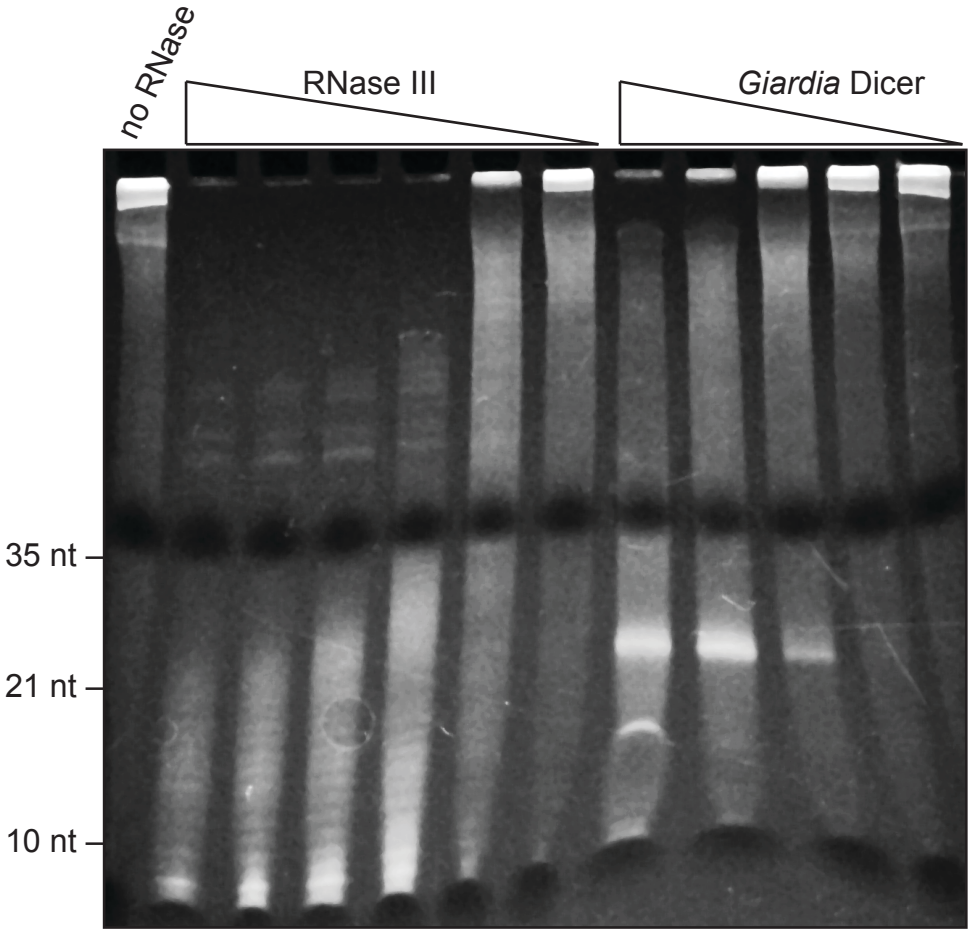

Figure S1. Co-transcriptional dsRNA cleavage with *E.coli* RNase III and *Giardia* Dicer. Decreasing amounts of nuclease were used in individual transcription reactions (each reaction contained 1/4 the amount of nuclease in the previous). The maximum amount of RNase III used was 5 µl of "Short Cut" RNase III from New England Biolabs in a 40 µl reaction volume. The maximum concentration of *Giardia* Dicer used was 50 ng/µl. We could identify no amount of RNase III that generated a clean sample of ~ 22 nt RNAs.

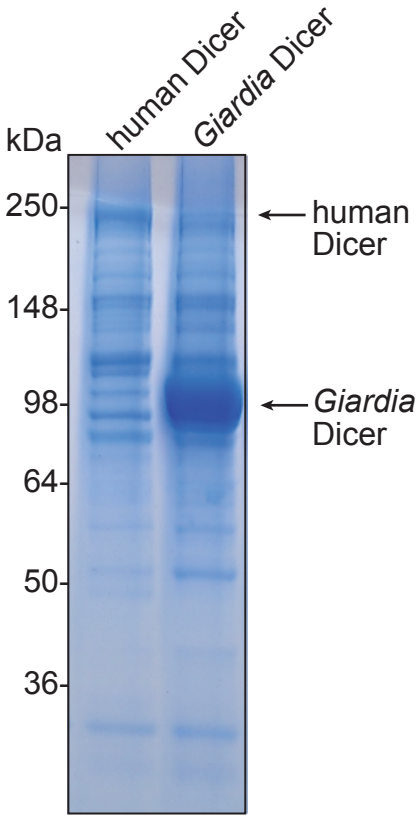

Figure S2. Expression levels of recombinant human and *Giardia* Dicer. Sf9 cells were infected with baculovirus bearing genes for His_6-tagged human Dicer or *Giarida* Dicer. Cells lysates were subjected to a crude Ni-NTA purification step and analyzed on SDS PAGE. The expression level of *Giardia* Dicer was more than 10-fold higher than human Dicer.

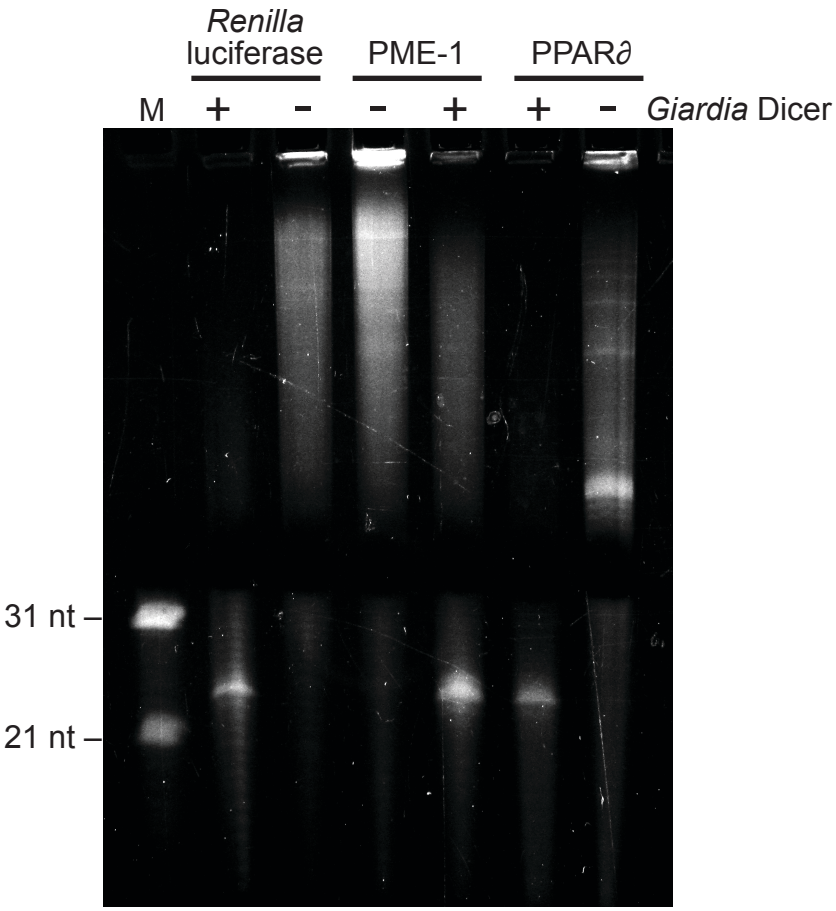

Figure S3. Generation of small RNA pools against three different targets. Transcription reactions, plus and minus Giardia Dicer, from three different DNA templates were resolved on a 14% denaturing gel. Transcription templates were generated by PCR amplifying 500 bp segments of Renilla luciferase, phosphatase methylesterase-1 (PME-1) or peroxisome proliferator-activated receptor delta (PPRə) cDNAs.