Science Advances
AAAS

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Supplementary Materials for

In situ recruitment of regulatory T cells promotes donor-specific tolerance in vascularized composite allotransplantation

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Published 13 March 2020, *Sci. Adv.* **6**, eaax8429 (2020)
DOI: 10.1126/sciadv.aax8429

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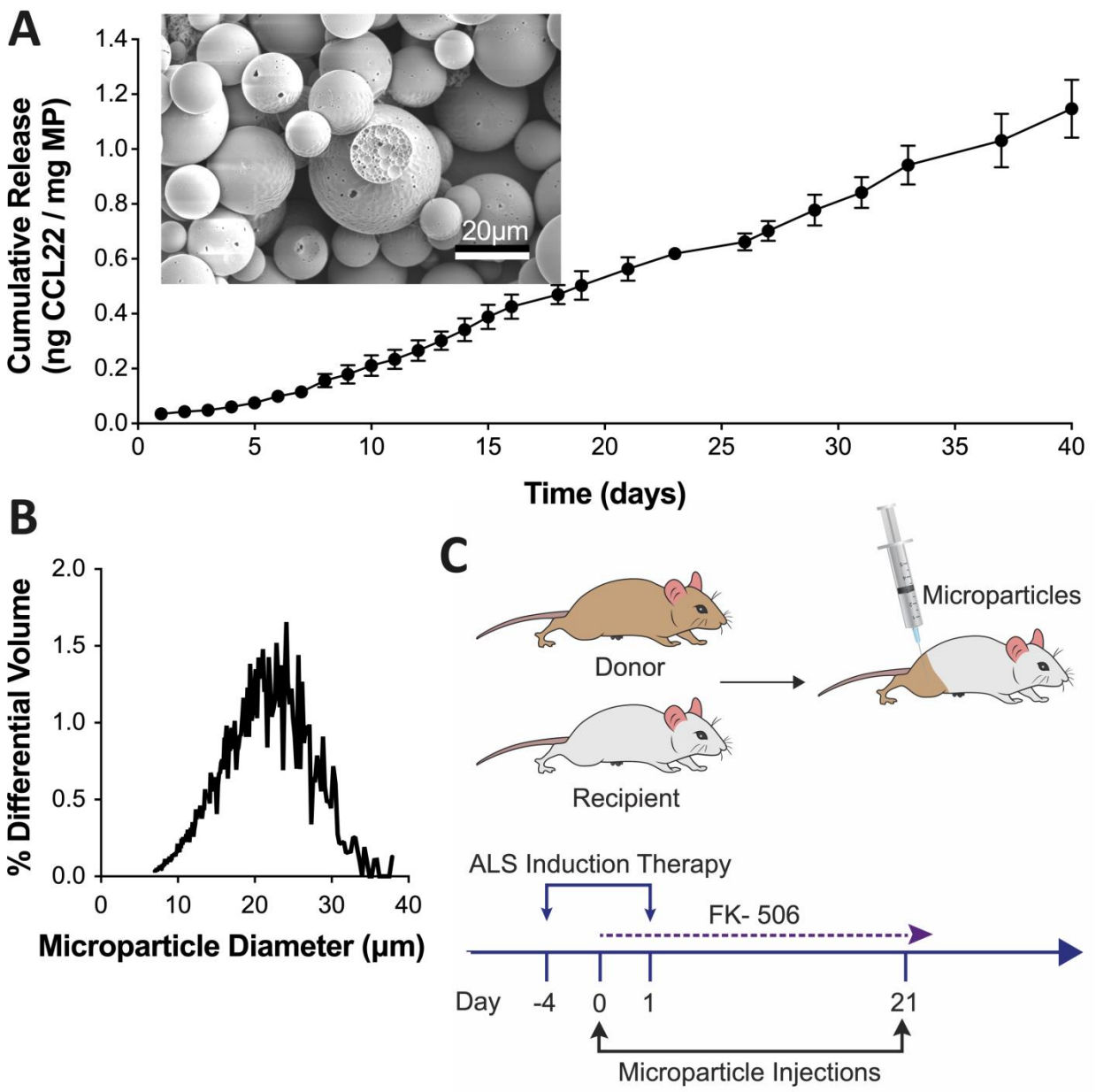

Fig. S1. Characterization of Recruitment-MP and experimental timeline for hindlimb transplantation. (**A**) Release profile shows that Recruitment-MP release CCL22 in a linear fashion for 40 days**.** Recruitment-MP are spherical in shape and slightly porous (inset), and (**B**) particle sizing reveals an average particle diameter of 18.3 μm. (**C**) All animals receive the same baseline immunosuppression protocol consisting of 21 days of FK506 (0.5 mg/kg IP daily), plus two doses of rabbit anti-rat lymphocyte serum (0.5 mL IP). Microparticles were administered subcutaneously in the transplanted graft (unless otherwise noted) on postoperative days (POD) 0 and 21.

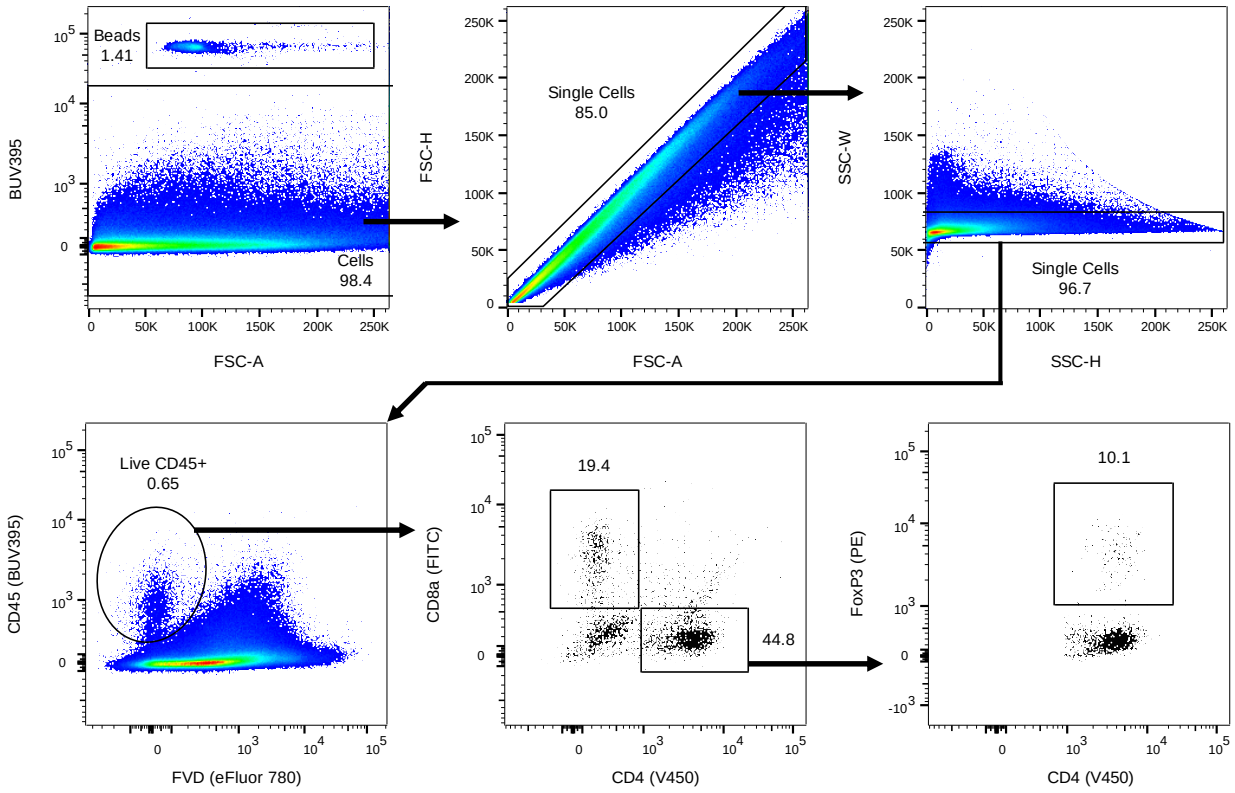

Fig. S2. Skin flow cytometry gating strategy used for Fig. 2C. Flow analysis of a single cell suspension from a 1cm^2 allograft skin biopsy. Gates identify total leukocytes (Live CD45$^+$), CD8$^+$ cytotoxic T cells, CD4$^+$ FoxP3$^+$ Treg, and CD4$^+$ FoxP3$^-$ helper T cells.

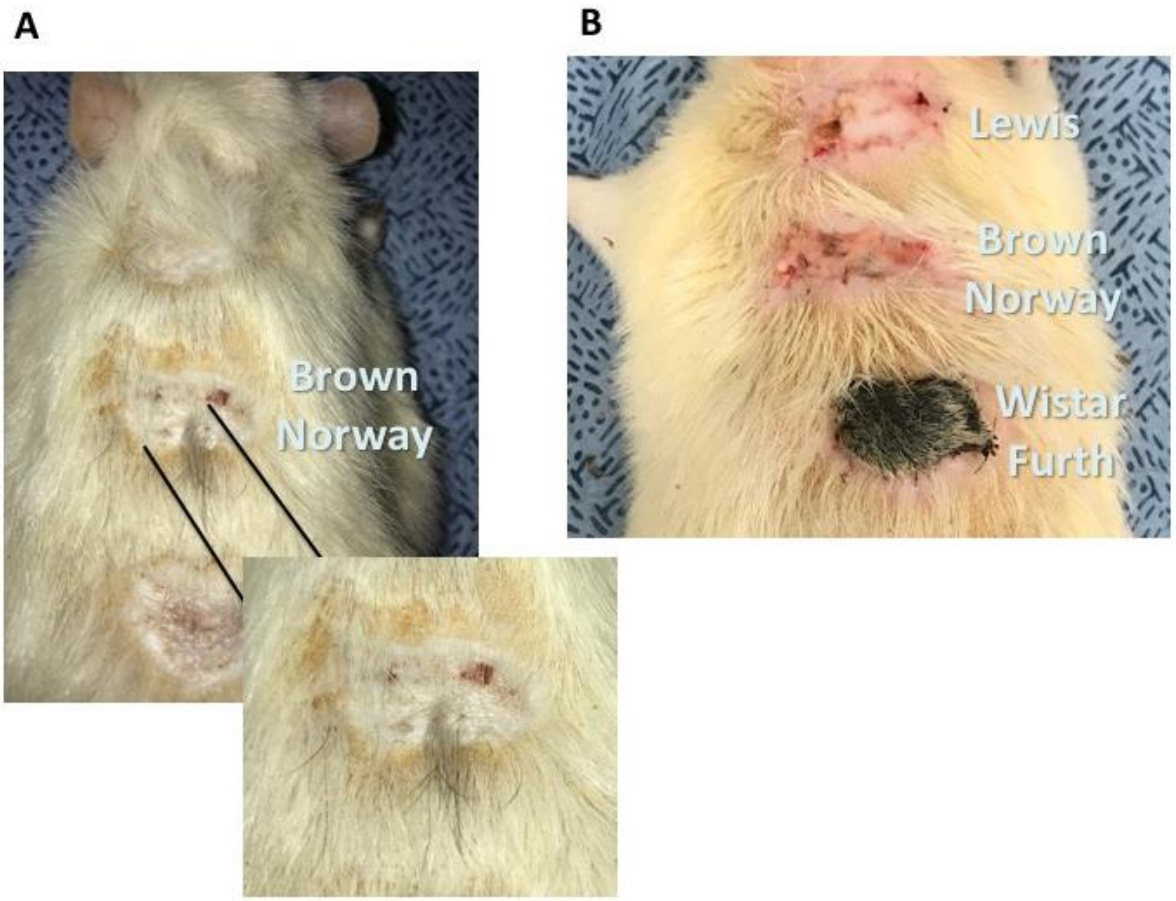

Fig. S3. Representative images of a hindlimb VCA recipient challenged with LEW, BN, and WF nonvascularized skin grafts. (**A**) Animals with long-term surviving grafts from the 50mg Recruitment-MP group were subject to a secondary skin graft challenge from same strain donors (BN) and third-party donors (WF), with autologous LEW grafts serving as controls. (A) Animals accept BN skin grafts, as evidenced BN hair growth (animal pictured 29 days after skin graft). (**B**) Conversely, animals fail to accept third-party (WF) grafts, as evidenced by complete graft necrosis (animal pictured 14 days after skin graft). WF grafts eventually slough off as an eschar, and only a contractured wound bed remains. Photo Credit: James D. Fisher, University of Pittsburgh.