Depletion of dendritic cells in perivascular adipose tissue improves arterial relaxation responses in type 2 diabetic mice

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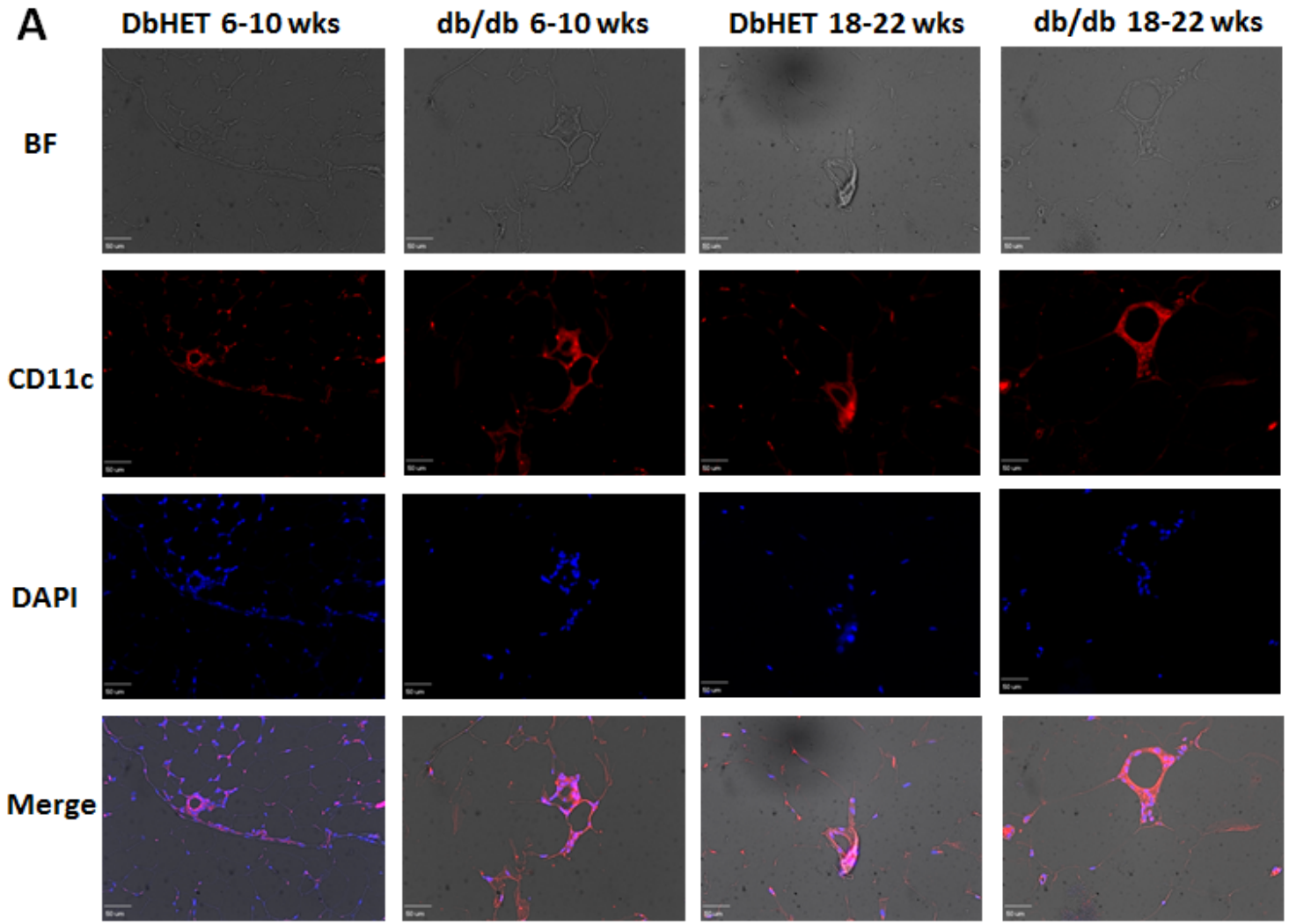
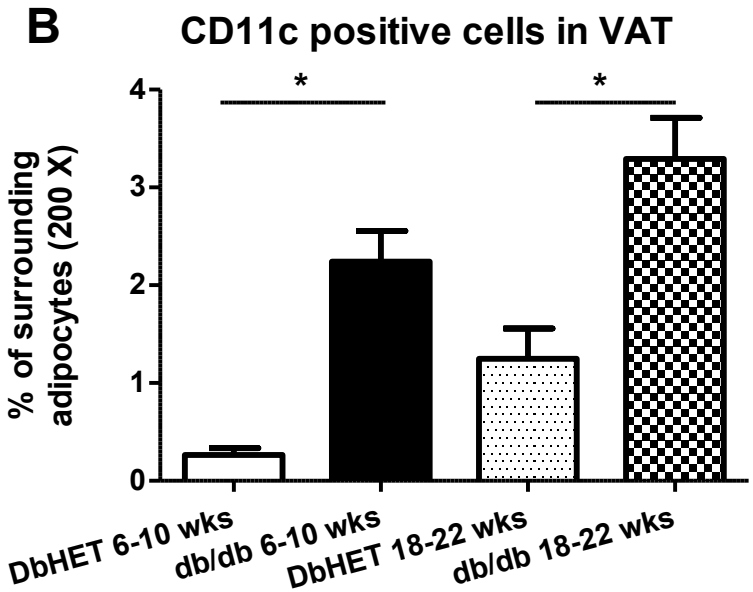
Supplementary Data File

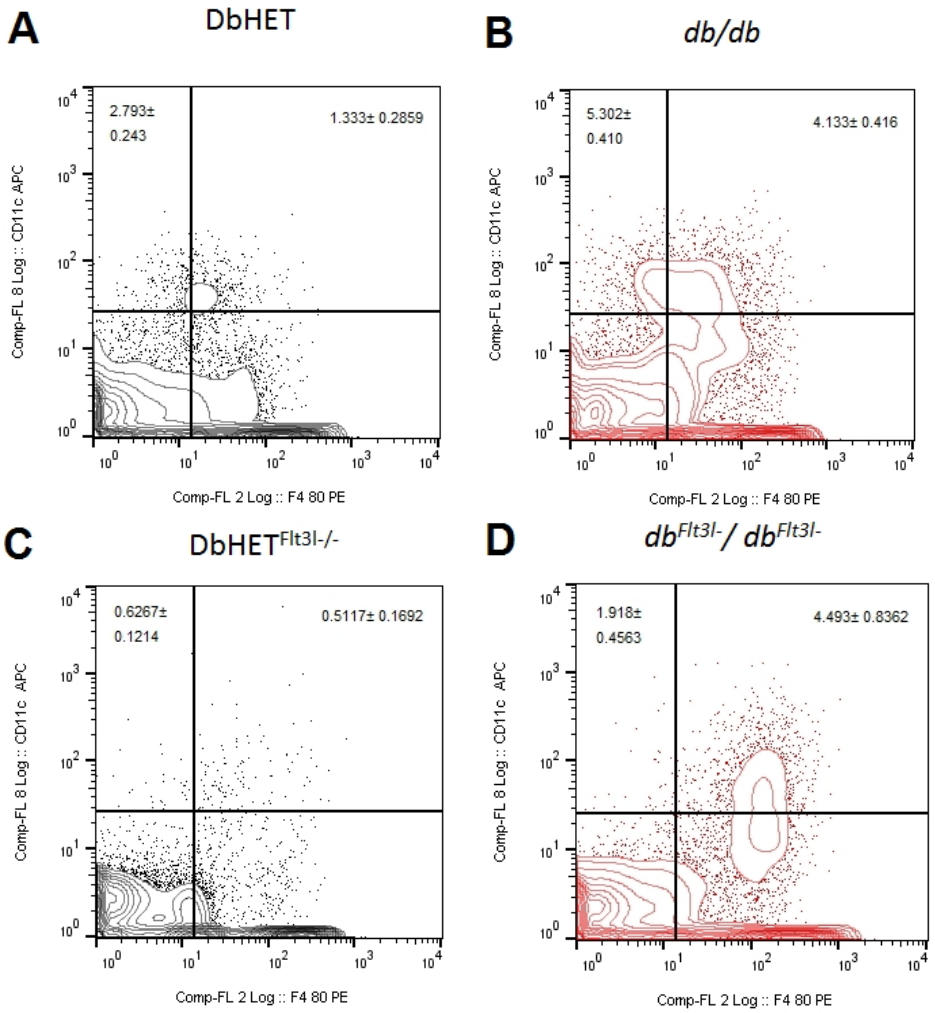


Supplementary Figure 1



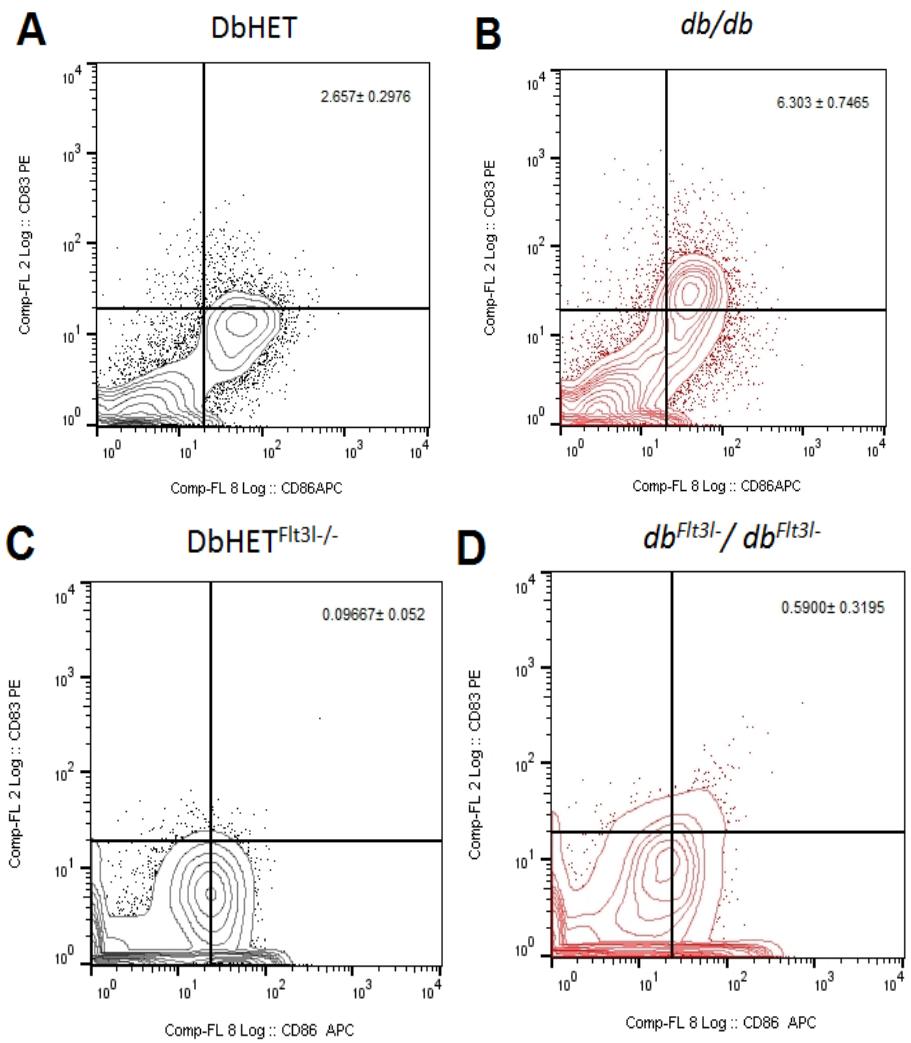
Supplementary Figure 1. Cellular distribution of CD11C positive dendritic cells in VAT of DbHET and *db/db* mice. (A): Immunofluorescence staining for CD11c in paraffin-embedded VAT from DbHET and *db/db* mice at ages 6-10 and 18-22 weeks. BF = bright field image of VAT (outlining the contour of adipocytes). CD11c was stained using Texas Red. Nuclei, stained with DAPI are shown as blue. Merged images, showing staining of the stromal vascular fraction, are shown in the bottom row. Scale bar represent 50 µm. Sections from six mice were analyzed for each staining. (**B)**: Quantitative analysis of CD11c positive staining in VAT. Data are presented as mean ± SEM. n=6. *: $P < 0.05$ between DbHET and *db/db* mice.

Supplementary Figure 1 (Cont)



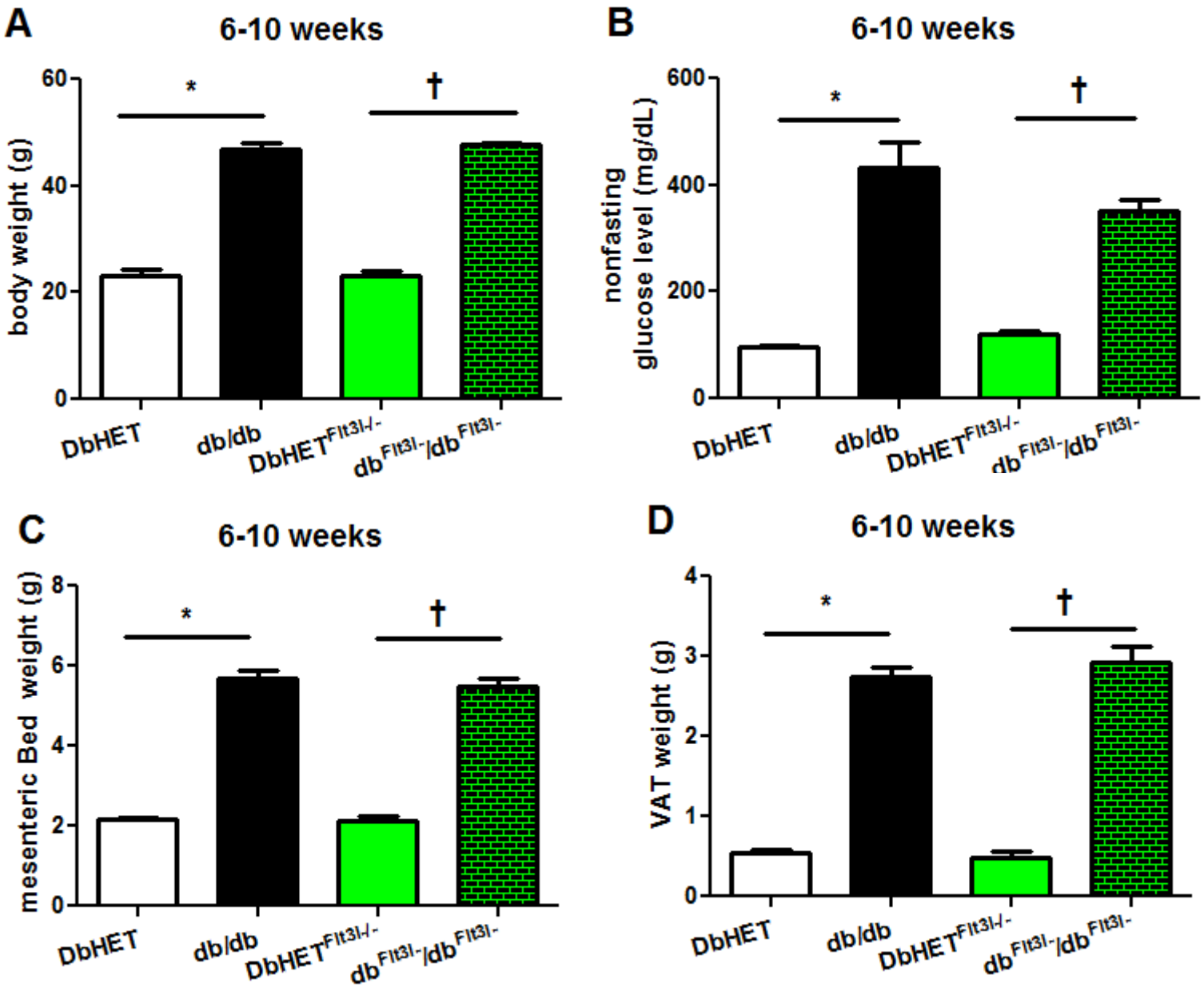
Supplementary Figure 2. Representative flow cytometry data for dendritic cell and macrophages populations in VAT from DbHET, *db/db,* DbHET $^{Flt3l-/-}$ and *db*$^{Flt3l-}$*/ db*$^{Flt3l-}$ mice at 6-10 weeks. Increased numbers of CD11c^{+}F4/80^{-} dendritic cells and CD11c^{+}F4/80^{+} macrophages, shown respectively in the upper left and right quadrants of data panel, were evident in VAT from *db/db* mice **(B)** than DbHET mice **(A)**. Genetic Flt3l depletion significantly decreased dendritic cell numbers in both DbHET$^{Flt3l-/-}$ **(C)** and *db*$^{Flt3l-}$*/ db*$^{Flt3l-}$ mice **(D)**. However, Flt3l depletion did not significantly change macrophage numbers in either DbHET $^{Flt3l-/-}$ **(C)** or *db*$^{Flt3l-}$*/ db*$^{Flt3l-}$ mice **(D)**.

Supplementary Figure 2

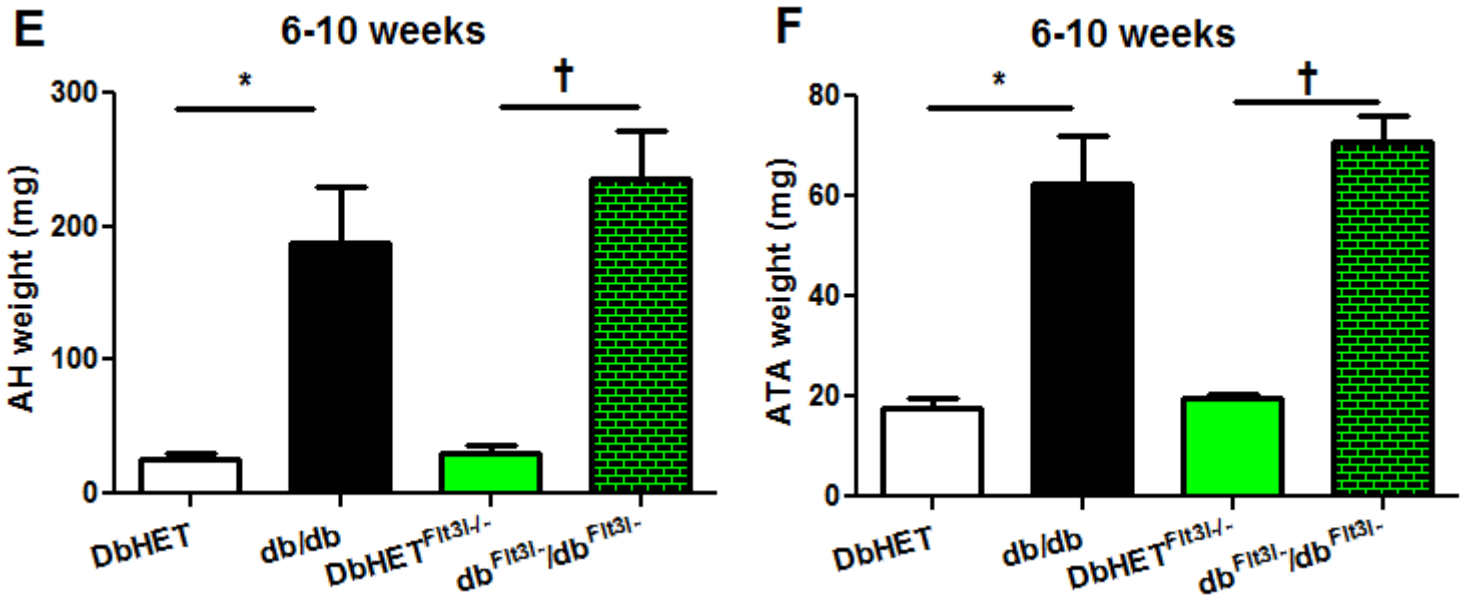


Supplementary Figure 3. . Representative flow cytometry data for dendritic cells populations in VAT from DbHET, *db/db,* DbHET $^{Flt3l-/-}$ and *db*$^{Flt3l-}$*/ db*$^{Flt3l-}$ mice at 6-10 weeks. More CD83^{+}CD86^{+} dendritic cells, shown in the upper right quadrant of of each data panel, accumulated in VAT of *db/db* mice **(B)**, compared to DbHET mice **(A)**. Genetic Flt3l depletion significantly decreased dendritic cell numbers in both DbHET$^{Flt3l-/-}$ **(C)** and *db*$^{Flt3l-}$*/ db*$^{Flt3l-}$ mice **(D).**

Supplementary Figure 3

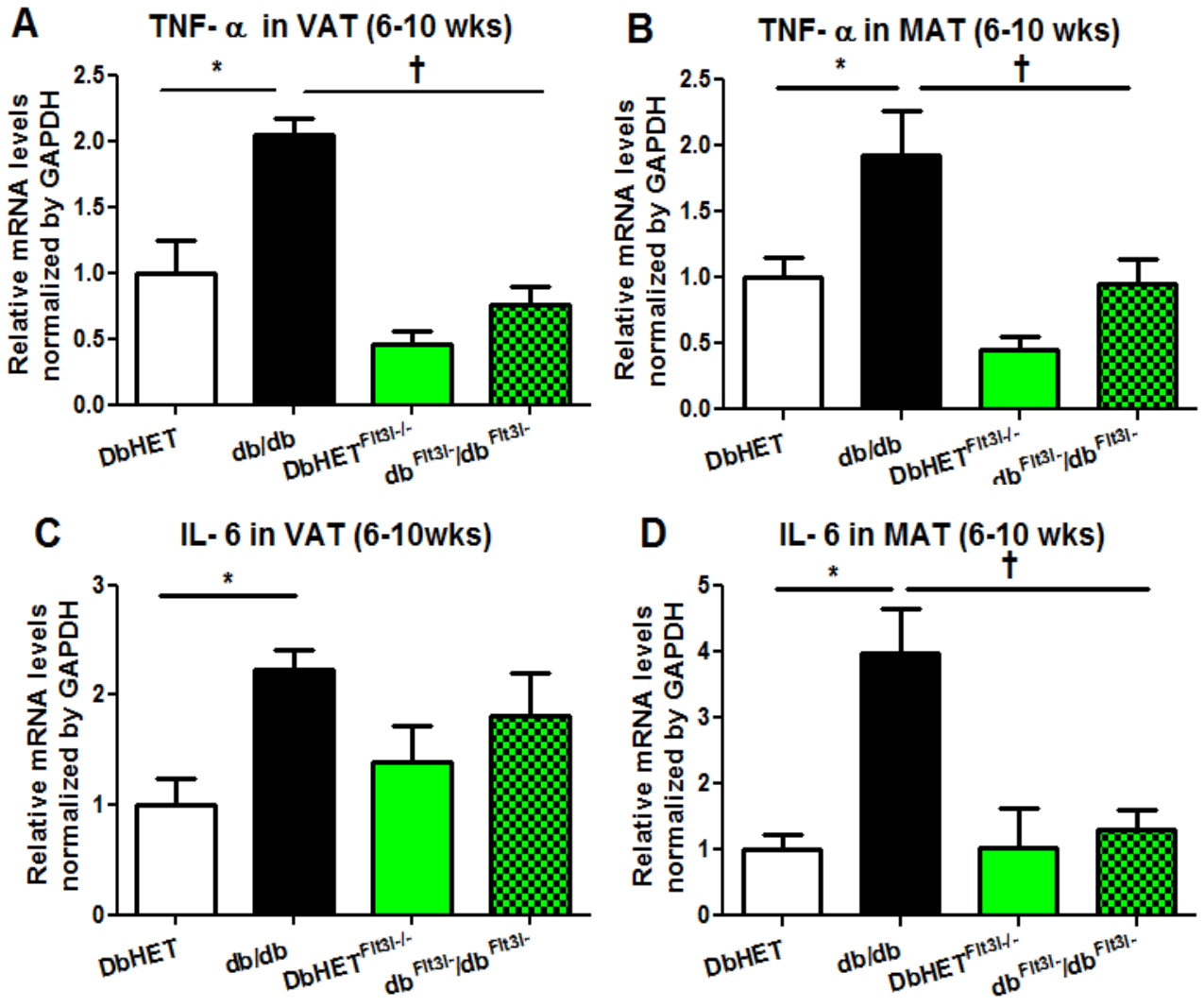


Supplementary Figure 4

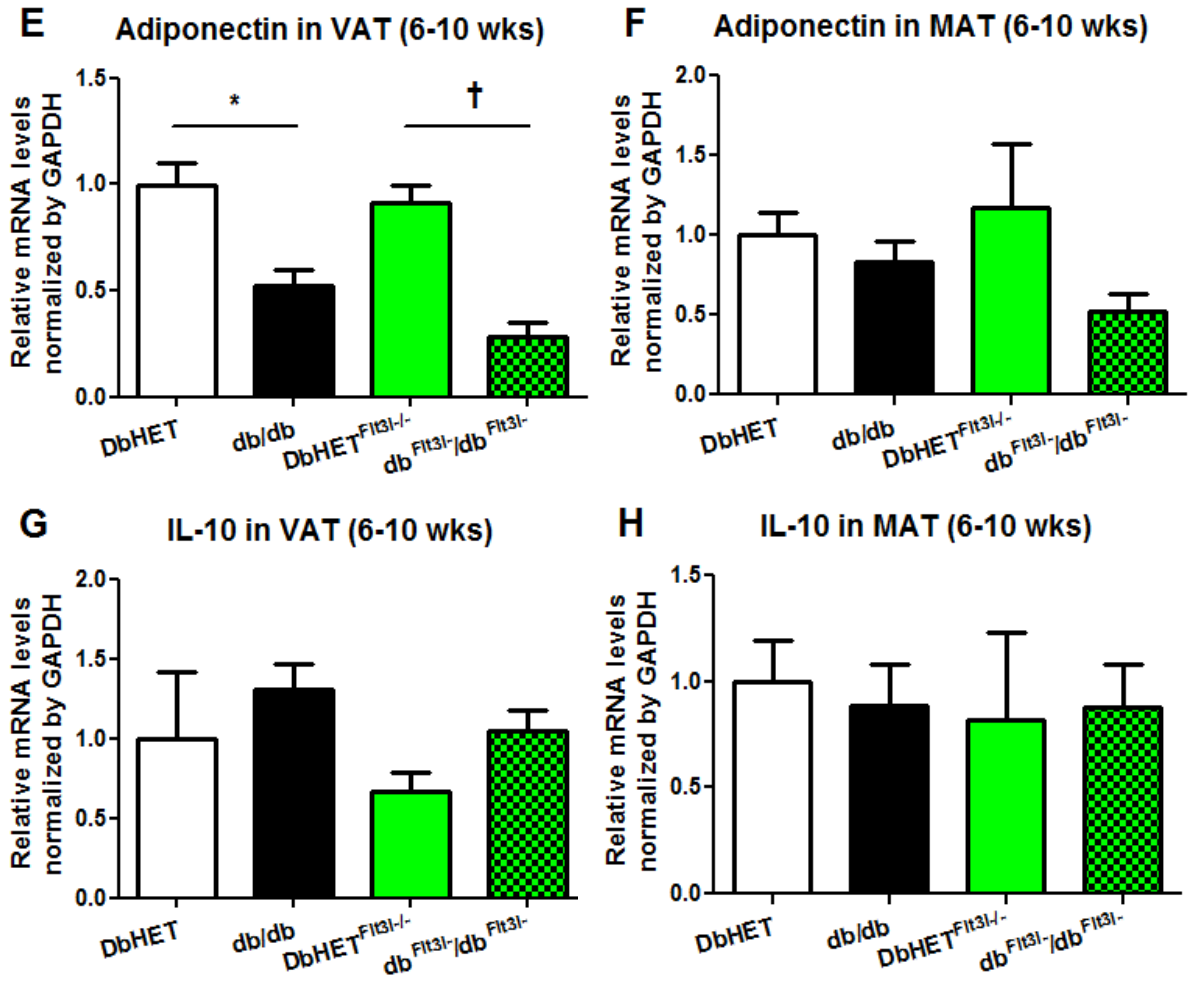


Supplementary Figure 4. Total body, mesenteric bed, VAT, AH, and ATA weights along with non-fasting glucose levels in all four groups of mice at 6-10 weeks. At the early stage of T2DM (6-10 weeks), *db/db* mice showed significantly greater body of body weights **(A)**, and increased weights of the mesenteric bed **(C)**, and PVAT, including VAT **(D)**, AH **(E)** and ATA **(F)**, compared to DbHET mice. In addition, *db/db* mice showed significantly higher non-fasting glucose levels **(B)** than controls. Depletion of Flt3l did not affect body/tissue weights or random glucose levels in DbHET $^{Flt3l-/-}$ or db^{Flt3l-}/db^{Flt3l-} mice. Data are shown as mean ± SEM. *, †: $P < 0.05$. *: between DbHET and *db/db* mice. †: between DbHET $^{Flt3l-/-}$ and db^{Flt3l-}/db^{Flt3l-} mice.

Supplementary Figure 4 (Cont)

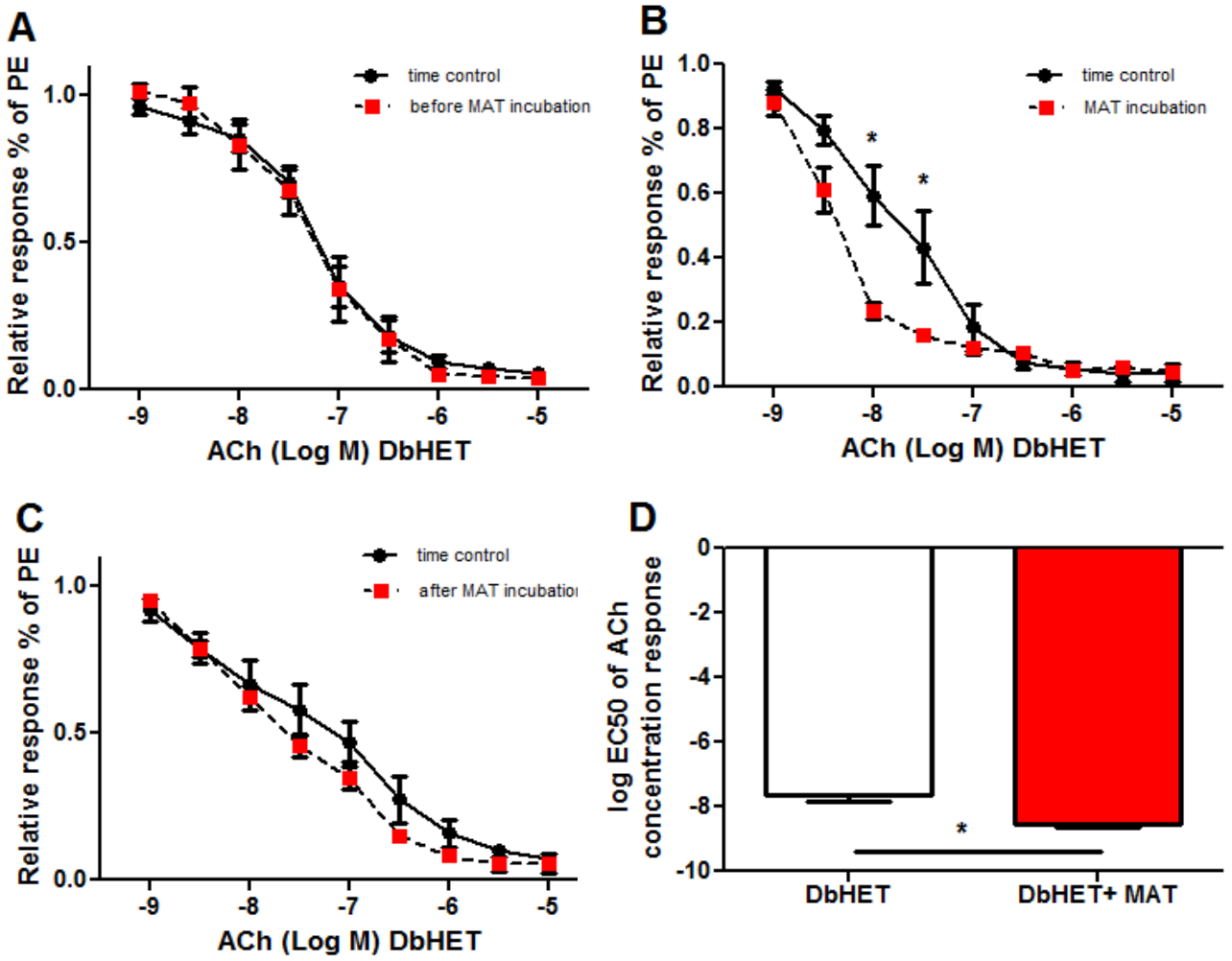


Supplementary Figure 5

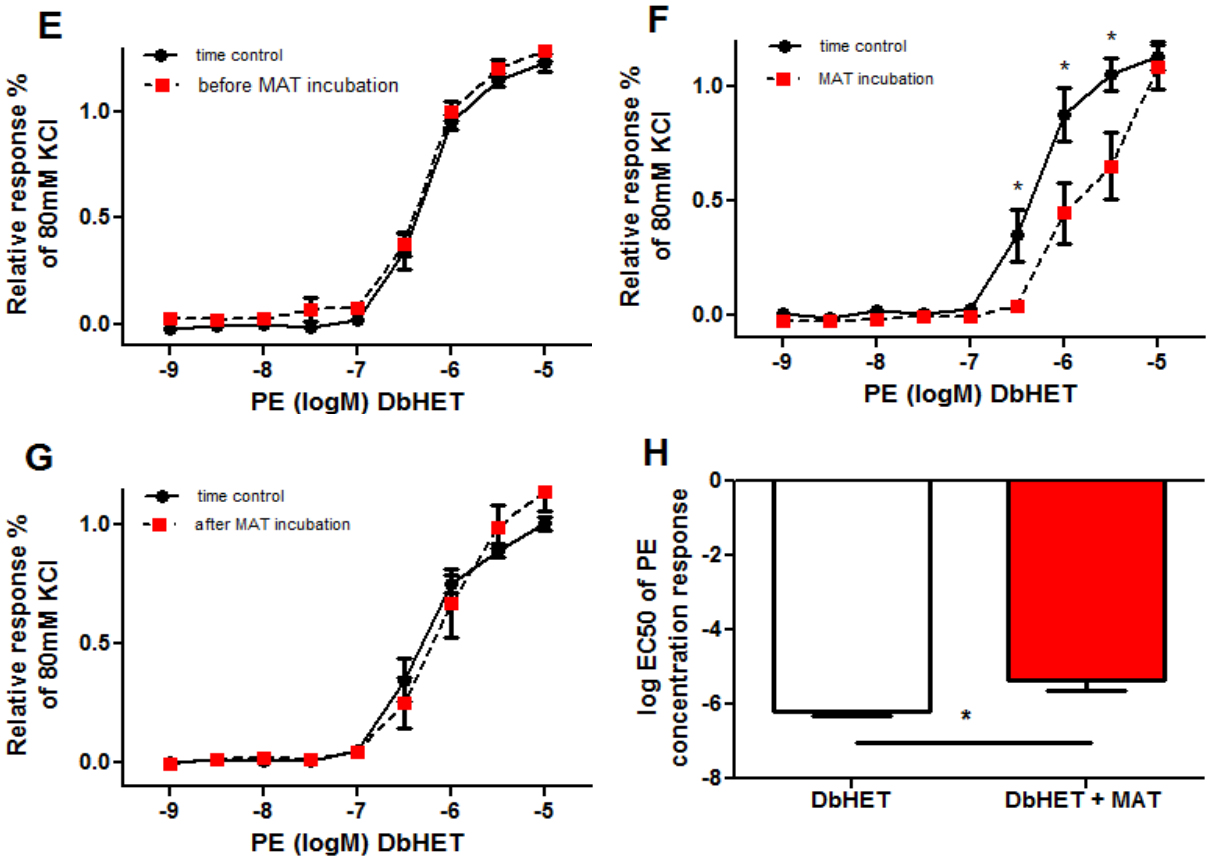


Supplementary Figure 5. mRNA expression for pro-inflammatory and anti-inflammatory factors in DbHET, *db/db,* DbHET $^{Flt3l-/-}$ and *db*$^{Flt3l-}$/ *db*$^{Flt3l-}$ mice (age 6-10 weeks). (A-D) Increased levels of mRNA expression for pro-inflammatory factors TNF-α **(A-B)** and IL-6 **(C-D)** were found in VAT and MAT of *db/db*, compared to DbHET mice. Flt3l depletion significantly decreased TNF-α mRNA levels in diabetic VAT and MAT, while IL-6 levels were decreased only in diabetic MAT. **(E-H)** Anti-inflammatory factor adiponectin **(E)** mRNA levels were decreased in VAT from *db/db*, compared to DbHET mice. However, Flt3l depletion did not restore adiponectin production in *db/db* mice. There was no significant difference of adiponectin mRNA levels in MAT **(F)** from all groups of mice. Similarly, at this age there was no significant difference in anti-inflammatory factor IL-10 mRNA levels in neither VAT **(G)** nor MAT **(H)** among all groups of mice. Data are shown as mean ± SEM. *, † : $P < 0.05$.

Supplementary Figure 5 (Cont)

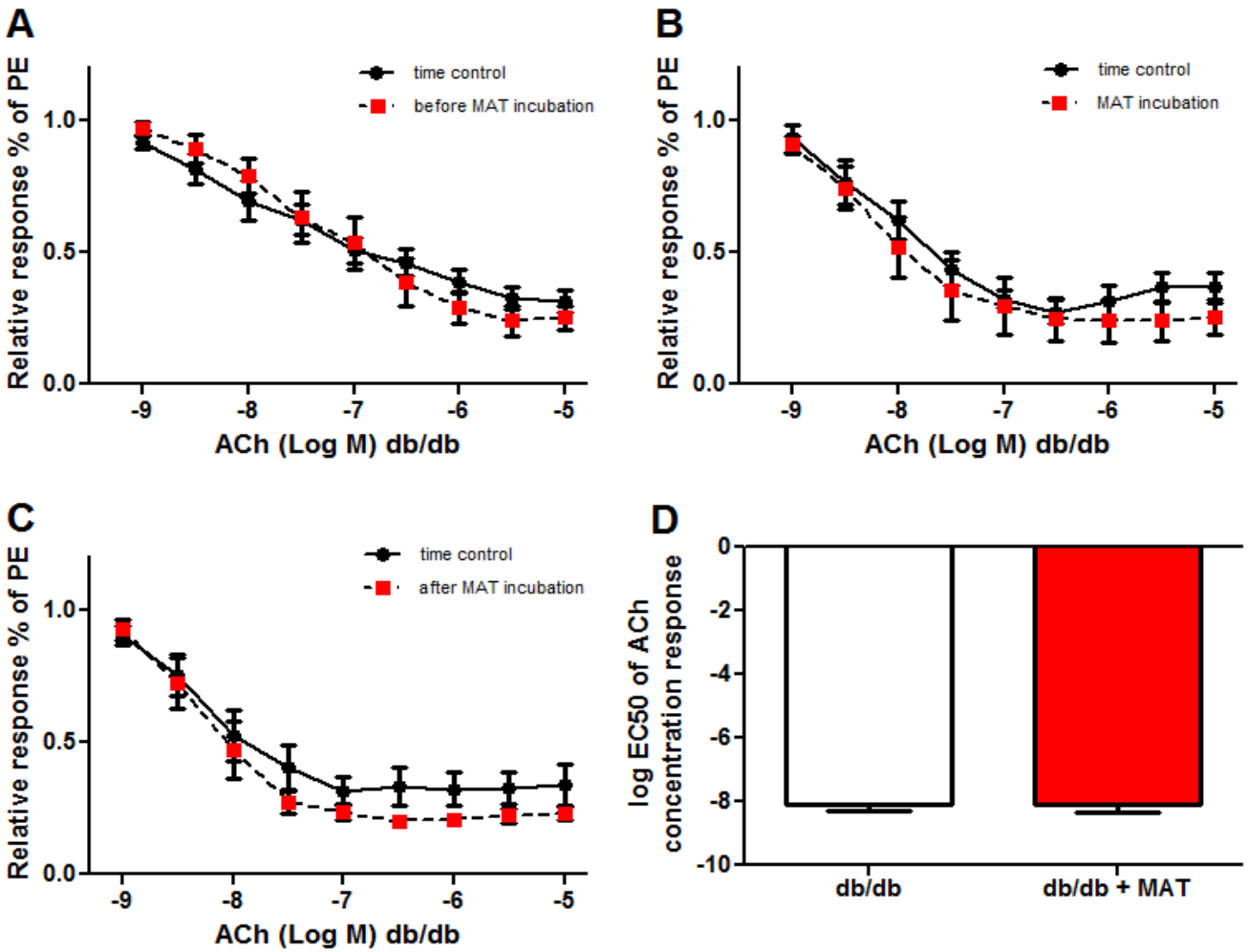


Supplementary Figure 6

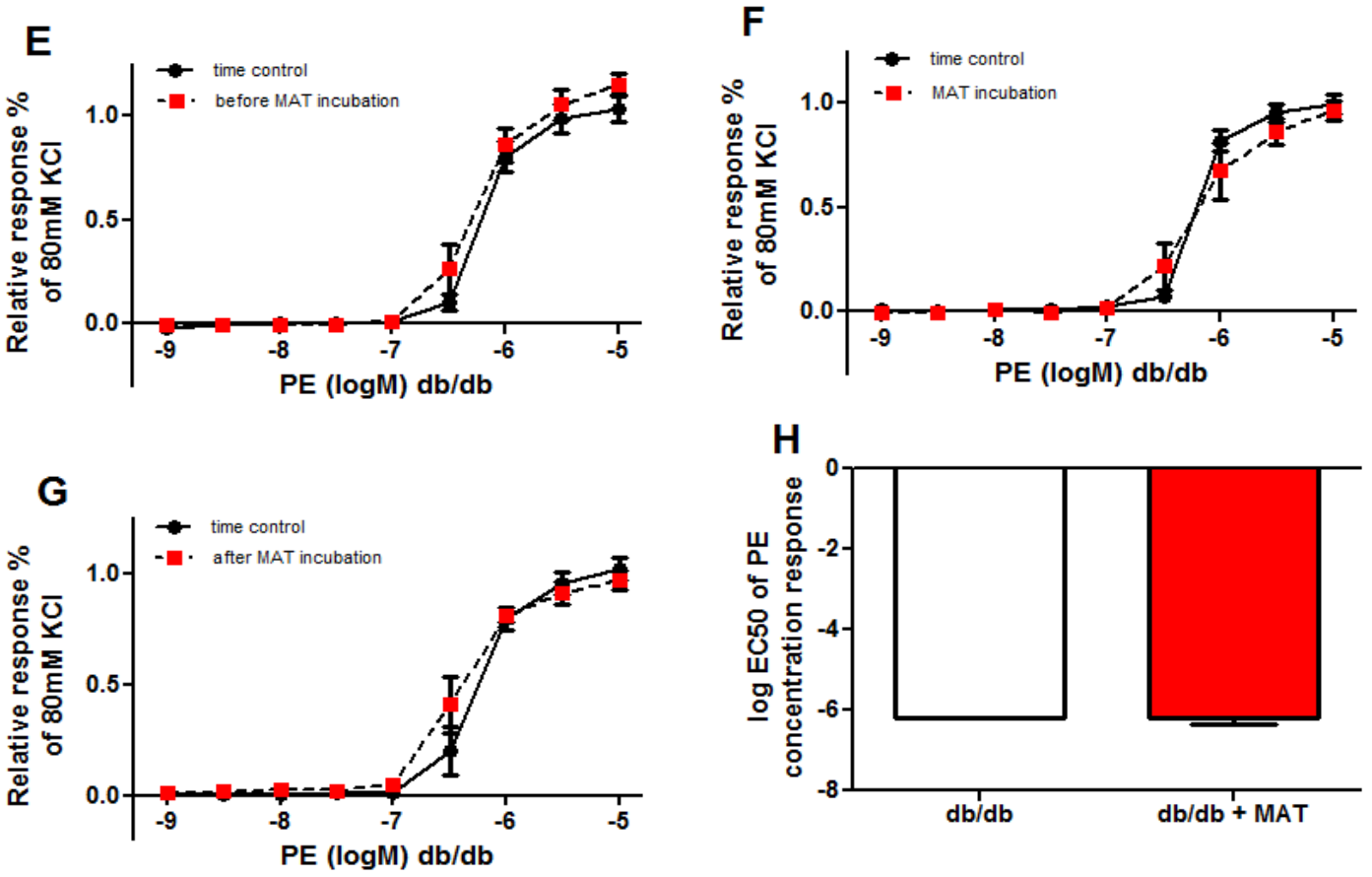


Supplementary Figure 6. Incubation of mesenteric arteries from DbHET mice (age 18-22 weeks) with (own) MAT enhances Ach responsiveness while attenuating responses to PE. (A and **E).** There was no significant difference of ACh induced vasorelaxation **(A)** or PE induced vasoconstriction **(E)** response between time control and "before MAT incubation" groups. **(B** and **F)** Significant increase of ACh induced vasorelaxation **(B)** and reduction of PE induced vasoconstriction **(F)** responses were observed following incubation with MAT incubation. **(C** and **G).** After 1 hour washing in PSS, the increase of ACh induced vasorelaxation **(C)** and reduction of PE induced vasoconstriction **(G)** were abolished in the MAT incubation group. **(D** and **H)** Significant logEC50 differences for ACh **(D)** and PE **(H)** concentration response curves were observed between time control and MAT incubation group. *: $P < 0.05$. Data in panels **(B)** and **(F)** were analyzed by Two-way ANOVA. Data in panels **(D)** and **(H)** panels were analyzed by student *t*-test. Data are presented as mean± SEM.

Supplementary Figure 6 (Cont)

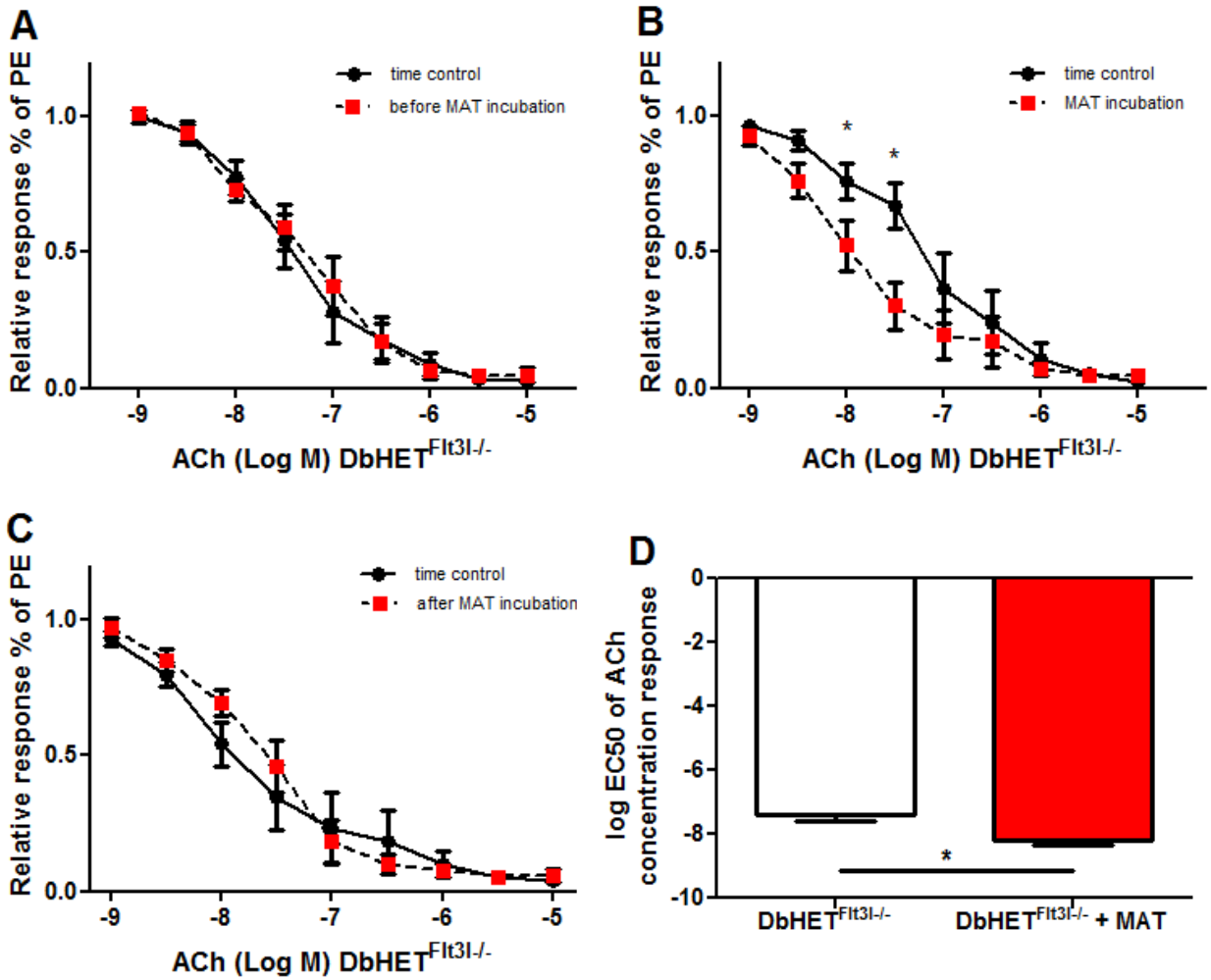


Supplementary Figure 7

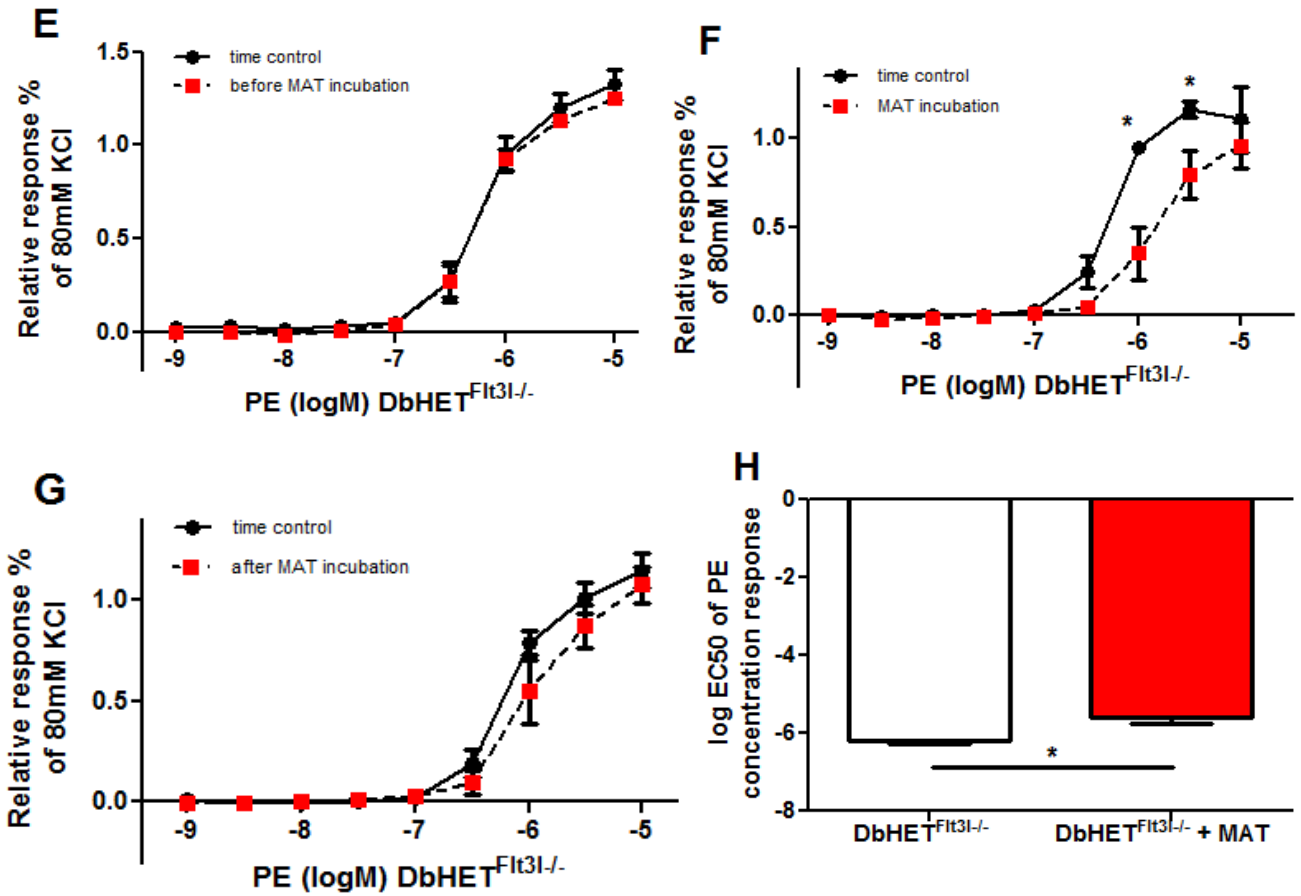


Supplementary Figure 7. Incubation of mesenteric arteries from from *db/db* mice (age 18-22 weeks) with (own) MAT does not affect responsiveness to Ach nor PE. (A and **E)** There were no significant differences in ACh **(A)** or PE **(E)** concentration responsiveness curve between time control and "before MAT incubation" MA. **(B** and **F).** Similarly, there was no significant differences in ACh induced vasorelaxation **(B)** or PE induced vasoconstriction **(F)** response between time control and following MAT incubation. **(C** and **G)** After 1 hour washing in fresh PSS, no significant differences were observed in ACh induced vasorelaxation **(C)** or PE induced vasoconstriction **(G)** responses between time controls and MAT incubation. **(D** and **H).** No significant differences in logEC50 values for ACh **(D)** or PE **(H)** concentration response curves were observed between time control and MAT incubation. All data presented as mean± SEM.

Supplementary Figure 7 (Cont)

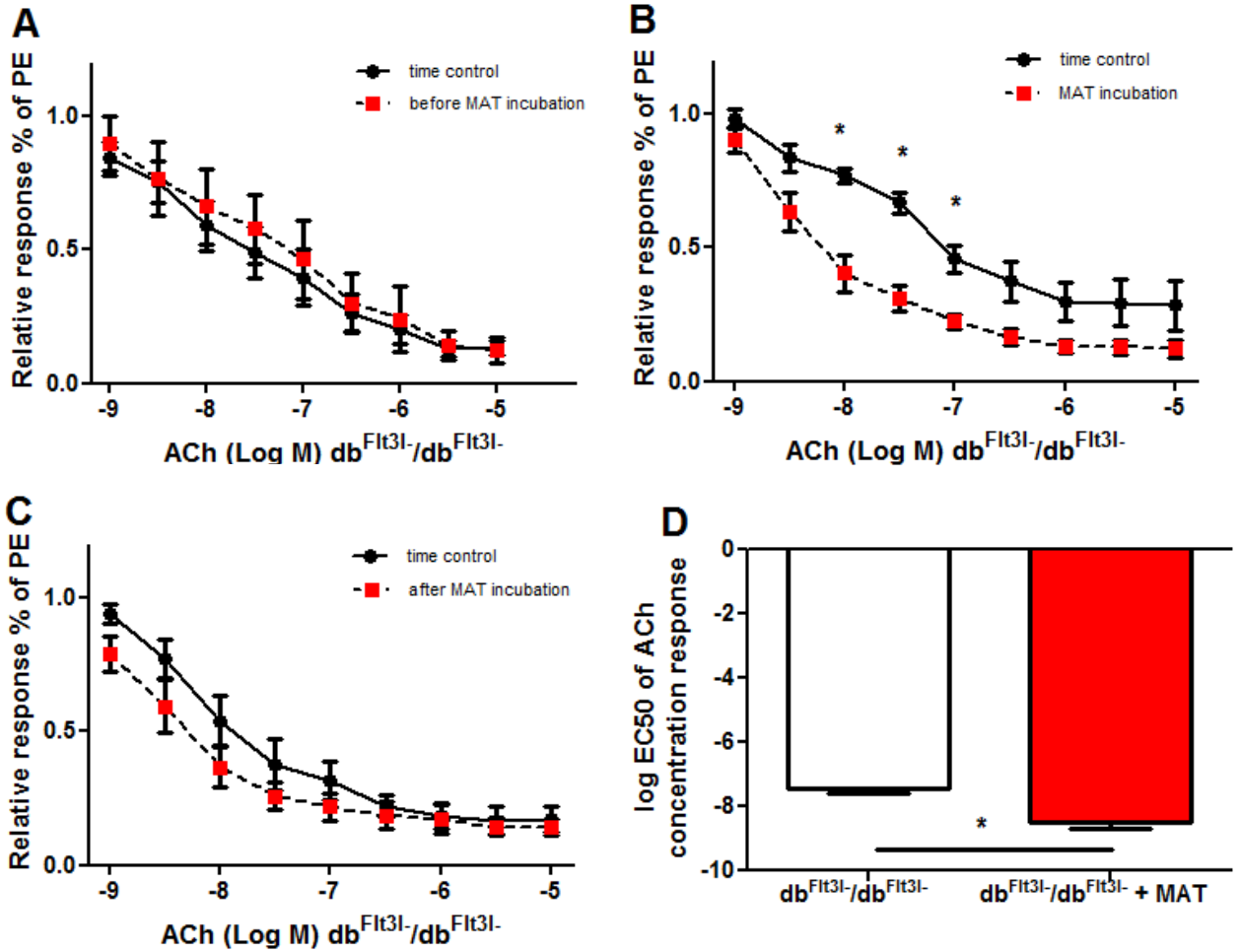


Supplementary Figure 8

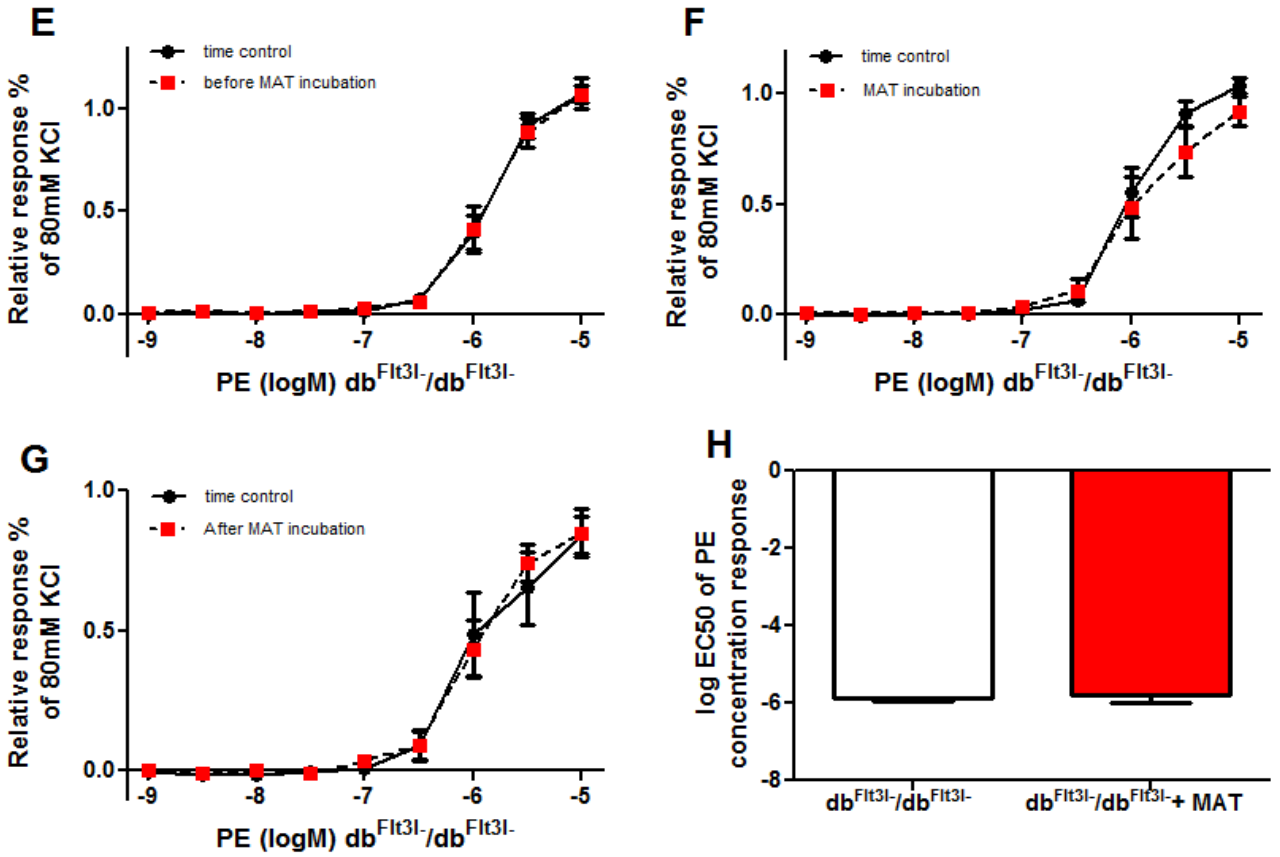


Supplementary Figure 8. Incubation of mesenteric arteries from DbHET $^{Flt3l-/-}$ mice (age 18-22 weeks) with (own) MAT enhances Ach responsiveness while attenuating responses to PE. (A and **E)** There was no significant difference of ACh induced vasorelaxation **(A)** or PE induced vasoconstriction **(E)** response between time control and "before MAT incubation" groups. **(B** and **F)** Significant increase of ACh induced vasorelaxation **(B)** and reduction of PE induced vasoconstriction **(F)** responses were found in MAT incubation, compared to time control. **(C** and **G)** 1 hour washing in fresh PSS abolished the MAT induced enhancement of ACh induced vasorelaxation **(C)** and inhibition of PE induced vasoconstriction **(G)**. **(D** and **H).** Significant logEC50 differences for ACh **(D)** and PE **(H)** concentration response curves were observed between time control and following MAT incubation. Panels **(B)** and **(F)** panels were analyzed by Two-way ANOVA. Panels **(D)** and **(H)** panels were analyzed by student *t*-test. *: $P < 0.05$. Data are presented as mean± SEM.

Supplementary Figure 8 (Cont)



Supplementary Figure 9



Supplementary Figure 9. Incubation of mesenteric arteries from *db^{Flt3l-}/db^{Flt3l-}* mice (age 18-22 weeks) with (own) MAT enhances Ach responsiveness while not affecting responses to PE. **(A** and **E)** There was no significant difference of ACh induced vasorelaxation **(A)** or PE induced vasoconstriction **(E)** response between time control and "before MAT incubation" groups. **(B)** A significant increase of ACh induced vasorelaxation response was found following MAT incubation. **(C** and **G).** After 1 hour washing procedure, there was no significant difference of ACh **(C)** or PE **(G)** concentration response curve between time control and MAT incubation. **(D)** A significant logEC50 difference for ACh concentration response curves was observed between time control and following MAT incubation **(F** and **H).** Interestingly, no significant difference of PE induced vasoconstriction was observed between time control and following MAT incubation MA **(F)**, which was supported by their logEC50 values **(H)**. Panels **(B)** and **(F)** panels were analyzed by Two-way ANOVA. Panels **(D)** and **(H)** panels were analyzed by student *t*-test.*: $P < 0.05$. Data are presented as mean± SEM.

Supplementary Figure 9 (Cont)