Figure 1 Free fatty acid (FFA) induced lipid accumulation and MD-1 differential expression in cardiomyocytes. (A) Proliferation curve of H9C2 measured with MTT assay. (B) Oil red O staining of H9C2. Cellular lipid accumulation was stained as bronzing droplets in the images. (C) qPCR and western blot analysis of MD-1 level. The mRNA and protein levels of MD-1 were determined following the treatment of FFA at the indicated doses. GAPDH was used as an internal control. (D) qPCR and western blot analysis of MD-1 level. The mRNA and protein levels of MD-1 were determined following the treatment of 1.0 mmol/L FFA for the indicated time. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 2 MD-1 protected cardiomyocytes to resist pathological remodelling induced by free fatty acid (FFA) stimulation in vitro. (A) qPCR and western blot analysis of MD-1 level in H9C2. The mRNA and protein levels of MD-1 in H9C2 were determined after cardiomyocytes transfection with MD-1 overexpression (Ad-MD-1) or knockdown (Ad-shMD-1) recombinant adenovirus for 48 h. (B) TRITC Phalloidin staining of H9C2 to measure the cross-section area of cardiomyocytes after adenovirus transfection and FFA treatment for 48 h. (C) qPCR and western blot analysis of ANP, BNP and β-MHC levels in H9C2 cells as described in B. (D) qPCR and western blot analysis of COL1A1, COL3A1 and CTGF expressions in H9C2 cells as described in B. * P < 0.05, ** P < 0.01, *** P < 0.001.
Figure 3 Silencing of MD-1 promoted high-fat diet induced myocardial damage. (A) Western blot validated the knockout efficiency in MD-1−/− mice compared to wild-type mice (WT). (B) The content of serum creatine kinase-MB (CK-MB), a common serum marker for myocardial damage in MD-1−/− mice and WT mice after high-fat diet feed for 20 weeks (n = 12). (C-F) BW, HW, HW/BW and HW/TL for the indicated groups (n = 12). (G) Glucose tolerance testing on MD-1−/− mice and WT mice after high-fat diet feed for 20 weeks (n = 12). (H) qPCR analysis of hypertrophy markers ANP, BNP and β-MHC in MD-1−/− hearts and WT mice hearts after high-fat diet feed for 20 weeks (n = 4). * P < 0.05, ** P < 0.01, *** P < 0.001.
Figure 4 Silencing of MD-1 accelerated high-fat diet induced myocardial dysfunction. (A-D) Echocardiographic results for MD-1−/− mice and wild-type (WT) mice after high-fat diet feed for 20 weeks (n = 12). (E) Masson's trichrome staining of heart tissues from MD-1−/− mice and WT mice after high-fat diet feed for 20 weeks (n = 4). (F) qPCR analysis of fibrosis markers COL1A1, COL3A1 and CTGF in MD-1−/− hearts and WT mice hearts after high-fat diet feed for 20 weeks (n = 4). *P < 0.05, **P < 0.01, ***P < 0.001
Figure 5 Overexpression of MD-1 alleviated high-fat diet induced myocardial damage. (A) Western blot validated the overexpression efficiency in MD-1 transgenic mice (TG) compared to wild-type mice (WT). (B) The level of serum CK-MB in TG and WT mice after high-fat diet feed for 20 weeks (n = 12). (C-F) BW, HW, HW/BW and HW/TL for the indicated groups (n = 12). (G) Glucose tolerance testing on TG and WT mice after high-fat diet feed for 20 weeks (n = 12). (H) qPCR analysis of hypertrophy markers ANP, BNP and β-MHC in TG and WT mice hearts after high-fat diet feed for 20 weeks (n = 4). *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 6 Overexpression of MD-1 protected myocardial function against high-fat stimulation. (A-D) Echocardiographic results for MD-1 transgenic mice (TG) and wild-type mice (WT) after high-fat diet feed for 20 weeks (n = 12). (E) Masson's trichrome staining of heart tissues from TG mice and WT mice after high-fat diet feed for 20 weeks (n = 4). (F) qPCR analysis of fibrosis markers COL1A1, COL3A1 and CTGF in TG hearts and WT mice hearts after high-fat diet feed for 20 weeks (n = 4). *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 7 MD-1 mediated high-fat diet-induced cardiac pathological remodelling via negatively regulating MAPK and NF-κB signalling. (A, C) Western blot analysis for the phosphorylation levels of MEK, ERK, JNK, p38, IκBα and p65, relative to levels of the total one, in MD-1−/− hearts and wild-type (WT) mice hearts after high-fat diet feed for 20 weeks. (B, D) Western blot analysis for the phosphorylation levels of MEK, ERK, JNK, p38, IκBα and p65, relative to levels of the total one, in TG hearts and WT mice hearts after high-fat diet feed for 20 weeks. (E, F) Representative images of NF-κB p65 immunohistochemistry analysis for heart tissues from TG or MD-1−/− mice models after high-fat diet feed for 20 weeks. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 8 Inactivation of MAPK and NF-kB signalling pathways by inhibitors rescues the adverse effects of MD-1 deficiency on high-fat stimulation induced cardiac remodelling. (A) Western blot analysis for phosphorylation levels of MEK1/2 and IκBα, relative to levels of the total one, in H9C2 cells pre-treated with 10 μmol/L U0126 and 10 μmol/L Bay 11-7082 for 30 min before FFA stimulation. (B) Western blot analysis for phosphorylation levels of MEK1/2, ERK1/2 and IκBα, relative to levels of the total one, in MD-1 deficient H9C2 cells (Ad-shMD-1) treated with U0126, Bay 11-7082 and FFA stimulation. (C) qPCR analysis for the mRNA levels of ANP, BNP, β-MHC, COL1A1, COL3A1 and CTGF in MD-1 deficient H9C2 cells (Ad-shMD-1) treated with U0126, Bay 11-7082 and FFA stimulation. *P < 0.05, **P < 0.01, ***P < 0.001.