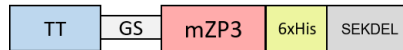


Supplementary Material

Plant-produced mouse-specific zona pellucida 3 peptide induces immune responses in mice

a

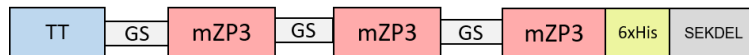


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M Q Y I K A N S K F I G I T E L R S G G
ATGCAATATATCAAGGCTAATTCCAAGTTCATAGGCATTACTGAACTTAGATCTGGTGGA
                                     mZP3-T
G G S G G G G S G G G G S L K C S N S S
GGAGGATCTGGAGGTGGTGGGTCAGGTGGAGGTGGGTCACTTAAGTGTAGCAACAGTTCT
mZP3-B
S S Q F Q I H G P R C T H H H H H H S E
AGTTCCTCAATTCAGATTTCATGGCCCAAGATGTACACACCATCATCACCATCACTCTGAG
K D E L *
AAAGATGAATTGTAA

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b



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M Q Y I K A N S K F I G I T E L G G G G
ATGCAATATATCAAGGCTAACTCTAAGTTTATCGGAATTACTGAACTTGGAGGAGGCGGG
                                     mZP3-T
S G G G G S G G G G G S C S N S S S S Q F
TCTGGTGGAGGAGGAGTGGAGGAGGAGGTCATGCTCTAATTCTTCTCATCTCAGTTT
                                     mZP3-B
Q I H G P R G G G G S G G G G S G G G G
CAGATACATGGTCCGAGAGGAGGAGGCGGTTTCAGGCGGAGGTGGTAGTGGTGGTGGTGGG
mZP3-T
S C S N S S S S Q F Q I H G P R G G G G
TCTTGTAGTAATTCAGCTCTTCTCAATTCCAAATTCATGGTCCACGTGGTGGCGGAGGC
mZP3-B
S G G G G S G G G G S C S N S S S S S Q F
TCAGGTGGTGGTGGCTCAGGAGGTGGCGGGTCTTGTTCCAATAGCAGTAGTTCACAATTC
mZP3-T
Q I H G P R H H H H H H S E K D E L *
CAGATTTCATGGGCTAGGCATCATCACCACCATCACAGCGAGAAAGATGAATTGTAA

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Figure S1. Protein and nucleotide sequences codon-optimized for *N. benthamiana*. The nucleotide and amino acid sequences of synthesized mZP3-1 (a) and mZP3-3 constructs (b). Nucleotides are represented in black and amino acids in blue. Mouse Zona pellucida 3-peptide (mZP3) sequences are underlined. The locations of the mouse ZP3 contraceptive T and B cell epitopes (mZP3-T and mZP3-B) are shown above the sequences.

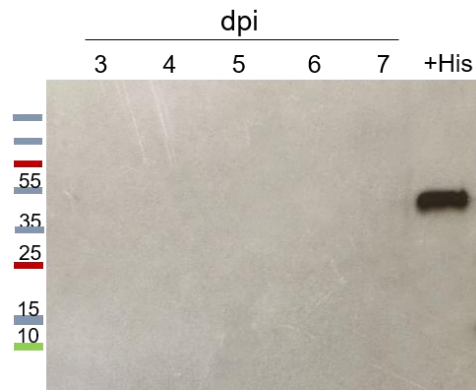


Figure S2. Western blot of crude leaf extracts of *N. benthamiana* leaves infiltrated with mZP3-1. No protein band corresponding to the expected size of the mZP3-1 protein was detected. Western blot was probed with anti-His monoclonal antibody. +His, a His-tagged protein as a positive western blot control. dpi, days post infiltration.

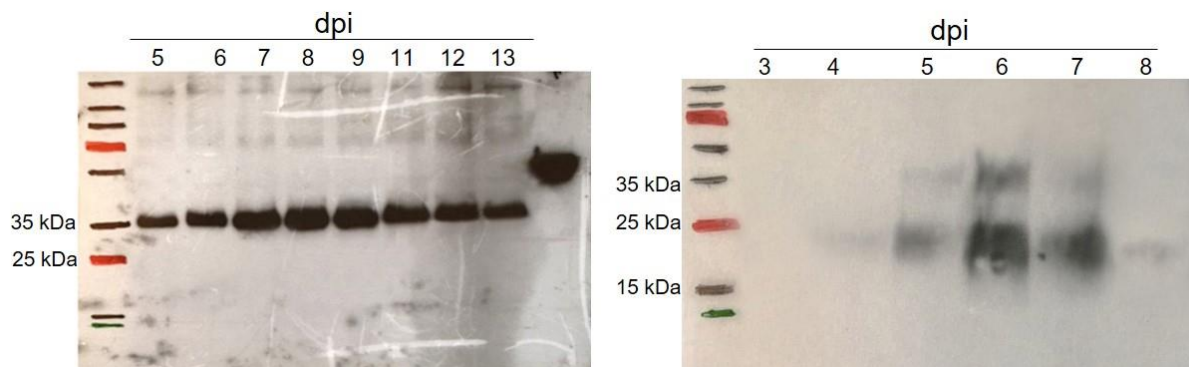


Figure S3. Western analysis of plant-produced GFP-mZP3-1 (left) and mZP3-3 (right). Figure 2 was cropped from these western blot images. dpi, days post infiltration.

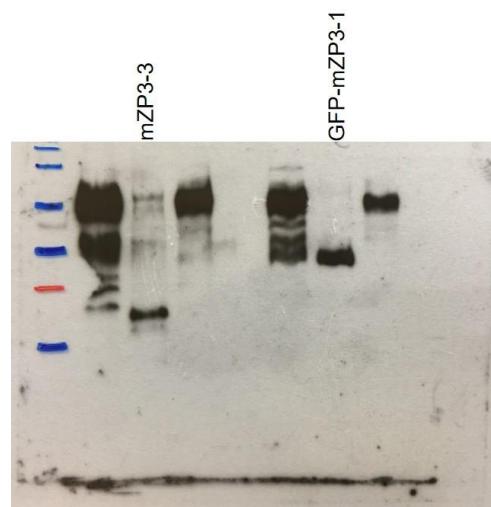


Figure S4. Profile of lectin binding to the *N. benthamiana*-expressed proteins. The panels of figure 3 were cropped from this lectin blot image.

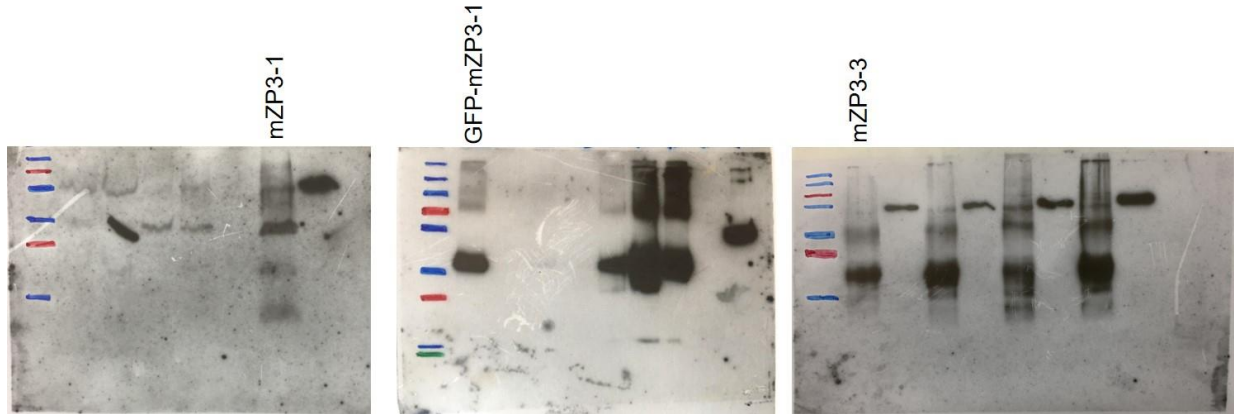


Figure S5. Detection of purified plant-expressed recombinant proteins. The panels of figure 5 were cropped from these western blot images.

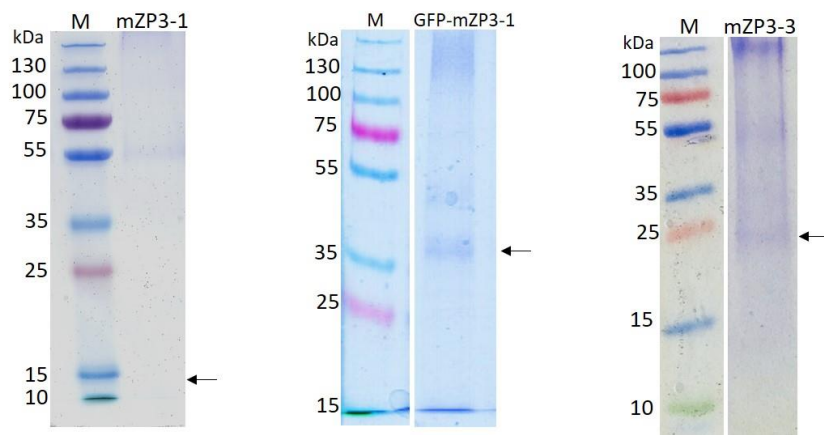


Figure S6. Coomassie-stained SDS-PAGE of purified plant-expressed recombinant proteins. M, Marker. Arrows indicate the expected size of the proteins.