A novel LIPS assay for insulin autoantibodies

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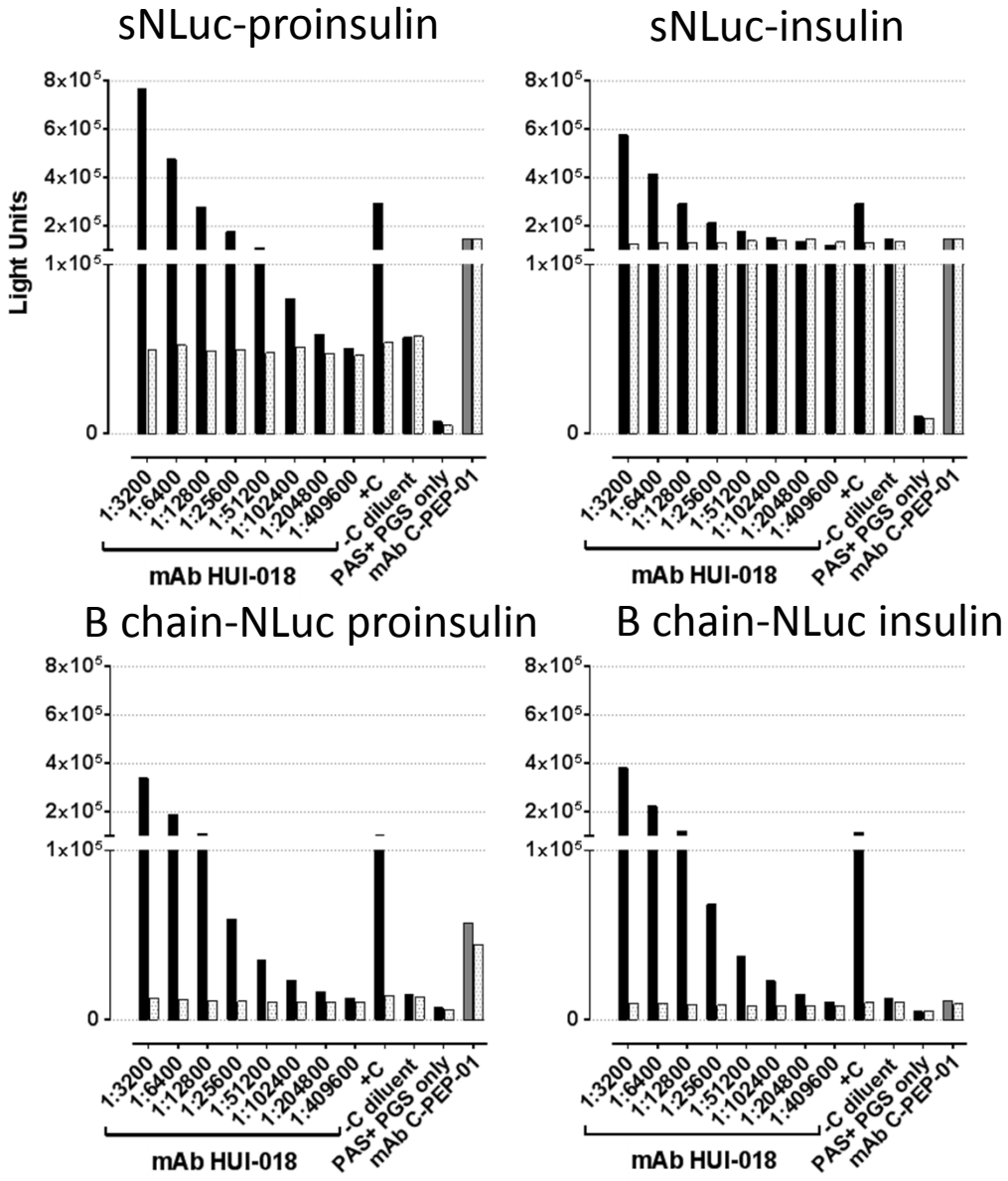
ESM Table 1

Sample Type	Source	Number	Age at sampling years median (IQR)	Follow-up duration years median (IQR)
new onset T1D	OSR	80	13.9 (7.9–14.9)	
Blood donors	OSR	123	22.1 (16.7–26.1)	
non-diabetic schoolchildren	BOX	186	11.0 (10.2-11.7)	
First degree relatives	BOX	53	21.0 (13.0-35.9)	21.8 (15.6-24.4)
First degree relatives	BDR	136		11.6 (5.29-18.1)
new onset T1D	IASP	50	n.a.	
Blood donors	IASP	90	n.a.	

IQR: Inter Quartile Range
OSR: San Raffele Scientific Institute
BOX: Bart's Oxford Famili Study
BDR: Belgian Diabetes Registry
n.a. = not available

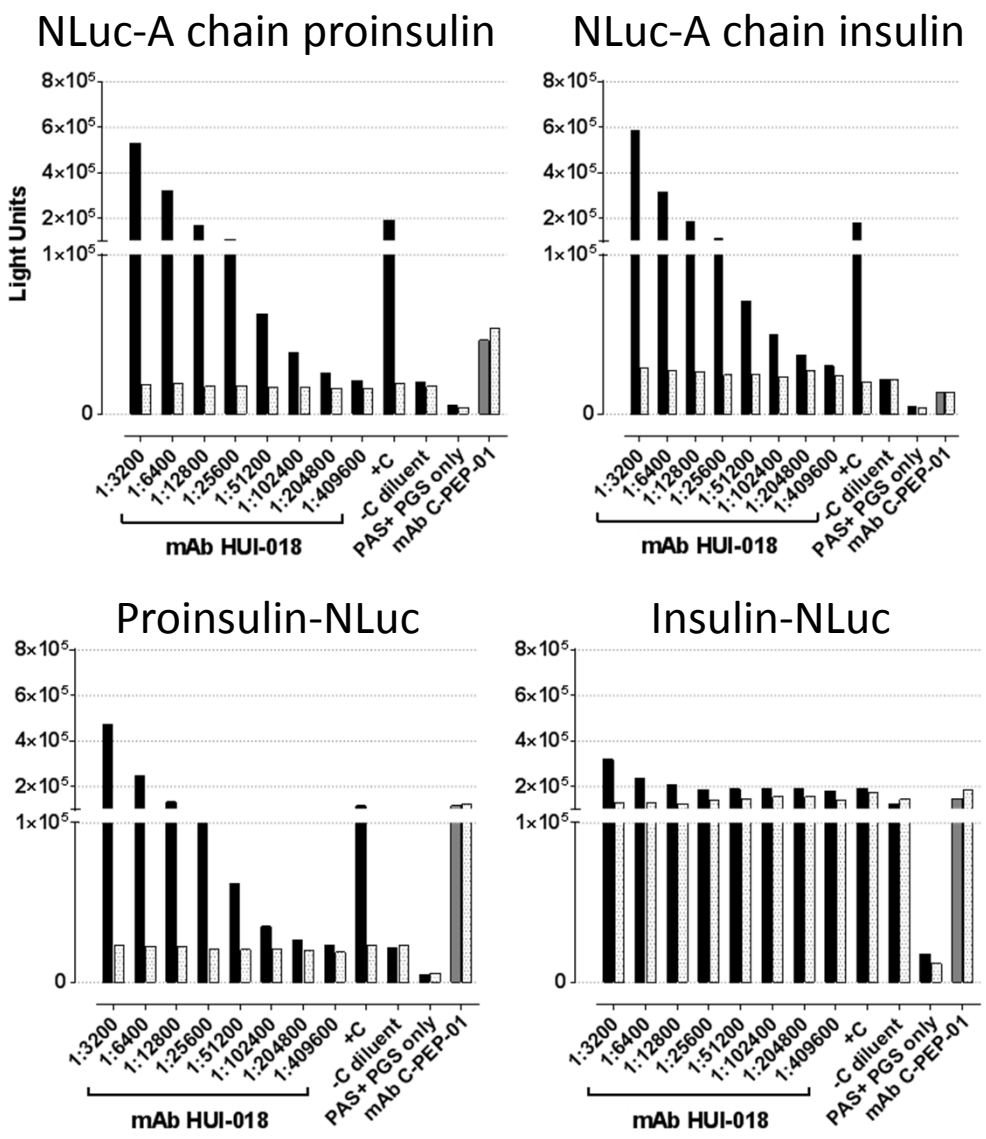
ESM Table 1 Demographic data of human subjects tested in the study

ESM Fig. 1a



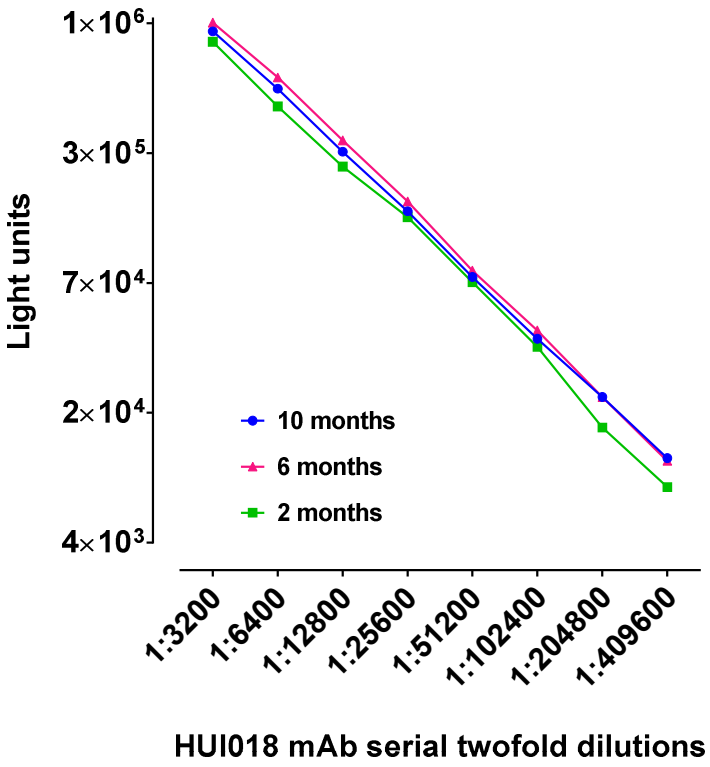
ESM Fig. 1a Effect of luciferase reporter placement on immunoprecipitation of (pro)insulin-luciferase antigens. The indicated serial dilutions of the anti-human insulin HUI-018 mAb in an IAA negative normal human serum were tested in LIPS using the indicated constructs as antigens. Tests were performed in parallel without (black bars) or with (dotted bars) competition with $4.5x10^{-5}$ moles/L of unlabeled ACTRAPID insulin. Also shown are results from an IAA positive serum, the IAA negative diluent human serum, protein A+G sepharose alone, and the anti C-peptide mAb C-PEP01 directed against an epitope in the middle region of the C-peptide

ESM Fig. 1b

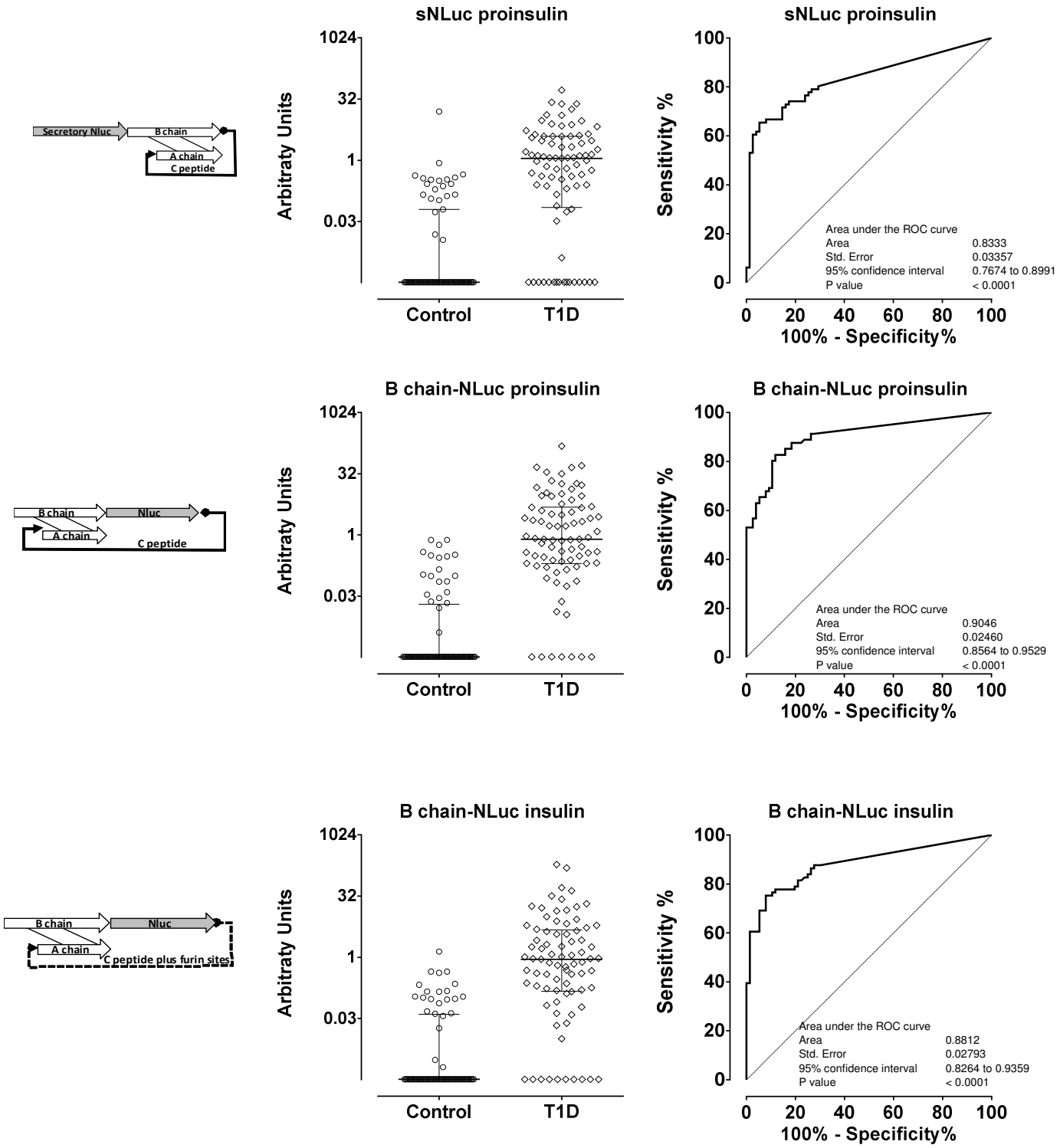


ESM Fig. 1b Effect of luciferase reporter placement on immunoprecipitation of (pro)insulin-luciferase antigens. The indicated serial dilutions of the anti-human insulin HUI-018 mAb in an IAA negative normal human serum were tested in LIPS using the indicated constructs as antigens. Tests were performed in parallel without (black bars) or with (dotted bars) competition with $4.5x10^{-5}$ moles/L of unlabeled ACTRAPID insulin. Also shown are results from an IAA positive serum, the IAA negative diluent human serum, protein A+G sepharose alone, and the anti C-peptide mAb C-PEP01 directed against an epitope in the middle region of the C-peptide

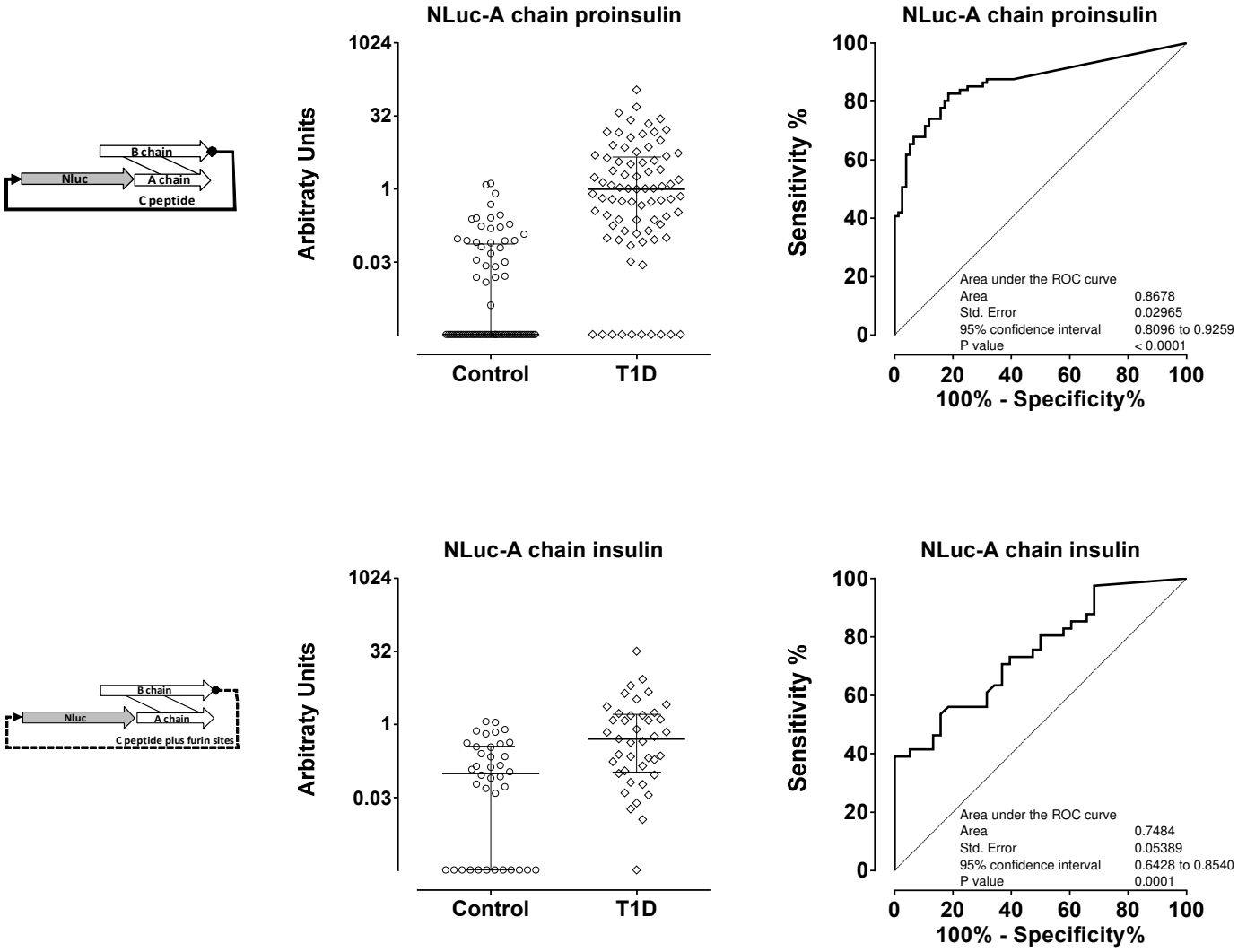
ESM Fig. 2



ESM Fig. 2 Prolonged storage of luciferase tagged antigens has minimal impact on immunoprecipitation and luciferase activity. Single use aliquots of B chain-Nluc proinsulin were thawed after 2, 6, 10 months of storage at -80°C and immunoprecipitated with the indicated serial dilutions of the anti-human insulin mAb HUI018. The delta of light units between replicates with and without competition with 4.5x10-5 mol/L of unlabeled ACTRAPID insulin is shown



ESM Fig. 3a Scatter plot of and ROC curve graphs of LIPS assays using alternative (pro)insulin-luciferase antigens. Human sera were used to immunoprecipitate the respective construct schematically depicted close to each graph. Tests were performed in parallel with or without competition with excess cold insulin and the delta of binding was used to calculate arbitrary units using serial dilutions of the HUI-018 mAb as a standard curve. Negative values were converted to 0.001 to plot on a log 2 scale. Results for the Insulin Nluc-A chain construct are incomplete. The dotted line in ROC graphs is the identity line



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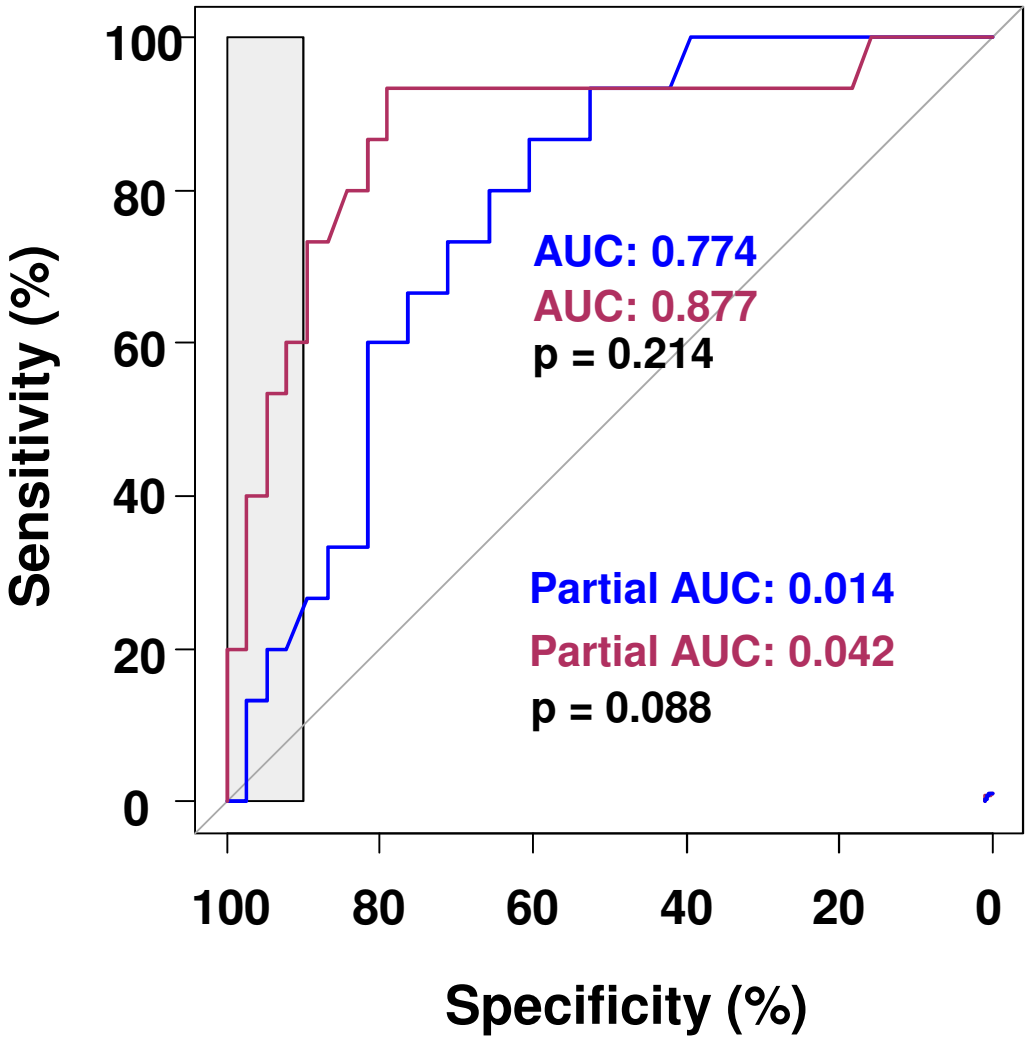
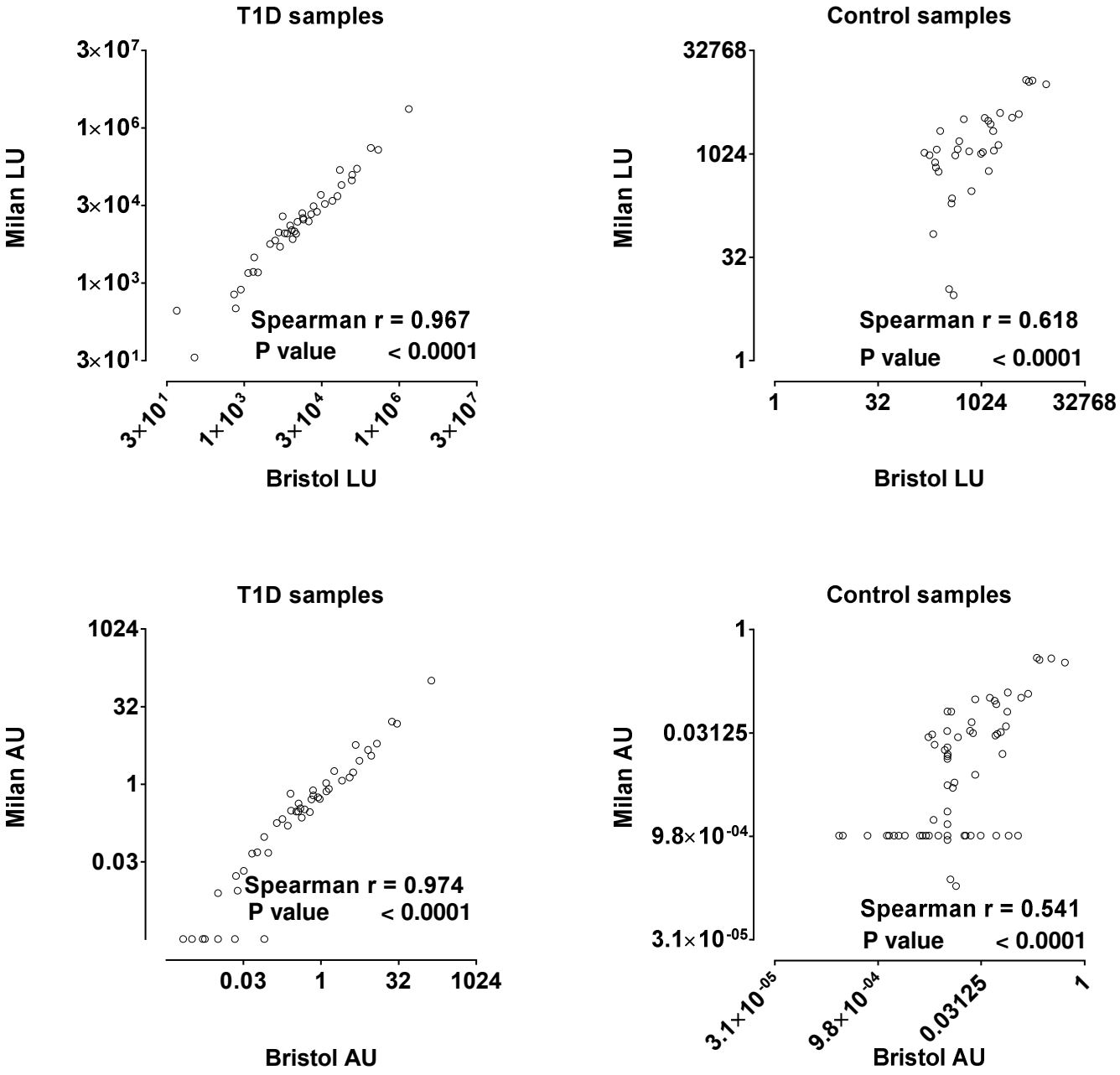
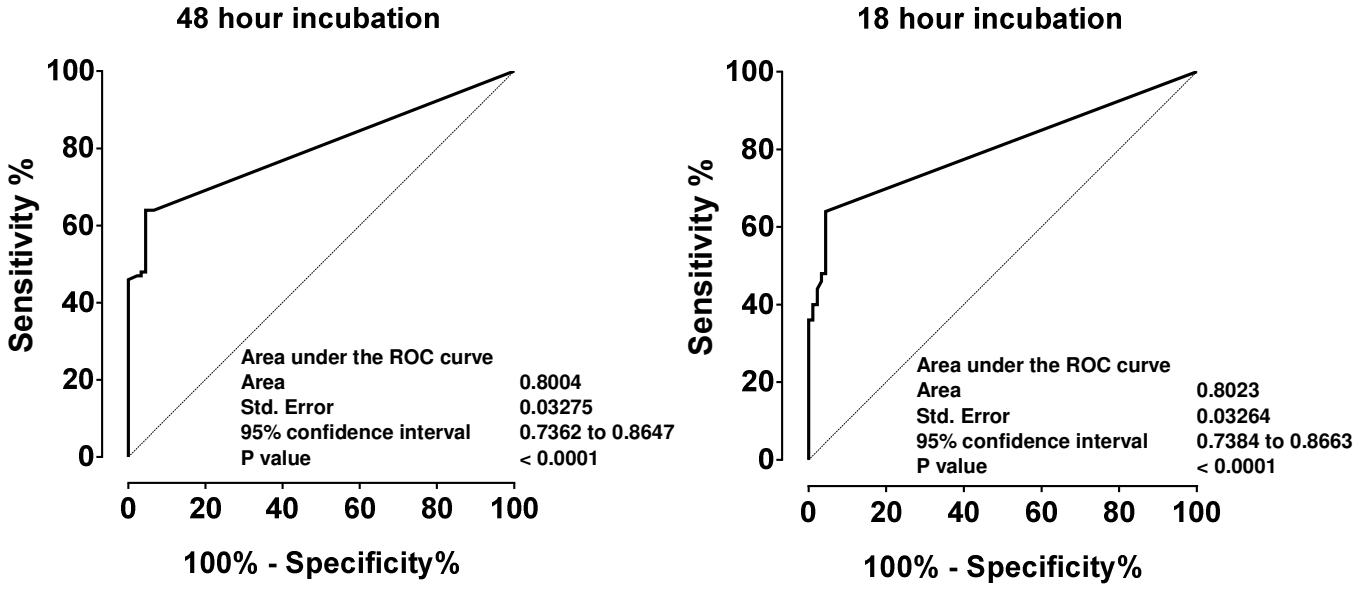


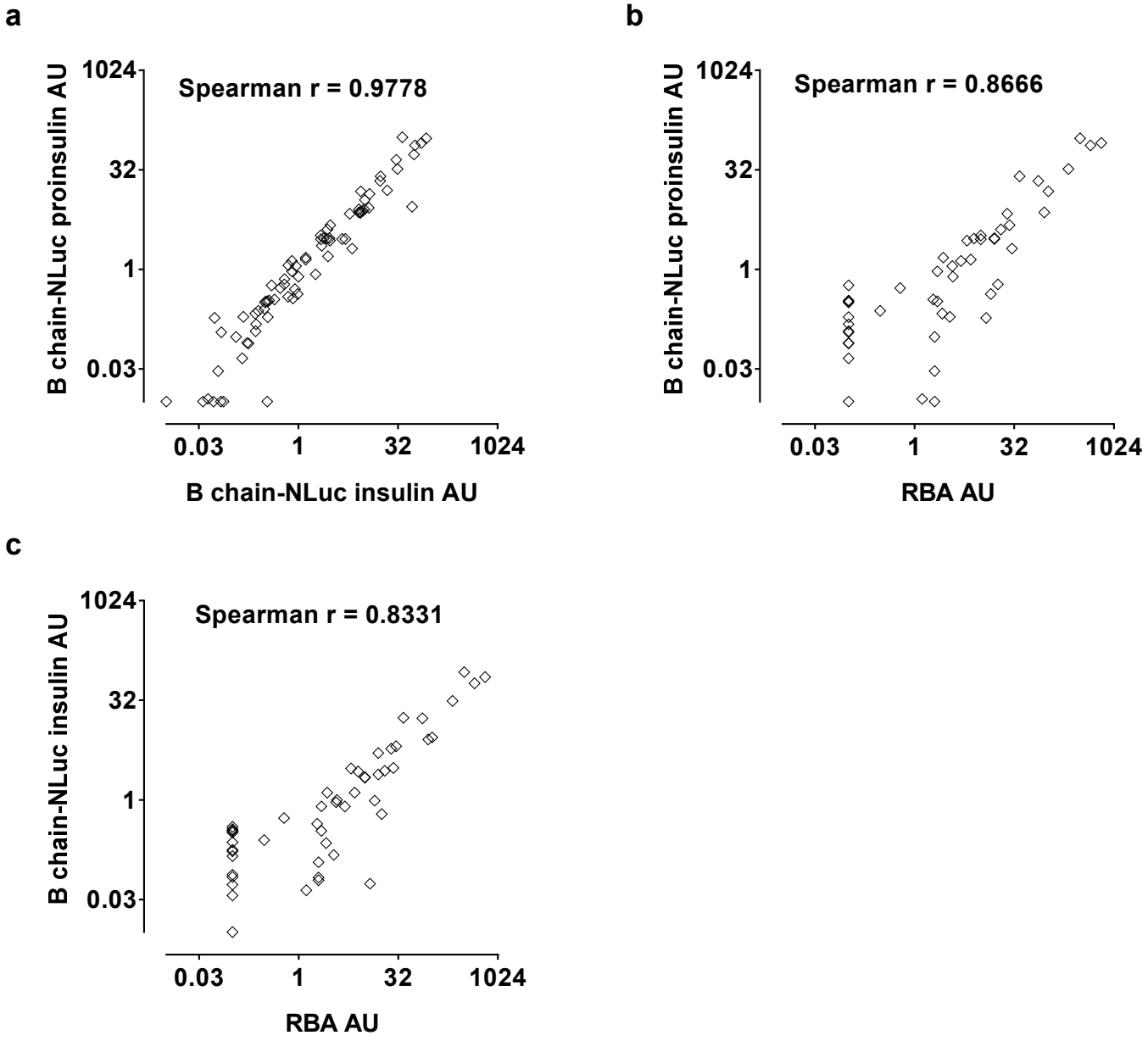
ESM Fig. 4 ROC curve analysis of high vs low risk relatives in RBA and in LIPS. Comparison of the classical IAA RBA assay (blue line) and the B chain-NLuc proinsulin LIPS (magenta line) in discriminating high risk (n=15) from low risk (n=38) first degree relatives of T1D patients from the BOX study. On the graph are shown the values of both the total ROC AUC and the partial AUC, corresponding to an assay specificity greater than 90% (grey rectangle), and the respective p values for the comparison between LIPS and RBA



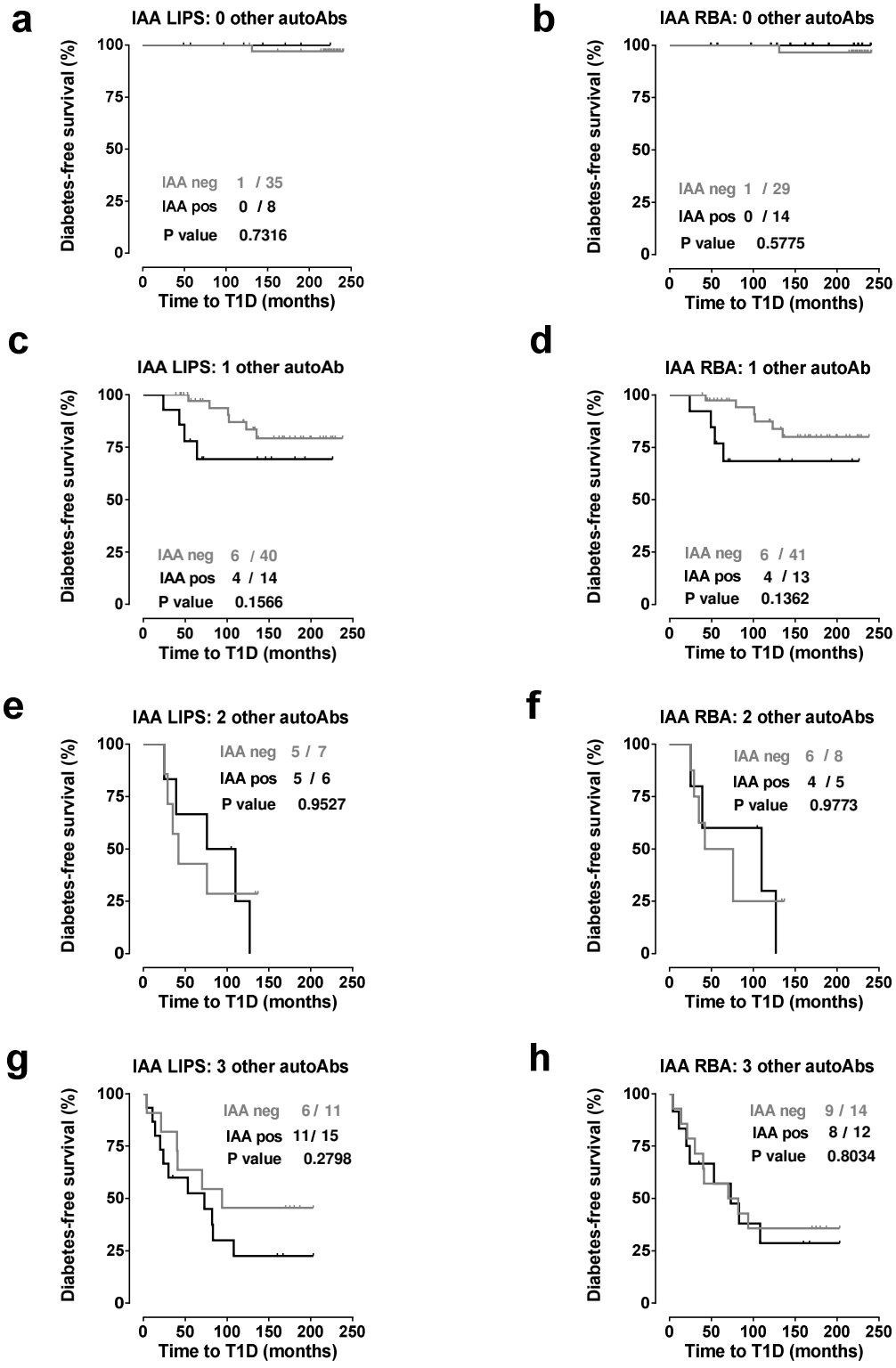
ESM Fig. 5 Correlation analysis of the B chain-NLuc proinsulin LIPS results in IASP2015 between the Bristol and Milan laboratories. Results from both T1D (left panels) and control (right panels) samples are shown. All results are expressed either in light units (LU, top row) or arbitrary units (AU, bottom row). LU values correspond to the delta of binding after competition, values that became negative were omitted from the graphs



ESM Fig. 6 ROC curve analysis of the B chain-NLuc proinsulin LIPS in IASP2015. Results are shown for two different protocols adopting a long (48 hours, left graph) or a short (18 hours, right graph) incubation



ESM Fig. 7 Optimized IAA LIPS results are highly correlated with RBA and between B chain-NLuc proinsulin or insulin antigens in T1D samples. IAA arbitrary units (AU) measured in the B chain-Nluc proinsulin or insulin LIPS were compared in new onset T1D samples (n=80) (panel a). B chain-Nluc proinsulin (panel b) or and B chain-Nluc insulin (panel c) LIPS results were then compared RBA in the same samples. The Spearman r values from a correlation analysis are shown in each graph



ESM Fig. 8: Diabetes-free survival in FDRs stratified according to IAA status and the number of other T1D autoantibodies. Shown are Kaplan-Meier curves based on the measurement of IAA in FDRs from the Belgian Diabetes Registry using LIPS (panels a-c-e-g) or RBA (panel b-d-f-h). Subjects in each graph are classified according to the presence of other antibodies (0 to 3 additional autoAbs). For IAA positives (black lines) and IAA negatives (grey lines) are shown the number of progressors, the total number of subjects in each group, and the P value of the Mantel-Haenszel Log-Rank test