Supplementary Information

Local immunomodulation with Fas ligand-engineered biomaterials achieves allogeneic islet graft acceptance

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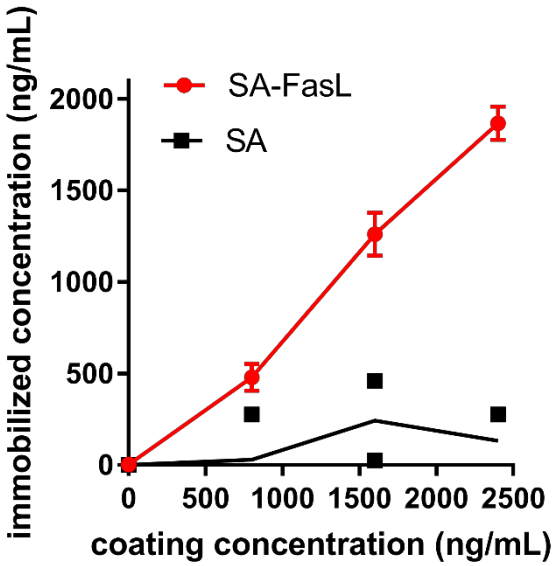
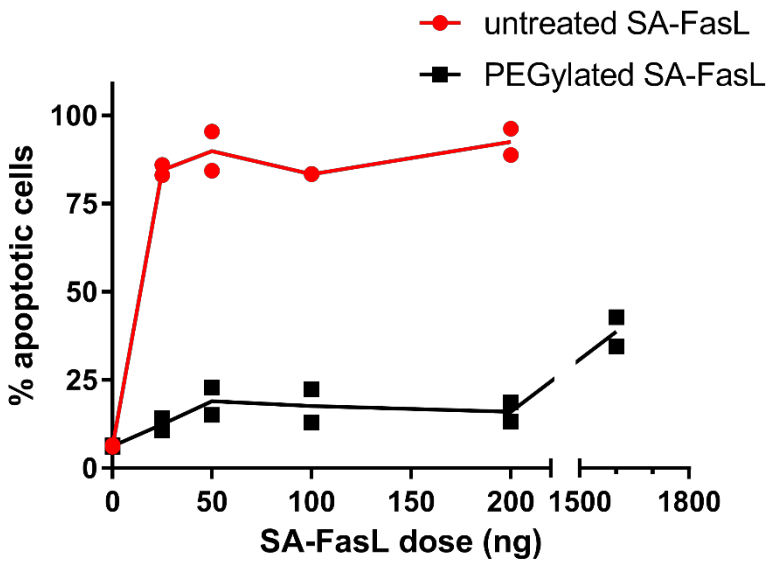
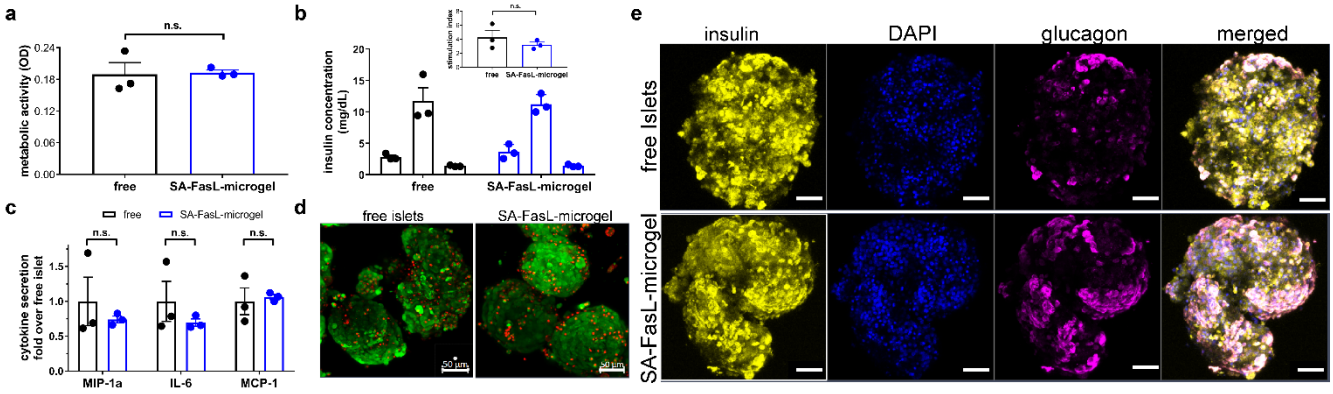
Supplementary Figures



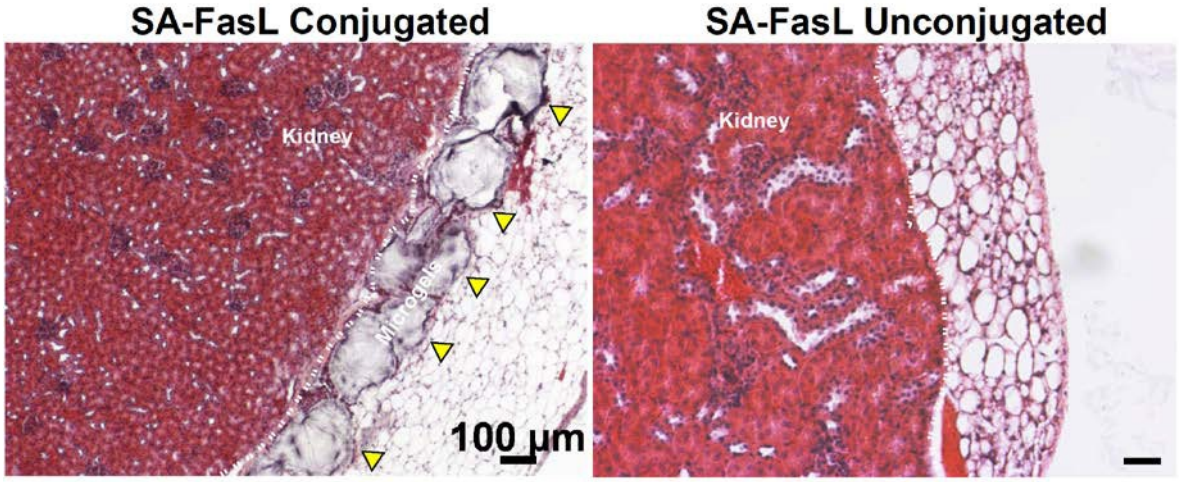
Supplementary Figure 1. SA-FasL is tethered to biotinylated microgels in a dose-dependent manner. Biotinylated microgels (10^4) were suspended in SA-FasL or SA only solution at the concentrations indicated for 1 h. Microgels were then washed by centrifugation 10 times in 1% bovine serum albumin in PBS to remove unbound protein. Functionalized microgels were incubated with a 1:100 dilution of fluorescently labelled anti-FasL antibody for 1 h, followed by 10 washes by centrifugation. Washed microgels were placed in a 96 well plate and read on Perkin Elmer HTS 7000 plate reader, and background signal (empty well) was subtracted from all values (n=3 biologically independent samples for SA-FasL, mean ± SE; n=2 independent samples for SA). Fluorescence values were converted to absolute concentrations using a standard curve.



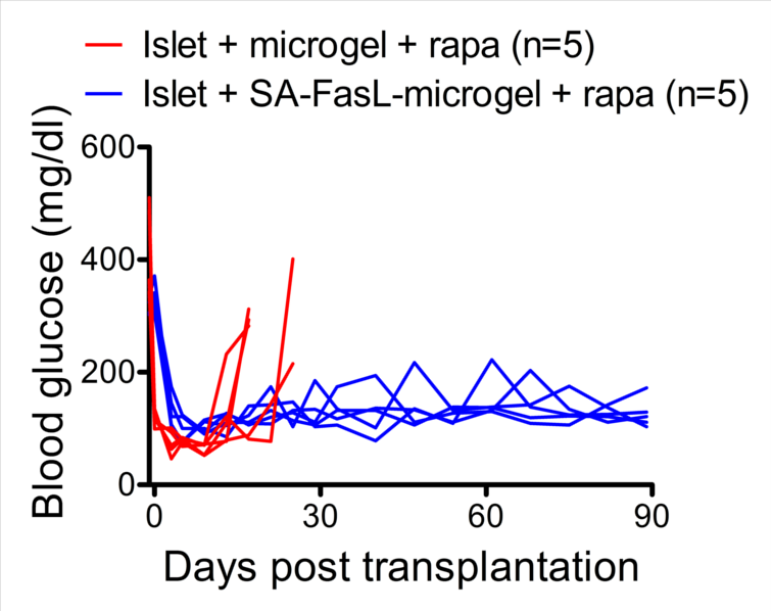
Supplementary Figure 2. Direct tethering of SA-FasL to PEG-4MAL macromer reduces bioactivity. Various doses of SA-FasL were reacted with PEG-4MAL macromer in solution for 1 h. Either soluble untreated SA-FasL or PEGylated SA-FasL was incubated with A20 cells overnight, and the number of apoptotic cells was determined by flow cytometry after staining with annexin V-APC and propidium iodide (n=2 biologically independent samples).



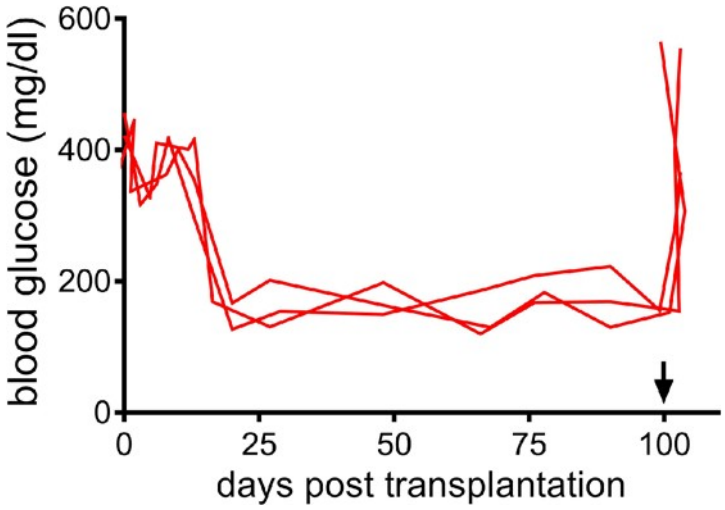
Supplementary Figure 3. SA-FasL-presenting microgels do not impact islet health or function. Rat islets were cultured with SA-FasL-presenting microgels (1:2 islet:microgel) for 24 h. (a) Metabolic activity (n=3 biological independent samples, mean ± SE, unpaired t test with Welch's correction, *p=0.89*). (b) Glucose-stimulated insulin secretion (n=3 biological independent samples, mean ± SE, unpaired t test with Welch's correction, *p=0.41*). (c) Secreted pro-inflammatory cytokines in media (n=3 biological independent samples, mean ± SE, unpaired t test with Welch's correction, MIP-1a [*p=0.54*], IL-6 [*p=0.40*], MCP-1 [*p=0.78*]). (d) Live-dead staining (scale bar 50 µm). Staining patterns are consistent for samples across 2 independent runs. (e) Immunostaining for insulin and glucagon and co-staining for DNA with DAPI (scale bar 50 µm). Staining patterns are consistent for samples across 2 independent runs.



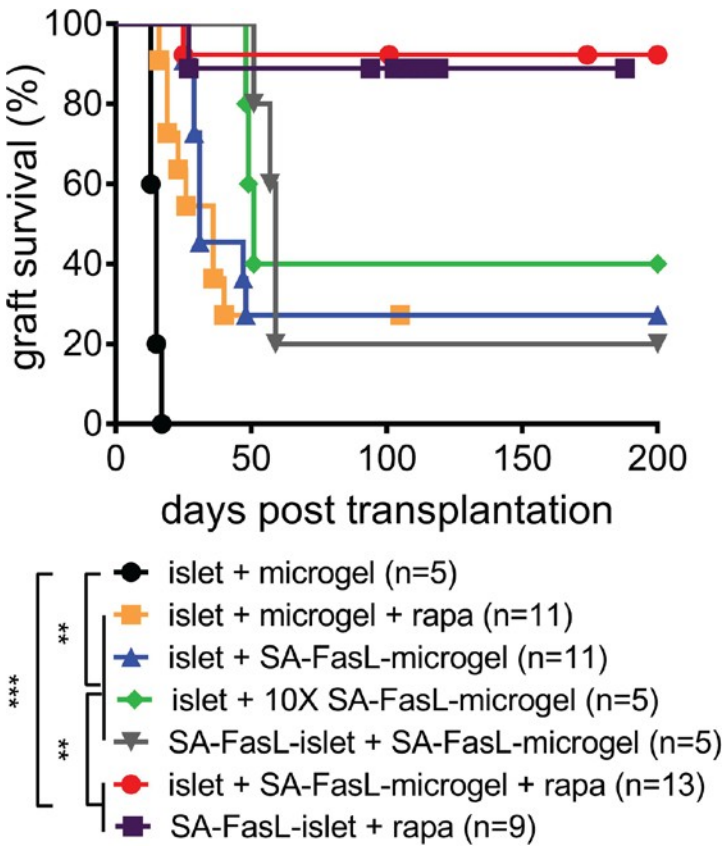
Supplementary Figure 4. H&E stained section of transplants in kidney capsule at 21 days post-transplantation. Yellow arrowheads indicate microgels. Staining patterns are consistent for 8 independent biological samples.



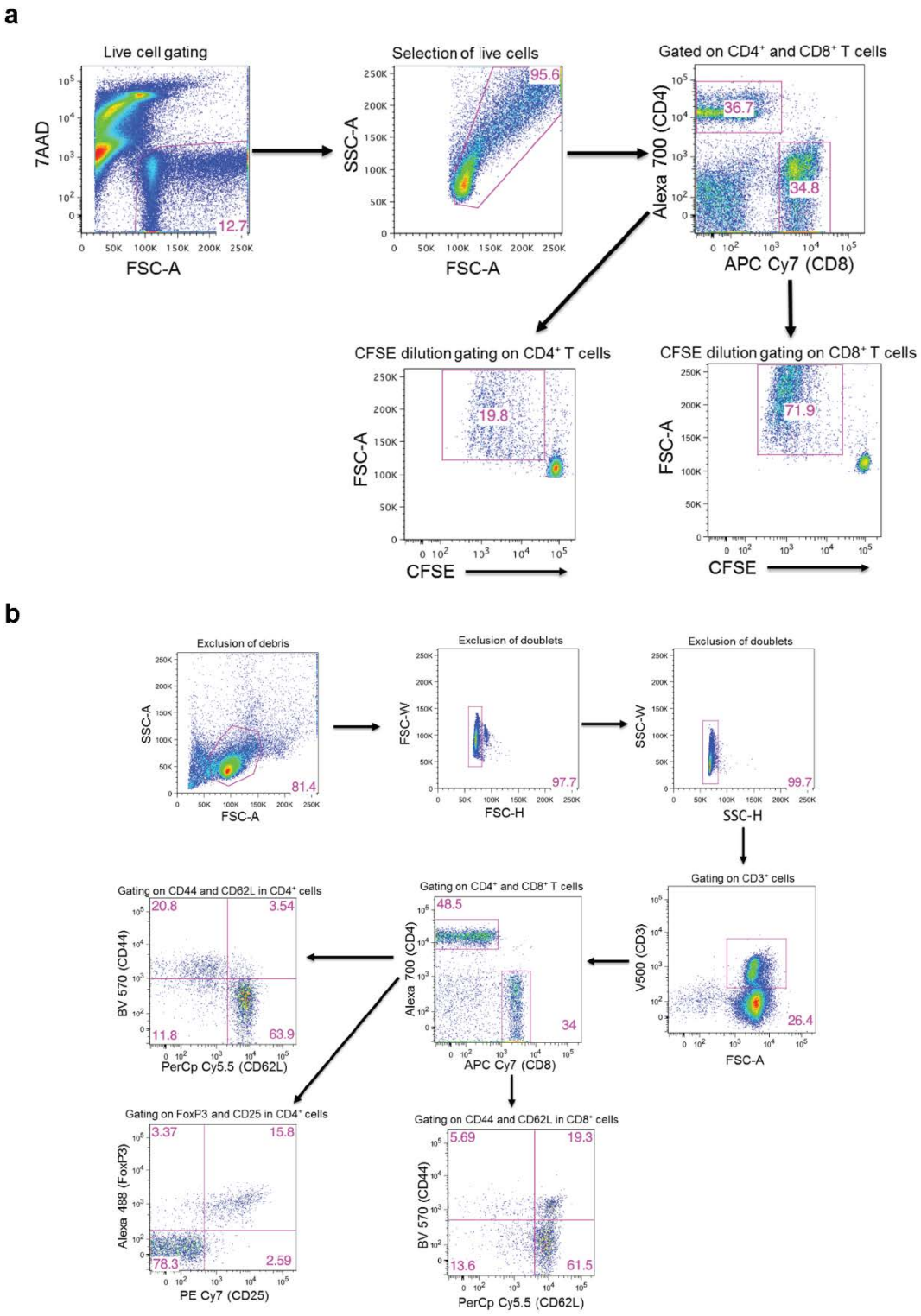
Supplementary Figure 5. Blood glucose levels. Readings were taken on chemically diabetic C57BL/6 mice transplanted with microgels presenting SA-FasL (1 µg protein/1000 microgels) and naïve BALB/c islet grafts (500) under a short cover of rapamycin (administered i.p. daily at 0.2 mg/kg for 15 doses). Controls included mice subjected to the same regimen, except receiving microgels without SA-FasL protein.



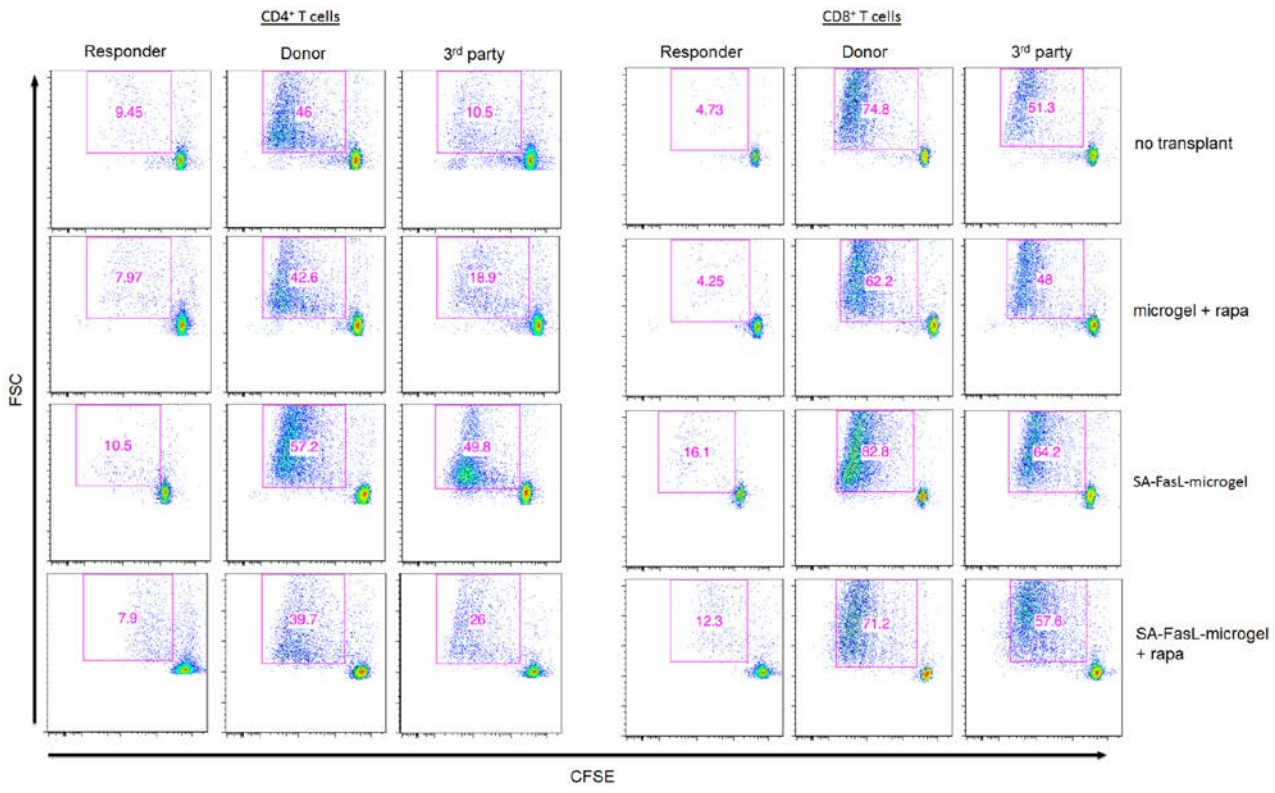
Supplementary Figure 6. Nephrectomy returns subjects transplanted with islets and SA-FasL-presenting microgels + rapamycin to hyperglycemic state. Kidneys were excised at day 100 post-transplantation (arrow), n=3 mice.



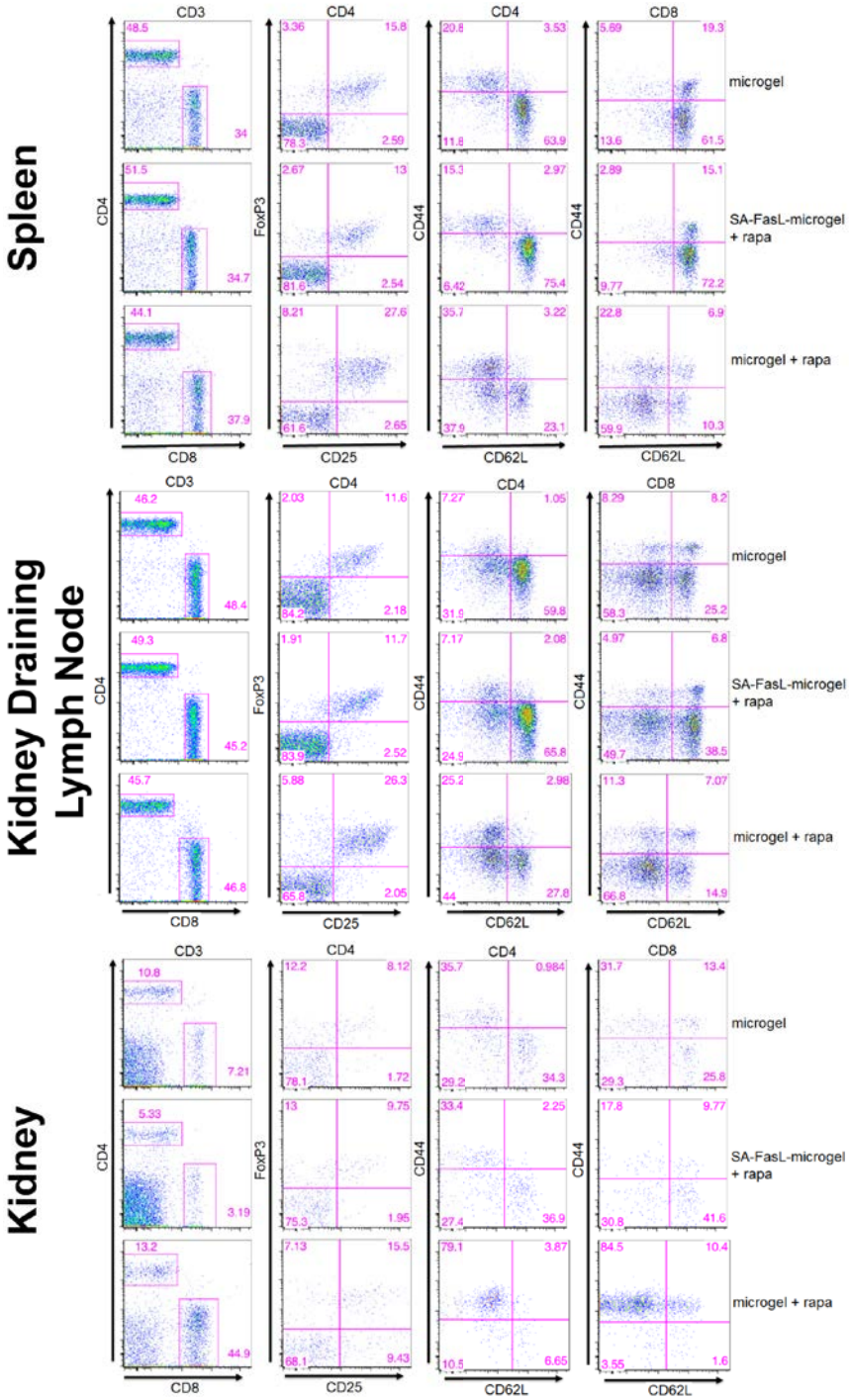
Supplementary Figure 7. Islet graft survival. Biotinylated microgels were engineered with SA-FasL (1 µg protein/1000 microgels, unless otherwise noted) and co-transplanted with unmodified or SA-FasL-engineered BALB/c islets (500/transplant) under the kidney capsule of chemically diabetic C57BL/6 recipients. Rapamycin was used at 0.2 mg/kg daily i.p. injection for 15 doses starting the day of transplantation in the indicated groups. Animals were monitored for blood glucose levels and two consecutive daily readings of ≥ 250 mg/dL were considered to be diabetic (rejection). Data comprise transplants performed from various islet isolations with sample size indicating number of treated mice. Survival curves were analyzed using Mantel-Cox test (χ^2 = 87.05, df = 6, $p < 0.0001$; two-tailed pair-wise comparisons with Bonferroni's correction, $^{**}p < 0.01$, $^{***}p < 0.001$).



Supplementary Figure 8. Flow cytometry gating strategy. (a) Strategy for Figure 4a and Supplementary Figure 9. (b) Strategy for Figure 4b and Supplementary Figure 10.

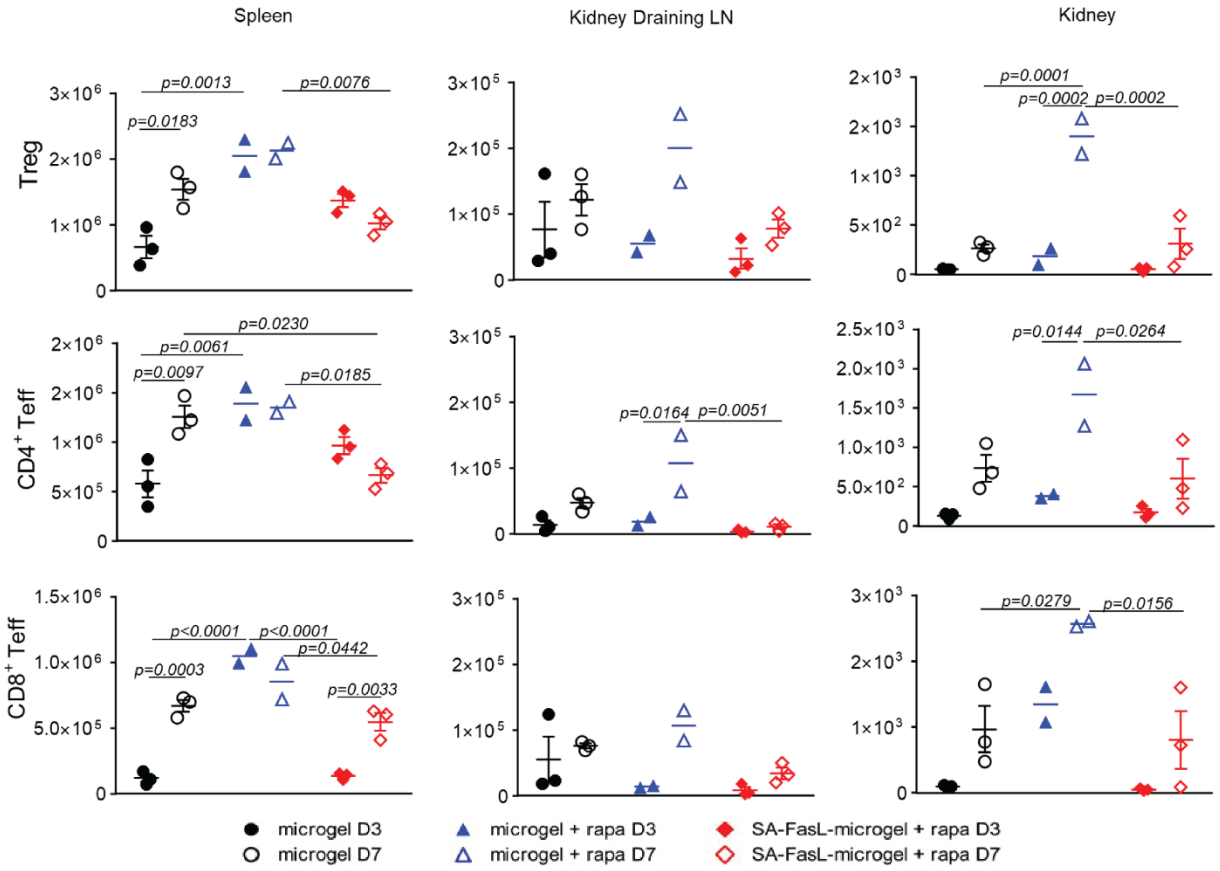


Supplementary Figure 9. Immune cell proliferation assay. Splenocytes harvested from selected group of transplant recipients were labeled with CFSE and used as responders to irradiated (2000 cGy) splenocytes from donor or third party C3H mice in a standard *in vitro* proliferation assay. After 4 days in culture, cells were stained with 7AAD and fluorescence-conjugated Abs against CD4 and CD8, and analyzed for CFSE dilution by gating on live cells using BD LSR II. Data was analyzed using Diva software. n=5 mice/group were tested for microgel + rapa and SA-FasL-microgel + rapa and n=3 mice were tested for the microgel group.

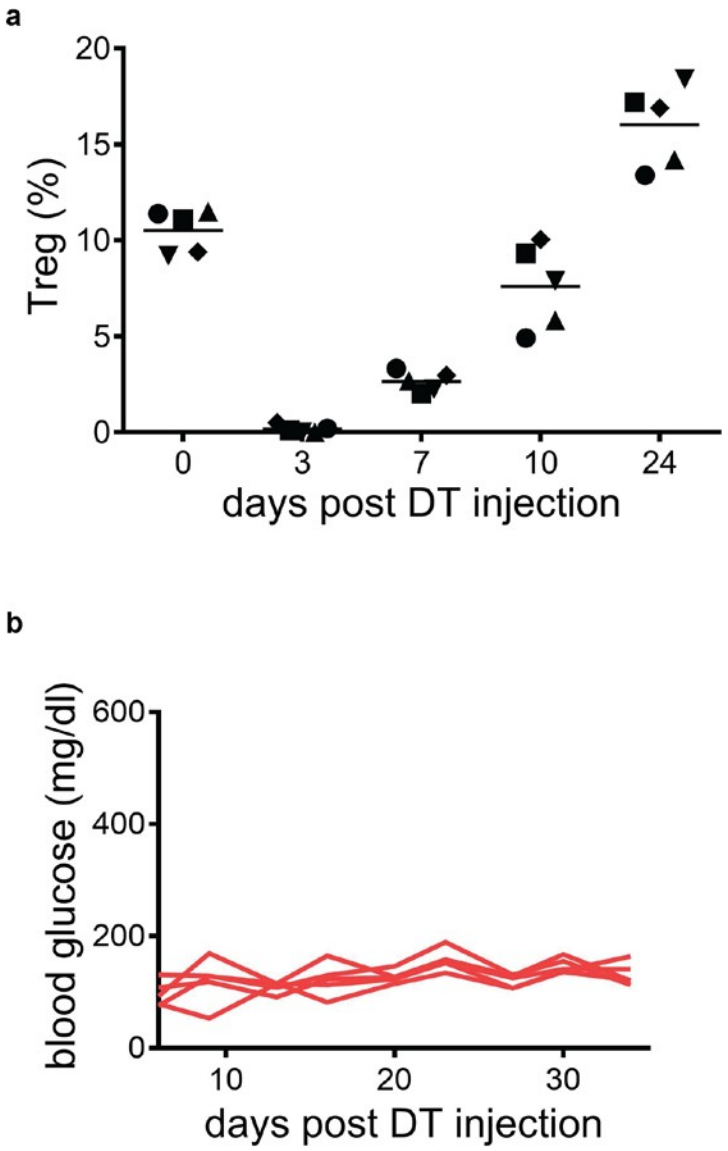


Supplementary Figure 10. Immune profiling. Spleen, kidney, and kidney draining lymph nodes were harvested from rejecting and long-term mice (> 200 days). Single cells were prepared from the spleen and lymph nodes by gentle mechanical dispersion and from islet harboring kidney by collagenase digestion. Cells were stained using antibodies to cell surface markers or intracellular

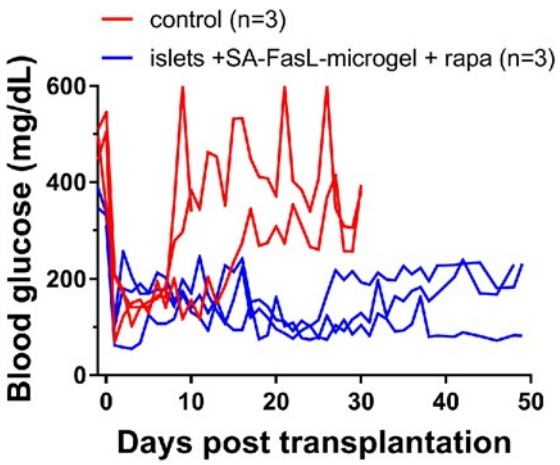
FoxP3. Data was collected using BD LSR II and analyzed using Diva software. n=3 mice/group were tested, except for microgel + rapa group in which 2 mice/group was tested.



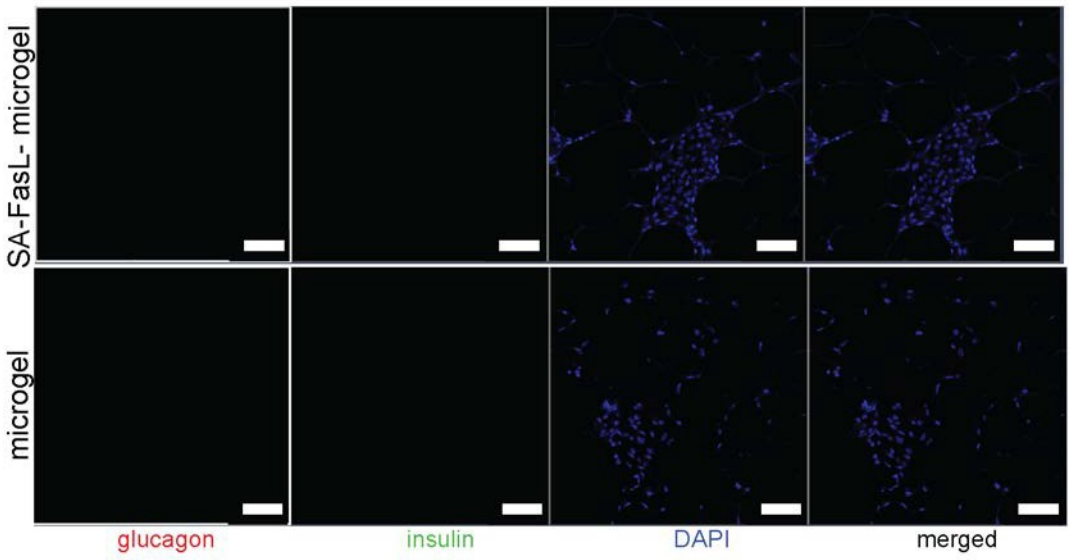
Supplementary Figure 11. Flow cytometric analysis of Teff and Treg cells in various tissues of islet graft recipients at early post-transplantation. Single cells prepared from the spleen, kidney, and kidney-draining lymph nodes of the indicated groups on day 3 and 7 post-islet transplantation were stained with fluorescence-labelled antibodies to cell surface molecules for $CD4^+$ Teff ($CD4^+CD44^{hi}CD62L^{lo}$), $CD8^+$ Teff ($CD8^+CD44^{hi}CD62L^{lo}$), and Treg ($CD4^+CD25^+FoxP3^+$) cells and analyzed using flow cytometry. Shown are absolute numbers of cells in indicated tissues (n=2 mice/group for microgel + rapa and n=3 mice/group for microgel and SA-FasL-microgel + rapa; mean ± SE; ANOVA with two-tailed pair-wise comparisons with Bonferroni's correction).



Supplementary Figure 12. DT administration to FoxP3/DTR mice. Mice were injected i.p. with diphtheria toxin (50 µg/kg body weight). (a) Transient Treg cell depletion analyzed by flow cytometry (n=5 mice, mean, each symbol corresponds to a particular subject). (b) Blood glucose levels following DT administration (n=5 mice).



Supplementary Figure 13. Blood glucose levels for epididymal fat pad transplants. Readings were taken on chemically diabetic C57BL/6 mice transplanted with microgels presenting SA-FasL (1 µg protein/1000 microgels) and unmodified BALB/c islet grafts under a short cover of rapamycin (administered i.p. daily at 0.2 mg/kg for 15 doses).



Supplementary Figure 14. Isotype control immunostaining for epididymal fat pad graft (scale bar 50 μm). Histological staining patterns are consistent for samples across 2 separate islet transplantations.