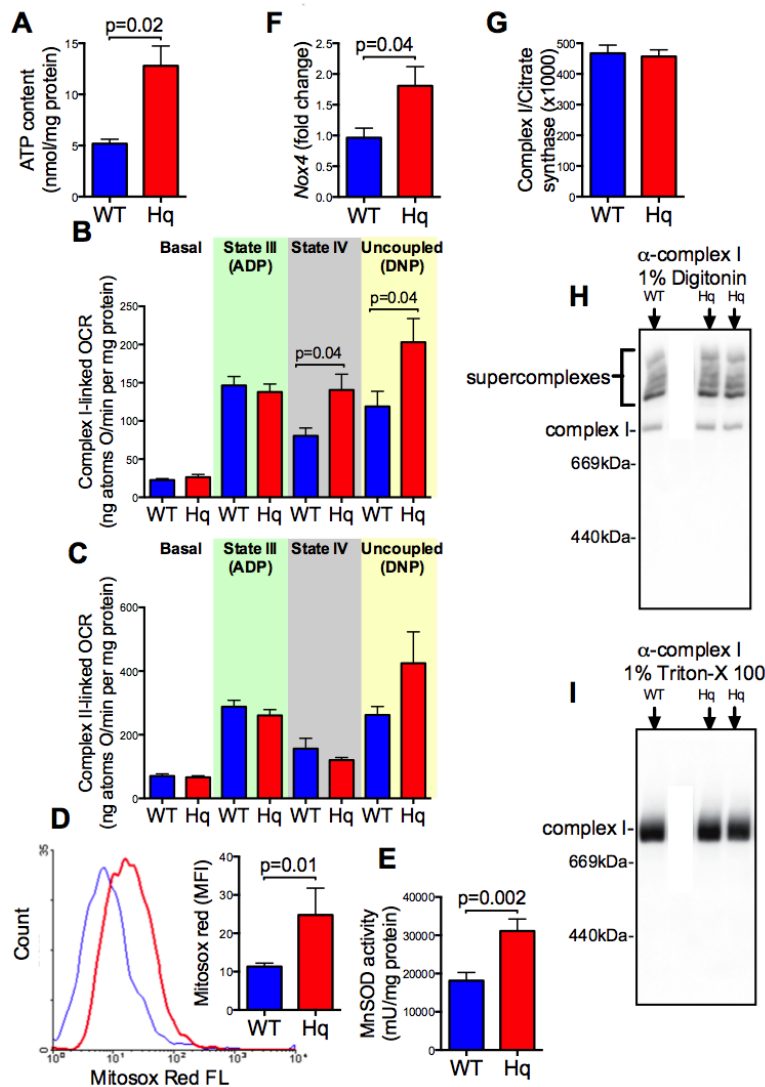


Supplementary Information Coughlan MT et al., **Deficiency in apoptosis inducing factor recapitulates chronic kidney disease via aberrant mitochondrial homeostasis**

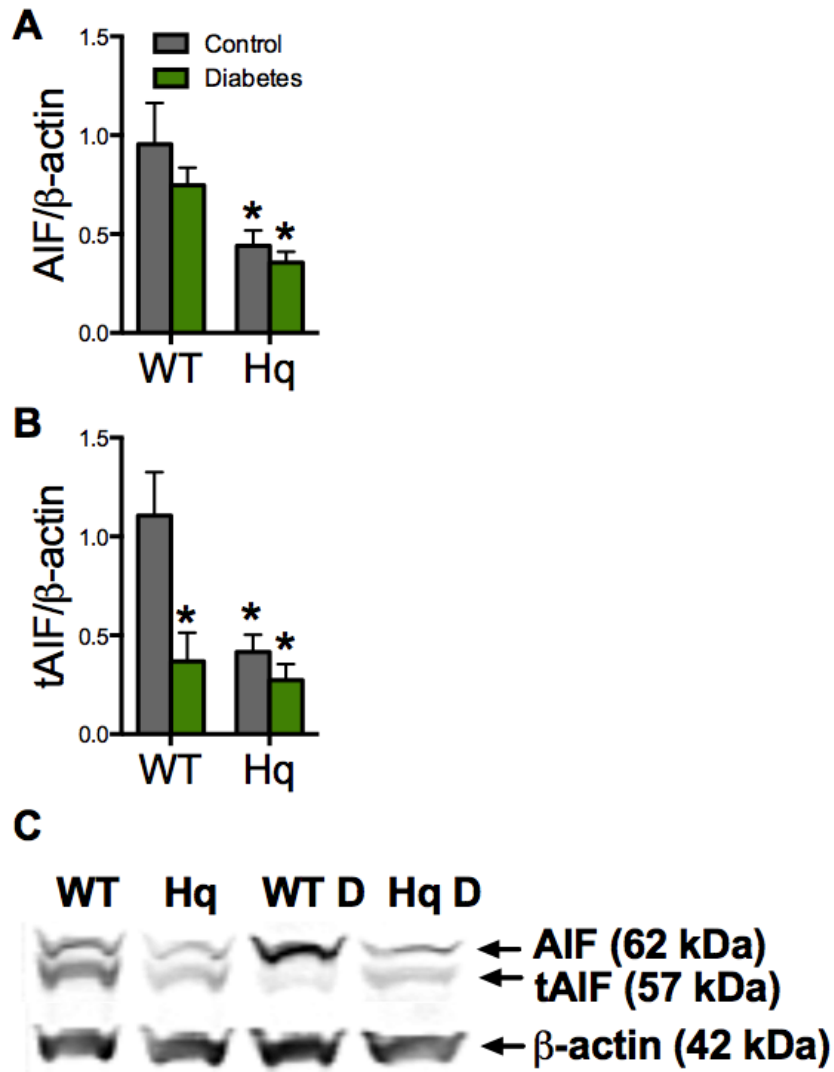
Supplementary Figure S1. AIF knockdown induces a shift in mitochondrial bioenergetics. (A) Mitochondrial ATP content. (B-C) Oxygen consumption rates were measured in isolated renal mitochondria from WT and Hq mice. Basal, ADP-stimulated State III (0.2mM), State IV and DNP-stimulated uncoupled respiration (0.06mM) were measured in the presence of (B) complex I substrates (5mM glutamate, 1mM malate) and (C) complex II substrates (5mM succinate) ± 1µM rotenone). (D) Representative flow cytometry experiment in isolated mitochondria treated with 5µM Mitosox red to determine superoxide production. WT (Blue histogram), Hq (red histogram). Inset: quantification of mitochondrial superoxide generation. (E) Manganese superoxide dismutase (SOD2) activity in isolated mitochondria. (F) Nox-4 mRNA expression. (G) Mitochondrial Complex I activity standardized to citrate synthase activity. Data are represented as mean±SEM, n=5 mice per group. Differences between samples were analyzed using a two-tailed Student’s t-test or a Mann-Whitney test. (H-I) Blue native (BN)-PAGE of mitochondrial extracts showing complex I assembly. (H) Solubilized in 1% (w/v) digitonin to retain complexes in supercomplex form and (I) Solubilized in 1% (w/v) Triton-X 100 to dissociate supercomplexes into smaller complexes and holocomplexes. Second lane on the gels shown in G and H contain an unrelated sample, which has been deleted from view.



SUPPLEMENTARY DATA

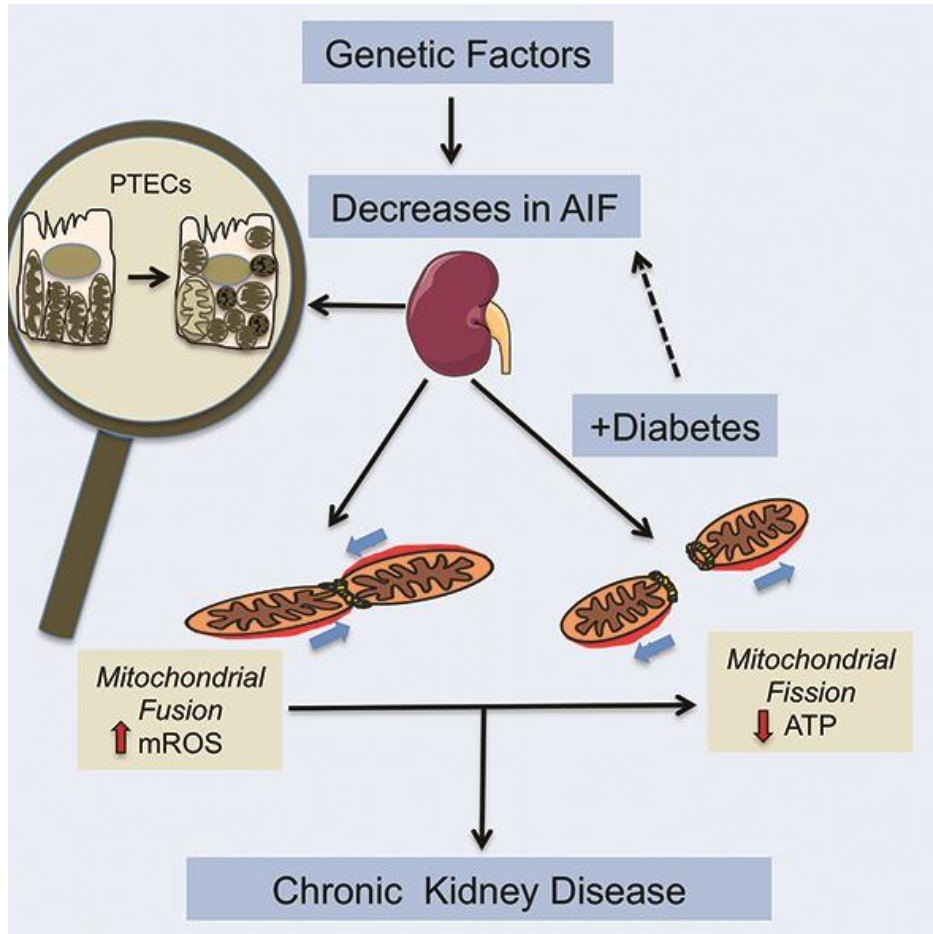
Supplementary Figure S2. tAIF protein content of renal cortices decreases in diabetes.

Western blots of renal cortical lysates showing quantitation of (A) the mature isoform of AIF (62 kDa) and (B) the truncated isoform of AIF (tAIF, 57 kDa). The grey bar represents control mice and the green bar represents mice with diabetes. * $p < 0.05$ compared to WT, $n = 5$ mice per group. (C) Representative western blot image. β -actin was used as a loading control (bottom).



Supplementary Figure S3. Schematic representation of the interplay between AIF and diabetes to promote renal injury.

Apoptosis inducing factor (AIF) is decreased in the kidney in human and experimental diabetes and its partial knockdown *in vivo* recapitulates chronic kidney disease (CKD). Once diabetes is superimposed onto AIF knockdown, the proximal tubule epithelial cell (PTEC) mitochondrial network is remodelled resulting in a shift towards fission, disrupting mitochondrial bioenergetics leading to a depleted ATP pool and an increase in mitochondrial ROS (mROS), thereby exacerbating the renal lesion.



SUPPLEMENTARY DATA

Supplementary Table S1. Primer sequences used for quantitative PCR

<i>Gene</i>	<i>Forward primer (5'-3')</i>	<i>Probe</i>
<i>Aifm1 (mouse)</i>	CCTCAGATCAGGGCACCAA	6-FAM CACGTCCCTTTCCTG
<i>AIFM1 (human, cell culture)</i>	GGGAGGACTACGGCAAAGGT	6-FAM TCATCTTCTACCTCAGGGAC
<i>AIFM1 (human, renal biopsies)</i>	CCAGTCATATTTTCTCCAGCCA	56-FAM/CT ACC CGC C/Zen/T CCT TCC CAA CTT T/3IABkFQ/
<i>Tfam (mouse)</i>	CCTCGGTCAGCATATAACATTT ACG	SYB
<i>Ppargc1α (mouse)</i>	TGAGGACCGCTAGCAAGTTT	SYB
<i>Mff (mouse)</i>	GCAGTTGGCAGGCTAAAAAG	SYB
<i>Nox4 (mouse)</i>	AAAAATATCACACACTGAATTC GAGACT	6- FAM CATTTTGCTATTTTCATCAAA
<i>Fn-1 (mouse)</i>	ACATGGCTTTAGGGCGGACAA	6- FAM CCCCCTCAGGCTTA

SUPPLEMENTARY DATA

Supplementary Table S2. Clinical characteristics of participants from which renal biopsies were obtained for AIF protein localisation.

	Control (n=2-6*)	Diabetic (n=15)
Gender (M/F)	3/3	12/3
eGFR (ml/min/1.73m²)	71±5	40±20
Urinary albumin (mg/L)	NA	1044±1213

*Four out of six of the non-diabetic controls were kidney donors, hence clinical data are not available (NA). It is presumed that the donor kidneys were normal. eGFR data for controls are n=2 only, therefore statistical analyses (*t*-test) cannot be performed.