Supplementary methods

Table SVI. A logistic regression model was constructed using the expression levels of LDHA and TXN, with controls coded as 0 and Lyme disease cases coded as 1. Model training was performed using repeated 10-fold cross-validation with three repeats. For classification reporting, the disease group (case=1) was predefined as the positive class. Accordingly, the confusion matrix and all derived performance metrics, including sensitivity, specificity, positive predictive value, negative predictive value and balanced accuracy, were calculated with 1 as the positive class. Receiver operating characteristic (ROC) analysis was based on the predicted probability of the case class, and the area under the ROC curve (AUC) with its 95% confidence interval (CI) was estimated by bootstrap resampling. In this analysis, the model yielded an AUC of 0.879 (95% CI, 0.761-0.967).

Table SVII. To reduce information leakage and obtain a less biased estimate of predictive performance, the final lactate dehydrogenase A-thioredoxin (LDHA-TXN) logistic regression model was evaluated using an outer 5-fold cross-validation framework. Within each outer split, LDHA and TXN were kept fixed as prespecified predictors, and the model was trained on the outer-training subset and tested on the held-out outer-test subset. Although an inner repeated 10-fold cross-validation structure with three repeats was predefined in the code, it was not ultimately used for additional feature selection or hyperparameter tuning in the final two-gene model.

Supplementary results

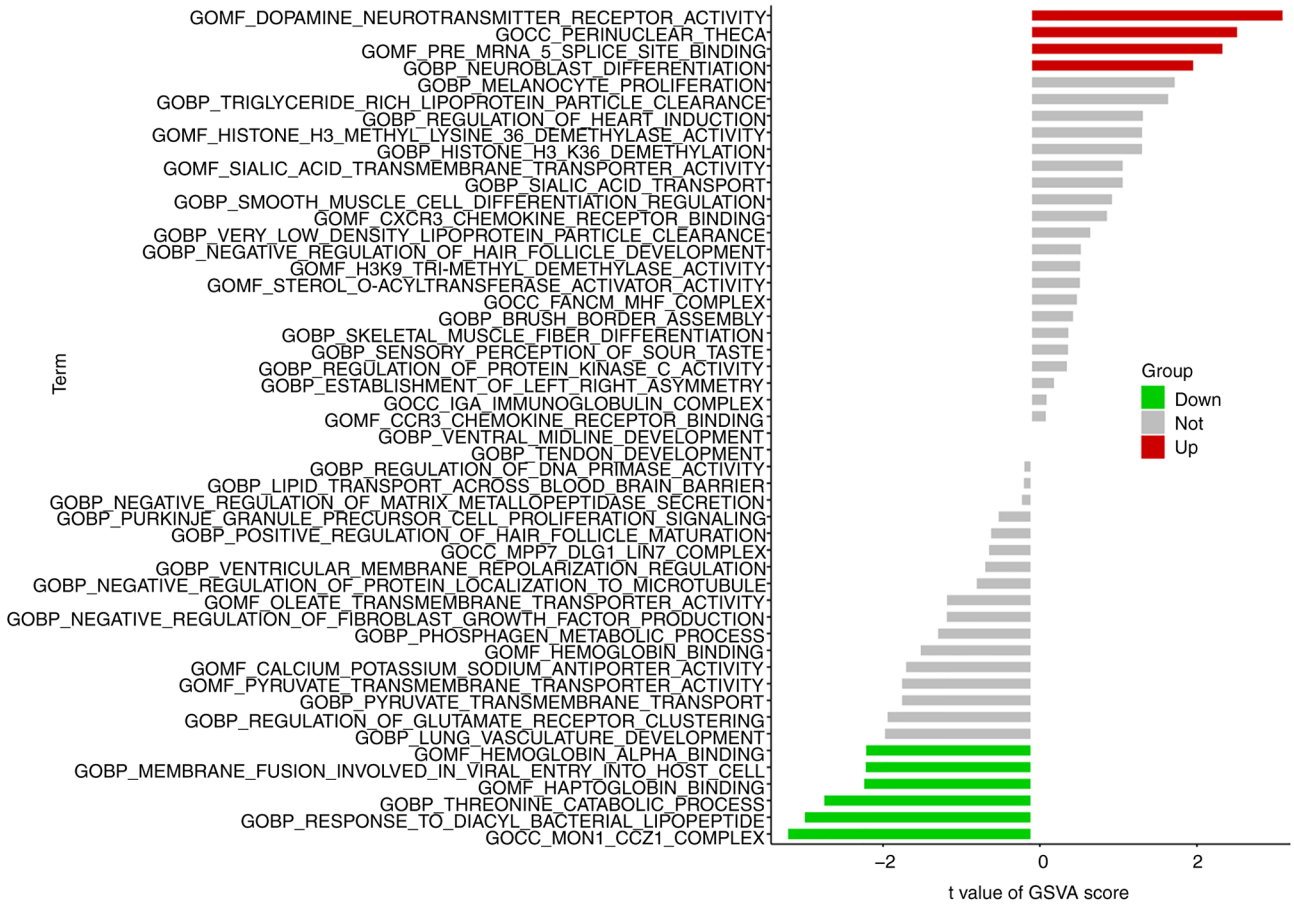
Table SVI. The LDHA-TXN logistic regression model showed good discriminative ability in the validation analysis, with an AUC of 0.879 (95% CI, 0.761-0.967). The confusion matrix showed 24 true-positives, 9 true-negatives, 4 false-positives and 4 false-negatives. The overall accuracy was 0.805 (95% CI, 0.651-0.912), with a κ value of 0.550. The sensitivity was 0.857, the specificity was 0.692, the positive predictive value was 0.857, the negative predictive value was 0.692 and the balanced accuracy was 0.775.

Table SVII. Across the five outer folds, the fold-specific AUCs were 1.000, 0.959, 1.000, 1.000 and 1.000, yielding a mean outer-fold AUC of 0.992. Based on the pooled out-of-fold predictions, the overall AUC was 0.974, with a bootstrap 95% CI of 0.918-1.000. These results indicate that the model retained strong discriminatory ability when evaluated on samples not used for model fitting in each outer fold.

In addition to discrimination, calibration and decision curve analyses were performed using pooled out-of-fold predicted probabilities to avoid optimistic bias. Fold-wise Brier scores ranged from 0.004 to 0.088, with a mean Brier score of 0.035. Calibration metrics showed variability across folds, with intercepts ranging from -7.423 to 2.846 and slopes ranging from 0.432 to 22.858. These findings suggest that, although the model showed excellent discrimination, fold-specific calibration was less stable.

Notably, the modeling process produced warnings indicating that the logistic regression algorithm did not fully converge in some folds and that fitted probabilities of 0 or 1 occurred. Together with the very high AUCs and large calibration slopes observed in several folds, this pattern is most consistent with near-complete separation and limited fold-wise sample size rather than truly perfect calibration. Therefore, the calibration findings should be interpreted cautiously, even though the discrimination results were strong.

Figure S1. GO-based GSVA between the high- and low-expression groups of LDHA and TXN. (A) GO-based GSVA results comparing the LDHA high- and low-expression groups. (B) GO-based GSVA results comparing the TXN high- and low-expression groups. GO, Gene Ontology; GSVA, gene set variation analysis; LDHA, lactate dehydrogenase A; TXN, thioredoxin.



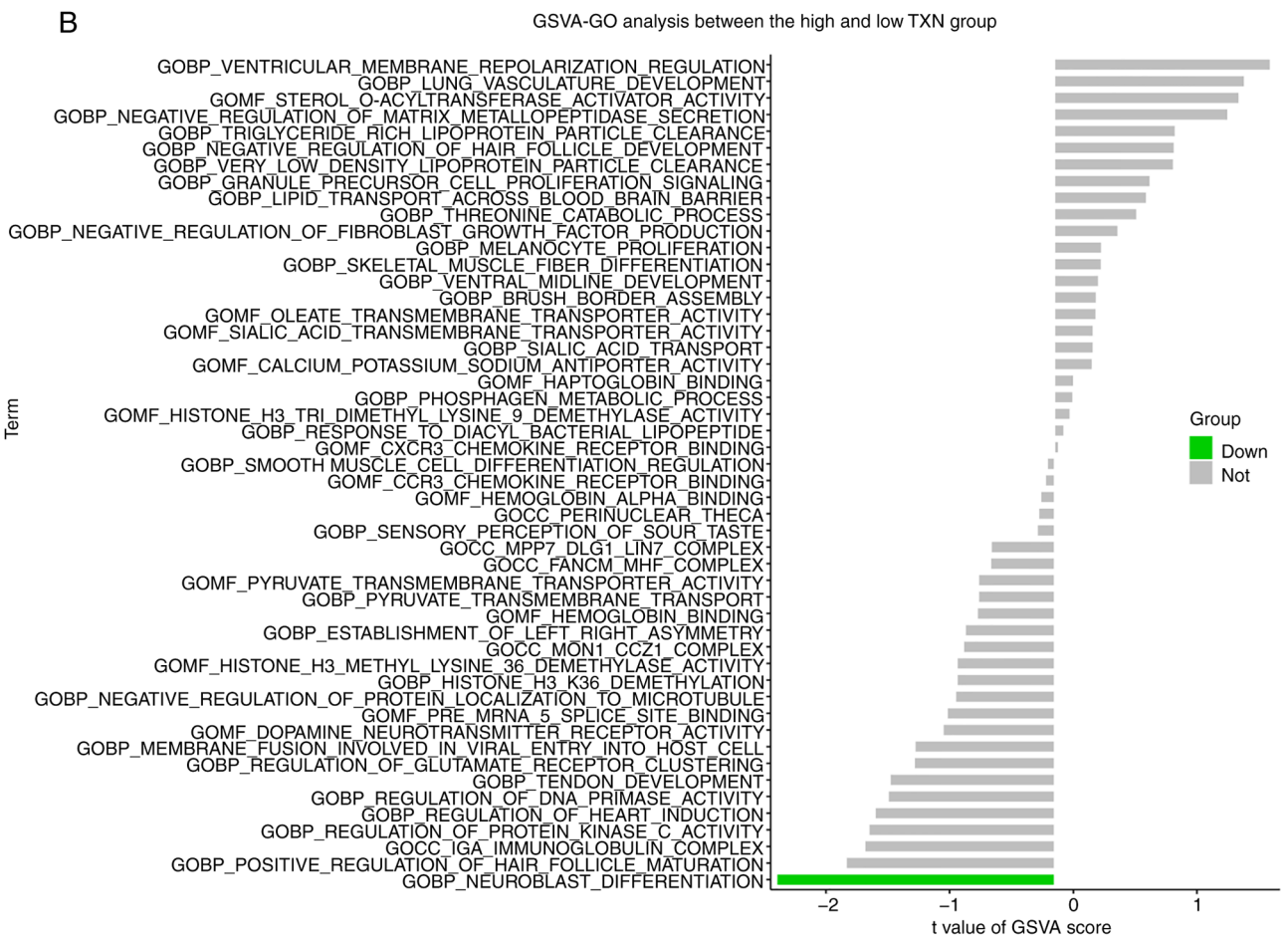


Figure S2. Validation performance of the LDHA-TXN model. In the validation analysis, the LDHA-TXN logistic regression model demonstrated good discriminative ability, with an AUC of 0.879 (95% CI, 0.761-0.967). When case=1 was treated as the positive class, the confusion matrix showed 24 true-positives, 9 true-negatives, 4 false-positives and 4 false-negatives. The overall accuracy was 0.805 (95% CI, 0.651-0.912), with a κ value of 0.549. The sensitivity was 0.857, the specificity was 0.692, the positive predictive value was 0.857, the negative predictive value was 0.692 and the balanced accuracy was 0.775. LDHA, lactate dehydrogenase A; TXN, thioredoxin.