Fix cells on coverslips:

1. Aspirate media and wash once with PBS
2. Add RT 4% paraformaldehyde in PBS for 10 minutes
3. Wash once with PBS
4. Add ice cold 0.5% TritonX-100 in PBS plus 1:200 VRC complex from NEB and let cells permeabilize on ice for 10 minutes
5. Wash with 70% EtOH
6. Store cells in 70% EtOH at -20 C indefinitely

RNA FISH only

1. Dehydrate in EtOH series, 3 min each: 75/85/95/100 % EtOH,
2. Air dry 5 min
3. FISH with 7ul of probe pair overnight in 50% formamide/2x SSC humidified chamber, 37 C
4. Wash @ 42 3x 5 min 50% formamide/2x SSC
5. Wash @50 3x 5 min in 1x SSC
6. Fix to slide with Prolong Gold DAPI antifade, let dry O/N, sop up extra with carefully placed kim wipe

coRNA FISH then IF

Previously fix and permeabilized TSCs, store @ -20C.

1. 1 hour block + 1:100 RNAsin
2. Primary antibody 1:400 H3K27me3 Cell signalling rabbit 1hour + 1:100 RNAsin
3. Wash in PBS 0.2% Triton X-100 + 1:100 RNAsin
4. Secondary antibody 1:400 goat anti-rabbit 488 (D5) in block 30 min @ RT + 1:100 RNAsin
5. Wash like step 3
6. Fix in 2% PFA for 3 minutes @ RT
7. Wash in 2x SSC (1 quick wash, 1 5 min wash)
8. Hyb O/N at 37
9. Wash @ 37 or 42:
   1. 3x 5 min 50% formamide/2x SSC
   2. 3x 5 min 2x SSC
   3. 2x 5 min 1x SSC
10. Fix to slide with Prolong Gold DAPI antifade, let dry O/N, sop up extra with carefully placed kim wipe

Block:

6mg/ml IgG-free BSA

1% donkey/goat serum

0.2% triton-X100

PBS