Microorganisms

Recombinant *Inga Laurina* Trypsin Inhibitor (ILTI) Production in *Komagataella Phaffii* Confirms its Potential Anti-Biofilm Effect and Reveals an Anti-Tumoral Activity

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Supplementary Materials:

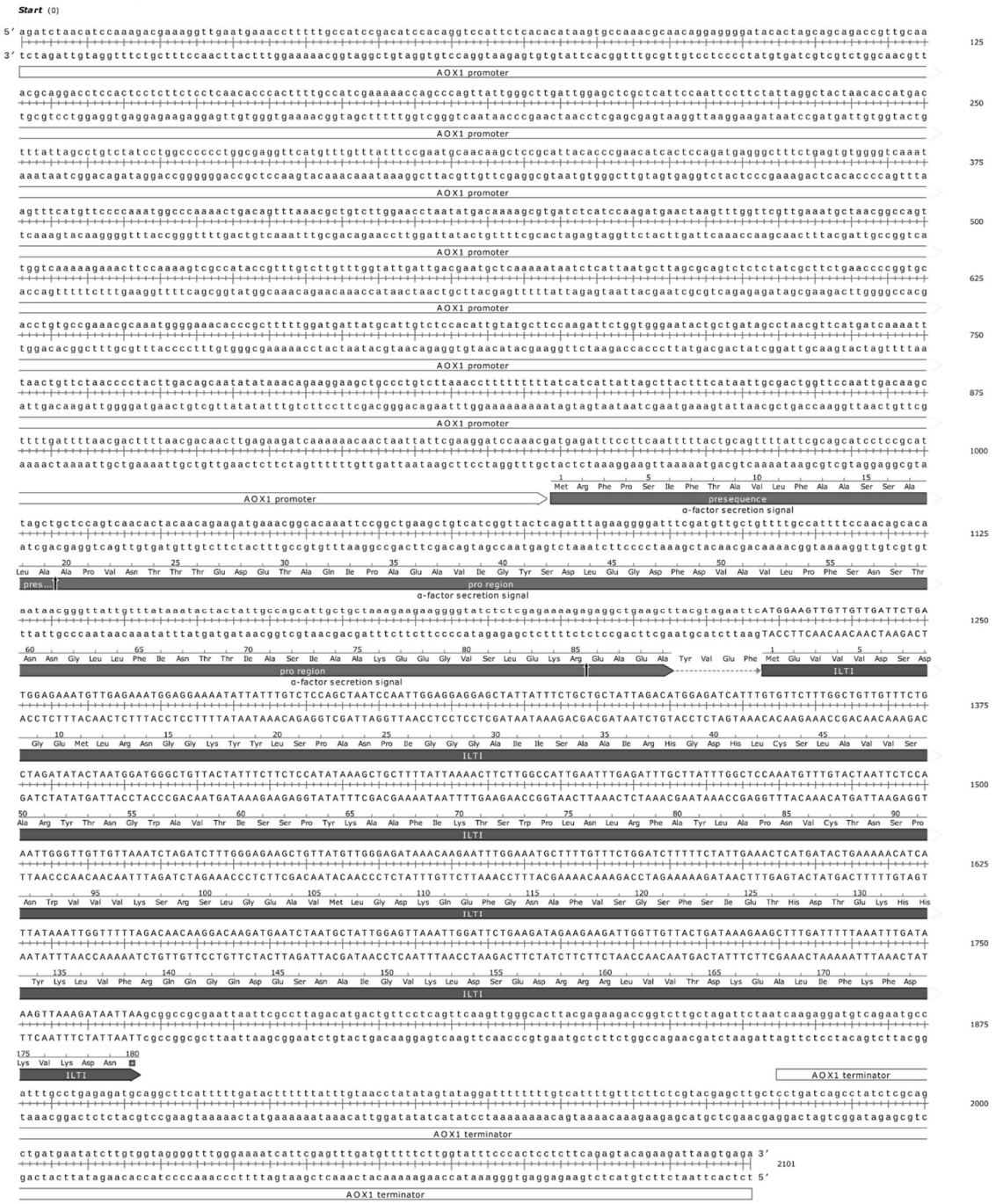

Figure S1. Nucleotide sequence of pPIC9K+ILTI expression cassette. In the figure it can be observed the sequence of AOX1 promoter region, followed by the α-factor secretion signal, showing the place of Kex2 cleavage sites by an arrow. The gene sequence can be observed right after the last Kex2 cleavage site, and was designed from native ILTI amino acid sequence (show in the figure), optimized for *K. phaffii* expression taking into account the published yeast codon usage. Lastly in can be observed the AOX1 terminator region.

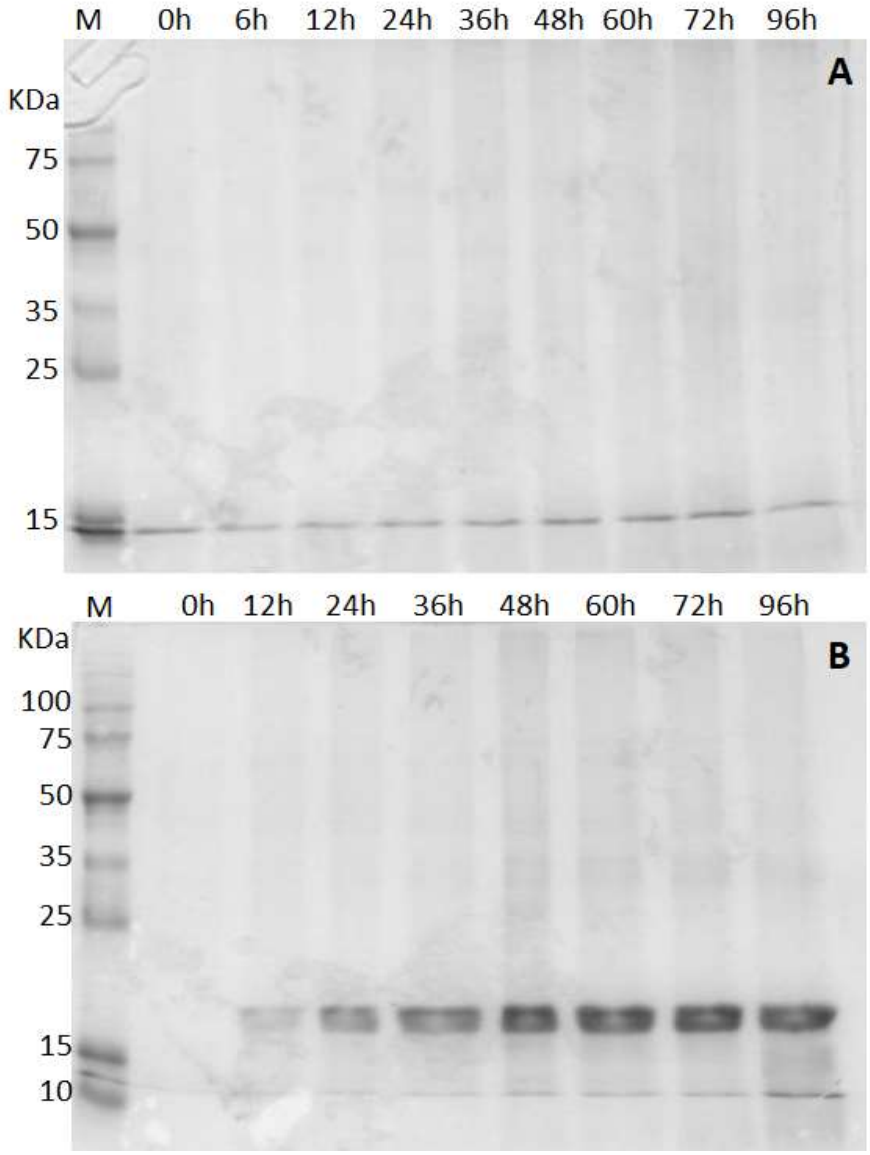

Figure S2. SDS-PAGE (12%) of cell-free samples collected during the 96 hours induction. Lane M contains the Broad Range Protein Molecular Weight Markers (Promega), at its side, it is possible to verify the molecular weight of each protein marker. In each of the followed lanes have the culture supernatant where the time of induction is indicated above. A) Secretory profile of the samples collected during the induction of GS9K strain; B) Secretory profile of the samples collected during the induction of GSILTIr strain.

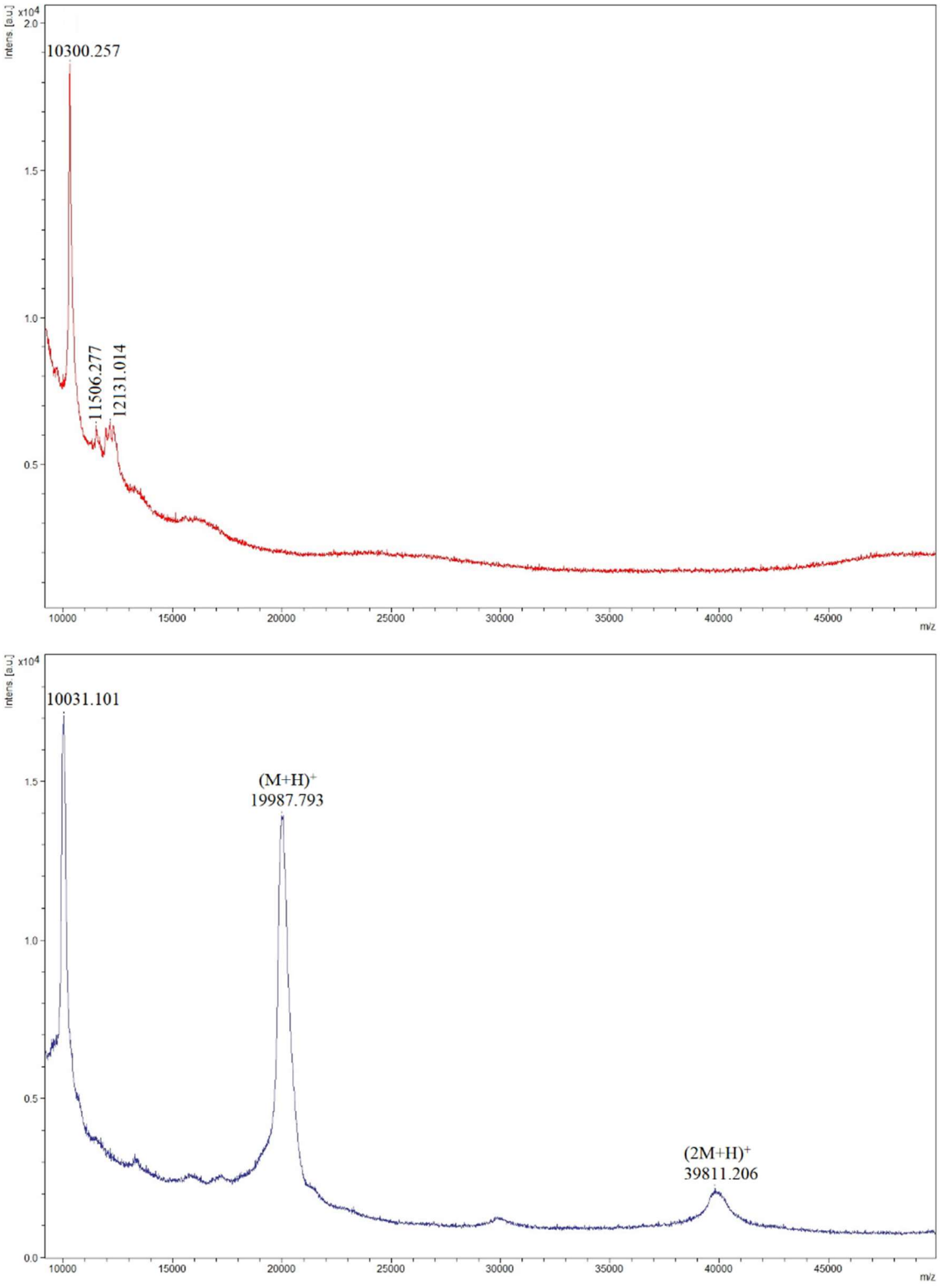

Figure S3. A) Spectrum obtained from the final fermentation precipitate of GS9K strain. The m/z correlation of each ion is indicated above itself; B) Spectrum obtained from the final fermentation precipitate of GSILTIr strain. The 19987.793 m/z ion corresponding to the recombinant ILTI (19.8 kDa), and the 39811.206 m/z ion corresponding to a single charged dimer of the recombinant ILTI.

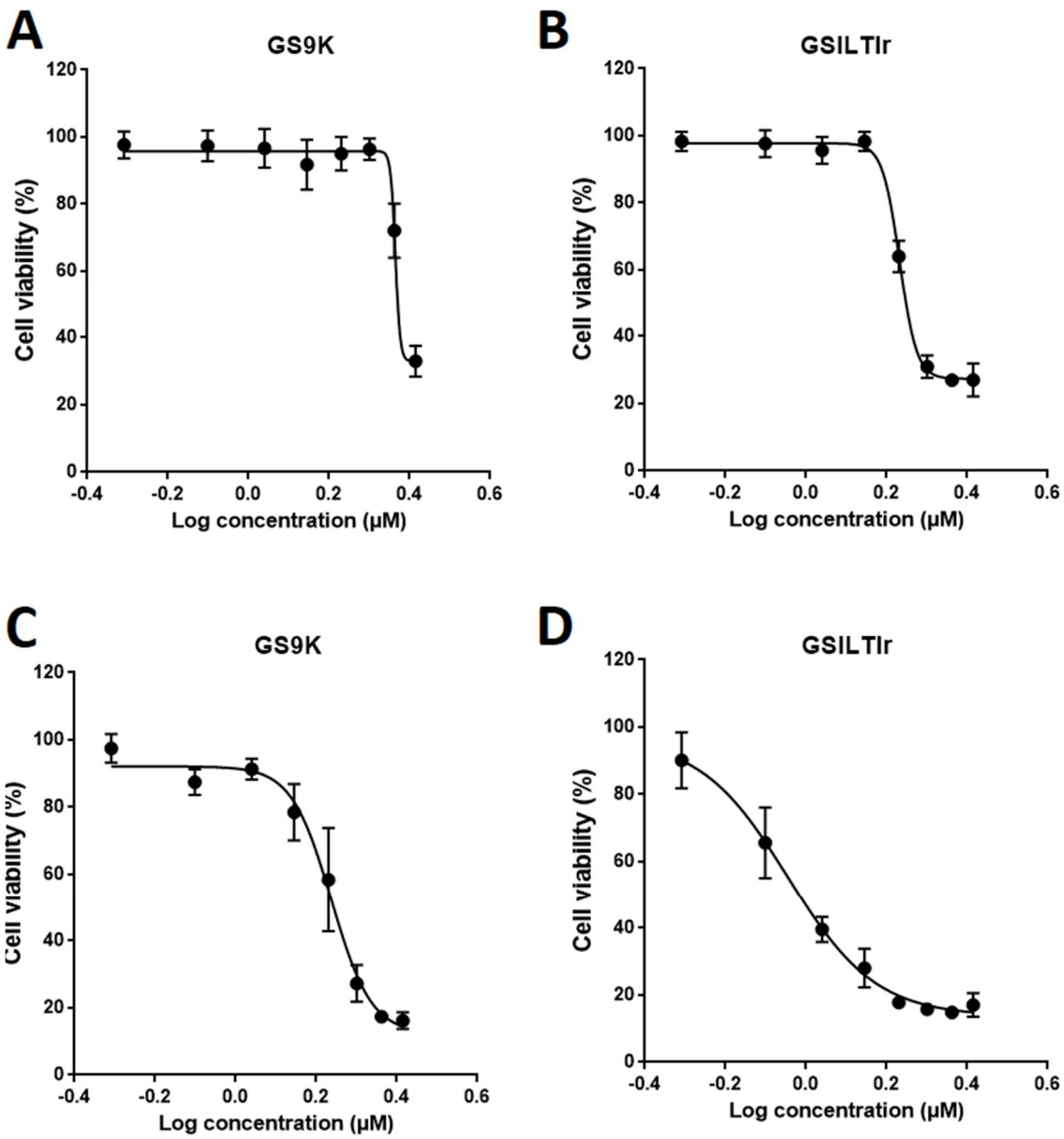

Figure S4. Determination of IC50 values of GSILTIr and GS9K final fermented broth against EAT cells after 24 (A and B) and 48 hours (C and D) of incubation. The graphic shows the fit of cell viability (%) vs a log concentration of inhibitor (μM) to IC50 value for the cell viability assay, implemented on GraphPad Prism v6.0 software.