# Enhancement of 26S Proteasome Functionality Connects Oxidative Stress and Vascular Endothelial Inflammatory Response in Diabetes



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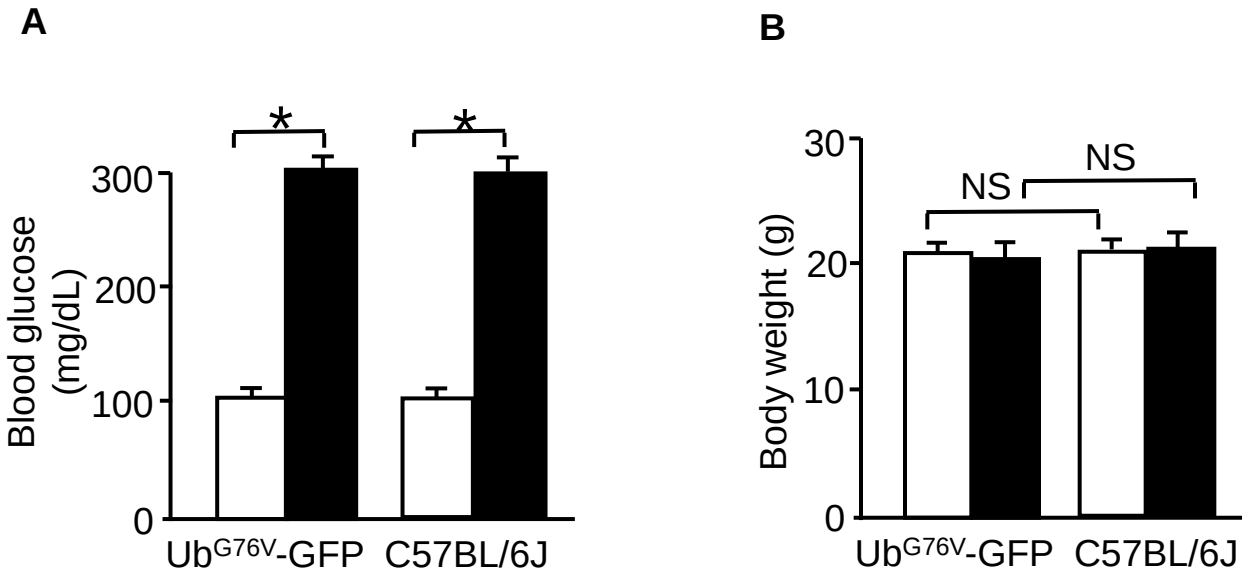
## Legends of Supplemental Figures

**Supplemental Figure I. $Ub^{G76V}$-GFP mice can be rendered diabetic by low dose regime of STZ, like their genetic control C57BL/6J mice.** Male and age matched (10 wks) wild type (C57BL/6J) and transgenic ($Ub^{G76V}$-GFP) mice received STZ-regimen (STZ: 50mg/kg/d; vehicle: sodium citrate, pH 4.5; for 5d; n=5/group). (A) Fasting (4h) blood glucose and (B) body weight shown here were obtained on $7^{th}$ day after the STZ regimen. The results (n=5/group) were analyzed with a one-way ANOVA. *$P$< 0.05.

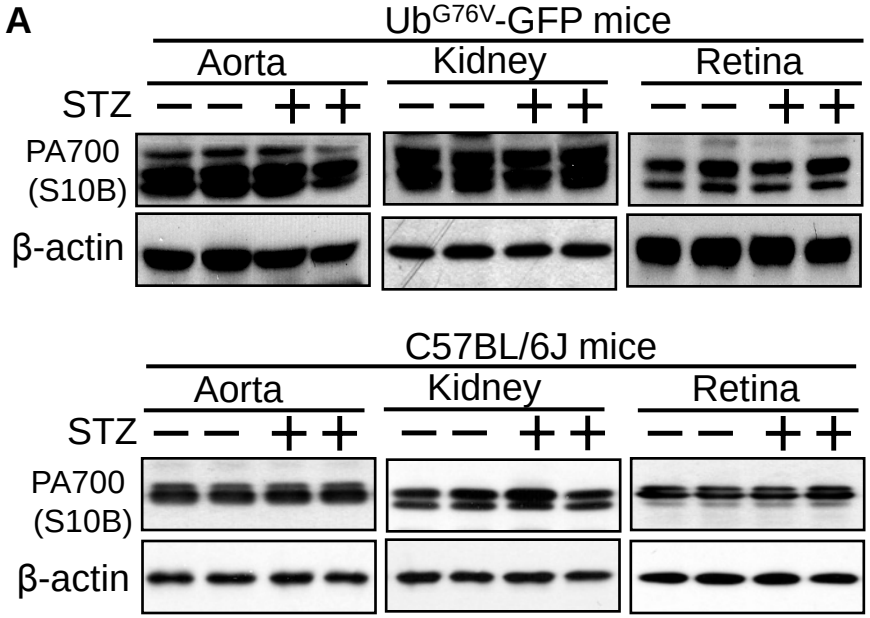
**Supplemental Figure II. Acute diabetes does not affect the protein levels of the PA700 (S10B), the regulatory sub-complex and activator of the 26S proteasome, in $Ub^{G76V}$-GFP mice.** Male and age matched (10 wks) wild type (C57BL/6J) and transgenic ($Ub^{G76V}$-GFP) mice received STZ-regimen (STZ: 50mg/kg/d; vehicle: sodium citrate, pH 4.5; for 5d; n=5/group). Tissue preparations 7d post STZ regimen were subjected to (A) Western blotting with a rabbit derived PA700 (S10B) antibody and a mouse derived β-actin antibody, followed by (B) quantification of protein band densitometry for levels of PA700 (S10B). The results (n=5/group) were analyzed with a one-way ANOVA. *$P$< 0.05 vs the vehicle-treated.

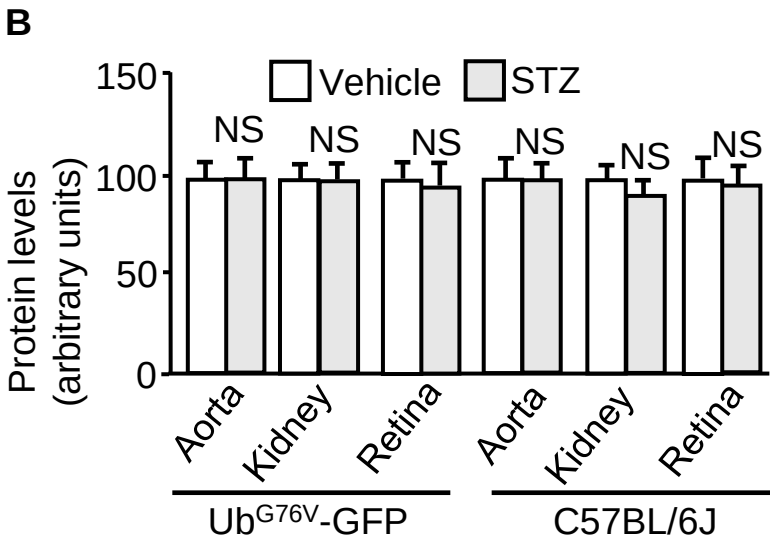
**Supplemental Figure III. The proposed scheme of early enhancement of 26S proteasome functionality and NF-κB-mediated inflammatory response in diabetes:** this study employed cell and mouse models to demonstrate that: (1) diabetes enhances 26S proteasome functionality in aortic, renal and retinal tissues; (2) such enhancement involves reactive oxidative species-

mediated 26S proteasome activation; (3) the enhancement operates as an early event in diabetes without affecting markers of autophagy and unfolded protein response (UPR); and (4) such an early event leads to activation of NF-κB pathway and pro-inflammatory responses. To the best of our knowledge, this is the first study on diabetic 26S proteasome functionality *in vivo* which is altered by acute hyperglycemia and may be the trigger of the increased inflammation observed in patients of diabetes. Notes, the ***models*** referred to cells ($Ub^{G76V}$-GFP cell/GFPu-1/HEK293, HUVEC, and BAEC/not shown) and mice (STZ-diabetic $Ub^{G76V}$-GFP mice and C57BL/6J mice, genetic diabetic OVE26 and Akita mice), the ***interventions*** included pharmaceutical and genetic approaches to inhibit or scavenge the generation of superoxide (mTEMPO), nitric oxide (L-NAME), or peroxynitrite (Uric acid); or to inhibit either 26S proteasome activity (MG132) or 26S proteasome activation (siRNA knockdown of 19S/PA700, the 26S proteasome activator). The ***checkpoints*** covered protein levels and cellular locations of poly-Ub-GFP and/or NF-κB and IκBα, promoter binding capacity of NF-κB (ChIP assay for promoters of Ccl5/RANTES, ICAM-1 and IκBα), as well as 26S proteasome activity and markers of autophagy and unfolded protein response (UPR).

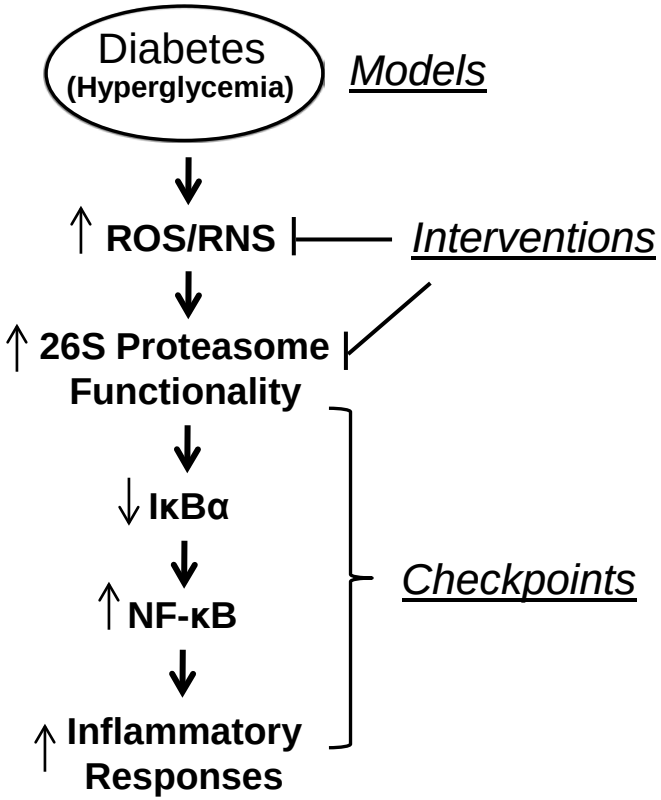



Liu *et al*. Supplemental Fig. I




Liu *et al*. Supplemental Fig. II




Liu *et al*. Supplemental Fig. III