

Supplemental material

Dambourne et al., <https://doi.org/10.1083/jcb.201710084>

hESC

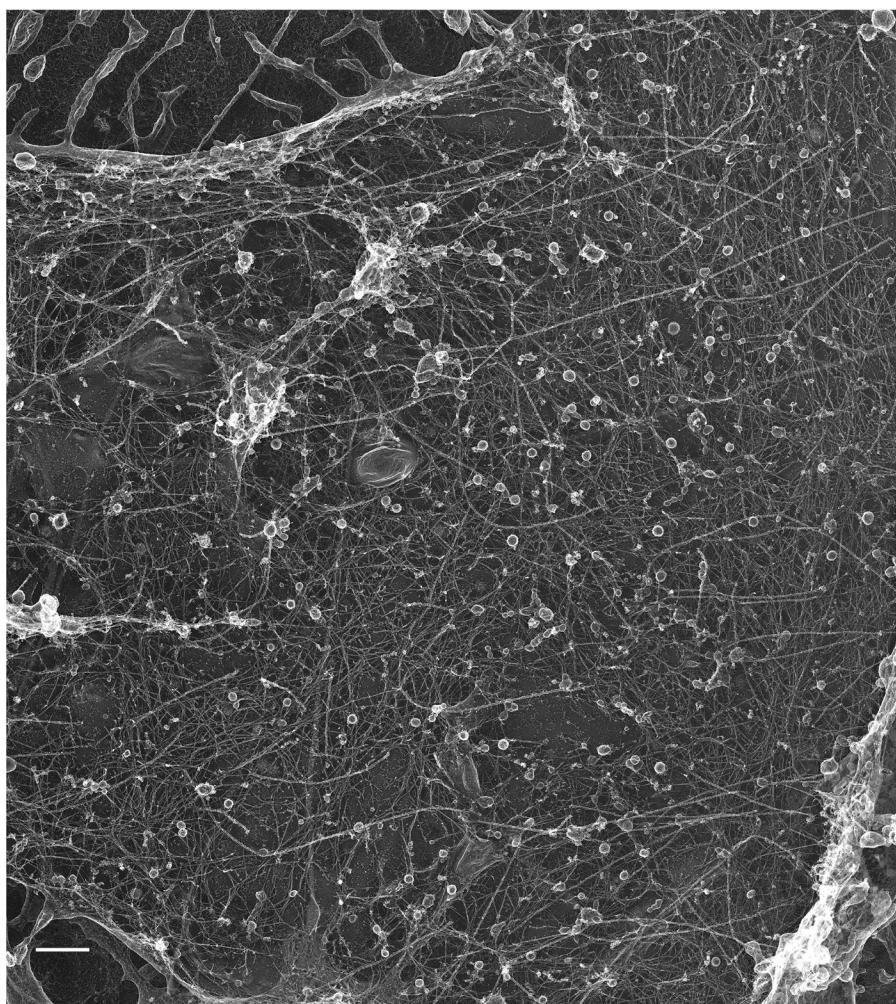


Figure S1. Wider view of platinum replica of hESC. Bar, 1 μm.

NPC

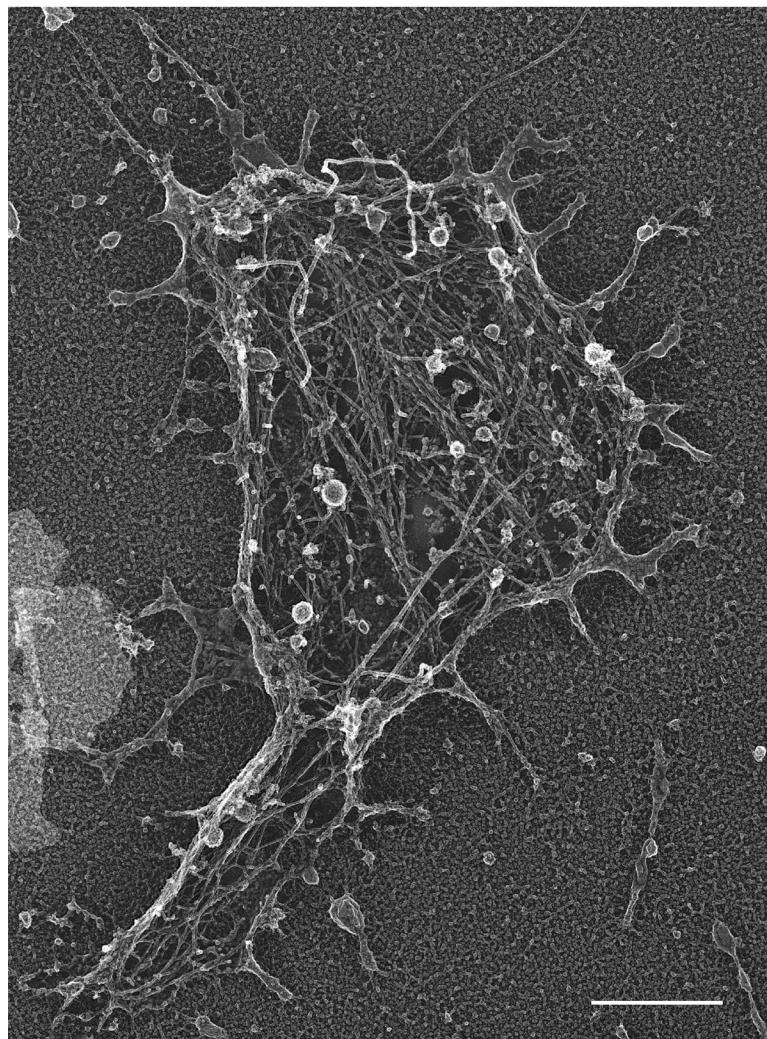


Figure S2. Wider view of platinum replica of NPC. Bar, 1 μ m.

Fibroblast

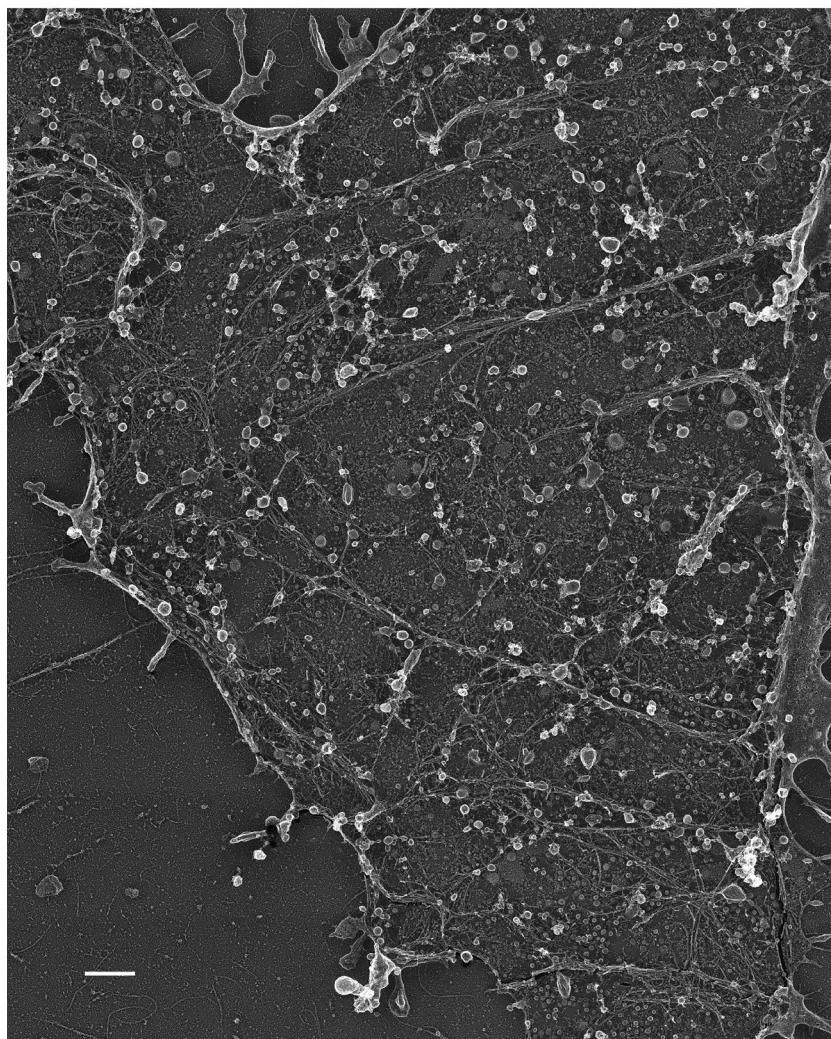


Figure S3. Wider view of platinum replica of fibroblast. Bar, 1 μm.

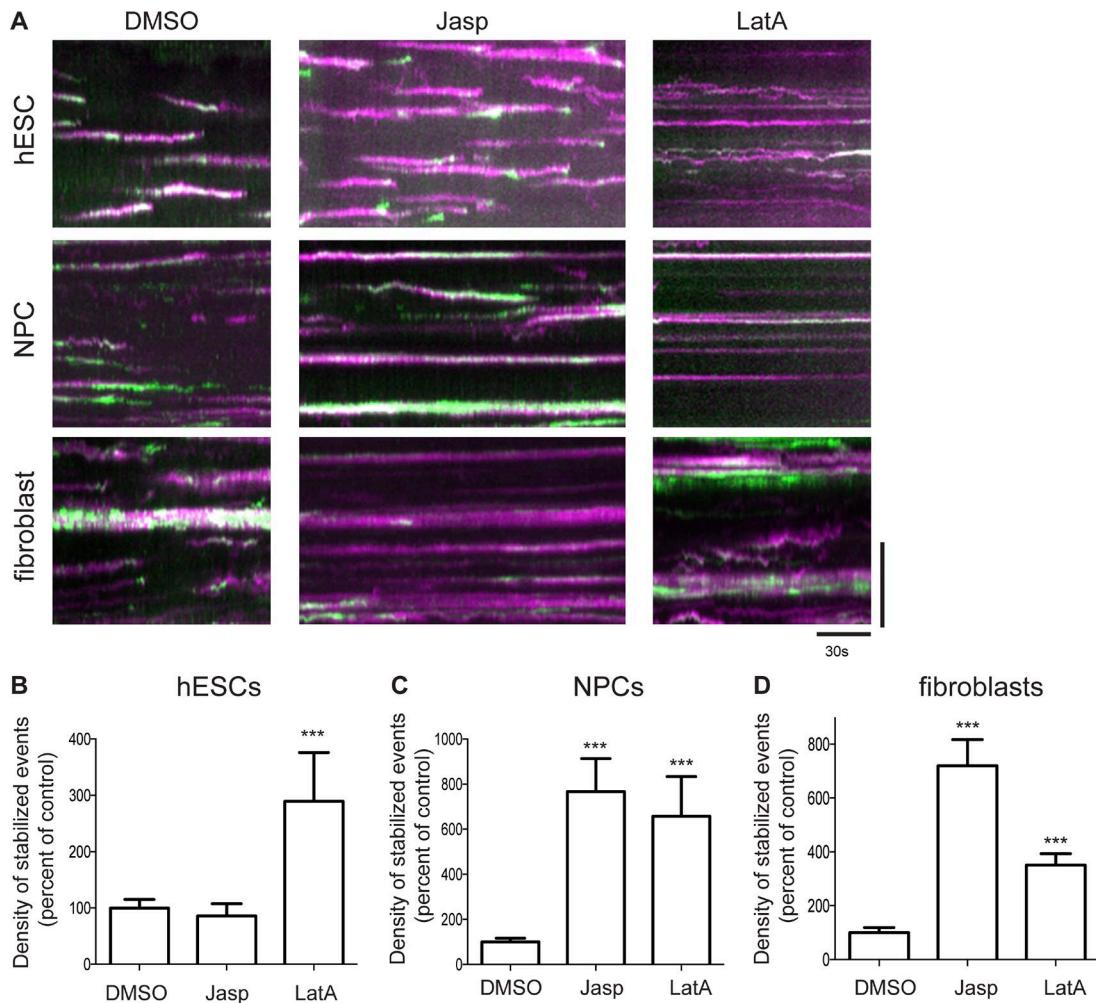


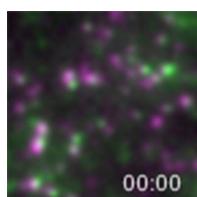
Figure S4. Differential effect of actin drugs on the three cell types. (A) Representative kymograph of hESCs, NPCs, and fibroblasts treated with DMSO, 1 μ M LatA (100 nM for the fibroblast), and 1 μ M Jasp. Bar, 5 μ m. **(B–D)** Density of stalled events in LatA- and Jasp-treated cells relative to respective control cells in hESCs, NPCs, and fibroblasts (mean \pm SEM). ***, P < 0.001, one-way ANOVA and Dunnett's test versus control (five independent experiments, three to four cells analyzed per experiment).

Table S1. List of reagents

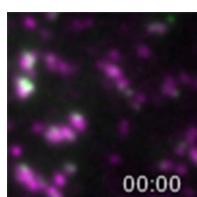
Reagent or resource	Source	Identifier
Antibodies		
Rabbit polyclonal anti-CLTA	Santa Cruz Biotechnology	sc-28276
Mouse monoclonal anti-AP2M1	Abcam	Ab-75995
Rabbit polyclonal anti-DNM2	Abcam	Ab-3457
Mouse monoclonal anti-GAPDH	Santa Cruz Biotechnology	sc-47724
Donkey anti-mouse IgG IRDye 800CW	LI-COR Biosciences	926-32212
Goat anti-rabbit IgG IRDye 680CW	LI-COR Biosciences	926-68071
Chemicals, peptides, and recombinant proteins		
Jasplakinolide	Molecular Probes	J7473
Latrunculin A	Molecular Probes	L12370
LY294002	Cell Signaling Technology	9901
Experimental models: Cell lines		
hESCs WIBR3	Soldner et al., 2011	RRID:CVCL9767
hESCs CLTA-Tag-RFP-t/DNM2-EGFP	This study	N/A
Recombinant DNA		
pX330-U6-Chimeric_BB-CBh-hSpCas9	Gift from F. Zhang ^a ; Cong et al., 2013	Addgene #42230
ZFN hCLTA eHF50	Doyon et al., 2011	N/A
ZFN hCLTA eHF51	Doyon et al., 2011	N/A
TALEN AP2	Hong et al., 2015	N/A
pCR8-CLTA-Tag-RFP-t	Doyon et al., 2011	N/A
pCR8-AP2μ1-Tag-GFP2	Fortian et al., 2015; Hong et al., 2015	N/A
pCR8-DNM2-EGFP	Doyon et al., 2011	N/A
Software and algorithms		
ImageJ	National Institutes of Health	https://imagej.nih.gov/ij/
GraphPad Prism 7	GraphPad software	https://www.graphpad.com/scientific-software/prism/
Matlab	Mathworks software	https://www.mathworks.com/products/matlab.html
cmeAnalysis software	Aguet et al., 2013	http://www.utsouthwestern.edu/labs/danuser/software/#cme-anchor
CME association software	Hong et al., 2015	N/A

This table lists and provides references for all the reagents used in this study. N/A, not applicable.

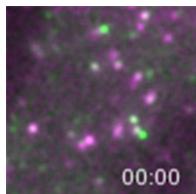
^aBroad Institute at MIT, Cambridge, MA.



Video 1. A TIRFM time-lapse video (4 min) of the hESC (hCLTA^{EN}/DNM2^{EN}) shown in Fig. 1 A. Arrows indicate productive events considered for analysis. Exposure time: 500 ms; acquisition: 2 s/frame. Frame rate: 10 frames/s.



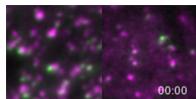
Video 2. A TIRFM time-lapse video (4 min) of the hNPC (hCLTA^{EN}/DNM2^{EN}) shown in Fig. 1 A. Arrows indicate productive events considered for analysis. Exposure time: 500 ms; acquisition: 2 s/frame. Frame rate: 10 frames/s.



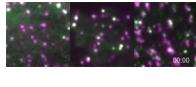
Video 3. A TIRFM time-lapse video (4 min) of the fibroblast ($\text{hCLTA}^{\text{EN}}/\text{DNM2}^{\text{EN}}$) shown in Fig. 1 A. Arrows indicate productive events considered for analysis. Arrowheads show DNM2 periodic recruitment to large clathrin structures representing periodic vesicle release. Exposure time: 500 ms; acquisition: 2 s/frame. Frame rate: 10 frames/s.



Video 4. TIRFM time-lapse videos (4 min) of the hESC (AP2μ2-tagGFP2), hNPC, and fibroblast related to Fig. 3 A. Exposure time: 500 ms; acquisition: 2 s/frame. Frame rate: 10 frames/s.



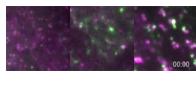
Video 5. TIRFM time-lapse videos (4 min) of control and AP2μ2-depleted fibroblast ($\text{hCLTA}^{\text{EN}}/\text{DNM2}^{\text{EN}}$) shown in Fig. 3 C. Exposure time: 500 ms; acquisition: 2 s/frame. Frame rate: 10 frames/s.



Video 6. TIRFM time-lapse videos (6 min) of the hESC ($\text{hCLTA}^{\text{EN}}/\text{DNM2}^{\text{EN}}$), hNPC, and fibroblast treated with LatA shown in Fig. S4 A. Exposure time: 500 ms; acquisition: 2 s/frame. Frame rate: 10 frames/s.



Video 7. TIRFM time-lapse videos (4 min) of the hESC ($\text{hCLTA}^{\text{EN}}/\text{DNM2}^{\text{EN}}$), hNPC, and fibroblast treated with Jasp shown in Fig. S4 A. Exposure time: 500 ms; acquisition: 2 s/frame. Frame rate: 10 frames/s.



Video 8. TIRFM time-lapse videos (4 min) of the hESC ($\text{hCLTA}^{\text{EN}}/\text{DNM2}^{\text{EN}}$), hNPC, and fibroblast treated with LY shown in Fig. 5 A. Exposure time: 500 ms; acquisition: 2 s/frame. Frame rate: 10 frames/s.

Provided online are five zipped txt files with image analysis programs used in this study. The following programs required Matlab 2013b or higher. ExtractXYcoordinates: Extracts XY coordinates of TIRFM videos analyzed by u-track. Associate_tracks: Associates two colors tracks from XY positions files. Cleanup_associated_tracks: Automatically rejects tracks that are within vicinity of each other. Plots_stats: Reads associated tracks files and extracts desired parameters. Read_me: Explains how to use the programs.

References

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