Supplementary Figures

Supplementary Figure S1. Balance between mitochondrial fusion/fission proteins is impaired in a murine model of kidney fibrosis and TGF-β1 treated macrophages. Western blot and densitometry analysis for the expression of OPA1 (large form, 92 KDa; small form, 72 KDa), phosphorylated (serine-616) form of DRP1 (82 KDa, p-DRP1-Ser-616), and DRP1 (82 KDa) in **a)** kidney tissue lysates from wild-type mice fed with control (Ctl) or adenine diet (AD) for 28 days, normalized to GAPDH; and **b)** Bone marrow-derived macrophages cultured in the absence (-) or presence (+) of TGF-β1 (5 ng/ml) for 48 hours, normalized to β-actin. Data are mean ± SEM, (n = 6 per group). *P < 0.05 and **P < 0.01, analyzed by student's unpaired 1-tailed t-test.

Supplementary Figure S2. Mitochondrial fusion protein expression and mitochondrial colocalization are repressed in experimental kidney fibrosis, and loss of PINK1 or Parkin reduces expression of MFN2 but not MFN1. a, b) Representative images of immunofluorescence staining showing colocalization of MFN1 (green) (a) or MFN2 (green) (b), with TIM23 expressing mitochondria (red), mounted with DAPI (blue) containing mountant media in the kidney sections from *Pink1*$^{+/+}$ and *Pink1*$^{-/-}$ mice harvested 7 days after sham or unilateral ureteral obstruction (UUO) surgeries. Scale bars: 200 μm. **c - f)** Representative histogram showing the expression of MFN1 and MFN2 in CD11b+ F4/80+ kidney macrophages (c, d) and megalin+ proximal tubules (e, f) from wild-type, *Pink1*$^{-/-}$, and *Prkn*$^{-/-}$ mice harvested 7 days after sham or UUO (n = 5 per group). *P < 0.05, **P < 0.01, and **P < 0.01, analyzed by one-way ANOVA followed by Newman-Keuls post-hoc test.

Supplementary Figure S3. Myeloid cell-specific *Mfn1*-deficient kidney macrophages display higher expression of MFN2. Representative flow cytometry plots and analysis showing the expression of MFN2 in the CD45+ Side scatter (SSC)-CD11b+ F4/80+ kidney macrophages from *Mfn1$^{fl/fl}$,LysM-Cre$^{-/-}$* and *Mfn1$^{fl/fl}$,LysM-Cre$^{+/-}$* mice fed with control (Ctl) or adenine (AD) diet for 28-days. Data are mean ± SEM, (*n* = 5 per group). **P* < 0.05 and ****P* < 0.001, analyzed by one-way ANOVA followed by Newman-Keuls post-hoc test.

Supplementary Figure S4. Myeloid cell-specific *Mfn1/Mfn2* double knockout (DKO) mice exert higher circulating levels of macrophage chemoattractants during kidney fibrosis. a, b) Plasma chemokine CCL2 (a) and CX3CL1 (b) levels in wild-type and DKO mice fed with Ctl or AD for 28 days, quantitated using ELISA. Data are mean ± SEM representative of 3 independent experiments, (*n* = 5 per group). **P* < 0.05, ***P* < 0.01 and ***P* < 0.01, analyzed by one-way ANOVA followed by Newman-Keuls post-hoc test.

Supplementary Figure S5. *Mfn2*-deficient macrophages display higher profibrotic response. a) Western blot for the expression of CD206, fibronectin (FN), arginase I (Arg-I), and GAPDH in monocyte colony-stimulating factor (M-CSF)-treated bone marrow-derived macrophages (BMDM) isolated from wild-type, *Mfn1$^{fl/fl}$,LysM-Cre$^{+/-}$* and *Mfn2$^{fl/fl}$,LysM-Cre$^{+/-}$* mice and western blot for Arg-I and corresponding GAPDH in BMDM (cultured in presence or absence of TGF-β1 (5 ng/ml) for 48 hours) from wild-type, double knockout (DKO), *Mfn1$^{fl/fl}$,LysM-Cre$^{+/-}$* and *Mfn2$^{fl/fl}$,LysM-Cre$^{+/-}$* mice. The same membrane as shown in Figure 2e was reprobed for Arg-I. **b)** Representative flow cytometric plots showing the numbers of CD206+ F4/80+ cells in BMDM from wild-type,

Mfn1$^{fl/fl}$*,LysM-Cre*$^{+/-}$, and *Mfn2*$^{fl/fl}$*,LysM-Cre*$^{+/-}$. Data are mean ± SEM representative of 3 independent experiments (*n* = 5 per group).

Supplementary Figure S6. MFN2 regulates antioxidant response in macrophages and mitochondrial injury marker increases after adenine-induced kidney fibrosis and in patients with chronic kidney disease (CKD). a, b) Western blot analysis for superoxide dismutase-2 (SOD-2) in the kidney lysates from wild-type mice fed with control (Ctl) or adenine (AD) diet, normalized to GAPDH (a) and bone marrow-derived macrophages (BMDM) isolated from wild-type, *Mfn1/Mfn2* double knockout (DKO), *Mfn1*$^{fl/fl}$*,LysM-Cre*$^{+/-}$, and *Mfn2*$^{fl/fl}$*,LysM-Cre*$^{+/-}$ mice treated with TGF-β1 (5 ng/ml) for 48 hours. The same membrane as shown in Figure 2e was reprobed for SOD-2. Data are mean ± SEM representative of 3 independent experiments (*n* = 6 per group). **c - f)** Cytochrome c levels in the urine (c) and plasma (d) samples from Ctl or AD fed *Mfn2*$^{fl/fl}$*,LysM-Cre*$^{-/-}$ and *Mfn2*$^{fl/fl}$*,LysM-Cre*$^{+/-}$ mice for 28 days (*n* = 5 per group) and urine (e) from patients with (CKD+, *n* = 7) or controls without (CKD-, *n* = 7) CKD and plasma (f) in CKD+ (*n* = 7) or CKD- (*n* = 9) were determined by ELISA. Data are mean ± SEM representative of 3 independent experiments. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, analyzed by student's unpaired 2-tailed *t*-test (a, e, f) or one-way ANOVA followed by Newman-Keuls post-hoc test (c, d).

Supplementary Figure S7. Mitochondrial fusion protein expression is downregulated in human CKD kidneys and is associated with increased urinary macrophage chemoattractant. a) Relative mRNA expression of MFN1 normalized with β-actin (ACTB) determined using TaqMan qPCR in kidney biopsy from patients with (CKD+, *n* = 6) and controls without (CKD-, *n* = 9) CKD. **b)** Urinary CX3CL1

normalized to urine creatinine in CKD+ (n = 6) and CKD- (n = 9) were quantitated using ELISA. Data are mean ± SEM. **P < 0.01 and ***P < 0.001, and analyzed by student's unpaired 2-tailed t-test.