MicroRNA-126 overexpression rescues diabetes-induced impairment in efferocytosis of apoptotic cardiomyocytes

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Figure S1

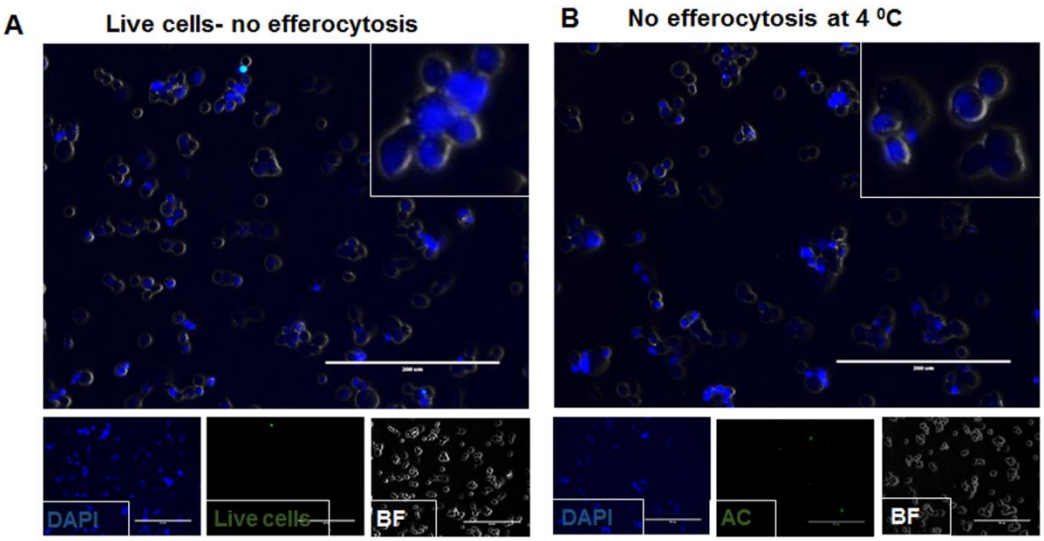

Figure S1: Fluorescent microscopy- efferocytosis assay under different experimental conditions. (A) Lack of engulfment of (calcein labeled green) live human ventricular cardiomyocytes by RAW 264.7 cells incubated at 37^{o}C. (B) Inhibition of efferocytosis of apoptotic cells (green) by RAW 264.7 cells at 4^{o}C. Macrophage nuclei is DAPI stained (blue).

Fig S2

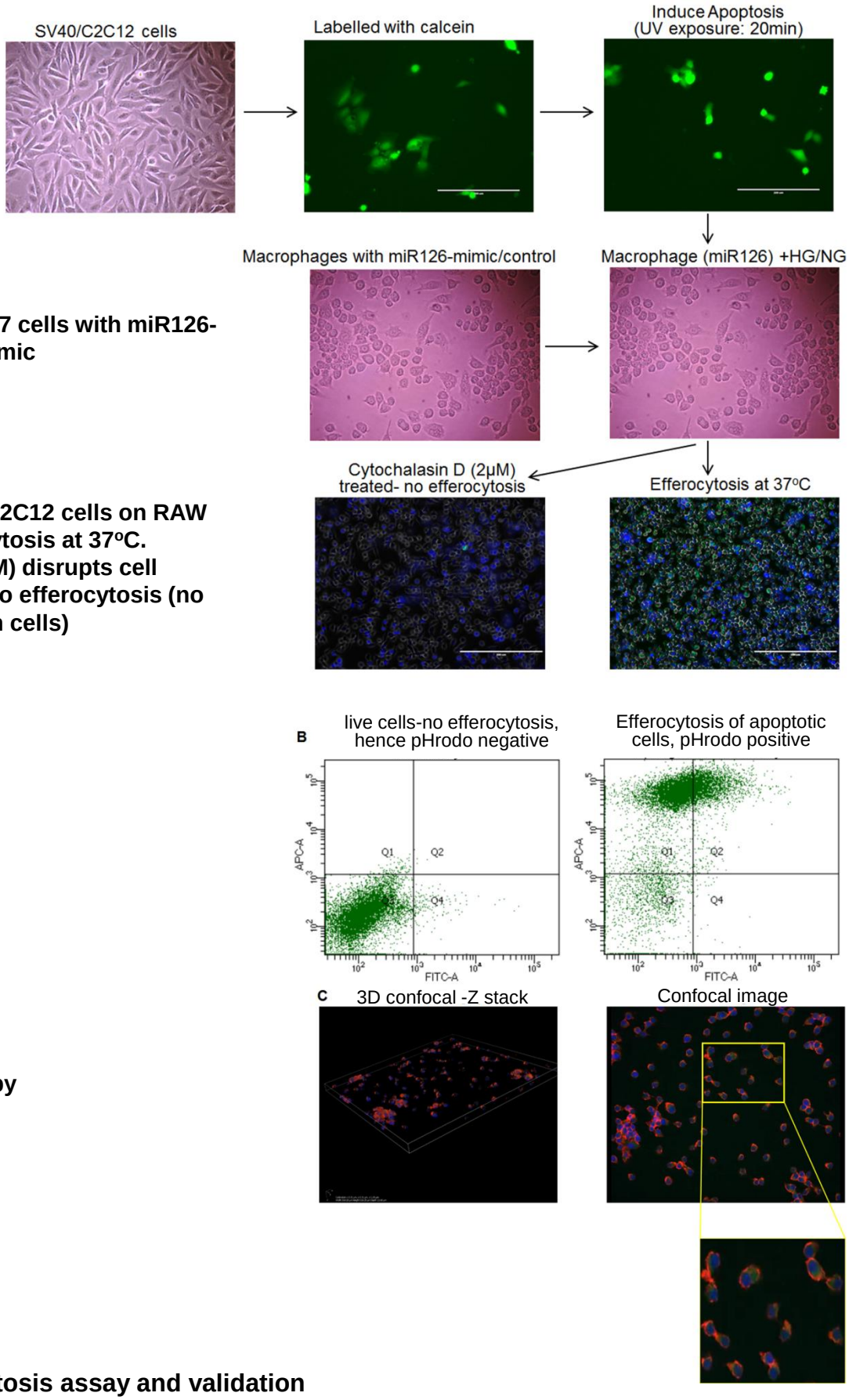

Figure S2: Efferocytosis assay and validation

(A) Efferocytosis assay. Pre-incubation of macrophages with cytochalasin D (2μM, to disrupt actin polymerization) inhibits efferocytosis and did not show engulfment of the apoptotic cells. (B) Flow cytometry analysis, engulfment of phRodo green labeled apoptotic cells by macrophages (stained with anti-CD11b-APC). Macrophages exhibit increasing fluorescence (green) due to acidification of endocytic compartments in comparison to the dye being nonfluorescent at neutral pH (or lack of efferocytosis of live cells). Representative z-stack image from 3D confocal imaging of corresponding video of phagocytic cells (Red: F-actin for macrophages, Green: calcein labeled apoptotic cells, blue nuclei: DAPI).

Figure S3

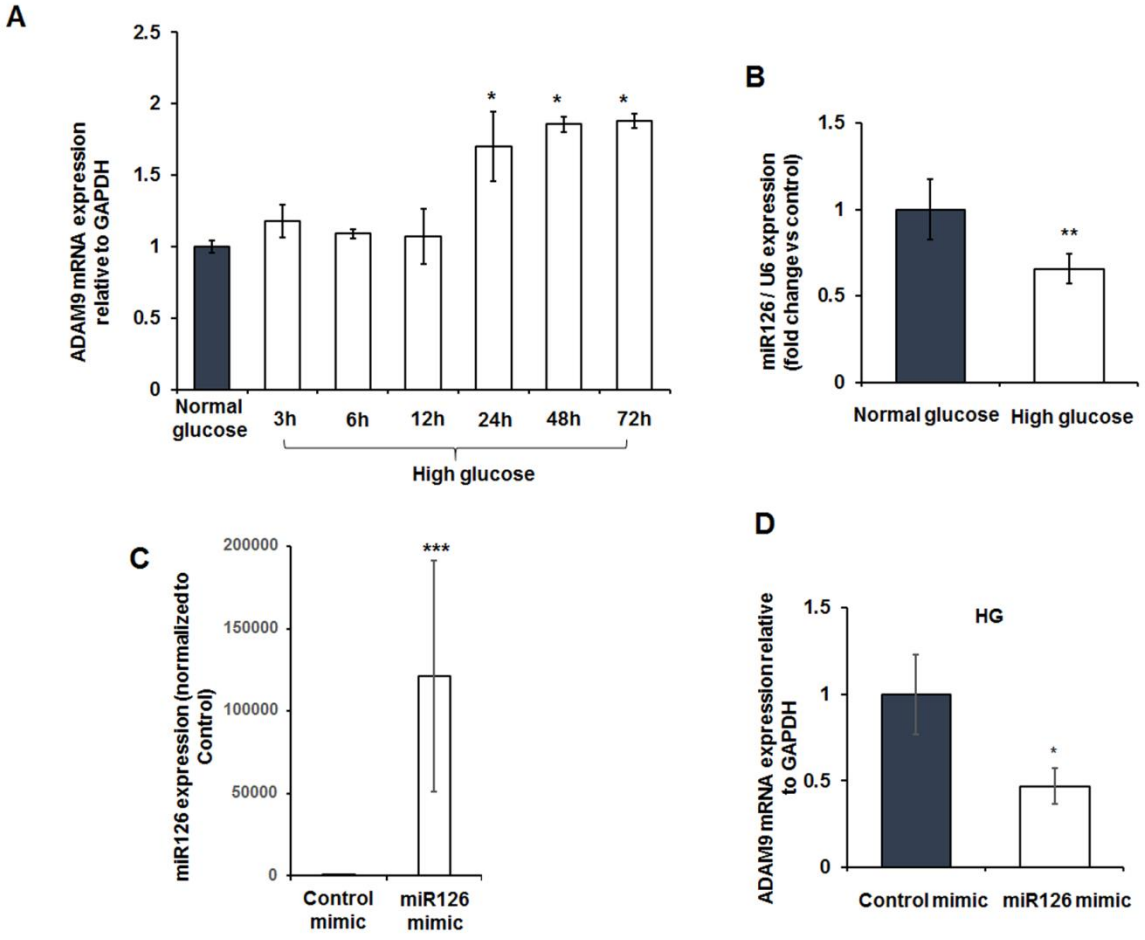

Figure S3: ADAM9 expression and miR-126 expression in RAW 264.7 cells.
(A) miR-126 expression measured in the RAW 264.7 cells subjected to normoglycemia (NG) and high glucose (HG) conditions (Normalized to U6, n = 5, **P < 0.01). (B) miR-126 mimic transfection in RAW 264.7 cells increases miR-126 expression as compared to control mimic transfections (normalized to U6, n = 5, ***P < 0.001). (C) ADAM9 mRNA expression in RAW cells subjected to NG and HG conditions at indicated time point (normalized to GAPDH, n = 3, *P < 0.05). (D) Under high glucose condition, miR-126 mimic treated RAW 264.7 cells show decrease in ADAM9 mRNA levels compared to control mimic transfected cells (n = 3. *P < 0.05, normalized to control GAPDH).

Figure S4

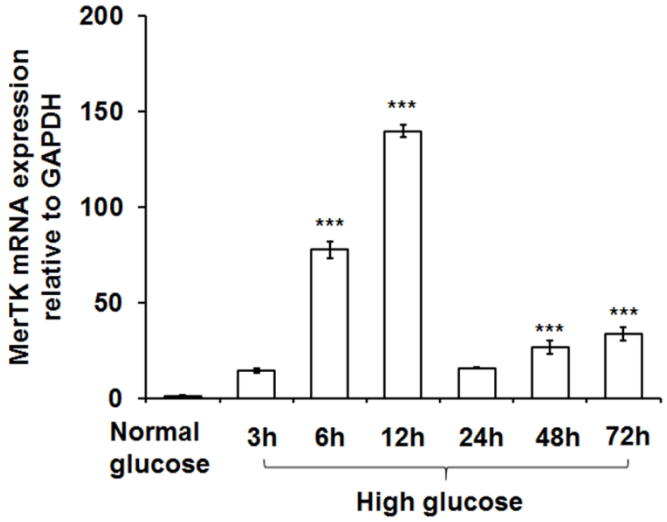

Figure S4: MerTK expression in RAW 264.7cells exposed to high glucose.
MerTK mRNA expression in RAW 264.7 cells subjected to NG and HG conditions at indicated time points (normalized to GAPDH, n = 3, ***P < 0.05).