3D MATRIGEL CULTURE PROTOCOL
based on McQualter et al. 2010 and Teisanu et al. 2010
revised by Joo-Hyeon Lee 1/2013

3D Media:(For 50 mL)
F12: 44 mL
Insulin/Transferrin/Selenium (Gibco,Cat# 41400-045): 500 uL
FBS 10%: 5 mL
P/S: 250 uL
L-glutamine: 250 uL
1 mM HEPES: 50 uL of the 1 M

**Plate stromal cells on 10 cm plates 2-4 days prior to planned FACS sorting date: MECs take about 3 days to reach confluence (70-90%). Thaw MECs or MLgs with MEC/MLg media in gelatinized plate (use passage number 2-8 of MECs). Make sure stromal cells are growing well. Critical step.

*Remember to thaw the growth factor reduced matrigel on ice 2-3 hours prior to plating!

1. Isolate BASCs (CD31-45-EpCAM+Sca1+) and AT2 cells (CD31-45-EpCAM+Sca1-) by Kim Lab general protocol.
2. During the sorting cells, prepare stromal cells for co-culture: Wash with PBS-> trypsinize well into single cells-> Wash with PBS once-> count the cells and take the cells as needed-> centrifuge and aspirate off the supernatant-> Resuspend with growth factor reduced matrigel (GFR matrigel): MECs at $1x10^6$ cells/ml GFR matrigel, MLg cells at $2x10^6$ cells/ml GFP matrigel:-> keep it on ice until ready
3. Centrifuge sorted BASCs and AT2 cells (10 sec) and aspirate off supernatant.
4. Resuspend cells well with cold 3D media at 2-3K cells/50ul 3D media
Optional: Aliquote it into e-tube as 50ul cells in one tube (keep on ice) * then you can get more consistent result compared to combine larger amount of volume of cells with stromal/matrigel
5. Mix stromal/matrigel well again and take 50ul stromal cells/GFR matrigel and put into e-tube that has 50ul BASCs/AT2 cells. Mix gentle, be careful not to make bubbles, plate on top of transwell insert (0.4um pore size).
6. Incubate in 37C incubator for 30 mins to set matrigel
7. Add 410 uL 3D media to bottom of well
8. Replace medium every other day