EC1 prep:

Introduction:

* Cancers that form into a metastatic form are deathly
* We focus on prostate cancer, with high incidence yet low mortalities.
* Metastatic form had been linked to the caveolae associated proteins.
  + Caveolin forms structural component that allows the membrane to curve only when cavin-1 is present.
  + In cancer there is a lack of cavin-1, where the lone caveolin exerts a secondary activity that regulates cancer-like properties.
  + Re-introduction of cavin-1 to this incomplete system truncates the aggressive nature. It was also found to regulate exosomal secretion of specific molecules.
* How caveolae are related to exosomes.
* Exosomes related to cancer.
* miRNAs differentially sorted into the exosomes, which exert down regulation of pathways in recipient cell to cause the establishment of a metastatic niche.
* Can’t shut off exosome production due to its natural roles in biology, but if we can find whats mediating the change in miRNAs which are exerting the negative behaviour, then we may find a new therapeutic to limit metastatic spread.

Methods:

* Aim1: compile extensive list of miRNAs being selectively exported by this system.
  + Bioinformatics on previous miRNA-seq experiment that found miRNAs expressed in the exosome fraction vs the cell pellet.
  + Confirmation by rt-qpcr
* Aim 2: find RNA-binding partners that may be the chaperone proteins that mediate this selective transport.
  + Prior proteomic data had been complied using MS which analyses proteins associated with the exosomes and the lipid raft fraction which is where chaperones are expected to be.
  + Added assessment of RNA-binding ability by gene ontology
  + Verification of protein presence, undecided method.
  + Further assess likelihood of miRNA binding by performing a motif discovery and the rationale behind that.
* Aim 3: verify the miRNA escort protein by co-localisation.
  + Co-localisation immunofluorescence, where the miRNA will be hybridised to a probe and the target protein will be associated with a GFP tag.



