Supplementary Table 1. Antibodies used for RPPA staining. The company name and catalog number for all antibodies are listed.

Protein	Company	Catalog #
14_3_3_beta	Santa Cruz	628
14_3_3_zeta	Santa Cruz	1019
4EBP1	Cell Signaling	9452
P-4EBP1_T37.46	Cell Signaling	9459
P-4EBP1_S65	Cell Signaling	9456
P-ACC_S79	Cell Signaling	3661
AIB1	BD Pharmingen	611105
AKT	Cell Signaling	9272
P-AKT_S473	Cell Signaling	9271
P-AKT_T308	Cell Signaling	9275
AMPK	Cell Signaling	2532
P-AMPK_T172	Cell Signaling	2535
Annexin1	Zymed	71-3400
AR	Epitomics	1852
b.catenin	Cell Signaling	9562
P-BRAF_S445	Cell Signaling	2696
BAD	Epitomics	1541-1
P-BAD_S112	Cell Signaling	9296
P-BAD_S136	Cell Signaling	9295
BCl.XL	Cell Signaling	2762
Bcl2	Dako	M0887
Beclin	Cell Signaling	3738
Bim	Epitomics	1036-1
BRAF	Santa Cruz	5284
c.KIT	Epitomics	1552
CRAF	Upstate	5-739
Caviolin	Cell Signaling	3238
CD31	Dako	M0823
IGF1R BETA	Cell Signaling	3027

Supplementary Table 1.
…Continued

Protein	Company	Catalog #
CDK4	Upstate	7659
Cl.Caspase7	Cell Signaling	9491
CollagenVI	Santa Cruz	20649
CyclinB1	Epitomics	1495
CyclinD1	Santa Cruz	718
CyclinE1	Santa Cruz	247
E-Cadherin	Cell Signaling	4065
EGFR	Santa Cruz	3
eIE4E	Cell Signaling	9741
ERK2	Santa Cruz	154
ETV	SDI	2626
FKHRL1	Cell Signaling	9467
P-FOXO3a_S318	Cell Signaling	9465
GSK	Santa Cruz	7291
P-GSK_S21.9	Cell Signaling	9331
IGFBP2	Cell Signaling	3922
IKKa	Epitomics	1615
P-IKKa_S176.S177	Cell Signaling	2078
IRS1	Upstate	6-248
P-JNK_T183.Y185	Cell Signaling	4671
LKB1	Abcam	15095
P-MAPK_T202.Y204	Cell Signaling	4377
MEK1	Epitomics	1235-1
P-MEK1,2_pS217.221	Cell Signaling	9121
MGMT	Chemicon	16200
MTOR	Cell Signaling	2983
N-Cadherin	Cell Signaling	4061
NOS2	Santa Cruz	651
OPN	Abcam	33046
P21	Santa Cruz	397
p27	Santa Cruz	528
p38	Cell Signaling	9212
P-P38_T180_Y182	Cell Signaling	9211
p53	Cell Signaling	9282

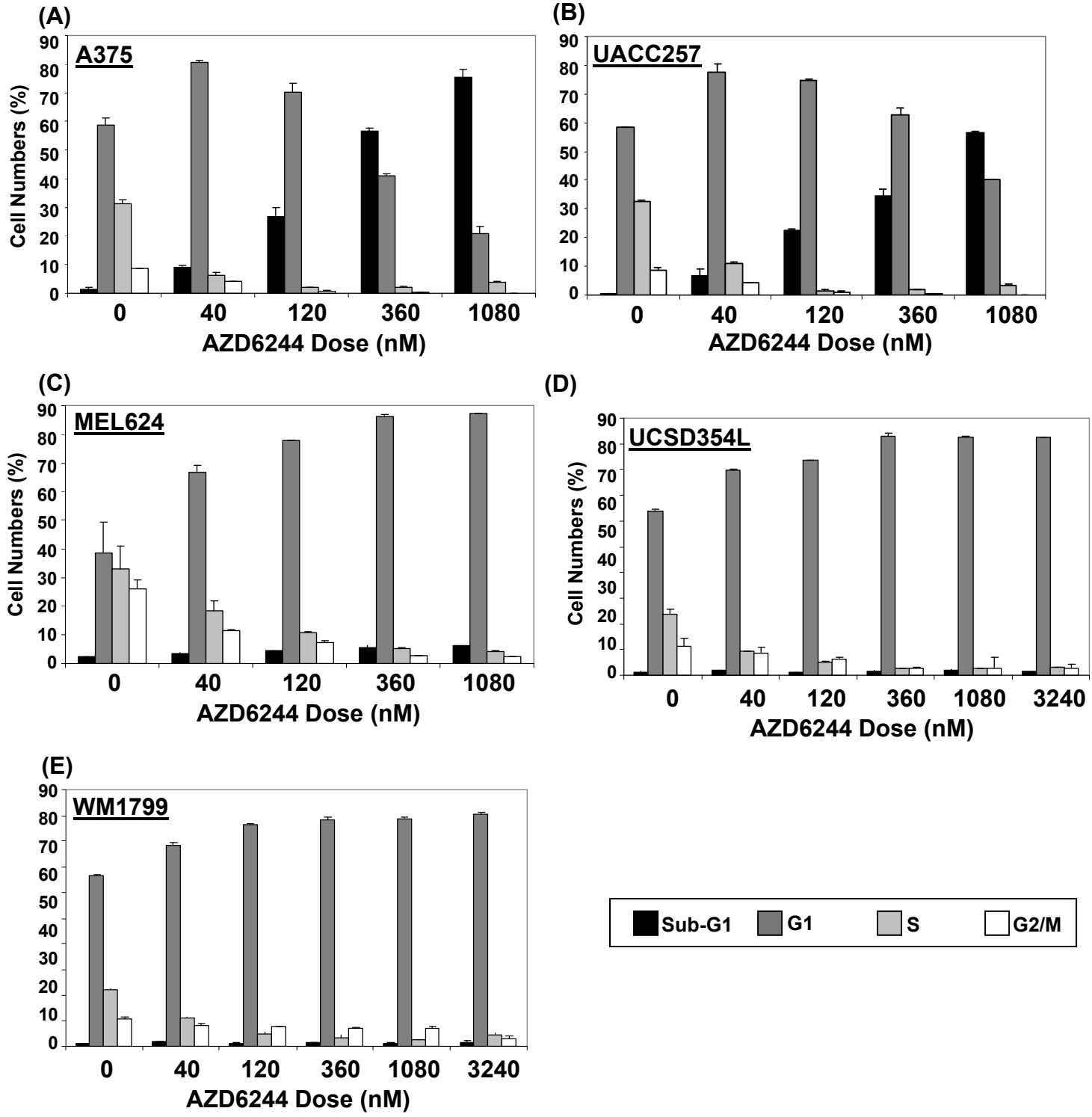
Supplementary Table 1.
…Continued

Protein	Company	Catalog #
P-p65.NFkB_S536	Cell Signaling	3033
p70s6k	Cell Signaling	9205
P-p70S6K_T389	Epitomics	1494
PDK1	Cell Signaling	3062
PDK1_pS241	Cell Signaling	3061
PKC	Upstate	5-154
P-PKC_S657	Upstate	6-822
PTEN	Cell Signaling	9552
Rb	Cell Signaling	9309
P-Rb_S807_811	Cell Signaling	9308
S6	Cell Signaling	2217
P-S6_S235_236	Cell Signaling	2211
P-S6_S240_244	Cell Signaling	2215
Smad3	Cell Signaling	9523
Smad5	Epitomics	1682
PSmad5_S463	Epitomics	2224
SOCS1	Zymed	38-5200
Src	Upstate	5-184
P-Src_Y527	Cell Signaling	2105
STAT1	Cell Signaling	9172
P-STAT3_Y705	Cell Signaling	9131
P-STAT5_Y694	Epitomics	1208
Stathmin	Epitomics	1972
TAZ	Abcam	3961
TGASE	Neomarkers	MS300P
TRAF3	Cell Signaling	4729B
TRAF6	Epitomics	1660-5
TSC2	Epitomics	1613-1
P-TSC2_S1462	Cell Signaling	3617
VEGFR2	Cell Signaling	2479
YAP	Santa Cruz	15407
P-YB1_S102	Cell Signaling	2900

Supplementary Table 2. Specific mutations in the cell lines were detected by mass spectroscopy genotyping. Relative IC50 values were determined from the dose response curves in Figure 1A using the 4PL and 3PLFT curve fit models as described in the NIH Chemical Genomics Center. (www.nih.ncgc.gov).

Cell Line	Mutation Status	AZD6244 IC50 (nM)
WM2664	BRAF, PTEN null	77
WM1799	BRAF, PTEN-null	1417
UCSD354L	BRAF, PTEN-null	524
WM1552	BRAF, PTEN null	10
WM46	BRAF, AKT3 E17K	9
MEL526	BRAF	3
A375	BRAF	3
SKMEL5	BRAF	1325
UACC257	BRAF	12
MEL624	BRAF	660
WM35	BRAF	0.1
Hs294T	WT	➢10000
MEWO	WT	26

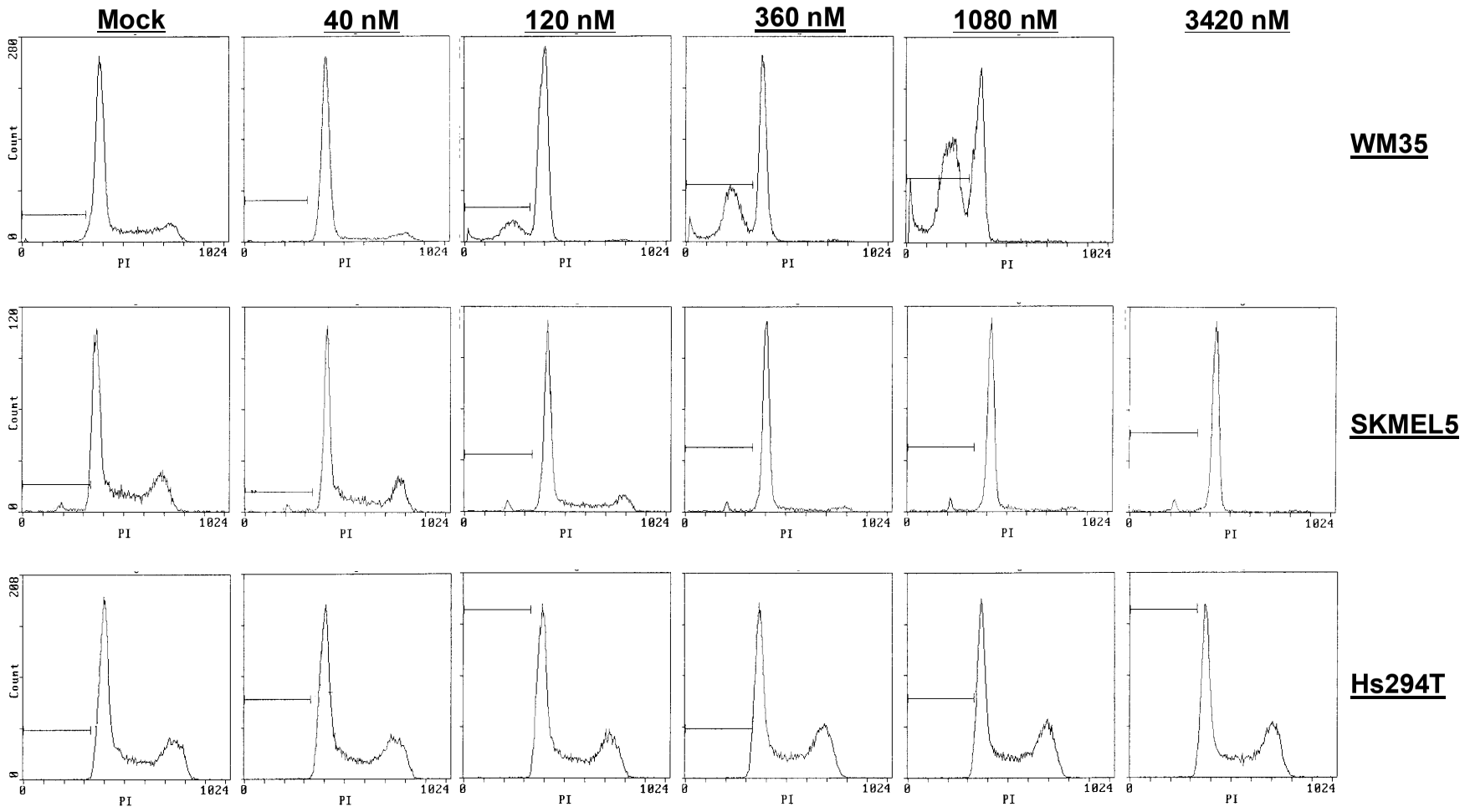
Figure S1



Cell cycle analysis of Group 1 (A & B), Group 2 (C, D, E) cells after 72h treatment with AZD6244. *X-axis,* concentration of AZD6244 (nM); *Y-axis,* % of cell population in different phases of the cell cycle. The phases of the cell cycle- Sub-G1 (dead cells), G1 , S, and G2/M are indicated by black, dark grey, light grey and white bars respectively. Each bar is the average of two or three replicates and error bars indicate standard deviation. Names of cell lines are in the upper left corner of each graph.

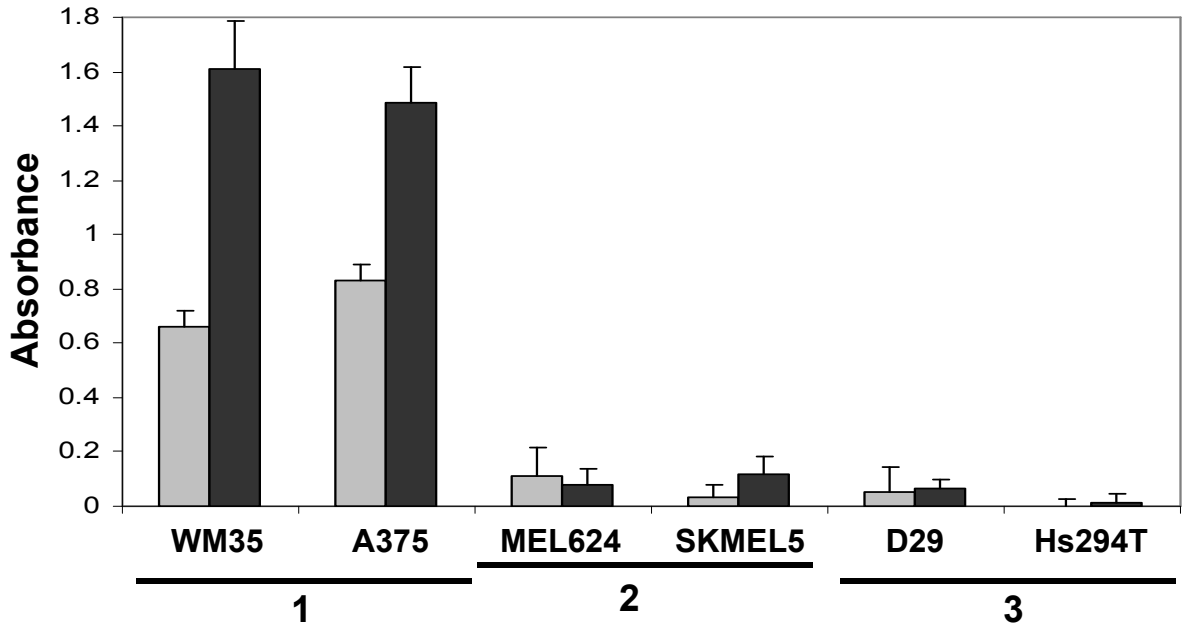
Figure S2

Cell Cycle Profiles of AZD6244 Treatments



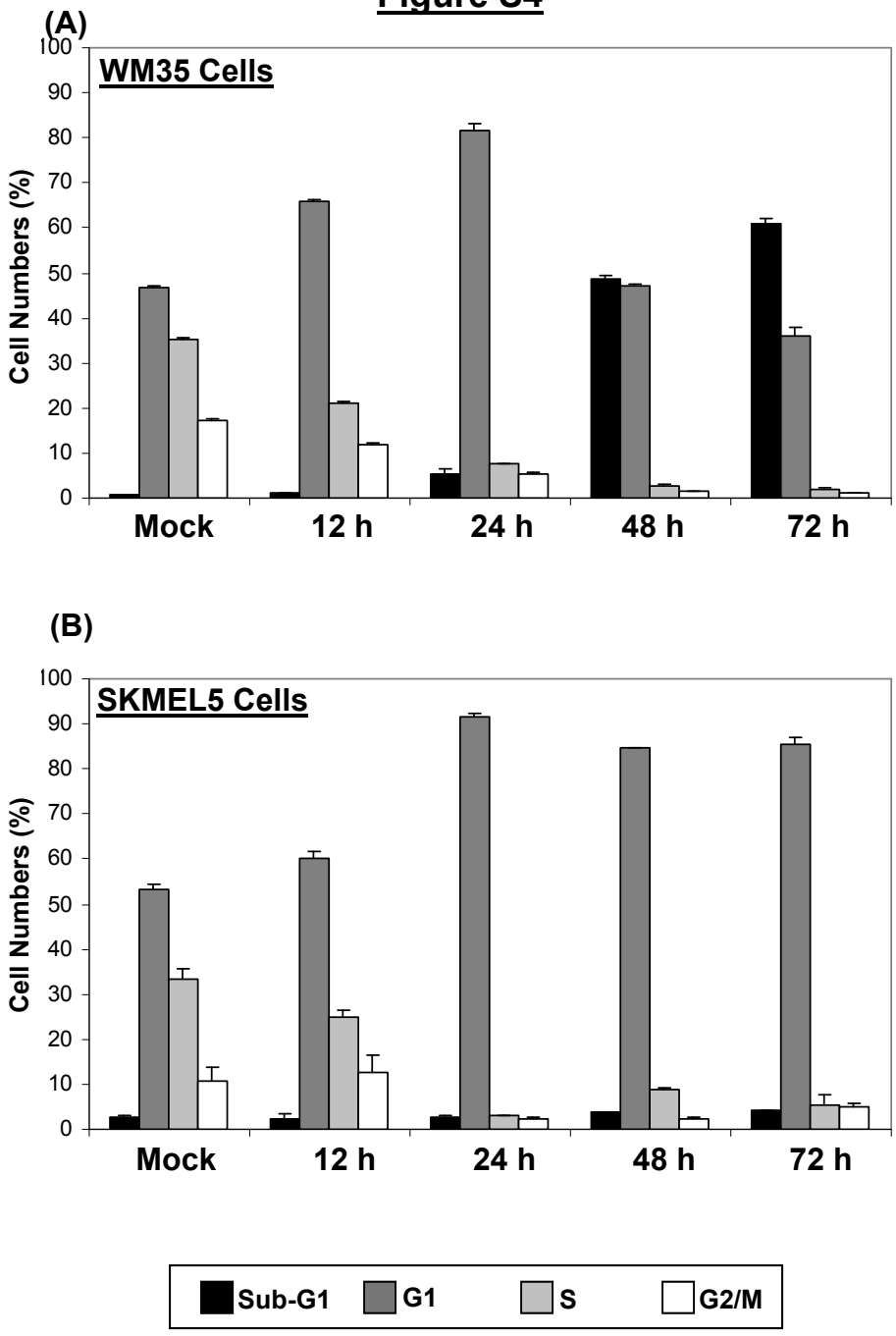
Cell cycle analysis histograms of WM35 (row 1), SKMEL5 (row 2) and Hs294T (row 3) cells after 72h treatment with AZD6244. *X-axis,* DNA content as stained by propidium iodide (PI); *Y-axis,* Cell numbers. The peaks represent sub-G1, G1, S and G2/M phases of the cell cycle. Horizontal lines inside the graphs represent the area of dead (sub-G1) cells. AZD6244 concentrations are indicated on the top of the histograms.

Figure S3



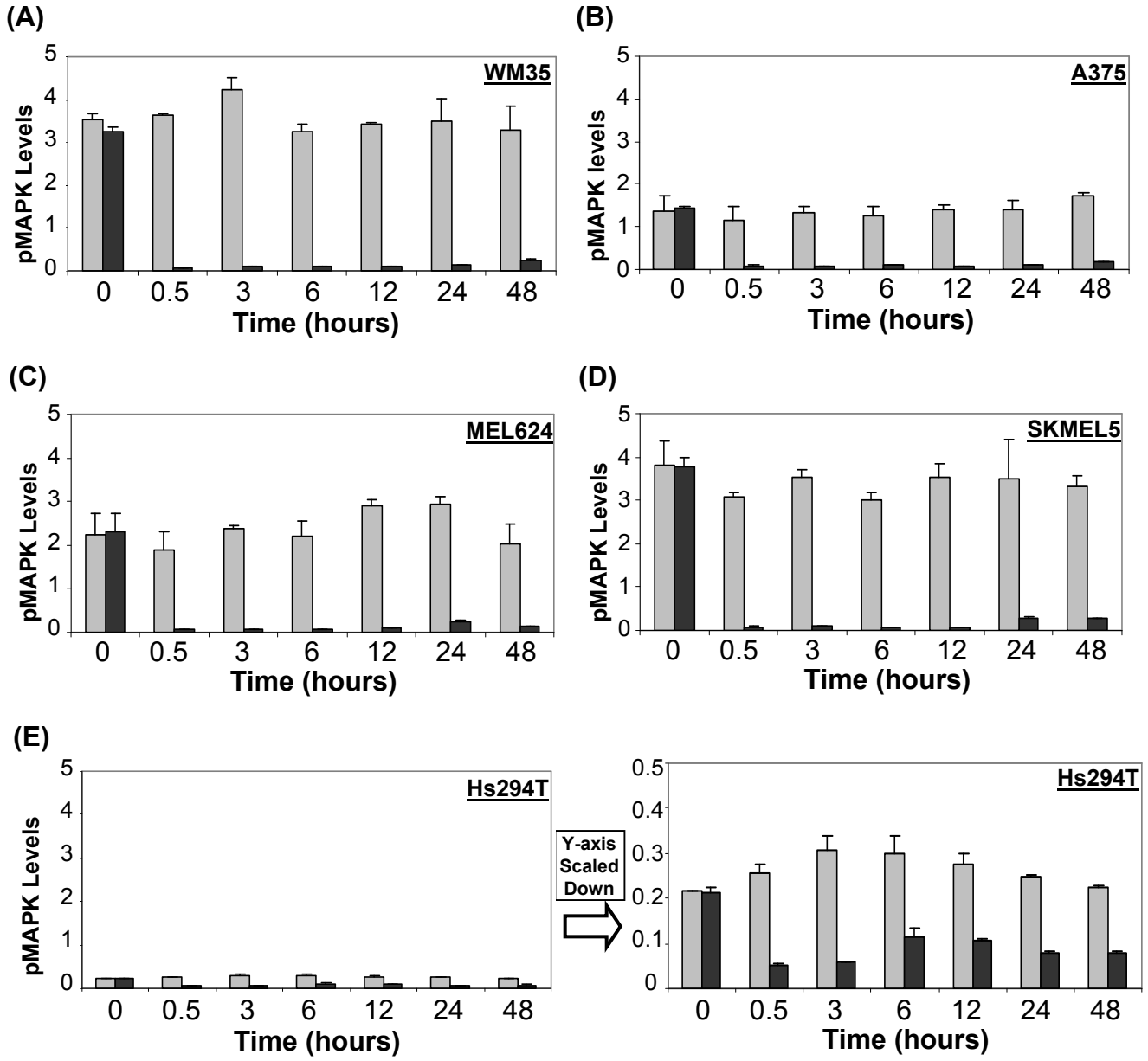
Two cell lines, each belonging to sensitivity groups 1, 2 and 3 were treated with vehicle or 360 nM AZD6244 for 24 h and 48 h. After incubation, cellular apoptosis was determined through cytoplasmic histone-DNA-fragment analysis. Increased absorbance at 405 nm in the treated samples relative to the untreated samples corresponded to increased apoptosis. The absorbance values of the AZD6244-treated samples were normalized against those of the vehicle-treated controls and plotted as bar graphs. Bars are average of triplicates and error bars represent standard deviation. 24h treatments are indicated by grey bars and 48 h treatments are indicated by black bars.

Figure S4



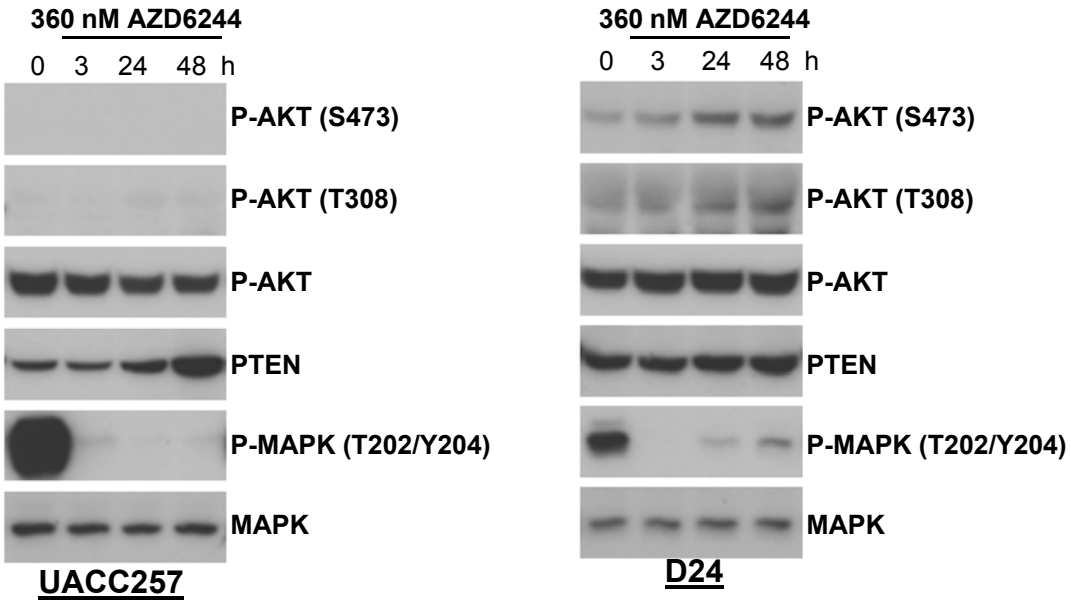
Time-course Cell cycle analysis of WM35 and SKMEL5 cells. *X-axis,* time of AZD6244 treatment; *Y-axis,* % of cell population. The cells were treated for the indicated periods of time, fixed and subjected to PI-cell cycle analysis. The phases of the cell cycle- Sub-G1 (dead cells), G1 , S, and G2/M are indicated by black, dark grey, light grey and white bars respectively.

Figure S5



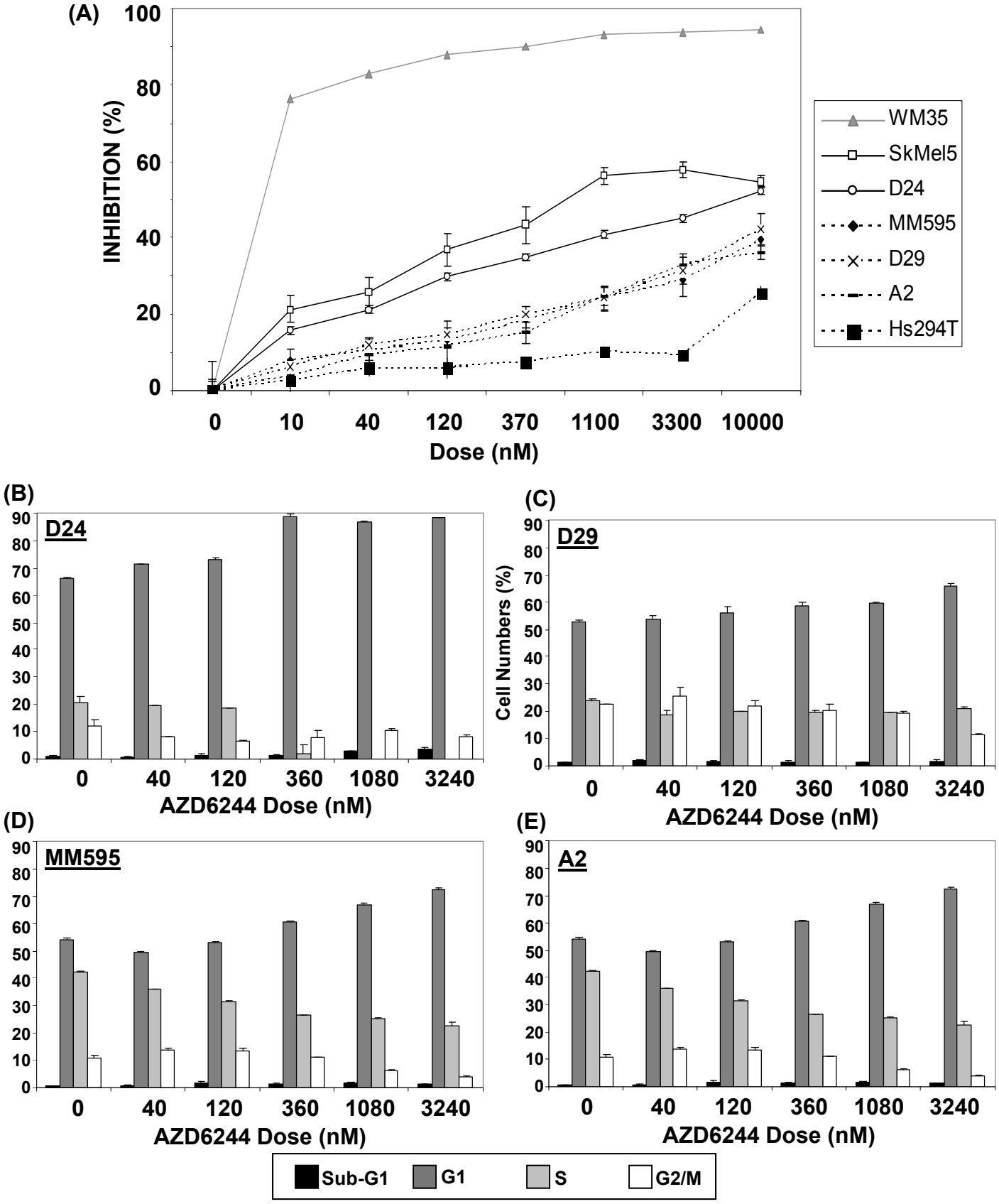
AZD6244 durably and potently inhibits P-MAPK in sensitive and resistant human melanoma cell lines. The load controlled linear intensity data of P-MAPK levels (Y-axis) were plotted against time of AZD6244 treatments (X-axis) for WM35 (**A**), A375 (**B**), MEL624(**C**), SKMEL5 (**D**) and Hs294T (**E**) cells respectively. Gray bar, DMSO treatment; Black bars, AZD6244 360 nM treatment. P-MAPK levels were equally inhibited in all cell lines, irrespective of the sensitivity to the growth and survival inhibitory effects of AZD6244. Note that Hs294T had extremely low levels of pMAPK levels, which were also inhibited (please see scaled Y-axis in the right panel of (**E**).

Figure S6



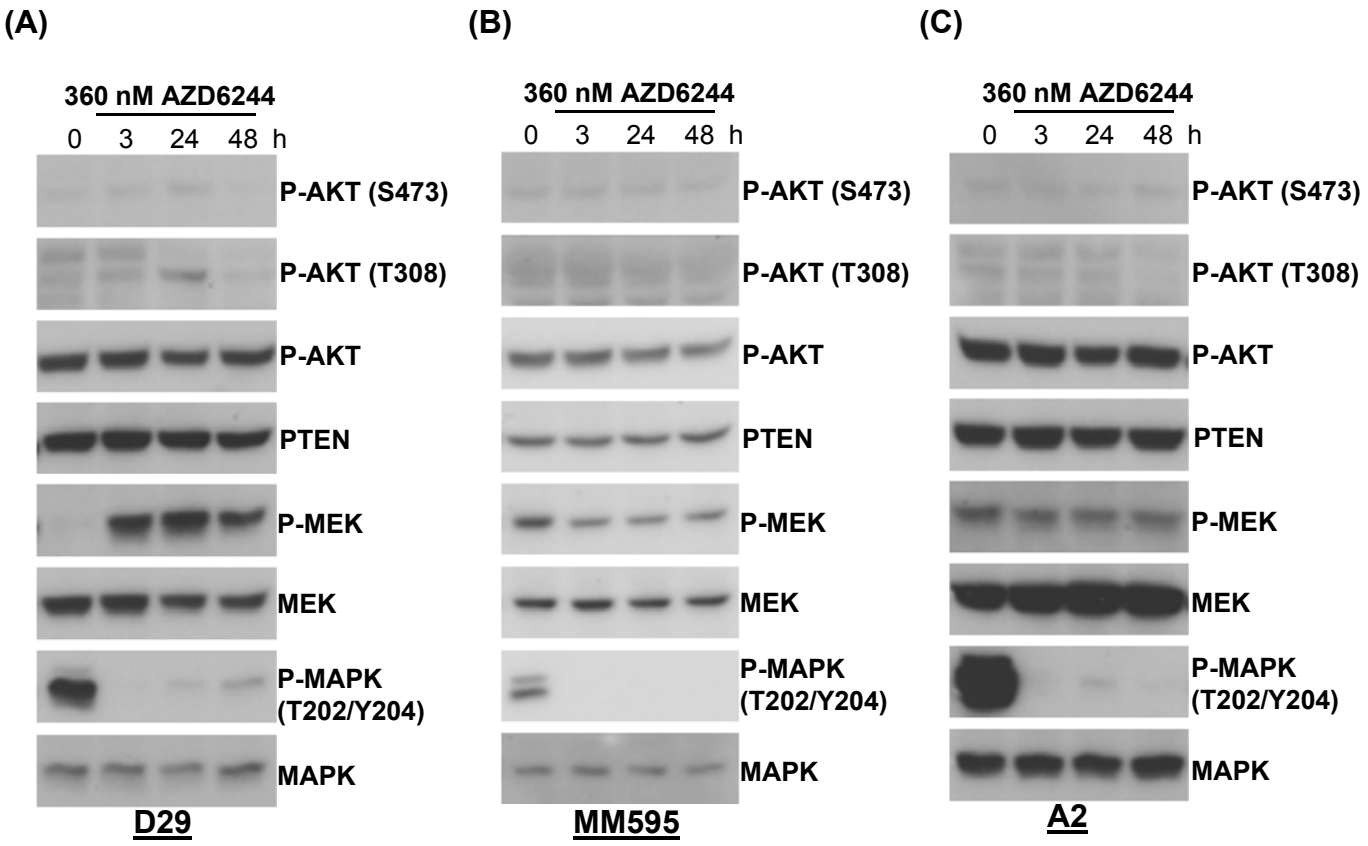
Effect of AZD6244 treatments on PTEN and P-AKT levels in sensitive UACC257 and the resistant D24 cells. The cells were treated with 360 nM AZD6244 for the indicated time pointes. Protein lysates were generated and western blotting with the indicated antibodies was performed.

Figure S7



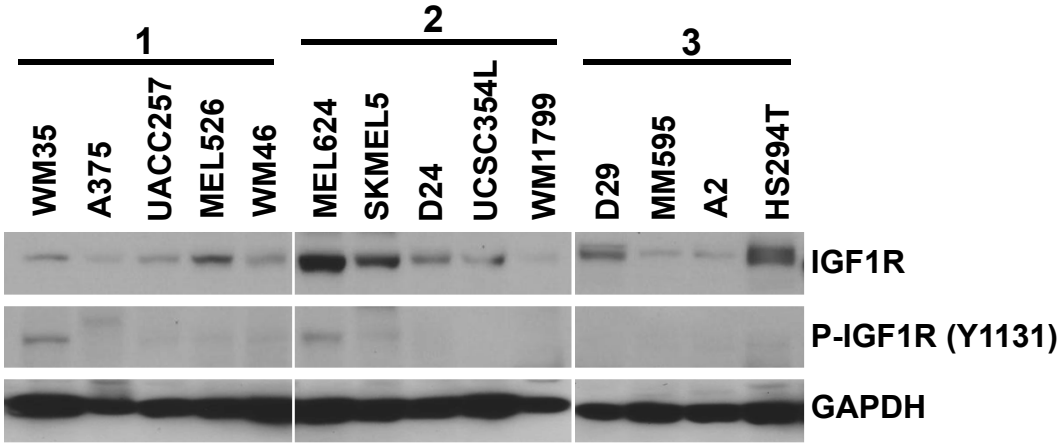
Growth and survival inhibition by AZD6244 in D24, D29, and MM595 cell lines. A, Growth inhibition in additional resistant lines D24, D29, MM595 and A2 treated with AZD6244 for 48 h. For comparison with Figure 1, results for the group 1 WM35, group 2 SKMEL5, and group 3 HS294T are shown. *X-axis,* concentration of AZD6244 (nM); *Y-axis,* % growth inhibition. Data points are the average of 3 replicates; error bars, standard deviation. B – D, cell cycle analysis of the resistant cell lines

Figure S8



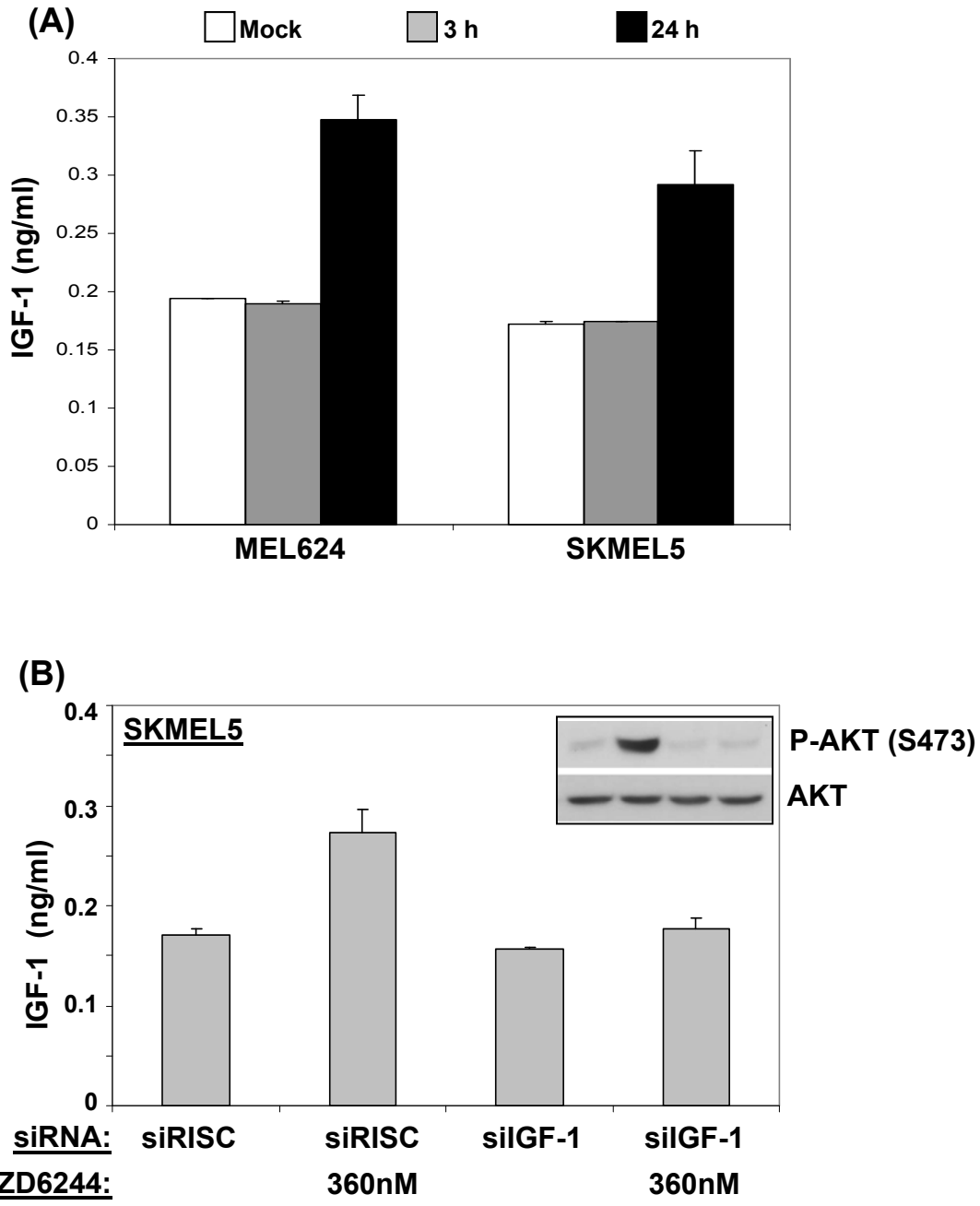
P-AKT and P-MAPK in the highly resistant D29, MM595 and A2 cells with AZD6244 treatments. The D29, MM595 and A2 cells were treated with 360 nM AZD6244 for the time periods shown, protein lysates were generated and analyzed by western blotting for the indicated proteins.

Figure S9



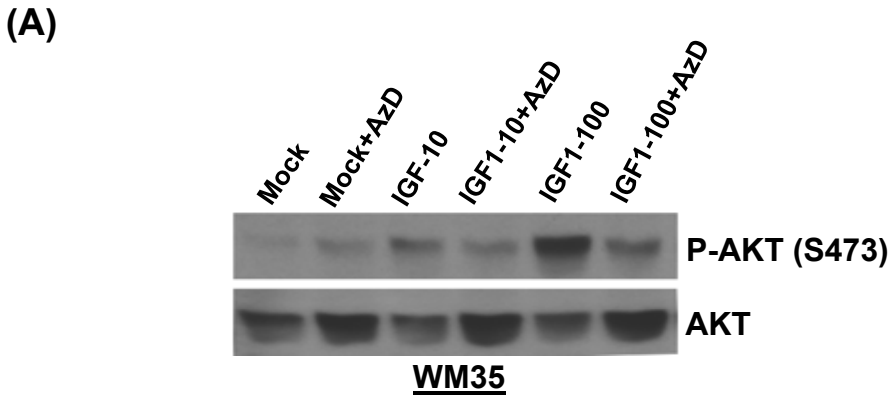
Basal IGF1R levels in melanoma cell lines. Melanoma cell lines were grown in 10 cm culture dishes to ~80% confluence in RPMI media with 5% serum. Protein lysates were generated, quantified, SDS-PAGE resolved and western blotted for IGF1R and P-IGF1R. P-IGF1R levels do not correlate with IGF1R levels.GAPDH was used as a loading control, Which is heterogeneously expressed in the different cell lines. The horizontal bars on the top represent cells in the sensitivity groups 1, 2 and 3.

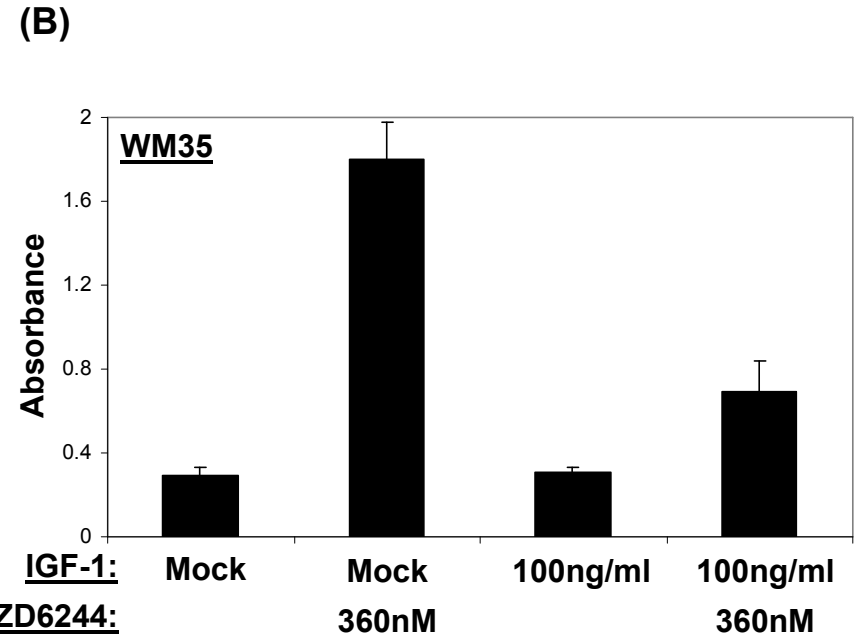
Figure S10



AZD6244-induced secretion of IGF-1. (**A**) The group 2 cells were treated with AZD6244 for 3 h or 24 h and the IGF-1 secreted into the medium was quantified by an IGF-1 ELISA assay. White bars represent basal IGF-1 levels in the media of mock (dmso) -treated cells, grey and black bars represent IGF-1 secretion after 3h and 24h of AZD6244 treatment. Fresh unspent media also showed similar levels of IGF-1 as the dmso controls (not shown). (**B**) SKMEL5 cells were transfected with siRisc or siIGF-1 siRNAs for 48 h, followed by 24 h of AZD6244 treatment. Lysates were generated and western blotted for P-AKT and AKT (inset picture). Treatments are shown on the X-axis of the bar-graph. Secreted IGF-1 levels in media from the same experiment were quantified against IGF-1 standards and plotted on the Y-axis. Bars represent average of triplicates and error bars represent standard deviation.

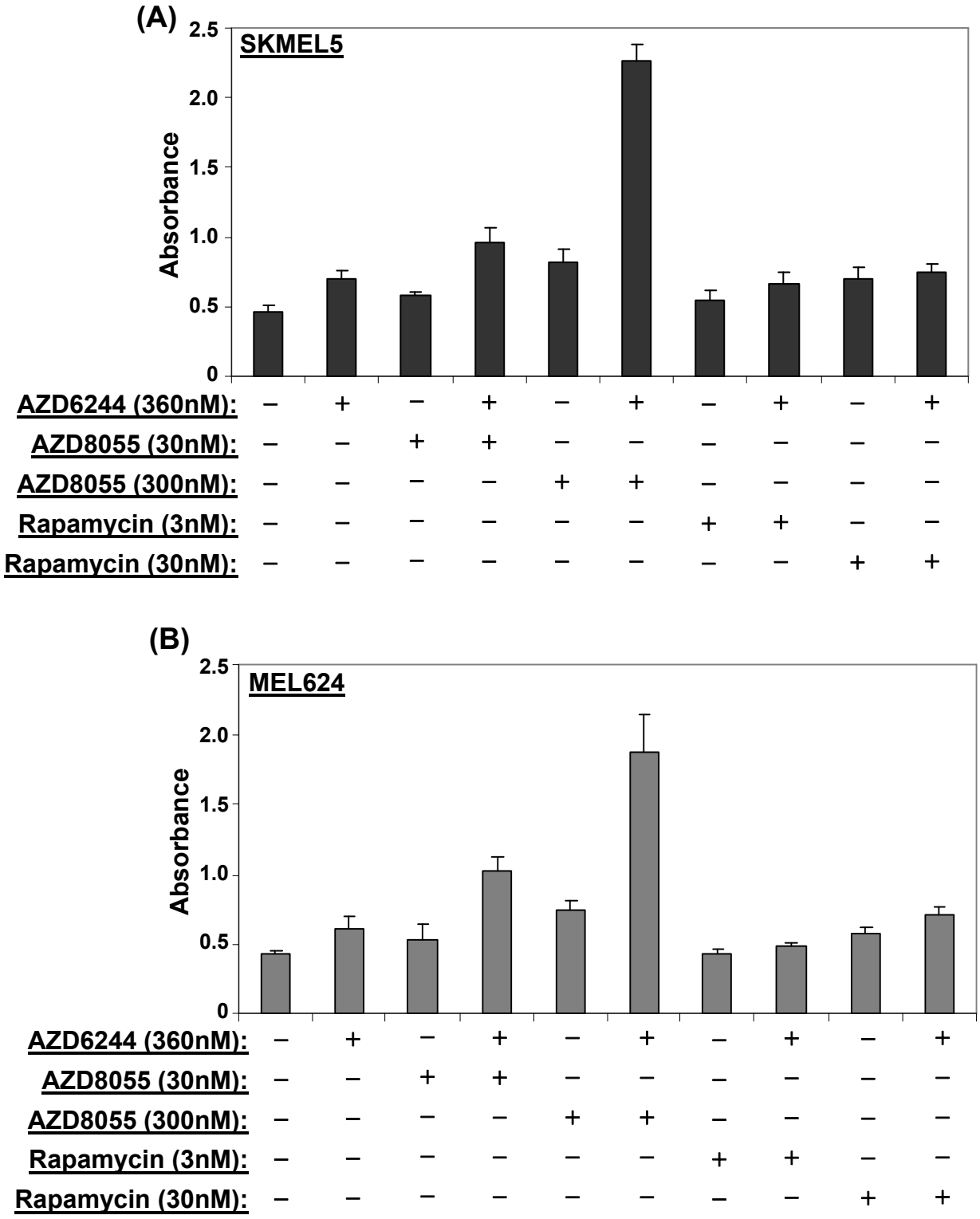
Figure S11





Effect of IGF-1 on AZD6244-treated WM35 Cells. (A) WM35 cells were treated with 360nM of AZD6244, 10ng/ml IGF-1, 100ng/ml IGF-1 or a combination of AZD6244 and 10ng/ml IGF-1 or 100ng/ml AZD6244 for 24 h. The cells were harvested and western blotted for P-AKT and AKT. (**B**) The WM35 cells were treated as above, incubated for 48 h and cellular apoptosis was detected using the cytoplasmic histone DNA fragment analysis. Absorbance of the mock treatments represent no- or basal levels of apoptosis in untreated cells.

Figure S12



Apoptosis induction in group 2 cells by AZD6244 combination treatments.
The (**A**) SKMEL5 and (**B**) MEL624 cells were treated with AZD6244 or AZD8055 or Rapamycin, either singly or in combinations. Untreated controls always contained equivalent amounts of dmso vehicle. Apoptosis induction was determined through the cytoplasmic histone-DNA fragment analysis. Absorbance of the control (dmso) treatments represent no- or basal levels of apoptosis in these cells. Bars represent average of triplicates and errors are indicated by standard deviation.