

ONLINE SUPPLEMENT

Altered distribution and loss of ACE2 into the urine in acute kidney injury

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Figure S1: ACE2 staining by IHC in uninjured control kidneys and kidneys 48 hours after IRI

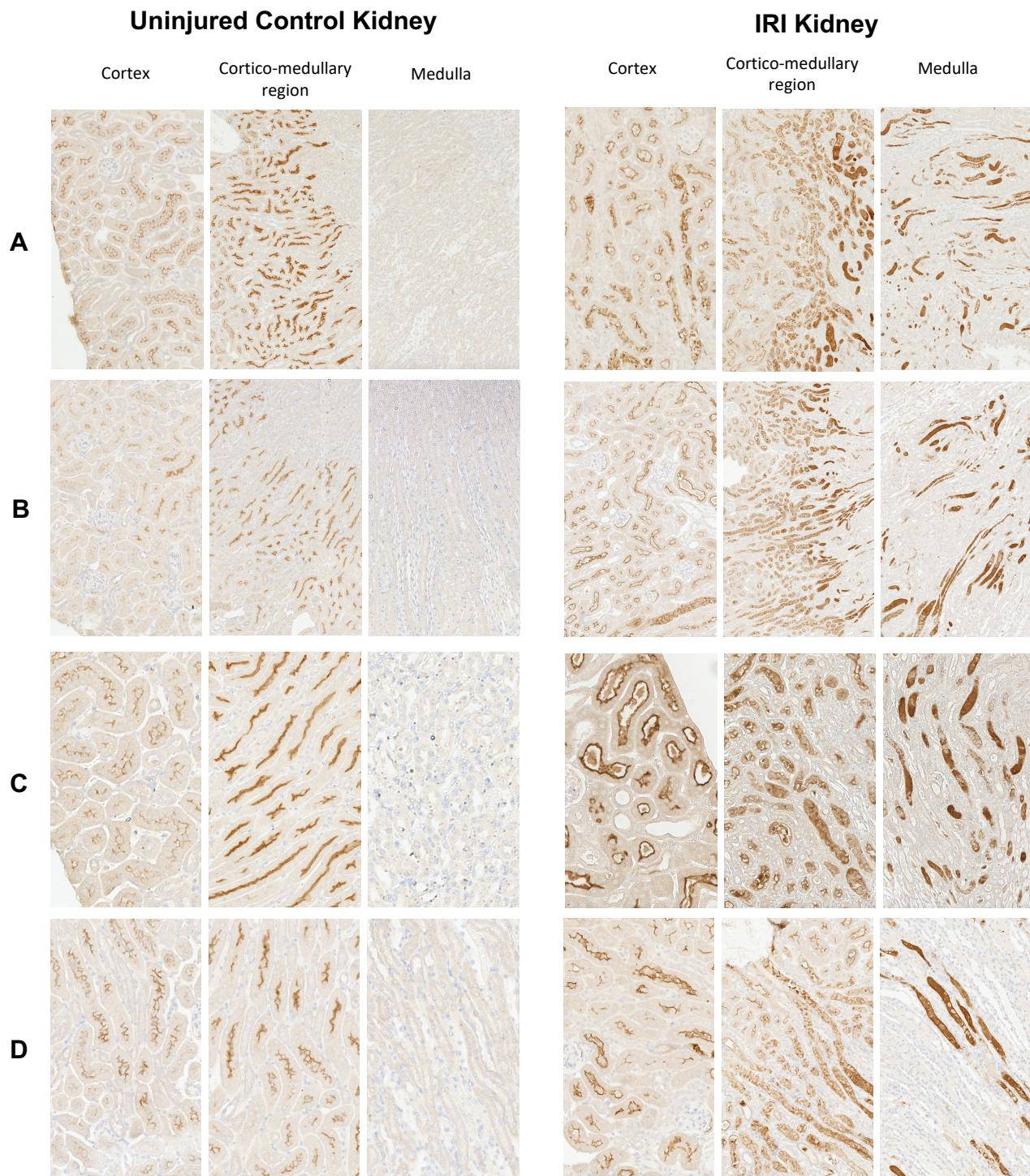


Figure S1: In the uninjured control kidneys (**Left panels A, B, C and D**) ACE2 staining is well delineated in the kidney cortex, corticomedullary region and absent in the medulla.

In kidneys from the same mice harvested 48 hours after IRI (**Right panels A, B, C and D**) tubules are widened with a loss of proximal brush border in cortex and corticomedullary region, with the lumens filled with ACE2 positive material. In the medullary region ACE2 casts are widely seen in the tubular lumens. Pictures taken at 200x magnification.

Figure S2: ACE2 staining by IF in uninjured control kidneys and kidneys 48 hours after IRI

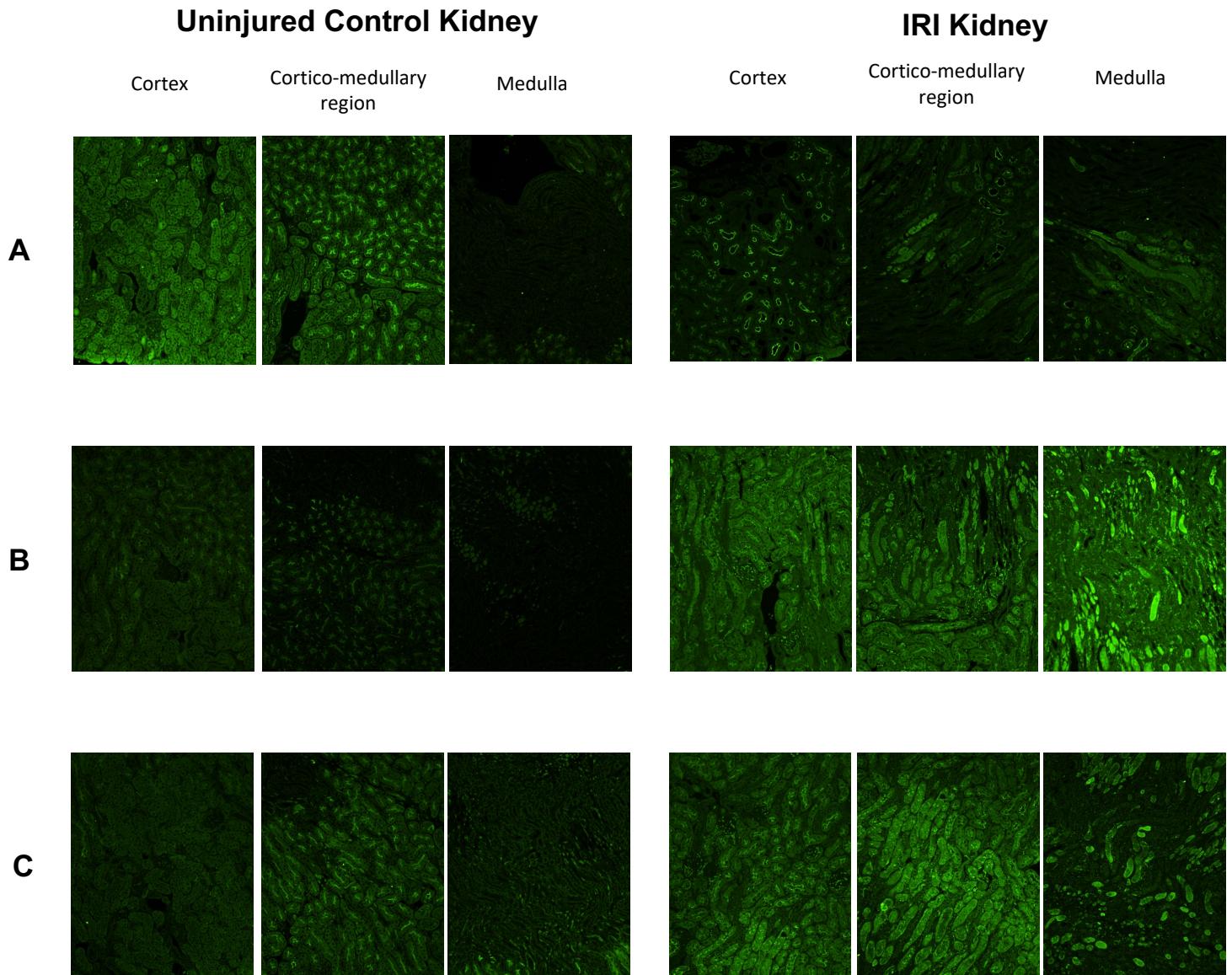
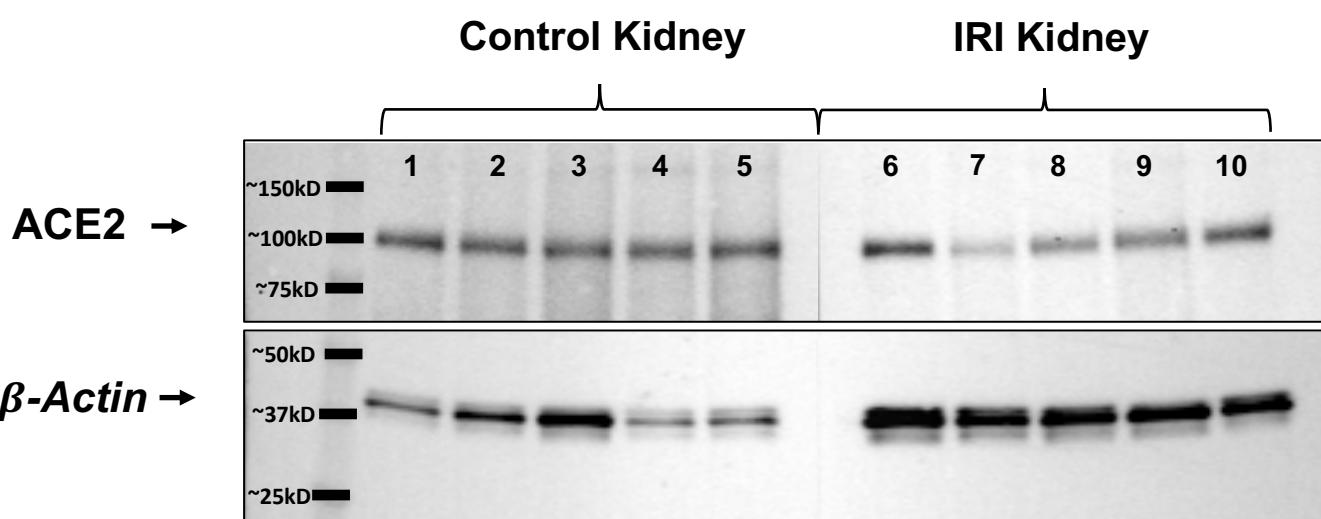


Figure S2: In uninjured control kidneys (**Left panels A, B and C**) IF staining for ACE2 is well delineated apically in cortex and corticomedullary region. By contrast in the medulla no staining can be detected.

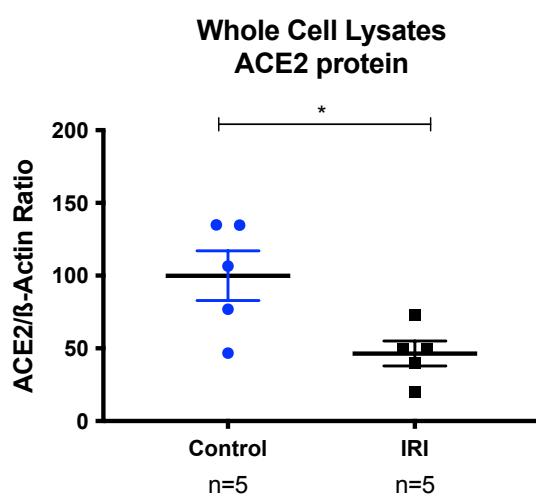
In kidneys from the same mice harvested 48 hours after IRI (**Right panels A, B and C**) staining for ACE2 in cortex and corticomedullary region shows widened tubules with ACE2 positive material inside the tubular lumen. ACE2 positive casts are seen in the medulla. Pictures taken at 200x magnification.

Figure S3: Kidney ACE2 protein and enzymatic activity in cortical whole cell lysates of uninjured controls and 48 hours post IRI

A.



B.



C.

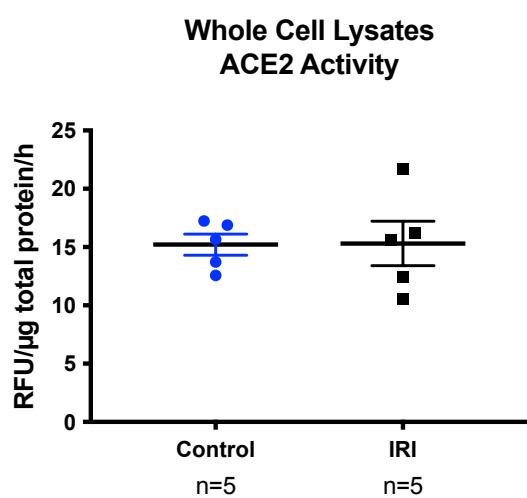


Figure S3: In cortical whole cell lysates (**A**) ACE2 protein by Western Blot showed a strong band at about 100 kD in control kidneys (Bands 1-5) which was reduced in kidneys from the same mice harvested 48 hours after IRI (Bands 6-10). This was further assessed by the ACE2/β-Actin-Ratio which was markedly reduced in IRI kidneys versus uninjured control kidneys (**B**) (46 ± 9 vs. 100 ± 17 ACE2/β-Actin, $P=0.02$).

ACE2 enzymatic activity in cortical whole cell lysates was not different in IRI kidneys as compared to uninjured control kidneys from the same mice (**C**) (15.3 ± 1.9 vs. 15.2 ± 0.9 RFU/μg total protein/h, $P=0.9$).

Figure S4: ACE staining by IHC in uninjured control kidneys and kidneys 48 hours after IRI

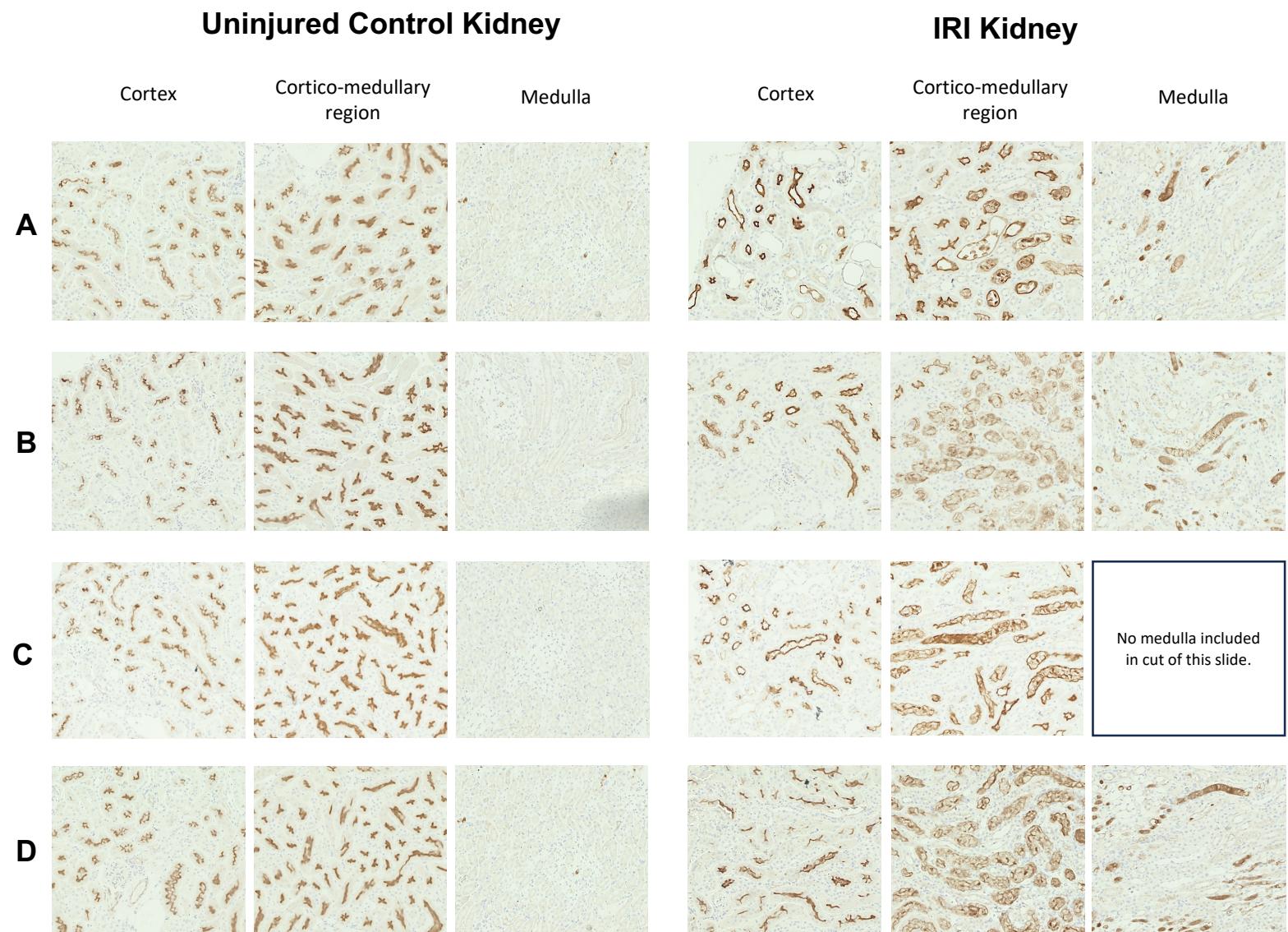


Figure S4: In uninjured control kidneys (**Left panels A, B and C**) ACE staining is delineated apically in the proximal tubule in the kidney cortex and corticomedullary region while no staining is seen in the medulla.

In the kidneys harvested 48 hours after IRI (**Right panels A, B and C**) ACE staining is seen in widened tubules in cortex and corticomedullary region and the lumens are filled with ACE positive material. In the medulla cast-like structures positive for ACE are frequently seen and occupy the tubular lumen. Pictures taken at 200x magnification.

Figure S5: Kidney ACE protein in cortical whole cell lysates of uninjured controls and 48 hours post IRI

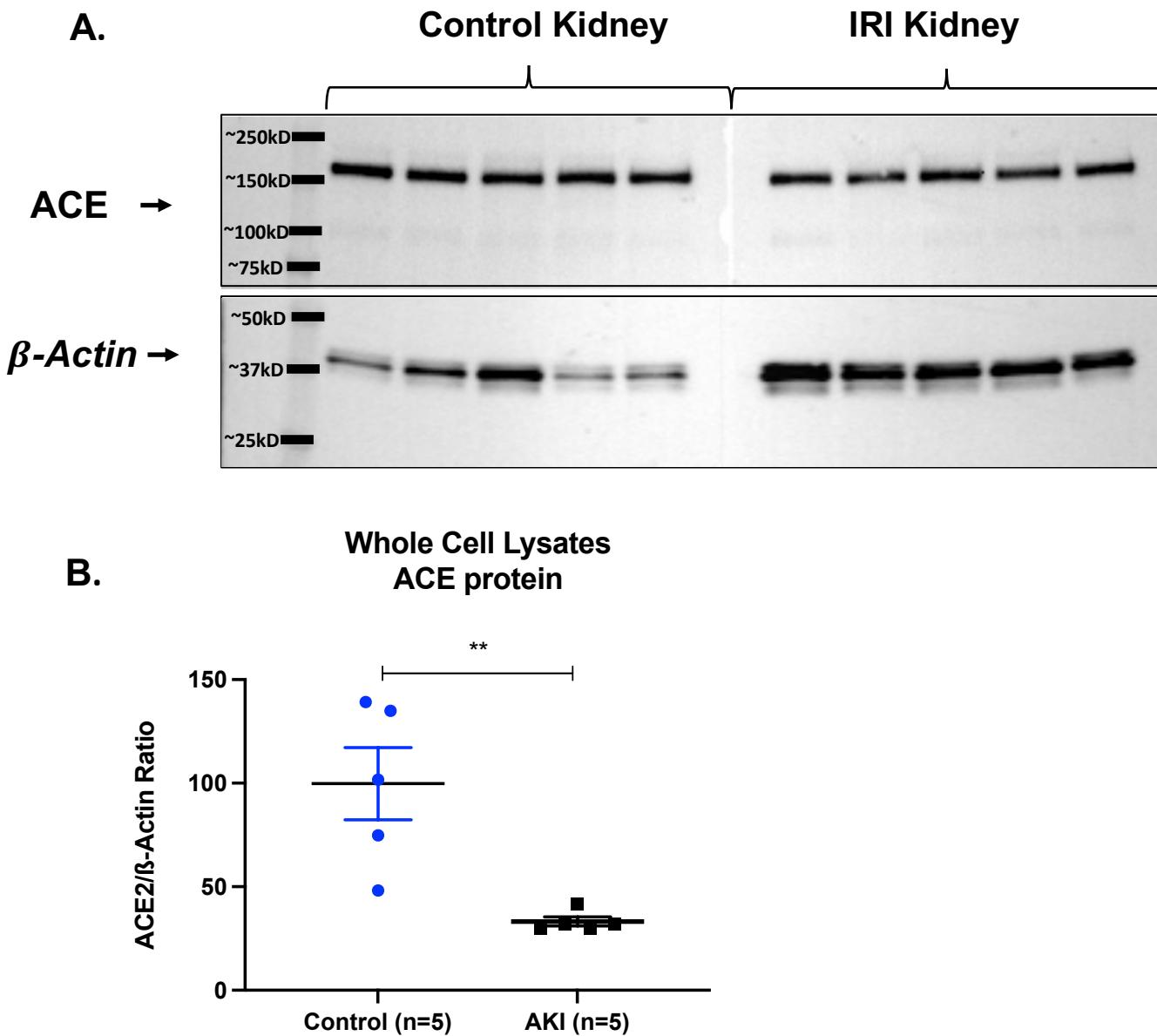


Figure S5: Western Blot of cortical whole cell lysates done on the same gel showing a strong band at about 170 kD corresponding to ACE in control kidneys (Bands 1-5) and kidneys from the same mice harvested 48 hours after IRI (Bands 6-10). The intensity of the band was quantified using the ACE2/β-Actin-Ratio which was reduced in IRI kidneys versus uninjured control kidneys (**B**) (33 ± 2 vs. 100 ± 17 ACE2/β-Actin, $P=0.008$).

Primers used for qPCR reactions:

Primer	Sequence 5'-3'	Accession Number
mACE2 Fwd	CCC AAA GAG CAG TGG ATG AA	NM_027286
mACE2 Rev	GAG ATG CAG GGT CAC AGT ATG	NM_027286
mACE Fwd	GCC ATA CGT CAG GTA CTT TGT	NM_001281819
mACE Rev	CTG CTT CCT TGG ATT GGT AGA	NM_001281819
mNGAL Fwd	GCC ACT CCA TCT TTC CTG TT	NM_008491
mNGAL Rev	GGA GTG CTG GCC AAA TAA GA	NM_008491
mKIM-1 Fwd	CAG GAA GAC CCA CGA CTA TTT C	NM_134248
mKIM-1 Rev	TTG TGA GTC CAT GTG TGT GTA G	NM_134248
mGAPDH Fwd	AAC AGC AAC TCC CAC TCT TC	AY618568
mGAPDH Rev	CCT GTT GCT GTA GCC GTA TT	AY618568

Table S1: Primers used for RT-qPCR reactions including their 5'-3' Sequence and accession numbers.