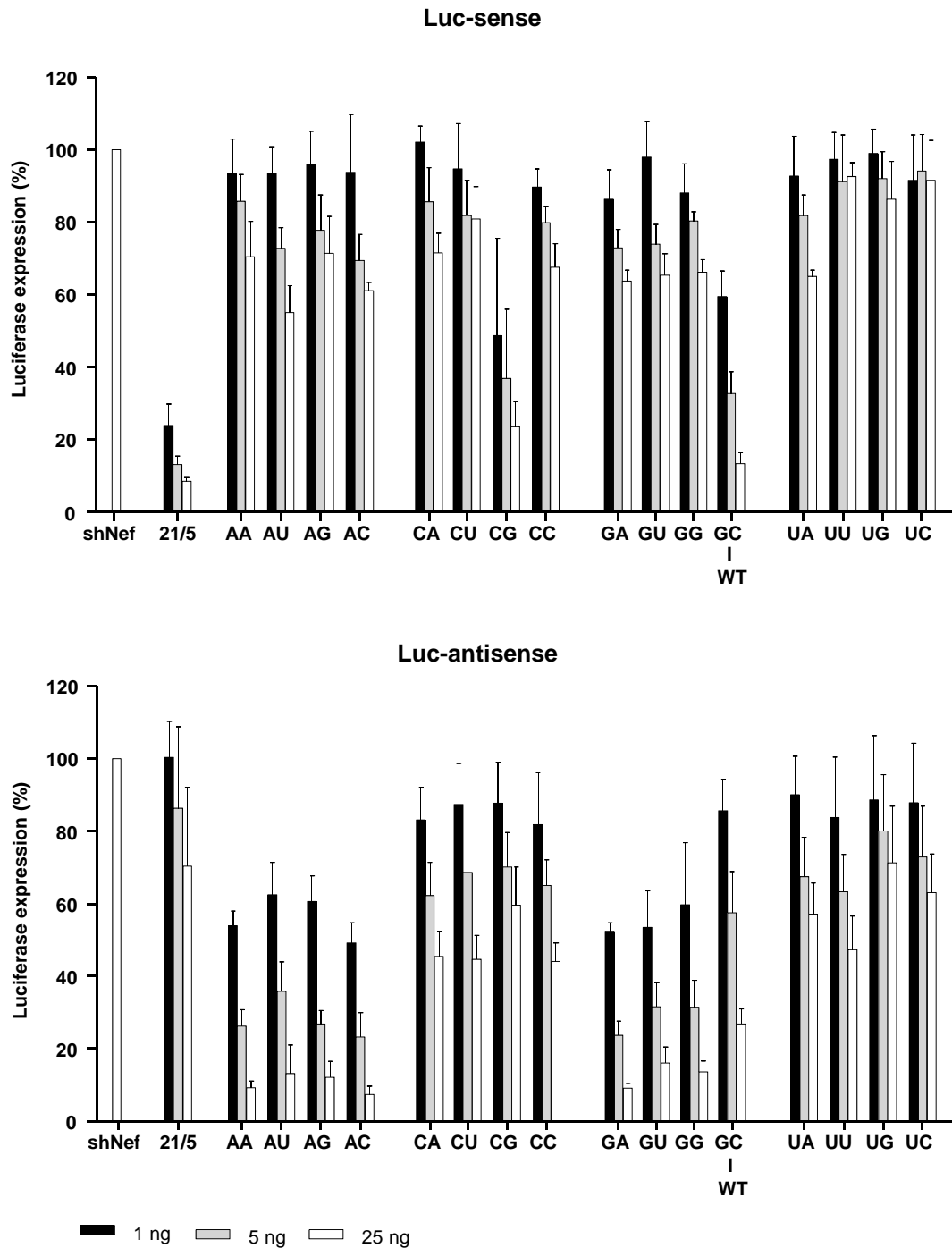
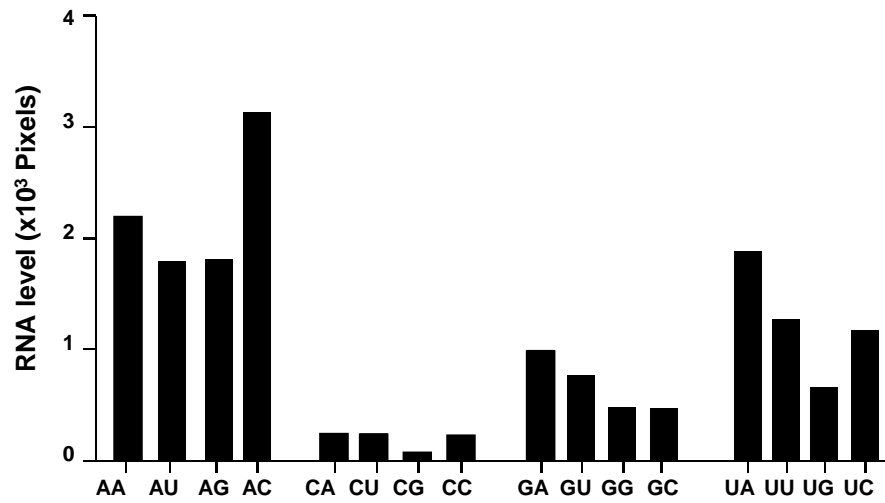
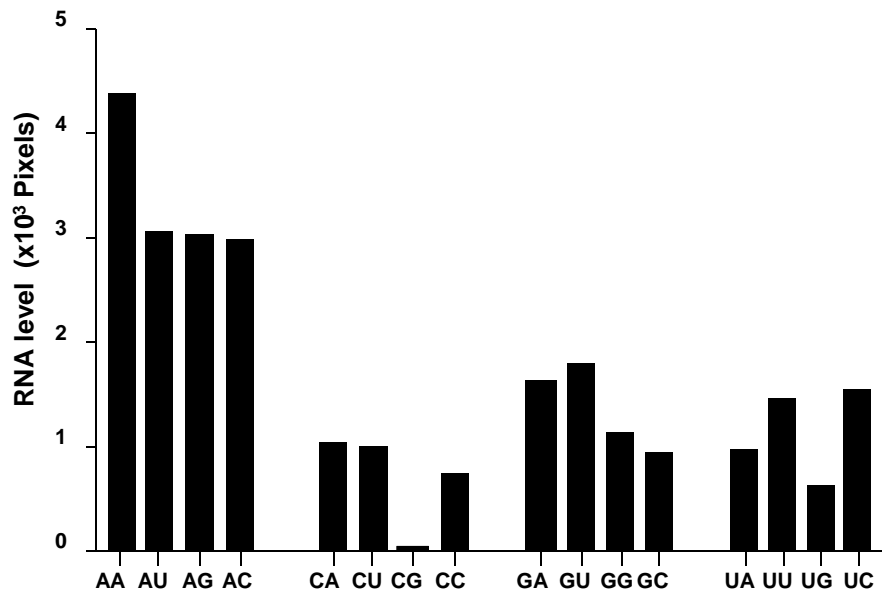


**Supplementary Figure S1.** Knockdown activity of AgoshRT5 variants. The knockdown activity of the different AgoshRT5 variants was determined by co-transfection with a luciferase reporter containing either the sense or antisense target sequence. HEK293T cells were co-transfected with 100 ng of the respective firefly luciferase reporter plasmid, 1 ng renilla luciferase plasmid as internal control and 1, 5 or 25 ng of the corresponding AgoshRNA construct. The regular shRT5 (21/5) was used as control. An unrelated shRNA (shNef) served as negative control, this activity was set at 100% luciferase expression. We performed three independent transfections, each in duplicate, and standard deviations were calculated. WT: wild-type.



**Supplementary Figure S2. Knockdown activity of AgoshPol47 variants.** The knockdown activity of the 3' strand on Luc-sense (upper panel) and the 5' strand on Luc-antisense (lower panel) was determined by co-transfection of a luciferase reporter encoding the sense and antisense target sequence in HEK293T cells. The regular shPol47 (21/5) was used as control. We performed three independent transfections, each in duplicate, and standard deviations were calculated. See supplementary figure 1 for additional details.

**A****B**

**Supplementary Figure S3. Quantification of AgoshRNA ~30 nt products.** Total RNA was analyzed by northern blot for processing products derived from 5' strand of AgoshRNA constructs (Figure 2B and 3B, lower panels). The ~30 nt RNA products derived from 5' strand of A) AgoshRT5 and B) AgoshPol47 variants were quantitated using ImageQuant.