Supplemental Figures

Figure S1. In vitro interaction analysis of RhoA and a Rho-binding-defective mDia2 mutant. GST fusion proteins of wild type or V180D mDia2 GBD-CC (71-533) were incubated with GSH-Sepharose beads and the recombinant RhoA-GDP or RhoA-GTP□S. Bound RhoA was then analyzed by SDS-PAGE and CBB staining (upper panel) or immunoblotting with an anti-RhoA anitibody (lower panel). Experiments were performed as described in the Material and Methods.

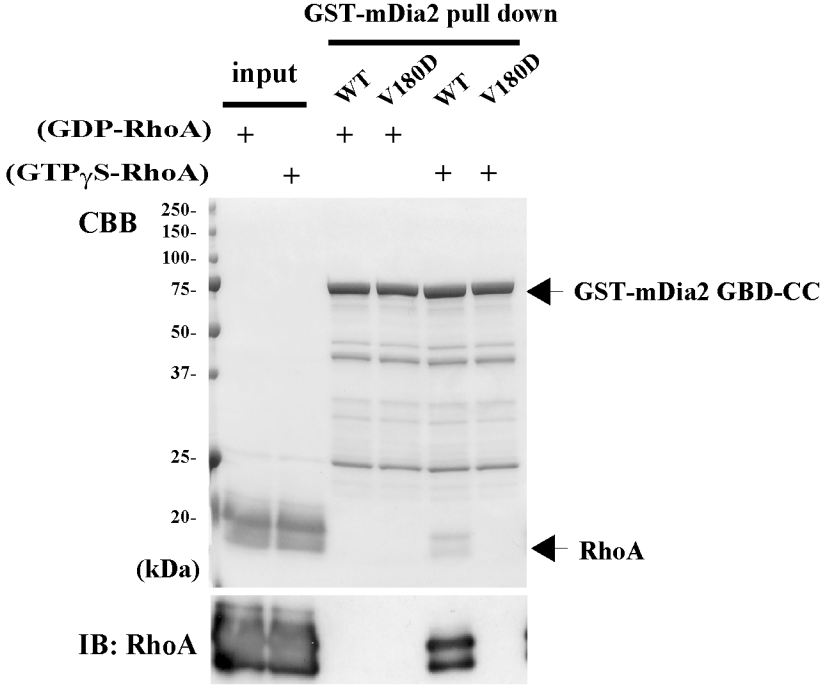
Figure S2. (A) Attenuated mDia2 localization in the cleavage furrow of Ect2-RNAi cells. NIH 3T3 cells were synchronized and treated for 36 h with control siRNA or siRNA specific for Ect2. The cells were then fixed and stained for microtubules (green), DNA (blue), and mDia2 (red). (B) Quantitative analysis of the mDia2 signal at the cleavage furrow in Ect2-RNAi cells. Experiments were performed as described in Figure S2A. The signal intensities of mDia2 at the cleavage furrow were quantified using MetaMorph software as described in the Materials and Methods. The results are from two independent experiments, in each of which N>7 cells were examined. *, $p<0.05$. (C) Depletion of anillin diminished the localization of mDia2 at the cleavage furrow in HT1080 cells. Immunofluorescence staining for mDia2 in HT1080 cells treated for 48 h with control siRNA or siRNA specific for anillin. The cells were then fixed and stained for microtubules (green), DNA (blue), and mDia2 (red). An arrow indicates the accumulation of mDia2 at the central spindle. Bar, 5 □m

Figure S3. (A) Biacore analysis of the binding of recombinant anillin and mDia proteins. Recombinant mDia2N (top) or mDia3N (bottom) proteins at different concentrations were infused over a GST-anillinN-captured sensor chip surface on a Biacore T100 instrument. Y-axis values are shown in response units (RU) as the difference between experimental and control GST-anillinN-uncaptured cells. (B) Fluorescence polarization analysis of the competition by anillin in binding of the DAD domain of mDia2 and the ARR-CC domain of mDia3. FITC-labelled mDia2-DAD peptides (1036-1051) were sequentially mixed with indicated concentrations of recombinant mDia3 and anillin, and the fluorescence polarization were measured with a PerkinElmer EnVision multilabel plate reader. An arrowhead indicates the addition of mDia3 ARR/DID-CC, and an arrow indicates the addition of anillinN. The results represent the average value from two independent experiments.

Figure S4. Anillin-GFP expression is able to rescue the loss of septin 7 at the cleavage furrow induced by depletion of anillin. (A and B) NIH 3T3 cells were synchronized and transfected with control siRNA or siRNAanillin#1 together with either RNAi-resistant anillin#1r-full-GFP or anillin#1r-(115-1086)-GFP. (A) The cells were synchronized in mitotic phase, and the cells were fixed and stained for septin 7 (upper panels) and GFP (lower panels). Bar, 5 □m. (B)

Quantitative analysis of the septin 7 signal at the cleavage furrow. Experiments were performed as described in Figure S4A. The signal intensities of septin 7 at the cleavage furrow were quantified using MetaMorph software as described in the Materials and Methods. The results are from two independent experiments, in each of which N>10 cells were examined. *, $p<0.05$. (C) Western blot analysis of NIH 3T3 cell lysates with anti-anillin and anti-septin 7 antibodies. NIH 3T3 cells were synchronized and transfected with control siRNA or siRNAanillin#1 for 36 h, and the cell lysates were subjected to Western bloting with anti-anillin (left) and anti-septin 7 (right). Positions of molecular mass markers are shown. Asterisks indicate predicted sizes of anillin and septin 7.

Figure S5. Localization of a series of anillin-GFP after progression through mitotic phase in the anillin-RNAi rescue experiment. NIH 3T3 cells were synchronized with thymidine and sequentially transfected with siRNAanillin#1 and then with either empty GFP vector or a series of RNAi-resistant anillin#1r-GFP constructs (full length, 115-1086, 600-1086, or 1-91+600-1086). The transfected cells were washed and released for 10 h, and the cells were then stained for GFP (green), microtubules (red), and DNA (blue). Arrows indicate the localization of the anillin-GFP at stress fibers. Arrow heads indicate the ectopic cortical foci containing the anillin-GFP. Bar, 20 □m.



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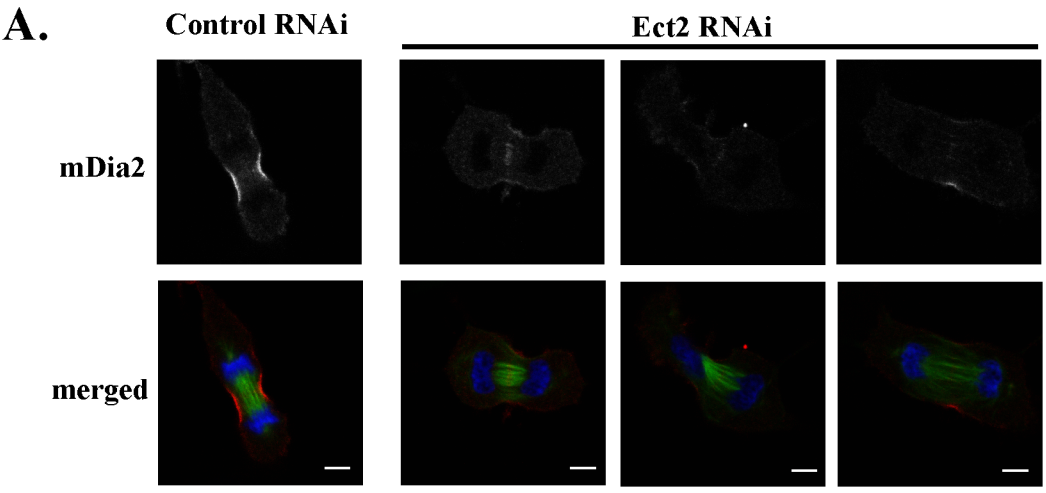
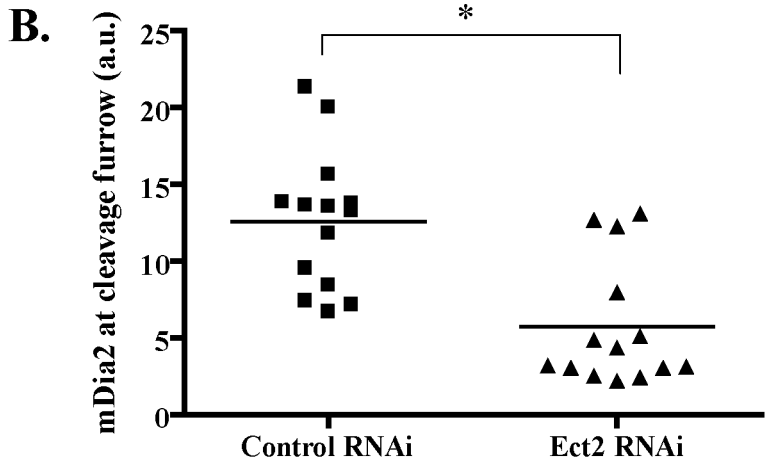
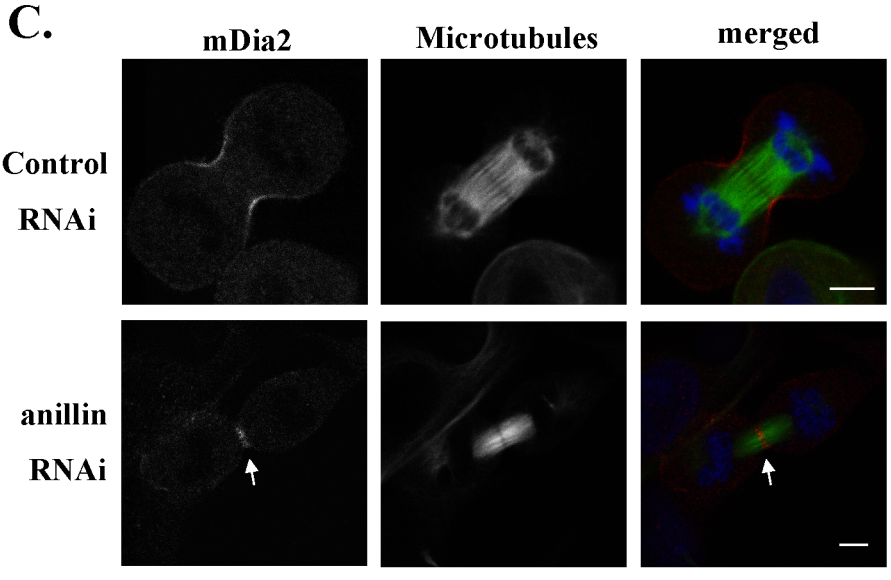
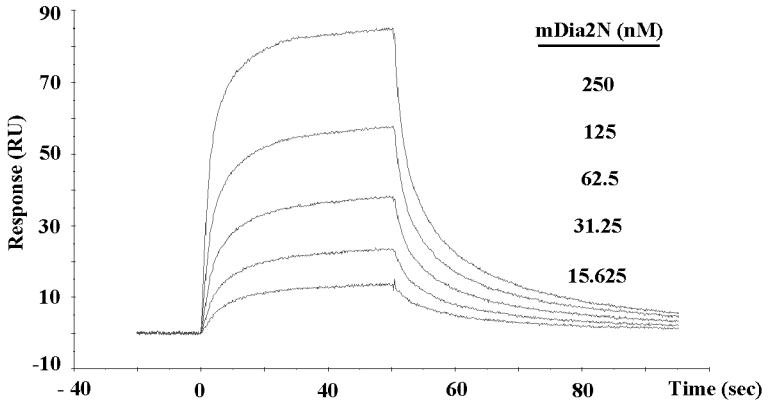


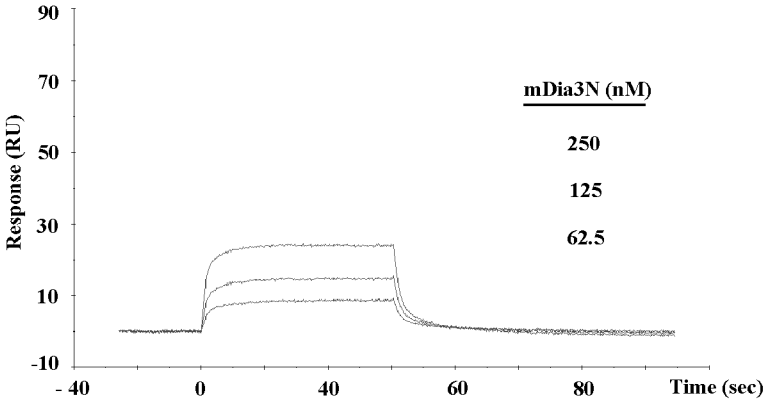




Watanabe S. et al. Figure S2

A.





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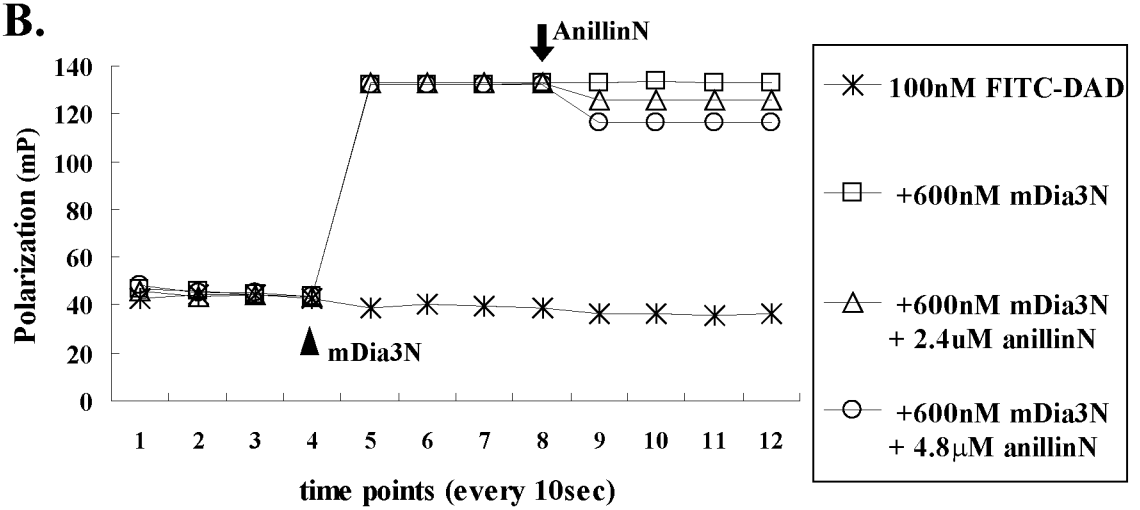
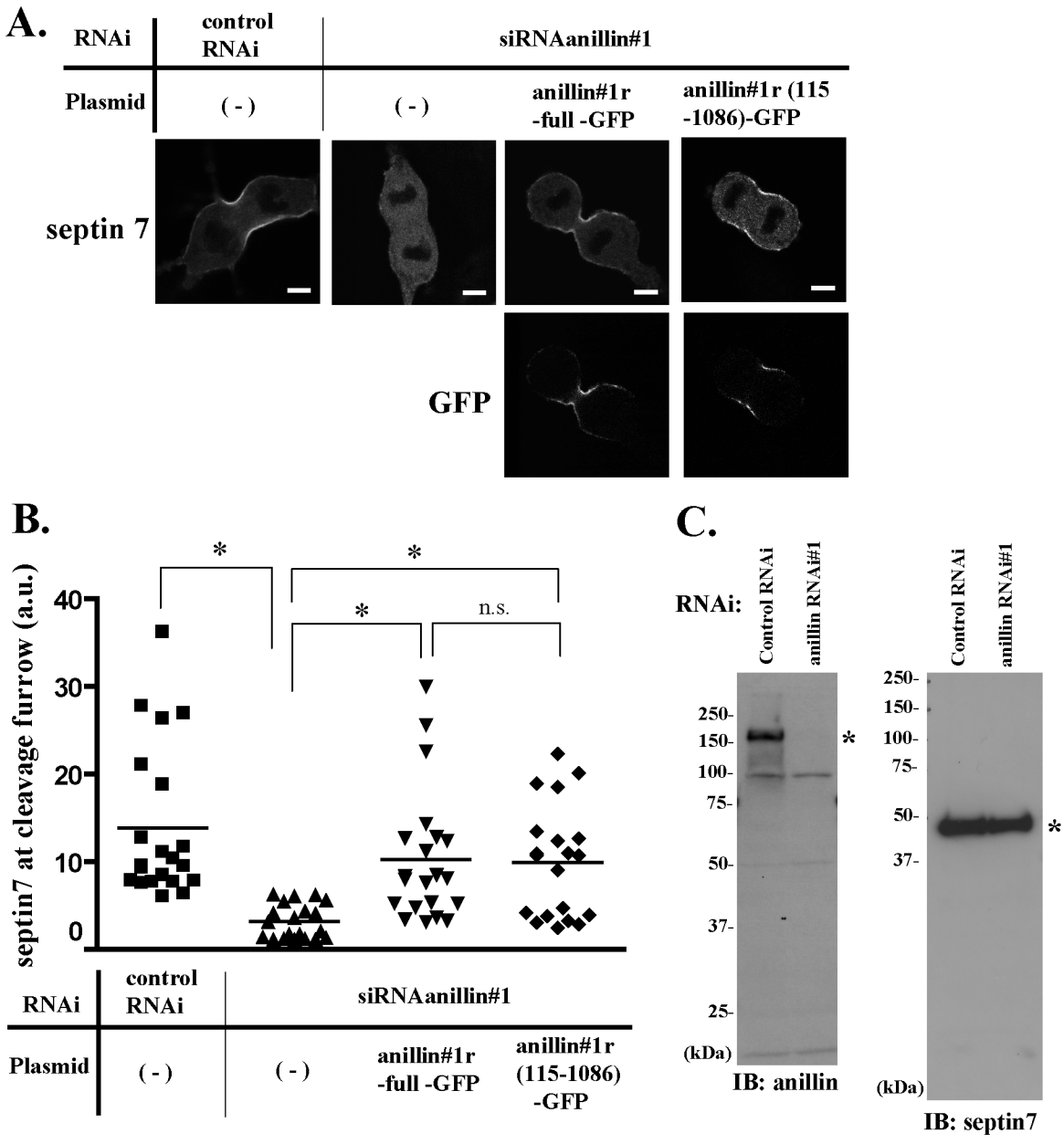
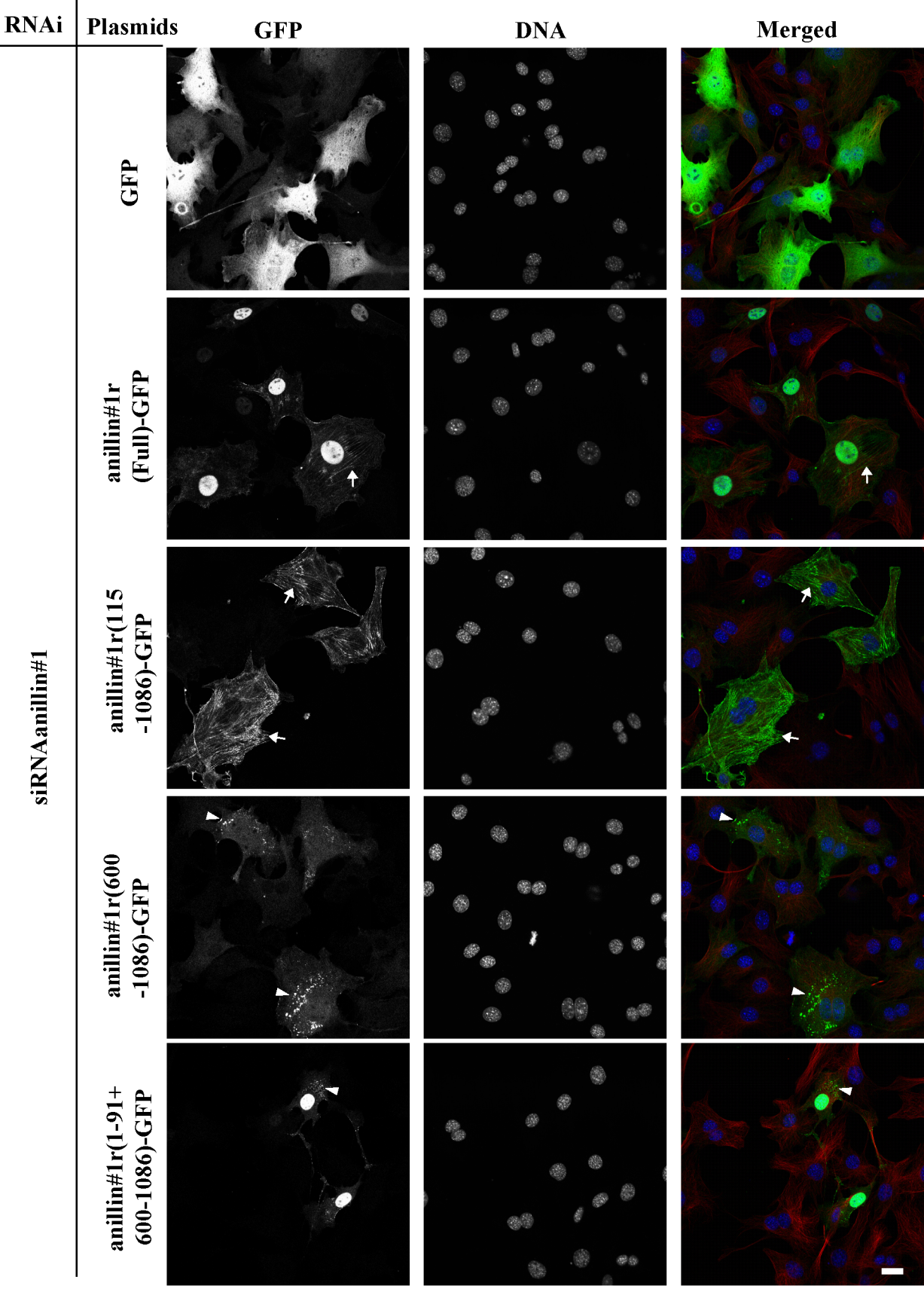


Watanabe S. et al. Figure S3



Watanabe S.et al. Figure S4



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