Multipronged activity of combinatorial miR-143 and miR-506 inhibits Lung Cancer cell cycle progression and angiogenesis *in vitro*

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Supplementary Table 1: Cell cycle distribution for cells treated with miR-143 and/or -506 at the 24 and 48 h time points post transfection

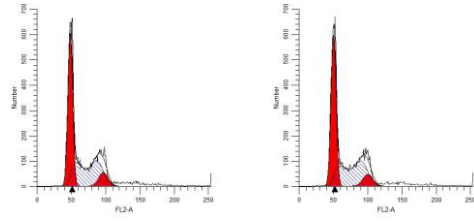|	G0/G1	S	G2/M
24h			
Untreated	47	41	11
miR-143	63	22	14
miR-506	63	23	14
miR-143+506	71	17	13
48h			
miR-143	66	21	13
miR-506	76	13	11
miR-143+506	75	12	13

Supplementary Table 2: Primer sequences for identifying different gene expressions by qRT-PCR

| Target Gene	Primer direction	Primer sequence
CDK1	Forward	TTTTCAGAGCTTTGGGCACT
CDK1	Reverse	CCATTTTGCCAGAAATTCGT
CDK4	Forward	TATGAACCCGTGGCTGAAAT
CDK4	Reverse	CATCAGCCGTACAACATTGG
CDK6	Forward	CGTGGTCAGGTTGTTTGATGTG
CDK6	Reverse	ACTCGGTGTGAATGAAGAAAGTCC
GAPDH	Forward	TGCACCACCAACTGCTTAGC
GAPDH	Reverse	GGCATGGACTGTGGTCATGAG
BCL2	Forward	CCTTGTGGATGACTGAGTACC
BCL2	Reverse	AGCCAGGAGAAATCAAACAGAG

Supplementary Figure 1: Cell cycle distribution for cells treated with scramble siRNA+lipofectamine compared to control
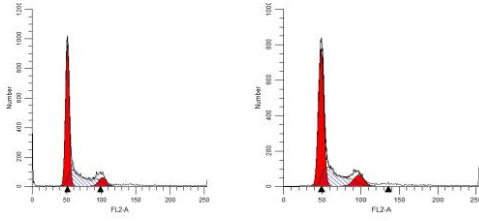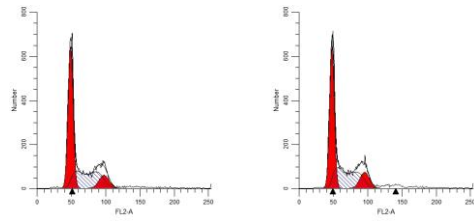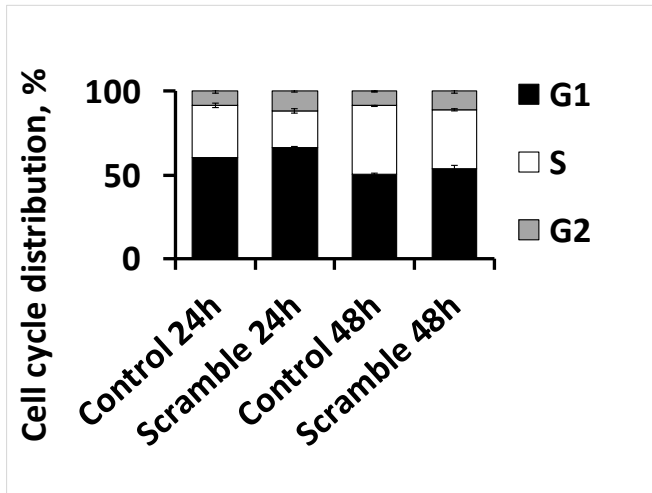

Control 24h

Control 48h
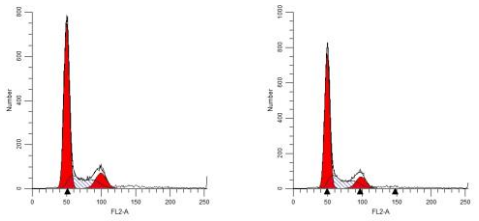
Scramble 24h

Scramble 48h

Sample	G0/G1	S	G2/M
Control 24h	60.27	32.54	7.19
Control 24h	60.48	30.01	9.51
Scramble 24h	67.12	20.7	12.17
Scramble 24h	65.38	23.66	10.96
Control 48h	49.81	41.33	8.86
Control 48h	51.3	40.62	8.08
Scramble 48h	55.65	34.2	10.16
Scramble 48h	52.24	35.95	11.81

Supplemental Figure 2: Annexin V/PI apoptosis assay for cells treated with scramble
siRNA+lipofectamine and lipofectamine alone compared to control
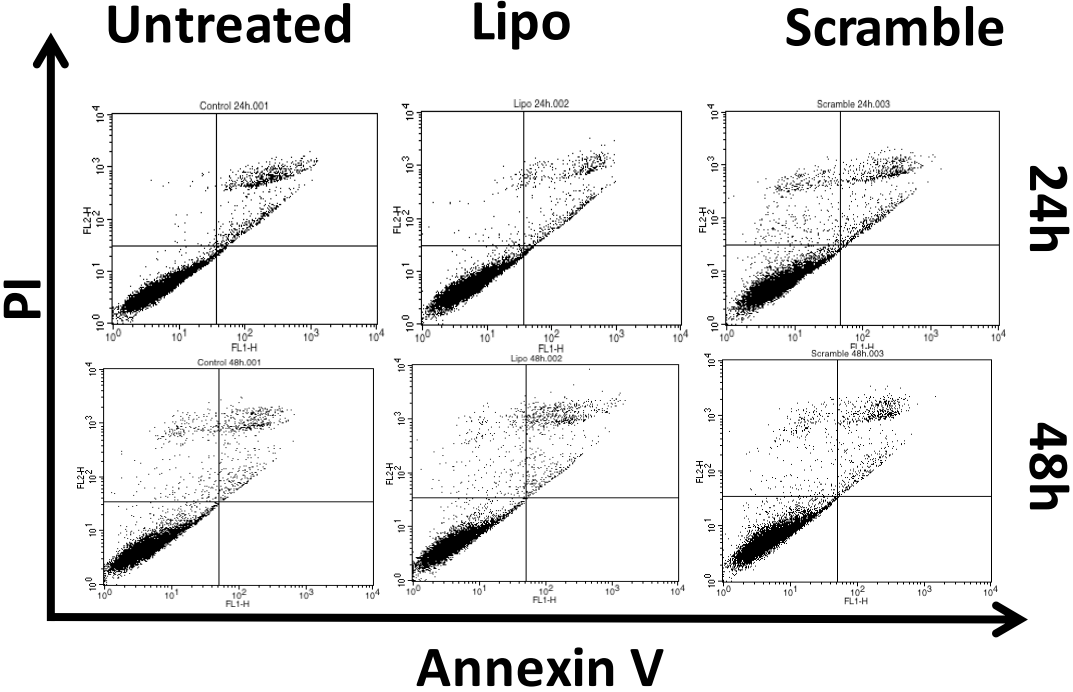
Supplementary Figure 3: RNA quality assessment prior RNA sequence (first duplicate for untreated and miR treated samples)
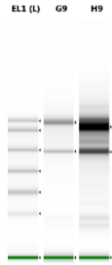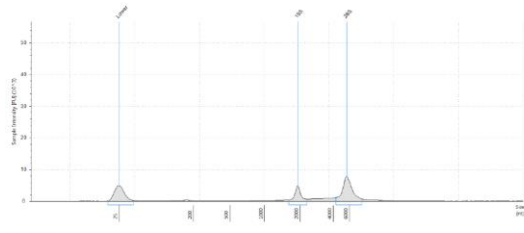

First Replicates

Untreated

miR-143+506

Untreated / miR-143+506

RIN: 10.0   RIN: 9.9

Untreated — Sample Table

Well	RINe	28S/18S (Area)	Conc. [ng/µl]	Sample Description	Alert	Observations
B1	10.0	3.5	221	NH-1		

Untreated — Peak Table

Size [nt]	Calibrated Conc. [ng/µl]	Assigned Conc. [ng/µl]	Peak Molarity [nmol/l]	% Integrated Area	Peak Comment	Observations
25	40.0	40.0	4710	-		Lower Marker
1778	38.0	-	62.9	22.15		18S
4279	134	-	91.9	77.85		28S

miR-143+506 — Sample Table

Well	RINe	28S/18S (Area)	Conc. [ng/µl]	Sample Description	Alert	Observations
C1	9.9	3.4	203	NH-2		

miR-143+506 — Peak Table

Size [nt]	Calibrated Conc. [ng/µl]	Assigned Conc. [ng/µl]	Peak Molarity [nmol/l]	% Integrated Area	Peak Comment	Observations
25	40.0	40.0	4710	-		Lower Marker
1793	34.2	-	56.2	22.74		18S
4347	116	-	78.7	77.26		28S

Supplemental Figure 4: RNA quality assessment prior RNA sequence (second duplicate for untreated and miR treated samples)
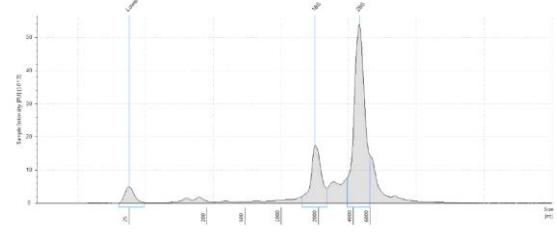
Second Replicates

Untreated

Sample Table

Well	RINe	28S/18S (Area)	Conc. [ng/µl]	Sample Description	Alert	Observations
G9	9.9	2.6	64.6	NH-1_2 1:10		

Peak Table

Size [nt]	Calibrated Conc. [ng/µl]	Assigned Conc. [ng/µl]	Peak Molarity [nmol/l]	% Integrated Area	Peak Comment	Observations
25	40.0	40.0	4710	-		Lower Marker
1923	12.4	-	19.0	27.96		18S
5616	31.9	-	16.7	72.04		28S

miR-143+506

Sample Table

Well	RINe	28S/18S (Area)	Conc. [ng/µl]	Sample Description	Alert	Observations
H9	9.6	3.1	490	2_20		

Peak Table

| Size [nt]	Calibrated Conc. [ng/µl]	Assigned Conc. [ng/µl]	Peak Molarity [nmol/l]	% Integrated Area	Peak Comment	Observations
25	40.0	40.0	4710	-		Lower Marker
1883	81.2	-	127	24.11		18S
4636	256	-	162	75.89		28S

Supplementary Information Figure 5: Representative heatmaps of the pathways associated with lung cancer
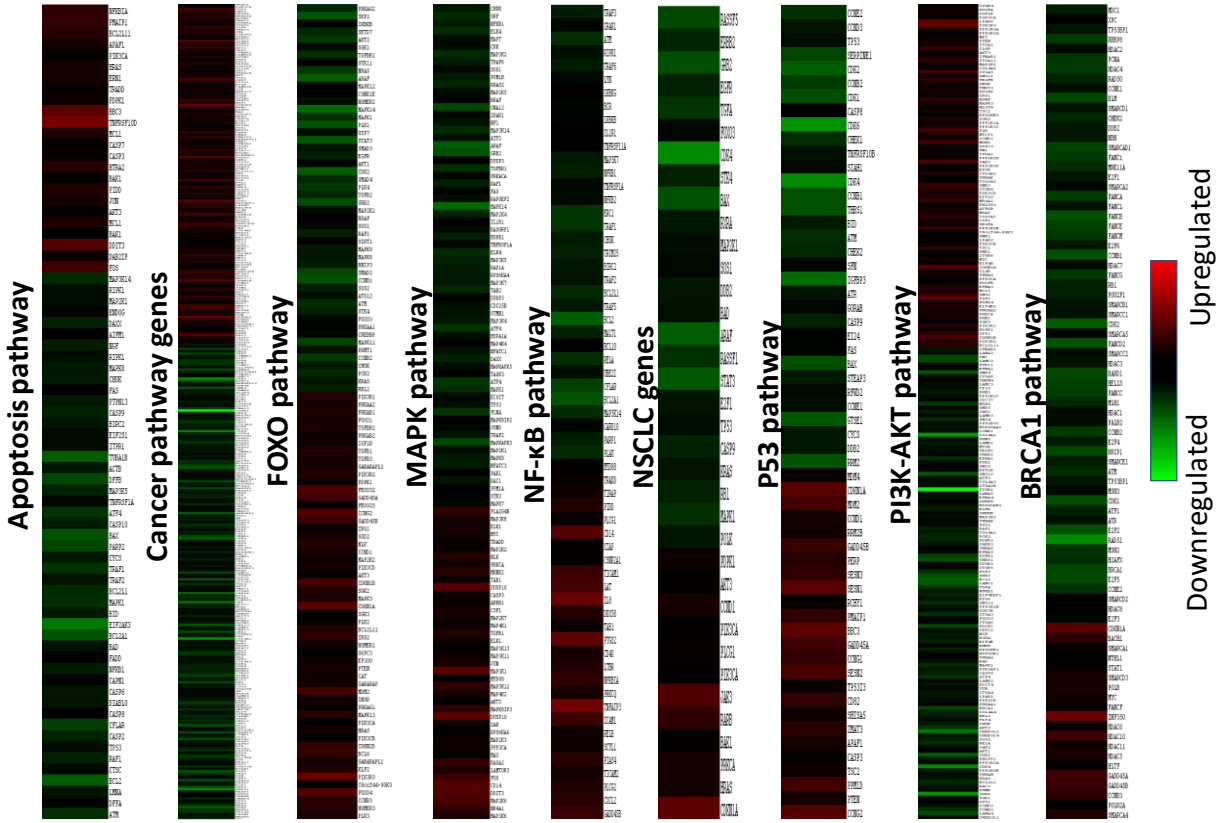
Supplementary Figure 6: Cell cycle pathway and predicted functions, as presented by IPA
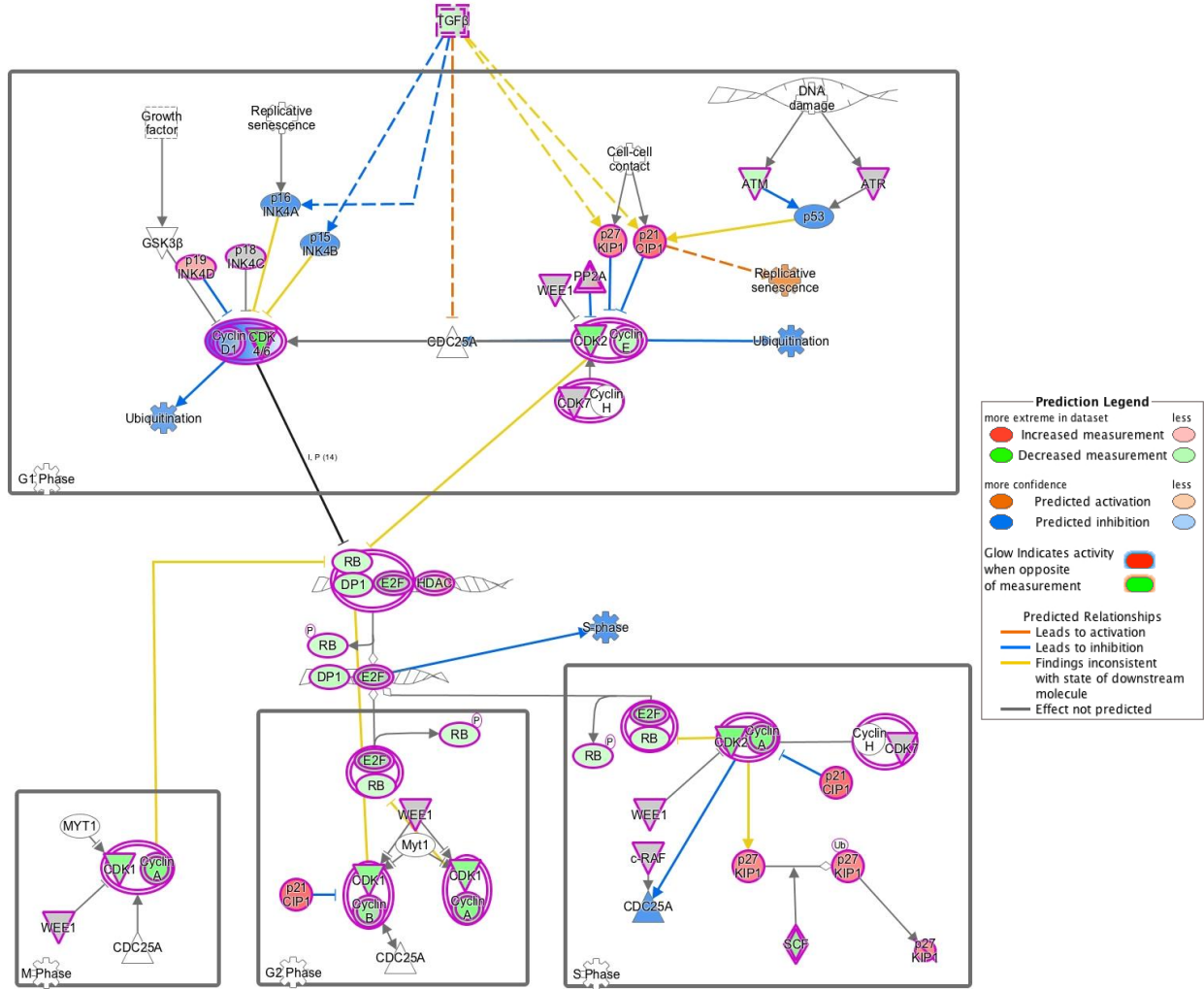
Supplementary Figure 7: Predicted functions due to miR-143 and miR-506 treatment, as predicted by IPA software
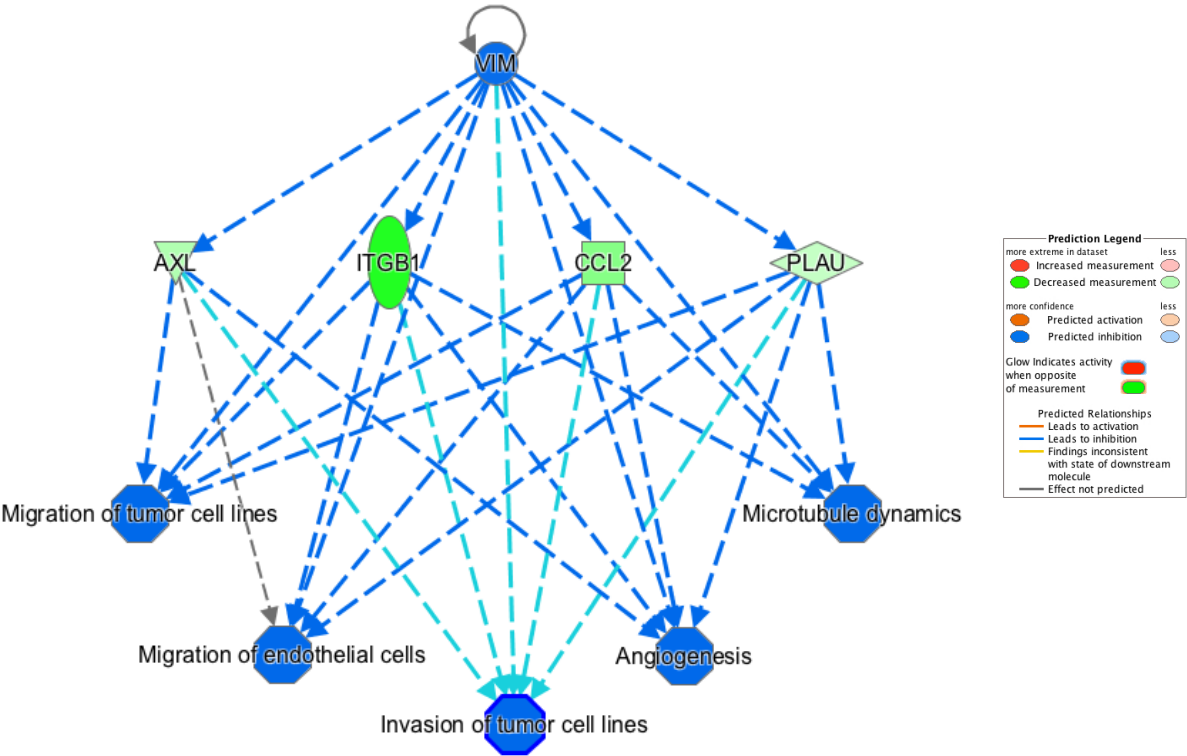
Supplementary Figure 8: BRCA1 pathway and predicted functions, as presented by IPA software, due to miR-143 and miR-506 treatment
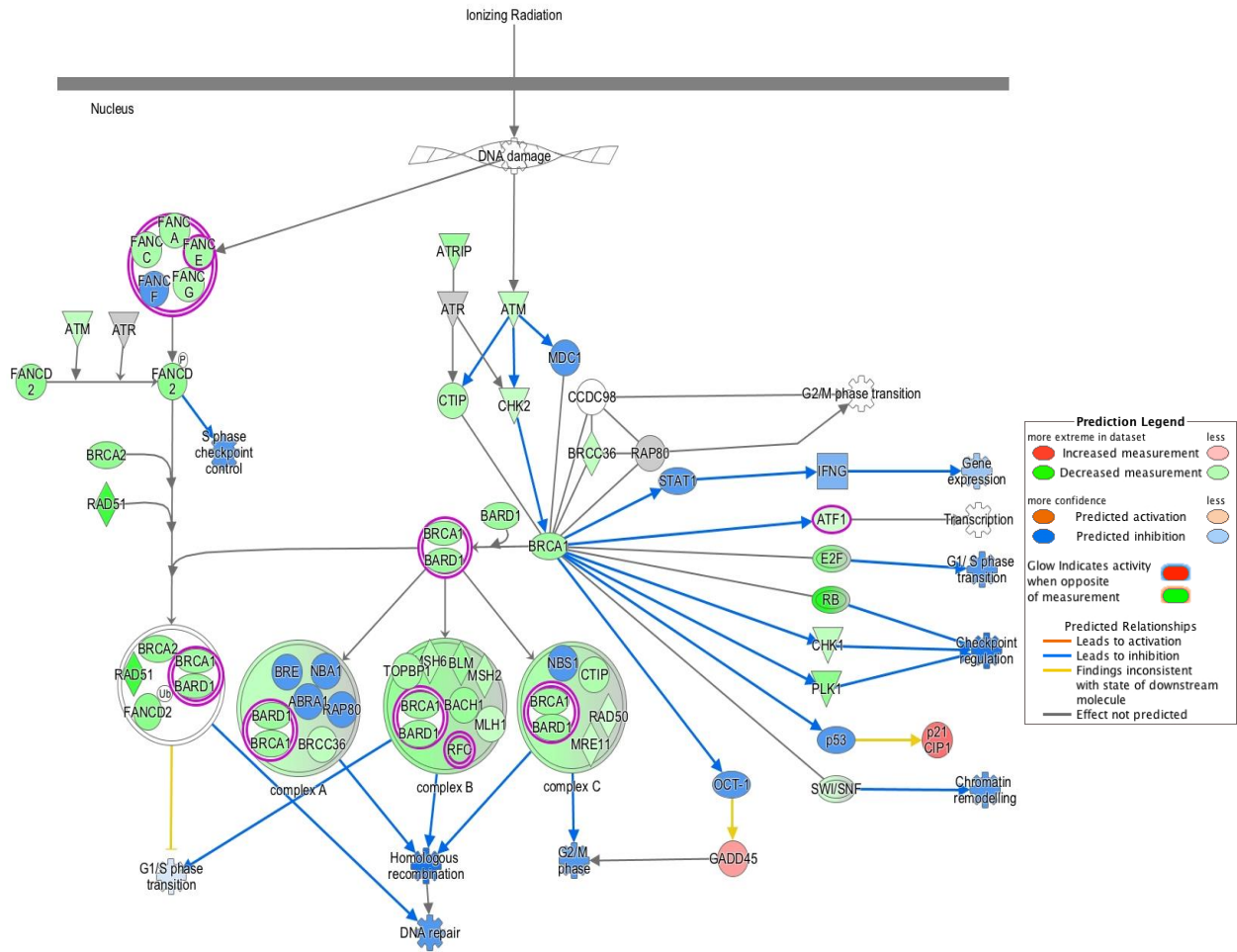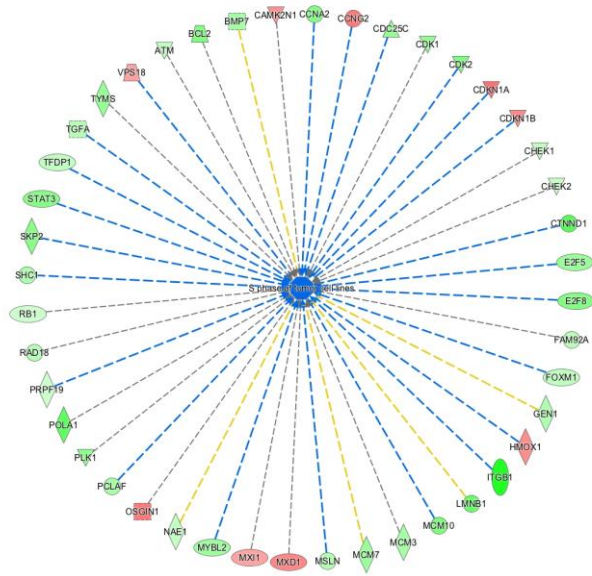
Supplementary Figure 9: Predicted apoptosis function in A549 cells due to the miR-143 and miR-506 treatment, as presented by IPA. (z-score = 3.6)
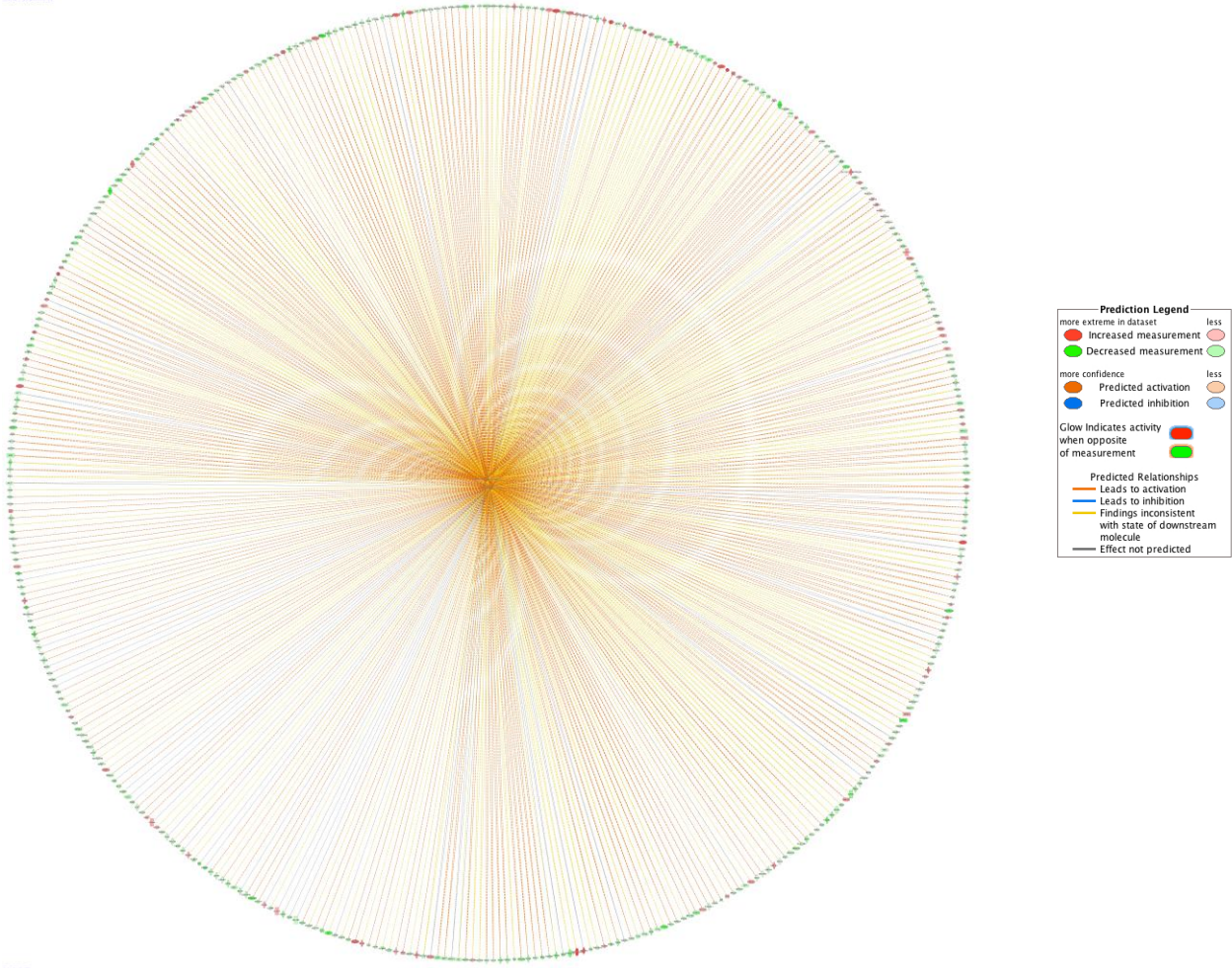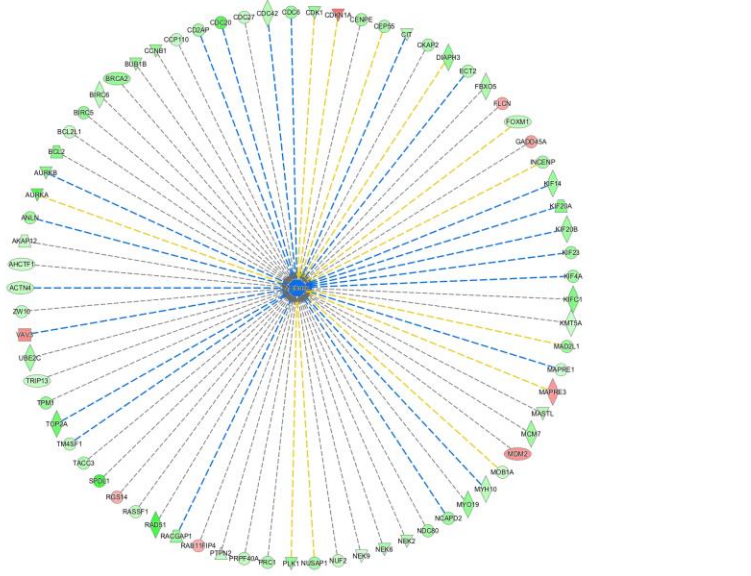
Supplementary Figure 10: *(left)* Predicted S phase of tumor cell lines (z-score = -3.118); *(right)* Predicted M phase (z-score = -2.069), as provided by IPA

Supplementary Figure 11: Full images from Western blot analysis

CDK1	b-tubulin for CDK1 blot
CDK4	b-tubulin for CDK4 blot