Tioconazole and chloroquine act synergistically to combat doxorubicin-induced toxicity via inactivation of PI3K/AKT/mTOR signaling mediated ROS dependent apoptosis and autophagic flux inhibition in MCF-7 breast cancer cells

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Supplementary Table 1: Data for best constant combination achieved high % of cell death, and combination index (CI) for DOX, CQ and TIC.

IC_{50} ratio	**DOX: CQ (1:2)**	**DOX: TIC (1:6)**	**DOX: CQ: TIC (1:1:1)**
Death (%) (ED85)	94%	93%	95%
CI	0.256	0.54	0.53

Supplementary Table 2: Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities in the different mice groups.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	LDH (U/L)
Control				
Mean± SD	22.4±1.6	93.4±3	137.3±9	790.4±16.8
MCF-7				
Mean± SD	74 ±4.5	139.4±2.4	170.1±6	575±17.5
***p* value**	< 0.0001	< 0.0001	>0.05	< 0.0001
% Change	230.35	49.25	23.8	-27.25
DOX				
Mean± SD	86±1.5	149.2±3.6	180.2±7	1492±10.5
***p* value**	< 0.0001	< 0.0001	< 0.01	< 0.0001
***p** value**	>0.05	>0.05	>0.05	< 0.0001
% Change	283.9	59.7	31.24	88.76
CQ				
Mean± SD	23±0.6	62±3.6	136±5	848.1±17
***p* value**	>0.05	< 0.0001	>0.05	>0.05
***p** value**	< 0.0001	< 0.0001	< 0.01	< 0.0001
pvalue**	< 0.0001	< 0.0001	< 0.01	< 0.0001
% Change	2.67	-33.61	-0.946	7.287
TIC				
Mean± SD	21.7±1.2	95±4	135.9±4	841±14.1
***p* value**	>0.05	>0.05	>0.05	>0.05
***p** value**	< 0.0001	< 0.0001	< 0.05	< 0.0001
pvalue**	< 0.0001	< 0.0001	< 0.01	< 0.0001
% Change	-3.12	1.71	-1.019	6.401
DOX&CQ				
Mean± SD	35±6.2	134±5.2	149±6	872±14
***p* value**	>0.05	< 0.0001	>0.05	< 0.01
***p** value**	< 0.0001	>0.05	>0.05	< 0.0001
pvalue**	< 0.0001	>0.05	>0.05	< 0.0001
% Change	56.25	43.46	8.521	10.32
DOX&TIC				
Mean± SD	32.8±4.8	111.8±4.7	145±12	862±16
***p* value**	>0.05	>0.05	>0.05	< 0.05
***p** value**	< 0.0001	<0.01	>0.05	< 0.0001
pvalue**	< 0.0001	< 0.0001	< 0.05	< 0.0001
% Change	46.42	19.70	5.608	9.05
TIC&DOX&CQ				
Mean± SD	29±2.7	109.2±7.6	144.5±8	855±15
***p* value**	>0.05	>0.05	>0.05	>0.05
***p** value**	< 0.0001	< 0.0001	>0.05	< 0.0001
pvalue**	< 0.0001	< 0.0001	< 0.05	< 0.0001
% Change	29.46	16.91	5.243	8.173

Values are expressed as mean ± SE, n = 8 for each group.
Means are significantly different as following; *p* value: versus normal control group *p** value: versus MCF-7 control group *p*** value: versus Doxorubicin group.

Supplementary Table 3: Serum total protein, albumin, urea, creatinine, and total lipid concentrations in the different mice groups.

Groups	Total protein (g/dl)	Albumin (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Total lipid (mg/dl)
Control					
Mean± SD	6.4±0.10	3.8±0.01	25.1±1.5	0.52±0.01	25.3±1.4
MCF-7					
Mean± SD	3.1±0.32	2.1±0.051	44.7±2.5	0.78±0.05	44±1.19
***p* value**	< 0.0001	< 0.01	< 0.0001	< 0.0001	< 0.0001
% Change	-51.56	-44.73	78.08	50	73.91
DOX					
Mean± SD	3.8±0.51	1.94±0.015	65.5±2.2	0.93±0.04	67±1.5
***p* value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
***p** value**	>0.05	>0.05	< 0.0001	< 0.01	< 0.0001
% Change	-40.62	-48.94	160.95	78.84	164.82
CQ					
Mean± SD	4.9±0.49	3.3±0.04	33.42±1.1	0.615±0.01	37±3.2
***p* value**	>0.05	>0.05	>0.05	>0.05	< 0.01
***p** value**	< 0.05	>0.05	< 0.01	< 0.0001	>0.05
p**value	>0.05	< 0.05	< 0.0001	< 0.0001	< 0.0001
% Change	-23.43	-13.15	33.14	18.26	46.24
TIC					
Mean± SD	4.5±0.3	3.6±0.025	31.5±1.3	0.64±0.02	35.3±2.2
***p* value**	< 0.05	>0.05	>0.05	< 0.05	< 0.05
***p** value**	>0.05	< 0.01	< 0.0001	< 0.01	< 0.05
p**value	>0.05	< 0.01	< 0.0001	< 0.0001	< 0.0001
% Change	-29.68	-5.26	25.49	23.07	39.5
DOX&CQ					
Mean± SD	5.7±0.53	3.42±0.012	32.15±1.3	0.603±0.019	38.3±2.18
***p* value**	>0.05	>0.05	>0.05	>0.05	< 0.0001
***p** value**	< 0.0001	< 0.05	< 0.01	< 0.0001	>0.05
p**value	< 0.05	< 0.05	< 0.0001	< 0.0001	< 0.0001
% Change	-10.93	-10	28.08	15.96	51.38
DOX&TIC					
Mean± SD	5.4±0.12	3.61±0.012	31.34±3.3	0.607±0.018	36.7±1.4
***p* value**	>0.05	>0.05	>0.05	>0.05	< 0.01
***p** value**	< 0.0001	< 0.01	< 0.0001	< 0.0001	>0.05
p**value	< 0.05	< 0.01	< 0.0001	< 0.0001	< 0.0001
% Change	-12.5	-5	24.86	16.73	45.05
TIC&DOX&CQ					
Mean± SD	5.9±0.29	3.68±0.032	30.41±2.3	0.564±0.014	33.4±1.6
***p* value**	>0.05	>0.05	>0.05	>0.05	>0.05
***p** value**	< 0.0001	< 0.01	< 0.0001	< 0.0001	< 0.01
p**value	< 0.01	< 0.01	< 0.0001	< 0.0001	< 0.0001
% Change	-7.81	-3.15	21.15	8.46	32.01

Values are expressed as mean ± SE, n = 8 for each group.
Means are significantly different as following; *p* value: versus normal control group *p** value: versus MCF-7 control group *p*** value: versus Doxorubicin group.

Supplementary Table 4: Hepatic total thiol concentrations, GPx, GST, and catalase activities and total antioxidant capacity in in the different mice groups (Antioxidant defense system markers).

Groups	Total Thiol (µmol/g tissue)	GPx (µmol/min/ mg protein)	GST (nmol/min/ mg protein)	Catalase (µmol/min/mg protein)	Total antioxidant capacity (µmol/ tissue)
Control					
Mean± SD	60±5	0.547±0.01	11.45±0.3	142±5	22.7±1.5
MCF-7					
Mean± SD	42.31±5.6	0.185±0.01	6.6±0.5	100.8±5.6	11.3±1.77
***p* value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
% Change	-29.48%	-66.17%	-42.3%	-28.5%	-50.22%
DOX					
Mean± SD	34±9	0.16±0.02	4.75±0.4	21.25±9	4.91±2.84
***p* value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
***p** value**	N.S	N.S	N.S	< 0.0001	< 0.0001
% Change	-43.3%	-69.1%	-58.5%	-84.9%	-78.37%
CQ					
Mean± SD	57.69±6	0.58±0.01	9.96±0.63	139±6	25.6±1.89
***p* value**	N.S	< 0.001	N.S	N.S	< 0.05
***p** value**	< 0.0001	< 0.0001	< 0.01	< 0.0001	< 0.0001
p value**	< 0.0001	<0.0001	< 0.0001	< 0.0001	< 0.0001
% Change	-3.85%	7.67	-13.01%	-1.41%	12.77%
TIC					
Mean± SD	57.9±5.3	0.56±0.01	10.2±0.47	140.25±5.3	33.2±1.67
***p* value**	N.S	N.S	N.S	N.S	< 0.0001
***p** value**	< 0.0001	< 0.0001	< 0.001	< 0.0001	< 0.0001
p value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
% Change	-3.5%	2.92%	-10.91%	-0.53%	46.2%
DOX&CQ					
Mean± SD	54.5±6.6	0.54±0.02	9.04±0.82	126.12±6.6	18.8±2.08
***p* value**	N.S	N.S	< 0.05	< 0.0001	< 0.001
***p** value**	< 0.001	< 0.0001	< 0.05	< 0.0001	< 0.0001
p value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
% Change	-9.16%	-1.27%	-21.04%	-10.54%	-17.18%
DOX&TIC					
Mean± SD	58.3±5.2	0.52±0.01	8.42±0.37	126±5.2	22.28±1.644
***p* value**	N.S	N.S	N.S	< 0.0001	N.S
***p** value**	< 0.0001	< 0.0001	< 0.05	< 0.0001	< 0.0001
p value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
% Change	-2.8%	-4.5%	-26.4%	-10.63%	-1.85%

TIC&DOX&CQ					
Mean± SD	55.69±6	0.66±0.01	10.58±0.6	138.8±6	29.8±1.89
***p* value**	N.S	< 0.0001	N.S	N.S	< 0.0001
***p** value**	< 0.001	<0.0001	< 0.0001	< 0.001	< 0.0001
***p**value**	< 0.0001	< 0.0001	< 0.0001	< 0.001	< 0.0001
% Change	-7.1%	22.12%	-7.59%	-1.56%	31.27%

Values are expressed as mean ± SE, n = 8 for each group.
Means are significantly different as following; *p* value: versus normal control group *p** value: versus MCF-7 control group *p*** value: versus Doxorubicin group.

Supplementary Table 5: Hepatic thiobarbituric acid reactive substance (TBARS), conjugated dienes, lipid hydroperoxide, total antioxidant capacity levels and Thioredoxin reductase activity in the different mice groups (Oxidative stress markers).

Groups	(TBARS) (nmol/g tissue)	Conjugated dienes (µmol/ g tissue)	Lipid hydroperoxide (µmol/ g tissue)	Thioredoxin reductase (µmol/min mg protein)	DNA Damage (%)
Control					
Mean± SD	55.85±7	1.57±0.1	5.32±1	1.48±0.05	9.25±0.47
MCF-7					
Mean± SD	68.7±5.6	1.84±0.14	7.8±1.4	0.91±0.056	15.85±0.56
***p* value**	< 0.01	< 0.0001	< 0.0001	< 0.0001	< 0.0001
% Change	23.0%	17.1%	46.6%	-38.70%	71.35%
DOX					
Mean± SD	98.03±8	2±0.13	8.16±1.3	0.86±0.09	8.4±0.411
***p* value**	< 0.0001	< 0.0001	< 0.0001	< 0.001	< 0.0001
***p** value**	< 0.0001	< 0.05	N.S	N.S	< 0.0001
% Change	75.5%	27.3%	53.3%	-41.93%	-9.18%
CQ					
Mean± SD	48.52±8.5	1.3±0.09	3.08±0.9	1.58±0.06	7.02±0.126
***p* value**	N.S	< 0.0001	< 0.0001	< 0.01	< 0.001
***p** value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
***p**value**	< 0.0001-	< 0.0001	< 0.0001	< 0.0001	< 0.0001
% Change	13.12%	-17.19%	-42.10%	6.67%	-24.10%
TIC					
Mean± SD	39.8±7.3	1.4±0.05	3.39±0.5	1.60±0.053	7.05±0.120
***p* value**	< 0.001	< 0.01	< 0.001	< 0.001	< 0.0001
***p** value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
***p**value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001	<0.01
% Change	-28.73%	-10.82%	-36.16%	7.76%	-23.78%
DOX&CQ					
Mean± SD	34.06±8.6	1.5±0.02	4.6±0.2	1.28±0.07	6.17±0.284
***p* value**	< 0.0001	N.S	N.S	< 0.0001	< 0.0001
***p** value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

pvalue**	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
% Change	-39.01%	-4.45%	-13.53%	-13.92%	-33.29%
DOX&TIC					
Mean± SD	31.29±5.2	1.4±0.08	4.52±0.8	1.401±0.052	5.65±0.094
***p* value**	< 0.0001	< 0.01	N.S	N.S	< 0.0001
***p** value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
pvalue**	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
% Change	-43.9%	-10.82%	-15.03%	-5.77%	-38.91%
TIC&DOX&CQ					
Mean± SD	34.66±7	1.4±0.12	3.86±0.12	1.57±0.06	5.22±0.053
***p* value**	< 0.0001	< 0.01	< 0.05	< 0.05	< 0.0001
***p** value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
pvalue**	< 0.0001	< 0.0001	< 0.0001	< 0.0001	<0.0001
% Change	-37.94%	-10.82%	-27.44%	5.86%	-43.56%

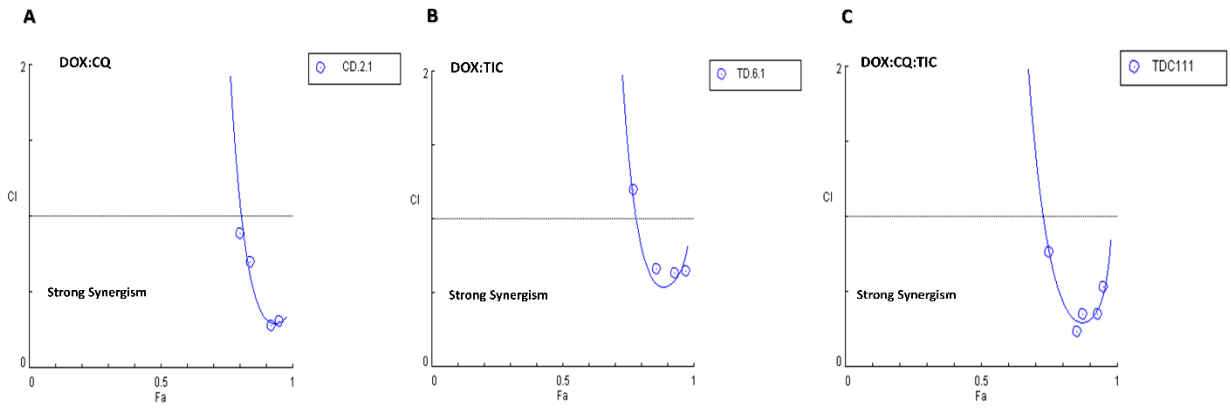
Values are expressed as mean ± SE, n = 8 for each group.
Means are significantly different as following; *p* value: versus normal control group *p** value: versus MCF-7 control group *p*** value: versus Doxorubicin group.

Supplementary Table 6: Hepatic Arylsulphatase A, Arylsulphatase B concentrations, Arylestrase and paraoxnase activities in in the different mice groups (Hepatic toxicity parameters)

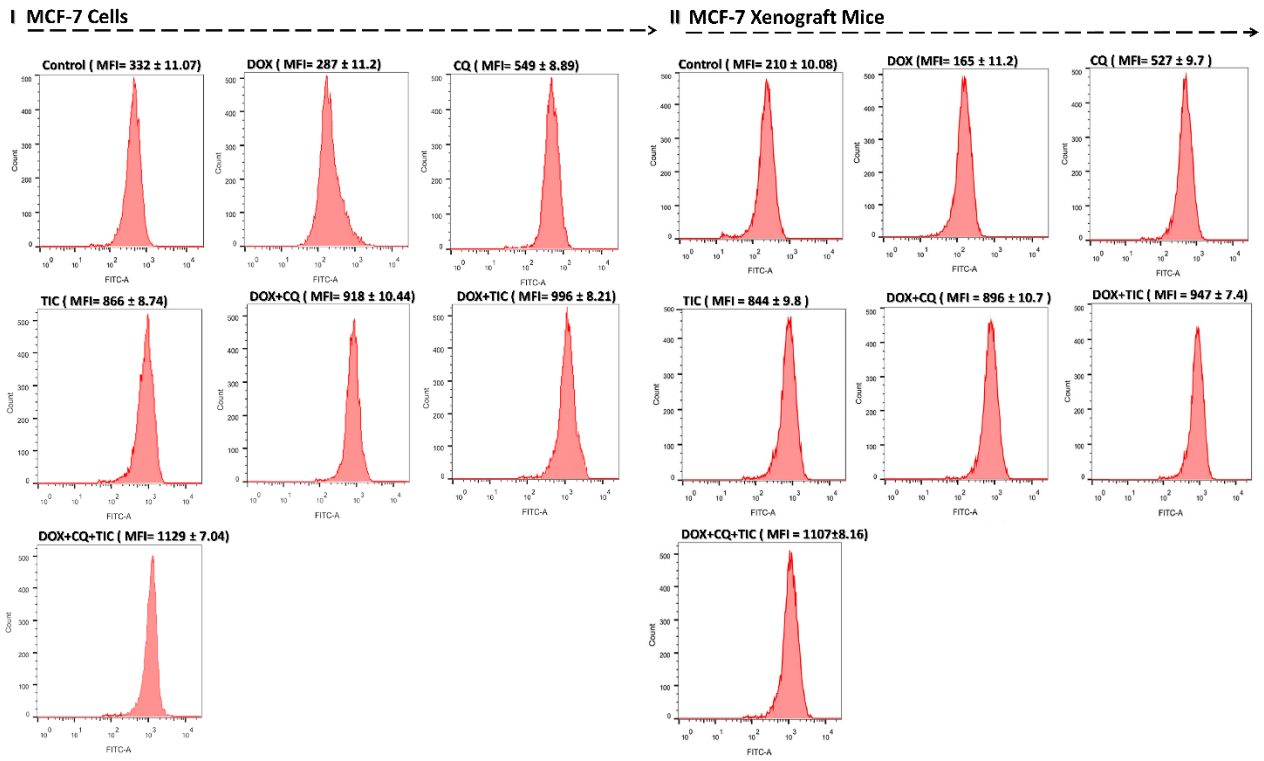
Groups	**ASA (µmol/h/mg protein)**	**ASB (µmol/h/mg protein)**	**Arylestrase (nmol/min/ml)**	**Paraoxnase (nmol/min/ml)**
Control				
Mean± SD	26.6±1	74.5±5	75.28±8	268.23±11
MCF-7				
Mean± SD	30±1.6	85.8±5.6	223.3±5.4	372.47±15.4
***p* value**	< 0.001	< 0.01	< 0.0001	< 0.0001
% Change	12.78%	15.16%	197.020%	38.8%
DOX				
Mean± SD	34.7±1.3	90.5±9	242.105±8	382.35±13
***p* value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001
***p** value**	< 0.0001	N.S	< 0.0001	N.S
% Change	30.45%	21.47%	222.03%	42.5%
CQ				
Mean± SD	20.8±2	67.43±6	77.81±8	182.82±9
***p* value**	< 0.0001	N.S	N.S	< 0.0001
***p** value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001
pvalue**	< 0.0001	< 0.0001	< 0.0001	< 0.0001
% Change	-21.8%	-9.48%	3.49	-31.84%
TIC				
Mean± SD	23.5±1.5	62.2±5.3	75.93±7.3	192.47±7.5
***p* value**	< 0.01	< 0.001	N.S	< 0.0001
***p** value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001
pvalue**	< 0.0001	< 0.0001	< 0.0001	< 0.0001

% Change	-11.6%	-16.5%	0.99%	-28.24%
DOX&CQ				
Mean± SD	19.4±2.6	69.14±6.6	76.31±6.6	172.23±11
***p* value**	< 0.0001	N.S	N.S	< 0.0001
***p** value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001
pvalue**	< 0.0001	< 0.0001	< 0.0001	< 0.0001
% Change	-27.06%	-7.19%	1.5%	-35.7%
DOX&TIC				
Mean± SD	18.5±1.2	64.26±5.2	76±4.2	164±8
***p* value**	< 0.0001	< 0.01	N.S	< 0.0001
***p** value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001
pvalue**	< 0.0001	< 0.0001	< 0.0001	< 0.0001
% Change	-30.45%	-13.74%	1.09%	-38.85%
TIC&DOX&CQ				
Mean± SD	16.53±2	58.33±6	78±8	240.94±12
***p* value**	< 0.0001	< 0.0001	N.S	< 0.0001
***p** value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001
pvalue**	< 0.0001	< 0.0001	< 0.0001	< 0.0001
% Change	-37.8%	-21.70%	3.75%	-10.17%

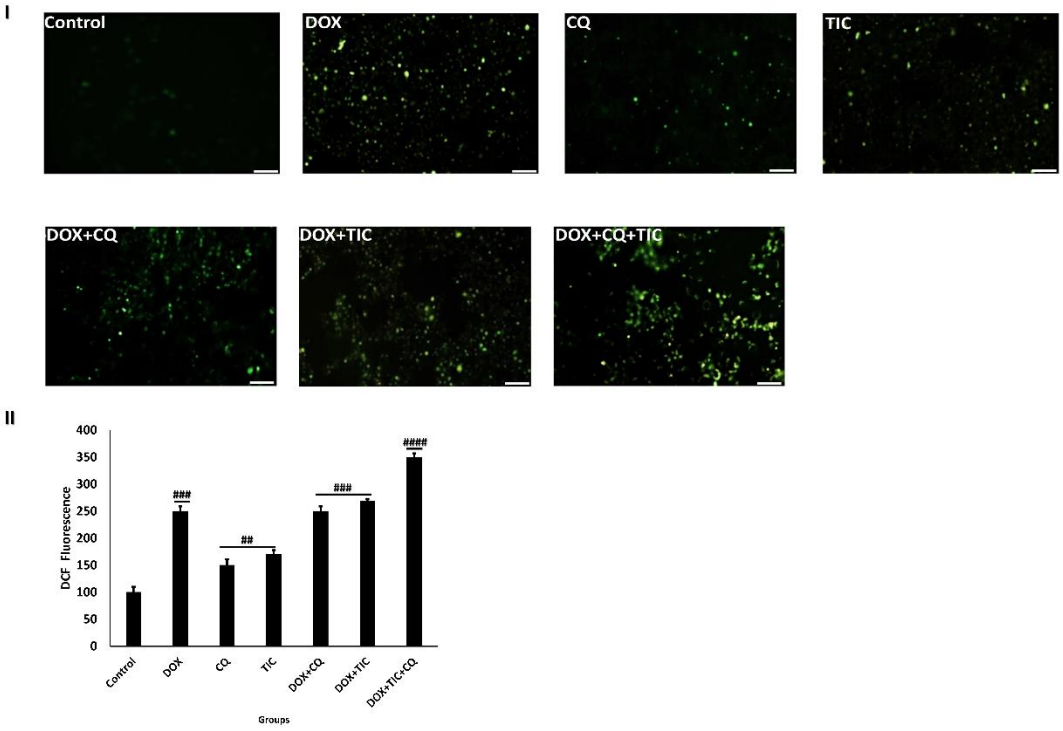
Values are expressed as mean ± SE, n = 8 for each group.
Means are significantly different as following; *p* value: versus normal control group *p** value: versus MCF-7 control group *p*** value: versus Doxorubicin group.



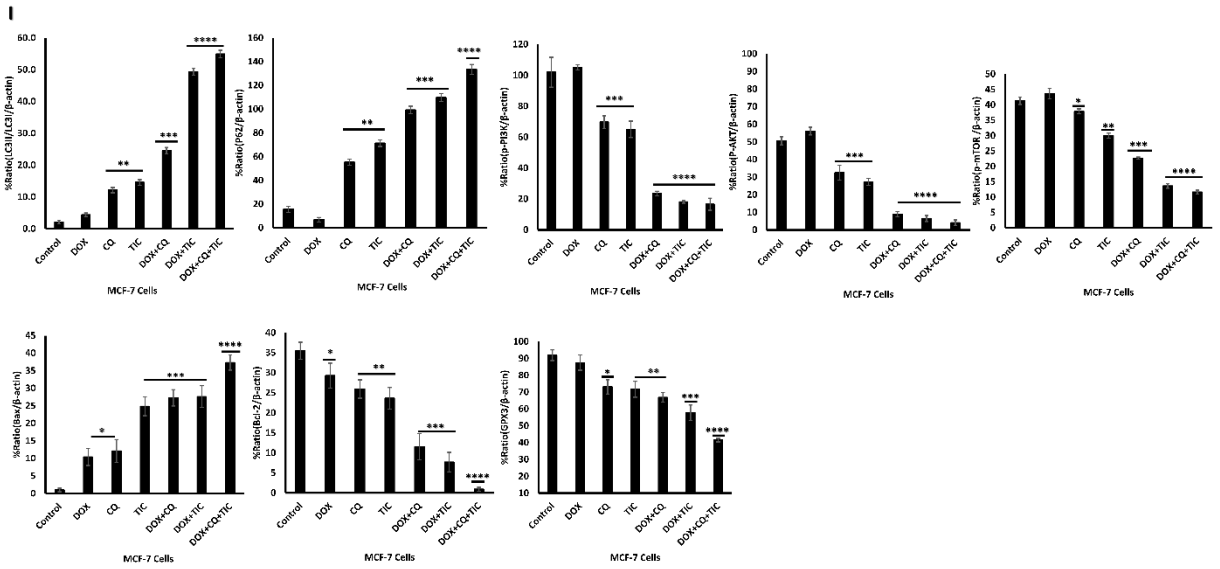
Supplementary Figure 1: Combination index plot of each selected combinatorial treatment ratio DOX+CQ (1:2) (A), DOX+TIC (1:6) (B) and DOX+CQ+TIC (1:1:1) (C) of MCF-7 cells. Effect of each dose (comes from triplicate assays). CI: Combination Index; CI< 1: Synergism, CI> 1: Antagonism, CI=1: additive.



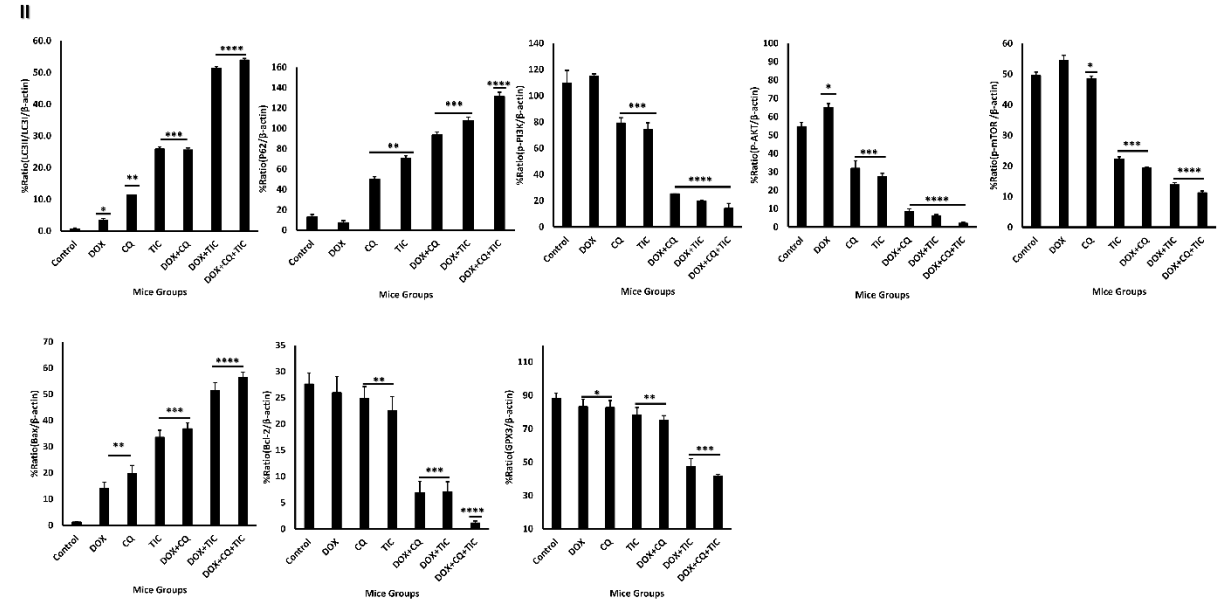
Supplementary Figure 2: Autophagy activity detection of MCF7- cells (I) and MCF-7 xenograft mice (II) treated with single or combinatorial treatments that induced autophagic cell death was investigated by flow cytometry analysis. Each value expressed as mean ± SE, (n=3).



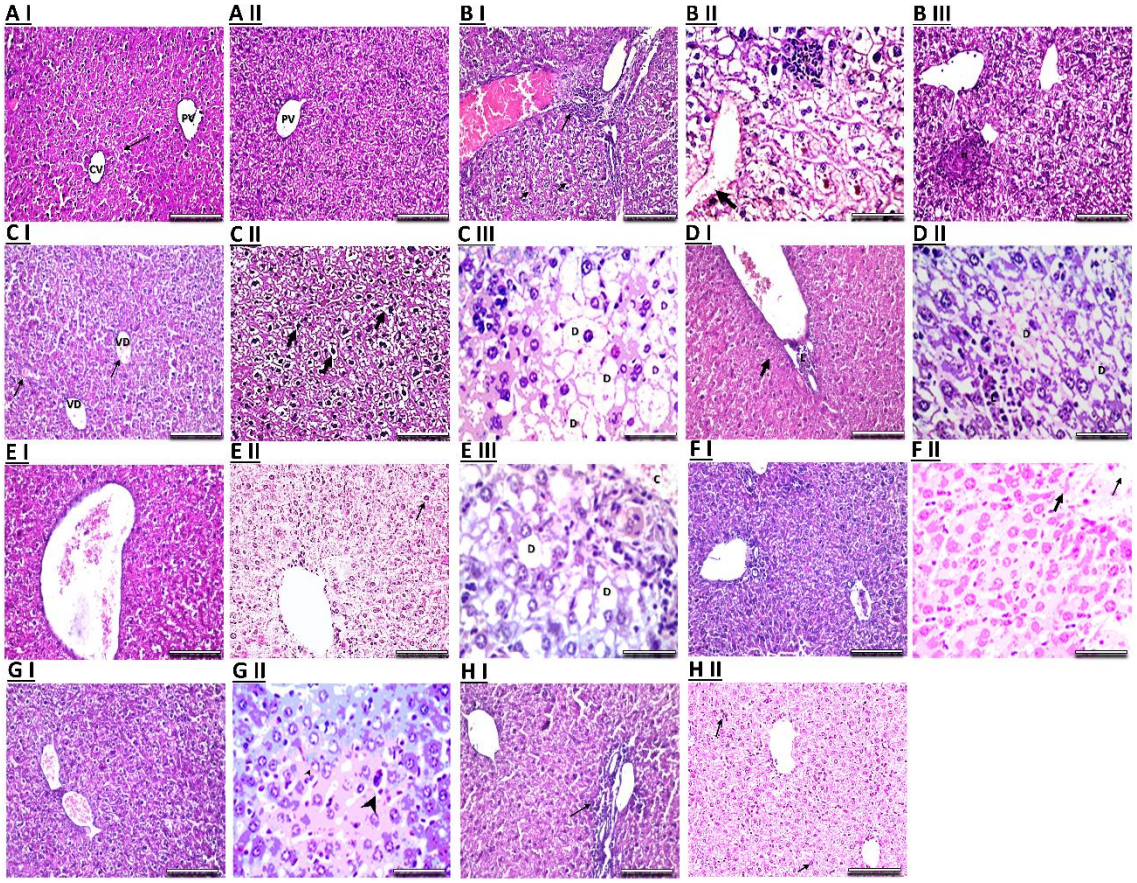
Supplementary Figure 3: Intracellular ROS generation accumulation visualized by fluorescence microscope by in MCF-7 cancer cells. MCF-7 cells were incubated with single and combinatorial treatments for 48hrs. The intracellular ROS generation in MCF-7 cancer cells was detected by fluorescence microscopy (I). Bar diagram showing DFC fluorescence level (II) of each cell line. Each value expressed as mean ± SE, (n=3). #$P < 0.05$, ##P <0 .01, ###P <0 .001, ####$P < 0.0001$ compared with control (untreated cells)(scale bar = 100 μm).



(Cont.)

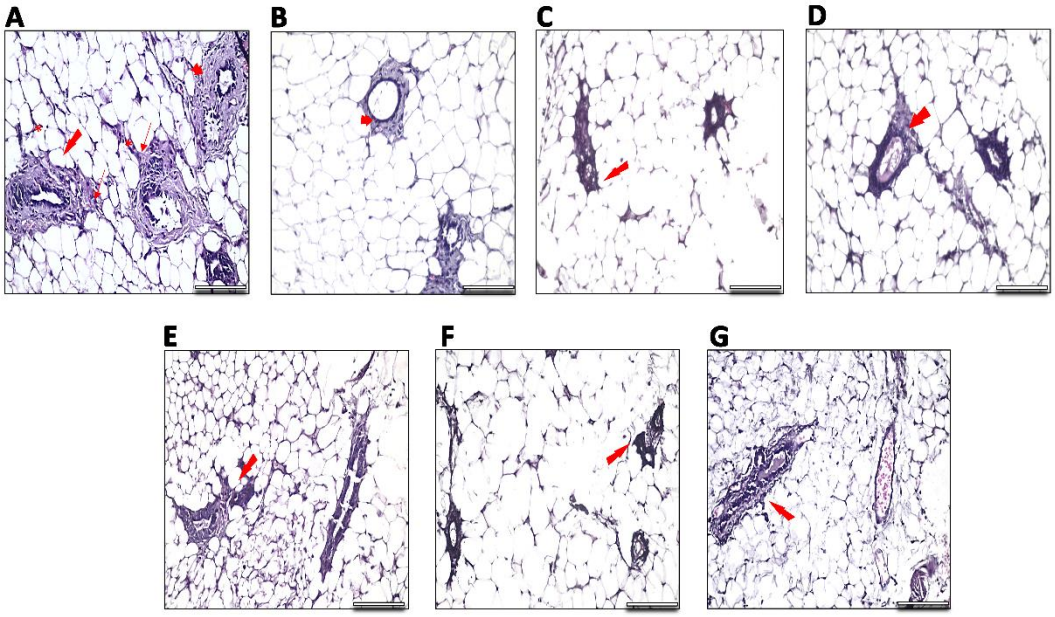


Supplementary Figure 4: Autophagy inhibition induces cell death dependent apoptosis. Quantification of western blotting changing for each protein levels using Image J software of autophagic markers (LC3I/II) and P62 , PI3K/Akt/mTOR pathway markers, apoptotic and anti- apoptotic markers (Bax, Bcl-2) , GPX3 (anti-oxidant marker) proteins expression levels of MCF7- cells **(I)** and MCF-7 xenograft mice **(II)** treated with single or combinatorial treatments. Date are presented as mean ± SE (error bar) of at least three independent experiments. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ and **** $P<0.0001$ compared with control (untreated groups).

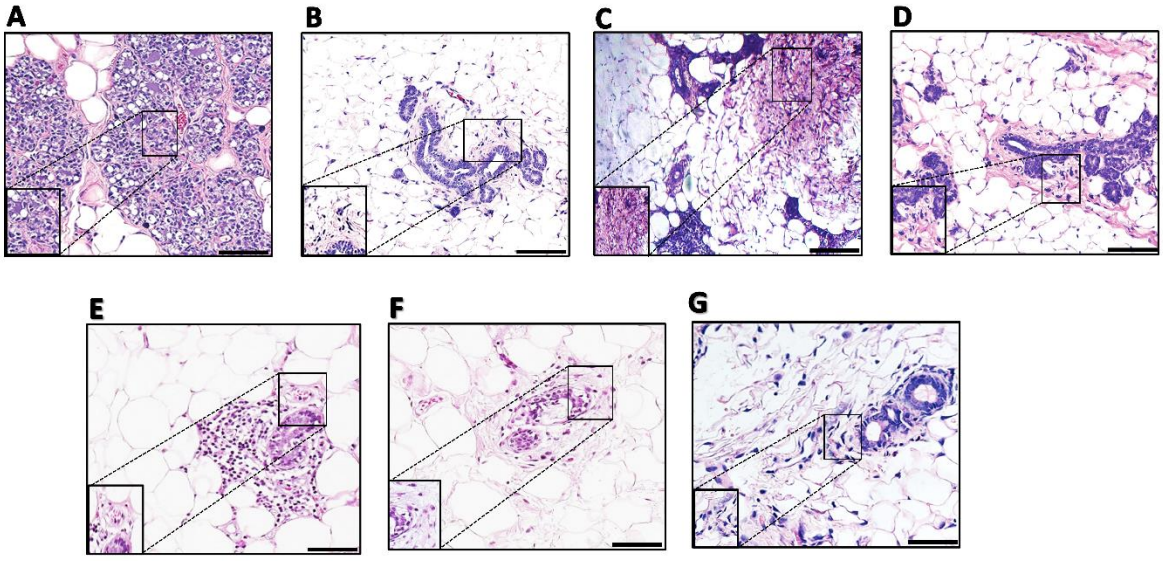


Supplementary Figure 5: Histopathological investigation for hepatic tissues of mice in each group. (AI, II); Liver of normal mice showing normal histological structure of central vein (CV) and surrounding hepatocytes (h) in the parenchyma. (B I) Liver of MCF-7 xenograft mice showing congestion in the portal vein with inflammatory cells infiltration associated with vacuolar degeneration in the hepatocytes. (B II) showing congestion with degeneration, necrosis, inflammatory cell infiltration and depletion of glycoprotein granules (thick arrow). (B III) Showing focal necrosis with inflammatory cells infiltration in hepatic parenchyma. (C I); Liver of tumor bearing mice treated with doxorubicin showing vacuolar degeneration (VD) in the hepatocytes all over the parenchyma. (C II); showing significant damaged cells in the liver (thick arrows). (C III); showing necrosis and hydropic degeneration (D) accompanied with inflammatory infiltrate. (D I); Liver of tumor bearing mice treated with Chloroquine showing a few inflammatory cells infiltration (lymphocytic infiltration (L)) in the portal area associated with dilatation in the portal vein. (D II); showing lymphocytic cells infiltration (L) and a few hydropic degeneration (D). (E I); Liver of tumor bearing mice treated with Tioconazole showing dilatation in the central vein. (EII); showing focal areas of vacuolar degeneration of hepatic cells (Thin arrow). (E III); showing moderate congestion (C) with mild hydropic degeneration (D). (F I); Liver of tumor bearing mice treated with doxorubicin with chloroquine showing dilatation in the central vein. (F II); showing liver with a few hydropic degeneration (Thin arrow), mild hyperemia in the sinusoids (Thick arrow). (G I); Liver of tumor bearing animals treated with doxorubicin with Tioconazole showing dilatation in the central vein. (G II); showing normal hepatocytes with moderate vacuolar degeneration (arrowhead). (H I); Liver of tumor bearing animals treated with doxorubicin, Tioconazole and Chloroquine showing a few Inflammatory cells infiltration (as good response) was observed in the portal area (Thin arrow). (H II); showing fairly normal tissue architecture and cellular details with mild vacuolar degeneration (black arrow). (H I); Liver of tumor bearing animals treated with doxorubicin, Tioconazole and Chloroquine showing a few Inflammatory cells infiltration (as good

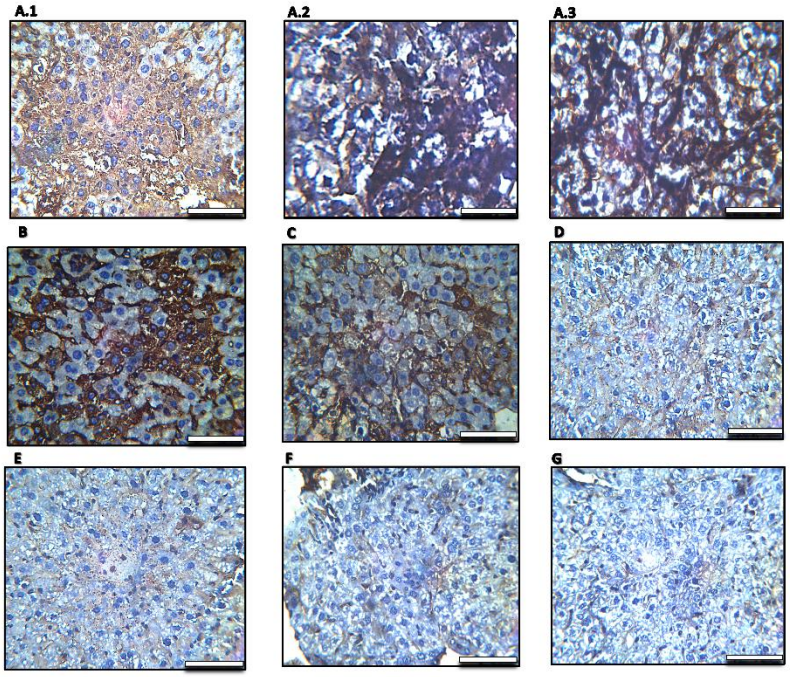
response) was observed in the portal area (Thin arrow). (H II); showing fairly normal tissue architecture and cellular details with mild vacuolar degeneration (black arrow) (H&E stain _scale bar = 50 μm).



Supplementary Figure 6 I: Histological structure of the mammary gland embedded in adipose tissue of mice groups (H&E stain, X 40). **A -MCF-7 xenograft:** showing the mammary ducts with a massive increase in the number of lining epithelial cells (thin arrow) , showing Periductal and peri acinar fibrosis was detected in most of the ducts and acini (thick arrow), also showing individual tumor cells that have invaded the stroma with variable-shaped dark- stained nuclei, asterisks represent the adipose connective with the massive fat cells tissues. **B- MCF-7 xenograft /DOX:** showing mammary acini and duct system embedded in adipose tissue, showing few scattered small- and medium-sized mammary ducts (thick arrow) **C- MCF-7 xenograft /CQ:** showing the normal histopathological structure, showing mild inflammatory cell with normal looking adipose tissue. **D- MCF-7 xenograft /TIC:** showing the normal histopathological structure with peritoneum tissue were orderly arranged and normal breast acini were found (thick arrows). **E- MCF-7 xenograft /DOX+CQ:** showing normal histopathological structure, showing tumor apoptosis with moderate peritumoral inflammatory response. **F- MCF-7 xenograft /DOX+TIC:** showing normal histopathological structure like a normal appearance without structural alternation **G- MCF-7 xenograft /DOX+CQ+TIC:** showing congestion in the blood vessels, showing apoptotic cells and dense peritumor inflammation as local immune response , scale bar = 30 μm.



Supplementary Figure 6II: Histological structure of the paraffin consecutive sections of xenograft MCF-7 tumors; **A -MCF-7 xenograft**: showing an increase in clusters of ER*α* positive cells was detected in ductal hyperplasia, proliferation of atypical ducts, associated with peritumoral inflammatory response. **B- MCF-7 xenograft /DOX**: showing apoptotic cells and dense peritumor inflammation. **C- MCF-7 xenograft /CQ**: showing tumor apoptosis with moderate peritumoral inflammatory response. **D- MCF-7 xenograft /TIC**: showing presence of apoptosis, with mild inflammatory response. **E- MCF-7 xenograft /DOX+CQ**: showing apoptotic individual cells have a moderate amount of slightly basophilic cytoplasm within abundant content of adipose connective tissues. **F- MCF-7 xenograft /DOX+TIC**: showing mononuclear cellular infiltration apoptotic cells of within the dense cellular adipose connective tissues. **G- MCF-7 xenograft /DOX+CQ+TIC**: showing cellular infiltration epithelial cells with and apoptotic cells with moderate basophilic scant cytoplasm, low-grade tubular tumors, scale bar = 50 μm.



Supplementary figure 7: Immunohistochemical expression of ER-α in Paraffin sections of mammary gland of xenograft mice experimental groups. A1-3- Mammary gland of MCF-7 xenograft: showing Invasive ductal mammary neoplasm within epithelial hyperplasia of this lineage (High-grade), showing intensive strong intense of cytoplasmic ERα immunoreaction in the form of a brown color in the cells. **B- Mammary gland of MCF-7 xenograft /DOX**: showing ductal mammary neoplasm (Moderate-grade) with a few intense of cytoplasmic ERα immunoreaction in the form of a brown color in the cells. **C- Mammary gland of MCF-7 xenograft /CQ**: showing ductal mammary neoplasm (slight moderate intense-grade) from mice in CQ treated group with a little bit high intense of cytoplasmic ERα immunoreaction in the form of a faint brown color in the cells. **D- Mammary gland of MCF-7 xenograft /TIC**: showing ductal mammary neoplasm low intense grade of cytoplasmic ERα immunoreaction in the form of a brown color in the cells. **E- Mammary gland of MCF-7 xenograft /DOX+CQ**: showing ductal mammary low- intense grade of cytoplasmic ERα immunoreaction in the form of slight faint brown color in the cells. **F- Mammary gland of MCF-7 xenograft /DOX+TIC**: showing ductal mammary neoplasm very low intense grade from of cytoplasmic ERα immunoreaction showing very low numbers of ER-α immunolabeled cells. **G- Mammary gland of MCF-7 xenograft /DOX+CQ+TIC**: showing ductal mammary neoplasm with almost loss of cytoplasmic ER-α immunolabeled cells, scale bar = 30 μm.