

1 **Supplemental Information**

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3 **A coronin-1C/RCC2 complex guides mesenchymal migration by trafficking Rac1**
4 **and controlling GEF exposure**

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10 **Fig. S1. RCC2 and Coro1C are Rac1 regulators**

11 (A) Syndecan-4-stimulated Rac1 activation in RCC2 knockdown MEFs. Experiment
12 uses an alternative oligo sequence to that shown in Fig. 1A. n=3.

13 (B) Pairwise comparison of syndecan-4-stimulated Rac1 activation at 0 and 10
14 minutes stimulation, in control and RCC2-knockdown MEFs (oligo #1), the complete
15 timecourses are shown in Fig. 1A. n =7.

16 (C) Comparison of Rac1 activation in unstimulated control MEFs and MEFs
17 overexpressing GFP-RCC2. n = 4.

18 (D) Distribution of Rac1, RhoGDI and RCC2 between soluble (CCT2) and total
19 membrane (β_1 -integrin) fractions using a Qproteome kit. n=7.

20 (E) Scores for *in silico* docking experiments between RCC2/Coro1C and GDP/GTP-
21 loaded Rac1.

22 (F) Binding interface between RCC2 and GTP-Rac1 from *in silico* docking
23 experiments, demonstrating that interactions are confined to the Switch 1 loop of
24 GTP-Rac1.

25 (G-H) Coro1C can be divided into functional subdomains. (E) Arp3 can be pulled-
26 down from lysate with GST-Coro1C-tail. (F) GFP-Coro1C full length and propeller
27 domain cosediment with filamentous actin. n=4.

28 (I) RCC2 coprecipitated from 293T lysates with GFP-Coro1C linker region (residues
29 351-435), but not the coiled-coil domain (residues 436-474). Further subdivision of
30 the linker into N- and C-terminal parts (residues 351-397 and 393-435 respectively)
31 also caused loss of binding. n=4.

32 (J) Knockdown of Coro1C had no effect on Rac1 activity in unstimulated MEFs. n=8.

33 (K) Syndecan-4-stimulated Rac1 activation in Coro1C knockdown MEFs.

34 Experiment uses an alternative oligo sequence to that shown in Fig. 3I. n=4.

35 Error bars indicate s.e.m.

36

37 **Fig. S2. Coro1C and RCC2 regulate Rac1 localization**

38 (A) Control, RCC2-knockdown, Coro1C-knockdown, RCC2/Rac1-knockdown, or
39 Coro1C/Rac1-knockdown MEFs were spread on fibronectin in the presence of serum.
40 Cells were fixed and stained with phalloidin and immunostained for endogenous
41 Rac1. Rac1 accumulated in actin-rich lamella (arrowheads) of RCC2-knockdown
42 MEFs and lateral membrane (arrows) of Coro1C-knockdown MEFs. Images are
43 representative of 100 cells on 4 separate occasions.

44 (B) GFP-Rac1 accumulation in protrusions of RCC2-knockdown MEFs and along the
45 sides of Coro1C-knockdown MEFs spread on fibronectin in the presence of serum.
46 Intensity profiles were measured across protrusions (red) and lateral membrane
47 (green). 13 profiles, randomly selected from 100 cells from 4 separate experiments
48 are displayed.

49

50 **Fig. S3. Competition between Coro1C and RCC2 cause Rac1 redistribution**

51 (A) Rac1 was restored to the detergent-soluble fraction of Coro1C-knockdown MEFs
52 by exogenous expression of Coro1C, but not Coro1A. n=4.

53 (B) Knockdown of Coro1C has no affect on total Rac1 protein levels, lysates prepared
54 with 0.1% SDS to ensure extraction of Rac1 from all membrane fractions. n=4.

55 (C) Immunofluorescent staining of endogenous Coro1C demonstrating localization to
56 both actin-rich ruffles (arrowheads) and lateral membrane (arrows). Staining is
57 ablated by knockdown of Coro1C. Images are representative of 100 cells on 3
58 separate occasions.

59 (D) Fibroblast expressing GFP-Coro1C following RCC2 knockdown, spread on
60 fibronectin with serum and fixed. Image representative of 50 cells on 2 separate
61 occasions.

62 (E) Protein distribution between soluble, total membrane, nuclear and cytoskeletal
63 fractions. Coro1C is still found in soluble, total membrane and cytoskeletal fractions
64 upon RCC2-knockdown. n=7.

65 (F) Fluorescent decay curves compare redistribution of photoactivated GFP-tagged
66 Rac1 from lateral membrane between control and Coro1C-knockdown MEFs.

67 (G) Rate constants of fluorescent decay, comparing Coro1C-knockdown with control
68 MEFs, using alternative oligos to those presented in Fig. 5G. n=18.

69 (H) Rate constants of fluorescent decay, comparing dispersion of PAGFP-Rac1 from
70 protrusive membrane Coro1C-knockdown with control MEFs, including dispersion
71 from lateral membrane of control cells for comparison. n=18.
72 (I) Binding interface between Coro1C and GDP-Rac1 from *in silico* docking
73 experiments, demonstrating that interactions include Thr35 and Asp38, similar to the
74 RCC2/GDP-Rac1 complex.
75 (J) RCC2 coprecipitates with GFP-Coro1C and GFP-Coro1C-R31E with similar
76 efficiency, using a GFP-Trap from 293T cells. n=3
77 (K) Wild type and R31E Coro1C cosediment with freshly polymerized filamentous
78 actin at 150,000xg, whereas the previously characterized actin-binding mutant of
79 Coro1C, R28D/2xKE, does not.
80 (L-M) Competition between RCC2 and Coro1C for binding to Rac1. Pull down
81 assays from lysates of control, RCC2-knockdown or Coro1C-knockdown cells using
82 GST or GDP-loaded GST Rac1 as bait. n=6
83 (N-O) Knockdown of both RCC2 and Coro1C results in a morphology that resembles
84 RCC2 knockdown. Cells form multiple lamellae (arrowheads) on fibronectin, to
85 which both immunostained endogenous Rac1 and GFP-Rac1 localize. Images are
86 reproduced, in part, in Fig. S2B. n=70.
87 Error bars indicate s.e.m. Significance was tested by T-test, ** p<0.005. Bar = 10 μ m.
88

89 **Fig. S4. RCC2 and Coro1C regulate migration**

90 (A) MEFs spread on CDM with serum, fixed and stained for cortactin and
91 fibronectin. Images representative of 100 cells on 2 separate occasions. Bar = 10 μ m.
92 (B) Fluorescent decay curves compare redistribution of photoactivated GFP-tagged
93 Rac1 from lateral membrane between control and Coro1C-knockdown MEFs plated
94 on CDM. n=18.
95 (C) Schematic of how the angle of each step of a migration path was assessed to
96 calculate curvature of path.
97 (D) Cell outlines illustrating a migration sequence, red>yellow>green>cyan>blue to
98 show that control cells are processive, while RCC2-depleted cells shunt. Individual
99 frames derived from Movies S8-9.
100 (E) Lysates of control, RCC2, and Coro1C knockdown and rescued MEFs, used in
101 Fig. 7C-F) were blotted for RCC2 to confirm knockdown and rescue.

102 (F) Lateral views of control, Coro1C or RCC2 morphants, at 4 dpf.
103 (G) Confocal stacks at 32 hours post fertilisation of the neural crest reporter line: Tg(-
104 4.9sox10:EGFP)ba2 (Wada et al, 2005). Images are all of left-facing zebrafish heads,
105 anterior to top. 1=neural crest stream from which 1st arch skeletal elements will be
106 derived, 2= neural crest stream from which 2nd arch skeletal elements are derived, 1/2
107 indicates failed separation of these two streams. Fish were staged as 32 hpf by
108 reference to migration of sox10:GFP labelled pigment precursors in the trunk. Ctrl are
109 control morpholino injected (n=70), Mo only= Coro1C morpholino injected only (at
110 2ng per embryo)(n=41), Mo+mut RNA = Coro1C morpholino (2ng) in addition to
111 200 pg truncated Coro1C RNA (n=37), Mo+wt RNA= Coro1C morpholino (2ng) in
112 addition to 200 pg full-length Coro1C RNA (n=30). All embryos were injected
113 directly into the first cell at the 1-cell stage of development.
114 Error bars indicate s.e.m. Significance was tested by T-test, ** p<0.005.

115

116 **Movie 1. RCC2 and Coro1C regulate Rac1 localization and membrane**
117 **protrusion.** Control, RCC2 or Coro1C knockdown MEFs were transfected with
118 GFP-Rac1 and filmed on a confocal microscope for 3.5 hours at 1 frame every 3
119 minutes. Red dots indicate protrusion between consecutive frames. Movie frames
120 reproduced in Fig. 4B.

121

122 **Movie 2. Localization of Rac1 activation is perturbed in RCC2 or Coro1C**
123 **knockdown MEFs.** Rac1 activity distribution was detected using a Raichu-Rac
124 FRET probe in cells spread on 50K before addition of H/0 (white flash). A non-
125 activatable mutant probe (Y40C) was used as a control to confirm that changes in
126 FRET signal are caused by changes in Rac1 activity, not relocalization. Movie
127 captured at 1 frame every 2 minutes, for 11 minutes prior to, and up to 49 minutes
128 after stimulation. Images are false-colored for FRET intensity. Movie frames
129 reproduced in Fig. 4C.

130

131 **Movie 3. Release of photoactivated GFP-tagged Rac1 from the membrane is**
132 **delayed in the absence of Coro1C expression.** PAGFP was photoactivated in a
133 1.5x1.5 µm or 1.5x4.5 µm box at the lateral edge of cells spread on fibronectin and
134 release of Rac1 followed by decay of GFP fluorescence. Fixed cells (no diffusion),
135 control MEFs (large and small boxes), Coro1C-depleted MEFs (large and small

136 boxes) and Coro1C-depleted MEFs using a -CAAX mutant Rac1 (no association of
137 PAGFP-Rac1 with membrane) were analyzed. Images are false-colored for
138 fluorescence intensity. Images were captured at 2 images per second for 5 seconds
139 prior to, and 15 seconds after photoactivation, and displayed at 2 frames per second.
140 Movie frames reproduced in Fig. 5F.

141

142 **Movie 4. Retrafficking of Rac1 from lateral to protrusive membrane is reliant on**
143 **Coro1C.** PAGFP-Rac1 was photoactivated in boxes at the lateral edge of control or
144 Coro1C KD cells spread on fibronectin, and arrival at protrusive membrane recorded.
145 Images are false-colored for fluorescence intensity. Images were captured at 1 image
146 per minute for 10 minutes following photoactivation and displayed a 1 frame per
147 second. Movie frames reproduced in Fig. 5H.

148

149 **Movie 5. Retrafficking of Coro1C from lateral to protrusive membrane.** PAGFP-
150 Coro1C was photoactivated by 3 pulses within 1 minute at boxes on the lateral
151 membrane of cells spread on fibronectin, and arrival at protrusive membrane
152 recorded over 10 minutes at 1 image per minute for 10 minutes following
153 photoactivation. Movie frames reproduced in Fig. 5I.

154

155 **Movie 6. RCC2 and Coro1C expression are necessary for processive migration.**
156 Control, RCC2 and Coro1C knockdown and *Sdc4* *-/-* MEFs migrating through a cell-
157 derived matrix. Movie captured with a 5x lens at 1 image every 10 minutes for 10
158 hours, displayed at 5 frames per second, bar = 100 μm .

159

160 **Movie 7. RCC2 and Coro1C expression are necessary for processive migration.**
161 Control, RCC2 and Coro1C knockdown MEFs migrating through a cell-derived
162 matrix. Movie captured with a 40x lens at 1 image every 10 minutes for 10 hours,
163 displayed at 5 frames per second, bar = 10 μm .

164

165 **Movie 8. Shunting migration of RCC2 knockdown MEFs is not due to a tail**
166 **retraction defect.** β_1 -integrin-GFP-expressing MEFs transfected with control oligos
167 were filmed migrating through a cell-derived matrix to allow the rearmost attachment
168 point to be seen. Movie captured at 1 frame every 10 minutes, displayed at 6 frames
169 per second.

170

171 **Movie 9. Shunting migration of RCC2 knockdown MEFs is not due to a tail**
172 **retraction defect.** β_1 -integrin-GFP-expressing MEFs transfected with RCC2-targeted
173 antisense oligo were filmed migrating through a cell-derived matrix to allow the
174 rearmost attachment point to be seen. Movie captured at 1 frame every 10 minutes,
175 displayed at 6 frames per second.

176

177 **Movie 10. RCC2 and Coro1C expression are necessary for processive migration.**
178 Control, RCC2 and Coro1C knockdown MEFs migrating along 5- μm fibronectin
179 stripes. Movie captured at 1 image every 10 minutes for 6.5 hours, displayed at 3
180 frames per second, bar = 50 μm .

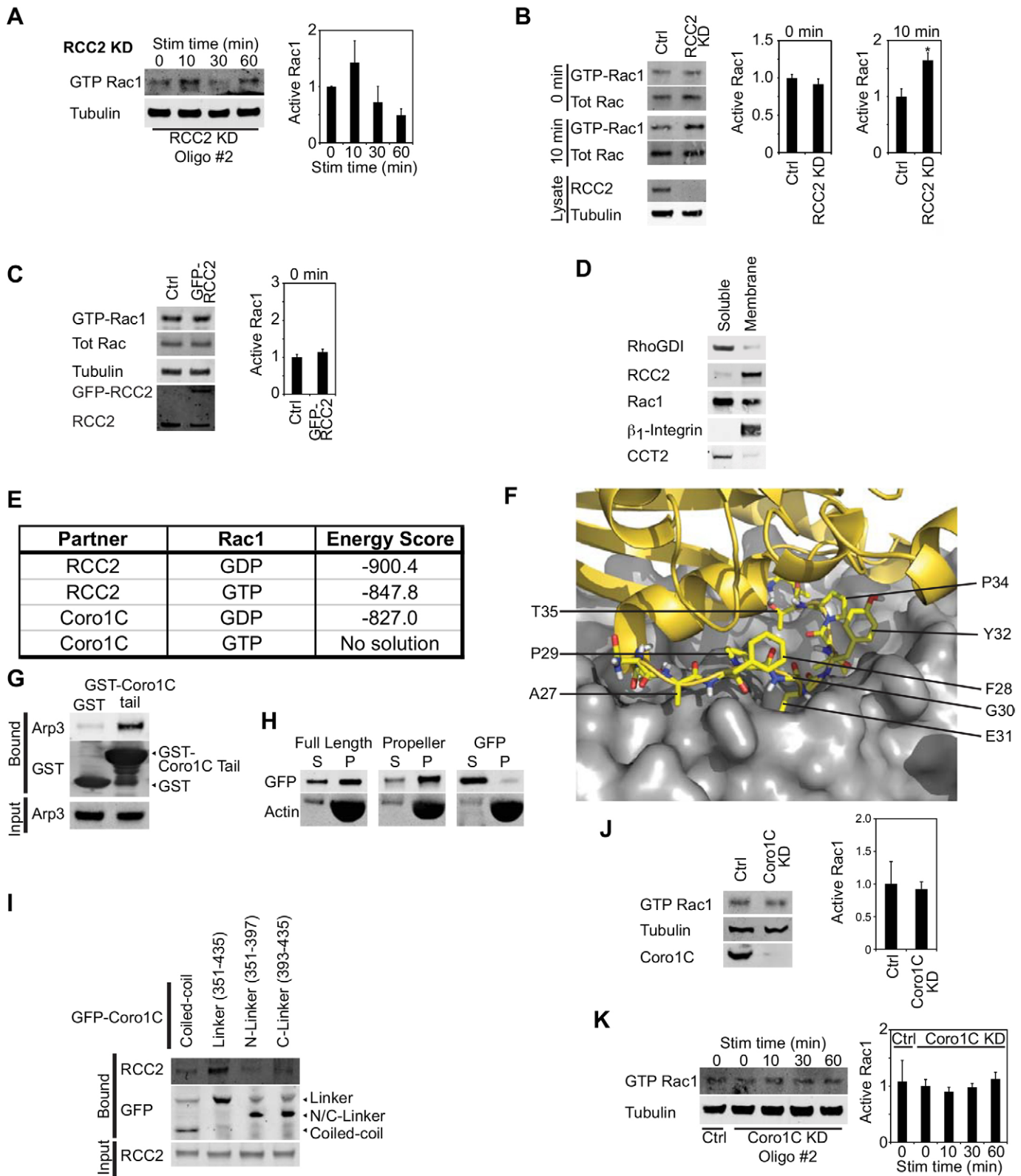


Figure S1.

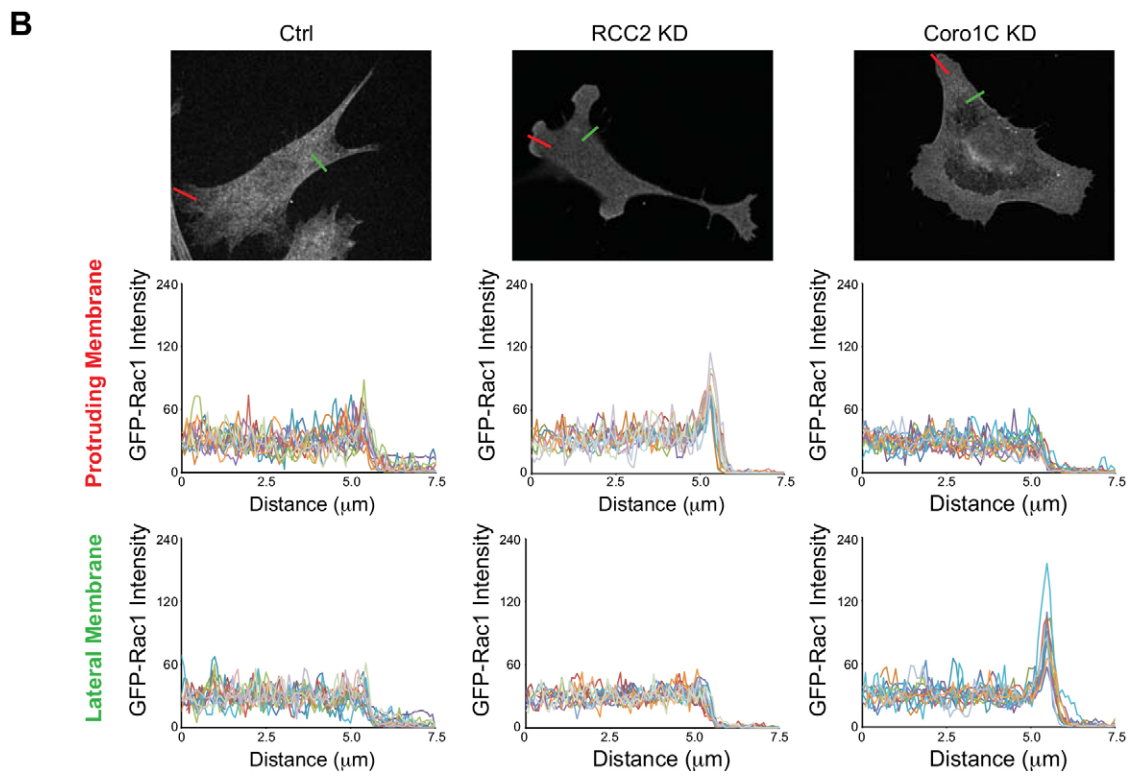
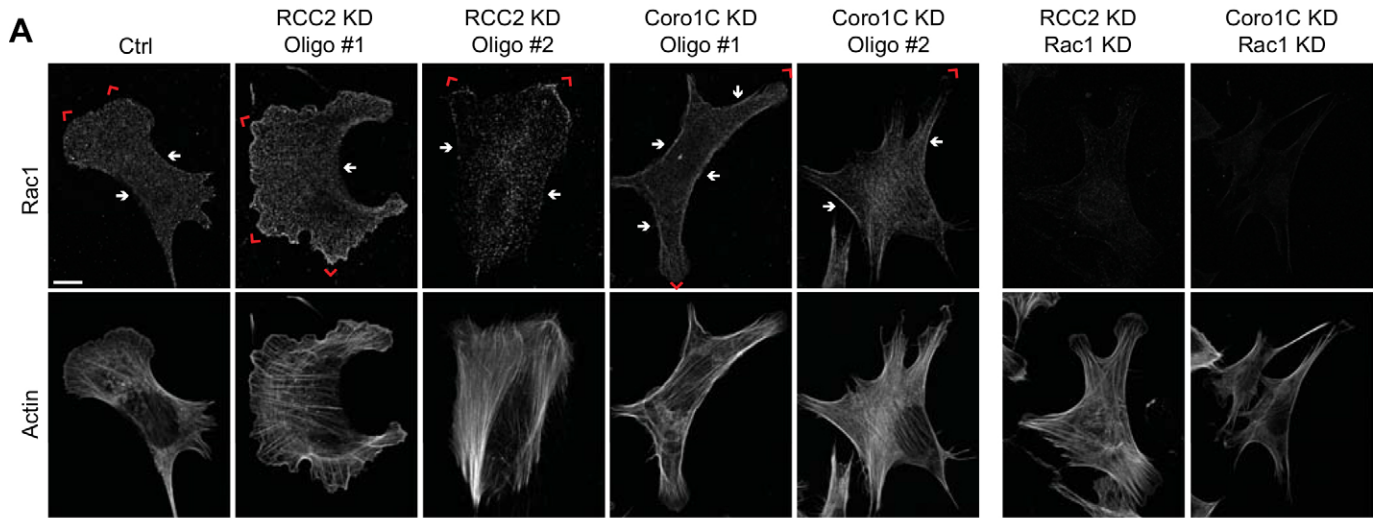


Figure S2.

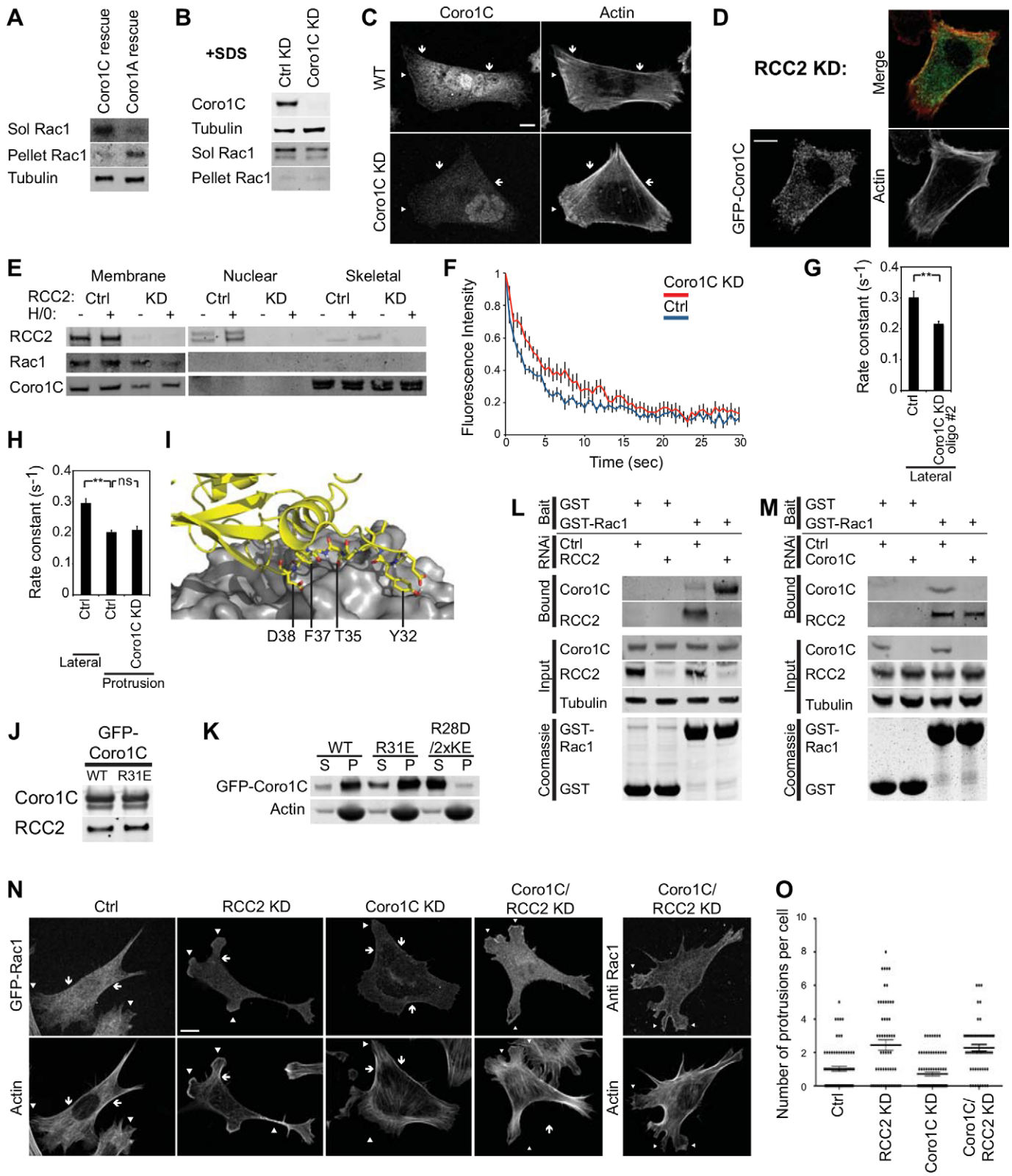


Figure S3.

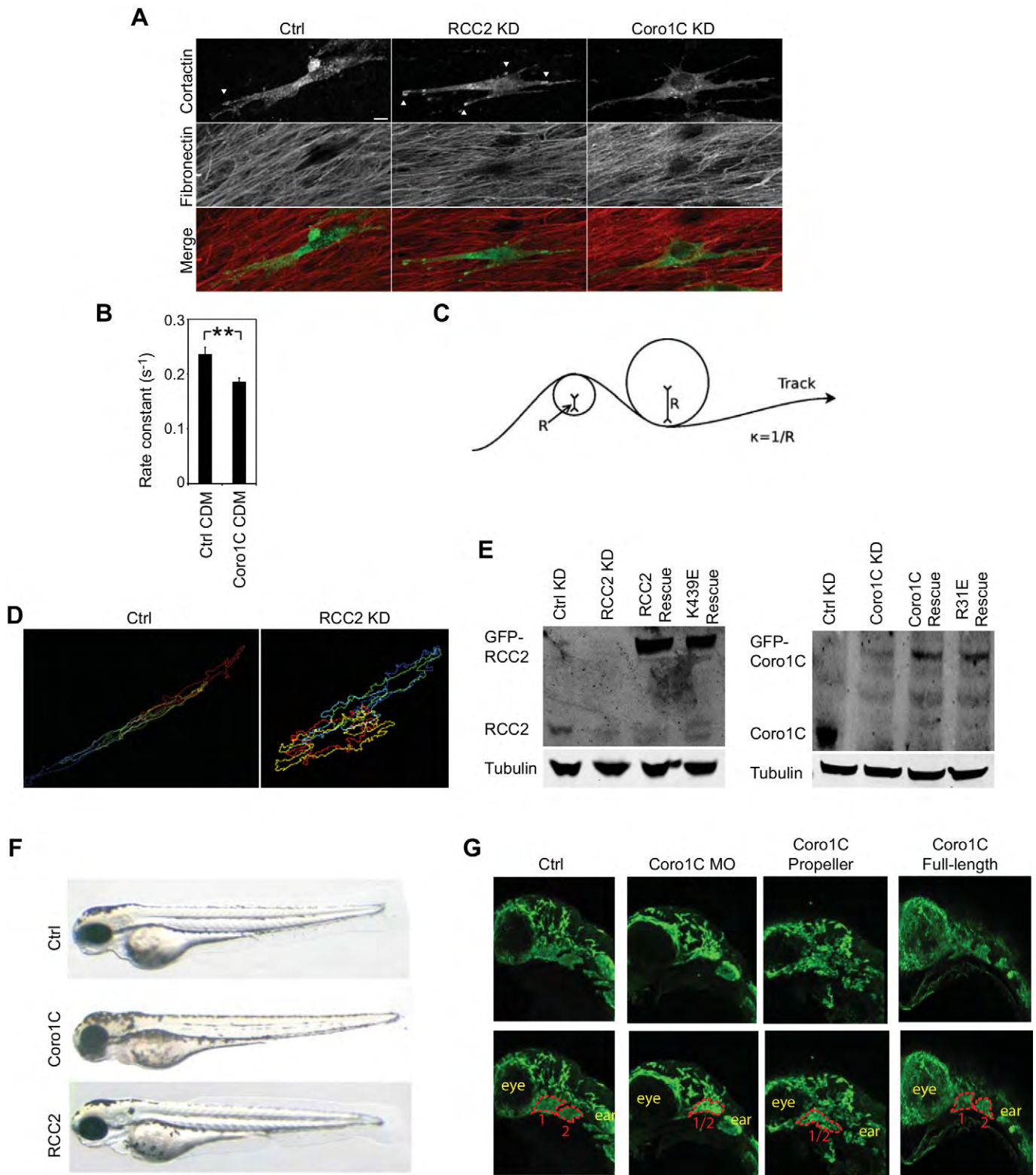
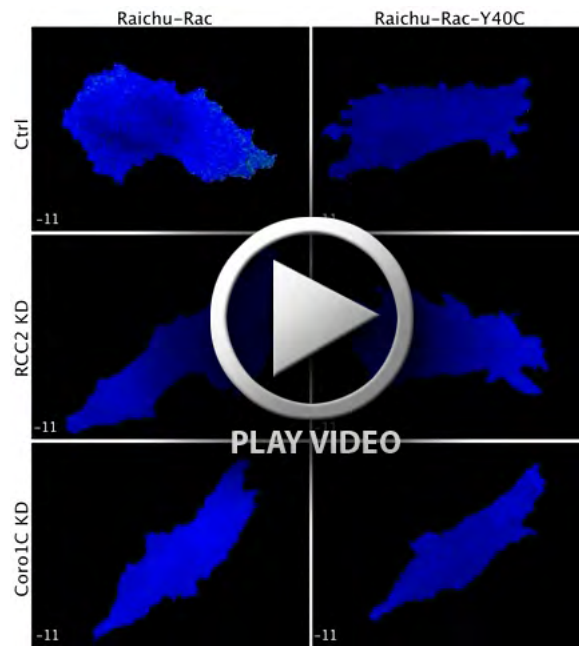


Figure S4.



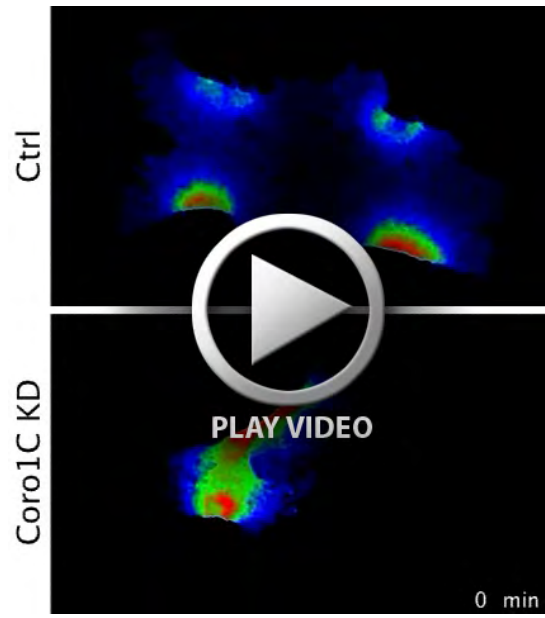
Movie 1.



Movie 2.



Movie 3.



Movie 4.



Movie 5.



Movie 6.



Movie 7.



Movie 8.



Movie 9.



Movie 10.