Background notes for Honours project:

From Application:

Why do we study this: prior research established importance of active protein, RNA and lipids transferred by long range inter-cellular communication. Using extracellular vesicles allows for stability in blood, and targeting which add to its involvement in cancers. For example, prostate cancer derived vesicles possess a homing mechanism to bone and lung, adding to its mortality rate.

Fundamental related biology: two types of extracellular vesicles, exosomes and microvesicles. Despite different production mechanisms, size and markers overlap. These vesicles are lipid bilayer, enriched in cholesterol, sphingolipids and ceramide, similar to lipid rafts. These cholesterol rich domains bind Caveolin-1 to form Caveolae. Addition of Cavins stabilize this interaction and regulate caveolae. How cargo is loaded have been somewhat investigated, regulation of this is not yet defined.

Linking to disease: Cavin-1 findings recently depict altered extracellular vesicle concentrations of miR148a which had been linked to osteoclastogenesis when active. However, the presence of cavin-1 had resulted in EV concentration decrease, to inhibit tumour progression. This indicates that cavins may have a role in EV-mediated transfer of miRNA, which in turn modulates biological progresses.

Outcome: reveal fundamental cell regulatory mechanisms that dictate extracellular vesicle cargo export and potentially translate into therapeutic targets for cancers and deformities of extracellular transport.

Background information to still look up/ write down: Cavin1, 2, 3 and Caveolin interactions. Prior evidence in their role in disease. How Caveolin alone produces a negative phenotype in PC3 cells. Explain why we use PC3 cells and HEK293.

Overall aim: assess the relationship of selectively exported miRNA species via extracellular vesicles and cavin family members relating to this selective export.

Aims: 1) determine if miRNA levels in extracellular vesicles correlate to cell line with different biological properties by computational analysis. Similarly, assess the differential levels of miRNA secretion correlating to the members of the cavin family. Using miRNA-seq data, DESeq2, R, lumi and edgeR programing.

1b) Validate the miRNAs of interest via wet-lab and rt-qPCR of said miRNAs. WILL REQUIRE CELL CULTURE

2) Using prior subcellular proteomics and miRNA data, assess potential interaction partners that may mediate this sorting mechanism. Using R, igraph and Cytoscape. Similar method/ analysis as Amanda’s thesis.

3) Observe and confirm miRNA and protein interactions that may reveal Escort protein functionality. This will use co-localization immunofluorescence microscopy, miRNA in-situ hybridization. Method may be similar to Amanda’s thesis.

Maybe) extend the understanding of this mechanism if time permitting. IE observe what occurs if you knockout this protein, is there conditions where the cavin/protein interactions is not adequate for expected function.

Cell Culture techniques you’ll need to exhibit: Cryopreserved cells will need to be thawed (SOP01), passaging (SOP02), probably counting cells to allow for the correct number of cells into qPCR (SOP05), probably whole cell lysate preparation (SOP06) for qPCR, exosome purification (SOP33), primer design (SOP34), Trizol RNA extraction or maybe a kit extraction (SOP35), immunofluorescence and precipitation(SOP18 and 13).

Aim1 method with data. Filter data (both PC3 and HEK together then a second filter of them seperate). Plug into DESeq2 method to find differences between Cavins in cell lines annnd assessment of cell lines against each other. Ask Alex if there is a method to assess the outcome of miRNA function. Not only get a list of important miRNAs but also get a few graphs. Maybe some MAplots with the high PADJ values highlighted. Run same analysis on lumi and EdgeR method to assess validity between replicates (dendrogram). Use miRNAs very different between cavin2 and 3 as candidates or just the cavins shared amongst them. The ones that are different could explain the difference in sorting mechanism potentially whereas any shared may mean the same. Need to make sure none of those candidate miRNA correlate similarly in HEK cells which is the control.

1b) get cell culture set up, extract Exosomes and maybe cell lysate if so inclined, RNA extraction, DNase treatment, cDNA conversion, qPCR.

2) Im not sure. Maybe load the Cytoscape from Bioconductor and run from there. Or Igraph. May need help for this one from Alex.

3) cell culture, co-IP, microscopy.