SUPPLEMENTAL METHODS

Materials. Acetonitrile (ACN), acetone, trifluoroacetic acid (TFA), DL-dithiothreitol (DTT), iodoacetamide (IAA), glycine, Ponceau S were purchased from Sigma (Sigma Aldrich St.Louis, MO, USA); urea, CHAPS, SDS, glycerol, acrylamide, Immobiline Dry-Strips, and ampholine were purchased from PlusOne Amersham Biosciences (Uppsala, Sweden); Agarose and TEMED from Invitrogen, UK; Tris from Chemie, UK, and piperazine di-acrylamide (PDA) from Bio-Rad Laboratories (Hercules, CA). Molecular Weight markers (ColorBurst electrophoresis, mol wt 8-220kDa), and Coomassie Blue G-250 were from Sigma-Aldrich, St Louis, MO. Trypsin (sequencing grade modified) was from Promega (Madison, Wisconsin, USA). All solvents used were Ultra-Resi-Analyzed grade.

SAMPLE MANAGEMENT AND SELDI-TOF/MS ANALYSIS

Supplemental Table 1. List of the 13 mass peaks marking the difference between IgAN and both CTRL and CKD.

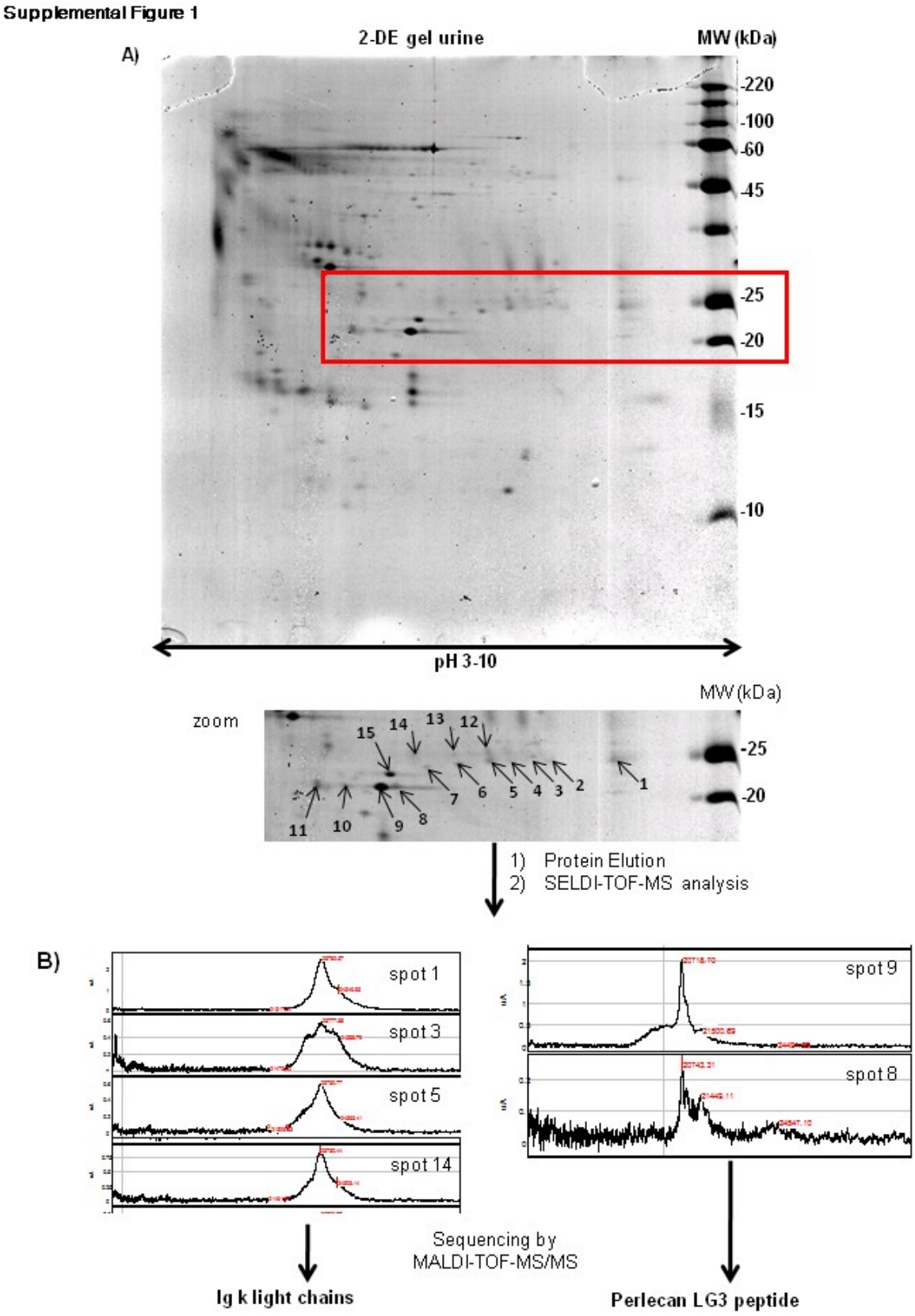
Mass (m/z)	p-value	trend in IgAN	Fold change (IgAN vs CTRL)	Fold change (IgAN vs CKD)
23458,79976	1,12E-10	reduced	-4,7	-1,6
21598,60778	7,34E-09	reduced	-2,5	-1,4
20969,49759	5,18E-09	reduced	-2,9	-1,4
19775,09674	1,43E-09	reduced	-3,8	-5
18075,93989	5,81E-10	reduced	-5	-12,8
11960,92877	0,004635	reduced	-2,2	-1,8
11770,88357	0,004626	reduced	-2,2	-1,4
10803,42267	5,43E-08	reduced	-2,8	-2,4
10549,03131	1,51E-10	reduced	-4,8	-3,4
10177,74748	3,85E-08	reduced	-2,2	-2,7
9769,716742	1,17E-07	reduced	-2,8	-2,8
9078,074797	9,03E-07	reduced	-1,2	-2,3
5868,339654	1,95E-04	reduced	-1,7	-1,9

IDENTIFICATION of ~23,458 and ~21,598 m/z by 2D-PAGE and MALDI-TOF-MS/MS.

1) **Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE)**. To identify the ~23,458 and ~21,598 m/z, urine proteins of two healthy subject and one IgAN patient were separated by 2D-PAGE. Isoelectrofocusing (IEF) was carried out using a 13 cm immobiline DryStrip of pH 3–10 nonlinear range. The IPG strips were rehydrated for 8–10 h at room temperature with 250 mL of rehydration solution (8 M urea, 2% w/v CHAPS, 0.5% ampholine (pH 3–10), 18 mM DTT, 0.002% w/v bromophenol blue). Five hundred micrograms of protein were loaded onto rehydrated IPG strips for preparative 2-D PAGE. IEF of the proteins was performed at 40 kVh total produced by overnight run. After IEF, IPG strips were incubated at room temperature for 15 min in 130 mM DTT equilibration buffer (75 mMTris-HCl, pH 8.8; 6 M urea; 30% v/v glycerol 87%; 2% w/v SDS; 0.002% bromophenol blue), then for 15 min in 270 mM IAA equilibration buffer. The second dimension was carried out on homemade 15% polyacrylamide/PDA slab gels in SDS-PAGE running buffer. The gels were stained by Colloidal Coomassie Blue G-250 and scanned with a flat-bed ImageScanner (Amersham Pharmacia Biotech) to generate digitized images. Supplemental Figure 1 shows a representative 2DE gel. The 2-DE gel images were analyzed using Image Master 2D Platinum software (Amersham Biosciences). At least three replicate gels were made from each urinary sample.

2) **Passive Elution of Proteins from Polyacrylamide Gels**. All protein spots (gel pieces) on 2-DE gels included in the 20-25 kDa mass range (outlined in the red box in Supplemental Figure 1) were manually excised and washed with H_2O for 2 h, and proteins were allowed to diffuse out of the gel overnight at 37 °C by incubation in 30 µl of 0.1M sodium acetate, 0.1% SDS, pH 8.2. Then, the surnatant containing the eluted proteins was analyzed by SELDI-TOF/MS, as described in the METHODS section of the main manuscript, to check for the presence of the proteins with ~23,458 and ~21,598 m/z. Spots number 1, 3, 5, 14 (left panel, Supplemental Figure 1) and 8, 9 (right panel,

Supplemental Figure 1), which showed a MW of ~23,458 and ~21,598 m/z, respectively, were subsequently identified by MALDI-TOF-MS/MS.



Supplemental Figure 1. Protein separation and identification. **A**. 2-DE urinary gel of a healthy subject. MW and pI values are indicated on the right side and at the bottom of the image, respectively. The area in the 20-25 kDa molecular weight range is highlighted by a red box and zoomed below the 2DE gel. The protein spots (from 1 to 15) were excised from the gel, eluted and analyzed by SELDI-TOF-MS. **B**. SELDI-TOF-MS spectra of the proteins eluted from 2DE gel, and corresponding to the interested m/z peaks (spots number 1, 3, 5, 14 in left panel and 8, 9 in right panel), that were subsequently identified by MALDI-TOF-MS/MS as Ig k light chains and perlecan LG3 peptide, respectively.

3) MALDI-TOF-MS/MS analysis.

The protein spots on 2-DE gels were manually excised, and underwent in-gel tryptic digestion by an adaptation of the procedure of Shevchenko *et al* [Shevchenko, A., Wilm, M., Vorm, O., Mann, M. et al. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal Chem 1996, 68, 850-858.]. The tryptic peptide mixture was eluted directly onto a Prespotted Anchor Chip^{TM} (PAC, Bruker Daltonics, Bremen, Germany) a MALDI sample carrier with ready spotted matrix (α-ciano-4-hydroxycinnaminic acid) positions besides the pre-spotted calibration point. After spotting the peptide mixture on the MALDI target plate it was dried under ambient conditions. The MALDI mass spectra were acquired on Autoflex III^{TM} TOF/TOF200 instrument with smartbeam^{TM} laser technology. All spectra were acquired in reflecting mode with 200 Hz laser frequency, a delayed extraction time of 10, in the 500-3500m/z range. LIFT^{TM} MS/MS spectra were externally calibrated using abundant fragment ion peaks derived from bradykinin(1-7), angiotensin I, angiotensin II, substance P, bombesin, ACTH 1-17, and ACTH 18-39, ACTH1_24, Insulin_B. Precursor ions for MS/MS analysis were selected with a timed ion selector at a resolution of approximately 450. All mass values are reported as monoisotopic masses. The program used to create the ”peak list” from the raw data acquired from the FlexControl 3.3 was FlexAnalysis 3.3 with the default parameters. Protein identification was achieved by database search via Biotools 3.2

and MASCOT search algorithm (http://www.matrix.science.com) against the MSDB, NCBInr and Swissprot databases using the following parameters: Homo Sapiens as taxonomic category, trypsin as enzyme, carbamidomethyl as fixed modification for cysteine residues, oxidation of methionine as variable modification, and one missing cleavage and 50 ppm as mass tolerance for the monoisotopic peptide masses and 0.3 Da mass tolerance for MS/MS analysis.

Supplemental Table 2. Protein sequence identified by Mascot Search Engine.

Spot n°	Entry name (Swiss-prot)	MASCOT score (Swiss-prot)	Sequence Coverage (%)	No. of peptides matched (PMF)[#]	Peptides sequenced
1	**KV302_HUMAN**	130	66%	12	**FSGSGSGTDFTLTISR** **SGTASVVCLLNNFYPR**
3	**KV302_HUMAN**	110	70%	12	**FSGSGSGTDFTLTISR** **SGTASVVCLLNNFYPR**
5	**IGKC_HUMAN**	82	49%	11	**SGTASVVCLLNNFYPR** **EIVLTQSPGTLSLSPGER**
14	**IGKC_HUMAN**	84	49%	8	**SGTASVVCLLNNFYPR**
8	**PGBM_HUMAN**	185	1%*	3	**YQLGSGEAR** **SLPEVPETIELEVR** **LVSEDPINDGEWHR**
9	**PGBM_HUMAN**	197	2%*	4	**YQLGSGEAR** **AQAGANTRPCPS** **SLPEVPETIELEVR** **GNVYIGGAPDVATLTGGR**

#:PMF: Peptide Mass Fingerprinting.

-Double Click on the corresponding icone "Mascot Search Results Protein View" or "Peptide Summary Report" or onthe corresponding link to visualize data-

1) Identification of Ig-kappa light chain spot 1, MS spectra

Mascot Search Results Protein View.url

http://www.matrixscience.com/cgi/protein_view.pl?file=../data/20111130/Ftoplesah.dat&hit=1

MS/MS spectra of 2 peptide of Ig-kappa light chain spot 1

Peptide Summary Report (lift 1632).url

http://www.matrixscience.com/cgi/master_results.pl?file=../data/20111130/FtopleeSm.dat

Peptide Summary Report (lift 1797).url

http://www.matrixscience.com/cgi/master_results.pl?file=../data/20111130/FtopleemT.dat

2) Identification of Ig-kappa light chain spot 3, MS spectra

Mascot Search Results Protein View.url

http://www.matrixscience.com/cgi/protein_view.pl?file=../data/20111130/FtopleTnm.dat&hit=2

MS/MS spectra of 2 peptide of Ig-kappa light chain spot 3

Peptide Summary Report (LIFT 1632).url

http://www.matrixscience.com/cgi/master_results.pl?file=../data/20111215/FtouSGanm.dat

Peptide Summary Report (lift 1797).url

http://www.matrixscience.com/cgi/master_results.pl?file=../data/20111130/FtopleYwh.dat

3) Identification of Ig-kappa light chain spot 5, MS spectra

Mascot Search Results Protein View.url

http://www.matrixscience.com/cgi/protein_view.pl?file=../data/20111216/FtouSzemR.dat&hit=1

MS/MS spectra of 2 peptide of Ig-kappa light chain spot 5

Peptide Summary Report (LIFT 1797.927).url

http://www.matrixscience.com/cgi/master_results.pl?file=../data/20111216/FtouSzSTE.dat

Peptide Summary Report (LIFT 1884.0269).url

http://www.matrixscience.com/cgi/master_results.pl?file=../data/20111216/FtouSbTnS.dat

4) Identification of Ig-kappa light chain spot 14, MS spectra

Mascot Search Results Protein View.url

http://www.matrixscience.com/cgi/protein_view.pl?file=../data/20111216/FtouinuET.dat&hit=2

MS/MS spectra of 1 peptide of Ig-kappa light chain spot 14

Peptide Summary Report (lift 1797.8966).url

http://www.matrixscience.com/cgi/master_results.pl?file=../data/20111216/Ftouinstt.dat

5) Identification of Perlecan LG3 peptide, spot 9 , Combination of MS and MS/MS spectra

Peptide Summary Report (combined spectra).url

http://www.matrixscience.com/cgi/master_results.pl?file=..%2Fdata%2F20111121%2FFtopoGsER.dat&REPTYPE=peptide&_sigthreshold=0.05&REPORT=5&_server_mudpit_switch=99999999&_ignoreionsscorebelow=0&_showsubsets=0&_showpopups=TRUE&_sortunassigned=scoredown&_requireboldred=0

6) Identification of Perlecan LG3 peptide, spot 8, Combination of MS and MS/MS spectra

Peptide Summary Report (combined spectra) (2).url

http://www.matrixscience.com/cgi/master_results.pl?file=..%2Fdata%2F20111128%2FFtopifHES.dat&REPTYPE=peptide&_sigthreshold=0.05&REPORT=20&_server_mudpit_switch=99999999&_ignoreionsscorebelow=0&_showsubsets=0&_showpopups=TRUE&_sortunassigned=scoredown&_requireboldred=0

*PGBM_HUMAN, P98160, Basement membrane-specific heparan sulfate proteoglycan core protein. Aminoacidic sequence:

```
  1-60   MGWRAAGALL LALLLHGRLL AVTHGLRAYD GLSLPEDIET VTASQMRWTH SYLSDDEDML
 61-120  ADSISGDDLG SGDLGSGDFQ MVYFRALVNF TRSIEYSPQL EDAGSREFRE VSEAVVDTLE
121-180  SEYLKIPGDQ VVSVVFIKEL DGWVFVELDV GSEGNADGAQ IQEMLLRVIS SGSVASYVTS
181-240  PQGFQFRRLG TVPQFPRACT EAEFACHSYN ECVALEYRCD RRPDCRDMSD ELNCEEPVLG
241-300  ISPTFSLLVE TTSLPPRPET TIMRQPPVTH APQPLLPGSV RPLPCGPQEA ACRNGHCIPR
301-360  DYLCDGQEDC EDGSDELDCG PPPPCEPNEF PCGNGHCALK LWRCDGDFDC EDRTDEANCP
361-420  TKRPEEVCGP TQFRCVSTNM CIPASFHCDE ESDCPDRSDE FGCMPPQVVT PPRESIQASR
421-480  GQTVTFTCVA IGVPTPIINW RLNWGHIPSH PRVTVTSEGG RGTLIIRDVK ESDQGAYTCE
481-540  AMNARGMVFG IPDGVLELVP QRGPCPDGHF YLEHSAACLP CFCFGITSVC QSTRRFRDQI
541-600  RLRFDQPDDF KGVNVTMPAQ PGTPPLSSTQ LQIDPSLHEF QLVDLSRRFL VHDSFWALPE
601-660  QFLGNKVDSY GGSLRYNVRY ELARGMLEPV QRPDVVLMGA GYRLLSRGHT PTQPGALNQR
```

661-720 QVQFSEEHWV HESGRPVQRA ELLQVLQSLE AVLIQTVYNT KMASVGLSDI AMDTTVTHAT

721-780 SHGRAHSVEE CRCPIGYSGL SCESCDAHFT RVPGGPYLGT CSGCNCNGHA SSCDPVYGHC

781-840 LNCQHNTEGP QCNKCKAGFF GDAMKATATS CRPCPCPYID ASRRFSDTCF LDTDGQATCD

841-900 ACAPGYTGRR CESCAPGYEG NPIQPGGKCR PVNQEIVRCD ERGSMGTSGE ACRCKNNVVG

901-960 RLCNECADGS FHLSTRNPDG CLKCFCMGVS RHCTSSSWSR AQLHGASEEP GHFSLTNAAS

961-1020 THTTNEGIFS PTPGELGFSS FHRLLSGPYF WSLPSRFLGD KVTSYGGELR FTVTQRSQPG

1021-1080 STPLHGQPLV VLQGNNIILE HHVAQEPSPG QPSTFIVPFR EQAWQRPDGQ PATREHLLMA

1081-1140 LAGIDTLLIR ASYAQQPAES RVSGISMDVA VPEETGQDPA LEVEQCSCPP GYRGPSCQDC

1141-1200 DTGYTRTPSG LYLGTCERCS CHGHSEACEP ETGACQGCQH HTEGPRCEQC QPGYYGDAQR

1200-1260 GTPQDCQLCP CYGDPAAGQA AHTCFLDTDG HPTCDACSPG HSGRHCERCA PGYYGNPSQG

1261-1320 QPCQRDSQVP GPIGCNCDPQ GSVSSQCDAA GQCQCKAQVE GLTCSHCRPH HFHLSASNPD

1321-1380 GCLPCFCMGI TQQCASSAYT RHLISTHFAP GDFQGFALVN PQRNSRLTGE FTVEPVPEGA

1381-1440 QLSFGNFAQL GHESFYWQLP ETYQGDKVAA YGGKLRYTLS YTAGPQGSPL SDPDVQITGN

1441-1500 NIMLVASQPA LQGPERRSYE IMFREEFWRR PDGQPATREH LLMALADLDE LLIRATFSSV

1501-1560 PLAASISAVS LEVAQPGPSN RPRALEVEEC RCPPGYIGLS CQDCAPGYTR TGSGLYLGHC

1561-1620 ELCECNGHSD LCHPETGACS QCQHNAAGEF CELCAPGYYG DATAGTPEDC QPCACPLTNP

1621-1680 ENMFSRTCES LGAGGYRCTA CEPGYTGQYC EQCGPGYVGN PSVQGGQCLP ETNQAPLVVE

1681-1740 VHPARSIVPQ GGSHSLRCQV SGSPPHYFYW SREDGRPVPS GTQQRHQGSE LHFPSVQPSD

1741-1800 AGVYICTCRN LHQSNTSRAE LLVTEAPSKP ITVTVEEQRS QSVRPGADVT FICTAKSKSP

1801-1860 AYTLVWTRLH NGKLPTRAMD FNGILTIRNV QLSDAGTYVC TGSNMFAMDQ GTATLHVQAS

1861-1920 GTLSAPVVSI HPPQLTVQPG QLAEFRCSAT GSPTPTLEWT GGPGGQLPAK AQIHGGILRL

1921-1980 PAVEPTDQAQ YLCRAHSSAG QQVARAVLHV HGGGGPRVQV SPERTQVHAG RTVRLYCRAA

1981-2040 GVPSATITWR KEGGSLPPQA RSERTDIATL LIPAITTADA GFYLCVATSP AGTAQARIQV

2041-2100 VVLSASDASP PPVKIESSSP SVTEGQTLDL NCVVAGSAHA QVTWYRRGGS LPPHTQVHGS

2101-2160 RLRLPQVSPA DSGEYVCRVE NGSGPKEASI TVSVLHGTHS GPSYTPVPGS TRPIRIEPSS

2161-2220 SHVAEGQTLD LNCVVPGQAH AQVTWHKRGG SLPARHQTHG SLLRLHQVTP ADSGEYVCHV

2221-2280 VGTSGPLEAS VLVTIEASVI PGPIPPVRIE SSSSTVAEGQ TLDLSCVVAG QAHAQVTWYK

2281-2340 RGGSLPARHQ VRGSRLYIFQ ASPADAGQYV CRASNGMEAS ITVTVTGTQG ANLAYPAGST

2341-2400 QPIRIEPSSS QVAEGQTLDL NCVVPGQSHA QVTWHKRGGS LPVRHQTHGS LLRLYQASPA

2401-2460 DSGEYVCRVL GSSVPLEASV LVTIEPAGSV PALGVTPTVR IESSSSQVAE GQTLDLNCLV

2461-2520 AGQAHAQVTW HKRGGSLPAR HQVHGSRLRL LQVTPADSGE YVCRVVGSSG TQEASVLVTI

2521-2580 QQRLSGSHSQ GVAYPVRIES SSASLANGHT LDLNCLVASQ APHTITWYKR GGSLPSRHQI

2581-2640 VGSRLRIPQV TPADSGEYVC HVSNGAGSRE TSLIVTIQGS GSSHVPSVSP PIRIESSSPT

2641-2700 VVEGQTLDLN CVVARQPQAI ITWYKRGGSL PSRHQTHGSH LRLHQMSVAD SGEYVCRANN

2701-2760 NIDALEASIV ISVSPSAGSP SAPGSSMPIR IESSSSHVAE GETLDLNCVV PGQAHAQVTW

2761-2820 HKRGGSLPSH HQTRGSRLRL HHVSPADSGE YVCRVMGSSG PLEASVLVTI EASGSSAVHV

2821-2880 PAPGGAPPIR IEPSSSRVAE GQTLDLKCVV PGQAHAQVTW HKRGGNLPAR HQVHGPLLRL

2881-2940 NQVSPADSGE YSCQVTGSSG TLEASVLVTI EPSSPGPIPA PGLAQPIYIE ASSSHVTEGQ

2941-3000 TLDLNCVVPG QAHAQVTWYK RGGSLPARHQ THGSQLRLHL VSPADSGEYV CRAASGPGPE

3001-3060 QEASFTVTVP PSEGSSYRLR SPVISIDPPS STVQQGQDAS FKCLIHDGAA PISLEWKTRN

3061-3120 QELEDNVHIS PNGSIITIVG TRPSNHGTYR CVASNAYGVA QSVVNLSVHG PPTVSVLPEG

3121-3180 PVWVKVGKAV TLECVSAGEP RSSARWTRIS STPAKLEQRT YGLMDSHAVL QISSAKPSDA

3181-3240 GTYVCLAQNA LGTAQKQVEV IVDTGAMAPG APQVQAEEAE LTVEAGHTAT LRCSATGSPA

3241-3300 PTIHWSKLRS PLPWQHRLEG DTLIIPRVAQ QDSGQYICNA TSPAGHAEAT IILHVESPPY

3301-3360 ATTVPEHASV QAGETVQLQC LAHGTPPLTF QWSRVGSSLP GRATARNELL HFERAAPEDS

3361-3420 GRYRCRVTNK VGSAEAFAQL LVQGPPGSLP ATSIPAGSTP TVQVTPQLET KSIGASVEFH

3421-3480 CAVPSDRGTQ LRWFKEGGQL PPGHSVQDGV LRIQNLDQSC QGTYICQAHG PWGKAQASAQ

3481-3540 LVIQALPSVL INIRTSVQTV VVGHAVEFEC LALGDPKPQV TWSKVGGHLR PGIVQSGGVV

3541-3600 RIAHVELADA GQYRCTATNA AGTTQSHVLL LVQALPQISM PQEVRVPAGS AAVFPCIASG

3601-3660 YPTPDISWSK LDGSLPPDSR LENNMLMLPS VRPQDAGTYV CTATNRQGKV KAFAHLQVPE

3661-3720 RVVPYFTQTP YSFLPLPTIK DAYRKFEIKI TFRPDSADGM LLYNGQKRVP GSPTNLANRQ

3721-3780 PDFISFGLVG GRPEFRFDAG SGMATIRHPT PLALGHFHTV TLLRSLTQGS LIVGDLAPVN

3781-3840 GTSQGKFQGL DLNEELYLGG YPDYGAIPKA GLSSGFIGCV RELRIQGEEI VFHDLNLTAH

3841-3900 GISHCPTCRD RPCQNGGQCH DSESSSYVCV CPAGFTGSRC EHSQALHCHP EACGPDATCV

3901-3960 NRPDGRGYTC RCHLGRSGLR CEEGVTVTTP SLSGAGSYLA LPALTNTHHE LRLDVEFKPL

3961-4020 APDGVLLFSG GKSGPVEDFV SLAMVGGHLE FRYELGSGLA VLRSAEPLAL GRWHRVSAER

4021-4080 LNKDGSLRVN GGRPVLRSSP GKSQGLNLHT LLYLGGVEPS VPLSPATNMS AHFRGCVGEV

4081-4140 SVNGKRLDLT YSFLGSQGIG QCYDSSPCER QPCQHGATCM PAGEYEFQCL CRDGFKGDLC

4141-4200 EHEENPCQLR EPCLHGGTCQ GTRCLCLPGF SGPRCQQGSG HGIAESDWHL EGSGGNDAPG

4201-4260 QYGAYFHDDG FLAFPGHVFS R**SLPEVPETI ELEVR**TSTAS GLLLWQGVEV GEAGQGKDFI

4261-4320 SLGLQDGHLV FR**YQLGSGEA R**LVSEDPIND GEWHRVTALR EGRRGSIQVD GEELVSGRSP

4321-4380 GPNVAVNAK**G SVYIGGAPDV ATLTGGR**FSS GITGCVKNLV LHSARPGAPP PQPLDLQHR**A**

4381-4390 **QAGANTRPCP S**

4197-4391: LG3 peptide (aminoacid sequence underlined)

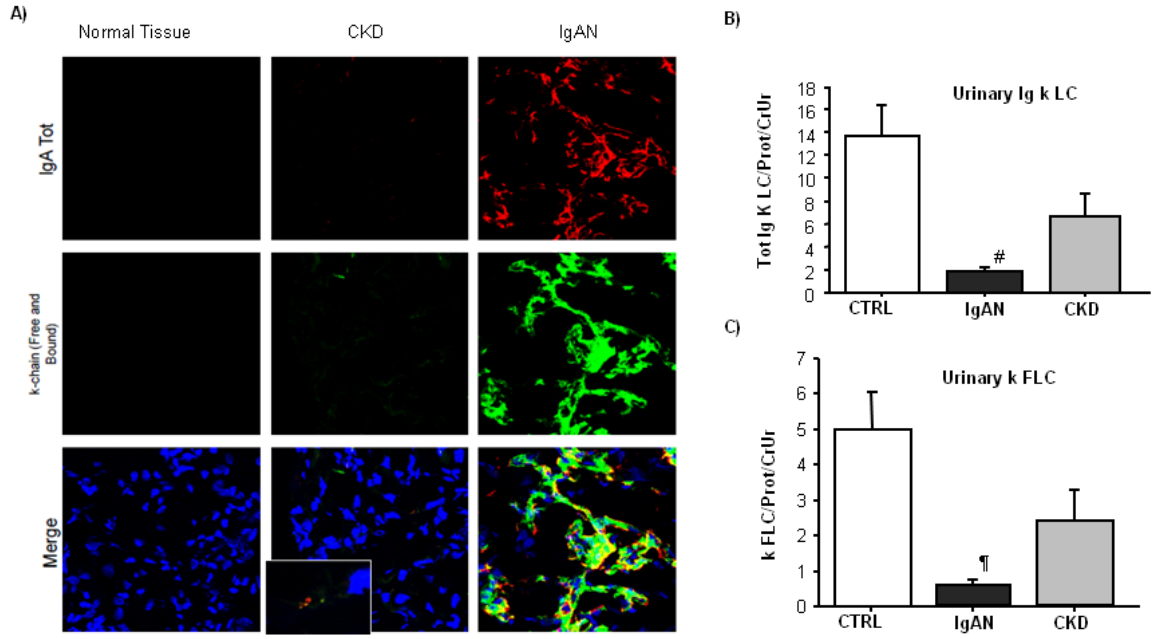
In red bold: sequenced peptides of LG3.

IMMUNOFLUORESCENCE AND CONFOCAL MICROSCOPY

Procedure used for Immunofluorescence and confocal microscopy for k FLC, LG3 and Perlecan on renal tissue biopsies. Tissue were washed twice for 5 min in PBS, then blocked with BSA 3% in PBS for 1h at RT and incubated overnight at +4°C with primary antibodies at the appropriate dilutions in blocking buffer. At the end of incubation time, sections were stained with Alexa Fluor 488 rabbit anti-goat IgG, CF 488A-labeled chicken anti-rabbit, and CF 555A-labeled goat anti-mouse antibody (Biotium, Hayward, CA), respectively. Nuclei were then counterstained with TOPRO-3 (Invitrogen, Grand Island, NY) before mounting in Fluorogel (EMS, Hatfield, PA) aqueous mounting media. Serial confocal images were captured at a total magnification of 400 x on a Leica TCS-SP5 confocal laser-scanning microscope (Leica Microsystems, Bensheim, Germany). Three-dimensional overlaid images were compressed using a maximum projection algorithm provided with the Leica TCS software.

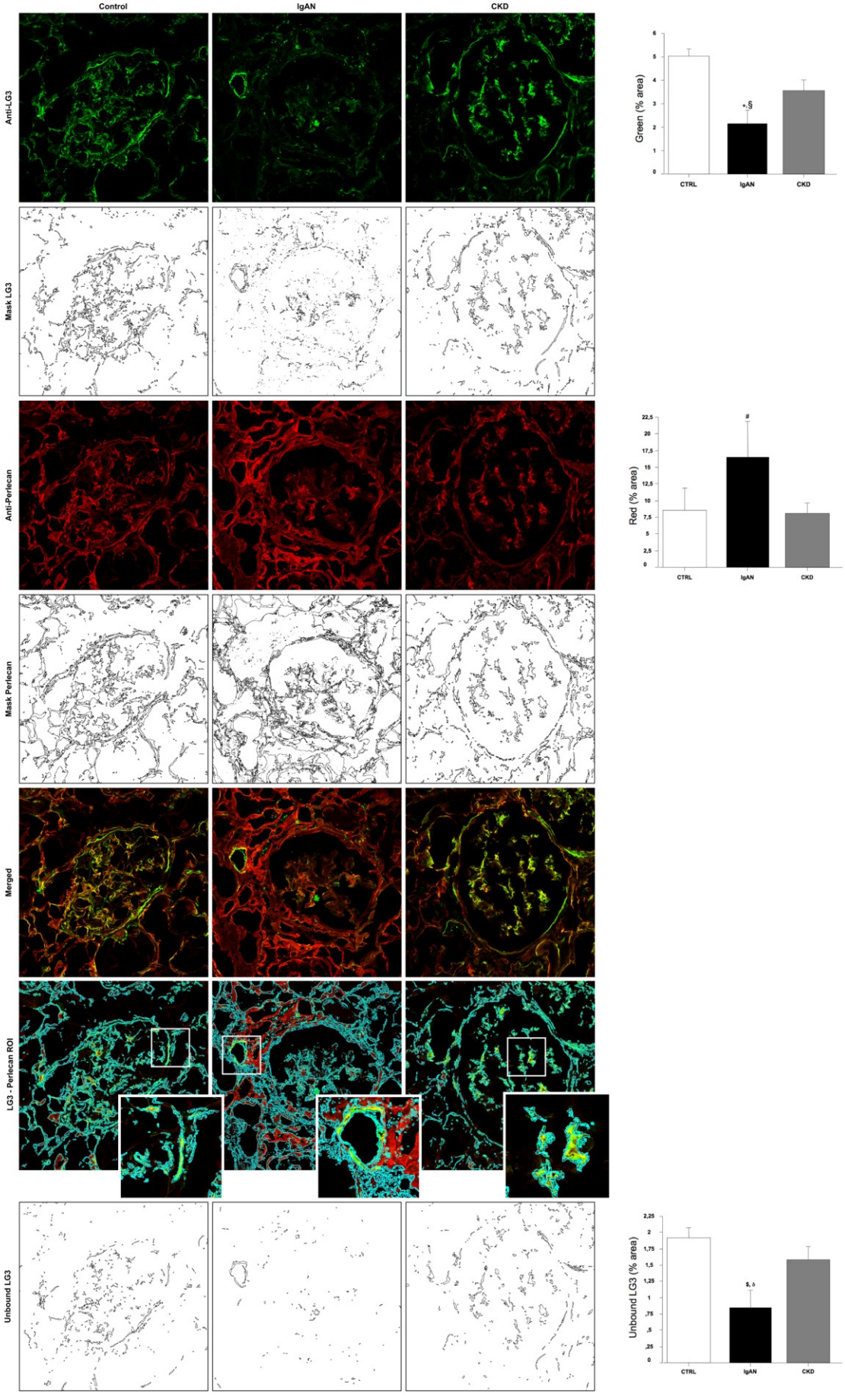
To investigate k FLC renal deposition in IgAN patients, a double immunostaining experiment for total Ig kappa light chains and for IgA was performed on renal biopsy specimens of IgAN patients. Immunostaining was performed using polyclonal mouse anti-human Kappa Light Chains/FITC (Dako Cytomation, Denmark A/S) and polyclonal rabbit anti-IgA (Cell Marque) antibodies on 4µm-thick sections cut from OCT-embedded frozen tissue stored in liquid nitrogen. To recognize anti-IgA a goat anti-rabbit 555 (Biotium Inc., Hayward, CA) was used as secondary antibody.

Supplemental Figure 2



Supplemental Figure 2. Tissue and urinary expression of Ig k light chains (LC) (free and bound). (**A**) Tissue expression of total IgA (upper panel), Ig k light chains (Ig k LC) (free and bound) (intermediate panel) and merging image (lower panel). The green spots in the lowest panel represent k free light chains (k FLC). The presence of Ig other than IgA was ruled out by immunohistochemistry. (**B**) Urine excretion of total Ig k LC (free and bound) and, (**C**) kappa free LC (reported in Figure 2), as measured by immunonephelometry analysis. Data are expressed as mean ±SEM. #: $p<0.0001$; ¶: $p<0.0001$ (ANOVA).

Supplemental Figure 3



Supplemental Figure 3. Confocal microscopy of LG3 and perlecan in renal tissues. **Anti-LG3, Anti-Perlecan**: LG3 (green fluorescence) and perlecan (red fluorescence) tissue expression in CTRL, patients with IgAN and patients with CKD. **Mask LG3, Mask Perlecan**: green channel (LG3) and red channel (perlecan) were transformed in RGB stack and converted to 8 bit images for the quantification of the fluorescence signal using the Analyze particle protocol in ImageJ software to obtain Total Area stained, Average Size of the particle measured (in pixel), and Area fraction (% area: percentage of the total area divided by the total pixels in the image). In brief, a constant value of threshold was applied for every channel and particle analysis was performed setting 50-infinity as limit and bare outliners as output results. The bare outlines were used to make a binary mask of each channel. **Merged**: merging of green and red fluorescence (Anti-LG3 + Anti-Perlecan). **LG3-Perlecan ROI** (region of interest): Red mask was added to green mask in order to create an overlapping signal. Selection in magenta (LG3+Perlecan) showed the overlapped area of merged image. **Unbound LG3**: LG3 resulting from the subtraction of the overlapped area (magenta) to Anti-LG3 image. (Magnification 400 ×). Data were expressed as area fraction ± SD. *: p=0.01; δ: p=0.04 (ANOVA). #: p=0.03, IgAN *vs* CKD (Mann-Whitney U test).