## Supplementary tables

**Supplementary table 1.** Estimation of the precision of the quantification of angiogenin in urines.
Absorbances are in Arbitratry Units.

| **Characteristic** | **Low concentration** | **High concentration** |
|---|---|---|
| Day 1 | 0.42 | 0.82 |
| | 0.47 | 0.79 |
| | 0.48 | 0.69 |
| | 0.42 | 0.77 |
| | 0.40 | 0.77 |
| Mean | 0.44 | 0 .77 |
| Standard deviation | 0.03 | 0.04 |
| CV% | **6.8** | **5.1** |
| Day 2 | 0.45 | 0.81 |
| | 0.43 | 0.83 |
| | 0.46 | 0.99 |
| | 0.45 | 0.77 |
| | 0.43 | 0.72 |
| Mean | 0.45 | 0.82 |
| Standard deviation | 0.01 | 0.1 |
| CV% | **2.2** | **12.1** |
| Day 3 | 0.47 | 0.77 |
| | 0.50 | 0.83 |
| | 0.46 | 0.79 |
| | 0.45 | 0.85 |
| | 0.44 | 0.81 |
| Mean | 0.46 | 0.81 |
| Standard deviation | 0.02 | 0.03 |
| CV% | **4.3** | **3.7** |
| Intralaboratory reproducibility (%CV) | ***4.4*** | ***7.5*** |

**Supplementary table 2.** Demographic and clinical characteristics of the cohort of the 192 patients referred for the exploration of a chronic kidney disease*.

| **Characteristic** | **Entire cohort** (n=192) |
|---|---|
| Age (yrs) | 54.9±18.2 |
| Male sex-n (%) | 115 (61) |
| Caucasian-n (%) | 96 (50) |
| Cause of CKD<br>• GN<br>• Diabetes<br>• ANCA vasculitidis<br>• FSGS<br>• Minimal changes disease<br>• Interstitial nephritis<br>• Myeloma<br>• CNI toxicity (heart transplantation)<br>• Others | <br>48 (25)<br>22 (10)<br>10 (5)<br>17 (9)<br>6 (3)<br>25 (13)<br>9 (4)<br>5 (2.6)<br>50 (26) |
| Fibrosis¶ (%) | 27±12 |
| Urinary angiogenin/creatinin (ng/mmol) | 112±228 |
| eGFR (ml/min/1.73m$^2$) | 57±23 |
| Proteinuria (g/mmol creat) | 0.35±0.65 |
| β2 microglobulin (mg/mmol creat) | 1.63±3.96 |
| α1 microglobulin (mg/mmol creat) | 8.41±12.04 |
| Retinol binding protein (mg/mmol creat) | 1.39±3.07 |
| Transferrin (mg/mmol creat) | 10.5±15.05 |
| Albumin (mg/mmol creat) | 185±243.7 |

* Plus-minus values are means±SD. CKD denotes chronic kidney disease, GN glomerulonephritis, ANCA anti-neutrophil cytoplasmic antibody, FSGS, focal segmental glomerulosclerosis, CNI calcineurin inhibitors, eGFR estimated glomerular filtration rate.

¶ Interstitial fibrosis was evaluated using automatic quantification by image analysis.

**Supplementary table 3.** Demographic and clinical characteristics of the cohort of the kidney transplant recipients who had a clinically indicated biopsy, according to the concentrations of angiogenin in urines*.

| **Characteristic** | **uANG 1th quartile (n=61)** | **uANG 2th quartile (n=61)** | **uANG 3th quartile (n=61)** | **uANG 4th quartile (n=59)** | **P value¶** |
|---|---|---|---|---|---|
| Urinary angiogenin/creatinin (ng/mmol) | 99±33 | 195±27 | 334±55 | 1214±1095 | <0.001 |
| Time interval after transplantation (months) | 37.7±36 | 49.5±74 | 39.8±54 | 33±63 | 0.48 |
| Age (yrs) | 43.6±14.2 | 42.7±17.1 | 47±17.7 | 48±16.5 | 0.45 |
| Male sex-n (%) | 37 (60) | 35 (57) | 42 (68) | 35 (59) | 0.57 |
| Cause of ESRD | | | | | 0.43 |
| • GN | 14 (2) | 10 (16) | 15 (25) | 14 (23) | |
| • Diabetes | 2 (3) | 7 (11.5) | 7 (11) | 6 (10) | |
| • Cystic/hereditary | 11 (18) | 12 (20) | 9 (15) | 10 (17) | |
| • Secondary GN | 4 (6.5) | 0 | 3 (5) | 2 (3) | |
| • Hypertension | 7 (11.5) | 8 (13) | 4 (6) | 3 (5) | |
| • Interstitial nephritis | 9 (14) | 10 (16) | 7 (11) | 15 (25) | |
| • Miscellaneous | 0 | 3 (5) | 2 (3) | 3 (5) | |
| • Uncertain | 14 (23) | 11 (18) | 13 (21) | 6 10) | |
| Donor age (yrs) | 53.3±13.5 | 54±16.5 | 54.3±19 | 51.6±17.6 | 0.84 |
| Living donor-n (%) | 15 (26) | 18 (29) | 13 (21) | 10 (16) | 0.37 |
| Expanded criteria donor-n (%) | 26 (43) | 25 43) | 24 (40) | 31 (52) | 0.55 |
| Retransplantation-n (%) | 9 (15) | 6 (10) | 12 (20) | 17 (28) | 0.04 |
| Preformed anti HLA DSA-n (%) | 15 (30) | 14 (29) | 15 (30) | 24 (48) | 0.55 |
| Cold ischemia time (hours) | 15.8±10.1 | 16.5±12.1 | 17.8±9.7 | 18.7±10.4 | 0.44 |
| Delayed graft function-n (%) | 14 (23) | 11 (19) | 16 (27) | 23 (40) | 0.05 |

* Plus-minus values are means±SD. ESRD denotes end stage renal disease, GN glomerulonephritis, DSA donor specific antibodies.

¶ The P value is for the comparison between patients across the quartiles of the distribution of uANG, which refers to Ln (urinary angiogenin/creatinin).

**Supplementary table 4.** Hazard ratio (univariate and multivariate models) for post-biopsy failure after 3 months according to individual clinical and histological parameters in Cox proportional hazards analysis*.

| Characteristic | Unadjusted Hazard ratio | P value | Adjusted Hazard ratio | P value |
|---|---|---|---|---|
| Recipient age, per increase of 1 yr | 0.96 (0.93-0.99) | 0.01 | 1 (0.97-1.05) | 0.73 |
| Time to transplantation, per increase of 1 yr | 1.09 (1.03-1.14) | 0.02 | 1.13 (1.01-1.25) | 0.02 |
| i+t ≥2 versus <2 | 3.44 (1.31-8.74) | 0.01 | 2.23 (0.82-5.61) | 0.11 |
| ci+ct ≥2 versus <2 | 1.52 (1.13-2.25) | 0.003 | 3.25 (0.82-14.6) | 0.09 |
| DSA at biopsy (Yes versus No) | 2.97 (0.99-10.8) | 0.05 | 2.11 (0.79-5.79) | 0.13 |
| Plasma creatinin at biopsy≥180 μmol/L versus <180 μmol/L¶ | 3.96 (1.42-14) | 0.007 | 3.57 (1.25-13.09) | 0.01 |
| Urine protein-to-creatinin ratio at biopsy ≥0.1g/mmol versus <0.1 g/mmol£ | 3.25 (1.28-8.51) | 0.01 | 1.88 (0.72-5.01) | 0.19 |
| uANG at biopsy (ng/mmol creat)$ | | | | |
| • ≥5.48 ng/mmol versus<5.48 ng/mmol | 5.41 (1.94-19.1) | 0.0009 | 3.37 (1.05-14.97) | 0.03 |
| • per unit increment | 2.95 (1.72-4.96) | 0.0001 | 2.60 (1.53-4.47) | 0.0005 |

*Adjusted hazard ratios were calculated with the use of a multivariate model incorporating all covariates listed in the table, and which were associated (p<0.1) with graft loss in the univariate analysis.

¶ Plasma Creatinin at biopsy≥180 μmol/L versus <180 μmol/L corresponds to the median of the distribution of the creatinin values in the cohort.

£ Urine protein-to-creatinin ratio at biopsy ≥0.1g/mmol versus <0.1 g/mmol which corresponds to 1g/24 hours, a validated cut-off for diagnosis, prognosis and management.

$ uANG at biopsy at biopsy ≥5.48 ng/mmol versus<5.48 ng/mmol L corresponds to the median of the distribution of the uANG values in the cohort.

## Supplementary methods

### *In vitro* studies

*Cell culture*- Human renal epithelial cells (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 was 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. The primers used for XBP1 have the following sequence: unspliced xbp1 Forward 5'-aaacagagtagcagctcagactgc, unspliced xbp1 Reverse 5'-tcctcctgggtagacctctgggag ; spliced XBP1 Forward 5'-tgctgagtccgcagcaggtg, spliced xbp1 Reverse 5'-gctggcaggctctggggaag.

*ELISA in vitro*-Subconfluent cells were grown in 6 wells plates for the indicated times under the indicated conditions. Secretion of angiogenin in the culture medium was quantified in the cell culture supernatant using the Quantikine® human Angiogenin immunoassay (RD Systems), according to the manufacturer's protocol.

*Protein extraction and Western blot analysis*

Total protein lysate from human renal epithelial cells was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis under denaturing conditions and transferred to a PVDF membrane (GE Healthcare). Primary antibodies were visualized using horseradish peroxidase-conjugated polyclonal secondary antibodies (Dako) and detected by ECL reagent® (GE Healthcare).

## Supplementary figure legends

### Supplementary figure 1

**A**. Calibrating curve of the ELISA method for urinary angiogenin monitoring. **B**. Distribution of the values of urinary angiogenin/creatinin and the log transformed values.

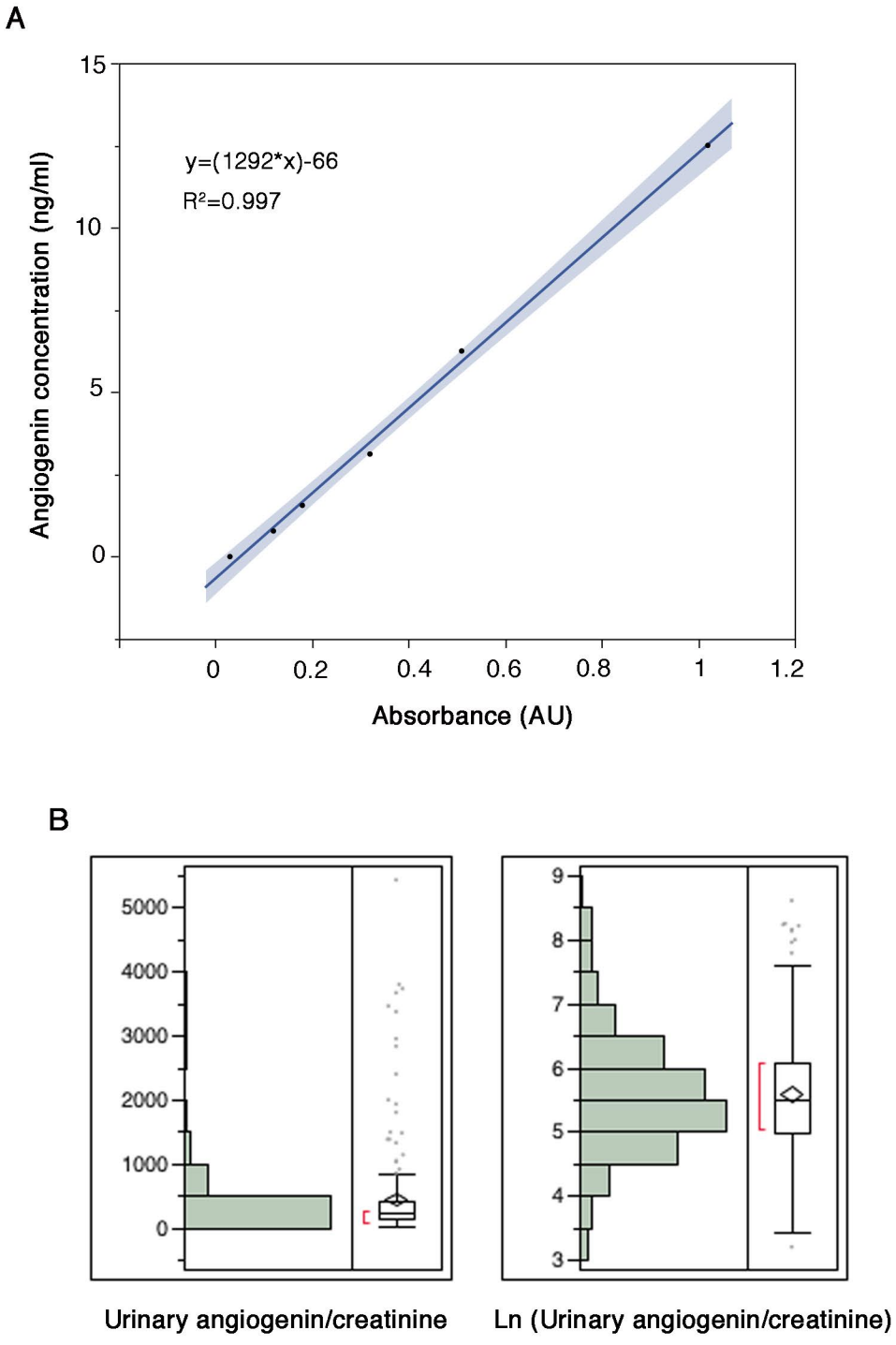
### Supplementary figure 2

Linear regression analysis of uANG with plasma creatinin and proteinuria in the cohort of 242 kidney transplant recipients with a clinically indicated biopsy.

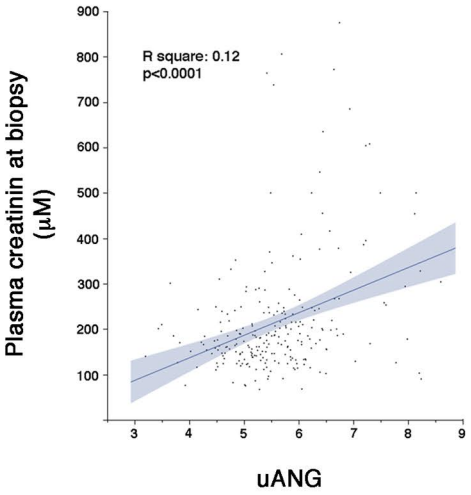
### Supplementary figure 3

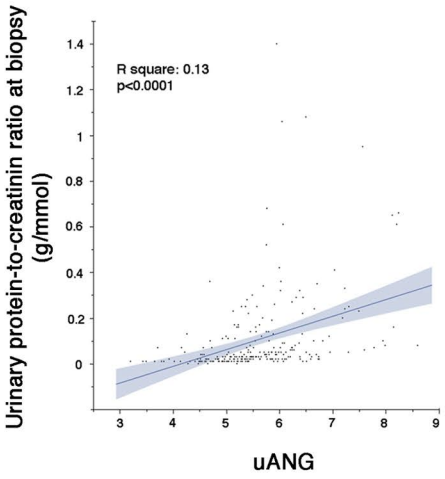
Box and whiskers plots representing the distribution of uANG according to the deceased donor and the period of time after transplantation during which uANG has been measured (before or after 3 months).*, p=0.03, Student's T test.

SUPPLEMENTARY FIGURE 1



SUPPLEMENTARY FIGURE 2



SUPPLEMENTARY FIGURE 3