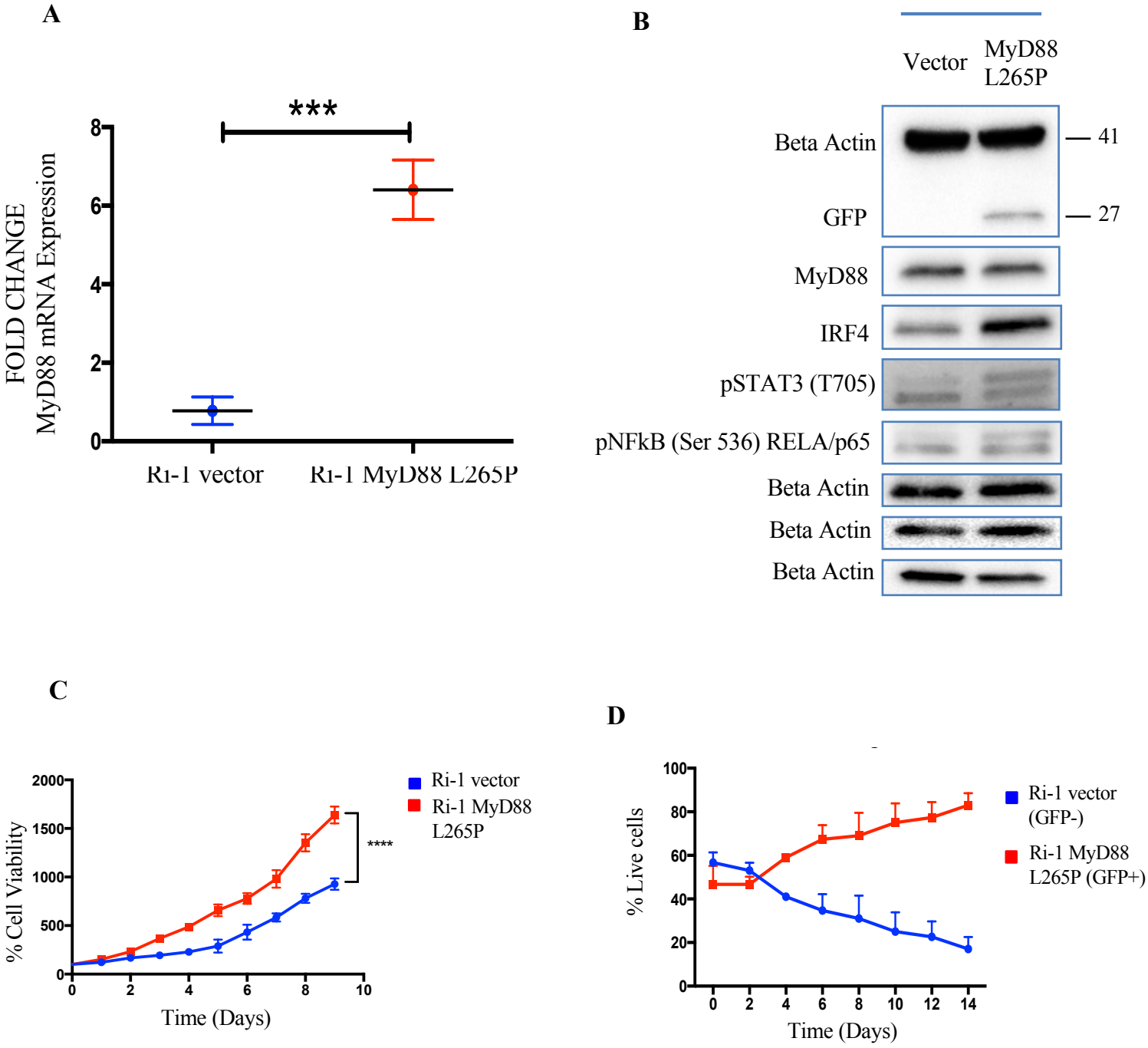

A
***
FOLD CHANGE
MyD88 mRNA Expression
8
6
4
2
0
Ri-1 vector
Ri-1 MyD88 L265P
B
Ri-1
Vector
MyD88 L265P
Beta Actin
41
GFP
27
MyD88
IRF4
pSTAT3 (T705)
pNFkB (Ser 536) RELA/p65
Beta Actin
Beta Actin
Beta Actin
C
% Cell Viability
2000
1500
1000
500
0
0
2
4
6
8
10
Time (Days)
****
Ri-1 vector
Ri-1 MyD88 L265P
D
% Live cells
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Time (Days)
Ri-1 vector (GFP-)
Ri-1 MyD88 L265P (GFP+)

Figure S3

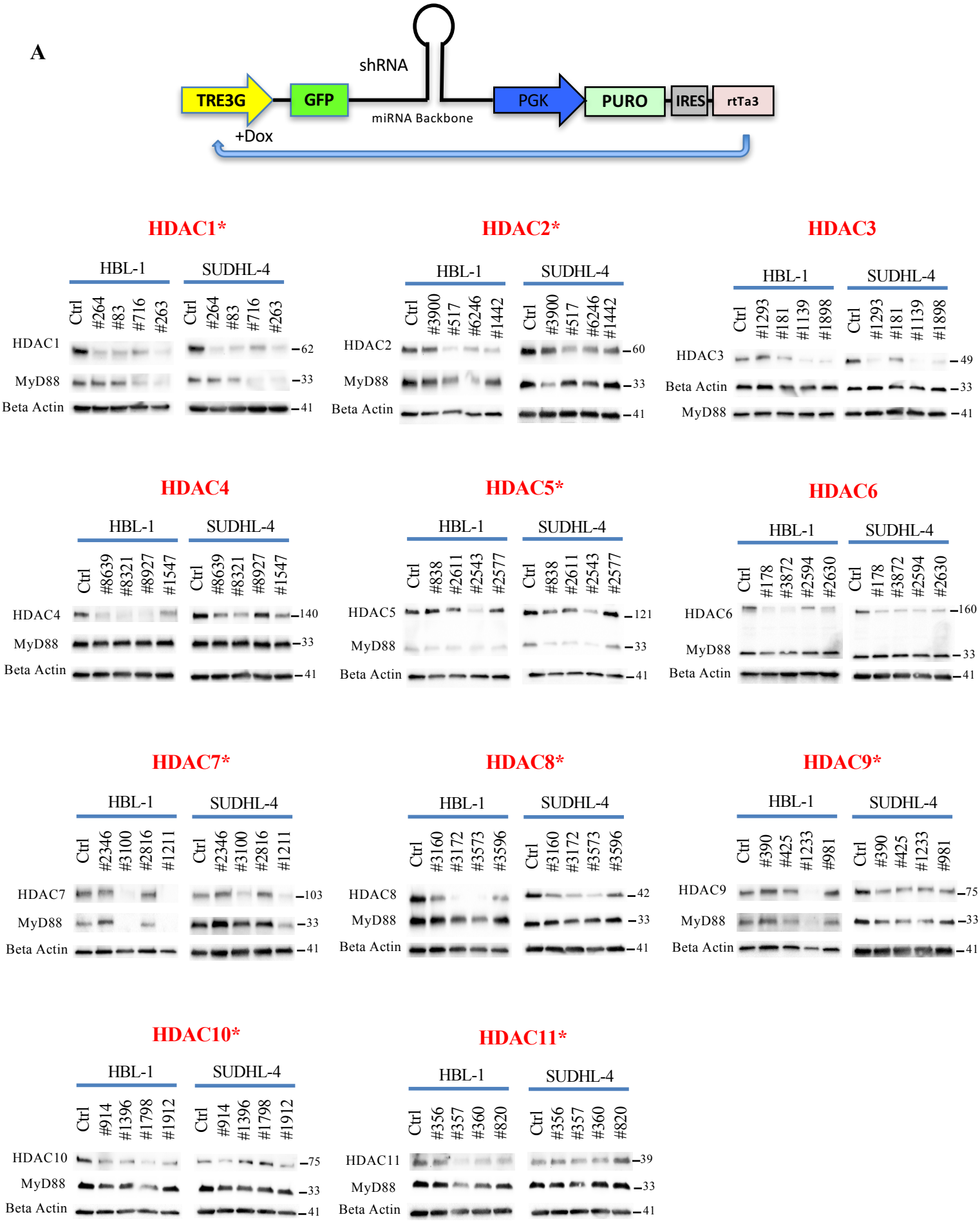

A
shRNA
TRE3G
GFP
miRNA Backbone
PGK
PURO
IRES
rtTa3
+Dox
HDAC1*
HBL-1
SUDHL-4
Ctrl
#264
#83
#716
#263
HDAC1
MyD88
Beta Actin
62
33
41
HDAC2*
Ctrl
#3900
#517
#6246
#1442
HDAC2
60
HDAC3
Ctrl
#1293
#181
#1139
#1898
49
HDAC4
Ctrl
#8639
#8321
#8927
#1547
HDAC4
140
HDAC5*
Ctrl
#838
#2611
#2543
#2577
HDAC5
121
HDAC6
Ctrl
#178
#3872
#2594
#2630
HDAC6
160
HDAC7*
Ctrl
#2346
#3100
#2816
#1211
HDAC7
103
HDAC8*
Ctrl
#3160
#3172
#3573
#3596
HDAC8
42
HDAC9*
Ctrl
#390
#425
#1233
#981
HDAC9
75
HDAC10*
Ctrl
#914
#1396
#1798
#1912
HDAC10
75
HDAC11*
Ctrl
#356
#357
#360
#820
HDAC11
39

Figure S4

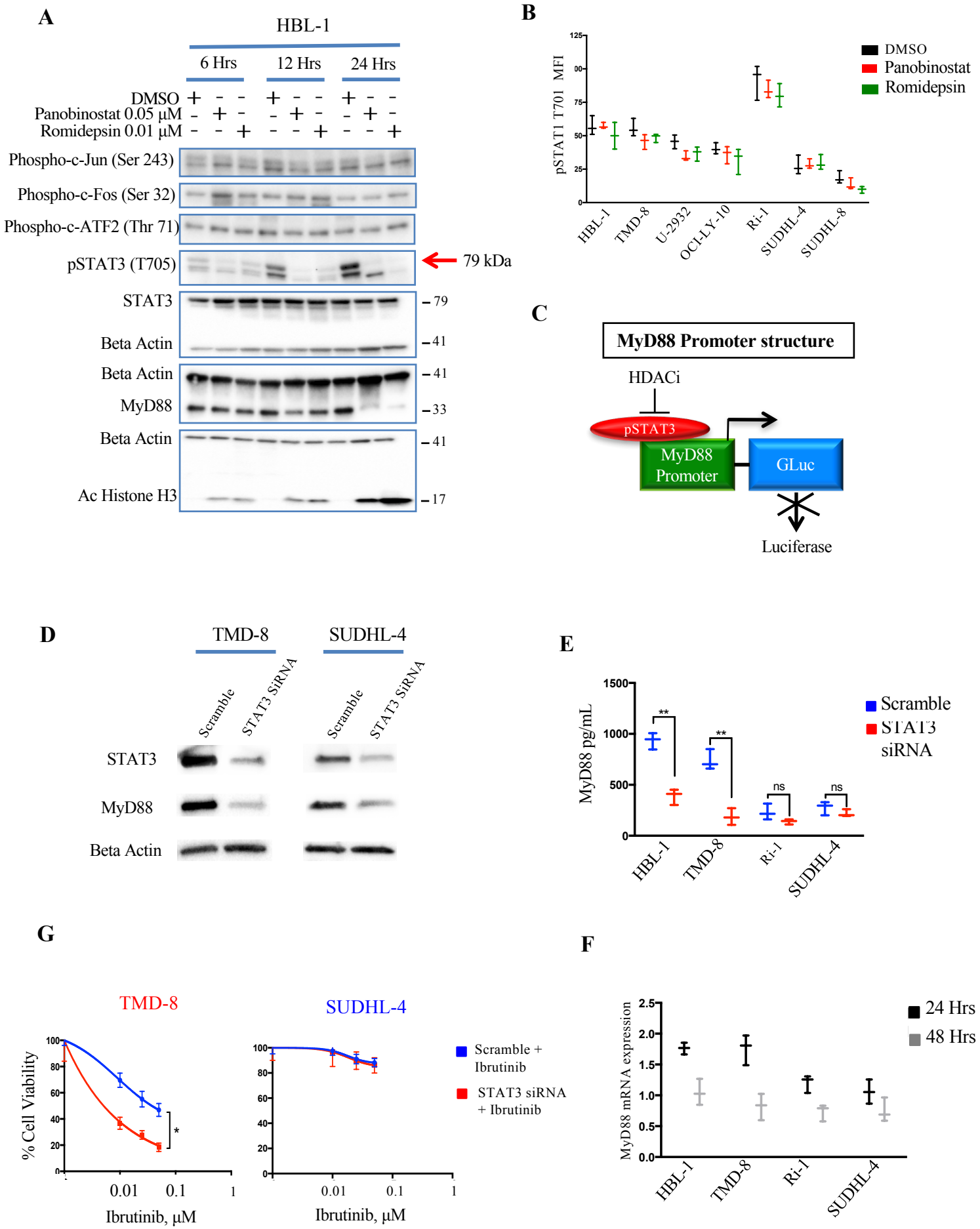
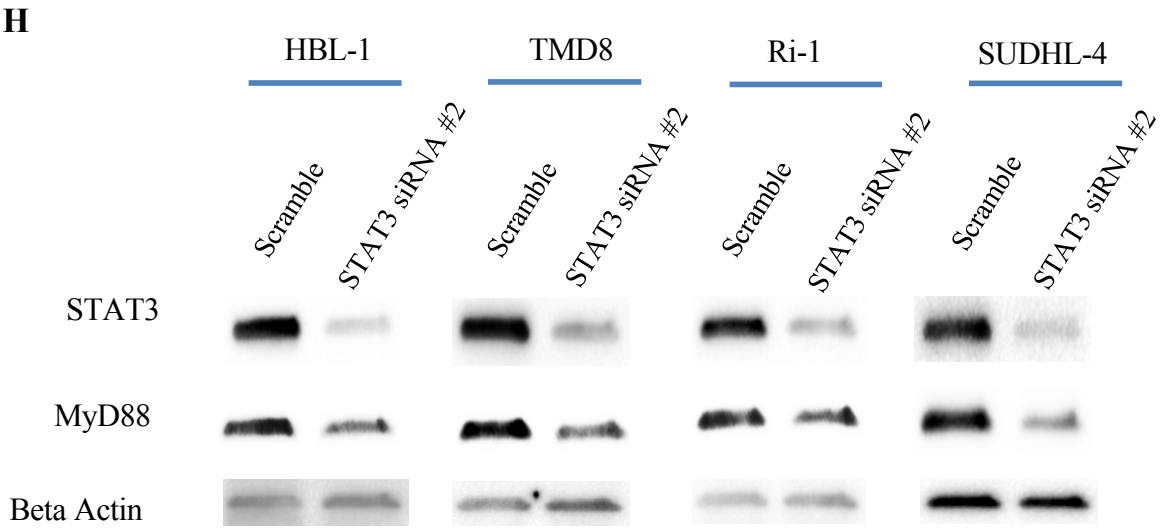
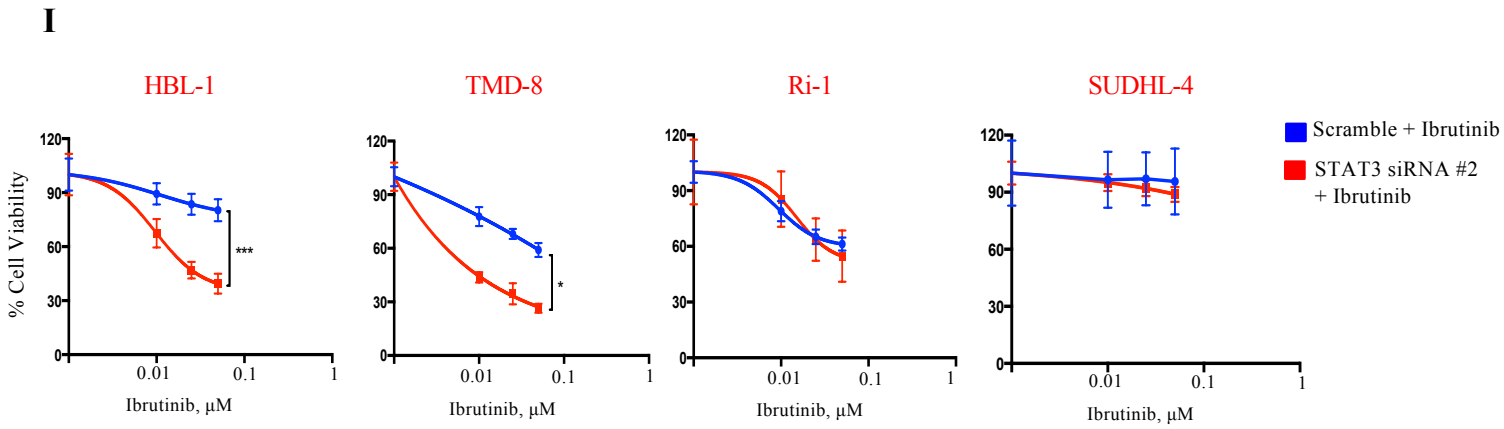

A
HBL-1
6 Hrs
12 Hrs
24 Hrs
DMSO
Panobinostat 0.05 µM
Romidepsin 0.01 µM
Phospho-c-Jun (Ser 243)
Phospho-c-Fos (Ser 32)
Phospho-c-ATF2 (Thr 71)
pSTAT3 (T705)
79 kDa
STAT3
Beta Actin
Beta Actin
MyD88
Beta Actin
Ac Histone H3
79
41
41
33
41
17
B
pSTAT1 T701 MFI
DMSO
Panobinostat
Romidepsin
HBL-1
TMD-8
U-2932
OCI-LY-10
Ri-1
SUDHL-4
SUDHL-8
C
MyD88 Promoter structure
HDACi
pSTAT3
MyD88 Promoter
GLuc
Luciferase
D
TMD-8
SUDHL-4
Scramble
STAT3 SiRNA
Scramble
STAT3 SiRNA
STAT3
MyD88
Beta Actin
E
MyD88 pg/mL
Scramble
STAT3 siRNA
**
**
ns
ns
HBL-1
TMD-8
Ri-1
SUDHL-4
G
TMD-8
SUDHL-4
% Cell Viability
Scramble + Ibrutinib
STAT3 siRNA + Ibrutinib
Ibrutinib, µM
Ibrutinib, µM
F
MyD88 mRNA expression
24 Hrs
48 Hrs
HBL-1
TMD-8
Ri-1
SUDHL-4

Figure S4


H
HBL-1
TMD8
Ri-1
SUDHL-4
Scramble
STAT3 siRNA #2
STAT3
MyD88
Beta Actin


I
HBL-1
TMD-8
Ri-1
SUDHL-4
% Cell Viability
Ibrutinib, µM
Scramble + Ibrutinib
STAT3 siRNA #2
+ Ibrutinib

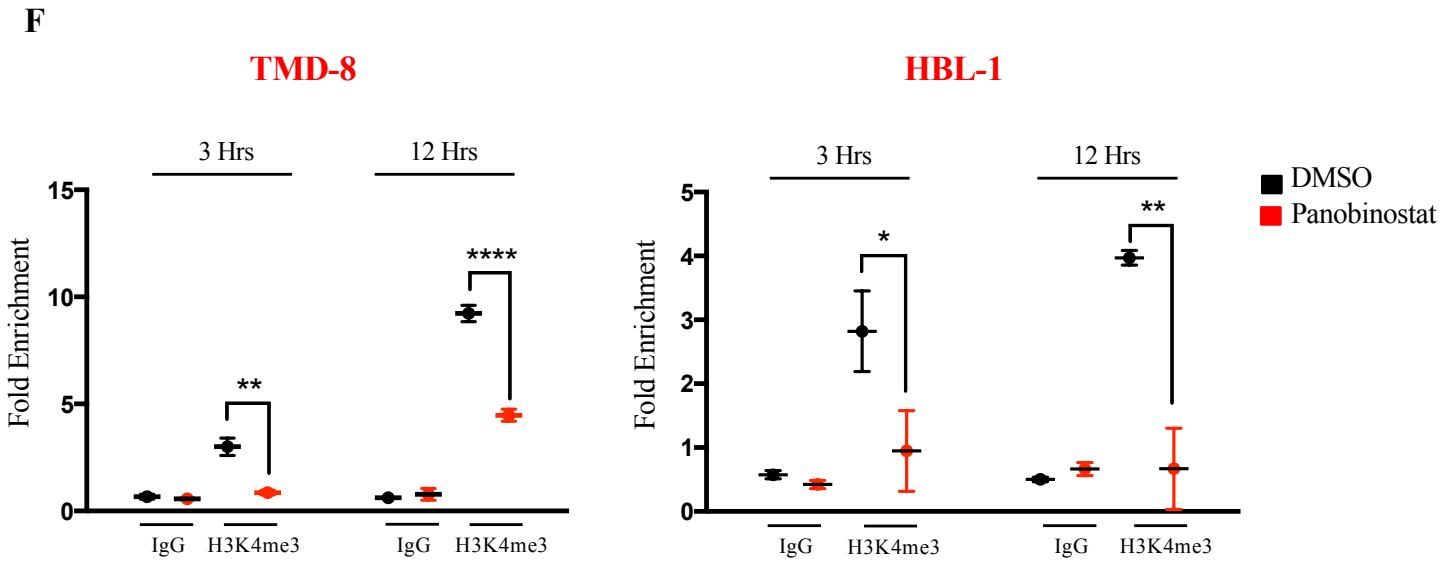

F
TMD-8
HBL-1
3 Hrs
12 Hrs
Fold Enrichment
IgG
H3K4me3
DMSO
Panobinostat

Figure S5

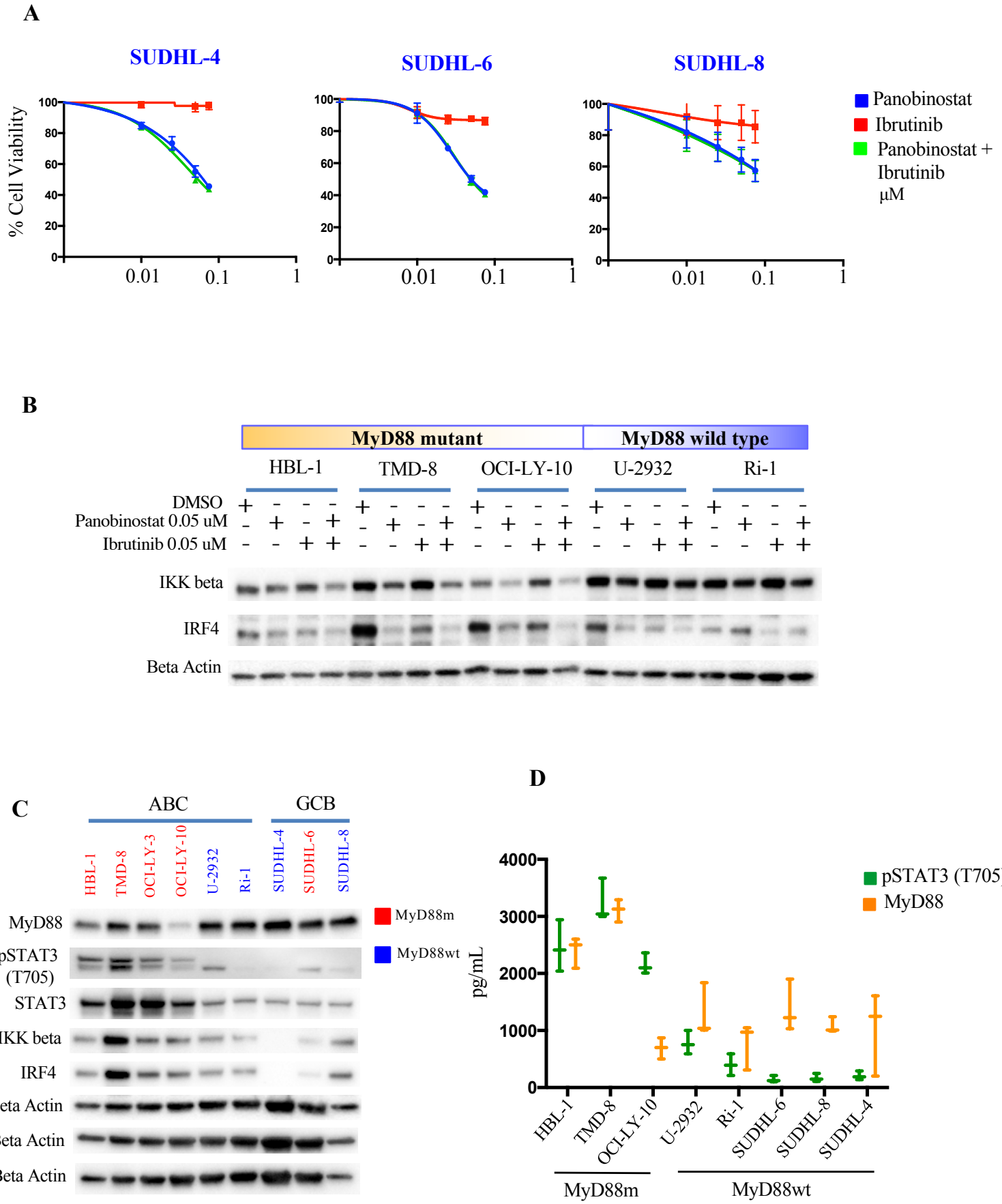
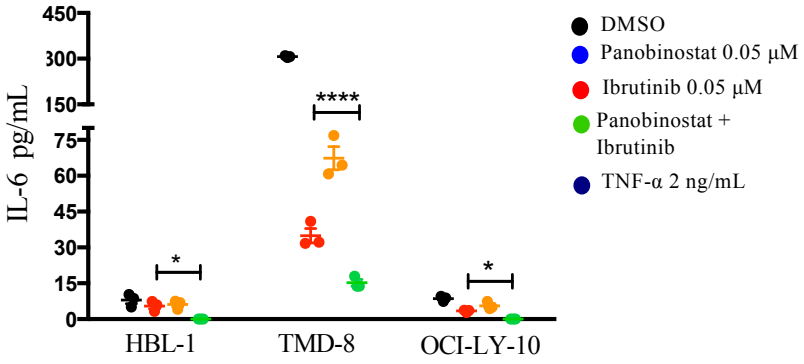

A
SUDHL-4
SUDHL-6
SUDHL-8
% Cell Viability
100
80
60
40
20
0
0.01
0.1
1
Panobinostat
Ibrutinib
Panobinostat + Ibrutinib
μM
B
MyD88 mutant
MyD88 wild type
HBL-1
TMD-8
OCI-LY-10
U-2932
Ri-1
DMSO
Panobinostat 0.05 uM
Ibrutinib 0.05 uM
IKK beta
IRF4
Beta Actin
C
ABC
GCB
HBL-1
TMD-8
OCI-LY-3
OCI-LY-10
U-2932
Ri-1
SUDHL-4
SUDHL-6
SUDHL-8
MyD88
pSTAT3 (T705)
STAT3
IKK beta
IRF4
Beta Actin
Beta Actin
Beta Actin
MyD88m
MyD88wt
D
pg/mL
4000
3000
2000
1000
0
pSTAT3 (T705)
MyD88
HBL-1
TMD-8
OCI-LY-10
U-2932
Ri-1
SUDHL-6
SUDHL-8
SUDHL-4
MyD88m
MyD88wt

Figure S5

E



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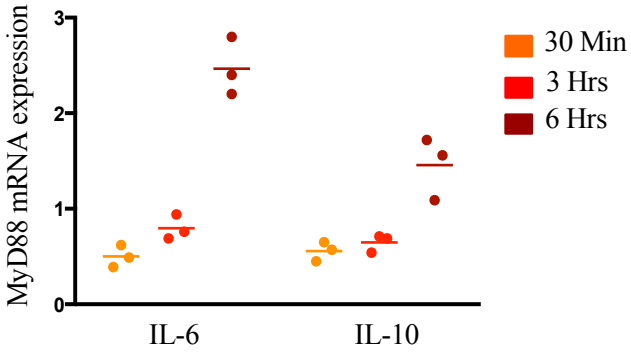

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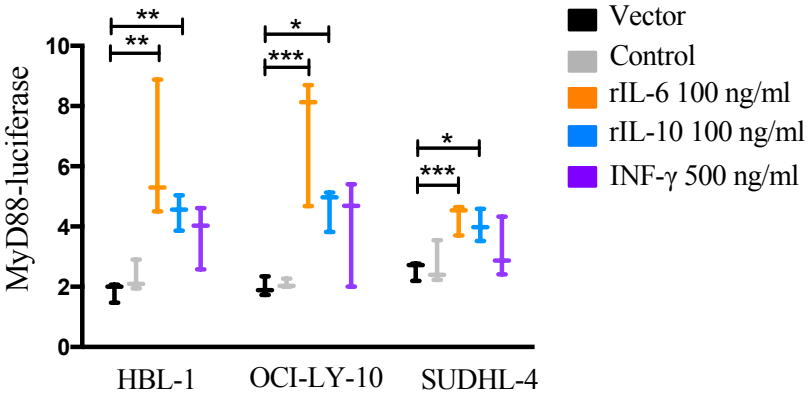


Figure S6

A

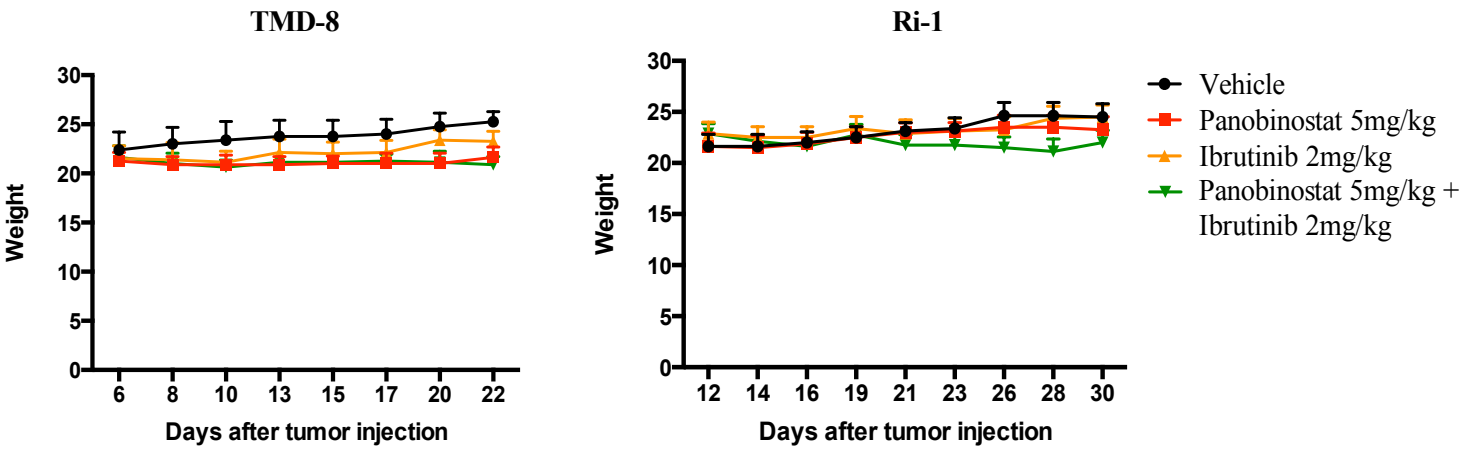
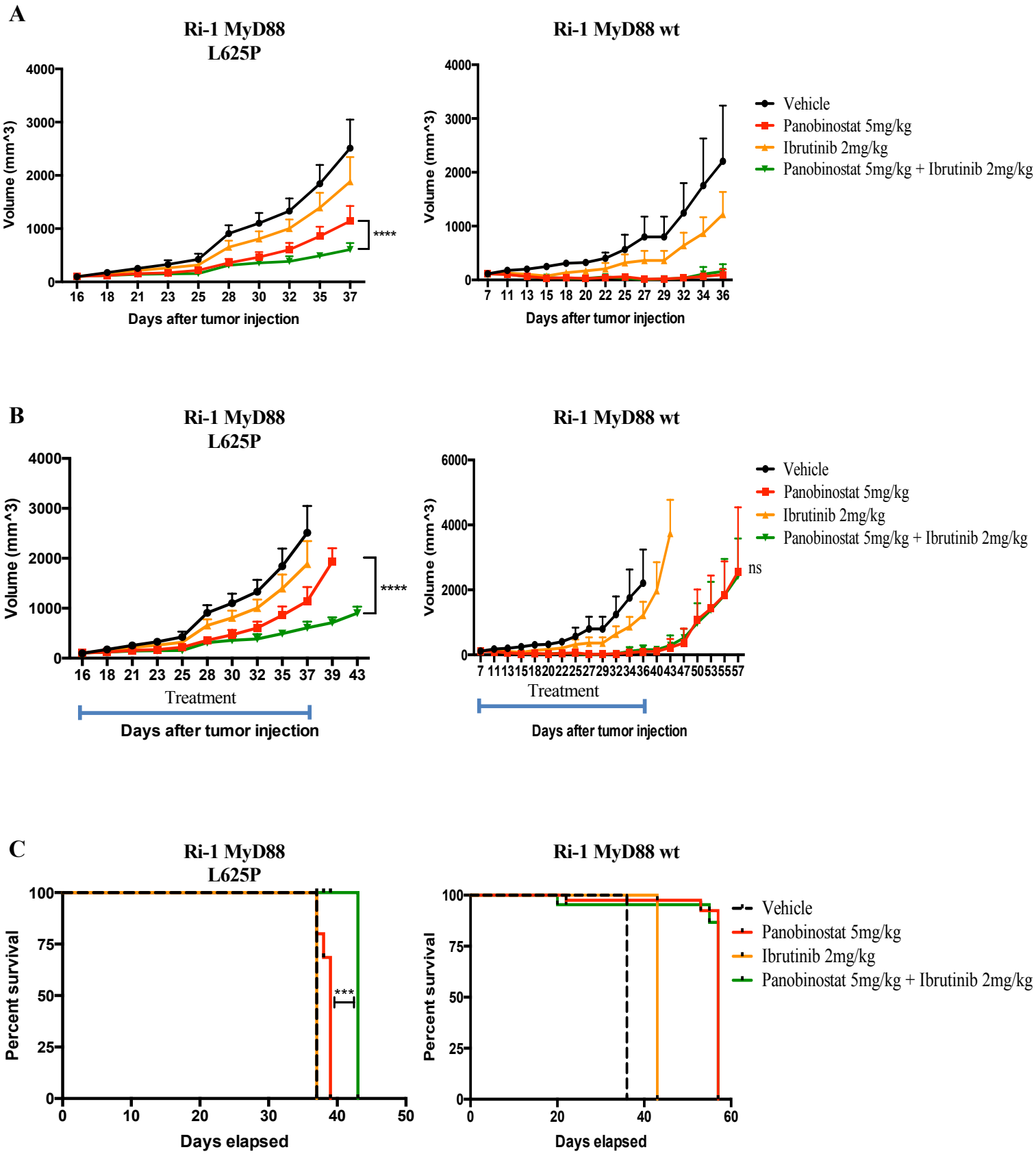

TMD-8
Weight
30
25
20
15
10
5
0
6
8
10
13
15
17
20
22
Days after tumor injection
Ri-1
Weight
30
25
20
15
10
5
0
12
14
16
19
21
23
26
28
30
Days after tumor injection
Vehicle
Panobinostat 5mg/kg
Ibrutinib 2mg/kg
Panobinostat 5mg/kg +
Ibrutinib 2mg/kg

Figure S7


A
Ri-1 MyD88 L625P
Volume (mm^3)
Days after tumor injection
****
Ri-1 MyD88 wt
Vehicle
Panobinostat 5mg/kg
Ibrutinib 2mg/kg
Panobinostat 5mg/kg + Ibrutinib 2mg/kg
B
Ri-1 MyD88 L625P
Treatment
Days after tumor injection
Ri-1 MyD88 wt
ns
C
Ri-1 MyD88 L625P
Percent survival
Days elapsed
***
Ri-1 MyD88 wt