Human Derma Fibroblast Scratch Assay

Day 1, Cell plating:

Complete medium:
DMEM supplimented with 10% FBS, 100 unit/ml penicillin, 100 ug/ml streptomycin, 1mM sodium pyruvate
Trypsin/EDTA (aliquot stored in -20 freezer)
Typan blue
ImageLock 96 well plate (EssenBio, 4739, Lot#17040501)

- → Wash the cells with 25 ml PBS
- → Detach the cells with trypsin (3.5 ml/T175 flask)
- → When cells have detached, add 7 ml complete medium, pipette up and down to disrupt cells, pool 2 flasks together, in total 20 ml
- → Count cells with cell count chamber at 40x
- → Spin down at 400 g for 10 min
- → Remove the medium, resuspend at 25000/ml with fresh complete medium, seed in ImageLock plate, 100 ul/well
- → Plate leftover cells in T25 flask, to use as standard for CellTitreGlo at day 4

Day 2, Wound scratch and addition of compounds:

Wound maker (IncuCyte S/N Nr.0666)
Cytotox Green (EssenBio, Cat# 4633, stored -20 freezer)
Nuclear Rapid Red(EssenBio, Cat# 4717, stored in fridge)

Dilute the probes to 10x final concentration in PBS
Prepare the wound maker following instructions from the manufacturer

- → Scratch the 1st plate seeded the day before using the Wound maker
- → Place the wound maker in 2nd boat container with fresh sterile MQ water
- → Remove the medium from the scratched cells carefully and wash with 200 ul PBS
- → Remove the PBS from cell plate, add 180 ul/well fresh complete medium with cytotox green and nuclear rapid red
- → Add 20 ul/well diluted compounds
- → Mix gently, put the plate back to incubator
- → Continue with plate 2 to 6, one plate at a time
- → Set up scan on IncuCyte every 2-3 hours

Day 4, End IncuCyte and collecting supnatant for ELISA:
CellTiterGlo (Premega, Cat# G7571, stored -20 freezer)
ATP (Sigma Aldrich, Cat# A7699-1G, stored -20 freezer)

Take out CellTiterGlo regents from freezer, let the buffer thaw at RT
Prepare the reagents by adding 10 ml buffer to the substrate bottle, and mix well

- → End the IncuCyte scan, analyze the data and export different read out, the relative wound density, cytotox green count, nuclear rapid red count in wound mask and total nuclear rapid red count was used to calculate the area under curve.

- → Centrifuge the plate at 500 g for 8 min
- → Take 135 ul/well supernatant (carefully, without touching the cells), store in -20 freezer for ELISAs
- → Prepare ATP standard curve from 10 uM to 10 nM
- → Prepare cell standard curve from 20 000 cells/well to 100 cells/well.
- → Add 50 ul/well CellTiterGlo reagent to the standards well
- → Add 50 ul/well CellTiterGlo reagent into the cells plate, gently shake the plate, put at RT for 10-15 min
- → Transfer the whole cell lysate to the opaque plates
- → Measure luminescence on GloMax®-Multi Detection System

ELISA for IL-6/ProCOL1A:

Human IL-6 ELISA development kit (HRP) (Mabtech, Cat#3460-1H-20)
COL1A ELISA (R&D, Cat#DY6220-05)
TMB substrates (Sigma Aldrich, T0440-1L)
Sulfate Acid (MERCK, 100731)

ELISA was run according to instruction from the manufacture