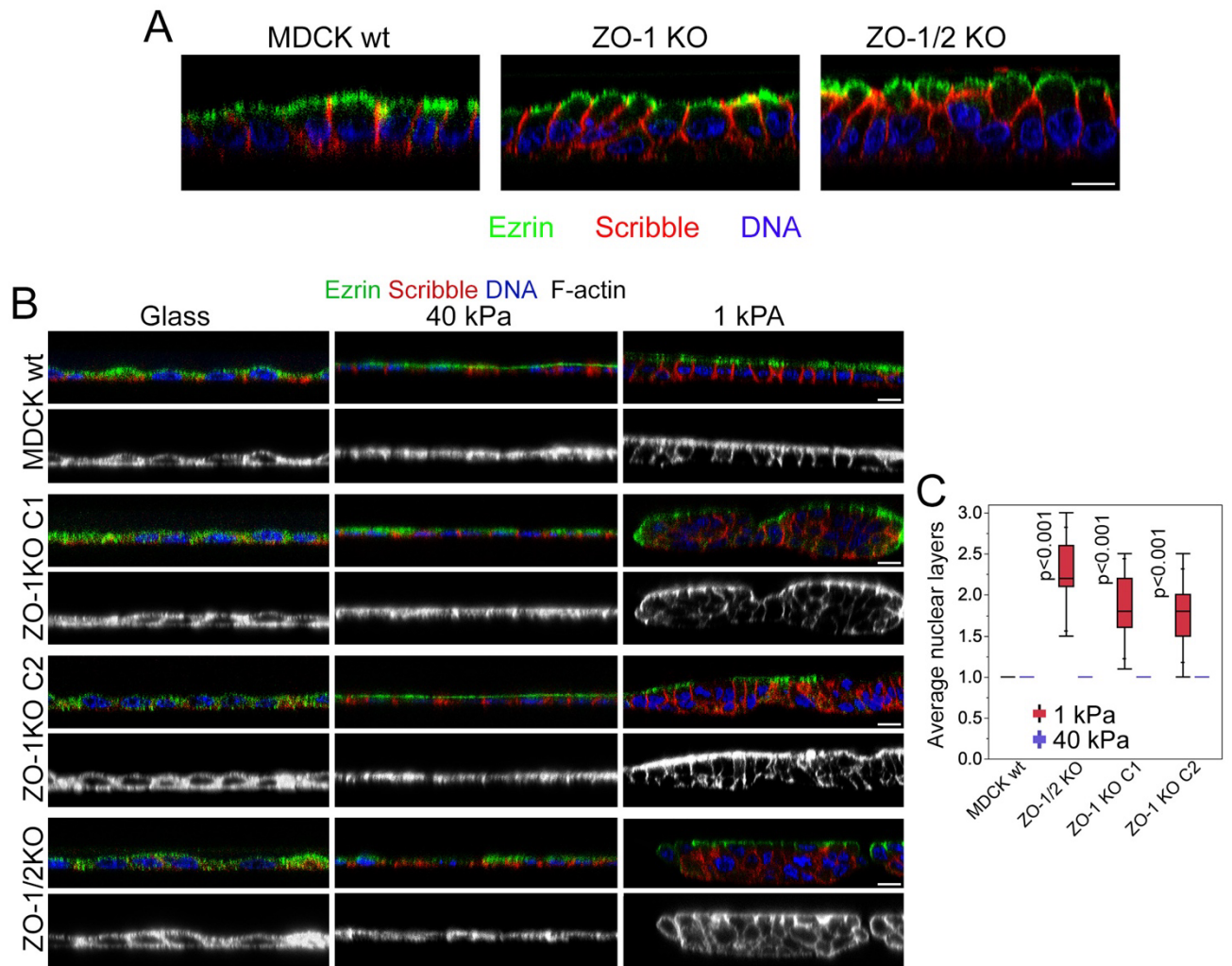


*Supplementary Figures*

**ZO-1 regulates Hippo-independent YAP activity and cell proliferation via a GEF-H1- and  
TBK1-regulated signalling network**

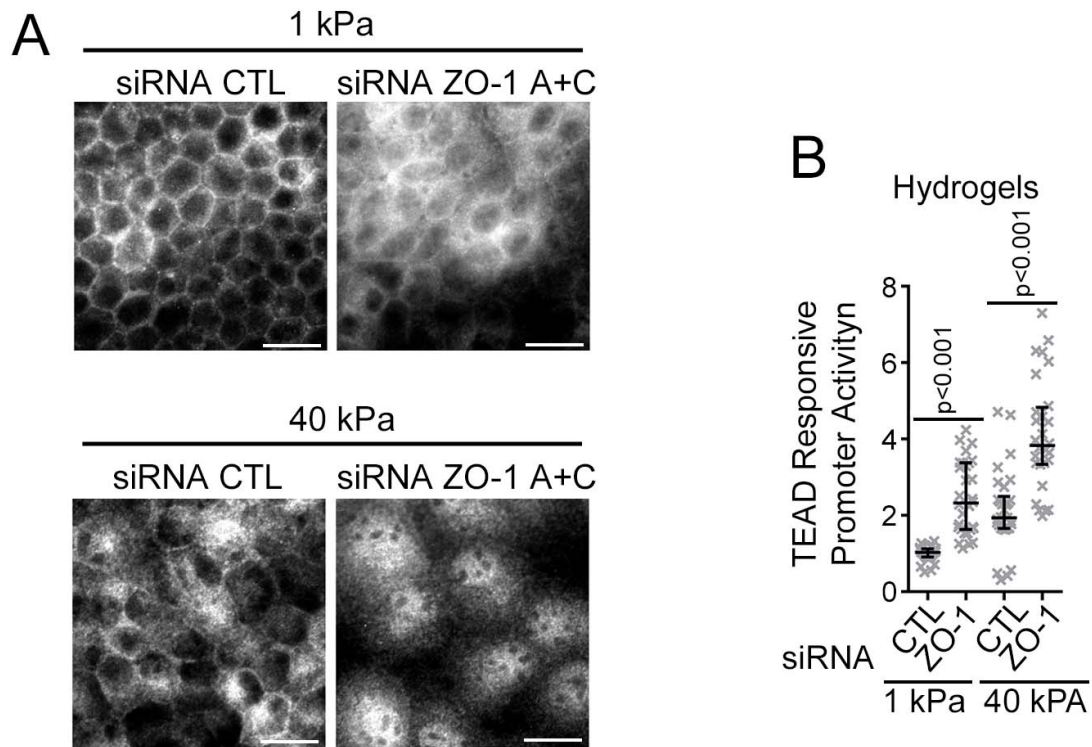
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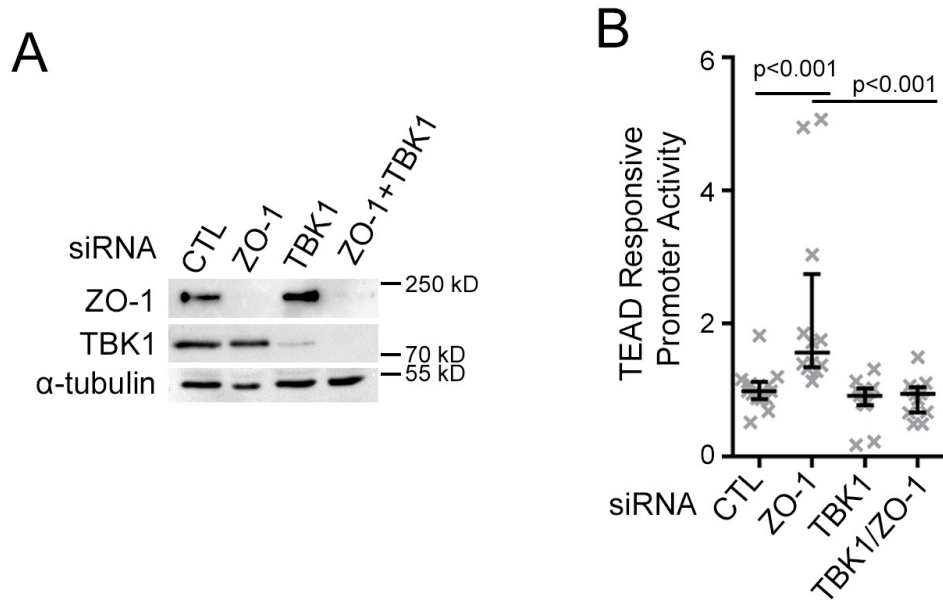
**Figure S1.** Knockout of ZO-1 increases cell proliferation and disrupts monolayer organization.

Expression of polarity markers and monolayer organization by control and knockout MDCK cells grown on filters (**A**) or hydrogels (**B,C**). Quantification shows means, interquartile ranges, and p-values derived from a signed-rank test ( $n=15$  images per cell line and condition). Magnification bars, 20  $\mu\text{m}$ .



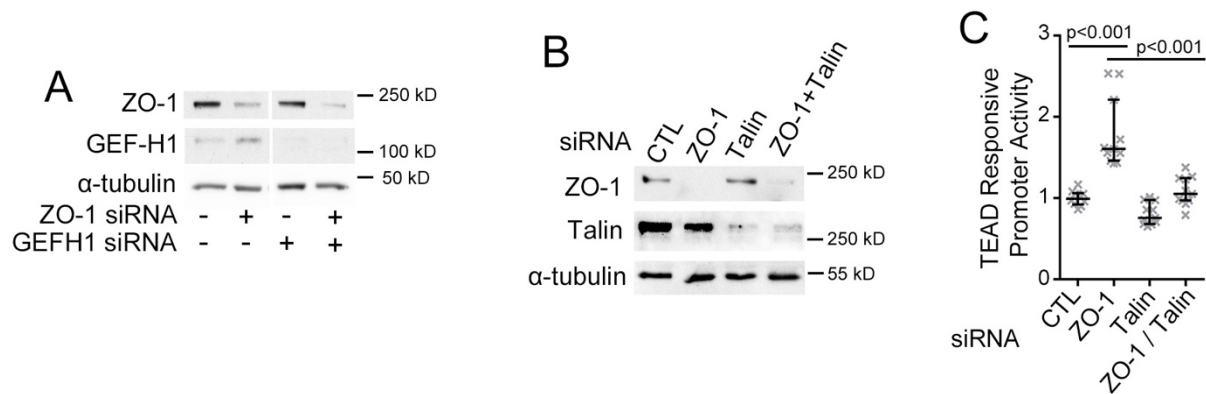
**Figure S2.** Knockdown of ZO-1 promotes nuclear translocation of YAP.

**A** siRNA-transfected MDCK cells grown on 40 and 1 kPa PAA hydrogels were fixed and immunostained for YAP. **B** TEAD transcriptional activity was analyzed by reporter gene assay in MDCK cells grown on hydrogels of 40 and 1 kPa. The quantification shows individual cells analyzed, medians, interquartile ranges, and p-values derived from Wilcoxon tests. Magnification bars, 20 μm.



**Figure S3.** Knockdown of TBK1 inhibits YAP/TEAD activation in ZO-1 depleted cells.

MDCK cells were transfected with siRNAs as indicated and then analyzed by immunoblotting (**A**) or a TEAD responsive promoter assay (**B**). The reporter assay shows individual determinations, medians, interquartile ranges, and p-values for the indicated pairs from Wilcoxon tests.



**Figure S4.** Analysis of siRNA transfected MDCK cells.

MDCK cells were transfected with siRNAs as indicated and then analyzed by immunoblotting to determine GEF-H1 and ZO-1 expression (**A**), knockdown of ZO-1 and talin (**B**), or the impact on the TEAD responsive promoter gene assay (**C**, shown are individual determinations, medians, interquartile ranges and p-values derived from Wilcoxon tests).