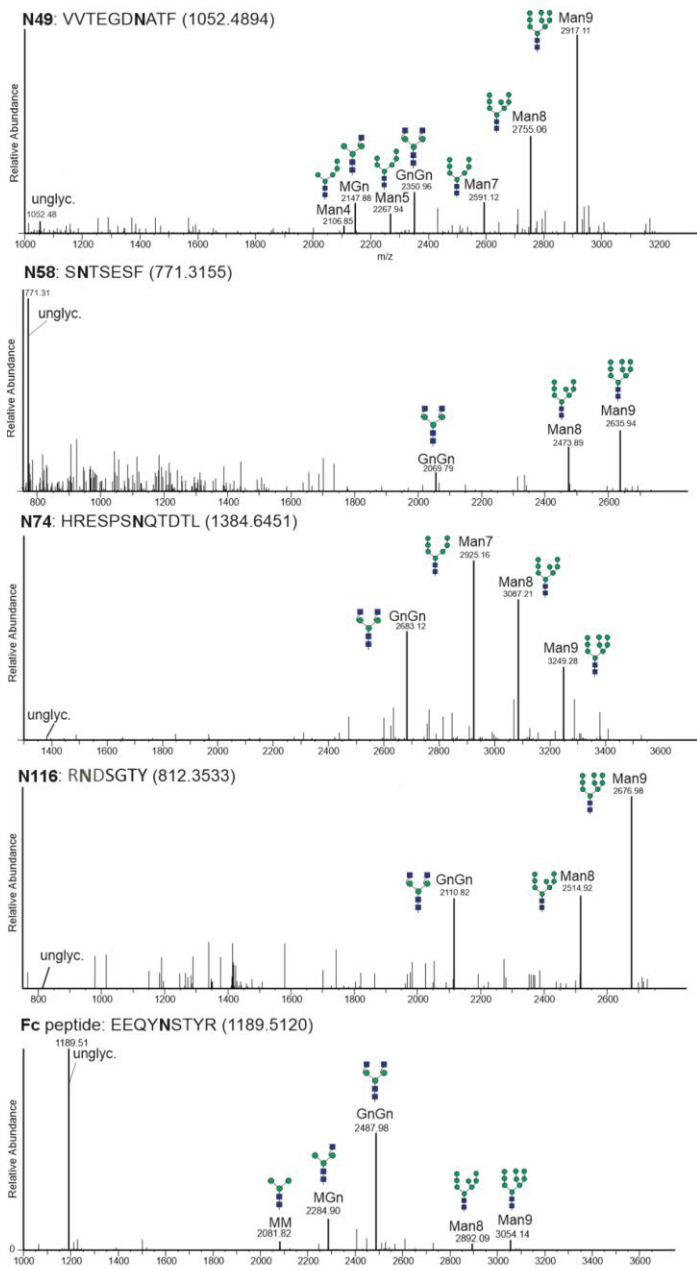


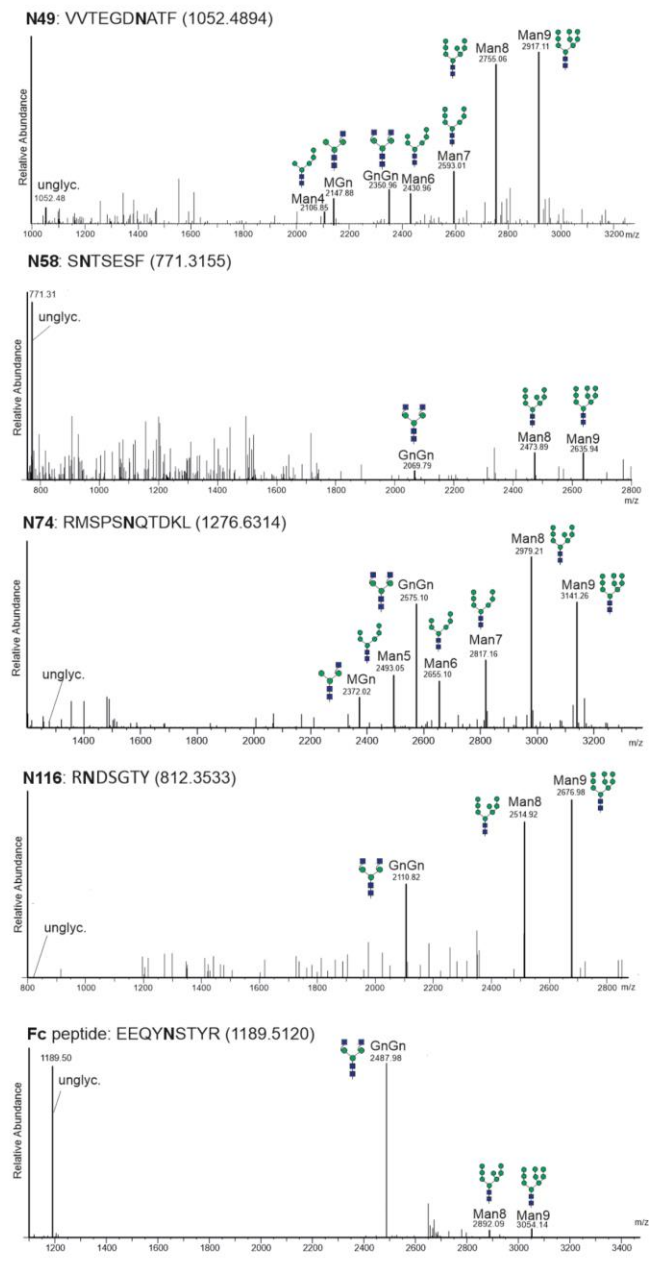
Supplementary figures

Fig. S1

PD1-<sup>T</sup>Fc

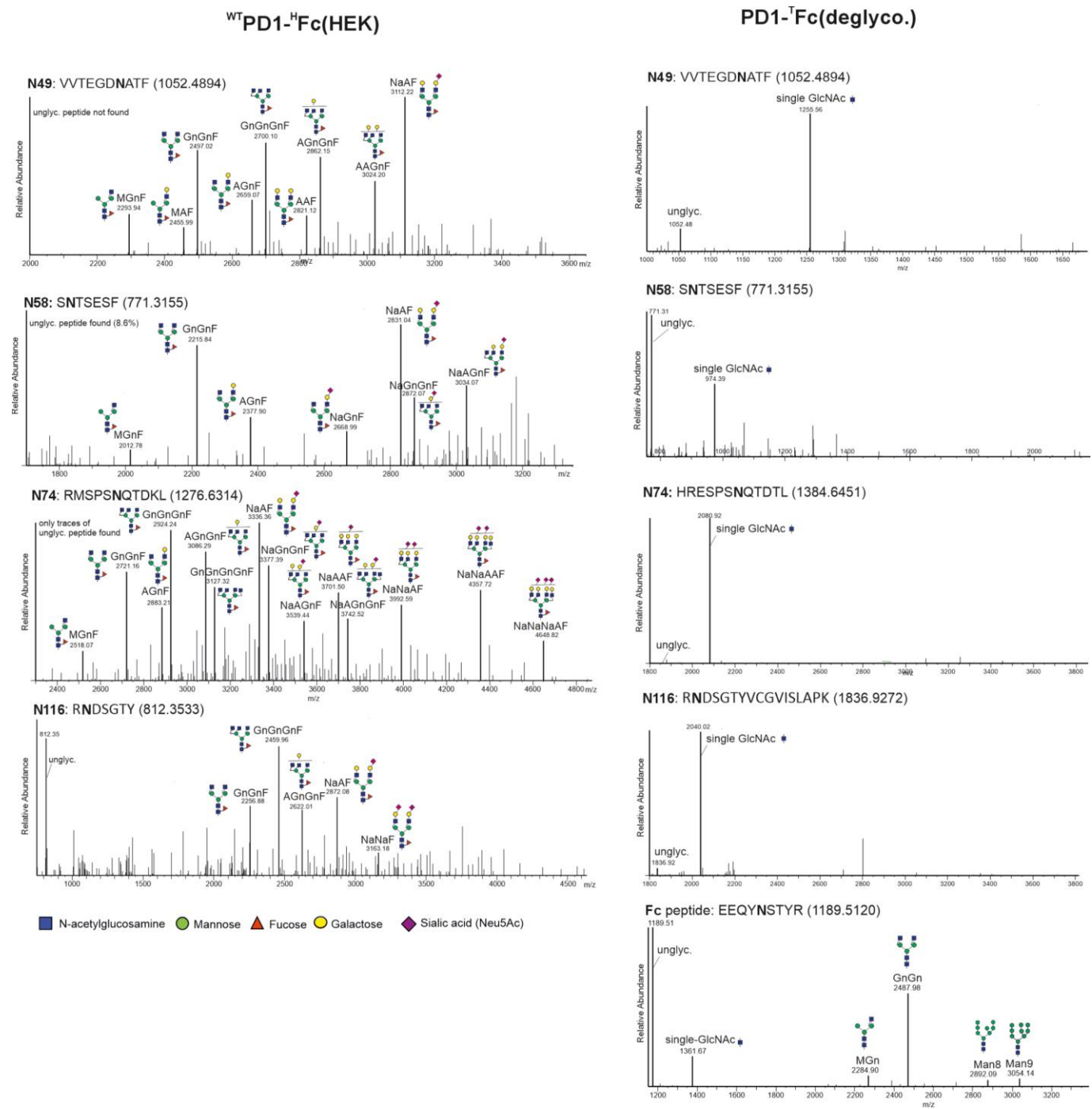


WT PD1-<sup>T</sup>Fc



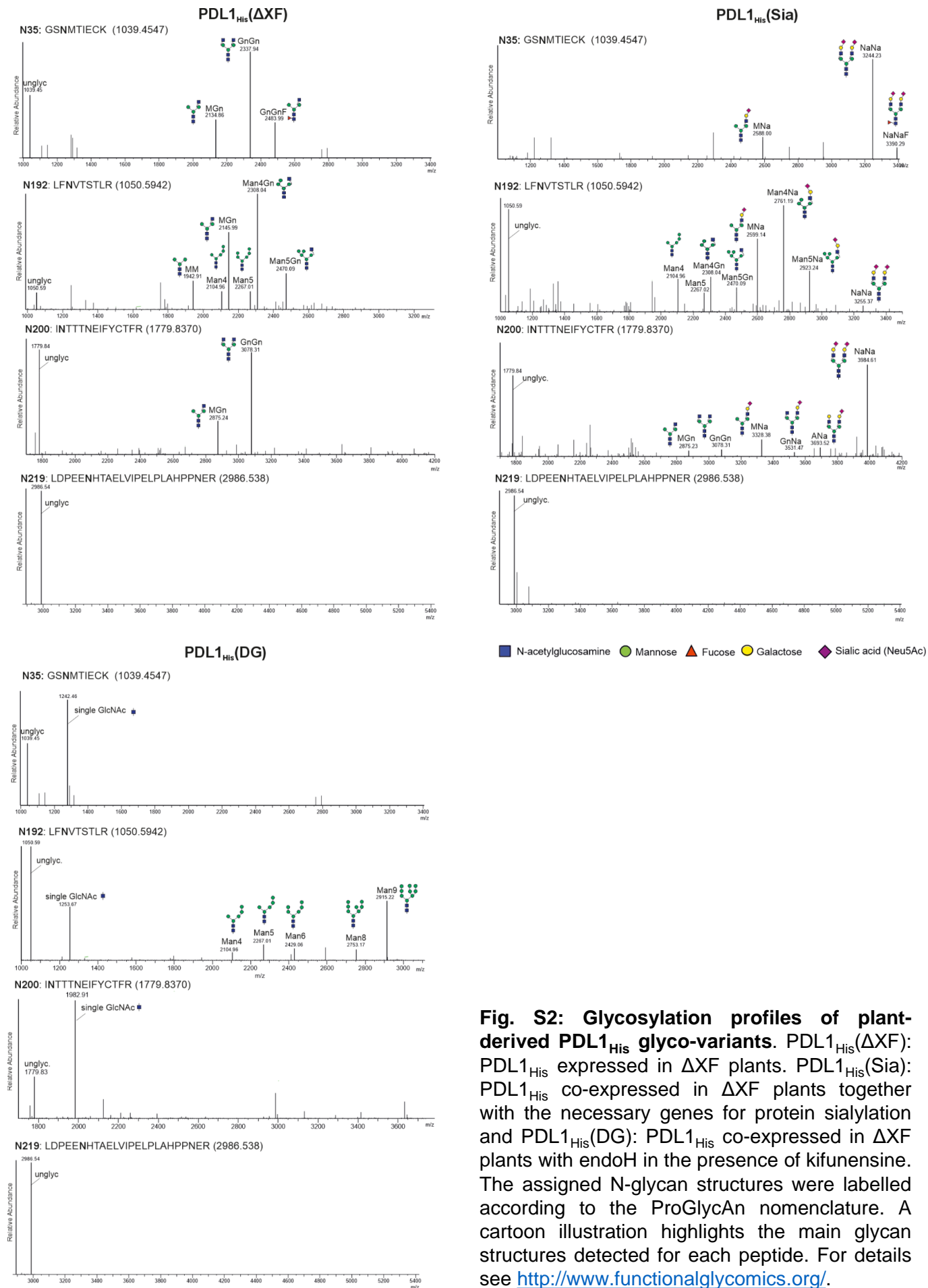
■ N-acetylglucosamine ● Mannose

Figure S1 continue

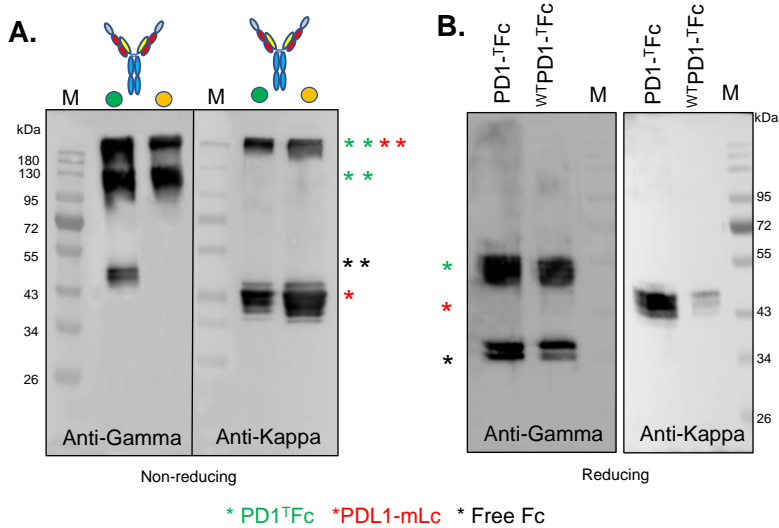


**Fig. S1: Glycosylation profiles of <sup>WT</sup>PD1-Fc(HEK) and plant-derived PD1-Fc variants expressed in *N. benthamiana* ΔXF plants.** The assigned N-glycan structures were labelled according to the ProGlycAn nomenclature. A cartoon illustration highlights the main glycan structures detected for each peptide. For details see <http://www.functionalglycomics.org/>.

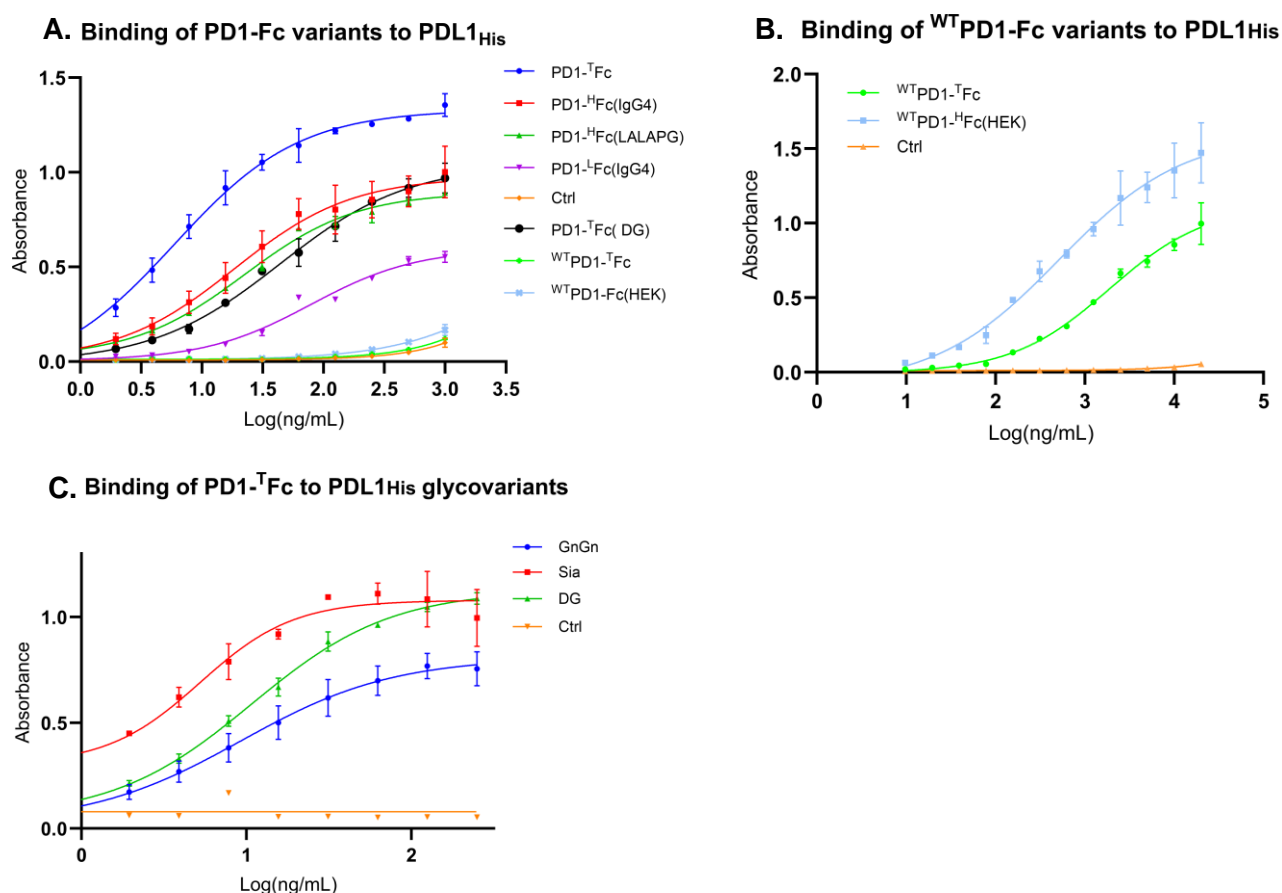
Figure S2:



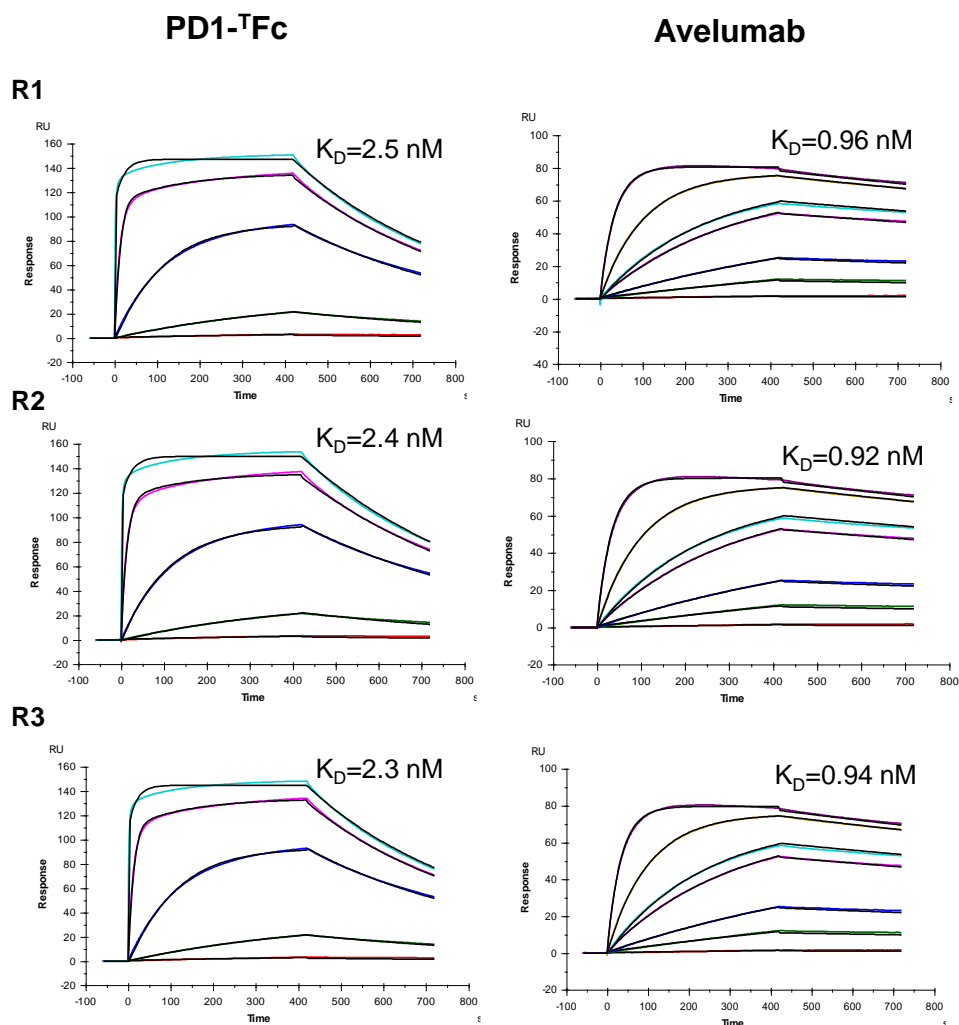
**Fig. S2: Glycosylation profiles of plant-derived PDL1<sub>His</sub> glyco-variants.** PDL1<sub>His</sub>(ΔXF): PDL1<sub>His</sub> expressed in ΔXF plants. PDL1<sub>His</sub>(Sia): PDL1<sub>His</sub> co-expressed in ΔXF plants together with the necessary genes for protein sialylation and PDL1<sub>His</sub>(DG): PDL1<sub>His</sub> co-expressed in ΔXF plants with endoH in the presence of kifunensine. The assigned N-glycan structures were labelled according to the ProGlycAn nomenclature. A cartoon illustration highlights the main glycan structures detected for each peptide. For details see <http://www.functionalglycomics.org/>.



**Fig. S3: In planta interaction of PDL1 and PD1-Fc fusions. (A)** Western blot analysis of proteins purified with protein A (green dot) and KappaSelect (orange dot) after co-expression of PDL1-mLc and PD1<sup>T</sup>Fc. Co-purified proteins were analysed in non-reducing conditions with anti-gamma (left) and anti-kappa (right) antibodies. **(B)** Western blot analysis of proteins purified with protein A after co-expression of PDL1-mLc with PD1<sup>T</sup>Fc and <sup>WT</sup>PD1<sup>T</sup>Fc. Co-purified proteins were analysed under reducing conditions with anti-gamma (left) and anti-kappa (right) antibodies. The apparent molecular mass of marker proteins (M) is shown in kilo Dalton (kDa).

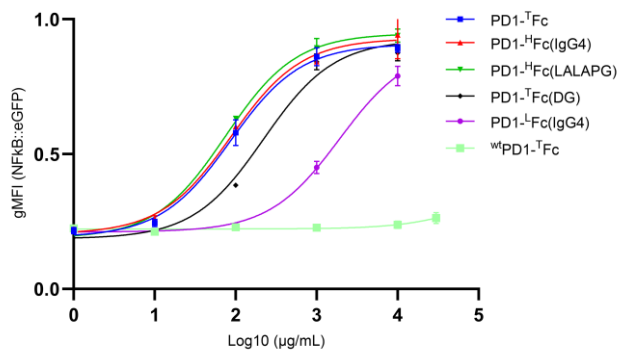


**Fig. S4: ELISA binding assays.** (A) Binding of plant-produced PD1-Fc fusion variants to recombinant PDL1<sub>His</sub>. Serial dilutions of PD1-Fc variants (1000 to 0.48 ng/mL) were added to plates coated with 200 ng of PDL1<sub>His</sub> and detected with anti-human IgG-HRP. (B) Binding of <sup>WT</sup>PD1-TFc and <sup>WT</sup>PD1-Fc(HEK) to recombinant PDL1<sub>His</sub>. Serial dilutions of PD1-Fc variants (20000 to 10 ng/mL) were added to plates coated with 1 µg of PDL1<sub>His</sub> and detected with anti-human IgG-HRP. An unrelated Fc fusion was used as negative control. Data represent the mean values of triplicates. (C) Binding of plant-produced PD1-TFc fusion to PDL1<sub>His</sub> glycovariants: PDL1<sub>His</sub> expressed in ΔXF plants. PDL1<sub>His</sub>(Sia): PDL1<sub>His</sub> co-expressed in ΔXF plants together with the necessary genes for protein sialylation and PDL1<sub>His</sub>(DG): PDL1<sub>His</sub> co-expressed in ΔXF plants with endoH in the presence of kifunensine. An unrelated His-tagged protein was used as negative control. Data represent the mean values of triplicates. Bars represent SD.



**Fig. S5: SPR sensorgrams for the binding of PD1-<sup>T</sup>Fc, <sup>WT</sup>PD1-Fc(HEK) and Avelumab to PDL1<sub>His</sub>.** Analyte serial dilutions of 250 nM-4000 nM, 0.1 nM-1000 nM and 0.1 nM-75 nM were used to probe affinity of PDL1<sub>His</sub> to <sup>WT</sup>PD1-Fc(HEK), PD1-<sup>T</sup>Fc and Avelumab, respectively. Experiments were done in three runs (R1-R3).  $K_D$  values are given for each sensorgram.

### Blocking PD1/PDL1 interaction



**Fig. S6: Blocking PD1/PDL1 interaction by PD1-Fc variants.** Inhibition curves used to determine functional half maximum effective concentrations ( $EC_{50}$ ). PD1-Fc variants were evaluated at different concentrations (ranging from 10 to 0.001  $\mu\text{g/mL}$ ) using PD1+NF- $\kappa$ B::eGFP T-cell reporters co-cultured with T-cell stimulator cells expressing PDL1. gMFI: geometric mean of fluorescence intensity. Data are derived from two independent experiments performed in triplicates ( $n=6$ ). Bars represent SD.