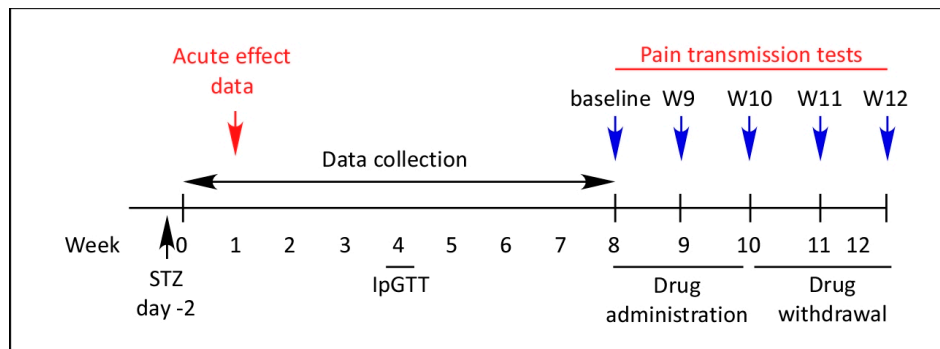


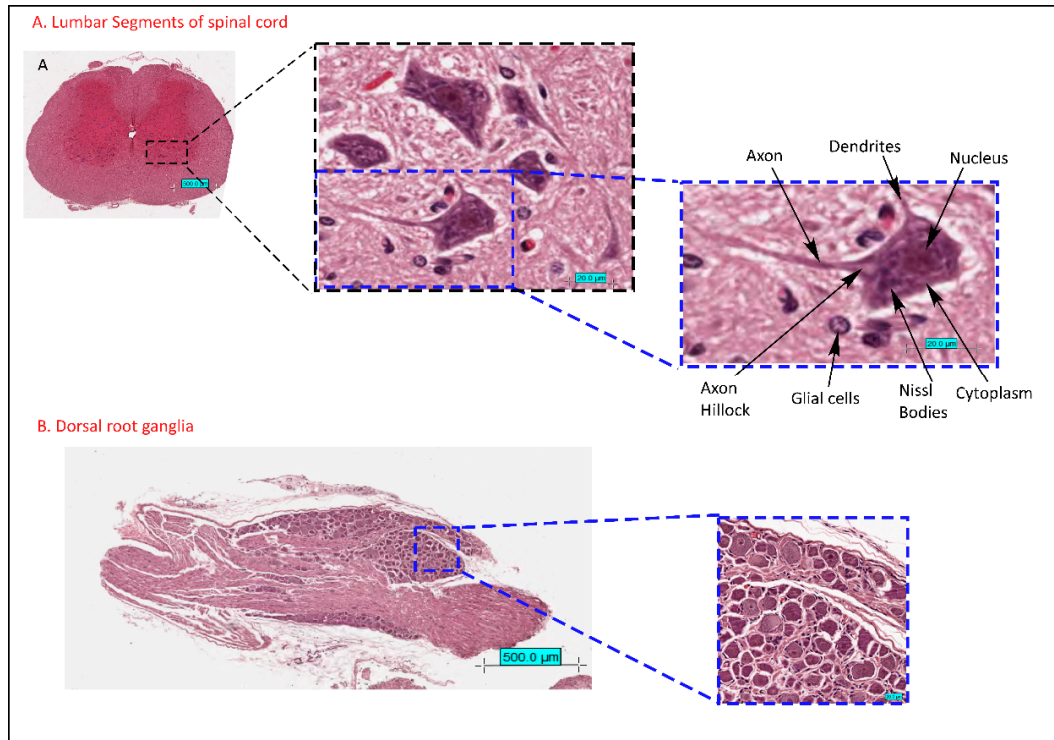
# Guanfacine Normalizes the Overexpression of Presynaptic $\alpha$ -2A Adrenoceptor Signaling and Ameliorates Neuropathic Pain in a Chronic Animal Model of Type 1 Diabetes

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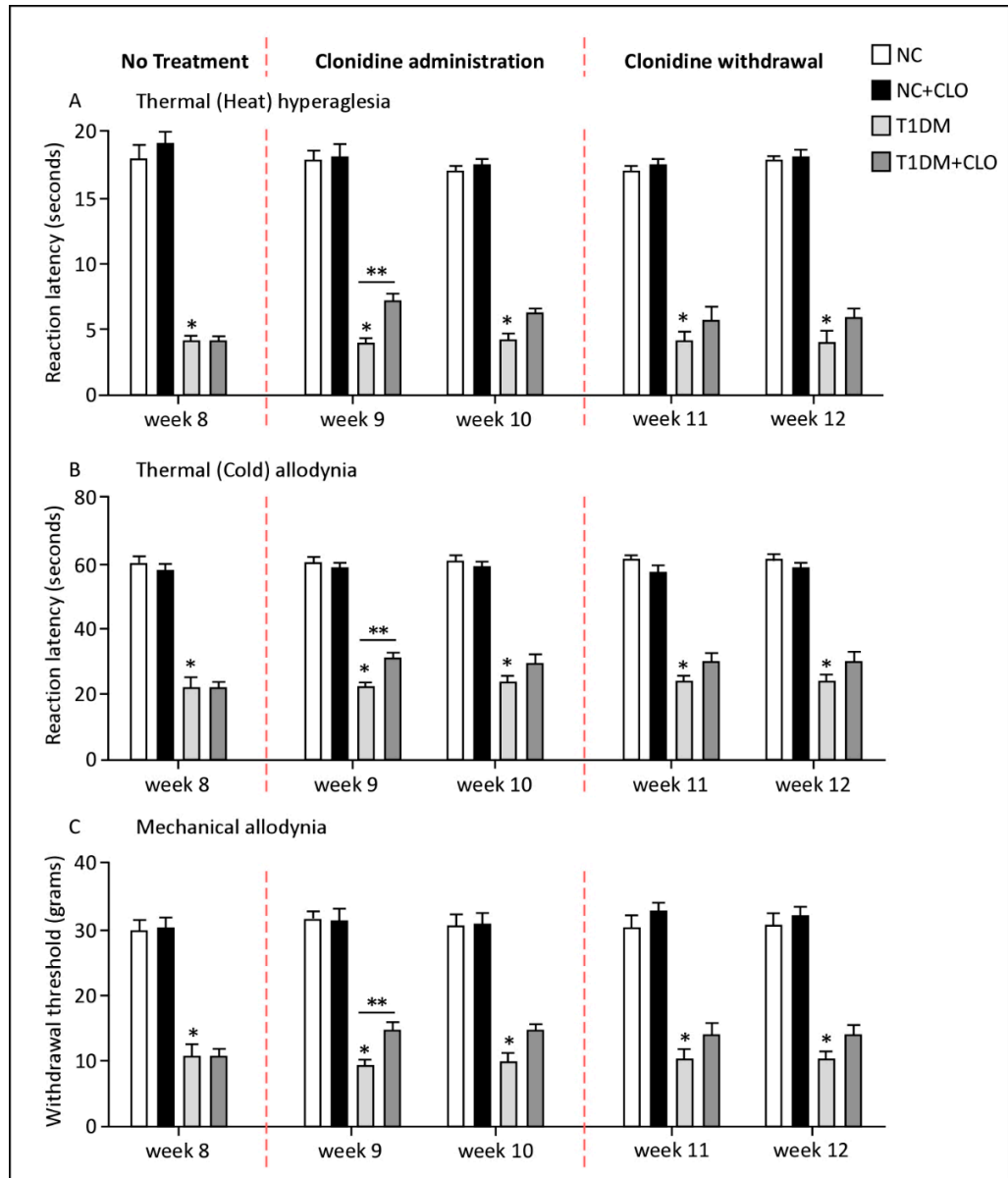
## Supplementary Figures and Legends



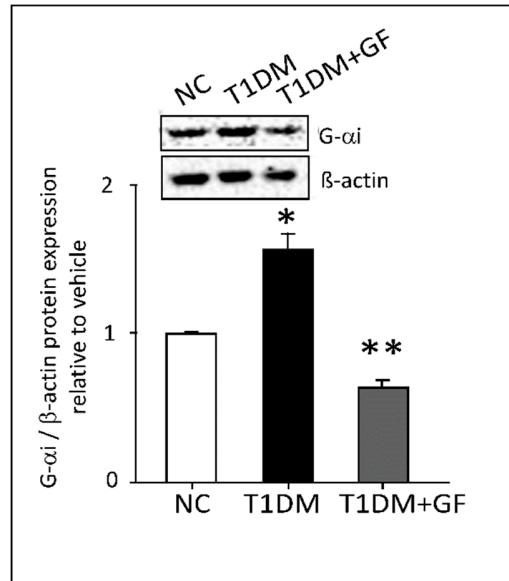
**Supplementary Figure S1.** Experimental procedures: the timeline of drugs administration and pain transmission tests conducted. Abbreviations: STZ, streptozotocin; and W, weeks.



**Supplementary Figure S2. Anatomy of H&E stained neuronal tissues of control rat showing the different type of cells. Lumbar segment of the spinal cord (A) and dorsal root ganglia (B).**



**Supplementary Figure S3. Clonidine's analgesic effects following two weeks of withdrawal.** Clonidine (0.1 mg/Kg) was administered intraperitoneally daily for a period of two weeks. Pain transmission tests were performed at weekly interval for two weeks of clonidine administration followed by two weeks of drug withdrawal. Data are expressed as means  $\pm$  SEM obtained from five animals/group. \* Significantly different from corresponding control values at  $p \leq 0.05$ . \*\* Significantly different from corresponding diabetic values at  $p \leq 0.05$ . Abbreviations: **NC**, Normal Control; **T1DM**, Type 1 Diabetes Mellitus; and **CLO**, clonidine.



**Supplementary Figure S4.** Chronically administered guanfacine **reversed** diabetes-induced dysregulation of neuronal spinal  $\alpha$ 2-AR signaling. GPCR-subtype: G- $\alpha_i$  in spinal cord lumbar region. Guanfacine (0.6 mg/Kg) was administered intraperitoneally daily for a period of two weeks. Protein bands imaged with the ChemiDoc™ MP were analyzed using the Image Lab software. Data are expressed as means  $\pm$  SEM from five animals/group. \* Significantly different from corresponding control values at  $p \leq 0.05$ . \*\* Significantly different from corresponding diabetic values at  $p \leq 0.05$ . Abbreviations: **NC**, Normal Control; **T1DM**, Type 1 Diabetes Mellitus; **GF**, guanfacine; **G- $\alpha_i$** , G-protein coupled receptor G-alpha I; and  **$\alpha$ 2-AR**, alpha-2A adrenoceptors.