Metastasis is the main cause of carcinoma-related death, yet we know little about how it initiates due to our inability to visualize stochastic invasion events. Classical models suggest that cells accumulate mutations that first drive formation of a primary mass, and then downregulate epithelia-specific genes to cause invasion and metastasis. Here, using transparent zebrafish epidermis to model simple epithelia, we can directly image invasion. We find that KRas-transformation, implicated in early carcinogenesis steps, directly drives cell invasion by hijacking a process epithelia normally use to promote death—cell extrusion. Cells invading by basal cell extrusion simultaneously pinch off their apical epithelial determinants, endowing new plasticity. Following invasion, cells divide, enter the bloodstream, and differentiate into stromal, neuronal-like, and other cell types. Yet, only invading KRasV12 cells deficient in p53 survive and form internal masses. Together, we demonstrate that KRas-transformation alone causes cell invasion and partial dedifferentiation, independently of mass formation.
Clinical cancer staging and most models suggest that metastasis arises as a sequential process, where cumulative mutations cause cells to form a primary mass, which then downregulate epithelia-specific genes to enable their detachment and invasion. Yet, metastases can arise independently of primary human tumours and mouse models of breast cancer and pancreatic cancer, indicating that metastases occur independently of primary masses. These findings and genetic signatures linked to poor prognosis suggest that some oncogenes are intrinsically invasive and that metastasis is not necessarily linked to primary tumours. However, due to our current inability to directly follow stochastic invasion events from epithelia where carcinomas originate, we lack insight into how cells invade. Our research on epithelial cell number homeostasis suggested an unexpected model. Normally, crowding forces trigger live cells to extrude out apically and die by contracting a ring of actomyosin basally, preserving both epithelial function and cell density.

However, Kras and other driver mutations of invasive cancers hijack extrusion, causing cells to instead form masses or abnormally induce basal cell extrusion (BCE), under the epithelium, where carcinomas initiate. We directly follow the fate of cells expressing or a loss-of-function mutant, and find that p53 expression did not significantly alter KRas-driven mass formation or BCE rates (Supplementary Fig. 3). By contrast, far more EGFP-CAAAX cells remained within the epidermis in p53 mutants, with none internalized by 5 dpf (Fig. 2c–e), suggesting that p53 mutation alone does not promote BCE and invasion. Thus, while KRas cells internalize, most die unless they lack functional p53.

Live imaging allowed us to test if BCE can drive cell invasion directly from epithelia. We found that KRas cells invade by BCE and then migrate throughout the zebrafish body (Fig. 3a, b, Supplementary Movie 8). These highly motile invaded cells do not represent macrophages that have engulfed EGFP-KRas cells, as movies labelling DNA indicate that internalized cells do not die (Supplementary Movie 9). When invaded cells do die, fragments that are engulfed lose EGFP signal rapidly (Supplementary Movie 7). Moreover, they do not colocalize with macrophages in movies or immunostained samples (Supplementary Movie 10 and Supplementary Fig. 2a, c). Following invasion, EGFP-KRas cells proliferate (Fig. 3c, Supplementary Movie 11, showing two divisions within 15 h). In addition, most EGFP-KRas cells (but not control EGFP-CAAX) embayed extruding cells, suggesting new plasticity. Around 50% of embryos contained neuron-like EGFP-KRas cells that elongate bidirectionally (Fig. 3e, h, Supplementary Fig. 4a and Supplementary Movie 15), rarely seen in EGFP-CAAX/eGFP embryos. While some neuron-like cells express the neuronal marker acetylated tubulin, others localizes along endogenous neurons (Fig. 3e, Supplementary Fig. 4b). In addition, ~15% invaded cells express the mesenchymal marker, N-cadherin (Fig. 3i, j). Notably, pancreatic cancer, also driven by Kras/p53 mutations, is predominantly stromal with significant neuronal involvement. Moreover, lineage-tracing studies of mouse pancreatic cancer suggest that stromal cells are partially derived from epithelia. Our data suggest that BCE drives not only cell invasion but can also enable new plasticity.

To determine how cells invading by BCE adopt new fates, we first assessed the epithelial markers, E-cadherin and ZO-1. We found all invaded EGFP-KRas/p53 cells lacked E-cadherin (340 cells in 25 fish), whereas those remaining in the epidermis retained E-cadherin (900 cells in 25 fish) (Fig. 4a). Interestingly, while most EGFP-CAAX cells remained at the epidermis (Fig. 4b, 13,006 cells in 30 fish), the few that invaded (Fig. 4c, 43 cells in 30 fish) were E-cadherin-negative, suggesting E-cadherin loss was tightly linked to BCE, rather than to oncogenic mutation per se. ZO-1 was similarly decreased in invaded EGFP-KRas/p53 cells (Supplementary Fig. 5a, b). E-cadherin expression remained absent in large internal masses in 5 dpf KRas/p53mut larvae (Fig. 4d). While much of EGFP signal within invading KRas cells is initially pinched off, its re-expression from the keratin 4 promoter suggests that cells invading by BCE continue to express this cytoplasmic epithelial marker as well as N-cadherin. Thus, cells invading by BCE lose cell surface epithelial determinants but retain cytoplasmic ones, with some adopting mesenchymal features. These findings could address why metastatic tumours can express both epithelial and mesenchymal markers.

How do invading EGFP-KRas/p53 cells lose apical epithelial markers? Dynasore treatment suggested that invasion and E-cadherin loss do not require dynamin-dependent endocytosis (Fig. 4e and Supplementary Fig. 4c, d). In addition, EGFP-KRas/p53 embryos rarely expressed the mesenchymal transcription factor.
factor snai1b in the epidermis or masses, compared to internalized cells, suggesting that BCE does not require snai1b-dependent transcription (Fig. 4f and Supplementary Fig. 5e).

While we cannot rule out by in situ hybridization that transient snai1b expression causes transcriptional downregulation of E-cadherin immediately before BCE, we think it unlikely for the following reasons: (i) reported E-cadherin protein perdurance of 3–5 h would predict some internalized cells would retain E-cadherin, (ii) all basally extruding CAAX cells also lose E-cadherin (Fig. 4c), (iii) only ~15% of invading cells express mesenchymal markers (Fig. 3j), suggesting invasion is independent of mesenchymal gene upregulation, and (iv) some wild-type epidermal cells also express snai1b (Supplementary Fig. 4 c, d), independent of KRasV12 expression.

To investigate how E-cadherin could suddenly disappear during BCE, we filmed cells expressing EGFP-directly tethered to KRasV12 (EGFP-dt-KRasV12) that localizes to the apical membrane. High-resolution imaging revealed that as a cell invades by BCE, the actin ring pinches off its entire apical membrane, releasing the cell underneath the epidermis (Fig. 4g, Fig. 1 KRasV12 induces formation of epidermal cell masses and basal extrusion at distinct sites. a 26 hpf wild-type zebrafish embryos expressing krt4:EGFP-CAAX or dt-KRasV12, masses indicated by black arrowheads and inset. b Mean of cell masses per embryo ± SEM, P < 0.0001 by a Mann-Whitney test, n = embryos. c Map of where cell masses occur, as observed at 28 hpf. d Stills from time-lapse imaging (hh:mm) of periderm:Lifeact-EGFP mosaically expressing EGFP-CAAX or EGFP-dt-KRasV12, showing hallmarks of apical and basal extrusion, with XZ sections beneath showing constriction at the base or apex, respectively (white arrows delimit ring constricting). e Number of apical and basal extrusions in EGFP-CAAX or dt-KRasV12 embryos. Data are mean ± SEM, **P < 0.02 by a Mann-Whitney test for apical and basal extrusions pooled together. f Maps indicating where apical and basal extrusions occur in 28-46 hpf embryos and 55–73 hpf embryos, where different colours represent different fish analyzed, n. Source data are provided as a Source Data file.
KRasV12-injected p53mut 48 hpf embryos. The white arrowhead indicates a KRasV12 cell internalized beneath p63+ basal keratinoctyes. **P < 0.001 by a Chi-Square test. Data are represented as boxplots where the middle line is the median, the lower and upper hinges correspond to the first and third quartiles, and the whiskers extend from the minimum to the maximum. All data points are shown. c Percentage of internalized or surface cells in CAAX or dt-KRasV12 48 hpf embryos compared to total EGFP+ cells in each embryo, n = embryos, ***P < 0.001 by a Fisher exact test. Source data are provided as a Source Data file.

Supplementary Movie16. This severing of the apical membrane explained why all cells invading by BCE experienced concomitant membrane blebbing and loss of green fluorescence before migrating from sites of extrusion (e.g. Supplementary Movies 8, 17, 18 and Fig. 3a). Moreover, E-cadherin colocalizes predominantly with apically localized actin in the periderm here and in other studies33 and becomes constricted into an apical point above a cell during BCE (Supplementary Fig. 5f and Movie 19 and Fig. 4h), suggesting it is also stripped from the invading cell. While BCE is reminiscent of cytokinesis, Fig. 4h and other images show the nucleus remains beneath the contractile ring, disfavouring asymmetric cell division as an invasion mechanism, seen in Src-expressing cells34.

To test if invasion and loss of epithelial determinants require BCE, we blocked this process using previously published methods. Because KRasV12 promotes basal extrusion through increased autophagic degradation of Sphingosine 1-Phosphate, a key signal for apical extrusion, we reported that blocking autophagy by ATG knockdown and several inhibitors rescues both S1P and apical extrusion16. To reverse autophagy in zebrafish without the lethality intrinsic to genetic methods35, we used chloroquine to impede KRasV12-dependent autophagy and BCE16. We find that chloroquine significantly decreased invasion of EGFP-KRasV12/p53MO zebrafish epidermal cells, compared to vehicle control (Fig. 4i and Supplementary Fig. 5g). In addition, Rockout, a Rho kinase inhibitor that blocks actomyosin contraction in zebrafish36,37, which is required for BCE, similarly impaired EGFP-KRasV12/p53MO cell invasion and E-cadherin loss compared to DMSO control treatment, supporting a role for BCE in both invasion and E-cadherin loss (Fig. 4j and Supplementary Movie2). Together, the tight link between BCE and E-cadherin loss suggests that BCE mechanically drives invasion of KRasV12 cells and simultaneous loss of apical epithelial determinants. While in our studies, E-cadherin and ZO-1 predominantly localize with cortical, apical actin and are stripped off by BCE, it is important to note that cells invading from epithelia where E-cadherin also localizes basally, such as stratified epithelia38,39, may not lose their epithelial determinants. In addition, cells could escape by extruding apically into the duct, in which case, we would expect them not to lose apically localized epithelial determinants as they invade. Future work will need to determine if cells invading with E-cadherin lose expression over time through different mechanisms or if the mechanism of invasion impacts later fate and tumour aggressiveness.

Here, we describe a new mechanism driving invasion of KRasV12-transformed cells that simultaneously mechanically pinches off the apex, containing most epithelial determinants, and endows escaping cells with new plasticity (Supplementary Fig. 6). In contrast to previous models where cells sequentially downregulate epithelial genes once they accumulate into a mass, we find that KRas-transformation alone, typically associated with early tumorigenesis steps, directly causes invasion through BCE,
Methods
Molecular biology. All cloning procedures followed protocols described previously34 the Invitrogen Gateway Technology Manual, and the Tol2kit wiki (http://tol2kit.genetics.utah.edu/index.php/Main_Page). We cloned the krt4 promoter sequence into pSE (gift from David Grunwald, University of Utah, Salt Lake City, UT, USA) using the BP Clonase II Enzyme mix (Thermo Fisher Scientific). Using BP recombination, we also cloned into pME the following constructs: human EGFP-dt-KRasV12 (isoform 4b) in a pEGFP-C3 backbone (gift from Channing J. Der, University of North Carolina, Chapel Hill, NC, USA), EGFP-CAAX (Tol2kit, gift from Kristen Kwan, University of Utah, Salt Lake City, UT, USA), and mCherry-T2A-cMyc (gift from Conan Kinsey, University of Utah, Salt Lake City, UT, USA). To generate constructs for microinjection, we recombined pSE-kr4t, pME-EGFP-dt-KRasV12 and pSE-polyA, or pSE-kr4t, pME-EGFP-CAAX and pSE-polyA or pSE-kr4t, pME-mCherry-T2A-cMyc and pSE-polyA, into pDestTol2CG2 using the LR Clonase II Plus Enzyme mix (Thermo Fisher Scientific) to produce kr4tEGFP-dt-KRasV12, krt4EGFP-CAAX or kr4t-mCherry-T2A-cMyc, respectively. The EGFP-T2A-KRasV12 construct was synthesized and cloned into pUC57 commercially (GenScript), then recombined into pME. Finally, pME-EGFP-T2A-KRasV12 was recombined with pSE-kr4t and pSE-polyA into pDestTol2CG2 to generate the kr4tEGFP-T2A-KRasV12 for microinjection.

Zebrafish Use. All zebrafish embryos and procedures were treated ethically, under approval of licenses issued from both the US Animal Welfare Act., and the UK the Animals in Scientific Procedures Act 1986 and animal use guidelines of the UK Home Office. In addition, all treatments and raising of fish were in compliance with the University of Utah - Centralized Zebrafish Animal Resource and the...
**Fig. 4** KRas$^{V12}$ cells invade by BCE and shed apical epithelial determinants. XZ sections of 48 hpf EGFP-dt-KRas$^{V12}$ embryos with E-cadherin$^-$ internalized cells (a, arrowheads) and EGFP-CAAX embryos, where cells remaining at epidermis and are E-cadherin$^+$ but the few that internalize are E-cadherin$^-$ (b, c, arrowhead). Those in an internal cell mass (T2A-KRas$^{V12}$, arrowhead) at 5 dpf also lacks E-cadherin (d). e XZ sections of DMSO or 100 μM Dynasore-treated T2A-KRas$^{V12}$/p53 MO-injected embryos showing internalized cells lack E-cadherin (arrowheads). f Percentage of invaded, surface, or mass-associated snai1b+ cells in 14 embryos, $P < 0.05$, **$P < 0.01$ by a Friedman test and Wilcoxon signed-rank test with the Holm-Sidak adjustment for pairwise comparisons, n = 11 embryos. Data are represented as boxplots where the middle (grey) line is the mean, the hinges indicate ±SD. All data points are shown. g Stills from Supplementary Movie 16 showing BCE of a EGFP-dt-KRas$^{V12}$ cell in a periderm:Lifeact-mCherry line (arrowheads pointing to ring contracting), clipping off the apex (green). h XZ section of a basally extruding EGFP-T2A-KRas$^{V12}$ cell (dashed) with E-cadherin constricted apically (arrowhead). Analysis of invasion in vehicle versus chloroquine-treated (i) or Rockout-treated (j) embryos at 48 hpf, ***$P < 0.001$, *$P < 0.05$ by a Fisher Exact Test. Source data are provided as a Source Data file.

Institutional Animal Care and Use Committee guidelines in compliance with King’s College London - Biological Services Unit.

**In vitro transcription.** Transposase and α-bungarotoxin mRNA were in vitro transcribed using the SP6 and T7 mMESSAGE mMACHINE Transcription Kits (Thermo Fisher Scientific), respectively, and purified using NucAway spin columns (Thermo Fisher Scientific). The concentrations of all nucleic acids used were measured using a NanoDrop 1000 (Thermo Fisher Scientific), an EPOCH 2 microplate (BioTek), or a NanoPhotometer N60 (Genflow) spectrophotometer.

**Microinjections and fluorescence sorting.** 2 μL of a 10-μL injection mix, comprised of 100 ng kr4EGFP-CAAX, 150 ng kr4EGFP-dt-KRas$^{V12}$, kr4EGFP-T2A-KRas$^{V12}$, or UAS:EGFP-KRas$^{V12}$, or 200 ng kr4:emCherry-T2A-cMyc + 200 ng transposase mRNA + 1 μL phenol red (Sigma) in nuclease-free dH$_2$O (Ambion), was microinjected into one-cell embryos. Some experiments included 0.2 pmol p53 morpholino (Gene Tools, 5'-GCGCCATTGCTTTGCAAGAATTG-3') or 25 ng a-bungarotoxin mRNA$^{59}$. Embryos were sorted for expression of transgenes at 1 dpf using a fluorescence dissection microscope, dechorionated with forceps at 1 or 2 dpf or with 1 mg/mL pronase, incubated in E3 with 0.003% N-phenylthiourea (PTU-E3, Merck), and prepared for live imaging or fixed and immunostained. Our studies were not blinded as injected embryos are very easy to visually distinguish, owing to the development of epidermal cell masses in EGFP-KRas$^{V12}$ embryos (both “dt” and “T2A” versions), absent in the majority of EGFP-CAAX embryos (see Results, Fig. 1A, and Supplementary Fig. 3A). All zebrafish embryos and adults were treated ethically in compliance with our UK Project Licence P946C972B.

**Time-lapse confocal imaging.** The protocol for mounting embryos for live imaging is described$^{55}$. Briefly, dechorionated embryos were anesthetized in 0.02% tricaine in PTU-E3 for 5 min or until no visible movement is observed, then mounted in 0.4–0.8% low-melt agarose as close as possible to the #1.5 glass coverslip within a slide chamber, covered with 0.02% tricaine in PTU-E3, and incubated in a controlled environment chamber at 28 °C and 85% humidity. Timelapse imaging of embryos were done for 18-20 h with 3-7 min time intervals overnight on an Andor Revolution spinning disk confocal microscope (Nikon CFi Plan Apo 20X/0.75 DIC M = 0.17 WD 1.0 objective), a Nikon A1R resonant scanning system (Nikon CFi Plan Apo 20X/0.75 objective), a Leica SP8 white light laser point-scanning confocal microscope, or a Yokogawa CSU-W1/Nikon Eclipse Ti2 spinning disk system equipped with an Andor iXon EMCCD camera. Nikon NIS Elements Advanced Research (v 4.60 & v 5.30), Leica Application Suite X (v3.4.2.18368), or Fiji (ImageJ 1.53c) were used for analysis. A Fiji plugin was used to track cells in movies with an arrow$^{60}.$
**Time-lapse light sheet imaging.** Selective Plane Illumination Microscopy (SPIM) was used for long-term in toto imaging using one of two custom-built setups: a multidirectional SPIM (mSPIM) or a four-directional SPIM setup. The mSPIM was equipped with an UMPAniL N Olympus 10 × 0.3 NA detection objective, a 488 nm and a 561 nm Coherent Sapphire laser, two Zeiss 10 × 0.2 illumination objectives and two Andor iXon 885 EMCCD cameras. The whole zebrafish embryo was imaged from several angles with 7 × overall magnification and a z-stack spacing of 5 μm every 4–7 min for up to 3 days. To image the whole embryo, several regions were acquired and stitched together using custom image processing plugins in Fiji and maximum intensity projections were generated and registered using SimpleElastiJ and GUI-based manual rigid registration.

**The four-lens SPIM.** consisted of four identical water-dipping Olympus UPLSAPO 10×/0.3 objectives, two for illumination and two for detection. Two Topica iGlow superluminescent diodes were externally triggered for alternating double-sided illumination. The laser beam was split 50/50 and directed onto a continuous running galvanometric mirror (1 kHz, EOPC), which pivoted the light sheet and reduced shadowing effects in the excitation paths due to absorption of the specimen. Light sheets were generated with cylindrical lenses and projected with telescopes and a 10× objective for time-lapse acquisition. A custom fusion program was used for visualization and generation of maximum intensity projections.

For long-term time-lapse acquisition, embryos were embedded in 0.1% low-melting point agarose inside fluorinated ethylene propylene (FEP) tubes as described in Supplementary Table 1. After six 20-minute washes with E3, embryos were imaged using a Zeiss 20× Plan Apo Achromat for 60 min in the E3-filled imaging chamber only when a-bungarotoxin mRNA was not co-injected with DNA constructs.

**Immunostaining and imaging of fixed embryos.** Embryos were immunostained as described previously. Briefly, dechorionated and anesthetized embryos were fixed in 4% paraformaldehyde, 4% sucrose, and 0.1% Triton X-100 in PBS overnight at 4 °C, blocked with 10% goat serum for 1 h, then incubated overnight at 4 °C using any of the following antibodies: chicken α-4 °C using any of the following antibodies: chicken α-4 °C using any of the following antibodies: chicken -E3 are indicated in the legend. After fixation, embryos were permeabilized with 0.5% PBST (PBS + 0.5% Triton-X), incubated with primary antibodies (goat α-chicken-AlexaFluor-488, goat α-rabbit-AlexaFluor-568, or goat α-mouse-AlexaFluor-647 (Thermo Fisher Scientific, 1:200) in 10% goat serum overnight at 4 °C, followed by four 20-minute washes with 0.5% PBST. Nuclei were stained with 1 μM DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) for 30 min and washed twice with 0.5% PBST. The immunostained embryos were taken through a glycerol series (25%, 50%, 70% glycerol in PBS), mounted in 70% glycerol–1.5% agarose inside FEP tubes as described above. Fluorescence was observed with a Nikon Ti2 spinning disk equipped with an Andor Zyla sCMOS camera. The whole embryo was imaged from several angles with a z-stack spacing of 2 μm every 30 s up to 5 min for up to 36 h. A custom LabVIEW (National Instruments) program was implemented to adjust stage parameters, stack correlation parameters for time-lapse acquisition. A custom fusion program was used for visualization and generation of maximum intensity projections.

**Drug treatments.** Rockout (30 mM), chloroquine (50 mM), dynasore (30 mM), and dextran-AlexaFluor-568 (10000 MW, 50 mg/mL) were dissolved in DMSO at the indicated concentrations, aliquoted, and stored at −20 °C. Working solutions in PTU-E3 are indicated in figures. Rockout and chloroquine: 24-hpf injected embryos were distributed into 6-well (30 embryos/well) or 24-well (10 embryos/well), E3 aspirated and quickly replaced with drug (or DMSO control) in PTU-E3 for 24 h (48 hpf), then washed, anesthetized with tricaine and fixed, and immunostained as described above. Dynasore: Injected 50%–epiboly or shield embryos in 6-well plates at 30 embryos/well were incubated with dynasore (or DMSO control) in PTU-E3. At 24 hpf, embryos were dechorionated, then incubated in dynasore and 2 mg/mL dextran-AlexaFluor-568 in the dark for 6 h. Embryos were rinsed 3X in E3, incubated in fresh PTU-E3 without drug or tracer in the dark for 2.5 h, anesthetized with tricaine, fixed, and immunostained, as described above.

**In situ mRNA hybridization and immunohistochemistry.** In situ mRNA hybridization for snai1b, previously described, was done as follows: 2 dpf PTU-E3–treated embryos fixed in 4% paraformaldehyde in PBS overnight at 4 °C, were treated with 50 μg/mL proteinase K (Sigma-Aldrich) for 17 min and hybridized with a digoxigenin (DIG)-tagged antisense snai1b probe61 at 60 °C overnight. After in situ hybridization, embryos were incubated with an α-digoxigenin–MAB conjugate directly conjugated to alkaline phosphatase (Roche, 1:5000) overnight at 4 °C and developed colorimetrically using NBT/BCIP solution (Roche). Finally, embryos were immunostained for GFP and E-cadherin and DNA (DAPI), as described above.

Embròys were mounted in ProLong Gold (Molecular Probes) with a #1.5 glass coverslip and imaged using a Nikon Eclipse Ti-E N-E1 FN Upright. snai1b-positive cells were identified and imaged using a colour camera and subsequently fluorescence and transmitted light images were acquired with the same pixel size to check for signal colocalization.

**Quantification and statistical analysis**

**Quantifications.** Cell masses: 1 dpf embryos were anesthetized in 0.02% tricaine and dechorionated to count cell masses manually using a brightfield dissection microscope. Live extrusions: Live cell extrusions were quantified from time-lapse movies and categorized as apical or basal using orthogonal slice projections. Internalized vs. surface cells: Internalized and surface cells were counted from confocal images in orthogonal slice projections, immunostained for p63 or E-cadherin to define the basal cell layer or the periderm, respectively, of the zebrafish epidermis. Cells were classified as live or dead using caspase-3 immunostaining and cell morphology in control- and drug-treated embryos. Cells expressing GFP (in muscle, notochord, melanocytes), an artifact of F0 transgenic, were recorded but excluded from invaded cell scoring and statistical analyses. Circulating tumour cells: CTCs were quantified in live, dechorionated embryos using a dissection microscope. Mapping of cell masses: The number and location of masses were counted using live, dechorionated embryos using a dissection microscope for cell masses, using a microscope tip to reposition embryos to observe both sides. Mapping of live extrusions: The numbers and locations of apical and basal extrusions were mapped from movies of injected E(PIerdem:Ga4); UAS:Lifeact-mCherry) embryos. For Supplementary Figs. 1 B–D, notochord, muscle, and melanocyte cells misexpressing GFP from E(PIerdem:Gal4) versus wild-type embryos were counted and compared to the number of cells scored as invading cells in our assay.

**Ectopic expression of snai1b.** To quantify if injection of embryos with EGFP-CAXA or EGFP-T2A-KrasV12 affects snai1b expression, each embryo was imaged from both lateral sides using a colour camera. Then images were randomized and, using the endogenous expression of snai1b in uninjected embryos as a guideline, three independent people assessed the expression of the transcription factor without knowing what was the construct injected in each embryo. If the expression of snai1b deviated from that of uninjected embryos, i.e., by the appearance of purple dots resembling to individual cells, embryos were quantified to have ectopic snai1b expression. In case of discrepancy, the embryo was quantified according to the criterion of the majority.

**Statistical analyses.** Statistics in all graphs were calculated using either Graphpad prism 9.0 or Matlab R2019b, both of which compute p values as an upper limit, rather than an exact number. All tests were run as two-sided tests. To evaluate sample means, we used a non-parametric Mann–Whitney test. To compare more than two groups, we used a Kruskal–Wallis test to reject the null hypothesis, and a Mann–Whitney test with the Holm-Sidak adjustment for pairwise comparisons. To analyze paired data, we used a Wilcoxon signed-rank test. For comparisons of more than two groups of paired data we used the Friedman test to reject the null hypothesis and a Wilcoxon signed-rank test with a Holm-Sidak correction for multiple pairwise comparisons.

For categorical data we used the Chi-Square test to reject the null hypothesis with the Yates correction when analyzing two populations and two categories and with the Holm-Sidak adjustment for pairwise comparisons when comparing more than two groups. For analysis of categorical data, when at least 20% of the groups presented frequencies lower than 5 for a given variable, we used the Fisher's Exact test.

**Statistics and reproducibility.** Fig. 4a, b, c, and d represent >30 embryos, whereas e is from 7 embryos, quantified in Supplementary Fig. 5d. Supplementary Fig. 1a represents >30 embryos. d represents 17 wild-type embryos and 14 periderm:Gal4 enhancer trap embryos, quantified in Supplementary Fig. 1b. Supplementary Fig. 2B is representative of 15 embryos, and c of 5 embryos. Supplementary Fig. 4a represents >30 embryos and b of 18 embryos. Supplementary Fig. 5e is from 7 embryos, quantified in Fig. 4f, f and g represent >30 embryos, and h of 5 or 8 embryos treated with vehicle control or Rockout, respectively, quantified in Fig. 4j.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The raw data that support the findings of this study are available from the corresponding author upon reasonable request. All the other data are available within the article and its Supplementary Information. Source data are provided with this paper.

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