Supplementary Materials

Expression of E-cadherin in epithelial cancer cells increases cell motility and directionality through the localization of ZO-1 during collective cell migration

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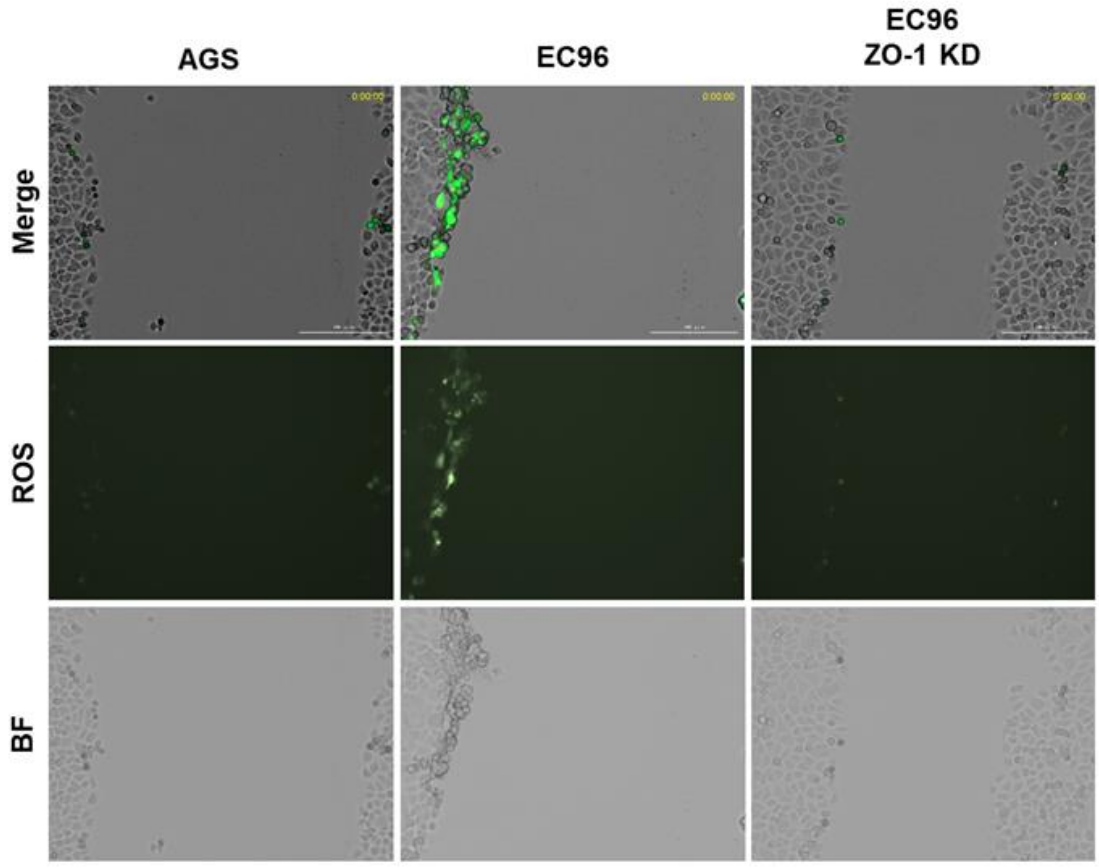


Figure S1. Detection of ROS in migrating cells. Intracellular ROS levels were also observed by live cell imager (magnification, ×100). The green fluorescence of DCF represents the levels of intracellular ROS.

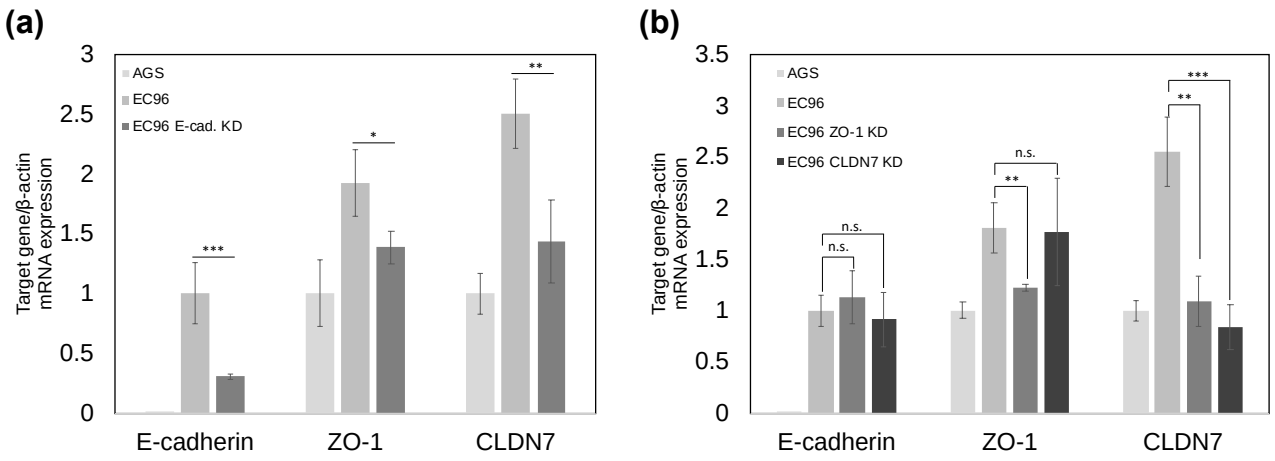


Figure S2. Re-expression of E-cadherin regulates ZO-1 and CLDN7 expression. (a) Total RNA extracted from AGS, EC96 and EC96 E-cad. KD cells were subjected to RT-PCR with E-cadherin, ZO-1 and CLDN7-specific primers. (b) Total RNA extracted from AGS, EC96, EC96 ZO-1 KD and EC96 CLDN7 KD cells were subjected to RT-PCR with E-cadherin, ZO-1 and CLDN7-specific primers. Averages of three independent experiments with error bars are presented. *P<0.05; **P<0.01; ***P<0.001; n.s., not significant.

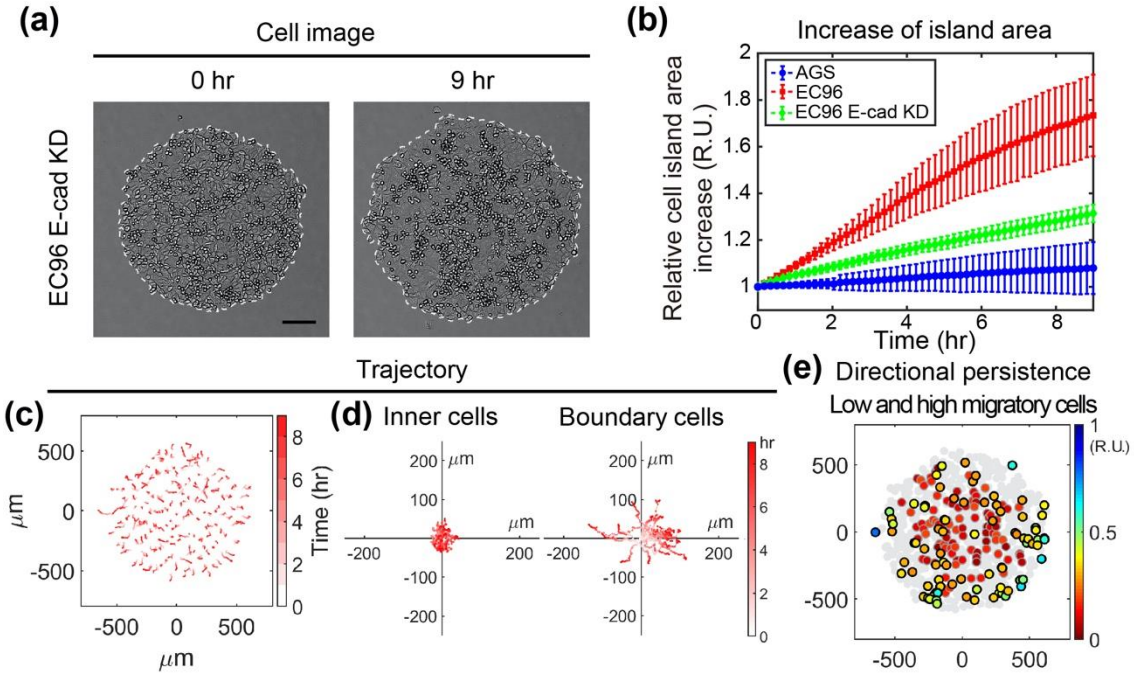
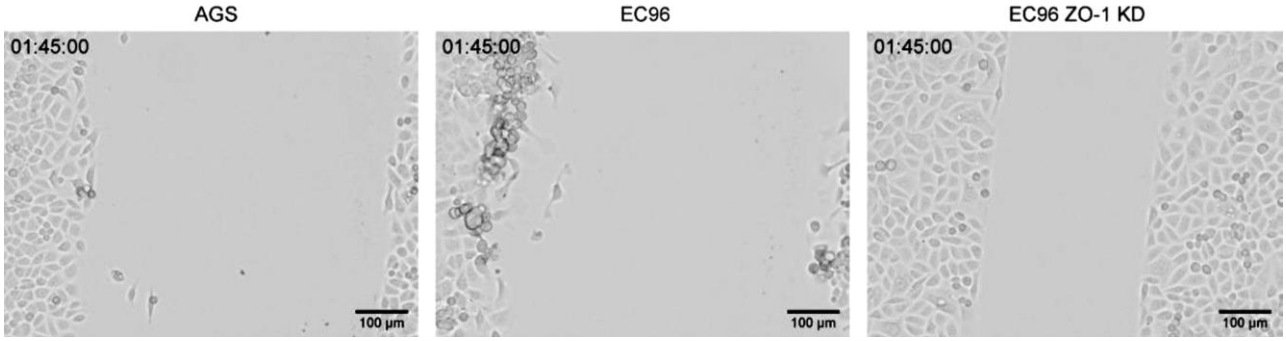


Figure S3. Analysis of motility and directionality of cell islands composed of E-cadherin knockdown cell line and comparison with AGS and EC96. (a) Images of E-cadherin KD cell islands at 0 hr and 9 hr after release (scale bar = 200 μm). (b) Relative area increase of cell islands composed of the AGS, EC96 and E-cadherin KD cells over time (n = 5). (c) Trajectory of the cells within the E-cadherin KD cell island. (d) Trajectory from the initial locations of the inner and boundary cells within the E-cadherin KD cell island. (e) Color-code map of the directional persistence of the lowest 25% and the highest 25% of motile cells within the E-cadherin KD cell island. Gray circles represent mid-quartile (25 – 75%) motile cells within each group population.

Table S1. Forward and reverse primers for real-time quantitative RT-PCR

Gene	Forward primer	Reverse primer
E-cadherin	ACC ATT AAC AGG AAC ACA GG	CAG TCA CTT TCA GTG TGG TG
ZO-1	TGC CAT TAC ACG GTC CTC TG	GGT TCT GCC TCA TTT CCT C
CLDN1	TTC TCG CCT TCC TGG GAT G	CTT GAA CGA TTC TAT TGC CAT ACC
CLDN7	TGA GAG CAA GGC TGG GTA C	TGG GAA TGA ATG TCG AGA TAC G
β-actin	ATC TAC GAG GGG TAT GCC	TAG CTC TTC TCC AGG GAG



Vides S1. Time-lapse microscopy of cell migration