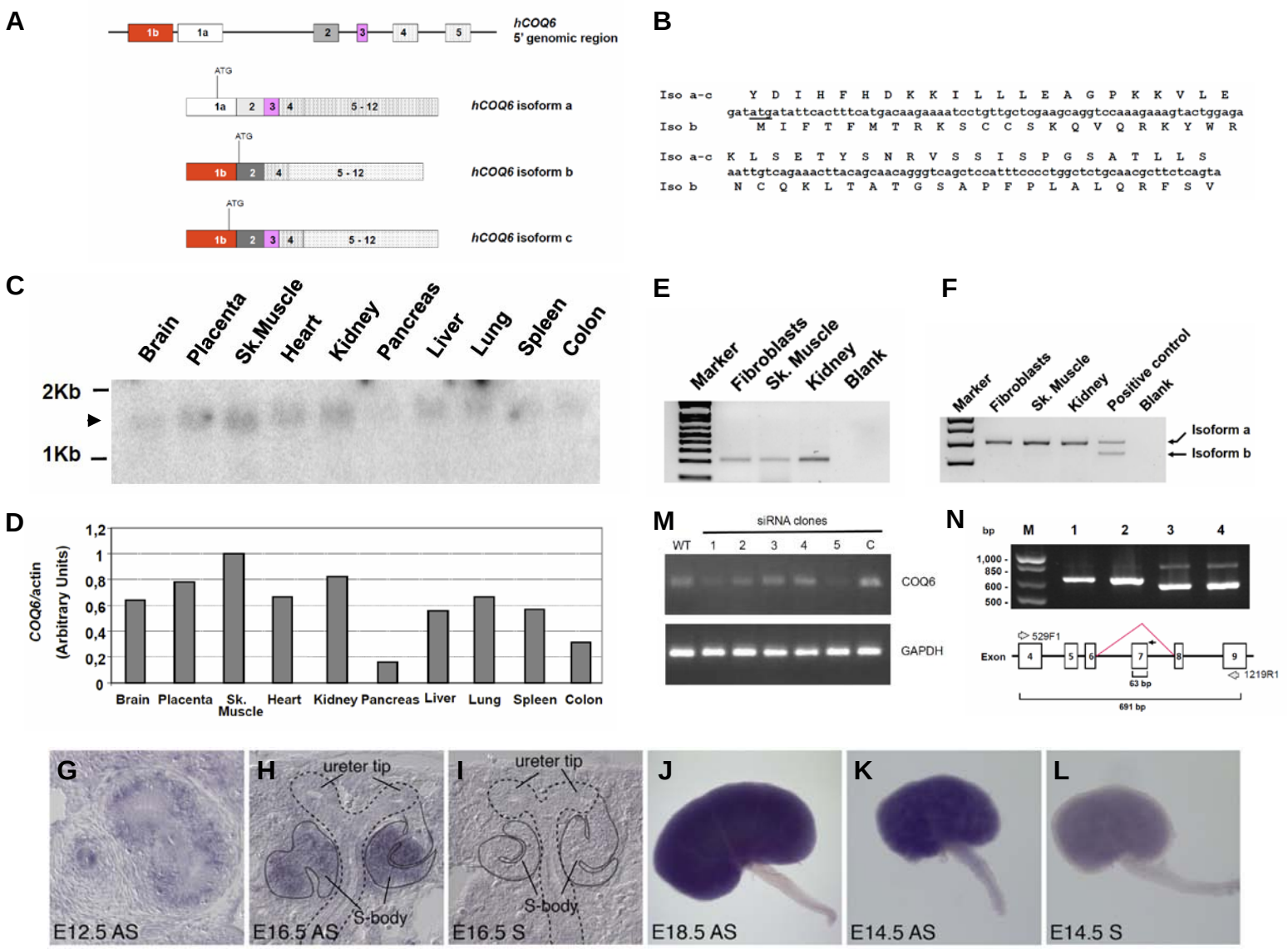
Supplementary Table 1. Yeast expression plasmids used in this study

Plasmid	Relevant description	Source
pSR1-1	Low copy, yeast COQ6 gene	Gin et al. *J Biol Chem* 278:25308-25316, 2003.
pQM	Low copy, yeast CYC1 promoter, mitochondrial leader sequence	Hsu et al. *Biochemistry* 35:9797-9806, 1996
pQM_hWTCOQ6-MLS	Human COQ6 wild-type	This study
pQM_COQ6-MLS_R162X	Human COQ6 with R162-Stop mutation	This study
pQM_COQ6-MLS_W188X	Human COQ6 with R188-Stop mutation	This study
pQM_COQ6-MLS_G255R	Human COQ6 with G255R substitution	This study
pQM_COQ6-MLS_A353D	Human COQ6 with A353D substitution	This study
pQM_COQ6-MLS_W447X	Human COQ6 with W447X-Stop mutation	This study
pQM_COQ6-MLS_Q461fsX478	Human COQ6 with Q461-Frame Shift	This study
pRS426	High copy yeast shuttle vector	Christianson et al. *Gene* 110:119-122, 1992
pRCM	High copy, yeast CYC1 promoter, mitochondrial leader sequence	Morvaridi S and Clarke CF (manuscript in preparation)
pRCM_hWTCOQ6-MLS	Human COQ6 wild-type	This study
pRCM_COQ6-MLS_R162X	Human COQ6 with R162-Stop mutation	This study
pRCM_COQ6-MLS_W188X	Human COQ6 with R188-Stop mutation	This study
pRCM_COQ6-MLS_G255R	Human COQ6 with G255R substitution	This study
pRCM_COQ6-MLS_A353D	Human COQ6 with A353D substitution	This study
pRCM_COQ6-MLS_W447X	Human COQ6 with W447X-Stop mutation	This study
pRCM_COQ6-MLS_Q461fsX478	Human COQ6 with Q461-Frame Shift	This study

Supplementary Table 2. *COQ6* exon-flanking primers.

Forward primer name and sequence (5'→ 3')	Reverse primer name and sequence (5'→ 3')	Length of PCR product [bp]
COQ6_Ex1F GCACTACGTAGGTGGGCCTG	COQ6_Ex1R CAAGTCGTGCTAGGGCTCTC	301
COQ6_Ex2F TGTTGTTTCTCTTGGTAATGGG	COQ6_Ex2R TGGGATACACTAGAAAGCTAAGTGG	272
COQ6_Ex3/4F GTAACAGGATGGAGGGACAAGG	COQ6_Ex3/4R TCTTCCAGTAAGTCCTAAGCAGTTC	618
COQ6_Ex5F TGGGACCTTGCTTTAGGTTTAG	COQ6_Ex5R CTGGCCTGAATAGGTACTGGTC	365
COQ6_Ex6/7F AACAATCAGAGCTGGAGGAAAC	COQ6_Ex6/7R GAAAGTGAAGAGGAAAGGCTTG	412
COQ6_Ex8F AGAGTTTCCAAGTGCAGCAGAG	COQ6_Ex8R CAACACCTTTCTGTATCTCCCC	224
COQ6_Ex9/10F GCTTTGGTTACAAACAAGGTTTC	COQ6_Ex9/10R CACTCCCTCTTGCTACTGTGG	771
COQ6_Ex11F TATCTGGCTTGCTAGGAGATGG	COQ6_Ex11R GGCGATAAGACCAAGACTCTG	326
COQ6_Ex12F GACACTTGGGAAGAATACCTACG	COQ6_Ex12R AATATGTATGATGGGTCCTGGG	198



Supplementary Figure 1. **Expression patterns of human *COQ6* isoforms 'a' and 'b' (A-F** and **G-L) and knockdown of murine podocyte *Coq6* (M) and zebrafish *coq6* (N).**

(**A**) Structure of the 5' genomic region of human *COQ6* and of the transcripts encoding the two isoforms 'a' and 'b' of the gene. Exons unique to isoform 'a' (white) or isoforms 'b' or 'c' (black) are shown. In isoforms 'a' and 'c' *versus* isoform 'b' alternative exon 2 (shades of grey) is translated from a different reading frame (see **B**). Exons 4-12 are common to all three isoforms (stippled).

(**B**) The two different reading frames of exon 2. The initiation codon of isoform 'b' is underlined.

(**C**) Northern blot analysis of *hCOQ6* expression in different tissues using a full length cDNA. Each lane contains 2 µg of poly(A)$^+$ RNA. Equal loading was reported previously by hybridization of the same membrane to a β-actin probe.[1] Note that a single transcript is seen at ~1,400 nt (arrow head), consistent with the full-length transcript of 1,407 nt.

(**D**) Densitometric analysis of Northern (**C**) blot in normalized against β-actin. Skeletal muscle is set to 1 arbitrary units.

(**E**) Amplification of isoform a from cDNA derived from different tissues (fibroblasts, skeletal muscle or kidney).

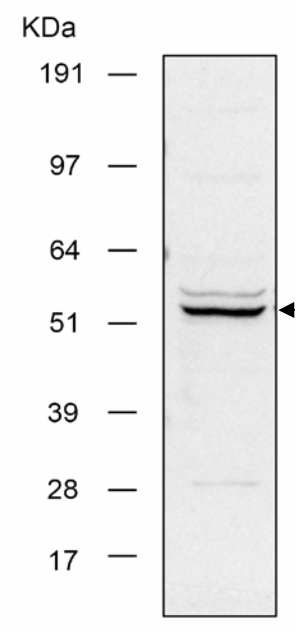
(**F**) Simultaneous amplification of isoform a and isoform b from the same cDNA as in (**E**) using primers on *COQ6* exons 2 and 4. A diluted 1:1 mixture of plasmids encoding isoforms 'a' or 'b' was used as positive

control. Note that, although isoform ‘b’ transcripts are present in all three tissues, they represent only a negligible fraction of the total *COQ6* transcripts.

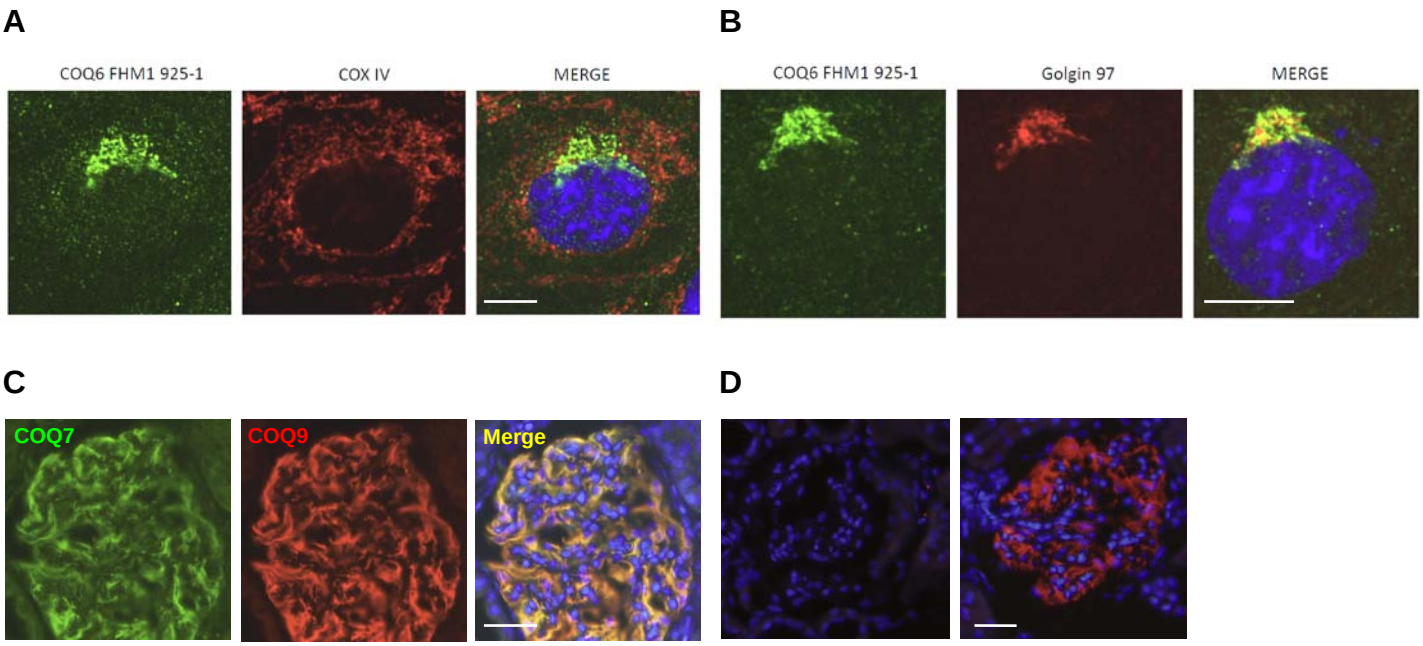
(**G-L**) *In situ* hybridization analysis of *Coq6* mRNA expression on transverse sections of mouse kidney demonstrates *Coq6* expression in the metanephric mesenchyme and forming nephrons (**G-I**) and in whole kidneys (**J-L**). *Coq6* is expressed in the condensing metanephric mesenchyme surrounding the ureter tips (**H**) and in the forming nephrons (**G**). Whole mount staining confirms *Coq6* expression in the metanephic mesenchyme (**J, K**). Sense probe is used as a negative control and shows weak staining (**L**). Stages are as indicated. AS antisense, S sense.

(**M**) Analysis of *Coq6* mRNA levels in undifferentiated mouse podocytes by RT-PCR. The *Coq6* siRNA clones express one of the five targeting sequences (lanes 1-5). A clone expressing a scrambled oligo (“C”) and untransfected wild-type podocytes (WT) serve as negative controls. The siRNA clones “1”, ”2” and ”5” showed lowest *Coq6* mRNA levels and were used for knockdown experiments.

(**N**) **Zebrafish *coq6* mRNA splicing is altered by spMO4.** RT-PCR was performed to detect the transcript of *coq6* in 24 hpf embryos. In wild-type embryos and embryos injected with 0.2 mM of the morpholino oligomucleotide (MO) spMO4mm (5-bp mismatch control) only the normal splicing product (691 bp) was detected (upper panel lanes 1 and 2). In contrast, in embryos injected with 0.1 mM spMO4 (black arrow) targeting the donor site of intron 7, a spliced product shorter by 63 bp (upper panel lanes 3 and 4) was detected that lacked exon 7 as confirmed by direct sequencing of the RT-PCR product. The schematic graph shows the structure of exons 4-9 of *coq6* and the location of the primers used in RT-PCR (grey arrows).



Supplementary Figure 2. **Immunoblot of HEK293 lysates with antibody α-COQ6-TPEP2.** Immunobloting of HEK293 lysates with antibody α-COQ6-TPEP2 reveals a major band (arrow head) close to the expected size of 50.8 kDa for full-length human COQ6.



Supplementary Fig. 3. Subcellular localization of COQ proteins in Cos7 cells and rat glomerular podocytes.

(**A-B**) **Subcellular localization of COQ6 to Golgi as determined with the α-COQ6-925-1 antibody (**see also **Fig. 3).**

(**A**) α-COQ6-925-1 antibody generated to an C-terminal peptide of human COQ6 (see **Fig. 1e**) does not detect endogenous COQ6 in mitochondria of Cos7 cells, which are labeled with an anti CoxIV antibody. (**B**) In contrast, the α-COQ6-925-1 antibody detects COQ6 in Golgi apparatus of Cos7 cells, which is labeled with an α-Golgin-97 antibody.

(**C**) **Colocalization of COQ7 and COQ9 in rat glomerular podocytes.** Upon immunofluorescence of rat renal glomeruli COQ7 (green) and COQ9 (red) colocalize in podocyte cytoplasm and cellular processes.

(**D**) **Preabsorption of the α-COQ6-TPEP2 antibody with cognate and non-cognate peptide.** Preabsorption of the α-COQ6-TPEP2 antibody with the cognate peptide abolishes the immunofluorescence signal (left panel), whereas the signal is present following preabsorption with the non-cognate peptide TPEP1 (right panel). Scale bars are 5 μm in (**A** and **B**) and 40 μm in (**C** and **D**).

REFERENCES

1. Casarin, A.*, et al.* Functional characterization of human COQ4, a gene required for Coenzyme Q10 biosynthesis. *Biochem Biophys Res Commun* **372**, 35-39 (2008).