

Supplementary Figure 1. Fluorescence immunohistochemistry of P2X7 receptor (green) in porcine bladder tissue with (A) $10 \times$ and (B) $40 \times$ magnification. Urothelium (U), suburothelium (SU) were labelled and DAPI (blue) is the nuclear marker. P2X7R antibody (1:100 dilution ab93354, Abcam).



Supplementary Figure 2. Western blot and densitometry analysis of P2X7 receptor protein expression in the mucosal layer of porcine bladder collected from *ex-vivo* experiments. A). A single band for P2X7 receptor (~68 kDa) and β -actin (~42 kDa) corresponding to their expected molecular weight (MW) was observed. B). P2X7 receptor expression levels in different treatment groups are expressed as a percentage of β -actin, a loading control. Densitometry analysis was performed using ImageJ. One way ANOVA analysis between fresh control, perfusion control, acrolein (0.05%), acrolein (0.05%) + A804598 (10 μ M) and A804598 (10 μ M) was performed and there was no significant difference among groups. Data are expressed as mean \pm SD (n = 6 - 9).



Supplementary Figure 3. ATP release in samples collected from the lumen of porcine bladders from *ex-vivo* experiments after 4 hours perfusion. ATP concentration in the perfusion media was measured using the ATP Bioluminescence Assay Kit (FLAA, Sigma-Aldrich) There was no significant difference between treatment groups. Data were expressed as scatter dot plot with the bar indicating the median (n = 8 -11, one-way ANOVA).