Supplementary Materials

MOVIE LEGENDS:

These are QuickTime format movie files (.mov extensions). View using the Apple QuickTime.

Movie1: MT and ER dynamics in growth cone of Aplysia bag cell neuron in CA Rac1 backgrounds. FSM time lapse of MT and ER dynamics in a growth cone injected with CA Rac1 showing DIC (upper left); MT (upper right); ER (lower left) and merge of MT (red) and ER (green) (lower right). Bar: 10μm; 12 s interval; elapsed time 120 s.

Movie2: MT and ER dynamics in growth cone of Aplysia bag cell neuron in DN Rac1 backgrounds. FSM time lapse of MT and ER dynamics in a growth cone injected with DN Rac1 showing DIC (upper left); MT (upper right); ER (lower left) and merge of MT (red) and ER (green) (lower right). Bar: 10μm; 12 s interval; elapsed time 120 s.

Movie3: 5-HT (10μM) triggers Ca^{2+} elevation in growth cone in CA Rac1 backgrounds. Ca^{2+} ratio image is coded by pseudo-colors in linear scale (see scale bar). Bar: 10μm; 20 s interval; elapsed time: 12 min.

Movie4: Taxol alters ER distribution in growth cone in CA Rac1 backgrounds. Left: Before; Right: 25 min in taxol (0.1 μM); Bar: 10μm; 12 s interval; elapsed time: 60 s.

Movie5: Taxol alters the spatial profile of 5-HT-evoked Ca^{2+} response in growth cone in CA Rac1 backgrounds. CA Rac1 injected neuron was pretreated with taxol (0.1 μM) for 20 min and then stimulated by 5-HT (10 μM) with the continued presence of taxol. Growth cone area is outlined by white line. Ca^{2+} ratio image is

coded by pseudo-colors in linear scale (see scale bar). Bar: 10μm; 10 s interval; elapsed time: 10 min.

SUPPLEMENTARY FIGURE LEGENDS:

Figure S1 Microtubule dynamics assessed with FSM. Two examples of single microtubule entering the P domain via plus end assembly (A and B) and an example of a microtubule catastrophe (B). Note microtubule transport due to microtubule association with retrograde actin flow (Schaefer *et al.*, 2002), which results in the microtubule kinking evident in the last frame of (A). Blue line: speckle at microtubule plus end; Yellow line: internal reference speckle.

Figure S2 Immunocytochemistry of actin filament and ER distribution in a growth cone. Left: actin; Right: ER. Red line: filopodial ends. Bar = 10 μm.

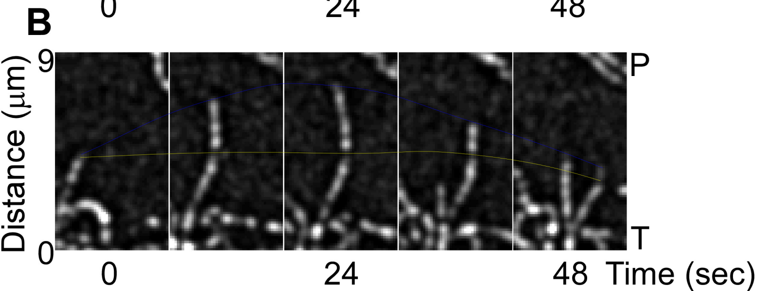
Figure S3 Immunocytochemistry of actin filament and microtubule distribution in a growth cone in CA Rac1 backgrounds. Left: Actin; Middle: Microtubule; Right: Merge of actin (red) and microtubule (green). Red line, green line and blue line: reference lines as showed in Figure 1 D. Bar = 10 μm.

Figure S4 Vinblastine alters the pattern of 5-HT-evoked Ca2+ release in CA Rac1 backgrounds. A) CA Rac1 injected neurons were pretreated with vinblastine (25 nM) for 20 min, followed by 5-HT (10μM) exposure with the continued presence of vinblastine. DIC (left) and Ca^{2+} (right) kymographs sampled from the region as indicated by white line in Figure 1 D before and after addition of 5-HT. Data acquired at 20 sec intervals. Blue line (75%P): three quarters of P-domain breadth from the red line; Red line (C-P interface): interface between C-domain and P-domain; Black line indicates 5-HT addition. P: P domain; C: C domain. Ca^{2+} kymograph is coded by pseudo-colors in linear scale (see scale bar). Bottom graph in A: the time course of 5-HT-evoked Ca^{2+} response sampled from blue line and red line (see above). B) Summary of $\Delta F/F_0$

plots recorded in the entire C-domain (red line) or P-domain (blue line) of growth cones pretreated with vinblastine (25 nM, 20-30 min) to quantify Ca^{2+} response to 5-HT (10μM) over time in CA Rac1 backgrounds. N=5. N denotes the number of growth cones tested. Values are expressed as mean ± SEM. Black bar shows 5-HT presence. C) Vinblastine (25 nM) effects on ER distribution in growth cones in CA Rac1 backgrounds. Top: Actin staining; Bottom: ER staining. Left: Vinblastine treatment for 20 min; Right: Vinblastine treatment for 30 min. Red line: filopodial ends. Bar = 10 μm.

Figure S5 Verification of ROS assay. DIC and ROS kymographs obtained from a control growth cone and sampled from the region as indicated by white line in Figure 1 D before and after H_2O_2 (10μM) addition. Data acquired at 10 sec intervals. Black line indicates H_2O_2 addition. P: P domain; C: C domain. ROS kymograph is coded by pseudo-colors in linear scale (see scale bar). Right: graph showing ROS level increase after addition of H_2O_2 sampled from the red line in kymograph.

Figure S6 ROS derived from Rac1 activation promotes 5-HT-evoked Ca2+ response. A and B) DIC (left) and Ca^{2+} (right) kymographs obtained from a line across GC (shown as the white line in Figure 1 D) in CA Rac1 backgrounds. Neurons were pretreated with NAC (1mM, 30min (A)) or Apocynin (1mM, 45min (B)) followed by 5-HT (10μM) exposure in the continued presence of NAC or Apocynin, respectively. Data acquired at 10 sec intervals. Black line indicates 5-HT addition. P: P domain; C: C domain. Ca^{2+} kymograph is coded by pseudo-colors in linear scale (see scale bars). C, D and E) DIC (left) and Ca^{2+} (right) kymographs from DN Rac1 injected cells before and after 5-HT (10μM) addition. Cells were pretreated with H_2O_2 (10μM) in the absence (D) or presence (E) of XeC (20 μM) for 20 min and treated continuously during 5-HT stimulation. Data acquired at 20sec or 10 sec intervals for (C and D) and (E), respectively. Black line indicates 5-HT addition. P: P domain; C: C domain. Ca^{2+} kymograph is coded by pseudo-colors in linear scale (see scale bars).


A
14
Distance (μm)
0
P
T
0
24
48
72
Time (sec)
B
9
Distance (μm)
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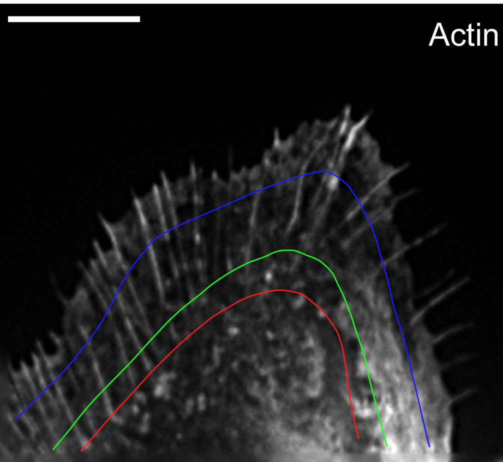
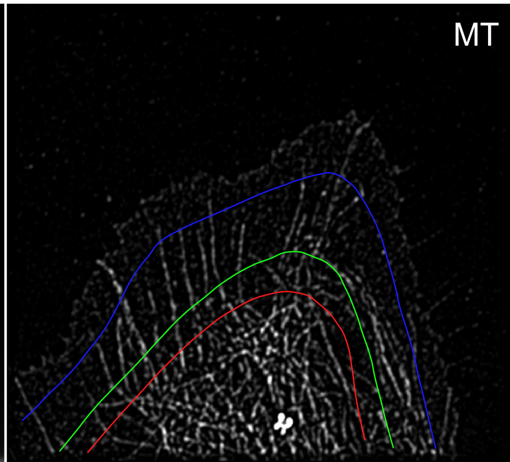
P
T
0
24
48
Time (sec)

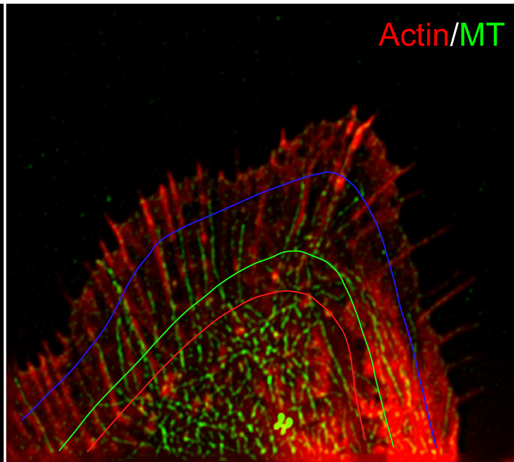
Actin

ER

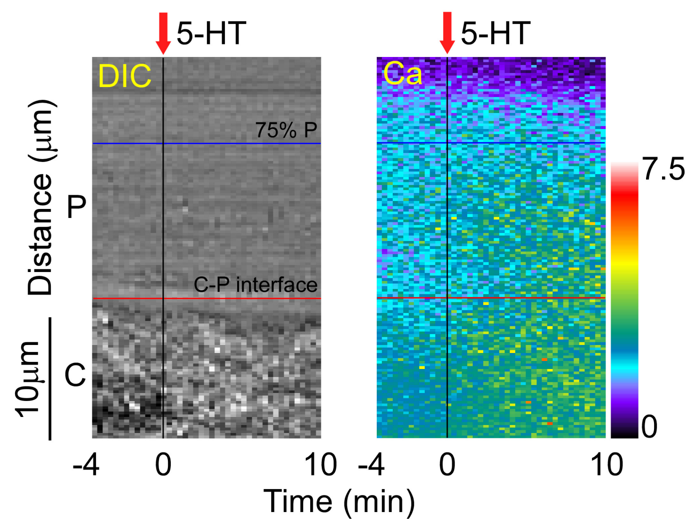
CA Rac1

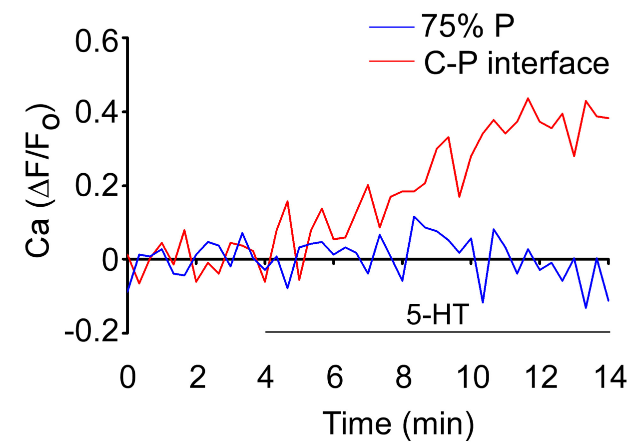






A





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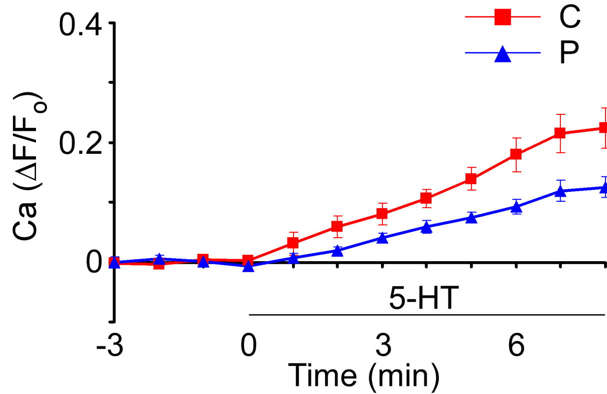

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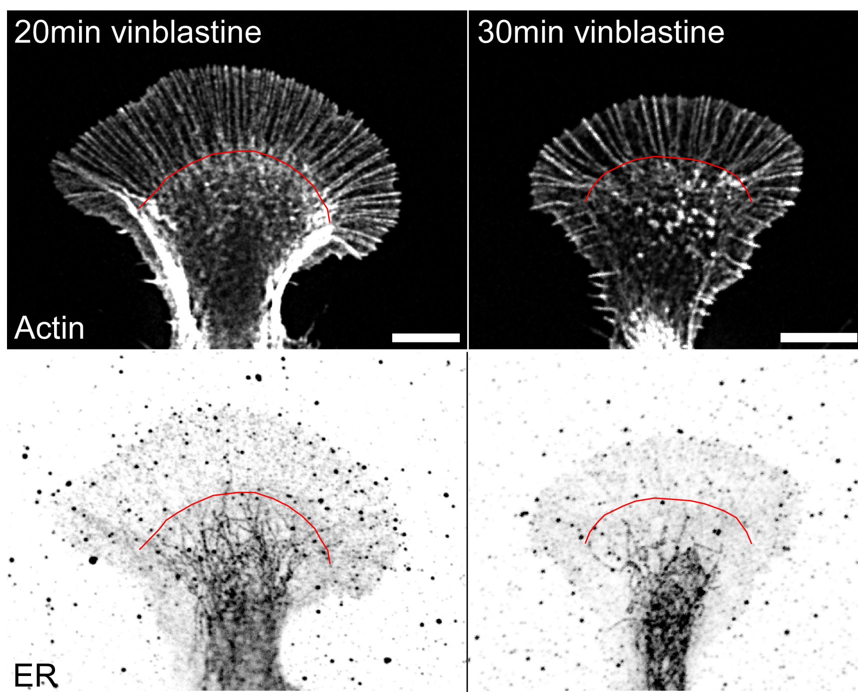


DIC

ROS

H_2O_2 H_2O_2

Distance (μm)

P

C

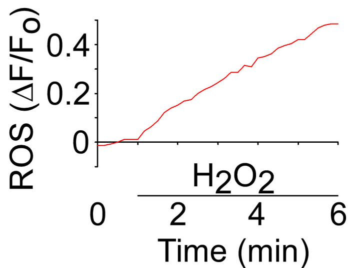
10μm

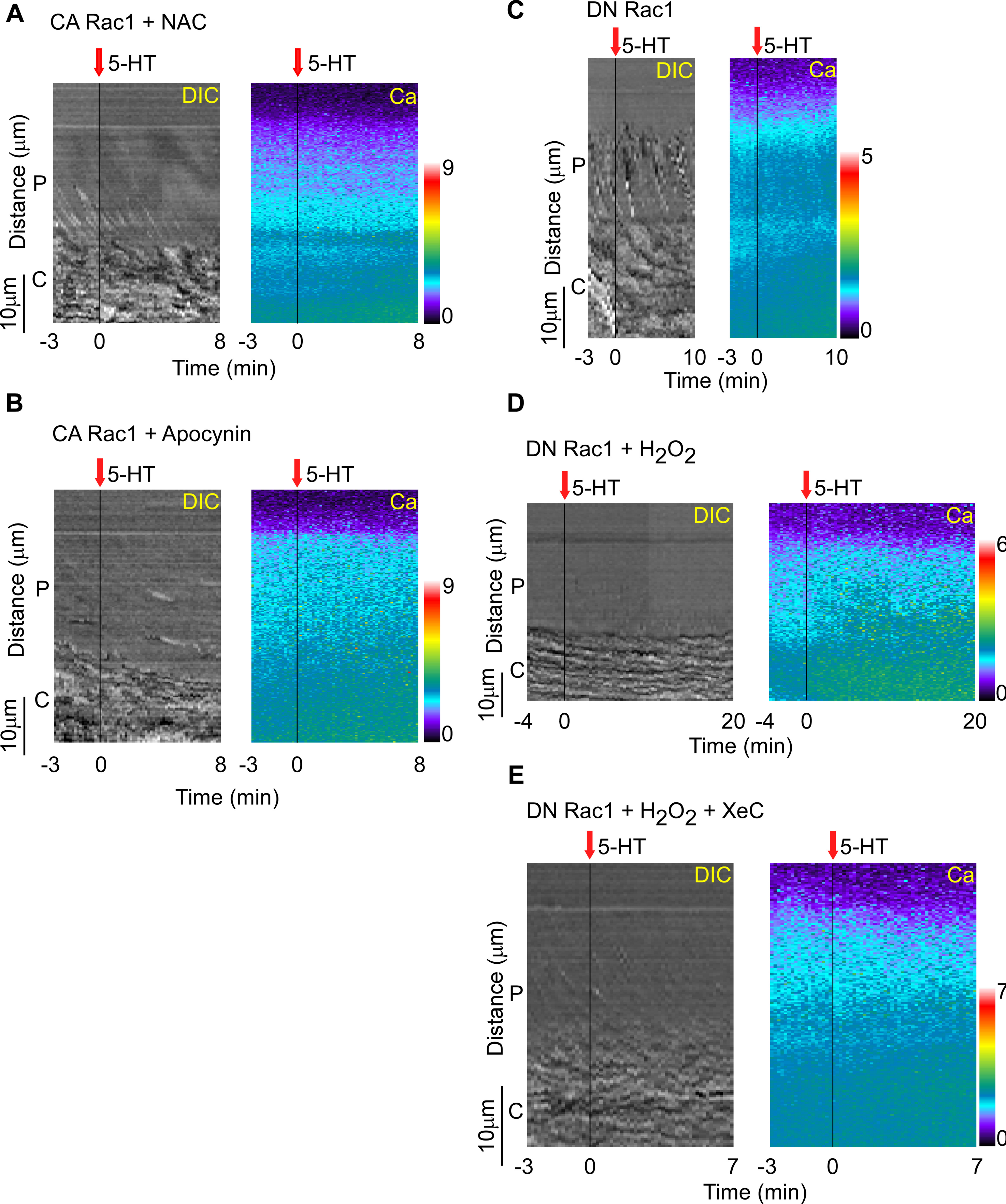
-1 0 5 -1 0 5

Time (min)

5.6

0




A
CA Rac1 + NAC
5-HT
DIC
5-HT
Ca
Distance (µm)
P
C
10µm
9
0
-3
0
8
-3
0
8
Time (min)
B
CA Rac1 + Apocynin
5-HT
DIC
5-HT
Ca
Distance (µm)
P
C
10µm
9
0
-3
0
8
-3
0
8
Time (min)
C
DN Rac1
5-HT
DIC
5-HT
Ca
P
Distance (µm)
C
10µm
5
0
-3
0
10
-3
0
10
Time (min)
D
DN Rac1 + H_2O_2
5-HT
DIC
5-HT
Ca
Distance (µm)
P
C
10µm
6
0
-4
0
20
-4
0
20
Time (min)
E
DN Rac1 + H_2O_2 + XeC
5-HT
DIC
5-HT
Ca
Distance (µm)
P
C
10µm
7
0
-3
0
7
-3
0
7
Time (min)