**Supplemental Data**

# Interleukin-1β Activates a MYC-Dependent Metabolic Switch in Kidney Stromal Cells Necessary for Progressive Tubulointerstitial Fibrosis

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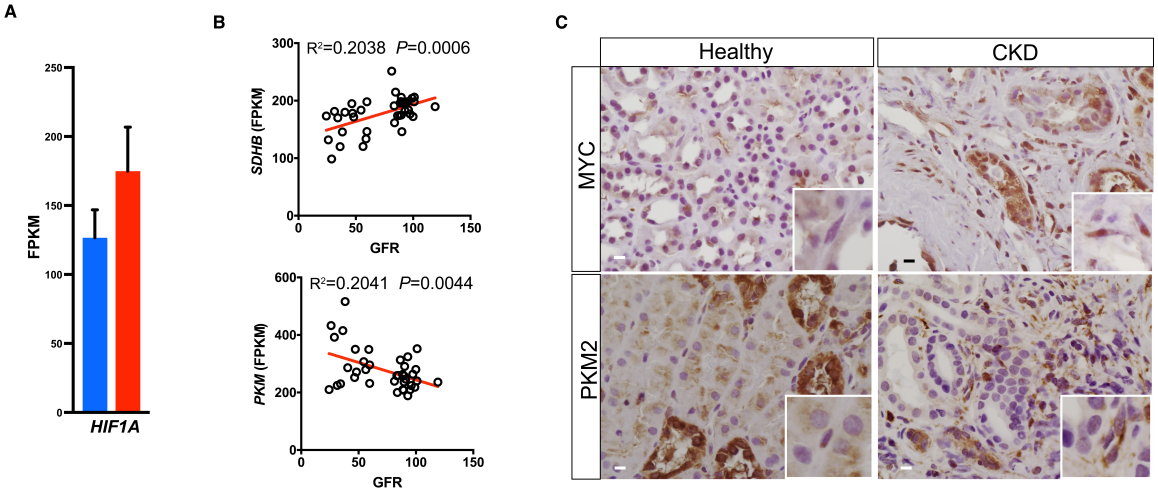
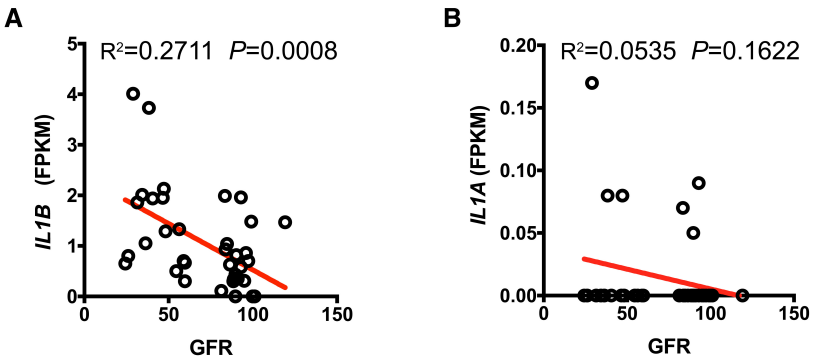
**Supplemental Figure 1**



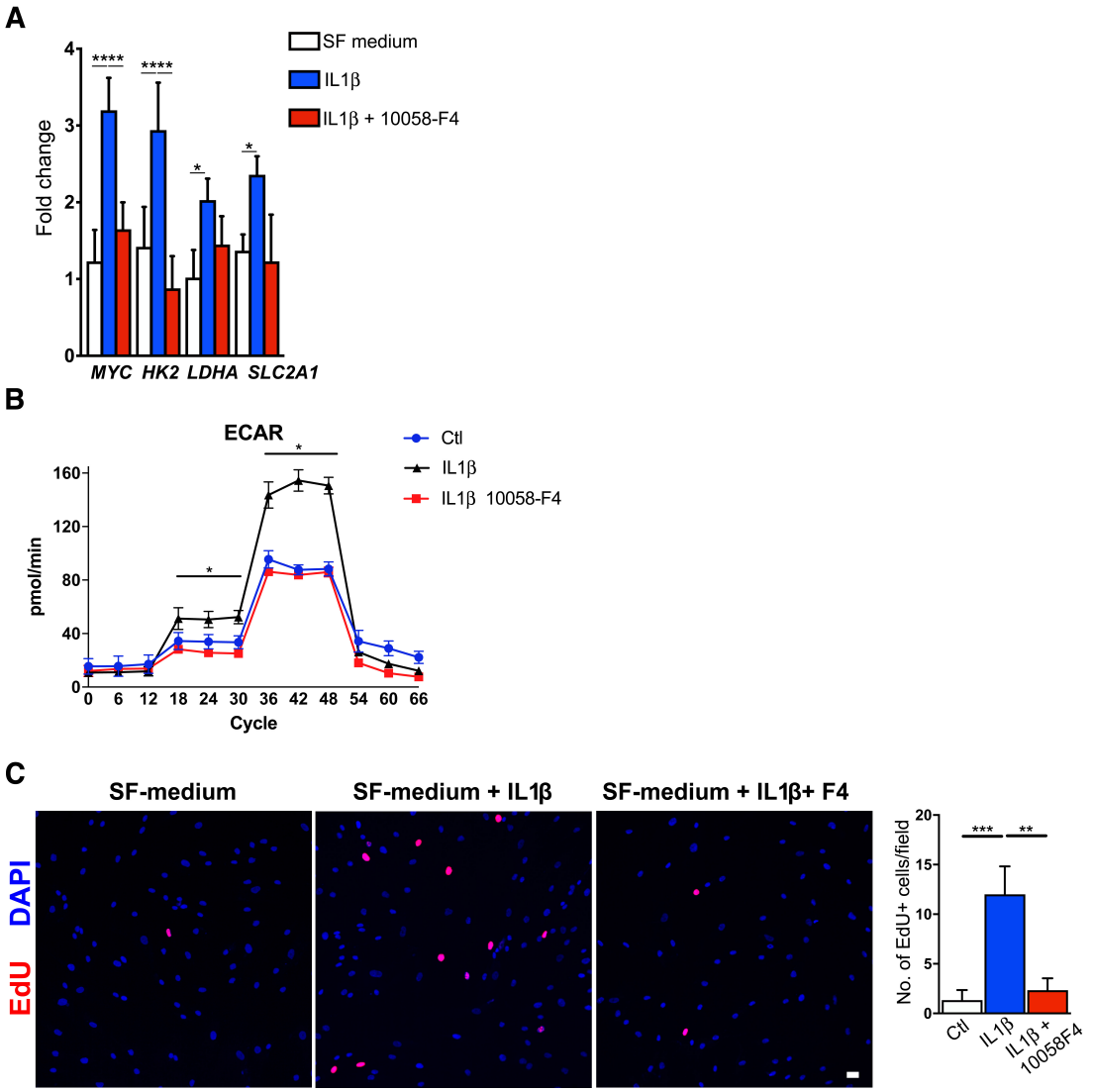

**Supplemental Figure 1.** Relationship of selected genes and proteins involved in metabolism to severity of CKD and cellular localization. **(**A) *HIF1A* expression in normal *versus* fibrotic human kidneys. Values are mean +/- SD. **P*< 0.05 *vs.* 10% fibrosis. Student's *t*-test. (B) Linear regression analysis of eGFR *versus SDHB* and *PKM* expression from a cohort of 38 healthy and CKD patients. (C) Representative immunostained images detecting MYC and PKM isoform 2 (brown stain, counterstained with hematoxylin to show nuclei [blue] in healthy and CKD kidney tissue sections. Insets show higher magnification of a single stromal cell (normal kidney) or SC-derived myofibroblast (CKD kidney). bar = 25μm.

## Supplemental Figure 2



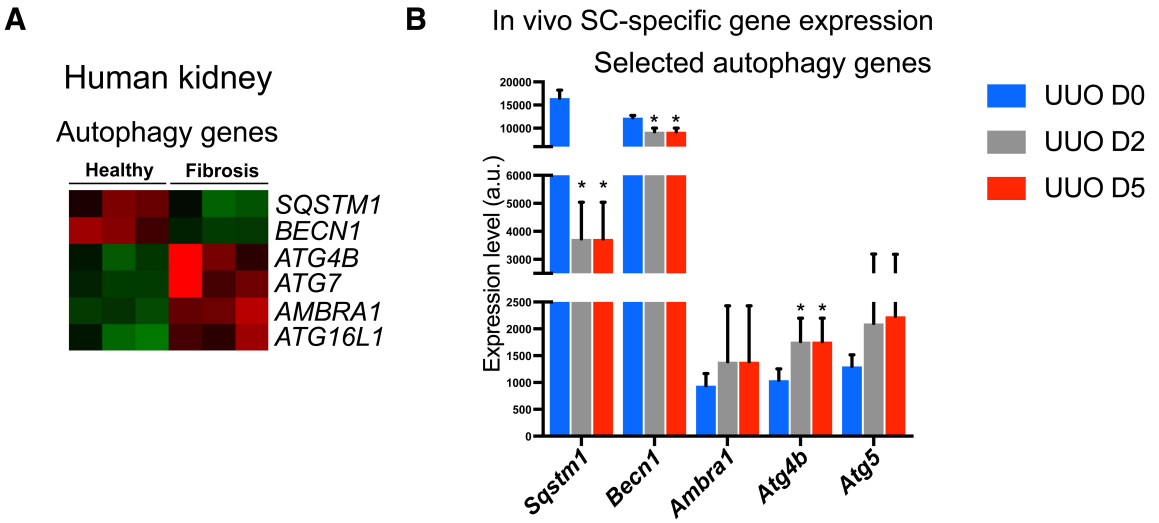

**Supplemental Figure 2.** Relationship of *IL1B* and *IL1A* levels to severity of CKD. **(**A-B) Linear regression analysis of eGFR *versus* *Il1B* (A) and *IL1A* (B) expression from a cohort of 38 healthy and CKD patients.

## Supplemental Figure 3



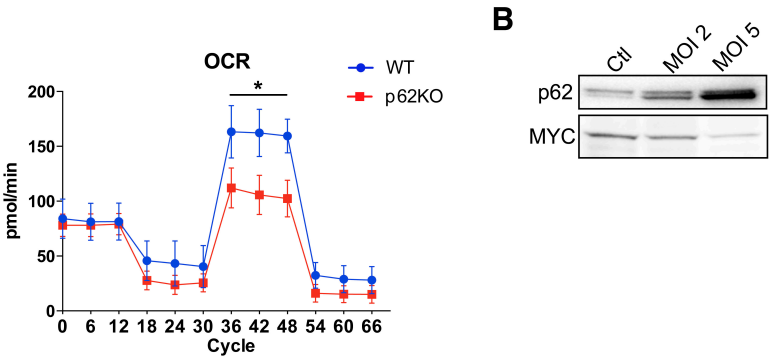

**Supplemental Figure 3.** Effect of the MYC inhibitor 10058-F4 on SC functions in response to IL1β. (A) Graph showing fold change in transcript levels of selected glycolytic pathway genes and MYC, following by IL1β +/- 10058-F4 (10μM) treatment of human kidney SCs. Values are mean +/- SD. * *P*< 0.05, ** *P*< 0.01. One-way ANOVA. **(**B) Extracellular acidification rate (ECAR) of human kidney PDGFRβ+ SCs incubated with IL1β for 48 hours in the presence or absence of 10 mM 10058-F4. Points on the curves represent mean +/- SD. **P*< 0.05. Student's *t*-test. *n* = 3/ point. (C) Images and graph demonstrating EdU incorporation into nuclei of human kidney PDGFRb+ SCs stimulated with IL1β for 48 hours in the presence or absence of 10058-F4, as a marker of proliferation. A four-hour EdU pulse was applied (bar = 25μm). Values are mean +/- SD. ** *P*< 0.01, *** *P*< 0.001. One-way ANOVA.

## Supplemental Figure 4



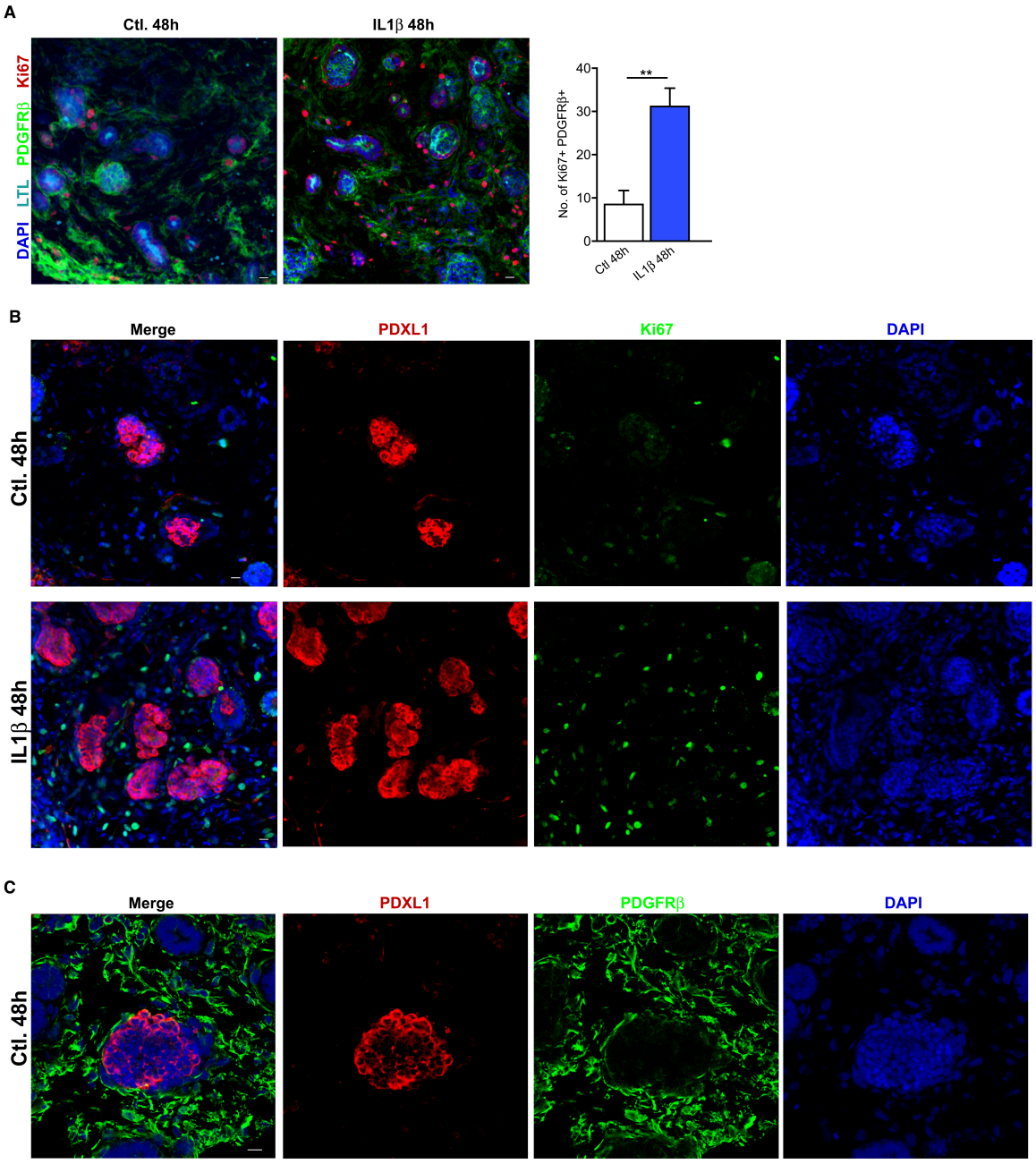

**Supplemental Figure 4.** Effect of kidney disease on transcripts for factors involved in autophagy in human kidney cortex and mouse kidney stromal cells in vivo. **(**A) Heatmap representing expression of selected transcripts of genes involved in autophagy in normal *versus* fibrotic human kidneys (red = high; green = low). (B) Graph showing in vivo SC-specific expression (absolute units [a.u.]) of translated mRNAs for factors involved in autophagy from UUO day 0 (D0), day 2 (D2) and day 5 (D5) purified from kidneys of *Col1a1-L10a-GFP* mice. Values are mean +/- SD. **P*< 0.05 *vs.* UUO D0. One-way ANOVA.

## Supplemental Figure 5




**Supplemental Figure 5.** Effect on human kidney SCs of deletion of SQSTM1 or lentiviral transduction with a *SQSTM1* transgene on oxygen consumption rate (OCR) and MYC protein levels respectively. **(**A) OCR measurement WT (blue curve) and SQSTM1/p62 KO (red curve) human kidney SCs. Points on the curves represent mean +/- SD. **P*< 0.05. Student's *t*-test. *n* = 3 points. Cycle 12-18 = treatment with oligomycin, cycle 30-36 = treatment with FCCP and cycle 48-54 = treatment with rotenone. (B) Representative western blot showing MYC and SQSTM1/p62 levels in human kidney SCs transduced with a lentivirus expressing SQSTM1/p62 under the CMV promoter (Ctl = control virus and MOI = multiplicity of infection of p62 producing virus where MOI = number of viral particles per cell).

**Supplemental Figure 6**




**Supplemental Figure 6.** Characterization of the effect of IL1β on proliferation in different cellular compartments of human kidney organoids. **(**A) Low magnification representative immunofluorescence photomicrographs showing Ki67 distribution in human kidney organoids treated with IL1b, where SCs express PDGFRβ, and proximal tubules express lotus tetragonolobus lectin (LTL) Graph (right panel) quantifiying Ki67+ cells in the SC compartment. (Values are mean +/- SD. ***P*< 0.01. Student's *t*-test. *n* = 3 organoids per treatment/time

point. (B) Representative split panel immunofluorescence photomicrographs showing the expression of Ki67 in glomeruli, where anti-podocalyxin antibodies (PDXL1) detect podocytes, in human kidney organoids treated with IL1b. Note podocytes to not express Ki67. (C) Representative immunofluorescence photomicrograph showing distribution of interstitial PDGFRb$^{+}$ SCs in relation to organoid glomeruli as detected by anti-podocalyxin antibodies. (bar = 25μm).

**Supplemental Table I**

| Gene | Taqman Probe | Species |
|---|---|---|
| *Acta2* | Mm00725412_s1 | Mouse |
| *Ccl2 (Mcp1)* | Mm00441242_m1 | Mouse |
| *Ccl3 (Mip1a)* | Mm00441259_g1 | Mouse |
| *Col1a1* | Mm00801666_g1 | Mouse |
| *Ctgf* | Mm01192932_g1 | Mouse |
| *Cxcl2 (Mip2)* | Mm00436450_m1 | Mouse |
| *Fn1* | Mm01256744_m1 | Mouse |
| *Gapdh* | Mm99999915_g1 | Mouse |
| *Havcr1 (Kim1)* | Mm00506686_m1 | Mouse |
| *Il1b* | Mm00434228_m1 | Mouse |
| *Il6* | Mm00446190_m1 | Mouse |
| *Myc* | Mm00487804_m1 | Mouse |
| *Sqstm1 (p62)* | Mm00448091_m1 | Mouse |
| *Tnf* | Mm00443258_m1 | Mouse |
| *Tgfb* | Mm01227699_m1 | Mouse |
| *GAPDH* | Hs02758991_g1 | Human |
| *ACTA2* | Hs00426835_g1 | Human |
| *COL1A1* | Hs00164004_m1 | Human |
| *FN1* | Hs01549976_m1 | Human |
| *HAVCR1 (KIM1)* | Hs00930379_g1 | Human |
| *HK2* | Hs00606086_,1 | Human |
| *LDHA* | Hs01378790_g1 | Human |
| *SQSTM1* | Hs01061917_g1 | Human |
| *TNF* | Hs00174128_m1 | Human |
| *MYC* | Hs00153408_m1 | Human |
| *SLC2A1* | Hs00892681_m1 | Human |
| *SLC2A3* | Hs00359840_m1 | Human |