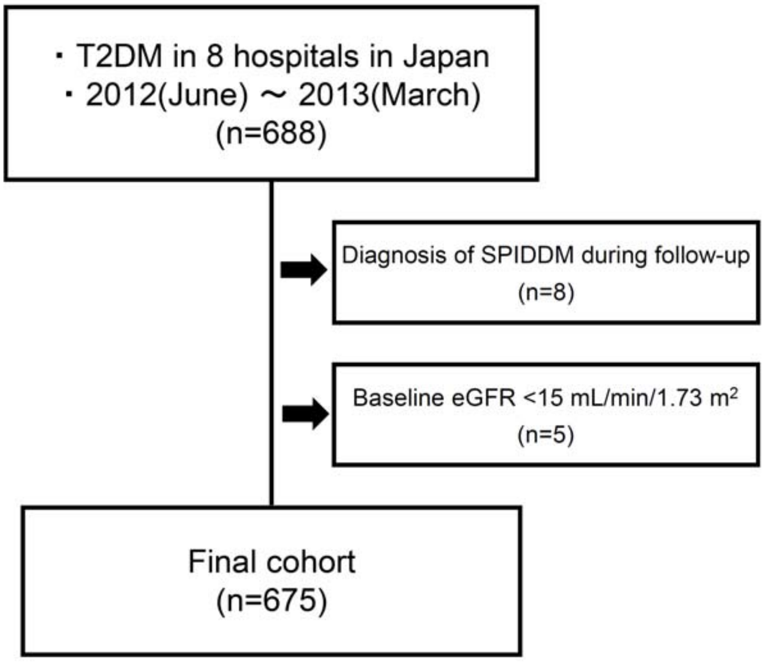
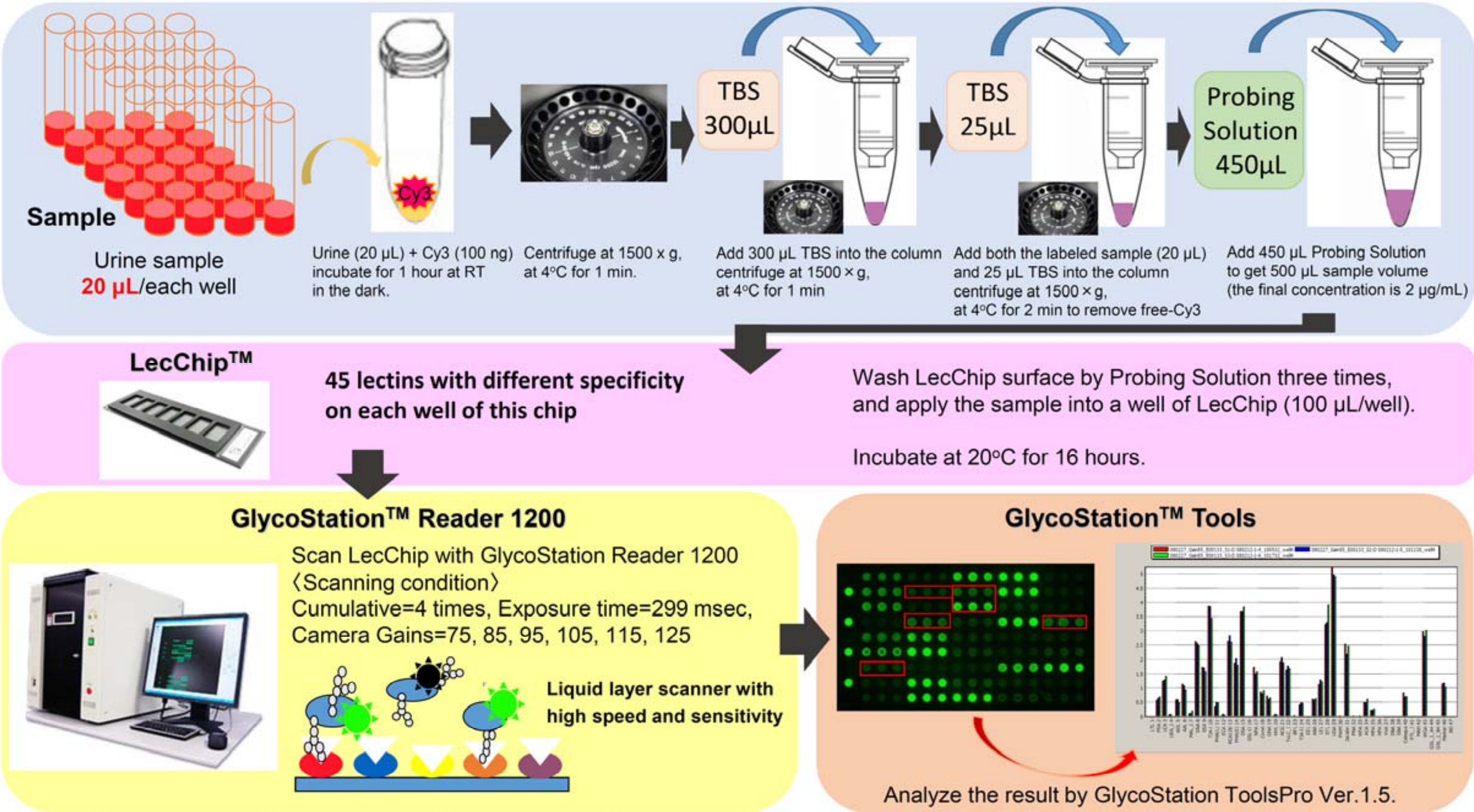
SUPPLEMENTARY DATA

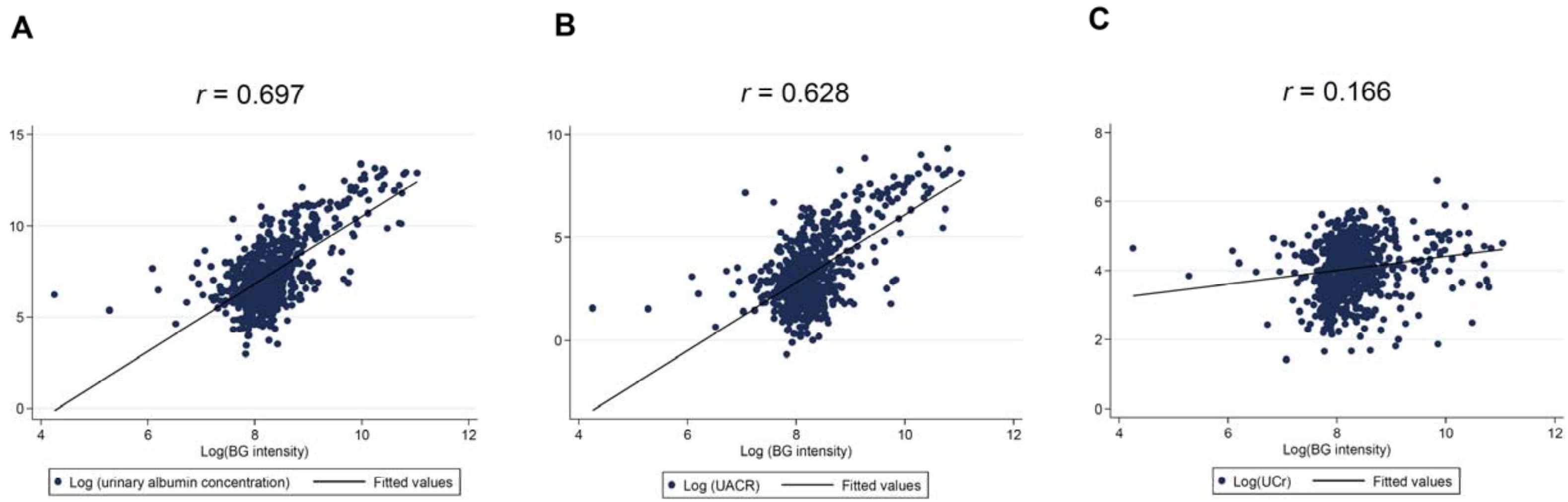
**Supplementary Fig. 1** Flowchart of study participants.
Eight hospitals include Okayama University Hospital, National Hospital Organization Okayama Medical Center, Okayama Saiseikai General Hospital, Kurashiki Central Hospital, The Sakakibara Heart Institute of Okayama, Tsuyama Chuo Hospital, Japanese Red Cross Okayama Hospital, and Okayama City General Medical Center. Abbreviations: T2DM, type 2 diabetes mellitus; SPIDDM, slowly progressive insulindependent (type 1) diabetes mellitus; eGFR, estimated glomerular filtration rate.



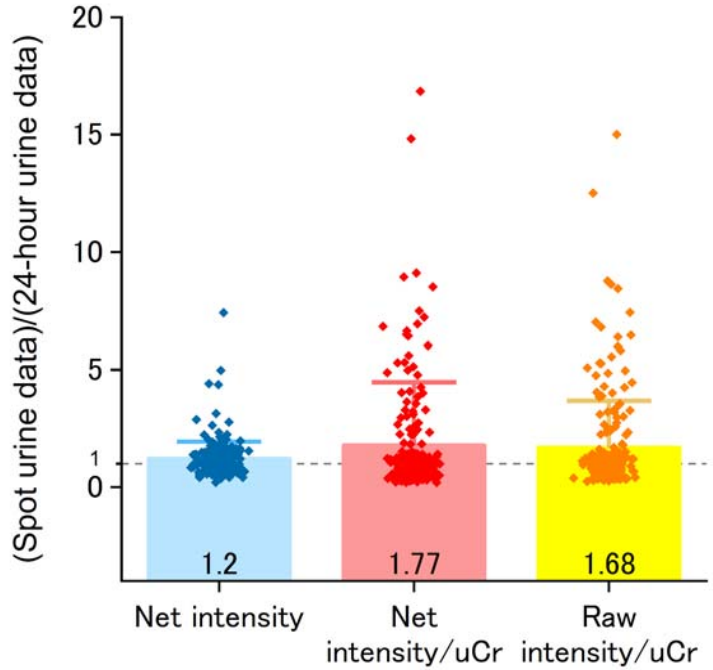
**Supplementary Fig. 2** Urinary glycan profiling system.
The protocol of measuring the intensity of urinary glycan is shown as follows. 1. Add 20 µL of urine sample into a tube containing 100 µg Cy3 (GE Healthcare), mix with a pipette, and incubate for 1 hour at RT in the dark. 2. Put a Zeba Desalt Spin Column (Pierce) in a 2 mL tube, and centrifuge at 1500×g, at 4$^{o}$C for 1 min. 3. Add 300 µL TBS into the column, and centrifuge at 1500×g, at 4$^{o}$C for 1 min. Repeat this process two times more. Put the column in a new 1.5 mL tube, add both the labeled sample (20 µL) and 25µL TBS into the column, and centrifuge at 1500×g, at 4$^{o}$C for 2 min to remove free-Cy3. 4. Recover the sample, and add 450 µL Probing Solution to get 500 µL sample volume (the final concentration is 2 µg/mL). 5. Wash LecChip surface by Probing Solution three times, and apply the sample into a well of LecChip (100 µL/well). 6. Incubate at 20$^{o}$C for 16 hours. 7. Scan LecChip with GlycoStation Reader 1200 (Scanning condition: Cumulative=4 times, Exposure time=299msec, Camera Gains=75, 85, 95, 105, 115, 125) Analyze the result by GlycoStation ToolsPro Ver.1.5. In this system, the measurement of glycan intensity of 300 urine samples required about three working days.

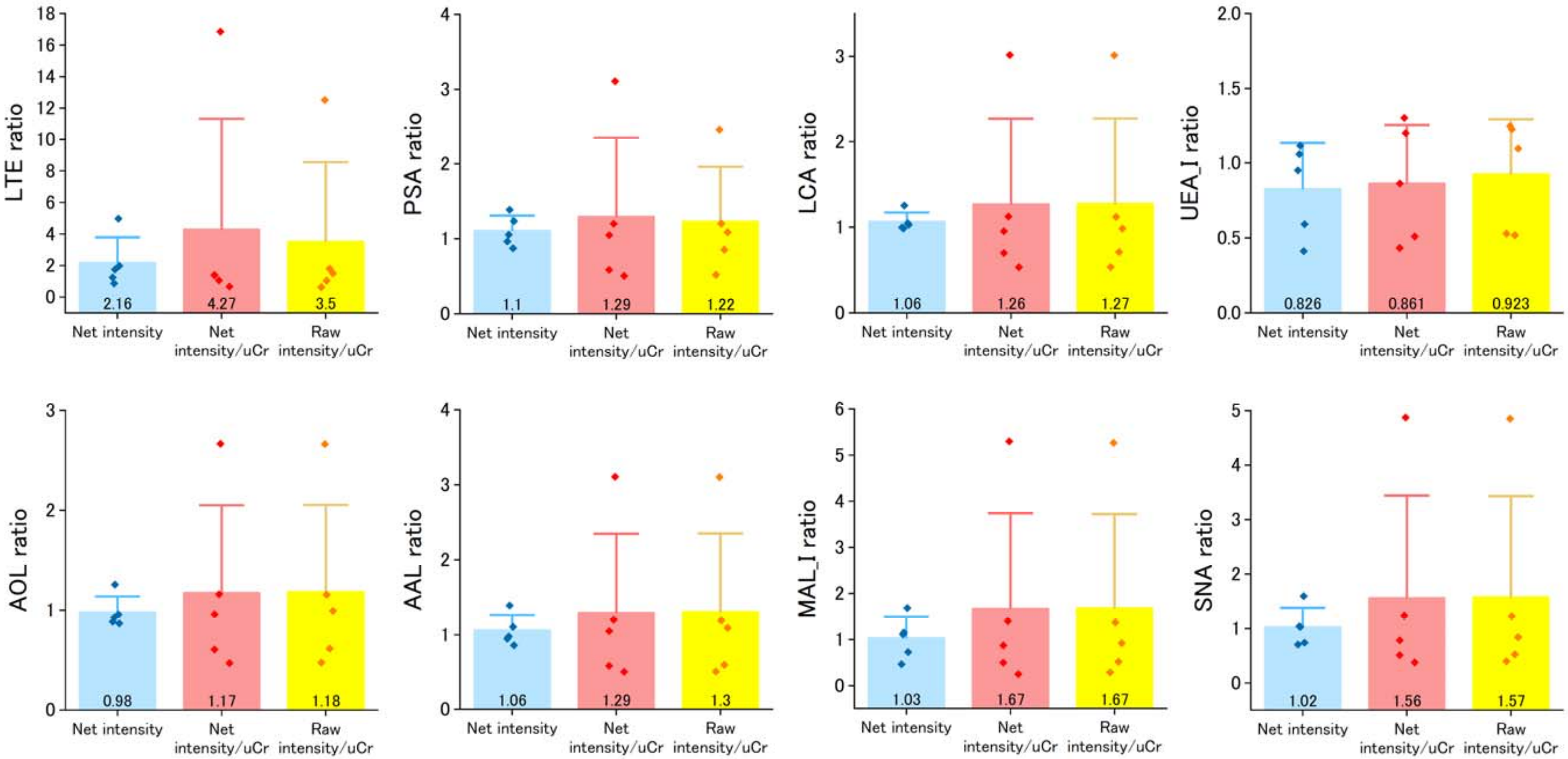


**Supplementary Fig. 3** Correlation coefficient between background intensity and urinary albumin concentration /urinary albumin excretion rate/urinary creatinine concentration. **A.** Correlation between background intensity and urinary albumin concentration (mg/dL). **B.** Correlation between background intensity and UACR (mg/gCr). **C**: Correlation between background intensity and UCr (mg/dL). Urinary albumin concentration (mg/dL) and urinary albumin excretion rate (mg/gCr) were significantly correlated with background intensity (r= 0.697 and 0.628, respectively). Urinary creatinine concentration (mg/dL) was also significantly but weakly correlated with background intensity than those two markers (r= 0.166). Pearson's correlation was employed. Abbreviations: BG, background; UACR, urinary albumin excretion rate; UCr, urinary creatinine concentration.

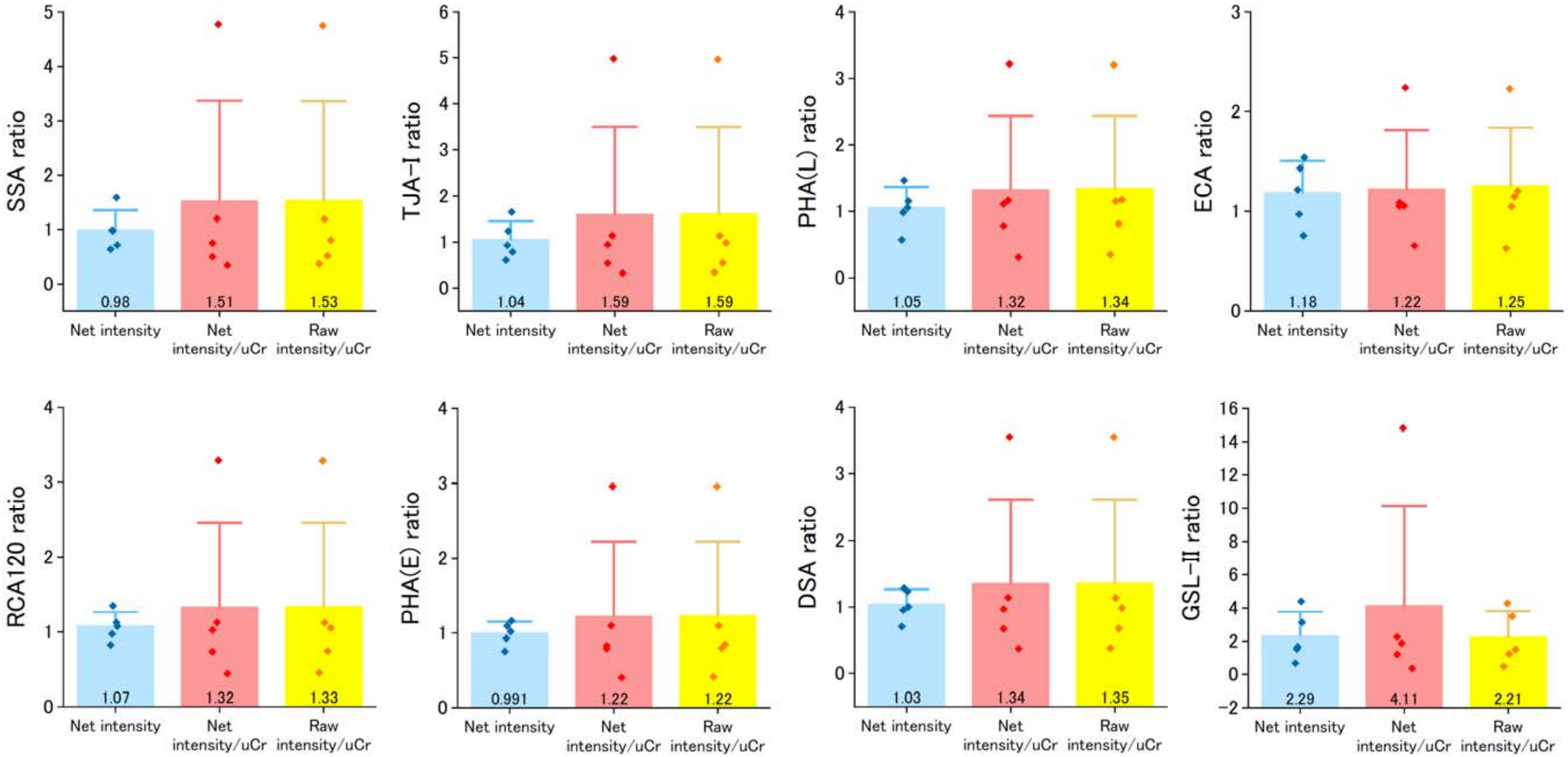


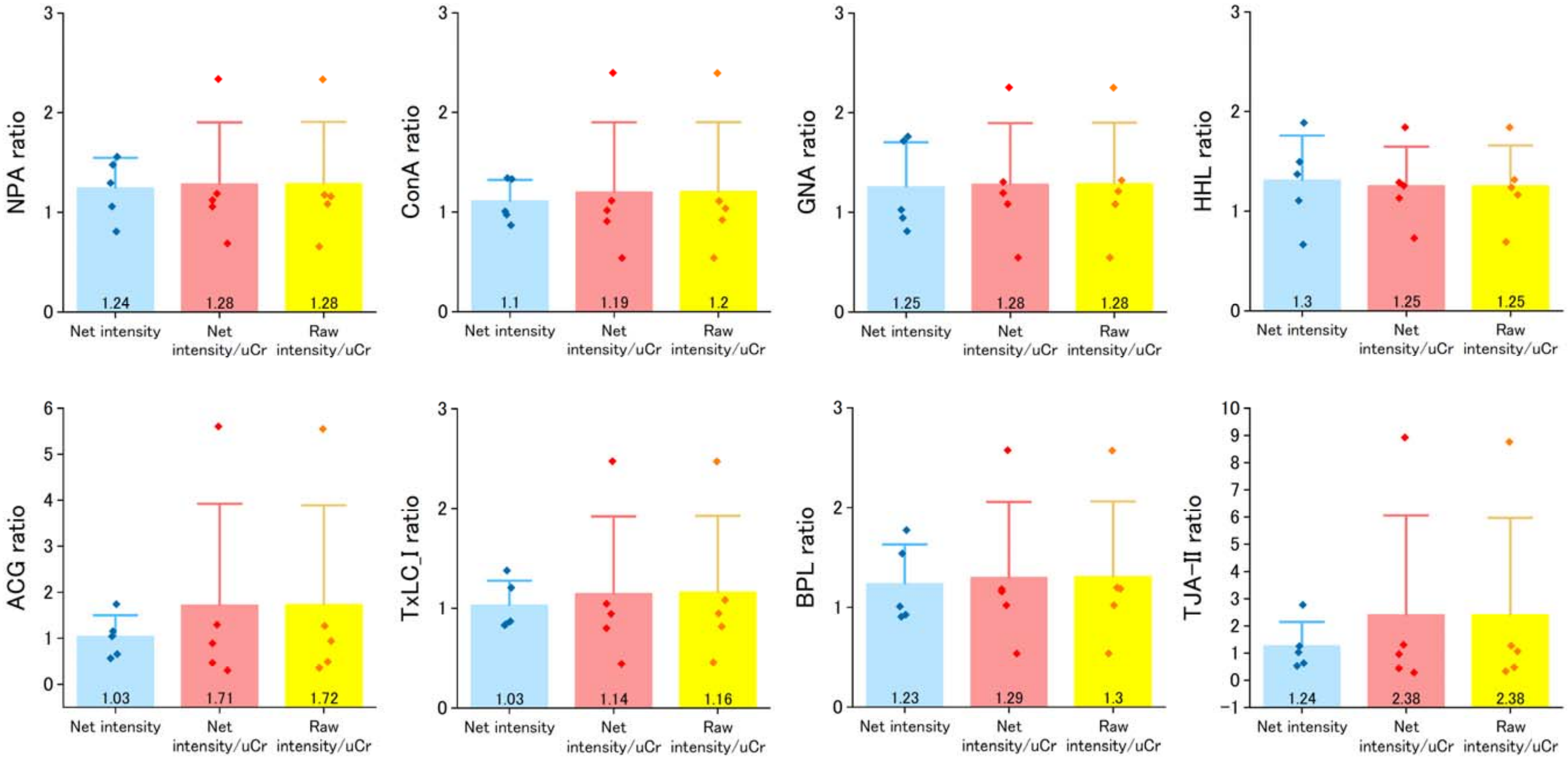
**Supplementary Fig. 4** The strongest correlation between spot urine samples and 24-hour urine samples in the glycan index using net intensity. In healthy 5 subjects, we investigated which spot urine data showed the strongest correlation with 24-hour urine data among the following glycan indexes: [Net intensity (raw glycan intensity – background intensity)], (net intensity)/ (urinary creatinine concentration), and (raw glycan intensity)/ (urinary creatinine concentration). Spot urine data were defined as the mean values from three urine samples collected in the early morning on three consecutive days. In addition, the glycan ratio was also defined as (spot urine data)/ (24-hour urine data), and we compared glycan ratios between the above three units. **A.** Total ratio of (net intensity), (net intensity)/(urinary creatinine concentration), and (raw intensity)/(urinary creatinine concentration) in 225 values (45 glycan intensities in 5 subjects). Total ratio of [net intensity (unit)] (mean ± SD) is 1.20 ± 0.74, total ratio of [net intensity (unit)]/ [urinary creatinine concentration (mg/dL)] 1.77 ± 2.69, and total ratio of [raw intensity (unit)]/ [urinary creatinine concentration (mg/dL)]: 1.68 ± 2.00. The bar and number at the bottom indicates the mean, and the whisker shows the standard deviation. Abbreviation: uCr: urinary creatinine concentration. **B.** Ratios [(spot urine data)/(24-hour urine data)] in glycan indexes for 45 lectins. In addition to the mean and standard deviation of the total glycan ratio, each glycan ratio was closest to "1" and the variability was smallest with net intensity. Therefore, we employed net intensity in all analyses. The bar and number at the bottom indicates the mean, and the whisker shows the standard deviation. Ratio indicates (spot urine data)/ (24-hour urine data). Abbreviation: uCr: urinary creatinine concentration.

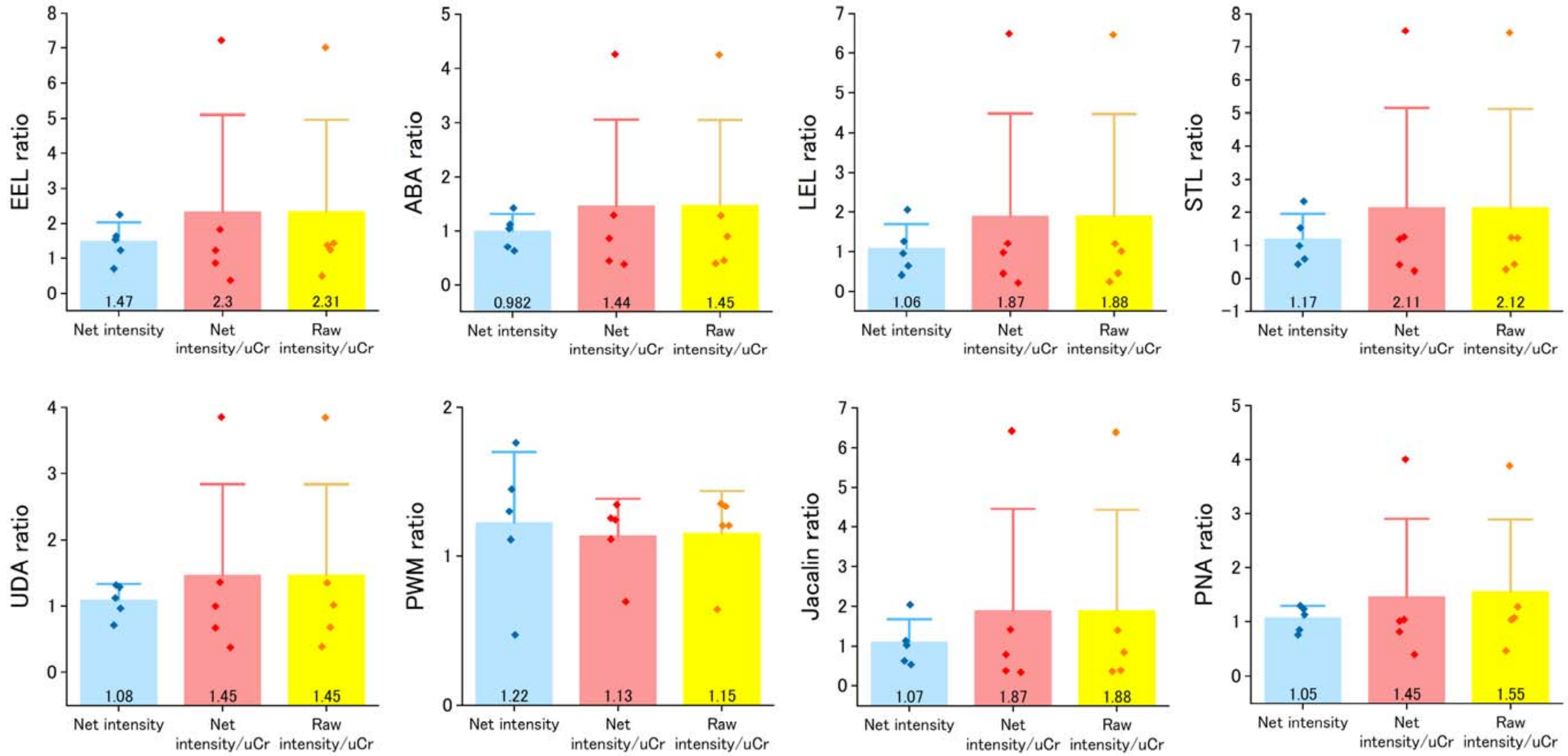


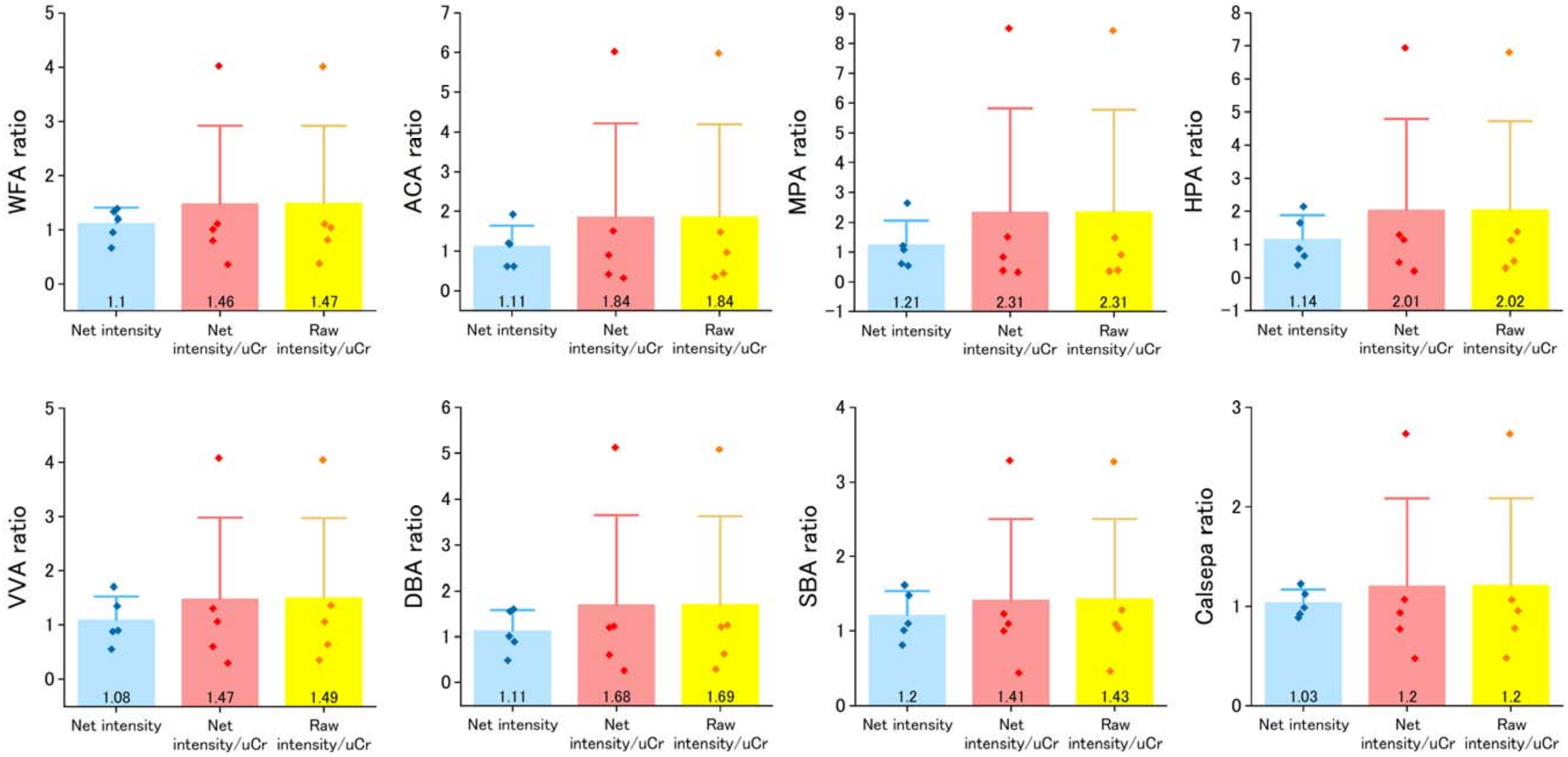

LTE ratio
2.16
4.27
3.5
PSA ratio
1.1
1.29
1.22
LCA ratio
1.06
1.26
1.27
UEA_I ratio
0.826
0.861
0.923
AOL ratio
0.98
1.17
1.18
AAL ratio
1.06
1.29
1.3
MAL_I ratio
1.03
1.67
1.67
SNA ratio
1.02
1.56
1.57
Net intensity
Net intensity/uCr
Raw intensity/uCr

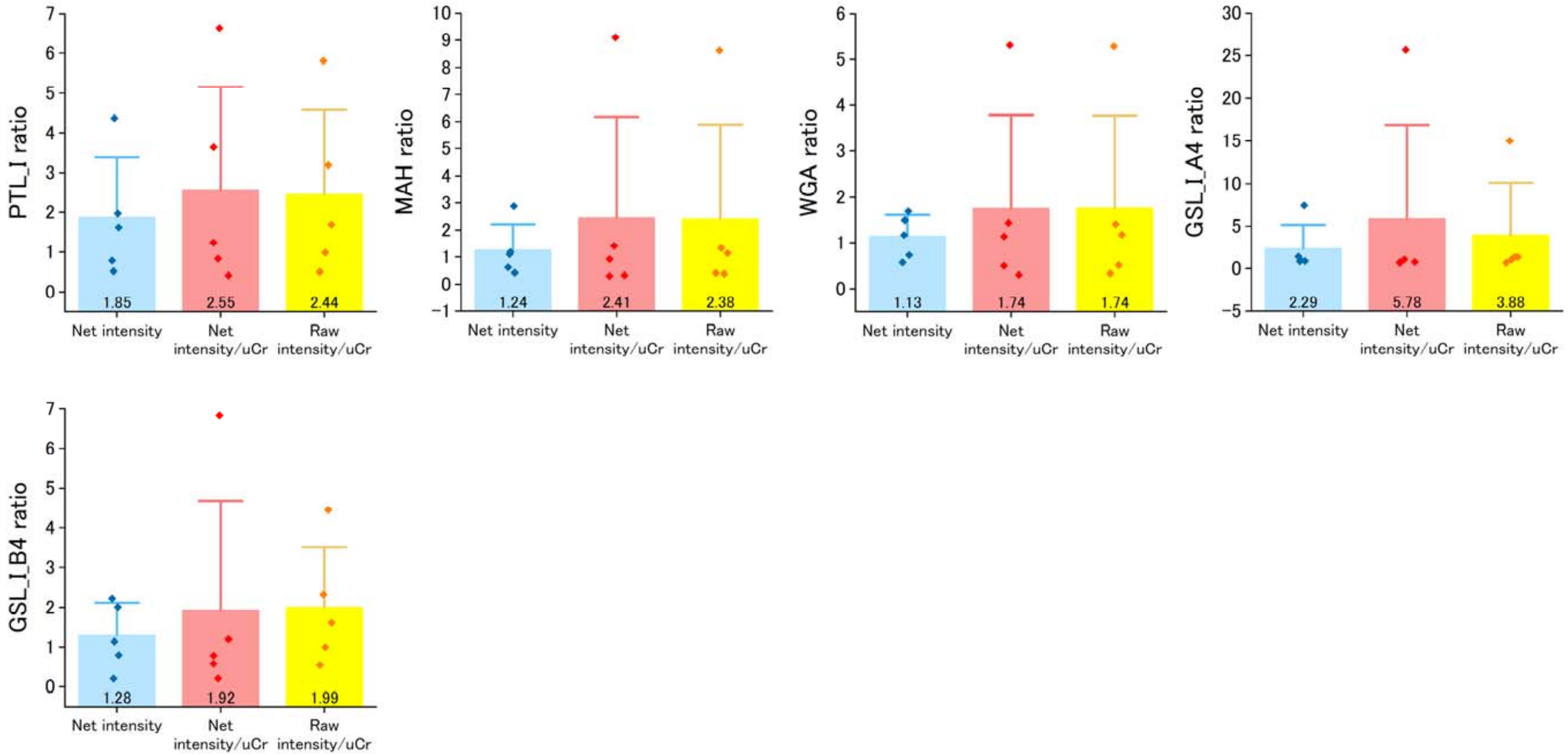
SUPPLEMENTARY DATA



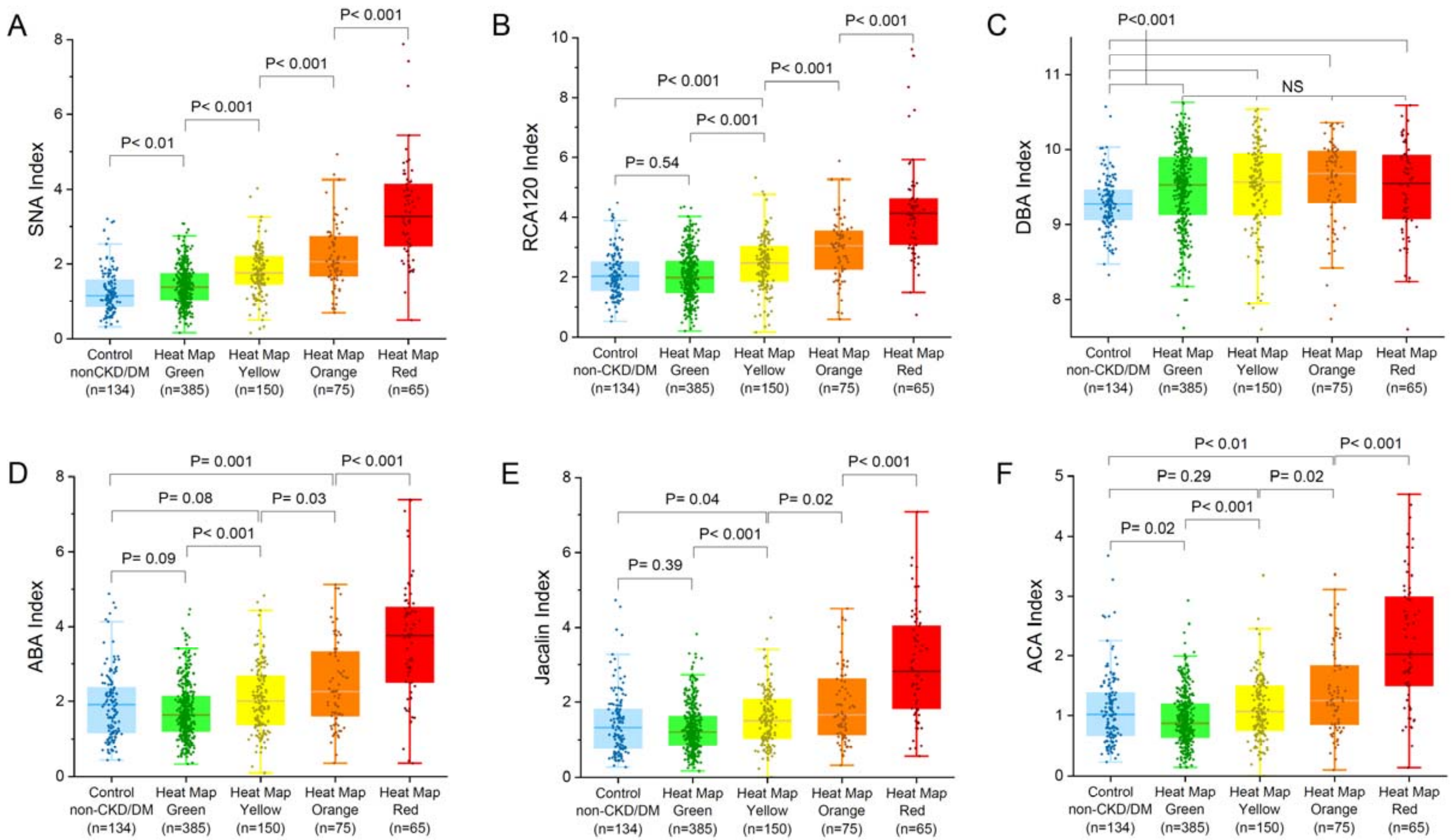

NPA ratio
1.24
1.28
1.28
ConA ratio
1.1
1.19
1.2
GNA ratio
1.25
1.28
1.28
HHL ratio
1.3
1.25
1.25
ACG ratio
1.03
1.71
1.72
TxLC_I ratio
1.03
1.14
1.16
BPL ratio
1.23
1.29
1.3
TJA-II ratio
1.24
2.38
2.38
Net intensity
Net intensity/uCr
Raw intensity/uCr


EEL ratio
1.47
2.3
2.31
ABA ratio
0.982
1.44
1.45
LEL ratio
1.06
1.87
1.88
STL ratio
1.17
2.11
2.12
UDA ratio
1.08
1.45
1.45
PWM ratio
1.22
1.13
1.15
Jacalin ratio
1.07
1.87
1.88
PNA ratio
1.05
1.45
1.55
Net intensity
Net intensity/uCr
Raw intensity/uCr


WFA ratio
1.1
1.46
1.47
ACA ratio
1.11
1.84
1.84
MPA ratio
1.21
2.31
2.31
HPA ratio
1.14
2.01
2.02
VVA ratio
1.08
1.47
1.49
DBA ratio
1.11
1.68
1.69
SBA ratio
1.2
1.41
1.43
Calsepa ratio
1.03
1.2
1.2
Net intensity
Net intensity/uCr
Raw intensity/uCr


PTL_I ratio
1.85
2.55
2.44
MAH ratio
1.24
2.41
2.38
WGA ratio
1.13
1.74
1.74
GSL_I_A4 ratio
2.29
5.78
3.88
GSL_I_B4 ratio
1.28
1.92
1.99
Net intensity
Net intensity/uCr
Raw intensity/uCr

**Supplementary Fig. 5** Urinary glycan intensities of SNA, RCA120, DBA, ABA, Jacalin, and ACA in classification of CKD based on eGFR and albuminuria categories. **A.** Glycan indexes for binding to SNA, RCA120, DBA, ABA, Jacalin, and ACA in the control subjects and patients with diabetes stratified according to CKD heat map. Glycan indexes for SNA, RCA120, ABA, Jacalin, and ACA were significantly higher in the severer CKD heat map group than in the milder groups. The glycan index of DBA was significantly higher in the diabetic patients than in the controls, but there were no significant differences among the CKD heat map groups. It is worth to note that the glycan index for SNA was significantly higher in Green group than in the controls (blue), even though both groups demonstrated normoalbuminuria and eGFR > 60 mL/min/1.73m$^2$. The control group included 134 volunteers without CKD or diabetes. Boxes show the interquartile range and whiskers show outliers with a coefficient of 1.5. **B.** Glycan indexes for binding to SNA, RCA120, DBA, ABA, Jacalin, and ACA in patients with diabetes stratified according to categories of baseline albuminuria and eGFR. Glycan indexes for SNA, RCA120, ABA, Jacalin, and ACA were higher in the severe categories of albuminuria and eGFR than in the milder categories. No one was classified into a category of normoalbuminuria and eGFR <30 mL/min/1.73m$^2$. Number in each category indicates median (interquartile range). Abbreviations: SNA, *Sambucus nigra*; RCA120, *Ricinus communis*; DBA, *Dolichos biflorus*; ABA, *Agaricus bisporus*; Jacalin, *Artocarpus integrifolia*; ACA, *Amaranthus caudatus*; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.



| SNA Index | | Albuminuria Categories (mg/gCr) | | |
|---|---|---|---|---|
| | | Normo (<30) | Micro (30-299) | Macro (>300) |
| eGFR Categories (ml/min/1.73$m^2$) | eGFR >60 | 1.38 (1.04, 1.74) | 1.82 (1.49, 2.21) | 2.28 (1.81, 3.11) |
| | eGFR 45-59 | 1.54 (0.92, 2.18) | 2.12 (1.78, 2.68) | 3.34 (2.70, 4.02) |
| | eGFR 30-44 | 1.62 (1.10, 1.92) | 2.08 (1.79, 2.23) | 3.53 (2.80, 3.84) |
| | eGFR <30 | – | 2.35 (2.17, 2.73) | 4.41 (3.81, 5.45) |

| RCA120 Index | | Albuminuria Categories (mg/gCr) | | |
|---|---|---|---|---|
| | | Normo (<30) | Micro (30-299) | Macro (>300) |
| eGFR Categories (ml/min/1.73$m^2$) | eGFR >60 | 1.98 (1.49, 2.53) | 2.49 (1.89, 2.93) | 3.09 (2.46, 3.74) |
| | eGFR 45-59 | 2.19 (0.85, 3.28) | 3.12 (2.61, 3.41) | 4.21 (3.32, 4.62) |
| | eGFR 30-44 | 2.20 (1.63, 3.07) | 2.78 (2.44, 3.08) | 4.06 (3.74, 4.40) |
| | eGFR <30 | – | 3.24 (2.97, 3.82) | 5.07 (4.35, 7.58) |

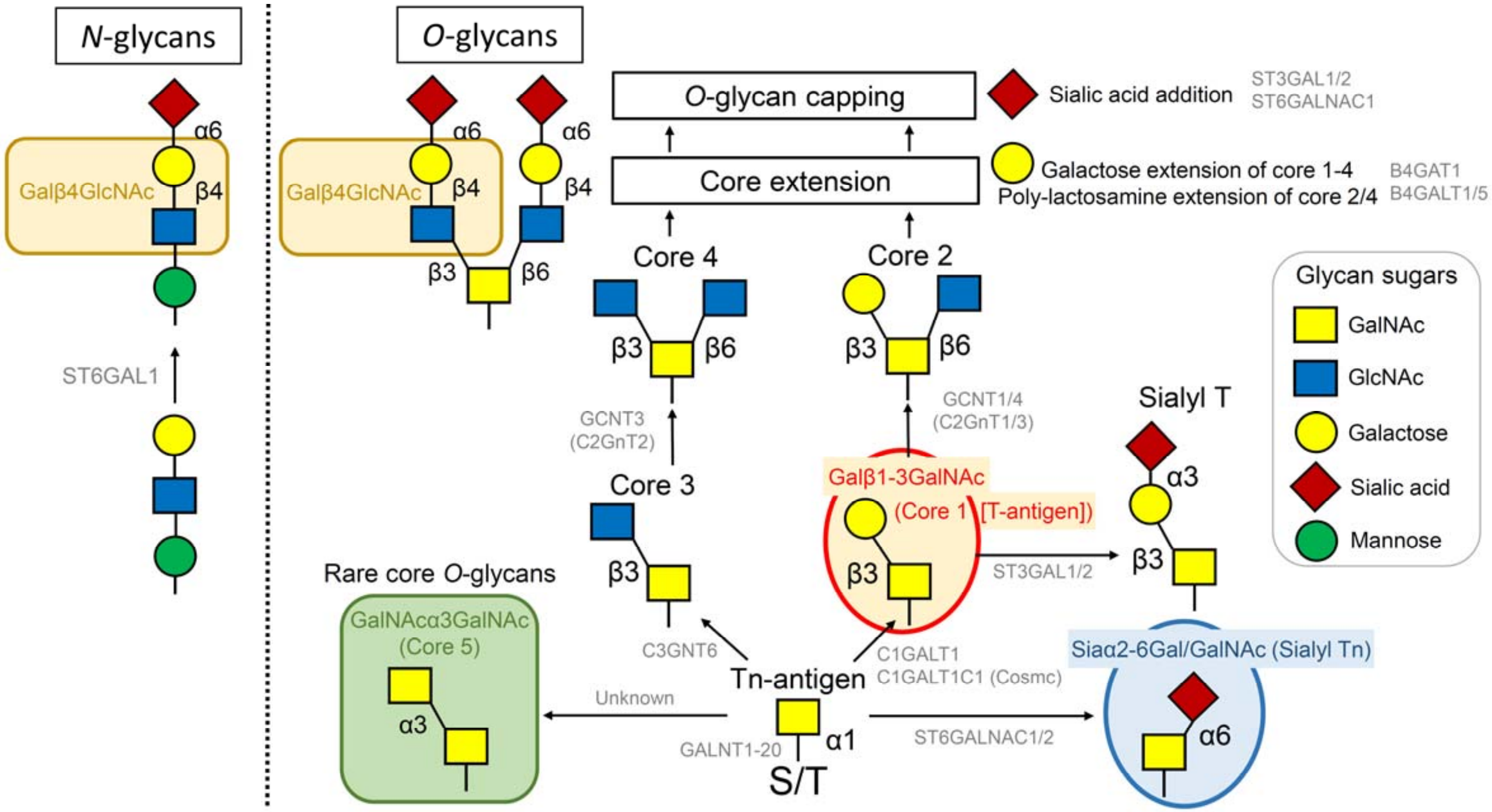
| DBA Index | | Albuminuria Categories (mg/gCr) | | |
|---|---|---|---|---|
| | | Normo (<30) | Micro (30-299) | Macro (>300) |
| eGFR Categories (ml/min/1.73$m^2$) | eGFR >60 | 9.53 (9.14, 9.89) | 9.57 (9.13, 9.93) | 9.68 (9.04, 9.91) |
| | eGFR 45-59 | 9.56 (8.57, 10.02) | 9.68 (9.48, 10.00) | 9.41 (9.07, 10.03) |
| | eGFR 30-44 | 9.50 (8.87, 9.85) | 9.91 (9.75, 10.00) | 9.40 (8.97, 10.09) |
| | eGFR <30 | – | 9.75 (9.24, 10.22) | 9.42 (8.94, 9.74) |

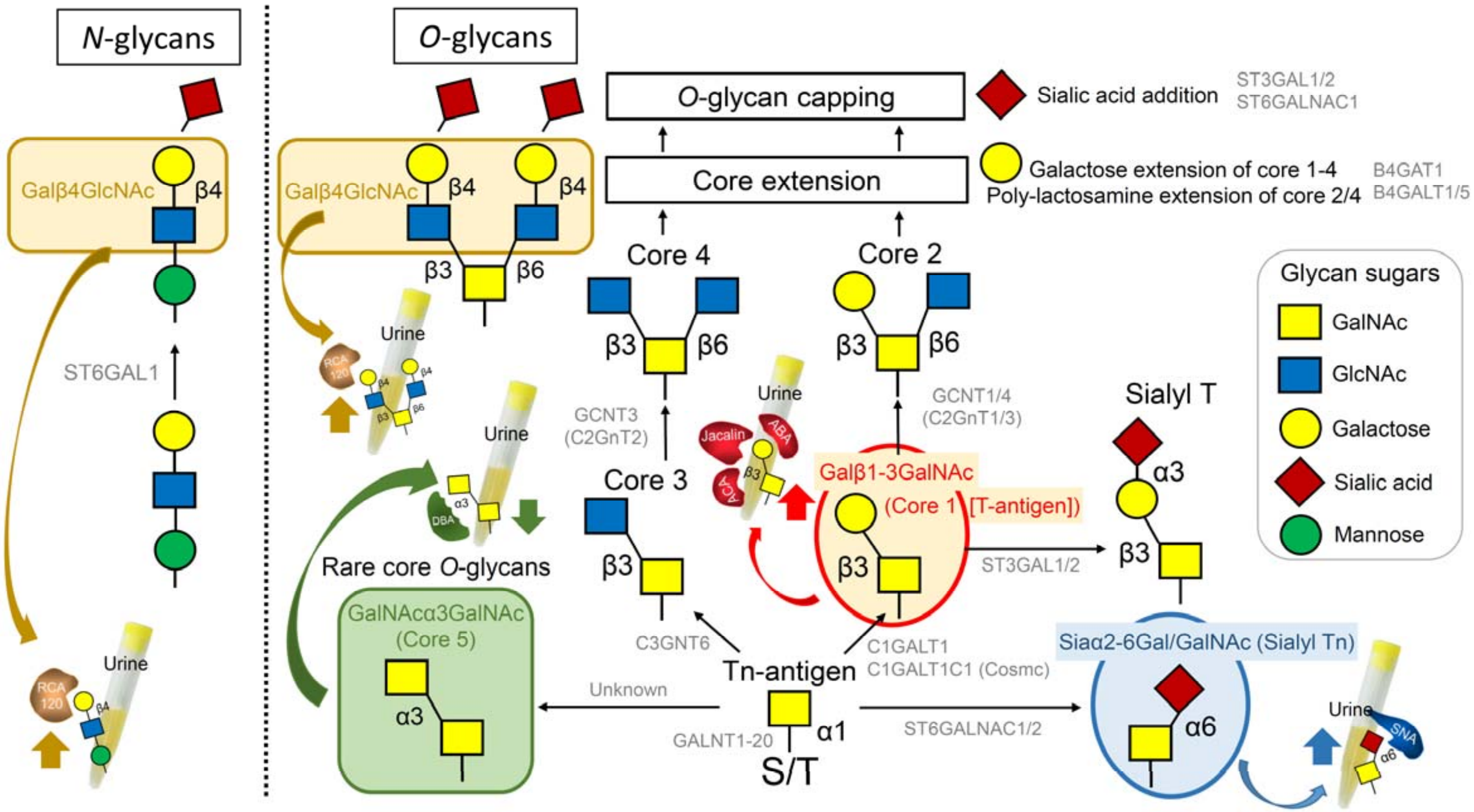
| ABA Index | | Albuminuria Categories (mg/gCr) | | |
|---|---|---|---|---|
| | | Normo (<30) | Micro (30-299) | Macro (>300) |
| eGFR Categories (ml/min/1.73$m^2$) | eGFR >60 | 1.63 (1.21, 2.13) | 1.99 (1.38, 2.68) | 1.87 (1.40, 2.64) |
| | eGFR 45-59 | 2.10 (1.21, 2.71) | 2.74 (1.67, 3.80) | 3.13 (2.23, 4.18) |
| | eGFR 30-44 | 2.17 (1.30, 3.25) | 2.50 (1.75, 2.90) | 4.14 (3.15, 4.47) |
| | eGFR <30 | – | 2.35 (1.80, 3.58) | 5.12 (3.93, 6.42) |

| Jacalin Index | | Albuminuria Categories (mg/gCr) | | |
|---|---|---|---|---|
| | | Normo (<30) | Micro (30-299) | Macro (>300) |
| eGFR Categories (ml/min/1.73$m^2$) | eGFR >60 | 1.21 (0.86, 1.61) | 1.51 (1.05, 2.13) | 1.61 (1.14, 2.15) |
| | eGFR 45-59 | 1.45 (0.83, 1.82) | 2.24 (1.28, 2.96) | 2.35 (1.57, 3.32) |
| | eGFR 30-44 | 1.55 (0.81, 2.20) | 1.83 (1.30, 2.48) | 3.42 (2.71, 4.22) |
| | eGFR <30 | – | 2.01 (1.57, 2.32) | 4.41 (3.82, 5.31) |

| ACA Index | | Albuminuria Categories (mg/gCr) | | |
|---|---|---|---|---|
| | | Normo (<30) | Micro (30-299) | Macro (>300) |
| eGFR Categories (ml/min/1.73$m^2$) | eGFR >60 | 0.87 (0.64, 1.19) | 1.10 (0.80, 1.50) | 1.17 (0.86, 1.81) |
| | eGFR 45-59 | 1.03 (0.60, 1.54) | 1.42 (0.89, 2.29) | 1.73 (1.09, 2.53) |
| | eGFR 30-44 | 1.23 (0.76, 1.50) | 1.15 (0.95, 1.73) | 2.62 (1.92, 3.14) |
| | eGFR <30 | – | 1.31 (1.04, 1.98) | 3.06 (2.75, 3.82) |

**Supplementary Fig. 6.** Mechanism of glycosylation and putative relationships between modification of glycans and urinary glycan excretion. **A**. Various glycosylation processes required to create mature *O*-glycans. If some of these glycosylation processes are impaired, normal *O*-glycans decrease, which could lead to increased urinary excretion of *O*-glycan components and the terminal epitopes of the immature *O*-glycans. **B**. Mechanisms for increased urinary excretions of recognized glycans. The components/epitopes of immature *O*-glycans, such as Siaα2-6Gal/GalNAc, Galβ3GalNAc, and Galβ4GlcNAc, could be recognized by specific lectins, such as SNA, ABA, Jacalin, ACA, and RCA120. Similarly, Galβ4GlcNAc (recognized by RCA120) could be the terminal epitope of immature *N*-glycans that would be excreted if sialylation is impaired. GalNAcα3GalNAc (Core 5) is one of the rare *O*-glycan cores and is recognized by DBA, although the relevant glycosyltransferase is unknown at present. The reason for lower urinary excretion of GalNAcα3GalNAc being associated with progression of DKD remains unclear, but the mechanism might not be related to abnormal glycosylation in the main process of *O*-glycan production. Gray fonts indicate glycosyltransferases. Abbreviations: SNA, *Sambucus nigra*; RCA120, *Ricinus communis*; DBA, *Dolichos biflorus*; ABA, *Agaricus bisporus*; Jacalin, *Artocarpus integrifolia*; ACA, *Amaranthus caudatus*; DKD, diabetic kidney disease.





N-glycans
O-glycans
O-glycan capping
Sialic acid addition
ST3GAL1/2
ST6GALNAC1
Core extension
Galactose extension of core 1-4
Poly-lactosamine extension of core 2/4
B4GAT1
B4GALT1/5
Galβ4GlcNAc
β4
Galβ4GlcNAc
β4
β4
β3
β6
Core 4
Core 2
β3
β6
β3
β6
Glycan sugars
GalNAc
GlcNAc
Galactose
Sialic acid
Mannose
ST6GAL1
Urine
Urine
Urine
GCNT3
(C2GnT2)
GCNT1/4
(C2GnT1/3)
Sialyl T
Jacalin
ABA
Galβ1-3GalNAc
(Core 1 [T-antigen])
α3
Core 3
Rare core O-glycans
β3
β3
ST3GAL1/2
β3
GalNAcα3GalNAc
(Core 5)
C3GNT6
C1GALT1
C1GALT1C1 (Cosmc)
Siaα2-6Gal/GalNAc (Sialyl Tn)
Urine
Tn-antigen
Unknown
α3
α1
α6
GALNT1-20
ST6GALNAC1/2
S/T
Urine
SNA
α6