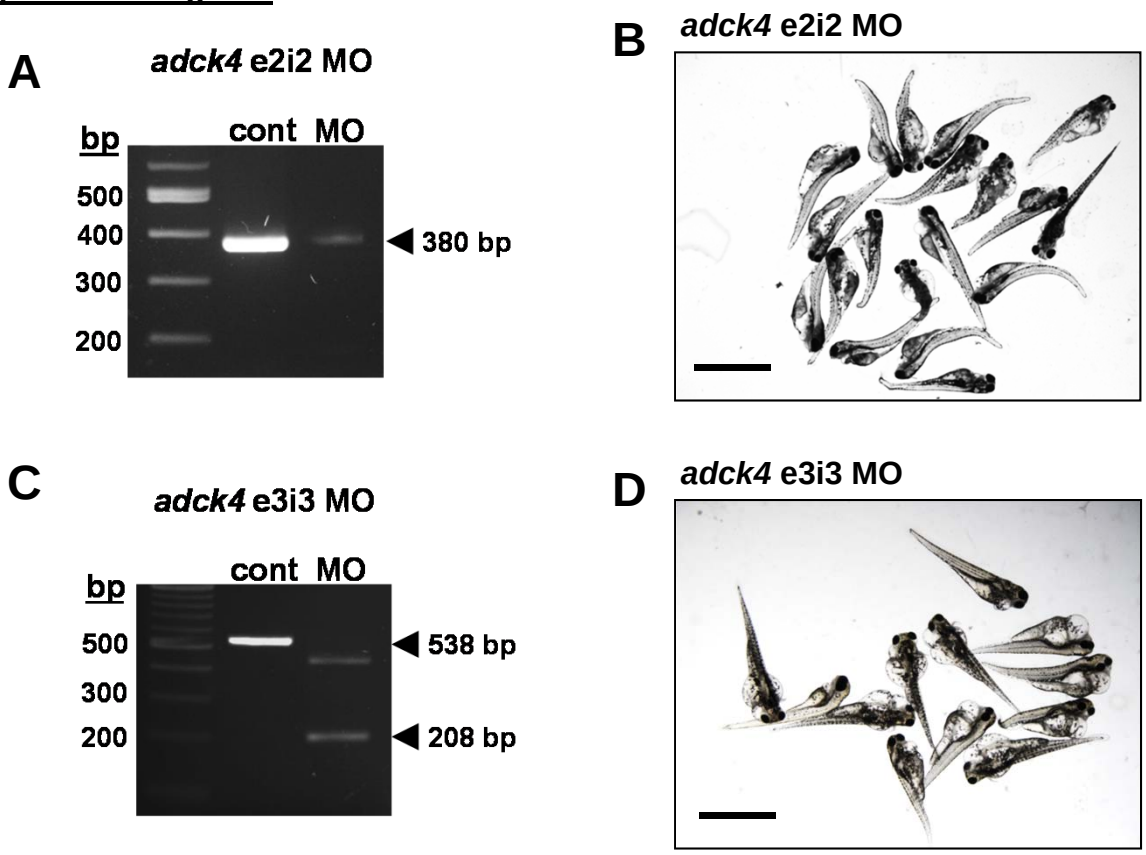
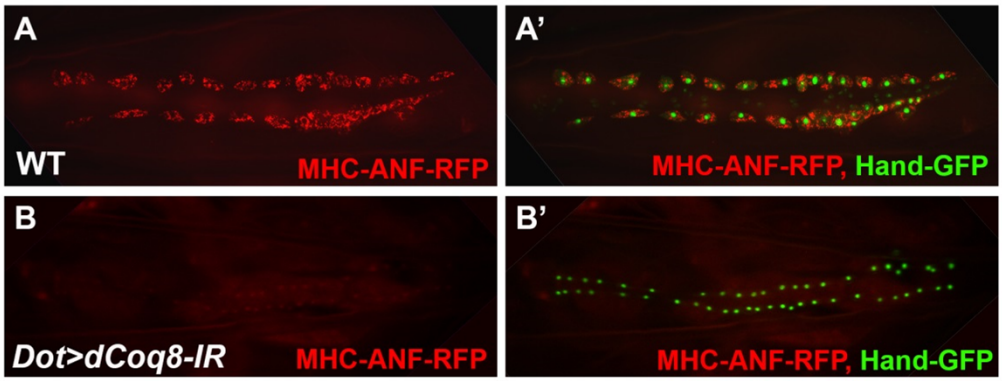
Supplemental Figures

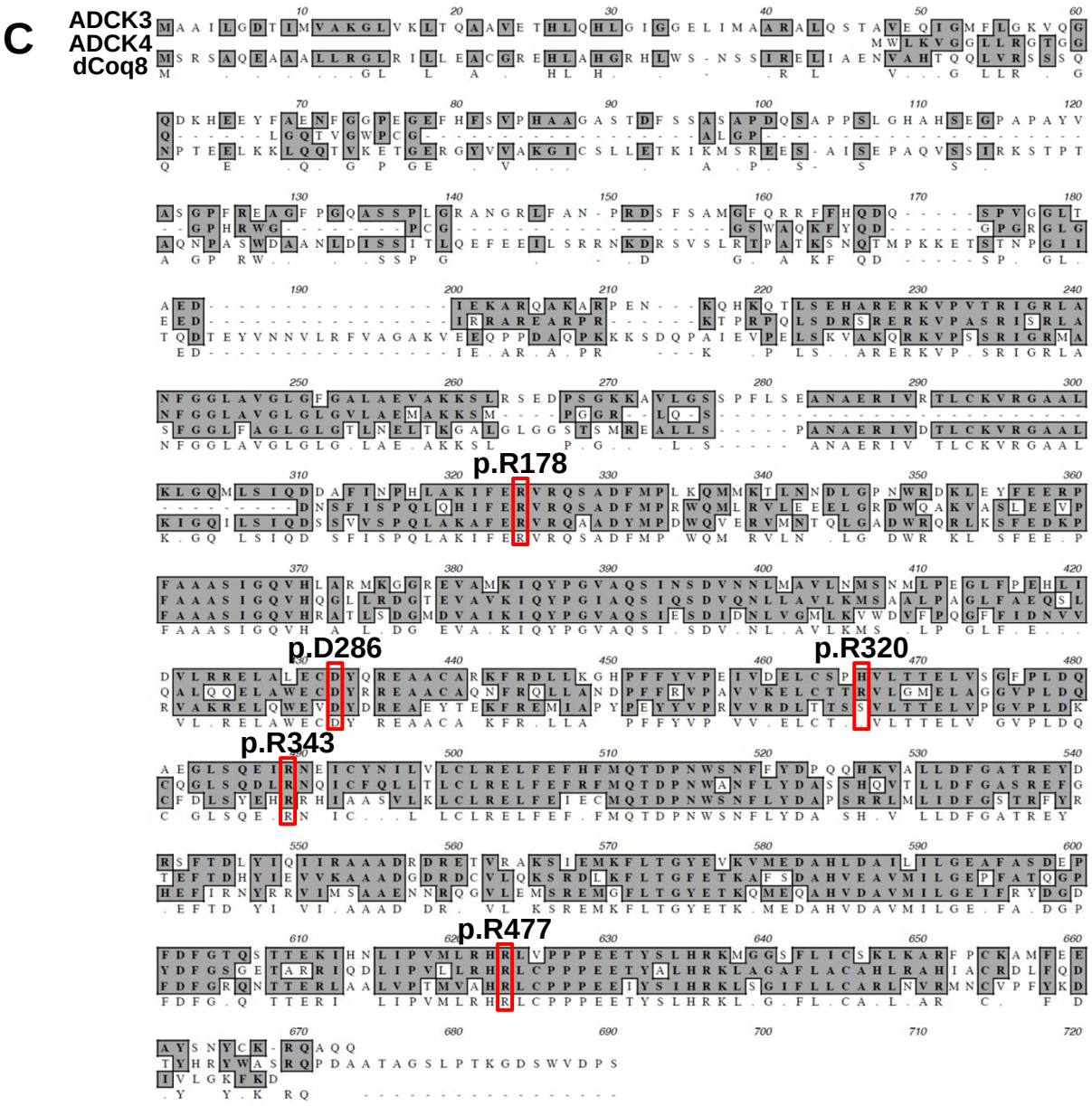


E

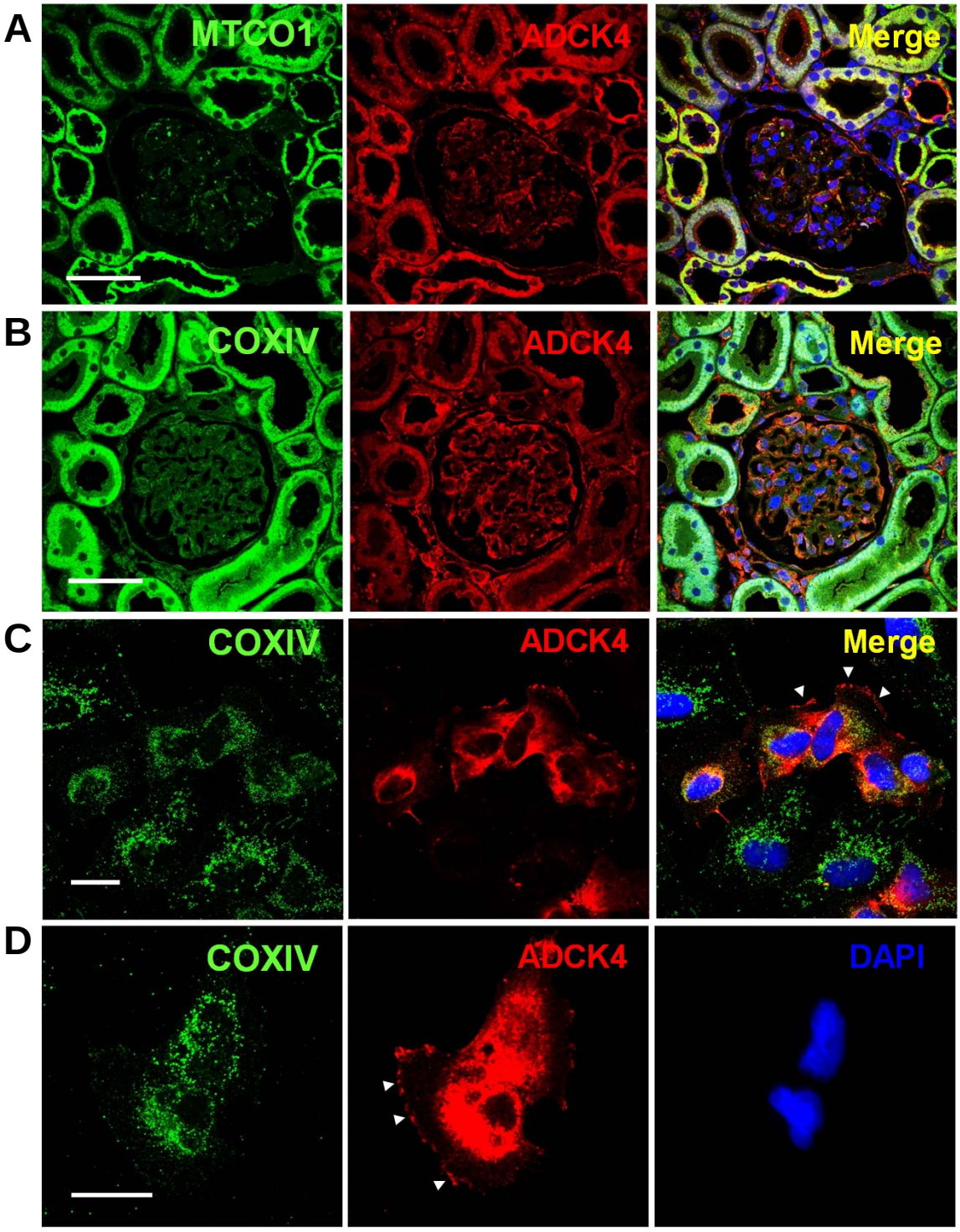
	ATG MO	e2i2 MO	e3i3 MO
edema phenotype	54.1% (193/357)	35.6% (112/315)	48.3% (274/567)

Supplemental Figure 1. *adkc4* knockdown by splicing-blocking MOs in zebrafish.
(A) RT-PCR was performed to detect the transcript of *adck4* in 48 hpf embryos. In embryos (cont) injected with 0.2 mM of negative control MO targeting *p53* (to control for unspecific apoptosis effects) (11), only the normal splicing product (380 bp) appeared. In contrast, in embryos (MO) injected with 0.2 mM MO (e2i2) targeting the donor site of intron 2 of *adck4*, the normal splicing product was significantly decreased. **(B)** Zebrafish coinjected with the e2i2 MO and with *p53* MO. At 120 hpf, *adck4* morphants displayed phenotype of periorbital edema and total body edema. Scale bar is 1 mm. **(C)** In embryos (cont) injected with 0.2 mM of *p53* MO, only the normal splicing product (538 bp) appeared. In contrast, in embryos (MO) injected with 0.2 mM MO (e3i3) targeting the donor site of intron 3 of *adck4*, a spliced product of 208 bp was detected that lacked exon 3 as confirmed by direct sequencing of the RT-PCR product. **(D)** Zebrafish coinjected with the e3i3 MO and with *p53* MO. The picture was also taken at 120 hpf. Scale bar is 1 mm. **(E)** Summary of *adck4* morphants. The 1st number in parentheses indicates number of embryos with edema phenotype and 2nd number denotes total number of embryos examined.



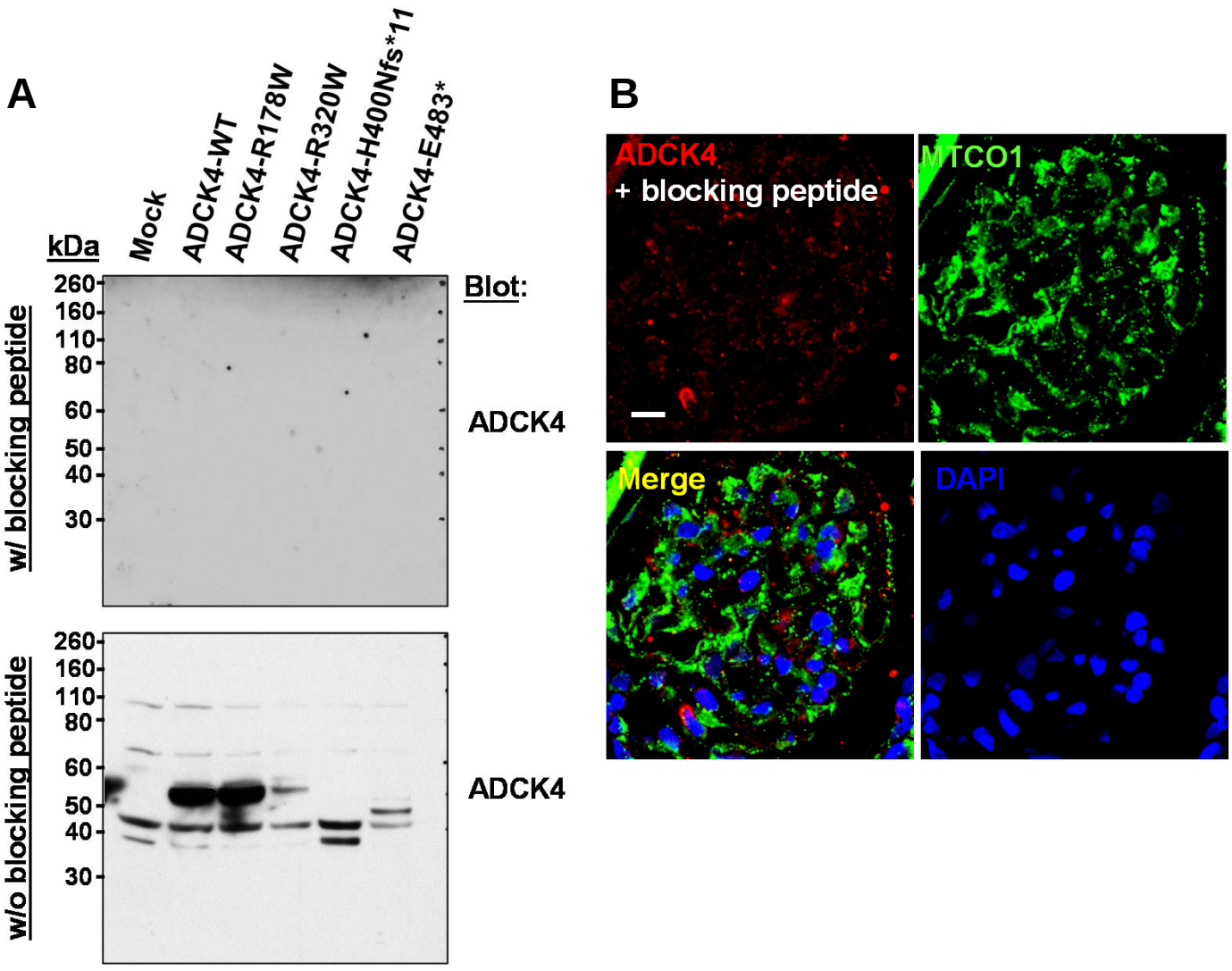


Supplemental Figure 2. *dCoq8* (CG32649) is required for pericardial nephrocyte function. (**A-A'**) Secreted ANF-RFP (red) is accumulated in pericardial nephrocytes labeled with Hand-GFP (green in **A'**). (**B-B'**) Pericardial nephrocyte specific RNAi knockdown of CG32649, which we named as *dCoq8* based on protein sequence homology to yeast *coq8*, blocked the accumulation of ANF-RFP (red in **B**, **B'**), but the survival of pericardial nephrocytes is not affected since Hand-GFP (green in **B'**) appears normal. (**C**) Amino acid sequence alignment of ADCK3, ADCK4 and dCoq8. dCoq8 shows 53% identity to both ADCK3 and ADCK4. Red boxes indicate the amino acid residue at which missense mutations in *ADCK4* were identified (**Table 1**).

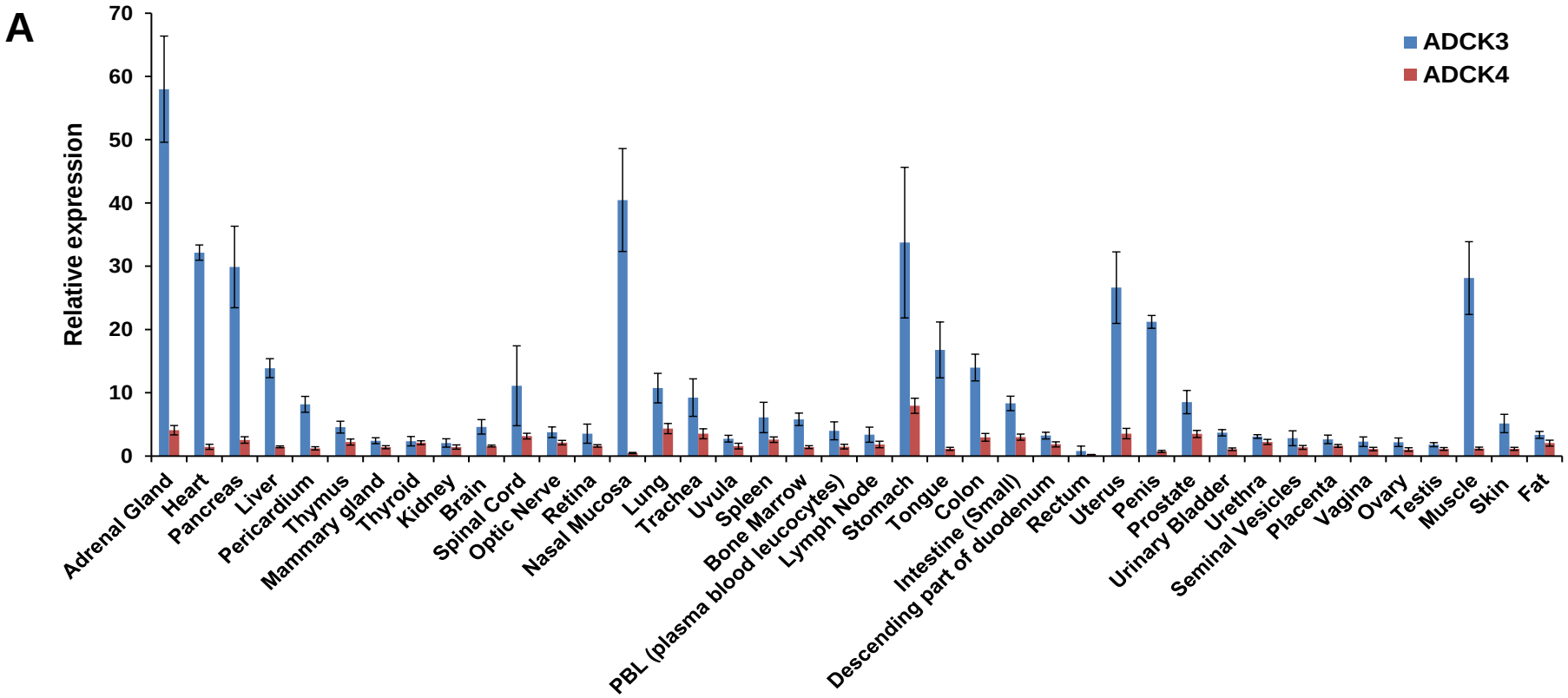


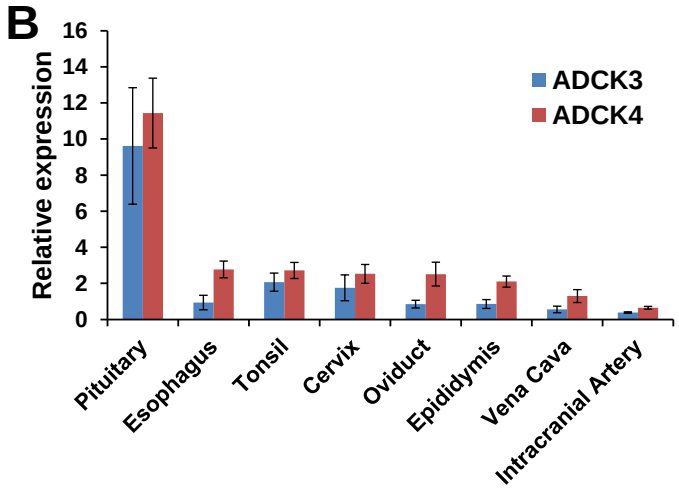
Supplemental Figure 3. Expression of ADCK4 in rat kidney and cultured human podocytes.

(A-B) Coimmunofluorescence of ADCK4 with MTCO1 (mitochondrially encoded cytochrome c oxidase 1) and COXIV (cytochrome oxidase subunit VI) in adult rat glomeruli. Scale bar is 50 μm. **(C-D)** Podocytes were transfected with ADCK4-RFP and stained with an anti-COXIV antibody. ADCK4-RFP partially colocalizes to mitochondria with COXIV. Note that ADCK4-RFP also localizes along the plasma membrane (white arrowheads) in undifferentiated podocytes. Scale bar is 25 μm.



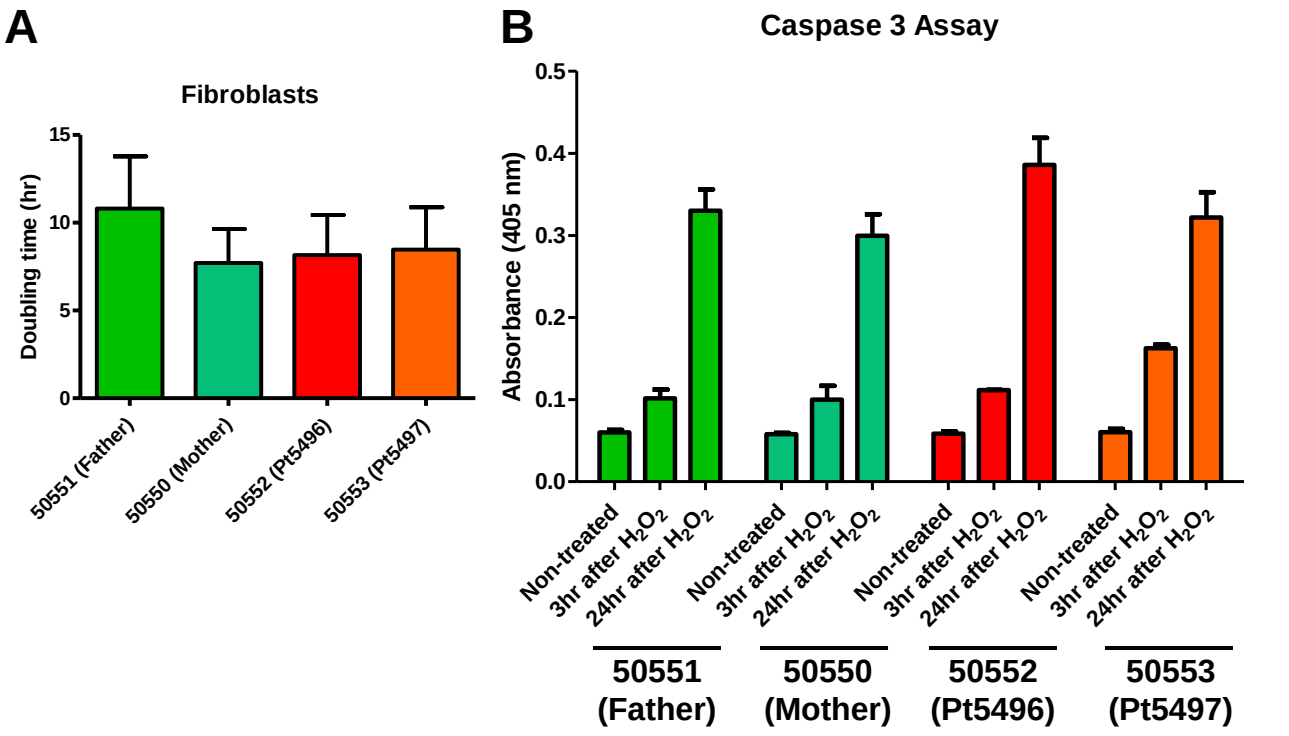
Supplemental Figure 4. Specificity of the anti-ADCK4 antibody used in this study.
(A) The wild type (WT) and mutant clones of ADCK4 were transfected into the undifferentiated podocytes and the cell lysates were immunoblotted with the anti-ADCK4 antibody which was mixed with the 2 μg/ml blocking peptide (GEEDIRRAREARPRK, 55-69 amino acids of ADCK4). The blot was exposed for 15 min. The membrane was stripped and immunoblotted with the anti-ADCK4 antibody without the blocking peptide. **(B)** A rat kidney section was incubated with the anti-ADCK4 antibody, the 2 μg/ml blocking peptide, and an anti-MTCO1 antibody.

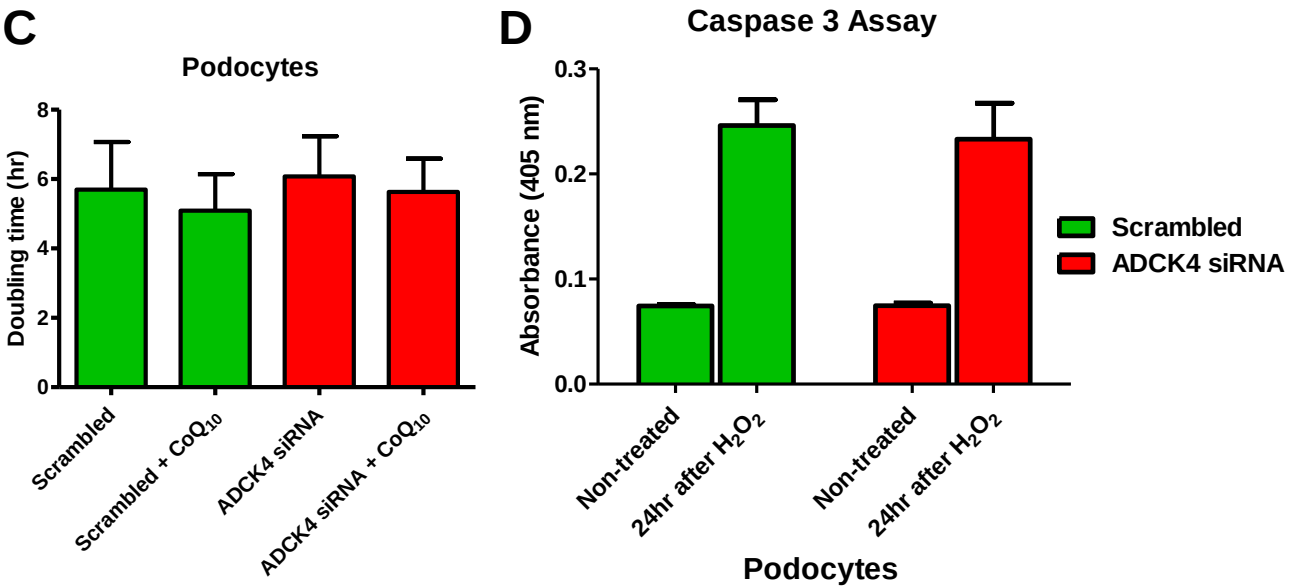




Supplemental Figure 5 . Differential expression of *ADCK3* and *ADCK4* in human tissues.

(A-B) *ADCK3* expression exceeds that of *ADCK4* in most of tissues. Expression measured by quantitative real-time PCR was normalized to GAPDH expression. cDNA from 48 human tissues was purchased form OriGene (Tissue SCAN™ Normal Tissue qPCR Arrays, HMRT502). Real-time PCR was performed using StepOnePlus™ Real-Time PCR System (Applied Biosystems). TaqMan probes for *ADCK3* (Hs00220382_m1), *ADCK4* (Hs00388243_m1), and *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) (Hs02758991_g1) were purchased from Applied Biosystems. The relative RNA expression levels were calculated via a comparative threshold cycle (Ct) method using *GAPDH* as control: ΔCt=Ct(*GAPDH*)-Ct(*ADCK3* or *ADCK4*). The gene expression fold change, normalized to the *GAPDH* and relative to the control sample (*ADCK4* expression in kidney), was calculated as $2^{-\Delta\Delta Ct}$. Error bars, standard deviation of four experiments.





Supplemental Figure 6. Loss of *ADCK4* does not induce defects in proliferation or apoptosis in fibroblasts and podocytes.
(A) Proliferation of fibroblasts of individuals with a *ADCK4* frameshift mutation did not show any difference from that of fibroblasts of their healthy parents. **(B)** Apoptosis induced by H_2O_2 was measured by caspase 3 assay. Caspase 3 assay was performed using a kit from Abcam. There was no difference in apoptosis induced by H_2O_2 between fibroblasts from parents and those from patients. **(C)** Knockdown of ADCK4 in podocytes did not affect proliferation. **(D)** Knockdown of ADCK4 in podocytes did not affect apoptosis induced by H_2O_2 .

Supplemental Table 1. Filtering process for variants from normal reference sequence (VRS) following whole exome resequencing (WER) in 5 sibling pairs or 7 single individuals with steroid resistant nephrotic syndrome.

FAMILY	[a]AFFECTED SIBS SENT FOR WER	Consanguinity	[b]# of homozygosity peaks	Cumulative homozygosity[c] [Mb]	Total sequence reads (Mill.)	Matched Reads[d]	Total variants (DIPs and SNPs)	Exonic variants (DIPs and SNPs)	% exonic / total variants	Variants not present in dbSNP129	Variants in mapped homozygous regions[c]	Variants after inspection and not present in dbSNP132 (MAF > 1%)	Total exonic variants shared by 2 affecteds from same family	Homozygous exonic variants shared by 2 affecteds from same family	[e]Unique homozygous exonic variants shared by 2 affecteds from same family	[f]Sanger confirmation / segregation	Nucleotide alteration (mutation) identified in *ADCK4*
A2338	A2338-21	Y	5	115	208	98.5%	374,304	5741	1.53%	575	23	1	–	–	–	1	c.532C>T(HOM)
231	231-21	Y	14	231.6	120	89.0%	62,153	12804	20.60%	9015	1304	2	–	–	–	2	c.645delT (het) c.1430G>A (het)
ABD	ABD-21	Y	19	61	197	71.7%	75,072	21159	28.20%	2677	200	1	–	–	–	1	c.958C>T (HOM)
Mek	1145	Y	–	–	141	89.5%	102,634	26287	25.60%	–	–	–	21182	94	1	1	c.1027C>T (HOM)
	1146	Y	–	–	93	86.6%	97,224	25874	26.60%	–	–	–					c.1027C>T (HOM)
Pt5496/97	Pt5496	Y	–	–	111	97.0%	57,537	11552	20.08%	–	–	–	862	67	3	1	c.1356_1362delGGGCCCT (HOM)
	Pt5497	Y	–	–	115	96.3%	57,077	11463	20.08%	–	–	–					c.1356_1362delGGGCCCT (HOM)

[a]see Table 1

[b]see Figure 1

[c]Evaluation for homozygous variants was done in regions of homozygosity for 2 affected siblings.

[d]Percent of total sequence reads that aligned to human reference genome assembly (hg19).

[e']Unique variants' are defined as the variants with damaging calls and not present in 'in-house' exomes of individuals with other diseases.

[f]Red numbers denote number of filtered-down variants that contained the disease causing gene.

"-" , not applicable; db, database; DIPs, deletion/insertion polymorphism; het, heterozygous; HOM, homozygous; MAF, Minor allele frequency; SNPs, single nucleotide polymorphism; WER, whole exome resequencing; Y, yes.

Supplemental Table 2. Primer sequences to amplify exons and exon-intron boundaries of *ADCK4*.

ADCK4 **coding exons**	**Forward primer sequence (5' ➞ 3')**	**Reverse primer sequence (5' ➞ 3')**	**Length of PCR product [bp]**
Ex 2	CCCACCTGAATAATCCCTCC	CCTCAGCCTCTTCCCCTTG	181
Ex 3	TCCTCCCCTCCTTATCAAAG	CAGCAGTATTCGTGGGGTG	200
Ex 4	ACACCCCACGAATACTGCTG	TGATTCATCCTCTCCCTTGC	270
Ex 5	CAAACGGGCACTCATGTTC	CAAAGCCAGGATCTGAACCC	153
Ex 6	ACCTCTTCGCTTACTTCCCC	AGCCTGCCTCCCTCACC	168
Ex 7	ATGATGGCTGTGACCACTCC	CACTTCTCTGGGCTAGCAGG	223
Ex 8	AGCCCAGAGAAGTGACTCCC	GTCAGCAGAGGAGTGGGTG	161
Ex 9	CCCTGGCTGACACACCTC	GAGGGAAGGAGGGAAGCTC	155
Ex 10	AAGTCCCCTTCCTCCCTTTC	CCCACTGTGCCACCAGAC	199
Ex 11	GATGCCTGTTAATTCTCCCC	CAGCAAATGTACCCAGACACAC	173
Ex 12	GATCCCAGCATTCCTTTCAC	TCGCCTTCTTACTCCTCTGC	181
Ex 13	TTGTCCCTCTAACTCCCACC	ACAGACACCAGCCCCTTTC	156
Ex 14_1	ACTGGCTGCCTTTGCTCTC	ACAGGCCAGGAAAGCCC	239
Ex 14_2	CGAGGAGACCTATGCCCTG	AGGCACTACAGCAGGGTACG	245

Supplemental Table 3. Morpholino (MO) and RT-PCR primer sequences for zebrafish MO knockdown.

	Primer sequence (5'→3')
adck4 ATG MO	GAGGACTTCAGACAGCAGCATCGTA
adck4 e2i2 MO	AATCTCTGTGTTCACTAACCATGTC
adck4 e2i2 RTPCR F	CTCAGGGTGAGCAGCTCCGA
adck4 e2i2 RTPCR R	CAACCTGGCCACTATAGAGTCATG
adck4 e3i3 MO	AATTCAGATGTCCTCACCTTTTGTC
adck4 e3i3 RTPCR F	TGCTGCTGTCTGAAGTCCTC
adck4 e3i3 RTPCR R	TTCCTTTCTTTGGCTCGTTC

(F, forward; R, reverse**)**