SUPPORTING INFORMATION

Methods S1

Measurement of trough levels of KRP-203

Twenty-three hours after the last dose of KRP-203 (1 or 3 mg/kg) or vehicle (n = 8, 7, and 4, respectively), blood samples from randomly selected mice were collected from the inferior vena cava in heparin-coated tubes. Plasma was separated by centrifugation at 2100g, 4°C for 5 min. Plasma samples were stored at -80°C until the analyses. Analyses were performed using liquid chromatography with tandem mass spectrometry (LC-MS). Briefly, 50 μL of plasma was added to 10 μL of internal standard (1 μg/mL d5-KRP203 and 1 μg/mL d5-KNF451) and 50 μL of acetonitrile. After 10 min of incubation, the mixture was mixed with a vortex mixer and centrifuged at 15 000g for 10 min at room temperature. A 5-μL aliquot of the solution was subjected to LC-MS. Chromatographic separation was performed using a Shimadzu Nexera HPLC System (Shimadzu, Kyoto, Japan) with an Inertsil ODS-3 column (50 mm × 2.0 mm i.d., 5 μm; GL Sciences, Tokyo, Japan), and the analytical column was kept at 40 °C. The mobile phase consisted of solution A (0.5% formic acid and 0.2% acetylacetone in water) and solution B (0.5% formic acid and 0.2% acetylacetone in acetonitrile), which formed the following gradient: 30%−80% B (0−4 min); 80% B (4−6 min); and 30% B (6−10 min). The flow rate was 0.2 mL/min. Positive ion electrospray tandem mass spectrometry was carried out using a TSQ Vantage EMR LC/MS/MS System (Thermo Scientific, Waltham, MA, USA) at unit resolution with selected reaction monitoring (SRM). The SRM transitions monitored were m/z 444.1 > 91.1 for KRP-203, m/z 524.1 > 426.1 for KNF-451, m/z 449.1 > 96.1 for d5-KRP-203 and m/z 529.1 > 431.2 for d5-KNF-451. Data were acquired and analyzed using the Xcalibur software program (version 2.1;

Thermo Scientific). The calibration curves for KRP-203 and KNF-451 were linear in the range of 1-500 ng/mL.

In vivo imaging of islets transplanted into the dorsal skin chamber

A 0.1-mL sample of Texas Red (10 mg/mL; Invitrogen, Leek, The Netherlands) was injected intravenously via the tail vein. The mice were anesthetized using the inhalation of isoflurane and then positioned in an acrylic resin tube with an inner diameter of 26 mm. The tube was fixed on an acrylic resin plate and then placed on the microscope stage. A multiphoton laser-scanning microscope (MPLSM; FluoView FV1000MPE; OLYMPUS, Tokyo, Japan) equipped with water-dipping lenses (OLYMPUS XLPLN25XWMP NA1.05; OLYMPUS) was used to image the transplanted islet grafts. Distilled water was used as immersion liquid. MPLSM imaging was performed under the minimum required laser-power and scan-time necessary. No signs of photo damage of the islet cells or blood vessels were observed throughout the study. We processed images and measured the newly formed vascular volume surrounding the islets using a Volocity 3D system (PerkinElmer, Waltham, MA, USA) to evaluate islet neovascularization (with fixed parameters for all measurements). The newly formed vascular volume surrounding the islets at each time point was calculated based on the rate of increase compared with day 1 after transplantation. GFP and Texas Red were excited at 890 nm and separated and collected emission light onto 2 nondescanned detectors using a dichroic mirror (FV10-MRG/R:DM570) and emission filters (FV10-MRG/R:BA495-540HQ and BA575-630).

Mixed lymphocyte reaction (MLR)

A MLR was performed to investigate the effect of KNF-451 (active metabolite of KRP-203) and Sirolimus alone or in combination on the lymphocyte proliferation in vitro. Peripheral blood mononuclear cells from the buffy coat obtained from peripheral blood samples of C57BL/6J mice were used as responders, while antigen-presenting-cells (APCs) isolated from a single-cell suspension of Balb/c mice spleen were irradiated and used as stimulators. Briefly, $1x10^5$ cells/well of responders (in 90 µL) were incubated at 37 ^oC with 5% CO_2 for 2.5 h, and then $7x10^5$ cells/well of APCs (in 90 µL) in addition to 1 of the following were added to the well content: 1) KNF-451 100 ng/mL, 2) low-dose Sirolimus (12 ng/mL), 3) high-dose Sirolimus (120 ng/mL), 4) combination of KNF-451 and low-dose Sirolimus, 5) combination of KNF-451 with high-dose Sirolimus, or 6) medium only (n = 5 per group). Appropriate controls were used for the assessment. The cells were cultured for 90±2 h, and then the proliferation was assessed using a cell proliferation ELISA kit (BrdU colorometric assay, cat. no. 11 647 229 001; Roche, Basel, Switezerland) following the manufacturer's protocol. Absorbances were measured using a microplate reader (Multiskan Go, Thermo scientific, Vanaa, Finland). The final concentrations of the medications were decided based on the corresponding trough levels in mice as measured in the in vivo study.

Table S1. Log-rank test for graft survival curve.

Log-rank (Mantel-Cox)										
Immunomodulation	**Vehicle only**		**KRP-203 1 mg/kg**		**KRP-203 3 mg/kg**		**Sirolimus 0.3 mg/kg + KRP-203 3 mg/kg**		**Sirolimus 0.3 mg/kg + vehicle**	
	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.
Vehicle only			7.329	.006784	3.777	.051969	19.044	.000013	9.647	.001897
KRP-203 1 mg/kg	7.329	.006784			.000	.991186	3.865	.049312	.138	.710497
KRP-203 3 mg/kg	3.777	.051969	.000	.991186			4.079	.043416	.101	.751209
Sirolimus 0.3 mg/kg + KRP-203 3 mg/kg	19.044	.000013	3.865	.049312	4.079	.043416			6.488	.010858
Sirolimus 0.3 mg/kg + vehicle	9.647	.001897	.138	.710497	.101	.751209	6.488	.010858		