**In Silico Wet Work**

**2D Culture**

**TGFβ1 Treatment**

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| **Fibroblast Lines** | | |
| **HDFn #1**  **A** | **HDFn #2**  **B** | **HDFn #3**  **C** |
| * Life Technologies * Cat no: C-004-5C * Lot no: #1366434 * Neonatal * Male * Caucasian | * Life Technologies * Cat no: C-004-5C * Lot no: #1366356 * Neonatal * Male * Caucasian | * Life Technologies * Cat no: C-004-5C * Lot no: #1206197 * Neonatal * Male * Caucasian |

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| **Fibroblast Lines** | | |
| **SEN #1**  **D** | **SEN #2**  **E** | **SEN #3**  **F** |
| * Life Technologies * Cat no: C-004-5C * Lot no: #1366434 * Neonatal * Male * Caucasian * **Irradiate with 20Gy, culture for 10 days before use.** | * Life Technologies * Cat no: C-004-5C * Lot no: #1366356 * Neonatal * Male * Caucasian * **Irradiate with 20Gy, culture for 10 days before use.** | * Life Technologies * Cat no: C-004-5C * Lot no: #1206197 * Neonatal * Male * Caucasian * **Irradiate with 20Gy, culture for 10 days before use.** |

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| **Fibroblast Lines** | | |
| **HDFa #1**  **G** | **HDFa #2**  **H** | **HDFa #3**  **I** |
| * Life Technologies * Cat no: C-013-5C * Lot no: #1528526 * 55 years * Female | * Life Technologies * Cat no: A11634 * Lot no: # 1090465 * 60 years * Male * Caucasian | * Life Technologies * Cat no A11634 * Lot no: # 200910-901 * 65 years * Female |

*\*All cells cultured in M106 + LSGS*

TGFWAF4

**Cells:** HDF, passage 5. Use 3x HDFn lots (A-C) 3x irradiated HDFn lots (D-F), 3x adult lots (G-I)

**Timecourse:** 0, 0.5, 1, 2, 3, 4, 8, 12, 24, 48, 72, 96 hours

**Technical repeats:** n=6

**Sample size:** 12 well plate

**Total wells required:** 144

**Total plates required:** 24

**Seeding density:** 10,000/cm2 (65,000/cm2 senescent cells)

**Cells per well:** 39,000 cells (253,500 senescent cells)

* **Cell Plating:** Seed at a density of **10,000 cells/cm2** in 3.5ml full M106 medium. Culture at 37°C, 5% CO2 for 4 days (cells are approx. 95% confluent).
  + For senescent cells seed at **65,000 cells/cm2** to achieve similar starting density.
* **Serum Starvation:** Remove full M106 media, wash cells 2 x with PBS, add 3.5ml serum free M106. Culture at 37°C, 5% CO2 for 24h.
* **Baseline:** t=0, after 24h serum starvation, prior to TGFb1 treatment baseline samples collected.
  + **1x 12 well plate, 6 x wells**
* **Final:** t=96h, keep in serum free media, no TGFb1 stimulation.
  + **1x 12 well plate, 6 x wells**
* **Treatment:** TGFb1 reconstituted in0.1% BSA/10mM citric acid, stock concentration 10ng/ul. After 24h serum starvation add 0.5ml serum free M106 containing 40ng/ml TGFb1.
  + **12x 12 well plates, 6 x wells each**
  + **1 x plate = 0hr no treatment**
  + **Need 35ml 40ng/ml TGFb1 in M106 (35ml + 140ul TGF)**
* **Control:** Media containing0.1% BSA/10mM citric acid for each time point.
  + **12x 12 well plates, 6 x wells each**
  + **1 x plate = 0hr no treatment**
  + **Need 35ml vehicle in M106 (35ml + 140ul citric acid + BSA)**
* **Harvest:** Aspirate media, wash with PBS, lyse in 350ul RLT buffer, snap freeze and store -80°C.