**Objectives**: Complex carbohydrates are major components of the fungal cell wall and serve as pathogen associated molecular patterns (PAMPs) to potentiate innate immunity. We recently reported that the glucose polymer glycogen and β-(1→3)-glucan form a covalently linked macromolecular complex at the *Candida albicans* cell wall. Therefore, we sought to determine whether disruption of glycogen synthesis altered fungal cell wall dynamics that could impact sensing by innate immune cells and mucosal colonization.

**Materials & Methods:** CRISPR-Cas9 gene editing was employed to create *C. albicans* deletion and overexpression (Pr*TEF1*) mutants of *GSY1*, encoding for glycogen synthase, in reference and clinical isolate backgrounds. Iodine staining and H nuclear magnetic resonance (NMR) spectroscopy was used to assess whole cell and cell wall glycogen content, respectively. Human THP1 macrophages were challenged with live or formalin-fixed parental and *gsy1*Δ/Δ mutants for 8 h at various MOI and the cytokine IL-1β measured by ELISA. In some experiments, blockade of the macrophage glucan receptor Dectin-1 was achieved by antibody-mediated neutralization prior to fungal challenge. Fungal cell wall carbohydrate content of parental and *gsy1*Δ/Δ strains was assessed by staining with calcofluor white (chitin), aniline blue (β-(1→3-glucan), concanavalin A-Texas Red (mannan), and anti-β-(1→3)-glucan IgG antibody + anti-IgG-FITC (exposed glucan). Neutrophil swarming assays were conducted by bioprinting *C. albicans* strains on glass slides and exposing them to Hoechst stained human neutrophils. Temporal images were capture by fluorescence microscopy. Immunocompetent and immunosuppressed (cortisone acetate, 225 mg/kg) C57BL/6 mice were sedated and sublingually incoulated with 2x107 *C. albicans* parental or *gsy1*Δ/Δ mutants constructed in pathogenic (SC5314) or commensal (529L) backgrounds. Body weight was monitored daily up to one week post-infection. For endpoint experiments, tongues were collected from euthanized mice at day 2 and 5 post-infection and CFUs enumerated to assess mucosal colonization.

**Results**: Using a combination of biochemical and genetic approaches, we confirmed that loss of *GSY1* ablated both cytoplasmic and cell wall glycogen content. Challenge of macrophages with fixed or live *gsy1*Δ/Δ led to exacerbated IL-1β secretion or neutrophil swarming. Analysis of cell wall components by fluorescence staining revealed that levels of total glucan, total mannan and total chitin remained similar, while reduced glycogen content significantly correlated with increased β-(1→3)-glucan exposure. Antibody-mediated blockade confirmed that exacerbated cytokine release observed in *gsy1*Δ/Δ was partially dependent on Dectin-1 signaling. To establish translational impact of our findings, a collection of *C. albicans* clinical isolates was screened for glycogen content, which revealed significant intra-species heterogeneity. Remarkably, overexpression of *GSY1* in a subset of reduced glycogen accumulation isolates reversed their hyperinflammatory phenotype and deletion of *GSY1* in a subset of WT-like glycogen accumulation isolates induced a hyperinflammatory phenotype during human macrophage challenge. Moreover, using an immunocompetent model of oropharyngeal candidiasis, mice inoculated with *gsy1*Δ/Δ in multiple strain backgrounds showed markedly decreased early fungal burdens. However, this phenotype was ameliorated in immunosuppressed mice.

**Conclusions**: Collectively, our data demonstrate that the glucan-glycogen macromolecular complex may be a novel cell wall determinant important for governing the host-*Candida* interaction and that fungal glycogen content contributes to commensalism in the oral cavity