Collective directional memory controls the range of epithelial cell migration

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Cell migration is a fundamental behavior involved in multicellular development, tissue regeneration and homeostasis, which is deregulated in cancer. Epithelial cells migrate individually when isolated and collectively in a tissue, and may be exposed to external guidance cues. How interactions between neighbors affect the efficiency of cell spatial exploration - the distance cells can reach in a given time - and sensitivity to external guidance cues is, however, poorly understood. We found that while isolated cells are persistent random walkers, they adopt a super-diffusive behavior within a confluent epithelium that dominates the efficiency of spatial exploration relative to guidance cues, and virtually allows cells to reach greater distances when surrounded by neighbors than when alone. This confluence-induced transition in directional memory emerges from a fractional Brownian motion that results from velocity coordination between neighboring cells and requires intact cytoskeletal connections to intercellular adhesion complexes. Furthermore, we show how the stability and mechanosensitivity of cell-matrix and cell-cell adhesions ultimately regulate the speed of collective migration and sensitivity to guidance cues in a non-cell-autonomous manner through their differential dependence on the dimerization of the adhesion protein vinculin. Overall, our results reveal how individual cell speed, tissue-dependent persistence, and guidance by the environment can limit the efficiency of cell spatial exploration at short, intermediate, and long time scales, respectively.

Cell migration | Epithelium | Cell adhesion

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Introduction

In multicellular organisms, cell migration is an essential process in tissue morphogenesis, homeostasis and repair. Deregulation of cell migration is also involved in metastatic invasion of cancers (1). Cell migration occurs in different ways, depending on the intrinsic properties of cells and how they interact with each other and their environment. As a result, cells may migrate individually or as a coordinated collective, guided by local or global cues (2).

For these reasons, how cells explore their environment and how cell interactions and guidance cues affect this exploration are fundamental questions that have motivated abundant research for over 70 years (3, 4). Since then, various models have proven useful in describing the exploration of space by individually migrating cells. Directional persistence has emerged as a consistent, though highly variable feature depending on cell type or context, and which could arise from the anomalous distribution of cell displacements, temporal velocity correlations, or both (5–8). When migrating collectively, neighbor cells typically exhibit correlated velocities that depend on their interactions (9–11). This coordination and the diverse mechanisms underlying it have been the subject of intense scrutiny for the past decade (12). In comparison, how each cell in the collective explores its environment has received far less attention, with a few exceptions (13–15). Remarkably, it remains unclear whether neighbor interactions actually help or hinder the exploratory capacity or sensitivity to guidance cues of each cell in the collective, much less whether and how they quantitatively or qualitatively affect the underlying cellular and molecular mechanisms of migration.

Adherent cells form Focal Adhesions (FAs) on their substrate, which they continuously assemble at their front and dissassemble at their back during migration (16). There, mechanosensory proteins such as vinculin provide force transmission from the contractile cytoskeleton (17). In cohesive tissues, cells additionally adhere to each other through cadherin-based Adherens Junctions (AJs) where α-catenin and vinculin enable dynamic connections with the cytoskeleton and force transmission (18). Vinculin comprises a head and a tail domain whose intramolecular interaction competes with intermolecular dimerization and binding to the actin cytoskeleton of its tail and with binding of its head to adaptor proteins in FAs and AJs (19). In AJs, α-catenin is this adaptor, which also binds actin by its own tail (20). Vinculin deficiencies impact the migration of cells as individuals, in a collective and with guidance cues, although in a cell type- and mutation-dependent manner (11, 21–32). Alpha-catenin deficiencies impact guided and coordinated collective migration, involving the interaction of α-catenin with vinculin at AJs (11, 26, 33, 34). However, the lack of comparable metrics, scales and molecular, cellular and migratory contexts between studies makes it difficult for a general picture to emerge, where the functions of proteins in regulating the stability and mechanosensitivity of adhesions could explain how they control the intrinsic speed of cells, their persistence, and their responses to interactions with neighbors and guidance cues.

Here, we address this issue by comparing the exploratory ability of epithelial cells as individuals, in the collective and exposed to a guidance cue, while genetically disrupting the interactions of cells with their substrate and neighbors and monitoring the associated effects on the dynamics and mechanics of adhesion complexes. We show that individual epithelial cells migrate with transient directional memory and that vinculin regulates their speed by synergizing FA stabil-

ity and cytoskeletal tension transmission through its dimerization and actin-binding abilities, respectively. In contrast, cells that migrate collectively acquire virtually infinite directional memory, which specifically requires α-catenin at AJs and results from velocity coordination between neighboring cells. In this context, vinculin additionally controls the speed of cells from AJs through its impact on their mobility and force transmission, but in a manner specifically dependent on its dimerization. Overall, this results in a spatial exploration that is less efficient than that of individual cells at time scales less than 3hrs but more efficient beyond that. Finally, cell guidance by an epithelial wound also requires α-catenin, but the directional bias is too small to significantly affect the efficiency of spatial exploration for hours. In this context of guided collective cell migration, vinculin dimerization further regulates cell speed from AJs by providing the whole epithelium mechanosensitivity to the wound.

Results

Single epithelial cells are persistent random walkers. To characterize the motion of single epithelial MDCK cells, we tracked nucleus-stained cells overnight, sparsely plated on collagen-coated glass coverslips (see Materials and Methods)(Fig. 1A). We found that a persistent random walk model (35, 36) was overwhelmingly more likely than a model of pure or super-diffusion to describe the average Mean Square Displacement (MSD) as function of time interval Δt: the MSD transitioned from ballistic (slope 2 for small Δt in ln-ln scale) to pure diffusion (slope 1 for large Δt) with a persistence time of the order of 10min (Fig. 1B; Table S1). To ascertain the underlying walk model, we examined the step size (R) for the time intervals $\Delta t = 10$min and $\Delta t = 3$hrs. In both cases, R followed a Rayleigh distribution, in agreement with a Gaussian walk regardless of the temporal resolution (Fig. 1C, S1A). Finally, we examined the deviation of each 10min-step from the direction of the previous step defined at every Δt (see Materials and Methods) and found that its distribution was skewed toward small angles at small Δt and uniform at large Δt (Fig. S1B). In other words, the angular memory decreased with increasing Δt (Fig. 1D). Overall, these results show that single epithelial cells migrate as persistent random walkers, displaying transient directional persistence overcome by pure diffusion at longer time scales.

To investigate the robustness of this behavior, we examined the effects of overexpressing full-length (FL) vinculin or dominant vinculin mutants, additionally bearing a tension sensor module (37, 38). Specifically, we considered the Tail-less mutant (TL) that impairs all tail interactions (39), the T12 mutant that impairs the head-tail interaction (40) and the Y1065E (YE) mutant that impairs the tail-tail interaction (41) (Fig. S2A). Stably expressing cell lines (Fig. S2B,C) all exhibited an MSD better described by a persistent random walk model than by a pure or super- diffusion model (Fig. 2A, Table S1). The effects of mutant expression on persistence times and diffusion coefficients were variable and showed no correlation (Fig. S2D). In addition, the diffusion coefficient scaled well with the squared apparent speed with a common persistence time for all cell lines (Fig. S2E), showing that cell lines differed primarily in apparent speed rather than persistence. Finally, we found that the mutants induced opposite changes in apparent speeds compared with FL vinculin (Fig. S2F). This confirms the effects being due to the dominant mutants rather than the overall level of all types of vinculin expressed together, consistent with the co-expression of vinculin splice variants in physiological situations (42). Specifically, the TL and YE mutants with impaired actin-tail or tail-tail interactions decreased cell speed, while the T12 mutant with altered head-tail interaction increased cell speed relative to FL vinculin (Fig. 2B). Overall, these results show that the persistence, but not speed of single migrating cells is robust to mutations in the FA protein vinculin.

The combination of Focal Adhesion stability and force transmission from the cytoskeleton predicts single cell migration speed. To identify the underlying cellular mechanisms and molecular determinants of speed regulation by FAs, we first took advantage of the tension sensor module in vinculin and its mutants to assess their mechanical state by Molecular Tension Microscopy (Fig. 2C) (see Materials and Methods). We found that alteration of the actin-tail and head-tail interactions (TL and T12) decreased and increased vinculin tension relative to WT vinculin, respectively (Fig. 2D), as seen in other cell types (37, 38). In contrast, alteration of the tail-tail interaction (YE) did not substantially affect vinculin tension, meaning that vinculin dimerization is unnecessary for force transmission (Fig. 2D). Next, we investigated a potential relationship between vinculin tension and single cell speed across mutants, but found no significant correlation (Fig. S2G), indicating that single cell speed is not simply a result of force transmission by vinculin.

We therefore investigated the effects of mutations on vinculin stability by Fluorescence Recovery After Photobleaching (FRAP, Fig. S2H) (see Materials and Methods). We found that TL and T12 mutants, both of which impair head-tail interaction, had a higher immobile fraction than WT vinculin (Fig. 2E), consistent with previous studies in other cell types (30, 39, 43–46). First, this shows that impaired head-tail interaction, regardless of tension, is sufficient for stability. Second, this shows that FA stability does not predict cell migration speed. On the other hand, the YE mutant that impairs the tail-tail interaction showed a smaller immobile fraction (Fig. 2E). This supports that vinculin dimerization promotes the stability of FAs.

Altogether, this reveals that migration speed decreases upon loss of force transmission or FA stability (TL and YE) and increases upon strengthening of both (T12). We therefore propose that single cell migration speed is the product of FA stability and force transmission, the former enabled by vinculin tail-tail interaction and the latter by vinculin-actin binding, and both competed for by its head-tail interaction (Fig.2F).

Collectively migrating epithelial cells are super-diffusive, fractional Brownian walkers. To assess whether the same behavior and mechanisms apply to a cell collective, we tracked overnight nucleus-stained cells plated at confluence

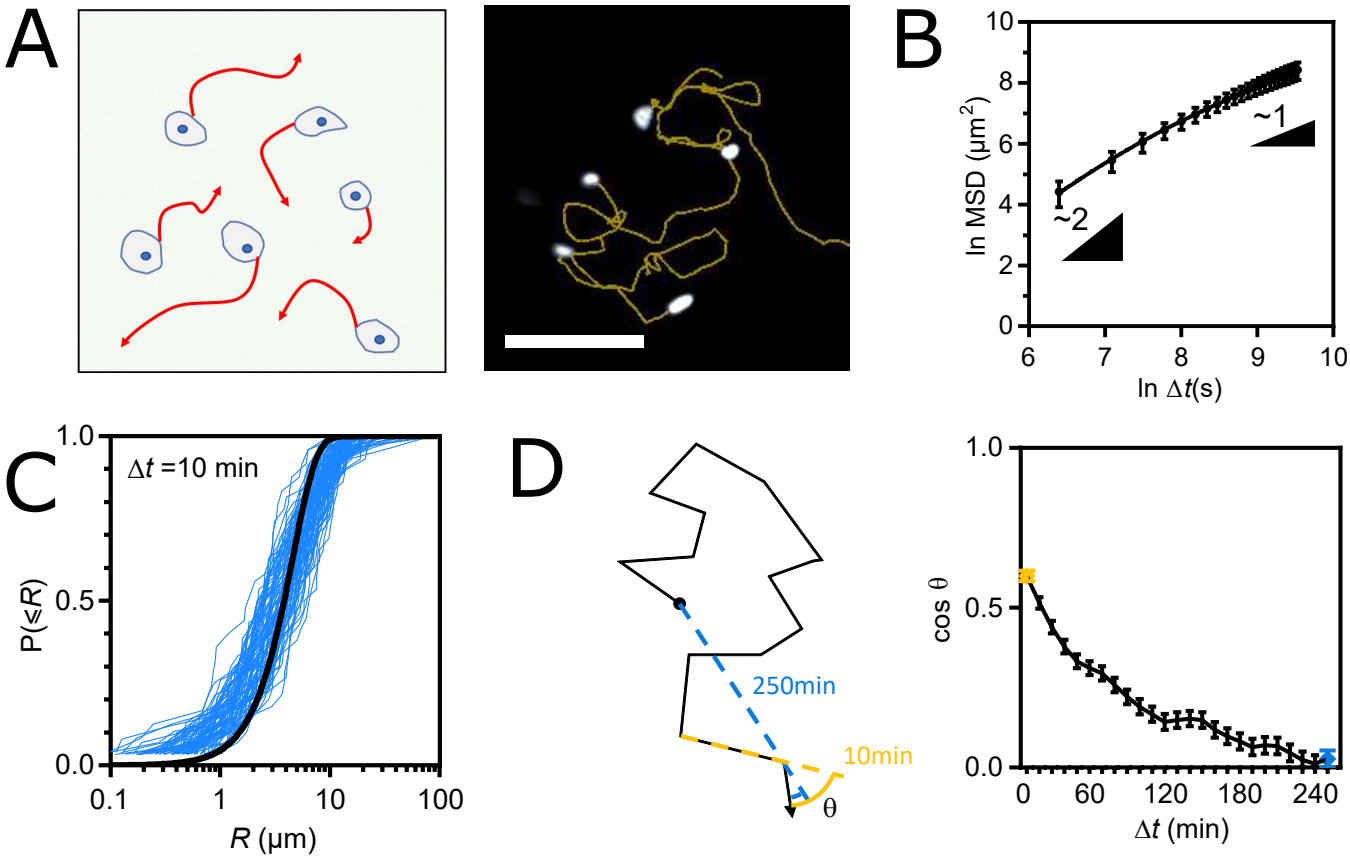

Fig. 1. (A) Left: Schematic representation of the single cell migration assay. Right: fluorescence image of nucleus-stained cells and their migration tracks in yellow. Scale bar = 100μm. (B) MSD(Δt) of WT cells fit with the Fürth equation. (C) Cumulative distributions of the step length R for $\Delta t = 10$min. One blue curve per frame pair along a movie. The black line is a global fit of all blue curves with a cumulative Rayleigh distribution function. (D) Left: Schematic migration track and angular memory ($\cos\theta(\Delta t)$, see Materials and Methods) at $\Delta t = 10$min (yellow) and $\Delta t = 250$min (blue). Right: Angular memory ($\cos\theta(\Delta t)$) between $\Delta t = 10$min (yellow dot) and $\Delta t = 250$min (blue dot). Data of (B-D) from 94 cell tracks of 2 independent experiments. Mean $\pm$ SEM.

on collagen-coated glass coverslips (see Materials and Methods)(Fig. 3A). We found that within the same time frame, cells no longer migrated as persistent random walkers, but more likely adopted super-diffusive behavior with an anomalous diffusion exponent of about 1.7 (Fig. 3B, Table S2). Remarkably, this super-diffusive behavior allowed the cells to eventually reach a greater distance than single cells in the same amount of time, even though their apparent speed was lower (Fig. S3A).

To distinguish between the various walk models that could produce such a super-diffusive behavior (Lévy flight, fractional Brownian motion, or their combinations (7, 47)), we examined the step size distributions and found that they were consistent with a Gaussian walk on any time scale up to $\Delta t = 3$hrs (Fig. 3C, S3B). However, we found that the angular deviation of any 10min-step from the direction of the previous step was skewed toward small angles and invariant with the time scale of the previous step up to $\Delta t = 250$min (Fig. S3C). In other words, the angular memory never disappeared within our experimental time frame, unlike that of single cells (Fig. 3D compare to Fig. 1D). Gaussianity and virtually infinite directional memory are characteristics of a pure fractional Brownian motion and thus rule out Lévy walks or other combination. Cell super-diffusion thus emerges from a fractional Brownian motion.

The transition in directional memory emerges from Adherens Junction-mediated intercellular adhesion. To assess whether the transition from finite to infinite directional memory observed upon confluence resulted from simple steric hindrance due to crowding by neighbor cells or from genuine AJ-mediated intercellular adhesion, we examined the migration of cells depleted of α-catenin (see Materials and Methods). Remarkably, the cells lost most of their super-diffusive behavior (with an anomalous exponent α dropping to about 1.2) despite being as crowded as WT cells (Fig. 3E). Consistently, angular memory vanished in the experimental time frame (Fig. 3F), as for single migrating cells. In contrast, cells depleted of E-cadherin (see Materials and Methods) exhibited a slightly higher anomalous diffusion coefficient than WT cells (Fig. 3E) and, consistently, infinite angular memory (Fig. 3F). This shows that E-cadherin specifically is largely dispensable, if not slightly detrimental, for these cells to migrate in a super-diffusive manner. Consistently, depletion of E-cadherin alone, unlike that of α-catenin, has little effect on the level or localization of other AJ proteins in this cell type, which also express cadherin-6 (48). Similarly, different cadherin subtypes can compensate for E-cadherin defficiency in other contexts (49–51).

The confluence-induced transition in directional memory thus results from *bona fide* AJ-mediated intercellular adhe-

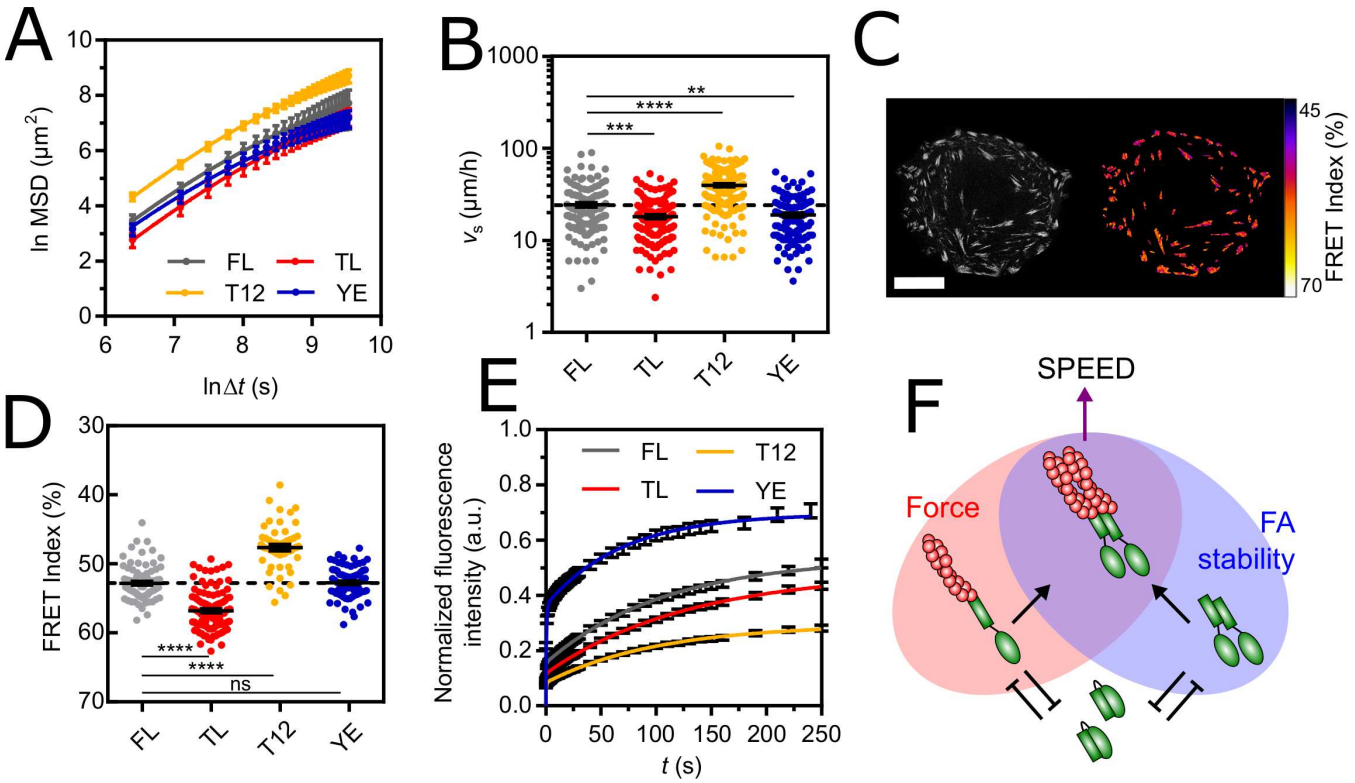

Fig. 2. (A) MSD(Δt) of vinculin FL and mutant cells fit with the Fürth equation. (B) Apparent speed of single, vinculin FL and mutant cells. (C) Left: direct fluorescence of vinculin TSMod in a single cell. Right: FRET Index map (see Materials and Methods). Scale bar = 20 μm. (D) FRET Index of TSMod in the FL, TL, T12 and YE constructs in FAs. (E) Fluorescence recovery after photobleaching of FL, TL, T12 and YE constructs in FAs. (F) Proposed model: at FAs, the head-tail interaction of vinculin (green) competes with both tail dimerization and actin filaments (red) binding, which provide FA stability and force transmission, respectively, together promoting cell speed. Data of (A,B) from 125 (FL), 129 (TL), 193 (T12) and 110 (YE) cell tracks of 3 independent experiments. Data of (D) from 81 (FL), 102 (TL), 52 (T12) and 84 (YE) cells of 4 (FL) and 3 (TL, T12, YE) independent experiments. Data of (E) from 105 (FL), 112 (TL), 118 (T12) and 65 (YE) cells of 2 independent experiments. Mean $\pm$ SEM. Two-tailed Mann-Whitney tests between each mutant and FL (B,D).

sion, but in an cadherin type-independent manner.

Velocity coordination between neighbors suffices to produce super-diffusion. To identify which features of intercellular adhesion affect the persistence of migrating cells, we turned to *in silico* simulations of interacting self-propelled particles (SPP). First, we modelled individual cells as persistent random walkers with the same apparent speed and intrinsic persistence P_0 in isolation for all cells, in compliance with single cell migration results. Accordingly, P_0 is directly proportional to the time τ needed to make a step and a function of the directional freedom between each step (see Materials and Methods). Then, we tested if super-diffusion could arise from the coordination between neighbor cell velocities that occurs upon intercellular adhesion (11). To do so, we added a classic Vicsek interaction within a cell-scale distance d (52), and explored the MSD of particles at densities ρ covering isolated cells up to beyond confluence. We found that as the number of interacting neighbors $n = \pi\rho d^2$ increased, the effective persistence P of particles increasingly diverged from P_0 before the migration behavior transitioned to super-diffusion (Fig. 3G). Strikingly, for a range of realistic values of interaction distance $d \sim 10 - 30\mu$m, confluent density $\rho \sim 2000 - 4000$ cells/μm^2 and various degrees of directional freedom $1 \lessapprox P_0/\tau \lessapprox 10$, the anomalous exponent fell around $\alpha \sim 1.7$ (yellow region), consistently with experimental observations (see Fig. 3). For $P_0 \sim 10$min, this implies a step time that can take values within 1-10 min. We conclude that velocity coordination is a feature of intercellular adhesion that suffices to produce super-diffusion at confluence.

Vinculin dimerization scales collective cell migration speed to single cell migration speed through the regulation of Adherens Junction stability. To further investigate the impact of intercellular adhesion on collective migration, we next examined the effects of vinculin mutations. All cell lines remained super-diffusive regardless of the expressed mutant (Fig. 4A, Table S3), showing that vinculin plays little to no role in regulating the persistence of collective migration. However, the mutants affected the apparent speed of cells in the collective in a way that could not be fully predicted by their effects on the migration speed of single cells, and this effect was primarily due to the YE mutant in particular (Fig. S4A). Indeed, whereas the TL and T12 mutants decreased and increased, respectively, both single and collective cell speed relative to FL vinculin, the YE mutant decreased single cell migration speed while increasing collective cell migration speed (Fig. 4B compared to Fig. 2B).

To investigate the mechanisms underlying this non-cell autonomous regulation of migration speed, we examined the tension experienced by vinculin and its mutants at AJs (Fig.

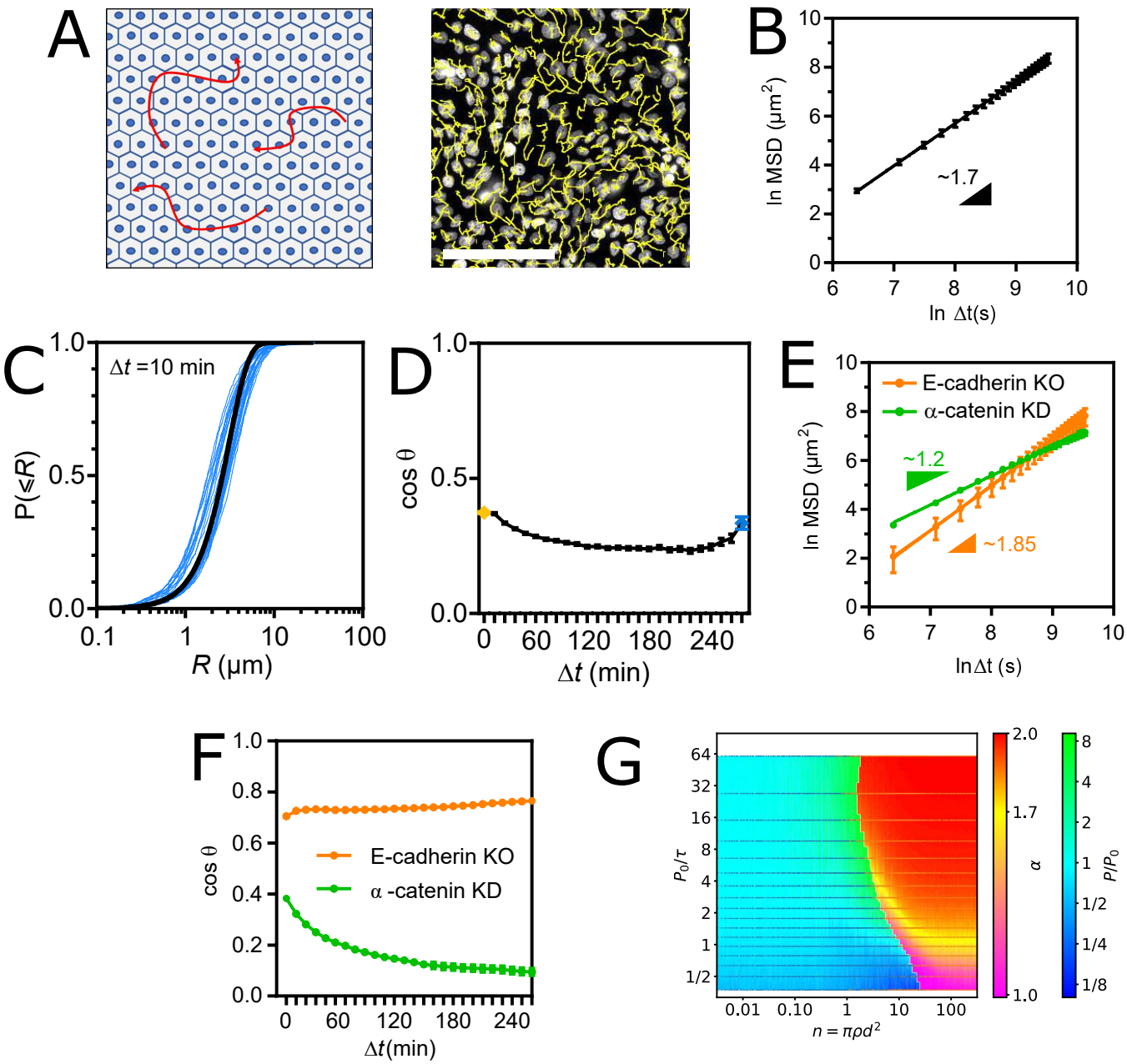

Fig. 3. (A) Left: Schematic representation of the collective cell migration assay. Right: fluorescence image of nucleus-stained cells and their migration tracks in yellow. Scale bar = 100μm. (B) MSD(Δt) of WT cells fit with a straight line in ln-ln scale . (C) Cumulative distributions of the step length R for $\Delta t = 10$min. One blue curve per frame pair along a movie. The black line is a global fit of all blue curves with a cumulative Rayleigh distribution function. (D) Angular memory ($\cos\theta(\Delta t)$) between $\Delta t = 10$min (yellow dot) and $\Delta t = 250$min (blue dot). (E) MSD(Δt) of E-cadherin KO and α-catenin KD cells fit with a straight line in ln-ln scale. (F) Angular memory ($\cos\theta(\Delta t)$) of E-cadherin KO and α-catenin KD cells. Data of (B-D) from 11 Fields Of View (FOV, 500-1000 tracks each) of 3 independent experiments. Data of (E-F) from 3 (E-cadherin KO) and 4 (α-catenin KD) FOV (500-100 tracks each) of 2 (E-cadherin KO) and 3 (α-catenin KD) independent experiments. Mean $\pm$ SEM. (G) Migration behavior of Self-Propelled Particles with Vicsek interactions as a function of their intrinsic directional freedom P_0/τ and number of interacting neighbors $n = \pi\rho d^2$. Blue and orange points are simulation coordinates that resulted in persistent and super-diffusive behavior, respectively. Cold and warm color backgrounds are interpolations from points above of P/P_0 and α values, respectively.

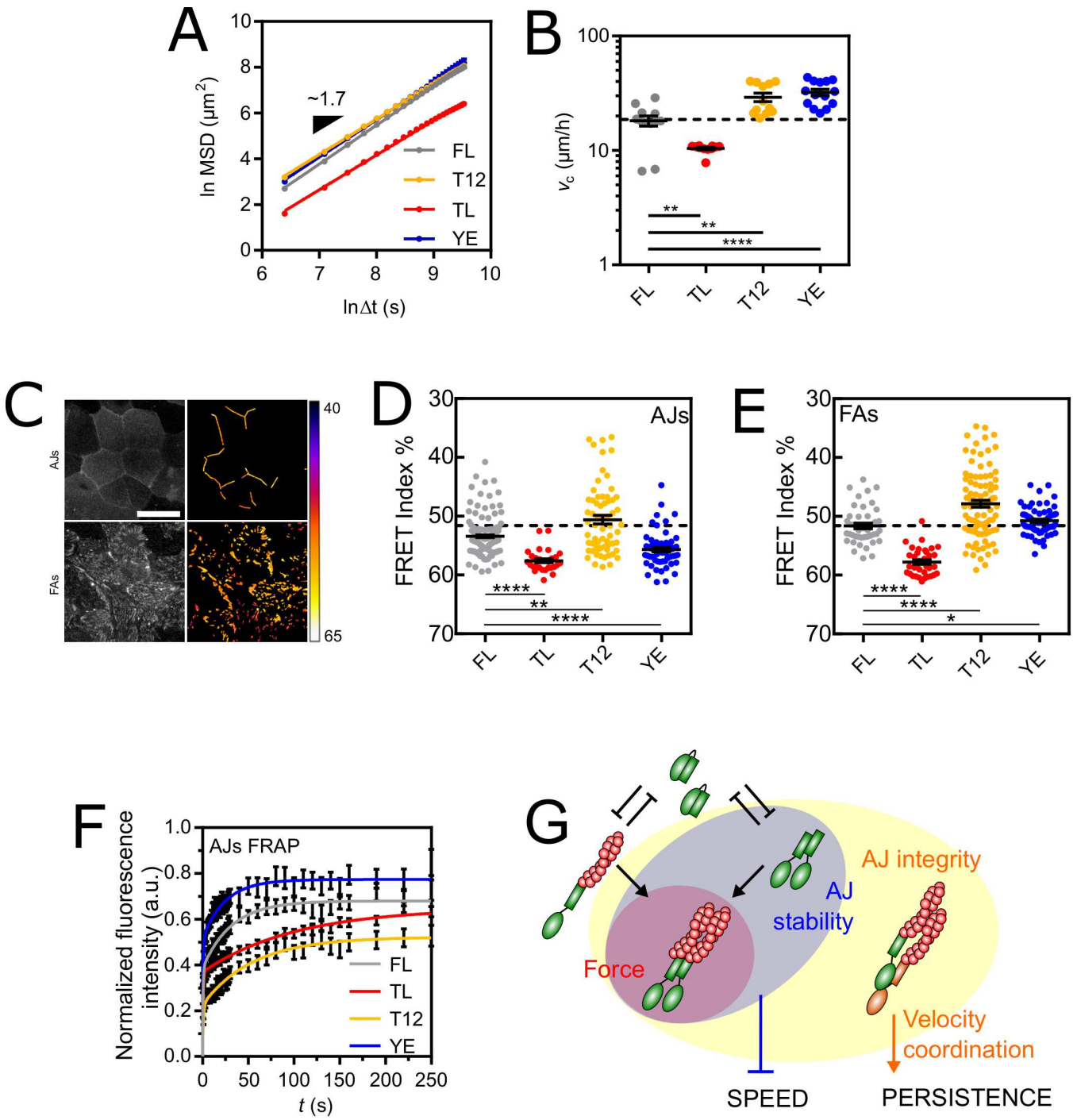

Fig. 4. (A) MSD(Δt) of vinculin FL and mutant cells fit with a straight line in ln-ln scale. (B) Apparent speed of vinculin FL and mutant cells at confluence. (C) Left: direct fluorescence of vinculin TSMod in a confluent monolayer. Right: FRET Index map (see Materials and Methods). Top: in the AJs plane. Bottom: in the FAs plane. Scale bar = 20μm. (D) FRET Index of TSMod in the FL, TL, T12 and YE constructs in AJs at confluence. (E) FRET Index of TSMod in the FL, TL, T12 and YE constructs in FAs at confluence. (F) Fluorescence recovery after photobleaching of FL, TL, T12 and YE constructs in AJs. (G) Proposed model: at AJs, α-catenin (orange) provides AJ integrity (connection to the cytoskeleton), which promotes persistence. The head-tail interaction of vinculin (green) competes with both tail dimerization and actin filaments (red) binding. Dimerization provides AJ stability, which slows cell down. Data of (A-B) from 12 (FL), 11 (TL, T12) and 14 (YE) FOV (500-1000 tracks each) of 3 independent experiments. Data of (D) from 169 (FL), 30 (TL) and 64 (T12, YE) contacts of 5 (FL) and 3 (TL, T12, YE) independent experiments, and (E) from 45 (FL), 35 (TL), 104 (T12) and 67 (YE) cells of 4 (FL) and 3 (TL, T12, YE) independent experiments. Data of (F) from 75 (FL), 137 (TL), 78 (T12) and 36 (YE) contacts of 4 (FL, TL, T12) and 3 (YE) independent experiments. Mean $\pm$ SEM. Two-tailed Mann-Whitney tests between each mutant and FL (B,D,E).

4C). We found that vinculin experienced slighly less tension overall in AJs compared with FAs at confluence (Fig. 4D,E). Furthermore, the effects of the mutants on tension at confluence could be predicted from their effects in single cells for the TL and T12 mutants, which decreased and increased tension, respectively, but not for the YE mutant, which had a stronger effect at confluence than in single cells and opposite effects between FAs and AJs at confluence: while it increased tension in FAs, it decreased tension in AJs (Fig. S4B). Furthermore, the YE mutant was responsible for the loss of correlation that now appeared at confluence between vinculin tension and cell migration speed (Fig. S4C).

To better understand how vinculin might combine its effects at FAs and AJs to regulate cell speed in a non-cell autonomous manner, we examined the stability of vinculin and its mutants in AJs (Fig. S4D). FRAP experiments revealed that the YE mutant had a smaller immobile fraction than WT vinculin, while the TL and T12 mutants had larger immobile fractions, just as in FAs. Thus, the same mutations regulate the stabilities of AJs and FAs in a similar manner (Fig. 4F).

Overall, these results reveal that when AJs are stable (TL, FL, T12), regardless of the tension they transmit via vinculin, cells collectively migrate more slowly but in proportion to their single cell migration speed. In contrast, when AJs are unstable (YE), cells collectively migrate proportionally faster than single cells. We therefore propose that vinculin dimerization ensures AJ stability, which, independent of vinculin tension, scales collective cell migration speed to single cell migration speed in a non-cell autonomous manner (Fig. 4G).

Super-diffusion overcomes weak intercellular adhesion-dependent sensitivity to external guidance cues. Here, we thought to evaluate how the addition of an external guidance cue would affect the spatial exploration efficiency of collectively migrating cells. To do this, we wounded a confluent epithelial monolayer and tracked nucleus-stained cells as they invaded the free surface to close the wound (Fig. 5A). The apparent speed of cells stabilized at approximately 3 hours after wounding (Fig. S5), consistent with previous reports (53). Thus, we thus examined the MSD from 3hrs post-wound. In principle, a directional bias eventually results in ballistic motion as Δt increases, so we expected the slope of the MSD to eventually tend toward 2 in ln-ln scale for large Δt. However, we found that the migration behavior remained super-diffusive with an anomalous diffusion coefficient around 1.6 (Fig. 5B). Furthermore, as in the case of unbiased collective migration, the anomalous diffusion coefficient fell to around 1.2 for cells depleted of α-catenin, whereas cells depleted of E-cadherin remained super-diffusive, (Fig. 5C). Similarly, vinculin mutants did not affect the super-diffusive behavior (Fig. 5D). Overall, these results show that the mechanisms governing the persistence of collective cell migration do not differ substantially in the presence or absence of an external guidance cue at the hours time scale.

We therefore sought to assess the time scale at which the guidance cue would significantly bias cell migration velocity, as well as the magnitude of this bias. In the steady-state speed regime (at 6hrs), a directional bias was already visible at the 10 min scale, i.e., on the apparent cell velocity (Fig. 5E). The bias appeared within 3hrs post-wound and remained stable throughout (Fig. 5F). This reveals that a directional bias of such magnitude at $\Delta t = 10$min that is maintained from 3hrs onwards is too weak to substantially improve the spatial reach of collectively migrating cells in the time range of the experiment. Finally, we asked whether disrupted AJs would impair the sensitivity of cells to directional bias in addition to migration persistence. Remarkably, cells depleted of α-catenin exhibited very little directional bias compared with WT cells (Fig. 5F). Taken together, this shows that AJ-mediated intercellular adhesion is not only required for cells to acquire virtually infinite directional memory, but also for them to perceive external guidance cues.

Vinculin dimerization scales wound healing rate to collective cell migration speed through the regulation of Adherens Junction mechanosensitivity. Because an external guidance cue did not affect the persistence of collective cell migration, we expected that the apparent speed of collectively migrating cells would predict the rate of wound healing, at which the wounded epithelium cover the free surface (see Materials and Methods). We found that this was mostly the case, with the exception of the YE mutant. Indeed, while most vinculin WT and mutant cells closed the wound at a rate proportional to their migration speed in the collective, YE mutant cells were less efficient at wound healing than expected (Fig. 6A). This supports that vinculin dimerization, in contrast to other vinculin tail interactions, affects collective migration speed in a manner dependent of external guidance cues, in addition to its role in scaling collective cell migration speed to single cell migration speed through AJs stability in an unbiased context (see Fig. 4).

To determine how such an effect might emerge from this mutant, we evaluated how adhesions mechanically perceived biased collective cell migration (Fig. 6B-E). Indeed, we had previously found that biased collective migration of epithelial cells imprints a transcellular gradient of molecular tension in E-cadherins, which are under higher tension away from the migration front (54). Consistently, we found that FL vinculin was increasingly recruited and was under higher tension at AJs away from the migrating front, as was the T12 mutant (Fig. 6C). As expected, the TL mutant showed no mechanosensitivity and, remarkably, neither did the YE mutant (Fig. 6C). In FAs, the TL mutant, which is insensitive to cytoskeletal tension by design, displayed a FRET differential suggesting a cytoskeleton-independent increase in compression of vinculin from the migration front, as previously seen within single fibroblasts on adhesive patterns (55). In contrast, FL vinculin displayed an opposite FRET differential, supporting that increased tension from front to back cells, as in AJs, overcompensates for cytoskeleton-independent compression specific to FAs. The T12 mutant showed no FRET gradient, suggesting that its increased cytoskeleton-independent compression from front to back cells is balanced by its increased cytoskeleton-dependent tension. Finally, the YE mutant exhibited mechanosensitivity similar to that of FL

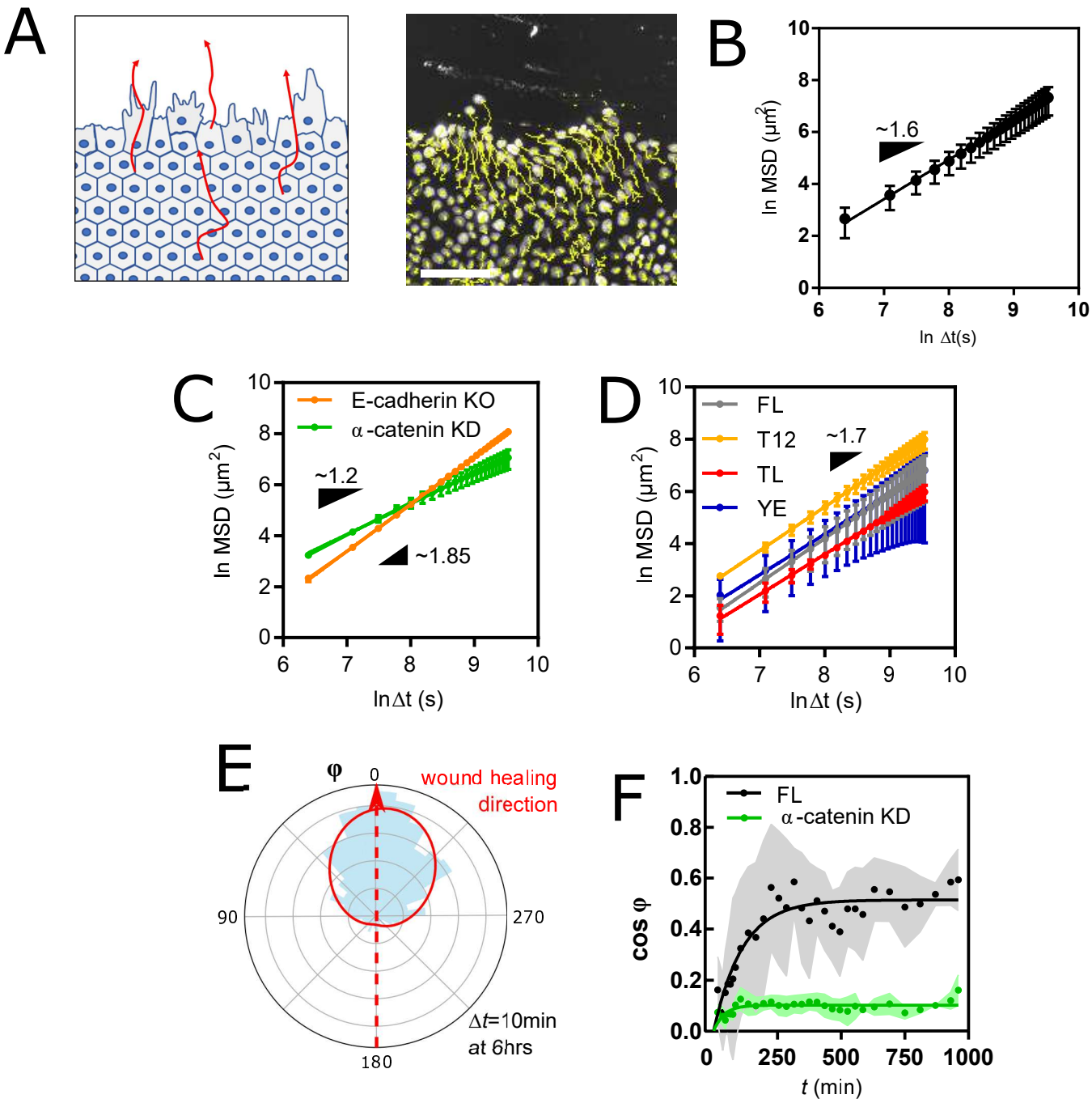

Fig. 5. (A) Left: Schematic representation of the guided collective cell migration assay. Right: fluorescence image of nucleus-stained cells and their migration tracks in yellow. Scale bar = 100μm. (B) MSD(Δt) of WT cells fit with a straight line in ln-ln scale. (C) MSD(Δt) of E-cadherin KO and α-catenin KD cells fit with a straight line in ln-ln scale. (D) MSD(Δt) of vinculin FL and mutant cells fit with a straight line in ln-ln scale. (E) Angular distribution of apparent velocities of vinculin FL cells at 6hrs post-wound fitted with a Von Mises distribution giving a concentration $\kappa \sim 1.3$ (solid red line). (F) Directionality ($\cos\phi(t)$, see Materials and Methods) of vinculin FL and α-catenin KD cells. All data from 4 (all but YE) and 3 (YE) FOV (500-1000 tracks each) of 4 (all but YE and α-catenin KD) and 3 (YE, α-catenin KD) independent experiments. Mean $\pm$ SEM.

vinculin (Fig. 3E). Altogether, this shows that the impaired mechanosensitivity of the YE mutant is specific to AJs, and that differential mechanosensitivity between FAs and AJs is specific to this mutant.

Overall, this supports that the wound healing rate scales with collective migration speed provided that the mechanosensitivity between AJs and FAs is balanced, a feature that depends on the dimerization of vinculin at AJs (Fig. 6F).

Discussion

In this work, we studied the exploratory capacity of epithelial cells, the influence of cell interactions and guidance cues on this exploration, and the dynamics and mechanics of the adhesion complexes that cells use in this process. Remarkably, we show that intercellular adhesion with neighbor cells within the epithelium provides virtually infinite directional memory for migration, as a result of velocity coordination. Mechanistically, we show that cell speed depends on the FAs

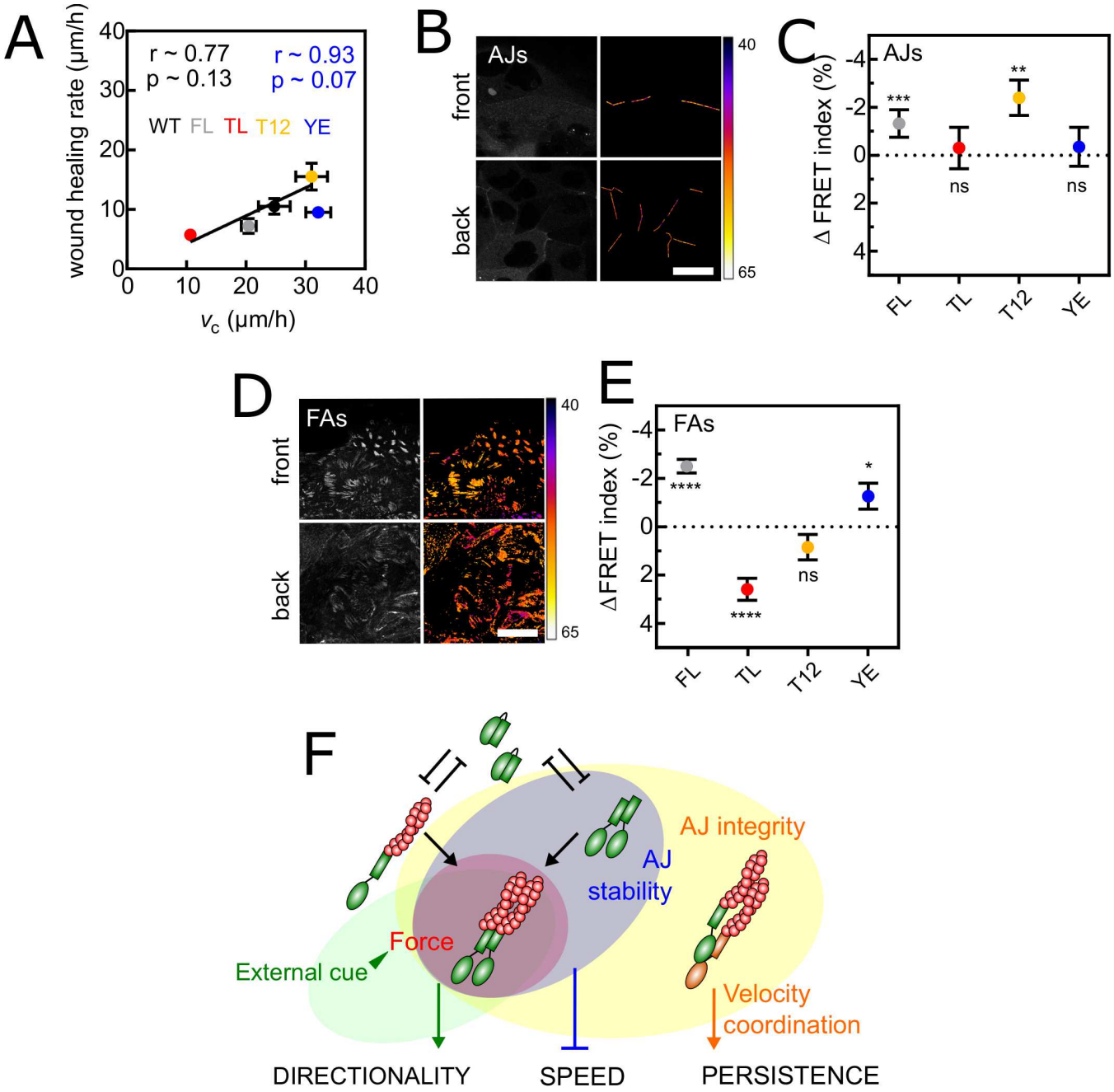

Fig. 6. (A) Wound healing rate (see Materials and Methods) as a function of cell speed at confluence for WT, vinculin FL and mutant cells. Pearson coefficients r and p-values in black for all data, in blue minus YE. (B) Left: direct fluorescence of vinculin TSMod in the AJs plane of cells at the wound front (top) and back (bottom). Right: corresponding FRET Index maps. (C) FRET Index difference between front and back cells at AJs of TSMod in vinculin FL and mutants. (D) Left: direct fluorescence of vinculin TSMod in the FAs plane of cells at the wound front (top) and back (bottom). Right: corresponding FRET Index maps. (E) FRET Index difference between front and back cells at FAs of TSMod in vinculin FL and mutants. (F) Proposed model: at AJs, vinculin dimerization and actin binding provide mechanosensitivity to stable AJs, which promotes wound healing. Data of (A) from 4 (all but YE) and 3 (YE) FOV of 4 (all but YE) and 3 (YE) independent experiments. Data of (C) from 55 (FL), 16 (TL), 56 (T12) and 26 (YE) front cells and 122 (FL), 30 (TL), 71 (T12) and 48 (YE) back cells of 4 (FL, T12) and 3 (TL, YE) independent experiments. Data of (E) from 144 (FL), 63 (TL), 124 (T12) and 46 (YE) front cells and 155 (FL), 84 (TL), 128 (T12) and 51 (YE) back cells of 7 (FL, T12), 4 (TL) and 3 (YE) independent experiments. Mean $\pm$ SEM. Two-tailed Mann-Whitney test between front and back cells (C,E). Scale bars = 50μm.

stability and force transmission and is facilitated by AJs mobility, both controlled by vinculin. Finally, we reveal a specific role for vinculin dimerization at AJs, which regulates the mechanosensitivity of the epithelium as a whole to the guidance cue of a wound, and the wound healing rate of the epithelium.

Locomotion patterns of single cells have been observed and quantified for decades and can can take many forms. When endowed with directional persistence arising from angular correlations between consecutive unit steps, cells adopt a so-called persistent random walk eventually converging toward a purely diffusive behavior, as initially observed in fibroblasts or single endothelial cells (4, 5). In contrast, in T cells and some metastatic cells that behave like Lévy walkers, the fat-tailed power-law distribution of step size is sufficient to support a super-diffusive behavior, even in the absence of temporal correlation in direction (7, 8). Here, our results are consistent with single cells undergoing a persistent random walk, with a persistence on the order of several minutes, beyond which cells purely diffuse (Fig. 1). The persistence of our epithelial cells is thus in the low range compared with individual endothelial cells, whose persistence time can reach several hours (5). Furthermore, by comparing cell lines expressing various forms of vinculin mutants, we observe that persistence does not correlate with cell speed, consistently with endothelial cells exposed to growth factors (5). Mechanistically, both cell speed and persistence are under the control of actin retrograde flow, via connections with cell-matrix adhesions for the former, and control of polarity factors for the latter (56). Our data are thus consistent with the idea that abnormal connection of cell-matrix adhesions to the cytoskeleton via vinculin uncouples speed from persistence.

Previously, fibroblasts or epithelial cells KO for vinculin have been shown to migrate faster than their WT counterparts (21, 22, 29). Here, we show that the migration speed of epithelial cells decreases upon expression of WT vinculin above endogenous levels (Fig. 1). Overall, this is consistent with the idea that the bulk effect of WT vinculin expression is a decrease in cell speed, as if vinculin was acting primarily as a migration moderator. Mechanistically, we show that the head-tail interaction of vinculin challenges both vinculin force transmission and stability in FAs, as has been observed in other cell types (30, 39, 43–46), and is countered by the actin-vinculin tail interaction, which provides force transmission but is unnecessary for stability, and the dimerization of tail, which promotes stability and is dispensable for force transmission (Fig. 2D,E). Overall, we show that the combination of maximal force transmission and stability predicts maximal cell speed (Fig. 2B). We therefore propose that the speed of single cells is the product of FA force transmission and stability (Fig. 2F, S5B).

At confluence, we show that epithelial cells adopt a super-diffusive behavior that a persistent random walk cannot explain (Fig. 2). Such behavior has previously been observed in single cells or in a collective for a variety of cell types (6–8, 13–15). However, whenever a model explained this behavior, it implied anomalous displacement distributions, which are sufficient to support super-diffusion without long-term memory. Moreover, it was not known whether super-diffusion in the collective originated from intercellular interactions or resulted from cells already walking alone in this manner. Here, we show that super-diffusion can be explained by a pure fractional Brownian motion, with no anomalous displacement distribution, but with virtually infinite directional memory (Fig. 2A-D).

Previously, sub-diffusive fractional Brownian motion has explained subcellular particles mobility in the crowded cytoplasm through interactions with the visco-elastic medium (57). Here in comparison, we show that a strong super-diffusive fractional Brownian motion emerges from *bona fide* intercellular adhesion mediated by cytoskeleton-bound but cadherin type-independent AJs, and not just steric hindrance due to the mere presence of neighboring cells (Fig. 3E,F). Remarkably, the same AJ-based mechanism also allows velocity coordination between neighboring cells (11), and our minimal agent-based model shows that such coordination is sufficient to explain the transition to super-diffusion in physiological conditions (Fig. 3G). Notably, the transition occurs for a number of interacting neighbors $n \sim 6$, about the number of direct neighbors at confluence, for a step time τ of a few minutes at experimentally observable persistence $P_0 \sim 10$min. Consequently, this means that cells can be endowed with a finite decision time τ between steps that is intrinsic to their persistent random walk but only revealed upon interaction with neighbors. Discovering its underlying mechanisms may be the subject of future research. Additionally, in the same modelling context, others have shown that velocity coordination also results in spontaneous particle density fluctuations (58). We thus propose that at confluence, such spatio-temporal fluctuations in the number of interacting neighbors generate variations in instantaneous persistence of cells along their trajectories, ultimately resulting in a distribution of persistence times across time-scales. Consistently, the slope of the MSD(Δt) (in log scale) of a persistent random walk at time P is $e-1$, which is about the anomalous exponent α we observe, as if super-diffusing cells experienced a distribution of persistence times that spanned all our experimentally accessible time-scales.

In addition to inducing a transition from finite to infinite directional memory, we show that intercellular adhesion globally decreases the apparent speed of cells provided that vinculin is stable at AJs, as if stable intercellular adhesion restricted cell motion at short time scales. Indeed, perturbation of vinculin dimerization leads to its low stability at AJs and faster than expected cell migration (Fig. 4B,F, S4A). This increase in collective cell speed upon destabilization of AJs is reminiscent of the phenomenon of epithelial unjamming previously observed in vitro and in vivo (59–64). Here, however, epithelial unjamming alone likely cannot fully explain the increase in cell speed, as cells end up migrating not only as fast collectively as alone, but faster. Instead, it is tempting to think that although MDCK cells cannot normally use AJs to propel themselves as, for example, Drosophila border-cells do (11, 65), they might in turn do so in the presence of a vin-

culin unable to dimerize at AJs. Nevertheless, the poor force transmission that the vinculin dimerization mutant exhibits specifically at AJs may not be expected in this case (Fig. 4D,E). Alternatively, we speculate that the vinculin dimerization mutant not only promotes epithelial unjamming by destabilizing AJs, but also subcellular unjamming, in a non-cell autonomous manner, possibly due to the impaired ability to bundle actin of this mutant (41). This could therefore decrease the cell's own resistance to the deformation required for migration but in a collective context only, and thus facilitate migration despite a less efficient FA machinery. Future studies may address this hypothesis.

Overall, intercellular adhesion normally impairs apparent cell speed but induces long-term directional persistence. As a result, while cells are collectively less efficient than single cells at exploring space at time scales of less than 3hrs, they become more efficient beyond that (Fig. S3A). This emergence of long-term collective persistence may therefore have a profound impact on tissue morphogenesis, regeneration and homeostasis in a variety of contexts. In the case of wound healing, cells are further exposed to an external guidance cue. Here we show that this signal is sufficient to bias the velocity of each cell at the smallest experimental time scale (Fig. 5E). Detection of this signal is an emergent property of the collective, as the disruption of intercellular adhesion renders cells nearly blind to free space (Fig. 5F). This is reminiscent of supracellular durotaxis, which is also lost upon the same perturbation (66). However, we show that this directional bias remains too small to drive ballistic motion in less than the longer experimental time range, such that cells remain super-diffusive throughout (Fig. 5A-D). Consistently, wound healing rates reach only a fraction of apparent cell speeds, and in a manner that depends on vinculin tail dimerization (Fig. 6A). In this context, vinculin tail dimerization provides equal mechanosensitivity to AJs and FAs, such that they transmit molecular tension gradients across the tissue (Fig. 6B-E). This is again consistent with the requirements for supracellular durotaxis, which emerges from intercellular force transmission (66), and may be related to the ability of cells to stiffen under force in a manner that depends on vinculin dimerization (41). Finally, we expect the directional bias to lead to more ballistic cell motion at longer time scales, with a lower limit of at least 3hrs.

In summary, we conclude that the spatial exploration efficiency of guided collective migration depends on the scale considered: at short time scales (<3hrs), the intrinsic migration speed of individual cells is the limit, which is overcome by collective directional memory at intermediate time scales (>3hrs), a tissue-level property that guidance cues from the environment must override to become significant at longer time scales (»3hrs) (Fig. S5C). We speculate that this generic framework applies qualitatively to guided collective migration in general and hope that future studies will test this prediction.

Materials and Methods

Live MDCK type II G cells stably expressing fluorescently tagged proteins were monitored on a wide-field epifluorescence inverted microscope for migration experiments, on a scanning spectral confocal microscope for FRET experiments, or on a spinning disk confocal microscope for FRAP experiments. Image analyses were performed with Imaris and ImageJ, data analyses with Matlab and statistics with Prism software. Full materials and methods are available in Supplementary Materials and Methods.

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Materials and Methods

Cells. MDCK type II G cells were cultured at 37°C and 5% CO2 in low glucose DMEM with phenol red supplemented with 10% v/v Fetal Bovine Serum (FBS) and Penicillin 10 U/mL + Streptomycin 10 µg/mL. Cells were transfected using Turbofect transfection reagent (Thermo Scientific) according to the manufacturer's instructions. 24 hours post-transfection, cells were collected and seeded for experimental use and/or cultured under drug selection (G418 sulfate 200 µg/mL) for 15 days and FACSed for intermediate expression to generate stable lines. Stable lines were cultured under drug selection. The α-catenin KD cell line was a kind gift of the laboratory of W. James Nelson (Stanford U.) (1). The E-cadherin KO cell line was a kind gift of the laboratory of B. Ladoux and R. M. Mège (Institut Jacques Monod) (2). Cells were imaged at 37°C and 5% CO2 in Fluorobrite medium supplemented with 10% v/v FBS, Penicillin 10 U/mL + Streptomycin 10 µg/mL, 2.5 mM L-Glutamine and 20mM HEPES on 1.0 borosilicate, 18 or 32mm Ø round glass coverslips or on 2- or 4-well Nunc™ Lab-Tek™ Chambered Coverglass supports (Thermo Scientific) previously coated with collagen IV by incubation with a solution (50 µg/mL in 0.5M acetic acid) of collagen from human placenta Bornstein and Traub Type IV (Sigma) for 10 minutes before air-drying.

Plasmids. VinculinTS (addgene #26019) and Tailless VinculinTS (#26020) were a kind gift of the laboratory of Carsten Grashoff (Westfälische Wilhelms-Universität Münster) (3). The following mutations were introduced with the QuikChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies):
T12 (5'-GAGGTGGCCAAGCAATGTACTGTCGCAGCCA
TTGCAACAAACCTC-3', 5'-CTCACAGACCTGTAAGA
GGTTTGTTGCAATGGCTGCAGCAGTACA-3'),
Y1065E (5'-ATCTGCAGAATTCTTACTGCTCCCATGGG
GTCTTTCTGACC-3', 5'-GGTCAGAAAGACCCCATGG
GAGCAGTAAGAATTCTGCAGAT-3'),

Migration assays. For single cell migration, 36 hours before imaging, cells were passaged at a 1:10 dilution with vigorous pipetting, then 4 to 6 hours before imaging cells were detached once again, resuspended with vigorous pipetting and plated at about 6 cells/mm². For collective migration, 24 hrs before imaging, cells were resuspended and plated at about 600 cells/mm². For Biased collective migration, cells were additionally incubated at 37°C in DPBS for 5 to 10 minutes before imaging to weaken calcium-dependent cell-cell contacts and a straight-lined scratch was made in the monolayer using a 200 µL pipette tip. DPBS was then replaced with imaging medium. Prior to imaging, nuclei were stained by incubation in Hoechst 0.1 µg/µl (Thermo Scientific) imaging medium for 20 minutes followed by further dilution to 0.05 µg/µl. Imaging was performed on a ZEISS Axio Observer Z1 widefield microscope with 20x Plan-Apochromat/0.8 dry objective. Hoechst fluorescence was imaged by excitation with a CoolLED pE-300 UV fluorescent lamp, excitation and emission filters 335-383 and 420-470, and an ORCA-Flash4.0 LT camera. Multi-position and multi-tile images were acquired every 10 minutes overnight.

Migration analyses. Nuclear staining detection and track generation were performed with Imaris 9.2 for single cell migration and Fiji with the ImageJ TrackMate plugin (4) for collective and biased collective migration. Visual inspection of tracks against raw movies enabled manual correction if necessary. Further data analyses were performed with Matlab.
Apparent cell speed is defined as the distance travelled during the smallest time interval (10min) and directly obtained from cell tracking in Imaris and TrackMate. Speed is averaged over each track, then over the cell population for single cells migration experiments. Speed is averaged over each field of view for each timepoint and over the duration of the experiment for collective and biased collective migration.
Mean square displacement (MSD) is: $\mathrm{MSD}(\Delta t) = \langle(\Delta\mathbf{r}_i(\Delta t))^2\rangle = \langle(\mathbf{r}_i(t+\Delta t) - \mathbf{r}_i(t))^2\rangle$, where $\mathbf{r}_i$ is the position of the cell i at time t and $\langle\rangle$ is the average for all cells and t. For each experiment comprising multiple field of view acquisitions, a weighed mean MSD was calculated to correct for differences in track duration. For each cell line and condition the average MSD was calculated as a weighed mean between experimental replicates to account for potential differences in experiment duration. $\mathrm{MSD}(\Delta t)$ curves were fit with the Fürth equation $\mathrm{MSD} = 4D(\Delta t - P(1 - \exp - \Delta t/P))$ where D is the coefficient of diffusion and P the persistence time, or with a straight line in $\ln - \ln$ scale where the slope α is the anomalous diffusion coefficient.
Step length $R_{i,t}(\Delta t)$ of a cell i is $\sqrt{(\mathbf{r}_i(t+\Delta t) - \mathbf{r}_i(t))^2}$. Step lengths cumulative distributions are displayed for all cells at every time point. Curves from all cells and all time points were fit together with the cumulative distribution function of the Rayleigh distribution $\mathrm{P}(< R) = 1 - \exp - R^2/2\sigma^2$ where σ is the scale parameter.
Angular memory $M_{i,t}(\Delta t)$ is defined as the cosine of the angle $\theta_{i,t}$ between the displacement vectors $\mathbf{L}_{i,t}(10\mathrm{min})$ between times t and $t + 10\mathrm{min}$, and $\mathbf{L}_{i,t-\Delta t}(\Delta t)$ between times $t - \Delta t$ and t of a cell i at time t: $M_{i,t}(\Delta t) = \cos\theta_{i,t}(\Delta t) = \cos(\mathbf{L}_{i,t}^{t+10\mathrm{min}}, \mathbf{L}_{i,t-\Delta t}^{t})$. Figures display $\langle M_{i,t}(\Delta t)\rangle$.
Directionality is defined as the cosine of the angle ϕ between the cell apparent velocity vector and the direction orthogonal to the wound. Figures display the population average and standard error. Curves were fit with a one-phase association equation:

$\cos\phi = \cos\phi_{\text{max}}(1-\exp(-t/\tau_{\text{d}}))$ where $\cos\phi_{\text{max}}$ is the average directionality at the infinite time limit and τ_{d} the characteristic time to reach it.
Wound closure speed is defined as the rate of wound half-area $A/2$ decrease in $\Delta t = 30$min normalized to wound length l: $\text{WC} = (A(t+\Delta t) - A(t))/2l\Delta t$.

Simulations. Self Propelled Particles (SPP) simulations were performed in R software (version 4.2.2) on $N = 200$ particles within a 2D square box of side L with periodic boundary conditions for $T = 600$ steps of duration τ. From an initial condition of random positions and orientations of all particles, the position of each particle i is updated according to $\mathbf{r}_i(t+\tau) = \mathbf{r}_i(t) + \mathbf{v}_i(t+\tau)\tau$, where $\mathbf{v}_i$ is the velocity of particle i with constant modulus v and an orientation $\theta_i(t+\tau) = \arg\left[\sum_{j,|\mathbf{r}_i(t)-\mathbf{r}_j(t)|<d}\mathbf{v}_j(t)\right] + \eta\xi_i$. The first term accounts for an interaction that causes velocity alignment between neighbors within a distance d (5). The second term is a white noise $\xi_i(t)$ over $[-\pi,\pi]$ bounded by $\eta \in [0,1]$, which defines the directional freedom of an isolated particle. The intrinsic cell persistence, i.e. that of an isolated cell, directly relates to η and τ through $P_0 = \tau(\overline{\cos\theta_i})^{1/2}/(1-\overline{\cos\theta_i})$ where $\overline{\cos\theta_i} = \sin\eta\pi/(\eta\pi)$, by generalization of Fürth's approach (6) to two dimensions. The apparent speed $v = 2.5$µm.min^{-1} was fixed in the typical range of experimental measurements. The total particle density $\rho = N/L$ was set to various values within $[10^{-5}, 10^{-2}]$cell.µm^{-2} to match single ($\rho \ll 10^{-3}$cell.µm^{-2}) or collective ($\rho \geq 10^{-3}$cell.µm^{-2}) cell migration assays. The interaction distance $d = 5-40$µm was varied around the cell size scale. The directional freedom η was varied within $]0,1[$ for the sake of exhaustiveness. Simulated tracks were analysed as live cell tracks to compute the $MSD(\Delta t)$ that was fitted in ln-ln scale with the minpack.lm R package. Fit parameters P and α were averaged over 5 simulations for each combination of parameter values and linearly interpolated on a 400x400 grid between extreme values of P_0/τ and $\pi\rho d^2$ for representation purposes. The $P-\alpha$ boundary was set at the transition in majority behavior within moving boxes of size 0.25 in log-log scale.

Molecular tension microscopy. Cells were imaged on a ZEISS LSM780 confocal microscope with a 63x/1.4NA oil immersion objective. The mTFP1 donor fluorophore was excited by a 458 nm Argon laser line. Fluorescence emission was detected in the 473nm-561nm spectrum with a spectral resolution of 8.8nm (10 channels) by a GaAsP (Gallium Arsenide Phosphide) detector. The pixel resolution was 0.086µm. Image analysis was performed in Fiji. Segmentation of FAs for FRET index measurements was performed by a custom macro that subtracts the background, applies a Gaussian filter, thresholds, localizes particles of an area of 0.7 µm² or more and a circularity factor between 0 and 0.95 and displays the final Region Of Interest (ROI) set with the HiLo look up table to enable manual removal of any spurious or saturated ROI. Segmentation of AJs was performed manually. FRET index = FRET/(Donor+FRET) is calculated using the PixFRET plugin in Fiji (7) from the channels around 494 and 530nm (Donor and FRET channels respectively) of the spectral stack, a 2px radius Gaussian filter and a threshold above 1x mean background intensity. Using the ROI set generated from the corresponding intensity image, the ROI pixels belonging to one cell are combined to obtain an average FRET index value of FAs or AJs per cell.

Fluorescence recovery after photobleaching. FRAP was performed on a Yokogawa Spinning Disk CSU-X1 microscope. Cells were imaged with a 63x/1.4NA oil immersion objective. Fluorescence was acquired by 491nm laser excitation with a GFP 525/50 emission filter and a sCMOS PRIME 95 camera (Teledyne-Photometrics). Photobleaching was achieved by a 476nm FRAP laser. Images were analyzed with ImageJ. Fluorescence in ROIs was background-subtracted, corrected for photobleaching due to imaging and normalized by the fluorescence before photobleaching. The average curve for each condition was then fitted with a two-phase association equation: $F = F_0 + F_{\tau_{\text{fast}}}(1-\exp(-t/\tau_{\text{fast}})) + F_{\tau_{\text{slow}}}(1-\exp(-t/\tau_{\text{slow}}))$ where F_0 is the fluorescence at the first timepoint at photobleaching, $F_{\tau_{\text{fast}}}$ the fluorescence of the mobile fraction characterized by the time τ_{fast} and $F_{\tau_{\text{slow}}}$ the fluorescence of the mobile fraction characterized by the time τ_{slow}.

Western Blot. Cells were washed with PBS at 4°C, lysed on ice in RIPA buffer (with protease and phosphatase inhibitors) by scraping, collected in centrifugation tubes, vortexed for 1 minute and centrifuged at 13000 rpm for 15 minutes at 4°C. Supernatant was then collected and diluted with an equal volume of Laemmli Buffer 2x (Sigma). Samples were loaded on a Mini-Protean TGX Precast gel (BioRad), ran for 1 hour at 100 V in running buffer (Tris-Glycine buffer 1x, SDS 0.005%) and transferred onto a nitrocellulose membrane in a 10% methanol transfer buffer (Tris-Glycine 1x, methanol 10%) for 1 hour at 100 V. The transfer was verified by staining with Ponceau S solution. The membrane was blocked for 30 minutes at room temperature in a 5% milk in TBST solution (Tris-buffer saline solution 1X, 0.05% Tween 20) followed by overnight incubation with the mouse anti-Vinculin V-9131 (Sigma) primary antibody in 5% milk in TBST solution. The membrane was then washed three times with TBST solution for 5 minutes each time and incubated for 1 hour at room temperature with HRP-Goat anti-Mouse (Invitrogen 626520) secondary antibody in 5% milk in TBST solution. Prior to revelation the membrane was once again washed three times with TBST solution for 5 minutes each time. Signal was developed using SuperSignal® West Femto Maximum Sensitivity Substrate Kit (Thermo Scientific) according to the manufacturer's instruction and a ChemiDoc™ MP Imaging system (BioRad).

Statistical analysis. Statistical analyses were performed mostly using the software GraphPad Prism VII or R. Data is represented with mean ± SEM unless stated otherwise. Difference between data sets was assessed with the non-parametric, unpaired, two-tailed Mann-Whitney test. p-values of linear regressions by the least-squares method were calculated using an extra sum-of-squares F test with slope = 0 as the null hypothesis. Correlations were calculated using the Pearson correlation. MSD models were compared using the Akaike Information Criterion (AIC) method. Tables show the score $1/(\exp \Delta \mathrm{AIC} + 1)$.

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Table 1. Most likely walk model for single cell migration

Cells	Relative likelihood of persistent diffusion vs. pure or super-diffusion
WT	>0.9999
FL	>0.9999
TL	>0.9999
T12	>0.9999
YE	>0.9999

Table 2. Most likely walk model for collective cell migration

Cells	Relative likelihood of persistent diffusion vs. pure or super-diffusion	Anomalous diffusion coefficient
WT	$< 10^{-4}$	1.73 ± 0.003
FL	$< 10^{-4}$	1.699 ± 0.008
TL	< 0.4172	1.52 ± 0.01
T12	$< 10^{-4}$	1.545 ± 0.007
YE	$< 10^{-4}$	1.636 ± 0.007
$\alpha-$catenin KD	< 0.1155	1.192 ± 0.009
E-cadherin KO	$< 10^{-4}$	1.847 ± 0.005

Table 3. Most likely walk model for directed collective cell migration

Cells	Relative likelihood of persistent diffusion vs. pure or super-diffusion	Anomalous diffusion coefficient
WT	$< 10^{-4}$	1.52 ± 0.01
FL	$< 10^{-4}$	1.718 ± 0.003
TL	$< 10^{-4}$	1.571 ± 0.008
T12	$< 10^{-4}$	1.687 ± 0.006
YE	$< 10^{-4}$	1.55 ± 0.01
$\alpha-$catenin KD	$< 3.10^{-4}$	1.199 ± 0.008
E-cadherin KO	$< 10^{-4}$	1.846 ± 0.004

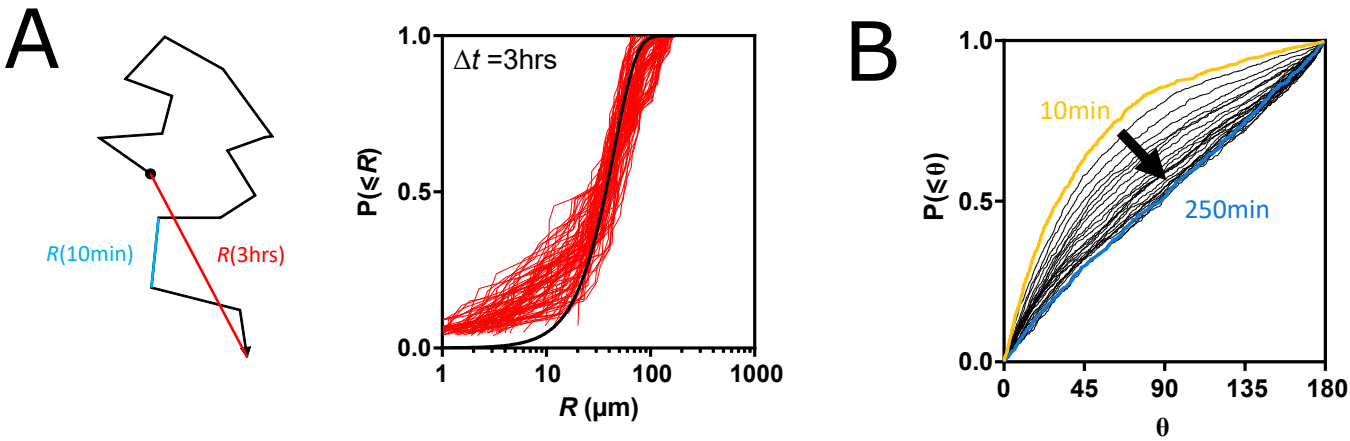

Fig. S 1. (A) Left: Schematic migration track and step length ($R(\Delta t)$, see Materials and Methods) at $\Delta t = 10$min (blue) and $\Delta t = 250$min (red). Right: cumulative distributions of the step length R for $\Delta t = 3$hrs of single cells. One red curve per frame pair along a movie. The black line is a global fit of all red curves with a cumulative Rayleigh distribution function. (B) Cumulative distributions of the angular deviation ($\theta(\Delta t)$, see Materials and Methods) between $\Delta t = 10$min (yellow) and $\Delta t = 250$min (blue) of single cells. Data from 94 cell tracks of 2 independent experiments (same as in Fig. 1).

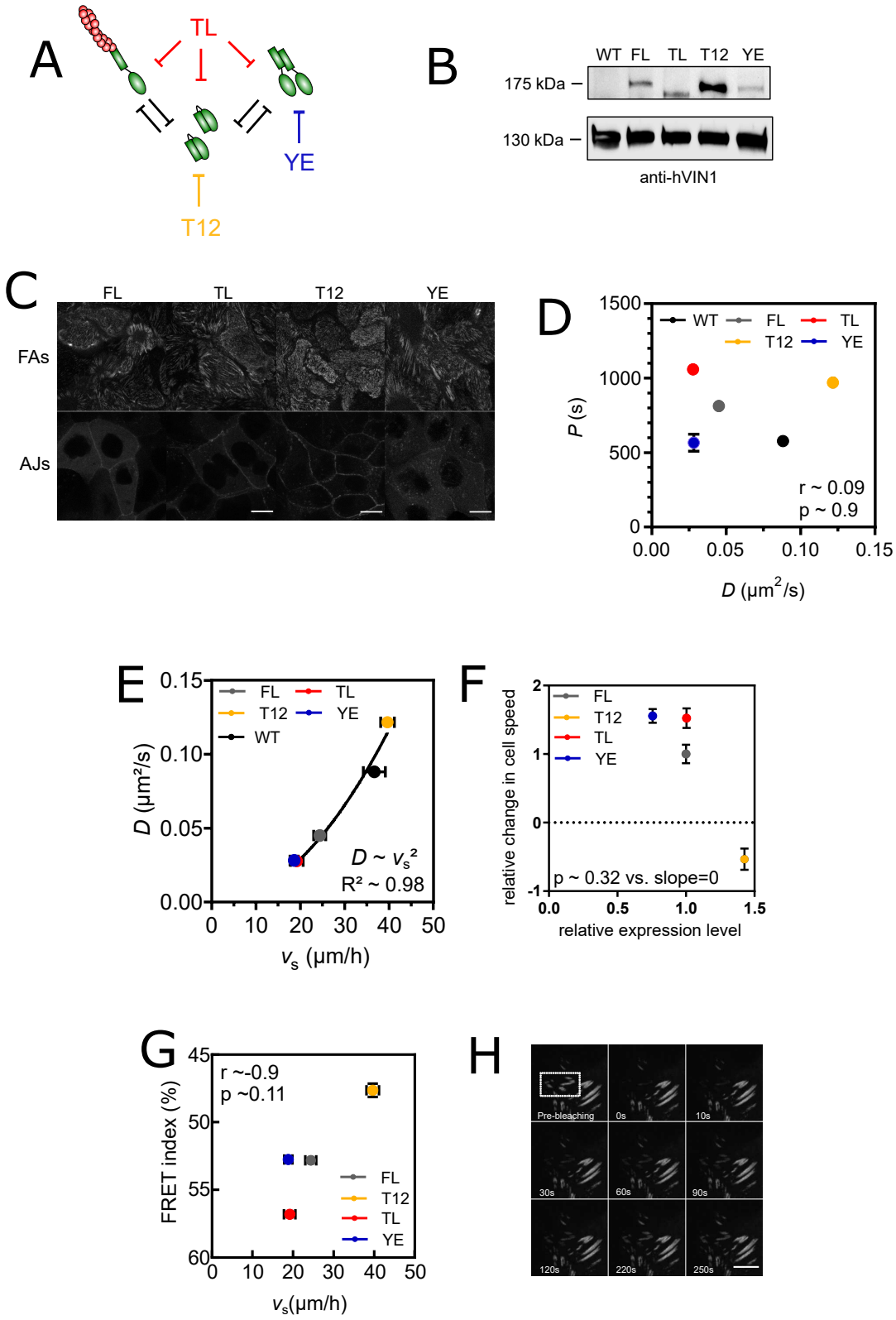

Fig. S 2. (A) Vinculin (green) and the different interactions (head-tail, tail-tail and actin-tail) and mutants (TL, T12 and YE) considered in this study. (B) Western Blot of the WT cells and cells expressing the FL, TL, T12, and YE vinculin TSMod constructs labelled for vinculin (WT at 130kDa, TSMod at 175kDa). (C) Direct fluorescence of the vinculin TSMod constucts in the FAs (top) and AJs (bottom) planes. Scale bar=20μm. (D) Persistence time P as a function of diffusion coefficient D for WT, vinculin FL and mutant single cells. Pearsons correlation coefficient r and p-value. (E) Diffusion coefficient D as a function of the apparent speed v_s of WT, vinculin FL and mutant single cells, globally fit the Fürth equation with $\Delta t = 10$min. (F) Relative change in single cell apparent speed as a function of vinculin FL or mutant expression level. p-value of an extra sum-of-squares F test on a linear regression with slope=0 as the null hypothesis. (G) FRET index as a function of apparent speed v_s for vinculin FL and mutant single cells. Pearsons correlation coefficient r and p-value. (H) Direct fluorescence of vinculin TSMod in FAs during FRAP. White Box shows the FRAPed region. Scale bar=5μm. Data of (D-F) from 94 (WT), 125 (FL), 129 (TL), 193 (T12) and 110 (YE) cell tracks of 2 (WT) and 3 (all others) independent experiments (same as in Figs. 1 and 2A,B). Data of (G) as above for v_s and from 81 (FL), 102 (TL), 52 (T12) and 84 (YE) cells of 4 (FL) and 3 (TL, T12, YE) independent experiments for FRET index (same as in Fig. 2B,D). Mean $\pm$ SEM.

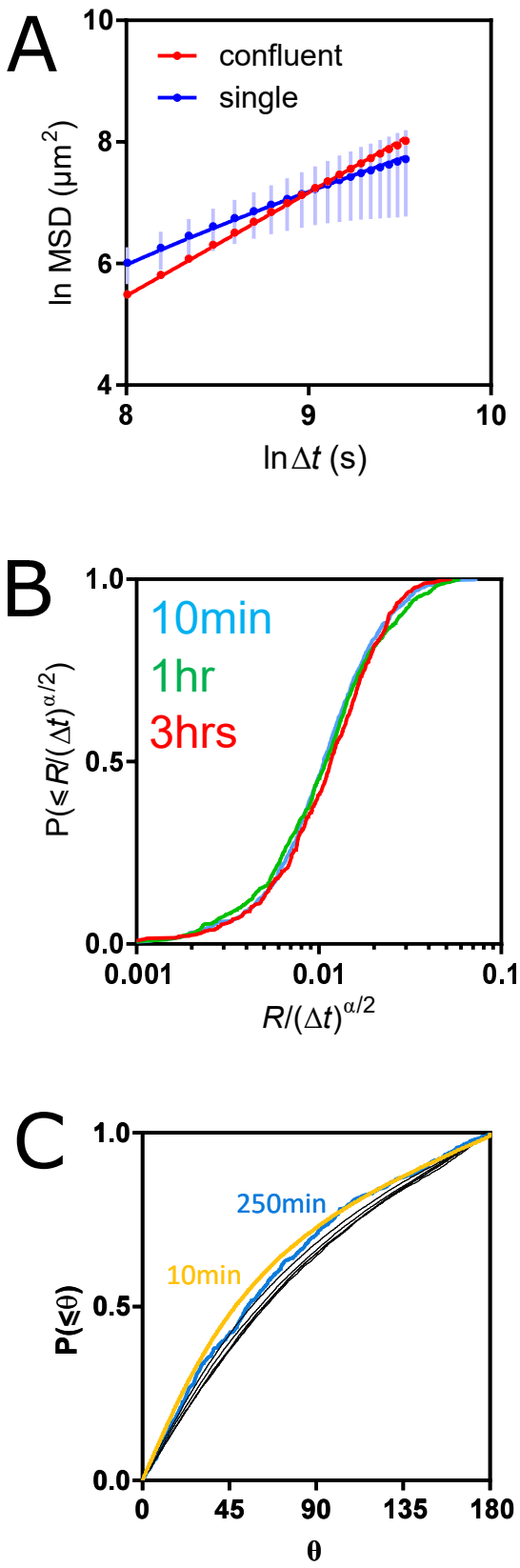

Fig. S 3. (A) MSD(Δt) of single (blue) and confluent (red) cells at long time scales. (B) Cumulative distributions of the normalized step length $R/(\Delta t)^{\alpha}$ where α is the anomalous coefficient, for $\Delta t = 10$min, 1hr and 3hrs at confluence. One representative frame pair per Δt. (C) Cumulative distributions of the angular deviation ($\theta(\Delta t)$, see Materials and Methods) between $\Delta t = 10$min (yellow) and $\Delta t = 250$min (blue) of confluent cells. Data from 11 FOV (500-1000 tracks each) of 3 independent experiments (same as in Fig. 3B-D). Mean $\pm$ SEM.

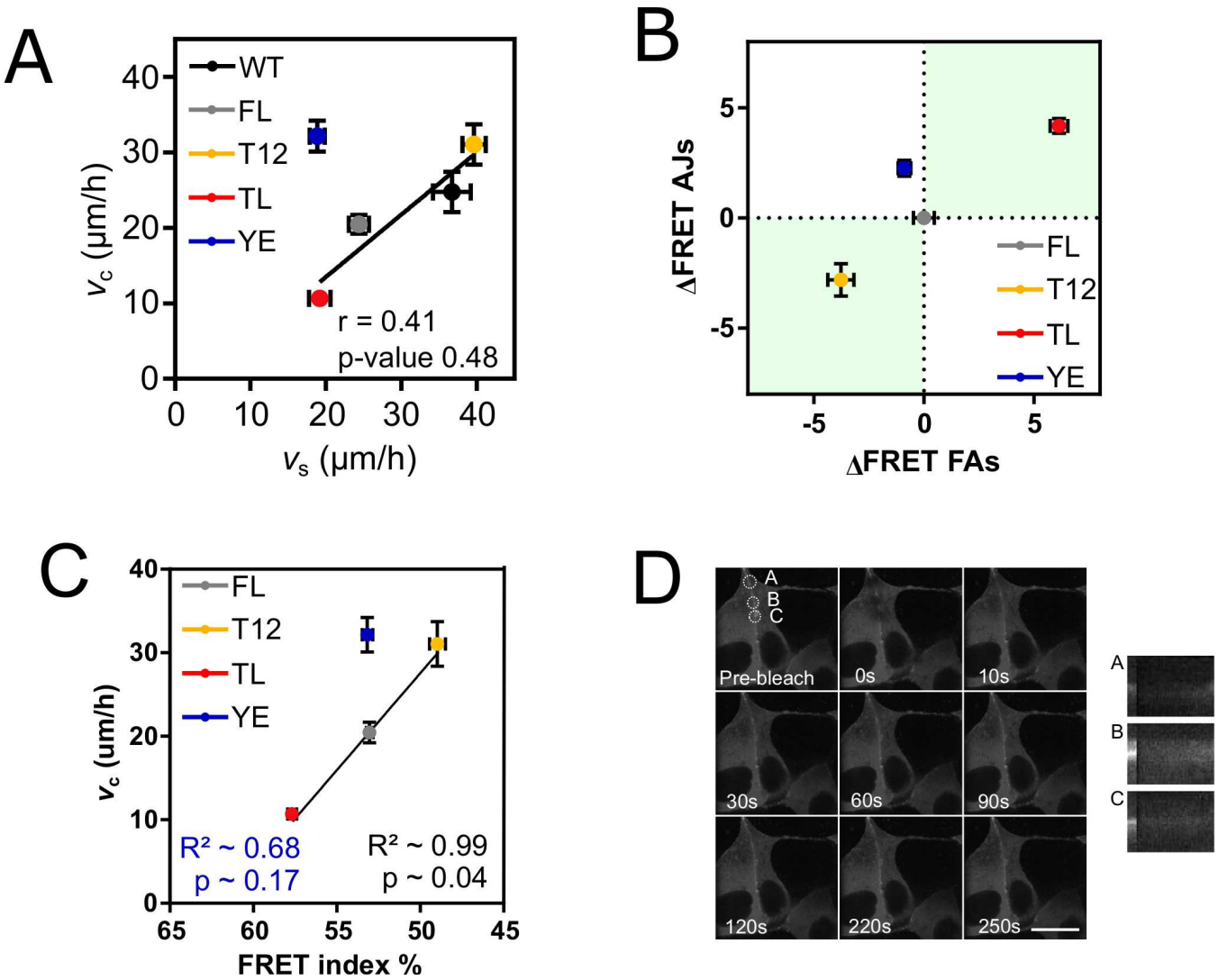

Fig. S 4. (A) Apparent collective cell speed (v_c) as a function of apparent single cell speed (v_s) for WT cells and cells expressing the vinculin FL and mutant constructs. Pearson coefficient r and p-value. (B) FRET index of the vinculin mutants relative to vinculin FL at AJs vs. FAs. (C) Apparent collective cell speed (v_c) as a function of FRET index of vinculin constructs. Pearson coefficient R^2 and p-value for all cell lines (blue) and without the YE mutant (black). (D) Left: direct fluorescence of vinculin TSMod in AJs during FRAP. Right: kymographs of the circled, FRAPed regions A, B, C on the left Scale bar=5μm. Data of (A) from 94 (WT), 125 (FL), 129 (TL), 193 (T12) and 110 (YE) cell tracks of 2 (WT) and 3 (all others) independent experiments for v_s) (same as in Figs. 1 and 2A,B) and from 11 (WT), 12 (FL), 11 (TL, T12) and 14 (YE) FOV (500-1000 tracks each) of 3 independent experiments for v_c) (same as in Figs. 3B-D and 4A,B). Data of (B) from 169 (FL), 30 (TL) and 64 (T12, YE) contacts of 5 (FL) and 3 (TL, T12, YE) independent experiments for AJs, and from 45 (FL), 35 (TL), 104 (T12) and 67 (YE) cells of 4 (FL) and 3 (TL, T12, YE) independent experiments for FAs (same as in Fig. 4D,E). Data of (C) as in (A) for v_c and in (B, FAs) for FRET index. Mean $\pm$ SEM.

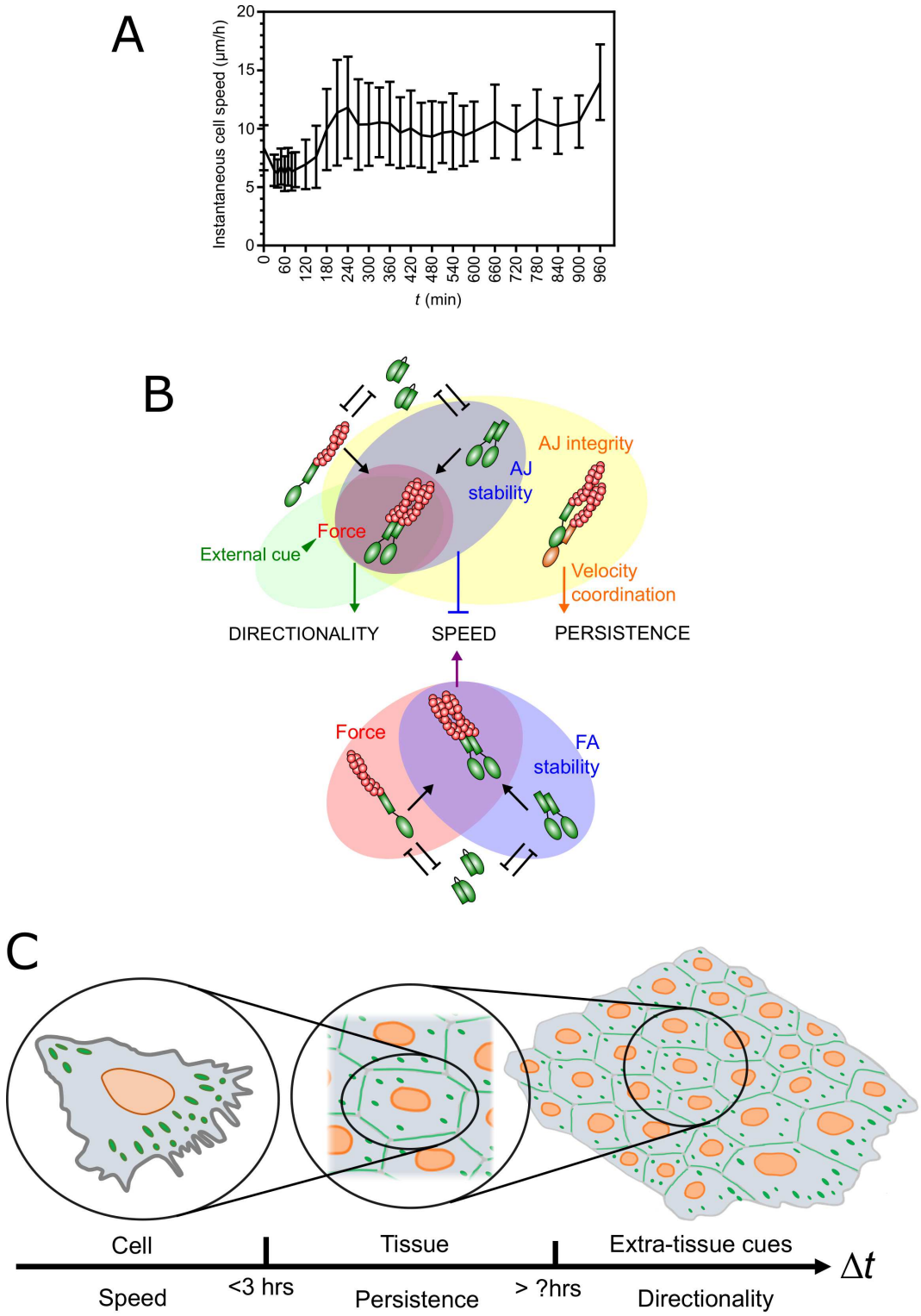

Fig. S 5. (A) Apparent cell speed as a function of time in a wound healing assay. (B) Complete model for the control of cell speed persistence and directionality by the stability and force transmission of adhesions, from Fig. 2F, 4G, 6F) (C) Working model for the control of cell spatial exploration efficiency as a function of time scales (see text for details). Data of (A) from 4 FOV (500-1000 tracks each) of 4 independent experiments (same as in Fig. 5B). Mean ± SEM.