A Novel benzopyrane derivative targeting cancer cell metabolic and survival pathways

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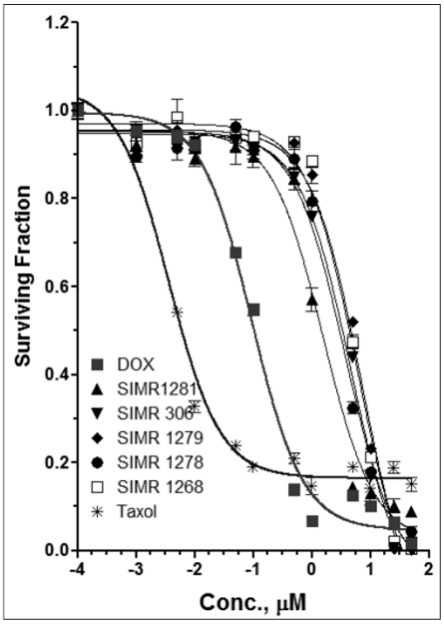
Supplementary Figure S1: Screening of a small library of polysubstituted benzopyranes, for potential anti-cancer activity on cancer cell lines. **(a)** Dose dependent effect of the synthesized SIMR compounds on MCF7, SKBR3 and HCT-116 after 48 h treatment, as analyzed by SRB assay. Points, mean; bars, SEM (n=6). **(b)** The IC_{50} of SIMR compounds in cancer cell lines after 48 h treatment. Data are mean ± SEM (*n=3)*. **(c)** Dose dependent effect of the synthesized SIMR -1281 on MCF7, MCF7 dox resistant, A549 and A549 dox resistant cell lines after 48 h treatment, as analyzed by MTT assay. Points, mean; bars, SEM (*n=6*).

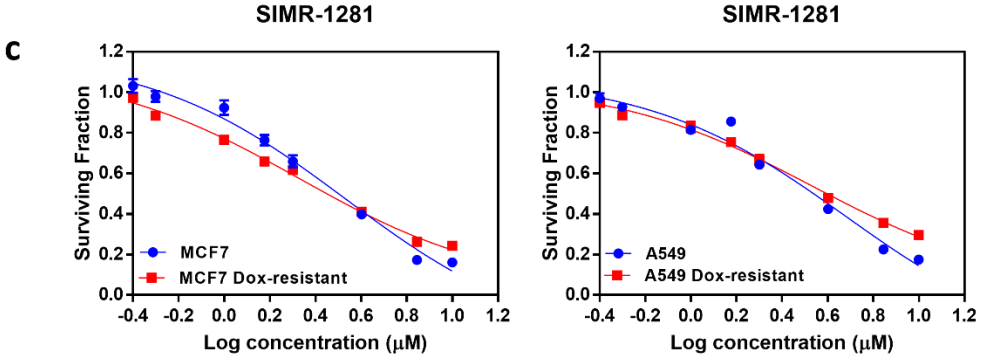
a



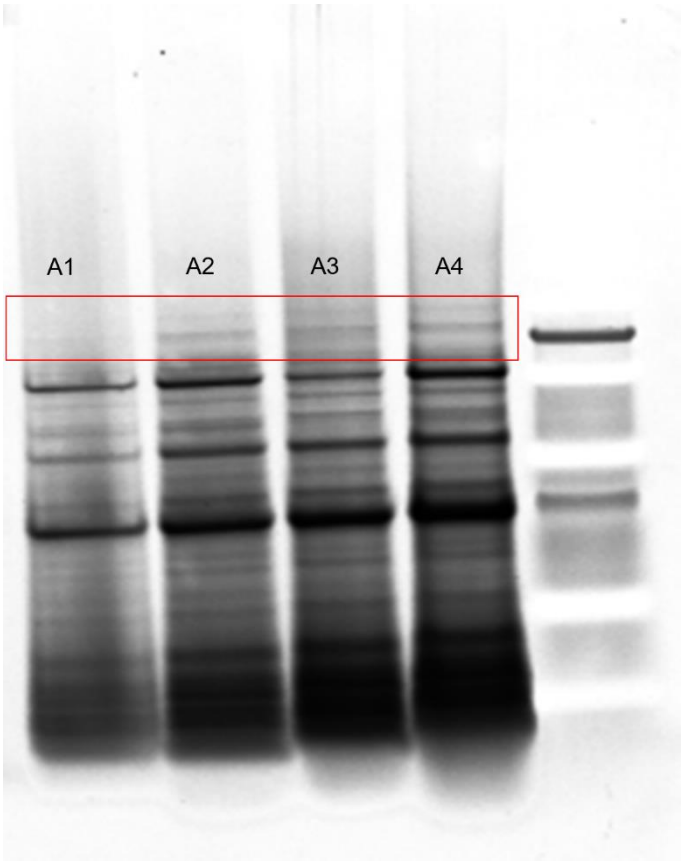
b

Compound	IC50(µM)		
	HCT116	MCF7	SKBR3
DOX	0.096	0.058	0.25
SIMR1281	1.554	1.91	1.9
SIMR306	4.739	11.8	9.4
SIMR1279	7.217	8.14	7.6
SIMR1278	3.733	4.5	6.4
SIMR1268	6.264	6.76	10.1

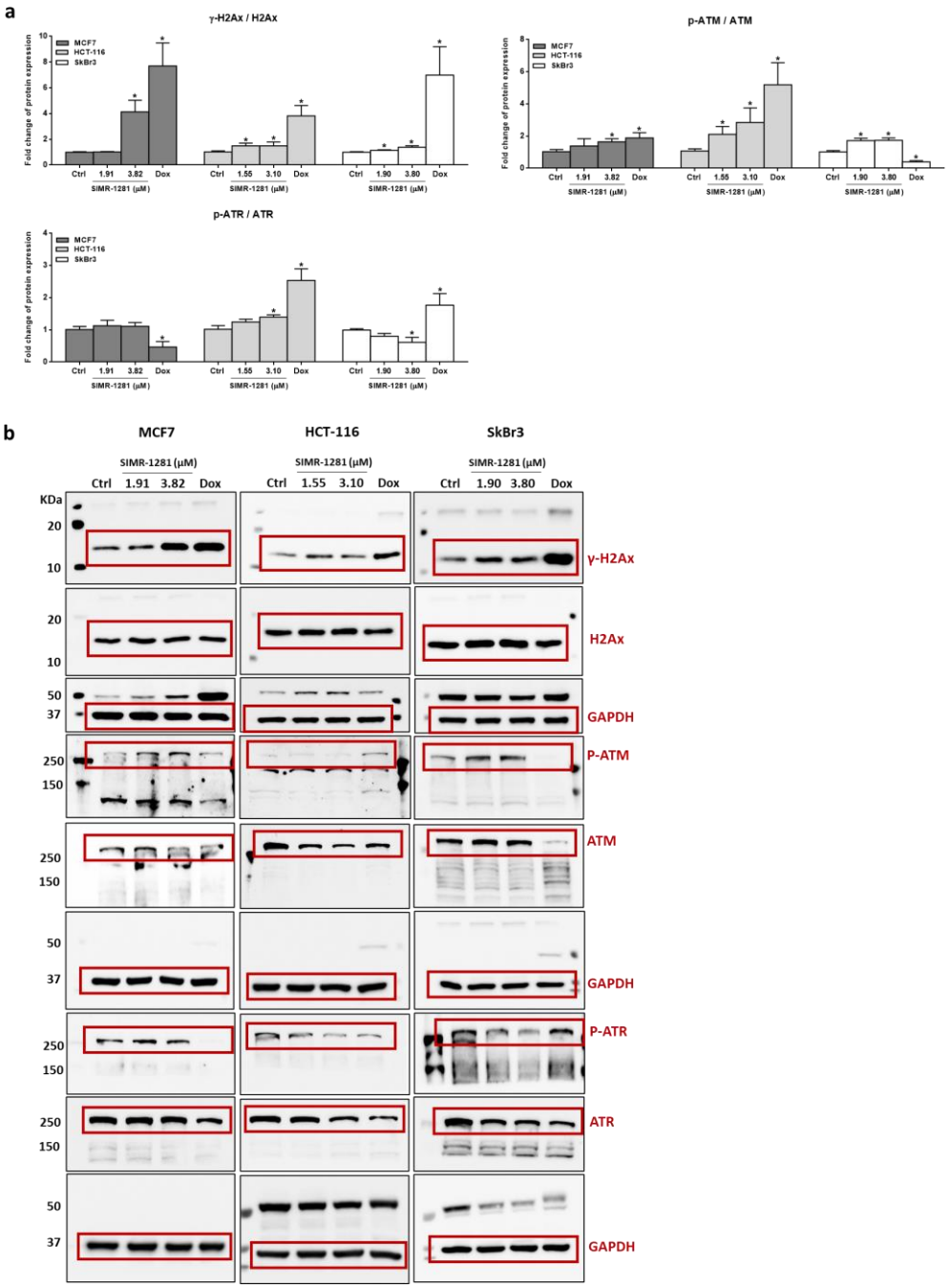
c



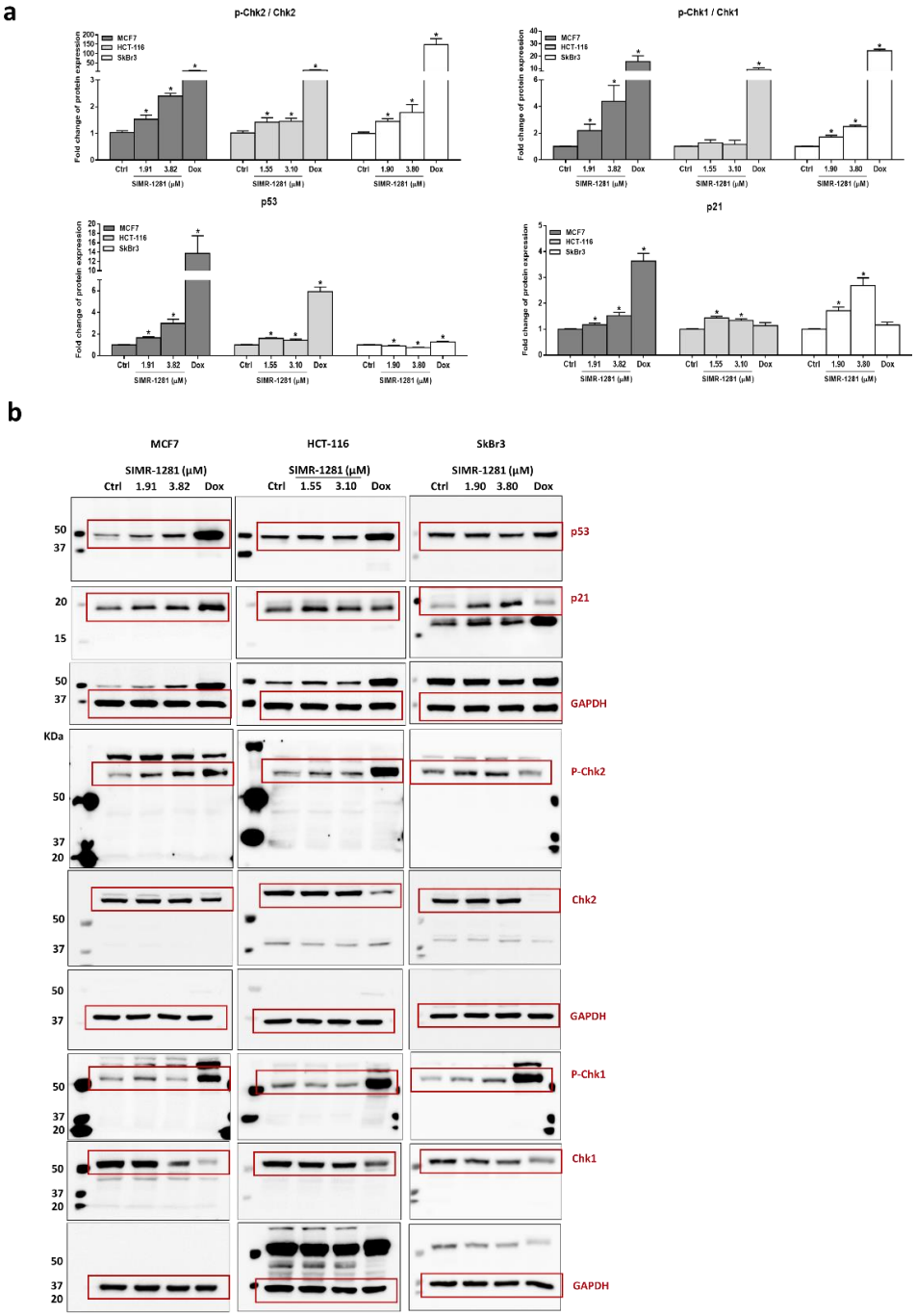
Supplementary Figure S2: DARST Assay showing the biding of SIMR1281 with protein targets



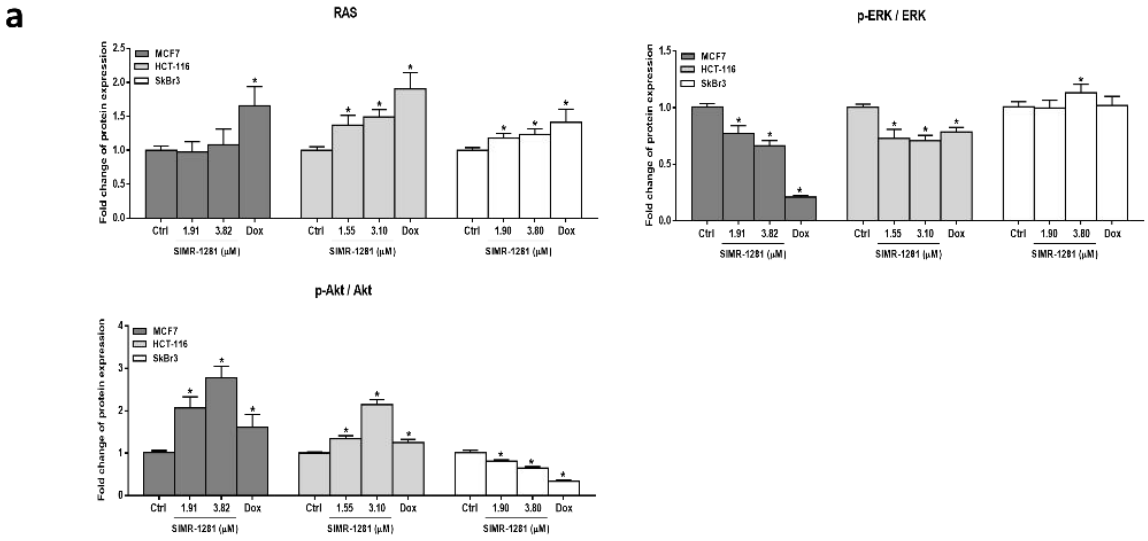
Supplementary Figure S3: Western blot analysis for γ-H2Ax/H2Ax, p-ATM/ATM and p-ATR/ATR proteins in MCF7, HCT116 and SKBR-3 cell lines after the treatment with SIMR-1281 and Dox for 24 h at the indicated concentrations (Whole blot corresponds to Fig3. a). **(a)** Quantification of the bands of γ-H2Ax, H2Ax, p-ATM, ATM, p-ATR and ATR proteins using Image lab software, Data expressed as mean ± SEM (*n=3*) independent experiments. *indicates significant difference versus control at p<0.05 determined by two-tailed unpaired *t*-test. **(b)** Full blot Western blot of the indicated proteins.

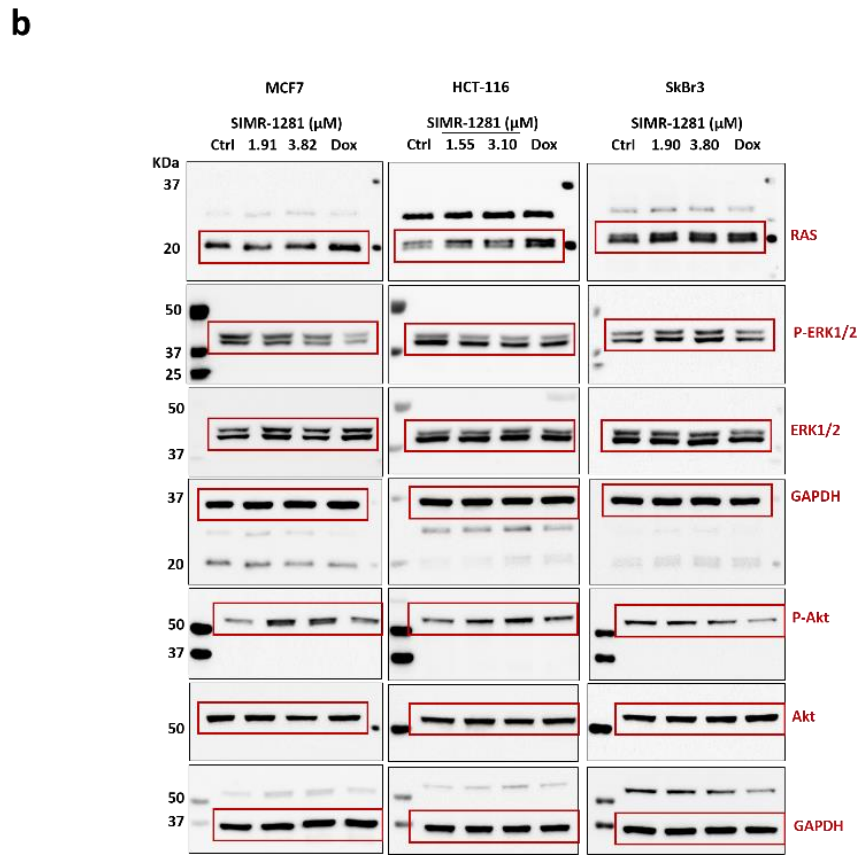


Supplementary Figure S4: Western blot analysis for p53, p21, p-Chk2/Chk2 and p-Chk1/Chk1 proteins in MCF7, HCT116 and SKBR-3 cell lines after the treatment with SIMR-1281 and Dox for 24 h at the indicated concentrations (Whole blot corresponds to Fig3. b). **(a)** Quantification of the bands of p53, p21, p-Chk2, Chk2, p-Chk1 and Chk1 proteins using Image lab software, Data expressed as mean ± SEM (*n=3*) independent experiments. *indicates significant difference versus control at p<0.05 determined by two-tailed unpaired *t*-test. **(b)** Full blot Western blot of the indicated proteins

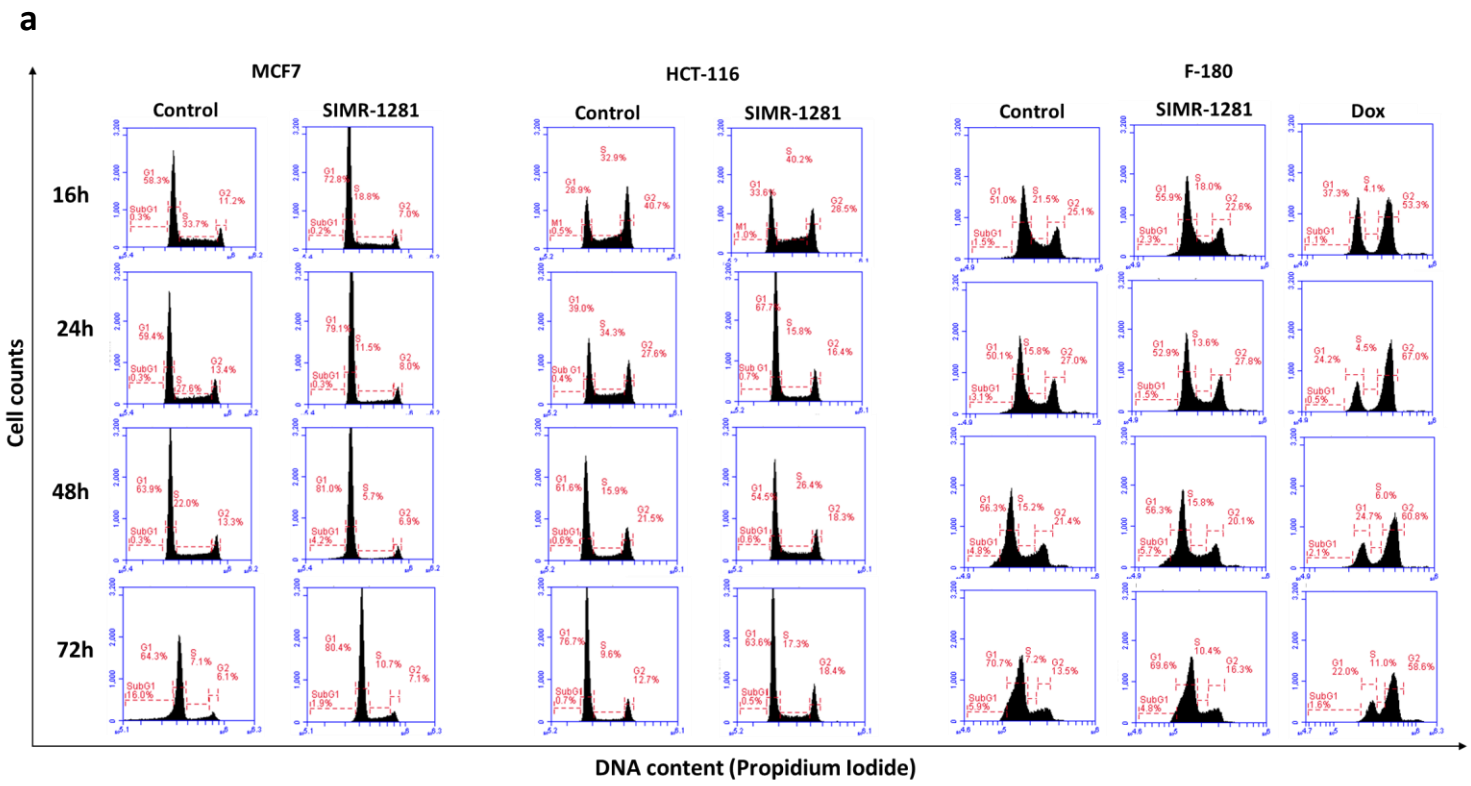


Supplementary Figure S5: Western blot analysis for Ras, p-ERK1/2, ERK1/2, p-Akt, Akt proteins in MCF7, HCT116 and SKBR-3 cell lines after the treatment with SIMR-1281 and Dox for 24 h at the indicated concentrations (Whole blot corresponds to Fig3. c). **(a)** Quantification of the bands of Ras, p-ERK1/2, ERK1/2, p-Akt, Akt proteins using Image lab software, Data expressed as mean ± SEM (*n=3*) independent experiments. *indicates significant difference versus control at p<0.05 determined by two-tailed unpaired *t*-test. **(b)** Full blot Western blot of the indicated proteins



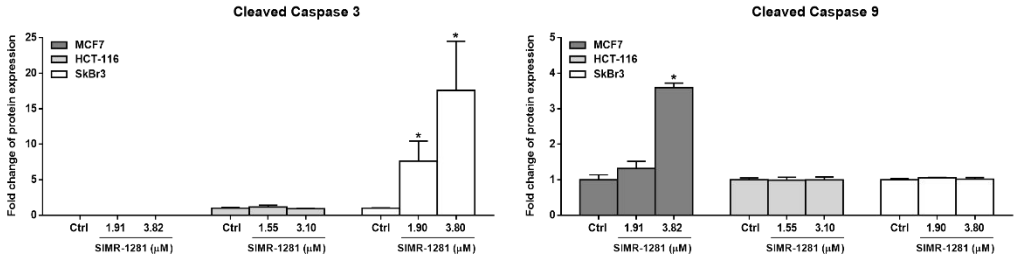


Supplementary Figure S6: Cell cycle analysis of MCF7, HCT116 and F-180 cells treated with SIMR-1281 at the indicated time points. (**a**) Histogram representation of the cell cycle distributions of MCF7, HCT116 and F-180 cells treated with SIMR-1281.



Supplementary Figure S7: Western blot analysis for caspase-3 and caspase-9 proteins in MCF7, HCT116 and SKBR-3 cell lines after the treatment with SIMR-1281 for 24 h at the indicated concentrations (Whole blot corresponds to Fig3. e). **(a)** Quantification of the cleavage bands of caspase 3 and caspase 9 proteins using Image lab software, Data expressed as mean ± SEM (*n=3*) independent experiments. *indicates significant difference versus control at p<0.05 determined by two-tailed unpaired *t*-test. **(b)** Full blot Western blot of the caspases proteins

a



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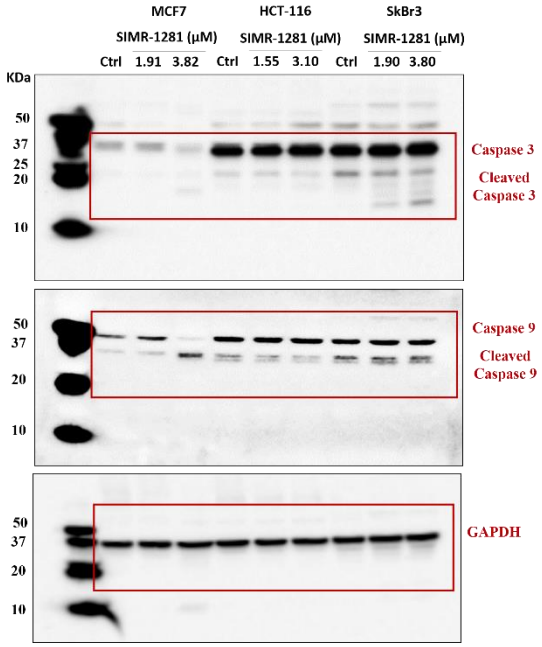
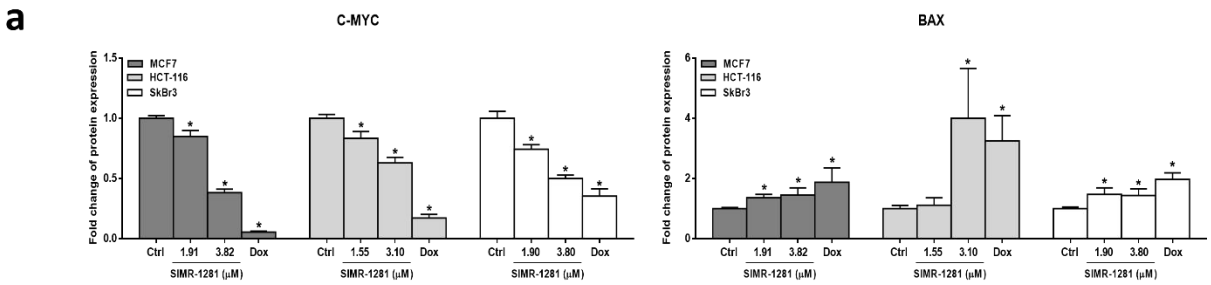
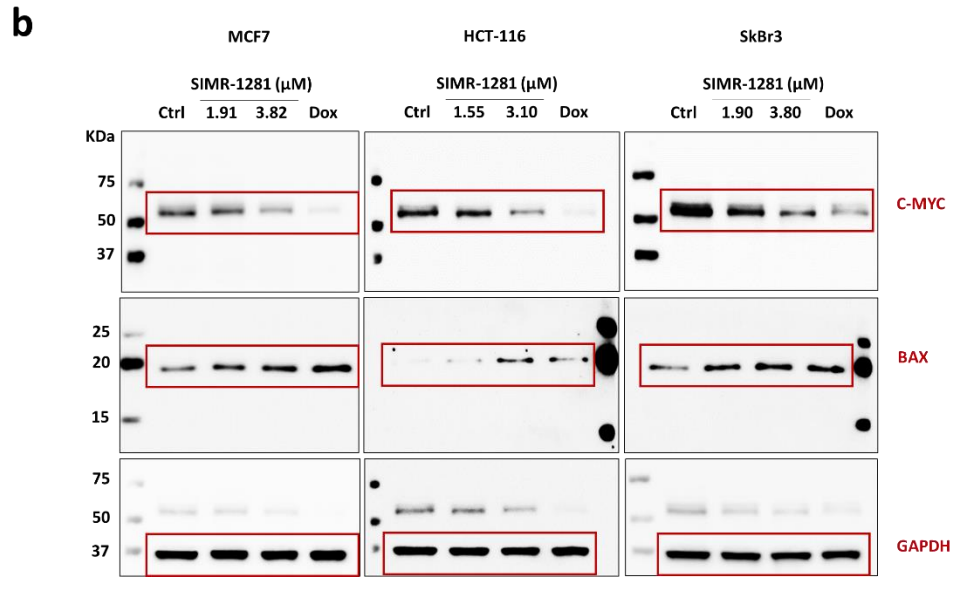
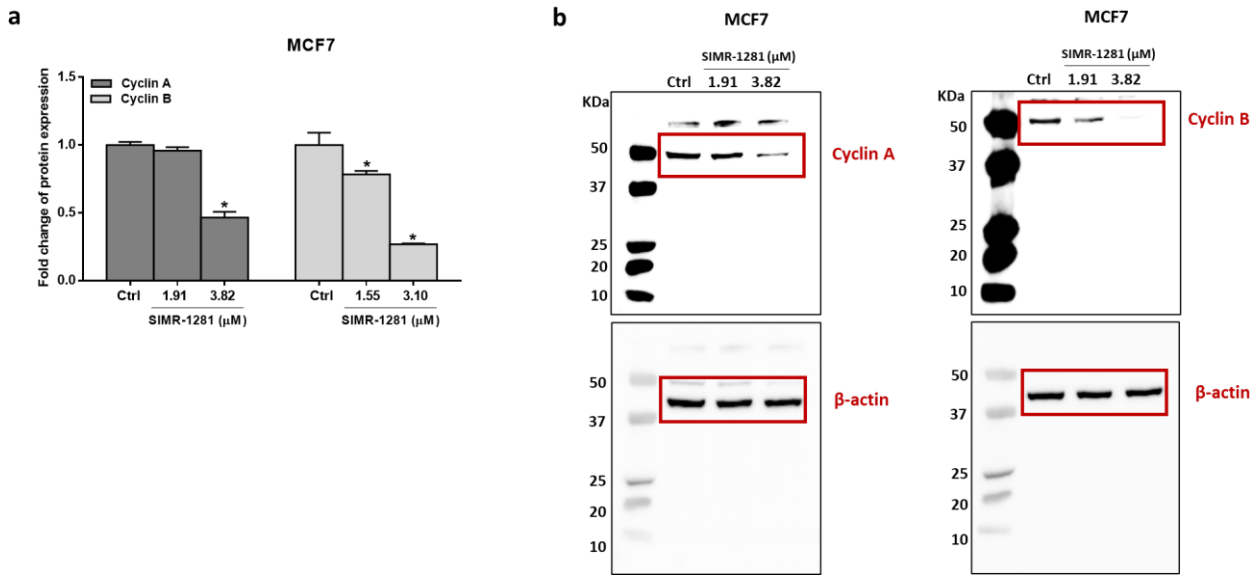


Supplementary Figure S8: Western blot analysis for apoptotic marker proteins in MCF7, HCT116 and SKBR-3 cell lines after the treatment with SIMR-1281 and Dox for 24 h at the indicated concentrations (Whole blot corresponds to Fig3. f). **(a)** Quantification of the apoptosis markers proteins bands using Image lab software, Data expressed as mean ± SEM (*n=3*) independent experiments. *indicates significant difference versus control at p<0.05 determined by two-tailed unpaired *t*-test. **(b)** Full blot Western blot of the apoptosis markers proteins

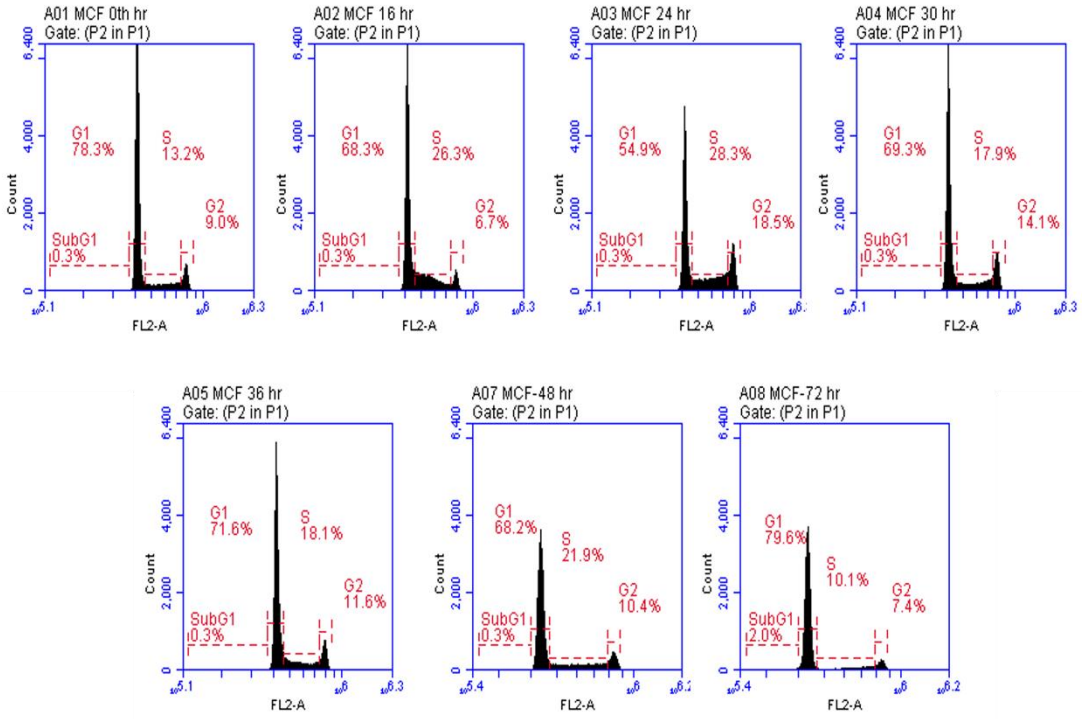




Supplementary Figure S9: Western blot analysis for cyclin A and cyclin B proteins in MCF7 cell line after the treatment with SIMR-1281 for 24 h at the indicated concentrations, (Whole blot corresponds to Fig4. c). **(a)** Quantification of cyclin A and cyclin B proteins using Image lab software, Data expressed as mean ± SEM (*n=3*) independent experiments. *indicates significant difference versus control at p<0.05 determined by two-tailed unpaired *t*-test. **(b)** Full blot Western blot of the cyclins proteins.



Supplementary Figure S10: Histogram representation of the cell cycle distributions of synchronized MCF7 cells at different time points.



Supplementary Figure S11: Hematology values[a] and serum chemistry values[a] from athymic nude mice bearing subcutaneous HCT116 xenograft tumors after 30 days of treatment with SIMR-1281. Values represent means ± SD. [a] WBC, white blood cells; RBC, red blood cells; HCT, Hematocrit; MCV, mean corpuscular volume; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; RDW, Red blood cell distribution width; RDWSD, Red blood cell distribution width; PLT, platelet; MPV, mean platelet volume; LY, leukocytes; MO, monocytes; NE, neutrophils; EO, eosinophils; BA, basophils. [B] ALT, Alanine aminotransferase; Aspartate Aminotransferase (AST); Alkaline Phosphatase (Alk Phos, ALP); Gamma Glutamyl Transpeptidase (GGT, GGTP or GTP); Significant difference versus control *** P<0.05 **P<0.005** determined by two-tailed unpaired *t*-test.

Hematology[b]	Units	Vehicle	SIMR-1281 (50mg/kg)
WBC	x10^3/µl	2.44±0.85	2.22±1.15
RBC	x10^3/µl	7.18±0.31	6.72±0.45
Hemoglobin	g/dL	12.42±0.75	11.53±0.76
HCT	%	32.34±2.11	30.89±2.18
MCV	fL	44.99±1.35	45.96±1.10
MCH	pg	17.28±0.35	17.16±0.25
MCHC	g/dL	38.42±0.58	37.36±1.06
RDW	%	23.61±1.26	26.04*±1.43
RDWSD	fL	26.14±0.5	29.46*±3.12
PLT	x10^3/µl	853.63±95.68	718.09±371.26
MPV	fL	6.30±0.40	7.69±1.96
LY	%	90.28±0.40	86.64±7.46
MO	%	5.68±2.81	8.03±6.73
NE	%	3.16±1.72	4.25±1.77
EO	%	0.52±0.27	0.81±0.57
BA	%	0.36±0.21	0.27±0.14
Serum Chemistry[c]			
Creatinine	mg/dL	1.04±0.89	1.176±0.398
ALT	iu/L	25.19±5.55	44.35±14.84
AST	iu/L	91.25±20.50	180.72±54.93
ALP	iu/L	33.42±19.21	25.722±13.25
GAMMA GT		11.88±9.20	16.45±2.041
Total Bilirubin	mg/dL	0.548±0.040	0.7±0.327
Total Protein	g/dL	7.136±0.157	10.124±4.383
Albumin	g/dL	3.277±0.168	2.660**±0.2478
Urea		51.22±2.469	57.094±12.29
Glucose	mg/dL	283.55±20.00	176.59±50.93

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