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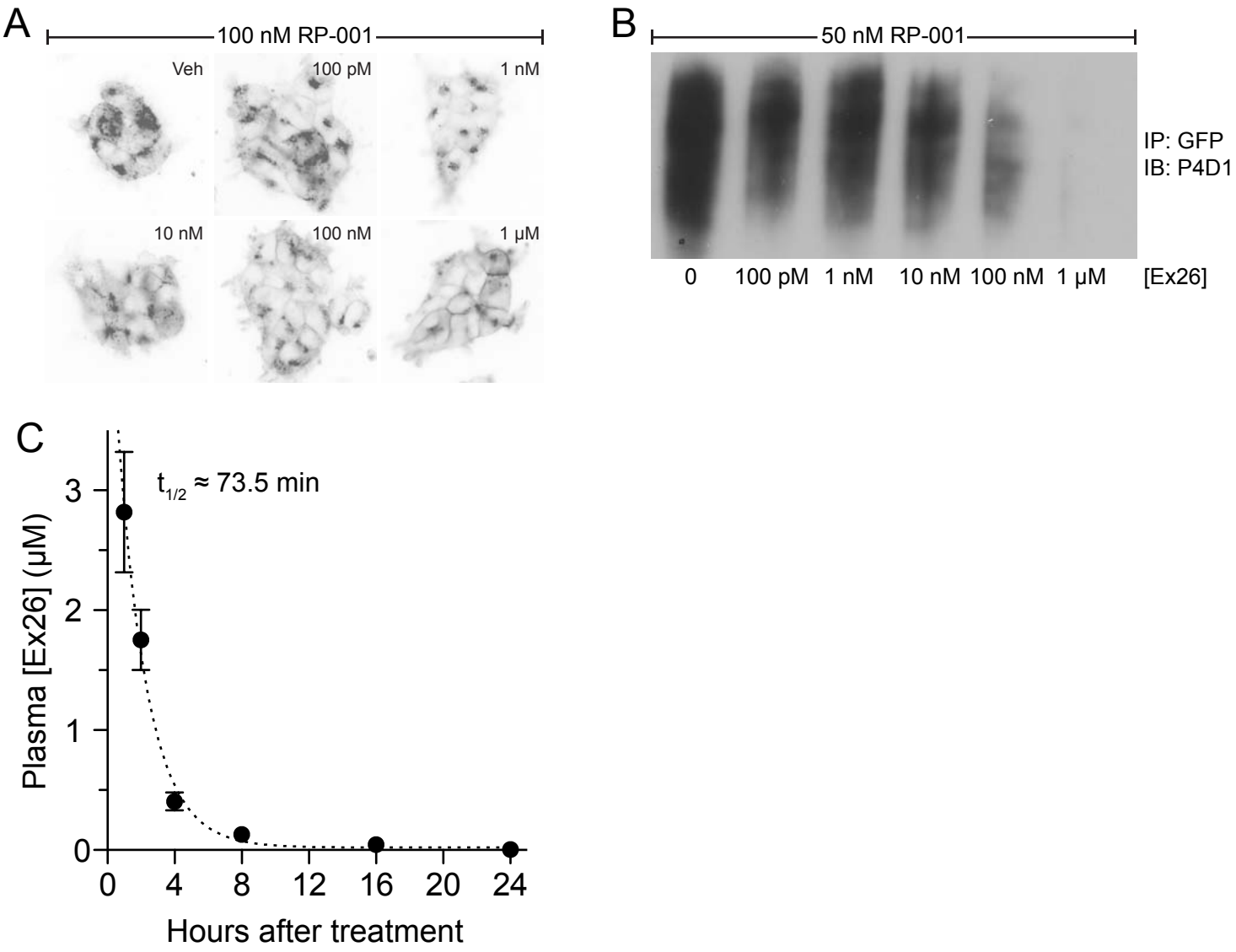
S1P1 Receptor Upregulation and Amelioration of
Experimental Autoimmune Encephalomyelitis by an S1P1 Antagonist
Molecular Pharmacology

Supplemental Figure 1



Supplemental Figure 1: Ex26 inhibits S1P1 internalization and polyubiquitinylation in vitro and exhibits a short half-life in vivo. **(A)** Ex26 inhibits S1P1 internalization induced by the S1P1 agonist RP-001 in vitro. HEK cells expressing S1P1-eGFP were pretreated with the indicated concentrations of Ex26 for 1 hour, and then were incubated in the presence of 100 nM RP-001 for an additional hour. **(B)** Ex26 inhibits RP-001-induced polyubiquitinylation. Cells were treated as in (A), but were incubated with 50 nM RP-001. Lysates were immunoprecipitated with an antibody specific to GFP, then blotted for P4D1 to detect ubiquitin. **(C)** Ex26 has a relatively short half-life following treatment with 3 mg/kg i.p. All data are representative of at least two experiments, with (C) utilizing 4 mice per group per experiment.

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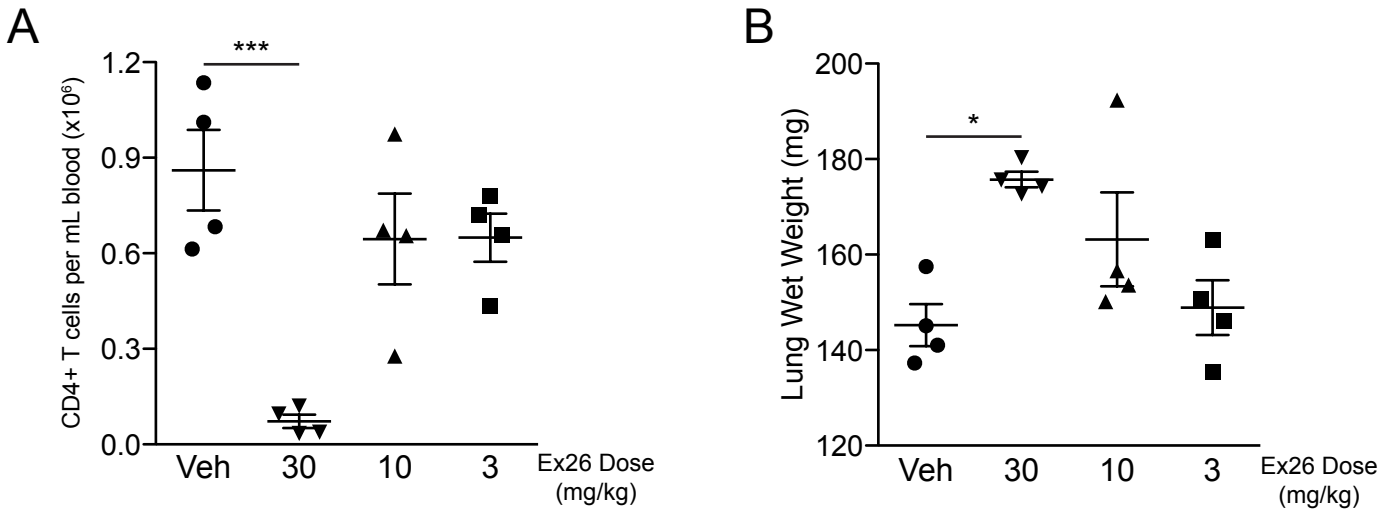
Supplemental Figure 2



Supplemental Figure 2: High dose Ex26 can cause sustained lymphocyte sequestration and pulmonary edema. **(A)** CD4+ T cell counts from the blood of mice treated i.p. 24 hours previously with the indicated doses of Ex26. **(B)** Lung wet weights of mice treated i.p. 24 hours previously with the indicated doses of Ex26. Graphs are representative of two experiments, with 4 mice per group per experiment and are plotted as mean ± S.E.M. * $p<0.05$, *** $p<0.001$ as determined by one-way ANOVA with Bonferroni's multiple comparison post-test.