1. Add 80 μl of cold liquid Matrigel into each well and incubate at 37 °C for 20 min to solidify Matrigel. We initially rinse the surface of each well with cold PBS to spread liquid Matrigel more evenly before its polymerization.
2. Combine 250 μl of a single-cell suspension in 2× organoid culture (with ROCKi) and 250 μl of viral particles in a 1.5 ml Eppendorf tube. Add Polybrene and mix well by tapping. Pre-mixing of cells and viruses before plating is critical because we observed lower infection efficiency when these two components were separately placed on polymerized Matrigel.
3. Plate 500 μl of mixture of viral particles and single cells on solidified Matrigel. Incubate overnight at 37 °C.
4. Next day (~16 h later), remove the medium with dead cells by aspiration. Overlay 60 μl of ice- cold Matrigel to cover the cells attached to Matrigel at the bottom. Incubate at 37 °C for 20 min to solidify Matrigel.
5. Resume 3D culture by overlaying 800 μl of 1× organoid culture media.

