Supplemental material table of contents:

Table S1. Clinical characteristics of patients

Patient	Sex	Age (years)	sCr (mg/dL)	Etiology
Healthy	M	76	0.9	
AKI 1	F	55	8.7	Renal vein thrombosis
AKI 2	M	52	8.1	Graft rejection

Legends of supplementary figures

Suppl. Figure 1. Colocalization of RIPK3 with neutrophils and tubular segments in mice**. A)** Colocalization of RIPK3 with neutrophil marker Ly6G in AKI at 48 hours. **B)** Colocalization of RIPK3 with proximal tubular marker (TLT: tetragonolobus lectin) or with distal tubular marker (DBA: Dolichos Biflorus Agglutinin)**. A, B)** Confocal microscopy. Magnification, 800x (scale bars: 5 μm).

Suppl. Figure 2. RIPK3 immunostaining in human AKI and control kidney. A) Immunofluorescence using anti-RIPK3 antibodies showed increased tubular cell RIPK3 expression in AKI, which was more prominent in some dilated injured tubules (arrow). Additionally, interstitial cells were also prominently stained in AKI kidneys (Asterisk). Staining in control kidney was faint. Nuclei were counterstained with DAPI. Magnification 100x, scale bars: 100 μm; magnification 200x, scale bars: 50 μm. **B)** Colocalization of RIPK3 with macrophage marker CD68 in human AKI. Confocal microscopy. Magnification, 800x (scale bars: 20 μm). Detail 1200x, (scale bars: 5 μm).

Suppl. Figure 3. WT bone marrow promotes inflammation in *Ripk3*-KO mice with AKI. A) Renal function was assessed by plasma creatinine and BUN levels at 48 hours after AKI induction in bone marrow chimera mice. WT→KO represents WT bone marrow to *Ripk3*-KO mice and KO→KO represents *Ripk3*-KO bone marrow to *Ripk3*-KO mice. Mean ± SD of 3-6 mice per group. *** p<0.001. **B)** Kidney *Mcp-1*, *IL-6 and Il-1β* mRNA levels assessed by RT-PCR at 48 hours of AKI in bone marrow chimera mice. **C)** Kidney interstitial infiltration by F4/80 positive interstitial macrophages, CD3+ T cells and Ly6G+ cells in FA-AKI at 48 hours in bone marrow chimera mice. Representative images (Magnification 200x, scale bars: 50 μm) and quantification. **B-C)** Mean ± SD of 6 mice per group. *p<0.05; **p<0.01; *** p<0.001.

Suppl. Figure 4. NLRP3 is not involved in RIPK3-induced kidney inflammation at 48 hours of AKI. **A)** Representative western blot and quantification of kidney NLRP3 and cleaved caspase 1. Mean ± SD of 6-8 animals per group. **p<0.01; ***p<0.001. **B)** Kidney function was assessed by plasma creatinine and BUN at 48 hours after AKI induction in WT and *Nlrp3*-KO mice. **C)** Kidney *Mcp-1*, *Il-6* and *Il-1β* mRNA level expression assessed by RT-PCR at 48 hours of AKI. **B, C)** Mean ± SD of 3-6 animals per group. ***p<0.001. **D)** Western blot of cleaved caspase 1. Note that cleaved caspase 1 is not observed in *Nlrp3*-KO mice with AKI, supporting the NLRP3-dependence of caspase 1 cleavage in this model and the lack of impact of NLRP3 and caspase 1 cleavage on kidney function and inflammation. Representative western blot.

Suppl. Figure 5. *Ripk3* expression is upregulated by TWEAK in cultured tubular cells and in BMDMs. MCT cells, TECs and BMDMs were stimulated with 100 ng/mL TWEAK for the indicated time periods and RIPK3 gene expression was assessed by RT-PCR. Mean ± SD of 4-5 independent experiments. **p<0.01 vs control.

Suppl. Figure 6. RIPK1 inhibition with Nec1 does not prevent TWEAK-induced cytokine gene expression in MCT cells or BMDMs. A, B) MCT cells and BMDMs were pretreated with Nec1 for 1 hour and then cells were stimulated with 100 ng/mL TWEAK for 3 hours. *Il-6* expression was assessed in MCT cells **(A)** and *Mcp-1, Il-6* and *Il-1β* expression were assessed in BMDMs **(B).** Mean ± SD of 3-4 independent experiments. *p<0.05; **p<0.01.

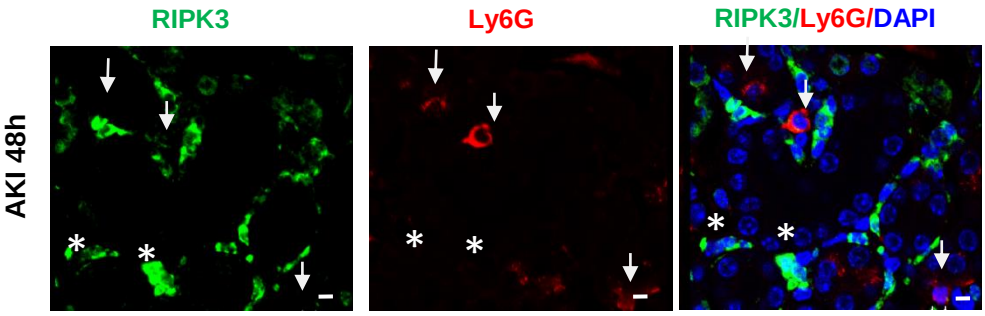
Suppl. Figure 7. RIPK3 partially mediates TWEAK-induced cytokine expression in BMDCs and in Jurkat cells. **A)** WT and *Ripk3*-KO BMDCs were stimulated with 100 ng/mL TWEAK for 3 hours and gene expression of *Mcp-1, Il-6* and *Il-1β* was assessed by RT-PCR. **B)** Jurkat cells were pre-treated with the RIPK3 inhibitor GSK´872 before stimulation with 100 ng/mL TWEAK for 3 hours. *Mcp-1* and *Il-6* mRNA was assessed by RT-PCR. Il-1β did not amplify. **A, B)** Mean ± SD 3 independent experiments. *p<0.05, **p<0.01. **C)** Immunofluorescence of NFκB p65 in Jurkat cells pretreated with the RIPK3 inhibitor GSK´872 and stimulated with 100 ng/mL TWEAK for 3 hours. Confocal microscopy. Magnification x800, scale bars 5 μm

Suppl. Figure 8. TWEAK injection does not increase cleaved caspase 1 in healthy kidneys. Western blot of cleaved caspase 1 in kidney protein extracts from WT and *Ripk3*-KO mice injected with 100 ng/mL TWEAK. Representative image of 2-4 animals per group. AKI WT mice were used as positive controls for cleaved caspase 1.

Suppl. Figure 9. Uncropped gel scans merged with molecular weight images for all presented Western blots. A) Corresponds to Fig. 1.C. **B-D)** Corresponds to Suppl. Fig. 2.

Supplementary figure 1

A)



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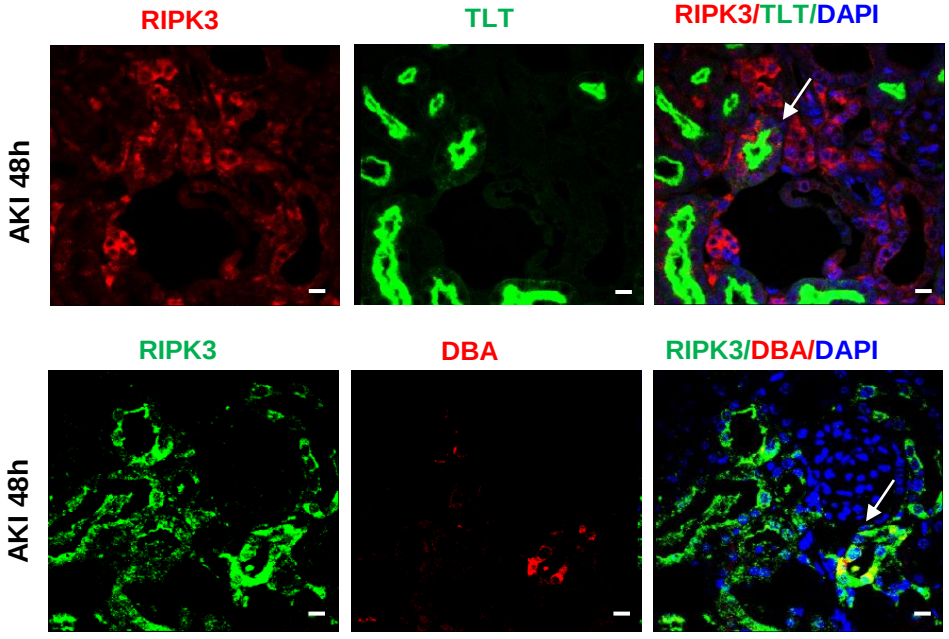

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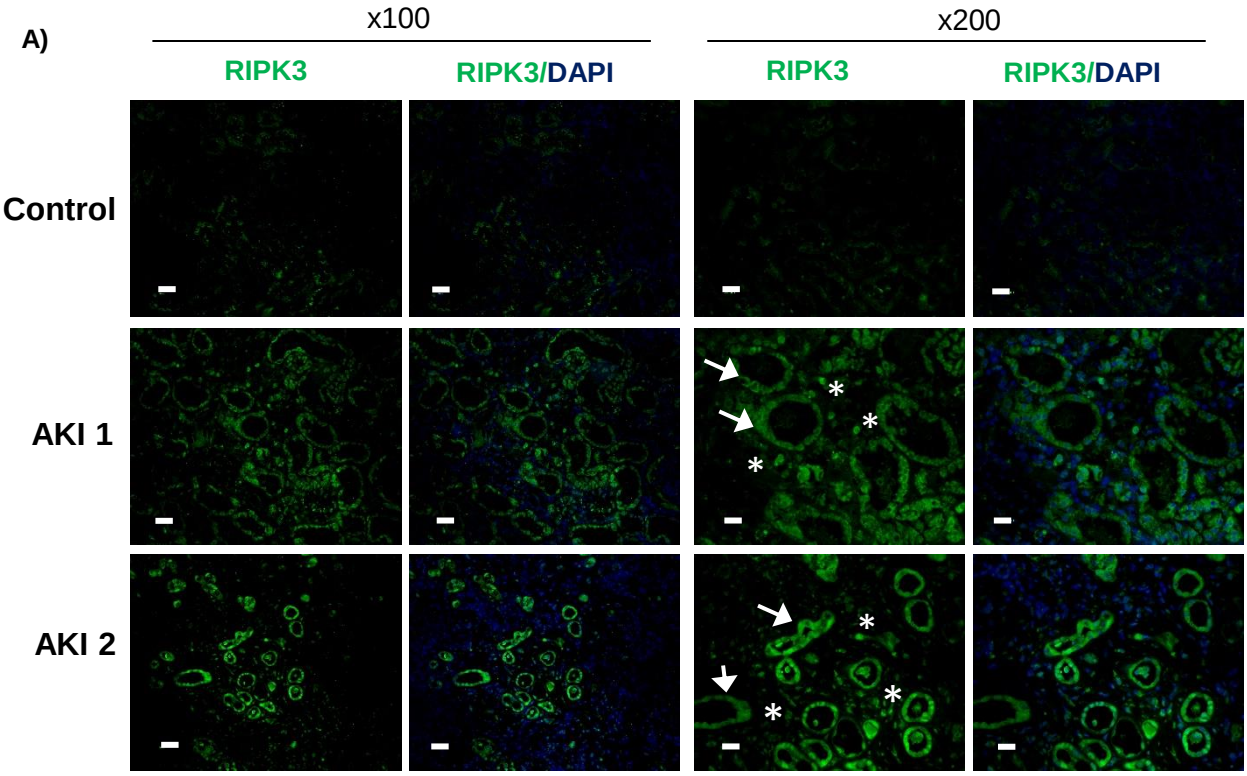

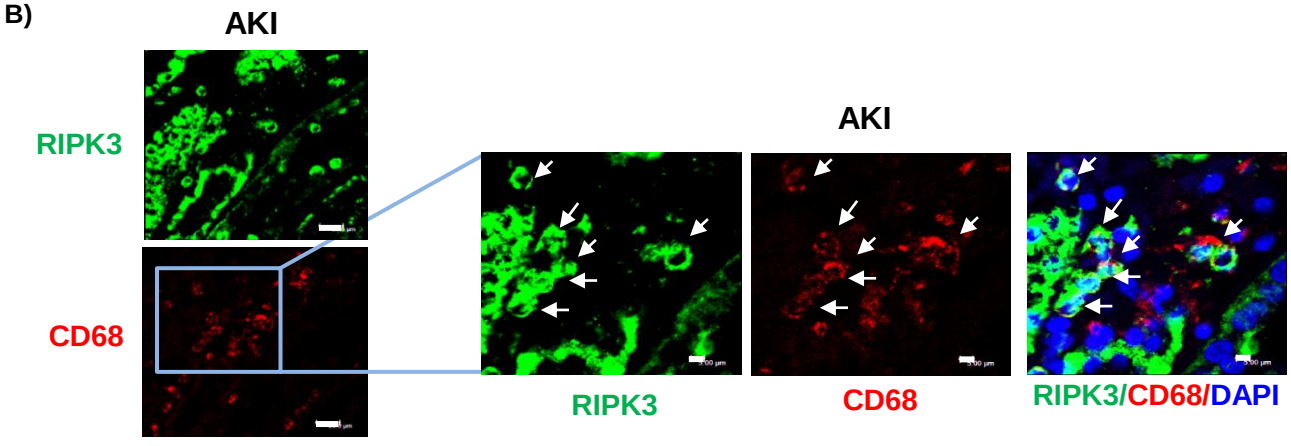

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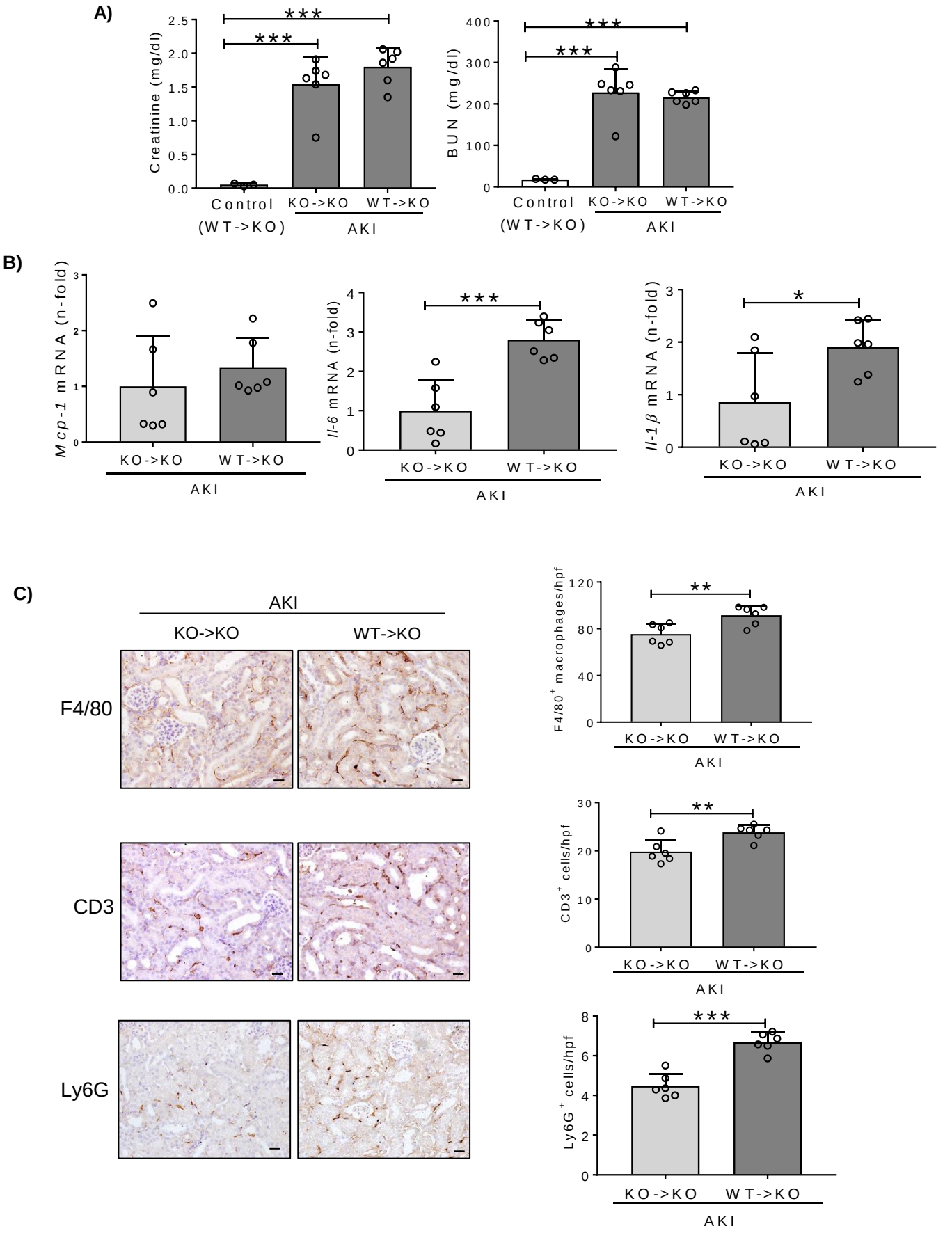

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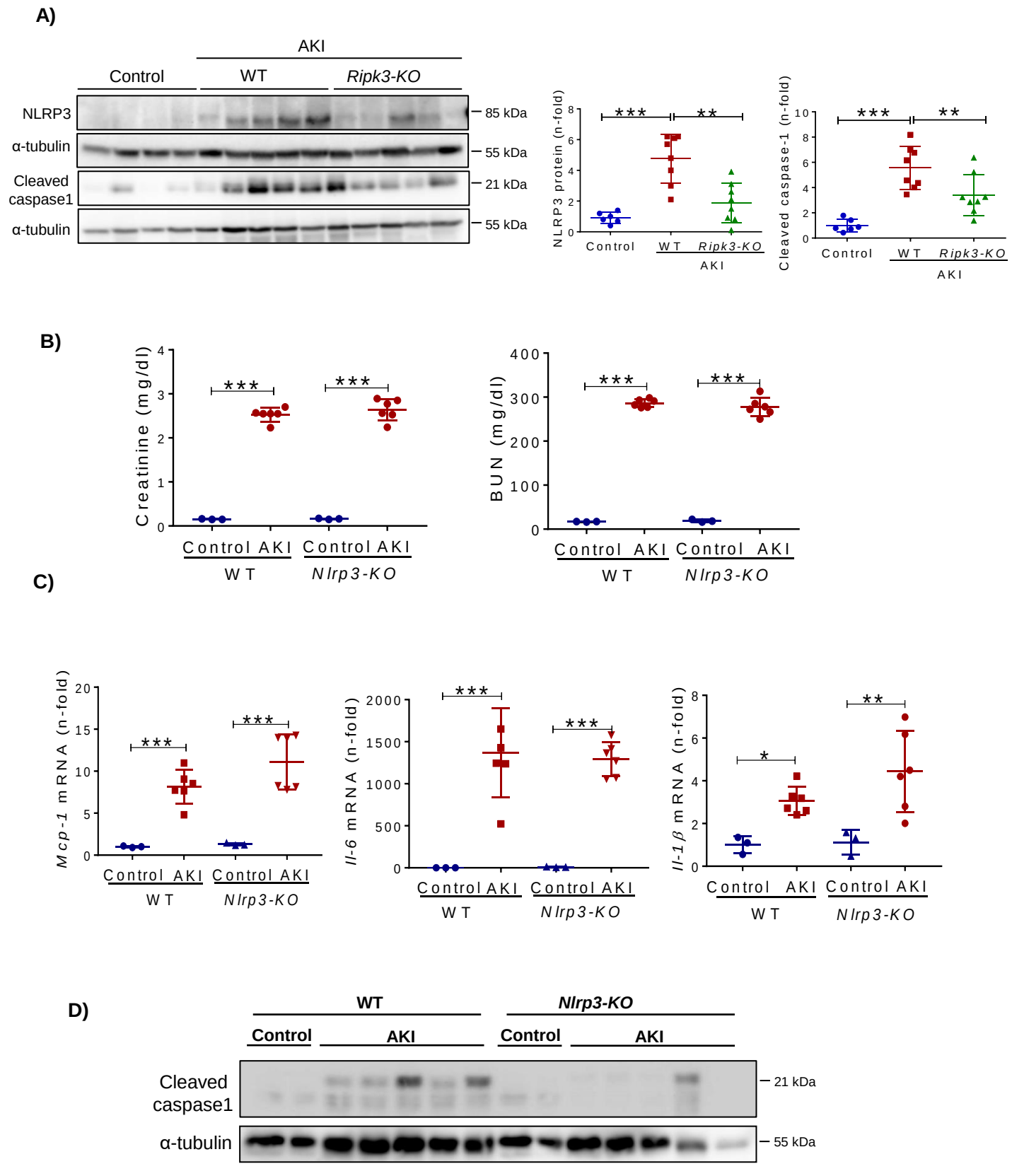

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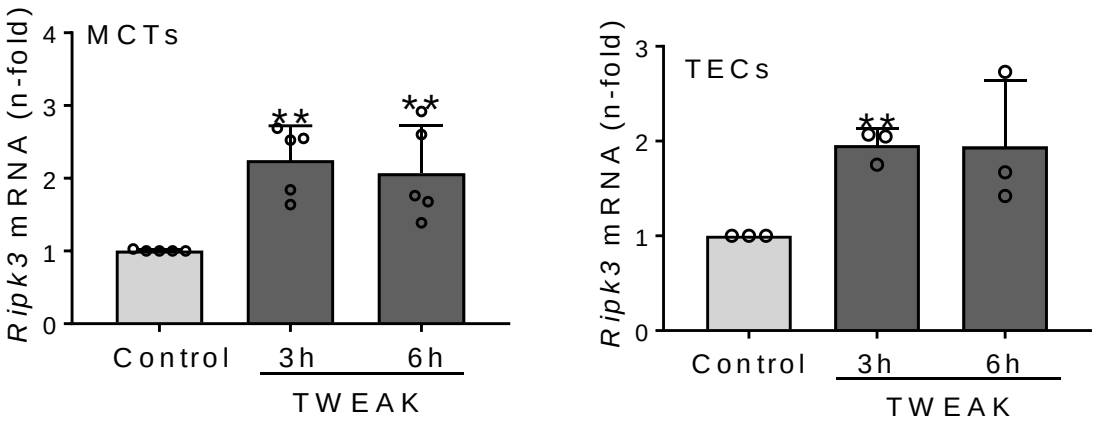

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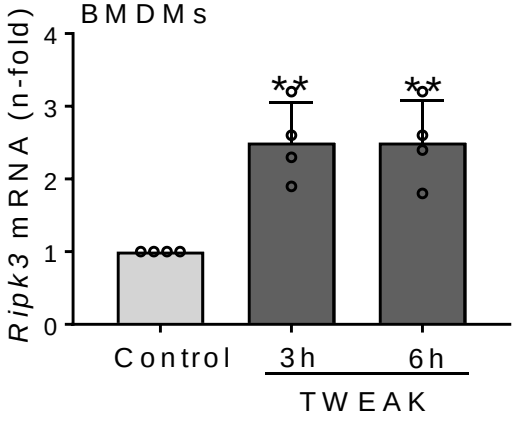

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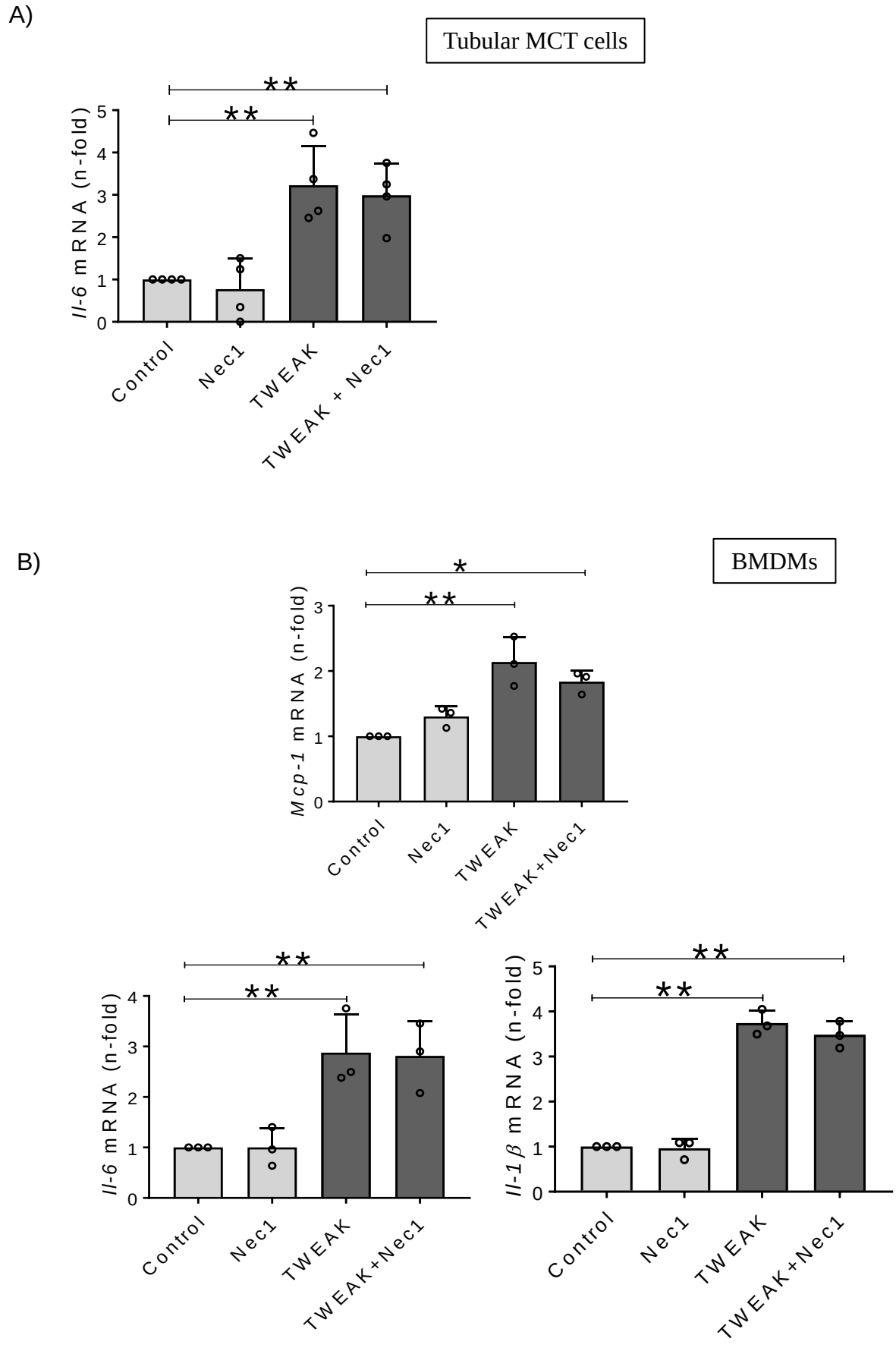

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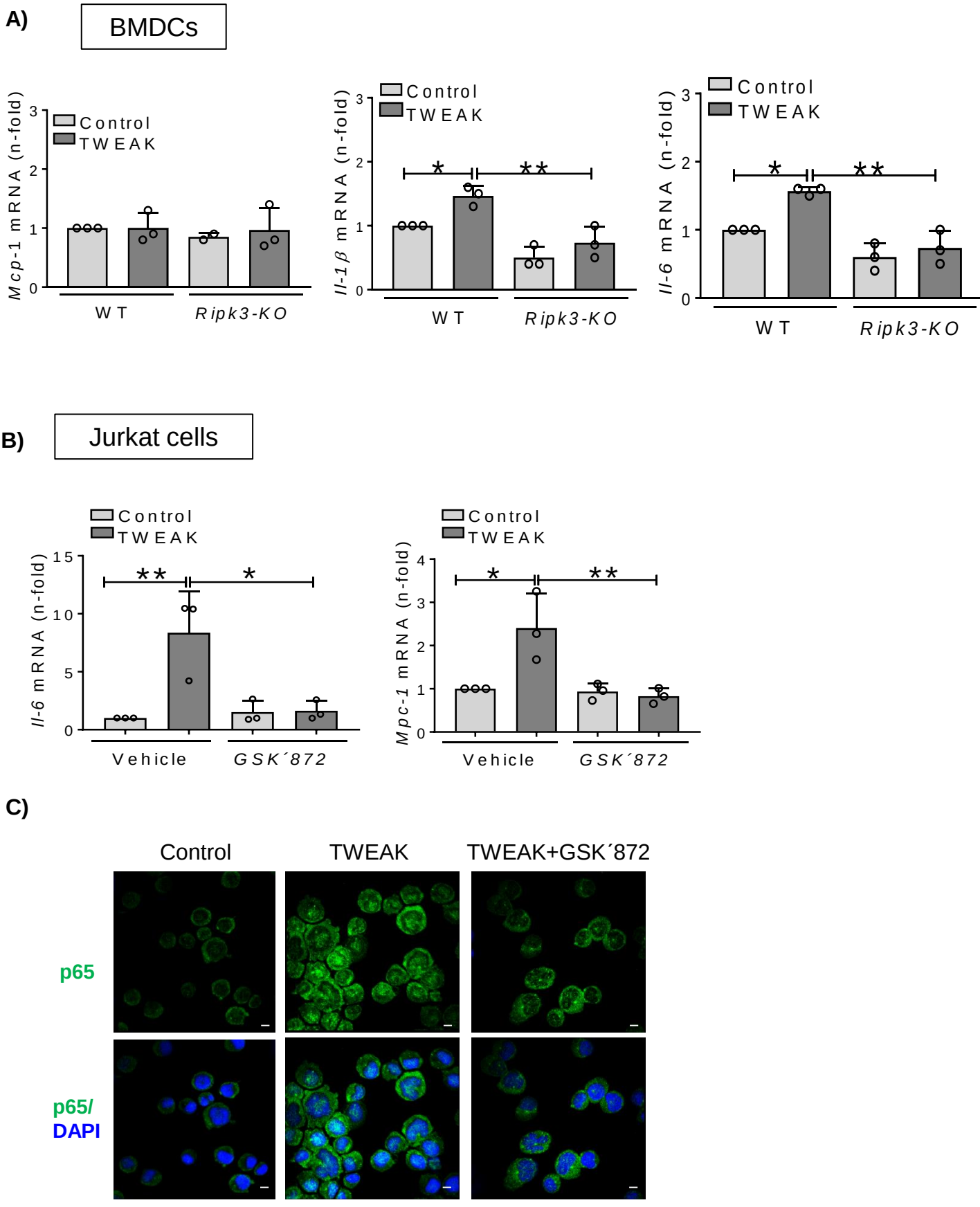

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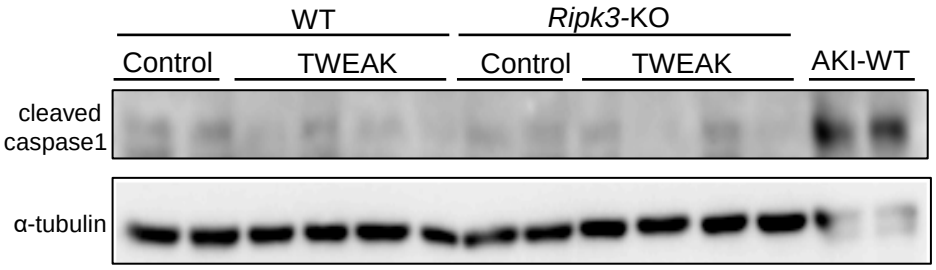
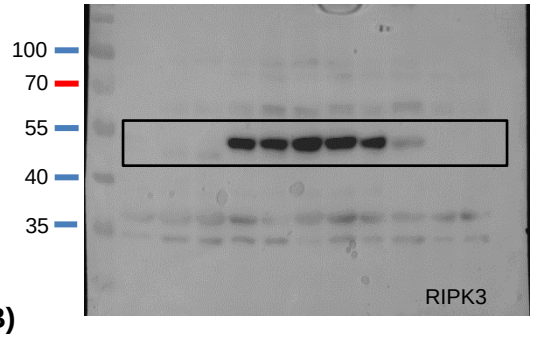
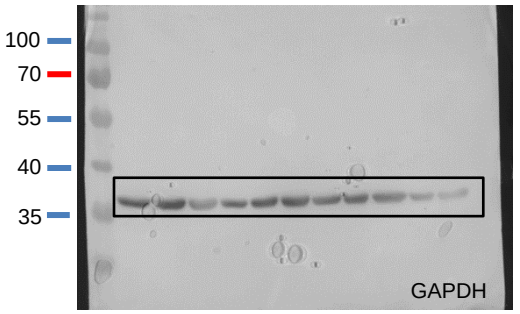


Supplementary figure 9

A)





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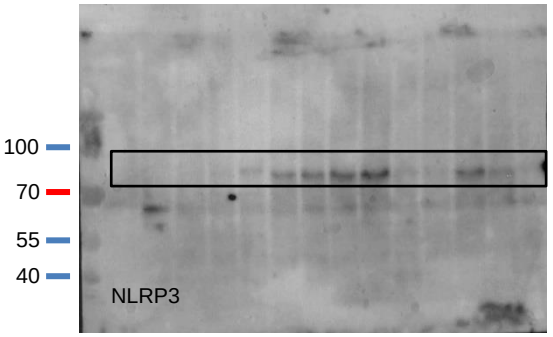

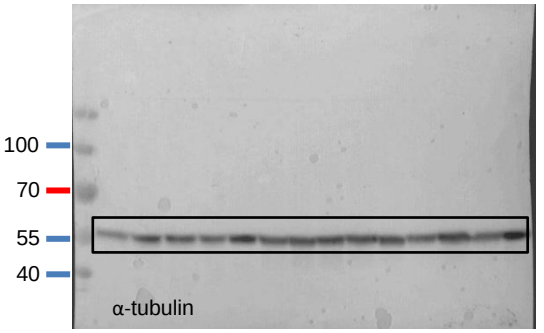

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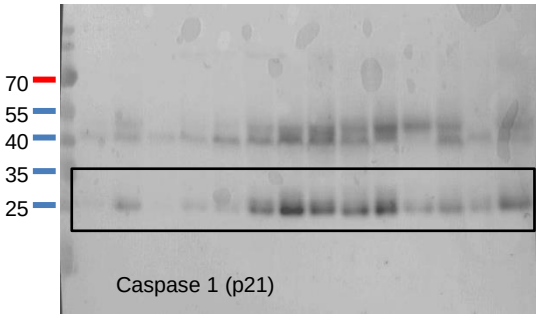

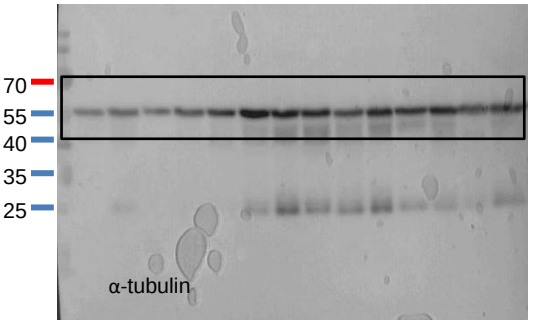

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