Supporting Information

Furry protein suppresses nuclear localization of yes-associated protein (YAP) by activating NDR kinase and binding to YAP

Kazuki Irie [1], Tomoaki Nagai [1], and Kensaku Mizuno [1,2,*]

From the [1]Department of Molecular and Chemical Life Sciences, Graduate School of Life Sciences, Tohoku University, Aobayama, Sendai, Miyagi 980-8578, Japan; [2]Institute of Liberal Arts and Sciences, Tohoku University, Kawauchi, Sendai, Miyagi 980-8576, Japan

*To whom correspondence should be addressed: Kensaku Mizuno: Department of Molecular and Chemical Life Sciences, Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi 980-8578, Japan; E-mail: kmizuno@biology.tohoku.ac.jp; Tel. (81)-22-795-6590, Fax. (81)-22-795-6594.

This PDF file includes:
Figure S1 to S4

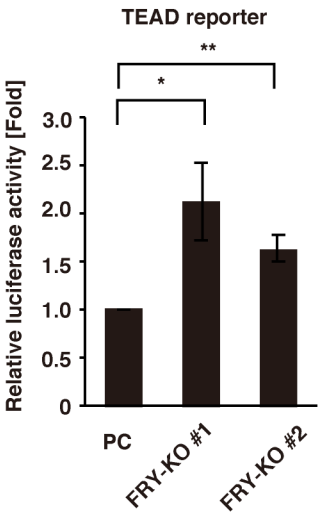

Fig. S1 FRY depletion promotes YAP/TAZ co-transcriptional activity. The parental and FRY-KO HEK293A cells were transfected with the plasmids for YAP/TAZ-responsive TEAD reporter (8xGTIIC-luciferase) and the control plasmids for the SV40 promoter driving Renilla luciferase. Cell extracts were subjected to the luciferase reporter assays. The level of firefly luciferase activity (YAP activity) was normalized to the level of Renilla luciferase activity. Data are the means ± SD from four independent experiments. Statistical analysis included one-way ANOVA followed by Dunnett' s test. *, $p < 0.05$, **, $p < 0.01$.

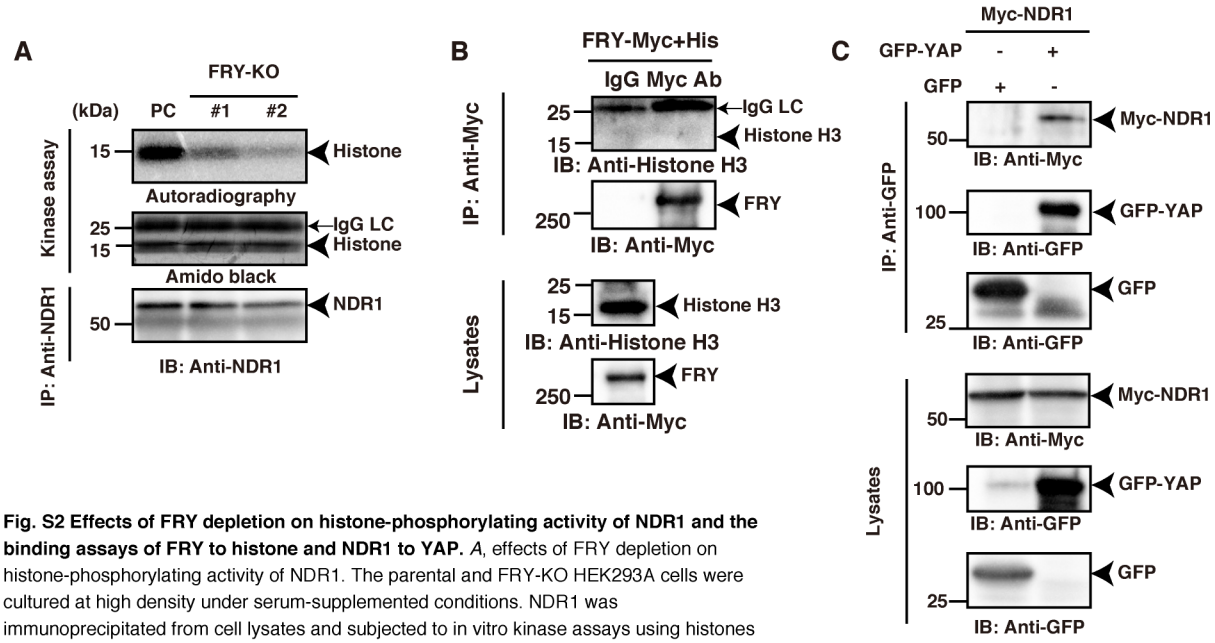

Fig. S2 Effects of FRY depletion on histone-phosphorylating activity of NDR1 and the binding assays of FRY to histone and NDR1 to YAP. *A*, effects of FRY depletion on histone-phosphorylating activity of NDR1. The parental and FRY-KO HEK293A cells were cultured at high density under serum-supplemented conditions. NDR1 was immunoprecipitated from cell lysates and subjected to in vitro kinase assays using histones as substrates. IgG LC, immunoglobulin light chain. *B*, co-precipitation assay of histone with FRY. HEK293T cells were transfected with FRY-(Myc+His). Cell lysates were immunoprecipitated using an anti-Myc antibody and the precipitates were immunoblotted using anti-histone H3 and anti-Myc antibodies. *C*, co-precipitation assay of NDR1 with YAP. HEK293T cells were transfected with Myc-tagged NDR1 and GFP-tagged YAP. Cell lysates were immunoprecipitated using an anti-GFP antibody and the precipitates were immunoblotted using anti-GFP and anti-Myc antibodies.

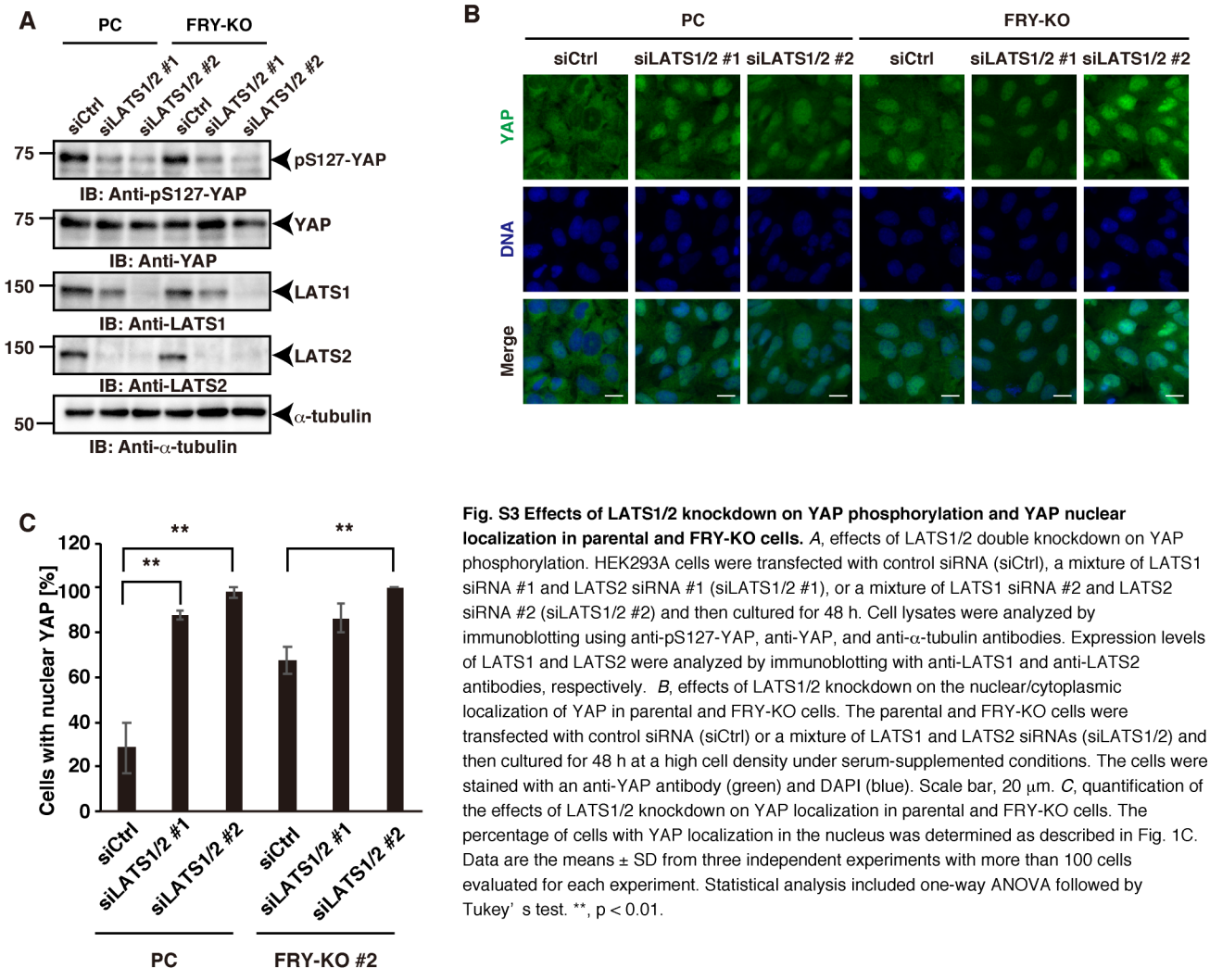

Fig. S3 Effects of LATS1/2 knockdown on YAP phosphorylation and YAP nuclear localization in parental and FRY-KO cells. *A*, effects of LATS1/2 double knockdown on YAP phosphorylation. HEK293A cells were transfected with control siRNA (siCtrl), a mixture of LATS1 siRNA #1 and LATS2 siRNA #1 (siLATS1/2 #1), or a mixture of LATS1 siRNA #2 and LATS2 siRNA #2 (siLATS1/2 #2) and then cultured for 48 h. Cell lysates were analyzed by immunoblotting using anti-pS127-YAP, anti-YAP, and anti-α-tubulin antibodies. Expression levels of LATS1 and LATS2 were analyzed by immunoblotting with anti-LATS1 and anti-LATS2 antibodies, respectively. *B*, effects of LATS1/2 knockdown on the nuclear/cytoplasmic localization of YAP in parental and FRY-KO cells. The parental and FRY-KO cells were transfected with control siRNA (siCtrl) or a mixture of LATS1 and LATS2 siRNAs (siLATS1/2) and then cultured for 48 h at a high cell density under serum-supplemented conditions. The cells were stained with an anti-YAP antibody (green) and DAPI (blue). Scale bar, 20 μm. *C*, quantification of the effects of LATS1/2 knockdown on YAP localization in parental and FRY-KO cells. The percentage of cells with YAP localization in the nucleus was determined as described in Fig. 1C. Data are the means ± SD from three independent experiments with more than 100 cells evaluated for each experiment. Statistical analysis included one-way ANOVA followed by Tukey's test. **, $p < 0.01$.

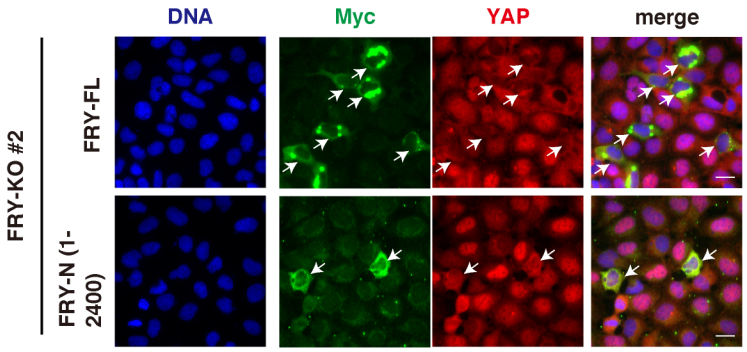

Fig. S4 Effects of expression of FRY-FL and FRY-N(1-2400) on YAP nuclear localization in FRY-KO cells. The Fry-KO HEK293A cells were transfected with (Myc+His)-tagged FRY-FL or FRY-N(1-2400) and then fixed and stained using anti-Myc (green) and anti-YAP antibodies (red). DNA was stained using DAPI (blue). Arrows indicate the Myc-positive cells. Scale bar, 20 μm.