Identification of circulating microRNA signatures as potential biomarkers in the serum of elk infected with chronic wasting disease

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<u>Further Information for Figure 1:</u> PMCA of CWD PrP^{Sc}.

- Cropped gels were from the 3rd round of PMCA were prepared for Figure 1 (below).
- Each panel represents a single gel/Western blot.
- Western blots for positives and negative samples were scanned together as a set of 6.
- Scans for each of these sets of Western blots are provided below representing the full gels.
- The control for each gel is the cellular prion protein blotted from the normal brain homogenate loaded in the final well of each gel.
- Gel 6 was omitted from the final version figure #1 as this was a repeat of the positive and negative controls provided on Gel #5.
- 6 blots were prepared and scanned together. The full-length scans for the positive and negative samples are provided below.

Full length Gel scans for PMCA Round #3 (positive samples plus controls).



Full length Gel scans for PMCA Round #3 (negative samples).



Supplementary Figure 1. The choice of human or mouse reference annotation does not influence serum miRNA abundance in elk. The mean log2 transformed normalized read count was plotted for 367 miRNAs detected in 70 elk serum samples using the human (GRCh38) and mouse (GRCm38) reference genome and accompanying miRBase annotation.







Supplementary Figure 3. Hierarchical clustering (A) and PCA (B) of the putative 21 serum miRNA biomarkers shows no relationship between IHC Scores reflecting prion staining intensity and individual elk samples.



Supplementary Figure 4. KEGG pathway for prion disease on which is marked those genes that are targeted by miRNAs altered in abundance in CWD IHC positive elk. MiRNAs marked in blue are those determined using the Pathway Union tool of miRPath v3.0 that identifies the significantly targeted pathways by the selected miRNA group, whereas those in green are all genes targeted by at least one selected miRNA within the pathway. Prion Diseases pathway map was retrieved with permission from KEGG, which was developed by Kanehisa Laboratories (See refs. 49-51 in main text).

