Table S1. The MEK1/2 inhibitor PD184352 synergizes with 17AAG to kill HEP3B cells that is abolished by over-expression of c-FLIP-s. HEP3B cells were infected 12h after plating at an approximate multiplicity of infection of 50 with either a control empty vector recombinant adenovirus (CMV) or a recombinant virus to express the caspase 8 inhibitor c-FLIP-s. Twenty four hours after infection, infected cells were plated as single cells (250-1500 cells/well) in sextuplicate and 12h after this plating the infected cells were treated with vehicle (VEH, DMSO), MEK1/2 inhibitor PD184352 (PD, 0.5-2.0 μM), a geldanamycin (17AAG, 0.25-1.00 μM) or with both drugs combined, as indicated at a fixed concentration ratio to perform median dose effect analyses for the determination of synergy. Forty eight hours after drug exposure, the media was changed and cells cultured in drug free media for an additional 10-14 days. Cells were fixed, stained with crystal violet and colonies of > 50 cells / colony counted. Colony formation data were entered into the Calcusyn program and combination index (CI) values determined. A CI value of less than 1.00 indicates synergy. These studies were repeated twice with near identical findings.

PD184352	17AAG	HEP3B (CMV)	
(μM)	(μM)	Fa	CI
0.5	0.25	0.52	0.16
1	0.5	0.58	0.24
1.5	0.75	0.65	0.26
2	1	0.72	0.25
PD184352	17AAG	HEP3B (c-FLIP-s)	
(μM)	(μM)	Fa	CI
0.5	0.25	0.20	0.99
1	0.5	0.31	0.89
1.5	0.75	0.35	1.06
2	1	0.40	1.10

Figure S1. Multiple MEK1/2 inhibitors interact with 17AAG in a greater than additive fashion to kill hepatoma cells. Human hepatoma cells (HEPG2, HEP3B) were treated 24 h after triplicate plating with vehicle (VEH, DMSO), a MEK1/2 inhibitor AZD6244 (AZD, 0.5 μM), a geldanamycin (17AAG, 0.5 μM) or with both drugs combined. Cell viability was determined 48 h after drug treatment as indicated by trypan blue exclusion visible light microscopy assays in triplicate using a hemacytometer (± SEM, n = 3, * $p < 0.05$ value greater amount of cell killing compared to VEH treated cells).

Figure S2. Expression of CRM A blocks pro-caspase 9 cleavage in MEK1/2 inhibitor and 17AAG treated hepatoma cells. HEP3B cells 12h after plating were infected with either a control empty vector recombinant adenovirus (CMV) or a recombinant virus to express the viral caspase 8 inhibitor CRM A. Twenty four hours after infection, as indicated, cells were treated with either vehicle (VEH, DMSO), MEK1/2 inhibitor PD184352 (PD, 2 μM), geldanamycin (17AAG, 1 μM) or both drugs combined. Cells were isolated 36h after drug exposure and the expression levels of pro-caspase 9 and cleaved caspase 9 fragments determined after SDS PAGE by immunoblotting (n = 2 independent experiments).

Figure S3. MEK1/2 inhibitors and 17AAG kill hepatoma cells in a CD95-FADD-caspase 8-BID-dependent manner. HEP3B cells were transfected with either a scrambled siRNA molecule (siSCR) or validated siRNA molecules to knock down the expression of CD95. Twelve hours after transfection, cells were re-plated. Twenty four hours after re-plating, cells were treated with vehicle (VEH, DMSO), MEK1/2 inhibitor PD184352 (PD, 1 μM), geldanamycin (17AAG, 1 μM) or both drugs combined. Cell viability was determined 48 h after drug treatment by annexin-proprdium iodide flow cytometry assay in triplicate using a hemacytometer, as indicated (± SEM, n = 2 independent studies, # $p < 0.05$ value lower amount of cell killing compared to corresponding value in siSCR treated cells).

Figure S4. Constitutive activation of MEK1 and AKT suppresses p38 MAPK activation following MEK1/2 inhibitor and 17AAG treatment in hepatoma cells. HEP3B cells 12h after plating were infected with either a control empty vector recombinant adenovirus (CMV), a recombinant virus to express constitutively active MEK1 EE, a recombinant virus to express constitutively active AKT, or both viruses to express activated AKT and MEK1 EE. Twenty four hours after infection, as indicated, cells were treated with either vehicle (VEH, DMSO), MEK1/2 inhibitor PD98059 (PD, 20 μM), geldanamycin (17AAG, 1 μM) or both drugs combined. Cells were isolated 48h after drug exposure and the phosphorylation of ERK1/2, AKT (S473) and p38 MAPK determined after SDS PAGE by immunoblotting (n = 2 independent experiments).

Figure S5. Combined activation of MEK1/2 and AKT suppresses 17AAG and MEK1/2 inhibitor lethality in HEPG2 cells. HEPG2 cells 12h after plating in triplicate were infected with either a control empty vector recombinant adenovirus (CMV), a recombinant virus to express constitutively active MEK1 EE, a recombinant virus to express constitutively active AKT, or both viruses to express activated AKT and MEK1 EE. Twenty four hours after infection, as indicated, cells were treated with either vehicle (VEH, DMSO), MEK1/2 inhibitor PD98059 (PD, 20 μM), geldanamycin (17AAG, 1 μM) or both drugs combined. Cells were isolated 48h after drug exposure and cell viability determined as indicated by trypan blue exclusion visible light microscopy assays in triplicate using a hemacytometer (± SEM, n = 3 independent experiments). # $p < 0.05$ value lower amount of cell killing compared to corresponding value in CMV infected cells; ## $p < 0.05$ value lower amount of cell killing compared to corresponding value in caAKT infected cells).

Figure S6. CD95 and FAS-L expression is not altered by modulation of AKT / MEK1/2 / p38 MAPK signaling. HEP3B cells 12h after plating into 4 chamber glass slides were infected with either a control empty vector recombinant adenovirus (CMV), a recombinant virus to express dominant

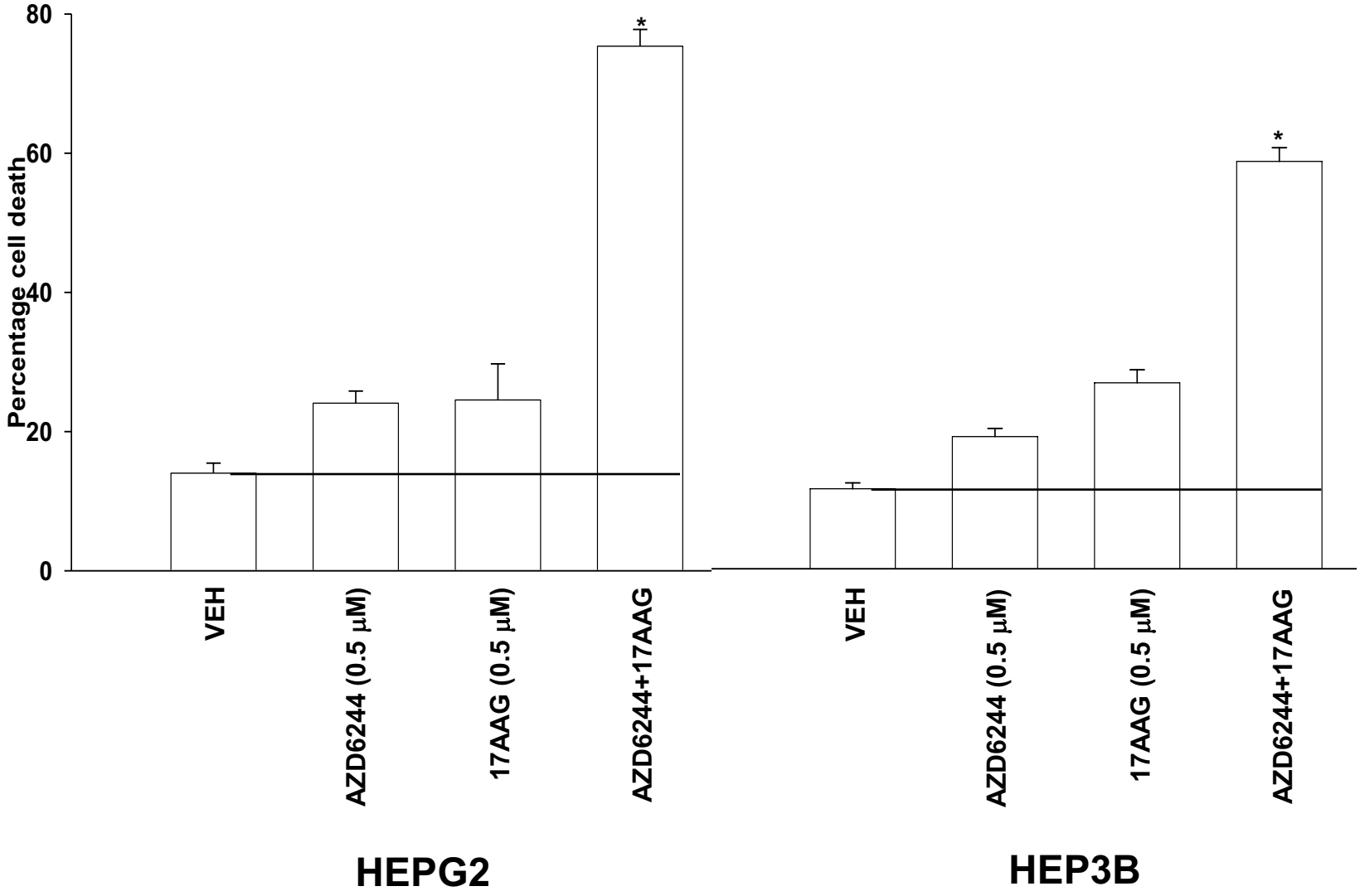
negative p38α MAPK, or viruses to express constitutively active forms of MKK1 EE and AKT. Twenty four hours after infection, as indicated, cells were treated with either vehicle (VEH, DMSO), or the combination of MEK1/2 inhibitor PD98059 (PD, 20 μM) and geldanamycin (17AAG, 1 μM). Six h after drug exposure, cells were lysed and lysates subjected to SDS PAGE followed by immunoblotting to determine the expression of CD95 and FAS ligand (n = 3 independent studies).

Figure S7. MEK1/2 inhibitors and 17AAG interact to promote cell death in human tumor cells via activation of p38 MAPK. HEP3B cells 12h after plating were infected with either a control empty vector recombinant adenovirus (CMV) or a recombinant virus to express dominant negative p38α MAPK. Twenty four hours after infection, as indicated, cells were treated with either vehicle (VEH, DMSO), MEK1/2 inhibitor PD184352 (1 μM), geldanamycin (17AAG, 1 μM) or both drugs combined. Cells were isolated 48h after drug exposure and annexin-propidium iodide flow cytometry assays performed in triplicate, as indicated (± SEM, n = 2, # $p < 0.05$ value lower amount of cell killing compared to corresponding value in CMV infected cells). Upper inset panel: HEP3B cells were infected as above and treated 24h after infection with 200 mM sorbitol and cells were isolated 15 min after treatment and p38 MAPK phosphorylation determined by immunoblotting (n = 2 independent studies).

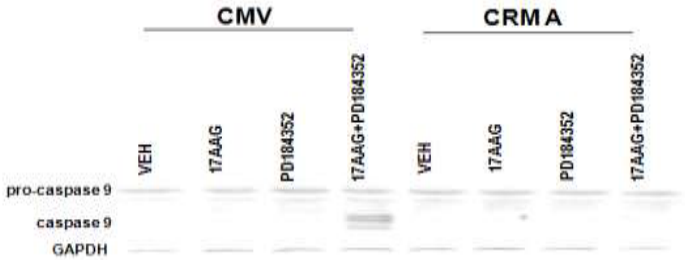
Figure S8. MEK1/2 inhibitors and 17AAG interact to promote cell death in human tumor cells. Treatment of hepatoma cells causes a rapid reduction in the activity of ERK1/2 and AKT which is responsible for reduced expression of c-FLIP-s, BCL-XL and XIAP, increased activation of p38 MAPK that in turn signals activation of CD95. Activation of CD95 promotes activation of caspase 8 and cleavage of BID. Cleavage of BID will promote activation of BAX and BAK by promoting their dissociation from proteins such as BCL-XL; phosphorylation of BAX and BAK by p38 MAPK will

also promote activation of BAX and BAK. Increased BID, BAX and BAK activity promotes cytochrome c translocation from the mitochondria into the cytosol where it can promote activation of caspase 9. Activation of caspase 9 is further promoted by reduced expression of XIAP. Caspase 9 promotes activation of effector caspases such as caspase 3.

Park et al. Figure S1



Park et al. Figure S2



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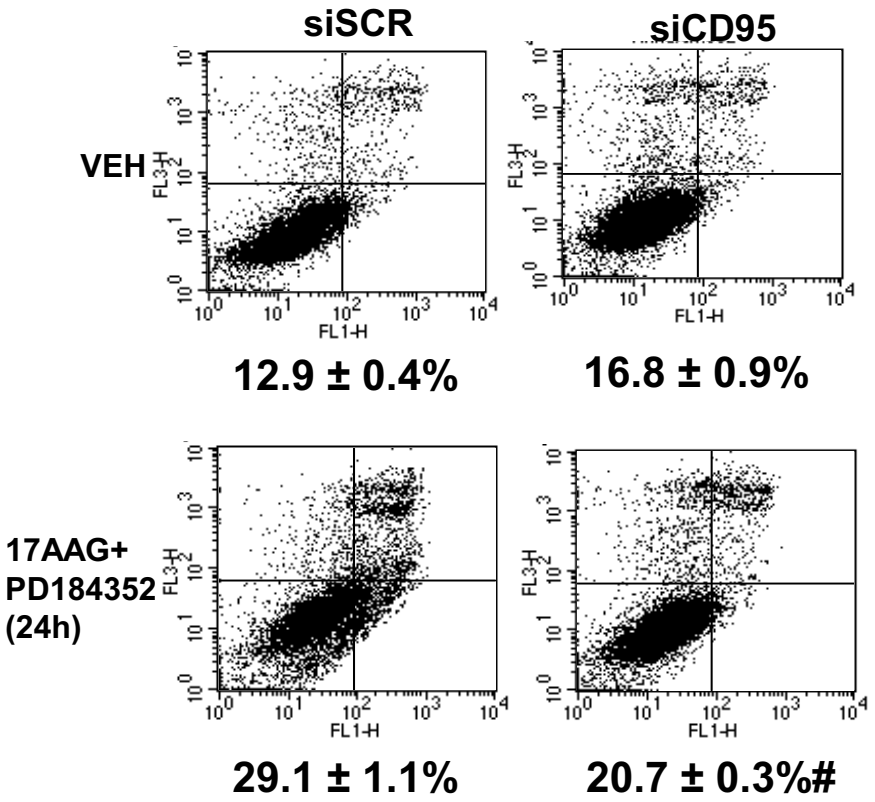

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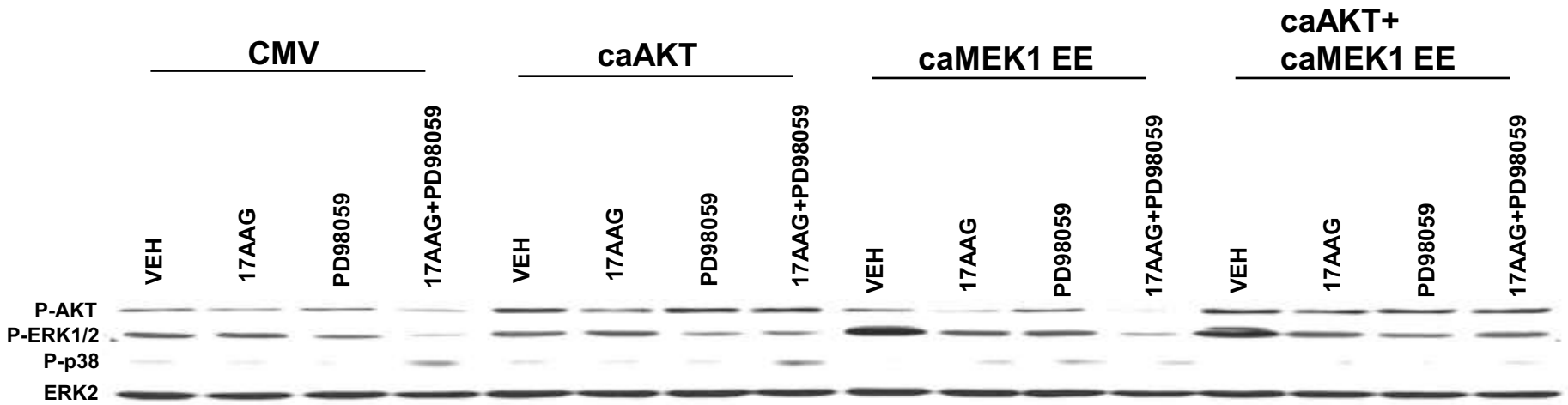

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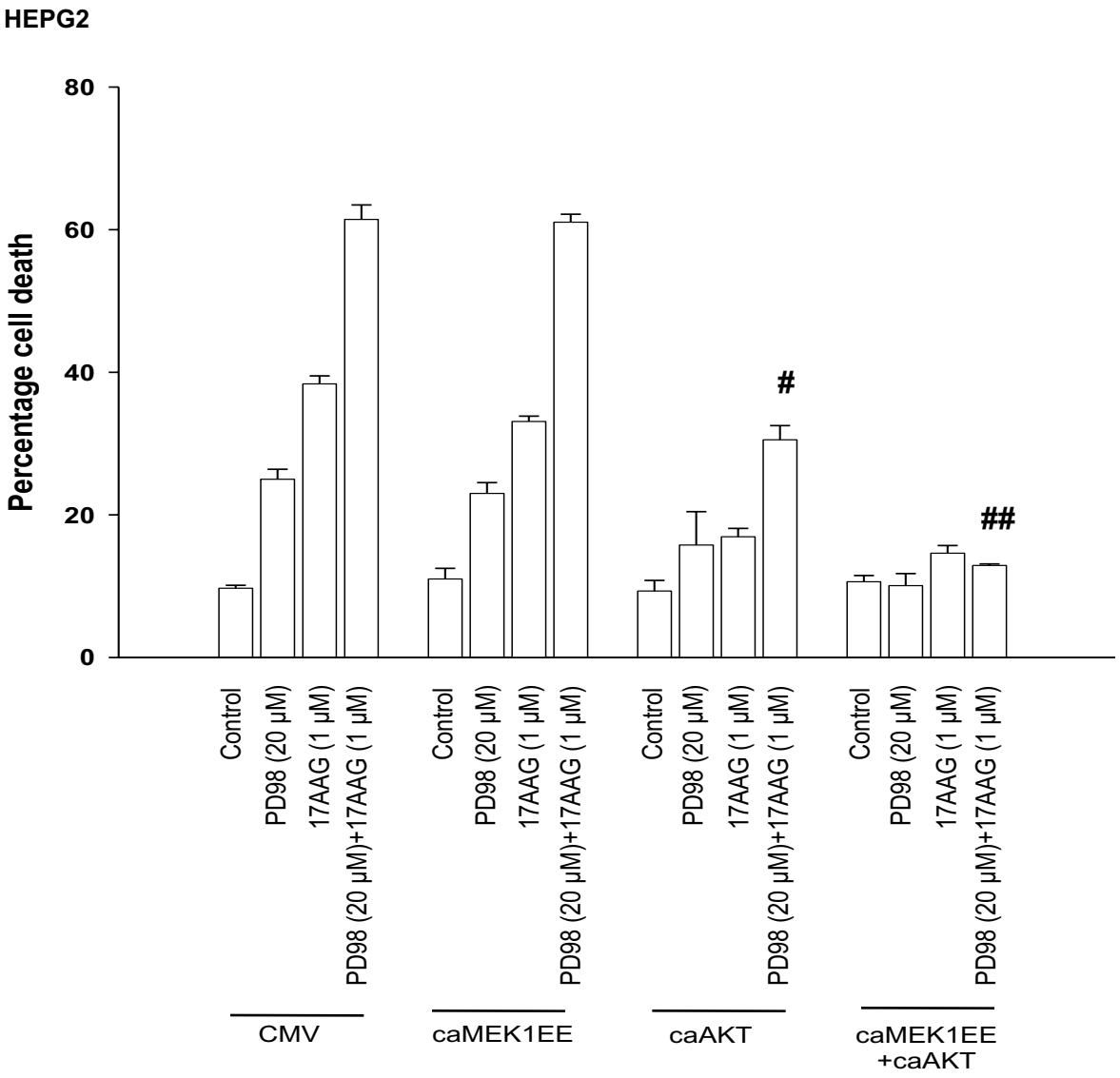

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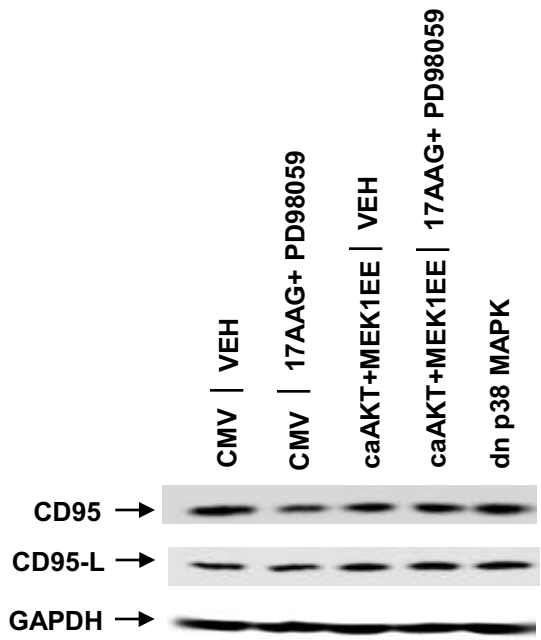

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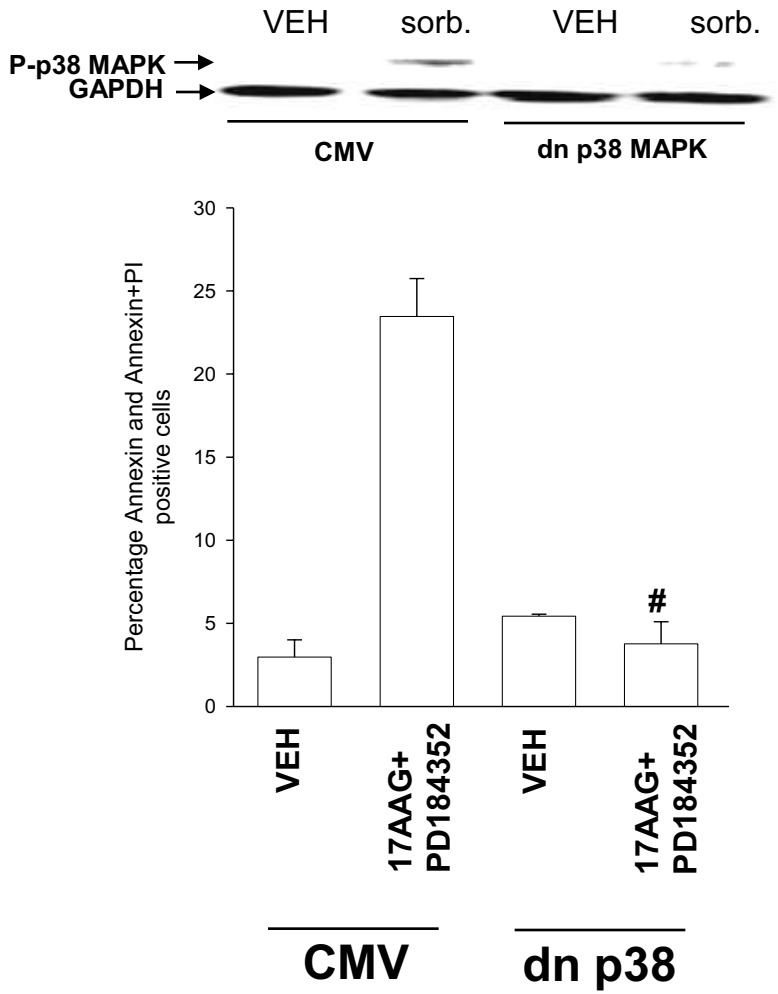

Park et al. Figure S8