Supplementary Data

Materials and Methods

Quantification of GFP-expressing cells in the spleen

Immunofluorescence of frozen sections was performed for Alexa Fluor 555–conjugated GFP Polyclonal Antibody (1:300) (Thermo Fisher Scientific, cat. no. A-31851). Frozen sections were washed three times with PBS, followed by a 1-h incubation in block solution and then incubation with Sudan black B (Sigma, cat. no. 199664) for 30 sec at room temperature. The sections were washed three times with PBS and one time with 70% ethanol and were incubated with the antibody for 1 h at room temperature before addition of DAPI for 5 min. Sections were analyzed by photomicroscopy using the EVOS FL Color Imaging System microscope. Imaging and densitometry analyses of at least 10 spleen images taken from each of the 12 animals described above at 4× and10× magnification were carried out as described in the materials and methods.

Supplementary Figures

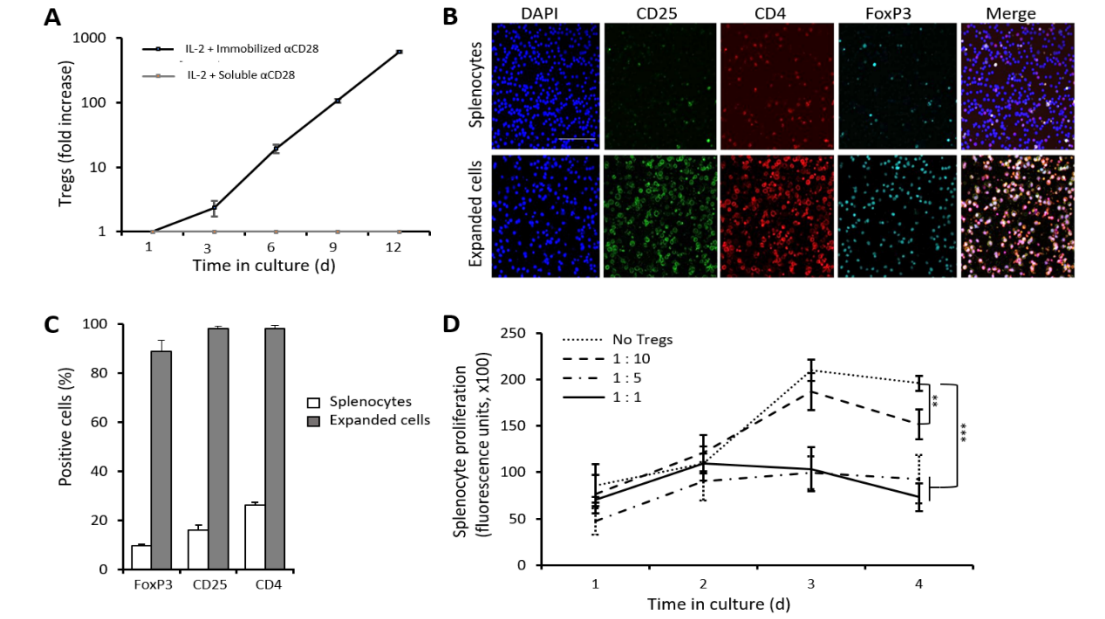

Fig. S1. Isolation, expansion and functional validation of Tregs. (**A**) Proliferation of Tregs depending on whether αCD28 superagonist antibody was surface-adsorbed via a secondary antibody or was provided in soluble form in the medium. (**B**) Representative images showing immunocytochemistry of *ex vivo*–expanded Tregs and rough splenocytes immunolabeled for CD25, CD4 and FoxP3, scale bar = 100 µm. (**C**) Quantification of the Treg immunophenotype shows expanded cells are >98% CD4+ and CD25+ and >89% FoxP3+. (**D**) Inhibition of conA-induced splenocyte proliferation by co-culturing with expanded Tregs. $N \geq 3$, $^{**}p < 0.005$, $^{***}p < 0.0005$ by Student's t-test. Error bars depict s.e.m.

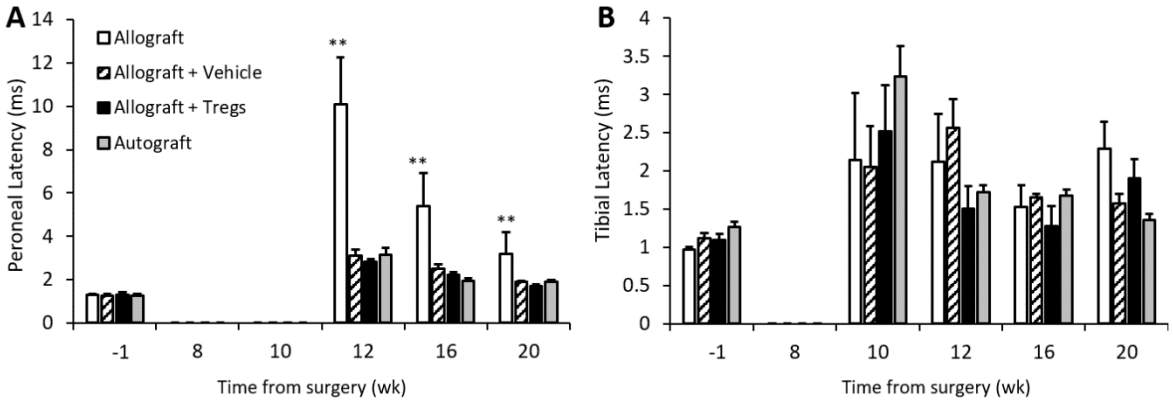

Fig. S2. CMAP latencies of regenerated sciatic nerves. (**A**) Latency of the peroneal branch of the sciatic nerve. (**B**) Latency of the tibial branch of the sciatic nerve. N = 6, **p < 0.005 by two-way ANOVA with Tukey's *post hoc* test. Error bars depict s.e.m.

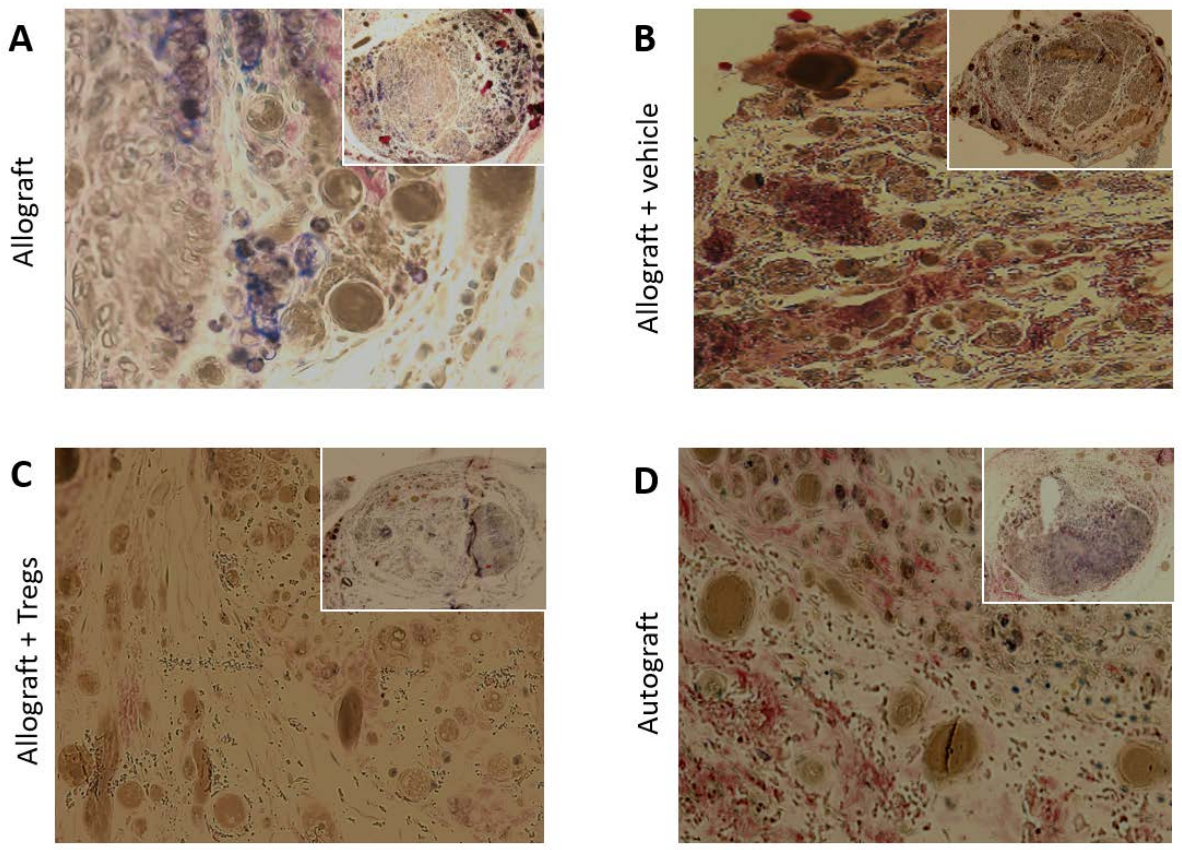

Fig. S3. Non-nerve tissue. (**A-D**) Representative images (40X and 10X) of nerve cross-sections from each of the four groups stained with Masson's trichrome.

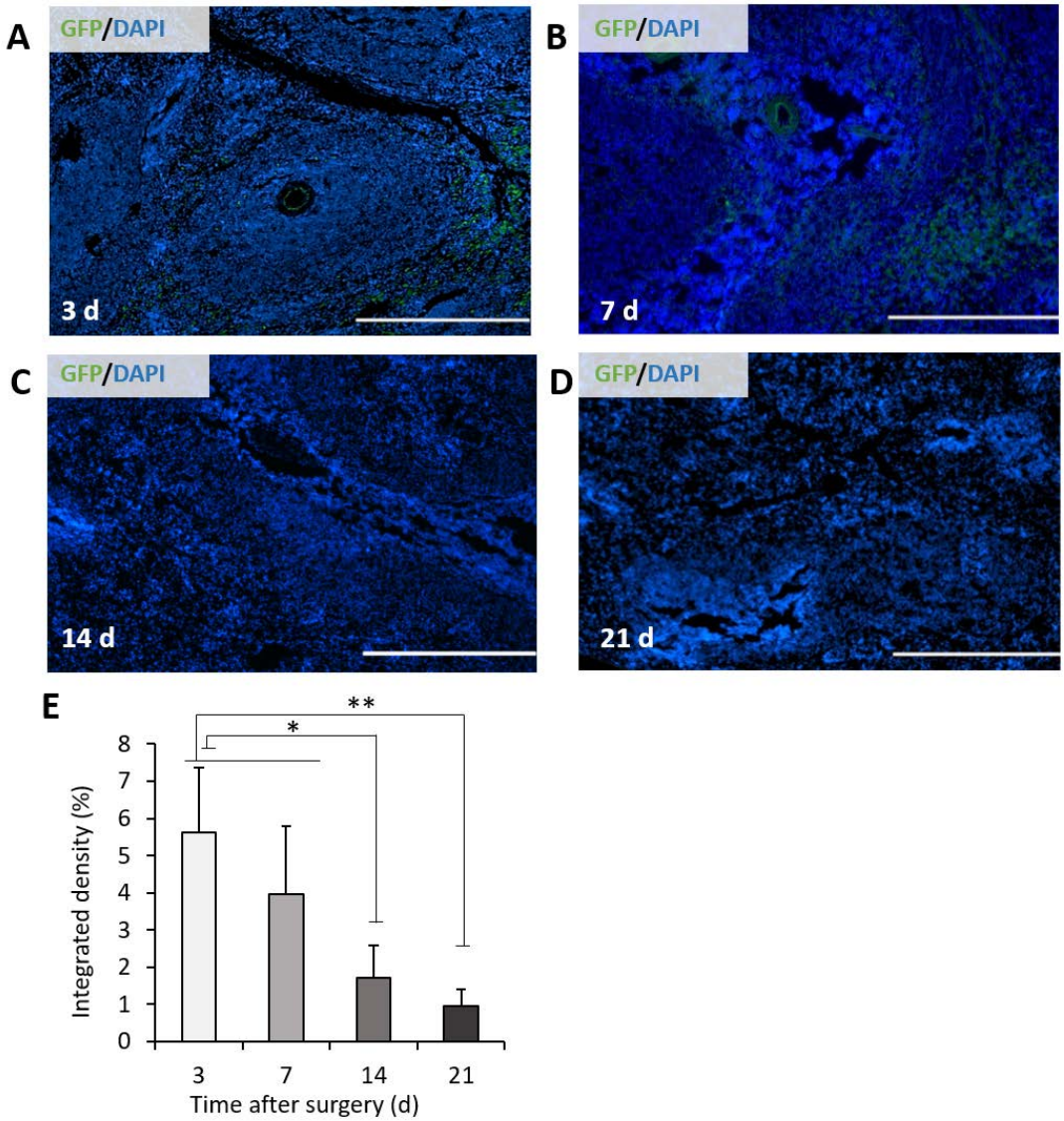

Fig. S4. Temporary splenic accumulation of GFP Tregs delivered local to the PN allograft. Images show GFP abundance in the region of the red pulp in proximity to the central arteriole or trabecular vein. (**A–D**) GFP-expressing cells at (**A**) 3 d, (**B**) 7 d, (**C**) 14 d and (**D**) 21 d. (**E**) Quantification demonstrates more GFP+ cells at 3 d and 7 d than 14 and 21 d. Images are representative of data from N = 3 rats per time point. Scale = 400 μm. *p = 0.0137, **p = 0.00172 by Tukey's multivariable test. Error bars depict s.e.m.