Logbook

<u>For a more detailed log without dates, ask to see my project construction document.</u>

Name: Sara Waqas
Grade: 11

Project Type: Study
Project Title: Neurobiologically Informed Schizophrenia Treatment: Multi-Modal Design and In-Vitro Testing of Targeted Therapeutics

June 29th, 2025	Brainstormed some ideas for new project. Not sure if I want to continue previous project, likely will not as I don't have a lot of direction with it. Feeling very lost honestly which is not great. Have an idea about virology and schizophrenia and seeing the impact of latent viral elements.
July 3rd, 2025	Want to integrate multiple omics level this time and have a more rigorous study where I analyze multiple datasets and not just one. Another cool lab idea would be to design a project around that like induces cells and seeing if they share characteristics w people with schizophrenia — but very unlikely to get actual good results. Maybe seeing if schizophrenia patients have increased expression of latent viral elements, which ones, and then how to address it.
July 4th, 2025	Began diving into some literature - "HSV-1 a neurotropic virus infecting specific limbic brain regions is a necessary—but not sufficient—cause of psychosis in a significant proportion of schizophrenia patients." That sounds very interesting, so actually seeing which brain regions are being affected by the latent virus.
July 5th, 2025	Figuring out which brain regions these viral elements are found in - seems like limbic and prefrontal cortex are regions talked about. Also got interested in maternal virology as exposure during birth has been noted as well. Interesting study about stem cells – stem cells in the brain are kept "stem-like" by epigenetic controls, which temporarily block neuron development.

	As needed, those controls ease off, letting the cells gradually mature into neurons. This precise regulation is critical to ensure the brain gets the right number of new neurons at the right time. Stem cells in the hippocampus stay "immature" with the help of REST, a protein that silences neuron-related genes. These stem cells may carry latent herpesvirus DNA. When the cells begin to mature into neurons, REST is removed—and this not only activates normal neuronal genes, but accidentally activates the viral DNA too. This abnormal viral activity, passed from stem cells to neuron precursors, can mess with brain development. But how to treat this? Well - The only thing to do to keep the virus silent is suppress the transcription by silencing the promoter region of the viral element of interest epigenetically: keeping the activity of REST sufficiently high, as shown to be effective in viral knock-in REST gene experiments, would suffice (21). However, in patients, this pharmacological way to improve suppression by REST needs stepping out of the usual antipsychotic therapy of schizophrenia. We would need trials with drugs that support the epigenetically silencing REST complex, such as the LSD inhibitor Tranylcypromine (35). Is it a coincidence that LSD inhibitors are by another mechanism—by monoamine-oxidase inhibition—the most potent drugs to treat therapy resistant depression? So this is the current MOA treatment.
July 10th, 2025	Continued reading into literature on virology. Read the JEI for new methodologies to utilize.
July 12th, 2025	Looking into PET scans as they would be good to add that multi-modal element to the analysis because it can show me neuroinflammation and where it is occurring on a macro level.
July 13th, 2025	Brain, csf and blood are very important, “Imaging and post-mortem analyses of brains from schizophrenia patients have revealed that: (a) endothelial cells expressed less of the chemokine receptor CX3CR1 (37) and more ICAM1 (10); (b) perivascular macrophages expressed more CD163 (44); and (c) microglia expressed less CD11c (44). However, microglia (45) and CD-3 T-cells (30) were found to be more prevalent in schizophrenia post-mortem brains than in unaffected controls (Table 1C).” Can look at Allen brain atlas (should look into that and how it can be useful and use tsne for cortex, and then explore protein level interactions to see how they interact. Found this idea from JEI.

July 27th, 2025	Looked into virology in galaxy because I was roughly excited about that - looked at tools offered – https://training.galaxyproject.org/training-material/tags/virology/ https://galaxyproject.org/use/virology/ Looked at another cool paper from JEI https://www.frontiersin.org/journals/genetics/articles/10.3389/fgene.2019.00232/full Found tools and datasets for PET analysis NETPET NIH-CIDI Segmentation of PET Images based on Affinity Propagation Clustering https://nda.nih.gov/edit_collection.html?id=2249 https://nda.nih.gov/ampscz https://nda.nih.gov/nda/webinars-and-tutorials#webinars https://neurovault.org/search?q=schizophrenia#gsc.tab=0&gsc.q=schizophrenia&gsc.page=1 Search Zenodo for “schizophrenia PET”, “raclopride PET schizophrenia” or similar to find data dumps. Gained access to OASIS 3 AND 4.
July 30th, 2025	Explored ways to analyze HERVs and viral elements in rna seq data (quantify it and find them): https://pubmed.ncbi.nlm.nih.gov/36339261/ https://pmc.ncbi.nlm.nih.gov/articles/PMC4410580/
August 1st, 2025	https://pubmed.ncbi.nlm.nih.gov/27540164/ Maternal, immune, virology - got interested in this because of Myka L Este’s grad work on maternal viral elements but this would just be hard to actually study.
August 2nd, 2025	https://pmc.ncbi.nlm.nih.gov/articles/PMC5733551/ - silencing viral elements for schizophrenia using RNAi treatment could work but this is very hard to validate.

	Exploring role of HERVs in individuals with schizophrenia For example, if scRNA was from organoids, infer that clusters correspond roughly to cortical or hippocampal-like cells. Alternatively, integrate scRNA cluster signatures with spatial transcriptomic atlases (via tools like Seurat's spatial mapping or Tangram) to estimate each cluster's anatomical bias. Then correlate the cell-type HERV pattern with PET findings: if microglia-like cells show high HERV and the PET shows elevated TSPO in hippocampus, infer that hippocampal microglia may be involved. In practice, compute a matrix of (region vs. cell-type) by propagating cluster identities to regions, then highlight overlaps where both HERV expression and TSPO are high. This multi-modal link will pinpoint where (which brain region) and in which cell type HERV-driven inflammation is likely greatest. Based on the above, select specific HERV transcripts driving the inflammation (e.g. HERV-W ENV mRNA or HERV-K ENV/GAG transcripts). Antisense Oligonucleotides (ASOs) can design and test affinity and do scoring, off target screening, and efficacy prediction using ASOptimizer - (https://pubmed.ncbi.nlm.nih.gov/38706632/) Found more medical imaging repositories but not helpful. Also put all my readings here: https://pubmed.ncbi.nlm.nih.gov/21936762/ https://pmc.ncbi.nlm.nih.gov/articles/PMC2639303/ https://pmc.ncbi.nlm.nih.gov/articles/PMC4707225/ https://www.nature.com/articles/s41467-024-48153-z https://psycnet.apa.org/record/2022-07279-009 https://link.springer.com/chapter/10.1007/978-3-030-71229-7_9
August 3rd, 2025	Explored the actual design of this project and essentially decided it will be like a meta analysis of rna seq data of different brain cells: i need to do an analysis of data and find out A. if HERVs are overexpressed

	Then B. which cells are they most expressed in and C. if this has a correlation to increased neuroinflammation, deterioration of cell viability, and immune responses. If this correlation is created, then we need to see which parts of brain have this issue in immune, neuroinflammation, and glutamate using PET This is to highlight specific cell populations and brain areas most vulnerable to viral mimicry associated dysfunction to create potential downstream interventions Then I looked into the data and compiled a list of all the bioprojects I can utilize: RNA SEQ DATA SETS: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE202537 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE292323 - human cortical cells and stuff tissue https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE80655 - just postmortem tissue. THIS. can be the exploratory thing. If it is general. Omg yes. Can compare amountto what is known as normal? And then also after PET look at the places PET identified and see if there is is more expression on thosetissues? ACTUALLY - this will happen last. So PET is MACRO. Very large scale. This will allow us to find the specific tissues affected. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE87194 if we find specifically for the DLPFC. Might not mix and match however. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE112523 - dna methylation post mortem SCRNA SEQ: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE250482 - parvalbumin-positive ($PVALB^{+}$) cortical interneurons https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE275064 ipcs based dopaminergic neurons

	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE213232 microglia - perfect so many samples https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE86906 - glial progenitors https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE106589 - neural progenitors https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE144639 - forebrain neurons https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE92874 - neural progenitors https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE42546 - dentate gyrus (DG) granule cells in postmortem hippocampus https://www.ncbi.nlm.nih.gov/bioproject/PRJNA779421 - glial progenitors and astrocytes https://www.ncbi.nlm.nih.gov/bioproject/PRJNA838554 - childhood onset schizo so not applicable Transcriptional signatures of schizophrenia in hiPSC-derived NPCs and neurons are concordant with signatures from post mortem adult brains. PROTEOMICS: METABOLOMICS: I had many questions about how this would actually work because there are just so many factors, for the treatment, could I target specific cells? No, but I would need to know so I could make a good proposal + be targeting the specific family/element that is most affecting the most affected cell. Many questions were asked and many articles were read today, very exhausted and quite confused - (like wondering if these induced cells still hold these elements - literature which i will link below state yes) but i must dive even further into literature and PET so i really understand this project Literature looked into and will continue to look into: Antisense oligonucleotide approach https://pubmed.ncbi.nlm.nih.gov/40420562/

	https://pmc.ncbi.nlm.nih.gov/articles/PMC9626538/ - applied HERVS in rna seq analysis https://www.sciencedirect.com/science/article/pii/S1471491418300315 HERVS in neurology https://www.nature.com/articles/nrneurol.2017.148 Oligonucleotides — READ THESE!!! https://learn.genetics.utah.edu/content/neuroscience/braincells/ - brain cells https://www.nature.com/articles/s41467-017-02330-5 – Transcriptional signatures of schizophrenia in hiPSC-derived NPCs and neurons are concordant with signatures from post mortem adult brains https://retrovirology.biomedcentral.com/articles/10.1186/1742-4690-10-115 - so the HERV expression is retained even in the models ::) https://molecularneurodegeneration.biomedcentral.com/articles/10.1186/s13024-018-0275-3?
August 11th-20th, 2025	Looking for data - I have good fluid biomarker data for individuals with schizophrenia so very happy with that BUT I don't have PET and I likely won't find any…. I'm thinking of using spectroscopy - MRS. So my current data is MRS for individuals with schizophrenia and then ofc fMRI if I want it, then fluid biomarkers (has essentially a regular baseline lol so no need for ctrl), PET for healthy controls. I want MRS that gives me information about inflammation and use that in conjunction with fluid biomarkers to locate inflammation, with fluid biomarkers giving me more insight.
August 21st, 2025	Found enigma project (https://enigma.ini.usc.edu/about-2/funding/rvi-project/) for data and saw the MCIC project - made COINS account and requested access to NK RSI, MCIC project, and Braingluschizophrenia project (will explore others in depth) Decided I should investigate diffusion weighted mri if I want to look into degeneration and neuroinflammation and can use NODDI or NODDI adjacent tools to do so

August 24th, 2025	Continued searching for data - Healthy controls - https://www.nitrc.org/projects/biggaba/ Followed up on data request on MCIC and downloaded FT browser needed for proteome serum data.
August 25th, 2025	Revised research plan with diffusion MRI embedded: If you see ↑ FW fraction only → could be inflammation (edema), but could also be atrophy/degeneration. "Not enough to confirm, essentially exploratory. If found, then move on to:" If you see ↑ SS3T GM-like fraction in WM regions → that suggests infiltration of isotropic "cell body–like" tissue → much more consistent with glial activation / inflammation. If both FW ↑ and SS3T GM-like ↑, and they colocalize to regions implicated in schizophrenia → that's converging evidence for inflammation. THESE FW UP AND SS3T GM-LIKE UP ARE NOW THE ROIS and we look at their expression IN BULK RNA SEQ DATA TRYING TO SEE A. IF INFLAMMATORY MAKRERS ARE NCREASED B WHICH ONES ARE C IF THERE ARE INTERESTING ONES IN SPECIFIC (IE HERV HYPOTHESIS) NOW WE HAVE VERY FOCUSED ROIS WHICH HAVE THE HERV THING (THE ONES WITH THAT HERV THING ARE THE ONE WE CONTINUE WITH DOWN THIS PATH, OTHERS MAY BE INFLAMMED FOR DIFFERENT REEASONS, OR NOT SHOW TOO ELEVATED INFLAMMATION) FINALLY, SCRNA SEQ OF ALL THOSE CELLS TO SEE WHICH ONES SPECIFICALLY (but whats the benefit if microglia are basically the one ones that would be yk? Lets ask.) Even if microglia dominate, other cell types may amplify or modulate the effect, and HERV expression may preferentially appear in non-microglial cells.

September 1st, 2025	Read more on PET data and the kind of inflammation I could look at: Approximately, 95% of the annihilated photons of 511 keV undergo interaction within a 3-cm thick block of BGO detector. https://www.sciencedirect.com/science/article/abs/pii/S0001299825000686 PET imaging has also revealed neuroinflammatory signatures, such as altered TSPO binding, as well as synaptic density reductions via SV2A tracers in early psychosis, emphasizing shared neurobiological underpinnings and disease progression markers. I In general, the primary objective of molecular neuroimaging and nuclear medicine exams is to confirm or exclude the presence of a degenerative condition. When this is present, the secondary objective is to direct a syndromic and pathological diagnosis. The term has lost practical relevance due to the modern understanding that all mental disorders have a neurobiological basis, even if not consistently detectable with current methods. for example, brain changes over time in schizophrenia. It can also be used to identify which patients will respond to medications or therapies, leading to personalized treatment based on individualized neuroimaging patterns, which can be called "precision psychiatry" conditions such as schizophrenia are better explained by disruptions in cortico-subcortical circuits than by damage to discrete cortical regions. A key clinical insight is that circuit-based disorders tend to be more amenable to pharmacological intervention than neurological disorders resulting from focal lesions. So discrete regions for schizophrenia not rlly This is because circuits involve neurotransmitter pathways, receptor subtypes, and second-messenger systems that are accessible to pharmacological modulation. Many psychiatric disorders are linked to disrupted glucose metabolism, and early dysregulation can occur at the initial disease stages.7 Thus, imaging glucose uptake holds the potential for early diagnosis and describing the course of various mental illnesses. FDG-PET has revealed metabolic alterations in disorders like schizophrenia and depression

	Functional PET (fPET) with [18F]FDG is a recent advancement that tracks stimulus-driven metabolic dynamics in a single scan. By infusing the [18F]FDG radiotracer at a constant rate, changes in glucose uptake are reflected as altered slopes in time-activity curves
September 2nd, 2025	Found the tutorial for DTI https://www.youtube.com/watch?v=OmzQYJrC_Mo&list=PLIQIswOrUH6_Osd3eVqbqHLH_Ipma4jYp&index=1 - will use this and Andy's brain book
September 10th, 2025	Trying to get DTI data, but it really is hard. Here is more information on some data I can use if MCIC doesn't provide, but I really want it. https://openneuro.org/search?query=%7B%22keywords%22%3A%5B%22diffusion+weighted+mri+schizophrenia%22%2C%22diffusion+tensor+mri%22%5D%7D So it seems like MCIC hates me
September 16th-20th, 2025	Went to EUCYS to represent Canada! - Judging was incredibly helpful in refining details as I asked field experts for criticism on the idea.
September 27th, 2025	Had some down time and I am working on my INKpro PROJECT in school which will be part of this project so I asked chat gpt for some kind of pipeline I could use for my project (didn't end up using it) Step 1: Map inflammation spatially (imaging / proxies) Produce a set of priority ROIs (brain regions) that show high inflammation signal. Output: ROI masks + a ranked list of "high-inflammation" regions per subject/cohort. Step 2: Quantify HERV expression by region & cell type Use single-cell / snRNA + bulk (and spatial if available) to measure which cell type(s) and tissue regions show elevated HERV expression. Use imaging ROIs from Step 1 to choose which samples to analyze or to match regions (e.g., Brodmann area 46 or hippocampal CA1).

	Output: locus-level HERV counts per cell type × region; statistics (which loci correlate with imaging signal). Step 3: Prioritize HERV targets and test in silico Pick loci that: (A) map to the imaging-defined ROIs, (B) are expressed in the implicated cell type(s), and (C) correlate with immune modules. Design ASOs for those loci and run the downstream impact simulations / LINCS comparisons you already learned.
October 1st, 2025	Drafting up email to send to my PI about data access to see if there's a way I can get access to restricted data since MCIC doesn't want to help me. Hi Sarah, I hope your trip was great and you got some rest as well! I apologize if you mentioned it, but I would love to hear about where you were and how it went! I'm very sorry to send such a lengthy email on a Sunday evening and completely understand if you need some time to respond. In terms of updates in the lab, Maggie got to apply the updates to the website so that has been done, and I am just waiting to do some work with the data for the new scoping review project once it is ready for me. I also got to review the recorded lab meeting on updates on lab operations which was good! As for my project, I have taken this month to do quite a bit of research, thinking, and emailing and I've come up with a plan and idea that I think is the most feasible and aligned with what I want to investigate. I refined the idea with the lead scientists at Youth Science Canada as they are working closely to help prepare my project for ISEF 2026. I was very lucky to get some really enthusiastic responses from professors working with cell lines for the second phase of my project that would (supposedly) be willing to provide me the training and lab space to conduct the small pilot I want to run. I will be meeting with most next week to see if it is a good fit. I've also taken the time to apply to a few grants and will apply to more during December when the bigger youth grants open up. Now that I have that part covered, the first phase I am thinking will remain computational but the data I require live in the NIMH Data

	Archive, which as far as I know, are mostly broad source sets without a pay wall, but require the PI of a lab to request the data (so they do not grant access to individuals). I was wondering if you would be willing to submit the data requests for the datasets I need. I understand that you are incredibly busy already, so I have compiled the links to the datasets that I need access to, and can send them over if/once you are able to make an NDA account for the NIMH. This link has the exact instruction for making an account: https://nda.nih.gov/nda/access-data-info and this link has step-by-step instructions for filling out data requests after your account has been made and approved https://s3.amazonaws.com/nda.nih.gov/cms/prod/Accessing%2BShared%2BData%2BApr2024.pdf (but if you are able to make an account I will send you the exact links to the datasets I need access to so you shouldn't need this document anyways!) If you are able to help me out with accessing the data, we could definitely meet to talk about what it will be used for and how I will use the data ethically according to the NIMH's provisions. Thank you so much for all your help!
October 10th, 2025	Looked more into ASO development - So it specifically will target that specific marker and thatmeans it is supposed to be directly for that cell, but still serves benefits for other cells which is honestly better yay!!! WHY ANTI INFLAMMATORY ISNT RLLY A GOOD IDEA: where chronic or repeated immunosuppression has yielded a significantly problematic number of side effects, including deathly encephalitis This is why this precise silencing mechanism is so crucial so i can test if it is good at doing its job tho, even if its not in a specific region. then just cite research that talks about targetin gthese in a specific regionm I can only test how well it will work in knockdown not rlly if it goes in the region but there are approaches to make it target theseregions so its not like its a non specific avenue It would be better to have a wet lab aspect

	Finding funding avenues: https://ingeniousplus.ca/faqs/ https://docs.google.com/forms/d/e/1FAIpQLSfeaKPZnS8dGo1MWS73YFMhRbLhWbXPBD_YTXSQEBNBa63YDA/viewform?pli=1 Nvm. I dont feel like doing the initial stuff wet lab would be good Would love to test my ASO and its potential as well as downstream effects through rna seq analysis
October 11th, 2025	Continued looking for funding avenues for the project - many are closed now which makes it difficult
October 12th, 2025	Trying to understand why I picked a certain modality - picked DWMRI as inflammation is well documented wanted to see if it induced real change structurally how this change was rather than just yeah it's inflammation as it could be a compensatory thkng Gathering tutorials: TUTORIALS http://mig.cs.ucl.ac.uk/index.php?n=Tutorial.NODDImatlab - NODDI Actually we will do andrew jahsn connectome thing + https://www.youtube.com/watch?v=kvBUzR5bKCs&list=PLIQIswOrUH68UhRtTlsjZR472nobKPD4i&index=1 Do the connectome one first since it will familiarize u and more follow alongable Omg this killed me but okay so that's all done we will find a way to connect this to the next step which is the amp scz stuff running t tests and quantification tests of that for CRP, all these things and then the correlation islike oh we found these brain regions and this and that and there salso this increase in neuroinflammation - it is shown that persistent neuroinflammation leads to white matter integrity loss blah blah and blah so what could it be caused by? Well lets firs tsee if this is consistent histologically and if so how? Which specific neurofinalmmatory elements - post mortem tissue analysis!

October 14th-16th	Cold emailed labs to try to get wet lab access - will update with any results
October 19th, 2025	Imaging analysis: https://www.youtube.com/watch?v=A9j9gbSwU2k&list=PLIQIswOrUH68Zi9SVDAdcUExpq2i6A2eD&index=1 Will do connectome analysis from here and then the fibre using the documentation from the beginning - can actually just start at the fibre stuff as it does it all for me lol 68 patient 68 control. Will finish scrna seq analysis this month Have post mortem analysis Dwmri analysis - Connectome Fixel based Scrna seq Continuing meeting with potential mentors - get to visit the Syed lab tomorrow. Also had ISEF 2026 info meeting but unfortunately because of the government shutdown its becoming super hard to do anything
October 22nd, 2025	Had really good meeting with Dr. Syed and had some good learnings about the lab and what I need to know to succeed in general. Still having data access issues.
October 30th, 2025	Had a cool idea about inducing cells with a virus and seeing if it shares characteristics with people with schizophrenia - just hard to do for a science fair project Loveee this https://pmc.ncbi.nlm.nih.gov/articles/PMC12055031/ https://pmc.ncbi.nlm.nih.gov/articles/PMC6970301/
November 3rd, 2025	Continued gathering tutorials for the DTI analysis https://www.youtube.com/watch?v=OmzQYJrC_Mo&list=PLIQIswOrUH6_Osd3eVqbqHLH_Ipma4jYp&index=1

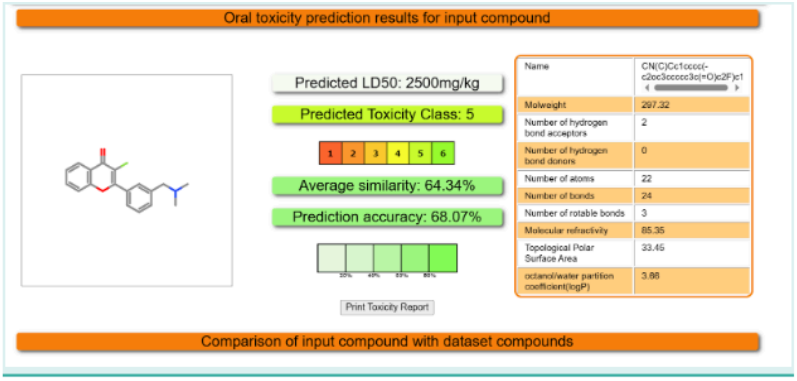
	And then https://www.youtube.com/watch?v=Xt42wDmdvKs&list=PLIQIswOrUH68Zi9SVDAdcUExpq2i6A2eD&index=8 but do fixel based https://andysbrainbook.readthedocs.io/en/latest/MRtrix/MRtrix_Course/MRtrix_11_FixelBasedAnalysis.html
November 3-9th, 2025	Working on DTI analysis and obtained external harddrive for data - using COBRE institute data as it is like MCIC and a good extensive of the last year work. Also collected data for the RNA-seq analysis. Results for DTI analysis: https://andysbrainbook.readthedocs.io/en/latest/MRIcroGL/MRIcroGL_2_dcm2nii.html Conv DICOM to NIFTI Then run all of the andys book stuff 9/14 statistically significant FDC based microstructural changes between control were related to the main regions in the fMRI analysis and 6/7 were connecting the main regions Not lat occipital cortex Anterior thalamic radiation - thalamus Retrolenticular limb – parietal and occipital cortex SLF 1 SLF I is the dorsal component and originates in the superior and medial parietal cortex, passes around the cingulate sulcus and in the superior parietal and frontal white matter, and terminates in the dorsal and medial cortex of the frontal lobe (Brodmann 6, 8, and 9) and in the supplementary motor cortex (M II).[3][1] SLF 3 SLF III is the ventral component and originates in the supramarginal gyrus (rostral portion of the inferior parietal lobe) and terminates in the ventral premotor and prefrontal cortex Short u fibre connecting inferior parietal and postcentral regions Arcuate fasciculus (posterior branch): Parietal ↔ temporal cortex (language and multimodal integration). Transcallosal fibers (via corpus callosum): Interhemispheric motor coordination. Precentral gyrus

	Uncinate fasciculus: Frontal ↔ anterior temporal/parahippocampal regions. Cingulum bundle: Parahippocampus ↔ cingulate cortex and other limbic areas. Uncinate fasciculus - amygdala Frontopontine fibers: Frontal cortex → pons Corticobulbar fibers: Motor cortex → cranial nerve nuclei in brainstem Short u fibre - insula - 1 Anterior insula ↔ middle insula and 1 Middle insula ↔ posterior insula Finding more data to help with as I got some odd fibres and want to make sure the work is grounded in the data: https://onlinelibrary.wiley.com/doi/10.1155/2020/6405930 https://www.biorxiv.org/content/10.1101/2025.06.19.660631v1.full https://pmc.ncbi.nlm.nih.gov/articles/PMC5671541/#Sec3 https://www.nature.com/articles/s41598-023-41359-z Started looking into ASO development and how I would go about it after analysis: https://synbio-tech.com/aso-synthesis-key-principles-applications https://www.ncbi.nlm.nih.gov/nuccore/X82272.1 https://rna.urmc.rochester.edu/RNAstructureWeb/Servers/AllSub/Example.php When you run a sequence in IDT OligoAnalyzer (or similar), it shows: Hairpins — intramolecular base-pairing within the same strand Self-dimers — pairing between two ASO molecules You'll see a predicted free energy (ΔG). Rules of thumb: Hairpin ΔG > –3 kcal/mol → fine (not very stable) Self-dimer ΔG > –7 kcal/mol → fine

	If ΔG is more negative (e.g., –10 kcal/mol), the self-pairing is strong → redesign that ASO (shift window or change length). 1. Avoid 5′ cap / 3′ UTR ends — Binding there often interferes less with translation; regions are usually better for knockdown. 2. Target accessible (unstructured) region — Use mRNA secondary structure predicti RNAfold) — choose loops, not stems. 3. Avoid runs of same base (e.g., AAAA, GGGG) — They make secondary structu synthesis. 4. GC ≈ 40–60 %, Tm ≈ 55–65 °C — Balances binding strength and sp 5. Avoid high self-complementarity (no strong hairpins/dimers) — Keeps ASO linear ar for binding. 6. Run BLAST (optional) — Make sure it doesn't match unintended R KEY CDS region atga acccatcaga gatgcaaaga aaagcacctc cgcggagacg 481 gagacatcgc aatcgagcac cgttgactca caagatgaac aaaatggtga cgtcagaaga 541 acagatgaag ttgccatcca ccaagaaggc agagccgcca acttgggcac aactaaagaa 601 gctgacgcag ttagctacaa aatatctaga gaacacaaag gtgacacaaa ccccagagag 661 tatgctgctt gcagccttga tgattgtatc aatggtggta agtctcccta tgcctgcagg 721 agcagctgca gctaactata cctactgggc ctatgtgcct ttcccgccct taattcgggc 781 agtcacatgg atggataatc ctacagaagt atatgttaat gatagtgtat gggtacctgg 841 ccccatagat gatcgctgcc ctgccaaacc tgaggaagaa gggatgatga taaatatttc 901 cattgggtat cattatcctc ctatttgcct agggagagca ccaggatgtt taatgcctgc 961 agtccaaaat tggttggtag aagtacctac tgtcagtccc atctgtagat tcacttatca 1021 catggtaagc gggatgtcac tcaggccacg ggtaaattat ttacaagact tttcttatca 1081 aagatcatta aaatttagac ctaaagggaa accttgcccc aaggaaattc ccaaagaatc

	1141 aaaaaataca gaagttttag tttgggaaga atgtgtggcc aatagtgcgg tgatattaca 1201 aaacaatgaa ttcggaacta ttatagattg ggcacctcga ggtcaattct accacaattg 1261 ctcaggacaa actcagtcgt gtccaagtgc acaagtgagt ccagctgttg atagcgactt 1321 aacagaaagt ttagacaaac ataagcataa aaaattgcag tctttctacc cttgggaatg 1381 gggagaaaaa ggaatctcta ccccaagacc aaaaatagta agtcctgttt ctggtcctga 1441 acatccagaa ttatggaggc ttactgtggc ctcacaccac attagaattt ggtctggaaa 1501 tcaaacttta gaaacaagag atcgtaagcc attttatact attgacctga attccagtct 1561 aacagttcct ttacaaagtt gcgtaaagcc cccttatatg ctagttgtag gaaatatagt 1621 tattaaacca gactcccaga ctataacctg tgaaaattgt agattgctta cttgcattga 1681 ttcaactttt aattggcaac accgtattct gctggtgaga gcaagagagg gcgtgtggat 1741 ccctgtgtcc atggaccgac cgtgggaggc ctcgccatcc gtccatattt tgactgaagt 1801 attaaaaggt gttttaaata gatccaaaag attcattttt actttaattg cagtgattat 1861 gggattaatt gcagtcacag ctacggctgc tgtagcagga gttgcattgc actcttctgt 1921 tcagtcagta aactttgtta atgattggca aaaaaattct acaagattgt ggaattcaca 1981 atctagtatt gatcaaaaat tggcaaatca aattaatgat cttagacaaa ctgtcatttg 2041 gatgggagac agactcatga gcttagaaca tcgtttccag ttacaatgtg actggaatac 2101 gtcagatttt tgtattacac cccaaattta taatgagtct gagcatcact gggacatggt 2161 tagacgccat ctacagggaa gagaagataa tctcacttta gacatttcca aattaaaaga 2221 acaaattttc gaagcatcaa aagcccattt aaatttggtg ccaggaactg aggcaattgc 2281 aggagttgct gatggcctcg caaatcttaa ccctgtcact tgggttaaga ccattggaag 2341 tactacgatt ataaatctca tattaatcct tgtgtgcctg ttttgtctgt tgttagtctg 2401 caggtgtacc caacagctcc gaagagacag cgaccatcga gaacgggcca tgatgacgat 2461 ggcggttttg tcgaaaagaa aaggggggaaa tgtggggaaa agcaagagag atcagattgt 2521 tactgtgtct gtgtag

November 10th, 2025	Running all RNA seq analysis - 9 additional tests spanning astrocytes, microglia, excitatory neurons and post mortem tissue (caudate, putamen, DLPFC) - takes a couple of days to finish all the aligning but aim to complete by the 16th of December so I can order my ASO. In terms of DTI analysis: Had more tracts but used other papers analyzing the data and literature to narrow list of likely disrupted tracts - this was to offset any analysis based errors Shows the functional differences are also shown structurally - this is supported widely by literature as well: https://pmc.ncbi.nlm.nih.gov/articles/PMC4207190/ https://pmc.ncbi.nlm.nih.gov/articles/PMC5148650/ https://pmc.ncbi.nlm.nih.gov/articles/PMC4707225/ Looking to revamp molecule as well: FINAL::: 3-fluoro-2-[3-methyl-4-(piperazin-2-yl)phenyl]-4H-chromen-4-one A7: Deleted hydroxide A8: Deleted hydroxide C3: Attached Fluorine C4: Attached a Piperazine Ring Need to optimize and see how i can fix - okay mostly we need to optimize for SAFETY. SWISSADME and https://tox.charite.de/protox3/index.php?site=compound_input for my drug. Trying out different alterations and how they preform in SWISSADME https://lmmd.ecust.edu.cn/admetsar2/admetopt2/ Optimizers too Seems too high in liphophicility? Investigate results given by admetai
November 11th, 2025	Looked into mechanism of ASO knockdown:

	https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0139504 Continued looking into drug design - CN(C)Cc1cccc(-c2oc3ccccc3c(=O)c2F)c1 - BEST ONE YET Scale: 25 nmol is fine. https://pfred.github.io/https://pfred.github.io/ https://pmc.ncbi.nlm.nih.gov/articles/PMC7822268/ Wowww do this for sure AND THEN ONCE DESIGN DISCOVERED: https://asoptimizer.s-core.ai/introduction https://www.cell.com/molecular-therapy-family/nucleic-acids/fulltext/S2162-2531(24)00073-8
November 12th, 2025	2-{3-[(dimethylamino)methyl]phenyl}-3-fluoro-4H-chromen-4-one We have a working prototype; can try other configurations just to make sure soon too Run this test too - wasnt able to as we have such a novel molecule https://tox.charite.de/protox3/index.php?site=compound_search_similarity  We have a working prototype; can try other configurations just to make sure soon too as neurotoxicity is weird

	Continued RNA-seq - finished 4 so far so we are on track, just 5 more with 3 being post mortem and then 1 from old project but still needs to be analyzed in the context of inflammation Used for docking: "C:\Program Files (x86)\The Scripps Research Institute\Vina\vina.exe" --receptor trkb.pdbqt --ligand liganddoit.pdbqt --config config.txt --log log.txt --out output.txt Created ASO targets: https://www.ncbi.nlm.nih.gov/nuccore/U32496.1 name parent_antisense_oligo Oligo_366 aguugguauaguuagcugc name parent_antisense_oligo Oligo_380 aggcacauaggcccaguug name parent_antisense_oligo Oligo_394 auuaagggcgggaaaggca name parent_antisense_oligo Oligo_408 augugacugcccgaauuaa name parent_antisense_oligo Oligo_422 aggauuauccauccaugug All human hits are BAC clones - no issue
November 16th, 2025	https://www.vegahub.eu/download/vega-qsar-download/ Based on research, doenst seem neurotoxic My toxicity class changes when I add hydroxyls - points to maybe using a less intense TRKB agonist W/ hydroxyl

	(2-{3-[(dimethylamino)methyl]phenyl}-3-fluoro-4-oxo-4H-chromen-7-yl)oxidanyl CN(C)CC=1C=C(C=CC1)C=1OC2=CC(=CC=C2C(C1F)=O)[O] https://cadd.nscc-tj.cn/deploy/chemmort/services/optimizer/tip.html https://academic.oup.com/bib/article/25/2/bbae008/7611273 BETTER: 2-[4-(dimethylamino)phenyl]-3-fluoro-7,8-dihydroxy-4H-chromen-4-one Test Novelty comes from: - addition of fluorine, placement of group thing May be hard to source, so what I would need to do would maybe be use something similar - EUTROPOFLAVIN VERY SIMILAR - difference of placement of group and not fluorinated however, but good comparison as showed good levels. Can dock both and see which does better as a way to compare too like optimization/similarity, etc etc CN(C1=CC=C(C=C1)C=1OC2=C(C(=CC=C2C(C1F)=O)O)O)C EUTROPOVLAVIN: CN(C)c1ccc(cc1)c2cc(=O)c3ccc(c(c3o2)O)O Docking results: C:\Users\Sara\Downloads\final>"C:\Program Files (x86)\The Scripps Research Institute\Vina\vina.exe" --receptor trkb.pdbqt --ligand updatedmol.pdbqt --config config.txt --log log.txt --out output.txt ## ####### # If you used AutoDock Vina in your work, please cite: # # # # O. Trott, A. J. Olson, # # AutoDock Vina: improving the speed and accuracy of docking # # with a new scoring function, efficient optimization and # # multithreading, Journal of Computational Chemistry 31 (2010) #

```
# 455-461                                        #
#                                                #
# DOI 10.1002/jcc.21334                          #
#                                                #
# Please see http://vina.scripps.edu for more information.      #
################################################################
#######

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 707068172
Performing search ...
0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************
done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -7.3      0.000      0.000
   2        -6.4     21.390     23.010
   3        -6.2      3.713      5.913
   4        -6.1     21.969     24.070
   5        -5.8      3.384      5.575
   6        -5.8     19.400     22.874
   7        -5.7     14.410     16.262
   8        -5.7     21.043     22.884
   9        -5.7      4.010      8.302
Writing output ... done.

C:\Users\Sara\Downloads\final>"C:\Program Files (x86)\The Scripps
Research Institute\Vina\vina.exe" --receptor trkb.pdbqt --ligand
updatedmol.pdbqt --config config.txt --log log.txt --out output.txt
################################################################
#######
# If you used AutoDock Vina in your work, please cite:          #
#                                                #
# O. Trott, A. J. Olson,                                 #
```

```
# AutoDock Vina: improving the speed and accuracy of docking    #
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
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#######

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1790714740
Performing search ...
0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************
done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -7.4      0.000      0.000
   2        -6.5     21.158     22.818
   3        -6.5      2.104      3.399
   4        -6.3     21.763     23.948
   5        -6.3      1.493      2.053
   6        -6.0      4.114      6.744
   7        -5.8     20.301     22.697
   8        -5.8     19.350     22.615
   9        -5.7      3.663      5.143
Writing output ... done.

C:\Users\Sara\Downloads\final>"C:\Program Files (x86)\The Scripps
Research Institute\Vina\vina.exe" --receptor trkb.pdbqt --ligand
updatedmol.pdbqt --config config.txt --log log.txt --out output.txt
#################################################################
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```

```
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# with a new scoring function, efficient optimization and       #
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#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################
#######

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 836799552
Performing search ...
0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************
done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -7.3      0.000      0.000
   2        -6.7      3.584      7.643
   3        -6.5     21.329     22.973
   4        -6.3      4.056      7.787
   5        -6.2      3.572      5.770
   6        -6.1     22.031     24.151
   7        -6.0     19.416     22.645
   8        -5.9      6.054      9.339
   9        -5.7     13.886     15.764
Writing output ... done.
```

```
C:\Users\Sara\Downloads\final>"C:\Program Files (x86)\The Scripps
Research Institute\Vina\vina.exe" --receptor trkb.pdbqt --ligand
updatedmol.pdbqt --config config.txt --log log.txt --out output.txt
#################################################################
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# with a new scoring function, efficient optimization and       #
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#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################
#######

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 844801068
Performing search ...
0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************
done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -7.4      0.000      0.000
   2        -6.7      3.548      7.657
   3        -6.5     21.053     22.696
   4        -6.4     21.667     23.573
   5        -6.4      4.109      7.896
   6        -6.2      3.512      7.796
   7        -6.1      3.450      5.621
   8        -5.9     19.181     22.542
```

```
   9        -5.9      4.044      8.287
Writing output ... done.

C:\Users\Sara\Downloads\final>"C:\Program Files (x86)\The Scripps
Research Institute\Vina\vina.exe" --receptor trkb.pdbqt --ligand
updatedmol.pdbqt --config config.txt --log log.txt --out output.txt
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# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################
#######

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -1997486828
Performing search ...
0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************
done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -7.5      0.000      0.000
   2        -6.6      3.550      7.661
   3        -6.4      2.058      3.295
   4        -6.4     21.142     22.814
   5        -6.4     21.861     23.683
```

```
   6        -6.4      1.661      2.079
   7        -6.3      3.900      7.832
   8        -6.0      3.374      5.472
   9        -6.0     19.393     22.506
Writing output ... done.

C:\Users\Sara\Downloads\final>"C:\Program Files (x86)\The Scripps
Research Institute\Vina\vina.exe" --receptor trkb.pdbqt --ligand
updatedmol.pdbqt --config config.txt --log log.txt --out output.txt
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#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################
#######

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1707902336
Performing search ...
0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************
done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -7.3      0.000      0.000
   2        -6.7      3.569      7.662
```

	3 -6.5 21.269 22.904 4 -6.4 1.965 2.504 5 -6.4 21.836 23.740 6 -6.4 3.848 7.848 7 -6.1 19.258 21.939 8 -5.9 19.334 22.613 9 -5.9 20.563 22.812 Writing output ... done. C:\Users\Sara\Downloads\final>"C:\Program Files (x86)\The Scripps Research Institute\Vina\vina.exe" --receptor trkb.pdbqt --ligand updatedmol.pdbqt --config config.txt --log log.txt --out output.txt ## # If you used AutoDock Vina in your work, please cite: # # # # O. Trott, A. J. Olson, # # AutoDock Vina: improving the speed and accuracy of docking # # with a new scoring function, efficient optimization and # # multithreading, Journal of Computational Chemistry 31 (2010) # # 455-461 # # # # DOI 10.1002/jcc.21334 # # # # Please see http://vina.scripps.edu for more information. # ## Settled on using Eutropoflavin since very similar and has not actually had the same testing I want to do done - also looked at in context of depression not schizophrenia.
November 28th, 2025	Worked on research plan for wet lab and proposed the following ideas: Aim 2: Use human derived stem cells to test ASO's knockdown abilities RT-qPCR on these to measure the effectiveness Grow the cells and then rip out cells and measure the DNA expression levels Cell viability test to ensure that the ASO doesn't affect the cell health Separate batch of cells Day 1: Apply ASO and ASO scramble (individually)

	Doses (for each): 1 nM 5 nM 10 nM 20 nM 30 nM Day 3 (48 hours later): Harvest batch 1 and perform RT-qPCR to quantify gene expression A separate batch treated identically will also be assessed after 48 hours for cell viability (Apply Calcein-AM and Ethidium Homodimer-1 dyes, image using fluorescent microscopy, quantify live/dead ratio using ImageJ) 1 µmol (around 0.3 mg) 5 µmol (around 1.5 mg) 10 µmol (around 3 mg) 20 µmol 50 µmol (around 15 mg) 100 µmol (around 30 mg) 150 µmol 200 µmol (around 60 mg) Control Negative control 6, +ctrl and neg ctrl = 9, DIV 0: Plate culture on chip

<table>
<tr><td></td><td>
At DIV 12, add doses of drug

DIV 14: Apply Calcein-AM and Ethidium Homodimer-1 dyes, image using fluorescent microscopy, quantify live/dead ratio using ImageJ

DIV 15: Immunocytochemistry - Primary antibodies:
Synaptophysin
PSD-95 (postsynaptic marker)
Neurofilament 160 kDa
Second batch of trays: Staining
Synaptophysin, PSD-95, and NF
<table>
<tr><td>Primary Ab</td><td>Animal</td><td>Secondary A</td></tr>
<tr><td>NF</td><td>Chicken</td><td>555</td></tr>
<tr><td>Synaptophysin</td><td>Rabbit</td><td>633</td></tr>
<tr><td>PSD-95</td><td>Mouse</td><td>488</td></tr>
</table>
Use MEAs at 24,48, and 72 hours to look at activity patterns.

Refined with Fahad and Zainab extensively.
</td></tr>
<tr><td>November 30th, 2025</td><td>
Continued working on improvements and decided to study specifically how it would work in the thalamus as I cannot get the gyrus without electrical stimulation and high probability of contamination.

Rationale for eutropoflavin:

I designed, optimized, and validated a novel candidate in silico, and tested it in vitro using the closest available analogue to model its biological effect.

Also finished RNA sequencing work and have elicited the role of HERVK - rationale is all in large document and Excel files - (available in next few pages printed). So ASO will be used to target and use the same method when creating the trial/prototype ASOs just for practice but this time the key coding region will be for HERVK.
</td></tr>
<tr><td>December 1st-16th, 2025</td><td>
Worked on ordering the ASO developed and the drug and met with Fahad to fill out ethics - had to get my IRISS account fixed as the ethics was not working well and I am not able to be added to the form. Overall a lot of logistics and planning my research plan.
</td></tr>
</table>

December 21st-24th, 2025	Continuing to apply for grants, working on collecting RNA-seq analysis data for my HERVK rationale and putting it together
December 25th, 2025	Continued working on RNA-seq analysis for HERVK rationale and finished final runs of data analysis today (had already looked through the data but not any specific and concrete analysis to provide credence to the rationale)
December 26th, 2025	Went to Dr. Syed's lab to get my cells plated beginning their development and preparing for tests to be conducted for early January (start DIV 14).
December 27th, 2025	Worked on in depth research of upregulated and downregulated genes and extracting duplicates to run further analysis to see if there are any more insights to glean.
December 28th, 2025	Finished in depth research and calculation of repeat upregulated and downregulated genes and preformed gene profiling of these repeats - TRKB was downregulated and repeatedly across experiments. Also worked on INGENIUS grant.
December 29th, 2025	Read up on qPCR experiment and how to store and resuspend oligo to ensure numbers are correct. Worked on ISEF 2026 research plan and finished it mainly (have to write up methodology for RNA seq)
December 30th, 2025	Finished INGENIOUS grant application, worked on dissolution table for both ASO and small molecule and read up more on HERVK and its mechanisms - how it induces inflammation and how it contributes dually - inflammation leads to increased HERV-K and HERV-K induces inflammation as well. Also read up on tyrosine kinase receptors that emerged in data.
December 31st, 2025	Finished drug and ASO dissolution tables and sent email to Fahad to check in (see if I need to come in for some kind of media change and when to create stock + clarification on forms).
January 1st, 2026	Completing any outstanding training needed and fixed drug and ASO dissolution (also worked on figuring out if I wanted to continue with gymnosis or lipofectamine - decided on gymnosis but then I need molecular grade water so will work on email to Zainab about it).
January 2nd, 2026	Worked on ISEF 2026 Research Plan and sent email to Zainab about water and primer for qPCR.

January 3rd, 2026	Error with drug filler impeded dissolving; scrapped original experiments to order pure compound from SCBT.
January 5th, 2026	Finished Ingenius application; submitted. Worked on revised experiments and design of experiments using cell lines instead of finicky primary culture.
January 6th-31st, 2026	Designed molecular (ASO) experiments and had numerous meetings with Frank Visser on how to best proceed with experiments - constant iteration of design and preparation of primers for this final plan: https://pmc.ncbi.nlm.nih.gov/articles/PMC9519970/ https://www.genecards.org/cgi-bin/carddisp.pl?gene=RPL36AL Standard housekeeping genes are not as stable in iPSCs and stem cell lines in general RPL36AL shows the most stability in the literature https://www.genecards.org/cgi-bin/carddisp.pl?gene=ERVK-6#expression Found to be expressed mostly in embryonic stem cell lines and malignant cancerous cell lines (melanoma, glioblastoma) Actively expressed in human iPSCs but rapidly becomes silenced and harder to quantity when these cells differentiate: (https://pubmed.ncbi.nlm.nih.gov/24156636/#:~:text=HERV%2DK(HML%2D2)%20RNA%20and%20protein%20expression%20is%20a%20marker%20for%20pluripotent%20human%20stem%20cells&text=human%20embryonic%20and%20induced%20pluripotent%20stem%20cells.) Found also to be overexpressed in cerebral spinal fluid Most healthy, somatic tissue show very little expression, and specifically this was studied in the context of liver, kidney, heart, skeletal muscle, pancreas, lung and adipose tissue https://pubmed.ncbi.nlm.nih.gov/24156636/ https://www.minipcr.com/how-to-order-pcr-primers/ RPL36AL : https://www.origene.com/catalog/gene-expression/qpcr-primer-pairs/hp200937-rpl36al-human-qpcr-primer-pair-nm-001001

Forward Sequence: ATCGGAAGCAGAGTGGCTATGG
Reverse Sequence: CAGCATCCTCTTGGATCTGCAG

Primer sequence for HERV-K (would have to custom-order from IDT, do not want to risk that for the housekeeper even though it would be cheaper so will just purchase the above):

HERV-K sequences:
https://pmc.ncbi.nlm.nih.gov/articles/PMC6421708/

HERV-K gag forwarda	AGCAGGTCAGGTGCCTGTAACATT	L
HERV-K gag reverse	TGGTGCCGTAGGATTAAGTCTCCT	L
HERV-K pol forward	TCACATGGAAACAGGCAAAA	L
HERV-K pol reverse	AGGTACATGCGTGACATCCA	L
HERV-K env forward	CTGAGGCAATTGCAGGAGTT	L
HERV-K env reverse	GCTGTCTCTTCGGAGCTGTT	L

NTRK2:

https://www.origene.com/catalog/gene-expression/qpcr-primer-pairs/hp231222-trkb-ntrk2-human-qpcr-primer-pair-nm-006180

Forward Sequence: ACAGTCAGCTCAAGCCAGACAC
Reverse Sequence: GTCCTGCTCAGGACAGAGGTTA
Day 0 - Plate cells (ASO and scramble ctrl)

3 replicates per condition, creating 24 dishes as we need 18 for qPCR (3 time points) and 6 for live/dead assay

Day 1 - Addition of ASO's in Lipofectamine

Should be dissolved in nuclease free water (pH 7.0 - use of HPLC- or molecular biology-grade water is preferable) and should centrifuge before opening

Make stock
60.5nmole - scramble
Dissolve in 605 ul

	64.1nmoles - main Dissolve in 641ul Makes 100um stock. Dose: 25nm for both https://www.researchgate.net/profile/Matthew-Adler-2/post/Do-B-lymphocytes-respond-to-foreign-DNA-during-transfections/attachment/5b6b0c273843b04aed792620/AS%3A657376248070144%401533742119579/download/FANA+-+Gymnotic+Delivery+-+in+vitro+protocol.pdf https://pmc.ncbi.nlm.nih.gov/articles/PMC2800216/ https://pubmed.ncbi.nlm.nih.gov/32865788/ Day 2 - Harvest for qPCR AND perform live-dead assay on the 6 dishes used for it Day 3 - Harvest for qPCR Day 4- Harvest for qPCR https://documents.thermofisher.com/TFS-Assets/LSG/manuals/lipofectamine3000_protocol.pdf https://www.youtube.com/watch?v=cPA2OQv8qA8 Found information online for it: “The 1:1 ratio is referring to the Opti-MEM that you will have diluted both your DNA and Lipofectamine in (separately) in the previous step/s. So for example if I was doing two transfections, both with 1 uL DNA, 25 uL Opti-MEM for each step, and one 1 transfection with 1 uL Lipofectamine and another with 2 uL then my steps would look like this. Add 1 uL DNA into 25 uL Opti-MEM (x2) Add 1 uL Lipofectamine to 25 uL Opti-MEM Add 2 uL Lipofectamine to 25 uL Opti-MEM Combine one of my DNA mixtures (1 uL DNA + 25 uL Opti-MEM) with my 1 uL Lipofectamine mixture (1 uL Lipofectamine to 25 uL Opti-MEM) and complex (total volume is now 52 uL)

	Combine my second DNA mixture (1 uL DNA + 25 uL Opti-MEM) with my 2 uL Lipofectamine mixture (2 uL Lipofectamine to 25 uL Opti-MEM) and complex (total volume is now 53 uL) Add each complexed mixture to the cells I am transfecting with it.” Materials Needed: Lipofectamine 3000 Diluted oligo solution Serum free cell medium Protocols: https://www.protocols.io/view/aso-transfection-of-ipsc-derived-cells-81wgbxbonlpk/v1 https://tools.thermofisher.com/content/sfs/manuals/lipofectamine3000_protocol.pdf https://www.protocol-online.org/biology-forums-2/posts/35487.html Day 0 1) "Seed cells to be 70–90% confluent* at transfection" You will know how fast your cells grow in your medium when passaged into a T-Flask from prior experience. If you do not have this prior experience you will need to look at published literature. You can also calculate the surface area of a 96-well, 24-well, or 6-well plate (area of a circle equation) versus the surface area of the T-flask you are using. See here for precalculated surface areas and number of cells at 100% confluency for HeLa cells: https://www.thermofisher.com/us/en/home/references/gibco-cell-culture-basics/cell-culture-protocols/cell-culture-useful-numbers.html So let's say your cells double once every 24 hours in the T-flask immediately after splitting*. The T-flask has a surface area of X, the well of the transfection plate has a surface area of 1/8th X. From a 70%-90% T-flask you would plate 1/16th of the total trypsinized volume into the well (assuming zero death from the trypsinizing and scraping, which isn't going to happen). Or ideally you'd place a known quantity of cells from a suspension culture since many fewer of them will be dead

	than after passage from a T-flask. You'd want to place enough cells to make your transfection well ~35%-45% confluent since you know that they will double to ~70%-90% confluent in 24 hours. You let them adhere to the surface for an hour or so and then swap the media with fresh media so that they will grow well (tip the well to the side and carefully pipette off the medium from the side of the well so that you don't disrupt the cells. Add fresh medium very soon after pulling the old medium off). If you do not know how fast your cell line doubles on a surface, to make everything simple I'd plate multiple wells of your cell line in a dilution series (e.g. 2x10^5 cells, 1.8x10^5, 1.6x10^5, 1.4x10^5, 1.2x10^5, 1.0x10^5, 0.8x10^5, 0.6x10^5, 0.4x10^5, 0.2x10^5 cells per well) in order to ensure that you will have at least one well around 70%-90% confluence on Day 1. I'd do this dilution series in duplicate or triplicate, and would likewise do the transfection in duplicate or triplicate, just to be safe. * - "Confluence" is how much of the surface of the well is covered with cells. See here: https://www.essenbioscience.com/media/uploads/files/HT1080_Timecourse_Plus_Images.jpg where the 6h time point is ~25% confluent, the 24h is ~50% confluent, and the 48h is >95% confluent. * - "Splitting" is when you trypsinize and scrape a T-Flask and then place a sub-portion of this into a new flask or suspension culture, or when you cell count from a suspension culture and then place a portion of that suspension culture into a T-flask or new suspension culture. Day 1 - This entire process should take 30 minutes to an hour, and will be done about 24 hours after the cells were seeded. !!!!Make sure the DNA you are transfecting is relatively free of endotoxins (by using an "endotoxin-free" DNA prep kit, or an endotoxin removal kit), and is free of other contaminants. As a precautionary measure it is a good idea to pass it through a sterile 0.22 um syringe filter into a new, sterile microcentrifuge tube, while in the laminar flow hood. The DNA concentration will stay mostly the same (a little loss), but you will lose some volume. I'd have at least 100 microliters of DNA and expect 50 - 60 microliter after syringe filtration. Make sure to "blow out" the syringe filter (unscrew the filter, pull out the plunger, rescrew the syringe filter, and plunge once more) to get as much of the DNA out as possible. Be careful that you do not push the DNA out fast enough that it ricochets out of the new tube!!!!

	"Dilute Lipofectamine™3000 Reagent in Opti-MEM™ Medium (2 tubes), mix well" Follow the instructions as indicated. You can generally use other serum-free* medium instead of Opti-MEM. The instructions are recommending that you do this in duplicate with the two indicated different concentrations of Lipofectamine 3000. "Prepare master mix of DNA by diluting DNA in Opti-MEM™ Medium, then add P3000™ Reagent, mix well" Follow the instructions. "Add Diluted DNA to each tube of Diluted Lipofectamine™ 3000 Reagent (1:1 ratio)" Follow the instructions. "Incubate - Incubate for 10–15 minutes at room temperature" Follow the instructions. "Add DNA-lipid complex to cells" Follow the instructions. * - "Serum-free" means it does not contain Fetal Bovine serum. Day 2-4 "Visualize/analyze transfected cells - Incubate cells for 2–4 days at 37°C. Then, analyze transfected cells." Follow the instructions. You'll wait 1 to 3 days after transfection before analyzing the cells. Analyze the cells whichever way you need to for whatever it is you transfected them with. If you can see successful transfection through a visual diagnostic (e.g. the cells change color) do this every days. If you cannot see this, but need to run SDS-PAGE + Coomassie stain or Western blot, I'd have multiple replicates of each transfection and harvest a replicate on each of these three days. You can resuspend* in gel loading dyesample buffer, boil, and then freeze this so that all samples can be loaded on the same gel on Day 4 or 5.

	* - To resuspend for SDS-PAGE: Remove all media from the well (slightly angle the well so the medium is on an edge. Use a pipette tip to pipette off the media without disrupting the cells. Add sample buffer and scrape the cell with the pipette tip while mixing them with the sample buffer until all have been resuspended, then pipette this into a microcentrifuge tube.
February 5th, 2026	Update on glioblastoma cells - difficult to transfect so will use the readily available HEK cells - testing viability and markers of good activity. Will also attempt preliminary qPCR work if expression can be extracted. Need to work on establishing protocol for cells.
February 6th-20th, 2026	Trouble shooted in vitro experiments and got information on how to transfect. Will use HEK cells and shadowed on work on HEK cells
February 21st, 2026	Worked on RNA isolation to see if baseline expression is present and knockdown can bemeasured
February 22nd, 2026	Worked on cDNA synthesis to see if baseline expression is present and knockdown can be measured
February 23rd, 2026	Performed qPCR and confirmed baseline HERV-K expression - will continue with validation experiments.

