Supplemental Figures

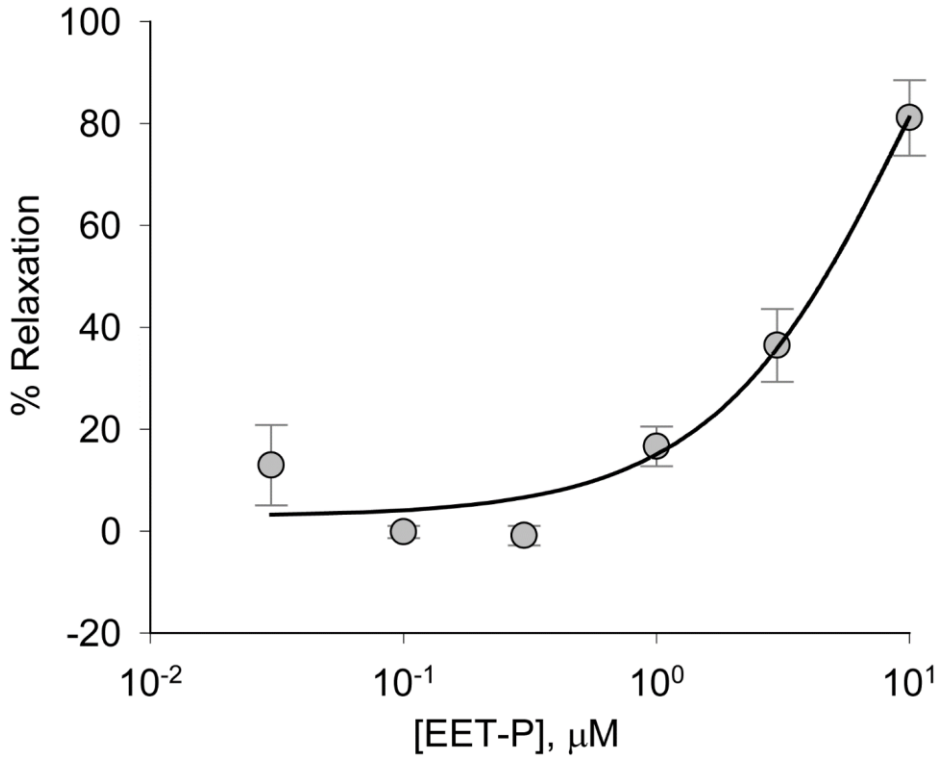

Fig S1. Characterization of the vasorelaxant effect of EET-P. Arteries were pre-constricted by superfusion with thromboxane A2 receptor agonist U-46619 (20-40 nM), then exposed to incremental concentrations of 14,15 EET-P (n=5).

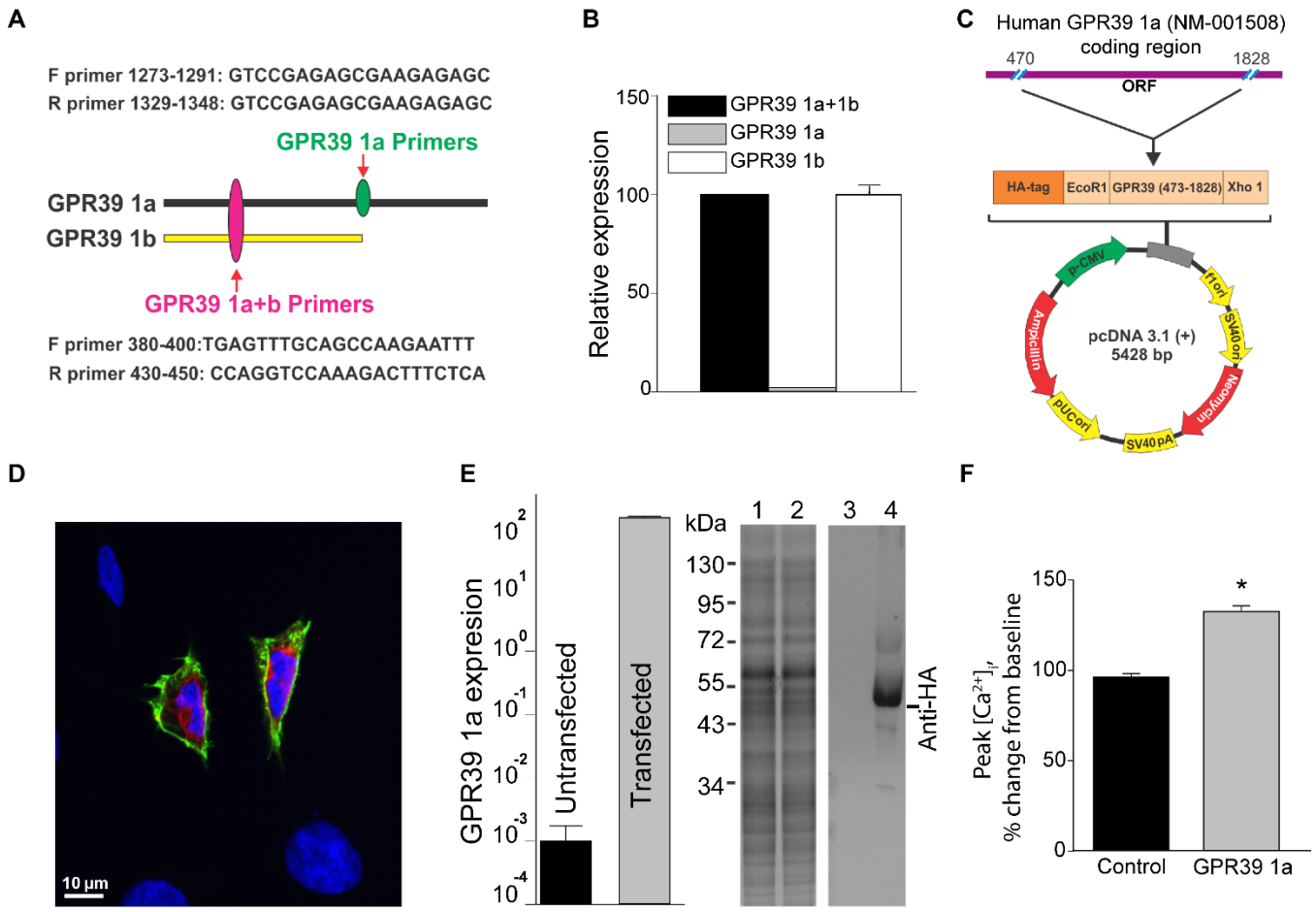

Fig. S2. Characterization of GPR39 1a expression in naïve and stably-expressing HEK293 cells. (A) Diagram depicting the sequence and location of the quantitative PCR primers used to distinguish GPR39 1a and 1b. (B) qPCR analysis of GPR39 isoform expression in naïve HEK293 cells (n=3). (C) Diagram of the expression vector used to create HEK293 cells stably expressing GPR39 1a. (D) Fluorescent labeling of transiently-expressed HA-tagged GPR39 in HEK-293 cells detected using a FITC-conjugated anti-HA antibody before (green) and after membrane permeabilization using Alexa Fluor 555-conjugated anti-HA (red). DNA is labled using DAPI (blue). (E) Expression of GPR39 1a confirmed by RT-PCR (left) and Western blot (right, lane 4; lane 3 untransfected). Coomassie blue staining gel used as a loading control (right, lanes 1 and 2). (F) Calcium imaging demonstrating an increase in $[Ca^{2+}]_i$ by 15-HETE (50 nM+4 µM zinc) in GPR39 1a-transfected (n=11), but not untransfected HEK cells (n=6).

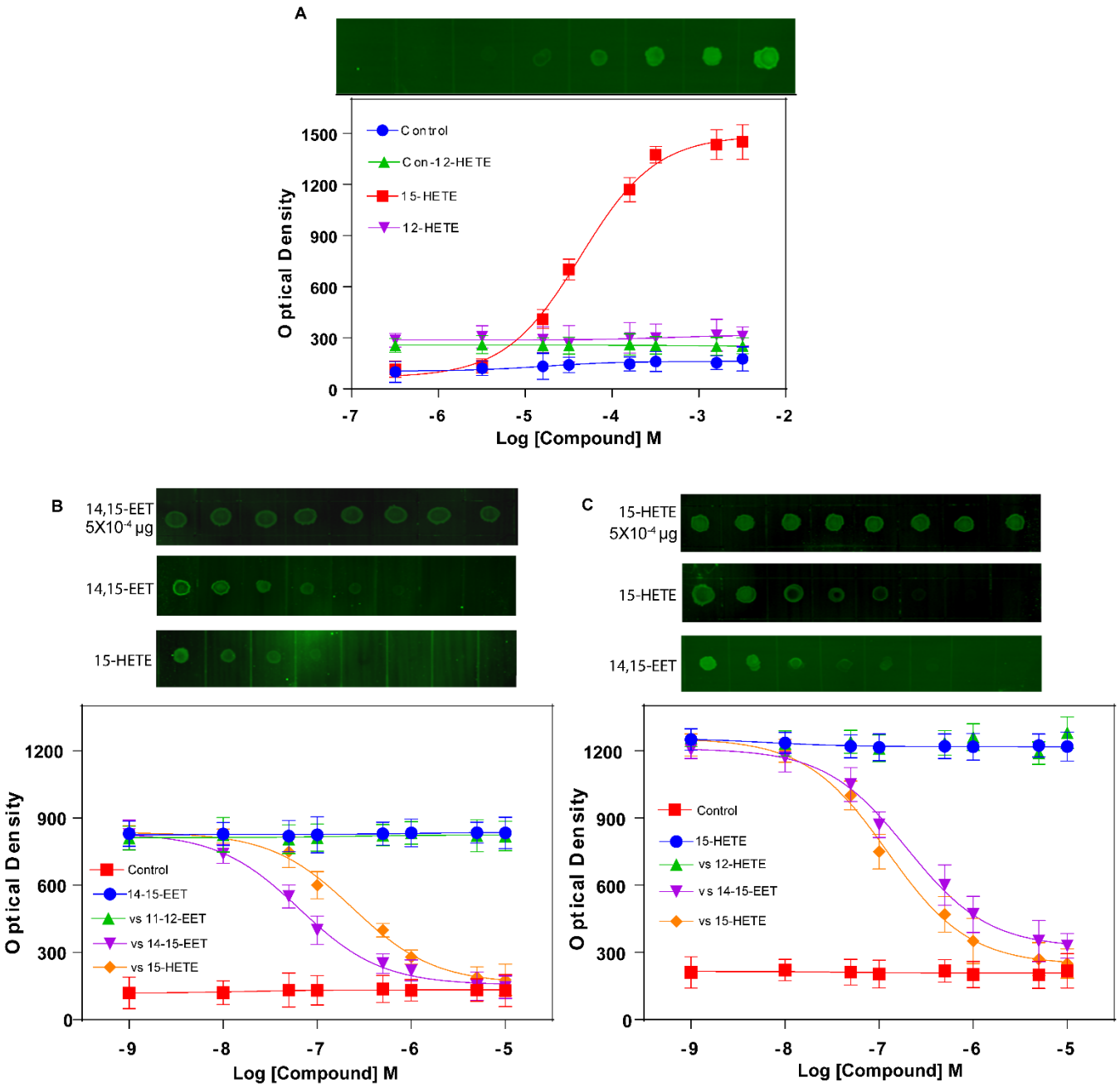

Fig. S3. Dot-blot assay analysis of eicosanoid-binding specificity and competition. (A) Comparison of 12-HETE and 15-HETE binding by membrane fraction lysates from control and GPR39 1a stably-expressing HEK293 cells (n=3). (B) Binding competition experiment indicating binding saturation of membrane fraction from GPR39 1a expressing, but not control HEK293 cells, to immobilized 14,15-EET, and displacement by 14,15-EET or 15-HETE, but not by 11,12-EET (n=3). (C) Dot-blot competition experiment demonstrating a similar saturation effect of membrane fraction from GPR39 1a expressing, but not control HEK293 cells, to immobilized 15-HETE, and displacement by 15-HETE or 14,15-EET, but not by 12-HETE (n=3).

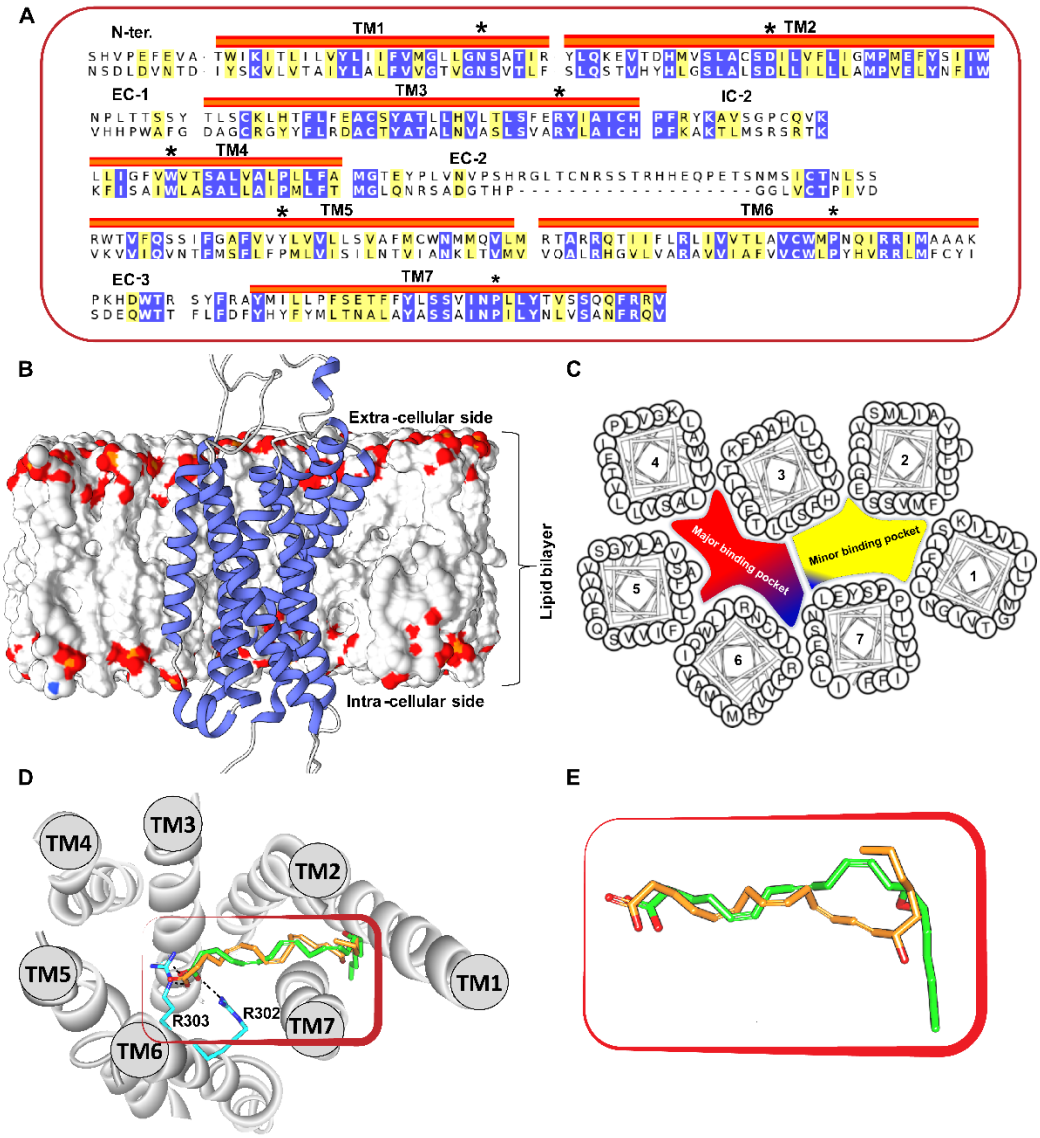

Fig. S4. Molecular modeling of GPR39 1a to characterize the eicosanoid binding pocket. (A) Sequence alignment between target (GPR39) and template (neurotensin receptor 1; 4GRV) used for generating homology model. Blue shades highlight identical residues, yellow shades highlight similar residues. Asterisks indicate conserved residues in the trans-membrane region. Conserved proline residue at TM5 is replaced by tyrosine in GPR39. (B) Visualization of the predicted structure of GPR39 1a in a lipid bilayer based on molecular modeling. (C) Map of the major and minor binding pockets formed by the transmembrane domains of GPR39 1a. (D) Localization of 14,15-EET (green) and 15-HETE (gold) relative to the transmembrane domains of GPR39 1a, indicating the carboxylate moieties interacting with arginine residues from TM6 and the lipid tail portion extend to minor binding pocket (E) Superimposition of 14,15-EET (green) and 15-HETE (gold) orientation in the putative GPR39 1a binding pocket.

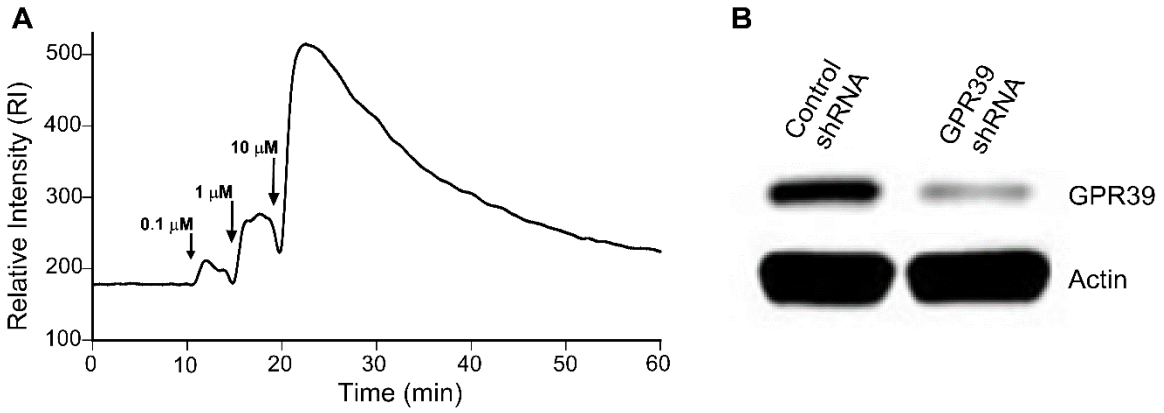

Fig. S5. Dose-dependent calcium response to 15-HETE and shRNA knockdown of GPR39 in mVSMCs. (A) Calcium imaging of primary mouse heart mVSMCs; 15-HETE (0.1, 1, 10 μM) increases $[Ca^{2+}]_i$ in mVSMCs. (B) Western blot confirmation of GPR39 knockdown in mVSMCs infected with a lentivirus containing a scrambled shRNA (control) or shRNA targeting GPR39.

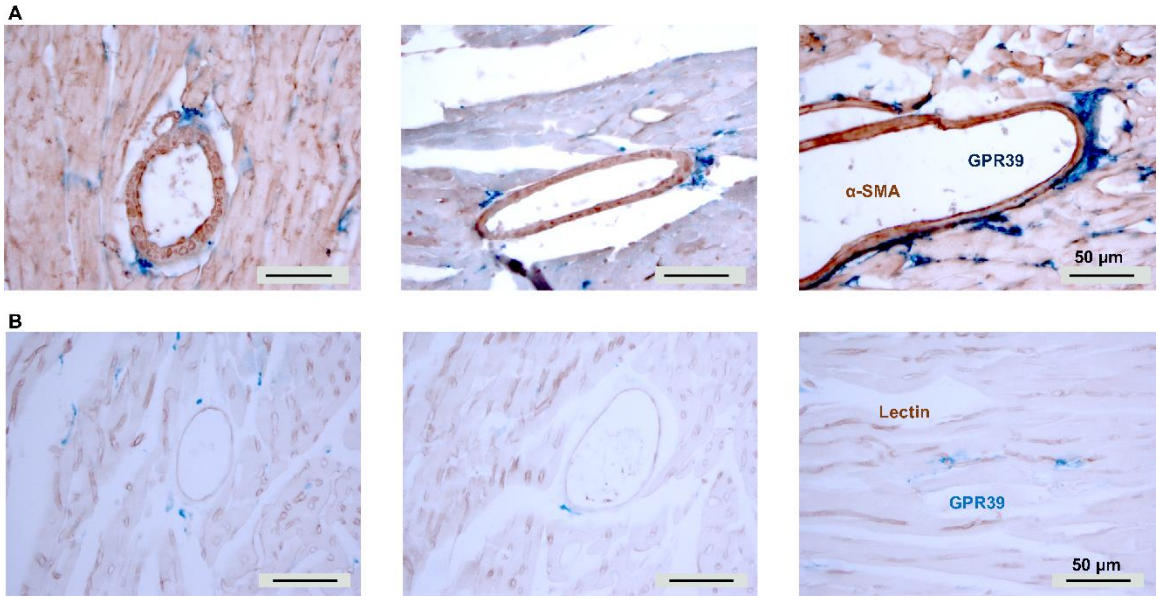

Fig. S6. Non-fluorescent immunohistochemical staining of GPR39 in mouse heart. A. Double immunostaining for GPR39 (blue) and α-smooth muscle actin (α-SMA, brown). B. Double staining for GPR39 (blue) and *Griffonia (Bandeiraea) Simplicifolia* Lectin I (brown). Scale bar: 50 microns.

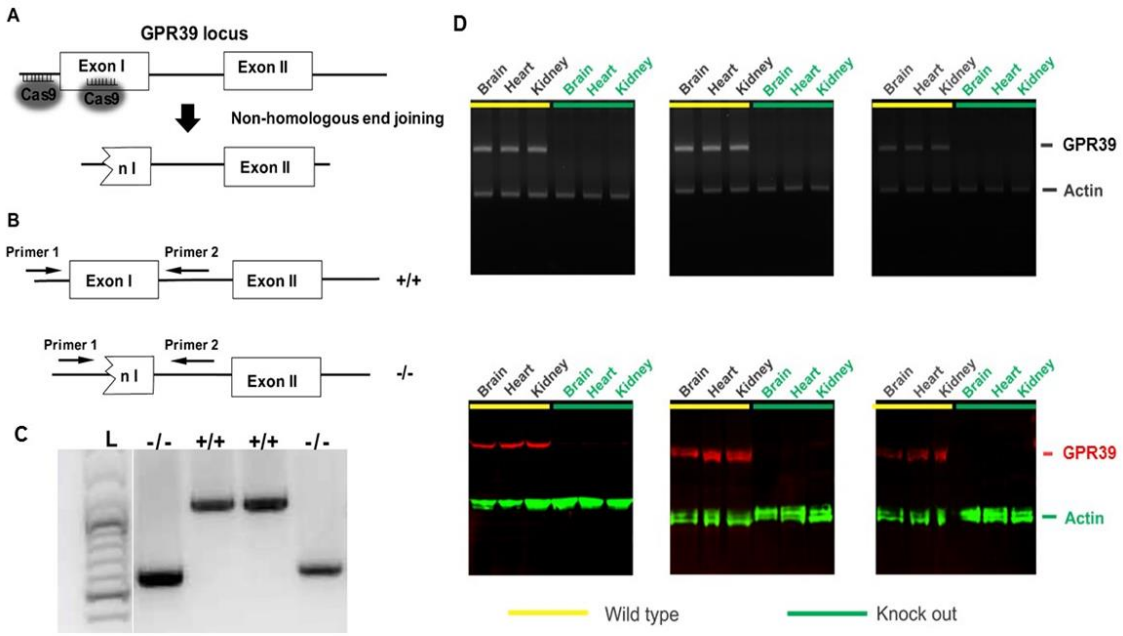

Fig. S7. CRISPR/Cas9-mediated disruption of the GPR39 locus. (A) Schematic depicting the strategy employed to delete the majority of the exon 1 coding sequence of GPR39. (B) Diagram of the position for the genotyping primers to detect both the mutant and wild type alleles of GPR39. (C) Representative results of genotyping indicating the presence of either the deleted or wild type PCR product. (D) RT-PCR and Western blot analysis of GPR39 1a expression in brain, heart and kidney from wild-type and GPR39 knockout mouse littermates.