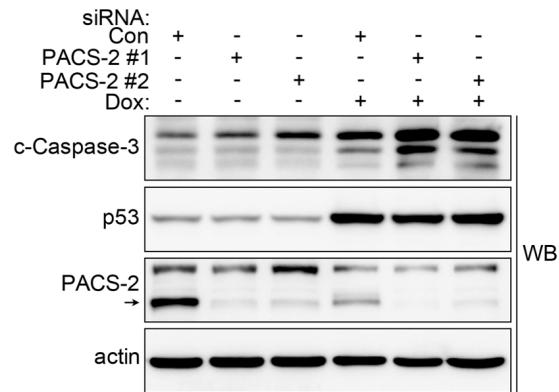
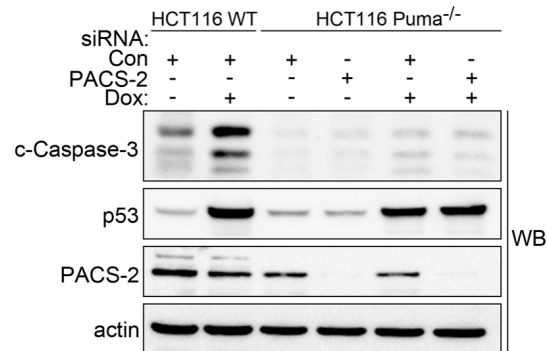


A



B



C

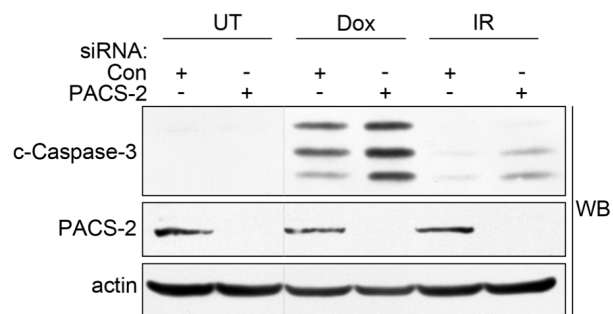
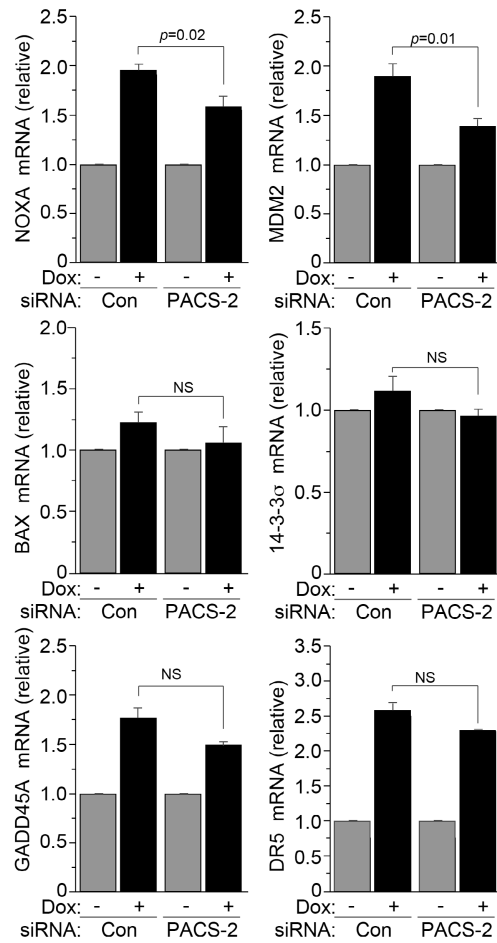
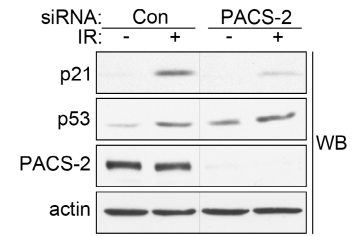


Figure S1, Related to Figure 1. (A) HCT116 cells were transfected with Control (Con) or PACS-2 siRNAs from Dharmacon (#1) or Qiagen (#2) for 48hr, treated for an additional 48hr with 0.5 μ M Dox, harvested and analyzed by western blot. Quantitation (AlphaView, Protein Simple) showed PACS-2 knockdown led to a 2.3-fold increase in cleaved caspase-3 compared to Dox-treated control cells. Arrow, PACS-2. **(B)** HCT116 WT or HCT116 Puma^{-/-} cells were transfected with Con or PACS-2 siRNAs for 48hr, treated for an additional 48hr with 0.5 μ M Dox, harvested and analyzed by western blot. **(C)** HCT116 WT cells were transfected with Con or PACS-2 siRNAs for 48hr, treated for an additional 48hr with 0.5 μ M Dox or 20 Gy IR, harvested and analyzed by western blot.

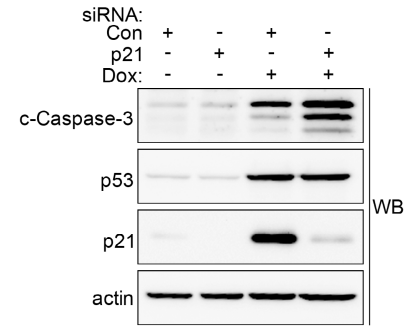
A



B



C



D

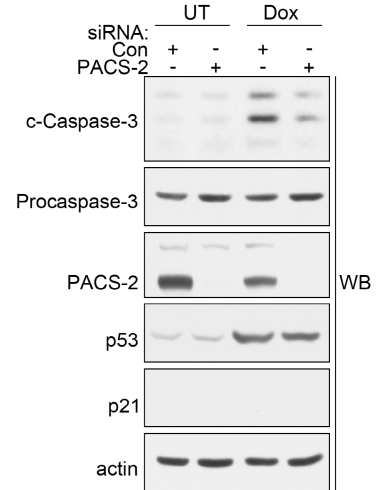


Figure S2, Related to Figure 1. (A) HCT116 WT cells from Figure 1, panel C were analyzed for the indicated transcripts by qRT-PCR (normalized to GAPDH). NS \geq 0.05. **(B)** U2OS cells were transfected with Con or PACS-2 siRNAs for 72hr, treated with 20 Gy IR 24hr prior to harvest and analyzed by western blot. **(C)** HCT116 WT cells were transfected with Con or p21 siRNAs for 48hr, treated for an additional 48hr with 0.5 μ M Dox and analyzed by western blot. Quantitation (AlphaView, Protein Simple) showed p21 knockdown led to a 2.2-fold increase in cleaved caspase-3 compared to p21 replete cells. **(D)** HCT116 p21^{-/-} cells were transfected with control or PACS-2 siRNAs for 48 hr and then treated for an additional 48 hr with 0.5 μ M Dox, harvested and analyzed by western blot.

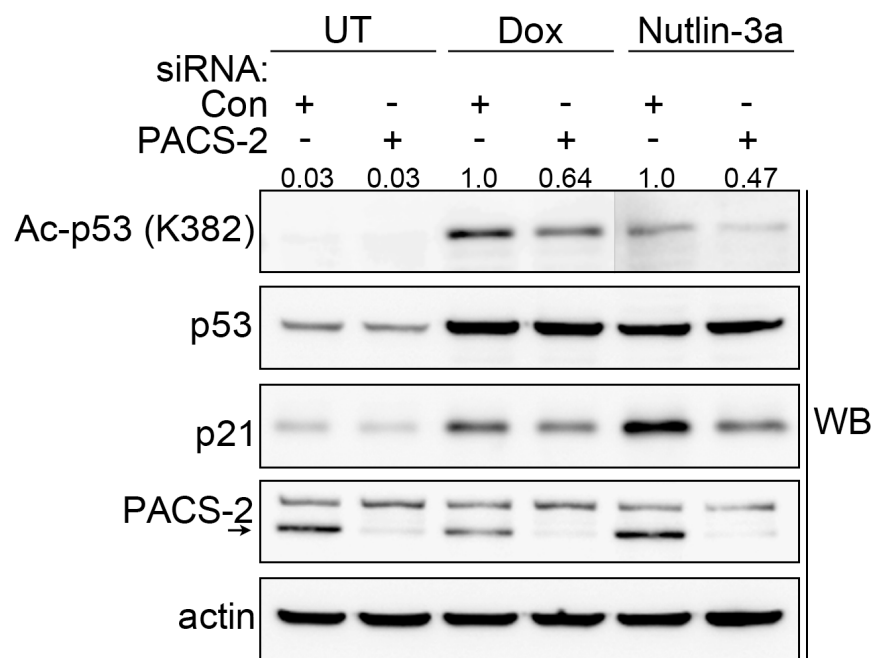


Figure S3, Related to Figure 2. HCT116 WT cells were transfected with Con or PACS-2 siRNAs for 48hr, treated for an additional 24hr with 0.5 μ M Dox or 4 μ M Nutlin-3a and analyzed by western blot. Ac-p53 (K₃₈₂) was quantified and normalized to total p53 (AlphaView, Protein Simple). A longer exposure of the Ac-p53 (K₃₈₂) blot for the Nutlin-3a treated samples is shown. Arrow, PACS-2.

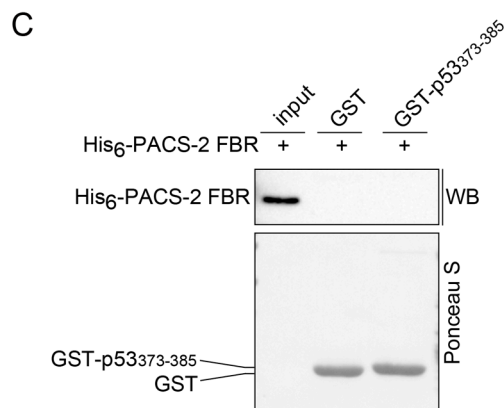
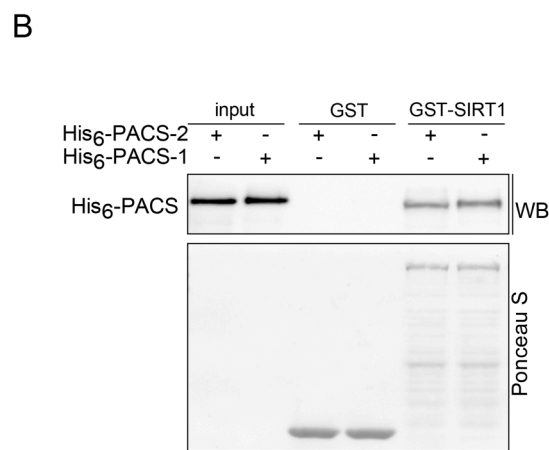
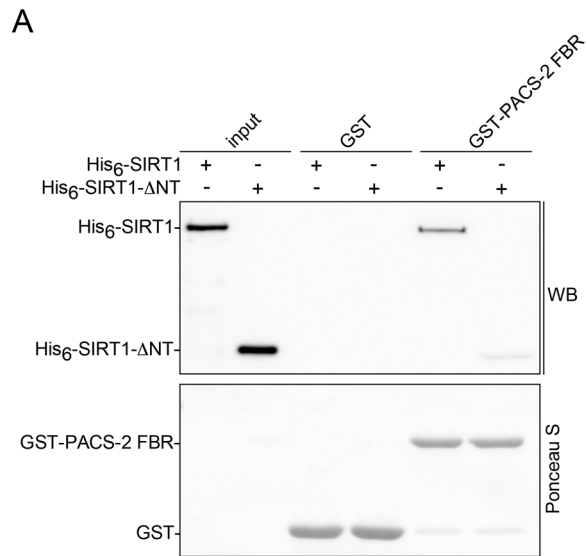


Figure S4, Related to Figure 7. (A) His₆-tagged full-length SIRT1 or SIRT1-ΔNT were incubated with GST-PACS-2_{FBR} or GST, captured with glutathione sepharose and bound SIRT1 or SIRT1-ΔNT were detected by western blot. Input GST and GST-PACS-2_{FBR} were detected with Ponceau S. **(B)** Full length His₆-tagged PACS-1 or PACS-2 were incubated with GST-SIRT1 or GST, captured with glutathione agarose and bound PACS-1 or PACS-2 were detected by western blot. Input GST and GST-SIRT1 were detected with Ponceau S. **(C)** His₆-tagged PACS-2_{FBR} was incubated with GST-p53₃₇₃₋₃₈₅ or GST, captured with glutathione sepharose and bound PACS-2_{FBR} was detected by western blot. Input GST and GST-p53₃₇₃₋₃₈₅ were detected with Ponceau S.

TABLE S1

Mean percentage of cells in each cell cycle phase \pm SD from 4 independent experiments. Statistical significance determined using an unpaired Student's t-test. Related to Figure 1.

	untreated		Doxorubicin (24 hr)		
siRNA:	Con	PACS-2	Con	PACS-2	<i>p</i> value
G ₀ /G ₁	7.4 \pm 0.4	6.8 \pm 1.4	24.7 \pm 3.1	15.3 \pm 1.7	0.004
S	87.7 \pm 0.5	88.6 \pm 3.4	63 \pm 3.1	70.5 \pm 1.6	0.012
G ₂ /M	3.8 \pm 0.5	3.6 \pm 2.1	10.1 \pm 0.3	11.6 \pm 0.7	0.013

TABLE S2

Mean percentage of cells in each cell cycle phase \pm SD from 3 independent experiments. Statistical significance determined using an unpaired Student's t-test to compare Dox vs. Dox + EX-527 for each siRNA condition. Related to Figure 5.

siRNA	Control			
	UT	Dox	Dox + EX-527	<i>p</i> value
G ₀ /G ₁	6.2 \pm 0.5	28.4 \pm 1.2	29.9 \pm 1.0	0.17
S	91.1 \pm 0.6	59.6 \pm 2.1	55.8 \pm 1.6	0.07
G ₂ /M	2.8 \pm 0.5	12 \pm 1.1	14.2 \pm 0.6	0.05
siRNA	PACS-2			
G ₀ /G ₁	4.9 \pm 0.6	18.0 \pm 0.9	25.7 \pm 0.9	<0.001
S	92.8 \pm 0.5	67.0 \pm 2.5	58.8 \pm 3.2	0.03
G ₂ /M	2.3 \pm 0.4	15 \pm 1.8	15.5 \pm 3.6	0.9
siRNA	p21			
G ₀ /G ₁	5.2 \pm 0.2	10.1 \pm 1.5	11.3 \pm 0.6	0.6
S	92.3 \pm 0.5	79.0 \pm 1.2	75.5 \pm 1.4	0.1
G ₂ /M	2.5 \pm 0.6	10.3 \pm 2.7	13.2 \pm 1.4	0.2