SUPPLEMENTAL FIGURES AND FIGURE LEGENDS

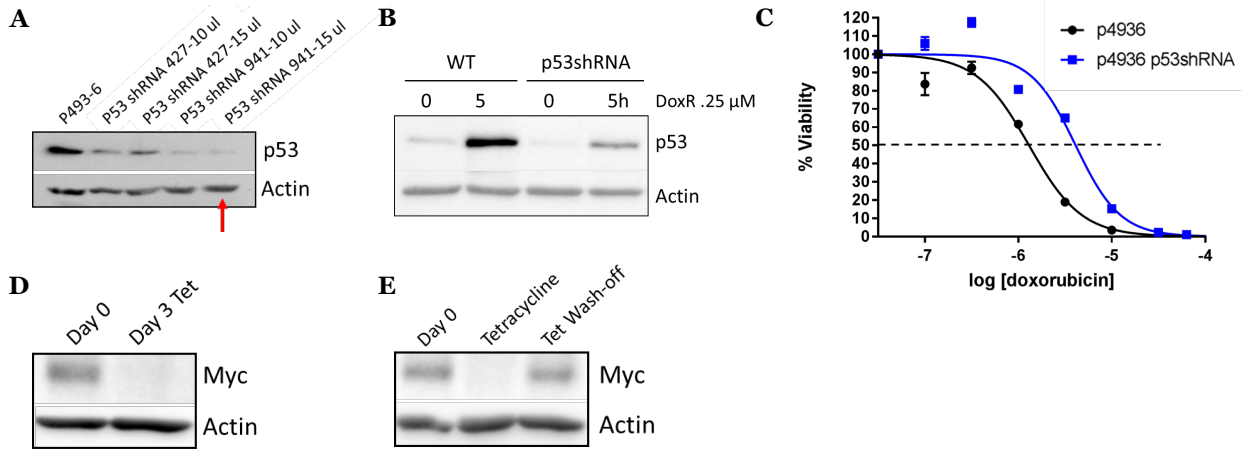
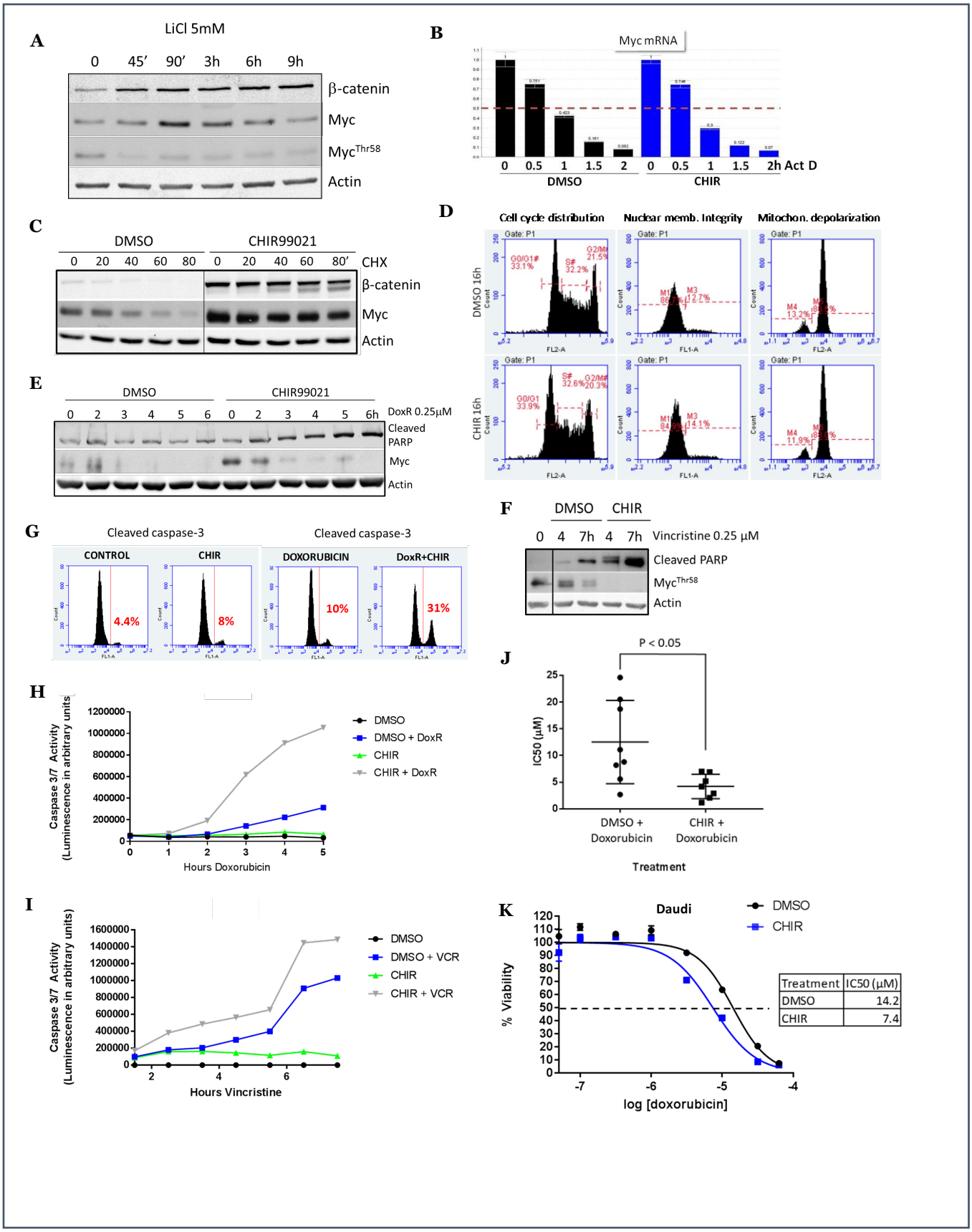


Figure S1. Generation and drug treatments of p493-6 p53shRNA cell line. A) P493-6 cells were infected with two p53shRNA at two viral concentrations. P53 knockdown was assessed by western blotting. **B)** P493-6 WT and p53shRNA cells were treated for 5 hours with .25 µM doxorubicin. Induction of p53 was assessed by western blotting. **C)** 72 hour Dox dose response curve of p493-6 WT and p53shRNA cells; survival was assessed using CellTiter-Glo and plotted using GraphPad Prism. **D)** P493-6 p53shRNA cells treated with 5 ng/mL tetracycline from Fig. 1B. Cells were harvested at day 0 and day 3 to assess Myc levels by western blotting. **E)** P493-6 p53shRNA cells treated overnight with 5 ng/mL tetracycline from Fig. 1C. The next day, tet was removed from half of the cells. Cells were harvested at day 0, after overnight tet, and 48 hours after tet wash-off to assess Myc levels by western blotting.

Figure S2 (following page). GSK-3β inhibition causes transient stabilization of Myc at the protein level and sensitizes multiple Burkitt lymphoma cell lines to chemotherapeutic drugs. A) Ramos cells were treated with LiCl as indicated. Western blotting was performed for β-catenin, Myc, and Myc^{Thr58} phosphorylation. **B)** Ramos cells were treated with DMSO or 3 µM CHIR99021 for 16 hours followed by actinomycin D. Bar graphs show qRT-PCR for Myc mRNA. **C)** Ramos cells were treated as in B) but followed by cycloheximide treatment. Western blotting was performed for Myc, β-catenin, and GAPDH control. **D)** Ramos cells were treated as in B), stained with PI, Yo-Pro, or TMRE (left, middle, and right columns), and analyzed by flow cytometry. Percentage of cells in each phase of cell cycle are shown (left column). Positive and negative cells are shown for Yo-Pro and TMRE staining. **E)** Ramos cells were treated with DMSO or 3 µM CHIR99021 for 2 hours followed by doxorubicin as indicated. Western blotting was performed for cleaved PARP, Myc, and actin control. **F)** Ramos cells were treated with DMSO or 3 µM CHIR99021 for 2 hours followed by vincristine. Western blotting was performed for cleaved PARP, Myc^{Thr58} phosphorylation, and actin control. **G)** Ramos cells were treated as in E). Cleaved caspase-3 expression was analyzed by flow cytometry after 6.5 hours of doxorubicin; percentages of positive cells are indicated. **H)** Ramos cells were treated as in G). Caspase 3/7 Glo assay was performed and luminescence data points for each treatment group are plotted. **I)** Ramos cells were treated with DMSO or 3 µM CHIR99021 for 2 hours followed by a 7.5 hour time course of .1 µM vincristine. Caspase 3/7 Glo assay was performed and luminescence data points plotted. **J)** Graph of means ± SEM analyzed by unpaired t-test for experimental repeats of Fig. 2C. **K)** Daudi cells were treated and analyzed as in Fig. 2C.


A
LiCl 5mM
0 45' 90' 3h 6h 9h
β-catenin
Myc
Myc^Thr58
Actin
B
Myc mRNA
0 0.5 1 1.5 2 0 0.5 1 1.5 2h Act D
DMSO
CHIR
C
DMSO
CHIR99021
0 20 40 60 80 0 20 40 60 80'
CHX
β-catenin
Myc
Actin
D
Cell cycle distribution
Nuclear memb. Integrity
Mitochon. depolarization
DMSO 16h
CHIR 16h
E
DMSO
CHIR99021
0 2 3 4 5 6 0 2 3 4 5 6h
DoxR 0.25μM
Cleaved PARP
Myc
Actin
F
DMSO CHIR
0 4 7h 4 7h Vincristine 0.25 μM
Cleaved PARP
Myc^Thr58
Actin
G
Cleaved caspase-3
Cleaved caspase-3
CONTROL
CHIR
DOXORUBICIN
DoxR+CHIR
4.4%
8%
10%
31%
H
Caspase 3/7 Activity (Luminescence in arbitrary units)
DMSO
DMSO + DoxR
CHIR
CHIR + DoxR
Hours Doxorubicin
I
Caspase 3/7 Activity (Luminescence in arbitrary units)
DMSO
DMSO + VCR
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CHIR + VCR
Hours Vincristine
J
P < 0.05
IC50 (μM)
DMSO + Doxorubicin
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Treatment
K
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% Viability
log [doxorubicin]
Treatment IC50 (μM)
DMSO 14.2
CHIR 7.4

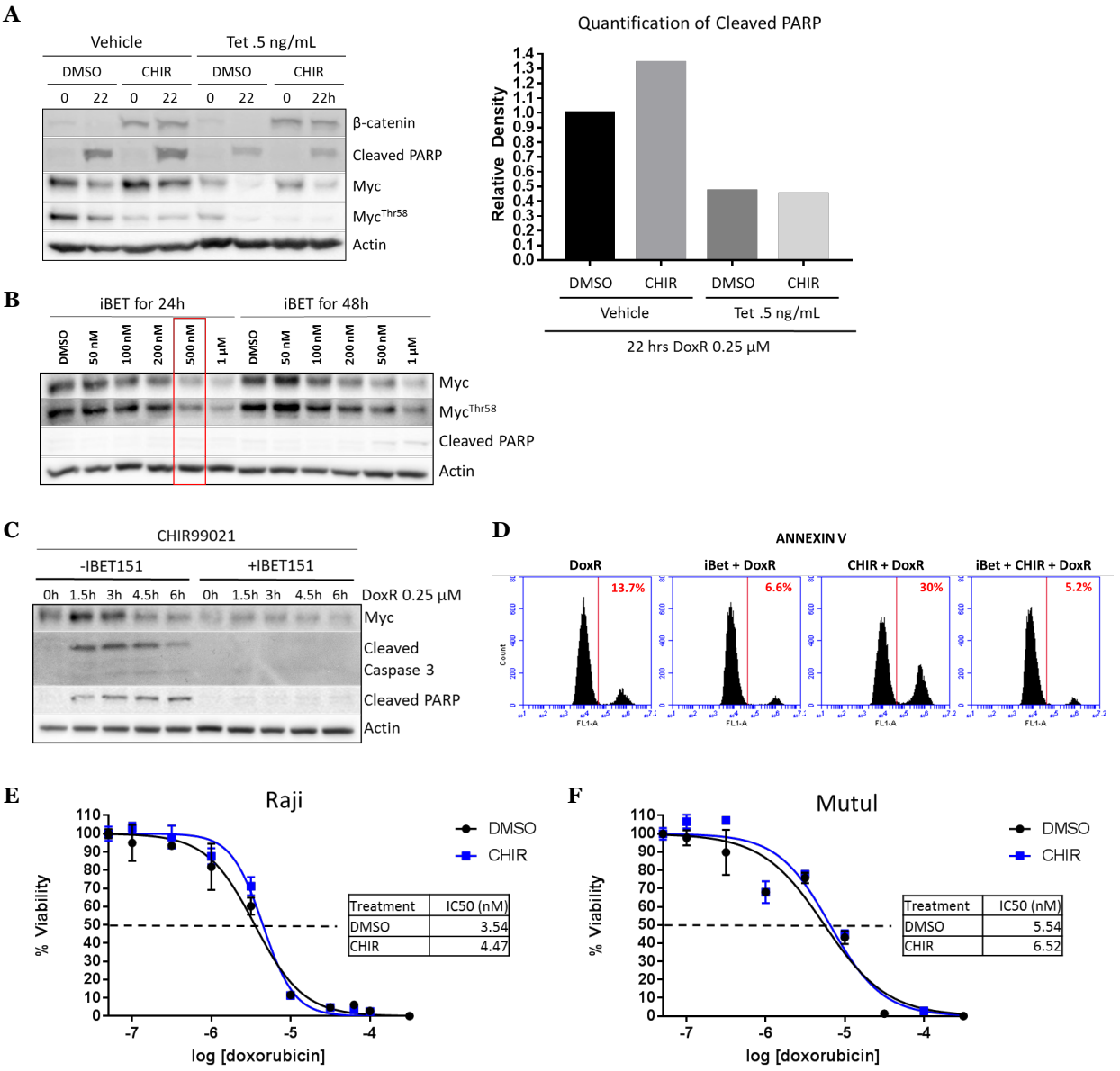

Figure S3. Myc is a key target of GSK-3β responsible for CHIR99021-mediated sensitization to doxorubicin. A) Left panel: p493-6 p53shRNA cells were treated with vehicle or tetracycline for 5 hours, followed by 2 hours or DMSO or CHIR and doxorubicin. Western blotting was done for β-catenin, cleaved PARP, Myc, Myc^{Thr58} phosphorylation and actin control. Right panel: Levels of cleaved PARP were quantified with Image J software and plotted with GraphPad Prism. **B)** Ramos cells were treated with increasing concentrations of iBet-151 or DMSO for 24-48 hours. Myc repression and cell death was assayed by western blotting. **C)** Ramos cells were treated with 500 nM iBet-151 or DMSO for 24 hours followed by 2 hours of 3 µM CHIR99021 and doxorubicin as indicated. Western blotting was performed for Myc and markers of cell death. **D)** Same experiment setup as in C). Annexin V expression was analyzed by flow cytometry after 6 hours of doxorubicin. Gates were drawn based on untreated cells. **E)** and **F)** Raji (E) and Mutul (F) cells were treated and cell survival assessed as in Fig. 2C.

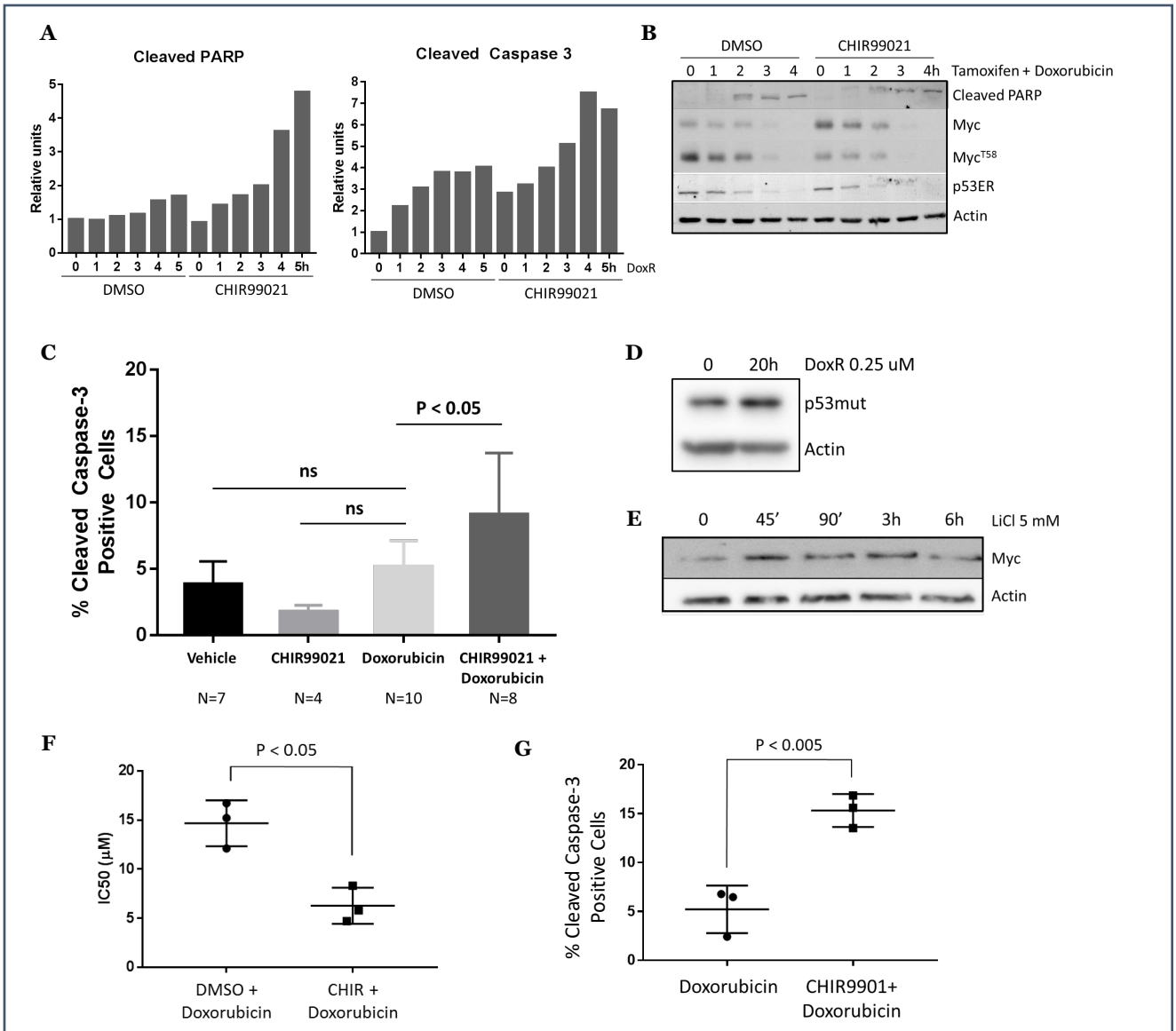

Figure S4. Responses of Burkitt lymphoma models/PDX to anti-cancer drugs and GSK3 inhibitors. A) Quantification of cleaved PARP and cleaved caspase-3 western blots in Fig. 3B. **B)** p53ER/MYC cells were treated with DMSO or 3 μM CHIR99021 for 2 hours followed by 250 nM 4OHT and .025 μM doxorubicin as indicated. Western blotting was performed for markers of GSK-3β inhibition, apoptosis, p53ER, and loading control actin. **C)** Graph of aperio positive pixel count quantification of cleaved caspase-3 IHC staining of p53ER/MYC tumors in Fig. 3C. Error bars represent mean + SEM analyzed by one-way ANOVA with correction for multiple comparisons. **D)** PDX MAP-GR-C95-BL-1 cells were treated with doxorubicin as indicated. P53 induction was measured by western blotting. **E)** PDX MAP-GR-C95-BL-1 cells were treated with LiCl as indicated, and Myc levels were assessed by western blotting. **F)** IC50s of multiple experiments performed as in Fig. 4B were statistically analyzed by unpaired t-test. **G)** Aperio positive pixel count quantification of cleaved caspase-3 IHC staining of MAP-GR-C95-BL-1 xenografts from Fig. 4C was statistically analyzed by unpaired t-test.

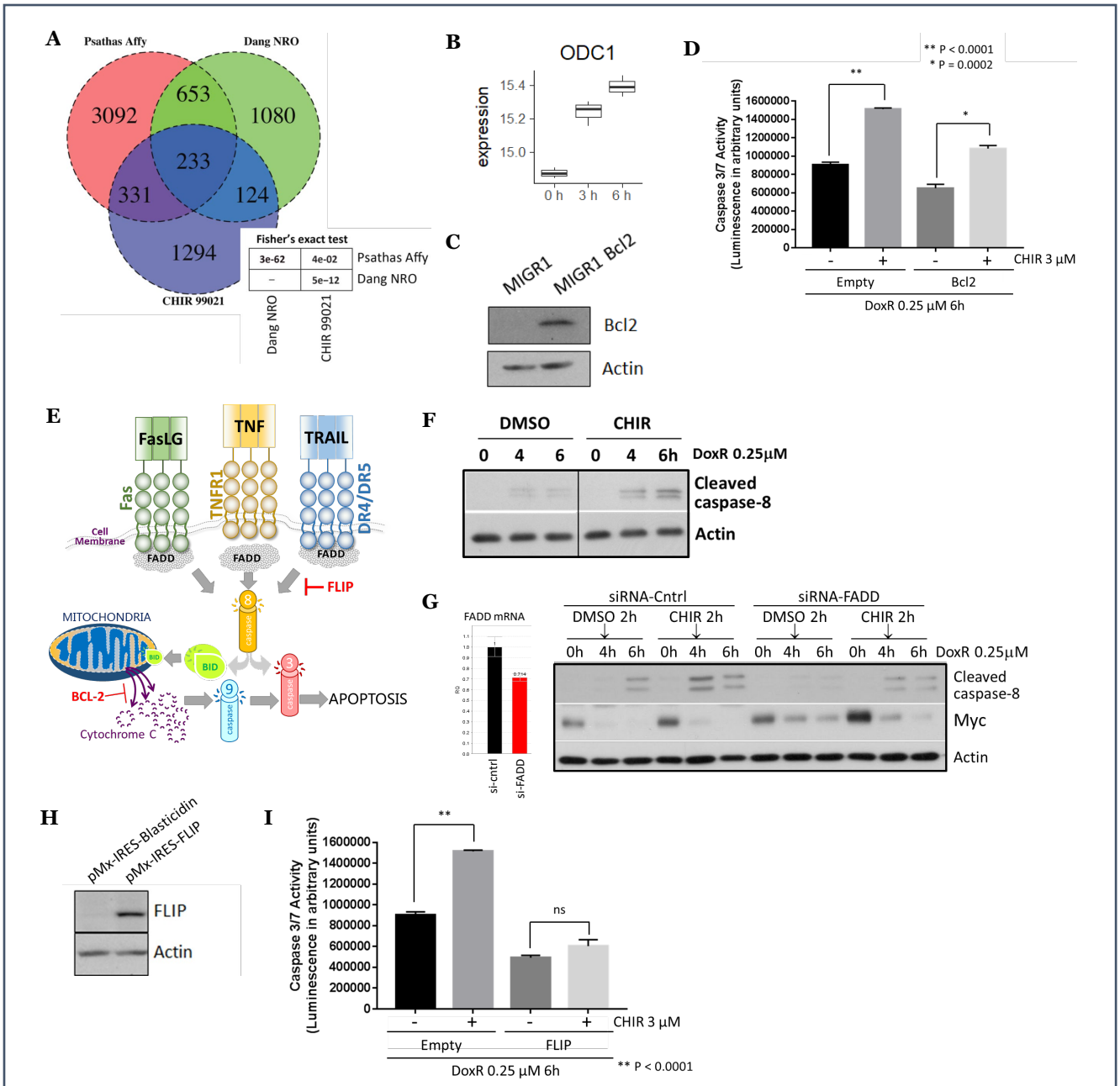

Figure S5. Anti-GSK-3β adjuvant therapy efficacy is abolished by disrupting extrinsic, not intrinsic, apoptosis. A) Venn diagrams showing overlap of activated genes between three datasets (CHIR99021, Psathas Affy, and Dang NRO). **B)** Change in expression level of ODC1 from RNA-sequencing. **C)** Western blotting confirming retroviral over-expression of Bcl-2 in Ramos cells. **D)** Caspase 3/7 Glo luminescence assay was performed on Ramos empty vector or Bcl-2 expressing cells treated as in Fig. 5B. Luminescence was plotted and statistically analyzed by unpaired t-test. **E)** Schematic of the extrinsic apoptotic pathway and its interplay with the intrinsic (mitochondrial) pathway. **F)** Ramos cells were treated for 2 hours with DMSO or 3 µM CHIR99021 followed by doxorubicin. Cleaved caspase-8 levels were assessed by western blotting. **G)** Left panel: qRT-PCR demonstrating modest knockdown of FADD mRNA with FADD-directed siRNA. Right panel: Ramos cells were treated with control or FADD siRNA for 24 hours followed by DMSO or CHIR and doxorubicin as indicated. Western blotting was used to assess activation of caspase-8 and Myc levels. **H)** Western blotting confirming retroviral over-expression of FLIP in Ramos cells. **I)** Caspase 3/7 Glo luminescence assay was performed on Ramos empty vector or FLIP expressing cells treated as in Fig. 6C. Luminescence was plotted and statistically analyzed by unpaired t-test.

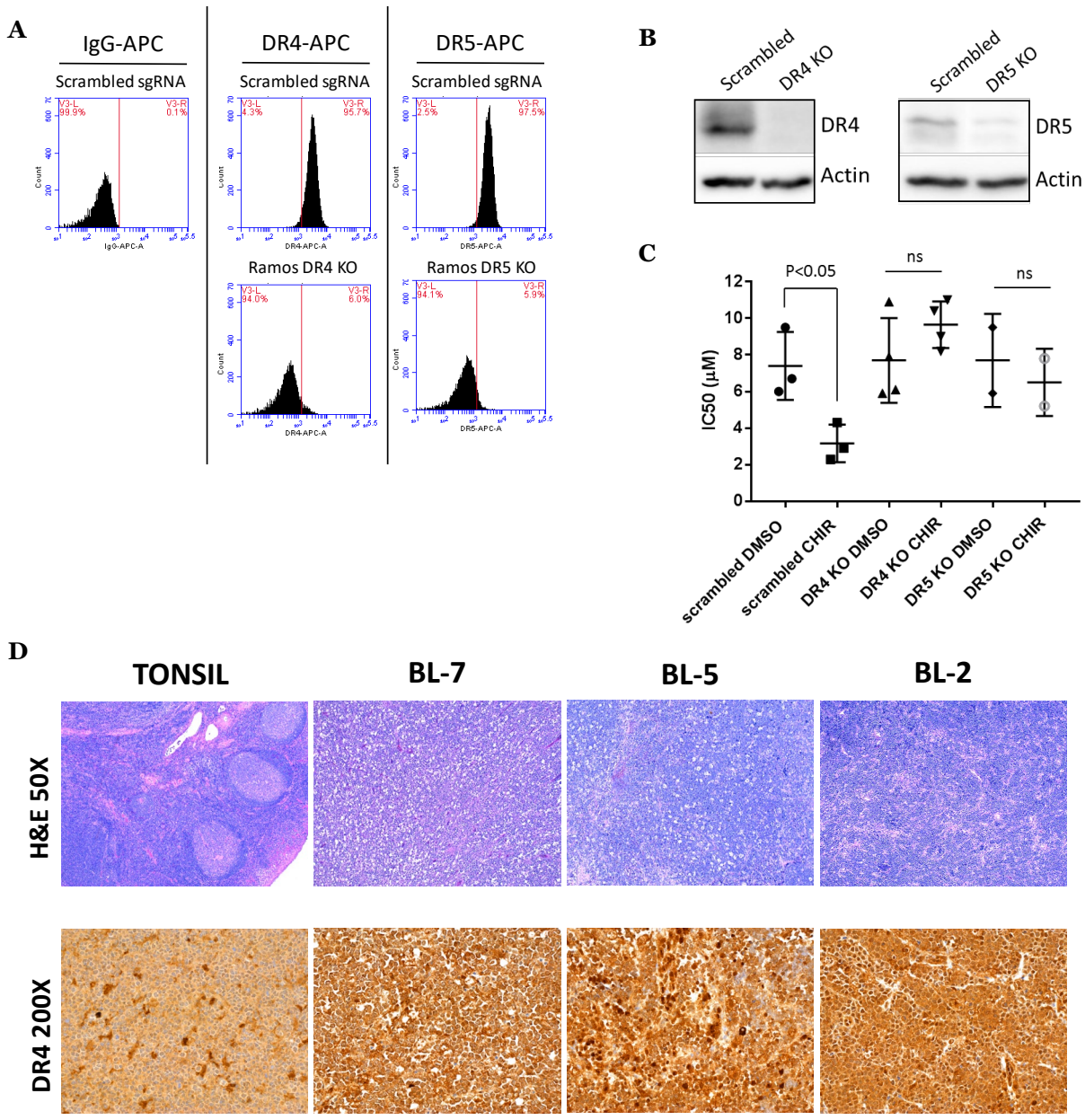

Figure S6. Generation and drug treatment of Ramos death receptor KO cell lines and expression of DR4 in Burkitt lymphoma clinical samples. A) Ramos derivatives with scrambled sgRNA, DR4 KO, and DR5 KO were stained for surface expression of DR4 (middle) or DR5 (right) and analyzed with flow cytometry. Gates were set based on scrambled cells stained with IgG-APC control (left). **B)** Western blotting confirming knockout of DR4 or DR5. **C)** IC50s of multiple experiments as done in Fig. 7B were statistically analyzed by unpaired t-test. **D)** Immunohistochemical staining was performed on human Burkitt lymphomas or normal tonsils. Representative images of hematoxylin and eosin (H&E) and DR4 stains at indicated magnification.