(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 June 2017 (01.06.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/091807 A1

(51) **International Patent Classification**:
A61K 38/17 (2006.01) *C12N 15/113* (2010.01)

(21) **International Application Number**:
PCT/US2016/063759

(22) **International Filing Date**:
25 November 2016 (25.11.2016)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
62/260,144 25 November 2015 (25.11.2015) US

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

[Continued on next page]

(54) **Title**: PEPTIDE INHIBITORS FOR CALCINEURIN

(57) **Abstract**: The present disclosure generally relates to compositions and methods for treating or preventing a calcineurin Aβ-mediated disorder. More particularly, the disclosure provides compositions that inhibit binding of calcineurin to a scaffold protein, or compositions that inhibit Cdc42-interacting protein 4 (CIP4).

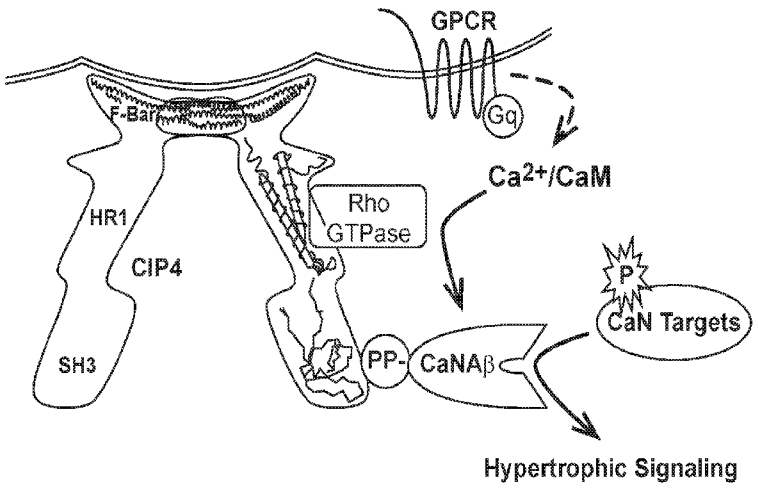


FIG. 1

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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

PEPTIDE INHIBITORS FOR CALCINEURIN

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 62/260,144, filed November 25, 2015, the disclosure of which is incorporated herein by reference in its entirety.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with government support under grant number RO1 HL075398 awarded by the National Institutes of Health. The government has certain rights in the invention.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0003] This application contains, as a separate part of the disclosure, a Sequence Listing in computer-readable form which is incorporated by reference in its entirety and identified as follows: Filename: 50044A_Seqlisting.txt; Size: 55,003 bytes, created : November 23, 2016.

FIELD OF THE INVENTION

[0004] The present disclosure generally relates to compositions and methods for treating or preventing a calcineurin Aβ-mediated disorder. More particularly, the disclosure provides compositions that inhibit binding of calcineurin to a scaffold protein, or compositions that inhibit Cdc42-interacting protein 4 (CIP4).

BACKGROUND

[0005] Heart failure is a syndrome of major public heath significance accountable for nearly 300,000 deaths each year. About 5.1 million US citizens suffer from heart failure, with nearly 670,000 new cases diagnosed annually. Despite current therapy, mortality for those with heart failure remains 50% at five years following diagnosis. It is estimated that by 2030, the prevalence of heart failure will increase by 25%, in part due to the ongoing obesity epidemic. This disease burden will cost the United States an estimated $70 billion per year. The discovery of new therapies to prevent or treat heart failure is, therefore, of paramount importance.

SUMMARY OF THE INVENTION

[0006] In some aspects, the disclosure provides a method of treating a patient suffering from a calcineurin Aβ-mediated disorder, comprising administering to the patient a composition that

inhibits binding of calcineurin Aβ (CaNAβ) amino-terminal polyproline domain to a scaffold protein. In some embodiments, the scaffold protein is Cdc42-interacting protein 4 (CIP4), profilin-1, profilin-2, sorbin, formin binding protein 11 (FBP11), or TOCA-1.

[0007] In further embodiments, the composition comprises a calcineurin Aβ (CaNAβ) peptide. In some embodiments, the CaNAβ peptide comprises a sequence from the amino-terminus polyproline domain of the CaNAβ amino acid sequence (SEQ ID NO: 1). In some embodiments, the CaNAβ peptide is amino acids 1-27 of the CaNAβ amino acid sequence (SEQ ID NO: 1).

[0008] In further embodiments, the composition comprises a Cdc42-interacting protein 4 (CIP4) peptide. In some embodiments, the CIP4 peptide comprises a sequence from the SRC Homology 3 (SH3) domain of the CIP4 amino acid sequence (SEQ ID NO: 2). In still further embodiments, the CIP4 peptide is amino acids 457-545 of the CIP4 amino acid sequence (SEQ ID NO: 2).

[0009] In various embodiments, the peptide is administered via a viral vector. In some embodiments, the viral vector is adeno-associated virus (AAV). In further embodiments, the viral vector comprises a cardiac muscle specific promoter. In still further embodiments, the promoter is cardiac troponin T promoter.

[0010] In some embodiments, the composition comprises a small interfering RNA (siRNA) that inhibits expression of CaNAβ. In further embodiments, the composition comprises a siRNA that inhibits expression of CIP4. In still further embodiments, the composition does not induce immunosuppression in the patient.

[0011] In various embodiments, the calcineurin Aβ-mediated disorder involves a T-cell, a neuron, a platelet, kidney, pancreas, or skeletal muscle.

[0012] It has previously been shown that calcineurin Aβ binds the scaffold protein mAKAPβ (SEQ ID NO: 3) in the heart. Evidence has also been acquired that the expression of mAKAPβ and calcineurin Aβ [Bueno *et al.*, Proc Natl Acad Sci USA 99(7): 4586-91 (2002)] in the heart, as well as the binding of calcineurin Aβ to mAKAPβ [Li *et al.*, Journal of Molecular and Cellular Cardiology, 48: 387-394 (2010)] is required for cardiac myocyte hypertrophy. Although calcineurin inhibition for treating heart failure has been proposed [Wilkins *et al.*, Biochem Biophys Res Commun. 322(4): 1178-91 (2004)], it is has not been therapeutically practical to target calcineurin because calcineurin inhibitors are potent immunosuppressants. It is known

that the expression of anchoring disruptor peptides in cultured myocytes inhibit cellular hypertrophy [Li *et al.*, Experimental Cell Research 319(4): 447-454 (2013)].

[0013] The present disclosure provides compositions that unexpectedly inhibit calcineurin *in vivo* without inducing immunosuppression.

[0014] In some aspects, a method of treating or preventing heart disease is provided comprising administering to a patient at risk of or suffering from heart disease a pharmaceutically effective amount of a composition that inhibits binding of calcineurin Aβ (CaNAβ) amino-terminal polyproline domain to a scaffold protein. In some embodiments, the scaffold protein is Cdc42-interacting protein 4 (CIP4), profilin-1, profilin-2, sorbin, formin binding protein 11 (FBP11), or TOCA-1. In further aspects, the disclosure provides a method of treating or preventing heart disease comprising administering to a patient at risk of or suffering from heart disease a pharmaceutically effective amount of a composition that inhibits Cdc42-interacting protein 4 (CIP4). In some embodiments, the composition comprises a calcineurin Aβ (CaNAβ) peptide. In further embodiments, the CaNAβ peptide comprises a sequence from the amino-terminus polyproline domain of the CaNAβ amino acid sequence (SEQ ID NO: 1). In still further embodiments, the CaNAβ peptide is amino acids 1-27 of SEQ ID NO: 1.

[0015] In some embodiments, the composition comprises a Cdc42-interacting protein 4 (CIP4) peptide. In further embodiments, the CIP4 peptide comprises a sequence from the SRC Homology 3 (SH3) domain of the CIP4 amino acid sequence (SEQ ID NO: 2). In still further embodiments, the CIP4 peptide is amino acids 457-545 of SEQ ID NO: 2.

[0016] Methods of the disclosure also include those wherein the peptide is encoded by a polynucleotide sequence incorporated into a viral vector. In some embodiments, the viral vector is adeno-associated virus (AAV). In some embodiments, the polynucleotide sequence encoding the peptide is operably linked to a cardiac muscle specific promoter. In related embodiments, the promoter is cardiac troponin T promoter.

[0017] In some embodiments, the composition comprises a small interfering RNA (siRNA) that inhibits expression of CaNAβ. In further embodiments, the composition comprises a siRNA that inhibits expression of CIP4. In some embodiments, the composition does not induce immunosuppression in the patient.

[0018] In some aspects, the disclosure provides a method of treating heart failure, comprising administering to a patient at risk of heart failure a pharmaceutically effective amount of a composition that inhibits binding of calcineurin Aβ (CaNAβ) amino-terminal polyproline domain to a scaffold protein. In various embodiments the scaffold protein is Cdc42-interacting protein 4 (CIP4), profilin-1, profilin-2, sorbin, formin binding protein 11 (FBP11), or TOCA-1. In some embodiments the composition comprises a calcineurin Aβ (CaNAβ) peptide. In further embodiments, the CaNAβ peptide comprises a sequence from the amino-terminus polyproline domain of the CaNAβ amino acid sequence (SEQ ID NO: 1). In still further embodiments, the CaNAβ peptide is amino acids 1-27 of the CaNAβ amino acid sequence (SEQ ID NO: 1). In some embodiments, the composition comprises a Cdc42-interacting protein 4 (CIP4) peptide. In further embodiments, the CIP4 peptide comprises a sequence from the SRC Homology 3 (SH3) domain of the CIP4 amino acid sequence (SEQ ID NO: 2). In still further embodiments, the CIP4 peptide is amino acids 457-545 of the CIP4 amino acid sequence (SEQ ID NO: 2). In some embodiments, the peptide is administered via a viral vector, and in additional embodiments the viral vector is adeno-associated virus (AAV). In further embodiments, comprises polynucleotide encoding the peptide is operably linked to a cardiac muscle specific promoter while in still further embodiments, the promoter is cardiac troponin T promoter.

[0019] In some embodiments, the composition comprises a small interfering RNA (siRNA) that inhibits expression of CaNAβ. In further embodiments, the composition comprises a siRNA that inhibits expression of CIP4. In still further embodiments, the composition does not induce immunosuppression in the patient.

[0020] In some aspects, the disclosure provides a composition comprising a peptide comprising a sequence from the amino-terminal polyproline domain of calcineurin Aβ (CaNAβ) amino acid sequence (SEQ ID NO: 1). In further embodiments, the CaNAβ peptide is amino acids 1-27 of SEQ ID NO: 1.

[0021] In further aspects, the disclosure provides a composition comprising a peptide comprising a sequence from the SRC Homology 3 (SH3) domain of Cdc42-interacting protein 4 (CIP4) (SEQ ID NO:2). In some embodiments, the CIP4 peptide is amino acids 457-545 of SEQ ID NO:2.

[0022] In some aspects, a composition comprising a viral vector is provided comprising a polynucleotide that encodes a peptide comprising a sequence from the amino-terminal polyproline domain of calcineurin Aβ (CaNAβ) amino acid sequence (SEQ ID NO: 1). In some embodiments, the CaNAβ peptide is amino acids 1-27 of SEQ ID NO: 1.

[0023] In further aspects, the disclosure provides a composition comprising a viral vector comprising a polynucleotide that encodes a peptide comprising a sequence from the SRC Homology 3 (SH3) domain of Cdc42-interacting protein 4 (CIP4) (SEQ ID NO: 2). In some embodiments, the CIP4 peptide is amino acids 457-545 of SEQ ID NO: 2. In further embodiments, the viral vector is adeno-associated virus (AAV). In still further embodiments, the viral vector comprises a cardiac muscle specific promoter. In some embodiments, the promoter is cardiac troponin T promoter.

[0024] In some aspects, the disclosure provides a method of treating or preventing heart disease by administering to a patient a pharmaceutically effective amount of a mAKAPβ peptide, wherein the mAKAPβ peptide is amino acids 1286-1346 of the mAKAPβ amino acid sequence (SEQ ID NO: 3). In some embodiments the mAKAPβ peptide is encoded by a polynucleotide sequence incorporated into a viral vector. In further embodiments, the viral vector is adeno-associated virus (AAV). In still further embodiments, the polynucleotide sequence is operably linked to a cardiac muscle specific promoter. In some embodiments, the promoter is cardiac troponin T promoter. In further embodiments, disclosure contemplates use of the corresponding mAKAP rat amino acid sequence (SEQ ID NO: 5) or a fragment thereof (*e.g.*, in some embodiments the peptide fragment is amino acids 1286-1345 of SEQ ID NO: 5).

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] **Figure 1** depicts a model for CIP4-CaNAβ signaling. G-protein coupled receptors (GPCR) increase intracellular Ca^{2+}, including through TRPC channels, activating the Ca^{2+}/calmodulin (CaM)-dependent phosphatase CaNAβ. CIP4 oligomers associate via the CIP4 F-BAR domain with membranes or the cytoskeleton and bind active Rho family GTPases. Through the SH3 domain, CIP4 can serve as a scaffold for CaNAβ, resulting in the activation of that CaN isoform at selective intracellular sites. Protein Data Bank (PDB) IDs for CIP4 structures are 2EFK, 2KE4, and 2CT4.

[0026] **Figure 2** shows the structure of CaNAβ [Rusnak *et al.*, Physiol Rev. 80(4): 1483-521 (2000)]. The Aβ-specific N-terminal polyproline domain (PP) and conserved catalytic, CaNB (B) and CaM binding, and autoinhibitory (AID) domains are indicated. Ca^{2+}/CaM binds CaNA, displacing the AID from the catalytic site, rendering it active. PxIxIT and LxVP binding sites are indicated.

[0027] **Figure 3** shows a diagram of CIP4a and CIP4h alternatively-spliced forms that are expressed in cardiac myocytes. CIP4h contains a 55 amino acid insert (4h) after the F-BAR domain. Binding partners are indicated next to their respective domains. The four CIP4a and CIP4h yeast 2-hybrid clones isolated with the CaNβ-PP bait are indicated.

[0028] **Figures 4A-4C** depicts a conditional CIP4 mouse allele. **4A.** Exon 1 encodes amino acids 1-8, Exon 2 – amino acids 9-47, Exon 3 - 47-66, and Exon 4 – amino acids 66-115. LoxP and residual FRT sites were inserted by homologous recombination and subsequent Flp recombinase-mediated deletion of a neo selection cassette. Cre-mediated recombination results in deletion of exons 2 and 3 and a frameshift in exon 4, removing all but the first 8 residues of CIP4. **4B.** Western blot for cardiac CIP4 using WT and $CIP4^{fl/fl}$;Tg(CMV-cre) KO mice. **4C.** Western blot for CIP4 in hearts from CIP4 CKO and Tg(Myh6-cre/Esr1*) control mice 3 weeks after tamoxifen administration.

[0029] **Figure 5** shows that CIP4 CKO attenuates transverse aortic constriction (TAC) induced hypertrophy. Tamoxifen-treated mice were subjected to 2 weeks of pressure overload by TAC or sham operation. *vs. Sham-operated mice, †vs. Tg(Myh6-cre/Esr1*) control mice (labeled as MCM+Tam); ‡vs. $CIP4^{fl/fl}$ control mice (labeled as FF+Tam). Increased lung weight is a sign of heart failure and was less in the CIP4 CKO mice. $n \geq 10$ for all cohorts.

[0030] **Figures 6A-6C** show that CIP4 and CaNAβ form a complex in cardiac myocytes. **6A.** Neonatal myocytes were infected with adenovirus expressing HA-tagged CaNAβ, CaNAα, or β-galactosidase, and protein complexes were immunoprecipitated using a CIP4 antibody or IgG control. **6B.** Extracts from COS-7 cells expressing GFP-CaNAβ and either wildtype myc-CIP4h or myc-CIP4h ΔSH3 were used for immunoprecipitation with α-GFP antibodies, showing that the SH3 domain of CIP4h is required for CaNAβ binding. **6C.** Cultured adult rat myocytes were infected with adenovirus expressing either GFP or GFP tagged-CaNAβ PP peptide. The next day PLA (Sigma Duolink kit) was performed using rabbit anti-CaNAβ antibody and either

mouse anti-CIP4 antibody or control mouse antibody. In PLA, discrete fluorescent spots represent the detection of single, endogenous protein-protein interaction events.

[0031] **Figure 7** shows CIP4 localization in myocytes. Adult rat myocytes cultured for 1 day +/- adenovirus expressing myc-CIP4h were stained as indicated and imaged by confocal microscopy. Note that the top panels show endogenous CIP4.

[0032] **Figure 8A** shows a diagram of the cTnT shuttle vector used to make AAV gene therapy vectors. **8B** shows the myc- and his-tagged CaN Aβ, CIP4 and mAKAP peptides expressed by AAV. **8C** shows that the CaNAβ PP and CIP4 SH3 fusion proteins are expressed in the left ventricle by AAV at comparable levels to control GFP peptide, as detected by this anti-myc tag western blot of whole ventricular extracts. Note that CIP4 SH3 migrates slower in SDS-PAGE than predicted by its MW.

[0033] **Figure 9** shows how AAV9-mediated expression of the mAKAPβ CaN-binding domain (CBD) attenuates TAC-induced hypertrophy. AAV-mAKAPβ CBD (mAKAP 1286-1345) was injected in neonatal C57BL/6 mice which were subjected to 2 weeks of pressure overload starting at 8 weeks of age before echocardiography and heart isolation. *vs. sham-operated mice; †vs. GFP control mice. $n > 16$ for TAC and > 7 for sham cohorts. Decreased End systolic LV Volume is an established marker for improved cardiac function in developing heart failure.

[0034] **Figure 10** shows that expression of CaNAβ PP and CIP4 SH3 peptides by AAV blocks CIP4-CaNAβ binding *in vivo*. Viruses were injected into neonatal C57BL/6 mice, and myocytes were isolated at 4 months of age. Proximity ligation assay (Sigma Duolink kit) was performed using rabbit anti-CaNAβ antibody and either mouse anti-CIP4 antibody or control mouse antibody. *vs. AAV-GFP injected mice; †vs. control. In PLA, discrete fluorescent spots represent the detection of single protein-protein interaction events. In fluorescent images: PLA - red, GFP - green, and Hoechst nuclear stain – blue.

[0035] **Figure 11** shows that AAV-CaNAβ PP-GFP attenuates pressure overload-induced cardiac hypertrophy, improving cardiac function. Virus was injected into neonatal C57BL/6 mice that at 2 months of age were subjected to 2 weeks of pressure overload before M-mode echocardiography (fractional shortening) and post-mortem gravimetric analysis (biventricular and atrial weights). *vs. sham-operated mice; † vs. GFP. n = 13-31.

DETAILED DESCRIPTION OF THE INVENTION

[0036] The present disclosure provides compositions and methods for treating or preventing a calcineurin Aβ-mediated disease process. The methods of the disclosure are enabled by the discovery, disclosed herein, of a method of anchoring (localization) for calcineurin Aβ involving binding of its unique N-terminal peptide. It is shown herein that this peptide, when separately expressed, delocalizes a phosphatase and improves cardiac function and structure in the face of pressure overload. Thus, calcineurin Aβ anchoring disruption via its N-terminus is contemplated herein to be useful as a general method for selectively inhibiting calcineurin Aβ function, either *in vitro* or *in vivo*. Because calcineurin Aβ is implicated in the regulation of multiple disease processes, it is contemplated that the anchoring disruptor compositions and methods disclosed herein are broadly useful for methods of calcineurin-based therapy.

[0037] For example, in addition to its role in mediating T cell Receptor (TCR) signals essential for T cell activation, calcineurin Aβ is also required for the homeostatic survival of naive T cells [Manicassamy et al., J Immunol. 180(1): 106-12 (2008)]. Calcineurin also plays a role in T cell regulation, which provides for therapeutic uses for treating, *e.g.*, inflammation and cancer [Bommireddy et al., Clin Exp Immunol. 158(3): 317-24 (2009)]. Calcineurin also mediates the immune response by regulating T cell development and activation [Bueno et al., Proc. Natl. Acad. Sci. USA 99: 9398-9403 (2002)].

[0038] Calcineurin Aβ has also been shown to be significantly up-regulated in pyramidal neurons of the hippocampus the brain of those suffering from Alzheimer's Disease (AD), indicating that calcineurin Aβ plays a crucial role in the pathophysiological mechanisms in AD [Hata et al., Biochem Biophys Res Commun. 284(2): 310-6 (2001)].

[0039] Calcineurin (also known as protein phosphatase 2B (PP2B)) has also been shown to limit platelet response to vascular injury [Khatlani et al., J Thromb Haemost. 12(12): 2089-101 (2014)]; thus, inhibiting calcineurin Aβ function via the calcineurin Aβ anchoring disruption methods disclosed herein is contemplated to be useful as a thrombotic therapy.

[0040] Calcineurin is also activated in diabetes and is required for glomerular hypertrophy and ECM accumulation [Williams et al., J Biol Chem. 289(8): 4896-905 (2014); Reddy et al., Am. J. Physiol. Renal Physiol. 284, F144–F154 (2011)]. It has also been shown that acinar cell calcineurin is activated in response to Ca^{2+} generated by bile acid exposure, and that bile acid-

induced pancreatic injury is dependent on calcineurin activation [Muili et al., J Biol Chem. 288(1): 570-80 (2013)]; thus, inhibiting calcineurin via the calcineurin Aβ anchoring disruption methods disclosed herein provides an adjunctive therapy for biliary pancreatitis.

[0041] Still further, it is known that calcineurin is a Ca^{2+}/calmodulin-dependent serine/threonine phosphatase that regulates differentiation-specific gene expression in diverse tissues, including the control of fiber-type switching in skeletal muscle [Parsons et al., J Biol Chem. 282(13): 10068-78 (2007)]. Thus, inhibiting calcineurin via the calcineurin Aβ anchoring disruption methods disclosed herein provides a benefit for, *e.g.*, muscular dystrophies including limb-girdle muscular dystrophy type 2F (delta-sarcoglycanopathy).

[0042] Heart failure, the common end-stage for cardiac disease, is a syndrome of major public health significance. Approximately 825,000 American adults are diagnosed annually with heart failure, such that 5.1 million are affected and 1 in 9 will ultimately die in heart failure [Go *et al.*, Circulation. 129(3): e28-e292 (2014)]. Despite modern therapy, 5-year mortality for heart failure remains about 50%. As a result, the discovery of new candidate drug targets remains an area of pressing concern, including those that might block pathological remodeling. Myocyte hypertrophy is the major intrinsic compensatory mechanism for chronic stress on the heart. In pathologic conditions, however, myocyte hypertrophy is typically accompanied by alterations in myocyte contractility and energy metabolism, the progressive loss of myocytes and the appearance of interstitial fibrosis that contribute to the development of heart failure. Within the individual myocyte, these changes are controlled by a network of mitogen-activated protein kinase (MAPK), cyclic nucleotide, Ca^{2+} and phosphoinositide-dependent intracellular signaling pathways [Heineke *et al.*, Nat Rev Mol Cell Biol. 7(8): 589-600 (2006); van Berlo *et al.*, J Clin Invest. 123(1): 37-45 (2013)]. Although progress has been made in defining the components of this network, it remains unclear how the member pathways act in concert to regulate overall cellular phenotype [Clerk *et al.*, J Cell Physiol. 212(2): 311-22 (2007)]. Moreover, while individual pathways may control specific cellular functions, enzymes such as calcineurin (CaN) that comprise these pathways often are pleiotropic, complicating therapeutic targeting.

[0043] The cardiac response to chronic stress involves the activation of a signal transduction network that induces myocyte hypertrophy and that in disease promotes myocardial apoptosis and interstitial fibrosis contributing to the development of heart failure [Burchfield *et al.*,

Circulation. 128(4): 388-400 (2013)]. The Ca^{2+}/calmodulin-dependent, serine/threonine phosphatase CaN plays a central role in these processes [Wilkins *et al.*, Biochem Biophys Res Commun. 322(4): 1178-91 (2004); Heineke *et al.*, J Mol Cell Cardiol. 52(1): 62-73 (2012)]. In cardiac myocytes, there are two different isoforms of the CaN catalytic subunit, Aα and Aβ, and yet only Aβ is required for myocyte hypertrophy [Bueno *et al.*, Proc Natl Acad Sci U S A. 99(7): 4586-91 (2002)]. Because CaN isoforms share a similar substrate specificity and common binding partners, it is unclear why there would be functional differences between the isoenzymes. One mechanism that confers specificity in signal transduction is the formation by scaffold proteins of localized multimolecular complexes containing select combinations of enzymes, their upstream activators and effector substrates. It is disclosed herein that by serving as a CaNAβ scaffold, Cdc42-interacting protein 4 (CIP4) contributes to the regulation of cardiac myocyte hypertrophy. This is due to the co-localization of CaNAβ by CIP4 with upstream receptors, ion channels and/or other signaling molecules that specifically confer CaNAβ function in pathologic remodeling.

Calcineurin

[0044] CaN is a heterodimer of catalytic (CaNA) and regulatory (CaNB) subunits. In mammals, there are three CaNA (α, β, and γ) and two CaNB isoforms (1 and 2). CaN has well-characterized binding partners that contact discrete domains on both subunits (Figure 2). Substrates such as NFATc and scaffold proteins such as AKAP79/150 that contain PxIxIT consensus sequences compete for a docking site on the CaNA catalytic core away from the active site [Li *et al.*, J Mol Biol. 369(5): 1296-306 (2007); Takeuchi *et al.*, Structure 15(5): 587-97 (2007); Martinez-Martinez et al., Current medicinal chemistry. 11(8): 997-1007 (2004)]. Via a second CaN-binding motif (LxVP), NFATc also contacts a pocket on Ca^{2+}/CaM-activated CaN located at the interface of the two CaN subunits, a site that is shared with immunophilin complexes [Rodriguez *et al.*, Mol Cell. 33(5): 616-26 (2009); Kissinger *et al.*, Nature 378(6557): 641-4 (1995)]. Targeting of the CaN LxVP site, but not the PxIxIT site, was recently shown to be anti-inflammatory [Escolano *et al.*, EMBO J. 33(10): 1117-33 (2014)]. Other proteins bind C-terminal domains of CaNA (*e.g.*, L-type Ca2+ channels [L_{ca}] and NCX1) and CaNB (*e.g.*, ASK1) [Liu *et al.*, Mol Cell Biol. 26(10): 3785-97 (2006); Katanosaka *et al.*, J Biol Chem. 280(7): 5764-72 (2005); Tandan *et al.*, Circ Res. 105(1): 51-60 (2009)].

[0045] CaN-dependent pathways are important for the induction of pathological remodeling in heart disease, including through the regulation of NFATc- and MEF2-dependent gene expression [Wilkins *et al.*, Circ Res. 94(1): 110-8 (2004); Kim *et al.*, J Clin Invest. 118(1): 124-32 (2008)]. Although CaNAα and CaNAβ are present at similar protein levels in the heart, the two isoforms appear to be differentially active *in vivo* [Bueno *et al.*, Proc Natl Acad Sci U S A. 99(7): 4586-91 (2002)]. Remarkably, sole CaNAβ knock-out attenuated the cardiac hypertrophy induced by pressure overload, angiotensin II (Ang II) and isoproterenol (Iso) infusion [Bueno *et al.*, Proc Natl Acad Sci U S A. 99(7): 4586-91 (2002)]. CaNAβ was also required for NFATc transcription factor activity in the heart [Bueno et al., Circ Res. 94(1): 91-9 (2004)]. These requirements for CaNAβ were despite the fact that the catalytic efficiencies for most substrates including NFATc are similar for CaN Aα and Aβ [Kilka *et al.*, Biochemistry 48(9): 1900-10 (2009)]. In addition, the CaN-binding proteins described above contact sites conserved in the two CaNA isoforms. The unique functions of CaNAβ in the heart are attributed to its binding to specific scaffold proteins. In order to identify CaNAβ-specific binding partners, a yeast 2-hybrid screen was performed using the CaNAβ-specific, N-terminal PP domain as bait and a human heart cDNA library (see Example 3). Of the greater than 900 known human proteins containing EVH1, GYF, profilin, WW and SH3 domains that bind PP sequences, cDNAs for only 11 proteins, including 4 clones for the SH3 domain protein CIP4, were isolated (Figure 3).

[0046] CaN is a problematic therapeutic target due to its important role in many organ systems, and it is impractical due to immunosuppression to use the immunophilin CaN inhibitors (*e.g.*, cyclosporin A) to treat heart disease. In addition, complete ablation of all CaN in the adult heart via CaN B1-subunit gene deletion depresses cardiac contractility, resulting in excess mortality both following stress and in unstressed mice [Maillet *et al.*, J Biol Chem. 285(9): 6716-24 (2010)]. CaNAβ itself is required for myocyte survival following ischemia-reperfusion and in a genetic model of dilated cardiomyopathy [Bueno *et al.*, Circ Res. 94(1): 91-9 (2004); Heineke *et al.*, J Mol Cell Cardiol. 48(6): 1080-7 (2010)]. The disruption of unique protein-protein interactions is an alternative strategy permitting the selective inhibition of pathological cellular processes, including those regulated by CaNAβ [Negro *et al.*, Prog Pediatr Cardiol. 25(1): 51-6 (2008)].

[0047] The main structural difference between CaN Aα and Aβ is the presence of a unique 23 amino acid, N-terminal proline-rich domain in CaNAβ. Since polyproline (PP) sequences often

mediate protein-protein interactions, a screen for binding partners for the CaNAβ PP domain was performed. One protein that bound CaNAβ specifically is CIP4 (Cdc42-interacting protein 4), a member of the F-BAR (Fer-CIP4 homology [**F**CH] – **B**in/**A**mphiphysin/**R**vs) family of membrane-associated proteins.

CIP4

[0048] CIP4 contains a N-terminal F-BAR domain that binds both cytoskeletal proteins and membrane phospholipids, a HR1 domain that binds Rho family small GTPases, and a C-terminal SH3 (SRC Homology 3) domain that binds CaNAβ and other regulatory proteins (Figure 1) [Suetsugu, J Biochem. 148(1): 1-12 (2010)]. The co-localization of CIP4 and CaNAβ in the myocyte is demonstrated herein and CIP4 localization is regulated by growth factor and G-protein coupled receptor (GPCR) stimulation and by Rho family GTPases, as in other cell types [Toguchi *et al.*, Biol Cell. 102(4): 215-30 (2010)].

[0049] CIP4 is a modular scaffold protein involved in the regulation of cellular morphology that can serve as an effector for the Rho family small GTPases Cdc42, TC10, and TCL [Suetsugu, J Biochem. 148(1):1-12 (2010)]. CIP4 contains a N-terminal F-BAR domain that binds both cytoskeletal proteins and negatively-charged membrane phospholipids, including phosphatidylinositol (3,4,5) trisphosphate. The F-BAR domain mediates CIP4 oligomerization at membrane surfaces, inducing a shallow curvature to the membrane [Daumke *et al.*, Cell 156(5): 882-92 (2014)]. CIP4 also has a HR1 domain that binds active, GTP-bound Rho family members and a C-terminal SH3 (Src Homology 3) domain that binds a variety of proteins involved in the control of the actin cytoskeleton and small GTPase signaling (Figure 3) [Saengsawang *et al.*, J Cell Sci. 126(Pt 11): 2411-23 (2013)]. CIP4 functions described in non-cardiac cell types include regulation of membrane tubulation, epidermal growth factor (EGF) and platelet-derived growth receptor endocytosis, vesicle trafficking, and dynamic remodeling of the actin cytoskeleton [Toguchi *et al.*, Biol Cell. 102(4): 215-30 (2010); Feng *et al.*, J Biol Chem. 285(7): 4348-54 (2010); Koduru *et al.*, Proc Natl Acad Sci U S A. 107(37): 16252-6 (2010); Hu *et al.*, Cell Signal 21(11): 1686-97 (2009)]. CIP4 also regulates filopodial and lamellipodial protrusion, affecting the invasiveness and metastasis of cancer cells and neurite extension in neurons [Saengsawang *et al.*, J Cell Sci. 126(Pt 11): 2411-23 (2013); Hu *et al.*, J Cell Sci. 124(Pt 10): 1739-51 (2011)]. Constitutive, global CIP4 knock-out mice have been described that were

overall normal in appearance and fertile, but that exhibited defects in skeletal muscle glucose transport and T-cell function and thrombocytopenia [Feng *et al.*, J Biol Chem. 285(7): 4348-54 (2010); Koduru *et al.*, Proc Natl Acad Sci U.S.A. 107(37): 16252-6 (2010); Chen *et al.*, Blood 122(10): 1695-706 (2013)].

Scaffold Proteins

[0050] The formation of multimolecular enzyme complexes by scaffold proteins is an important mechanism responsible for specificity in intracellular signal transduction [Scott *et al.*, Science 326(5957): 1220-4 (2009)]. Many signaling enzymes have broad substrate specificity. The co-localization of an enzyme with its substrate by a scaffold protein can selectively enhance the post-translational modification of that substrate, providing a degree of specificity that may not be intrinsic to the enzyme's active site [Scott *et al.*, Science 326(5957): 1220-4 (2009)]. For example, specificity is generally conferred to the PP1 and PP2A serine/threonine protein phosphatases by the binding of scaffolding or regulatory subunits [Virshup *et al.*, Mol Cell. 33(5): 537-45 (2009)]. In addition, since many scaffolds are multivalent, scaffold binding can orchestrate the co-regulation of a phosphatase substrate by additional enzymes responsible for determining effector function. As the organizers of "nodes" in the intracellular signaling network, these scaffold proteins may be of interest as potential therapeutic targets [Negro *et al.*, Prog Pediatr Cardiol. 25(1): 51-6 (2008)].

Disruption of CaNAβ Anchoring

[0051] Anchoring proteins are therapeutic targets for the treatment of cardiac hypertrophy and heart failure. In particular, it is disclosed herein that the multivalent scaffold protein CIP4 is important for the induction of pathological hypertrophy by the phosphatase calcineurin Aβ (CaNAβ).

[0052] It is also disclosed herein that the unique role of CaNAβ in pathological myocyte hypertrophy is due to CaNAβ anchoring through its PP domain. CIP4-CaNAβ complexes are identified herein as a therapeutic target for the prevention of pathological cardiac hypertrophy and heart failure.

[0053] Accordingly, in some aspects the disclosure provides a method of treating or preventing heart disease comprising administering to a patient at risk of or suffering from heart disease a pharmaceutically effective amount of a composition that inhibits binding of calcineurin

Aβ (CaNAβ) amino-terminal polyproline domain to a scaffold protein. In some embodiments, the scaffold protein is profilin-1, profilin-2, sorbin, formin binding protein 11 (FBP11), or TOCA-1. In further aspects, a method of treating or preventing heart disease is provided comprising administering to a patient at risk of or suffering from heart disease a pharmaceutically effective amount of a composition that inhibits Cdc42-interacting protein 4 (CIP4).

[0054] In particular embodiments, these inhibiting compositions or "inhibitors" include peptide inhibitors, which can be administered by any known method, including by gene therapy delivery. In further embodiments, the inhibitors are small molecule inhibitors.

[0055] In various embodiments, the composition comprises a calcineurin Aβ (CaNAβ) peptide. In further embodiments, the CaNAβ peptide comprises a sequence from the amino-terminus polyproline domain of the CaNAβ amino acid sequence (SEQ ID NO: 1). In some embodiments, the CaNAβ peptide is amino acids 1-27 of SEQ ID NO: 1. The disclosure also contemplates use of the corresponding calcineurin Aβ mouse amino acid sequence (SEQ ID NO: 4) or a fragment thereof (*e.g.*, in some embodiments the peptide fragment is amino acids 1-27 of SEQ ID NO: 4).

[0056] In further embodiments, the composition comprises a Cdc42-interacting protein 4 (CIP4) peptide which, in some embodiments, comprises a sequence from the SRC Homology 3 (SH3) domain of the CIP4 amino acid sequence (SEQ ID NO: 2). In further embodiments, the CIP4 peptide is amino acids 457-545 of SEQ ID NO: 2.

[0057] Delivery of peptides that inhibit CaNAβ-scaffold protein interaction can be enhanced by the use of cell-penetrating sequences such as the transactivator of transcription peptide and polyarginine tails, or conjugation with lipid-derived groups such as stearate. Stability may also be enhanced by the use of peptidomimetics (*i.e.*, peptides with structural modifications in the original sequence giving protection against exo- and endoproteases without affecting the structural and functional properties of the peptide). Alternatively, as described herein, peptides can be delivered by intracellular expression via viral-based gene therapy vectors, such as, without limitation, AAV vectors.

[0058] The present disclosure relates to methods of treating any cardiac condition, which is initiated through the interaction of a scaffold protein and CaNAβ. Such cardiac dysfunction can

result in signs and symptoms such as shortness of breath and fatigue, and can have various causes, including, but not limited to hypertension, coronary artery disease, myocardial infarction, valvular disease, primary cardiomyopathy, congenital heart disease, arrhythmia, pulmonary disease, diabetes, anemia, hyperthyroidism and other systemic diseases.

[0059] It should be appreciated that various amino acid substitutions, deletions or insertions with a peptide sequence is permitted in the context of the disclosure and such modifications optionally enhance the ability of an inhibiting peptide of the disclosure to inhibit the interaction of a scaffold protein and CaNAβ. A substitution mutation of this sort can be made to change an amino acid in the resulting protein in a non-conservative manner (*i.e.*, by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to another grouping) or in a conservative manner (*i.e.*, by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to the same grouping). The present disclosure optionally includes sequences containing conservative changes, which do not significantly alter the activity, or binding characteristics, of the resulting protein.

[0060] The following is one example of various groupings of amino acids:

[0061] Amino Acids with Nonpolar R Groups: Alanine, Valine, Leucine, Isoleucine, Proline, Phenylalanine, Tryptophan, Methionine.

[0062] Amino Acids with Uncharged Polar R Groups: Glycine, Serine, Threonine, Cysteine, Tyrosine, Asparagine, Glutamine.

[0063] Amino Acids with Charged Polar R Groups (negatively charged at pH 6.0): Aspartic acid, Glutamic acid.

[0064] Basic Amino Acids (positively charged at pH 6.0): Lysine, Arginine, Histidine (at pH 6.0).

[0065] Another grouping may be those amino acids with phenyl groups: Phenylalanine, Tryptophan, Tyrosine.

[0066] Another grouping may be according to molecular weight (*i.e.*, size of R groups): Glycine (75), Alanine (89), Serine (105), Proline (115), Valine (117), Threonine (119), Cysteine (121), Leucine (131), Isoleucine (131), Asparagine (132), Aspartic acid (133), Glutamine (146),

Lysine (146), Glutamic acid (147), Methionine (149), Histidine (at pH 6.0) (155), Phenylalanine (165), Arginine (174), Tyrosine (181), Tryptophan (204).

[0067] Contemplated substitutions include: Lys for Arg and vice versa such that a positive charge may be maintained; Glu for Asp and vice versa such that a negative charge may be maintained; Ser for Thr such that a free --OH can be maintained; and Gln for Asn such that a free NH_2 can be maintained.

[0068] Amino acid substitutions may also be introduced to substitute an amino acid with a particularly preferable property. For example, a Cys may be introduced a potential site for disulfide bridges with another Cys. A His may be introduced as a particularly "catalytic" site (*i.e.*, His can act as an acid or base and is the most common amino acid in biochemical catalysis). Pro may be introduced because of its particularly planar structure, which induces-turns in the protein's structure. Two amino acid sequences are "substantially identical" when at least about 70% of the amino acid residues (preferably at least about 80%, and most preferably at least about 90 or 95%) are identical, or represent conservative substitutions.

[0069] Likewise, nucleotide sequences utilized in accordance with the disclosure can also be subjected to substitution, deletion or insertion. Where codons encoding a particular amino acid are degenerate, any codon which codes for a particular amino acid may be used. In addition, where it is desired to substitute one amino acid for another, one can modify the nucleotide sequence according to the known genetic code.

[0070] Two nucleotide sequences are "substantially identical" when at least about 70% of the nucleotides (preferably at least about 80%, and most preferably at least about 90 or 95%) are identical.

[0071] The term "standard hybridization conditions" refers to salt and temperature conditions substantially equivalent to 5XSSC and 65° C for both hybridization and wash. However, one skilled in the art will appreciate that such "standard hybridization conditions" are dependent on particular conditions including the concentration of sodium and magnesium in the buffer, nucleotide sequence length and concentration, percent mismatch, and percent formamide. Also important in the determination of "standard hybridization conditions" is whether the two sequences hybridizing are RNA-RNA, DNA-DNA or RNA-DNA. Such standard hybridization conditions are easily determined by one skilled in the art according to well-known formulae,

wherein hybridization is typically 10-20° C below the predicted or determined T_m with washes of higher stringency, if desired.

[0072] The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable when administered to a human.

[0073] The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to prevent or treat, and preferably reduce by at least about 5%, or at least 10%, or at least 20%, or at least 30%, or at least 50%, or at least 90%, a clinically significant change in a cardiac myocyte feature.

[0074] The preparation of therapeutic compositions which contain polypeptides, analogs, or active fragments as active ingredients is well understood in the art. As used herein, an "analog" refers to a polypeptide substantially similar in structure and having the same or essentially the same biological activity, albeit in certain instances to a differing degree, to a naturally-occurring molecule. Analogs differ in the composition of their amino acid sequences compared to the naturally-occurring polypeptide from which the analog is derived, typically by way of insertion, deletion, or substitution of amino acid(s) as described herein.

[0075] Typically, such therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

[0076] A polypeptide, analog, or active fragment, as well as a small molecule inhibitor, can be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example and without limitation,

sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, and histidine.

[0077] The therapeutic compositions of the disclosure are conventionally administered intravenously, as by injection of a unit dose, for example. The term "unit dose" when used in reference to a therapeutic composition of the present disclosure refers to physically discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent, *i.e.*, carrier, or vehicle.

[0078] The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to utilize the active ingredient, and degree of inhibition of a scaffold protein-CaNAβ binding desired. However, suitable dosages may range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations of ten nanomolar to ten micromolar in the blood are contemplated.

[0079] In various embodiments, it is necessary for the inhibitor to reach the cytosol. Accordingly, a peptide in accordance with the disclosure is, in some embodiments, modified in order to allow its transfer across cell membranes, or expressed by a vector which encodes the peptide inhibitor. Likewise, a nucleic acid inhibitor (including but not limited to siRNAs and antisense nucleic acids) can be expressed by a vector. Any vector capable of entering the cells to be targeted may be used in accordance with the invention. In particular, viral vectors are able to "infect" the cell and express the desired nucleic acid (*e.g.*, RNA) or peptide. Any viral vector capable of "infecting" the cell may be used. In some embodiments, the viral vector is adeno-associated virus (AAV).

[0080] With respect to small molecule inhibitors, any small molecule that inhibits the interaction of CaNAβ and a scaffold protein may be used. In addition, any small molecule that inhibits the activity of CIP4 may be used.

EXAMPLES

[0081] Using an adeno-associated virus gene therapy vector that is genetically engineered to expressing the calcineurin-binding domain of mAKAP in only heart myocytes, data was acquired showing that this method will block the development of heart failure in mice (Figure 9). Others have expressed calcineurin binding peptides in mice before blocking hypertrophy [DeWindt *et al.*, Proc Natl Acad Sci USA. 98(6): 3322-7 (2001)], but these peptides appear to target a different domain of calcineurin, have different effects on calcineurin phosphatase activity, and may have more broad effects. In addition, the present disclosure demonstrates delivery of AAV to selectively target these peptides to the heart.

[0082] The present disclosure makes use of several technologies. First, a new conditional CIP4 mouse allele is described that allows for the testing of myocyte autonomous function of an F-BAR protein in the heart. Second, AAV is employed to express in myocytes new anchoring disruptor peptides that inhibit endogenous CIP4-CaNAβ binding *in vivo*. This technology was not only used to test whether CaNAβ anchoring through its PP domain is functionally significant for pathological remodeling, but also provided proof-of-concept for a new therapeutic strategy, as AAV gene therapy is now established as a safe approach in human patients [Jessup *et al.*, Circulation. 124(3): 304-13 (2011)].

Example 1

[0083] CIP4 is a known regulator of cellular structure, including the actin cytoskeleton [Saengsawang *et al.*, J Cell Sci. 126(Pt 11): 2411-23 (2013)]. In order to study CIP4 *in vivo*, a new CIP4 conditional mouse allele was generated (Figure 4). $CIP4^{fl}$ mice have been crossed with two cre recombinase deleter mice: Tg(CMV-cre) conferring global, constitutive knock-out and Tg(Myh6-cre/Esr1*) conferring tamoxifen-inducible, cardiac myocyte-specific knock-out via expression of an estrogen receptor-cre fusion protein [Sohal *et al.*, Circ Res. 89(1): 20-5 (2001)]. All mice are C57BL/6. No CIP4 is detectable by western blot in hearts isolated from $CIP4^{fl/fl}$;Tg(CMV-cre) global knock-out mice (Figure 4B). To induce conditional knock-out (CIP CKO), $CIP4^{fl/fl}$;Tg(Myh6-cre/Esr1*) mice were fed tamoxifen-containing chow (125 mg/kg

chow to provide ~20 mg tamoxifen/kg body weight/day, Harlan Teklad) for one week. CIP4h protein expression is diminished >95% in the hearts of CIP4 CKO mice, while the expression of CIP4a is not significantly reduced (Figure 4C). These data show that although both isoforms are expressed in neonatal (and potentially adult) myocytes, CIP4h is expressed only in the cardiac myocytes of the adult heart.

[0084] Based on preliminary data regarding CIP4 in neonatal myocytes hypertrophy [Rusconi *et al.*, J Biomed Sci. 20: 56 (2013)], the relevance of CIP4 to pathological cardiac remodeling in the adult heart *in vivo* was established. In particular, whether CIP4 was required for the induction of cardiac remodeling in response to surgically-induced pressure overload (transverse aortic constriction, TAC) was tested. Mice subjected to two weeks of pressure overload by TAC survival surgery revealed that CIP4 CKO attenuated cardiac hypertrophy (increase in indexed biventricular weight over Sham-operated mice) when compared to two different control cohorts: tamoxifen-treated Tg(Myh6-cre/Esr1*) control mice and tamoxifen-treated $CIP4^{fl/fl}$ control mice (Figure 5). Left atrial hypertrophy (a sign of diastolic dysfunction) was also significantly attenuated (data not shown, $p < 0.001$), while wet lung weight (an indicator of heart failure) was less for the CIP4 CKO mice ($p < 0.001$ vs. tamoxifen-treated Tg(Myh6-cre/Esr1*) control mice and $p = 0.08$ vs. tamoxifen-treated $CIP4^{fl/fl}$ control mice, Figure 5). Notably, no significant differences were detected between sham-operated CIP4 CKO and control mice.

Example 2

Mechanisms Underlying CIP4-CaNAβ Signaling in Myocytes

[0085] CIP4 was identified in a yeast 2-hybrid screen using the CaNAβ-specific N-terminal PP domain as bait (Figures 2 and 3). To show that CIP4 binds CaNAβ in cardiac myocytes, myocytes were infected with adenovirus expressing HA-tagged CaNA proteins. HA-CaNAβ, but not HA-CaNAβ, was immunoprecipitated with the CIP4 antibody (Figure 6A). CIP4h binding to CaNAβ required the CIP4h SH3 domain (Figure 6B). The association of endogenous CaNAβ and CIP4 in adult rat myocytes was confirmed by detection of endogenous protein complexes in situ by proximity ligation assay (PLA, Figure 6C) [Soderberg *et al.*, Nat Methods 3(12): 995-1000 (2006)]. Importantly, adenoviral-mediated expression of a CaNAβ PP – GFP fusion protein in adult myocytes successfully competed endogenous CaNAβ-CIP4 binding.

[0086] Following expression by adenoviral infection in neonatal myocytes, myc-CIP4h was expressed throughout the cell in a reticular/punctate pattern that did not coincide with Z-line, α-actinin antibody staining [Rusconi *et al.*, J Biomed Sci. 20: 56 (2013)]. A qualitatively similar pattern was observed in adult rat ventricular myocytes using a rabbit anti-CIP4 antibody to detect endogenous CIP4, as well as using a myc antibody to stain adult myocytes infected with an adenovirus that expresses myc-tagged CIP4h (Figure 7).

[0087] **Rationale**: Without being bound to a particular theory, it is believed that CIP4 binds CaNAβ conferring specificity to CaNAβ function by organizing a compartment specialized for the regulation of hypertrophy. CaNAβ is required *in vivo* for both the morphologic changes in cardiac hypertrophy (increased myocyte size) and the increased expression of hypertrophy-associated genes [Bueno *et al.*, Proc Natl Acad Sci U.S.A. 99(7): 4586-91 (2002)]. CIP4 was also found to be required for cardiac hypertrophy *in vivo* (Figure 5).

Example 3

Therapeutic Disruption of CaNAβ Anchoring *in vivo*

[0088] The use of both immunophilin CaN inhibitors and CaNAβ knock-out mice has established a requirement for CaN activity in pathological cardiac hypertrophy [Wilkins *et al.*, Biochem Biophys Res Commun. 322(4): 1178-91 (2004)]. Hypertrophy has also been inhibited by transgenic expression of CaN binding domains from AKAP79 and CAIN that compete for the allosteric CaNA PxIxIT site [De Windt *et al.*, Proc Natl Acad Sci U S A. 98(6): 3322-7 (2001); Martinez-Martinez *et al.*, Proc Natl Acad Sci U S A. 106(15): 6117-22 (2009)]. Nevertheless, therapeutic intervention in humans based on CaN inhibition has remained elusive due to CaN pleiotropism. In order to overcome the limitations of conventional approaches for CaN-targeted interventions, the use of AAV gene therapy vectors to deliver to the myocyte anchoring disruptor peptides has been developed that will inhibit the binding of CaN to scaffold proteins (Figure 8). An AAV serotype 9 that is cardiotropic [Prasad *et al.*, Gene Ther. 18(1): 43-52 (2011)] is used herein. In addition, the viruses are engineered to direct mini-gene expression under the control of the cardiac myocyte-specific, cardiac troponin T (cTNT) promoter [Prasad *et al.*, Gene Ther. 18(1): 43-52 (2011)]. Thus, the anchoring disruptor peptides are expressed in a highly cell-type selective manner.

[0089] Data regarding CIP4-CaNAβ binding show that the CaNAβ PP domain unique to that phosphatase isoform is a novel scaffold docking domain. The yeast 2-hybrid screen identifying CIP4 as a CaNAβ scaffold resulted in the isolation of a remarkably small set of PP-binding proteins that almost exclusively have been implicated in control of the cytoskeleton. Besides the CIP4 clones, we isolated multiple cDNAs for profilin-1, profilin-2, sorbin and SH3 domain-containing protein 2 (sorbin), and formin binding protein 11 (FBP11) were isolated, as well as a single clone for the F-BAR protein TOCA-1. The relevance of CaNAβ PP domain-based anchoring was tested *in vivo* via AAV-mediated expression of anchoring disruptor peptide – GFP fusion proteins (Figure 8). Using proximity ligation assay to detect endogenous CIP4-CaNAβ complexes *in situ*, the ability of both CaNAβ PP-GFP and GFP-CIP4 SH3 to block CIP4-CaNAβ binding in adult mouse myocytes *in vivo* has been verified (Figure 10). The efficacy of AAV-CaNAβ PP-GFP in preventing remodeling has been tested, and results show that expression of the anchoring disruptor peptide will like CIP4 CKO prevent cardiac dysfunction (Figure 11).

Gravimetric analysis showed that compared to AAV-GFP controls, AAV-CaNAβ PP-GFP will attenuate cardiac hypertrophy by approximately 36% (increase in indexed biventricular weight). Importantly, cardiac contractility (fractional shortening) in the presence of pressure overload was significantly improved by the gene therapy vector (Figure 11).

[0090] In this Example, AAV is used to deliver anchoring disruptor peptides that will inhibit CaNAβ binding *in vivo* to CIP4 and any other CaNAβ PP scaffolds. AAV is also used for gene transduction due to its ease of delivery and the availability of vectors containing cardiac myocyte-specific promoters that confer cell type-specific expression [Prasad *et al.*, Gene Ther. 18(1): 43-52 (2011)]. AAV transduction has the benefit of long-term expression following a single injection and has been used successfully in projects involving the expression of recombinant proteins and siRNA in the heart [Tilemann *et al.*, Circ Res. 110(5): 777-93 (2012)]. For the studies described above, 10^{11} AAV were injected intraperitoneally into neonates, obviating concerns regarding expression latency. This dose has proven effective for CaNAβ anchoring disruption in adult mice (Figures 6C and 10). In addition, there have been no deleterious effects detected for any of the viruses in unstressed mice.

[0091] Expression driven by the cTnT promoter has proven sufficient for anchoring disruptor peptide studies (Figures 9 and 11), even though expression is presumably less than expected when using stronger promoters such as the CMV immediate early promoter [Prasad *et al.*, Gene Ther. 18(1): 43-52 (2011)]. This is reasonable especially in the case of CaNAβ PP anchoring, since inhibition of CaNAβ PP anchoring should not require targeting of all CaN in the cell and since many scaffolds are relatively low in abundance. If increased expression is desired, then it is contemplated that the dose of the virus injected is increased or that a switch is made to a CMV promoter vector. Further, self-complementary AAV vectors are also contemplated for use, which can increase fusion protein expression and decrease expression latency, potentially increasing the efficacy of anchoring disruption [Andino *et al.*, Genetic vaccines and therapy. 5: 13 (2007)]. These vectors are useful due to the small size of the encoded peptides and would be particularly useful for any reverse remodeling experiments.

[0092] Together, the experiments in this Example provide *in vivo* evidence for a mechanism conferring specificity to CaN signaling, isoform-specific anchoring of CaNAβ through its unique N-terminal PP domain. Using the anchoring disruptor peptides, inhibition of CaNAβ function in

cardiac myocytes is expected, whether anchored by CIP4 or by other scaffolds responsible for regulating the cytoskeleton. The systemic effects of CaN inhibition are avoided, including immunosuppression, as well as the inhibition of CaNAα that may serve other useful functions in the myocyte. A major advantage of the AAV system is that successful results with AAV can be rapidly translated to the human setting. Phase 2 clinical trials (CUPID and AGENT-HF) are currently underway for MYDICAR in which intracoronary gene transfer of SERCA2A via AAV is being tested for efficacy in heart failure [Jessup *et al.*, Circulation. 124(3):304-13 (2011)]. The MYDICAR studies have established AAV as a safe cardiac drug delivery system.

WHAT IS CLAIMED IS:

1. A method of treating a patient suffering from a calcineurin Aβ-mediated disorder, comprising administering to the patient a composition that inhibits binding of calcineurin Aβ (CaNAβ) amino-terminal polyproline domain to a scaffold protein.

2. The method of claim 1 wherein the scaffold protein is Cdc42-interacting protein 4 (CIP4), profilin-1, profilin-2, sorbin, formin binding protein 11 (FBP11), or TOCA-1.

3. The method of claim 1 or claim 2, wherein the composition comprises a calcineurin Aβ (CaNAβ) peptide.

4. The method of claim 3, wherein the CaNAβ peptide comprises a sequence from the amino-terminus polyproline domain of the CaNAβ amino acid sequence (SEQ ID NO: 1).

5. The method of claim 4, wherein the CaNAβ peptide is amino acids 1-27 of the CaNAβ amino acid sequence (SEQ ID NO: 1).

6. The method of any one of claims 1-5 wherein the composition comprises a Cdc42-interacting protein 4 (CIP4) peptide.

7. The method of claim 6, wherein the CIP4 peptide comprises a sequence from the SRC Homology 3 (SH3) domain of the CIP4 amino acid sequence (SEQ ID NO: 2).

8. The method of claim 7, wherein the CIP4 peptide is amino acids 457-545 of the CIP4 amino acid sequence (SEQ ID NO: 2).

9. The method of any one of claims 3-8, wherein the peptide is administered via a viral vector.

10. The method of claim 9, wherein the viral vector is adeno-associated virus (AAV).

11. The method of claim 9 or claim 10 wherein the viral vector comprises a cardiac muscle specific promoter.

12. The method of claim 11 wherein the promoter is cardiac troponin T promoter.

13. The method of any one of claims 1-12 wherein the composition comprises a small interfering RNA (siRNA) that inhibits expression of CaNAβ.

14. The method of any one of claims 1-13 wherein the composition comprises a siRNA that inhibits expression of CIP4.

15. The method of any one of claims 1-14 wherein the composition does not induce immunosuppression in the patient.

16. The method of any one of claims 1-15 wherein the calcineurin Aβ-mediated disorder involves a T-cell, a neuron, a platelet, kidney, pancreas, or skeletal muscle.

17. A method of treating or preventing heart disease comprising administering to a patient at risk of or suffering from heart disease a pharmaceutically effective amount of a composition that inhibits binding of calcineurin Aβ (CaNAβ) amino-terminal polyproline domain to a scaffold protein.

18. The method of claim 17 wherein the scaffold protein is Cdc42-interacting protein 4 (CIP4), profilin-1, profilin-2, sorbin, formin binding protein 11 (FBP11), or TOCA-1.

19. A method of treating or preventing heart disease comprising administering to a patient at risk of or suffering from heart disease a pharmaceutically effective amount of a composition that inhibits Cdc42-interacting protein 4 (CIP4).

20. The method of any one of claims 17-19, wherein the composition comprises a calcineurin Aβ (CaNAβ) peptide.

21. The method of claim 20, wherein the CaNAβ peptide comprises a sequence from the amino-terminus polyproline domain of the CaNAβ amino acid sequence (SEQ ID NO: 1).

22. The method of claim 21, wherein the CaNAβ peptide is amino acids 1-27 of SEQ ID NO: 1.

23. The method of any one of claims 17-22 wherein the composition comprises a Cdc42-interacting protein 4 (CIP4) peptide.

24. The method of claim 23, wherein the CIP4 peptide comprises a sequence from the SRC Homology 3 (SH3) domain of the CIP4 amino acid sequence (SEQ ID NO: 2).

25. The method of claim 24, wherein the CIP4 peptide is amino acids 457-545 of SEQ ID NO: 2.

26. The method of any one of claims 20-25, wherein the peptide is encoded by a polynucleotide sequence incorporated into a viral vector.

27. The method of claim 26, wherein the viral vector is adeno-associated virus (AAV).

28. The method of claim 26 or claim 27 wherein the polynucleotide sequence is operably linked to a cardiac muscle specific promoter.

29. The method of claim 28 wherein the promoter is cardiac troponin T promoter.

30. The method of any one of claims 17-29 wherein the composition comprises a small interfering RNA (siRNA) that inhibits expression of CaNAβ.

31. The method of any one of claims 17-30 wherein the composition comprises a siRNA that inhibits expression of CIP4.

32. The method of any one of claims 17-31 wherein the composition does not induce immunosuppression in the patient.

33. A method of treating heart failure, comprising administering to a patient at risk of heart failure a pharmaceutically effective amount of a composition that inhibits binding of calcineurin Aβ (CaNAβ) amino-terminus polyproline domain to a scaffold protein.

34. The method of claim 33, wherein the composition comprises a calcineurin Aβ (CaNAβ) peptide.

35. The method of claim 34, wherein the CaNAβ peptide comprises a sequence from the amino-terminus polyproline domain of the CaNAβ amino acid sequence (SEQ ID NO: 1).

36. The method of claim 35, wherein the CaNAβ peptide is amino acids 1-27 of the CaNAβ amino acid sequence (SEQ ID NO: 1).

37. The method of any one of claims 33-36 wherein the composition comprises a Cdc42-interacting protein 4 (CIP4) peptide.

38. The method of claim 37, wherein the CIP4 peptide comprises a sequence from the SRC Homology 3 (SH3) domain of the CIP4 amino acid sequence (SEQ ID NO: 2).

39. The method of claim 38, wherein the CIP4 peptide is amino acids 457-545 of the CIP4 amino acid sequence (SEQ ID NO: 2).

40. The method of any one of claims 34-39, wherein the peptide is administered via a viral vector.

41. The method of claim 40, wherein the viral vector is adeno-associated virus (AAV).

42. The method of claim 40 or claim 41 wherein the viral vector comprises a cardiac muscle specific promoter.

43. The method of claim 42 wherein the promoter is cardiac troponin T promoter.

44. The method of any one of claims 33-43 wherein the composition comprises a small interfering RNA (siRNA) that inhibits expression of CaNAβ.

45. The method of any one of claims 33-44 wherein the composition comprises a siRNA that inhibits expression of CIP4.

46. The method of any one of claims 33-45 wherein the composition does not induce immunosuppression in the patient.

47. A composition comprising a peptide comprising a sequence from the amino-terminal polyproline domain of calcineurin Aβ (CaNAβ) amino acid sequence (SEQ ID NO: 1).

48. The composition of claim 47, wherein the CaNAβ peptide is amino acids 1-27 of SEQ ID NO: 1.

49. A composition comprising a peptide comprising a sequence from the SRC Homology 3 (SH3) domain of Cdc42-interacting protein 4 (CIP4) (SEQ ID NO: 2).

50. The composition of claim 49, wherein the CIP4 peptide is amino acids 457-545 of SEQ ID NO: 2.

51. A composition comprising a viral vector comprising a polynucleotide that encodes a peptide comprising a sequence from the amino-terminal polyproline domain of calcineurin Aβ (CaNAβ) amino acid sequence (SEQ ID NO: 1).

52. The composition of claim 51, wherein the CaNAβ peptide is amino acids 1-27 of SEQ ID NO: 1.

53. A composition comprising a viral vector comprising a polynucleotide that encodes a peptide comprising a sequence from the SRC Homology 3 (SH3) domain of Cdc42-interacting protein 4 (CIP4) (SEQ ID NO: 2).

54. The composition of claim 53, wherein the CIP4 peptide is amino acids 457-545 of SEQ ID NO: 2.

55. The composition of any one of claims 51-54, wherein the viral vector is adeno-associated virus (AAV).

56. A method of treating or preventing heart disease by administering to a patient a pharmaceutically effective amount of a mAKAPβ peptide, wherein the mAKAPβ peptide is amino acids 1286-1346 of the mAKAPβ amino acid sequence (SEQ ID NO: 3).

57. The method of claim 56 wherein the mAKAPβ peptide is encoded by a polynucleotide sequence incorporated into a viral vector.

58. The method of claim 57, wherein the viral vector is adeno-associated virus (AAV).

59. The method of claim 57 or claim 58 wherein the polynucleotide sequence is operably linked to a cardiac muscle specific promoter.

60. The method of claim 59 wherein the promoter is cardiac troponin T promoter.

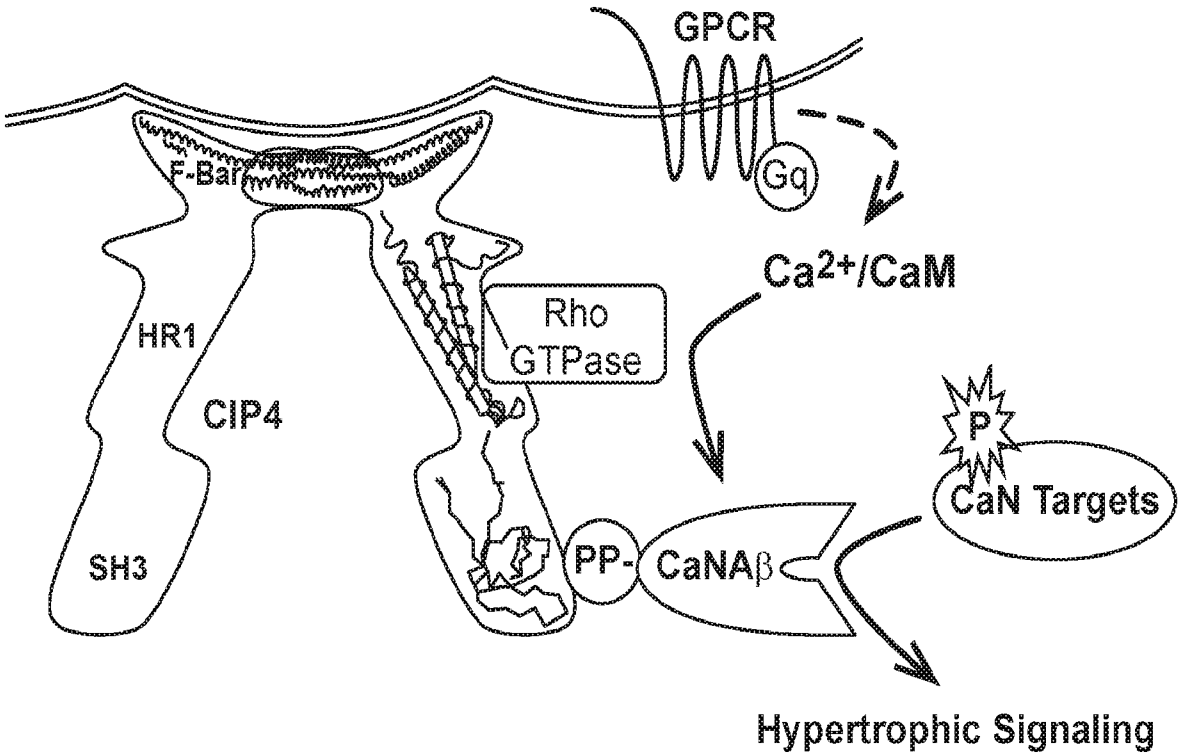

GPCR
Gq
F-Bar
HR1
CIP4
SH3
Rho
GTPase
Ca2+/CaM
P
CaN Targets
PP-
CaNAβ
Hypertrophic Signaling

FIG. 1

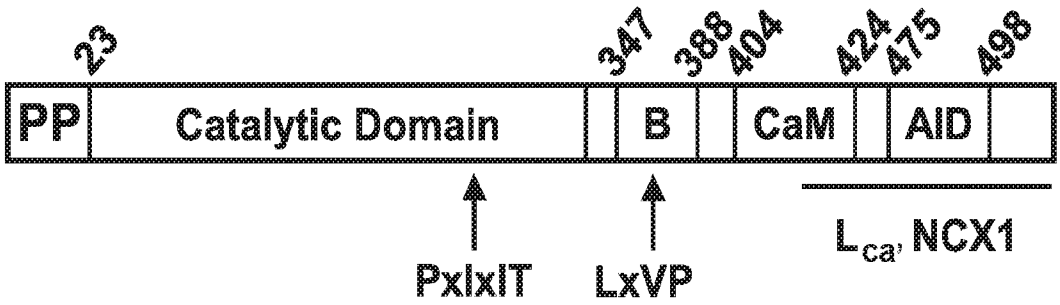

23
347
388
404
424
475
498
PP
Catalytic Domain
B
CaM
AID
PxIxIT
LxVP
L_{ca}, NCX1

FIG. 2

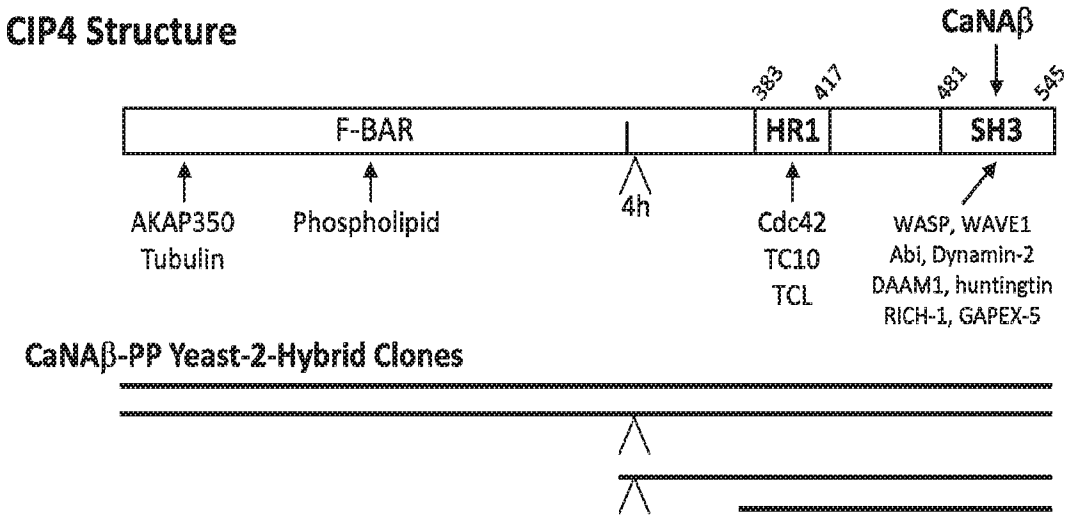

CIP4 Structure
CaNAβ
383
417
481
545
F-BAR
HR1
SH3
AKAP350
Tubulin
Phospholipid
4h
Cdc42
TC10
TCL
WASP, WAVE1
Abi, Dynamin-2
DAAM1, huntingtin
RICH-1, GAPEX-5
CaNAβ-PP Yeast-2-Hybrid Clones

FIG. 3

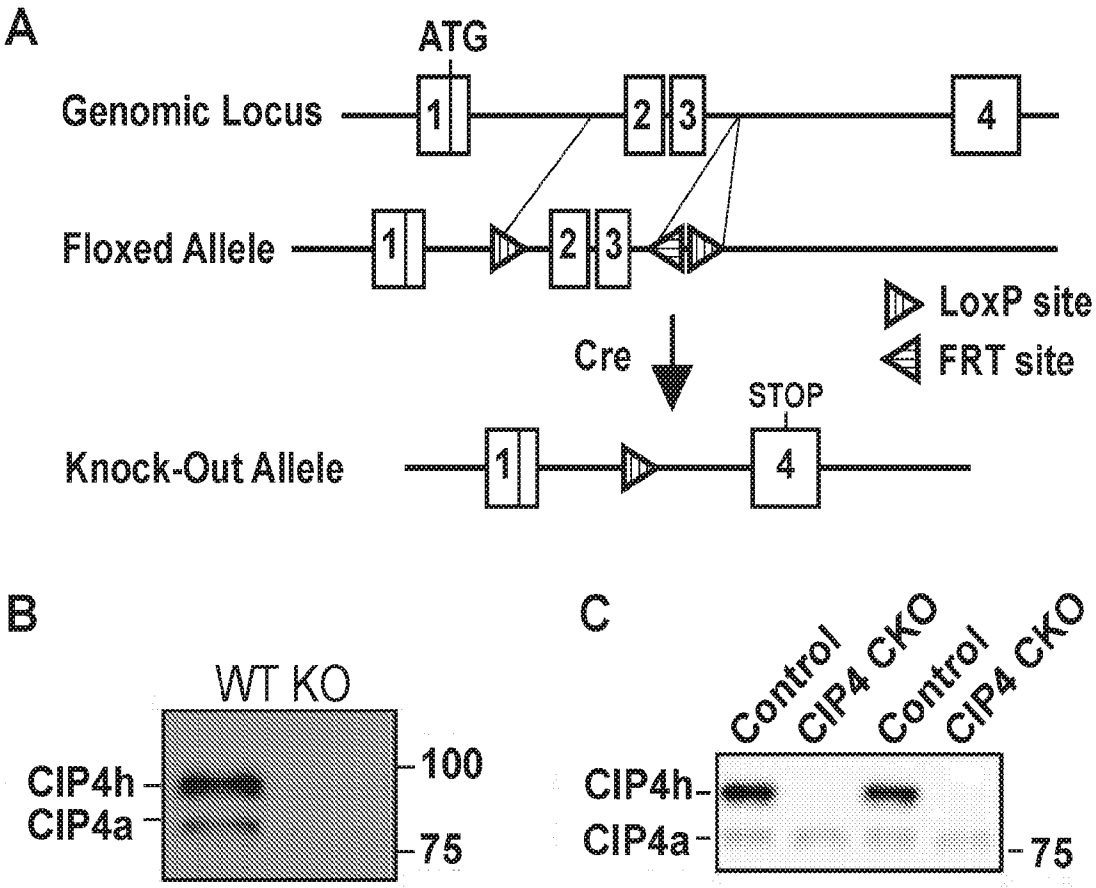

A
ATG
Genomic Locus
1
2
3
4
Floxed Allele
LoxP site
FRT site
Cre
STOP
Knock-Out Allele
B
WT KO
CIP4h
CIP4a
100
75
C
Control
CIP4 CKO
Control
CIP4 CKO
CIP4h
CIP4a
75

FIG. 4

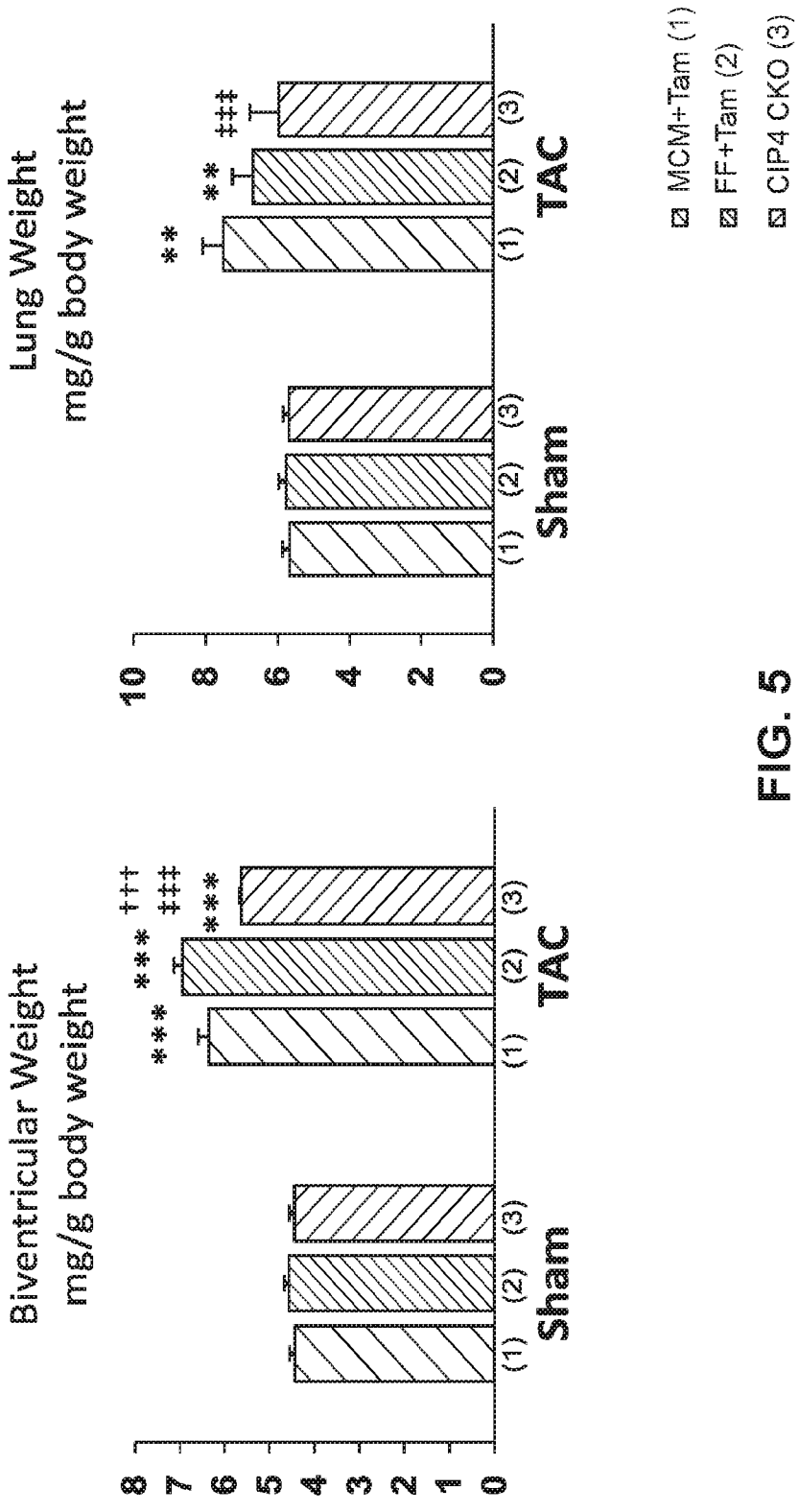

Biventricular Weight
mg/g body weight
8
7
6
5
4
3
2
1
0
***
***
***
†††
‡‡‡
(1)
(2)
(3)
Sham
TAC
Lung Weight
mg/g body weight
10
8
6
4
2
0
**
**
‡‡‡
(1)
(2)
(3)
Sham
TAC
MCM+Tam (1)
FF+Tam (2)
CIP4 CKO (3)

FIG. 5

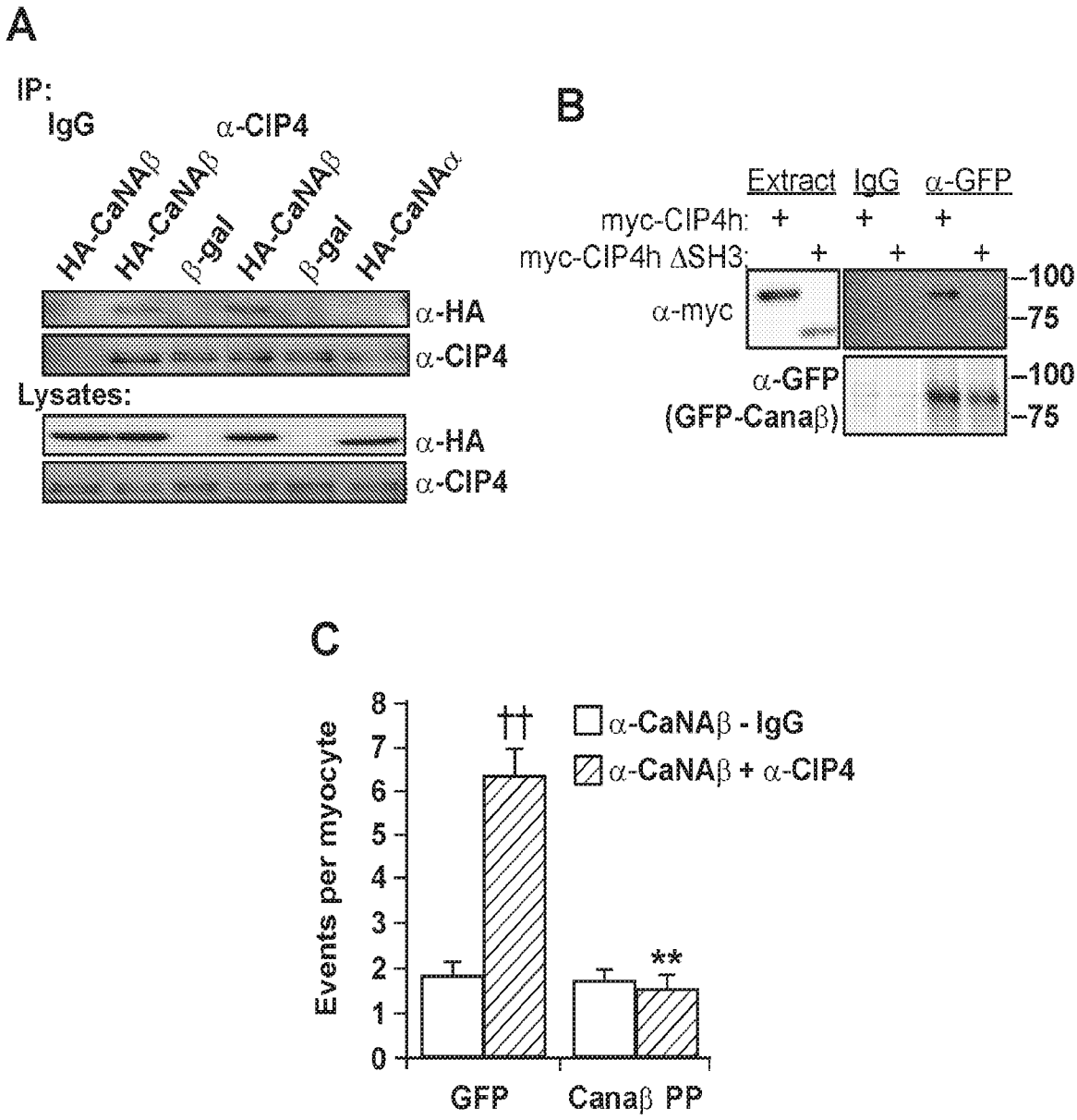

A
IP:
IgG
α-CIP4
HA-CaNAβ
HA-CaNAβ
β-gal
HA-CaNAβ
β-gal
HA-CaNAα
α-HA
α-CIP4
Lysates:
α-HA
α-CIP4
B
Extract
IgG
α-GFP
myc-CIP4h: + + +
myc-CIP4h ΔSH3: + + +
α-myc
α-GFP
(GFP-Canaβ)
100
75
100
75
C
Events per myocyte
0
1
2
3
4
5
6
7
8
††
**
α-CaNAβ - IgG
α-CaNAβ + α-CIP4
GFP
Canaβ PP

FIG. 6

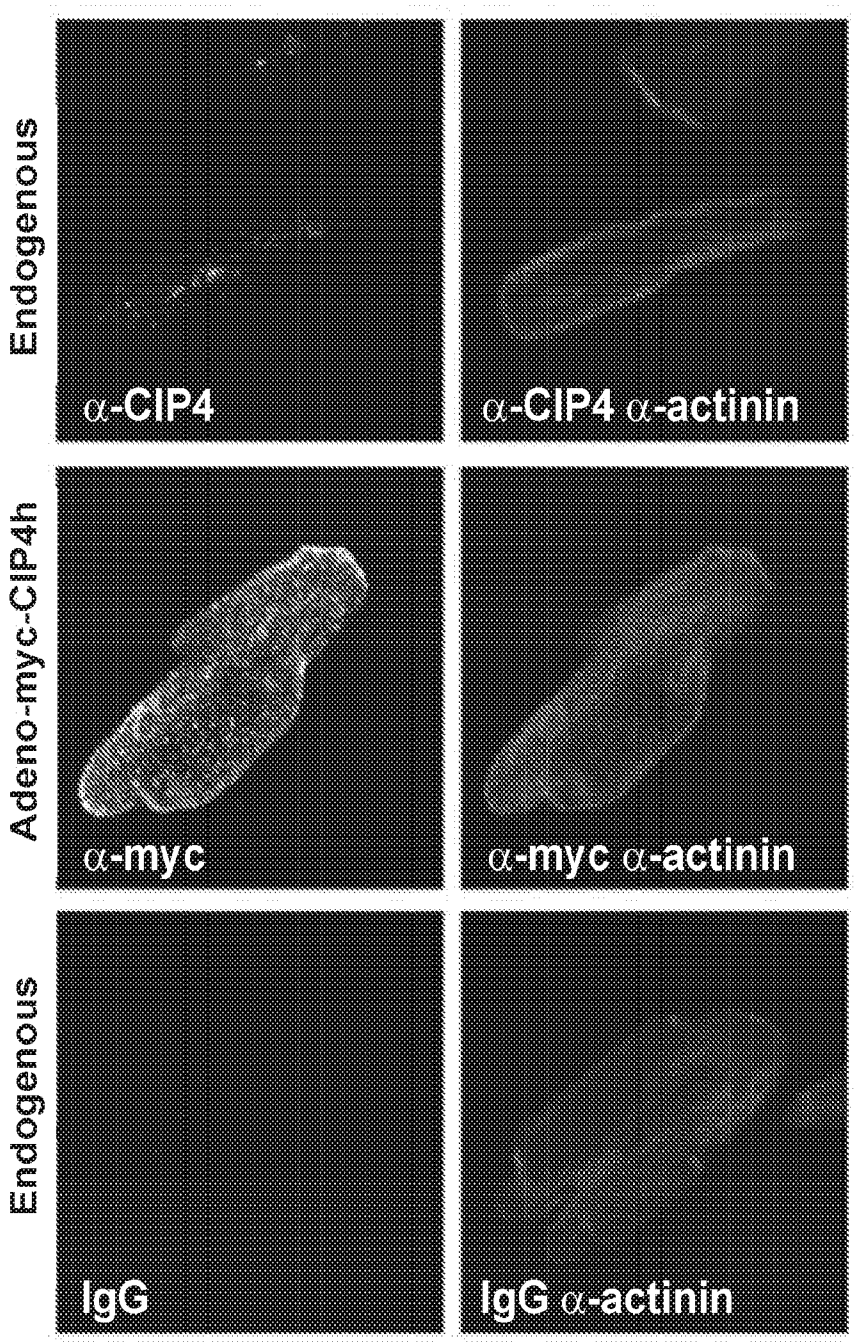

Endogenous
α-CIP4
α-CIP4 α-actinin
Adeno-myc-CIP4h
α-myc
α-myc α-actinin
Endogenous
IgG
IgG α-actinin

FIG. 7

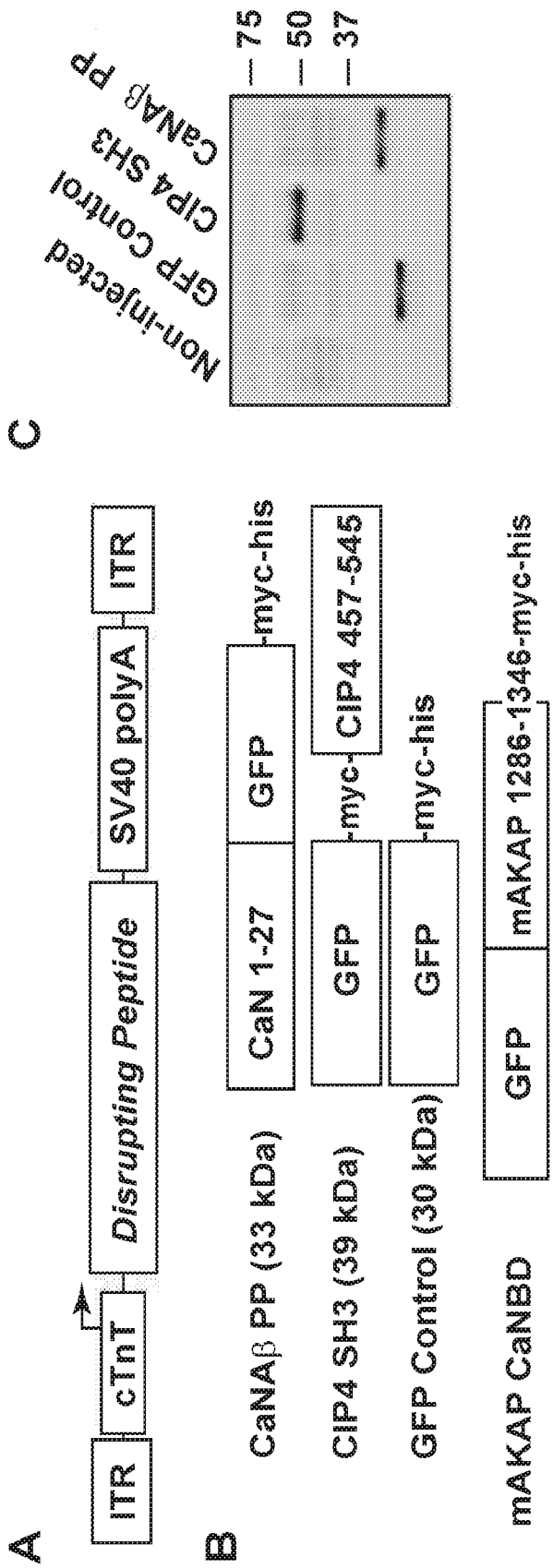

A
ITR
cTnT
Disrupting Peptide
SV40 polyA
ITR
B
CaNAβ PP (33 kDa)
CaN 1-27
GFP
myc-his
CIP4 SH3 (39 kDa)
GFP
myc-
CIP4 457-545
GFP Control (30 kDa)
GFP
myc-his
mAKAP CaNBD
GFP
mAKAP 1286-1346-myc-his
C
75
50
37
Non-injected
GFP Control
CIP4 SH3
CaNAβ PP

FIG. 8

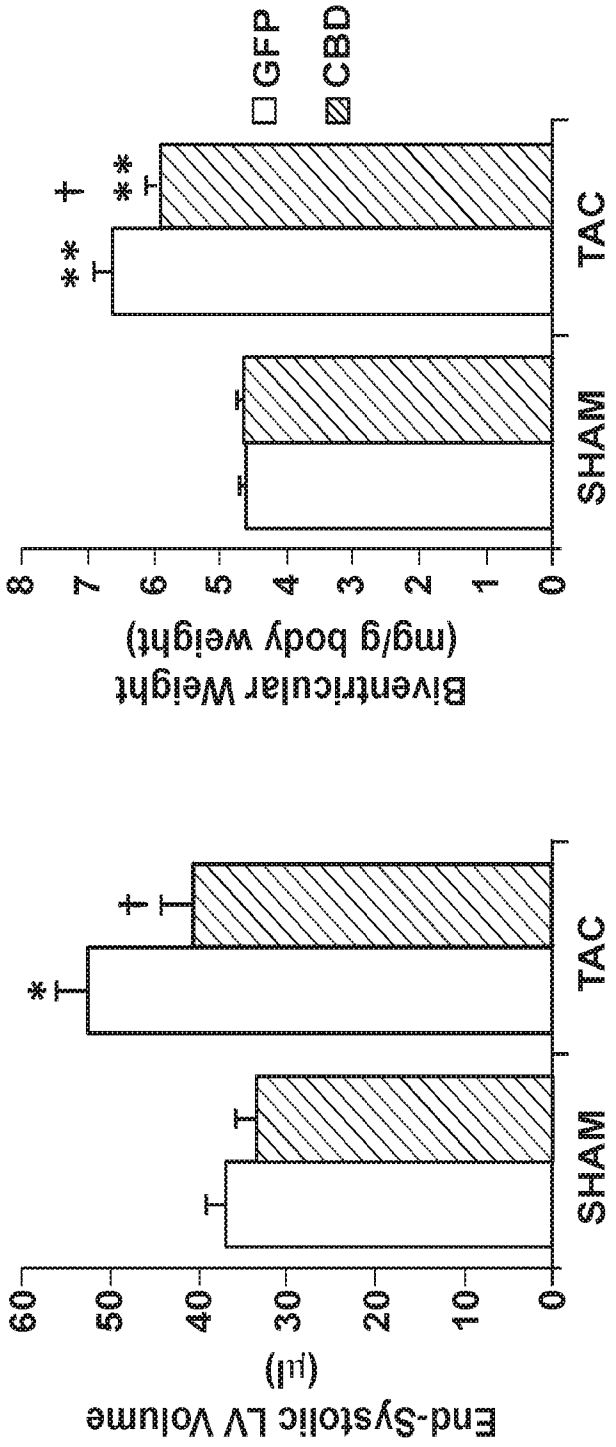
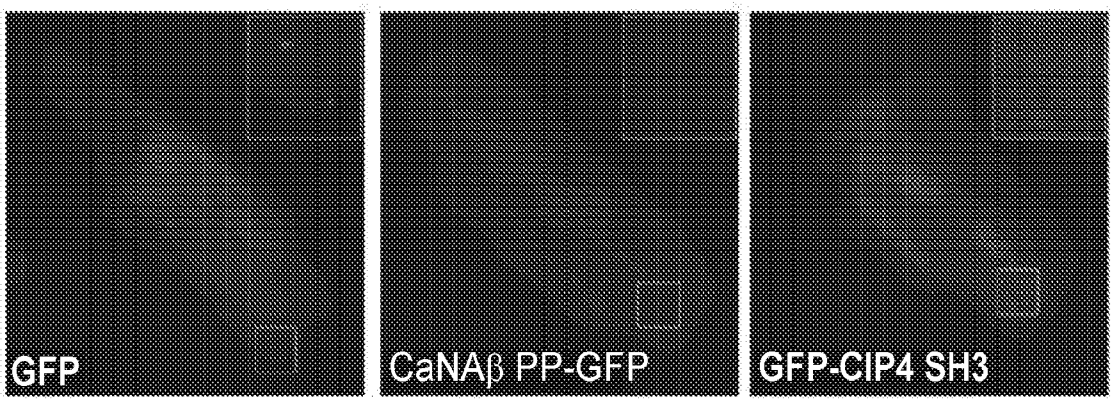

GFP
CBD
Biventricular Weight
(mg/g body weight)
0
1
2
3
4
5
6
7
8
SHAM
TAC
**
**
†
End-Systolic LV Volume
(μl)
0
10
20
30
40
50
60
SHAM
TAC
*
†

FIG. 9


GFP
CaNAβ PP-GFP
GFP-CIP4 SH3

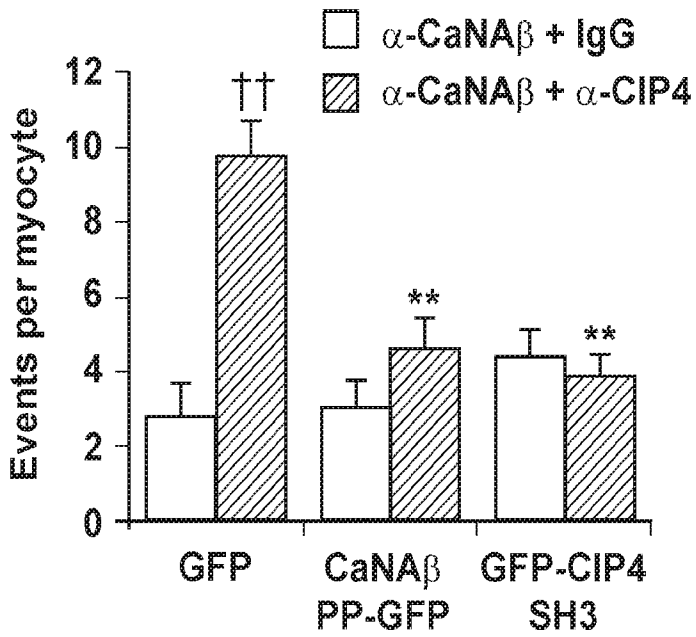

α-CaNAβ + IgG
α-CaNAβ + α-CIP4
Events per myocyte
0
2
4
6
8
10
12
††
**
**
GFP
CaNAβ PP-GFP
GFP-CIP4 SH3

FIG. 10

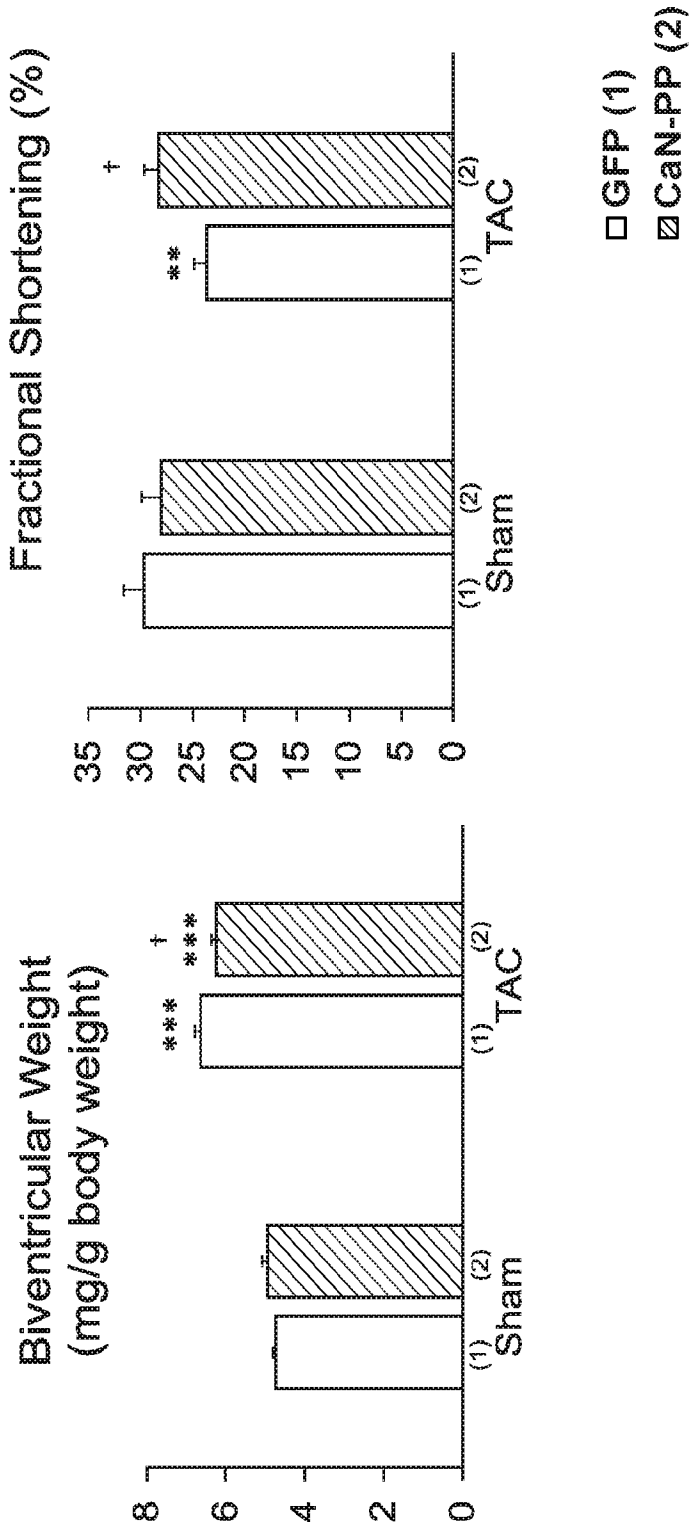

Fractional Shortening (%)
35
30
25
20
15
10
5
0
**
†
(1) Sham
(2)
(1) TAC
(2)
GFP (1)
CaN-PP (2)
Biventricular Weight (mg/g body weight)
8
6
4
2
0
***
***
†
(1) Sham
(2)
(1) TAC
(2)

FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/63759

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/17, C12N 15/113 (2017.01)
CPC - A61K 38/45, A61K 31/7048
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 38/17, C12N 15/113 (2017.01)
CPC - A61K 38/45, A61K 31/7048

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/16.4, 514/44A, 514/21.3
(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar, Google Web
Search terms: calcineurin, protein phosphatase 2B, PP2B, PPP3, Cdc42 interacting protein 4, CIP4, TRIP10, F BAR, Toca family, profilin 1, profilin 2, sorbin, FBP11, TOCA 1, scaffold, bind, polyproline, proline rich, SRC homology 3, SH3, nfat, heart disease, cdc42, wasp

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 1281756 A1 (GENOPIA BIOMEDICAL GMBH) 05 February 2003 (05.02.2003) para [0009], [0012], [0017], [0018], [0021], Fig. 4, SEQ ID NO: 7, SEQ ID NO: 8	1-5, 17, 18, 20-22, 33-39
Y	KILKA et al. The proline-rich N-terminal sequence of calcineurin Abeta determines substrate binding. Biochemistry. (10 March 2009) Vol 48, No 9, pp 1900-10, title, pg 1908 col 2 para 2	1-5, 17, 18, (20-22)/(17,18), 30-39
Y	WO 2002/046219 A2 (LUDWIG INSTITUTE FOR CANCER RESEARCH) 13 June 2002 (13.06.2002) pg 28 ln 28-30, pg 32 ln 1-3, pg 32 ln 26-32	19, (20-22)/19
Y	RUSCONI et al. CIP4 is required for the hypertrophic growth of neonatal cardiac myocytes. J Biomed Sci. (3 August 2013) Vol 20, No 56, pp 1-7, title, abstract, pg 1 col 2 para 1, pg 2 Fig 1A	2, (3-5)/2, 18, 19, (20-22)/(18,19)
Y	US 2002/0102708 A1 (BANDMAN et al.) 01 August 2002 (01.08.2002) para [0004], [0009], [0024], SEQ ID NO: 3	37-39
A	CEGLIA et al. Signaling pathways controlling the phosphorylation state of WAVE1, a regulator of actin polymerization. J. Neurochem. (July 2010) Vol 114, No 1, pp 182-190, pg 183 col 1 para 3	2, (3-5)/2, 18, (20-22)/(18,19)

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 March 2017	10 APR 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/63759

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-16, 23-32 and 40-46
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-5, 17-22 and 33-39, directed to a method of treating or preventing a disorder comprising administering a composition that inhibits binding of calcineurin Abeta (CaNAbeta) amino-terminal polyproline domain to a scaffold protein.

Group II, claims 47-55, directed to a composition comprising a peptide or a polynucleotide encoding thereof.

Group II, claims 56-60, directed to a method of treating or preventing heart disease by administering to a patient a mAKAPbeta peptide.

---please see continuation on extra sheet---

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5, 17-22 and 33-39

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/63759

Continuation of Box No. III Observations where unity of invention is lacking

The inventions listed as Groups I, II and III do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features
Group I has the special technical feature of a treatment method comprising administering a composition that inhibits binding of CaNAbeta amino-terminal polyproline domain to a scaffold protein, that is not required by Groups II or III.
Group II has the special technical feature of a composition comprising a peptide or a polynucleotide encoding, that is not required by Groups I or III.
Group II has the special technical feature of a method of treating heart disesase by administering to a patient a mAKAPbeta peptide, that is not required by Groups I or II.

Common technical features:
Groups I-II share the common technical feature of a composition that inhibits binding of CaNAbeta amino-terminal polyproline domain to a scaffold protein.
Groups I-II share the common technical feature of CIP4.
Groups I and III share the common technical feature of a method of treating or preventing heart disease by administering to a patient a pharmaceutically effective amount of a peptide inhibitor.
Groups II and III share the common technical feature of a peptide encoding a scaffold binding protein of fragment thereof (mAKAPbeta is a scaffold protein).

However, these shared technical features do not represent a contribution over prior art, because these shared technical features are made obvious over US 2014/0286928 A1 to ANCHORED RSK3 INHIBITORS, LLC, (hereinafter ARI), in view of US 2010/0035963 A1 to Chajut et al., (hereinafter Chajut).

ARI teaches a method of treating or preventing heart disease by administering to a patient a pharmaceutically effective amount of a composition that inhibits binding of CaNAbeta amino-terminal polyproline domain to a scaffold protein and a peptide encoding a scaffold binding protein of fragment thereof (claim 27, A method of treating or preventing heart disease, by administering to a patient a pharmaceutically effective amount of a composition which inhibits the activity of mAKAPbeta. Claim 28. The method of claim 27, wherein the composition is a mAKAPbeta peptide.)

Chajut teaches CIP4 is a cardiovascular-related gene (para [0026] "The present invention further relates to compounds which down-regulate expression of the cardiovascular-related genes listed in Table O and to the use of these compounds in the treatment of various pathologies, in particular pathologies related to cardiovascular diseases or medical conditions accompanied by an elevated level of the cardiovascular-related genes"; Table O "Homo sapiens thyroid hormone receptor interactor 10 (TRIP10), mRNA NM_004240 42516568 TRIP10"; TRIP10 is a synonym for CIP4). Thus it would have been obvious to one of the ordinary skill in the art to apply CIP4 to methods of treating or preventing heart disease, in order to effect expression or activity of CIP4 related pathology.

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I-III inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.