

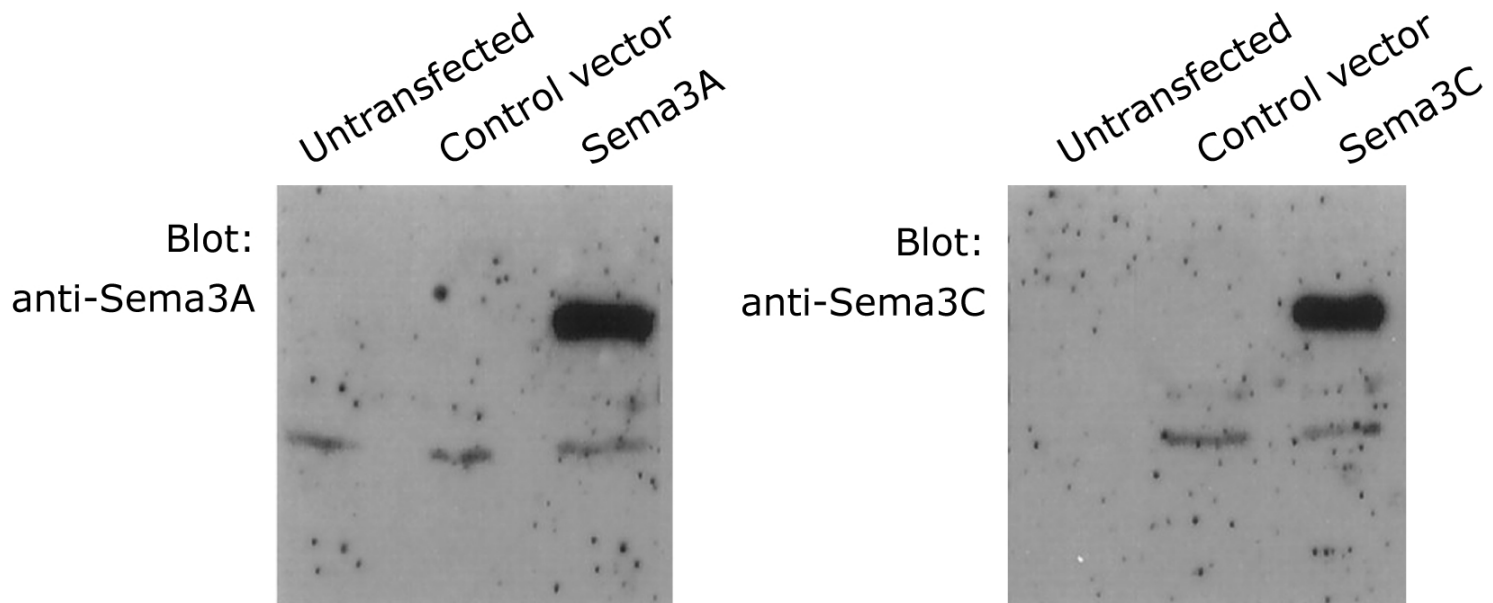
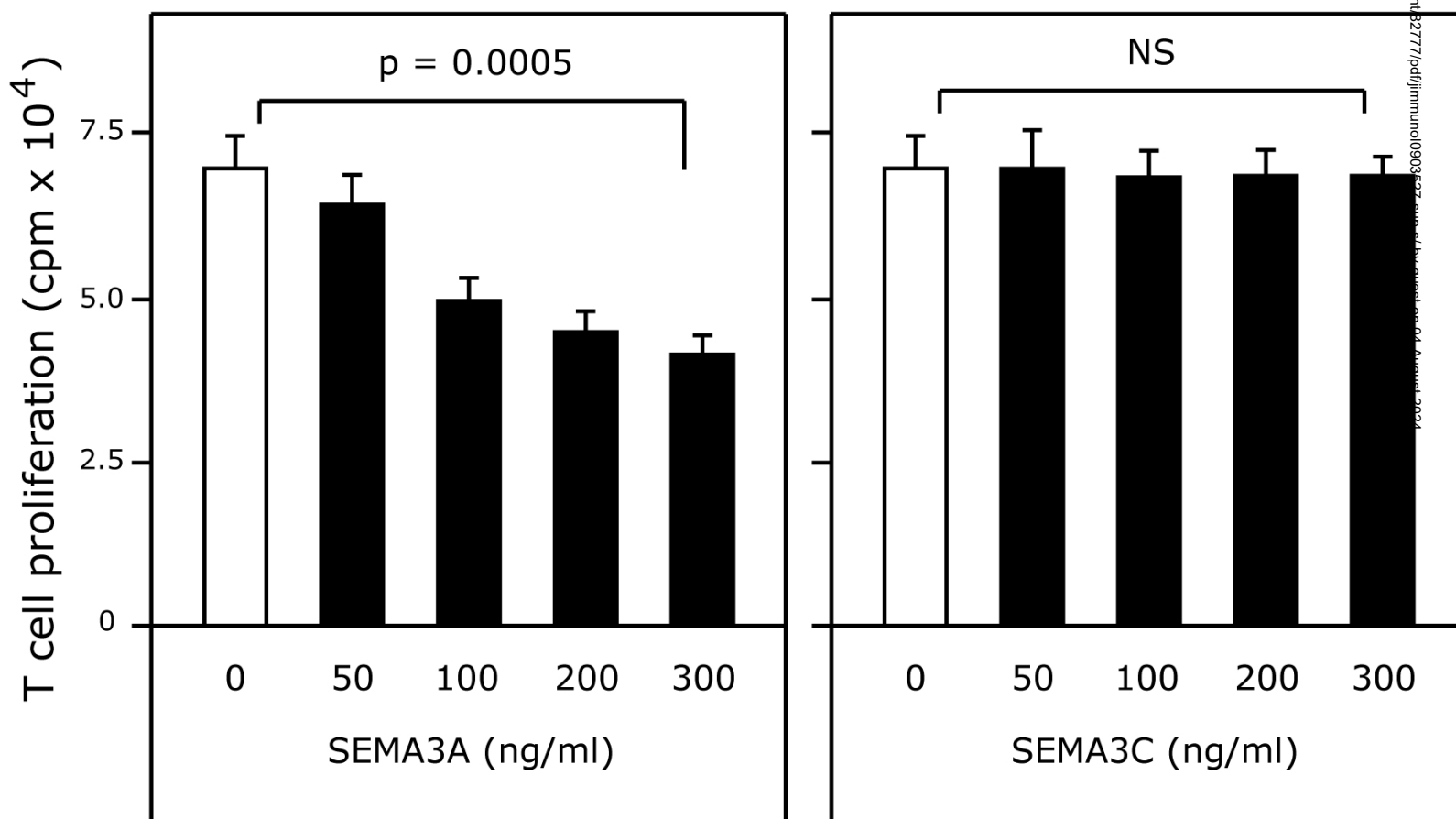
Supplementary Figure Legend

Supplementary Figure 1. Purification of recombinant Sema3A and Sema3C and their effects on anti-CD3/CD28-induced T cell proliferation. **(A)** Immuno-blot containing the fraction derived from an anti-FLAG affinity column that was loaded with serum-free conditioned medium from Sema3A-expressing HEK293 cells (Sema3A) or Sema3C-expressing cells (Sema3C). A purified corresponding fraction eluted from a similar affinity column loaded with conditioned medium of empty vector-transfected HEK293 cells (Control vector) is also shown. **(B)** T cells were activated by anti-CD3/anti-CD28 antibodies in the absence or in the presence of recombinant semaphorins at different doses as indicated. T cell proliferation was then measured by [³H]thymidine incorporation. Results are representative of triplicate values of one representative experiment out of four. *p* values from Student's unpaired *t*-test are indicated. *ns*, not significant.

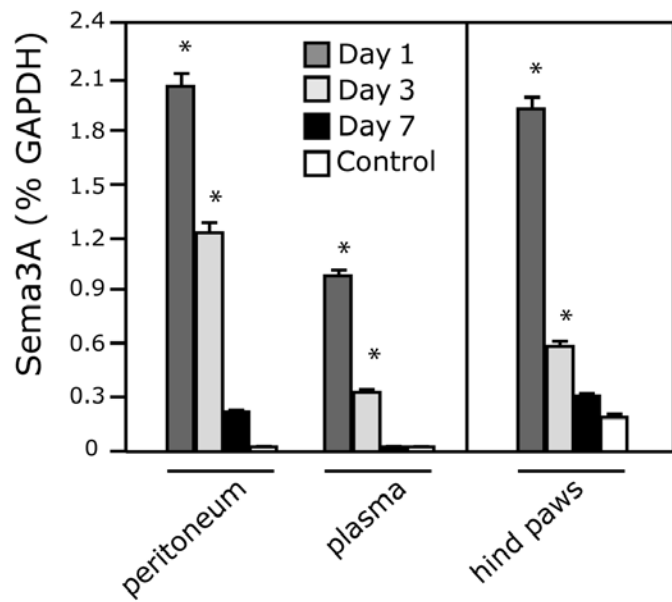
Supplementary Figure 2. **(A)** Sema3A protein levels in nonarthritic DBA/1 mice following i.p. Sema3A administration. Mice were sacrificed at 1, 3 and 7 days (*n* = 4 mice/time point). Following i.p. Sema3A administration, significantly elevated levels of Sema3A in peritoneal lavage, blood plasma, and hind paw homogenates were demonstrated as determined by densitometry analysis (% GAPDH expression) of gel data. Equal loading of proteins was confirmed using GAPDH as an internal control. Values were recorded as the mean ± s.e.m. **(B)** The mean weights of mice in each group of the experiment described in the legend of Figure 3 (prevention study) are presented. **(C)** Male DBA/1 WT mice were immunized i.d. with CII emulsified in CFA at day 0 and treated with Sema3A-encoding plasmid or control vector. At the indicated time points, PBMC were isolated, whole cell protein extracts were generated and western blotting with a Sema3A-specific Ab was performed. After stripping, reblotting with a GAPDH-specific Ab was performed to demonstrate equal protein loading. The results are representative for two independent experiments with three

mice per time point. **(D)** Up-regulation of Sema3A protein expression in mice treated with Sema3A-encoding plasmid. Spleen cell lysates isolated from mice ($n = 5$) were harvest 24 h after the last DNA treatment (day 48) and Sema3A protein expression was examined by Western blot as described in Materials and Methods. Equal loading of proteins was confirmed using GAPDH as an internal control. Blot shown is a representative from three independent experiments with similar results.

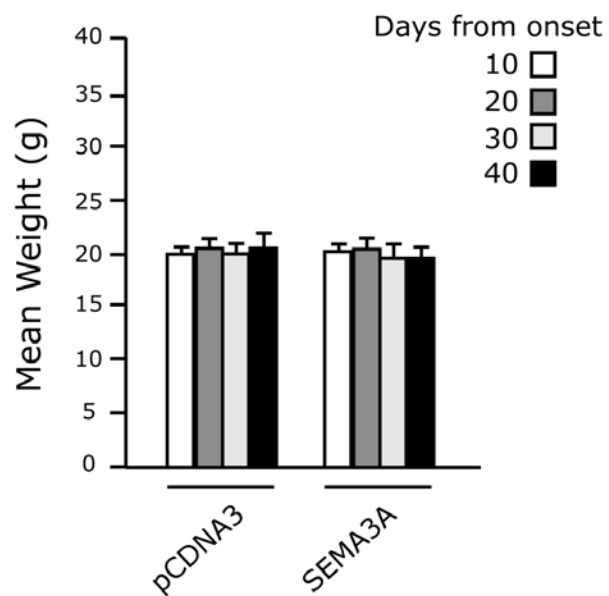
Supplementary Figure 3. **(A)** Representative H&E-stained joint tissue sections from DBA/1 mice from a CIA prevention study. Scale bars, 40 μm . **(B)** Histopathological scores of inflammation (e.g. synovitis and the inflammatory infiltrate), pannus formation, and bone and cartilage erosions in DBA/1 mice with CIA in the prevention studies ($n = 8$ per group). Values are mean \pm s.e.m. from one independent representative experiment of two. * $P < 0.05$ by Mann-Whitney U test.

A**B**

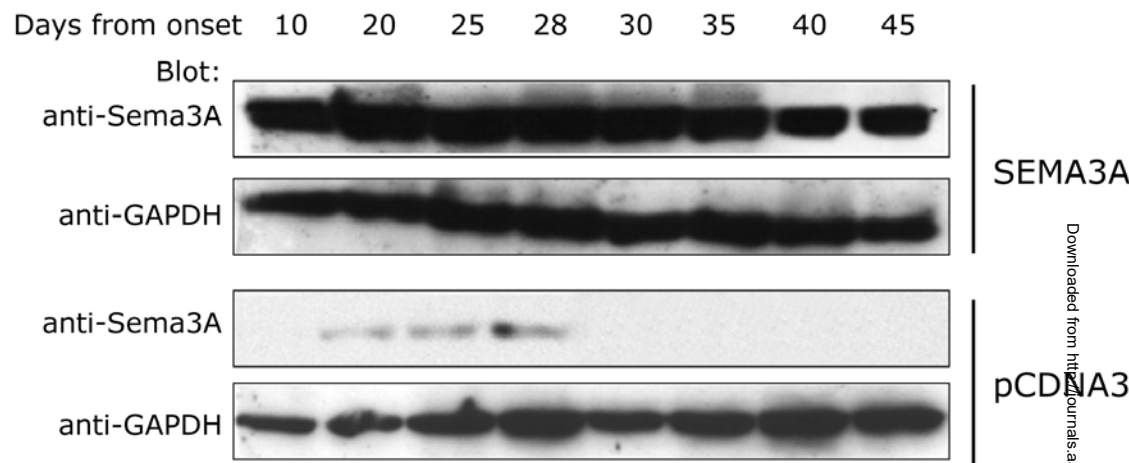
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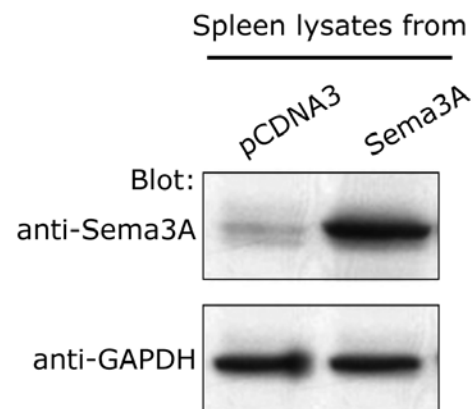
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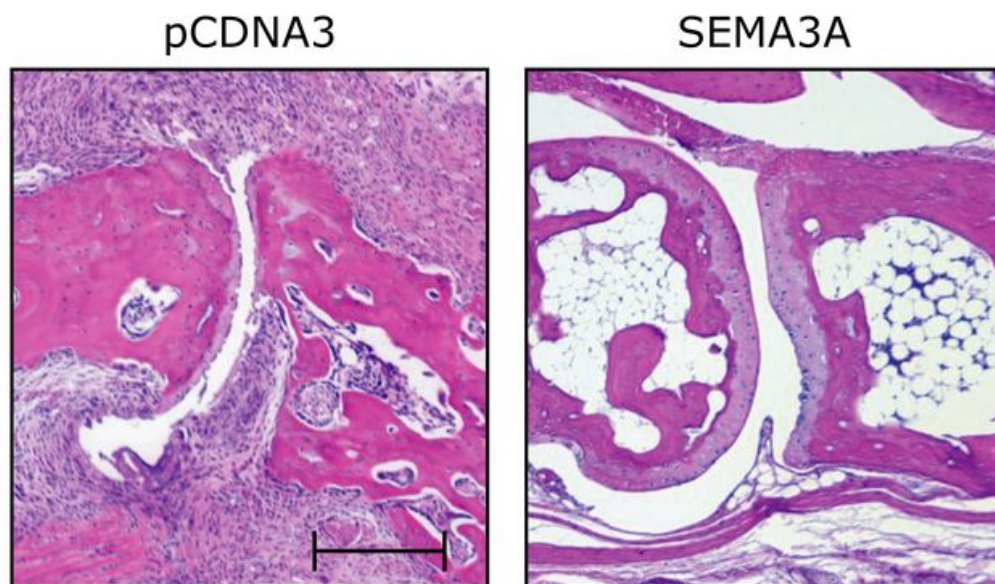
C



D



A



B

