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Supplemental Information

An Integrated Stochastic Model of Matrix-Stiffness-Dependent Filopodial Dynamics

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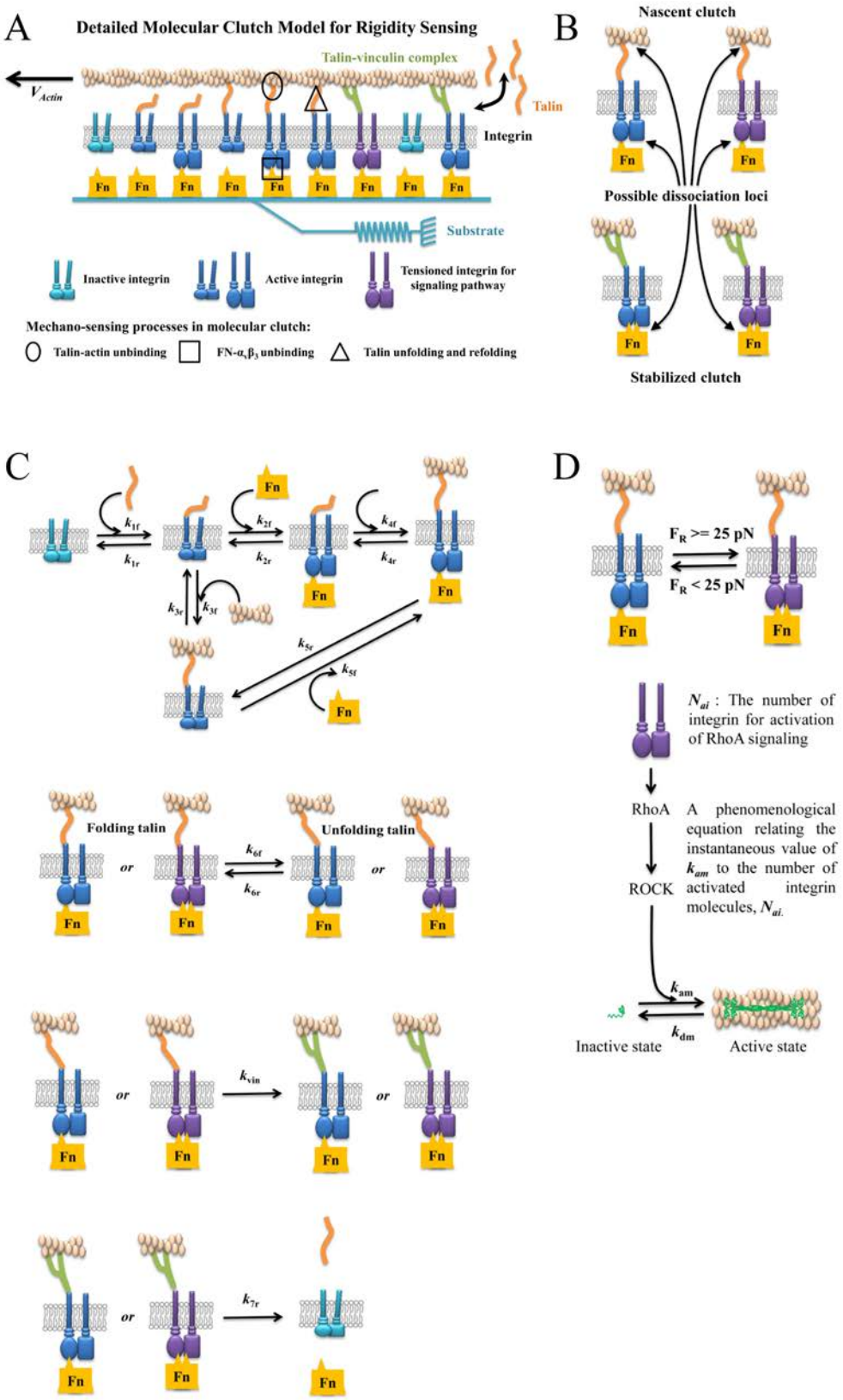

Figure S1. The schematic figure of molecular clutch.(A) Detailed clutch model for rigidity sensing. (B) Possible dissociation loci in molecular clutch for nascent and stabilized clutch. (C) Formation and rupture of nascent and stabilized clutch. (D) Activation of Rho signaling pathway.

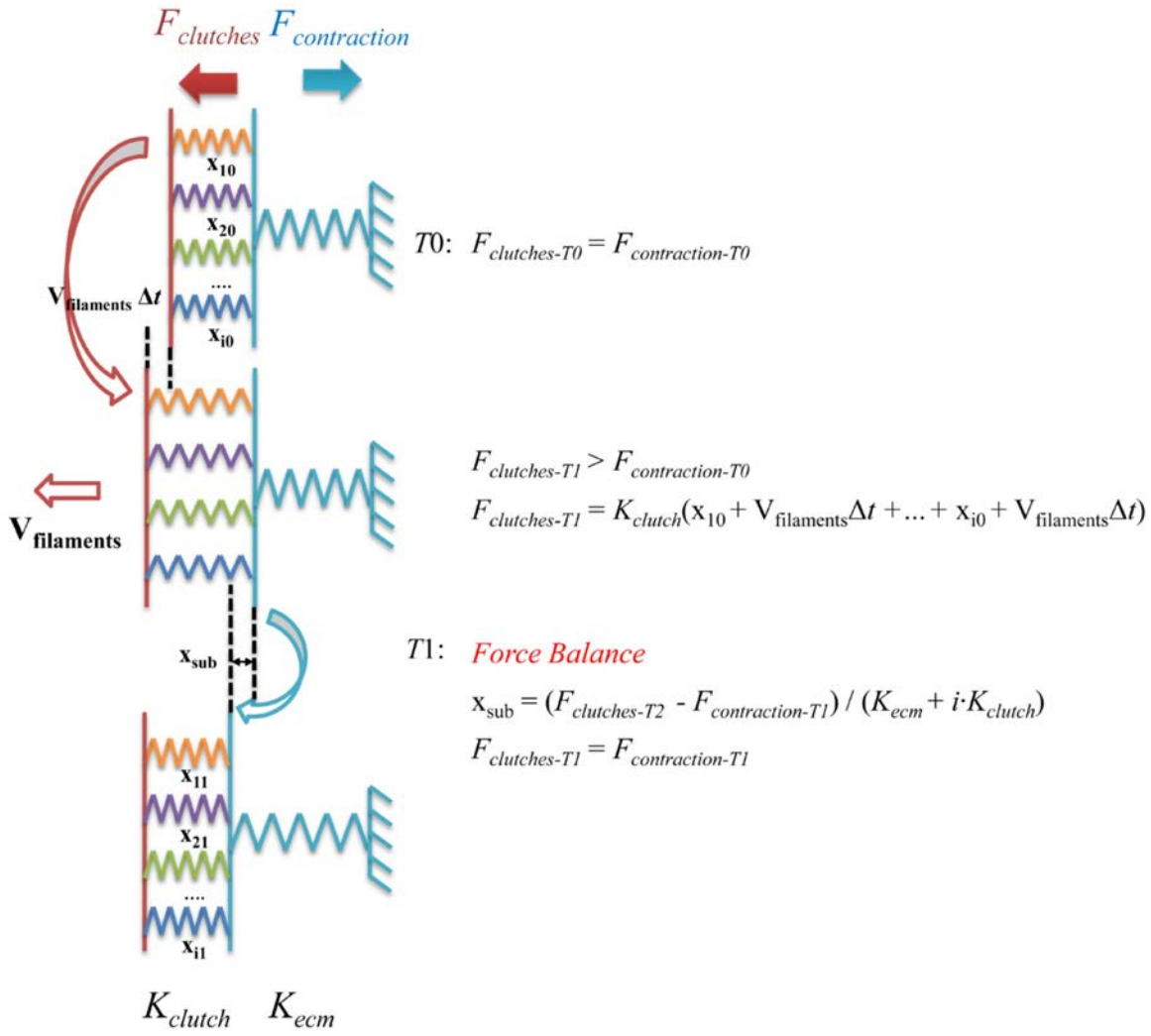

Figure S2. Diagram for calculation of force balance between molecular clutch and substrate.

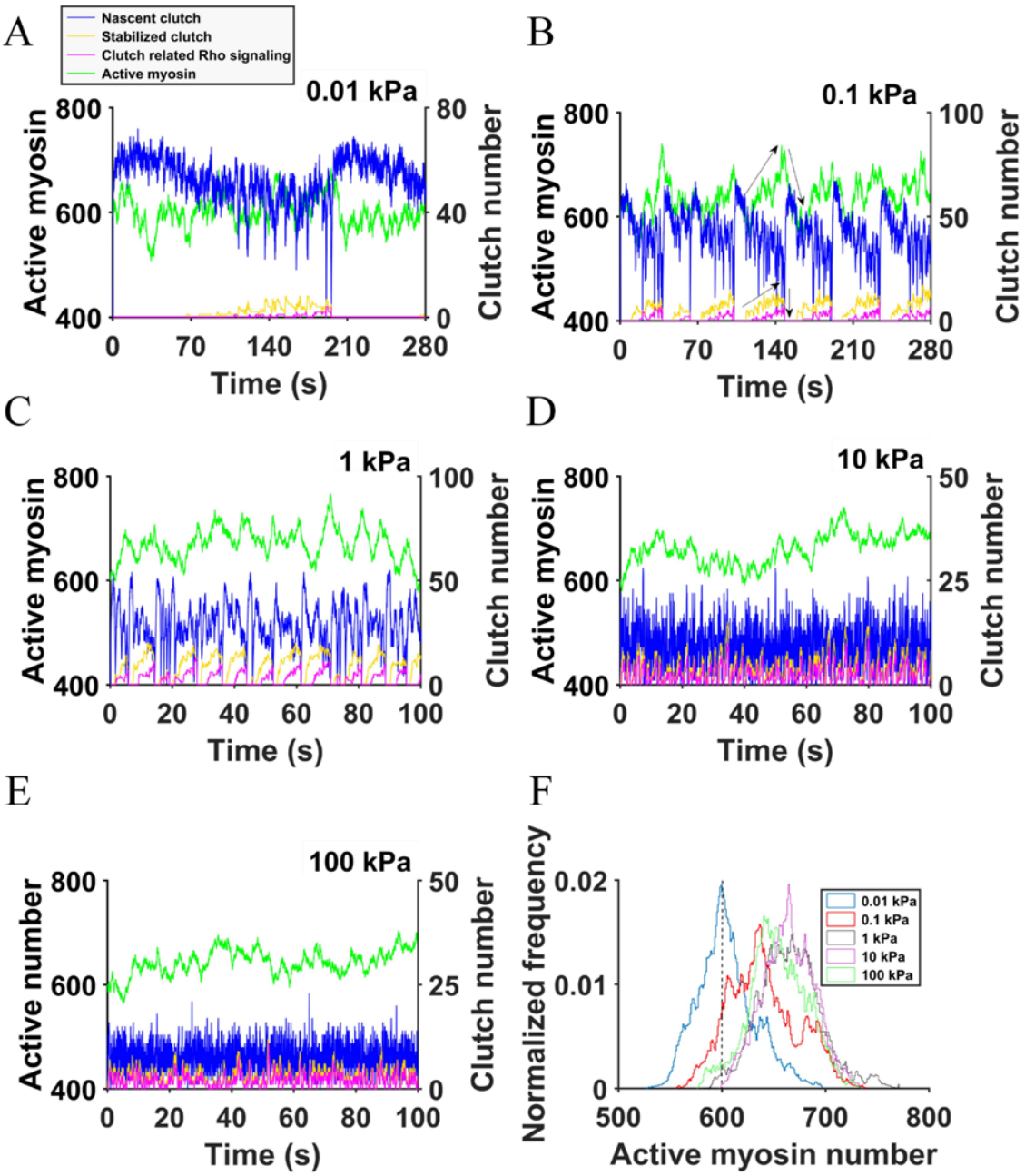

Figure S3.The effect of Rho signaling on myosin motor activation on substrate with different stiffness (0.01 kPa ~ 100 kPa).

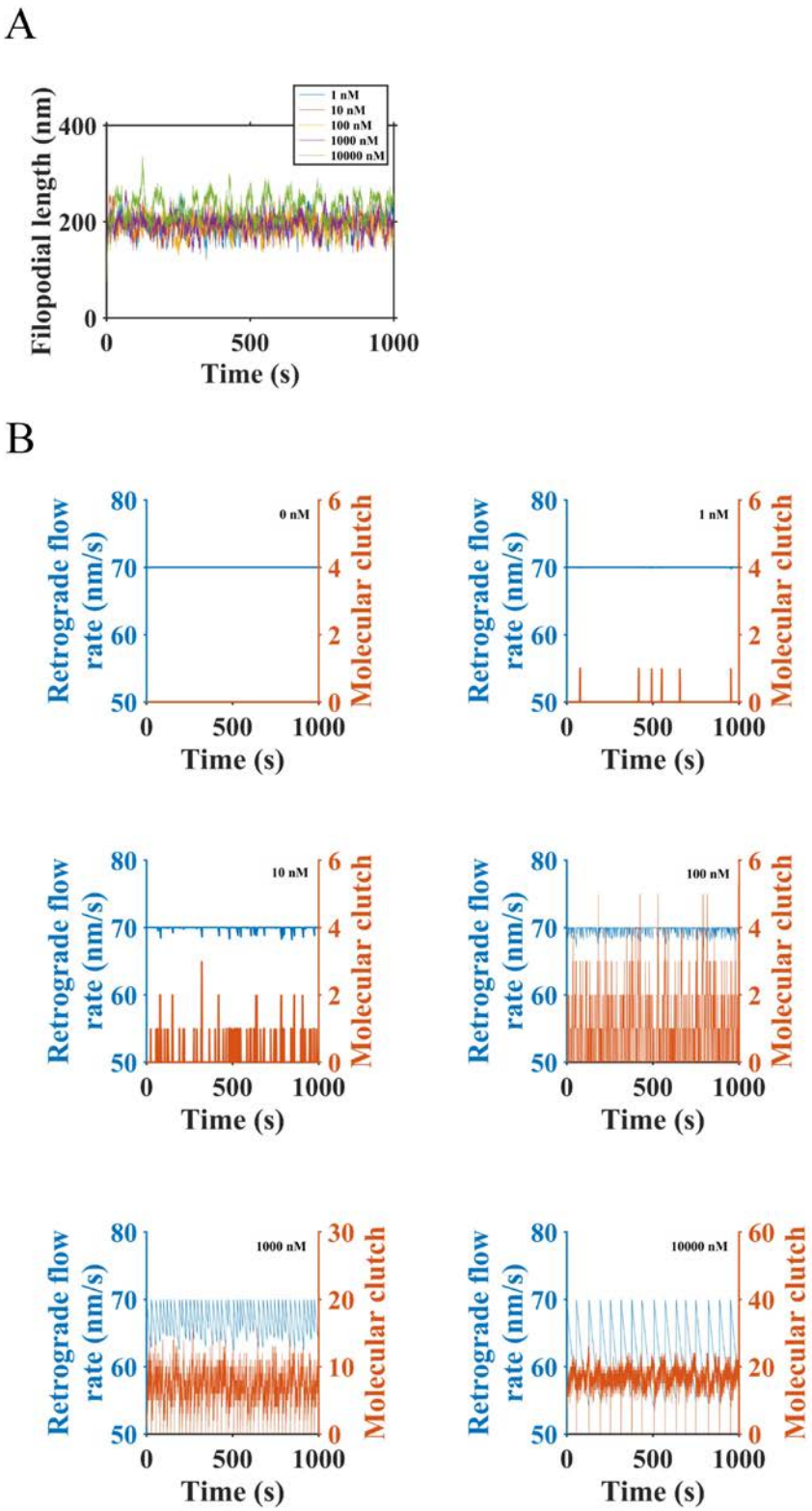

Figure S4. The effect of talin concentration on filopodial dynamics.(A) Five individual trajectories are shown from stochastic simulations within 0 uM ~10 uM talin. (B) Retrograde flow is strongly influenced by the molecular clutch formation at 1 and 10 uM talin.

Simulation algorithm:

The stochastic filopodial dynamics model was implemented using a variable time step Gillespie Stochastic Simulation Algorithm (SSA), also known as a Kinetic Monte Carlo simulation. The simulation is allowed to run for 10^8 time steps (>1000s) to ensure the simulation has reached steady-state before statistics are calculated in **Fig. 2-4**. 20 trajectories were generated for each set of parameter values in **fig. 5** for different substrate stiffness. For most results, we report the mean values obtained by averaging over the duration of at least one simulation.

1. The order of events in filopodial dynamics model was:

(1) Calculate the unbinding rate for each nascent and stabilized clutch, based on the current tension on clutch (product of clutch deformation and clutch stiffness). Talin-actin bond was modeled as a slip bond (one exponential with a positive exponent) and integrin-FN bond was modeled as a catch bond (the sum of two exponentials, one with positive exponent and the other with negative exponent). The parameters of such bonds were obtained after fitting the curve to previously experimental data (Fig1C, lifetime data in the figure correspond to the inverse of k_{off}).

(2) Calculate the event time t_i for each possible reaction in each loop by Eq.S1

$$t_i = \frac{-\ln(RAN_i)}{k_i}, \tag{S1}$$

where RAN_i is a uniformly distributed random number between zero and one, and k_i is the kinetic rate for the following reactions:

a) Active talin binding with inactive integrin forming talin-integrin complexes; talin-integrin complexes dissociation.

b) Talin-integrin complexes binding with FN forming talin-integrin-FN complexes; talin-integrin-FN complexes dissociation.

c) Talin-integrin complexes binding with actin forming talin-integrin-actin complexes; talin-integrin-actin complexes dissociation.

d) Talin-integrin-FN complexes binding with actin forming actin-talin-integrin-FN complexes (nascent clutch); actin-talin-integrin-FN complexes unbinding at talin-actin interface.

e) Talin-integrin-actin complexes binding with FN forming actin-talin-integrin-FN complexes (nascent clutch); actin-talin-integrin-FN complexes unbinding at integrin-FN interface.

f) Talin in molecular clutch unfolding and refolding.

g) Vinculin binding with unfolding talin's VBS site resulting in Actin-talin-vinculin-integrin-FN complexes (stabilized clutch).

h) Integrin-FN interface in stabilized clutch unbinding leading to clutch bond break.

i) Activation or inactivation of myosin.

j) Actin and talin diffusion within in filopodium.

k) Actin polymerization and depolymerization. Actin polymerization under membrane force was derived earlier (3),

$$k_{ona} = k_{ona0} e^{\left(-\frac{F_{mem}\delta}{k_B T}\right)}. \quad \text{(S2)}$$

(3) Advance time by the minimum calculated event time.

(4) Execute the reaction corresponding to the minimum calculated event time.

(5) Calculate F-actin retrograde flow rate based on the current substrate deformation and

membrane force using the linear force-velocity relationship,

$$V_{retro}=V_u\left(1-\frac{F_{substrate}}{N_m F_b+F_{mem}}\right), \quad \text{(S3)}$$

$$F_{substrate}=K_{substrate}X_{substrate}. \quad \text{(S4)}$$

(6) Advance engaged clutch positions by the product of the F-actin retrograde flow rate and time step.

(7) Calculate substrate position through a force balance between the substrate and clutch springs (**Fig.S2**).

(8) Calculate filopodial length based on retrograde flow. If the barbed end resides beyond the last compartment, then update the number of compartments.

(9) Return to step 1.

Reactions of clutch adhesions in Gillespie set:

1. Inside-out activation of integrins by active talin

(1) $talin_{fold} + integrin \underset{k_{1r}}{\overset{k_{1f}}{\rightleftarrows}} talin_{fold} \cdot integrin$

2. Formation of Mechanosensing links

(2) $talin_{fold} \cdot integrin + FN \underset{k_{2r}}{\overset{k_{2f}}{\rightleftarrows}} talin_{fold} \cdot integrin \cdot FN$

(3) $talin_{fold} \cdot integrin + actin \underset{k_{3r}}{\overset{k_{3f}}{\rightleftarrows}} actin \cdot talin_{fold} \cdot integrin$

(4) $talin_{fold} \cdot integrin \cdot FN + actin \underset{k_{4r}}{\overset{k_{4f}}{\rightleftarrows}} actin \cdot talin_{fold} \cdot integrin \cdot FN$

(5) $actin \cdot talin_{fold} \cdot integrin + FN \underset{k_{5r}}{\overset{k_{5f}}{\rightleftarrows}} actin \cdot talin_{fold} \cdot integrin \cdot FN$

3. Talin unfolding/refolding, vinculin binding, integrin-FN break and vinculin dissociation

(6) $actin \cdot talin_{fold} \cdot integrin \cdot FN \underset{k_{6r}}{\overset{k_{6f}}{\rightleftarrows}} actin \cdot talin_{unfold} \cdot integrin \cdot FN$

(7) $actin \cdot talin_{unold} \cdot integrin \cdot FN + vinculin \xrightarrow{k_{vin}} actin \cdot talin_{unfold} \cdot$ vinculin $\cdot integrin \cdot FN$

(8) $actin \cdot talin_{unfold} \cdot$ vinculin $\cdot integrin \cdot FN \xrightarrow{k_{7r}} talin_{fold} + integrin + FN$

4. Myosin activation

(9) $myosin_{inavitve} \underset{k_{dm}}{\overset{k_{am}}{\rightleftarrows}} myosin_{active}$

The mathematical representation of reactions in clutch model:

$$\frac{\mathrm{d}C_{T\cdot I}}{\mathrm{d}t} = k_{1f}C_T C_{ti} + k_{2r}C_{TIF} + k_{3r}C_{ATI} - \left(k_{1r} + k_{2f} + k_{3f}\right)C_{T\cdot I} \quad \text{(S5)}$$

$$\frac{\mathrm{d}C_{TIF}}{\mathrm{d}t} = k_{2f}C_{T\cdot I} + k_{4r}C_{A\cdot T\cdot I\cdot F} - \left(k_{2r} + k_{4f}\right)C_{TIF} \quad \text{(S6)}$$

$$\frac{\mathrm{d}C_{ATI}}{\mathrm{d}t} = k_{3f}C_{T\cdot I} + k_{5r}C_{A\cdot T\cdot I\cdot F} - \left(k_{3r} + k_{5f}\right)C_{ATI} \quad \text{(S7)}$$

$$\frac{\mathrm{d}C_{A\cdot T\cdot I\cdot F}}{\mathrm{d}t} = k_{4f}C_{TIF} + k_{5f}C_{ATI} + k_{6r}C_{A\cdot T\cdot I\cdot F} - \left(k_{4r} + k_{5r} + k_{6f}\right)C_{A\cdot T\cdot I\cdot F} \quad \text{(S8)}$$

$$\frac{\mathrm{d}C_{A\cdot TU\cdot I\cdot F}}{\mathrm{d}t} = k_{6f}C_{A\cdot T\cdot I\cdot F} - k_{6r}C_{A\cdot T\cdot I\cdot F} - k_{vin}C_{A\cdot TU\cdot I\cdot F} \quad \text{(S9)}$$

$$\frac{\mathrm{d}C_{A\cdot T\cdot V\cdot I\cdot F}}{\mathrm{d}t} = k_{vin}C_{A\cdot TU\cdot I\cdot F} - k_{7r}C_{A\cdot T\cdot V\cdot I\cdot F} \quad \text{(S10)}$$

$$\frac{\mathrm{d}C_{Ma}}{\mathrm{d}t} = k_{am}C_{Mina} - k_{dm}C_{Ma} \quad \text{(S11)}$$

In these equations, C_T represents the concentration of folding talin, C_{ti} represents the concentration of integrin, C_{TI} represents the concentration of talin·integrin complexes, C_{TIF} represents the concentration of talin·integrin·fibronectin complexes, C_{ATI} represents the concentration of actin·talin·integrin complexes, $C_{A\cdot T\cdot I\cdot F}/C_{A\cdot TU\cdot I\cdot F}$ represents the concentration of actin·talin(fold/unfold)·integrin·fibronectin complexes, $C_{A\cdot T\cdot V\cdot I\cdot F}$ represents the concentration of actin·talin·vinculin·integrin·fibronectin complexes, C_{Mina}/C_{Ma} represents the concentration of inactive/active myosin.

Table S1.Baseline model parameters

Parameter	Symbol	Value	Source	sensitivity
Integrin activation by talin	k_{1f}	3.3 s^{-1}	(1)	
Talin·integrin dissociation	k_{1r}	0.0042 s^{-1}	(1)	
Talin·integrin binding with FN	k_{2f}	1.5 s^{-1}	(1)	
Talin·integrin·FN dissociation	k_{2r}	0.1 s^{-1}	(1)	
Talin·integrin binding with actin	k_{3f}	1.5 s^{-1}	(1)	
Talin·integrin·actin dissociation	k_{3r}	0.1 s^{-1}	(1)	
Talin·integrin·FN binding with actin	k_{4f}	1.5 s^{-1}	(1)	
Actin·talin bond dissociation	k_{4r}	*slip bond*	(2)	
Talin·integrin·FN binding with actin	k_{5f}	1.5 s^{-1}	(1)	
Integrin·FN bond dissociation in nascent clutch	k_{5r}	*catch bond*	(2)	
Talin unfolding	k_{6f}	*slip bond*	(2)	
Talin refolding	k_{6r}	*slip bond*	(2)	
Vinculin on-rate	k_{onv}	1 s^{-1}	0.1~10 s^{-1}	1.04
Integrin·FN bond dissociation in stabilized clutch	k_{7r}	*catch bond*	(2)	
Actin·talin bond rupture force	F_{tb}	2 pN	0.5~2.5 pN	0.86
Clutch spring constant	K_t	1 pN/nm	(2)	
Concentration of actin at the filopodial base	C_A	10 μM	(3)	
Concentration of talin at the filopodial base	C_T	0 ~ 10 μM	(3)	
Integrin density per compartment	C_{ti}	0.1	(1)	
Half of actin monomer size	δ	2.7 nm	(3)	
Diffusion rate	k_D	5/16 μm^2 s^{-1}	(4)	
Actin polymerization rate	k_{ona}	50 s^{-1}	(5)	
Actin depolymerization rate	k_{offa}	1.4 s^{-1}	(6)	
Membrane force	F_{mem}	10 pN	(3)	
Initial number of active motors	C_a	600	set	
Number of inactive motors in cytoplasm	C_{ina}	120	set	
Motor stall force	F_b	2 pN	(2)	

Motor unloaded retrograde flow velocity	V_u	110 nm/s	(2)	
Maximum motor binding rate	αk_{am0}	0.05 s^{-1}	(7)	
Minimum motor binding rate	k_{am0}	0.01 s^{-1}	(7)	
Motor unbinding rate	k_{dm0}	0.002 s^{-1}	(7)	
Characteristic force, activated adhesion signalling	F_R	10~25 pN	(7)	
Saturation constant, Rho effect on myosin activation	λ	0.1	0~1	0.0
Radius of circular adhesion site	a	550 nm	(8)	

Table S2.Model parameters range for Fig. 2-5and Fig. S3

Figure	Actin-talin bond rupture force F_{tb}(pN)	Vinculin binding k_{vin}(s^{-1})	Integrin clustering 1. linearity function 2. Gaussian function -. no clustering	Rho effect on myosin activation λ	Integrin density C_{ti}	Clutch spring constant K (pN/nm)
Fig.2	0.5~2.5	-	-	-	0.1	1
Fig.3A	1.5	0.1/1/10	-	-	0.1	1
Fig. 3B	1.5	0.1/1/10	-	-	1	1
Fig. 3C	1.5	10	-	-	0.1/1	1
Fig. 3D	1.5	10	-	-	0.1/1	1
Fig. 3H (red line)	1.5	10	1	-	-	1
Fig. 3H (blue line)	1.5	10	2	-	-	1
Fig. 3H (black line)	5	10	-	-	0.1	1700
Fig. 4	1.5	10	-	0~1	0.1	1
Fig. S3	1.5	10	-	0.1	0.1	1

Table S3.Statistical significance tests for Fig. 5

Figure panel	Statistical significance
Fig. 5A	P<0.05 between E_{ecm} = 0.1 kPa, 1 kPa, 10 kPa, 100 kPa only above 1000 nM
Fig. 5B	P<0.02 between F_{mem} = 0 pN, 5 pN, 10 pN in the range of 0.1 kPa ~ 100 kPa
Fig. 5C	P<0.05 between F_{unf} = 5 pN, 10 pN, 30 pN only below15.8 kPa
Fig. 5D	P<0.05 between F_{Rho} = 10 pN, 15 pN, 20 pN only below 4 kPa

Supplementary References

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