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Neuraminidase 1 is a driver of experimental cardiac hypertrophy

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Aims Despite considerable therapeutic advances, there is still a dearth of evidence on the molecular determinants of cardiac hypertrophy that culminate in heart failure. Neuraminidases are a family of enzymes that catalyze the cleavage of terminal sialic acids from glycoproteins or glycolipids. This study sought to characterize the role of neuraminidases in pathological cardiac hypertrophy and identify pharmacological inhibitors targeting mammalian neuraminidases.

Methods and results Neuraminidase 1 (NEU1) was highly expressed in hypertrophic hearts of mice and rats, and this elevation was confirmed in patients with hypertrophic cardiomyopathy ($n = 7$) compared with healthy controls ($n = 7$). The increased NEU1 was mainly localized in cardiomyocytes by co-localization with cardiac troponin T. Cardiomyocyte-specific NEU1 deficiency alleviated hypertrophic phenotypes in response to transverse aortic constriction or isoproterenol hydrochloride infusion, while NEU1 overexpression exacerbated the development of cardiac hypertrophy. Mechanistically, co-immunoprecipitation coupled with mass spectrometry, chromatin immunoprecipitation, and luciferase assays demonstrated that NEU1 translocated into the nucleus and interacted with GATA4, leading to Foetal gene (*Nppa* and *Nppb*) expression. Virtual screening and experimental validation identified a novel compound C-09 from millions of compounds that showed favourable binding affinity to human NEU1 (KD = 0.38 μM) and effectively prevented the development of cardiac remodelling in cellular and animal models. Interestingly, anti-influenza drugs zanamivir and oseltamivir effectively inhibited mammalian NEU1 and showed new indications of cardio-protection.

Conclusions This work identifies NEU1 as a critical driver of cardiac hypertrophy and inhibition of NEU1 opens up an entirely new field of treatment for cardiovascular diseases.

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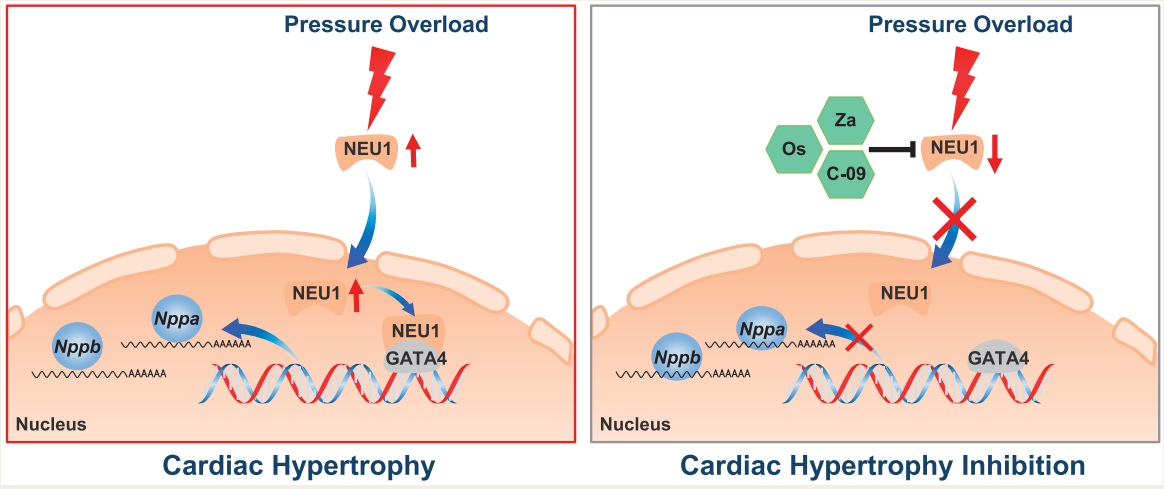
Graphical Abstract



NEU1 promotes cardiac hypertrophy by interacting with transcriptional factor GATA4 (left), and inhibiting NEU1 by small-molecule compounds attenuates cardiac remodelling (right).

Keywords Cardiac hypertrophy • GATA4 • Neuraminidase 1 • Neuraminidase inhibitors

Translational perspective

This work identified neuraminidase 1 (NEU1) as a crucial inducer of cardiac hypertrophy using different models ranging from rodents to humans. Inhibiting NEU1 opens up an entirely new field of treatment for cardiovascular diseases. Compound-09 was screened as a novel candidate compound that specifically targets human NEU1 and attenuates cardiac remodelling. The existing anti-viral drugs (zanamivir and oseltamivir) with well-established safety and pharmacokinetic profiles show novel indications for cardiovascular diseases.

Introduction

Pathological cardiac hypertrophy, characterized by myocyte enlargement and dysfunctional cardiac contractility, is a hallmark of numerous cardiovascular diseases.[1] Hypertrophic transformation is viewed as a fundamental and indispensable step in myocardial response to pressure overload. In the long term, myocardial hypertrophy is a major predisposing factor for heart failure, arrhythmia, and sudden death.[2] Increasing body of evidence shows that inhibiting such hypertrophy is beneficial for preventing its transition to heart failure.[3]

Molecular mechanisms contributing to the development of cardiac hypertrophy and heart failure are very complex.[3,4] The hypertrophic process involves a vast array of structure, calcium handling, metabolism, gene transcription, inflammation, autophagy, and other functional events within the growing cell.[5–9] Pharmacotherapy targeting cardiomyocyte cell-surface neurohormones and receptors are effective in limiting progression of cardiac hypertrophy and heart failure, including β-adrenergic receptor blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers.[10,11] Despite considerable therapeutic advances, there is still a dearth of evidence on the molecular determinants of cardiac hypertrophy that culminate in decompensated heart failure.

Characterization of new signalling mediators associated with cardiac hypertrophy and remodelling is a key focus in the exploration of novel drug targets that improve clinical outcomes. Neuraminidases (NEUs), also known as sialidases, are a family of enzymes that cleave sialic acid on the surfaces of cells.[12] The functions of viral NEUs have been well documented in the influenza virus replication.[13] The biological effects of mammalian NEUs, however, tend to be underestimated and are less characterized. Mammalian NEUs catalyze the cleavage of terminal sialic acids from glycoproteins or glycolipids.[12] Four types of mammalian NEUs (NEU1, NEU2, NEU3, and NEU4) have been identified to date, differing in their subcellular localization and enzymatic properties.[12] Of these, NEU1 is the most abundantly expressed. In addition to its typical catabolic function in lysosomes, NEU1 can also translocate to the cell surface, where it is involved in structural and functional modulation of cellular receptors. Some examples of receptors and cellular signalling modulated by NEU1 desialylation at cell membrane include TLR4-NFκB-associated response, insulin receptor-related glucose uptake, Fc receptors for immunoglobulin G in macrophage phagocytosis, and epidermal growth factor receptor and insulin-like growth factor-2 in proliferation.[14–18]

With regard to the effects of NEU1 in cardiovascular diseases, a few studies have focused on blood vessel disorders such as

atherosclerosis.[19] Neuraminidase 1 was highly expressed in monocytes and macrophages, promoting atherosclerosis, and plaque inflammation.[20] Down-regulation of NEU1 reduced very-low-density lipoprotein and attenuated atherosclerosis.[20,21] Investigations on the effects of NEU1 in cardiomyocytes are very few. A recent published study demonstrated that an up-regulation of NEU1 in invading monocytes and macrophages after ischaemia/reperfusion contributed to heart failure by promoting inflammation.[22] It is well known that cardiomyocytes account for >50% of an adult human heart ventricular region, much higher than the ~3% for cardiac monocytes and macrophages.[23] This therefore begs the question, 'what role does the cardiomyocyte-localized NEU1 play in cardiac hypertrophy and remodelling?'. Compared with the ischaemia-induced model such as ischaemia/reperfusion, the activation of the immune system is less robust in non-ischaemic cardiac hypertrophy and heart failure models.[24] Selective depletion of mononuclear phagocytes in pressure overload-induced heart failure had no obvious effects on subsequent cardiac remodelling or dysfunction.[25] From this point, long-term pressure-overload stimulation seems to be an ideally experimental non-ischaemic model to characterize the role of cardiomyocyte-localized NEU1 in cardiac hypertrophy.

Here, we proposed the hypothesis that cardiomyocyte-localized NEU1 is a mediator of pressure overload-induced cardiac hypertrophy. We employed several experimental models in mice and rats using long-term transverse aortic constriction (TAC) or isoproterenol hydrochloride (ISO) infusion. The increased level of NEU1 was further confirmed in heart tissues of patients with hypertrophic cardiomyopathy. We used neonatal rat primary cardiomyocytes (NRCMs) in response to angiotensin II (Ang II) stimulation, a commonly used stimulator to induce cardiac hypertrophy through angiotensin II type 1 receptors[26] to study the effects of cardiomyocyte-localized NEU1 and its underlying mechanism. To explore the function of cardiomyocyte-localized NEU1 *in vivo*, we further generated cardiomyocyte-specific NEU1 knockout and overexpression mice. Subsequently, novel compounds targeting mammalian NEU1 were screened to effectively prevent cardiac hypertrophy. Additionally, we tested new indications for viral NEU inhibitors in cardio-protection.

Methods

A detail description of the methods is provided in the Supplementary material online.

Results

Neuraminidase 1 is elevated in cardiomyocytes of hypertrophic hearts in rodents and humans

Neuraminidase 1 expression was significantly elevated in hypertrophic heart tissue of mice in response to TAC stimulation (*Figure 1A*). The expressions of other three isoforms (NEU2, NEU3, and NEU4) were not altered markedly between the sham and TAC groups (*Figure 1A*). The increased level of NEU1 was replicated in two rat models of TAC- and ISO-induced hypertrophy by western blots (*Figure 1B*). Immunohistochemistry further confirmed the increased NEU1 (*Figure 1C*), in which IgG was used as the negative control to exclude non-specific staining (Supplementary material online, *Figure S1A*). The NEU activity was elevated in TAC- or ISO-induced hypertrophic hearts compared with the control as a result (*Figure 1D*).

Healthy controls and diseased patients are the most appropriate subjects to obtain tissue samples for target discovery. We analysed 14 human heart samples collected from subjects with normal cardiac function ($n = 7$) and hypertrophic cardiomyopathy ($n = 7$). Detailed information of human heart samples is provided in Supplementary material online, *Table S1*. Western blots and immunohistochemistry revealed that NEU1 protein levels in the patients were significantly higher than the healthy controls (*Figure 1E and F*).

The elevated NEU1 was mainly localized in cardiomyocytes based on co-localization with cardiac troponin T (*Figure 1G*). Non-specific staining was excluded by co-staining with IgG and alpha-sarcomeric actinin (Supplementary material online, *Figure S1B*). We further compared NEU1 expression in cardiomyocytes and non-cardiomyocytes of heart tissue from mice subjected to TAC. The NEU1 mRNA (Supplementary material online, *Figure S1C*) and protein expression (*Figure 1H*) were markedly increased in the cardiomyocyte fraction and not the non-cardiomyocyte fraction.

Neuraminidase 1 promotes cardiomyocyte hypertrophy at the cellular level

We isolated NRCMs (*Figure 2A*) and observed that NEU1 expression was increased in response to Ang II treatment (*Figure 2B and C*). Knockdown of NEU1 (*Figure 2D–F*) notably ameliorated Ang II-induced NEU enzyme activation, sialic acid releasing, and cardiomyocyte enlargement compared with the negative controls (*Figure 2G–I*). Hypertrophic markers *Nppa, Nppb*, and *Myh7* were also significantly decreased (*Figure 2J*). Conversely, NEU1 overexpression without any pathological stimulus up-regulated enzyme activity, sialic acid level, the expression of *Nppa, Nppb*, and *Myh7*, and induced cardiomyocyte enlargement (Supplementary material online, *Figure S2*).

*Neu1*CKO alleviates cardiac hypertrophy and remodelling in mice

To explore the function of NEU1 *in vivo*, we generated mice with cardiomyocyte-specific deletion of NEU1 using a Cre/loxP-dependent conditional gene-targeting approach. Mice homozygous for the *Neu1*-loxp (fl)-targeted allele ($Neu1^{fl/fl}$) were crossed with cardiomyocyte-specific tamoxifen-inducible Myh6 Cre lines (Supplementary material online, *Figure S3A and B*). The mRNA, protein, and enzyme activity of NEU1 were efficiently deleted from the hearts of Myh6-Cre, $Neu1^{fl/fl}$ mice (*Neu1* conditional knockout, *Neu1*CKO) (Supplementary material online, *Figure S3C–E*). Immunofluorescence results showed that NEU1 was significantly knocked down in the cardiomyocytes (Supplementary material online, *Figure S3F*). These mice and their age-matched $Neu1^{fl/fl}$ littermates without Cre recombinase activity (control mice) were then challenged with TAC surgery (*Figure 3A*). The *Neu1*-deleted group showed a significantly ameliorated phenotype with reduced heart size, heart weight, and lung weight (*Figure 3B and C*; Supplementary material online, *Figure S4A and B*). Cardiac sections stained with wheat germ agglutinin (WGA)

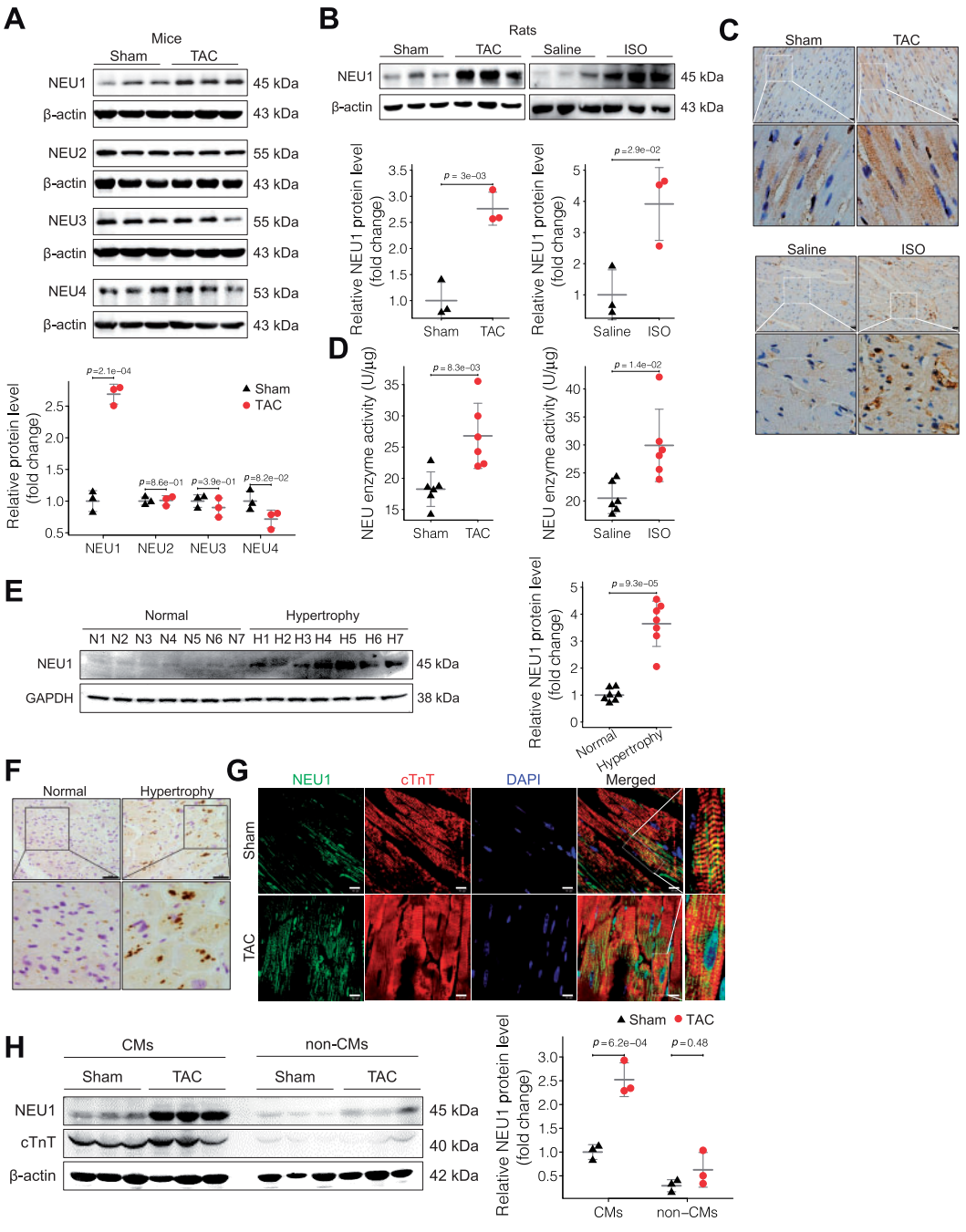


Figure 1 NEU1 is elevated in cardiac hypertrophic heart tissues. (*A*) Representative western blots and quantitative results of NEU1, NEU2, NEU3, and NEU4 levels in hearts of mice subjected to transverse aortic constriction for 4 weeks. $n = 3$ mice per group. (*B*) NEU1 levels in hearts of rats subjected to transverse aortic constriction or isoproterenol (ISO, 2.5 mg/kg/d, s.c.) treatment for 4 weeks. $n = 3$ rats per group. (*C*) Immunohistochemistry with an anti-NEU1 antibody in slices from the indicated rat hearts. $n = 3$ rat hearts per group; scale bar, 20 μm. (*D*) The neuraminidase enzyme activity of hypertrophic heart in mice by enzyme-linked immunosorbent assay. $n = 6$ hearts per group. (*E*) Representative western blots (left) and quantitative results (right) of NEU1 levels in human heart samples of healthy donors (N1–N7) and patients with hypertrophic cardiomyopathy (H1–H7). $n = 7$ samples per group. (*F*) Immunohistochemistry of NEU1 from human hearts. Scale bar, 50 μm. (*G*) Immunofluorescence images of NEU1 in hearts from mice subjected to TAC for 4 weeks. Cardiac troponin T is used as a cardiomyocyte marker. $n = 3$ mice per group; scale bar, 10 μm. (*H*) NEU1 levels in cardiomyocytes and non-cardiomyocytes from the adult mice subjected to transverse aortic constriction for 4 weeks. β-actin as an internal control, cardiac troponin T as cardiomyocyte marker. $n = 3$ mice per group. Data are presented as mean ± SD. *A, B, D,* and *E*, unpaired two-tailed *t*-test; *H*, two-way ANOVA. CMs, cardiomyocytes; cTnT, cardiac troponin T; TAC, transverse aortic constriction.

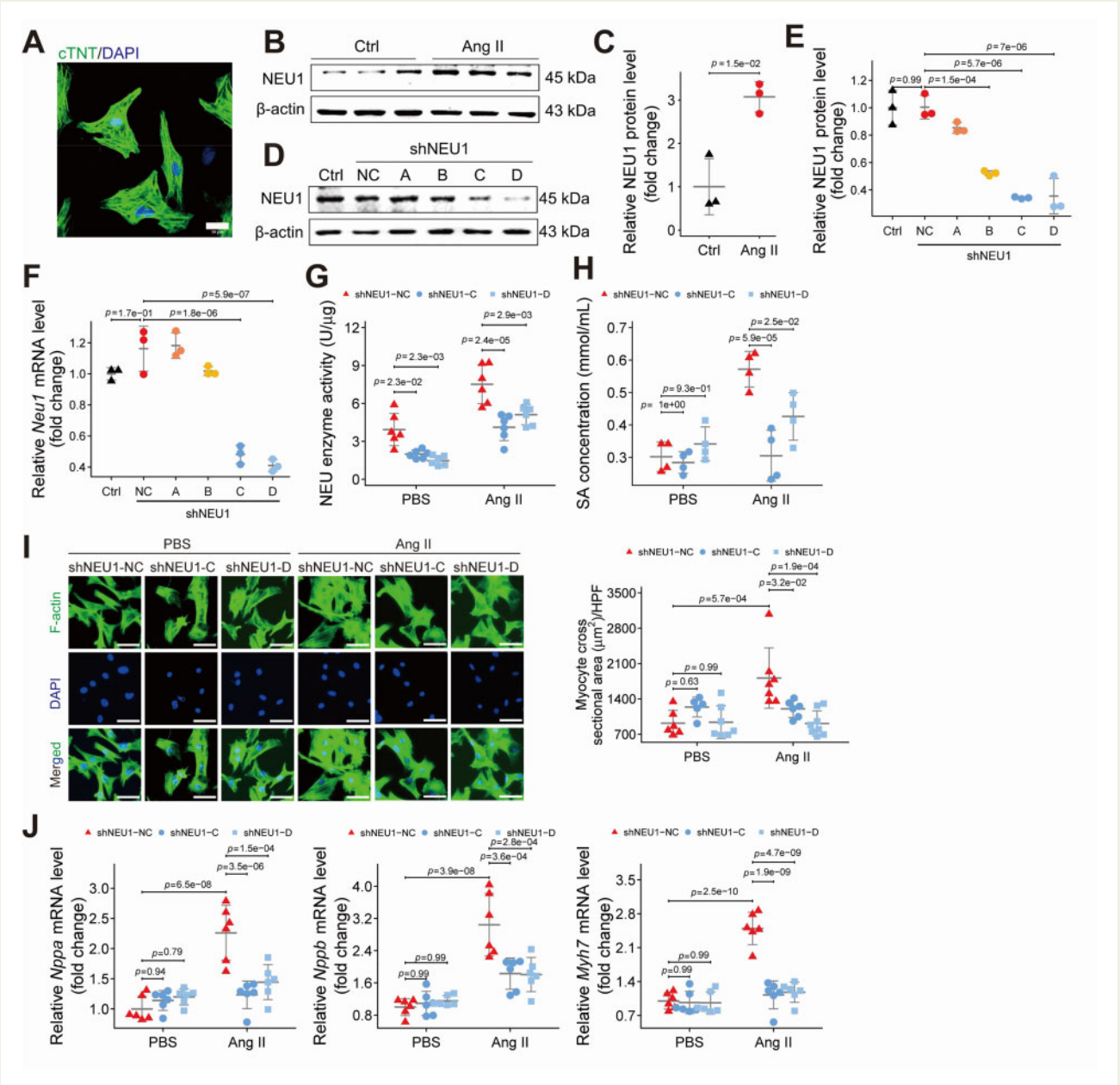


Figure 2 Knockdown of NEU1 protects neonatal rat primary cardiomyocytes from angiotensin II-induced cardiomyocyte hypertrophy. (*A*) Representative immunofluorescence images of neonatal rat primary cardiomyocytes stained with cardiac troponin T. Scale bar, 20 μm. (*B* and *C*) Western blots and quantitative results of NEU1 in neonatal rat primary cardiomyocytes stimulated with or without angiotensin II (1μM) for 48 h. n = 3 samples per group. (*D* and *E*) Representative western blots and quantitative results of NEU1 in neonatal rat primary cardiomyocytes. n = 3 samples per group. Neonatal rat primary cardiomyocytes were transduced with lentivirus-encoded scrambled (shNEU1-NC) or NEU1 shRNA for 48 h. shNEU1-A, shNEU1-B, shNEU1-C, and shNEU1-D are four different shRNA sequences. (*F*) Relative mRNA levels of *Neu1* in neonatal rat primary cardiomyocytes. n = 3 samples per group. (*G*) Neuraminidase enzyme activity of neonatal rat primary cardiomyocytes determined by enzyme-linked immunosorbent assay. Neonatal rat primary cardiomyocytes were transduced with or without NEU1 shRNA for 24 h and then the cells were stimulated with angiotensin II (1μM) for 48 h. n = 6 samples per group. (*H*) The sialic acid (SA) concentration in neonatal rat primary cardiomyocytes was determined by kit. n = 4 samples per group. (*I*) Neonatal rat primary cardiomyocytes morphology stained by F-actin (green). The nuclei were stained with DAPI (blue). n = 3 samples per group; scale bar, 50 μm. HPF, high power field. (*J*) mRNA levels of the indicated genes in neonatal rat primary cardiomyocytes. All normalized to *18s* rRNA, n = 6 samples per group. Data are presented as mean ± SD for *C*, *E*, and *F*–*J*. *C*, unpaired two-tailed *t*-test; *E* and *F*, one-way ANOVA; *G*–*J*, two-way ANOVA. Ang II, angiotensin II; *Nppa*, atrial natriuretic peptide; *Nppb*, brain natriuretic peptide; NRCMs, neonatal rat primary cardiomyocytes; *Myh7*, β-myosin heavy chain.

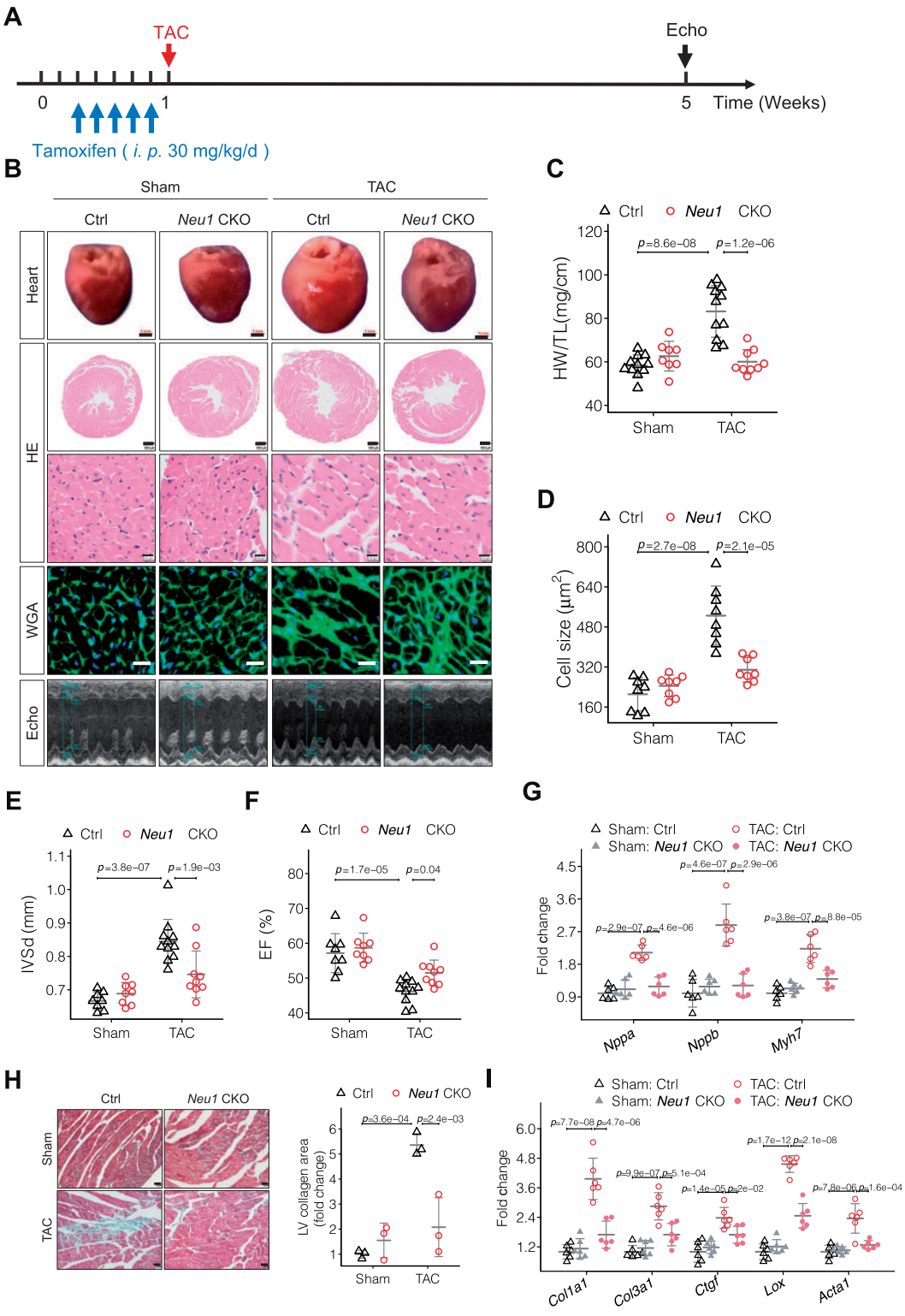


Figure 3 Cardiomyocyte-specific deletion of NEU1 blocks transverse aortic constriction-induced cardiac hypertrophy and remodelling. (*A*) Treatment regimen for Ctrl and *Neu1* CKO mice *in vivo* studies. Tamoxifen (30 mg/kg/day) was injected into mice by intraperitoneal injection (*i.p.*) for 5 days. The mice were then subjected to transverse aortic constriction surgery for 4 weeks. (*B*) Representative gross appearance of whole hearts (scale bar, 1 mm), heart cross-sections stained with haematoxylin-eosin (scale bar, 500 and 10 μm), cell boundaries demarcated with fluorescein isothiocyanate-wheat germ agglutinin (scale bar, 20 μm) and M-mode echocardiography from *Neu1* CKO and Ctrl littermate mice 4 weeks after transverse aortic constriction surgery. (*C*) The ratio of heart weight to tibia length (HW/TL). n = 11, 8, 11, 9 mice from left to right. (*D*) Statistical results for the cell cross-sectional area, n = 8. (*E* and *F*) Interventricular septum diameter in diastole (IVSd, *E*) and ejection fraction (EF, *F*) by echocardiography. n = 8, 8, 11, 9 mice from left to right. (*G* and *I*) mRNA levels of the indicated genes in heart samples, all normalized to *18s* rRNA. n = 6 samples per group. (*H*) Histological analysis of heart slices by Masson's trichrome (left), scale bar, 20 μm. Statistical results for myocardial interstitial collagen were analysed by Image Pro-Plus software (right). n = 3 mice per group. Data are presented as mean ± SD; two-way ANOVA. TAC, transverse aortic constriction; WGA, wheat germ agglutinin.

showed that *Neu1* deficiency decreased cardiomyocyte cross-sectional area (*Figure 3B and D*). Echocardiography indicated that the hearts of *Neu1*CKO mice developed less dilation and dysfunction after TAC based on analysis of interventricular septum diameter in diastole (IVSd), interventricular septum diameter in systole (IVSs), left ventricular posterior wall diameter in diastole (LVPWd), left ventricular posterior wall diameter in systole (LVPWs), ejection fraction (EF), left ventricular mass, and fractional shortening (FS) (*Figure 3E and F*; Supplementary material online, *Figure S4C–F*). Transverse aortic constriction surgery appeared not to affect diastolic function in terms of E/A and left ventricular isovolumic relaxation time (Supplementary material online, *Figure S4G and H*). The TAC-induced foetal gene expressions were greatly down-regulated in the hearts of the *Neu1*CKO mice compared with control (*Figure 3G*). Heart sections were stained with Masson to evaluate the degrees of fibrosis. The results revealed that cardiac fibrosis was improved in *Neu1*CKO mice compared with control (*Figure 3H*). The markers of fibrosis were also significantly down-regulated in *Neu1*CKO mice (*Figure 3I*). Additionally, RT–qPCR showed that NEU1 knockdown inhibited TAC-induced *IL-6, TNF-α*, and *IL-1β* increase (Supplementary material online, *Figure S4I–K*). TUNEL assay showed that NEU1 knockdown reduced TAC-induced apoptosis rates (Supplementary material online, *Figure S4L*). Isolectin B4 staining showed that capillary density was not altered by TAC stimulation or NEU1 knockdown (Supplementary material online, *Figure S4M*).

In another hypertrophic model induced by ISO (Supplementary material online, *Figure S5A*), *Neu1*CKO mice also showed significantly improved cardiac hypertrophy (Supplementary material online, *Figure S5B–E*) and ventricular thickening (Supplementary material online, *Figure S5F and G*). Cardiac function-associated EF and FS showed no significant alteration in ISO-induced *Neu1*CKO mice (Supplementary material online, *Figure S5H and I*). Hypertrophic and fibrosis marker gene expression were moderately reduced in *Neu1*CKO mice compared with littermate controls (Supplementary material online, *Figure S5J and K*). Apoptosis rates of cardiomyocytes were reduced in *Neu1*CKO mice (Supplementary material online, *Figure S5L*). Capillary density was not changed by ISO stimulation or NEU1 knockdown (Supplementary material online, *Figure S5M*).

Neuraminidase 1 overexpression promotes cardiac hypertrophy and remodelling in mice

Besides loss-of-function, a gain-of-function approach was performed using adeno-associated virus serotype 9 encoding NEU1 (AAV9-NEU1) and AAV9-Ctrl (Supplementary material online, *Figure S6A*). The mRNA, protein, and enzyme activity of NEU1 were significantly increased in cardiomyocytes after injection of AAV9-NEU1 for 4 weeks (Supplementary material online, *Figure S6B–E*). The immunofluorescence results showed that AAV9-NEU1 was transduced into cardiomyocytes (Supplementary material online, *Figure S6F*). We investigated the effects of NEU1 overexpression on cardiac hypertrophic phenotypes in mice under both pathological and physiological conditions (Supplementary material online, *Figure S6G*). As expected, NEU1 overexpression aggravated the development of TAC-stimulated cardiac hypertrophy and remodelling (Supplementary material online, *Figure S7*). Surprisingly, under physiological condition without any pathological stimulus, NEU1 overexpression for 13 weeks led to significant increase in heart size, heart weight, and cardiomyocyte cross-sectional area (*Figure 4A–D*). Echocardiography indicated that NEU1 overexpression triggered slight myocardial injury at 8 weeks (Supplementary material online, *Figure S8*), and significant ventricular thickening and cardiac dysfunction at 13 weeks (*Figure 4E–G*, Supplementary material online, *Figure S9*). In addition, cardiac hypertrophy foetal gene and fibrosis markers were also significantly up-regulated in hearts of mice subjected to NEU1 overexpression for 13 weeks (*Figure 4H–J*). These results suggest that cardiomyocyte-specific NEU1 overexpression promotes cardiac hypertrophy and remodelling under both pathological and physiological conditions.

Neuraminidase 1 interacts with GATA4 in the nucleus

To investigate the underlying mechanism by which NEU1 promotes cardiac damage, we determined the subcellular localization of NEU1 in cardiomyocytes. Of note, we observed that NEU1 is able to translocate into the nucleus in response to pressure overload, as measured by subcellular fractionation and immunofluorescence imaging (*Figure 5A–C*). This observation is different from the generally held view that NEU1 is located in the lysosome.[15]

To identify potential NEU1-interacting proteins in the nucleus, the nuclear protein extraction of NRCMs was co-immunoprecipitated with an anti-NEU1 antibody. The immunoprecipitate was analysed by liquid chromatography-tandem mass spectrometry (Supplementary material online, *Figure S10A*). A total of 12 transcription factors were identified, and among them, GATA4 and PURA are reported to be linked to cardiac injury (*Figure 5D*).[27,28] GATA4 is a zinc finger-containing transcription factor that acts a key transcriptional regulator of numerous cardiac genes including *Nppa* and *Nppb*.[28] The binding sites between GATA4 and the promoter of *Nppa* or *Nppb* are evolutionarily conserved (Supplementary material online, *Figure S10B*). We confirmed that NEU1 binds to GATA4 (*Figure 5E*) but could not bind to PURA (data not shown) in the NRCMs by co-immunoprecipitation (CoIP). The immunofluorescence results showed that NEU1 was able to translocate into the nucleus and co-localize with GATA4 in response to Ang II stimulation (Supplementary material online, *Figure S10C*). Surface plasmon resonance indicated that NEU1 exhibited strong binding affinity to GATA4 with an estimated equilibrium dissociation constant of 0.35 nM, suggesting that NEU1 directly targets GATA4 (*Figure 5F*). The interaction between NEU1 and PURA is too weak to fit the equilibrium dissociation constant (Supplementary material online, *Figure S10D*). The potential interactions between NEU1 and cardiac protein synthesis-associated transcription factors were also detected by CoIP. The results showed that NEU1 could not bind to MYOCARDIN, MYOGENIN, SRF, MEF2C, and MYOD1 (Supplementary material online, *Figure S10E*). These results suggest that NEU1 selectively binds to GATA4.

We next determined the effect of NEU1 on the transcriptional activity of GATA4. ChIP-qPCR results demonstrated that the binding of GATA4 to the promoter regions of *Nppa* and *Nppb* was significantly decreased in cardiomyocytes upon NEU1 silencing (*Figure 5G and H*, Supplementary material online, *Figure S10F and G*). On the contrary, NEU1 overexpression facilitated the interaction between GATA4 and *Nppa* or *Nppb* promoter (Supplementary material

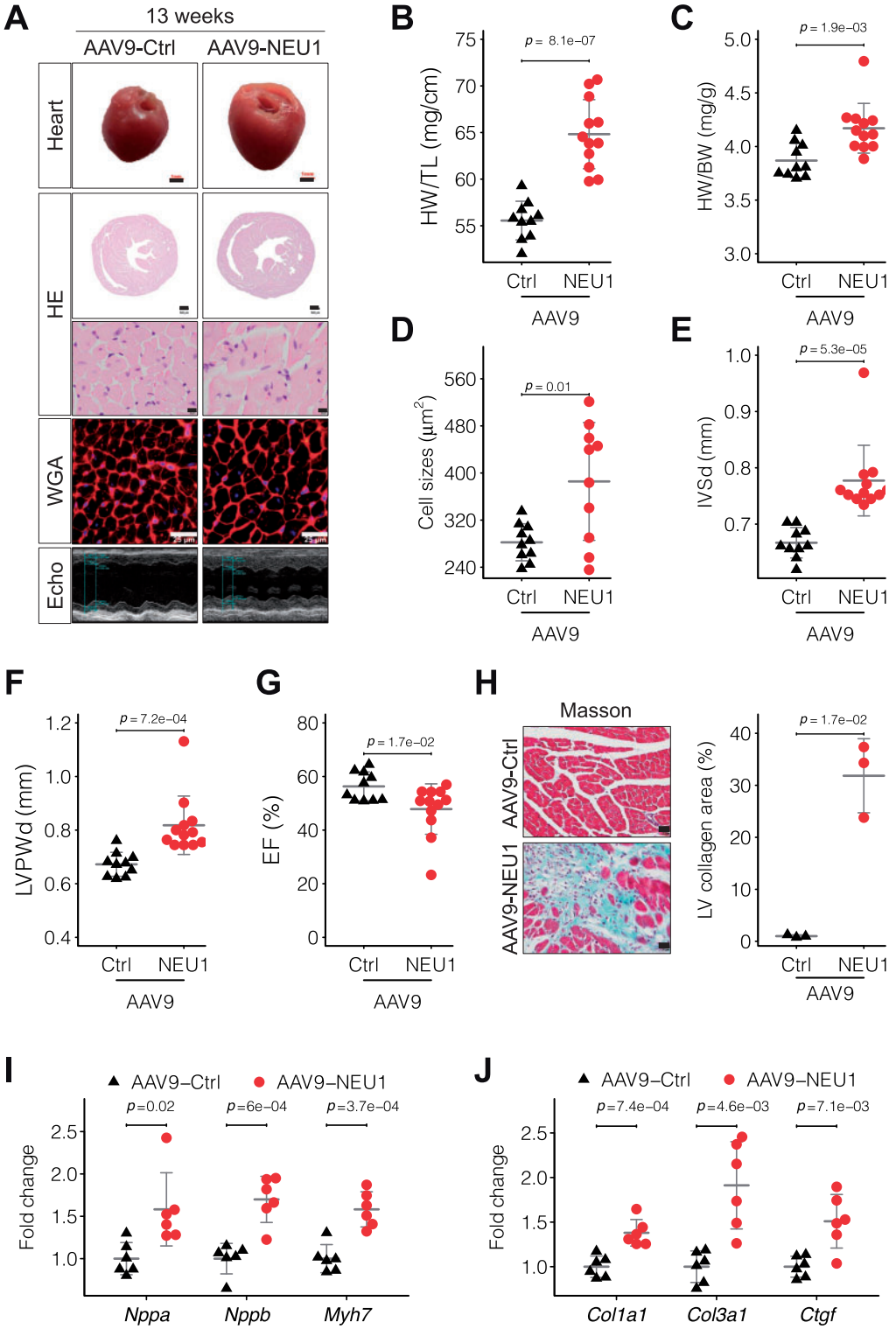


Figure 4 NEU1 overexpression in heart of mice causes cardiac hypertrophy and remodelling under physiological conditions. (*A*) The representative gross appearance of whole hearts (scale bar, 1 mm), heart cross-sections stained with haematoxylin-eosin (scale bar, 500 and 10 μm), cell boundaries demarcated with rhodamine-wheat germ agglutinin (scale bar, 20 μm) and M-mode echocardiography (the bottom row) from mice 13 weeks after infection with AAV9-NEU1. (*B* and *C*) The ratio of heart weight to tibia length (HW/TL, *B*) and heart weight to body weight (HW/BW, *C*). AAV9-Ctrl, $n = 10$; AAV9-NEU1, $n = 12$. (D) Statistical results for the cell cross-sectional area. $n = 10$. (*E-G*) Interventricular septum diameter in diastole (IVSd, *E*), left ventricular posterior wall thickness in diastole (LVPWd, *F*), and ejection fraction (EF, *G*) by echocardiography. AAV9-Ctrl, $n = 10$; AAV9-NEU1, $n = 12$. (*H*) Masson's trichrome staining (scale bars, 20 μm) and statistical results for the myocardial interstitial collagen of mice. (*I* and *J*) Hypertrophic marker and fibrosis genes in the indicated groups determined by RT–qPCR. All normalized to *18s* rRNA. $n = 6$ per group. Data are presented as mean ± SD; unpaired two-tailed *t*-test.

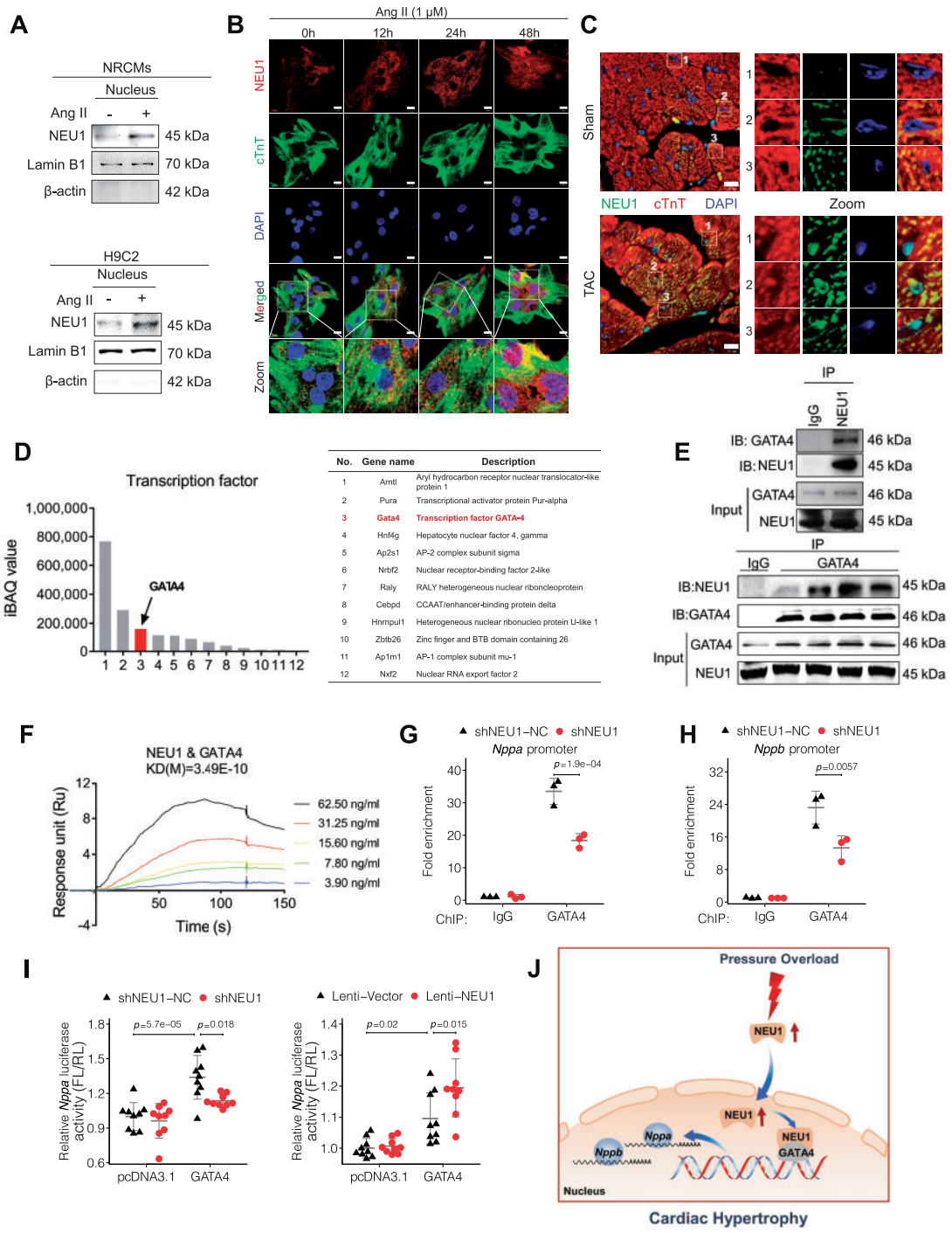


Figure 5 NEU1 translocates into the nucleus and interacts with GATA4. (*A*) NEU1 expression in the nucleus of neonatal rat primary cardiomyocytes and H9C2 cells in response to angiotensin II (1 μM) for 48 h. (*B*) Confocal images of NEU1 localization in the nucleus of neonatal rat primary cardiomyocytes. Neonatal rat primary cardiomyocytes in response to angiotensin II (1 μM) for 0, 12, 24, and 48 h, respectively. $n = 3$ per group; scale bar, 10 μm. (*C*) Immunofluorescence images of NEU1 and cardiac troponin T in hearts from mice subjected to transverse aortic constriction for 4 weeks. Scale bar, 10 μm. $n = 3$ per group. (*D*) Relative abundance of 12 transcription factors that could interact with NEU1. (*E*) Co-immunoprecipitation of NEU1 and GATA4 in neonatal rat primary cardiomyocytes. (*F*) The NEU1-GATA4 interaction tested by Surface plasmon resonance. (*G* and *H*) GATA4 occupancy at the promoters of *Nppa* (*G*) and *Nppb* (*H*) in neonatal rat primary cardiomyocytes transfected with NEU1 shRNA plasmid by ChIP. $n = 3$ per group. (*I*) Luciferase activity of the HEK293T cell extracts was determined. The cells were transfected with GATA4 plasmid, *Nppa* promoter luciferase reporter plasmid, and NEU1 shRNA (left) or NEU1 full-length plasmid (Lenti-NEU1, right). $n = 9$ per group. (*J*) The proposed mechanisms of NEU1-mediated cardiac hypertrophy. Data are presented as mean ± SD; two-way ANOVA. cTnT, cardiac troponin T; NRCMs, neonatal rat primary cardiomyocytes; TAC, transverse aortic constriction.

online, *Figure S10H and I*). Luciferase reporter assays further showed that NEU1 knockdown inhibited the GATA4-mediated *Nppa* and *Nppb* transcription, while NEU1 overexpression significantly promoted GATA4-induced *Nppa* and *Nppb* transcriptions (*Figure 5I*, Supplementary material online, *Figure S10J and K*). When GATA4 was knocked down (Supplementary material online, *Figure S10L*), NEU1 overexpression-induced *Nppa, Nppb*, and *Myh7* transcriptions were blocked in cardiomyocyte (Supplementary material online, *Figure S10M–O*), indicating that NEU1 promoted cardiac hypertrophy in a GATA4 dependent manner. Mechanism-wise, NEU1 translocated into the nucleus in response to Ang II stimulation and selectively interacted with the transcriptional factor GATA4, promoting hypertrophy-related gene transcription (*Figure 5J*).

C-09 is screened as a novel human neuraminidase 1 inhibitor

As NEU1 is a potential target for pathological cardiac hypertrophy, we proposed a computer-aided drug design strategy to screen novel human NEU1 inhibitors. The strategy involves homology modelling, molecular dynamics simulation, virtual screening, and experimental validation (*Figure 6A*). Since the precise crystal structure of mammalian NEU1 is not reported yet, SWII-MODEL, a protein structure homology modelling, was employed to produce the three-dimensional structure of human NEU1 based on the structure of influenza A virus NEU. Molecular dynamics simulation was carried out to produce tens of thousands of time-dependent protein frames. Cluster analysis showed the four dominant conformational states accounting for 65%, 22%, 4%, and 3%, respectively. Molecular operating environment was used to dock 1.6 million compounds from the ChemDiv library into the most populated NEU1 structure and to score their potential complementarity with putative binding sites. After docking, the top 500 ligands based on the lowest binding energies were selected for further analysis. Through inspecting the interaction patterns of each ligand with the binding and active sites at Arg78, Arg97, Asp103, Glu264, Arg280, Arg341, Tyr370, and Glu394, 16 compounds (C-01 to C-16) that formed reasonable hydrogen bonds with NEU1 were obtained. The binding modes of C-09 and C-12 with NEU1 are shown in *Figure 6B*. The biolayer interferometry assay (BLI) was performed to measure the binding ability of the two compounds with human recombinant NEU1. C-09 and C-12 showed strong binding affinity to human recombinant NEU1 with the estimated equilibrium dissociation constant at 0.38 and 2.3 μM, respectively (Supplementary material online, *Figure S11A and B*).

In NRCMs, C-09 and C-12 showed protective effects on cardiomyocytes (Supplementary material online, *Figure S11C–E*). Next, we studied the effects of the two compounds in mice with TAC-induced cardiac hypertrophy (*Figure 6C*). C-09 but not C-12 effectively inhibited NEU enzyme activation *in vivo* (Supplementary material online, *Figure S11F*). Of note, oral administration of C-09 (7.5 mg/kg/d) alleviated TAC-induced cardiac injury, with significantly improved heart morphology and function (*Figure 6D–F*, Supplementary material online, *Figure S11G–L*). The Masson staining showed the extent of cardiac fibrosis was improved after C-09 treatment (*Figure 6D and G*). RT–qPCR results demonstrated that the TAC-induced *Nppa, Nppb, Myh7, Col1a1, Col3a1, Ctgf, Lox*, and *Acta1* expressions were greatly down-regulated in the hearts of C-09-treated mice (*Figure 6H and I*). C-12 (7.5 mg/kg/d) showed moderate improvements in left ventricular mass, LVPWd, hypertrophic marker genes, and cardiac fibrosis, but no significant effects on heart weight, IVSd, IVSs, and EF. These data suggest that C-09 may be a novel NEU1 inhibitor and is effective in preventing the progression of cardiac hypertrophy and remodelling.

Anti-viral drugs zanamivir and oseltamivir protect cardiomyocytes from hypertrophy

Zanamivir and oseltamivir, two widely used anti-influenza drugs, are viral NEU inhibitors.[29,30] In addition to targeting viral NEU, by their nature NEU inhibitors have the potential to interfere with human NEU. As expected, the two drugs significantly inhibited TAC-induced NEU enzyme activation in mice (Supplementary material online, *Figure S11F*). Both of them significantly alleviated TAC-induced cardiac injury, with significantly reduced heart size (*Figure 6D and F*) and heart weight (*Figure 6E*). Echocardiography demonstrated that left ventricular mass, IVSs, IVSd, LVPWd, LVPWs, and EF were significantly improved by treatment with the two drugs (Supplementary material online, *Figure S11G–L*). Masson staining showed the extent of cardiac fibrosis was improved after zanamivir or oseltamivir treatment (*Figure 6D and G*). RT–qPCR results demonstrated that TAC-induced hypertrophic and fibrotic marker expression were greatly down-regulated in the hearts of zanamivir and oseltamivir-treated mice (*Figure 6H and I*).

In order to confirm that anti-influenza drugs have potential new indications, we evaluated the effects of zanamivir and oseltamivir in hypertrophic rat models. In TAC-induced hypertrophic rat models (Supplementary material online, *Figure S12A*), zanamivir and oseltamivir markedly inhibited NEU1 expression (Supplementary material online, *Figure S12B*). They protected the myocardium from cardiac hypertrophy as evidenced by improvement in the morphological structure (Supplementary material online, *Figure S12C–H*), heart function (Supplementary material online, *Figure S12I and J*), haemodynamics (Supplementary material online, Supplementary *Figure S13A–E*), and pathological changes (Supplementary *Figure S13F–H*). The anti-hypertrophic effects of the two drugs were also replicated in ISO-induced cardiac hypertrophy models in rats (Supplementary material online, *Figures S14 and S15*).

Discussion

This study is the first, to our knowledge, to characterize a crucial role for cardiomyocyte-localized NEU1 in pressure overload-induced cardiac hypertrophy and remodelling. The major findings of this study include the following: (i) we observed that NEU1 is significantly elevated in cardiomyocytes of hypertrophic hearts of rodents and humans; (ii) we identified NEU1 as a key driver of cardiac hypertrophy using genetically engineered animal models; (iii) in terms of mechanism, NEU1 translocated into the nucleus and interacted with transcriptional factor GATA4, leading to increased transcription of hypertrophy-related genes, *Nppa* and *Nppb*; (iv) a novel compound C-09 screened from millions of compounds showed favourable binding affinity to human NEU1 and effectively prevented the development of cardiac hypertrophy and remodelling; and (v) we showed

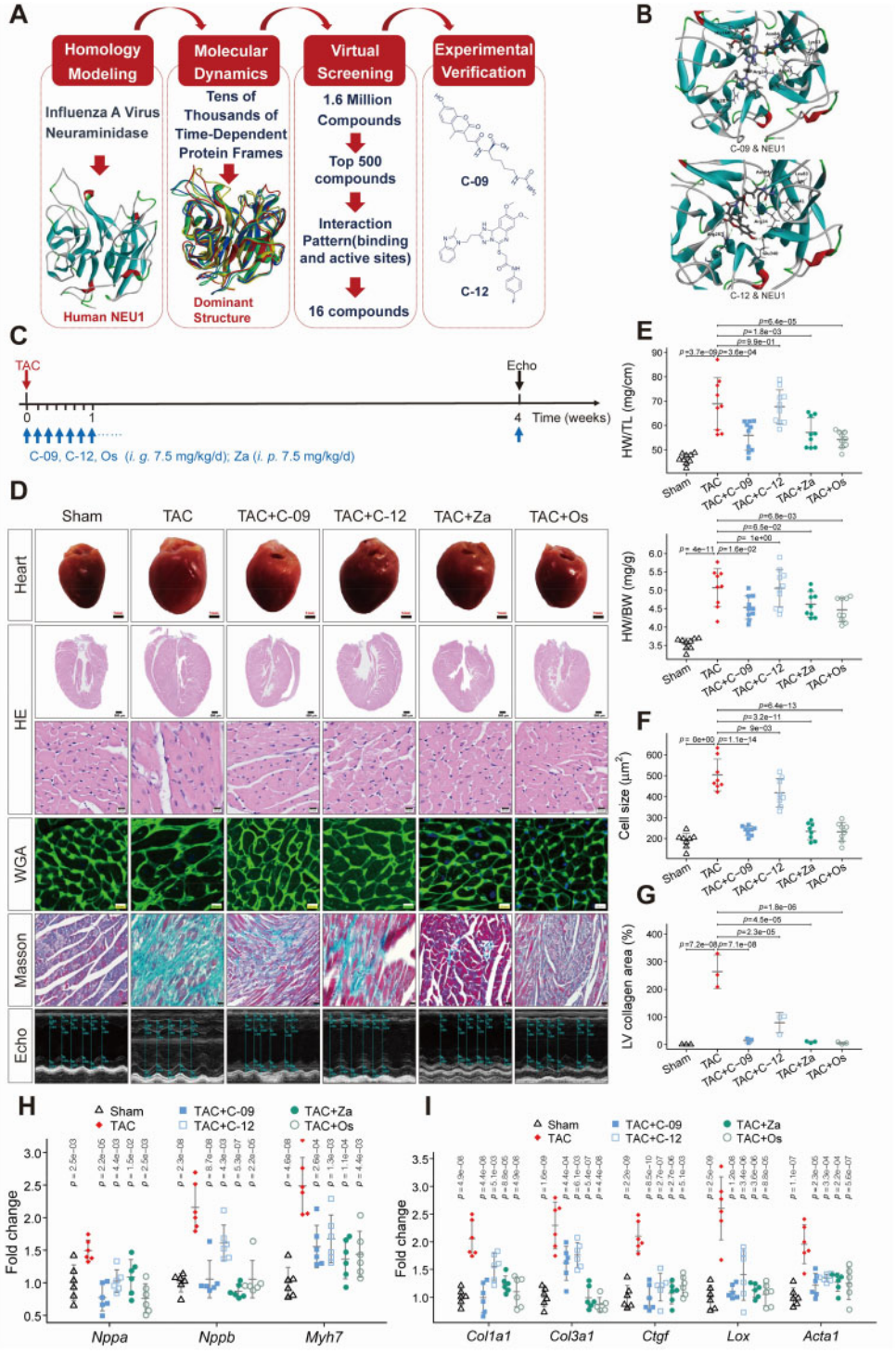


Figure 6 C-09 and C-12 prevent transverse aortic constriction-induced cardiac hypertrophy and remodelling in mice. (*A*) Schematic diagram displaying a strategy to screen human NEU1 inhibitors. In dominant structure image, red conformational state accounting for 65% frequency; green, 22% frequency; blue, 4% frequency; and yellow, 3% frequency. (*B*) Binding modes of C-09 and C-12 with human NEU1 predicted by AutoDock. (*C*) Treatment regimen. (*D*) Representative gross appearance of whole hearts (scale bar, 1 mm), heart vertical-sections stained with haematoxylin-eosin (scale bar, 500 or 10 μm), cell boundaries demarcated with fluorescein isothiocyanate-wheat germ agglutinin (scale bar, 10 μm), Masson's trichrome (scale bar, 10 μm), and M-mode echocardiography from mice. (*E*) The ratio of heart weight to tibia length (HW/TL, up) and heart weight to body-weight (HW/BW, down). n = 9, 9, 10, 10, 9, 9 mice from left to right. (*F*) Statistical results for the cell cross-sectional area. n = 8. (*G*) Statistical results for myocardial interstitial collagen. n = 3 samples per group. (*H* and *I*) mRNA levels of the indicated genes in heart samples, all normalized to *18s* rRNA. n = 6 samples per group. Data are presented as mean ± SD; one-way ANOVA. TAC, transverse aortic constriction; WGA, wheat germ agglutinin.

new indications of cardio-protection for anti-influenza drugs (zanamivir and oseltamivir) that directly inhibited mammalian NEU1 (*Graphical abstract*).

We previously identified a role for the metabolic marker *N*-acetylneuraminic acid in acute myocardial ischaemia, and NEU1 was shown to act as an upstream enzyme to regulate release of free *N*-acetylneuraminic acid.[31] In this work, we explored the role of NEU1 in pressure overload-induced cardiac hypertrophy and remodelling. Unlike from our previous findings, here, NEU1 plays a non-canonical role as a co-activator to interact with transcriptional factors GATA4, promoting cardiac hypertrophy and remodelling. This study combined with our previous work showed that NEU1 is a key driver of cardiovascular disease, and is therefore a potential therapeutic target.

NEU1 was observed to be highly expressed in different immune cell types, such as macrophages within atherosclerotic arteries, circulating monocytes, and invading monocytes/macrophages in the heart.[20,22] It has been reported that NEU1 is involved in the development of inflammatory responses and contributes to atherosclerosis and heart failure.[20,21] This work showed that cardiomyocyte-localized NEU1 was increased significantly in pressure overload-induced cardiac hypertrophy in mice and rats and was confirmed in heart tissues of patients with hypertrophic cardiomyopathy. Cardiomyocyte-specific NEU1 knockout or overexpression effectively counteracted or aggravated the development of TAC-stimulated cardiac hypertrophy and remodelling. Our findings suggest NEU1 in cardiomyocytes as an inducer of cardiac hypertrophy.

Mammalian NEU1 is typically located in the lysosome, where it has a well-defined catabolic function in removing terminal sialic acid residues of glycoproteins and oligosaccharides by forming a multienzyme complex along with β-galactosidase and protective protein/cathepsin A (PPCA).[12] In line with a previous report,[22] we also found that PPCA expression and the interstitial sialylglycoproteins were regulated by NEU1 (Supplementary material online, *Figure S16*). Emerging data have demonstrated that NEU1 can be sorted to plasma membrane of many cell types upon activation.[17–22] It thereby modulates the structure and function of cellular receptors by desialylation.[12] Interestingly, our work showed that NEU1 translocated into the nucleus, and acted as a transcriptional co-activator. Co-immunoprecipitation and liquid chromatography-tandem mass spectrometry identified a transcriptional factor GATA4 that strongly binds to NEU1. Transcriptional factors and their cofactors are key regulatory molecules in determining reactivation of cardiac hypertrophy-related 'Foetal genes', including *Nppa, Nppb*, and *Myh7*.[32] The zinc finger protein GATA4 is one such transcription factors, and a number of its cofactors have been implicated in cardiac hypertrophy, most notably Nkx-2.5, myocyte enhancer factor 2, and serum response factor.[33,34] Our work showed that the binding of GATA4 to the promoter region of *Nppa* and *Nppb* depended on NEU1, suggesting NEU1 is a novel co-transcriptional factor of GATA4.

Molecular mechanisms regarding the NEU1-involved cardiac hypertrophy and heart failure are very complex. Interestingly, we observed that NEU1 was up-regulated not only in nucleus but also in cytoplasm in response to pressure overload. We cannot exclude the fact that alteration of NEU1 in other subcellular organelles also contributes to cardiac hypertrophy. It has been reported that cAMP enhanced ISO-stimulated sialidase activity.[35] In line with this, we also observed that the cAMP level was increased in hypertrophic cardiomyocytes (Supplementary material online, *Figure S17A* and *B*). It was possible that activated NEU1 partly resulted from increased cAMP levels. It was reported that NEU activity affected calcium handling of cardiomyocytes.[36] In close agreement, by knockdown or overexpression of NEU1, we found that NEU1 positively regulated intracellular calcium levels in NRCMs (Supplementary material online, *Figure S17C* and *D*).

Given the critical role of NEU1 in cardiac hypertrophy and remodelling as characterized in this work, it is of importance to discover novel human-specific and organ-specific inhibitors. Because of unavailability of the three-dimensional structure of human NEU1, very few studies focus on designing human-specific NEU inhibitors. Based on the influenza virus NEU structure and binding/active sites of human NEU1, we developed a strategy to screen small-molecule inhibitors of human NEU1. From millions of compounds, we discovered the novel compound C-09, showing favourable binding affinity to NEU1 and significant protective effects against cardiac hypertrophy and remodelling through virtual screening and cellular/animal validation. In terms of amino acid sequence, NEU2, NEU3, and NEU4 show large similarity, while NEU1 shares <10% identities. Since the amino acid sequence of NEU1 is quite different from NEU2-NEU4, we speculated that C-09 selectively inhibited NEU1 activity. The determination of the three-dimensional structure of the NEU1 and its complex in the near future would contribute to the discovery of stronger inhibitors and explain its interdependent mode of action. Also, NEU1 inhibitors could complement existing therapies, such as β-adrenergic receptor blockers and angiotensin-converting enzyme inhibitors in an effort to efficiently treat heart failure.

Repositioning existing drugs for new indications has the potential to shorten development timelines and lower overall costs. Use of NEU inhibitors is recommended in expert consensus statement for the treatment of fulminant myocarditis.[37] In close with this, this work showed that zanamivir and oseltamivir, two first-line anti-virus drugs, effectively inhibited mammalian NEU enzyme. As a result, the two drugs elicited cardio-protection against TAC- and ISO-induced cardiac damage. It is known that marked reduction in NEU1 activity leads to sialidosis, an inherited disease characterized by coarse facial features and developmental delay.[38] As of now, there is no case report on sialidosis syndrome caused by oseltamivir or zanamivir use for up to 6 weeks. We did not observe any side effects of oseltamivir, zanamivir, and C-09 during the entire experimental period. Because of their well-established safety and pharmacokinetic profiles, it looks promising to repurpose anti-viral drugs for new indications of ischaemic and non-ischaemic cardiovascular diseases.

This study has some limitations. First, we observed that NEU1 expression in the nucleus was elevated in response to Ang II challenge. Further investigations are required to address how NEU1 was elevated and how it translocated from the lysosome to the nucleus. Second, in addition to acting as a co-activator to interact with transcriptional factor GATA4, NEU1 may also be involved in other signalling pathways or interact with other transcription factors that remain to be investigated. Third, this work highlights a role for NEU1 in maladaptive hypertrophy. It is not clear whether NEU1 also plays a role in adaptive hypertrophy. Fourth, some haemodynamic parameters were not determined such as mean arterial pressure and systemic vascular resistance of mice receiving ISO owing to our technical limitation. Fifth, because of lacking data on diastolic function in

hypertrophic cardiomyopathy patients, it is likely that the magnitude of diastolic impairment has impact on myocardial molecular profile investigated in cells and animal model. Sixth, we showed obvious cardio-protective effects for zanamivir and oseltamivir in animal models. Repurposing this new medical indication for existing anti-viral drugs requires clinical trials to confirm their efficacy.

In conclusion, this work identifies NEU1 as a critical driver of pressure overload-induced cardiac hypertrophy by interacting with the transcriptional factor GATA4 and inhibiting NEU1 opens up an entirely new field of treatment for cardiovascular diseases. It has the clinical potential to develop small-molecule compounds targeting human NEU1 and repurpose novel cardio-protective indications for existing anti-viral drugs.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

References

1. Schiattarella GG, Hill JA. Inhibition of hypertrophy is a good therapeutic strategy in ventricular pressure overload. *Circulation* 2015;**131**:1435–1447.
2. Maron BJ, Maron MS. Hypertrophic cardiomyopathy. *Lancet* 2013;**381**:242–255.
3. Nakamura M, Sadoshima J. Mechanisms of physiological and pathological cardiac hypertrophy. *Nat Rev Cardiol* 2018;**15**:387–407.
4. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling-concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. *J Am Coll Cardiol* 2000;**35**:569–582.
5. Lan F, Lee AS, Liang P, Sanchez-Freire V, Nguyen PK, Wang L, Han L, Yen M, Wang Y, Sun N, Abilez OJ, Hu S, Ebert AD, Navarrete EG, Simmons CS, Wheeler M, Pruitt B, Lewis R, Yamaguchi Y, Ashley EA, Bers DM, Robbins RC, Longaker MT, Wu JC. Abnormal calcium handling properties underlie familial hypertrophic cardiomyopathy pathology in patient-specific induced pluripotent stem cells. *Cell Stem Cell* 2013;**12**:101–113.
6. Lee DI, Zhu G, Sasaki T, Cho GS, Hamdani N, Holewinski R, Jo SH, Danner T, Zhang M, Rainer PP, Bedja D, Kirk JA, Ranek MJ, Dostmann WR, Kwon C, Margulies KB, Van Eyk JE, Paulus WJ, Takimoto E, Kass DA. Phosphodiesterase 9A controls nitric-oxide-independent cGMP and hypertrophic heart disease. *Nature* 2015;**519**:472–476.
7. Han P, Li W, Lin CH, Yang J, Shang C, Nuernberg ST, Jin KK, Xu W, Lin CY, Lin CJ, Xiong Y, Chien H, Zhou B, Ashley E, Bernstein D, Chen PS, Chen HV, Quertermous T, Chang CP. A long noncoding RNA protects the heart from pathological hypertrophy. *Nature* 2014;**514**:102–106.
8. Song L, Wang L, Li F, Yukht A, Qin M, Ruther H, Yang M, Chaux A, Shah PK, Sharifi BG. Bone marrow-derived tenascin-c attenuates cardiac hypertrophy by controlling inflammation. *J Am Coll Cardiol* 2017;**70**:1601–1615.
9. Ranek MJ, Kokkonen-Simon KM, Chen A, Dunkerly-Eyring BL, Vera MP, Oeing CU, Patel CH, Nakamura T, Zhu G, Bedja D, Sasaki M, Holewinski RJ, Van Eyk JE, Powell JD, Lee DI, Kass DA. PKG1-modified TSC2 regulates mTORC1 activity to counter adverse cardiac stress. *Nature* 2019;**566**:264–269.
10. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, González-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P; ESC Scientific Document Group. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J* 2016;**37**:2129–2200.
11. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ; National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National High Blood Pressure Education Program Coordinating Committee. The seventh report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. *JAMA* 2003;**289**:2560–2572.
12. Miyagi T, Yamaguchi K. Mammalian sialidases: physiological and pathological roles in cellular functions. *Glycobiology* 2012;**22**:880–896.
13. McAuley JL, Gilbertson BP, Trifkovic S, Brown LE, McKimm-Breschkin JL. Influenza virus neuraminidase structure and functions. *Front Microbiol* 2019;**10**:39.
14. Amith SR, Jayanth P, Franchuk S, Finlay T, Seyrantepe V, Beyaert R, Pshezhetsky AV, Szewczuk MR. Neu1 desialylation of sialyl alpha-2,3-linked beta-galactosyl residues of TOLL-like receptor 4 is essential for receptor activation and cellular signaling. *Cell Signal* 2010;**22**:314–324.
15. Dridi L, Seyrantepe V, Fougerat A, Pan X, Bonneil E, Thibault P, Moreau A, Mitchell GA, Heveker N, Cairo CW, Issad T, Hinek A, Pshezhetsky AV. Positive regulation of insulin signaling by neuraminidase 1. *Diabetes* 2013;**62**:2338–2346.
16. Seyrantepe V, Iannello A, Liang F, Kanshin E, Jayanth P, Samarani S, Szewczuk MR, Ahmad A, Pshezhetsky AV. Regulation of phagocytosis in macrophages by neuraminidase 1. *J Biol Chem* 2010;**285**:206–215.
17. Lillehoj EP, Hyun SW, Feng C, Zhang L, Liu A, Guang W, Nguyen C, Luzina IG, Atamas SP, Passaniti A, Twaddell WS, Puché AC, Wang LX, Cross AS, Goldblum SE. NEU1 sialidase expressed in human airway epithelia regulates epidermal growth factor receptor (EGFR) and MUC1 protein signaling. *J Biol Chem* 2012;**287**:8214–8231.
18. Hinek A, Bodnaruk TD, Bunda S, Wang Y, Liu K. Neuraminidase-1, a subunit of the cell surface elastin receptor, desialylates and functionally inactivates adjacent receptors interacting with the mitogenic growth factors PDGF-BB and IGF-2. *Am J Pathol* 2008;**173**:1042–1056.
19. White EJ, Gyulay G, Lhoták Š, Szewczyk MM, Chong T, Fuller MT, Dadoo O, Fox-Robichaud AE, Austin RC, Trigatti BL, Igdoura SA. Sialidase down-regulation reduces non-HDL cholesterol, inhibits leukocyte transmigration, and attenuates atherosclerosis in ApoE knockout mice. *J Biol Chem* 2018;**293**:14689–14706.
20. Sieve I, Ricke-Hoch M, Kasten M, Battmer K, Stapel B, Falk CS, Leisegang MS, Haverich A, Scherr M, Hilfiker-Kleiner D. A positive feedback loop between IL-1β, LPS and NEU1 may promote atherosclerosis by enhancing a pro-inflammatory state in monocytes and macrophages. *Vascul Pharmacol* 2018;**103–105**:16–28.
21. Yang A, Gyulay G, Mitchell M, White E, Trigatti BL, Igdoura SA. Hypomorphic sialidase expression decreases serum cholesterol by downregulation of VLDL production in mice. *J Lipid Res* 2012;**53**:2573–2585.
22. Heimerl M, Sieve I, Ricke-Hoch M, Erschow S, Battmer K, Scherr M, Hilfiker-Kleiner D. Neuraminidase-1 promotes heart failure after ischemia/reperfusion injury by affecting cardiomyocytes and invading monocytes/macrophages. *Basic Res Cardiol* 2020;**115**:62.
23. Litviňuková M, Talavera-López C, Maatz H, Reichart D, Worth CL, Lindberg EL, Kanda M, Polanski K, Heinig M, Lee M, Nadelmann ER, Roberts K, Tuck L, Fasouli ES, DeLaughter DM, McDonough B, Wakimoto H, Gorham JM, Samari S, Mahbubani KT, Saeb-Parsy K, Patone G, Boyle JJ, Zhang H, Zhang H, Viveiros A, Oudit GY, Bayraktar OA, Seidman JG, Seidman CE, Noseda M, Hubner N, Teichmann SA. Cells of the adult human heart. *Nature* 2020;**588**:466–472.
24. Adamo L, Rocha-Resende C, Prabhu SD, Mann DL. Reappraising the role of inflammation in heart failure. *Nat Rev Cardiol* 2020;**17**:269–285.

25. Patel B, Ismahil MA, Hamid T, Bansal SS, Prabhu SD. Mononuclear phagocytes are dispensable for cardiac remodeling in established pressure-overload heart failure. *PLoS One* 2017;**12**:e0170781.
26. Heineke J, Molkentin JD. Regulation of cardiac hypertrophy by intracellular signalling pathways. *Nat Rev Mol Cell Biol* 2006;**7**:589–600.
27. Herzig TC, Jobe SM, Aoki H, Molkentin JD, Cowley AW Jr, Izumo S, Markham BE. Angiotensin II type1a receptor gene expression in the heart: AP-1 and GATA-4 participate in the response to pressure overload. *Proc Natl Acad Sci USA* 1997;**94**:7543–7548.
28. Zhang A, David JJ, Subramanian SV, Liu X, Fuerst MD, Zhao X, Leier CV, Orosz CG, Kelm RJ Jr, Strauch AR. Serum response factor neutralizes Pur alpha- and Pur beta-mediated repression of the fetal vascular smooth muscle alpha-actin gene in stressed adult cardiomyocytes. *Am J Physiol Cell Physiol* 2008;**294**:702–714.
29. Liang Q, De Windt LJ, Witt SA, Kimball TR, Markham BE, Molkentin JD. The transcription factors GATA4 and GATA6 regulate cardiomyocyte hypertrophy in vitro and in vivo. *J Biol Chem* 2001;**276**:30245–33053.
30. Uyeki TM, Bernstein HH, Bradley JS, Englund JA, File TM, Fry AM, Gravenstein S, Hayden FG, Harper SA, Hirshon JM, Ison MG, Johnston BL, Knight SL, McGeer A, Riley LE, Wolfe CR, Alexander PE, Pavia AT. Clinical practice guidelines by the Infectious Diseases Society of America: 2018 update on diagnosis, treatment, chemoprophylaxis, and institutional outbreak management of seasonal influenzaa. *Clin Infect Dis* 2019;**68**:895–902.
31. Zhang L, Wei TT, Li Y, Li J, Fan Y, Huang FQ, Cai YY, Ma G, Liu JF, Chen QQ, Wang SL, Li H, Alolga RN, Liu B, Zhao DS, Shen JH, Wang XM, Zhu W, Li P, Qi LW. Functional metabolomics characterizes a key role for N-acetylneuraminic acid in coronary artery diseases. *Circulation* 2018;**137**:1374–1390.
32. Barry SP, Davidson SM, Townsend PA. Molecular regulation of cardiac hypertrophy. *Int J Biochem Cell Biol* 2008;**40**:2023–2039.
33. Morin S, Charron F, Robitaille L, Nemer M. GATA-dependent recruitment of MEF2 proteins to target promoters. 2000;**19**:2046–2055.
34. Sepulveda JL, Vlahopoulos S, Iyer D, Belaguli N, Schwartz RJ. Combinatorial expression of GATA4, Nkx2-5, and serum response factor directs early cardiac gene activity. *J Biol Chem* 2002;**277**:25775–25782.
35. Saito M, Sakiyama K, Shiota T, Ito M. Isoproterenol produces a rapid increase in sialidase activity in rat heart tissue and cardiomyocyte-derived H9c2 cells in culture. *FEBS Lett* 2003;**542**:105–108.
36. Yee, HF Jr, Kuwata JH, Langer GA. Effects of neuraminidase on cellular calcium and contraction in cultured cardiac myocytes. *J Mol Cell Cardiol* 1991;**23**:175–185.
37. Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, Doig A, Guilliams T, Latimer J, McNamee C, Norris A, Sanseau P, Cavalla D, Pirmohamed M. Drug repurposing: progress, challenges and recommendations. *Nat Rev Drug Discov* 2019;**18**:41–58.
38. Franceschetti S, Canafoglia L. Sialidoses. *Epileptic Disord* 2016;**18**:89–93.