Supporting Information S7 Fig. Morphology and ultrastructure of *Komagataella pastoris* (Saccharomycetales, syn. *Pichia pastoris*) expressing *Trichoderma* HFB-encoding genes.

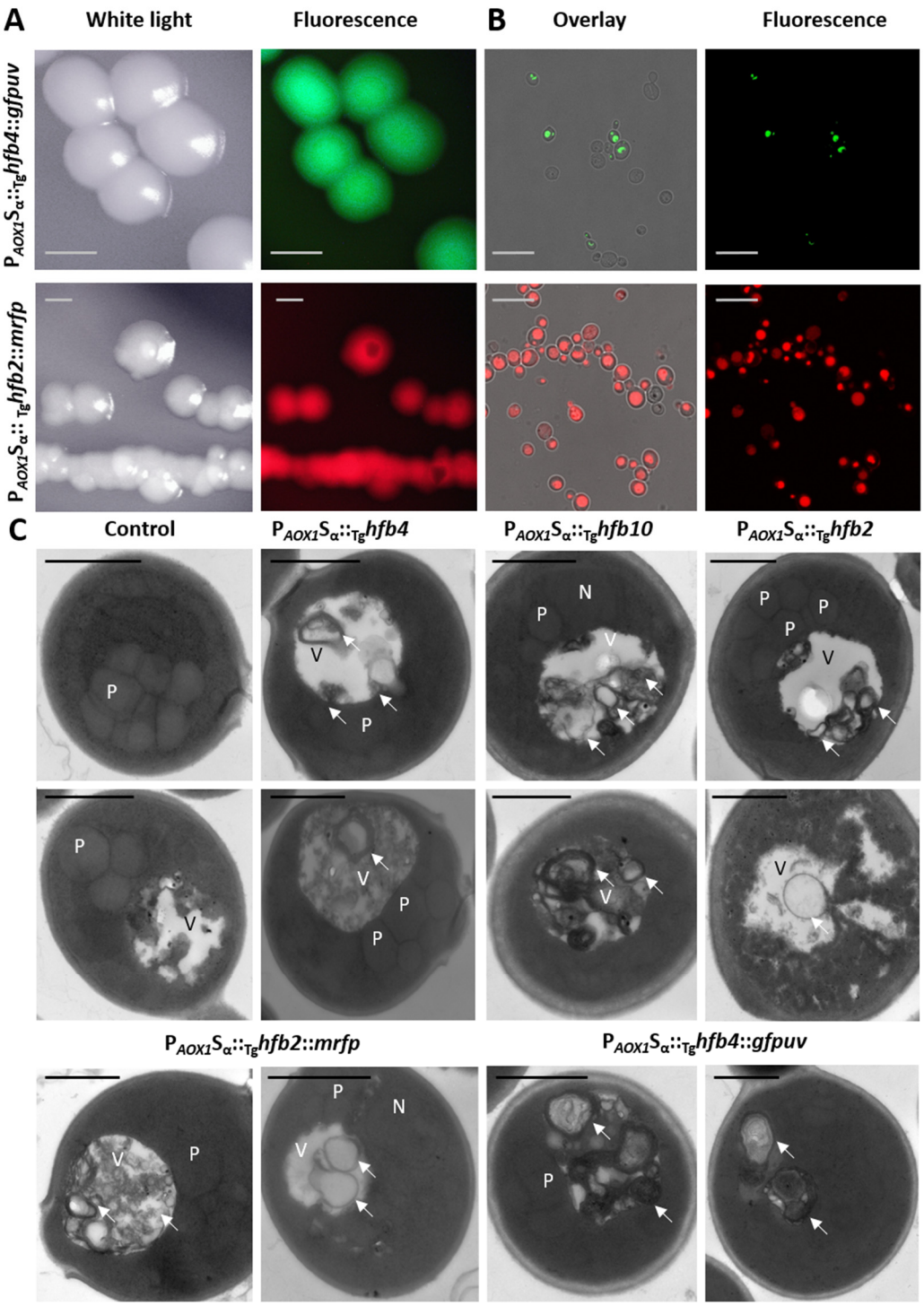

Fig A (A) Stereo confocal microscopy *of P. pastoris* producing fluorescently labeled HFBs from *Trichoderma* using the signal peptide of *Saccharomyces cerevisiae* α-mating factor (Sα) under the control of the AOX1 promoter ($P_{AOX1}$). *P. pastoris* were cultivated on BMM agar plates for four days, and HFB expression was induced by methanol. Scale bar = 5 mm. (B) Intracellular accumulation of HFBs in the same strains as in (A) fermented in BMM liquid medium. Scale bar = 10 µm. (C) TEM micrographs of the *P. pastoris* mutants overexpressing HFBs shown in (B). The control corresponds to the strain transformed with the HFB-free vector (pPICZαA); see the Materials and Methods for the details. P – peroxisome, V – vacuole, N – nucleus. Arrows point to putative HFB vesicles in VMSs that correspond to HFB-rich organelles in (B). Representative images were selected from total 139 images. Samples for TEM were prepared with at least two mutants and 15 images studied per each haplotype.

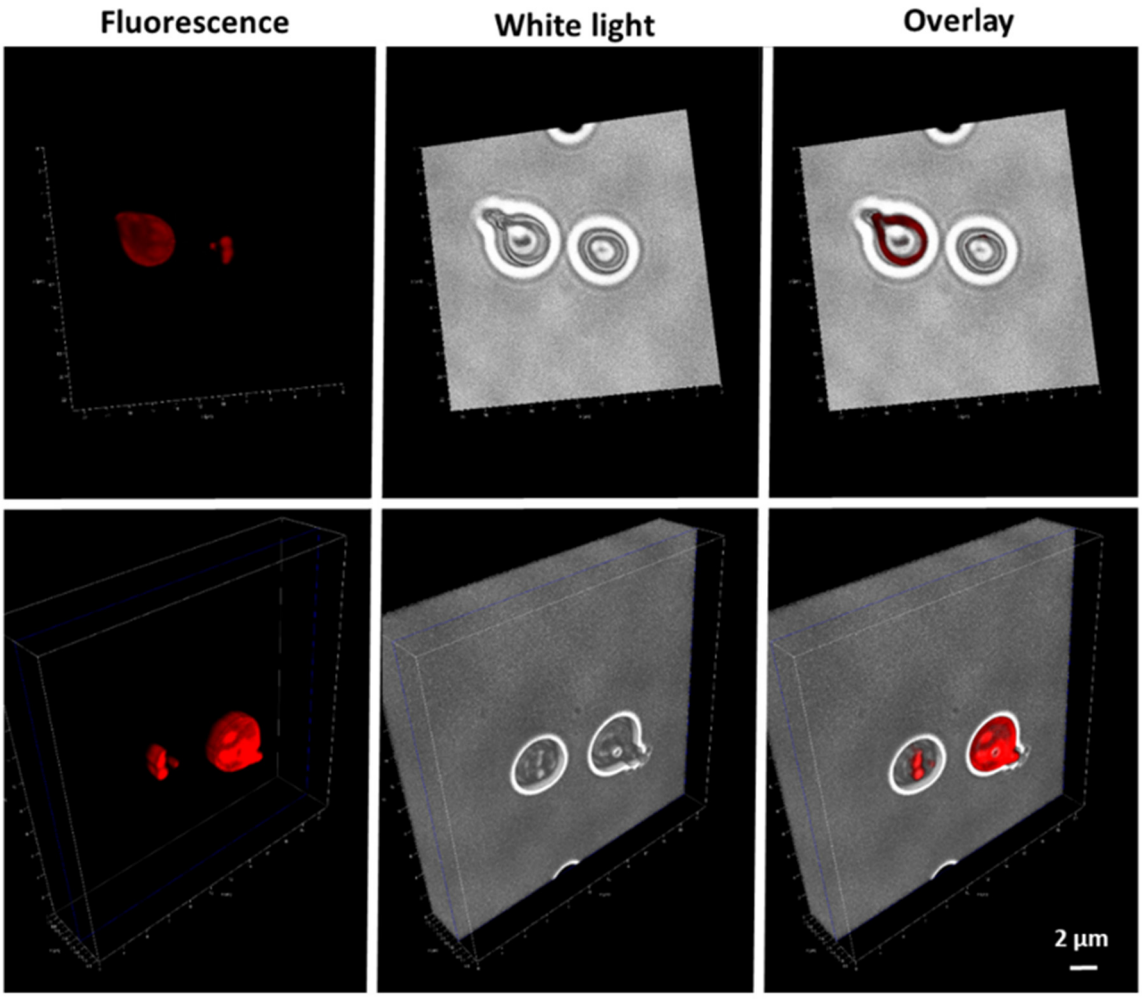


Fig B 3D visualization of HFB intracellular accumulation in the *P. pastoris* strain $P_{aox1}S_{\alpha}::_{Tg}hfb2::mrfp$ overexpressing $_{Tg}hfb2$ was imaged using a Leica DMi8 microscope (Leica, Germany). The strain was cultivated in BMM medium for HFB induction by 0.5% methanol (see the detailed procedure in Material & Method). mRFP was fused to the C-terminus of $_{Tg}$HFB2 for protein localization.