Supplementary Data for

Aggressive B-cell Lymphoma with MYC/TP53 Dual Alterations Displays Distinct Clinicopathobiological Features and Response to Novel Targeted Agents

Supplementary Table S1. Clinicopathologic characteristics of patients with *de novo* DLBCL with dual MYC/TP53 aberrations

	Total N	Myc^{high} Mut-*TP53* n (% of total N)	*P*	Myc^{high}p53^{high} n (% of total N)	*P*	*MYC*-R Mut-*TP53* n (% of total N*)	*P*	Total N*
Patients	480	69 (14.4)		78 (16.5)		15 (4.7)		320
Sex								
F	198	28 (14.1)	1.0	35 (17.7)	.53	5 (3.7)	.60	136
M	282	41 (14.5)		43 (15.2)		10 (5.4)		184
Age, years								
≤ 60	171	25 (14.6)	.89	25 (14.6)	.52	7 (6.5)	.27	107
> 60	309	44 (14.2)		53 (17.2)		8 (3.8)		213
Stage								
I - II	216	27 (12.5)	.19	30 (13.9)	.14	4 (3.0)	.19	134
III–IV	246	42 (17.1)		48 (19.5)		11 (6.4)		173
B symptom								
Yes	132	16 (12.1)	.37	21 (15.9)	.78	7 (6.9)	.40	102
No	282	45 (16.0)		49 (17.4)		8 (4.0)		198
LDH level								
Normal	165	24 (14.5)	1.0	25 (14.3)	.60	2 (1.8)	**.05**	114
Elevated	268	39 (14.6)		47 (26.0)		12 (7.1)		168
ECOG performance status								
< 2	357	52 (14.6)	.84	57 (16.0)	.13	6 (2.6)	.58	228
≥2	57	7 (12.3)		14 (24.6)		1 (3.3)		31
Extranodal sites								
0 - 1	357	46 (13.4)	.26	57 (14.3)	**.0096**	6 (2.6)	**.0039**	235
≥ 2	100	18 (18.0)		14 (26.0)		8 (11.9)		67
Tumor size								
< 5 cm	219	28 (12.8)	.18	30 (13.7)	.067	1 (0.7)	**.044**	149
≥ 5 cm	151	27 (17.9)		32 (21.2)		6 (5.6)		108
IPI score								
0 - 2	266	33 (12.4)	.26	35 (13.2)	**.012**	6 (3.5)	26	171
3 - 5	165	27 (16.4)		38 (23.0)		8 (7.1)		112
Cell of origin								
ABC	231	28 (12.1)	.19	33 (18.1)	.26	3 (1.9)	**.031**	161

GCB	248	41 (16.5)		45 (14.3)		12 (7.5)		159
Ki-67								
< 70%	163	13 (8.0)	**.0038**	17 (10.4)	**.013**	1 (1)	.44	98
≥ 70%	312	55 (17.6)		60 (19.2)		7 (3.3)		215
Therapy response								
CR	364	36 (9.9)	**<.0001**	45 (12.4)	**.0001**	2 (0.8)	**<.0001**	239
PR+PD+SD	116	33 (28.4)		33 (28.4)		13 (16.0)		81
Recurrence								
Yes	194	43 (22.2)	**<.0001**	43 (22.2)	**.0001**	6 (4.8)	.34	125
No	285	24 (8.4)		35 (12.3)		2 (2.3)		88
***MYC*/*BCL2* double-hit**								
No	389	50 (12.9)	.13	58 (14.9)	**.0066**	10 (3.3)	**<.0001**	306
Yes	10	3 (30.0)		5 (50.0)		5 (41.7)		12
MYC/BCL-2 double-expression								
No	315	30 (9.5)	**<.0001**	35 (11.1)	**<.0001**	9 (3.4)	.16	306
Yes	157	38 (24.2)		41 (26.1)		6 (7.2)		12

* Number of cases with *MYC* rearrangement data available. Detailed numbers for single and combinational markers are in Supplementary Table S2.

P values are by the Fisher's exact test for difference between DLBCL cases with concurrent MYC/TP53 aberrations (shown in the table) and those without (not shown).

Abbreviation: *MYC*-R, *MYC* rearrangement; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; CR, complete remission; PR, partial remission; PD, progressive disease; SD, stable disease.

Supplementary Table S2. Frequency of single or dual abnormal Myc protein, p53 protein, BCL-2 protein, *MYC* gene, and *TP53* gene in the studied patients with DLBCL treated with R-CHOP

Biomarker	Number of positive cases/total tested cases	Positive rate
In 480 cohort		
Protein expression marker		
Myc^{high} (Myc >40%)	307/475	64.6%
$p53^{high}$ (p53 >30%)	105/474	22.2%
BCL-2^{high} (BCL-2 >50%)	230/473	48.6%
Genetic lesion markers		
TP53 mutation (Mut-*TP53*)	108/480	22.5%
MYC rearrangement (*MYC*-R)	34/313	10.9%
Combinational markers		
Myc^{high} $p53^{high}$	78/474	16.5%
Myc^{high} Mut-*TP53*	69/480	14.4%
MYC-R $p53^{high}$	13/310	4.2%
MYC-R Mut-*TP53*	8/313	2.6%
In 487 cohort*		
MYC-R $p53^{high}$	18/315	5.7%
MYC-R Mut-*TP53*	15/320	4.7%

* The post-hoc 7 cases were all *MYC*-rearranged *TP53*-mutated cases (*MYC*-R Mut-*TP53*), added to the 480 cohort for sake of better prognostic analysis which however increased the "natural" frequency of *MYC*-R Mut-*TP53*.

Supplementary Table S3. Prognostic factors by univariate analysis and multivariate analysis in DLBCL

A. Univariate analysis

	Overall survival			Progression-free survival		
	HR	**95% CI**	***p***	**HR**	**95% CI**	***p***
B symptom	1.61	1.17-2.21	.004	1.60	1.17-2.18	.003
IPI >2	3.13	2.15-4.56	<.001	2.73	1.92-3.87	<.001
ABC subtype	1.52	1.13-2.04	.005	1.45	1.09-1.93	.01
MYC-R Mut-*TP53*	3.71	1.62-8.48	.002	3.30	1.44-7.55	.005
MYC-R $p53^{high}$	2.61	0.92-7.45	.072	2.88	1.05-7.84	.039
Myc^{high} Mut-*TP53*	2.33	1.65-3.29	<.001	2.45	1.75-3.43	<.001
$Myc^{high}p53^{high}$	1.85	1.37-2.51	<.001	1.89	1.41-2.53	<.001

B. Multivariate analysis

	Overall survival			Progression-free survival		
	HR	**95% CI**	***p***	**HR**	**95% CI**	***p***
B symptom	1.40	.93-2.11	.11	1.52	1.02-2.25	.04
IPI score >2	2.60	1.59-4.26	<.001	2.16	1.37-3.41	.001
ABC subtype	1.39	.92-2.12	.12	1.30	.87-1.95	.20
MYC-R Mut-*TP53*	4.40	1.82-10.65	.001	3.86	1.60-9.32	.003

Abbreviation: HR, Hazard Ratio; CI, confidence interval; IPI, International Prognostic Index; ABC, activated B-cell like; *MYC*-R, *MYC* rearrangement; Mut, mutant.

Supplementary Table S4. Gene expression signatures identified by comparing DLBCL patients with concurrent *MYC*-R Mut-*TP53* or Myc^{high} Mut-*TP53* alterations (double-positive) with DLBCL patients with none or the alterations (double-negative)

Upregulated genes	Downregulated genes
Integrated Mut-*TP53 MYC*-R signature: *MYC*-R Mut-*TP53* vs. Non-*MYC*-R Wt-*TP53* (FDR 0.0005)	
POLR3B, LOC100507266, ***CDKN2A****, WASF1, DNMT3B, UNG*, UHRF1*,* ***TERT****,* <u>*NCR3LG1*</u>*, SMARCA4, E2F2, FZD3, UBE3D,* ***BMP3****, RAG2,* ***TFDP2****,* ***ZNF385B****, BEST3, XYLB, NTF3, C7orf33, PHOX2A, LOC101928191, NR1I3,* ***SLC25A27****, CYP39A1, LZTS1, ART1, NFE2, LINC00550, LOC102725116,* ***OR51B6****,* ***NAA11****, LOC284561, LINC01360, OR2F1,* ***APOH****, MOGAT1,* ***PLA2G2F****, DKFZp434E1119, C1QTNF6, LRRC48, LOC101930593,* ***HMGN5****, ACP6,* ***SLITRK3****, CELSR2, FOXO6,* ***GJB4****, TBX20, STRBP,* ***DIP2C***	*NPLOC4, SP140L, MDFIC, LOC285628,* <u>*TICAM2*</u>*, LRP10, UNC50, SPATA20, RAC2,* ***FAS****,* <u>*NFAT5*</u>*, IL10RA, MPEG1,* ***RASSF5****,* ***IL24,*** *YIPF5, PIGF,* <u>*IL21R*</u>*, ZBTB4, PBXIP1, CMTM6, TNFRSF1B, LOC541471,* <u>*SERPINB1*</u>*, IDS,* <u>*JAK2*</u>*, NCOA1, RAP2C, F8A1, DOCK8, REEP5, MGEA5,* ***SH3GLB1****, TRIM21, SNX18,* <u>*MIR155HG*</u>*, AHR,* ***CFLAR****,* ***SP100****, SP110, CD44, STAT3,* <u>*JAK1*</u>*,* ***BCL2A1****, UGCG, TMSB4X, RNASEK, NFKBIA,* ***MCL1****, YPEL5, PTTG1IP, WSB2, LOC100507463, HCP5,* ***CASP4****,* ***LITAF****, CDC42SE1,* ***RASSF4****,* <u>*IL2RG*</u>*,* ***VMP1****,* <u>*HLA-E*</u>*,* <u>*HLA-F*</u>*,* <u>*HLA-B*</u>*,* <u>*HLA-C*</u>*,* <u>*HLA-A*</u>*,* <u>*HLA-G*</u>*,* <u>*TAP1*</u>*, TPP1,* <u>***HIPK3***</u>*, PLEK, EDF1,* ***PEA15****, ANXA7, ATP6V0E1, CTSH, CAP1, ARPC2*, ARPC5*,* <u>*LCP1*</u>*, SH3BGRL3, TMSB10, UBB*, LGALS8, IL6ST, KIAA1551, GVINP1, N4BP2L2, ARL6IP5, UBD,* <u>*FYB*</u>*,* <u>*CD3D*</u>*, SLFN5,* <u>*LCP2*</u>*, RAB27A,* <u>*SLAMF7*</u>*, GBP2,* <u>*STAT1*</u>*,* ***TNFSF10****, KCTD12, ITM2B, ASAH1, ADAMDEC1, LYZ, GBP3,* ***DAPK1****, GIMAP2, CHI3L1, ENPP2, PTGDS, PLA2G2D,* <u>*PTPRC*</u>*, SPPL2A, SAT1,* ***SRGN****, CDC42SE2, NLRC5,* ***SAMD9L****, PLSCR1, RNF19A, PNRC1,* ***TNFAIP3****,* <u>*STAM2*</u>*, IRF2BP2,* <u>*HLA-DMA*</u>*,* <u>*HLA-DRA*</u>*,* <u>*HLA-DMB*</u>*,* ***RFFL****, FNBP1,* <u>*CD58*</u>*, CD53,* ***RASSF2****, TACC1, PIP4K2A, RNF111, WDR26,* <u>*IL13RA1*</u>*, CXCL13,* ***BIRC3****, PARP12, TMEM2,* <u>*APOBEC3G*</u>*, TINF2, VOPP1, CLIP1, ZFP36, PPAPDC1B, RGS1, AHNAK, UBALD2, ELL2, DOCK10, CCR7, SOS1, TNIP1,* ***FAM129A****,* ***RUNX3****, FAM65B,* <u>*CD99*</u>*, ZNF224, OAZ2, MAFF*
Integrated Myc^{high} Mut-*TP53* signature: Myc^{high} Mut-*TP53* vs. Myc^{low} Wt-*TP53* (FDR 0.0001)	
CDKN2A*, OR7E12P, ALMS1-IT1, UBE3D, XPO5, MTAP, KIAA1958, WASF1,* ***TFDP2****, SRM, MPP6, APLP2, ZNF259,* ***NAA40****, FLJ41455, NREP,* ***HK2****, CCNE1, UNG*, POLR3B, MTHFD2L, ACACA,* <u>*MIR17HG*</u>*,* ***PEG10****,* ***EI24****, KIAA0020, CLSPN*, CEP152, FAM216A, AKAP1, SMARCA4, MCM7, EIF3B,* ***TERT****, C17orf75, EXOSC2, TIMM8B, WDR75, PUS7, DPY19L2P2, GART, ZNF639,* ***MYC****, DKC1, WDR77, IARS, DCUN1D5,* ***WDR4****, FARSB, KLHL23, GCSH, CDC25A, CDC7, SLC16A1, WDR43, BZW2, ASF1B, MZT1, PRKDC*,* ***SNHG1****, SKP2, TMEM48,*	*ARID5B, AHNAK, ZFP36L2, FLII, NCOA1, PPM1M, ZBTB4, HCP5, SOD3, TGOLN2,* <u>*HLA-F*</u>*,* <u>*HLA-E*</u>*, PBXIP1, LGALS8, ARL4C, GVINP1, TRAF3IP3, N4BP2L2, CYLD,* <u>*LCP2*</u>*, SLFN5,* <u>*FYB*</u>*,* <u>*CD3D*</u>*,* <u>*CD3E*</u>*,* <u>***BCL11B***</u>*,* <u>*TRBC1*</u>*, ITM2A, AAK1, FYN,* ***MAF****, GPR174, GBP2, FGL2, RNF213,* <u>*B2M*</u>*, IL6ST, ITM2B, GGTA1P, EVI2B, WIPF1, ATP2B4, INPP4A,* <u>*CD226*</u>*, LBH, DAZAP2,* ***RASSF5****,* <u>*JAK2*</u>*,* <u>*CD58*</u>*,* ***ZBTB38****,* ***TRAF1****, ITGB2-AS1, FGFR1,*

CDT1, SRSF1, TMEM97, PPAT, PAICS, RAD51AP1, CHEK1*, UHRF1*, KIF20A, MCM4, DEPDC1, GMNN, ATAD2, FAM72C, TOP2A, NCAPG, CKS2, MND1*, CENPF, E2F8, RFC4,* ***AURKA****,* ***BUB1B*****, DDX21, MCM8,* ***VRK1****, HSPD1, SERBP1, TOPBP1, DCAF13,* ***BIRC5****, TAF1D, SRPK1, NME2, NCL, SHMT2, LYAR, TFDP1, CMSS1, DIAPH3, SPC25, WHSC1, CAPRIN1, NDC80, POLR3G, POLE2*, KIAA1524, CENPK, CHAF1A*, CTPS1, UCHL3, CENPJ, GTPBP4, SSX2IP, CHCHD3, POLR1B,* ***ARMC10****, DNAJC2, DNMT1, SLC25A33, CCDC150,* ***USP13****, ACN9, PM20D2, LRRC41, ACTL6A, NUFIP1, IPO4, PRMT3, FBXO45, PFKM, NCR3LG1,* ***AIMP2****, MTCH2, PNPT1, SNORA71A, PNN, PGAM5, ASUN, DNAAF2, C11orf57, MORC2*, PIGW, DTYMK, TAF4B, STRBP, PSPH, CYP39A1, RAE1, C15orf41, MARS2, TRIT1, PPP1R8, FZD3, DNMT3B, XYLB, CBX2,* ***E2F1****, SLC19A1, RCL1, EBPL, SQLE, EHBP1, UMODL1, PEX13, SLC25A37, C11orf85*	*ALPK1, IDS, MIR155HG,* ***VMP1****, DOCK10, IL10RA, RGS3, VAMP2*

Abbreviation: FDR, false discovery rate.

Notes: Genes involved in apoptosis, cell death, p53 pathway regulation, or tumor suppression are in bold; genes involved in DNA repair or DNA damage response are marked by *; genes indicating impaired immune responses (such as MHC/TCR/CD3 components) are underlined by straight underlines; and micorRNA genes are underlined with wavy underlines.

Supplementary Table S5. Molecular, genetic and phenotypic status of 8 DLBCL/HGBCL cell lines by targeted next-generation sequencing, fluorescence in situ hybridization (FISH), and immunohistochemistry analysis

Cell line	**Subtype**	***MYC* rearrangement**	**Myc expression**	***TP53* mutation**	**p53 expression**
OCI-LY10	HGBCL-DH	*MYC*-R$^+$	30**%**	Wild type	0%
OCI-LY19	HGBCL-DH	*MYC*-R$^+$	80%	Wild type	10%
MCA	HGBCL-DH	*MYC*-R$^+$	50%	Wild type	100%
RC	HGBCL-DH	*MYC*-R$^+$	60%	Wild type	0%
GR	GCB	FISH failed	50%	Mutated (p.G245A)	90%
HBL1	ABC	Non-*MYC*-R	80%	Mutated (p.V157A)	70%
MZ	HGBCL-DH	*MYC*-R$^+$	40%	Mutated (p.Y126N)	30%
TMD8	HGBCL-DH	*MYC*-R$^+$	60%	Mutated (p.F134C)	90%

Abbreviation: HGBCL-DH, high-grade B cell lymphoma with *MYC*/*BCL2* double-hit; GCB, germinal-center B-cell–like; ABC, activated B-cell–like; *MYC*-R, *MYC* rearrangement.

Supplementary Figure Legends

Supplementary Figure S1. Morphologic and immunophenotypic features of DLBCL with *MYC* rearrangement (*MYC*-R) and *TP53* mutation (Mut-*TP53*) dual-alterations and high-grade B-cell lymphoma (HGBCL) with *MYC*/*BCL2* double-hit (DH) in representative patients. (A) A representative case with both *MYC*-R and Mut-*TP53* but not *BCL2*-R (*MYC*/*TP53* double-hit), characterized by monotonous sheets of medium or large-sized B-cells, with expression of Myc and p53, but negative for BCL-2. (B) A representative case with *MYC*-R, Mut-*TP53*, and *BCL2*-R (*MYC*/*BCL2*/*TP53* triple-hit), characterized by monotonous sheets of large-sized B-cells, with expression of Myc, BCL-2 and p53. (C) A representative case of HGBCL-DH (*MYC*/*BCL2* double-hit), characterized by monotonous sheets of large-sized B-cells, with expression of Myc and BCL-2, but negative for p53.

Supplementary Figure S2. Prognostic impact of p53 and Myc protein overexpression as single or double abnormalities in overall DLBCL and GCB/ABC subtypes.

Supplementary Figure S3. Heatmap for gene expression signatures of MYC/TP53 dual alterations. (A) Differentially expressed genes (DEGs) between the *MYC*-R/Mut-*TP53* and *MYC*-R/Wt-*TP53* groups. (B) DEGs between the *MYC*-R/Mut-*TP53* and non-*MYC*-R/Mut-*TP53* groups. (C) DEGs between the Myc^{high}/Mut-*TP53* and Myc^{low}/Mut-*TP53* Groups. (D) DEGs between Myc^{high}/Mut-*TP53* and Myc^{low}/Wt-*TP53 g*roups.

Supplementary Figure S4. Representative figures of flow cytometric analysis indicating G2/M phase-cell cycle arrest by NCB057643 treatment for 24 hours in cells with *MYC*-R or Myc overexpression with Wt-*TP53* (OCI-LY19) or Mut-*TP53* (GR and TMD8).

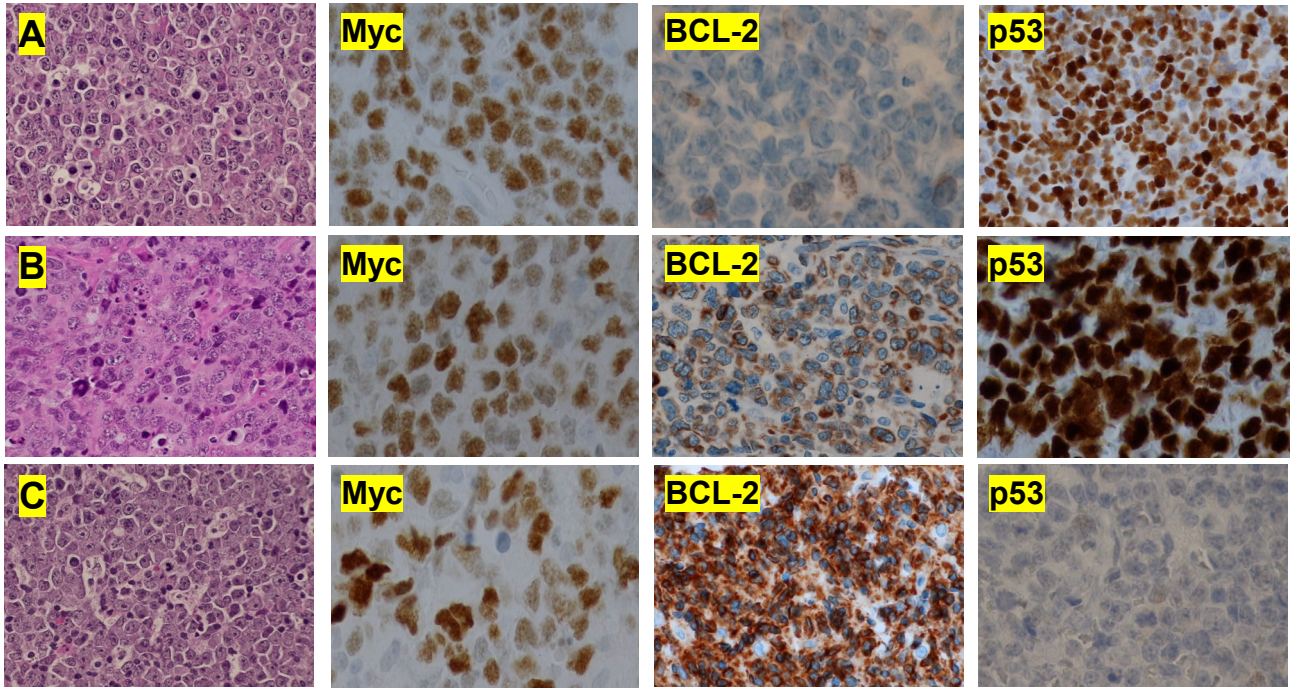
Supplementary Figure S5. Heatmap for significantly up- or downregulated proteins after INCB057643 treatment (5μM, 24 hours) in OCI-LY19 cells. The protein expression levels were measured by RPPA assays performed in three independent experiments.

Supplementary Figure S6. A MDM2 inhibitor DS3032b shows cytotoxic effects selectively in high-grade B-cell lymphoma (HGBCL) with *MYC*/*BCL2* double-hit (DH) and wild-type (Wt) *TP53*. (A) Levels of p53 protein were markedly upregulated by DS3032b treatment (24 hours) in HGBCL-DH cells with Wt-*TP53* (OCI-LY19, OCI-LY10, MCA and RC) but not in those with *TP53* mutation (Mut-*TP53*, TMD8 and MZ). (B) DS3032b significantly suppressed cell viability of HGBCL-DH cells with Wt-*TP53* but not in cell lines with Mut-*TP53*. CellTiter-Glo® 2.0 Assays were performed after treatment for 72 hours. (C) The IC50 values of DS3032b for four HGBCL-DH cell lines with Wt-*TP53* as calculated by GraphPad Prism 8. (D) Exposure with DS3032b for 48 hours induced apoptosis by Annexin V/PI dual staining in four HGBCL-DH cell lines with Wt-*TP53* but not in a HGBCL-DH cell line with Mut-*TP53*. (E) DS3032b treatment for 24 hours blocked the cell cycle at G0/G1 phase, in parallel with the reduction of cell percentage at S phase in Wt-*TP53* cell lines. Values indicate mean ± SD for at least three independent experiments performed in triplicate.

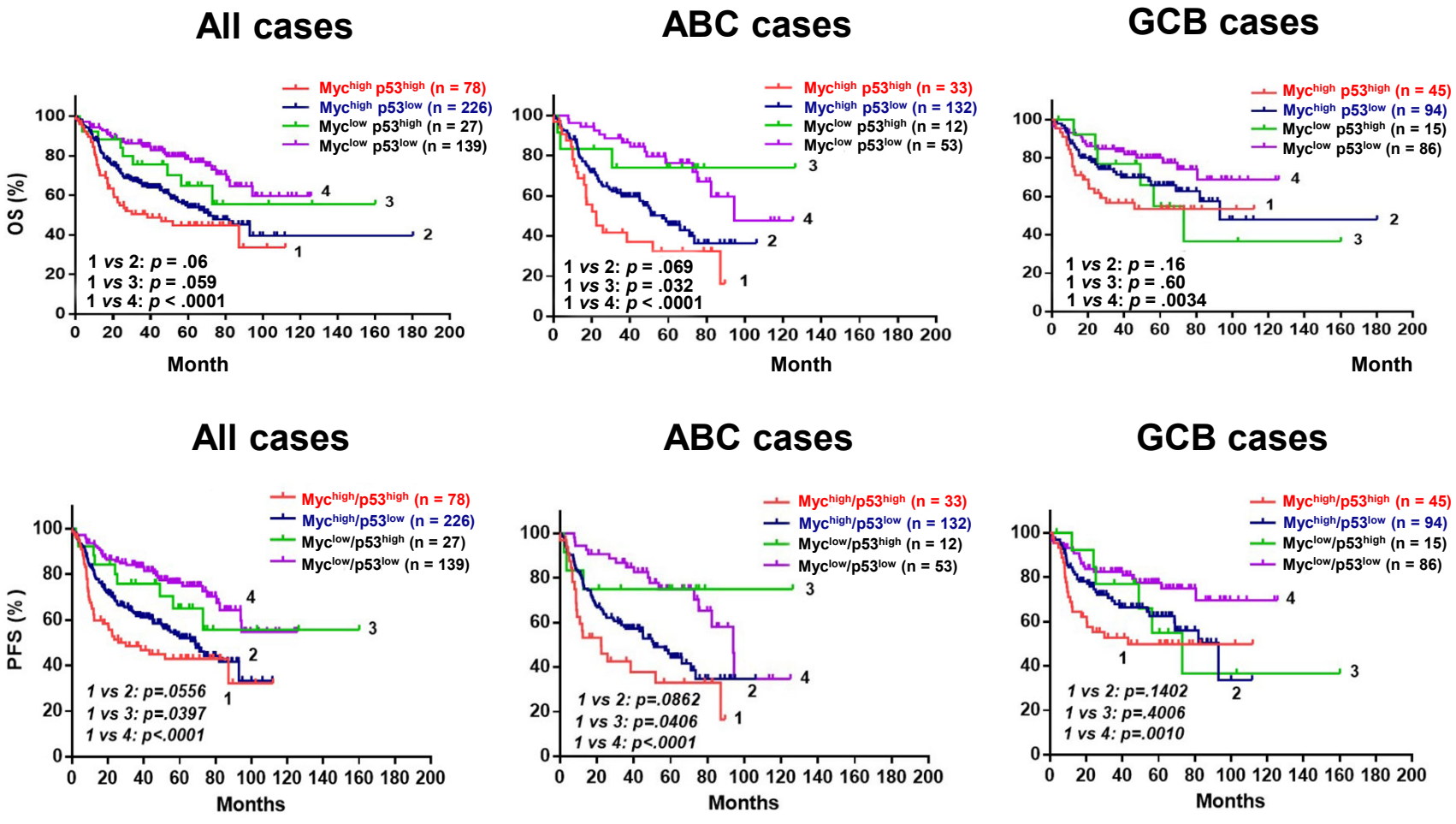
Supplementary Figure S7. Combined DS3032b and INCB057643 treatment has no synergistic cytotoxicity in DLBCL cell lines with MYC aberrations and *TP53* mutation (Mut-*TP53*). Cell viability was measured by CellTiter-Glo® 2.0 Assay at 72 hours. Values indicate mean ± SD for at least three independent experiments performed in triplicate.

Supplementary Figure S8. INCB057643 treatment (1.25 μM) alone or in combination with ABT-199 (venetoclax, 6.25 mM) for 24 hours induced p21 expression in TMD8 cells, whereas had no effect on p53 (mutant) expression levels.

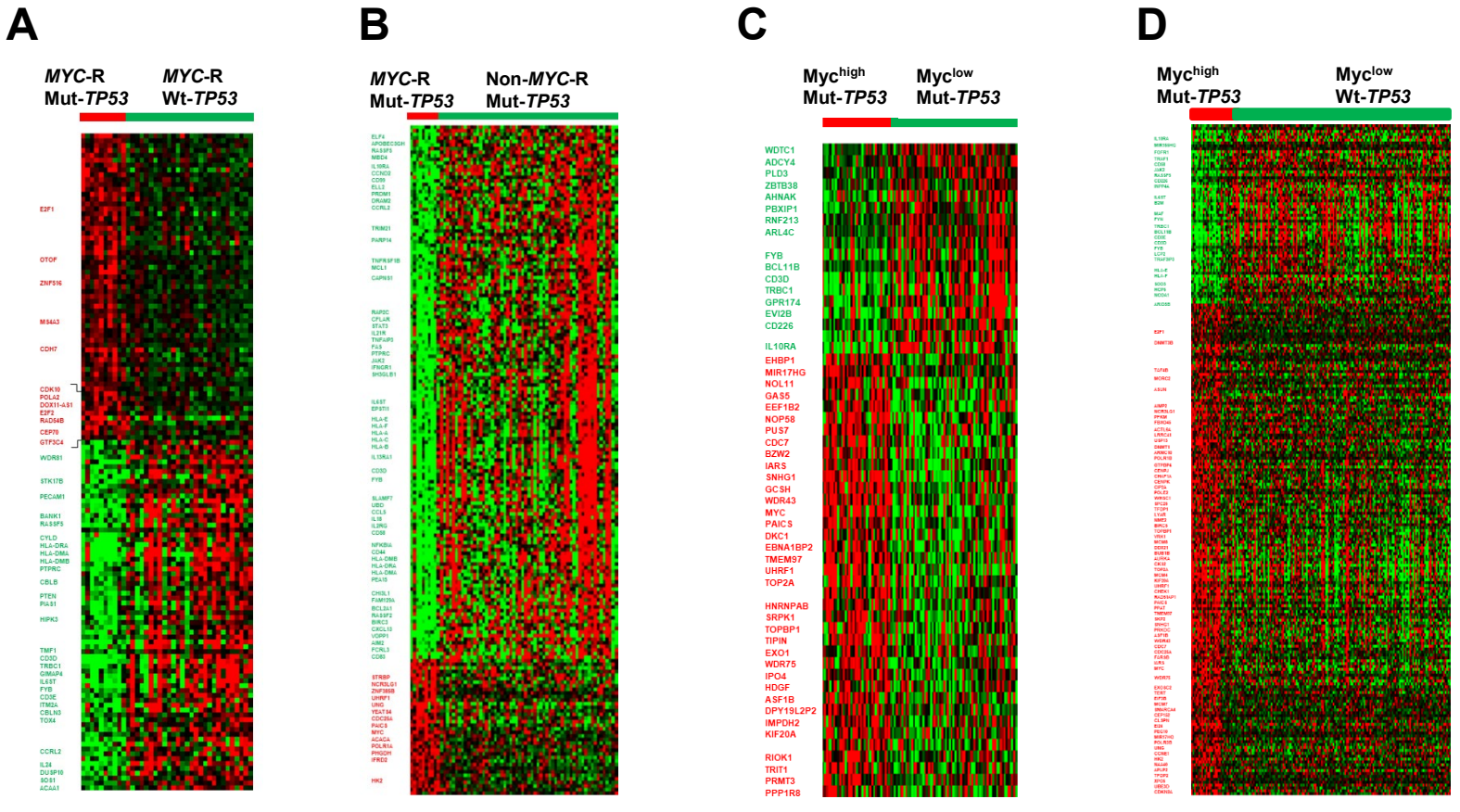
Supplementary Figure S1



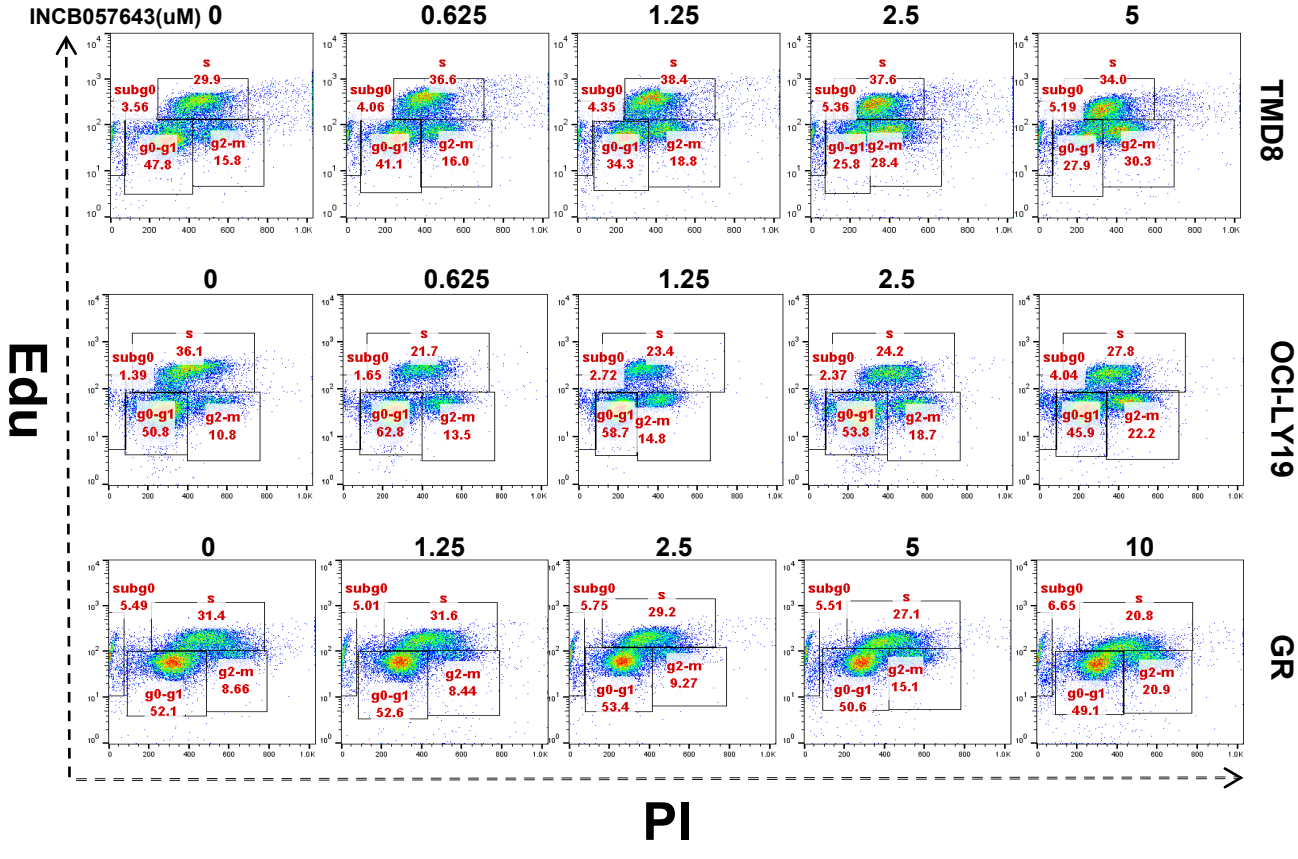
Supplementary Figure S2



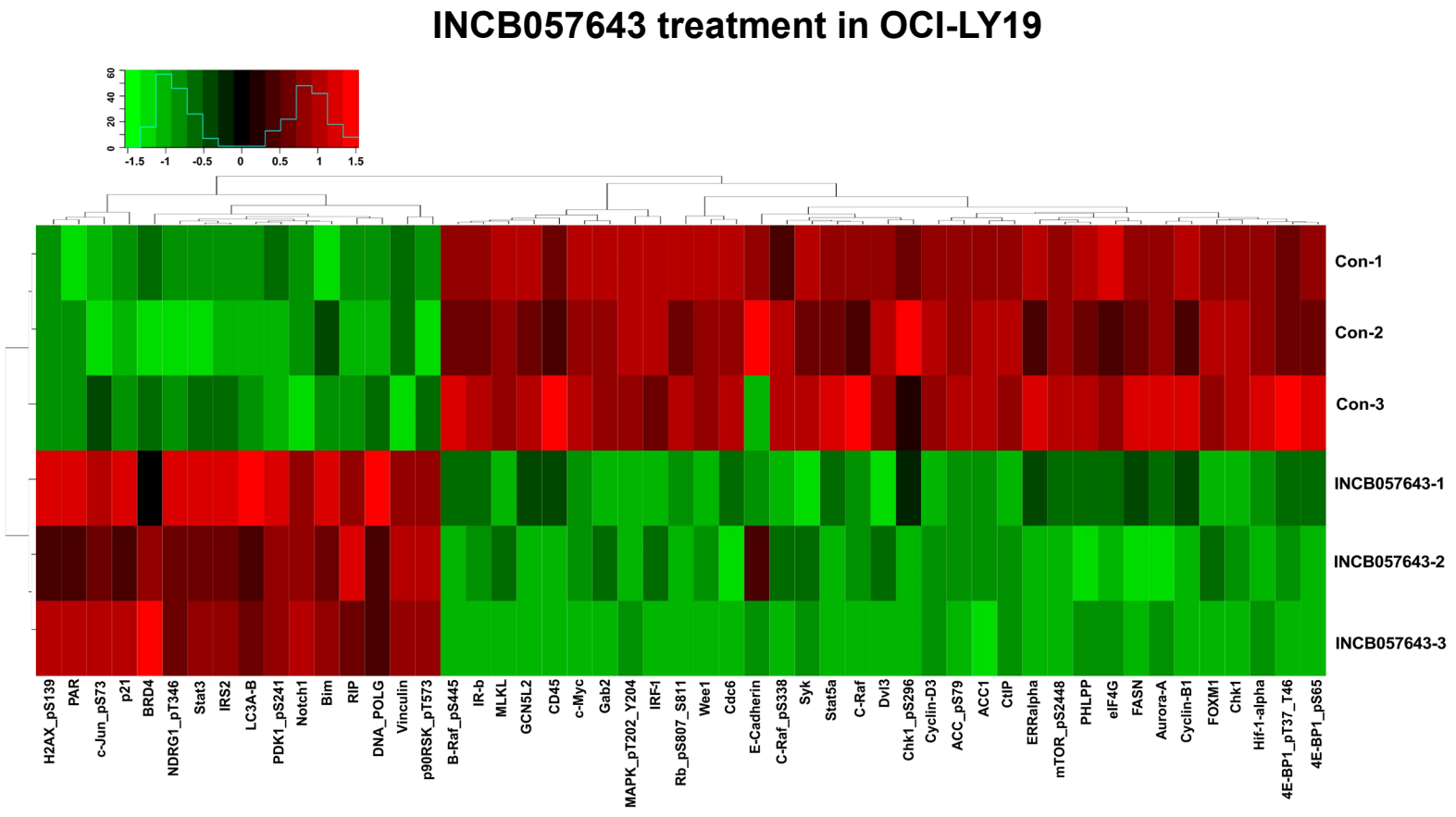
Supplementary Figure S3



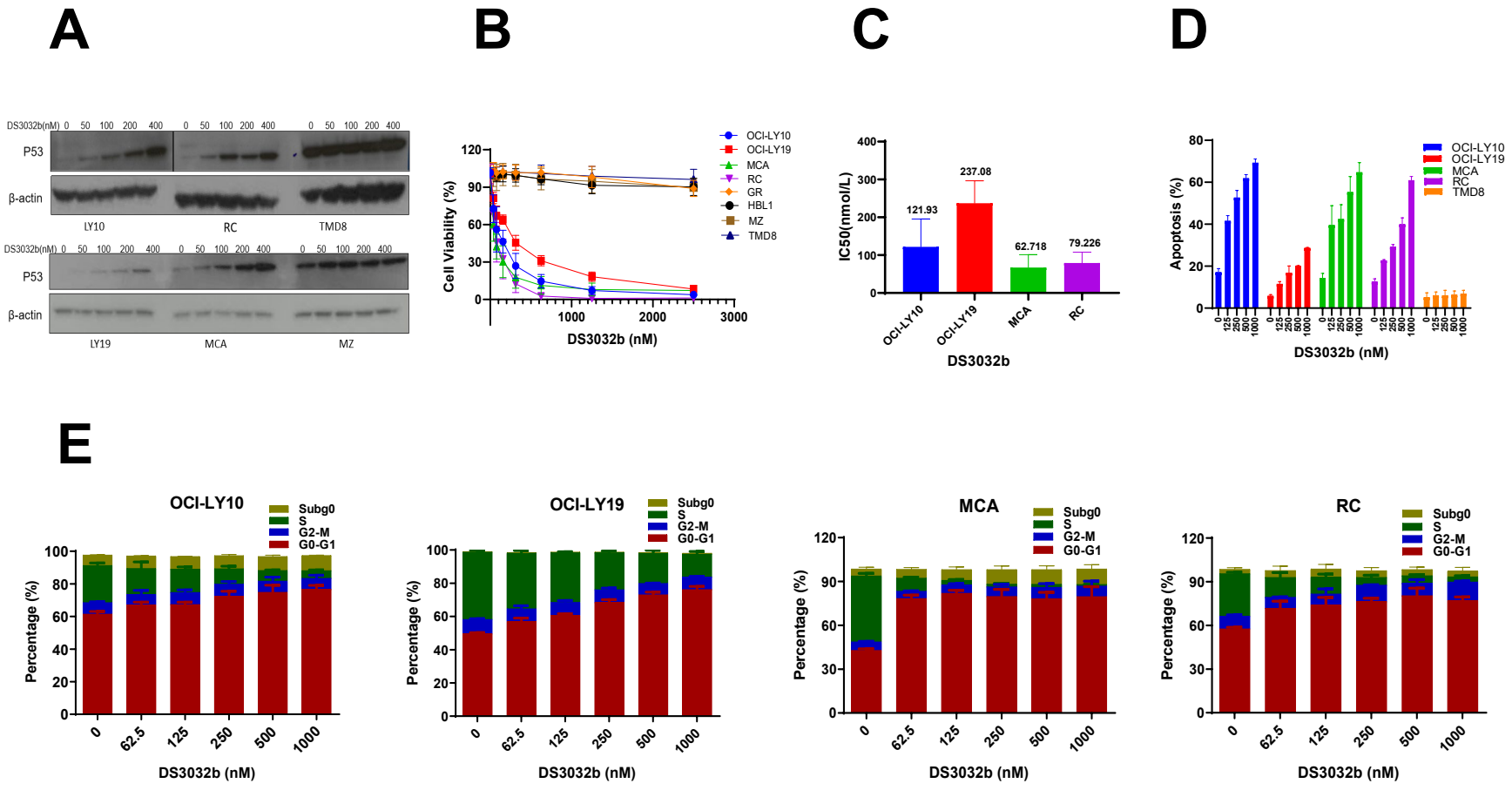
Supplementary Figure S4



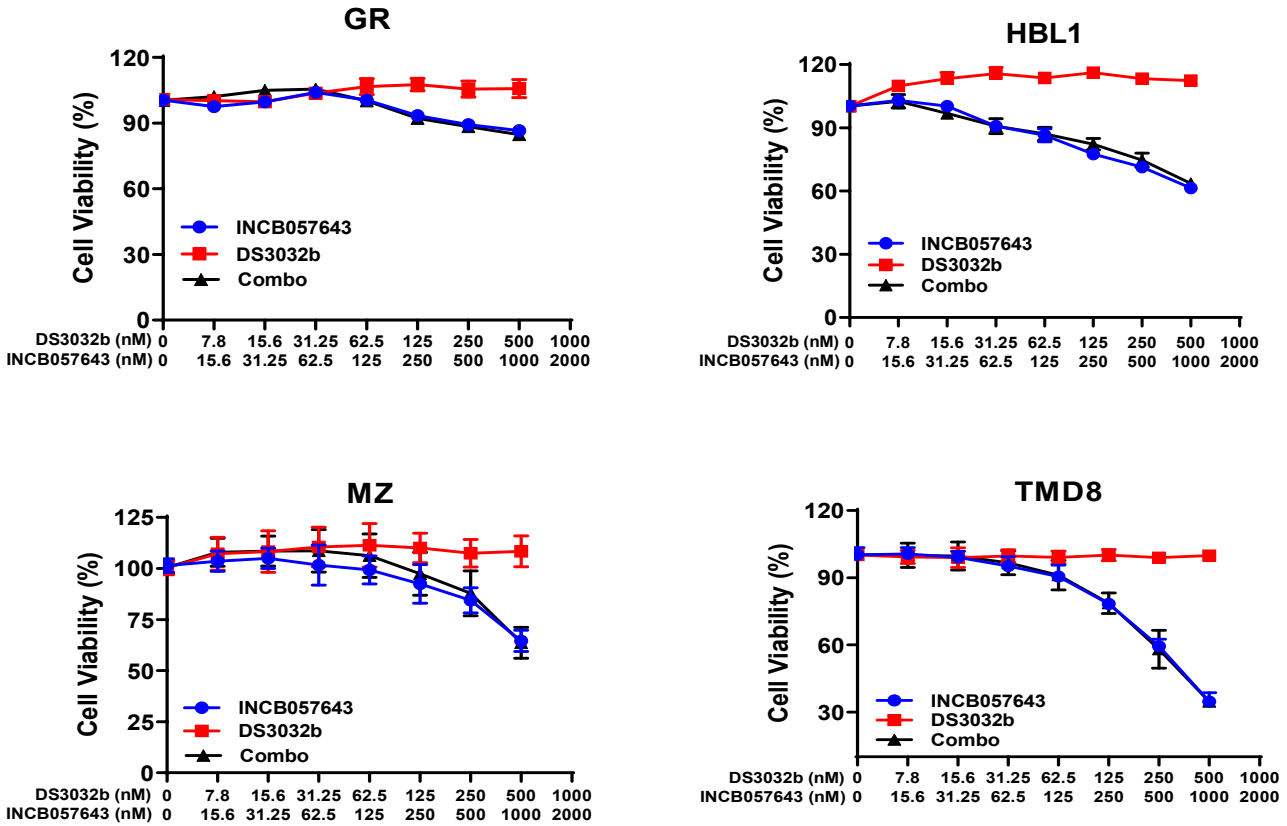
Supplementary Figure S5



Supplementary Figure S6



Supplementary Figure S7



Supplementary Figure S8