Transwell Invasion / Migration Assay Protocol (Everything the same for migration, just skip the Matrigel)

* For YUMMs: cells serum-starved in F12/DMEM (serum-free) (1% BSA) (0.1% DMSO) media 24h prior to seeding
* Prep upper and lower media
  + Upper media
    - F12/DMEM (serum-free) (1% BSA)
  + Lower media
    - F12/DMEM (10% FBS)
* Day of
  + Thaw Matrigel on ice prior to experiment, put tips in 4C (tips contacting Matrigel should be cool in temp), get 24 well transwell plates out, along with additional 24 well plates
  + Prep F12/DMEM + Matrigel media in conical (keep on ice) (how much you prep depends on how many plates/conditions you have, each well gets 50 uL total)
    - Media mastermix makeup per transwell:
      * 35.92 uL of F12/DMEM (serum-free) (1% BSA) + 14.08 uL Matrigel = 50 uL per well (this is 2 mg/mL Matrigel) (consider using 1 mg/mL Matrigel in a parallel experiment)
  + Add 50 uL mastermix to transwells (put in middle and slowly spiral outwards till all edges of transwell are covered)
  + Incubate plate at 37C for 2hr
  + Add 800 uL ‘Lower media’ to bottom of all wells of a separate 24 well-plate (this is the chemoattractant plate) (transwells will eventually be inserted into these), cover and place in incubator
  + Harvest cells
    - Count cells & add appropriate # to ‘Upper media’ conical 🡪 typically want ~50k cells per 250 uL media (note – if testing drugs, partition into multiple conicals and add appropriate drug conditions, along with whatever % DMSO will be vehicle, typically 0.1%)
    - Insert transwells into chemoattractant plate
    - Plate 250 uL of ‘Upper media’ w/cells into transwells for each condition
    - Do a minimum of 3 replicates per condition (the variance in results can be substantial so the more replicates the better if you can afford it $$)
    - Plates will be Incubated 37C for 5 days (Invasion) or 3 days (Migration) 🡪 If drug study, add 75 uL “Upper media” w/drugs daily
      * Note – different cell types will move at different rates, adjust days accordingly depending on cell type, pilot experiments may be necessary to gauge optimal endpoint (if any condition produces a confluent monolayer on the basolateral side of the well, the experiment has gone too long, ideally want <50% confluency for condition with the most cells)
* Day 5 (Invasion) or Day 3 (Migration)
  + Rinse transwells 2x in PBS (can just dip gently with forceps in flask of PBS)
  + Fill new 24 well plate with 500 uL MeOH, insert transwells, fix for 10 min, rock gently on orbital
  + Fill new 24 well plate with 500 uL of 0.5% crystal violet, 25% MeOH, ddH2O, insert transwells, fix for 10 min, rock gently on orbital
  + Rinse transwells 2x in PBS as before
  + Use cotton swab to remove cells & Matrigel on **TOP** of transwell only (apical side) – Don’t touch cells that made their way to the basolateral side
  + Dry overnight & image the next day (3 representative images per)
    - To image, cut the top of the transwells off at the 3 break points using pilers, turn right-side up and place on a glass slide to image
  + Quantification
    - Solubilize crystal violet w/20% acetic acid
      * Fill new 24 well plate with 800 uL ddH20 + 200 uL acetic acid, add transwells, set on orbital 10 min, pipette 150 uL from each transwell into 96 well plate, read absorbance @590 nm, plot results