Supplemental Materials and Methods

Short hairpin RNA (shRNA) against MMP14. Lentiviral plasmids containing shRNA (Mission shRNA, Sigma) against mouse MMP14 were transfected into HEK293 cells using FuGene6 (Roche). Transfected cells were cultured in DMEM containing 5% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin. Lentivirus was concentrated from filtered culture media (0.45 µm filters) by ultracentrifugation at 25,000 rpm for 90 min. To infect EpH4 cells, 1.0×10^5 cells were plated in each well of a 6-well plate, infected with the lentivirus, treated with polybrene for 30 min, and selected over 4 days with 5 µg/ml puromycin.

Immunofluorescence analysis of E-cadherin and cleaved caspase-3. Samples were washed in PBS and fixed in 4% paraformaldehyde for 15 min at room temperature, permeabilized 3 x 5 min in 0.5% Igepal Ca-630 and incubated in 0.1% Triton X-100 in PBS for 15 min. Samples were blocked overnight at 4°C in 10% goat serum in PBS (PBS-S), and incubated overnight at 4°C in primary antibody recognizing E-cadherin (Cell Signaling) or cleaved caspase-3 (Cell Signaling) accordingly, at 1:100 dilution in PBS-S. Samples were washed extensively with PBS and incubated in secondary antibody at 1:1000 in PBS-S overnight at 4°C. Frequency maps of the proteins were constructed from fluorescence images as described in the main text.

EdU proliferation assay. Proliferating cells were visualized by utilizing the Click-iT EdU Imaging Kit (Invitrogen). Samples were incubated for 20 hours (starting 4 hours after micropatterning) in 10 µM 5-ethynyl-2-deoxyuridine (EdU). They were subsequently fixed in 4% paraformaldehyde for 15 min at room temperature and permeabilized for 15 min in 0.1% Triton X-100 in PBS. EdU incorporation was detected by incubation in the Click-iT reaction

cocktail (as prescribed by the kit) at room temperature. The samples were then washed 3 x 5 min in PBS. Frequency maps of the cell proliferation were constructed from fluorescence images as described in the main text.

Supplemental Figure Legends

Fig. S1. Engineered tissue model to study cell sorting and morphogenesis. **(A)** Schematic of engineered tissue model. Branching morphogenesis of engineered tissues requires expression of MMP14. Shown are quantification of branching from 50 tubules of **(B)** control cells, **(C)** MMP14-overexpressing cells, and **(D)** cells treated with shRNA against MMP14 (shMMP14). Scale bars, 50 μm.

Fig. S2. Mosaic expression of MMP14 does not affect proliferation or apoptosis in the tubules. Immunofluorescence analysis of EdU incorporation in **(A)** one tubule and quantification of EdU incorporation in 50 tubules mosaic for **(B)** vector control, **(C)** MMP14, **(D)** si control, or **(E)** siMMP14. Immunofluorescence analysis of cleaved caspase-3 in **(F)** one tubule and quantification of cleaved caspase-3 in 50 tubules mosaic for **(G)** vector control, **(H)** MMP14, **(I)** si control, or **(J)** siMMP14. Scale bars, 50 μm.

Fig. S3. Inhibition of Erk does not affect cell motility. **(A)** Average speed of individual control cells and cells treated with the MEK inhibitor, PD98059. **(B)** Cumulative distribution of cell speed amongst populations in (A). **(C)** Persistence time of control cells and cells treated with the MEK inhibitor, PD98059. **(D)** Distribution of persistence time amongst populations in (C). For (A, C), error bars indicate s.e.m. For (B, D), edges represent 25th and 75th percentiles and error bars represent 10th and 90th percentiles. (n.s.), not significantly different, as determined by t-test.

Fig. S4. Self-organization of MMP14 mosaic tubules is not affected by TGFβ gradient. We showed previously that the position of branches was dependent on a gradient in concentration of TGFβ (27). Endogenous gradient of TGFβ, however, has no effect on sorting, as demonstrated by frequency maps of YFP-expressing cells co-transfected with **(A)** vector and **(B)** MMP14, and constructed into tubules which increase the local concentration of TGFβ. Exogenous expression of TGFβ also has no effect on sorting, as demonstrated by frequency maps of YFP-expressing cells co-transfected with MMP14 and **(C)** latent TGFβ1 or **(D)** active TGFβ1. Scale bars, 50 μm.

Fig. S5. Tubules mosaic for MMP3 do not sort. Immunofluorescence analysis of MMP3 in **(A)** one tubule and **(B)** quantification of immunofluorescence intensity from 50 tubules, represented as a frequency map. Frequency map quantifying location of labeled cells co-expressing **(C)** control vector and **(D)** MMP3. Frequency map quantifying location of YFP-expressing cells co-transfected with **(E)** control siRNA (si control) and **(F)** siRNA against MMP3 (siMMP3). Scale bars, 50 μm.

Fig. S6. MMP14-mediated self-organization depends on activate ROCK. Frequency maps quantifying location of YFP-expressing cells co-transfected with dominant negative ROCK^{KDIA} and **(A)** vector and **(B)** MMP14, or co-transfected with constitutively active ROCK$^{\Delta 3}$ and **(C)** si control and **(D)** siMMP14. Scale bars, 50 μm.

Fig. S7. MMP14-mediated self-organization requires CD44. Immunofluorescence analysis of CD44 in **(A)** one tubule and **(B)** quantification of immunofluorescence intensity from 50 tubules. Frequency maps quantifying location of YFP-expressing cells co-transfected with MMP14 and

(C) si control or **(D)** siRNA against CD44 (siCD44). **(E)** Quantitative RT/PCR analysis for CD44 expression in cells transfected with control vector, CD44, si control and siCD44, normalized to levels of 18S rRNA. Frequency maps quantifying location of YFP-expressing cells co-transfected with **(F)** CD44 and **(G)** siCD44 demonstrate that CD44 expression phenocopies MMP14 sorting. CD44-induced self-organization requires expression of the MMP14 hemopexin domain, as demonstrated by frequency maps of YFP-expressing cells co-transfected with CD44 and **(H)** si control, **(I)** siMMP14, **(J)** human MMP14 deleted of the catalytic domain (huΔCAT), and **(K)** siMMP14 plus huΔCAT. CD44 expression activates ROCK signaling, as shown in **(L)** western blots for phosphorylated LIMK (pLIMK) and total LIMK. CD44-induced self-organization requires activation of ROCK, as demonstrated by frequency maps of YFP-expressing cells co-transfected with CD44 and treated with **(M)** vehicle or **(N)** Y27632 (10 μM). Scale bars, 50 μm.

Fig. S8. CD44-mediated self-organization depends on active ROCK. Frequency maps quantifying location of YFP-expressing cells co-transfected with dominant negative ROCK^{KDIA} and **(A)** vector and **(B)** CD44, or co-transfected with constitutively active ROCK$^{\Delta3}$ and **(C)** control siRNA (si control) and **(D)** siRNA against CD44 (siCD44). Scale bars, 50 μm.

Fig. S9. Increasing initial the number of MMP14^{hi} cells per tissue affects kinetics of sorting. **(A)** Average percentage of sorted simulated tissues as a function of time. Each curve represents data from 40 simulated tissues with a different number of MMP14^{hi} cells. Shown are mean ± s.e.m. **(B)** Experimental validation of simulation results, represented as percentage of sorted tissues as a function of time. Shown are mean ± s.e.m. for 3 independent experiments.

Fig. S10. Expression of MMP14 does not affect expression or distribution of E-cadherin.

(A) Quantitative RT/PCR analysis for E-cadherin expression in cells transfected with control vector, MMP14, ΔCAT, or ΔPEX, normalized to levels of β-actin. Shown are mean ± s.e.m. for 6 experiments. **(B)** Immunofluorescence analysis of E-cadherin in tubules at 8, 12, and 24 hr time points. **(C)** Frequency maps of E-cadherin staining at 8, 12, and 24 hr time points. Scale bars, 50 μm.
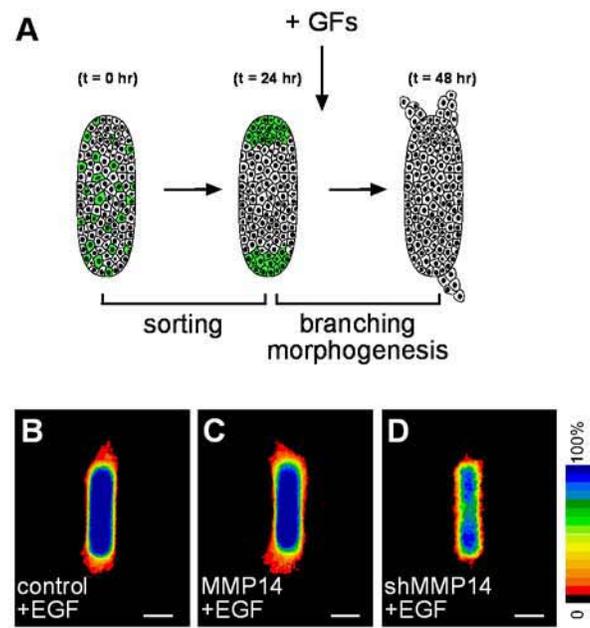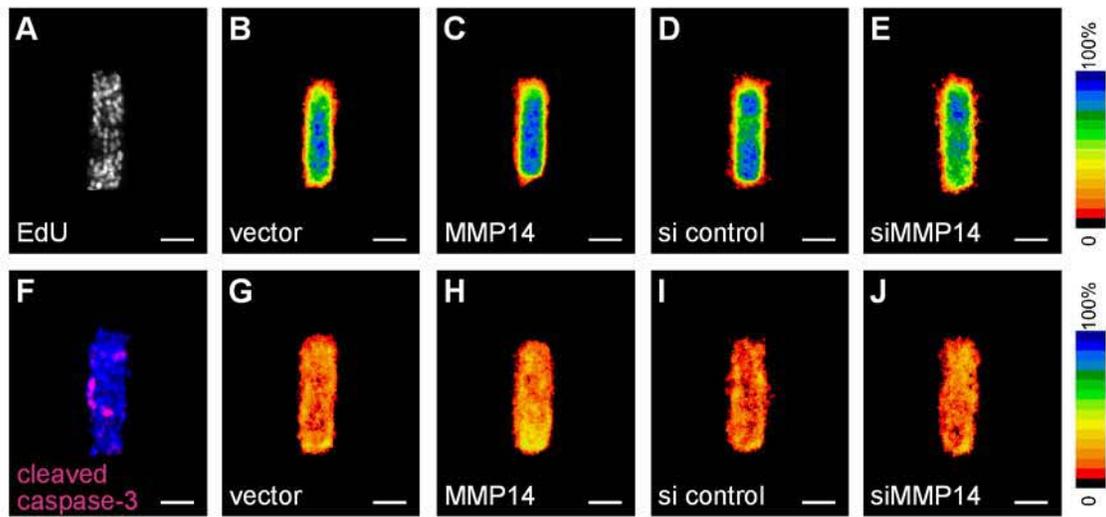
Figure S1

A

(t = 0 hr)   (t = 24 hr)   + GFs   (t = 48 hr)

sorting   branching morphogenesis

B control +EGF   **C** MMP14 +EGF   **D** shMMP14 +EGF

100%

0

Figure S2

Figure S3

Figure S4

A vector

B MMP14

C MMP14 latent TGFβ
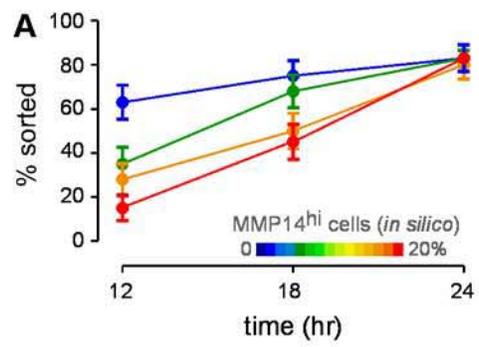
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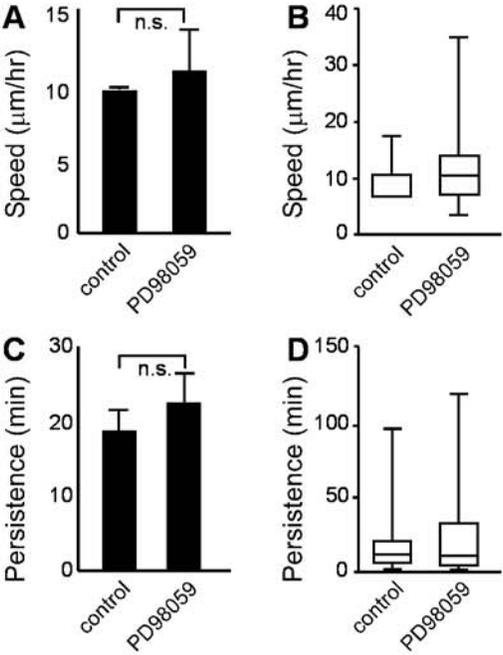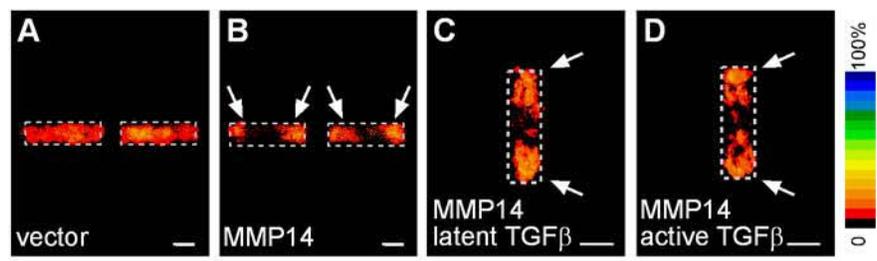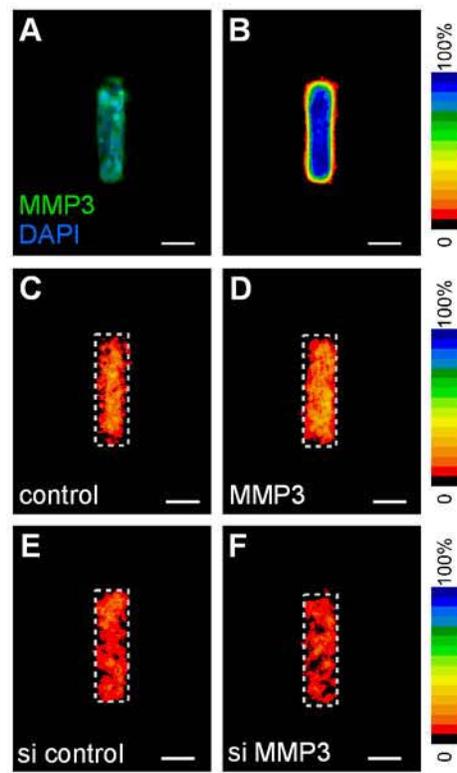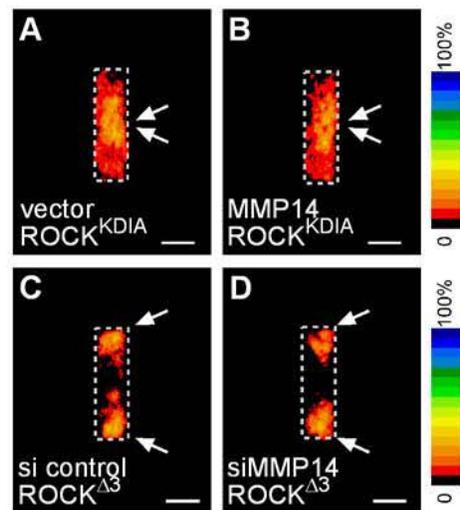
Figure S5

Figure S6

Figure S7
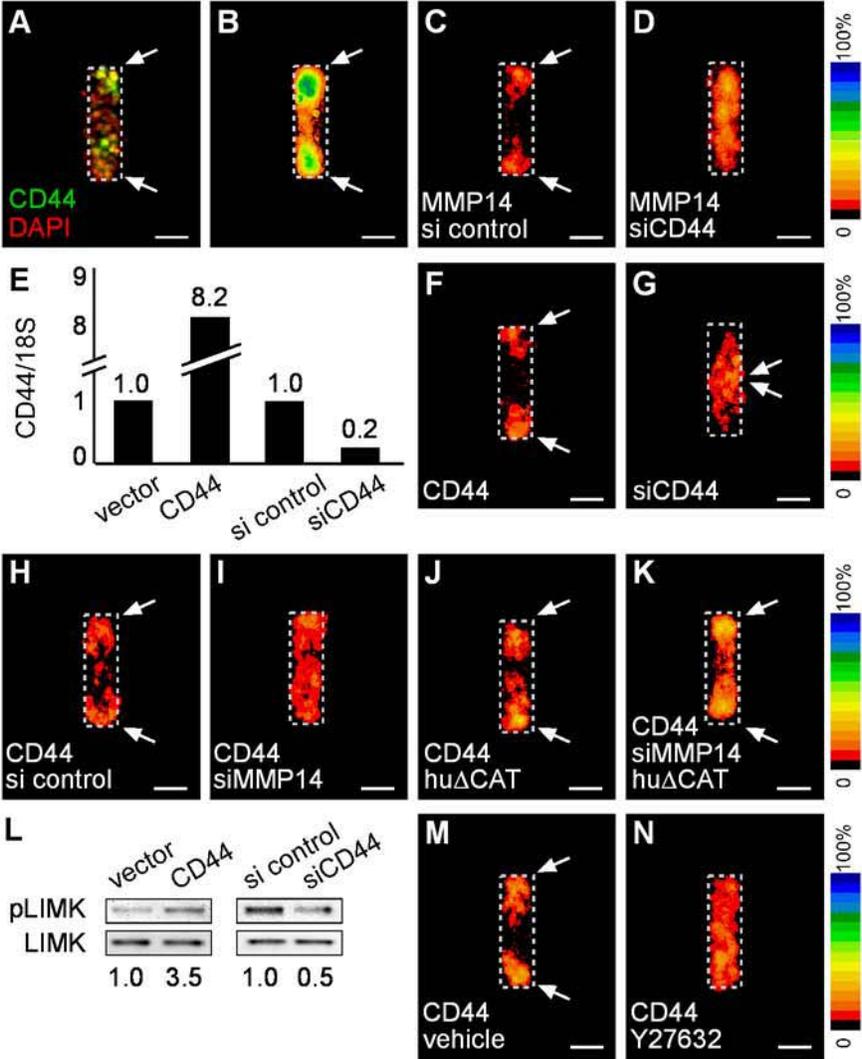
Figure S8
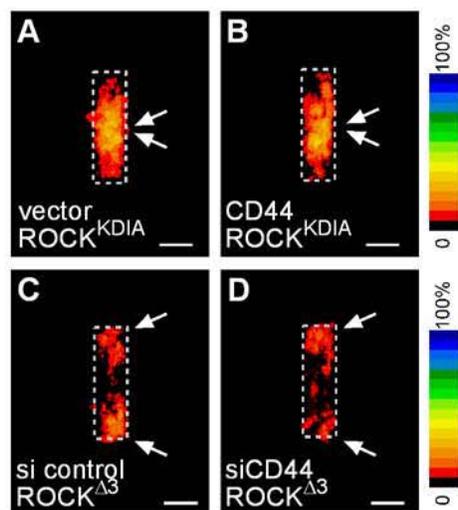
Figure S8

Figure S9

Figure S10