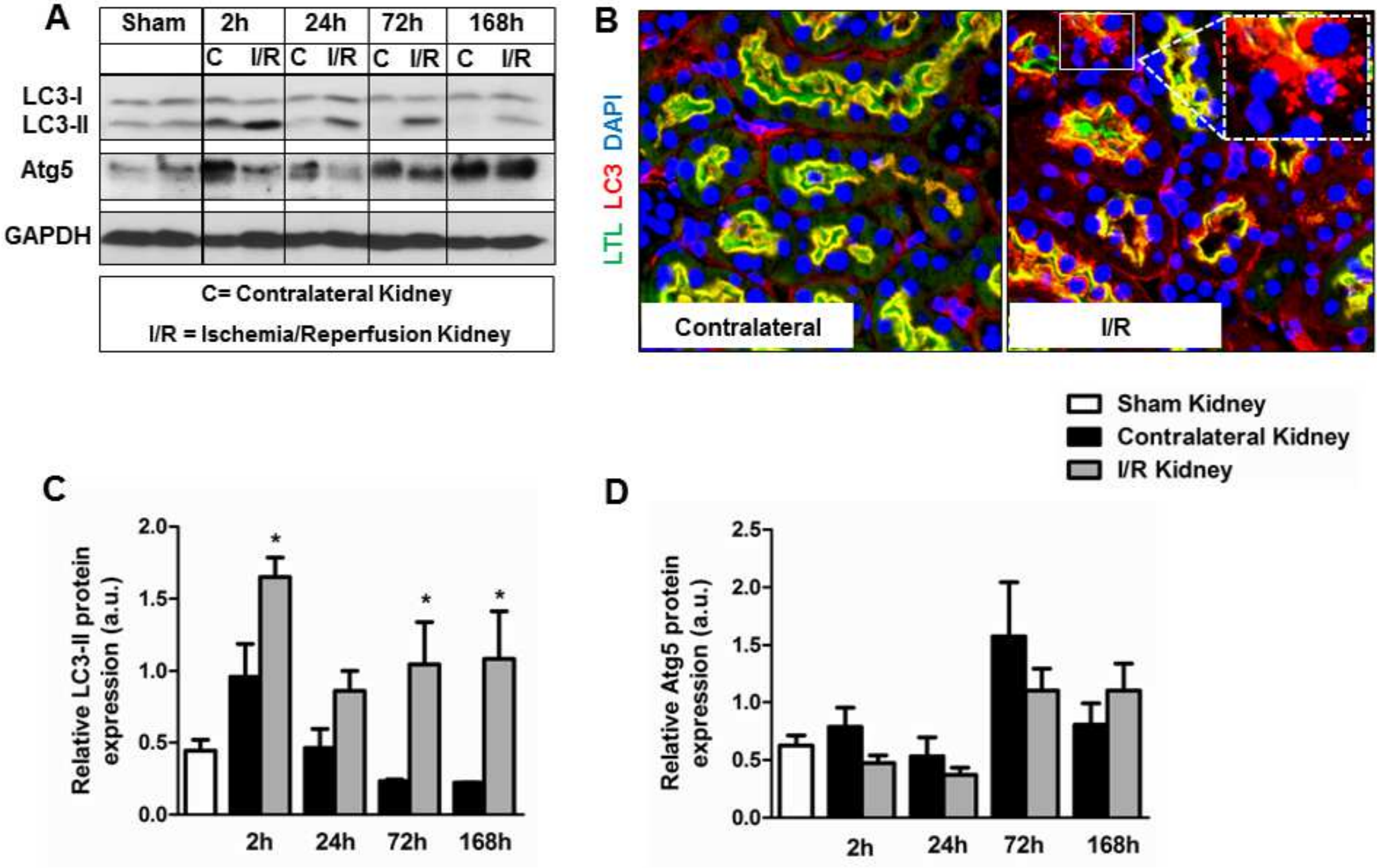
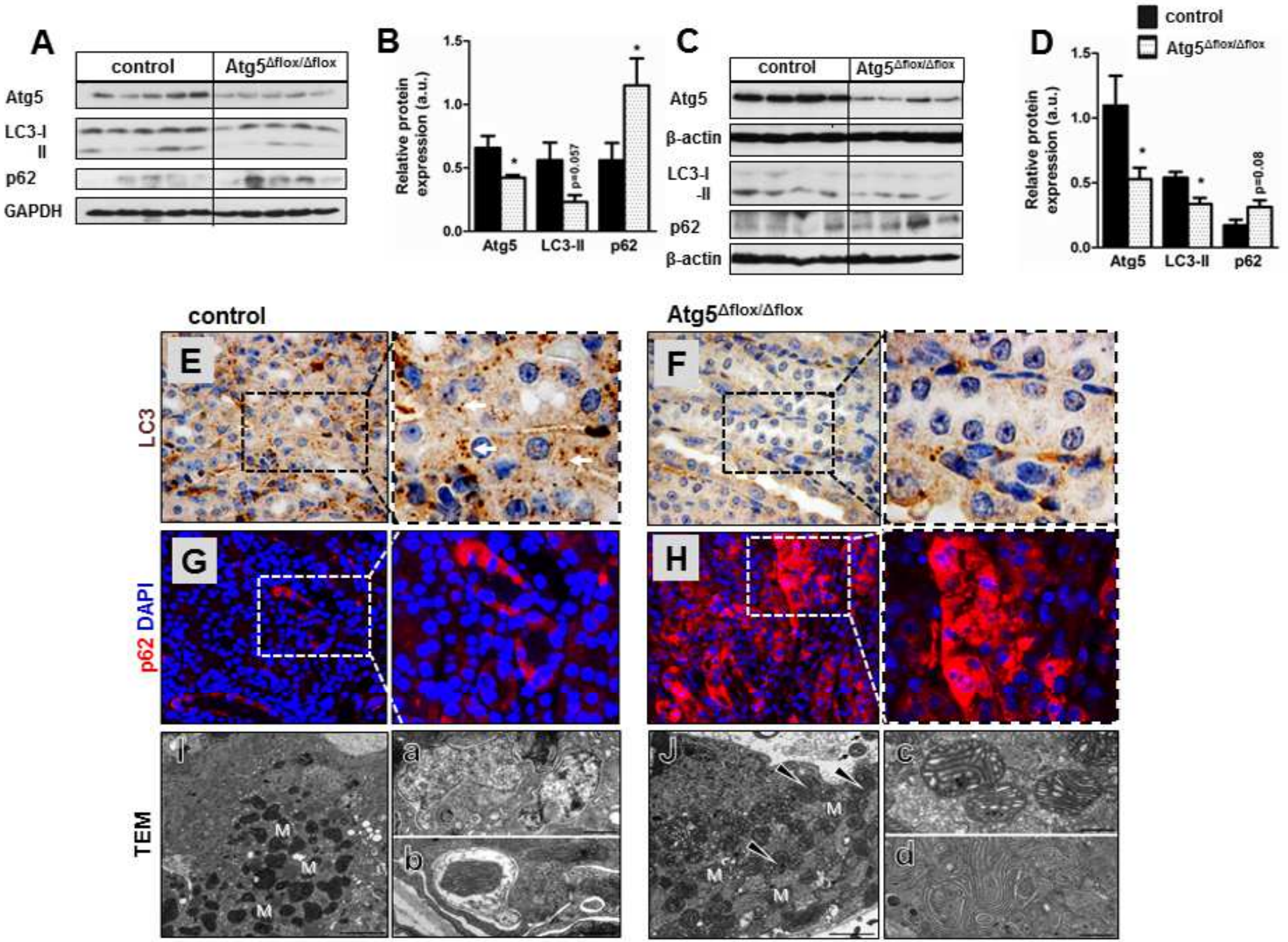
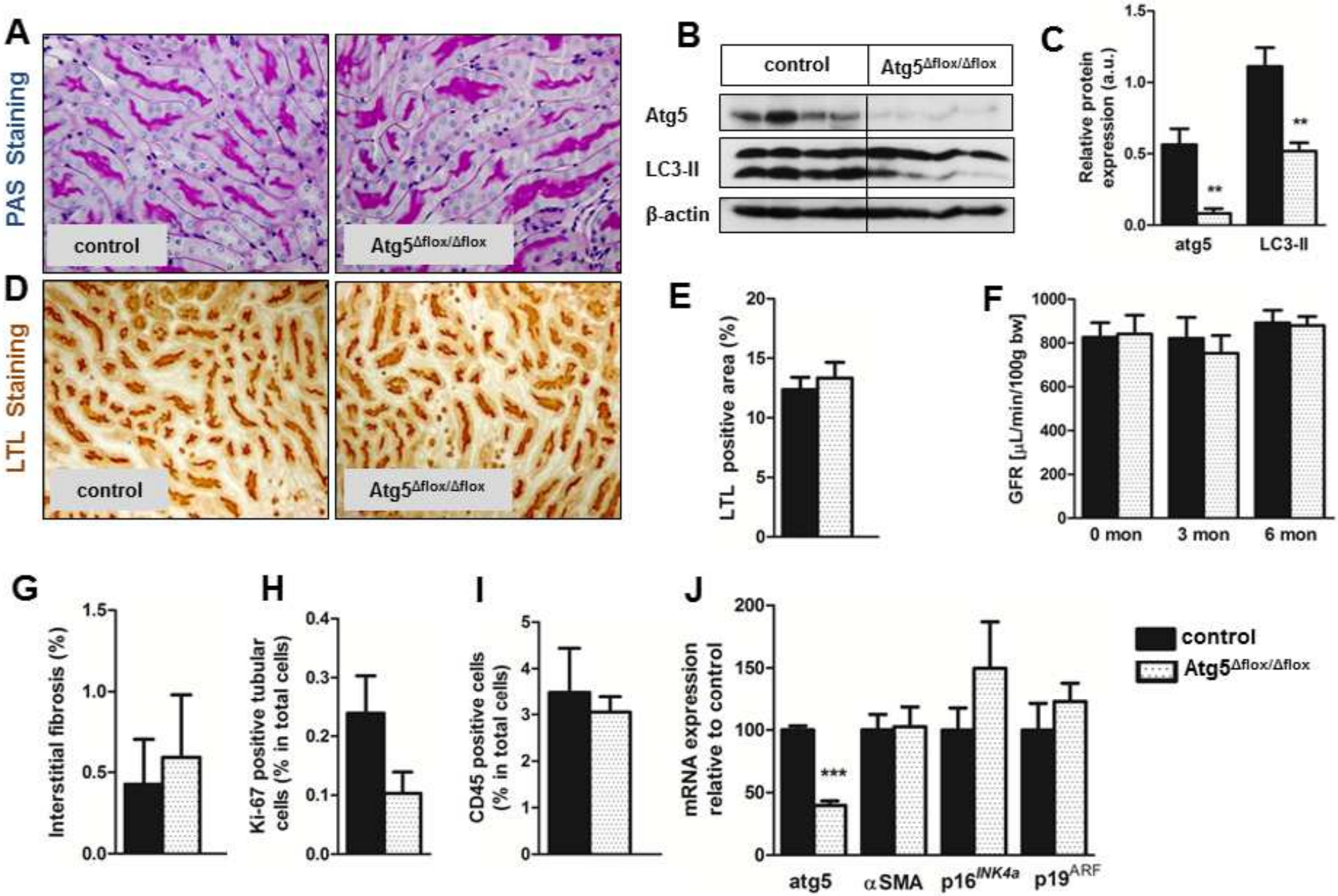


Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3

Supplementary Figure 1: Renal ischemia-reperfusion (I/R) induces a rapid and sustained activation of proximal tubular autophagy. (A) Representative immunoblots for LC3-II and Atg5 using kidney lysates of wild-type mice sacrificed at indicated time points after 27 min of unilateral I/R. **(B)** Co-immunostaining of brush border marker Lotus tetragonolobus lectin (LTL, green) and LC3 (red) showing enhanced LC3-II punctae at 6 hours after I/R (enlarged) during autophagy induction in post-ischemic kidneys. **(C-D)** Densitometry analysis of LC3-II and Atg5 protein relative to glyceraldehyde 3-phosphate dehydrogenase (GADPH) at the indicated time points after I/R. Original magnifications x 630 in B. Data is presented as mean values ± SEM. n=5-8. *P<0.05.

Supplementary Figure 2: Targeted Atg5 deletion impairs activation of autophagy after ischemia-reperfusion (I/R) in S3 segments of kidney proximal tubules. Control and $Atg5^{\Delta flox/\Delta flox}$ mice underwent 27 min or 16 min of renal I/R. Representative immunoblots for Atg5, LC3-II, p62, GAPDH and β-actin and densitometry analysis of kidney lysates enriched for outer medulla **(A-B)** at day 3 and **(C-D)** at day 30 after I/R. Representative immunohistochemistry of **(E-F)** LC3-II and **(G-H)** p62 in outer medulla revealing diminished LC3-II punctae (arrows) and p62 accumulation in $Atg5^{\Delta flox/\Delta flox}$ kidneys as compared to control kidneys. **(I-J)** Representative electron microscopy of S3 segment cells in control and $Atg5^{\Delta flox/\Delta flox}$ kidneys. While cells in control kidneys contain normal looking mitochondria (M), autolysosomes (inset a) and autophagosomes (inset b) altered mitochondria with swollen cristae (arrowheads, inset c) and multilamellar structures surrounding autophagosomal content (inset d) were detected in cells of $Atg5^{\Delta flox/\Delta flox}$ kidneys. Original magnifications x 400 in E and F; x 200 in G and H. Scale bars 2 µm in I and J; 500 nm in insets a-d. Data is presented as mean values ± SEM. n=5. *P<0.05.

Supplementary Figure 3: S3 segment specific Atg5 deletion does not result in structural or functional alterations in naïve unstressed kidneys. Unstressed control and $Atg5^{flox/flox}$ mice were followed until 9 months after tamoxifen injection. **(A)** Representative PAS staining from outer medulla of unstressed control and $Atg5^{flox/flox}$ mice **(B-C)** Representative immunoblots from kidney lysates enriched for outer medulla confirmed a reduction in Atg5 protein and loss of LC3 lipidation in $Atg5^{flox/flox}$ mice. **(D)** Representative Lotus tetragonolobus lectin (LTL) staining and **(E)** quantification revealed no significant changes in tubular integrity. No significant differences were observed in **(F)** GFR, **(G)** interstitial fibrosis, **(H)** proliferation, **(I)** infiltrating leukocytes and **(J)** fibrosis and senescence markers. Original magnifications x 400 in A; x 200 in D. Data is presented as mean values ± SEM. n=5. **P<0.005; ***P<0.001.