Introduction:

* Name, Group, Institute, title: control of selective export of miRNAs, via exosomes, in prostate cancer.
* This focuses in on the miRNA export mechanisms which haven’t been extensively researched.
* Describe why miRNAs are important; down regulate entire pathways and how.
* On top of this, exosomes and other extracellular vesicles allow for the long-range spread of these miRNAs.
  + Uptake into new cell exerts the down regulation process as described which helps in trans- communication.
  + Issues arise however in cancers, as these methods allow for cancer like properties to be exerted in the recipient cells. This is one method of metastasis.
* Metastasis is an issue, clinically, as it prolongs cancer treatments, spreads to other organs, increases the mortality rates. Hence the need for treatments that target metastasis.
* Previously miRNA export was really only considered to be non-selective where the miRNAS in the exosomes are representative of the cellular miRNAs. Yet, results from our lab had found something more selective.
* We focus on an advanced prostate cancer cell, called PC3, which exhibits this metastatic activity.
* It was found that caveolin-1 expression, which isn’t usually expressed in prostate epithelia, is correlated with metastatic and aggressive behaviour.
* Caveolin will associate to the lipid raft fraction, which is a region on the membrane, and endosome membrane that is enriched for a particular lipid type; cholesterol
* Caveolin usually associates with cavins, which initiates caveolae formation. It also down regulates the aggressive nature of the cancer model.
* This interaction causes a change in composition of the lipid raft fraction which was found to mediate part of the flux in behaviour.
* When further assessing the difference in molecular consequence between caveolin only and with the cavin-1, it was that there was a change in exosome cargo. Particularly mirnas.
* On top of this, further assessment revealed that this was selective due to unchanging the miRNA concentration in the cell, but changing it in the exosomes.
* This indicates that in this cell model, that cavin-1 appear to be mediating some sort of miRNA binding protein that is mediating export.

Methods:

* Aim 1; Finding the comprehensive list of miRNAs that are selectively exported; prior work wasn’t comprehensive as they were trying to find proteins, not miRNA, where the methods they used filtered out the lowly expressed, compared to protein, miRNAs. This will assess previously compiled miRNA-seq data, comparing the miRNAs in the exosome and cell pellet fractions and between the model cancer cell line and the cavin-1 transformed cancer cells.
* Aim 2: given the list of the miRNAs and prior proteomic data from MS, we should be able to predict potential RNA binding proteins that may be associated with the process. Will use bioinformatics to assess differential presence of the proteins correlated to the miRNA presence. Verify with rt-qpcr.
* Perform a motif discovery on the miRNAs, though Gibbs sampling, then perform a literature search for any proteins that bind that that particular motif.
* Aim3: Verify the protein-miRNA interaction by co-localisation immunofluorescence.