SUPPLEMENTAL MATERIAL

SUPPLEMENTAL FIGURE LEGENDS

Supplementary Figure 1—Time course of serum MG53 levels in non-diabetic and diabetic mice after injection of rhMG53-WT or rhMG53-C14A. A: The circulating MG53 levels at the indicated time points in *db/+* mice after the injection of BSA, rhMG53-WT or rhMG53-C14A at different doses. **B**: The circulating MG53 levels at the indicated time points in *db/+* and *db/db* mice after the injection of BSA, rhMG53-WT or rhMG53-C14A (1×10^6 ng/kg body weight). The circulating MG53 levels were determined by an ELISA assay.

Supplementary Figure 2 — Experimental procedures in diabetic mice and cultured cardiomyocytes. A and B: Experimental procedures for the pretreatment (**A**) and post-treatment (**B**) of hSA, rhMG53-WT, or rhMG53-C14A in non-diabetic *db/+* mice or diabetic *db/db* mice subjected to I/R *in vivo*. **C**: Experimental procedures for treatment of hSA, rhMG53-WT, or rhMG53-C14A in cultured cardiomyocytes subjected to H/R.

Supplementary Figure 3—Injection of rhMG53-WT does not cause acute hyperglycemia or influence glucose tolerance and insulin sensitivity in non-diabetic mice. A and B: Alterations of blood glucose in normal mice injected *i.p.* with 1 mg/kg hSA or rhMG53-WT in *db/+* mice (n = 4 per group; **A**) and *KK* mice (n = 5 per group; **B**). **C and D**: Glucose tolerance (GTT; n = 12; **C**) and insulin sensitivity (ITT; n = 6; **D**) of contol *db/+* mice after *i.p.* injection with hSA or

rhMG53-WT. All data are presented as mean ± SEM. Statistical analysis was conducted by two-way ANOVA with Sidak's multiple comparisons test (**A-D**). NS, not significant.

Supplementary Figure 4—Chronic treatment of rhMG53-WT or rhMG53-C14A does not cause metabolic side-effects in *db/db* mice. A and B: Fed blood glucose levels (**A**) and insulin tolerance tests (ITTs; **B**) of the diabetic *db/db* mice after the treatment with hSA, rhMG53-WT, or rhMG53-C14A for 2 weeks as the protocol in **Fig. 4A**. All data are presented as mean ± SEM. Statistical analysis was conducted by one-way ANOVA with Tukey's multiple comparisons test (**A**) or two-way ANOVA with Tukey's multiple comparisons test (**B**).

Supplementary Figure 5 — Adv-null, Adv-tPA-MG53-WT and Adv-tPA-MG53-C14A treatment of *db/db* mice. A: Experimental procedures for elevating circulating MG53 levels in mice by adenovirus. **B**: Representative western blots and statistical analysis of MG53 levels in the hearts and livers of diabetic *db/db* mice 1 week after infection with adenovirus overexpressing circulating wild type (Adv-tPA-MG53-WT) or C14A mutated (Adv-tPA-MG53-C14A) human MG53 or control adenovirus (Adv-null) (n = 4).

Supplementary Figure 6—Experimental procedures for diet intervention on MG53-C14A (ki/ki) mice and their WT littermates. Mice were fed on chow diet or HFD from 4 weeks of age. GTTs were performed after diet intervention for 8, 15, and 30 weeks and ITTs were performed

after diet intervention for 9, 16, and 31 weeks. All mice were sacrificed and sampled at 36 weeks of age.

Supplementary Figure 7－Metabolic cage analysis of WT and MG53-C14A knock-in mice fed with chow or high fat diet (HFD). A-C: O_2 consumption (n = 6, 6, 11, and 8 for WT chow, MG53-C14A (ki/ki) chow, WT HFD, and MG53-C14A (ki/ki) HFD, respectively; the same group order is shown below; **A**), energy expenditure (n = 8, 8, 9, and 9, respectively; **B**), and food and water intake (n = 4, 4, 8, and 7, respectively; **C**) in WT and MG53-C14A (ki/ki) mice on chow diet or HFD for 32 weeks beginning at 4 weeks of age. All data are presented as mean ± SEM. Statistical analysis was conducted by two-way ANOVA with Tukey's multiple comparisons test (**A-C**). NS, not significant.

Supplementary Figure 8－MG53-C14A mutant prevents both I/R-induced myocardial injury and HFD-induced metabolic disorders in female diabetic mice. A-C: Representative images and statistical data of myocardial infarct size (n = 8, 9, 8; **A**), serum LDH level (n = 7, 10, 8; **B**) and mortality (n = 10, 11, 8; **C**) in female diabetic *db/db* mice subjected to I/R *in vivo* with post-treatment of hSA, rhMG53-WT, or rhMG53-C14A. **D-F**: Body weights (n = 3, 3, 4, and 3 for WT chow, MG53-C14A (ki/ki) chow, WT HFD, and MG53-C14A (ki/ki) HFD, respectively; **D**), fasted and fed blood glucose levels after diet intervention for 30 weeks (n = 3; **E**), glucose tolerance test (GTT) after diet intervention for 28 weeks and insulin tolerance test (ITT) after diet

intervention for 30 weeks (n = 3; **F**) in female MG53-C14A (ki/ki) mice and their WT littermates on chow diet or HFD from 4 weeks of age. All data are presented as mean ± SEM. Statistical analysis was conducted by one-way ANOVA with Tukey's multiple comparisons test (**A**) or two-way ANOVA with Tukey's multiple comparisons test (**B, and D-F**). For panels **D and F**, **P* < 0.05, ***P* < 0.01 WT HFD vs. WT chow, #*P* < 0.05, ##*P* < 0.01 MG53-C14A (ki/ki) HFD vs. WT HFD. NS, not significant.