Materials Needed:

1. General Medium: α-MEM, containing L-glutamine and nucleosides (Cellgro#MT10-022-CV or GIBCO#12571-063); supplemented with heat inactivated 10% Fetal Bovine Serum; penicillin-streptomycin at 100 U/ml-100ug/ml.

2. Recombinant Mouse Interferon-gamma (INF-γ) (Invitrogen cat#PMC4031). **Necessary for cell proliferation

3. Collagen coated plates, dishes, or flasks. Buy pre-coated or coat yourself (see below) with Rat tail type I collagen (Becton Dickson Bioscience, cat # 354236; or cat # CB-40236 if purchased through Fisher Scientific)- It is already sterile.

4. To split cells, we generally use a 0.05% Trypsin/ 0.53mM EDTA solution.

5. Freezing medium: 60% α-MEM, 30%FBS, 10%DMSO, at 1-2 x 10⁶ cells/vial/1ml

Reviving frozen IDG-SW3 cells

- Prepare Proliferation Media by adding approx. 2500U** of IFN-γ to 100ml of general media as described above. Warm the media to ~33°C before use. **INF-γ is required to induce expression of the SV40 large tumor antigen and maintain proliferation of this cell line. The accompanying Data Sheet lists a wide range of Specific Activity: 1.6 x 10⁶ to 1.0 x 10⁷ units/mg = 800-5000 U/ul. - We typically dilute 1ul of the stock IFN-γ into 100ml, so theoretically, the final concentration could range from 8-50 U/ml. Others report using 20-100U/ml.

- The INF-γ does degrade, so use media within 2wks, and limit exposure to heat.

- Thaw a vial of IDG-SW3 cells and add the cells to a 15ml Falcon tube.

- Add 5-10ml of media to the tube and mix gently.

- Centrifuge the tube at 500 x g for 5 minutes.

- Remove the media and gently re-suspend the cell pellet in proliferation media.

- Plate the cell suspension into a rat tail type I collagen-coated cell culture dish.
• Gently rock the culture dish to evenly distribute the cells.

• Incubate the cells at 33°C with 5% CO₂ and maintain at sub-confluence.

• The media should be replaced every 2-3 days. The cells will initially grow slowly, but will begin to rapidly proliferate 2-3 days after revival.

• Note: you can monitor expression of the SV40 large T antigen via western blot analysis using Santa Cruz cat# sc-58665 SV40 T Ag (Pab 419), mouse monoclonal IgG₂a ,200 µg/ml

• It is best to keep your stock dish subconfluent ~80-85%.

• You can split at ~1:6 to ~1:15, depending if you need the cells in ~2 to 3 days, respectively.

Differentiation of IDG-SW3 cell line

• Plate the cells at a density of 4x10⁴ cells/cm², in proliferation media, so they will be confluent in ~2 days. Incubate at 33°C. Using vastly different densities may affect cell differentiation, so try to be consistent.

• To induce differentiation of the cell line, the cells must be cultured in media WITHOUT the INF-γ and incubated at 37°C. Differentiation is induced by adding 50µg/ml Ascorbic Acid and 4mM β-glycerophosphate to the general media, and adding it to the cultures at confluence. We call this Day 0.

• The differentiation media should be replaced every 2-3 days.

• Culturing the cells on collagen-coated plates is important for maintaining the osteocyte phenotype.

• Differentiation of the cell line may be assessed by examining the cultures via fluorescence microscopy. The cells express GFP under control of the Dmp1 promoter. GFP expression is usually initially observed at 3-4 days of culture in osteogenic conditions and should be strong by day 10-14 of culture.

• We typically harvest our time course studies at ~28-30days. Since there can be laboratory variability, especially with the serum, you may need to optimize conditions for your lab.
Collagen Coating of plates-to be done in a sterile Tissue Culture hood!

- Dilute the sterile collagen in previously filter sterilized 0.02 M Acetic acid to a final concentration of 0.15 mg/ml. Use a chilled pipet so the collagen does not stick.

- This solution can be reused approx. 6 times and should be kept in the refrigerator when not in use. I generally use 8 or 14ml for coating a 100mm or 150mm dish.

- After coating plates for 1 hour at room temperature, tilt the plate for several minutes to remove excess collagen and save.

- To use immediately, it is best to rinse the plate with PBS to remove residual acid.

- Prior to storage at 4 °C, make sure to completely dry the plates (~1 hour) with the lid off.

Preparation of INF-γ:

Our INF-γ comes at a concentration of [0.5mg/ml] = [0.0005mg/ul], and we dilute 1ul in 100ml media.

If the specific activity is reported to be in the following range:

a. \((1.6 \times 10^6 \text{ U/mg}) \times [0.0005\text{mg/ul}] = 800 \text{ U/ul}\)

b. \((1.0 \times 10^7 \text{ U/mg}) \times [0.0005\text{mg/ul}] = 5000 \text{ U/ul}\)

- We decided to assume it is somewhere in the middle, so approximately 2500 U/ul

Here is some info on how specific activity is determine.


Ascorbic acid (Sigma A-4544)- Make a [10mg/ml] stock solution in media, no serum, and syringe filter sterilize (2um). Make small aliquots and freeze at -20C, shielded from light. Once an aliquot is thawed & used, we usually discard the rest.

β-Glycerophosphate (Sigma G-9422)- Make a [400mM] stock solution, and follow as above. However, once thawed, you can keep the remainder at 4C, and use for the week.

Typically, on the day of feeding, we add the Ascorbic acid & β-GP to the media that day.