1: Prkd2 inhibitor experiment

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**Overview and goals:**

The goal of this experiment is to see how two prkd2 inhibitors affect lipid uptake of zebrafish embryos from the yolk. We specifically will test how Prkd2 inhibitors affect ApoB levels, as the literature suggests that the inhibitor affects chylomicron size, which is linked to the levels of that protein. We will be using 2 drugs: CRT0066101 and CID 2011756. The first drug was used in Trujillo-Viera et al. 2021 The second we selected because it has the most specificity to Prkd2. It was generated in 2011 by Sharlow et al. We will be looking for both lower levels of ApoB using the nanoluc assay, which suggest small chylomicrons and/or inhibition of lipid uptake pathways, and a dark yolk phenotype, which would indicate defective lipid mobilization by the fish.

**Four days before Experiment(6/3):**

* Self nluc(-/-) fish. Setup 4-5 pairs.

**Three days before the experiment (6/4):**

* Collect eggs from crosses. We will have four doses for four different time points. 8 fish for each condition (maybe should do 10-16 in case eggs are bad). This means we need 132-264 fish total for each drug condition. At the end of the experiment, we should have 4-96 well plates (so about ~400 fish). Assuming 50% of the eggs die, we perhaps want 800 eggs going into the experiment.

**Day 1(6/7):**

* Prepare drug aliquots of 600uM for CID 2011756 in DMSO. 2.5 mM for CRT0066101. Prepare 2x, 1/2x, ¼x dilutions of this stock.
* Give drug to 3 DPF larva at concentrations ¼, ½, 1 and 2x IC50. Aim to have 25 mL in petri dish so would dilute MM 1000x.
* Harvest and freeze 8 larvae at 0 and 6 hrs post-treatment. Count dark-yolk phenotype when doing so.

**Day 2 (6/8):**

* Harvest and freeze 8 larvae 24hrs post-treatment. Count dark-yolk phenotype when doing so.

**Day 3 (6/9):**

* Harvest and freeze 8 larvae 24hrs post-treatment. Count dark-yolk phenotype when doing so.

**Day 4 onward**:

* Make homogenate of each sample. Nluc assay. Confirm genotype of each fish. Quantification and analysis.