Fig. 1: Cavin-1 expression modifies selective export of specific microRNA species. A) Cellular and EV miR content modified upon expression of cavin-1. RNA-seq experiment was performed on small RNAs extracted from GFP-PC3-EVs and cavin-1-PC3-EVs and whole cell lysates. Points on the correlation graph represent the mean log2 fold change of RNA species (n=95) upon cavin-1 expression. Lines roughly approximate the 95% confidence intervals of a linear regression. MiRs outside of the CI are considered selectively exported. B) MiRs significantly (p≤0.05) modified within the EVs upon cavin-1 expression where closer examined to determine selective export. Log2fold change for cell (grey) and EV (black) for each interesting miRs were plotted and compared with a Mann-Whitney U-test.

Fig. 2: Confirmation of selective miRNA export upon cavin-1 expression. RT-qPCR measured cDNA synthesised from total RNA from GFP-PC3 cell, cavin-1-PC3 cells and the EVs derived from these cells. Bar graphs represent the normalised change for each miR between the cell lines. Mann Whitney U-test was used to determine significant change.

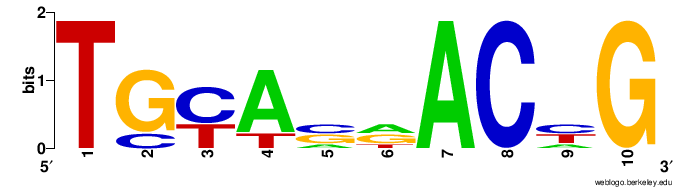


Fig. 3: Exported miRs contain specific motifs that correlate to sorting. A) TAMO software suite was used MEME algorithm to identify stretches of nucleotides shared within the selectively exported miRs. These motifs are expressed as sequence logos. B) Position of the motifs overlaps with the seed region.

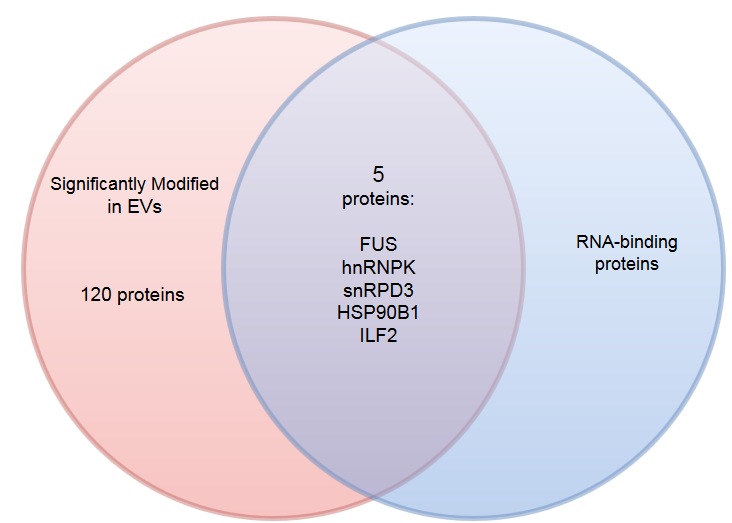
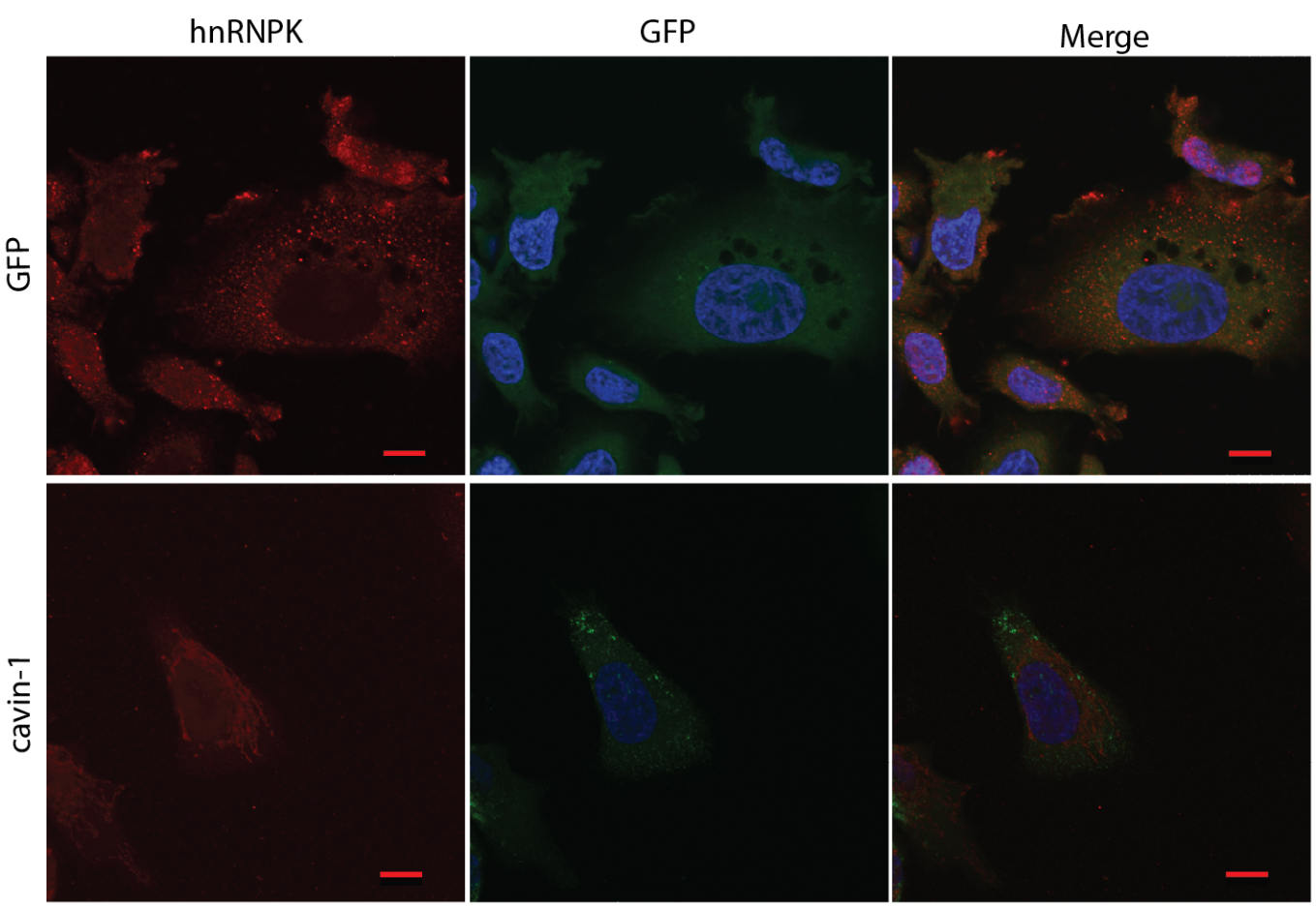


Fig. 4: Exported RNA-binding proteins may mediate miR export. Mass spectrometry was completed to determine the proteomic content of EVs from GFP-PC3 and cavin-1-PC3 combined with gene ontology assessment to determine RNA binding capabilities. Venn diagram represents number of proteins that correspond to each criteria. 5 proteins fulfil both criteria and may be candidate miR export proteins.



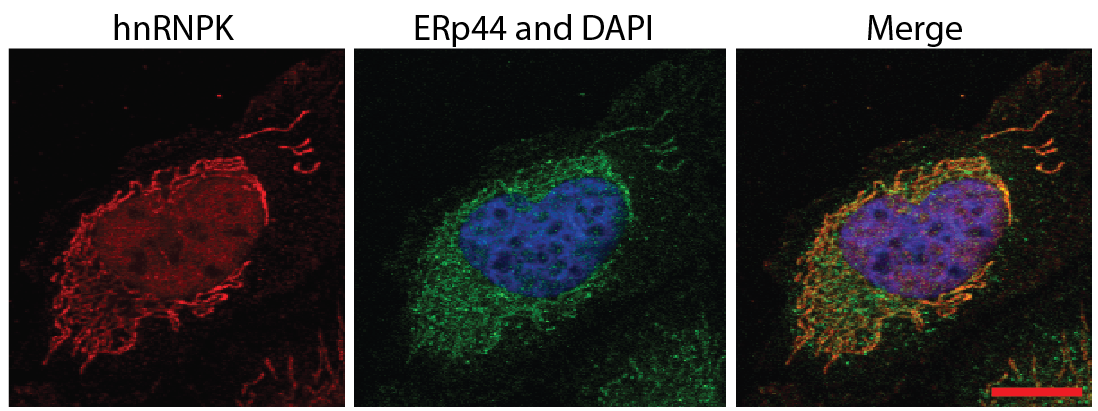


Fig. 5: hnRNPK changes from vesicular to endoplasmic reticular localisation in cavin-1 expressive cells. A) Confocal immunofluorescence of GFP-PC3 and cavin-1-PC3 shows subcellular localisation of endogenous hnRNPK (red). DAPI (blue) stain and GFP (Green) expressivity also visualised. Bar scale is 10um. B) Identification of the subcellular fraction that hnRNPK (red) localises to was completed using CD9 and ERp44 (green) and a nuclear DAPI stain. hnRNPK localised partially to CD9 positive vesicles in GFP-PC3 cells, whereas cavin-1-PC3 cells modified localisation to endoplasmic reticulum.

Fig. 6: hnRNPK co-localizes with selectively exported miRs and not non-selective miRs.

Fig. 7???: hnRNPK pulls down microRNAs or RNAs