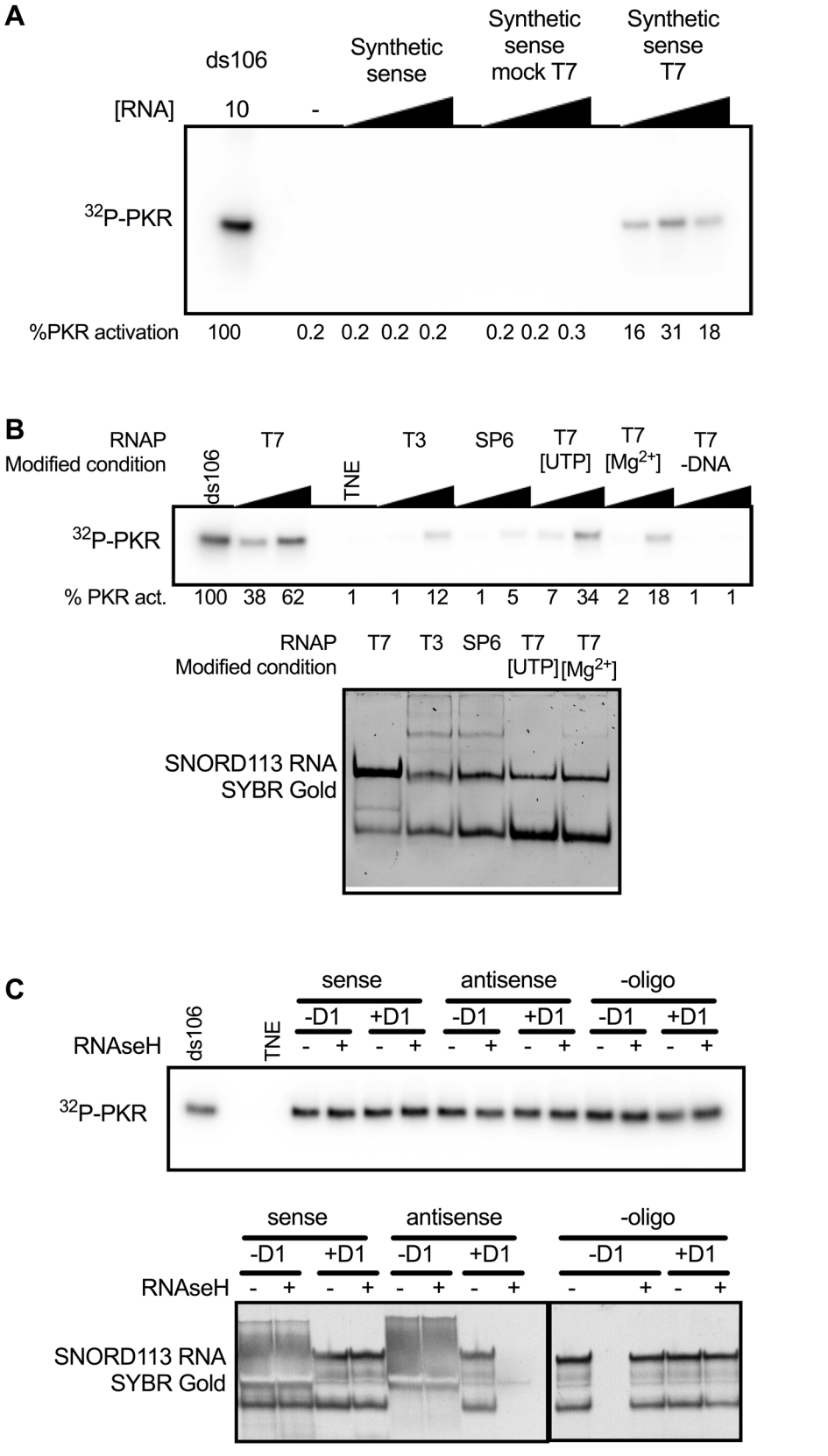
Safran et al. Supplemental Figure S6



Supplemental Figure S6. PKR activation by SNORD113 is a product of in vitro transcription with T7 RNAP. A) Synthetic sense SNORD113 RNA was gel purified after denaturing PAGE and used in a PKR activation assay directly (sense), or after incubation in high yield transcription conditions without (mock T7), or with (T7), T7 RNAP. RNA concentrations were 10 nM, 100 nM, and 500 nM. PKR activation, normalized to reactions with 10 nM ds106 (100%) is denoted below gel. B) PKR activation by SNORD113 RNA transcribed using different transcription conditions (top gel). After transcription, each RNA was purified as a single band from a denaturing gel prior to using in the PKR activation assay. RNA concentrations used were 10 nM and 100 nM. As in (A), PKR activation was normalized to activation with 10 nM ds106 and percentages are noted under the gel. The bottom gel shows 300 fmoles of each RNA from the different transcription reactions visualized after 8% native PAGE and staining with SYBR Gold. C) PKR activation by SNORD113 RNA treated with RNAseH and different DNA oligos. T7 in vitro-transcribed SNORD113 RNA was refolded without oligo, or in the presence of full-length sense SNORD113 DNA oligo, or full-length antisense SNORD113 DNA oligo. Samples were then mock (-) or treated (+) with RNaseH, followed by mock (-D1) or treatment with DNAseI (+D1). Enzymes were removed by Zymogen clean and concentrate columns, the samples were eluted in 10 uL of water, and used in PKR activation assays (top gel) or visualized after 8% native PAGE and staining with SYBR Gold (bottom gel).