Prognostic and biological relevance of collagen-related genes in prostate cancer

Supplementary material, transcriptome part

2025-02-21

# Supplementary Figures



Figure 1: Co-expression networks of the collagen-related transcripts in PCA tissue.

**Supplementary Figure S1. Co-expression networks of the collagen-related transcripts in PCA tissue.**

*Co-expression networks of the collagen-related transcripts in PCA were constructed in the pooled GEO (cancer samples: n = 501), TCGA (n = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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The network edges were defined by pairwise correlations between ComBat-adjusted transcript levels in the cancer tissue with Spearman’s 0.5. Isolated vertices, i.e. transcripts without correlation partners, were removed.* *The co-expression networks were visualized with Fruchterman-Reingold algorithm. Each point represents a single transcript in the network; point color codes for functional classification of the transcript. Edges, i.e. correlations of expression levels with 0.5, are depicted as gray lines. Numbers of transcripts (vertices) and correlations with 0.5 (edges) are displayed in the plot captions.*

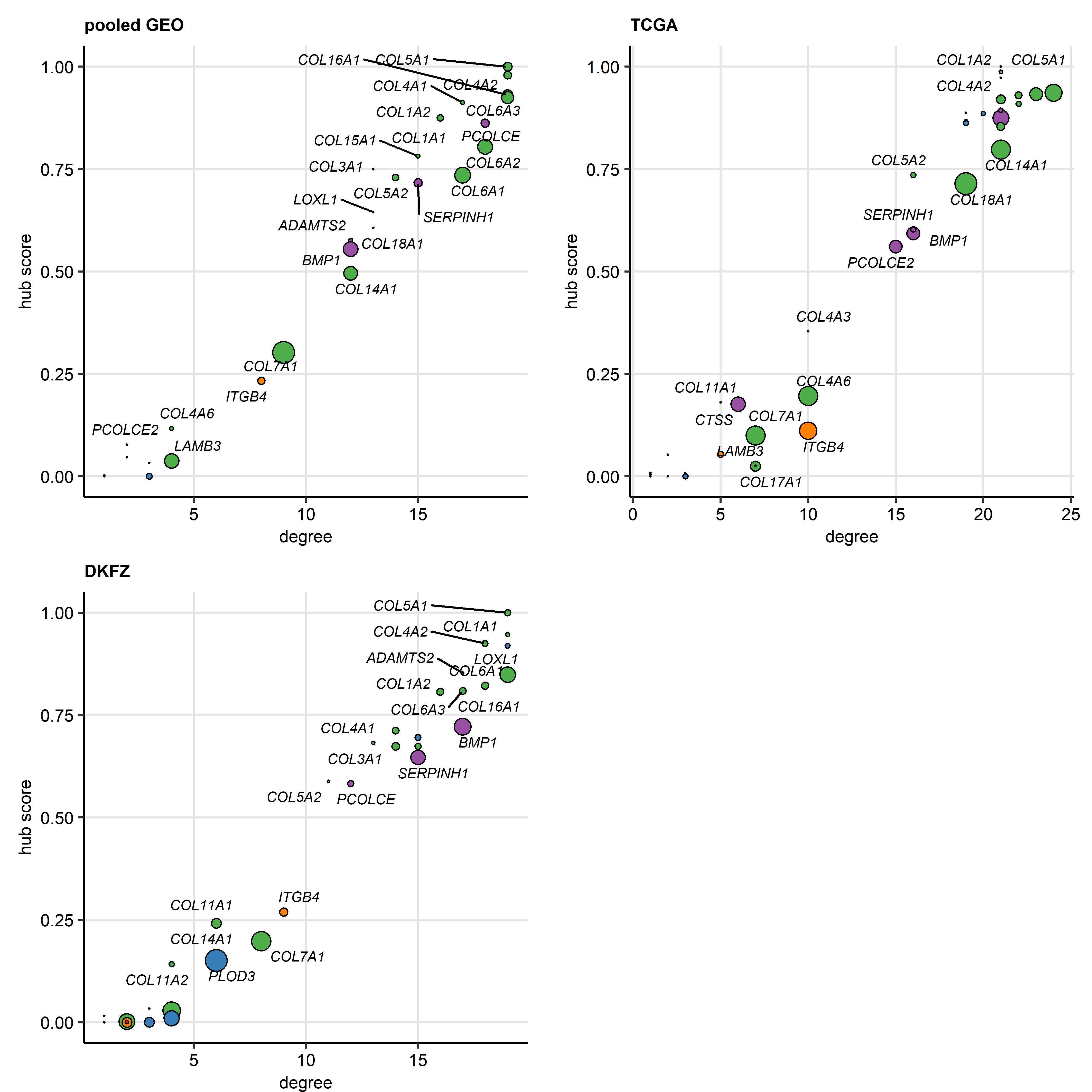


Figure 2: Vertex importance statistics for co-expression networks of the collagen-related transcripts in PCA.

**Supplementary Figure S2. Vertex importance statistics for co-expression networks of the collagen-related transcripts in PCA.**

*Co-expression networks of the collagen-related transcripts were constructed in the pooled the pooled GEO (cancer samples: n = 501), TCGA (n = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 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importance metrics for the collagen-related transcripts in the co-expression networks are shown in dot plots. Each point represents a single collage-related transcript, point color codes for functional classification of the transcript.*

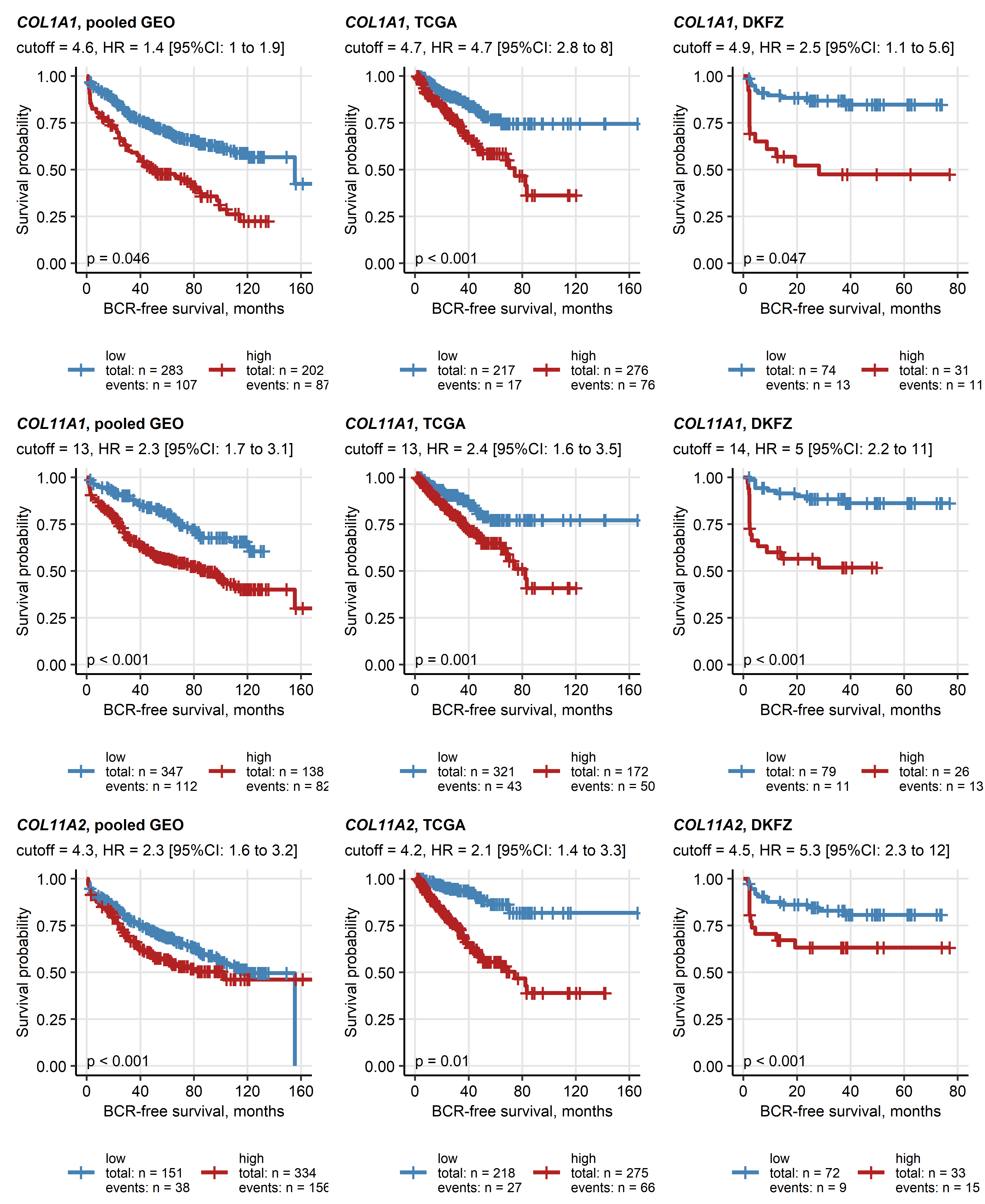


Figure 3: Top three unfavorable standalone collagen-related transcriptional markers of BCR-free survival in PCA.

**Supplementary Figure S3. Top three unfavorable standalone collagen-related transcriptional markers of BCR-free survival in PCA.**

*Prostate cancer (PCA) patients in the pooled GEO (total: n = 485, biochemical relapse [BCR]: n = 194), TCGA (total: n = 493, BCR: n = 93), and DKFZ cohort (total: n = 105, BCR: n = 24) were classified as high and low expressors for each of the rlength(globals$genes)` collagen-related transcripts with the expression cutoffs corresponding to the largest differences in survival between the high and low expressors.* *Collagen-related transcripts associated with unfavorable and favorable BCR prognosis were identified by univariable Cox proportional hazard regression as presented in Figure 1.* *COL1A1, COL11A1, and COL11A2 were identified as the strongest unfavorable BCR risk markers, as measured by mean Harrell’s concordance index (measure of model accuracy) in the investigated cohorts. Fractions of BCR-free patients in the high and low expression strata are visualized in Kaplan-Meier plots. The log2 expression cutoffs used for the high/low expressor classification, hazard-ratios (HR) of BCR risk in the high as compared with the low expression strata with 95% confidence intervals (95% CI) are displayed in the plot captions. P values (p(HR = 0)) corrected for multiple testing with the false discovery rate method are shown in the plots. Numbers of patients and BCR cases in the expression strata are indicated in the plot legends.*

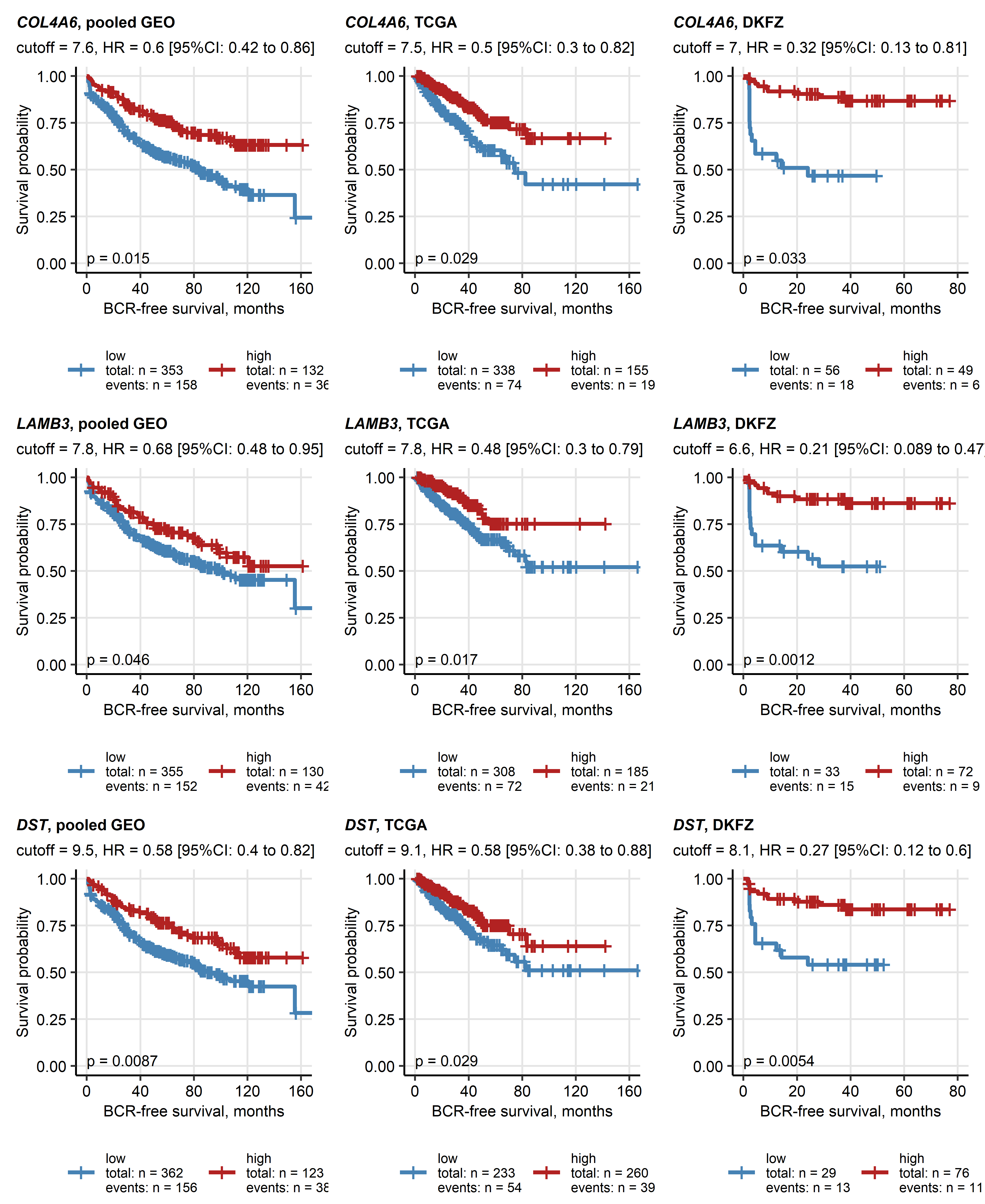


Figure 4: Top three favorable standalone collagen-related transcriptional markers of BCR-free survival in PCA.

**Supplementary Figure S4. Top three favorable standalone collagen-related transcriptional markers of BCR-free survival in PCA.**

*Prostate cancer (PCA) patients in the pooled GEO (total: n = 485, biochemical relapse [BCR]: n = 194), TCGA (total: n = 493, BCR: n = 93), and DKFZ cohort (total: n = 105, BCR: n = 24) were classified as high and low expressors for each of the rlength(globals$genes)` collagen-related transcripts with the expression cutoffs corresponding to the largest differences in survival between the high and low expressors.* *Collagen-related transcripts associated with unfavorable and favorable BCR prognosis were identified by univariable Cox proportional hazard regression as presented in Figure 1.* *COL4A6, LAMB3, and DST were identified as the strongest favorable BCR risk markers, as measured by mean Harrell’s concordance index (measure of model accuracy) in the investigated cohorts. Fractions of BCR-free patients in the high and low expression strata are visualized in Kaplan-Meier plots. The log2 expression cutoffs used for the high/low expressor classification, hazard-ratios (HR) of BCR risk in the high as compared with the low expression strata with 95% confidence intervals (95% CI) are displayed in the plot captions. P values (p(HR = 0)) corrected for multiple testing with the false discovery rate method are shown in the plots. Numbers of patients and BCR cases in the expression strata are indicated in the plot legends.*

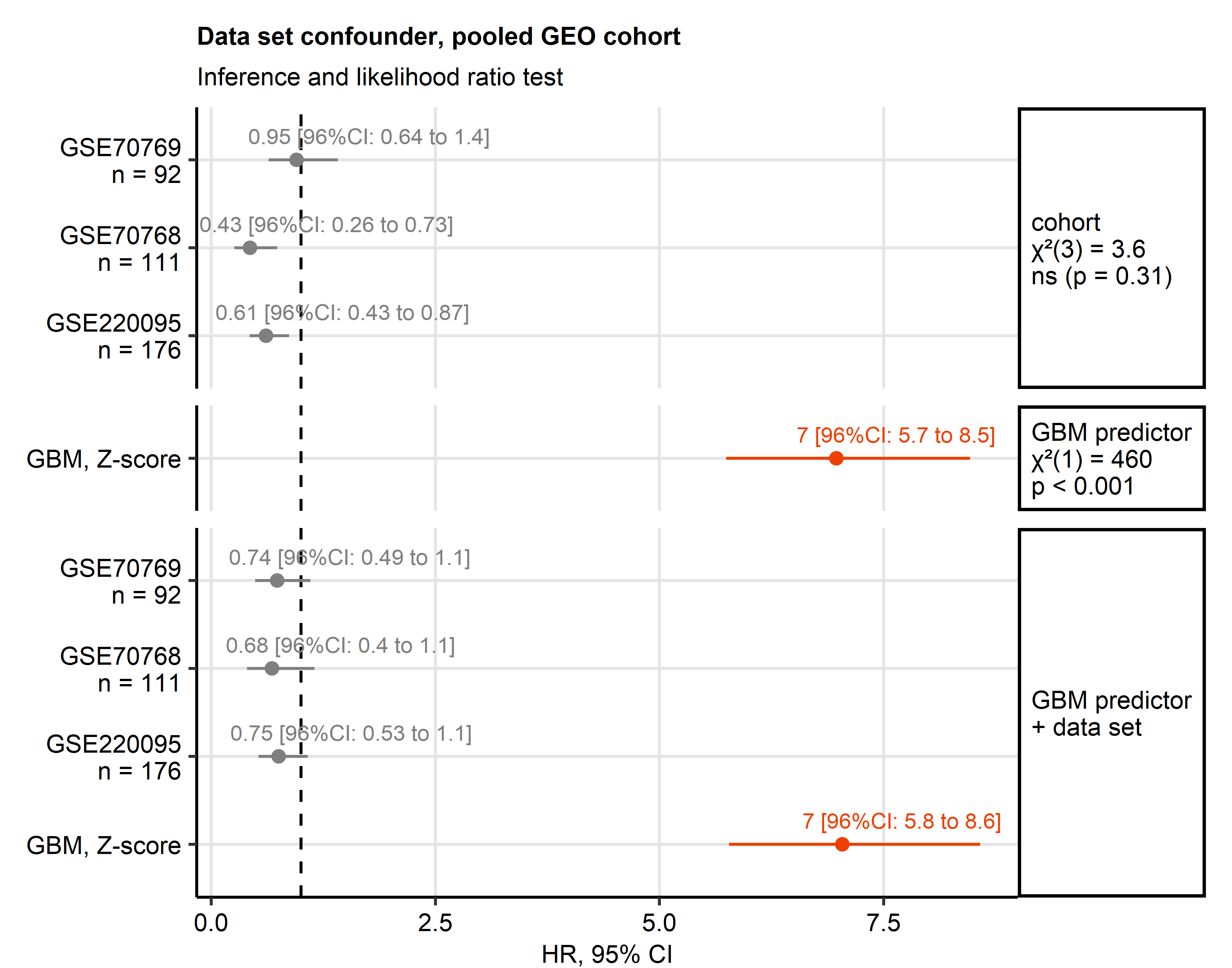


Figure 5: Investigation of confounding effect of the cohort on prediction of BCR-free survival by the GBM model in the pooled GEO cohort.

**Figure 5. Investigation of confounding effect of the cohort on prediction of BCR-free survival by the GBM model in the pooled GEO cohort.**

*Biochemical relapse (BCR) free survival was modeled by the GBM algorithm with ComBat-adjusted -transformed expression levels of the collagen-related genes as explanatory factors. The Gradient Boosted Machines (GBM) model was trained in the pooled GEO cohort (total: n = 485, BCR: n = 194).* *To investigate if and to which extend the BCR risk prediction depends on the confounding effect of single data sets constituting the pooled GEO cohort (GSE54460, GSE70768, GSE70769, and GSE220095), we constructed a canonical Cox proportional hazard regression model of BCR-free survival with the GBM predictor score and assignment to the data sets as independent variables. Performance and inference of this data set-adjusted GBM model was compared with performance and inference of the initial GBM model.*

*Coefficient estimates expressed as hazard ratios (HR) with 95% confidence intervals of the data set-adjusted, the initial and the data set-only survival models are presented in a Forest plot. Significance of contribution of the cohort and GBM predictor score to the BCR-free survival prediction were assessed by likelihood ratio test (LRT), whose results are presented in the plot facets. Note the significant contribution of the GBM predictor score to the prediction of BCR-free survival and the non-significant effect of the data set.*

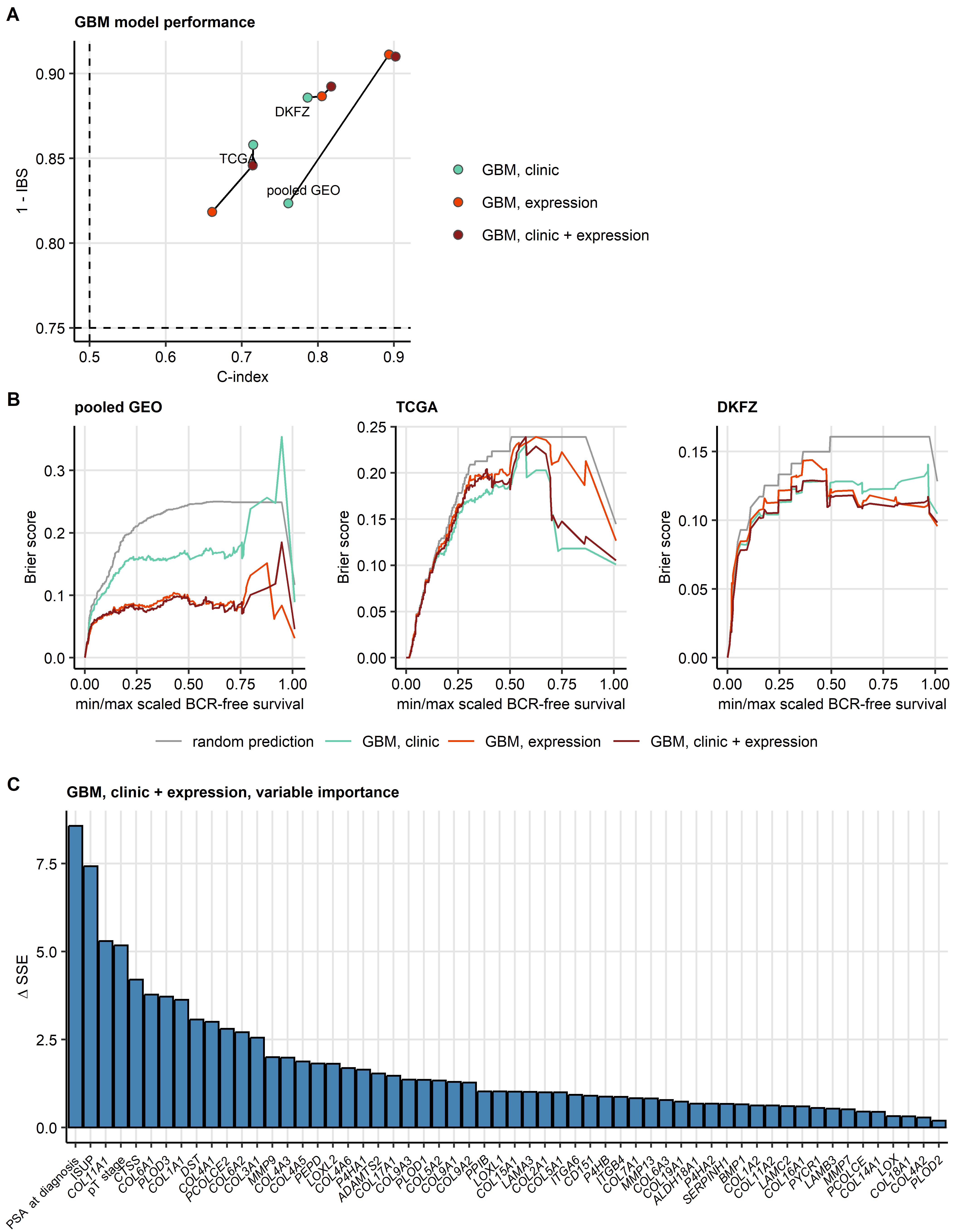


Figure 6: Modeling of BCR-free survival by GBM algorithm with clinical predictors and mRNA expression of the collagen-related genes.

**Figure 6. Modeling of BCR-free survival by GBM algorithm with clinical predictors and mRNA expression of the collagen-related genes.**

*Three Gradient Boosted Machines (GBM) models of biochemical relapse (BCR) free survival were trained in the pooled GEO cohort: a model with clinical predictors (age, pathological tumor stage, ISUP grade, and blood PSA concentration at diagnosis), a model with ComBat-adjusted expression levels of the collagen-related transcripts, and a model with the clinical and transcriptomic predictors. Performance of the GBM models was evaluated in the pooled GEO training collective (total: n = 460, BCR: n = 180), and TCGA (total: n = 422, BCR: n = 84) and DKFZ (total: n = 98, BCR: n = 18) validation cohorts.*

*(A) Numeric metrics of survival model performance, Harrell’s concordance index (C-index, measured of model accuracy) and Integrated Brier Score (IBS, measure of overall calibration, low values are characteristic for well calibrated models), are presented in a dot plot. Each point represents a single GBM model, point color codes for the model type. Models fit in the same cohort are connected with lines. Note the substantial improvement of the GBM model accuracy and calibration upon inclusion of the collagen-related transcript expression levels in the explanatory variable set in the pooled GEO and DKFZ cohorts.*

*(B) Brier Scores for unique survival time points for the GBM models in the pooled GEO, TCGA, and DKFZ cohort. Low Brier Scores at a particular time point indicate accurate and confident prediction of the BCR risk. Note: gray lines represent random survival predictions by a null model.*

*(C) Importance of the explanatory variables for BCR-risk prediction by the GBM model with the clinical and transcriptional predictors. The variable importance was computed as difference in sum of squared error () attributed to inclusion of a particular explanatory factor in the GBM learner ensemble. High values are characteristic for highly influential variables. values for the variables that contributed substantially to the BCR risk prediction ( > 0) are presented in a bar plot.*

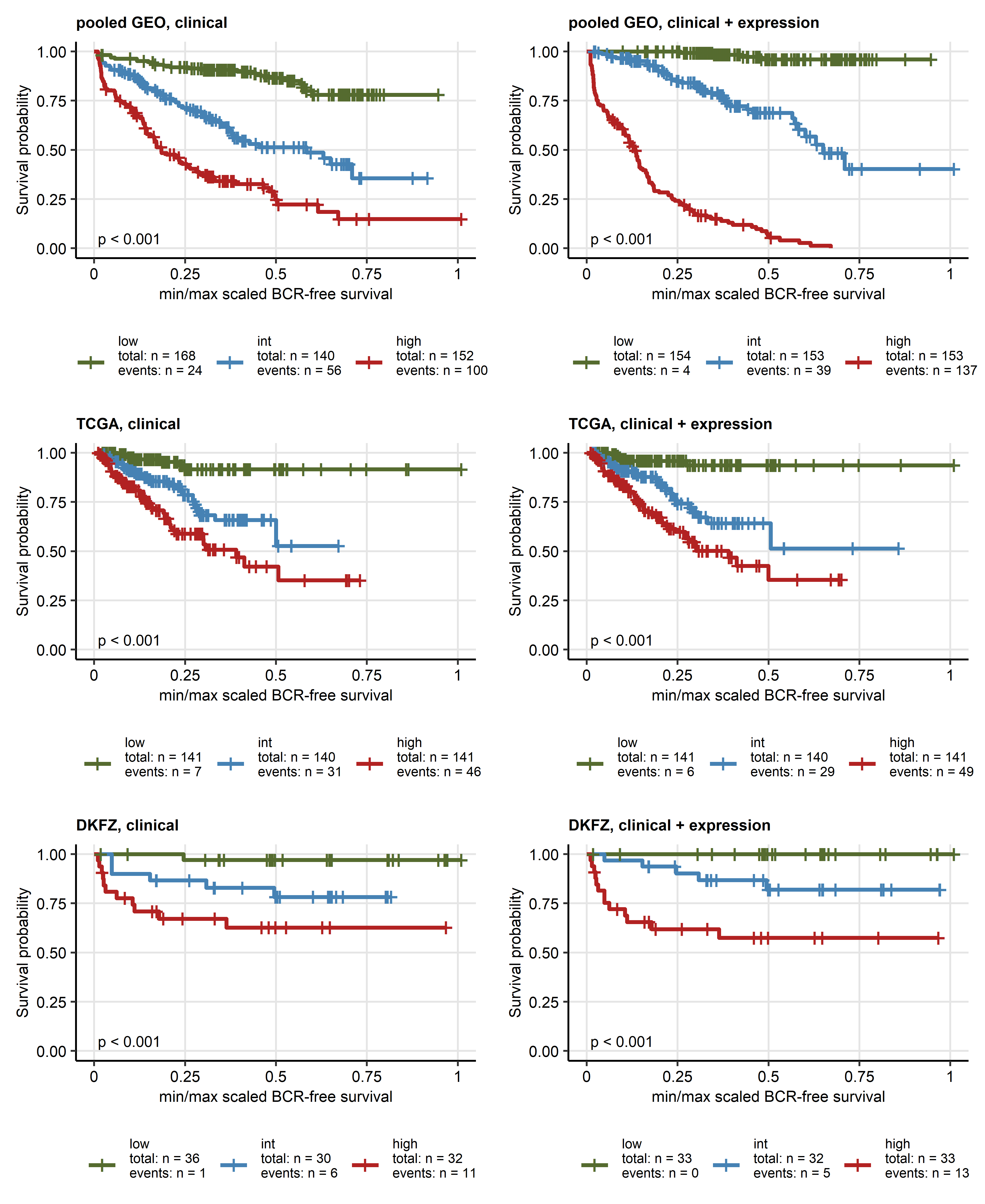


Figure 7: GBM modeling of BCR-free survival in PCA with clinical prediction and expression of collagen-related transcripts: survival in tertiles of predictor scores.

**Figure 7. GBM modeling of BCR-free survival in PCA with clinical prediction and expression of collagen-related transcripts: survival in tertiles of predictor scores.**

*Three Gradient Boosted Machines (GBM) models of biochemical relapse (BCR) free survival were trained in the pooled GEO cohort: a model with clinical predictors (age, pathological tumor stage, ISUP grade, and blood PSA concentration at diagnosis), a model with ComBat-adjusted expression levels of the collagen-related transcripts, and a model with the clinical and transcriptomic predictors, as presented in Supplementary Figure S7.* *BCR-free survival in tertiles of the predictor scores of the clinical-only and the combined clinical - expression GBM models was visualized with Kaplan-Meier plots. Numbers of patients and BCR cases in the predictor score tertiles are indicated in the figure legend. P values for differences in survival between the tertiles obtained by Peto-Peto test are displayed in the plots. Note improved resolution of the survival curves for the combined clinical/expression model as compared with the clinical-only model in the pooled GEO and DKFZ cohort.*

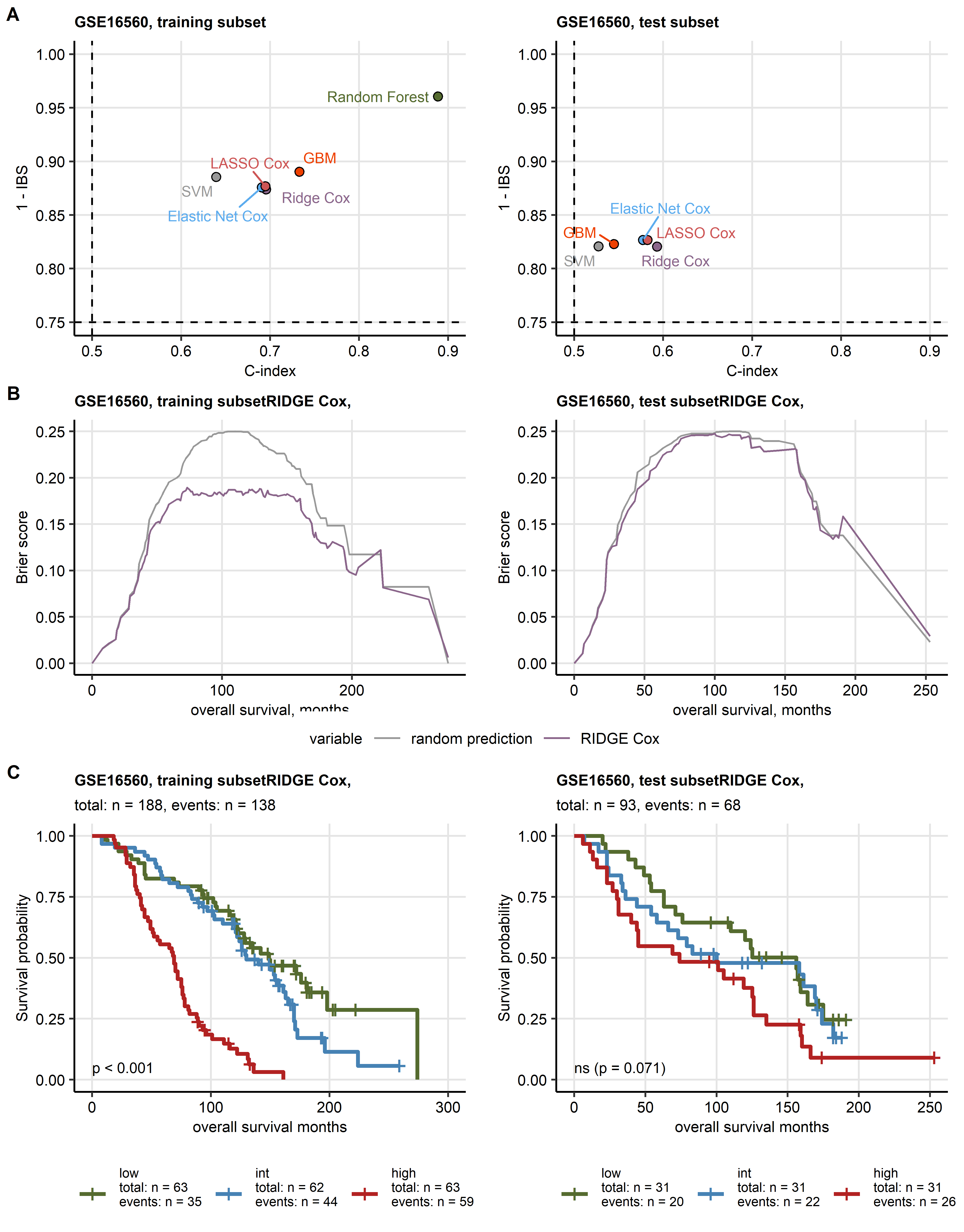


Figure 8: Modeling of overall survival in PCA with expression levels of the collagen-related transcripts as explanatory factors.

**Figure 8. Modeling of overall survival in PCA with expression levels of the collagen-related transcripts as explanatory factors.**

*Overall survival in prostate cancer (PCA) was modeled by multi-parameter machine learning models with ComBat-processed -transformed expression levels of 55 collagen-related transcripts as explanatory factors.* *The models were trained in the training subset of the GSE16560 cohort with six machine learning algorithms (RIDGE Cox regression, Elastic Net Cox regression, LASSO Cox regression, survival Support Vector Machines [SVM], survival Random Forest, and survival Gradient Boosted Machines [GBM]).* *Performance of the models was evaluated in the training (total patients: n = 188, deaths: n = 138) and test (total patients: n = 93, deaths: n = 68) subsets of the GSE16560 cohort.*

*(A) Metrics of performance of the survival models, Harrell’s concordance index (C-index, measure of model accuracy) and Integrated Brier Score (IBS, measure of overall model calibration, low values are characteristic for good calibration), are presented in dot plots. Dashed lines represent C-index and IBS values expected for random death risk prediction. Note the superior accuracy and calibration of the GBM model in the validation collectives.*

*(B) Brier Scores for unique survival time points for the best performing RIDGE Cox model. Low Brier Scores at a particular time point indicate accurate and confident prediction of the BCR risk. Note: gray lines represent random survival predictions by a null model.*

*(C) Overall survival of PCA patients stratified by tertiles of the predictor scores of the best performing RIDGE Cox model. Statistical significance of differences between the predictor score tertiles was determined by Peto-Peto test corrected for multiple testing wit the false discovery rate method. Fractions of alive patients and time after diagnosis are visualized in Kaplan-Meier plots. Numbers of patients and deaths in the predictor score tertiles are indicated in the legends. P values are displayed in the plots.*

# References