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Supplemental information

Double-stranded RNA reduction by chaotropic agents during *in vitro* transcription of messenger RNA

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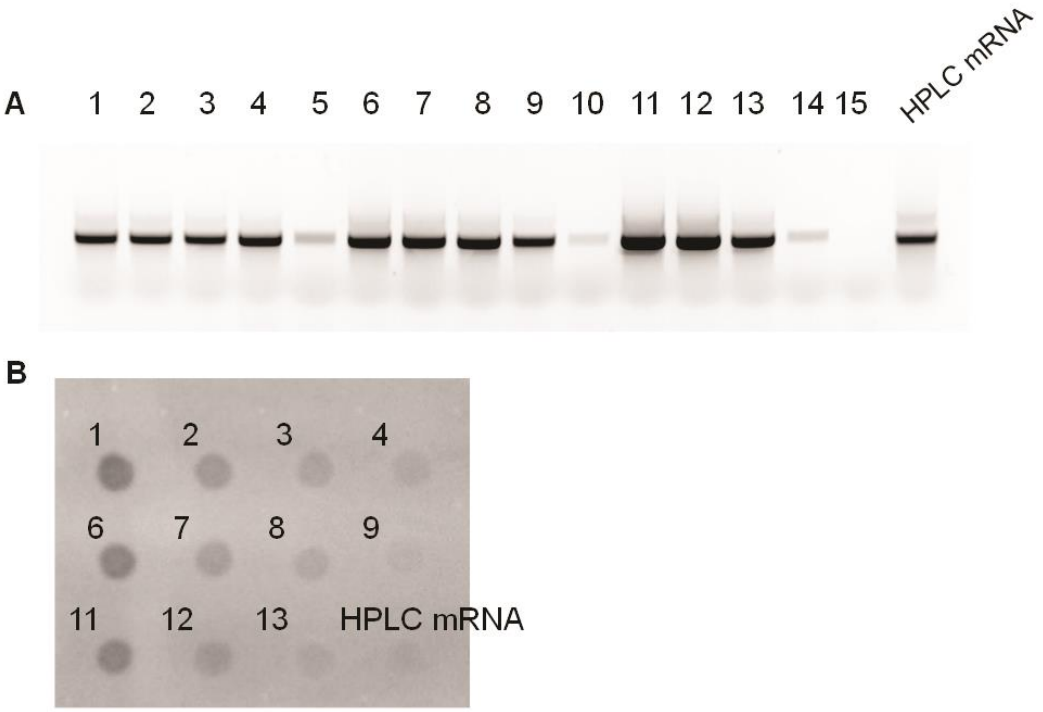

Figure S1. Impacts of temperature and urea concentration on mRNA yield and dsRNA byproducts. (A) Agarose gel image to show higher temperature improves yield. (B) Dot blot to show increasing urea concentration reduces dsRNA. Sample 1-5 contained 0M, 0.4M, 0.8M, 1.2M, and 1.6M urea, and the IVT reactions were performed at 34ºC for 2 hours. Sample 6-10 contained 0M, 0.4M, 0.8M, 1.2M, and 1.6M urea, and the IVT reactions were performed at 37ºC for 2 hours. Sample 11-15 contained 0M, 0.4M, 0.8M, 1.2M, and 1.6M urea, and the IVT reactions were performed at 40ºC for 2 hours. Sample 5, 10, 14, and 15 are not shown on dot blot due to low IVT yield.

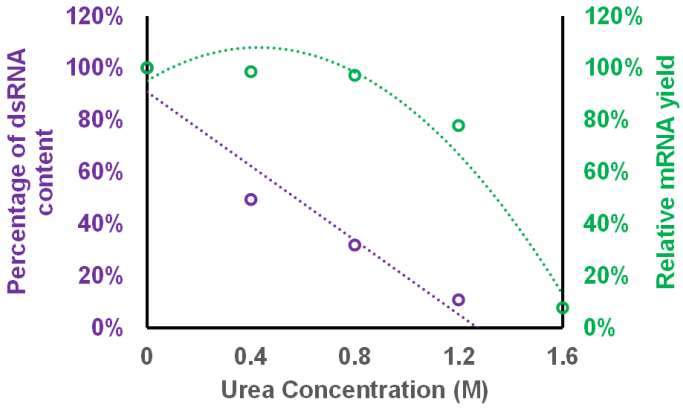

Figure S2. Correlations between urea concentration, dsRNA reduction, and mRNA yield. Purple color is relative percentage of dsRNA content and green color is relative mRNA yield.

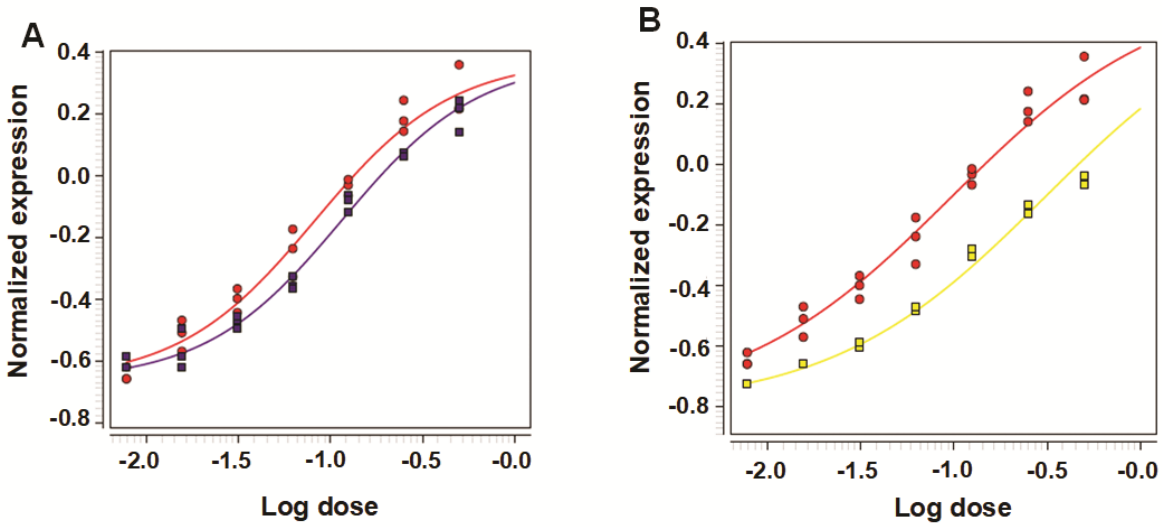

Figure S3. Dose-dependent ICW potency. Y axis is the normalized protein expression transformed from natural logarithm. (A) mRNA1 vs mRNA2, purple color is mRNA1 and red color is mRNA2. (B) HPLC mRNA vs mRNA2, yellow is HPLC mRNA and red is mRNA2.

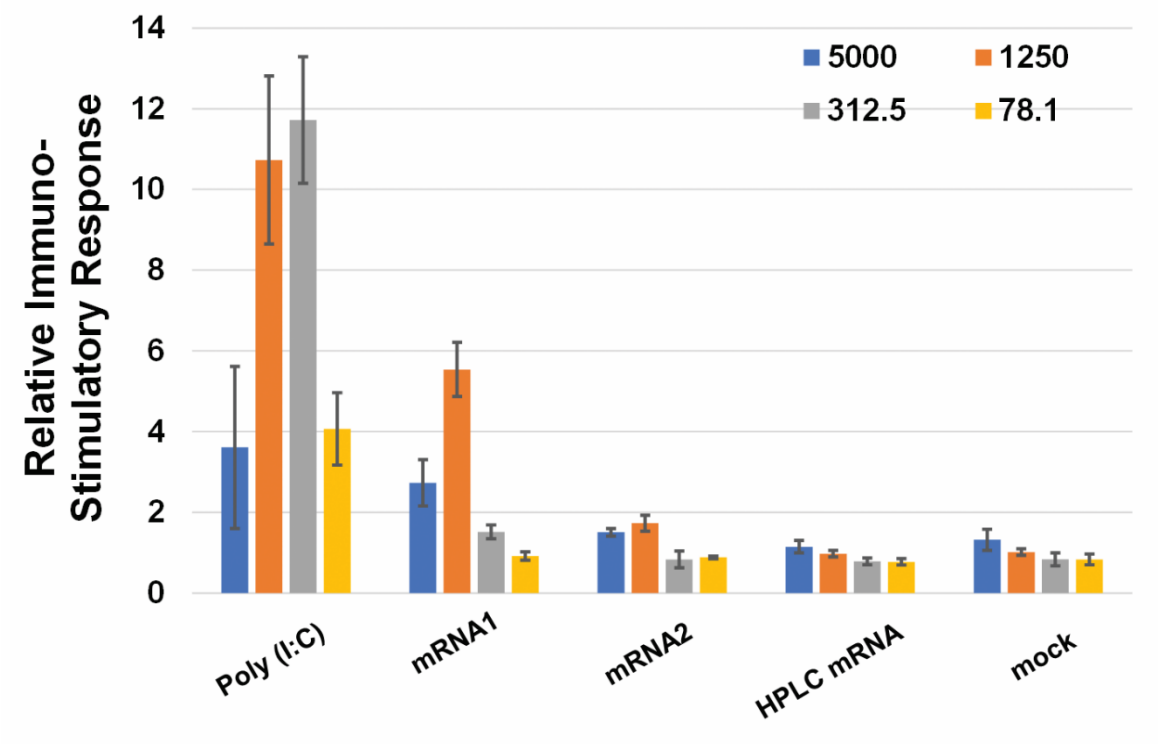

Figure S4. Dose-dependent immuno-stimulatory (IS) response. Unit is ng. Error bars are standard error of the mean. Mock sample contains only transfection reagent.

Table S1. Primer sequences used for Sanger sequencing

dT18	5’ – TTTTTTTTTTTTTTTTT – 3’
Seq1a	5’ – ACAAACGAATCTCAAGCAATCA – 3’
Seq2a	5’ – GCGACAAGTACTGCAAGCTG – 3’
Seq3a	5’ – ATCGAGAACGACCACCACAT – 3’
Seq4a	5’ – GGACATCACCCACGACAAC – 3’
Seq5a	5’ – AGAGCGAGGAGAAGGAGGAC – 3’
Seq6a	5’ – GACGAGGGCTTCAACATCAC – 3’
Seq7a	5’ – GCTGAAGAAGGAGTGGAACG – 3’
Seq8a	5’ – AACAAGGCCTGCTTCCTGAT – 3’
Seq9a	5’ – GGAAGGACGAGAACAGCATC – 3’
Seq10a	5’ – CATCTACGCCCGGTACAACT – 3’
Seq11a	5’ – GTACTGCGGCATCTACGAC – 3’
Seq12a	5’ – CTGGTGAAGAACGTGCTGAG – 3’
Template switch oligo	5’ – CCCATGTACTCTGCGTTGATACCACTGCTTrGrGrG – 3’
5’ end RT primer	5’ – TTCTCCCGGTTGAAGGTCTC – 3’
Seq0R	5’ – ACAGGGTCTTCTCCAGCTTC – 3’