**Lentiviral Infection of Organoids**

Cultured organoids were transferred in suspension (in 50% L-WRN conditioned media) into a 15 ml tube, centrifuged at 200 x g for 5 min, resuspended in 200 μl trypsin-EDTA, and incubated at 37°C for 5 min. Washing media (1 ml) was added and the organoids were dissociated by vigorous pipetting.

Organoids were centrifuged again at 200 x g for 5 min and then the cells were resuspended in 250 μl solution containing lentivirus, 8 μg/ml polybrene and 10 μM Y27632 (R&D). Each suspension with a single lentivirus was then transferred to a single well of a 48 well plate, which was sealed with Parafilm (Bemis Co.) and centrifuged at 600 x g at 32°C for 1 h.

Plates were then incubated at 37°C for 6 h to allow transduction.

Cells were resuspended in conditioned organoid media (1 ml per well) and transferred to a 1.5 ml tube for centrifugation at 200 x g for 5 min.

Cells were resuspended in 20 μl Matrigel and cultured via our organoid culture method as listed above, except that the medium was supplemented with the appropriate antibiotics (e.g., puromycin) for 7 days to deplete non-transduced organoid cells.

**Human organoid version (From PB, Sep 11, 2023)**

1. **Do all this in lenti-room!!!**
2. AK and AKS human organoids in falcon, seed on 24-well NUNC plate
3. Choose 1 NTC and 2 shRNA
4. Add 500ml virus + 0.8 ul Polybrene + 1 ul Rocki (prevent cell death) (1 ml in total)
5. Centrifuge 600g/1hr in 32 degree, tape on plate
6. Put to 37 degree/CO2 for 4 hrs
7. Collect in falcons, 6 tubes
8. Spin down 900 g/5 min
9. Remove supernatant
10. Plate the organoids + Matrigel
11. 100 ml / 6 well
12. ERN media (not WRN)
13. Culture for 2 days
14. Puro selection, add normal organoids, select until they all NA die