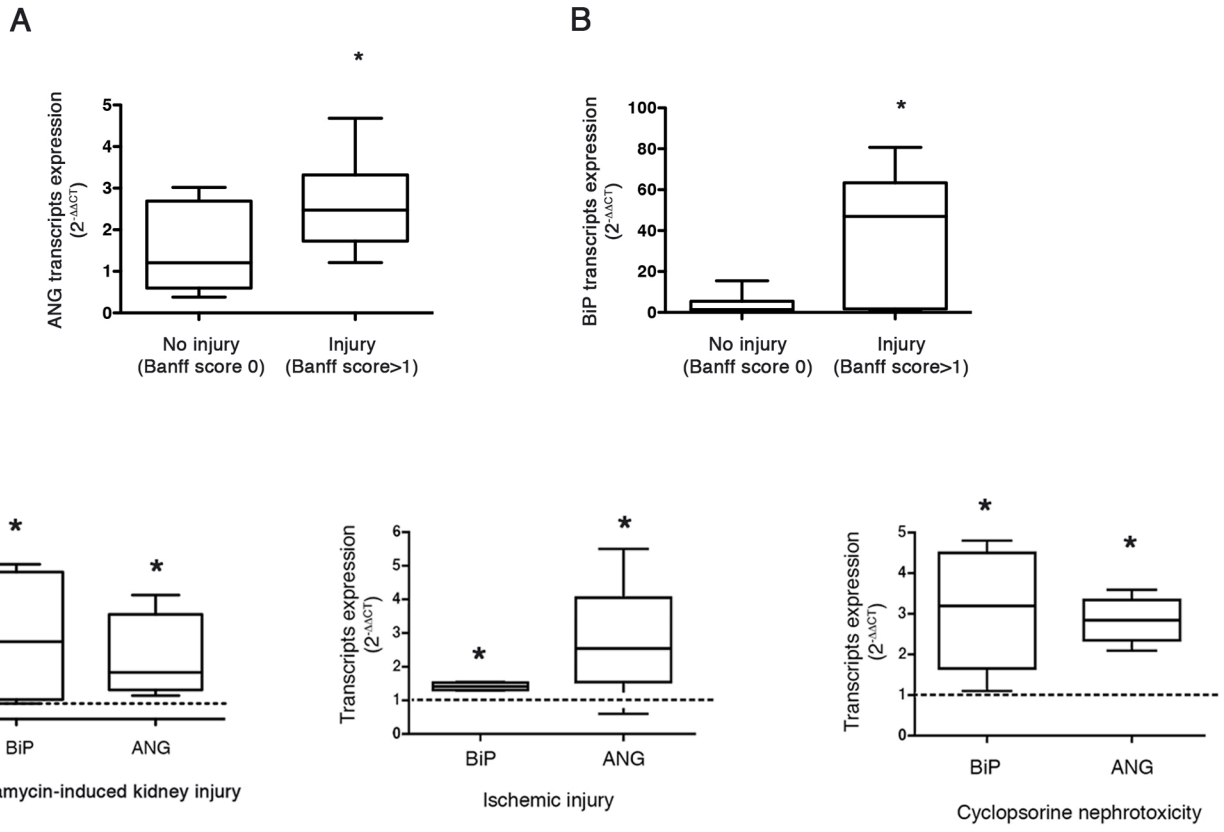
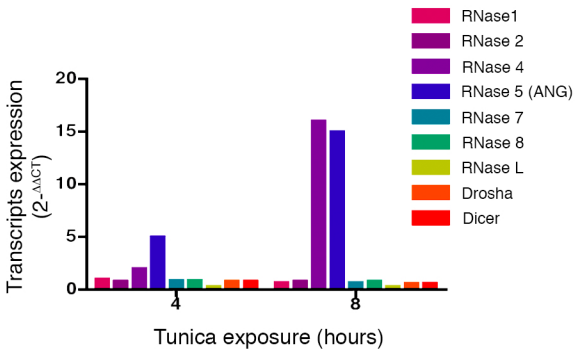
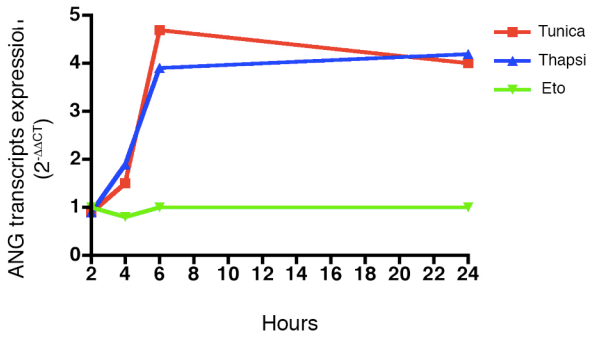
SUPPLEMENTARY FIGURE 1



SUPPLEMENTARY FIGURE 2

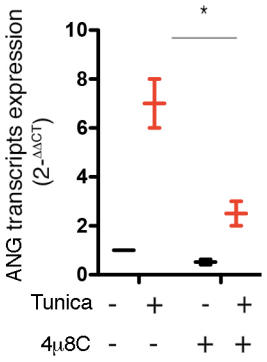


SUPPLEMENTARY FIGURE 3

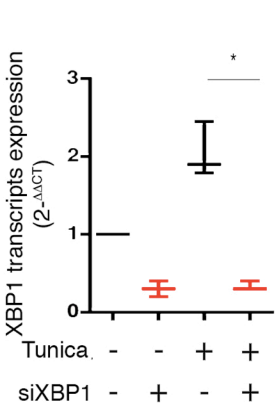


SUPPLEMENTARY FIGURE 4

A

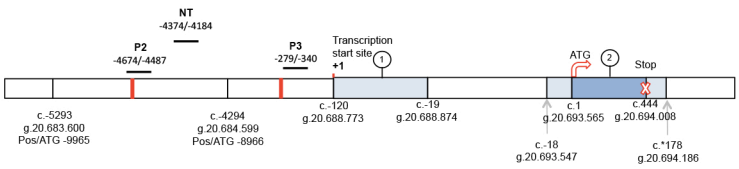


B

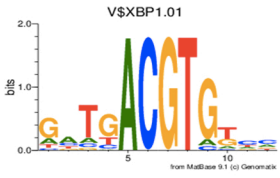


C

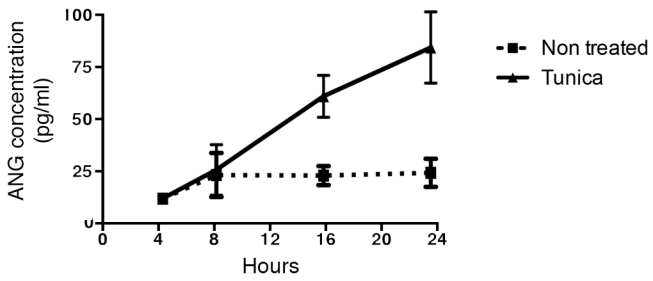
ANG, Rnase 5, NM_001097577, (14q11.2), GRCh 38



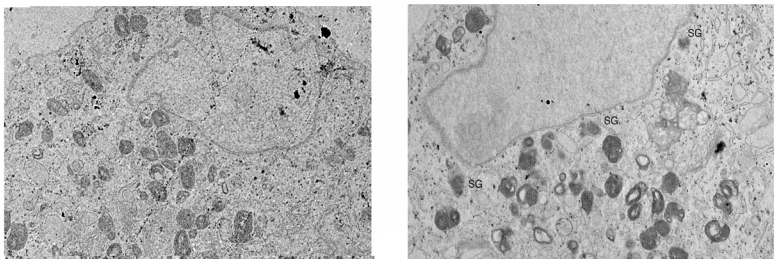
XBP1 binding site (-4565 and - 475)



SUPPLEMENTARY FIGURE 5

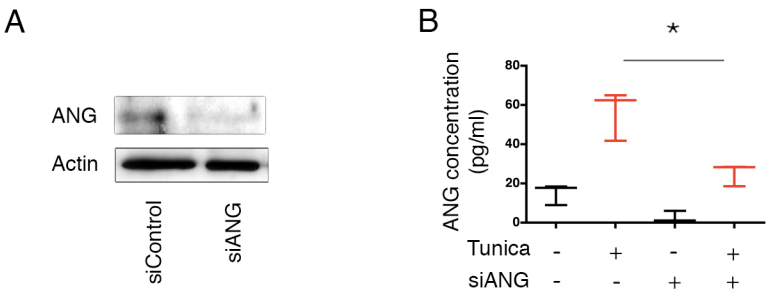


SUPPLEMENTARY FIGURE 6

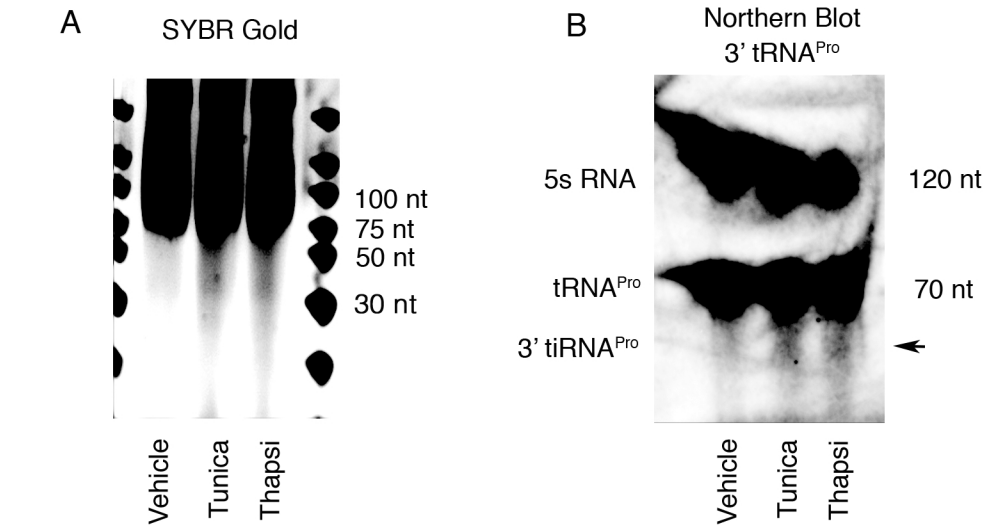


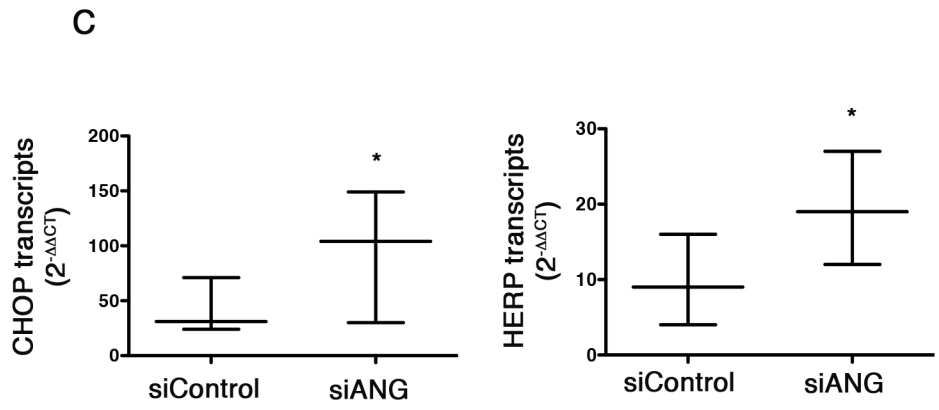
Vehicle Tunica

SUPPLEMENTARY FIGURE 7



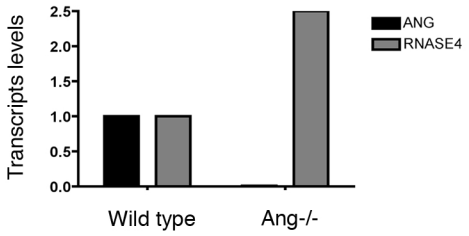
SUPPLEMENTARY FIGURE 8





SUPPLEMENTARY FIGURE 9

A



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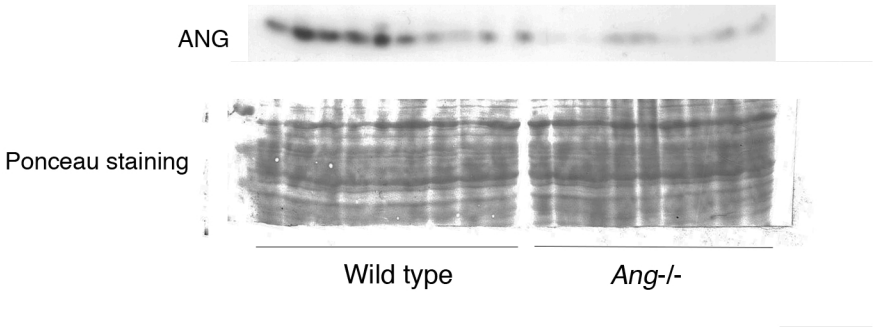
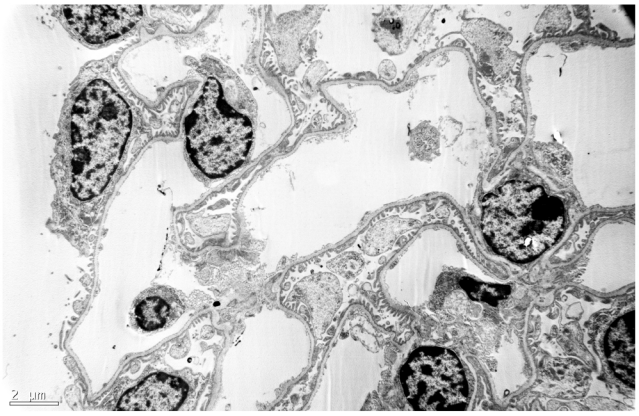


SUPPLEMENTARY FIGURE 10

A



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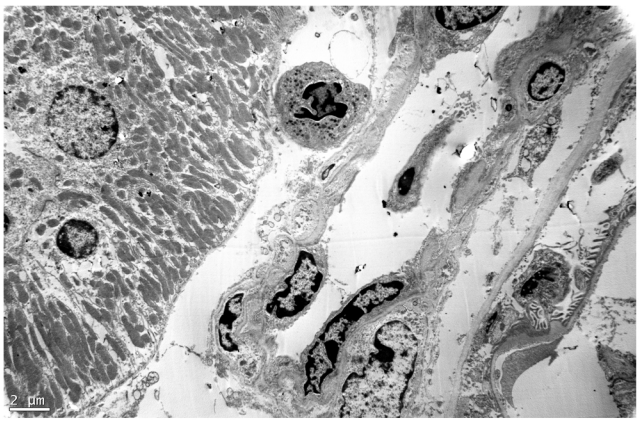
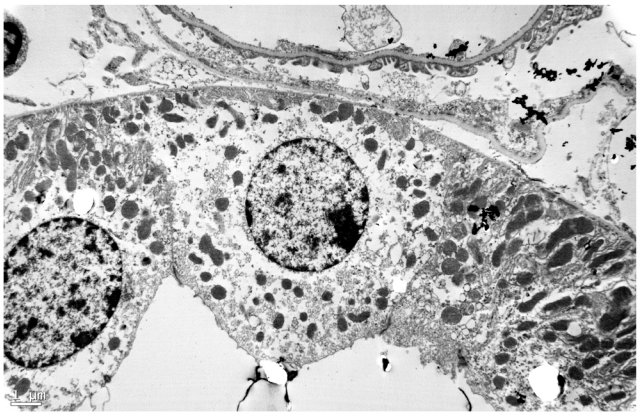
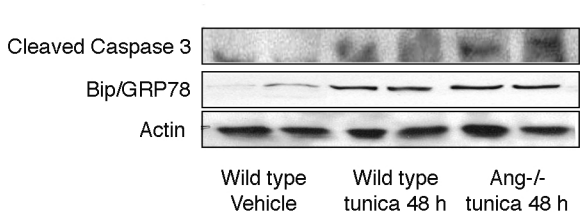


C



SUPPLEMENTARY FIGURE 11

A



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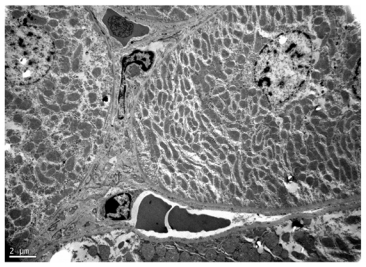
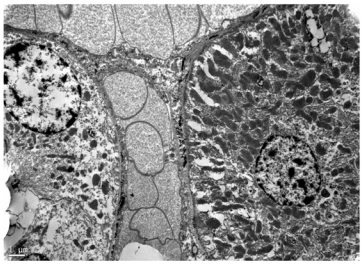
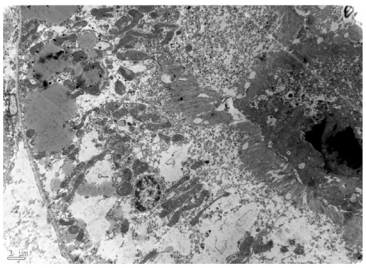


Wild type
Vehicle
(6000X)



Wild type
Tunica
(7500X)



Ang-/-
Tunica
(7500X)

Supplementary table 1. In silico analysis of ANG putative transcription factors (Genomatix and Cscan)

Abbreviation	Gene name
ATF4	Activating transcription factor 4
BATF	Basic leucine zipper transcription factor
BCL3	B-cell CLL/lymphoma 3
BCLAF1	BCL2-associated transcription factor 1
BDP1	Subunit of RNA polymerase III transcription initiation factor IIIB
BHLHE40	Basic helix-loop-helix family, member e40
BRCA1	Breast cancer 1, early onset
BRF1	BRF1 homolog, subunit of RNA polymerase III transcription initiation factor IIIB
CEBPB	CCAAT/enhancer binding protein (C/EBP), beta
CHD2	Chromodomain helicase DNA binding protein 2
CREB1	cAMP-responsive element-binding protein 1
E2F1	E2F transcription factor 1
E2F4	E2F transcription factor 4
E2F6	E2F transcription factor 6
EBF1	Early B-cell factor 1
EGR1	Early growth response 1
ELF1	E74-like factor 1 (ets domain transcription factor)
EP300	E1A-binding protein p300
ESR1	Estrogen receptor 1
ETS1	v-ets erythroblastosis virus E26 oncogene homolog 1
FOS	FBJ murine osteosarcoma viral oncogene homolog
FOSL2	FOS-like antigen 2
FOXA1	Forkhead box A1
FOXA2	Forkhead box A2
FOXO1	Forkhead box O1
GATA2	GATA-binding protein 2
GATA3	GATA-binding protein 3
GTF2F1	General transcription factor IIF, polypeptide 1
HDAC2	Histone deacetylase 2
HEY1	Hairy/enhancer-of-split related with YRPW motif 1
HIF1	Hypoxia-inducible factor 1
HNF1	Hepatocyte nuclear factor 1
HNF4A	Hepatocyte nuclear factor 4

HNF4G	Hepatocyte nuclear factor 4
HSF1	Heat shock transcription factor 1
IRF4	Interferon regulatory factor 4
JUN	jun oncogene
JunD	jun D proto-oncogene
LHX1	LIM homeobox 1
LYL1	Lymphoblastic leukemia-derived sequence 1
MAFF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F
MAFK	v-maf musculoaponeurotic fibrosarcoma oncogene homolog K
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)
NANOG	Nanog homeobox
NFKB1	Nuclear factor of light polypeptide gene enhancer in B-cells 1
NR3C1	Nuclear receptor subfamily 3, group C, member 1
PAX5	Paired box 5
PBX1	Pre-B-cell leukemia homeobox 1
PBX3	Pre B cell leukemia homeobox 3
PGR	Progesterone receptor
POLR3A	Polymerase (RNA) III (DNA directed) polypeptide A
POU5F1	POU class 5 homeobox 1
PPAR	Peroxisome proliferator-activated receptor
PPAR	Peroxisome proliferator-activated receptor
PPARGC1A	Peroxisome proliferator-activated receptor , coactivator 1
RAD21	RAD21 homolog
RB1	Retinoblastoma 1
RFX5	Regulatory factor X, 5
RXRA	Retinoid X receptor
SIN3A	SIN3 transcription regulator homolog A (yeast)
SMARCC2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin
SMC3	Structural maintenance of chromosomes 3
SP1	Sp1 transcription factor
SREBF2	Sterol regulatory element binding transcription factor 2
SRF	Serum response factor
STAT3	Signal transducer and activator of transcription 3
TCF3	Transcription factor 3
TFAP2A	Transcription factor AP-2
TFAP2C	Transcription factor AP-2

TP53	Tumor protein p53
TRIM28	Tripartite motif containing 28
VDR	1,25- dihydroxyvitamin D3 receptor
XBP1	X-box binding protein 1

Supplementary table 2A. Primer sequences used for RT-PCR analysis

Gene name	Primer sequence
ANG	F 5'-tgtcctgcccgtttctgcgg-3' R 5'-ccggccctgtggtttggcat-3'
GRP78	F 5'-ggtgaaagacccctgacaaa-3' R 5'-gtcaggcgattctggtcatt-3'
CHOP	F 5'-tggaagcctggtatgaggac-3' R 5'-tgtgacctctgctggttctg-3'
RNASE 1	F 5'-ccacctcacaacccactctt-3' R 5'-ggtcagagcagcgagatacc-3'
RNASE 3	F 5'-aggtgaactggaaccacagg-3' R 5'-agattccgggtgcctttact-3'
RNASE 4	F 5'-ccaaccccacctagatgcaa-3' R 5'-tatcttagaggtgcctgcctcggcct-3'
RNASE 7	F 5'- tcttggggataagcatctgg-3' R 5'-tttggctgaccttcaattcc-3'
RNASE 8	F 5'-ctgccagaccccaacatag-3' R 5'-agtgcacaggaacaatggg-3'
RNASE L	F 5'-ggagatccacaggaagtcaagaga-3' R 5'-caggatggaagagacgatgaatg-3'
Drosha	F 5'-gctacgaacggagcagagag-3' R 5'-acgaggtcaggaacaaccg-3'
Dicer	F 5'-gccattggacacatcaatagatactg-3' R 5'-ggttctgcatttaggagtagatga-3'
GRP78 *(rattus norvegicus)*	F 5'-ggaggaggacaagaagg-3' R 5'-acatacgacggtgtgatg-3'
ANG Non target (NT) -9393/-9539	F 5'-atcccaggctcgttctttg-3' R 5'-tgtgtacacggacggagatg-3'
ANG promoter 2 (P2) -4674/-4487	F 5'-gtttttccacccccttttgt-3' R 5'cagagactacccctggctga -3'
ANG promoter 3 (P3) -279/-340	R 5' tgccttggaagatgtcacag-3' F 5' tggctgtatgaaatcgatgg-3'
IL-6	F 5'-aggagacttgcctggtgaaa-3' R 5'-ggatgcagggtcaagagtagtg-3'
CHOP	F 5'- tggaagcctggtatgaggac -3' R 5'- tgtgacctctgctggttctg -3'
HERP	F 5'- agaacttgcggatgaatgc -3' R 5'- gaggaaaacggaaaatgtcg -3'

Supplementary table 2B. Primary antibodies used for Western blot.

Antibody	Dilution	Reference
Anti-β Actin	1:1000	A2668[1]
Anti-Angiogenin (human)	1 :500	ab-10600[2]
Anti-Angiogenin (mouse)	1 :500	sc-9044[3]
Anti-ATF6	1 :500	IMG-273[5]
Anti-eIF2α	1:1000	2103[4]
Anti-phospho-eIF2α	1 :1000	3597[4]
Anti-GRP78	1:1000	sc-1050[3]
Anti-IRE1α	1:500	3294[4]
Anti-PERK	1:500	3192[4]
Anti PARP	1 :1000	9542[4]
Anti RNH1	1 :1000	sc-49699[3]
Anti Cleaved caspase 3	1 :1000	9661[4]

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

Supplementary figures legends

Supplementary figure 1. ANG and BiP expression in kidneys under ER stress.

A,B. Box and whiskers plots representing ANG and BiP transcripts expression in 16 kidney transplant biopsies using RT-qPCR. The ANG/RPL13A and BiP/RPL13A ratio were calculated compared with a normal kidney cDNA. *, p<0.05, Mann Withney test. **C**. Box and whiskers plots representing BiP and ANG transcripts relative expression measured by RT-qPCR in in kidneys of 5 mice treated with 1 mg/kg tunicamycin for 24 hours; or 5 rats subjected to 24 hours of cold ischemic injury, or 5 rats treated with 15 mg/kg/day cyclosporine for 28 days. Mann-Whitney U test: *P<0.05. **D.** Representative photomicrographs of ANG and BiP coexpression in kidneys of 5 mice treated with 1 mg/kg tunicamycin for 24 hours, being evaluated by immunohistochemistry and immunofluorescence on frozen sections. Magnification, x200.

Supplementary figure 2. Expression of ribonucleases in HREC under ER stress.

Graph representing the expression levels of the transcripts of various ribonucleases measured by RT-qPCR in cultured HREC, incubated with 2 μg/ml tunicamycin or vehicle for 4 or 8 hours. The graph represents two independent experiments.

Supplementary figure 3. Expression of ANG in primary cultured human renal epithelial cells.

Graph representing the relative expression of ANG transcripts measured by RT-qPCR during a time course experiment on primary cultured renal epithelial cells incubated with 2 μg/ml tunicamycin, 0.25 μM thasigargin, 100 μM etoposide or vehicle. The graph represents two independent experiments.

Supplementary figure 4. Expression of XBP1 after transfection of HREC with XBP siRNA.

A. Box and whiskers plots representing ANG transcripts relative expression in HREC incubated with 10μM 4μ8C, or vehicle, and with 2 μg/ml tunicamycin or vehicle, for 24 hours, measured using RT-qPCR. Data are from three independent experiments. Mann-Whitney U test: *P<0.05. **B**. Box and whiskers plots representing XBP1 transcripts relative expression in HREC transfected with a siRNA targeting XBP1, or a scrambled siRNA, and incubated with 2 μg/ml tunicamycin or vehicle for 24 hours, measured using RT-qPCR. Data are from three independent experiments. Mann-Whitney U test: *P<0.05. **C**. Graph representing the sXBP1 binding sites on the ANG promoter marked in red. P2, P3 and NT denote the binding site of the primer used for CHIP.

Supplementary figure 5. Secretion of ANG under ER stress.

Graph representing the ANG concentration (means±sem), measured using ELISA in the extracellular medium obtained from a time course experiment on HREC incubated with 2 μg/ml tunicamycin, or vehicle. The data are representative of four independent experiments.

Supplementary figure 6. Production of stress granules during ER stress.

A. Electron microscopy of HREC incubated with 2 μg/ml tunicamycin or vehicle. SG denotes stress granules.

Supplementary figure 7. ANG expression after transfection of ANG siRNA of HREC under ER stress.

A. Immunoblot representing ANG and actin protein expression in HREC 24 hours after transfection with a siRNA targeting ANG mRNA. The immunoblot represents three independent experiments. **B**. Box and whiskers plots representing the ANG concentration in the extracellular medium of HREC transfected with a siRNA targeting ANG, or a scrambled siRNA, and incubated with 2 μg/ml tunicamycin or vehicle for 24 hours, measured using ELISA. Mann-Whitney U test: *P<0.05.

Supplementary figure 8. Production tiRNA in HREC under ER stress.
A. Polyacrylamide gel electrophoresis of small RNA extracted from HREC after 2 hours of incubation with 2 μg/ml tunicamycin, 0.25 μM thapsigargin or vehicle followed by staining with SYBR gold. The data were obtained from 3 independent experiments**. B.** Northern blotting analysis of RNA extracted from HREC cells incubated with 2 μg/ml tunicamycin, 0.25 μM thapsigargin or vehicle for 2 hours. Blots were hybridized to cDNA complementary to the 3′ fragments of the $tRNA^{Pro}$ and 5S RNA as a loading control. The blot represents two independent experiments. **C**. Box and whiskers plots representing CHOP and HERP transcripts relative expression in HREC incubated with a siRNA targeting ANG mRNA or siRNA control, and with 2 μg/ml tunicamycin or vehicle, for 24 hours, measured using RT-qPCR. Data are from three independent experiments. Mann-Whitney U test: **P*<0.05.

Supplementary figure 9. ANG expression in *Ang*-/- and wild type mice.

A. Graph representing the expression of ANG and RNAse 4 transcripts measured by RT-qPCR in *Ang*-/- and wild type mice. The graph represents two independent experiments. **B**. Immunoblot representing ANG expression in *Ang*-/- and wild type mice.

Supplementary figure 10. Electron microscopy of *Ang*-/- mouse kidneys.

Electron microscopy photomicrographs of elementary kidney structures from *Ang*-/- mice. **A**, glomerulus; **B**, proximal tubular cells and peritubular capillaries; **C**, distal tubular cells.

Supplementary figure 11. Tubular injury in *Ang*-/- mouse kidneys under ER stress

A. Immunoblot representing cleaved caspase 3 and BiP expression in *Ang*-/- and wild type mice 48 h after treatment with 1 mg/kg tunicamycin or vehicle. **B**. Electron microscopy photomicrographs of tubules from wild type and *Ang*-/- mice under ER stress.

Supplementary methods

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.

Immunohistochemistry of human kidney biopsies

Kidney biopsies were fixed in alcohol-formalin-acetic acid, dehydrated with ethanol and xylene, embedded in paraffin, and cut into 3-μm sections. The samples were subsequently deparaffinized, rehydrated and heated for 20 minutes at 97°C in citrate buffer. Endogenous peroxidase was inactivated through incubation for 10 minutes at room temperature in 3% H_2O_2. The sections were incubated with PBS containing 1:500 anti-BiP antibody (sc-1050, Santa Cruz Biotechnology) and 1:50 anti-ANG (ab-125231, Abcam). Subsequently, the sections were incubated with anti-goat or anti-rabbit antibodies conjugated to a peroxidase-labeled polymer (Dako) and visualized with a peroxidase kit (Dako). Moreover, the tissue sections were counterstained with hematoxylin.

Animal studies

Cyclosporine nephrotoxicity:
Adult male Sprague-Dawley rats (Charles River laboratories, L'Arbresle, France) weighing 325-350g were allowed free access to tap water. CsA was diluted in olive oil and administered intraperitoneally at a dose of 15 mg/kg/day. The vehicle-treated group received olive oil at 1 ml/kg/day. The animals were sacrificed at 28 days. Kidneys were processed for RNA extraction.

Ang knockout (Ang-/-) mice
For electron microscopy of *Ang*-/- mouse kidneys, the samples were fixed in a solution of 2% glutaraldehyde 0.1 M sodium cacodylate, post-fixed in 1% OsO4, and dehydrated in alcohol. They were then processed for flat embedding in Epon 812 and observed in a Zeiss CEM 902 electron microscope. TUNEL staining was performed with the *In Situ* Cell Death Detection Kit, Fluorescein (Roche), following the manufacturer instructions. Samples were counterstained with DAPI to visualize nuclei. The number of TUNEL positive nuclei per high power field was reported to the number of DAPI positive nuclei to calculate the percentage of TUNEL positive nuclei, and calculated on at least 4 fields (x100) per kidney sample.

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.

RNA extraction and real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted using the RNeasy Mini Kit® (Qiagen) according to the manufacturer's protocol. Transcript expression levels were quantified through SYBR green RT-qPCR using an ABI PRISM 7900 sequence detector system (Applied Biosystems). Vehicle-treated samples were used as controls, and the fold-changes for each tested gene were normalized to the Ribosomal Protein L13A (RPL13A) housekeeping gene. The relative

expression levels were calculated using the $2^{(-\Delta\Delta CT)}$ method (43). By definition, the expression level of a given gene in control sample, using the $2^{-\Delta\Delta CT}$ method to calculate relative expression levels, is 1. Primer sequences are listed in the **Supplementary table 2A**.

Protein extraction and Western blot analysis

Total protein lysate from HREC was separated through sodium dodecyl sulfate polyacrylamide gel electrophoresis under denaturing conditions and transferred to a PVDF membrane (GE Healthcare). Primary antibodies (listed in the **Supplementary table 2B**) were visualized using horseradish peroxidase-conjugated polyclonal secondary antibodies (Dako) and detected using ECL reagent® (GE Healthcare).

siRNA transfections

Two different siRNA directed against the same target were transfected: PERK (Hs_EIF2AK3_5; Hs_EIF2AK3_6), IRE1α (Hs_ERN1_17; Hs_ERN1_17), ATF6 (Hs_ATF6_5; Hs_ATF6_5), ANG (Hs_ANG_6; Hs_ANG_7), XBP1 (Hs_XBP1_7; Hs_XBP1_10). Cells were incubated with siRNA for 24 hours before conducting the experiments.

Enzyme-linked immunosorbent assays

Subconfluent cells were grown on 6-well or 96 wells plates for the indicated times under the indicated conditions. The secretion of ANG was quantified in the cell culture supernatant using the Quantikine® human ANG immunoassay (R and D Systems) according to the manufacturer's protocol.

Immunofluorescence analyses

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), or 1:50 anti-eIF4E (sc-9976, Santa Cruz Biotechnology), 1:50 anti-eIF3B (ab40799, Abcam) antibodies, 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 © 488 Goat anti-mouse IgG and 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). Alexa Fluor© 488, AlexaFluor© 555, and DAPI were imaged using a Zeiss LSM 710 imaging fluorescence microscope.
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 I = 360-425 nm). An investigator blinded to the experimental conditions estimated the percentages of HO-PI positive cells adhering to the dishes in 3 random fields per condition.

Transfection of transcription factors

Transient transfections were performed using Lipofectamine 2000 (Invitrogen) according to manufacturer instructions. The following plasmids were used: ATF4 (Addgene Inc, ID 21845), ATF6 (Addgene Inc, ID 11975), and XBP1s (Addgene Inc, ID 21833). pcDNA3.1 vector was used as a control. After 36 hours of transfection, cells were harvested for mRNA preparation.

Northern Blotting for the identification tiRNA

Total RNA (20 µg per well) was analyzed using TBE-urea gels, transferred to Hybond N+ membranes (Amersham), and hybridized overnight at 50° with digoxigenin (DIG)-labeled

DNA probes in DIG Easy Hyb solution (Roche). After washing, the membranes were blocked in blocking solution (Roche) for 30 min at room temperature, probed with alkaline phosphatase-labeled anti-digoxigenin antibody (Roche), and washed for 30 min with washing buffer (Roche). Signals were visualized with CSPD, ready-to-use (Roche). DIG-labeled probes for 5S rRNA, tRNA, and tiRNAs were prepared using the DIG Oligonucleotide 3′-End Labeling kit, second generation (Roche) according to the manufacturer's instructions.

Co-immunoprecipitation assay

Specifically, total protein lysates from HREC were extracted using ice-cold M-PER (Mammalian Protein Extraction Reagent, Thermo Scientific) containing protease inhibitor cocktail (Roche applied science) then incubated on ice for 10 minutes. The lysates were pre-cleared for 30 minutes at 4°C with 50 µL of Immobilized Protein G-Agarose (Roche diagnostics). A fraction of 50 µL was taken and used as an input control. Whole cell lysates were rotated overnight at 4°C with 5 µg anti-ANG antibody (sc-9044, Santa Cruz Biotechnology) and 50 µl of protein A-agarose beads (cat. no. 16–156, Upstate). The next day, the agarose beads were washed four times in the extraction buffer solution. The immunoprecipitated fractions were denatured then analyzed by SDS-PAGE and immunoblotted for ANG and RNH1.