SUPPLEMENTARY MATERIAL

Figure Legends

Figure S1. Verifying enzymatic capping of mRNA.
RNA transcribed in the presence of γ-^{32}P-GTP was capped enzymatically, separated on a denaturing agarose gel, stained with ethidium bromide and UV illuminated. RNA was then transferred to a nylon membrane and exposed to film. Capping efficiently removes the γ-^{32}P from the RNA.

Figure S2. Activation of purified PKR by *in vitro* transcribed RNA containing modified nucleosides.
(**A**) Purified PKR was mixed with γ-^{32}P-ATP and *in vitro* transcribed firefly luciferase mRNA that contained the indicated modified nucleosides. Reaction products were separated by SDS-PAGE and imaged using phosphor storage radiography. A representative of three independent experiments is shown. (**B**) Quantification of PKR activation by mRNA containing modified nucleosides. Data represented as mean value ± SEM from five independent experiments using 5–25 ng/μL RNA, normalized to PKR activation by unmodified RNA. Asterisks indicate P-values <0.05 calculated by two-tailed Student's *t*-test.

Figure S3. PKR activation in cells by *in vitro* transcribed mRNA containing modified nucleosides.
In vitro transcribed firefly luciferase mRNA incorporating the indicated modified nucleoside was delivered to HEK293T cells by lipofection. Following cell lysis at 4 hours after transfection, proteins were separated by SDS-PAGE, and phosphorylation of PKR (**A**) and eIF-2α (**B**) was assessed by western blotting. Arrowhead in (B) indicates the eIF-2α band below a heavier non-specific band. Poly(dC) and poly(I:C) were tested as controls. Relative band densities compared to unmodified RNA are presented below each gel lane. Representative images of at least three independent experiments are shown.

Figure S4. RNAs containing modified nucleosides do not inhibit PKR activation.
An activating 200 bp dsRNA was mixed with a 125-fold mass excess of *in vitro* transcribed firefly luciferase mRNA containing the indicated modifications prior to mixing with purified PKR. Reaction products were separated by SDS-PAGE. Relative band densities compared to unmodified RNA are presented below each gel lane. Representative data of three independent experiments is shown.

Figure S5. Double-stranded characteristics of mRNA.
mFold web server prediction of firefly and *Renilla* luciferase mRNA secondary structures.

Figure S1

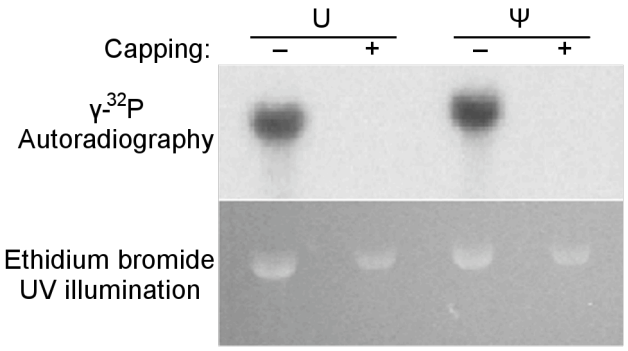

Figure S2

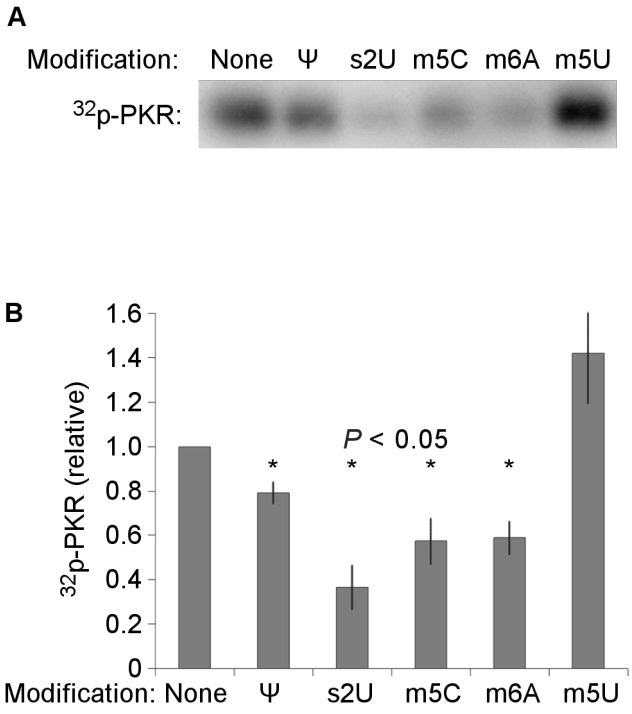

Figure S3

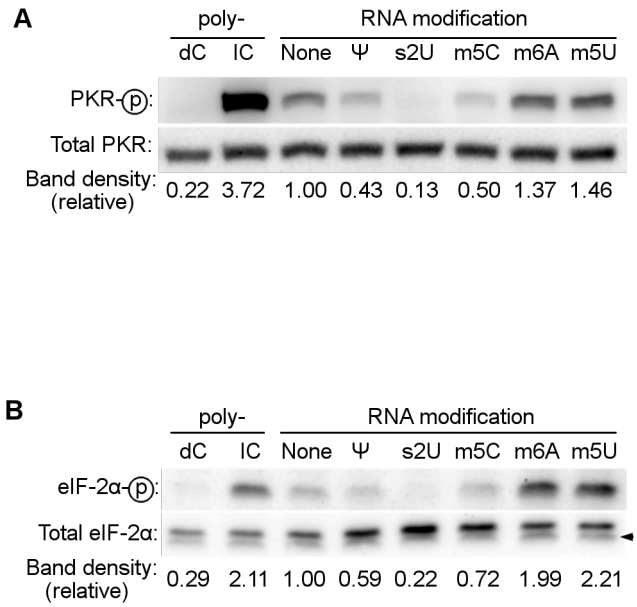

Figure S4

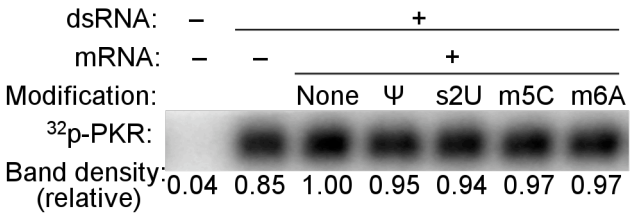

Figure S5