Supplemental Methods

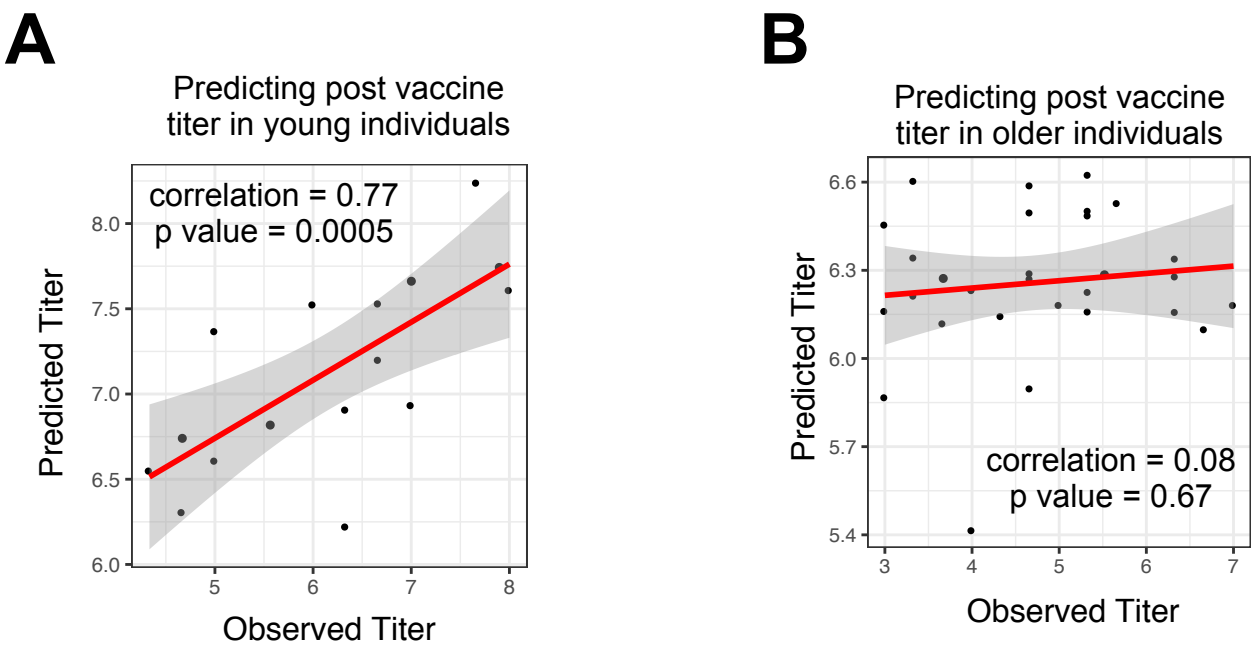
Predicting HAI titer with cydar

To compare CytoDx with an existing method, cydar (Lun *et al.*, 2017), we applied cydar on the rank transformed data. Briefly, the local density around each cell was estimated using countCells function from cydar R package. The package edgeR was used to identify the regions significantly different between high titer group and low titer group. spatialFDR function from cydar R package was used to control for the false discover rate.

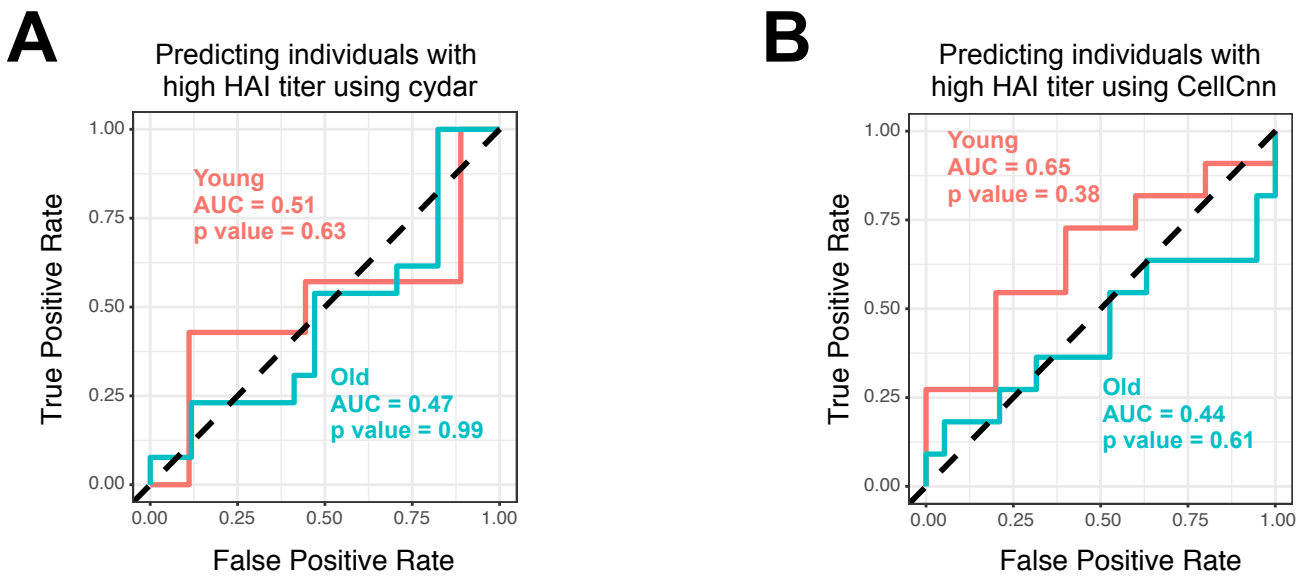
The procedure did not identify any regions significantly different between high and low titer groups. Therefore, we used the cell densities in all regions to predict HAI titer. Because the regularized generalized linear regression was used in CytoDx, we applied the same regression method on the cell densities from cydar to ensure a fair comparison. We trained a logistic regression model using the training data from SDY112 and applied the model to testing data from SDY404 to predict HAI titer.

Predicting HAI titer with CellCnn

To compare CytoDx with an existing method, CellCnn (Arvaniti and Claassen, 2017), we applied CellCnn on the rank transformed data. Briefly, we used two thirds of samples from SDY112 to train CellCnn models with different hyper-parameter combinations (maxpool percentage ranges from 0.01 to 100.0; number of filters ranges from 3 to 9). The performance of each model is evaluated using the rest of the samples in SDY112 to choose the best performing model. The chosen model is then used to predict HAI titer in SDY404.



Supplementary Fig. 1. CytoDx is used to predict the post-vaccine titer (in log scale) as a continuos variable in young (**A**) and older individuals (**B**). The scatter plots shows the correlation between the observed titer and predicted titer.



Supplementary Fig. 2. Performance of existing methods on rank data. Cydar (**A**) and CellCnn(**B**) was applied on the rank transformed data from SDY404 and SDY112. The performance is visualized by ROC and measured by AUC.