Table S1 Human cancer genes harbouring somatic mutations in the three cell lines Hep3B, HepG2 and Huh-7

Mutated cancer genes [61] were identified using the COSMIC Cell Lines database for Hep3B and Huh7. For HepG2, only mutations in CTNNB1 (β-catenin) and NRAS have been reported in the literature.

Hep3B	**HepG2**	**Huh-7**
AXIN1	CTNNB1	FOXP1
BCR	NRAS	ABI1
CD74		ALK
CDKN2A		AMER1
CREB3L2		CYLD
CREBBP		FAM22D
ERCC3		FANCD2
HIST1H3B		KMT2D
MLL3		MAX
NFE2L2		MLL3
NUP214		MYH9
RB1		PTPRC
TRRAP		RANBP17
		ROS1
		SYK
		TP53
		TPR
		UBR5

Figure S1 Effects of AZD6244 or AZD8055 treatment on intracellular signaling

HCC cell lines were incubated with increasing concentrations of AZD6244 or AZD8055 as indicated over 24 h. PI3K-AKT-mTOR and RAF-MEK-ERK signaling pathway activity was analyzed by western blot with antibodies directed against the indicated targets. HSC70 served as loading control.

Figure S2 Effects of AZD6244 or AZD8055 proliferation of the three HCC cell lines

HCC cells were seeded into 96-well plates and incubated with increasing concentrations of MEK inhibitor AZD6244 or mTOR inhibitor AZD8055. Controls were treated with DMSO only. Proliferation was analyzed after 72h by BrdU incorporation. Each data point represents mean of three independent experiments measured in triplicated, normalized to controls.

Figure S3 Quantification of synergistic interactions between inhibitors targeting AKT (MK-2206), mTOR (AZD8055) and MEK (AZD6244)

The interaction between two compounds was analysed using the method proposed by Chou and Talalay, as described in the Material and Methods section. Fractional Effect blots were computed based on the experiment as described in Fig. 1 for each drug combination and cell line. Combination Index (CI) values greater 1 indicate an antagonistic interaction; CI values below 1 indicate synergism.

Figure S4 Relief of feedback inhibition on CRAF restores pERK1/2 signaling

Huh7 cells were treated with AZD6244 for up to 48 hours, and cell lysates were prepared at the indicated time points, controls were treated with DMSO. RAF-MEK-ERK pathway activity was analyzed by western blot, and HSC70 served as loading control.

Figure S5 HepG2 R6244 cell are cross resistant against allosteric MEK inhibitor PD0325901, but not against tyrosine kinase inhibitor Sorafenib

HepG2 cells, as well as their AZD6244 resistant derivatives (HepG2 R6244) were seeded into 96-well plates and treated with increasing concentrations of Sorafenib or PD0325901, controls were treated with DMSO. Proliferation was analyzed after 72h by BrdU incorporation. Each data point represents three independent experiments, each performed in triplicates. Bars: SD.

Fig. S1

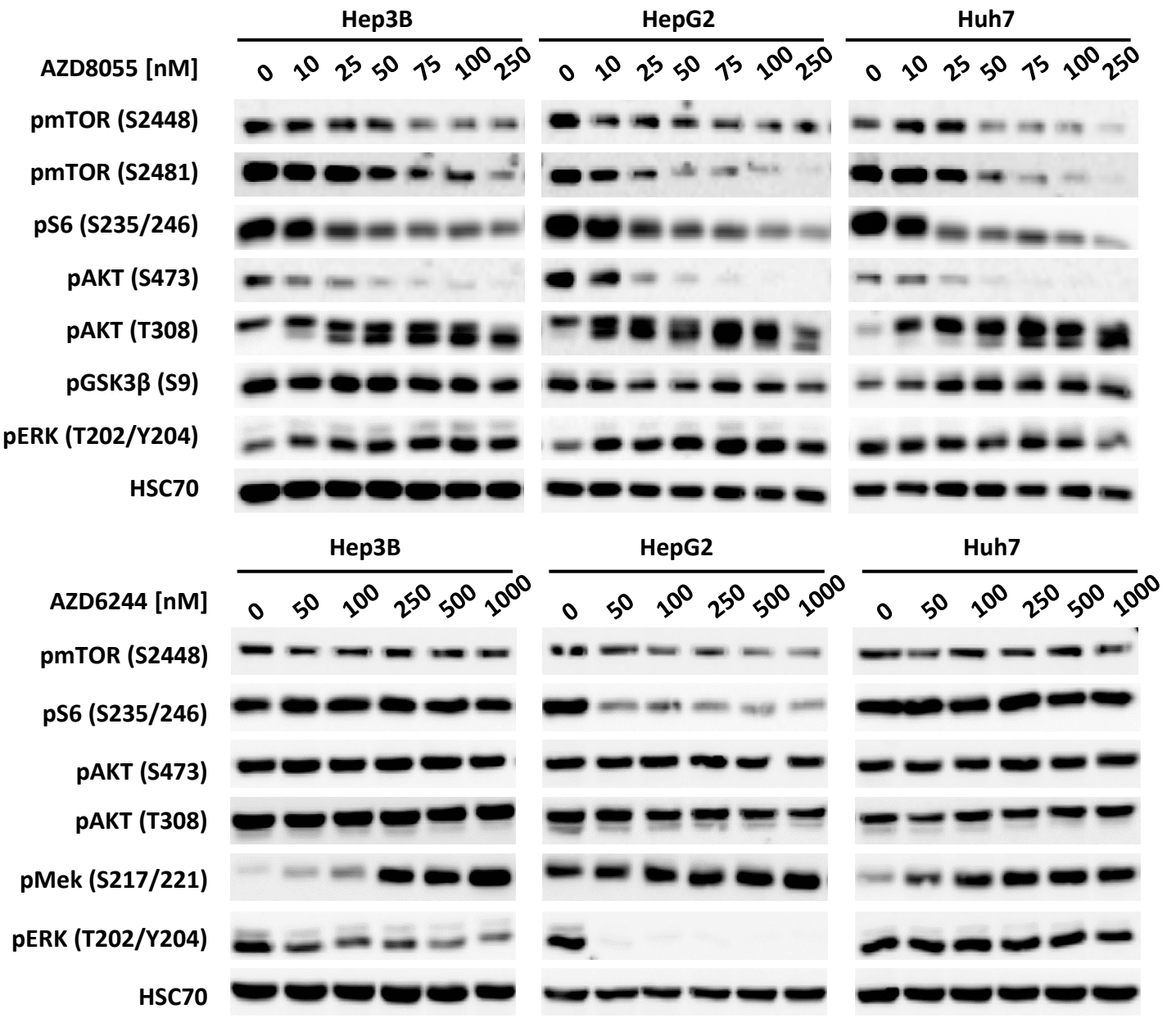

Hep3B
HepG2
Huh7
AZD8055 [nM]
0 10 25 50 75 100 250
pmTOR (S2448)
pmTOR (S2481)
pS6 (S235/246)
pAKT (S473)
pAKT (T308)
pGSK3β (S9)
pERK (T202/Y204)
HSC70
Hep3B
HepG2
Huh7
AZD6244 [nM]
0 50 100 250 500 1000
pmTOR (S2448)
pS6 (S235/246)
pAKT (S473)
pAKT (T308)
pMek (S217/221)
pERK (T202/Y204)
HSC70

Fig. S2

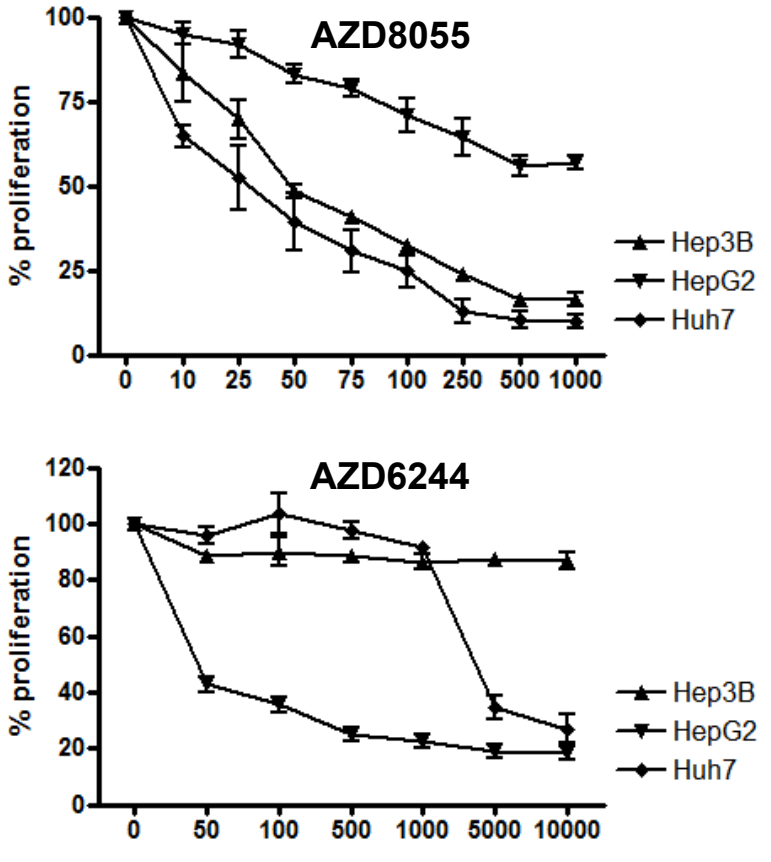

AZD8055
% proliferation
100
75
50
25
0
0 10 25 50 75 100 250 500 1000
Hep3B
HepG2
Huh7
AZD6244
% proliferation
120
100
80
60
40
20
0
0 50 100 500 1000 5000 10000
Hep3B
HepG2
Huh7

Fig. S3

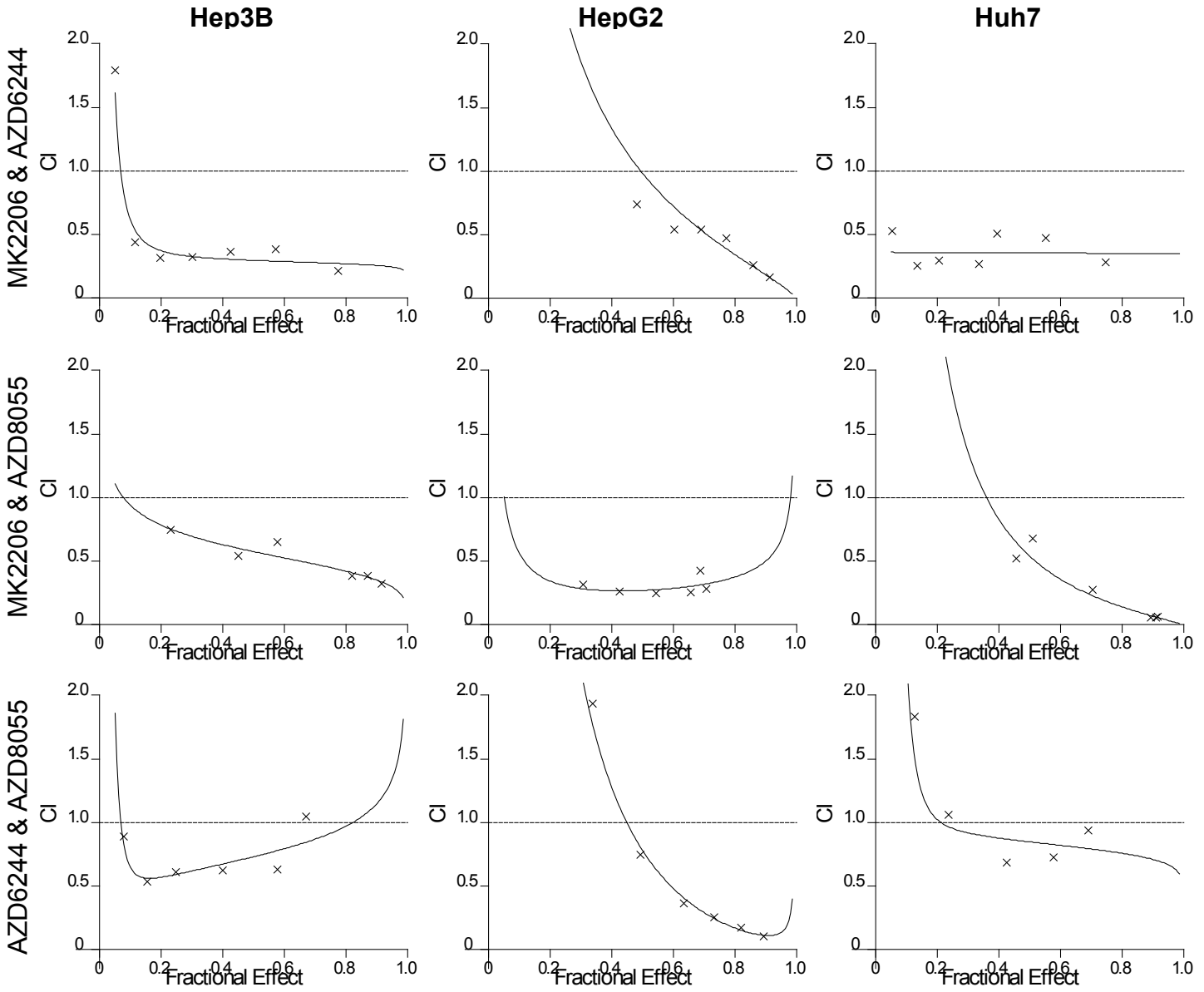

Hep3B
HepG2
Huh7
MK2206 & AZD6244
MK2206 & AZD8055
AZD6244 & AZD8055
CI
Fractional Effect
0
0.5
1.0
1.5
2.0
0.2
0.4
0.6
0.8

Fig. S4

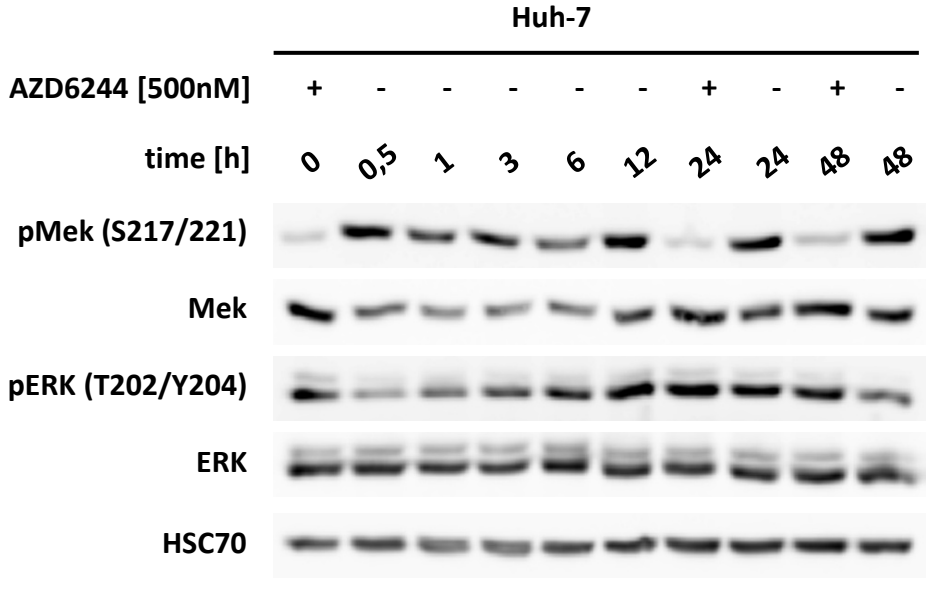
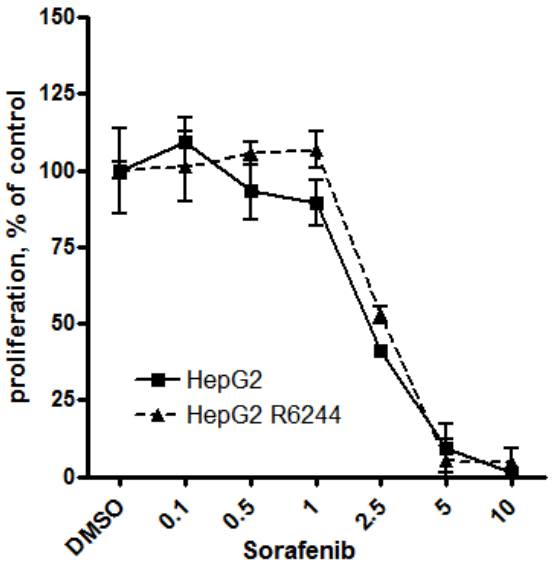
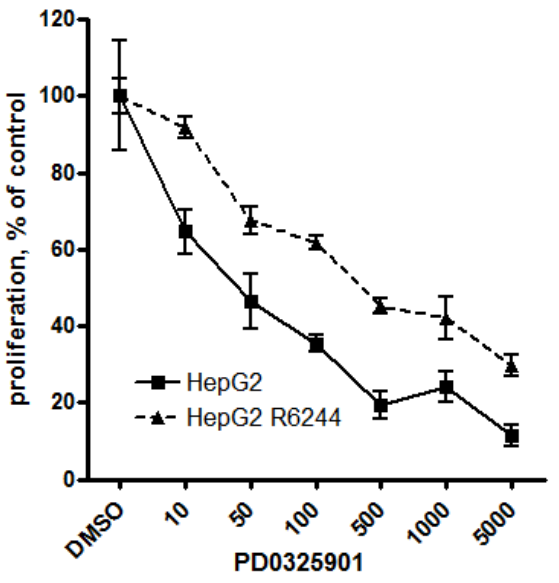

Huh-7
AZD6244 [500nM] + - - - - - + - + -
time [h] 0 0,5 1 3 6 12 24 24 48 48
pMek (S217/221)
Mek
pERK (T202/Y204)
ERK
HSC70

Fig. S5


150
125
100
75
50
25
0
proliferation, % of control
HepG2
HepG2 R6244
DMSO
0.1
0.5
1
2.5
5
10
Sorafenib


120
100
80
60
40
20
0
proliferation, % of control
HepG2
HepG2 R6244
DMSO
10
50
100
500
1000
5000
PD0325901