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online data

SUPPLEMENTARY ONLINE DATA

Coronin 1C harbours a second actin-binding site that confers co-operative binding to F-actin

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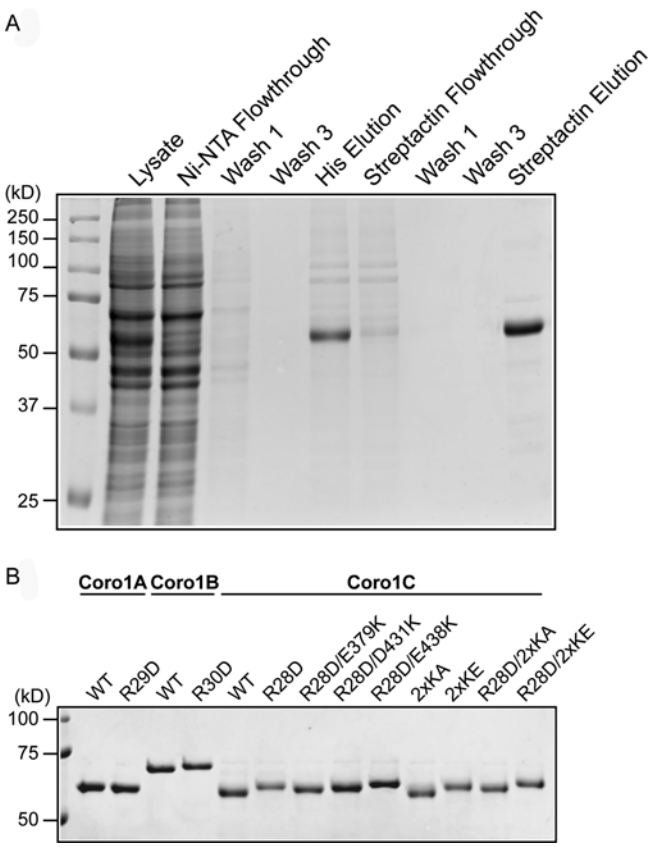

Figure S1 Purification of mammalian Coro1 proteins

(**A**) Coomassie Blue-stained gel shows purification steps for recombinant Coro1C, which contains a C-terminal Strep–His_8 tag. (**B**) Purified wild-type (WT) and mutant coronin proteins (0.5 μg) used for *in vitro* studies were separated by SDS/PAGE and subjected to Coomassie Blue staining. Molecular masses are shown in kDa on the left-hand side. Ni-NTA, Ni^{2+}-nitrilotriacetate.

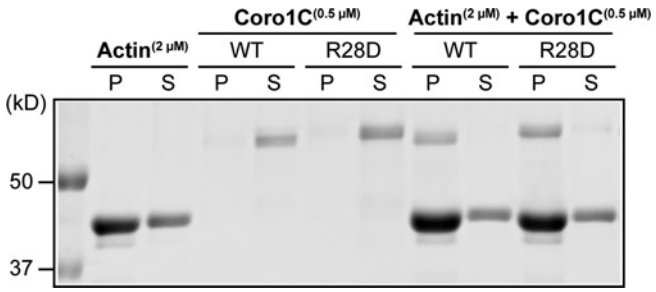

Figure S2 Actin cosedimentation control for Coro1C and Coro1C–R28D

Cosedimentation assays were performed using 0.5 μM Coro1C or Coro1C–R28D with and without 2 μM actin. Pellet (P) and supernatant (S) fractions were analysed by SDS/PAGE and subjected to Coomassie Blue staining. Molecular masses are shown in kDa on the left-hand side. WT, wild-type.

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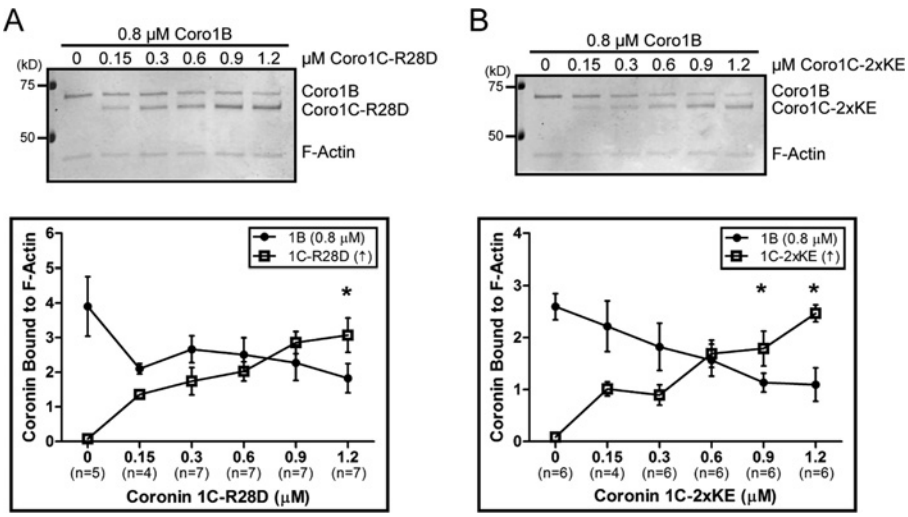

Figure S3 Coro1B competes with Coro1C–R28D and Coro1C–2×KE for binding to F-actin

Coro1B (0.8 μM) was incubated with actin (0.3 μM) for 1 h followed by incubation with increasing concentrations of Coro1C–R28D (**A**) or Coro1C–2×KE (**B**). Actin cosedimentation was performed and pellet fractions were subjected to SDS/PAGE and Coomassie Blue staining. Quantification of the relative amount of Coronin bound to F-actin from the indicated number of independent experiments (*n*) is shown as means ± S.E.M. $^*P < 0.05$ determined by one-way ANOVA as compared with the initial concentration of coronin. Molecular masses are shown in kDa on the left-hand side of the gels.

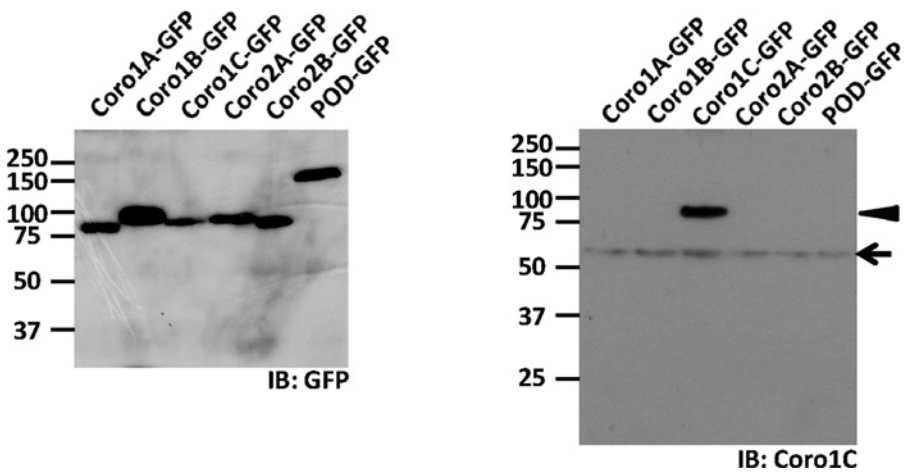

Figure S4 Specificity of anti-Coro1C antibody

(**A**) Lysates of HEK (human embryonic kidney)-293FT cells expressing GFP fusions of Coro1A, Coro1B, Coro1C, Coro2A, Coro2B or POD (polarity-osmotic defective) were separated by SDS/PAGE and analysed by immunoblotting (IB) with anti-GFP or anti-Coro1C antibodies. Arrow indicates endogenous Coro1C and arrowhead denotes Coro1C–GFP. Molecular masses are shown in kDa on the left-hand side.

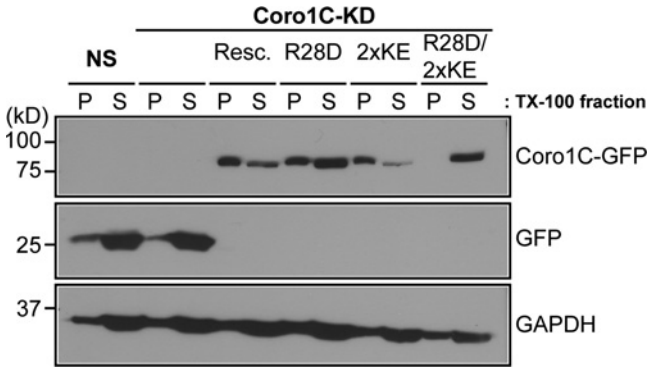

Figure S5 Analysis of Coro1C association with the detergent-insoluble cytoskeleton

IA32 mouse fibroblasts were infected with lentivirus expressing NS shRNA (GFP), Coro1C shRNA (Coro1C-KD) or Coro1C shRNA and re-expressing Coro1C–GFP (resc.) or R28D, 2×KE, R28D/2×KE mutants. Cells were lysed in RIPA buffer containing 1% Triton X-100 (TX-100) and subjected to centrifugation at 14000 ***g*** for 10 min. Detergent-insoluble pellets (P) and supernatants (S) were separated by SDS/PAGE and analysed by immunoblotting with an anti-GFP antibody. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was probed as a loading control. Molecular masses are shown in kDa on the left-hand side.

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