

Expanded View Figures

Figure EV1. Finding novel regulators of Hippo signaling by analyzing the TCGA database.

(A) List of the top fifteen differentially expressed genes with poor prognosis at high expression levels in stomach adenocarcinoma (STAD). Genes with a high correlation with YAP target genes (*CTGF* and *CYR61*) are highlighted in light yellow. Genes with increased expression in tumors are highlighted in apricot. Genes corresponding to both of the above conditions are highlighted in yellow. (B) Kaplan–Meier plots show that high expression of *RAI14* in gastric cancer patients is associated with poor prognosis. The upper 50th percentile ($n = 189$) and the lower 50th percentile ($n = 189$) were analyzed in gastric cancer patients. HR, hazard ratio. (C) The violin plot shows a high expression of *RAI14* in gastric tumor samples. The Y-axis indicates the log base 2 of *RAI14* expression. The analysis included normal samples from the GTEx database ($n = 172$) and the TCGA database ($n = 36$) as well as tumor samples from the TCGA database ($n = 414$). (D) The mRNA expression of gastric cancer patients ($n = 450$) from the TCGA database shows a positive correlation between *RAI14* and YAP-target genes (*CTGF* and *CYR61*). Data information: In (B), the hazard ratio (HR) was calculated using the Mantel–Haenszel method. In (D), the correlation coefficient (r) was calculated using Pearson's linear correlation. Statistical analysis was performed using log-rank test (B) and two-tailed unpaired t test (C), or calculating right-tailed F probability distribution (D), and exact P values are shown in each figure; $P < 0.05$, statistically significant. Source data are available online for this figure.

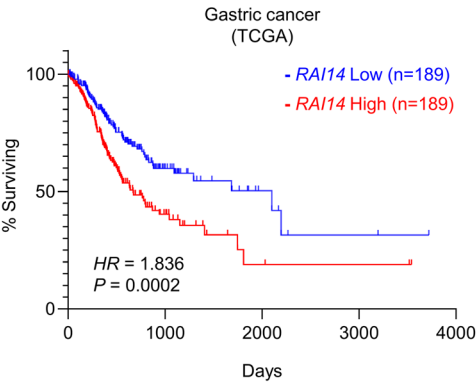
A

Top15 differential Survival Genes in STAD (gastric cancer)

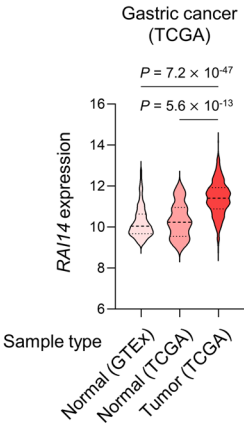
RANK	Gene name	Gene ID	P-Value (Survival os)	Prognosis	Correlation with CTGF (Pearson correlation R)	Correlation with CYR61 (Pearson correlation R)	Increased expression in tumor?
1	GFAP	ENSG00000131095.11	2.12×10^{-5}	unfavorable	0.23	0.2	
2	RP11-497E19.1	ENSG00000205562.2	2.33×10^{-5}	unfavorable	0.28	0.3	
3	ASPA	ENSG00000108381.10	2.69×10^{-5}	unfavorable	0.42	0.36	
4	SERPINE1	ENSG00000106366.8	3.41×10^{-5}	unfavorable	0.39	0.43	O
5	ZNF883	ENSG00000228623.3	3.59×10^{-5}	unfavorable	-0.026	-0.0087	
6	AOC4P	ENSG00000260105.6	3.86×10^{-5}	unfavorable	0.36	0.31	
7	CBLN4	ENSG00000054803.3	3.97×10^{-5}	unfavorable	0.11	0.15	
8	NT5E	ENSG00000135318.11	4.74×10^{-5}	unfavorable	0.082	0.044	O
9	AC002480.3	ENSG00000232759.1	6.37×10^{-5}	unfavorable	0.062	0.11	
10	MEI4	ENSG00000269964.2	6.39×10^{-5}	unfavorable	0.065	0.054	
11	CTD-2054N24.2	ENSG00000259363.5	6.93×10^{-5}	unfavorable	0.3	0.24	
12	RP11-1069G10.2	ENSG00000259727.1	9.09×10^{-5}	unfavorable	0.23	0.18	
13	EMX2OS	ENSG00000229847.8	9.24×10^{-5}	unfavorable	0.16	0.16	
14	ZNF192P1	ENSG00000226314.7	9.45×10^{-5}	unfavorable	0.023	-0.0096	
15	RAI14	ENSG00000039560.13	9.67×10^{-5}	unfavorable	0.49	0.4	O

- Genes having a high correlation with CTGF, CYR61 (R > 0.3)
- Genes with increased expression in tumors
- Genes having a high correlation with CTGF, CYR61 and increased expression in tumors

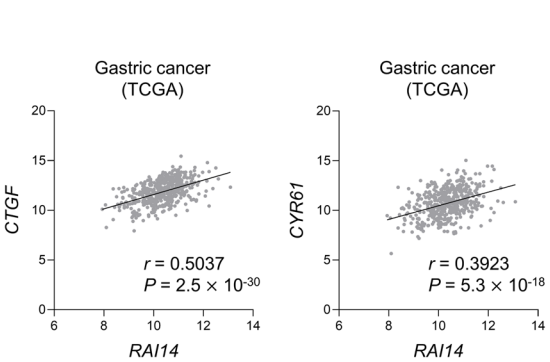
B



C



D



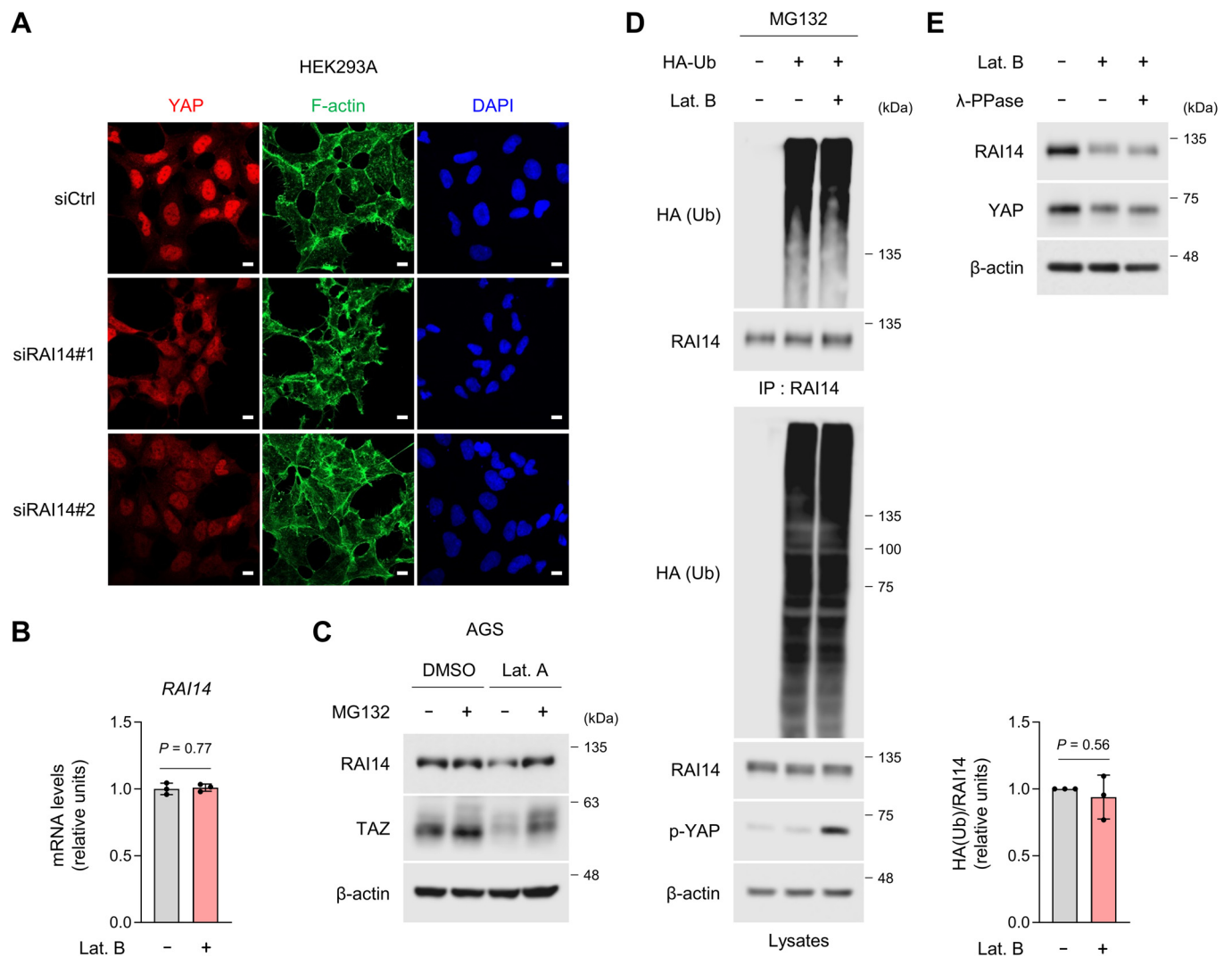


Figure EV2. Destabilization of F-actin induces proteasomal degradation of RAI14.

(A) Marginal effect of RAI14 knockdown on F-actin formation. HEK293A cells were used. For F-actin staining, phalloidin was used. Scale bars: 10 μ m. (B) Destabilization of F-actin by Latrunculin B (Lat. B) does not reduce RAI14 mRNA expression. HEK293A cells were treated with DMSO or Lat. B (2 μ M) for 6 h. (C) F-actin destabilization-mediated downregulation of RAI14 is dependent on the proteasomal degradation pathway. AGS cells were treated with Lat. A (2 μ M) and MG132 (25 μ M) for 6 h as indicated in the figure. (D) Destabilization of F-actin does not induce polyubiquitination of RAI14. HEK293A cells were transfected and treated with Lat. B (2 μ M) and MG132 (25 μ M) for 6 h as indicated in the figure. The HA(Ub)/RAI14 ratio from three immunoblot bands was quantified. (E) The mobility shift of RAI14 by F-actin destabilization is due to its phosphorylation. HEK293A cells were treated with Lat. B for 6 h and lambda phosphatase (λ -PPase) according to the manufacturer's instructions. Data information: In (B, D), the error bars indicate $\pm$ s.d. of triplicate measurements (B is technical replicates, and D is biological replicates). Statistical analysis was performed using two-tailed unpaired *t* test, and exact *P* values are shown in each figure; *P* < 0.05, statistically significant. Black dots on the graphs indicate individual measurements. Source data are available online for this figure.

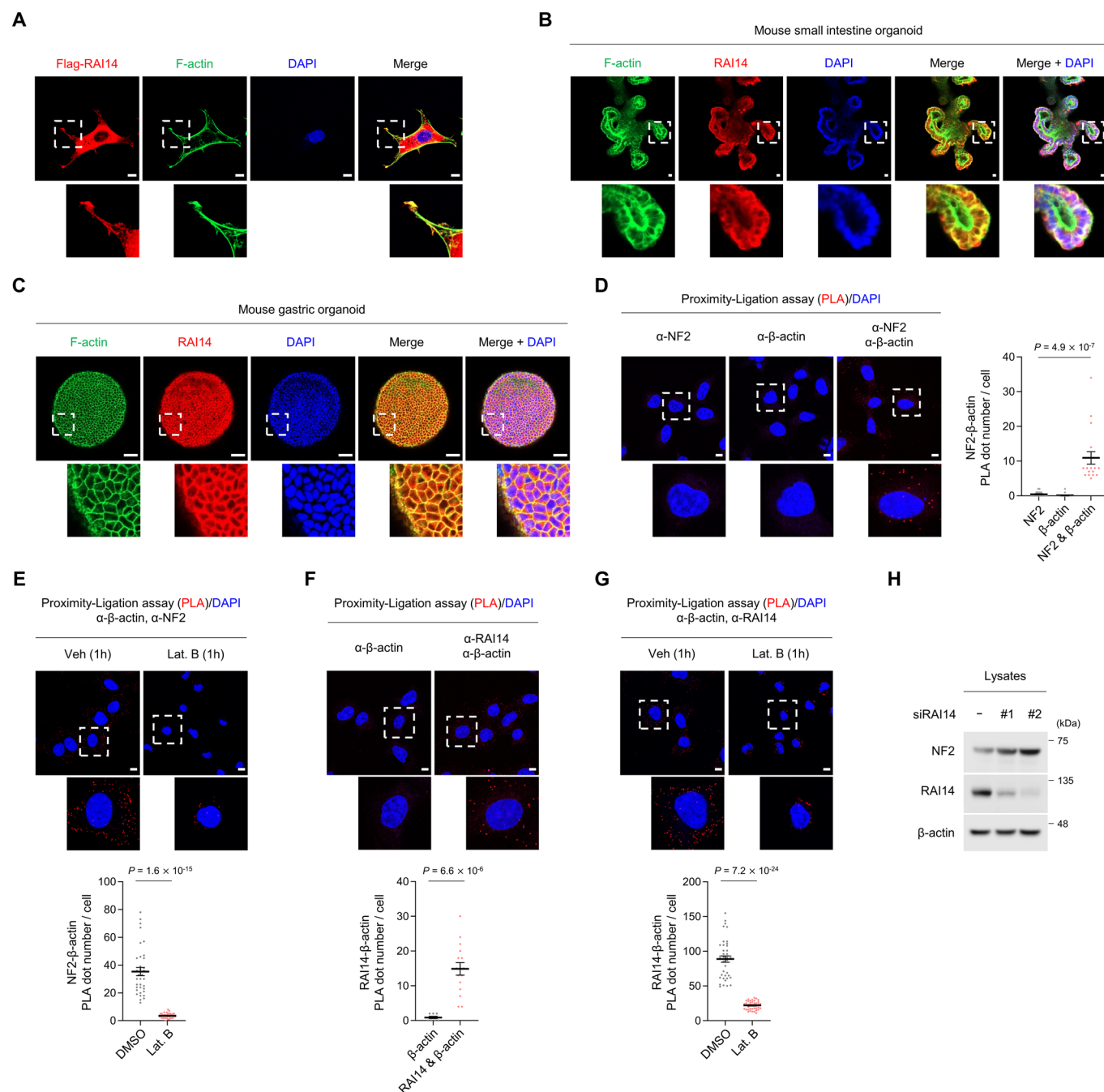


Figure EV3. RAI14 and NF2 interaction occurs on F-actin.

(A) Overexpressed Flag-RAI14 colocalizes with F-actin. HEK293A cells were used. Scale bars: 10 μ m. (B, C) RAI14 colocalizes with F-actin at the endogenous level in organoid models. Mouse small intestine organoids (B, scale bars: 10 μ m) or gastric organoids (C, scale bars: 50 μ m) were used. (D–G) Endogenous NF2 and β -actin (D, E), and RAI14 and β -actin (F, G) are in proximity. HEK293A cells were treated with Lat. B (2 μ M) for 1 h. The quantification of the number of dots in a single cell is shown. In (D), 20, 21, and 18 cells were used for NF2, β -actin, and NF2& β -actin, respectively. In (E), 35 and 33 cells were used for DMSO and Lat. B, respectively. In (F), 9 and 16 cells were used for β -actin and RAI14& β -actin, respectively. In (G), 40 cells were used for DMSO and Lat. B, respectively. Scale bars: 10 μ m. (H) Immunoblot of lysates used for Fig. 5E. Data information: In (D–G), the error bars indicate $\pm$ s.e.m. of the measurements (technical replicates). Statistical analysis was performed using two-tailed unpaired t-test, and exact P values are shown in each figure; $P < 0.05$, statistically significant. Colored dots on the graphs indicate individual measurements. Source data are available online for this figure.

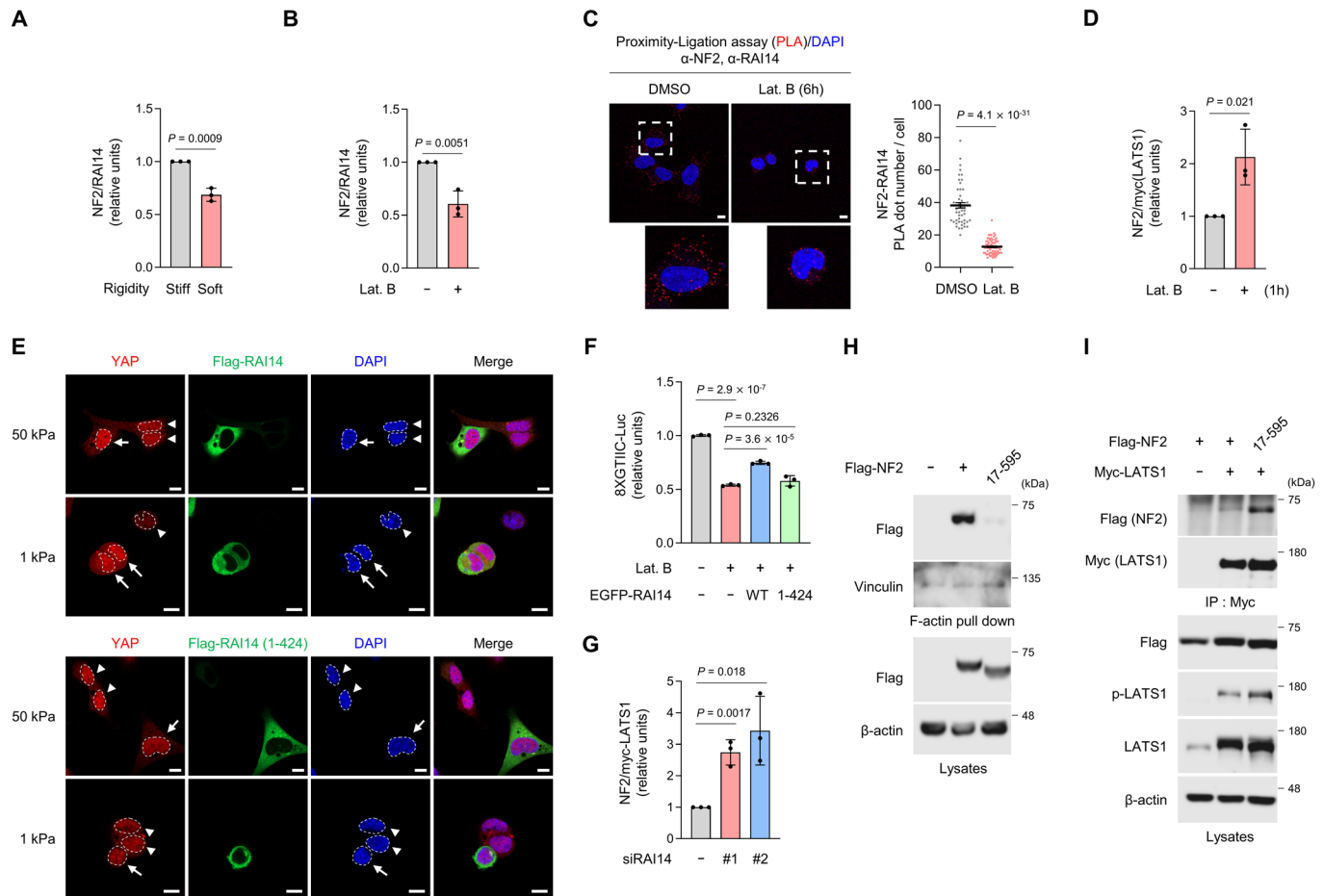


Figure EV4. ECM stiffness and F-actin integrity regulate the interaction between RAI14, NF2 and LATS.

(A, B) Quantification of immunoprecipitated NF2/RAI14 ratio from three immunoblot bands in Fig. 6B,C, respectively. (C) PLA assays show that the interaction between NF2 and RAI14 is inhibited by Lat. B treatment. HEK293A cells were treated with Lat. B (2 μ M) and MG132 (25 μ M) for 6 h. The quantification of the number of dots in a single cell is shown in the right panel. 52 and 70 cells were used for DMSO and Lat. B, respectively. Scale bars: 10 μ m. (D) Quantification of immunoprecipitated NF2/Myo(LATS1) ratio from three immunoblot bands in Fig. 6H. (E) Overexpression of RAI14, but not the RAI14(1-424) mutant form, promotes nuclear localization of YAP under both hard and soft matrix conditions. HEK293A cells were transfected and incubated on a stiff (50 kPa) or soft (1 kPa) matrix for one day. Nuclei of Flag-RAI14 (upper panel) or Flag-RAI14(1-424) (lower panel) overexpressing cells are marked with a white arrow. Nuclei of un-transfected cells (upper and lower panel) are marked with a white arrowhead. Scale bars: 10 μ m. (F) Overexpression of RAI14 partially rescues the YAP reporter activity but not in RAI14 (1-424) form. HEK293A cells were transfected and treated with Lat. B (2 μ M) for 6 h. (G) Quantification of immunoprecipitated NF2/Myo(LATS1) ratio from three immunoblot bands in Fig. 6K. (H) The NF2 (17-595) form fails to bind F-actin. HEK293A cells were used. (I) The NF2 (17-595) form shows increased interaction with LATS1 and phosphorylation of LATS1 compared to wild-type NF2. HEK293A cells were used. Data information: In (A, B, D, F, G), the error bars indicate $\pm$ s.d. of triplicate measurements (biological replicates). In (C), the error bars indicate $\pm$ s.e.m. of the measurements (technical replicates). Statistical analysis was performed using two-tailed unpaired *t* test, and exact *P* values are shown in each figure; $P < 0.05$, statistically significant. Black or colored dots on the graphs indicate individual measurements. Source data are available online for this figure.

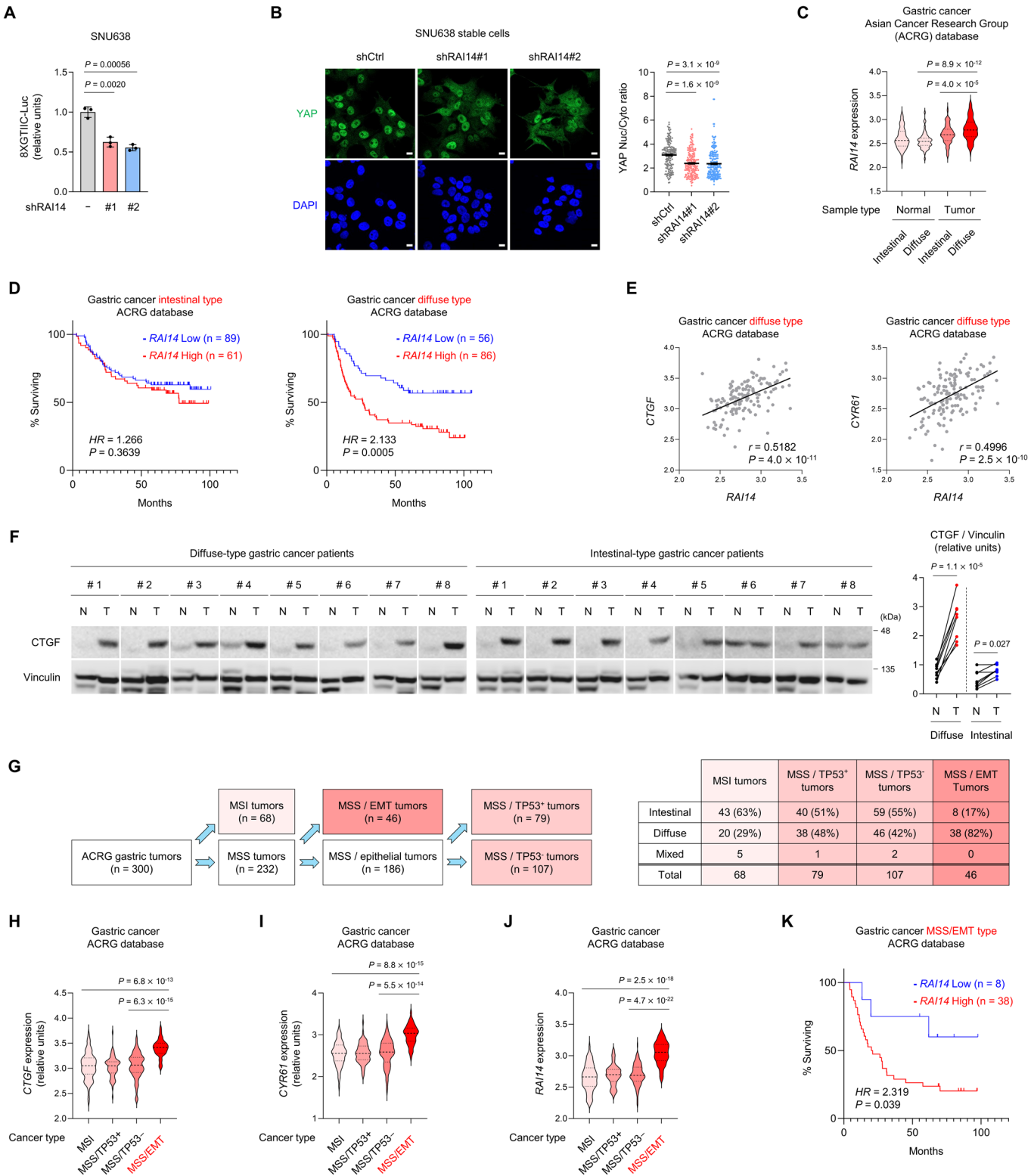


Figure EV5. RAI14-Hippo signaling promotes cell proliferation and growth, and is implicated in gastric cancer progression.

(A) Downregulation of RAI14 by shRNA reduces YAP-reporter activity. SNU638 cells were used. (B) Knockdown of RAI14 reduces the nuclear localization of YAP. SNU638 cells were used. Scale bars: 10 μ m. Quantification of the nuclear/cytoplasmic ratio in a single cell is shown in the right panel. 170, 187, and 203 cells were used for shCtrl, shRAI14#1, and shRAI14#2, respectively. (C) The violin plot shows a high expression of *RAI14* in gastric tumor samples, especially in the diffuse-type. The y-axis indicates the log base 10 of *RAI14* expression. Adjacent normal tissue samples from intestinal-type ($n = 39$) and diffuse-type ($n = 58$) patients and tumor tissue samples from intestinal-type ($n = 150$) and diffuse-type ($n = 142$) patients were analyzed. (D) Kaplan-Meier plots show that high expression of *RAI14* in diffuse-type GC patients is associated with poor prognosis. The upper 41st ($n = 61$) and lower 59th ($n = 89$) percentiles were analyzed for intestinal-type GC patients, and the upper 61st ($n = 86$) and lower 39th ($n = 56$) percentiles were analyzed for diffuse-type GC patients. HR, hazard ratio. (E) The mRNA expression of diffuse-type GC ($n = 142$) from the ACRG database shows a positive correlation between *RAI14* and YAP-target genes (*CTGF* and *CYR61*). (F) *CTGF* level were high in GC tissues and significantly higher in DGC. Quantification of *CTGF*/Vinculin ratios from immunoblots was performed as in Fig. 7K. (G) Schematic representation of the molecular classification of GC from the ACRG database (left panel). Patients with MSS/EMT-type mainly belong to the diffuse-type (right panel). (H-J) The violin plot shows high expression of *CTGF*, *CYR61* and *RAI14* in MSS/EMT-type gastric tumor samples. The y-axis indicates the log base 10 of *RAI14* expression. Tissue samples from MSI ($n = 68$), MSS/TP53⁺ ($n = 79$), MSS/TP53⁻ ($n = 107$) and MSS/EMT-type patients ($n = 46$) were analyzed. (K) Kaplan-Meier plots show that high expression of *RAI14* in MSS/EMT-type GC patients is associated with a worse prognosis. The upper 17th percentile ($n = 8$) and the lower 83rd percentile ($n = 38$) were analyzed in patients with intestinal-type gastric cancer. HR, hazard ratio. Data information: In (A), the error bars indicate $\pm$ s.d. of triplicate measurements (biological replicates). In (B), the error bars indicate $\pm$ s.e.m. of the measurements (technical replicates). In (D, K), the hazard ratio (HR) was calculated using the Mantel-Haenszel method. In (E), the correlation coefficient (r) was calculated using Pearson's linear correlation. Statistical analysis was performed using two-tailed unpaired t test (A-C, F, H-J) and log-rank test (D, K), or calculating right-tailed F probability distribution (E), and exact P values are shown in each figure; $P < 0.05$, statistically significant. Black or colored dots on the graphs indicate individual measurements. Source data are available online for this figure.