

MG53 Mutants, Methods of Making the Same, and Uses Thereof

TECHNICAL FIELD

[0001] The present invention relates to the biomedical field. In particular, the present invention relates to MG53 mutants, pharmaceutical compositions comprising the MG53 mutants, nucleic acids encoding the MG53 mutants, methods for preparing the MG53 mutants, and uses of the MG53 mutants in the manufacture of medicaments for treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage.

BACKGROUND ART

[0002] Mitsugumin 53 (MG53), also known as TRIM72, is a member of the Tripartite motif-containing proteins (TRIM) family. MG53 comprises a TRIM motif at the N-terminus and a SPRY motif at the C-terminus. The TRIM motif consists of successively linked Ring, B-box and coiled-coil domains (see Chuanxi Cai et al., *the Journal of Biological Chemistry*, Vol. 284 (5), 3314-3322 (2009)). MG53 plays a variety of roles throughout the body, but it is mainly expressed in striated muscles, and is essential for maintaining the homeostasis of skeletal muscle and the heart. MG53 was previously found to have cell membrane repair function and cardioprotective function (see, e.g., Chuanxi Cai et al., *Nature Cell Biology*, Vol. 11, 56-64 (2009); CN101797375B). In addition, further studies have found that MG53 also plays a protective role in ischemic preconditioning (IPC) and ischemic postconditioning (PostC), by activation of the reperfusion injury salvage kinase (RISK) pathway. The N- and C-termini of the MG53 molecule can bind to Caveolin-3 and p85-PI3K kinases respectively to form a complex, which activates the RISK pathway to elicit cardiac protection (see Chun-Mei Cao et al., *Circulation* 121, 2565-2574, (2010)).

[0003] Although MG53 has cell membrane repair function and cardioprotective function, previous studies also found that MG53 has E3 ubiquitin ligase activity, which contributes to the development of insulin resistance and metabolic syndrome. The Ring domain of the TRIM motif at the N-terminus of MG53 binds to insulin receptor (IR) and insulin receptor substrate-1 (IRS1) and mediates the ubiquitination and subsequent degradation of these proteins by the proteasome, thereby blocking the insulin signaling pathway and leading to insulin resistance and

associated metabolic diseases such as obesity, diabetes, hypertension, dyslipidemia, etc. (see, for example, R. Song et al. *Nature* 494, 375-379, (2013); J. S. Yi et al., *Nature Communications* 4, 2354 (2013)). Thus, the wild-type MG53 elicits cell membrane repair function and cardioprotective function, but also causes deleterious side effects, such as insulin resistance and its associated metabolic diseases.

SUMMARY OF THE INVENTION

[0004] The present invention relates to MG53 mutants, pharmaceutical compositions comprising the MG53 mutants, nucleic acids encoding the MG53 mutants, methods for preparing the MG53 mutants, and uses of the MG53 mutants in the manufacture of medicaments for treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage. In particular, the MG53 mutants may avoid or reduce metabolic side effects, such as insulin resistance, obesity, diabetes, hypertension, dyslipidemia, etc., while still retaining cell membrane repair function and/or cardioprotective function.

[0005] In one aspect, the present invention relates to an MG53 mutant, wherein the MG53 mutant is identical to the amino acid sequence of a wild-type MG53 except for at least one serine in the coiled-coil-SPRY region of the wild-type MG53, which is deleted and/or mutated into any other non-serine or non-threonine amino acid(s). In certain embodiments, the coiled-coil-SPRY region is located at positions 122-477 of the amino acid sequence of the wild-type MG53. In certain embodiments, the wild-type MG53 is derived from an animal, preferably from a mammal, e.g., human, mouse, rat, monkey, swine, dog, etc. In certain embodiments, the amino acid sequence of the wild type MG53 is set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, or SEQ ID NO: 142.

[0006] In certain embodiments, the MG53 mutant is identical to the amino acid sequence of a wild-type MG53 except for at least one serine in the coiled-coil-SPRY region of the wild-type MG53, which is mutated into a non-polar amino acid. In certain embodiments, the non-polar amino acid is selected from the group consisting of glycine, alanine, leucine, isoleucine, valine, proline, phenylalanine, methionine, and tryptophan. Preferably, in certain embodiments, the non-polar amino acid is alanine. In certain embodiments, the MG53 mutant is identical to the

amino acid sequence of a wild-type MG53 except for at least one serine in the coiled-coil-SPRY region of the wild-type MG53, which is mutated into any non-serine or non-threonine polar amino acid(s). In certain embodiments, the polar amino acid is selected from the group consisting of glutamine, cysteine, asparagine, tyrosine, aspartic acid, glutamic acid, lysine, arginine, and histidine. Preferably, in certain embodiments, the polar amino acid is cysteine.

[0007] In certain embodiments, the deleted or mutated serine is located at one or more of positions 150, 189, 211, 214, 246, 255, 269, 296, 297, 301, 305, 306, 307, 314, 341, 377, 405, 418, 425, or 430 of the amino acid sequence set forth in SEQ ID NO: 1 of a wild-type MG53.

[0008] In certain embodiments, the deleted or mutated serine is located at one or more of positions 150, 189, 211, 214, 246, 255, 269, 296, 297, 301, 305, 306, 307, 314, 341, 377, 405, 418, 425, or 430 of the amino acid sequence set forth in SEQ ID NO: 139 of a wild-type MG53.

[0009] In certain embodiments, the deleted or mutated serine is located at one or more of positions 150, 189, 211, 214, 246, 255, 269, 296, 297, 301, 305, 306, 307, 314, 341, 377, 405, 418, 425, or 430 of the amino acid sequence set forth in SEQ ID NO: 140 of a wild-type MG53.

[0010] In certain embodiments, the deleted or mutated serine is located at one or more of positions 150, 189, 211, 214, 246, 255, 269, 296, 297, 301, 305, 306, 307, 314, 341, 377, 405, 418, 425, or 430 of the amino acid sequence set forth in SEQ ID NO: 141 of a wild-type MG53.

[0011] In certain embodiments, the deleted or mutated serine is located at one or more of positions 150, 189, 211, 214, 246, 255, or 269 of the amino acid sequence set forth in SEQ ID NO: 142 of a wild-type MG53.

[0012] In certain embodiments, the deleted or mutated serine is located at one or more of positions 188, 189, 210, 211, 214, 246, 253, 255, 269, 296, 297, 301, 305, 306, 307, 314, 341, 367, 377, 418, 430, or 440 of the amino acid sequence set forth in SEQ ID NO: 2 of a wild-type MG53.

[0013] In certain embodiments, the deleted or mutated serine is located at one or more of positions 150, 188, 189, 210, 211, 214, 246, 253, 255, 269, 296, 297, 301, 305, 307, 314, 341, 367, 377, 418, 430, 440, 464, or 474 of the amino acid sequence set forth in SEQ ID NO: 3 of a wild-type MG53.

[0014] In certain embodiments, the deleted or mutated serine is located at one or more of positions 150, 189, 211, 214, 246, 255, 269, 296, 297, 301, 305, 306, 307, 341, 377, 405, 418, 425, 430, or 464 of the amino acid sequence set forth in SEQ ID NO: 4 of a wild-type MG53.

[0015] In certain embodiments, the deleted or mutated serine is located at one or more of positions 150, 189, 211, 214, 246, 255, 269, 296, 301, 305, 307, 314, 341, 377, 411, 418, 425, 430, or 474 of the amino acid sequence set forth in SEQ ID NO: 5 of a wild-type MG53.

[0016] In certain embodiments, the deleted or mutated serine is located at one or more of positions 150, 189, 211, 214, 246, 255, 269, 296, 297, 301, 305, 307, 314, 341, 367, 377, 418, 425, or 430 of the amino acid sequence set forth in SEQ ID NO: 6 of a wild-type MG53.

[0017] In certain embodiments, the deleted or mutated serine is located at one or more of positions 150, 188-189, 210-211, 214, 246, 253-255, 269, 296-297, 301, 305-307, 314, 341, 367, 377, 405, 411, 418, 425, 430, 440, 464, or 474 of the amino acid sequence (e.g., SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, or SEQ ID NO: 142) of a wild-type MG53.

[0018] In certain embodiments, the deleted or mutated serine is located at one or more of positions 189, 211, 214, 246, 253-255, 269, 296, 301, 305, 307, 341, 377, 418, or 430 of the amino acid sequence (e.g., SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, or SEQ ID NO: 142) of a wild-type MG53.

[0019] In certain embodiments, the deleted or mutated serine is located at one or more of positions 211, 214, 246, 253-255, 269, 296, or 297 of the amino acid sequence (e.g., SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, or SEQ ID NO: 142) of a wild-type MG53.

[0020] In certain embodiments, the deleted or mutated serine is located at one or more of positions 211, 214, 246, 255, 269, 296, or 297 of the amino acid sequence (e.g., SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, or SEQ ID NO: 142) of a wild-type MG53.

[0021] In certain embodiments, the deleted or mutated serine is located at one or more of positions 253-255 of the amino acid sequence (e.g., SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, or SEQ ID NO: 142) of a wild-type MG53. In certain embodiments, the deleted or mutated serine is located at position 253 of the amino acid sequence of a wild-type MG53. In certain embodiments, the deleted or mutated serine is located at position 255 of the amino acid sequence of a wild-type MG53. In certain embodiments, the deleted or mutated serine is located at position 255 of the amino acid sequence set forth in SEQ ID NO: 1 of a wild-type MG53. In certain embodiments, the deleted or mutated serine is located at position 255 of the amino acid sequence set forth in SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141 or SEQ ID NO: 142 of a wild-type MG53.

[0022] In certain embodiments, the MG53 mutant comprises two or more serine mutations. In certain embodiments, two or more serine mutations of the MG53 mutant comprise serine mutation at one or more of positions 253-255. In certain embodiments, two or more serine mutations of the MG53 mutant comprise serine mutation at position 253. In certain embodiments, two or more serine mutations of the MG53 mutant comprise serine mutation at position 255. In certain embodiments, two or more serine mutations of the MG53 mutant comprise serine mutations at positions 253 and 255.

[0023] A person skilled in the art will comprehend that serine residues in the wild-type MG53 proteins of different species may be located at different positions, and thus the positions of the deleted or mutated serine may also differ. In certain embodiments, the deleted or mutated serine is within 1 to 10 amino acids, 1 to 5 amino acids, or 1 to 3 amino acids upstream or downstream of the corresponding serine position in the amino acid sequence of the wild-type MG53 set forth in SEQ ID NO: 1.

[0024] In certain embodiments, the amino acid sequence of the MG53 mutant is the amino acid sequence set forth in SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149 or SEQ ID NO: 150. Preferably, in certain embodiments, the amino acid sequence of the MG53 mutant is the amino acid sequence set forth in SEQ ID NO: 7. In certain embodiments, the amino acid sequence of the MG53 mutant has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%,

at least 98%, or at least 99% amino acid sequence homology to one of the amino acid sequences set forth in SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO:11, SEQ ID NO: 12, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO:150, and the MG53 mutant avoids metabolic side effects caused by wild-type MG53 while still retaining cell membrane repair function and/or cardioprotective function.

[0025] In another aspect, the present invention relates to a pharmaceutical composition comprising the MG53 mutant and a pharmaceutically acceptable carrier.

[0026] In another aspect, the present invention relates to an isolated nucleic acid comprising a nucleic acid sequence encoding the amino acid sequence of the MG53 mutant. In certain embodiments, the nucleic acid comprises any one of the nucleic acid sequences set forth in SEQ ID NOS: 13-18. In certain embodiments, the nucleic acid comprises any one of the nucleic acid sequences set forth in SEQ ID NOS: 151-154.

[0027] In another aspect, the present invention relates to an expression vector comprising a nucleic acid sequence encoding the amino acid sequence of the MG53 mutant. In certain embodiments, the nucleic acid comprises any one of the nucleic acid sequences set forth in SEQ ID NOS: 13-18. In certain embodiments, the nucleic acid comprises any one of the nucleic acid sequences set forth in SEQ ID NOS: 151-154.

[0028] In yet another aspect, the present invention relates to a host cell comprising the expression vector described herein.

[0029] In still another aspect, the present invention relates to a method for preparing the MG53 mutant, comprising determining one or more serine positions for mutation, performing site-directed mutagenesis at said position on the full-length sequence of a plasmid comprising a nucleic acid sequence encoding the amino acid sequence of a wild-type MG53, transfecting the plasmid with site-directed mutagenesis into a host cell, and inducing the host cell to produce the MG53 mutant. In certain embodiments, the site-directed mutagenesis comprises the following steps: (1) determining the corresponding nucleotide site of the amino acid targeted for site-directed mutagenesis in the cDNA sequence; modifying the nucleotide sequence at the mutation site according to the target amino acid; and designing primers by intercepting a sequence of 20-40 bp in length comprising the mutation site; (2) performing PCR reaction by using the primers

of step (1) and taking a wild type MG53 plasmid as a template, performing agarose gel electrophoresis for the PCR product, and purifying the PCR product; (3) performing enzymatic reaction for the purified PCR product of step (2) by using restriction endonuclease, ligating the enzyme-digested product with a suitable plasmid expression vector, transforming and cultivating the ligation product in bacterial competent cells. In certain embodiments, the site-directed mutagenesis further comprises the following step: (4) selecting the clones of step (3) to perform colony PCR identification by using the primers of step (1), performing agarose gel electrophoresis for the PCR product, and then performing DNA sequencing identification to identify positive clones with the site-directed mutagenesis.

[0030] In another aspect, the present invention relates to use of the MG53 mutant in the manufacture of a medicament for treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage. In certain embodiments, the medicament may avoid or reduce metabolic side effects, such as insulin resistance, obesity, diabetes, hypertension, dyslipidemia, while treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage. In certain embodiments, the heart diseases are diseases associated with myocardial damage, including but not limited to, diabetic heart disease, myocardial ischemia, cardiac ischemia/reperfusion injury, myocardial infarction, heart failure, arrhythmia, heart rupture, angina, myocarditis, coronary heart disease, and pericarditis. In certain embodiments, the diabetic cerebrovascular diseases include, but are not limited to, cerebral arteriosclerosis, ischemic cerebrovascular disease, cerebral hemorrhage, cerebral atrophy, and cerebral infarction. In certain embodiments, the diabetic ocular complications include, but are not limited to, diabetic retinopathy, diabetic cataract, diabetic associated uveitis, and blindness. In certain embodiments, the diabetic neuropathy includes, but is not limited to, diabetic peripheral neuropathy. In certain embodiments, the kidney diseases include, but are not limited to, acute glomerulonephritis, chronic glomerulonephritis, nephrotic syndrome, acute kidney injury, diabetic nephropathy, etc. In certain embodiments, the diseases associated with cellular and/or tissue damage include, but are not limited to, diseases associated with the cellular and/or tissue damage of kidney, brain, lung, liver, heart, spleen, digestive tract, and skin, such as brain injury, lung injury, spleen injury, splenic rupture, gastric ulcer, gastritis, gastric perforation,

gastrointestinal mucosal injury, trauma, burns, ulcers, mucositis, asthma, chronic obstructive pulmonary disease (COPD), stroke, skin aging, etc. In certain embodiments, the present invention relates to use of a polypeptide set forth in SEQ ID NO: 7 in the manufacture of a medicament for treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage. In certain embodiments, the present invention relates to use of a polypeptide set forth in SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149 or SEQ ID NO: 150 in the manufacture of a medicament for treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage.

[0031] In yet another aspect, the present invention relates to a method of treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage, comprising administering to a subject in need thereof a therapeutically effective amount of the MG53 mutant. In certain embodiments, the MG53 mutant may avoid or reduce metabolic side effects, such as insulin resistance, obesity, diabetes, hypertension, and dyslipidemia, while treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage. In certain embodiments, the heart diseases are diseases associated with myocardial damage, including but not limited to, diabetic heart disease, myocardial ischemia, cardiac ischemia/reperfusion injury, myocardial infarction, heart failure, arrhythmia, heart rupture, angina, myocarditis, coronary heart disease, and pericarditis. In certain embodiments, the diabetic cerebrovascular diseases include, but are not limited to, cerebral arteriosclerosis, ischemic cerebrovascular disease, cerebral hemorrhage, cerebral atrophy, and cerebral infarction. In certain embodiments, the diabetic ocular complications include, but are not limited to, diabetic retinopathy, diabetic cataract, diabetic associated uveitis, and blindness. In certain embodiments, the diabetic neuropathy includes, but is not limited to, diabetic peripheral neuropathy. In certain embodiments, the kidney diseases include, but are not limited to, acute glomerulonephritis, chronic glomerulonephritis, nephrotic syndrome, acute kidney injury, diabetic nephropathy, etc. In certain embodiments, the diseases associated with cellular and/or tissue damage include, but are not limited to, diseases associated with the cellular and/or

tissue damage of kidney, brain, lung, liver, heart, spleen, digestive tract, and skin, such as brain injury, lung injury, spleen injury, splenic rupture, gastric ulcer, gastritis, gastric perforation, gastrointestinal mucosal injury, trauma, burns, ulcers, mucositis, asthma, chronic obstructive pulmonary disease (COPD), stroke, skin aging, etc. In certain embodiments, the present invention relates to a method of treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage, comprising administering to a subject in need thereof a therapeutically effective amount of the polypeptide set forth in SEQ ID NO: 7. In certain embodiments, the present invention relates to a method of treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage, comprising administering to a subject in need thereof a therapeutically effective amount of a polypeptide set forth in SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, or SEQ ID NO: 150.

[0032] In yet other aspect, the present invention relates to an MG53 mutant for use in treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage. In certain embodiments, the MG53 mutant may avoid or reduce metabolic side effects, such as insulin resistance, obesity, diabetes, hypertension, and dyslipidemia, while treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage. In certain embodiments, the heart diseases are diseases associated with myocardial damage, including but not limited to, diabetic heart disease, myocardial ischemia, cardiac ischemia/reperfusion injury, myocardial infarction, heart failure, arrhythmia, heart rupture, angina, myocarditis, coronary heart disease, and pericarditis. In certain embodiments, the diabetic cerebrovascular diseases include, but are not limited to, cerebral arteriosclerosis, ischemic cerebrovascular disease, cerebral hemorrhage, cerebral atrophy, and cerebral infarction. In certain embodiments, the diabetic ocular complications include, but are not limited to, diabetic retinopathy, diabetic cataract, diabetic associated uveitis, and blindness. In certain embodiments, the diabetic neuropathy includes, but is not limited to, diabetic peripheral neuropathy. In certain embodiments, the kidney diseases include, but are not limited to, acute glomerulonephritis, chronic glomerulonephritis, nephrotic syndrome, acute kidney injury,

diabetic nephropathy, etc. In certain embodiments, the diseases associated with cellular and/or tissue damage include, but are not limited to, diseases associated with the cellular and/or tissue damage of kidney, brain, lung, liver, heart, spleen, digestive tract, and skin, such as brain injury, lung injury, spleen injury, splenic rupture, gastric ulcer, gastritis, gastric perforation, gastrointestinal mucosal injury, trauma, burns, ulcers, mucositis, asthma, chronic obstructive pulmonary disease (COPD), stroke, skin aging, etc. In certain embodiments, the present invention relates to a polypeptide set forth in SEQ ID NO: 7 for use in treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage. In certain embodiments, the present invention relates to a polypeptide set forth in SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, or SEQ ID NO: 150 for use in treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage.

BRIEF DESCRIPTION OF THE FIGURES

[0033] Figure 1 illustrates the molecular structure of a wild-type MG53.

[0034] Figure 2 illustrates the amino acid sequence SEQ ID NO: 1 of human wild-type MG53 and its encoding nucleic acid sequence SEQ ID NO: 19.

[0035] Figure 3 illustrates the amino acid sequence SEQ ID NO: 2 of mouse wild-type MG53 and its encoding nucleic acid sequence SEQ ID NO: 20.

[0036] Figure 4 illustrates the amino acid sequence SEQ ID NO: 3 of rat wild-type MG53 and its encoding nucleic acid sequence SEQ ID NO: 21.

[0037] Figure 5 illustrates the amino acid sequence SEQ ID NO: 4 of monkey wild-type MG53 and its encoding nucleic acid sequence SEQ ID NO: 22.

[0038] Figure 6 illustrates the amino acid sequence SEQ ID NO: 5 of swine wild-type MG53 and its encoding nucleic acid sequence SEQ ID NO: 23.

[0039] Figure 7 illustrates the amino acid sequence SEQ ID NO: 6 of dog wild-type MG53 and its encoding nucleic acid sequence SEQ ID NO: 24.

[0040] Figure 8 illustrates the amino acid sequence SEQ ID NO: 7 of human MG53 S255A mutant and its encoding nucleic acid sequence SEQ ID NO: 13.

[0041] Figure 9 illustrates the amino acid sequence SEQ ID NO: 8 of mouse MG53 S255A mutant and its encoding nucleic acid sequence SEQ ID NO: 14.

[0042] Figure 10 illustrates the amino acid sequence SEQ ID NO: 9 of rat MG53 S255A mutant and its encoding nucleic acid sequence SEQ ID NO: 15.

[0043] Figure 11 illustrates the amino acid sequence SEQ ID NO: 10 of monkey MG53 S255A mutant and its encoding nucleic acid sequence SEQ ID NO: 16.

[0044] Figure 12 illustrates the amino acid sequence SEQ ID NO: 11 of swine MG53 S255A mutant and its encoding nucleic acid sequence SEQ ID NO: 17.

[0045] Figure 13 illustrates the amino acid sequence SEQ ID NO: 12 of dog MG53 S255A mutant and its encoding nucleic acid sequence SEQ ID NO: 18.

[0046] Figure 14 illustrates the amounts of intracellular ATP and LDH release after adenovirus is used to overexpress mouse wild-type MG53 and mouse MG53 S255A mutant in primarily cultured cardiomyocytes of neonatal rats. The upper panel shows the detected amount of intracellular ATP after hypoxia and reoxygenation treatment when adenovirus is used to overexpress mouse wild-type MG53 and mouse MG53 S255A mutant in primarily cultured cardiomyocytes of neonatal rats, wherein Adv- β -gal represents a control virus expressing galactosidase, but not expressing mouse wild-type MG53 or mouse MG53 S255A mutant; Adv-MG53-myc represents adenovirus expressing mouse wild-type MG53; Adv-MG53-S255A-myc represents adenovirus expressing mouse MG53 S255A mutant (n=7, *p < 0.05, **p < 0.01). The lower panel shows the detected amount of LDH release after hypoxia and reoxygenation treatment when adenovirus is used to overexpress mouse wild-type MG53 and mouse MG53 S255A mutant in primarily cultured cardiomyocytes of neonatal rats, wherein Adv- β -gal represents a control virus expressing galactosidase, but not expressing mouse wild-type MG53 or mouse MG53 S255A mutant; Adv-MG53-myc represents adenovirus expressing mouse wild-type MG53; Adv-MG53-S255A-myc represents adenovirus expressing mouse MG53 S255A mutant (n=7, **p < 0.01, ***p < 0.005).

[0047] Figure 15 illustrates that mouse MG53 S255A mutation does not affect the activation of AKT by mouse wild-type MG53. The upper panel shows the amount of intracellular p-AKT⁴⁷³ detected by Western Blot when adenovirus is used to overexpress mouse wild-type MG53 and mouse MG53 S255A mutant in primarily cultured cardiomyocytes of neonatal rats, wherein β -gal represents control adenovirus expressing galactosidase, but not expressing mouse wild-type MG53 or mouse MG53 S255A mutant; MG53 represents adenovirus expressing mouse wild-type MG53; S255A represents adenovirus expressing mouse MG53 S255A mutant. The lower panel is a statistic diagram of the upper panel, wherein Adv β -gal represents control adenovirus expressing galactosidase, but not expressing mouse wild-type MG53 or MG53 S255A mutant; Adv MG53 represents adenovirus expressing mouse wild-type MG53; Adv MG53 S255A represents adenovirus expressing mouse MG53 S255A mutant (n = 5, * p < 0.05).

[0048] Figure 16 illustrates that mouse MG53 S255A mutation inhibits MG53-mediated substrate degradation. The upper panel of Figure 16A shows IRS1 protein content detected by Western Blot in plasmids overexpressing IRS1, mouse wild-type MG53, mouse MG53 S255A mutant, or mouse MG53-D-RING truncated mutant in HEK293T cell line, wherein CON represents an empty vector plasmid control without expression of mouse wild-type MG53, mouse MG53 S255A mutant, and mouse MG53-D-RING truncated mutant; MG53 represents a vector plasmid expressing mouse wild-type MG53; S255A represents a vector plasmid expressing mouse MG53 S255A mutant; D-RING represents a vector plasmid expressing mouse MG53 truncated mutant MG53-D-RING, in which mouse RING domain is truncated. The lower panel of Figure 16A is a statistic diagram of the upper panel (n=5, *p < 0.05; **p < 0.01). The upper panel of Figure 16B shows IR protein content detected by Western Blot in plasmids overexpressing IR, mouse wild-type MG53, mouse MG53 S255A mutant, or mouse MG53-D-RING truncated mutant in HEK293T cell line, wherein CON represents an empty vector plasmid control without expression of mouse wild-type MG53, mouse MG53 S255A mutant, and mouse MG53-D-RING truncated mutant; MG53 represents a vector plasmid expressing mouse wild-type MG53; S255A represents a vector plasmid expressing mouse MG53 S255A mutant; D-RING represents a vector plasmid expressing mouse MG53-D-RING truncated mutant. The lower panel of Figure 16B is a statistic diagram of the upper panel (n=5, **p < 0.01).

[0049] Figure 17 illustrates that human MG53 S255A mutant inhibits MG53-mediated substrate degradation. The upper panel shows IRS1 protein content detected by Western Blot in plasmids

plasmids overexpressing IRS1, human wild-type MG53, human MG53 S255A mutant, or human MG53-D-RING truncated mutant in HEK293T cell line, wherein CON represents an empty vector plasmid control without expression of human wild-type MG53, human MG53 S255A mutant, and human MG53-D-RING truncated mutant; MG53 represents a vector plasmid expressing human wild-type MG53; MG53-dR represents a vector plasmid expressing human MG53-D-RING truncated mutant. The lower panel is a statistic diagram of the upper panel (n=5, **p <0.01).

[0050] Figure 18 illustrates that mouse MG53 S255A mutant inhibits the binding of MG53 to substrate IRS1. Figure 18A shows the content of MG53-IRS1 complex detected by immunoprecipitating IRS1 protein and performing Western Blot after co-expressing mouse wild-type MG53 and MG53 substrate IRS1, or co-expressing mouse MG53 S255A mutant and MG53 substrate IRS1 in HEK293T cell line. In Figure 18A, MG53 represents mouse wild-type MG53, MG53 S255A represents mouse MG53 S255A mutant, IRS1 represents mouse insulin receptor substrate. Figure 18B shows the binding intensity of immobilized and purified protein IRS1 to purified protein mouse wild-type MG53 or mouse MG53 S255A mutant in the mobile phase during surface plasmon resonance (SPR) test, wherein MG53 represents mouse wild-type MG53, MG53-S255A represents mouse MG53 S255A mutant, and Rat IRS1 represents rat insulin receptor substrate.

[0051] Figure 19 illustrates the effect of mouse MG53 S255A mutant on the amount of protein IRS1. The upper panel shows the content of protein IRS1 detected by Western Blot in plasmids overexpressing IRS1, Ub, MG53, or MG53 S255A mutant in HEK293T cell line, wherein Ub represents ubiquitin, MG53 represents mouse wild-type MG53, and MG53-S255A represents mouse MG53 S255A mutant. The lower panel is a statistic diagram of the upper panel, wherein CON represents an empty vector plasmid control without expression of mouse wild-type MG53 and mouse MG53 S255A mutant, MG53-S255A represents a vector plasmid expressing mouse MG53 S255A mutant (n=3, *p <0.05; ***p <0.005).

[0052] Figure 20 illustrates the amino acid sequence SEQ ID NO: 139 (which corresponds to NCBI number BAD18630.1) of a human MG53 subtype and its encoding nucleic acid sequence SEQ ID NO: 143.

[0053] Figure 21 illustrates the amino acid sequence SEQ ID NO: 140 (which corresponds to NCBI Accession No. XP_016878743.1) of a human MG53 subtype and its encoding nucleic acid sequence in SEQ ID NO: 144.

[0054] Figure 22 illustrates the amino acid sequence SEQ ID NO: 141 (which corresponds to NCBI BAC03506.1) of a human MG53 subtype and its encoding nucleic acid sequence SEQ ID NO: 145.

[0055] Figure 23 illustrates the amino acid sequence SEQ ID NO: 142 (which corresponds to NCBI number AAH33211.1) of a human MG53 subtype and its encoding nucleic acid sequence SEQ ID NO: 146.

[0056] Figure 24 illustrates the amino acid sequence SEQ ID NO: 147 of a human MG53 subtype S255A mutant and its encoding nucleic acid sequence SEQ ID NO: 151.

[0057] Figure 25 illustrates the amino acid sequence SEQ ID NO: 148 of a human MG53 subtype S255A mutant and its encoding nucleic acid sequence SEQ ID NO: 152.

[0058] Figure 26 illustrates the amino acid sequence SEQ ID NO: 149 of a human MG53 subtype S255A mutant and its encoding nucleic acid sequence SEQ ID NO: 153.

[0059] Figure 27 illustrates the amino acid sequence SEQ ID NO: 150 of a human MG53 subtype S255A mutant and its encoding nucleic acid sequence in SEQ ID NO: 154.

[0060] Figure 28 illustrates the nomenclature schematic of the amino acid positions of full-length MG53 proteins and fragments thereof for each species in the present invention.

DETAILED DESCRIPTION

[0061] Although various aspects and embodiments of the present invention will be disclosed in the following, a person skilled in the art can make various equivalent changes and modifications without departing from the spirit and scope of the subject matter of the application. The various aspects and embodiments disclosed herein are given by way of illustration only, and are not intended to limit the present invention. The actual protection scope of the present application is defined by the claims. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by a person having ordinary skills

in the art to which this invention pertains. All references, patents, patent applications cited in the present application are hereby incorporated by reference in their entireties.

[0062] In one aspect, the present invention relates to an MG53 mutant, wherein the MG53 mutant is identical to the amino acid sequence of a wild-type MG53 except for at least one serine in the coiled-coil-SPRY region of the wild-type MG53, which is deleted and/or mutated into any other non-serine or non-threonine amino acid(s).

[0063] As used herein, the term "wild-type MG53" or "wild-type MG53 protein" refers to the natural sequence of a full-length MG53 protein or fragments thereof expressed in a subject. MG53 protein is a multi-functional protein with a structure shown in Figure 1. The full-length MG53 proteins of different species are slightly different in length, but generally have about 477 amino acids, comprising a TRIM motif at the N-terminus and a SPRY motif at the C-terminus. The TRIM motif consists of successively linked Ring, B-box and coiled-coil domains (RBCC). MG53 protein is one of the important components for membrane repair, which plays an essential role in the pre-conditioning and post-conditioning protection of ischemia-reperfusion injury. Meanwhile, the high expression of MG53 protein may also cause insulin resistance and metabolic syndrome. The structure and function of MG53, as well as its interaction with other proteins have been reported in detail in the art (see, e.g., Chuanxi Cai et al., *Journal of Biological Chemistry*, 284 (5), 3314-3322, (2009); Xianhua Wang et al., *Circulation Research* 107, 76-83, (2010); Eun Young Park et al., *Proteins*, 790-795 (2009)).

[0064] As used herein, the term "subject" includes both human and non-human animals. Non-human animals include all vertebrates, such as mammals and non-mammals. The "subject" may also be a domestic animal such as cow, swine, sheep, poultry and horse; or rodent such as rat, mouse; or a primate such as ape, monkey; or domesticated animal such as dog or cat. "Subject" may be male or female, and may be elderly, adult, adolescent, child or infant. A human "subject" may be Caucasian, African, Asian, Semitic, or other races, or a mixture of the racial backgrounds above.

[0065] In certain embodiments, the wild-type MG53 is preferably derived from a mammal, e.g., human, ape, monkey, mouse, rat, swine, dog, etc. A person skilled in the art may, from an open channel (e.g., National Center for Biotechnology Information (NCBI)), obtain the amino acid sequence of wild-type MG53 of each species, which is incorporated herein by reference. In

certain embodiments, the amino acid sequence of the wild-type MG53 is set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6, which corresponds to the human, mouse, rat, monkey, swine, and dog wild-type MG53 full length protein, respectively.

[0066] As used herein, the terms "protein", "polypeptide" and "peptide" are used interchangeably to refer to a polymer of amino acids. The proteins, polypeptides or peptides described herein may contain natural amino acids, non-natural amino acids, or analogs or mimetics of amino acids. The proteins, polypeptides or peptides described herein may be obtained by any known method in the art, such as, but not limited to, by natural isolation, recombinant expression, chemical synthesis, etc.

[0067] As used herein, the term "coiled-coil-SPRY region" is a corresponding region of the amino acid sequence of a wild-type MG53. The coiled-coil domain, existing in most TRIM family proteins, mediates homologous or heterologous association between TRIM family members or between TRIM members and other proteins to form complexes such as dimers, polymers, etc., thereby eliciting cell membrane repair (see, e.g., Ozato et al., *Nature Review Immunology*, 8: 849-860 (2008); Sanchez S. et al., *PNAS*, 111: 2494-2499 (2014)). The SPRY domain is located at the C-terminus of the amino acid sequence of a wild-type MG53 and is typically located at positions 288-477 of the amino acid sequence of a wild-type MG53. In certain embodiments, the SPRY domain described herein includes a PRY motif and a SPRY motif. The SPRY domain is evolutionarily conserved and is expressed throughout fungi to higher animals (see, e.g., Ozato et al., *Nature Review Immunology*, 8: 849-860 (2008)). So far, about 60 TRIM family members are identified in different mammalian genomes, among which 15 members carry a similar SPRY domain after the TRIM domain (i.e., the Ring-B-box-Coiled-Coil domain), and MG53 exhibits a conserved primary structure with these TRIM subfamily proteins (see, e.g., WO2009/073808). The specific amino acid positions corresponding to the coiled-coil-SPRY region may be slightly different among different species, but a person skilled in the art may obtain the specific amino acid positions corresponding to the coiled-coil-SPRY region of wild-type MG53 of different species through the prior art (e.g., the information as disclosed in NCBI) and/or routine experimental methods. In certain embodiments, the coiled-coil-SPRY region as used herein refers to amino acid positions 122-477 of a wild-type MG53 or a structurally similar region. In certain embodiments, the structurally similar region may be a region comprising 70%, 80% or 90% contiguous amino acid sequence of amino acid positions 122-477. In certain embodiments, the

N-terminal initiation site of the structurally similar region may have about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 amino acids longer or shorter than the N-terminus of the amino acid positions 122-477, and/or the C-terminal termination site of the structurally similar region may have about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 amino acids longer or shorter than the C-terminus of the amino acid positions 122-477. For example, in certain embodiments, the coiled-coil-SPRY region as used herein refers to the coiled-coil-SPRY region of human wild-type MG53 (i.e., SEQ ID NO: 1), which corresponds to amino acid positions 122-477 of SEQ ID NO: 1.

[0068] As used herein, the term "MG53 mutant" or "MG53 protein mutant" refers to an MG53 protein variant or fragment in which the natural amino acid sequence of a wild-type MG53 protein is modified. Such modifications include, but are not limited to, deletion and/or substitution of one or more amino acids. In certain embodiments, the MG53 mutant of the present invention is identical to the amino acid sequence of a wild-type MG53 except for at least one serine in the coiled-coil-SPRY region of the wild-type MG53, which is deleted and/or mutated into any other non-serine or non-threonine amino acid(s).

[0069] As used herein, "at least one serine" refers to one or more serine, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 or more serine within the coiled-coil-SPRY region of a wild-type MG53.

[0070] As used herein, "other non-serine or non-threonine amino acids" refers to any other natural amino acids, substituted natural amino acids, non-natural amino acids, substituted non-natural amino acids, or any combination thereof, that are not serine or threonine. The names of natural amino acids are represented as standard single letter or three-letter codes in the present application. Natural amino acids include non-polar amino acids and polar amino acids. Unless otherwise specified, any of the amino acids described herein may be in the D- or L-configuration.

[0071] In certain embodiments, at least one serine (Ser or S) within the coiled-coil-SPRY region of the wild-type MG53 is deleted or mutated into a non-polar amino acid. The non-polar amino acids include glycine (Gly or G), alanine (Ala or A), leucine (Leu or L), isoleucine (Ile or I), valine (Val or V), proline (Pro or P), phenylalanine (Phe or F), methionine (Met or M), tryptophan (Trp or W). In certain embodiments, at least one serine within the coiled-coil-SPRY region of the wild-type MG53 is deleted or mutated into glycine or alanine. Preferably, in certain

embodiments, at least one serine within the coiled-coil-SPRY region of the wild-type MG53 is deleted or mutated into alanine. More preferably, in certain embodiments, at least one serine within the coiled-coil-SPRY region of the wild-type MG53 is mutated into alanine.

[0072] In certain embodiments, at least one serine within the coiled-coil-SPRY region of the wild-type MG53 is deleted or mutated into a polar amino acid. The polar amino acids include glutamine (Gln or Q), cysteine (Cys or C), asparagine (Asn or N), tyrosine (Tyr or Y), aspartic acid (Asp or D), glutamic acid (Glu or E), lysine (Lys or K), arginine (Arg or R), histidine (His or H). In certain embodiments, at least one serine within the coiled-coil-SPRY region of the wild-type MG53 is deleted or mutated into cysteine or histidine. Preferably, in certain embodiments, at least one serine within the coiled-coil-SPRY region of the wild-type MG53 is deleted or mutated into cysteine. More preferably, in certain embodiments, at least one serine within the coiled-coil-SPRY region of the wild-type MG53 is mutated into cysteine.

[0073] In certain embodiments, the serine mutations described herein include one serine is substituted with one or more other non-serine or non-threonine amino acids, for example, one serine may be substituted with 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 other non-serine or non-threonine amino acids. If two or more serine are substituted, then each serine may be independently substituted with one or more other non-serine or non-threonine amino acids, respectively.

[0074] The specific serine positions of the wild-type MG53 of each species may vary from species to species; however, once the amino acid sequence of the wild-type MG53 of a species and the amino acid region corresponding to its coiled-coil-SPRY region is known, a person skilled in the art can determine the specific amino acid positions to which the serine within the coiled-coil-SPRY region corresponds. For example, a person skilled in the art knows that the amino acid sequence of human wild-type MG53 is set forth in SEQ ID NO: 1 and its coiled-coil-SPRY region corresponds to amino acid positions 122-477 of SEQ ID NO: 1. Under such circumstances, a person skilled in the art can determine that the specific amino acid positions corresponding to serine within the coiled-coil-SPRY region of human wild-type MG53 are positions 150, 189, 211, 214, 246, 255, 269, 296, 297, 301, 305, 306, 307, 314, 341, 377, 405, 418, 425, 430 of SEQ ID NO: 1, respectively.

[0075] In certain embodiments, the deleted or mutated serine is located at one or more of the following positions of human wild-type MG53 amino acid sequence set forth in SEQ ID NO: 1:

positions 150, 189, 211, 214, 246, 255, 269, 296, 297, 301, 305, 306, 307, 314, 341, 377, 405, 418, 425, 430.

[0076] In certain embodiments, the MG53 protein as described herein includes a full-length MG53 protein, or a truncated mutant thereof (i.e., a truncated fragment of the full-length MG53 protein) or a mutant having mutation, addition or deletion of one or more amino acids compared to the full-length MG53 protein or the truncated mutant thereof. In this case, in order to unify the nomenclature of amino acid positions for the amino acid sequences of different MG53 protein subtypes, when the amino acid position of a certain MG53 protein subtype is mentioned in the present invention, the amino acid sequence of the MG53 protein subtype shall be aligned with the amino acid sequence of the full-length MG53 protein (e.g., SEQ ID NO: 1) and if necessary, gaps shall be introduced into the relevant amino acid sequence so as to maximize the number of identical amino acids. The position No. of the first amino acid in the amino acid sequence of the MG53 subtype is designated as the position No. of the full-length MG53 protein that corresponds to said amino acid, so that when reference is made to the amino acid positions of the wild-type MG53 of a species, both the full-length MG53 protein and the truncated mutant thereof refer to the position No. in the amino acid sequence of the full-length MG53 protein.

[0077] For example, as shown in Figure 28, the full-length MG53 protein of a species has n amino acids and the corresponding amino acid positions are positions 1- n . The amino acid sequence of MG53 subtype 1, compared to the amino acid sequence of the full-length MG53 protein, contains a truncated mutant corresponding to the amino acids located at positions m - n of the full-length sequence. Thus, the first amino acid position of the MG53 subtype 1 amino acid sequence is designated as position m , and the second amino acid position of the MG53 subtype 1 amino acid sequence is designated as position $m+1$, analogously, the last amino acid position of the MG53 subtype 1 amino acid sequence is designated as position n . For another example, the amino acid sequence of MG53 subtype 2, compared to the amino acid sequence of the full-length MG53 protein, contains a truncated mutant corresponding to the amino acids located at positions 1- s of the full-length sequence. Thus, the first amino acid position of the MG53 subtype 2 amino acid sequence is designated as position 1, and the second amino acid position of the MG53 subtype 2 amino acid sequence is designated as position 2, analogously, the last amino acid position of the MG53 subtype 2 amino acid sequence is designated as position s . For yet another example, the amino acid sequence of MG53 subtype 3, compared to the

amino acid sequence of the full-length MG53 protein, contains a truncated mutant corresponding to the amino acids located at positions p - q of the full-length sequence. Thus, the first amino acid position of the MG53 subtype 3 amino acid sequence is designated as position p , and the second amino acid position of the MG53 subtype 3 amino acid sequence is designated as position $p+1$, analogously, the last amino acid position of the MG 53 subtype 3 amino acid sequence is designated as position q .

[0078] For example, SEQ ID NO: 140 is one of the human wild-type MG53 subtypes, having 333 amino acids corresponding to amino acid positions 145-477 of the human wild-type MG53 full-length sequence set forth in SEQ ID NO: 1. Under this circumstance, the first amino acid (methionine) position of the amino acid sequence SEQ ID NO: 140 is designated as position 145 of SEQ ID NO: 140, and the second amino acid position is designated as position 146 of SEQ ID NO: 140, analogously, the last amino acid position of the amino acid sequence SEQ ID NO: 140 is designated as position 477 of SEQ ID NO: 140. As another example, SEQ ID NO: 141 is one of the human wild-type MG53 subtypes, which also has 333 amino acids, and only one amino acid differs from amino acid positions 145-477 of the human wild-type MG53 full-length sequence set forth in SEQ ID NO: 1, that is, position 315 of SEQ ID NO: 1 is glutamic acid, while the corresponding position of SEQ ID NO: 141 is glycine. Under this circumstance, the first amino acid position (methionine) of the amino acid sequence set forth in SEQ ID NO: 141 is designated as position 145 of SEQ ID NO: 141, and the second amino acid position is designated as position 146 of SEQ ID NO: 141, analogously, the last amino acid position is designated as position 477 of SEQ ID NO: 141. As yet another example, SEQ ID NO: 142 is one of the human wild-type MG53 subtypes, having 269 amino acids corresponding to amino acid positions 1-269 of the human wild-type MG53 full-length sequence set forth in SEQ ID NO: 1. Under this circumstance, the first amino acid (methionine) position of the amino acid sequence SEQ ID NO: 142 is designated as position 1 of SEQ ID NO: 142, the second amino acid position of the amino acid sequence SEQ ID NO: 142 is designated as position 2 of SEQ ID NO: 142, analogously, the last amino acid position of the amino acid sequence SEQ ID NO: 142 is designated as position 269 of SEQ ID NO: 142.

[0079] In certain embodiments, the deleted or mutated serine is located at one or more of the following positions of human wild-type MG53 subtype amino acid sequence set forth in SEQ ID NO: 139: positions 150, 189, 211, 214, 246, 255, 269, 296, 297, 301, 305, 306, 307, 314, 341, 377, 405, 418, 425, or 430.

[0080] In certain embodiments, the deleted or mutated serine is located at one or more of the following positions of human wild-type MG53 subtype amino acid sequence set forth in SEQ ID NO: 140: positions 150, 189, 211, 214, 246, 255, 269, 296, 297, 301, 305, 306, 307, 314, 341, 377, 405, 418, 425, or 430.

[0081] In certain embodiments, the deleted or mutated serine is located at one or more of the following positions of human wild-type MG53 subtype amino acid sequence set forth in SEQ ID NO: 141: positions 150, 189, 211, 214, 246, 255, 269, 296, 297, 301, 305, 306, 307, 314, 341, 377, 405, 418, 425, or 430.

[0082] In certain embodiments, the deleted or mutated serine is located at one or more of the following positions of human wild-type MG53 subtype amino acid sequence set forth in SEQ ID NO: 142: positions 150, 189, 211, 214, 246, 255, or 269.

[0083] In certain embodiments, the deleted or mutated serine is located at one or more of the following positions of mouse wild-type MG53 amino acid sequence set forth in SEQ ID NO: 2: positions 188, 189, 210, 211, 214, 246, 253, 255, 269, 296, 297, 301, 305, 306, 307, 314, 341, 367, 377, 418, 430, or 440.

[0084] In certain embodiments, the deleted or mutated serine is located at one or more of the following positions of rat wild-type MG53 amino acid sequence set forth in SEQ ID NO: 3: positions 150, 188, 189, 210, 211, 214, 246, 253, 255, 269, 296, 297, 301, 305, 307, 314, 341, 367, 377, 418, 430, 440, 464, or 474.

[0085] In certain embodiments, the deleted or mutated serine is located at one or more of the following positions of monkey wild-type MG53 amino acid sequence set forth in SEQ ID NO: 4: positions 150, 189, 211, 214, 246, 255, 269, 296, 297, 301, 305, 306, 307, 341, 377, 405, 418, 425, 430, or 464.

[0086] In certain embodiments, the deleted or mutated serine is located at one or more of the following positions of swine wild-type MG53 amino acid sequence set forth in SEQ ID NO: 5: positions 150, 189, 211, 214, 246, 255, 269, 296, 301, 305, 307, 314, 341, 377, 411, 418, 425, 430, or 474.

[0087] In certain embodiments, the deleted or mutated serine is located at one or more of the following positions of dog wild-type MG53 amino acid sequence set forth in SEQ ID NO: 6: positions 150, 189, 211, 214, 246, 255, 269, 296, 297, 301, 305, 307, 314, 341, 367, 377, 418, 425, or 430.

[0088] The specific positions of the deleted or mutated serine may vary from species to species, however, the serine position of wild-type MG53 protein is highly conserved among various species. For example, positions 189, 211, 214, 246, 255, 269, 296, 301, 305, 307, 341, 377, 418, and 430 of amino acid sequences of the human, mouse, rat, monkey, swine and dog wild-type MG53 proteins are all serine. In some embodiments, the deleted or mutated serine is located at one or more of the following positions of a wild-type MG53 amino acid sequence (e.g., SEQ ID NOs: 1-6): positions 189, 211, 214, 246, 255, 269, 296, 301, 305, 307, 341, 377, 418, or 430.

[0089] In certain embodiments, the deleted or mutated serine is located at one or more of the following positions of a wild-type MG53 amino acid sequence (e.g., SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, or SEQ ID NO: 142): positions 150, 188-189, 210-211, 214, 246, 253-255, 269, 296-297, 301, 305-307, 314, 341, 367, 377, 405, 411, 418, 425, 430, 440, 464, or 474.

[0090] In certain embodiments, the deleted or mutated serine is located at one or more of the following positions of a wild-type MG53 amino acid sequence (e.g., SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, or SEQ ID NO: 142): positions 189, 211, 214, 246, 253-255, 269, 296, 301, 305, 307, 341, 377, 418, or 430.

[0091] In certain embodiments, the deleted or mutated serine is located at one or more of the following positions of a wild-type MG53 amino acid sequence (e.g., SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, or SEQ ID NO: 142): positions 211, 214, 246, 253-255, 269, 296, or 297.

[0092] In certain embodiments, the deleted or mutated serine is located at one or more of the following positions of a wild-type MG53 amino acid sequence (e.g., SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, or SEQ ID NO: 142): positions 211, 214, 246, 255, 269, 296, or 297.

[0093] In certain embodiments, the deleted or mutated serine is located at one or more of positions 253-255 of a wild-type MG53 amino acid sequence (e.g., SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, or SEQ ID NO: 142). In certain embodiments, the deleted or mutated serine is located at position 253 of a wild-type MG53 amino acid sequence. In certain embodiments, the deleted or mutated serine is located at position 255 of a wild-type MG53 amino acid sequence. In certain embodiments, the deleted or mutated serine is located at position 255 of the wild-type MG53 amino acid sequence SEQ ID NO: 1. In certain embodiments, the deleted or mutated serine is located at position 255 of the wild-type MG53 amino acid sequence SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, or SEQ ID NO: 142.

[0094] In certain embodiments, the MG53 mutant has two or more serine mutations. In certain embodiments, two or more serine mutations of the MG53 mutant include serine mutation at one or more of positions 253-255. In certain embodiments, two or more serine mutations of the MG53 mutant include serine mutation at position 253. In certain embodiments, two or more serine mutations of the MG53 mutant include serine mutation at position 255. In certain embodiments, two or more serine mutations of the MG53 mutant include serine mutation at positions 253 and 255.

[0095] A person skilled in the art will comprehend that serine residues among the wild-type MG53 proteins of different species may be located at different positions, and thus the positions of the deleted or mutated serine may also differ. In certain embodiments, the deleted or mutated serine is within 1 to 10 amino acids, 1 to 5 amino acids, or 1 to 3 amino acids upstream or downstream of the corresponding serine position in the amino acid sequence of the wild-type MG53 set forth in SEQ ID NO: 1.

[0096] In certain embodiments, the amino acid sequence of the MG53 mutant is the amino acid sequence set forth in SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, or SEQ ID NO: 150. More preferably, in certain embodiments, the amino acid sequence of the MG53 mutant is the amino acid sequence set forth in SEQ ID NO: 7. In certain embodiments, the amino acid sequence of the MG53 mutant has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% amino acid sequence homology to one of the amino acid

sequences set forth in SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO:11, SEQ ID NO: 12, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO:150, and the MG53 mutant avoids metabolic side effects caused by wild-type MG53 while still retaining cell membrane repair function and/or cardioprotective function.

[0097] As used herein, the percent (%) "sequence homology to" refers to, for amino acid sequences, the percentage of identity between two amino acid sequences after aligning the candidate and the reference sequences, and if necessary introducing gaps, to achieve the maximum number of identical amino acids; for nucleotide sequence, the percentage of identity between two nucleotide sequences after aligning the candidate and the reference sequences, and if necessary introducing gaps, to achieve the maximum number of identical nucleotides.

[0098] The percentage of homology can be determined by various well-known methods in the art. For example, the comparison of sequences can be achieved by the following publically available tools: BLASTp software (available from the website of National Center for Biotechnology Information (NCBI): <http://blast.ncbi.nlm.nih.gov/Blast.cgi>, also see, Altschul SF et al., *J. Mol. Biol.*, 215: 403-410 (1990); Stephen F. et al., *Nucleic Acids Res.*, 25: 3389-3402 (1997)), ClustalW2 (available from the website of European Bioinformatics Institute: <http://www.ebi.ac.uk/Tools/msa/clustalw2/>, see Higgins DG et al., *Methods in Enzymology*, 266: 383-402 (1996); Larkin MA et al., *Bioinformatics* (Oxford, England), 23 (21): 2947-8 (2007)), and Toffee (available from the Swiss Institute of Bioinformatics website, also see Poirot O. et al., *Nucleic Acids Res.*, 31 (13) : 3503-6 (2003); Notredame C. et al., *J. Mol. Boil.*, 302 (1): 205-17 (2000)). If the alignment of the sequences is performed using software, the default parameters available in the software may be used, or otherwise the parameters may be customized to suit the alignment purpose. All of these are within the scope of knowledge of a person skilled in the art.

[0099] In certain embodiments, SEQ ID NOs: 7-12, SEQ ID NOs: 147-150 and the MG53 mutants that have amino acid sequence homology to SEQ ID NOs: 7-12 and SEQ ID NOs: 147-150 avoid metabolic side effects caused by wild-type MG53, while still retaining cell membrane repair function and/or cardioprotective function.

[0100] The inventors of the present invention have found that when at least one serine in the coiled-coil-SPRY region of a wild-type MG53 is deleted or mutated into any other non-serine or

non-threonine amino acid(s), the metabolic side effects caused by the wild-type MG53 can be avoided or reduced, without any impact on the cell membrane repair function and/or cardioprotective function of MG53. While not wishing to be bound by any theory, the above results may be due to the fact that the phosphorylation of serine within the coiled-coil-SPRY region of a wild-type MG53 may significantly regulate the E3 ubiquitin ligase activity of MG53 and modulate the regulation function of MG53 on insulin signaling system through substrates IR β and IRS1. However, the phosphorylation of serine within this region does not regulate the cell membrane repair function and/or cardioprotective function of MG53.

[0101] As used herein, "cell membrane repair function" refers to the ability of wild-type MG53 or MG53 mutants to repair and restore the damaged cell membrane during cell injury, especially acute cell injury, through activating relevant signaling pathways (e.g. the RISK pathway) to reduce cell death, promote cell survival, and thereby restore cellular functions. In certain embodiments, the wild-type MG53 or MG53 mutants of the present invention can repair viable cells, cells *in vitro*, or cells *in vivo*. The wild-type MG53 or MG53 mutants of the present invention can also repair different types of cells, such as, but not limited to, cardiomyocytes, striated muscle cells, skeletal muscle cells, renal proximal tubular epithelial cells, alveolar epithelial cells, gastrointestinal epithelial cells (e.g., oral epithelial cells, esophageal epithelial cells, gastric epithelial cells, duodenal epithelial cells, small intestinal epithelial cells, jejunal epithelial cells, ileum epithelial cells, colonic epithelial cells), mucosal cells (e.g., oral mucosal cells, nasal mucosal cells, gastric mucosal cells, small intestinal mucosal cells, colonic mucosal cells, duodenal mucosal cells), skin cells (e.g., epidermal cells, epithelial cells, dermal cells, endothelial cells), vascular cells (e.g., vascular parietal cells, vascular endothelial cells, vascular endothelial cells, vascular smooth muscle cells), etc. In certain embodiments, the wild-type MG53 or MG53 mutants of the present invention can repair cardiomyocytes, skeletal muscle cells, striated muscle cells, proximal renal tubular epithelial cells, alveolar epithelial cells, etc.

[0102] The cell membrane repair function of the MG53 mutants as described herein can be determined using methods well known in the art. For example, the cell membrane repair function of the MG53 mutants of the present invention can be determined by overexpressing wild-type MG53 and the MG53 mutants of the present invention using adenovirus in neonatal rat ventricular myocytes (NRVMs), using hypoxia to stimulate cells and detecting the survival of cells (for example, by MTT method, ATP and LDH concentration measurements in medium,

TUNEL staining (for detailed steps please refer to Zhang. T et al., *Nature Medicine*, 175-184 (2016)), etc.), and then comparing the indicators (e.g. intracellular ATP level, LDH releasing level, etc.) of control groups (e.g. empty vector negative control groups without the expression of wild-type MG53 and MG53 mutants, positive control group overexpressing wild-type MG53) and test group (i.e. the group overexpressing the MG53 mutants of the present invention).

[0103] As used herein, "cardioprotective function" refers that the wild-type MG53 or MG53 mutants can repair myocardial cell membrane damage during myocardial injury, especially acute myocardial injury, optionally, through activating myocardial cell-related signaling pathways (e.g., the RISK pathway) to achieve the protection of cardiomyocyte and thereby enhance the cardiac protection. The cardioprotective function of the MG53 mutants as described herein can be determined using methods well known in the art. For example, the cardioprotective function of the MG53 mutants of the present invention can be determined by overexpressing wild-type MG53 and the MG53 mutants of the present invention using adenovirus in neonatal rat ventricular myocytes (NRVMs), using hypoxia to stimulate cells and detecting the survival of cells (for example, by MTT method, ATP and LDH concentration measurements in medium, TUNEL staining (for detailed steps please refer to Zhang. T et al., *Nature Medicine*, 175-184 (2016)), etc.), and then comparing the indicators (e.g. intracellular ATP level, LDH releasing level, etc.) of control groups (e.g. empty vector negative control groups without the expression of wild-type MG53 and MG53 mutants, positive control group overexpressing wild-type MG53) and test group (i.e. the group overexpressing the MG53 mutants of the present invention). For another example, cardiomyocytes are incubated with wild-type MG53 and the MG53 mutants as described herein, respectively, various stimuli (e.g., hypoxia, H₂O₂) are used to lead to cell death, and the cardioprotective function of the MG53 mutants as described herein are evaluated by comparing the survival rates in control groups (e.g., negative control group that is neither incubated with wild-type MG53 nor MG53 mutants, positive control group overexpressing wild-type MG53) and test group (i.e., the group incubated with MG53 mutants of the present invention). The cell survival rate can be determined by MTT cell count, LDH, ATP, or TUNEL staining measurements.

[0104] As used herein, "metabolic side effects" refers to diseases or discomforts due to metabolic disorders that are outside of the therapeutic purpose after administration of a therapeutic amount of a drug, including, but not limited to, insulin resistance, obesity, diabetes, high blood pressure, dyslipidemia, etc. Without wishing to be bound by any theory, it is

believed that the severity of metabolic side effects can be assessed by measuring E3 ubiquitin ligase activity of MG53. In certain embodiments, when at least one serine in the coiled-coil-SPRY region of the wild-type MG53 is deleted and/or mutated into any other non-serine or non-threonine amino acid(s), at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or even 100% of the E3 ubiquitin ligase activity of a wild-type MG53 would be inhibited, thereby avoiding or reducing the metabolic side effects caused by wild-type MG53 without having any impact on the cell membrane repair function and/or cardioprotective function of MG53. "Avoiding or reducing the metabolic side effects caused by wild-type MG53" refers to the absence of metabolic side effects caused by wild-type MG53 or the reduction in severity of metabolic side effects caused by wild-type MG53 by at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or even 100% compared to the corresponding wild-type MG53. "Without having any impact on the cell membrane repair function and/or cardioprotective function of MG53" refers the complete retention of cell membrane repair function and/or cardioprotective function of MG53, or the reduction of cell membrane repair function and/or cardioprotective function of MG53 by up to 5%, up to 10%, up to 15%, up to 20%, up to 25%, up to 30%, etc.

[0105] In certain embodiments, the MG53 mutants provided herein comprises their analogs. The MG53 mutant analog refers to a polypeptide having a functional or structural characteristic that is substantially similar to all or part of the MG53 mutant provided herein, however, the amino acid sequence of the MG53 mutant analog differs from the amino acid sequence of the wild-type MG53 by at least one amino acid position. The MG53 mutant analog may be a partial fragment, derivative or variant of the MG53 mutant, and may comprise chemical or biological modifications. The MG53 mutant analog may have conservative substitutions, additions, deletions, insertions, truncations, modifications (e.g., phosphorylation, glycosylation, labeling, etc.) or any combination thereof on one or more amino acids of the MG53 mutant. The MG53 mutant analog may include naturally-occurring variants and artificially-produced variants of the MG53 mutant, such as artificial polypeptide sequences obtained by recombinant methods or chemical synthesis. The MG53 mutant analogs may comprise non-naturally occurring amino acid residues. A person skilled in the art will comprehend that the MG53 mutant analogs described herein still retain substantially similar functions as the MG53 mutants, for example,

the MG53 mutant analogs may avoid or reduce the metabolic side effects caused by wild-type MG53, such as insulin resistance, obesity, diabetes, hypertension, dyslipidemia, etc., while still retaining cell membrane repair function and/or cardioprotective function.

[0106] Conservative substitutions of amino acid residues refer to substitutions between amino acids with similar characteristics, such as substitutions between polar amino acids (e.g. substitutions between glutamine and asparagine), substitutions between hydrophobic amino acids (e.g. substitution among arginine, isoleucine, methionine and valine), and substitutions between amino acids with the same charge (e.g. substitutions among arginine, lysine and histidine, or between glutamine and aspartate), etc. In certain embodiments, the sequence of the MG53 mutant described herein has conservative substitution at only one or more non-serine positions compared to the sequence set forth in SEQ ID NOs: 7-12, or SEQ ID NOs: 147-150. In certain embodiments, the sequence of the MG53 mutant described herein has conservative substitution at 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 non-serine positions compared to the sequence set forth in SEQ ID NOs: 7-12, or SEQ ID NOs: 147-150.

[0107] The MG53 mutants described herein may also contain non-natural amino acids, on the premises that the activity is not affected. Non-natural amino acids include, for example, β -fluoroalanine, 1-methylhistidine, γ -methylene glutamate, α -methyl leucine, 4,5-dehydrolysine, hydroxyproline, 3-fluorophenylalanine, 3-aminotyrosine, 4-methyltryptophan, etc.

[0108] MG53 mutants of the present invention also can be modified using methods well known in the art. For example, but not limited to, PEGylation, glycosylation, amino-terminal modification, fatty acylation, carboxyl-terminal modification, phosphorylation, methylation, etc.

[0109] A person skilled in the art will comprehend that the MG53 mutants of the present invention still retain functions substantially similar to the MG53 mutants after being modified by methods well known in the art. For example, the modified MG53 mutants may avoid or reduce metabolic side effects caused by wild-type MG53, such as insulin resistance, obesity, diabetes, hypertension, dyslipidemia, etc., while still retaining cell membrane repair function and/or cardioprotective function.

[0110] In another aspect, the present invention relates to a pharmaceutical composition comprising the MG53 mutant described herein and a pharmaceutically acceptable carrier.

[0111] As used herein, the term "pharmaceutically acceptable carrier" refers to a pharmaceutically acceptable solvent, suspending agent, or any other pharmacologically inert vehicles used to deliver MG53 mutants to a subject without interfering the structure and properties of MG53 mutants. Some of such carriers may enable MG53 mutants to be formulated, for example, as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and troches for oral administration to the subject. Some of such carriers enable MG53 mutants to be formulated as injections, infusions or topical administration.

[0112] The pharmaceutically acceptable carriers for use in the pharmaceutical compositions of the present invention may include, but are not limited to, for example, pharmaceutically acceptable liquids, gels, or solid carriers, aqueous vehicles (e.g., sodium chloride injection, Ringer's injection, isotonic glucose injection, sterile water injection, or Ringer's injection of glucose and lactate), non-aqueous vehicles (e.g., fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil, or peanut oil), antimicrobial agents, isotonic agents (such as sodium chloride or dextrose), buffers (such as phosphate or citrate buffers), antioxidants (such as sodium bisulfate), anesthetics (such as procaine hydrochloride), suspending/dispersing agents (such as sodium carboxymethylcellulose, hydroxypropyl methylcellulose, or polyvinylpyrrolidone), chelating agents (such as EDTA (ethylenediamine tetraacetic acid) or EGTA (ethylene glycol tetraacetic acid)), emulsifying agents (such as Polysorbate 80 (TWEEN-80)), diluents, adjuvants, excipients, or non-toxic auxiliary substances, other components known in the art, or various combinations thereof. Suitable components may include, for example, fillers, binders, disintegrants, buffers, preservatives, lubricants, flavorings, thickeners, coloring agents, or emulsifiers.

[0113] In certain embodiments, the pharmaceutical composition is an oral formulation. The oral formulations include, but are not limited to, capsules, cachets, pills, tablets, troches (for taste substrates, usually sucrose and acacia or tragacanth), powders, granules, or aqueous or non-aqueous solutions or suspensions, or water-in-oil or oil-in-water emulsions, or elixirs or syrups, or confectionery lozenges (for inert bases, such as gelatin and glycerin, or sucrose or acacia) and / or mouthwash and its analogs.

[0114] In certain embodiments, the oral solid formulation (e.g., capsules, tablets, pills, dragees, powders, granules, etc.) includes the MG53 mutant and one or more pharmaceutically

acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or the followings: (1) fillers or extenders such as starch, lactose, sucrose, glucose, mannitol and/or silicic acid; (2) binders such as, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and/or acacia; (3) humectants such as glycerol; (4) cleaving agents such as agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) retarder solutions such as paraffin; (6) accelerating absorbers such as quaternary ammonium compounds; (7) lubricants such as acetyl alcohol and glycerol monostearate; (8) absorbents such as kaolin and bentonite; (9) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycol, sodium sulfate, and mixtures thereof; and (10) colorants.

[0115] In certain embodiments, the oral liquid formulation includes pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs, etc. In addition to the MG53 mutant, the liquid dosage forms may also contain conventional inert diluents such as water or other solvents, solubilizers and emulsifiers such as ethanol, isopropanol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzene (meth) acrylate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, olive, castor and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycol and fatty acid sorbitol esters, and mixtures thereof. Besides inert diluents, the oral compositions may also contain adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, flavoring and preserving agents.

[0116] In certain embodiments, the pharmaceutical composition may be an injectable formulation, including sterile aqueous solutions or dispersions, suspensions or emulsions. In all cases, the injectable formulation should be sterile and should be liquid to facilitate injections. It should be stable under the conditions of manufacture and storage, and should be resistant to the infection of microorganisms (such as bacteria and fungi). The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyols (e.g., glycerol, propylene glycol, and liquid polyethylene glycols, etc.) and suitable mixtures and / or vegetable oils thereof. The injectable formulation should maintain proper fluidity, which may be maintained in a variety of ways, for example, using a coating such as lecithin, using a surfactant, etc. Antimicrobial contamination can be achieved by the addition of various antibacterial and antifungal agents (e.g., parabens, chlorobutanol, phenol, sorbic acid, thimerosal, etc.).

[0117] In certain embodiments, the pharmaceutical composition is an oral spray formulation or nasal spray formulation. Such spray formulations include, but are not limited to, aqueous aerosols, non-aqueous suspensions, liposomal formulations, or solid particulate formulations, etc. Aqueous aerosols are formulated by combining an aqueous solution or suspension of the agent with a conventional pharmaceutically acceptable carrier and stabilizer. The carrier and stabilizer may vary according to the needs of specific compounds, but generally include nonionic surfactants (Tweens, or polyethylene glycol), oleic acid, lecithin, amino acids such as glycine, buffers, salts, sugar or sugar alcohol. Aerosols are usually prepared from isotonic solutions and can be delivered by nebulizers.

[0118] In certain embodiments, the pharmaceutical compositions may be used in combination with one or more other drugs. In certain embodiments, the composition comprises at least one other drug. In certain embodiments, the other drugs are cardiovascular drugs, drugs for treating kidney diseases, drugs for cell membrane repair, etc.

[0119] In certain embodiments, the pharmaceutical compositions may be delivered to the subject by suitable routes including, but not limited to, the oral route, injection route (e.g., intravenous injection, intramuscular injection, subcutaneous injection, intradermal injection, intracardiac injection, intrathecal injection, intrapleural injection, intraperitoneal injection, etc.), mucosal route (e.g., intranasal administration, oral administration, etc.), sublingual route, rectal route, transdermal route, intraocular route, pulmonary route. In certain embodiments, the pharmaceutical compositions can be administered by injection route.

[0120] In another aspect, the present invention relates to an isolated nucleic acid comprising a nucleic acid sequence encoding the amino acid sequence of the MG53 mutant described herein.

[0121] As used herein, the term "isolated" refers to a substance (such as a polypeptide or a nucleic acid) is separated from the environment in which it is normally present in nature or in an environment different from the environment in which it is normally found in nature.

[0122] As used herein, the term "nucleic acid" or "polynucleotide" refers to ribonucleic acid (RNA), deoxyribonucleic acid (DNA), or a mixture of ribonucleoside-deoxyribonucleic acids such as DNA-RNA hybrids. The nucleic acids or polynucleotides can be single- or double-stranded DNA, RNA, or DNA-RNA hybrids. Nucleic acids or polynucleotides may be linear or cyclic. As

used herein, the term "encoding" or "encoding for....." means capable of transcription into mRNA and/or translation into a peptide or protein. The term "encoding sequence" or "gene" refers to a polynucleotide sequence encoding an mRNA, peptide or protein. These two terms can be used interchangeably in the present application.

[0123] In certain embodiments, the isolated nucleic acid comprises any one of the nucleic acid sequences set forth in SEQ ID NOs: 13-18.

[0124] SEQ ID NO: 13 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 7, and its specific sequence is shown in Fig. 8.

[0125] SEQ ID NO: 14 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 8, and its specific sequence is shown in Fig. 9.

[0126] SEQ ID NO: 15 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 9, and its specific sequence is shown in Fig. 10.

[0127] SEQ ID NO: 16 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 10, and its specific sequence is shown in Fig. 11.

[0128] SEQ ID NO: 17 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 11, and its specific sequence is shown in Fig. 12.

[0129] SEQ ID NO: 18 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 12, and its specific sequence is shown in Fig. 13.

[0130] In certain embodiments, the isolated nucleic acid comprises any one of the nucleic acid sequences set forth in SEQ ID NOs: 151-154.

[0131] SEQ ID NO: 151 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 147, and its specific sequence is shown in Fig. 24.

[0132] SEQ ID NO: 152 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 148, and its specific sequence is shown in Fig. 25.

[0133] SEQ ID NO: 153 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 149, and its specific sequence is shown in Fig. 26.

[0134] SEQ ID NO: 154 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 150, and its specific sequence is shown in Fig. 27.

[0135] In certain embodiments, the isolated nucleic acids provided herein comprise the nucleic acid sequence that has at least 70% homology, e.g., at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% homology to any one of the nucleic acid sequences set forth in SEQ ID NOs: 13-18, SEQ ID NOs: 151-154, and can still encode one of the amino acid sequences set forth in SEQ ID NOs: 7-12, SEQ ID NOs: 147-150.

[0136] In certain embodiments, the present invention provides nucleic acid sequences encoding SEQ ID NOs: 7-12, SEQ ID NOs: 147-150, but they are different from any one of the nucleic acid sequences set forth in SEQ ID NOs: 13-18, SEQ ID NOs: 151-154 due to the degeneracy of the genetic code.

[0137] As used herein, the term "degeneracy of the genetic code" refers to a phenomenon that one amino acid has two or more corresponding genetic codons. For example, proline has 4 synonymous codons CCU, CCC, CCA, and CCG. It is well-known in the art that due to the degeneracy of genetic codes, it is possible to replace nucleic acids in certain positions in a given nucleic acid sequence without changing the encoded amino acid sequence. It is trivial for a person skilled in the art to conduct the replacement of degeneracy of the genetic code by, for example, the site-directed mutagenesis of bases. Different organisms have developed different preferences for different codons. In order to express the polypeptide of the present invention in a selected biological cell, the preferred codon of the biological cell can be selected to obtain the corresponding coding sequence, and the MG53 mutant sequence (e.g., SEQ ID NOs: 7-12, SEQ ID NOs: 147-150) of the present invention can be obtained by recombinant expression.

[0138] In another aspect, the present invention related to an expression vector comprising the encoding sequence of the amino acid sequence of the MG53 mutant described herein.

[0139] The expression vector in the present invention may be, for example, a DNA plasmid, a bacterial plasmid, a virus, etc. Non-limiting examples of expression vectors are described in, e.g. Paul et al., 2002, *Nature Biotechnology*, 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology*, 19, 497; Lee et al., 2002, *Nature Biotechnology*, 19, 500; and Novina et al. 2002, *Nature Medicine*, advance online publication doi: 10.1038 / nm725. The expression vector may

further contain a promoter operably linked to the encoding sequence of the amino acid sequence of the MG53 mutant, so that the promoter may initiate the expression of the encoding sequence after the expression vector enters the host cell. The expression vector can be introduced into the host cell by a suitable method such as, but not limited to, calcium phosphate transfection, lipofection transfection, electroporation transfection, bacterial heat shock, etc. For details, please refer to Sambrook et al. *Molecular Cloning* (a laboratory manual, Cold Spring Harbor, 1989). In certain embodiments, the expression vectors described herein comprise any one of the nucleic acid sequences set forth in SEQ ID NOs: 13-18.

[0140] In another aspect, the present invention relates to a host cell comprising the expression vector described herein.

[0141] The host cell described in the present invention can be a eukaryotic cell or a prokaryotic cell. Suitable eukaryotic cells may include, for example, mammalian cells such as Chinese hamster ovary cells (CHO). Suitable prokaryotic cells may include, for example, bacteria such as *E. coli*.

[0142] In another aspect, the present invention relates to a method for preparing the MG53 mutants, and the MG53 mutants provided herein can be prepared by techniques known in the art. For example, they can be prepared by chemical synthesis or genetic engineering.

[0143] Chemical synthesis methods mainly include solid-phase synthesis and liquid-phase synthesis. Solid-phase polypeptide synthesis methods include, for example, the Merrifield solid-phase synthesis method, which is described in detail in the literature "Merrifield, *J. Am. Chem. Soc.* 85: 2149-2154" and "M. Bodanszky et al., *Peptide Synthesis*, John Wiley & Sons, Second Edition, 1976 " and "J. Meienhofer, *Hormonal Proteins and Peptides*, Vol. 2, p. 46, Academic Press (New York), 1983". The entire contents of these documents are hereby incorporated into this application as references. The Merrifield solid-phase synthesis mainly includes the following steps: attaching the protected C-terminal amino acid of the peptide to the resin based on the amino acid sequences of the target protein. After attachment the resin is filtered, washed and the protecting group (e.g. t-butyloxycarbonyl) on the alpha amino group of the C-terminal amino acid is removed. The removal of this protecting group must take place, of course, without breaking the bond between that amino acid and the resin. The resulting resin peptide is then coupled to the penultimate C-terminal protected amino acid. This coupling

coupling takes place by the formation of an amide bond between the free carboxy group of the second amino acid and the amino group of the first amino acid attached to the resin. This sequence of events is repeated with successive amino acids until all amino acids of the peptide are attached to the resin. Finally, the protected peptide is cleaved from the resin and the protecting groups removed to obtain the desired peptide. The polypeptides disclosed herein can also be prepared by liquid-phase synthesis, for example, by the standard solution peptide synthesis, which has been described in "E. Schroder and K. Kubke, *The Peptides*, Vol. 1, Academic Press (New York), 1965" in detail, which is incorporated herein in its entirety by reference. Liquid-phase synthesis mainly includes coupling amino acids or peptide fragments step by step by chemical or enzymic methods that form amide bonds.

[0144] The genetic engineering method is a method of expressing a nucleic acid sequence encoding the corresponding MG53 mutant in a proper host cell to generate the corresponding mutant. For a detailed description of this method, see Sambrook et al. *Molecular Cloning* (a laboratory manual, Cold Spring Harbor, 1989). In certain embodiments, the method for preparing the MG53 mutant provided herein comprises determining one or more serine positions for mutation, performing site-directed mutagenesis at said position on the full-length sequence of a plasmid comprising a nucleic acid sequence encoding the amino acid sequence of a wild-type MG53, transfecting the plasmid with site-directed mutagenesis into a host cell, and inducing the host cell to produce the MG53 mutant.

[0145] As used herein, the term "site-directed mutagenesis" refers to the introduction of an interested change into a target DNA fragment, including additions, deletions, and substitutions of bases, etc. In certain embodiments, the target DNA fragment is the encoding sequence of a wild-type MG53, i.e. SEQ ID NOs: 19-24 and SEQ ID NOs: 143-146. The mutated position is located at one or more serine positions in the encoding sequence of the coiled-coil-SPRY region of the wild-type MG53.

[0146] SEQ ID NO: 19 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 1, and its specific sequence is shown in Fig. 2.

[0147] SEQ ID NO: 20 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 2, and its specific sequence is shown in Fig. 3.

[0148] SEQ ID NO: 21 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 3, and its specific sequence is shown in Fig. 4.

[0149] SEQ ID NO: 22 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 4, and its specific sequence is shown in Fig. 5.

[0150] SEQ ID NO: 23 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 5, and its specific sequence is shown in Fig. 6.

[0151] SEQ ID NO: 24 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 6, and its specific sequence is shown in Fig. 7.

[0152] SEQ ID NO: 143 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 139, and its specific sequence is shown in Fig. 20.

[0153] SEQ ID NO: 144 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 140, and its specific sequence is shown in Fig. 21.

[0154] SEQ ID NO: 145 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 141, and its specific sequence is shown in Fig. 22.

[0155] SEQ ID NO: 146 is a nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO: 142, and its specific sequence is shown in Fig. 23.

[0156] In certain embodiments, the site-directed mutagenesis comprises the following steps:

[0157] (1) determining the corresponding nucleotide site of the amino acid targeted for site-directed mutagenesis in the cDNA sequence, modifying the nucleotide sequence at the mutation site according to the target amino acid, and designing primers by intercepting a sequence of 20-40 bp in length comprising the mutation site;

[0158] (2) performing PCR reaction by using the primers of step (1) and taking a wild type MG53 plasmid as a template, performing agarose gel electrophoresis for the PCR product, and purifying the PCR product;

[0159] (3) performing enzymatic reaction for the purified PCR product of step (2) by using restriction endonuclease, ligating the enzyme-digested product with a suitable plasmid expression vector, transforming and cultivating the ligation product in bacterial competent cells.

[0160] In certain embodiments, the site-directed mutagenesis further comprises the following step:

[0161] (4) selecting the clones of step (3) to perform colony PCR identification by using the primers of step (1), performing agarose gel electrophoresis for the PCR product, and then performing DNA sequencing identification to identify positive clones with the site-directed mutagenesis.

[0162] Site-directed mutagenesis of a wild-type MG53 can be carried out using a variety of commercially available site-directed mutagenesis kits, for example, with reference to the instructions for the Easy Mutagenesis System Kit from Beijing Transgen Biotech, which details the method and procedures for site-directed mutagenesis.

[0163] In another aspect, the present invention relates to use of the MG53 mutant in the manufacture of a medicament for treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage. In certain embodiments, the medicament may avoid or reduce metabolic side effects, such as insulin resistance, obesity, diabetes, hypertension, dyslipidemia, while treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage. In certain embodiments, the heart diseases are diseases associated with myocardial damage, including but not limited to, diabetic heart disease, myocardial ischemia, cardiac ischemia/reperfusion injury, myocardial infarction, heart failure, arrhythmia, heart rupture, angina, myocarditis, coronary heart disease, and pericarditis. In certain embodiments, the diabetic cerebrovascular diseases include, but are not limited to, cerebral arteriosclerosis, ischemic cerebrovascular disease, cerebral hemorrhage, cerebral atrophy, and cerebral infarction. In certain embodiments, the diabetic ocular complications include, but are not limited to, diabetic retinopathy, diabetic cataract, diabetic associated uveitis, and blindness. In certain embodiments, the diabetic neuropathy includes, but is not limited to, diabetic peripheral neuropathy. In certain embodiments, the kidney diseases include, but are

not limited to, acute glomerulonephritis, chronic glomerulonephritis, nephrotic syndrome, acute kidney injury, diabetic nephropathy, etc. In certain embodiments, the diseases associated with cellular and/or tissue damage include, but are not limited to, diseases associated with the cellular and/or tissue damage of kidney, brain, lung, liver, heart, spleen, digestive tract, and skin, such as brain injury, lung injury, spleen injury, splenic rupture, gastric ulcer, gastritis, gastric perforation, gastrointestinal mucosal injury, trauma, burns, ulcers, mucositis, asthma, chronic obstructive pulmonary disease (COPD), stroke, skin aging, etc. In certain embodiments, the present invention relates to use of a polypeptide set forth in SEQ ID NO: 7 in the manufacture of a medicament for treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage. In certain embodiments, the present invention relates to use of a polypeptide set forth in SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149 or SEQ ID NO: 150 in the manufacture of a medicament for treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage.

[0164] In yet another aspect, the present invention relates to a method of treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage, comprising administering to a subject in need thereof a therapeutically effective amount of the MG53 mutant. In certain embodiments, the MG53 mutant may avoid or reduce metabolic side effects, such as insulin resistance, obesity, diabetes, hypertension, and dyslipidemia, while treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage. In certain embodiments, the heart diseases are diseases associated with myocardial damage, including but not limited to, diabetic heart disease, myocardial ischemia, cardiac ischemia/reperfusion injury, myocardial infarction, heart failure, arrhythmia, heart rupture, angina, myocarditis, coronary heart disease, and pericarditis. In certain embodiments, the diabetic cerebrovascular diseases include, but are not limited to, cerebral arteriosclerosis, ischemic cerebrovascular disease, cerebral hemorrhage, cerebral atrophy, and cerebral infarction. In certain embodiments, the diabetic ocular complications include, but are not limited to, diabetic retinopathy, diabetic cataract, diabetic associated uveitis, and blindness. In

certain embodiments, the diabetic neuropathy includes, but is not limited to, diabetic peripheral neuropathy. In certain embodiments, the kidney diseases include, but are not limited to, acute glomerulonephritis, chronic glomerulonephritis, nephrotic syndrome, acute kidney injury, diabetic nephropathy, etc. In certain embodiments, the diseases associated with cellular and/or tissue damage include, but are not limited to, diseases associated with the cellular and/or tissue damage of kidney, brain, lung, liver, heart, spleen, digestive tract, and skin, such as brain injury, lung injury, spleen injury, splenic rupture, gastric ulcer, gastritis, gastric perforation, gastrointestinal mucosal injury, trauma, burns, ulcers, mucositis, asthma, chronic obstructive pulmonary disease (COPD), stroke, skin aging, etc. In certain embodiments, the present invention relates to a method of treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage, comprising administering to a subject in need thereof a therapeutically effective amount of the polypeptide set forth in SEQ ID NO: 7. In certain embodiments, the present invention relates to a method of treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage, comprising administering to a subject in need thereof a therapeutically effective amount of a polypeptide set forth in SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, or SEQ ID NO: 150.

[0165] As used herein, the term "therapeutically effective amount" refers to the amount of a medicament which achieves a therapeutic effect by inhibiting or alleviating a disease and disorder of a subject, or by prophylactically inhibiting or preventing the onset of a disease or disorder. A therapeutically effective amount may be the amount of the medicament which relieves to some extent one or more symptoms of a disease or disorder in a subject; returns to normal either partially or completely one or more physiological or biochemical parameters associated with or causative of the disease or disorder; and/or reduces the likelihood of the onset of the disease or disorder.

[0166] In yet other aspect, the present invention relates to an MG53 mutant for use in treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage. In certain embodiments, the MG53 mutant may avoid or reduce metabolic side effects, such as insulin resistance, obesity, diabetes, hypertension, and dyslipidemia, while

treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage. In certain embodiments, the heart diseases are diseases associated with myocardial damage, including but not limited to, diabetic heart disease, myocardial ischemia, cardiac ischemia/reperfusion injury, myocardial infarction, heart failure, arrhythmia, heart rupture, angina, myocarditis, coronary heart disease, and pericarditis. In certain embodiments, the diabetic cerebrovascular diseases include, but are not limited to, cerebral arteriosclerosis, ischemic cerebrovascular disease, cerebral hemorrhage, cerebral atrophy, and cerebral infarction. In certain embodiments, the diabetic ocular complications include, but are not limited to, diabetic retinopathy, diabetic cataract, diabetic associated uveitis, and blindness. In certain embodiments, the diabetic neuropathy includes, but is not limited to, diabetic peripheral neuropathy. In certain embodiments, the kidney diseases include, but are not limited to, acute glomerulonephritis, chronic glomerulonephritis, nephrotic syndrome, acute kidney injury, diabetic nephropathy, etc. In certain embodiments, the diseases associated with cellular and/or tissue damage include, but are not limited to, diseases associated with the cellular and/or tissue damage of kidney, brain, lung, liver, heart, spleen, digestive tract, and skin, such as brain injury, lung injury, spleen injury, splenic rupture, gastric ulcer, gastritis, gastric perforation, gastrointestinal mucosal injury, trauma, burns, ulcers, mucositis, asthma, chronic obstructive pulmonary disease (COPD), stroke, skin aging, etc. In certain embodiments, the present invention relates to a polypeptide set forth in SEQ ID NO: 7 for use in treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage. In certain embodiments, the present invention relates to a polypeptide set forth in SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, or SEQ ID NO: 150 for use in treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage.

EMBODIMENTS

[0167] The biological materials used in all examples such as *E. Coli* strains, various clones and expression plasmids, media, enzymes, buffer solutions, and various culturing methods, protein extraction and purification methods, and the other molecular biological operation methods, are all well-known to persons skilled in the art. For more details, please refer to the “Molecular

Cloning: A Laboratory Manual” edited by Sambrook, et al. (Cold Spring Harbor, 1989) and “Short Protocols in Molecular Biology” (Frederick M. Ausubel, et al., translated by Yan Ziyang et al., Science Press (Beijing), 1998).

[0168] Example 1: Preparation of MG53 Mutants

[0169] 1. Preparation of human MG53 mutants

[0170] Human MG53 S255A Mutant

[0171] (1) Primer design: determining the corresponding nucleotide site of the amino acid targeted for site-directed mutagenesis in the cDNA sequence, modifying the nucleotide sequence at the mutation site according to the mutated amino acid, and designing primers by intercepting a sequence of 20 bp upstream and 10 bp downstream the mutation site. The sequences of the primers are as follows:

Forward primer of human MG53 S255A: tgcagaagatcctggcagaggctccccacccg (SEQ ID NO: 25)

Reverse primer of human MG53 S255A: tccagacgggcgggtgggggagcctctgccagg (SEQ ID NO: 26)

[0172] (2) Polymerase chain reaction (PCR) of plasmid full-length clone: performing PCR reaction by using the designed primers for site-directed mutagenesis, using *Taq* Polymerase High Fidelity, and taking the original gene expression plasmid as a template. The reaction system and conditions are as follows:

template	x μ l	95°C	5min	} 30 cycles
primer-F	0.5 μ l	95°C	5min	
primer-R	0.5 μ l	55°C	30sec	
2 × Taq Mix	10 μ l	72°C	Ymin	
ddH ₂ O	9-x μ l	72°C	10min	
total	20 μ l	4°C	∞	

[0173] Agarose gel electrophoresis of the PCR product: performing agarose gel electrophoresis for the PCR product to identify its purity and quantity.

[0174] Ethanol precipitation of the PCR product: purifying the PCR product with correct size and a single band by the method of ethanol precipitation.

[0175] Dpn I digestion: performing enzymatic reaction for the purified PCR product by using restriction endonuclease Dpn I.

[0176] Transformation of enzyme-digested product: transforming the Dpn I-digested product in bacterial competent cells, coating onto an appropriate screen plate for cultivation.

[0177] Identification of positive clones: selecting the clones with proper size to perform colony PCR identification, performing agarose gel electrophoresis for the PCR product, performing small-volume shaking amplification cultivation for the clone with a clear band and correct size, and then performing sequencing identification.

[0178] The same method was used to prepare human MG53 S255G, S255L, S255V, S255P, S255F, S255W, S255Q, S255C, S255Y, S255D, S255R, S211A, S214A, S246A, S269A, S296A, S297A mutants. Except for the primers, all of the other steps are the same as the steps for preparing human MG53 S255A mutant. The primers of each mutant are as follows:

[0179] (i) the forward and reverse primers of human MG53 S255G, which represents an MG53 mutant in which serine at position 255 of human wild-type MG53 (i.e. SEQ ID NO: 1) is mutated into glycine.

Forward primer of human MG53 S255G: tgcagaagatcctggcagagggtccccaccgc (SEQ ID NO: 27)

Reverse primer of human MG53 S255G: tccagacgggcgggtgggggaccctctgccagg (SEQ ID NO: 28)

[0180] (ii) the forward and reverse primers of human MG53 S255L, which represents an MG53 mutant in which serine at position 255 of human wild-type MG53 (i.e. SEQ ID NO: 1) is mutated into leucine.

Forward primer of human MG53 S255L: tgcagaagatcctggcagagcttccccaccgc (SEQ ID NO: 29)

Reverse primer of human MG53 S255L: tccagacgggcgggtgggggaagctctgccagg (SEQ ID NO: 30)

[0181] (iii) the forward and reverse primers of human MG53 S255V, which represents an MG53 mutant in which serine at position 255 of human wild-type MG53 (i.e. SEQ ID NO: 1) is mutated into valine.

Forward primer of human MG53 S255V: tgcagaagatcctggcagaggttccccaccgc (SEQ ID NO: 31)

Reverse primer of human MG53 S255V: tccagacgggcgggtgggggaacctctgccagg (SEQ ID NO: 32)

[0182] (iv) the forward and reverse primers of human MG53 S255P, which represents an MG53 mutant in which serine at position 255 of human wild-type MG53 (i.e. SEQ ID NO: 1) is mutated into proline.

Forward primer of human MG53 S255P: tgcagaagatcctggcagagcctccccaccg (SEQ ID NO: 33)

Reverse primer of human MG53 S255P: tccagacgggcgggtgggggaggctctgccagg (SEQ ID NO: 34)

[0183] (v) the forward and reverse primers of human MG53 S255F, which represents an MG53 mutant in which serine at position 255 of human wild-type MG53 (i.e. SEQ ID NO: 1) is mutated into phenylalanine.

Forward primer of human MG53 S255F: tgcagaagatcctggcagagtttccccaccg (SEQ ID NO: 35)

Reverse primer of human MG53 S255F: tccagacgggcgggtgggggaaactctgccagg (SEQ ID NO: 36)

[0184] (vi) the forward and reverse primers of human MG53 S255W, which represents an MG53 mutant in which serine at position 255 of human wild-type MG53 (i.e. SEQ ID NO: 1) is mutated into tryptophan.

Forward primer of human MG53 S255W: tgcagaagatcctggcagagtggccccaccg (SEQ ID NO: 37)

Reverse primer of human MG53 S255W: tccagacgggcgggtgggggccactctgccagg (SEQ ID NO: 38)

[0185] (vii) the forward and reverse primers of human MG53 S255Q, which represents an MG53 mutant in which serine at position 255 of human wild-type MG53 (i.e. SEQ ID NO: 1) is mutated into glutamine.

Forward primer of human MG53 S255Q: tgcagaagatcctggcagagcaacccccaccg (SEQ ID NO: 39)

Reverse primer of human MG53 S255Q: tccagacgggcgggtgggggttgctctgccagg (SEQ ID NO: 40)

[0186] (viii) the forward and reverse primers of human MG53 S255C, which represents an MG53 mutant in which serine at position 255 of human wild-type MG53 (i.e. SEQ ID NO: 1) is mutated into cysteine.

Forward primer of human MG53 S255C: tgcagaagatcctggcagagtggtccccaccg (SEQ ID NO: 41)

Reverse primer of human MG53 S255C: tccagacgggcgggtgggggacactctgccagg (SEQ ID NO: 42)

[0187] (ix) the forward and reverse primers of human MG53 S255Y, which represents an MG53 mutant in which serine at position 255 of human wild-type MG53 (i.e. SEQ ID NO: 1) is mutated into tyrosine.

Forward primer of human MG53 S255Y: tgcagaagatcctggcagagtatccccaccg (SEQ ID NO: 43)

Reverse primer of human MG53 S255Y: tccagacgggcgggtgggggatactctgccagg (SEQ ID NO: 44)

[0188] (x) the forward and reverse primers of human MG53 S255D, which represents an MG53 mutant in which serine at position 255 of human wild-type MG53 (i.e. SEQ ID NO: 1) is mutated into aspartic acid.

Forward primer of human MG53 S255D: tgcagaagatcctggcagaggatccccaccg (SEQ ID NO: 45)

Reverse primer of human MG53 S255D: tccagacgggcgggtgggggctactctgccagg (SEQ ID NO: 46)

[0189] (xi) the forward and reverse primers of human MG53 S255R, which represents an MG53 mutant in which serine at position 255 of human wild-type MG53 (i.e. SEQ ID NO: 1) is mutated into arginine.

Forward primer of human MG53 S255R: tgcagaagatcctggcagagcgtccccaccg (SEQ ID NO: 47)

Reverse primer of human MG53 S255R: tccagacgggcgggtgggggacgctctgccagg (SEQ ID NO: 48)

[0190] (xii) the forward and reverse primers of human MG53 S255A, which represents an MG53 mutant in which serine at position 255 of human wild-type MG53 (i.e. SEQ ID NO: 1) is mutated into alanine.

Forward primer of human MG53 S211A: ccttgccgggagctggggccctgaactctt (SEQ ID NO: 49)

Reverse primer of human MG53 S211A: gctgctccaggaagagttcaggccccagctcc (SEQ ID NO: 50)

[0191] (xiii) the forward and reverse primers of human MG53 S214A, which represents an MG53 mutant in which serine at position 214 of human wild-type MG53 (i.e. SEQ ID NO: 1) is mutated into alanine.

Forward primer of human MG53 S214A: gggagctggggagcctgaacgcttacctggagc (SEQ ID NO: 51)

Reverse primer of human MG53 S214A: tgccgcagctgctccaggaagcgttcaggctc (SEQ ID NO: 52)

[0192] (xiv) the forward and reverse primers of human MG53 S246A, which represents an MG53 mutant in which serine at position 246 of human wild-type MG53 (i.e. SEQ ID NO: 1) is mutated into alanine.

Forward primer of human MG53 S246A: tgaaatactgcctggtgaccgccaggctgcaga (SEQ ID NO: 53)

Reverse primer of human MG53 S246A: gccaggatcttctgcagcctggcggtcaccagg (SEQ ID NO: 54)

[0193] (xv) the forward and reverse primers of human MG53 S269A, which represents an MG53 mutant in which serine at position 269 of human wild-type MG53 (i.e. SEQ ID NO: 1) is mutated into alanine.

Forward primer of human MG53 S269A: aggagctgacctttgacccggcctctgcgcacc (SEQ ID NO: 55)

Reverse primer of human MG53 S269A: accaggctcggtgctgcagaggccgggtcaaag (SEQ ID NO: 56)

[0194] (xvi) the forward and reverse primers of human MG53 S297A, which represents an MG53 mutant in which serine at position 297 of human wild-type MG53 (i.e. SEQ ID NO: 1) is mutated into alanine.

Forward primer of human MG53 S297A: agctgacctttgacccgagcgtgcgcacccga (SEQ ID NO: 57)

Reverse primer of human MG53 S297A: accaccaggctcggtgctgcagcgtcggtca (SEQ ID NO: 58)

[0195] 2. Preparation of mouse MG53 mutants

[0196] Mouse MG53 S255A, S255G, S255L, S255W, S255Q, S255Y, S255D, and S255R mutants were prepared according to the method for preparing human MG53 S255A mutant. Except for the primers, all of the other steps are the same as the steps for preparing human MG53 S255A mutant. The primers of each mutant are as follows:

[0197] (i) the forward and reverse primers of mouse MG53 S255A, which represents an MG53 mutant in which serine at position 255 of mouse wild-type MG53 (i.e. SEQ ID NO: 2) is mutated into alanine.

Forward primer of mouse MG53 S255A: tgcagaagatcctgtcagaggcaccaccaccgg (SEQ ID NO: 59)

Reverse primer of mouse MG53 S255A: tctagccttgccggtggtggtgcctctgacagg (SEQ ID NO: 60)

[0198] (ii) the forward and reverse primers of mouse MG53 S255G, which represents an MG53 mutant in which serine at position 255 of mouse wild-type MG53 (i.e. SEQ ID NO: 2) is mutated into glycine.

Forward primer of mouse MG53 S255G: tgcagaagatcctgtcagaggaccaccaccgg (SEQ ID NO: 61)

Reverse primer of mouse MG53 S255G: tctagccttgccggtggtggtccctctgacagg (SEQ ID NO: 62)

[0199] (iii) the forward and reverse primers of mouse MG53 S255L, which represents an MG53 mutant in which serine at position 255 of mouse wild-type MG53 (i.e. SEQ ID NO: 2) is mutated into leucine.

Forward primer of mouse MG53 S255L: tgcagaagatcctgtcagagttaccaccaccgg (SEQ ID NO: 63)

Reverse primer of mouse MG53 S255L: tctagccttgccggtggtggttaactctgacagg (SEQ ID NO: 64)

[0200] (iv) the forward and reverse primers of mouse MG53 S255W, which represents an MG53 mutant in which serine at position 255 of mouse wild-type MG53 (i.e. SEQ ID NO: 2) is mutated into tryptophan.

Forward primer of mouse MG53 S255W: tgcagaagatcctgtcagagtggccaccaccgg (SEQ ID NO: 65)

Reverse primer of mouse MG53 S255W: tctagccttgccggtggtggccactctgacagg (SEQ ID NO: 66)

[0201] (v) the forward and reverse primers of mouse MG53 S255Q, which represents an MG53 mutant in which serine at position 255 of mouse wild-type MG53 (i.e. SEQ ID NO: 2) is mutated into glutamine.

Forward primer of mouse MG53 S255Q: tgcagaagatcctgtcagagcaaccaccaccgg (SEQ ID NO: 67)

Reverse primer of mouse MG53 S255Q: tctagccttgccggtggtggtgctctgacagg (SEQ ID NO: 68)

[0202] (vi) the forward and reverse primers of mouse MG53 S255Y, which represents an MG53 mutant in which serine at position 255 of mouse wild-type MG53 (i.e. SEQ ID NO: 2) is mutated into tyrosine.

Forward primer of mouse MG53 S255Y: tgcagaagatcctgtcagagtatccaccaccgg (SEQ ID NO: 69)

Reverse primer of mouse MG53 S255Y: tctagccttgccggtggtggatactctgacagg (SEQ ID NO: 70)

[0203] (vii) the forward and reverse primers of mouse MG53 S255D, which represents an MG53 mutant in which serine at position 255 of mouse wild-type MG53 (i.e. SEQ ID NO: 2) is mutated into aspartic acid.

Forward primer of mouse MG53 S255D: tgcagaagatcctgtcagaggatccaccaccgg (SEQ ID NO: 71)

Reverse primer of mouse MG53 S255D: tctagccttgccggtggtggatcctctgacagg (SEQ ID NO: 72)

[0204] (viii) the forward and reverse primers of mouse MG53 S255R, which represents an MG53 mutant in which serine at position 255 of mouse wild-type MG53 (i.e. SEQ ID NO: 2) is mutated into arginine.

Forward primer of mouse MG53 S255R: tgcagaagatcctgtcagagcgaccaccaccgg (SEQ ID NO: 73)

Reverse primer of mouse MG53 S255R: tctagccttgccggtggtggtcgctctgacagg (SEQ ID NO: 74)

[0205] 3. Preparation of rat MG53 mutants

[0206] Rat MG53 S255A, S255G, S255L, S255W, S255Q, S255Y, S255D, and S255R mutants were prepared according to the method for preparing human MG53 S255A mutant. Except for the primers, all of the other steps are the same as the steps for preparing human MG53 S255A mutant. The primers of each mutant are as follows:

[0207] (i) the forward and reverse primers of rat MG53 S255A, which represents an MG53 mutant in which serine at position 255 of rat wild-type MG53 (i.e. SEQ ID NO: 3) is mutated into alanine.

Forward primer of rat MG53 S255A: tgcagaagattctgtcagaggcaccacccccag (SEQ ID NO: 75)

Reverse primer of rat MG53 S255A: tctagccttgctgggggtggcctctgacaga (SEQ ID NO: 76)

[0208] (ii) the forward and reverse primers of rat MG53 S255G, which represents an MG53 mutant in which serine at position 255 of rat wild-type MG53 (i.e. SEQ ID NO: 3) is mutated into glycine.

Forward primer of rat MG53 S255G: tgcagaagattctgtcagaggaccacccccag (SEQ ID NO: 77)

Reverse primer of rat MG53 S255G: tctagccttgctgggggtggcctctgacaga (SEQ ID NO: 78)

[0209] (iii) the forward and reverse primers of rat MG53 S255L, which represents an MG53 mutant in which serine at position 255 of rat wild-type MG53 (i.e. SEQ ID NO: 3) is mutated into leucine.

Forward primer of rat MG53 S255L: tgcagaagattctgtcagagttaccacccccag (SEQ ID NO: 79)

Reverse primer of rat MG53 S255L: tctagccttgctgggggtggtaactctgacaga (SEQ ID NO: 80)

[0210] (iv) the forward and reverse primers of rat MG53 S255W, which represents an MG53 mutant in which serine at position 255 of rat wild-type MG53 (i.e. SEQ ID NO: 3) is mutated into tryptophan.

Forward primer of rat MG53 S255W: tgcagaagattctgtcagagtggccacccccag (SEQ ID NO: 81)

Reverse primer of rat MG53 S255W: tctagccttgctgggggtggccactctgacaga (SEQ ID NO: 82)

[0211] (v) the forward and reverse primers of rat MG53 S255Q, which represents an MG53 mutant in which serine at position 255 of rat wild-type MG53 (i.e. SEQ ID NO: 3) is mutated into glutamine.

Forward primer of rat MG53 S255Q: tgcagaagattctgtcagagcaaccacccccag (SEQ ID NO: 83)

Reverse primer of rat MG53 S255Q: tctagccttgctgggggtggttgctctgacaga (SEQ ID NO: 84)

[0212] (vi) the forward and reverse primers of rat MG53 S255Y, which represents an MG53 mutant in which serine at position 255 of rat wild-type MG53 (i.e. SEQ ID NO: 3) is mutated into tyrosine.

Forward primer of rat MG53 S255Y: tgcagaagattctgtcagagtatccacccccag (SEQ ID NO: 85)

Reverse primer of rat MG53 S255Y: tctagccttgctgggggtggatactctgacaga (SEQ ID NO: 86)

[0213] (vii) the forward and reverse primers of rat MG53 S255D, which represents an MG53 mutant in which serine at position 255 of rat wild-type MG53 (i.e. SEQ ID NO: 3) is mutated into aspartic acid.

Forward primer of rat MG53 S255D: tgcagaagattctgtcagaggatccacccccag (SEQ ID NO: 87)

Reverse primer of rat MG53 S255D: tctagccttgctgggggtggatcctctgacaga (SEQ ID NO: 88)

[0214] (viii) the forward and reverse primers of rat MG53 S255R, which represents an MG53 mutant in which serine at position 255 of rat wild-type MG53 (i.e. SEQ ID NO: 3) is mutated into arginine.

Forward primer of rat MG53 S255R: tgcagaagattctgtcagagcgaccacccccag (SEQ ID NO: 89)

Reverse primer of rat MG53 S255R: tctagccttgctgggggtggcgcctctgacaga (SEQ ID NO: 90)

[0215] 4. Preparation of monkey MG53 mutants

[0216] Monkey MG53 S255A, S255G, S255L, S255W, S255Q, S255Y, S255D, and S255R mutants were prepared according to the method for preparing human MG53 S255A mutant. Except for the primers, all of the other steps are the same as the steps for preparing human MG53 S255A mutant. The primers of each mutant are as follows:

[0217] (i) the forward and reverse primers of monkey MG53 S255A, which represents an MG53 mutant in which serine at position 255 of monkey wild-type MG53 (i.e. SEQ ID NO: 4) is mutated into alanine.

Forward primer of monkey MG53 S255A: aagatcctggcagaggctccccacccgccgtctg (SEQ ID NO: 91)

Reverse primer of monkey MG53 S255A: cagacgggcgggtgggggagcctctgccaggatctt (SEQ ID NO: 92)

[0218] (ii) the forward and reverse primers of monkey MG53 S255G, which represents an MG53 mutant in which serine at position 255 of monkey wild-type MG53 (i.e. SEQ ID NO: 4) is mutated into glycine.

Forward primer of monkey MG53 S255G: aagatcctggcagaggggtcccccacccgccgtctgg (SEQ ID NO: 93)

Reverse primer of monkey MG53 S255G: ccagacgggcggtgggggaccctctgccaggatctt (SEQ ID NO: 94)

[0219] (iii) the forward and reverse primers of monkey MG53 S255L, which represents an MG53 mutant in which serine at position 255 of monkey wild-type MG53 (i.e. SEQ ID NO: 4) is mutated into leucine.

Forward primer of monkey MG53 S255L: agatcctggcagagttacccccacccgccgtctgga (SEQ ID NO: 95)

Reverse primer of monkey MG53 S255L: tccagacgggcggtgggggtaactctgccaggatct (SEQ ID NO: 96)

[0220] (iv) the forward and reverse primers of monkey MG53 S255W, which represents an MG53 mutant in which serine at position 255 of monkey wild-type MG53 (i.e. SEQ ID NO: 4) is mutated into tryptophan.

Forward primer of monkey MG53 S255W: agatcctggcagagtggccccacccgccgtctgga (SEQ ID NO: 97)

Reverse primer of monkey MG53 S255W: tccagacgggcggtgggggccactctgccaggatct (SEQ ID NO: 98)

[0221] (v) the forward and reverse primers of monkey MG53 S255Q, which represents an MG53 mutant in which serine at position 255 of monkey wild-type MG53 (i.e. SEQ ID NO: 4) is mutated into glutamine.

Forward primer of monkey MG53 S255Q: aagatcctggcagagcaacccccacccgccgtctgga (SEQ ID NO: 99)

Reverse primer of monkey MG53 S255Q: tccagacgggcggtgggggtgctctgccaggatctt (SEQ ID NO: 100)

[0222] (vi) the forward and reverse primers of monkey MG53 S255Y, which represents an MG53 mutant in which serine at position 255 of monkey wild-type MG53 (i.e. SEQ ID NO: 4) is mutated into tyrosine.

Forward primer of monkey MG53 S255Y: agatcctggcagagtatccccacccgccgtctgg (SEQ ID NO: 101)

Reverse primer of monkey MG53 S255Y: ccagacgggcgggtgggggatactctgccaggatct (SEQ ID NO: 102)

[0223] (vii) the forward and reverse primers of monkey MG53 S255D, which represents an MG53 mutant in which serine at position 255 of monkey wild-type MG53 (i.e. SEQ ID NO: 4) is mutated into aspartic acid.

Forward primer of monkey MG53 S255D: aagatcctggcagaggatccccacccgccgtctgg (SEQ ID NO: 103)

Reverse primer of monkey MG53 S255D: ccagacgggcgggtgggggacacctctgccaggatctt (SEQ ID NO: 104)

[0224] (viii) the forward and reverse primers of monkey MG53 S255R, which represents an MG53 mutant in which serine at position 255 of monkey wild-type MG53 (i.e. SEQ ID NO: 4) is mutated into arginine.

Forward primer of monkey MG53 S255R: aagatcctggcagagcgtccccacccgccgtctgg (SEQ ID NO: 105)

Reverse primer of monkey MG53 S255R: ccagacgggcgggtgggggacacctctgccaggatctt (SEQ ID NO: 106)

[0225] 5. Preparation of swine MG53 mutants

[0226] Swine MG53 S255A, S255G, S255L, S255W, S255Q, S255Y, S255D, and S255R mutants were prepared according to the method for preparing human MG53 S255A mutant. Except for the primers, all of the other steps are the same as the steps for preparing human MG53 S255A mutant. The primers of each mutant are as follows:

[0227] (i) the forward and reverse primers of swine MG53 S255A, which represents an MG53 mutant in which serine at position 255 of swine wild-type MG53 (i.e. SEQ ID NO: 5) is mutated into alanine.

Forward primer of swine MG53 S255A: aagatcctggcagaggcggggcacctgcccgcctg (SEQ ID NO: 107)

Reverse primer of swine MG53 S255A: caggcgggcaggtgggggcgcctctgccaggatctt (SEQ ID NO: 108)

[0228] (ii) the forward and reverse primers of swine MG53 S255G, which represents an MG53 mutant in which serine at position 255 of swine wild-type MG53 (i.e. SEQ ID NO: 5) is mutated into glycine.

Forward primer of swine MG53 S255G: aagatcctggcagagggggcccccacctgcccgcctgg (SEQ ID NO: 109)

Reverse primer of swine MG53 S255G: ccaggcgggcaggtgggggcccctctgccaggatctt (SEQ ID NO: 110)

[0229] (iii) the forward and reverse primers of swine MG53 S255L, which represents an MG53 mutant in which serine at position 255 of swine wild-type MG53 (i.e. SEQ ID NO: 5) is mutated into leucine.

Forward primer of swine MG53 S255L: agatcctggcagagttggggcccccacctgcccgcctgg (SEQ ID NO: 111)

Reverse primer of swine MG53 S255L: ccaggcgggcaggtgggggcaactctgccaggatct (SEQ ID NO: 112)

[0230] (iv) the forward and reverse primers of swine MG53 S255W, which represents an MG53 mutant in which serine at position 255 of swine wild-type MG53 (i.e. SEQ ID NO: 5) is mutated into tryptophan.

Forward primer of swine MG53 S255W: agatcctggcagagtggggcccccacctgcccgcctgg (SEQ ID NO: 113)

Reverse primer of swine MG53 S255W: ccaggcgggcaggtgggggcccactctgccaggatct (SEQ ID NO: 114)

[0231] (v) the forward and reverse primers of swine MG53 S255Q, which represents an MG53 mutant in which serine at position 255 of swine wild-type MG53 (i.e. SEQ ID NO: 5) is mutated into glutamine.

Forward primer of swine MG53 S255Q: aagatcctggcagagcagcccccacctgcccgcctgg (SEQ ID NO: 115)

Reverse primer of swine MG53 S255Q: ccaggcgggcaggtgggggctgctctgccaggatctt (SEQ ID NO: 116)

[0232] (vi) the forward and reverse primers of swine MG53 S255Y, which represents an MG53 mutant in which serine at position 255 of swine wild-type MG53 (i.e. SEQ ID NO: 5) is mutated into tyrosine.

Forward primer of swine MG53 S255Y: agatcctggcagagtatcccccacctgcccgcctgga (SEQ ID NO: 117)

Reverse primer of swine MG53 S255Y: tccaggcgggcaggtgggggatactctgccaggatct (SEQ ID NO: 118)

[0233] (vii) the forward and reverse primers of swine MG53 S255D, which represents an MG53 mutant in which serine at position 255 of swine wild-type MG53 (i.e. SEQ ID NO: 5) is mutated into aspartic acid.

Forward primer of swine MG53 S255D: aagatcctggcagaggatccccacctgcccgcctgga (SEQ ID NO: 119)

Reverse primer of swine MG53 S255D: tccaggcgggcaggtgggggatactctgccaggatctt (SEQ ID NO: 120)

[0234] (viii) the forward and reverse primers of swine MG53 S255R, which represents an MG53 mutant in which serine at position 255 of swine wild-type MG53 (i.e. SEQ ID NO: 5) is mutated into arginine.

Forward primer of swine MG53 S255R: aagatcctggcagagcggccccacctgcccgcctgg (SEQ ID NO: 121)

Reverse primer of swine MG53 S255R: ccaggcgggcaggtgggggatactctgccaggatctt (SEQ ID NO: 122)

[0235] 6. Preparation of dog MG53 mutants

[0236] Dog MG53 S255A, S255G, S255L, S255W, S255Q, S255Y, S255D, and S255R mutants were prepared according to the method for preparing human MG53 S255A mutant. Except for the primers, all of the other steps are the same as the steps for preparing human MG53 S255A mutant. The primers of each mutant are as follows:

[0237] (i) the forward and reverse primers of dog MG53 S255A, which represents an MG53 mutant in which serine at position 255 of dog wild-type MG53 (i.e. SEQ ID NO: 6) is mutated into alanine.

Forward primer of dog MG53 S255A: aagatcctggcagaagcaccaccgcctgcccgtttg (SEQ ID NO: 123)

Reverse primer of dog MG53 S255A: caaacgggcaggcggtggtgcttctgccaggatctt (SEQ ID NO: 124)

[0238] (ii) the forward and reverse primers of dog MG53 S255G, which represents an MG53 mutant in which serine at position 255 of dog wild-type MG53 (i.e. SEQ ID NO: 6) is mutated into glycine.

Forward primer of dog MG53 S255G: aagatcctggcagaaggaccaccgcctgcccgtttg (SEQ ID NO: 125)

Reverse primer of dog MG53 S255G: ccaaacgggcaggcggtggtccttctgccaggatctt (SEQ ID NO: 126)

[0239] (iii) the forward and reverse primers of dog MG53 S255L, which represents an MG53 mutant in which serine at position 255 of dog wild-type MG53 (i.e. SEQ ID NO: 6) is mutated into leucine.

Forward primer of dog MG53 S255L: agatcctggcagaattaccaccgcctgcccgtttgg (SEQ ID NO: 127)

Reverse primer of dog MG53 S255L: ccaaacgggcaggcggtggttaattctgccaggatct (SEQ ID NO: 128)

[0240] (iv) the forward and reverse primers of dog MG53 S255W, which represents an MG53 mutant in which serine at position 255 of dog wild-type MG53 (i.e. SEQ ID NO: 6) is mutated into tryptophan.

Forward primer of dog MG53 S255W: agatcctggcagaatggccaccgcctgcccgtttgga (SEQ ID NO: 129)

Reverse primer of dog MG53 S255W: tcaaacgggcaggcggtggcattctgccaggatct (SEQ ID NO: 130)

[0241] (v) the forward and reverse primers of dog MG53 S255Q, which represents an MG53 mutant in which serine at position 255 of dog wild-type MG53 (i.e. SEQ ID NO: 6) is mutated into glutamine.

Forward primer of dog MG53 S255Q: aagatcctggcagaacaaccaccgcctgcccgtttgg (SEQ ID NO: 131)

Reverse primer of dog MG53 S255Q: ccaaacgggcaggcggtggttcttctgccaggatctt (SEQ ID NO: 132)

[0242] (vi) the forward and reverse primers of dog MG53 S255Y, which represents an MG53 mutant in which serine at position 255 of dog wild-type MG53 (i.e. SEQ ID NO: 6) is mutated into tyrosine.

Forward primer of dog MG53 S255Y: agatcctggcagaatatccaccgcctgcccgtttgga (SEQ ID NO: 133)

Reverse primer of dog MG53 S255Y: tcaaacgggcaggcggtggatattctgccaggatct (SEQ ID NO: 134)

[0243] (vii) the forward and reverse primers of dog MG53 S255D, which represents an MG53 mutant in which serine at position 255 of dog wild-type MG53 (i.e. SEQ ID NO: 6) is mutated into aspartic acid.

Forward primer of dog MG53 S255D: aagatcctggcagaagatccaccgcctgcccgtttgga (SEQ ID NO: 135)

Reverse primer of dog MG53 S255D: tccaaacgggcaggcggtggatcttctgccaggatctt (SEQ ID NO: 136)

[0244] (viii) the forward and reverse primers of dog MG53 S255R, which represents an MG53 mutant in which serine at position 255 of dog wild-type MG53 (i.e. SEQ ID NO: 6) is mutated into arginine.

Forward primer of dog MG53 S255R: aagatcctggcagaacgaccaccgcctgcccgtttgg (SEQ ID NO: 137)

Reverse primer of dog MG53 S255R: ccaaacgggcaggcggtggtcgttctgccaggatctt (SEQ ID NO: 138)

[0245] MG53 mutants of various species with serine deletion and/or mutation at each serine position may be prepared according to the method for preparing human MG53 S255A mutant. Except for the primers, all of the other steps are the same as the steps for preparing human MG53 S255A mutant. A person skilled in the art may design the primers of each mutant by conventional technical means in the art.

[0246] Example 2: Impact of mouse MG53 S255A mutant on the cell membrane repair function and cardioprotective function of mouse wild-type MG53.

[0247] In order to evaluate the impact of mouse MG53 S255A mutant (its amino acid sequence is set forth in SEQ ID NO: 8) on the cell membrane repair function and cardioprotective function of mouse wild-type MG53, the inventors overexpressed mouse wild-type MG53 and mouse MG53 mutant using adenovirus in neonatal rat ventricular myocytes (NRVMs) that were primarily cultured, and detected the survival rate of cells. The results are shown in Figure 14, which indicates that hypoxia/reoxygenation-simulating ischemia-reperfusion injury result in the massive release of lactate dehydrogenase (LDH) and decreased intracellular ATP in NRVMs, the over-expression of mouse wild-type MG53 and mouse MG53 S255A mutant can similarly inhibit LDH release and ATP reduction. This suggests that mouse wild-type MG53 has a protective effect on ischemia-reperfusion induced apoptosis and necrosis, while the mouse MG53 S255A mutant does not affect the cell membrane repair function and cardioprotective function of the mouse wild-type MG53, i.e. the phosphorylation of mouse MG53 S255 position does not regulate the cell membrane repair function and cardioprotective function of MG53.

[0248] Mouse MG53 S255A mutation does not affect MG53's activation on the RISK signaling pathway.

[0249] The mechanism of MG53's cardioprotective function is as follows: MG53 is an essential component in the RISK signaling pathway of cardiac protection, and mediates the interaction between caveolin-3 and p85-PI3K proteins to activate the RISK signaling pathway, for example, an important downstream signaling molecule AKT (see Zhang Y. et al., *Cardiovascular Research* 91, 108-115 (2011)). Therefore, the inventors further studied the mechanism by which the mouse MG53 S255A mutation does not affect MG53's cardioprotective function to identify whether it is related to the fact that mouse MG53 S255A mutant and mouse wild-type MG53 can similarly activate the RISK signaling pathway. The experimental results are shown in Figure 15, which indicates that the over-expression of mouse wild-type MG53 in NRVMs enhances the phosphorylation of serine at position 473 of AKT, which is a downstream signaling molecule of the RISK signaling pathway, i.e. can activate AKT. The mouse MG53 S255A mutant and mouse wild-type MG53 can similarly enhance the phosphorylation of serine at position 473 of AKT, i.e. can activate AKT. This suggests that the mouse MG53 S255A mutant does not affect mouse wild-type MG53's activation of the RISK signaling pathway, i.e. the regulation on the phosphorylation of mouse MG53 S255 does not affect the RISK signaling cardioprotective pathway.

[0250] Example 3: Impact of human or mouse MG53 S255A mutant on E3 ubiquitin ligase activity of wild-type MG53.

[0251] The repression of insulin signaling pathway induced by high-expression of MG53 is one of the important mechanisms for the development of insulin resistance and metabolic syndrome, and it is also one of the important functions of MG53. First, the inventors constructed the expression plasmid of serine mutation of mouse wild-type MG53 --- mouse MG53 S255A (its amino acid sequence is set forth in SEQ ID NO: 8). In addition, the inventors also constructed the expression plasmids of IRS1, ubiquitin, and mouse wild-type MG53. Second, the inventors' previous study suggests that, MG53 is an E3 ubiquitin ligase of insulin substrate IRS1, and mediates proteasomal pathway degradation of proteins (Song, R. et al., *Nature* 494, 375-379,(2013)). Therefore, the inventors co-transfected HEK293T cell line with the expression plasmids of IRS1, ubiquitin, and the plasmid of mouse wild-type MG53 or mouse MG53 S255A

mutant, and evaluated the impact of mouse MG53 S255A mutation on MG53 functions through the changes in IRS1 protein contents. The experimental results are shown in Figure 19, which suggests that mouse wild-type MG53 can significantly reduce IRS1 expression, while mouse MG53 S255A mutant can significantly inhibit MG53-mediated IRS1 protein reduction. This suggests that, in the co-expression system of HEK293T cell line, the mouse wild-type MG53 can exert normal E3 ubiquitin ligase activity, and mediate the ubiquitin degradation of IRS1. This also suggests that the detected mouse MG53 S255A mutant inhibited the E3 ubiquitin ligase activity of MG53, i.e. the phosphorylation status of MG53 S255 position can regulate the E3 ubiquitin ligase activity of MG53.

[0252] Human or mouse MG53 S255A mutant inhibits MG53-mediated substrate degradation

[0253] MG53 is an E3 ubiquitin ligase with a RING domain, which is associated with E2 ubiquitin conjugating enzyme to mediate the ubiquitination of substrate and further proteasomal degradation. Therefore, the MG53-D-RING truncated mutant, which is without the RING domain, loses the E3 ubiquitin ligase activity of MG53. Further, in order to quantitatively evaluate the impact of human MG53 S255A mutant (its amino acid sequence is set forth in SEQ ID NO: 7) and mouse MG53 S255A mutant (its amino acid sequence is set forth in SEQ ID NO: 8) on the E3 ubiquitin ligase activity of human or mouse wild-type MG53, the inventors, by taking human or mouse MG53-D-RING as a positive control, co-expressed human or mouse wild-type MG53, MG53 S255A mutant, or MG53-D-RING truncated mutant, and the substrate IRS1 of MG53 or insulin receptor IR in human embryonic kidney epithelial cell line HEK293T, and detected the impact of different mutations on MG53-mediated substrate degradation. The results are shown in Figure 16 (mouse) and Figure 17 (human), which indicate that human or mouse wild-type MG53 can significantly mediate IRS1 degradation, human or mouse MG53-D-RING truncated mutant can hardly mediate IRS1 degradation, while mouse MG53 S255A mutant can only mediate about 40% degradation of IRS1, and human MG53 S255A mutant can only mediate about 10% degradation of IRS1. Mouse wild-type MG53 can mediate about 50% degradation of IR β , mouse MG53-D-RING truncated mutant and mouse MG53 S255A mutant can hardly mediate IR β degradation. In addition, in HEK293T co-expression system, mouse wild-type MG53 can mediate the degradation of precursor protein of IR β , while mouse MG53-D-RING truncated mutant and mouse MG53 S255A mutant almost completely inhibited MG53-mediated degradation. This study suggests that human or mouse MG53-D-RING truncated mutant

significantly inhibit the E3 ubiquitin ligase activity of MG53, while human or mouse MG53 S255A mutant inhibits about at least 50% E3 ubiquitin ligase activity of MG53.

[0254] Mouse MG53 S255A mutant inhibits the binding of MG53 to the substrate IRS1 protein

[0255] In order to study whether mouse MG53 S255A mutant (its amino acid sequence is set forth in SEQ ID NO: 8) may affect the recognition and binding of MG53 to the substrate IRS1 protein, the inventors, in one aspect, detected the binding intensity of substrate IRS1 and mouse wild-type MG53 or mouse MG53 S255A mutant under active status in *ex vivo* HEK293T cell line through co-immunoprecipitation, in another aspect, detected the direct binding intensity of purified protein IRS1 and mouse wild-type MG53 or mouse MG53 S255A mutant through surface plasmon resonance (SPR) test. The experimental results are shown in Figure 18. Figure 18A shows that IRS1 can physically bind to mouse wild-type MG53, while the same amount of IRS1 can only bind to extremely small amount of mouse MG53 S255A mutant; Figure 18B shows that mouse wild-type MG53 protein can bind to IRS1 protein very well, with a KD of 64.2 nM, while mouse MG53 S255A mutant cannot bind to IRS1 protein. In light of the above, mouse MG53 S255A mutation inhibited the recognition and binding of MG53 to the substrate IRS1 protein, so as to inhibit the E3 ubiquitin ligase activity of MG53.

[0256] Example 4: the *in vivo* activity of human MG53 S255A mutant

[0257] In order to validate whether human MG53 S255A mutant (its amino acid sequence is set forth in SEQ ID NO: 7) *in vivo* may still avoid or reduce metabolic side effects caused by wild-type MG53, while retaining cell membrane repair function and cardioprotective function, the inventors introduce human MG53 S255A mutant and human wild-type MG53 into the body of rats, respectively, to further analyze the *in vivo* activity of human MG53 S255A mutant. First, the inventors prepare human MG53 S255A mutant according to Example 1 of the present application. Second, 15 male Sprague-Dawley (SD) rats (about 250g/rat, provided by Beijing Vital River Laboratory Animal Technology Co., Ltd.) are selected and grouped into three groups, i.e. test group, positive control group, and negative control group, 5 rats/group. The rats are anaesthetized via 30mg/kg pentobarbital sodium (intraperitoneal injection), and then thoracotomy is performed in the left third rib to expose the heart. Then, human MG53 S255A mutant protein (6mg/kg, iv) is intravenously injected into the hearts of rats in the test group, human wild-type MG53 protein (its amino acid sequence is set forth in SEQ ID NO: 1) (6mg/kg,

iv) is intravenously injected into the hearts of rats in the positive control group, and bovine serum albumin (BSA) (6mg/kg, iv) is intravenously injected into the hearts of rats in the negative control group. The ligation of the left anterior descending arteries of the rats in each group are conducted at 5 minutes after the intravenous injections of the proteins above, and lasted for 45 minutes. Before the ending of the ligation, the rats of each group are again intravenously injected with the corresponding human MG53 S255A mutant protein, human wild-type MG53 protein and BSA (6mg/kg, iv), respectively, and then the coronary arteries are released. After a 24 hour reperfusion period, survival rates are compared between the test group, positive control group, and negative control group. Insulin is used to stimulate the rats in each group, and the rats are then sacrificed to compare the infarct size, serum LDH concentration, and TUNEL staining of myocardial sections of the rats in the test group, positive control group, and negative control group. Taken together, these indicators measure the level of cardiac injury of rats in each group. If the infarct size of the rats in the test group is significantly smaller than the infarct size of the rats in the negative control group, or the rats in test group demonstrate significantly inhibited LDH release compared to the rats in negative control group, or the TUNEL staining results of the myocardial sections of the rats in test group indicates decreased cell death compared to that of the rats in negative control group, then it suggests that human MG53 S255A mutant does not affect the cell membrane repair function and cardioprotective function of human wild-type MG53, and can be used for treating heart diseases, such as myocardial infarction, cardiac ischemia/reperfusion injury, etc. At last, p-AKT/t-AKT changes in each tissue (e.g. myocardium, skeletal muscle, liver, etc.) of the rats in test group, positive control group and negative control group are compared to evaluate the insulin responsiveness of each tissue of the rats in test group, positive control group and negative control group. If the ratio of p-AKT/t-AKT in the tissues of the rats in positive control group is decreased, then it suggests that human wild-type MG53 protein may induce the reduction of insulin sensitivity; if the ratio of p-AKT/t-AKT in the tissues of the rats in negative control group is normal, then it suggests that BSA has no effect on insulin sensitivity; if the ratio of p-AKT/t-AKT in the tissues of the rats in test group is higher than positive control group, then it suggests that human MG53 S255A mutant may eliminate or weaken the reduction of insulin sensitivity induced by human wild-type MG53 protein.

[0258] In summary, if at least one serine (especially serine at position 255) within the MG53 coiled-coil-SPRY region is deleted or mutated into any other non-serine or non-threonine (e.g.

alanine) amino acid, the resulting MG53 mutant may avoid or reduce metabolic side effects, such as insulin resistance, caused by wild-type MG53, while still retaining cell repair function and/or cardioprotective function.

[0259] Although the present invention is disclosed and described by introducing specific embodiments, a person skilled in the art will comprehend that various formal and detailed modifications can be made to the contents above without departing from the spirit and scope of the subject matter of the present invention.

WHAT CLAIMED IS:

1. An MG53 mutant, wherein the MG53 mutant is identical to the amino acid sequence of a wild-type MG53 except for at least one serine in the coiled-coil-SPRY region of the wild-type MG53, which is deleted and/or mutated into any other non-serine or non-threonine amino acid(s).
2. The MG53 mutant according to claim 1, wherein the coiled-coil-SPRY region is located at positions 122-477 of the amino acid sequence of the wild-type MG53.
3. The MG53 mutant according to claim 1 or 2, wherein the amino acid sequence of the wild-type MG53 is set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6.
4. The MG53 mutant according to any one of claims 1 to 3, wherein the serine is mutated into a non-polar amino acid.
5. The MG53 mutant according to claim 4, wherein the non-polar amino acid is selected from the group consisting of glycine, alanine, leucine, isoleucine, valine, proline, phenylalanine, methionine and tryptophan.
6. The MG53 mutant according to claim 5, wherein the non-polar amino acid is alanine.
7. The MG53 mutant according to any one of claims 1 to 6, wherein the serine is mutated into a non-serine or non-threonine polar amino acid.
8. The MG53 mutant according to claim 7, wherein the polar amino acid is selected from the group consisting of glutamine, cysteine, asparagine, tyrosine, aspartic acid, glutamic acid, lysine, arginine and histidine.
9. The MG53 mutant according to claim 8, wherein the polar amino acid is cysteine.
10. The MG53 mutant according to any one of claims 1 to 9, wherein the deleted or mutated serine is located at one or more of the following positions of the amino acid sequence of the wild-type MG53: positions 189, 211, 214, 246, 255, 269, 296, 301, 305, 307, 341, 377, 418, 430.
11. The MG53 mutant according to any one of claims 1 to 10, wherein the deleted or mutated serine is located at one or more of the following positions of the amino acid sequence of the wild-type MG53: positions 211, 214, 246, 255, 269, 296, 297.

12. The MG53 mutant according to any one of claims 1 to 11, wherein the deleted or mutated serine is located at position 255 of the amino acid sequence of the wild-type MG53.
13. The MG53 mutant according to claim 12, wherein the MG53 mutant has two or more serine mutations.
14. The MG53 mutant according to any one of claims 1 to 13, wherein the MG53 mutant has an amino acid sequence having at least 70% homology to any one of the amino acid sequences set forth in SEQ ID NOs: 7-12, and the MG53 mutant avoids metabolic side effects caused by wild-type MG53, while still retaining cell repair function and/or cardioprotective function.
15. The MG53 mutant according to any one of claims 1 to 14, wherein the amino acid sequence of the MG53 mutant is any one of the amino acid sequences set forth in SEQ ID NOs: 7-12.
16. The MG53 mutant according to any one of claims 1 to 15, wherein the amino acid sequence of the MG53 mutant is the amino acid sequence set forth in SEQ ID NO: 7.
17. A pharmaceutical composition comprising the MG53 mutant of any one of claims 1 to 16 and a pharmaceutically acceptable carrier.
18. An isolated nucleic acid comprising a nucleic acid sequence encoding the amino acid sequence of the MG53 mutant of any one of claims 1 to 16.
19. The nucleic acid according to claim 18 comprising any one of the nucleic acid sequences set forth in SEQ ID NOs: 13-18.
20. An expression vector comprising the nucleic acid sequence according to claim 18.
21. The expression vector according to claim 20 comprising any one of the nucleic acid sequences set forth in SEQ ID NOs: 13-18.
22. A host cell comprising the expression vector according to claim 20 or 21.
23. A method for preparing the MG53 mutant according to any one of claims 1 to 16, comprising determining one or more serine positions for mutation, performing site-directed mutagenesis at the position on the full-length sequence of a plasmid comprising the nucleic acid sequence encoding the amino acid sequence of wild-type MG53, transfecting the plasmid with site-directed mutagenesis into a host cell, and inducing the host cell to produce the MG53 mutant.
24. The method according to claim 23, wherein the site-directed mutagenesis comprises the following steps:
 - (1) determining the corresponding nucleotide site of the amino acid targeted for site-directed mutagenesis in the cDNA sequence, modifying the nucleotide sequence at the

- mutation site according to the target amino acid, and designing primers by intercepting a sequence of 20-40 bp in length comprising the mutation site;
- (2) performing PCR reaction by using the primers of step (1) and taking a wild-type MG53 plasmid as a template, performing agarose gel electrophoresis for the PCR product, and purifying the PCR product;
- (3) performing enzymatic reaction for the purified PCR product of step (2) by using restriction endonuclease, ligating the enzyme-digested product with a suitable plasmid expression vector, transforming and cultivating the ligation product in bacterial competent cells;
- (4) selecting the clones of step (3) to perform colony PCR identification by using the primers of step (1), performing agarose gel electrophoresis for the PCR product, and then performing DNA sequencing identification to identify positive clones with the site-directed mutagenesis.
25. Use of the MG53 mutant according to any one of claims 1 to 16 in the manufacture of a medicament for treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage.
26. The use according to claim 25, wherein the medicament avoids metabolic side effects while treating heart diseases, diabetic cerebrovascular diseases, diabetic ocular complications, diabetic neuropathy, diabetic foot, kidney diseases, and diseases associated with cellular and/or tissue damage.
27. The use according to claim 25 or 26, wherein the heart diseases are diseases associated with myocardial damage.
28. The use according to any one of claims 25 to 27, wherein the heart diseases comprise diabetic heart disease, myocardial ischemia, cardiac ischemia/reperfusion injury, myocardial infarction, heart failure, arrhythmia, heart rupture, angina, myocarditis, coronary heart disease and pericarditis.
29. The use according to claim 25 or 26, wherein the diabetic cerebrovascular diseases comprise cerebral arteriosclerosis, ischemic cerebrovascular disease, cerebral hemorrhage, cerebral atrophy and cerebral infarction.
30. The use according to claim 25 or 26, wherein the diabetic ocular complications comprise diabetic retinopathy, diabetic cataract, diabetic associated uveitis and blindness.

31. The use according to claim 25 or 26, wherein the diabetic neuropathy comprises diabetic peripheral neuropathy.
32. The use according to claim 25 or 26, wherein the kidney diseases include acute glomerulonephritis, chronic glomerulonephritis, nephrotic syndrome, acute kidney injury and diabetic nephropathy.
33. The use according to claim 25 or 26, wherein the diseases associated with cellular and/or tissue damage comprise diseases associated with the damage of kidney, brain, lung, liver, heart, spleen, digestive tract and skin.
34. The use according to claim 33, wherein the diseases associated with cellular and/or tissue damage comprise trauma, burns, ulcers, mucositis, asthma, chronic obstructive pulmonary disease, stroke and skin aging.
35. The use according to any one of claims 25 to 34, wherein the amino acid sequence of the MG53 mutant is the amino acid sequence set forth in SEQ ID NO: 7.