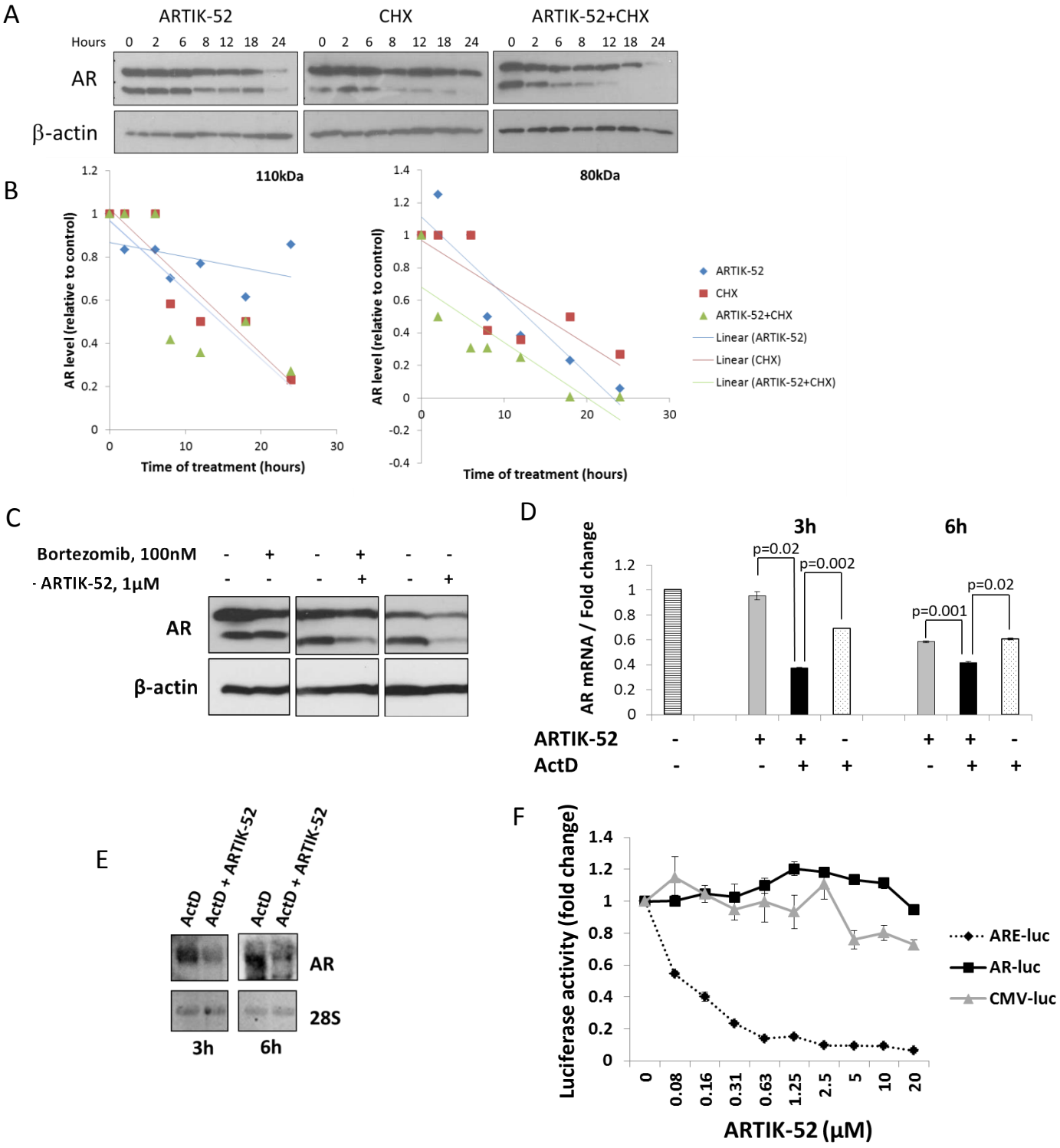


Figure S1. ARTIK-52 treatment leads to the reduction of AR on mRNA level. A. CWR22R cells were treated with 50 μg/ml of cycloheximide (CHX) alone or together with 1μM of ARTIK-52 for the indicated periods of time. Western blotting of total cell extracts. B. Quantitation of data shown on A. C. CWR22R cells were treated either with bortezomib or ARTIK-52 (1μM) alone or in combination for 8 hours. Western blotting of total cell extracts. D. Q- PCR analysis of AR mRNA levels in CWR22R cells treated with ARTIK-52 (1μM) or actinomycin D (ActD), 100ng/ml alone or in combination for 3 or 6h. E. Results of Northern Blot hybridization, CWR22R cells were treated with 100ng/ml ActD alone or in combination with 1μM ARTIK-52 for 3h or 6h. F. Activity of luciferase reporters regulated by different promoters (ARE - AR-responsive elements, AR - AR gene promoter; CMV-cytomegalovirus promoter) in CWR22R cells treated with the indicated amount of ARTIK-52 for 16 hours.

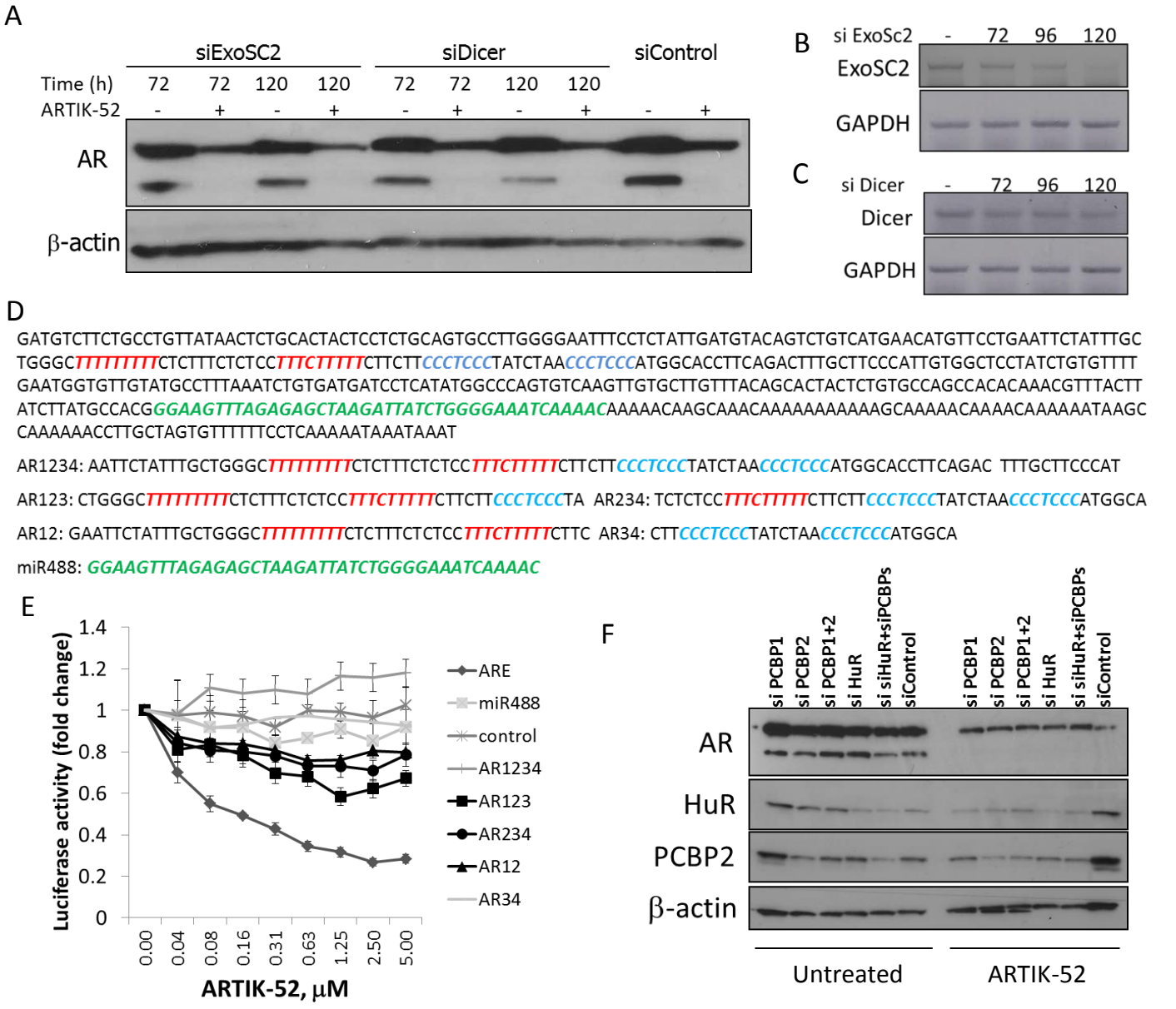


Figure S2. Effect of modulation of different RNA degradation mechanisms on ARTIK-52 induced AR mRNA reduction. A. Inhibition of RNA degradation using siRNA to ExoSC2 or DICER does not abrogate ARTIK-52 effect on AR mRNA. Western blotting of total cell extracts of CWR22R cells transfected with control siRNA or siRNA to ExoSC2 or DICER at different time point before treatment and treated with ARTIK-52 (1μM, 24hrs). B, C. Reduction of ExoSC2 (B) or Dicer (C) mRNAs at different time points (hours) after transfection of CWR22R cells with corresponding siRNAs. D. Fragment of AR 3'UTR mRNA with possible docking sites for HuR (red), PCBP1 and 2 (blue) and target site for miR 488 (green). Below are elements used for the cloning into reporter vectors. E. Effect of ARTIK-52 treatment on the activity of different luciferase reporters in CWR22R cells: ARE – with AR-responsive promoter, control – CMV promoter without any 3'UTR, others – with CMV promoter and 3'UTR elements of AR gene shown on panel D. Error bars are standard deviation between three replicates. F. Knockdown of HuR, PCBP1, PCBP2 or their combinations does not affect decrease of AR caused by ARTIK-52 treatment. Western blotting of total cell extracts of CWR22R cells transfected with siRNAs to corresponding RNA binding protein and treated 72 hours later with 3μM of ARTIK-52 for 16h.

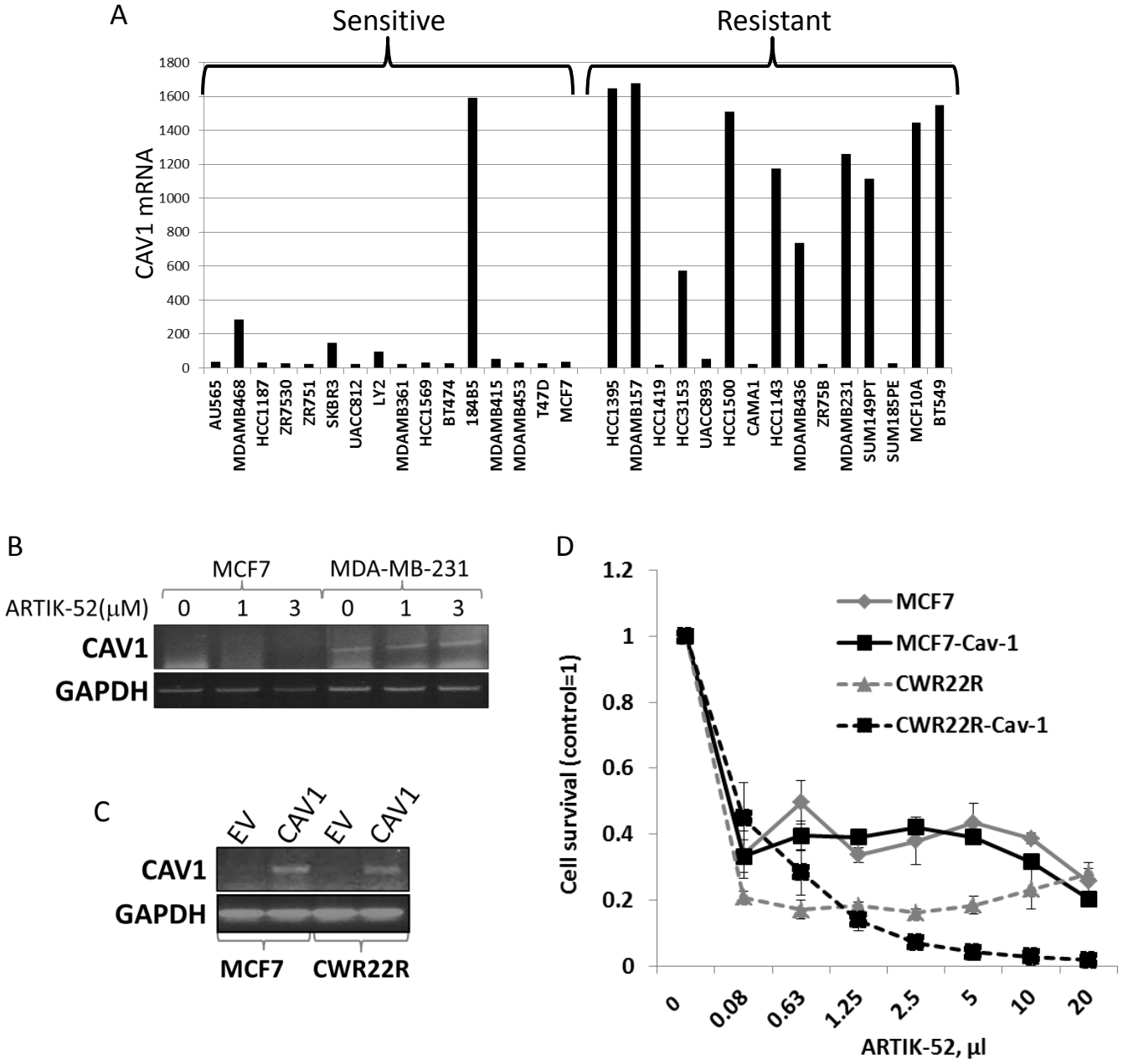


Figure S3. Overexpression of Caveolin 1 (CAV1) does not abrogate ARTIK-52 toxicity to sensitive cells. A. Caveolin 1 (CAV1) is predominantly expressed in resistant and not sensitive BC cell lines. Normalized signal intensity of microarray hybridization. B. RT-PCR analysis of CAV1 and GAPDH expression in MCF7 and MDA-MB-231 cells treated with ARTIK-52 for 24 hours. C. RT-PCR analysis of CAV1 and GAPDH expression in MCF7 and CWR22R cells transduced with CAV1 cDNA or empty vector (EV). D. Toxicity of ARTIK-52 to MCF7 or CWR22R transduced with CAV1 or empty vector. Error bars represent standard deviation between three replicates.

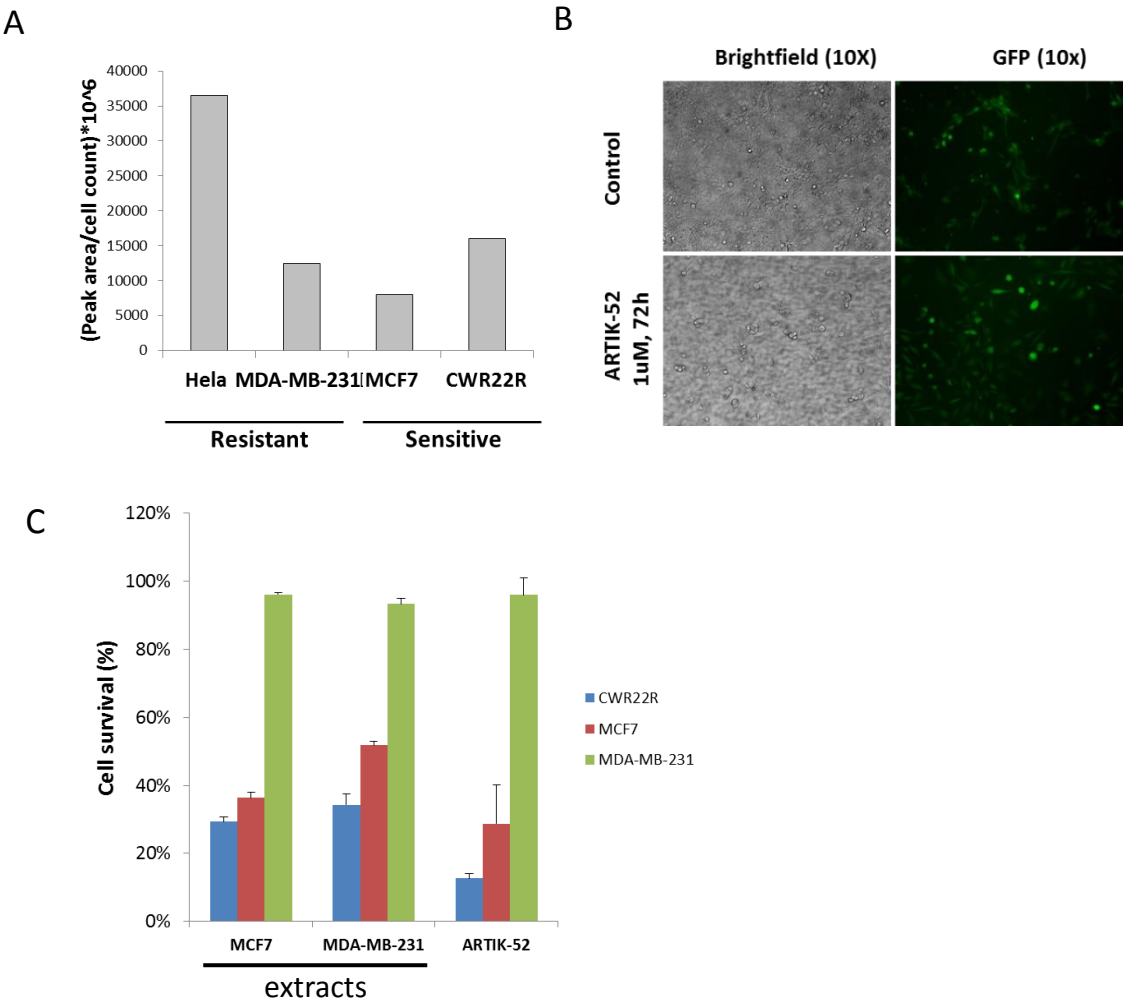


Figure S4. Comparison of ARTIK-52 uptake and potential metabolic conversion in sensitive and resistant cells. A. LC-MS detection of ARTIK-52 corresponding peaks in extracts of cells treated with 5μM of ARTIK-52 for 6 hours. Peak area was normalized by the amount of cells collected at the end of incubation with ARTIK-52. B. Co-culture of ARTIK-52 sensitive MCF7 cells with resistant MDA-MB-231 cells did not change pattern of ARTIK-52 sensitivity of either of cells. GFP labeled MDA-MB-231 cells were mixed with GFP-negative MCF7 cells and incubated with or without 3μM of ARTIK-52 for 24 hours. Photograph of cells at the end of incubation demonstrating no difference in the number of green cells between conditions, while complete loss of GFP-negative cells after treatment. C. Survival of cells in the presence of extracts from MCF7 or MDA-MB-231 cells incubated with ARTIK-52 during 20 hours or in medium with freshly added ARTIK-52 (1 μM) assessed 72 hours after start of incubation. Error bars are standard deviation between three replicates.

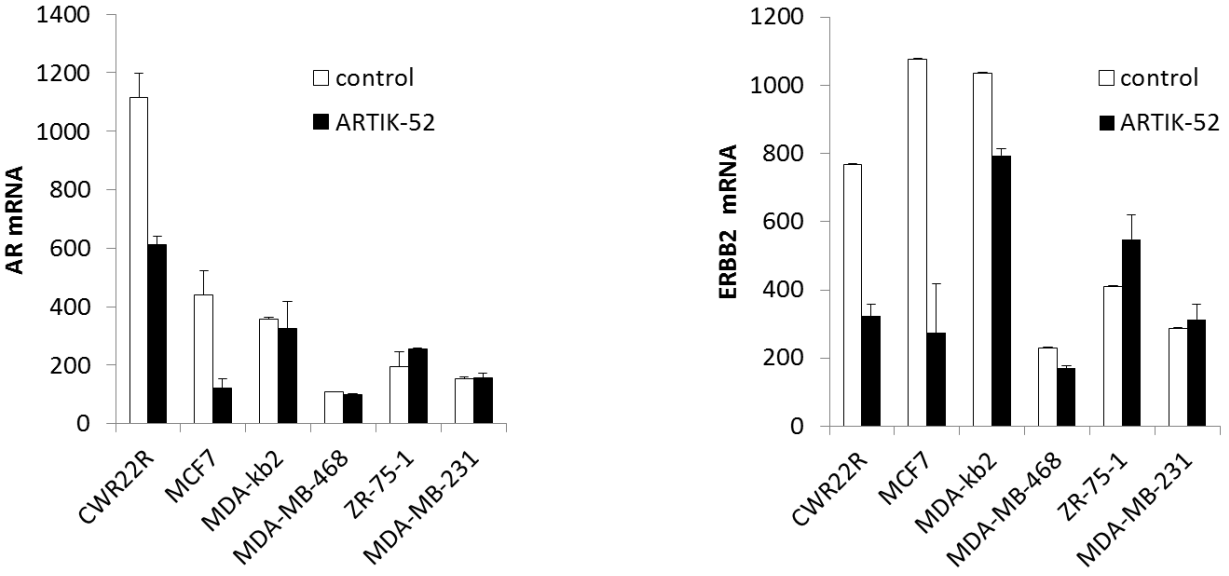
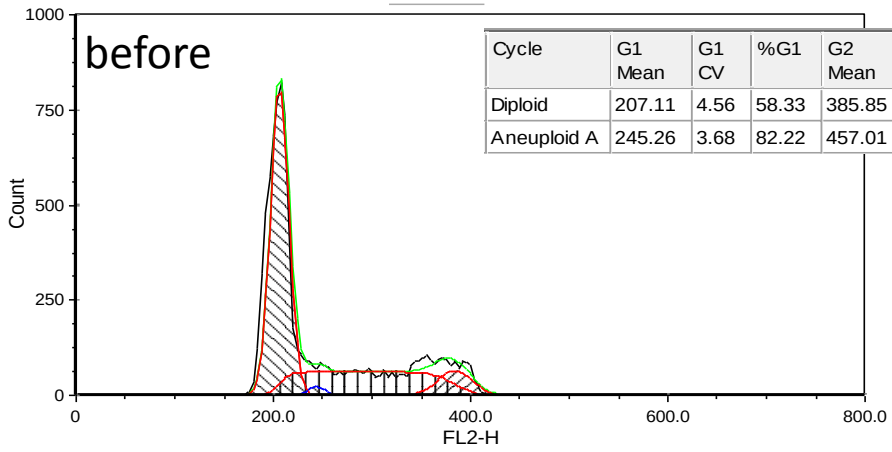
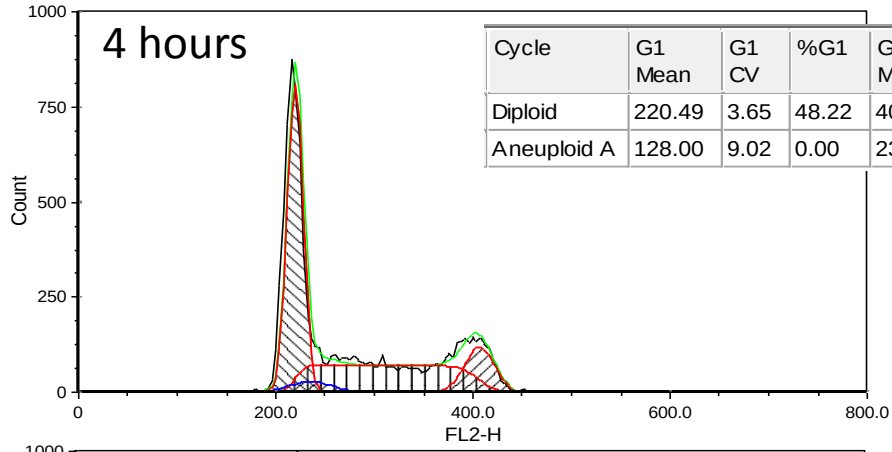


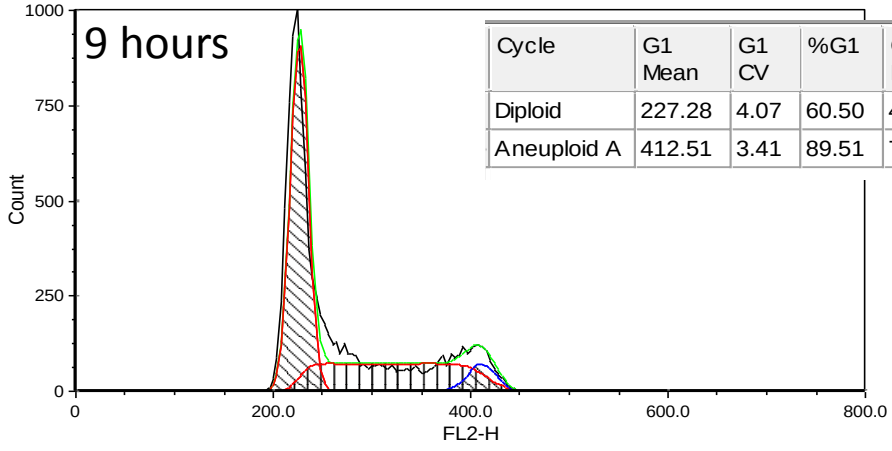
Figure S5. ARTIK-52 treatment leads to the reduction of AR and ERBB2 mRNA levels in sensitive (CWR22R, MCF7, MDA-kb2, MDA-MB-468), but not resistant cells (ZR-75-1, MDA-MB-231). Average normalized signal intensity of microarray hybridization. Error bars – deviation between three biological replicates.



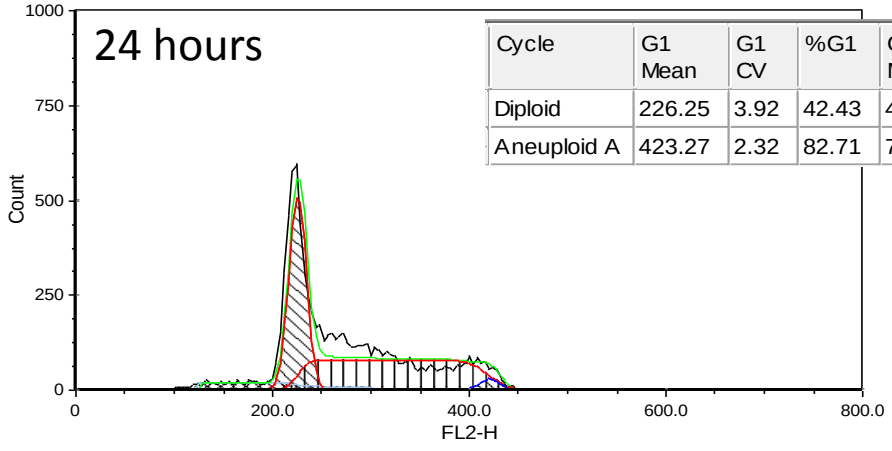
Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	207.11	4.56	58.33	385.85	4.56	8.56	33.11	1.86	98.18	n/a	0.23
Aneuploid A	245.26	3.68	82.22	457.01	3.68	17.78	0.00	1.86	1.82	1.18	0.30



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total
Diploid	220.49	3.65	48.22	408.13	3.65	13.11	38.67	1.85	95.82
Aneuploid A	128.00	9.02	0.00	236.93	9.02	100.00	0.00	1.85	4.18



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total
Diploid	227.28	4.07	60.50	419.57	4.07	0.00	39.50	1.85	92.57
Aneuploid A	412.51	3.41	89.51	761.46	3.41	0.00	10.49	1.85	7.43



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total
Diploid	226.25	3.92	42.43	422.86	3.92	0.00	57.57	1.87	96.78
Aneuploid A	423.27	2.32	82.71	791.01	2.32	0.00	17.29	1.87	3.22

Figure S6. Histograms and analyses of cell cycle distribution of CWR22R cells before treatment and at different time points after start of treatment with ARTIK-52 (1μM) analyzed using ModFit software.