APB staining protocol (AG Rippe)

- Seed cells on cover slips and let cells attach for at least 5 h
- Wash cells once in 1X PBS
- Fix cells for 12 min in 4% PFA in PBS (under hood!)
- Wash cells 3x 5 min in PBS
- Permeabilize for 10 min in cold 0.2% Triton-X/PBS
- Wash cells in 3x 5 min in PBS
- Optional: Incubate cells in 100 µg/ml RNase A in 2X SSC at 37°C for 1h
- Wash cells in PBS
- Dehydrate slides: 2 min each in 70% -> 85% -> 100% EtOH
- Hybridize with telomeric PNA probe:
  Prepare PNA probe TelC or TelG Cy3 probe (100 µM) 1:1000 in hybridization buffer (75% formamide, 0.1% BSA, 20 mM NaCl, 20 mM Tris)
  Put appropriate volume of hybridization mix on a microscopy slide and add coverslip (cell side facing down) on top
- Seal cover slip with fixogum and let fixogum dry in dark for a few minutes
- Denature for 3 min at 80 °C
- Hybridize slides in wet chamber for O/N at 37 °C

Next day:

- 2x 15 min in 70% formamide in 10 mM Tris pH 7.4
- 1 min in 2X SSC
- 5 min at 55 °C in 0.1X SSC
- 2x 5 min in 2X SSC with 0.05% Tween-20
- 2x 5 min in PBS
- Block with 10% goat serum (GS) in PBS for >15 min in wet chamber
- Incubate with anti-PML mouse antibody (Santa Cruz sc-966, 1:100 in 10% GS) at RT for 1 h in wet chamber
- Wash cells 3x 5 min in 0.002% NP40 in PBS
- Incubate with anti-rabbit Alexa488 antibody (1:300 in 10% GS) at RT for 30 min in wet chamber
- Wash cells 3x 5 min in PBS
- Shortly wash with H2O
- Dehydrate slides for 1 min in 100% EtOH and air dry slides shortly
- mount slides with Prolong + DAPI