

Longitudinal serum proteomics analyses reveal discriminatory biomarkers for porcine influenza and coronavirus infections

Figure legends

Figure S1: PCA plots of pre-infection, pH1N1, and PRCV-infected serum samples analysed across nine batches demonstrate minimal segregation in the pre-infection control samples **(A)**. Post-normalisation, minimal batch effects were observed, evidenced by the tight clustering of the total pools **(B)**. **(C)** Venn diagram depicting the number of unique and shared differentially regulated proteins ($P_{adj} < 0.05$) between PRCV-infected pigs and pH1N1-infected pigs.

Figure S2: PCA plots reveal differences in protein expression across groups, with post-infection samples (1, 5, and 12 DPI) for both PRCV **(A)** and pH1N1 **(B)** infections exhibiting distinct patterns compared to pre-infection samples, largely driven by the 5 DPI samples. Volcano plots comparing serum proteome in PRCV and pH1N1 versus pre-infection at 1 DPI **(C-D)** and 12 DPI **(E-F)**. Differential expression analysis was performed using linear modelling with empirical Bayes moderation in the *limma* R package. Orange and blue dots represent statistically significant proteins (adjusted p -value < 0.05 and $\log_2FC > |0.6|$).

Figure S3: Protein-trait association maps for all differentially expressed significant proteins in PRCV **(A)** and pH1N1 **(B)**, showing regression coefficients (β) between protein abundance, viral load and pathology data (Halbur and Iowa scores).

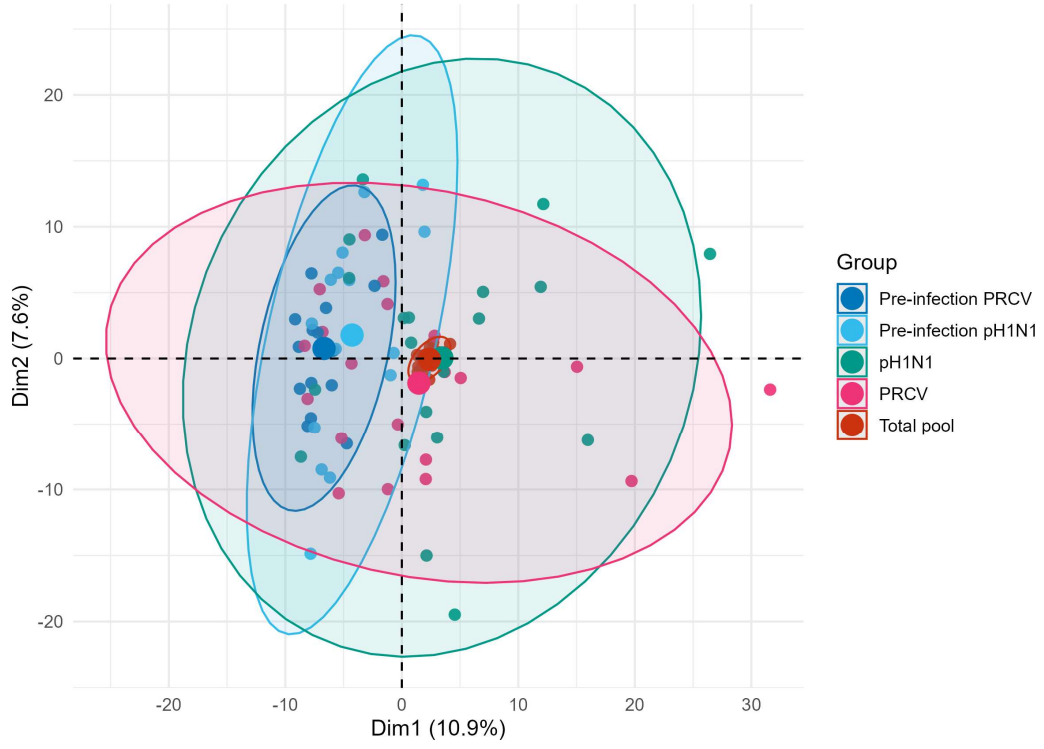
Figure S4: Viral load correlation for all differentially expressed significant proteins in PRCV **(A)** and pH1N1 **(B)** across all time points. Heatmaps show standardised protein abundance (Z-score, day mean).

Figure S5: Evaluation of analytical performance of selected peptides for targeted MRM assay development. **(A-L)** Linearity curve to determine the limits of detection and quantitation of selected peptides mapping to CRP, LTF, PPIA, PRDX2, RAN, SOD1 and SRGN. A dilution series of the peptides was carried out by spiking a pooled set of 12 isotopically labelled synthetic peptides into a pooled serum matrix at different concentrations (5–500 fmol). Linear regression analysis was performed using Analyst software, with a maximum LOQ bias of xx and an LOQ CV of 20%. Analyses were performed in triplicate on a Sciex ZenoToF 7600 mass spectrometer using MRM-HR mode. Error bars represent SD for triplicates. All peptides showed strong linearity ($R^2 > 0.98$), with limits of detection of 1 – 5 pg and limits of quantification of 5 – 10 pg. **(M)** Log₂-transformed peptide abundances for the 12 monitored peptides plotted across injection order before (Pre, red) and after (Post, blue) total pool normalisation. Each panel represents a single peptide and illustrates improved signal stability across injections after normalisation.

Figure S6: Centred time-course profiles of serum protein abundance for all 7 candidate markers for PRCV **(A)** and pH1N1 **(B)**. Protein abundances from the discovery (DIA-MS) and validation (MRM) cohorts were centred on the median pre-infection baseline, separately for each cohort, virus, and protein. Values represent mean \log_2 change from pre-infection \pm standard error across days post-infection. **(C)** Viral load kinetics in the validation cohort were quantified and expressed as \log_{10} TCID₅₀ per 100 mg of tissue over the infection time course for PRCV and pH1N1.

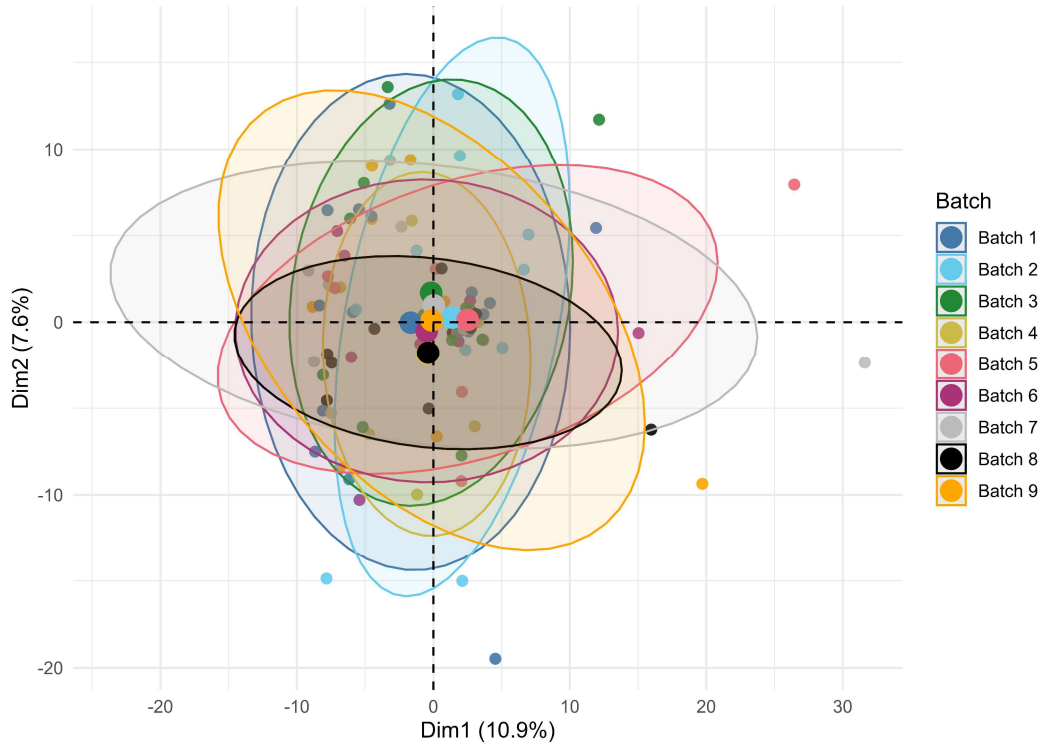
Figure S7: Receiver operating characteristic (ROC) curves for individual peptides discriminating infected versus pre-infection samples in PRCV and pH1N1 across all post-infection timepoints (1, 5 and 12 DPI; **A – K**) and restricted to 1 DPI (**L – V**). Each panel corresponds to a single peptide mapping to one of the candidate proteins (CRP, LTF, PPIA, SRGN, SOD1, RAN or PRDX2). Discriminatory performance is reported as the area under the curve (AUC) for both PRCV and pH1N1.

A PCA — Pre and post infection with total pool

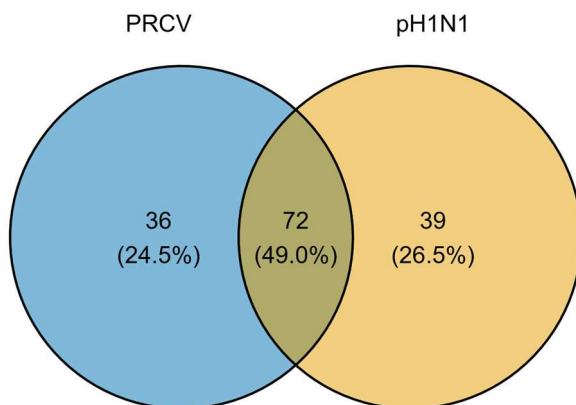


B

PCA — coloured by Batch



C



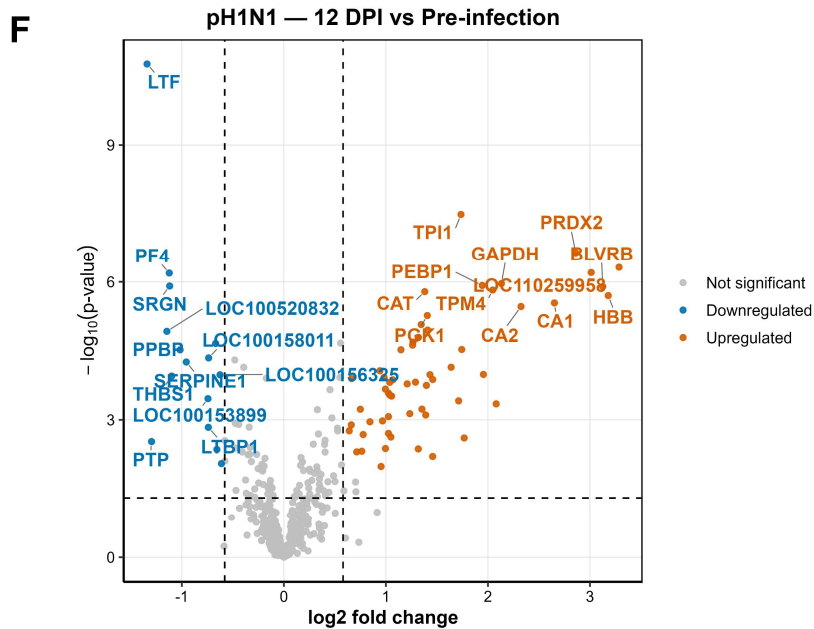
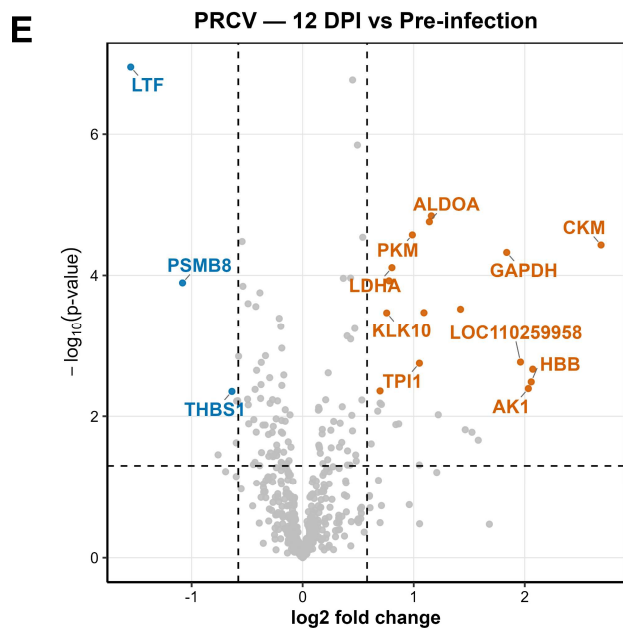
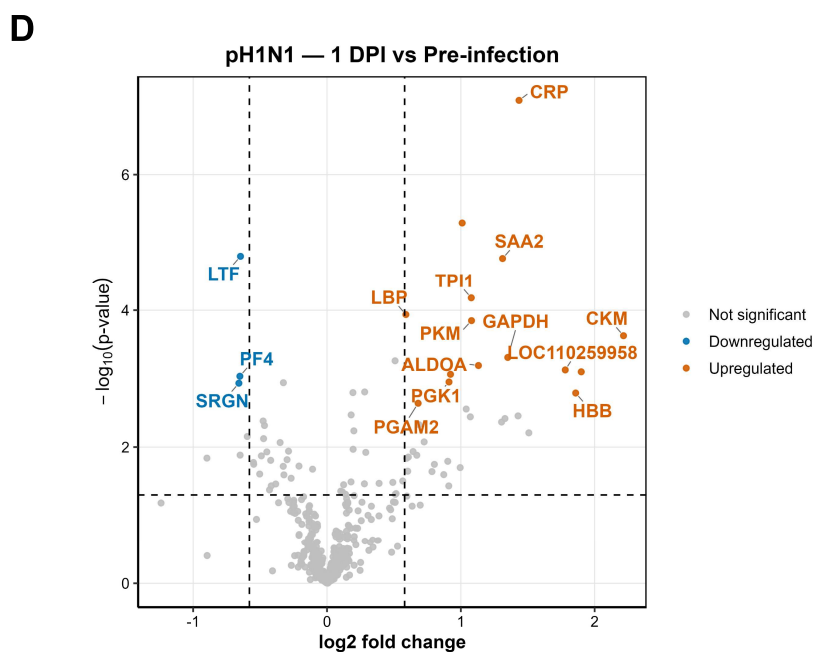
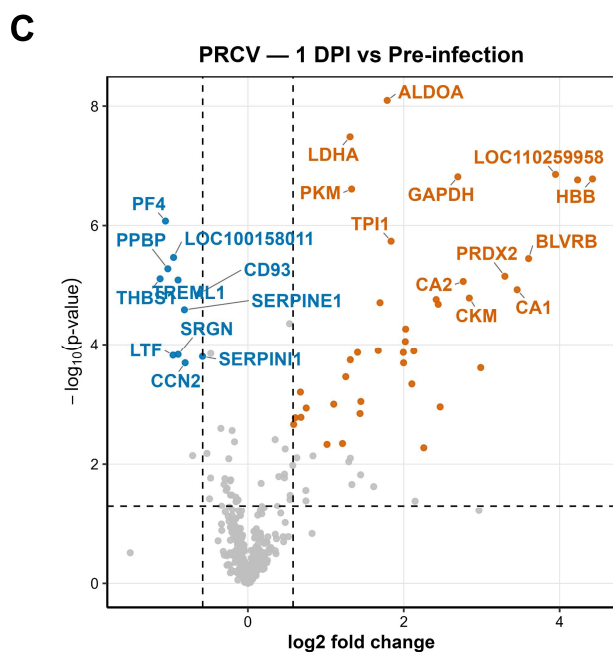
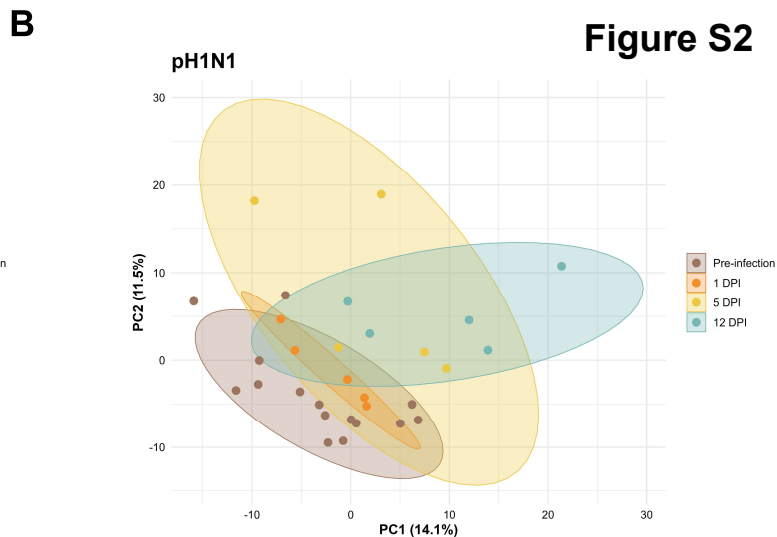
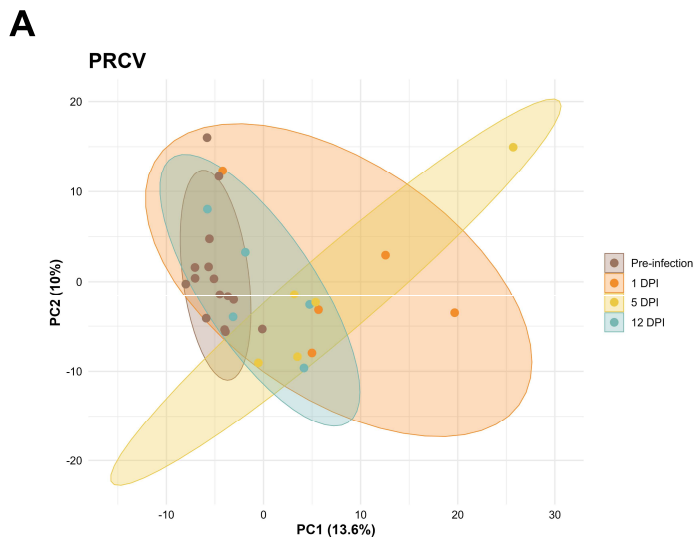
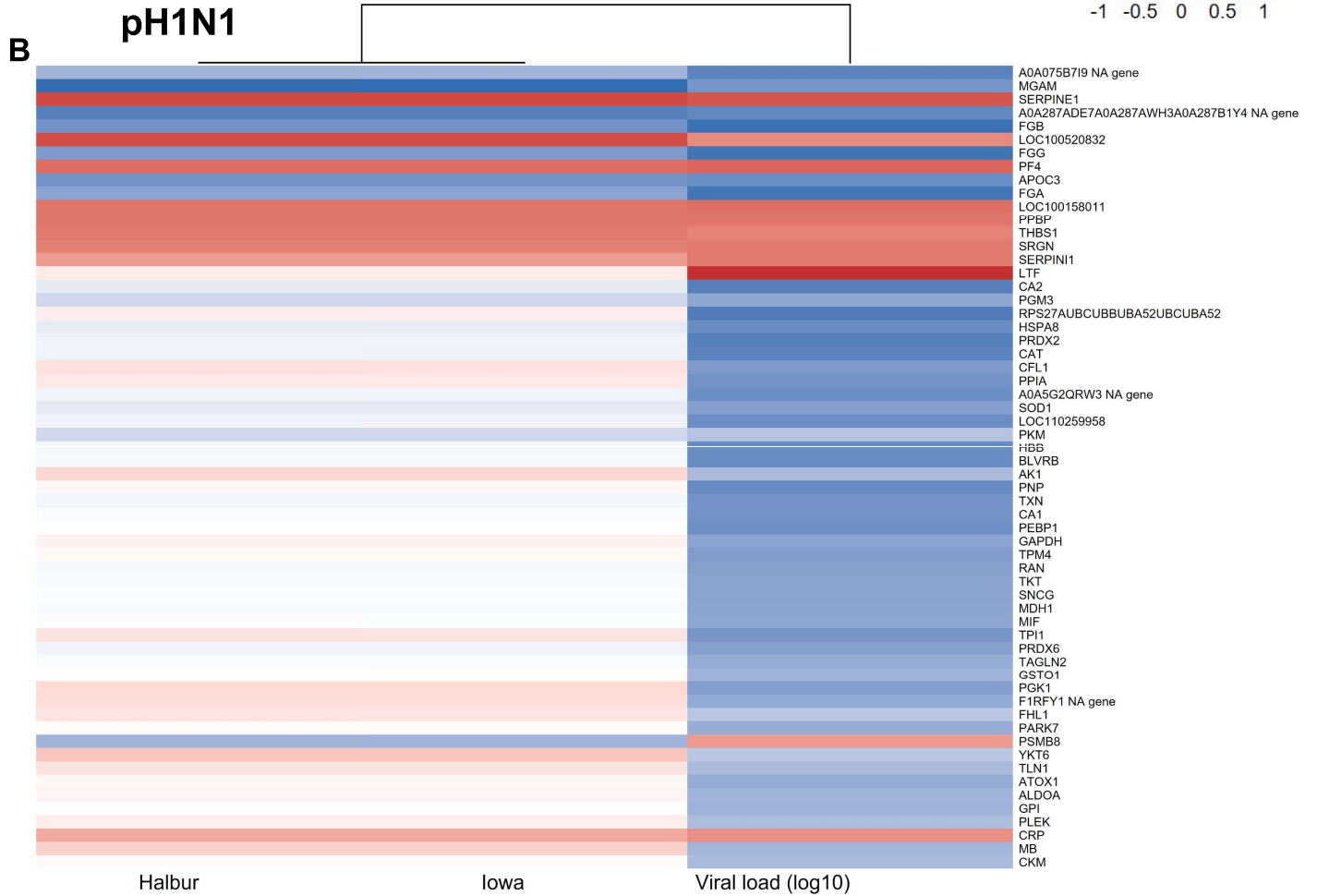
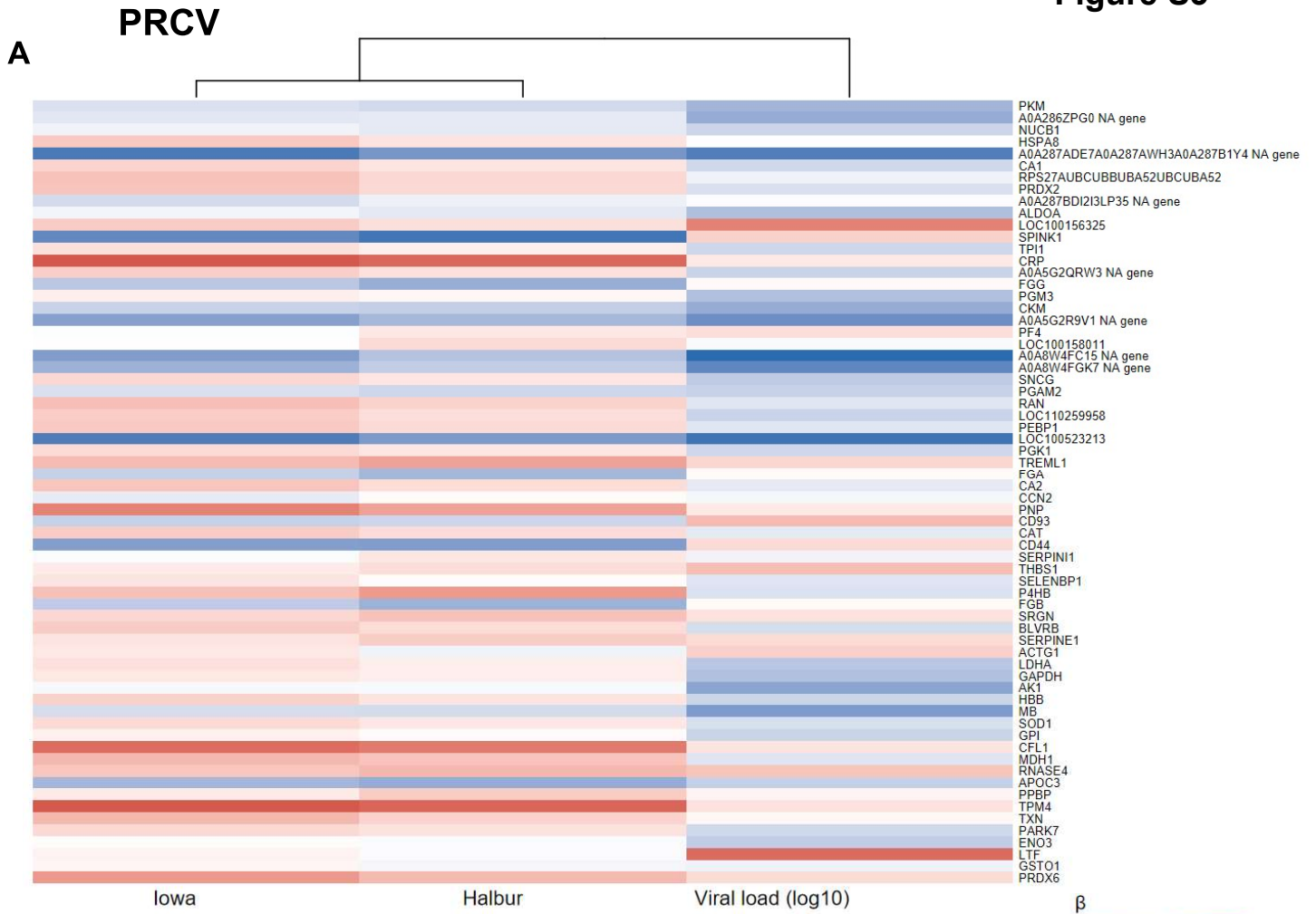
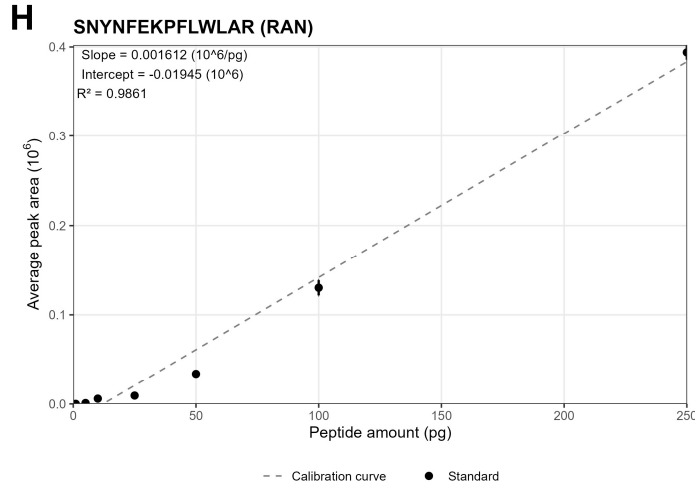
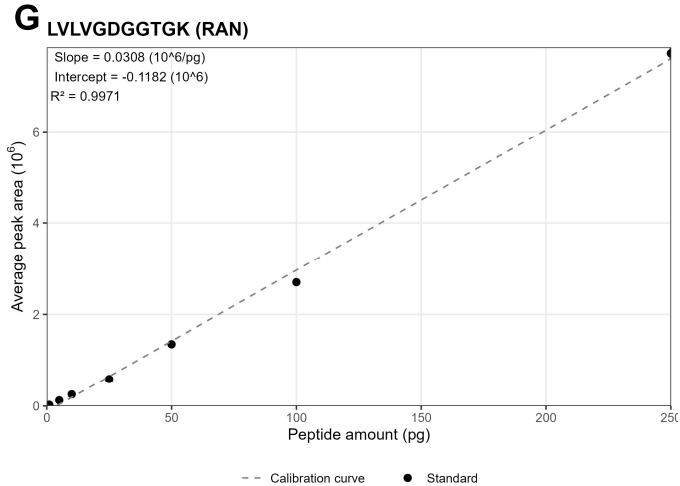
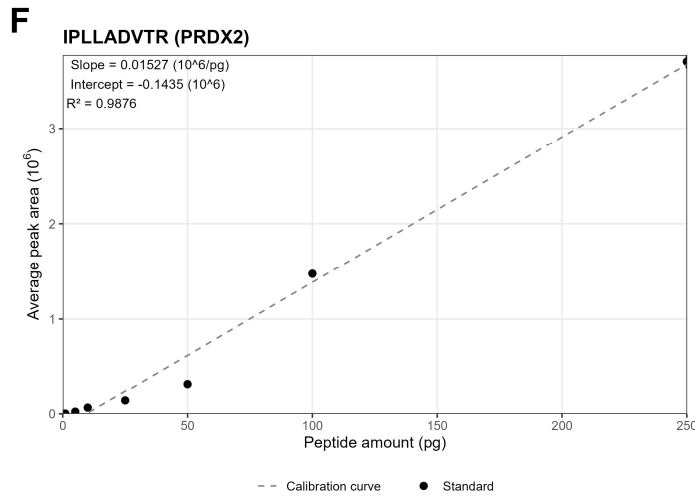
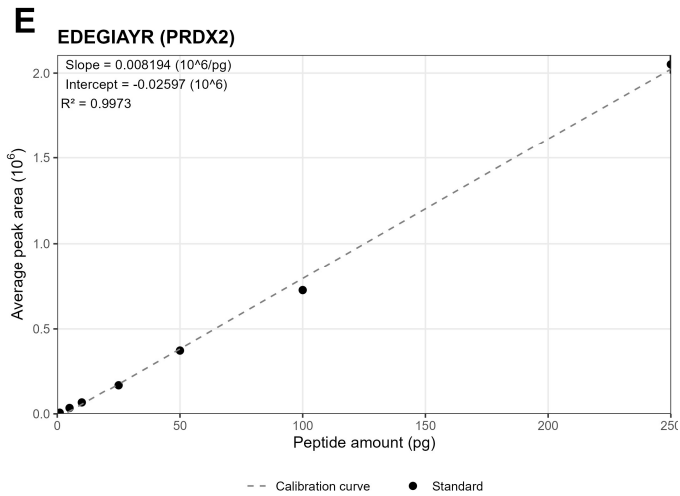
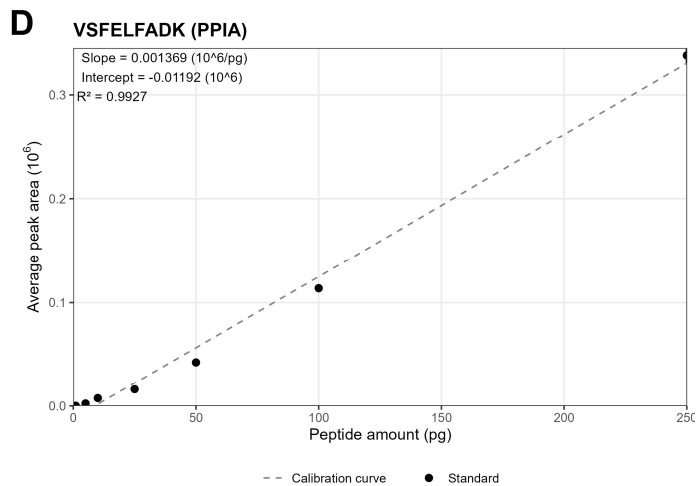
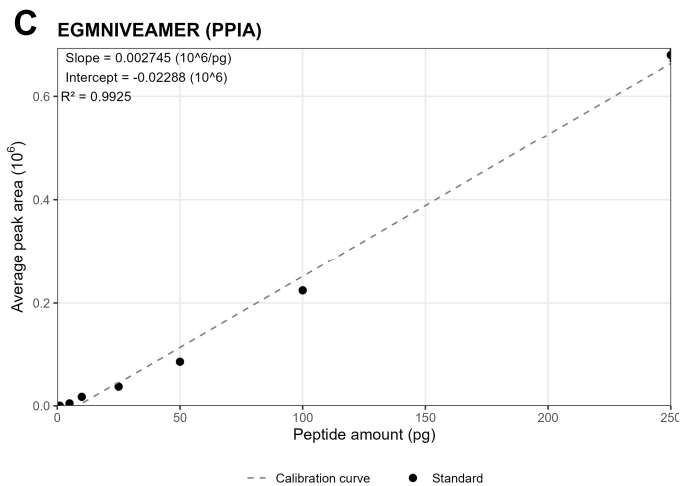
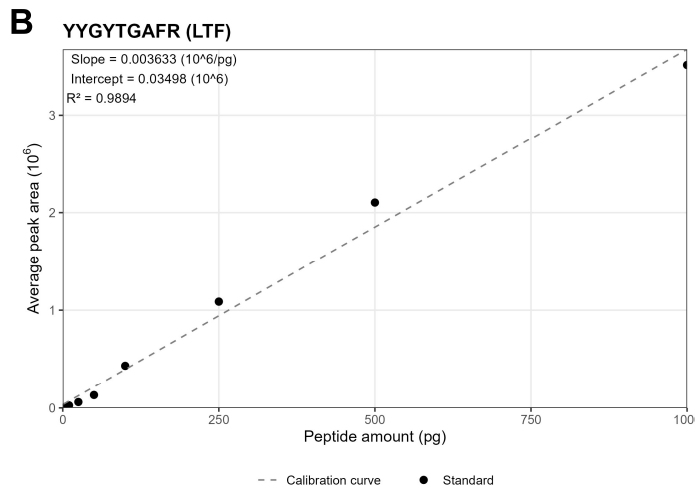
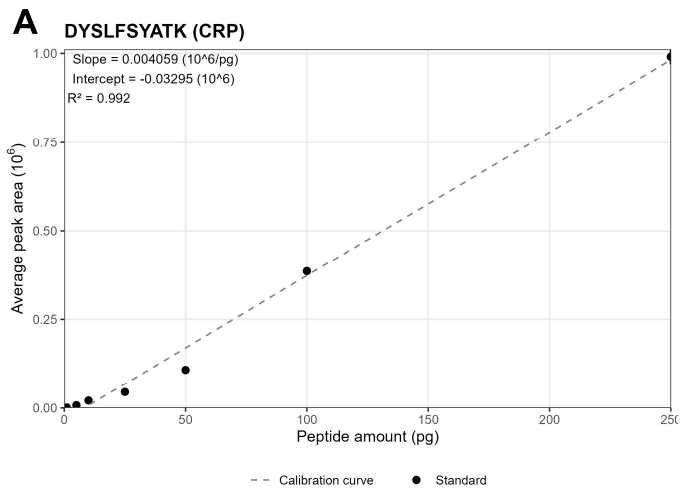
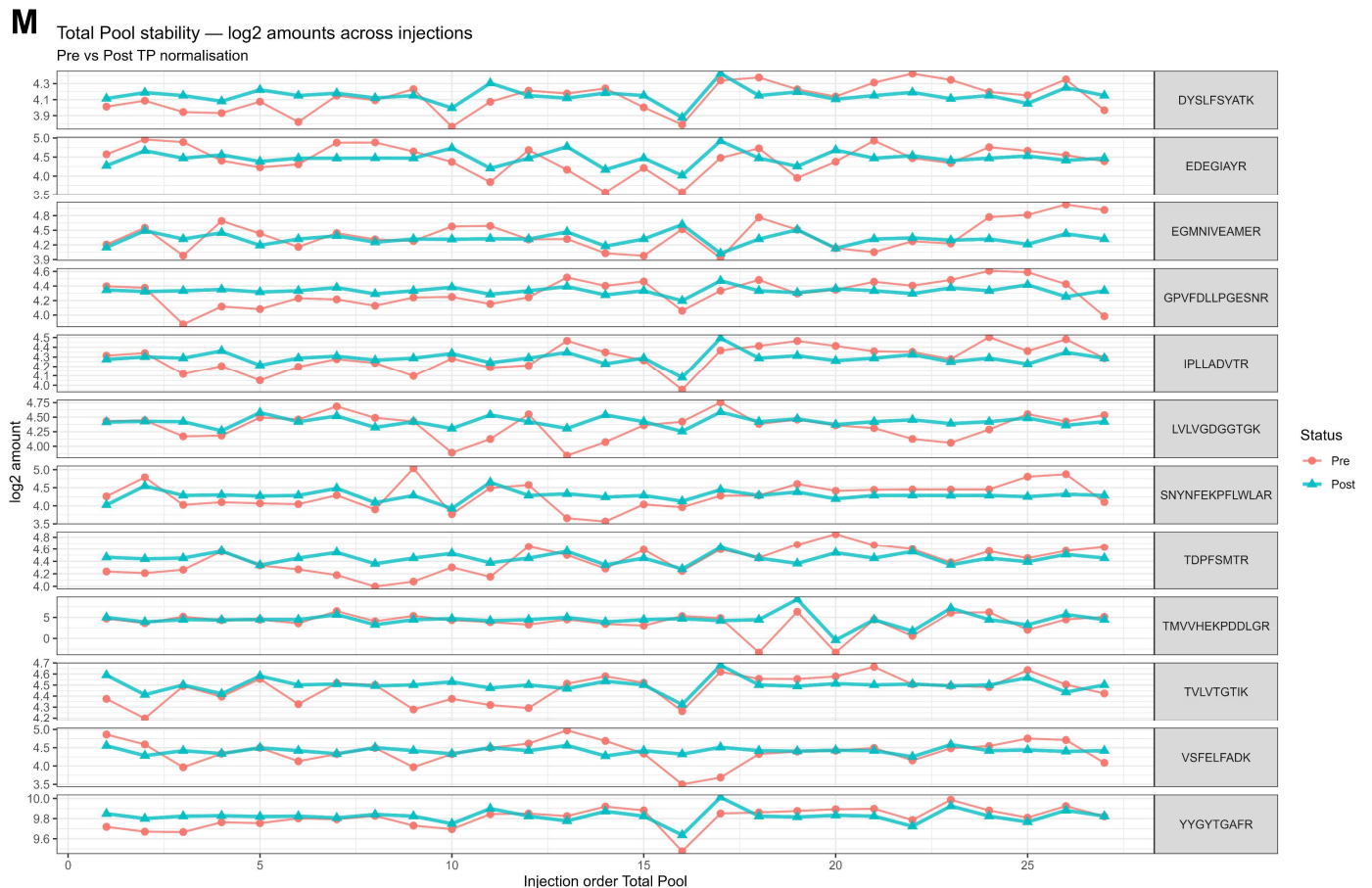
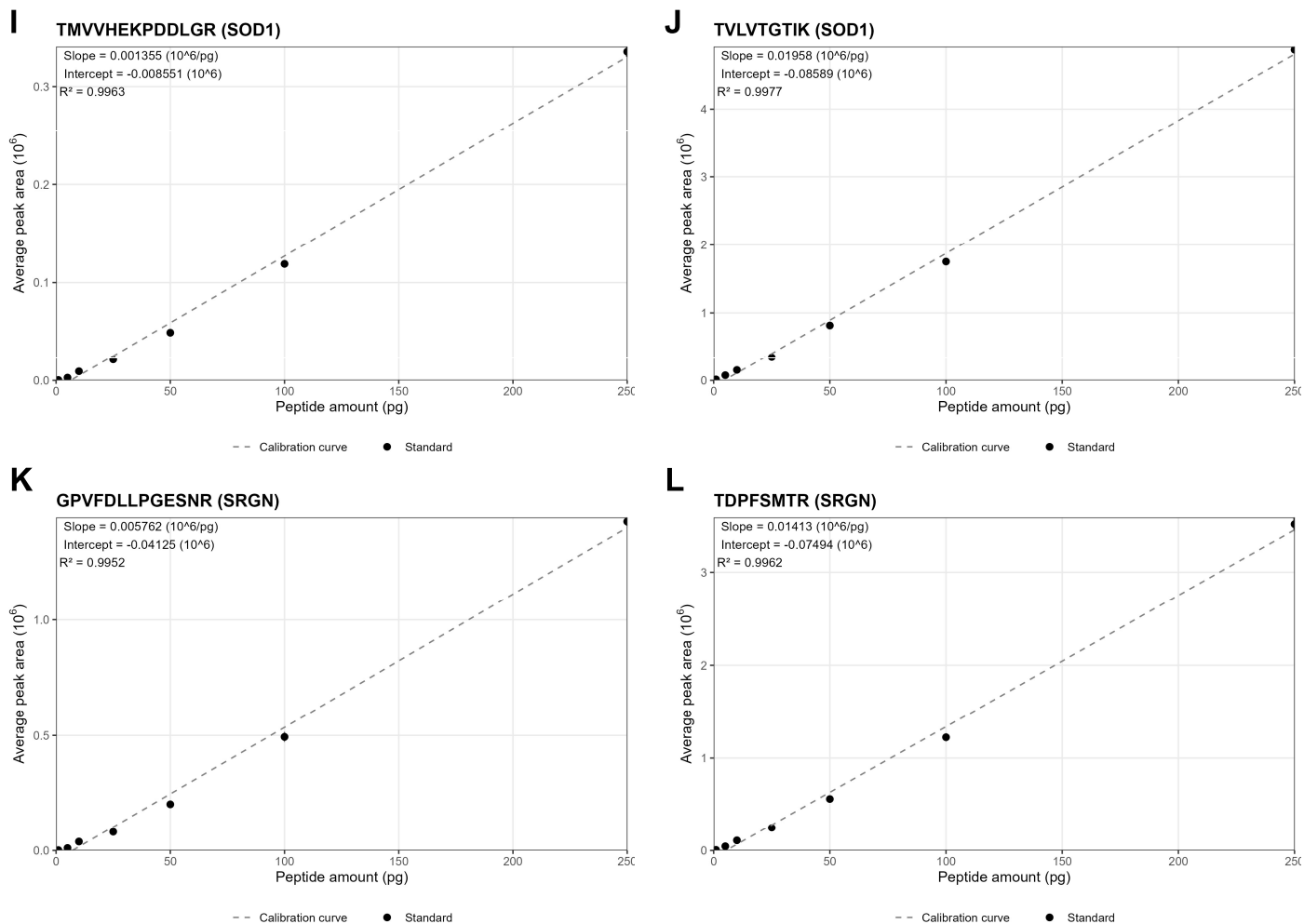


Figure S3



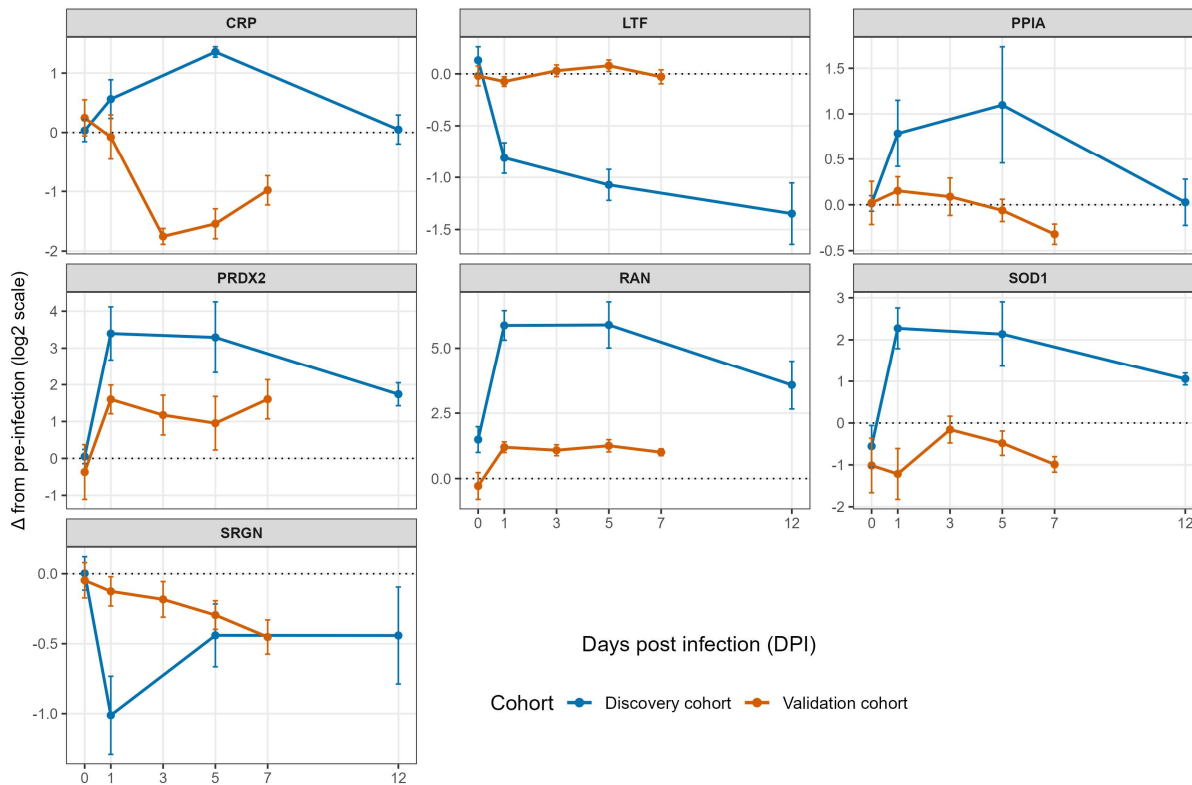




A

PRCV — centered protein time-course

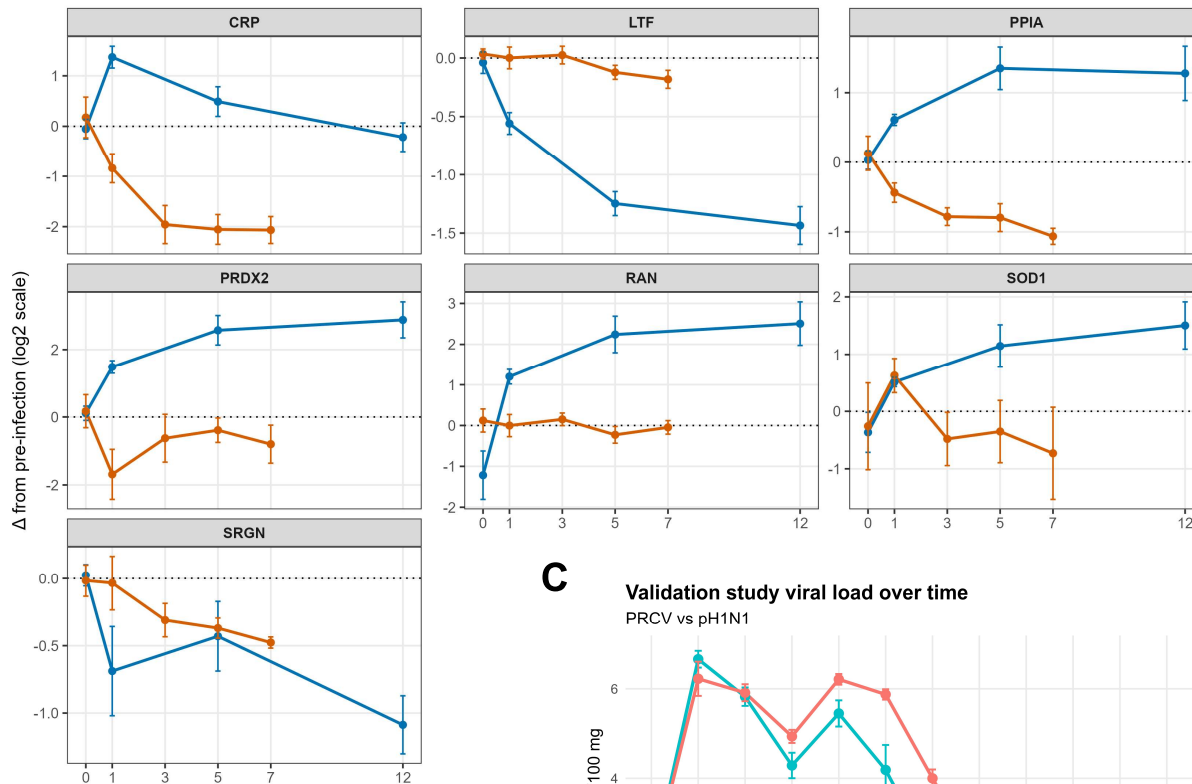
All selected proteins; centered to pre-infection baseline



B

pH1N1 — centered protein time-course

All selected proteins; centered to pre-infection baseline



C

Validation study viral load over time
PRCV vs pH1N1

