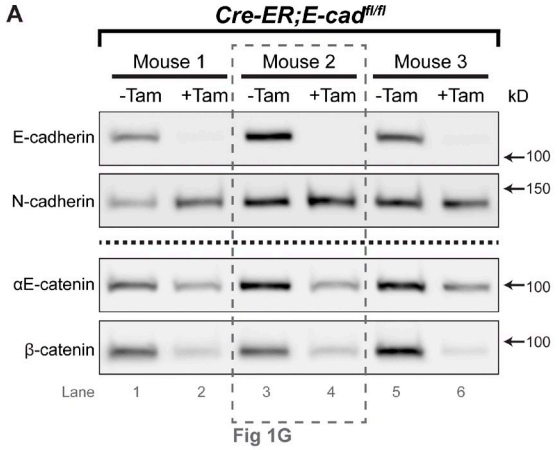
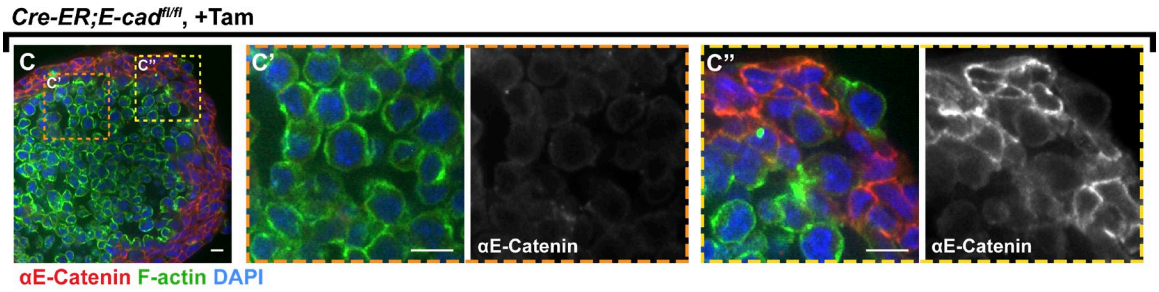
Shamir et al., http://www.jcb.org/cgi/content/full/jcb.201306088/DC1



B

	Band Intensity						P-value
	Mouse 1		Mouse 2		Mouse 3		
	-Tam	+Tam	-Tam	+Tam	-Tam	+Tam	
E-cadherin	3395	77	6931	0	4984	88	**0.004**
N-cadherin	3129	6336	7820	9378	8068	6667	**0.58**
αE-catenin	4865	2532	6874	2816	6616	3173	**0.008**
β-catenin	7954	2485	7468	2551	9788	1177	**0.002**



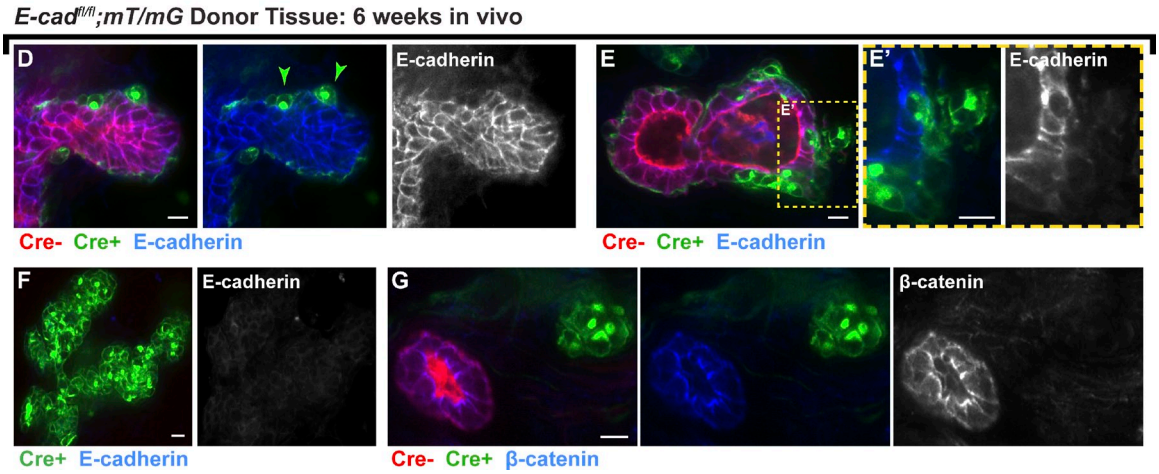


Figure S1. ***E-cad* deletion induced loss of adherens junction proteins in 3D culture and in vivo.** (A) Organoids were isolated from three *Cre-ER;E-cad*$^{fl/fl}$ mice, and *E-cad* deletion was induced with tamoxifen in half of the organoids from each mouse. Protein was extracted on day 6 in culture and assayed for levels of adherens junction components by Western blot. Whole cell lysate samples were loaded for equal protein based on BCA analysis. The dotted line indicates two separate blots, prepared from the same samples in parallel, each probed with different antibodies. (B) Fiji was used to quantify intensity of all bands in A. E-cad was essentially absent after gene deletion ($P = 0.004$, one-tailed Student's *t* test with equal variance). αE- and β-catenin were significantly reduced, whereas N-cad did not change (two-tailed Student's *t* test with equal variance). (C) Tamoxifen-treated *Cre-ER;E-cad*$^{fl/fl}$ organoids on day 6 had membrane-localized αE-catenin in a subset of cells near the basal surface. Most internal cells were αE-catenin^{-}. (D–G) In genetic mosaic *E-cad*$^{fl/fl}$*;mT/mG* outgrowths in vivo, green, Cre^{+} cells reliably lacked membrane-localized E-cad (D–F) and β-catenin (G). E-cad^{-} luminal cells were excluded from buds (D, arrowheads) and from polarized epithelium (E and E′) and were observed as unpolarized clusters at the injection site (F and G). Bars, 10 µm.

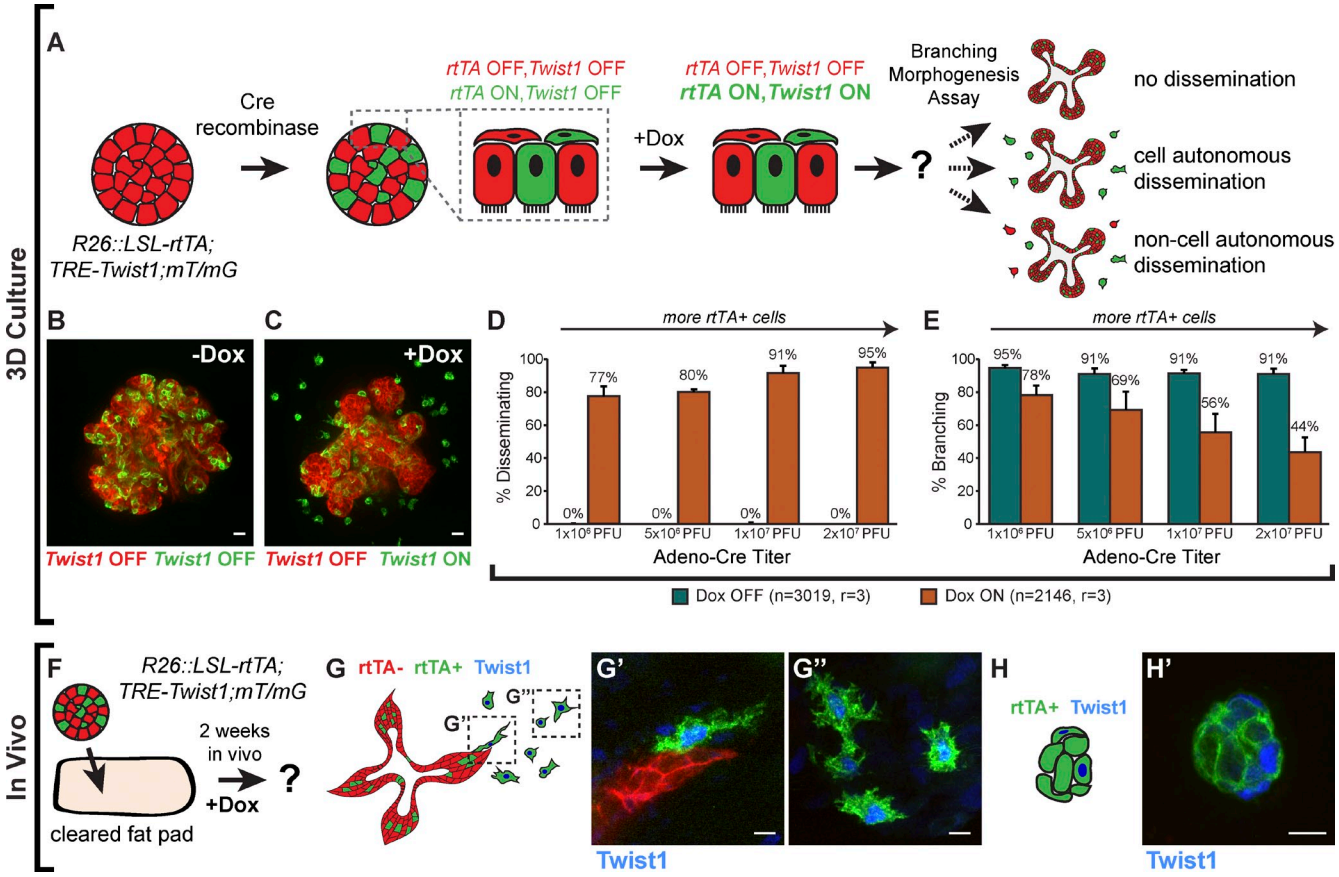
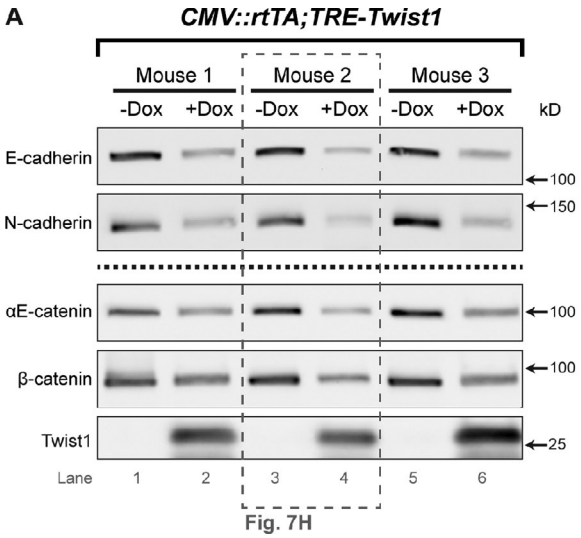


Figure S2. **Twist1-induced dissemination was cell autonomous, and Twist1 was sufficient for dissemination in vivo.** (A) A Cre-inducible rtTA (*R26::Lox-Stop-Lox-rtTA-IRES-EGFP*) and varying titers of Adeno-Cre were used to activate *rtTA* and *Twist1* expression in a labeled subset of epithelial cells. The *mT/mG* biosensor was used as an indirect marker of rtTA$^+$ cells (green), and dissemination was monitored in the branching morphogenesis assay. (B) Without doxycycline, *Twist1* expression was off, and organoids branched normally, with a mixture of red and green cells. (C) With doxycycline, *Twist1* expression was induced in green, rtTA$^+$ cells, and organoids exclusively disseminated green cells. Red, rtTA$^-$Twist1$^-$ cells formed normal branched structures and did not disseminate. (D and E) The dose of Adeno-Cre was titrated to vary the number of rtTA$^+$ cells per organoid, and branching and dissemination were quantified on day 7 in culture. With doxycycline, a high percentage of organoids disseminated cells, even at Adeno-Cre titers that produced few rtTA$^+$Twist1$^+$ cells per organoid (D). Increasing the number of rtTA$^+$Twist1$^+$ cells per organoid resulted in a decrease in branching (E). *n*, total number of organoids; *r*, number of biological replicates. Error bars indicate SD. (F) Adeno-Cre–transduced *R26::LSL-rtTA;TRE-Twist1;mT/mG* organoids were transplanted into cleared mammary fat pads of 3-wk-old NOD/SCID mice. *Twist1* expression was induced before transplantation by overnight incubation with doxycycline and maintained in vivo with doxycycline feed for 2 wk. (G) Red, Twist1$^-$ cells maintained epithelial organization. Green, Twist1$^+$ cells appeared mesenchymal and protrusive and disseminated locally into the fat pad. (H) Small, disorganized clusters of exclusively green cells containing Twist1$^+$ cells. Gamma adjustments were performed in G and H to improve image clarity. Bars: (B and C) 20 µm; (G′, G″, and H′) 10 µm.



B

	Band Intensity						P-value
	Mouse 1		Mouse 2		Mouse 3		
	-Dox	+Dox	-Dox	+Dox	-Dox	+Dox	
E-cadherin	5618	2086	4941	1002	4725	2025	**0.002**
N-cadherin	4854	2237	4831	1109	7006	2091	**0.01**
αE-catenin	4163	2724	4940	1626	5876	3468	**0.03**
β-catenin	8057	5683	7208	3499	7299	5397	**0.02**
Twist1	218	9331	113	7443	175	12348	**0.001**

Figure S3. **Adherens junction protein levels were partially reduced in *Twist1*-expressing tissue.** (A) Organoids were isolated from three *CMV::rtTA;TRE-Twist1* mice, and *Twist1* expression was induced with doxycycline in half of the organoids from each mouse. Protein was extracted after 5 d of *Twist1* induction and assayed for levels of adherens junction components by Western blot. Whole cell lysate samples were loaded for equal protein based on BCA analysis. The dotted line indicates two separate blots, prepared from the same samples in parallel, each probed with different antibodies. (B) Fiji was used to quantify intensity of all bands in A. Twist1 protein was verified to be absent without doxycycline ($P = 0.001$, one-tailed Student's *t* test with equal variance). E-cad, N-cad, αE-catenin, and β-catenin were all significantly reduced in *Twist1*-expressing tissue (two-tailed Student's *t* test with equal variance).

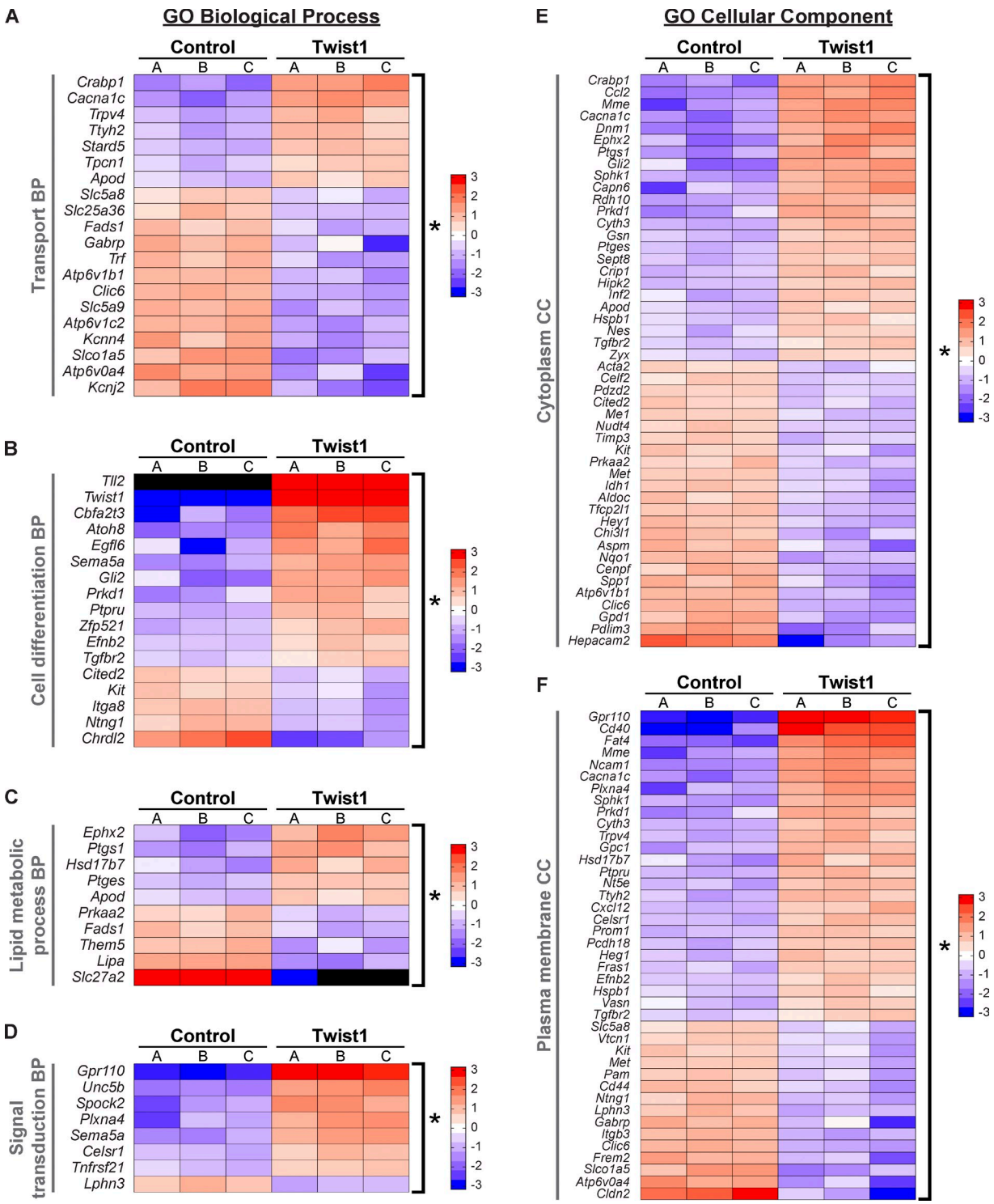


Figure S4. **Heat maps of DE genes within relevant GO Slim biological process and cellular component categories.** (A–D) Heat maps of DE genes associated with the GO biological process categories: transport (A), cell differentiation (B), lipid metabolic process (C), and signal transduction (D). (E and F) Heat maps of DE genes associated with the GO cellular component categories: cytoplasm (E) and plasma membrane (F). Genes are sorted by descending fold change. BP, biological process; CC, cellular component.

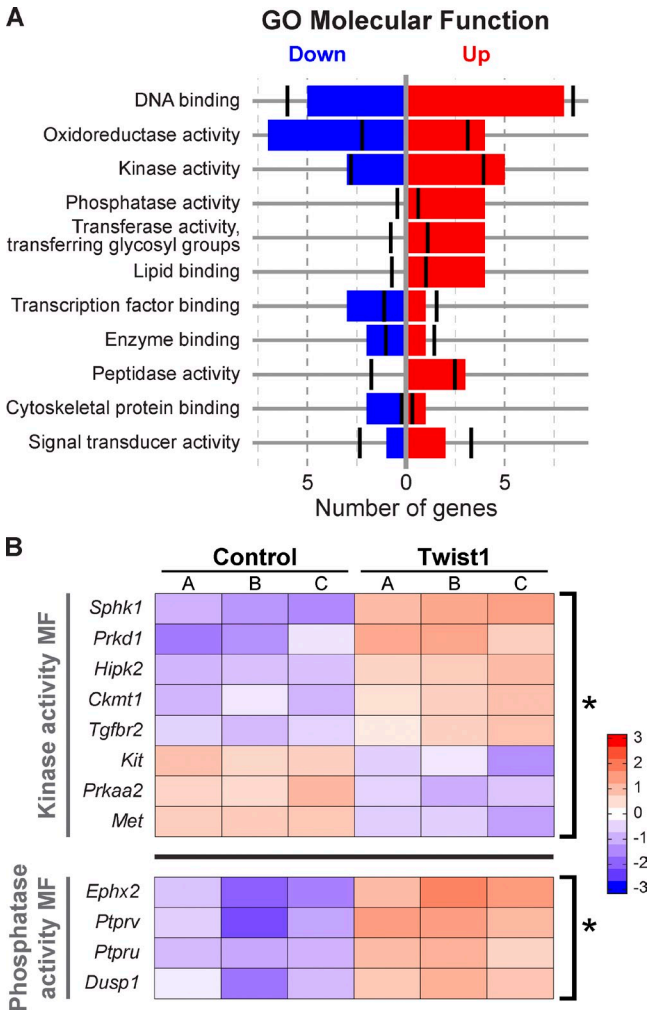
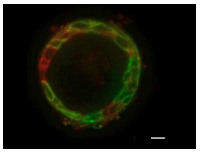
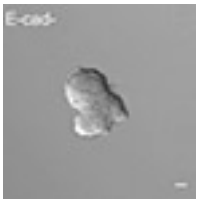


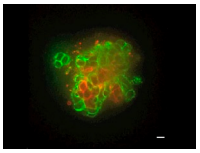
Figure S5. **Enzymatic activities up-regulated by Twist1 offer candidate targets for blocking dissemination.** (A) The 183 DE genes were mapped to direct associations with GO Slim molecular function terms. Black vertical bars indicate the expected number of DE genes per category. No terms were significantly enriched. (B) Heat map of DE genes associated with kinase activity and phosphatase activity. Genes are sorted by descending fold change. MF, molecular function.



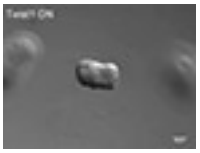
Video 1. ***E-cad* deletion induced a transition from simple to multilayered epithelium.** Representative confocal time-lapse movie (2D slice) of cell shape changes after *E-cad* deletion in a *Cre-ER;E-cad$^{fl/fl}$;mT/mG* organoid (corresponds to Fig. 1 H). E-cad^{-} cells (green) changed from simple columnar to round and shifted apically, abolishing simple architecture. Frames were collected every 20 min for 42 h (displayed at 15 frames/s) using a spinning-disk confocal microscope (Solamere Technology Group Inc.) with an LD C-Apochromat 40×/1.1 W Korr objective lens (Carl Zeiss). Bar, 10 µm.



Video 2. ***E-cad* deletion blocked branching morphogenesis in response to FGF2 and induced disorganization at the basal surface.** Representative DIC time-lapse movies of an E-cad^{+} organoid and an E-cad^{-} organoid cultured in 3D Matrigel in medium with 2.5 nM FGF2. Normal epithelium (left) initiated and elongated multiple epithelial buds that maintained a smooth basal surface with the ECM (corresponds to Fig. 3 B). E-cad^{-} epithelium (right) failed to initiate and elongate buds (corresponds to Fig. 3 C). Instead, E-cad^{-} cells displayed extensive uncoordinated motility at the basal surface. Frames were collected every 20 min for 108 h (displayed at 24 frames/s) using a Cell Observer system with an AxioObserver Z1 and an AxioCam MRM camera (Carl Zeiss). Bars, 50 µm.

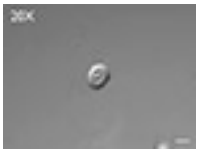


Video 3. **Branching morphogenesis in genetic mosaic *E-cad$^{fl/fl}$* epithelium was autonomous to E-cad^{+} cells.** Representative confocal time-lapse movie (2D slice, followed by 3D reconstruction) of a genetic mosaic *E-cad$^{fl/fl}$;mT/mG* organoid cultured in 3D Matrigel in medium with 2.5 nM FGF2 (corresponds to Fig. 3 H). E-cad^{+} cells (red) initiated buds from disorganized E-cad^{-} cell groups (green). Frames were collected every 20 min for 31 h (displayed at 15 frames/s) using a spinning-disk confocal microscope (Solamere Technology Group Inc.) with an LD C-Apochromat 40×/1.1 W Korr objective lens (Carl Zeiss). Bar, 10 µm.



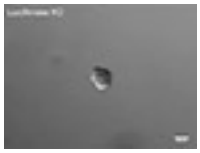
Video 4. ***Twist1* induction induced rapid and robust epithelial cell dissemination.** Representative DIC time-lapse movies of *CMV::rtTA;TRE-Twist1* organoids isolated from the same mouse cultured in 3D Matrigel in basal medium. Without *Twist1* induction (no doxycycline, left), the epithelium maintained a smooth basal surface with the ECM (corresponds to Fig. 5 B). With *Twist1* induction (5 µg/ml doxycyline, right), single cells rapidly disseminated into the ECM with extensive protrusions (corresponds to Fig. 5 C). Videos start 2 h after doxycyline addition. Frames were collected every 20 min for 161 h (displayed at 24 frames/s) using a Cell Observer system with an AxioObserver Z1 and an AxioCam MRM camera (Carl Zeiss). Bars, 50 µm.

Video 5. ***Twist1* induction blocked branching morphogenesis and induced robust cell dissemination and secondary site formation.** Representative DIC time-lapse movies of *CMV::rtTA;TRE-Twist1* organoids isolated from the same mouse cultured in 3D Matrigel in medium with 2.5 nM FGF2. Without *Twist1* induction (no doxycycline, left), the epithelium branched efficiently, and no cells disseminated (corresponds to Fig. 5 D). With *Twist1* induction (5 µg/ml doxycyline, right), the epithelium failed to branch, and cells rapidly disseminated and formed secondary epithelial sites (corresponds to Fig. 5 E). Videos start 2 h after doxycycline addition. Frames were collected every 20 min for 161 h (displayed at 24 frames/s) using a Cell Observer system with an AxioObserver Z1 and an AxioCam MRM camera (Carl Zeiss). Bars, 50 µm.

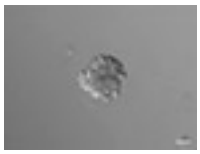


Video 6. **Disseminated epithelial cells stopped migrating once *Twist1* expression ceased.** Representative DIC time-lapse movie of a *CMV::rtTA;TRE-Twist1* organoid cultured in 3D Matrigel in basal medium with a 48-h pulse of doxycycline (corresponds to Fig. 6 B). When *Twist1* expression ceased, disseminated cells stopped migrating through the ECM. Video starts 2 h after doxycycline addition. Frames were collected every 20 min for 161 h (displayed at 24 frames/s) using a Cell Observer system with an AxioObserver Z1 and an AxioCam MRM camera (Carl Zeiss). Bar, 50 µm.

Video 7. **Epithelium initiated branching morphogenesis once *Twist1* expression ceased.** Representative DIC time-lapse movie of a *CMV::rtTA;TRE-Twist1* organoid cultured in 3D Matrigel in medium with 2.5 nM FGF2 and a 48-h pulse of doxycycline (corresponds to Fig. 6 C). When *Twist1* expression ceased, the epithelium initiated new buds, and disseminated cells reintegrated with the main epithelial group. Video starts 2 h after doxycyline addition. Frames were collected every 20 min for 161 h (displayed at 24 frames/s) using a Cell Observer system with an AxioObserver Z1 and an AxioCam MRM camera (Carl Zeiss). Bar, 50 µm.



Video 8. ***E-cad* KD blocked Twist1-induced single cell dissemination.** Representative DIC time-lapse movies of *CMV::rtTA;TRE-Twist1* organoids isolated from the same mouse treated with lentiviral shRNA against *Luc* or *E-cad*. Organoids were cultured in 3D Matrigel in medium with 2.5 nM FGF2, and *Twist1* expression was induced with doxycycline. With *Luciferase* KD (left), the epithelium robustly disseminated (corresponds to Fig. 10 C). With *E-cad* KD (clone #1, middle; clone #2, right), the epithelium disseminated significantly fewer cells (corresponds to Fig. 10, D and E). Videos start 3 h after doxycycline addition. Frames were collected every 20 min up to 100 h (displayed at 24 frames/s) using a Cell Observer system with an AxioObserver Z1 and an AxioCam MRM camera (Carl Zeiss). Bars, 50 µm.



Video 9. ***E-cad* KD in *Twist1*-expressing tissue promoted collective epithelial migration.** Representative DIC time-lapse movie of a *CMV::rtTA;TRE-Twist1* organoid treated with lentiviral shRNA against *E-cad* (clone #2) and cultured in 3D Matrigel in medium with 2.5 nM FGF2 and doxycycline (corresponds to Fig. 10 G). Concurrent *E-cad* KD and *Twist1* expression induced collective migration of long chains of cells. Video starts 7 h after doxycycline addition. Frames were collected every 20 min for 119 h (displayed at 24 frames/s) using a Cell Observer system with an AxioObserver Z1 and an AxioCam MRM camera (Carl Zeiss). Bar, 50 µm.

Table S1 lists all genes sequenced by RNA-seq, sorted by p-value, and the 183 DE genes along with their associated GO Slim terms, and is provided as a Microsoft Excel file.

Table S2 lists RNA-seq raw counts and p-values for 127 epithelial genes involved in cell–cell adhesion and intercellular junctions, and is provided as a Microsoft Excel file.

Table S3 lists the significant canonical pathways and curated gene sets identified by gene set enrichment analysis, and is provided as a Microsoft Excel file.