Non-invasive humanized readout of diabetic nephropathy in mice using urinary peptidomics

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ABSTRACT

Diabetic nephropathy (DN) is among the most frequent complications of diabetes and the first cause of end-stage renal disease. Despite being successful in animal models, the majority of clinical trials for novel drugs targeting DN failed. This lack of translational value may in part be due to an inadequate comparability of human disease and animal models that often capture only a few aspects of disease. Here we overcome this limitation by developing a multi-molecular non-invasive humanized readout of DN based on urinary peptidomics. The disease-modified urinary peptides of two type 2 diabetic (T2D) DN mouse models were identified and compared with previously validated urinary peptide markers of DN in humans to generate a classifier composed of 21 ortholog peptides. This classifier predicted the response to disease and treatment with inhibitors of the renin-angiotensin system (RASi) in mice. The humanized classifier was significantly correlated with glomerular lesions. Using a human T2D validation cohort consisting of 207 patients, the classifier also distinguished between patients with and without DN, and response to RASi. Our approach demonstrates that a combination of multiple molecular features similar in both human and animal disease could provide a step change in translational drug discovery research in T2D-DN nephropathy.

Keywords: diabetic nephropathy, urine, proteomic analysis, albuminuria, ACEi.

INTRODUCTION

Diabetic nephropathy (DN) is among the most frequent complications of diabetes [1] and recent evidence shows that kidney involvement is the main determinant of the increased risk of death in diabetic patients [2]. Despite significant advances in the understanding of the molecular mechanisms of type 2 diabetic (T2D, accounting for 90-95% of the diabetes cases) DN, the current standard-of-care is, however, still based on achieving blood pressure control by use of inhibitors of the renin-angiotensin system (RASi) on top of glycemic control. While slowing down the progression of DN in most cases, this treatment does not halt the ineluctable progression to end stage renal disease (ESRD, [3]). Therefore new drugs specifically targeting the DN pathophysiology are needed to reduce the burden of this disease. The translation of the extensive knowledge and plethora of potential targets [4] obtained in animal models of DN and other diseases to the clinic has been extremely limited [3, 4]. This has been attributed to the fact that: i) human pathophysiology and drug susceptibility are often not adequately captured by animal models [5, 6], ii) readouts used in preclinical studies are usually different from the required endpoints in clinical trials [7], or, iii) those readouts are based on a single molecule or phenotypic trait, not sufficient to assess pathology in detail and identify clinically valuable potential drug target (Fig. 1*A*) [8, 9]. Ways have been developed to circumvent these concerns mainly in the field of cancer and immunology by the development of humanized mouse models [10]. An alternative to the development of these humanized animal models, which is technically complicated and not always feasible in the context of many complex diseases such as DN, might be the optimization of the experimental readout based on a panel of molecular features that are

identical in both human and animal disease ignoring the non-human animal disease related traits (Fig. 1*B*).

While a multitude of animal models of DN exist [11], the most frequently used models of T2D DN are the BTBRob/ob mice [12] and uninephrectomized db/db mice on a C57BLKS background (UNxdb/db, [11]). These two models are obese insulin resistant DN models due to leptin or leptin receptor deficiency respectively, displaying renal features similar to that observed in humans, including progressive albuminuria with advanced glomerular lesions and mild focal interstitial fibrosis. In addition, standard-of-care treatment using RASi confers renoprotection in these models [13, 14]. However this resemblance to human DN relies on albuminuria and histology, and often lacks any established molecular similarity; moreover, access to histological data requires animal sacrifice, which is costly, time consuming, and does not allow for longitudinal follow-up.

In humans, current non-invasive readouts of progression of DN are based on the urinary albumin excretion rate (AER) and/or estimation of the glomerular filtration rate (eGFR) using serum creatinine. However, reduction of AER is not always correlated with complete reversal of all phenotypic and *in situ* changes observed in preclinical mouse models of DN [13]. Moreover, while routinely performed in humans, eGFR assessment in mice is of little comparative value, because in mice 35-50% of the creatinine is secreted and not attributable to the glomerular filtration [15]. Although methods for GFR measurement, based on fluorescent inulin clearance, have been developed for mice, these are still invasive (and cannot be repeated frequently, [16]) and will not detect small changes in renal function.

We and others have shown that urine is a rich and non-invasive source of biomarkers of disease in humans [17-19]. Mass spectrometry-based analysis of the endogenous urinary peptidome (i.e.,

not generated by trypsin digestion) allows the assessment of thousands of urinary peptides at once. Combined in multi-molecular classifiers of tens to hundreds of peptides, these urinary peptides have proven their ability to faithfully describe complex diseases including kidney disease [17, 20], cardiovascular disease [21], graft-versus-host disease [22], and cholangiocarcinoma [23]. A successful example of the use of urinary peptides was the development of a urinary peptide based classifier for chronic kidney disease (CKD). Capillary electrophoresis coupled to mass spectrometry (CE-MS) was used to study the urinary peptidome of 230 patients compared to 379 healthy control subjects and led to the identification of a panel of 273 peptides associated with advanced CKD [24]. These 273 peptides combined into a classifier, termed CKD273, was subsequently validated for its capacity to detect CKD in independent cross-sectional studies with over 2000 patients including a significant number of patients (~70%) with diabetes [25-28]. Although initially designed for the detection of CKD, employment of the classifier also allowed prediction of progression of CKD in patients with CKD derived from different aetiologies [28, 29], including patients with DN [30, 31].

Based on this knowledge and the available urinary peptide classifiers in human for DN, the aim of the current study was to develop the first humanized multimarker readout in animal models of DN, using urinary peptidome analysis to improve on the translatability of the results obtained in animal models to the clinic for this disease. We showed that this multimarker model predicted the response to disease and standard-of-care treatment of DN in mice and in diabetic patients and outperformed the standard readout of DN in the mouse models.

RESULTS

The biological parameters, histology and urinary peptidome are highly comparable in response to DN and standard-of-care treatment in BTBRob/ob and UNxdb/db mice.

With the aim to avoid strain- or mutation specific urinary peptide markers and to determine whether a generic DN peptidome readout in mice exists, we first analyzed the similarity of BTBRob/ob and the UNxdb/db mouse. BTBRob/ob and UNxdb/db mice demonstrated hyperglycemia with elevated blood glucose and glycated haemoglobin levels (Table 1) and increased body weights (not shown). This was accompanied by renal lesions as shown by the increased urinary albumin excretion rate (AER) (Fig. 2*A*) and glomerular PAS-positive material, indicative of glomerulosclerosis (Fig. 2*B* and *C*). In both models enalapril, a standard-of-care drug inhibiting the renin-angiotensin system (RASi), significantly reduced AER and glomerular lesions (Fig. 2*A*-*C*), without altering blood glucose or glycosylated haemoglobin levels (Table 1). The urinary peptidome of these two mouse strains was analyzed using CE-MS leading to the identification of 129 and 158 (Fig. 2*D*) sequenced peptides with significantly altered urinary excretion levels when employing multiple testing in the BTBRob/ob and the UNxdb/db DN mouse models compared to non diabetic control mice, respectively (Supplementary Table 1 and 2). Forty-eight identical peptides were modified with same regulation pattern in both models representing 30-37% of the observed peptides with different urinary abundance in DN (Fig. 2*E*). Most of the urinary peptides were collagen fragments (collagen type I alpha-1 and type III alpha-1 chains), similar to what has been found in humans with DN and CKD [24, 32]. Peptide fragments from other proteins, including complement factor D, uromodulin and pro-epidermal growth factor were also among the peptides of which the urinary abundance was modified in

DN (Supplementary Table 3). Using an approach that we have previously developed for the rat [33], we identified 64 and 72 similar (orthologs) peptides in the human CKD273 classifier and in BTBRob/ob and UNxdb/db mice respectively, showing high molecular similarity between the mouse models and human DN. Treatment with RASi modified the urinary secretion of 11 peptides in BTBRob/ob mice, 18 peptides in the UNxdb/db DN mouse model (Supplementary Table 1 and 2). Four of the peptides modified by RASi were identical in both strains (Fig. 2*F* and Supplementary Table 4). Counterintuitive regulation of 2 of these 4 peptides was observed after RASi as RASi amplified the DN-induced changes for Napsin-A or SOX-6 fragments. Similar observations were reported in the past, where RASi was shown to exacerbate disease-induced changes while improving kidney function and histology [34].

Development of a humanized urinary peptide based classifier in T2D nephropathy mice.

These data indicate that consistent changes were induced both by disease and treatment in these different strains. To obtain a generic (*eg* mutation independent) DN mouse urinary peptidome profile, we combined the data from the BTBRob/ob and UNxdb/db mice and their respective non-diabetic controls. Individual samples from non-diabetic and diabetic mice of the two strains were randomly assigned to a discovery or validation cohort (Table 2). The discovery cohort consisted of 10 non-diabetic, 10 DN, and 8 DN mice treated with RASi (Table 2). Ninety-two sequenced peptides displayed significantly altered urinary excretion levels between DN and non-diabetic and/or between treated and untreated DN mice (Supplementary Table 5), including 42 out of the 51 overlapping peptides previously identified in the two strains separately (Fig. 2*E*). These 92 peptides were confirmed to be mainly fragments of collagens. We

next identified the mouse peptides that could consistently reflect human disease by comparing the orthology of mouse urine peptide sequences to that of the 273 peptides in the CKD273-classifier (Fig. 1*B*, left panel). Among the 92 peptides significantly associated with DN in the mouse, 40 were orthologs (44%, Supplementary Table 5), again supporting high molecular similarity between human disease and these preclinical DN mouse models. Using support vector machines [24], 21 mouse orthologs that had the best association to DN or RASi treatment were combined in a urine peptide classifier (Supplementary Table 6). This 21 peptide classifier included four peptides (Supplementary Table 6) which were uniquely changed after RASi and were not modified by DN. In this mouse discovery cohort, the humanized peptide classifier was able to differentiate between non-diabetic and DN mice (Fig. 3*A*). The classifier could also significantly discriminate between RASi treated and untreated DN mice (Fig. 3*A*). This humanized readout was next tested to validate its accuracy in diagnosing disease and detecting drug treatment changes in both an independent cohort of mice and humans.

Validation of the humanized urinary peptide based classifier in an independent set of T2D nephropathy mice.

We further validated the classifier using the additional independent cohort of non-diabetic (n=7), untreated DN (n=10) and RASi-treated DN mice (n=10) (Table 2). In this separate validation set, significant differences were seen not only between non-diabetic and DN animals, but also between untreated DN mice and DN mice treated with RASi (Fig. 3*B*).

As the aim of the classifier was prediction of DN and not T2D *per se*, we assessed the association of the humanized classifier with the development of glomerular lesions in the

validation cohort. The classifier was highly correlated with glomerular PAS-positive matrix accumulation (r=0.6420, p=0.0003, Fig. 3*C*) and outperformed albuminuria, the standard readout, that showed a weaker association with renal lesions (r=0.4839, p=0.0105, Fig. 3*D*). This result supports the hypothesis that multimolecular humanized readouts can better describe complex disease phenotypes [9] than individual biomarkers such as albuminuria routinely used as a biomarker of DN in man.

Validation of the humanized urinary peptide based classifier in humans.

Since the CKD273 classifier was based on 273 urine peptides of human origin, we verified that the selected reduced subset of mouse ortholog peptides used for the humanized urine peptide classifier could efficiently translate to the clinic and predict the presence of DN and respond to RASi treatment in humans. Using a discovery cohort of 25 non-diabetic healthy controls and 25 patients with DN available in the human urinary peptide database [35] (Table 3), we built a classifier based on the orthologs, which significantly distinguished between the 2 groups in the discovery set (Fig. 4*A*). For a first independent validation of the classifier we scored a small cohort of 42 T2D patients with or without DN, treated either with placebo or with irbesartan, an angiotensin II receptor antagonist representing another class of RASi. In this additional independent validation cohort, the ortholog-based classifier was able to discriminate T2D patients with DN treated with placebo or the RASi (Fig. 4*B*). As expected, a significant difference was also observed between T2D-patients with and without DN (Fig. 4*B*). We further validated the classifier using a previously published cohort [27] of 165 T2D patients among which 87 displayed DN as defined by macroalbuminuria and/or eGFR <45 mL/min/1.73 m^2 [27]. By this

design, macroalbuminuria and eGFR were strongly associated to the presence of DN (AUC of 0.94 and 0.88 for albuminuria and eGFR, respectively). Patient treatment information was not available in this study [27]. The humanized classifier clearly separated the 165 T2D-patients with and without DN, although with lower sensitivity and specificity than the original CKD273 classifier (AUC of 0.73 and 0.95 for the humanized- and CKD273-classifier, respectively) (Fig. 4*C*).

DISCUSSION

The limited success rate of cross-species translation of results from preclinical models to the patient still hampers the pharmaceutical development for many complex diseases including DN. One major hurdle appears that disease progression and therapeutic effects are not adequately captured by animal models [5]. Moreover, readouts used in preclinical studies are usually different from the required endpoints in clinical trials [7]. In animals, the readouts are designed to give the highest discriminative power between healthy and diseased animal, e.g., rely on tissue examination at termination of the study. In contrast, treatment efficiency assessed in clinical trials is often limited to the use of a non- or minimally invasive approach, especially in chronic diseases where biomarkers are used to follow disease progression. This limitation can be exemplified in the DN area, where phase II studies largely depend on changes in albuminuria, which cannot always predict progression of renal lesions or drug treatment benefits.

Here, using urinary peptidomics, we have developed and demonstrated the validity of a non-invasive, humanized multimarker readout of DN. This classifier was shown to correlate with the renal lesions in two prototypic T2D-DN mouse models and also shown to respond to RASi treatment in both mouse models and in a cohort of diabetic patients. Therefore, the classifier tool defined holds great potential for building the translational bridge between a drug candidate's effects in a mouse DN model to human disease. Although we developed this approach for urinary peptides, this concept can, in theory, be applied to any non- or minimal-invasive biological trait including miRNAs, mRNAs, proteins or metabolites. However the availability of reference panels for human disease is a prerequisite.

Reliable markers of DN are needed to characterize the severity of disease and to monitor drug treatment effects in improving kidney health and function. RASi are drugs routinely used to slow-down the progression of DN following the landmark study by Lewis et al. in 1993 [36]. Therefore, we decided to use RASi to validate our strategy that drugs that are effective in humans will lead to a positive humanized urinary peptidome readout in selected mouse models, and *vice versa*. We observed that the treatment with RASi (enalapril) moderately, but significantly, reduced the score of the humanized urinary peptide classifier, and that reduced histopathological lesions accompanied the lower scores. In addition, the classifier score was lower in humans with DN treated with irbesartan, an angiotensin II receptor antagonist that represents another class of RASi, validating not only the translational value of the classifier, but also its sensitivity to different drugs from the same class.

Our results strengthen the notion that urine is a rich source of biomarkers and support the hypothesis that multi-peptide panels/readouts can better describe complex disease phenotypes [8, 9]. This is further exemplified by the observation that this novel humanized urine peptide classifier based on 21 urinary peptides displayed a strong and highly significant correlation with lesions *in-situ*, whereas the correlation and significance of albuminuria alone with the score of glomerular lesions in mice, routinely used as a biomarker of DN in man, were much lower. These results are in accordance with previous observations that albuminuria does not always correlates with disease progression in man [37-39], while multimarker urinary peptidome classifiers have been suggested to better depict disease progression[28, 31, 40].

The use of body fluid-based humanized classifiers also has the benefit of being non-invasive and would permit longitudinal follow-up and *on-route* adjustment during the drug evaluation

process; no animal sacrifice is needed. Furthermore, such non-invasive assessment of progression of disease in animals will also more closely mimic the surrogate endpoints in human clinical trials where evaluation of renal lesions using kidney biopsies, especially in DN, is not routinely performed. In addition, the option to sample multiple times from the same animal will significantly reduce the number of animals required for long-term studies.

Although our model is based upon multiple markers, all markers in the model were derived from fragments of either collagen α-1(I) or (III). This can be considered as a limitation as to the concept of a multimarker panel to represent multiple disease mechanisms in complex diseases. Indeed the collagen-based urinary markers most likely represent the development of fibrosis [41] that is considered a hallmark of kidney disease [42, 43]. When taking into account the non-ortholog peptides, some of the corresponding proteins could also be linked to human chronic kidney disease or diabetic nephropathy. These include pro-epidermal growth factor [44], uromodulin [45], meprin A [46], osteopontin [47], complement factor D [48] and α-1-antitrypsin [49] (Supplementary Table 5). Improvement of the applied strategy will come from new sequence information on the disease-associated peptides in both mice and humans. Updated orthology models based on larger panels of different peptides (i.e. not only composed of collagen I and III fragments) are anticipated to be more resistant to variability, provide an optimized description of molecular mechanisms of disease and hence to improve the confidence in prediction of disease progression and treatment effects. Finally, a validated humanized urine peptide classifier may also facilitate decision making about the preclinical model to use for a specific human disease. In this context it could add to a recent transcriptomics studies [50] using renal tissue that identified cross-species shared transcriptional networks of DN allowing identification of

pathways unique to each of the human-mouse strain networks. Such approaches could reduce the number of different animal models currently used for drug discovery today.

In conclusion we have developed, based on a new concept, the first humanized non-invasive multi-peptide classifier that could provide a step change in translational drug discovery research in T2D-DN.

RESEARCH DESIGN AND METHODS

Animals and experimental design

Male diabetic db/db mice (BKS.Cg-Dock7m +/+ Leprdb/J stock no. 000642) and female BTBRob/ob mice (BTBR.V(B6)-Lepob/WiscJ stock no. 004824) and their respective lean littermates db/m and BTBR were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). All mice had free access to food and water and were maintained on a 12-h light/dark cycle at 21-22°C. Db/m and db/db mice were subjected to right side uninephrectomy (UNx) under anesthesia at 8 weeks of age to accelerate development of diabetic nephropathy as described previously [51]. The following groups of mice were studied: (i) untreated BTBRob/ob diabetic mice (n=9); (ii) BTBRob/ob diabetic mice treated with enalapril (Sigma E6888-5G) admixtured in drinking water 200 mg/l with a non-caloric sweetener (Splenda, 240mg/l) for 12 weeks from age 8 to 20 weeks (n=9); (iii) untreated BTBR non-diabetic lean mice (n=9); (iv) untreated UNxdb/db diabetic mice (n=11); (v) UNxdb/db diabetic mice treated with enalapril for 4 weeks from age 18 to 22 weeks (n=9); (vi) untreated UNxdb/m non-diabetic lean mice (n=8). Mice were sacrificed under isoflurane anesthesia at 20 weeks of age in BTBRob/ob and at 22 weeks of age in UNxdb/db. Kidneys were collected for histologic analyses. The experimental protocols were approved by the Animal Care Committee at the University of Utah (UNxdb/db) and the Local Ethics Review Committee on Animal Experiments in the Göteborg Region (BTBRob/ob).

Blood and urine chemistry

Blood glucose and HbA1c levels were measured in fasted awake mice using an Accu-Chek (Roche, REF 05599415370; BTBRob/ob) or a glucose meter (Glucometer Elite XL, Bayer Healthcare, Elkhart, IN, USA; UNxdb/db) and HbA1cNow+ (Bayer, REF 81611409-3038; BTBRob/ob) or DC 2000+ HbA1C kit (Bayer Healthcare; UNxdb/db). To determine albumin excretion rates (AER, µg/24 h) urine was collected from metabolic cages. During urine collection mice had free access to food and water. Urine albumin concentration was measured using a mouse specific albumin ELISA (ICL, E-90AL; BTBRob/ob) or a laser nephelometer (Hyland, Deerfield, Illinois; UNxdb/db) using a monospecific antibody to rat serum albumin (Cappel Laboratories, West Chester, Pennsylvania).

Histological analysis

Kidneys were fixed in Carnoy solution, dehydrated in alcohol, and embedded in paraffin. Two-micrometer sections were stained with periodic acid-Schiff reagent. At least 50 glomeruli, including superficial and juxtaglomerullary cortical area, were examined for each animal. The extent of glomerular damage was expressed as the percentage of glomerular area fraction occupied by PAS-positive matrix [51].

Urine peptidomics

Sample preparation: Thirteen hour-urine obtained from BTBRob/ob mice at 20 weeks age and bladder urine collected from UNxdb/db mice at 22 weeks age at termination were used for peptide profiling, respectively. One hundred fifty µl of mouse urine sample was diluted with the same volume of a solution composed of 2 M urea, 10 mM NH4OH and 0.2% SDS. Subsequently,

samples were ultrafiltered using a Centristat 20 kDa cut-off centrifugal filter device (Satorius, Göttingen, Germany) to eliminate high molecular weight molecules. The filtrate was desalted using a NAP5 gel filtration column (GE Healthcare Bio Sciences, Uppsala, Sweden) to remove urea and electrolytes, and thereby to decrease matrix effects. The sample was lyophilized in a Savant speedvac SVC100H connected to a Virtis 3L Sentry freeze dryer (Fischer Scientific, Illkirch, France) and stored at 4°C until use. Shortly before CE-MS analysis, the samples were re-suspended in 10µL HPLC grade H2O.

CE-MS Analysis: CE-MS analysis was performed as previously described [52, 53]. Briefly, CE-MS analyses were performed using a Beckman Coulter Proteome Lab PA800 capillary electrophoresis system (Beckman Coulter, Fullerton, USA) on-line coupled to a micrOTOF II MS (Bruker Daltonic, Bremen, Germany). The electro-ionization sprayer (Agilent Technologies, Palo Alto, CA, USA) was grounded, and the ion spray interface potential was set to –4.5 kV. Data acquisition and MS acquisition methods were automatically controlled by the CE via contact-close-relays. Spectra were accumulated every 3 s, over a range of m/z 350 to 3000.

Data Processing and Cluster Analysis: MosaiquesVisu software package was applied to deconvolve mass spectral ion peaks representing identical molecules at different charge states into single masses [54]. Migration time and ion signal intensity (amplitude) were normalized using internal polypeptide standards [55]. Each polypeptide present in the list was defined by its normalized migration time [min], molecular mass [kDa], and signal intensity detected. Using a Microsoft SQL database, all detected polypeptides were deposited, matched, and annotated in order to allow for further comparison between the groups. The criteria applied to considered a polypeptide identical was that within different samples the mass deviation was lower than 50

ppm for masses <4kDa, 150 ppm for masses >6kDa, and between 50-150 ppm for masses between 4-6kDa. Acceptable migration time deviation was between 1 and 2.5 minutes.

Peptide sequencing: For sequencing, processed urine samples were also separated on a Dionex Ultimate 3000 RSLS nano flow system (Dionex, Camberly UK). A 5 µl sample was loaded onto a Dionex 5 mm C18 nano trap column at a flowrate of 5 µl/min. Elution was performed on an Acclaim PepMap 75 mm C18 nano column over 100 min. The sample was ionised in positive ion mode using a Proxeon nano spray ESI source (Thermo Fisher Hemel UK) and analyzed in an Orbitrap Velos FTMS (Thermo Finnigan, Bremen, Germany). The MS was operated in data-dependent mode to switch between MS and MS/MS acquisition and parent ions were fragmented by (high-) energy collision-induced dissociation and also electron transfer dissociation. Data files were searched against *Mus musculus* entries in the Swiss-Prot database without any enzyme specificity using Open Mass Spectrometry Search Algorithm (OMSSA, http://pubchem.ncbi.nlm.nih.gov/omssa) with an e-value cut-off of 0.1. No fixed modification and oxidation of methionine as variable modifications were selected. Mass error windows of 10 ppm for MS and 0.05 Da (HCD) or 0.5 Da (CID, ETD) for MS/MS were allowed. For further validation of obtained peptide identifications, the strict correlation between peptide charge at pH 2 and CE-migration time was utilized to minimize false-positive identification rates [56]. Calculated CE-migration time of the sequence candidate based on its peptide sequence (number of basic amino acids) was compared to the experimental migration time. Peptides were accepted only if they had a mass deviation below ± 80 ppm and a CE-migration time deviations below ± 2 min.

Human data CE-MS and patient data

Human CE-MS data and general patient characteristics (Table 5) were extracted from the human urinary peptide database [35] and from [27]. The study was designed and conducted fulfilling all of the requisites of the laws on the protection of individuals collaborating in medical research and was in accordance with the principles of the Declaration of Helsinki. All datasets included in the study derived from previous studies and all data were anonymized. The approach employing anonymized proteomics data from previous studies was approved by the local ethics committee.

Orthology

To examine the orthology between mouse peptides and human peptides from CKD273 [24] we applied three criteria: first, we looked for identical fragments; secondly, we looked for fragments with one identical cleavage site (N or C-term) and third, we looked for peptides from the same protein area with a minimum overlap in 6 amino acids.

Statistics

Comparisons and correlations: Statistical comparisons between two groups were assessed using a Mann-Whitney test for independent samples. Correlations were assessed using a non-parametric Spearman correlation test. All statistics were performed using the Prism 5 GraphPad software.

Biomarker selection and classifier modelling: For the identification of potential biomarkers, the reported p-values were calculated using the Wilcoxon Rank-Sum test (R-based statistic software, version 2.15.3) followed by adjustment for multiple testing using the method described by Benjamini and Hochberg [57]. Peptides that were detectable in >70% of mice in at least one group and reached an adjusted p-value of <0.05 were further considered as relevant. Using MosaCluster software package [52] we next used a support vector machine (SVM)-based approach [58] to generate a prognostic biomarker classifier based on 21 mice ortholog biomarkers associated with DN and that were sequenced. The parameters of the kernel function for the 21-dimensional hyperplane were: penalty parameter (C) of 4 and kernel width (γ) of 0.00819. The ortholog-based human model was formed by 19 biomarkers and the parameters used for the hyperplane were: penalty parameter (C) of 1024 and kernel width (γ) of 0.00012. Sensitivity and specificity were calculated based on the number of properly classified samples. The overall yield of the polypeptide pattern was evaluated by the receiver operating characteristic (ROC) and area under curve (AUC) plots with MedCalc software (MedCalc version 8.1.1.0, MedCalc Software, Belgium).

DISCLOSURE

AE and XRP are employees of Astra-Zeneca. JS and ART are/were employees of Mosaiques Diagnostics GmbH. HM is cofounder and a shareholder of Mosaiques Diagnostics GmbH.

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ACKNOWLEDGMENTS

We are grateful to Pernilla Håkansson for performing the BTBRob/ob experimental work and to Ulrika Johansson for facilitating the UNxdb/db studies. Gerhard Böttcher and Ann-Katrin Andersson for assisting with histopathology. This work was supported in part by the « Fondation du Rein sous égide de la Fondation pour la Recherche Médicale et ses partenaires », grant number GENZYME 2014 FDR-SdN/FRM for JK.

FIGURE LEGENDS

Fig. 1. Improving the translatability of animal models of disease by the development of a multimolecular humanized readout. *A*: Disease readouts are often based on a single phenotypic trait or biomarker similar, if possible, in animals and humans. Such single feature does not capture the complexity of most disorders. Hence novel drugs, although successful in the preclinical phase based on such single feature readout in animal models, often fail in humans. *B*: In contrast, the analysis of multimolecular changes using omics approaches, by for example urinary peptidomics, allows a more complete description of complex phenotypes. Furthermore the combination of these multiple features similar (ortholog) in human disease and animal models, leading to a "humanized" readout, most likely will more efficiently translate the effects of new drugs in preclinical models to the clinic.

Fig. 2. The biological response, histology and urinary peptidome are highly comparable in two frequently used T2D nephropathy mouse strains. *A*: albumin excretion rate, *B*: glomerular periodic acid Schiff staining -positive matrix fraction and *C*: representative histology sections of glomerular mesangial matrix deposition stained with periodic acid Schiff staining; Scale bars: 100µm. *D*: Representation of urinary peptides significantly modified between non-diabetic and mice with DN. Each peptide is identified using CE-MS by a unique identifier on the basis of the migration time (min) and specific mass (kDa), with a peak height representing the relative abundance. *E* and *F*: Venn diagrams showing the overlap of urinary peptides significantly modified in the two T2D mouse strains with DN and overlap in response to RASi. BTBRob/ob, T2D mice with leptin-deficiency mutation; Unxdb/db uninephrectomized T2D mice with a

mutation in the leptin receptor; No diab, lean control mice; DN, T2D mice with diabetic nephropathy; DN+RASi, T2D mice with diabetic nephropathy treated with inhibitors of the renin angiotensin system (RASi, enalapril); PAS^+, periodic acid Schiff positive; AER, urinary albumin excretion rate. *p<0.05 versus No diab. °p<0.05 versus non treated DN. Mann-Whitney test for independent samples. Data presented as mean ± SEM.

Fig. 3. Development and validation of a humanized urinary peptide based classifier in T2D nephropathy mice. *A*: scores of the humanized classifier in the discovery cohort consisting of 10 non-diabetic, 10 untreated diabetic and 8 diabetic mice treated with RASi and in the validation cohort B: consisting of 7 non-diabetic, 10 untreated diabetic and 10 RASi-treated diabetic mice. *C-D*: Correlation of the humanized urine peptide classifier score (*C*) and albumin excretion rate (*D*) with the glomerular mesangial matrix deposition assessed by periodic acid Schiff staining using the Spearman correlation test. No. diab, lean control mice; DN, T2D mice with diabetic nephropathy; RASi: enalapril; PAS^+, periodic acid Schiff positive; AER, urinary albumin excretion rate; BTBRob/ob, type 2 diabetic mice; UNxdb/db mice, type 2 diabetic mice. *p<0.05 versus No diab. °p<0.05 versus DN. Mann-Whitney test for independent samples. Data presented as mean ± SEM.

Fig. 4. Validation of the humanized urinary peptide based classifier in humans. *A*: Scores of the humanized urine peptide classifier in a discovery human cohort consisting of 25 healthy controls and 25 patients with T2D and DN. *B*: Scores of the humanized urine peptide classifier in a validation cohort consisting of 14 patients with T2D without DN, 14 patients with DN treated

with placebo and 14 patients with DN treated with RASi. *C*: Score, AUC, sensitivity and specificity of the humanized urine peptide classifier and the CKD273 classifier in a supplementary human cohort consisting of 165 T2D-patients with (n=87) and without DN (n=78), for patient details see [27]. No. diab, healthy humans; DN, diabetic humans with diabetic nephropathy; Diab. noDN, diabetic humans without diabetic nephropathy; RASi: irbesartan; Pla, placebo; AUC, area under the curve. *p<0.05 versus No diab. or Diab. noDN. °p<0.05 versus DN+Pla. Mann-Whitney test for independent samples. Data presented as mean ± SEM.

TABLES

Table 1. Blood chemistry data of BTBRob/ob and UNxdb/db mice.

	BTBR			Unxdb		
	No diab. (BTBR)	**DN (BTBRob/ob)**	**DN+RASi**	**No diab. (Unxdb/m)**	**DN (Unxdb/db)**	**DN+RASi**
Glucose (mM)	9.1 ±0.2	24.3 ±2,8*	26.3 ±2.9*	5.7 ±0.5	33.3 ±4.7*	33.3 ±0.0*
HbA1c (%)[mmol/mol]	4.7 ±0.1 [28 ±1]	11.5 ±0.4 [103 ±4]*	11.5 ±0.4 [102 ±4]*	4.0 ±0.1 [20 ±1]	13.3 ±0.4 [123 ±4]*	13.4 ±0.4 [123 ±5]*

Data presented as mean ± SEM (n=5-11/ group). BTBRob/ob, T2D mice with leptin-deficiency mutation; Unxdb/db uninephrectomized T2D mice with a mutation in the leptin receptor; No diab., lean control mice; DN, T2D mice with diabetic nephropathy; DN+RASi, T2D mice with diabetic nephropathy treated with inhibitors of the renin angiotensin system (RASi, enalapril); HbA1c, glycated haemoglobin A1c. *$p<0.05$ versus No diab. Mann-Whitney test for independent samples.

Table 2. Biochemistry data of mouse discovery and validation cohorts.

	Discovery cohort			Validation cohort		
	No diab.	**DN**	**DN+RASi**	**No diab.**	**DN**	**DN+RASi**
Number (BTBRob/ob/Unxdb/db)	10 (5/5)	10 (5/5)	8 (4/4)	7 (4/3)	10 (4/6)	10 (5/5)
Albumin excretion rate (mg/24h)	0.03 ±0.01	1.4 ±0.4*	0.4 ±0.11*,°	0.1 ±0.1	1.1 ±0.3*	0.7 ±0.3*
Glomerular PAS+ (%)	19.6 ±4.0	50.1 ±2.5*	25.5 ±1.4°	14.1 ±1.1	44.5 ±1.2*	32.5 ±3.6*,°
Blood glucose (mM)	7.2 ±0.7	25.6 ±3.2*	27.3 ±3.0*	7.8 ±0.8	30.6 ±2.1*	31.8 ±1.6*
HbA1c (%)[mmol/mol]	4.4 ±0.1 [24 ±1]	11.8 ±0.5 [105 ±6]*	12.3 ±0.6 [111 ±7]*	4.5 ±0.2 [25 ±2]	12.8 ±0.4 [117 ±4]*	12.5 ±0.5 [114 ±5]*

Data presented as mean ± SEM. No diab., lean control mice; DN, T2D mice with diabetic nephropathy; RASi: enalapril; AER, urinary albumin excretion rate; PAS^{+}, periodic acid Schiff positive; HbA1c, glycated haemoglobin A1c; BTBRob/ob, type 2 diabetic mice; UNxdb/db mice, type 2 diabetic mice. *p<0.05 versus No diab. °p<0.05 versus DN. Mann-Whitney test for independent samples.

Table 3. Biochemistry data of human discovery and validation cohorts.

	Discovery cohort		Validation cohort			Supplementary cohort	
	No diab.	**DN**	**Diab. noDN**	**DN+Placebo**	**DN+RASi**	**Diab. noDN**	**DN**
Gender (M/F)	20/5	25/0	11/3	14/0	12/2	19/28 (for 31 n.a.)	35/23 (for 29 n.a.)
Age (years)	62.4 ±4.0	65.1 ± 5.5	63.9 ± 4.7	67.3 ± 4.7	65.4 ± 4.6	58.1 ± 14.2 (for 33 n.a)	62.6 ± 14.7 (for 28 n.a.)
eGFR (mL/min/1.73m2)	75.3 ±7.5	55.5 ± 4.6*	80.1 ± 5.4	53.1 ± 3.1*	53.9 ± 4.8*	90.1 ± 19.0	47.6 ± 27.6*
HbA1c (%)[mmol/mol]	n.a.	6.5 ± 0.7 [48 ±2]	7.8 ± 0.9 [63 ±3]	7.2 ± 0.8 [55 ±2]	8.5 ± 0.6 [69 ±2]°	n.a.	n.a.
U-Albumin							
mg/mL	2.3 ± 5.3	228.2 ± 241.6 *	41.1 ± 27.7	71.6 ± 76,3		11.8 ± 7.2 (n=28)	994.4 ± 2224.5 (n=32)
mg/g creatinine						6.3 ± 5.8 (n=31)	655.4 ± 714.3 (n=30)
mg/24h					241.0 ± 282.3	7.4 ± 5.7 (n=19)	3682.5 ± 2999.3 (n=10)
U-Protein (mg/24h)							2914.0 ±2249.9 (n=10)

Data presented as mean ± SEM. No diab., healthy humans; DN, diabetic humans with diabetic nephropathy; Diab. noDN, diabetic humans without diabetic nephropathy; RASi: irbesartan; Pla, placebo; n.a, not available; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin A1c; U-albumin or U-protein, urinary albumin or urinary protein excretion, respectively. Since data were collected from different cohorts, urinary albumin was measured using different collection-and normalisation-methods. For 5 patients U-albumin or protein was not available. *p<0.05 versus No diab. or Diab. noDN. °p<0.05 versus DN+Pla. Mann-Whitney test for independent samples.

A

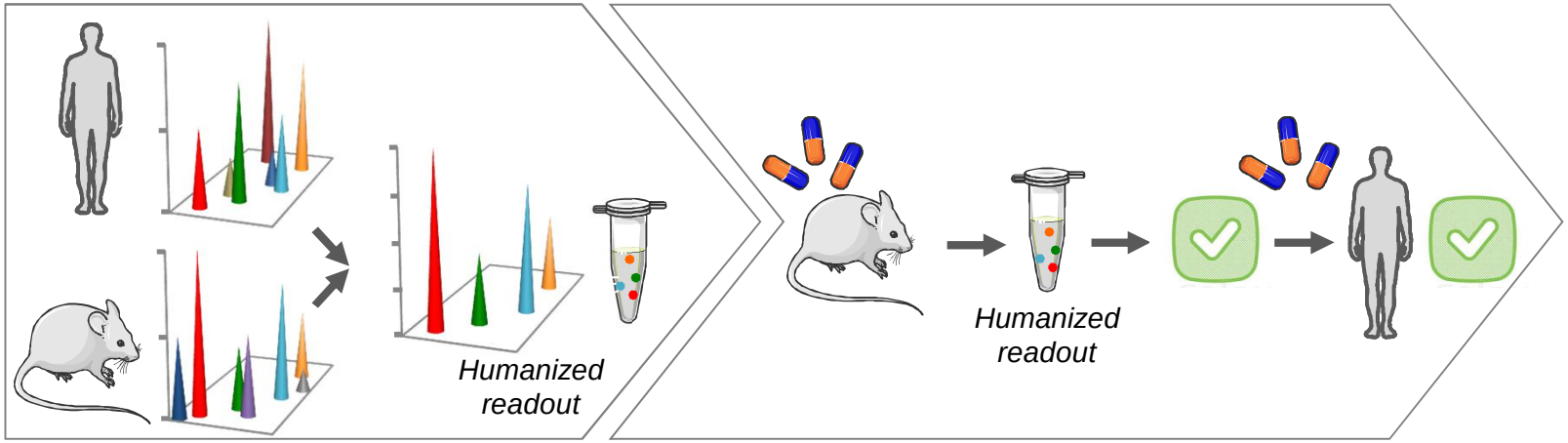
Analysis of single phenotypic trait or single biomarker change in mouse preclinical models

Development/ Evaluation of drugs in mouse preclinical models

Assessment of disease progression based on single phenotypic trait or single biomarker

Limited translatability of drugs effects observed in preclinical models

B



Analysis of multimolecular changes in human disease and mouse preclinical models (e.g. urinary peptidomics)

Identification of multimolecular similarity (orthology) → Humanized readout

Development/ Evaluation of drugs in mouse preclinical models

Assessment of disease progression based on multimolecular humanized readout

Improved translatability

Figure 1

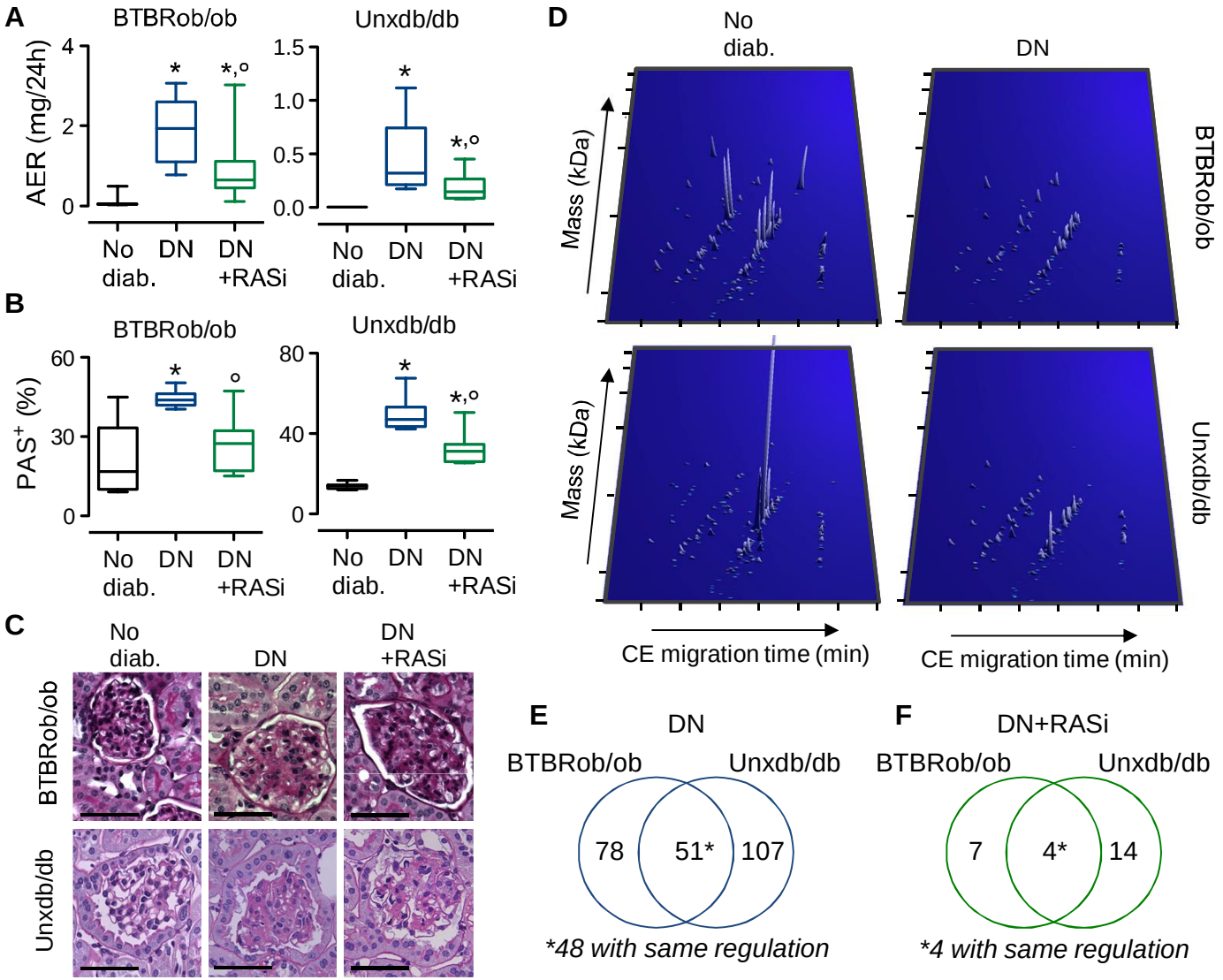

A
BTBRob/ob
Unxdb/db
AER (mg/24h)
No diab.
DN
DN +RASi
B
PAS+ (%)
C
D
Mass (kDa)
CE migration time (min)
E
DN
78
51*
107
*48 with same regulation
F
DN+RASi
7
4*
14
*4 with same regulation

Figure 2

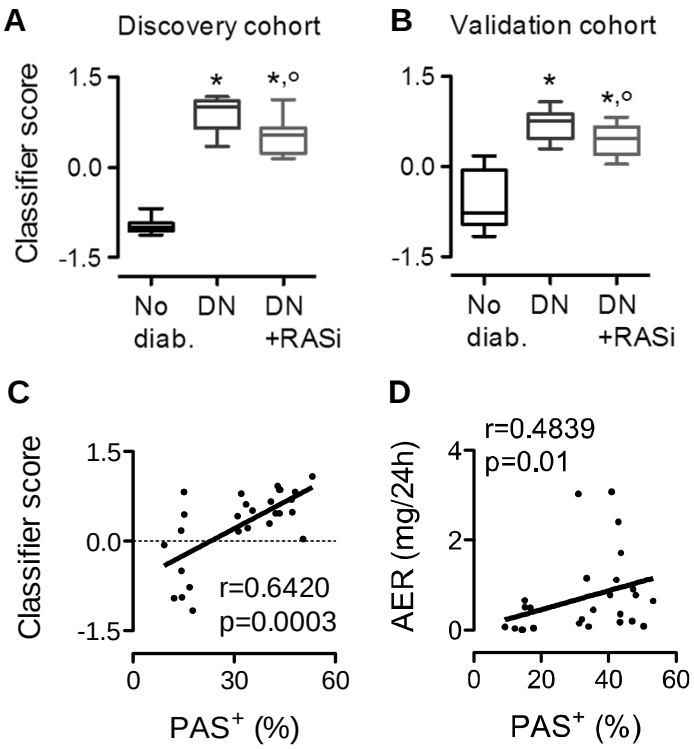
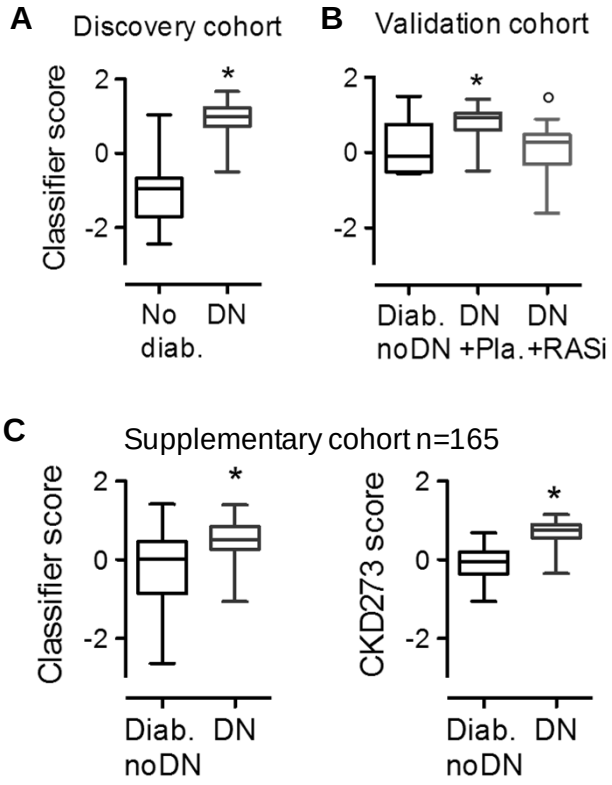

A
Discovery cohort
B
Validation cohort
Classifier score
1.5
0.0
-1.5
*
*,°
No diab.
DN
DN +RASi
C
D
r=0.6420
p=0.0003
r=0.4839
p=0.01
AER (mg/24h)
4
2
0
0
30
60
20
40
PAS+ (%)

Figure 3



	Classifier	CKD273
AUC [CI 95%]	0.729 [0.654-0.795]	0.948 [0.902-0.977]
Sensitivity [CI 95%] (%)	81 [76-92]	90 [81-95]
Specificity [CI 95%] (%)	59 [47-76]	92 [84-97]

Figure 4

Supplementary Table 1: List of urinary peptides associated to i) T2D nephropathy and ii) treatment with RASi (enalapril) in the BTBRob/ob mouse model.

Peptide_ID	Sequence	Protein name	Start	Stop	Uniprot	Masse	CE-Time	Adjusted pValue	Freq BTBR	mean Amp. BTBR	Freq BTBRob/ob	mean Amp. BTBRob/ob	FOLD CHANGE BTBRob/ob/BTBR	DIRECTION	Adjusted pValue	Freq BTBRob/ob	mean Amp. BTBRob/ob	Freq BTBRob/ob+ RASi	mean Amp. BTBRob/ob+ RASi	FOLD CHANGE BTBRob/ob+RASi/ BTBRob/ob	DIRECTION	
710	SpGRDGApG	Collagen alpha-1(I) chain	1012	1020	P11087	844,43	33,46	0,01840412	1	247,45	0,33	68,87	0,278318852	down-regulated in ob/ob								
1127	ApGERGPpG	Collagen alpha-1(III) chain	678	686	P08121	868,45	33,7	0,004385248	1	307,74	0,22	20,91	0,067946968	down-regulated in ob/ob								
2465	QVSPGAKmP	Transcription factor SOX-6	378	386	P40645	929,47	35,74								0,02548084	0,11	9,45	0,89	1230,7	130,2328042	up-regulated in ACEi	
2756	GPpGENGKpG	Collagen alpha-1(III) chain	647	656	P08121	940,48	34,25	0,012142689	0,78	103,41	0,11	2,62	0,025336041	down-regulated in ob/ob								
2806	YRELVHQ	Alpha-1-antitrypsin 1-1	56	62	P07758	943,5	28,34	0,049524642	0,78	72,76	0,11	74,71	1,02680044	up-regulated in ob/ob								
3062	GQpGEKGPpG	Collagen alpha-1(III) chain	926	935	P08121	954,47	34,61	0,007164724	0,89	165,18	0,22	11,39	0,068955079	down-regulated in ob/ob	0,01921313	0,22	11,39	1	1327,35	116,5364355	up-regulated in ACEi	reversed by ACEi treatment
3144	GPpGESGREG	Collagen alpha-1(I) chain	996	1005	P11087	957,46	34,3	0,003740306	1	82,52	0	0	0	down-regulated in ob/ob								
3268	FVNNDLKL	Alpha-1-antitrypsin 1-1	137	144	P07758	961,5	34,74	0,00717354	1	556,21	0,78	275,02	0,494453534	down-regulated in ob/ob	0,03642468	0,78	275,02	0	0	0	down-regulated in ACEi	
3438	DGKTGPpGPAG	Collagen alpha-1(I) chain	539	549	P11087	968,51	37,23	0,004385248	1	434,59	0,33	23,6	0,054304057	down-regulated in ob/ob								
3736	VLNLGPITR	Uromodulin	599	607	Q91X17	981,62	35,78	0,014035669	0,89	428,51	0,11	68,63	0,160159623	down-regulated in ob/ob								
4385	GPpGERGGpGP	Collagen alpha-1(III) chain	503	513	P08121	1008,51	35,45	0,004385248	1	698,93	0,56	113,81	0,162834619	down-regulated in ob/ob								
4520	ASLQPETLR	Serine protease inhibitor A3K	288	296	P07759	1013,55	38,53	0,004385248	0,89	502,01	0	0	0	down-regulated in ob/ob								
4856	ApGEKGEGGpP	Collagen alpha-1(III) chain	828	838	P08121	1026,53	36,03	0,020852404	0,89	162,82	0,11	40,59	0,249293699	down-regulated in ob/ob								
4882	GQPGAKGEpGD	Collagen alpha-1(I) chain	810	820	P11087	1027,51	36,35	0,017010274	0,22	23,65	0,78	452,11	19,1167019	up-regulated in ob/ob								
5204	SpGPDGKTGPp	Collagen alpha-1(I) chain	535	545	P11087	1040,53	36,08	0,009919353	1	1273,46	0,78	365,04	0,286652113	down-regulated in ob/ob								
5229	SpGAKGEVGPAG	Collagen alpha-1(III) chain	345	356	P08121	1041,49	37,04	0,003740306	1	178,89	0	0	0	down-regulated in ob/ob								
5599	ApGKDGPpGPAG	Collagen alpha-1(III) chain	897	908	P08121	1051,54	36,38								0,01921313	1	1147,13	0	0	0	down-regulated in ACEi	
5677	ApGDRGEAGPp	Collagen alpha-1(I) chain	787	797	P11087	1054,52	36,74	0,002949087	1	934,56	0,89	332,3	0,355568396	down-regulated in ob/ob	0,03777412	0,89	332,3	0,33	17,36	0,05224195	down-regulated in ACEi	
5807	SpGGKGEMGPAG	Collagen alpha-1(III) chain	390	401	P08121	1059,5	37,01	0,005095706	1	800,29	0,89	358,58	0,448062577	down-regulated in ob/ob								
5886	GPpGPTGPTGPp	Collagen alpha-1(I) chain	321	332	P11087	1062,53	47,72	0,005037542	1	558,21	0,44	38,15	0,068343455	down-regulated in ob/ob								
6895	RpGEVGPpGPp	Collagen alpha-1(I) chain	907	917	P11087	1106,59	35,07	0,004385248	0,89	168,93	0	0	0	down-regulated in ob/ob								
6927	KPGppGEpGPQ	Collagen alpha-1(VIII) chain	289	299	Q00780	1107,57	38,4	0,005095706	1	640,91	0,78	229,8	0,358552683	down-regulated in ob/ob								
6997	SISGEYNLKT	Alpha-1-antitrypsin 1-1	310	319	P07758	1110,58	36,36	0,00768409	0,89	401,38	0,33	68,79	0,171383726	down-regulated in ob/ob								
7020	DQTRVLNLGP	Uromodulin	595	604	Q91X17	1111,62	36,1	0,00768409	0,78	265,87	1	1753,49	6,595290932	up-regulated in ob/ob								
7064	GpPGSpGSpGEQ	Collagen alpha-1(I) chain	1107	1118	P11087	1113,49	47,49	0,003344693	1	427,02	0,11	6,82	0,015971149	down-regulated in ob/ob								
7194	GPpGPTGPTGPpG	Collagen alpha-1(I) chain	321	333	P11087	1119,54	47,64	0,014205913	0,89	521,69	0,33	60,8	0,116544308	down-regulated in ob/ob								
7213	GpGERGEHGPp	Collagen alpha-1(III) chain	795	805	P08121	1120,55	28,62	0,023549868	1	553,03	0,44	192,41	0,347919643	down-regulated in ob/ob								
7284	GGKGEQGPAGpPG	Collagen alpha-2(I) chain	547	559	Q01149	1123,55	36,25	0,041310707	1	332,29	0,44	109,65	0,329982846	down-regulated in ob/ob								
7366	FLEQIHVLE	Major urinary protein 1	59	67	P11588	1126,63	38,99	0,004385248	1	598,37	0,22	27,34	0,045690793	down-regulated in ob/ob								
7489	VSINKELQNS	Kidney androgen-regulated protein	25	34	P61110	1130,63	36,82	0,012142689	0,78	1181,69	0,11	5,28	0,004468177	down-regulated in ob/ob								
7700	SpGKAGEDGHpG	Collagen alpha-2(I) chain	143	154	Q01149	1139,55	29,02	0,004385248	1	1987,99	0,89	922,37	0,463971147	down-regulated in ob/ob								
7719	GLpGAAGERGPSG	Collagen alpha-1(III) chain	476	488	P08121	1140,56	38,23	0,00768409	1	469,88	0,78	178,78	0,380480123	down-regulated in ob/ob								
7786	GPpGPLGpPGLPG	Collagen alpha-1(XI) chain	1427	1439	Q61245	1143,56	47,71	0,002949087	1	5555,04	1	1332,1	0,239800253	down-regulated in ob/ob								
7814	DGApGAKGDRGE	Collagen alpha-1(I) chain	1016	1027	P11087	1144,57	31,02	0,031121543	1	586,1	1	383,27	0,653932776	down-regulated in ob/ob								
8166	GPpGPpGPpGPPS	Collagen alpha-1(I) chain	1170	1182	P11087	1157,54	47,77	0,009799137	0,89	1090,03	0,44	132,24	0,121317762	down-regulated in ob/ob								
8240	SpGPAGPRGPVGP	Collagen alpha-1(III) chain	1131	1143	P08121	1160,59	37,28	0,042327425	0,33	30,79	0,78	219,78	7,138031829	up-regulated in ob/ob	0,02744468	0,78	219,78	1	877,1	3,990808991	up-regulated in ACEi	
8738	ApGEKGEGGPpGP	Collagen alpha-1(III) chain	828	840	P08121	1180,58	37,84	0,012479191	1	1637,58	1	807,95	0,493380476	down-regulated in ob/ob								
9077	SpGPDGKTGPpGP	Collagen alpha-1(I) chain	535	547	P11087	1194,61	37,73	0,048043989	1	4026,93	1	2969,04	0,737296154	down-regulated in ob/ob								
9139	SpGQRGEpGPQG	Collagen alpha-1(III) chain	363	374	P08121	1197,58	37,06	0,014018563	0,89	1383,69	0,78	761,42	0,550282216	down-regulated in ob/ob								
9415	VGPRVGLARAQS	Napsin-A	387	398	O09043	1209,74	30,12	0,004385248	1	2059,96	0,22	18,38	0,008922503	down-regulated in ob/ob								
9460	DGKTGPpGPAGQD	Collagen alpha-1(I) chain	539	551	P11087	1211,61	39,95	0,012142689	0,78	228,89	0,11	7,7	0,033640613	down-regulated in ob/ob								
9523	GPpGESGREGSpG	Collagen alpha-1(I) chain	996	1008	P11087	1214,56	37,26	0,004385248	1	2120,9	1	1384,76	0,6529115	down-regulated in ob/ob								
9525	GPpGPpGPpGPSGP	Collagen alpha-1(XIII) chain	277	290	Q9R1N9	1214,59	47,91	0,006027174	1	1188,18	0,67	321,62	0,270682893	down-regulated in ob/ob								
9735	GPPGEpGEpGGSGP	Collagen alpha-1(I) chain	192	205	P11087	1222,58	48,01	0,012479191	1	905,24	0,89	242,5	0,26788476	down-regulated in ob/ob								
9877	PGAKGEpGDTGVK	Collagen alpha-1(I) chain	812	824	P11087	1227,65	31,3	0,048052934	1	362,87	0,33	128,33	0,353652823	down-regulated in ob/ob								
10074	DpGKPGERGHpG	Collagen alpha-2(I) chain	509	520	Q01149	1234,64	25,97	0,036469953	1	558,83	0,78	276,39	0,494586905	down-regulated in ob/ob								
10156	GPpGEpGEpGGSGP	Collagen alpha-1(I) chain	192	205	P11087	1238,56	47,93	0,004385248	1	2233,37	0,78	809,17	0,362308977	down-regulated in ob/ob								
10290	VSINKELQNSI	Kidney androgen-regulated protein	25	35	P61110	1243,71	37,9	0,005541668	1	3103,25	0,11	232,88	0,075043906	down-regulated in ob/ob								
10361	KGEpGDTGVKGDA	Collagen alpha-1(I) chain	815	827	P11087	1245,62	31,92	0,003485285	1	2947,74	1	1170,01	0,396917639	down-regulated in ob/ob	0,01921313	1	1170,01	0	0	0	down-regulated in ACEi	
10497	DpGKpGERGHpG	Collagen alpha-2(I) chain	509	520	Q01149	1250,63	26,16	0,003485285	1	4719,03	0,89	1997,48	0,423281903	down-regulated in ob/ob								
10514	ApGEKGEGGPpGPA	Collagen alpha-1(III) chain	828	841	P08121	1251,61	38,23	0,039808966	0,89	1566,24	0,89	997,53	0,636894729	down-regulated in ob/ob								
10734	RpGEVGPpGPpGP	Collagen alpha-1(I) chain	907	919	P11087	1260,65	38,35	0,003485285	1	2843,92	1	5027,56	1,767827506	up-regulated in ob/ob								
10940	DGKTGPpGPAGQDG	Collagen alpha-1(I) chain	539	552	P11087	1268,62	40,04	0,013313568	1	439,65	0,44	99,96	0,227362675	down-regulated in ob/ob								
11018	GpPGPpGPpGPPSGG	Collagen alpha-1(I) chain	1170	1184	P11087	1271,62	47,98	0,003740306	1	687,19	0	0	0	down-regulated in ob/ob								
11248	EDVQETDTSQK	Alpha-1-antitrypsin 1-1	25	35	P07758	1278,64	38,43								0,02548084	1	1580,89	0,89	488,09	0,308743809	down-regulated in ACEi	
12099	LGPYVAVFDRGD	Napsin-A	373	384	O09043	1307,69	39,1								0,02206446	0	0	0,89	1521,42	1521,42	up-regulated in ACEi	
12726	GGpGERGEHGPpGP	Collagen alpha-1(III) chain	794	807	P08121	1331,65	30,51	0,014018563	0,78	795,79	0,89	2718,6	3,416227899	up-regulated in ob/ob								
12934	KLGVGSLQELAPQ	Sperm flagellar protein 1	119	131	Q99JL1	1338,8	39,93	0,007501881	0,78	1457,66	0	0	0	down-regulated in ob/ob								
13399	NGDDGEAGKpGRpG	Collagen alpha-1(I) chain	218	231	P11087	1357,65	31,57	0,012479191	1	4439,71	1	2522,84	0,568244322	down-regulated in ob/ob								
13533	SGNFIDQTRVLN	Uromodulin	590	601	Q91X17	1362,72	38,83	0,006027174	1	2824,95	0,22	343,66	0,121651711	down-regulated in ob/ob								
13637	QTRVLNLGPITR	Uromodulin	596	607	Q91X17	1366,85	33,58	0,02550148	0,78	387,01	0,22	36,75	0,094958787	down-regulated in ob/ob								
13692	GpPGEpGEpGGSGPM	Collagen alpha-1(I) chain	192	206	P11087	1369,62	48,02	0,00768409	0,89	445,2	0,33	28,86	0,064824798	down-regulated in ob/ob								
13864	WTDVGMSPRIES	Pro-epidermal growth factor	620	631	P01132	1376,73	37,67	0,012142689	0,78	114,1	0,11	6,53	0,0572305	down-regulated in ob/ob								
14045	PGAKGEPGDTGVKGD	Collagen alpha-1(I) chain	812	826	P11087	1383,69	32,05	0,007501881	0,78	86	0	0	0	down-regulated in ob/ob								
14400	INKELQNSIIDL	Kidney androgen-regulated protein	27	38	P61110	1398,79	38,95	0,004385248	1	3872,94	0,11	252,71	0,065250172	down-regulated in ob/ob								
14644	QGELGEVGAQGpPGP	Collagen alpha-1(IX) chain	453	467	Q05722	1407,7	47,91	0,003740306	1	536,54	0	0	0	down-regulated in ob/ob								
14770	GQPGAKGEpGDTGVK	Collagen alpha-1(I) chain	810	824	P11087	1412,74	31,8	0,005095706	1	1986,91	0,89	1144,64	0,576090512	down-regulated in ob/ob								
14987	GPpGPpGpAGEKGSpG	Collagen alpha-1(I) chain	912	927	P11087	1421,72	39,36	0,005095706	1	7477,24	1	4524,82	0,605145749	down-regulated in ob/ob								
15063	GPpGPAGPAGPPGPIGN	Collagen alpha-1(I) chain	828	844	P11087	1424,7	48,22	0,027033297	0,89	5564,19	0,89	2188,46	0,393311515	down-regulated in ob/ob								
15130	GQpGAKGEpGDTGVK	Collagen alpha-1(I) chain	810	824	P11087	1428,74	31,96	0,004385248	1	2860,63	1	1495,87	0,522916281	down-regulated in ob/ob								
15221	GEVGPpGPpGPAGEKG	Collagen alpha-1(I) chain	909	924	P11087	1433,72	38,81								0,01921313	1	3377,27	0,11	22,88	0,006774703	down-regulated in ACEi	
15293	GpPGPTGPTGPpGPpG	Collagen alpha-1(I) chain	321	336	P11087	1436,7	48,08	0,004385248	1	1797,88	0,56	200,6	0,111575856	down-regulated in ob/ob								
15342	SpGKAGEDGHPGkpG	Collagen alpha-2(I) chain	143	157	Q01149	1437,72	26,48	0,004385248	1	1795,05	0,89	702,97	0,391615832	down-regulated in ob/ob								
15427	GPpGPAGPAGPpGPIGN	Collagen alpha-1(I) chain	828	844	P11087	1440,7	48,22	0,016072859	0,89	1120,36	0,56	318,47	0,284256846	down-regulated in ob/ob								
15429	FLEQIHVLENSL	Major urinary protein 1	59	70	P11588	1440,7	38,72								0,03124018	0,89	498,55	0,22	38,2	0,076622204	down-regulated in ACEi	
15539	GPpGERGGpGPAGPRG	Collagen alpha-1(III) chain	503	518	P08121	1446,74	31,55	0,00705176	0,89	111,77	0,11	7,86	0,070322985	down-regulated in ob/ob								
15855	GRpGPpGPSGPRGQp	Collagen alpha-1(III) chain	560	574	P08121	1460,74	32,22	0,009919353	1	1037,2	0,22	174,22	0,167971462	down-regulated in ob/ob								
16362	DQTRVLNLGPITR	Uromodulin	595	607	Q91X17	1481,88	33,69	0,004385248	1	6703,59	0,67	544,39	0,081208725	down-regulated in ob/ob								
16415	KNGDDGEAGKpGRpG	Collagen alpha-1(I) chain	217	231	P11087	1485,73	27,52	0,004385248	1	3759,82	1	1554,16	0,413360214	down-regulated in ob/ob								
16425	SINKELQNSIIDL	Kidney androgen-regulated protein	26	38	P61110	1485,84	39,49	0,004344693	1	18784,43	0,11	8,15	0,00043387	down-regulated in ob/ob								
16583	GSpGSPGPDGKTGPpGP	Collagen alpha-1(I) chain	531	547	P11087	1492,74	39,9	0,016072859	1	4432,49	1	6727,79	1,517835348	up-regulated in ob/ob								
16733	GPpGPpGPpGpGVSGGG	Collagen alpha-2(I) chain	1096	1113	Q01149	1500,73	48,28	0,012142689	0,89	2054,34	0,44	383,86	0,186853199	down-regulated in ob/ob								
16741	AHLINEKDGETFQ	Major urinary protein 1	121	133	P11588	1500,77	32,08	0,019906338	1	797,99	1	1488,35	1,865123623	up-regulated in ob/ob								
16857	KpGEQGVPGDLGApGP	Collagen alpha-1(I) chain	646	661	P11087	1506,78	40,49	0,039110041	1	2170,84	1	4362,62	2,009646036	up-regulated in ob/ob								
17232	KpGEQGVpGDLGApGP	Collagen alpha-1(I) chain	646	661	P11087	1522,78	40,4	0,007501881	1	2366,4	1	4196,38	1,77331812	up-regulated in ob/ob								
17342	DGQPGAKGEpGDTGVK	Collagen alpha-1(I) chain	809	824	P11087	1527,78	33,82	0,010033808	1	1016,73	0,89	590,94	0,581216252	down-regulated in ob/ob								
17585	GPpGPpGppGLGGNFAS	Collagen alpha-1(I) chain	135	151	P11087	1538,73	48,37	0,013313568	0,89	6546,4	0,56	1514,25	0,231310339	down-regulated in ob/ob								
18066	SpGSPGPDGKTGPPGpAG	Collagen alpha-1(I) chain	532	549	P11087	1563,79	40,57	0,012479191	1	911,52	1	2220,7	2,436260312	up-regulated in ob/ob								
18443	ILGGQEAAAHARPYM	Complement factor D	26	40	P03953	1583,81	33,09	0,017010274	0,78	3792,01	0,22	118,11	0,03114707	down-regulated in ob/ob								
18466	VSINKELQNSIIDL	Kidney androgen-regulated protein	25	38	P61110	1584,92	40,18	0,005603303	0,89	20465,8	0,11	229,53	0,011215296	down-regulated in ob/ob								

19285	EVGPpGPpGPAGEKGSpG	Collagen alpha-1(I) chain	910	927	P11087	1633,82	41,12	0,003740306	1	8059,6	1	3507,43	0,43518661	down-regulated in ob/ob							
19345	GSpGSpGPDGKTGPpGPAG	Collagen alpha-1(I) chain	531	549	P11087	1636,83	40,73	0,012479191	1	4111,71	0,89	6559,88	1,595414073	up-regulated in ob/ob							
19367	APEPYKRWYDVQS	Complement factor D	88	100	P03953	1637,8	32,93	0,005603303	0,89	694,47	0,11	8,19	0,011793166	down-regulated in ob/ob							
19606	EVGPpGPpGpAGEKGSpG	Collagen alpha-1(I) chain	910	927	P11087	1649,81	41,29	0,012479191	1	16535	1	9453,33	0,571716359	down-regulated in ob/ob							
19814	GLpGPAGPpGEAGKpGEQ	Collagen alpha-1(I) chain	633	650	P11087	1662,83	41	0,00294987	1	3541,81	1	6708,74	1,894155813	up-regulated in ob/ob							
20085	TGPIGPpGPAGApGDKGEA	Collagen alpha-1(I) chain	755	773	P11087	1676,84	41,36	0,004385248	1	1202,25	1	2971,04	2,471233105	up-regulated in ob/ob							
20385	GEVGPpGPpGPAGEKGSpG	Collagen alpha-1(I) chain	909	927	P11087	1690,83	41,14	0,016072859	0,44	78,84	1	348,68	4,422628108	up-regulated in ob/ob							
20503	VSINKELQNSIIDLL	Kidney androgen-regulated protein	25	39	P61110	1697,99	40,83	0,003740306	1	26893,79	0	0	0	down-regulated in ob/ob							
20763	SPRIESASLQGSDRVL	Pro-epidermal growth factor	626	641	P01132	1713,91	34,12	0,012142689	0,78	4136,56	0,11	11,04	0,002668884	down-regulated in ob/ob							
21009	ASHEIATNLGDFALRL	Alpha-1-antitrypsin 1-4	40	55	Q00897	1726,88	34,18	0,02550148	0,33	164,23	0,89	1183,23	7,204712903	up-regulated in ob/ob							
21108	PGQTGAAGTPGKMGPPGPPG	EMI domain-containing protein 1	232	251	Q91VF5	1730,89	39,23	0,039110041	1	1534,05	0,89	939,39	0,612359441	down-regulated in ob/ob							
21455	pTGARGAPGDRGEAGPPGp	Collagen alpha-1(I) chain	781	799	P11087	1747,81	36,04	0,04691826	0,89	171,09	0,22	47,6	0,278216144	down-regulated in ob/ob							
21893	DGQPGAKGEpGDTGVKGDA	Collagen alpha-1(I) chain	809	827	P11087	1770,89	35,47	0,003485285	1	466,91	1	1073,81	2,299822236	up-regulated in ob/ob							
22065	YLNRDSEGSDGGELVLG	Napsin-A	215	231	O09043	1779,88	41,85	0,003740306	1	1575,48	1	2981,4	1,892375657	up-regulated in ob/ob							
22220	DGQpGAKGEpGDTGVKGDA	Collagen alpha-1(I) chain	809	827	P11087	1786,86	35,6	0,004385248	1	1371,97	1	2276	1,658928402	up-regulated in ob/ob							
22880	VGEKGPSGEpGTAGApGTAGP	Collagen alpha-2(I) chain	849	869	Q01149	1824,89	42,14	0,02550148	1	1030,46	1	2207,02	2,14178134	up-regulated in ob/ob							
22917	SPRIESASLQGSDRVLI	Pro-epidermal growth factor	626	642	P01132	1826,96	35,11	0,004385248	0,89	2980,38	0	0	0	down-regulated in ob/ob							
23042	GIpGTGGPpGENGKpGEpGP	Collagen alpha-1(III) chain	641	660	P08121	1834,89	41,58	0,012479191	1	3253,68	0,89	5840,31	1,794985985	up-regulated in ob/ob							
23356	IELSKASDYVDRNVEL	ATP-binding cassette sub-family A member	1292	1307	Q5SSE9	1849,93	34,64	0,012479191	1	965,05	0,89	5251,04	5,4412103	up-regulated in ob/ob							
23770	GPIGPpGPAGQpGDKGEGGSp	Collagen alpha-1(III) chain	764	784	P08121	1875,92	42,43	0,007164724	1	1160,08	0,67	297,86	0,256758155	down-regulated in ob/ob							
24239	ETGPAGRpGEVGPpGPpGPAG	Collagen alpha-1(I) chain	901	921	P11087	1900,94	42,53	0,003740306	1	1750,2	1	4283,88	2,447651697	up-regulated in ob/ob							
24713	ALDYDPVESKIYFAQTA	Pro-epidermal growth factor	522	538	P01132	1929,97	42,48	0,005603303	0,89	6125,23	0,11	18,25	0,00297948	down-regulated in ob/ob							
25110	VVPHPGSRPDSLEDDLIL	Complement factor D	101	118	P03953	1958,05	35,31	0,004385248	1	25452,72	0,22	31,58	0,001240732	down-regulated in ob/ob							
25169	QGIpGTGGPpGENGKpGEpGP	Collagen alpha-1(III) chain	640	660	P08121	1962,95	42,24	0,019906338	1	2370,47	1	3687,96	1,555792733	up-regulated in ob/ob							
25395	RpGEVGPpGPpGpAGEKGSpG	Collagen alpha-1(I) chain	907	927	P11087	1975,97	35,73	0,004385248	0,89	2080,23	0	0	0	down-regulated in ob/ob							
25689	GPKGSpGEAGRpGEAGLpGAKG	Collagen alpha-1(I) chain	507	528	P11087	1994,03	29,7	0,012479191	1	919,01	0,89	2294,12	2,496294926	up-regulated in ob/ob							
25768	SGNFIDQTRVLNLGPITR	Uromodulin	590	607	Q91X17	2000,1	35,77	0,019906338	1	27322,53	0,56	2810,49	0,102863461	down-regulated in ob/ob							
26052	GRpGEVGPpGPpGPAGEKGSpG	Collagen alpha-1(I) chain	906	927	P11087	2017,01	35,92	0,007501881	0,89	374,55	0,11	19,79	0,052836737	down-regulated in ob/ob							
26273	GRpGEVGPpGPpGpAGEKGSpG	Collagen alpha-1(I) chain	906	927	P11087	2033,01	36,09	0,007501881	0,78	168,66	0	0	0	down-regulated in ob/ob							
26458	SVVPHPGSRPDSLEDDLIL	Complement factor D	100	118	P03953	2045,07	35,9	0,031121543	0,78	1276,6	0,44	149,95	0,117460442	down-regulated in ob/ob							
26537	KNGETGPQGPpGPTGPAGDKGD	Collagen alpha-1(III) chain	609	630	P08121	2049,01	36,9	0,007164724	0,89	366,3	0,22	19,23	0,052497952	down-regulated in ob/ob							
27104	AGRpGEVGPpGPpGPAGEKGSpG	Collagen alpha-1(I) chain	905	927	P11087	2088,05	36,3	0,012142689	0,89	348	0,44	59,64	0,17137931	down-regulated in ob/ob							
27322	AGRpGEVGPpGPpGpAGEKGSpG	Collagen alpha-1(I) chain	905	927	P11087	2104,02	36,19	0,014018563	1	481,07	0,67	125,88	0,261666701	down-regulated in ob/ob							
27962	GFpGPKGPSGDpGKpGERGHpG	Collagen alpha-2(I) chain	499	520	Q01149	2148,08	28,16	0,020886117	1	331,28	0,22	66,41	0,200464864	down-regulated in ob/ob							
28672	TTGEVGKpGERGLpGEFGLpGP	Collagen alpha-2(I) chain	569	590	Q01149	2199,05	34,97	0,02550148	1	1454,78	1	975,7	0,670685602	down-regulated in ob/ob							
30714	SGTTGEVGKpGERGLpGEFGLpGP	Collagen alpha-2(I) chain	567	590	Q01149	2343,17	37,04	0,039808966	0,89	2527,56	0,89	5404,25	2,138129263	up-regulated in ob/ob							
30739	PGSpGNVGPSGKEGpVGLpGIDGRP	Collagen alpha-2(I) chain	459	483	Q01149	2345,07	34,61	0,007501881	1	956,73	1	541,31	0,565791812	down-regulated in ob/ob							
32649	AVGGVQPPLDAMVEQGLLEKPVFS	Napsin-A	190	213	O09043	2480,32	45,06	0,007501881	0,78	505,29	0	0	0	down-regulated in ob/ob							
34635	AVGGVQPPLDAMVEQGLLEKPVFSF	Napsin-A	190	214	O09043	2627,39	45,64	0,012142689	0,78	17651,2	0,11	9,66	0,000547272	down-regulated in ob/ob							
34793	AVGGVQPPLDAmVEQGLLEKPVFSF	Napsin-A	190	214	O09043	2643,38	45,91	0,004385248	0,89	4457,79	0	0	0	down-regulated in ob/ob							
35125	REGSpGAEGSpGRDGApGAKGDRGETGP	Collagen alpha-1(I) chain	1003	1030	P11087	2669,26	29,68	0,048043989	1	3031,72	0,89	5197,89	1,714501999	up-regulated in ob/ob							
36104	KNGETGPQGPpGPTGPAGDKGDSGPpGPQG	Collagen alpha-1(III) chain	609	638	P08121	2742,29	39,65	0,031121543	1	3463,84	1	5616,43	1,621446141	up-regulated in ob/ob							
36597	GLpGPSGTTGEVGKpGERGLpGEFGLpGP	Collagen alpha-2(I) chain	562	590	Q01149	2780,39	38,92	0,048043989	0,89	664,51	0,89	1583,27	2,382612752	up-regulated in ob/ob							
41481	YLNRDSEGSDGGELVLGGSDPAHYVPPLTFIP	Napsin-A	215	246	O09043	3371,6	41,55	0,007501881	0,78	2389,84	0	0	0	down-regulated in ob/ob							
43263	SAPEPYKRWYDVQSVVPHPGSRPDSLEDDLIL	Complement factor D	87	118	P03953	3664,78	33,12	0,007501881	0,78	8344,63	0	0	0	down-regulated in ob/ob							

Supplementary Table 2: List of urinary peptides associated to i) T2D nephropathy and ii) treatment with RASi (enalapril) in the Unxdb/db mouse model.

Peptide_ID	Sequence	Protein name	Start	Stop	Uniprot	Masse	CE-Time	Adjusted pValue	Freq Unxdb/m	mean Amp. Unxdb/m	Freq Unxdb/db	mean Amp. Unxdb/db	FOLD CHANGE db/db / db/m	DIRECTION	Adjusted pValue	Freq Unxdb/db	mean Amp. Unxdb/db	Freq Unxdb/db+ RASi	mean Amp. Unxdb/db+RASi	FOLD CHANGE Unxdb/db+RASi/ Unxdb/db	DIRECTION	
486	AVFEDHL	Pro-epidermal growth factor	697	703	P01132	829,44	36,03	0,014560573	1	249,38	0,82	146,97	0,589341567	down-regulated in db/db								
710	SpGRDGApG	Collagen alpha-1(I) chain	1012	1020	P11087	844,43	33,46								0,018081205	0	0	0,78	125,06	125,06	up regulated in ACEi	
878	DPPKINDG	Complement decay-accelerating factor transmembrane isoform	232	239	Q61476	854,44	33,86	0,004667295	0,25	14,14	0,91	335,54	23,72984441	up-regulated in db/db								
931	ApGApGGKGD	Collagen alpha-1(III) chain	666	675	P08121	857,45	34,81	0,004667295	1	136,98	0,27	23,6	0,172287925	down-regulated in db/db								
1127	ApGERGPpG	Collagen alpha-1(III) chain	678	686	P08121	868,45	33,7	0,01324474	1	207,52	0,45	72,07	0,347291827	down-regulated in db/db								
1215	GEpGDTGVK	Collagen alpha-1(I) chain	816	824	P11087	874,42	36,76	0,002348934	0,88	20,58	0	0	0	down-regulated in db/db								
1283	SmLKPSSL	Pro-epidermal growth factor	730	737	P01132	877,47	33,6	0,004667295	0,75	48,98	0	0	0	down-regulated in db/db								
1724	GPmGPRGpP	Collagen alpha-1(I) chain	204	212	P11087	896,48	34,05	0,005633844	0	0	0,82	148,88	148,88	up-regulated in db/db								
1760	GPpGEAGKpG	Collagen alpha-1(I) chain	639	648	P11087	897,47	33,68	0,005633844	1	176,22	0,09	28,52	0,161843151	down-regulated in db/db								
2081	IAVDPIER	Pro-epidermal growth factor	170	177	P01132	911,46	33,71	0,021057571	0,88	109,16	1	238,48	2,184683034	up-regulated in db/db	0,018081205	1	238,48	0,67	62,94	0,263921503	down regulated in ACEi	reversed by ACEi treatment
2465	QVSPGAKmP	Transcription factor SOX-6	378	386	P40645	929,47	35,74								0,006917625	0,36	54,13	1	1285,6	23,75023093	up regulated in ACEi	
2988	pPGERGGpGP	Collagen alpha-1(III) chain	504	513	P08121	951,49	35,01								0,048419994	0,91	242,86	1	612,82	2,523346784	up regulated in ACEi	
3062	GQpGEKGPpG	Collagen alpha-1(III) chain	926	935	P08121	954,47	34,61	0,008031871	0,88	115,09	0,18	14,54	0,126335911	down-regulated in db/db								
3144	GPpGESGREG	Collagen alpha-1(I) chain	996	1005	P11087	957,46	34,3	0,046851511	0,88	38,73	0,27	16,72	0,431706687	down-regulated in db/db								
3705	PGSPGEKGVPG	Collagen alpha-1(IV) chain	1019	1029	P02463	980,51	36,61	0,036037801	1	324,84	0,82	171,72	0,528629479	down-regulated in db/db								
4209	GEpGPAGSKGE	Collagen alpha-2(I) chain	352	362	Q01149	1000,5	36,97	0,046851511	0,88	99,4	0,27	30,68	0,308651911	down-regulated in db/db								
4497	SpGQRGEpGP	Collagen alpha-1(III) chain	363	372	P08121	1012,5	35,68	0,019594272	1	108,12	0,45	39,97	0,369681835	down-regulated in db/db	0,006917625	0,45	39,97	1	980,33	24,52664498	up regulated in ACEi	reversed by ACEi treatment
4520	ASLQPETLR	Serine protease inhibitor A3K	288	296	P07759	1013,55	38,53	0,004667295	0,75	399,24	0	0	0	down-regulated in db/db								
4882	GQPGAKGEpGD	Collagen alpha-1(I) chain	810	820	P11087	1027,51	36,35	0,006274818	1	335,27	1	716,71	2,137709905	up-regulated in db/db								
5070	PGpEGGKGPAGP	Collagen alpha-1(III) chain	700	711	P08121	1035,54	35,71	0,002921538	1	475,8	0,64	135,75	0,285308953	down-regulated in db/db								
5204	SpGPDGKTGPp	Collagen alpha-1(I) chain	535	545	P11087	1040,53	36,08	0,004786026	1	1023,77	1	500,55	0,488928177	down-regulated in db/db								
5677	ApGDRGEAGPp	Collagen alpha-1(I) chain	787	797	P11087	1054,52	36,74	0,010321988	1	527,03	0,82	235,82	0,447450809	down-regulated in db/db	0,018081205	0,82	235,82	0	0	0	down regulated in ACEi	
5763	PpGSAGSpGKDG	Collagen alpha-1(I) chain	1132	1143	P11087	1057,52	36,16								0,018081205	0,91	1368,75	0,22	28,52	0,02083653	down regulated in ACEi	
5886	GPpGPTGPTGPp	Collagen alpha-1(I) chain	321	332	P11087	1062,53	47,72	0,010955367	1	234,98	0,45	58,18	0,24759554	down-regulated in db/db								
6095	EVLASGRAIR	Apoptosis-inducing factor 3	572	581	Q3TY86	1070,6	28,8	0,006274818	1	208,44	0,91	529,87	2,542074458	up-regulated in db/db								
6403	VFDQLGSYR	Kidney androgen-regulated protein	42	50	P61110	1083,52	37,6	0,027189316	0,25	18,14	0,73	399,86	22,0429989	up-regulated in db/db								
6763	VGEpGPAGSKGE	Collagen alpha-2(I) chain	351	362	Q01149	1099,56	37,4	0,005633844	1	87	0,09	20,74	0,238390805	down-regulated in db/db								
6921	GPpGPpGPpGSSG	Collagen alpha-1(III) chain	176	188	P08121	1107,53	47,75	0,03034805	0,75	57,17	0,09	18,96	0,33164247	down-regulated in db/db								
6952	GPpGPAGApGDKG	Collagen alpha-1(I) chain	759	771	P11087	1108,61	38,1	0,003307534	1	659,67	0,27	62,47	0,094698865	down-regulated in db/db								
7020	DQTRVLNLGP	Uromodulin	595	604	Q91X17	1111,62	36,1	0,012123266	0,63	172,85	1	652,15	3,772924501	up-regulated in db/db								
7050	YSLHPDPIAT	Osteopontin	32	41	P10923	1112,63	39,74	0,002902818	1	2600,18	0,18	71,89	0,027648086	down-regulated in db/db								
7064	GpPGSpGSpGEQ	Collagen alpha-1(I) chain	1107	1118	P11087	1113,49	47,49	0,035804475	1	516,47	0,73	286,49	0,554707921	down-regulated in db/db	0,030751954	0,73	286,49	0	0	0	down regulated in ACEi	
7091	SpGEAGRpGEAG	Collagen alpha-1(I) chain	511	522	P11087	1115,53	36,49								0,018010925	0,18	19,83	0,89	1131,39	57,05446293	up regulated in ACEi	
7194	GPpGPTGPTGPpG	Collagen alpha-1(I) chain	321	333	P11087	1119,54	47,64	0,002348934	1	1084,75	0,64	130,17	0,12	down-regulated in db/db								
7213	GpGERGEHGPp	Collagen alpha-1(III) chain	795	805	P08121	1120,55	28,62	0,006274818	1	759,67	1	480,48	0,632485158	down-regulated in db/db	0,006917625	1	480,48	0	0	0	down regulated in ACEi	
7366	FLEQHVLE	Major urinary protein 1	59	67	P11588	1126,63	38,99	0,00842438	0,75	438,91	0,09	4,74	0,010799481	down-regulated in db/db								
7394	IDQTRVLNLG	Uromodulin	594	603	Q91X17	1127,6	37,67	0,001509036	1	77,04	0	0	0	down-regulated in db/db								
7545	GpPGPpGSpGPpG	Collagen alpha-2(I) chain	60	72	Q01149	1133,52	47,71	0,002348934	0,88	279,77	0	0	0	down-regulated in db/db								
7629	GQpGPpGPpGTAG	Collagen alpha-1(III) chain	329	341	P08121	1136,51	47,74	0,012873829	0,88	510,34	0,36	86,37	0,169240114	down-regulated in db/db								
7687	NIAVDPIERL	Pro-epidermal growth factor	169	178	P01132	1138,68	39,09	0,004667295	0,75	283,41	0	0	0	down-regulated in db/db								
7785	EpGRDGTpGGpG	Collagen alpha-1(III) chain	522	533	P08121	1143,54	37,18	0,011462987	1	840,03	0,55	373,65	0,444805543	down-regulated in db/db								
7814	DGApGAKGDRGE	Collagen alpha-1(I) chain	1016	1027	P11087	1144,57	31,02	0,004466958	1	1192,56	1	777,11	0,651631784	down-regulated in db/db								
8188	NIGFpGpKGPSG	Collagen alpha-2(I) chain	497	508	Q01149	1158,55	38,76	0,033066705	1	410,57	0,45	132,46	0,322624644	down-regulated in db/db								
8447	KpGEQGVPGDLG	Collagen alpha-1(I) chain	646	657	P11087	1168,61	37,46	0,016775791	1	1006,91	1	1942,09	1,928762253	up-regulated in db/db								
8738	ApGEKGEGGPpGP	Collagen alpha-1(III) chain	828	840	P08121	1180,58	37,84	0,001509036	1	3489,85	1	1837,6	0,526555583	down-regulated in db/db								
8774	KpGEpGPKGEVG	Collagen alpha-1(III) chain	654	665	P08121	1182,63	29,56	0,00193307	1	1581,07	1	942,48	0,596102639	down-regulated in db/db	0,006917625	1	942,48	0	0	0	down regulated in ACEi	
8824	TRVSSYRMW	Complement factor D	240	248	P03953	1184,61	29,34								0,024837209	0,82	706,18	0,11	16,58	0,023478433	down regulated in ACEi	
9139	SpGQRGEpGPQG	Collagen alpha-1(III) chain	363	374	P08121	1197,58	37,06	0,001509036	1	2201,62	1	1200,39	0,54523033	down-regulated in db/db								
9197	GEpGRDGTpGGpG	Collagen alpha-1(III) chain	521	533	P08121	1200,57	37,45	0,021057571	1	1464,99	0,91	1056,65	0,721267722	down-regulated in db/db								
9234	KpGERGLPGEF	Collagen alpha-2(I) chain	575	585	Q01149	1201,64	29,65	0,001509036	1	4494,09	1	1424,54	0,316980746	down-regulated in db/db								
9385	ApGDRGEAGPpGP	Collagen alpha-1(I) chain	787	799	P11087	1208,6	38,36	0,002358894	0,75	7627,11	1	20826,57	2,730597828	up-regulated in db/db								
9523	GPpGESGREGSpG	Collagen alpha-1(I) chain	996	1008	P11087	1214,56	37,26	0,038867999	1	1454,77	1	2738,8	1,882634368	up-regulated in db/db								
10139	GPpGPTGPAGDKGD	Collagen alpha-1(III) chain	617	630	P08121	1237,61	38,32	0,041353671	1	1122,39	0,55	498,08	0,443767318	down-regulated in db/db								
10236	LNSVFDQLGSY	Kidney androgen-regulated protein	39	49	P61110	1241,63	47,95	0,002348934	0,88	551,44	0	0	0	down-regulated in db/db								
10290	VSINKELQNSI	Kidney androgen-regulated protein	25	35	P61110	1243,71	37,9	0,00842438	1	2770,56	0,82	570,25	0,205824815	down-regulated in db/db								
10361	KGEpGDTGVKGDA	Collagen alpha-1(I) chain	815	827	P11087	1245,62	31,92								0,006917625	1	1281,27	0	0	0	down regulated in ACEi	
10386	PLHGGDWAVAPQ	Synaptotagmin-15	87	98	Q8C6N3	1246,54	37,94	0,035804475	0,75	784,6	0,45	134,58	0,171526893	down-regulated in db/db								
10734	RpGEVGPpGPpGP	Collagen alpha-1(I) chain	907	919	P11087	1260,65	38,35	0,004466958	1	2514,8	1	4190,9	1,666494353	up-regulated in db/db								
10940	DGKTGPpGPAGQDG	Collagen alpha-1(I) chain	539	552	P11087	1268,62	40,04	0,00226331	1	201,44	0,18	16,45	0,081662033	down-regulated in db/db								
11018	GpPGPpGPpGPPSGG	Collagen alpha-1(I) chain	1170	1184	P11087	1271,62	47,98	0,004466958	0,88	92,16	0,09	4,14	0,044921875	down-regulated in db/db								
11025	SPGpPGSPGPRGPP	Collagen alpha-1(XIV) chain	1729	1742	Q80X19	1271,66	38	0,004786026	1	2288,99	1	4753,22	2,076557783	up-regulated in db/db								
11248	EDVQETDTSQK	Alpha-1-antitrypsin 1-1	25	35	P07758	1278,64	38,43	0,002348934	1	1068,41	1	2219,29	2,077189468	up-regulated in db/db								
11326	SpGSPGPDGKTGPp	Collagen alpha-1(I) chain	532	545	P11087	1281,66	38,78	0,005633844	1	97,53	0,09	32,65	0,334768789	down-regulated in db/db								
11438	NKELQNSIIDL	Kidney androgen-regulated protein	28	38	P61110	1285,72	38,39	0,004667295	0,75	1445,81	0	0	0	down-regulated in db/db								
11688	GLpGERGRPGppG	Collagen alpha-1(I) chain	294	306	P11087	1293,69	29,92	0,002348934	1	793,12	1	1811,93	2,284559714	up-regulated in db/db								
11797	LILPDQGRMQQ	Serine protease inhibitor A3K	275	285	P07759	1297,64	38,4	0,002348934	1	955,25	0,27	157,84	0,165234232	down-regulated in db/db								
11919	RLITEGVDTLEG	Pro-epidermal growth factor	554	565	P01132	1301,64	38,91	0,004667295	1	245,49	0,45	48,28	0,196667889	down-regulated in db/db								
11936	GAKGEpGDTGVKGD	Collagen alpha-1(I) chain	813	826	P11087	1302,66	31,79	0,006927736	1	211,4	0,36	55,56	0,2628193	down-regulated in db/db								
12096	GDpGKpGERGHpG	Collagen alpha-2(I) chain	508	520	Q01149	1307,65	25,46	0,01324474	1	1754,53	1	3035,54	1,730115757	up-regulated in db/db								
12099	LGPYVAVFDRGD	Napsin-A	373	384	O09043	1307,69	39,1								0,006917625	0	0	1	1405,12	1405,12	up regulated in ACEi	
12174	QILSLTGDRVPL	Nucleoporin Nup43	209	220	P59235	1310,79	40,19	0,001509036	1	319,48	0	0	0	down-regulated in db/db								
12270	IGFpGPKGPSGDpG	Collagen alpha-2(I) chain	498	511	Q01149	1313,67	39,06	0,022429314	1	173,76	0,36	54,44	0,313305709	down-regulated in db/db								
12325	GSRPDSLEDDLI	Complement factor D	106	117	P03953	1315,64	38,84	0,027878213	0,88	688,46	0,91	1573,78	2,285942538	up-regulated in db/db								
12554	GPpGPTGPAGDKGDS	Collagen alpha-1(III) chain	617	631	P08121	1324,64	38,85	0,030021618	0,75	59,47	0,82	284,97	4,791827812	up-regulated in db/db	0,024837209	0,82	284,97	0,11	19,58	0,068708987	down regulated in ACEi	reversed by ACEi treatment
12605	GPpGPAGPAGPpGPIG	Collagen alpha-1(I) chain	828	843	P11087	1326,69	48,06	0,01066665	0,88	2230,21	1	4029,04	1,806574269	up-regulated in db/db								
12624	FLEQHVLENS	Major urinary protein 1	59	69	P11588	1327,66	38,59	0,00226331	1	910,73	0,18	60,84	0,066803553	down-regulated in db/db								
12726	GGpGERGEHGPpGP	Collagen alpha-1(III) chain	794	807	P08121	1331,65	30,51	0,046851511	1	988,2	0,91	1480,66	1,498340417	up-regulated in db/db								
12934	KLGVGSLQELAPQ	Sperm flagellar protein 1	119	131	Q99JL1	1338,8	39,93	0,002658092	1	5906,82	0,27	158,67	0,026862169	down-regulated in db/db								
12996	NGDDGEAGKPGRpG	Collagen alpha-1(I) chain	218	231	P11087	1341,65	31,48	0,002348934	1	954,61	1	2898,26	3,036067085	up-regulated in db/db								

13399	NGDDGEAGKpGRpG	Collagen alpha-1(I) chain	218	231	P11087	1357,65	31,57	0,01066665	1	1564,23	1	3037,37	1,941766876	up-regulated in db/db							
13864	WTDVGMSPRIES	Pro-epidermal growth factor	620	631	P01132	1376,73	37,67	0,002921538	1	96,82	0,09	10,07	0,104007436	down-regulated in db/db							
14373	AGPpGPAGPAGPpGPIG	Collagen alpha-1(I) chain	827	843	P11087	1397,73	48,13	0,01066665	1	809,54	1	1768,29	2,184314549	up-regulated in db/db							
14420	PGAKGEpGDTGVKGD	Collagen alpha-1(I) chain	812	826	P11087	1399,71	33,42	0,00193307	1	3061,85	1	4985,28	1,628192106	up-regulated in db/db							
14770	GQPGAKGEpGDTGVK	Collagen alpha-1(I) chain	810	824	P11087	1412,74	31,8	0,046851511	1	2239,14	1	1880,29	0,839737578	down-regulated in db/db							
14858	GpRGLpGPpGAPGpQ	Collagen alpha-1(I) chain	174	188	P11087	1417,73	39,72	0,046000405	0,75	400,45	0,55	128,67	0,321313522	down-regulated in db/db							
14924	GPpGPTGPTGPpGFPG	Collagen alpha-1(I) chain	321	336	P11087	1420,69	48,02	0,004667295	0,75	521,12	0	0	0	down-regulated in db/db							
15221	GEVGPpGPpGPAGEKG	Collagen alpha-1(I) chain	909	924	P11087	1433,72	38,81	0,035804475	0,75	702,07	0,91	1537,19	2,189511017	up-regulated in db/db							
15293	GpPGPTGPTGPpGFpG	Collagen alpha-1(I) chain	321	336	P11087	1436,7	48,08	0,001509036	1	3355,44	1	722	0,215172973	down-regulated in db/db							
15530	GRpGEVGPpGPpGPAG	Collagen alpha-1(I) chain	906	921	P11087	1445,74	39,5	0,006554586	0,5	672,46	1	2168,98	3,225440918	up-regulated in db/db							
16118	ESGSPGENGSpGPMGP	Collagen alpha-1(II) chain	310	325	P28481	1471,67	48,22	0,03034805	0,75	245,51	0,18	31,66	0,128956051	down-regulated in db/db							
16128	VSINKELQNSIID	Kidney androgen-regulated protein	25	37	P61110	1471,82	39,58	0,004309984	1	26067,23	0,55	675,95	0,025931025	down-regulated in db/db							
16332	GPpGEAGKpGEQGVpG	Collagen alpha-1(I) chain	639	654	P11087	1480,71	39,19	0,004487973	1	1216,77	0,73	590,98	0,485695735	down-regulated in db/db							
16425	SINKELQNSIIDL	Kidney androgen-regulated protein	26	38	P61110	1485,84	39,49	0,002348934	1	5638,18	0,18	241,96	0,042914558	down-regulated in db/db							
16668	QQVEASLQPETLR	Serine protease inhibitor A3K	284	296	P07759	1497,81	37,83	0,030028334	1	74,58	0,27	21,15	0,283588093	down-regulated in db/db							
16733	GPpGPpGPpGpPGVSGGG	Collagen alpha-2(I) chain	1096	1113	Q01149	1500,73	48,28	0,01066665	1	2831,95	1	1854,09	0,654704356	down-regulated in db/db							
16741	AHLINEKDGETFQ	Major urinary protein 1	121	133	P11588	1500,77	32,08	0,006274818	1	383,34	1	897,59	2,341498409	up-regulated in db/db							
17232	KpGEQGVpGDLGApGP	Collagen alpha-1(I) chain	646	661	P11087	1522,78	40,4	0,031508511	1	2912,19	0,91	3798,09	1,304204053	up-regulated in db/db							
17342	DGQPGAKGEpGDTGVK	Collagen alpha-1(I) chain	809	824	P11087	1527,78	33,82	0,046851511	1	702,02	1	1094,05	1,558431384	up-regulated in db/db							
17350	ALAHFDGILGLGFPT	Napsin-A	174	188	O09043	1527,82	38,48	0,046851511	1	1590,89	0,91	394,29	0,247842403	down-regulated in db/db							
17924	RDGApGAKGDRGETGP	Collagen alpha-1(I) chain	1015	1030	P11087	1555,8	28,13	0,021057571	1	1009,83	1	2316,36	2,29381183	up-regulated in db/db							
18066	SpGSPGPDGKTGPPGpAG	Collagen alpha-1(I) chain	532	549	P11087	1563,79	40,57	0,002348934	0,13	4,18	1	1069,27	255,8062201	up-regulated in db/db							
18174	GQPGAKGEPGDTGVKGD	Collagen alpha-1(I) chain	810	826	P11087	1568,8	33,54	0,001509036	1	1062,57	1	2485,05	2,338716508	up-regulated in db/db							
18466	VSINKELQNSIIDL	Kidney androgen-regulated protein	25	38	P61110	1584,92	40,18	0,00193307	1	66953,46	1	6520,21	0,097384213	down-regulated in db/db							
18821	GIpGEKGPpGERGGPGp	Collagen alpha-1(III) chain	497	513	P08121	1605,82	32,95	0,01066665	1	2130	1	3514,86	1,650169014	up-regulated in db/db							
18875	TGSpGSpGPDGKTGPpGP	Collagen alpha-1(I) chain	530	547	P11087	1609,79	40,88	0,003271116	1	2758,37	1	4373,74	1,585624844	up-regulated in db/db							
19279	GApGpSGFQGLPGPPGPp	Collagen alpha-1(III) chain	657	674	P28481	1633,72	48,23	0,001509036	1	331,23	0	0	0	down-regulated in db/db							
19345	GSpGSpGPDGKTGPpGPAG	Collagen alpha-1(I) chain	531	549	P11087	1636,83	40,73	0,006274818	1	3894,08	1	5988,93	1,537957618	up-regulated in db/db							
19556	ATLNKTGKAVSYLGPK	Copper transport protein ATOX1	53	68	O08997	1646,9	28,02	0,001509036	1	154,05	0	0	0	down-regulated in db/db							
19559	GQAGPpGppGLpGRTGAP	Collagen alpha-1(VII) chain	1267	1284	Q63870	1646,84	40,3	0,004487973	1	8357,77	0,73	825,11	0,098723703	down-regulated in db/db							
19701	SGVGGmGGYPGPAGpPGPp	Collagen alpha-1(III) chain	163	181	P08121	1655,75	48,34	0,004973959	1	1191,2	0,45	188,59	0,158319342	down-regulated in db/db							
19814	GLpGPAGPpGEAGKpGEQ	Collagen alpha-1(I) chain	633	650	P11087	1662,83	41	0,031508511	1	4052,25	1	5189,94	1,280755136	up-regulated in db/db							
19837	GpPGPTGPTGpPGFpGAVG	Collagen alpha-1(I) chain	321	339	P11087	1663,79	48,38	0,002962923	1	769,6	0,36	118,25	0,153651247	down-regulated in db/db							
19995	DpGFVGpEGLAGEpGpp	Collagen alpha-1(XVI) chain	436	452	Q8BLX7	1671,65	48,03	0,004487973	0,75	1030,59	1	2987,63	2,898951086	up-regulated in db/db							
20402	DGKTGPpGPAGQDGRPGp	Collagen alpha-1(I) chain	539	556	P11087	1691,85	35,18	0,001509036	1	340,67	1	1320,92	3,877418029	up-regulated in db/db							
20503	VSINKELQNSIIDLL	Kidney androgen-regulated protein	25	39	P61110	1697,99	40,83	0,003593092	1	19694,1	0,82	2691,01	0,136640415	down-regulated in db/db							
20534	DGQpGAKGEPGDTGVKGD	Collagen alpha-1(I) chain	809	826	P11087	1699,82	35,9	0,014560573	1	685,41	0,82	355,45	0,51859471	down-regulated in db/db							
20552	EDGKDGSpGEpGANGLpG	Collagen alpha-1(III) chain	462	479	P08121	1700,78	41,84	0,014560573	1	616,26	0,82	352,8	0,572485639	down-regulated in db/db							
20580	pGEPGQTGPAGPRGPAGSP	Collagen alpha-2(I) chain	126	144	Q01149	1701,86	38,55	0,002348934	1	283	0,27	29,17	0,103074205	down-regulated in db/db							
20728	GmPGSpGpGNDGKpGpPG	Collagen alpha-1(III) chain	536	554	P08121	1711,79	40,67	0,030021618	0,5	976,2	1	3138,62	3,21514034	up-regulated in db/db							
20796	DGQpGAKGEpGDTGVKGD	Collagen alpha-1(I) chain	809	826	P11087	1715,82	35,95	0,014505996	1	921,43	0,73	436,77	0,474013219	down-regulated in db/db	0,030751954	0,73	436,77	0	0	0	down regulated in ACEi
21257	TGSpGSpGPDGKTGpPGPAG	Collagen alpha-1(I) chain	530	549	P11087	1737,84	41,61	0,046851511	0,88	3616,54	0,91	5530,04	1,529096872	up-regulated in db/db							
21347	SGNFIDQTRVLNLGPI	Uromodulin	590	605	Q91X17	1742,91	41,19	0,046851511	0,13	120,61	0,73	764,51	6,338694967	up-regulated in db/db							
21455	pTGARGAPGDRGEAGPPGp	Collagen alpha-1(I) chain	781	799	P11087	1747,81	36,04	0,031508511	0,88	76,87	0,18	27,91	0,363080526	down-regulated in db/db							
21480	KNGETGPQGPpGPTGPAGD	Collagen alpha-1(III) chain	609	627	P08121	1748,85	42,07	0,008235741	1	394,81	0,64	173,96	0,440617006	down-regulated in db/db							
21517	LSAPEPYKRWYDVQ	Complement factor D	86	99	P03953	1750,85	33,74								0,03637941	0,91	893,67	0,56	268,98	0,300983585	down regulated in ACEi
21729	GpPGEPGPpGPPGpPGHLT	Collagen alpha-2(V) chain	1210	1228	Q3U962	1761,89	40,36	0,022100272	0,88	406,46	0,18	199,06	0,489740688	down-regulated in db/db							
21733	TTGEVGKpGERGLpGEF	Collagen alpha-2(I) chain	569	585	Q01149	1761,9	33,73	0,006274818	1	1026,22	0,91	1836,43	1,789509072	up-regulated in db/db							
21810	EDHLWVSDWAIPSVI	Pro-epidermal growth factor	700	714	P01132	1765,89	41,6	0,03034805	0,75	214,86	0,09	43,72	0,203481337	down-regulated in db/db							
21893	DGQPGAKGEpGDTGVKGDA	Collagen alpha-1(I) chain	809	827	P11087	1770,89	35,47	0,021057571	1	679,12	1	920,15	1,354915184	up-regulated in db/db							
22065	YLNRDSEGSDGGELVLG	Napsin-A	215	231	O09043	1779,88	41,85	0,001509036	1	490,82	1	1687,45	3,438022085	up-regulated in db/db							
22220	DGQpGAKGEpGDTGVKGDA	Collagen alpha-1(I) chain	809	827	P11087	1786,86	35,6	0,00193307	1	932	1	1636,7	1,75611588	up-regulated in db/db							
22305	IGAHGEDWDPAYVIHL	Vacuolar protein sorting-associated protein 13C	2646	2661	Q8BX70	1791,9	35	0,00842438	0,75	231,9	0,09	10,08	0,043467012	down-regulated in db/db							
22694	SpGRDGApGAKGDRGETGP	Collagen alpha-1(I) chain	1012	1030	P11087	1812,91	29,3	0,043022835	1	203,26	0,82	419,34	2,063071928	up-regulated in db/db							
22729	pGpAGpKGETGEmGLSGLP	Collagen alpha-1(XXIII) chain	357	375	Q8K4G2	1814,9	41,54	0,021057571	1	1394,75	1	937,78	0,672364223	down-regulated in db/db							
22880	VGEKGPSGEpGTAGApGTAGP	Collagen alpha-2(I) chain	849	869	Q01149	1824,89	42,14	0,004786026	0,88	563,1	1	1687,86	2,997442728	up-regulated in db/db							
23042	GIpGTGGPpGENGKpGEpGP	Collagen alpha-1(III) chain	641	660	P08121	1834,89	41,58	0,001509036	1	2510,82	1	5195,54	2,069260242	up-regulated in db/db							
23356	IELSKASDYVDRNVEL	ATP-binding cassette sub-family A member 13	1292	1307	Q5SSE9	1849,93	34,64	0,002358894	0,75	442,18	1	3655,45	8,266882265	up-regulated in db/db							
23484	ADGQpGAKGEpGDTGVKGDA	Collagen alpha-1(I) chain	808	827	P11087	1857,92	35,06	0,038867999	1	291,29	0,91	671,06	2,303752274	up-regulated in db/db							
23764	SSLKHPSNIAVDPIERL	Pro-epidermal growth factor	162	178	P01132	1875,07	29,58	0,001509036	1	346,52	0	0	0	down-regulated in db/db							
23770	GPIGPpGPAGQpGDKGEGGSp	Collagen alpha-1(III) chain	764	784	P08121	1875,92	42,43	0,014505996	1	1102,74	0,73	600,28	0,544353157	down-regulated in db/db							
24239	ETGPAGRpGEVGPpGPpGPAG	Collagen alpha-1(I) chain	901	921	P11087	1900,94	42,53	0,01324474	0,88	876,13	1	2061,45	2,352904249	up-regulated in db/db							
24713	ALDYDPVESKIYFAQTA	Pro-epidermal growth factor	522	538	P01132	1929,97	42,48	0,002348934	0,88	910,69	0	0	0	down-regulated in db/db							
24883	EESSSMERNFNVEQISG	Major urinary protein 3	23	39	P04939	1941,95	42,94	0,002348934	1	320,25	0,18	16,14	0,050398126	down-regulated in db/db							
24907	RpGEVGPpGPpGPAGEKGSPG	Collagen alpha-1(I) chain	907	927	P11087	1943,98	35,51	0,038867999	1	1272,48	0,91	724,78	0,569580661	down-regulated in db/db							
25110	VVPHPGSRPDSLEDDLIL	Complement factor D	101	118	P03953	1958,05	35,31	0,039008995	0,88	612,86	0,36	103,96	0,169630911	down-regulated in db/db							
25579	LSSLKHPSNIAVDPIERL	Pro-epidermal growth factor	161	178	P01132	1988,15	30,27	0,001509036	1	183,12	0	0	0	down-regulated in db/db							
25611	ApGApGPVGPAGKNGDRGETGP	Collagen alpha-1(I) chain	1039	1060	P11087	1990,01	35,87	0,031508511	1	1087,81	1	1877,48	1,725926403	up-regulated in db/db							
25859	SSLKHPSNIAVDPIERLM	Pro-epidermal growth factor	162	179	P01132	2006,1	30,24	0,004667295	0,75	156,44	0	0	0	down-regulated in db/db							
26103	EDHLWVSDWAIPSVIRV	Pro-epidermal growth factor	700	716	P01132	2021,01	35,74	0,004786026	1	677,13	1	1275,21	1,883257277	up-regulated in db/db							
26537	KNGETGPQGPpGPTGPAGDKGD	Collagen alpha-1(III) chain	609	630	P08121	2049,01	36,9	0,00226331	1	377,91	0,18	39,03	0,103278558	down-regulated in db/db							
26581	WTDQDSKDSTYSMSSTLT	Ig kappa chain C region	55	72	P01837	2051,92	43,31	0,004667295	0,75	65,31	0	0	0	down-regulated in db/db							
27007	SQGKSISRIDPDGTNHQQL	Pro-epidermal growth factor	58	76	P01132	2080,08	30,51	0,001509036	1	719,5	0	0	0	down-regulated in db/db							
27161	GQpGAKGEpGDTGVKGDAGPpGP	Collagen alpha-1(I) chain	810	832	P11087	2093,01	36,72	0,031508511	1	730,82	1	1319,69	1,805766126	up-regulated in db/db							
27354	mSKNMLETSPQSLPQTPTT	Forkhead box protein P2	430	448	P58463	2106,02	42,1	0,002348934	0,88	236,81	0	0	0	down-regulated in db/db							
27944	EEHTQSPIFVGKVVDPTHK	Alpha-1-antitrypsin 1-4	395	413	Q00897	2147,13	28,35	0,007084217	0,88	406,7	0,09	46,41	0,114113597	down-regulated in db/db							
28103	NGIpGEKGPpGERGGpGPAGPRG	Collagen alpha-1(III) chain	496	518	P08121	2158,1	30,75	0,00193307	1	326,36	0,09	10,84	0,033214855	down-regulated in db/db							
28963	GPIGPpGPAGQpGDKGEGGSpGLpG	Collagen alpha-1(III) chain	764	788	P08121	2216,07	43,88	0,016775791	1	637,26	0,91	341,08	0,535228949	down-regulated in db/db							
29213	IASSNLLEPSGITIDYLTDTL	Pro-epidermal growth factor	642	662	P01132	2235,18	50,34	0,004667295	0,75	415,91	0	0	0	down-regulated in db/db							
30714	SGTTGEVGKpGERGLpGEFGLpGP	Collagen alpha-2(I) chain	567	590	Q01149	2343,17	37,04	0,025952657	1	1463,62	1	2096,59	1,43246881	up-regulated in db/db							
31518	WTDVGMSPRIESASLQGSDRVL	Pro-epidermal growth factor	620	641	P01132	2403,22	37,53	0,001509036	1	929,76	0	0	0	down-regulated in db/db							
32253	FQSPTYTQPHQGPSVMLSTEAF	Uromodulin	464	485	Q91X17	2452,17	44,6	0,004667295	0,75	37,81	0	0	0	down-regulated in db/db							
34667	EEASSTGRNFNVEKINGEWHTII	Major urinary protein 1	19	41	P11588	2630,31	32,52	0,001509036	1	1368,32	0	0	0	down-regulated in db/db							
35125	REGSpGAEGSpGRDGApGAKGDRGETGP	Collagen alpha-1(I) chain	1003	1030	P11087	2669,26	29,68	0,009219572	0,88	684,2	0,91	2804,12	4,098392283	up-regulated in db/db							
40511	EDVQETDTSQKDQSPASHEIATNLGDFAIS	Alpha-1-antitrypsin 1-1	25	54	P07758	3232,46	41,87	0,017898821	0,88	1264,04	0,18	222,84	0,176291889	down-regulated in db/db							

1502027	SNIAVDPIERL	Pro-epidermal growth factor	168	178	P01132	1225,72	39,95	0,002348934	1	3806,75	0,18	88,1	0,023143101	down-regulated in db/db							
1502180	LKWIERANMD	Pro-epidermal growth factor	539	548	P01132	1274,63	29,99	0,00193307	1	954,92	1	2645,97	2,77088133	up-regulated in db/db							
1504057	KNGDDGEAGKpGRpGERGPPGp	Collagen alpha-1(I) chain	217	238	P11087	2192,09	27,74	0,001509036	1	577,86	1	1322,75	2,289049251	up-regulated in db/db	0,030751954	1	1322,75	0,89	788,79	0,596325836	down regulated in ACEi

reversed by ACEi treatment

Supplementary Table 3: Sequences of the 51 identical peptides in the two T2D nephropathy mouse strains.

Peptide_ID	Sequence	Protein name	Adjusted pValue BTBRob/ob/BTBR	FOLD CHANGE BTBRob/ob/BTBR	DIRECTION BTBRob/ob/BTBR	Adjusted pValue db/db / db/m	FOLD CHANGE db/db / db/m	DIRECTION db/db / db/m
1127	ApGERGPpG	Collagen alpha-1(III) chain	0,004385248	0,067946968	down-regulated in ob/ob	0,01324474	0,347291827	down-regulated in db/db
3062	GQpGEKGPpG	Collagen alpha-1(III) chain	0,007164724	0,068955079	down-regulated in ob/ob	0,00803187	0,126335911	down-regulated in db/db
3144	GPpGESGREG	Collagen alpha-1(I) chain	0,003740306	0	down-regulated in ob/ob	0,04685151	0,431706687	down-regulated in db/db
4520	ASLQPETLR	Serine protease inhibitor A3K	0,004385248	0	down-regulated in ob/ob	0,0046673	0	down-regulated in db/db
4882	GQPGAKGEpGD	Collagen alpha-1(I) chain	0,017010274	19,1167019	up-regulated in ob/ob	0,00627482	2,137709905	up-regulated in db/db
5204	SpGPDGKTGPp	Collagen alpha-1(I) chain	0,009919353	0,286652113	down-regulated in ob/ob	0,00478603	0,488928177	down-regulated in db/db
5677	ApGDRGEAGPp	Collagen alpha-1(I) chain	0,002949087	0,355568396	down-regulated in ob/ob	0,01032199	0,447450809	down-regulated in db/db
5886	GPpGPTGPTGPp	Collagen alpha-1(I) chain	0,005037542	0,068343455	down-regulated in ob/ob	0,01095537	0,24759554	down-regulated in db/db
7020	DQTRVLNLGP	Uromodulin	0,00768409	6,595290932	up-regulated in ob/ob	0,01212327	3,772924501	up-regulated in db/db
7064	GpPGSpGSpGEQ	Collagen alpha-1(I) chain	0,004344693	0,015971149	down-regulated in ob/ob	0,03580448	0,554707921	down-regulated in db/db
7194	GPpGPTGPTGPpG	Collagen alpha-1(I) chain	0,014205913	0,116544308	down-regulated in ob/ob	0,00234893	0,12	down-regulated in db/db
7213	GpGERGEHGPp	Collagen alpha-1(III) chain	0,023549868	0,347919643	down-regulated in ob/ob	0,00627482	0,632485158	down-regulated in db/db
7366	FLEQIHVLE	Major urinary protein 1	0,004385248	0,045690793	down-regulated in ob/ob	0,00842438	0,010799481	down-regulated in db/db
7814	DGApGAKGDRGE	Collagen alpha-1(I) chain	0,031121543	0,653932776	down-regulated in ob/ob	0,00446696	0,651631784	down-regulated in db/db
8738	ApGEKGEGGPpGP	Collagen alpha-1(III) chain	0,012479191	0,493380476	down-regulated in ob/ob	0,00150904	0,526555583	down-regulated in db/db
9139	SpGQRGEpGPQG	Collagen alpha-1(III) chain	0,014018563	0,550282216	down-regulated in ob/ob	0,00150904	0,54523033	down-regulated in db/db
9523	GPpGESGREGSpG	Collagen alpha-1(I) chain	0,004385248	0,6529115	down-regulated in ob/ob	0,038868	1,882634368	up-regulated in db/db
10290	VSINKELQNSI	Kidney androgen-regulated protein	0,005541668	0,075043906	down-regulated in ob/ob	0,00842438	0,205824815	down-regulated in db/db
10734	RpGEVGPpGPpGP	Collagen alpha-1(I) chain	0,003485285	1,767827506	up-regulated in ob/ob	0,00446696	1,666494353	up-regulated in db/db
10940	DGKTGPpGPAGQDG	Collagen alpha-1(I) chain	0,013313568	0,227362675	down-regulated in ob/ob	0,00226331	0,081662033	down-regulated in db/db
11018	GpPGPpGPpGPPSGG	Collagen alpha-1(I) chain	0,003740306	0	down-regulated in ob/ob	0,00446696	0,044921875	down-regulated in db/db
12726	GGpGERGEHGPpGP	Collagen alpha-1(III) chain	0,014018563	3,416227899	up-regulated in ob/ob	0,04685151	1,498340417	up-regulated in db/db
12934	KLGVGSLQELAPQ	Sperm flagellar protein 1	0,007501881	0	down-regulated in ob/ob	0,00265809	0,026862169	down-regulated in db/db
13399	NGDDGEAGKpGRpG	Collagen alpha-1(I) chain	0,012479191	0,568244322	down-regulated in ob/ob	0,01066665	1,941766876	up-regulated in db/db
13864	WTDVGMSPRIES	Pro-epidermal growth factor	0,012142689	0,0572305	down-regulated in ob/ob	0,00292154	0,104007436	down-regulated in db/db
14770	GQPGAKGEpGDTGVK	Collagen alpha-1(I) chain	0,005095706	0,576090512	down-regulated in ob/ob	0,04685151	0,839737578	down-regulated in db/db
15293	GpPGPTGPTGPpGFpG	Collagen alpha-1(I) chain	0,004385248	0,111575856	down-regulated in ob/ob	0,00150904	0,215172973	down-regulated in db/db
16425	SINKELQNSIIDL	Kidney androgen-regulated protein	0,004344693	0,00043387	down-regulated in ob/ob	0,00234893	0,042914558	down-regulated in db/db
16733	GPpGPpGPpGpPGVSGGG	Collagen alpha-2(I) chain	0,012142689	0,186853199	down-regulated in ob/ob	0,01066665	0,654704356	down-regulated in db/db
16741	AHLINEKDGETFQ	Major urinary protein 1	0,019906338	1,865123623	up-regulated in ob/ob	0,00627482	2,341498409	up-regulated in db/db
17232	KpGEQGVpGDLGApGP	Collagen alpha-1(I) chain	0,007501881	1,77331812	up-regulated in ob/ob	0,03150851	1,304204053	up-regulated in db/db
17342	DGQPGAKGEpGDTGVK	Collagen alpha-1(I) chain	0,010033808	0,581216252	down-regulated in ob/ob	0,04685151	1,558431384	up-regulated in db/db
18066	SpGSPGPDGKTGPPGpAG	Collagen alpha-1(I) chain	0,012479191	2,436260312	up-regulated in ob/ob	0,00234893	255,8062201	up-regulated in db/db
18466	VSINKELQNSIIDL	Kidney androgen-regulated protein	0,005603303	0,011215296	down-regulated in ob/ob	0,00193307	0,097384213	down-regulated in db/db

19345	GSpGSpGPDGKTGPpGPAG	Collagen alpha-1(I) chain	0,012479191	1,595414073	up-regulated in ob/ob	0,00627482	1,537957618	up-regulated in db/db
19814	GLpGPAGPpGEAGKpGEQ	Collagen alpha-1(I) chain	0,002949087	1,894155813	up-regulated in ob/ob	0,03150851	1,280755136	up-regulated in db/db
20503	VSINKELQNSIIDLL	Kidney androgen-regulated protein	0,003740306	0	down-regulated in ob/ob	0,00359309	0,136640415	down-regulated in db/db
21455	pTGARGAPGDRGEAGPPGp	Collagen alpha-1(I) chain	0,04691826	0,278216144	down-regulated in ob/ob	0,03150851	0,363080526	down-regulated in db/db
21893	DGQPGAKGEpGDTGVKGDA	Collagen alpha-1(I) chain	0,003485285	2,299822236	up-regulated in ob/ob	0,02105757	1,354915184	up-regulated in db/db
22065	YLNRDSEGSDGGELVLG	Napsin-A	0,003740306	1,892375657	up-regulated in ob/ob	0,00150904	3,438022085	up-regulated in db/db
22220	DGQpGAKGEpGDTGVKGDA	Collagen alpha-1(I) chain	0,004385248	1,658928402	up-regulated in ob/ob	0,00193307	1,75611588	up-regulated in db/db
22880	VGEKGPSGEpGTAGApGTAGP	Collagen alpha-2(I) chain	0,02550148	2,14178134	up-regulated in ob/ob	0,00478603	2,997442728	up-regulated in db/db
23042	GIpGTGGPpGENGKpGEpGP	Collagen alpha-1(III) chain	0,012479191	1,794985985	up-regulated in ob/ob	0,00150904	2,069260242	up-regulated in db/db
23356	IELSKASDYVDRNVEL	ATP-binding cassette sub-family A member	0,012479191	5,4412103	up-regulated in ob/ob	0,00235889	8,266882265	up-regulated in db/db
23770	GPIGPpGPAGQpGDKGEGGSp	Collagen alpha-1(III) chain	0,007164724	0,256758155	down-regulated in ob/ob	0,014506	0,544353157	down-regulated in db/db
24239	ETGPAGRpGEVGPpGPpGPAG	Collagen alpha-1(I) chain	0,003740306	2,447651697	up-regulated in ob/ob	0,01324474	2,352904249	up-regulated in db/db
24713	ALDYDPVESKIYFAQTA	Pro-epidermal growth factor	0,005603303	0,00297948	down-regulated in ob/ob	0,00234893	0	down-regulated in db/db
25110	VVPHPGSRPDSLEDDLIL	Complement factor D	0,004385248	0,001240732	down-regulated in ob/ob	0,039009	0,169630911	down-regulated in db/db
26537	KNGETGPQGPpGPTGPAGDKGD	Collagen alpha-1(III) chain	0,007164724	0,052497952	down-regulated in ob/ob	0,00226331	0,103278558	down-regulated in db/db
30714	SGTTGEVGKpGERGLpGEFGLpGP	Collagen alpha-2(I) chain	0,039808966	2,138129263	up-regulated in ob/ob	0,02595266	1,43246881	up-regulated in db/db
35125	REGSpGAEGSpGRDGApGAKGDRGETGP	Collagen alpha-1(I) chain	0,048043989	1,714501999	up-regulated in ob/ob	0,00921957	4,098392283	up-regulated in db/db

Supplementary Table 4: Overlap between peptides responding to RASi in two different T2D mouse strains. RASi treatment modified the abundance of 4 identical peptides (out of 11 and 18 peptides for the BTBRob/ob and UNxdb/db strains, respectively) that were all changed in the same direction in the two T2D strains. RASi, enalapril.

PeptideID	Sequence	Protein name	Start	Stop	Uniprot	Adjusted pValue	Fold change BTBRob/ob+RASi	Adjusted pValue	Fold change Unxdb/db+RASi	Regulation by RASi
2465	QVSPGAKmP	Transcription factor SOX-6	378	386	P40645	0,025	130,23	0,007	23,75	Up
5677	ApGDRGEAGPp	Collagen alpha-1(I) chain	787	797	P11087	0,038	0,05	0,018	Absent in UNxdb/db+RASi	Down
10361	KGEpGDTGVKGDA	Collagen alpha-1(I) chain	815	827	P11087	0,019	Absent in BTBRob/ob+RASi	0,007	Absent in UNxdb/db+RASi	Down
12099	LGPYVAVFDRGD	Napsin-A	373	384	O09043	0,022	1521,42	0,007	1405,12	Up

Supplementary Table 5: Similarity of urinary peptides associated to mouse and human T2D nephropathy and peptides used in the SVM model.

Peptide_ID	Sequence	Protein name	Start	Stop	Uniprot	Masse	CE-Time	Adjusted pValue	Freq Non diab.	mean Amp. Non diab.	Freq DN	mean Amp. DN	FOLD CHANGE DN/Non diab	DIRECTION	Adjusted pValue	Freq DN	mean Amp. DN	Freq DN+RASi	mean Amp. DN+RASi	FOLD CHANGE DN+RASi/DN	DIRECTION	Ortholog	CKD273 ID	Human sequence	Human protein name	Human peptide start	Human peptide stop	Human uniprot	DIRECTION	Mouse peptide
1127	ApGERGPpG	Collagen alpha-1(III) chain	678	686	P08121	868,45	33,7	0,003	1	301,35	0,4	49,89	0,17	Down in Diab.		0,4	49,89	0,25	40,71			C Term	84440	KGDAGApGApGpGGKGDAGApGERGPpG	Collagen alpha-1 (III) chain	662	687	P02461	Down in patients	Present in model
4497	SpGQRGEpGP	Collagen alpha-1(III) chain	363	372	P08121	1012,5	35,68								0,02714521			1	1204,24	14,80	Up in Acei	C Term	55523	SpGSNGApGQRGEpGPQG	Collagen alpha-1 (III) chain	358	375	P02461	Down in patients	Present in model
5599	ApGKDGPpGPAG	Collagen alpha-1(III) chain	897	908	P08121	1051,54	36,38								0,04554068			0,38	255,56	0,19	Down in Acei	C Term	76415	SNGNpGpPGPSGPGKDGPpGpAG	Collagen alpha-1 (III) chain	886	909	P02461	Down in patients	Present in model
9139	SpGQRGEpGPQG	Collagen alpha-1(III) chain	363	374	P08121	1197,58	37,06	0,015	0,9	1796,45	0,9	937,73	0,52	Down in Diab.		0,9	937,73	0,88	938,65			C Term	55523	SpGSNGApGQRGEpGPQG	Collagen alpha-1 (III) chain	358	375	P02461	Down in patients	Present in model
19345	GSpGSpGPDGKTGPpGPAG	Collagen alpha-1(I) chain	531	549	P01087	1636,83	40,73	0,022	1	3801,59	0,9	6393,43	1,68	Up in Diab		0,9	6393,43	1	8346,86			C Term	48106	SpGSpGPDGKTGPPGpAG	Collagen alpha-1 (I) chain	543	560	P02452	Up in patients	Present in model
3062	GDpGEKGPpG	Collagen alpha-1(III) chain	926	935	P08121	954,47	34,61	0,004	1	175,65	0,3	22,11	0,13	Down in Diab.		0,3	22,11	0,63	727,98			Cluster	133849	NTGApGSpGVSGPKGDAGQPGEkGSPGAQGPpGApGpLG	Collagen alpha-1 (III) chain	910	948	P02461	Down in patients	Present in model
8738	ApGEKGEGGPpGP	Collagen alpha-1(III) chain	828	840	P08121	1180,58	37,84	0,014	1	2769,32	1	1321,03	0,48	Down in Diab.		1	1321,03	1	1059,1			Cluster	77184	NGEpGGKGERGApGEKGEGGPpG	Collagen alpha-1 (III) chain	818	840	P02461	Down in patients	Present in model
10361	KGEpGDTGVKGDA	Collagen alpha-1(I) chain	815	827	P11087	1245,62	31,92								0,02666596		0	0	0	0,00	Down in Acei	Cluster	97506	GPpGADGQpPGAKGEpGDAGAKGDAGPpGP	Collagen alpha-1 (I) chain	815	843	P02452	Up in patients	Present in model
10514	ApGEKGEGGPpGPA	Collagen alpha-1(III) chain	828	841	P08121	1251,61	38,23	0,016	1	1661,82	0,7	808,67	0,49	Down in Diab.		0,7	808,67	0,75	626,12			Cluster	87460	GQNGEpGGKGERGApGEKGEGGPpG	Collagen alpha-1 (III) chain	816	840	P02461	Down in patients	Present in model
12554	GPpGPTGPAGDKGDS	Collagen alpha-1(III) chain	617	631	P08121	1324,64	38,85								0,04554068			0,13	22,03	0,05	Down in Acei	Cluster	32343	TGPGGDKGDTGPpGP	Collagen alpha-1 (III) chain	623	637	P02461	Up in patients	Present in model
16857	KpGEQGVPGDLGApGP	Collagen alpha-1(I) chain	646	661	P11087	1506,78	40,49	0,022	0,9	955,06	0,9	3264,15	3,42	Up in Diab		0,9	3264,15	0,88	3730,51			Cluster	58941	GPpGEAGKpGEQGVpGDLG	Collagen alpha-1 (I) chain	650	668	P02452	Up in patients	Present in model
19814	GLpGPAGPpGEAGKpGEQ	Collagen alpha-1(I) chain	633	650	P11087	1662,83	41	0,012	1	3628,89	1	5518,2	1,52	Up in Diab		1	5518,2	0,88	4460,04			Cluster	58941	GPpGEAGKpGEQGVpGDLG	Collagen alpha-1 (I) chain	650	668	P02452	Up in patients	Present in model
21893	DGQPGAKGEpGDTGVKGDA	Collagen alpha-1(I) chain	809	827	P11087	1770,89	35,47	0,005	1	534,46	1	994,8	1,86	Up in Diab		1	994,8	0,88	1037,79			Cluster	80891	ADGQPGAKGEpGDAGAKGDAGPPGp	Collagen alpha-1 (I) chain	819	843	P02452	Up in patients	Present in model
27104	AGRpGEVGPpGPpGPAGEKGSpG	Collagen alpha-1(I) chain	905	927	P11087	2088,05	36,3	0,034	0,9	386,22	0,4	56,83	0,15	Down in Diab.		0,4	56,83	0,38	264,94			Cluster	108724	KEGGKGPRGETGPAGRpGEVGpPGPpGPAG	Collagen alpha-1 (I) chain	903	932	P02452	Down in patients	Present in model
710	SpGRDGApG	Collagen alpha-1(I) chain	1012	1020	P11087	844,43	33,46	0,033	0,8		0,1	44,16	0,32	Down in Diab.		0,1	44,16	0,63	107,29			N Term	39163	SpGRDGSpGAKGDRG	Collagen alpha-1 (I) chain	1023	1037	P02452	Down in patients	Present in model
3438	DGKTGPpGPAG	Collagen alpha-1(I) chain	539	549	P11087	968,51	37,23	0,035	0,8	302,22	0,3	51,98	0,17	Down in Diab.		0,3	51,98	0,38	45,93			N Term	5675	DGKTGPpGPA	Collagen alpha-1 (I) chain	550	559	P02452	Down in patients	Present in model
11018	GpPGPpGPpGPPSGG	Collagen alpha-1(I) chain	1170	1184	P11087	1271,62	47,98	0,003	1	373,15	0	0	0,00	Down in Diab.		0	0	0	0			N Term	20756	GPpGpPGPPGPPS	Collagen alpha-1 (I) chain	1181	1193	P02452	Down in patients	Present in model
22220	DGQpGAKGEpGDTGVKGDA	Collagen alpha-1(I) chain	809	827	P11087	1786,86	35,6	0,005	1	1101,23	1	1925,46	1,75	Up in Diab		1	1925,46	1	2200,93			N Term	77018	DGQPGAKGEpGDAGAKGDAGPPGp	Collagen alpha-1 (I) chain	820	843	P02452	Up in patients	Present in model
26537	KNGETGPQGPpGPTGPAGDKGD	Collagen alpha-1(III) chain	609	630	P08121	2049,01	36,9	0,003	1	410,04	0,1	19,33	0,05	Down in Diab.		0,1	19,33	0,25	38,55			N Term	107460	KNGETGPQGPPGPTGPGGDKGDTGPpGpQG	Collagen alpha-1 (III) chain	610	639	P02461	Down in patients	Present in model
5677	ApGDRGEAGPp	Collagen alpha-1(I) chain	787	797	P11087	1054,52	36,74	0,005	1	705,69	0,8	255,54	0,36	Down in Diab.		0,8	255,54	0,13	7,3			Same	17694	ApGDRGEpGpP	Collagen alpha-1 (I) chain	798	808	P02452	Down in patients	Present in model
23042	GIpGTGGPpGENGKpGEpGP	Collagen alpha-1(III) chain	641	660	P08121	1834,89	41,58	0,003	1	2988,1	1	5594,12	1,87	Up in Diab		1	5594,12	1	7466,49			Same	61304	GLpGTGGPpGENGKPGEPGp	Collagen alpha-1 (III) chain	642	661	P02461	Up in patients	Present in model
21455	pTGARGAPGDRGEAGPPGp	Collagen alpha-1(I) chain	781	799	P11087	1747,81	36,04	0,012	0,9	122,13	0,1	17,81	0,15	Down in Diab.		0,1	17,81	0	0			C Term	15593	GDRGEpGPpGP	Collagen alpha-1 (I) chain	800	810	P02452	Down in patients	
24239	ETGPAGRpGEVGPpGPpGPAG	Collagen alpha-1(I) chain	901	921	P11087	1900,94	42,53	0,014	0,9	1210,1	1	3022,32	2,50	Up in Diab		1	3022,32	0,75	2705,68			C Term	108724	KEGGKGPRGETGPAGRpGEVGpPGPpGPAG	Collagen alpha-1 (I) chain	903	932	P02452	Down in patients	
35125	REGSpGAEGSpGRDGApGAKGDRGETGP	Collagen alpha-1(III) chain	1003	1030	P11087	2669,26	29,68	0,050	0,9	1372,85	0,9	4247,82	3,09	Up in Diab		0,9	4247,82	0,75	3238,81			C Term	70674	EGSpGRDGSpGAKGDRGETGP	Collagen alpha-1 (I) chain	1021	1041	P02452	Up in patients	
1724	GPmGPRGpP	Collagen alpha-1(I) chain	204	212	P11087	896,48	34,05								0,04554068			1	337,27	4,98	Up in Acei	Cluster	14906	MGPRGPpGPpG	Collagen alpha-1 (I) chain	217	227	P02452	Down in patients	
1760	GPpGEAGKpG	Collagen alpha-1(I) chain	639	648	P11087	897,47	33,68	0,005	0,8	152,95	0	0	0,00	Down in Diab.		0	0	0	0			Cluster	78332	AGPpGEAGKpGEQGVPGDLGAPGp	Collagen alpha-1 (I) chain	646	669	P02452	Down in patients	
4882	GQPGAKGEpGD	Collagen alpha-1(I) chain	810	820	P11087	1027,51	36,35								0,04554068			1	1079,67	2,29	Up in Acei	Cluster	101157	GPpGADGQpGAKGEpGDAGAKGDAGPpGPA	Collagen alpha-1 (I) chain	815	844	P02452	Down in patients	
5229	SpGAKGEVGPAG	Collagen alpha-1(III) chain	345	356	P08121	1041,49	37,04	0,003	1	217,76	0,3	41,35	0,19	Down in Diab.		0,3	41,35	0	0			Cluster	132383	ARGNDGARGSDGQPGPpGppGTAGFpGSpGAKGEVGP	Collagen alpha-1 (III) chain	319	355	P02461	Down in patients	
7785	EpGRDGTpGGpG	Collagen alpha-1(I) chain	522	533	P08121	1143,54	37,18	0,032	0,9	776,31	0,6	354,91	0,46	Down in Diab.		0,6	354,91	0,75	511,36			Cluster	124268	GEpGRDGVpGGpGMRGmpGSpGGpGSDGKpGPpG	Collagen alpha-1 (III) chain	522	555	P02461	Down in patients	
10734	RpGEVGPpGPpGP	Collagen alpha-1(I) chain	907	919	P11087	1260,65	38,35	0,005	1	2489,18	1	4463,62	1,79	Up in Diab		1	4463,62	1	4614,38			Cluster	108724	KEGGKGPRGETGPAGRpGEVGpPGPpGPAG	Collagen alpha-1 (I) chain	903	932	P02452	Down in patients	
15427	GPpGPAGPAGPpGPIGN	Collagen alpha-1(I) chain	828	844	P11087	1440,7	48,22	0,032	1	860,37	0,5	348,32	0,40	Down in Diab.		0,5	348,32	0,25	187,89			Cluster	121241	ADGQPGAkGEPGDAGAKGDAGPPGpAGPAGPPGPIG	Collagen alpha-1 (I) chain	819	854	P02452	Down in patients	
19701	SGVGGmGGYPGPAGpPGPp	Collagen alpha-1(III) chain	163	181	P08121	1655,75	48,34	0,003	1	957,99	0,1	12,71	0,01	Down in Diab.		0,1	12,71	0	0			Cluster	19085	GpAGPpGPpGPpG	Collagen alpha-1 (III) chain	174	186	P02461	Down in patients	
26052	GRpGEVGPpGPpGPAGEKGSpG	Collagen alpha-1(I) chain	906	927	P11087	2017,01	35,92	0,005	1	330,9	0,1	29,98	0,09	Down in Diab.		0,1	29,98	0,13	10,29			Cluster	108724	KEGGKGPRGETGPAGRpGEVGpPGPpGPAG	Collagen alpha-1 (I) chain	903	932	P02452	Down in patients	
3144	GPpGESGREG	Collagen alpha-1(I) chain	996	1005	P11087	957,46	34,3	0,005	0,9	61,28	0,1	4,94	0,08	Down in Diab.		0,1	4,94	0	0			N Term	127432	GPpGESGREGAPGAEGSpGRDGSpGAKGDRGETGp	Collagen alpha-1 (I) chain	1007	1041	P02452	Down in patients	
7020	DQTRVLNLGP	Uromodulin	595	604	Q91X17	1111,62	36,1	0,004	0,7	172,48	1	1474,1	8,55	Up in Diab		1	1474,1	0,88	866,01			N Term	41513	DQSRVLNLGPITR	Uromodulin	594	606	P07911	Up in patients	
7213	GpGERGEHGPp	Collagen alpha-1(III) chain	795	805	P08121	1120,55	28,62	0,011	1	675,01	0,5	213,06	0,32	Down in Diab.		0,5	213,06	0	0			N Term	28747	SpGERGETGPpGP	Collagen alpha-1 (III) chain	796	808	P02461	Up in patients	
10940	DGKTGPpGPAGQDG	Collagen alpha-1(I) chain	539	552	P11087	1268,62	40,04	0,005	1	332,14	0,3	55,01	0,17	Down in Diab.		0,3	55,01	0	0			N Term	5675	DGKTGPpGPA	Collagen alpha-1 (I) chain	550	559	P02452	Down in patients	
17585	GPpGPpGppGLGGNFAS	Collagen alpha-1(III) chain	135	151	P11087	1538,73	48,37	0,013	1	4789,87	0,5	1749,62	0,37	Down in Diab.		0,5	1749,62	0,63	1364,48			N Term	3508	GPpGPpGPpG	Collagen alpha-1 (I) chain	145	154	P02452	Down in patients	
5204	SpGPDGKTGPp	Collagen alpha-1(I) chain	535	545	P11087	1040,53	36,08	0,004	1	1199,02	0,8	345,47	0,29	Down in Diab.		0,8	345,47	0,88	437,45			Same	14478	SpGPDGKTGPp	Collagen alpha-1 (I) chain	546	556	P02452	Down in patients	
18066	SpGSPGPDGKTGPPGpAG	Collagen alpha-1(I) chain	532	549	P11087	1563,79	40,57	0,004	0,6	286,16	1	1548,7	5,41	Up in Diab		1	1548,7	0,88	1207,1			Same	48106	SpGSpGPDGKTGPPGpAG	Collagen alpha-1 (I) chain	543	560		Up in patients	
2465	QVSPGAKmP	Transcription factor SOX-6	378	386	P40645	929,47	35,74								0,02590209			1	1342,35	1342,35	Up in Acei	No ortholog								
2806	YRELVHQ	Alpha-1-antitrypsin 1-1	56	62	P07758	943,5	28,34	0,033	0,8	52,47	0,1	114,27	2,18	Up in Diab		0,1	114,27	0	0			No ortholog								
4385	GPpGERGGpGP	Collagen alpha-1(III) chain	503	513	P08121	1008,51	35,45	0,045	1	874,13	0,8	445,26	0,51	Down in Diab.		0,8	445,26	0,75	515,26			No ortholog								
4520	ASLQPETLR	Serine protease inhibitor A3K	288	296	P07759	1013,55	38,53	0,005	0,8	487,11	0	0	0,00	Down in Diab.		0	0	0	0			No ortholog								
4801	IAVDPIERL	Pro-epidermal growth factor	170	178	P01132	1024,52	36,98	0,005	1	421,78	0,5	80,37	0,19	Down in Diab.		0,5	80,37	0,5	88,3			No ortholog								
5807	SpGGKGEMGPAG	Collagen alpha-1(III) chain	390	401	P08121	1059,5	37,01	0,006	1	779,35	0,9	392,63	0,50	Down in Diab.		0,9	392,63	0,88	539,22			No ortholog								
5886	GPpGPTGPTGPp	Collagen alpha-1(I) chain	321	332	P11087	1062,53	47,72	0,003	1	370,72	0,4	20,43	0,06	Down in Diab.		0,4	20,43	0,13	23,88			No ortholog								
6927	KPGppGEpGPQ	Collagen alpha-1(VIII) chain	289	299	Q00780	1107,57	38,4	0,014	1	547,72	0,8	253,32	0,46	Down in Diab.		0,8	253,32	0,63	169,13			No ortholog								
7050	YSLHPDPIAT	Osteopontin	32	41	P10923	1112,63	39,74	0,005	0,9	1070,3	0,1	23,5	0,02	Down in Diab.		0,1	23,5	0,25	58,77			No ortholog								
7064	GpPGSpGSpGEQ	Collagen alpha-1(I) chain	1107	1118	P11087	1113,49	47,49	0,015	1	484,73	0,4	155,97	0,32	Down in Diab.		0,4	155,97	0,13	40,07			No ortholog								
7194	GPpGPTGPTGPpG	Collagen alpha-1(I) chain	321	333	P11087	1119,54	47,64	0,005	1	862,23	0,5	106,65	0,12	Down in Diab.		0,5	106,65	0,5	84,37			No ortholog								
7366	FLEQIHVLE	Major urinary protein 1	59	67	P11588	1126,63	38,99	0,007	0,8	545,85	0,1	5,21	0,01	Down in Diab.		0,1	5,21	0	0			No ortholog								
7489	VSINKELQNS	Kidney androgen-regulated protein	25	34	P61110	1130,63	36,82	0,020	0,9	1397,81	0,5	207,81	0,15	Down in Diab.		0,5	207,81	0,38	326,85			No ortholog								
7719	GLpGAAGERGPSG	Collagen alpha-1(III) chain	476	488	P08121	1140,56	38,23	0,006	1	376,95	0,7	133,98	0,36	Down in Diab.		0,7	133,98	0,63	182,67			No ortholog								
7786	GPpGPLGpPGLPG	Collagen alpha-1(X) chain	1427	1439	Q61245	1143,56	47,71	0,012	1	4131,35	1	1743,9	0,42	Down in Diab.		1	1743,9	1	1505,82			No ortholog								
8188	NIGPpGpKGPSG	Collagen alpha-2(I) chain	497	508	Q01149	1158,55	38,76	0,032	1	323,7	0,5	94,3	0,29	Down in Diab.		0,5	94,3	0,5	61,69			No ortholog								
10085	DpGGRLGpPGLpG	Collagen alpha-1(XIX) chain	556	568	Q1VF58	1235,41	47,31	0,008	0,4	35,94	0,9	1025,82	28,54	Up in Diab		0,9	1025,82	1	1120,73			No ortholog								
10290	VSINKELQNSI	Kidney androgen-regulated protein	25	35	P61110	1243,71	37,9	0,006	1	2594,53	0,4	386,35	0,15	Down in Diab.		0,4	386,35	0,38	383,21			No ortholog								
11919	RLITEGVDTLEG	Pro-epidermal growth factor	554	565	P01132	1301,64	38,91	0,014	0,9	164,6	0,3	37,77	0,23	Down in Diab.		0,3	37,77	0	0			No ortholog								
12099	LGPYVAVFDRGD	Napsin-A	373	384	O09043	1307,69	39,1								0,02590209			1	1632,45	1632,45	Up in Acei	No ortholog								
12174	QILSLTGDRVPL	Nucleoporin Nup43	209	220	P59235	1310,79	40,19	0,003	0,9	192,68	0	0	0,00	Down in Diab.		0	0	0	0			No ortholog								
12934	KLGVGSLQELAPQ	Sperm flagellar protein 1	119	131	Q99JL1	1338,8	39,93	0,004	0,9	2949,01	0,1	48,85	0,02	Down in Diab.		0,1	48,85	0,25	117,12			No ortholog								
13700	QVEASLQPETLR	Serine protease inhibitor A3K	285	296	P07759	1369,74	39,25								0,04496663			0,88	533,13	14,20	Up in Acei	No ortholog								
13864	WTDVGMSPRIES	Pro-epidermal growth factor	620	631	P01132	1376,73	37,67	0,003	1	120,6	0	0	0,00	Down in Diab.		0	0	0,13	7,05			No ortholog								
14400	INKELQNSIIDL	Kidney androgen-regulated protein	27	38	P61110	1398,79	38,95	0,009	0,8	3425,5	0,1	227,44	0,07	Down in Diab.		0,1	227,44	0,25	195,99			No ortholog								
15293	GpPGPTGPTGPpGPpG	Collagen alpha-1(I) chain	321	336	P11087	1436,7	48,08	0,004	1	2355,02	0,8	415,14	0,18	Down in Diab.		0,8	415,14	0,38	243,16			No ortholog								
15539	GPpGERGGpGPAGPRG	Collagen alpha-1(III) chain	503	518	P08121	1446,74	31,55	0,032	0,8	124,11	0,2	27,96	0,23	Down in Diab.		0,2	27,96	0	0			No ortholog								
15855	GRpGPpGPSGPRGQp	Collagen alpha-1(III) chain	560	574	P08121	1460,74	32,22	0,018	0,8	530,74	0,2	61,16	0,12	Down in Diab.		0,2	61,16	0,25	528,92			No ortholog								
16425	SINKELQNSIIDL	Kidney androgen-regulated protein	26	36	P61110	1485,84	39,49	0,003	1	14665,03	0,1	185,59	0,01	Down in Diab.		0,1	185,59	0,13	501,58			No ortholog								
16733	GPpGPpGPpGpPGVSGGG	Collagen alpha-2(I) chain	1096	1113	Q01149	1500,73	48,28	0,016	1	2313,98	0,6	976,35	0,42	Down in Diab.		0,6	976,35	0,5	780,08			No ortholog								
16741	AHLINEKDGETFQ	Major urinary protein 1	121	133	P11588	1500,77	32,08	0,008	1	443,37	1	1086,99	2,45	Up in Diab		1	1086,99	0,88	1253,08			No ortholog								
18466	VSINKELQNSIIDL	Kidney androgen-regulated protein	25	38	P61110	1584,92	40,18	0,007	0,9	36532,78	0,5	2450,16	0,07	Down in Diab.		0,5	2450,16	0,5	4700,94			No ortholog								
18821	GIpGEKGPpGERGGPGp	Collagen alpha-1(III) chain	497	513	P08121	1605,82	32,95	0,040	1	2655,77	0,9	3850,57	1,45	Up in Diab		0,9	3850,57	0,88	4172,82			No ortholog								
19279	GApGpSGFQGLPGPPGPp	Collagen alpha-1(II) chain	657	674	P28481	1633,72	48,23	0,003	0,9	235,96	0	0	0,00	Down in Diab.		0	0	0	0			No ortholog								
19367	APEPYKRWYDVQS	Complement factor D	88	100	P03953	1637,8	32,93	0,005	0,8	459,85	0	0	0,00	Down in Diab.		0	0	0	0			No ortholog								
19559	GQAGPpGpGpGLqGRTGAP	Collagen alpha-1(VII) chain	1267	1284	Q63870	1646,84	40,3	0,008	0,9	5054,6	0,4	482,93	0,10	Down in Diab.		0,4	482,93	0,38	596,92			No ortholog								
20503	VSINKELQNSIIDLL	Kidney androgen-regulated protein	25	39	P61110	1697,99	40,83	0,004	1	30586,5	0,4	1040,98	0,03	Down in Diab.		0,4	1040,98	0,38	1367,12			No ortholog								
20580	pGEPGQTGPAGPRGPAGSP	Collagen alpha-2(I) chain	126	144	Q01149	1701,86	38,55	0,011	0,8	189,32	0,1	16,43	0,09	Down in Diab.		0,1	16,43	0,25	47,02			No ortholog								
22065	YLNRDSEGSDGGELVLG	Napsin-A	215	231	O09043	1779,88	41,85	0,003	1	923,54	1	2338,12	2,53	Up in Diab		1	2338,12	1	2588,15			No ortholog								
23280	MSPRIESASLQGSDRVL	Pro-epidermal growth factor	625	641	P01132	1844,98	34,5	0,006	0,9	2467,45	0,3	47,79	0,02	Down in Diab.		0,3	47,79	0,38	105,09			No ortholog								
23356	IELSKASDYVDRNVEL	ATP-binding cassette sub-family A member 13	1292	1307	Q5SSE9	1849,93	34,64	0,011	0,9	619,55	0,9	4548,52	7,34	Up in Diab		0,9	4548,52	0,88	4364,73			No ortholog								
23764	SSLKHPSNIAVDPIERL	Pro-epidermal growth factor	162	178	P01132	1875,07	29,58	0,003	0,9	386,07	0	0	0,00	Down in Diab.		0	0	0,13	391,8			No ortholog								
23770	GPtGPpGPAGQpGDKGEGGSp	Collagen alpha-1(III) chain	764	784	P08121	1875,92	42,43	0,014	1	1158,81	0,8	581,88	0,50	Down in Diab.		0,8	581,88	0,75	809,16			No ortholog								
24713	ALDYDPVESKIYFAQTA	Pro-epidermal growth factor	522	538	P01132	1929,97	42,48	0,003	0,9	4497,86	0	0	0,00	Down in Diab.		0	0	0	0			No ortholog								
25110	VVPHPGSRPDSLEDDLIL	Complement factor D	101	118	P03953	1958,05	35,31	0,004	1	16798,18	0,2	29,52	0,00	Down in Diab.		0,2	29,52	0,38	154,92			No ortholog								
25579	LSSLKHPSNIAVDPIERL	Pro-epidermal growth factor	161	178	P01132	1988,15	30,27	0,003	0,9	525,59	0	0	0,00	Down in Diab.		0	0	0	0			No ortholog								
27962	GFpGPKGPSGDpGKpGERGHpG	Collagen alpha-2(I) chain	499	520	Q01149	2148,08	28,16	0,034	0,9	311,04	0,4	80,38	0,26	Down in Diab.		0,4	80,38	0,25	37,21			No ortholog								
28103	NGIpGEKGPpGERGGpGPAGPRG	Collagen alpha-1(III) chain	496	518	P08121	2158,1	30,75	0,030	0,8	368,78	0,3	58,02	0,16	Down in Diab.		0,3	58,02	0,5	215,61			No ortholog								
31518	WTDVGMSPRIESASLQGSDRVL	Pro-epidermal growth factor	620	641	P01132	2403,22	37,53	0,003	0,9	1314,01	0	0	0,00	Down in Diab.		0	0	0	0			No ortholog								
32253	FQSPTYTQPHQGPSVMLSTEAF	Uromodulin	464	485	Q91X17	2452,17	44,6	0,025	0,9	267,68	0,2	40,28	0,15	Down in Diab.		0,2	40,28	0,63	3885,79			No ortholog								
1502027	SNIAVDPIERL	Pro-epidermal growth factor	168	178	P01132	1225,72	39,95	0,004	0,9	2002,59	0,1	23,2	0,01	Down in Diab.		0,1	23,2	0,13	25,56			No ortholog								
1503005	ADNLELNAKGAILHA	Meprin A subunit alpha	83	97	P28825	1548,8	32,63	0,040	1	696,44	0,9	1203,06	1,73	Up in Diab		0,9	1203,06	0,88	857,44			No ortholog								

Supplementary Table 6: The 21 differentially excreted peptides identified and used for the generation of the humanized classifier and their respective human ortholog identified in CKD273. Bold: orthology. RASi, enalapril.

Peptide ID	Sequence	Protein name	Regulation Diab/Non diab	Regulation Diab+RASi/Diab	Ortholog CKD273		
					Regulation in patients	CKD273 ID	Human sequence
710	SpGRDGApG	Collagen alpha-1(I) chain	Down		Down	39163	**SpGRDGSpG**AKGDRG
1127	ApGERGPpG	Collagen alpha-1(III) chain	Down		Down	84440	KGDAGApGApGGKGDAG**ApGERGPpG**
3062	GQpGEKGPpG	Collagen alpha-1(III) chain	Down		Down	133849	NTGApGSpGVSGPKGDA**GQPGEkGSPG**AQGPpGApGpLG
3438	DGKTGPpGPAG	Collagen alpha-1(I) chain	Down		Down	5675	**DGKTGPpGPA**
4497	SpGQRGEpGP	Collagen alpha-1(III) chain		Up	Down	55523	SpGSNG**ApGQRGEpGP**QG
5599	ApGKDGPpGPAG	Collagen alpha-1(III) chain		Down	Down	76415	SNGNpGpPGPSG**SPGKDGPpGpAG**
5677	ApGDRGEAGPp	Collagen alpha-1(I) chain	Down		Down	17694	**ApGDRGEpGpP**
8738	ApGEKGEGGPpGP	Collagen alpha-1(III) chain	Down		Down	77184	NGEpGGKGERG**ApGEKGEGGPpG**
9139	SpGQRGEpGPQG	Collagen alpha-1(III) chain	Down		Down	55523	SpGSNG**ApGQRGEpGPQG**
10361	KGEpGDTGVKGDA	Collagen alpha-1(I) chain		Down	Up	97506	GPpGADGQpPGA**KGEpGDAGAKGDA**GpPGP
10514	ApGEKGEGGPpGPA	Collagen alpha-1(III) chain	Down		Down	87460	GQNGEpGGKGERG**ApGEKGEGGPpG**
11018	GpPGPpGPpGPPSGG	Collagen alpha-1(I) chain	Down		Down	20756	**GPpGpPGPPGPPS**
12554	GPpGPTGPAGDKGDS	Collagen alpha-1(III) chain		Down	Up	32343	**TGPGGDKGDT**GPpGP
16857	KpGEQGVPGDLGApGP	Collagen alpha-1(I) chain	Up		Up	58941	GPpGEAG**KpGEQGVpGDLG**
19345	GSpGSpGPDGKTGPpGPAG	Collagen alpha-1(I) chain	Up		Up	48106	**SpGSpGPDGKTGPPGpAG**
19814	GLpGPAGPpGEAGKpGEQ	Collagen alpha-1(I) chain	Up		Up	58941	**GPpGEAGKpGEQ**GVpGDLG
21893	DGQPGAKGEpGDTGVKGDA	Collagen alpha-1(I) chain	Up		Up	80891	A**DGQPGAKGEpGDAGAKGDA**GPPGp
22220	DGQpGAKGEpGDTGVKGDA	Collagen alpha-1(I) chain	Up		Up	77018	**DGQPGAKGEpGDAGAKGDA**GPPGp
23042	GIpGTGGPpGENGKpGEpGP	Collagen alpha-1(III) chain	Up		Up	61304	**GLpGTGGPpGENGKPGEPGp**
26537	KNGETGPQGPpGPTGPAGDKGD	Collagen alpha-1(III) chain	Down		Down	107460	**KNGETGPQGPPGPTGPGGDKGD**TGPpGpQG
27104	AGRpGEVGPpGPpGPAGEKGSpG	Collagen alpha-1(I) chain	Down		Down	108724	KEGGKGPRGETGP**AGRpGEVGpPGPpGPAG**