SUPPLEMENTARY MATERIALS

Suppression of autoimmune demyelinating disease by preferential stimulation of CNS-specific CD8 T cells using Listeria-encoded neuroantigen

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Supplementary Figure Legends:

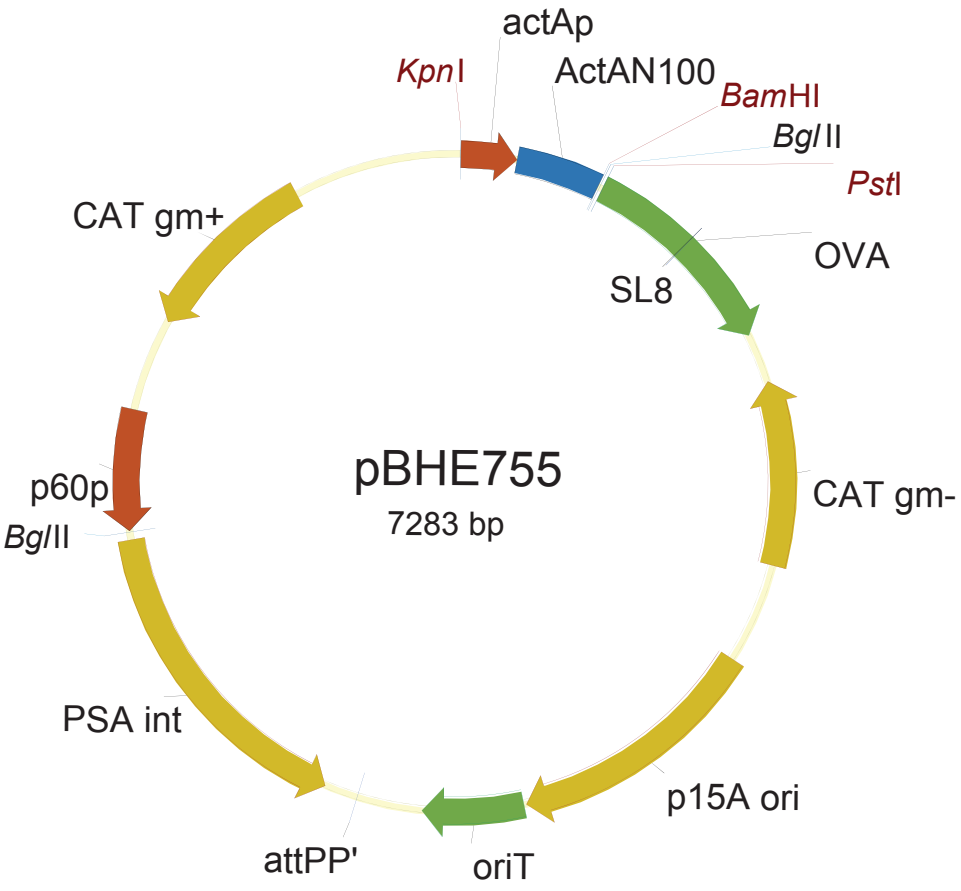
Figure S1. Vector used to create recombinant *Listeria* expressing PLP175-194, adapted from *Lauer et. al*. The CNS peptide sequence was inserted between the *Bam*HI and *Pst*I restriction sites.

Figure S2. Peptide dose-response identifies PLP-specific CD8 T cells at various stimulatory concentrations. Intracellular cytokine staining was performed on bulk splenocytes harvested from LM-PLP infected B6 mice on d7 post infection. Splenocytes were left unstimulated, or stimulated with either OVA257-264 or varying concentrations of PLP178-191. Flow plots show antigen-specific IFN-γ production by CD8 (top panels) and CD4 (bottom panels) T cells.

Figure S3. LM-PLP infection of SJL/J mice leads to attenuation of EAE. Female 6-8 wk old SJL/J mice were infected with LM-PLP or LM-OVA on d-7 and immunized with PLP178-191/CFA on d0 and monitored for clinical disease. Data are representative of three independent experiments with at least seven mice per group per experiment. *$p < 0.05$, ***$p < 0.001$.

Figure S4. Histological analysis of the spinal cord reveals less inflammation in LM-PLP infected animals immunized with PLP178-191/CFA.
H&E staining of lumbar spinal cord from infected and immunized B6 mice on d18 post-immunization depicts differences in inflammation (thick arrows) and axonal sheath swelling (thin arrows). Data are representative of at least two independent experiments.

Figure S1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KpnI
ActAN100
BamHI
BglII
PstI
CAT gm+
OVA
SL8
pBHE755
7283 bp
p60p
BglII
CAT gm-
PSA int
p15A ori
attPP'
oriT

Figure S2

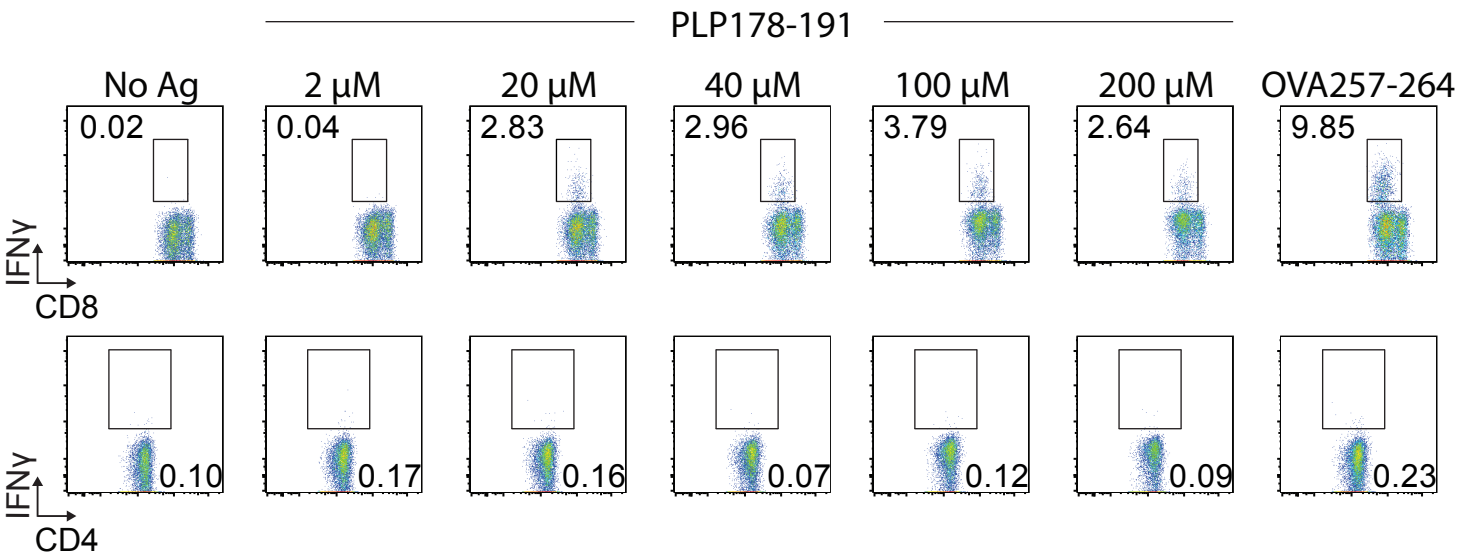
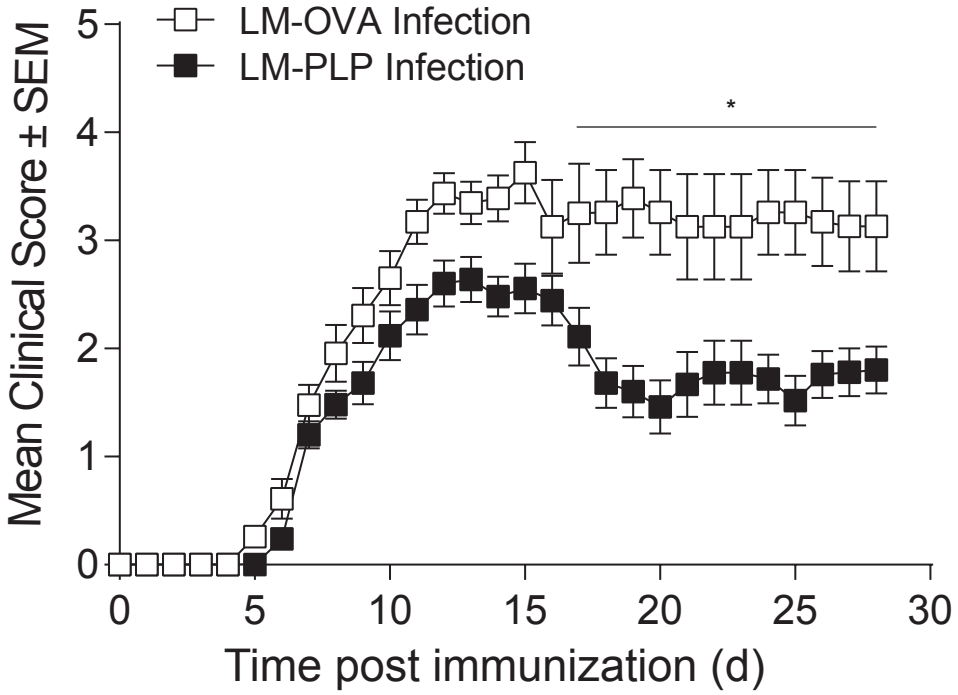

PLP178-191
No Ag
2 µM
20 µM
40 µM
100 µM
200 µM
OVA257-264
0.02
0.04
2.83
2.96
3.79
2.64
9.85
IFNγ
CD8
0.10
0.17
0.16
0.07
0.12
0.09
0.23
IFNγ
CD4

Figure S3



Infection	Onset	Peak***	Mortality***	CDI ***
LMOVA121-386	6.6 ± 1.2	4.2 ± 0.9	12/23	62.0 ± 22.9
LMPLP175-194	7.0 ± 0.8	3.24 ± 0.8	2/25	39.0 ± 16.4

Figure S4