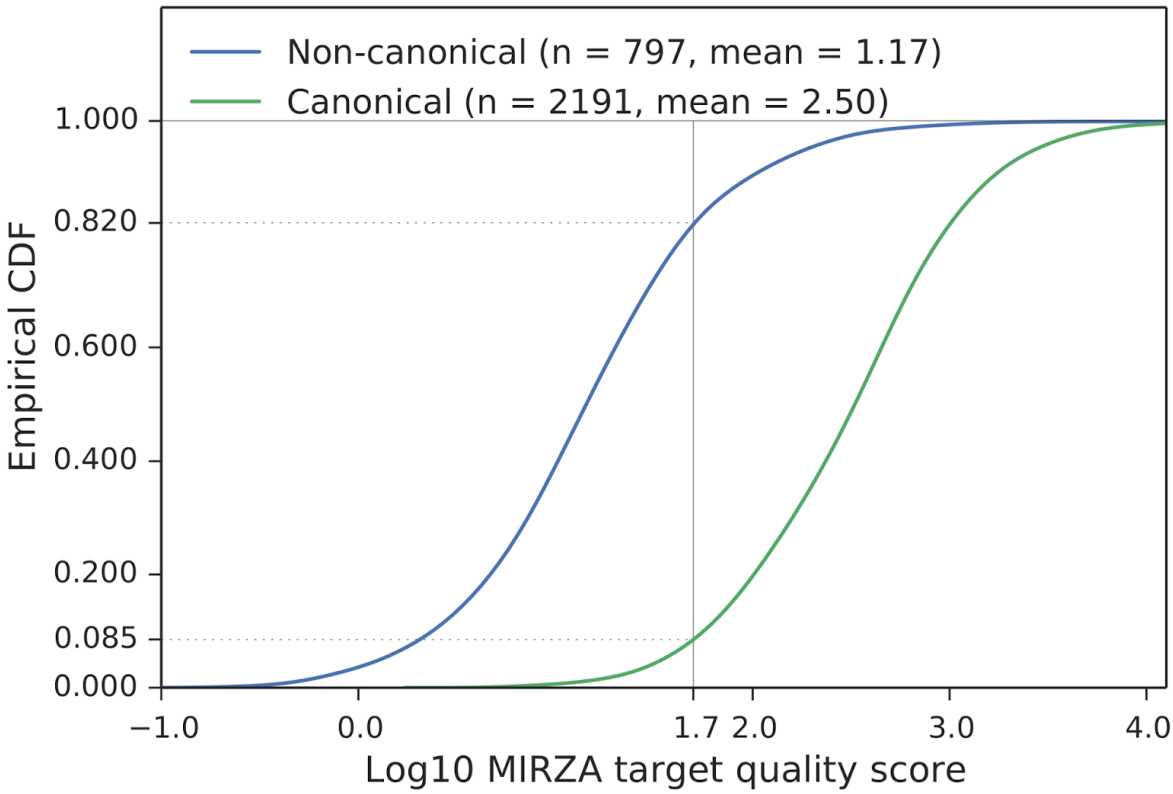
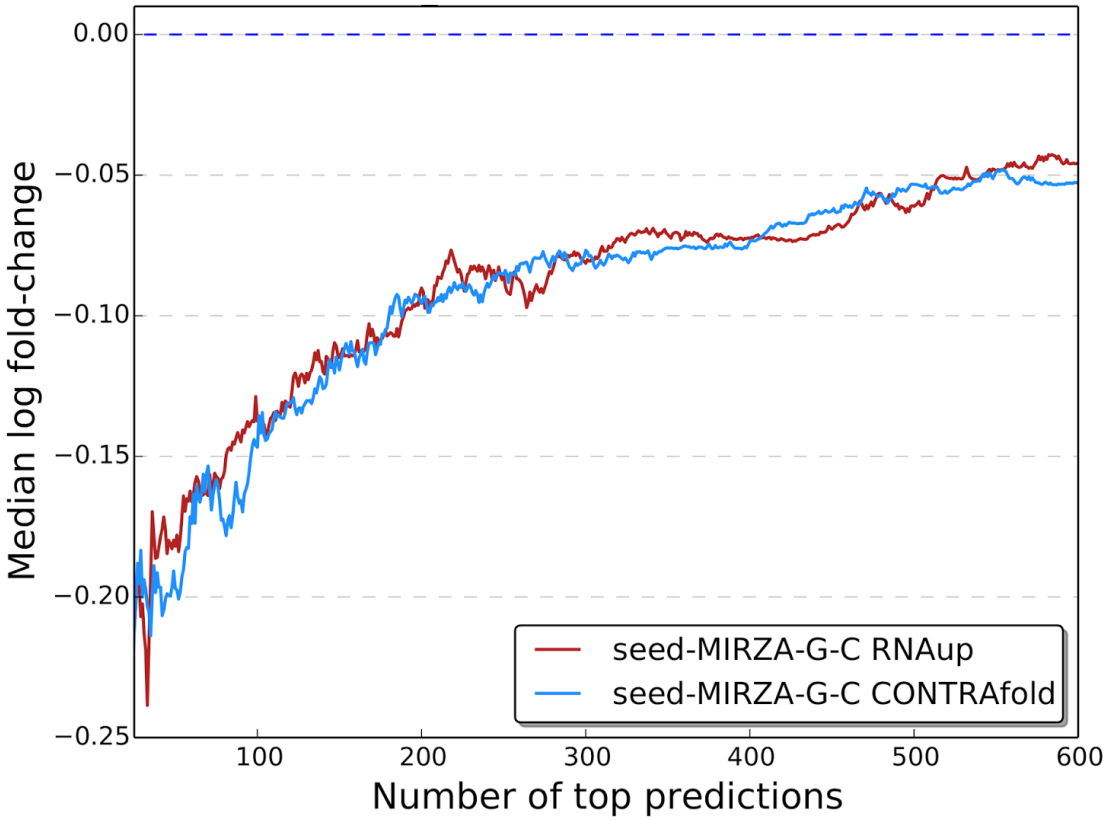
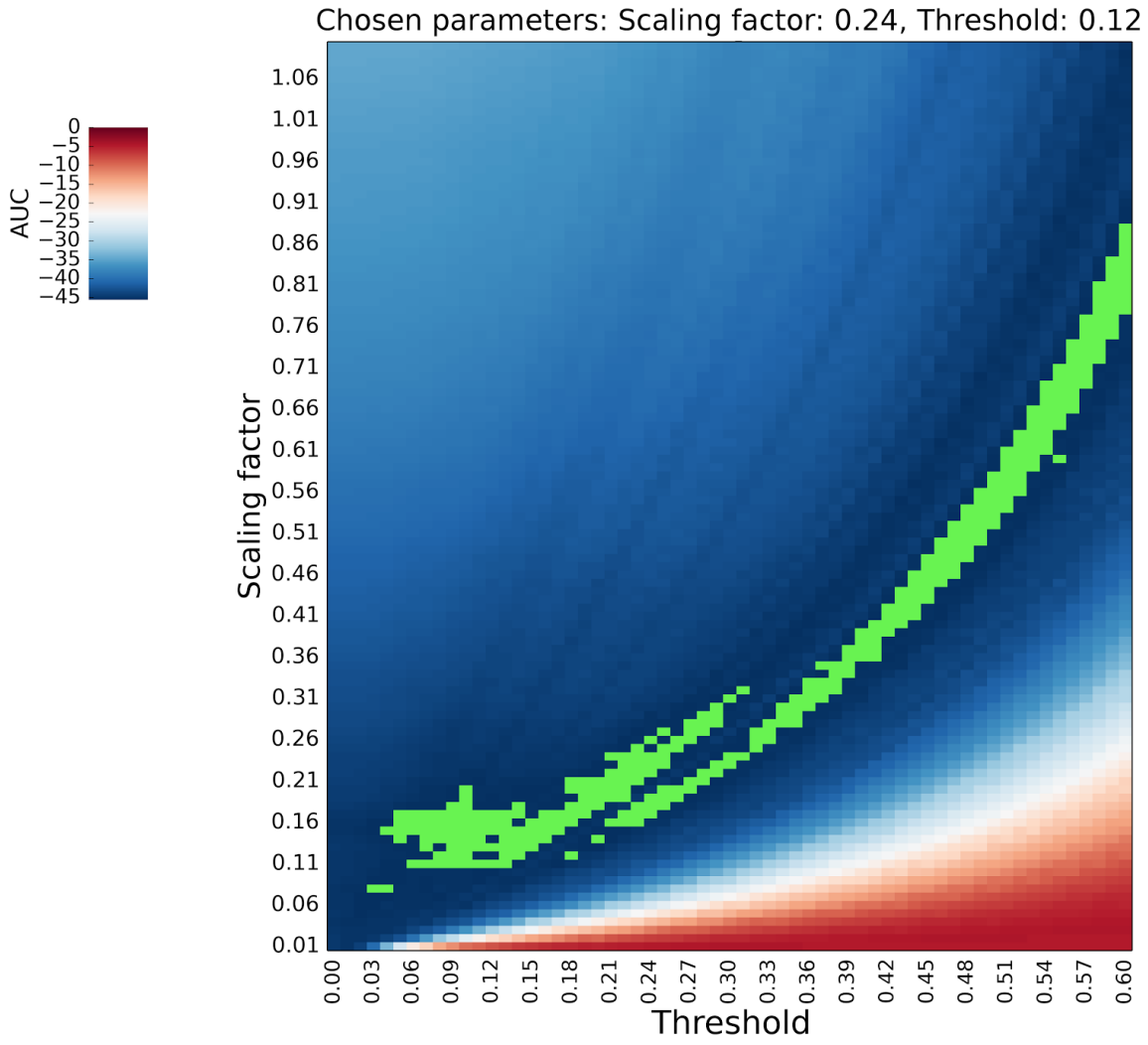


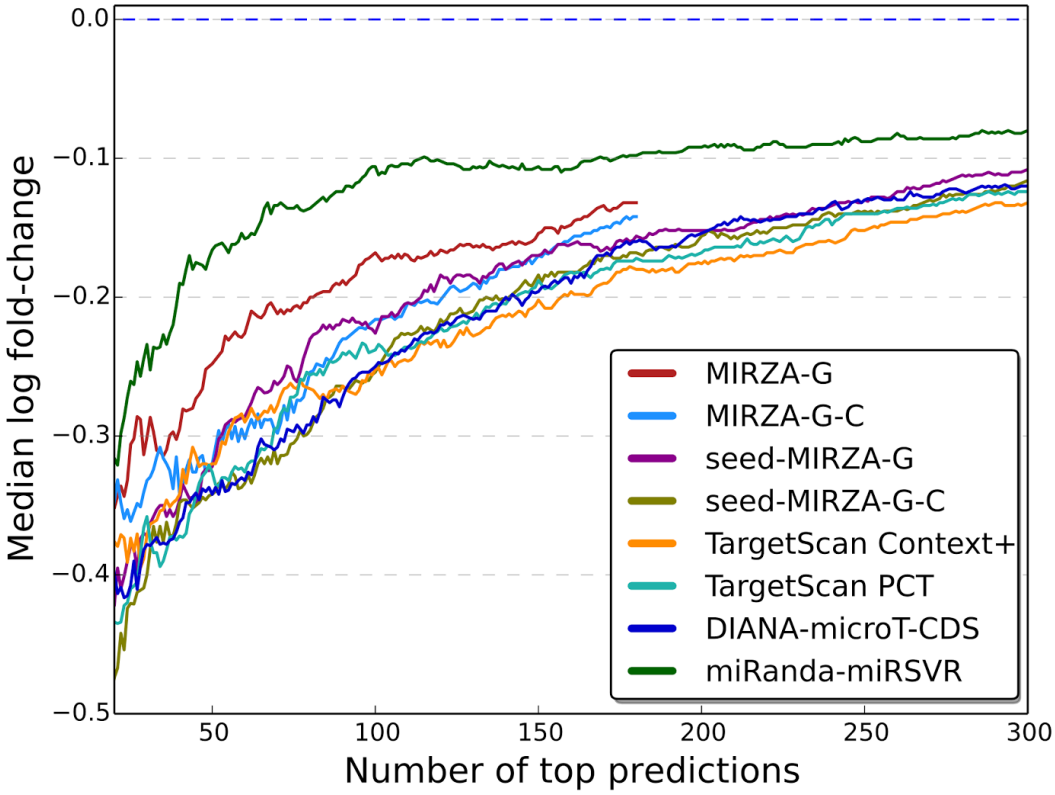
Supplementary Figure 1. Empirical cumulative distribution function of MIRZA target quality scores for canonical (green) and non-canonical (blue) miRNA binding sites. The binding sites were obtained with Argonaute 2 crosslinking and immunoprecipitation interactions and binding sites of individual miRNAs were predicted with the MIRZA method (1).



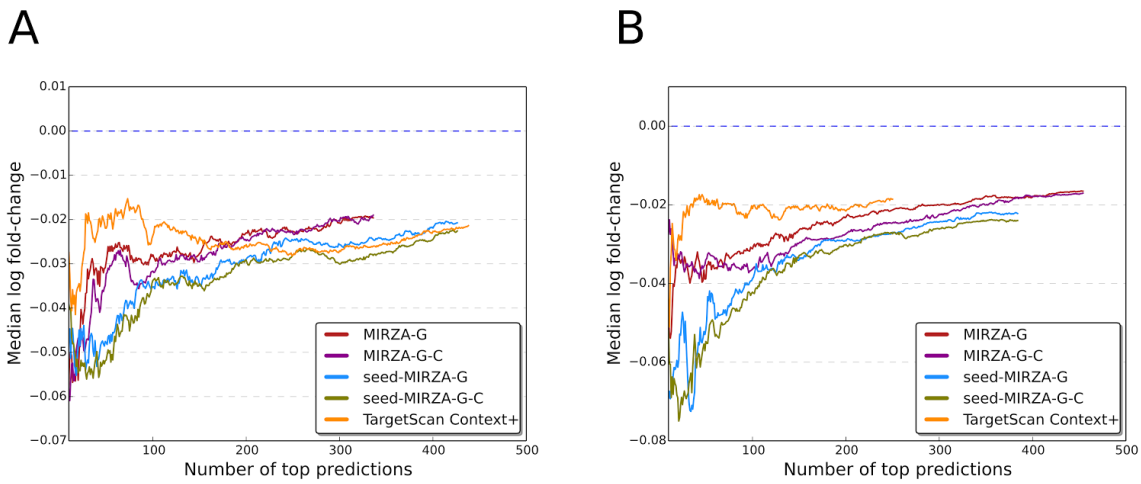
Supplementary Figure 2. Comparison of the down-regulation of targets predicted with models that either used RNAup or CONTRAfold to estimate target site accessibility upon miRNA transfection. Both models were trained as described in the Methods section on the 'training set' of miRNA transfection data and were then tested on the 'test' set of experiments.



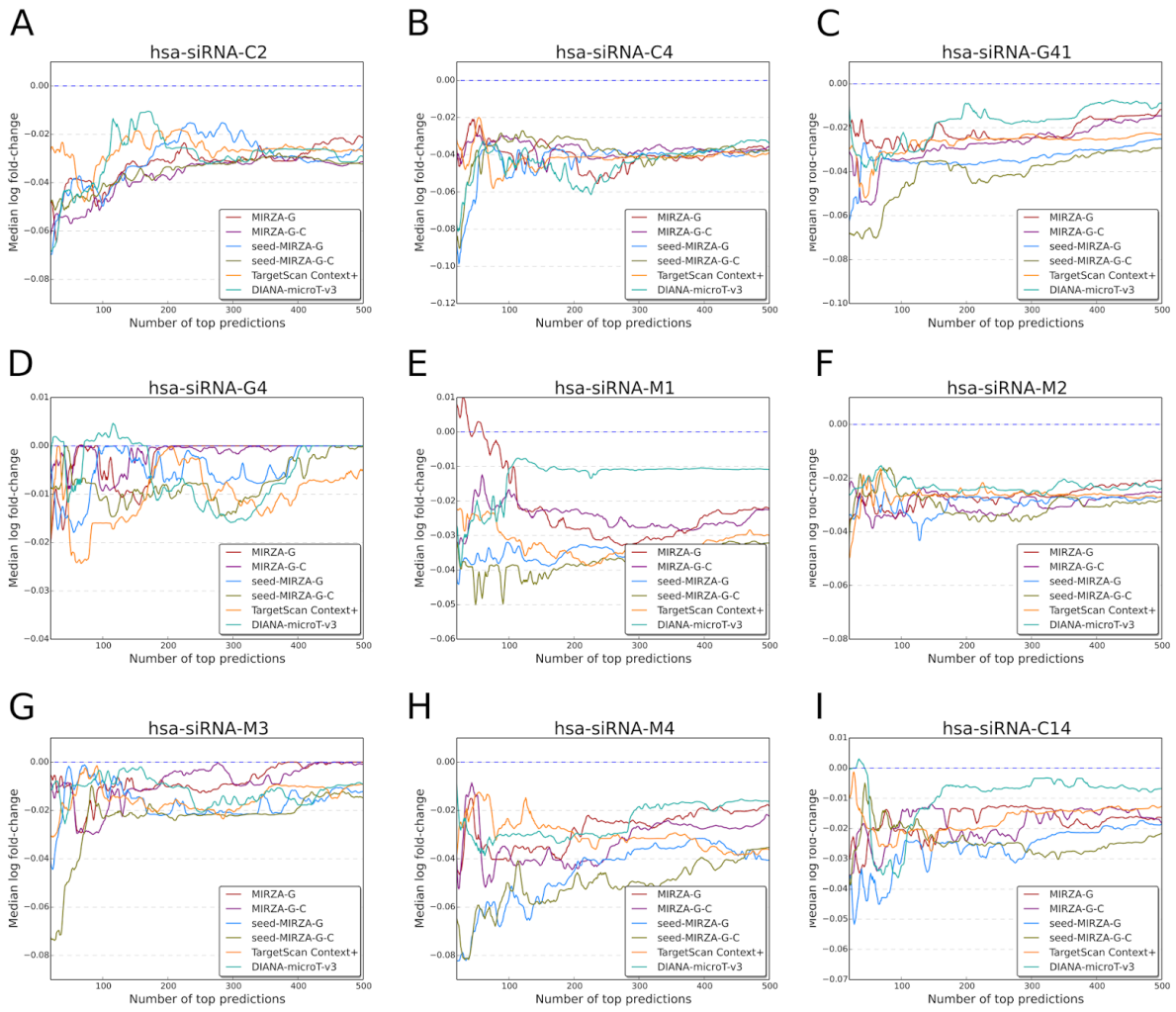
Supplementary Figure 3. Optimization of scaling factor (K) used to score individual sites and threshold (τ) used to compute gene-level scores. Predictions were made using individual K - τ pairs, the median down-regulation as a function of the number of top predictions considered was computed, and then the total down-regulation over the entire range of targets ('AUC') was calculated. The optimal values of the parameters were considered those that lead to predictions with the strongest overall down-regulation and were highlighted in green.



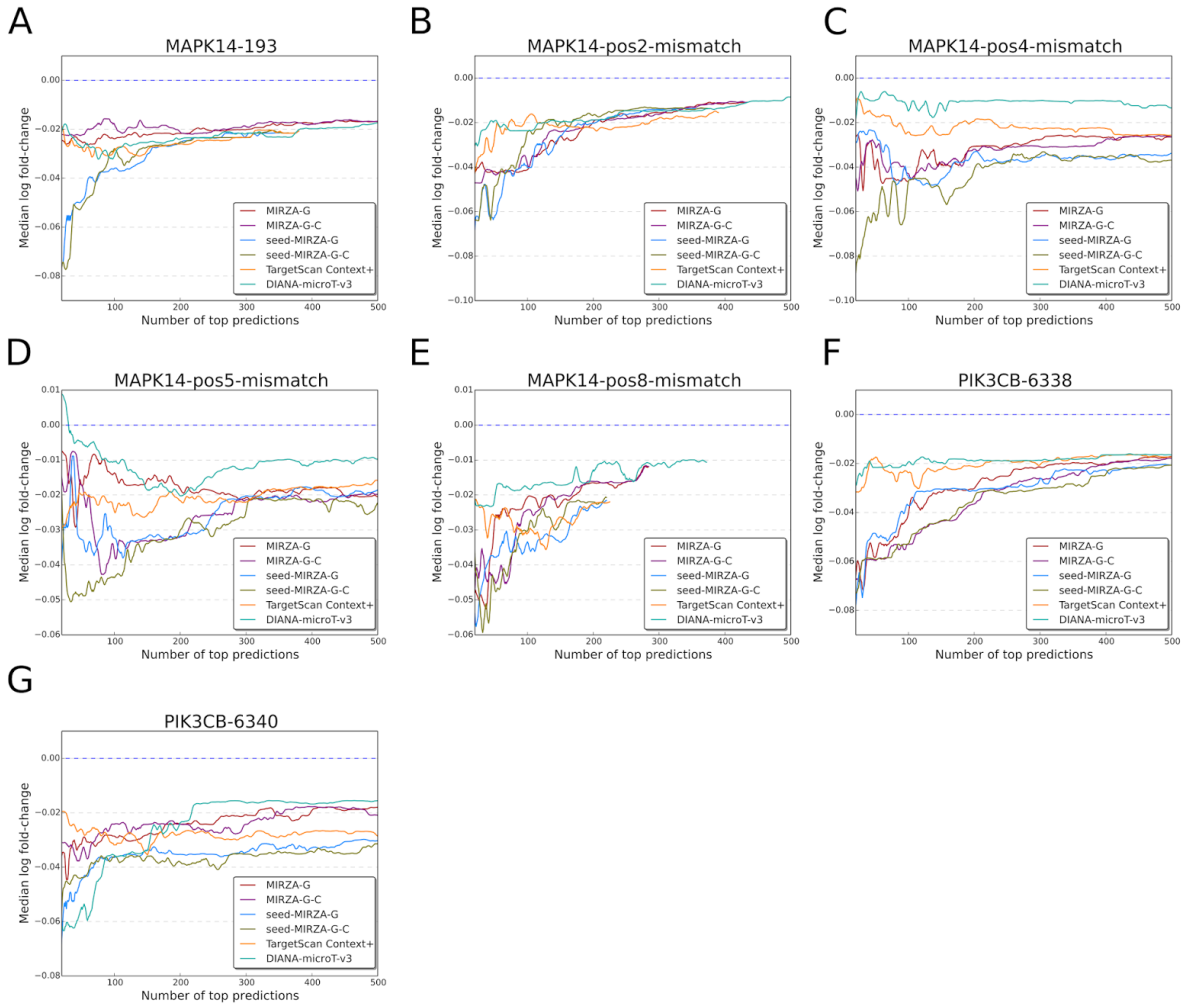
Supplementary Figure 4. Comparative evaluation of the performance of various models in predicting protein down-regulation following miRNA transfection. Variants of the MIRZA-G model are described in Table 2. The other tested models are TargetScan Context+, TargetScan PCT, DIANA-microT (the newest version), and miRanda-mirSVR (the most conservative predictions).



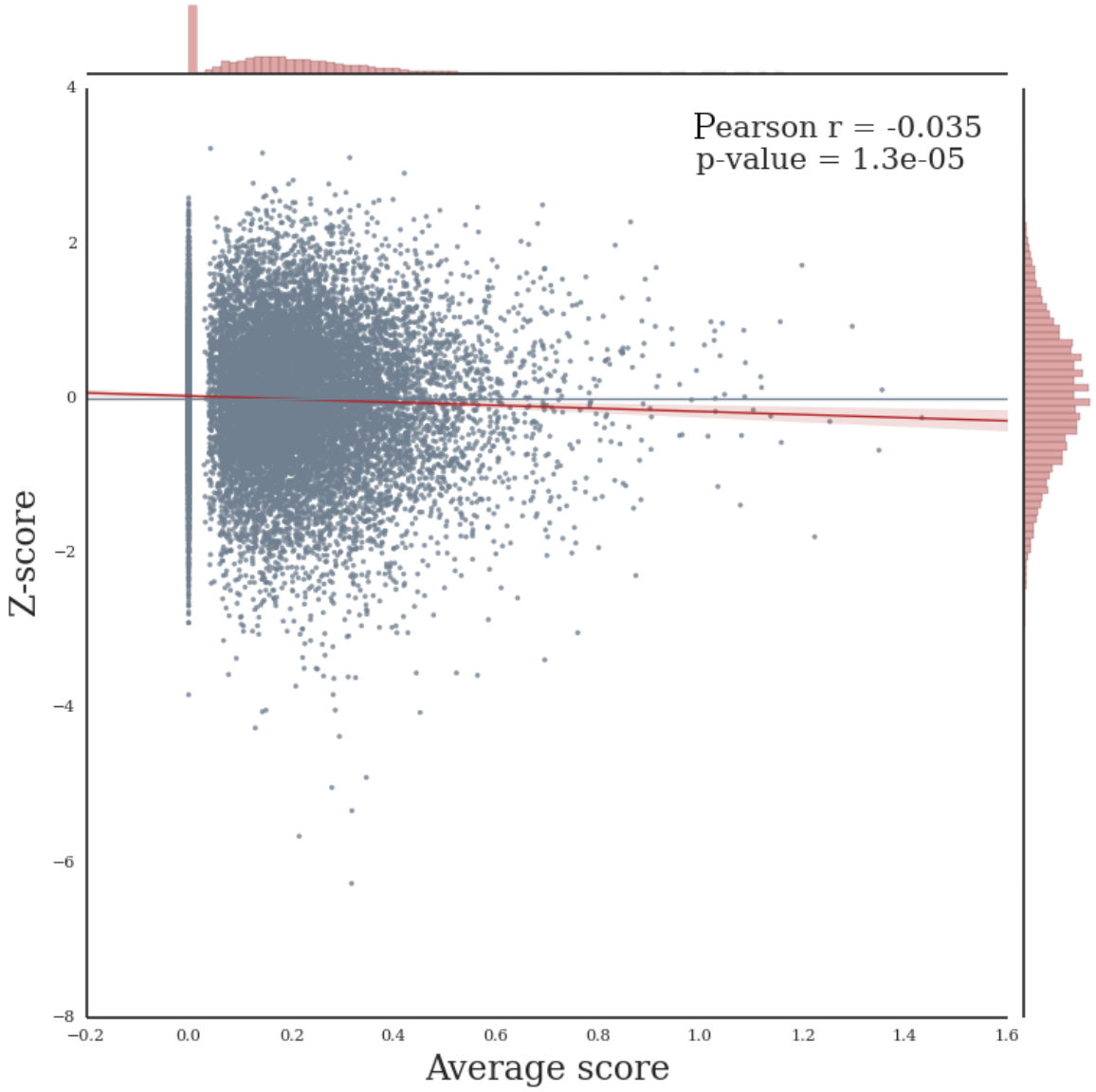
Supplementary Figure 5. Comparison of performance of the different models in predicting off-targets of siRNAs from the Birmingham et al. (2) (**A**) and Jackson et al. (3) (**B**) studies. Only siRNAs that did not share 6 or more nucleotides in the seed region with a known miRNA were used.



Supplementary Figure 6. Performance comparison of various models on individual siRNA transfections (siRNAs labeled on the top of each panel) from the Birmingham et al. (2) dataset.



Supplementary Figure 7. Performance comparison of various models on individual siRNA transfections (siRNA labeled on the top of each panel) from the Jackson et al. (3) dataset.



Supplementary Figure 8. Correlation between the z-score of an siRNA in the TGF-β screen and the average score that our model assigns to the interaction of core components of the TGF-beta pathway (TGFBR2, TGFBR1, SMAD2 and SMAD4) with the siRNA.

1. Khorshid, M., Hausser, J., Zavolan, M. and van Nimwegen, E. (2013) A biophysical miRNA-mRNA interaction model infers canonical and noncanonical targets. *Nat. Methods*, **10**, 253–255.

2. Birmingham, A., Anderson, E.M., Reynolds, A., Ilsley-Tyree, D., Leake, D., Fedorov, Y., Baskerville, S., Maksimova, E., Robinson, K., Karpilow, J., et al. (2006) 3′ UTR seed matches, but not overall identity, are associated with RNAi off-targets. *Nat. Methods*, **3**, 199–204.

3. Jackson, A.L., Burchard, J., Schelter, J., Chau, B.N., Cleary, M., Lim, L. and Linsley, P.S. (2006) Widespread siRNA “off-target” transcript silencing mediated by seed region sequence complementarity. *RNA*, **12**, 1179–1187.