Supporting information

Oral nano-curcumin alone or in combination with insulin alleviates STZ-induced diabetic neuropathy in rats

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Figure S1

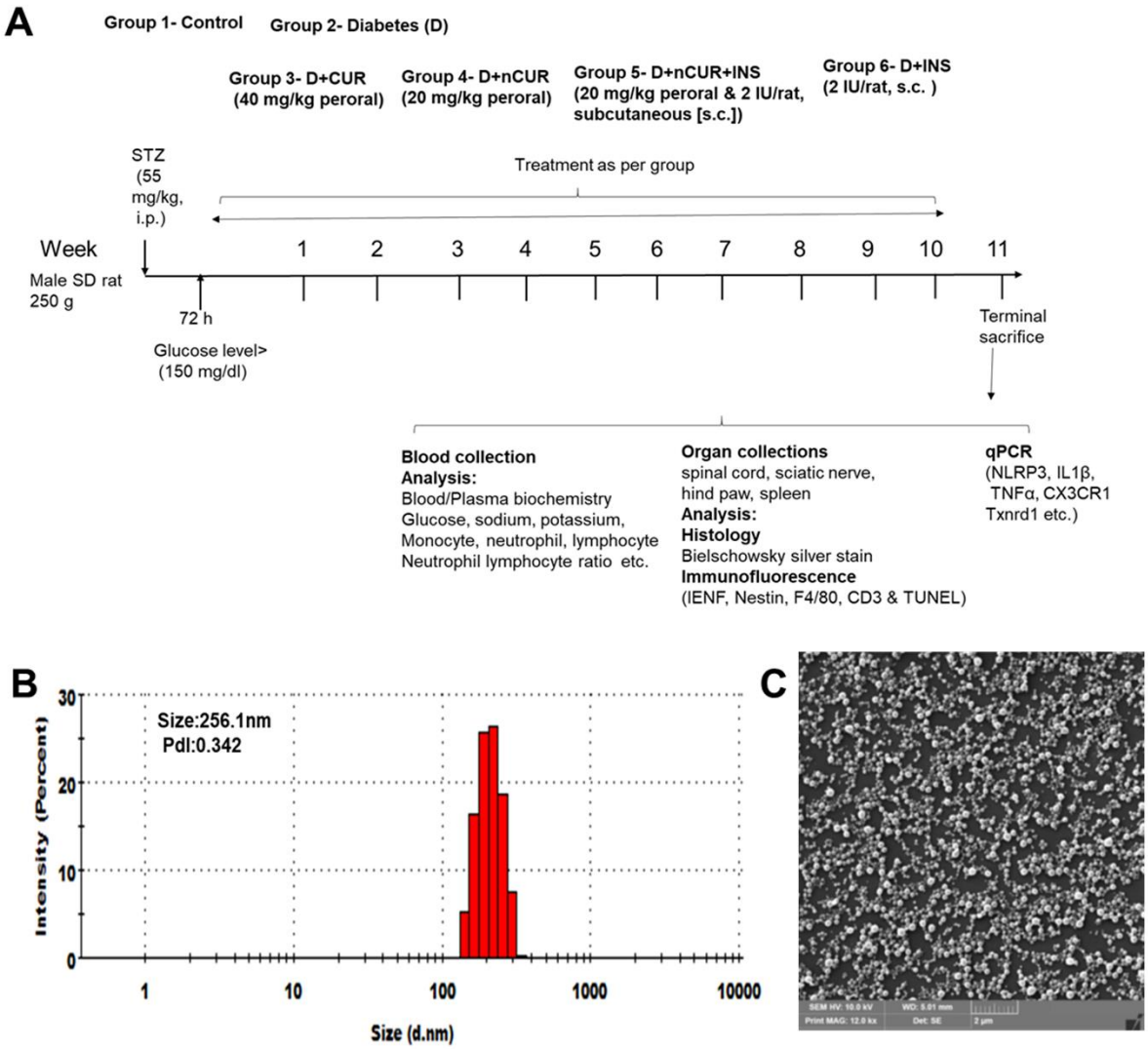

Figure S1. Study design and characterization of representative nCUR preparations. (A) Schematic representation of study design and treatment plan, treatments were performed in the morning around 9 am each day, insulin was injected (subcutaneously) first and an hour later nCUR administered orally. (B) A representation of particle size distribution measured by DLS and (C) representative scanning electron micrograph of nCUR.

Figure S2

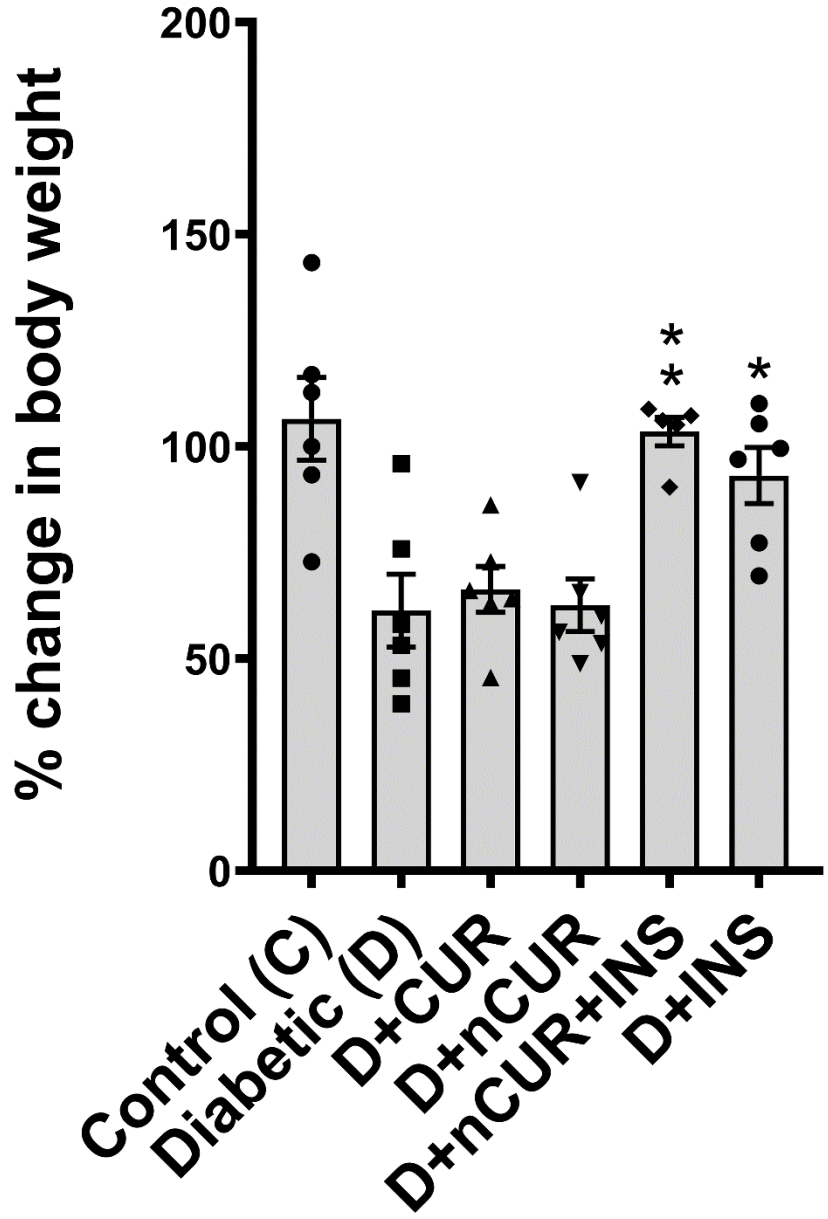

Figure S2. Effect of treatment on body weights. Data represents percent change in body weights in different groups. Statistical analysis was carried out using one-way ANOVA and Tukey post-hoc. Significant values were compared against the diabetic group. * $P < 0.05$, ** $P < 0.01$.

Figure S3

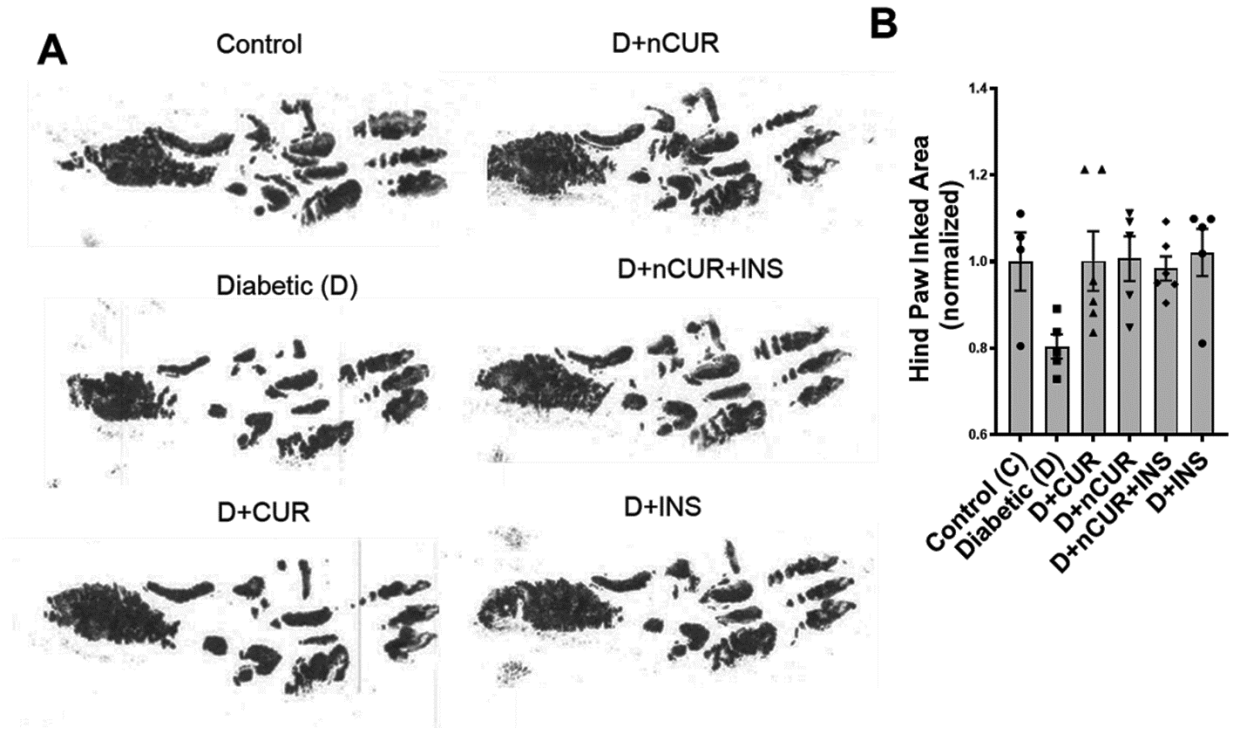

Figure S3. Effect of treatment on hind paw morphology in diabetic rats. (A). Representative images of right hind paw. (B). The inked pad area (n=8 images) was measured for quantification using image J. Statistical analysis was carried out using one-way ANOVA and Tukey post-hoc. Significant values were compared against the diabetic group.

Figure S4

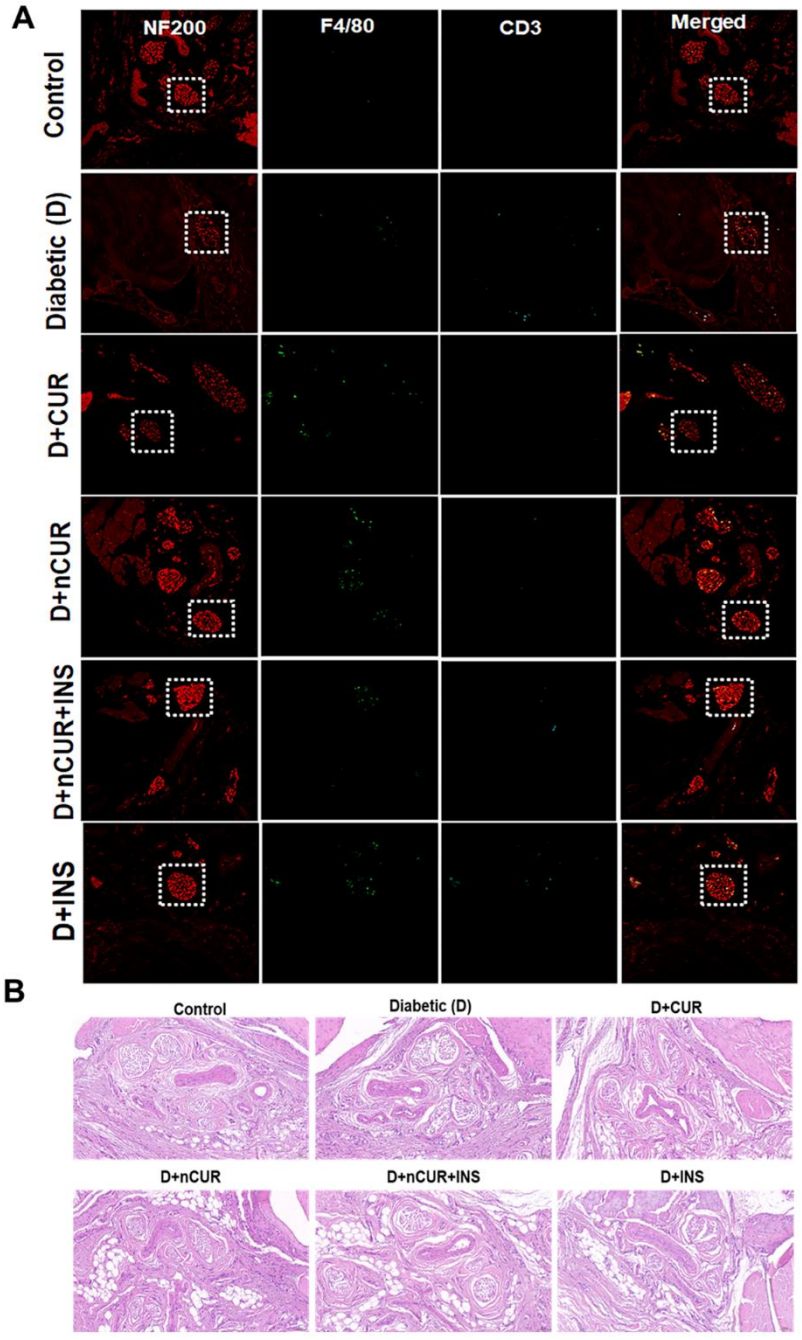

Figure S4. Effect of treatment on peripheral nerves in diabetic rats (A) Representative confocal images (20x magnification) of peripheral nerves of right hind paw stained for NF200 (red), F4/80^{+} (green), and CD3^{+} (turquoise). (B) Representative H & E staining of peripheral nerves localization (red arrow) in right hind paw (20x magnification).

Figure S5

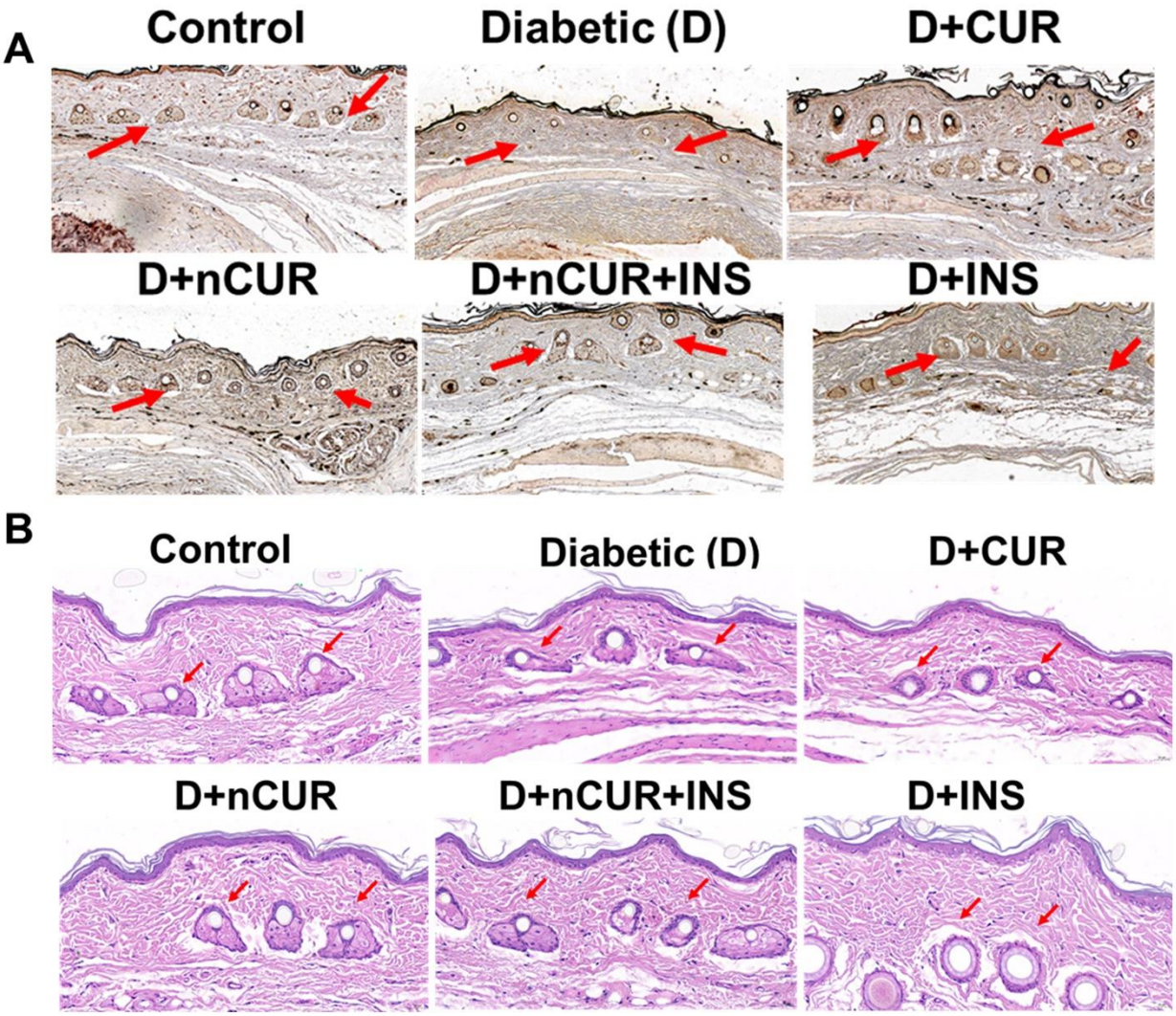

Figure S5. Effect of treatment on Bielschowsky's silver and H & E staining of skin in diabetic rats (A) Representative Bielschowsky's silver staining of skin of right hind paw (20x magnification) with possible nerve innervation around sebaceous gland. Red arrow indicating the sebaceous gland. (B) Representative H &E staining of sebaceous gland in right hind paw (40x magnification). Red arrow indicating the sebaceous gland.

Figure S6

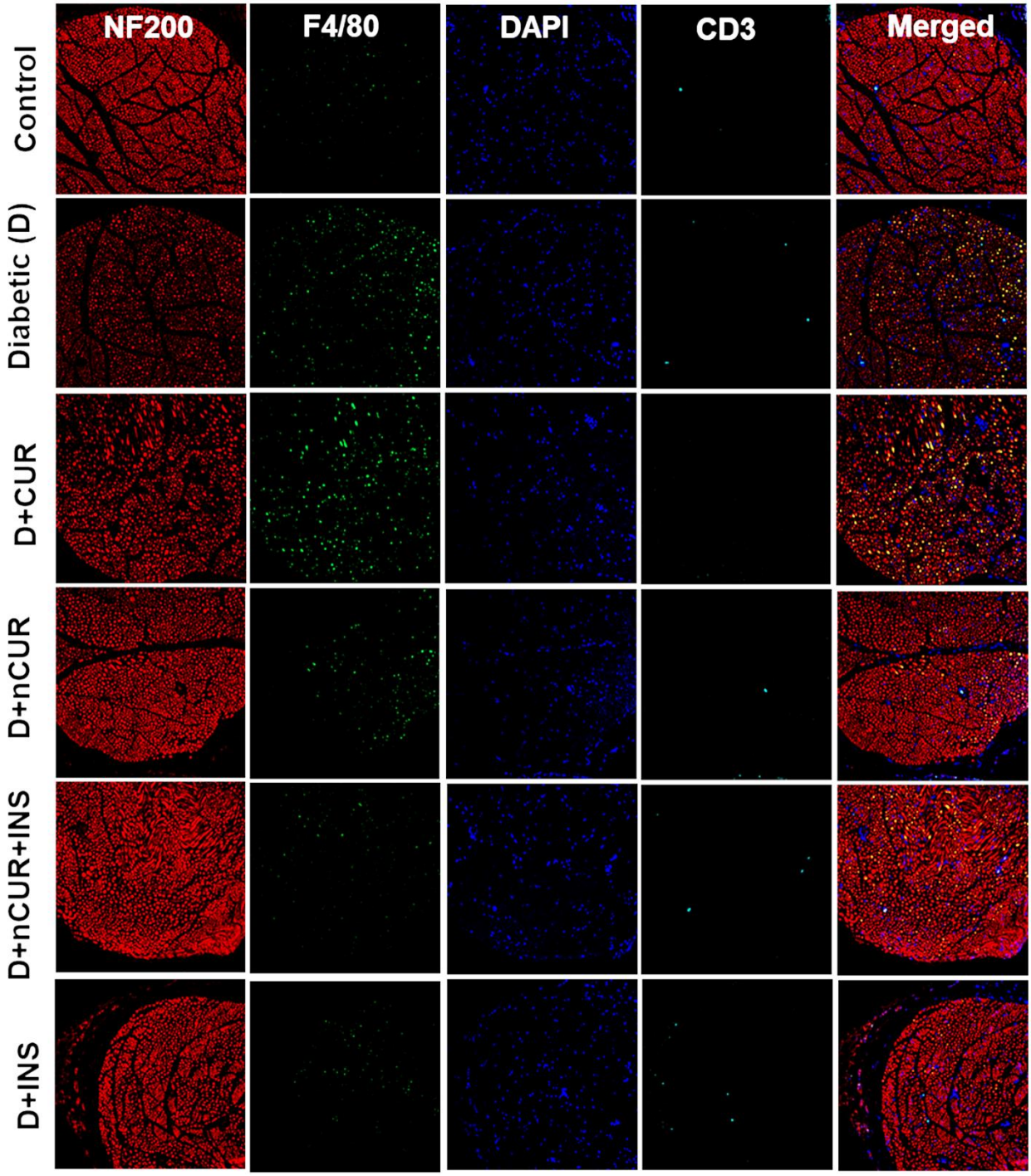

Figure S6. Representative confocal images (20 x magnification) of sciatic nerve stained for NF200 (red), F4/80^{+} (green), and CD3^{+} (turquoise).

Figure S7

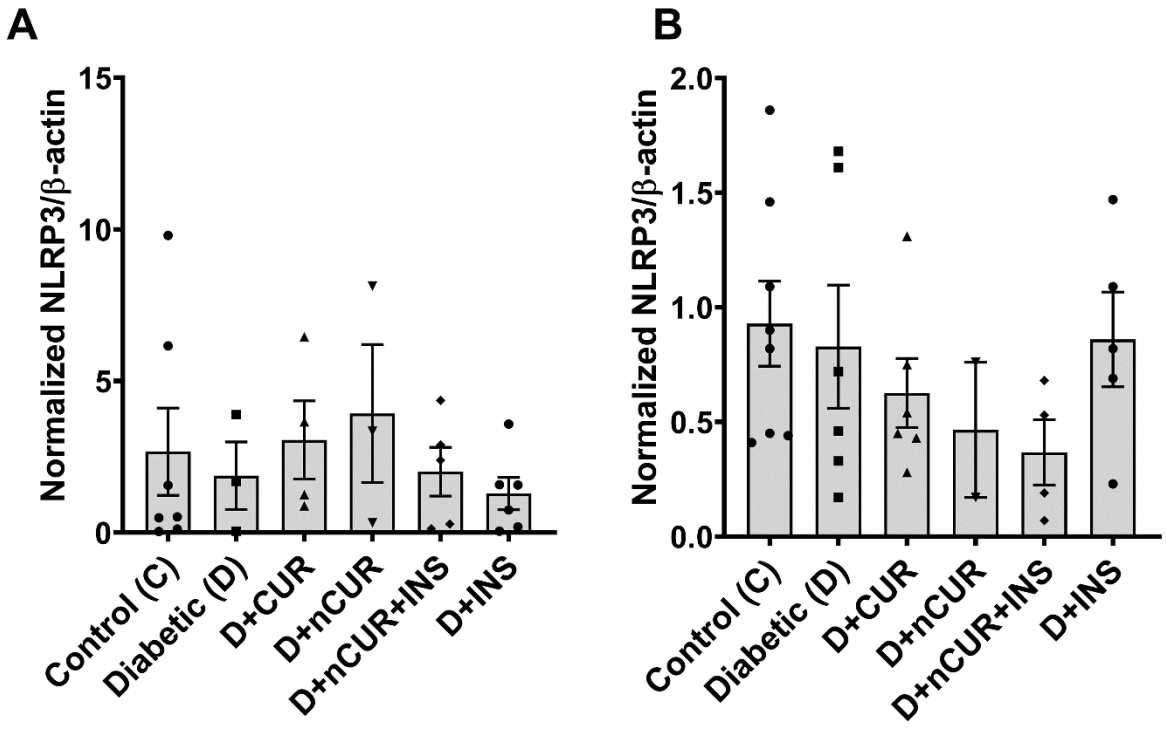

Figure S7. Effect of treatment on NLRP3 inflammasome in hind paw of diabetic rats. Real-time PCR mRNA level of NLRP3 in hind paw of (A) bulb region and (B) ventral nerve region. Statistical analysis was carried out using one-way ANOVA and Tukey post-hoc test.

Figure S8

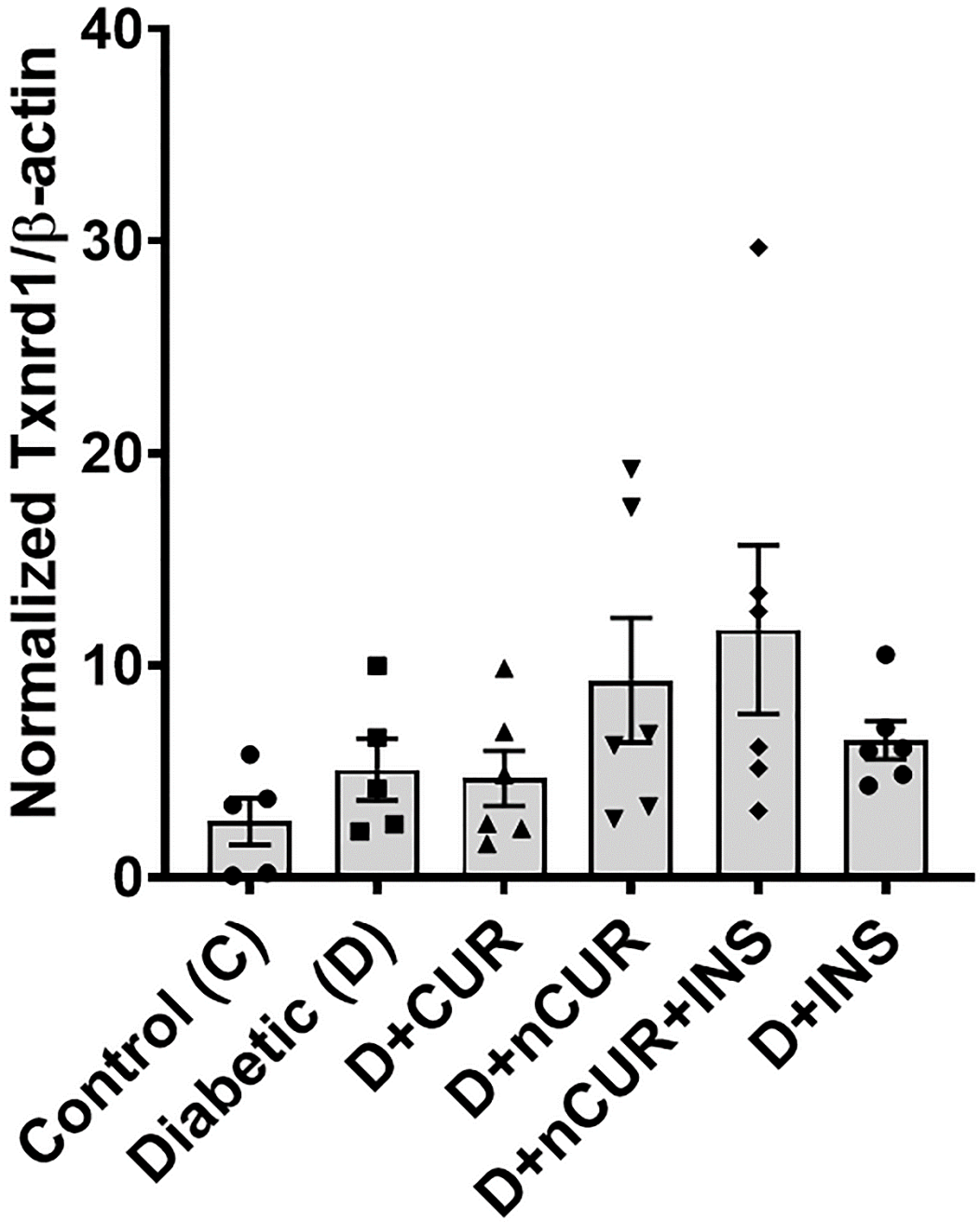

Figure S8. Real time PCR mRNA level of Txnrd1 in sciatic nerve. Statistical analysis was carried out using one-way ANOVA and Tukey post-hoc test.

Figure S9

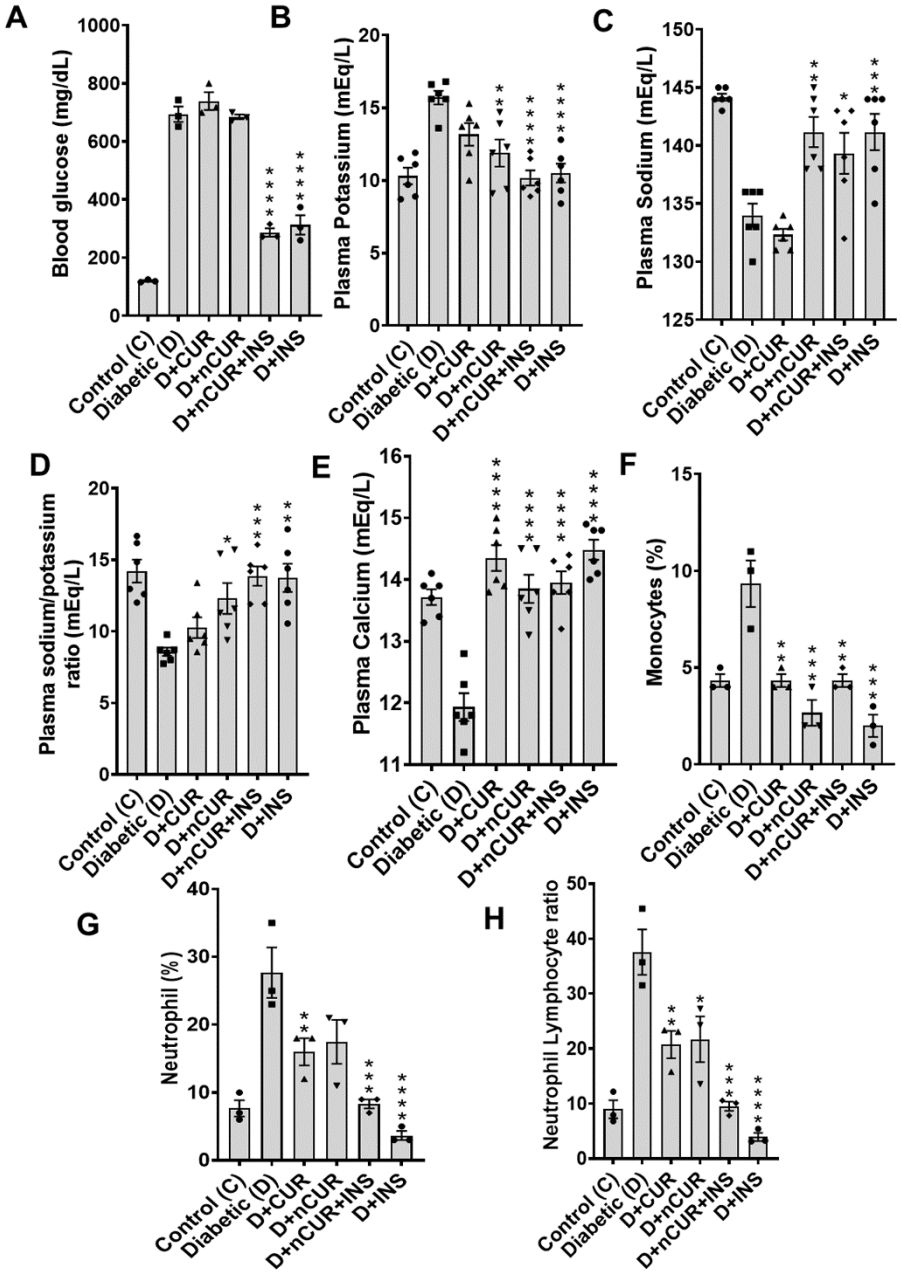

Figure S9. Effect of treatment on terminal blood/plasma biochemistry in diabetic rats. (A) blood glucose, (B), potassium (C) sodium, (D) sodium-potassium ratio (E) calcium and percentage of (F) monocytes (G) neutrophils and (H) Neutrophil-lymphocyte ratio (NLR) was presented. Statistical analysis was carried out using one-way ANOVA and Tukey post-hoc. Significant values were compared against the diabetic group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$.

Figure S10

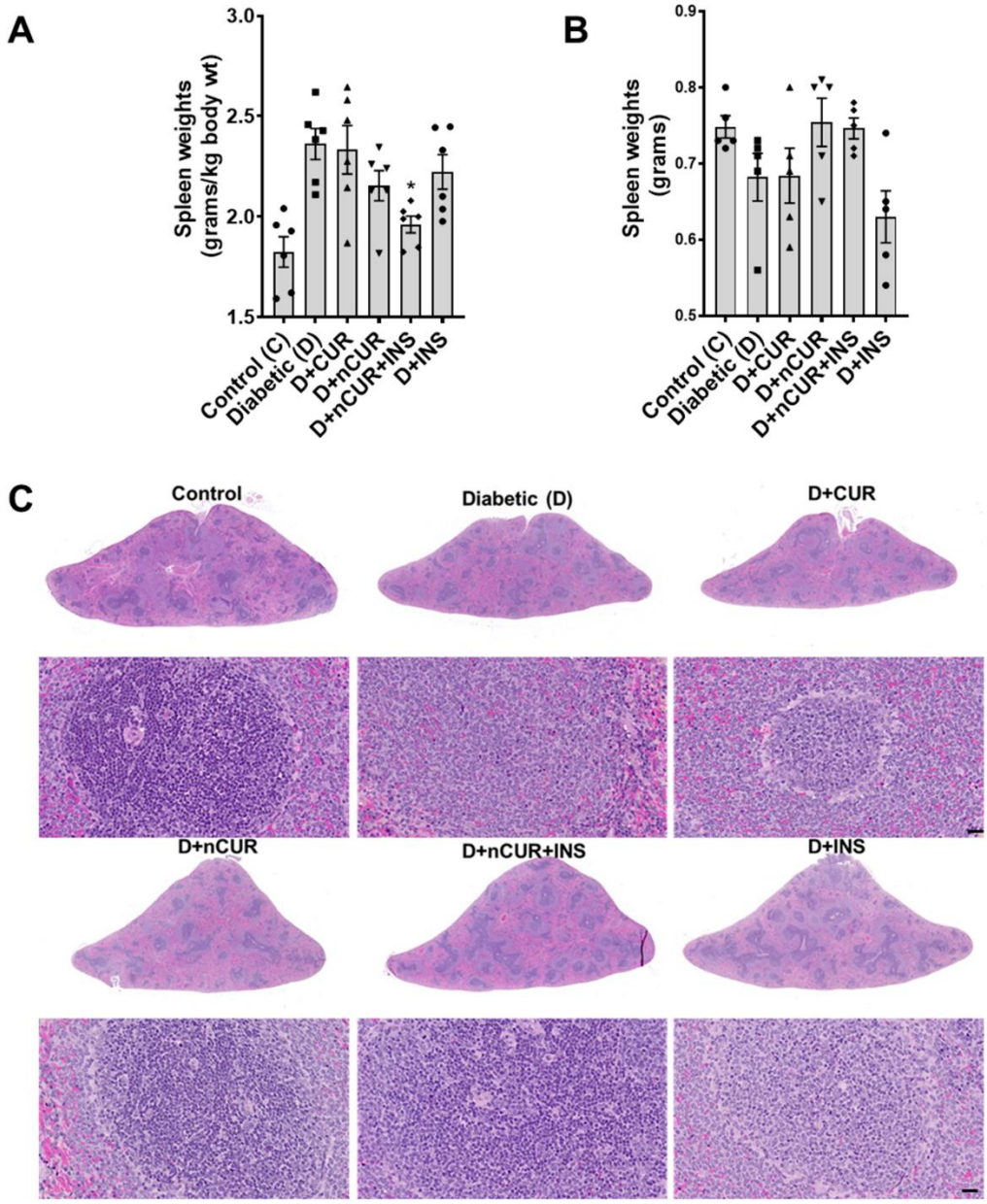

Figure S10. Effect of treatment on spleen in diabetic rats (A). Spleen weight per kg of body weight was increased overall in untreated diabetes and only combination treatment is comparable to non-diabetic control. (B). Absolute spleen weight was reduced in untreated diabetes and improvement was not significant in different treatment group. (C). Representative histological sections (1.5x magnification scale bar represents 500µm for whole stitched images and remaining higher magnified images are at 40x magnification scale bar represents 20µm) of spleen stained with H & E showing wide distributions of white pulp in comparison to nondiabetic controls. Statistical analysis was carried out using one-way ANOVA and Tukey post-hoc. Significant values were compared against the diabetic group, * $P < 0.05$.

Figure S11

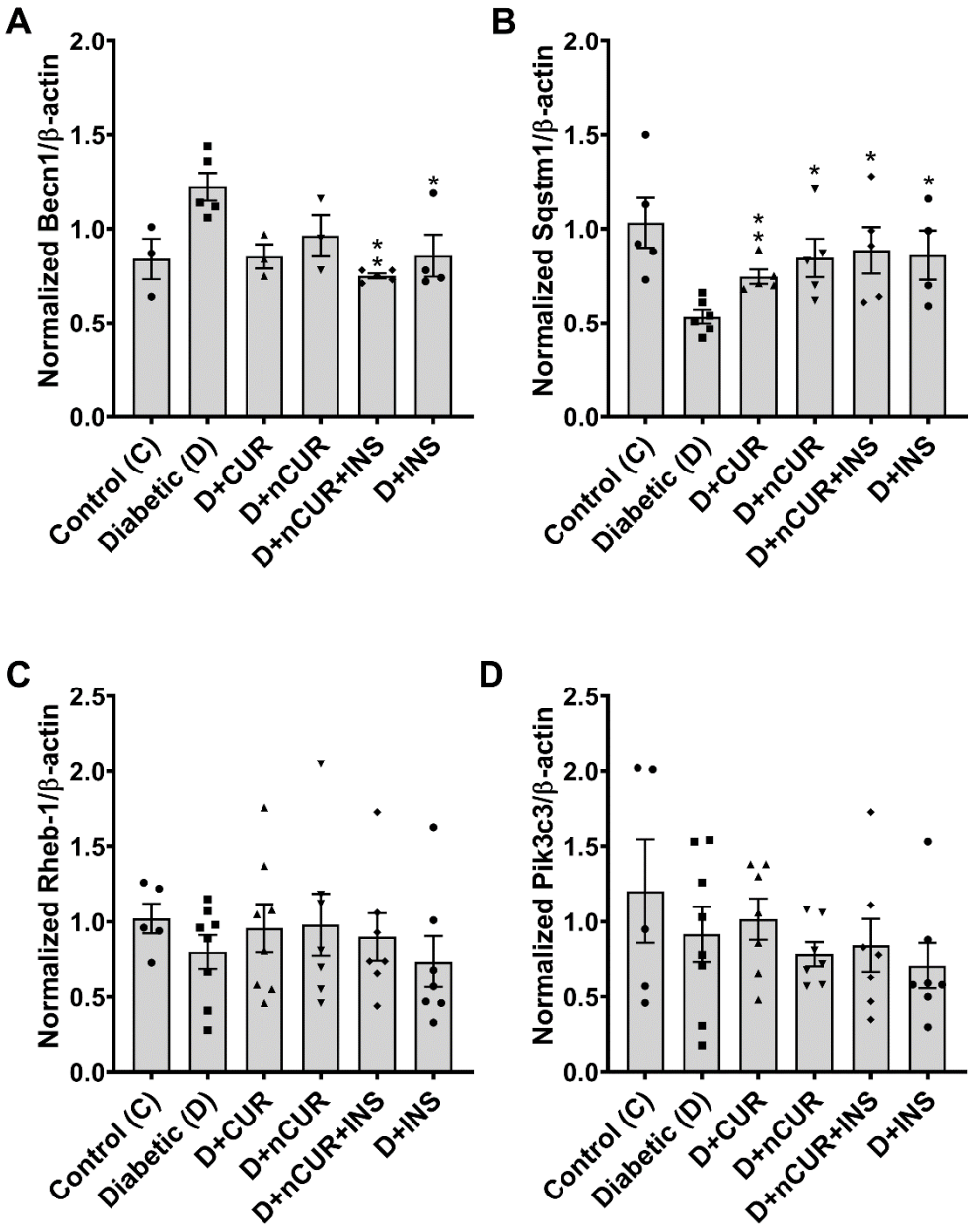

Figure S11. Real time PCR mRNA levels of autophagy markers in spinal cord (A) Becn1; (B) Sqstm1; (C) Rheb-1; and (D) Pik3C3. Statistical analysis was carried out using one-way ANOVA and Tukey post-hoc test. Significant values were compared against the diabetic group, * $P < 0.05$ and ** $P < 0.01$.

Figure S12

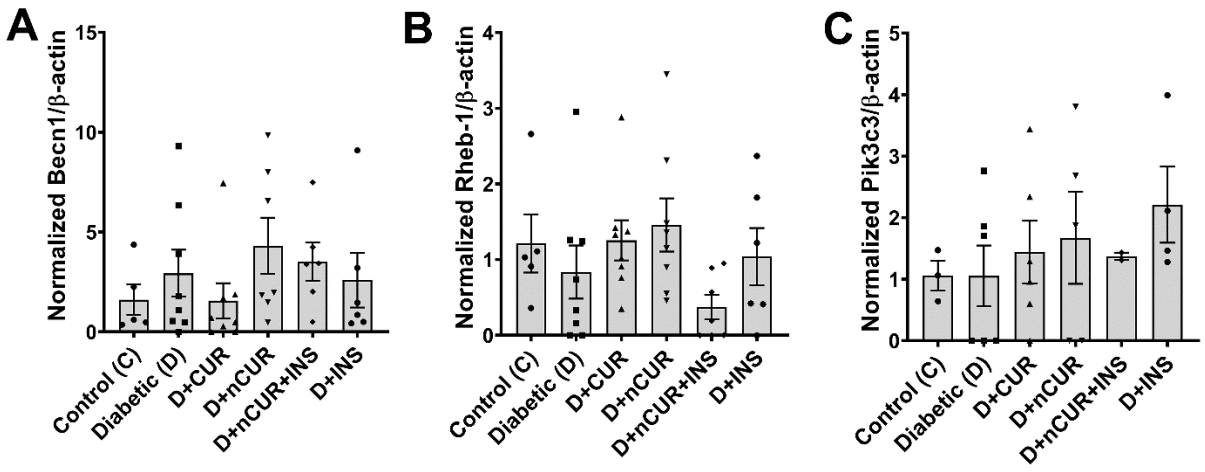

Figure S12. Real time PCR mRNA levels of autophagy markers in sciatic nerve (A) Becn1, (B) Rheb-1, and (C) Pik3C3. Statistical analysis was carried out using one-way ANOVA and Tukey post-hoc test for Becn1, Rheb and Pik3C3. Significant values were compared against the diabetic group.