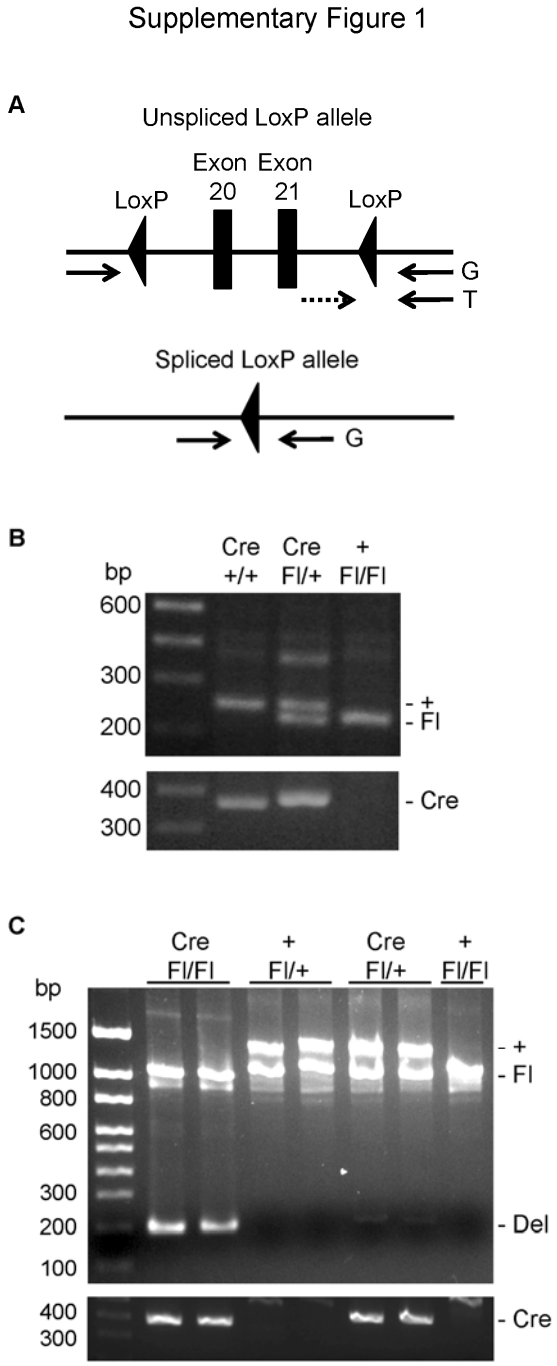
# Supplemental Materials

***Molecular Biology of the Cell***

Kaufman et al.

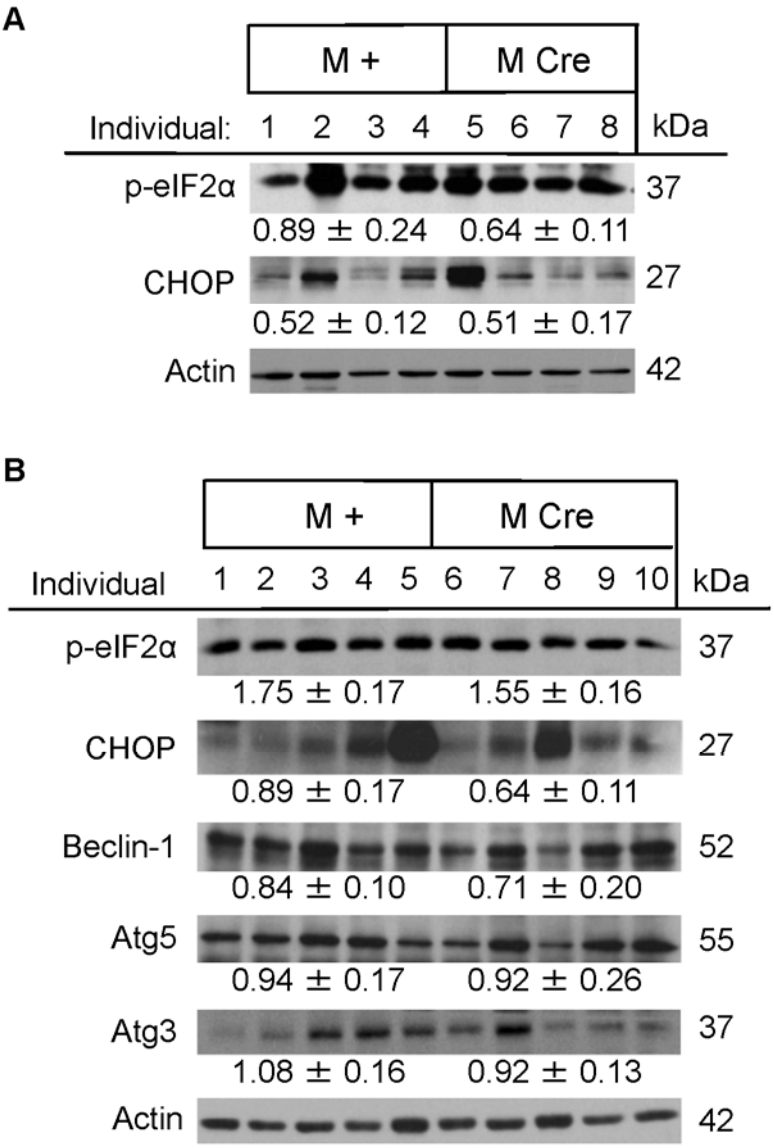
**Supplementary Materials**




Supplementary Figure 1. Podocin-Cre recombinase removes IRE1α exons 20-21 in podocytes. A) IRE1α allele containing LoxP sites (floxed or Fl allele), and positions of PCR primers used

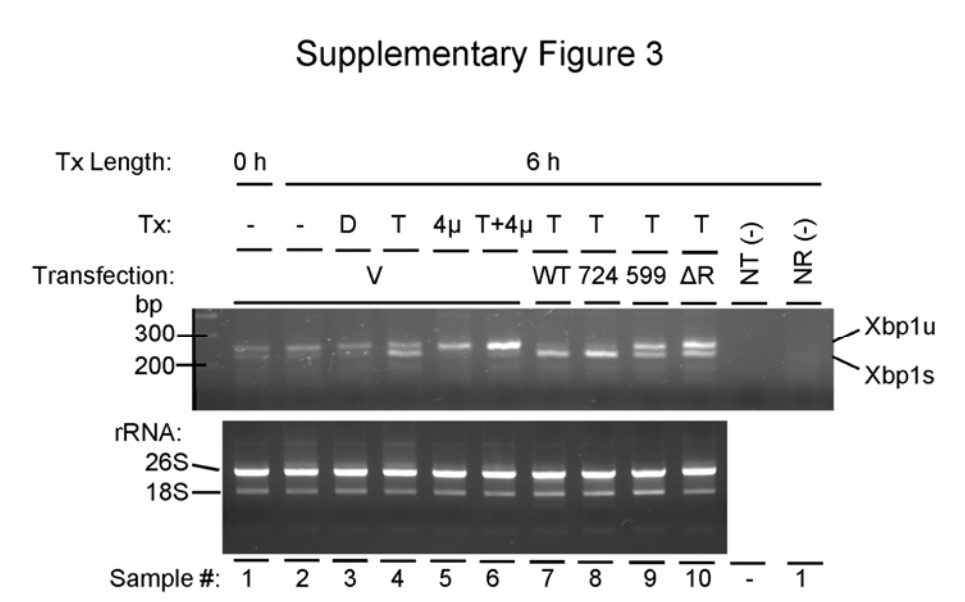
for tail (T) and glomerular (G) PCR reactions. B) Representative mouse tail DNA PCR shows a 254 bp IRE1α WT band (+/+ and Fl/+) and a 229 bp LoxP-containing unspliced band (Fl/+ and Fl/Fl). The lower panel shows a representative PCR for the podocin-Cre gene (374 bp). C) Glomerular DNA PCR shows a 1294 bp WT (+) allele product in Cre;Fl/+ and +;Fl/+ mice, a 1025 bp LoxP-containing unspliced-product (Fl) in all mice, and a 189 bp Cre-deletion product (Del) in Cre;Fl/Fl mice and to a lesser extent in Cre;Fl/+ mice. The 374 bp bands in the lower panel represent the podocin-Cre recombinase gene.

Supplementary Figure 2




Supplementary Figure 2. Effect of podocyte IRE1α deletion on markers of the UPR and autophagy. A) 4-5 month pre-albuminuric mice (4 per group).B) 9 month albuminuric mice (5 per group). Glomeruli were isolated from IRE1α$^{flox/flox;+}$ (M +) and IRE1α$^{flox/flox;Cre}$ (M Cre) mice. Lysates were immunoblotted with antibodies, as indicated. Densitometry measurements are

presented under each panel. There are no statistically significant differences between groups. (p-eIF2α, phospho-eIF2α).




Supplementary Figure 3 .Inhibition of IRE1α with IRE1α dominant negative mutants and 4μ8C. COS-1 cells were transiently transfected with vector (V), or FLAG-IRE1α WT or mutants, as indicated. After 24 h, cells were treated with vehicle (DMSO, D), tunicamycin (T, 10 μg/ml), the RNAse inhibitor, 4μ8c (4μ, 10 μM), or tunicamycin + 4μ8c, for 6 h. Xbp1 splicing was assessed with RT-PCR. In addition to unspliced (u) Xbp1 (247 bp), a small amount of spliced (s) Xbp1 (221 bp) is detectable in vector-transfected, untreated or DMSO-treated cells, which is reduced in the presence of 4μ8c. Tunicamycin induced Xbp1 splicing, which was abolished by 4μ8c. The tunicamycin-induced splicing was potentiated by IRE1α WT or S724A overexpression. Overexpression of IRE1α K599A or ΔR inhibited tunicamycin-induced Xbp1 splicing. Tx, treatment; rRNA, ribosomal RNA; NT, no template negative control; NR, no reverse transcriptase negative control.