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(54) **Title**: MUSCLE FUNCTION ENHANCING PEPTIDE

FIGURE 1

SEQ ID NO: 1 EF-Hand EF-Hand
S100A1 protein: MGSELETAMETLINVFHAHSGKEGDKYKLSKKELKELLQTELSGFLDAQKDVDAVDKVMKELDENGDGEVDFQEYVVLVAALTVACNNFFWENS
(aa) 1 10 20 30 40 50 60 70 80 90

SEQ ID NO: 2
S100A1-ct peptide: YVVLVAALTVACNNFFWENS

(57) **Abstract**: The present invention relates to a peptide comprising a muscle function enhancing amino acid sequence which is derived from the S100 calcium binding protein family. Furthermore, the present invention provides said peptide for medical use, in particular, for treating or preventing disorders associated with muscular malfunction, such as skeletal muscle or cardiac muscle disorders. The present invention also provides a pharmaceutical composition comprising said peptide and a method for treating or preventing disorders associated with muscular malfunction using said peptide or said pharmaceutical composition.

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MUSCLE FUNCTION ENHANCING PEPTIDE

TECHNICAL FIELD OF INVENTION

The present invention relates to a peptide comprising a muscle function enhancing amino acid sequence derived from an S100 protein, which can be used for treating or preventing myopathies, in particular for treating or preventing skeletal muscle or cardiac muscle disorders, a pharmaceutical composition comprising said peptide, and a method for treating or preventing such myopathies.

BACKGROUND OF THE INVENTION

Muscle tissue is subdivided into skeletal muscle, cardiac muscle, and smooth muscle tissue and can be considered the biggest organ of a vertebrate. For example, an average adult human male is made up of 40 to 50% skeletal muscle. Skeletal muscle and cardiac muscle belong to the striated muscle tissue and share many functional aspects. For example, the process of excitation-contraction coupling in skeletal muscle cells and cardiac muscle cells (cardiomyocytes) is very similar. Membrane depolarization of the myocytes causes calcium influx via activated voltage-gated L-type calcium channels into the cytoplasm (sarcoplasm) of the myocyte. The rise of the cytoplasmic calcium concentration leads to calcium release from the sarcoplasmic reticulum (SR) by activation of ryanodine receptors (RyR) through the calcium-induced calcium release (CICR) mechanism, and thus, to a further rapid rise of the cytoplasmic calcium concentration. Calcium molecules diffuse through the cytoplasm and bind to the contractile proteins such as troponin C which causes contraction of the myocytes. After contraction, calcium is cleared from the cytoplasm by re-uptake of calcium into the sarcoplasmic reticulum mainly by the action of a sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA). These events are essentially identical in skeletal muscle cells and cardiac muscle cells with minor differences in the isoforms of the involved proteins. For example, while RyR1 is the predominant sarcoplasmic reticulum calcium release channel in skeletal muscle cells, RyR2 is predominant in cardiomyocyte. Similarly, the skeletal muscle sarcoplasmic/endoplasmic reticulum calcium ATPase is SERCA1a, whereas SERCA2a is cardiomyocyte-specific.

Calcium cycling in myocytes is regulated by a plethora of proteins. For example, S100A1 belonging to the S100 protein family (the largest EF-hand calcium-binding protein subfamily) has been reported to interact with both the RyR calcium release channel and

SERCA. S100A1 stabilizes RyR in diastole reducing the frequency of calcium sparks and augments calcium release during systole. Furthermore, S100A1 increases SERCA activity during the relaxation phase and it was found to increase contractile function in cardiac muscle as well as skeletal muscle cells. It has been shown that a carboxy-terminal peptide derived from the S100A1 protein mimics the inotropic effect of the full-length S100A1 protein (Most P. et al., 2007, Am. J. Physiol. Regul. Integr. Comp. Physiol. 293:R568-577; Voelkers M. et al., 2007, Cell Calcium 41:135-143).

Defective calcium cycling in myocytes, for example, reduced calcium release from the sarcoplasmic reticulum during contraction, aberrant calcium release events, calcium leakage from the sarcoplasmic reticulum, or slowed calcium clearance from the cytoplasm, results in a variety of myopathies, i.e., diseases associated with muscular malfunction. For example, cardiac insufficiency, contractile ventricular dysfunction, arrhythmias, heart failure, cardiogenic shock, myocardial infarction, and dysfunction of heart valves have been associated with dysregulation of calcium handling in cardiomyocytes. Analogously, defective calcium cycling in skeletal muscle fibers has been linked with muscular dystrophy (Hopf F.W. et al., 2007, Subcell. Biochem. 45:429-64). Furthermore, mutations in the RyR calcium release channels causing disruption of calcium signaling in muscle cells have been associated with myopathies. In particular, more than 80 mutations have been identified in the skeletal muscle RyR1 calcium release channel and have been linked to malignant hyperthermia, central core disease, or multi-minicore disease. Furthermore, more than 40 mutations in the cardiac RyR2 calcium release channel leading to ventricular arrhythmias and sudden cardiac death have been reported (Dulhunty A.F. et al., 2006, J. Muscle Res. Cell Motil. 27:351-365).

At present, there are no clinical inotropic therapies available for skeletal muscle disorders. Approved therapeutics currently available for the inotropic treatment of cardiomyopathies, such as glycoside derivatives, catecholamines, and phosphodiesterase inhibitors, are afflicted with severe side effects such as increased heart rate and life threatening proarrhythmogenic potential. Besides these approved therapeutics, the S100A1 protein has been suggested as therapeutic in cardiomyopathies, since it was shown that myocardial levels of S100A1 are decreased in heart failure and that S100A1 delivery to cardiomyocytes results in an increase of isometric contraction followed by an increase in the amount of calcium pumped into the sarcoplasmic reticulum. However, the administration of S100A1 to a patient with the purpose of treating myopathies requires the delivery route of gene therapy, for example, using viral delivery, with all its well-known side effects and

disadvantages (Most P. et al., 2007, Am. J. Physiol. Regul. Integr. Comp. Physiol. 293:R568-577, WO 2008/054713, and Vinge L.E. et al., 2008, Circ. Res. 102:1458-1470).

Therefore, there is an urgent need for novel therapeutics for the inotropic treatment of myopathies, preferably myopathies associated with dysregulation of calcium cycling in muscle cells, which do not exhibit the severe side effects observed for the approved therapeutics and which do not require the high risk delivery route of gene therapy. Regarding skeletal muscle diseases, there is an urgent need for any inotropic therapeutics having the ability to increase the contractile performance of skeletal muscle cells and/or reducing calcium-induced apoptotic cell death in skeletal muscle cells.

The present inventors have surprisingly found that peptides according to the present invention which are derived from the calcium binding protein S100 exhibit inotropic effects when administered parenterally and are useful for the treatment of myopathies, such as cardiac and skeletal muscle disorders, without exhibiting mentionable side effects and without requiring gene therapy. For example, the peptides according to the present invention enhance and restore inotropy in normal and failing myocardium as well as in normal and diseased skeletal muscle, enhance and restore sarcoplasmic reticulum calcium handling, prevent calcium induced apoptotic cell death in myocytes, protect from proarrhythmogenic sarcoplasmic reticulum calcium leak and tachyarrhythmias, and prevent cardiac death due to protection from pump failure and tachyarrhythmias. The peptides of the present invention are particularly useful for enhancing contractile performance of cardiac and skeletal muscle tissue without major side effects.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides a peptide comprising a muscle function enhancing amino acid sequence comprising, essentially consisting or consisting of the amino acid motif

$$\Phi_4\text{-X-}\Psi\text{-L-[T/A]-}\Psi_2,$$

wherein Φ and Ψ are in each instance an independently selected hydrophobic non-aromatic amino acid, and X is any amino acid, preferably a small amino acid, wherein the muscle function enhancing amino acid sequence does not contain more than 18 continuous amino acids of the carboxy-terminal amino acids of an S100A1 protein, preferably of any S100 calcium binding protein, the peptide has a total length of maximally 100 amino acids, and the peptide exhibits a positive inotropic action. Preferably, said peptide is capable of penetrating cell membranes. Preferably, said peptide exhibits the ability to enhance contractile

performance and/or calcium cycling in myocytes. In a preferred embodiment, the muscle function enhancing amino acid sequence comprises, essentially consists or consists of the amino acid sequence [V/I]-[V/I]-[L/M]-[V/I/M]-[A/G/S]-[A/V]-L-[T/A]-[V/A/I]-[A/M/V], preferably comprises, essentially consists or consists of the amino acid sequence V-V-L-V-A-A-L-T-V-A (SEQ ID NO: 4). As indicted above it is preferred in this context that the muscle function enhancing amino acid sequence does not contain more than 18 continuous amino acids of the carboxy-terminal amino acids of an S100A1 protein. Preferably, said peptide further comprises one or more of the elements selected from the group consisting of a membrane penetration enhancing motif, one or more epitope-tag(s), a hydrophilic motif, and a peptide targeting motif, wherein preferably the hydrophilic motif comprises, essentially consists or consists of the hydrophilic amino acid motif Λ_4-Θ_2, wherein Λ is in each instance independently selected from aspartate, glutamate, lysine, and arginine, and Θ is an α-helix interrupter, preferably is in each instance independently selected from proline or glycine. Preferably the hydrophilic motif comprises, essentially consists or consists of the amino acid sequence [D/E]-[K/R]-[D/E]-[D/E]-[P/G]-[P/G], more preferably comprises, essentially consists or consists of the amino acid sequence D-K-D-D-P-P (SEQ ID NO: 354). In a particular preferred embodiment, the peptide according to the present invention comprises, essentially consists or consists of the amino acid sequence D-K-D-D-P-P-V-V-L-V-A-A-L-T-V-A (SEQ ID NO: 370), or an amino acid sequence which is at least 60% identical to said amino acid sequence.

In a second aspect, the present invention provides the peptide of the first aspect for medical use.

In a third aspect, the present invention provides the peptide of the first or second aspect for therapeutic use in treating or preventing disorders associated with muscular malfunction, wherein preferably the disorder is a cardiac and/or skeletal muscle disorder, wherein preferably the muscular malfunction is associated with defective calcium cycling and/or defective contractile performance in muscle cells. Preferably, the peptide is for enhancing and/or restoring calcium cycling and/or for enhancing and/or restoring contractile performance in muscle cells. The cardiac muscle disorder may be selected from the group consisting of postischemic contractile dysfunction, congestive heart failure, cardiogenic shock, septic shock, myocardial infarction, cardiomyopathy, dysfunction of heart valves, and ventricular disorder, the skeletal muscle disorder may be selected from the group consisting of muscular dystrophy, muscle weakness, muscular atrophy, myositis, central core disease,

nemaline rod myopathy, centronuclear myotubular myopathy, ophthalmoplegia of the eye, mitochondrial myopathy.

In a fourth aspect, the present invention provides a pharmaceutical composition comprising the peptide of the first aspect of the present invention and a pharmaceutically acceptable excipient, carrier, and/or diluent. In a preferred embodiment, the pharmaceutical composition is for treating or preventing disorders associated with muscular malfunction.

In a fifth aspect, the present invention relates to a use of the peptide according to the first aspect of the present invention for the preparation of a pharmaceutical composition for treating or preventing disorders associated with muscular malfunction.

In a sixth aspect, the present invention provides a method for treating or preventing disorders associated with muscular malfunction comprising administering to an individual in need thereof the peptide or the pharmaceutical composition according to the present invention in an amount sufficient to ameliorate the disease condition of said individual.

In a seventh aspect, the present invention provides a composition comprising the peptide according to the first aspect of the present invention and a medicament selected from the group consisting of catecholamines, β-adrenergic receptor agonists, and β-adrenergic receptor blockers.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1: Characterization of the S100A1 protein C-terminus as the bioactive lead structure.

Figure 1 shows the primary structure of native human S100A1 protein (S100A1, 94 amino acids; SEQ ID NO: 1) in the upper row and the S100A1 C-terminus in the lower row as a 20-mer peptide (S100A1-ct peptide) encompassing amino acids 75-94 (SEQ ID NO: 2) devoid of the C-terminal calcium binding EF-hand.

FIG. 2: Hydrophobicity plot of human S100A1.

The S100A1 C-terminal domain encompassing amino acids 75-94 is the most hydrophobic region of the protein. The Kyte-Doolittle Plot was performed by the inventors with the published cDNA sequence of the human *s100a1* gene (GenBank accession number: NM006271) employing a hydrophobicity plot accessible at http://www.vivo.colostate.edu/molkit/hydropathy/index.html. A y-axis score > 0 depicts increasing hydrophobicity. The S100A1 C-terminus including amino acids 75-94 is marked by a grey bar.

FIG. 3: Tertiary/quaternary structure of human S100A1 protein.

Figure 3A visualizes the tertiary/quaternary structure of human S100A1 showing that the hydrophobic C-terminus is buried inside the calcium-unbound form and apo-state, respectively, of the homodimeric protein. Figure 3B shows that calcium binding to both EF-hand motives results in exposure of the S100A1 C-terminus to the molecule surface rendering the hydrophobic domain accessible for protein-protein interactions. It has therefore been suggested that the S100A1 C-terminus including amino acids 75-94 accounts for target protein binding and modulation of target protein function/activity in the calcium-bound and "activated" dimeric S100A1 protein. Calcium binding to S100A1 confers a conformational change rendering the C-terminal domain (amino acids 75-94) (dashed box) accessible for protein-protein interaction.

FIG. 4: Human S100A1 protein and the 20-mer C-terminal peptide in chemically permeabilized cardiac and skeletal muscle cell preparations.

Equivalent bioactivity of native human S100A1 protein and the 20-mer C-terminal peptide was shown by the inventors in chemically permeabilized cardiac and skeletal muscle cell preparations enabling intracellular access and regulation of RyR2 and RyR1 function. Figure 4 depicts a similar intracellular binding pattern for rhodamine-labeled recombinant human S100A1 protein (10415 Mw) (Figure 4 A/B) and the 20-mer FITC-labeled synthetic S100A1 C-terminal peptide (2258 Mw) (Figure 4 C/D). Neither rhod-S100A1 protein nor FITC-S100A1 C-terminus (amino acids 75-94) is able to permeate the cell membrane of adult intact cardiomyocytes.

FIG. 5: S100A1 protein decreases diastolic calcium spark frequency and RyR2 activity in permeabilized cardiomyocytes (A) and enhances isometric twitch force in permeabilized skeletal muscle fibers (B). S100A1 protein does not alter calcium homeostasis in adult intact cardiomyocytes or skeletal muscle fibers.

FIG. 6: The 20-mer S100A1 C-terminal peptide (amino acids 75 to 94 of the S100A1 protein) decreases diastolic calcium spark frequency and RyR2 activity in permeabilized cardiomyocytes (A) and enhances isometric twitch force in permeabilized skeletal muscle fibers (B), but does not alter calcium homeostasis in adult intact cardiomyocytes or skeletal muscle fibers.

FIG. 7: Both S100A1 protein (B1) and the 20-mer S100A1 C-terminal peptide (B3) have equivalent biological potency to enhance isometric force in permeabilized EDL murine skeletal muscle fibers. B2 and B3 confirm that solely the S100A1 C-terminus mediates the inotropic effect. S100A1 peptides (N/H/C) refer to the N-terminal peptide (N) G-S-E-L-E-T-A-M-E-T-L-I-N-V-F (amino acids 2 to 16 of S100A1, SEQ ID NO: 388), the hinge-region peptide (H) L-S-G-F-L-D-A-Q-K-D-V-D-A (amino acids 42 to 54 of S100A1, SEQ ID NO: 389), and the C-terminal 20-mer (C) (SEQ ID NO: 2).

FIG. 8: Cell permeable S100A1ct$_{6/11}$ sequence and intracellular accumulation in normal and diseased cardiomyocytes.

S100A1ct$_{6/11}$ refers to the peptide D-K-D-D-P-P-Y-V-V-L-V-A-A-L-T-V-A (SEQ ID NO: 372), wherein the sequence D-K-D-D-P-P (SEQ ID NO: 354) is a hydrophilic motif and the sequence Y-V-V-L-V-A-A-L-T-V-A (SEQ ID NO: 390) are amino acids 75 to 85 of the human S100A1 protein. The S100A1ct$_{6/11}$ peptide is cell permeable and accumulates in the intracellular space of cardiomyocytes in contrast to the cell-impermeable 20-mer C-terminal S100A1 peptide. Figure 8 shows that FITC-coupled S100A1ct$_{6/11}$ enriches in the intracellular space of intact rat ventricular cardiomyocytes resulting in a striated pattern (B, confocal image taken after 15 min of extracellular exposure) similar to endogenous S100A1 protein assessed by anti-S100A1 immunofluorescence staining (C), while the control (A) did not exhibit a specific labeling pattern.

FIG. 9: Time- and dose-dependent positive inotropic effect of S100A1ct$_{6/11}$ in electrical field stimulated isolated ventricular rat cardiomyocytes.

S100A1ct$_{6/11}$ mimics the inotropic effect both of viral-mediated and cardiac-targeted transgenic S100A1 over-expression in adult ventricular cardiomyocytes in a dose-dependent and time-dependent manner. Figure 9 shows representative tracings of the dose-dependent (upper panel) and time-dependent (lower panel) positive inotropic effect of S100A1ct$_{6/11}$ in isolated electrical-field stimulated (2 Hz) rat ventricular cardiomyocytes. Note the onset of S100A1ct$_{6/11}$ inotropic actions between 10 and 20 minutes in line with its intracellular accumulation after 15 minutes extracellular exposure (Figure 8B). Calculated EC50% is 87±6 nM S100A1ct$_{6/11}$. Calcium transients were assessed in FURA2-AM field-stimulated cardiomyocytes employing epifluorescent digitalized microscopy.

FIG. 10: Neither the synthetic peptide DKDDPP-YVVLVA (amino acids 75-80 of human S100A1 fused to a hydrophilic motif, SEQ ID NO: 382) nor the synthetic peptide DKDDPP-AALTVA (amino acids 80-85 of human S100A1 fused to a hydrophilic motif, SEQ ID NO: 383) is sufficient to mimic or reproduce inotropic effects of $S100A1ct_{6/11}$. Calcium transients were assessed in FURA2-AM field-stimulated cardiomyocytes employing epifluorescent digitalized microscopy (n = 60 in each group).

FIG. 11: Peptides encompassing amino acids 75-85 derived from S100 paralogs A4 and B are not sufficient to mimic $S100A1ct_{6/11}$ mediated inotropy.
Both the synthetic peptide DKDDPP-YCVFLSCIAMM (amino acids 75-85 of S100A4 fused to a hydrophilic motif, SEQ ID NO: 386) and DKDDPP-FMAFVAMVTTA (amino acids 75-85 of S100B fused to a hydrophilic motif, SEQ ID NO: 387) fail to reproduce $S100A1ct_{6/11}$ inotropic actions. (A) shows that neither S100A4ct nor S100Bct mimic $S100A1ct_{6/11}$ inotropic effects under basal (left panel) and β-AR stimulated conditions (right panel). (B) depicts primary sequence alignment of DKDDPP (SEQ ID NO: 354) coupled amino acids 75-85 peptides derived from S100A1 (top), S100A4 (middle) and S100B (down). Identical amino acids between S100A1ct and S100A4ct and S100Bct are underlined. (n = 60 cells in each group, *P<0.05 vs. control, S100A4ct and S100Bct, 2-way ANOVA). 10^{-9} Iso means 10^{-9} M Isoproterenol.

FIG. 12: The inotropic effect of $S100A1ct_{6/11}$ is associated with control and regulation of the sarcoplasmic reticulum (SR) calcium content.
Inotropic effects of $S100A1ct_{6/11}$ are conveyed by enhanced SR calcium load similar to effects of native human S100A1 protein employing viral-mediated and cardiac-targeted transgenic S100A1 over-expression in adult ventricular cardiomyocytes. Figure 12 depicts representative tracings of a field-stimulated (2 Hz) control (black, left trace) and $S100A1ct_{6/11}$ (1000 nM, light grey, right trace) ventricular cardiomyocyte subjected to 10 mM caffeine *in vitro* resulting in complete release of SR calcium. The amplitude of the caffeine evoked calcium transient serves as an indirect measure of the SR calcium content being greater in $S100A1ct_{6/11}$ treated cardiomyocytes. These data indicate that $S100A1ct_{6/11}$ inotropic effects are associated with control and enhanced SR calcium storage and content.

FIG. 13: The positive inotropic effect of $S100A1ct_{6/11}$ in cardiomyocytes is additive and independent of β-adrenergic stimulation and signaling, respectively.

(A) shows a representative Western blot employing phospho-specific antibodies revealing that S100A1ct$_{6/11}$ neither recruits nor alters β-adrenergic receptor (βAR) signaling including cAMP-dependent kinase (PKA) activity at sarcoplasmic reticulum (phospholamban, PLB) and sarcomeric (troponin I, TnI) targets under basal conditions and βAR stimulation. In support of this, Figure 13 B shows that S100A1ct$_{6/11}$ inotropic effects are additive and preserved under βAR stimulation assessed in FURA2-AM field-stimulated cardiomyocytes employing epifluorescent digitalized microscopy. Note that the major inotropic effect of the βAR-PKA axis is conveyed by enhanced PLB-ser16 phosphorylation. S100A1ct$_{6/11}$ neither includes nor alters this mechanism explaining its additive inotropic effect on βAR stimulation. (n = 60 cells in each group, *P<0.05 vs. control, 2-way ANOVA). 10^{-9} Iso means 10^{-9} M Isoproterenol.

FIG. 14: S100A1ct$_{6/11}$ controls diastolic RyR2 function and modulates physiologic diastolic SR calcium spark frequency in ventricular cardiomyocytes.

S100A1ct$_{6/11}$ modulates diastolic SR calcium spark frequency in intact ventricular cardiomyocytes and mimics the effect of cell-impermeable native S100A1 protein and the 20-mer S100A1 C-terminal domain peptide in permeabilized ventricular cardiomyocytes. Figure 14 A shows representative confocal tracings of calcium sparks in Fluo-3 AM loaded control and S100A1ct$_{6/11}$ treated quiescent ventricular rat cardiomyocytes. Figure 14 B-D depict that S100A1ct$_{6/11}$ differentially controls diastolic SR calcium spark frequency and amplitude. While 100 nM S100A1ct$_{6/11}$ decreases calcium spark frequency under basal conditions, a ten-fold greater S100A1ct$_{6/11}$ concentration (1000 nM) enhances calcium spark frequency (n = 60 cells in each group, 2-way ANOVA).

FIG. 15: The molecular mechanism conveying S100A1ct$_{6/11}$ inotropic effects concurrently protects cardiomyocytes from pro-arrhythmic store overload-induced calcium release (SOICR) and calcium waves, respectively.

SOICR, being a critical pathomechanism for arrhythmogenic sudden cardiac death, was evoked *in vitro* employing a previously published protocol by Isner and co-workers (Venetucci et al., 2007, Circ Res 100:105-111). Figure 15 A shows representative confocal tracings of calcium sparks in a Fluo-3 AM loaded control cardiomyocyte (left) which frequency and spatial characteristics are dramatically increased under conditions (βAR stimulation + 0.5 mM caffeine) resulting in SOICR (middle) as described by (Venetucci et al., 2007, Circ Res 100:105-111). Note that treatment with 1000 nM S100A1ct$_{6/11}$ (Figure 15 A,

right) effectively antagonizes the SR calcium leak. Figure 15 B reveals statistical analysis of the therapeutic impact of S100A1ct$_{6/11}$ normalizing abnormal calcium spark frequency and spatial characteristics in the presence of isoproterenol/caffeine. Figure 15 C depicts the potent anti-arrhythmic effect of S100A1ct$_{6/11}$ with representative tracings of a control cardiomyocyte exhibiting SOICR triggered calcium waves in the presence of βAR stimulation + 0.5 mM caffeine that are completely prevented by 100 nM and 1000 nM S100A1ct$_{6/11}$. Given that SOICR and subsequent calcium waves are molecular substrates for lethal ventricular arrhythmias and sudden cardiac death, these experiments uncover the unique molecular profile of S100A1ct$_{6/11}$ combining inotropy with protection from calcium-induced arrhythmias in cardiomyocytes (n = 60 cells in each group, 2-way ANOVA). It is important to note that the protective effect of S100A1ct$_{6/11}$ is effective at concentrations (100 nM and 1000 nM) that exert inotropic actions in cardiomyocytes (Figure 9) due to enhanced SR calcium load. Thus, despite its own enhancing effect on SR calcium resequestration, S100A1ct$_{6/11}$ effectively antagonizes βAR-triggered SOICR highlighting the unique molecular profile combining inotropic actions with anti-arrhythmic potency. Akin S100A1ct$_{6/11}$, viral-mediated S100A1 over-expression also prevented βAR-triggered pro-arrhythmic SR calcium leak in adult ventricular cardiomyocytes with leaky RyR2s indicating that cell-permeable S100A1ct$_{6/11}$ mimics the anti-arrhythmic effect of over-expressed S100A1 protein.

FIG. 16: The molecular mechanism conveying S100A1ct$_{6/11}$ inotropic effects concurrently protects cardiomyocytes from apoptotic cell death due to the prevention of SR calcium leak.
S100A1ct$_{6/11}$ protects adult ventricular cardiomyocytes with leaky RyR2s that are sensitized to luminal calcium by long-term caffeine exposure from apoptotic cell death. Figure 16 A shows representative images of control and S100A1ct$_{6/11}$ treated cardiomyocytes exposed to caffeine. Black arrowheads highlight dead cells due to SR calcium leak induced apoptosis facilitated by leaky RyR2. Statistical analysis revealed significantly less apoptotic cardiomyocytes in the S100A1ct$_{6/11}$ treated group. Figure 16 B shows a representative DNA gel of two independent experiments with laddered DNA in control but not S100A1ct$_{6/11}$ treated cardiomyocytes indicative of a prevention of apoptosis.

FIG. 17: S100A1ct$_{6/11}$ resists cleavage and degradation in human serum enabling application and long-term biological effectiveness *in vivo*.
Human serum spiked with S100A1ct$_{6/11}$ *in vitro* (1 μM) shows uncleaved S100A1ct$_{6/11}$ for up to 3 hours indicating high serum stability as a prerequisite for *in vivo* administration and

biological long-term effectiveness. A-D show representative tracings of MALDI-TOF analyses of human serum samples spiked with 1 µM S100A1ct$_{6/11}$ *in vitro* at different time points. Note that Figure A throughout D reveals no cleavage and degradation of S100A1ct$_{6/11}$ indicating high stability in a protease rich environment.

FIG. 18: S100A1ct$_{6/11}$ exerts significant *in vivo* hemodynamic effects resulting in enhanced contractile performance under basal and βAR-stimulated conditions.

Anesthetized adult C57/B6 male mice (30 g BW) receiving a single intravenous (i.v.) application of 225 ng S100A1ct$_{6/11}$ (squares) exhibited a 3-hour lasting enhancement of left ventricular contractile performance (left panel) that was preserved and additive to i.v. isoproterenol application (250 pg) (compare to solid diamonds of control animals). Note that the *in vivo* effect reflects *in vitro* actions of S100A1ct$_{6/11}$ under basal and βAR stimulated conditions. The inotropic effect of S100A1ct$_{6/11}$ *in vivo* was independent of heart rate and its responsiveness to βAR stimulation (right panel). S100A1ct$_{6/11}$ is also effective with delayed onset after intraperitoneal and subcutaneous use. Figure 18 shows significantly enhanced basal contractile performance assessed by left ventricular catherization in anesthetized mice after i.v. S100A1ct$_{6/11}$ injection. The gain in function was preserved under βAR stimulation and independent of heart rate (n=7 animals in each group, *P<0.05 vs. corresponding control animal, 2-way ANOVA).

FIG. 19 S100A1ct$_{6/11}$ exerts significant *in vivo* hemodynamic effects which are effective in response to the β1AR-blocker metoprolol.

Anesthetized adult C57/B6 male mice (30 g BW) receiving a single intravenous (i.v.) application of 225 ng S100A1ct$_{6/11}$ 15 min after intraperitoneal (i.p.) administration of metoprolol (62.5 µg) showed similar slowing in heart rate (Figure 19) without ECG abnormalities but preserved S100A1ct$_{6/11}$ mediated gain in function (Figure 20). Figure 19 shows representative telemetric ECG recordings (DSI systems, Einthoven lead II) in a control (i.v. vehicle) and i.v. treated S100A1ct$_{6/11}$ mice with similar slowing in heart rate without conduction abnormalities in response to metoprolol.

FIG. 20: shows preserved S100A1ct$_{6/11}$ inotropic effectiveness in presence of the β1AR blocker metoprolol in anesthetized mice (left panel). Note that S100A1ct$_{6/11}$ antagonized the negative inotropic but not the negative chronotropic effect of metoprolol (right panel) highlighting feasibility of combined S100A1ct$_{6/11}$ and metoprolol therapy for cardiac

dysfunction (n = 7 animals in each group, *P<0.05 vs. corresponding control animal, 2-way ANOVA).

FIG. 21: S100A1ct$_{6/11}$ exerts significant therapeutic effects *in vivo* restoring hemodynamic function in an experimental mouse heart failure model.

Daily S100A1ct$_{6/11}$ i.p. treatment of adult C57/B6 male/female mice with postischemic contractile dysfunction at 225 ng (30 g BW) for 2 weeks results in significantly improved cardiac performance and survival. Figure 21 A depicts the therapeutic effect of 2-week i.p. S100A1ct$_{6/11}$ heart failure treatment restoring left ventricular performance in mice with contractile dysfunction assessed by serial echocardiography. Figure 21 B depicts that improved contractile performance in S100A1ct$_{6/11}$ treated heart failure mice is translated in significantly improved survival. (A, n = 10 animals in each group; B, 18 animals in each group, *P<0.05 vs. pre-MI, †P<0.01 vs. control heart failure animals, 2-way ANOVA).

FIG. 22: S100A1ct$_{6/11}$ exerts significant therapeutic effects *in vivo* preventing apoptotic cell death in failing myocardium in an experimental heart failure animal model.

Daily S100A1ct$_{6/11}$ i.p. treatment of adult C57/B6 male/female mice with postischemic contractile dysfunction at 225 ng (30 g BW) for 2 weeks resulted in significantly diminished apoptosis in failing hearts. Note that the *in vivo* effect reflects the anti-apoptotic action of S100A1ct$_{6/11}$ in cardiomyocytes *in vitro*. Figure 22 shows representative TUNEL stainings of a heart failure (HF) control and an S100A1ct$_{6/11}$ treated failing heart (2-week i.p.) where green nuclei indicate DNA strand breaks labeled by a FITC-coupled probe. Note that the S100A1ct$_{6/11}$ treated failing heart exhibits less apoptotic nuclei (middle panel). Statistical analysis revealed a significant reduction of apoptosis in S100A1ct$_{6/11}$ treated failing hearts contributing to the overall therapeutic effect on survival (n = 6 animals in each group, P<0.01 vs. control hearts, 2-way ANOVA).

FIG. 23: S100A1ct$_{6/11}$ exerts significant therapeutic effects *in vivo* protecting heart failure mice from βAR triggered lethal ventricular tachyarrhythmias.

Daily S100A1ct$_{6/11}$ i.p. treatment of adult C57/B6 male/female mice with postischemic contractile dysfunction at 225 ng (30 g BW) for 2 weeks protects from βAR triggered ventricular fibrillations in hearts with calcium sensitized leaky RyR2 channels by caffeine. The pro-arrhythmogenic protocol in heart failure mice was adapted from the previously published protocol by Wayne Chen and co-workers (Xiao et al., 2007, JBC 282:34828-

34838). Figure 23 shows representative ECG tracings in a heart failure control and S100A1ct$_{6/11}$ treated mouse (2-week i.p.) exposed to i.p. epinephrin/caffeine injection resulting in abrupt onset of lethal ventricular fibrillation (left panel). Note that lethal ventricular fibrillation only occurred in 2 out of 10 animals in the S100A1ct$_{6/11}$ treated group whereas control heart failure mice showed 80% mortality (Contingency tested by Fischer's exact test).

FIG. 24: S100A1ct$_{6/11}$ significantly enhances isometric twitch force in normal and diseased skeletal muscle.

Incubation of intact extensor digitorum longum (EDL) skeletal muscle isolated from 12 weeks old C57/B6 male mice with S100A1ct$_{6/11}$ (1 μM) for 45 min resulted in significantly enhanced specific isometric and tetanic twitch force as shown in Figure 24 A applying a method for muscle isolation and isometric tension measurement as previously published by the inventors (Most et al., 2003, J. Biol. Chem. 278:26356-26364). Tetanic train was applied at 125 Hz for 175 ms reaching a stable force plateau. Moreover, post-myocardial infarction heart failure mice generated by the inventors as described previously (Most et al., 2006, Circulation 114:1258-1268) presented with improved skeletal muscle function after a 2-week i.p. S100A1ct$_{6/11}$ (225 ng, daily injections) treatment as shown in Figure 24 B. This is a significant finding as major clinical symptoms such as fatigue and impaired exercise capacity in heart failure patients are caused by impaired skeletal muscle function and are not directly related to cardiac output. Figure 24 A shows that extracellular application of S100A1ct$_{6/11}$ (0.1-1μM) significantly enhances EDL isometric and tetanic twitch force in a dose-dependent manner. Figure 23 B depicts that systemic (i.p.) S100A1ct$_{6/11}$ administration in heart failure mice attenuates skeletal muscle dysfunction and significantly improves contractile performance (n = 5 muscles/animals in each group, *P<0.05 vs. corresponding control, †P<0.01 vs. control, 2-way ANOVA).

FIG. 25: Effect of S100A1 peptides N-75-85-C (amino acids 75 to 85 of human S100A1 protein set forth in SEQ ID NO: 1), N-76-85-C (amino acids 76 to 85 of human S100A1 protein set forth in SEQ ID NO: 1), N-77-85-C (amino acids 77 to 85 of human S100A1 protein set forth in SEQ ID NO: 1), N-78-85-C (amino acids 78 to 85 of human S100A1 protein set forth in SEQ ID NO: 1), N-79-85-C (amino acids 79 to 85 of human S100A1 protein set forth in SEQ ID NO: 1) on calcium transient amplitudes in field-stimulated (1 Hz) isolated rat ventricular cardiomyocytes. Note that N-75-85-C and N-76- 85-C have similar

potency in enhancing the calcium transient. Any further deletion of N-terminal amino acids abolishes the inotropic effect of the peptide. n equals the number of tested cells from three different preparations. * $P < 0.05$ vs. linker and vehicle, ANOVA.

DETAILED DESCRIPTION OF THE INVENTION

Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

In the following, the elements of the present invention will be described. These elements are listed with specific embodiments, however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise. For example, if in an embodiment of the peptide according to the present invention the muscle function enhancing amino acid sequence comprises, essentially consists or consists of the amino acid motif [V/I]-[V/I]-[L/M]-[V/I/M]-[A/G/S]-[A/V]-L-[T/A]-[V/A/I]-[A/M/V], and in another embodiment the hydrophilic motif comprises, essentially consists or consists of the hydrophilic amino acid motif Λ_4-Θ_2, wherein Λ and Θ are as defined herein below and the hydrophilic motif is preferably directly linked to the amino terminus of the amino acid motif comprised by the muscle function enhancing amino acid sequence, a peptide comprising the amino acid sequence Λ_4-Θ_2-[V/I]-[V/I]-[L/M]-[V/I/M]-[A/G/S]-[A/V]-L-[T/A]-[V/A/I]-[A/M/V] is an embodiment of the peptide according to the present invention.

Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", H.G.W. Leuenberger, B. Nagel, and H. Kölbl, Eds., Helvetica Chimica Acta, CH-4010 Basel, Switzerland, (1995).

To practice the present invention, unless otherwise indicated, conventional methods of chemistry, biochemistry, cell biology, and recombinant DNA techniques are employed which are explained in the literature in the field (cf., e.g., *Molecular Cloning: A Laboratory Manual*, 2nd Edition, J. Sambrook et al. eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1989). Furthermore, conventional methods of clinical cardiology are employed which are also explained in the literature in the field (cf., e.g., *Practical Methods in Cardiovascular Research*, S. Dhein et al. eds., Springer Verlag Berlin Heidelberg, 2005).

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps. As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural referents, unless the content clearly dictates otherwise.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether *supra* or *infra*, are hereby incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

A “peptide” according to the present invention refers to a chain of amino acid residues which may be naturally occurring or derivatives of naturally occurring amino acid residues and which are preferably linked via peptide bonds, wherein the peptide consists of not more than 100 amino acid residues or amino acid residue derivatives. The term “amino acid” encompasses naturally occurring amino acids as well as amino acid derivatives. A “small amino acid” in the context of the present invention is preferably an amino acid having a molecular weight of less than 125 Dalton. Preferably, a small amino acid in the context of the present invention is selected from the group consisting of the amino acids glycine, alanine, serine, cysteine, threonine, and valine, or derivatives thereof. A hydrophobic non-aromatic amino acid in the context of the present invention, is preferably any amino acid which has a Kyte-Doolittle hydropathy index of higher than 0,5, more preferably of higher than 1,0, even more preferably of higher than 1,5 and is not aromatic. Preferably, a hydrophobic non-aromatic amino acid in the context of the present invention, is selected from the group consisting of the amino acids alanine (Kyte Doolittle hydropathy index 1.8), methionine (Kyte Doolittle hydropathy index 1.9), isoleucine (Kyte Doolittle hydropathy index 4.5), leucine

Kyte Doolittle hydropathy index 3.8), and valine (Kyte Doolittle hydropathy index 4.2), or derivatives thereof having a Kyte Doolittle hydropathy index as defined above.

"Muscle" in the context of the present invention means preferably striated muscle tissue or muscle cells derived from striated muscle tissue such as skeletal muscle cells/tissue and cardiac muscle cells (cardiomyocytes) and cardiac muscle tissue.

According to the present invention, the term "muscle function enhancing amino acid sequence" refers to an amino acid sequence that is capable of enhancing and/or restoring any muscle-specific function, for example, enhancing the contractile performance of muscle cells and muscle tissue, preferably of striated muscle tissue, most preferably of cardiac and skeletal muscle cells and tissues. Since it is assumed that proper muscle function is tightly dependent on a functioning calcium handling within the muscle cell, the term "muscle function enhancing amino acid sequence" also refers to an amino acid sequence that is capable of enhancing and/or restoring the calcium handling/cycling, preferably the sarcoplasmic reticulum calcium handling/cycling in muscle cells, preferably skeletal muscle cells and/or cardiomyocytes. Contractile performance in myocytes can be directly measured, for example, by assessing single cardiomyocyte electrical field stimulated contractility using the video-edge-detection (VED) technique (Most et al., 2004, J. Clin. Invest. 114:1550-1563, page 1561). Calcium handling can be determined by assessing calcium transients using fluorescent calcium indicators (Most et al., 2004, J. Clin. Invest. 114:1550-1563, page 1561).

The term "enhancing" in the context of the present invention, e.g., enhancing muscle function, contractile performance, and/or calcium handling, means that the particular function is increased/enhanced independently of whether the function is normal or defective, i.e., the muscle cell is healthy or diseased. Preferably, "enhancing" means that the particular function is enhanced by at least 15%, preferably by at least 25%, preferably by at least 35%, more preferably by at least 45%, and most preferably by at least 50% compared to a control setting. Preferably the control setting is the muscle function, contractile performance, and/or calcium handling of a healthy patient or the average of a group of healthy patients.

The term "restoring" in the context of the present invention, e.g., restoring muscle function, contractile performance, and/or calcium handling, preferably means that a defective function is brought back to at least 50% of the normal function, preferably to at least 60% of the normal function, preferably to at least 70% of the normal function, more preferably to at least 80% of the normal function, more preferably to at least 85% of normal function, even more preferably to at least 90% of the normal function, even more preferably to at least 95% of the normal function, and most preferably to at least 100% of the normal function, wherein

"normal function" means an average value of the function exhibited by muscle cells derived from an individual who does not suffer from any muscle diseases. For example, in left ventricular catheterization, force development is assessed by the first derivative of pressure rise in the left ventricle, +dp/dt [mmHg/sec], in echocardiography, contractile performance is assessed by fractional shortening (FS%) in M-Mode or the calculated ejection fraction (EF%) (Most et al., 2004, J. Clin. Invest. 114: 1550-1563; Most et al., 2006, Circulation 114, 1258-1268, Material and Methods in on-line supplement), in VED, contractility is assessed by fractional shortening (FS%) and velocity of contraction (μm/sec). Calcium cycling can only be assessed in single cells – if calibrated – it is measured in nM free calcium concentrations. Roughly "normal" +dp/dt in anesthetized mice can range from 5000-8000 mmHg/sec, "normal" Echo EF% 60-80% / FS% 40-70%, "normal" cellular FS% can range from 5-12% and calibrated calcium transients might range from 200 to 400 nM.

The term "inotropic action" with respect to an agent means that said agent affects the force of muscle contraction irrespective of the muscle type. "Positive inotropic action" means that the force of muscle contraction is increased, wherein "negative inotropic action" means that the force of muscle contraction is decreased. The peptide of the present invention exhibits a positive inotropic action, preferably *in vitro* as well as *in vivo*. The inotropic effect of an agent, e.g., of the peptide of the present invention, can be readily determined *in vitro*, for example, by determining calcium transients in stimulated myocytes with and without the agent/peptide to be tested. For example, calcium transients can be assessed in FURA2-AM field-stimulated cardiomyocytes employing epifluorescence digitalized microscopy (Most et al., 2004, J. Clin. Invest. 114: 1550-1563, page 1561). Any fluorescent calcium indicator can be used instead of FURA-2AM such as a member of the Fluo calcium indicator family or Rhod-2AM. The underlying principal remains the same. Alternatively, calcium transient measurements in patch-clamped isolated cardiomyocytes (Kettlewell/Most et al., 2005, J. Mol. Cell. Cardiol., 200: 900-910, page 901) may also be used. The positive inotropic effect of a peptide can also be tested *in vivo*, for example, by determining the contractile performance by left ventricular catherization in anesthetized mice with and without administration of the peptide. Usually, in this experiment, contractility is described as the first derivative of maximal left ventricular pressure rise (+dp/dt max) (Most et al., 2004, J. Clin. Invest. 114: 1550-1563; Most et al., 2006, Circulation 114;1258-1268) Alternatively, echocardiography (Most et al., 2006, Circulation 114;1258-1268) can be used.

The term "enhancing and/or restoring calcium cycling" in the context of the present invention means that either calcium cycling in myocytes, preferably sarcoplasmic reticulum

calcium cycling, is improved under normal/non-pathological conditions or restored to normal function as specified above under pathological conditions, i.e., if calcium cycling is defective. Defective calcium cycling may be a result of reduced calcium content in the sarcoplasmic reticulum, reduced release of calcium from the sarcoplasmic reticulum during excitation-contraction coupling, calcium leakage from the sarcoplasmic reticulum in quiescent muscle cells, for example, due to leaky RyR sarcoplasmic reticulum calcium release channels, increased calcium spark frequency, or reduced/slowed re-uptake of calcium into the sarcoplasmic reticulum and/or the mitochondria after contraction, for example, due to a malfunctioning or non-functioning sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA). Therefore, according to the present invention the calcium cycling can preferably be enhanced or restored by improving said parameters, e.g., increasing sarcoplasmic reticulum calcium content, increasing release of calcium from the sarcoplasmic reticulum during excitation-contraction coupling, reducing calcium leakage from the sarcoplasmic reticulum in quiescent muscle cells, reducing calcium spark frequency, and/or improving calcium re-uptake into the sarcoplasmic reticulum or the mitochondria. Without being bound to this theory, it is assumed that defective calcium cycling is one of the major reasons for defective contractile performance, e.g., contractile dysfunction, of muscle cells. Thus, it is assumed that enhancing or restoring calcium cycling also enhances and/or restores contractile performance

In the context of the present invention, the term "contractile performance" encompasses any function that is associated with muscle contraction, for example, the force of muscle contraction or the timing of muscle contraction. In case of skeletal muscle tetanic contractions fall also within the term "contractile performance". "Defective contractile performance" refers to contractile dysfunction when compared to average values for normal/healthy muscle cells or tissue. For example, the contractile performance of a muscle cell or tissue is considered defective if, for example, the force of contraction of a given muscle cell or tissue deviates from the average value for normal/healthy muscle cells or tissue by at least 10%, preferably at least 20%, preferably at least 30%, more preferably at least 40%, and most preferably at least 50%, wherein the term "deviate" can refer to values less than the normal average value or to values higher than the normal average value, preferably it refers to values less than the normal average value. For example, for conscious humans an echocardiographic cardiac EF% below 50% is considered as beginning heart failure. Normal human cardiac conscious EF% is around 65-70%. Preferably, the term "enhancing and/or restoring contractile performance" means the increase of contractile force of muscle cells or tissue, preferably skeletal muscle cells or tissue or cardiac muscle cells or tissue, as well as

the correction of defective timing of muscle cell contractions. In this context, the term "defective timing" refers to inappropriately timed muscle contraction events such as arrhythmias in the heart muscle or tremor or twitching of skeletal muscle tissue.

"Anti-arrhythmic potential" in the context of the peptide according to the present invention means that the peptide is capable of reducing inappropriately timed muscle contractions, i.e., arrhythmic events in myocytes, preferably in cardiomyocytes and cardiac tissue. The peptide of the present invention preferably protects cardiomyocytes from pro-arrhythmic store overload-induced calcium release (SOICR) which is a critical pathomechanism underlying arrhythmogenic sudden cardiac death, e.g., by lethal ventricular arrhythmias. In a preferred embodiment, the peptide according to the present invention combines the inotropic action with protection from arrhythmias, preferably calcium-induced arrhythmias in cardiomyocytes. The skilled person can readily determine whether a peptide exhibits anti-arrhythmic potency, for example, by assessing whether the peptide to be tested is capable of protecting cardiomyocytes, preferably ventricular cardiomyocytes, with leaky RyR2s sensitized to luminal calcium from βAR-triggered pro-arrhythmogenic SOICR and calcium waves. For example, normal ventricular cardiomyocytes may be treated with 10^{-7} M Isoproterenol and 0.5 mM caffeine with and without the peptide potentially exhibiting anti-arrhythmic potency and monitor the diastolic calcium concentration. In failing cardiomyocytes treatment with 10^{-7} M Isoproterenol or an equi-effective catecholamine (e.g., dobutamine, noradrenaline, adrenaline) alone can be used to unmask pro-arrhythmic molecular alterations with respect to calcium handling. In addition, other agents enhancing the β-adrenergic receptor downstream second messenger cyclic adenosine mono-phosphate (cAMP) such as phosphodiesterase inhibitors (rolipram, enoximon) at appropriate equi-effective dosages can be used with or without caffeine. SOICR can be identified by confocal microscopic calcium wave and spark measurements in fluorescent calcium indicator loaded quiescent cardiomyocytes (Voelkers et al., 2007, Cell Calcium 41:135-143, page 136) or as diastolic calcium waves/release in fluorescent calcium indicator loaded electrical field stimulated (Most et al., 2004, J. Clin. Invest. 114:1550-1563) and patch clamped (Kettlewell et al., 2005, J. Mol. Cell. Cardiol. 39:900-910, page 901) cardiomyocytes by epifluorescent microscopy. Alternatively, SOICR and calcium wave equivalents such as delayed or early after-contractions can be assessed by diastolic contractions in electrical field stimulated cardiomyocytes by VED (Most et al., 2004, J. Clin. Invest. 114:1550-1563).

In the context of the present invention, the term "carboxy-terminal amino acids of an S100 protein" preferably refers to the carboxy-terminal 20 amino acids of an S100 protein,

e.g., to amino acids 75 to 94 of the amino acid sequence set forth in SEQ ID NO: 1, i.e., the amino acid sequence Y-V-V-L-V-A-A-L-T-V-A-C-N-N-F-F-W-E-N-S (SEQ ID NO: 2), more preferably to the carboxy-terminal 25 amino acids of an S100 protein, and most preferably to the carboxy-terminal 30 amino acids of an S100 protein.

The term "capable of penetrating cell membranes" in the context of the peptide according to the present invention means that the peptide is able to traverse cell membranes of intact cells, wherein preferably the cell is a vertebrate cell, more preferably a mammalian cell, such as a mouse, rat, goat, sheep, dog, cat, pig, cow, or horse cell etc., most preferably a human cell, Preferably, a cell in the context of the present invention is a muscle cell, preferably a skeletal muscle cell or a cardiomyocyte. Thus, most preferably the cell in the context of the present invention is a mammalian muscle cell. The skilled person can readily assess whether a peptide is capable of penetrating cell membranes, e.g., by labeling said peptide, for example, with a radioactive or fluorescent marker, and incubating the labeled peptide with intact cells, preferably mammalian muscle cells, for example, rat ventricular cardiomyocytes, and assessing whether the labeled peptide can be detected inside the cells, for example, in the cytoplasm of the intact cells, e.g., by fluorescence microscopy (Most et al., 2005, J. Cell Sci. 118:421-431, page 422; Voelkers et al., 2007, Cell Calcium 41:135-143, page 136).

An S100 calcium binding protein in the context of the present invention is preferably selected from the group consisting of S100 calcium binding protein A1, S100 calcium binding protein Z, S100 calcium binding protein T, S100 calcium binding protein S, and the S100 protein α-chain. Most preferably the S100 calcium binding protein in the context of the present invention is S100 A1. The S100 calcium binding protein in the context of the present invention may be of any species, for example, human or other primate, mouse, or rat S100 protein etc, and is preferably of human origin. Preferred examples of S100 calcium binding proteins are those accessible by the following GenBank or Ref Seq accession numbers: XP_001494920.1, XP_001365057.1, XP_001140144, XP_513820.2, XP_001111052.1, CAI19674.1, XP_537265.1, NP_001092512.1, NP_006262.1, NP_001127319.1, AAB20539.2, NP_001007637.1, NP_035439.1, XP_002196029.1, XP_001332692.1, NP_001082820.1, XP_001504000.2, NP_570128.2, XP_526887.2, XP_226710.1, XP_607154.2, XP_853219.1, NP_001074628.1, NP_001013513.1, AAN63527.1, ACI68060.1, and XP_001344575.2.

In the context of the present invention the term "treating" a disease or disorder means that a disease condition is ameliorated independently whether the cause of the disease is

eliminated, i.e., the individual having the disease is cured, or only the symptoms are diminished. Thus, even though it is assumed that the peptide according to the present invention exerts its therapeutic effects by stabilizing and/or restoring the calcium cycling/handling in muscle cells, and thereby, improving contractile performance of said cells, the peptide may also be used for the treatment of muscle diseases which are not caused by defective calcium cycling. For example, the symptoms of a skeletal muscle disorder, such as muscle weakness, which are not caused by or are not associated with defective calcium cycling in the muscle cells, are also diminished by the peptide according to the present invention.

The term "individual" in the context of the present invention preferably refers to an animal patient, preferably suffering from a cardiac muscle disorder or a skeletal muscle disorder or suffering from both. An animal patient is preferably a vertebrate patient, more preferably a mammalian patient, such as a domesticated animal, e.g., a mouse, rat, cat, guinea pig, rabbit, dog, pig, cow, or horse. Most preferably an animal patient is a human patient and the term "individual" refers to a human patient suffering from a muscle disorder, preferably from a cardiac muscle disorder and/or a skeletal muscle disorder. In the context of assessing functional features of the peptide according to the present invention, the term "individual" preferably refers to an experimental animal, such as a mouse, rat, rabbit, or primate, most preferably said term in this context refers to a heart failure animal model such as the post-myocardial infarction mouse or rat model (mouse: Most et al., 2006, Circulation 114:1258-1268, supplement; rat: Most et al., 2004, J. Clin. Invest. 114:1550-1563).

In a first aspect, the present invention provides a peptide comprising a muscle function enhancing amino acid sequence comprising, essentially consisting or consisting of the amino acid motif

$$\Phi_4\text{-X-}\Psi\text{-L-[T/A]-}\Psi_2,$$

wherein Φ and Ψ are in each instance an independently selected hydrophobic non-aromatic amino acid, and X is any amino acid, preferably a small amino acid, wherein the muscle function enhancing amino acid sequence does not contain more than 18 continuous amino acids, e.g., not more than 18, 17, 16, 15, 14, 13, 12, 11 or 10 continuous amino acids, of the carboxy-terminal amino acids of an S100 calcium binding protein A1, the peptide has a total length of maximally 100 amino acids, e.g., maximally 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, or 10 amino acids, and the peptide exhibits a positive inotropic action. L refers to the amino acid leucine, T to the amino acid threonine, and A to the amino acid alanine. In a preferred embodiment, the peptide of the

present invention has a length of between 10 and 80 amino acids, more preferably of between 10 and 70 amino acids, more preferably of between 10 and 60 amino acids, more preferably between 10 and 50 amino acids, even more preferably between 10 and 40 amino acids, even more preferably between 10 and 30 amino acids, most preferably the peptide has a length of between 10 and 20 amino acids. In a preferred embodiment, the peptide is 15 or 16 amino acids long.

Preferably, the peptide according to the present invention with the exception of the muscle function enhancing amino acid sequence significantly differs from the carboxy-terminal amino acids of an S100 calcium binding protein A1, preferably from an S100 calcium binding protein selected from the group consisting of S100 calcium binding protein A1, S100 calcium binding protein Z, S100 calcium binding protein T, S100 calcium binding protein S, and S100 protein α-chain, and most preferably significantly differs from the carboxy-terminus of any S100 calcium binding protein. More preferably, the peptide according to the present invention with the exception of the muscle function enhancing amino acid sequence significantly differs from the amino acid sequence of an S100 calcium binding protein A1, preferably from an S100 calcium binding protein selected from the group consisting of S100 calcium binding protein A1, S100 calcium binding protein Z, S100 calcium binding protein T, S100 calcium binding protein S, and S100 protein α-chain, and most preferably significantly differs from the amino acid sequence of any S100 calcium binding protein. The term "significantly differs" means that the amino acid sequences are at least 80% different, more preferably at least 85%, more preferably at least 90%, even more preferably at least 95%, and most preferably 100% different. The difference in the sequences may be assessed by aligning the polypeptide sequences. Such alignment tools are well known to the person skilled in the art and can be, for example, obtained on the World Wide Web, e.g., ClustalW (www.ebi.ac.uk/clustalw) or Align (http://www.ebi.ac.uk/ emboss/align/ index.html) using standard settings, preferably for Align EMBOSS::needle, Matrix: Blosum62, Gap Open 10.0, Gap Extend 0.5. Residues in two or more polypeptide sequences are said to differ from each other if the residues which are aligned in the best sequence alignment differ from each other. The "best sequence alignment" between two polypeptides is defined as the alignment that produces the largest number of aligned identical residues.

In a preferred embodiment of the peptide according to the present invention, the muscle function enhancing amino acid sequence comprises, essentially consists or consists of the amino acid motif

$$[Y/F/W]\text{-}\Phi_4\text{-}X\text{-}\Psi\text{-}L\text{-}[T/A]\text{-}\Psi_2,$$

wherein Φ and Ψ are in each instance an independently selected hydrophobic non-aromatic amino acid, and X is any amino acid, preferably a small amino acid. The hydrophobic non-aromatic amino acid and the small amino acid are as defined above.

In a preferred embodiment, the muscle function enhancing amino acid sequence of the peptide according to the present invention forms an α-helical structure.

In a particularly preferred embodiment, the peptide of the present invention is capable of penetrating cell membranes, preferably vertebrate cell membranes, even more preferably mammalian cell membranes, even more preferably mammalian muscle cell membranes, and most preferably mammalian skeletal muscle cell membranes and membranes of mammalian cardiomyocytes. Preferably, the peptide of the present invention is capable of penetrating cell membranes as defined above in a physiological environment such as in culture medium, for example, for mammalian tissue culture, and/or in body fluids such as in blood. Thus, most preferably, the peptide of the present invention is capable of penetrating cell membranes *in vivo* when it is administered by a parenteral administration route such as by intravenous injection.

In a preferred embodiment, the peptide according to the present invention does not contain more than 18, 17, 16, 15, 14, 13, 12, 11, or 10 continuous amino acids of the carboxy-terminal amino acids of an S100 calcium binding protein selected from the group consisting of S100 calcium binding protein A1, S100 calcium binding protein Z, S100 calcium binding protein T, S100 calcium binding protein S, and S100 protein α-chain, wherein the S100 calcium binding protein is preferably of human origin, most preferably of any species. Thus, preferably the peptide according to the present invention preferably does not contain more than 18, 17, 16, 15, 14, 13, 12, 11, or 10 continuous amino acids of the carboxy-terminal amino acids of an S100 calcium binding protein A1, does not contain more than 18, 17, 16, 15, 14, 13, 12, 11, or 10 continuous amino acids of the carboxy-terminal amino acids of an S100 calcium binding protein Z, does not contain more than 18, 17, 16, 15, 14, 13, 12, 11, or 10 continuous amino acids of the carboxy-terminal amino acids of an S100 calcium binding protein T, does not contain more than 18, 17, 16, 15, 14, 13, 12, 11, or 10 continuous amino acids of the carboxy-terminal amino acids of an S100 calcium binding protein S, and does not contain more than 18, 17, 16, 15, 14, 13, 12, 11, or 10 continuous amino acids of the carboxy-terminal amino acids of an S100 protein alpha chain, wherein the S100 calcium binding protein is preferably of human origin, most preferably of any species. In a most preferred embodiment, the peptide of the present does not contain more than 18, 17, 16, 15, 14, 13, 12, 11, or 10 continuous amino acids of the carboxy-terminal amino acids of any S100 calcium

binding protein preferably of human origin, more preferably of any species. In particular, the peptide according to the present invention does not comprise or consist of the sequence Y-V-V-L-V-A-A-L-T-V-A-C-N-N-F-F-W-E-N-S (SEQ ID NO: 2), i.e., amino acids 75 to 94 of the amino acid sequence set forth in SEQ ID NO: 1.

In a particular preferred embodiment, the peptide of the present invention exhibits the ability to enhance contractile performance and/or calcium cycling in myocytes, preferably in skeletal muscle cells or cardiomyocytes.

In a particularly preferred embodiment, the peptide of the present invention exhibits anti-arrhythmic potential on myocytes, preferably on cardiomyocytes, and thus, is preferably capable of protecting myocytes and heart tissue from arrhythmias, preferably from catecholamine triggered arrhythmias, preferably from ventricular arrhythmias which frequently are the cause of sudden cardiac death. Preferably, the peptide of the present invention exhibits the anti-arrhythmic potential *in vitro* as well as *in vivo*. Preferably, the peptide of the present invention exhibits the ability of protecting an individual from lethal ventricular tachyarrhythmias, preferably from β-adrenergic receptor (βAR) triggered lethal ventricular tachyarrhythmias, preferably from catecholamine triggered lethal ventricular tachyarrhythmias. Preferably, the *in vivo* anti-arrhythmic potential is observed when the peptide is administered via a parenteral administration route. The anti-arrhythmic potential of a peptide can be assessed *in vitro*, for example, by examining whether the peptide protects cardiomyocytes from SOICR as described above. The anti-arrhythmic potential of a peptide can be assessed *in vivo*, for example, by examining the effect of a treatment with the peptide on mortality caused by βAR triggered tachyarrhythmias in a heart failure animal model, for example, in a post myocardial infarction mouse model (Most et al., 2006, Circulation 114:1258-1268, supplement). For example, the peptide may be administered to mice with postischemic contractile dysfunction, preferably parenterally, such as intraperitoneally, intravenously, or subcutaneously, daily or every second day for several days, such as 6, 7, 8, or 9 days, up to a few weeks, such as 2, 3, or 4 weeks, preferably 2 weeks. The lethal ventricular tachyarrhythmias may be triggered in the animals after a certain period of treatment with the peptide, for example after 7, 8, 9, 10 days or after 2 weeks, by administration of epinephrine, e.g., at a concentration in the range of 1.5 to 2.5 mg/kg, preferably at a concentration of 2 mg/kg, in combination of caffeine, e.g., at a concentration in the range of 100 to 140 mg/kg, preferably at a concentration of 120 mg/kg. The lethal ventricular fibrillation may be monitored by telemetric ECG (cf., for example, Xiao et al., 2007, J. Biol. Chem. 282:34828-34838).

In another preferred embodiment, the peptide of the present invention has the ability to reduce calcium spark frequency in myocytes such as skeletal muscle cells and cardiomyocytes, preferably in cardiomyocytes. Preferably, the peptide of the present invention exhibits the ability to reduce calcium spark frequency *in vitro* as well as *in vivo*. Preferably, the *in vivo* effect is observed when the peptide is administered via a parenteral administration route. "Reducing" in this context preferably means that the calcium spark frequency in myocytes treated with the peptide is at least 15%, more preferably at least 25%, even more preferably at least 30%, and most preferably at least 40% reduced compared to control myocytes that have not been treated with the peptide. Preferably, this ability is dependent on the concentration of the peptide applied to the cardiomyocytes. Preferably, the peptide of the invention has the ability of reducing calcium spark frequency in intact cardiomyocytes when added to the liquid in which the cardiomyocytes are present. For example, the peptide of the present invention preferably reduces calcium spark frequency in quiescent cardiomyocytes, e.g., in cultured quiescent rat ventricular cardiomyocytes, when added to the medium of the cardiomyocytes at a concentration in the range of 50 nM to 500 nM, preferably, when applied at a concentration in the range of 50 nM to 250 nM, more preferably when applied at a concentration in the range of 75 to 150 nM, and most preferably when applied at 100 nM, whereas the calcium spark frequency is increased when applied at a concentration of 600 nM or higher, preferably at a concentration of 700 mM or higher, more preferably at a concentration of 800 nM or higher, even more preferably at a concentration of 900 nM or higher, and most preferably at a concentration of 1000 nM or higher (Voelkers M. et al., 2007, Cell Calcium 41:135-143). Thus, the skilled person can readily determine whether a peptide has the ability to reduce calcium spark frequency. In a particularly preferred embodiment, the peptide of the present invention exhibits an anti-arrhythmic potential and the ability to reduce calcium spark frequency as described above.

In another preferred embodiment, the peptide of the present invention protects myocytes, preferably skeletal muscle cells and/or cardiomyocytes from apoptotic cell death, preferably from calcium-induced apoptotic cell death, preferably from sarcoplasmic reticulum calcium leakage triggered apoptotic cell death. Thus, preferably, the peptide of the present invention exhibits anti-apoptotic potential. Preferably, the peptide of the present invention exhibits this anti-apoptotic effect *in vitro* as well as *in vivo*. Preferably, the peptide of the present invention prevents apoptotic cell death in failing myocardium *in vivo*, i.e., protects cardiomyocytes in failing myocardium from apoptotic cell death *in vivo*. Preferably, the *in vivo* protective effect is observed when the peptide is administered via a parenteral

administration route. "Protecting" in this context means that the extent of apoptotic cell death is reduced in the cells treated with the peptide according to the present invention compared to a control group by at least 20%, preferably by at least 30%, more preferably by at least 40%, and even more preferably by at least 50%, and most preferably by at least 60%. The skilled person is able to test for this feature *in vitro*, for example, by observing the extent of apoptosis in myocytes, preferably in ventricular cardiomyocytes, with leaky RyR calcium release channels that are sensitized to luminal calcium by long-term caffeine exposure with and without the peptide. An indication for apoptosis is, for example, a fragmented genome which can be examined, e.g., by DNA laddering (Liu et al., 2005, Circulation 111:90-96), cytochrom-c release, or caspase 3 activity (Most et al., 2003, J. Biol. Chem. 278:48404-48412). The anti-apoptotic effect of a peptide may be assessed *in vivo* in an experimental heart failure animal model. For example, mice with postischemic contractile dysfunction may be treated with the peptide and cardiac tissue of treated and control mice may be assessed for the extent of apoptotic cardiomyocytes. The peptide may be administered preferably parenterally, such as intraperitoneally, intravenously, or subcutaneously, daily or every second day for several days, such as 6, 7, 8, or 9 days, up to a few weeks, such as 2, 3, or 4 weeks, preferably 2 weeks. The extent of apoptotic cells may be assessed by TUNEL staining of TnI and CD31 counterstained heart tissue sections (Most et al., 2006, Circulation 114:1258-1268). In a particularly preferred embodiment, the peptide of the invention exhibits anti-arrhythmic potential and protects myocytes from apoptotic cell death as described above.

In another preferred embodiment, the peptide of the present invention has the ability to prevent and/or reduce calcium leakage from the sarcoplasmic reticulum, preferably in quiescent myocytes such as skeletal muscle cells and cardiomyocytes. Preferably, the peptide of the present invention exhibits this effect *in vitro* and *in vivo*. Preferably, this *in vivo* effect is observed when the peptide is administered via a parenteral administration route. Without being bound by this theory, it is assumed that the peptide of the present invention stabilizes RyR sarcoplasmic reticulum calcium release channels in their closed conformation, and thereby reduces calcium leakage from these channels (Most et al., 2006, Circulation 114:1258-1268; Voelkers M. et al., 2007, Cell Calcium 41:135-143). In a particularly preferred embodiment, the peptide of the present invention exhibits anti-arrhythmic potential and prevents and/or reduces calcium leakage as described above.

In another preferred embodiment, the peptide of the present invention exhibits the ability of restoring hemodynamic function *in vivo*. Preferably, the peptide of the present invention restores hemodynamic function in an individual suffering from heart failure such as

during or after myocardial infarction. Preferably, this effect is observed when the peptide is administered via a parenteral administration route. The skilled person can readily test a peptide for this function, e.g., by using an experimental mouse heart failure model. For example, the skilled person may determine cardiac performance and survival rate in mice with postischemic contractile dysfunction with and without administration of the peptide. The peptide may be administered preferably parenterally, such as intraperitoneally, intravenously, or subcutaneously, daily or every second day for several days, such as 6, 7, 8, or 9 days, up to a few weeks, such as 2, 3, or 4 weeks, preferably 2 weeks. The left ventricular performance of the experimental animals may be assessed by serial echocardiography (Most et al., 2003, J. Bio. Chem. 278; 33809-33817; Most et al., 2006, Circulation 114:1258-1268). Preferably, the peptide of the present invention exhibits anti-arrhythmic potency and the ability of restoring hemodynamic function *in vivo*.

In another preferred embodiment, the peptide of the present invention enhances the isometric and/or tetanic twitch force in skeletal muscle tissue, such as skeletal muscle fibers. Preferably, the peptide of the present invention exhibits this effect *in vitro* and *in vivo*. Preferably, this *in vivo* effect is observed when the peptide is administered via a parenteral administration route. The skilled person can readily assess this function for a given peptide, for example, by isometric tension measurement in peptide treated and untreated intact muscles or muscle fibers, e.g., intact extensor digitorum longum skeletal muscles, isolated from an experimental animal. For example, the isolated muscle may be incubated for a certain period of time, such as 30 to 60 minutes, preferably 45 minutes, with the peptide at different concentrations, for example at a concentration in the range of 500 nM to 4 μM, preferably at a concentration of 1 μM. The isolated muscle may then be stimulated with a tetanic train, for example, applied at 125 Hz for 175 ms and the isometric tension may be measured (Weisleder et al., 2006, J. Cell Biol. 174:639-654). Preferably, the enhancing effect on isometric and/or tetanic twitch force is also observed for muscle fibers isolated from an experimental animal which was treated systemically with the peptide, wherein preferably the peptide was administered parenterally. Thus, in a preferred embodiment, the peptide of the present invention attenuates skeletal muscle dysfunction and enhances contractile performance in skeletal muscle cells *in vivo* when administered systemically, preferably parenterally, such as intraperitoneally, intravenously, or subcutaneously. In a particularly preferred embodiment, the peptide of the present invention exhibits the ability to enhance isometric and tetanic twitch force in skeletal muscle cells, the ability to increase contractile performance in cardiomyocytes, and the anti-arrhythmic potential described above.

In a particularly preferred embodiment, the peptide of the present invention exhibits two or more, e.g., 2, 3, 4, or 5, preferably all of the above functions, i.e., anti-arrhythmic potential, anti-apoptotic potential, the ability to reduce calcium spark frequency, the ability to prevent and/or reduce calcium leakage from the sarcoplasmic reticulum, the ability to restore hemodynamic function preferably in an individual suffering from heart failure, and the ability to enhance isometric and/or tetanic twitch force in skeletal muscle cells and/or fibers. Preferably, said functions can be observed *in vitro* and *in vivo*. Preferably, said *in vivo* effects can be observed when the peptide is administered via a parenteral administration route.

In a preferred embodiment of the peptide according to the present invention, Φ is in each instance independently selected from the group of amino acids consisting of alanine, methionine, isoleucine, leucine, and valine, preferably Φ is in each instance independently selected from methionine, isoleucine, leucine, and valine.

In another preferred embodiment of the peptide according to the present invention, Ψ is in each instance independently selected from the group of amino acids consisting of alanine, methionine, isoleucine, leucine, and valine, preferably Ψ is in each instance independently selected from alanine, methionine, isoleucine, and valine. In a particularly preferred embodiment of the peptide according to the present invention, Φ is in each instance independently selected from methionine, isoleucine, leucine, and valine, and Ψ is in each instance independently selected from alanine, methionine, isoleucine, and valine.

In another preferred embodiment of the peptide according to the present invention, X is a small amino acid, wherein the small amino acid is preferably not proline. Preferably, X is selected from the group of amino acids consisting of glycine, alanine, serine, cysteine, threonine, and valine, more preferably X is selected from the group consisting of glycine, alanine, and serine. In a particularly preferred embodiment of the peptide according to the present invention, Φ is in each instance independently selected from methionine, isoleucine, leucine, and valine, Ψ is in each instance independently selected from alanine, methionine, isoleucine, and valine, and X is selected from glycine, alanine, serine, cysteine, threonine, and valine, preferably from glycine, alanine, and serine.

In a particularly preferred embodiment of the peptide according to the present invention, the muscle function enhancing amino acid sequence comprises, essentially consists or consists of the amino acid sequence [V/I]-[V/I]-[L/M]-[V/I/M]-[A/G/S]-[A/V]-L-[T/A]-[V/A/I]-[A/M/V]. This means that the amino acid motif is preferably the sequence [V/I]-[V/I]-[L/M]-[V/I/M]-[A/G/S]-[A/V]-L-[T/A]-[V/A/I]-[A/M/V].

In a particularly preferred embodiment of the peptide according to the present invention, the muscle function enhancing amino acid sequence comprises or consists of the amino acid sequence V^1-V^2-L^3-V^4-A^5-A^6-L^7-T^8-V^9-A^{10} (SEQ ID NO: 3), wherein V^1 and V^2 may be independently replaced by isoleucine, preferably V^1 is not replaced, L^3 may be replaced by methionine, but is preferably not replaced, V^4 may be replaced by isoleucine or methionine, preferably by isoleucine, A^5 may be replaced by glycine or serine, preferably by serine, A^6 may be replaced by valine, T^8 may be replaced by alanine, V^9 may be replaced by alanine or isoleucine, preferably by alanine, and A^{10} may be replaced by methionine or valine, but is preferably not replaced, wherein preferably maximally 5, preferably maximally 4, more preferably maximally 3, even more preferably maximally 2, and most preferably maximally 1 amino acid of the amino acid sequence V^1-V^2-L^3-V^4-A^5-A^6-L^7-T^8-V^9-A^{10} are/is replaced with another amino acid as specified above. In a preferred embodiment of the peptide according to the present invention, maximally 3, e.g., 1, 2, or 3 amino acids are replaced in the most preferred amino acid sequence, i.e., V-V-L-V-A-A-L-T-V-A (SEQ ID NO: 4) as described above.

In a particular preferred embodiment of the peptide according to the present invention, the muscle function enhancing amino acid sequence comprises or consists of an amino acid sequence selected from the group consisting of V-V-L-V-A-A-L-T-V-A (SEQ ID NO: 4), I-V-L-V-A-A-L-T-V-A (SEQ ID NO: 5), V-I-L-V-A-A-L-T-V-A (SEQ ID NO: 6), V-V-M-V-A-A-L-T-V-A (SEQ ID NO: 7), V-V-L-I-A-A-L-T-V-A (SEQ ID NO: 8), V-V-L-M-A-A-L-T-V-A (SEQ ID NO: 9), V-V-L-V-G-A-L-T-V-A (SEQ ID NO: 10), V-V-L-V-S-A-L-T-V-A (SEQ ID NO: 11), V-V-L-V-A-V-L-T-V-A (SEQ ID NO: 12), V-V-L-V-A-A-L-A-V-A (SEQ ID NO: 13), V-V-L-V-A-A-L-T-A-A (SEQ ID NO: 14), V-V-L-V-A-A-L-T-I-A (SEQ ID NO: 15), V-V-L-V-A-A-L-T-V-M (SEQ ID NO: 16), V-V-L-V-A-A-L-T-V-V (SEQ ID NO: 17), I-I-L-V-A-A-L-T-V-A (SEQ ID NO: 18), I-V-M-V-A-A-L-T-V-A (SEQ ID NO: 19), I-V-L-I-A-A-L-T-V-A (SEQ ID NO: 20), I-V-L-M-A-A-L-T-V-A (SEQ ID NO: 21), I-V-L-V-G-A-L-T-V-A (SEQ ID NO: 22), I-V-L-V-S-A-L-T-V-A (SEQ ID NO: 23), I-V-L-V-A-V-L-T-V-A (SEQ ID NO: 24), I-V-L-V-A-A-L-A-V-A (SEQ ID NO: 25), I-V-L-V-A-A-L-T-A-A (SEQ ID NO: 26), I-V-L-V-A-A-L-T-I-A (SEQ ID NO: 27), I-V-L-V-A-A-L-T-V-M (SEQ ID NO: 28), I-V-L-V-A-A-L-T-V-V (SEQ ID NO: 29), V-I-M-V-A-A-L-T-V-A (SEQ ID NO: 30), V-I-L-I-A-A-L-T-V-A (SEQ ID NO: 31), V-I-L-M-A-A-L-T-V-A (SEQ ID NO: 32), V-I-L-V-G-A-L-T-V-A (SEQ ID NO: 33), V-I-L-V-S-A-L-T-V-A (SEQ ID NO: 34), V-I-L-V-A-V-L-T-V-A (SEQ ID NO: 35), V-I-L-V-A-A-L-A-V-A (SEQ ID NO: 36), V-I-L-V-A-A-L-T-A-A (SEQ ID NO: 37), V-I-L-V-A-A-L-T-I-A (SEQ ID NO: 38), V-I-L-V-A-A-L-

T-V-M (SEQ ID NO: 39), V-I-L-V-A-A-L-T-V-V (SEQ ID NO: 40),V-V-M-I-A-A-L-T-V-A (SEQ ID NO: 41), V-V-M-M-A-A-L-T-V-A (SEQ ID NO: 42), V-V-M-V-G-A-L-T-V-A (SEQ ID NO: 43), V-V-M-V-S-A-L-T-V-A (SEQ ID NO: 44), V-V-M-V-A-V-L-T-V-A (SEQ ID NO: 45), V-V-M-V-A-A-L-A-V-A (SEQ ID NO: 46), V-V-M-V-A-A-L-T-A-A (SEQ ID NO: 47), V-V-M-V-A-A-L-T-I-A (SEQ ID NO: 48), V-V-M-V-A-A-L-T-V-M (SEQ ID NO: 49), V-V-M-V-A-A-L-T-V-V (SEQ ID NO: 50), V-V-L-I-G-A-L-T-V-A (SEQ ID NO: 51), V-V-L-I-S-A-L-T-V-A (SEQ ID NO: 52), V-V-L-I-A-V-L-T-V-A (SEQ ID NO: 53), V-V-L-I-A-A-L-A-V-A (SEQ ID NO: 54), V-V-L-I-A-A-L-T-A-A (SEQ ID NO: 55), V-V-L-I-A-A-L-T-I-A (SEQ ID NO: 56), V-V-L-I-A-A-L-T-V-M (SEQ ID NO: 57), V-V-L-I-A-A-L-T-V-V (SEQ ID NO: 58), V-V-L-M-G-A-L-T-V-A (SEQ ID NO: 59), V-V-L-M-S-A-L-T-V-A (SEQ ID NO: 60), V-V-L-M-A-V-L-T-V-A (SEQ ID NO: 61), V-V-L-M-A-A-L-A-V-A (SEQ ID NO: 62), V-V-L-M-A-A-L-T-A-A (SEQ ID NO: 63), V-V-L-M-A-A-L-T-I-A (SEQ ID NO: 64), V-V-L-M-A-A-L-T-V-M (SEQ ID NO: 65), V-V-L-M-A-A-L-T-V-V (SEQ ID NO: 66), V-V-L-V-G-V-L-T-V-A (SEQ ID NO: 67), V-V-L-V-G-A-L-A-V-A (SEQ ID NO: 68), V-V-L-V-G-A-L-T-A-A (SEQ ID NO: 69), V-V-L-V-G-A-L-T-I-A (SEQ ID NO: 70), V-V-L-V-G-A-L-T-V-M (SEQ ID NO: 71), V-V-L-V-G-A-L-T-V-V (SEQ ID NO: 72), V-V-L-V-S-V-L-T-V-A (SEQ ID NO: 73), V-V-L-V-S-A-L-A-V-A (SEQ ID NO: 74), V-V-L-V-S-A-L-T-A-A (SEQ ID NO: 75), V-V-L-V-S-A-L-T-I-A (SEQ ID NO: 76), V-V-L-V-S-A-L-T-V-M (SEQ ID NO: 77), V-V-L-V-S-A-L-T-V-V (SEQ ID NO: 78), V-V-L-V-A-V-L-A-V-A (SEQ ID NO: 79), V-V-L-V-A-V-L-T-A-A (SEQ ID NO: 80), V-V-L-V-A-V-L-T-I-A (SEQ ID NO: 81), V-V-L-V-A-V-L-T-V-M (SEQ ID NO: 82), V-V-L-V-A-V-L-T-V-V (SEQ ID NO: 83), V-V-L-V-A-A-L-A-A-A (SEQ ID NO: 84), V-V-L-V-A-A-L-A-I-A (SEQ ID NO: 85), V-V-L-V-A-A-L-A-V-M (SEQ ID NO: 86), V-V-L-V-A-A-L-A-V-V (SEQ ID NO: 87), V-V-L-V-A-A-L-T-A-M (SEQ ID NO: 88), V-V-L-V-A-A-L-T-A-V (SEQ ID NO: 89), V-V-L-V-A-A-L-T-I-M (SEQ ID NO: 90), V-V-L-V-A-A-L-T-I-V (SEQ ID NO: 91), I-I-M-V-A-A-L-T-V-A (SEQ ID NO: 92), I-I-L-I-A-A-L-T-V-A (SEQ ID NO: 93), I-I-L-M-A-A-L-T-V-A (SEQ ID NO: 94), I-I-L-V-G-A-L-T-V-A (SEQ ID NO: 95), I-I-L-V-S-A-L-T-V-A (SEQ ID NO: 96), I-I-L-V-A-V-L-T-V-A (SEQ ID NO: 97), I-I-L-V-A-A-L-A-V-A (SEQ ID NO: 98), I-I-L-V-A-A-L-T-A-A (SEQ ID NO: 99), I-I-L-V-A-A-L-T-I-A (SEQ ID NO: 100), I-I-L-V-A-A-L-T-V-M (SEQ ID NO: 101), I-I-L-V-A-A-L-T-V-V (SEQ ID NO: 102), I-V-M-I-A-A-L-T-V-A (SEQ ID NO: 103), I-V-M-M-A-A-L-T-V-A (SEQ ID NO: 104), I-V-M-V-G-A-L-T-V-A (SEQ ID NO: 105), I-V-M-V-S-A-L-T-V-A (SEQ ID NO: 106), I-V-M-V-A-V-L-T-V-A (SEQ ID NO: 107), I-V-M-V-A-A-L-A-V-A (SEQ ID NO: 108), I-V-M-V-A-A-L-T-A-A (SEQ ID NO: 109), I-V-M-V-A-A-L-T-I-A

(SEQ ID NO: 110), I-V-M-V-A-A-L-T-V-M (SEQ ID NO: 111), I-V-M-V-A-A-L-T-V-V (SEQ ID NO: 112), I-V-L-I-G-A-L-T-V-A (SEQ ID NO: 113), I-V-L-I-S-A-L-T-V-A (SEQ ID NO: 114), I-V-L-I-A-V-L-T-V-A (SEQ ID NO: 115), I-V-L-I-A-A-L-A-V-A (SEQ ID NO: 116), I-V-L-I-A-A-L-T-A-A (SEQ ID NO: 117), I-V-L-I-A-A-L-T-I-A (SEQ ID NO: 118), I-V-L-I-A-A-L-T-V-M (SEQ ID NO: 119), I-V-L-I-A-A-L-T-V-V (SEQ ID NO: 120), I-V-L-M-G-A-L-T-V-A (SEQ ID NO: 121), I-V-L-M-S-A-L-T-V-A (SEQ ID NO: 122), I-V-L-M-A-V-L-T-V-A (SEQ ID NO: 123), I-V-L-M-A-A-L-A-V-A (SEQ ID NO: 124), I-V-L-M-A-A-L-T-A-A (SEQ ID NO: 125), I-V-L-M-A-A-L-T-I-A (SEQ ID NO: 126), I-V-L-M-A-A-L-T-V-M (SEQ ID NO: 127), I-V-L-M-A-A-L-T-V-V (SEQ ID NO: 128), I-V-L-V-G-V-L-T-V-A (SEQ ID NO: 129), I-V-L-V-G-A-L-A-V-A (SEQ ID NO: 130), I-V-L-V-G-A-L-T-A-A (SEQ ID NO: 131), I-V-L-V-G-A-L-T-I-A (SEQ ID NO: 132), I-V-L-V-G-A-L-T-V-M (SEQ ID NO: 133), I-V-L-V-G-A-L-T-V-V (SEQ ID NO: 134), I-V-L-V-S-V-L-T-V-A (SEQ ID NO: 135), I-V-L-V-S-A-L-A-V-A (SEQ ID NO: 136), I-V-L-V-S-A-L-T-A-A (SEQ ID NO: 137), I-V-L-V-S-A-L-T-I-A (SEQ ID NO: 138), I-V-L-V-S-A-L-T-V-M (SEQ ID NO: 139), I-V-L-V-S-A-L-T-V-V (SEQ ID NO: 140), I-V-L-V-A-V-L-A-V-A (SEQ ID NO: 141), I-V-L-V-A-V-L-T-A-A (SEQ ID NO: 142), I-V-L-V-A-V-L-T-I-A (SEQ ID NO: 143), I-V-L-V-A-V-L-T-V-M (SEQ ID NO: 144), I-V-L-V-A-V-L-T-V-V (SEQ ID NO: 145), I-V-L-V-A-A-L-A-A-A (SEQ ID NO: 146), I-V-L-V-A-A-L-A-I-A (SEQ ID NO: 147), I-V-L-V-A-A-L-A-V-M (SEQ ID NO: 148), I-V-L-V-A-A-L-A-V-V (SEQ ID NO: 149), I-V-L-V-A-A-L-T-A-M (SEQ ID NO: 150), I-V-L-V-A-A-L-T-A-V (SEQ ID NO: 151), I-V-L-V-A-A-L-T-I-M (SEQ ID NO: 152), I-V-L-V-A-A-L-T-I-V (SEQ ID NO: 153), V-I-M-I-A-A-L-T-V-A (SEQ ID NO: 154), V-I-M-M-A-A-L-T-V-A (SEQ ID NO: 155), V-I-M-V-G-A-L-T-V-A (SEQ ID NO: 156), V-I-M-V-S-A-L-T-V-A (SEQ ID NO: 157), V-I-M-V-A-V-L-T-V-A (SEQ ID NO: 158), V-I-M-V-A-A-L-A-V-A (SEQ ID NO: 159), V-I-M-V-A-A-L-T-A-A (SEQ ID NO: 160), V-I-M-V-A-A-L-T-I-A (SEQ ID NO: 161), V-I-M-V-A-A-L-T-V-M (SEQ ID NO: 162), V-I-M-V-A-A-L-T-V-V (SEQ ID NO: 163), V-I-L-I-G-A-L-T-V-A (SEQ ID NO: 164), V-I-L-I-S-A-L-T-V-A (SEQ ID NO: 165), V-I-L-I-A-V-L-T-V-A (SEQ ID NO: 166), V-I-L-I-A-A-L-A-V-A (SEQ ID NO: 167), V-I-L-I-A-A-L-T-A-A (SEQ ID NO: 168), V-I-L-I-A-A-L-T-I-A (SEQ ID NO: 169), V-I-L-I-A-A-L-T-V-M (SEQ ID NO: 170), V-I-L-I-A-A-L-T-V-V (SEQ ID NO: 171), V-I-L-M-G-A-L-T-V-A (SEQ ID NO: 172), V-I-L-M-S-A-L-T-V-A (SEQ ID NO: 173), V-I-L-M-A-V-L-T-V-A (SEQ ID NO: 174), V-I-L-M-A-A-L-A-V-A (SEQ ID NO: 175), V-I-L-M-A-A-L-T-A-A (SEQ ID NO: 176), V-I-L-M-A-A-L-T-I-A (SEQ ID NO: 177), V-I-L-M-A-A-L-T-V-M (SEQ ID NO: 178), V-I-L-M-A-A-L-T-V-V (SEQ ID NO: 179), V-I-L-V-G-V-L-T-V-A (SEQ ID NO: 180), V-I-L-V-G-A-L-A-V-A

(SEQ ID NO: 181), V-I-L-V-G-A-L-T-A-A (SEQ ID NO: 182), V-I-L-V-G-A-L-T-I-A (SEQ ID NO: 183), V-I-L-V-G-A-L-T-V-M (SEQ ID NO: 184), V-I-L-V-G-A-L-T-V-V (SEQ ID NO: 185), V-I-L-V-S-V-L-T-V-A (SEQ ID NO: 186), V-I-L-V-S-A-L-A-V-A (SEQ ID NO: 187), V-I-L-V-S-A-L-T-A-A (SEQ ID NO: 188), V-I-L-V-S-A-L-T-I-A (SEQ ID NO: 189), V-I-L-V-S-A-L-T-V-M (SEQ ID NO: 190), V-I-L-V-S-A-L-T-V-V (SEQ ID NO: 191), V-I-L-V-A-V-L-A-V-A (SEQ ID NO: 192), V-I-L-V-A-V-L-T-A-A (SEQ ID NO: 193), V-I-L-V-A-V-L-T-I-A (SEQ ID NO: 194), V-I-L-V-A-V-L-T-V-M (SEQ ID NO: 195), V-I-L-V-A-V-L-T-V-V (SEQ ID NO: 196), V-I-L-V-A-A-L-A-A-A (SEQ ID NO: 197), V-I-L-V-A-A-L-A-I-A (SEQ ID NO: 198), V-I-L-V-A-A-L-A-V-M (SEQ ID NO: 199), V-I-L-V-A-A-L-A-V-V (SEQ ID NO: 200), V-I-L-V-A-A-L-T-A-M (SEQ ID NO: 201), V-I-L-V-A-A-L-T-A-V (SEQ ID NO: 202), V-I-L-V-A-A-L-T-I-M (SEQ ID NO: 203), V-I-L-V-A-A-L-T-I-V (SEQ ID NO: 204), V-V-M-I-G-A-L-T-V-A (SEQ ID NO: 205), V-V-M-I-S-A-L-T-V-A (SEQ ID NO: 206), V-V-M-I-A-V-L-T-V-A (SEQ ID NO: 207), V-V-M-I-A-A-L-A-V-A (SEQ ID NO: 208), V-V-M-I-A-A-L-T-A-A (SEQ ID NO: 209), V-V-M-I-A-A-L-T-I-A (SEQ ID NO: 210), V-V-M-I-A-A-L-T-V-M (SEQ ID NO: 211), V-V-M-I-A-A-L-T-V-V (SEQ ID NO: 212), V-V-M-M-G-A-L-T-V-A (SEQ ID NO: 213), V-V-M-M-S-A-L-T-V-A (SEQ ID NO: 214), V-V-M-M-A-V-L-T-V-A (SEQ ID NO: 215), V-V-M-M-A-A-L-A-V-A (SEQ ID NO: 216), V-V-M-M-A-A-L-T-A-A (SEQ ID NO: 217), V-V-M-M-A-A-L-T-I-A (SEQ ID NO: 218), V-V-M-M-A-A-L-T-V-M (SEQ ID NO: 219), V-V-M-M-A-A-L-T-V-V (SEQ ID NO: 220), V-V-M-V-G-V-L-T-V-A (SEQ ID NO: 221), V-V-M-V-G-A-L-A-V-A (SEQ ID NO: 222), V-V-M-V-G-A-L-T-A-A (SEQ ID NO: 223), V-V-M-V-G-A-L-T-I-A (SEQ ID NO: 224), V-V-M-V-G-A-L-T-V-M (SEQ ID NO: 225), V-V-M-V-G-A-L-T-V-V (SEQ ID NO: 226), V-V-M-V-S-V-L-T-V-A (SEQ ID NO: 227), V-V-M-V-S-A-L-A-V-A (SEQ ID NO: 228), V-V-M-V-S-A-L-T-A-A (SEQ ID NO: 229), V-V-M-V-S-A-L-T-I-A (SEQ ID NO: 230), V-V-M-V-S-A-L-T-V-M (SEQ ID NO: 231), V-V-M-V-S-A-L-T-V-V (SEQ ID NO: 232), V-V-M-V-A-V-L-A-V-A (SEQ ID NO: 233), V-V-M-V-A-V-L-T-A-A (SEQ ID NO: 234), V-V-M-V-A-V-L-T-I-A (SEQ ID NO: 235), V-V-M-V-A-V-L-T-V-M (SEQ ID NO: 236), V-V-M-V-A-V-L-T-V-V (SEQ ID NO: 237), V-V-M-V-A-A-L-A-A-A (SEQ ID NO: 238), V-V-M-V-A-A-L-A-I-A (SEQ ID NO: 239), V-V-M-V-A-A-L-A-V-M (SEQ ID NO: 240), V-V-M-V-A-A-L-A-V-V (SEQ ID NO: 241), V-V-M-V-A-A-L-T-A-M (SEQ ID NO: 242), V-V-M-V-A-A-L-T-A-V (SEQ ID NO: 243), V-V-M-V-A-A-L-T-I-M (SEQ ID NO: 244), V-V-M-V-A-A-L-T-I-V (SEQ ID NO: 245), V-V-L-I-G-V-L-T-V-A (SEQ ID NO: 246), V-V-L-I-G-A-L-A-V-A (SEQ ID NO: 247), V-V-L-I-G-A-L-T-A-A (SEQ ID NO: 248), V-V-L-I-G-A-L-T-I-A (SEQ ID NO: 249), V-V-L-I-G-A-L-T-V-M (SEQ ID NO: 250),

V-V-L-I-G-A-L-T-V-V (SEQ ID NO: 251), V-V-L-I-S-V-L-T-V-A (SEQ ID NO: 252), V-V-L-I-S-A-L-A-V-A (SEQ ID NO: 253), V-V-L-I-S-A-L-T-A-A (SEQ ID NO: 254), V-V-L-I-S-A-L-T-I-A (SEQ ID NO: 255), V-V-L-I-S-A-L-T-V-M (SEQ ID NO: 256), V-V-L-I-S-A-L-T-V-V (SEQ ID NO: 257), V-V-L-I-A-V-L-A-V-A (SEQ ID NO: 258), V-V-L-I-A-V-L-T-A-A (SEQ ID NO: 259), V-V-L-I-A-V-L-T-I-A (SEQ ID NO: 260), V-V-L-I-A-V-L-T-V-M (SEQ ID NO: 261), V-V-L-I-A-V-L-T-V-V (SEQ ID NO: 262), V-V-L-I-A-A-L-A-A-A (SEQ ID NO: 263), V-V-L-I-A-A-L-A-I-A (SEQ ID NO: 264), V-V-L-I-A-A-L-A-V-M (SEQ ID NO: 265), V-V-L-I-A-A-L-A-V-V (SEQ ID NO: 266), V-V-L-I-A-A-L-T-A-M (SEQ ID NO: 267), V-V-L-I-A-A-L-T-A-V (SEQ ID NO: 268), V-V-L-I-A-A-L-T-I-M (SEQ ID NO: 269), V-V-L-I-A-A-L-T-I-V (SEQ ID NO: 270), V-V-L-M-G-V-L-T-V-A (SEQ ID NO: 271), V-V-L-M-G-A-L-A-V-A (SEQ ID NO: 272), V-V-L-M-G-A-L-T-A-A (SEQ ID NO: 273), V-V-L-M-G-A-L-T-I-A (SEQ ID NO: 274), V-V-L-M-G-A-L-T-V-M (SEQ ID NO: 275), V-V-L-M-G-A-L-T-V-V (SEQ ID NO: 276), V-V-L-M-S-V-L-T-V-A (SEQ ID NO: 277), V-V-L-M-S-A-L-A-V-A (SEQ ID NO: 278), V-V-L-M-S-A-L-T-A-A (SEQ ID NO: 279), V-V-L-M-S-A-L-T-I-A (SEQ ID NO: 280), V-V-L-M-S-A-L-T-V-M (SEQ ID NO: 281), V-V-L-M-S-A-L-T-V-V (SEQ ID NO: 282), V-V-L-M-A-V-L-A-V-A (SEQ ID NO: 283), V-V-L-M-A-V-L-T-A-A (SEQ ID NO: 284), V-V-L-M-A-V-L-T-I-A (SEQ ID NO: 285), V-V-L-M-A-V-L-T-V-M (SEQ ID NO: 286), V-V-L-M-A-V-L-T-V-V (SEQ ID NO: 287), V-V-L-M-A-A-L-A-A-A (SEQ ID NO: 288), V-V-L-M-A-A-L-A-I-A (SEQ ID NO: 289), V-V-L-M-A-A-L-A-V-M (SEQ ID NO: 290), V-V-L-M-A-A-L-A-V-V (SEQ ID NO: 291), V-V-L-M-A-A-L-T-A-M (SEQ ID NO: 292), V-V-L-M-A-A-L-T-A-V (SEQ ID NO: 293), V-V-L-M-A-A-L-T-I-M (SEQ ID NO: 294), V-V-L-M-A-A-L-T-I-V (SEQ ID NO: 295), V-V-L-V-G-V-L-A-V-A (SEQ ID NO: 296), V-V-L-V-G-V-L-T-A-A (SEQ ID NO: 297), V-V-L-V-G-V-L-T-I-A (SEQ ID NO: 298), V-V-L-V-G-V-L-T-V-M (SEQ ID NO: 299), V-V-L-V-G-V-L-T-V-V (SEQ ID NO: 300), V-V-L-V-G-A-L-A-A-A (SEQ ID NO: 301), V-V-L-V-G-A-L-A-I-A (SEQ ID NO: 302), V-V-L-V-G-A-L-A-V-M (SEQ ID NO: 303), V-V-L-V-G-A-L-A-V-V (SEQ ID NO: 304), V-V-L-V-G-A-L-T-A-M (SEQ ID NO: 305), V-V-L-V-G-A-L-T-A-V (SEQ ID NO: 306), V-V-L-V-G-A-L-T-I-M (SEQ ID NO: 307), V-V-L-V-G-A-L-T-I-V (SEQ ID NO: 308), V-V-L-V-S-V-L-A-V-A (SEQ ID NO: 309), V-V-L-V-S-V-L-T-A-A (SEQ ID NO: 310), V-V-L-V-S-V-L-T-I-A (SEQ ID NO: 311), V-V-L-V-S-V-L-T-V-M (SEQ ID NO: 312), V-V-L-V-S-V-L-T-V-V (SEQ ID NO: 313), V-V-L-V-S-A-L-A-A-A (SEQ ID NO: 314), V-V-L-V-S-A-L-A-I-A (SEQ ID NO: 315), V-V-L-V-S-A-L-A-V-M (SEQ ID NO: 316), V-V-L-V-S-A-L-A-V-A-V (SEQ ID NO: 317), V-V-L-V-S-A-L-T-A-M (SEQ ID NO: 318), V-V-L-V-S-A-L-T-A-V (SEQ ID NO:

319), V-V-L-V-S-A-L-T-I-M (SEQ ID NO: 320), V-V-L-V-S-A-L-T-I-V (SEQ ID NO: 321), V-V-L-V-A-V-L-A-A-A (SEQ ID NO: 322), V-V-L-V-A-V-L-A-I-A (SEQ ID NO: 323), V-V-L-V-A-V-L-A-V-M (SEQ ID NO: 324), V-V-L-V-A-V-L-A-V-V (SEQ ID NO: 325), V-V-L-V-A-V-L-T-A-M (SEQ ID NO: 326), V-V-L-V-A-V-L-T-A-V (SEQ ID NO: 327), V-V-L-V-A-V-L-T-I-M (SEQ ID NO: 328), V-V-L-V-A-V-L-T-I-V (SEQ ID NO: 329), V-V-L-V-A-A-L-A-A-M (SEQ ID NO: 330), V-V-L-V-A-A-L-A-A-V (SEQ ID NO: 331), V-V-L-V-A-A-L-A-I-M (SEQ ID NO: 332), and V-V-L-V-A-A-L-A-I-V (SEQ ID NO: 333). These amino acid sequences are preferred specific embodiments of the amino acid motif comprised in the peptide according to the present invention. In another embodiment, the muscle function enhancing amino acid sequence comprises or consists of an amino acid sequence selected from the group consisting of the above amino acid sequences, i.e., SEQ ID NO: 4 to 333, wherein said amino acid sequences further have or comprise a tyrosine residue directly attached at their N-terminus. Thus, for example, in a preferred embodiment, the muscle function enhancing amino acid sequence comprises or consists of the amino acid sequence Y-V-V-L-V-A-A-L-T-V-A (SEQ ID NO: 390), i.e., the amino acid sequence set forth in SEQ ID NO: 4 having a tyrosine directly attached to its amino-terminus.

In another particularly preferred embodiment of the peptide according to the present invention, the muscle function enhancing amino acid sequence comprises, essentially consists or consists of the amino acid sequence V-[V/I]-L-[V/I]-[A/S]-[A/V]-L-[T/A]-[V/A]-A (SEQ ID NO: 374), wherein the preferred sequence is V-V-L-V-A-A-L-T-V-A (SEQ ID NO: 4), wherein preferably maximally 4, more preferably maximally 3, even more preferably maximally 2, and most preferably maximally 1 amino acid is replaced as specified above. Thus, in a particularly preferred embodiment of the peptide according to the present invention, the amino acid motif consists of the amino acid sequence V-[V/I]-L-[V/I]-[A/S]-[A/V]-L-[T/A]-[V/A]-A (SEQ ID NO: 374), wherein the preferred sequence is V-V-L-V-A-A-L-T-V-A (SEQ ID NO: 4), wherein preferably maximally 4, more preferably maximally 3, even more preferably maximally 2, and most preferably maximally 1 amino acid is replaced with another amino acid as specified above.

In a preferred embodiment of the peptide according to the present invention, the muscle function enhancing amino acid sequence comprises, essentially consists or consists of an amino acid sequence selected from the group consisting of the amino acid sequences V-V-L-V-A-A-L-T-V-A (SEQ ID NO: 4), V-I-L-V-A-A-L-T-V-A (SEQ ID NO: 6), V-V-M-V-A-A-L-T-V-A (SEQ ID NO: 7), I-I-L-V-G-A-L-T-V-A (SEQ ID NO: 95), V-V-L-I-A-A-L-A-A-A (SEQ ID NO: 263), V-I-L-V-S-V-L-T-V-A (SEQ ID NO: 186), I-I-L-M-G-A-L-T-V-A

(SEQ ID NO: 334), and V-V-M-V-A-A-L-T-V-V (SEQ ID NO: 50). These amino acid sequences are particularly preferred specific embodiments of the amino acid motif comprised by the peptide according to the present invention.

In another embodiment of the peptide according to the present invention, an amino acid or an amino acid sequence selected from the group consisting of C, C-N, C-N-[N/D/E], C-N-[N/D/E]-[F/Y], C-N-[N/D/E]-[F/Y]-F, C-N-[N/D/E]-[F/Y]-F-[W/L/Q], C-N-[N/D/E]-[F/Y]-F-[W/L/Q]-E (SEQ ID NO: 375), C-N-[N/D/E]-[F/Y]-F-[W/L/Q]-E-[N/T] (SEQ ID NO: 376), preferably selected from the group consisting of C, C-N, C-N-N, C-N-N-F (SEQ ID NO: 335), C-N-N-F-F (SEQ ID NO: 336), C-N-N-F-F-W (SEQ ID NO: 337), C-N-N-F-F-W-E (SEQ ID NO: 338), and C-N-N-F-F-W-E-N (SEQ ID NO: 339), is directly linked to the carboxy-terminus of the amino acid motif. In a preferred embodiment of the peptide according to the present invention, the muscle function enhancing amino acid sequence comprises, essentially consists or consists of an amino acid sequence selected from the group consisting of [V/I]-[V/I]-[L/M]-[V/I/M]-[A/G/S]-[A/V]-L-[T/A]-[V/A/I]-[A/M/V]-C, [V/I]-[V/I]-[L/M]-[V/I/M]-[A/G/S]-[A/V]-L-[T/A]-[V/A/I]-[A/M/V]-C-N, [V/I]-[V/I]-[L/M]-[V/I/M]-[A/G/S]-[A/V]-L-[T/A]-[V/A/I]-[A/M/V]-C-N-[N/D/E], [V/I]-[V/I]-[L/M]-[V/I/M]-[A/G/S]-[A/V]-L-[T/A]-[V/A/I]-[A/M/V]-C-N-[N/D/E]-[F/Y], [V/I]-[V/I]-[L/M]-[V/I/M]-[A/G/S]-[A/V]-L-[T/A]-[V/A/I]-[A/M/V]-C-N-[N/D/E]-[F/Y]-F (SEQ ID NO: 377), [V/I]-[V/I]-[L/M]-[V/I/M]-[A/G/S]-[A/V]-L-[T/A]-[V/A/I]-[A/M/V]-C-N-[N/D/E]-[F/Y]-F-[W/L/Q] (SEQ ID NO: 378), [V/I]-[V/I]-[L/M]-[V/I/M]-[A/G/S]-[A/V]-L-[T/A]-[V/A/I]-[A/M/V]-C-N-[N/D/E]-[F/Y]-F-[W/L/Q]-E (SEQ ID NO: 379), and [V/I]-[V/I]-[L/M]-[V/I/M]-[A/G/S]-[A/V]-L-[T/A]-[V/A/I]-[A/M/V]-C-N-[N/D/E]-[F/Y]-F-[W/L/Q]-E-[N/T] (SEQ ID NO: 380).

Preferably, the muscle function enhancing amino acid sequence comprises or consists of any of the above specifically disclosed amino acid sequences, i.e., specifically disclosed amino acid sequences of the amino acid motif, to which an amino acid or an amino acid sequence selected from the group specified above is linked directly to the carboxy-terminus. For example, preferably the muscle function enhancing amino acid sequence comprises or consists of an amino acid sequence selected from the group consisting of V-V-L-V-A-A-L-T-V-A-C (SEQ ID NO: 340), V-V-L-V-A-A-L-T-V-A-C-N (SEQ ID NO: 341), V-V-L-V-A-A-L-T-V-A-C-N-N (SEQ ID NO: 342), V-V-L-V-A-A-L-T-V-A-C-N-N-F (SEQ ID NO: 343), V-V-L-V-A-A-L-T-V-A-C-N-N-F-F (SEQ ID NO: 344), V-V-L-V-A-A-L-T-V-A-C-N-N-F-F-W (SEQ ID NO: 345), V-V-L-V-A-A-L-T-V-A-C-N-N-F-F-W-E (SEQ ID NO: 346), and V-V-L-V-A-A-L-T-V-A-C-N-N-F-F-W-E-N (SEQ ID NO: 347).

In another embodiment of the peptide according to the present invention, an aromatic amino acid, preferably tyrosine, phenylalanine, or tryptophan, more preferably tyrosine or phenylalanine, most preferably tyrosine, is directly linked to the amino-terminus of the amino acid motif. Preferably, the muscle function enhancing amino acid sequence comprises or consists of one of the above specifically disclosed amino acid sequences extended at their amino-terminus by a tyrosine.

In another preferred embodiment, the peptide of the present invention further comprises one or more, e.g., one, two, three, or four, of the elements selected from the group consisting of a membrane penetration enhancing motif, one or more epitope-tag(s), a hydrophilic motif, and a peptide targeting motif.

A membrane penetration enhancing motif may be any amino acid sequence that is capable of penetrating membranes as specified above, e.g., a cell-penetrating peptide (CCP). Such a motif may enable other macromolecules, such as peptides, proteins or nucleic acids, which normally do not possess the ability to traverse cell membranes, to penetrate intact cell membranes when said membrane penetration enhancing motif is attached to said macromolecule. Such membrane penetration enhancing motifs may be derived from protein transduction domains, may be amphipathic peptides, or may be any other penetrating peptide. For example, the membrane penetration enhancing motif may be derived from the HIV Tat peptide, e.g., G-R-K-K-R-R-Q-R-R-R (SEQ ID NO: 348), the penetratin peptide, e.g., R-Q-I-K-I-W-F-Q-N-R-R-M-K-W-K-K (SEQ ID NO: 349) or K-K-W-K-M-R-R-N-Q-F-W-V-K-V-Q-R-G (SEQ ID NO: 350), the transportan peptide, e.g., G-W-T-L-N-S-A-G-Y-L-L-G-K-I-N-L-K-A-L-A-A-L-A-K-K-I-L (SEQ ID NO: 351), an MPG/Pep family member peptide, e.g., G-A-L-F-L-G-F-L-G-A-A-G-S-T-M-G-A-W-S-QP-K-K-K-R-K-V (SEQ ID NO: 352) or K-E-T-W-W-E-T-W-W-T-E-W-S-Q-P-K-K-K-R-K-V (SEQ ID NO: 353), or arginine rich peptides etc. (Deshayes et al., 2005, Cell. Mol. Life Sci. 62:1839-1849). Such a membrane penetration enhancing motif may be located amino-terminally or carboxy-terminally to the muscle function enhancing amino acid sequence within the peptide according to the present invention. Furthermore, the peptide according to the present invention may comprise more than one membrane penetration enhancing motif, for example, the peptide according to the present invention may contain 2, 3, 4, or 5 such motifs.

An epitope is a portion of a molecule to which an antibody binds. In the context of the present invention, an epitope is preferably a peptide-tag, for example, hemagglutinin-(HA-), FLAG-, myc-, or a poly-His-tag. Such an epitope tag may be used to locate the peptide of the present invention within a cell, for example, for determining whether the peptide penetrates,

i.e., traverses, cell membranes and can be found inside an intact cell incubated with said peptide.

In a particular preferred embodiment, the peptide according to the present invention further comprises a hydrophilic motif. In a preferred embodiment, said hydrophilic motif comprises acidic, basic, and/or otherwise negatively or positively charged amino acids. In a particular preferred embodiment of the peptide according to the present invention, the hydrophilic motif comprises or consists of the amino acid motif Λ_4-Θ_2, wherein Λ is in each instance independently selected from aspartate, glutamate, lysine, and arginine and Θ is an α-helix interrupter, preferably proline or glycine. Preferably, the hydrophilic motif comprises or consists of an amino acid sequence selected from the group consisting of [D/E]-[D/E]-[D/E]-[D/E]-[P/G]-[P/G], [K/R]-[D/E]-[D/E]-[D/E]-[P/G]-[P/G], [D/E]-[K/R]-[D/E]-[D/E]-[P/G]-[P/G], [D/E]-[D/E]-[K/R]-[D/E]-[P/G]-[P/G], [D/E]-[D/E]-[D/E]-[K/R]-[P/G]-[P/G], [K/R]-[K/R]-[D/E]-[D/E]-[P/G]-[P/G], [K/R]-[D/E]-[K/R]-[D/E]-[P/G]-[P/G], [K/R]-[D/E]-[D/E]-[K/R]-[P/G]-[P/G], [D/E]-[K/R]-[K/R]-[D/E]-[P/G]-[P/G], [D/E]-[K/R]-[D/E]-[K/R]-[P/G]-[P/G], [D/E]-[D/E]-[K/R]-[K/R]-[P/G]-[P/G], [K/R]-[K/R]-[K/R]-[D/E]-[P/G]-[P/G], [K/R]-[K/R]-[D/E]-[K/R]-[P/G]-[P/G], [K/R]-[D/E]-[K/R]-[K/R]-[P/G]-[P/G], [D/E]-[K/R]-[K/R]-[K/R]-[P/G]-[P/G], and [K/R]-[K/R]-[K/R]-[K/R]-[P/G]-[P/G]. Preferably, the hydrophilic motif comprises or consists of the amino acid sequence [D/E]-[K/R]-[D/E]-[D/E]-[P/G]-[P/G]. More preferably, the hydrophilic motif comprises or consists of an amino acid sequence selected from the group consisting of D-K-D-D-P-P (SEQ ID NO: 354), E-K-D-D-P-P (SEQ ID NO: 355), D-R-D-D-P-P (SEQ ID NO: 356), D-K-E-D-P-P (SEQ ID NO: 357), D-K-D-E-P-P (SEQ ID NO: 358), E-R-D-D-P-P (SEQ ID NO: 359), E-K-E-D-P-P (SEQ ID NO: 360), E-K-D-E-P-P (SEQ ID NO: 361), D-R-E-D-P-P (SEQ ID NO: 362), D-R-D-E-P-P (SEQ ID NO: 363), D-K-E-E-P-P (SEQ ID NO: 364), E-R-E-D-P-P (SEQ ID NO: 365), E-R-D-E-P-P (SEQ ID NO: 366), D-R-E-E-P-P (SEQ ID NO: 367), E-K-E-E-P-P (SEQ ID NO: 368), and E-R-E-E-P-P (SEQ ID NO: 369), wherein P-P in said sequences may be exchanged for G-G. Most preferably, the hydrophilic motif comprises or consists of the amino acid sequence D-K-D-D-P-P (SEQ ID NO: 354), wherein P-P in said sequences may also be G-G. Preferably, the hydrophilic motif is located within the peptide according to the present invention amino-terminally to the muscle function enhancing amino acid sequence, but could also be located carboxy-terminally to the muscle function enhancing amino acid sequence. In a particularly preferred embodiment, the hydrophilic motif is directly linked to the amino-terminus of the muscle function enhancing amino acid sequence, preferably is directly linked to the amino-terminus of the amino acid motif.

Thus, in a particular preferred embodiment of the present invention, the peptide comprises, essentially consists, preferably consists of the amino acid sequence Λ_4-Θ_2-Φ_4-X-Ψ-L-[T/A]-Ψ_2, wherein Λ is in each instance independently selected from aspartate, glutamate, lysine, and arginine, Θ is an α-helix interrupter, preferably is in each instance independently selected from proline or glycine, Φ and Ψ are in each instance an independently selected hydrophobic non-aromatic amino acid, preferably Φ is in each instance independently selected from methionine, isoleucine, leucine, and valine, Ψ is in each instance preferably selected from alanine, methionine, isoleucine, and valine, and X is any amino acid, preferably a small amino acid, preferably selected from glycine, alanine, serine, cysteine, threonine, and valine, more preferably selected from glycine, alanine, and serine. Preferably said peptide exhibits one or more, e.g., 1, 2, 3, 4, or 5, preferably all of the above defined functional characteristics, i.e., anti-arrhythmic potential, anti-apoptotic potential, the ability to reduce calcium spark frequency, the ability to prevent and/or reduce calcium leakage from the sarcoplasmic reticulum, the ability to restore hemodynamic function preferably in an individual suffering from heart failure, and the ability to enhance isometric and/or tetanic twitch force in skeletal muscle cells and/or fibers. Preferably, said functions can be observed *in vitro* and *in vivo*. Preferably, said *in vivo* effects can be observed when the peptide is administered via a parenteral administration route.

In an even more preferred embodiment, the peptide according to the present invention comprises, preferably consists of the amino acid sequence Λ_4-Θ_2-[V/I]-[V/I]-[L/M]-[V/I/M]-[A/G/S]-[A/V]-L-[T/A]-[V/A/I]-[A/M/V], wherein Λ is in each instance independently selected from aspartate, glutamate, lysine, and arginine and Θ is an α-helix interrupter, preferably proline or glycine. Preferably said peptide exhibits one or more, e.g., 1, 2, 3, 4, or 5, preferably all of the above defined functional characteristics, i.e., anti-arrhythmic potential, anti-apoptotic potential, the ability to reduce calcium spark frequency, the ability to prevent and/or reduce calcium leakage from the sarcoplasmic reticulum, the ability to restore hemodynamic function preferably in an individual suffering from heart failure, and the ability to enhance isometric and/or tetanic twitch force in skeletal muscle cells and/or fibers. Preferably, said functions can be observed *in vitro* and *in vivo*. Preferably, said *in vivo* effects can be observed when the peptide is administered via a parenteral administration route.

In an even more preferred embodiment, the peptide according to the present invention comprises, preferably consists of the amino acid sequence [D/E]-[K/R]-[D/E]-[D/E]-[P/G]-[P/G]-[V/I]-[V/I]-[L/M]-[V/I/M]-[A/G/S]-[A/V]-L-[T/A]-[V/A/I]-[A/M/V]. Preferably said peptide exhibits one or more, e.g., 1, 2, 3, 4, or 5, preferably all of the above defined

functional characteristics, i.e., anti-arrhythmic potential, anti-apoptotic potential, the ability to reduce calcium spark frequency, the ability to prevent and/or reduce calcium leakage from the sarcoplasmic reticulum, the ability to restore hemodynamic function preferably in an individual suffering from heart failure, and the ability to enhance isometric and/or tetanic twitch force in skeletal muscle cells and/or fibers. Preferably, said functions can be observed *in vitro* and *in vivo*. Preferably, said *in vivo* effects can be observed when the peptide is administered via a parenteral administration route.

In an even more preferred embodiment, the peptide according to the present invention comprises, preferably consists of the amino acid sequence [D/E]-[K/R]-[D/E]-[D/E]-P-P-V-[V/I]-L-[V/I]-[A/S]-[A/V]-L-[T/A]-[V/A]-A (SEQ ID NO: 381). Preferably said peptide exhibits one or more, e.g., 1, 2, 3, 4, or 5, preferably all of the above defined functional characteristics, i.e., anti-arrhythmic potential, anti-apoptotic potential, the ability to reduce calcium spark frequency, the ability to prevent and/or reduce calcium leakage from the sarcoplasmic reticulum, the ability to restore hemodynamic function preferably in an individual suffering from heart failure, and the ability to enhance isometric and/or tetanic twitch force in skeletal muscle cells and/or fibers. Preferably, said functions can be observed *in vitro* and *in vivo*. Preferably, said *in vivo* effects can be observed when the peptide is administered via a parenteral administration route.

In a most preferred embodiment, the peptide according to the present invention comprises, essentially consists, preferably consists of the amino acid sequence D^1-K^2-D^3-D^4-P^5-P^6-V^7-V^8-L^9-V^{10}-A^{11}-A^{12}-L^{13}-T^{14}-V^{15}-A^{16} (SEQ ID NO: 370) or an amino acid sequence that is preferably at least 60%, preferably at least 65%, preferably at least 70%, more preferably at least 75%, more preferably at least 80%, even more preferably at least 85%, even more preferably at least 90%, and most preferably at least 95% identical to the amino acid sequence set forth in SEQ ID NO: 370, i.e., D^1-K^2-D^3-D^4-P^5-P^6-V^7-V^8-L^9-V^{10}-A^{11}-A^{12}-L^{13}-T^{14}-V^{15}-A^{16}. Preferably, the amino acid replacements are as specified above, e.g., D^1 may be replaced by glutamate, arginine, or lysine, preferably by glutamate, K^2 may be replaced by arginine, glutamate, or aspartate, preferably by arginine, D^3 may be replaced by glutamate, arginine, or lysine, preferably by glutamate, D^4 may be replaced by glutamate, arginine, or lysine, preferably by glutamate, P^5 and P^6 may be independently replaced by glycine, V^7 and V^8 may be independently replaced by isoleucine, preferably V^7 is not replaced, L^9 may be replaced by methionine, but is preferably not replaced, V^{10} may be replaced by isoleucine or methionine, preferably by isoleucine, A^{11} may be replaced by glycine or serine, preferably by serine, A^{12} may be replaced by valine, T^{14} may be replaced by alanine, V^{15} may be replaced

by alanine or isoleucine, preferably by alanine, and A^{16} may be replaced by methionine or valine, but is preferably not replaced. Preferably said peptide exhibits one or more, e.g., 1, 2, 3, 4, or 5, preferably all of the above defined functional characteristics, i.e., anti-arrhythmic potential, anti-apoptotic potential, the ability to reduce calcium spark frequency, the ability to prevent and/or reduce calcium leakage from the sarcoplasmic reticulum, the ability to restore hemodynamic function preferably in an individual suffering from heart failure, and the ability to enhance isometric and/or tetanic twitch force in skeletal muscle cells and/or fibers. Preferably, said functions can be observed *in vitro* and *in vivo*. Preferably, said *in vivo* effects can be observed when the peptide is administered via a parenteral administration route. Thus, in a preferred embodiment, the peptide according to the present invention comprises, essentially consists, preferably consists of the amino acid sequence D^1-K^2-D^3-D^4-P^5-P^6-V^7-V^8-L^9-V^{10}-A^{11}-A^{12}-L^{13}-T^{14}-V^{15}-A^{16}, wherein the amino acid residues may be replaced as specified above (SEQ ID NO: 371), wherein preferably the amino acid sequence is at least 60%, preferably at least 65%, preferably at least 70%, more preferably at least 75%, more preferably at least 80%, even more preferably at least 85%, even more preferably at least 90%, and most preferably at least 95% identical to the amino acid sequence set forth in SEQ ID NO: 370, wherein preferably one or more, preferably all of the above functions can be observed, and wherein most preferably the peptide is cell permeable.

In another preferred embodiment, the peptide according to the present invention comprises, essentially consists, preferably consists of the amino acid sequence D^1-K^2-D^3-D^4-P^5-P^6-Y^7-V^8-V^9-L^{10}-V^{11}-A^{12}-A^{13}-L^{14}-T^{15}-V^{16}-A^{17} (SEQ ID NO: 372) or an amino acid sequence that is preferably at least 60%, preferably at least 65%, preferably at least 70%, more preferably at least 75%, more preferably at least 80%, even more preferably at least 85%, even more preferably at least 90%, and most preferably at least 95% identical to the amino acid sequence set forth in SEQ ID NO: 372, i.e., D^1-K^2-D^3-D^4-P^5-P^6-Y^7-V^8-V^9-L^{10}-V^{11}-A^{12}-A^{13}-L^{14}-T^{15}-V^{16}-A^{17}. Preferably, the amino acid replacements are as specified above, e.g., D^1 may be replaced by glutamate, arginine, or lysine, preferably by glutamate, K^2 may be replaced by arginine, glutamate, or aspartate, preferably by arginine, D^3 may be replaced by glutamate, arginine, or lysine, preferably by glutamate, D^4 may be replaced by glutamate, arginine, or lysine, preferably by glutamate, P^5 and P^6 may be independently replaced by glycine, Y^7 may be replaced by phenylalanine or tryptophan, preferably by phenylalanine, V^8 and V^9 may be independently replaced by isoleucine, preferably V^8 is not replaced, L^{10} may be replaced by methionine, but is preferably not replaced, V^{11} may be replaced by isoleucine or methionine, preferably by isoleucine, A^{12} may be replaced by glycine or serine, preferably

by serine, A^{13} may be replaced by valine, T^{15} may be replaced by alanine, V^{16} may be replaced by alanine or isoleucine, preferably by alanine, and A^{17} may be replaced by methionine or valine, but is preferably not replaced. Preferably said peptide exhibits one or more, e.g., 1, 2, 3, 4, or 5, preferably all of the above defined functional characteristics, i.e., anti-arrhythmic potential, anti-apoptotic potential, the ability to reduce calcium spark frequency, the ability to prevent and/or reduce calcium leakage from the sarcoplasmic reticulum, the ability to restore hemodynamic function preferably in an individual suffering from heart failure, and the ability to enhance isometric and/or tetanic twitch force in skeletal muscle cells and/or fibers. Preferably, said functions can be observed *in vitro* and *in vivo*. Preferably, said *in vivo* effects can be observed when the peptide is administered via a parenteral administration route. Thus, in a preferred embodiment, the peptide according to the present invention comprises, essentially consists, preferably consists of the amino acid sequence D^1-K^2-D^3-D^4-P^5-P^6-Y^7-V^8-V^9-L^{10}-V^{11}-A^{12}-A^{13}-L^{14}-T^{15}-V^{16}-A^{17}, wherein the amino acid residues may be replaced as specified above (SEQ ID NO: 373), wherein preferably the amino acid sequence is at least 60%, preferably at least 65%, preferably at least 70%, more preferably at least 75%, more preferably at least 80%, even more preferably at least 85%, even more preferably at least 90%, and most preferably at least 95% identical to the amino acid sequence set forth in SEQ ID NO: 372, wherein preferably one or more, preferably all of the above functions can be observed, and wherein most preferably the peptide is cell permeable.

A peptide targeting motif in the context of the present invention may be any moiety that is suitable for targeting a peptide *in vivo* to a specific organ or specific cells. For example, a peptide targeting motif may be a peptide that specifically binds to a particular receptor which is specific for certain cells or a certain organ. Preferably, the presence of a peptide targeting motif within the peptide according to the present invention allows for specific targeting of cells or organs in a patient to which the peptide was administered systemically.

In another embodiment, the peptide of the present invention further comprises a marker moiety. A marker moiety in the context of the present invention may be any moiety that allows for a straightforward detection of the peptide, such as a fluorescent label, e.g., fluorescein (for example, fluorescein isothiocyanate FITC), rhodamine (for example, tetramethylrhodamine TAMRA or its isothiocyanate derivative TRITC, sulforhodamine 101 and its sulfonylchloride form Texas Red™, and Rhodamine Red), or Alexa Fluor® dyes, a radioactive label, e.g., a radioactively labeled amino acid, or biotin. In one embodiment, the peptide of the present invention comprises a hydrophilic motif, preferably D-K-D-D-P-P

(SEQ ID NO: 354), and a marker moiety, preferably FITC or rhodamine, wherein preferably the muscle function enhancing amino acid sequence is V-V-L-V-A-A-L-T-V-A (SEQ ID NO: 4), and preferably the hydrophilic motif is directly linked to the amino-terminus of the muscle function enhancing amino acid sequence.

The skilled person is well aware of methods for producing peptides according to the present invention. For example, the peptide may be chemically synthesized, e.g., by liquid phase or solid phase peptide synthesis, or the peptide may be genetically engineered using recombinant DNA techniques and a cellular expression system, such as bacteria (e.g., *Escherichia coli*), yeast cells, insect cells, mammalian cells etc, or an *in vitro* expression system.

In a second aspect, the present invention provides the peptide according to the first aspect of the present invention for medical use.

In a third aspect, the present invention provides the peptide according to the first and second aspects of the present invention for therapeutic use for treating and/or preventing a disorder associated with muscular malfunction, e.g., a myopathy. Preferably, said disorder is a cardiac and/or skeletal muscle disorder. The disorder may be acquired or congenital. In this context, the term "acquired" means that the medical condition, i.e., the disorder, developed post-fetally. Such an acquired disorder in the context of the present invention may be a myocardial infarction. An example for an acquired skeletal muscle disorder is myositis. Congenital disorders involve defects to a developing fetus which may be the result of genetic abnormalities, errors of morphogenesis, or chromosomal abnormalities. Genetic diseases or disorders are all congenital, though they may not be expressed or recognized until later in life. Congenital disorders in the context of the present invention are, for example, Nemaline myopathy, Myotubular myopathy, or Centronuclear myopathy. Furthermore, in the context of the present invention, the cardiac or skeletal muscle disorder may be acute or chronic. For example, an acute cardiac muscle disorder is acute heart failure, an acute skeletal muscle disorder is Rhabdomyolysis. A chronic skeletal muscle disorder is, for example, Dermatomyositis. A chronic cardiac muscle disease is, for example, chronic heart failure.

In a preferred embodiment of the third aspect of the present invention, the muscular malfunction is associated with defective calcium cycling and/or defective contractile performance in muscle cells, preferably in skeletal muscle cells or cardiomyocytes. Preferably, the peptide is for enhancing and/or restoring calcium cycling and/or for enhancing and/or restoring contractile performance in muscle cells. Defective calcium cycling in myocytes may be a result of reduced calcium content in the sarcoplasmic reticulum, reduced

release of calcium from the sarcoplasmic reticulum during excitation-contraction coupling, calcium leakage from the sarcoplasmic reticulum, for example, due to a leaky RyR sarcoplasmic reticulum calcium release channel, increased calcium spark frequency, or reduced or slowed re-uptake of calcium into the sarcoplasmic reticulum and/or the mitochondria after contraction, for example, due to a malfunctioning or non-functioning sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA). Without being bound to this theory, it is assumed that a defective calcium cycling is one of the major reasons for defective contractile performance, e.g., contractile dysfunction, of muscle cells. Thus, it is assumed that enhancing or restoring calcium cycling also enhances and/or restores contractile performance. Almost all cardiac and skeletal muscle disorders/diseases are a result of contractile dysfunction of the respective muscle cells. For example, in cardiac arrhythmias, the cardiac muscle contraction is not precisely timed. This may have lethal consequences. In most of the skeletal muscle disorders, the contractile performance is reduced which has the consequence of muscle weakness such as in various types of dystrophies. It is assumed that the peptide according to the first aspect of the present invention is capable of enhancing and/or restoring calcium cycling in myocytes, and thereby, enhances and/or restores contractile performance. However, it is emphasized that the peptide of the present invention is not only suitable for treating disorder associated with muscular malfunction, wherein the muscular malfunction is associated with defective calcium cycling, but also muscular diseases which are not based on malfunctioning calcium handling. In these diseases the peptide of the present invention may relief the symptoms such as muscle weakness.

In a preferred embodiment of the second and third aspect of the present invention, the peptide is for protecting myocytes, preferably skeletal muscle cells and/or cardiomyocytes, more preferably heart tissue from arrhythmias, preferably from catecholamine triggered arrhythmias, preferably for protecting an individual from ventricular arrhythmias, preferably from lethal ventricular tachyarrhythmias, and thus, preferably from sudden cardiac death. Preferably, said function is exhibited *in vivo*, preferably when the peptide is applied parenterally without the need for gene therapy.

In a further preferred embodiment of the second and third aspect of the present invention, the peptide is for reducing calcium spark frequency in myocytes, preferably in skeletal muscle cells and/or cardiomyocytes, and/or for preventing and/or reducing calcium leakage from the sarcoplasmic reticulum of myocytes, preferably in skeletal muscle cells and/or cardiomyocytes. Preferably, said function is exhibited *in vivo*, preferably when the peptide is applied parenterally without the need for gene therapy.

In a further preferred embodiment of the second and third aspect of the present invention, the peptide is for preventing or reducing calcium leakage from the sarcoplasmic reticulum of muscle cells, preferably of skeletal muscle cells and/or cardiomyocytes. Preferably, the peptide is for preventing or reducing calcium leakage from the sarcoplasmic reticulum due to leaky RyR sarcoplasmic reticulum calcium release channels. Preferably, said function is exhibited *in vivo*, preferably when the peptide is applied parenterally without the need for gene therapy.

In another preferred embodiment of the second and third aspect of the present invention, the peptide is for protecting myocytes, preferably skeletal muscle cells and/or cardiomyocytes from apoptotic cell death, preferably from calcium-induced apoptotic cell death, preferably from sarcoplasmic reticulum calcium leakage triggered apoptotic cell death. Preferably, the peptide is for preventing apoptotic cell death in failing myocardium, i.e., protecting cardiomyocytes from apoptotic cell death in failing myocardium. Preferably, said function is exhibited *in vivo*, preferably when the peptide is applied parenterally without the need for gene therapy.

In another particularly preferred embodiment of the second and third aspect of the present invention, the peptide is for restoring and/or enhancing hemodynamic function, for example, cardiac performance such as contractile performance of cardiomyocytes, preferably the peptide is for restoring and/or enhancing hemodynamic function in an individual suffering or has suffered from heart failure such as from myocardial infarction. Preferably, said function is exhibited *in vivo* when the peptide is applied parenterally without the need for gene therapy.

In another preferred embodiment of the second and third aspect of the present invention, the peptide is for enhancing and/or restoring contractile performance in skeletal muscle cells, preferably for enhancing and/or restoring isometric and/or tetanic twitch force in skeletal muscle cells, preferably in skeletal muscle tissue. The isometric twitch force is tension development without muscle shortening, the tetanic twitch force is the maximal isometric force development, normally, when single contractions start to merge above 50 Hz stimulation. Preferably, said function is exhibited *in vivo*, preferably when the peptide is applied parenterally without the need for gene therapy.

It is emphasized that the disclosure on functional characteristics of specific embodiments of the peptide according to the present invention in the first aspect of the present invention also applies to the second and third aspects of the present invention.

In a preferred embodiment of the third aspect of the present invention, the cardiac muscle disorder is selected from the group consisting of postischemic contractile dysfunction, preferably postischemic contractile right and/or left ventricular dysfunction, congestive heart failure, preferably compensated and/or decompensated congestive heart failure, cardiogenic shock, septic shock, myocardial infarction, cardiomyopathy, dysfunction of heart valves, and ventricular disorder, such as acute or chronic right ventricular disorder.

In a preferred embodiment of the third aspect of the present invention, the skeletal muscle disorder is selected from the group consisting of muscular dystrophy, muscle weakness, muscular atrophy, myositis, central core disease, nemaline (rod) myopathy, centronuclear myopathy, myotubular myopathy, centronuclear myotubular myopathy, ophthalmoplegia of the eye, and mitochondrial myopathy. The muscular dystrophy may be selected from the group consisting of Becker's muscular dystrophy, congenital muscular dystrophy, Duchenne muscular dystrophy, distal muscular dystrophy, Emery-Dreifuss muscular dystrophy, facioscapulohumeral muscular dystrophy, Limb-girdle muscular dystrophy, myotonic muscular dystrophy, and oculopharyngeal muscular dystrophy. The myositis may be selected from the group consisting of myositis ossificans, fibromyositis, idiopathic inflammatory myopathies (such as dermatomyositis, polymyositis, and inclusion body myositis), and pyomyositis.

In a fourth aspect, the present invention provides a pharmaceutical composition comprising the peptide of the first aspect or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient, carrier, and/or diluent.

The term "pharmaceutically acceptable salt" refers to a salt of the peptide of the present invention. Suitable pharmaceutically acceptable salts include acid addition salts which may, for example, be formed by mixing a solution of the peptide of the present invention with a solution of a pharmaceutically acceptable acid such as hydrochloric acid, sulfuric acid, fumaric acid, maleic acid, succinic acid, acetic acid, benzoic acid, citric acid, tartaric acid, carbonic acid or phosphoric acid. Furthermore, where the peptide carries an acidic moiety, suitable pharmaceutically acceptable salts thereof may include alkali metal salts (e.g., sodium or potassium salts); alkaline earth metal salts (e.g., calcium or magnesium salts); and salts formed with suitable organic ligands (e.g., ammonium, quaternary ammonium and amine cations formed using counteranions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl sulfonate and aryl sulfonate). Illustrative examples of pharmaceutically acceptable salts include, but are not limited to, acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate,

bromide, butyrate, calcium edetate, camphorate, camphorsulfonate, camsylate, carbonate, chloride, citrate, clavulanate, cyclopentanepropionate, digluconate, dihydrochloride, dodecylsulfate, edetate, edisylate, estolate, esylate, ethanesulfonate, formate, fumarate, gluceptate, glucoheptonate, gluconate, glutamate, glycerophosphate, glycolylarsanilate, hemisulfate, heptanoate, hexanoate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, hydroxynaphthoate, iodide, isothionate, lactate, lactobionate, laurate, lauryl sulfate, malate, maleate, malonate, mandelate, mesylate, methanesulfonate, methylsulfate, mucate, 2-naphthalenesulfonate, napsylate, nicotinate, nitrate, N-methylglucamine ammonium salt, oleate, oxalate, pamoate (embonate), palmitate, pantothenate, pectinate, persulfate, 3-phenylpropionate, phosphate/diphosphate, picrate, pivalate, polygalacturonate, propionate, salicylate, stearate, sulfate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide, undecanoate, valerate, and the like (see, for example, S. M. Berge et al., "Pharmaceutical Salts", J. Pharm. Sci., 66, pp. 1-19 (1977)).

The term "excipient" when used herein is intended to indicate all substances in a pharmaceutical formulation which are not active ingredients such as, e.g., carriers, binders, lubricants, thickeners, surface active agents, preservatives, emulsifiers, buffers, flavoring agents, or colorants.

In a preferred embodiment, the pharmaceutical composition of the present invention is for treating or preventing disorders associated with muscular malfunction as specified above for the peptide of the invention in the third aspect of the present invention.

In another preferred embodiment, the pharmaceutical composition of the present invention is for protecting myocytes from arrhythmias, protecting an individual from ventricular arrhythmias, preferably from lethal ventricular tachyarrhythmias, and thus, preferably from sudden cardiac death, for reducing calcium spark frequency in myocytes, for preventing and/or reducing calcium leakage from the sarcoplasmic reticulum of myocytes, for protecting myocytes from apoptotic cell death, preferably protecting cardiomyocytes from apoptotic cell death in failing myocardium, for restoring and/or enhancing hemodynamic function, preferably enhancing hemodynamic function in an individual suffering from heart failure, and/or for enhancing and/or restoring contractile performance in skeletal muscle cells, preferably for enhancing and/or restoring isometric and/or tetanic twitch force in skeletal muscle cells as described above for the peptide according to the present invention in the third aspect of the present invention.

The pharmaceutical composition contemplated by the present invention may be formulated in various ways well known to one of skill in the art. For example, the pharmaceutical composition of the present invention may be in liquid form such as in the form of solutions, emulsions, or suspensions. Preferably, the pharmaceutical composition of the present invention is formulated for parenteral administration, preferably for intravenous, intramuscular, subcutaneous, transdermal, intrapulmonary, intraperitoneal, intracardiac administration, or administration via mucous membranes, preferably for intravenous, subcutaneous, or intraperitoneal administration. Preferably, the pharmaceutical composition of the present invention is in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9, more preferably to a pH of from 5 to 7), if necessary.

The pharmaceutical composition is preferably in unit dosage form. In such form the pharmaceutical composition is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of pharmaceutical composition such as vials or ampoules.

In a fifth aspect, the present invention provides a use of the peptide according to the first aspect of the present invention for the preparation of a pharmaceutical composition for treating or preventing disorders associated with muscular malfunction, wherein said disorder is preferably as specified above in the third aspect of the present invention. In a preferred embodiment, the use of the peptide is for the preparation of a pharmaceutical composition for protecting myocytes from arrhythmias, protecting an individual from ventricular arrhythmias, preferably from lethal ventricular tachyarrhythmias, and thus, preferably from sudden cardiac death, for reducing calcium spark frequency in myocytes, for preventing and/or reducing calcium leakage from the sarcoplasmic reticulum of myocytes, for protecting myocytes from apoptotic cell death, preferably protecting cardiomyocytes from apoptotic cell death in failing myocardium, for restoring and/or enhancing hemodynamic function in an individual suffering from heart failure, and/or for enhancing and/or restoring contractile performance in skeletal muscle cells, preferably for enhancing and/or restoring isometric and/or tetanic twitch force in skeletal muscle cells as described above for the peptide according to the present invention in the third aspect of the present invention. In a preferred embodiment of the fifth aspect of the present invention, the use is for the preparation of a pharmaceutical composition for ameliorating a disease condition associated with a muscular disorder, preferably a skeletal

muscle disorder and/or a cardiac muscle disorder, wherein the term "ameliorating a disease condition" is as defined for the sixth aspect of the present invention.

In a sixth aspect, the present invention provides a method for treating or preventing disorders associated with muscular malfunction comprising administering to an individual in need thereof the peptide of the first aspect of the present invention or the pharmaceutical composition of the fourth aspect of the present invention in an amount sufficient to ameliorate the disease condition of said individual, preferably the patient. In this context, "ameliorating the disease condition" means, for example, that the individual has a subjective sensation of improvement after a certain period of time after the peptide or the pharmaceutical composition has been administered to the patient, or that the function of the diseased muscle has been measurably improved after treatment with the peptide or the pharmaceutical composition of the present invention. For example, if the contractile performance such as the contractile force of a muscular tissue, e.g. a diseased heart muscle or a diseased skeletal muscle, deviated from an average normal contractile function by 50%, the disease condition is ameliorated by the treatment if, after the treatment, the contractile performance of said musclular tissue deviates less than 50%, e.g. less than 40%, less than 30%, less than 20%, less than 10%, or not at all, from the average normal contractile function of a corresponding healthy muscular tissue. The contractile performance may also be improved compared to the average cardiac performance of a healthy cardiac tissue. The term "individual in need thereof" preferably refers to an animal patient, more preferably to a mammalian patient, most preferably to a human patient as defined above.

The disorder associated with muscular malfunction is preferably as defined for the third aspect of the present invention.

In a preferred embodiment, the method according to the sixth aspect of the present invention is for protecting myocytes from arrhythmias, protecting an individual from ventricular arrhythmias, preferably from lethal ventricular tachyarrhythmias, and thus, preferably from sudden cardiac death, for reducing calcium spark frequency in myocytes, for preventing and/or reducing calcium leakage from the sarcoplasmic reticulum of myocytes, for protecting myocytes from apoptotic cell death, preferably protecting cardiomyocytes from apoptotic cell death in failing myocardium, for restoring and/or enhancing hemodynamic function in an individual suffering from heart failure, and/or for enhancing and/or restoring contractile performance in skeletal muscle cells, preferably for enhancing and/or restoring isometric and/or tetanic twitch force in skeletal muscle cells as described above for the peptide according to the present invention in the third aspect of the present invention.

For treating or preventing a disorder associated with muscular malfunction as specified in the fifth and sixth aspect of the present invention, the peptide or the pharmaceutical composition according to the present invention can be administered to an animal patient, preferably a mammalian patient, preferably a human patient, preferably via a parenteral administration route, for example, intravenously, intramuscularly, subcutaneously, transdermally, intrapulmonary, intraperitoneally, intracardiacally, or via mucous membranes, preferably intravenously, subcutaneously, or intraperitoneally. Administration may be by infusion or classical injection, for example, using cannulas, or by needleless injection techniques.

In a seventh aspect, the present invention provides a composition comprising, essentially consisting or consisting of the peptide according to the first aspect of the present invention in combination with another medicament usually administered for treating or preventing diseases associated with muscular malfunction, preferably skeletal muscle diseases, more preferably cardiac muscle diseases. Preferably, said composition is a pharmaceutical composition which may also comprise one or more pharmaceutically acceptable diluent(s), carrier(s), and/or excipient(s). In a preferred embodiment, said medicament exhibits pro-arrhythmogenic potential, preferably on cardiomyocytes. In a preferred embodiment of this aspect of the present invention, the peptide according to the present invention reduces the pro-arrhythmogenic potential of said medicament. Preferably, said medicament is a catecholamine, e.g., a direct β-mimetics such as endogenous or synthetic catecholamine or an indirect β-mimetics such as a phosphodiesterase inhibitor β-mimetics or another agent enhancing RyR2 calcium-sensitivity such as caffeine or similar chemicals, e.g., purine alkaloids or dimethylxanthines. In a preferred embodiment of the seventh aspect of the present invention, said medicament is selected form the group consisting of a catecholamine, a β-adrenergic receptor agonist, and a β-adrenergic receptor blocker. In one embodiment, said medicament is a catecholamine such as dobutamine, noradrenaline, adrenaline, dopamine, or isoprenalin, preferably dobutamine, noradrenaline, or adrenaline. In another embodiment, said medicament is a β-adrenergic receptor agonist such as isoproterenol, salbutamol, fenoterol, formoterol, metaproterenol, salmeterol, terbutaline, clenbuterol, arbutamine, befunolol, bromoacetylalprenololmenthane, broxaterol, cimaterol, cirazoline, denopamine, epinephrine, etilefrine, hexoprenaline, higenamine, isoetharine, isoxsuprine, mabuterol, methoxyphenamine, nylidrin, oxyfedrine, pirbuterol, prenalterol, procaterol, ractopamine, reproterol, rimiterol, ritodrine, tretoquinol, tolubuterol, xamoterol, zilpaterol, or zinterol, preferably isoproterenol, salbutamol, fenoterol, formoterol, metaproterenol, salmeterol,

terbutaline, clenbuterol, more preferably isoproterenol. In another embodiment, said medicament is a β-adrenergic receptor blocker such as metoprolol, atenolol, bisoprolol, nebivolol, esmolol, betaxolol, acebutolol, celiprolol, bupranolol, propranolol, timolol, carvedilol, sotalol, pindolol, oxprenolol, or alprenolol, preferably metoprolol, atenolol, bisoprolol, nebivolol, esmolol, or betaxolol, most preferably metoprolol. The composition according to the seventh aspect of the invention may comprise one or more, e.g., 1, 2, 3, or 4 different medicaments either of the same category or of different categories, in combination with the peptide according to the first aspect of the present invention.

The present inventors have surprisingly found that the peptides according to the present invention are useful for treating or preventing disorders associated with muscular malfunction as specified throughout the description, that the peptides according to the present invention have the ability to reduce the pro-arrhythmogenic potential of medicaments such as catecholamines, β-adrenergic receptor agonists, or β-adrenergic receptor blocker without counteracting their beneficial effects, and that these therapeutic effects of said peptides are exerted even if the peptides are administered parenterally, preferably via an intravenous, intraperitoneal, or subcutaneous administration route, without the need for genetic modification by gene therapy and without causing major side effects.

EXAMPLES

The Examples are designed in order to further illustrate the present invention and serve a better understanding. They are not to be construed as limiting the scope of the invention in any way.

Example 1: Inotropic effects of S100A1 protein and S100A1 C-terminal 20-mer peptide on permeabilized cardiomyocytes and skeletal muscle fibers.

Adult ventricular rabbit cardiomyocytes were isolated from four different animals as previously described (Loughrey C.M. et al., 2004, J. Physiol. 556:919-934) and permeabilized using ß-escin (0.1 mg/ml). The permeabilized cells were incubated for 1 minute with rhodamine-labeled human S100A1 protein (0.1 µM) or FITC-labeled S100A1 C-terminal 20-mer peptide (0.1 µM) fused to a hydrophilic linker (amino acids 75 to 94 of human S100A1 fused to D-K-D-D-P-P (SEQ ID NO: 354)). Cells were monitored using a Bio-Rad 2000 laser scanning confocal microscope (LSCM). A striated staining pattern can be observed that resembles the ryanodine staining pattern (Figure 4).

Furthermore, Ca^{2+}-spark frequency has been assessed in the permeabilized cells treated with S100A1 protein or the S100A1 C-terminal 20-mer peptide fused to a hydrophilic linker and it was shown that both, the full-length protein as well as the C-terminal fragment, decrease calcium spark frequency in permeabilized cardiomyocytes (Figures 5 and 6). Isolated cardiomyocytes were perfused with a mock intracellular solution and permeabilized using ß-escin (0.1 mg/ml). Fluo-3 free acid (10 µM) present in the perfusing solution was excited at 488 nm (Kr-laser) and measured at >515 nm applying epifluorescence optics of an inverted microscope with a ×60-1.2 NA water-immersion objective lens. Fluorescence was acquired in line scan mode at 2 ms $line^{-1}$; pixel dimension was 0.3 µm (512 pixels $scan^{-1}$; zoom = 1.4). The scanning laser line orientated parallel with the long axis and placed approximately equidistant between the outer edge of the cell and the nucleus/nuclei, to ensure the nuclear area was not included in the scan line. To enable this trace to be converted to free calcium concentration($[Ca^{2+}]$) a series of calibration solutions were used at the end of each Ca^{2+}-spark measurement period incorporating 10 mM EGTA. In all experiments concerning Ca^{2+}-sparks, the $[Ca^{2+}]$ in the test solution was 145-160 nM. Ca^{2+}-sparks recorded in Fluo-3-containing solutions were quantified using an automated detection and measurement algorithm. All Ca^{2+}-spark measurements were made within 7-8 min of cell permeabilization. This time was standardized to minimize loss of soluble proteins. S100A1 protein or S100A1-ct peptide was applied in mock solution using a gravity-fed perfusion system. Effects were compared to permeabilized control cardiomyocytes perfused with mock-solution without addition of S100A1. Up to four different cells from each animal were used for Ca^{2+}-spark measurements.

Muscle Fiber Preparation and Experimental Solutions. All of the animals were handled according to the guidelines of the animal care committee of the University of Heidelberg. Male BALB/c mice (3-6- months-old) were sacrificed by an overdose of carbon dioxide, and muscle fiber preparation was carried out as previously described (Fink R.H. and Stephenson D.G., 1987, Pflugers Arch. Eur. J. Physiol. 409:374-380; Makabe M. et al., 1996, Pflugers Arch. Eur. J. Physiol. 432: 717-726). Either EDL (M. ext. dig. longum) or Soleus was isolated, and a small fiber bundle containing two to four single fibers (between 80 and 150 µm in diameter and 3-4-mm-long) was dissected in paraffin oil. The fiber preparation was glued between a force transducer pin (AE801, Senso-Noras, Horton, Norway) and a micrometer-adjustable screw. All of the experiments were carried out at room temperature (23–25 °C). All of the solutions were adjusted to pH 7.0. The free ion concentrations were

calculated with the computer program REACT (version 2.0) from G. L. Smith (Glasgow, Scotland). Table I shows the concentrations of the solution used in the experiments.

TABLE I

Total concentration, in brackets is free concentration

LR, low relaxing solution; HR, high relaxing solution; HA, high activation solution; SK, skinning solution; LS, loading solution.

	LR	HR	HA	SK	LS
ATP (mM)	8	8	8	8	8
CP (mM)	10	10	10	10	10
CK (unit/ml)	150	150	150	150	150
Ca^{2+} (mM)		0.01	49.5		[4×10^{-4}]
Mg^{2+} (mM)	[0.5]	[0.5]	[0.5]	[0.5]	[0.5]
Na^{+} (mM)	36	36	36	36	36
K^{+} (mM)	117	117	117	117	117
HEPES (mM)	60	60	60	60	60
EGTA (mM)	0.5	50	50	0.5	50
HDTA (mM)	49.5			49.5	
Saponin (mg/ml)				50	

The high relaxation and the high activation solution contained 50 mM EGTA to buffer free Ca^{2+}, whereas the low relaxing solution contained 0.5 mM EGTA and 49.5 mM 1,6-diamino hexane-*N,N,N,N*-tetraacetic acid (HDTA), which in contrast to EGTA has very low affinity to Ca^{2+}. The skinning solution is obtained by the addition of 50 µg/ml saponin to the low relaxing solution. The release solution consisted of the low relaxing solution with 5 mM caffeine added. Loading solution contained 50 mM EGTA to clamp free Ca^{2+} to 0.4 µM (pCa 6.4). The solutions to measure the pCa-force relation were obtained by mixing high relaxing solution with appropriate amounts of high activating solution, and 5 mM caffeine added. All of the experiments were recorded using a strip chart recorder and were simultaneously digitally converted with an Axon Instruments Digidata 1200 board and interface (using the Axotape Software, version 2.0). For muscle fiber preparation and force measurements see also Weisleder N. et al., 2006, J. Cell Biol. 174:639-645.

Assessment of Ca^{2+}-induced Isometric Twitch Force and Ca^{2+} Transients in skeletal muscle fibers.

It was shown that both, the full-length S100A1 protein as well as the C-terminal fragment, has a potency to enhance isometric twitch force in permeabilized murine skeletal muscle fibers (Figure 7). Muscle fibers were skinned for 5 min in skinning solution while the

sarcomere length was adjusted to 2.6 ± 0.1 μm using the diffraction pattern of a helium-neon laser. Before loading the SR with the loading solution (pCa 6.4) for 1 min, the fibers were shortly immersed in release solution and high relaxing solution and then equilibrated for 2 min in low relaxing solution. Subsequently, the preparation was dipped for 1 s into the high relaxing solution and again for 2 min in low relaxing solution. The fibers were exposed to the release solution containing 5 mM caffeine until the initial force transient returned to the resting force level. Maximum force was measured in the high activating solution at pCa 4.28 and 5 mM caffeine. The fibers then were relaxed in high relaxing solution for 1 min to buffer Ca^{2+}. Several control transients were recorded before the fiber was exposed to the S100A1 protein or the S100A1 peptide mixture (N/H/C) or the C-terminal 20-mer alone, and the experiment was repeated as outlined above. S100A1 protein or peptides were added to the low relaxing solution before and during release and to the high activating solution. The pCa-force relation in response to S100A1 interventions (0.001–10 μM) was measured with six different Ca^{2+} concentrations (EDL, pCa 9.07, 5.91, 5.72, 5.49, 5.17, and 4.28), each containing 5 mM caffeine. The EC50 and the Hill coefficient were obtained from a Hilltype fit. The EC50 value indicates the Ca^{2+} concentration needed for half-maximal isometric force activation, which is as a measure of Ca^{2+} sensitivity of the contractile apparatus. The Hill coefficient gives an indication of the maximum steepness of the sigmoidal curve. The correlation coefficients were calculated to determine the accuracy of the fit. The force transient was transformed into the corresponding free Ca^{2+} transient by using the individual pCa^{2+} force relation as a Ca^{2+} indicator and reversing each point of the force transients into the corresponding free Ca^{2+} level as previously described. Based on the fact that sensitivity of the Ca^{2+}-regulatory proteins and the corresponding force development directly provide a measure of the free myofibrillar Ca^{2+}, the pCa force relation relates free Ca^{2+} and force. Thus, the pCa-force relation can be used as a bioassay, which converts the rather slow force transients from the Ca^{2+} release from the SR into apparent Ca^{2+} transients.

Example 2: Cell-permeability of the S100A1$ct_{6/11}$ peptide

Neither rhodamine-labeled S100A1 protein nor the FITC-labeled S100A1 C-terminal 20-mer peptide with or without a hydrophilic motif such as D-K-D-D-P-P (SEQ ID NO: 354) are able to penetrate the cell membrane of adult intact cardiomyocytes. However, the present inventors surprisingly found that a peptide having the sequence D-K-D-D-P-P-Y-V-V-L-V-A-A-L-T-V-A (SEQ ID NO: 372) referred to as S100A1$ct_{6/11}$ is cell permeable. FITC-labeled S100A1$ct_{6/11}$ was incubated with intact rat ventricular cardiomyocytes for 15 minutes before

the cells were monitored using confocal laser scanning microscopy. Endogenous S100A1 protein was stained using a conventional immunofluorescence protocol. The intracellular staining pattern of FITC-labeled S100A1ct$_{6/11}$ resembles that of endogenous S100A1 (Figure 8).

Example 3: Functional characterization of the S100A1ct$_{6/11}$ peptide in cardiomyocytes

All experiments performed for the functional characterization of the S100A1ct$_{6/11}$ peptide were performed on intact, i.e., non-permeabilized cardiomyocates. It was shown that the S100A1ct$_{6/11}$ peptide exerts positive inotropic effects on stimulated isolated ventricular cardiomyocytes (Figure 9), while fragments thereof (Figure 10) or corresponding peptides derived from the carboxy-terminus of S100A4 or S100B (Figure 11) do not show this ability. Calcium transients were assessed in FURA2-AM field-stimulated cardiomyocytes employing epifluorescent digitalized microscopy and sarcoplasmic reticulum calcium load was determined (Figure 12).

Calibration and Measurement of Ca^{2+} Transients and SR Ca^{2+} Load in Cardiomyocytes. Intracellular Ca^{2+} transients of mouse ventricular cardiomyocytes were calibrated and measured as previously described (Remppis A. et al., 2002, Basic Res. Cardiol. 97: I/56-I/62). Briefly, isolated cells were washed in HEPES-modified medium 199 (M199) (Sigma), incubated in 1 ml of M199 (2 mM $[Ca^{2+}]_e$) with 2 µM Fura2-AM for 20 min at room temperature. Calibration and fluorescence measurements were carried out using an inverse Olympus microscope (Ix70) with a UV filter connected to a monochromator (Polychrome II, T.I.L.L. Photonics GmbH, Germany). Cells were electrically stimulated with 1 Hz and excited at 340/380 nm. Fluorescence emission was detected at 510 nm, digitized, and analyzed with T.I.L.L.VISION software (v. 3.3). Baseline data from five consecutive steady-state transients were averaged for analysis of transient amplitude (Ca^{2+} amplitude; (nM)), time to peak (ms), and time to 50% decline (ms). Calibration for Fura2-AM loaded mouse ventricular myocytes on 50 cells yielded a minimal ratio (R_{min}) of 0.38 ± 0.05 and a maximal ratio (R_{max}) of 3.36 ± 0.21, whereas β and *Kd* were estimated to amount to 5.21 ± 0.24 and 236 ± 29 nM, respectively. Free intracellular Ca^{2+} concentration $[Ca^{2+}]i$ was calculated by the equation of Grynkiewicz *et al.* (Grynkiewicz G. et al., 1985, J. Biol. Chem. 260:3440–3450). Ca^{2+} transients were investigated at baseline and throughout a stepwise increase of isoproterenol concentrations (10^{-9}-10^{-5} M) under electrical stimulation at 1 Hz and 2 mM $[Ca^{2+}]_e$ in M199. SR Ca^{2+} load was assessed using a standard caffeine pulse protocol. After 2 min of electrical

stimulation (1 Hz), myocytes were abruptly exposed to 0 Na^+/0 Ca^{2+} solution with caffeine (10 mM). The peak of the caffeine-induced Ca^{2+} transient was used as an index of the SR Ca^{2+} load.

Myocyte Contractile Parameters.

Contractility studies of isolated ventricular myocytes were performed as recently described (Most P. et al., 2001, Proc. Natl. Acad. Sci. U. S. A. 98:13889–13894) with a video-edge detection system (Crescent Electronics, Sandy, UT). In brief, myocytes were electrically stimulated to contract at 1 Hz in M199 at room temperature; edge detection measurements were obtained under basal condition and incremental isoproterenol concentrations (10^{-9}- 10^{-5} M). Data from five consecutive steady-state twitches were averaged for analysis of fractional cellular shortening (%CS (%)), shortening velocity (-dL/dt, (μm/s)) and relengthening velocity (+dL/dt, (μm/s)).

Example 4: $S100A1ct_{6/11}$ does not alter β-adrenergic receptor signaling and protects cardiomyocytes from pro-arrhythmic store-overload-induced calcium release (SOICR)

The inotropic effect of $S100A1ct_{6/11}$ is additive to and independent of β-adrenergic stimulation (Figure 13). Ventricular cardiomyocytes have been isolated as described above and the calcium transient amplitude has been assessed in presence and absence of isoproterenol and in presence or absence of the $S100A1ct_{6/11}$ peptide, respectively.

Furthermore, the $S100A1ct_{6/11}$ peptide protects cardiomyocytes from pro-arrhythmic store-overload-induced calcium release (SOICR) (Figure 15). Calcium sparks were assessed in Fluo-3 AM loaded cardiomyocytes under control and βAR (10^{-7} M Isoproterenol + 0.5 mM caffeine) as described in Ventucci et al., 2007, Circ. Res. 100:105-111). It is important to note that the protective effect of $S100A1ct_{6/11}$ is effective at concentrations (100 and 1000 nM) that exert inotropic actions in cardiomyocytes due to enhanced SR calcium load. Thus, despite its own enhancing effect on SR Ca resequestration, $S100A1ct_{6/11}$ effectively antagonizes βAR-triggered SOICR highlighting the unique molecular profile combining inotropic actions with anti-arrhythmic potency.

Example 5: functional characterization of the $S100A1ct_{6/11}$ peptide in normal and disease hearts.

The S100A1ct$_{6/11}$ peptide exerts significant *in vivo* hemodynamic effects resulting in enhanced contractile performance under basal and βAR-stimulated conditions (Figure 18). These hemodynamic effects are effective in response to the β1AR-blocker metoprolol (Figures 19 and 20). Furthermore, the S100A1ct$_{6/11}$ peptide exerts significant therapeutic effects *in vivo* restoring hemodynamic function in an experimental heart failure mouse model (Figure 21) and preventing apoptotic cell death in failing myocardium in said mouse model (Figure 22). Furthermore, the S100A1ct$_{6/11}$ peptide protects the heart failure mice from βAR-triggered lethal ventricular tachyarrhythmias (Figure 22).

Transthoracic Echocardiography.

Two-dimensional guided M-mode and Doppler echocardiography was carried out using an HDI 5000 echocardiograph (ATL, Bothell, WA) in conscious mice as previously described (Kohout et al., 2001, Circulation 104:2485-2491). Three independent echocardiographic measurements were taken in both modes. Left ventricular chamber diameter in endsystole (LVESD) and end-diastole (LVEDD), interventricular septum (IVSth), LV posterior (LVPth) wall thickness in end-diastole, and LV fractional shortening (FS%) were determined in a short-axis M-modeview at the level of the papillary muscles; FS% = LVEDD - LVESD/ LVEDD x 100; (%). LV ejection time (LVET) and heart rate (bpm) taken from aortic valve Doppler measurements were used to assess heart rate corrected mean velocity of circumferential fiber shortening: mean Vcfc = FS%/ET x √60/bpm x 10; (circ/s).

Cardiac Catheterization and Hemodynamic Assessment.

Transthoracic two-dimensional echocardiography (TTE) in lightly anesthesized mice (tribromoethanol/amylene hydrate; Avertin; 2.5% wt/vol, 8µl/g IP) with spontaneous respiration was performed with a 12-MHz probe both in sham and infarcted mice (TTE in M-mode was carried out in the parasternal short axis before and after (7 and 28 days) surgical procedure to assess LV diameter and subsequently fractional shortening (FS% = [(LVEDD - LVESD)/LVEDD) X 100]). Under the same anesthesia, a 1.4 French micromanometer-tipped catheter (SPC-320, Millar instruments, Inc.) was inserted into the right carotid artery and then advanced into the LV. Hemodynamic analysis, including heart rate (beats/min^{-1}), LV end-diastolic pressure (LVEDP) and maximal (LV +dp/dt$_{max.}$) and minimal (LV dp/dt$_{min}$) first derivate of LV pressure.

Myocardial Histopathology and Apoptosis

LV tissue was cryosectioned (5 μm) and stained with hematoxylin-eosin (HE) to measure myocyte width in the remote, non-infarcted area of the LV, and measures were obtained at the level of the nucleus in longitudinally sectioned myocytes using NIH image software (ImageJ 1.34; http:/rsb.info.nih.gov/ij). Terminal deoxy-nucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining was carried out according to the manufacturers protocol (Roche, 11684795001). The number of TUNEL-positive cardiac myocyte nuclei in the remote area were counted a IX 70 inverse Olympus microscope (T.I.L.L. Vision software, version 3.3) and normalized per 10^5 total nuclei identified by HE staining in the same section. To identify cells or bodies of cardiac origin, the sections were double stained with a cardiac specific anti-troponin C antibody (Santa Cruz, sc-8117, 1:50 dilution) and a corresponding pair of donkey anti-goat Alexa Fluor 568 (Molecular Probes, 1:100) (data not shown). Caspase 3 activity in myocardial tissue was measured using a Caspase-Glo assay kit (Promega). Briefly, the proluminescent substrate is cleaved by caspase-3. After caspase cleavage, a substrate for luciferase (aminoluciferin) is released resulting in the luciferase reaction and the production of luminescent signal. Cytosolic extracts from heart tissue were prepared by homogenization in hypotonic extraction buffer (25 mM HEPES, pH 7.5, 5 mM $MgCl_2$, 1 mM EGTA) containing protease inhibitor mix (1 tablet/5ml) (Roche; Mini complete EDTA free protease inhibitor) and subsequently centrifuged (15 min, 13.000 rpm, 4°C). The protein concentration of the supernatant was adjusted to 1 mg/ml with extraction buffer and stored at -80°C. An equal volume of reagents and 10μg/ml cytosolic protein were added to a white-walled 96-well plate and incubated at room temperature for 1h. The luminescence of each sample was measured in triplicates in a plate-reading luminometer.

The pro-arrhythmic protocol was adapted from the previously published protocol by Wayne Chen and co-workers (Xiao et al., 2007, J. Biol. Chem. 282:34828-34838).

Example 6: Functional characterization of the S100A1ct$_{6/11}$ peptide in normal and skeletal muscle

The S100A1ct$_{6/11}$ peptide significantly enhances isometric twitch force in normal and diseased skeletal muscle (Figure 24). The protocol used for assessing isometric twitch force in skeletal muscle fibers is described in Example 1. For the experiment S100A1ct$_{6/11}$ peptide, intact (non-permeabilized) extensor digitorum longum (EDL) skeletal muscles fibers have been used. The twitch force of the isolated muscle fibers were enhanced upon S100A1ct$_{6/11}$ treatment, irrespective of whether the peptide was incubated with the isolated muscle fiber

(Figure 24 A) or whether the peptide was administered systemically before the muscle fiber was isolated (Figure 24 B).

Example 7: The inotropic effect of the S100A1ct$_{6/11}$ peptide is also exerted by a shorter peptide.

A peptide consisting of amino acids 76 to 85 of human S100A1 fused to a hydrophilic linker (D-K-D-D-P-P, SEQ ID NO: 354) exerts the same inotropic function as the S100A1ct$_{6/11}$ peptide (Figure 25). The protocol for assessing the calcium transient amplitude is described above. The linker alone, the vehicle alone, or amino-terminal deletion peptides lacking more than the amino acid 76 do not exhibit the inotropic effect. This experiment demonstrates that the tyrosine at position 76 is not essential for the inotropic function and cell permeability of the S100A1ct$_{6/11}$ peptide.

Claims

1. A peptide comprising a muscle function enhancing amino acid sequence comprising or consisting of the amino acid motif:

$$\Phi_4\text{-X-}\Psi\text{-L-[T/A]-}\Psi_2,$$

wherein Φ and Ψ are in each instance an independently selected hydrophobic non-aromatic amino acid, and X is any amino acid,
wherein the muscle function enhancing amino acid sequence does not contain more than 18 continuous amino acids of the carboxy-terminal amino acids of an S100A1 protein, the peptide has a total length of maximally 100 amino acids, and the peptide exhibits a positive inotropic action.

2. The peptide of claim 1, which is capable of penetrating cell membranes.

3. The peptide of claim 1 or 2, wherein the muscle function enhancing amino acid sequence does not contain more than 18 continuous amino acids of the carboxy-terminal amino acids of an S100 protein selected from the group consisting of S100 calcium binding protein A1, S100 calcium binding protein Z, S100 calcium binding protein T, S100 calcium binding protein S, and S100 protein α-chain.

4. The peptide of any one of claims 1 to 3, wherein the peptide exhibits the ability to enhance contractile performance and/or calcium cycling in myocytes.

5. The peptide on any one of claims 1 to 4, which exhibits the ability of protecting myocytes from arrhythmias, reducing calcium spark frequency in myocytes, preventing and/or reducing calcium leakage from the sarcoplasmic reticulum of myocytes, protecting myocytes from apoptotic cell death, restoring and/or enhancing hemodynamic function, and/or enhancing and/or restoring isometric and/or tetanic twitch force in skeletal muscle cells.

6. The peptide of any one of claims 1 to 5, wherein Φ is in each instance independently selected from the group of amino acids consisting of alanine, methionine, isoleucine, leucine, and valine, preferably methionine, isoleucine, leucine, and valine.

7. The peptide of any one of claims 1 to 6, wherein Ψ is in each instance independently selected from the group consisting of alanine, methionine, isoleucine, leucine, and valine, preferably alanine, methionine, isoleucine, and valine.

8. The peptide of any one of claims 1 to 7, wherein said muscle function enhancing amino acid sequence comprises or consists of the amino acid sequence:

[V/I]-[V/I]-[L/M]-[V/I/M]-[A/G/S]-[A/V]-L-[T/A]-[V/A/I]-[A/M/V].

9. The peptide of any one of claims 1 to 8, wherein said muscle function enhancing amino acid sequence comprises or consists of an amino acid sequence selected from the group consisting of the amino acid sequences V-V-L-V-A-A-L-T-V-A (SEQ ID NO: 4), V-I-L-V-A-A-L-T-V-A (SEQ ID NO: 6), V-V-M-V-A-A-L-T-V-A (SEQ ID NO: 7), I-I-L-V-G-A-L-T-V-A (SEQ ID NO: 95), V-V-L-I-A-A-L-A-A-A (SEQ ID NO: 263), V-I-L-V-S-V-L-T-V-A (SEQ ID NO: 186), and I-I-L-M-G-A-L-T-V-A (SEQ ID NO: 334), and V-V-M-V-A-A-L-T-V-V (SEQ ID NO: 50).

10. The peptide of any one of claims 1 to 9, wherein an amino acid or an amino acid sequence selected from the group consisting of C, C-N, C-N-[N/D/E], C-N-[N/D/E]-[F/Y], C-N-[N/D/E]-[F/Y]-F, C-N-[N/D/E]-[F/Y]-F-[W/L/Q], C-N-[N/D/E]-[F/Y]-F-[W/L/Q]-E (SEQ ID NO:), C-N-[N/D/E]-[F/Y]-F-[W/L/Q]-E-[N/T] (SEQ ID NO:) is directly linked to the carboxy-terminus of the amino acid motif.

11. The peptide of any one of claims 1 to 10, wherein an aromatic amino acid is directly linked to the amino-terminus of the amino acid motif.

12. The peptide of any one of claims 1 to 11 further comprising one or more of the elements selected from the group consisting of a membrane penetration enhancing motif, one or more epitope-tag(s), a hydrophilic motif, and a peptide targeting motif.

13. The peptide of any one of claims 1 to 12 further comprising a marker moiety.

14. The peptide of claim 12 or 13, wherein the hydrophilic motif comprises acidic, basic, and/or otherwise negatively or positively charged amino acids.

15. The peptide of any one of claims 12 to 15, wherein the hydrophilic motif comprises or consists of the hydrophilic amino acid motif:

$$\Lambda_4\text{-}\Theta_2,$$

wherein Λ is in each instance independently selected from aspartate, glutamate, lysine, and arginine and Θ is an α-helix interrupter, preferably is in each instance independently selected from proline or glycine.

16. The peptide of any one of claims 12 to 15, wherein the hydrophilic motif comprises or consists of the amino acid sequence:

[D/E]-[K/R]-[D/E]-[D/E]-[P/G]-[P/G].

17. The peptide of any one of claims 1 to 16 comprising or consisting of the amino acid sequence D-K-D-D-P-P-V-V-L-V-A-A-L-T-V-A (SEQ ID NO: 370), or an amino acid sequence which is at least 60% identical to said amino acid sequence.

18. The peptide of any one of claims 1 to 17 for medical use.

19. The peptide of any one of claims 1 to 18 for therapeutic use in treating or preventing disorders associated with muscular malfunction.

20. The peptide of claim 19, wherein the disorder is a cardiac and/or skeletal muscle disorder.

21. The peptide of claim 19 or 20, wherein the muscular malfunction is associated with defective calcium cycling and/or defective contractile performance in muscle cells.

22. The peptide of any one of claims 19 to 21, which is for enhancing and/or restoring calcium cycling and/or for enhancing and/or restoring contractile performance in muscle cells.

23. The peptide of any one of claims 20 to 22, wherein the cardiac muscle disorder is selected from the group consisting of postischemic contractile dysfunction, congestive heart failure, cardiogenic shock, septic shock, myocardial infarction, cardiomyopathy, dysfunction of heart valves, and ventricular disorder.

24. The peptide of any one of claims 20 to 22, wherein the skeletal muscle disorder is selected from the group consisting of muscular dystrophy, muscle weakness, muscular atrophy, myositis, central core disease, nemaline rod myopathy, centronuclear myotubular myopathy, ophthalmoplegia of the eye, mitochondrial myopathy.

25. A pharmaceutical composition comprising the peptide of any one of claims 1 to 17 and a pharmaceutically acceptable excipient, carrier, and/or diluent.

26. The pharmaceutical composition of claim 25 for treating or preventing disorders associated with muscular malfunction.

27. The pharmaceutical composition of claim 26, wherein the disorder is a cardiac and/or skeletal muscle disorder.

28. The pharmaceutical composition of claim 26 or 27, wherein the muscular malfunction is associated with defective calcium cycling and/or defective contractile performance in muscle cells.

29. The pharmaceutical composition of any one of claims 26 to 28, which is for enhancing and/or restoring calcium cycling and/or for enhancing and/or restoring contractile performance in muscle cells.

30. The pharmaceutical composition of any one of claims 27 to 29, wherein the cardiac muscle disorder is selected from the group consisting of postischemic contractile dysfunction, congestive heart failure, cardiogenic shock, septic shock, myocardial infarction, cardiomyopathy, dysfunction of heart valves, and ventricular disorder.

31. The pharmaceutical composition of any one of claims 27 to 29, wherein the skeletal muscle disorder is selected from the group consisting of muscular dystrophy, muscle

weakness, muscular atrophy, myositis, central core disease, nemaline rod myopathy, centronuclear myotubular myopathy, ophthalmoplegia of the eye, mitochondrial myopathy.

32. Use of the peptide according to any one of claims 1 to 17 for the preparation of a pharmaceutical composition for treating or preventing disorders associated with muscular malfunction.

33. The use of claim 32, wherein the disorder is a cardiac and/or skeletal muscle disorder.

34. The use of claim 32 or 33, wherein the muscular malfunction is associated with defective calcium cycling and/or defective contractile performance in muscle cells.

35. The use of any one of claims 32 to 34, wherein the pharmaceutical composition is for enhancing and/or restoring calcium cycling and/or for enhancing and/or restoring contractile performance in muscle cells.

36. The use of any one of claims 33 to 35, wherein the cardiac muscle disorder is selected from the group consisting of postischemic contractile dysfunction, congestive heart failure, cardiogenic shock, septic shock, myocardial infarction, cardiomyopathy, dysfunction of heart valves, and ventricular disorder.

37. The use of any one of claims 33 to 35, wherein the skeletal muscle disorder is selected from the group consisting of muscular dystrophy, muscle weakness, muscular atrophy, myositis, central core disease, nemaline rod myopathy, centronuclear myotubular myopathy, ophthalmoplegia of the eye, mitochondrial myopathy.

38. A method for treating or preventing disorders associated with muscular malfunction comprising administering to an individual in need thereof the peptide of any one of claims 1 to 17 or the pharmaceutical composition of any one of claims 25 to 31 in an amount sufficient to ameliorate the disease condition of said individual.

39. The method of claim 38, wherein the disorder associated with muscular malfunction is a cardiac or skeletal muscle disorder.

40. The method of claim 38 or 39, wherein the muscular malfunction is associated with defective calcium cycling and/or defective contractile performance in muscle cells.

41. The method of any one of claims 38 to 40, wherein the method is for enhancing and/or restoring calcium cycling and/or for enhancing and/or restoring contractile performance in muscle cells.

42. The method of any one of claims 39 to 41, wherein the cardiac muscle disorder is selected from the group consisting of postischemic contractile dysfunction, decompensated and compensated congestive heart failure, cardiogenic shock, septic shock, myocardial infarction, cardiomyopathy, dysfunction of heart valves, and right ventricular disorder.

43. The method of any one of claims 39 to 41, wherein the skeletal muscle disorder is selected from the group consisting of muscular dystrophy, muscle weakness, muscular atrophy, myositis, central core disease, nemaline rod myopathy, centronuclear myotubular myopathy, ophthalmoplegia of the eye, mitochondrial myopathy.

44. The method of any one of claims 54 to 63, wherein the peptide or the pharmaceutical composition is administered via a parenteral administration route.

45. A composition comprising the peptide of any one of claims 1 to 17 and a medicament selected from the group consisting of catecholamines, β-adrenergic receptor agonists, and β-adrenergic receptor blockers.

FIGURE 1

SEQ ID NO: 1

S100A1 protein:

EF-Hand EF-Hand

MGSELETAMETLINVFHAHSGKEGDKYKLSKKELKELLQTELSGFLDAQKDVDAVDKVMKELDENGDGEVDFQEYVVLVAALTVACNNFFWENS

(aa) 1 10 20 30 40 50 60 70 80 90

SEQ ID NO: 2

S100A1-ct peptide:

YVVLVAALTVACNNFFWENS

FIGURE 2

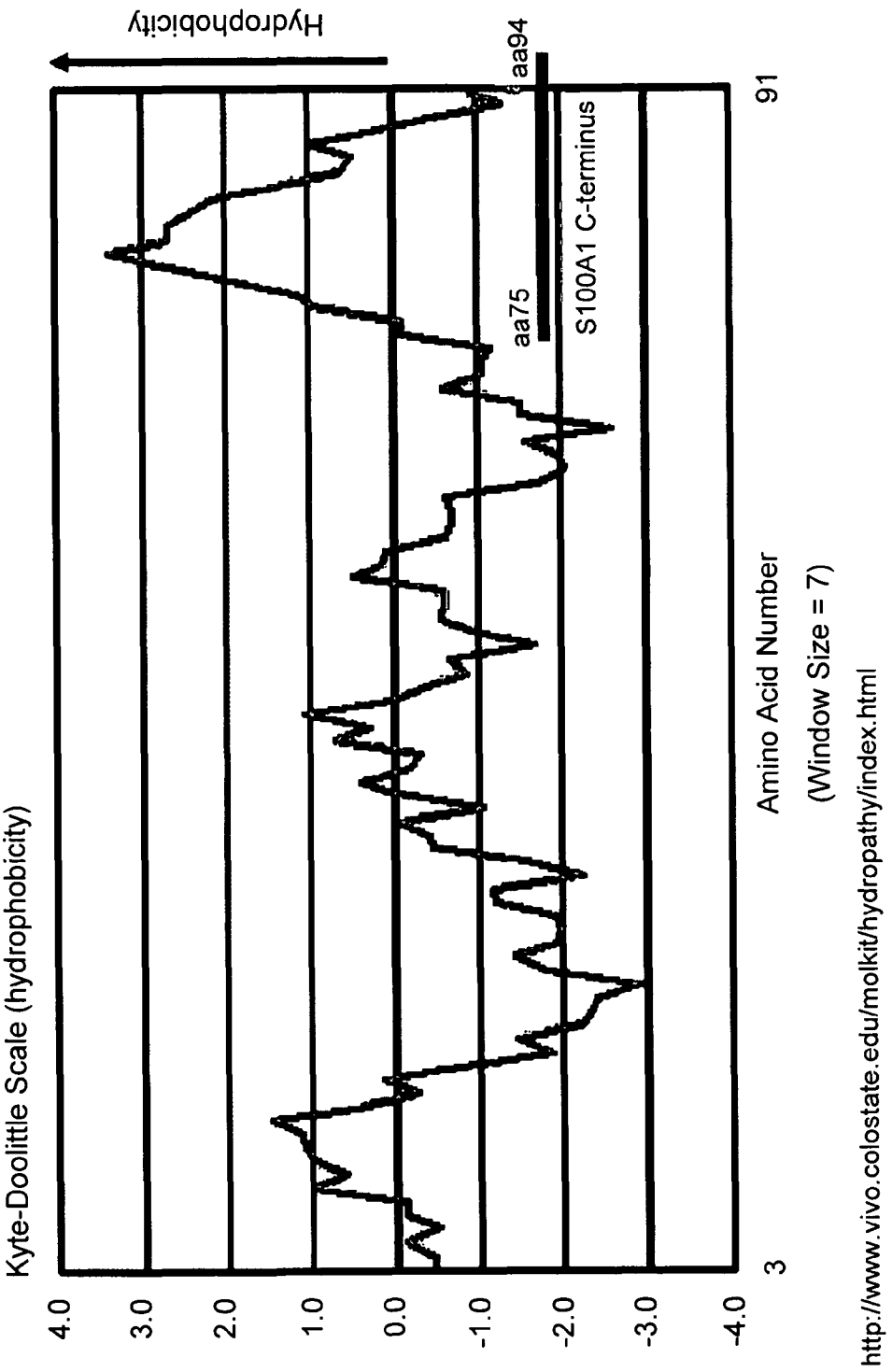

Hydrophobicity
aa94
aa75
S100A1 C-terminus
91
3
Amino Acid Number
(Window Size = 7)
Kyte-Doolittle Scale (hydrophobicity)
4.0
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
-4.0
http://www.vivo.colostate.edu/molkit/hydropathy/index.html

FIGURE 3

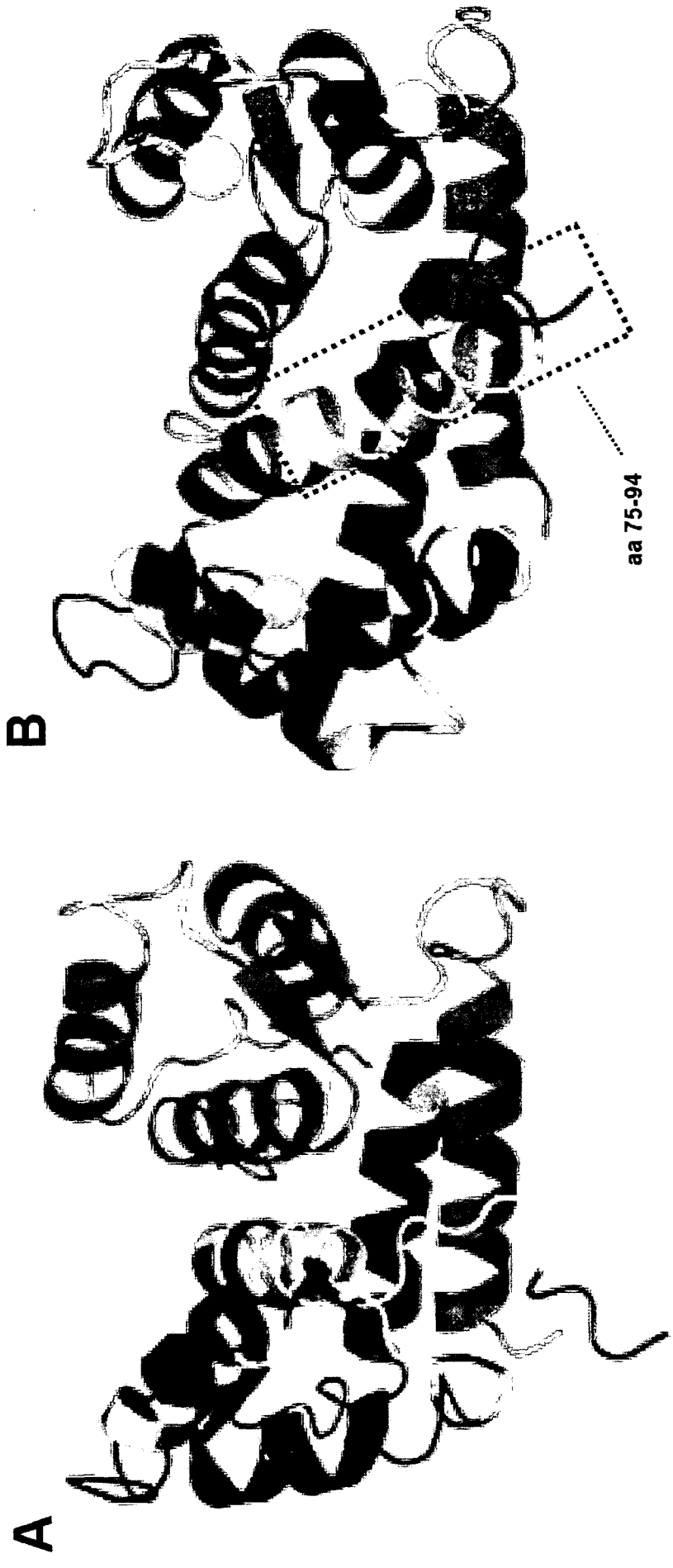


FIGURE 4

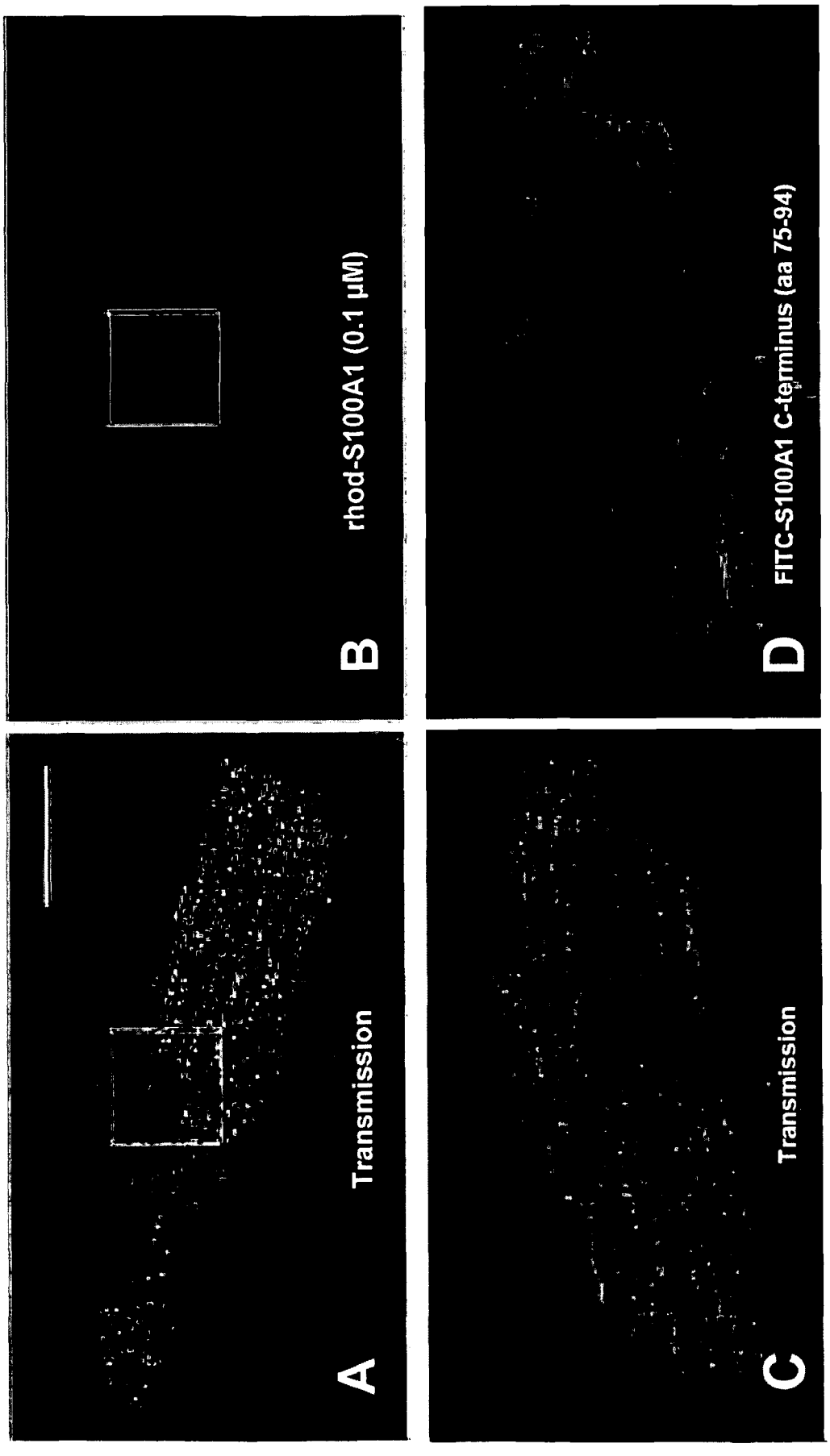

A
Transmission
B
rhod-S100A1 (0.1 µM)
C
Transmission
D
FITC-S100A1 C-terminus (aa 75-94)

FIGURE 5

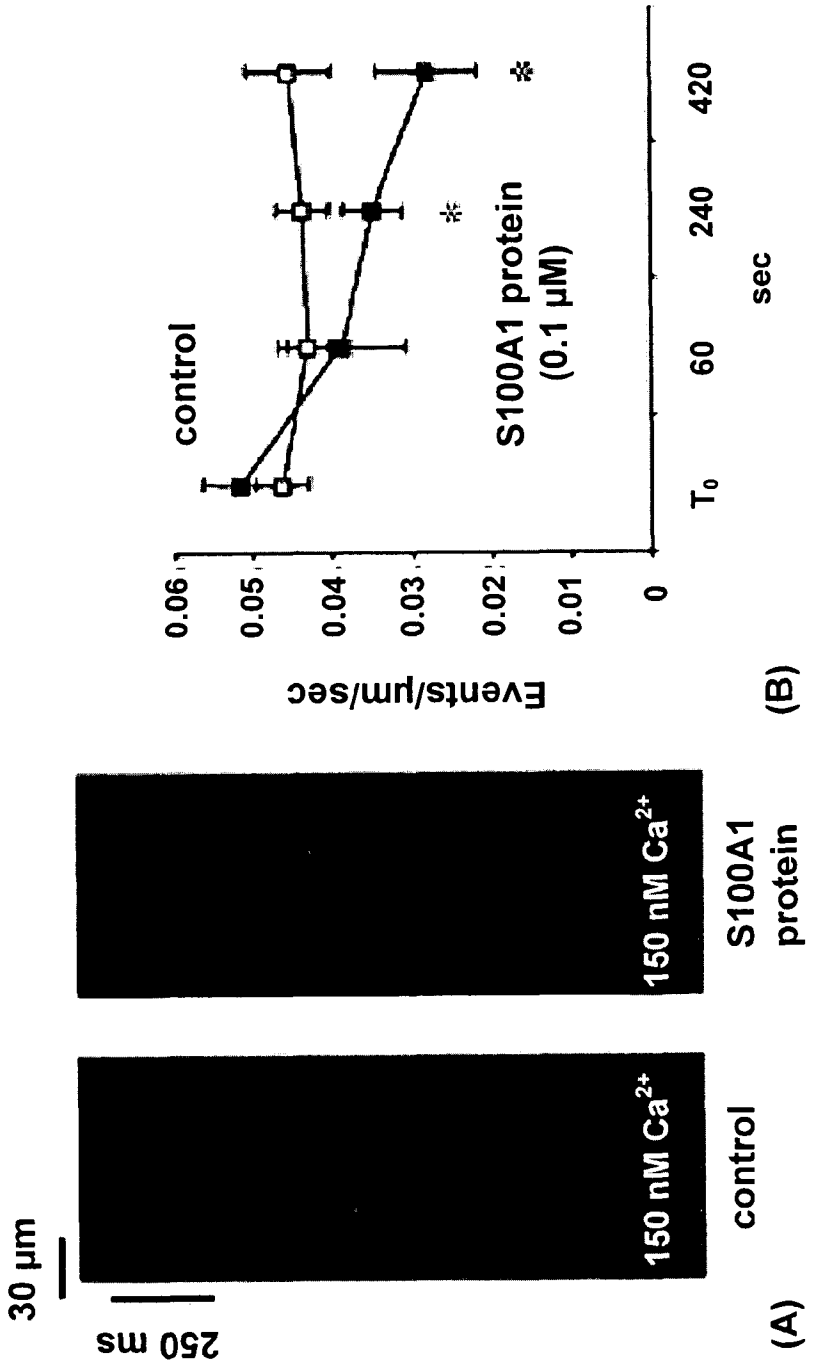

30 µm
250 ms
150 nM Ca2+
150 nM Ca2+
control
S100A1 protein
(A)
Events/µm/sec
0.06
0.05
0.04
0.03
0.02
0.01
0
control
S100A1 protein (0.1 µM)
T0
60
240
420
sec
(B)

FIGURE 6

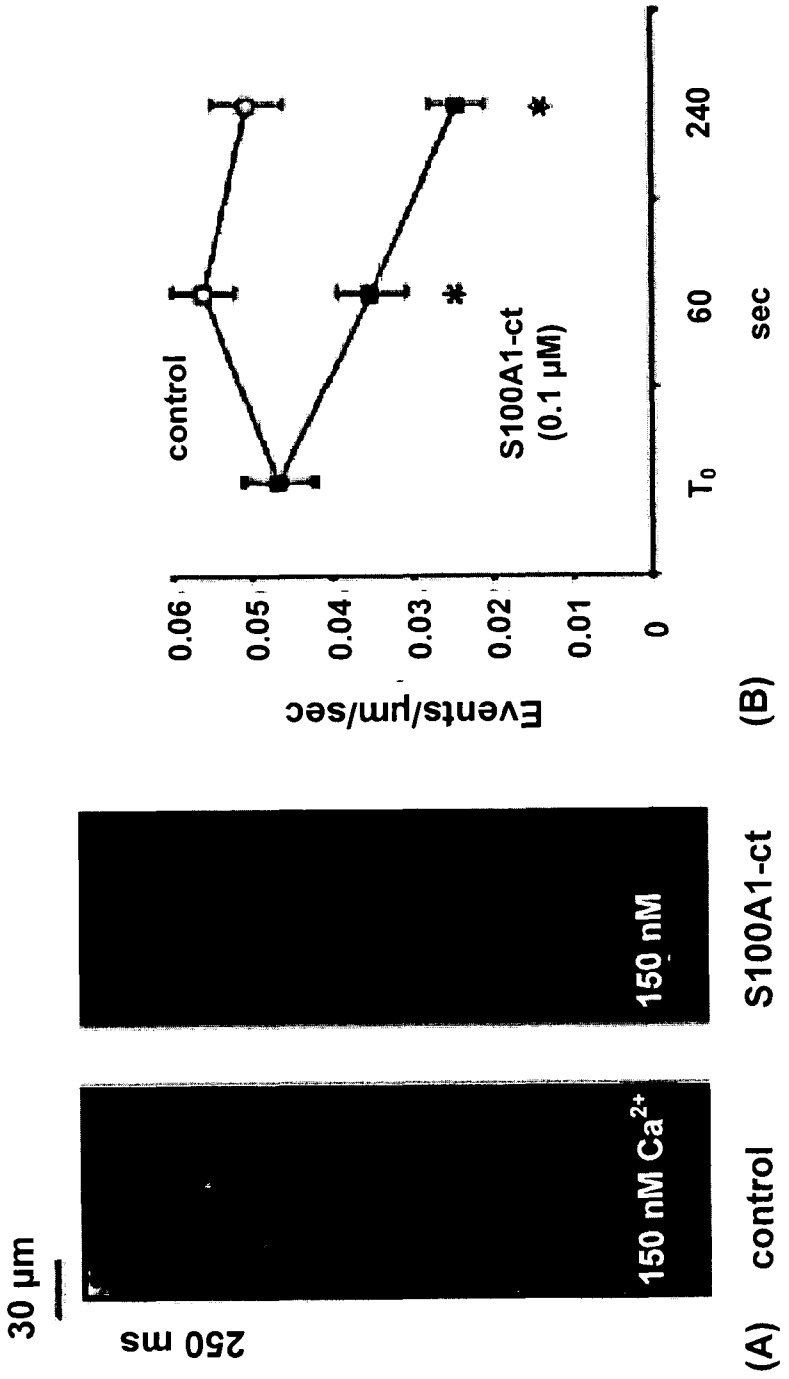

(A)
control
150 nM Ca2+
S100A1-ct
150 nM
30 µm
250 ms
(B)
Events/µm/sec
0.06
0.05
0.04
0.03
0.02
0.01
0
control
S100A1-ct
(0.1 µM)
T0
60
240
sec

FIGURE 7

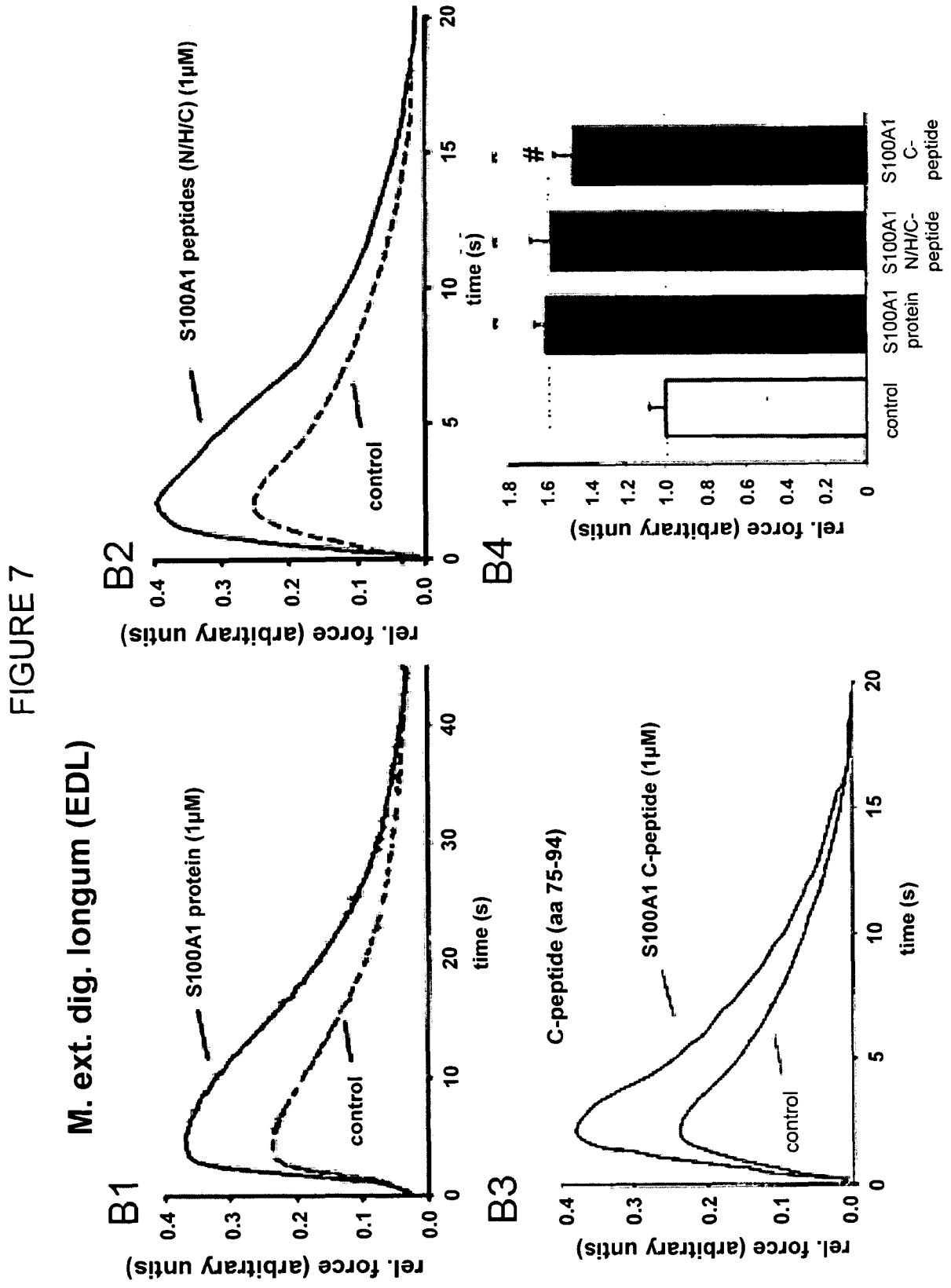
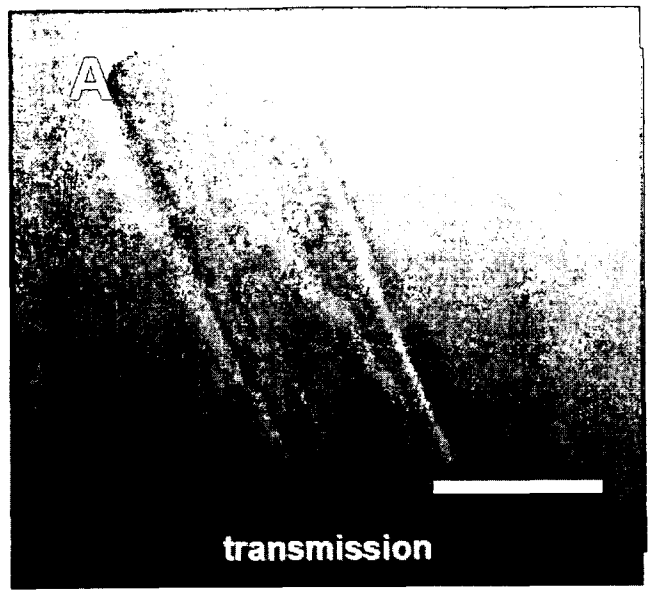
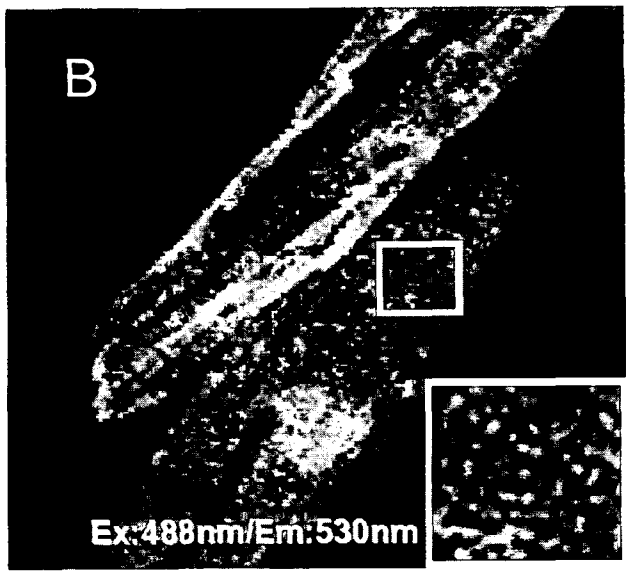
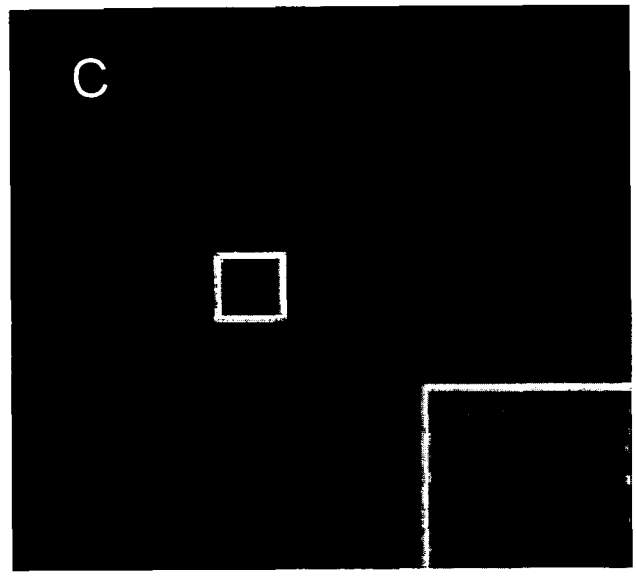

M. ext. dig. longum (EDL)
B1
S100A1 protein (1µM)
control
rel. force (arbitrary untis)
time (s)
B2
S100A1 peptides (N/H/C) (1µM)
control
rel. force (arbitrary untis)
time (s)
B3
C-peptide (aa 75-94)
S100A1 C-peptide (1µM)
control
rel. force (arbitrary untis)
time (s)
B4
#
control
S100A1 protein
S100A1 N/H/C-peptide
S100A1 C-peptide
rel. force (arbitrary untis)

FIGURE 8





S100A1ct$_{6/11}$-FITC



Endogenous S100A1

FIGURE 9

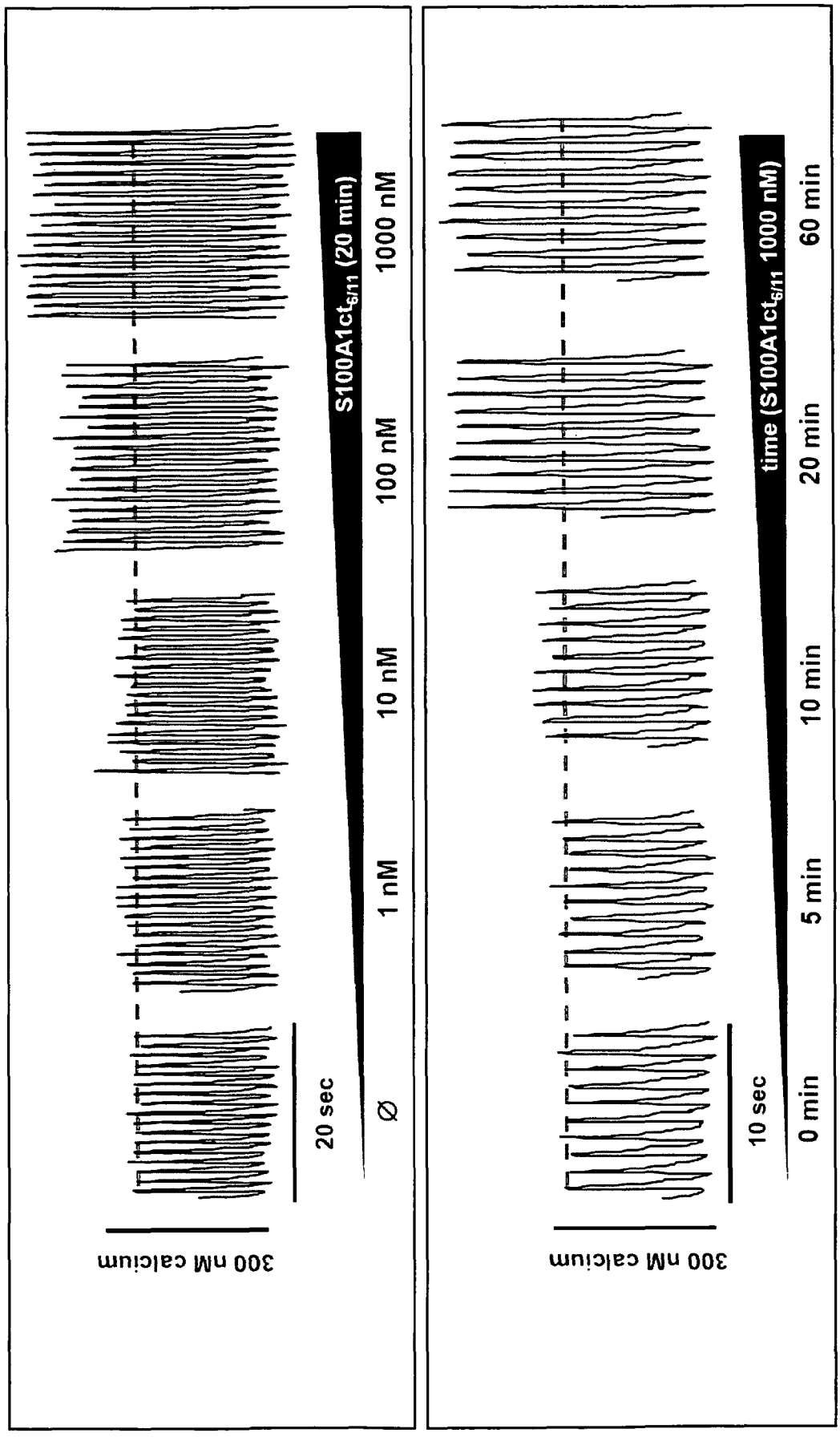

300 nM calcium
20 sec
S100A1ct6/11 (20 min)
Ø
1 nM
10 nM
100 nM
1000 nM
300 nM calcium
10 sec
time (S100A1ct6/11 1000 nM)
0 min
5 min
10 min
20 min
60 min

FIGURE 10

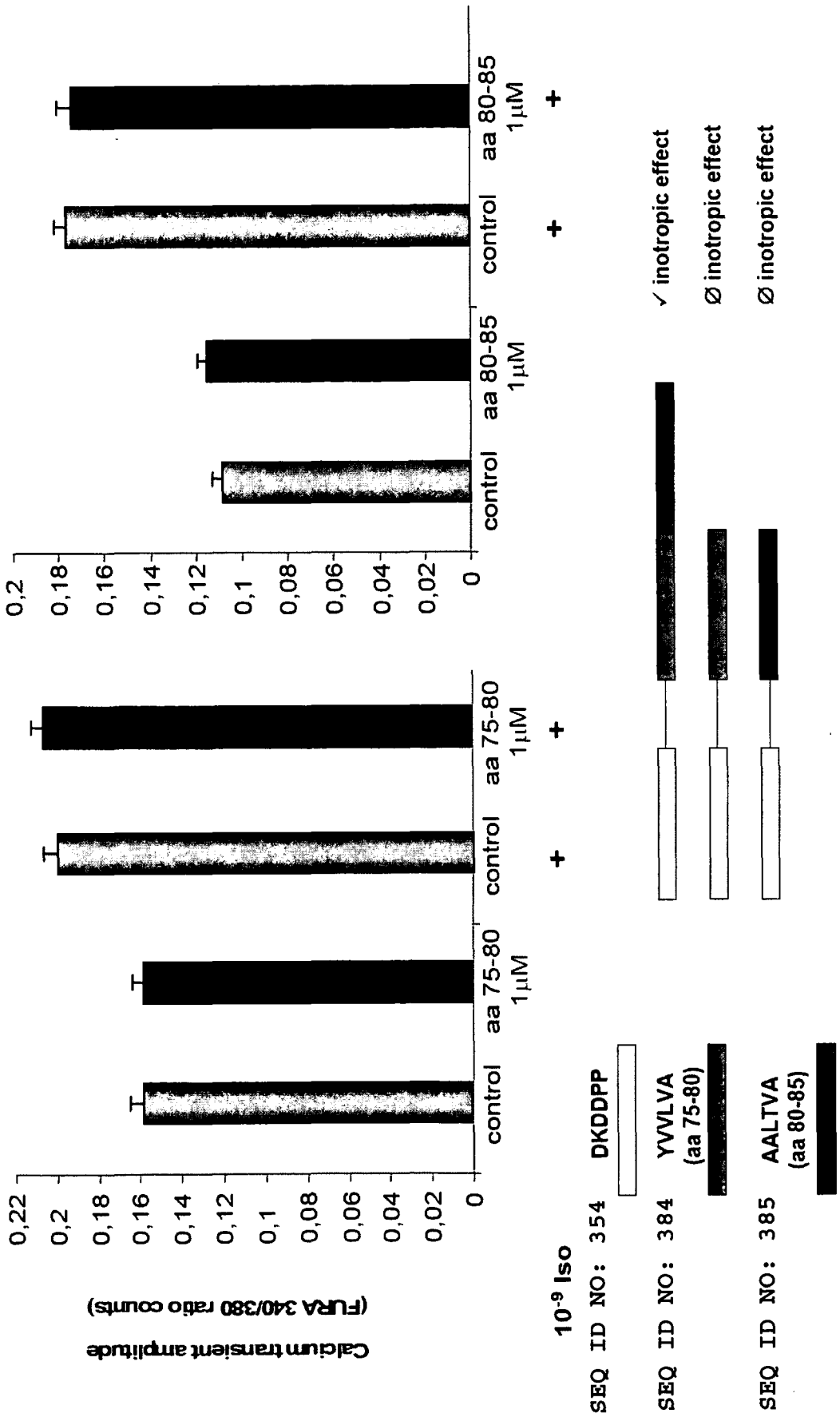
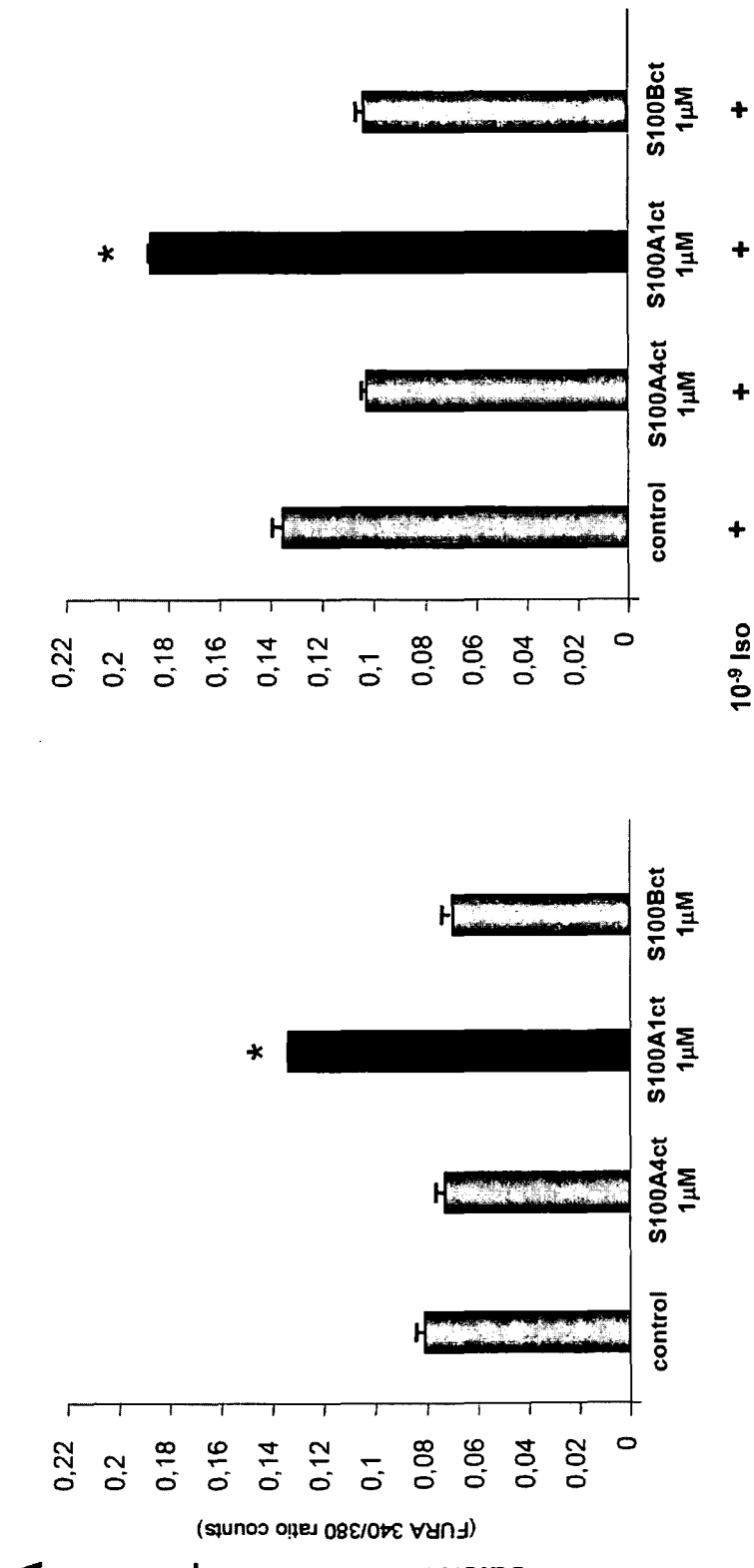

SEQ ID NO: 382 DKDDPP-YVVLVA (aa 75-80)
SEQ ID NO: 383 DKDDPP-AALTVA (aa 80-85)
Calcium transient amplitude
(FURA 340/380 ratio counts)
0,22
0,2
0,18
0,16
0,14
0,12
0,1
0,08
0,06
0,04
0,02
0
control
aa 75-80 1µM
aa 80-85 1µM
+
10-9 Iso
SEQ ID NO: 354 DKDDPP
SEQ ID NO: 384 YVVLVA (aa 75-80)
SEQ ID NO: 385 AALTVA (aa 80-85)
✓ inotropic effect
Ø inotropic effect
Ø inotropic effect

FIGURE 11



B

S100A1ct (aa 75-85) DKDDPP-YVVLVAALTVA SEQ ID NO: 372

S100A4ct (aa 75-85) DKDDPP-YCVFLSCIAMM SEQ ID NO: 386

S100Bct (aa 75-85) DKDDPP-FMAFVAMVTTA SEQ ID NO: 387

FIGURE 12

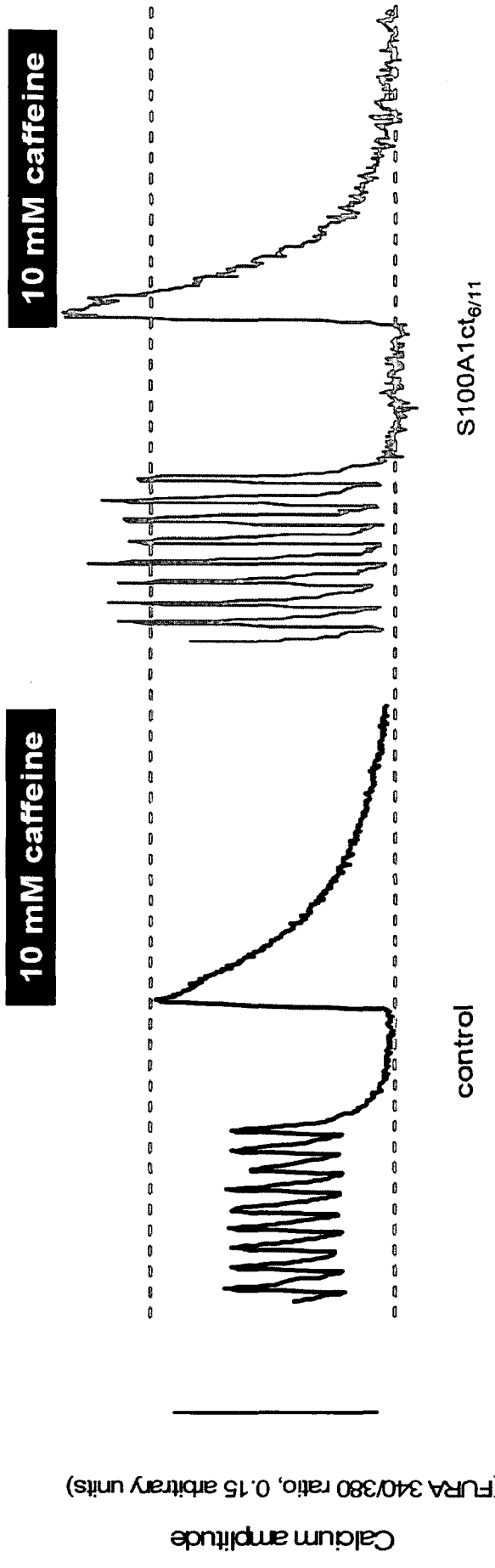
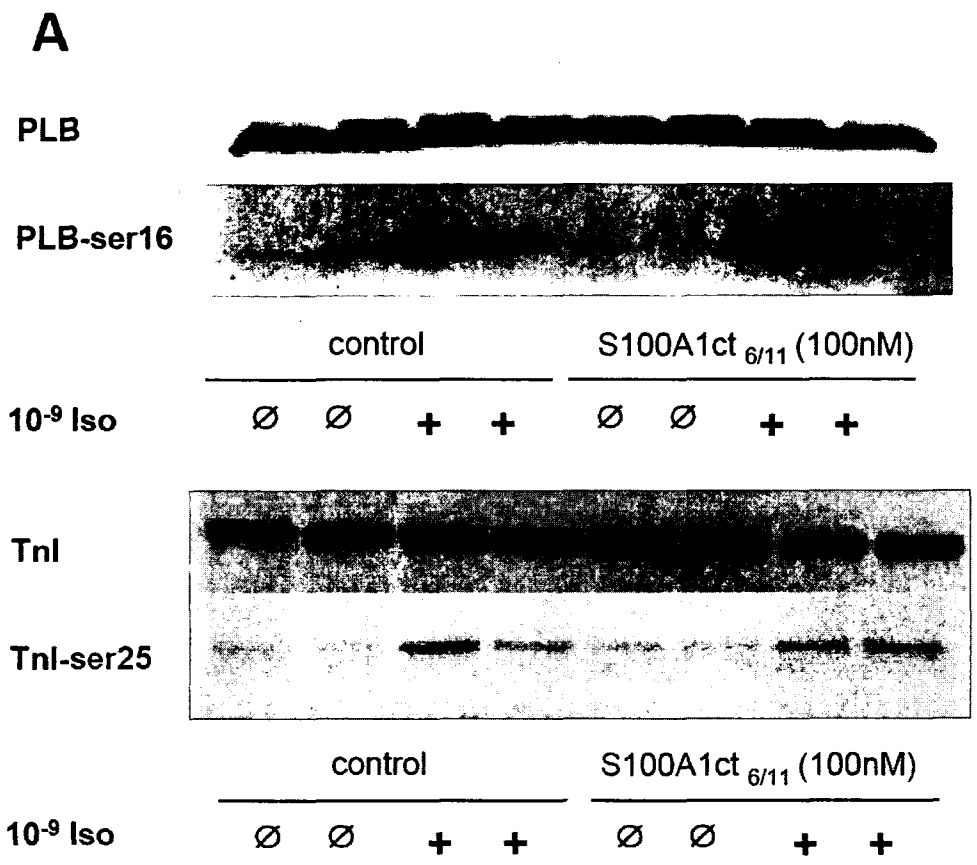

10 mM caffeine
10 mM caffeine
S100A1ct6/11
control
Calcium amplitude
(FURA 340/380 ratio, 0.15 arbitrary units)

FIGURE 13


A
PLB
PLB-ser16
control
S100A1ct 6/11 (100nM)
10-9 Iso
Ø Ø + + Ø Ø + +
TnI
TnI-ser25
control
S100A1ct 6/11 (100nM)
10-9 Iso
Ø Ø + + Ø Ø + +

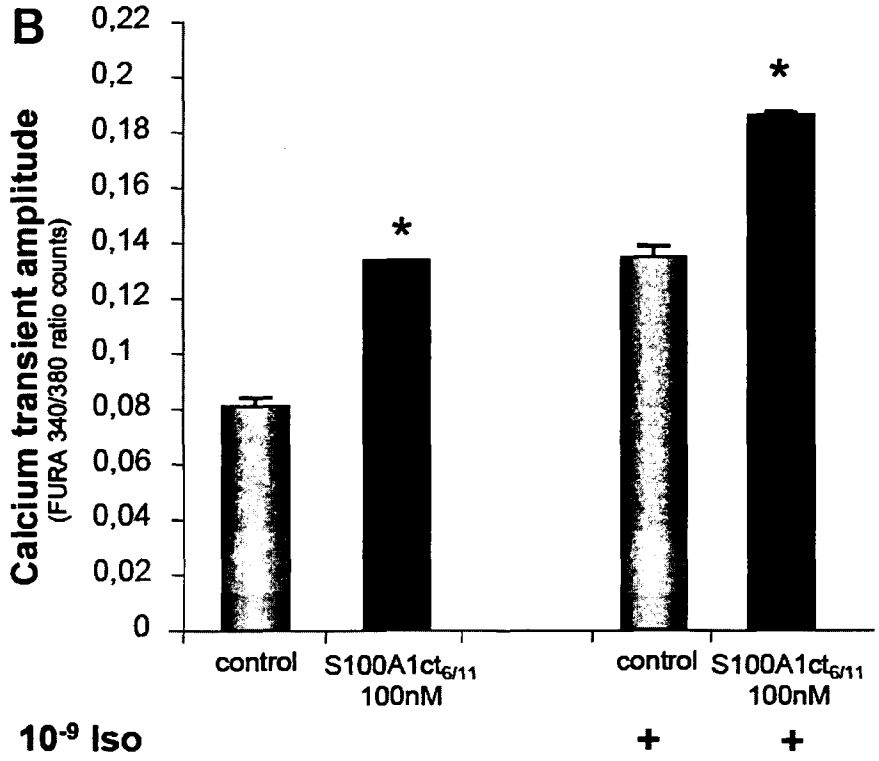

B
Calcium transient amplitude
(FURA 340/380 ratio counts)
0,22
0,2
0,18
0,16
0,14
0,12
0,1
0,08
0,06
0,04
0,02
0
*
*
control
S100A1ct6/11
100nM
control
S100A1ct6/11
100nM
10-9 Iso
+ +

FIGURE 14

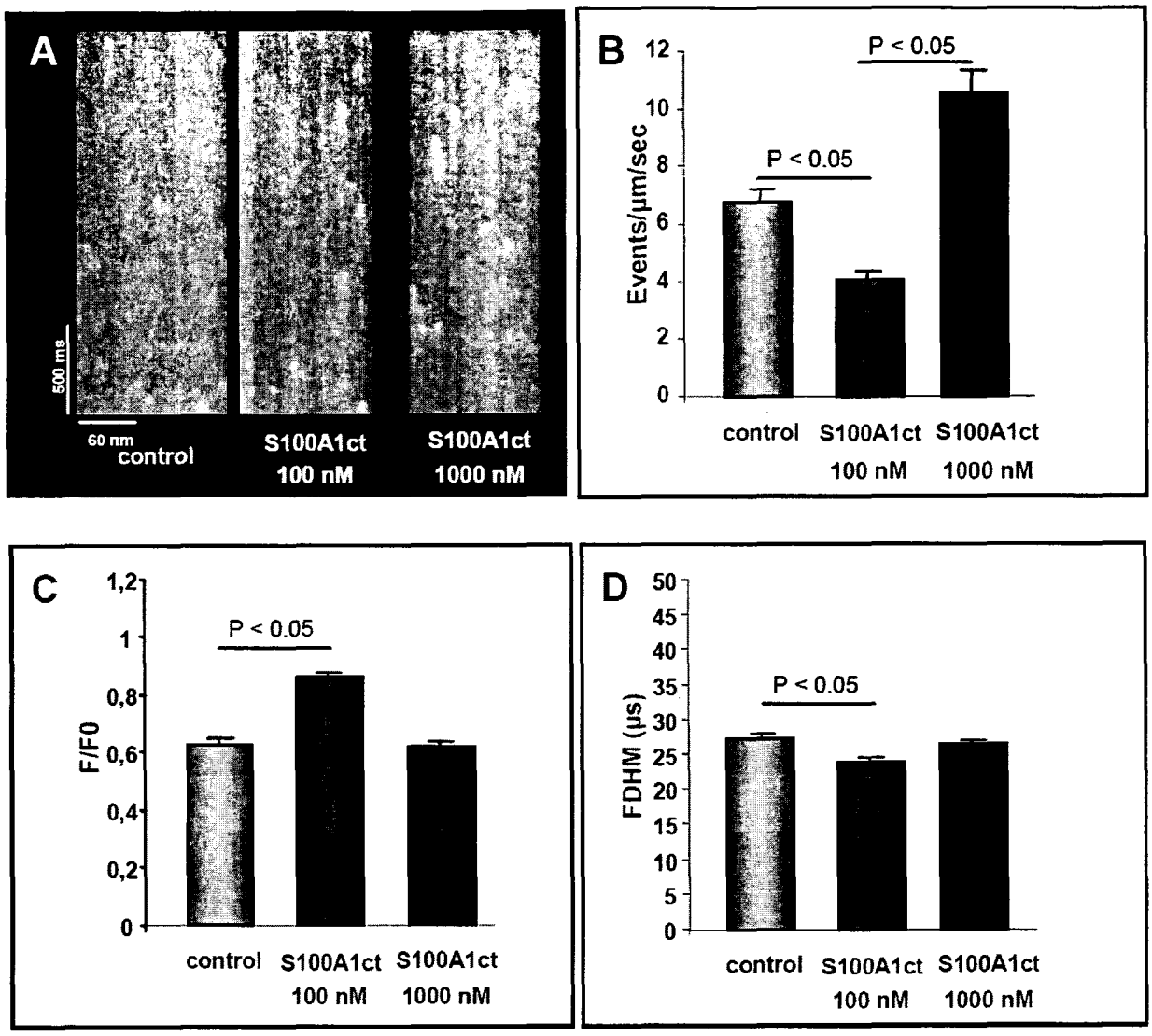

A
500 ms
60 nm
control
S100A1ct
100 nM
S100A1ct
1000 nM
B
Events/µm/sec
P < 0.05
P < 0.05
control
S100A1ct
100 nM
S100A1ct
1000 nM
C
F/F0
P < 0.05
control
S100A1ct
100 nM
S100A1ct
1000 nM
D
FDHM (µs)
P < 0.05
control
S100A1ct
100 nM
S100A1ct
1000 nM

FIGURE 15

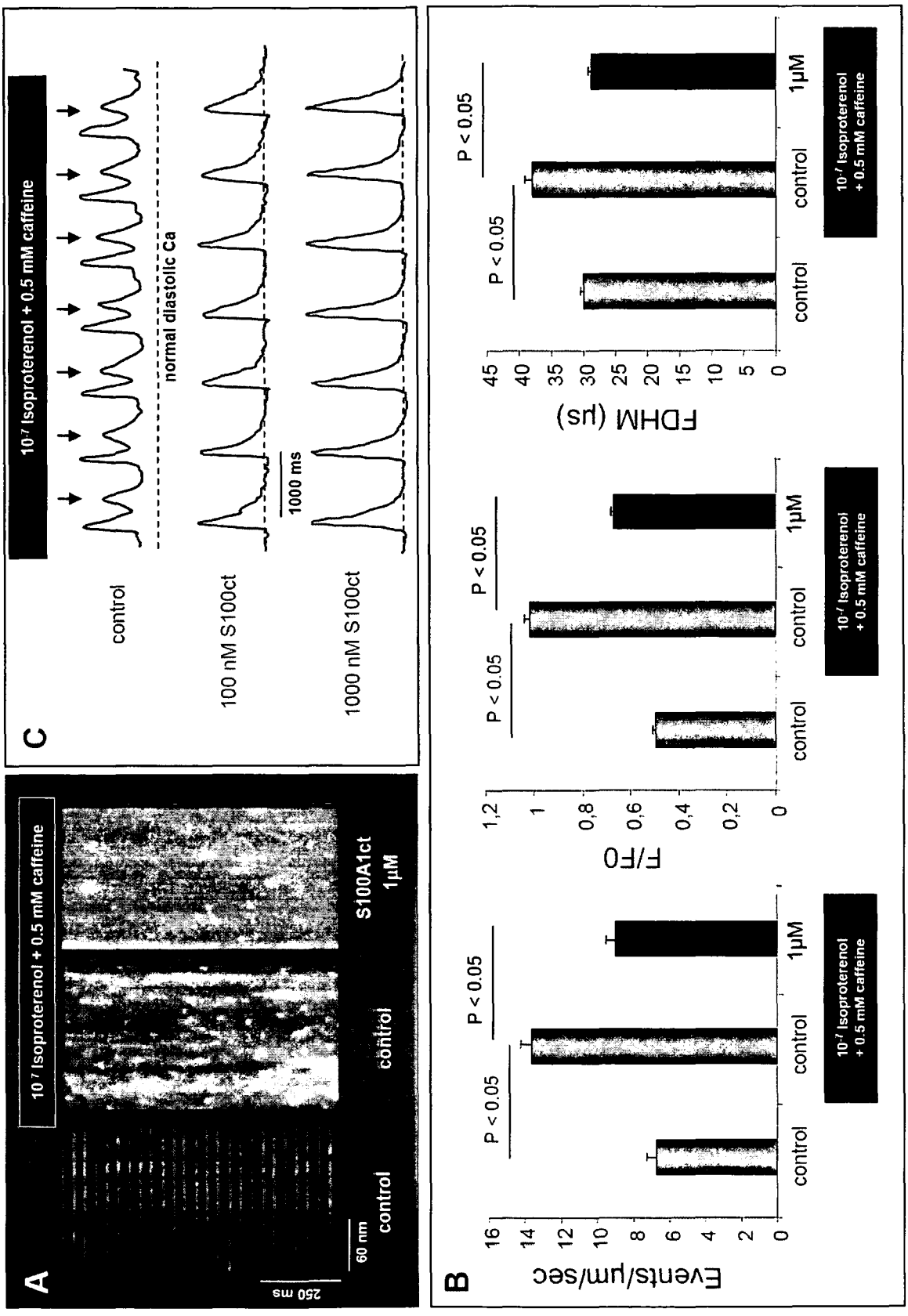

A
10⁻⁷ Isoproterenol + 0.5 mM caffeine
control
S100A1ct 1µM
60 nm
250 ms
C
10⁻⁷ Isoproterenol + 0.5 mM caffeine
normal diastolic Ca
control
100 nM S100ct
1000 ms
1000 nM S100ct
B
Events/µm/sec
P < 0.05
P < 0.05
control
control
1µM
10⁻⁷ Isoproterenol + 0.5 mM caffeine
F/F0
P < 0.05
P < 0.05
control
control
1µM
10⁻⁷ Isoproterenol + 0.5 mM caffeine
FDHM (µs)
P < 0.05
P < 0.05
control
control
1µM
10⁻⁷ Isoproterenol + 0.5 mM caffeine

FIGURE 16

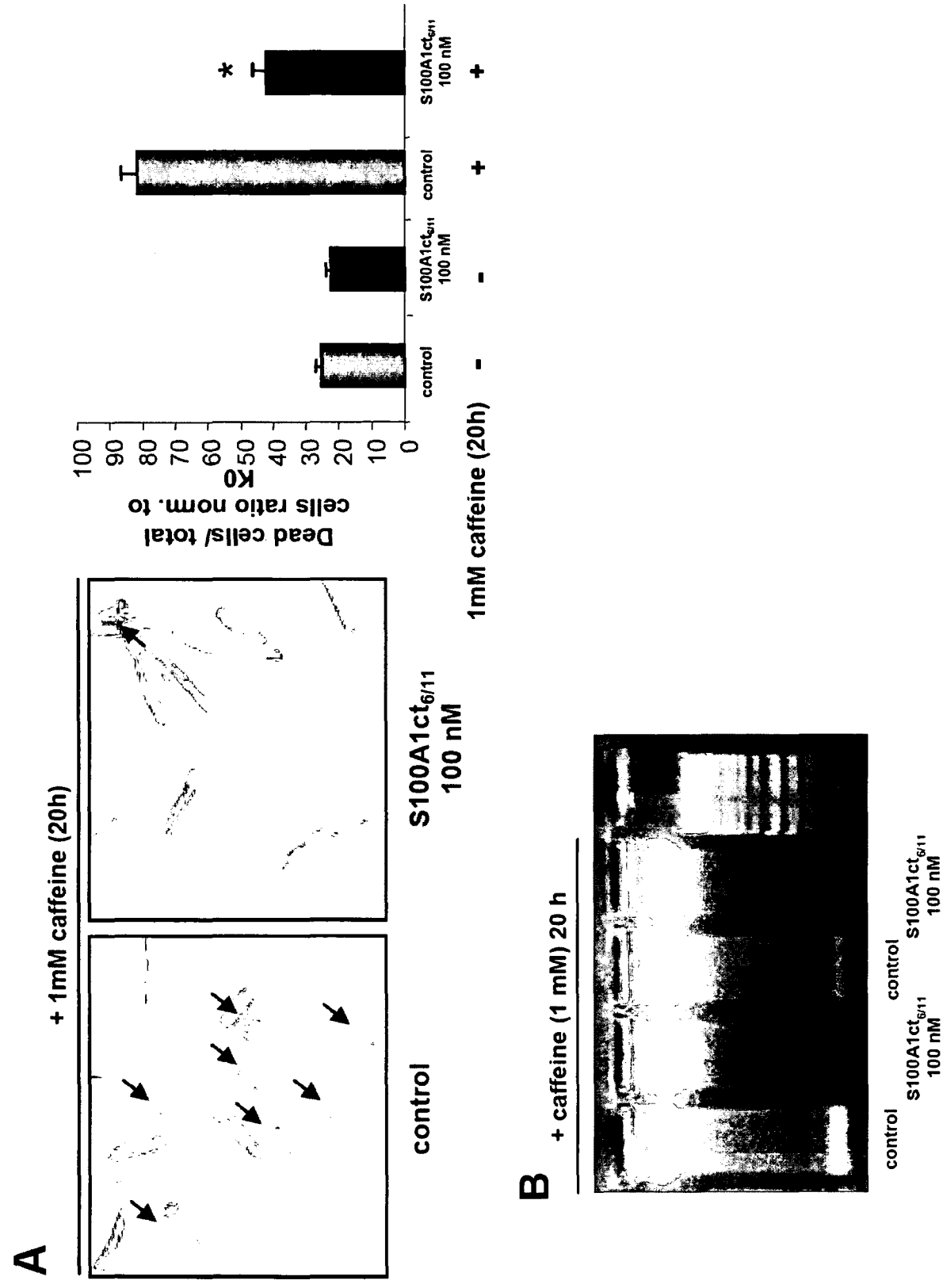

A
+ 1mM caffeine (20h)
control
S100A1ct6/11
100 nM
Dead cells/ total cells ratio norm. to KO
100
90
80
70
60
50
40
30
20
10
0
control
S100A1ct6/11 100 nM
control
S100A1ct6/11 100 nM
1mM caffeine (20h) - - + +
*
B
+ caffeine (1 mM) 20 h
control
S100A1ct6/11 100 nM
control
S100A1ct6/11 100 nM

FIGURE 17

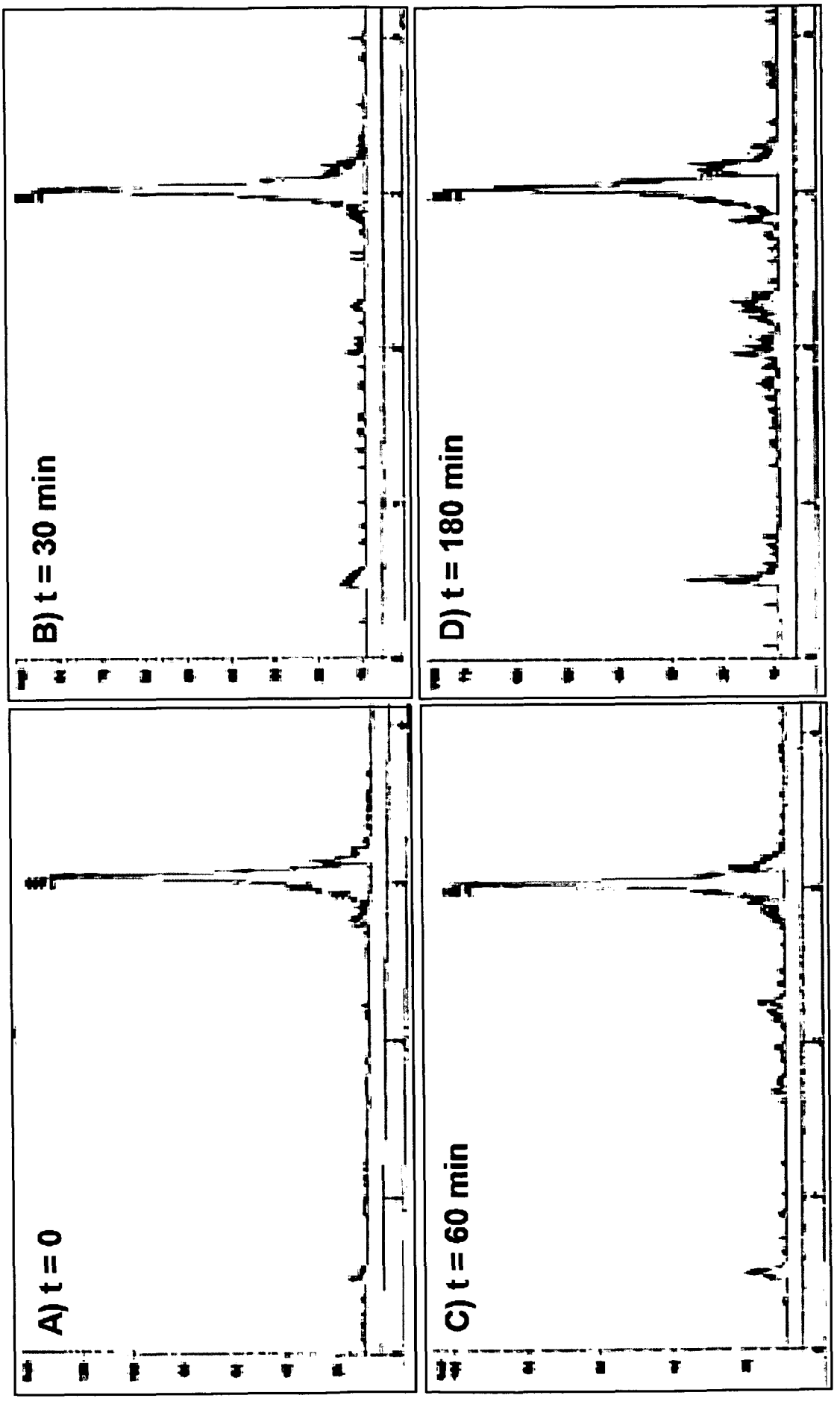


FIGURE 18

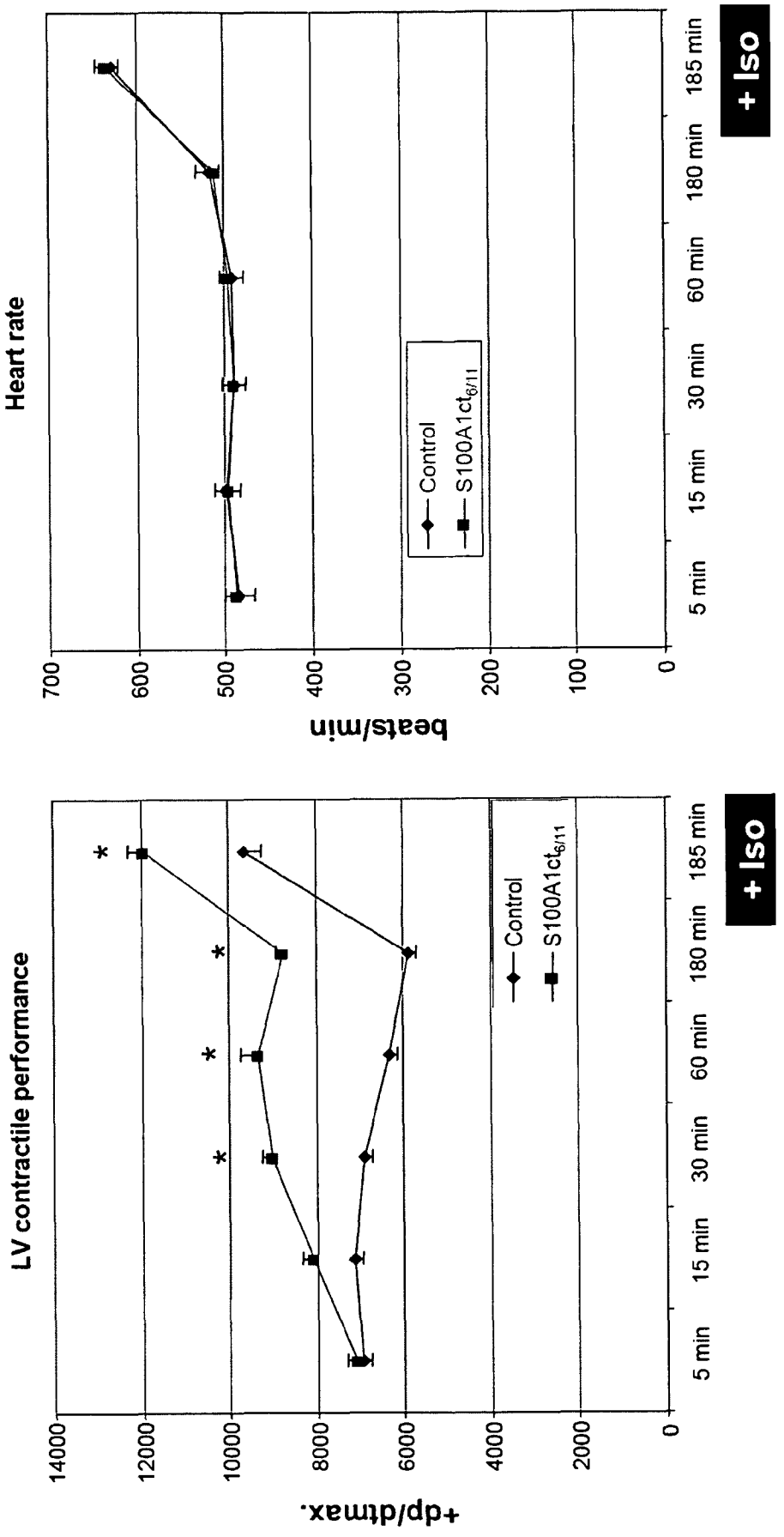

Heart rate
beats/min
700
600
500
400
300
200
100
0
5 min
15 min
30 min
60 min
180 min
185 min
+ Iso
Control
S100A1ct6/11
LV contractile performance
+dp/dtmax.
14000
12000
10000
8000
6000
4000
2000
0
*
*
*
*
5 min
15 min
30 min
60 min
180 min
185 min
+ Iso
Control
S100A1ct6/11

FIGURE 19

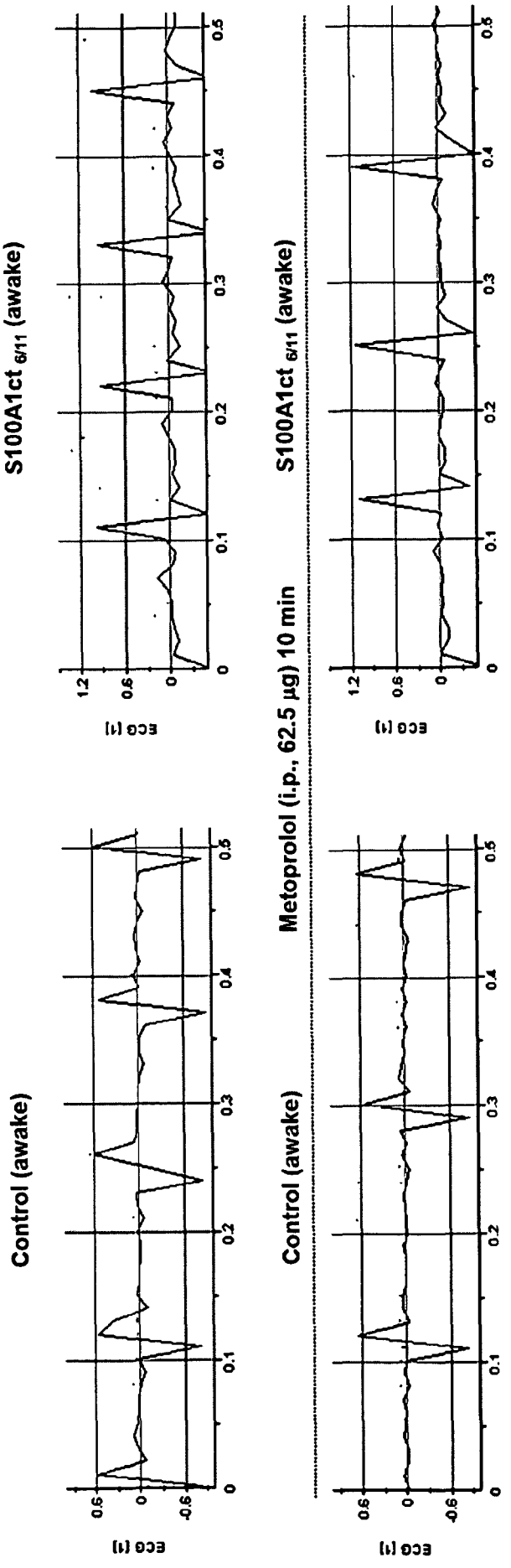

S100A1ct 6/11 (awake)
S100A1ct 6/11 (awake)
Metoprolol (i.p., 62.5 µg) 10 min
Control (awake)
Control (awake)
ECG (1)
ECG (1)
ECG (1)
ECG (1)

FIGURE 20

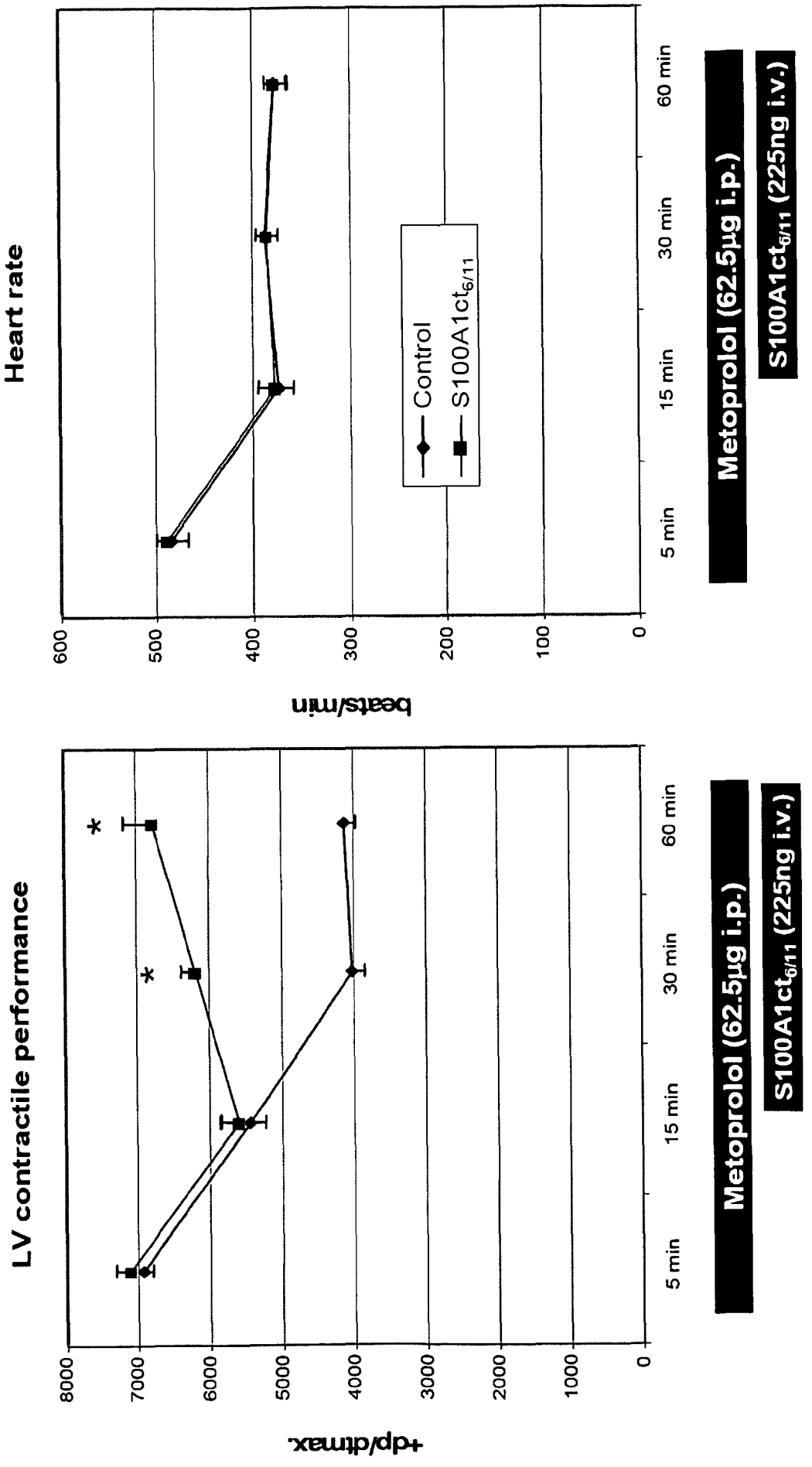

Heart rate
beats/min
600
500
400
300
200
100
0
5 min
15 min
30 min
60 min
Control
S100A1ct6/11
Metoprolol (62.5µg i.p.)
S100A1ct6/11 (225ng i.v.)
LV contractile performance
+dp/dtmax
8000
7000
6000
5000
4000
3000
2000
1000
0
*
*
5 min
15 min
30 min
60 min
Metoprolol (62.5µg i.p.)
S100A1ct6/11 (225ng i.v.)

FIGURE 21

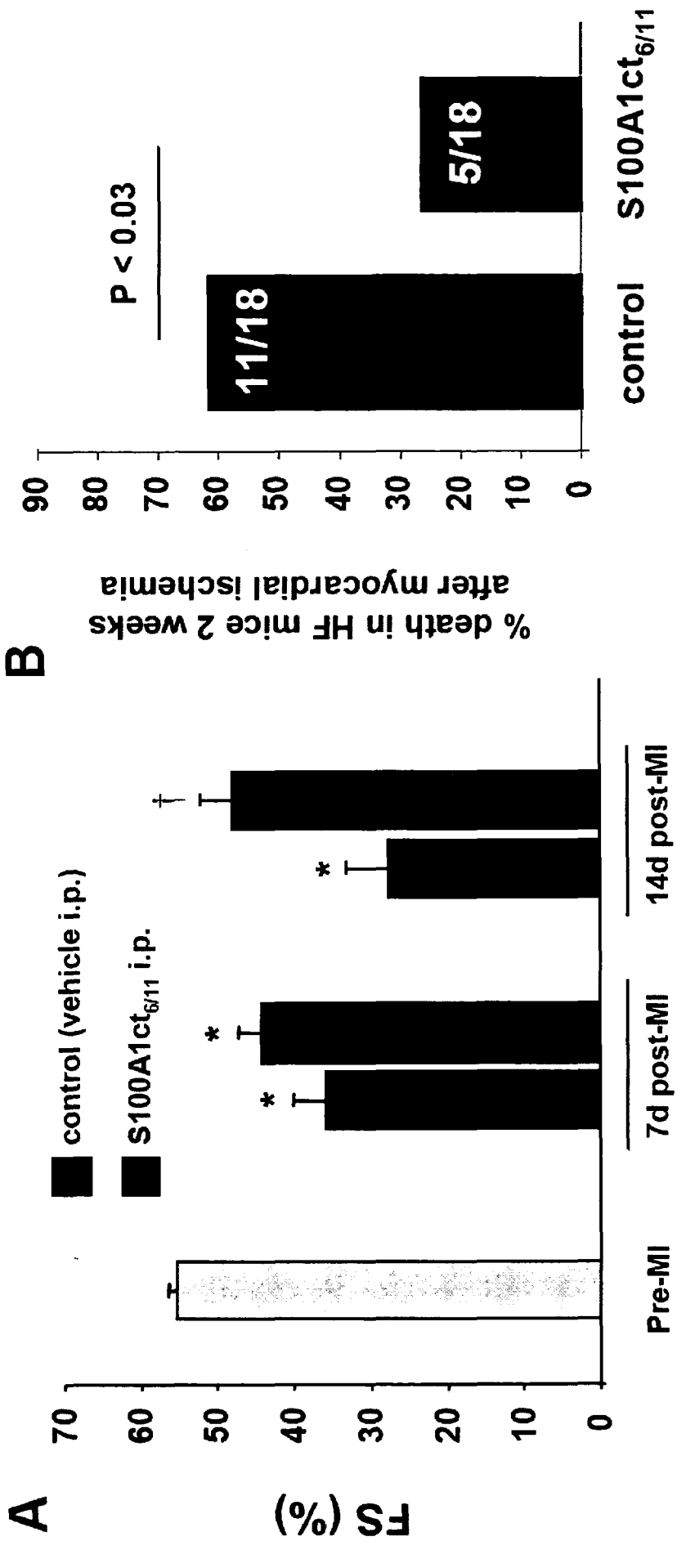

A
FS (%)
70
60
50
40
30
20
10
0
control (vehicle i.p.)
S100A1ct6/11 i.p.
*
*
*
Pre-MI
7d post-MI
14d post-MI
B
% death in HF mice 2 weeks after myocardial ischemia
90
80
70
60
50
40
30
20
10
0
P < 0.03
11/18
5/18
control
S100A1ct6/11

FIGURE 22

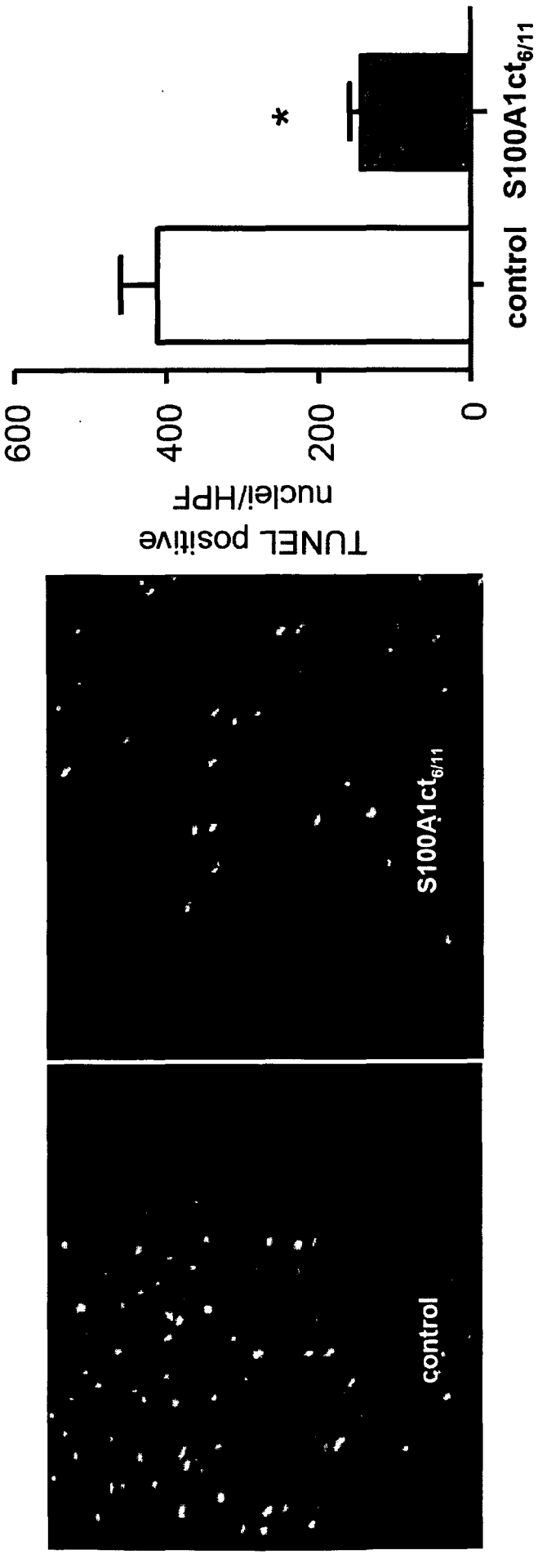

TUNEL positive
nuclei/HPF
600
400
200
0
*
control
S100A1ct6/11
control
S100A1ct6/11

FIGURE 23

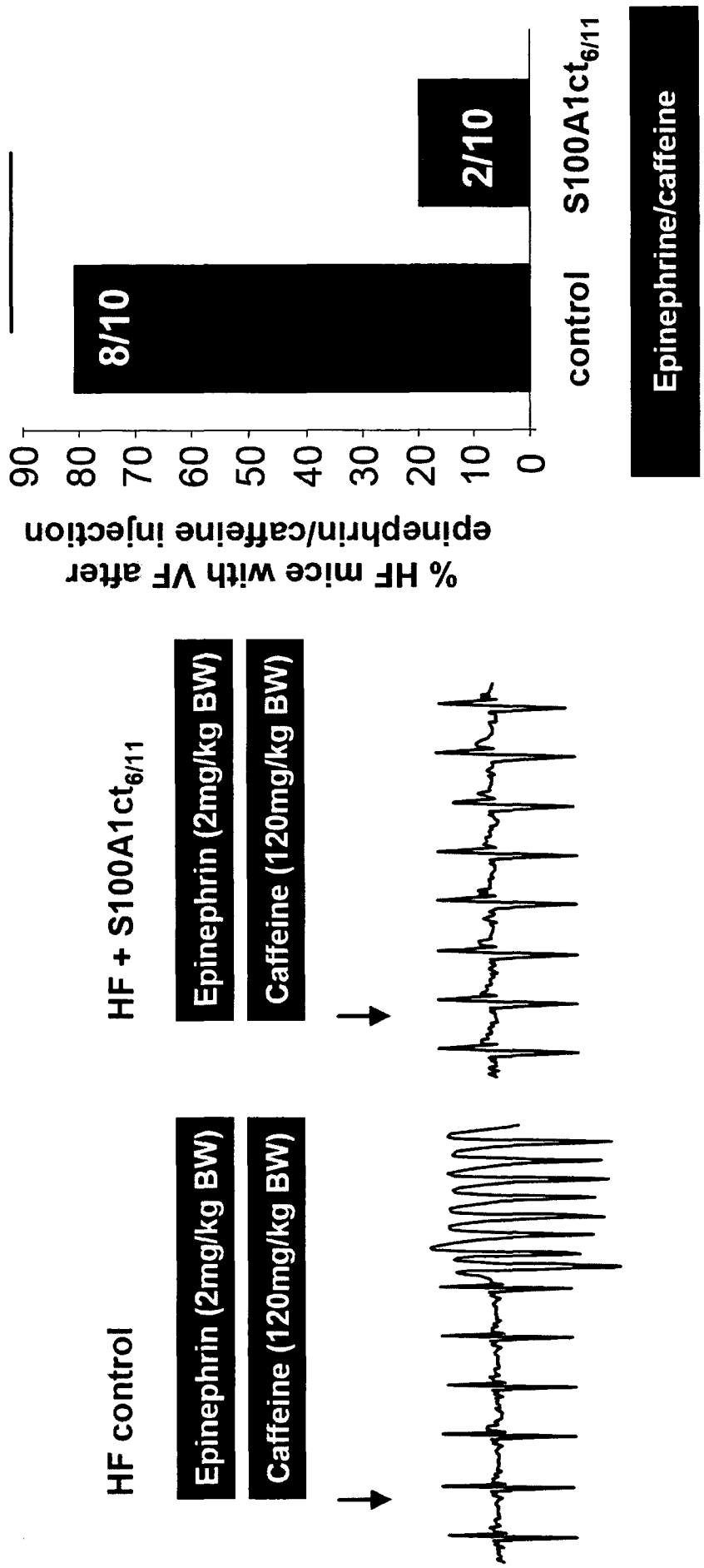

HF control
Epinephrin (2mg/kg BW)
Caffeine (120mg/kg BW)
HF + S100A1ct6/11
Epinephrin (2mg/kg BW)
Caffeine (120mg/kg BW)
% HF mice with VF after epinephrin/caffeine injection
90
80
70
60
50
40
30
20
10
0
P < 0.01
8/10
2/10
control
S100A1ct6/11
Epinephrine/caffeine

FIGURE 24

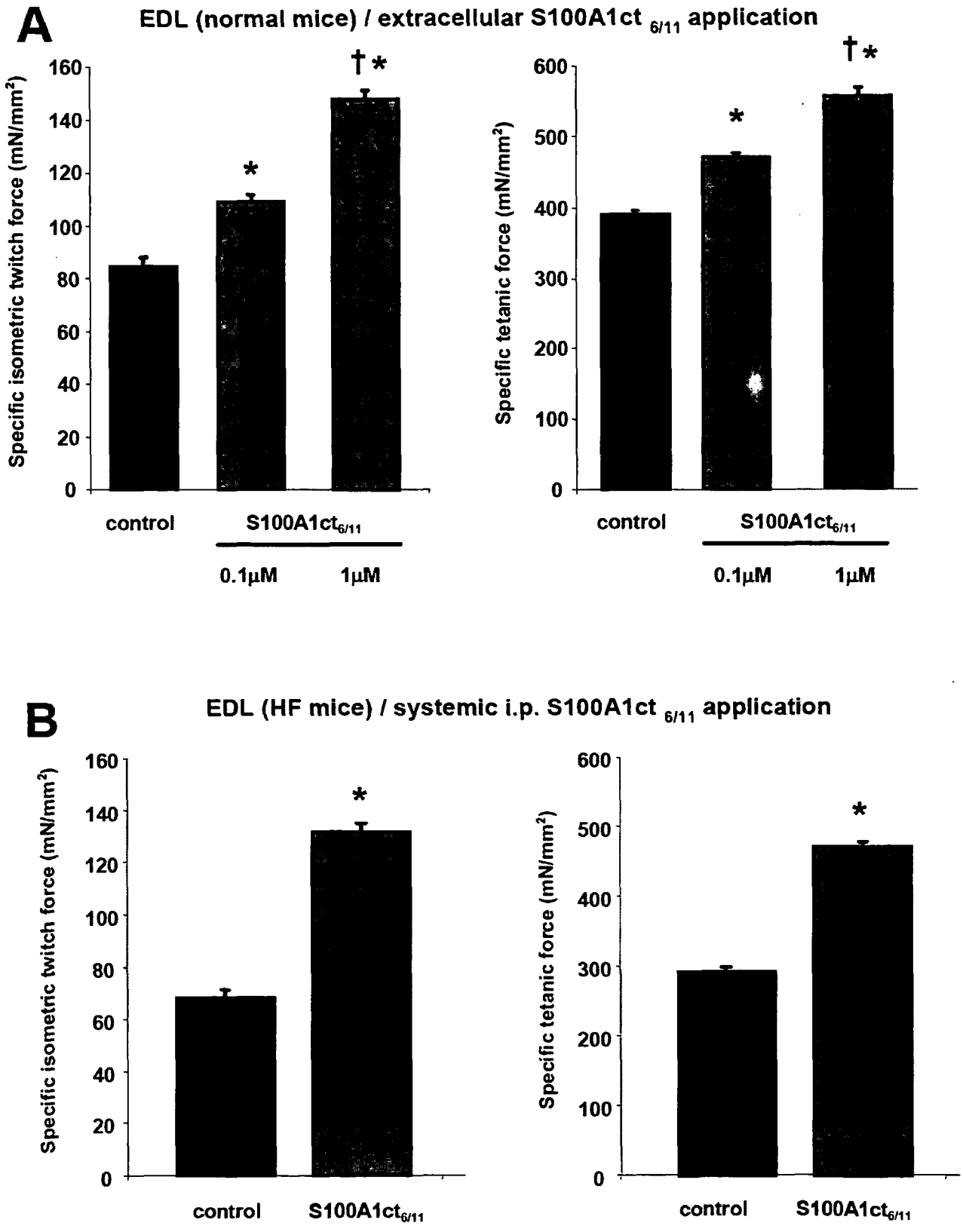
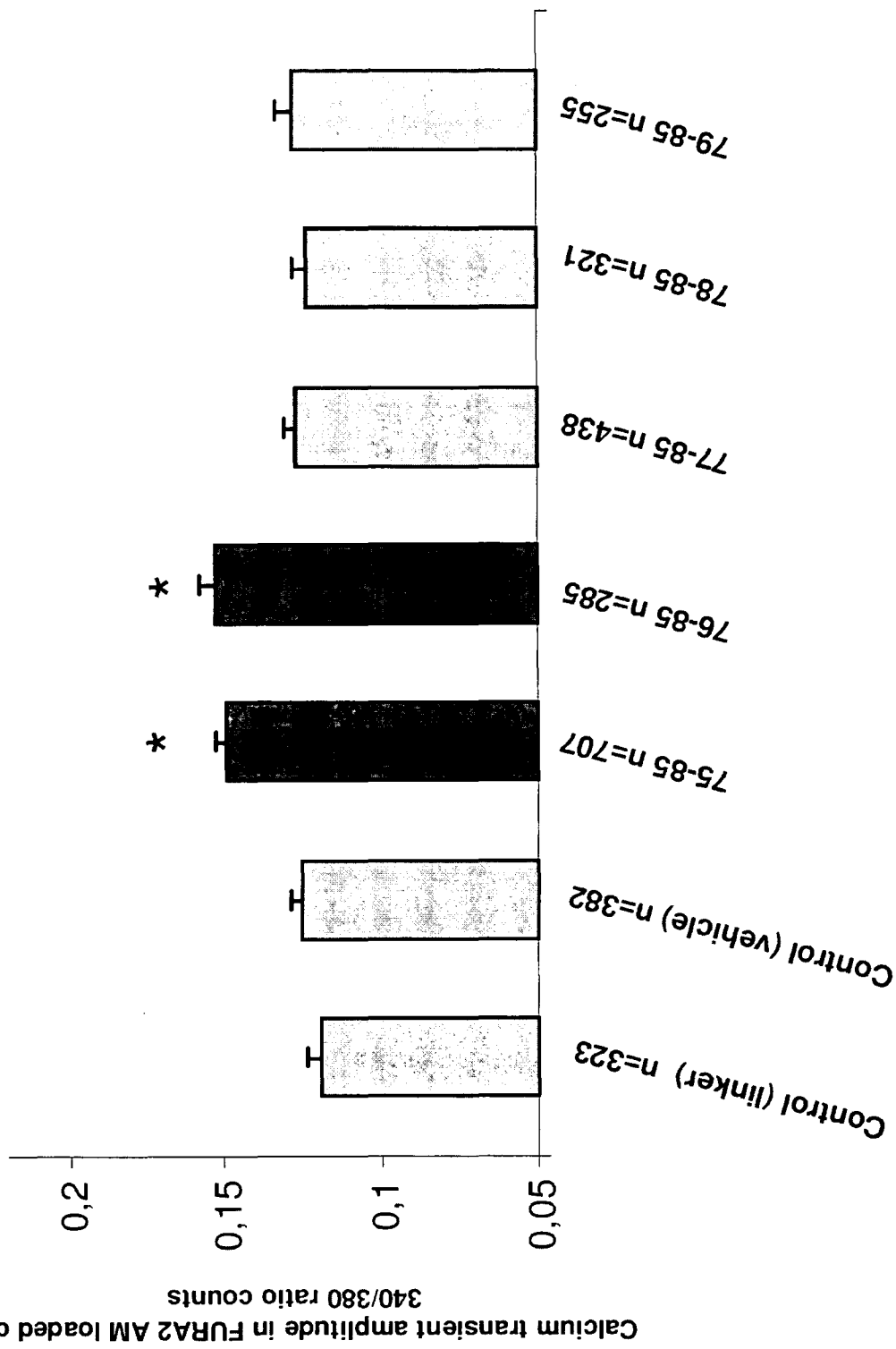

A
EDL (normal mice) / extracellular S100A1ct 6/11 application
Specific isometric twitch force (mN/mm²)
160
140
120
100
80
60
40
20
0
*
†*
control
S100A1ct6/11
0.1µM
1µM
Specific tetanic force (mN/mm²)
600
500
400
300
200
100
0
*
†*
control
S100A1ct6/11
0.1µM
1µM
B
EDL (HF mice) / systemic i.p. S100A1ct 6/11 application
Specific isometric twitch force (mN/mm²)
160
140
120
100
80
60
40
20
0
*
control
S100A1ct6/11
Specific tetanic force (mN/mm²)
600
500
400
300
200
100
0
*
control
S100A1ct6/11

FIGURE 25


Calcium transient amplitude in FURA2 AM loaded cells
340/380 ratio counts
0,2
0,15
0,1
0,05
Control (linker) n=323
Control (vehicle) n=382
75-85 n=707
76-85 n=285
77-85 n=438
78-85 n=321
79-85 n=255
*
*

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/002343

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/17 C07K14/47
ADD.

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)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/61742 A2 (KATUS HUGO A [DE]; REMPPIS ANDREW [DE]) 19 October 2000 (2000-10-19) the whole document sequences 36, 39	1-45
X	GRIBENKO ALEXEY V ET AL: "Molecular characterization tissue distribution of a novel member of the S100 family of EF-hand proteins" BIOCHEMISTRY, vol. 40, no. 51, 25 December 2001 (2001-12-25), pages 15538-15548, XP002586473 ISSN: 0006-2960 the whole document figure 2	1-45

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* 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 document 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
13 September 2010	20/09/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Petri, Bernhard

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MOST PATRICK ET AL: "S100A1: a novel inotropic regulator of cardiac performance. Transition from molecular physiology to pathophysiological relevance" AMERICAN JOURNAL OF PHYSIOLOGY: REGULATORY, INTEGRATIVE AND COMPARATIVE PHYSIOLOGY, AMERICAN PHYSIOLOGICAL SOCIETY, US LNKD- DOI:10.1152/AJPREGU.00075.2007, vol. 293, no. 2, 1 August 2007 (2007-08-01), pages R568-R577, XP009134588 ISSN: 0363-6119 [retrieved on 2007-04-25] the whole document	1-45
A	WO 98/02454 A2 (ADPROTECH PLC [GB]; SMITH RICHARD ANTHONY GODWIN [GB]; DODD IAN [GB];) 22 January 1998 (1998-01-22) claim 11	11-17
X	MOST PATRICK ET AL: "Cardiac adenoviral S100A1 gene delivery rescues failing myocardium" JOURNAL OF CLINICAL INVESTIGATION, AMERICAN SOCIETY FOR CLINICAL INVESTIGATION, US, vol. 114, no. 11, 1 December 2004 (2004-12-01), pages 1550-1563, XP002493143 ISSN: 0021-9738 the whole document	18-45
X	VOLKERS ET AL: "S100A1 decreases calcium spark frequency and alters their spatial characteristics in permeabilized adult ventricular cardiomyocytes" CELL CALCIUM (EDINBURGH), CHURCHILL LIVINGSTONE MEDICAL JOURNALS, EDINBURGH, GB LNKD- DOI:10.1016/J.CECA.2006.06.001, vol. 41, no. 2, 28 December 2006 (2006-12-28), pages 135-143, XP005833808 ISSN: 0143-4160 page 141, right-hand column, lines 1-2	11-45

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2010/002343

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.:
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:

see additional 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 an 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.:

1-45(partially)

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.:

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-45(partially)

Inotropic peptides comprising [VI][VI][LM][VIM][AGS][AV]L[TA][VAI][AMV] and not more than 18 continous amino acids of the C-terminus of an S100A1 protein of maximally 100 aa length, wherein the peptide comprises Seq.Id.No 4.

2-8. claims: 1-45(partially)

Inotropic peptides comprising [VI][VI][LM][VIM][AGS][AV]L[TA][VAI][AMV] and not more than 18 continous amino acids of the C-terminus of an S100A1 protein of maximally 100 aa length, wherein the peptide comprises Seq.Id.No 6, 7, 95, 263, 186, 334, 50, respectively

---

9. claims: 1-45(partially)

Inotropic peptides comprising [VI][VI][LM][VIM][AGS][AV]L[TA][VAI][AMV] and not more than 18 continous amino acids of the C-terminus of an S100A1 protein of maximally 100 aa length, wherein C, is linked at the C-terminal part of the molecule

---

10. claims: 1-45(partially)

Inotropic peptides comprising [VI][VI][LM][VIM][AGS][AV]L[TA][VAI][AMV] and not more than 18 continous amino acids of the C-terminus of an S100A1 protein of maximally 100 aa length, wherein an aromatic amino acid is directly linked to the amino terminus of the peptide.

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11. claims: 1-45(partially)

Inotropic peptides comprising [VI][VI][LM][VIM][AGS][AV]L[TA][VAI][AMV] and not more than 18 continous amino acids of the C-terminus of an S100A1 protein of maximally 100 aa length, further comprising membrane penetration enhancing motif, one or more epitope tags a hydrophilic motif or a peptide targeting motif

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12. claims: 1-45(partially)

Inotropic peptides comprising [VI][VI][LM][VIM][AGS][AV]L[TA][VAI][AMV] and not more than

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

18 continous amino acids of the C-terminus of an S100A1 protein of maximally 100 aa length, further comprising a marker moiety

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2010/002343

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0061742 A2	19-10-2000	AT 297464 T	15-06-2005
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