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Supplementary Materials for

Ski regulates Hippo and TAZ signaling to suppress breast cancer progression

Juliet Rashidian, Erwan Le Scolan, Xiaodan Ji, Qingwei Zhu, Melinda M. Mulvihill, Daniel Nomura, Kunxin Luo*

*Corresponding author. E-mail: kluo@berkeley.edu

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This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
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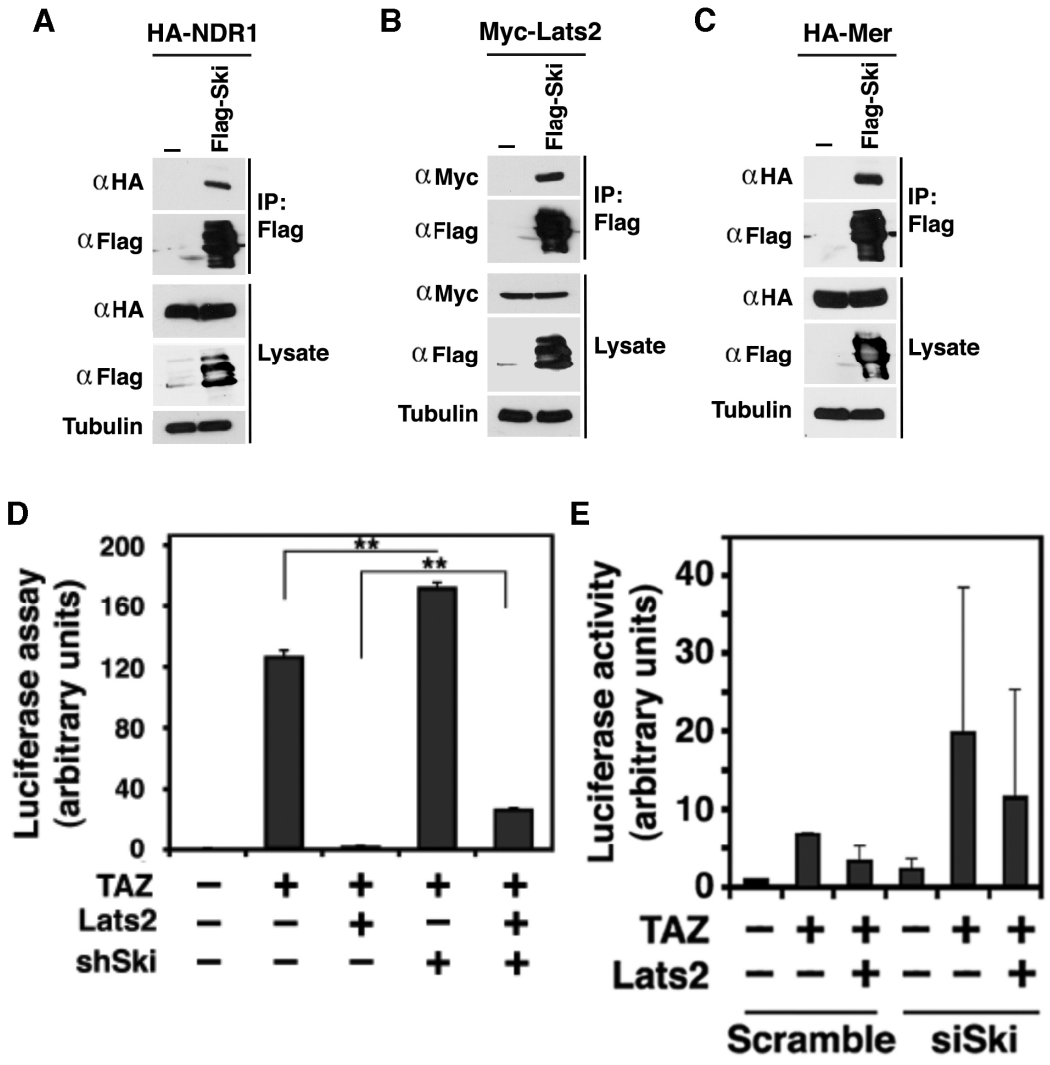
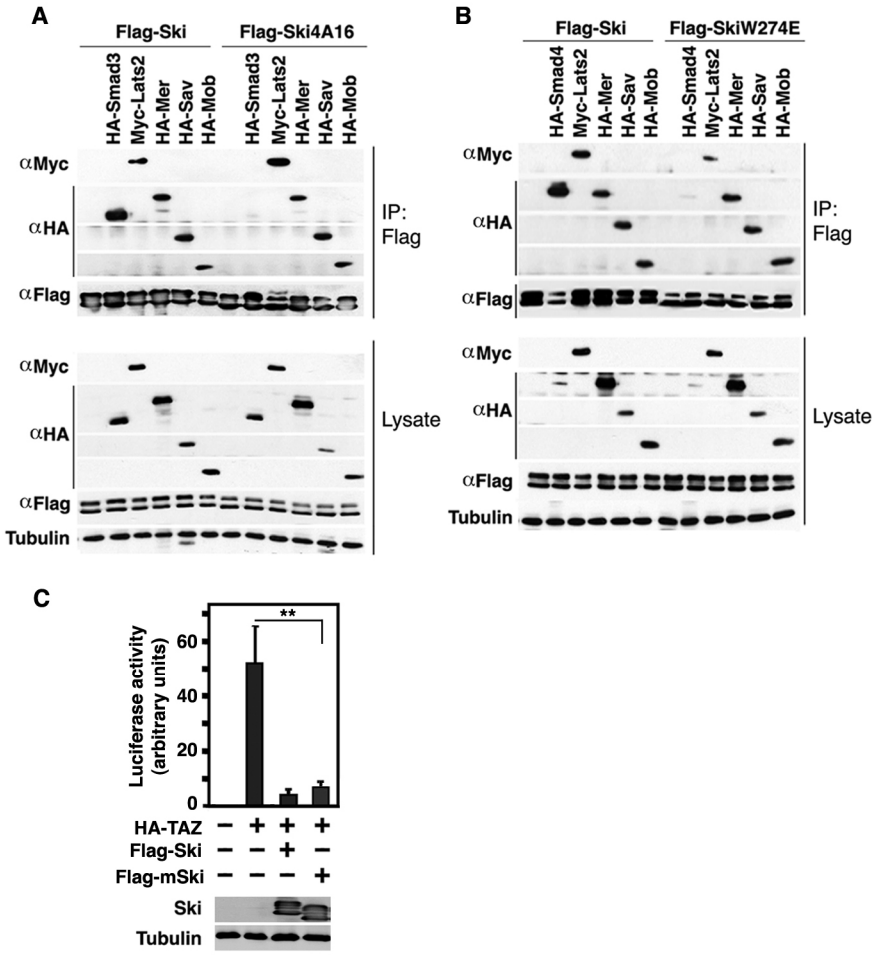
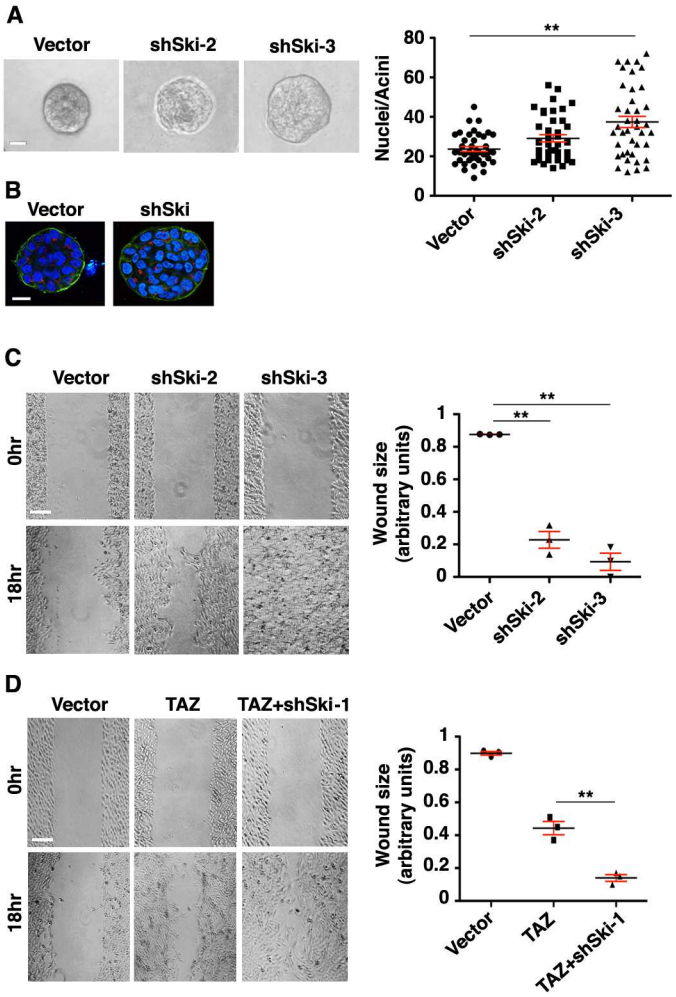


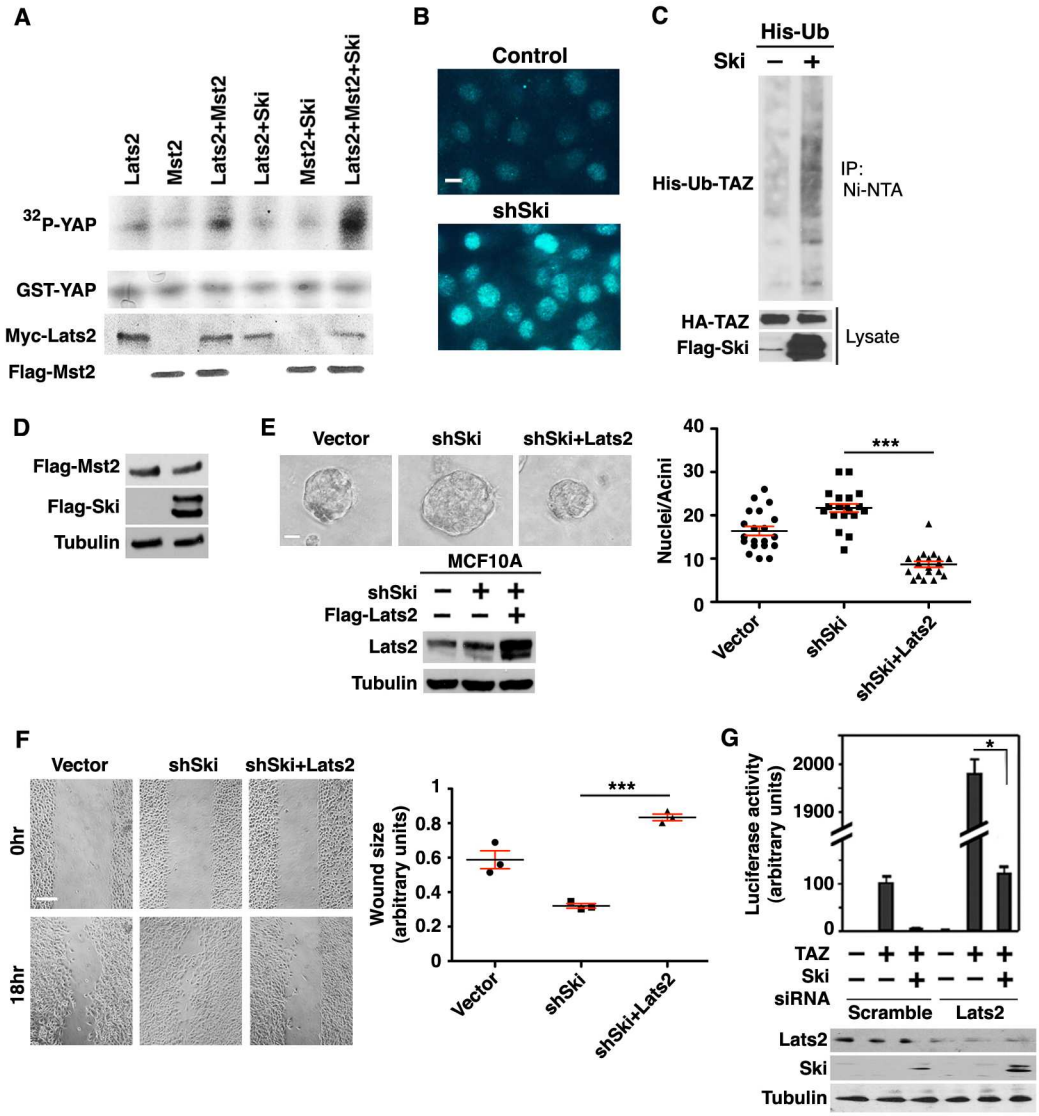
Supplemental Figure S1. **Ski specifically interacts with Hippo signaling components to inhibit TAZ transcriptional activity. A-C**: Anti-Flag immunoprecipitation was carried out in cells expressing HA-NDR1 alone (**A**), Myc-Lats2 alone (**B**), HA-Mer alone (**C**) or together with Flag-Ski and subjected to Western blotting with the indicated antibodies. The Western blots are representatives of 2 independent experiments. **D-E**: Reducing endogenous Ski with shSki (D) and a different set of siRNA pool (E) increased the transcription activity of transfected TAZ. The graphs are representative of 3 independent experiments. top: presented as mean ± SEM (Student's t-test, **P<0.01).



Supplemental Figure S2. Ski interacts with the Hippo pathway and inhibits TAZ independently of its antagonism of TGF-β/Smad signaling. (A) Mutant Ski defective in binding to Smad2 and Smad3 (4A16) or **(B)** Smad 4 (W274) associated with Lats2, Mer, Sav and Mob in co-immunoprecipitation assays. The Western blots are representative of 3 independent experiments. **(C)** Mutant Ski defective in binding to all the Smad proteins (mSki) inhibited transcription activation by TAZ as efficiently as wild-type Ski. The graph is derived from 3 independent experiments and presented as means ± SEM (Student's t-test, $^{**}P < 0.01$).

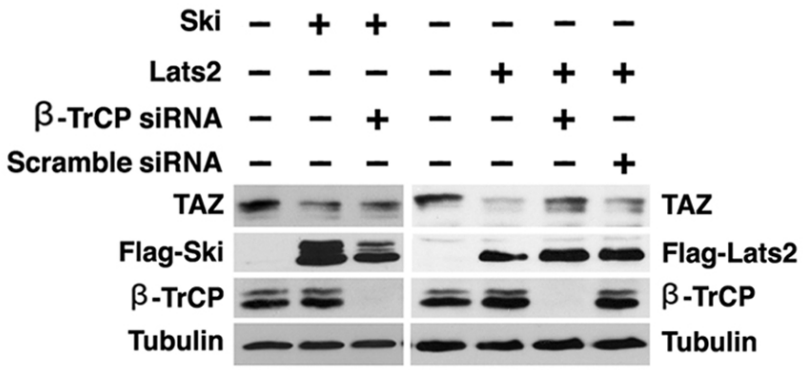


Supplemental Figure S3. Multiple shSki MCF10A clones all displayed increased acinar size and cell mobility. Different shSki MCF10A clones displayed an increase in acinar size **(A, B)** and cell mobility (**C)**. The large acini had similar polarity and structural organization to those derived from control cells **(B)**. Green: α6 integrin staining; blue: DAPI. Images are representative of 3 independent experiments. **(D)** An additional TAZ+shSki clone showed increased cell mobility. Scale bar in A and B: 40μm, in C and D: 300 μm. The graphs are derived from 3 independent experiments and presented as means ± SEM (ANOVA, Newman–Keuls Multiple Comparison Test on logarithmic conversions of the nuclei counts (A) or wound size measurements (C and D), **P <0.01). In A, each point represents a separate acinus.



Supplemental Figure S4. Ski suppressed YAP/TAZ signaling by promoting their phosphorylation by Lats2. (A) Ski increased the phosphorylation of YAP in the presence of Lats2 and Mst2 in an in vitro kinase assay (top panel). The abundance of YAP, Lats2 and Mst2 was examined by Western blotting (bottom). Western blots are representative of 3 independent experiments. **(B)** Ski knockdown increased the abundance of TAZ without altering its localization in MDA-MB-231 cells. Images are representative of 2 independent experiments. **(C)** Ski overexpression promoted polyubiquitination of TAZ. Ubiquitinated TAZ was pulled down from cells transfected with His-Ub, HA-TAZ, Lats2 and Mst2 in the absence or presence of Flag-Ski by Ni-NTA and detected by Western blotting with anti-TAZ. Western blots are representative

of 3 independent experiments. **(D)** Ski did not affect the abundance of Mst2. Western blots are representative of 3 independent experiments. **(E)** Overexpression of Lats2 in MCF10A cells with Ski knockdown reduced the size of the acini to that of the control cells. The graph shows the size of acini as determined by the number of nuclei in each acinus from 40 acini derived from 2 independent experiments and presented as means ± SEM (ANOVA, Newman–Keuls Multiple Comparison Test on logarithmic conversions of the nuclei counts, ***P <0.001). Scale bar: 40μm. The abundance of ectopic Lats2 was shown by Western blotting. **(F)** Overexpression of Lats2 in MCF10A/shSki cells reduced cell motility. The graph was derived from 3 independent experiments and presented as means ± SEM (ANOVA, Newman–Keuls Multiple Comparison Test ***P <0.001). Scale bar: 300 μm. **(G)** Reducing Lats2 by an additional siRNA in TAZ+Ski cells enhanced transcription by TAZ, but did not prevent Ski-mediated inhibition of TAZ. Data in the graph was derived from at least 3 independent experiments and presented as mean ± SEM (Student's t-test, *P<0.05).



Supplemental Figure S5. Ski-induced TAZ degradation is not mediated by the β-TrCP E3 ligase. siRNA-mediated knockdown of β-TrCP did not block TAZ degradation by Ski (left panels), but blocked Lats2-induced degradation of TAZ. 293T cells were transfected with Ski, Lats2 and the indicated siRNA. The abundance of endogenous TAZ was detected by Western blotting. Western blots are representative of 2 independent experiments.