AC16 Differentiation Protocol Entered by Millie Shah
Janes Lab Protocols 11/1/17

1. Coat a 12-well culture dish with 300 µL/well of 0.02% gelatin for 1–2 hours in a humidified, CO_2-controlled 37ºC incubator.
2. Aspirate the gelatin solution and wash coated plates with 1 mL room temp PBS.
3. Plate AC16 cells at 70,000 cells per well on the precoated 12-well dish.
   - *This protocol should, in principle, scale to larger plates, but scale-up has not been tested explicitly.*
   - *It is best to maintain AC16 cells within 10 passages for formal studies.*
4. After 24 hrs, wash the plate with 1 mL of PBS per well and add 1 mL of differentiation medium per well.
   - *If the cells are grown with a selection antibiotic, refeed with differentiation medium containing the appropriate concentration of antibiotic.*
5. After 24 hrs, wash the plate with 1 mL of PBS per well and transduce cells with shSV40 neo lentivirus.
   - *Each well should get 125 µL of unconcentrated lentivirus as described in Janes_Virusprep.pdf*
   - *The final volume in each well should be 0.5 mL containing 8 µg/ml polybrene (1×).*
   - *For this step, do not add selection antibiotics.*
6. 18–21 hrs after transduction, wash the plate with 1 mL of PBS per well and refeed with 1 mL of differentiation medium.
   - *Include selection antibiotics if the cells require.*
7. After 24 hrs, wash the plate with 1 mL of PBS per well and refeed with 1 mL of differentiation medium.
8. After 24 hrs, the cells are ready for use. The differentiated cells will survive for up to 48 hrs longer.
   - *After 48 hrs, differentiated cells will gradually undergo apoptosis.*

Buffer recipes

- 0.02% (w/v) sterile gelatin
  0.02 g gelatin (Sigma #G9391)
  100 mL of ddH_2O
  autoclave on small liquid setting (120°C, 40 min)
  Store at 4°C

- Differentiation medium (make fresh before each use)
  2% Horse Serum
  1× ITS supplement (100x stock: 41400-045 Life Technologies)
  in DMEM/F12 HEPES (+ 1% pen/strep)