Figure S1: Bovine tracheal epithelial organoids differentiation in **(A)** BME2 matrix-embedded, and **(B)** ALI cultures. Differentiation is characterized by immunofluorescent confocal microscopy imaging for α-tubulin (AcTub, green), DAPI (nuclei, blue) and F-actin (phalloidin, grey-white), or control antibody (Control Ab). Scale bar is 50μm.
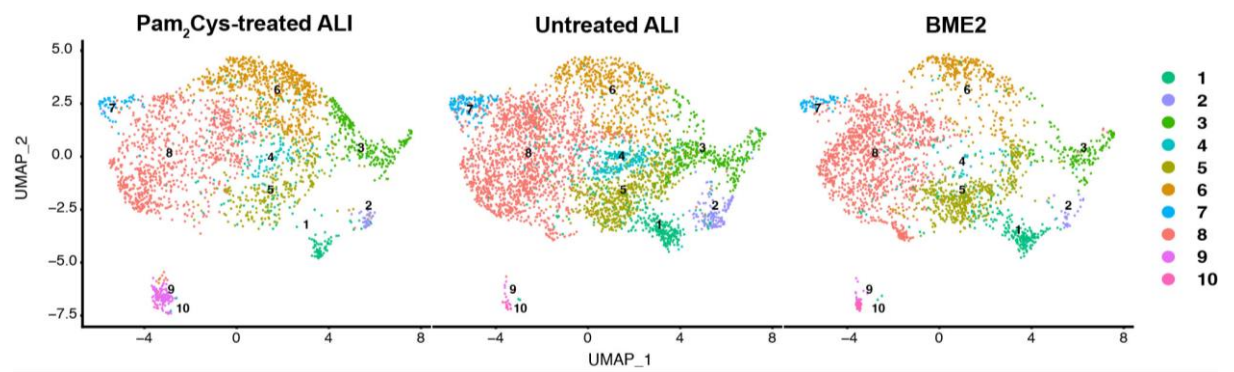
Figure S2: Single-cell transcriptomic analysis identifying bovine organoid cell types in Pam$_2$Cys treated ALI organoid, untreated ALI organoid and BME2 organoid. UMAP plot shows clustering of cell type classification as determined by Seurat analysis (Table 2).
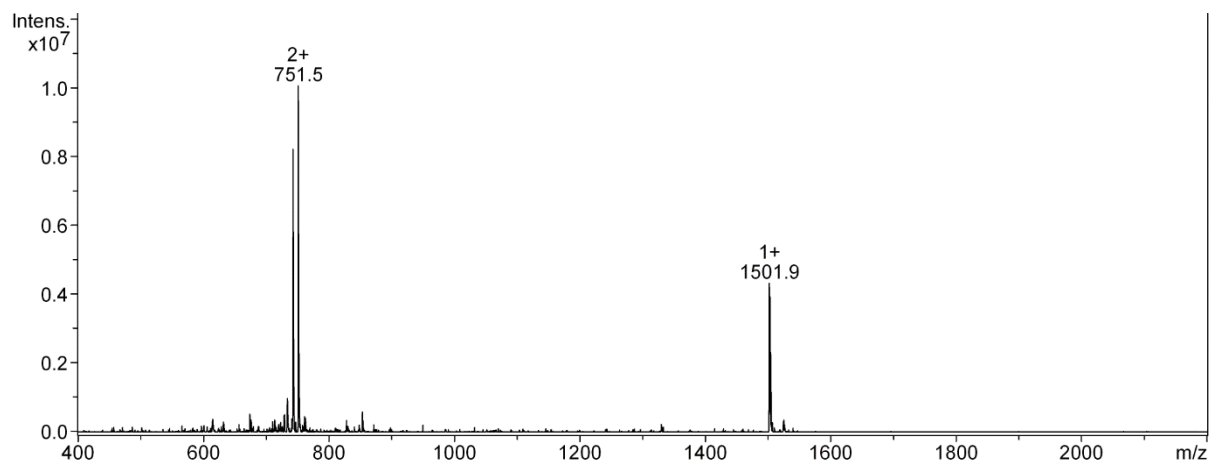
Figure S3: Mass spectrometry validation of Pam2Cys. Agilent 1100 series capillary LC system in-line with an Agilent 1100 series LC/MSD ion-trap mass spectrometer (Santa Clara, CA, USA) was used. The mass spectrometer was operated with electrospray ionisation configured in the positive ion mode. Data analysis software from Agilent Technologies was used to de-convolute the charged ion series for identification of the protein. Mass calculated: 1501.1Da and Found 1501.9 Da (M+1).
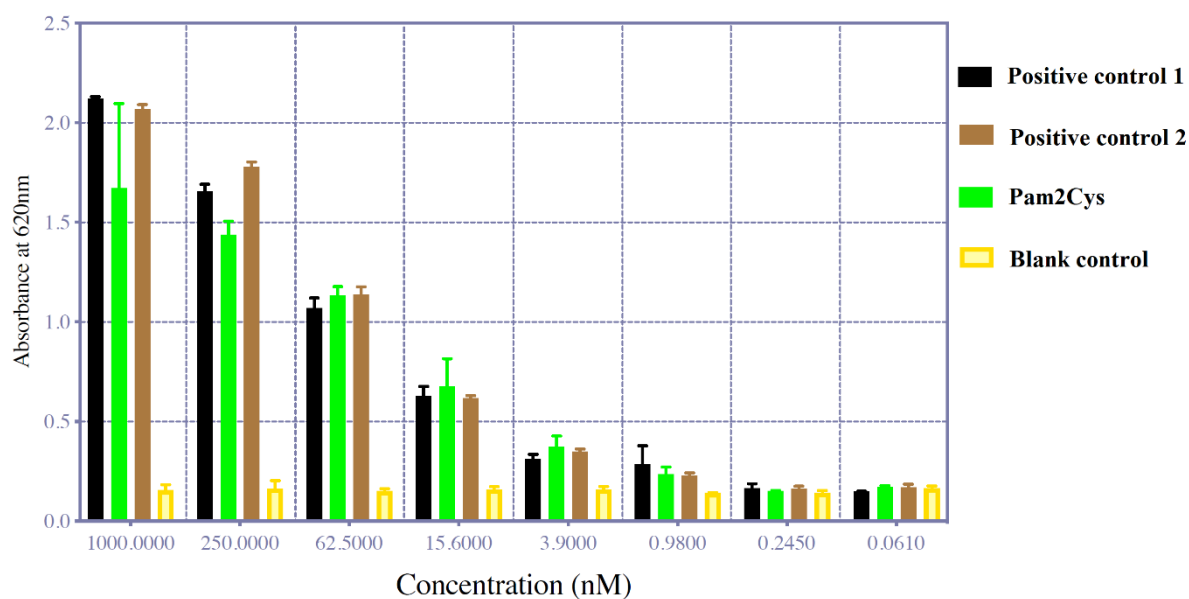
Figure S4: HEK-Blue™ hTLR2 assay of investigated Pam₂Cys compound at serial dilution concentrations for stimulatory effect on TLR2 signalling. HEK-Blue™ hTLR2 cells which have been stably transfected with human TLR2 and express TLR1 and TLR6 endogenously, were purchased from InvivoGen (San Diego, CA, USA). The cells were cultured, passaged and then seeded at a density of 2.8×10^5 cells per well. and Pam₂Cys and the positive controls' activities on TLR2 signaling were then evaluated at eight different concentrations as shown in the X axis and performed in triplicate as per the manufacturer's instructions.