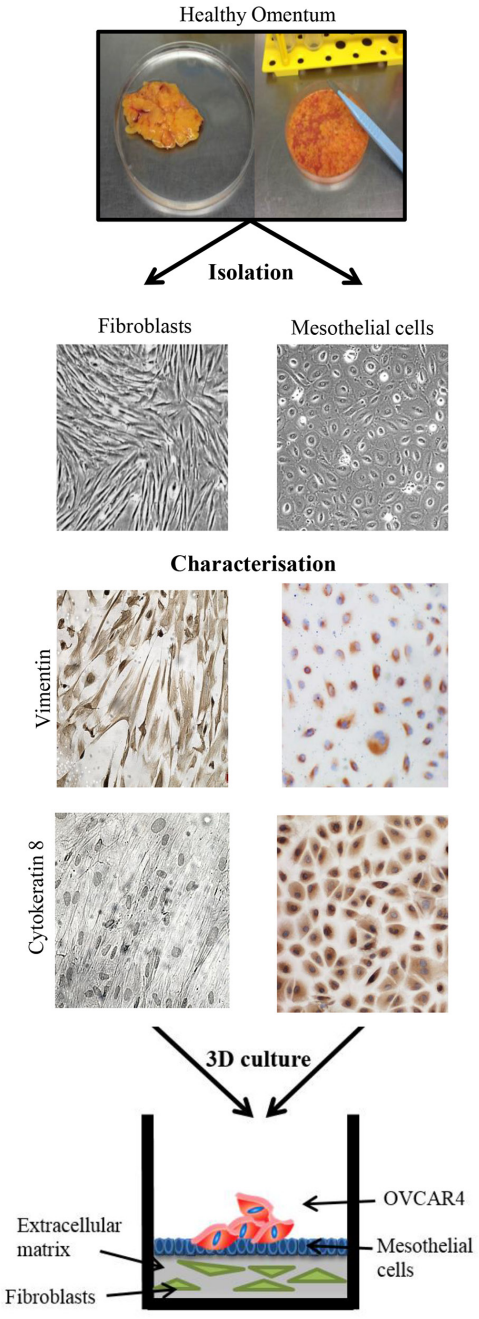
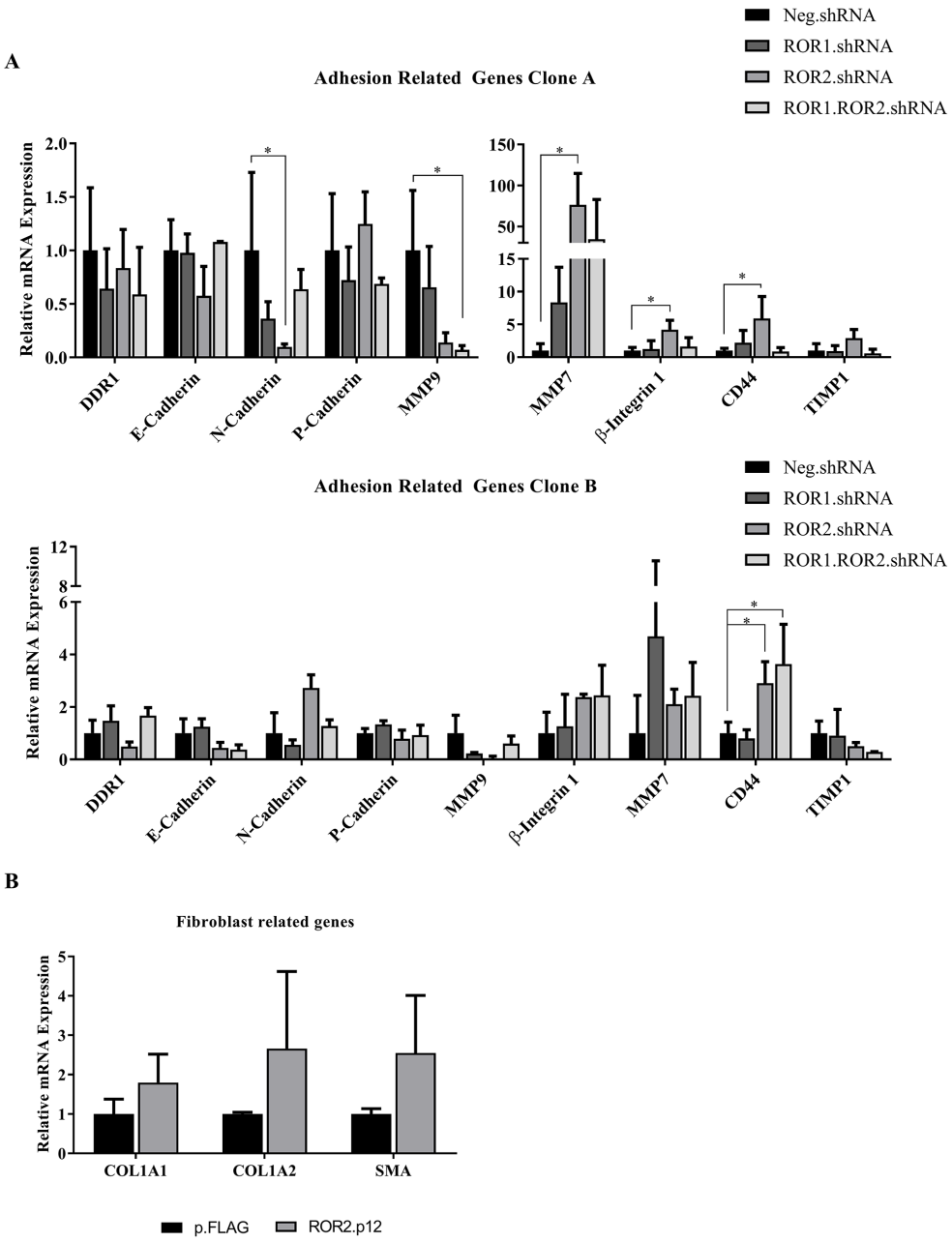
Silencing ROR1 and ROR2 inhibits invasion and adhesion in an organotypic model of ovarian cancer metastasis

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Flow chart of omentum processing. Omentum is washed and scrapped to isolate HPMCs and further digested with collagenase to digest NOFs. HPMCs exhibit a cuboidal, round shape whereas NOFs exhibit long, elongated shapes. Confirming immunocytochemistry shows HPMCs express vimentin and cytokeratin 8 whereas NOFs only express vimentin. NOFs (green triangles) mixed with collagen (grey) are plated first in the 3D culture, followed by HPMCs (blue) to create a 3D organotypic model of omentum metastasis with cancer cells (red) added on top.



Supplementary Figure 2: RNA analysis of associated genes. (A) mRNA expression relative to negative control OVCAR4 cells of adhesion associated genes in shRNA clone A (top two panels) and shRNA clone B (bottom panel). qRT-PCR was performed in triplicate and normalised to three different housekeeping genes (SDHA, HSPCB, RPL13A). Results represent an average of three experiments. Error bars represent the s.d of the mean. **(B)** mRNA expression of Smooth Muscle Actin (SMA), Collagen 1 A1 (COL1A1) and Collagen 1 A2 (COL1A2) in ROR2 overexpressing NOFs, relative to p.FLAG control. qRT-PCR was performed in triplicate and normalised to three different housekeeping genes (SDHA, HSPCB, RPL13A). Results represent an average of three experiments. Error bars represent the s.d of the mean.

Supplementary Table 1: Primer sequences used for qRT-PCR

Gene	Forward primer 5'-3'	Reverse primer 5'-3'
ROR1	CAACAAGAAGCCTCCCTAATGG	CCTGAGTGACGGCACCTAGAA
ROR2	CGACTGCGAATCCAGGACC	GGCAGAACCCATCCTCGTG
DDR1	AATCGCAGACTTTGGCATGAG	CGTGAACTTCCCCATGAGGAT
CDH1	AGGCCAAGCAGCAGTACATT	ATTCACATCCAGCACATCCA
CDH2	ACAGTGGCCACCTACAAACG	CCGAGATGGGGTTGATAATG
CDH3	CACGACCTCATGTTCACCAT	CTCAGGGACTTTTTCCCGGT
INTEGRINβ1	GGATTCTCCAGAAGGTGGTT	GGTAAAACAATGCCACCAAG
MMP9	ACTGAGAGGCTCCGAGAAATG	GAACCCCGCATCTTGGCTT
MMP7	GATGAGGATGAACGCTGGAC	GCTAAATGGAGTGGAGGAACAG
CD44	CCTCTTGGCCTTGGCTTTG	TCCATTGCCACTGTTGATCA
TIMP1	GGGGCTTCACCAAGACCTAC	GGAAGCCCTTTTCAGAGCCT
COL1A1	ACGAAGACATCCCACCAATCAC	CGTTGTCGCAGACGCAGAT
COL1A2	GAGGGCAACAGCAGGTTCACTTA	TCAGCACCACCGATGTCCAA
SMA	CCGACCGAATGCAGAAGGA	ACAGAGTATTTGCGCTCCGAA
RPL13A	CCTGGAGGAGAAGAGGAAAGAGA	TTGAGGACCTCTGTGTATTTGTCAA
SDHA	TGGGAACAAGAGGGCATCTG	CCACCACTGCATCAAATTCATG
HSPCB	TCTGGGTATCGGAAAGCAAGCC	GTGCACTTCCTCAGGCATCTTG