Nov.1st, 2019 Xiaobo Wang

NASH mouse model based on FPC-NASH diet

1. Ordering diet from Envigo: TD.160785 (now Cat#: TD.190142). ($40/kg, 10kg is enough for 10 mice x 16 wks feeding)
2. Ordering Fructose (F2543, $177/kg) and Glucose (49159, $50/kg) from Sigma.
3. Ordering C57BL/6J mice from Jax lab (Cat#: 000664, 10-11 weeks old)
4. One week after mice arriving, changing the diet to FPC, at the same time, changing the water to sugar water (23.1g Fructose + 18.9g glucose in 1 Liter water), labeling the water bottle with "special water" tape.
5. Changing the diet and water each week, otherwise, food and water will be contaminated.
6. After 8 wks NASH diet, start the shRNA injection or durg treatment.
7. After 16 wks NASH diet, harvesting liver for analysis.

Liver harvesting:

1. Measuring body weight, then fasting the mice.
2. 5 hrs later, measuring blood glucose.
3. Euthanizing mice by isoflurane, open chest, collecting blood by preloaded 20ul Citrate-dextrose solution syringe. Centrifuging blood @ 2000g x 15min to collect plasma for ALT analysis later.
4. Taking liver down and separating the lobes, saving the biggest lobe in 10% formalin for 24hs, then sending to histology facility embedding and cutting sections. (for lipid droplet staining, one lobe should be embedded in OCT)
5. For other lobes, snap freezing in liquid nitrogen and saving in -80C. (you may cut small pieces off for RNA and protein analysis before freezing them)

Liver analysis:

1. ALT measuring according to kit instruction. (TECO, A526-120, scale down to 10ul plasma sample)
2. HE staining and Sirius red staining (Polysciences, 24901) or/and Masson trichrome staining.
3. RNA extraction and qRT-PCR for inflammatory and fibrotic genes.
4. Western blot for related gene expression.
5. F4/80 staining for macrophage.
6. aSMA staining for stellate cells.
7. Tunnel staining for cell death.