FLOW SORTING PREP  
  
Prepare ahead of time:

* Large centrifuge to 4C, petri dishes for flushing
* Ice cold 5 mM EDTA (0.5M can be 100 times diluted) in PBS (around 60 mL per mouse)
* Ice cold PBS 60 mL per mouse
* 10x TryplE diluted to 4x (20 mL each mouse) in phenol red free DMEM/RPMI; pre warm in water bath
* RT DMEM complete
* FACS tubes, FACS buffer; DAPI stain viability; 70 μM filters for falcon tubes

Cell isolation

1. Sacrifice mice and isolate desired section of intestine (duodenum for tomato). Ethanol spray the belly before opening it up. Hold the middle part of the skin (between torso and belly), make incision with sharp scissors. Hold two pieces (upper and lower) and pull them open.
2. If needed, flush intestine with PBS (if it has a lot of stool in it) and cut open along longitudinal axis.
3. Rinse in ~30 mL ice cold PBS on a petri dish.
4. Get fresh petri dish plate and put rinsed intestine parts, scrape villi off by using two glass slides by holding with one and scraping with the other. Repeat several times until all cells are scraped off from intestine.
5. Pour ice cold 5 mM EDTA in PBS to a petri dish plate with cells and transfer to 50 mL Falcon tube.
6. Rock tubes at 4C (cold room rocker) for 20 minutes, shaking every 5-7 mins for ~30 seconds.
7. After incubation, shake vigorously for 30 seconds before removing intestine and spinning down the epithelial fraction in EDTA at 1400 rpm (originally 800 rpm) for 5 mins.
   1. **Note:** after this point, work in TC.
8. Decant or aspirate EDTA supernatant and resuspend cell pellet in 20 mL of pre~warmed 4x TryplE.
   1. **Note:**If there are lots of cells (or a big chunk of the intestine), digest them in 2 or more batches.
   2. **Note:** slowly add and pipette to make sure it's homogenous.
9. Place cells in 37C water bath (or warm room 37C in a shaker) to digest for 30 mins, gently shaking tubes every 10 mins.
10. Neutralize with RTP DMEM complete (equal amount to tryplE), slowly making sure the solution is homogenous. Pass cells through 70 μL filter.
11. Spin cells at 2000 rpm (originally 1200 rpm) for 5 minutes.
    1. Aspirate supernatant, add ~150 μL PBS to resuspend (or ~2 mL complete DMEM with 2 uL DAPI)
    2. Take 100 μL for antibody experiment + 100 uL PBS + 2 uL antibody if only primary is needed. Incubate for 30 minutes.
    3. Add 1 mL PBS to wash the antibody, 1200 rpm for 5 minutes spin down. Aspirate supernatant.
    4. Prepare post-FACS collection tubes (could be eppendorf tubes) with 5 mL of 50% FBS + 50% DMEM complete with Rock inh added.
12. Re-suspend cells in 1-2 mL FACS buffer with DAPI and pass through 40 μM filtered FACS tubes. Keep cells on ice until sorting.