OMTN, Volume *9*

Supplemental Information

miR-3174 Contributes to Apoptosis and Autophagic Cell Death Defects in Gastric Cancer Cells by Targeting ARHGAP10

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Supplemental Figures

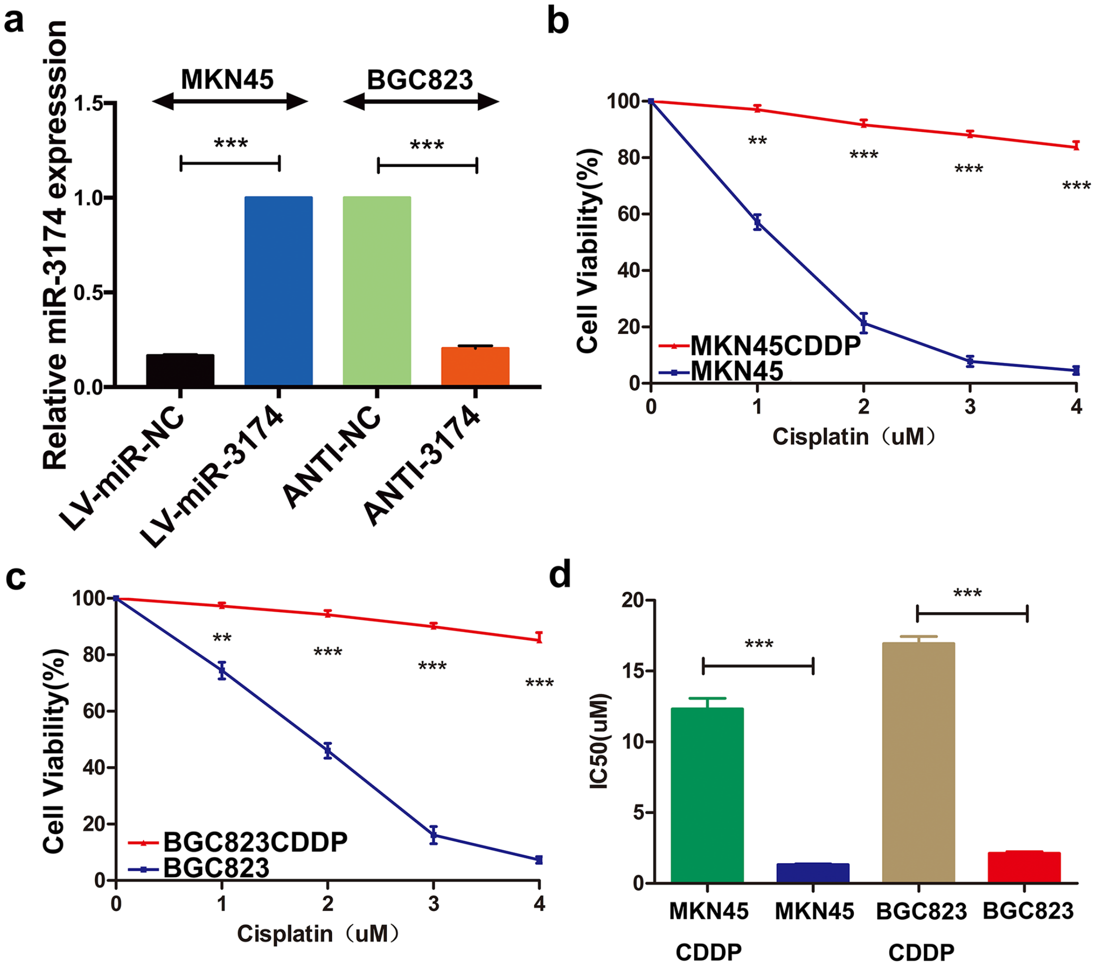

Figure S1. Lentivirus effectiveness detection and CDDP resistant characteristics of cell lines validation. (**a**) Expression of miR-3174 was validated after infecting MKN45 cells with lentivirus carrying LV-miR-3174 or LV-miR-NC and BGC823 cells with ANTI-3174 or ANTI-NC. (**b,c**) CDDP resistance features of MKN45CDDP and BGC823CDDP cells were verified by CCK-8 cell viability assays, and IC50 of each cell line was showed in (**d**). Graph represents mean ± SEM; *=P<0.05, **=P<0.01, and ***=P<0.001.

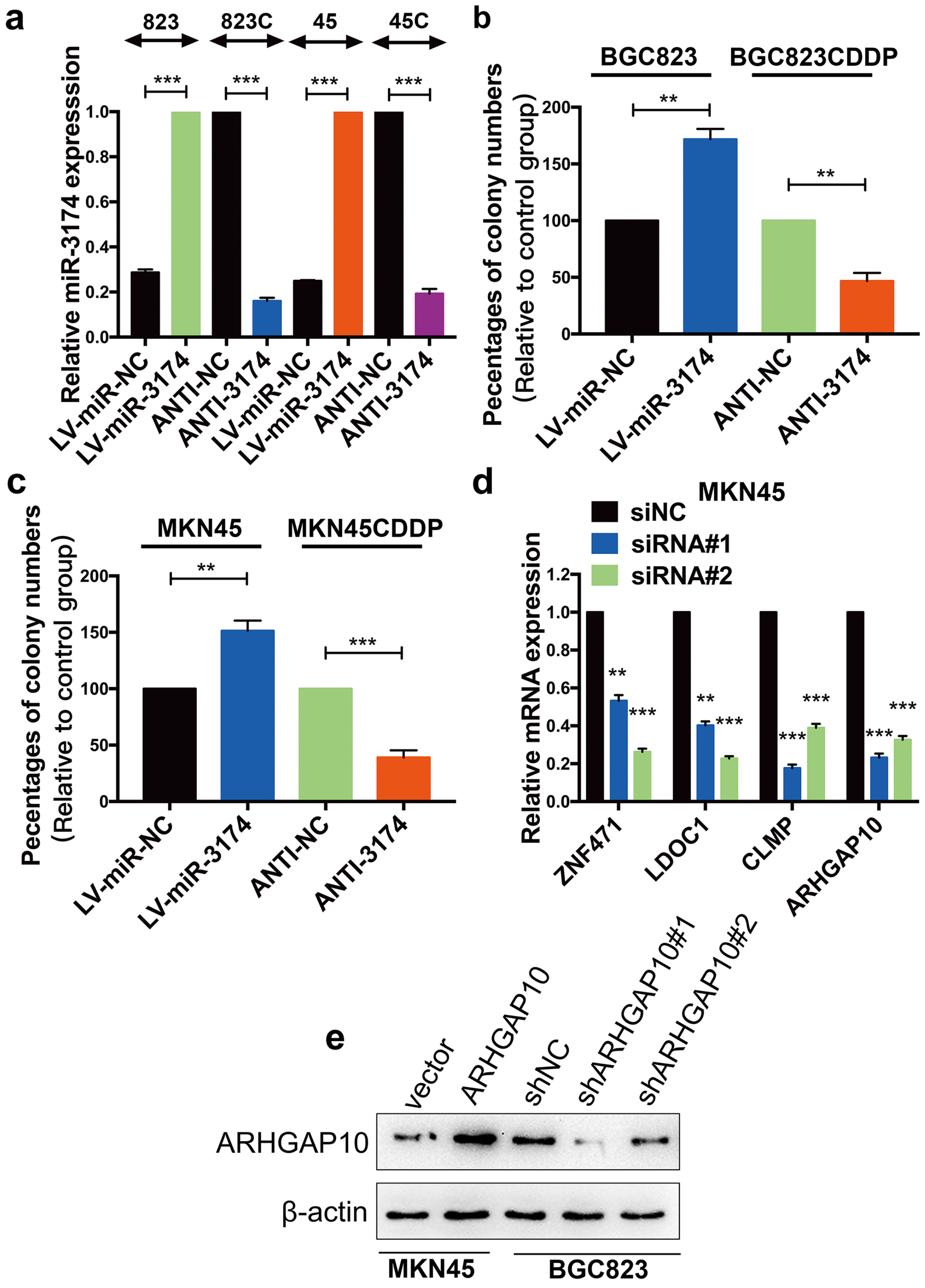

Figure S2. Colony numbers quantitation and target genes' siRNA effectiveness verification. (**a**) miR-3174 expression was detected after BGC823 or MKN45 cells was infected with lentivirus carrying LV-miR-3174 or LV-miR-NC and BGC823CDDP or MKN45CDDP cells was infected with lentivirus carrying ANTI-3174 or ANTI-NC. (**b,c**) The colony units in Figure 5j,k were counted, and the data were converted to percentages and compared with data for the control groups. (**d**) mRNA expression of ZNF471, LDOC1, CLMP and ARHGAP10 was determined respectively by RT-PCR after MKN45 cells were treated with their specific RNA interference sequences. (**e**) Protein levels of ARHGAP10 were detected by Western blot after transfecting MKN45 cells with plasmids overexpressed ARHGAP10 or empty vector plasmids, and BGC823 cells with ARHGAP10 inhibited or control plasmids. Graph represents mean ± SEM; $*=P<0.05$, $**=P<0.01$, and $***=P<0.001$.

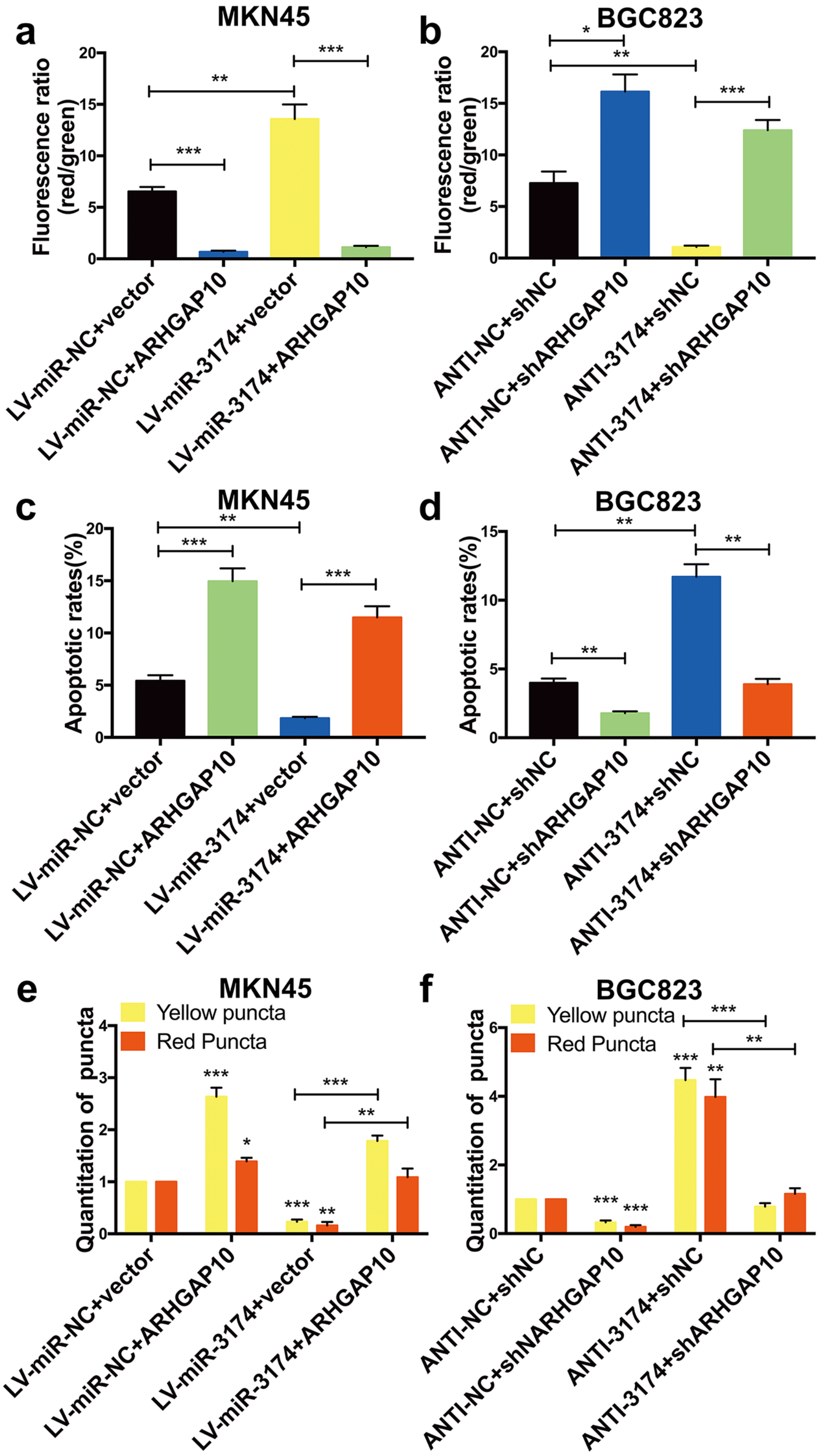

Figure S3. Mitochondria membrane potential level, apoptosis and autophagosome quantitation. (**a**,**b**) Fluorescence intensity ratios of red vs green in Figure 8a were calculated in MKN45 and BGC823 cells. (**c**,**d**) Apoptotic rates in cells as mentioned in Figure 8b,c were quantitated and transformed to histograms. (**e**,**f**) Red and yellow puncta after GFP-mRFP-LC3 transfection and detection as shown in Figure 8d,e were also quantitated with the same method in Figure 4b. Graph represents mean ± SEM; *=P<0.05, **=P<0.01, and ***=P<0.001.

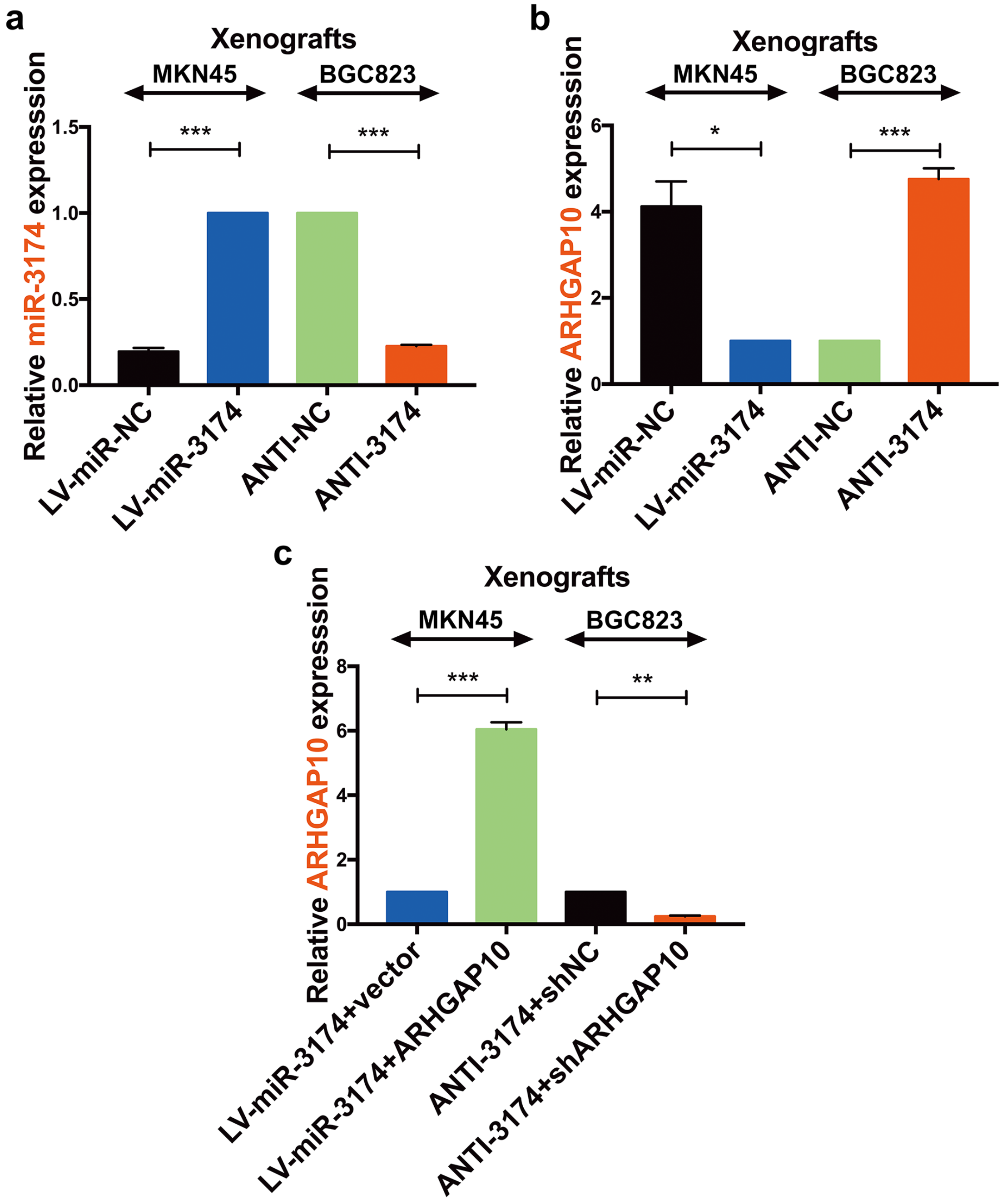

Figure S4. miR-3174 and ARHGAP10 expression in xenograft tumors. (**a**) miR-3174 and (**b**) ARHGAP10 expression in xenografts as displayed in Figure 9a was determined by RT-PCR. (**c**) Expression of ARHGAP10 was also measured in xenografts as showed in Figure 9d. Graph represents mean ± SEM; *=P<0.05, **=P<0.01, and ***=P<0.001.

Supplemental Tables

miRNA expression in 50 paired GC tissues				
	miR-17-5p		miR-19-3p	
	Normal	Tumor	Normal	Tumor
Mean±SD	3.00±1.79	4.02±2.26	0.53±0.46	0.73±0.57
	miR-20a-5p		miR-93-5p	
	Normal	Tumor	Normal	Tumor
Mean±SD	0.69±0.73	0.83±0.77	2.51±1.53	3.46±2.47
	miR-125b-1-3p		miR-148a-3p	
	Normal	Tumor	Normal	Tumor
Mean±SD	1.04±0.90	0.68±0.62	2.26±1.26	1.60±1.13
	miR-4732		miR-3174	
	Normal	Tumor	Normal	Tumor
Mean±SD	0.09±0.07	0.06±0.05	0.08±0.06	0.13±0.08

miRNA expression in 100 paired GC tissues		
	miR-3174	
	Normal	Tumor
Mean±SD	0.09±0.08	0.14±0.11
miR-3174 grouping		
	miR-3174（Median）	
	High	Low
Mean	0.23	0.07
Variance value	0.0059	0.0016

Table S1. Tendency of dispersion of each miRNA profile in GC, and means or variance values of two miR-3174 groups. The means and standard deviations of eight miRNAs' expression in 50-paired gastric cancer patients, and miR-3174's expression in 100-paired gastric cancer patients, and the means or variance values of miR-3174's expression in two groups of survival curve or clinicopathological features analysis cut-off by median expression. SD = standard deviation.

Clinicopathological features		**Number**	**No. of patients**		**Pvalue**
			miR-3174^{high}	**miR-3174^{low}**	
Age(y)	≥ 60	84	47	37	0.084
	< 60	56	23	33	
Gender	Male	105	56	49	0.172
	Female	35	14	21	
Tumor size	≥ 3.0cm	84	51	33	0.002**
	< 3.0cm	56	19	37	
Lauren's classification	Intestinal-type	92	50	42	0.154
	Diffuse-type	48	20	28	
Histological grade	Well	47	26	21	0.371
	Moderately and Poorly	93	44	49	
TNM stage	I	31	10	21	0.005**
	II	38	15	23	
	III	71	45	26	
T stage	T1-T2	48	18	30	0.033
	T3-T4	92	52	40	
N stage	N0	55	29	26	0.604
	N1-N3	85	41	44	

Table S2. Correlation of relative miR-3174 expression with the clinicopathological characteristics of 140 patients with gastric cancer. *=P<0.05, **=P<0.01, and ***=P<0.001.

Table S3. Potential genes associated with GC cells CDDP sensitivity or resistance on GDSC. The correlations between gene expressions with IC50 values of CDDP in 28 kinds of GC cells based on GDSC database were measured using Pearson correlation coefficient analysis in R language library followed by Z-test to acquire P-value. Genes meeting the following requirements were showed: P-value < 0.05. “1005” represents Cisplatin (CDDP) on GDSC. **(the table was supplied separately as an Excel file: Supplemental Table S3)**

Table S4. Candidate drugs whose IC50 in GC cells were related to ARHGAP10 expression in GDSC. The correlations between IC50 values of all types of anti-cancer drugs with ARHGAP10 expressions in 28 kinds of GC cells based on GDSC database were calculated using Pearson correlation coefficient analysis in R language library followed by Z-test to acquire P-value. Genes meeting the following requirements were showed: P-value < 0.05. “1005” represents Cisplatin (CDDP) on GDSC. **(the table was supplied separately as an Excel file: Supplemental Table S4)**

Abbreviations

GC: Gastric cancer; miRNA: microRNA; mRNA: messenger RNA; CDDP: cis-diamminedichloroplatinum (Cisplatin); ARHGAP10: Rho GTPase activating protein 10; mTOC1: rapamycin complex 1; PCD: Programmed cell death; ACD: autophagic cell death; AP: autophagosome; AL: autolysosome; SH3 domain: SRC hoology 3 domain; PH domain: Pleckstrin homology domain; FBS: fetal bovine serum; BECN1: Beclin 1; SQSTM1: sequestosome 1; RT-PCR: Quantitative real-time PCR; TEM: Transmission electron microscopy; DMSO: dimethyl sulfoxide; PVDF: polyvinylidene difluoride; HRP: horseradish peroxidase; UTR: untranslated region; ZNF471: zine finger 471; LDOC1: leucine zipper down-regulated in cancer 1; CLMP: CXADR like membrane protein; IC50: half maximal inhibitory concentration; CQ: Chloroquine; 3-

MA: 3-methyladenine; WMT: Wortmannin; MMP: mitochondria membrane potential