

Cows were milked before biopsy and sampled within 5 hours of milking. Biopsy site was clipped and given aseptic skin preparation (povidone iodine base scrub, and iodine tincture). Local anesthetic was injected subcutaneously (approximately 4ml per site). Core biopsies were taken using a powered sampling cannula of a larger bore (4.5mm internal diameter) inserted into a 2cm incision, collecting a core sample (70 x 4mm diameter) of mammary gland parenchyma.

Tissue was minced to a fine slurry in freshly prepared Collagenase solution (0.2% Collagenase A (Roche), 0.05% Trypsin (1:250 powder, 100U/ ml Gibco/life sciences), Hyaluronidase (Sigma, St Louis MO), 5% Fetal Calf serum, Pen/Strep/Fung (hyclone) or 5µg/ml Gentamycin (Sigma), in DMEM/F12 (Gibco/Life technologies) 10 ng/ml Insulin). Minced tissue was incubated in Collagenase solution (10 ml solution/g tissue) for 3.5 hours at 37C in 50 ml conical tube with slow shaking (120 rpm). Digested tissue was centrifuged for 10 minutes at 453xg at 4°C, supernatant and fat layer discarded, pellet was gently resuspended by pipetting in 5 ml DMEM/F12 without serum. A further 5 ml DMEM/F12 without serum was added and resuspended sample was centrifuged for 10 minutes at 453xg at 4°C, media was discarded and pellet resuspended gently in 10 ml DMEM/F12 and centrifuged for 10 minutes at 453xg at 4°C . Pellet was resuspend gently in 10 ml followed by brief pulls spins at 453xg (centrifuge set to 1 minute @ 453 x g, hit the brakes 2 seconds after 453 xg is reached). Pulls spins were repeated at least 4 time or until very few single cells and mainly epithelial organoid clusters were observed under a microscope. Organoid pellet for RNA-analysis was resuspended in 1 ml TRIzol and stored at -80C.