Supplemental Figure Legends

Supplemental Figure S1. %NTA observed in prostate tissue miRNAs is not related to miRNA abundance. A. The %NTA for the 167 shared miRNAs with 10 or more reads in both synthetic miRNA and prostate tissue small RNA sequencing datasets are plotted along the x-axis, sorted with respect to increasing total number of reads in the normal prostate tissue dataset. Normal prostate tissue data is plotted in black and synthetic miRNA data is in red.

Supplemental Figure S2. Histograms of %NTA observed in **(A)** human, **(B)** mouse cerebellum and medulloblastoma, and **(C)** *C. elegans* small RNA sequencing datasets. MicroRNAs were required to have 10 or more reads in a given dataset to be included, and the number of miRNAs that qualified is given in parentheses. The y-axis scale is the same for all histograms in each species. Parenthetical labels are dataset identifiers that refer to the datasets listed in **Supplemental Table S1**.

Supplemental Figure S3. Distribution of added nucleotides for individual datasets in (A) mouse cerebellum (datasets m1-11) and (B) *C. elegans* (datasets c1-12). Parenthetical labels refer to dataset identifiers given in **Supplemental Table S1**. Distribution was calculated based on miRNAs with an abundance of at least 10 reads, and the number of miRNAs that qualified is given in parentheses. For the mouse cerebellum and medulloblastoma samples, each pie chart typically represents biological replicates from which the data were combined before calculating nucleotide distribution. Fractional contribution of each nucleotide addition was calculated for each miRNA, and then averaged for all the abundant miRNAs (i.e., at least 10 reads) in each dataset.

Supplemental Figure S4. Specificity of nucleotidyl transferase knockdown. qRT-PCR was used to assess the expression of each nucleotidyl transferase enzyme after knockdown of each of the eight enzymes. Bars represent the mean relative expression (± SD) across two biological replicates of a given enzyme in each knockdown compared to

control cells (treated with siCyclophilin). Expression of the endogenous control gene *GUSB* was used to normalize for technical variation in RNA input. Knockdown was highly specific to each enzyme and did not decrease the expression of the other nucleotidyl transferase enzymes. In a few cases, however, knockdown of a given nucleotidyl transferase increased the expression of one or more of the other enzymes. This suggests that, in such cases, effects of the gene knockdown might be functionally compensated by an increase in expression of other nucleotidyl transferase family members.

Supplemental Figure S5. Specificity of NanoString nCounter miRNA 3' variant assay for miRNA variants presented in Figure 7. A. Mixtures of 13 canonical synthetic miRNAs, or of 13 variant synthetic miRNAs of interest were individually assayed with the canonical and variant bridge pools. The miRNAs and variants chosen for validation were ones that were significantly affected by nucleotidyl transferase knockdown as shown in **Figure 7B**. The variant mixture comprises 9 miRNAs that have variants of interest that correspond to the variant 1 bridge pool (i.e., miR-200a, let-7b, miR-100, let-7d, let-7c, let-7a, miR-31, let-7g and miR-15a) and 4 miRNAs that have variants that correspond to the variant 2 bridge pool (let-7i, let-7e, miR-1246 and miR-106b). The graphs display the miRNA counts resulting when each of the two mixtures was assayed in the **(B)** canonical, **(C)** variant 1, and **(D)** variant 2 bridge pools.

Supplemental Figure S6. Validation of the linear range of the nCounter assay. Standard curves of synthetic oligonucleotides corresponding to the canonical and variant versions of 12 miRNAs of interest from **Figure 7B** were assayed in their appropriate bridge pool. The graph displays the counts resulting from technical duplicates of standard curves ranging from $1x10^5$ to $1x10^8$ input copies of miRNA per reaction.