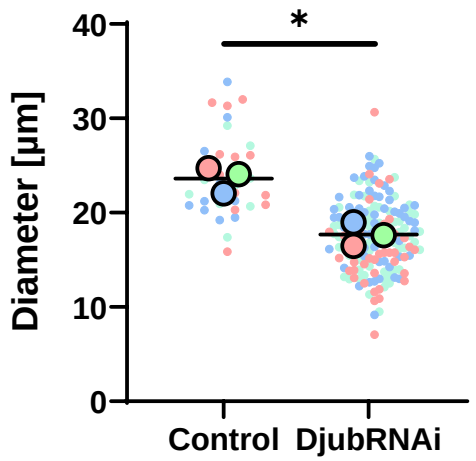
Supplemental Table of Contents

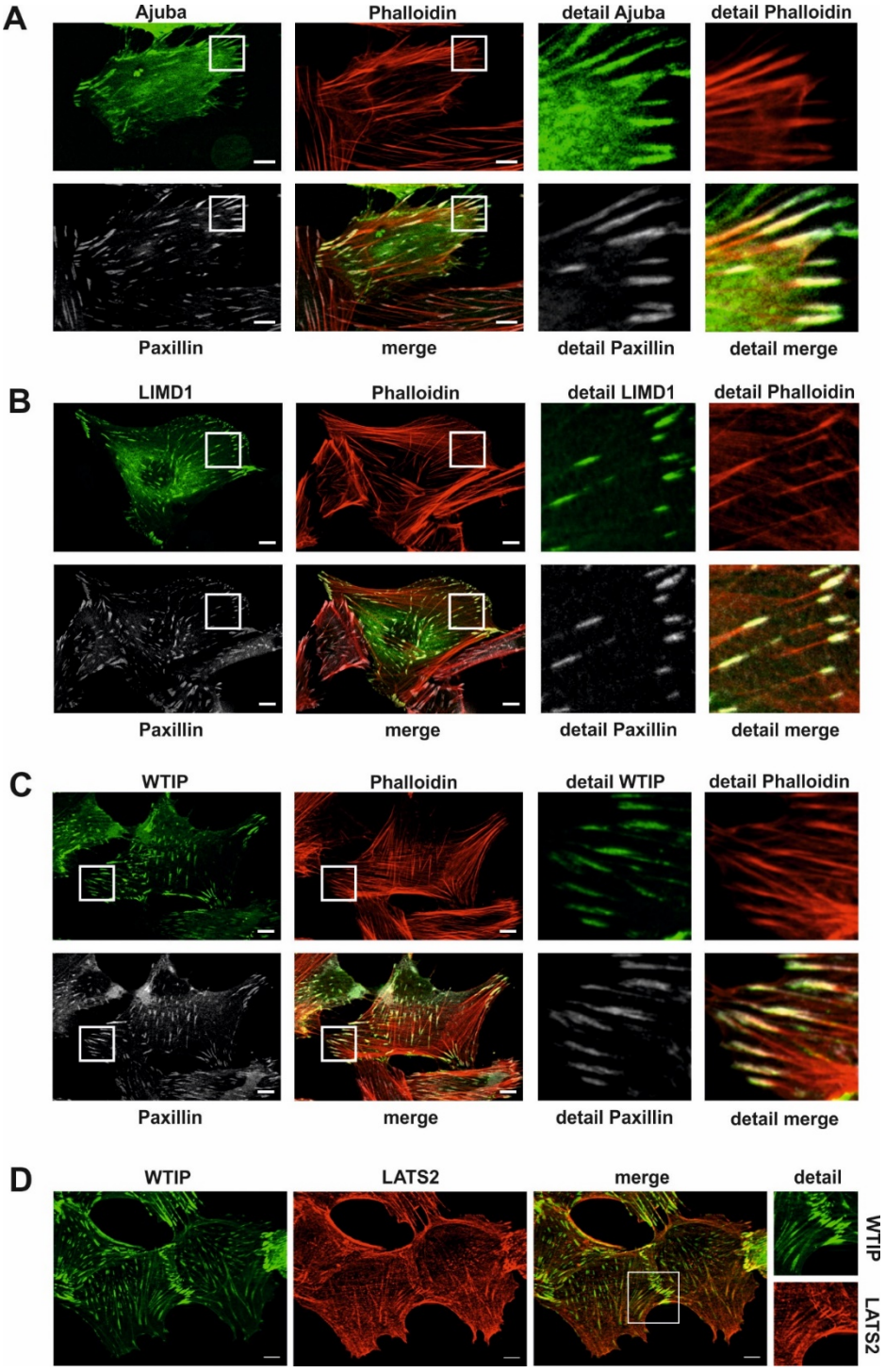
Suppl. Fig. 1



Suppl. Fig. 1: Djub knockdown decreases diameter of nephrocytes

Comparison of nephrocyte diameters, Djub knockdown versus control. 3rd instar larvae garland nephrocytes with Djub knockdown are significantly smaller than wildtype nephrocytes.

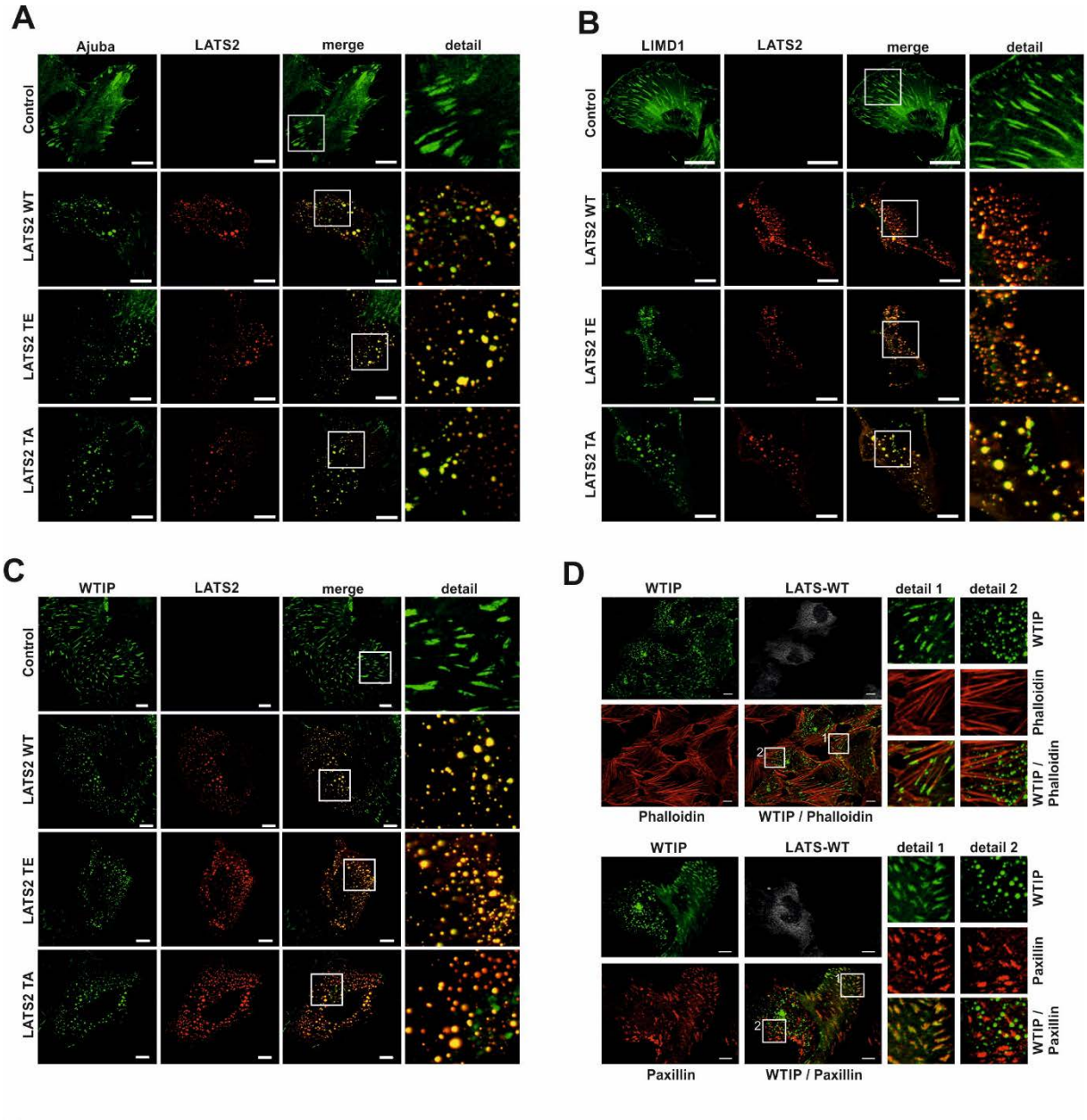
Suppl. Fig. 2



Suppl. Fig. 2: Localization of Ajuba proteins at focal adhesions and cell-cell contacts in podocytes

A: Overexpression of N-terminal EGFP-tagged Ajuba in AB8/13 cells and immunostaining of Actin (Phalloidin, red) and Paxillin (grey). Additional to diffuse cytoplasmic staining, Ajuba co-localizes with Paxillin in focal adhesions at the end of the Actin fibers. **B-C:** Immunostaining of EGFP-LIMD1 (B) and EGFP-WTIP (C) also shows co-localization with Paxillin in focal adhesions at the end of the Actin fibers. **D:** Immunostaining of endogenous LATS2 (red) in EGFP-WTIP overexpressing cells demonstrates partial co-localization especially at cell-cell contacts and focal adhesions. Scale bar 10 µm.

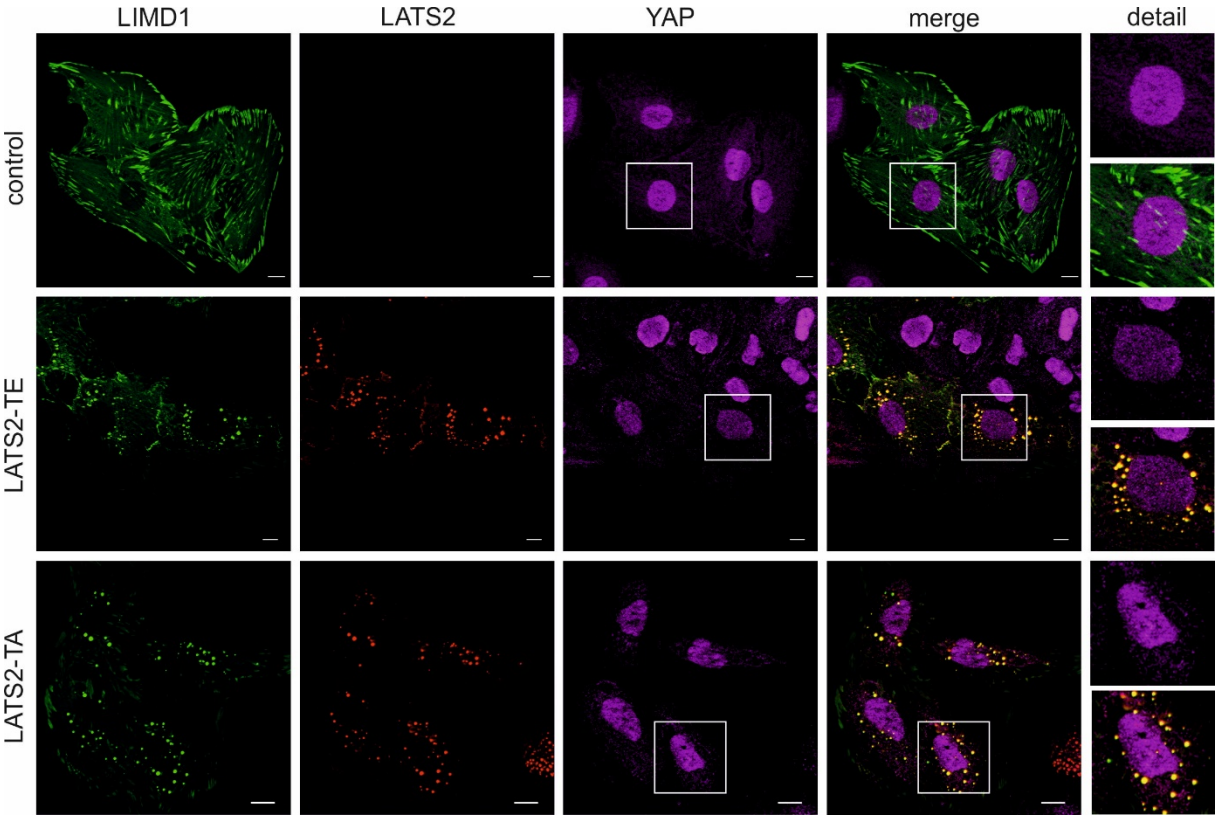
Suppl. Fig. 3



Suppl. Figure 3: Increased LATS2 expression results in recruitment of Ajuba proteins in punctate structures in the cytoplasm independent from kinase activity

A-C: AB8/13 cells overexpressing EGFP-Ajuba (A), EGFP-LIMD1 (B), or EGFP-WTIP (C) after Dox induction (green) were transiently transfected with RFP-tagged LATS2-WT, LATS2-TE or LAT2-TA (red). Immunofluorescence analyses show recruitment of all three Ajuba proteins from focal adhesions into Ajuba protein/LATS2 agglomerates independent from LATS2 kinase activity. **D:** EGFP-WTIP overexpressing AB8/13 cells were transiently transfected with RFP-LATS2-WT and additionally stained for Actin (Phalloidin) or Paxillin. Cells overexpressing LATS2-WT show redistribution of WTIP from focal adhesion at the end of Actin stress fibers into agglomerates while the strong Actin fibers of cultivated podocytes and Paxillin staining remained unchanged. Scale bars 10 µm.

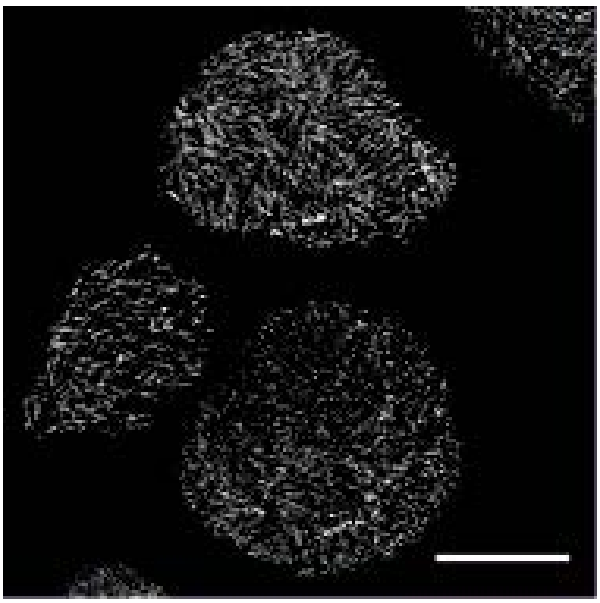
Suppl. Fig. 4



Suppl. Fig. 4: Redistribution of Ajuba proteins occurs independent from YAP localization

AB8/13 cells stably expressing C-terminal tagged LIMD1-EGFP (green) were transfected with RFP-LATS2-TE or RFP-LATS2-TA (red), protein expression induced by Dox treatment. Immunostaining of YAP (magenta) shows predominant nuclear YAP localization in mock transfected control cells. Expression of kinase active LATS2-TE leads to reduction of nuclear YAP, cells expressing kinase inactive LATS2-TA keep predominant nuclear YAP localization. Independent form LATS2 activity, LIMD1 is redistributed into agglomerates in LATS2 expressing cells. Scale bars 10 µm.

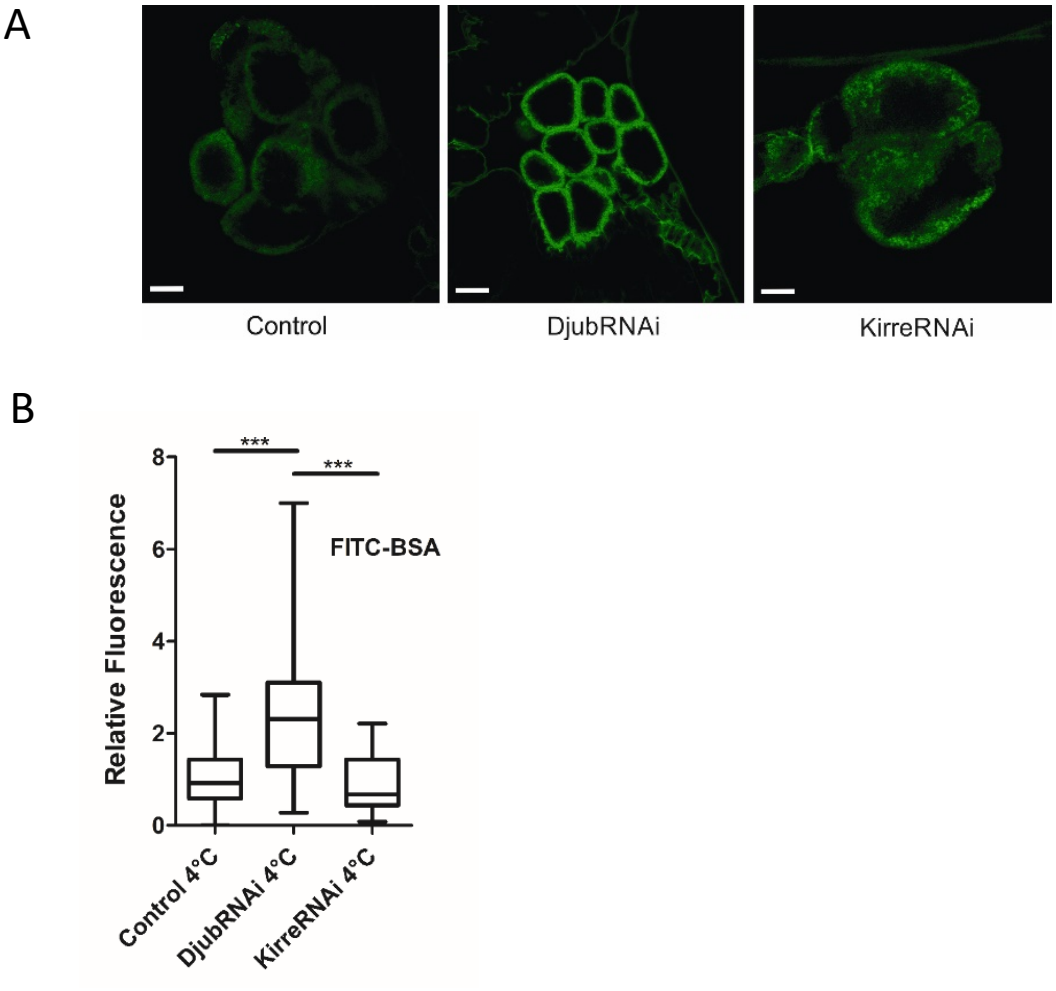
Suppl. Fig. 5



Suppl. Fig. 5: Second DjubRNAi line shows comparable SD phenotype

Immunostaining of Sns in 3rd instar larvae garland nephrocytes as slit diaphragm marker. A second DjubRNAi line (#32923) reduced the density of SD on nephrocytes surface comparable to the DjubRNAi line (#41938) shown in Fig. 5. Scale bars 10 µm.

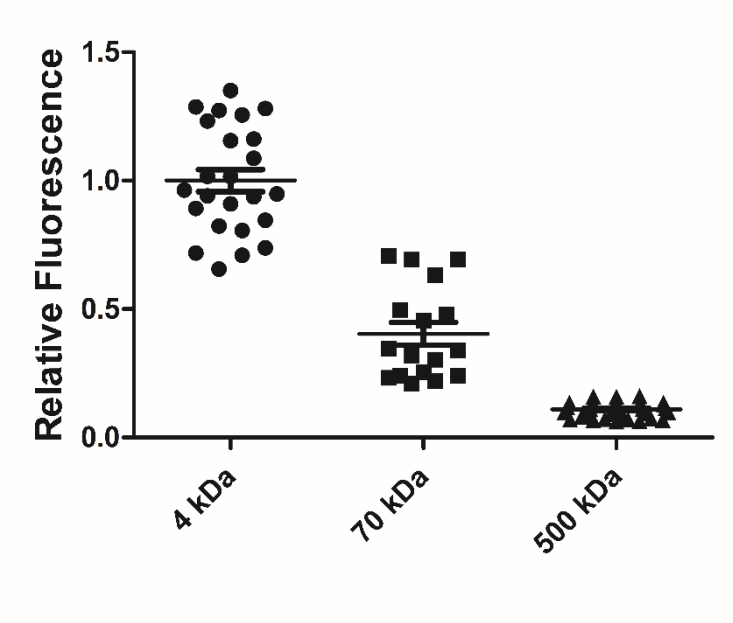
Suppl. Fig. 6



Suppl. Fig. 6: Djub knockdown increases uptake of FITC-BSA

A: Representative images of uptake assays with FITC-BSA (1 mg/ml, 1 min incubation) performed at 4° C to inhibit active uptake processes. **B:** The quantification of the uptake experiments shown in A proves comparable uptake efficiencies in control GCN and nephrocytes with Kirre knockdown. The uptake efficiency of Djub knockdown nephrocytes remains on a higher level. $n > 25$, *** $p < 0.001$. Scale bars 10 µm.

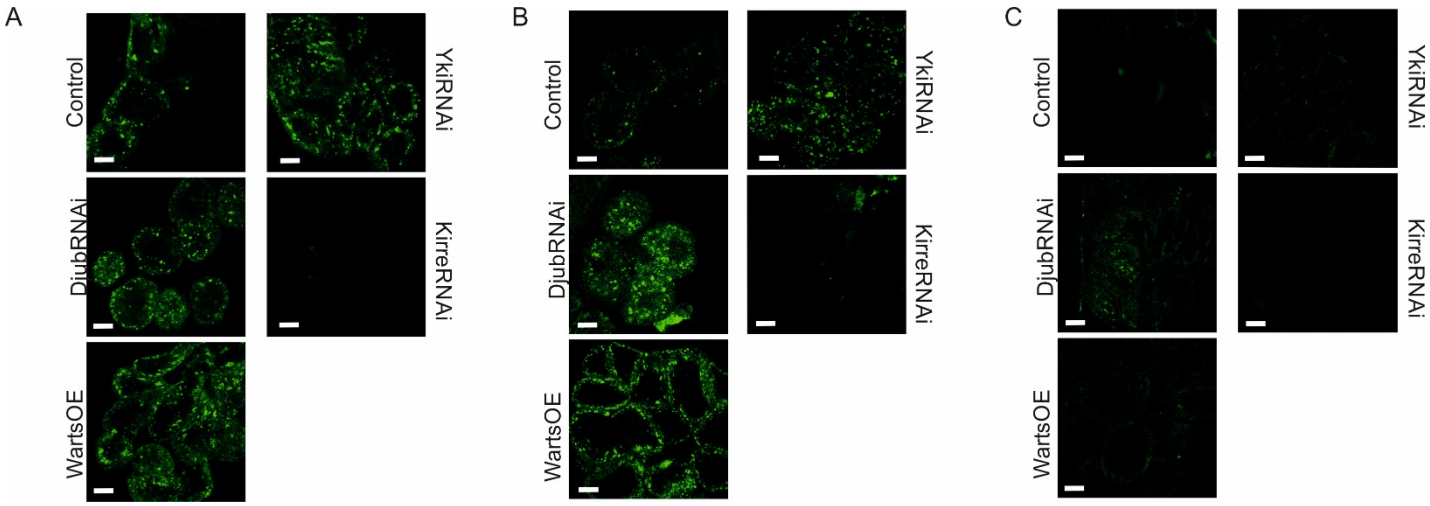
Suppl. Fig. 7



Suppl. Fig. 7: Uptake efficiency of FITC-Dextran depends on molecular size.

Measurement of the uptake efficiency of FITC-dextran in 3rd instar larvae garland nephrocytes in dependency of molecular size at room temperature. For the experiments dextran solutions with equal concentrations (1mg/ml) were used, which were assured to have equal fluorescence levels. The uptake of 70 kDa FITC-Dextran is about 40 % and that of 500 kDa FITC-Dextran about 10 % as efficient as the uptake of 4 kDa FITC-Dextran. This reduction of uptake efficiency visualizes the size selectivity of nephrocyte filtration. 4 kDa Dextran can freely pass SD, 70 kDa Dextran is filtrated by SD, but diffusion is limited, 500 kDa Dextran is excluded from filtration. The basal uptake level of 500 kDa dextran can be explained by endocytosis independent from SD filtration.

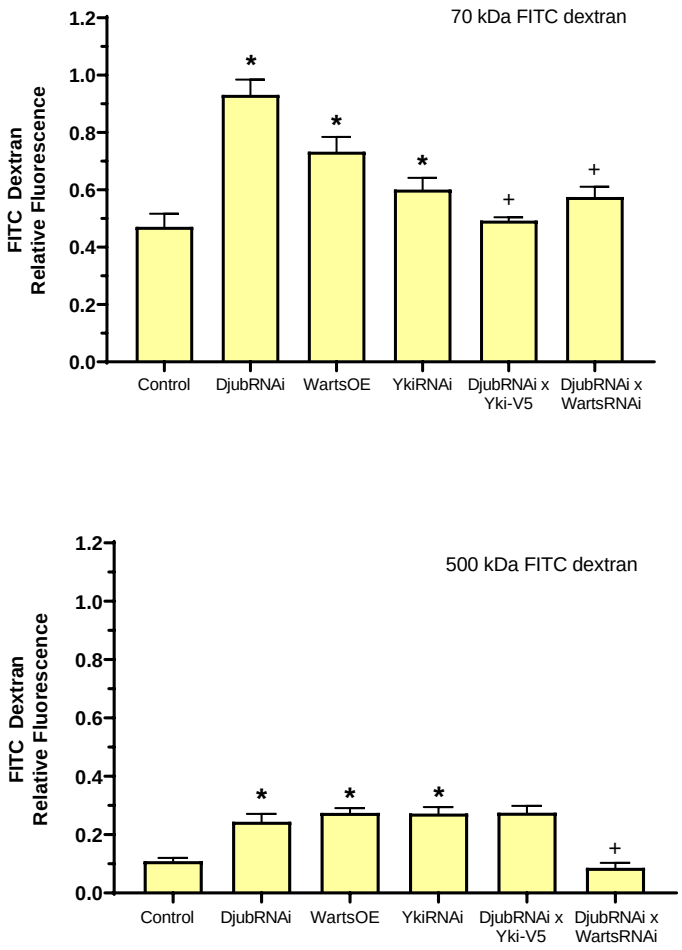
Suppl. Fig. 8



Suppl. Fig. 8: Representative images of Dextran uptake experiments

Uptake assays performed at room temperature with FITC-Dextran (1 mg/ml, 15 min incubation) of molecular sizes 4 kDa (**A)**, 70 kDA (**B**) and 500 kDa (**C**). Djub or Yki knockdown (DjubRNAi, YkiRNAi) and Warts overexpression (WartsOE) increase accumulation of all three FITC-dextrans in lacunae. Scale bars 10 µm.

Suppl. Fig. 9



Suppl. Fig. 9 Increased uptake efficiency especially of 70 kDa FITC-Dextran after Djub knockdown or Hippo activation demonstrates changes in size selectivity

Uptake assays with FITC-dextran of different sizes (4 kDA, 70 kDa and 500 kDa, 1 mg/ml, RT). Presuming that 4 kDa dextran molecules freely pass SD, normalization on the 4 kDa uptake value measured for each condition indicates the respective selectivity of filtration for 70 and 500 kDA and reveals a significantly increased uptake of bigger dextran molecules for Djub and Yki knockdown and Warts overexpression nephrocytes in comparison to control. The effect of Djub knockdown was significantly reduced by simultaneous Yki-V5 overexpression (DjubRNAixYki-V5) or Warts knockdown (DjubRNAixWartsRNAi). n>20, * $p < 0.05$ compared to control, +$p < 0.05$ compared to DjubRNAi.

Since 4 kDa dextran should freely pass the SD and changes in its accumulation should mainly represent SD-independent changes, e.g. change in size of lacunae or endocytosis rate, this normalization gives a better idea how the filter properties for higher molecular weight dextrans have been changed. In case of DjubRNAi uptake of 70 kDa dextran was almost as high as the uptake of 4 kDa dextran (normalized value reaching almost 1.0), suggesting that the SD provides essentially no resistance for 70 kDa dextran anymore. At the same time, uptake of 500 kDa dextran, while increased, was still much lower than that of 4 kDa dextran, indicating that the filtration barrier preserved though its filtration properties have been impaired.

Supplemental Video 1 Description:

Live cell imaging after induction of RFP-LATS2-TA expression (red) in a mixed AB8/13 cell culture with cells stably expressing EGFP-LIMD1 (green) and others without EGFP-LIMD1 overexpression. All cells express LATS2-TA over time after induction. The film starts 4 hours after induction of LATS2-TA expression. The time stamp on the film indicates the period after film start. Before expression of LATS2-TA in EGFP-LIMD1 expressing cells, EGFP-LIMD1 was distributed at focal adhesions, cell-cell contacts and diffusely around the nucleus. Expression of LATS2-TA in these cells led to appearance of RFP-LATS2-TA at cell-cell junctions, visible by the green line between the cells becoming more and more yellow due to co-localization of LIMD1 and LATS2-TA. At later time points, both LATS2-TA and LIMD1 redistributed to punctate structures and the junctional localization of the proteins seemed to be increasingly lost. Cells without LIMD1 expression show unspecific cytoplasmic LATS2-TA localization. Scale bar 10 µm.