# ELECTRONIC SUPPLEMENTARY MATERIAL (ESM)

## ESM METHODS

**Animals and reagents.** Six to ten week old mice (C57BL6; B6 and DBA/2) were purchased from JAX laboratory (USA) and the baboon (Papio hamadryas) was obtained from the Mannheimer Foundation, Inc. (Homestead, FL). Animals were housed with free access to food and water during the studies in dedicated facilities under the supervision of the University of Miami's department of veterinary resources (DVR).

**Diabetes induction with streptozotocin (STZ).** Diabetes was induced in male and female B6 mice with a single dose of STZ (200 mg/kg) administered intravenously (i.v.) and frank diabetes was confirmed by 3 consecutive daily readings of glycemia ≥300 mg/dl in all diabetic mice before transplantation. The recipient baboon was rendered diabetic by partial pancreatectomy performed 557 prior to islet transplantation (tail and >50% of pancreas body removed) followed with a single i.v. dose of STZ (1,250 mg/m$^2$ of body surface area; Sigma). Lack of residual islet function afterwards was confirmed by persistent negative fasting c-peptide measurements, two negative glucagon challenges, and a negative response in an IVGTT performed 48 prior to transplantation. In both mouse and non-human primate models, STZ treatment was administered following overnight fasting.

**Aqueous humor collection and cytokine measurements.** Aqueous humor samples ranging from 5 – 15 μL in volume were collected from euthanized mice by aspiration using 31G disposable insulin syringe and frozen immediately on dry ice and kept at -80$^0$ C until further use. The samples were collected from tolerant mice after confirming prolonged survival for no less than 3 months and from mice that either had rejected their grafts (>20 days post rejection) or during ongoing acute rejection whenever it occurred in the different mice. Aqueous humor samples (50 – 100 μL) from the baboon were collected by paracentesis under sterile conditions during the eye examinations. Cytokine measurements in aqueous humor were done by Bio-Plex assay (Bio-Rad, USA) and measurements were acquired by a *Luminex* instrument using *xPonent* software and analyzed using *Milliplex Analyst* software (*Luminex,* USA).

**Pancreatic islet isolation and islet transplantation.** Islet isolation from donor mice (males/females) or baboon (male), and transplantation into the anterior chamber of the eye or under

the kidney capsule of recipient mice (males/females) or the eye of the baboon (female) were performed as previously described in detail [1-5]. Diabetic B6 (male/female) recipient mice received 200 – 300 islet equivalents (IEQ) into the anterior chamber of one eye. Mice transplanted under the kidney capsule received 500 – 600 IEQs. We have previously demonstrated that less islets are needed to correct hyperglycemia in diabetic recipients when transplanted in the eye anterior chamber compared to kidney. In mice challenged with repeat transplant to assess tolerance, only ~50 IEQs were transplanted in the eye to avoid correcting hyperglycemia and the mice were maintained on insulin therapy (subcutaneous sustained release insulin pellets; Linplant; LinShin Canada Inc., Toronto) until re-transplantation of 500 – 600 IEQs under the kidney capsule. Control mice initially transplanted under the kidney capsule underwent nephrectomy prior to re-transplantation of 500 – 600 IEQs in the remaining contralateral kidney. Hyperglycemia was confirmed in these mice prior to re-transplantation.

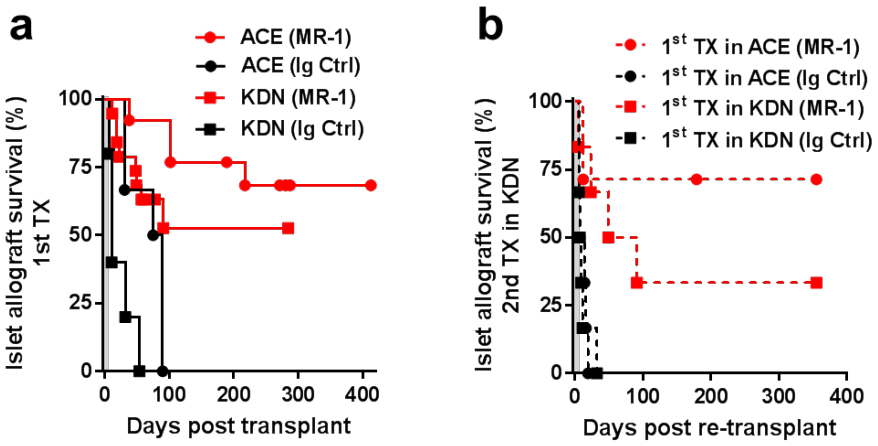
***Trans-vivo* DTH assay.** *Trans-vivo* DTH (delayed-type hypersensitivity) assay was performed as previously described in detail [6, 7]. To assess DTH reactivity, peripheral blood mononuclear cells (PBMCs from the recipient baboon or the non-transplanted untreated control female baboon) or PBMCs + lymph node cells were subcutaneously injected along with the donor antigen or third-party control antigen(s) into footpads of naïve SCID mice. The recipient and untreated control baboons were immunized for tetanus toxoid and diphtheria (TT/D). The response to the recall antigen (TT/D; (Sanofi Pasteur Inc., PA, USA) alone + PBMCs was used as a positive control. PBMCs + PBS was used as a negative control. To test for antigen-specific bystander suppression, the recall antigen (TT/D) was co-injected with test soluble antigen(s) derived from the islet donor or third-party control male/female baboons. Antigen-driven swelling was measured after 24 h using a dial thickness gauge (Mitutoyo, Japan). DTH reactivity was expressed as the change in footpad thickness (swelling), using units of $10^{-4}$ inches.

**ESM Table 1**

| | **Spearman's Correlation Coefficient** | | | | |
|---|---|---|---|---|---|
| **Aq. humor c-peptide** | 1 | 1 | - | 1 | 1 |
| **Plasma c-peptide** | 0.4306 ($p$<0.0001) | 0.4038 ($p$<0.0001) | - | 1 | - |
| **Plasma/Aq. h. c-peptide** | 1 | 1 | 1 | - | - |
| **FBG** | 0.5167 ($p$<0.0001) | 1 | - | - | - |
| **PBG** | 1 | - | - | - | - |
| | **PBG** | **FBG** | **Plasma/Aq. h. c-peptide** | **Plasma c-peptide** | **Aq. humor c-peptide** |

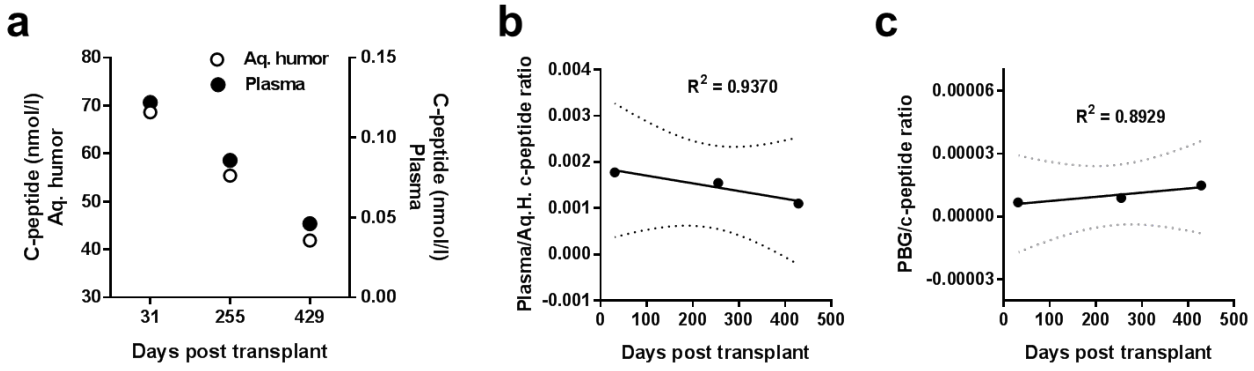
**Spearman's correlation analysis.** Pairwise correlation analysis between aqueous humor (Aq. h.) c-peptide, plasma c-peptide, fasting blood glucose (FBG), and postprandial blood glucose levels (PBG) in the diabetic baboon after intraocular islet transplantation. Reported is the nonparametric Spearman's correlation coefficient with the corresponding $p$ values. A Spearman's correlation coefficient of +1 (or -1) indicates perfect correlation, and any deviation from 1 with a p value ≥0.05 indicates random correlation between the two compared parameters; p values ≤0.05 indicate non-random correlation.

**ESM Fig. 1**



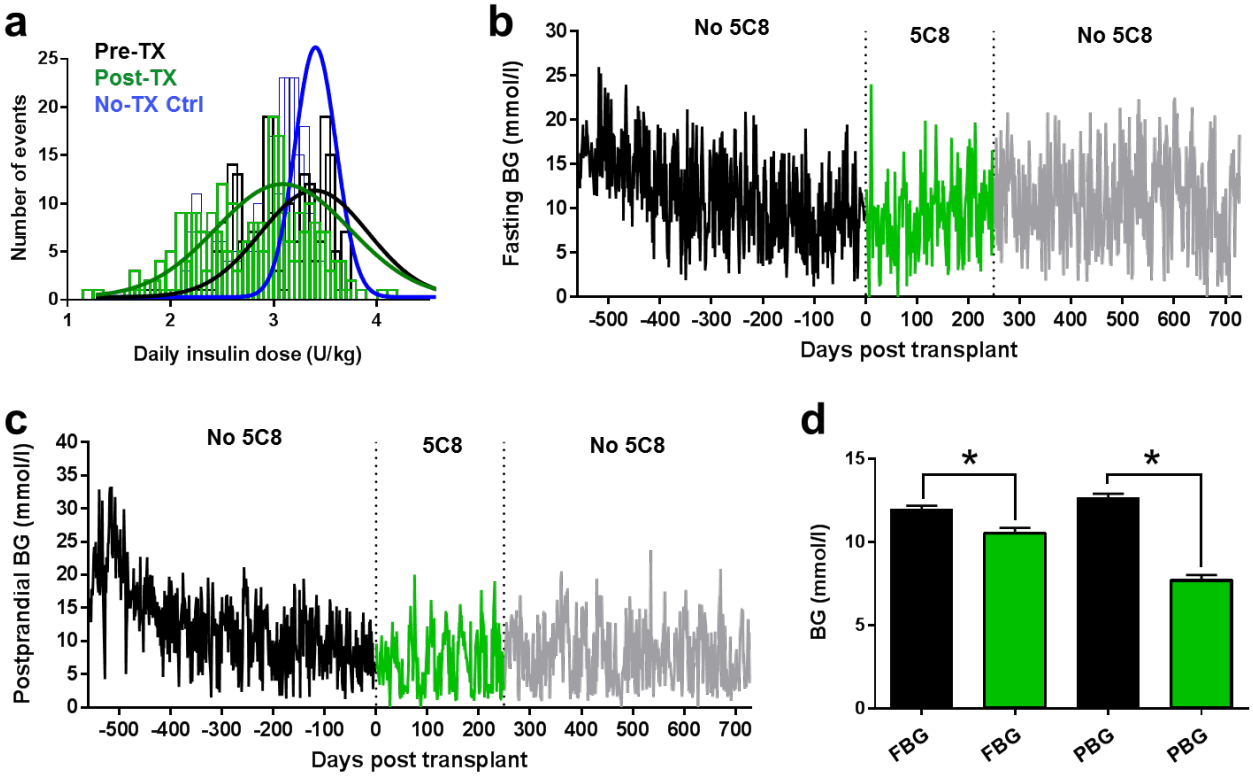

**Peri-transplant immune intervention with MR-1 leads to long-term survival of islet allografts but with evident advantage in the eye compared to the kidney.** (**a**) Kaplan-Meyer survival curves of islet allografts either in the eye (ACE; circles) or kidney (KDN; square symbols) of diabetic recipients treated with MR-1 (red) or Ig isotype control (black) antibodies. (**b**) Kaplan-Meyer survival curves of islet allografts in the kidney of tolerant recipients upon repeat transplantation ($2^{nd}$ TX) following initial transplant either in the eye (ACE; circles) or kidney (KDN; square symbols) and treatment with MR-1 (red) or Ig isotype control (black) antibodies. Grey areas near zero indicate the transient peri-transplant treatment period.

**ESM Fig. 2**




**Plasma and aqueous humor c-peptide levels correlated well with each other and with postprandial blood glucose levels.** (**a**) C-peptide levels in the aqueous humor (Aq. H.) and the corresponding plasma levels on the same time points after transplantation [also see ref. [5]]. (**b**) Linear regression analysis of the correlation between c-peptide levels in the plasma and aqueous humor (expressed as ratio) of the diabetic baboon, which was transplanted with allogeneic islets into the anterior chamber of one eye. Dotted lines denote 95% confidence intervals. (**c**) Linear regression analysis of the correlation between the c-peptide ratio (plasma/aqueous humor) and the corresponding postprandial blood glucose (PBG) levels (nonfasting glycemia) (also see **ESM Table 1** for additional correlations).

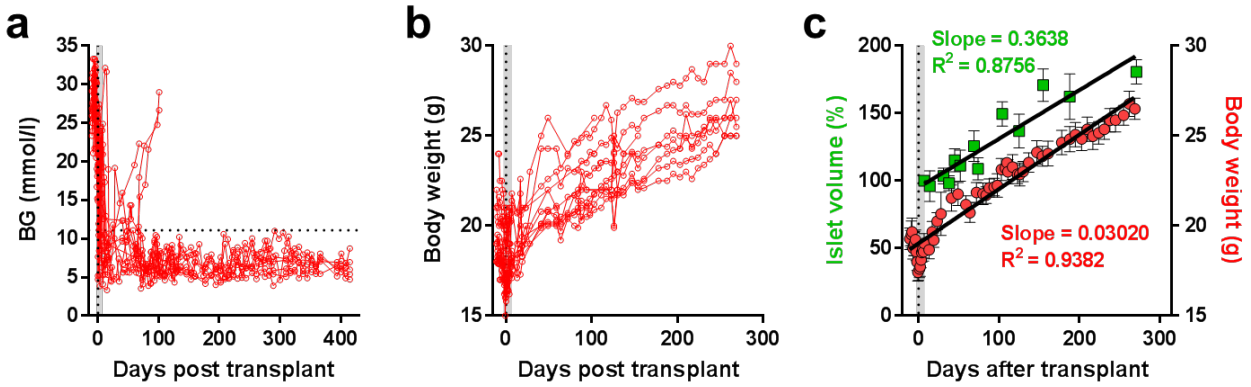
**ESM Fig. 3**




**Improved overall blood sugar control following intraocular islet transplantation in the diabetic baboon.** (**a**) Frequency analysis of the daily insulin dose [units (U) per kg B.W.] that was needed in the diabetic baboon before and after intraocular islet transplantation. Data presented as histograms (binned at 0.5) of the daily insulin dose before (Pre-TX; black bars) and after transplant (Post-TX; green). Also shown (in blue bars) are the daily insulin requirements of a non-transplanted diabetic control baboon (No-TX Ctrl) that was housed with the recipient under identical conditions to rule out potential changes in the insulin requirements in association with environmental conditions. Superimposed onto the histograms are also non-linear function (Gaussian) best-fits to the data in the recipient baboon before (black line) and after (green) transplantation, and in the non-transplanted diabetic control (blue). These data highlighted the left-shift tendency in the daily insulin requirements following islet transplantation in the recipient, despite the minimal functional islet mass, which was not observed in the control non-transplanted baboon as evident by the narrow distribution of the daily insulin dose throughout the same follow up period. (**b, c**) Comprehensive records of daily (**b**) fasting and (**c**) postprandial blood sugar levels

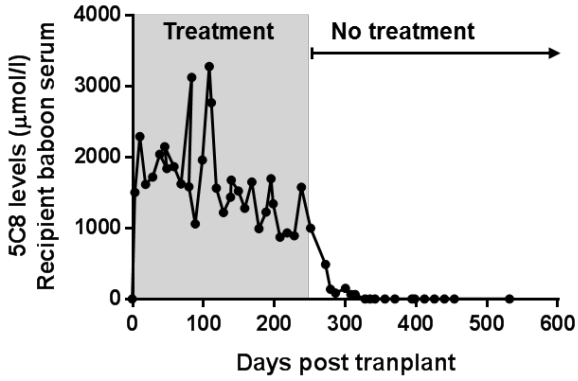
before (black lines) and after transplantation (green and grey, respectively, during and after stopping 5C8 treatment). The functional islet mass in the diabetic recipient baboon was estimated at ~600 islet equivalents (IEQ)/kg after islet clumping (see **Methods**). (**d**) Daily mean fasting blood glucose (FBG) and postprandial blood glucose (PBG) levels before (black) and after (green) transplantation. Data shown as means ± SEM (n=556 pre-TX and n=724 post-TX; $p<0.0001$ by ANOVA followed by Tukey's multiple comparison).

**ESM Fig. 4**




**Longitudinal volume analysis of tolerated intraocular islet allografts in MR-1 treated recipients showed islet growth coincident with increased body weight of the recipients.** (**a, b**) Longitudinal measurements of (**a**) nonfasting glycemia and (**b**) body weight of individual diabetic B6 mice before/after transplantation (dotted vertical line) of allogeneic islets in the eye anterior chamber (see **Methods**). Transplantation was done under transient treatment with anti-CD154 antibody (MR-1) in the peri-transplantation period (grey areas around day 0). Most of the recipients treated with MR-1 maintained normoglycemia [≤11.11 mmol/l (200 mg/dl); horizontal dotted line] long after stopping treatment. The mice exhibited transient reduction in body weight during diabetes induction with STZ but recovered their body mass thereafter and continued to grow normally during the extended follow up. (**c**) Longitudinal analysis of the volume of islets exhibiting long-term survival in the tolerant mice showed coincident increase in islet volume (green symbols; normalized to baseline on POD7) in association with body weight (red symbols). Data shown as mean ± SEM (n=8 mice in **a**, **b**, and **c**; n=19 islets in **c**). Slopes and $R^2$ values were derived by linear regression analysis of the means (black solid lines).

**ESM Fig. 5**




**Serum levels of anti-CD154/CD40L mAb (5C8) in the transplanted baboon before and after stopping treatment.** Longitudinal measurements of 5C8 trough-levels indicated that its concentration in the transplanted baboon's circulation dropped below therapeutic levels within 30 days after stopping treatment (treatment period shown as grey area). Consequently, full rebound and recovery of its immune competence was well-established during the >300 days follow-up after stopping the treatment.

## ESM REFERENCES

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