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The fusion of *Toxoplasma gondii* SAG1 vaccine candidate to *Leishmania infantum* heat shock protein 83-kDa improves expression levels in tobacco chloroplasts

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chLiHsp83-SAG1 sequence

MSKGEELFTGVVPILPGTETFAFQAEINQLMSLIINTFYSNKEIFLRELISNASDA
CDKIR**YQSLTDPSVLGESPR**LCIRVVPDKENKTLTVEDNGIGMTK**ADLVNNLGTIA**
RSGTKAFMEALEAGGDMSMIGQFGVGFYSAYLVADRVTVTSKNNSDESYVWESSAC
GTFTITSTPESDMKRGTRITLHLK**EDQMEYLEPR**RLKELIKKHSEFIGYDIELMVE
KTTEKEVTDEDEEDTKKADEDEEPKVEEVREGDEGEKKKTKKVKEVTKEYEVQNK**H**
KPLWTRDPK**DVTKEEYAAFYK**AISNDWEDPRATK***HFSVEGQLEFR***SIMFVPK**RAPF**
DMFEPNKKRNNIKLYVRRVFIMDNCEDLCPDWLGFVK***GVVDSEDLPLNISR***ENLQQ
NKILKVIRKNIVKKCLEMFDEVAENKEDYK**QFYEQFGK**NIKLGIHQDTANRKKLME
FVRFYSSESGEEMTTLKDYVTRMKAGQKSIYYITGDSKK**KLESSPFIEQAK**RRGLE
VLFMTEPIDEYVMQQVKDFEDKKFACLTK**EGVHFEESEEEKQQR**EEEKAACEKLCK
TMKEVLGDKVEKVIVSECLSTSPCILVTSEFGWSAHMEQIMRNQALR**DSSMAQYMM**
SKKTMELNPRHPIIKELRR**RVDADENDKAVK**DLVFLLFDTSLLTSGFQLEDPTGYA
ERINRMIKLGLSLDEEEEVVAAEATVAETAPAEVTAGTSSMEQVDEFFTLKCPK***TA***
LTEPPTLAYSPNRQICPAGTTSSCTSKAVTLSSLIPEAEDSWWTGDSASLDTAGIK
LTVPIEKFPVTTQTFVVGCIKGDDAQSCMVTVTVQARASSVVNNVARCSYGADSTL
GPVKLSAEGPTTMTLVCGKDGVKVPQDNNQYCSGTTLTGCNEKS**FKDILPKLTENP**
WQGNASSDKGATLTIKKEAFPAESKSVIIGCTGGSPEKHHCTVK**LEFAGAAGSAK**S
AAGTASHVSIFAMVDLDKPLDGEYFTLQIRGR**ERFEMFR**ELNEALELKDAQAGKEP
GAAAHHHHHH

Figure S1: Identity of chLiHsp83-SAG1 protein using MALDI TOF/TOF mass spectrometry. All fragments in bold letters and underlined were identified using Peptide Mass Fingerprinting (PMF) method and the fragments that also are showed in italic letters were validated by Peptide Fragmentation Fingerprinting (PFF) method.

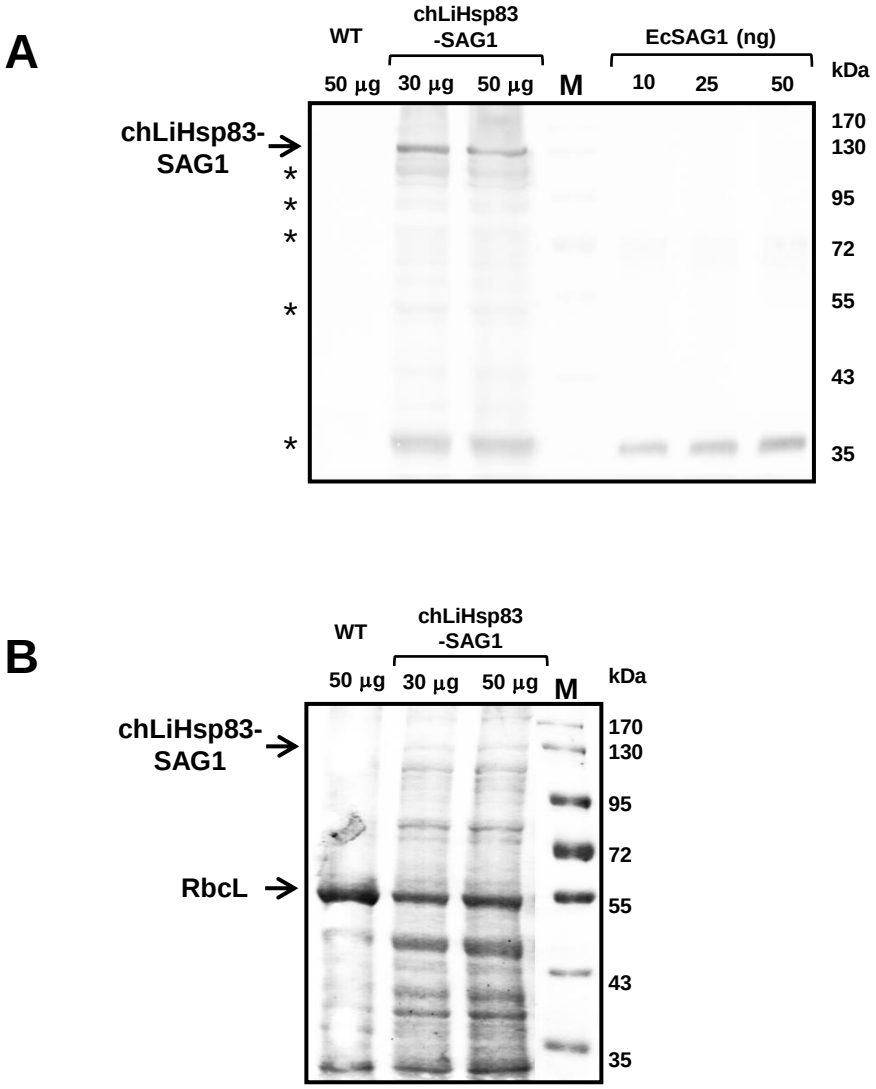

Figure S2: Western bot analysis and coomassie blue-stained SDS-PAGE of mature and old lyophilized leaves expressing LiHsp83-SAG1. The equivalent to 0.5 mg and 0.3 mg of lyophilized leaf fresh weight (50 and 30 µg of total protein per well, respectively) were separated by 10% SDS-PAGE and immunoblotted with an anti-SAG1 polyclonal antibody (A) or analyzed by coomassie blue (B). A dilution series of purified *E. coli*-derived SAG1 (EcSAG1; 10, 25 and 50 ng) were used as reference for protein quantification. chLiHsp83-SAG1: transplastomic plant expressing LiHsp83-SAG1 protein; WT: wild-type tobacco plant; RbcL: Ribulose bisphosphate carboxylase large subunit; M: molecular weight marker (Fermentas). chLiHsp83-SAG1 protein migrates as a 130-kDa band. The asterisks indicate proteolytic degradation products.