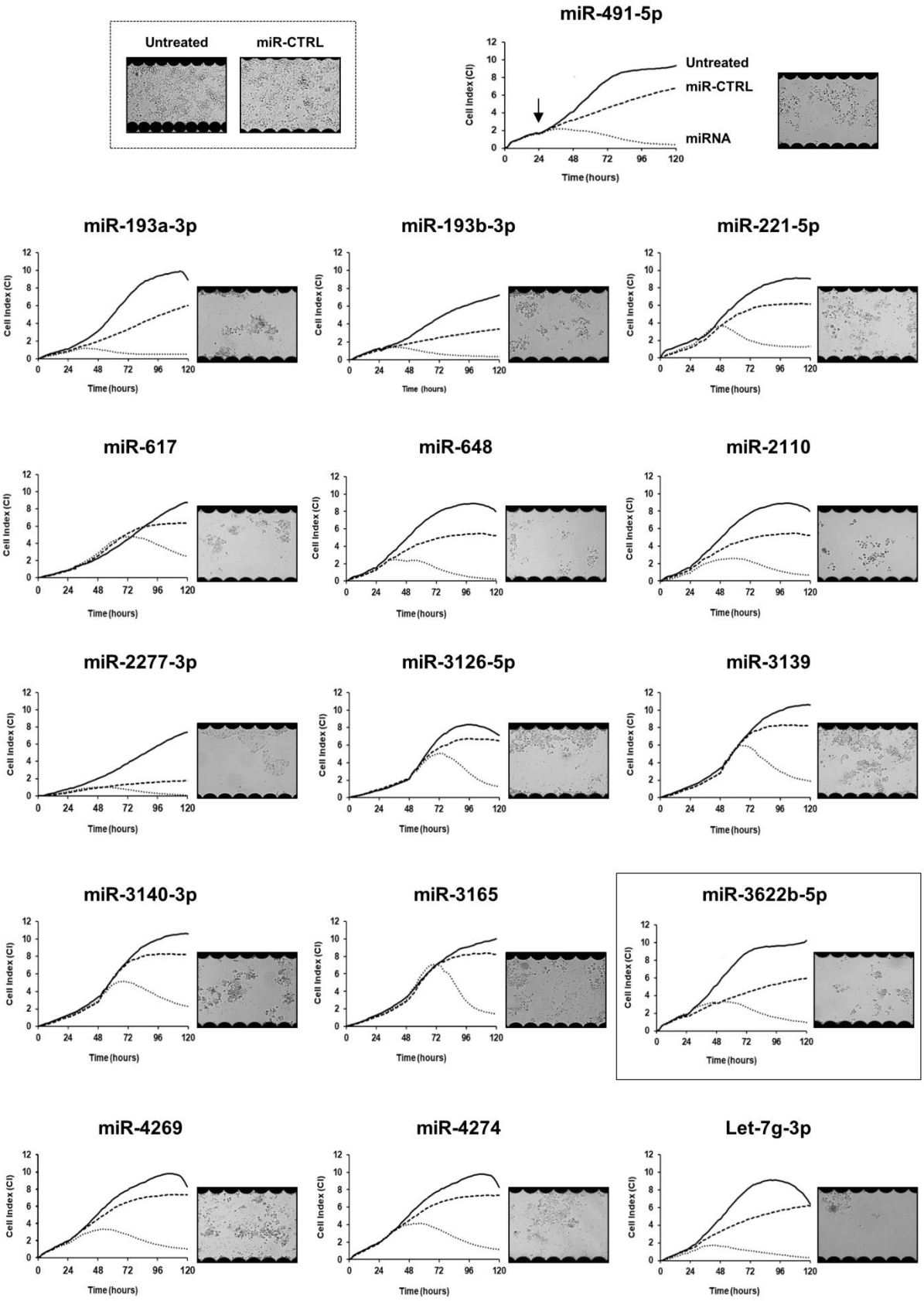
Supplementary Data

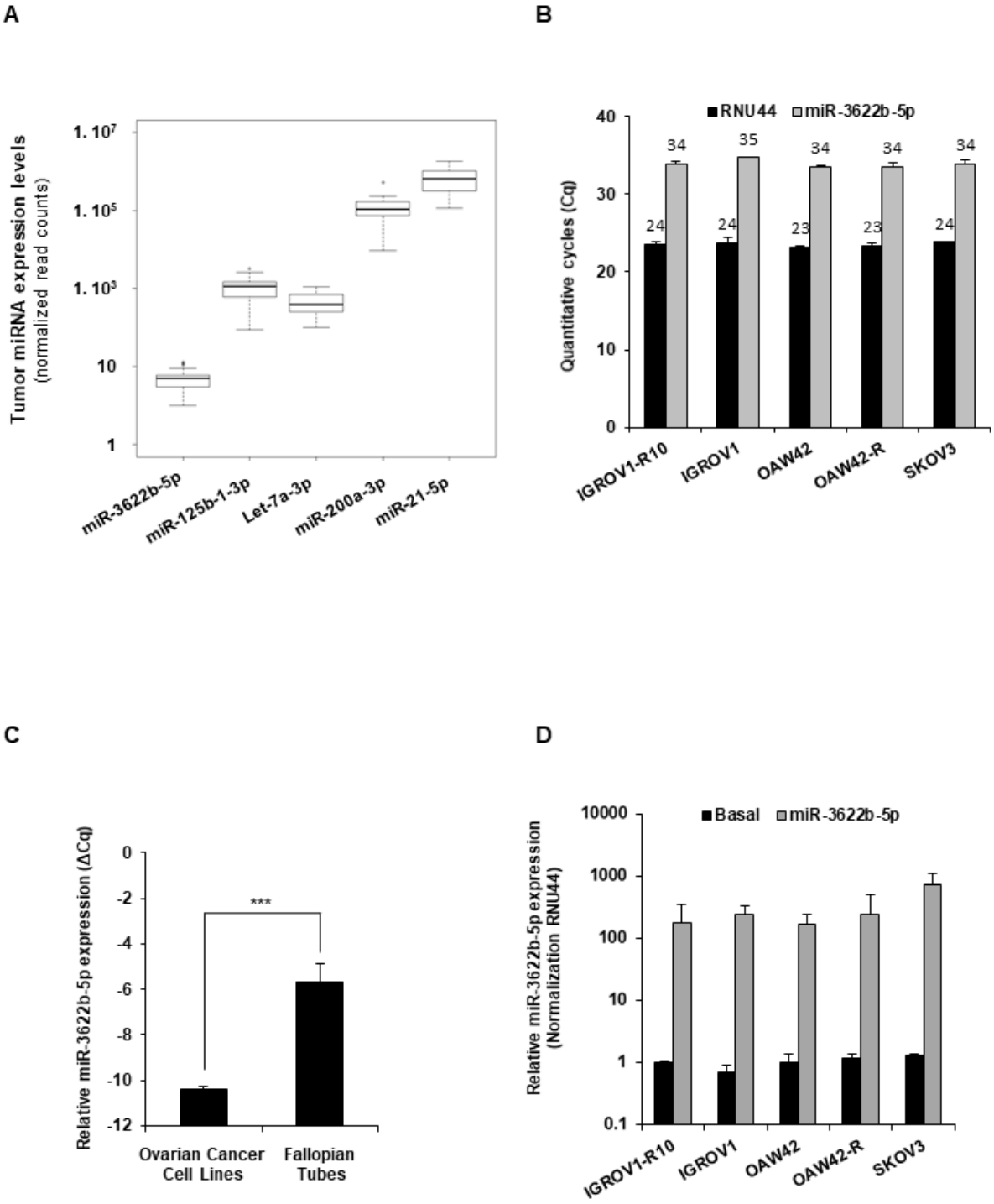
Supplementary Figures



Supplementary Figure S1. ***Vernon et al.***

Supplementary Figure S1. Impedance profile and morphological observation of the 15 candidate cytotoxic miRNAs in IGROV1-R10 ovarian cancer cells.

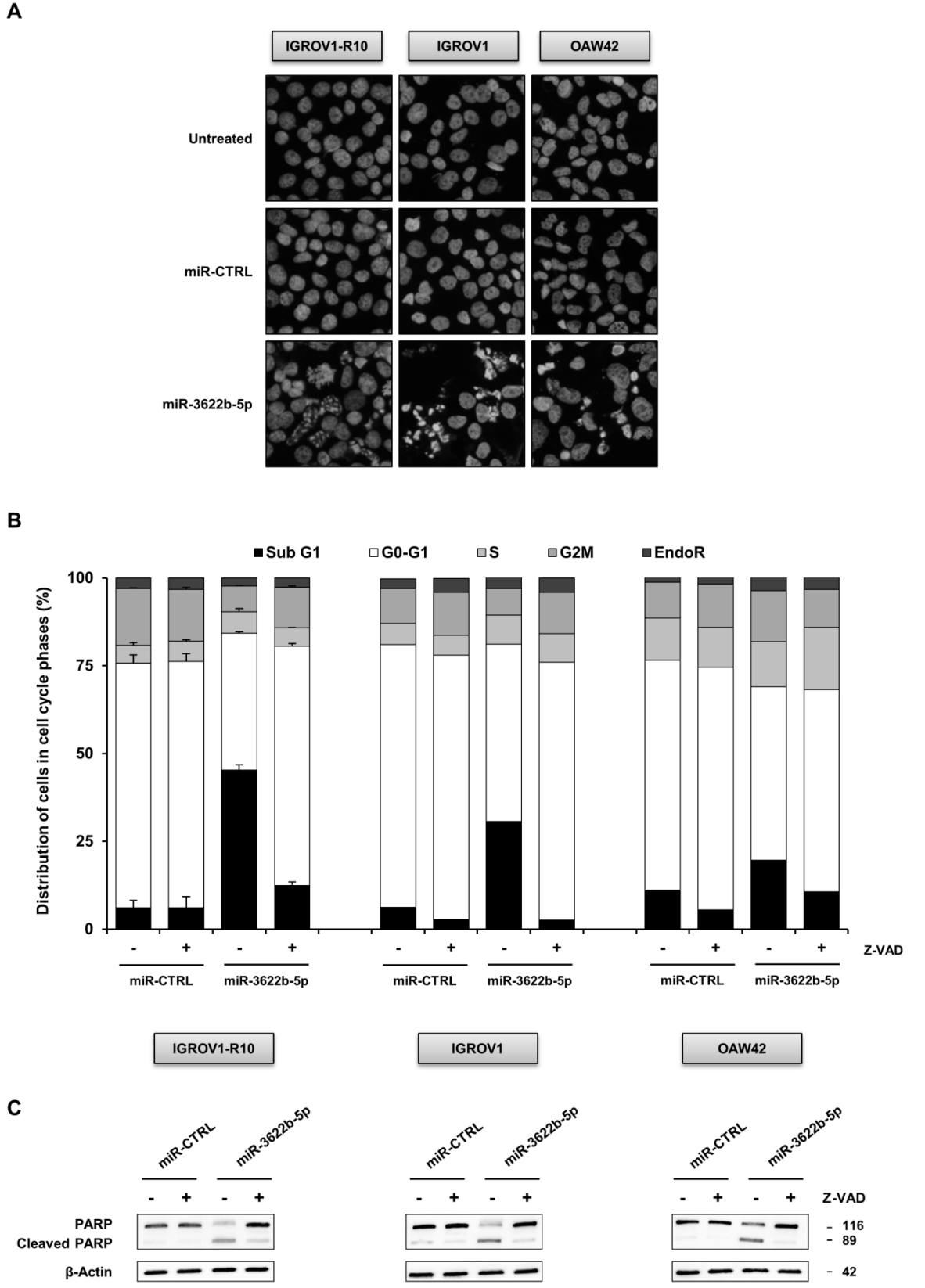
Real-time curves were monitored by impedance measurement (xCELLigence technology). IGROV1-R10 ovarian cancer cells were seeded into 96-well E-Plates VIEW allowing the measurement of CI based on impedance. Cells were grown for 24 hours before transfection with indicated miRNA (arrow). CI was recorded every 2 hours for 120 hours. Morphology of IGROV1-R10 cells was observed by a fully automated high-throughput cell imaging system. Impedance profile and endpoint morphological observation for miR-491-5p was used as a positive control of cytotoxicity (top panel) and 15 candidates selected from screening (lower panel) are shown (surrounded figure focus on miR-3622b-5p). After transfection of miR-491-5p in IGROV1-R10 cells, we observed the beginning of cytotoxicity by modification in the shape of the curve. At 48 hours post-transfection, the CI values decreased to 50% of the maximum value (CI50) and then continued to decline until the experiment endpoint at 96 hours post-transfection, indicating a potential massive cell death. The morphological observation (decrease in cell number and presence of cellular debris) confirmed (or not) the cytotoxic effect of the candidate miRNAs.



Supplementary Figure S2. ***Vernon et al.***

Supplementary Figure S2. Low expression level of miR-3622b-5p in ovarian tumor and in a panel of ovarian cancer cell lines.

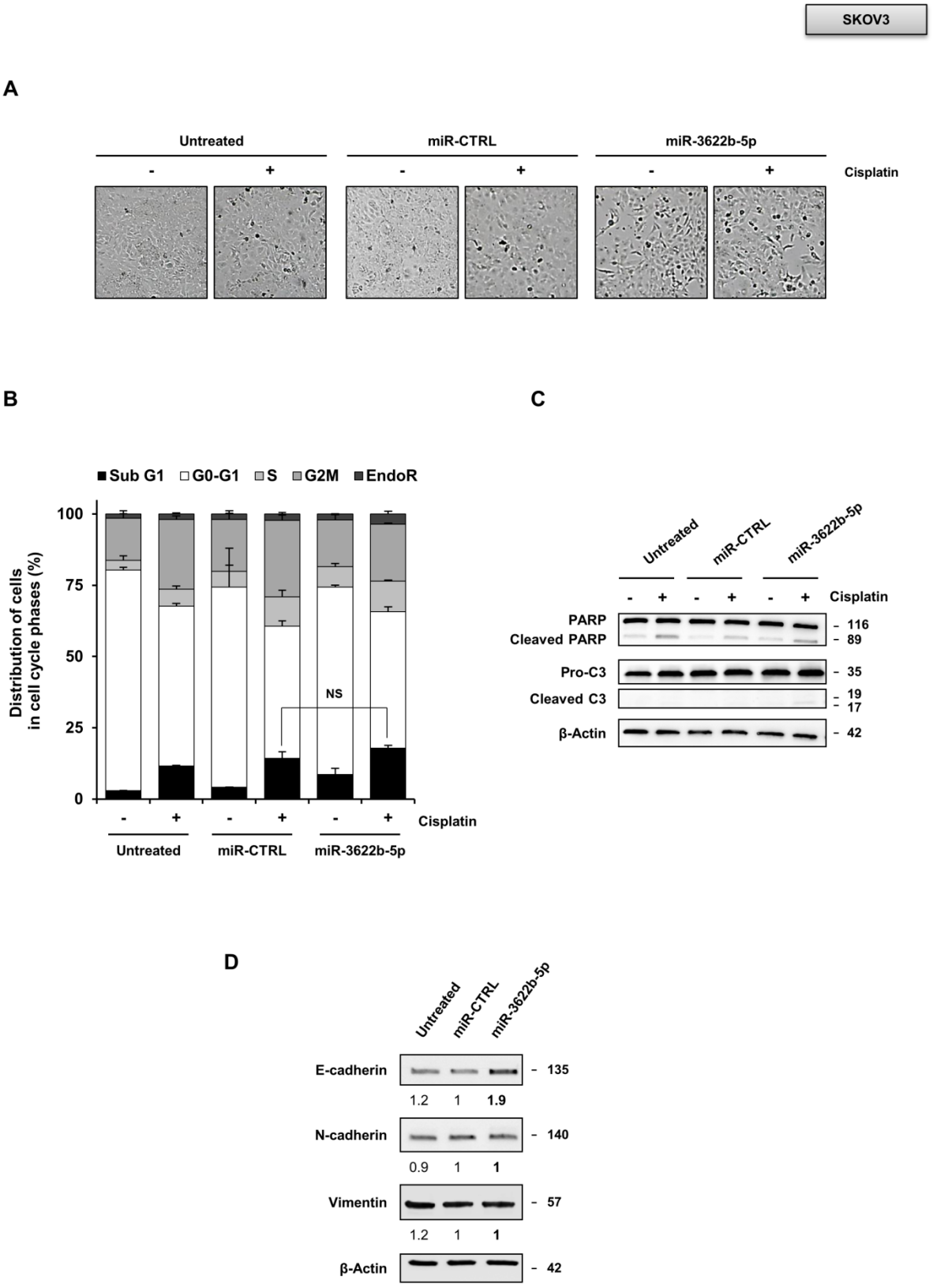
(**A**) Expression level of miR-3622b-5p and of clinically relevant underexpressed (miR-125b-1-3p, let-7a-3p) or overexpressed (miR-200a-3p, miR-21-5p) miRNAs analyzed by RNA-Seq in 50 ovarian tumor samples and represented in boxplot. MiRNA reads count normalized to library size are presented in log2 scale. (**B**) Endogenous expression levels of miR-3622b-5p were analyzed by RT-qPCR in a panel of ovarian cancer cell lines. Results are expressed in Cycle quantitative (Cq). Data are representative of three independent experiments performed in triplicate and no template controls were included for each assay. (**C**) Relative expression level of miR-3622b-5p normalized to RNU44 was studied by RT-qPCR in human ovarian cancer cell lines (see above, S2B) and human fallopian tubes samples (*N=10*). Data are presented as mean ± SEM of ΔCq (Cq_RNU44 - Cq_miR-3622b-5p). ***p< 0.001 by paired Student's t test. (**D**) Ectopic expression levels of miR-3622b-5p by RT-qPCR in a panel of ovarian cancer cell lines. Relative expression of miR-3622b-5p normalized to RNU44 was studied in untransfected cells (baseline) or 24 hours after transfection with miR-3622b-5p. The $2^{-\Delta\Delta}$Cq method was used to calculate relative changes in gene expression determined from real-time quantitative PCR experiments. Data are representative of three independent experiments performed in triplicate and are presented as means ± SEM in log scale.



Supplementary Figure S3. ***Vernon et al.***

Supplementary Figure S3. Z-VAD-FMK protects against miR-3622b-5p-induced apoptosis in ovarian cancer cells.

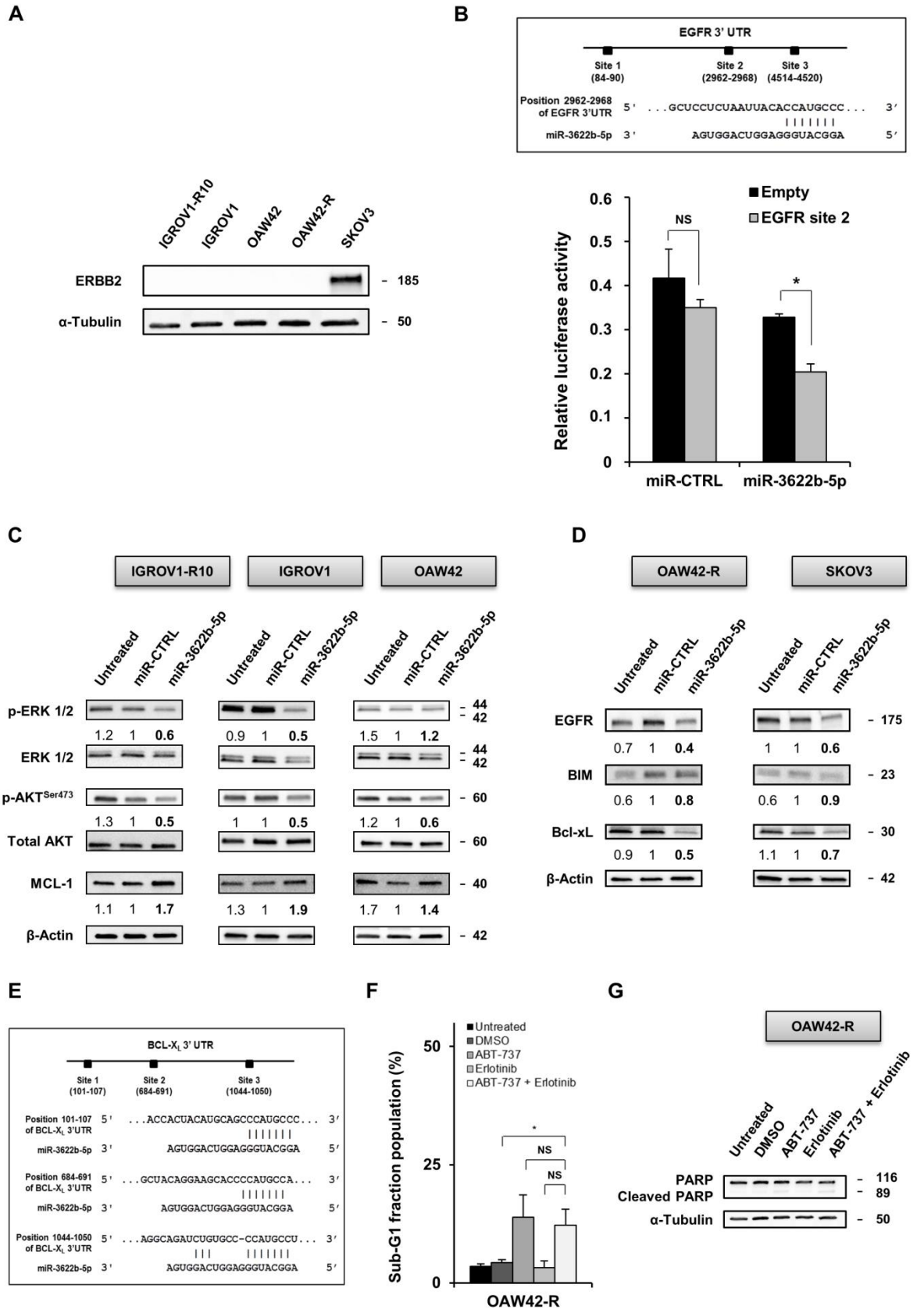
IGROV1-R10, IGROV1 and OAW42 cancer cells were transfected with miR-CTRL or miR-3622b-5p. Twenty-four hours later, Z-VAD-FMK (50 µM) or DMSO were added for 48 hours. (**A**) Nuclear morphology was revealed after DAPI staining. (**B**) DNA content histograms were obtained by flow cytometry. Data are expressed as mean ± SEM of two independent experiments. (**C**) PARP (native and cleaved forms) protein expression levels were determined by western blot. β-Actin level is shown as loading control.



Supplementary Figure S4. ***Vernon et al.***

Supplementary Figure S4. MiR-3622b-5p does not sensitize SKOV3 cancer cells to cisplatin.

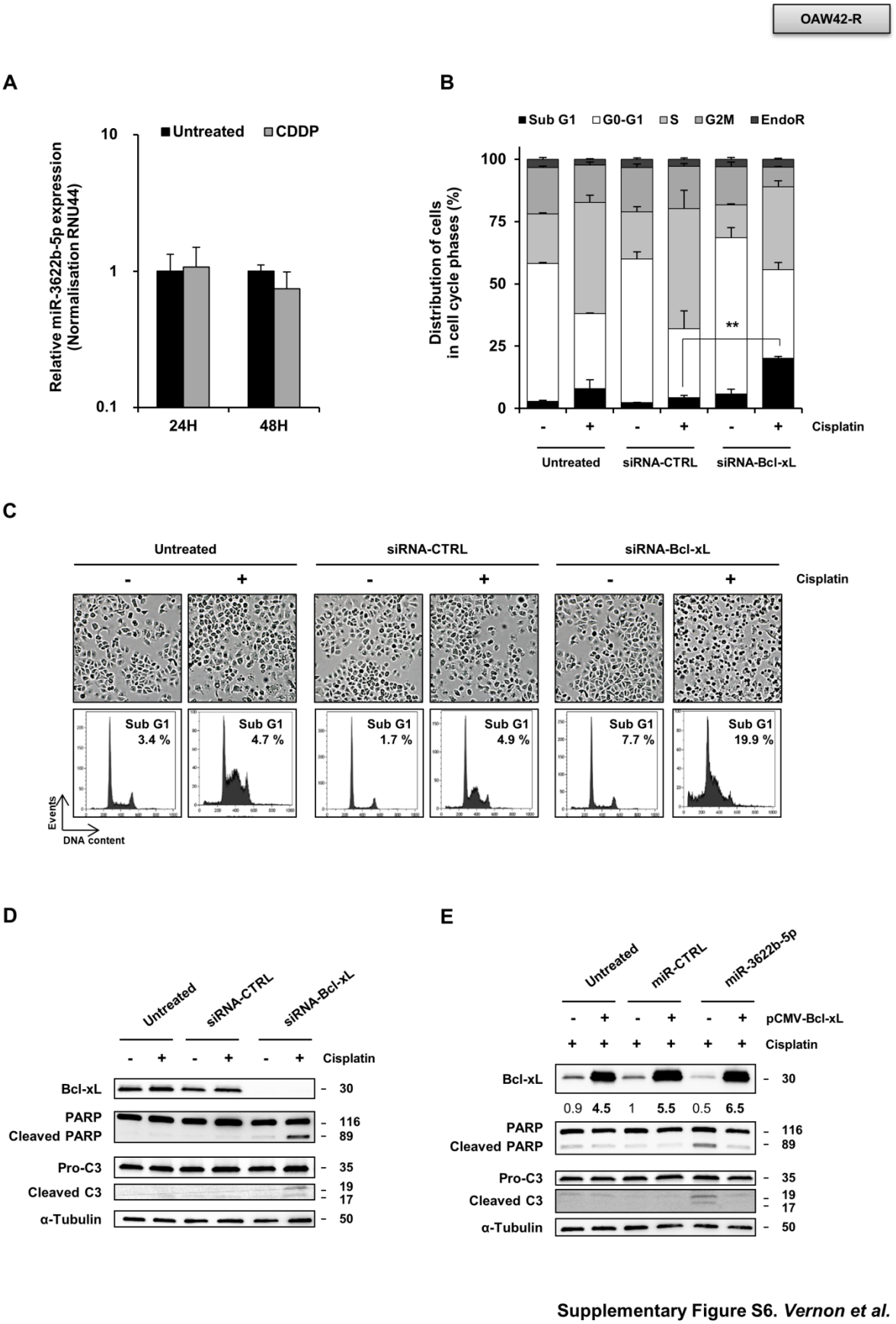
(**A** and **B**) Cells were grown for 24 hours before transfection with indicated miR-CTRL or miR-3622b-5p. SKOV3 cancer cells were treated for 2 hours with cisplatin 48 hours post-transfection. Twenty-four hours later, representative (**A**) cell morphology is shown and (**B**) DNA content histograms were obtained by flow cytometry. Data are presented as mean ± SEM of three independent experiments. NS $p > 0.05$ by paired Student's t test. (**C**) Seventy-two hours after transfection of SKOV3 cancer cells, protein levels of caspase-3 [pro and cleaved (active) forms] were analyzed by western blotting. Representative one of three independent experiments is shown. (**D**) Forty-eight hours after transfection of SKOV3 cancer cells with miR-CTRL or miR-3622b-5p, protein levels of E-cadherin, N-cadherin and Vimentin were analyzed by western blotting. Representative one of two independent experiments is shown. β-Actin level is shown as loading control and the relative densitometry values (calculated relative to β-Actin) were determined using ImageJ software and are shown at the bottom of each western blot.



Supplementary Figure S5. ***Vernon et al.***

Supplementary Figure S5. Identification of potential targets of miR-3622b-5p in ovarian cancer cell lines.

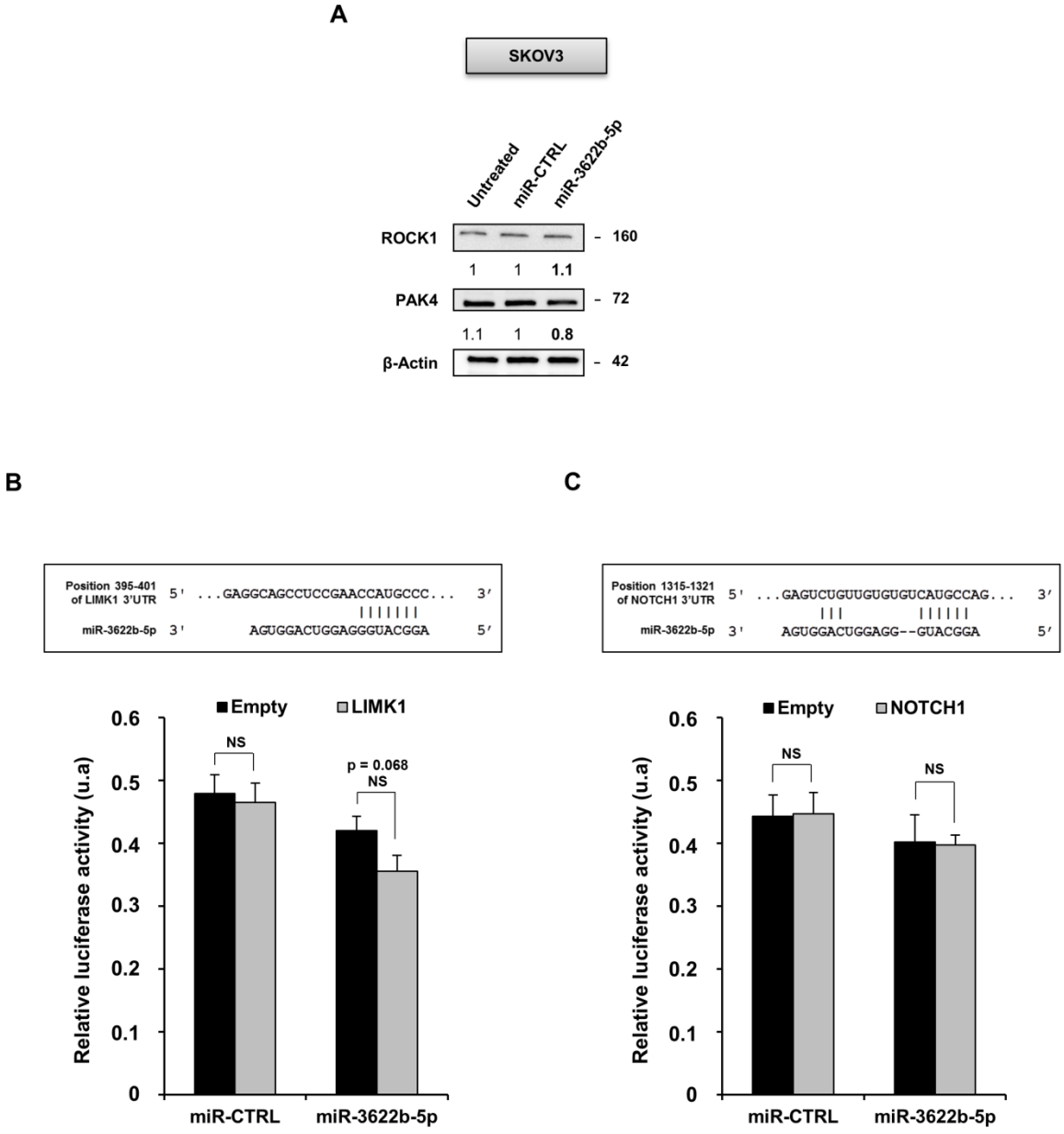
(**A**) Endogenous expression of ERBB2 protein level in a panel of ovarian cancer cell lines (SKOV3 cancer cells were used as positive control) was analyzed by western blotting. α-Tubulin level is shown as loading control. (**B**) Schematic representation of 3′-UTR EGFR mRNA with three predicted binding sites of miR-3622b-5p (complementarity is presented for the tested site 2). SKOV3 cancer cells were transfected with miR-CTRL or miR-3622b-5p. Twenty-four hours after transfection, luciferase reporter constructs containing human EGFR putative binding site 2 or empty construct (control) were transfected into SKOV3 ovarian cancer cells. Luciferase assays were carried out 24 hours after plasmid transfection. Firefly luciferase activity was normalized to renilla luciferase. Data are presented as mean ± SEM of two independent experiments. NS $p > 0.05$, *$p \leq 0.05$ by paired Student's t test. (**C**) IGROV1-R10, IGROV1 and OAW42 ovarian cancer cell lines were transfected with miR-CTRL or miR-3622b-5p. Forty-eight hours post-transfection, total and/or phosphorylated protein expression levels were determined by western blot. The relative densitometry values (calculated relative to β-Actin) were determined using ImageJ software and are shown at the bottom of each western blot. (**D**) OAW42-R and SKOV3 cancer cell lines were transfected with miR-CTRL or miR-3622b-5p. Forty-eight hours post-transfection, EGFR, Bcl-xL and BIM protein expression levels were determined by western blot. The relative densitometry values (calculated relative to β-Actin) were determined using ImageJ software and are shown at the bottom of corresponding western blot. (**E**) Schematic representation of 3′-UTR Bcl-xL mRNA with three predicted binding sites of miR-3622b-5p. (**F** and **G**) OAW42-R cancer cells were treated with or without 1 μM Erlotinib for 48 hours and then with or without 5 μM ABT-737 for an additional 6 hours. (**F**) The percentage of sub-G1 population is expressed as mean ± SEM of three independent experiments. NS $p > 0.05$ and *$p \leq 0.05$ by paired Student's t test. (**G**) PARP (native and cleaved forms) protein expression levels were determined by western blot. α-Tubulin level is shown as loading control.



Supplementary Figure S6. ***Vernon et al.***

Supplementary Figure S6. Inhibition of Bcl-xL by miR-3622b-5p is required to induce chemosensitivity to cisplatin in OAW42-R chemoresistant ovarian cancer cells.

(**A**) OAW42-R cancer cells were grown for 24 hours before treatment with or without cisplatin. Relative expression of miR-3622b-5p normalized to RNU44 was studied by RT-qPCR in OAW42-R cancer cells, 24 hours or 48 hours after cisplatin treatment. The $2^{-\Delta\Delta}$Cq method was used to calculate relative changes in gene expression determined from real-time quantitative PCR experiments. Data are representative of two independent experiments performed in triplicate and are presented as means ± SEM in log scale. (**B** and **C**) Forty-eight hours post-transfection with indicated siRNAs, OAW42-R cancer cells were treated for 2 hours with cisplatin and 24 hours later (**B**) DNA content histograms were obtained by flow cytometry. Data are expressed as mean ± SEM of three independent experiments. **p ≤ 0.01 by paired Student's t test. (**C**) Cell morphology was analyzed. One of three representative experiments is shown. (**D**) Forty-eight hours post-transfection with siRNA, OAW42-R cancer cells were treated for 2 hours with cisplatin. Twenty-four hours later, OAW42-R cancer cells were assessed for protein levels of Bcl-xL, PARP (native and cleaved forms) and caspase-3 [pro and cleaved (active) forms] by western blotting. (**E**) OAW42-R cancer cells were transfected for 24 hours with pCMV containing Bcl-xL (pCMV-Bcl-xL). Cells were then transfected with miR-CTRL or miR-3622b-5p for 24 hours. Cells were treated for 2 hours with cisplatin and 24 hours later (*i.e.*, 72 hours after miRNA transfection), protein levels of Bcl-xL, PARP (native and cleaved forms) and caspase-3 [pro and cleaved (active) forms] were studied by western blotting. The relative densitometry values of Bcl-xL (calculated relative to α-Tubulin) were determined using ImageJ software and are shown at the bottom of corresponding western blot.



Supplementary Figure S7. ***Vernon et al.***

Supplementary Figure S7. Identification of potential targets of miR-3622b-5p in SKOV3 ovarian cancer cell line.

(**A**) SKOV3 ovarian cancer cell line was transfected with control (miR-CTRL) or miR-3622b-5p. Forty-eight hours after transfection, ROCK1 and PAK4 protein expression levels were determined by western blot. The relative densitometry values of each protein (calculated relative to β-Actin) were determined using ImageJ software and are shown at the bottom of corresponding western blot. (**B** and **C**) Schematic representation of 3′-UTR LIMK1 mRNA or 3′-UTR NOTCH1 mRNA with the predicted binding site of miR-3622b-5p. SKOV3 cancer cells were transfected with miR-CTRL or miR-3622b-5p. Twenty-four hours post-transfection, luciferase reporter constructs containing human LIMK1 (**B**) or NOTCH1 (**C**) putative binding site or empty construct (control) were transfected into SKOV3 cancer cells. Luciferase assays were carried out 24 hours after plasmid transfection. Firefly luciferase activity was normalized to renilla luciferase. Data are presented as mean ± SEM of three independent experiments. NS $p > 0.05$ by paired Student's t test.

Supplementary Table

A

Antibody		Host species	Source	Source code
Primary				
	Anti-α-Tubulin	Mouse	Sigma Aldrich	T6199
	Anti-β-Actin	Mouse	EDM Millipore, Fisher Scientific	MAB1501
	BCL-X_L (54H6) mAb	Rabbit	Cell Signaling Technology	2764
	BIM	Rabbit	Cell Signaling Technology	2819
	Cleaved NOTCH1 (Val1744) (D3B8) mAb	Rabbit	Cell Signaling Technology	4147
	E-Cadherin (24E10) mAb	Rabbit	Cell Signaling Technology	3195
	EGFR (D38B1) XP® mAb	Rabbit	Cell Signaling Technology	4267
	HER2 / ErbB2 (D8F12) XP® mAb	Rabbit	Cell Signaling Technology	4290
	LIMK1	Rabbit	Cell Signaling Technology	3842
	MCL-1 (D35A5) mAb	Rabbit	Cell Signaling Technology	5453
	N-Cadherin (D4R1H) XP® mAb	Rabbit	Cell Signaling Technology	13116
	NOTCH1 (D6F11) XP® mAb	Rabbit	Cell Signaling Technology	4380
	PAK4	Rabbit	Cell Signaling Technology	3242
	PARP	Rabbit	Cell Signaling Technology	9542
	Phospho p44/42 MAPK (Erk 1/2) (Thr202/Tyr204) (D13.14.4E) XP® mAb	Rabbit	Cell Signaling Technology	4370
	Phospho-AKT Ser473 (D9E) XP® mAb	Rabbit	Cell Signaling Technology	4060
	ROCK1 (C8F7) mAb	Rabbit	Cell Signaling Technology	4035
	Total AKT	Rabbit	Cell Signaling Technology	9272
	Total and cleaved Caspase-3	Rabbit	Cell Signaling Technology	9662
	Total ERK (p44/42 MAPK) (Erk 1/2)	Rabbit	Cell Signaling Technology	9102
	Vimentin (D21H3) XP® mAb	Rabbit	Cell Signaling Technology	5741
Secondary				
	Anti-Mouse IgG		GE HealthCare Europe GmbH	NA931
	Anti-Rabbit IgG		Cell Signaling Technology	7074

B

Binding site of miR-3622b-5p	Oligonucleotide pairs sequences	
EGFR site 2	Fwd	5' **TCGA**GCTCCTCTAATTACACCATGCCC 3'
	Rev	5' **CTAG**GGGCATGGTGTAATTAGAGGAGC 3'
BCL-X_L site 1	Fwd	5' **TCGA**ACCACTACATGCAGCCCATGCCC 3'
	Rev	5' **CTAG**GGGCATGGGCTGCATGTAGTGGT 3'
BCL-X_L site 2	Fwd	5' **TCGA**GCTACAGGAAGCACCCCATGCCA 3'
	Rev	5' **CTAG**TGGCATGGGGTGCTTCCTGTAGC 3'
BCL-X_L site 3	Fwd	5' **TCGA**AGGCAGATCTGTGCCCCATGCCT 3'
	Rev	5' **CTAG**AGGCATGGGGCACAGATCTGCCT 3'
NOTCH1	Fwd	5' **TCGA**GAGTCTGTTGTGTGTCATGCCAG 3'
	Rev	5' **CTAG**CTGGCATGACACACAACAGACTC 3'
LIMK1	Fwd	5' **TCGA**GAGGCAGCCTCCGAACCATGCCC 3'
	Rev	5' **CTAG**GGGCATGGTTCGGAGGCTGCCTC 3'

Supplementary Table S1. ***Vernon et al.***

Supplementary Table S1. Supplementary Methods.

(**A**) Primary and secondary antibodies used in this study. (**B**) Oligonucleotide pairs corresponding to miR-3622b-5p binding sites from TargetScan. Sequences in bold are additional bases to constitute overhang for cloning into XhoI and XbaI restricted pMIRGLO.

Supplementary Material and Methods information.

Human material for patient-derived tumor organoids (PDOs) establishment and culture

The medium used for the culture of ovarian PDOs was the following: Advanced DMEM supplemented with 100 UI/mL of penicillin, 100 µg/mL of streptomycin, 1% GlutaMAX, 1x B27 (Gibco), 1.25 mM NAC (Sigma-Aldrich), 50 ng/mL EGF (PeproTech), 20ng/mL FGF-10 (PeproTech), 1ng/mL FGF-2 (PeproTech), 500 nM A-83-01 (PeproTech), 10 µM Y27632 (Interchim), 1 µM SB202190 (PeproTech), 10 mM Nicotinamide (Sigma-Aldrich), 1 µM PGE2 (PeproTech), 100 µg/mL Primocin (InvivoGen), 50% Wnt3a, RSPO3, Noggin-conditioned media (L-WRN, ATCC), and 10% RSPO1-conditioned media (Cultrex HA-R-Spondin-1-Fc 293T, Amsbio).

Drugs

Stock solutions were prepared in DMSO according to the manufacturer's instructions. Cisplatin (CDDP) was purchased from Mylan (Merck). For cisplatin treatment, cells were incubated in serum free medium supplemented with CDDP for 2 hours (20 µg/mL for OAW42-R or 5 µg/mL for SKOV3 cancer cell lines), rinsed with PBS and incubated in complete medium for the indicated time.

MiRNA mimic screening using real-time cell analysis by impedance measurement

The RTCA xCELLigence system monitors cellular events in real time by measuring electrical impedance across interdigitated micro-electrodes integrated at the bottom of 96 well E-plates VIEW (ACEA). These electrodes measure a Cellular Index (CI) based on impedance, which correlates with the area of attached cells. Standard deviations of well replicates were analyzed with the RTCA 2.1.0 Software. For each plate, miR-CTRL and miR-491-5p were use as negative and positive controls of cytotoxicity, respectively. The AUC was measured by the trapeze method according to the following formula:

$$Area = \sum_{n=0}^{N} \frac{CI_{t_n} + CI_{t_{n+1}}}{2} \times (t_n - t_{n+1})$$

Real-time cell migration and invasion analyses by impedance measurement

Twenty-four hours after transfection, SKOV3 cells were serum-starved for approximately 4 hours prior to conducting the experiments. The upper chamber of the CIM-plates was coated with a solution of 1:40 Matrigel® (BD Biosciences) for invasion assays. A total of 5×10^4 cells

were seeded in the upper chamber of CIM-plates in serum-free media. Medium with 10% serum added to the well of the lower chamber was used as a chemoattractant.

RNA isolation and miRNA expression assays

RNA quantity and quality were assessed using the NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific). For RT-qPCR experiments, 5 µL of isolated RNA were mixed with 10 µL of RT master mix. 1.33 µL of cDNA were mixed with 18.7 µL of qPCR master mix (Universal Master Mix II without UNG). PCR amplification was performed using a LightCycler® 480 Instrument II Software Version 1.5 (Roche).

RNA sequencing of ovarian tumor samples

For each patient, 32 slices (10 µm thick) were cut in a cryo-microtome. First and last slices were mounted on a microscope slide and HES-stained to estimate the proportion of tumor and stroma in the fragment. Only patients for whom the tumor fraction was >50% were selected. Thirty remaining slices were kept on dry ice until RNA extraction. RNA extraction was performed with the Nucleospin miRNA extraction kit. Long and short RNA fractions were pooled together according to the manufacturer's protocol. RNA integrity and quality were analyzed with the Agilent RNA 6000 Pico kit (Agilent Technologies).

Western blotting

Cells were scraped and lysed in ice-cold RIPA lysis buffer. After 30 min on ice, lysates were clarified (10,000 x g, 4°C, 10 min) and protein concentrations were determined using the Bradford assay (Bio-Rad). Equal amount of proteins (30 µg) were separated by SDS-PAGE on a 4-15% gradient polyacrylamide gel (Bio-Rad) and transferred to PVDF membranes using the Trans Blot®Turbo™ Transfer system (Bio-Rad). Protein levels were analyzed by immunoblotting with antibodies from Cell Signaling Technology (CST) unless otherwise indicated according to the manufacturer's instructions and appropriate horseradish peroxidase conjugated secondary antibodies, and signals were detected using enhanced chemiluminescence (GE HealthCare). Blots were hybridized with β-Actin or α-Tubulin monoclonal antibodies to control protein loading.

Nuclear morphology and cell cycle analysis by DNA content

For DAPI staining, both detached and adherent cells were pooled, washed with 1X PBS and then either applied to a polylysine coated glass slide by cytocentrifugation and fixed with a solution of ethanol/chloroform/acetic acid (6:3:1). The preparations were then incubated for 15 min at room temperature with 1 µg/mL DAPI solution (Boehringer), washed in distilled water, mounted on a coverslip in Mowiol (Calbiochem) and analyzed under a fluorescence

microscope (BX51, Olympus). For cell cycle analysis, adherent and detached cells were pooled, fixed in 70% ethanol and stored at -20°C until analysis. Fixed cells were centrifuged (4000 rpm, 5 min) and incubated for 30 min at 37°C in PBS to allow the release of low-molecular weight DNA characteristic of apoptotic cells. After centrifugation, cell pellets were resuspended and incubated with RNAse and propidium iodide (Thermo Fisher Scientific) and were analyzed using a Gallios flow cytometer (Beckman Coulter). Computerized gating was applied on the side and forward scatter to exclude small debris and on a pulse width and integral peak of red fluorescence to eliminate aggregates. The data were analyzed by Kaluza® acquisition software.

Plasmid constructs

pMIRGLO plasmid (Promega) was double-restricted by XhoI and XbaI and gel-purified. Oligonucleotide pairs (Supplementary Table S1B) were phosphorylated and annealed as described previously. The overhangs created by oligonucleotide pairs are complementary to those generated by the XhoI-XbaI double-restricted pMIRGLO plasmid. Ligation of inserts and plasmid was performed using T7 DNA ligase. JM109-competent cells were transformed with ligation products according to the distributor's instructions (Promega). Carbenicillin was used for the selection of resistant clones. All the constructions were checked by sequencing.

MiRNA target predictions

The Targetscan 7.2 algorithm (http://www.targetscan.org) was used in this study.