Results and methods from PC3/HEK293 pellet miRNA-seq analysis.

Lumi/EdgeR analysis:

Methods: Use normalized (counts per million) filtered and unfiltered data from raw counts to find significantly up and downregulated miRNAs in the PC and HEK data between cavin protein expressed and GFP (caveolin+ in PC3). Produced Dendrograms, which show the ‘distance’ between samples. Produced .txt files that list top10/20 significant miRNAs for each GFP/Cavin comparison.

Results: Unfiltered/filtered HEK: dendrogram, quite a lot of variance between samples of the same condition unfortunately. Yet, filtered of the same conditions yields slightly better similarities. This is probably due to outliers where small counts for certain miRNAs are only coming up for some conditions due to random error? Comparing filtered to unfiltered does not yield any similarities. So, from here on out, will use filtered data only.

HEK, PN4vs Cavins: no miRs were differentially expressed across all three cavins which may indicate a lack of robustness of this process. CAV1vsCavins: this shows the same thing. As Im pretty sure this involves the expression of cavins without the addition of caveolin, this may indicate that this differential activity only occurs when these proteins interact.

PC3 Unfiltered: again, high variance between samples of the same condition in the unfiltered for probably the same reason. Top 10 includes, 363-3p, 574-5p, 146a-5p, 6744-5p, 10b-3p, 1291-5p, 1268b and a-5p, 629-5p and 18a-5p between GFP-Cav1. Cav2 has a butttonne more significant values, some of which overlap with cavin1, especially 363 and 574, but also 10b, 146a and 6744. Cav3 really only shared 574 and 363 with the other two.

Filtered PC3: dendrogram is better again, where cav1 roughly group etc etc. when it comes to universally shared across the three cavins, 574 and 363 are the only significant ones, annnd 20b-5p. Several others are shared between two, but not all.

DESeq2:

Methods: requires unfiltered/filtered raw count files to assess the same data and result. Produced MA and PCA plots to assess the likeliness of the results. Produces .txt files showing the significant, either by pvalue or padjusted value above the cutoff.

Unfiltered: most of the data from unfiltered did not yield P-adj significant values. Hereby, use only the filtered data.

Filtered:

HEK: No significant miR changes observed by p-adj values for any of the condition comparisons.

PC3: CAV1vGFP: 363 and 574…… CAV2vGFP: 18 miRs, 363 and 574 overlap with all cavins…. CAV3vsGFP: share 363 and 574 with all, but share some additional ones with cavin2. Don’t know if this is anything significant.

Therefore, current hypothesis is that miR363 downregulation and miR574 upregulation appears to be exerting tumour suppressor functions. This process does not appear to be robust, but then again it could only occur when caveolin and cavins are interacting.

Pickle and Cytoscape:

Sorted the miRs into condition specific regulation and up or down regulation. Then found gene targets based on miRanda and RNAhybrid predictions. Plugging these files into cytoscape shows shared gene targets amongst miRs differentially regulation in the presence of the cavins/caveolin interaction.

So this doesn’t work with cavin 1 as it only has 1 miR up and 1 down. So, cavin2: UP: 5miRNAs in this network, 1 gene shared all 5mirs, and several share 4 connections. Should run those through GO to find similar mechanisms are shared. Cav2down: have 13miRs in this network, 1 gene shared 7 connections, two with 6 and several with 5. Cavin3UP: only two mirs so yeah. Cavin3Down: 6mirs in this one, several genes shared 6, heaps shared 5 etc.

Where to go next? Need mRNA quanitification data to see if any of these proteins are actually present in the cells. Or find associated prediction values to assess whether or not these miR interactions are likely. Then you do the qPCR to verify.

^^ all pellet. For exosome, methods are similar, however for the protein gene target prediction, youll need to consider cell localization and membrane interactions as well as you will be looking for ESCRT-like proteins.