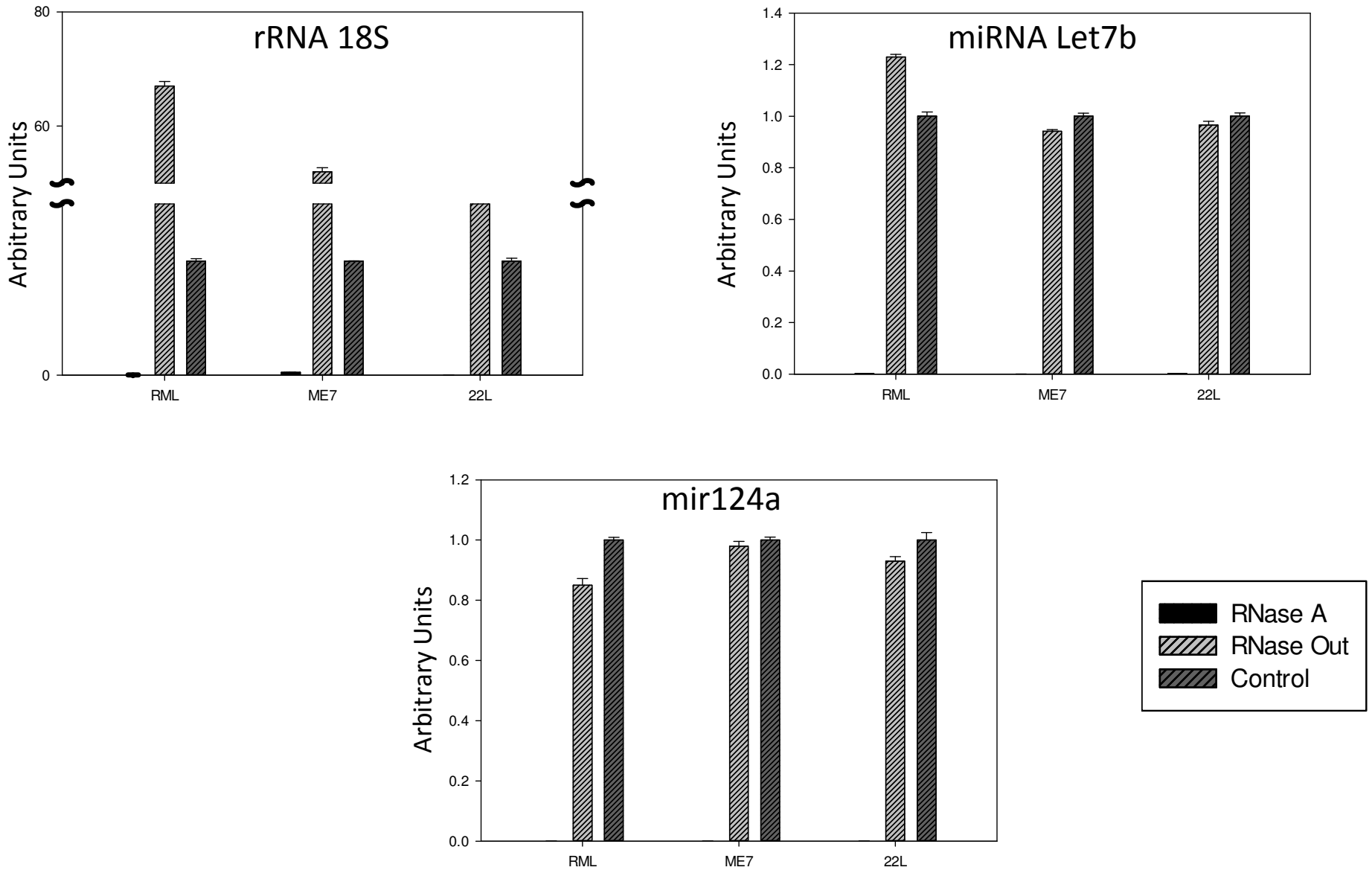
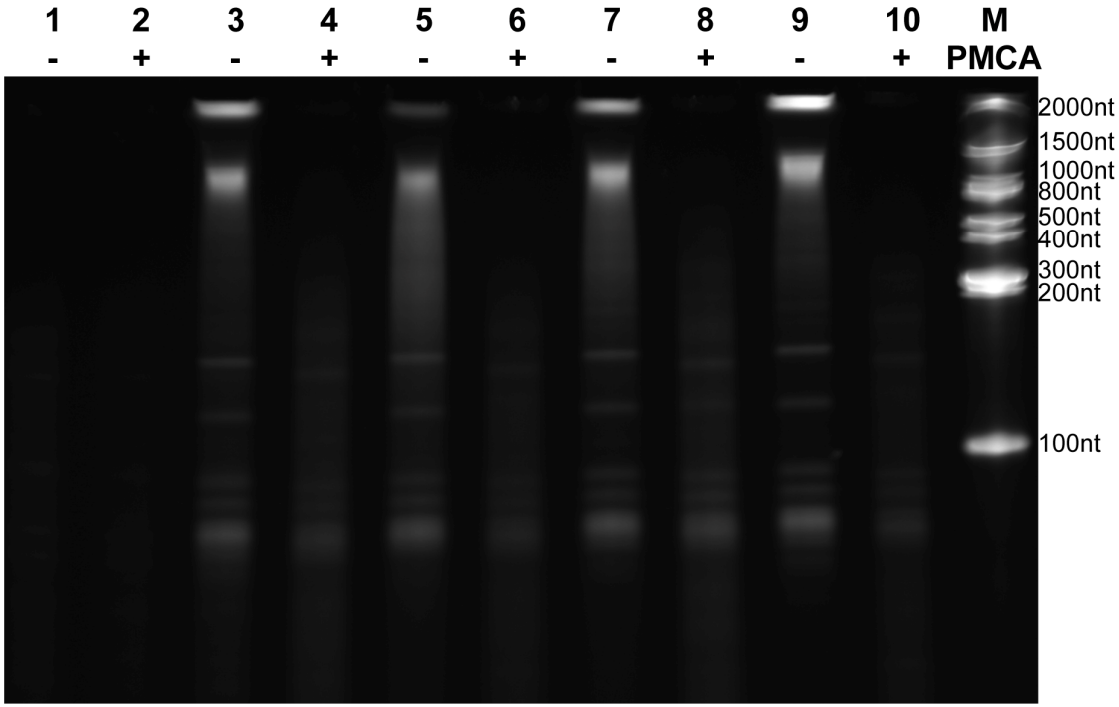


Figure S1 RT-qPCR analysis of PMCA substrates demonstrates RNA removal by RNase A treatment. Total and small RNAs were purified from PMCA substrates after RNase A and RNase Out treatments. The relative abundance of the ubiquitous miRNA Let7b as well as the brain specific miRNA mir124a and the rRNA 18S was determined by RT-qPCR.



Lane #	Sample
1 & 2	RNase A Treated NBH + RNase Out
3 & 4	RNase A Treated NBH + RNase Out + RNA
5 & 6	RNase A + RNase Out Treated NBH
7 & 8	RNase Out Treated NBH
9 & 10	NBH
M	RNA Ladder

Figure S2 Qualitative evaluation of RNA in PMCA samples. RNA was purified before and after PMCA from NBHs subjected to different treatments and reconstituted with total RNA (where indicated). These preparations were fractionated in 15% TBE-Urea Polyacrylamide gels to evaluate the quality of the RNA. RNA extracted from NBHs treated with RNase A is shown in lanes 1 and 2. RNA extracted from NBHs treated with RNase A whose activity was stopped with RNase Out prior to total RNA reconstitution is shown in lanes 3 and 4. RNA extracted from NBHs simultaneously treated with RNase A and RNase Out is shown in lanes 5 and 6. RNA extracted from NBHs treated with RNase Out is shown in lanes 7 and 8. RNA extracted from untreated NBHs is shown in lanes 9 and 10. Odd and even lanes correspond to RNA samples obtained before and after PMCA respectively.

Experiment A	LogRI					$Log[RI_{PK1}/RI_{PK1+swa}]$	$Log[RI_{LD9}/RI_{LD9+curc}]$	$RI_{PK1}/RI_{PK1+swa}$	$RI_{LD9}/RI_{LD9+curc}$
	PK1	PK1+swa	LD9	LD9+curc	$R33_{2H11}$				
RML-P-RNaseA	4.78	4.09	3.82	< 3.5	< 3.5	0.69	> 0.32	4.90	> 2.09
RML-P-RNaseOut	4.82	3.90	3.71	< 3.5	< 3.5	0.93	> 0.21	8.43	> 1.62
RML-B	5.10	4.26	4.34	< 3.5	< 3.5	0.84	> 0.84	6.86	> 6.92

Experiment B	LogRI					$RI_{PK1}/RI_{PK1+swa}$	$RI_{LD9}/RI_{LD9+curc}$	$RI_{PK1}/RI_{PK1+swa}$	$RI_{LD9}/RI_{LD9+curc}$
	PK1	PK1+swa	LD9	LD9+curc	$R33_{2H11}$				
RML-B	6.60	4.77	4.98	< 3	< 3	4.90	> 2.09	68.27	> 95.5
ME7-B	< 3	< 3	5.55	5.05	< 3	8.43	> 1.62	n.d.	3.16
22L	6.41	6.34	6.33	6.54	5.62	6.86	> 6.92	1.18	0.62

Table S1. Response Index (RI) of a cell line for a prion strain. The RI of a cell line for a prion strain is the reciprocal of the dilution required to yield an arbitrary number (here A) 750 spots and B) 300 spots) of positive cells ("spots") per 20,000 cells after the third split. RI ratios between different cell lines as well as RI ratios between cells subjected or not to a specific pharmacological treatment are characteristic for different prion strains. The differences in RI ratios (with or without swainsonine treatment) of the brain-derived RML and the PMCA material generated in the presence or absence of RNA and passaged in Tga20 mice were smaller than a log. In addition, replication of RML from all three types of samples was abolished by curcumin treatment. These data show that the three RML samples correspond to the same prion strain. Cell lines: PK1, LD9, $R_{33}2H11$. Prion inhibitors: cur: curcumin, swa: swainsonine.

Movie S1 and S2 Videos of *Tga20* mice infected with RML-P^{RNaseA} and RML-B samples. Movie S1 clinically sick RML-P^{RNaseA}-inoculated *Tga20* mouse showing an abnormal disease phenotype characterized by forelimb paresis. **Movie S2** clinically sick RML-B-inoculated *Tga20* mouse showing classical signs of scrapie.