**List of supplementary data:**

*Label-free study*
*MRM study*

**Supplementary Data 1**: List of peptides and transitions monitored during the MRM verification study

**Supplementary Data 2**: Assessment of the repeatability of the experimental workflow used for the MRM verification study (test1)

**Supplementary Data 3**: Assessment of the repeatability of the experimental workflow used for the MRM verification study (test2)

**Supplementary Data 4**: Signal response curve for EGF (peptide YPANVAVDPVER)

**Supplementary Data 5**: Signal response curve for ARG1 (peptide TIGIIGAPFSK)

**Supplementary Data 6**: Assessment of instrument performances during the MRM verification study using injection of a quality control external standard

**Supplementary Data 7**: Evaluation of the repeatability of the MRM measurements during the verification study using spiked exogenous yeast proteins

**Supplementary Data 8**: Relative quantification of the candidate proteins across clinical samples using MRM data

**Supplementary Data 9:** Variation in LC-MS/MS analyses and improvement of protein coverage.

**Supplementary Data 10**: Chromatographic repeatability of the label-free LC-MS discovery study

**Supplementary Data 11:** Evaluation of the LC-MS random measurements and the inter-individual variation obtained in the label-free quantitative analysis.

**Supplementary Data 12**: Mode of selection of urinary proteins specifically modulated in obstructed kidney

**Supplementary Data 13:** Significant proteins that were assigned as specifically modulated in the contralateral kidney

**Supplementary Data 14**: MRM analysis of urinary EGF concentrations in the verification cohort.

**Supplementary Data 15**: MRM chromatograms for peptide YPANVAVDPVER of EGF acquired across the 31 samples 31 samples of the clinical study (4 sheet)

**Supplementary Data 16**: MRM chromatograms for peptide TIGIIGAPFSK of ARG1 acquired across the 31 samples of the clinical study (4 sheet)

**Supplementary Data 17:** MRM chromatograms for peptide DVDPGEHYILK of ARG1 acquired across the 31 samples of the clinical study (4 sheet)

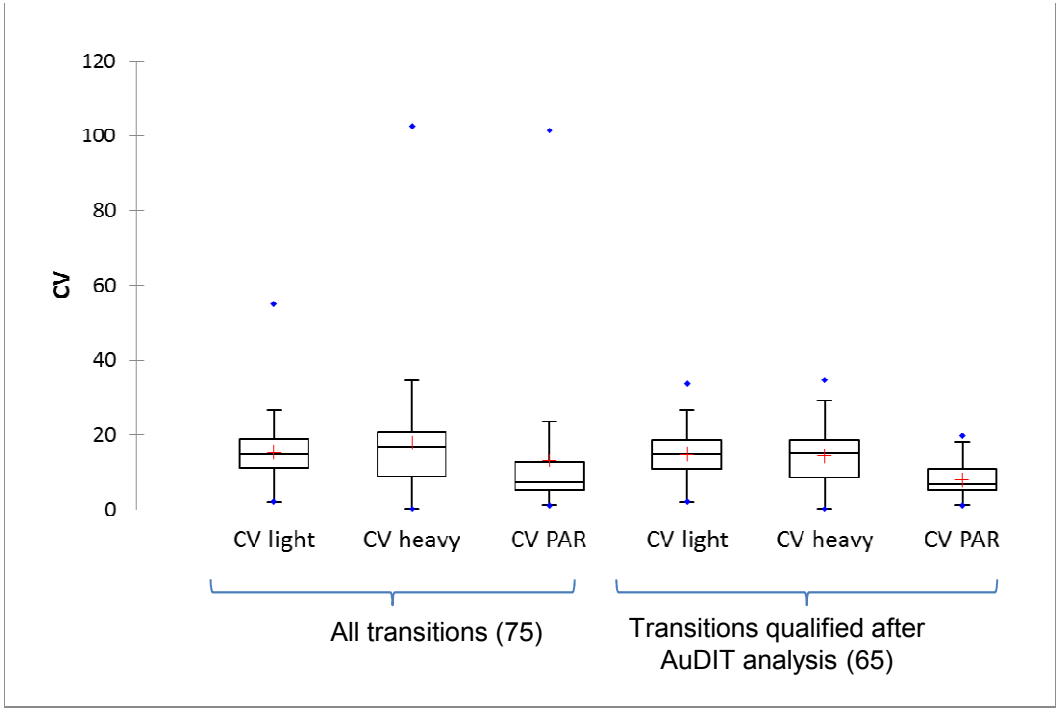
**Supplementary Data 18**: Correlation between the age of the patients and the level of urinary ARG1 found in the discovery and verification studies

| Protein name | Peptide sequence | z(Q1) | Q1 (m/z) | Q3 (m/z) | CE (V) |
|---|---|---|---|---|---|
| **Human targeted proteins** | | | | | |
| ARG1 | TIGIIGAPFSK | 2 | 552.33 | 889.51 / 719.41 / 606.32 | 27.2 / 27.2 / 24.2 |
| | DVDPGEHYILK | 2 | 643.32 | 478.76 / 956.52 / 859.47 | 32.4 / 32.4 / 35.4 |
| EGF | NQVTPLDILSK | 2 | 614.35 | 886.52 / 985.59 / 342.18 | 27.8 / 24.8 / 27.8 |
| | YPANVAVDPVER | 2 | 665.34 | 500.28 / 785.42 / 998.53 | 45.7 / 36.7 / 39.7 |
| | LFWIQYNR | 2 | 570.30 | 879.45 / 580.28 / 693.37 | 28.2 / 28.2 / 31.2 |
| | ADLDGVGVK | 2 | 437.24 | 687.40 / 574.32 / 459.29 | 20.7 / 23.7 / 26.7 |
| VCAN | LLASDAGLYR | 2 | 539,80 | 852.42 / 781.38 / 579.32 | 26.5 / 26.5 / 38.5 |
| | YEINSLIR | 2 | 504.28 | 293.11 / 715.45 / 602.36 | 24.5 / 24.5 / 27.5 |
| | YTLNFEAAQK | 2 | 592.80 | 920.48 / 807.40 / 693.36 | 29.5 / 29.5 / 29.5 |
| LRRC15 | NWLLLNQPR | 2 | 577.33 | 853.53 / 740.44 / 627.36 | 28.6 / 31.6 / 31.6 |
| | YLSLANNK | 2 | 461.75 | 759.44 / 646.35 / 446.24 | 22.1 / 22.1 / 28.1 |
| | LTLFGNSLK | 2 | 496.79 | 778.45 / 665.36 / 518.29 | 24.1 / 24.1 / 27.1 |
| PTGDS | AQGFTEDTIVFLPQTDK | 2 | 955.48 | 947.52 / 848.45 / 588.30 | 44.2 / 44.2 / 47.2 |
| | AQGFTEDTIVFLPQTDK | 3 | 637.32 | 947.52 / 848.45 / 588.30 | 23.8 / 23.8 / 32.8 |
| | GPGEDFR | 2 | 389.18 | 623.28 / 322.19 / 360.67 | 20.9 / 29.9 / 20.9 |
| CDH13 | YEVSSPYFK | 2 | 560.27 | 827.43 / 728.36 / 293.11 | 27.7 / 27.7 / 27.7 |
| | VNSDGGLVALR | 2 | 550.81 | 887.49 / 800.46 / 685.44 | 30.1 / 30.1 / 33.1 |
| | SIVVSPILIPENQR | 2 | 782.96 | 300.19 / 869.48 / 643.32 | 40.4 / 43.4 / 31.4 |
| HSPA5 | TWNDPSVQQDIK | 2 | 715.85 | 288.13 / 914.49 / 260.20 | 36.5 / 36.5 / 51.5 |
| | NQLTSNPENTVFDAK | 2 | 839.41 | 243.11 / 356.19 / 480.25 | 40.6 / 40.6 / 37.6 |
| | ITPSYVAFTPEGER | 3 | 522.93 | 906.43 / 688.33 / 587.28 | 26.3 / 23.3 / 23.3 |
| **Yeast internal standards proteins** | | | | | |
| ENO-1 | VNQIGTLSESIK | 2 | 644.86 | 834.46 / 342.18 / 947.54 | 32.1 / 32.1 / 29.1 |
| | NVNDVIAPAFVK | 2 | 643.86 | 745.46 / 561.34 / 632.38 | 29 / 41 / 29 |
| ADH-1 | ANELLINVK | 2 | 507.30 | 586.39 / 473.31 / 828.52 | 27.1 / 24.1 / 24.1 |
| | DIVGAVLK | 2 | 407.76 | 586.39 / 487.32 / 260.20 | 20.5 / 20.5 / 29.5 |

**<u>Supplementary Data 1</u>: List of peptides and transitions monitored during the MRM verification study**

The final optimized assay consisted of 25 peptides corresponding to the 7 urinary candidate proteins, plus the 2 yeast proteins used as internal standards (total: 24 target peptides, 25 target peptide ions). The corresponding heavy forms (AQUA or PEPotec synthetic peptides) of these 25 target peptide ions were also monitored. Three transitions were monitored for each of the 50 peptide ion forms (total: 150 transitions).

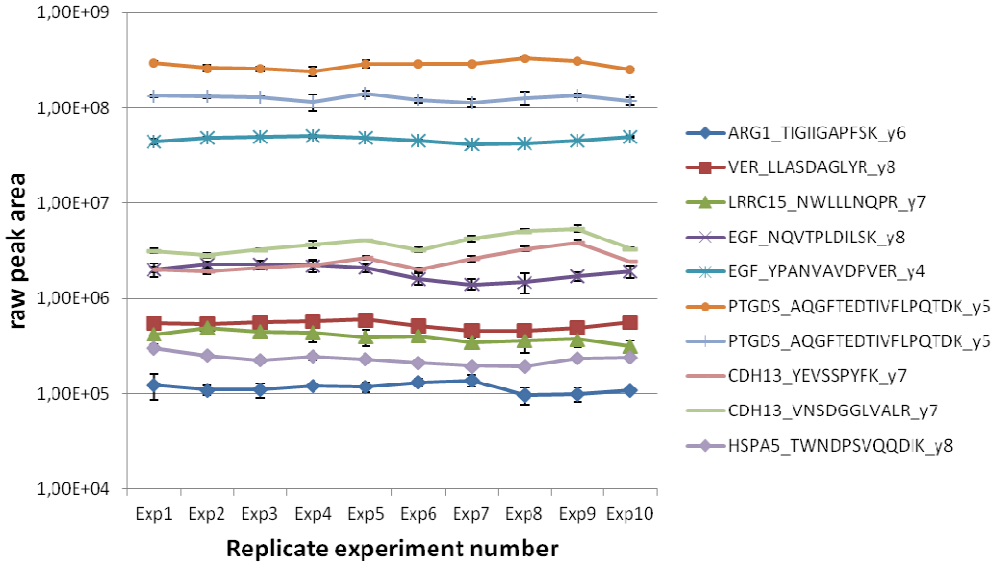
| | All transitions | | | AuDIT controlled transitions | | |
|---|---|---|---|---|---|---|
| Statistic | CV light | CV heavy | CV PAR | CV light | CV heavy | CV PAR |
| No. of observations | 75 | 75 | 75 | 65 | 65 | 65 |
| Minimum | 2,052 | 0,351 | 1,235 | 2,052 | 0,351 | 1,235 |
| Maximum | 55,045 | 102,613 | 101,392 | 33,723 | 34,693 | 19,766 |
| 1st Quartile | 10,983 | 8,963 | 5,446 | 10,853 | 8,665 | 5,277 |
| Median | 15,068 | 16,590 | 7,558 | 15,068 | 15,151 | 6,970 |
| 3rd Quartile | 19,017 | 20,995 | 12,914 | 18,715 | 18,770 | 10,730 |
| Mean | 15,462 | 17,920 | 13,254 | 14,819 | 14,476 | 8,067 |




**<u>Supplementary Data 2</u>: Assessment of the repeatability of the experimental workflow used for the MRM verification study (test1)**

Triplicate aliquots from a unique healthy urinary sample from the cohort of the verification study were processed in parallel (10µg total protein concentrated on SDS-PAGE, in-gel digestion and peptide extraction) and submitted to LC-MRM analysis using the MRM method described in Sup. Table 1. The boxplots illustrates the CVs across the triplicate measurements, calculated for all measured transitions (75 transitions of the candidate biomarker proteins and spiked yeast standard proteins), or only for the 65 transitions qualified as « good » after AuDIT analysis (Sup. Table 2). CVs across the triplicate experiments are shown for the light target peptides, the heavy internal standards, and for the peak area ratio calculated for light/heavy pairs.

A

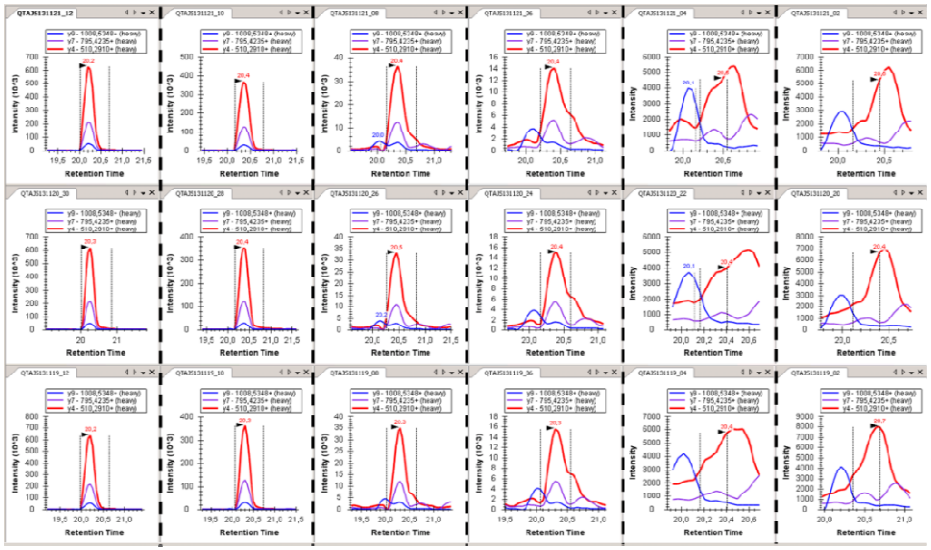



B

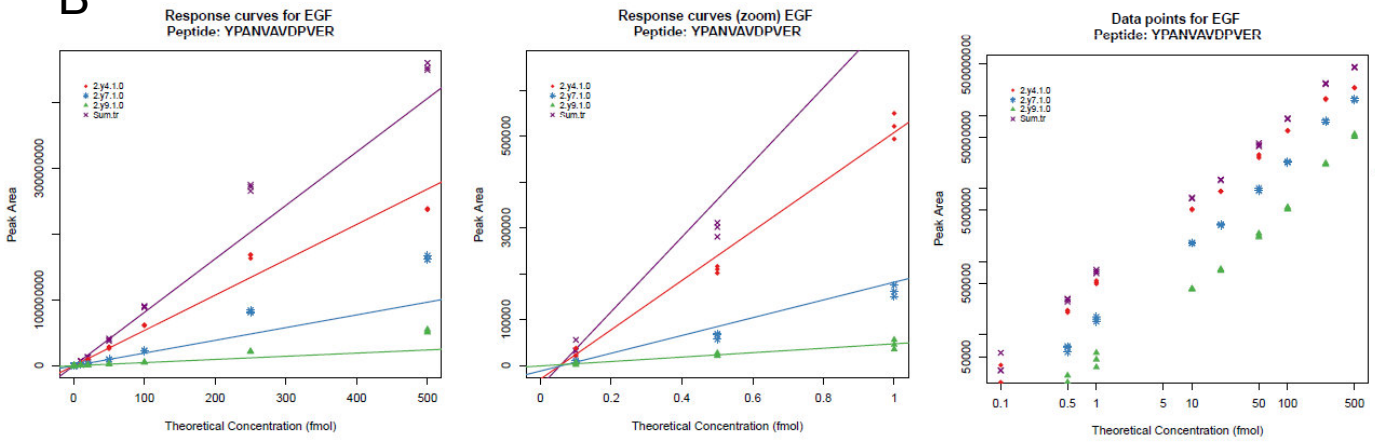
| Name | Peptide | charge | fragment ion | Exp1 | Exp2 | Exp3 | Exp4 | Exp5 | Exp6 | Exp7 | Exp8 | Exp9 | Exp10 | CV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ARG1 | TIGIIGAPFSK | 2 | y6 | 1,24E+05 | 1,09E+05 | 1,10E+05 | 1,20E+05 | 1,17E+05 | 1,32E+05 | 1,38E+05 | 9,67E+04 | 9,90E+04 | 1,09E+05 | 11,6 |
| VER | LLASDAGLYR | 2 | y8 | 5,51E+05 | 5,42E+05 | 5,67E+05 | 5,80E+05 | 5,95E+05 | 5,19E+05 | 4,63E+05 | 4,59E+05 | 4,89E+05 | 5,67E+05 | 9,1 |
| LRRC15 | NWLLLNQPR | 2 | y7 | 4,23E+05 | 4,89E+05 | 4,50E+05 | 4,43E+05 | 3,91E+05 | 4,06E+05 | 3,43E+05 | 3,61E+05 | 3,72E+05 | 3,13E+05 | 13,5 |
| EGF | NQVTPLDILSK | 2 | y8 | 2,00E+06 | 2,29E+06 | 2,31E+06 | 2,21E+06 | 2,08E+06 | 1,61E+06 | 1,41E+06 | 1,48E+06 | 1,72E+06 | 1,90E+06 | 17,5 |
| EGF | YPANVAVDPVER | 2 | y4 | 4,40E+07 | 4,80E+07 | 4,91E+07 | 5,05E+07 | 4,80E+07 | 4,54E+07 | 4,15E+07 | 4,20E+07 | 4,48E+07 | 4,92E+07 | 6,8 |
| PTGDS | AQGFTEDTIVFLPQTDK | 2 | y5 | 2,96E+08 | 2,60E+08 | 2,56E+08 | 2,42E+08 | 2,87E+08 | 2,86E+08 | 2,87E+08 | 3,29E+08 | 3,06E+08 | 2,52E+08 | 9,7 |
| PTGDS | AQGFTEDTIVFLPQTDK | 3 | y5 | 1,33E+08 | 1,31E+08 | 1,29E+08 | 1,15E+08 | 1,42E+08 | 1,20E+08 | 1,12E+08 | 1,26E+08 | 1,35E+08 | 1,18E+08 | 7,6 |
| CDH13 | YEVSSPYFK | 2 | y7 | 2,01E+06 | 1,92E+06 | 2,09E+06 | 2,25E+06 | 2,68E+06 | 2,02E+06 | 2,62E+06 | 3,29E+06 | 3,86E+06 | 2,46E+06 | 24,9 |
| CDH13 | VNSDGGLVALR | 2 | y7 | 3,14E+06 | 2,83E+06 | 3,23E+06 | 3,64E+06 | 4,07E+06 | 3,22E+06 | 4,23E+06 | 5,05E+06 | 5,37E+06 | 3,30E+06 | 22,5 |
| HSPA5 | TWNDPSVQQDIK | 2 | y8 | 3,04E+05 | 2,50E+05 | 2,25E+05 | 2,45E+05 | 2,30E+05 | 2,12E+05 | 1,95E+05 | 1,94E+05 | 2,37E+05 | 2,43E+05 | 13,6 |

**<u>Supplementary Data 3</u>: Assessment of the repeatability of the experimental workflow used for the MRM verification study (test2)**
Ten aliquots from a unique pooled urinary sample from healthy donors were processed in parallel (10µg total protein concentrated on SDS-PAGE, in-gel digestion and peptide extraction) and submitted to LC-MRM analysis (triplicate injections). Ten best-responding endogenous transitions corresponding to the candidate biomarkers protein were monitored. **A,** plot of raw intensity values for the monitored transitions (mean raw peak area across triplicate injections, error bars showing standard deviation across triplicate injections); **B,** raw intensity values and CVs calculated for the transitions of the candidate biomarker proteins detected in the pooled urine sample. CVs across the 10 replicate experiments are in the range 6-24%, median value 12%.

A



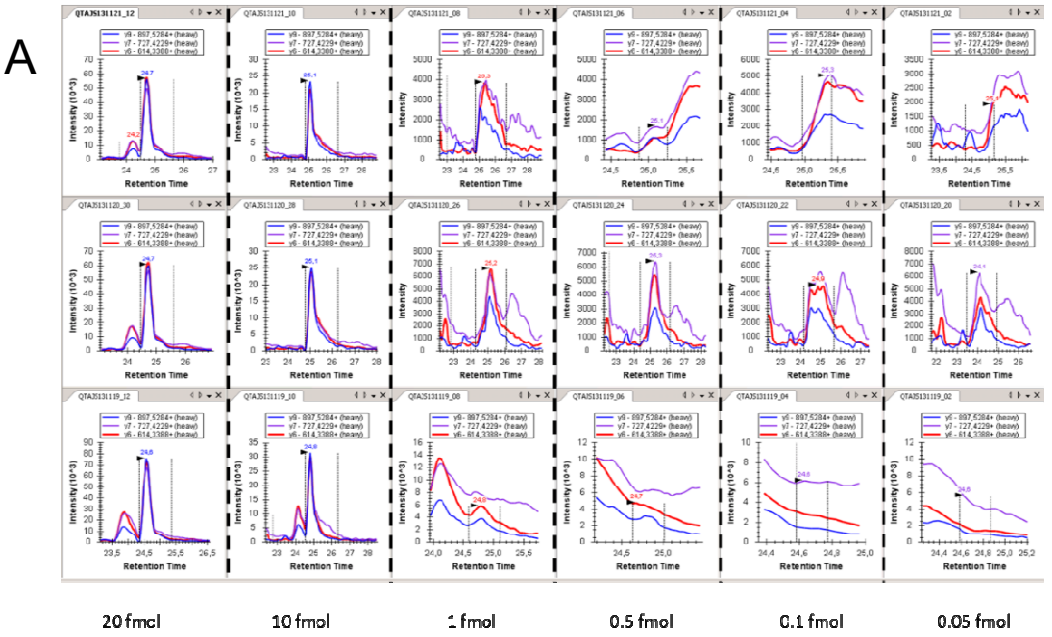

B



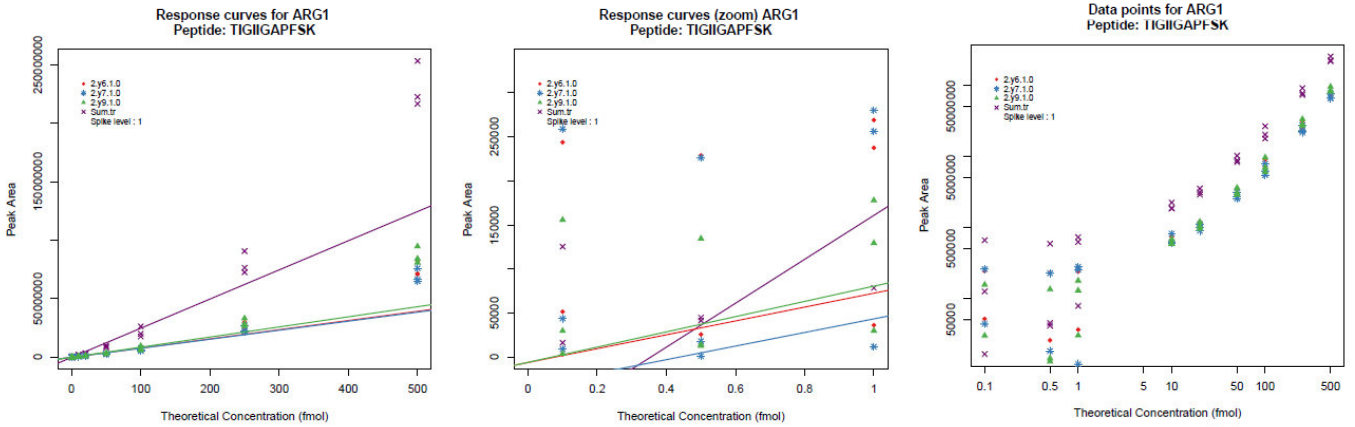

| Transition | LOD | LOD (fmol OC) | LOD (fmol /µg) |
|---|---|---|---|
| 2.y4.1.0 | 164740.31 | 0,361 | 0,101 |
| 2.y7.1.0 | 28860.95 | 0,208 | 0,058 |
| 2.y9.1.0 | 12765.04 | 0,273 | 0,076 |
| Sum.tr | 68318.38 | 0,307 | 0,086 |

**<u>Supplementary Data 4</u>: Signal response curve for EGF (peptide YPANVAVDPVER)**

The isotopically labeled AQUA peptide YPANVAVDPVER was spiked in increasing amounts in a pooled urine sample from healthy donors. Samples were processed according to the analytical protocol used for the verification study. **A,** MRM chromatograms of the monitored product ions (y9: blue; y4: red; y7: purple), for the five last points of the concentration range, showing extinction of the peptide signal. Spiked amounts are indicated in fmol on column. **B,** Calibration curve across the concentration range generated with QuaSAR. Raw peak area values for each transition were plotted against the spiked amount of AQUA peptide, in normal (left: whole concentration range; middle: zoom on low concentration spikes) or logarithmic scale (right). LOD and LLOQ were retrieved as peak area values from QuaSAR and converted to fmol on column using the linear regression fitting of the response curve, and then to fmol per total amount of urinary protein.




B



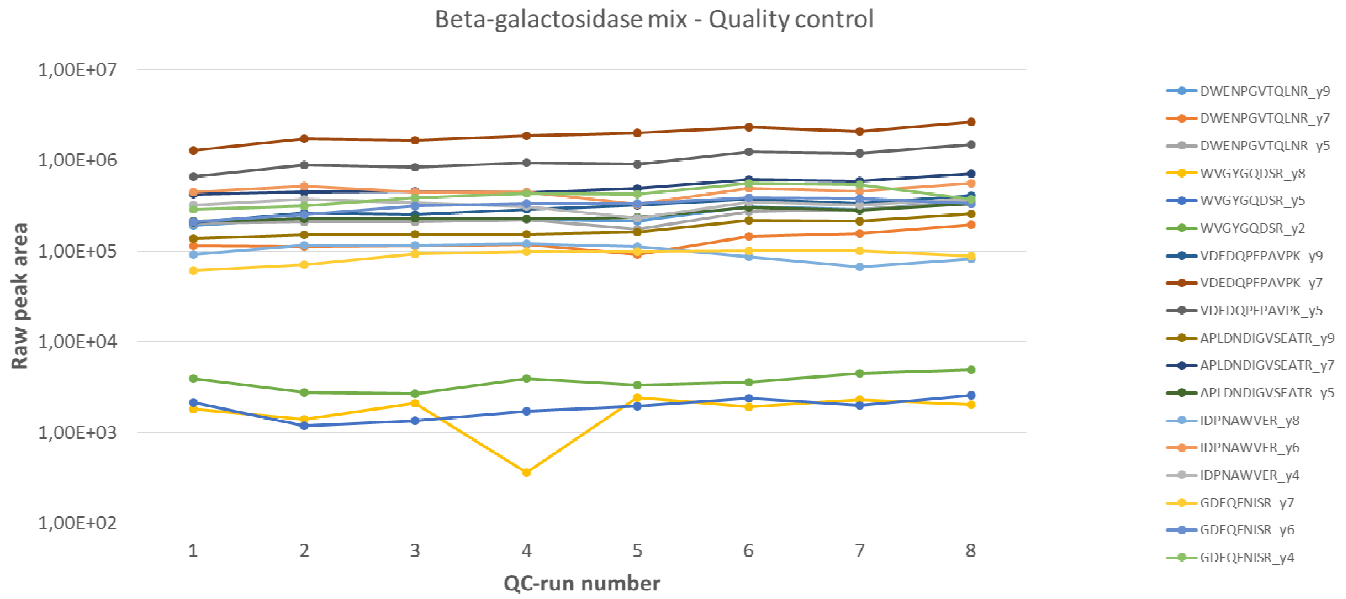

| Transition | LOD | LOD (fmol OC) | LOD (fmol /µg) |
|---|---|---|---|
| 2.y6.1.0 | 719901.31 | 9,23 | 2,58 |
| 2.y7.1.0 | 876243.90 | 11,79 | 3,30 |
| 2.y9.1.0 | 458811.81 | 5,37 | 1,50 |
| Sum.tr | 684312.81 | 8,59 | 2,40 |

**<u>Supplementary Data 5</u>: Signal response curve for ARG1 (peptide TIGIIGAPFSK)**

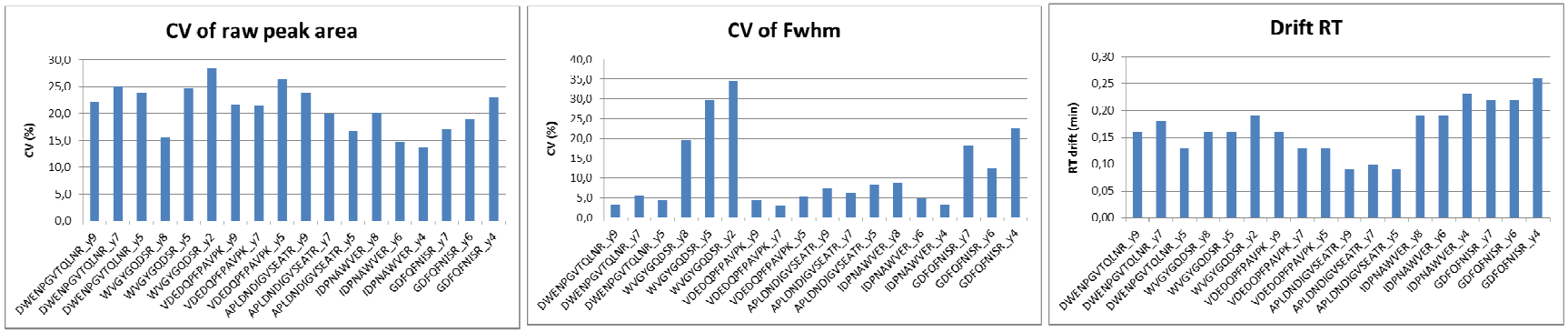
The isotopically labeled AQUA peptide TIGIIGAPFSK was spiked in increasing amounts in a pooled urine sample from healthy donors. Samples were processed according to the analytical protocol used for the verification study. **A,** MRM chromatograms of the monitored product ions (y9: blue; y6: red; y7: purple), for the five last points of the concentration range, showing extinction of the peptide signal. Spiked amounts are indicated in fmol on column.

**B,** Calibration curve across the concentration range generated with QuaSAR. Raw peak area values for each transition were plotted against the spiked amount of AQUA peptide, in normal (left: whole concentration range; middle: zoom on low concentration spikes) or logarithmic scale (right). LOD and LLOQ were calculated as peak area values from QuaSAR and converted to fmol on column using the linear regression fitting of the response curve, and then to fmol per total amount of urinary protein.

A

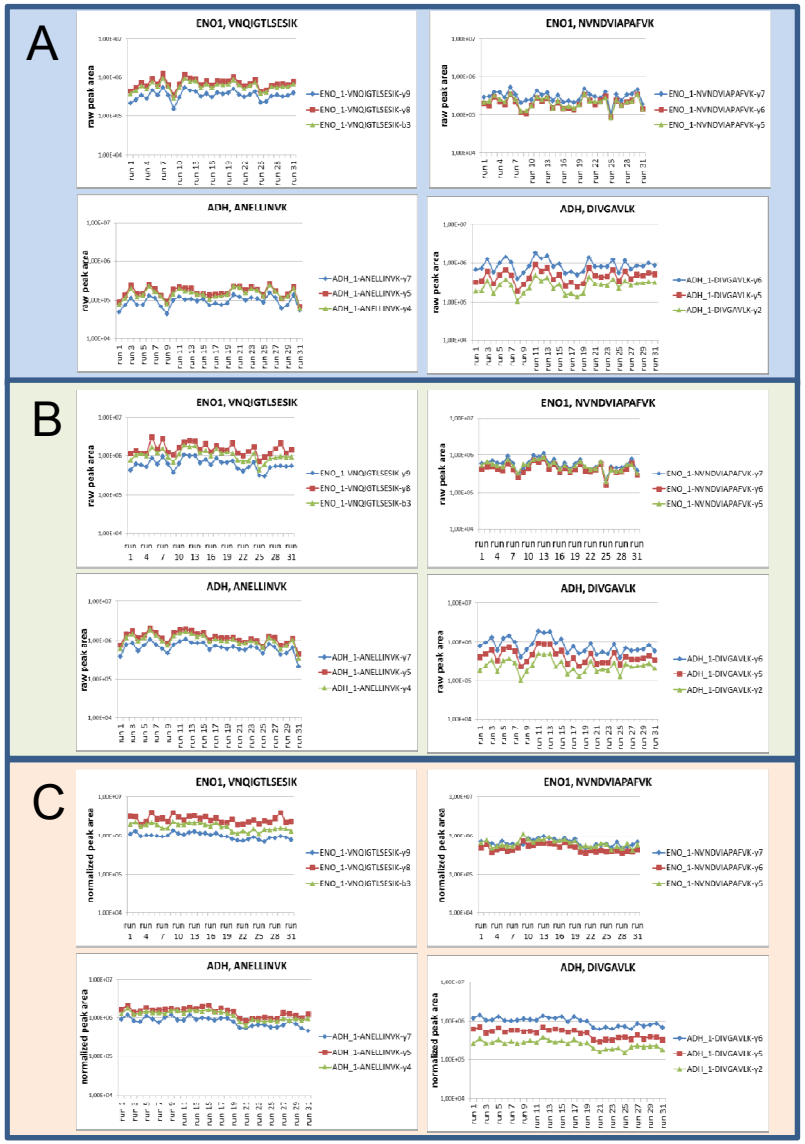



B




**<u>Supplementary Data 6</u>: Assessment of instrument performances during the MRM verification study using injection of a quality control external standard**

A peptide mixture (beta-galactosidase digest) was injected regularly in the time course of the verification study in between samples (8 QC runs). Eighteen transitions corresponding to 6 peptides were monitored. **A,** plot of raw peak area values for the transitions of the QC sample; **B,** CVs of raw peak area values and of full width at half maximum (Fwhm), and retention time drift (difference between maximal and minimal retention time values) were calculated across the 8 QC runs for the transitions of the QC sample, for each monitored transitions.




| PeptideSequence | ProteinName | FragmentIon | CV heavy (raw areas) |
|---|---|---|---|
| VNQIGTLSESIK | ENO_1 | y9 | 25,7 |
| VNQIGTLSESIK | ENO_1 | y8 | 27,2 |
| VNQIGTLSESIK | ENO_1 | b3 | 26,0 |
| NVNDVIAPAFVK | ENO_1 | y7 | 30,4 |
| NVNDVIAPAFVK | ENO_1 | y6 | 31,5 |
| NVNDVIAPAFVK | ENO_1 | y5 | 31,0 |
| ANELLINVK | ADH_1 | y7 | 28,9 |
| ANELLINVK | ADH_1 | y5 | 29,4 |
| ANELLINVK | ADH_1 | y4 | 29,8 |
| DIVGAVLK | ADH_1 | y6 | 38,3 |
| DIVGAVLK | ADH_1 | y5 | 36,4 |
| DIVGAVLK | ADH_1 | y2 | 35,2 |

| PeptideSequence | ProteinName | FragmentIon | CV light (raw areas) |
|---|---|---|---|
| VNQIGTLSESIK | ENO_1 | y9 | 30,9 |
| VNQIGTLSESIK | ENO_1 | y8 | 36,2 |
| VNQIGTLSESIK | ENO_1 | b3 | 33,8 |
| NVNDVIAPAFVK | ENO_1 | y7 | 32,8 |
| NVNDVIAPAFVK | ENO_1 | y6 | 30,3 |
| NVNDVIAPAFVK | ENO_1 | y5 | 28,1 |
| ANELLINVK | ADH_1 | y7 | 29,0 |
| ANELLINVK | ADH_1 | y5 | 31,6 |
| ANELLINVK | ADH_1 | y4 | 31,7 |
| DIVGAVLK | ADH_1 | y6 | 47,8 |
| DIVGAVLK | ADH_1 | y5 | 42,3 |
| DIVGAVLK | ADH_1 | y2 | 40,4 |

| PeptideSequence | ProteinName | FragmentIon | CV light (normalized areas) |
|---|---|---|---|
| VNQIGTLSESIK | ENO_1 | y9 | 17,9 |
| VNQIGTLSESIK | ENO_1 | y8 | 20,8 |
| VNQIGTLSESIK | ENO_1 | b3 | 20,6 |
| NVNDVIAPAFVK | ENO_1 | y7 | 21,3 |
| NVNDVIAPAFVK | ENO_1 | y6 | 21,5 |
| NVNDVIAPAFVK | ENO_1 | y5 | 24,5 |
| ANELLINVK | ADH_1 | y7 | 24,6 |
| ANELLINVK | ADH_1 | y5 | 25,6 |
| ANELLINVK | ADH_1 | y4 | 25,2 |
| DIVGAVLK | ADH_1 | y6 | 24,1 |
| DIVGAVLK | ADH_1 | y5 | 25,3 |
| DIVGAVLK | ADH_1 | y2 | 21,7 |

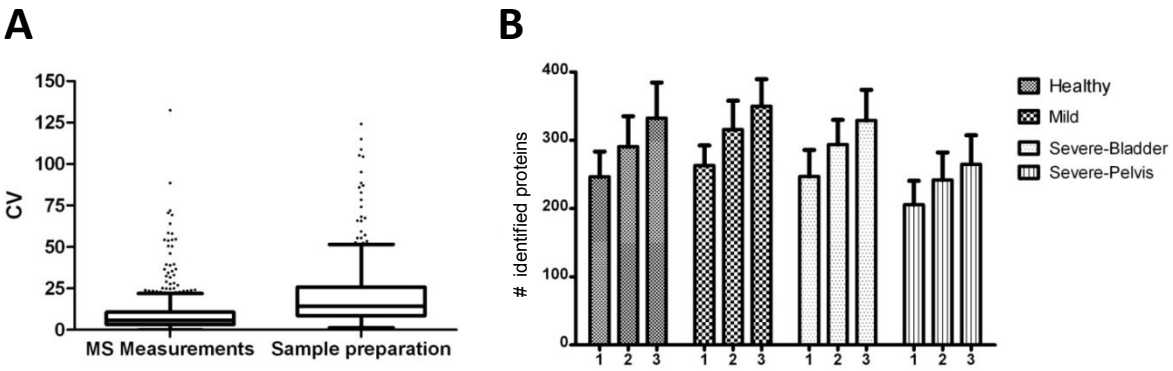
**<u>Supplementary Data 7</u>: Evaluation of the repeatability of the MRM measurements during the verification study using spiked exogenous yeast proteins**

Two exogenous yeast proteins (ADH_1 and ENO_1) were spiked into all the clinical samples to be measured, before SDS-PAGE and in-gel digestion. During analysis of the clinical samples, these yeast proteins were monitored together with the candidate biomarkers, using 2 peptides / protein, and 3 transitions / peptide for both light and isotopic heavy forms (PEPotec peptides). **A,** Raw areas of the 12 transitions monitored for the heavy peptides; **B,** Raw areas of the 12 transitions monitored for the light peptides; **C,** areas of the 12 transitions for the light peptides normalized across all the runs using a correction factor calculated from the signal of the heavy peptides.

| ProteinName | PeptideSequence | z | Transistion used for quantification | Internal standard peptide | Estimated fmol/µg (mean/group) | | | Mild vs Healthy | | | Severe-Bladder vs Healthy | | | Severe-Baldder vs Mild | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Healthy (n=10) | Mild (n=13) | Severe-Bladder (n=8) | ttest pvalue | pvalue BH | Fold Change | ttest pvalue | pvalue BH | Fold Change | ttest pvalue | pvalue BH | Fold Change |
| **Human targeted proteins** | | | | | | | | | | | | | | | | |
| **ARG1** | **TIGIIGAPFSK** | **2** | **y9** | **Purified** | **2,89** | **0,96** | **0,74** | **0,000728*** ** | **0,007409** ** | **-3,00** | **0,003194** ** | **0,011431* ** | **-3,89** | **0,479889** | **0,924980** | **-1,30** |
| **ARG1** | **DVDPGEHYILK** | **2** | **y8++** | **Crude** | **6,78** | **1,85** | **1,72** | **0,000434*** ** | **0,007409** ** | **-3,67** | **0,004157** ** | **0,011431* ** | **-3,95** | **0,872662** | **0,924980** | **-1,08** |
| **EGF** | **YPANVAVDPVER** | **2** | **y7** | **Purified** | **67,99** | **32,35** | **8,84** | **0,001363** ** | **0,007498** ** | **-2,10** | **0,000016*** ** | **0,000178*** ** | **-7,69** | **0,009046** ** | **0,085629** | **-3,66** |
| **EGF** | **NQVTPLDILSK** | **2** | **y8** | **Purified** | **31,25** | **18,20** | **4,86** | **0,020614* ** | **0,064788** | **-1,72** | **0,000005*** ** | **0,000111*** ** | **-6,44** | **0,017342* ** | **0,095382** | **-3,75** |
| **EGF** | **LFWIQYNR** | **2** | **y5** | **Crude** | **558,93** | **273,79** | **82,66** | **0,002783** ** | **0,012246* ** | **-2,04** | **0,000046*** ** | **0,000251*** ** | **-6,76** | **0,041621* ** | **0,183131** | **-3,31** |
| **EGF** | **ADLDGVGVK** | **2** | **y6** | **Crude** | **176,56** | **82,27** | **22,97** | **0,001010** ** | **0,007409** ** | **-2,15** | **0,000039*** ** | **0,000251*** ** | **-7,69** | **0,011677* ** | **0,085629** | **-3,58** |
| LRRC15 | YLSLANNK | 2 | y6 | Crude | 2,19 | 1,07 | 0,66 | 0,018853* | 0,064788 | -2,05 | 0,002804** | 0,011431* | -3,31 | 0,090042 | 0,235559 | -1,62 |
| LRRC15 | LTLFGNSLK | 2 | y7 | Crude | 11,40 | 6,27 | 3,91 | 0,047373* | 0,129366 | -1,82 | 0,004094** | 0,011431* | -2,92 | 0,096365 | 0,235559 | -1,60 |
| LRRC15 | NWLLLNQPR | 2 | y6 | Purified | 27,17 | 21,87 | 8,59 | 0,319260 | 0,396692 | -1,24 | 0,013713* | 0,033521* | -3,16 | 0,073909 | 0,232287 | -2,54 |
| VER | LLASDAGLYR | 2 | y7 | Purified | 0,72 | 0,60 | 2,00 | 0,742137 | 0,760060 | -1,21 | 0,019952* | 0,043894 | 2,79 | 0,007445** | 0,085629 | 3,36 |
| VER | YEINSLIR | 2 | y5 | Crude | 1,03 | 0,65 | 1,47 | 0,324566 | 0,396692 | -1,59 | 0,450307 | 0,762057 | 1,43 | 0,061340 | 0,224915 | 2,28 |
| PTGDS | AQGFTEDTIVFLPQTDK | 3 | y5 | Purified | 16011,04 | 16996,01 | 25644,90 | 0,489268 | 0,538195 | 1,06 | 0,843758 | 0,940203 | 1,60 | 0,815690 | 0,924980 | 1,51 |
| CDH13 | YEVSSPYFK | 2 | b2 | Purified | 19,31 | 26,81 | 32,08 | 0,091591 | 0,175380 | 1,39 | 0,854730 | 0,940203 | 1,66 | 0,579198 | 0,924980 | 1,20 |
| CDH13 | VNSDGGLVALR | 2 | y7 | Purified | 13,57 | 18,88 | 21,83 | 0,095662 | 0,175380 | 1,39 | 0,939280 | 0,984008 | 1,61 | 0,542929 | 0,924980 | 1,16 |
| CDH13 | SIVVSPILIPENQR | 2 | y7 | Crude | 60,79 | 28,25 | 54,27 | 0,319822 | 0,396692 | -2,15 | 0,494477 | 0,777035 | -1,12 | 0,796101 | 0,924980 | 1,92 |
| HSPA5 | TWNDPSVQQDIK | 2 | y8 | Purified | 5,62 | 2,42 | 3,10 | 0,052922 | 0,129366 | -2,33 | 0,222354 | 0,407648 | -1,81 | 0,773269 | 0,924980 | 1,28 |
| HSPA5 | NQLTSNPENTVFDAK | 2 | b2 | Crude | 5,85 | 3,26 | 6,81 | 0,080246 | 0,175380 | -1,79 | 0,854695 | 0,940203 | 1,16 | 0,236212 | 0,519666 | 2,09 |
| HSPA5 | ITPSYVAFTPEGER | 3 | y8 | Crude | 7,74 | 5,39 | 7,02 | 0,475912 | 0,538195 | -1,44 | 0,666782 | 0,862894 | -1,10 | 0,981443 | 0,981443 | 1,30 |
| **Yeast internal standards proteins** | | | | | | | | | | | | | | | | |
| ENO_1 | VNQIGTLSESIK | 2 | b3 | Crude | 23,19 | 26,27 | 26,87 | 0,150799 | 0,255198 | 1,13 | 0,162484 | 0,324968 | 1,16 | 0,841787 | 0,924980 | 1,02 |
| ENO_1 | NVNDVIAPAFVK | 2 | y6 | Crude | 29,58 | 32,15 | 32,08 | 0,303608 | 0,396692 | 1,09 | 0,529838 | 0,777096 | 1,08 | 0,882935 | 0,924980 | -1,00 |
| ADH_1 | ANELLINVK | 2 | y7 | Crude | 200,83 | 204,44 | 200,61 | 0,760060 | 0,760060 | 1,02 | 0,989521 | 0,989521 | -1,00 | 0,774395 | 0,924980 | -1,02 |
| ADH_1 | DIVGAVLK | 2 | y6 | Crude | 6,32 | 6,98 | 6,74 | 0,281411 | 0,396692 | 1,11 | 0,581769 | 0,799932 | 1,07 | 0,678408 | 0,924980 | -1,04 |

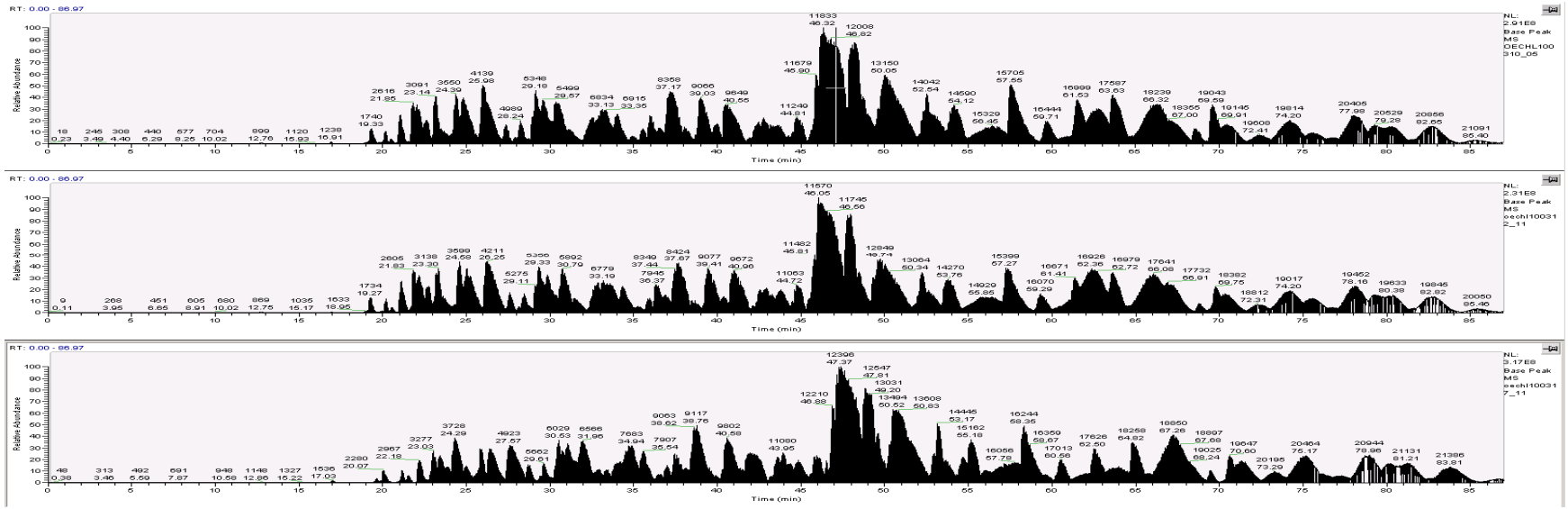
**<u>Supplementary Data 8</u>**: **Relative quantification of the candidate proteins across clinical samples using MRM data**

Quantitative analysis of the MRM data were performed with the QuaSAR software. Endogenous protein concentrations were estimated by QuaSAR from the calculation of peak area ratio (PAR) values between light and heavy peptides, and from the known spiked amounts of the heavy internal standard peptides (approximate amounts were used in the case of crude PEPotec peptides) using the best transition of the targeted peptide. The mean of concentrations values (fmol/weight of total protein) estimated for each target peptide per group are reported here. Statistical analysis between sample groups was performed by unpaired *t* test assuming unequal variances (ttest pvalue) using the endogenous amounts calculated by the software for each targeted peptide. Pvalues were corrected for multiple testing using the Benjamini-Hochberg procedure (pvalue BH) (22 pvalues corrected corresponding to 22 peptides quantified per comparison) (*<0,05; **<0,01; ***<0,001). The fold change per group comparison using the mean estimated concentration values was also calculated.

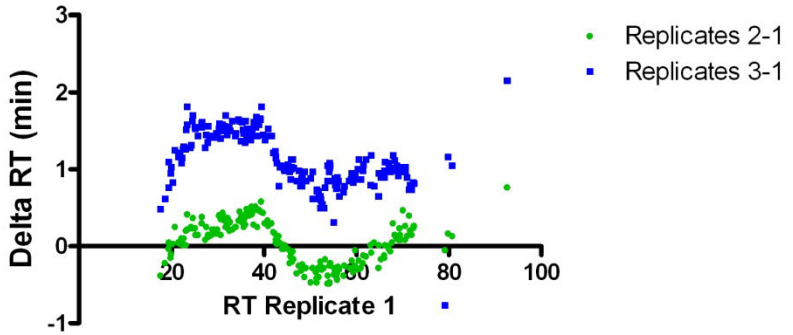



**Supplementary Data 9**: **Variation in LC-MS/MS analyses and improvement of protein coverage.**
**A**, Box plots analysis showing the distribution of PAI CVs for the sample preparation method performed in triplicate using a urinary sample of a control individual and LC-MS triplicate measurements of one of these sample preparations. Each box represents the range between the 25th and the 75th percentiles with the median value shown as an intersect line. The minimum and maximum values were within 1.5 x interquartile range. **B**, Cumulative increase in the number of proteins identified when performing a single (1), duplicate (2) or triplicate (3) runs. Means +/- S.D. of identified proteins for the 5 samples per group are shown.

A

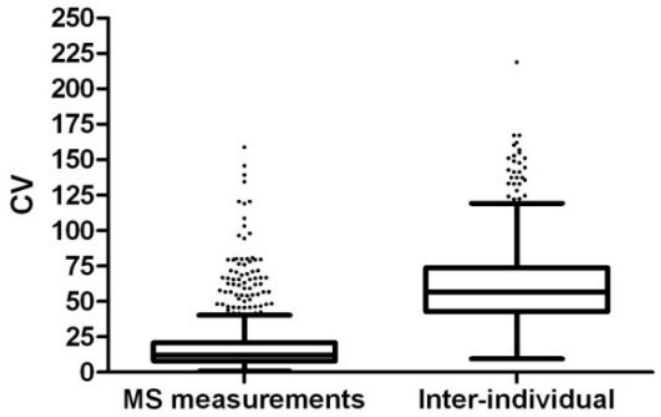


B




**<u>Supplementary Data 10</u>: Chromatographic repeatability of the label-free LC-MS discovery study**

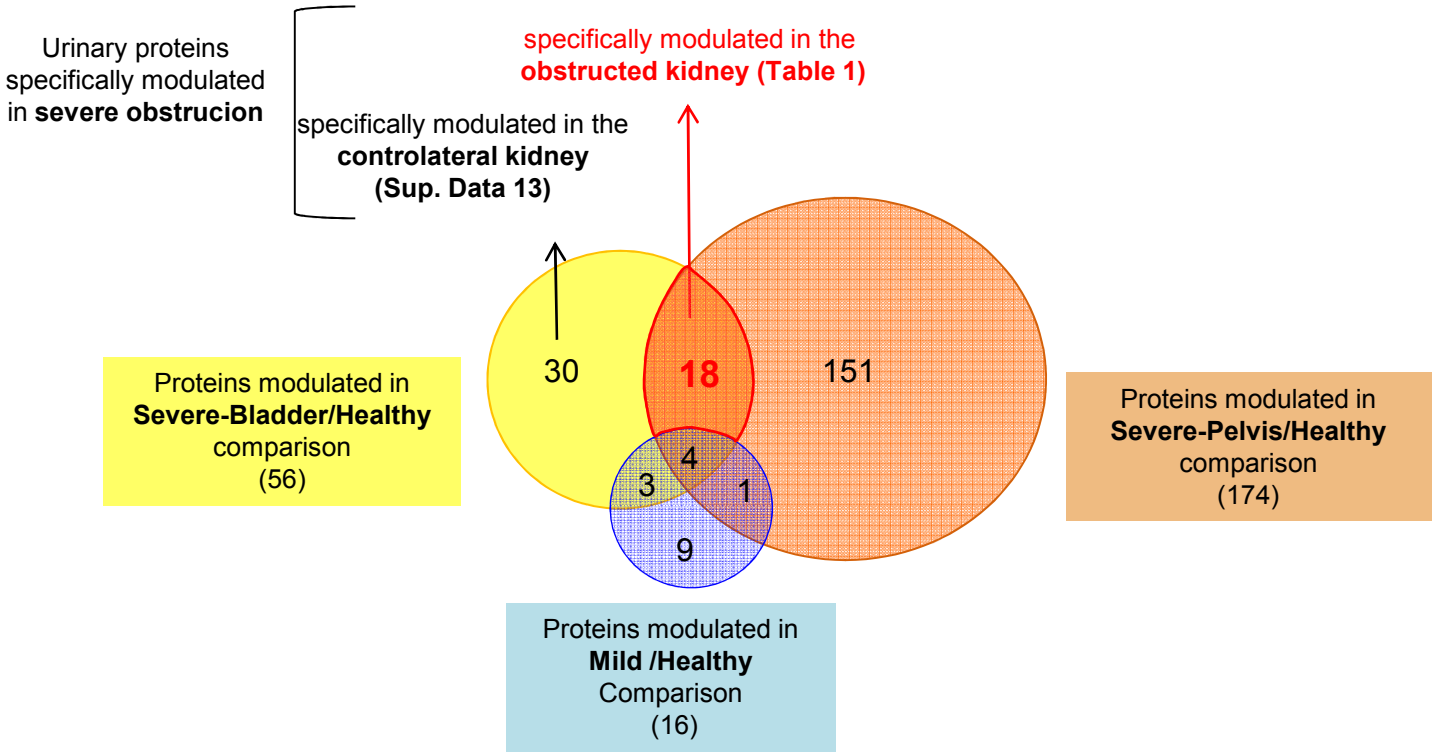
The total analysis thus resulted in 60 LC-MS/MS runs, representing about 10 days of mass spectrometry measurement. All along this period, good chromatographic reproducibility was observed across random triplicate injections, with retention times on average deviating in the order of 1 min . **A,** Base peak chromatograms between the 3 LC-MS replicates runs obtained from one patient of the cohort (Severe-Bladder A patient, LC-MS running order : days 2, 4 and 8), illustrating that good chromatographic reproducibility is observed between random replicates of injection. **B,** Difference in retention time (in minutes) between the first replicate (day 2) and the second (2-1) or the third (3-1) replicates (days 4 and 8 respectively) is shown.




**<u>Supplementary Data 11:</u> Evaluation of the LC-MS random measurements and the inter-individual variation obtained in the label-free quantitative analysis.**

We calculated protein PAI CVs across LC-MS/MS triplicates. As the triplicate measurements were randomly performed during the 10-day mass spectrometry measurement period, variability was expected to be higher than in the case of consecutive LC-MS/MS runs. Indeed we observed a median CV of 13% in the random LC-MS measurements. However, this was still significantly lower than the inter-individual variability measured for samples of the same group that typically gave median protein CV around 50-60% in our study (e.g. median CV of 56% in the Severe-Bladder group)

Representative Box plots analysis of CVs of the PAI values is shown from the Severe-Bladder group. Each box represents the range between the 25th and the 75th percentiles with the median value shown as an intersect line. The minimum and maximum values were within 1.5 x interquartile range.

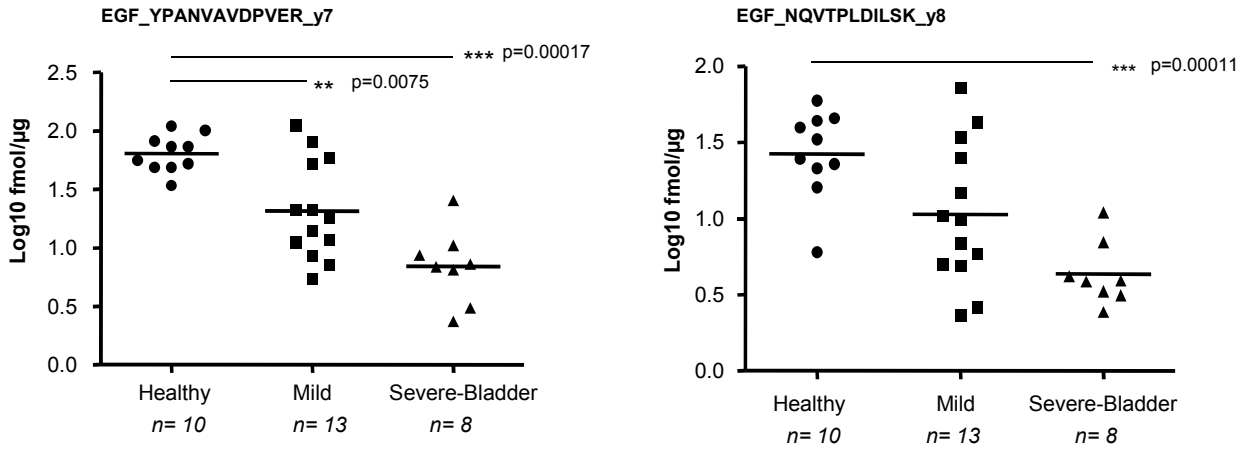



**Supplementary Data 12:** Mode of selection of urinary proteins specifically modulated in obstructed kidney

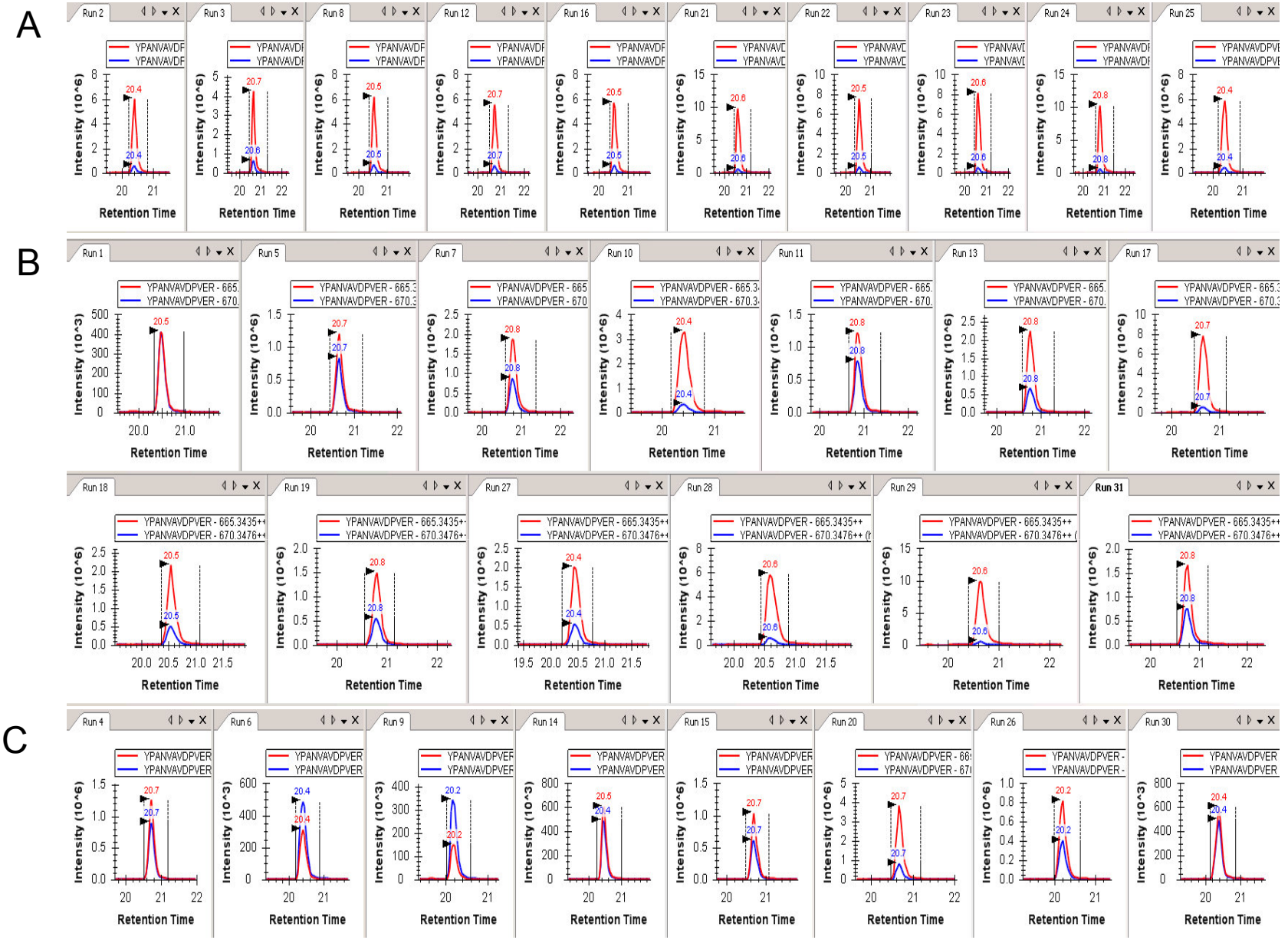
| AC | Protein description | Gene name | Severe-Pelvis/Healthy | | Severe-Bladder/Healthy | | Mild/Healthy | |
|---|---|---|---|---|---|---|---|---|
| | | | P value | Fold change | P value | Fold change | P value | Fold change |
| IPI00871537 | interferon, gamma-inducible protein 30 preproprotein | *IFI30* | 0.888479 | 1.01 | 0.003902 | 2.92 | 0.363219 | 1.14 |
| IPI00645614 | Isoform 2 of Cadherin-3 | *CDH3* | 0.898318 | -1.12 | 0.008584 | 2.44 | 0.269954 | 1.55 |
| IPI00006988 | Resistin | *RETN* | | | 0.011083 | 2.94 | 0.169922 | 1.75 |
| IPI00024046 | cDNA FLJ52398, highly similar to Cadherin-13 | *CDH13* | 0.87387 | -1.06 | 0.011830 | 2.10 | 0.056452 | 1.6 |
| IPI00003362 | HSPA5 protein | *HSPA5* | 0.712979 | 1.46 | 0.013145 | -2.12 | 0.141180 | -1.84 |
| IPI00176193 | Isoform 1 of Collagen alpha-1(XIV) chain | *COL14A1* | 0.752205 | -1.01 | 0.013613 | 2.05 | 0.175621 | 1.15 |
| IPI00290856 | Lymphatic vessel endothelial hyaluronic acid receptor 1 | *LYVE1* | 0.819059 | 2.32 | 0.018286 | 2.89 | 0.285866 | 1.62 |
| IPI00939673 | cDNA FLJ51061, highly similar to Opioid-binding protein/cell adhesion molecule | *OPCML* | 0.05522 | -2.27 | 0.020482 | -2.51 | 0.300234 | -1.79 |
| IPI00395488 | Vasorin | *VASN* | 0.426283 | -1.05 | 0.022222 | -2.14 | 0.697949 | -1.27 |
| IPI00025204 | CD5 antigen-like | *CD5L* | 0.474149 | -1.06 | 0.024666 | 2.61 | 0.058992 | 2.29 |
| IPI00293276 | Macrophage migration inhibitory factor | *MIF* | | | 0.027621 | 9.68 | 0.251364 | 2.25 |
| IPI00032328 | Isoform HMW of Kininogen-1 | *KNG1* | 0.088819 | -1.41 | 0.028083 | -1.51 | 0.095400 | -1.55 |
| IPI00018236 | Ganglioside GM2 activator | *GM2A* | 0.470957 | 8.41 | 0.028084 | 7.10 | 0.265810 | 3.28 |
| IPI00215894 | Isoform LMW of Kininogen-1 | *KNG1* | 0.129495 | -1.31 | 0.029426 | -1.49 | 0.110828 | -1.43 |
| IPI00102543 | SLIT and NTRK-like protein 1 | *SLITRK1* | 0.663712 | -1.01 | 0.029847 | -2.80 | 0.945348 | 1.8 |
| IPI00009866 | Isoform 1 of Keratin, type I cytoskeletal 13 | *KRT13* | 0.160072 | -2.56 | 0.032094 | -4.01 | 0.267497 | -3.97 |
| IPI00166729 | alpha-2-glycoprotein 1 zinc precursor | *AZGP1* | 0.104823 | 2.22 | 0.038908 | 2.80 | 0.190472 | 1.09 |
| IPI00878953 | MRNA for apolipoprotein E | *APOE* | 0.197498 | 1.05 | 0.038941 | -2.41 | 0.214412 | -1.84 |
| IPI00006705 | Uteroglobin | *SCGB1A1* | 0.936548 | 2.39 | 0.039353 | 6.96 | 0.082352 | 5.7 |
| IPI00290077 | Keratin, type I cytoskeletal 15 | *KRT15* | 0.331046 | -1.38 | 0.039515 | -4.10 | 0.318082 | -3.13 |
| IPI00789324 | cDNA FLJ60424, highly similar to Junction plakoglobin | *JUP* | 0.449258 | -1.08 | 0.040426 | -3.86 | 0.314250 | -3.19 |
| IPI00554788 | Keratin, type I cytoskeletal 18 | *KRT18* | 0,509019 | -1,01 | 0,041532 | -3,97 | 0,316127 | -3,09 |
| IPI00012887 | Cathepsin L1 | CTSL1 | 0,880644 | 1,02 | 0,042986 | 2,97 | 0,108161 | 2,51 |
| IPI00007797 | Fatty acid-binding protein, epidermal | *FABP5* | | | 0,043595 | -2,20 | 0,137584 | -31,19 |
| IPI00000861 | Isoform 1 of LIM and SH3 domain protein 1 | *LASP1* | 0,123856 | 2,46 | 0,044399 | 3,60 | 0,094305 | 3,16 |
| IPI00003919 | Isoform 1 of Glutaminyl-peptide cyclotransferase | *QPCT* | 0,146783 | -1,34 | 0,044808 | -2,16 | 0,614584 | 1,13 |
| IPI00026303 | Peptidase inhibitor 15 | *PI15* | 0,915957 | -1,99 | 0,046914 | 2,80 | 0,860642 | -1,27 |
| IPI00917299 | Isoform 1 of Gamma-glutamylcyclotransferase | *GGCT* | | | 0.047362 | -3.32 | 0.073043 | -2.53 |
| IPI00479145 | Keratin, type I cytoskeletal 19 | *KRT19* | 0,524974 | 1,02 | 0,047756 | -3,97 | 0,248917 | -3,71 |
| IPI00014964 | lymphocyte antigen 6 complex, locus H isoform b | *LY6H* | 0,811924 | 1,22 | 0,049694 | 1,93 | 0,090982 | 1,63 |

**<u>Supplementary Data 13</u>: Significant proteins that were assigned as specifically modulated in the contralateral kidney**

Proteins that were found significantly modulated in the Severe-Bladder/Healthy comparison but not in Severe-Pelvis/Healthy and Mild/Healthy comparisons. P value was determined using Student's *t* test and Fold changes between the patient and the control groups were calculated using the median normalized area of the 5 samples per group.



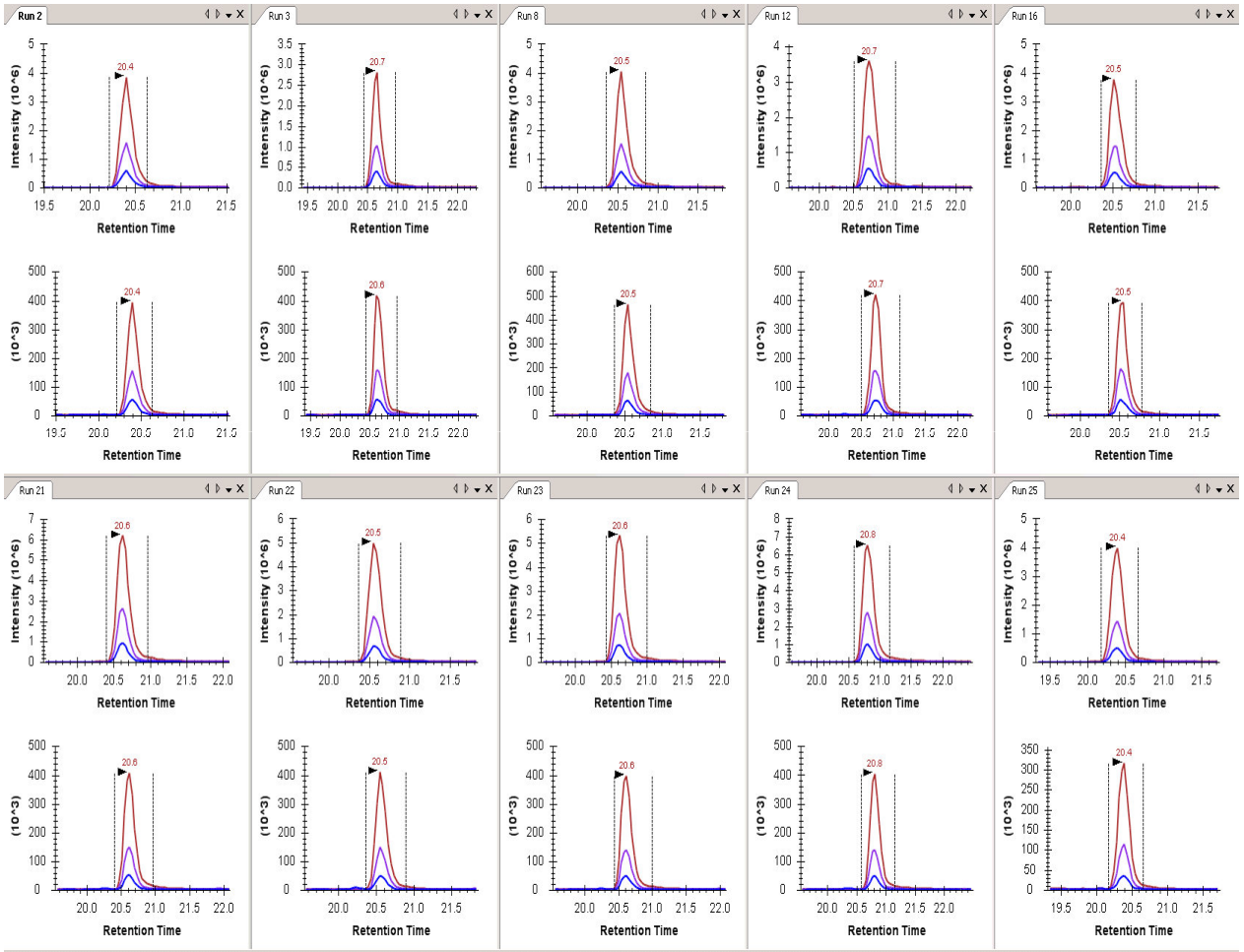

**<u>Supplementary Data 14</u>**: **MRM analysis of urinary EGF concentrations in the verification cohort.**
LC-MRM analysis shows that the urinary EGF concentration is lower in UPJ obstruction patients. Logarithmic plots of the EGF protein concentration values calculated from 2-targeted peptides, for each group of patients are shown. Concentrations were calculated either based on the peptide YPANVAVDPVER, product ion y7 (calibration curve shown in Sup. Data 4), AQUA peptide spiked at 20 fmol, or on peptide NQVTPLDILSK, product ion y8, AQUA peptide spiked at 20 fmol. Analysis of the samples was performed using 3.57 µg of total urinary proteins and concentrations were calculated in fmol/µg of total protein. Mean values shown as an intersect line and significance of the difference between groups based on unpaired *t* test assuming unequal variances corrected for multiple testing using the Benjamini-Hochberg procedure are shown. Adjusted pvalue (pvalue BH) **<0,01; ***<0,001.




**<u>Supplementary Data 15</u>: MRM chromatograms for peptide YPANVAVDPVER of EGF acquired across the 31 samples of the clinical study**
The summed of the 3 transitions monitored for the endogenous peptide (red) is displayed together with the corresponding sum of transitions for the heavy AQUA standard (blue). **A,** samples from healthy group (n=10); **B,** samples from mild group (n=13); **C,** samples from severe group (n=8).
In the 3 slides below, individual transitions for this peptide and for the heavy standard are represented for the 3 different groups.

**YPANVAVDPVER, EGF**

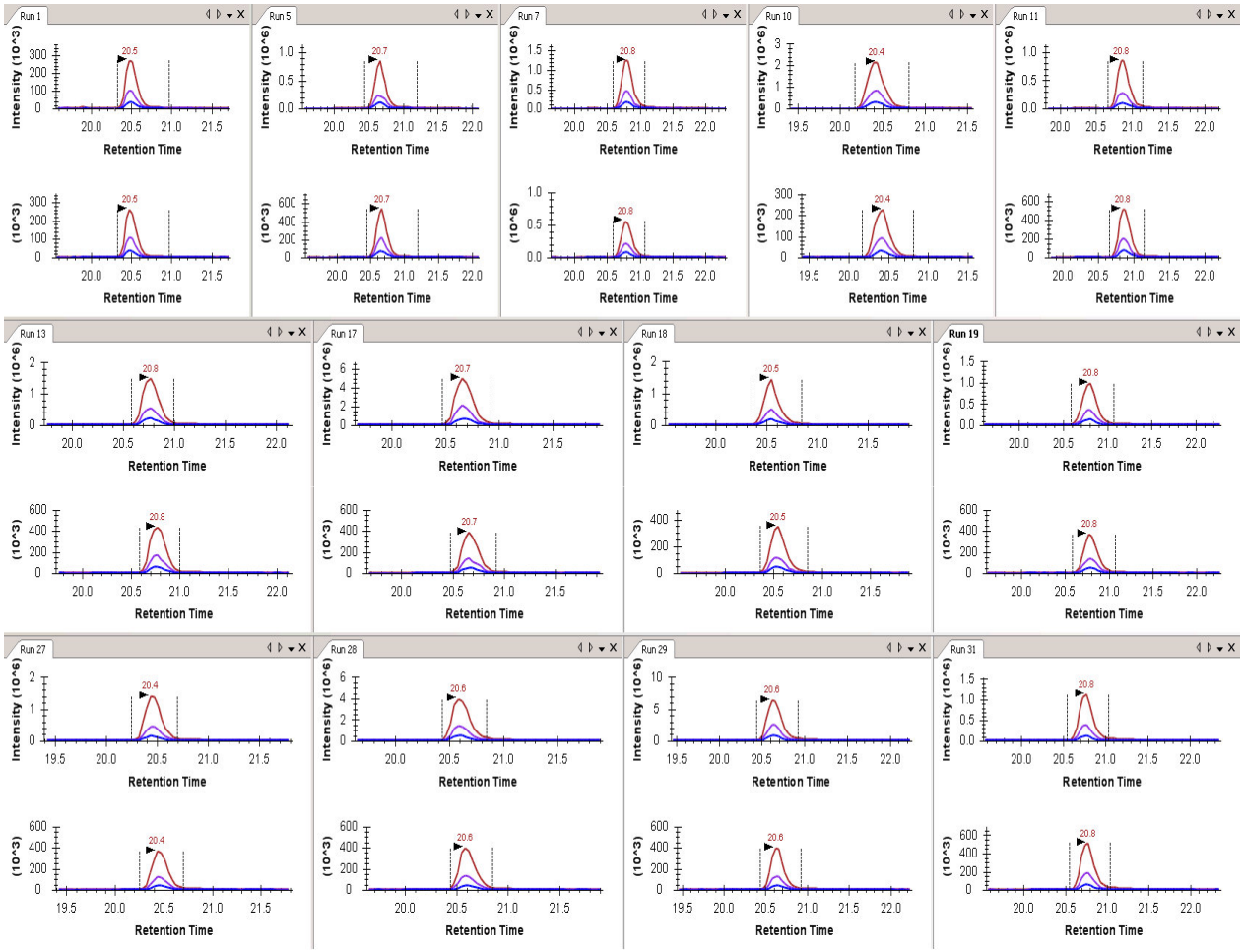
Transitions: y9: blue; y4: red; y7: purple **Group Healthy (n=10)**



**YPANVAVDPVER, EGF**

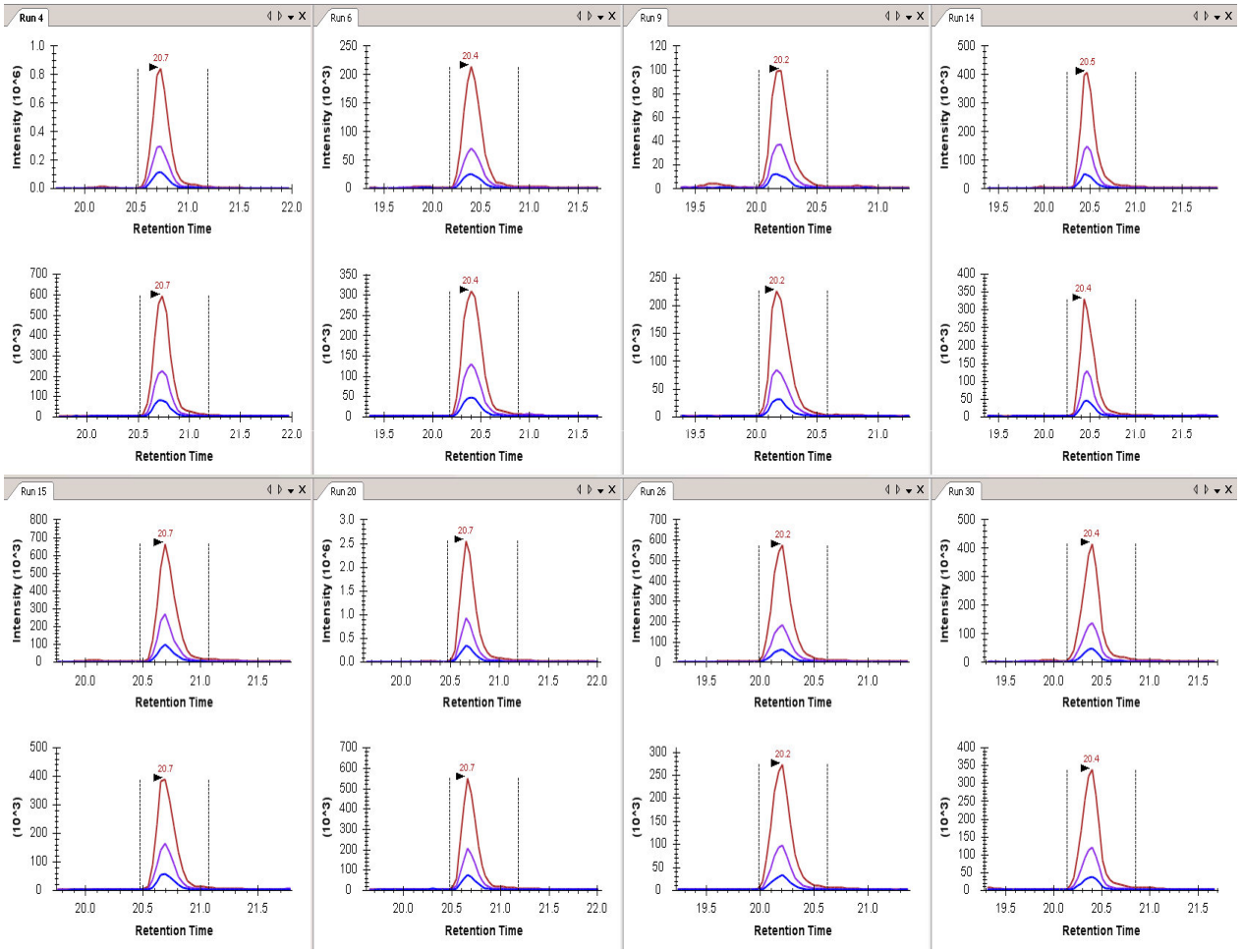
Transitions: y9: blue; y4: red; y7: purple

**Group Mild (n=13)**

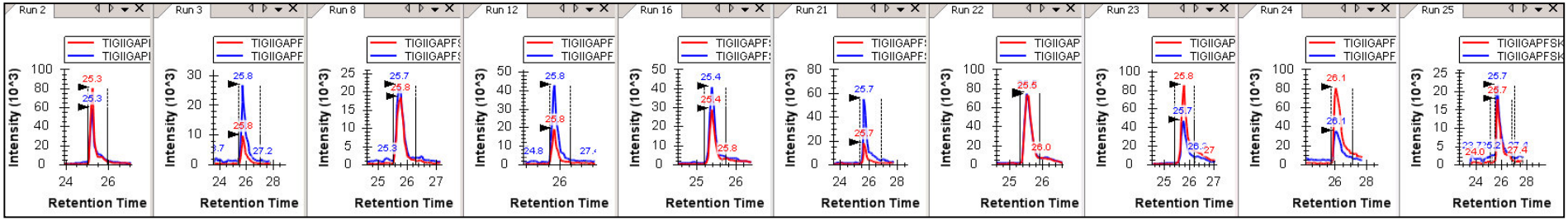


**YPANVAVDPVER, EGF** **Group Severe-Bladder (n=8)**

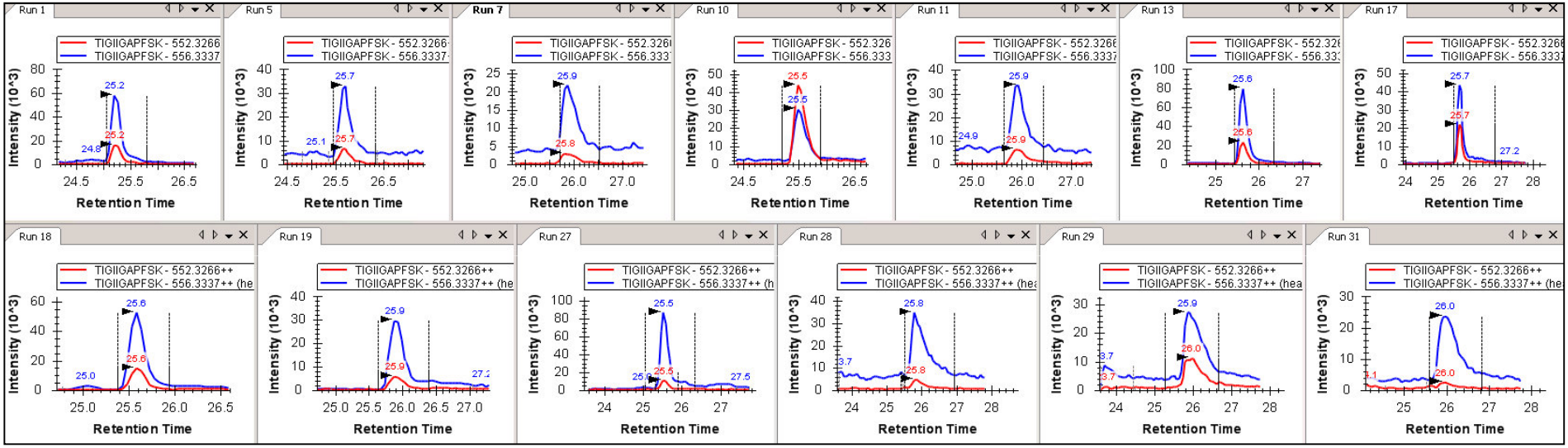
Transitions: y9: blue; y4: red; y7: purple



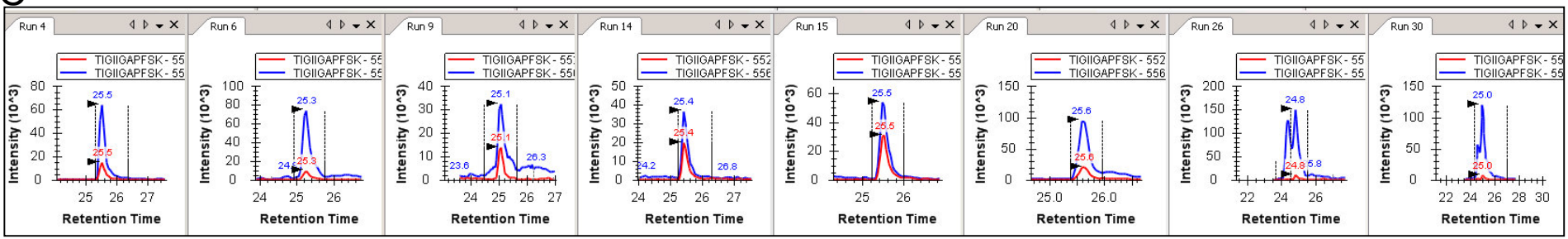
A




B




C




**<u>Supplementary Data 16</u>: MRM chromatograms for peptide TIGIIGAPFSK of ARG1 acquired across the 31 samples of the clinical study**

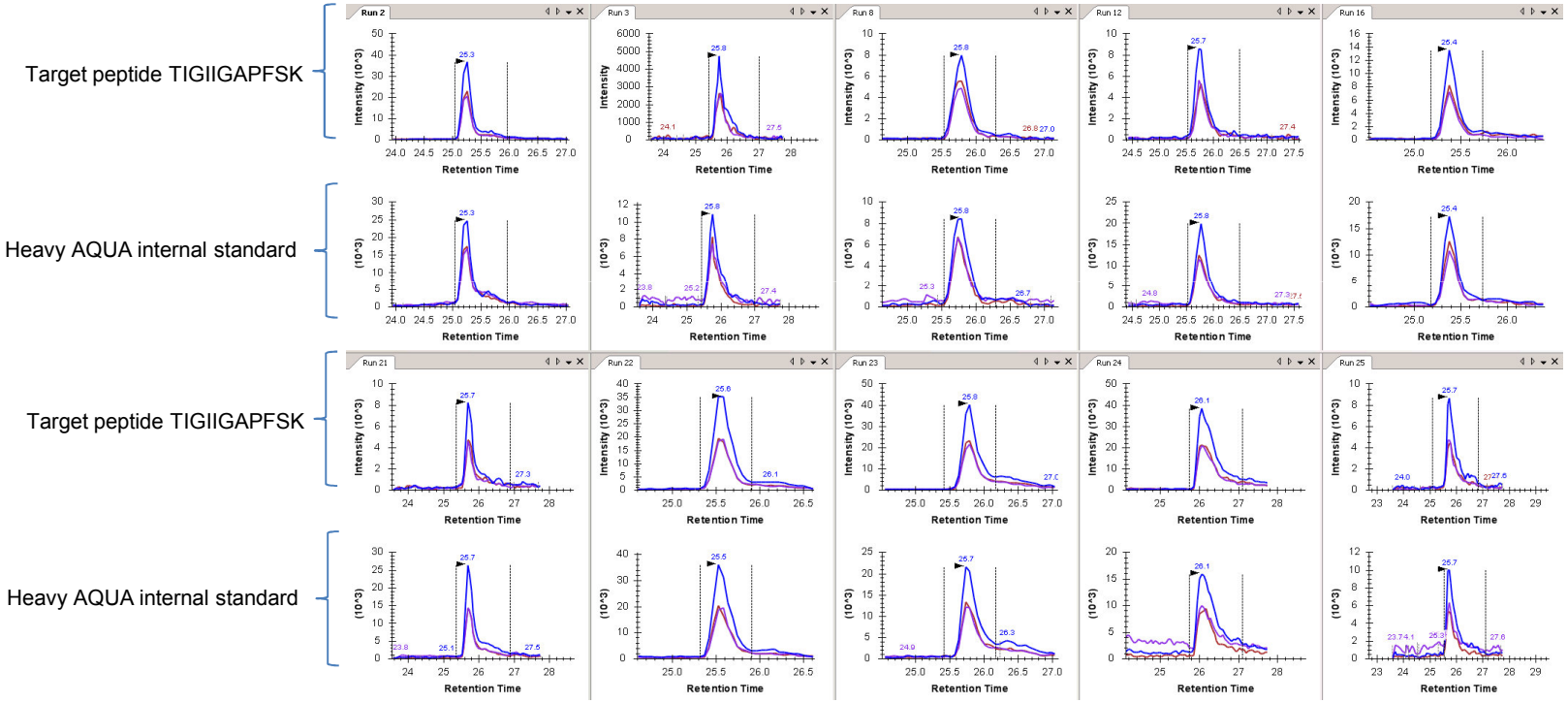
The summed of the 3 transitions monitored for the endogenous peptide (red) is displayed together with the corresponding sum of transitions for the heavy AQUA standard (blue). **A,** samples from healthy group (n=10); **B,** samples from mild group (n=13); **C,** samples from severe group (n=8).

In the 3 slides below, individual transitions for this peptide and for the heavy standard are represented for the 3 different groups.

# TIGIIGAPFSK, ARG1

Transitions: y9: blue; y6: red; y7: purple

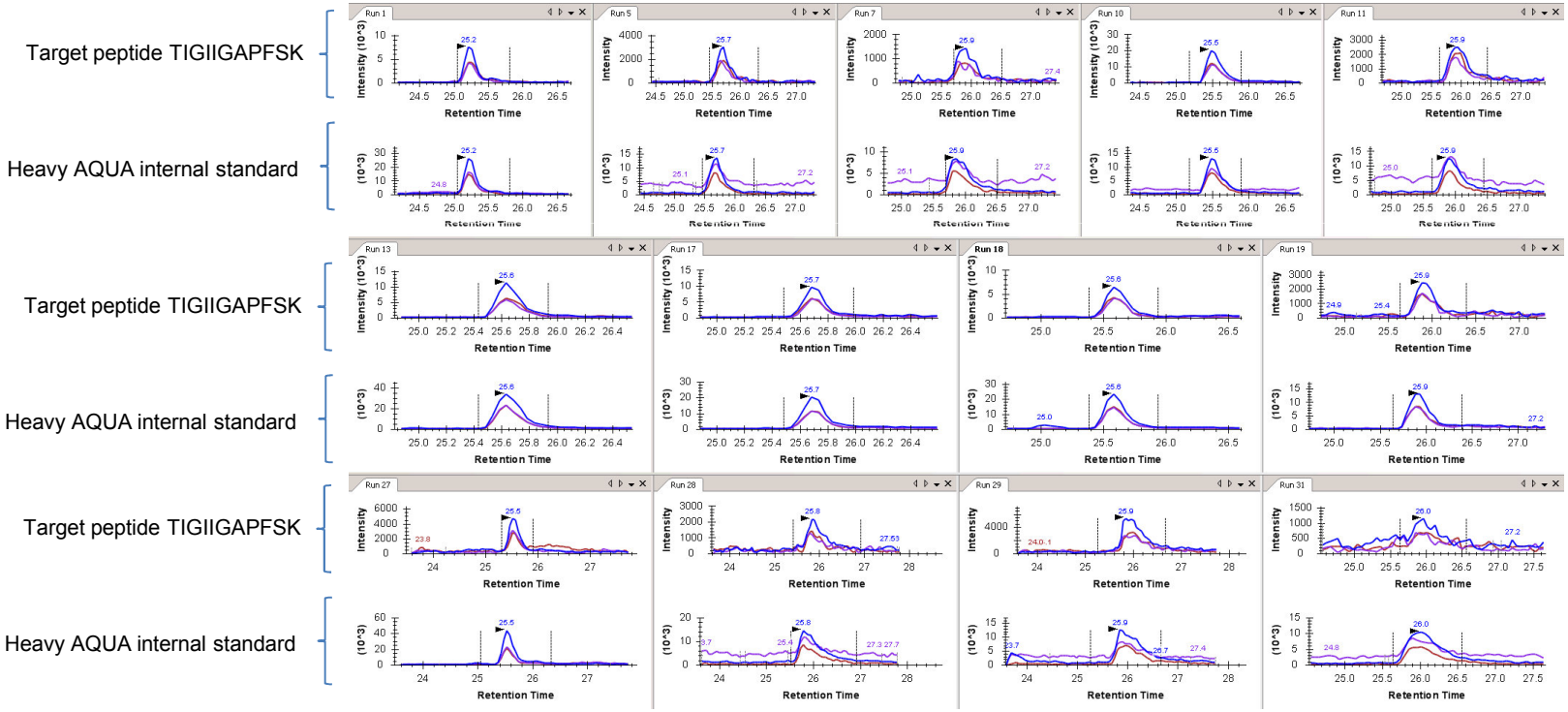
## Group Healthy (n=10)



# TIGIIGAPFSK, ARG1

Transitions: y9: blue; y6: red; y7: purple

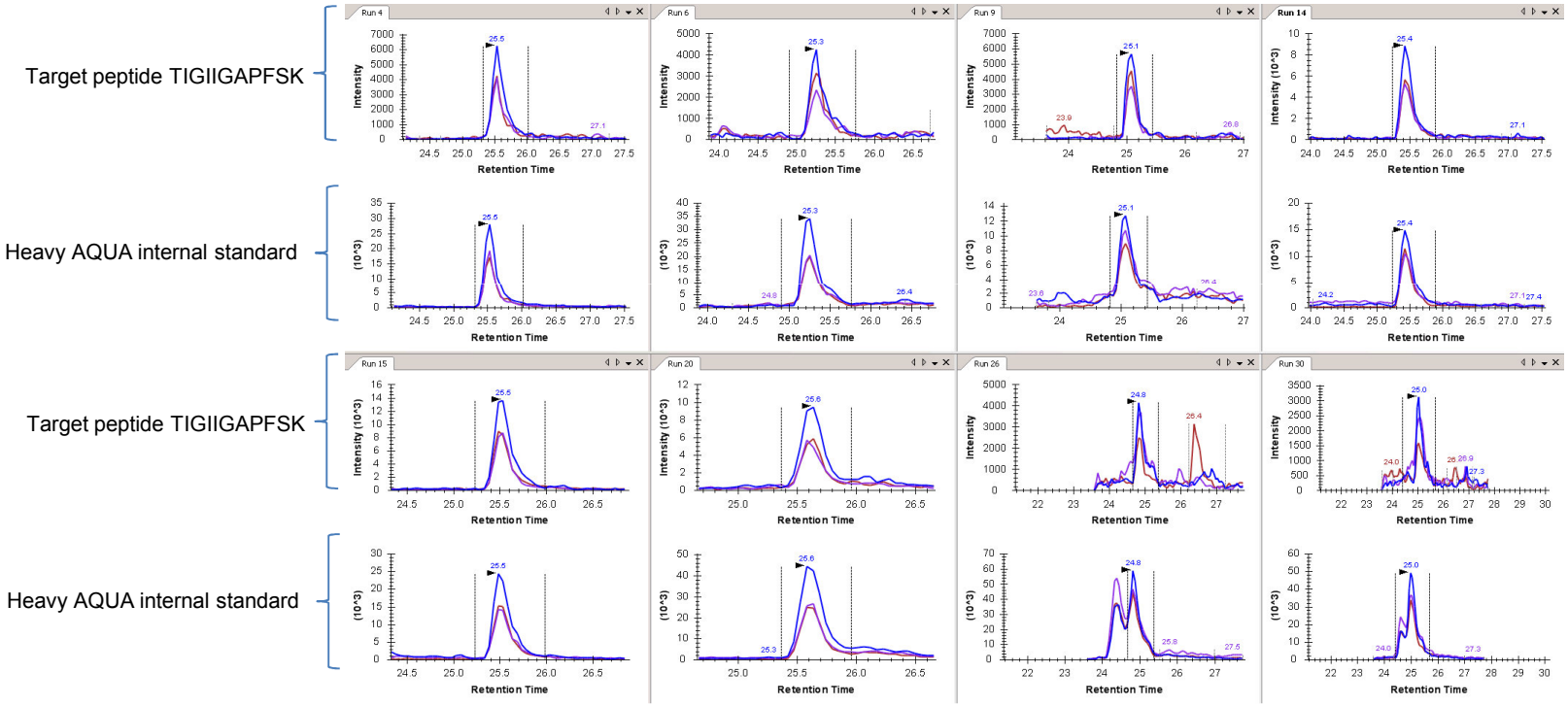
## Group Mild (n=13)



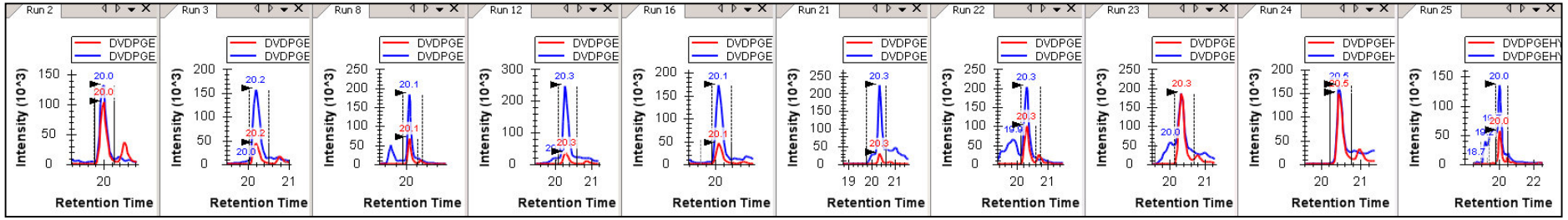
# TIGIIGAPFSK, ARG1

Transitions: y9: blue; y6: red; y7: purple

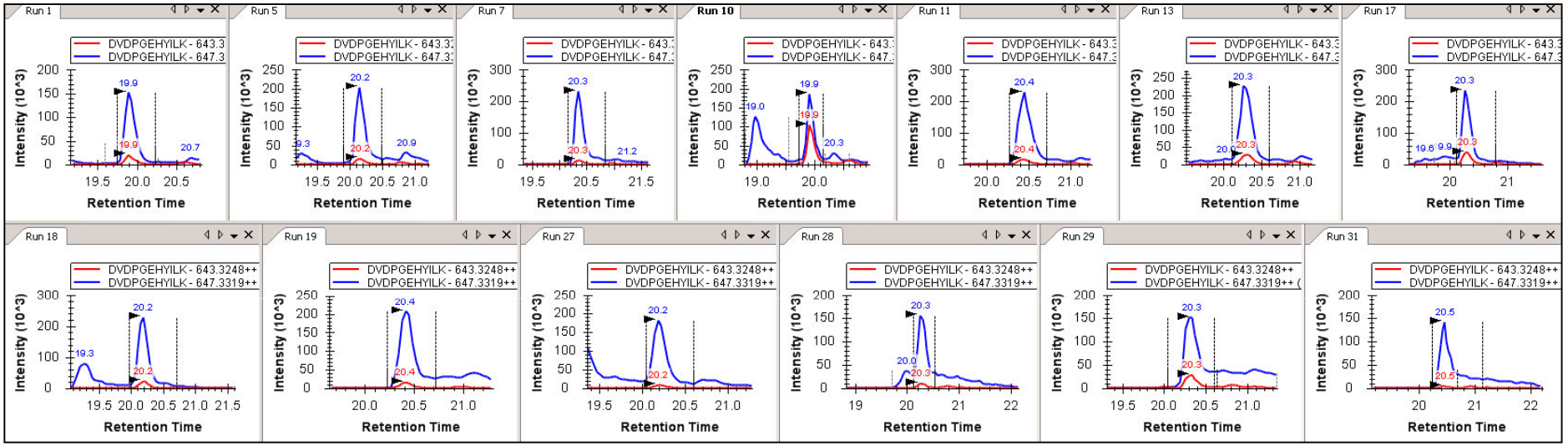
## Group Severe-Bladder (n=8)



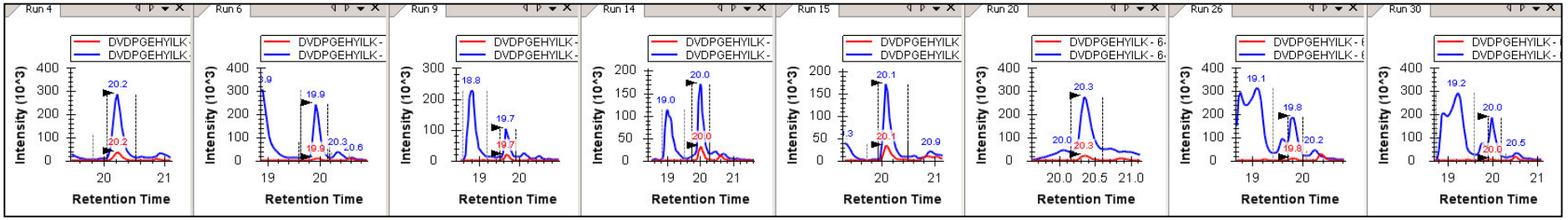
A




B




C




**<u>Supplementary Data 17</u>: MRM chromatograms for peptide DVDPGEHYILK of ARG1 acquired across the 31 samples of the clinical study**

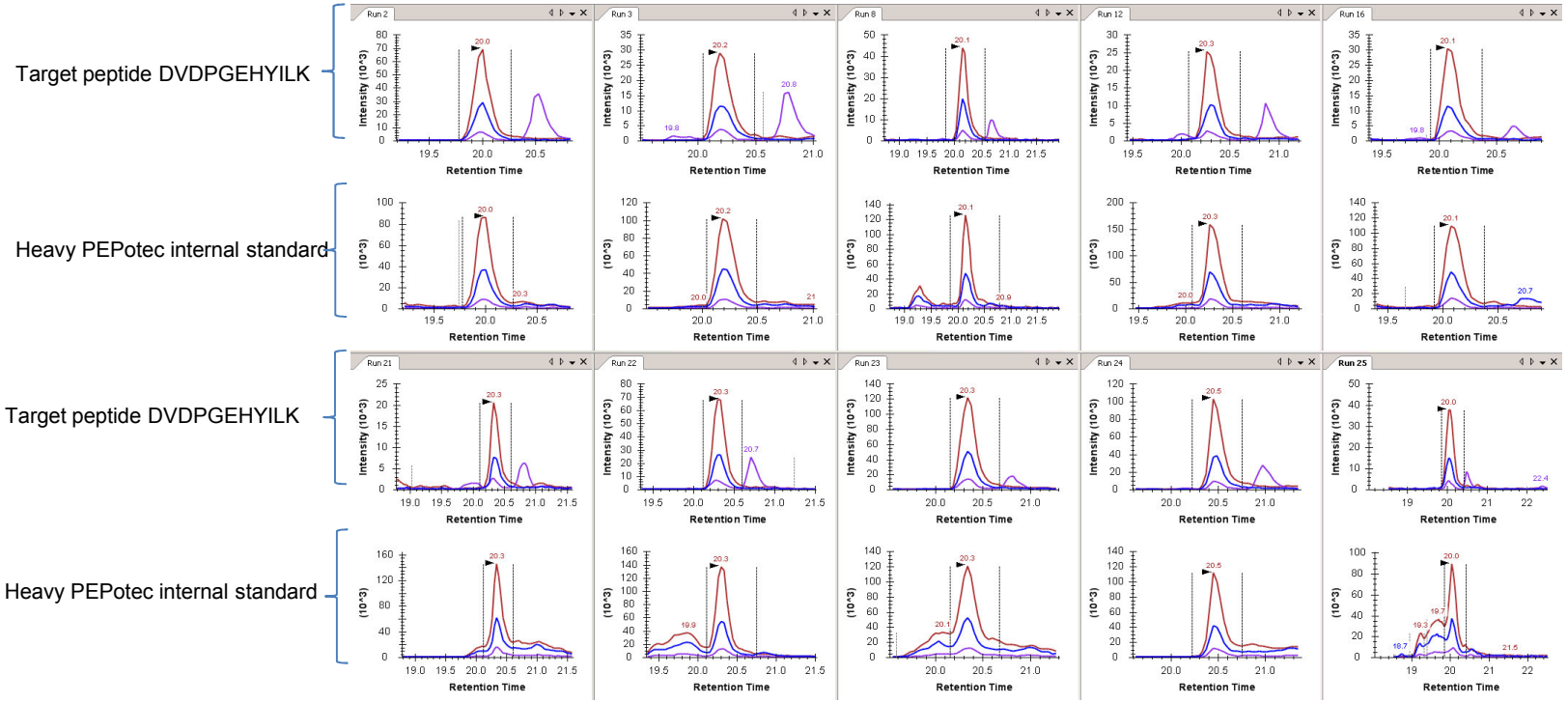
The summed of the 3 transitions monitored for the endogenous peptide (red) is displayed together with the corresponding sum of transitions for the heavy PEPotec standard (blue). **A,** samples from healthy group (n=10); **B,** samples from mild group (n=13); **C,** samples from severe group (n=8).

In the 3 slides below, individual transitions for this peptide and for the heavy standard are represented for the 3 different groups.

# DVDPGEHYILK, ARG1

Transitions: y8++: red; y8: blue; y7: purple

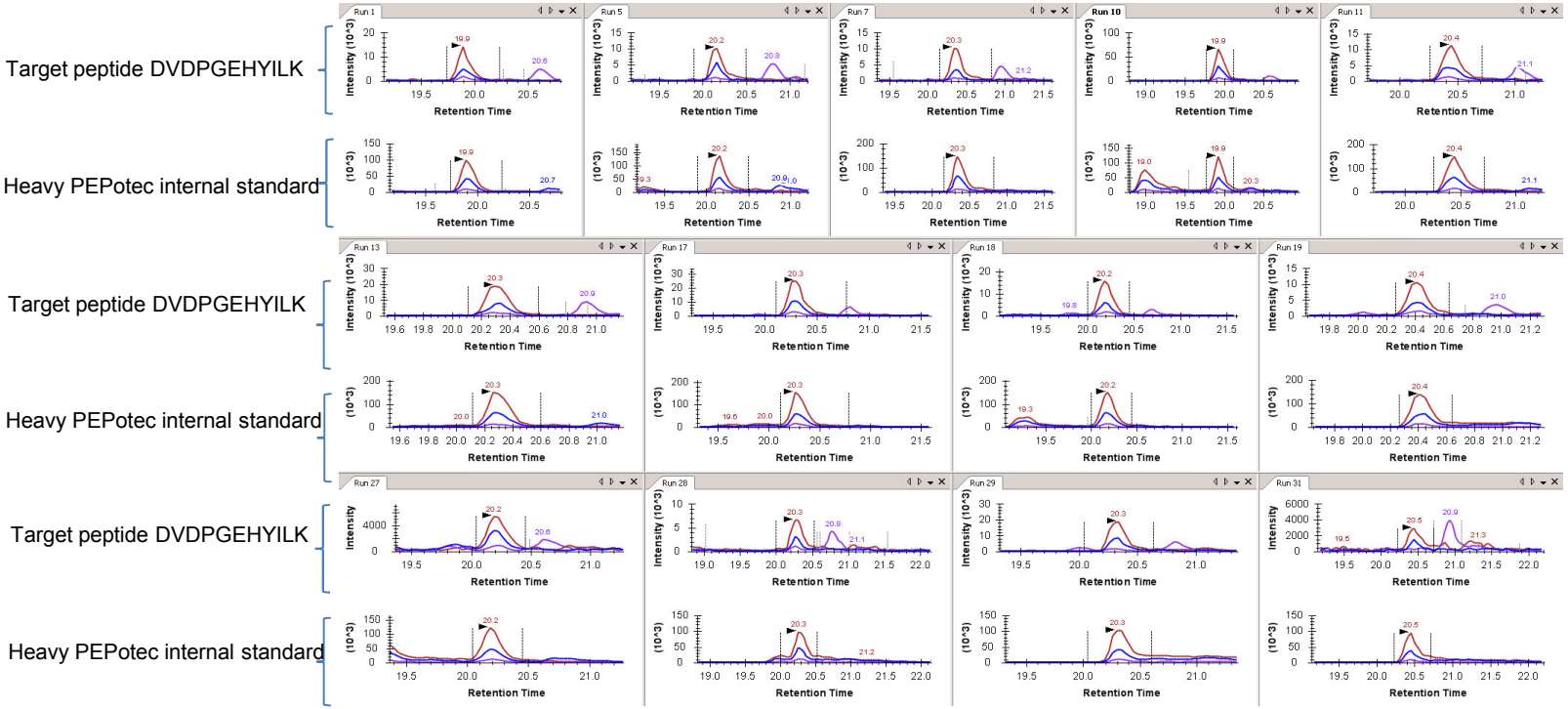
## Group Healthy (n=10)



# DVDPGEHYILK, ARG1

Transitions: y8++: red; y8: blue; y7: purple

## Group Mild (n=13)



# DVDPGEHYILK, ARG1

Transitions: y8++: red; y8: blue; y7: purple

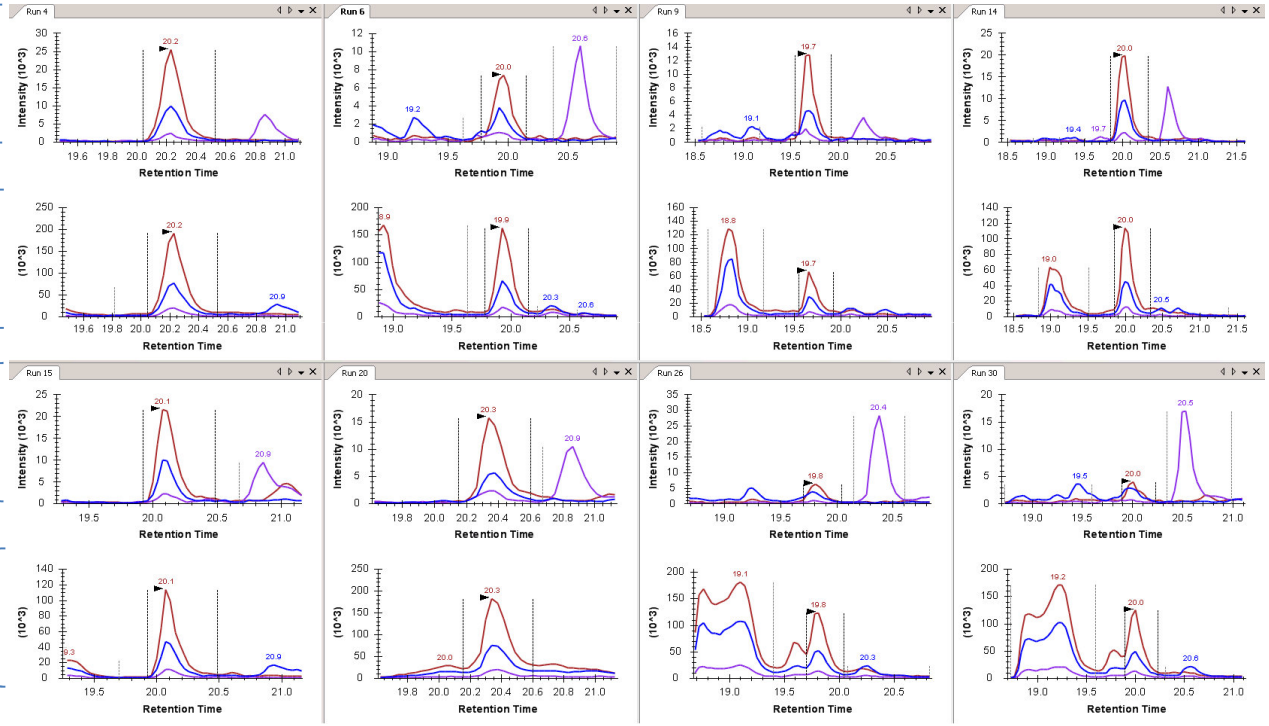
**Group Severe-Bladder (n=8)**

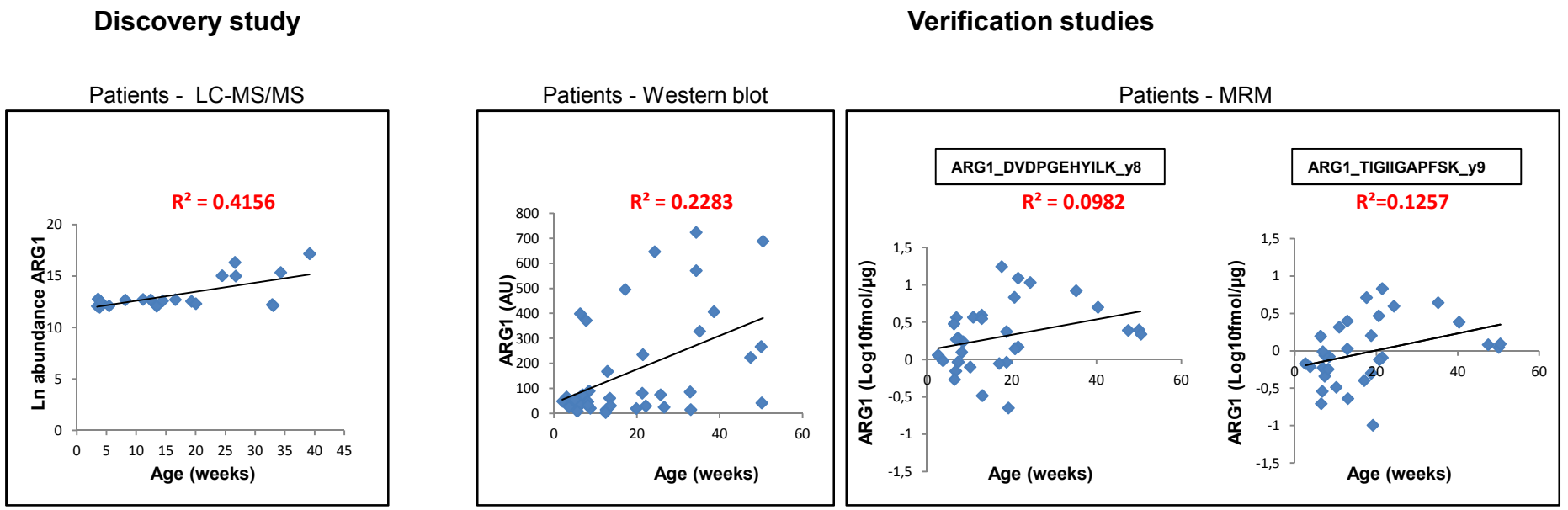
Target peptide DVDPGEHYILK

Heavy PEPotec internal standard

Target peptide DVDPGEHYILK

Heavy PEPotec internal standard






**Supplementary Data 18**: Correlation between the age of the patients and the level of urinary ARG1 found in the discovery and verification studies.