

Supplementary information for

Antifungal Activity, Structural Stability, and Immunomodulatory Effects on Human Immune Cells of Defensin from the Lentil *Lens culinaris*

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Table S1. List of overlapping primers.

Nº	primer sequences (5'-3')
s1	CCTCGACGCTAACCTGGCCGGATCTATGAAAACCTTGGAACACTTGGCTGATACCT A
s2	CTTGGCTGATACCTACCGTGGAGTTTGTTCACCTAATGCTAGTTGTGACGATCACTG TAAAAA
ans3	CATTTCOAATTATGACACGTGCCTGATATTAGATGTGCTTTATTTTACAGTGATCG
ans4	GGTGCTCGAGAGAATTCGCGGATCCTTAACAGTTTTGGGTACAGAAGCATTTCCAA TTATGACAC

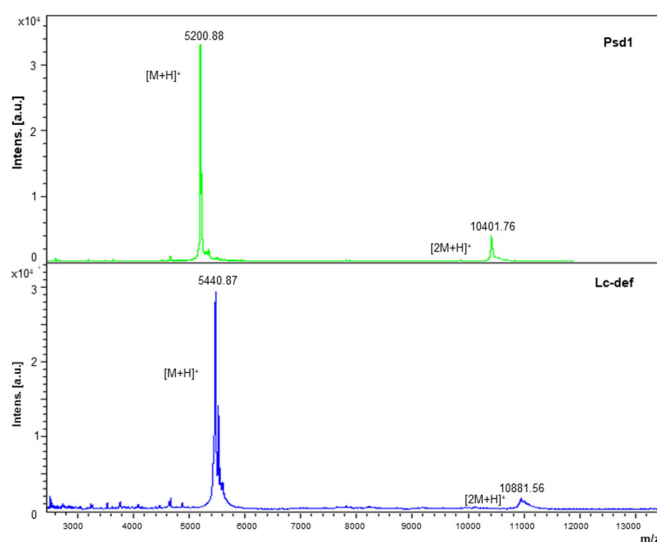


Figure S1. MALDI mass spectra of the recombinant defensins.

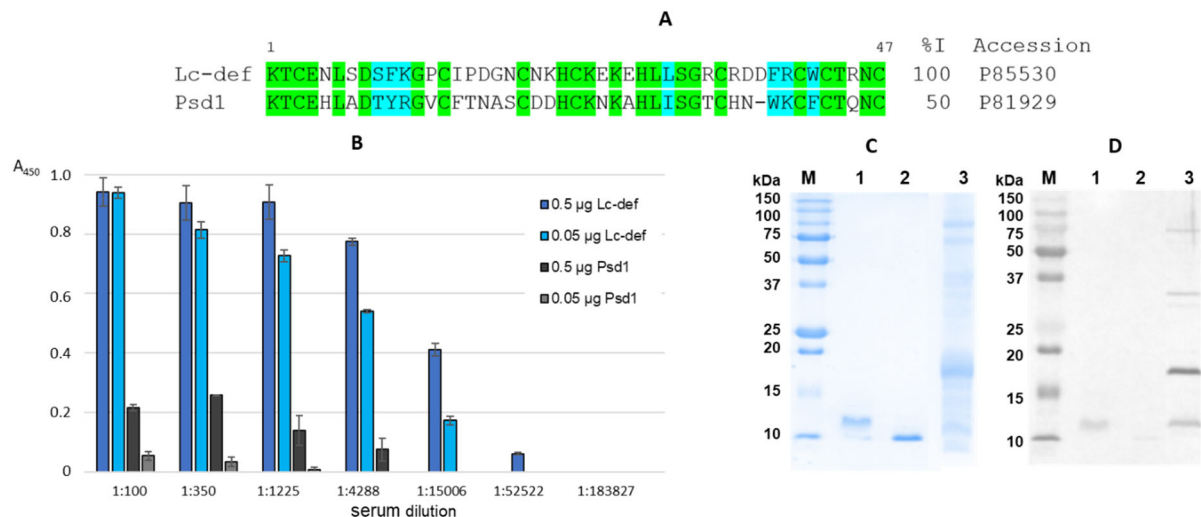


Figure S2. (A) Comparison of the amino acid sequences of two legumes defensins – lentil Lc-def and pea Psd1. Identical and similar amino acids are shown in green and blue, respectively. %I, percentage of sequence identity. (B) ELISA with lentil Lc-def and pea Psd1 using rabbit polyclonal anti-Lc-def antibodies. Error bars represent standard deviation between technical replications. (C) SDS-PAGE analysis or (D) immunoblotting with rabbit polyclonal anti-Lc-def antibodies of Lc-def in the absence (1) or present (2) of BME and lysate of *E. coli* cells (3) expressing the fusion protein 8His-Trx1-Lc-def (MW 19kDa).

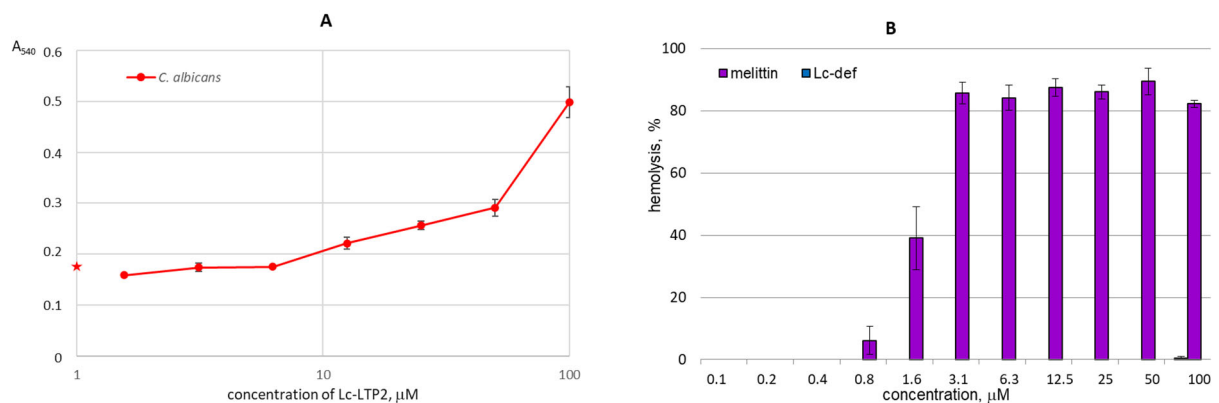


Figure S3. (A) The growth of the clinical isolate of *C. albicans* in the presence of different concentrations of lentil lipid transfer protein Lc-LTP2 (100, 50, 25, 12.5, 6.25, 3.125 and 1.56 µM). Asterisk (red) shows optical density of the control without peptide. (B) Hemolytic assay of different concentrations of Lc-def and melittin as positive control. Error bars represent standard deviation between technical replications.

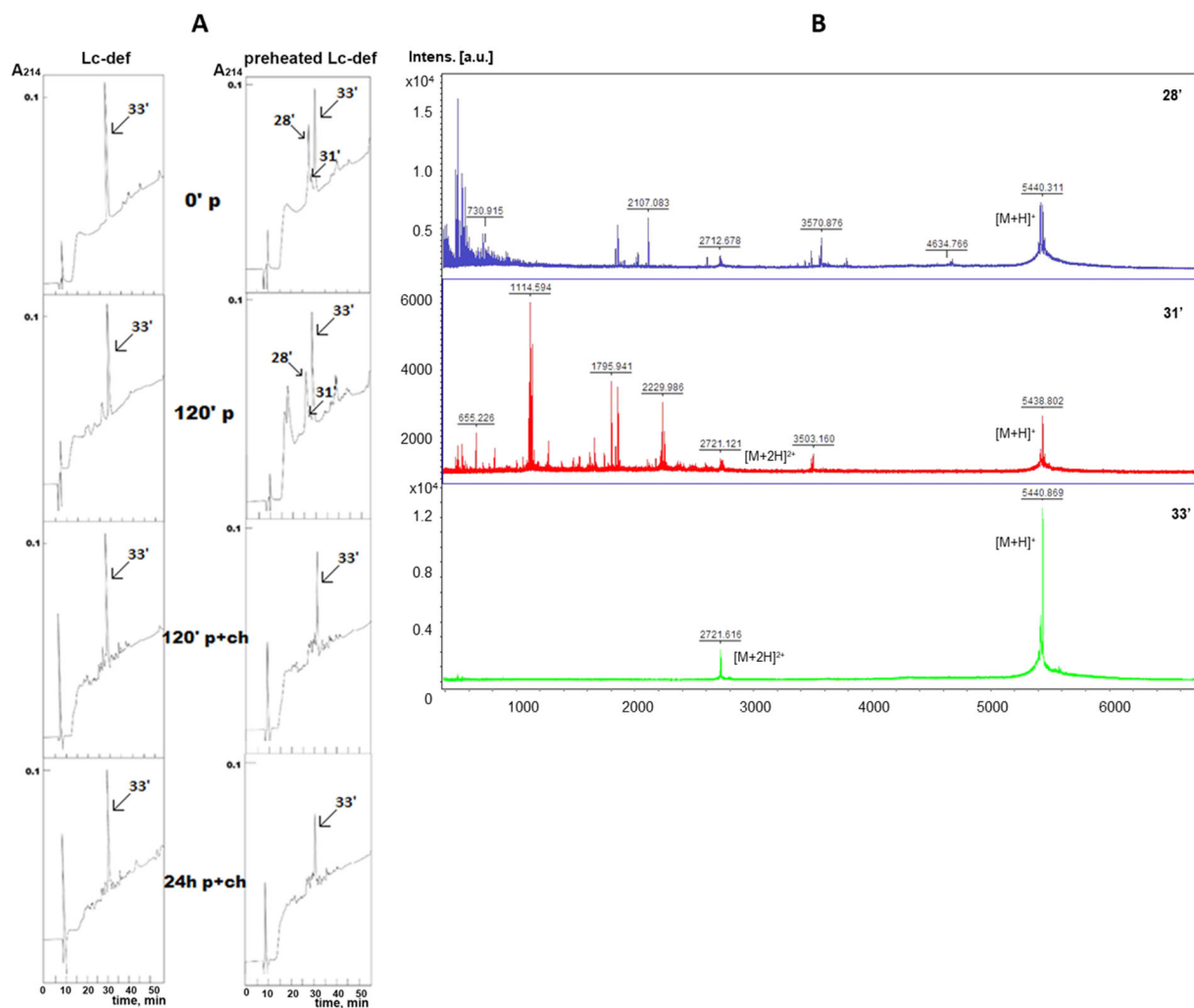


Figure S4. (A) Effect of heating on the sensitivity of Lc-def to digestive enzymes as detected by means of RP-HPLC on Luna C₁₈ column (5 μ m, 250 \times 4.6 mm; Phenomenex) at a flow rate of 0.5 mL/min, using a linear gradient of acetonitrile concentration from 5 to 80% for 60 min in 0.1% trifluoroacetic acid (0' and 120' p— pepsin digestion during 10 s and 120 min; 120' and 24 h p+ch—subsequent digestion by mixture trypsin/ α -chymotrypsin during 120 min and 24 h). (B) MALDI mass spectra of the RP-HPLC fractions (from 28, 31 and 33 min) of Lc-def digests.