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Identification of TLR2 Signalling Mechanisms Which Contribute to Barrett's and Oesophageal Adenocarcinoma Disease Progression

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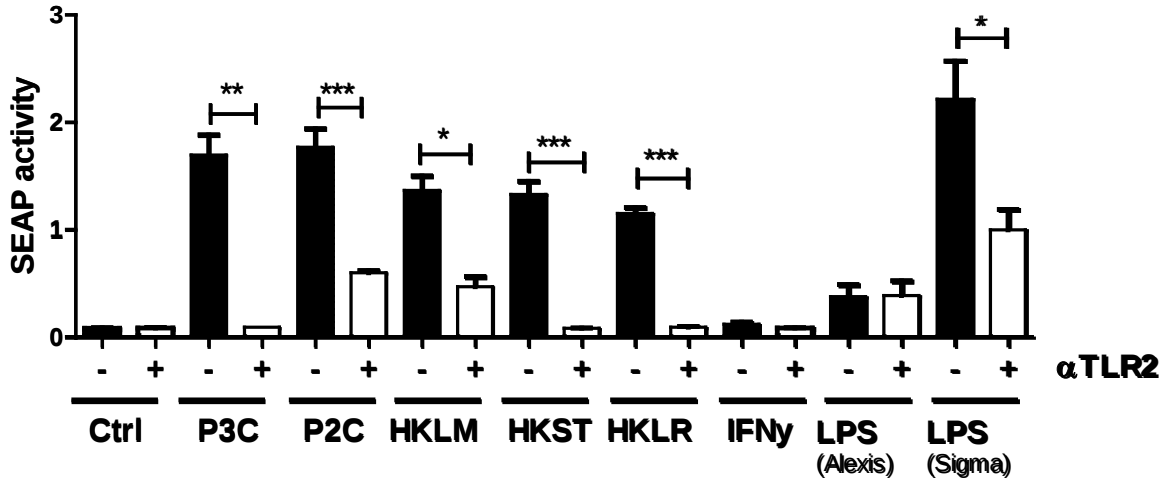

Figure S1. Direct TLR2 stimulation of the TLR reporter cell line. THP1-XBlue-CD14, is inhibited by the neutralising antibody, αTLR2. THP1-XBlue-CD14 were left untreated or pre-treated for 1 h with αTLR2 (10 μg/ml) prior to stimulation with P3C (0.05 μg/ml, Invivogen, tlrl-pms); P2C (0.05 μg/ml, Invivogen, tlrl-pm2s-1); heat-killed bacteria: *Listeria monocytogenes* (HKLM, 10^7 cells/ml, Invivogen, tlrl-hklm); *Salmonella typhimurium* (HKST, 10^6 cells/ml, Invivogen, tlrl-hkst2); *Lactobacillus rhamnosus* (HKLR, 10^7 cells/ml, Invivogen, tlrl-hklr); IFNγ (40 ng/ml, PeproTech, 300-02); LPS from *Escherichia coli*, Serotype R515 (Alexis, TLRGRADE, 1 μg/ml, ALX-581-007-L001) or LPS from *Escherichia coli* O111:B4 (Sigma, 1 μg/ml, L4391) for 24 h. TLR stimulation was assessed by measuring supernatant levels of SEAP using QUANTI-Blue reagent. Values represent the mean ± SEM of three independent experiments. Statistical analysis was performed using unpaired 2-tailed Student t-test to compare mean values between αTLR2 treated and untreated cells, *$p < 0.05$; ** $p < 0.01$; ***$p < 0.001$.

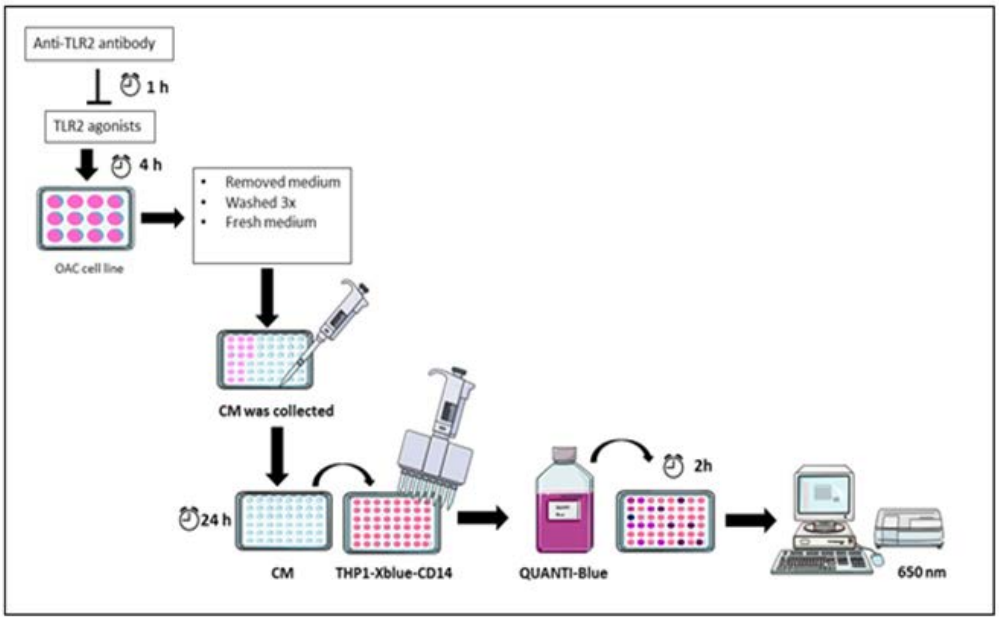

Figure S2. Experimental scheme. Oesophageal cell lines (GO, SK-GT 4 and OE33) (seeded at 2×10^5, 1.6×10^5, 2×10^5 cell/mL, respectively) were left unstimulated or stimulated with αTLR2 (10 μg/mL) for 1 h prior to stimulation with P3C (0.05 μg) / P2C (0.05 μg)/ or LPS (1 μg/mL) for 4 h Media were removed, cells were washed ×3 with PBS and fresh media was added/well. After 24 h, 50 μL CM was collected and added to 50 μL THP1-XBlue-CD14 (seeded at 4×10^6 cells/mL) and incubated for 24 h at 37°C. 40 μL resulting supernatant was added to 160 μL QUANTI Blue™ and incubated for 2 h at RT in a 96 well plate prior to absorbance measurement at 650 nm.

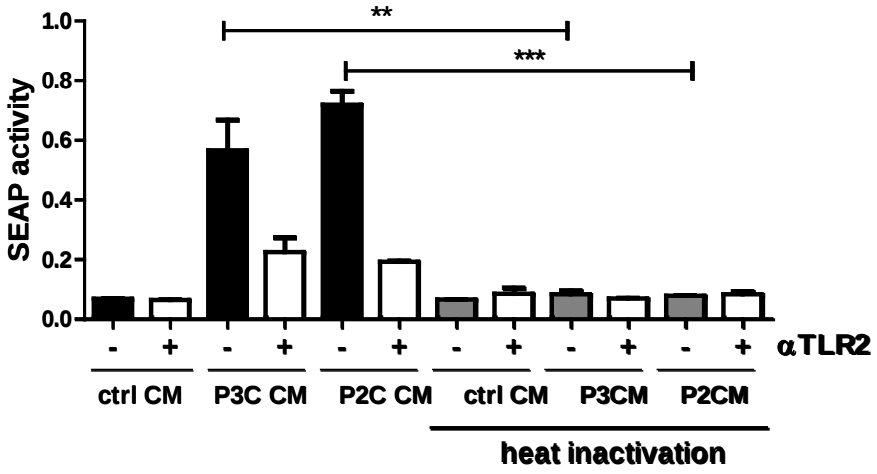

Figure S3. Heat-inactivated CM is unable to activate TLRs**.** CM was collected from unstimulated or αTLR2 stimulated SK-GT4 (ctrl CM), P2C-pretreated SK-GT 4 (P2C CM) and P3C-pretreated SK-GT4 (P3C CM). Half of the collected CM were heat-inactivated by incubating for 1 h at 75° C. 50 µL ctrl CM, P3C CM, P2C CM and heat-inactivated ctrl CM, P3C CM and P2C CM were added to THP1-Xblue-CD14 and incubated for 24 h at 37°C. 40 µL supernatant was added to 160 µL QUANTI-Blue™ and incubated for 2 h at RT in a 96 well plate prior to absorbance measurement at 650nm. Values represent the mean ± SEM of two independent experiments (run in. duplicate). Statistical analysis was performed using 2-way *ANOVA* test to compare mean values between CM and heat-inactivated CM, ** $p < 0.01$;***$p < 0.001$.