Supplementary Materials and Methods

DNA methylation of miR-7 is a mechanism involved in platinum response through *MAFG* overexpression in cancer cells

Cell culture, treatments and viability to CDDP

Cell viability comparing sensitive with resistant cell lines was estimated relative to the density recorded over the same experimental group without drug exposure during the same period of time (0 µg/ml). The epigenetic reactivation drugs 5Aza-2deoxycytidine (5Aza-dC) and trichostatin A (TSA) (Sigma-Aldrich, Spain) were used at 5µM and at 300nM respectively. The resistant cells H23R, H460R, OVCAR3R and A2780R were maintained in the absence of cisplatin for 20 days before the epigenetic treatment. For reexpression studies, cells were seeded at 700,000 cells/p60 and exposed to 5Aza-dC/TSA or to PBS/Ethanol (mock cells) as described(1). DNA and RNA were extracted from all the experimental groups to perform the miRNA array assays, the confirmation of the expression changes by qRT-PCR and the epigenetic validations.

RNA extraction and miRNA/mRNA array preprocessing

Total RNA from sensitive (S), resistant (R) and resistant under epigenetic reactivation treatment (RT) cells was extracted by the guanidine thiocyanate method using TRIZOL reagent (Invitrogen, CA) and purified with the miRNeasy mini kit (Quiagen, CA), combined with DNAsa treatment as recommended by Agilent. RNA Integrity was determined by running samples in a 2100 Bioanalyzer (Agilent Technologies).

miRNA microarrays: Labeling Kit (Agilent Technologies) was used to label RNA. Basically, 100 ng of total RNA were dephosphorylated and Cyanine 3-pCp molecule was ligated to the 3´ end of each RNA molecule by using T4 RNA ligase. For hybridization, 100 ng of Cy3 labelled RNA were hybridized onto Human miRNA Microarray v2, 8x15K (G4470B) for 20 hours at 55ºC in a hybridization oven (G2545A, Agilent Technologies) set to 15 rpm in a final concentration of 1X GE Blocking Agent and 1X Hi-RPM Hybridization Buffer, according to manufacturer's instructions (miRNA Microarray System Protocol, Agilent Technologies). Arrays were washed according to manufacturer's instructions (miRNA Microarray System Protocol, Agilent Technologies) and dried out using a centrifuge at 1000 rpm for 2 min. Finally, arrays were scanned at 5mm resolution on an

Agilent DNA Microarray Scanner (G2565BA, Agilent Technologies) using the default settings for miRNA Microarray (miRNA Microarray System Protocol, Agilent Technologies) and image provided by the scanner was analyzed using Feature Extraction software version 10 (Agilent Technologies). *mRNA microarrays:* One-Color Microarray-Based Gene Expression Analysis Protocol (Agilent Technologies, Palo Alto, CA, USA) was used to amplify and label RNA. Briefly, 1 µg of total RNA was reverse transcribed using T7 promoter Primer and MMLV-RT. Then cDNA was converted to aRNA using T7 RNA polymerase, which simultaneously amplifies target material and incorporates cyanine 3-labeled CTP. Subsequently, samples were hybridized to Whole Human Genome Microarray 4 x 44K (G4112F, Agilent Technologies). 1.65 mg of Cy3 labeled aRNA were hybridized for 17 hours at 65ºC in a hybridization oven (G2545A, Agilent) set to 10 rpm in a final concentration of 1X GEx Hybridization Buffer HI-RPM, according to manufacturer's instructions (One-Color Microarray-Based Gene Expression Analysis, Agilent Technologies). Arrays were washed according to manufacturer's instructions (One-Color Microarray-Based Gene Expression Analysis, Agilent Technologies) and were dried out using a centrifuge. Finally, Arrays were scanned at 5mm resolution on an Agilent DNA Microarray Scanner (G2565BA, Agilent Technologies) using the default settings for 4x44k format one-color arrays and the images provided by the scanner were analyzed using Feature Extraction software (Agilent Technologies).

mRNA array data were normalized using quantiles normalization(2) while miRNA array data were normalized using the VSN method(3) which preserves the biological characteristics of the data while stabilizing the variance across the entire intensity range. Quality control was based on statistical outlier criteria implemented in the Bioconductor package ArrayQualityMetrics; there were no statistical outliers in terms of MA plots, dendrogram or boxplots. After normalization, only those probes present in at least one sample from the microRNA-microarrays and at least 50% of all the samples from the mRNA-microarrays were considered for further analysis. Fifteen different bioinformatics algorithms were developed from GeneCard (www.genecards.org), miRBase (www.mirbase.org), mirwalk (www.umm.uni-heidelberg.de/apps/zmf/mirwalk) and Web gestalt (www.bioinfo.vanderbilt.edu/webgestalt) databases for studying the “in silico” complementarity between miRNAs/mRNA (4-6). (GEO reference: GSE84201. Referees accession link upon request).

Epigenetic validation: CpG island identification, DNA extraction, bisulfite modification, bisulfite sequencing and methylation-specific PCR

The occurrence of CpG islands (CGIs) encompassing microRNA genes or being located nearby (2000 bp 5-upstream) was assessed using various CGI-revealing programs; we first used CGI Searcher, (http://bioinfo.itb.cnr.it/cgi-bin/wwwcpg.pl) under Takai and Jones parameters: GC ≥55%; Obs/Exp ≥65; and length ≥200 bp, because these situations exclude most of the Alu- repetitive elements (7, 8). To confirm the CGI position, we used ENCODE annotation data (http://www.genome.ucsc.edu/index.html). CGIs containing repetitive elements were detected using the RepeatMasker Web Server (http://www.repeatmasker.org) and then excluded from the study. The possible gene in which the miRNA was encoded was also analyzed, followed by an analysis of the presence of 5´ CGIs located in the transcriptional site and at least 1000 bp upstream. For BS, primers were designed, when possible, to exclude binding to any CpG dinucleotide to ensure amplification of either methylated or unmethylated sequences (Supplementary Table 3). PCR reactions were used for cell lines and control studies, and performed under the following conditions: (a) 1 cycle of 95°C for 5 min; (b) 40–42 cycles of 95°C for 1 min, 56°–62°C for 1 min, 72°C for 1 min; (c) an extension of 8 min at 72°C. The PCR products were run on a 1.5% agarose gel, using the 100 bp Molecular size Marker (Invitrogen, USA) for appropriate identification of band size, then cut and cleaned by the MinElute gel extraction kit (Qiagen, USA) and direct sequencing was performed on all the genes. For MSP, primers were design to bind specifically to methylated or unmethylated modified DNA (Supplementary Table 4). PCR reactions were used for the primary tumor and control samples and performed for 35 cycles at 95°C denaturing, 57–59°C annealing and 72°C extension with a final extension step of 5 minutes. Each set of DNAs modified and PCR amplified, includes lymphocyte DNA from healthy donors as a negative control, and as a positive control DNA *in vitro* methylated with *Sss* I methylase (IVD) (New England Biolabs, USA). Water with no DNA template was used as a control for contamination. After PCR, samples were run on a 6% nondenaturing acrylamide gel with 10 bp Molecular size marker (Invitrogen, USA), and the presence or absence of a PCR product was analyzed. For Image acquisition we used the Vision-Capt Software v16.11a.

References

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Supplementary Figures and Tables

Name of Cell line	Cancer type	Testing Date											REF	Match/Not Match
Sample			*M musculus*	D5S818	D13S317	D7S820	D16S539	VWA	TH01	AMEL	TPOX	CSF1PO	D21S11		Match/Not Match
REF NCI-H23			Negative	12,13	12	9,10	11	16,17	6	X	8,9	10		ATCC ® CRL-5800	
H23	Lung	06/22/2016	********	12,13	12	9,10	11	16,17	6	X	8,9	10	*30*		Match
REF NCI-H460			Negative	9,10	13	9,12	9	17	9.3	X,Y	8	11,12		ATCC ® HTB-177	
H460	Lung	06/22/2016	********	9,10	13	9,12	9	17	9.3	X,Y	8	11,12	*30*		Match
REF A2780			Negative	11,12	12,13	10	11,13	15,16	6	X	8,10	10,11	*27,28*	SIGMA	
A2780	Ovary	06/22/2016	********	11,12	12,13	10	11,12,13	15,16	6	X	8,10	10,11	*28*		Match
REF OVCAR3			Negative	11,12	12	10	12	17	9,9,3	X	8	11,12		ATCC ® HTB-161	
OVCAR3	Ovary	06/22/2016	********	11,12	12	10	12	17	9,9,3	X	8	11,12	*29,31,2*		Match
REF PC3			Negative	13	11	8,11	11	17	6,7	X	8,9	11	*29,31,2*	ATCC ® CRL-1435	
PC3	Prostate	06/22/2016	********	13	11	8,12	11	17	6,8	X	8,10	12	*29,31,2*		Match
REF LNCAP			Negative	11,12	10,12	9.1,10.3	11	16,18	9	X,Y	8,9	10,11		ATCC ® CRL-1740	
LNCAP	Prostate	06/22/2016	********	11,12	10,12	9.1,10.4	11	16,18	9	X,Y	8,9	10,11	*29,31,2*		Match
REF H727			Negative	11,12	11	8,10	11,13	14,15	8	X	8	11,12		ATCC ® CRL-5815	
H727	Lung	06/22/2016	********	11,12	11	8,10	11,13	14,15	8	X	8	11,12	*29,32,2*		Match
REF HT29			Negative	11,12	11,12	10	11,12	17,19	6,9	X	8,9	11,12	*29,30*	ATCC ® HTB-38	
HT29	Colorectal	06/22/2016	********	11,12	11	10	11,12	17	6,9	X	8,9	11,12	*29*		Match
REF A549			Negative	11	11	8,11	11,12	14	8,9.3	X,Y	8,11	10,12	*29*	ATCC ® CCL-185	
A549	Lung	06/22/2016	********	11	11	8,11	11,12	14	8,9.3	X,Y	8,11	10,12	*29*		Match
REF BT474			Negative	11,13	11	9,12	9,11	15,16	7	X	8	10,11	*28,32.2*	ATCC® HTB-20	
BT474	Mama	06/22/2016	********	11,13	11	9,12	9,11	15,16	7	X	8	10,11	*28,32.2*		Match
REF LoVo			Negative	11,12,13	8,11	9.3,10,11	9,12	17,18	9.3	X,Y	8,9	11,13,14		ATCC® CCL-229	
LoVo	Colorectal	06/22/2016	********	11,13	8,11	10,11	9,12	17,18	9.3	X,Y	8,9	10,11,13,14	*29,31,2*		Match
REF IMIM PC2			Negative											Dr. F.X. Real (#)	No Match neither in ATCC nor the DSMZ
IMIM PC2	Pancreas	06/22/2016	********	12	11	8,1	11	14,16	7,9	X	11	11	*29,30*		
REF SKOV3			Negative	11	8, 1	13, 14	12	17, 18	9, 9.3	X	8, 11	11		ATCC® HTB-77	
SKOV3	Ovary	06/29/2016	********	11	8, 1	13, 14	12	17, 18	9, 9.3	X	8, 11	11	*30,31.2*		Match
REF SW780			Negative	11,12	11,12	9,10	9,11	16,19	6	X	8	10,11	*29,30*	ATCC ® CRL-2169	
SW780	Bladder	06/22/2016	********	11,12	11,12	9,10	9,11	16,19	6	X	8	10,11	*29,30*		Match
REF IMR90			Negative	12,13	11,13	9,12	10,13	16,19	8,9.3	X	8,9	11,13	30.2, 31	ATCC ® CRL-186	
IMR90	Lung fibroblast	19/07/2016	********	12,13	11,13	9,12	10,13	16,19	8,9.3	X	8,9	11,13	*30.2, 31*		Match

Supplementary Table 1. Cell Authentication. Genomics Core Facility. IIBm CSIC-UAM. (Each cell line individual report, with registered number, is available upon request).

Method:

STR amplification kit	GenePrintR 10 System (Promega)
STR profile analysis software	GeneMapper® v3.7 (LifeTechnologies)
Genomic Analyzer System	ABI 3130 XL (Applied Biosystems)
DNA source	Cultured cells; cultured cells pellet
DNA isolation method	DNeasy blood and tissue kit (Qiagen)
DNA quantification method	Qubit 2.0 Fluorometer (Life Technologies)
Amount of DNA/amplification	4 ng

The GenePrint® 10 System allows co-amplification and three-color detection of ten human loci: TH01, TPOX, vWA, Amelogenin, CSF1PO, D16S539, D7S820, D13S317, D21S11 and D5S818. These loci collectively provide a genetic profile with a random match probability of 1 in 2.92 × 109 and are used for human cell line and tissue authentication and identification and human cell line cross-contamination determination. STRs profiles are sent for comparison against cell line date bases like ATCC (American Type Culture Collection), DSMZ (Deutsche Sammlung von Mikrorganismen and Zellkulturen). # Vila MR, Lloreta J, Schussler MH, Berrozpe G, Welt S, and Real FX. New pancreas cancers cell lines that represent distinct stages of ductal differentiation. Lab Invest. 1995; 72(4):395-404.

miRNA Name	Accession Number	Statistical contrast	FDR RvsS	FDR RTvsR	Assay Number	Validation confirmed	CpG Island position/ Methylation status
hsa-miR-7-5p	MIMAT0000252	Lung & Ovarian	0.0000	0.0000	268	H23/A2780	Intergenic/ M
hsa-miR-132-3p	MIMAT0000426	Lung & Ovarian	0.0207	0.0000	457	H23/A2780	Intergenic/ U
hsa-miR-335-5p	MIMAT0000765	Lung & Ovarian	0.0159	0.0850	546	H23/H460	Gene promoter/M
hsa-miR-148a-3p	MIMAT0000243	Lung	0.0017	0.1007	470	H23/H460	Intergenic/U
hsa-miR-10a-5p	MIMAT0000253	Lung & Ovarian	0.0159	0.0761	387	A2780	Gene promoter
hsa-miR-124-3p	MIMAT0000422	Lung & Ovarian	0.0442	0.0000	1182	H23	Intergenic
hsa-miR-9-5p	MIMAT0000441	Lung	0.0067	0.0112	583	H23	Intergenic

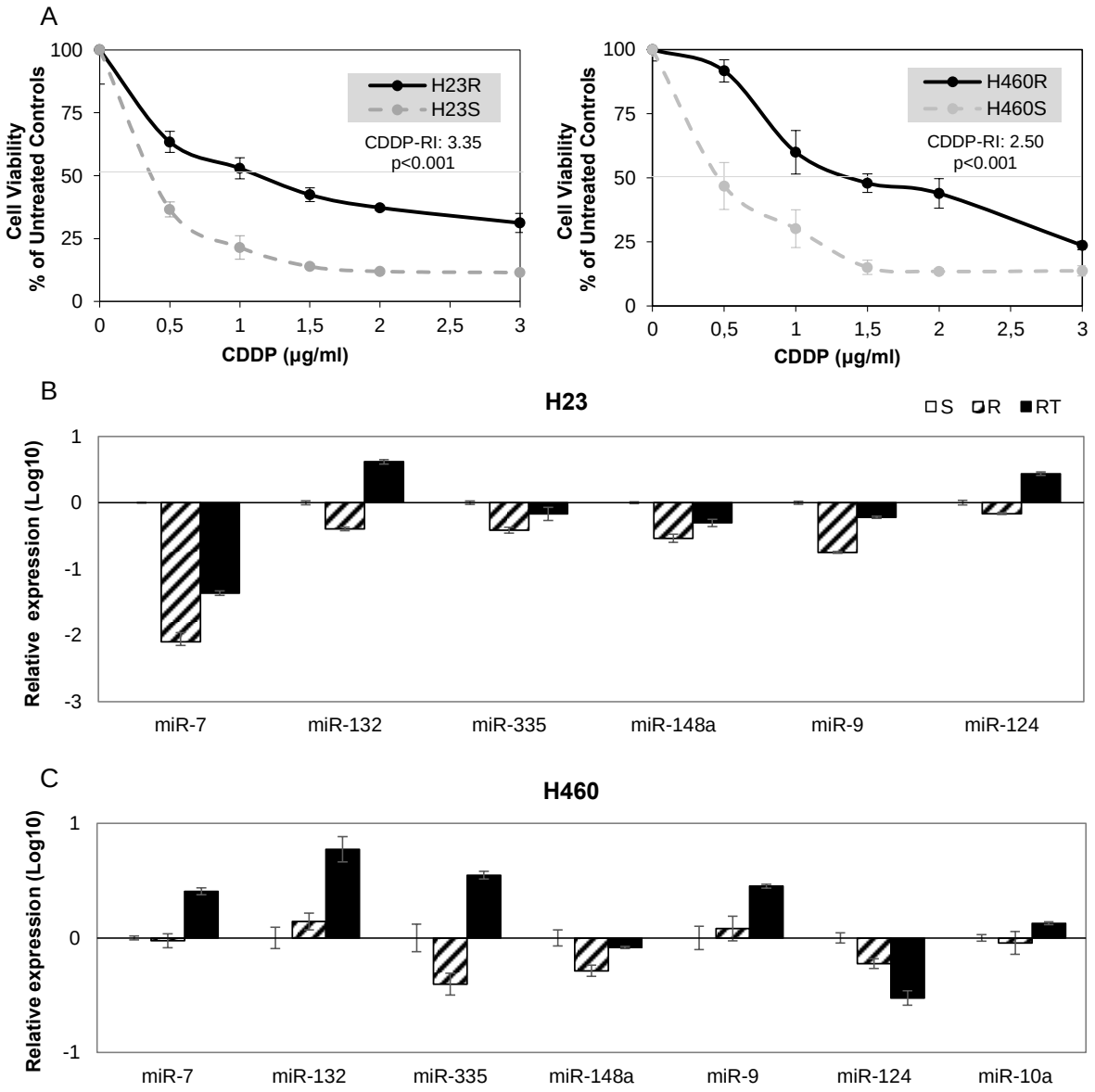
Supplementary Table 2. Baseline and statistical characteristics associated with the selected miRNAs. The table shows the list of miRNAs with accession number, which were selected following the cutoff indicated in the FDR columns according to the statistical analysis performed in two contrasts: for both tumor types (lung and ovarian) or for each tissue origin (lung or ovarian). The assay numbers used for the qRT-PCR validation and the cell lines in which the changes in the expression were confirmed are also indicated. Finally, the CGI positions were obtained from CGI Searcher (http://cpgislands.usc.edu) and the ENCODE annotation data (http://www.genome.ucsc.edu/index.html). M: Methylated; U: Unmethylated.

PRIMERS		miR-7-3	miR-132	miR-335	miR-148	C19MC
Bisulfite Sequencing	SENSE	5-TTAGGAAGAAGTTAGGAGGGGAAA-3	5-GTTTTAGGTTTTTAYGGGAGTTTA-3	5-TGGGAAAGAGGAGGTGAGAAA-3	5-GTYGTTTTTTTTTTAGTTAGGAGATA-3	5-GTAAGGTTGGTTTTTTTATTTGTAAA-3
	ANTISENSE	5-CAAACACCTCAAACCACCCTCT-3	5-TACRACCRCRACTCCTACACACT-3	5-CGCTTCCTAAAACCAAAAATTCT-3	5-CCRCTCCCTTCCATCTTAACT-3	5-ATTCCAATTAAACAAATTCTAATCCCT-3
Size		372bp	441bp	528bp	560bp	394bp
Bisulfite Sequencing	SENSE	5-TAGTTGGGGAAGTTTTTTTTAGGA-3	5-AGGAGTYGYGGTYGTATGAATGA-3			
	ANTISENSE	5-TTTCCCCTCCTAACTTCTTCCT-3	5-GTCTCCTAAAACRCCAACACCTT-3			
Size		428bp	441bp			
MSP Unmethylated	SENSE	5-GGGGTGGGGTTTTTTAAGAATT-3				
	ANTISENSE	5-ATAACATTCTCCTCCTTCAATCA -3				
Size		131bp				
MSP Methylated	SENSE	5-GGGTGGGGTTTTTTAAGAATC -3				
	ANTISENSE	5-ACATTCTCCTCCTTCGATCG-3				
Size		127bp				

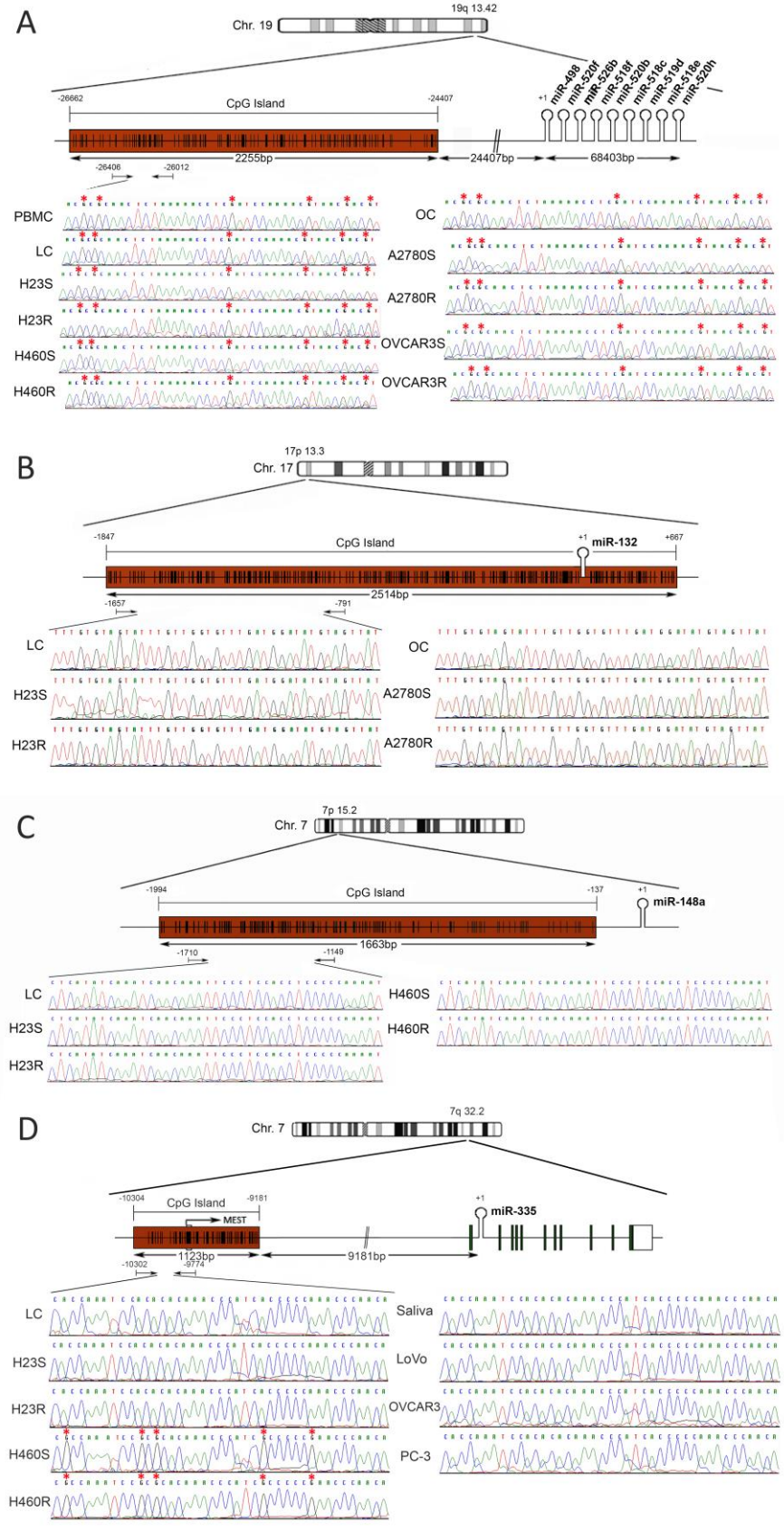
Supplementary Table 3. Bisulfite sequencing and methylation specific PCR (MSP) primers designed for the study of miR-7, -132, -335, -148a and the C19MC cluster selected in this study, as well as the amplicon size for each PCR reaction.

miRNAS in at least two cell lines (*P*<0.05)	miRNAs that match both conditions (RvsS < 0, RTvsR >0 and *FDR* < 0.1)	miRNAs that show a regulatory CpG island	miRNAs with candidate target genes involved in tumor progression
87	28	10	7
hsa-miR-10a	hsa-miR-10a	hsa-miR-10a	hsa-miR-10a
hsa-miR-124	hsa-miR-124	hsa-miR-124	hsa-miR-124
hsa-miR-132	hsa-miR-132	hsa-miR-132	hsa-miR-132
hsa-miR-148a	hsa-miR-148a	hsa-miR-148a	hsa-miR-148a
hsa-miR-335	hsa-miR-335	hsa-miR-335	hsa-miR-335
hsa-miR-7	hsa-miR-7	hsa-miR-7	hsa-miR-7
hsa-miR-9	hsa-miR-9	hsa-miR-9	hsa-miR-9
hsa-miR-498	hsa-miR-212	hsa-miR-212	
hsa-miR-518c	hsa-miR-363	Hsa-miR-363	
hsa-miR-518e	hsa-miR-95	Hsa-miR-95	
hsa-miR-518f	hsa-miR-132*		
hsa-miR-519d	hsa-miR-140-5p		
hsa-miR-520b	hsa-miR-146b-5p		
hsa-miR-520f	hsa-miR-150*		
hsa-miR-520h	hsa-miR-154*		
hsa-miR-526b	hsa-miR-192		
hsa-miR-1225-5p	hsa-miR-195		
hsa-miR-132*	hsa-miR-199a-3p		
hsa-miR-134	hsa-miR-21*		
hsa-miR-135a*	hsa-miR-215		
hsa-miR-139-5p	hsa-miR-222		
hsa-miR-146b-5p	hsa-miR-301b		
hsa-miR-149	hsa-miR-338-3p		
hsa-miR-150*	hsa-miR-379		
hsa-miR-185	hsa-miR-449a		
hsa-miR-188-5p	hsa-miR-497		
hsa-miR-198	hsa-miR-630		
hsa-miR-199a-3p	hsa-miR-9*		
hsa-miR-199b-5p			
hsa-miR-200c			
hsa-miR-203			
hsa-miR-212			
hsa-miR-215			
hsa-miR-22			
hsa-miR-221			
hsa-miR-222			
hsa-miR-23b			
hsa-miR-30a			
hsa-miR-30c-1*			
hsa-miR-31			
hsa-miR-31*			
hsa-miR-320a			
hsa-miR-324-5p			
hsa-miR-340			
hsa-miR-34b*			
hsa-miR-371-3p			
hsa-miR-371-5p			
hsa-miR-372			
hsa-miR-373			
hsa-miR-376a			
hsa-miR-379			
hsa-miR-425			
hsa-miR-451			
hsa-miR-454			
hsa-miR-483-5p			
hsa-miR-503			
hsa-miR-512-3p			
hsa-miR-512-5p			
hsa-miR-513c			
hsa-miR-515-3p			
hsa-miR-515-5p			
hsa-miR-516b			
hsa-miR-517b			
hsa-miR-517c			
hsa-miR-518f*			
hsa-miR-519c-3p			
hsa-miR-520a-5p			
hsa-miR-520c-3p			
hsa-miR-520d-3p			
hsa-miR-523			
hsa-miR-524-5p			
hsa-miR-525-5p			
hsa-miR-548c-3p			
hsa-miR-574-5p			
hsa-miR-625			
hsa-miR-629*			
hsa-miR-630			
hsa-miR-658			
hsa-miR-660			
hsa-miR-663			
hsa-miR-758			
hsa-miR-765			
hsa-miR-886-3p			
hsa-miR-9*			
hsa-miR-940			
hsa-miR-95			
hsa-miR-98			

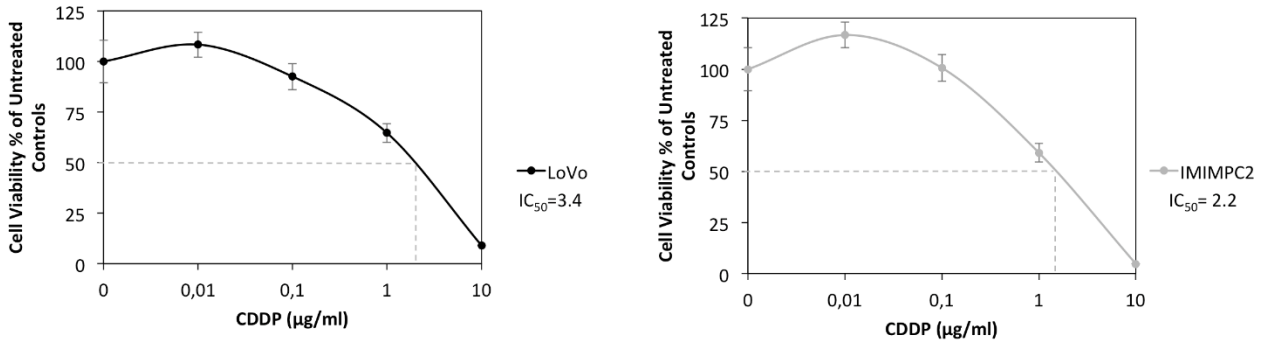
Supplementary Table 4. miRNAs selection. Note: yellow, final miRNAs selected; orange, miRNAs included in the C19MC cluster



Supplementary Figure 1: (A) Effect of cisplatin on cell viability. Viability curves showing the acquired resistance of H23 and H460 cell lines; Cells were exposed for 72 h to each drug concentration. Data were normalized to the untreated control, which was set at 100% and represent the mean + SD of at least 3 independent experiments performed in quadruplicate at each drug concentration tested for every one cell analyzed. IC50, is the inhibitory concentration that kills 50% of the cell population. Resistant index (RI) calculated as IC50 resistant / IC50 sensitive cell line. $p<0.001$ was considered as a significant change in drug sensitivity (Student's t-test). (B-C) Relative expression levels of the selected miRNAs measured by qRT-PCR. Data are represented in log10 scale and are expressed using the corresponding sensitive (S) line as a calibrator. Each miRNA level was normalized to RNU48 as an endogenous control. Assays were made in the lung cancer cell lines H23 (B) and H460 (C) in all experimental conditions: S, R and RT. S: sensitive; R: resistant; RT: resistant treated with epigenetic reactivation drugs (5-Aza and TSA). The expression number assays for each miRNA are indicated in Supplementary Table 2.



Supplementary Figure 2. Bisulfite sequencing (BS) of the miRNA potential regulatory CGIs. The figure shows the schemas of chromosome CGIs and miRNA location as well as representative images of corresponding BS. CGIs are represented in red boxes; each CpG position is represented by vertical black lines inside the boxes. The first nucleotide of each miRNA is indicated by +1. Facing arrows mark the primers position. Asterisks indicate methylated positions. LC: lung control; OC: ovary Control (A) Analysis of the potential regulatory C19MC cluster's CGI. All the samples were fully methylated in all tested CpG positions. (B) The miR-132 CGI was unmethylated in all samples tested, with the presence of a T instead of a C previous to G. (C) The CGI located near miR-148a was also unmethylated in all analyzed positions. (D) The miR-335 CGI was methylated in H460 cells.



Supplementary Figure 3. Effect of cisplatin on cell viability. Viability curves showing the acquired resistance of LoVo and IMIM-PC2 cell lines; Cells were exposed for 72 h to each drug concentration. Data were normalized to the untreated control, which was set at 100% and represent the mean + SD of at least 3 independent experiments performed in quadruplicate at each drug concentration tested for every one cell analyzed. IC50, is the inhibitory concentration that kills 50% of the cell population

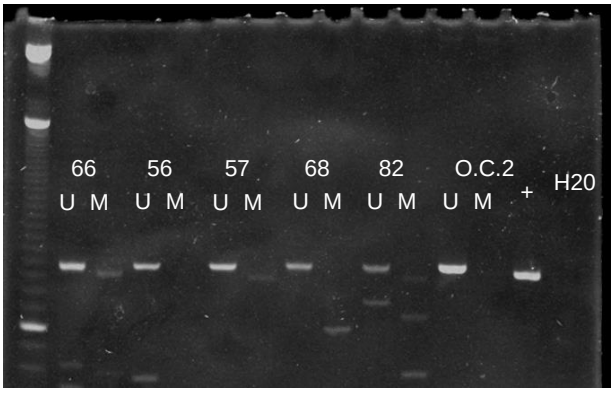
Characteristics		Complete Series (n=55)		Unmethylated (n=35)		Methylated (n=20)		*p*
		No. of Patients	%	No. of Patients	%	No. of Patients	%	
Type								0.520
	Adenocarcinoma	23	41.8	15	42.9	8	40.0	
	Carcinoma	19	34.5	10	28.6	9	45.0	
	Cystadenocarcinoma	12	21.8	9	25.7	3	15.0	
	Undetermined	1	1.8	1	2.9	0	0.0	
Tumor Grade								0.483
	I	5	9.1	2	5.7	3	15.0	
	II	13	23.6	9	25.7	4	20.0	
	III	13	23.6	7	20.0	6	30.0	
	Undetermined	24	43.6	17	48.6	7	35.0	
Histology								0.125
	Serous	30	54.5	19	54.3	11	55.0	
	Mucinous	3	5.5	1	2.9	2	10.0	
	Endometrioid	8	14.5	7	20.0	1	5.0	
	Clear Cell	3	5.5	1	2.9	2	10.0	
	Other	11	20.0	7	20.0	4	20.0	
Chemotherapy								0.474
	Platinum+Taxane	37	67.3	23	65.7	14	70.0	
	Platinum+CTX	8	14.5	7	20.0	1	5.0	
	Platinum	3	5.5	1	2.9	2	10.0	
	Other	3	5.5	2	5.7	1	5.0	
	No	4	7.3	2	5.7	2	10.0	
Platinum Sensitivity								0.625
	Sensitive	20	36.4	12	34.3	8	40.0	
	Refractory/Resistant	26	47.3	16	45.7	10	50.0	
	Undetermined	9	16.4	7	20.0	2	10.0	
Relapse								0.436
	No	17	30.9	13	37.1	4	20.0	
	Yes	29	52.7	19	54.3	10	50.0	
Death					0.0		0.0	0.438
	No	23	41.8	16	45.7	7	35.0	
	Yes	32	58.2	19	54.3	13	65.0	

Supplementary Table 5. Clinicopathological characteristics in 55 patients from IDIS-CHUS/HULP biobank with ovarian cancer.

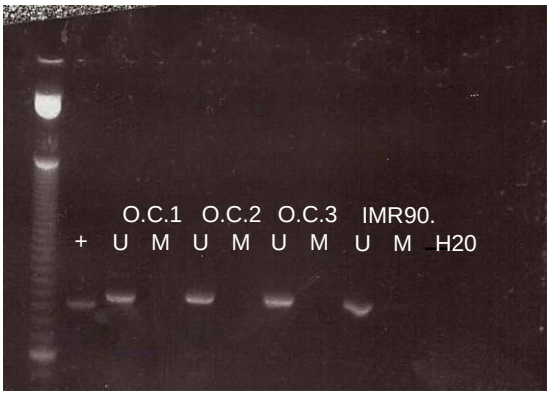
	Complete Series (n=22)		Unmethylated (n=11)		Methylated (n=11)		
CNIO biobank	No. of Patients	%	No. of Patients	%	No. of Patients	%	*P*
Age (mean)	58.6		55.1		62.1		0.236
Familial Type							
Familial	7	31.2	5	45.5	2	18.2	0.360
Sporadic	15	68.2	6	54.5	9	81.8	
Grade							
I	3	13.6	1	9.1	2	18.2	0.070
II	4	18.2	0	0.0	4	36.4	
III	9	40.9	6	54.5	3	27.3	
IV	3	13.6	3	27.3	0	0.0	
Undetermined	3	13.6	1	9.1	2	18.2	
Relapse							
No	7	31.8	3	27.3	4	36.4	0.990
Yes	15	68.2	8	72.7	7	63.6	
Death							
No	11	50.0	4	36.4	7	63.6	0.394
Yes	11	50.0	7	63.6	4	36.4	

Supplementary Table 6. Clinicopathological characteristics in 22 patients from National Cancer Research Center (CNIO) biobank with HGS ovarian cancer.

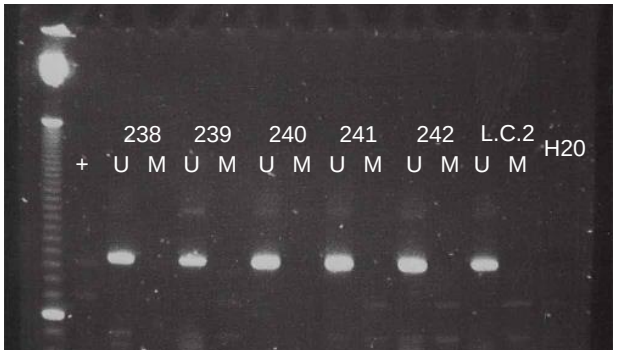
“Tumor ovarian tissues (167)”:



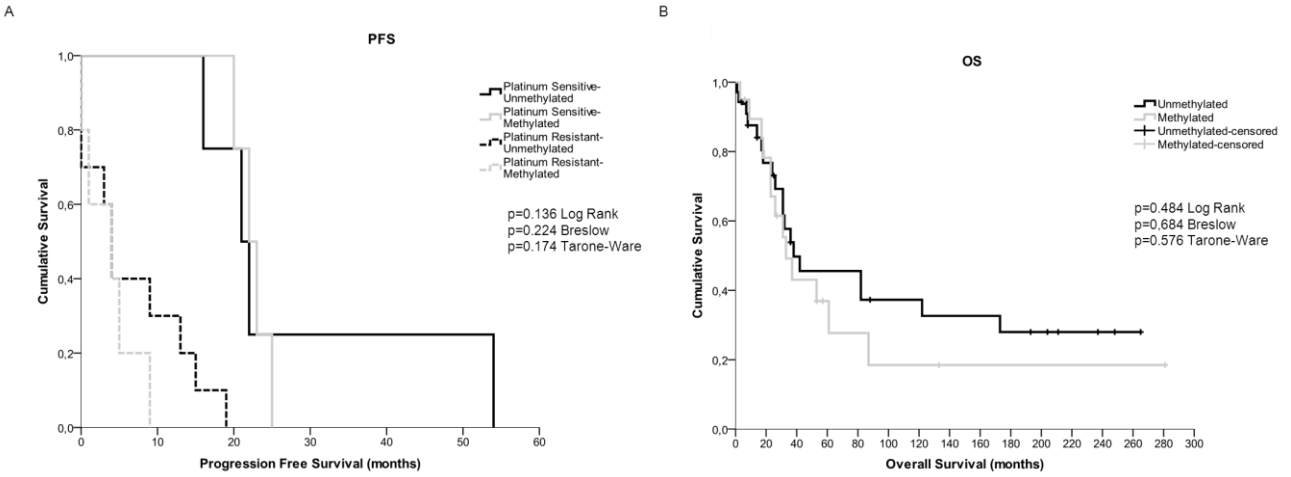
“Normal ovarian tissue (10) + IMR90 cell line :



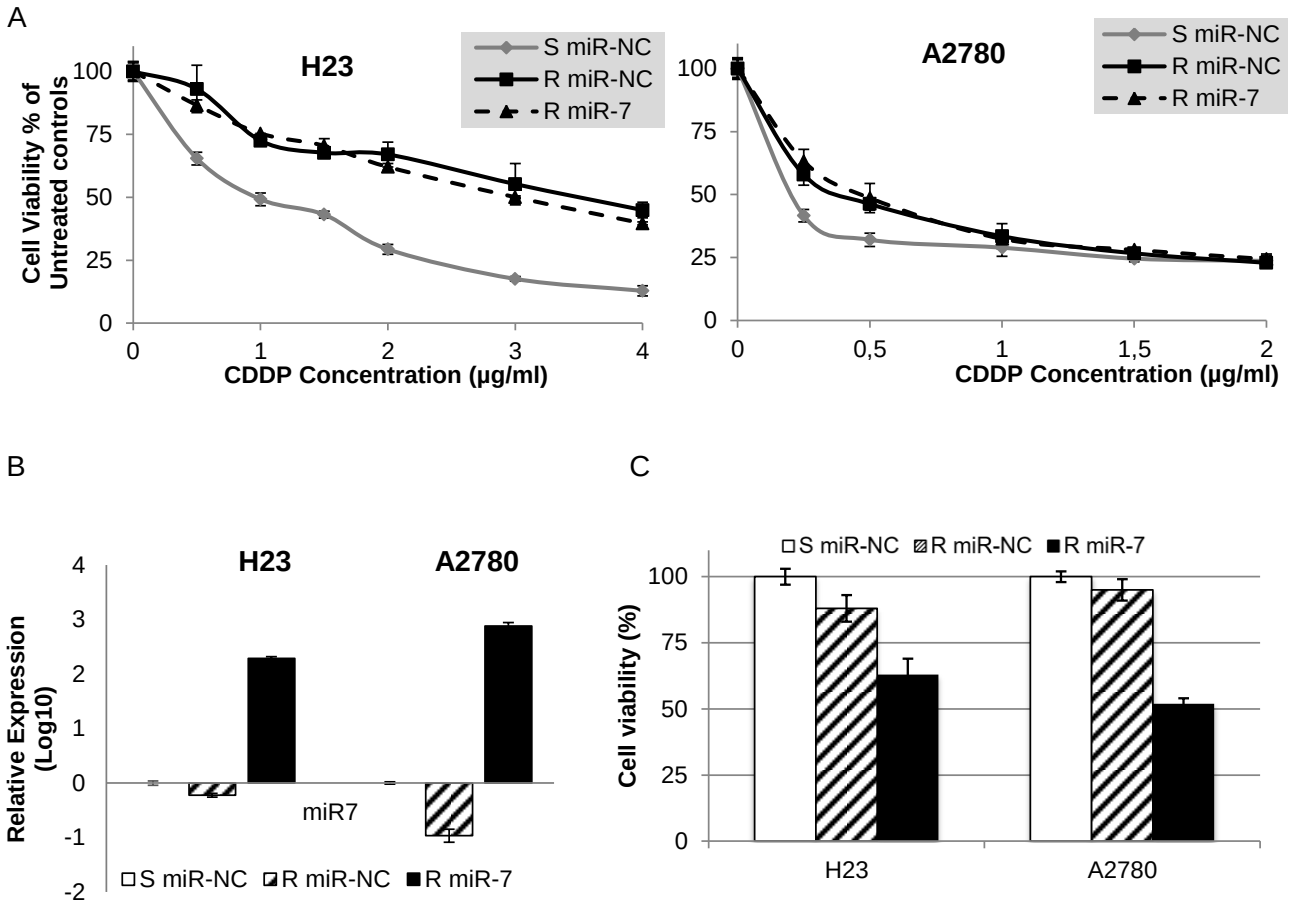
“PBMCs (10)”:



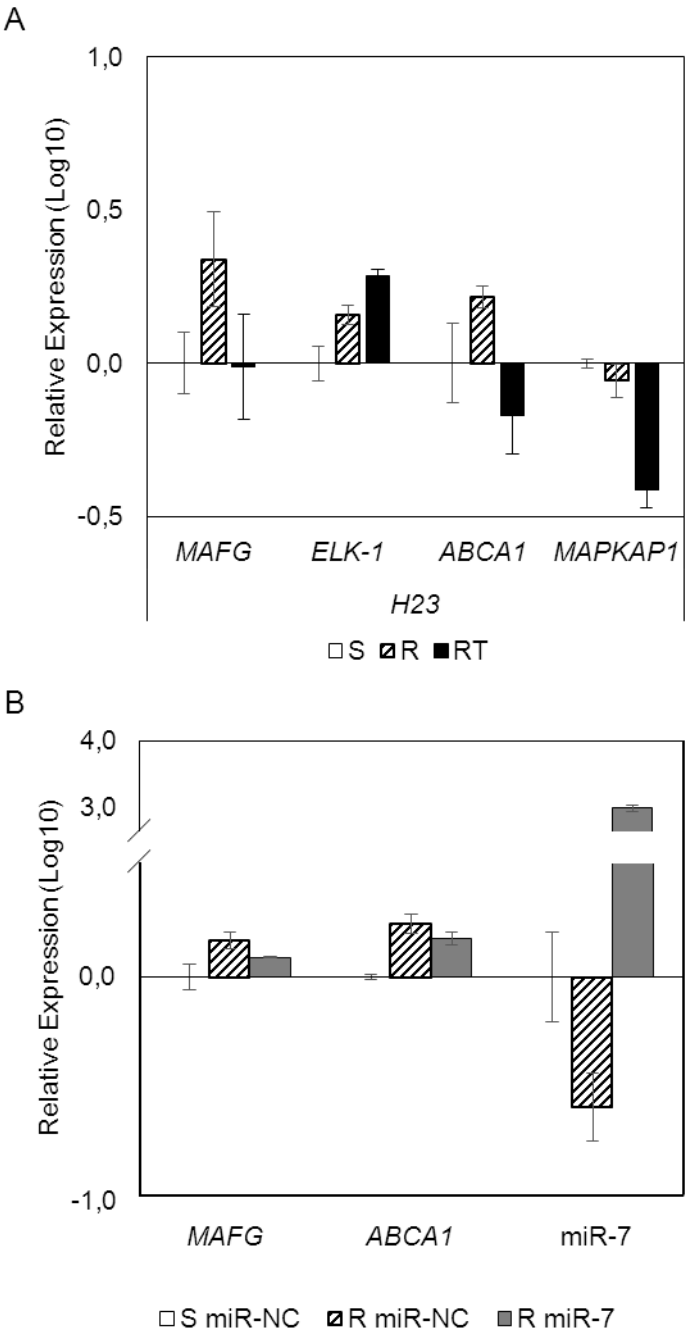
Supplementary Figure 4. Full unedited gels for Figure 4.



Supplementary Figure 5. Kaplan-Meier comparison between cisplatin treatment and miR-7 proximal island methylation in 55 ovarian cancer patients treated with platinum in terms of progression free survival (A) and overall survival in months (B). LogRank, Breslow and Tarone-Ware tests were used for comparisons and $p<0.05$ was considered as a significant change in OS or PFS. p values in (A) represent the significative difference between sensitive-unmethylated and sensitive-methylated patients



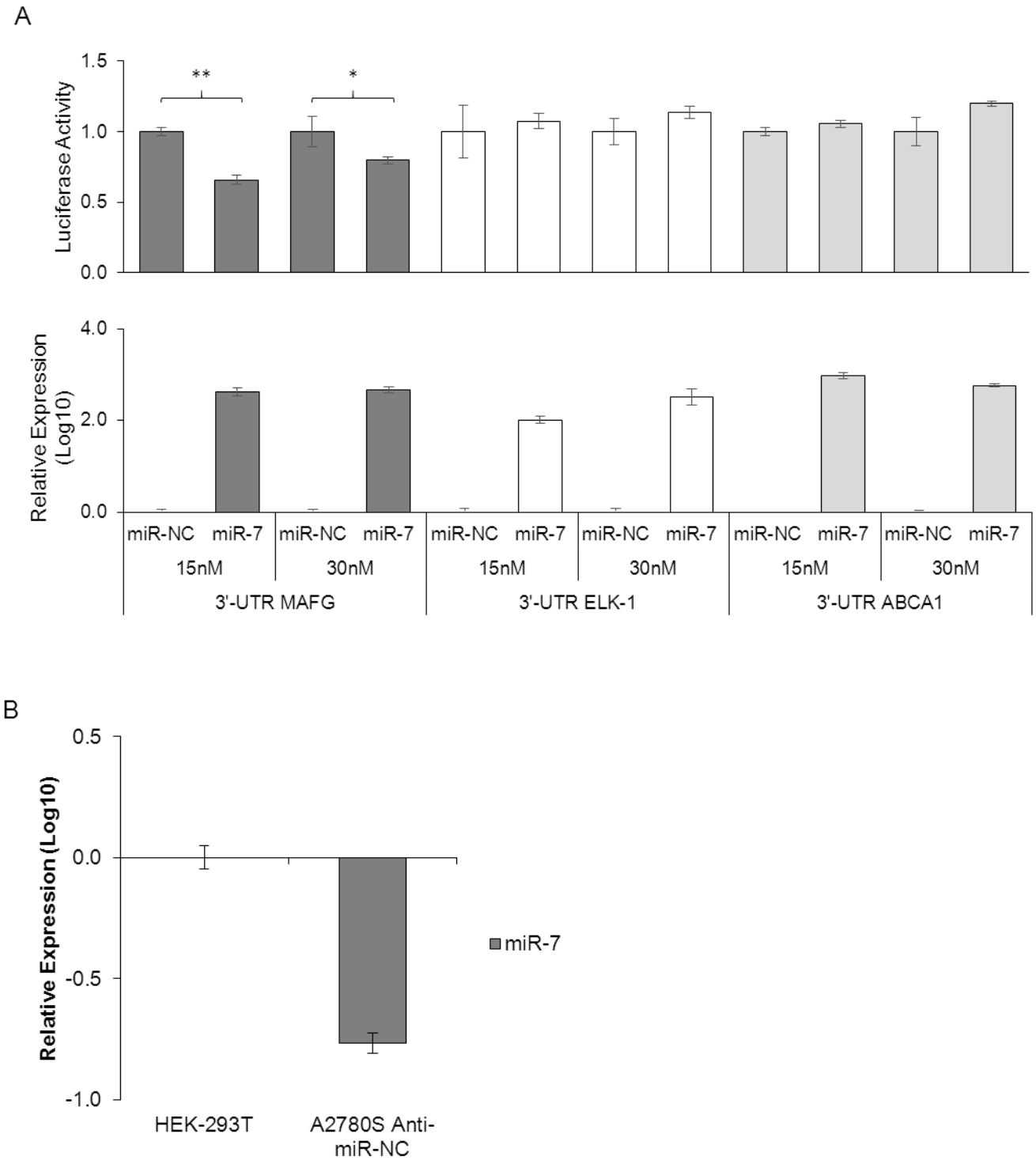
Supplementary Figure 6. Effect of miRNA-7 overexpression on cell viability at 40 and 50nM of microRNA precursor. (A) Cell viability assay on H23 and A2780 cell lines transfected with 40 nM of the negative control (white, S miR-Control; striped, R miR-Control) and overexpressing miR-7 precursor (black, R miR-7). Data were normalized to each untreated control and represent the mean ± SD of at least 3 independent experiments performed in quadruplicate for each cell line analyzed. (B) Relative miR-7 expression levels measured by qRT-PCR in the H23 and A2780 cell lines, represented in log10 scale. The resistant cell line transfected with the mimic negative control was used as a calibrator. (C) Cell viability assay on H23 and A2780 cell lines transfected with 50 nM of the negative control (white, S miR-Control; striped, R miR-Control) and overexpressing miR-7 precursor (black, R miR-7). Data were normalized to each sensitive subtype and represent the mean ± SD of at least 3 independent experiments performed in quadruplicate for each cell line analyzed.



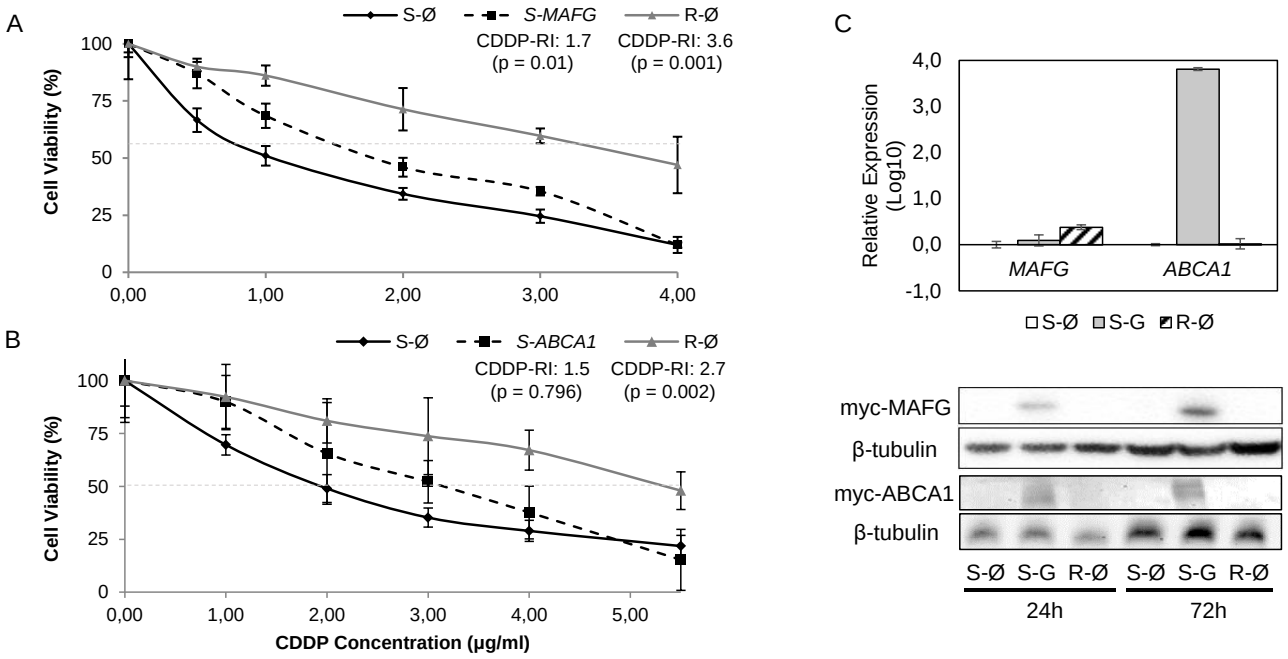
Supplementary Figure 7. mRNAarrays data validation and effect of miRNA-7 over-expression on candidate target genes in the lung cancer cell line H23. (A) Relative expression levels of the selected genes measured by qRT-PCR. Assays were made in all experimental conditions: S, R and RT. S: sensitive; R: resistant; RT: resistant treated with epigenetic reactivation drugs (5-Aza and TSA). Sensitive cells were used as calibrator. (B) Relative expression levels of *MAFG*, *ABCA1* and miR-7 measured by quantitative RT-PCR after miR-7 overexpression. The sensitive cell line transfected with the mimic negative control was used as a calibrator (S miR-NC, white). H23R cells were transfected with same negative control (R miR-NC, stripped) or with miR-7 precursor (R miR-7, grey). For both (A) and (B), data are represented in Log10 scale obtained from the combined relative expression of 2 independent experiments measured in triplicate. Each gene expression level was normalized to *GAPDH* or *B-actin* as an endogenous control.

Gene		*MAFG*	*ELK1*	*ABCA1*	*MAPKAP1*
Accession Number		NM_002359	NM_005229	NM_005502	NM_001006617
Name/Function		v-maf musculoaponeurotic fibrosarcoma	ETS domain-containing protein Elk-1	ATP-binding cassette transporter A1	Mitogen-Activated Protein Kinase Associated Protein 1
Correlation		-0.4266	-0.4545	-0.4615	-0.5175
miR-7 FDR		0.0845	0.0702	0.0669	0.0443
H23	Array	**X**	X	**X**	X
	qRT-PCR	**X**	-	**X**	-
	After miR-7 overexpression	**X**		**X**	
A2780	Array	**X**	**X**	X	X
	qRT-PCR	**X**	**X**	-	-
	After miR-7 overexpression	**X**	**X**		

Supplementary Table 7. Summary of the main characteristics of the candidate target genes and the followed selection steps. Note. In bold X are indicated the three validations needed as a criteria for inclusion for further analysis.



Supplementary Figure 8. Effect of miR-7 over its candidate target genes. (A) Co-transfection of mimic miR-7 (miR-7) or mimic control (miR-NC) with the 3' UTR of MAFG, ELK-1 and ABCA1. Two different concentrations were tested 15nM and 30nM. Data was analyzed after 24h of co-transfection. (Upper panel) Relative luciferase activity. The figures represent the mean ± SD of at least 3 independent experiments after data normalization with Renilla and the data from the negative control 3'-UTR. *p=0.004; **p=0.003 (Student's t-test) p<0.01 was considered as significant change in Luciferase activity. (Lower panel) relative miR-7 expression levels measured by qRT-PCR after co-transfection. Each bar represents the combined relative expression of 2 independent experiments measured in triplicate. The miR-NC co-transfected with the 3'-UTR of the candidate genes was used as calibrator. (B) Relative basal expression levels of miR-7 of A2780S cells compared with a normal HEK293T cells.



Supplementary Figure 9. Effect of overexpression of *MAFG* and *ABCA1* on cell sensitivity to CDDP in H23 cell line. (A-B) Viability curves of H23 cell line transfected with pCMV6 (S-Ø and R-Ø) and with the overexpression vectors (S-*MAFG* and S-*ABCA1*). Each experimental group was exposed for 48 h to 6 different test CDDP concentrations, and data were normalized to each untreated control, set to 100%. The data represent the mean ± SD of at least 3 independent experiments performed in quadruplicate at each drug concentration for each cell line analyzed. The CDDP-RI (Resistant Index to CDDP) was calculated as "IC50 from the R-Ø / IC50 from the S-Ø" and "IC50 from the S-transfected with the gene / IC50 from the S-Ø" ± SD. $p<0.01$ was considered as significant change in drug sensitivity (Student's t-test). (C) Validation of the transfection efficacy at mRNA and protein levels. Top, Relative expression levels of *MAFG* and *ABCA1* measured by quantitative RT-PCR, in the cell line H23 represented in Log10 scale; In each experimental group, the sensitive cell line transfected with pCMV6 plasmid was used as a calibrator. Each bar represents the combined relative expression of 2 independent experiments measured in triplicate. Bottom, total cell protein (20µg) at 24 and 72 hours was subjected to WB, membranes were hybridized with antibodies against c-Myc and β-tubulin as loading control. S: Sensitive; S-G: Sensitive transfected with the gene; R: Resistant; β-tub: β-tubulin.