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1 ***Classification*:**

2 **BIOLOGICAL SCIENCES: Cell Biology**

3

4 # Ras Inhibitor CAPRI Enables Neutrophils to Chemotax Through a Higher-Concentration
5 # Range of Gradients

6

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8

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14

15 **Key Words:**

16 Chemotaxis, neutrophils, G protein-coupled receptor (GPCR), adaptation, Ras, Ras guanine
17 nucleotide exchange factor (Ras GEF), Ras GTPase-activating proteins (RasGAPs), polarity,
18 directional cell migration.

19

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21 and analyzed data; X.X. and T.J. wrote the paper.

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22

23 The authors declare no competing interest.

24 Abstract

25 Neutrophils sense and migrate through an enormous range of chemoattractant gradients through
26 adaptation. Here, we reveal that, in human neutrophils, Calcium-promoted Ras inactivator
27 (CAPRI) locally controls the GPCR-stimulated Ras adaptation. Human neutrophils lacking
28 CAPRI (*capri*^{kd}) exhibit chemoattractant-induced non-adaptive Ras activation; significantly
29 increased phosphorylation of AKT, GSK3α/3β, and cofilin; and excessive actin polymerization.
30 *capri*^{kd} cells display defective chemotaxis in response to high-concentration gradients but exhibit
31 improved chemotaxis in low- or subsensitive-concentration gradients of various chemoattractants
32 as a result of their enhanced sensitivity. Taken together, our data reveal that CAPRI controls
33 GPCR activation-mediated Ras adaptation and lowers the sensitivity of human neutrophils so
34 that they are able to chemotax through a higher concentration range of chemoattractant gradients.

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35 **Significance Statement**

36 Neutrophils provide first-line host defense by migrating through chemoattractant gradients to the
37 sites of inflammation. Inappropriate recruitment and mis-regulated activation of neutrophils
38 contribute to tissue damage and cause autoimmune and inflammatory disease. One fascinating
39 feature of chemotactic neutrophils is their ability to migrate through an enormous concentration
40 range of chemoattractant gradients ($10^{-9} \sim 10^{-5}$ M) through "adaptation," in which cells no longer
41 respond to the present stimuli, but remain sensitive to stronger stimuli. The inhibitory
42 mechanism largely remains elusive, although many molecules of the excitatory signaling
43 pathway have been identified. Our study reveals, for the first time, that the inhibitory component,
44 CAPRI, is essential for both the sensitivity and the GPCR-mediated adaptation of human
45 neutrophils.

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46 **/body**

47 Introduction

48 Neutrophils provide first-line host defense and play pivotal roles in innate and adaptive
49 immunity (1-3). Inappropriate recruitment and dysregulated activation of neutrophils contribute
50 to tissue damage and cause autoimmune and inflammatory diseases (1, 4). Neutrophils sense
51 chemoattractants and migrate to sites of inflammation using G protein-coupled receptors
52 (GPCRs). To accurately navigate through an enormous concentration-range gradient of various
53 chemoattractants ($10^{-9} \sim 10^{-5}$ M) (Fig. S1), neutrophils employ a mechanism called adaptation, in
54 which they no longer respond to present stimuli but remain sensitive to stronger stimuli.
55 Homogeneous, sustained chemoattractant stimuli trigger transient, adaptive responses in many
56 steps of the GPCR-mediated signaling pathway downstream of heterotrimeric G proteins (5, 6).
57 Adaptation provides a fundamental strategy for eukaryotic cell chemotaxis through large
58 concentration-range gradients of chemoattractants. Abstract models and computational
59 simulations have proposed mechanisms generating the temporal dynamics of adaptation: an
60 increase in receptor occupancy activates two antagonistic signaling processes, namely, a rapid
61 “excitation” that triggers cellular responses and a temporally delayed “inhibition” that terminates
62 the responses and results in adaptation (5, 7-13). Many excitatory components have been
63 identified during last two decades; however, the inhibitor(s) have just begun to be revealed (11,
64 14-17). It has been recently shown that an elevated Ras activity increases the sensitivity and
65 changes migration behavior (18, 19). However, the molecular connection between the GPCR-
66 mediated adaptation and the cell sensitivity remains missing.
67 The small GTPase Ras mediates multiple signaling pathways that control directional cell
68 migration in both neutrophils and *Dictyostelium discoideum* (17, 20-24). In *D. discoideum*, Ras

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69 is the first signal event that displays GPCR-mediated adaptation (20). Ras signaling is mainly
70 regulated through its activator, guanine nucleotide exchange factor (GEF), and its inactivator,
71 GTPase-activating proteins (GAP) (16, 17, 25). In *D. discoideum*, the roles of DdNF1 and an F-
72 actin-dependent negative feedback mechanism have been previously reported (14, 17). We have
73 previously demonstrated the involvement of locally recruited inhibitors that act on upstream of
74 PI_3K in sensing of chemoattractant gradients (11, 26). Recently, we identified a locally recruited
75 RasGAP protein, C2GAP1, that is essential for F-actin-independent Ras adaptation and long-
76 range chemotaxis in *Dictyostelium* (16). Active Ras proteins enrich at the leading edge in both *D.*
77 *discoideum* cells and neutrophils (17, 27, 28). It has been reported that a RasGEF, RasGRP4,
78 plays a critical role in Ras activation in murine neutrophil chemotaxis (21, 29). However, the
79 components involved in the GPCR-mediated deactivation of Ras and their function in neutrophil
80 chemotaxis are still not known.

81 In the present study, we show that CAPRI (a Calcium-promoted Ras inactivator) locally controls
82 the GPCR-mediated Ras adaptation in human neutrophils. In response to high-concentration
83 stimuli, cells lacking CAPRI ($capri^{kd}$) exhibit non-adaptive Ras activation; significantly
84 increased activation of AKT, GSK3α/3β, and cofilin; excessive actin polymerization; and
85 subsequent defective chemotaxis. Unexpectedly, $capri^{kd}$ cells display enhanced sensitivity
86 toward chemoattractants and an improved chemotaxis in low- or subsensitive-concentration
87 gradients. Taken together, our findings show that CAPRI Functions as an inhibitory component
88 of Ras signaling, plays a critical role in controlling the concentration range of chemoattractant
89 sensing, and is important for the proper adaptation during chemotaxis.

90

91 Results

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92 **CAPRI regulates GPCR-mediated Ras adaptation in human neutrophils.** Chemoattractants
93 induce robust, transient Ras activation in mammalian neutrophils (21, 30). To identify which
94 RasGAP proteins that deactivate Ras in neutrophils, we examined the expression of potential
95 RasGAPs in mouse and human neutrophils (Fig. S2*A*) (31, 32). We found that human and mouse
96 neutrophils highly expressed CAPRI, also called RASA4, consistent with previous reports (33-
97 35). The human neutrophil-like (HL60) cell line provides a useful model to study mammalian
98 neutrophils (22, 36). The differentiated HL60 cell also highly expresses CAPRI (Fig. S2*B*),
99 consistent with a previous report (37), and provides a suitable cell system in which to study
100 CAPRI's function in human neutrophils. We found that chemoattractant fMLP (N-formyl-L-
101 methionyl-L-leucyl-phenylalanine) stimulation promoted the association between CAPRI and N-
102 Ras/Rap1(Fig 1*A*), suggesting a role of CAPRI in the regulation of chemoattractant-induced Ras
103 and Rap1 signaling. To determine the function of CAPRI, we stably knocked down the
104 expression of *capri* ($capri^{kd}$) in HL60 cells using *capri*-specific shRNA lentiviral particles (Fig.
105 1*B*). We first biochemically determined the dynamics of fMLP-induced Ras activation in both
106 CTL and $capri^{kd}$ cells using a pull-down assay with a large population of cells (Fig. 1*C*). In
107 resting $capri^{kd}$ cells, there was a notably higher level of active Ras, indicating CAPRI's function
108 in regulating basal Ras activity in the cells. In response to 1 μM fMLP stimulation, we detected a
109 transient Ras activation followed by a secondary reactivation in CTL cells as previously reported
110 (14, 16, 30), but a significantly stronger, prolonged Ras activation in $capri^{kd}$ cells (Fig. 1*D*). We
111 further monitored fMLP-induced Ras activation by visualizing the membrane translocation of a
112 fluorescent active Ras probe, the active Ras binding domain of human Raf1 tagged with RFP
113 (RBD-RFP, red), in single live cells using fluorescence microscopy (Fig. 1*E*). In the present
114 study, we used three plasma membrane (PM) markers, Mem-cerulean, C1AC1A-YFP, and

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115 CAAX-mCherry, to track the cell membrane in live cell experiment (Fig. S3). We monitored Ras
116 activation in CTL and *capri*^{kd} cells expressing both PM marker (green) and active Ras probe
117 RBD-RFP (red) in response to uniformly applied 1 μM fMLP stimulation. We found that RBD-
118 RFP translocated to and colocalized with PM marker (green) and then returned to the cytoplasm,
119 followed by a second translocation to the protrusion sites of CTL cells (Fig. 1E, upper panel, and
120 see Video S1 for a complete, longer time period). In *capri*^{kd} cells, the same fMLP stimulation
121 induced persistent membrane translocation and accumulation on the continuously expanding and
122 broadened leading front of the cell (Fig. 1*E*, middle panel, and Video S2). To further examine
123 CAPRI's function in Ras deactivation, we expressed CAPRI-tagged turboGFP (CAPRI-tGFP),
124 RBD-RFP, and PM marker (Mem-cerulean) in *capri*^{kd} cells (*capri*^{kd}/^{OE}) and monitored fMLP-
125 induced Ras activation in these cells. 1 μM fMLP stimulation triggered a clear membrane
126 translocation of CAPRI-tGFP but much weaker or no membrane translocation of RBD-RFP in
127 *capri*^{kd}/^{OE} cells (Fig. 1*E*, lower panel, Fig. S4, and Video S3), suggesting that CAPRI
128 translocates to the plasma membrane to inhibit Ras activation (33). Quantitative measurement of
129 RBD-RFP membrane translocation confirmed that fMLP stimulation induced a biphasic Ras
130 activation in CTL cells as previously reported (30), a prolonged Ras activation in *capri*^{kd} cells,
131 and a reduced Ras activation in *capri*^{kd}/^{OE} cells (Fig. 1*F*). Taken together, our results indicate
132 that CAPRI is a RasGAP protein and is required for chemoattractant-mediated Ras adaptation in
133 neutrophils.
134
135 **Membrane targeting of CAPRI in response to chemoattractant stimulation.** To reveal how
136 CAPRI functions during neutrophil chemotaxis, we examined cellular localization of CAPRI-
137 tGFP in HL60 cells. We found that CAPRI-tGFP colocalized with active Ras (RBD-RFP) and

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138 actin at the leading edge of chemotaxing HL60 cells in a fMLP gradient (Fig. 2*A*), consistent
139 with previous reports (16, 22, 35). Chemoattractant fMLP and IL-8 induced membrane
140 translocation of CAPRI-tGFP (Fig. 1E and Fig. S4-S5). The G protein inhibitor pertussis toxin
141 (PT) blocked the membrane translocation of CAPRI (Fig. S6), indicating that chemoattractant
142 GPCR/G protein-mediated signaling is required for CAPRI membrane targeting. To reveal the
143 molecular mechanism of CAPRI membrane targeting, we investigated the domain requirement
144 for its membrane translocation and interaction with Ras. We expressed tGFP-tagged WT and
145 mutants of ΔC2, ΔPH, and a GAP-inactive mutant R472A, and determined their ability for Ras
146 interaction (Fig. 2*B*-2*C* and Fig. S7). We found that ΔC2 did not interact with Ras and R472A
147 showed a decreased interaction with Ras (Fig. 2*C*), consistent with previous reports (33, 38-41).
148 We next monitored the membrane translocation ability of CAPRI WT and its mutant in response
149 to fMLP stimulation (Fig. 2*D*). Upon fMLP stimulation, WT and R472A clearly translocated to
150 and colocalized with PM marker (Fig. 2*D*, top two panels and Video S4-S5), while ΔPH and
151 ΔC2 showed significantly decreased or little membrane translocation (Fig. 2*D*, lower two panels,
152 and Video S6-S7). Using total internal reflections fluorescent (TIRF) microscopy, we confirmed
153 the membrane-translocation behavior of CAPRI WT and its mutants (Fig. S8 and Fig. 2*E*) (42).
154 Our results indicate that both the C2 and PH domains are crucial for chemoattractant-induced
155 membrane targeting.
156
157 **Chemoattractant induces hyperactivation of Ras effectors in *capri*^{kd} cells.** PI3Kγ is a direct
158 effector of Ras, which synthesizes lipid phosphatidylinositol (3,4,5)-trisphosphate
159 (PtdIns(3,4,5)P_3, PIP_3) and activates the PIP_3-binding protein AKT in neutrophils (21, 29, 43).
160 To examine the consequence of non-adaptive Ras activation, we monitored fMLP-induced PIP_3

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161 production using a biosensor PH-GFP (PH-domain of human AKT tagged with GFP, green) in
162 both CTL and *capri*^{kd} cells (Fig. 3*A*) (44). We found that 1 μM fMLP trigged a robust
163 translocation of PH-GFP to the entire plasma membrane followed by a partial return to the
164 cytosol and a gradual accumulation in the protrusion site of CTL cell (Fig. 3*A*, upper panel, and
165 Video S8). The same stimulation induced a stronger and persistent accumulation of PH-GFP all
166 around the periphery of *capri*^{kd} cells (Fig. 3*A*, lower panel, and Video S9), indicating a
167 hyperactivation of PI3K in cells lacking CAPRI. We further determined the activation profile of
168 $PI_3K\gamma$ downstream effectors in CTL and *capri*^{kd} cells. AKT and GSK are well-known effectors of
169 Ras/PI3Kγ signaling and are critical for the reorganization of actin cytoskeleton in neutrophil
170 chemotaxis (22, 45). Therefore, we examined fMLP-induced PI3Kγ activation by measuring the
171 phosphorylation of AKT on residues T308 and T473 in both CTL and *capri*^{kd} cells. We found
172 that 1 μM fMLP triggered a transient phosphorylation on T308 and T473 of AKT in CTL cells,
173 while it induced a persistent and significantly increased phosphorylation on both residues of
174 AKT in *capri*^{kd} cells (Fig. 3*B*-3*C*), consistent with previous reports (46-48). Cofilin, an F-actin
175 depolymerization factor (ADF), is essential for depolymerization of F-actin in dynamic
176 reorganization of the actin cytoskeleton during cell migration (49). Its activity is regulated
177 mainly through a phosphorylation event: phosphorylation on Ser-3 inhibits its actin binding,
178 severing, and depolymerizing activities; and dephosphorylation on Ser-3 by slingshot proteins
179 (SSHs) reactivates it. Slingshot 2 is a direct substrate of GSK3 and chemoattractants induce
180 phosphorylation and inhibition of GSK3α/3β partially through PI3Kγ-AKT pathways in
181 neutrophils (45). As previously reported (45), GSK3α/3β is constitutively active and
182 dephosphorylated in resting cells, and fMLP induced a transient phosphorylation and

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183 deactivation of GSK3α/3β and led to a transient dephosphorylation of cofilin (Fig. 3*D*-3*E*). We
184 found that 1 μM fMLP stimulation triggered significantly stronger, prolonged phosphorylation of
185 GSK3α/3β and persistent dephosphorylation of cofilin in $capri^{kd}$ cells. Taken together, these
186 results indicate that CAPRI is essential for the proper activation/deactivation dynamics of the
187 PI3Kγ/AKT/GSK3/cofilin signaling pathway in response to chemoattractant stimulations.
188

189 **fMLP stimulation induces an increased activation of Rap1 and its effector in $capri^{kd}$ cells.**
190 Rap1 is a close relative of Ras initially described as a competitor of Ras by directly interacting
191 with Ras effectors (50, 51). Later studies indicate that Rap1 also functions in independent
192 signaling pathways to control diverse processes, such as cell adhesion, cell-cell junction
193 formation, and cell polarity (52, 53). It has been shown that CAPRI also interacts with Rap1 in
194 CHO cells (54). We found that fMLP stimulation promoted the interaction between CAPRI and
195 Rap1 in neutrophils (Fig. 1*A*) and that CAPRI was recruited to adhesion sites and colocalized
196 with the PM marker during cell migration (Fig. S9). To understand CAPRI's function in Rap1
197 activation in human neutrophils, we determined fMLP-induced Rap1 activation in both CTL and
198 $capri^{kd}$ cells by a pull-down assay using the Rap-GTP binding domain of human RalGDS
199 (RBD_{RalGDS}) (Fig. 4*A*). There was a higher level of active Rap1 in resting $capri^{kd}$ cells. fMLP
200 stimulation (1 μM) induced a transient Rap1 activation in CTL cells, while it triggered a
201 significantly increased and prolonged Rap1 activation in $capri^{kd}$ cells (Fig. 4*B*). We further
202 monitored Rap1 activation using an active Rap1 probe, GFP-tagged RBD_{RalGDS} (RBD_{RalGDS}-
203 GFP) (52), using live cell fluorescence microscopy (Fig. 4*C*). We found that RBD_{RalGDS}-GFP
204 colocalized with PM marker at the adhesion/protrusion sites of the resting cells (Fig. 4*C*),

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205 suggesting a potential function of Rap1 in the adhesion of neutrophils. Upon 1 μM fMLP
206 stimulation, RBD_{RalGDS}-GFP (green) transiently translocated to and colocalized with PM marker
207 (red), then returned to the cytoplasm, and then translocated to and colocalized with the PM
208 marker again at the protruding sites of CTL cells (Fig. 4C, upper panel, and Video S10). In
209 resting *capri*^{kd} cells, there was notably more membrane localization of RBD_{RalGDS}-GFP, which is
210 consistent with the notion that there is a higher basal Rap1 activity in *capri*^{kd} cells (Fig. 4*C*,
211 lower panel). We further found that 1 μM fMLP triggered prolonged translocation of RBD_{RalGDS}-
212 GFP (green) to and colocalization with the PM marker (red) during a continuous expansion of
213 *capri*^{kd} cells (Video S11), suggesting that the fMLP induces a hyperactivation of Rap1 in cells
214 lacking CAPRI. To understand the effects of Rap1 hyperactivation, we examined Rap1 effector
215 Erk42/44 phosphorylation (Fig. 4*D*). We detected higher basal Erk42/44 phosphorylation in the
216 non-stimulated *capri*^{kd} cells. In contrast to a rapid maximum phosphorylation of Erk 42/44 at 30
217 s in CTL cells, same stimulation induced an increasing dynamics of Erk42/44 phosphorylation in
218 *capri*^{kd} cells (Fig. 4*E*), consistent with previous reports (33, 35, 54). Our results suggest that
219 CAPRI deactivates Rap1 activation to facilitate an appropriate Rap1 activation and its effector
220 for chemotaxis in neutrophils.

221 **Excessive polymerization of actin impairs the polarization and migration of *capri*^{kd} cells.**

222 The chemoattractant GPCR/G-protein signaling regulates spatiotemporal activities of Ras and
223 Rap1 that mediate multiple signaling pathways to control the dynamics of actin cytoskeleton that
224 drives cell migration. To evaluate the role of CAPRI in chemoattractant GPCR-mediated actin
225 assembly in neutrophils, we determined fMLP-mediated polymerization of actin in CTL and
226 *capri*^{kd} cells using a centrifugation assay of actin filaments (F-actin) (Fig. 5A). In CTL cells, 10

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227 μM fMLP stimulation induced a transient polymerization of actin. In *capri^{kd}* cells, the same
228 stimulation also triggered the initial, transient actin polymerization followed by a much stronger
229 and persistent actin polymerization (Fig. 5*A*-5*B*). To understand the temporospatial dynamics of
230 actin polymerization, we next monitored actin polymerization using the membrane translocation
231 of an actin filament probe, F-tractin-GFP, in live cells by fluorescence microscopy (55). We
232 found that, in response to uniformly applied 1 μM fMLP, F-tractin-GFP (green) translocated to
233 and colocalized with the PM marker (red) along the entire periphery of CTL cells, then
234 withdrew, and accumulated at the protruding sites of CTL cells (Fig. 5*C*, upper panel and Video
235 S12), indicating an initial overall actin polymerization on the entire periphery followed by a
236 localized polymerization on the protrusion sites. In *capri^{kd}* cells, the same stimulation trigged a
237 stronger and prolonged membrane translocation of F-tractin-GFP, followed by a slight
238 withdrawal, and then continuous accumulation on multiple expending/ruffling sites of cells (Fig.
239 5*C*, lower panel and Video S13). To understand the effect of excessive actin polymerization on
240 chemotaxis, we visualized the distribution of F-actin in cells migrating toward a 1 μM fMLP
241 gradient using another F-actin probe (SiR-actin, a live-cell staining probe) (Fig. 5*D*). As
242 expected, CTL cells displayed a clearly polarized actin polymerization: a protruding leading
243 front (pseudopod) and a contracting trailing edge (uropod), and they chemotaxed through the
244 gradient and accumulated at the source of the fMLP gradient (Fig. 5*D*, left panel and Video S14).
245 However, the *capri^{kd}* cells, especially those close to the source of the 1 μM fMLP gradient,
246 displayed an overall excessive F-actin distribution, poor polarization, and significantly slow
247 migration during chemotaxis (Fig. 5*D*, right panel and Video S15). Notably, some of the *capri^{kd}*
248 cells located relatively far away from the source of fMLP showed polarized morphology and
249 migrated effectively toward the fMLP source, but gradually lost their polarity and stopped

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250 moving when they were close to the source of the fMLP (Video S15). The above results together
251 indicate that chemoattractant stimuli at high concentrations induce excessive polymerization of
252 actin that impairs the polarization and migration of *capri^{kd}* cells.

253 ***capri^{kd}* cells display improved chemotaxis in low- or subsensitive-concentration gradients of**
254 **chemoattractants, but defective chemotaxis in high-concentration gradients.** To further
255 determine the function of CAPRI in chemotaxis of neutrophils, we monitored chemotaxis
256 behavior of CTL and *capri^{kd}* cells in gradients of fMLP at different concentrations using an *EZ-*
257 *TAXIScan* analysis (56). In a 1 μM fMLP gradient, *capri^{kd}* cells, in a clear contrast to CTL cells,
258 displayed a significant decrease in migrating speed (CTL: 20.86 ± 3.12 μm/min; *caprikd*: 12.16
259 ± 3.73 μm/min), directionality (CTL: 0.86 ± 0.09; *caprikd*: 0.73 ± 0.14 μm/min), total path
260 length (CTL: 140.56 ± 27. 7 μm; *caprikd*: 86.74 ± 13.75 μm), and polarity measured as
261 roundness of a cell (%) (CTL: 76.2 ± 6.81; *caprikd*: 84.82 ± 5.76) (Fig. 6*A*-6*B*). To understand
262 chemotaxis behavior over a large concentration range, we further monitored the chemotaxis
263 behavior of both CTL and *capri^{kd}* cells in the fMLP gradients at lower concentrations (Fig. 6*C*).
264 We found that severe defects in chemotaxis in *capri^{kd}* cells were clearly observed when they
265 experienced fMLP gradients at high concentration (>100 nM). When exposed to a gradient
266 generated from a lower concentration (10 nM fMLP source, both CTL and *capri^{kd}* cells displayed
267 similar directionality, although *capri^{kd}* cells still displayed decreased speed and total path length
268 (Fig. 6*D*). In response to a gradient generated from a 1 nM fMLP source, *capri^{kd}* cells displayed
269 significantly better directionality, but similar migration speed, in comparison with CTL cells. In
270 a 0.1 nM fMLP gradient, most CTL cells displayed random migration, while the majority of
271 *capri^{kd}* cells displayed improved directionality and migration speed. Without a gradient, *capri^{kd}*

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272 cells displayed a bigger random walk compared to CTL cells. That is, *capri^{kd}* cells display
273 defective chemotaxis in high-concentration gradients but improved chemotaxis in low- or
274 subsensitive-concentration gradients of chemoattractants. We also observed this concentration-
275 dependent change of chemotaxis capability of *capri^{kd}* cells in response to gradients of IL-8,
276 another chemoattractant for neutrophils (Fig. S10). The above result indicates that CTL cells
277 chemotaxed efficiently through gradients of various chemoattractants ranging from 10^{-9} to 10^{-6}
278 M, while *capri^{kd}* cells did so from 10^{-10} to 10^{-7} M.

279 **An increased sensitivity of *capri^{kd}* cells toward chemoattractants.** Using TIRF imaging, we
280 noticed that CAPRI-tGFP localized on the membrane of resting cells, suggesting its potential
281 role in inhibiting basal Ras activity (Fig. 7*A*). As expected, fMLP stimulation induced a strong,
282 quick membrane translocation of CAPRI followed by a slow, gradual withdrawal with a notable
283 fraction of CAPRI remaining on the plasma membrane for quite a long period of time. We also
284 observed a higher basal Ras activity in *capri^{kd}* cells (Fig. 1*C*), indicating a potential role of
285 CAPRI in maintaining a low basal Ras activity in the resting cells. The basal activity of Ras is
286 part of a positive feedback mechanism that promotes actin dynamics and, more importantly,
287 chemotaxis in a shallow chemoattractant gradient in *D. discoideum* (24). We speculated that the
288 higher basal Ras activity in *capri^{kd}* cells might contribute to their enhanced random walk without
289 a gradient or to their improved chemotaxis in a low- or subsensitive-concentration gradient (Fig.
290 6). To determine whether *capri^{kd}* cells have become more sensitive to the stimulus, we
291 determined Ras and Rap1 activation in response to fMLP stimulation at different concentrations
292 using a pull-down assay (Fig. 7*B*). Comparing to the CTL cells, we detected a stronger Ras/Rap1
293 activation in *capri^{kd}* cells in response to all three different concentrations (Fig. 7*C*). In response
294 to 0.1 nM fMLP stimulation, CTL cells did not show a clear Ras or Rap1 activation but

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295 displayed a weak oscillation, while *capri*^{kd} cells showed a clear transient Ras activation. In
296 response to 10 nM and 1 μM fMLP stimulation, *capri*^{kd} cells showed a stronger, longer
297 activation of both Ras and Rap1 than CTL cells did.

298 We further determined the responsiveness of both CTL and *capri*^{kd} cells to either 0.1 nM or 10
299 nM fMLP stimulation by monitoring actin polymerization through the membrane translocation
300 of F-tractin-GFP (Fig. 8). In response to 0.1 nM fMLP stimulation, most CTL cells (~ 90 %) did
301 not show clear membrane translocation of F-tractin-GFP to the plasma membrane, while they
302 showed a continuous translocation of F-tractin-GFP to the protrusion sites (Fig. 8*A*, top panel,
303 and Video S16). In contrast, more than 80 % of the *capri*^{kd} cells showed clear membrane
304 translocation of F-tractin upon 0.1 nM fMLP stimulation (Video S17 and Fig. 8*B*), indicating a
305 higher sensitivity of *capri*^{kd} cells. Not surprisingly, 10 nM fMLP induced robust membrane
306 translocation of F-tractin in both CTL and *capri*^{kd} cells with different dynamics: CTL cells
307 showed a clear transient translocation of F-tractin-GFP to plasma membrane (~ 5-40 s), followed
308 by a withdrawal of most F-tractin from the plasma membrane (~ 50-60 s) and then a second
309 translocation to the protrusion sites (> 60 s) (Video S18); while *capri*^{kd} cells showed a stronger
310 and longer membrane translocation of F-tractin-GFP to plasma membrane (~ 5-90 s) followed by
311 a gradual withdrawal (~ 90-130 s) (Video S19), indicating a stronger response and a longer
312 period of time required to adapt the stimulation. Consistent with the results above, a prolonged,
313 non-adaptive actin polymerization was observed in *capri*^{kd} cells in response to stimuli at higher
314 concentrations (Fig. 5 and Fig. 8*C*). These results together demonstrate that *capri*^{kd} cells, lacking
315 Ras inhibitor, are sensitive to subsensitive-concentration chemoattractant stimuli for CTL cells,
316 while they fail to chemotax through a high-concentration gradient as CTL cells do.

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317 **Discussion**

318 Here, we show that CAPRI is a locally membrane-recruited negative regulator of Ras signaling
319 and enables neutrophils to chemotax through a higher concentration range of chemoattractant
320 gradients by lowering their sensitivity and through GPCR-mediated adaptation.

321 **Chemoattractant-induced deactivation of Ras is regulated through the local membrane**
322 **recruitment of CAPRI.** The membrane recruitment of CAPRI and its function in Ras
323 deactivation have been previously studied (33, 39). CAPRI, CAlcium-Promoted Ras Inactivator,
324 was first characterized by its calcium-dependency on deactivation of Ras (33). In this study, we
325 found that the ΔC2 mutant of CAPRI did not translocate in response to chemoattractant
326 stimulation, indicating an essential role of C2/calcium binding in the membrane targeting of
327 CAPRI (Fig. 2*E*). In addition to the C2 domain, we found that the ΔPH mutant showed
328 significantly decreased membrane translocation in response to chemoattractant stimulation (Fig.
329 2*D*-2*E*). This result is consistent with the previous reports that the PH domain of the GAP1
330 family is responsible for, or plays an important role, in their membrane translocation (34, 39, 57,
331 58). We also found that deactivation of GAP activity of the GAP domain (R472A mutant) had no
332 effect on CAPRI's membrane targeting (Fig. 2*D*), although it might affect its interaction with
333 Ras (Fig. 2E), consistent with the notion that the arginine residue of the GAP domain not only is
334 responsible for its GAP activity, but also stabilizes its interaction with Ras (41). We also found
335 that $capri^{kd/OE}$ cells, which displayed little or no Ras activation, still exhibited a strong CAPRI
336 membrane translocation, indicating that active status of Ras is not required for CAPRI membrane
337 translocation. These results indicate that membrane translocation of CAPRI and its function as a
338 GAP protein are sequential and yet independent.

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339 **Inhibitor for the GPCR-mediated adaptation regulates the sensitivity of a eukaryotic cell.**

340 Motile Escherichia coli (*E. coli*) provides the simplest and the best understood model of
341 chemosensing system that is also mediated by chemoreceptors (59, 60). Modification of the
342 chemoreceptors and interactions among the chemoreceptors have been proposed for a robust
343 response, a precise adaptation, and a high sensitivity of chemoreceptors in bacteria (61-63).
344 However, the molecular mechanism by which to control the GPCR-mediated adaption and the
345 sensitivity of a eukaryotic cell in chemotaxis are not fully understood. A local excitation and
346 global inhibition (LEGI) model explained how a eukaryotic cell responds to chemoattractant
347 stimuli with a wide-concentration range, achieves adaptation, and establishes the intracellular
348 polarization (10, 64). Different from *E. coli*, chemoattractant stimulation induces a persistent
349 activation of heterotrimeric G protein in *Dictyostelium* (6, 11, 26), indicating that adaptation
350 occurs downstream of GPCR/G protein in the eukaryotic cell. We constructed a detailed model
351 to explain adaptation and chemosensing of *Dictyostelium* cells (15, 65), which led us to uncover
352 the essential role of Ras inhibitors in the GPCR-mediated adaptation (11, 16). Recently, it has
353 been shown that reducing the $PI(3,4)P_2$ level on the plasma membrane increases Ras activity and
354 enhances the excitability in *D. discoideum* (18, 19) and that active Ras plays a role in basal
355 locomotion and in chemotaxing through very shallow gradients (24). We also detected a higher
356 basal activity of Ras and Rap1 in *capri*^{kd} cells. These reports inspired us to test the chemotaxis
357 capability of both CTL and *capri*^{kd} cells using wide concentration-range gradients of various
358 chemoattractants (Fig. 6 and Fig. S10). In response to fMLP gradients, CTL cells chemotaxed
359 better than *capri*^{kd} cells did in gradients at high concentrations (> 100 nM), while they displayed
360 similar chemotaxis capability to *capri*^{kd} cells in mild gradients (10 nM of fMLP). In weak
361 gradients (< 1 nM), *capri*^{kd} cells chemotaxed better than CTL cells did. Similar chemotaxis

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362 behavior was also observed when CTL and *capri*^{kd} cells were exposing to gradients of IL-8.
363 Interestingly, *capri*^{kd} cells sensed and responded to weak gradients of fMLP and IL-8 at the
364 concentration that are subsensitive to CTL cells due to a higher sensitivity of *capri*^{kd} cells (Fig. 7
365 and Fig. 8). Thus, our study provides evidence that human neutrophils locally recruits Ras
366 inhibitor, CAPRI, to regulate Ras adaptation and their sensitivity. CAPRI enables human
367 neutrophils to chemotax through a higher-concentration range of chemoattractant gradients by
368 lowering its sensitivity and by mediating the GPCR-mediated adaptation.

369 **Multiple RasGAP proteins might be involved in Ras deactivation in chemoattractant-**
370 **induced adaptation of neutrophils.** In the present study, we have shown that CAPRI mediates
371 chemoattractant-induced Ras adaptation and sensitivity of neutrophils. Human neutrophils
372 without CAPRI fail in chemoattractant-induced adaptive Ras activation; have significantly
373 increased phosphorylation of AKT, GSK3α/3β, and cofilin; demonstrate excessive actin
374 polymerization; and exhibit a subsequent defect in chemotaxis in response to high-concentration
375 gradients. However, in response to a low-concentration (either 0.1 nM or 10 nM fMLP)
376 stimulation, *capri*^{kd} cells displayed transient Ras/ Rap1 activation and actin polymerization,
377 although they displayed prolonged, non-adaptive responses upon stimulation with higher
378 concentrations (Fig. 1, Fig. 7, and Fig. 8). HL60 cells also expressed RASAL1 and RASAL2
379 (Fig. S2)(37), which also deactivate Ras (34, 39). In mouse and human neutrophils, there are
380 other RasGAP proteins expressed in addition to CAPRI (Fig. S2). p120 GAP is present in both
381 human and mouse neutrophils but absent in HL60 cells (66) and its role has been implicated in
382 cell migration (67, 68). While we revealed a major role of CAPRI in mediating the GPCR-

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383 mediated Ras adaptation in HL60 cells, it is also crucial to determine potentially roles of other
384 RasGAP proteins in Ras adaptation and chemotaxis in primary mammalian neutrophils.

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388 Allergy and Infectious Diseases, National Institutes of Health.

389 **Materials and Methods**

390 Detailed descriptions of cells, cell lines, cell culture, and differentiation, reagents and antibodies,
391 plasmids and transfection of cells, immunoblotting of fMLP-mediated signaling components,
392 Ras and Rap1 activation assay, actin polymerization assay, immunoprecipitation assay, imaging
393 and data processing, TAXIScan chemotaxis assay and data analysis, and SiR-actin staining are in
394 Supplementary information.

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546

547 **Figure legends**

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548 **Fig. 1.** CAPRI interacts with Ras and regulates chemoattractant GPCR-induced Ras adaptation.
549 (A) fMLP-stimulated association between Ras/Rap1/Rac1 and CAPRI detected by co-
550 immunoprecipitation assay. (B) Expression of *capri* in HL60 cells transfected with non-specific
551 (CTL) or *capri* specific (*capri*^{kd}) shRNA virus particles. CAPRI was detected by antibodies
552 against human CAPRI. Actin was detected as a loading control; fMLP receptor 1 FPR1 was also
553 detected in both CTL and *capri*^{kd} cells. (C) fMLP-induced Ras activation in CTL and *capri*^{kd}
554 cells determined by a pull-down assay. Upon stimulation with 10 μM fMLP at time 0", cells
555 were collected, lysed at the indicated time points, and then centrifuged at 10,000 *g* for 10 min at
556 4 °C. Agarose beads pre-conjugated with RBD-GST (active Ras binding domain of Raf1 tagged
557 with GST) were incubated with the supernatants of the lysates for 2 hours at 4 °C, then washed
558 with lysis buffer. The protein bound to agarose beads was eluted with 2X SDS loading buffer
559 (SLB). Aliquots of cells at the indicated time points were also mixed with the same volume of
560 SLB for the detection of total Ras protein. The elutes of RBD-GST beads and the aliquots of the
561 cells were analyzed by immunoblotting with anti-pan Ras antibody to detect either active Ras or
562 total Ras protein. (D) Normalized quantitative densitometry of the active Ras from three
563 independent experiments, including the result presented in **C**. The intensity ratio of active Ras
564 and total Ras in WT at time 0 s was normalized to 1. Mean ± SD from three independent
565 experiments is shown. (E) Ras activation in CTL, *capri*^{kd}, and *capri*$^{kd/OE}$ cells monitored by the
566 membrane translocation of the mRFP-tagged active Ras binding domain of Raf1 (RBD-RFP) in
567 response to 1 μM fMLP stimulation. CTL (top panel and Video S1) and *capri*^{kd} (middle panel
568 and Video S2) cells expressed RBD-RFP (red) and a plasma membrane maker (PM, green). The
569 *capri*$^{kd/OE}$ cell (bottom panel and Video S3) is a *capri*^{kd} cell expressing a PM marker (cyan),
570 CAPRI tagged with turboGFP (CAPRI-tGFP, green), and RBD-RFP (red). fMLP was applied to

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571 the cells after 0 s. Scale bar, 10 μm. (F) The quantitative measurement of Ras activation as the
572 membrane translocation of RBD-RFP in E is shown. Mean ± SD is shown; n = 3, 4, and 4 for
573 CTL, $capri^{kd}$, and $capri^{kd/OE}$ cells, respectively.

574 **Fig. 2.** Chemoattractant-induced membrane targeting of CAPRI. (A) Colocalization of active Ras
575 and Rac1 with CAPRI in the leading edge of a chemotaxing cell. HL60 cells expressed a PM
576 marker (cyan), both tGFP-tagged CAPRI (green), and RBD-RFP (red) or actin-mCherry (red)
577 were exposed to a 100 nM fMLP gradient (dark blue). To visualize the fMLP gradient, 100 nM
578 fMLP was mixed with Alexa 633 (dark blue). Scale bar = 10 μm. (B) The scheme shows the
579 domain composition of tGFP-tagged wild-type (WT) or mutants of CAPRI. (C) Domain
580 requirement for the interaction of CAPRI and Ras determined by co-immunoprecipitation. HL60
581 cells expressing either tGFP alone or tGFP-tagged WT or mutants of CAPRI were lysed with
582 immunoprecipitation buffer with 1 mM GTPγS and went through the coimmunoprecipitation
583 process and western blotting using anti-GFP or Ras antibodies. (D) Montage shows the
584 membrane translocation of CAPRI and its mutants upon uniform application of 1 μM fMLP.
585 Cells expressing tGFP-tagged WT or mutants of CAPRI (green) and PM marker (red) were
586 imaged in time-lapse and 100 nM fMLP (red) was applied to the cells after 0 s. Scale bar = 10
587 μm. Videos S4-S7 are CTL cells expressing PM marker (red) and tGFP-tagged WT, R472A,
588 ΔPH or ΔC2 of CAPRI (green), respectively. (E) The quantitative measurement of the
589 membrane translocation of CAPRI or its mutants is shown. Mean ± SD is shown, n = 5, 4, 4, and
590 4 for WT, ΔC2, ΔPH, or R472A of CAPRI, respectively. The quantitative measurement of
591 intensity changes was described in the Materials and Methods section.

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592 **Fig. 3.** Enhanced chemoattractant-induced activation of PI_3K signaling in *capri*^{kd} cells. (A)
593 Montage shows the membrane translocation of PIP_3 biosensor PH-GFP in CTL and *capri*^{kd} cells
594 in response to 1 μM fMLP stimulation. Cells expressing PH-GFP (GFP-tagged PH domain of
595 human AKT, green) and PM marker (red) were stimulated with 1 μM fMLP at time 0 s. Scale
596 bar = 10 μm. Videos S8 and S9 are CTL and *capri*^{kd} cells, respectively. (B) fMLP-induced
597 phosphorylation of AKT in CTL and *capri*^{kd} cells. A final concentration of 10 μM fMLP
598 stimulation was added to the cells at time 0 s. Aliquots of cells were sampled at the indicated
599 time points and subjected to western blot detection of the phosphorylated and total proteins of
600 interest in **A** and **C**. (C) Normalized quantitative densitometry of phosphorylated AKT by total
601 AKT protein in **B**. Mean ± SD of three independent experiments is shown. The intensity ratio of
602 the phosphorylated versus total AKT in CTL cells at time 0 s is normalized to 1. (D) fMLP-
603 induced phosphorylation of GSK3α/3β and cofilin in CTL and *capri*^{kd} cells. (E) Normalized
604 quantitative densitometry of the phosphorylated GSK3α/3β, and cofilin in **D**. The intensity ratio
605 of the phosphorylated versus total GSK3α/β and cofilin in CTL cells at time 0 s is normalized to
606 1. Mean ± SD of three independent experiments is shown.

607 **Fig. 4.** Increased activation of Rap1 and its effector induced by fMLP stimulation in *capri*^{kd}
608 cells. (A) fMLP-induced Rap1 activation in CTL and *capri*^{kd} cells determined by a pull-down
609 assay using GST-RBD_{RalGDS} agarose beads. (B) Normalized quantitative densitometry of the
610 active Rap1 from three independent experiments, including the result presented in A. The
611 intensity ratio of active Rap1 and total Rap1 in WT at time 0 s was normalized to 1. Mean ± SD
612 from three independent experiments is shown. (C) fMLP-induced Rap1 activation monitored by
613 the membrane translocation of the active Rap1 probe GFP-RBD_{RalGSD}. Cells expressing

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614 RBD_{RalGSD}-GFP (green) and PM marker (red) were stimulated with 1 μM fMLP at 0 s. Scale bar
615 = 10 μm. Videos S11-S12 are CTL and *capri*^{kd} cells, respectively. (D) fMLP-induced
616 phosphorylation of Erk42/44 in CTL and *capri*^{kd} cells. (E) Normalized quantitative densitometry
617 of the phosphorylated Erk42/44 in E. The intensity ratio of the phosphorylated versus total
618 Erk42/44 in CTL cells at time 0 s is normalized to 1. Mean ± SD of three independent
619 experiments is shown.

620 **Fig. 5.** Elevated polymerization of actin in response to a high concentration of fMLP stimulation
621 impairs the polarity and migration of *capri*^{kd} cells. (A) The amount of globular (G) and
622 filamentous (F) actin in CTL and *capri*^{kd} cells was determined by a centrifugation assay of F-
623 actin. Cells were stimulated with 10 μM fMLP at time 0 s and aliquots of cells at the indicated
624 time points were analyzed. (B) Normalized quantitative densitometry of the F/G-actin ratio in the
625 CTL and of *capri*^{kd} cells in (A). Mean ± SD from three independent experiments is shown. The
626 F/G ratio of CTL cells at 0 s was normalized to 1. (C) fMLP-induced actin polymerization by
627 monitoring F-actin probe GFP-F-tractin using live cell confocal microscopy in CTL and *capri*^{kd}
628 cells. Cells expressing tractin-GFP (green) and PM marker (red) were stimulated with 1 μM
629 fMLP at time 0 s. Scale bar = 10 μm. Videos S12-S13 are CTL and *capri*^{kd} cells, respectively.
630 (D) F-actin distribution in chemotaxing CTL and *capri*^{kd} cells in a 1 μM fMLP gradient. The
631 differentiated cells were stained with the actin filament probe, SiR-actin (red). Cells were
632 exposed to a 1 μM fMLP gradient and allowed to chemotax for 5 min. Arrows point to the cells
633 with a polarized, chemotaxing morphology. To visualize the gradient, fMLP was mixed with
634 Alexa 488 (green, 1 μg/ml). Scale bar = 20 μm. Videos S14-S15 are CTL and *capri*^{kd} cells,
635 respectively. (E) The graph shows the roundness of CTL and *capri*^{kd} cells shown in **D**. The

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636 roundness was measured as the ratio of the width vs the length of the cell. That is, the roundness
637 for a circle is 1 and for a line is 0. Student's *t*-test was used to calculate the *p* value.

638 **Fig. 6.** The concentration range of fMLP gradients in which neutrophils chemotax efficiently is
639 upshifted in *capri*^{kd} cells. (A) Montages showing the travel path of chemotaxing CTL or *capri*^{kd}
640 cells in a gradient generated from a 1 μM fMLP source. Cell movement was analyzed by DIAS
641 software. (B) Chemotaxis behaviors measured from A are described as four parameters:
642 directionality, which is "upward" directionality, where 0 represents random movement and 1
643 represents straight movement toward the gradient; speed, defined as the distance that the centroid
644 of the cell moves as a function of time; total path length, the total distance the cell has traveled;
645 and roundness (%) for polarization, which is calculated as the ratio of the width to the length of
646 the cell. Thus, a circle (no polarization) is 1 and a line (perfect polarization) is 0. Thirteen cells
647 from each group were measured and the mean ± SD was shown. Student's *t*-test was used to
648 calculate the *p* value. (C) Montages show the travel path of chemotaxing CTL or *capri*^{kd} cells in
649 response to a wide range of fMLP gradients. (D) Chemotaxis behaviors measured from **C** are
650 described by three parameters: directionality, speed, and total path length as described in **B**.
651 Thirteen cells from each group were measured. Student's *t*-test was used to calculate the *p* value.

652 **Fig. 7.** Activity of Ras and Rap1 is increased in *capri*^{kd} cells. (A) CAPRI localizes at the plasma
653 membrane before and after fMLP stimulation. (B) Ras and Rap1 activation in CTL and *capri*^{kd}
654 cells in response to 0.1 nM, 10 nM, or 1 μM fMLP stimulation determined by a pull-down assay.
655 (C) Normalized quantitative densitometry of the active Ras and Rap1 from three independent
656 experiments, including the result presented in **B**. The intensity ratio of active Ras and Rap1 in
657 WT at time 0 s was normalized to 1. Mean ± SD from three independent experiments is shown.

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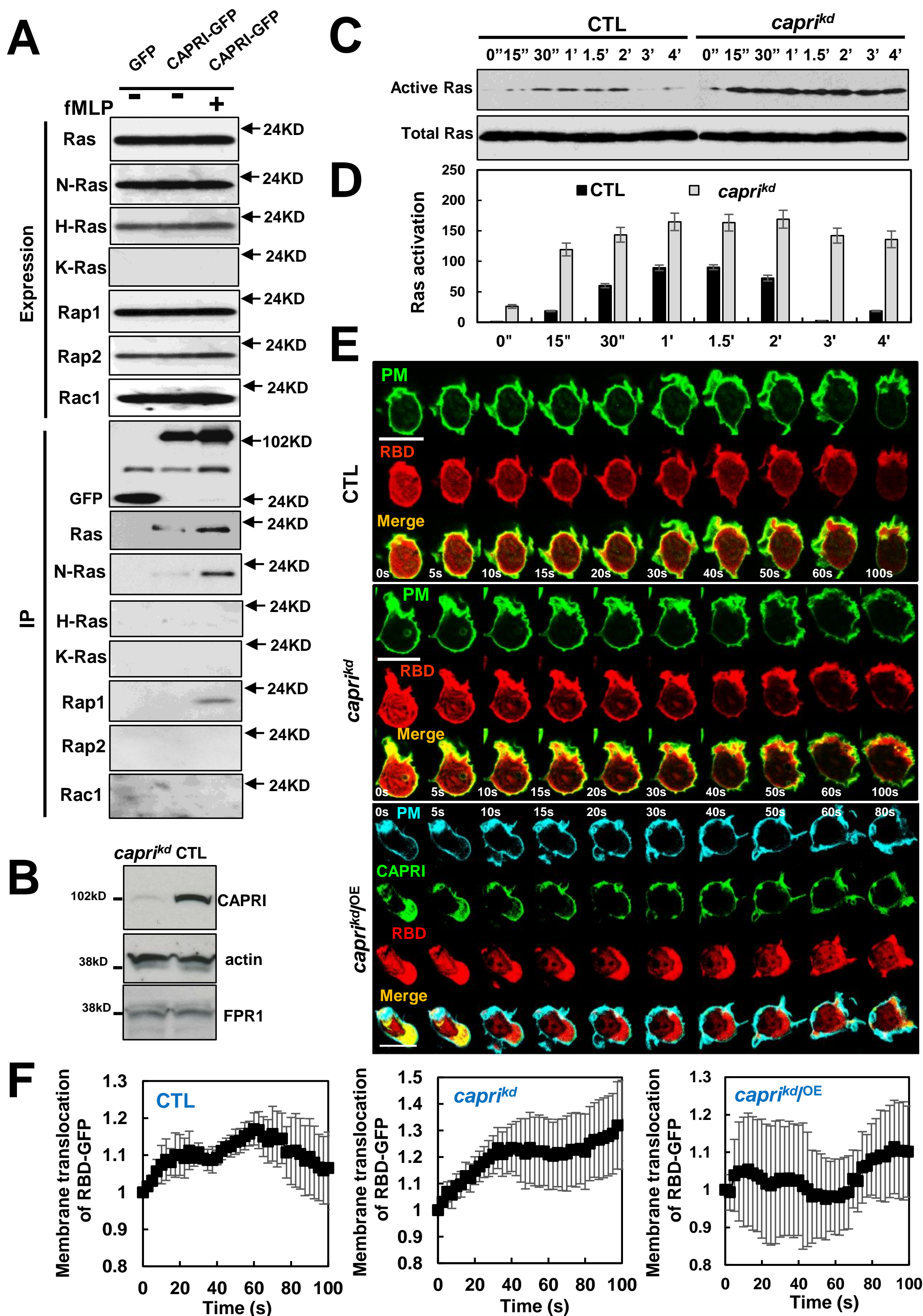
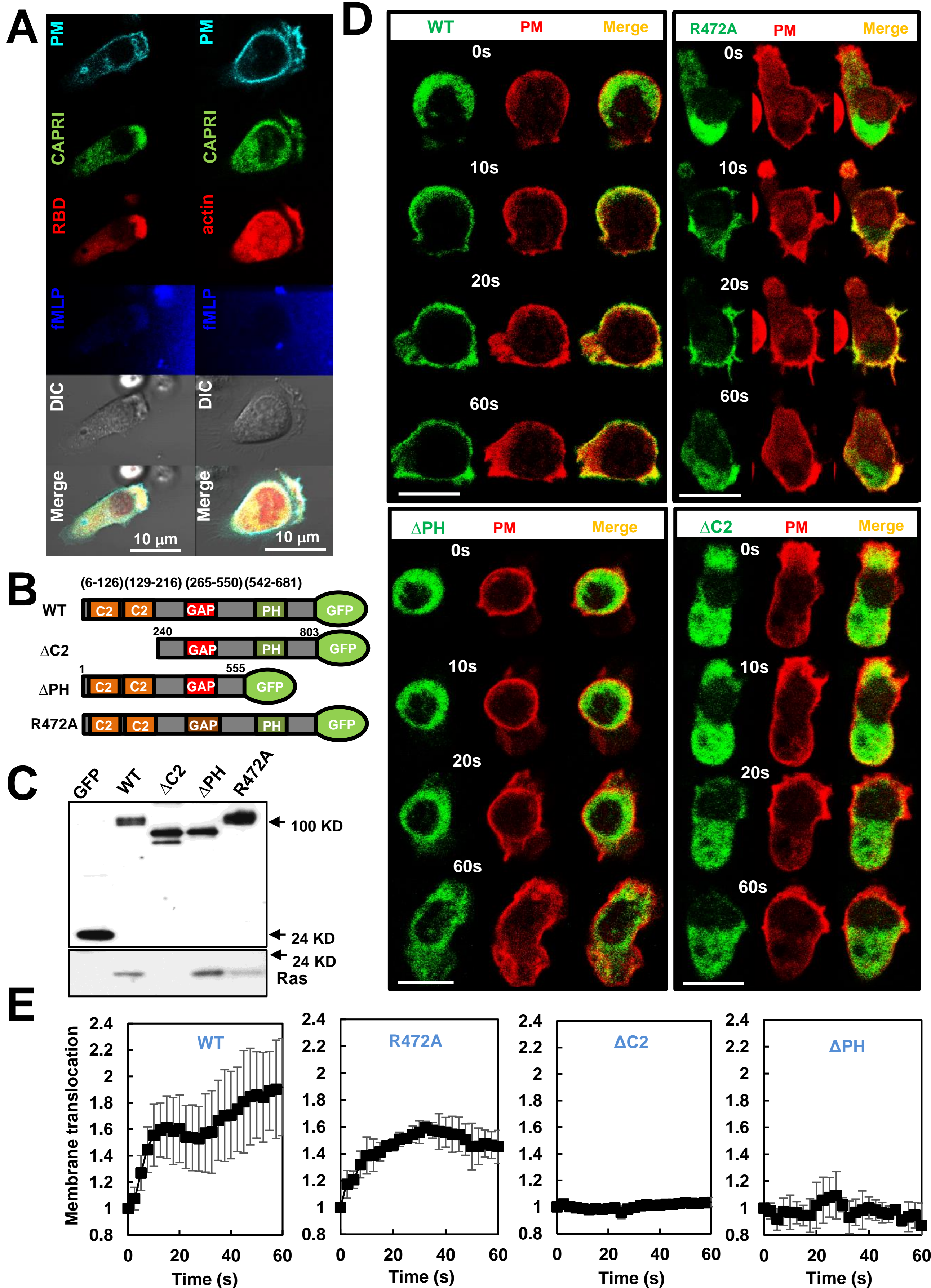
658 **Fig. 8.** *capri*^{kd} cells are more sensitive to the stimuli at low concentration and fail to adapt to the
659 stimuli at high concentrations. (A) Montage shows cell response as actin polymerization
660 monitored through the membrane translocation of F-actin probe, Ftractin-GFP, in both CTL or
661 *capri*^{kd} HL60 cells using fluorescent microscopy. Cells expressing GFP-F-tractin (green) and PM
662 marker (red) were stimulated with either 0.1 nM or 10 nM fMLP stimulation. Scale bar = 10 µm.
663 Videos S16 and S18 are CTL cells and Videos S17 and S19 are *capri*^{kd} cells. Videos S16-S17
664 and Videos S18-19 were cells stimulated with either 0.1 nM or 10 nM fMLP, respectively. (B)
665 The percentage of cells that respond to the indicated concentration of fMLP stimuli in CTL and
666 *capri*^{kd} cells is shown. (C) Percentage of cells with adaptation in response to fMLP stimulation.
667 **B** and **C** were measured with the same sets of data. The numbers of independent experiments for
668 CTL and *capri*^{kd} cells for 0.1 nM, 10 nM, and 1 µM fMLP stimuli are 11 and 7, 8 and 10, and 5
669 and 9, respectively. Student's *t*-test was used to calculate the *p* value.


A
GFP
CAPRI-GFP
CAPRI-GFP
fMLP
Expression
Ras
N-Ras
H-Ras
K-Ras
Rap1
Rap2
Rac1
24KD
IP
GFP
102KD
B
caprikd CTL
102kD
CAPRI
38kD
actin
FPR1
C
CTL
caprikd
0" 15" 30" 1' 1.5' 2' 3' 4'
Active Ras
Total Ras
D
CTL
caprikd
Ras activation
E
PM
RBD
Merge
CAPRI
caprikd/OE
0s 5s 10s 15s 20s 30s 40s 50s 60s 100s 80s
F
Membrane translocation of RBD-GFP
Time (s)


A
PM
CAPRI
RBD
actin
fMLP
DIC
Merge
10 μm
B
(6-126)(129-216) (265-550) (542-681)
WT
C2
GAP
PH
GFP
ΔC2
240
803
ΔPH
1
555
R472A
C
GFP
WT
ΔC2
ΔPH
R472A
100 KD
24 KD
24 KD
Ras
D
WT
PM
Merge
R472A
ΔPH
ΔC2
0s
10s
20s
60s
E
Membrane translocation
Time (s)
WT
R472A
ΔC2
ΔPH
0
20
40
60
0.8
1
1.2
1.4
1.6
1.8
2
2.2
2.4

Xu et al., Figure 3

A

CTL

PH

PM

Merge

0s 5s 15s 25s 35s 45s 60s 70s 80s 90s 110s

capri^{kd}

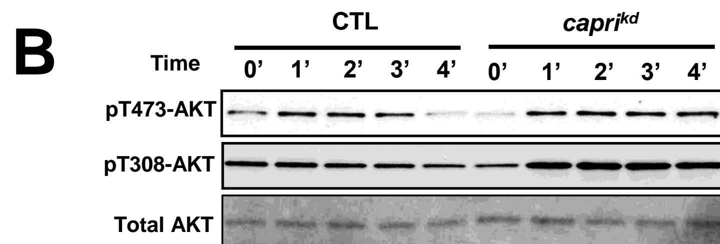
PH

PM

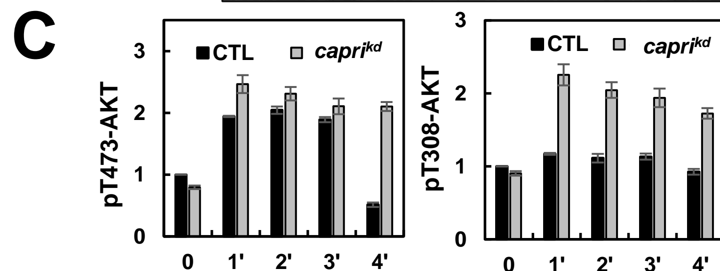
Merge

0s 5s 15s 25s 35s 45s 60s 70s 80s 90s 110s

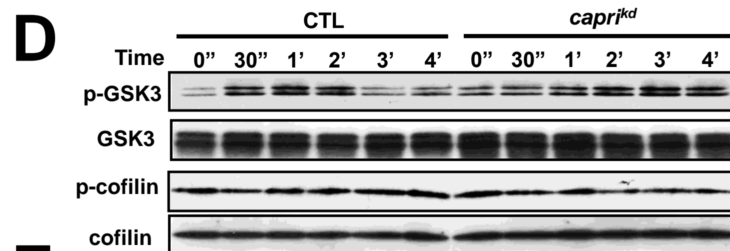
B



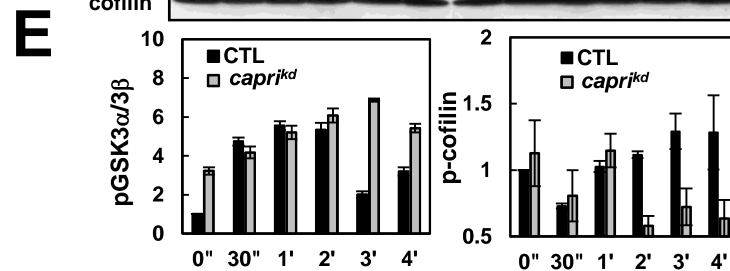
C

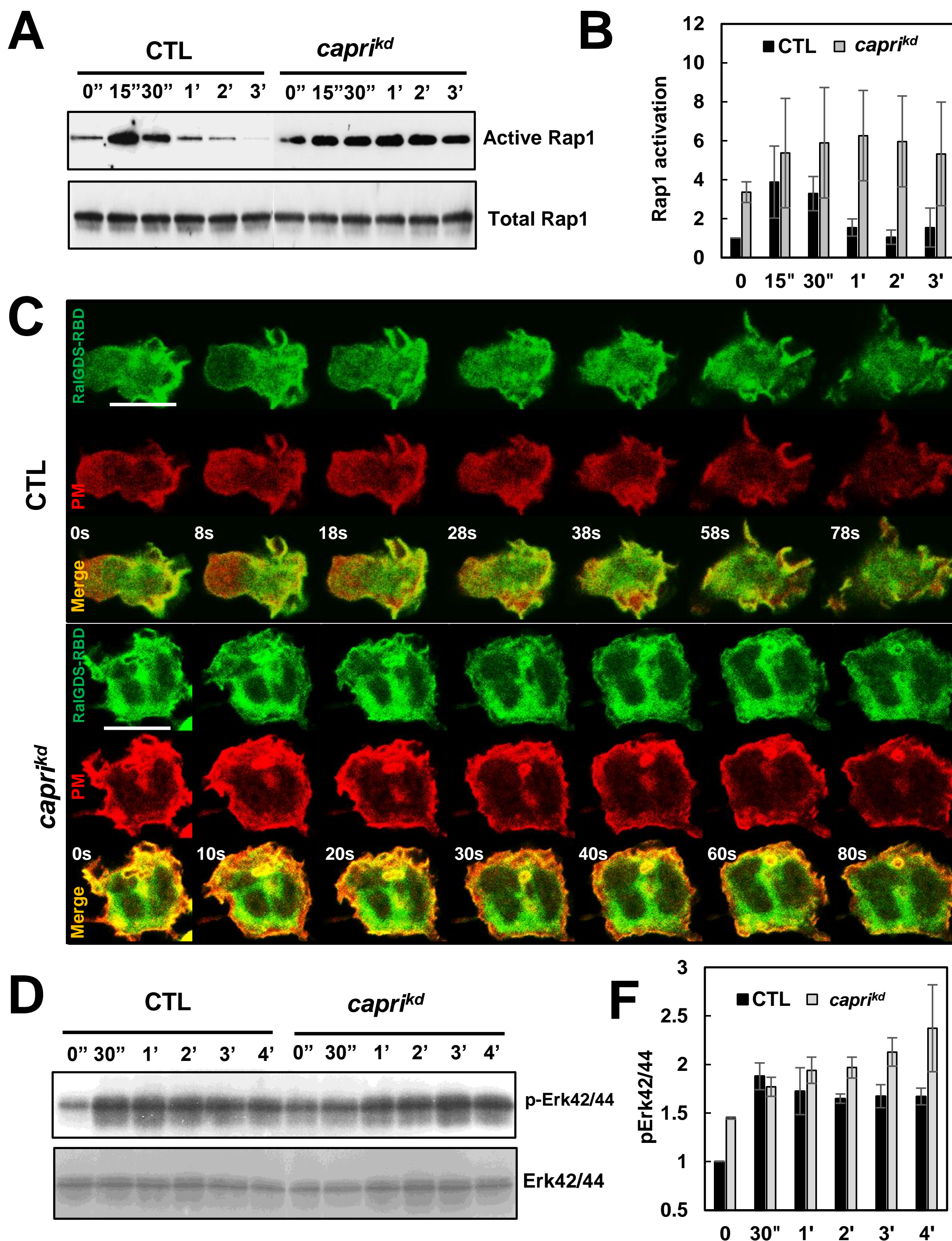


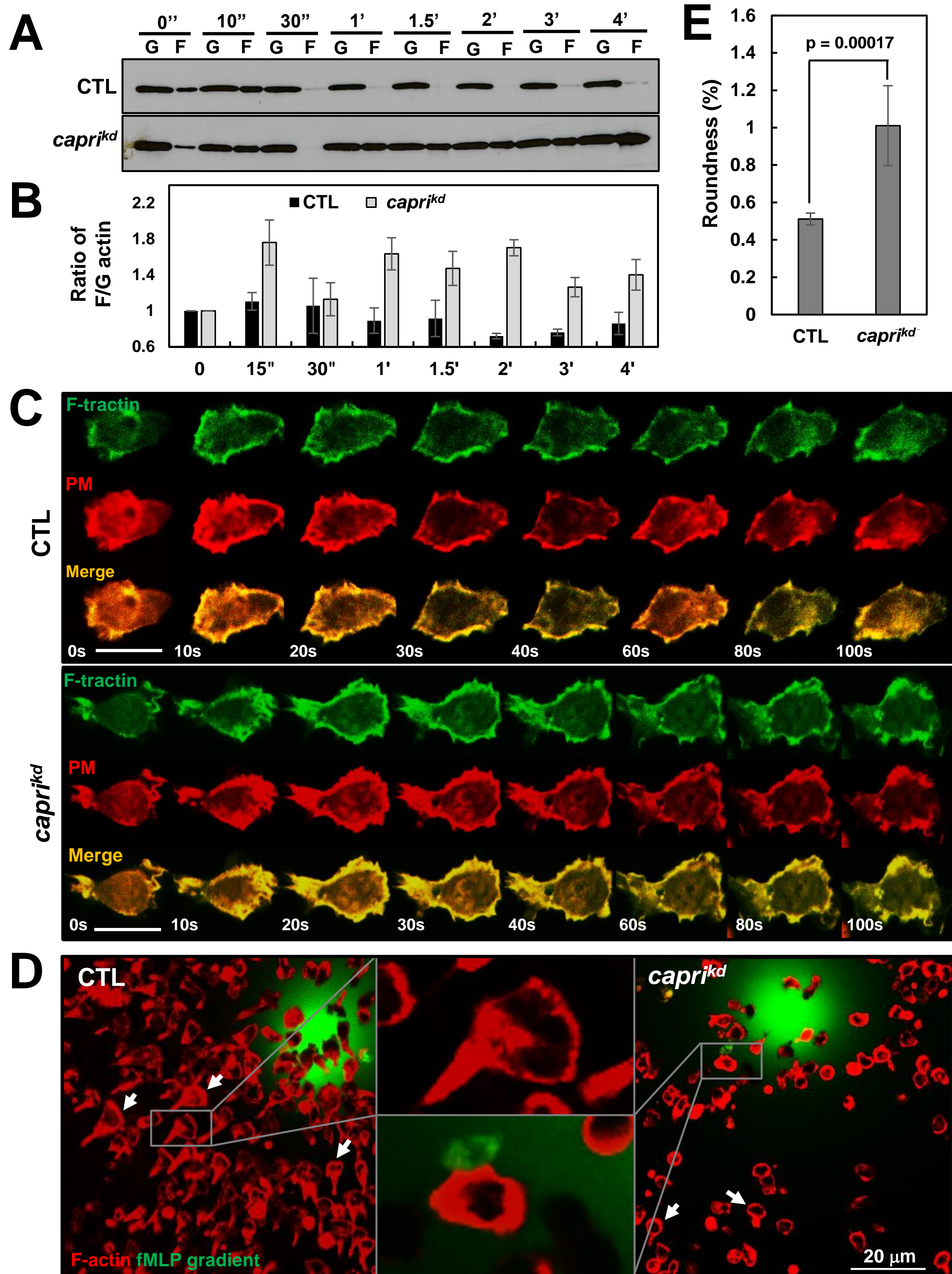
D

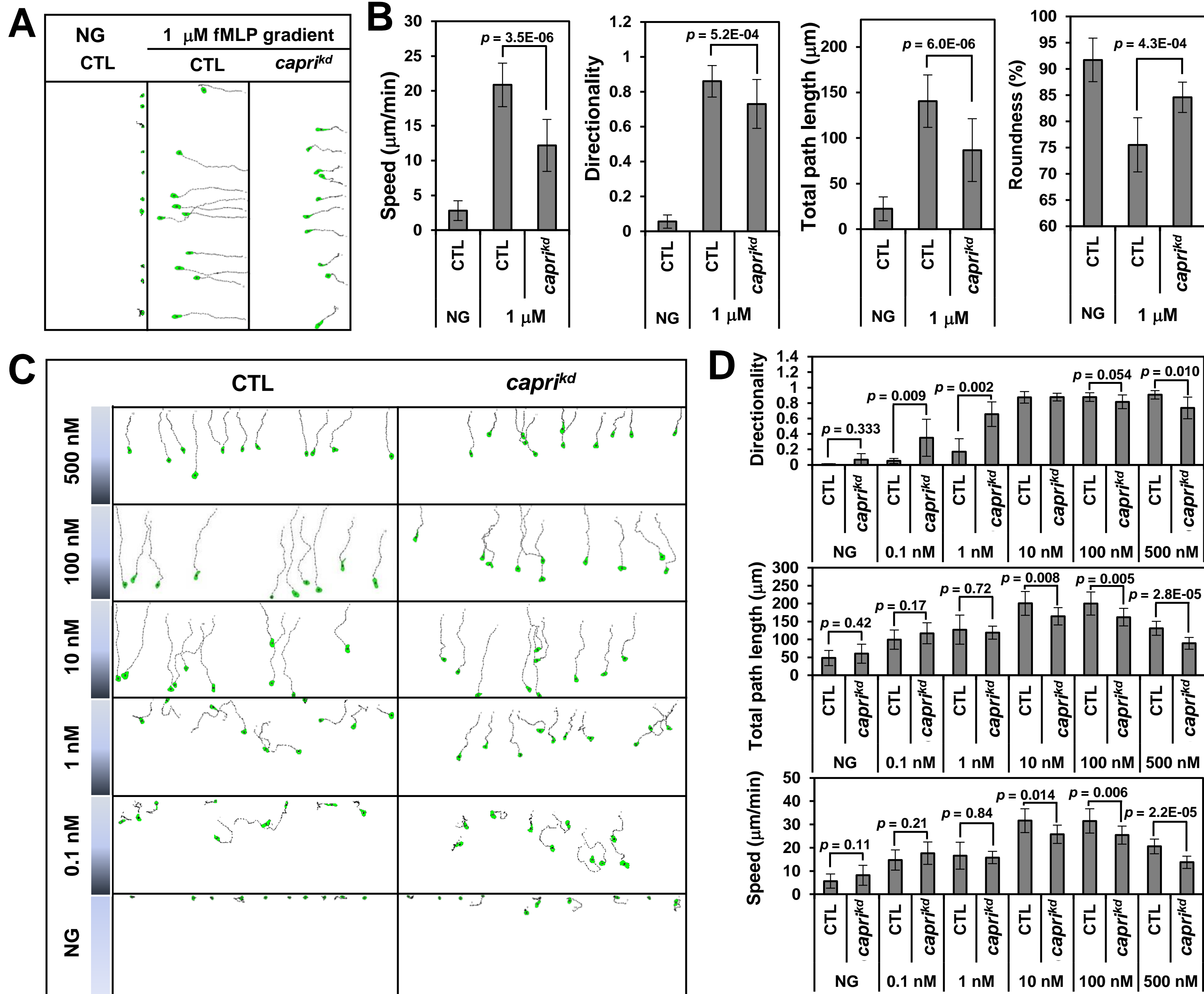


E

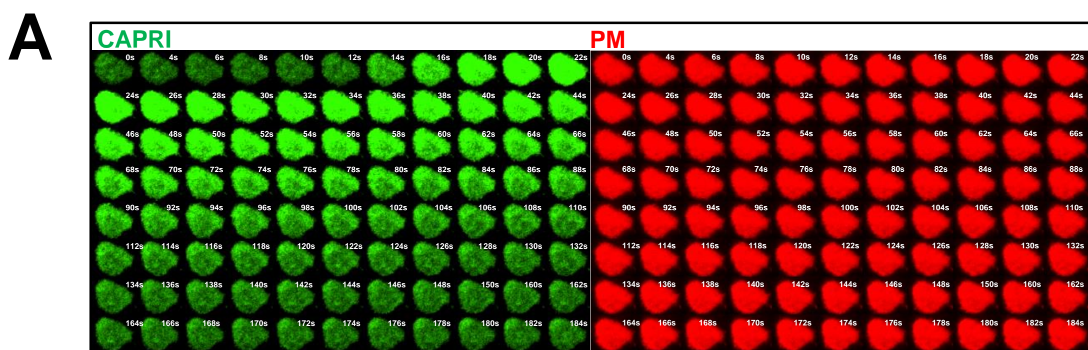



A
CTL
capri^kd
0" 15" 30" 1' 2' 3'
0" 15" 30" 1' 2' 3'
Active Rap1
Total Rap1
B
Rap1 activation
CTL
capri^kd
0 15" 30" 1' 2' 3'
C
CTL
RalGDS-RBD
PM
Merge
0s 8s 18s 28s 38s 58s 78s
capri^kd
0s 10s 20s 30s 40s 60s 80s
D
CTL
capri^kd
0" 30" 1' 2' 3' 4'
p-Erk42/44
Erk42/44
F
pErk42/44
0 30" 1' 2' 3' 4'

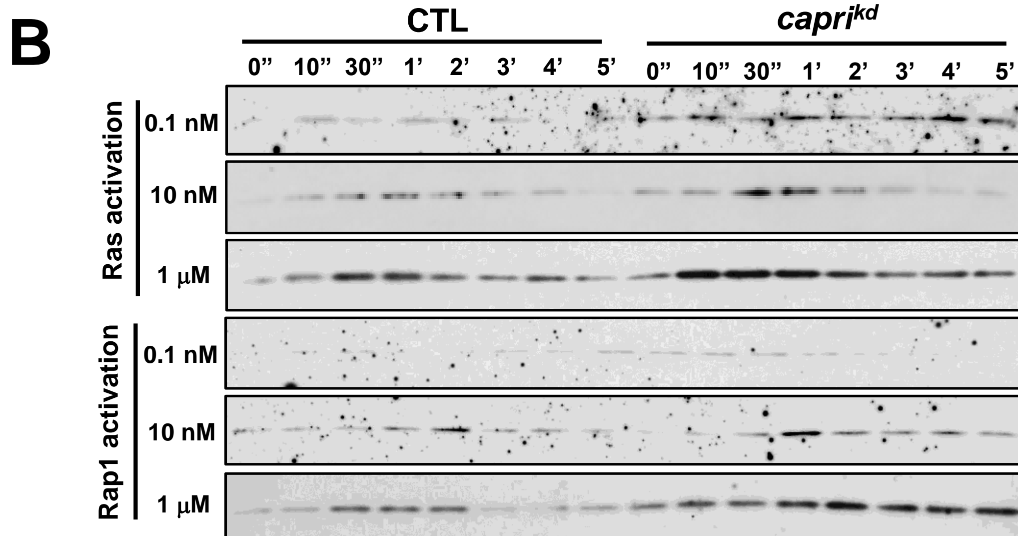

A
0''
10''
30''
1'
1.5'
2'
3'
4'
G F G F G F G F G F G F G F G F
CTL
capri^kd
B
Ratio of F/G actin
CTL
capri^kd
2.2
1.8
1.4
1
0.6
0
15''
30''
1'
1.5'
2'
3'
4'
E
Roundness (%)
p = 0.00017
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
CTL
capri^kd
C
CTL
F-tractin
PM
Merge
0s
10s
20s
30s
40s
60s
80s
100s
capri^kd
F-tractin
PM
Merge
0s
10s
20s
30s
40s
60s
80s
100s
D
CTL
capri^kd
F-actin fMLP gradient
20 μm


A
NG
CTL
1 μM fMLP gradient
CTL
capri^kd
B
Speed (μm/min)
p = 3.5E-06
Directionality
p = 5.2E-04
Total path length (μm)
p = 6.0E-06
Roundness (%)
p = 4.3E-04
CTL
capri^kd
NG
1 μM
C
CTL
capri^kd
500 nM
100 nM
10 nM
1 nM
0.1 nM
NG
D
Directionality
p = 0.333
p = 0.009
p = 0.002
p = 0.054
p = 0.010
Total path length (μm)
p = 0.42
p = 0.17
p = 0.72
p = 0.008
p = 0.005
p = 2.8E-05
Speed (μm/min)
p = 0.11
p = 0.21
p = 0.84
p = 0.014
p = 0.006
p = 2.2E-05
NG
0.1 nM
1 nM
10 nM
100 nM
500 nM

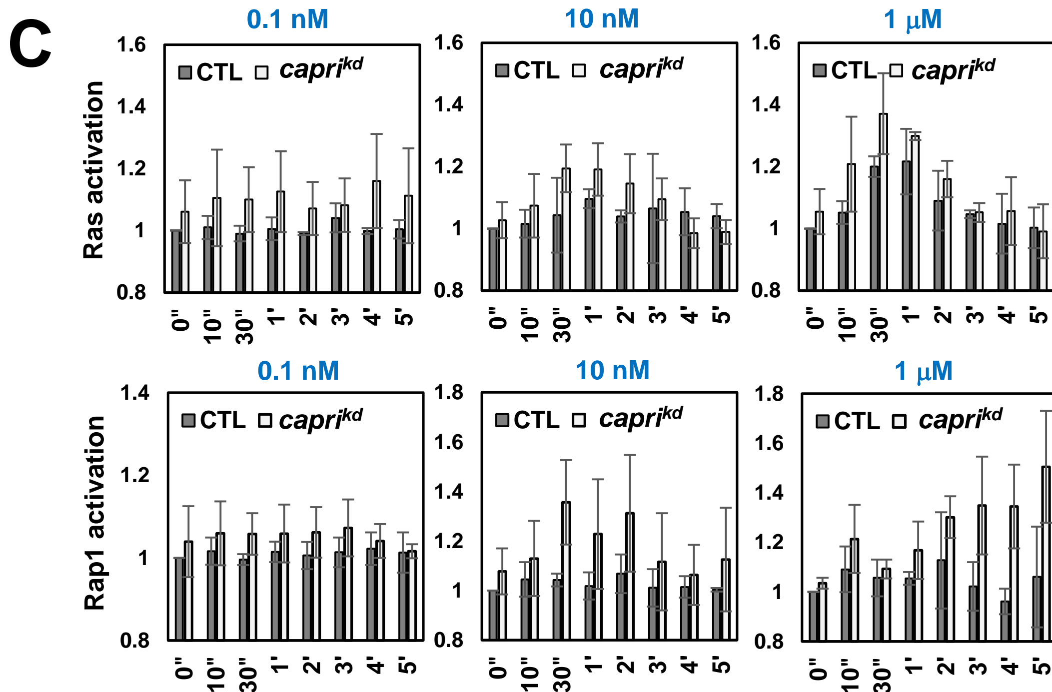
A



B



C



A

0.1 nM fMLP

CTL

F-tractin
PM
Merge

0s 5s 10s 20s 30s 40s 50s 70s 90s 110s 130s

capri^{kd}

F-tractin
PM
Merge

0s 5s 10s 20s 30s 40s 50s 70s 90s 110s 130s

10 nM fMLP

CTL

F-tractin
PM
Merge

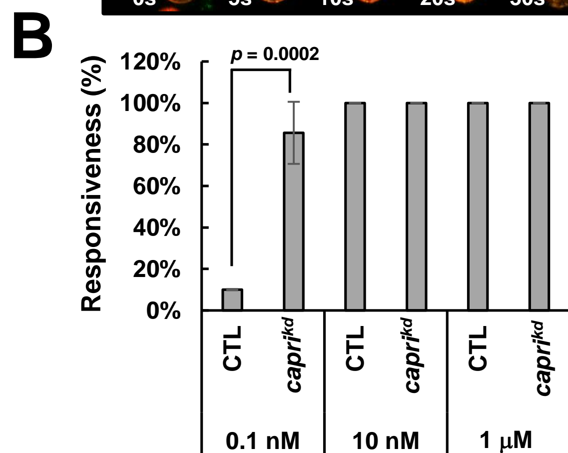
0s 5s 10s 20s 30s 40s 50s 70s 90s 110s 130s

capri^{kd}

F-tractin
PM
Merge

0s 5s 10s 20s 30s 40s 50s 70s 90s 110s 130s

B



C