Supplemental Material for:

Activation of PKR by short stem-loop RNAs containing single stranded arms

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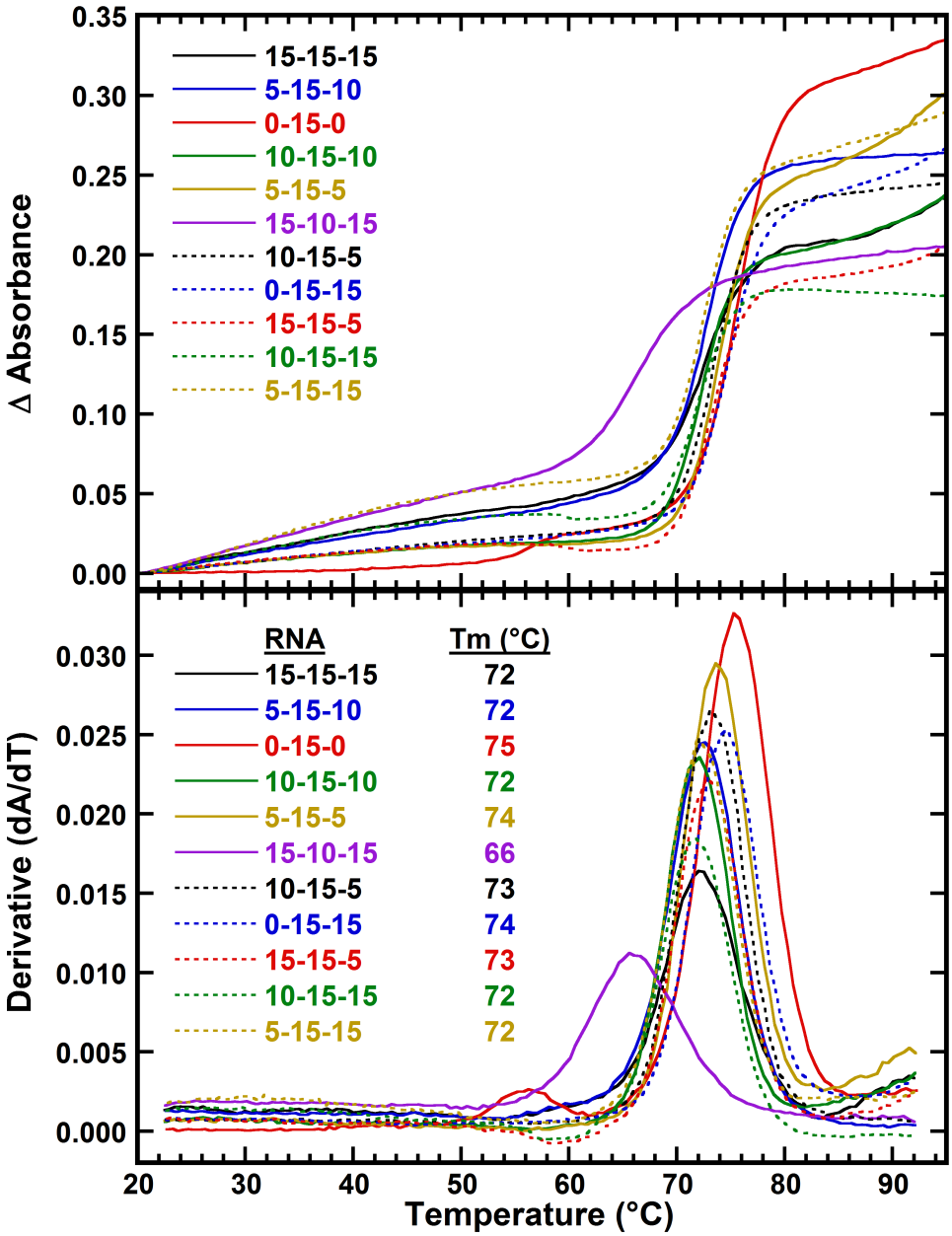

Figure S1. Thermal denaturation analysis of ss-dsRNAs. (A) Absorbance melting curves. Thermal denaturation was analyzed by monitoring the absorbance change at 260 nm at a heating rate of 0.5°C/min. Data were collected every 0.5°C. The measurements were conducted in 10 mM $NaPO_4$ (pH 7.0) with 0.1 mM EDTA. The absorbance of each sample was 0.5 OD. (B) First derivative of the melting curves. The derivative curves were smoothed by boxcar averaging over a 5°C window. T_m values were determined by a Gaussian fit to the derivative curves.

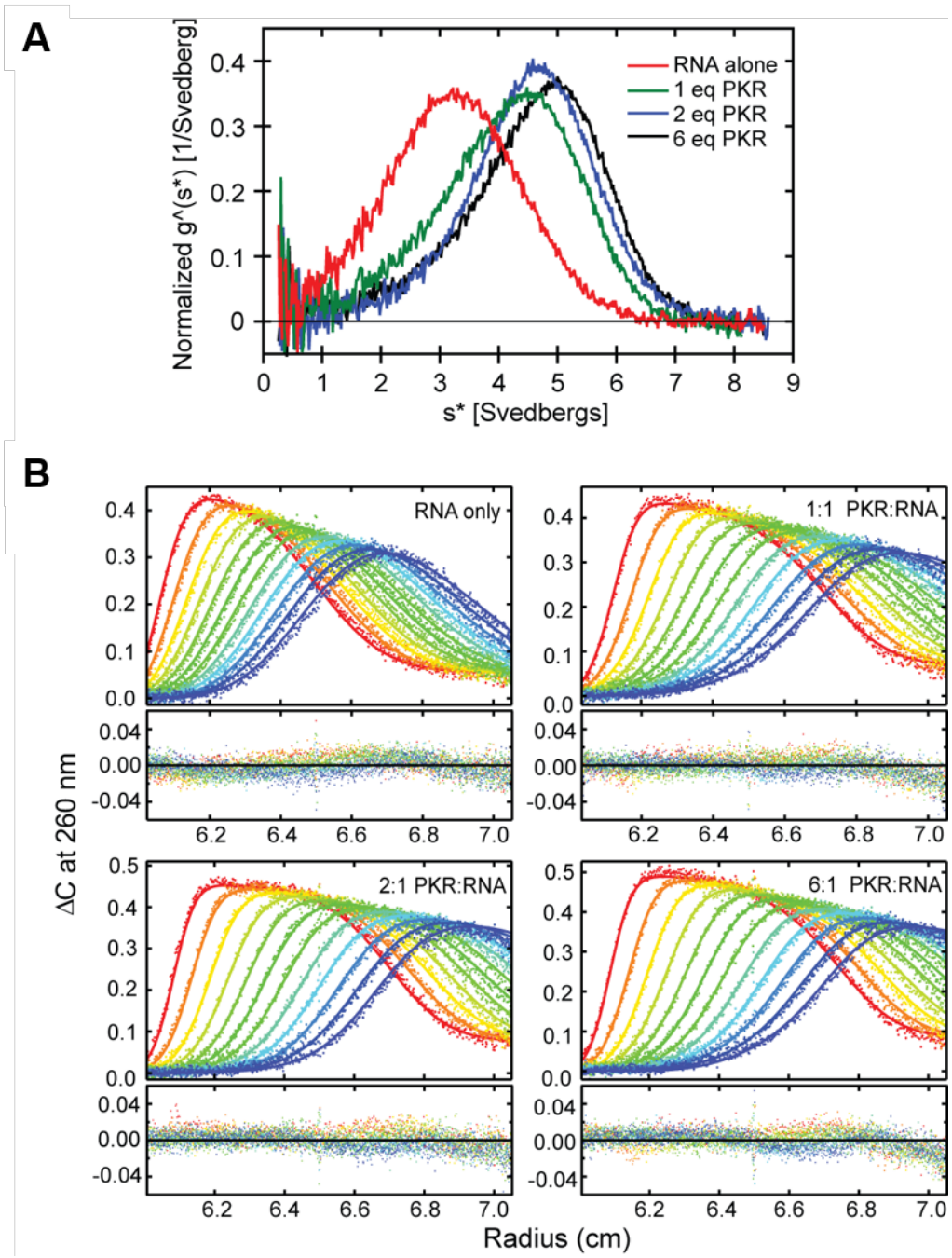

Figure S2. Sedimentation velocity analysis of PKR binding to ppp-15-15-15 in AU200. A) Plots of normalized g^(s*) distributions for 0.7 μM RNA (red) and 0.7 μM RNA plus 1 eq. (green), 2 eq. (blue) and 6 eq. PKR (black). The distributions are normalized by area. B) Global analysis of the sedimentation difference curves. Scans within each data set were subtracted in pairs to remove time-invariant background and fit to a 1:1 binding model using SEDANAL. The top panels show the data (points) and the fit (continuous lines). The bottom panels show residuals. The resulting fit parameters are in Table 1. Only every third difference curve is shown for clarity. Measurement were performed in AU200 buffer at 20°C and 40,000 rpm using absorbance detection at 260 nm.

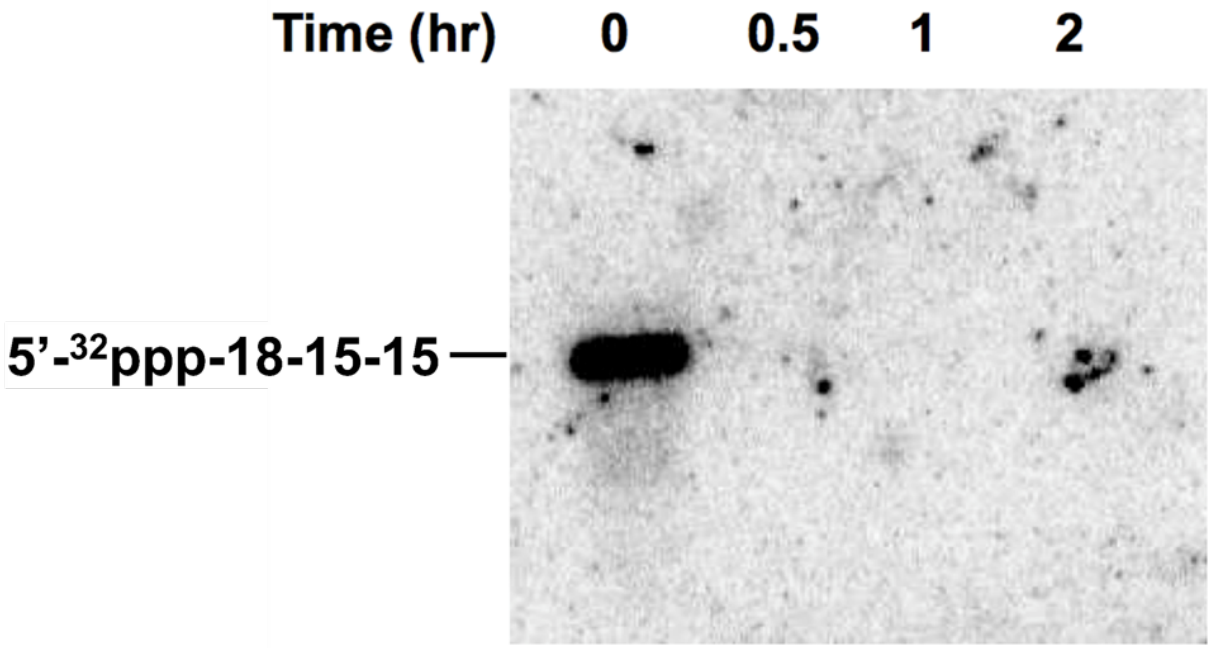

Figure S3. Analysis of dephosphorylation of 5'-ppp ss-dsRNAs. A ppp-18-15-15 RNA containing a ^{32}P at the 5'-γ-phosphate was incubated with Antarctic phosphatase at 37°C. Aliquots were removed at time points as indicated in the figure and quenched with denaturing gel loading buffer and resolved by 10% TBE-Urea PAGE. The gel was exposed to a phosphor screen and scanned on a Typhoon phosphoimager. Removal of 5'-ppp is complete at 30 min. $\gamma^{32}P$-labeled 18-15-15 was made by *in vitro* transcription in the presence of γ-^{32}P GTP. RNA dephosphorylated by Antarctic phosphatase activates PKR.

Table S1. Sequences of ss-dsRNAs.

ss-dsRNA	Sequence[a]
15-15-15	GGAGAACUUAAUGCCGGAGACCAAAGACUUUCCGAAGUCUUUGGUCUCCCUUACAAACCUGACU
15-15-10	GGAGACAAUAAUACAGGAGACCAAAGACUUUCCGAAGUCUUUGGUCUCCAAACCUGACU
15-15-5	GGAGAACUUAAUGCCGGAGACCAAAGACUUUCCGAAGUCUUUGGUCUCCAGACU
10-15-15	GGACAUAAUAGGAGACCAAAGACUUUCCGAAGUCUUUGGUCUCCCAAACAAACAUAACU
10-15-10	GGAGAACUUAGGAGACCAAAGACUUUCCGAAGUCUUUGGUCUCCAAACCUGACU
10-15-5	GGACAAUAUAGGAGACCAAAGACUUUCCGAAGUCUUUGGUCUCCAAACU
5-15-15	GGACAGGAGACCAAAGACUUUCCGAAGUCUUUGGUCUCCCAAACAAACAUAACU
5-15-10	GGAUAGGAGACCAAAGACUUUCCGAAGUCUUUGGUCUCCAAACAUAACU
5-15-5	GGAGAGGAGACCAAAGACUUUCCGAAGUCUUUGGUCUCCAGACU
15-15-0	GGAGAACUUAAUGCCGGAGACCAAAGACUUUCCGAAGUCUUUGGUCUCC
0-15-15	GGAGACCAAAGACUUUCCGAAGUCUUUGGUCUCCCUUACAAACCUGACU
0-15-0	GGAGACCAAAGACUUUCCGAAGUCUUUGGUCUCC
15-10-15	GGAGACAAUAAUACAGGAGACCAAAUCCGUUUGGUCUCCCAAACAAACAUAACG
15-5-15	GGAGAAUAGAAUACAGCGACUCCGGUCGCACAUAAGAUAAGAGG

[a] Sequences are written 5' to 3' with duplex regions underlined.

Table S2. Hydrodynamic properties of ss-dsRNAs.

ss-dsRNA	Buffer	Mass[a]	s (Svedbergs)[b]	$s_{20,w}$ (Svedbergs)[c]	f/f_o
ppp-15-15-15	AU75	20,659	3.12	3.21	1.38
HO-15-15-15	AU75	20,359	3.19	3.28	1.34
ppp-15-15-10	AU75	19,138	2.87	2.95	1.69
ppp-15-15-5	AU75	17,532	2.82	2.90	1.78
ppp-10-15-15	AU75	19,106	2.93	3.01	2.05
ppp-10-15-10	AU75	17,516	2.93	3.01	1.88
ppp-10-15-5	AU75	15,909	2.77	2.85	1.84
ppp-5-15-15	AU75	17,506	2.90	2.97	1.87
ppp-5-15-10	AU75	15,909	2.80	2.88	1.64
ppp-5-15-5	AU75	14,366	2.73	2.80	1.76
ppp-15-15-0	AU75	15,917	2.80	2.88	1.86
ppp-0-15-15	AU75	15,798	2.66	2.73	1.59
ppp-0-15-0	AU75	11,057	2.46	2.52	1.65
ppp-15-10-15	AU75	17,577	2.66	2.74	1.53
ppp-15-5-15	AU75	14,522	2.43	2.50	1.82
ppp-15-15-15	AU200	20,659	3.21	3.36	1.58
ppp-15-15-0	AU200	15,917	2.90	3.03	1.73
ppp-0-15-15	AU200	15,798	2.66	2.78	1.83
ppp-0-15-0	AU200	11,057	2.47	2.58	1.55
ppp-15-15-15	AU75+Mg^{2+}	20,659	3.36	3.45	1.78
ppp-0-15-0	AU75+Mg^{2+}	11,057	2.54	2.61	1.60
ppp-15-15-15	AU60+Mg^{2+}	20,659	3.43	3.53	1.74
ppp-0-15-0	AU60+Mg^{2+}	11,057	2.53	2.6	1.90

[a] Masses predicted based on composition.

[b] Uncorrected sedimentation coefficient obtained by fitting to a single discrete species model.

[c] Sedimentation coefficient corrected to standard conditions (water at 20°C).

Table S3. Hydrodynamic properties of ss-dsRNA · PKR complexes.

Species	Buffer	Mass[a]	s (Svedbergs)[b]	$s_{20,w}$ (Svedbergs)[c]	f/f_o[d]
ppp-15-15-15·PKR	AU75	82,754	4.88	5.04	1.60
ppp-15-15-15·PKR_2	AU75	144,848	8.02	8.30	1.33
HO-15-15-15·PKR	AU75	82,454	4.76	4.91	1.64
HO-15-15-15·PKR_2	AU75	144,548	8.39	8.68	1.27
ppp-15-15-10·PKR	AU75	81,233	5.01	5.17	1.61
ppp-15-15-10·PKR_2	AU75	143,327	7.57	7.83	1.44
ppp-15-15-5·PKR	AU75	79,627	4.71	4.86	1.71
ppp-15-15-5·PKR_2	AU75	141,721	7.86	8.13	1.39
ppp-10-15-15PKR	AU75	81,201	4.84	4.99	1.78
ppp-10-15-15·PKR_2	AU75	143,295	7.80	8.06	1.45
ppp-10-15-10·PKR	AU75	79,611	4.88	5.04	1.70
ppp-10-15-10·PKR_2	AU75	141,705	7.36	7.61	1.50
ppp-10-15-5·PKR	AU75	78,004	4.80	4.96	1.67
ppp-10-15-5·PKR_2	AU75	140,098	7.40	7.65	1.47
ppp-5-15-15·PKR	AU75	79,601	4.43	4.57	1.86
ppp-5-15-15·PKR_2	AU75	141,695	7.13	7.37	1.55
ppp-5-15-10·PKR	AU75	78,004	4.64	4.79	1.68
ppp-5-15-10·PKR_2	AU75	140,098	6.94	7.18	1.54
ppp-5-15-5·PKR	AU75	76,460	4.67	4.82	1.67
ppp-5-15-5·PKR_2	AU75	138,555	7.12	7.36	1.50

[a] Masses predicted based on composition.

[b] Uncorrected sedimentation coefficient obtained by fitting to a single discrete species model.

[c] Sedimentation coefficient corrected to standard conditions (water at 20°C).

[d] Frictional ratio.

[e] The value of s(RP) and s(RP_2) could not be measured for these RNAs and were fixed to the values measured in AU75 buffer.

Table S3: Continued. Hydrodynamic properties of ss-dsRNA · PKR complexes.

Species	Buffer	Mass[a]	s (Svedbergs)[b]	$s_{20,w}$ (Svedbergs)[c]	f/f_o[d]
ppp-15-15-0·PKR	AU75	78,012	4.75	4.90	1.70
ppp-15-15-0·PKR_2	AU75	140,106	7.19	7.43	1.52
ppp-0-15-15·PKR	AU75	77,893	4.95	5.11	1.54
ppp-0-15-15·PKR_2	AU75	139,987	6.82	7.05	1.54
ppp-0-15-0·PKR	AU75	73,151	4.58	4.73	1.60
ppp-0-15-0·PKR_2	AU75	135,246	6.19	6.40	1.66
ppp-15-10-15·PKR	AU75	79,671	4.76	4.92	1.60
ppp-15-10-15·PKR_2	AU75	141,766	6.48	6.70	1.62
ppp-15-5-15·PKR	AU75	76,616	4.71	4.87	1.63
ppp-15-5-15·PKR_2	AU75	138,711	6.87	7.10	1.54
ppp-15-15-15·PKR	AU200	82,754	5.17	5.46	1.60
ppp-15-15-0·PKR	AU200	78,012	4.96	5.23	1.62
ppp-0-15-15·PKR	AU200	77,893	4.72	4.98	1.68
ppp-0-15-0·PKR	AU200	73,151	4.52	4.78	1.60
ppp-15-15-15·PKR	AU75+Mg^{2+}	82,754	5.48	5.73	1.60
ppp-15-15-15·PKR_2	AU75+Mg^{2+}	144,848	6.98	7.23	1.63
ppp-0-15-0·PKR	AU75+Mg^{2+}	73,151	4.58 (fixed)[e]	4.80	1.62
ppp-0-15-0·PKR_2	AU75+Mg^{2+}	135,246	6.19 (fixed)[e]	6.42	1.66
ppp-15-15-15·PKR	AU60+Mg^{2+}	82,754	5.08	5.30	1.73
ppp-15-15-15·PKR_2	AU60+Mg^{2+}	144,848	6.75	6.98	1.69
ppp-0-15-0·PKR	AU60+Mg^{2+}	73,151	4.58 (fixed)[e]	4.73	1.66
ppp-0-15-0·PKR_2	AU60+Mg^{2+}	135,246	6.19 (fixed)[e]	6.40	1.70

[a] Masses predicted based on composition.

[b] Uncorrected sedimentation coefficient obtained by fitting to a single discrete species model.

[c] Sedimentation coefficient corrected to standard conditions (water at 20°C).

[d] Frictional ratio.

[e] The value of s(RP) and s(RP_2) could not be measured for these RNAs and were fixed to the values measured in AU75 buffer.

Table S4. Effect of Mg^{2+} on PKR binding.

RNA	Buffer	K_{d1} (nM)	K_{d2} (nM)	Maximum % RP_2	Relative RP_2[a]	RMSD[b]
ppp-15-15-15	AU75	4	294	12.9	-	0.0082
ppp-15-15-15	AU60 / 5 Mg[c]	21	2,104	2.3	18	0.0101
ppp-15-15-15	AU75 / 5 Mg[d]	42	3,704	1.3	10	0.0108
ppp-0-15-0	AU75	138	1,662	2.8	-	0.0090
ppp-0-15-0	AU60 / 5 Mg[c]	331	11,627	0.4	14	0.0110
ppp-0-15-0	AU75 / 5 Mg[d]	267	13,918	0.4	14	0.0880

Parameters obtained by global nonlinear least square analysis of sedimentation velocity experiments.
[a] Maximum % RP_2 relative to AU75 (in percent).
[b] Root mean square deviation in absorbance units
[c] 20 mM HEPES pH 7.5, 60 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM TCEP.
[d] 20 mM HEPES pH 7.5, 75 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM TCEP.