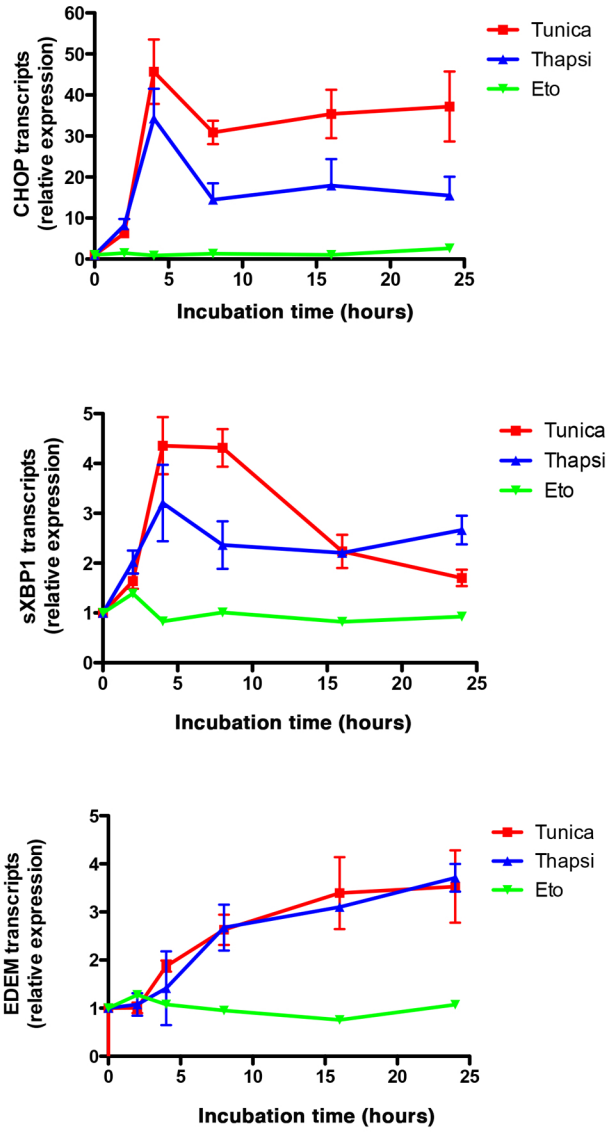
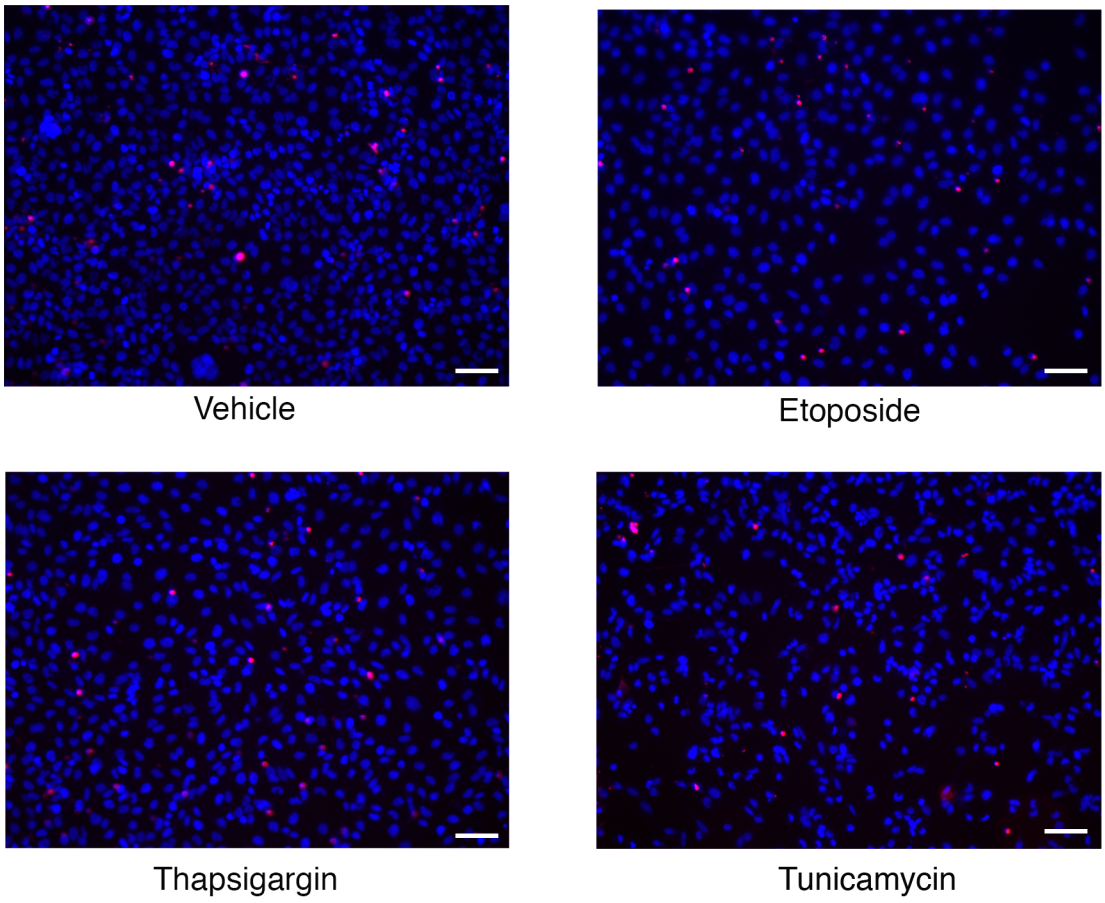
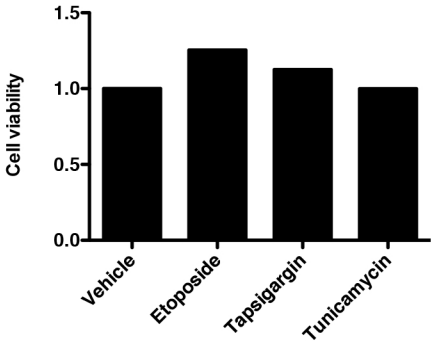
SUPPLEMENTARY FIGURE 1



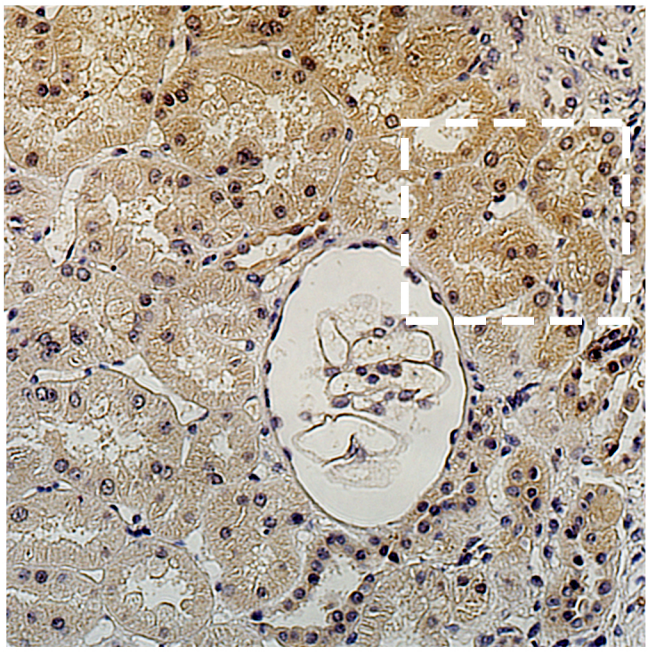
SUPPLEMENTARY FIGURE 2

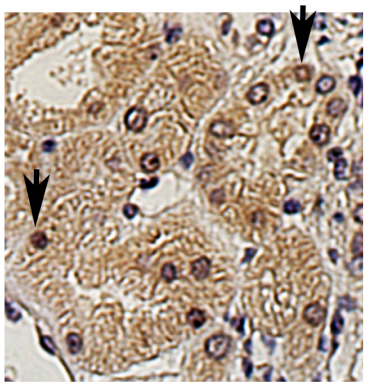




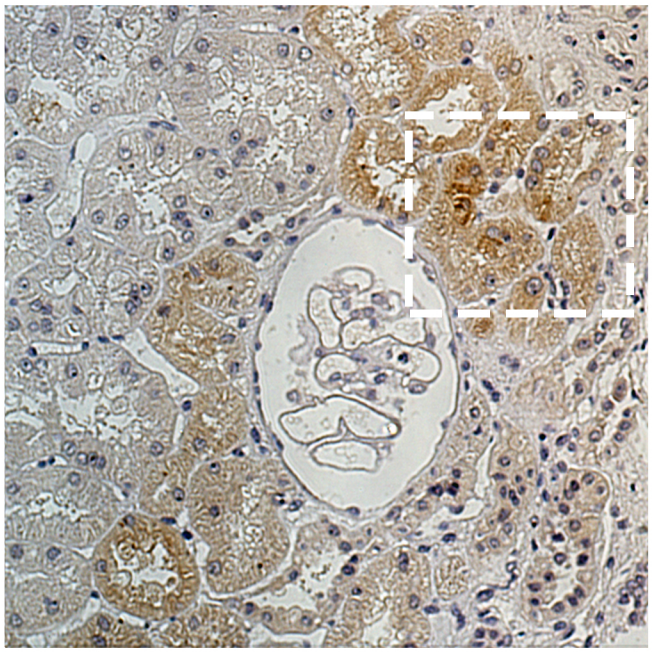
SUPPLEMENTARY FIGURE 3

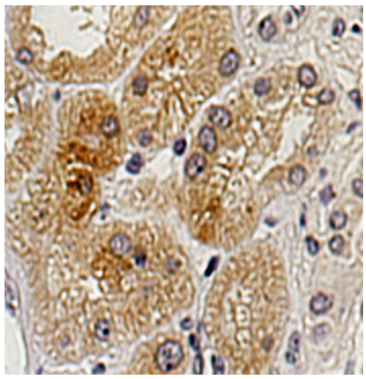
sXBP1





ANG





Supplementary figure legends

Supplementary figure 1

Box and whiskers plots representing CHOP, sXBP1 and EDEM transcripts relative expression analyzed by RT-qPCR in HREC incubated with 2.5 μg/mL Tunicamycin, 0.25μM Thapsigargin or vehicle, for a various periods of time. Data are from 3 independent experiments.

Supplementary figure 2

A. Representative epifluorescence photomicrograph of HREC incubated 8 hours with 2.5 μg/mL Tunicamycin, 0.25 μM thasigargin, 100μM etoposide or vehicle, and stained with 1 μg/ml Hoechst 33342 (HO) and 5 μg/ml propidium iodine (PI). The bar represents 50 μm. The data represent three independent experiments. **B.** Cell viability was determined using the MTS assay 8 hours after incubation with 2.5 μg/mL Tunicamycin, 0.25 μM Thasigargin, 100μM Etoposide. Values are ratio of cell viability reported to vehicle-treated cells.

Supplementary figure 3

Representative photomicrograph of sXBP1 and ANG expression evaluated by immunohistochemistry on consecutive slices in kidney allograft biopsy. Original magnification, x40. Black arrows denote sXBP1 positive epithelial cells nuclei, and blue arrow denotes a sXBP1 negative nuclei.

Supplementary methods

Cell culture

HREC (HK-2) cells were established by transduction with human papilloma virus (HPV 16) E6/E7 genes from a primary proximal tubule cells culture from normal adult human renal cortex exposed to a recombinant retrovirus containing the HPV 16 E6/E7 genes. HK-2 cells are cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 5 μg/mL insulin, 10 μg/mL human apotransferrin, 500 ng/mL hydrocortisone, 10 ng/mL Epithelial growth factor, 6.5 ng/mL triiodothyronin, 5 ng/mL sodium selenite, 1% fetal calf serum, 25 IU/mL penicillin, 25 μg/mL streptomycin and 10 mM HEPES buffer. These cells lines are Mycoplasm free (Mycoalert Mycoplasma Detection Kit, Lonza). Tunicamycin, thapsigargin and etoposide were from Sigma Aldrich.

Hoechst Propidium iodine staining

Epifluorescence microscopy was performed on unfixed/unpermeabilized adherent cells stained with Hoechst 33342 (Invitrogen) and propidium iodide (Invitrogen) as previously described (44). Briefly, the cells were grown to confluence on 6-well polycarbonate culture plates. Hoechst 33342 (1 μg/ml) was added for 10 min at 37°C. Propidium Iodide was added at a final concentration of 5 μg/ml immediately prior to the fluorescence microscopy analysis (excitation filter l = 360-425 nm).

Immunofluorescence analysis

For confocal microscopy, the cells were fixed in 2% paraformaldehyde for 1 hour on ice and permeabilized with 0.1% Triton X-100 in PBS for 1 hour. Prior to overnight incubation with 1/50 anti ANG (sc-9044, Santa Cruz Biotechnology), the slides were blocked for 1 hour in a solution comprising 2% goat serum (Sigma Aldrich), 1% bovine serum albumin (Sigma Aldrich), and 0.1% Tween-20 in PBS. Alexa Fluor 555© donkey anti-rabbit IgG (Invitrogen) were used as secondary antibodies. The nuclear counterstain used for visualization was 4',6-diamidino-2-phenylindole (DAPI, 0.05 μg/ml) (Invitrogen, D3571). AlexaFluor© 555 and DAPI were imaged using a Zeiss LSM 710 imaging fluorescence microscope.

Chromatin immunoprecipitation assay

Briefly, HREC were incubated with Tunicamycin for 1 hour. Subsequently, the cells were washed with PBS and cross-linked with 1% formaldehyde at 37°C. After terminating the crosslinking reaction, the cells were collected, washed, and resuspended in the SDS lysis buffer containing protease inhibitor cocktail (Roche). The lysates were sonicated five times followed by cooling on ice. The cell debris was cleared, and the supernatant was diluted in a ChIP dilution buffer. After a brief centrifugation, 1% of the total supernatant was put aside and one-tenth of this material was used as input control. Half of the remaining supernatant was incubated with ananti-sXBP1 or anti-p65 antibody, and the other half of the supernatant was incubated with a non-immune rabbit immunoglobulin G and protein G magnetic beads at 4°C overnight with rotation. The beads were washed and pelleted using a magnetic separator. After elution, DNA fragments were purified using a spin column. For PCR reactions, 10% of the immunoprecipitated materials were used as the DNA template in 40 cycles of amplification using the following primer sets. The primers sequences are listed in the **Supplementary table 1A**. The results of the ChIP analysis were calculated and recorded as fold-enrichment values. The default Input fraction was 1%, which represents a dilution factor of 100 or 6.644 cycles (i.e., log2 of 100).

RNA isolation from kidney transplant biopsies

Total RNA was extracted using TriPure Isolation® reagent (Roche Applied Science). The expression levels ANG mRNA were quantified through RT-qPCR. The fold-changes were normalized to the RPL13A housekeeping gene and compared with the results obtained from a normal kidney.

Clinical chemistry analyses

The urine protein measurements were performed at the Clinical Chemistry Department of the European Georges Pompidou Hospital. The urinary total protein concentration was quantified by measuring pyrogallol red at 600/800 nm absorbance (urinary CSF protein assay, Beckman Coulter), and the Albumin concentration was measured by immunoturbidimetry analysis (DIAgAM assay, Beckman Coulter) using a Beckman Coulter AU680 analyzer. The urinary levels of RBP were measured using a Siemens BN II nephelometer Analyzer II and kits from Siemens. The urine protein concentrations were corrected for creatinin levels, which were measured using a colorimetric assay (modified kinetic Jaffe method) on a Beckman Coulter AU680 analyzer (urine).

Viability studies

HRECs were seeded in 96-well plates (10^4 cells/ml). Twenty-four hours later, cells were treated. Three days afterwards, the relative number of living cells per well was determined on the basis of mitochondrial integrity by assay with 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sul-fophenyl)-2H-tetrazolium (MTS) (Promega, Charbonnieres, France), according to the manufacturer's instructions.

Supplementary table 1A. Primer sequences used for RT-PCR analysis

Gene name	Primer sequensce
ANG	F 5'-tgtcctgcccgtttctgcgg-3' R 5'-ccggccctgtggtttggcat-3'
GRP78/BiP	F 5'-ggtgaaagacccctgacaaa-3' R 5'-gtcaggcgattctggtcatt-3'
IL-6	F 5'-aggagacttgcctggtgaaa-3' R 5'-ggatgcagggtcaagagtagtg-3'
IL-8	F 5'-caggaattgaatgggtttgc-3' R 5'-aaaccaaggcacagtggaac-3'
TNFα	F 5'-tccttcagacaccctcaacc-3' R 5'-cagggatcaaagctgtaggc-3'
IDO	F 5'-ggcacacgctatggaaaact-3' R 5'-cgctgtgacttgtggtctgt-3'
INFα	F 5'-acctagagcccaaggttca-3' R 5'-cccagagagcagcttgact-3'
INFβ	F 5'-cattacctgaaggccaagga-3' R 5'-cagcatctgctggttgaaga-3'
IL1β	F 5'-cagccaatcttcattgctca-3' R 5'-gcatcttcctcagcttgtcc-3'
iNOS	F 5'-aagccccacagtgaagaaca-3' F 5'-tccaaggacaggccatctct-3'
sXBP1	F 5'-tgctgagtccgcagcaggtg-3' F 5'-gctggcaggctctggggaag-3'
ANG promoter primer 1 (XBP1 CHIP)	F 5'- acaaaaggggtggaaaaac -3' R 5'- agctacagtggctcgttggt -3'
ANG promoter primer 2 (XBP1 CHIP)	F 5'- cagagactacccctggctga-3' R 5'- gtttttccacccccttttgt-3'
ANG promoter primer 1 (p65 CHIP)	F 5'- acaaaaggggtggaaaaac -3' R 5'- agctacagtggctcgttggt -3'
ANG promoter primer 2 (p65 CHIP)	F 5'- tgtgtacacggacggagatg-3' R 5' atcccaggctcgttctttg -3'
IL6 promoter (p65 CHIP)	F 5'-tagagcttctctttcgttcccggt-3' R 5'-tgtgtcttgcgatgctaaaggacg-3'

Supplementary table 1B. Primary antibodies used for Western blot.

Antibody	Dilution	Reference
Anti-β Actin	1:1000	A2668[1]
Anti-ATF6	1 :500	IMG-273[2]
Anti-IRE1α	1:500	3294[4]
Anti-PERK	1:500	3192[4]
Anti-XBP1	1 :1000	37152[4]
Anti IκB	1 :1000	9542[4]
Anti phospho JNK	1 :1000	9251[4]
Anti JNK	1 :1000	3708[4]
Anti phosphoERK	1 :1000	4376[4]
Anti ERK	1 :1000	4372[4]
Anti phosphoAkt	1 :1000	8200[4]
Anti Akt	1 :1000	9272[4]
Anti P65	1 :1000	SC-8008[5]
Anti phosphoMEK	1 :1000	9121[4]
Anti PhosphoP70S6Kinase	1 :1000	9205[4]

[1] Sigma-Aldrich
[2] Imgenex
[3] Abcam
[4] Cell Signaling Technology
[5] Santa Cruz Biotechnology