**Supplementary Figure Legends:**

**Supplementary Figure 1:** Pre-clustered spheroids from 20 established ovarian cancer cell lines incubated with mesothelial monolayers. Images were taken 0, 4 and 8 hours after co-incubation. Phase-contrast images show ovarian cancer spheroids attached to mesothelial monolayers. GFP images of mesothelial monolayers (green) were used to measure clearance area at 8 hours post co-culture. Scale bars = 100 μm.

**Supplementary Figure 2:** Over-expression of EMT transcription factors in OVCA433 cells promotes mesothelial clearance – Average of 3 experiments. A. Normalized average clearance area of ZT mesothelial monolayers 8 hours after co-culture with 20nM 4-OHT-induced MCAS spheroids carrying control empty vector, inducible WZL-TWIST or WZL-SNAIL expression vectors. B. Normalized average clearance area of ZT mesothelial monolayers 8 hours after co-culture with 1μg/ml Doxycycline -induced MCAS spheroids carrying control empty vector, inducible FUW-LPT2 Zeb1 expression vectors. >60 spheroids averaged per condition. Results are shown as averages from three replicate experiments in which triplicate samples were analyzed. Error bars denote SEM. *, $p<0.05$ using Student's t-test.

**Supplementary Figure 3:** TWIST1, TWIST2, and ZEB1 siRNA deconvolution. A. qRT-PCR measurements of mRNA levels of TWIST1 in OVCA433 cells treated with control (luciferase), TWIST1 SMARTpool, and four individual siRNA hairpins that

comprise the SMARTpool. B. Normalized average clearance area of ZT mesothelial monolayers 8 hours after co-culture with OVCA433 ovarian cancer spheroids treated with control (luciferase), TWIST1 SMARTpool, and four individual siRNA hairpins that comprise the SMARTpool. C. E-cadherin expression in OVCA433 ovarian cancer spheroids treated with control (luciferase), TWIST1 SMARTpool, and four individual siRNA hairpins that comprise the SMARTpool by Western Blot. D. qRT-PCR measurements of mRNA levels of TWIST1 in OVCA433 cells treated with control (luciferase), ZEB1 SMARTpool, and four individual siRNA hairpins that comprise the SMARTpool. E. Normalized average clearance area of ZT mesothelial monolayers 8 hours after co-culture with OVCA433 ovarian cancer spheroids treated with control (luciferase), ZEB11 SMARTpool, and four individual siRNA hairpins that comprise the SMARTpool. F. E-cadherin expression in OVCA433 ovarian cancer spheroids treated with control (luciferase), ZEB11 SMARTpool, and four individual siRNA hairpins that comprise the SMARTpool by Western Blot. G. qRT-PCR measurements of mRNA levels of TWIST1 in OVCA433 cells treated with control (luciferase), TWIST2 SMARTpool, and four individual siRNA hairpins that comprise the SMARTpool. H. Normalized average clearance area of ZT mesothelial monolayers 8 hours after co-culture with OVCA433 ovarian cancer spheroids treated with control (luciferase), TWIST2 SMARTpool, and four individual siRNA hairpins that comprise the SMARTpool. I. E-cadherin expression in OVCA433 ovarian cancer spheroids treated with control (luciferase), TWIST2 SMARTpool, and four individual siRNA hairpins that comprise the SMARTpool. >60 spheroids

averaged per condition over three independent experiments in mesothelial clearance experiments. Error bars denote SEM. *, $p<0.05$ using Student's t-test.

**Supplementary Figure 4:** Long-term TWIST knockdown using shRNAs promote decreased mesothelial clearance and increased epithelial character. A. qRT-PCR measurements of mRNA levels of TWIST1 in OVCA433 cells infected with control (pLKO), or one of two hairpins targeting TWIST (G9 and G11). B. Phase-contrast images of OVCA433 cells transfected with control (pLKO), or one of two hairpins targeting TWIST (G9 and G11). 10x Magnification C. Normalized average clearance area of ZT mesothelial monolayers at 8 hours after co-incubation with OVCA433 spheroids transfected with control (LKO), or one of two hairpins targeting TWIST (G9 and G11). D. qRT-PCR measurements of mRNA levels of EMT markers in OVCA433 cells transfected with TWIST shRNA, normalized to control (LKO) marker expression.

**Supplementary Figure 5:** Knockdown of TWIST1 and ZEB1 in OVCA432 ovarian cancer spheroids using siRNA SMARTpools attenuates mesothelial clearance. Normalized average clearance area of ZT mesothelial monolayers 8 hours after co-culture with OVCA432 ovarian cancer spheroids transfected with siRNA SMARTpools targeting luciferase (control), TWIST1, and ZEB1. >60 spheroids averaged per condition in three independent experiments. Error bars denote SEM. *, $p<0.05$ using Student's t-test.

**Supplementary Figure 6:** Knockdown of vimentin in OVCA433 ovarian cancer spheroids using shRNA hairpins attenuates mesothelial clearance. A. Western Blot analysis of vimentin in OVCA433 cells carrying 'Control' LKO empty vector, LKO-Vimetin All, B1, B2 or B3. B. Normalized average clearance area of ZT mesothelial monolayers 8 hours after co-culture with OVCA433 spheroids carrying 'Control' LKO empty vector, LKO-Vimetin All, B1, B2 or B3. >60 spheroids averaged per condition in three independent experiments. Error bars denote SEM. *, $p<0.05$ using Student's t-test.

**Supplementary Figure 7:** Supplementary Figure 7: Knockdown of vimentin in CP70 cells inhibits mesothelial clearance. A. Normalized average clearance area of ZT mesothelial monolayers at 8 hours after co-incubation with CP70 spheroids transfected with siRNAs targeting luciferase (control), an siRNA smartpool targeting vimentin, or three individual hairpins from the siRNA smartpool targeting vimentin. 20 positions scored per condition. Error bars denote SEM. *, $p<0.05$ using Student's t-test. B. Western blot analysis of vimentin in CP70 cells transfected with siRNAs targeting luciferase (control), an siRNA smartpool targeting vimentin, or three individual hairpins from the siRNA smartpool targeting vimentin.

**Supplementary Figure 8:** Pre-clustered spheroids from 21 primary ovarian cancer cell lines incubated with mesothelial monolayers. Images were taken 0, 4 and 8 hours after co-incubation. Phase-contrast images show ovarian cancer spheroids attached to mesothelial monolayers. GFP images of mesothelial monolayers (green)

were used to measure clearance area at 8 hours post co-culture. Scale bars = 100 μm.

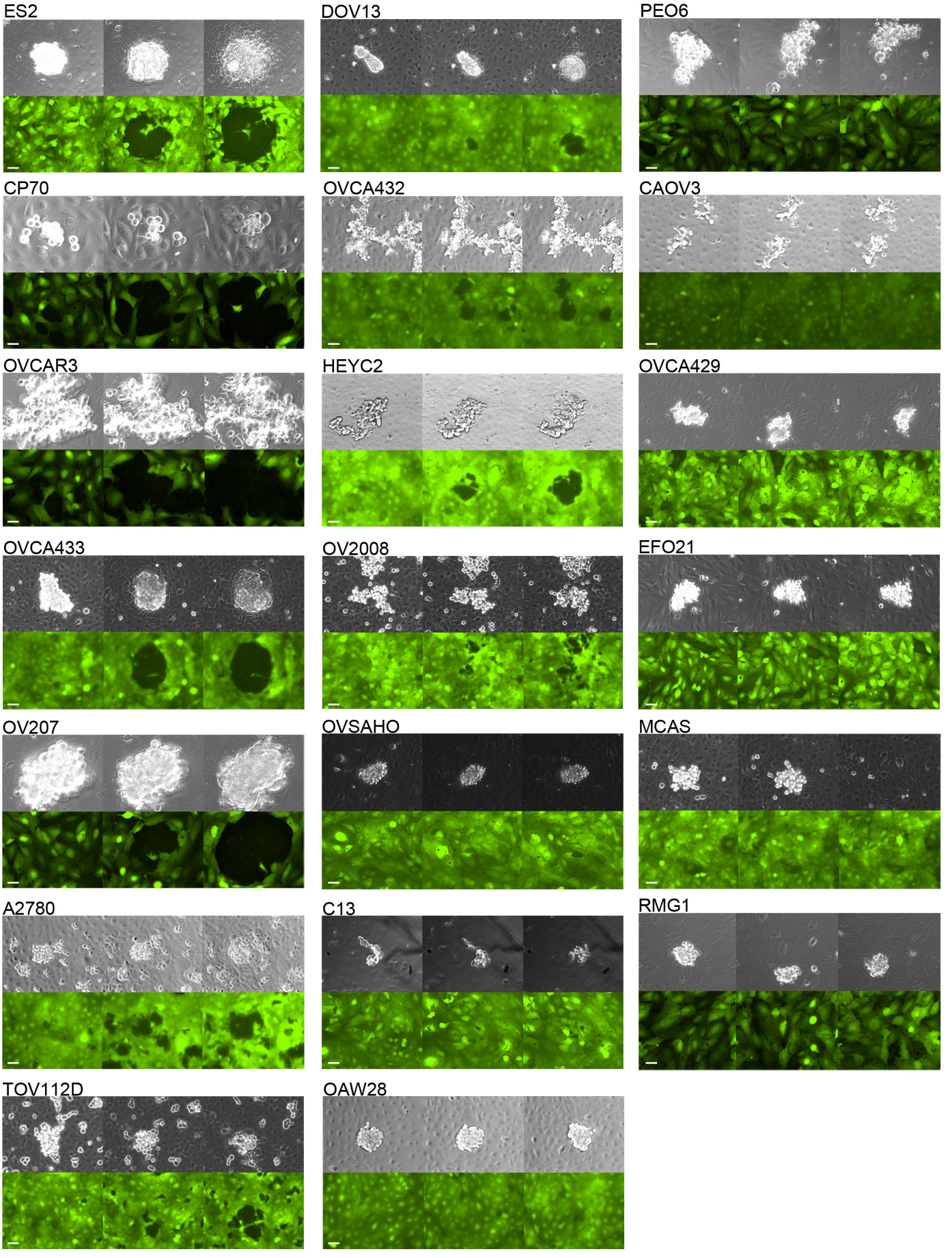
**Supplementary Figure 9:** Representative images of immunofluorescence for PAX8 (red) and DAPI (blue) in OVCA433 spheroids invading GFP (green) expressing mesothelial monolayers and DF164, DF143, DF163 spheroids spreading on glass for 8 hours. MCF10A cells were used as a control for negative Pax8 staining. Scale bars = 100 μm.

**Supplementary Table 1:** Genes identified that distinguish the clearance-competent cell lines from the clearance-incompetent cell lines.

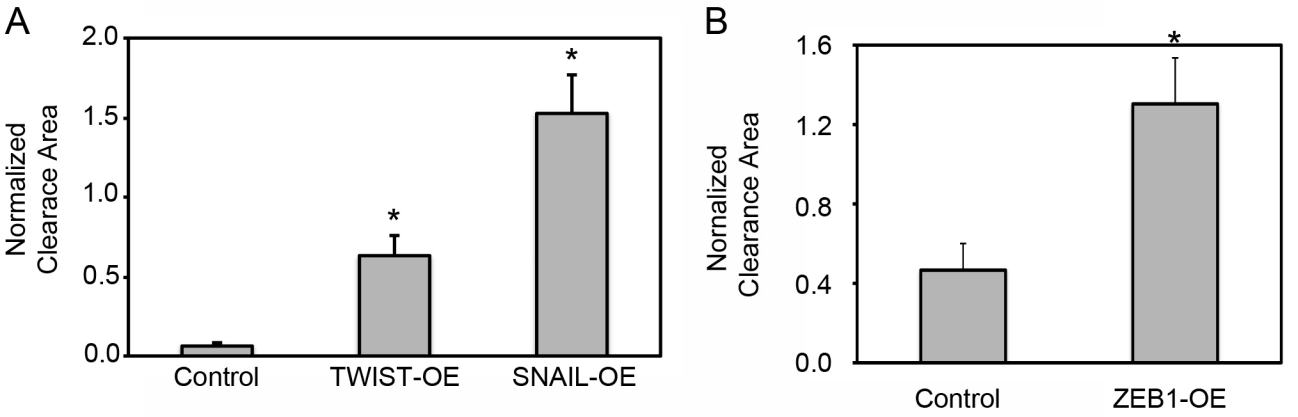
**Supplementary Movie 1:** Composite of representative movies of mesothelial clearance by ovarian cancer cell lines in order of clearance ability from left to right, top to bottom: ES2, CP70, OVCAR3, OVCA433, OV207, A2780, TOV112D, DOV13, OVCA432, HEYC2, OV2008, OVSAHO, C13, OAW28, PE06, CAOV3, OVCA429, EFO21, MCAS, RMG1.

**Supplementary Movie 2:** Composite of representative movies of mesothelial clearance by primary ovarian cancer cell samples in order of clearance ability from left to right, top to bottom: DF168, DF106, DF164, DF29, DF163, DF24, DF43, DF173, DF143, DF155, DF166, DF118, DF59, DF141, DF68, DF160, DF176, DF9, DF172, DF147, DF14.

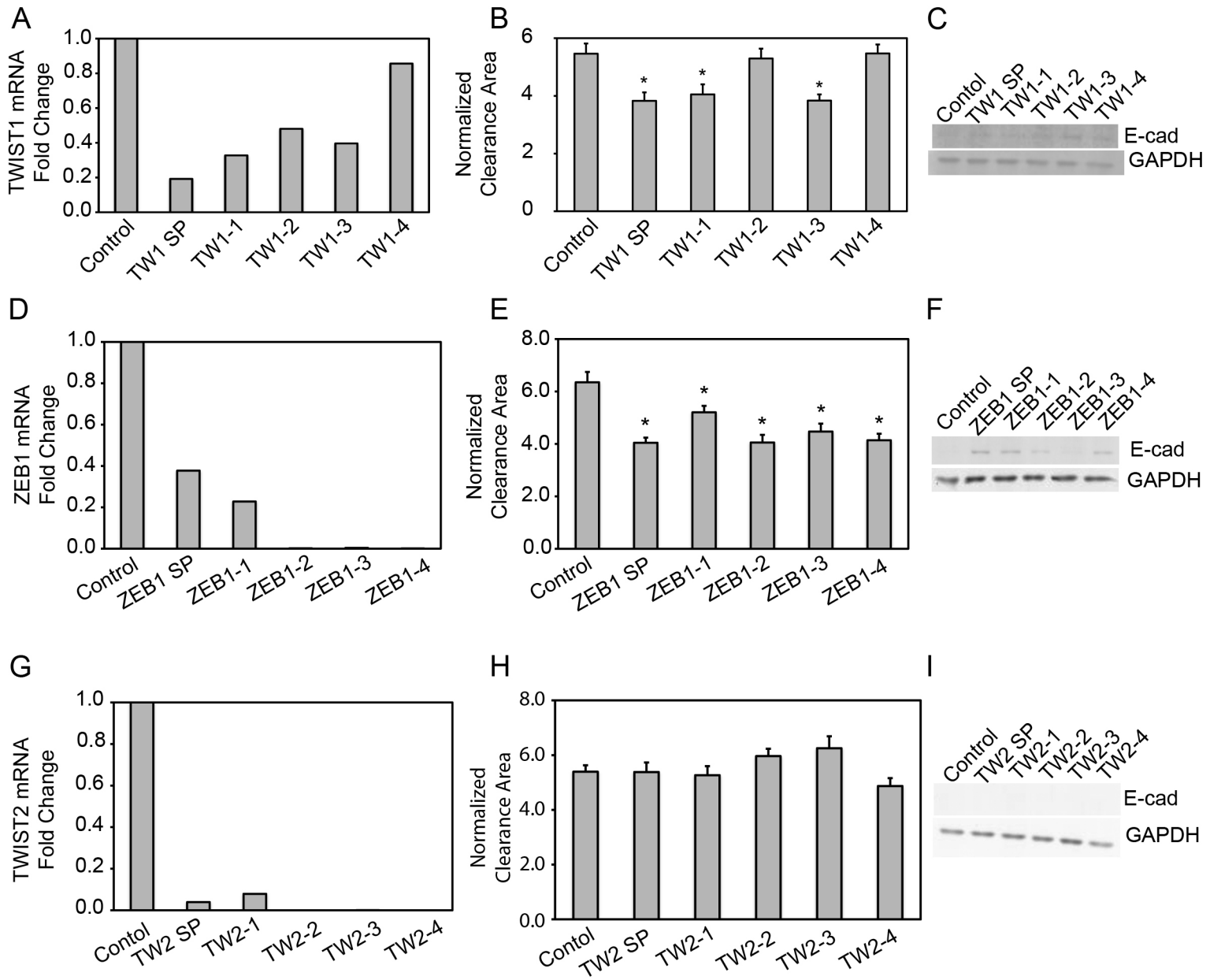
Supplementary Figure 1:



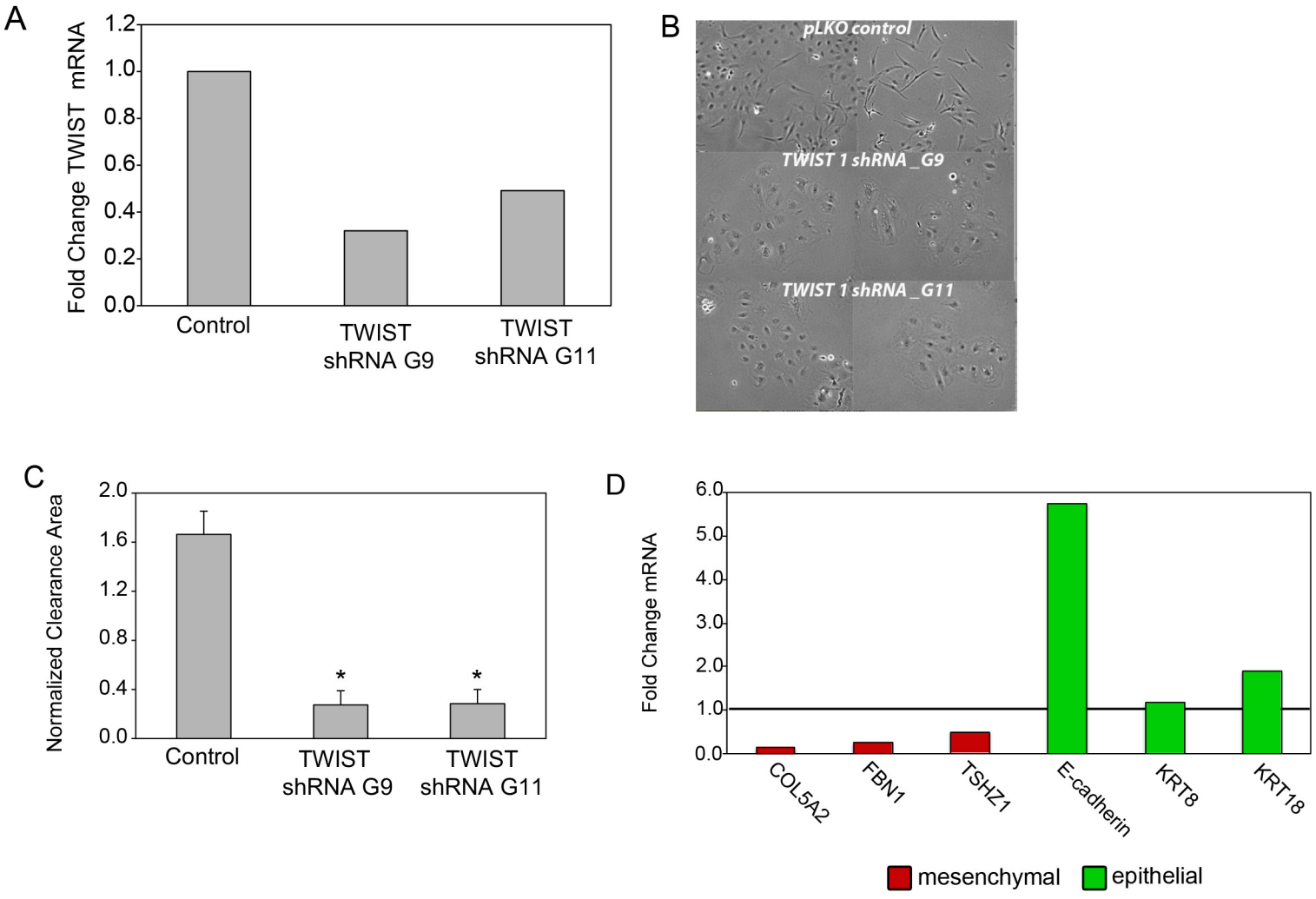
Supplementary Figure 2



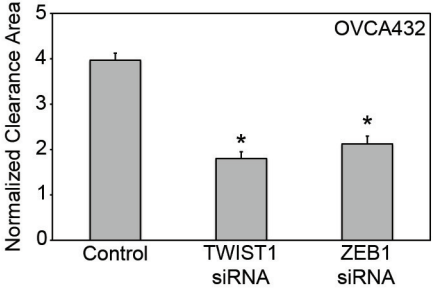
Supplementary Figure 3



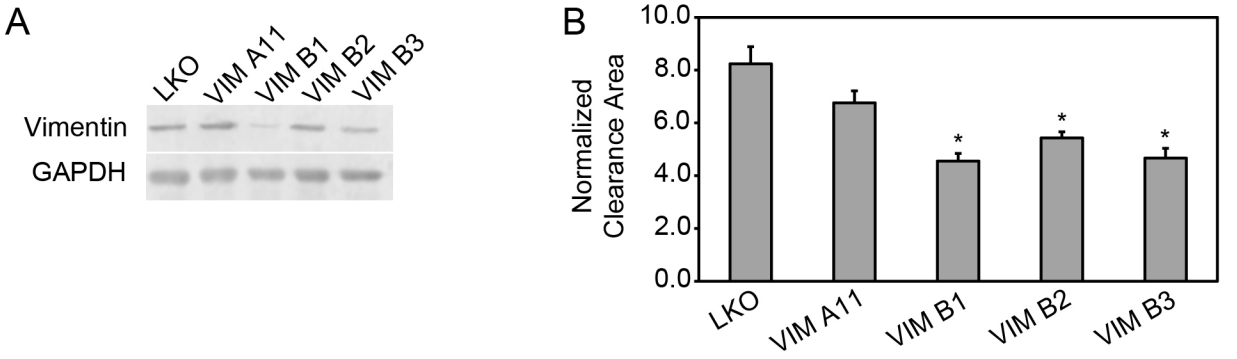
Supplementary Figure 4



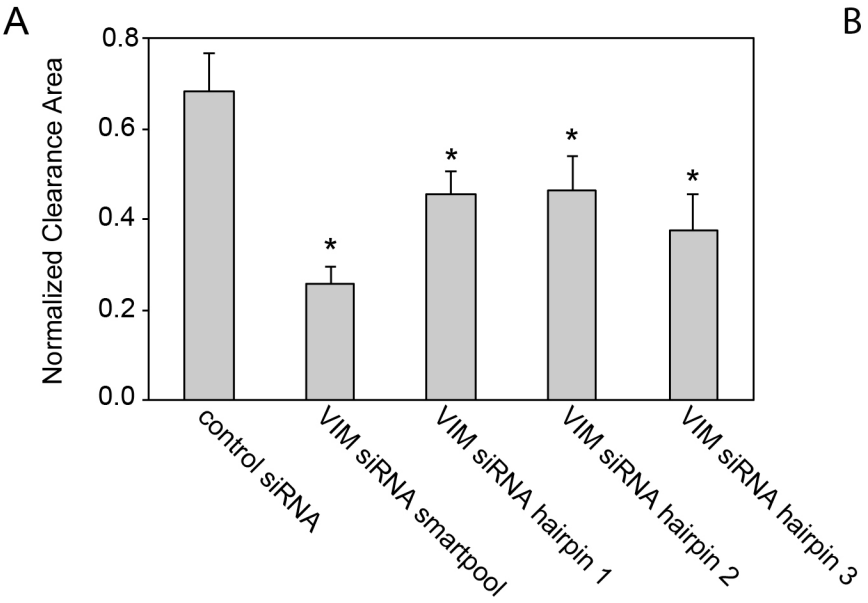
Supplementary Figure 5

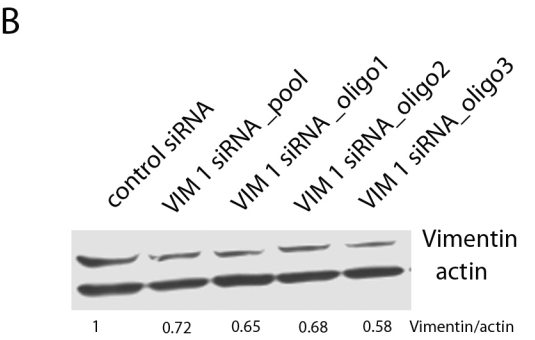


Supplementary Figure 6



Supplementary Figure 7:



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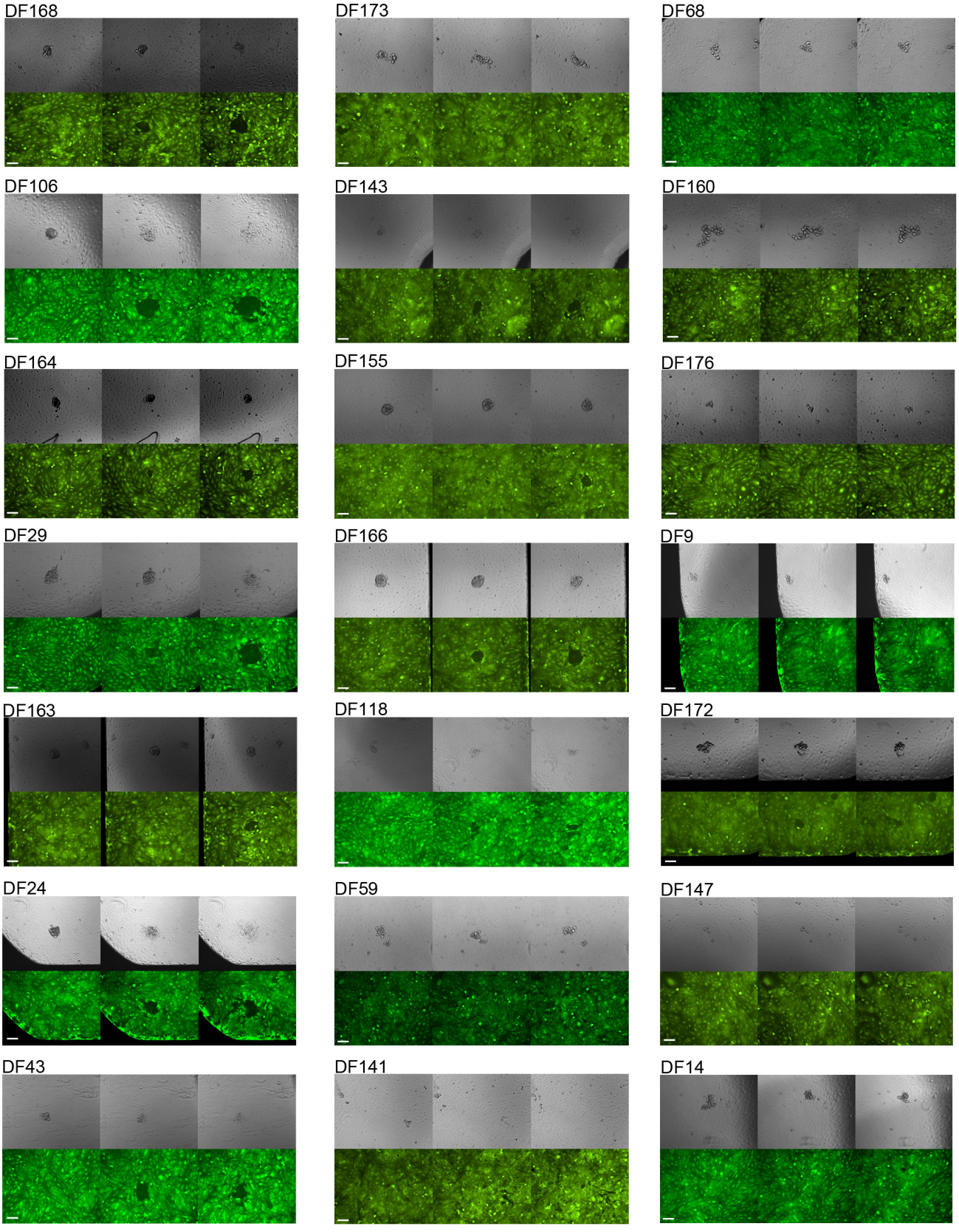

Supplementary Figure 9