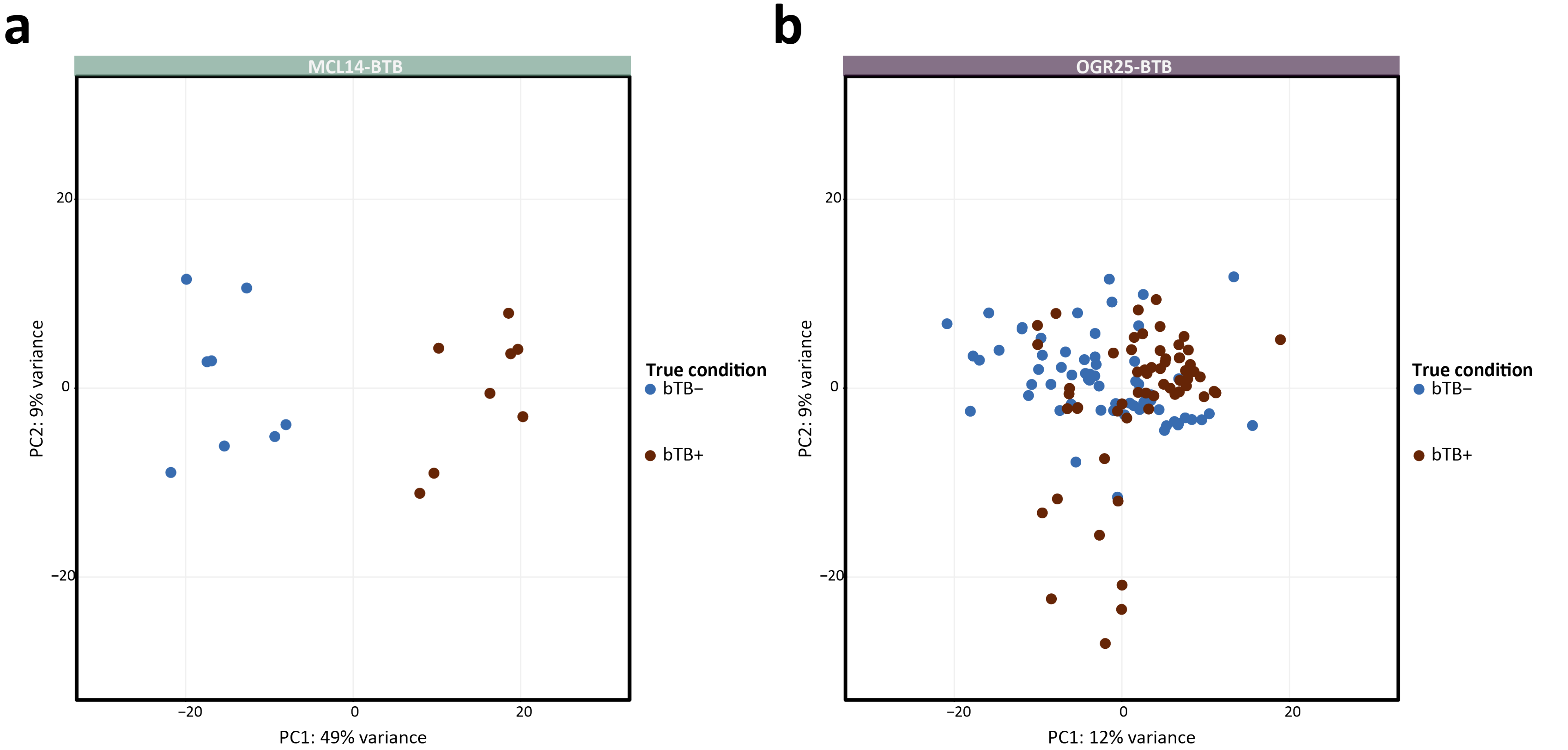
Supplementary Information for:

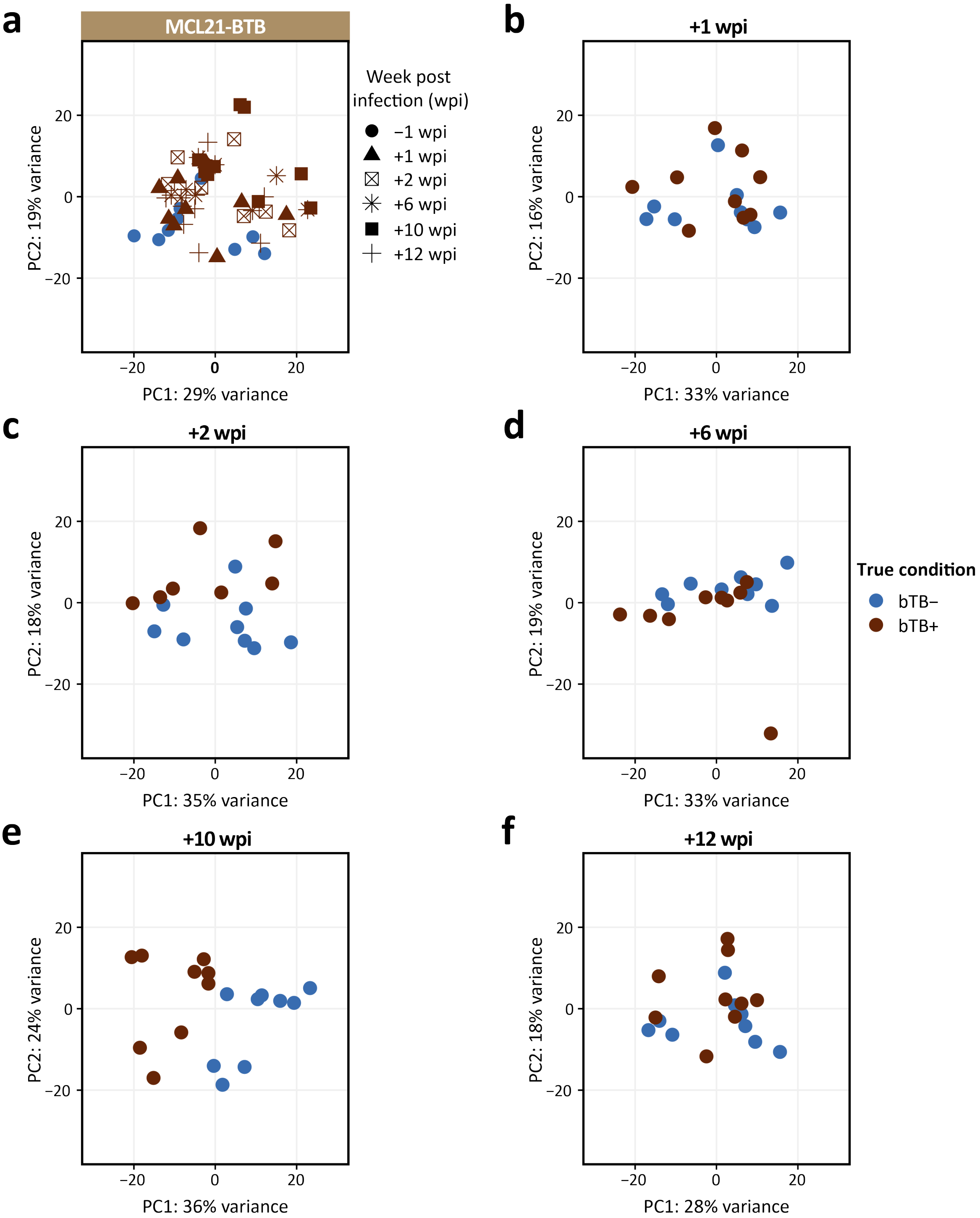
Chapter 4 | Accurate and robust classification of *M. bovis*-infected cattle using peripheral blood RNA-seq data

This PDF file includes:

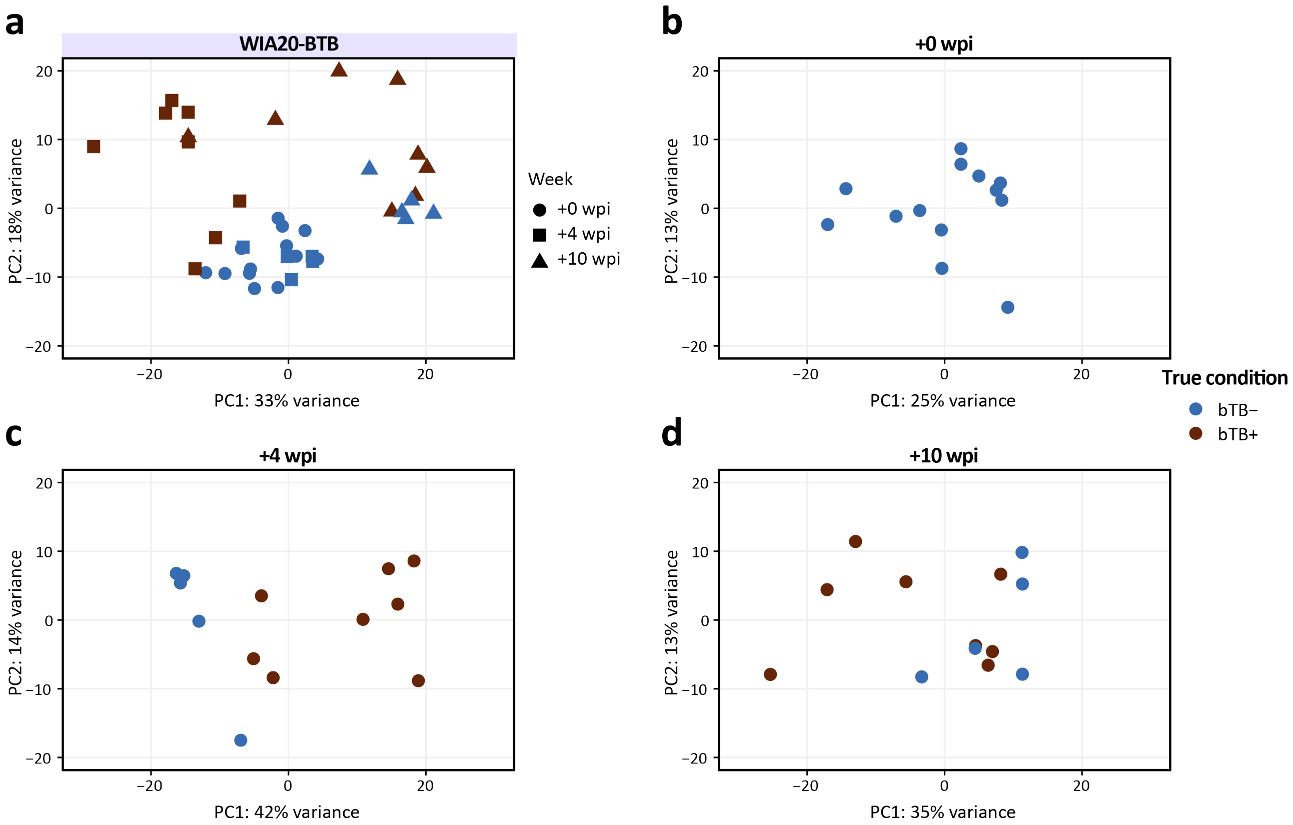
Supplementary Figures



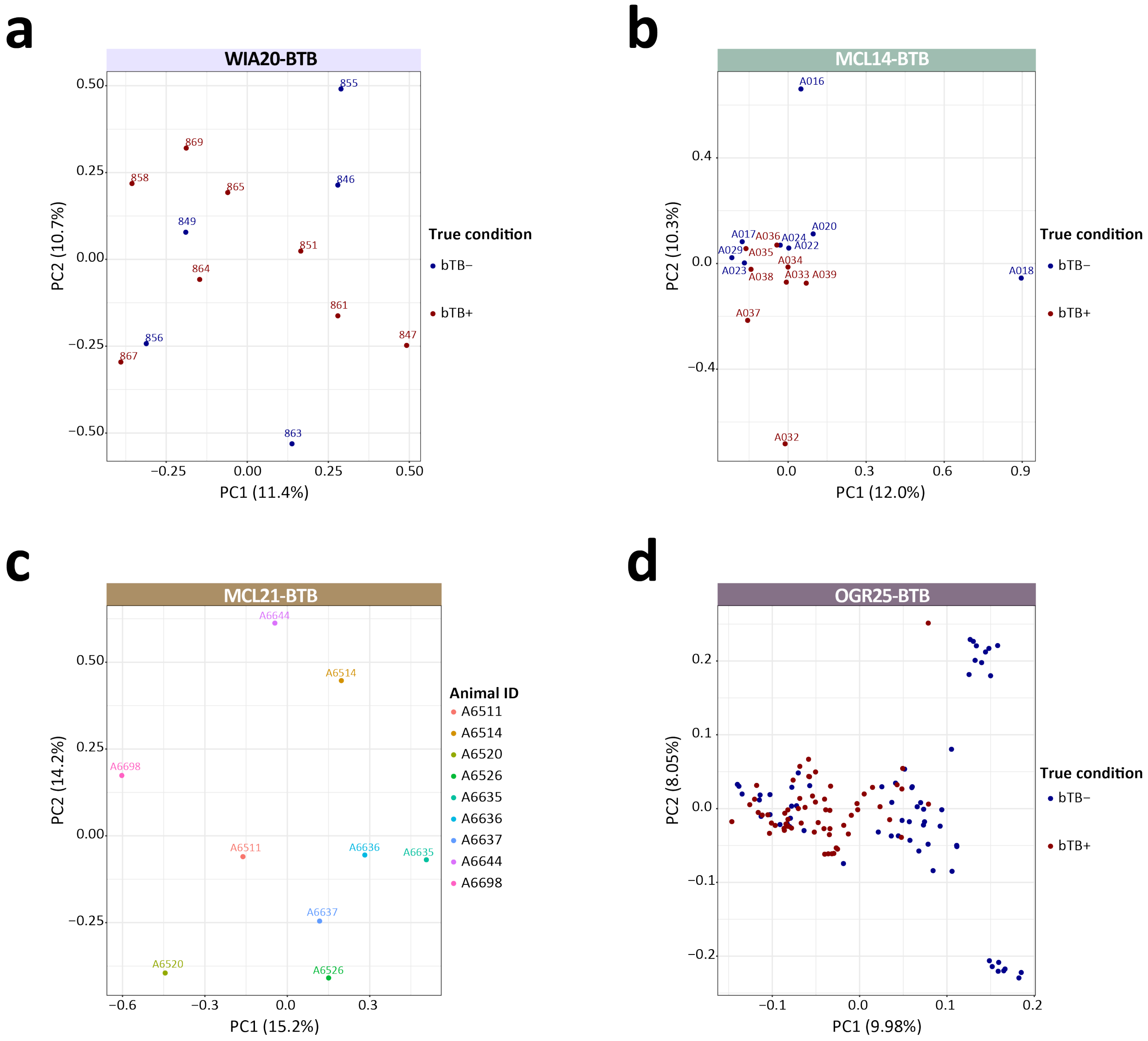
Supplementary Figure S4.1: Principal component analysis (PCA) of the top 750 most variable genes identified in (**a**) all *n* = 16 RNA-seq samples from the MCL14-BTB dataset and (**b**) all *n* = 123 RNA-seq samples from the OGR25-BTB dataset after variance stabilising transformation (VST) using DESeq2 (Love *et al.* 2014). Principal components 1 and 2 (PC1 and PC2) are plotted. Animal data points are shaped based on their experimental condition.



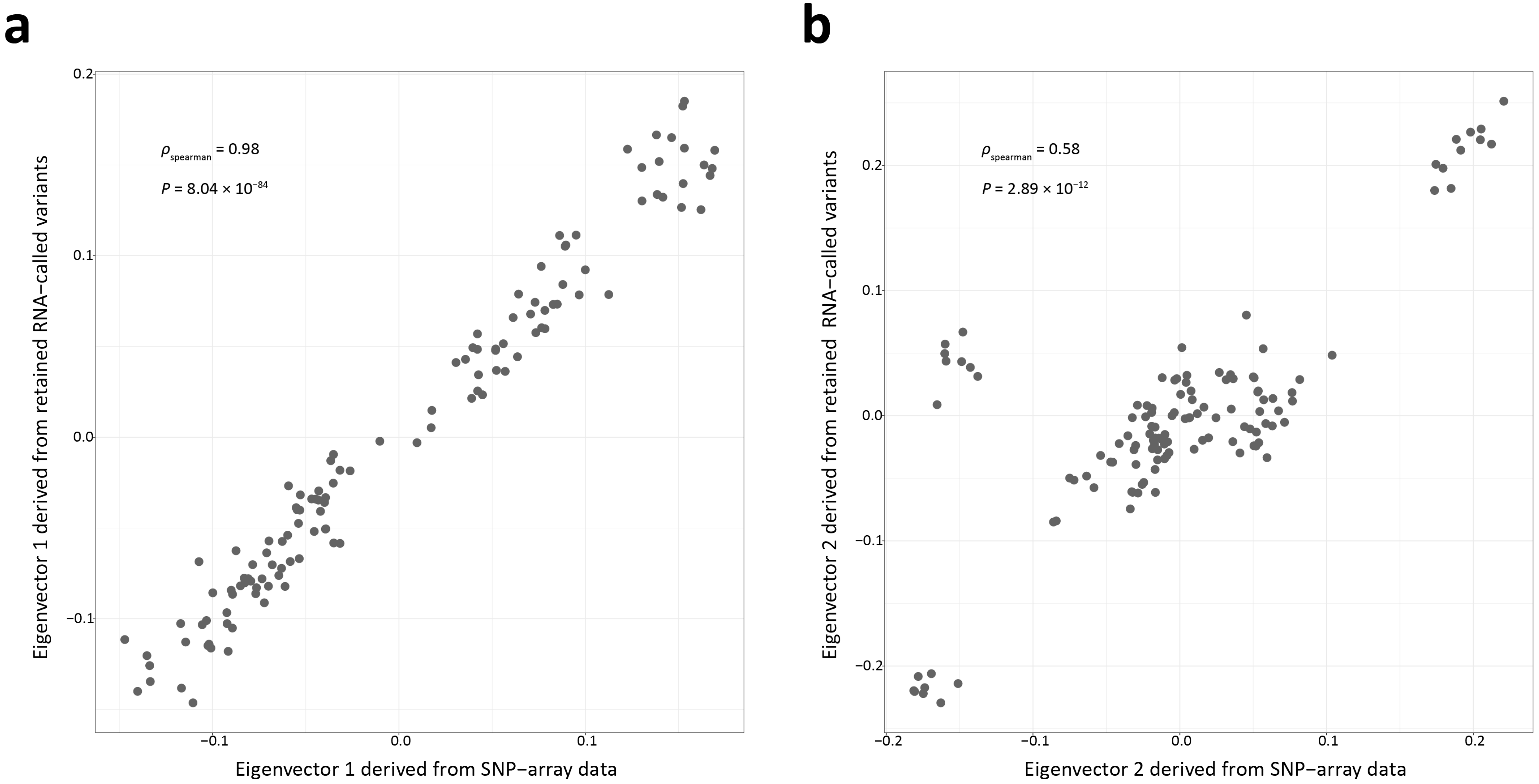
Supplementary Figure S4.2: Principal component analysis (PCA) of the top 750 most variable genes identified for (**a**) all *n* = 52 RNA-seq samples from the MCL21-BTB dataset after variance stabilising transformation (VST) using DESeq2. Principal components 1 and 2 (PC1 and PC2) are plotted. Animal data points are coloured based on their experimental condition and shaped based on their sampling time point, measured in terms of weeks post-infection (wpi). (**b**) PCA of animals sampled at +1 wpi compared to animals sampled at −1 wpi. (**c**) PCA of animals sampled at +2 wpi compared to animals sampled at −1 wpi. (**d**) PCA of animals sampled at +6 wpi compared to animals sampled at −1 wpi. (**e**) PCA of animals sampled at +10 wpi compared to animals sampled at −1 wpi. (**f**) PCA of animals sampled at +12 wpi compared to animals sampled at −1 wpi.



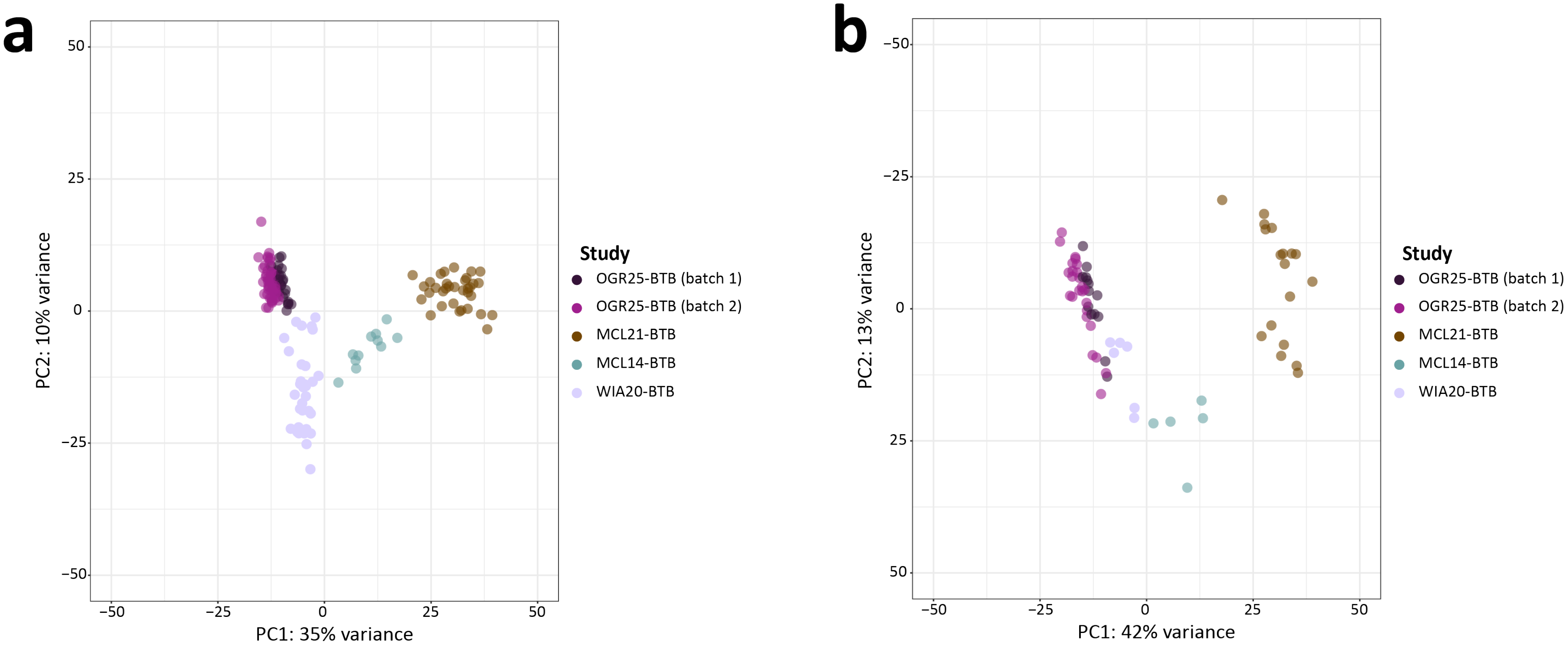
Supplementary Figure S4.3: Principal component analysis (PCA) of the top 750 most variable genes identified for (**a**) all *n* = 39 RNA-seq samples from the WIA20-BTB dataset after variance stabilising transformation (VST) using DESeq2. Principal components 1 and 2 (PC1 and PC2) are plotted. Animal data points are coloured based on their experimental condition and shaped based on their sampling time point, measured in terms of weeks post-infection (wpi). (**b**) PCA of animals sampled at +0 wpi. Note: all *n* = 8 animals experimentally infected with *M. bovis* were sampled prior to inoculation and are therefore considered control (bTB−) animals at this time point. (**c**) PCA of animals sampled at +4 wpi compared to animals sampled at 0 wpi. (**d**) PCA of animals sampled at +10 wpi compared to animals sampled at 0 wpi.



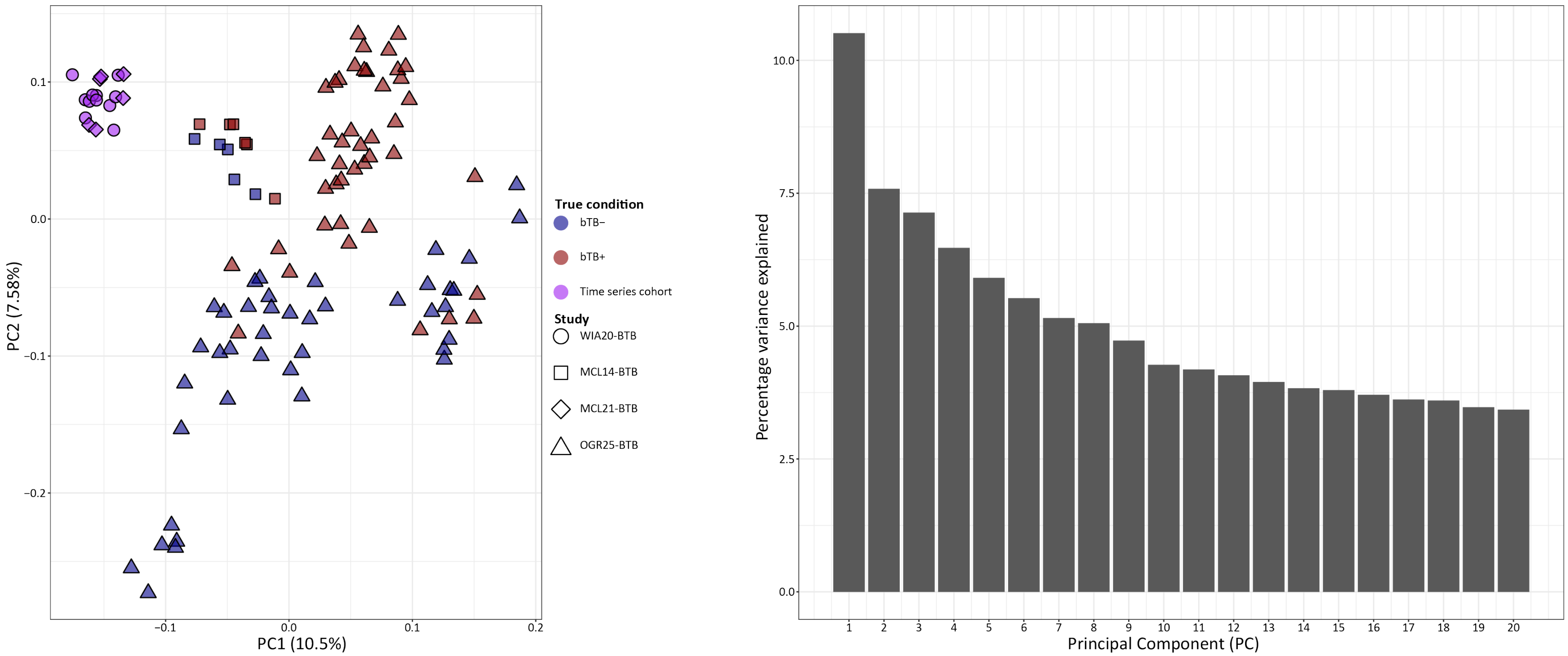
Supplementary Figure S4.4: (**a**) Genotype principal component analysis (PCA) of 1,048 genome-wide pruned SNPs called from the RNA-seq data in the WIA20-BTB dataset. Animals are coloured based on whether they were experimentally infected with *M. bovis* or not. (**b**) Genotype PCA of 917 genome-wide pruned SNPs called from the RNA-seq data in the MCL14-BTB dataset. Animals are coloured based on their experimental designation. (**c**) Genotype PCA of 1,022 genome-wide pruned SNPs called from the RNA-seq data in the MCL21-BTB dataset. Animals are coloured based on their designated animal ID. (**d**) Genotype PCA of 2,280 genome-wide pruned SNPs called in all other RNA-seq only datasets and present in a set of 31,213 imputed WGS SNPs from the OGR25-BTB dataset. Animals are coloured based on their experimental designation. For all panels principal components 1 and 2 (PC1 and PC2) are plotted.



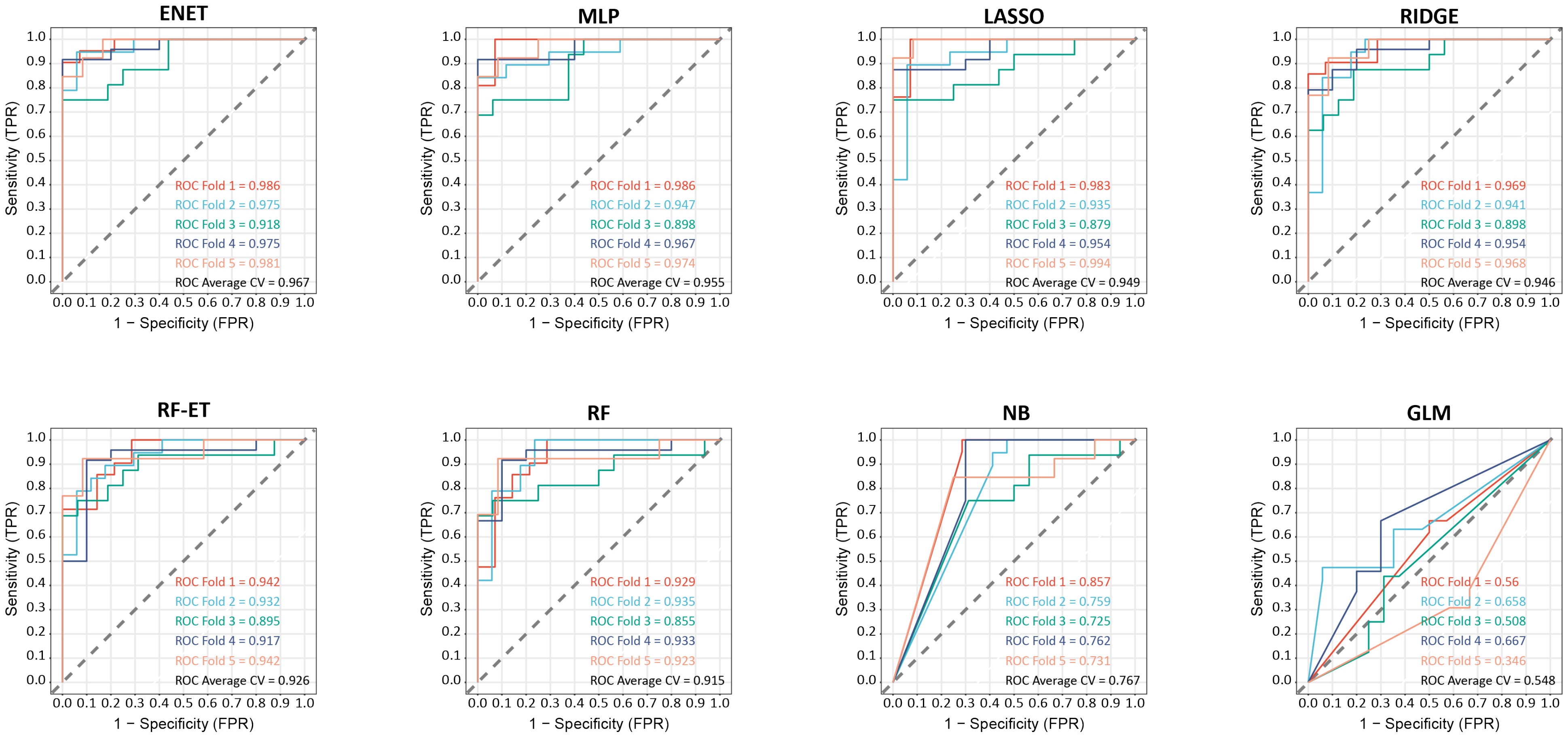
Supplementary Figure S4.5: (**a**) Spearman correlation (*ρ*) between Eigenvector 1 coordinates for all *n* = 123 animals from the OGR25-BTB dataset derived from a principal component analysis (PCA) of 2,280 pruned genome-wide SNPs and Eigenvector 1 derived from a PCA of 34,272 pruned genome-wide SNP array data reported for the same set of animals in O'Grady *et al.* (2025) . (**b**) Same as **a** but for Eigenvector 2 coordinates.



Supplementary Figure S4.6: (**a**) Principal component analysis (PCA) of the top 750 most variable genes identified in the training set after variance stabilising transformation (VST) using DESeq2. Principal components 1 and 2 (PC1 and PC2) are plotted. Animals are coloured based on their designated study. (**b**) A PCA of the top 750 most variable genes identified in the testing set after VST using DESeq2. PC1 and PC2 are plotted and animals are coloured based on their designated study.



Supplementary Figure S4.7: Genotype principal component analysis (PCA) of 2,136 pruned genome-wide SNPs identified in the training set. Principal components 1 and 2 (PC1 and PC2) are plotted. Animals are coloured depending on their experimental designation or if they are derived from a time series experiment. Animals are shaped based on their designated study. The barplot depicts the proportion of variance explained by each PC out of the top 20 PCs.



Supplementary Figure S4.8: Area-under receiver operating characteristic curve (AUROC) plots for eight machine learning (ML) models in each cross-validation fold in the training set. ENET, elastic-net; MLP, multi-layered perceptron; LASSO, least absolute shrinkage and selection operator; RIDGE, ridge-penalised regression; RF-ET, random forest (extra trees); RF, random forest; NB, naïve Bayes; and GLM, generalised unpenalized logistic regression model.

References

Love M.I., Huber W. & Anders S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15, 550.

O'Grady J.F., McHugo G.P., Ward J.A., Hall T.J., Faherty O'Donnell S.L., Correia C.N., Browne J.A., McDonald M., Gormley E., Riggio V., Prendergast J.G.D., Clark E.L., Pausch H., Meade K.G., Gormley I.C., Gordon S.V. & MacHugh D.E. (2025) Integrative genomics sheds light on the immunogenetics of tuberculosis in cattle. *Commun Biol* 8, 479.