Abstract: no more than two pages ~500words

Introduction: Brief summary of the literature leading up to rationale and statement of aims and hypotheses. ~1000w

Methods and Materials: 5-10 pages. Include description of techniques and sources of materials. Including controls, technical controls and biological controls. 2000w

## Reagents:

Trypsin-EDTA (Gibco), Roswell Park Memorial Institute (RPMI) 1640 media, Fetal Bovine Serum (FBS) (Bovogen), Phosphate Buffered Saline (PBS) (Amresco Inc), Geneticin G418 Antibiotic and Lipofectamine 2000 (Invitrogen). Rabbit anti-FUS and rabbit anti-hnRNP K (Abcam).

## Cell culture:

Previously generated PC3 cell lines, containing GFP or GFP tagged Cavin-1, were assessed through Western immunoblotting for GFP to determine stable GFP expressivity. These cell lines were cultured in 5% FBS/RPMI1640 media in a 5% CO2 incubator set to 37°C. G418 antibiotic was added to these cultured cells to select for GFP expressing cells, making a total concentration of 0.1mg/mL. Detachment of the cells during passaging was completed using 0.25% Trypsin-EDTA solution.

## Differential miRNA expression:

Describe what data youre using. Utilizing an R package, DESeq2. Normalized to a negative binomial as per the code, filtered out low/no count miRs and then applied to a function. Returned pval, as per a negative binomial Wald statistic, pval adjusted by false discovery rate. By using the log2FC values.

## Extracellular Vesicle Extraction:

Using either Ultracentrifugation or exoRNeasy kit.

## Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) and preparation:

RNA extraction methods for cellular RNA. Poly-A addition, first strand synthesis, cDNA conversion. Then RT-qPCR.

## Bioinformatics Analysis:

The data being used, make sure you reference it. Comparison between data sets using R in conjunction with biomaRt for Gene ontology assessment. Followed by PPI investigation to identify interacting proteins that integral membrane proteins.

Motif Discovery and Assessment:

A open source script

## Pull down assay:

List antibodies etc set up, controls.

## Transfection of Biotinylated miRNA:

Basically a lipofectamine process and any optimization test.

## Colocalization by Immunofluorescence Confocal Microscopy:

All the things.

Results: 10pages. Experimental data with explanations to make the data comprehendible with stats. 2000w

Discussion: 5-10pages. Interpret and critical review of the results in relation to the published body of knowledge. 2000w

# References:

Appendices: large bits of data in here with summary in result section.