Supporting Information 1

Optimization of tetrahydroindazoles as inhibitors of human dihydroorotate dehydrogenase and evaluation of their activity and in vitro metabolic stability

Gergana Popova,[†*] Marcus J. G. W. Ladds,[†,#,∂] Lars Johansson,[§] Aljona Saleh,[‖] Johanna Larsson,[‡] Lars Sandberg,[‡,∫] Sara Häggblad Sahlberg,[£] Weixing Qian,[&] Hjalmar Gullberg,[£] Neeraj Garg,[‡] Anna-Lena Gustavsson,[§] Martin Haraldsson,[§] David Lane,[†] Ulrika Yngve,[‡] Sonia Lain[†,#]

[†]Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Solnavägen 9, SE-171 65 Solna, Stockholm, Sweden.

[#]SciLifeLab, Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Tomtebodavägen 23, SE-171 21 Solna, Stockholm, Sweden.

[§]Chemical Biology Consortium Sweden, SciLifeLab, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, SE-171 21 Stockholm, Sweden.

[‖] SciLifeLab, Drug Discovery and Development Platform, ADME of Therapeutics Facility, Department of Pharmacy, Uppsala University, Box 580, SE-751 23 Uppsala, Sweden.

[‡]SciLifeLab, Drug Discovery and Development Platform, Department of Medicinal Chemistry, Uppsala University, Box 574, SE-751 23, Uppsala, Sweden

[∫] SciLifeLab, Drug Discovery and Development Platform, Department of Organic Chemistry, Stockholm University, Box 1030, SE-171 21 Solna, Sweden.

[£]SciLifeLab, Drug Discovery and Development Platform, Department of Biochemistry and Biophysics, Stockholm University, SE-171 21 Solna, Stockholm, Sweden

[&]Chemical Biology Consortium Sweden, Laboratories for Chemical Biology Umeå, Umeå University, SE-901 87 Umeå, Sweden.

[*]*corresponding author*

Content of Supporting Information:

Supporting Information 1:

Supplementary Figures S1 – S14.

Supplementary Tables S1 - S3.

Supporting Information 2:

A pdf file containing the HPLC and HRMS raw data of selected compounds.

Supporting Information 3:

Molecular formula strings table.

Supplementary Information Figures:

Figure S1.

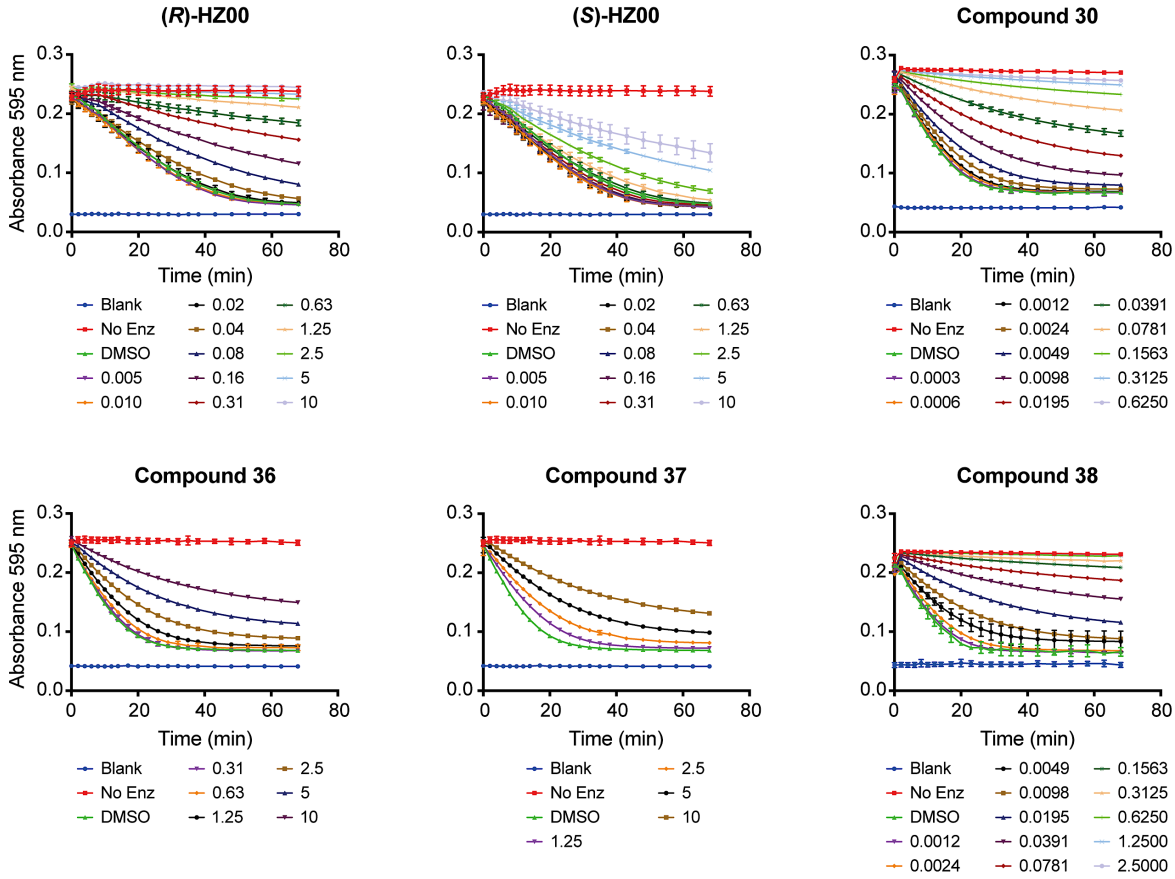

Figure S1. Inhibitory activity of HZ00 analogues. The inhibitory activity of the HZ00 analogues was measured in an enzymatic assay using DCIP as a final electron acceptor and 4 nM of recombinant DHODH. Several concentrations (µM) of each compound were tested in three technical replicates. The IC_{50} was calculated based on V_{max} estimation using linear regression analysis within the linear range of the reaction. The mean IC_{50} values in Table 1 are calculated based on the IC_{50} obtained from three independent biological replicates ± SD.

Figure S2.

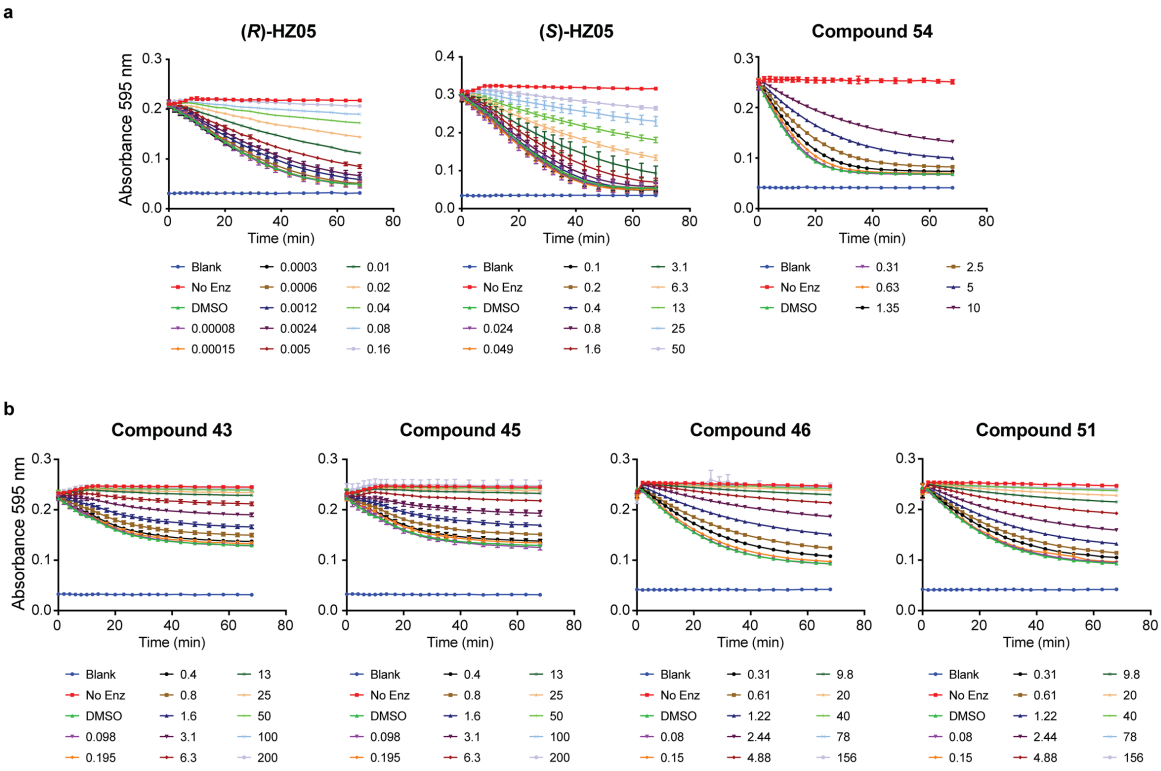

Figure S2. Inhibitory activity of HZ05 analogues. The inhibitory activity of the HZ05 analogues was measured in an enzymatic assay using DCIP as a final electron acceptor. Several concentrations of each compound were tested in three technical replicates. The IC_{50} was calculated based on V_{max} estimation using linear regression analysis within the linear range of the reaction. (a) The enzymatic assay was performed with 4 nM of recombinant DHODH. The inhibitor concentrations in the legend is in µM. (b) The enzymatic assay was performed with 2 nM of recombinant DHODH. The inhibitor concentrations in the legend is in nM. The mean IC_{50} values in Table 2 of the main text are calculated based on the IC_{50} obtained from three independent biological replicates ± SD.

Figure S3.

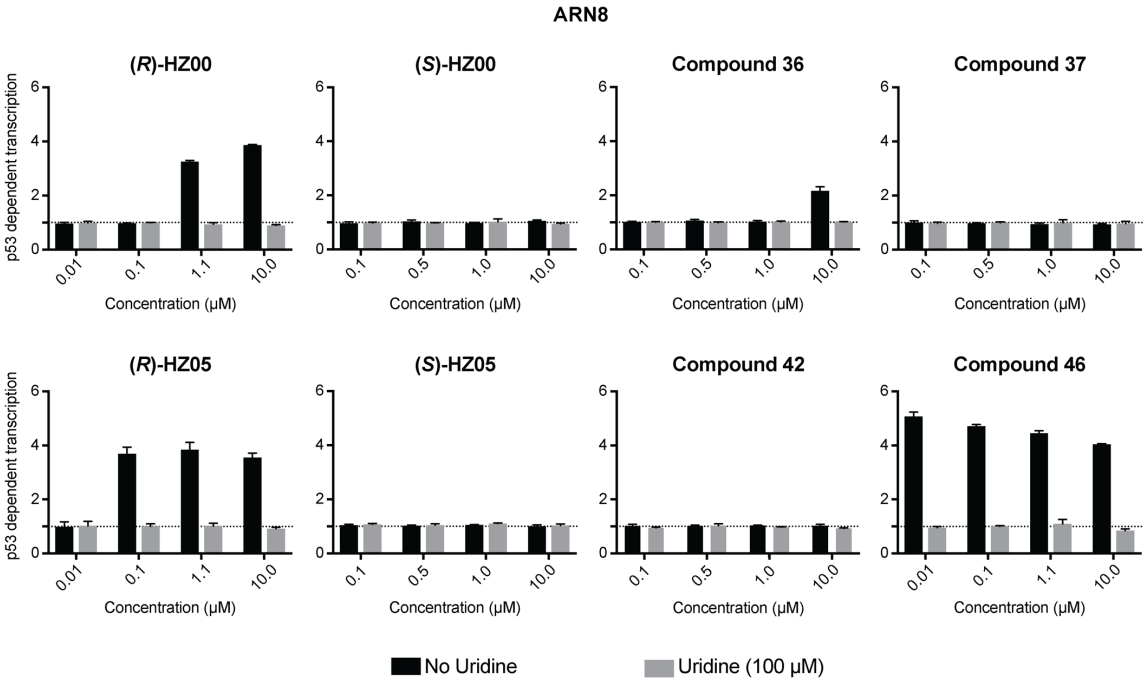

Figure S3. Effect of HZ00 and HZ05 enantiomers and analogues on p53 transcription factor activity. ARN8 cells were treated for 16 h with the indicated compounds in a medium supplemented with 100 μM uridine or without supplementation. The induction of p53-dependent transcription was measured using β-galactosidase CPRG substrate as described in the Experimental section. All assays were performed with 2 μM nutlin-3a as a positive control, excluded from the graphs. Values correspond to the average of three technical replicates ± SD. The experiments are representative of at least two independent biological replicates.

Figure S4.

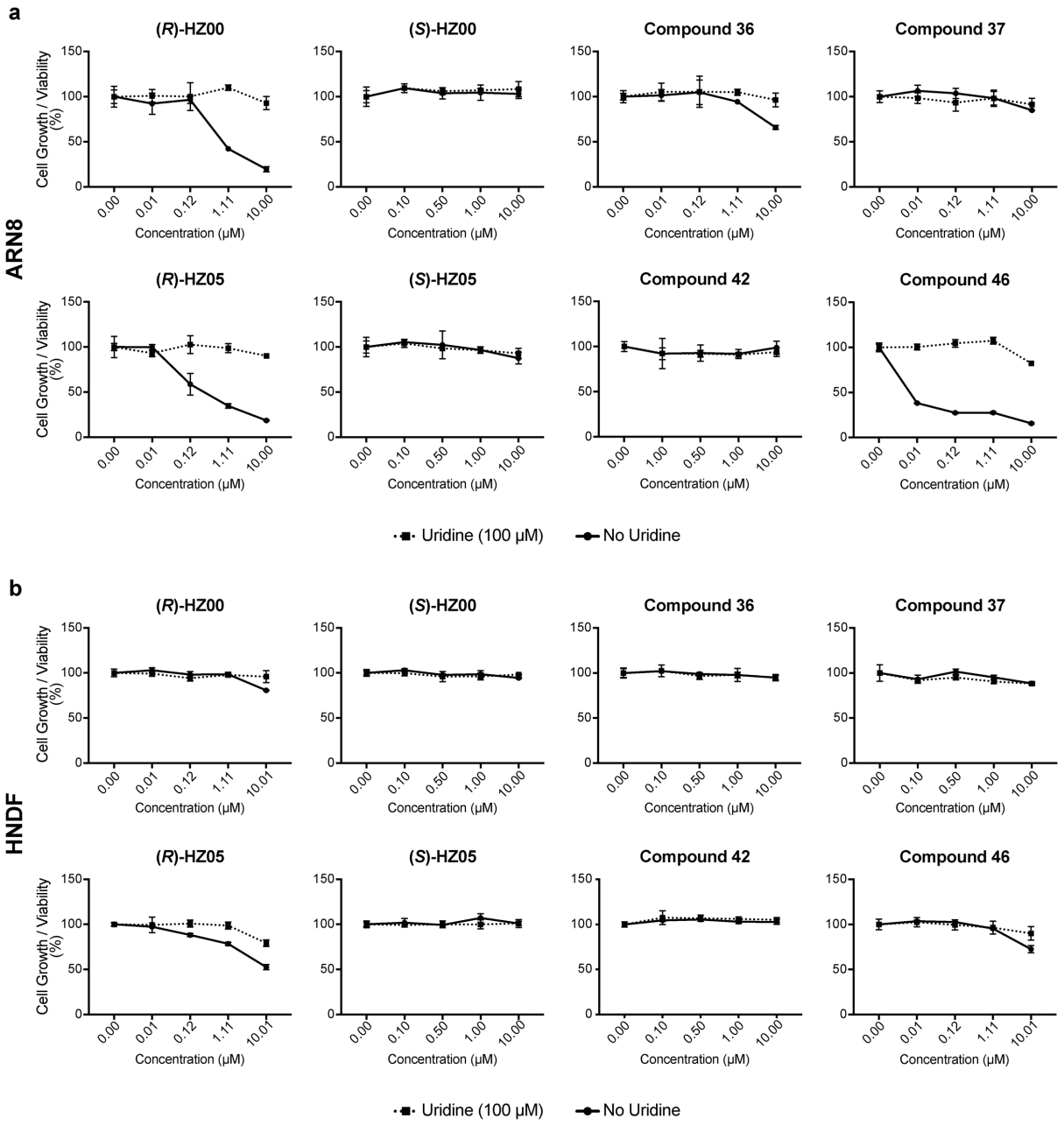

Figure S4. Effect of HZ00 and HZ05 enantiomers and analogues on cell growth/viability. **(a)** ARN8 cells or **(b)** HNDFs, were treated with the indicated HZ00 and HZ05 analogues for 72 h in medium supplemented with 100 μM uridine or without supplementation. The effect of the compounds on cell growth/viability was measured by sulforhodamine B staining. Values

correspond to the average of three technical replicates ± SD. The experiments are representative of at least two independent biological replicates.

Figure S5.

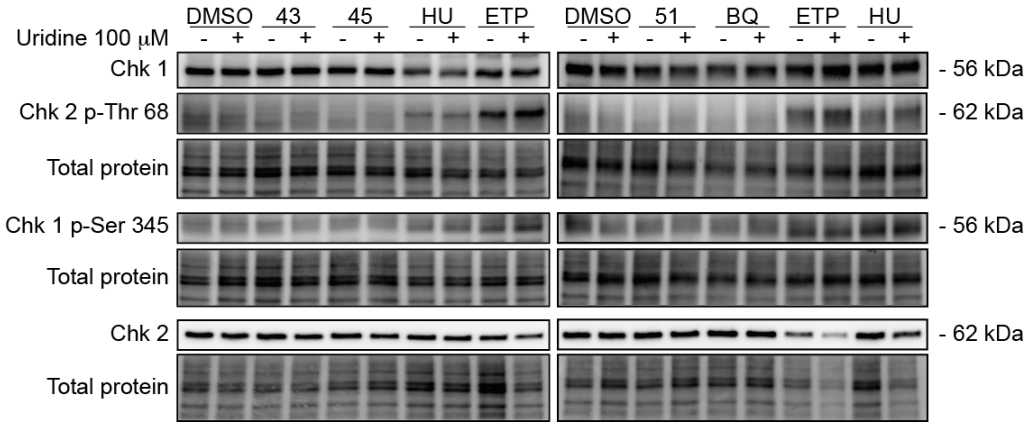

Figure S5. DHODH inhibitors do not activate markers of DNA damage. ARN8 cells were treated for 24 h with 20 nM of the corresponding HZ compounds or 250 nM brequinar (BQ). Where indicated, medium was supplemented with 100 μM uridine. The protein levels of Chk-1, Chk-2, Chk-1 p-Ser345 and Chk-2 p-Thr68 were analyzed by western blotting. Total protein was used as a loading control. Treatment with 2 mM hydroxyurea (HU) for 4 h was used as positive control for single strand DNA breaks. Treatment with 20 μM etoposide (ETP) for 1 h was used as a positive control for DNA double strand breaks.

Figure S6.

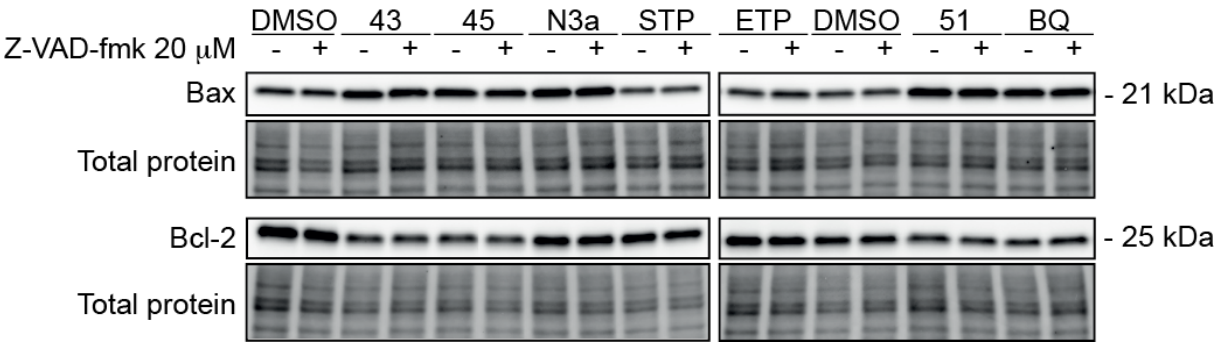

Figure S6. Effect of DHODH inhibition on proteins upstream the caspase cascade. ARN8 cells were treated for 24 h with 20 nM of compounds **43**, **45**, **51**, 250 nM brequinar (BQ) or 2 μM nutlin-3a (N3a). 20 μM Z-VAD-FMK or vehicle control were added 4.5 h prior to harvesting. Treatment with 20 μM etoposide (ETP) was for 1 h and treatment with 1 μM staurosporine (STP) was for 4.5 h, with 20 μM Z-VAD-FMK or vehicle added for 1 h and 4.5 h respectively. The protein levels of Bax and Bcl-2 were analyzed by western blotting. Total amount of protein was used as a loading control.

Figure S7.

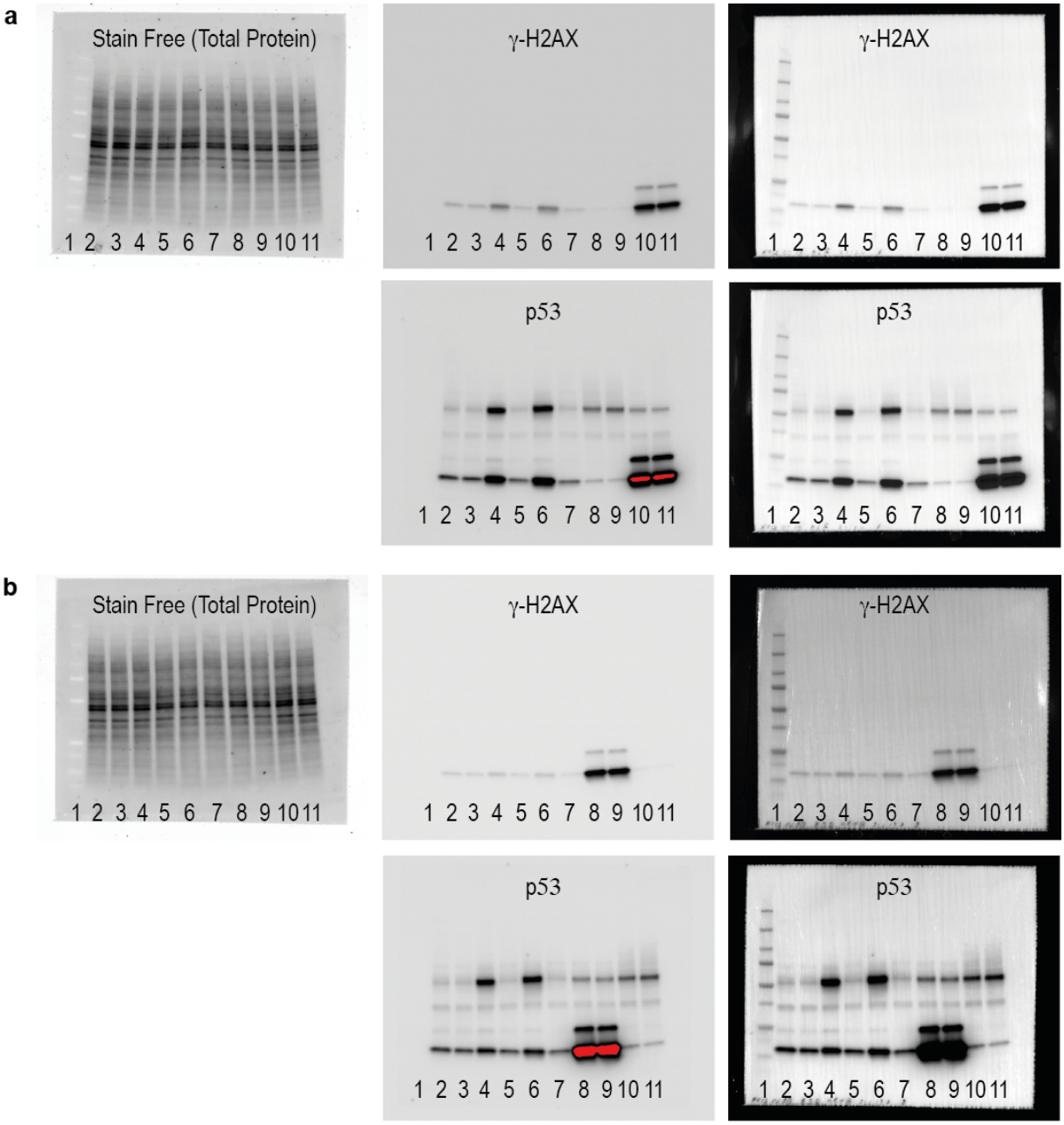

Figure S7. Whole blots from Figure 6a (γ-H2AX and p53). Left - stain free membrane (total protein levels), middle - chemiluminescence image (antibodies), right - merged chemiluminescence and colorimetric image (protein marker). **(a)** Lane 1: Precision Plus Protein All Blue Standards (BioRad #161-0373); Lane 2: DMSO; Line 3: DMSO plus 100 μM uridine;

Lane 4: compound **43**; Lane 5: compound **43** plus 100 μM uridine; Lane 6: compound **45**; Lane 7: compound **45** plus 100 μM uridine; Lane 8: nutlin-3a (N3a); Lane 9: N3a plus 100 μM uridine; Lane 10: etoposide (ETP); Lane 11: ETP plus 100 μM uridine; **(b)** Lane 1: Precision Plus Protein All Blue Standards (BioRad #161-0373); Lane 2: DMSO; Lane 3: DMSO plus 100 μM uridine; Lane 4: compound **51**; Lane 5: compound **51** plus 100 μM uridine; Lane 6: brequinar (BQ); Lane 7: BQ plus 100 μM uridine; Lane 8: ETP; Lane 9: ETP plus 100 μM uridine; Lane 10: N3a; Lane 11: N3a plus 100 μM uridine.

Figure S8.

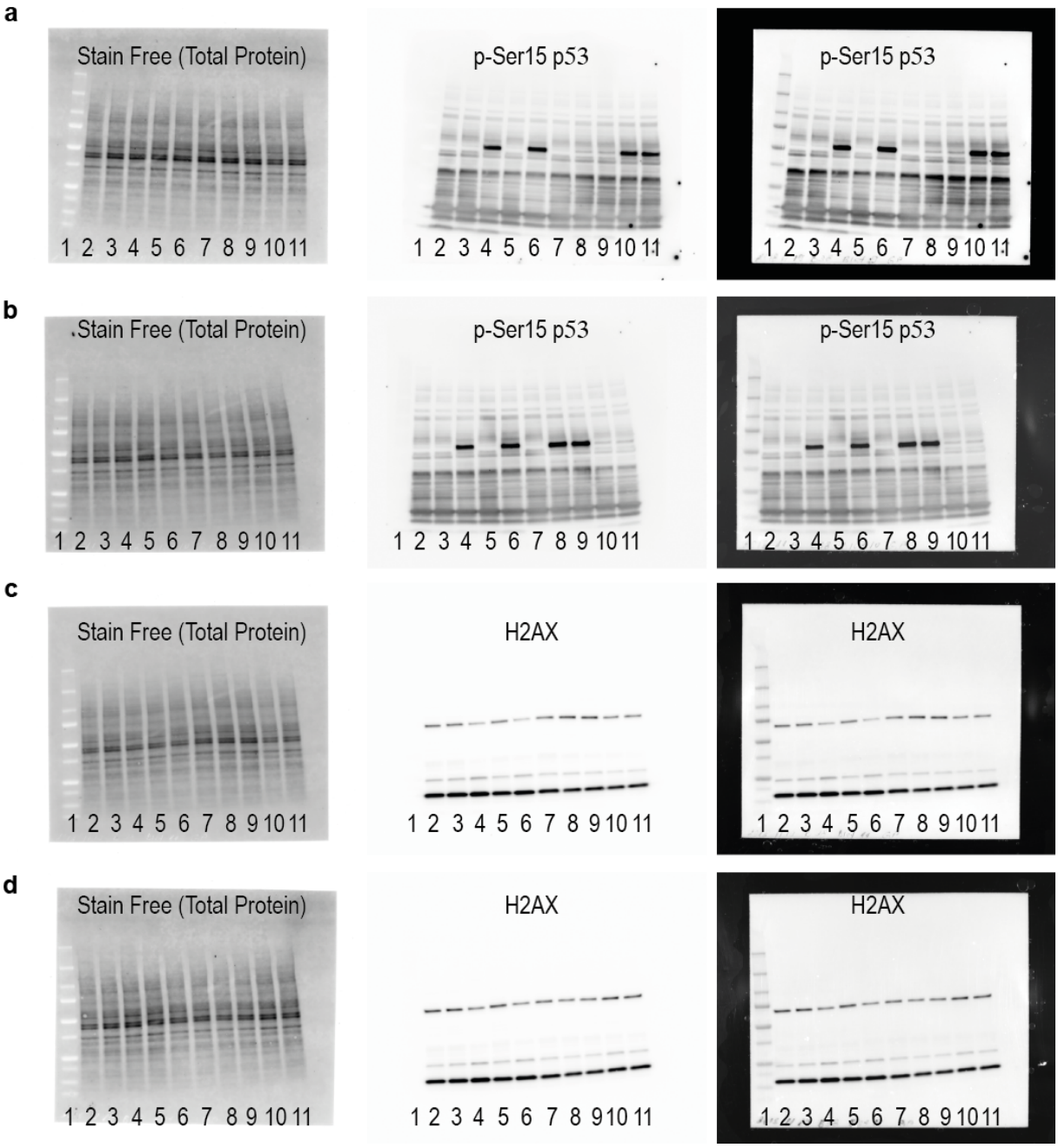

Figure S8. Whole blots from Figure 6a (p-Ser15 p53 and H2AX). Left - stain free membrane (total protein levels), middle - chemiluminescence image (antibodies), right - merged chemiluminescence and colorimetric image (protein marker). **(a)** and **(c)** Lane 1: Precision Plus Protein All Blue Standards (BioRad #161-0373); Lane 2: DMSO; Lane 3: DMSO plus 100 μM

uridine; Lane 4: compound **43**; Lane 5: compound **43** plus 100 μM uridine; Lane 6: compound **45**; Lane 7: compound **45** plus 100 μM uridine; Lane 8: nutlin-3a (N3a); Lane 9: N3a plus 100 μM uridine; Lane 10: etoposide (ETP); Lane 11: ETP plus 100 μM uridine; **(b)** and **(d)** Lane 1: Precision Plus Protein All Blue Standards (BioRad #161-0373); Lane 2: DMSO; Lane 3: DMSO plus 100 μM uridine; Lane 4: compound **51**; Lane 5: compound **51** plus 100 μM uridine; Lane 6: brequinar (BQ); Lane 7: BQ plus 100 μM uridine; Lane 8: ETP; Lane 9: ETP plus 100 μM uridine; Lane 10: N3a; Lane 11: N3a plus 100 μM uridine.

Figure S9.

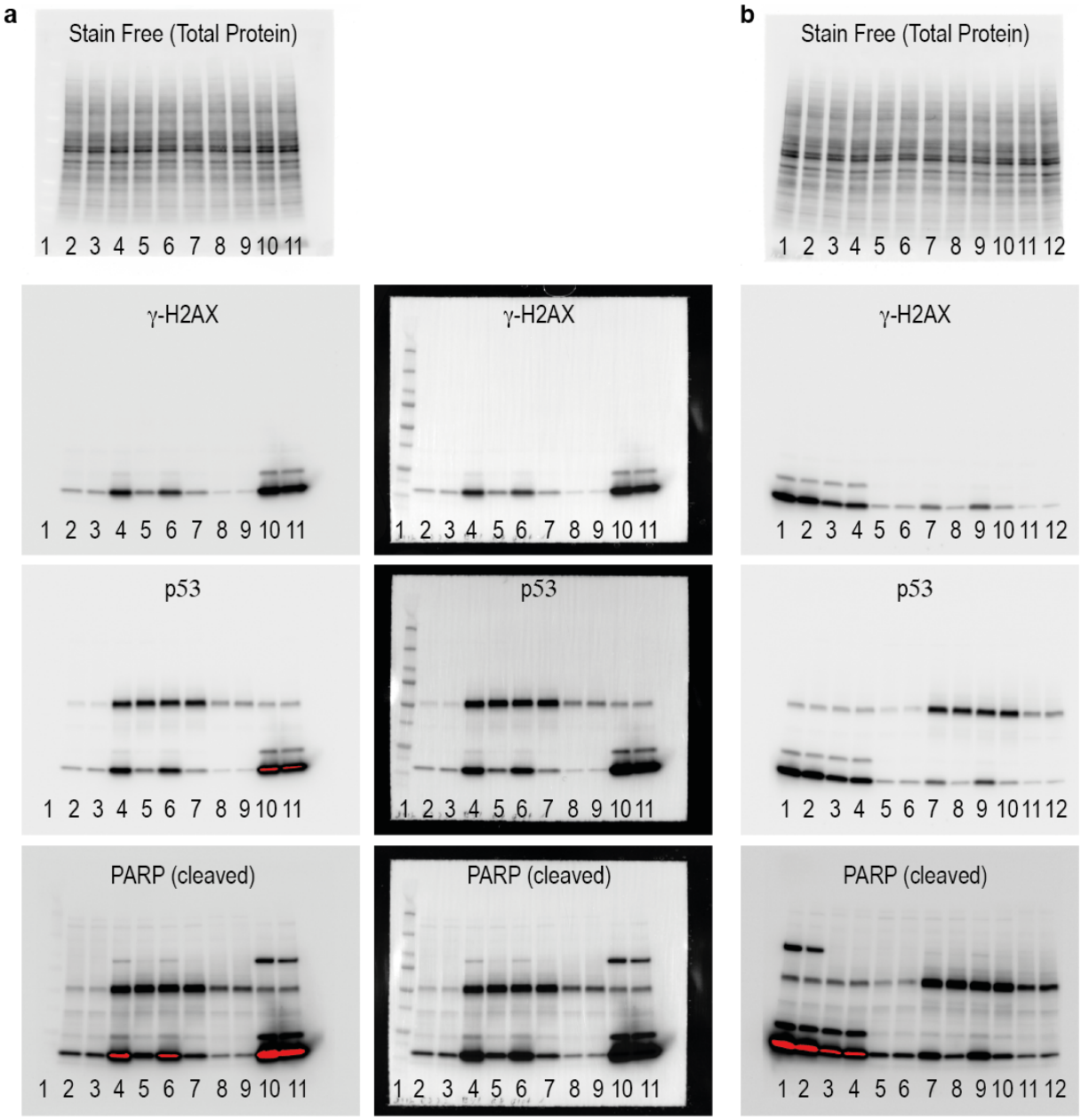

Figure S9. Whole blots from Figure 6b (γ-H2AX, p53 and PARP cleaved). (a) Top - stain free membrane (total protein levels), underneath left - chemiluminescence image (antibodies), right - merged chemiluminescence and colorimetric image (protein marker). Lane 1: Precision Plus Protein All Blue Standards (BioRad #161-0373); Lane 2: DMSO; Lane 3: DMSO plus 20 μM Z-VAD-FMK; Lane 4: compound **43**; Lane 5: compound **43** plus 20 μM Z-VAD-FMK;

Lane 6: compound **45**; Lane 7: compound **45** plus 20 μM Z-VAD-FMK; Lane 8: nutlin-3a (N3a); Lane 9: N3a plus 20 μM Z-VAD-FMK; Lane 10: staurosporine (STP); Lane 11: STP plus 20 μM Z-VAD-FMK; **(b)** Top - stain free membrane (total protein levels), underneath - chemiluminescence image (antibodies). Lane 1: STP; Lane 2: STP plus 20 μM Z-VAD-FMK; Lane 3: etoposide (ETP); Lane 4: ETP plus 20 μM Z-VAD-FMK; Lane 5: DMSO; Lane 6: DMSO plus 20 μM Z-VAD-FMK; Lane 7: compound **51**; Lane 8: compound **51** plus 20 μM Z-VAD-FMK; Lane 9: brequinar (BQ); Lane 10: BQ plus 20 μM Z-VAD-FMK; Lane 11: N3a; Lane 12: N3a plus 20 μM Z-VAD-FMK.

Figure S10.

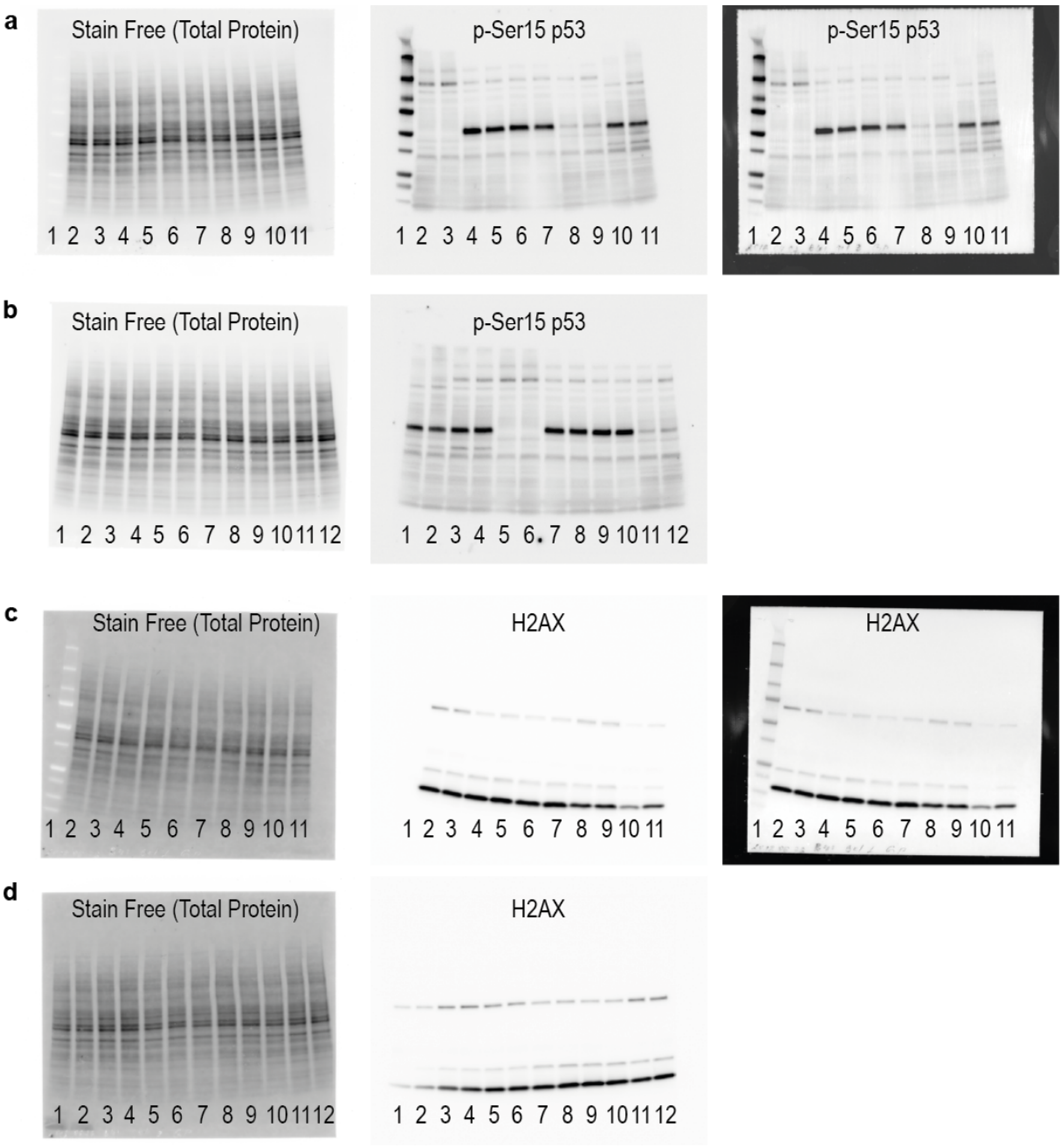

Figure S10. Whole blots from Figure 6b (p-Ser15 p53 and H2AX). (a) and **(c)** left - stain free membrane (total protein levels), middle - chemiluminescence image (antibodies), right - merged chemiluminescence and colorimetric image (protein marker). Lane 1: Precision Plus Protein All Blue Standards (BioRad #161-0373); Lane 2: DMSO; Lane 3: DMSO plus 20 μM

Z-VAD-FMK; Lane 4: compound **43**; Lane 5: compound **43** plus 20 μM Z-VAD-FMK; Lane 6: compound **45**; Lane 7: compound **45** plus 20 μM Z-VAD-FMK; Lane 8: nutlin-3a (N3a); Lane 9: N3a plus 20 μM Z-VAD-FMK; Lane 10: staurosporine (STP); Lane 11: STP plus 20 μM Z-VAD-FMK; **(b)** and **(d)** left - stain free membrane (total protein levels), right - chemiluminescence image (antibodies). Lane 1: STP; Lane 2: STP plus 20 μM Z-VAD-FMK; Lane 3: etoposide (ETP); Lane 4: ETP plus 20 μM Z-VAD-FMK; Lane 5: DMSO; Lane 6: DMSO plus 20 μM Z-VAD-FMK; Lane 7: compound **51**; Lane 8: compound **51** plus 20 μM Z-VAD-FMK; Lane 9: brequinar (BQ); Lane 10: BQ plus 20 μM Z-VAD-FMK; Lane 11: N3a; Lane 12: N3a plus 20 μM Z-VAD-FMK.

Figure S11.

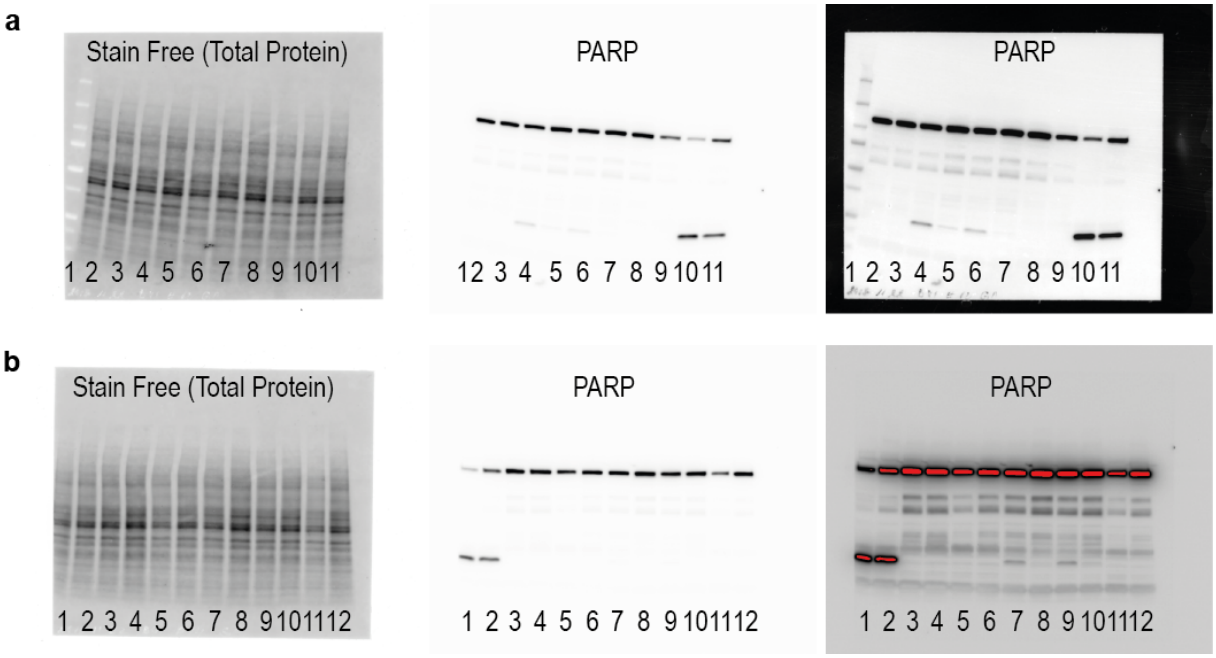

Figure S11. Whole blots from Figure 6b (PARP full length and 25 kDa cleaved fragment). **(a)** left - stain free membrane (total protein levels), middle - chemiluminescence image (antibodies), right - merged chemiluminescence and colorimetric image (protein marker). Lane 1: Precision Plus Protein All Blue Standards (BioRad #161-0373); Lane 2: DMSO; Lane 3: DMSO plus 20 μM Z-VAD-FMK; Lane 4: compound **43**; Lane 5: compound **43** plus 20 μM Z-VAD-FMK; Lane 6: compound **45**; Lane 7: compound **45** plus 20 μM Z-VAD-FMK; Lane 8: nutlin-3a (N3a); Lane 9: N3a plus 20 μM Z-VAD-FMK; Lane 10: staurosporine (STP); Lane 11: STP plus 20 μM Z-VAD-FMK; **(b)** left - stain free membrane (total protein levels), middle - chemiluminescence image (antibodies), right – chemiluminescence overexposed image (antibodies). Lane 1: STP; Lane 2: STP plus 20 μM Z-VAD-FMK; Lane 3: etoposide (ETP); Lane 4: ETP plus 20 μM Z-VAD-FMK; Lane 5: DMSO; Lane 6: DMSO plus 20 μM Z-VAD-FMK; Lane 7: compound **51**; Lane 8: compound **51** plus 20 μM Z-VAD-FMK; Lane 9:

brequinar (BQ); Lane 10: BQ plus 20 μM Z-VAD-FMK; Lane 11: N3a; Lane 12: N3a plus 20 μM Z-VAD-FMK.

Figure S12.

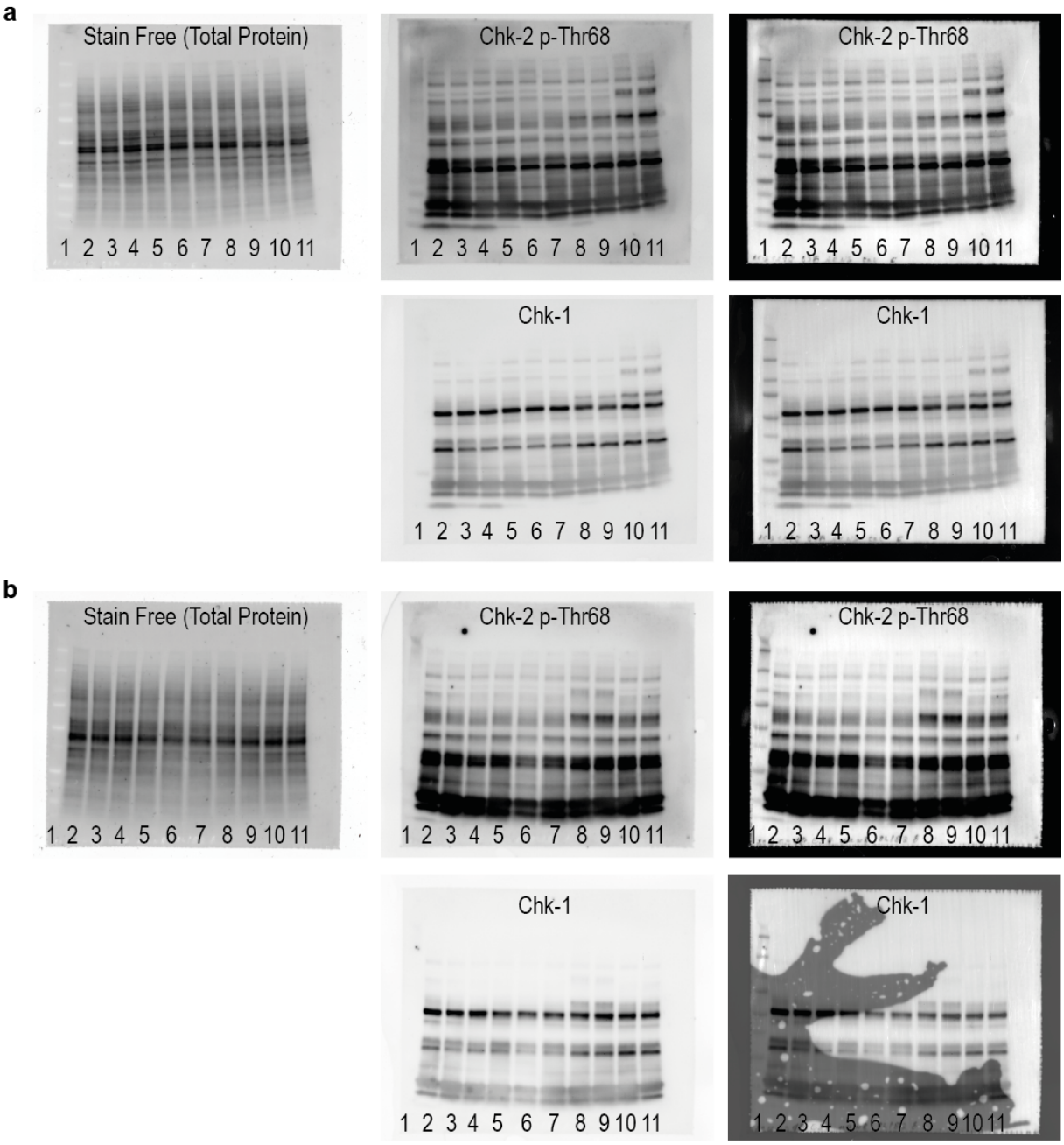

Figure S12. Whole blots from Supplementary Figure S2 (Chk-2 p-Thre68 and Chk-1). Left - stain free membrane (total protein levels), middle - chemiluminescence image (antibodies), right - merged chemiluminescence and colorimetric image (protein marker). **(a)** Lane 1: Precision Plus Protein All Blue Standards (BioRad #161-0373); Lane 2: DMSO; Lane

3: DMSO plus 100 μM uridine; Lane 4: compound **43**; Lane 5: compound **43** plus 100 μM uridine; Lane 6: compound **45**; Lane 7: compound **45** plus 100 μM uridine; Lane 8: hydroxyurea (HU); Lane 9: HU plus 100 μM uridine; Lane 10: etoposide (ETP); Lane 11: ETP plus 100 μM uridine; **(b)** Lane 1: Precision Plus Protein All Blue Standards (BioRad #161-0373); Lane 2: DMSO; Lane 3: DMSO plus 100 μM uridine; Lane 4: compound **51**; Lane 5: compound **51** plus 100 μM uridine; Lane 6: brequinar (BQ); Lane 7: BQ plus 100 μM uridine; Lane 8: ETP; Lane 9: ETP plus 100 μM uridine; Lane 10: HU; Lane 11: HU plus 100 μM uridine.

Figure S13.

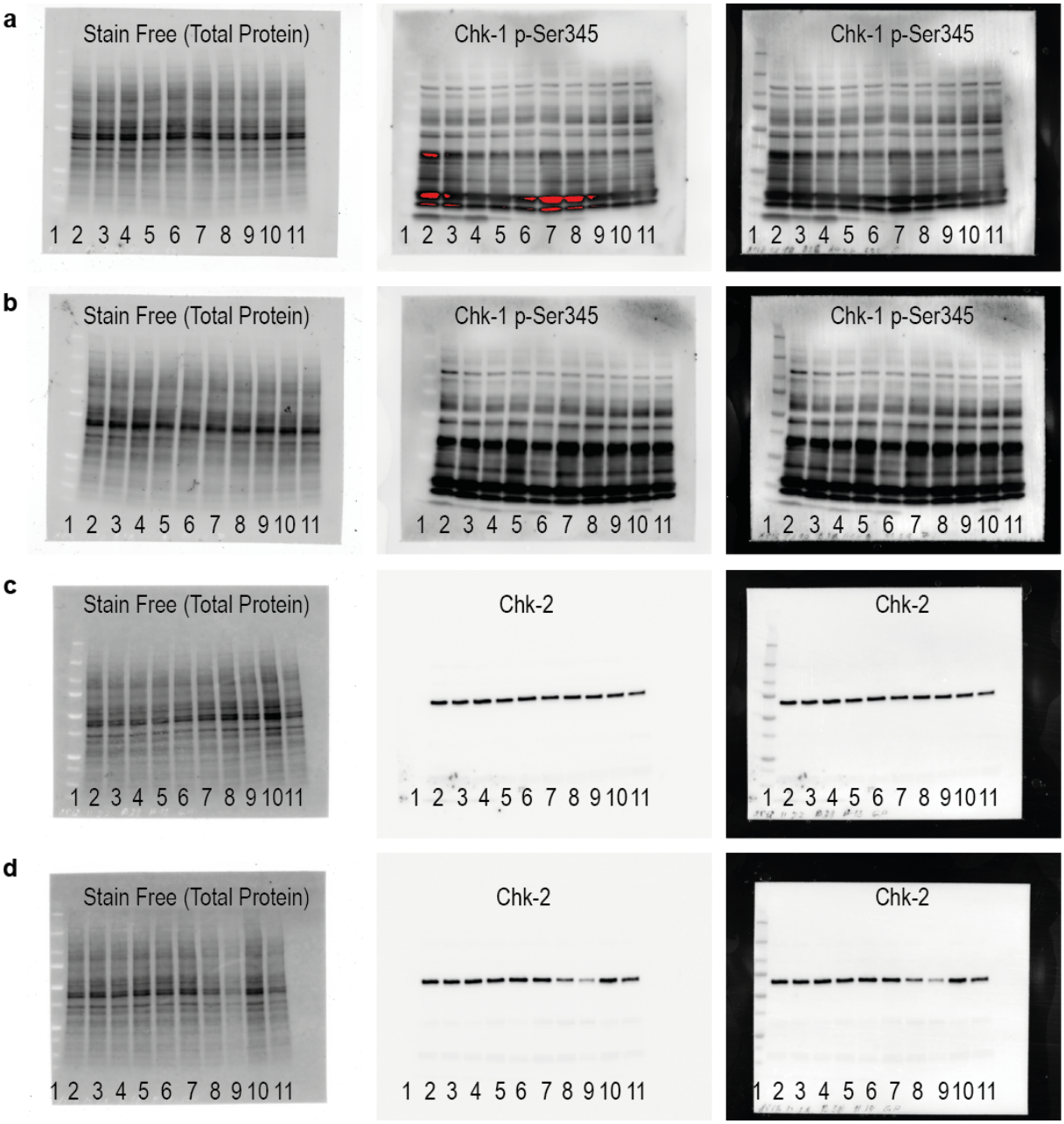

Figure S13. Whole blots from Figure S5 (Chk-1 p-Ser345 and Chk-2). Left - stain free membrane (total protein levels), middle - chemiluminescence image (antibodies), right - merged chemiluminescence and colorimetric image (protein marker). **(a)** and **(c)** Lane 1: Precision Plus Protein All Blue Standards (BioRad #161-0373); Lane 2: DMSO; Lane 3: DMSO plus 100 μM uridine; Lane 4: compound **43**; Lane 5: compound **43** plus 100 μM uridine;

Lane 6: compound **45**; Lane 7: compound **45** plus 100 μM uridine; Lane 8: hydroxyurea (HU); Lane 9: HU plus 100 μM uridine; Lane 10: etoposide (ETP); Lane 11: ETP plus 100 μM uridine; **(b)** and **(d)** Lane 1: Precision Plus Protein All Blue Standards (BioRad #161-0373); Lane 2: DMSO; Lane 3: DMSO plus 100 μM uridine; Lane 4: compound **51**; Lane 5: compound **51** plus 100 μM uridine; Lane 6: brequinar (BQ); Lane 7: BQ plus 100 μM uridine; Lane 8: ETP; Lane 9: ETP plus 100 μM uridine; Lane 10: HU; Lane 11: HU plus 100 μM uridine.

Figure S14.

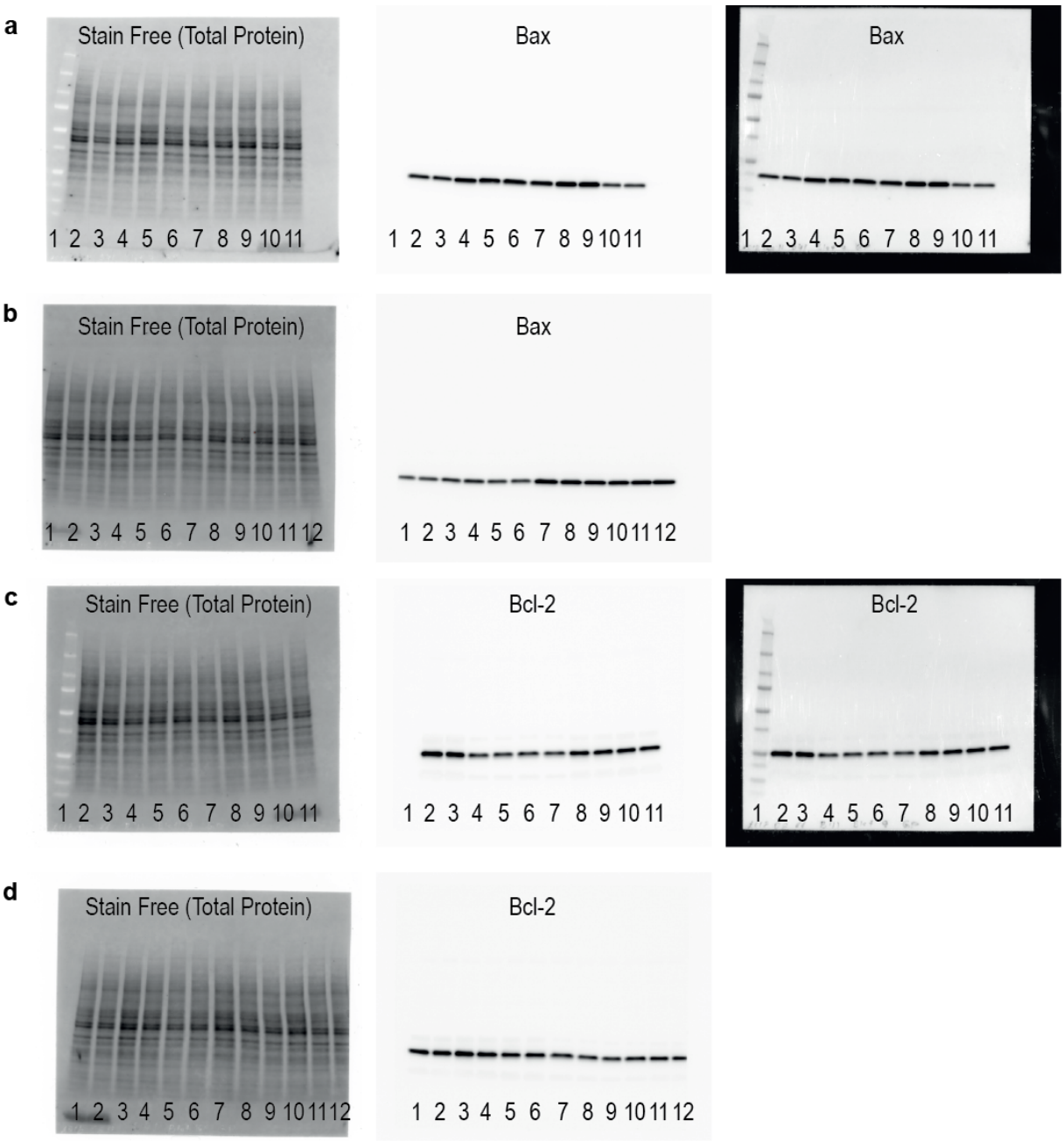

Figure S14. Whole blots from Figure S6 (Bax and Bcl-2). (a) and **(c)** left - stain free membrane (total protein levels), middle - chemiluminescence image (antibodies), right - merged chemiluminescence and colorimetric image (protein marker). Lane 1: Precision Plus Protein All Blue Standards (BioRad #161-0373); Lane 2: DMSO; Lane 3: DMSO plus 20 μM

Z-VAD-FMK; Lane 4: compound **43**; Lane 5: compound **43** plus 20 μM Z-VAD-FMK; Lane 6: compound **45**; Lane 7: compound **45** plus 20 μM Z-VAD-FMK; Lane 8: nutlin-3a; Lane 9: nutlin-3a plus 20 μM Z-VAD-FMK; Lane 10: staurosporine (STP); Lane 11: STP plus 20 μM Z-VAD-FMK; **(b)** and **(d)** left - stain free membrane (total protein levels), right - chemiluminescence image (antibodies). Lane 1: STP; Lane 2: STP plus 20 μM Z-VAD-FMK; Lane 3: etoposide (ETP); Lane 4: ETP plus 20 μM Z-VAD-FMK; Lane 5: DMSO; Lane 6: DMSO plus 20 μM Z-VAD-FMK; Lane 7: compound **51**; Lane 8: compound **51** plus 20 μM Z-VAD-FMK; Lane 9: brequinar (BQ); Lane 10: BQ plus 20 μM Z-VAD-FMK; Lane 11: nutlin-3a (N3a); Lane 12: N3a plus 20 μM Z-VAD-FMK.

Supplementary Information Tables

Table S1. Ligand-lipophilic efficiency (LLE). The logarithm of the octanol/water partition coefficient (LogP), the distribution coefficient at pH 7.4 (LogD), and the molecular heavy atom count (HAC), i.e. all non-hydrogen atom count, is predicted with ChemAxon. Ligand efficiency at room temperature (LE) and ligand-lipophilic efficiency (LLE) are defined as LE = 1.364 pIC50/HAC and LLE = pIC50 – LogP.

Compound	LogP	LogD	HAC	IC_{50} [nm]	pIC_{50}	LE	LLE
(*R*)-HZ05	3.4	3.4	28	11 ± 0.94	8.0	0.39	4.6
51	2.9	2.9	28	2.3 ± 0.27	8.6	0.42	5.7
52	2.6	2.6	28	6.3 ± 0.48	8.2	0.40	5.6

Table S2. Effect of compound 51 on 468 kinases. The interaction between 1 μM of compound **51** with 450 kinases was measured through KINOME*scan*™ screening platform. The results are represented as percent of control; therefore, lower numbers indicate stronger inhibition of kinase activity.

Target (gene symbol)	% Ctrl	Target (gene symbol)	% Ctrl	Target (gene symbol)	% Ctrl
AAK1	98	FES	100	PCTK3	91
ABL1(E255K)-phosphorylated	100	FGFR1	89	PDGFRA	100
ABL1(F317I)-nonphosphorylated	100	FGFR2	100	PDGFRB	100
ABL1(F317I)-phosphorylated	100	FGFR3	100	PDPK1	91
ABL1(F317L)-nonphosphorylated	100	FGFR3(G697C)	99	PFCDPK1(P.falciparum)	69
ABL1(F317L)-phosphorylated	90	FGFR4	100	PFPK5(P.falciparum)	90

ABL1(H396P)-nonphosphorylated	79	FGR	100	PFTAIRE2	100
ABL1(H396P)-phosphorylated	92	FLT1	100	PFTK1	97
ABL1(M351T)-phosphorylated	100	FLT3	100	PHKG1	98
ABL1(Q252H)-nonphosphorylated	100	FLT3(D835H)	100	PHKG2	100
ABL1(Q252H)-phosphorylated	87	FLT3(D835V)	99	PIK3C2B	100
ABL1(T315I)-nonphosphorylated	100	FLT3(D835Y)	96	PIK3C2G	68
ABL1(T315I)-phosphorylated	95	FLT3(ITD)	100	PIK3CA	96
ABL1(Y253F)-phosphorylated	100	FLT3(ITD,D835V)	86	PIK3CA(C420R)	75
ABL1-nonphosphorylated	62	FLT3(ITD,F691L)	47	PIK3CA(E542K)	63
ABL1-phosphorylated	74	FLT3(K663Q)	100	PIK3CA(E545A)	81
ABL2	100	FLT3(N841I)	100	PIK3CA(E545K)	50
ACVR1	100	FLT3(R834Q)	82	PIK3CA(H1047L)	100
ACVR1B	100	FLT3-autoinhibited	100	PIK3CA(H1047Y)	100
ACVR2A	100	FLT4	100	PIK3CA(I800L)	93
ACVR2B	96	FRK	100	PIK3CA(M1043I)	100
ACVRL1	100	FYN	100	PIK3CA(Q546K)	88
ADCK3	100	GAK	100	PIK3CB	98
ADCK4	100	GCN2(Kin.Dom.2,S808G)	100	PIK3CD	72
AKT1	86	GRK1	65	PIK3CG	80
AKT2	100	GRK2	55	PIK4CB	73
AKT3	100	GRK3	90	PIKFYVE	100
ALK	92	GRK4	100	PIM1	100
ALK(C1156Y)	99	GRK7	97	PIM2	98
ALK(L1196M)	100	GSK3A	100	PIM3	100
AMPK-alpha1	100	GSK3B	92	PIP5K1A	100
AMPK-alpha2	63	HASPIN	98	PIP5K1C	100
ANKK1	100	HCK	99	PIP5K2B	100
ARK5	100	HIPK1	84	PIP5K2C	97
ASK1	95	HIPK2	86	PKAC-alpha	100
ASK2	99	HIPK3	84	PKAC-beta	100

AURKA	91	HIPK4	94	PKMYT1	100
AURKB	71	HPK1	97	PKN1	100
AURKC	100	HUNK	100	PKN2	100
AXL	100	ICK	79	PKNB(M.tuberculosis)	90
BIKE	100	IGF1R	100	PLK1	83
BLK	59	IKK-alpha	86	PLK2	97
BMPR1A	91	IKK-beta	73	PLK3	83
BMPR1B	99	IKK-epsilon	82	PLK4	93
BMPR2	97	INSR	80	PRKCD	83
BMX	100	INSRR	96	PRKCE	100
BRAF	76	IRAK1	64	PRKCH	82
BRAF(V600E)	87	IRAK3	100	PRKCI	100
BRK	100	IRAK4	65	PRKCQ	91
BRSK1	100	ITK	100	PRKD1	100
BRSK2	87	JAK1(JH1domain-catalytic)	100	PRKD2	98
BTK	95	JAK1(JH2domain-pseudokinase)	100	PRKD3	100
BUB1	68	JAK2(JH1domain-catalytic)	81	PRKG1	100
CAMK1	100	JAK3(JH1domain-catalytic)	65	PRKG2	100
CAMK1B	70	JNK1	55	PRKR	97
CAMK1D	100	JNK2	55	PRKX	100
CAMK1G	100	JNK3	56	PRP4	100
CAMK2A	100	KIT	100	PYK2	100
CAMK2B	100	KIT(A829P)	88	QSK	52
CAMK2D	100	KIT(D816H)	69	RAF1	100
CAMK2G	100	KIT(D816V)	100	RET	100
CAMK4	80	KIT(L576P)	100	RET(M918T)	99
CAMKK1	100	KIT(V559D)	100	RET(V804L)	100
CAMKK2	100	KIT(V559D,T670I)	81	RET(V804M)	100
CASK	74	KIT(V559D,V654A)	100	RIOK1	100
CDC2L1	100	KIT-autoinhibited	100	RIOK2	90
CDC2L2	94	LATS1	98	RIOK3	100
CDC2L5	70	LATS2	100	RIPK1	93
CDK11	96	LCK	92	RIPK2	98
CDK2	100	LIMK1	100	RIPK4	72
CDK3	99	LIMK2	92	RIPK5	93
CDK4	76	LKB1	100	ROCK1	68
CDK4-cyclinD1	82	LOK	95	ROCK2	92
CDK4-cyclinD3	89	LRRK2	96	ROS1	91
CDK5	100	LRRK2(G2019S)	92	RPS6KA4(Kin.Dom.1-N-terminal)	100
CDK7	85	LTK	97	RPS6KA4(Kin.Dom.2-C-terminal)	98

CDK8	100	LYN	100	RPS6KA5(Kin. Dom.1-N-terminal)	76
CDK9	100	LZK	78	RPS6KA5(Kin. Dom.2-C-terminal)	100
CDKL1	78	MAK	100	RSK1(Kin.Dom. 1-N-terminal)	93
CDKL2	100	MAP3K1	85	RSK1(Kin.Dom. 2-C-terminal)	100
CDKL3	93	MAP3K15	100	RSK2(Kin.Dom. 1-N-terminal)	71
CDKL5	90	MAP3K2	69	RSK2(Kin.Dom. 2-C-terminal)	93
CHEK1	100	MAP3K3	86	RSK3(Kin.Dom. 1-N-terminal)	99
CHEK2	100	MAP3K4	99	RSK3(Kin.Dom. 2-C-terminal)	100
CIT	96	MAP4K2	100	RSK4(Kin.Dom. 1-N-terminal)	90
CLK1	100	MAP4K3	100	RSK4(Kin.Dom. 2-C-terminal)	100
CLK2	100	MAP4K4	100	S6K1	74
CLK3	99	MAP4K5	100	SBK1	93
CLK4	88	MAPKAPK2	95	SGK	100
CSF1R	70	MAPKAPK5	100	SgK110	100
CSF1R-autoinhibited	99	MARK1	82	SGK2	78
CSK	100	MARK2	100	SGK3	100
CSNK1A1	100	MARK3	100	SIK	92
CSNK1A1L	100	MARK4	60	SIK2	100
CSNK1D	100	MAST1	100	SLK	91
CSNK1E	100	MEK1	95	SNARK	100
CSNK1G1	100	MEK2	92	SNRK	95
CSNK1G2	99	MEK3	98	SRC	99
CSNK1G3	100	MEK4	84	SRMS	96
CSNK2A1	84	MEK5	63	SRPK1	100
CSNK2A2	89	MEK6	89	SRPK2	87
CTK	86	MELK	100	SRPK3	100
DAPK1	100	MERTK	100	STK16	99
DAPK2	100	MET	100	STK33	88
DAPK3	100	MET(M1250T)	100	STK35	100
DCAMKL1	77	MET(Y1235D)	100	STK36	90
DCAMKL2	96	MINK	93	STK39	98
DCAMKL3	86	MKK7	72	SYK	74
DDR1	100	MKNK1	64	TAK1	84
DDR2	84	MKNK2	63	TAOK1	100
DLK	80	MLCK	100	TAOK2	91
DMPK	100	MLK1	91	TAOK3	97
DMPK2	84	MLK2	71	TBK1	76

DRAK1	100	MLK3	89	TEC	100
DRAK2	100	MRCKA	100	TESK1	94
DYRK1A	75	MRCKB	100	TGFBR1	99
DYRK1B	85	MST1	83	TGFBR2	100
DYRK2	83	MST1R	100	TIE1	100
EGFR	100	MST2	100	TIE2	86
EGFR(E746-A750del)	99	MST3	100	TLK1	100
EGFR(G719C)	95	MST4	85	TLK2	95
EGFR(G719S)	95	MTOR	94	TNIK	100
EGFR(L747-E749del, A750P)	100	MUSK	100	TNK1	100
EGFR(L747-S752del, P753S)	96	MYLK	56	TNK2	100
EGFR(L747-T751del,Sins)	91	MYLK2	93	TNNI3K	100
EGFR(L858R)	100	MYLK4	100	TRKA	75
EGFR(L858R,T790M)	66	MYO3A	100	TRKB	65
EGFR(L861Q)	56	MYO3B	42	TRKC	79
EGFR(S752-I759del)	100	NDR1	83	TRPM6	94
EGFR(T790M)	87	NDR2	94	TSSK1B	100
EIF2AK1	100	NEK1	97	TSSK3	100
EPHA1	100	NEK10	65	TTK	100
EPHA2	100	NEK11	100	TXK	100
EPHA3	100	NEK2	100	TYK2(JH1domain-catalytic)	91
EPHA4	100	NEK3	52	TYK2(JH2domain-pseudokinase)	88
EPHA5	100	NEK4	69	TYRO3	100
EPHA6	100	NEK5	100	ULK1	81
EPHA7	100	NEK6	100	ULK2	79
EPHA8	97	NEK7	92	ULK3	71
EPHB1	98	NEK9	96	VEGFR2	100
EPHB2	87	NIK	83	VPS34	63
EPHB3	96	NIM1	70	VRK2	73
EPHB4	100	NLK	100	WEE1	100
EPHB6	100	OSR1	90	WEE2	100
ERBB2	81	p38-alpha	99	WNK1	100
ERBB3	50	p38-beta	84	WNK2	66
ERBB4	100	p38-delta	100	WNK3	98
ERK1	100	p38-gamma	100	WNK4	62
ERK2	96	PAK1	87	YANK1	100
ERK3	96	PAK2	79	YANK2	97
ERK4	100	PAK3	100	YANK3	100
ERK5	88	PAK4	100	YES	100

ERK8	89	PAK6	100	YSK1	76
ERN1	92	PAK7	100	YSK4	67
FAK	97	PCTK1	91	ZAK	100
FER	82	PCTK2	100	ZAP70	100

Table S3. List of antibodies used for western blotting.

Antibody	Source	Clone	MW	Manufacturer	Product number
p53	Mouse monoclonal	DO-1	53 kDa	Made inhouse [a]	
γ-H2AX (phospho-Ser139)	Mouse monoclonal	JBW301	17 kDa	Millipore	#05-636
p-Ser15 p53	Rabbit polyclonal		53 kDa	Cell Signaling	#9284S
H2AX	Rabbit polyclonal		17 kDa	AbCam	#ab20669
PARP cleaved	Rabbit monoclonal	Y34	85 kDa	AbCam	#ab32561
PARP	Rabbit monoclonal	E102	113 kDa; 25 kDa	AbCam	#ab32138
Bax	Rabbit monoclonal	E63	21 kDa	AbCam	#ab32503
Bcl-2	Rabbit monoclonal	EPR17909	26 kDa	AbCam	#ab182858
Chk-1	Mouse monoclonal	G-4	56 kDa	Santa Cruz	#SC-8408
Chk-1 (pospho-Ser345)	Rabbit polyclonal		56 kDa	Cell Signaling	#2341
Chk-2	Rabbit monoclonal	ERP4325	62 kDa	AbCam	#ab109413
Chk-2 (phospho-Thr68)	Rabbit polyclonal		62 kDa	Cell Signaling	#2661

All dilutions were prepared according to manufacturer recommendations. [a] Vojtesek et al. [1]

References

1. Vojtesek, B.; Bartek, J.; Midgley, C. A.; Lane, D. P. An immunochemical analysis of the human nuclear phosphoprotein p53. New monoclonal antibodies and epitope mapping using recombinant p53. *J. Immunol. Methods* **1992,** 151, 237-44.