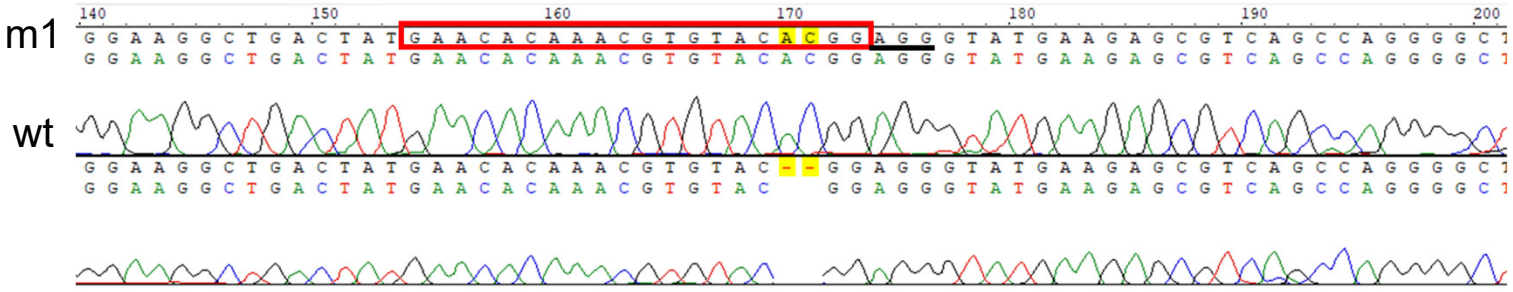
Supplementary Figure 1

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CLUSTAL 2.1 multiple sequence alignment


WT              MGPGRLLLVAVGLSLCGPLSSRVPMRQPESERMYATPYATPNPRSFFLRNPSEDTFEQFP
-m1             MGPGRLLLVAVGLSLCGPLSSRVPMRQPESERMYATPYATPNPRSFFLRNPSEDTFEQFP
                ************************************************************

WT              LGDEEEKNESIPLEGRAVYLNKSRFPPMPPPPFISEDASGYLTSPWLTLFIPSVYTFVFI
-m1             LGDEEEKNESIPLEGRAVYLNKSRFPPMPPPPFISEDASGYLTSPWLTLFIPSVHVCVHS
                ************************************************:. *.

WT              VSLPLNILAIAVFVFRMKVKKPAVVYMLHLAMADVLFVSVLPFKISYYFSGTDWQFGSGM
-m1             QPSPE-------------------------------------HPGHRCVCLSDEGQEAGRGV
                 . *                                     * : .  :*. . : * *:

WT              CRFATAAFYCNMYASIMLMTVISIDRFLAVVYPIQSLSWRTLGRANFTCVVIWVMAIMGV
-m1             HAAPGHGRCPLRVRAPLQDQLLLLRHRLAVRVRNVSLRHRSVLL----------------
                   .  .       : :   :: : : ***     **  *::

WT              VPLLLKEQTTQVPGLNITTCHDVLNETLLHGFYSYYFSAFSAIFFLVPLIISTVCYTSII
-m1             ------------------------------------------------------------


WT              RCLSSSAVANRSKKSRALFLSAAVFCIFIVCFGPTNVLLIVHYLLLSDSPGTETAYFAYL
-m1             ------------------------------------------------------------


WT              LCVCVSSVSCCIDPLIYYYASSECQKHLYSILCCRESSDSNSCNSTGQLMPSKMDTCSSH
-m1             ------------------------------------------------------------


WT              LNNSIYKKLLA
-m1             -----------
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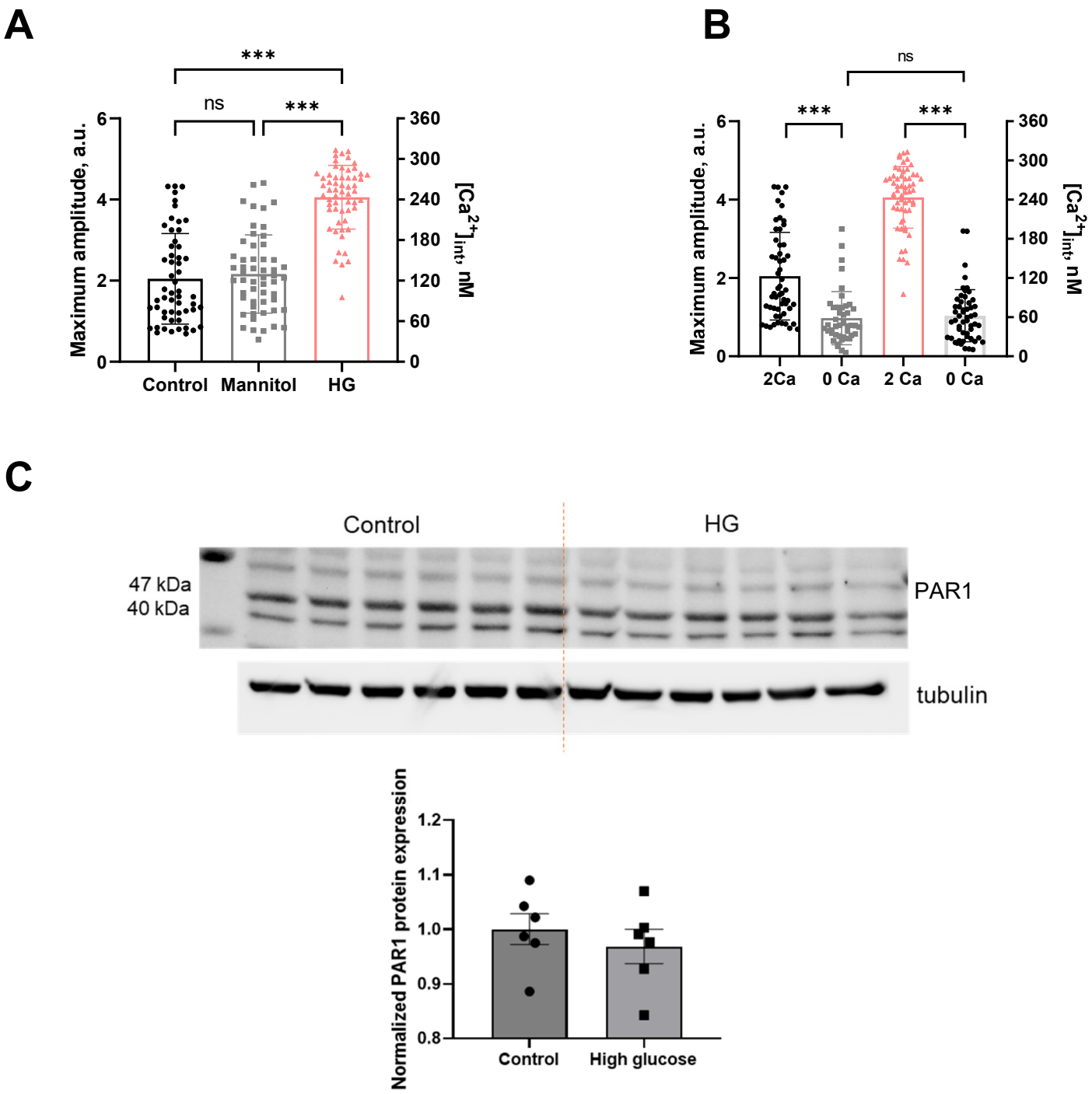
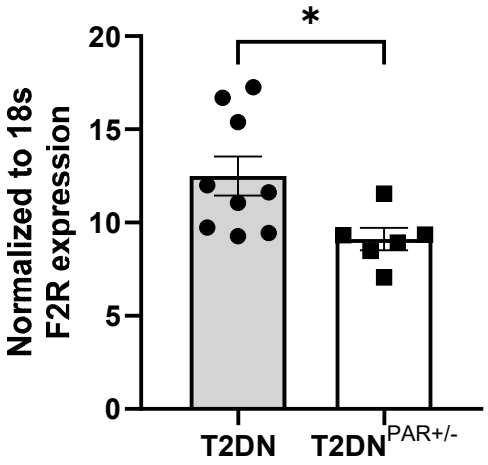
Supplementary Figure 1. (A) Sanger sequencing alignments of a WT and –m1 mutant sequence. A CRISPR targeting GAACACAAACGTGTACACGG, in red box above, PAM is underlined. Sanger read from mutant allele shows 2bp deletion. (B) Predicted protein alignment between WT and –m1 from translation of DNA. The CRISPR was introduced into T2DN rat embryos via RNP pro nuclear injection into one cell embryos. The founder animals were screened using fluorescent fragment analysis genotyping assay for the targeted locus (Primers: 5' AAGGGGAGCACGGACACGAA 3' and 5' CCCTAGGGGATGAGGAGGAGAAA 3'). The genotyping results were further verified by Sanger sequencing to confirm the accuracy of the introduced mutations. The mutation induced in F2r resulted in a 2 base pair deletion within exon 7. After confirming the desired mutation in the F2r gene, the founders were crossed back with the parental strain to establish a stable genetic line. Subsequent litters were screened using fluorescent fragment analysis to ensure the presence of the introduced mutations.

Supplementary Figure 2



Supplementary Figure 2. (A) Summary of the $[Ca^{2+}]_i$ amplitudes in response to PAR1 activation in control, Mannitol (20 mM) and high glucose (HG) (2.05 ± 1.12 vs. 2.16 ± 0.97 vs. 4.06 ± 0.79 a.u., for Control vs. Mannitol vs. HG; n≥55 cells, N≥4 dishes, ANOVA, Holm-Sidak, ****P*<0.001). Right Y axis shows actual calcium concentration in podocytes. (B) Summary of the $[Ca^{2+}]_i$ amplitudes in response to PAR1 activation in 2 mM and 0 mM Ca^{2+} conditions (2.05 ± 1.12 vs. 2.16 ± 0.97 vs. 4.06 ± 0.79 a.u., for Control vs. Mannitol vs. HG; n≥55 cells, N≥4 dishes, ANOVA, Holm-Sidak, ****P*<0.001). (C) Western blot analysis of PAR1 expression in cultured human podocytes after 12 hrs incubation in control or high glucose solutions.

Supplementary Figure 3



Supplementary Figure 3. mRNA expression of F2R (PAR1) estimated by RT-qPCR in >48 weeks old rats. Quantification of mRNA was determined by normalizing to 18S (t-test, *P*<0.05). For real-time quantitative RT-qPCR analysis, total RNA was extracted with TRIzol reagent (ThermoFisher Scientific) from renal cortical tissue. The quality and quantity of the individual samples were determined by spectrophotometry (multimode microplate reader BioTek Synergy Neo2). Primers (forward: AATTGGCAAGGGAGGGGATG, reverse: TGTGTCTGTCTGTGCAAGGG) for F2r gene were designed using National Center for Biotechnology Information Primer3 and BLAST and purchased from Invitrogen (Waltham, MA).

Supplementary Tables

Blood electrolytes analysis of 12-wk-old T2DN and T2DN$^{PAR1+/-}$ rats

BLOOD		T2DN	T2DN$^{PAR1+/-}$	*P* value
pH		7.37±0.07	7.37±0.03	0.88
Calcium	mmol/L	1.23±0.05	1.22±0.07	0.91
Sodium	mmol/L	137±2	135±2	0.26
Potassium	mmol/L	3.75±0.33	3.81±0.33	0.69
Chloride	mmol/L	100±3	98±4	0.35
Glucose	mg/dL	463±77	463±84	0.96
Creatinine	mg/dL	0.29±0.09	0.35±0.3	0.59

Blood electrolytes analysis of >48-wk-old T2DN and T2DN$^{PAR1+/-}$ rats

BLOOD		T2DN	T2DN$^{PAR1+/-}$	*P* value
pH		7.4±0.04	7.4±0.04	0.94
Calcium	mmol/L	1.26±0.03	1.27±0.06	0.99
Sodium	mmol/L	136±1.3	137±3.4	0.24
Potassium	mmol/L	4.4±0.3	4.1±0.4	0.1
Chloride	mmol/L	104±7	109±31	0.5
Glucose	mg/dL	440±57	473±128	0.4
Creatinine	mg/dL	0.42±0.13	0.49±0.19	0.31

Data are presented as means ± SE. T2DN, type 2 diabetic nephropathy. T2DN (n=10) and T2DN$^{PAR1+/-}$ (n=6) groups were not significantly different (t-test).