Supplemental figures

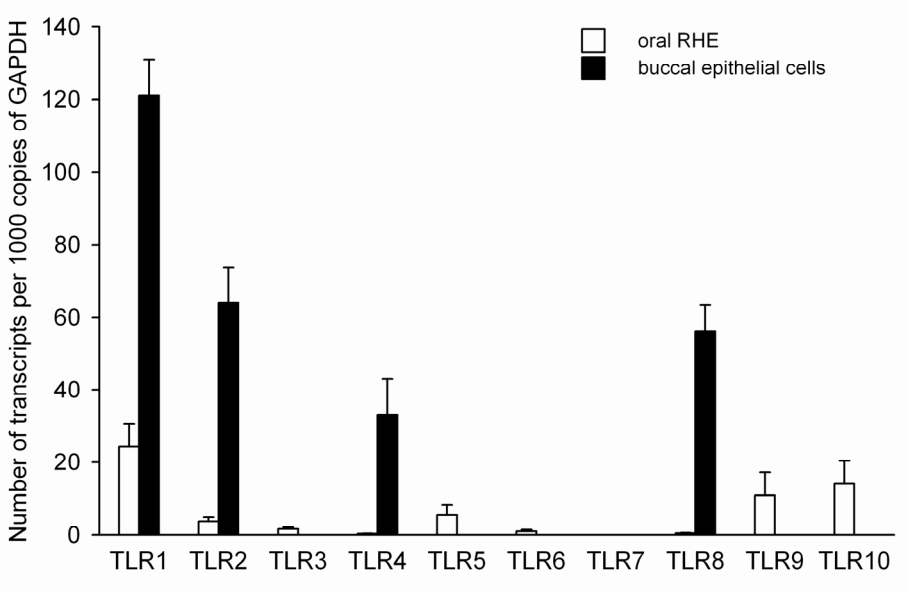

Figure S1

Quantitative expression of TLR in oral RHE and TLR1, 2, 4, 8 in buccal epithelial cells in vivo. For analysis unstimulated oral RHE samples were used (n = 6), while buccal epithelial scrape samples were obtained from healthy individuals (n = 17). RNA was isolated and TLR1-10 expression profiles determined. All the TLRs except TLR7 are expressed. The most commonly expressed epithelial TLR genes in vivo are TLR1, 2, 4, 8, which are present in 82%, 100%, 88% and 79% of subjects, respectively. Relative expression of TLRs has been normalized to the housekeeping gene GAPDH (arbitrary value of 1000 mRNA transcripts). TLR1 is the most highly expressed gene in vivo and in vitro.

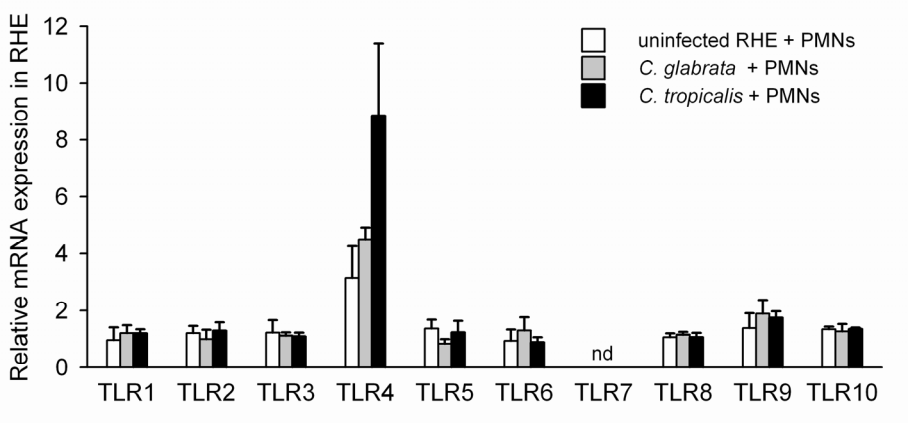

Figure S2

PMNs slightly induce epithelial TLR4 gene expression in the presence of *C. glabrata* and *C. tropicalis*. TLR mRNA expression after 24h in uninfected and infected oral RHE in the presence of PMNs (n = 3). Expression values are normalized to YWHAZ and G6PD and relative to uninfected RHE without PMNs (control, assigned as 1.0). nd, not detected.

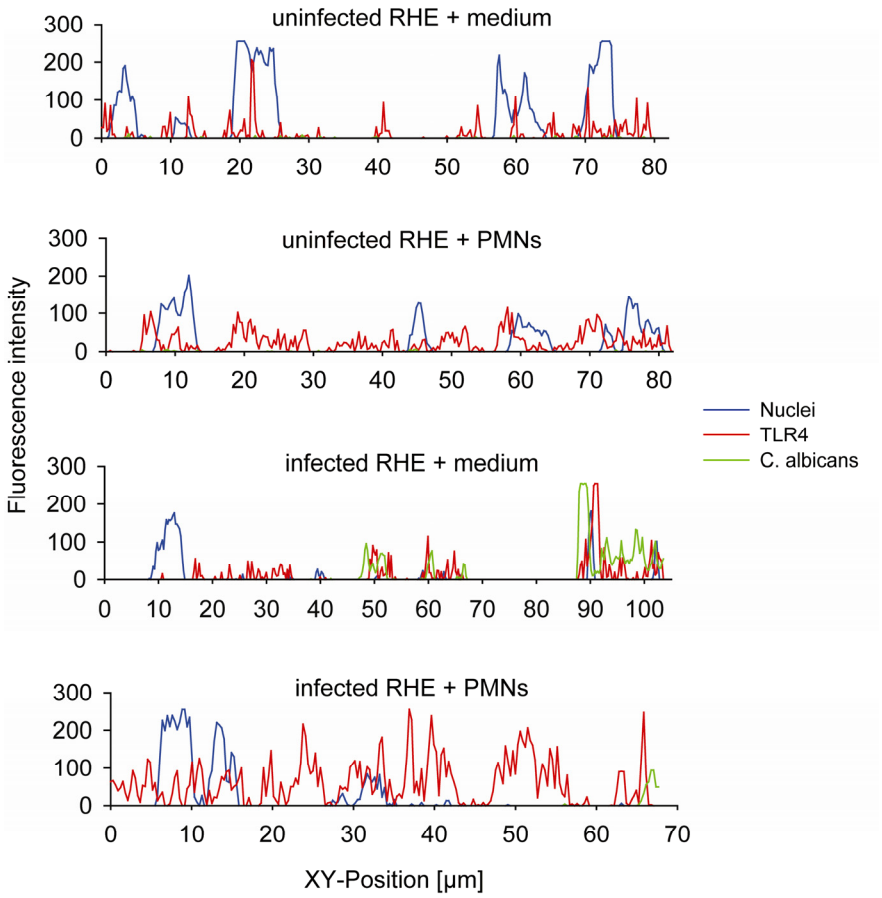

Figure S3
Increased TLR4 expression in the infected RHE in the presence of PMNs. In Figure 3, A-D vertical lines were drawn through the sections and the fluorescence intensity profile was measured along each of these lines. Fluorescence intensity (in arbitrary units) is plotted against the XY-position in µm from the basal (0 µm) to apical side of the oral RHE. Data are representative of 3 independent experiments.

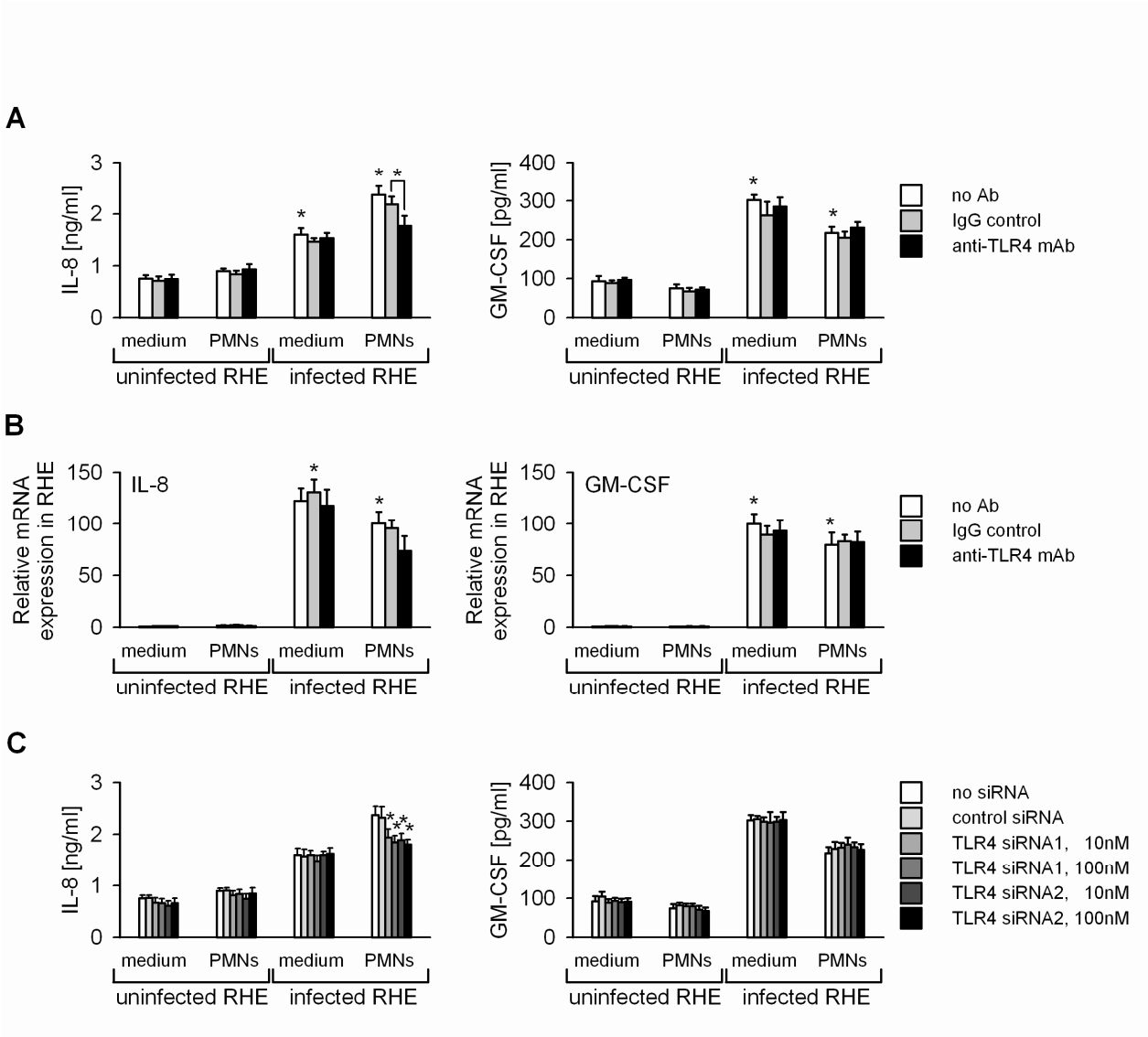

Figure S4

Effect of TLR4 blocking antibody and siRNA on epithelial IL-8 and GM-CSF production. (**A**) PMN supplementation of *C. albicans*-infected RHE further stimulates IL-8 secretion. In the presence of PMNs, co-incubation with anti-TLR4 monoclonal antibodies 1 h prior to *C. albicans* infection significantly reduces IL-8 protein production, but not GM-CSF. (**B**) *C. albicans* induces significant upregulation of epithelial IL-8 and GM-CSF gene expression in the presence or absence of PMNs. Expression values are normalized to YWHAZ and G6PD and relative to uninfected RHE without PMNs (control, assigned as 1.0). (**C**) Knockdown of TLR4 with two separate siRNAs significantly reduces IL-8 production but not GM-CSF (n = 6). **P* < 0.05, 2-tailed paired Student's t test.

Supplemental Methods

Human samples. Epithelial samples of the buccal mucosa were obtained from 17 healthy individuals attending the Oral Medicine clinic at Guy's Hospital, London. Buccal cells were collected using a plastic spatula by soft scraping action on buccal epithelial surfaces on both sides of the oral cavity and immediately frozen on dry ice to preserve RNA integrity. Total RNA was isolated from patient buccal samples using the RNeasy system (Qiagen). After digestion with Turbo DNase (Ambion) real-time analysis was performed on TLR1-10 using the ABI 5700 thermal cycler (Perkin Elmer) using the SYBR Green RT-PCR kit (Qiagen). Fold difference in TLR1-10 gene expression was normalized to the housekeeping gene GAPDH. To ensure TLR expression was of epithelial origin, patient RNA samples were also analyzed for CD66b and myeloperoxidase (neutrophils), CD11c (dendritic cells), and CD3 (T cells). In addition to the specified TLR primer pairs, the following primers were used: GAPDH, 5′-CTCTCTGCTCCTCCTGTTCGAC-3′ and 5′-TGAGCGATGTGGCTCGGCT-3′; MPO, 5′-GTGGCATTGACCCCATCCTC-3′ and 5′-CGCCTCCAGGCATTGTATCC-3′. Primer pairs for CD66b, CD11c, and CD3 were obtained by Assay-on-Demand (Applied Biosystems) with assay IDs Hs00167901_ml, Hs00174217_ml, and Hs00266198_ml, respectively.

Candida strains. *C. tropicalis* DSM4959 (Deutsche Stammsammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany) and a *C. glabrata* strain originally isolated from a female patient with oral candidiasis were used.