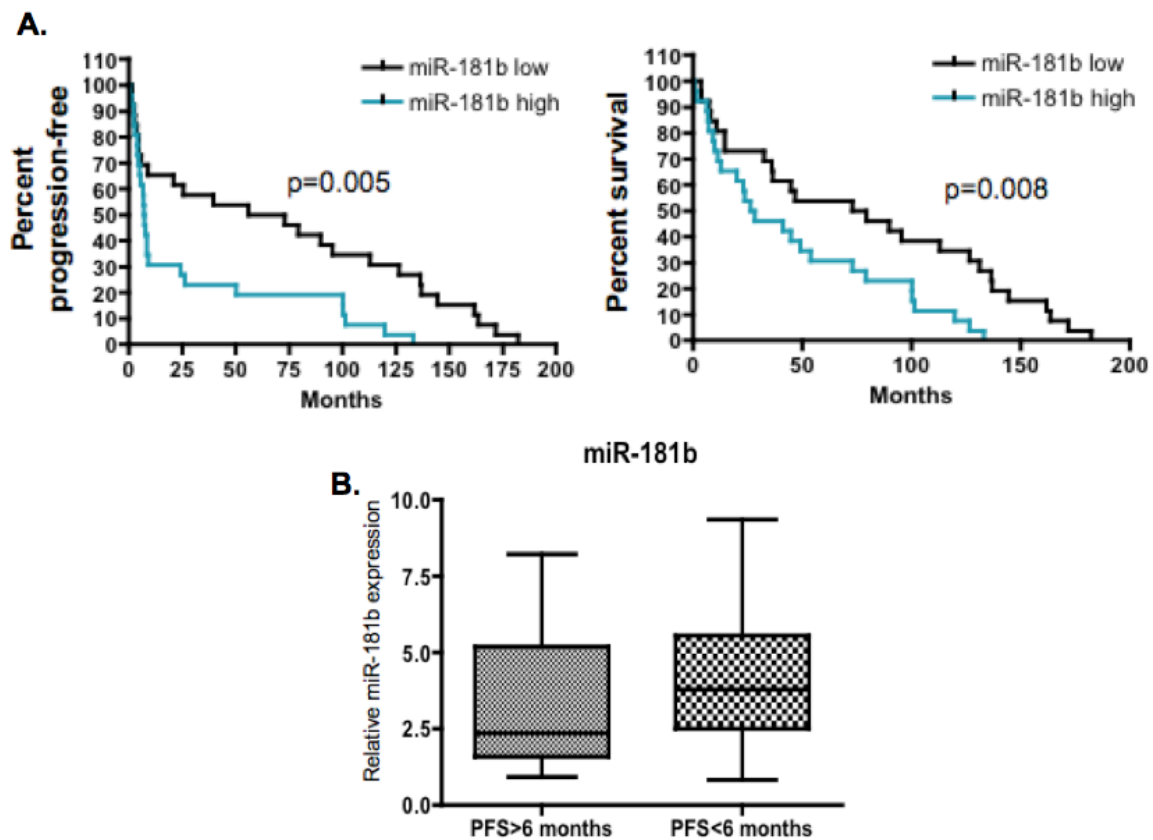
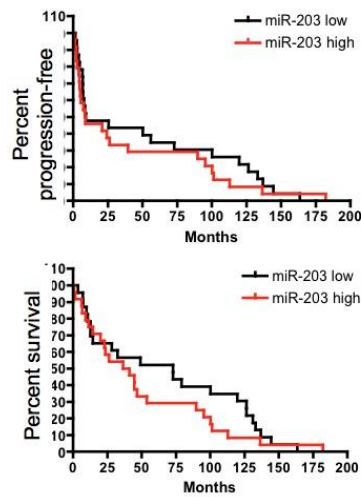


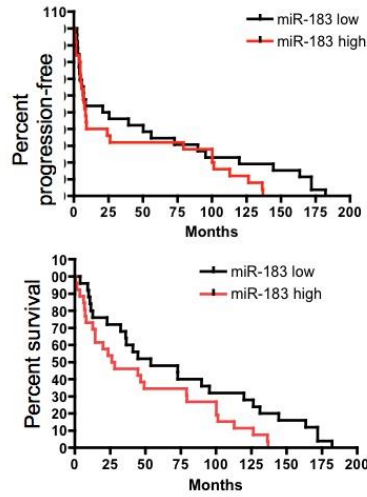
Supplementary Figure S1 Expression of miR-181b in EOC (A) Kaplan-Meier curves for progression-free survival (PFS) and overall survival (OS) in a cohort of patients (N=52) with stage III primary ovarian serous adenocarcinoma according to relative expression levels of miR-181b. Relative expression levels were dichotomized at the median. Both PFS and overall survival showed divergent Kaplan-Meier curves according to miR-181b status. P-values determined by Log rank statistical test. **(B)** miR-181b expression in patients with PFS<6 months (clinically described as platinum resistant) compared to patients with PFS>6 months (platinum sensitive), $P=0.19$, Two-tailed Student *t*-test). (Box and Whiskers plot: min to max) Error bars =s.d.



Supplementary Figure S2 miR-203 and miR-183 expressions in advanced staged epithelial ovarian cancer patient cohort. Kaplan-Meier survival curve generated from Taqman-based qRT-PCR analysis of miR-203 and -183 expressions in the same patient cohort described in Figure 1. (p-values were calculated using the Log Rank Mantel Cox test)



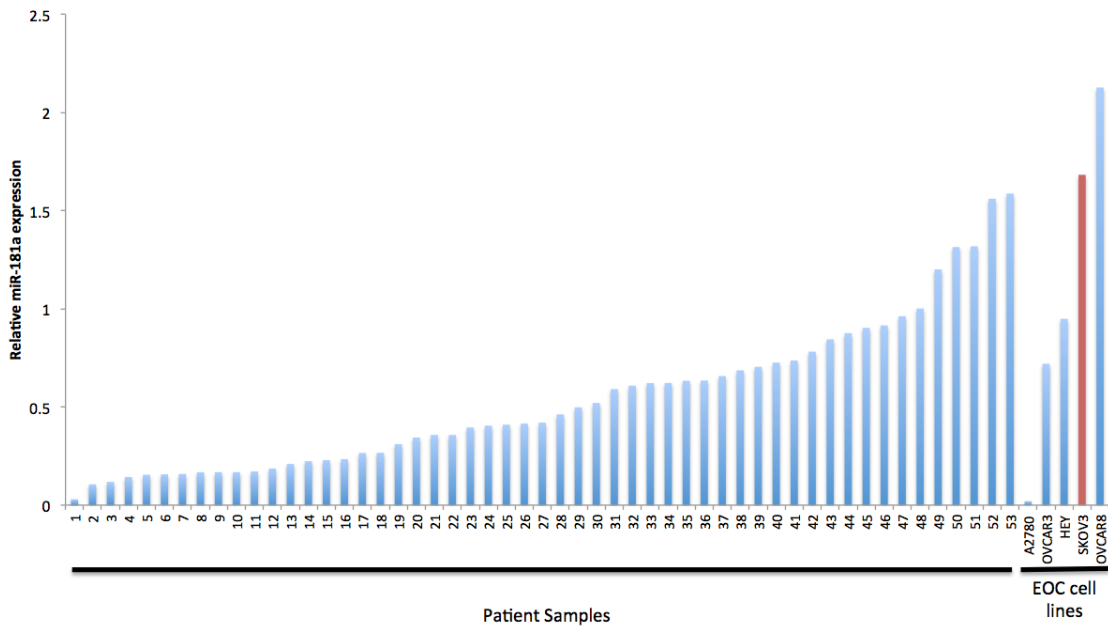
	miR-203 low	miR-203 high
Median PFI	9.0	8.7
P=0.48, OR 1.0; 95% CI: 0.5 to 1.6		
Median Survival	72.8	38.8
P=0.31, OR 1.9; 95% CI: 1.3 to 2.8		



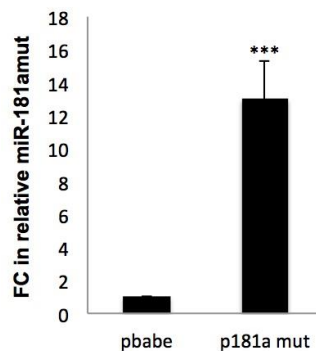
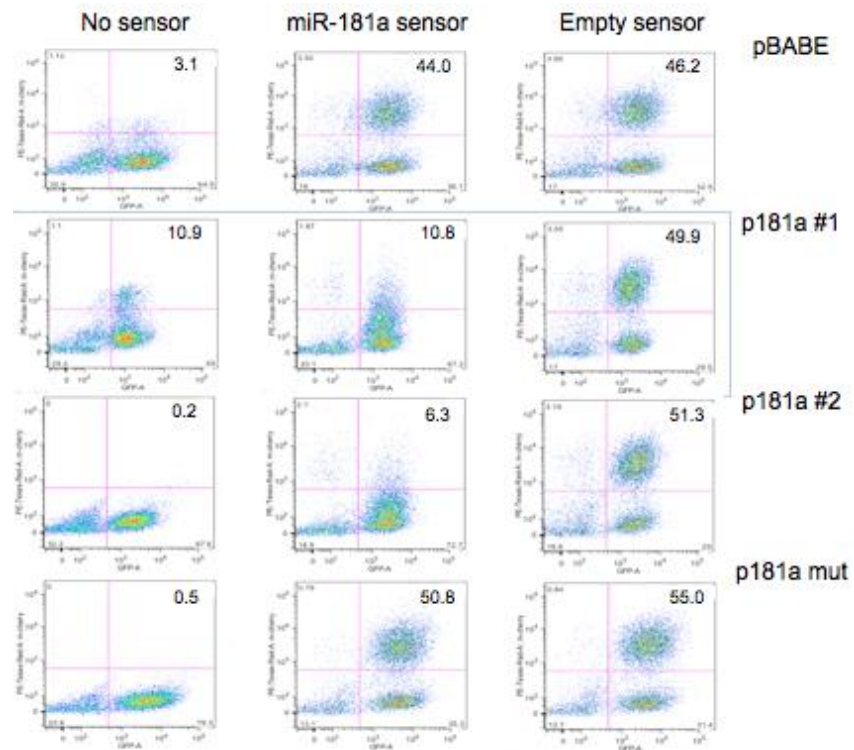
	miR-183 low	miR-183 high
Median PFI	23.2	8.4
P=0.23, OR 2.8; 95% CI: 2.2 to 3.3		
Median Survival	53.8	27.2
P=0.06, OR 2.0; 95% CI: 1.4 to 2.6		

Supplementary Figure S3 Relative expression of miR-181a in primary EOC tumors and cells lines.

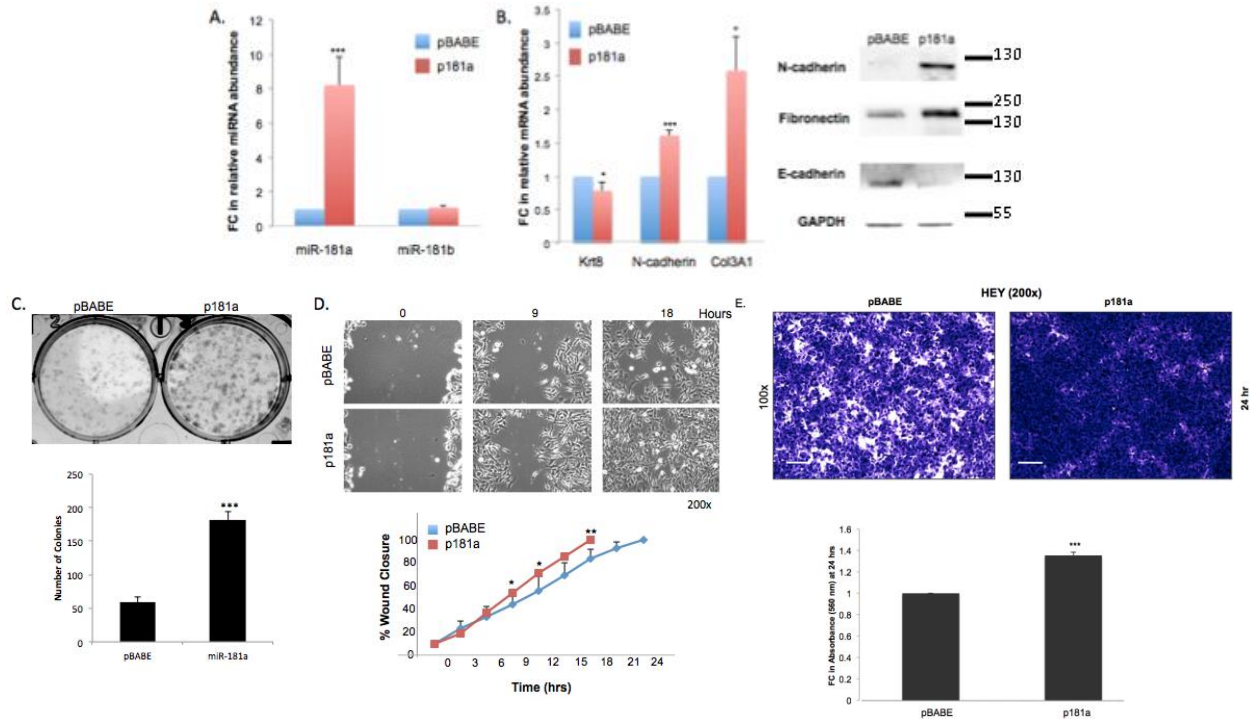
In order to determine which EOC cell lines to use for gain- and loss- of function analysis of miR-181a, we directly compared the expression of miR-181a in all patient samples and established EOC cell lines. A2780 (blue bar, "EOC cell lines") represented a relatively low expressing miR-181a cell lines whereas SKOV3 (red bar) represented a high expressing miR-181a cell line therefore gain- and loss- of function assays were performed in these lines respectively.



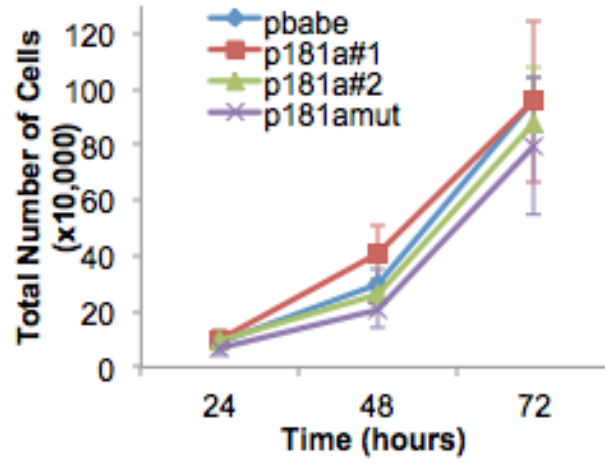
Supplementary Figure S4 Functional characterization of miR-181a in overexpression cell lines. The functional activity of miR-181a was monitored via a lentiviral-based mCherry sensor construct containing miR-181a binding sites in the 3'UTR and a control vector without miR-181a binding sites. Both stable cell lines expressing miR-181a revealed a downregulation of mCherry expression (middle panel). Additionally, through Taqman-based qRT-PCR analysis we have also confirmed expression of the mature form of the mutant miR-181a (bottom chart). Student's *t*-test, *** $P \leq 0.001$ ($n=3$). Error bars: s.d.



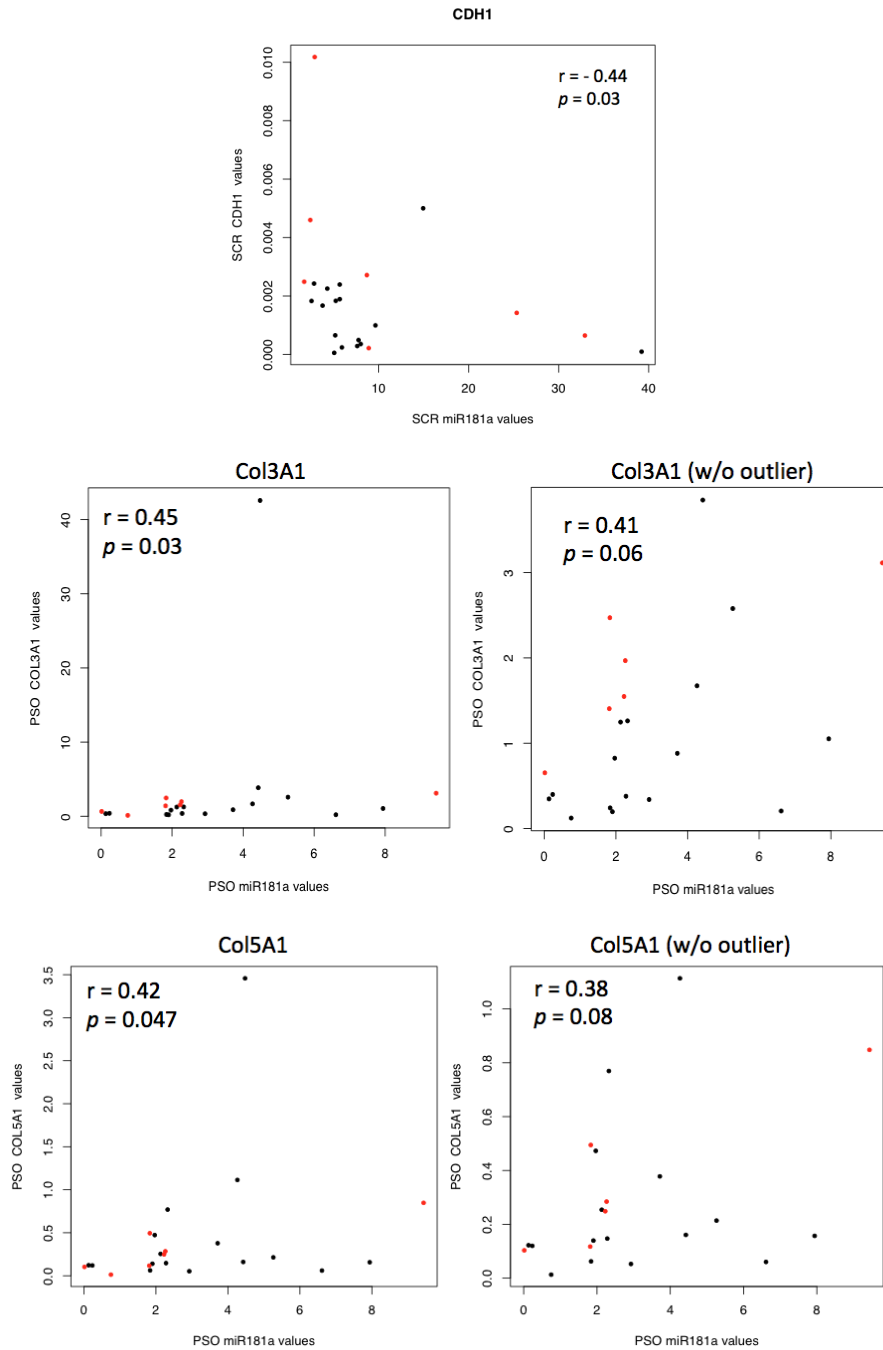
Supplementary Figure S5 Enhanced expression of miR-181a results in an EMT-like phenotype and increases cellular survival and migration in the HEY serous ovarian adenocarcinoma cell line. (A) Taqman-based qRT-PCR analysis revealing stable overexpression of miR-181a in the HEY cell line. Student's *t*-test $*P \leq 0.001$ ($n=3$). Error bars: s.d **(B)** SYBR-based qRT-PCR and western blot analyses show epithelial and mesenchymal marker differences consistent with an EMT-like phenotype in the miR-181a expressing HEY cells. Student's *t*-test $*P \leq 0.05$, $***P \leq 0.001$ ($n=3$). Error bars: s.d **(C)** miR-181a overexpression increased cellular survival as assessed through the colony formation assay **(D)** and cellular migration measured by wound healing scratch assay (200x, Scale bars (black), 50 μ M), as well as **(E)** transwell motility assay (200x, Scale bars (white), 50 μ M), which both reveal that miR-181a enhances cellular motility. Student's *t*-test $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$. ($n=3$) Error bars: s.d.



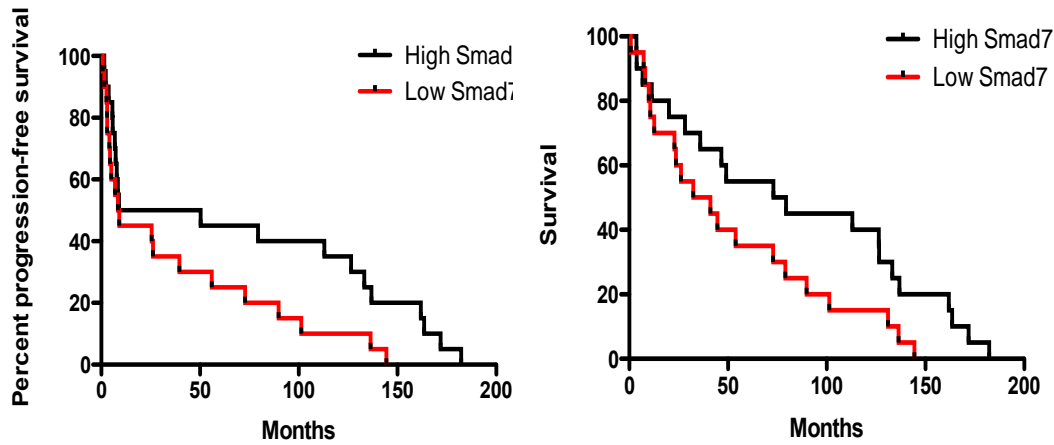
Supplementary Figure S6 Effects of miR-181a on proliferation. Cell doubling time in miR-181a-overexpressing and control lines via cell counting. (n=3) Error bars: s.d.



Supplementary Figure S7 Correlation of miR-181a with EMT markers in patient samples Pearson correlation between miR-181a and mesenchymal markers (COL5A1, COL3A1) and epithelial markers (E-cadherin) in patient samples. Fibronectin, Analysis of Vimentin and Col6A3 correlation to miR-181a expression shown as well.



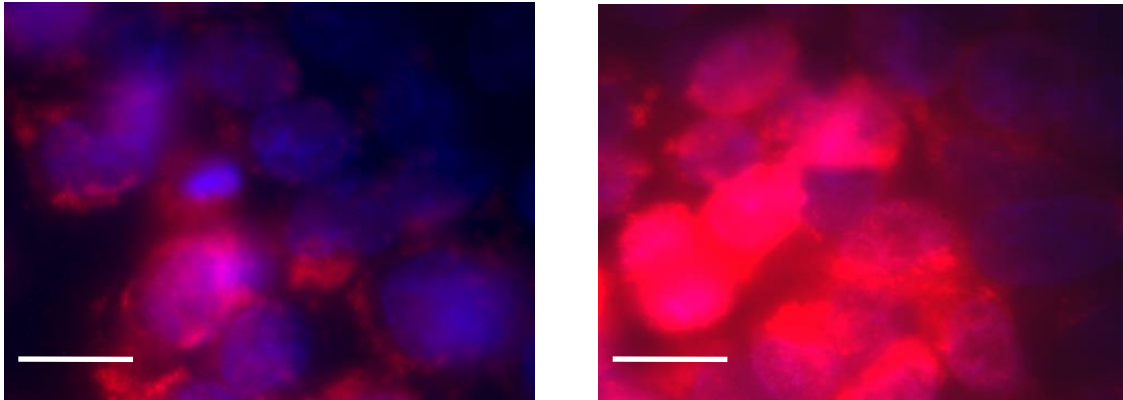
Supplementary Figure S8 Smad7 expression correlates with patient outcome. Kaplan-Meier survival curves generated from qRT-PCR analysis of Smad7 expressions in a cohort of high-grade serous ovarian tumors. (p-values were calculated using the Log Rank Mantel Cox test)



	Smad7 low	Smad7 high
Median PFI	8.7	29.6
*P=0.05, OR 3.4; 95% CI: 2.9 to 3.9		
Median Survival	36.8	76.2
*P=0.05, OR 2.1; 95% CI: 1.5 to 2.6		

Supplementary Figure S9 Smad7 localization in the pBABE and pSmad7 cell lines.

Immunofluorescence of Smad7 (shown in Texas Red, DAPI for nuclear stain) in the cell lines stably expressing Smad7 reveals that Smad7 is localized in the cytoplasm. (1000x, Scale bar (white), 5 μ M).

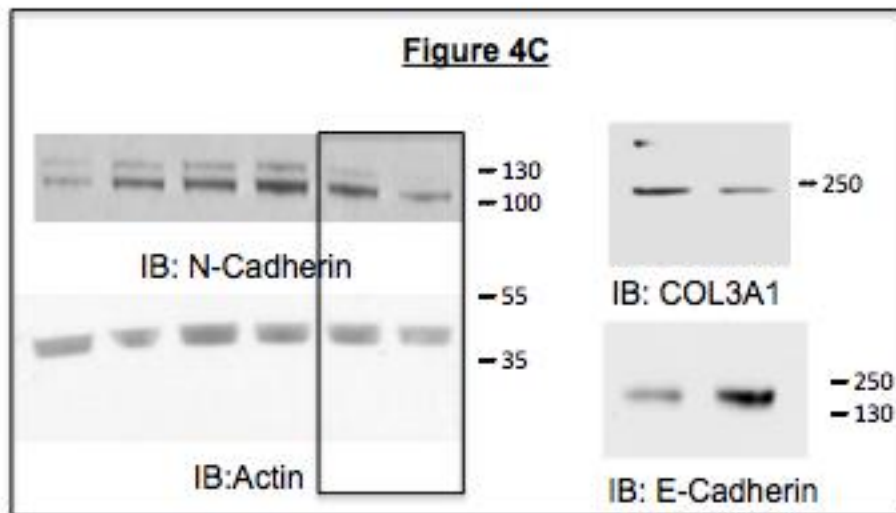
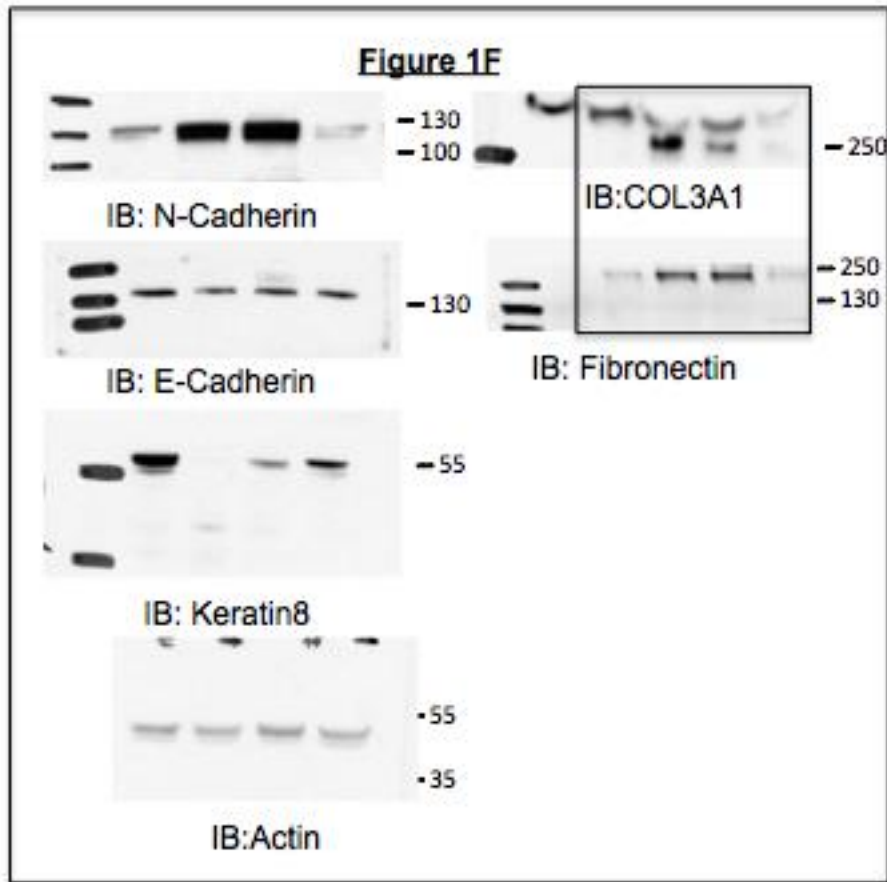


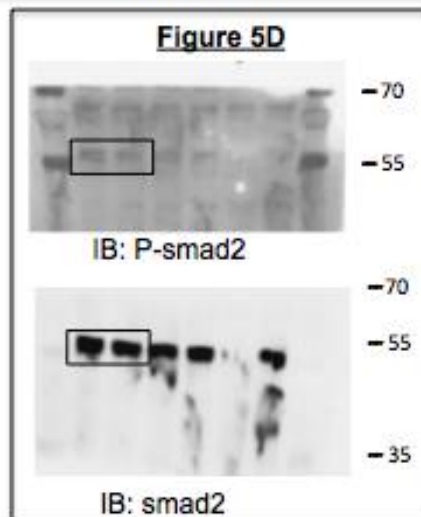
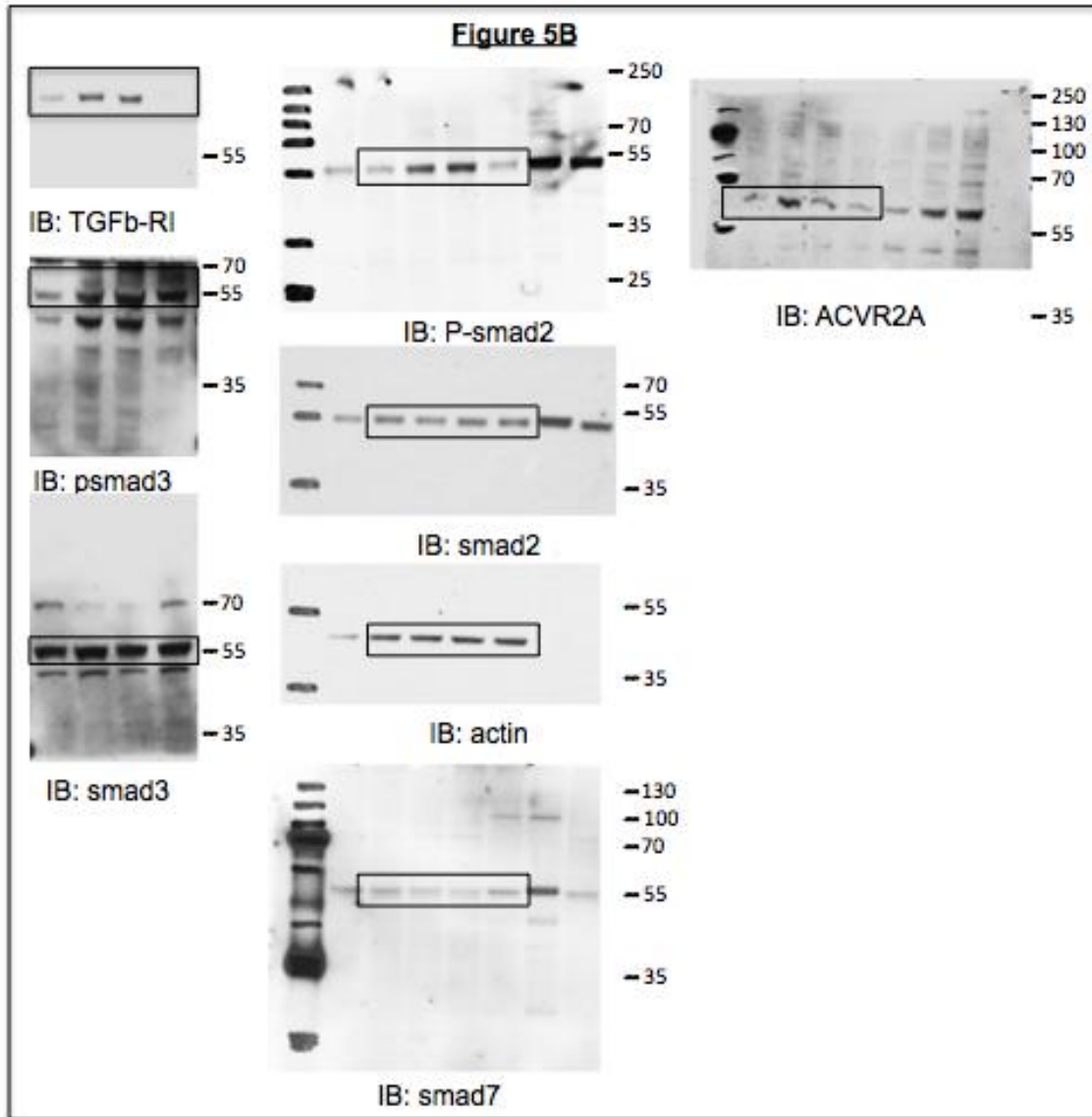
pBABE

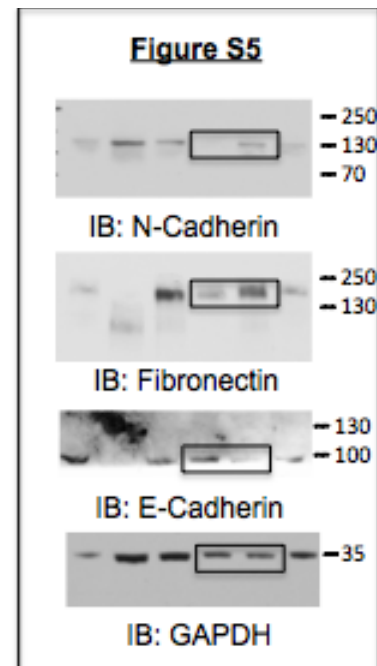
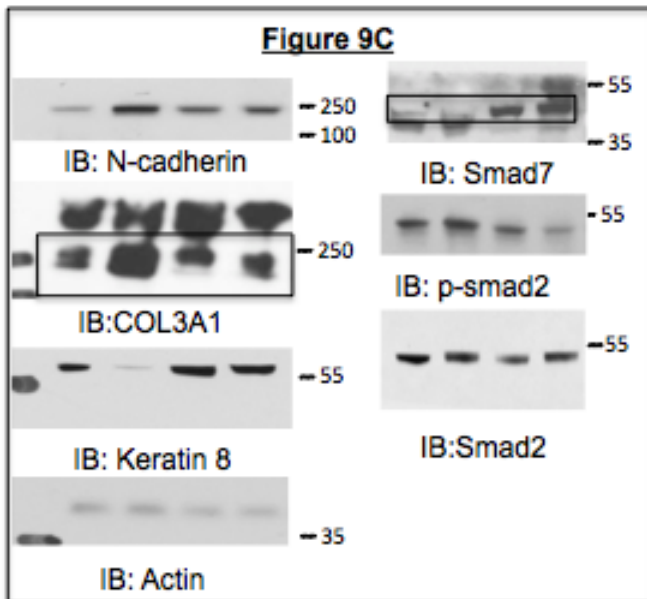
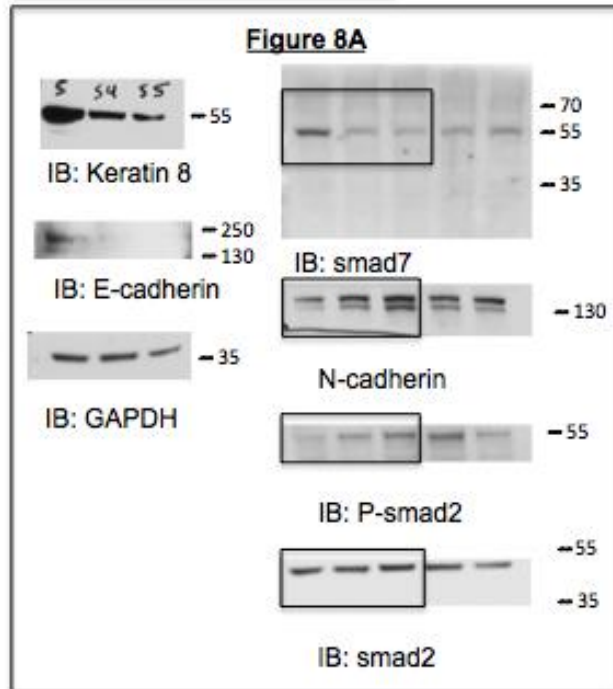
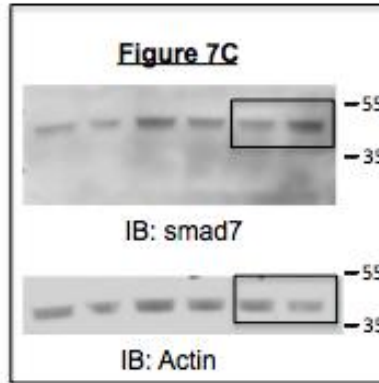
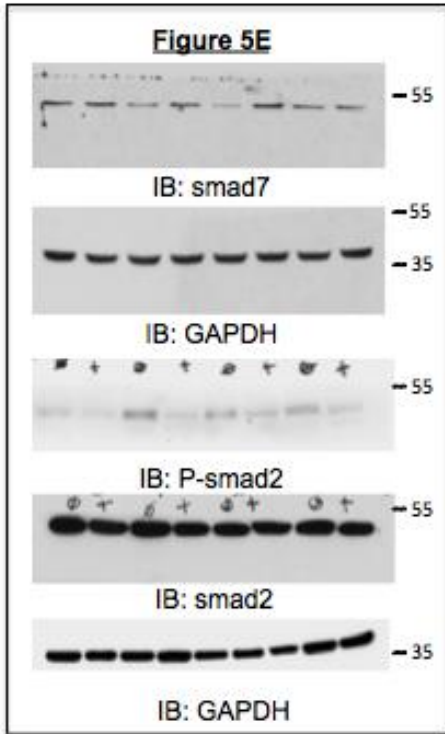
1000x

pSmad7

Supplementary Figure S10 Full Scans of Western Blots







Supplementary Tables

Supplementary Table S1: Clinicopathological characteristic of HGSOC patients with either high or low miR-181a expression. (N= 52)

		miR-181a low	miR-181a high
Age (yrs.)		59.7±12.9	60.5±13.8
Stage	III	26 (100%)	26 (100%)
Grade	1	1 (3.8%)	3 (11.5%)
	2	5 (19.2%)	5 (19.2%)
	3	19 (73.0%)	15 (57.7%)
	4	0 (0%)	1 (3.8%)
Ascites volume (L)		3.0±1.9	3.0±2.0

Supplementary Table S2. Summary of survival differences between miR-181a high and low tumours. (p-values were calculated using the Log Rank Mantel Cox test)

	miR-181a low	miR-181a high
Median PFS	55.9	7.1
**P=0.002, OR 7.8; 95% CI: 7.2 to 8.4		
Median survival	66.6	24.9
**P=0.002, OR 2.7; 95% CI: 2.1 to 3.3		

Supplementary Table S3. Distribution of intraperitoneal nodules (%) in the pBabe and p181a#1 mice and specific sites of metastasis. Fisher Exact Test (** $P < 0.01$, * $P < 0.05$)

Sites of metastasis	pBabe	p181a#1	
Peritoneum	25%	100%	**
Liver	25%	60%	
Diaphragm	0%	40%	
Omentum	25%	100%	**
Spleen	0%	60%	*

Supplementary Table S4. Summary of mRNA sequencing analysis from A2780 miR-181a overexpressing cells

	Total Reads	Max. Reads	Min. Reads	Average Reads	Genes down-regulated \geq 5fold	Genes up-regulated \geq 5fold	Predicted miR181 targets down-regulated \geq 5fold
pBABE	26,240,674	99,713	15	2,281			
p181a#1	32,225,698	219,630	3	2,801	705	34	49

Supplementary Table S5. Enhanced miR-181a expression induces global changes in gene expression consistent with TGF- β mediated EMT. Exogenous miR-181a expression leads to differential transcriptomic changes of EMT markers, such as downregulation of Keratin 8/18, NUDT13, and others as well as upregulation of Col3A1.

Gene symbol	RefSeq	Fold Change
Genes downregulated during EMT		
KRT18*	NM_199187	-5.80
NUDT13	NM_015901	-4.97
OCLN	NM_002538	-2.16
KRT8*	NM_002273	-2.02
RGS2	NM_002923	-1.21
Genes upregulated during EMT		
CDH2*	NM_001792	7.3
TIMP1	NM_003254	4.6
COL3A1*	NM_000090	4.0
TGFB2	NM_001135599	4.0
SNAI2*	NM_003068	3.2
ITGAV	NM_001145000	2.7
SPARC	NM_003118	2.7
COL5A2	NM_000393	2.2
ITGA5	NM_002205	2.1
TGFBR1	NM_001130916	2.1
TGFBR1	NM_004612	2.0
VIM*	NM_003380	2.0
SNAI3*	NM_178310	2.0
COL1A2	NM_000089	1.8
TMEFF1	NM_003692	1.5
STEAP1	NM_012449	1.4

Supplementary Table S6. P-Smad2 expression in advanced ovarian cancer patients negatively correlates with progression-free interval.

Immune Score	PFI< 6 months	PFI> 6 months
<2	11	17
>2	14	4
N=	25	21

Fisher Exact Test ($P=0.016$)**

Supplementary Table S7. Summary of survival differences between the p-Smad2 high and low tumours (left panel) and the combined pSmad2/miR-181a high and low tumours. (p-values were calculated using the Log Rank Mantel Cox test)

	P-Smad2 (I.S. < 2)	P-Smad2 (I.S. ≥ 2)		low miR-181a/ low P-Smad2	high miR-181a/ high P-Smad2
Median PFS	53.1	6.7	Median PFS	123.1	6.6
* <i>P</i> =0.03, OR 7.9; 95% CI: 7.4 to 8.5			*** <i>P</i> =0.0007, OR 18.7; 95% CI: 9.2 to 37.8		
Median Survival	72.8	25.5	Median Survival	132.2	22.8
* <i>P</i> =0.05, OR 2.9; 95% CI: 2.3 to 3.4			*** <i>P</i> =0.0006, OR 5.8; 95% CI: 2.6 to 12.8		

Supplementary Table S8. Summary of miR-181a expression in primary (PS-O) and recurrent tumours (SCR). (p-values were calculated using the Wilcoxon signed rank test)

Gene name	Status	All dataset (n = 23)			A Group (EMT enriched; n=16)			B Group (n=7)		
		Median (IQ-range)	R	p	Median (IQ-range)	R	p	Median (IQ-range)	R	p
miR-181a	PS-O	2.263 (1.831-4.425)	2.51	0.0024	2.626 (1.919-4.464)	2.16	0.0006	1.831 (0.750-2.263)	4.74	0.063
	SCR	5.685 (3.774-8.904)			5.682 (4.492-7.953)			8.695 (2.410-25.34)		

Supplementary Table S9. Main clinicopathological features of EOC patients enrolled in the study from which biopsies were taken at primary surgery (PS-O) and at time of relapse (SCR)

Clinicopathologic Variable		Overall (n=23)
Age (years)	<i>Median Range</i>	55.4 (28.5 - 72.2)
Stage		
	III	21 (91.3%)
<i>Sub-stage</i>	b	3 (14.3%)
	c	18 (85.7%)
	IV	2 (8.7%)
Grade		
	1	4 (17.4 %)
	2	3 (13.0%)
	3	16 (69.6)
Histotype		
	Serous	18 (78.3%)
	Endometrioid	3 (13.1%)
	Undifferentiated	0
	Clear cell	1 (4.3%)
	Other	1 (4.3%)