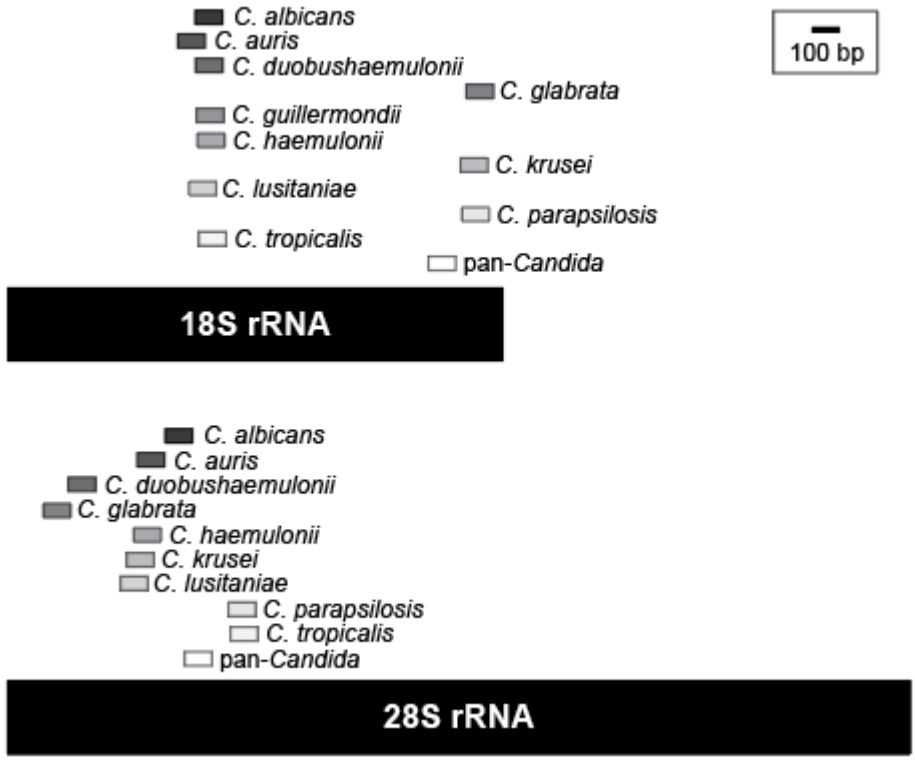
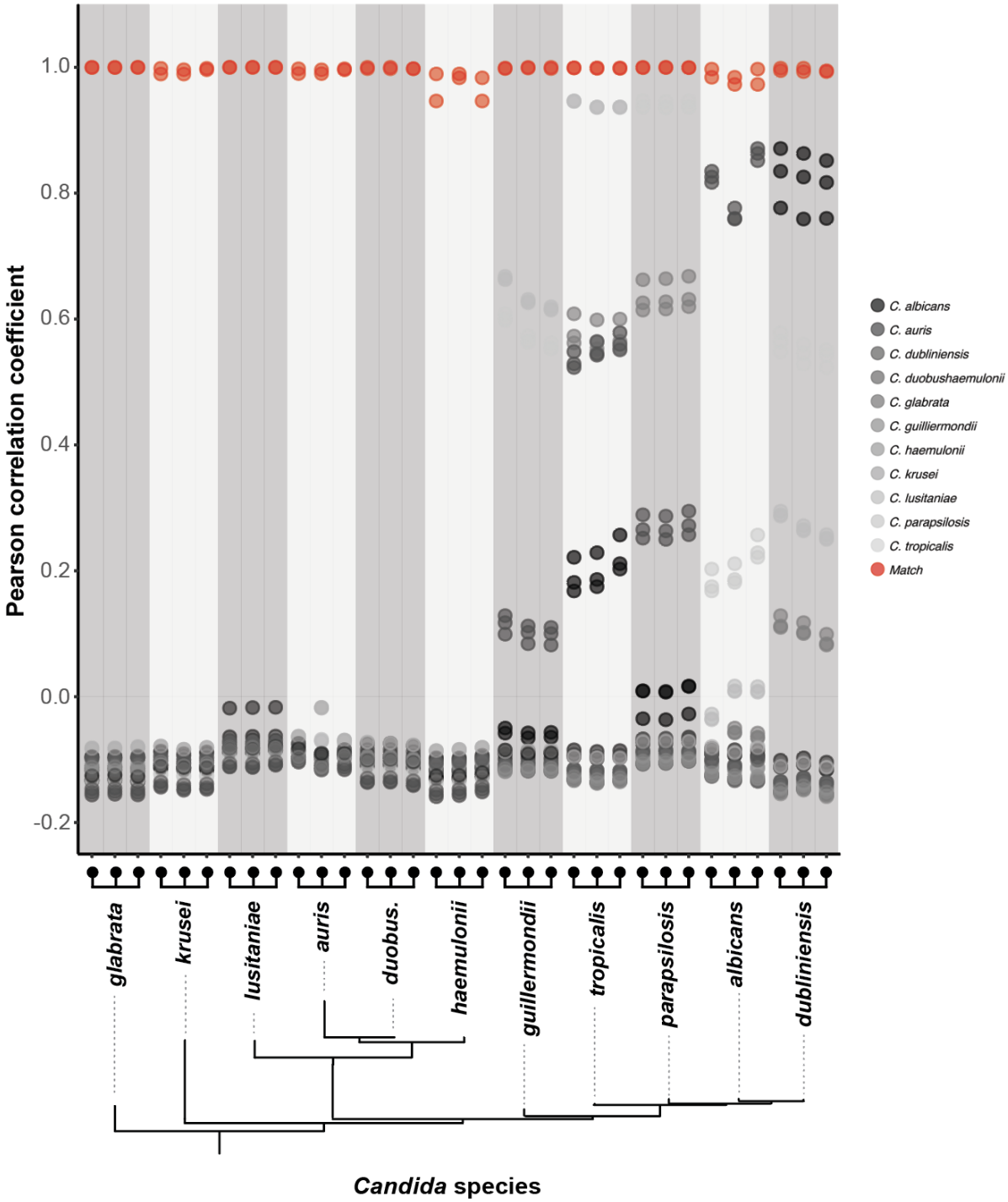
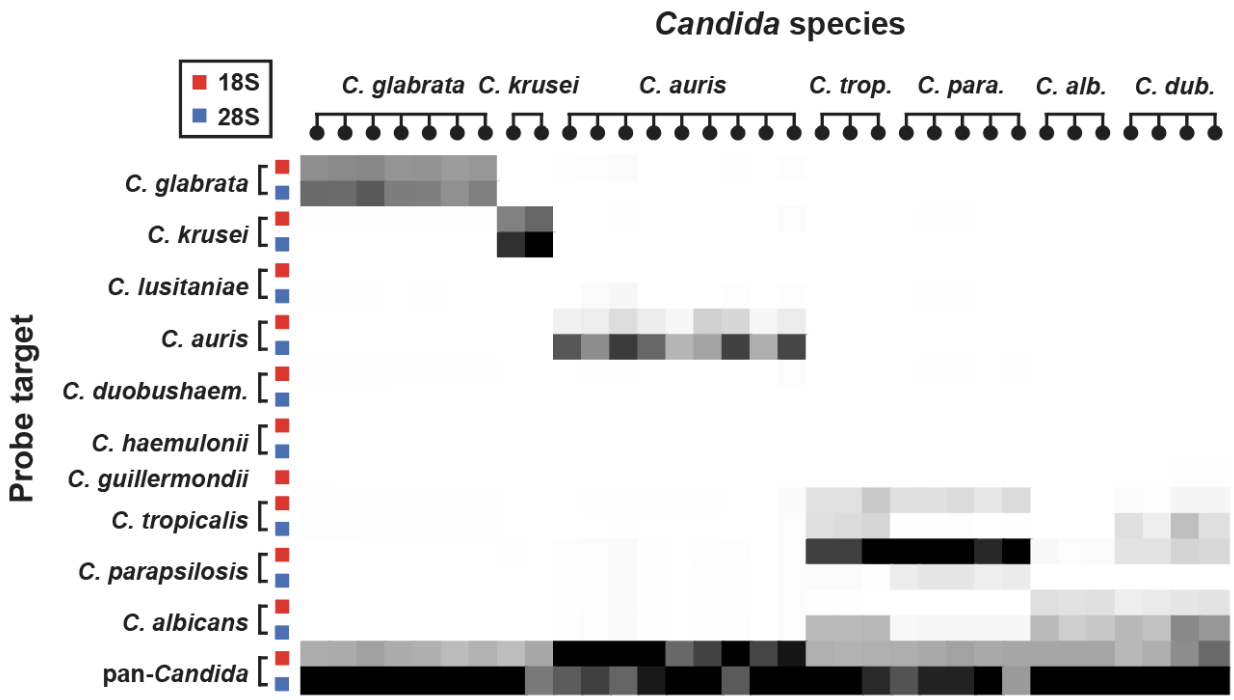


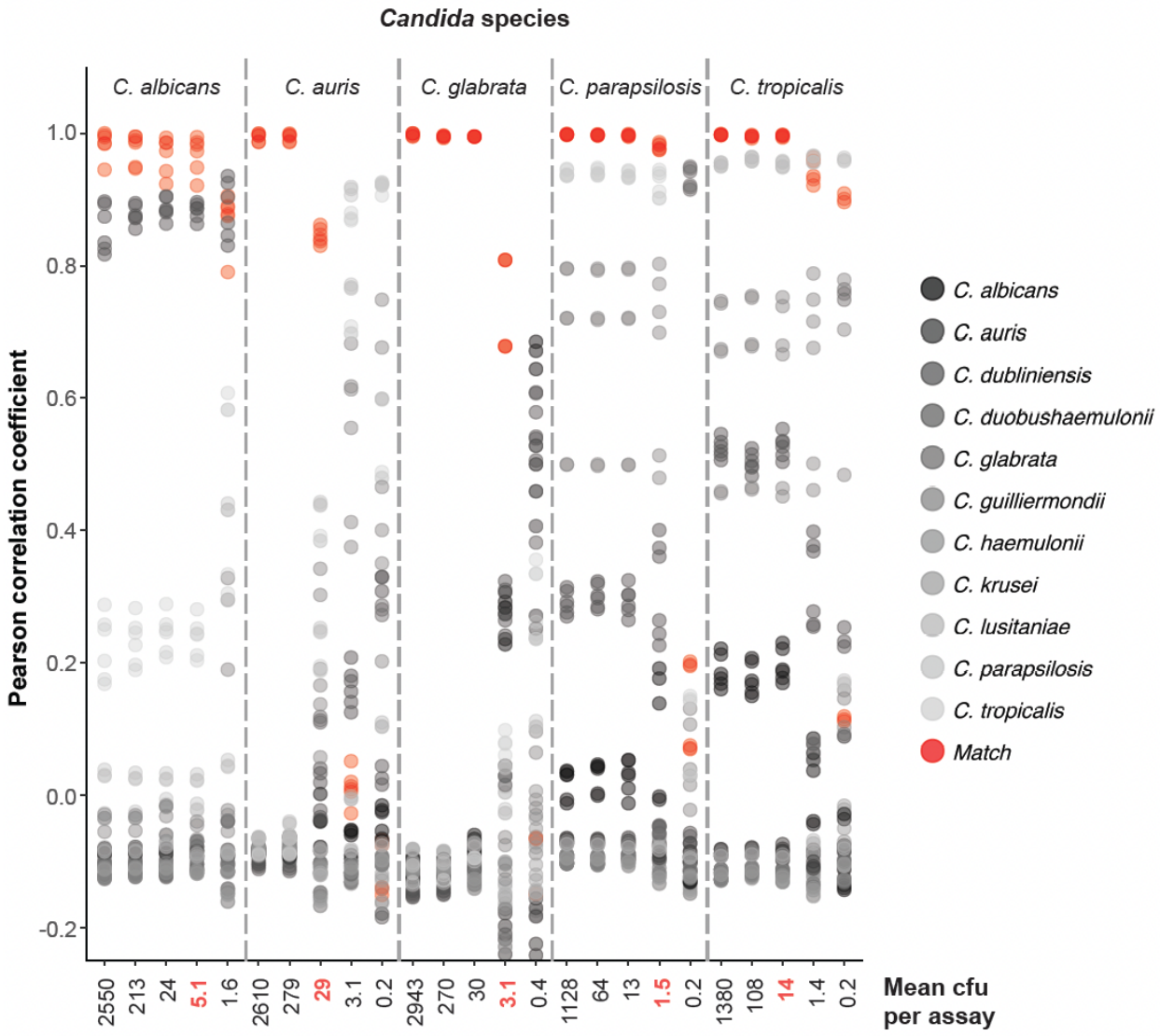
Supplementary Figure S1. Target regions of 21 *Candida* Phirst-ID probes on 18S and 28S rRNA subunits. Each of the 21 probe pairs in the *Candida* Phirst-ID probeset are mapped onto their complementary regions in schematic representations of the 18S or 28S rRNA subunits.



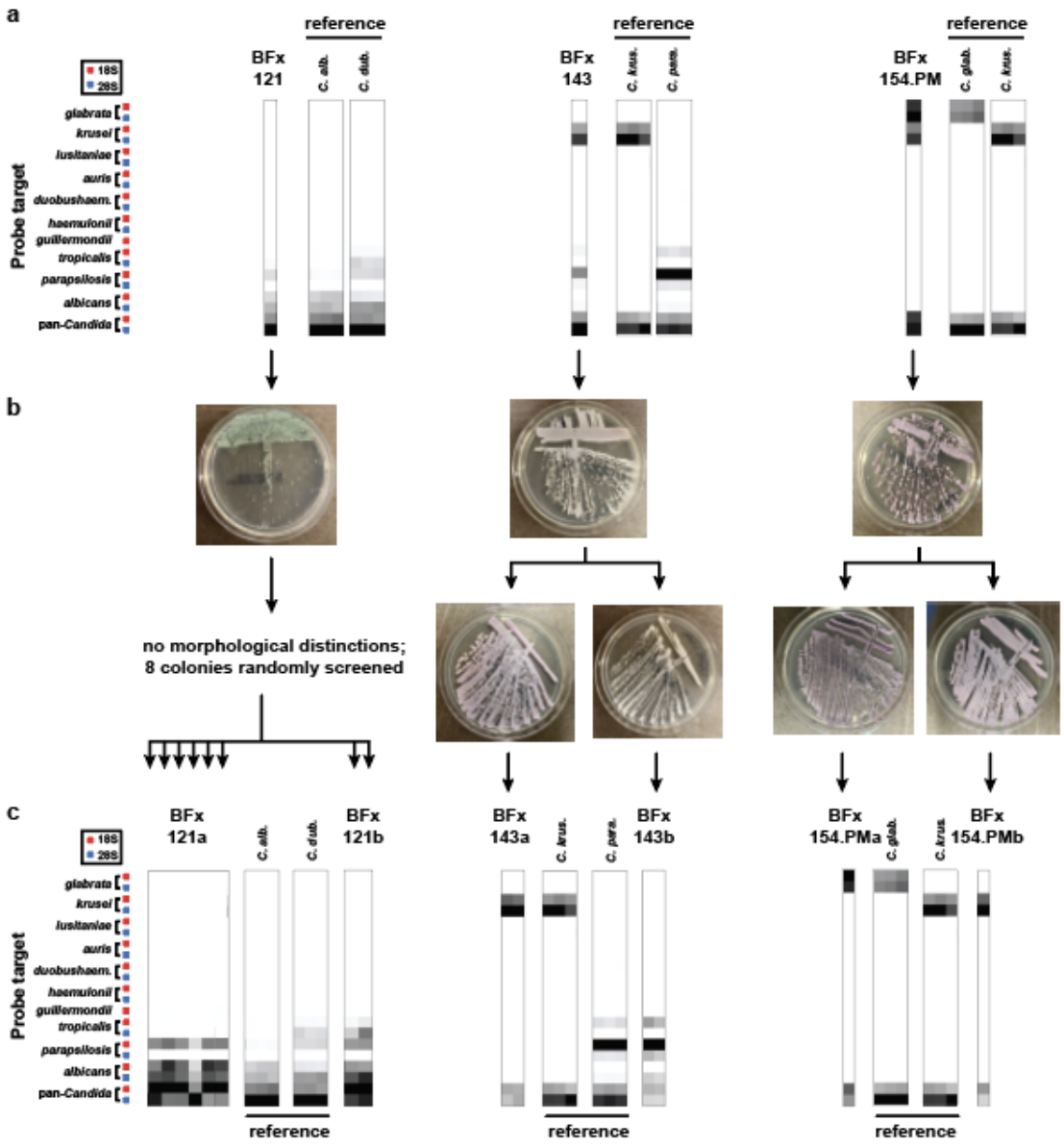
Supplementary Figure S2. Non-self Pearson correlation coefficients of *Candida* Phirst-ID profiles identify species in a reference panel. Pearson correlation coefficients of probeset reactivity profiles of species-specific probes from 3 isolates of each of 11 species in a reference panel, against each other panel member, are plotted. Shading of data points indicate the comparison species; species matches are shown in red.



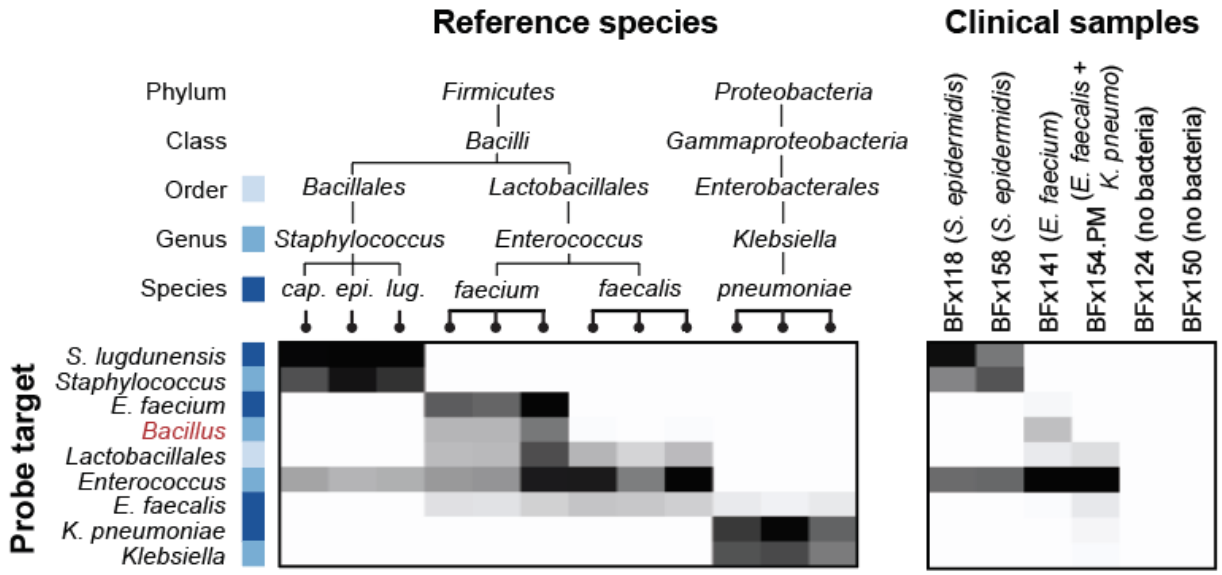
Supplementary Figure S3. Independent validation of *Candida* Phirst-ID probeset. Heatmap of normalized, background-subtracted binding intensities show Phirst-ID probeset reactivity profiles of 33 isolates from 7 *Candida* species grown in laboratory culture, independent of the reference panel in Figure 1a. Heatmap intensity is normalized within each sample to the maximum signal for that sample.



Supplementary Figure S4. Phirst-ID limits of distinction for 5 *Candida* species. Pearson correlation coefficients of Phirst-ID probeset reactivity profiles from serial dilutions of each indicated *Candida* species (upper horizontal axis label) versus the reference panel, with cfu for each sample as indicated (lower horizontal axis label). Shading of data points indicate the comparison species from the reference panel; species matches are shown in red. The lowest cfu per assay at which the highest Pearson correlation coefficient corresponded to the correct species is indicated in red as the measured limit of distinction for that species.



Supplementary Figure S5. *Candida* Phirst-ID revealed mixed blood cultures that were missed by standard clinical microbiology workflows. (a) Phirst-ID probeset reactivity profiles (PSRPs) for two clinical blood cultures (BFx121 and BFx143) and one post-mortem blood culture (BFx154.PM) appeared to be a linear combination of two species. PSRPs from three reference panel isolates from the two species that appeared to be represented are shown at right. **(b)** Blood culture broth from each of these three samples was streaked onto ChromAgar *Candida* plates, in two cases revealing two distinct culture morphologies (top row) that could be restreaked to homogeneity from single colonies of each morphotype (bottom row). **(c)** Phirst-ID PSRPs from individual colonies unambiguously matched only one reference species, confirming that each original blood culture broth represented a mixture of two species. PSRPs from three reference panel isolates of each matching species are again provided for comparison.



Supplementary Figure S6. Bacterial Phirst-ID recognizes bacteria from mixed fungal and bacterial blood cultures. Left panel: bacterial Phirst-ID data from selected probes for reference strains are shown at left (from Bhattacharyya et al, *Sci Rep* 2019, reference 18), with phylogenetic hierarchy of probe target encoded in color. Red probe = off-target probe designed against *Bacillus* genus that fortuitously distinguished *E. faecium* from *E. faecalis* due to unanticipated cross-reactivity. Right panel: data from the same selected bacterial Phirst-ID probes for 6 clinical or post-mortem blood cultures that grew yeast, 4 of which also grew bacteria, and 2 of which did not. Bacterial identifications for each sample from the clinical microbiology laboratory are shown in parentheses. Heatmaps display normalized, background-subtracted read intensities for each probe, scaled to the maximum signal in each sample. (*cap*. = *capitis*, *epi*. = *epidermidis*, *lug*. = *lugdunensis*)