Supporting Information

METHODS

Materials. Hyclone fetal bovine serum (FBS) was purchased from Fisher Chemicals. IRDye800-NHS ester was purchased from Licor Biosciences. GAD65 was purchased from Diamyd Medical and IA2 (ICA512) was purchased from Kronus Inc. Goat anti-human IgG antibody was purchased from Vector Lab. Plasmonic gold chips (pGOLD) were purchased from Nirmidas Inc. Human serum Institutional Review Board approval for this study was obtained from Stanford University. And human serum samples were provided from University of Florida and Islet Autoantibody Standardization Program (IASP). Samples were aliquoted and stored at -80 ^{o}C until processing.

Preparation of recombinant antigens. The 325R polymorphic variant of human ZnT8 isoform-2 was over-expressed in HEK293 cells, and purified in reconstituted proteoliposomes as described previously (18). Briefly, full-length ZnT8 was solubilized by n-dodecyl β-D-maltoside (DDM), partially purified via Ni-NTA affinity to a C-terminal polyhistidine tag, and then further purified by reconstitution into proteoliposomes (the lipids containing 1,2-dioleoyl-*sn*-glycero-3-phosphocholine, 1,2-dioleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol), and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine at a ratio of 2:1:1 were premixed, dried, sonicated and detergent destabilized with DDM before reconstitution of full-length ZnT8) followed by proteoliposome washing with a detergent-free assay buffer (20 mM HEPES, 100 mM NaCl, 1 mM TCEP, pH 7.0). The C-terminal domain (275-369 aa) of the 325R variant was His-tagged, over-expressed in HEK293 cells, and Ni-NTA affinity purified, followed by dialysis against the assay buffer. Purified full-length ZnT8 in proteoliposomes were suspended in the same assay buffer. Full-length ZnT8 in detergent micelles were prepared by solubilizing proteoliposomes with 0.1% DDM in the assay buffer. The purified full-length ZnT8, either in detergent micelles or proteoliposomes, was analyzed by SDS-PAGE with Coomassie blue staining.

HPLC analysis of vacuum-dried full-length ZnT8. Purified full-length ZnT8 either in detergent micelles or proteoliposomes was dried by nitrogen gas evaporation, and then kept under a vacuum for over 60 min to remove a trace amount of liquids. The completely dried full-length ZnT8 samples were rehydrated by adding ddH_2O with 0.1% lauryl maltose-neopentyl glycol (LMNG) and 0.1 mM fluorescein-5-maleimide (FM), a thiol-reactive fluorescence label. Unreacted FM was removed by passing the rehydrated mixture through a desalting column pre-equilibrated with the assay buffer. The resultant LMNG-solubilized and FM-labeled full-length ZnT8 were analyzed by size-exclusion HPLC using the FM fluorescence readout to facilitate the detection of full-length ZnT8 and lipids. An aliquot of proteoliposome suspension or DS-ZnT8 in the assay buffer was HPLC-analyzed in parallel as a control.

Preparation of T1D-related antigens microarray on pGOLD. As-prepared PLR-ZnT8 (0.2 mg/mL), IA2 (0.088 mg/mL), and GAD (0.13 mg/mL) antigens were printed onto pGOLD using GeSiM Nano-Plotter 2.1. Each microarray consisted of three spots of PLR-ZnT8, IA2, and GAD antigens. ~ 5 nL of antigen solution was delivered to each spot. Microarray followed a 3 x 3 layout. Spot diameter was ~ 400 μm and the distance between each spot was ~ 1000 μm. 16 identical microarrays were formed on each pGOLD. The prepared pGOLD were vacuum-sealed and stored at -20 ℃ before use. In a different microarray preparation process, as-prepared DS-ZnT8 and CTD were also immobilized on pGOLD under the same experimental conditions with PLR-ZnT8 for antibody detection.

Multiplexed detection of T1D-related autoantibodies. The prepared pGOLD was integrated in a module in which 16 identical microarrays on each biochip were separated into 16 wells to process 16 samples. Each well was incubated with 5 μL human serum diluted with 100 μL FBS (20-fold) for 1.5 h, followed by incubation with anti-human IgG-IRDye800 conjugates at the final concentration of 4 nM diluted by FBS for 45 min. Each well was washed with washing buffer between each incubation procedure. 14 samples, together with two reference samples (one serum sample, IgG

positive for GAD, and one serum sample with negative IgG binding to all T1D-related antigens) were applied to each biochip. The assay system was composed of the printed antigen array on a pGOLD slide, slide frame, buffers, plate washer and a dual-channel (700/800 nm) scanner, which can be easily deployed in clinical and public-health laboratories.

Data analysis. After the assay process, each biochip was scanned with a MidaScan-IR near-infrared scanner. MidaScan-IR is a dual channel (700 and 800 nm) near-infrared confocal microscope scanner for imaging tissues, cells and microarrays on standard glass or plasmonic slides. IRDye680 and IRDye800 fluorescence images were generated, and the median fluorescence signal for each channel on each microarray spot was quantified by MidaScan software. For each sample, each antigen and each channel, the average of the three median fluorescence signals for three spots was calculated and normalized by reference samples through a two-point calibration. On each pGOLD we added one T1D positive sample to be used as the reference for normalization of other samples' MFI signal to attenuate the difference caused by experimental conditions. Cutoff was determined by the ROC curve analysis. Measurements were performed three times for all values presented in this work.

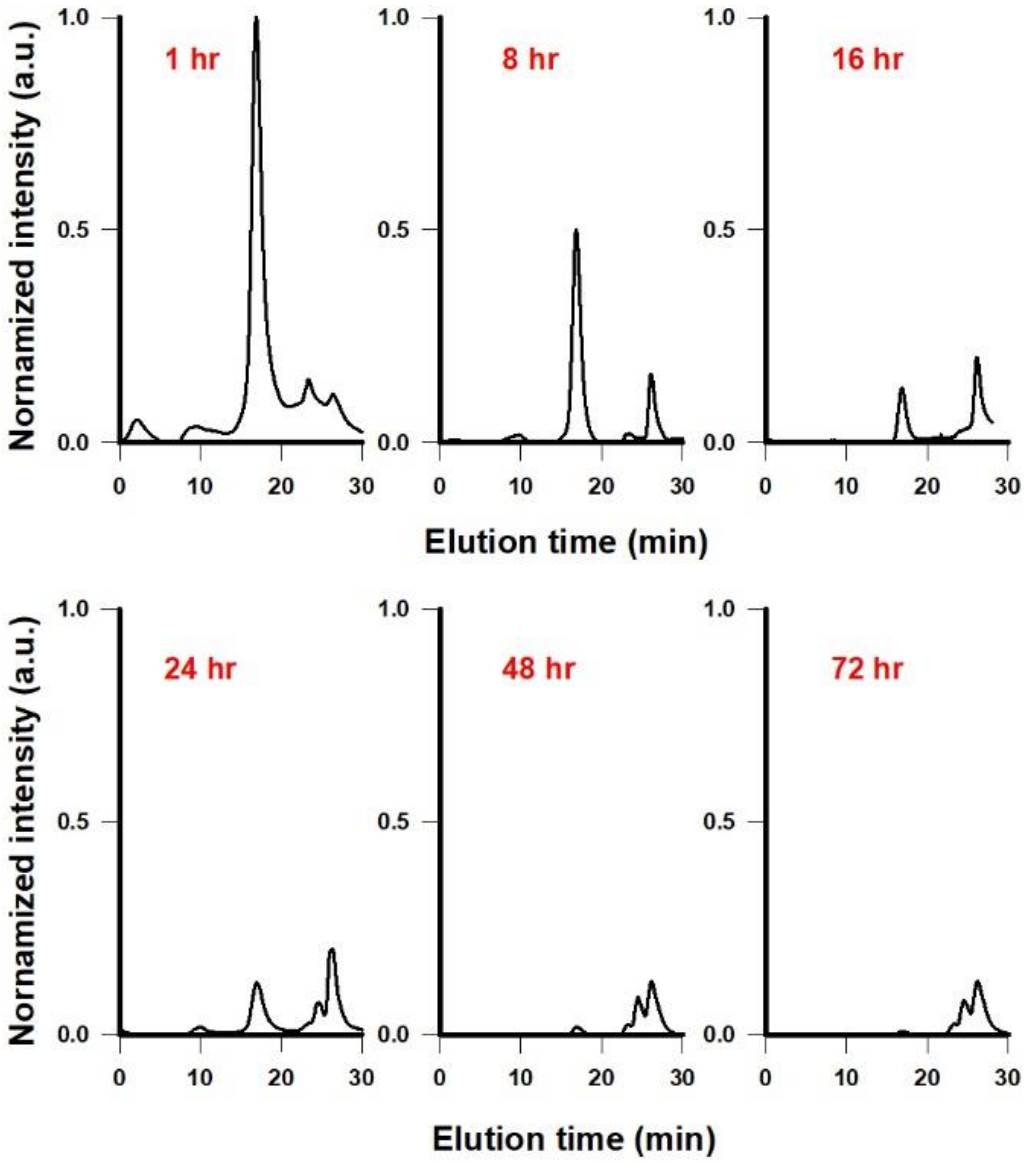

Fig. S1. Rapid denaturation of DS-ZnT8 at various time points as indicated after solubilization of proteoliposomes with detergents. Peak heights are normalized to the initial DS-ZnT8 intensity at 1 hr.

Detection method	Used domains	Used variants	Mean AS95	Max AS95
PLR-ZnT8/pGOLD	Full length	325R	76%	76%
ELISA	CTD	325R+325W	75%	78%
RBA	CTD	325R	48%	66%
RBA	CTD	325W	54%	56%
RBA	CTD	325R+325W	65%	76%
LIPS	CTD	325R	56%	56%
LIPS	CTD	325W	46%	46%
LIPS	CTD	325R+325W	70%	70%

Table S1. Summary of assay performance of ZnT8A detection among all participating assays in 2016 IASP workshop. ELISA stands for enzyme-linked immunosorbent assay; LIPS stands for luciferase immunoprecipitation systems; RBA stands for radioimmuoassay; AS95 means sensitivity adjusted to 95% specificity; CTD stands for C- terminal domain of ZnT8.

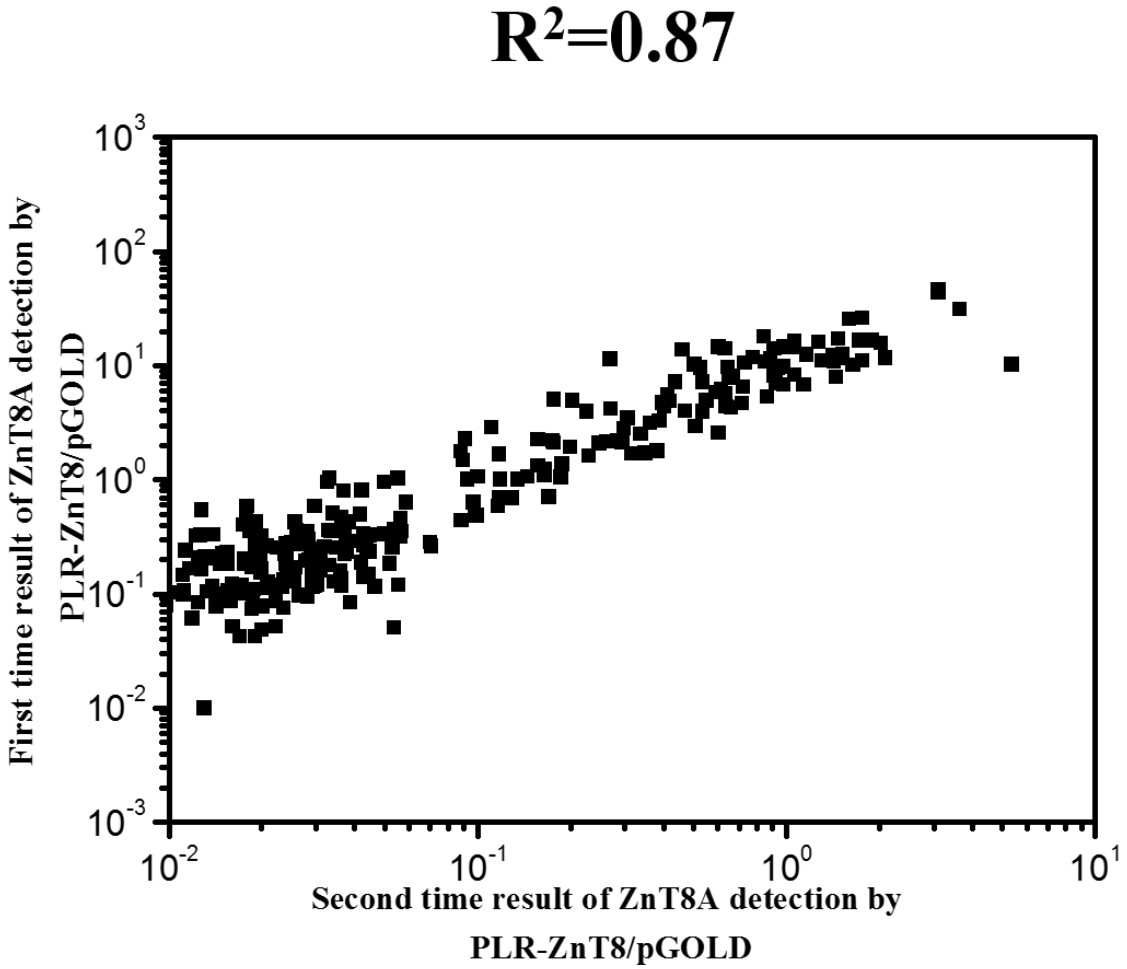

Fig. S2. Correlation tests. Correlation of two technical replicates of ZnT8A detection using PLR-ZnT8/pGOLD, demonstrating the good reproducibility of our assay

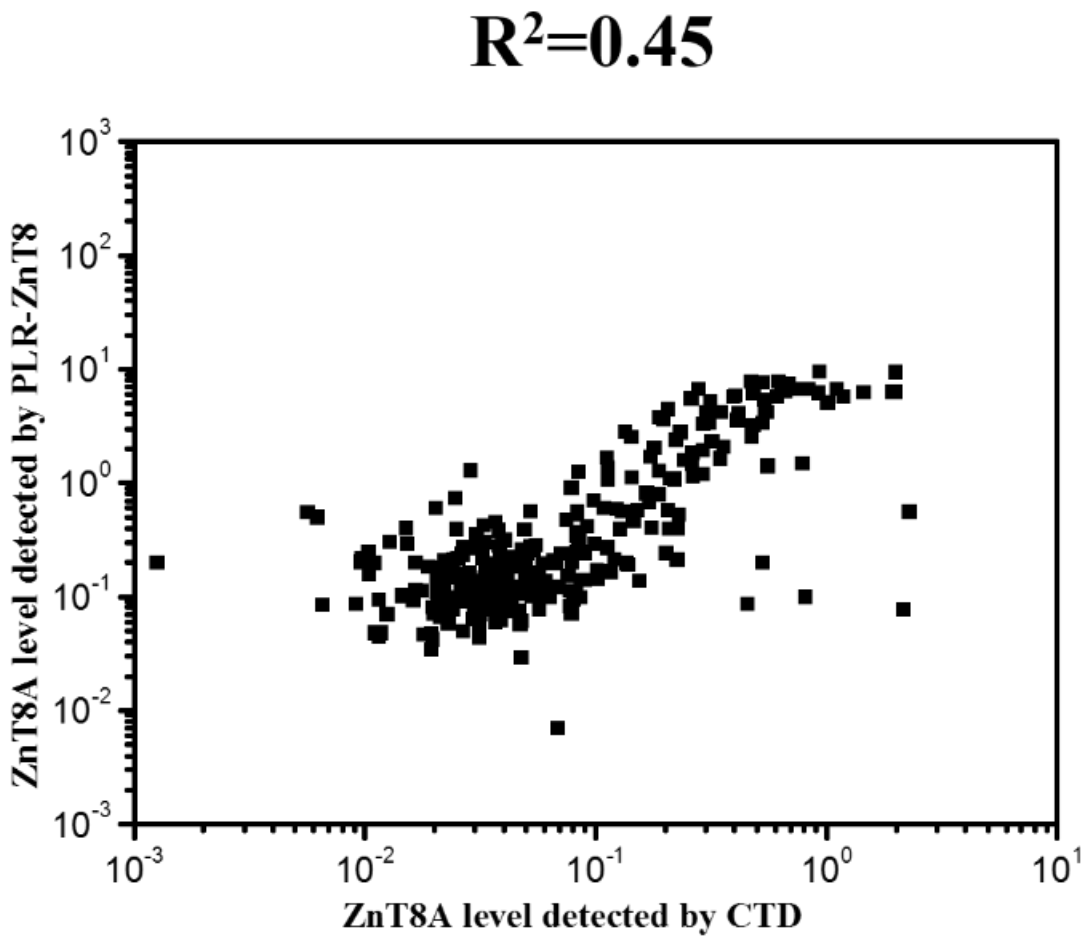

Fig. S3. Correlation tests. Correlation of ZnT8A level detected by PLR-ZnT8 and CTD, implying besides CTD some other auto-reactive sites on PLR-ZnT8 also contributed to ZnT8A detection.

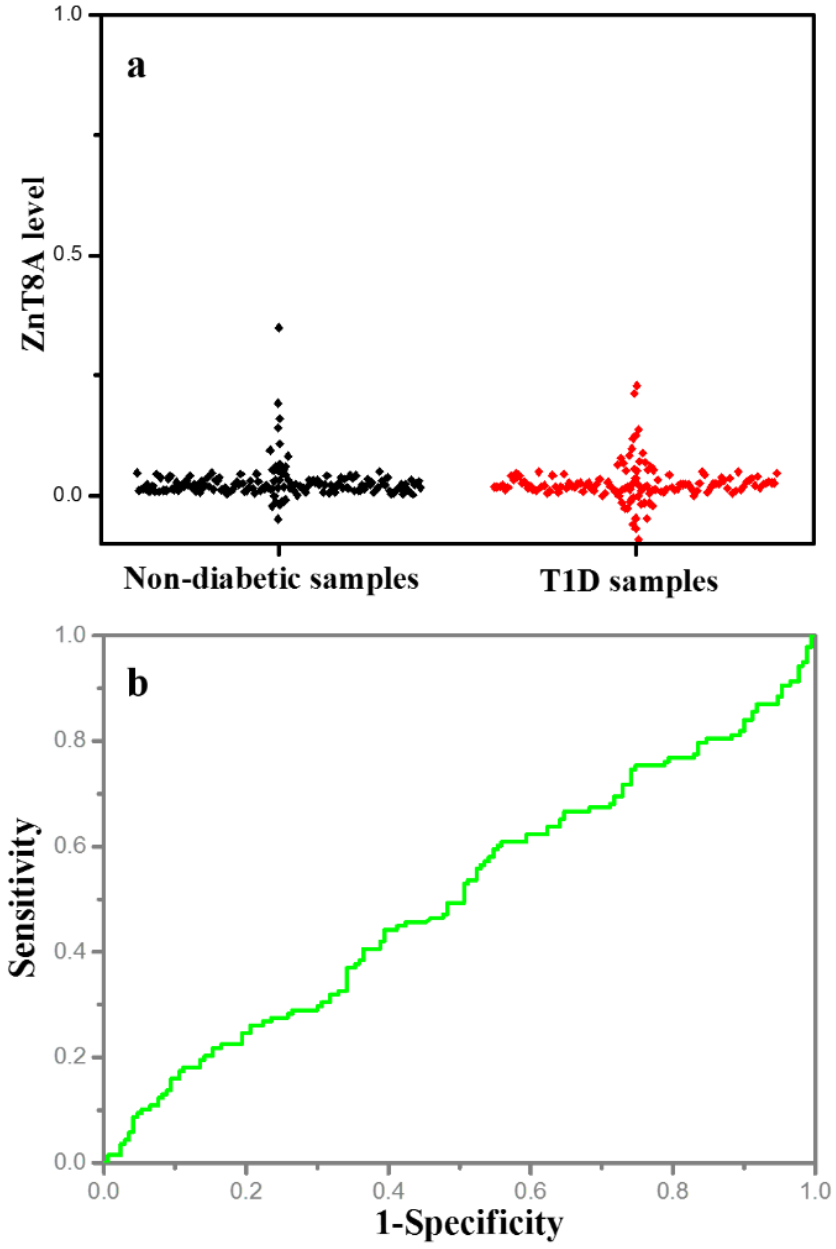

Fig. S4. Scattering plot and ROC analysis of ZnT8A level detected by free liposomes for 307 human sera from 138 T1D patients and 169 healthy individuals provided by IASP and University of Florida.

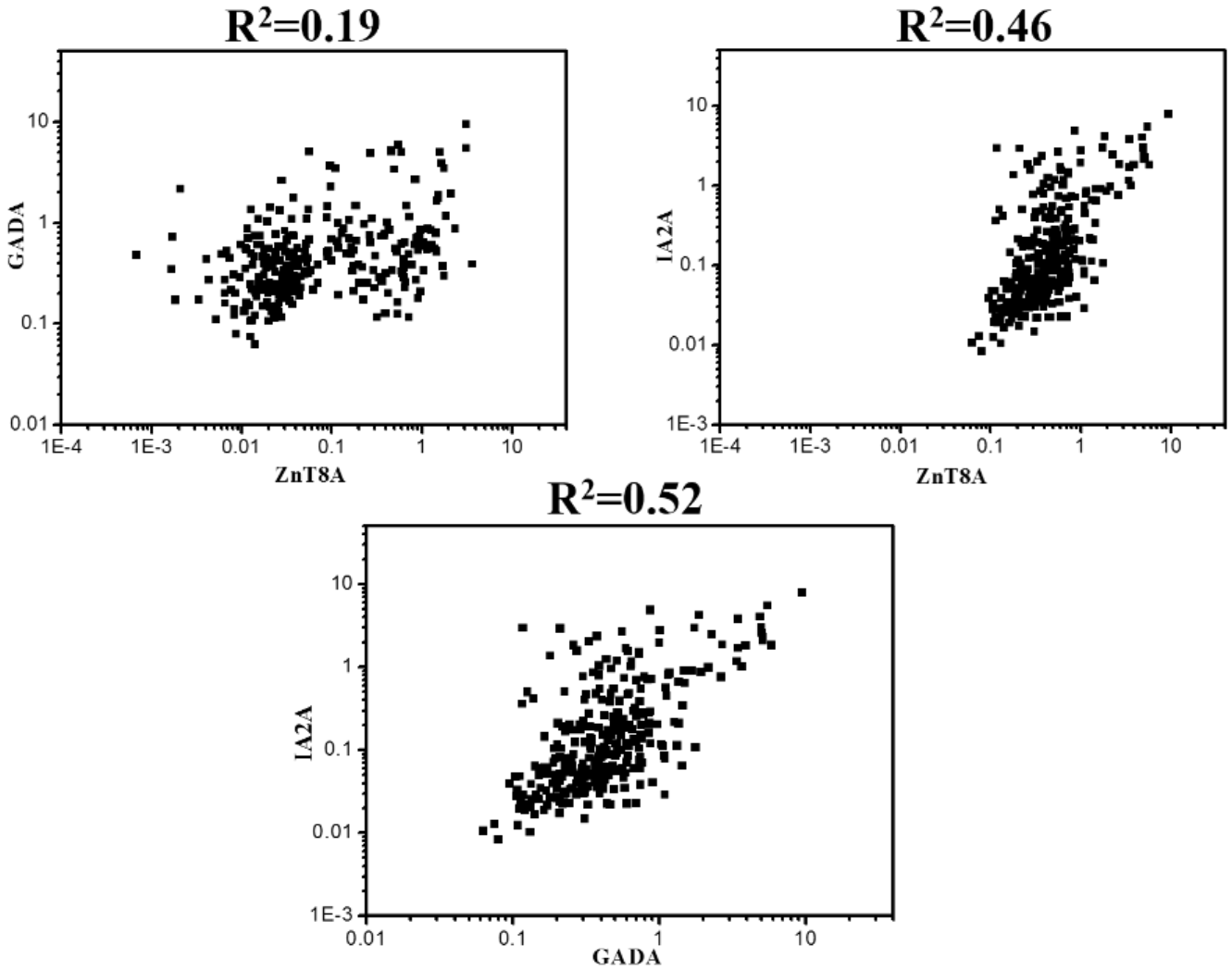

Fig. S5. Correlation among different T1D autoantibodies detected by corresponding antigens for 307 human sera.

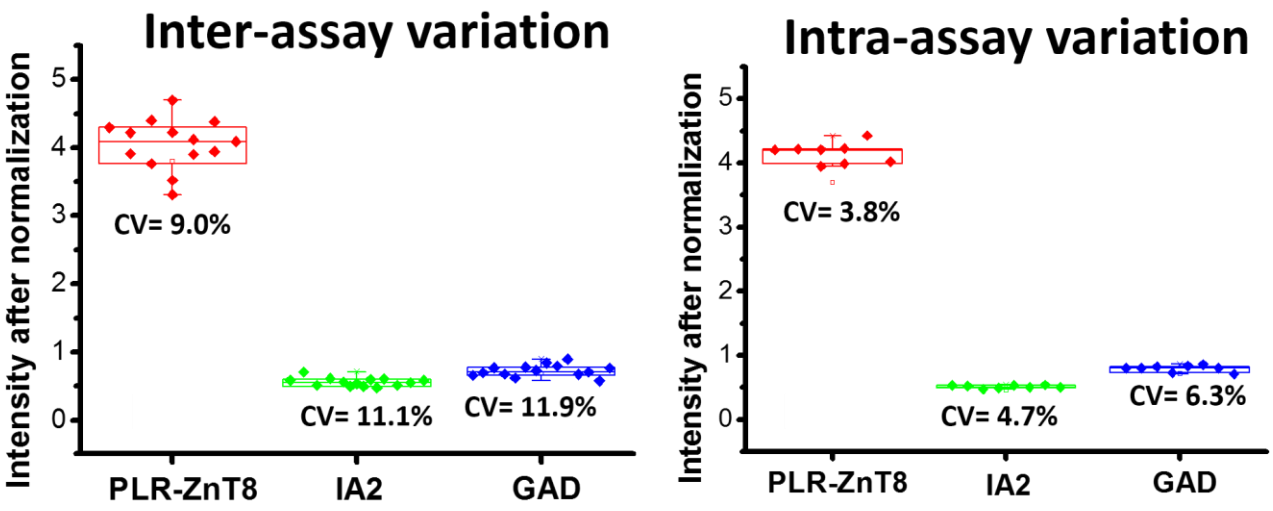

Fig. S6. Intra- and inter-assay variation of multiplexed antibody detection using pGOLD.