Snail knockdown reverses stemness and inhibits tumour growth in ovarian cancer

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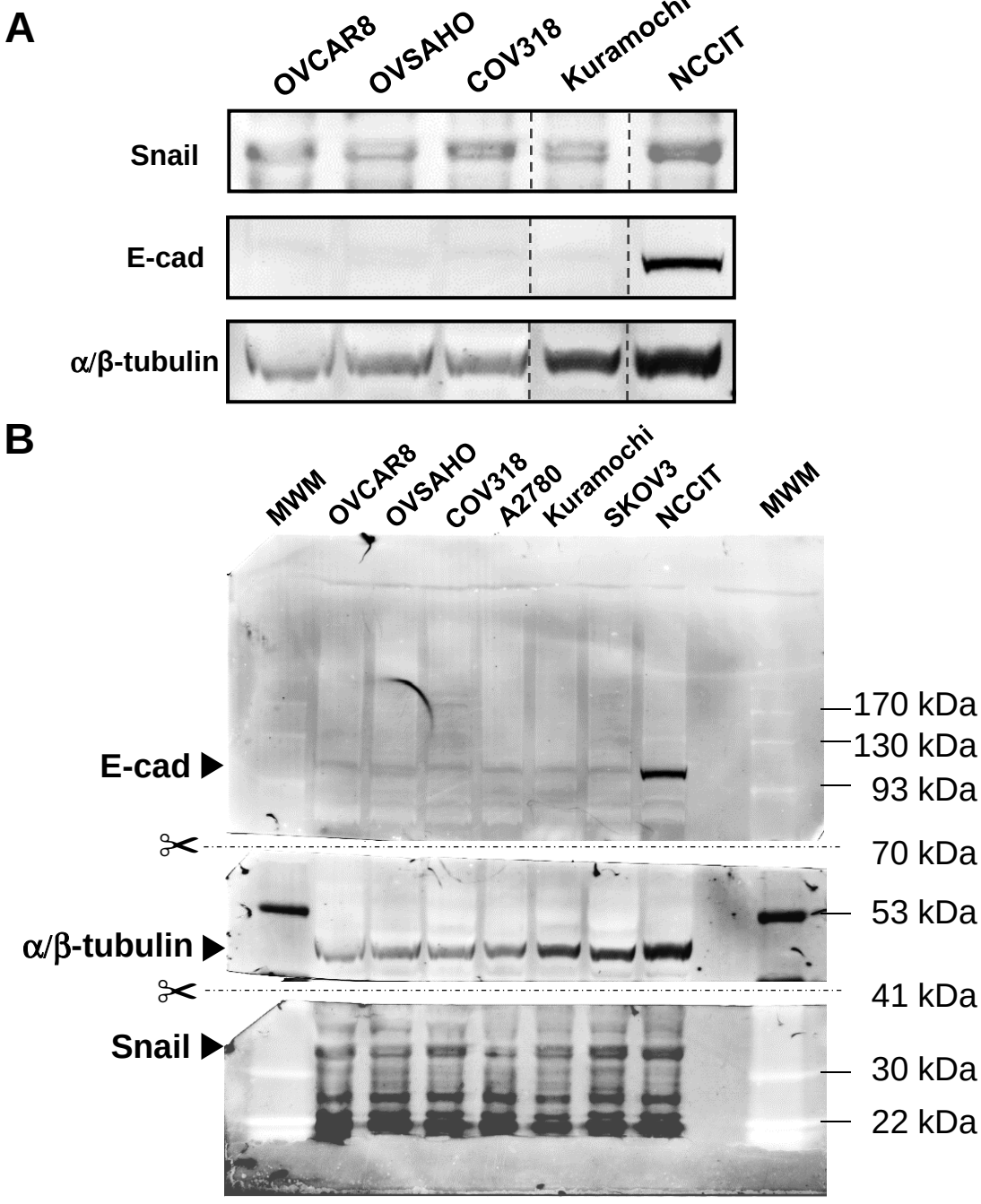
Supplementary Table S1

Primer for	Forward	Reverse
β-Actin (ACTB)	5'-TGAAGTGTGACGTGGACATC-3'	5'-GGAGGAGCAATGATCTTGAT-3'
Snail (SNAI1)	5'-CACTATGCCGCGCTCTTTC-3'	5'-GGTCGTAGGGCTGCTGGAA-3'
E-cadherin (CDH1)	5'-TGCCCAGAAAATGAAAAAGG-3'	5'-GTGTATGTGGCAATGCGTTC-3'
N-cadherin (CDH2)	5'-GAGGAGTCAGTGAAGGAGTCA-3'	5'-GGGAAGTTGATTGGAGGGATG-3'
Nanog	5'-CAAAGGCAAACAACCCACTT-3'	5'-TCTGCTGGAGGCTGAGGTAT-3'
Lin28A	5'-GAGCATGCAGAAGCGCAGATCAAA-3'	5'-TATGGCTGATGCTCTGGCAGAAGT-3'

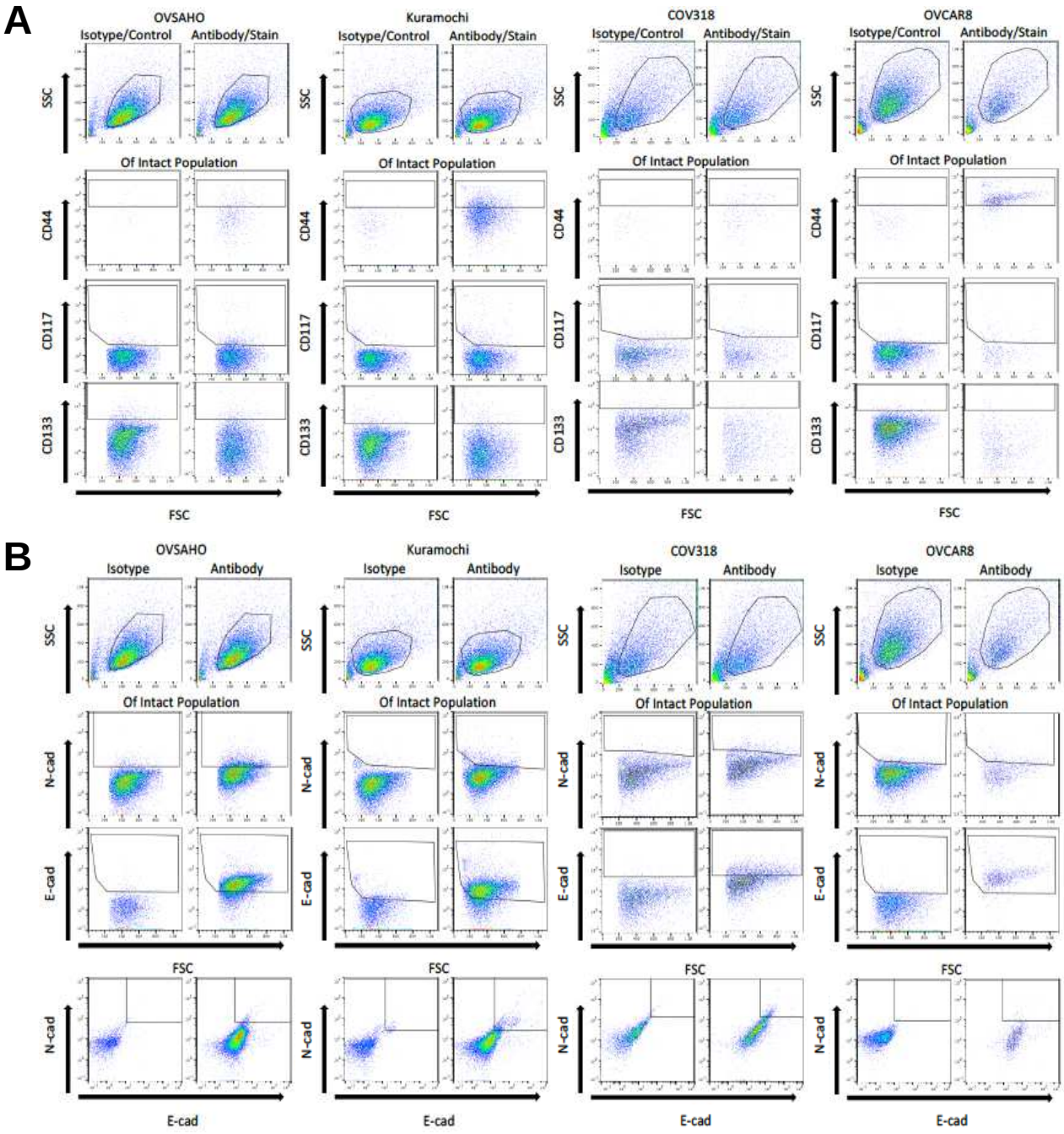
Supplementary Table 1. Primer sequences used for qRT-PCR.

Supplementary Figure S1



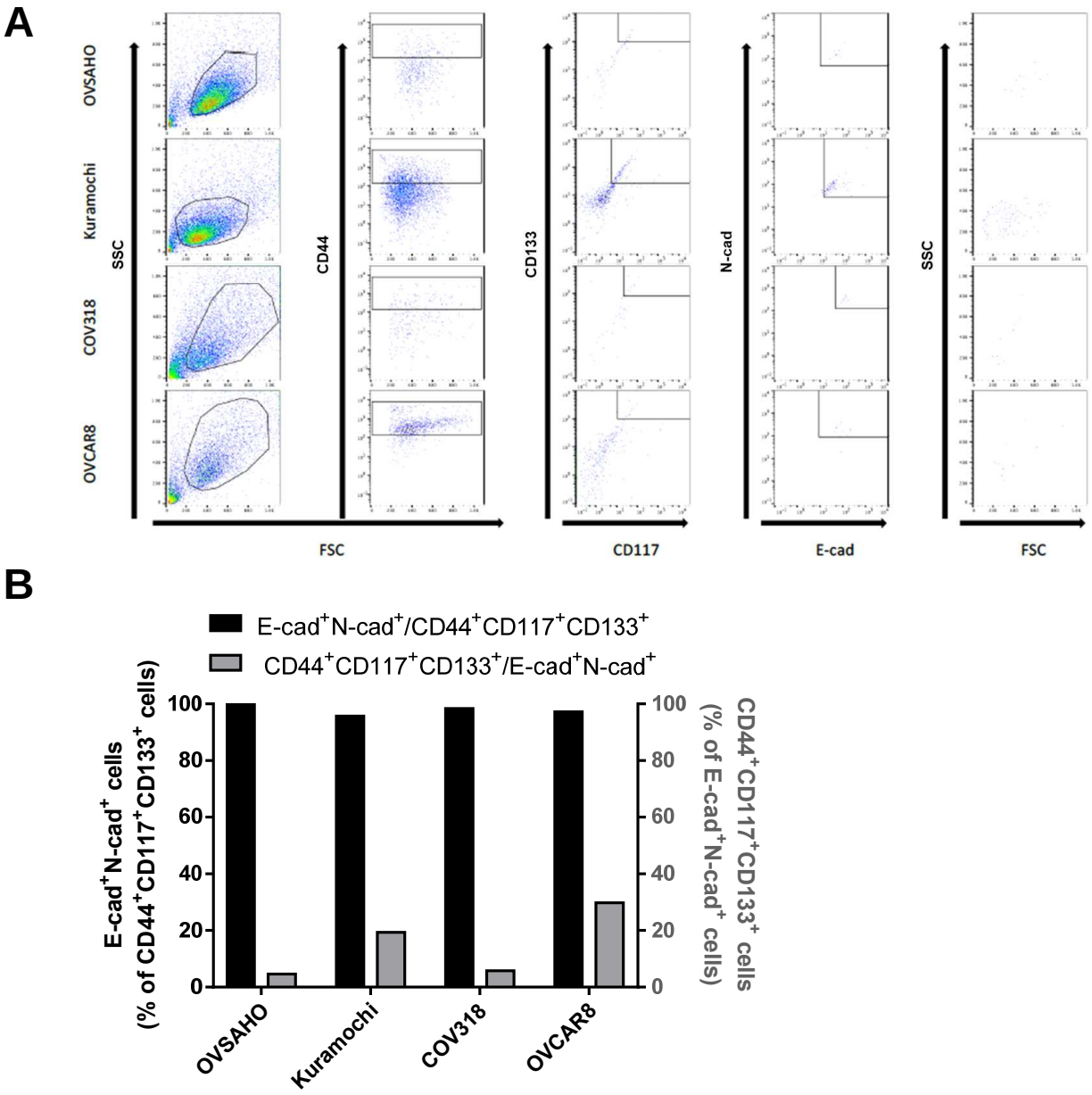
Supplementary Figure S1. Western blot analysis for the quantitation of Snail and E-cadherin protein in HGSOC cell lines. α/β-tubulin was used as housekeeping protein. A. Cropped image. B. Full-length blots of A. After transferring proteins from gel to membrane, the membrane was cut into 3 pieces at the height of 70kDa and 41kDa. Upper, middle, and lower membranes were incubated with anti-E-cad, anti-α/β tubulin and anti-Snail antibodies, respectively.

Supplementary Figure S2



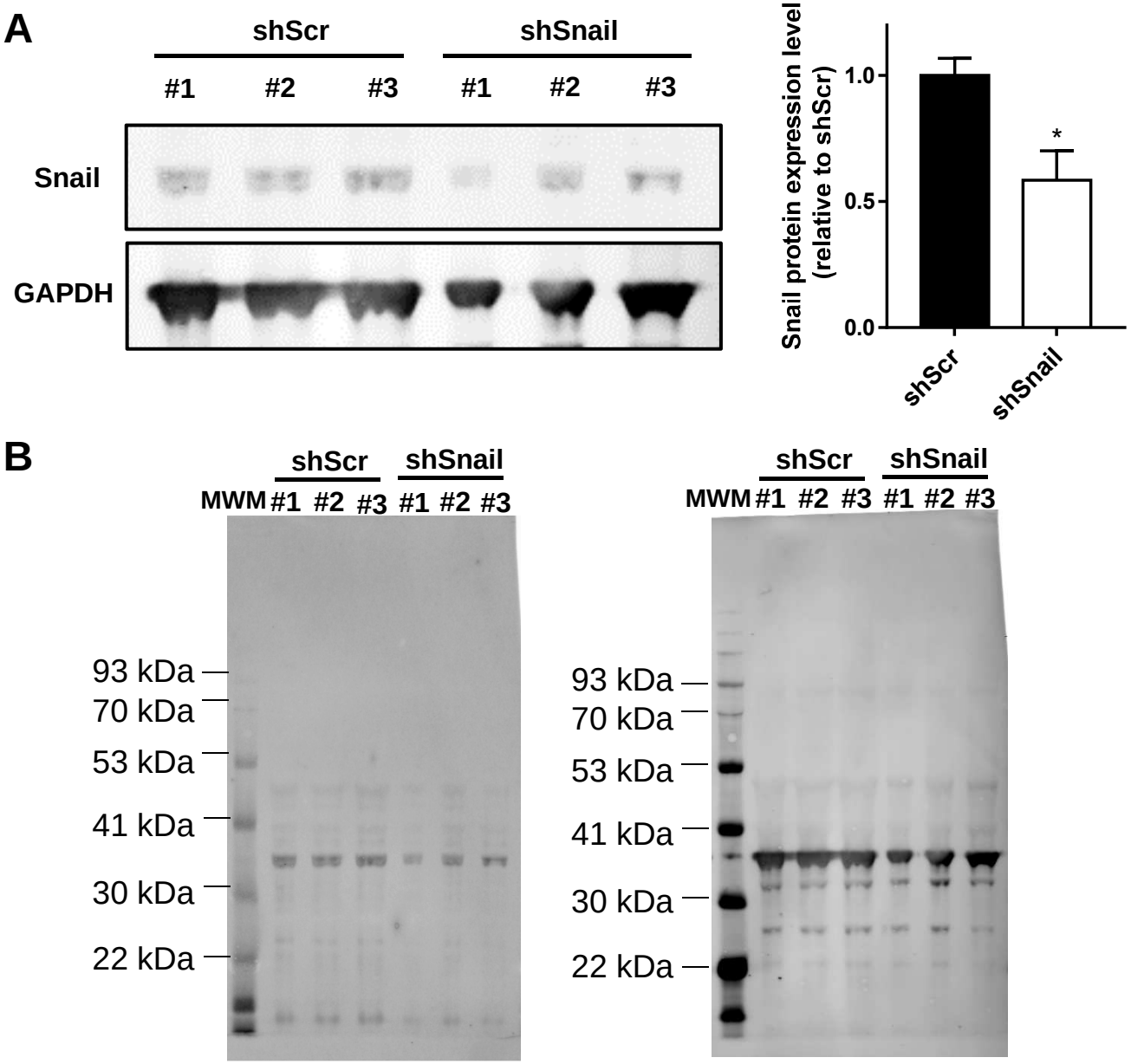
Supplementary Figure S2. Flow cytometry analysis for HGSOC cell lines.
A. Cancer stem cell markers (CD44, CD117, and CD133).
B. Cell surface E-cadherin and N-cadherin. For each antibody, gating was determined based on appropriate isotype-stained controls.

Supplementary Figure S3



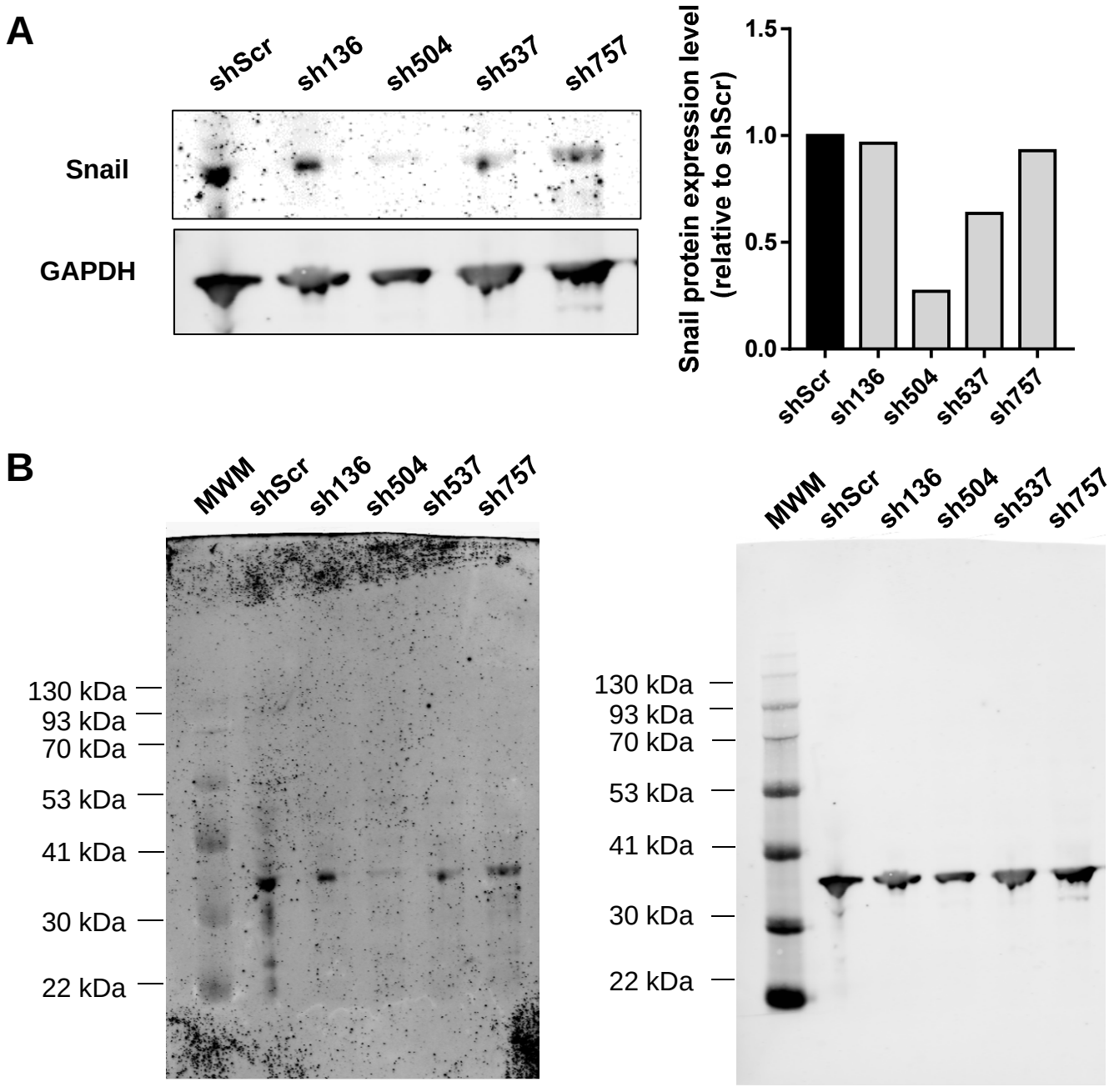
Supplementary Figure S3. Multicolour flow cytometry analysis for HGSOC cell lines.
A. Sequential gating strategy for quantitation of E-cad$^+$N-cad$^+$ cell population in CSC marker positive cell population (CD44$^+$CD117$^+$CD133$^+$).
B. Black bar, percentage of E-cad$^+$N-cad$^+$ cells in CD44$^+$CD117$^+$CD133$^+$ cell population. Grey bar, percentage of CD44$^+$CD117$^+$CD133$^+$ cells in E-cad$^+$N-cad$^+$ cell population.

Supplementary Figure S4



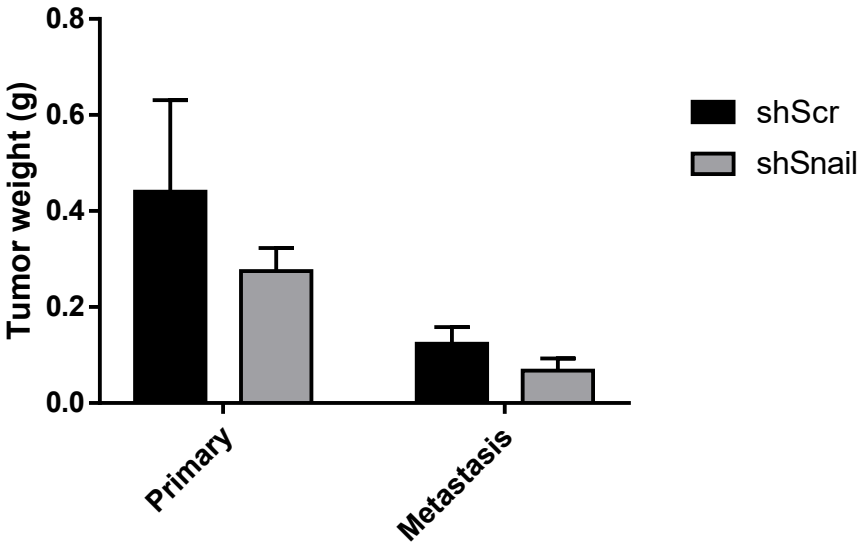
Supplementary Figure S4. Snail knockdown by shRNA in OVCAR8 cells.
A. Left, western blot image for the quantitation of Snail and GAPDH (housekeeping protein). Right, quantification of western blot signals. Data are the mean of biological triplicates. Error bars, SEM; *, $P < 0.05$ by unpaired Student's t-test.
B. Full-length blots of A. The membrane was incubated with anti-Snail mouse antibody and anti-GAPDH rabbit antibody simultaneously. Left, Snail signal was detected by 800nm channel. Right, GAPDH signal was detected by 700nm channel.

Supplementary Figure S5



Supplementary Figure S5. Snail knockdown by 4 different shRNAs in OVCAR8 cells.
A. Left, western blot image for the quantitation of Snail and GAPDH (housekeeping protein). Right, quantification of western blot signals.
B. Full-length blots of A. The membrane was incubated with anti-Snail mouse antibody and anti-GAPDH rabbit antibody simultaneously. Left, Snail signal was detected by 800nm channel. Right, GAPDH signal was detected by 700nm channel.

Supplementary Figure S6



Supplementary Figure S6 Tumour weight in orthotopic xenograft mouse model. Tumour weight in grams for primary and metastatic tumour burden as observed during necropsy. Error bars, SEM.