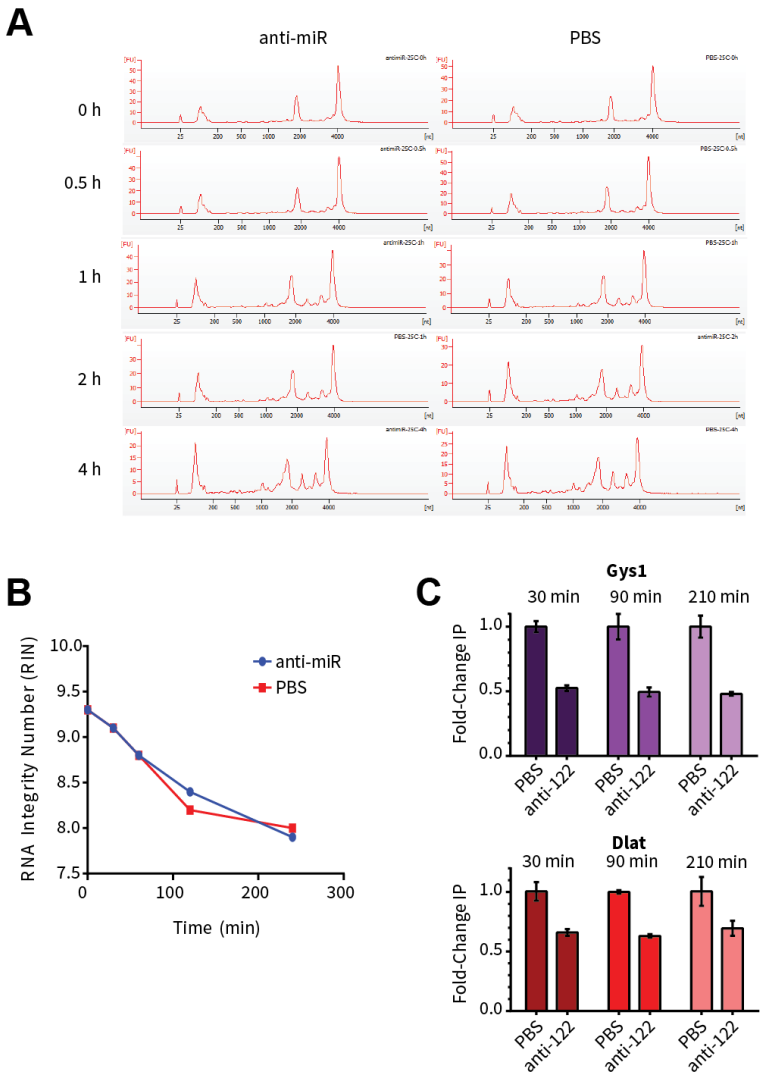
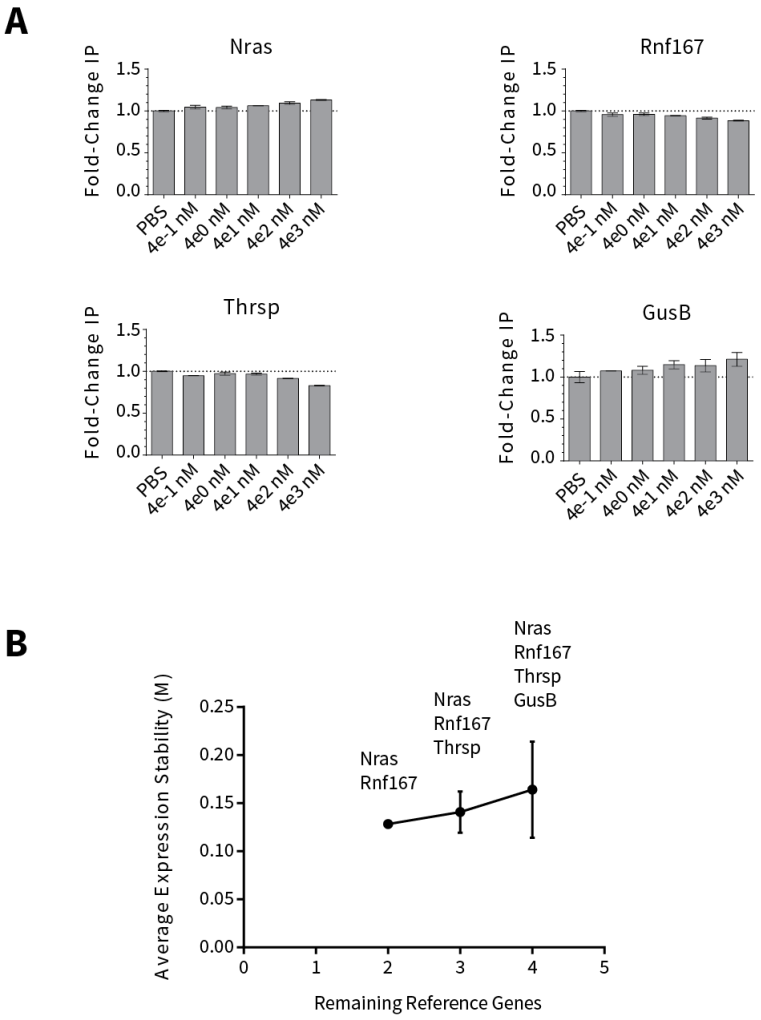
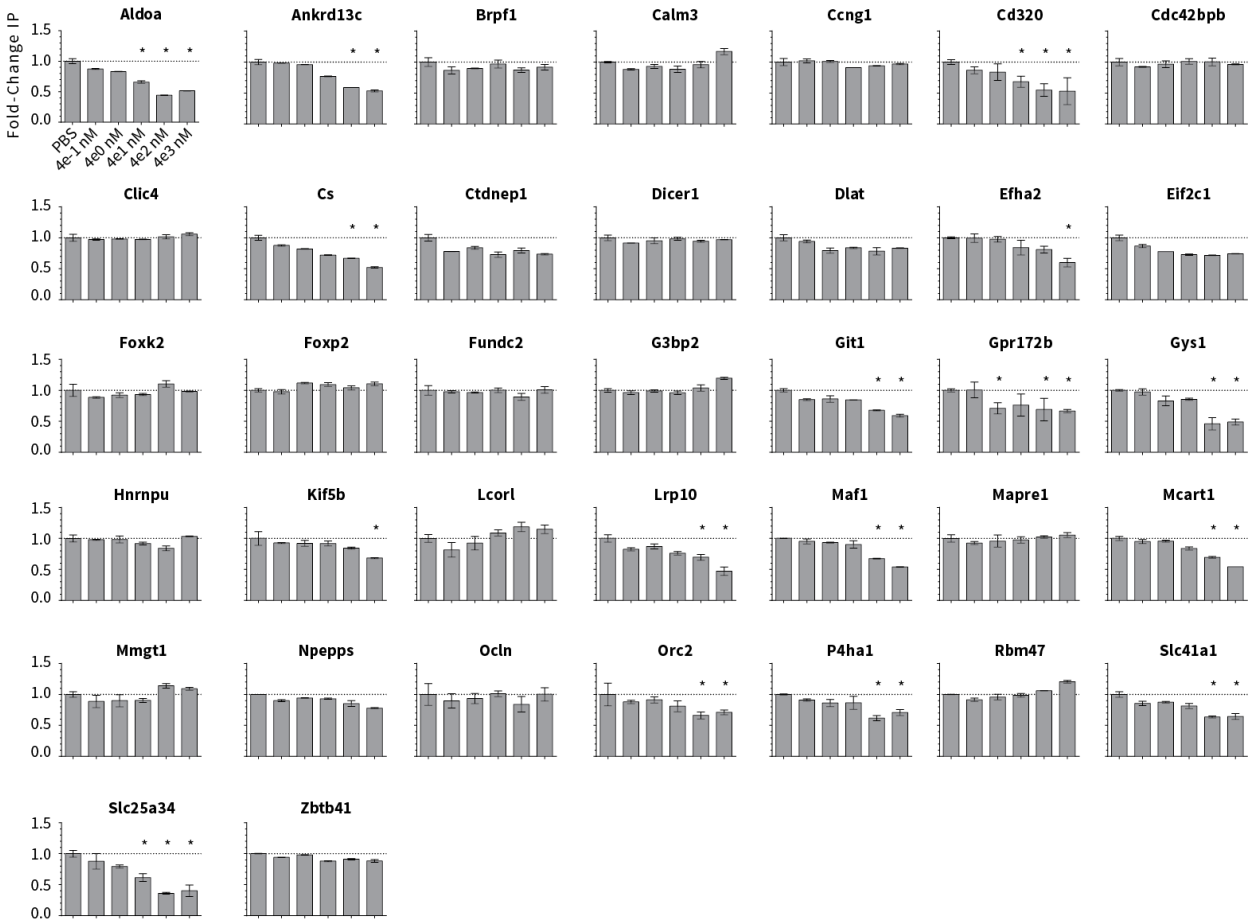
Supplementary Data



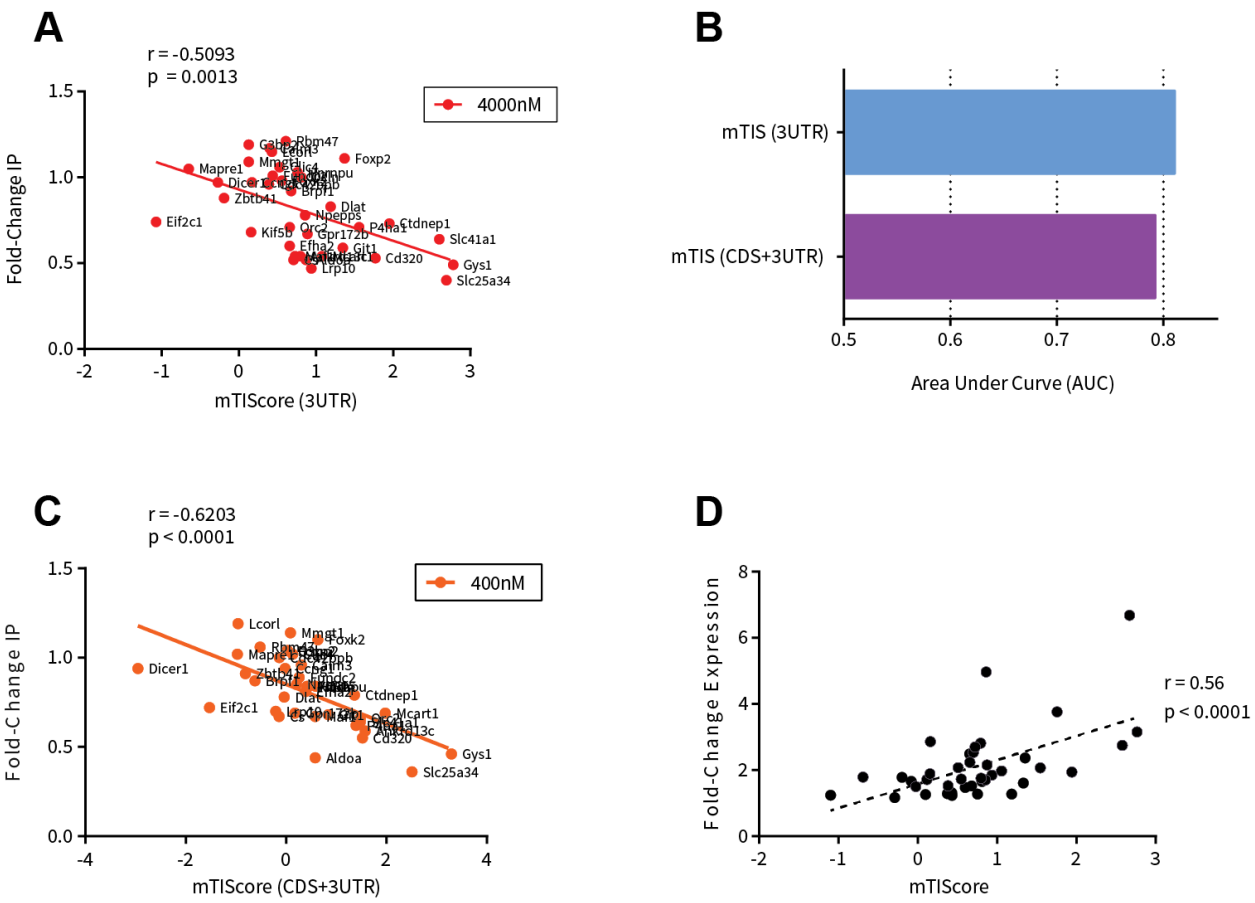
Supplementary Figure S1. Optimizing duration of anti-miR competition pre-incubation. (**A-B**) RNA integrity after anti-miR competition pre-incubation. (**A**) Electropherograms for RNA isolated from lysate samples incubated at 25°C for varying lengths of time. Lysates were spiked with either 400 nM anti-122 (anti-miR) or an equivalent volume of PBS. No difference was observed with either treatment. RNA degradation was evident at later time points based on decreased amplitude of the 28S peak (at ~4000 nt marker) and presence of additional peaks between 2000 – 4000 nt range. (**B**) Quantitative scoring of RNA integrity based on electropherograms in **A**, where RIN values range from 1 (poor quality) to 10 (high quality). (**C**) Prolonged pre-incubation with 400 nM anti-122 did not enhance target release of Gys1 (top) or Dlat (bottom). Errors bars represent standard deviation (n = 2). Based on these results, 30 minute pre-incubation was selected as the optimal time point to minimize any effects of RNA degradation.



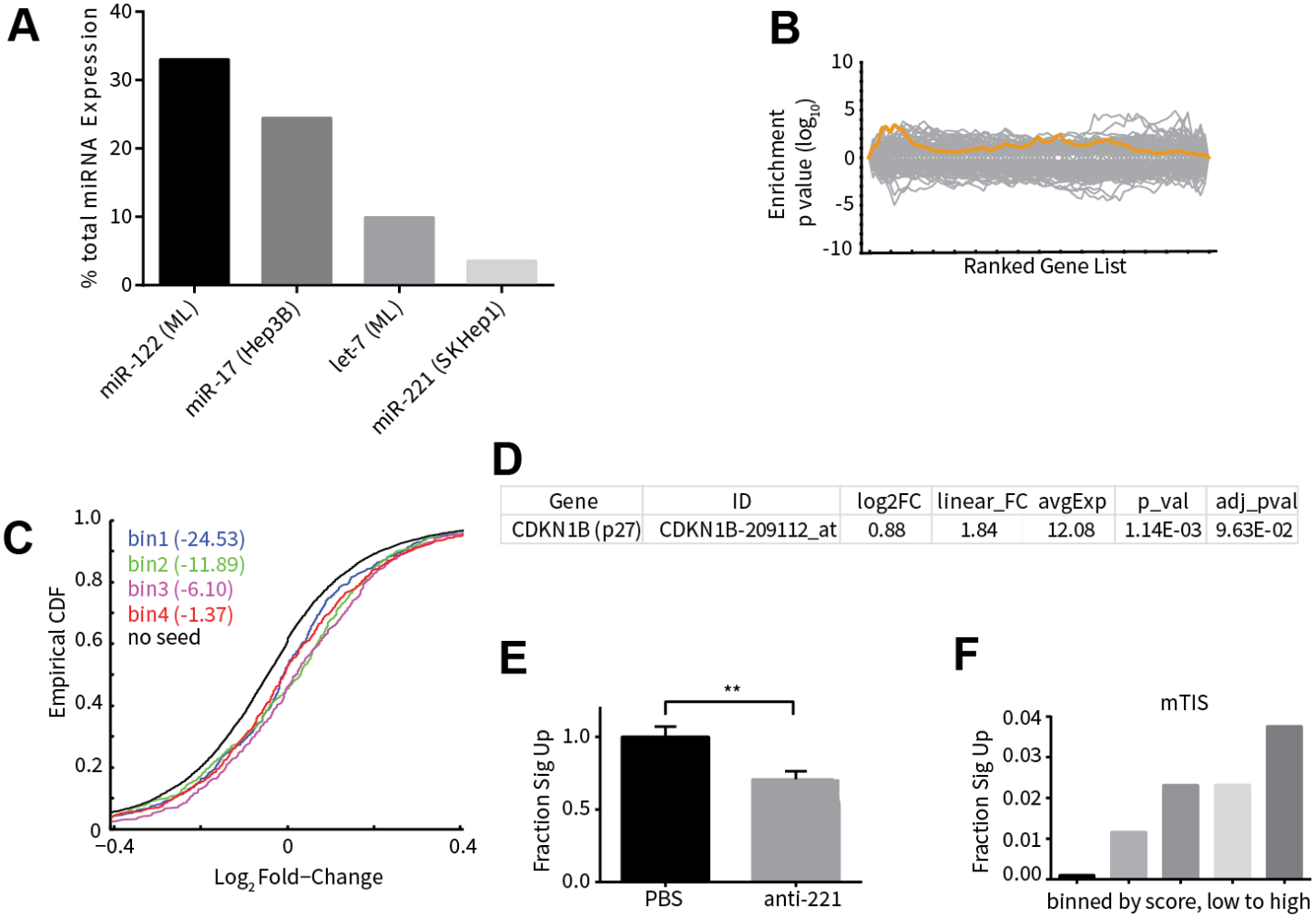
Supplementary Figure S2. Selection of reference genes for IP normalization. (**A**) RIP competition results for four control genes lacking miR-122 canonical sites. Error bars represent SEM. (**B**) The most stabile pair of control genes—Rnf167 and Nras— was identified empirically by stepwise exclusion. For each step, the pairwise variation of all remaining genes was calculated, and the gene with the highest variability was excluded from the next iteration. As a result, the average expression stability factor (M) decreases after each round. In the plot, remaining genes are listed in decreasing order of stability. The geometric mean of Rnf167 and Nras was used to normalize IPs.



Supplementary Figure S3. Complete dose-response RIP competition results for miR-122 candidate targets (source data for Figure 2A). All axes are identical and are as indicated for Aldoa (top left). Fold-changes are relative to PBS. Asterisks mark samples with statistically significant reduction of mRNA in IP with treatment ($p < 0.05$ by ANOVA with Dunnett correction for multiple comparisons, $n = 3$, error bars represent SEM).



Supplementary Figure S4. Additional analyses of mTIScores and target responses. (**A**) Correlation between mTIScore computed using 3'UTR sites alone and RIP responses at 4000 nM anti-miR (compare with Figure 3C). (**B**) Inclusion of CDS site sites did not enhance mTIScore performance in predicting RIP response as measured by area under curve (AUC; compare with Figure 3D); however (**C**) it did lead to very slight improvement in correlation. (**D**) Correlation between mTIScore using 3'UTR sites only and expression changes induced by *in vivo* dosing of anti-miR-122.



Supplementary Figure S5. mTIScore analysis of miR-221/222 inhibition profiling data. (A) Estimated expression levels for each of the tested miRNAs, calculated as percentage of total miRNA expression in mouse liver (ML), Hep3B cells, or SKHep1 cells. (B) Sylamer analysis for miR-221/222 inhibition. Genes were ranked from most upregulated (left hand side of x-axis) to most downregulated (right hand side), and hypergeometric enrichment scores were calculated for each possible 8mer nucleic acid sequence based on their frequency of occurrence in the 3'UTRs of the ranked gene list (y-axis). The miR-221/222 8mer seed sequence is highlighted and grey lines represent the background for all other 8mer sequences. No significant enrichment was observed, indicating a weak miR-221/222 repression signature. (C) Cumulative distribution frequencies (CDFs) for miR-221/222 seed-matched genes binned by descending mTIScores into equal sized groups. Median mTIScores for each bin are indicated in parentheses. CDF for non-seed containing genes is shown in black. (D) Array profiling results for previously well-validated miR-221/222 target CDKN1B (p27). FC = fold change; avgExp = average expression; adj_pval = p-value adjusted for multiple comparisons. (E) RIP competition results for CDKN1B (p27) using 4000 nM anti-221/222 compound spiked into SKHep-1 lysates. qPCR values were

normalized to the geometric mean of non-miR-221/222 targets HMGA2 and IGF2BP2. p = 0.0049 by unpaired t test, *n* = 3, error bars represent SD. (F) Fraction of miR-122 seed-matched genes significantly upregulated (FDR = 20%) in equal sized ranked bins of mTIScores computed using all seed sequences.