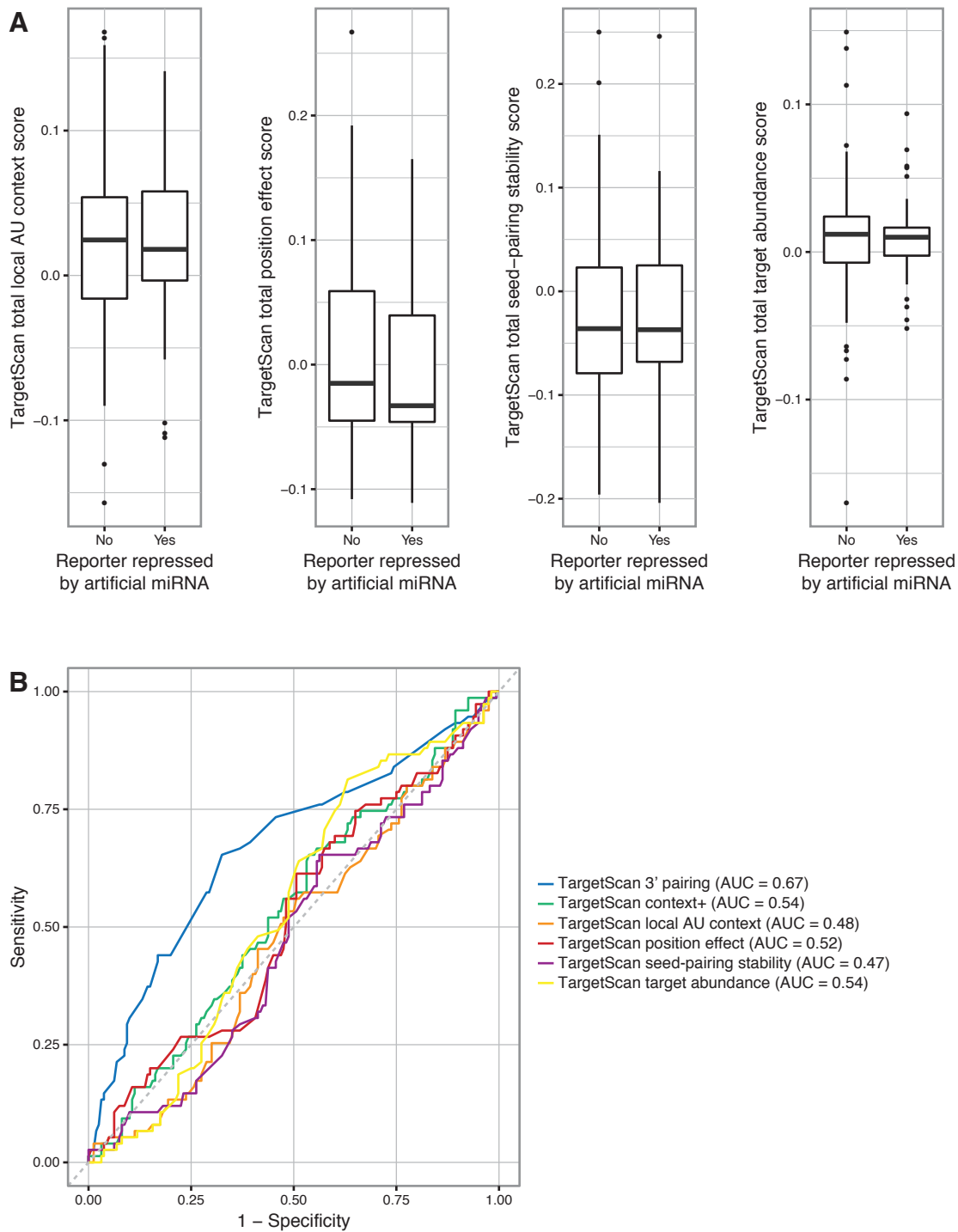
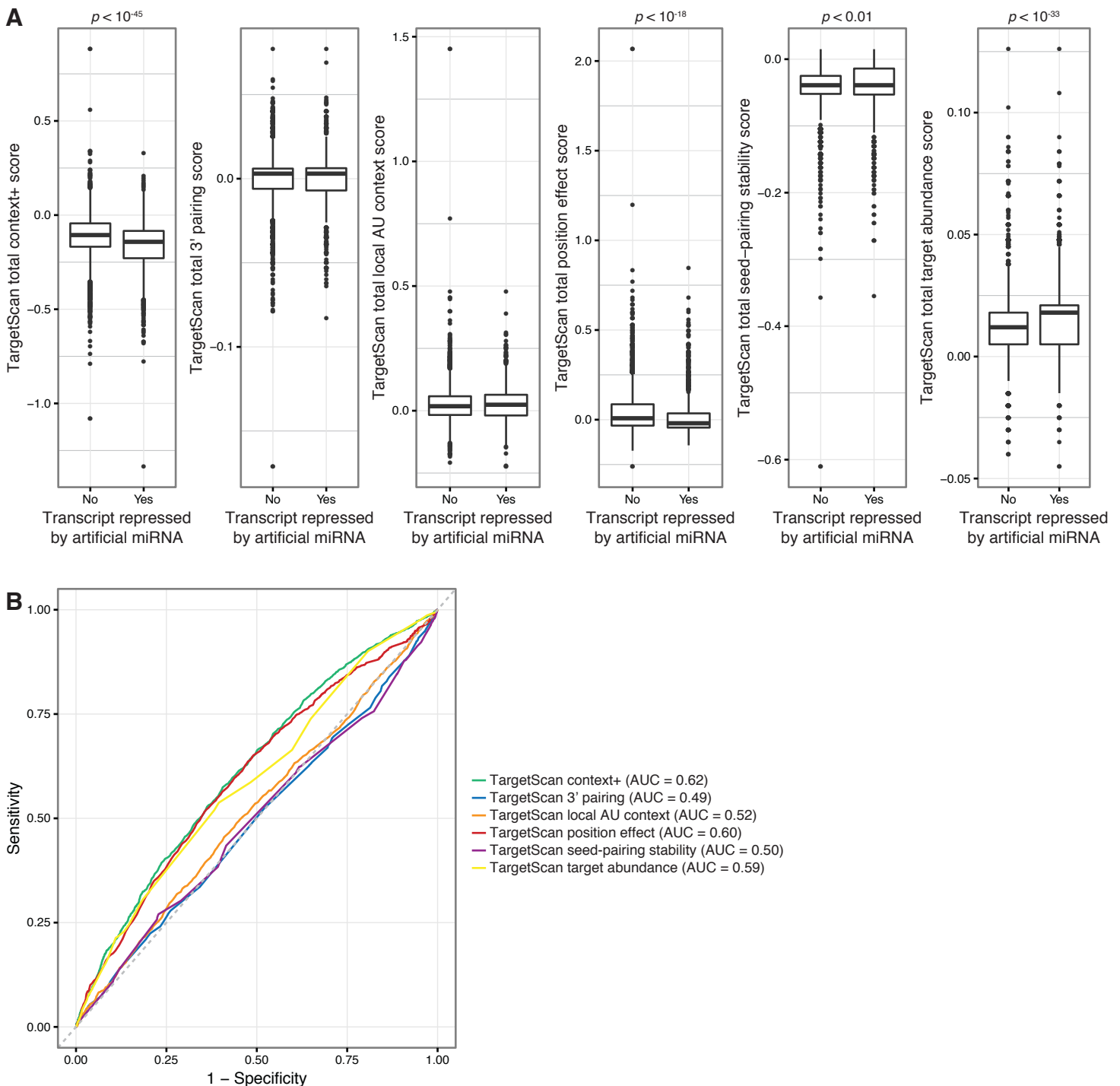


Supplemental Figures



Supplemental Figure 1. MicroRNA targeting determinants measured by TargetScan are weak predictors of artificial miRNA reporter activity. A) TargetScan scores for local AU context, site position effects, seed-pairing stability, and target abundance were calculated for each artificial miRNA and the PC and GLS 3' UTRs. When multiple sites were present in a 3' UTR, the scores for each site were added to produce the total score. Boxplots represent the indicated scores for miRNA/gene pairs in which the miRNA did or did not repress the corresponding 3' UTR reporter. For each of the scores shown, a t -test yielded $p > 0.05$. **B)** The ability of the indicated measures of miRNA targeting to predict repression of PC and GLS 3' UTR reporter genes by each artificial miRNA were compared by plotting sensitivity versus specificity. Results for the total context+ score and total 3' pairing score are reproduced from **Figure 5E**. The area under the curve (AUC) for each prediction method is indicated. A random prediction is shown as a dashed line.



Supplemental Figure 2. TargetScan scores for miRNA targeting determinants are weak predictors of artificial miRNA activity captured by microarray profiling. A) Microarray profiling data from transfection of PC/GLS-targeting artificial miRNAs (presented in **Figure 4**) was used to define transcripts that were repressed or unchanged by a given artificial miRNA. TargetScan context+ scores as well as the component scores for 3' pairing, local AU context, site position effects, seed-pairing stability, and target abundance were then calculated for each artificial miRNA and the 3' UTR of each transcript. When multiple sites were present in a 3' UTR, the scores for each site were added to produce the total score. Boxplots represent the indicated scores for miRNA/transcript pairs in which the miRNA did or did not repress the corresponding transcript. A *t*-test was conducted for each score, and tests that yielded $p < 0.05$ are indicated. **B)** The ability of the indicated measures of miRNA targeting to predict repression of endogenous transcripts by artificial miRNAs were compared by plotting sensitivity versus specificity. The area under the curve (AUC) for each prediction method is indicated. A random prediction is shown as a dashed line.