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Supplemental information

Candida albicans oscillating *UME6* expression during intestinal colonization primes systemic Th17 protective immunity

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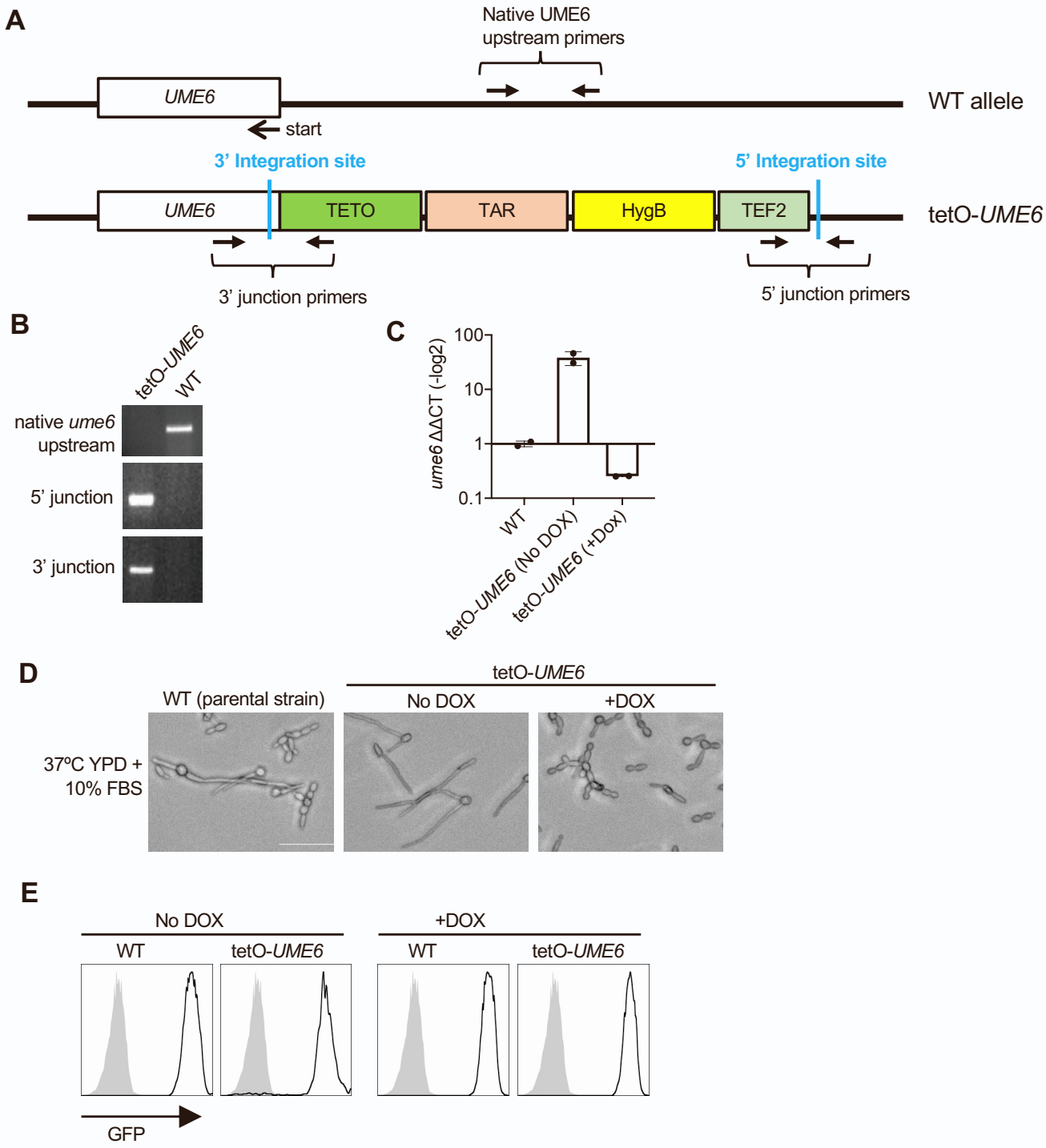
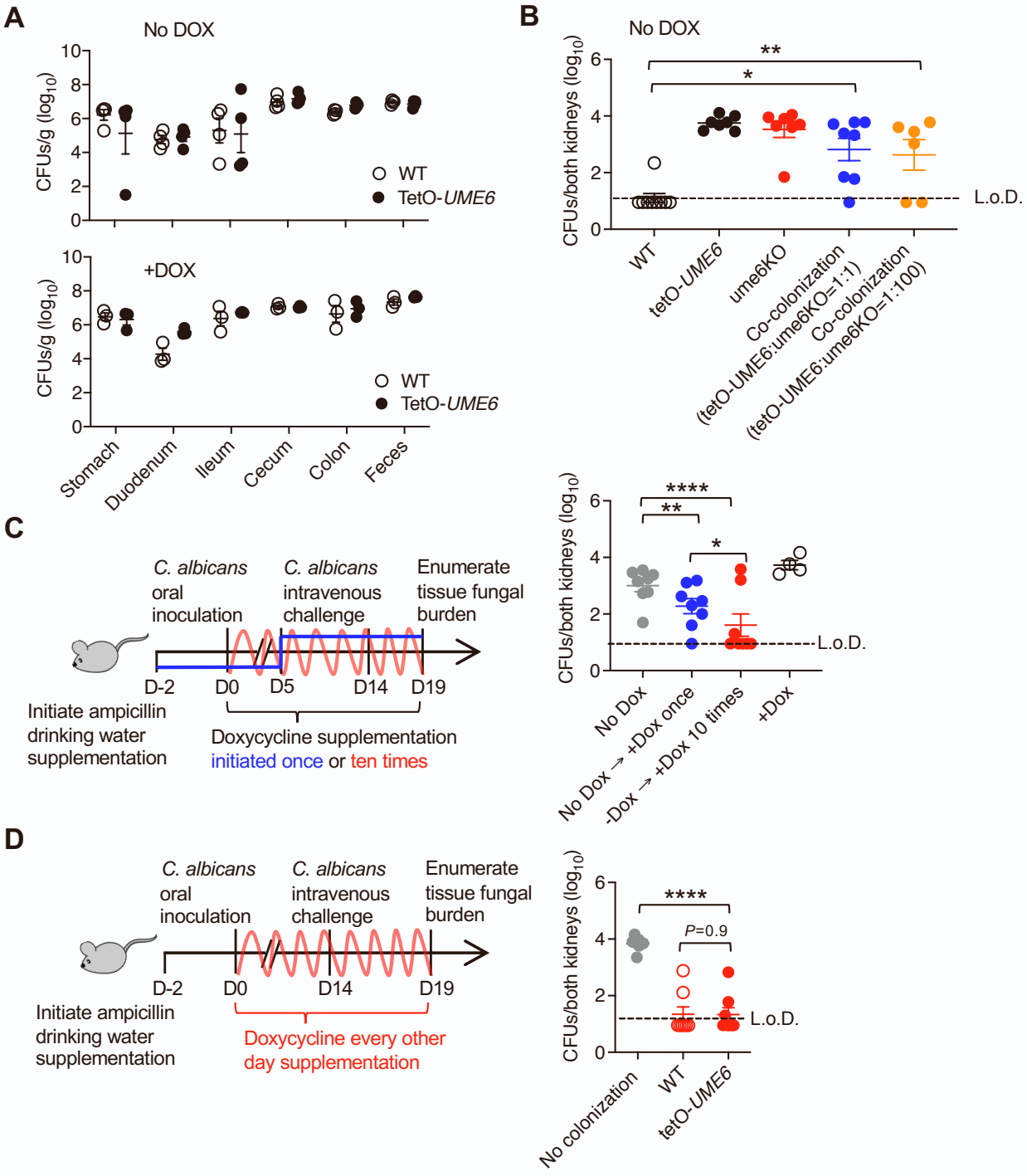
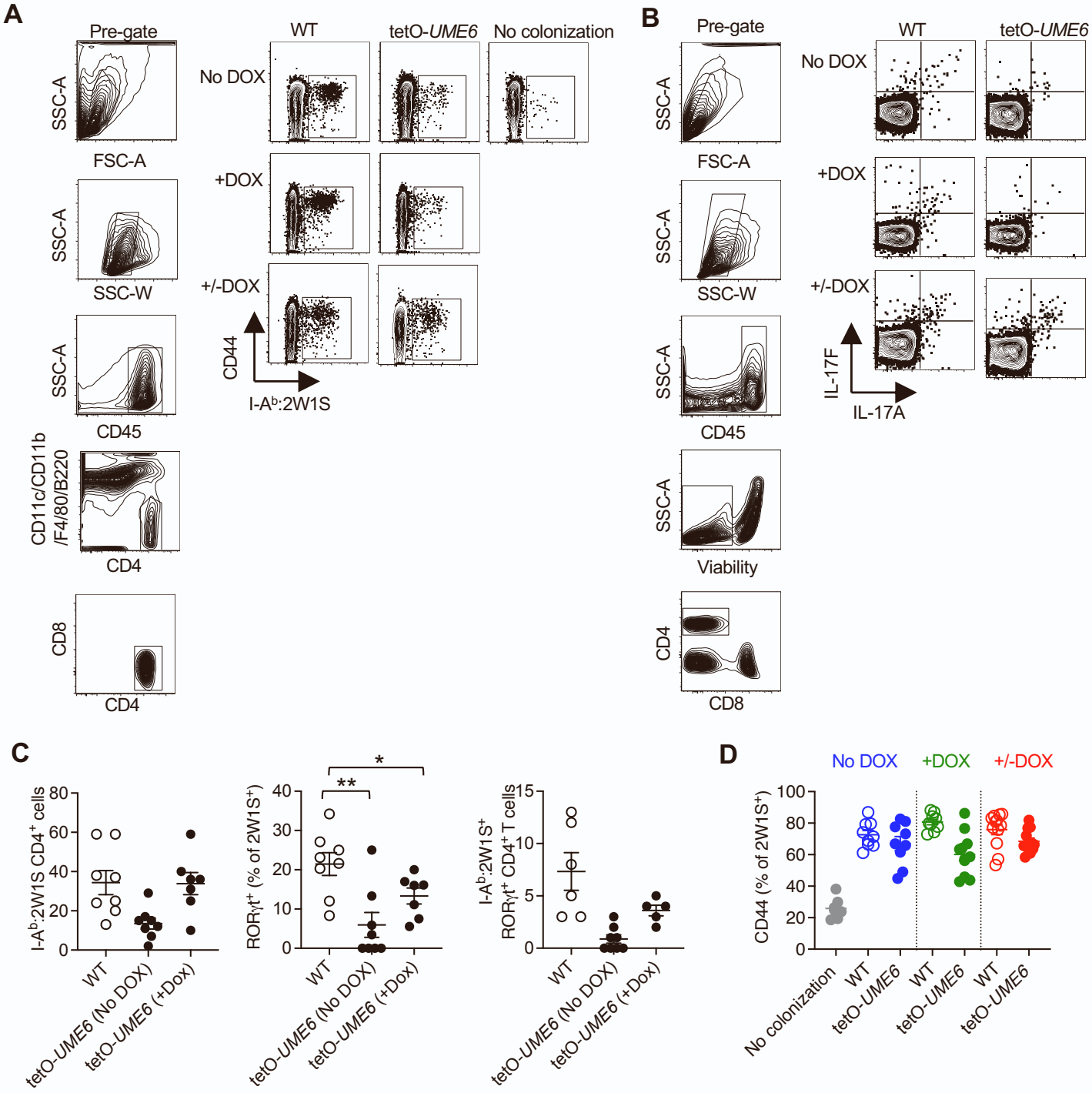


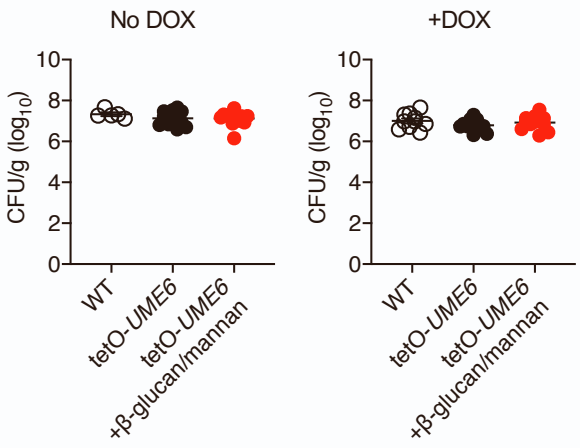
Supplementary Figure 1. Generation and verification of Ca-2W1S-tetO-*UME6* strain (linked with Figure 1). (**A**) TetO-*UME6* construct schematic illustrating replacement of the endogenous *ume6* upstream regulatory loci with translational elongation factor EF-1 alpha (TEF2) promotor driving hygromycin resistance (HygB), tetracycline repressible transactivator (TAR) and activator binding sequence (TETO). (**B**) PCR verification showing replacement of both *ume6* alleles by the tetO-*UME6* construct in Ca-2W1S-tetO-*UME6* compared with the parental Ca-2W1S (WT) strain. (**C**) Relative *ume6* compared with act1 expression levels by Ca-2W1S (WT), Ca-2W1S-tetO-*UME6* strain cultured at 37°C in YPD media supplemented with 10% fetal bovine serum without (No DOX) or with DOX (+DOX) (2 mg/ml). (**D**) Morphology of Ca-2W1S (WT) compared with Ca-2W1S-tetO-*UME6* strain cultured at 37°C in YPD media supplemented with 10% fetal bovine serum without (No DOX) or with DOX (+DOX) (2 mg/ml). (**E**) GFP fluorescence intensity of Ca-2W1S (WT) or Ca-2W1S-tetO-*UME6* strain (black line), compared with the parental non-recombinant *C. albicans* strain SC5314 (grey shaded) cultured in YPD media without (No DOX) or with DOX (2 mg/ml).



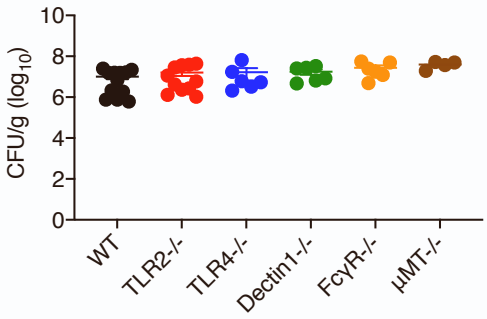
Supplementary Figure 2. Intestinal colonization density, and persistent susceptibility to intravenous infection in mice co-colonized with Ca-2W1S-tetO-*UME6 (UME6-on)* and *UME6*-deficient *C. albicans* (linked with Figure 1). (**A**) *C. albicans* in each intestinal segment or the feces 14 days after oral inoculation with Ca-2W1S (WT) or Ca-2W1S-tetO-*UME* for mice maintained on ampicillin drinking water plus continuous DOX drinking water supplementation (+DOX) or no DOX supplementation (No DOX). (**B**) *C. albicans* kidney CFUs 5 days after Ca-2W1S intravenous challenge for mice colonized individually with Ca-2W1S (WT) or Ca-2W1S-tetO-*UME6* or *UME6-deficient C. albicans* (ume6KO), or co-colonized with Ca-2W1S-tetO-*UME6* and *UME6*-deficient Ca (ume6KO) at the indicated ratios for mice maintained on ampicillin drinking water. (**C**) *C. albicans* kidney CFUs 5 days after Ca-2W1S intravenous challenge for mice colonized with Ca-2W1S-tetO-*UME6* maintained on ampicillin drinking water without DOX supplementation (No DOX, gray), DOX supplementation initiated once day 5 (blue), alternating DOX supplementation every other day (red), or continuous DOX supplementation (+DOX). (**D**) *C. albicans* kidney CFUs 5 days after Ca-2W1S intravenous challenge for mice colonized with Ca-2W1S (WT) compared with Ca-2W1S-tetO-*UME6*, or no colonization controls each maintained on ampicillin drinking water with DOX supplementation every other day. *p < 0.05, **p < 0.01, ****p < 0.0001; Bar, mean ± SEM; L.o.D., limit of detection.



Supplementary Figure 3. *UME6* expression oscillations during *C. albicans* colonization primes protective Th17 immunity (linked with Figure 1). (**A**) Representative FACS plots showing gating strategy for CD4+ T cells, and I-A^b:2W1S CD4+ T cells with surrogate fungal specificity in the spleen and peripheral lymph nodes for mice 14 days after Ca-2W1S (WT) or Ca-2W1S-tetO-*UME6* or no colonization controls maintained on ampicillin drinking water without (No DOX) or with (+DOX) continuous supplementation, or oscillating DOX supplementation every other day. (**B**) Representative FACS plots showing gating strategy for CD4+ T cells, and IL-17A and/or IL-17F production by CD4+ T cells in the spleen and peripheral lymph nodes after heat-killed WT *C. albicans* stimulation for mice described in panel (A). (**C**) Number I-A^b:2W1S CD4+ T cells with surrogate fungal specificity, and percent RORγt+ and number RORγt+ I-A^b:2W1S CD4+ T cells in the mesenteric lymph nodes for mice described in panel (A). (**D**) CD44 expression by I-A^b:2W1S CD4+ T cells with surrogate fungal specificity in the spleen and peripheral lymph nodes for mice described in panel (A). Each data point represents the results from an individual mouse, representative of at least two independent experiments. *$p < 0.05$, **$p < 0.01$; Bar, mean ± SEM.



Supplementary Figure 4. Intestinal colonization density by Ca-2W1S-tetO-*UME6* with β-glucan and mannan supplementation (linked with Figure 2). *C. albicans* in feces 14 days after oral inoculation with Ca-2W1S (WT) or Ca-2W1S-tetO-*UME* with or without β-glucan and mannan daily treatment for mice maintained on ampicillin drinking water plus continuous DOX drinking water supplementation (+DOX) or no DOX supplementation (No DOX). Each data point represents the results from an individual mouse, representative of at least two independent experiments.



Supplementary Figure 5. Intestinal colonization density by Ca-2W1S (linked with Figure 4). *C. albicans* in feces 14 days after Ca-2W1S oral inoculation for each group of mice maintained on ampicillin drinking water. Each data point represents the results from an individual mouse, representative of at least two independent experiments.