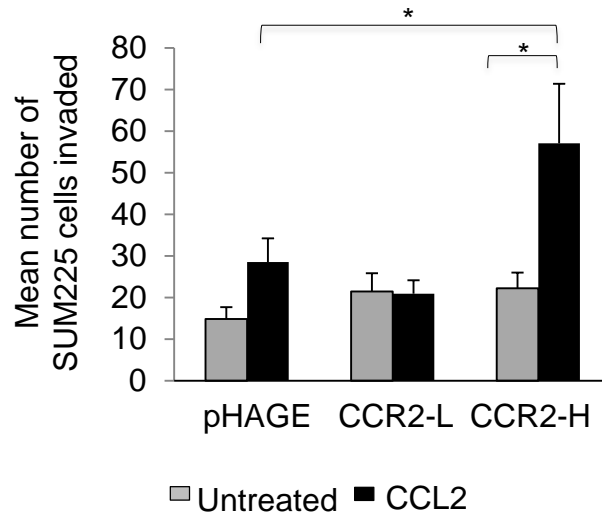
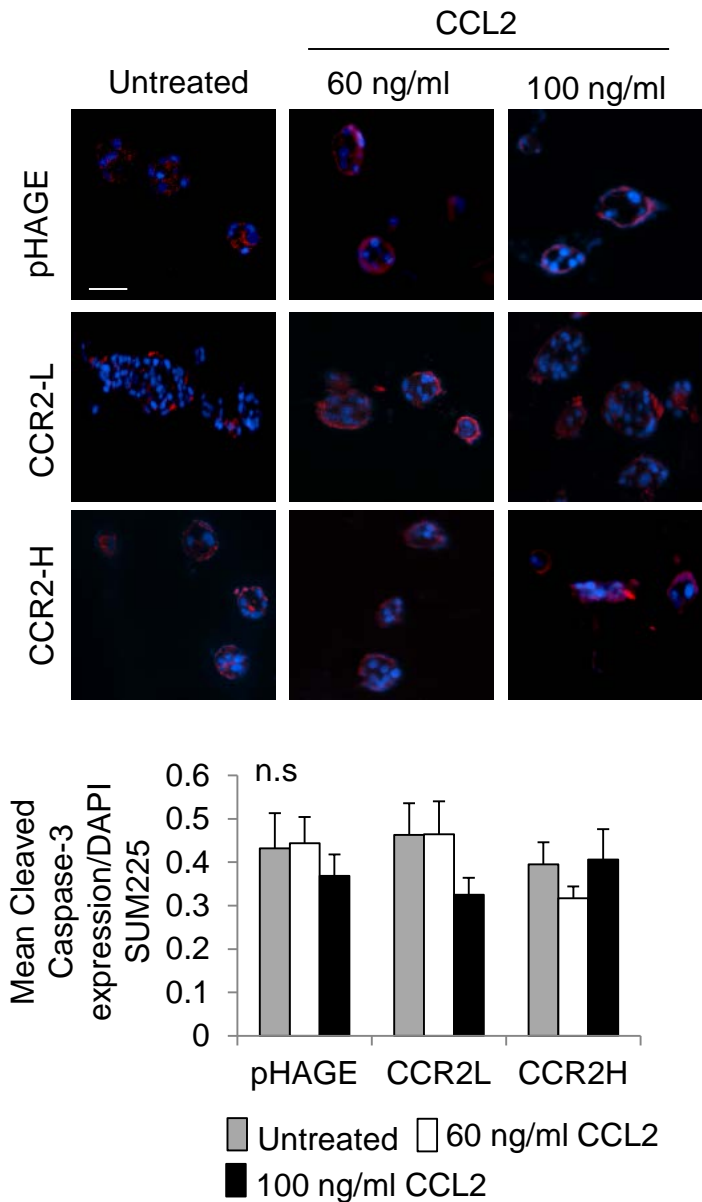


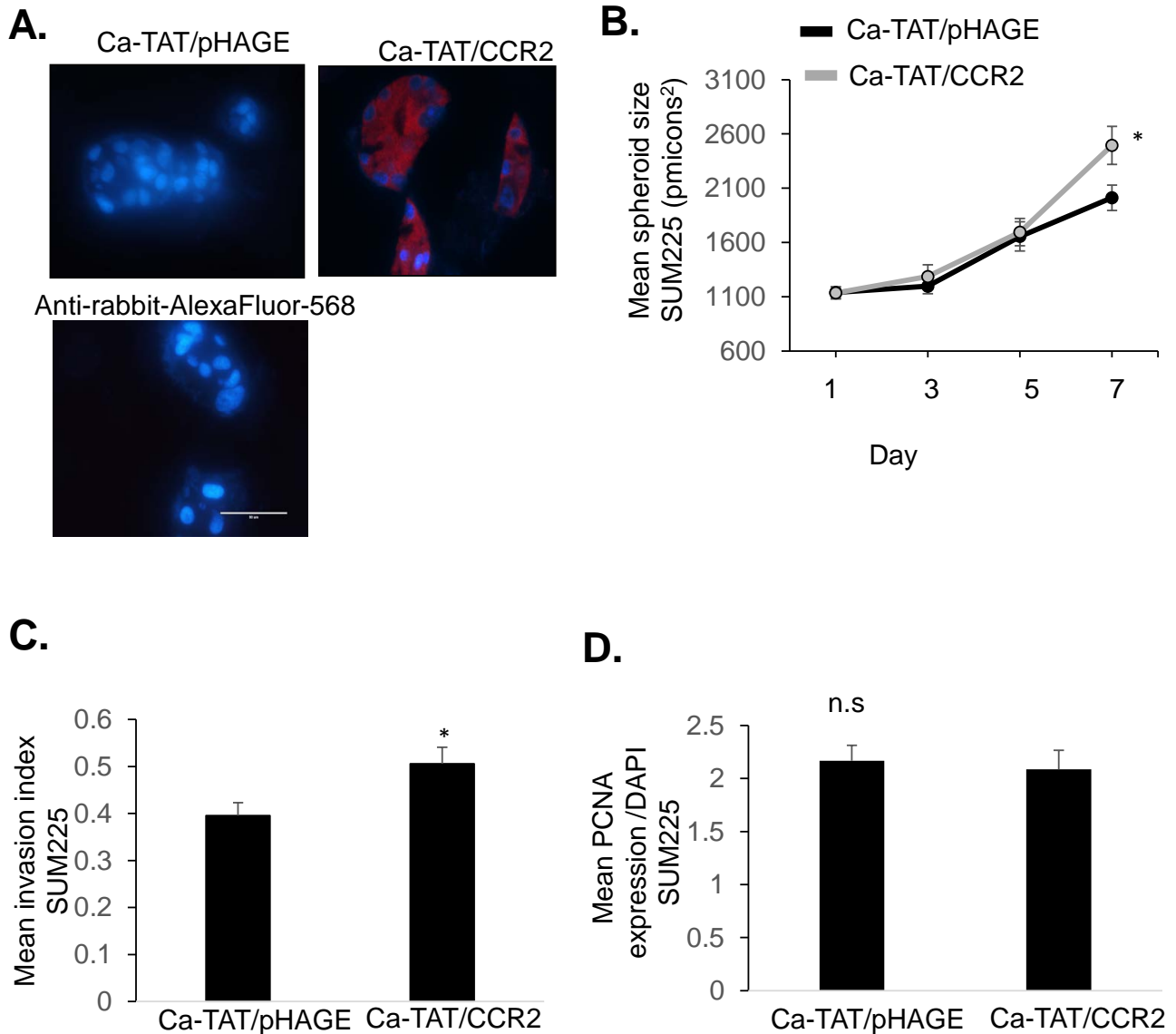
**Figure S1. Effect of CCR2 overexpression on CCL2 expression.** Conditioned medium from pHAGE or CCR2-H SUM225 cells were analyzed for CCL2 expression by ELISA. Statistical analysis was performed by Two Tailed T-test. Statistical significance was determined by \* $p < 0.05$ . Mean  $\pm$  SEM are shown.



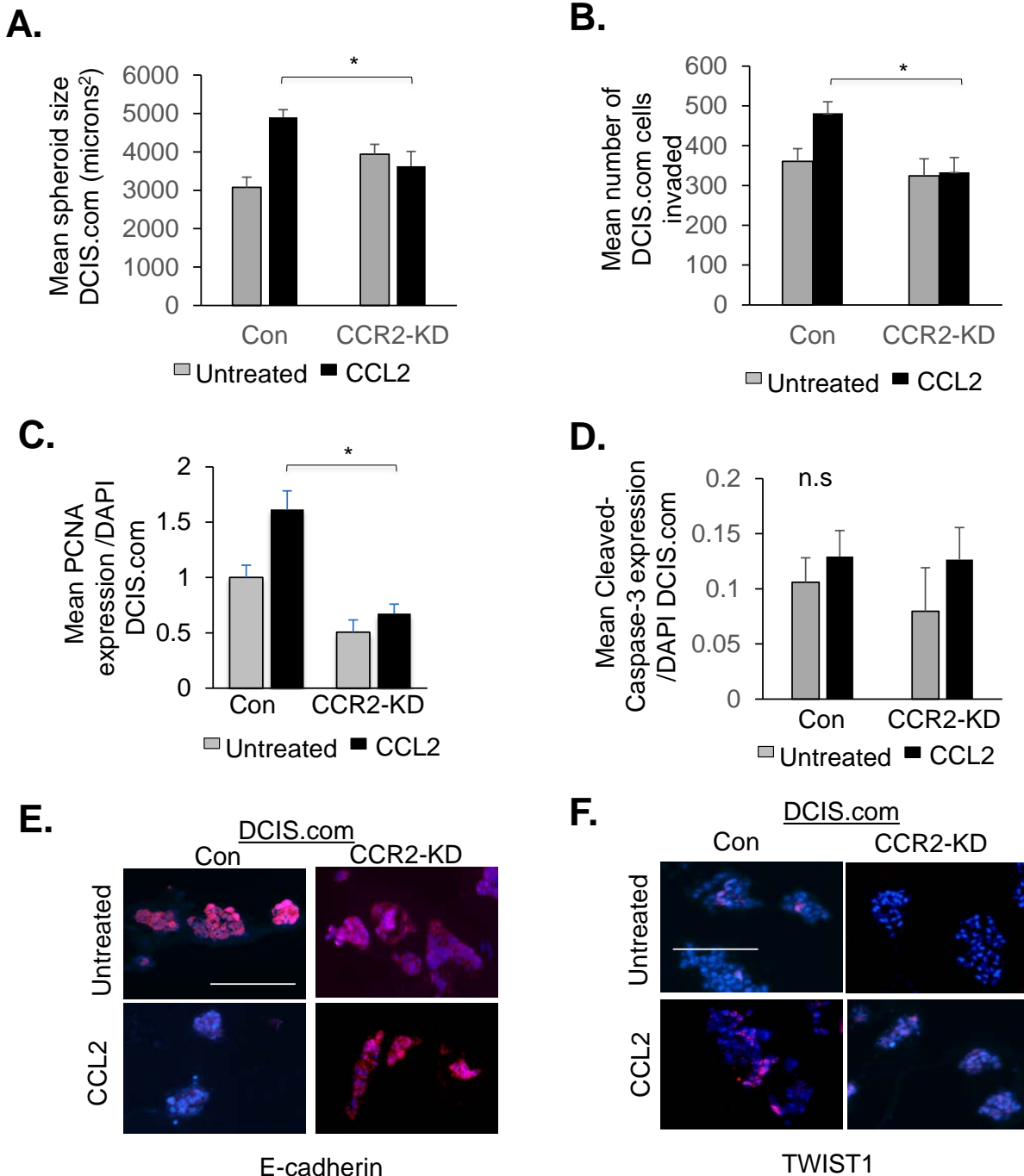
**Figure S2. CCL2 enhances transwell invasion of CCR2 overexpressing SUM225 cells.** pHAGE control or CCR2 overexpressing SUM225 breast cancer cells were treated with 100 ng/ml CCL2 and analyzed for invasion through Matrigel coated transwells. Statistical analysis was performed using One Way ANOVA with Bonferroni post-hoc comparison. Statistical significance was determined by  $p < 0.05$ . \* $p < 0.05$ . Mean  $\pm$  SEM are shown.



**Figure S3. CCR2 overexpression in SUM225 cells does not affect apoptosis.** SUM225 spheroids were immunofluorescence stained for Cleaved caspase-3 expression. Expression was quantified by Image J, and normalized to DAPI. Statistical analysis was performed using One Way ANOVA with Bonferroni post-hoc comparison. Statistical significance was determined by  $p < 0.05$ . ns.=not significant. Scale bar=100 microns. Mean+SEM are shown.

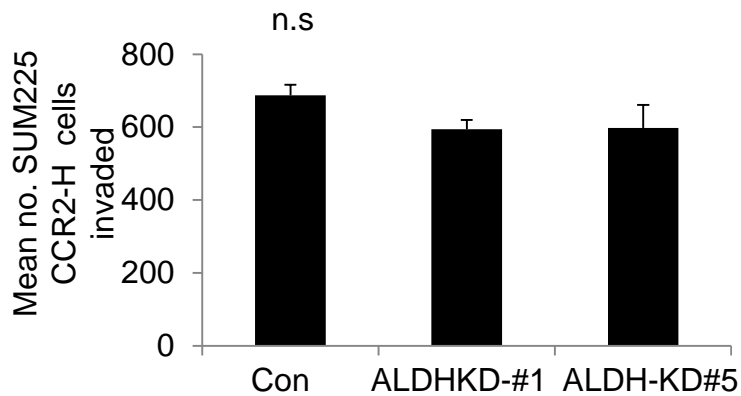


**Figure S4. Ca-TAT mediated induction of CCR2 expression enhances growth of SUM225 spheroids.** SUM225 breast cancer cells spheroids were seed in Matrigel:Collagen. After 2 days, cells were transfected with Ca-TAT peptides complexed to pHAGE vehicle control or CCR2 overexpression plasmid, and cultured for up to an additional 5 days. Cultures were analyzed by **A.** immunofluorescence staining for CCR2 expression, **B.** spheroid size over time, **C.** Invasion index and **D.** PCNA expression by IF staining. Spheroid size and invasion index are normalized to sphere number. Expression was quantified by Image J, and normalized to DAPI/sphere. Mean number  $\pm$  SEM spheroids analyzed: Ca-TAT/pHAGE=  $78 \pm 25$ , Ca-TAT/CCR2=  $79 \pm 18$ . Statistical analysis was performed using Two Tailed T-test. Statistical significance was determined by  $p < 0.05$ . \* $p < 0.05$ , n.s= not significant. Scale bar=50 microns. Mean  $\pm$  SEM are shown.

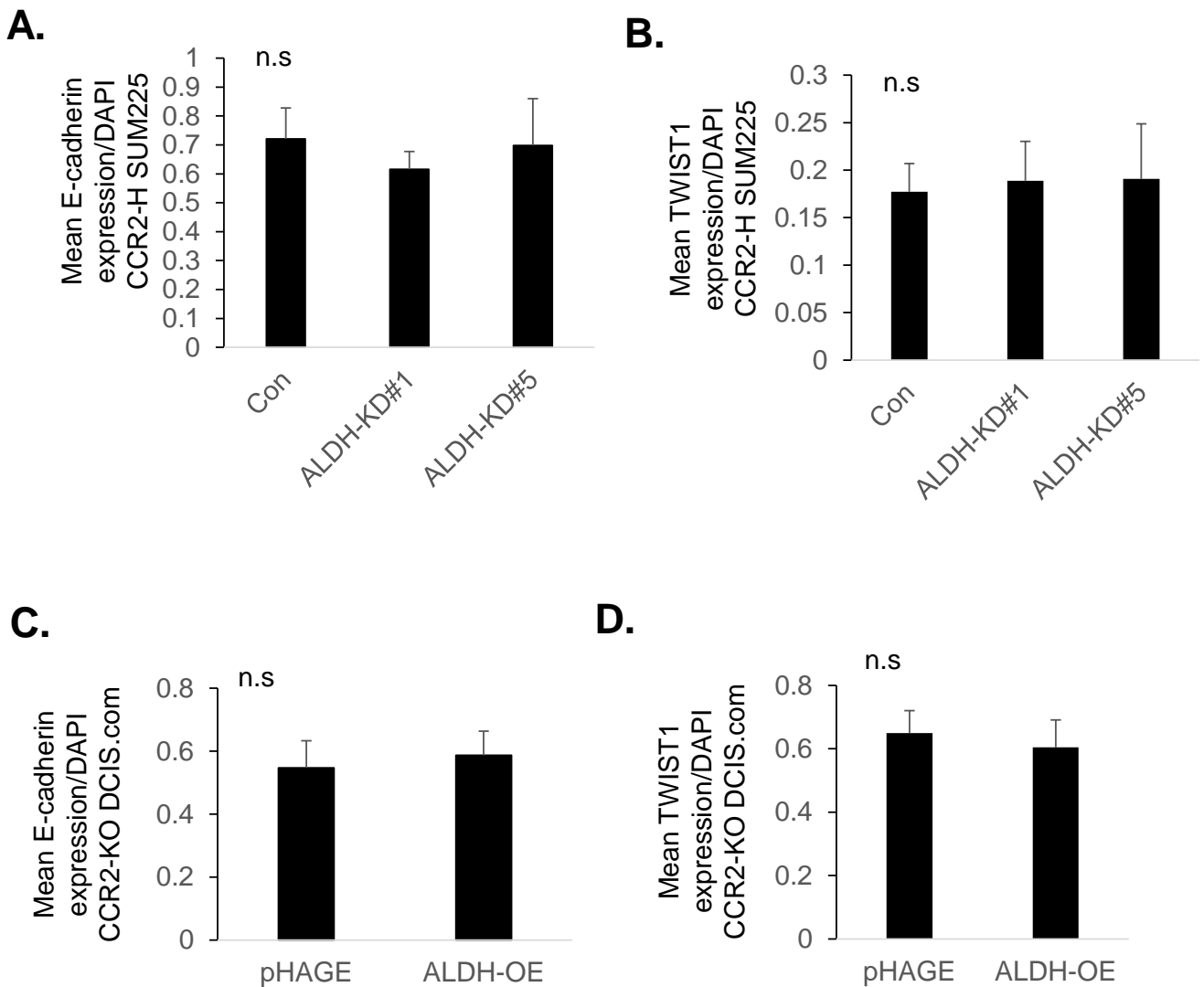


**Figure S5. Stable CCR2 shRNA expression in DCIS.com cells inhibits growth and invasion.** DCIS.com cells expressing Control (Con) CCR2 shRNAs (CCR2-KD) were treated with or without 60 ng/ml CCL2 and analyzed for **A.** growth in Matrigel: Collagen, **B.** transwell invasion, and expression of **C.** PCNA, **D.** Cleaved caspase-3, **E.** E-cadherin and **F.** TWIST1. Mean number of spheroids analyzed/group  $\pm$ SEM: Con Untreated= 248 $\pm$ 28, Con $\pm$ CCL2=243 $\pm$ 21, CCR2-KD Untreated=243 $\pm$ 41, CCR2-KD $\pm$ CCL2= 181 $\pm$ 3. Expression was quantified by Image J, and normalized to DAPI. Statistical analysis was performed using One Way ANOVA with Bonferroni post-hoc comparison. Statistical significance was determined by  $p < 0.05$ . Statistical significance was determined by  $p < 0.05$ .

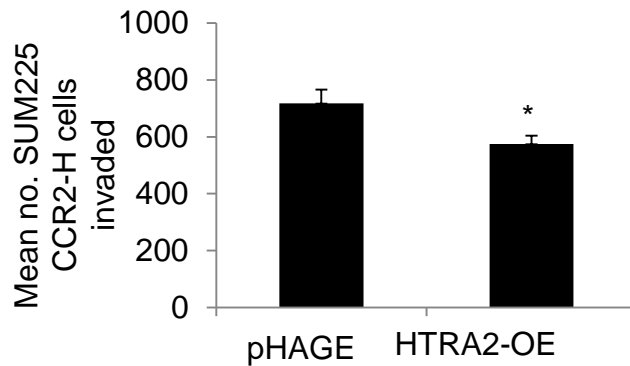
\* $p < 0.05$ , n.s.=not significant. Scale bar=100 microns. Mean $\pm$ SEM are shown.



**Figure S6. Effect of ALDH1 knockdown in SUM225 CCR2-H cells on transwell invasion.** SUM225 CCR2-H cells stably expressing control shRNAs (Con) or ALDH1 shRNAs (ALDH1-KD#1, ALDH1-KD#5) were analyzed for transwell invasion. Statistical analysis was performed using One Way ANOVA with Bonferroni post-hoc comparison. Statistical significance was determined by  $p < 0.05$ . n.s=not significant.

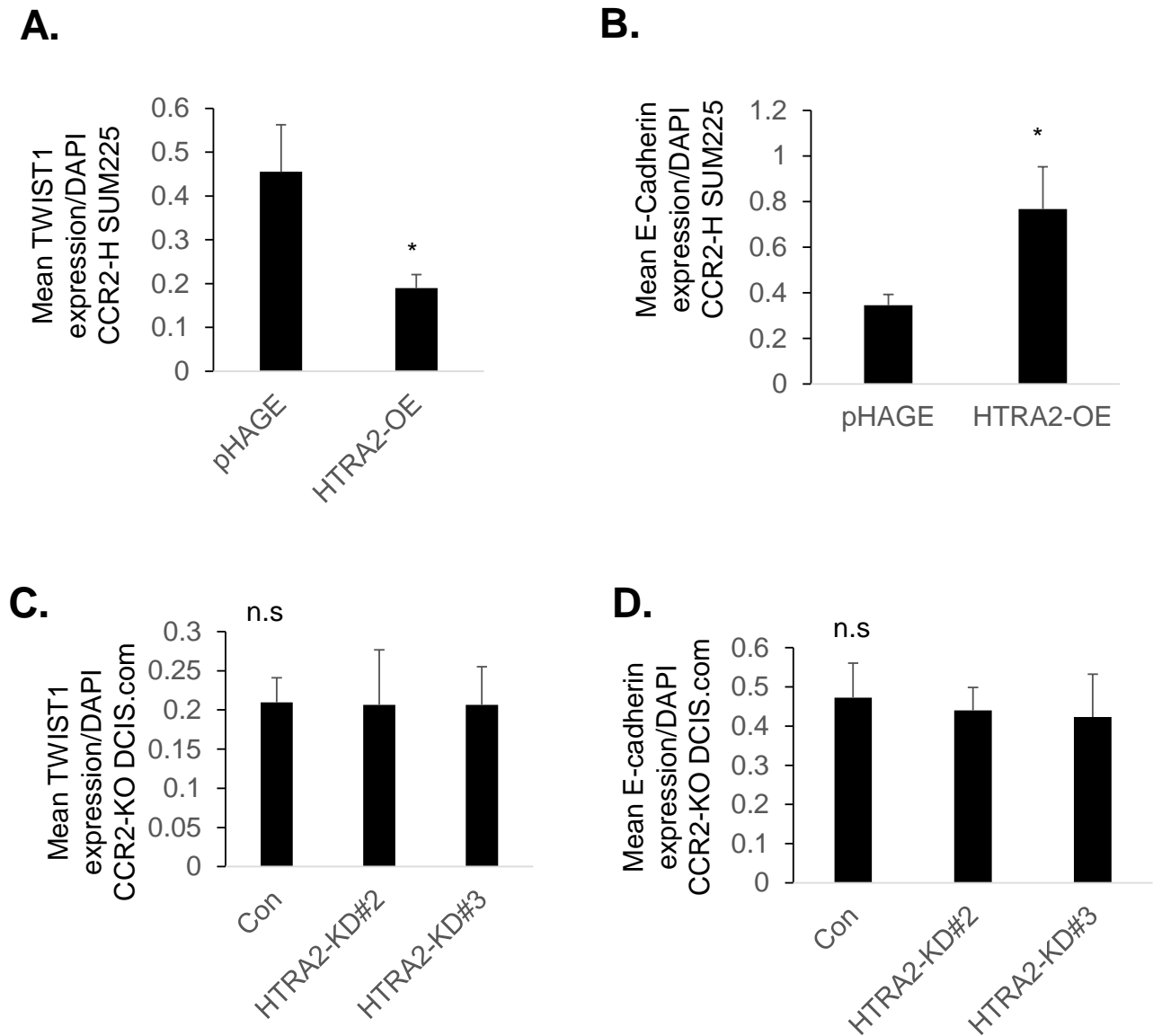


**Figure S7. Effect of ALDH1A1 knockdown and overexpression on TWIST1 and E-cadherin expression.** CCR2-H SUM225 cells expressing control shRNAs or shRNAs to ALDH1A1 were cultured in 3D Matrigel: Collagen for 10 days, and were analyzed for expression of **A.** E-cadherin and **B.** TWIST1 by immunofluorescence staining. CCR2-KO DCIS.com cells expressing pHAGE control or ALDH1A1 were cultured in 3D Matrigel: Collagen for 10 days, and were analyzed for expression of **C.** E-cadherin and **D.** TWIST1 expression. Expression was quantified by Image J, and normalized to DAPI. Statistical analysis was performed using One Way ANOVA with Bonferroni post-hoc comparison (A,B) or Two Tailed T-test (C,D). Statistical significance was determined by  $p < 0.05$ . Statistical significance was determined by  $p < 0.05$ . n.s.=not significant. Mean  $\pm$  SEM are shown.



**Figure S8. Effect of HTRA2 overexpression in SUM225 CCR2-H cells on transwell invasion.** SUM225 CCR2-H cells stably expressing pHAGE vehicle control or overexpressing HTRA2 were analyzed for transwell invasion. Statistical analysis was performed using Two Tailed T-test. Statistical significance was determined by  $p < 0.05$ . \* $p < 0.05$ . Mean  $\pm$  SEM are shown.





**Figure S9. Effect of HTRA2 overexpression and knockdown on TWIST1 and E-cadherin expression.** CCR2-H SUM225 cells expressing pHAGE control or HTRA2 were cultured in 3D Matrigel: Collagen for 10 days, and were analyzed for expression of **A.** TWIST1 and **B.** E-cadherin expression. CCR2-KO DCIS.com cells expressing control shRNAs or shRNAs to HTRA2 were cultured in 3D Matrigel: Collagen for 10 days, and were analyzed for expression of **C.** TWIST1 and **D.** E-cadherin by immunofluorescence staining. Expression was quantified by Image J, and normalized to DAPI. Statistical analysis was performed using Two Tailed T-test (A,B) or One Way ANOVA with Bonferroni post-hoc comparison (C,D). Statistical significance was determined by  $p < 0.05$ . \* $p < 0.05$ , n.s=not significant. Mean  $\pm$  SEM are shown.