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Use of HMGB polypeptides for increasing immune responses

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(56) Related Art
Wen et al, A human placental cDNA clone that encodes non-histone chromosomal protein HMG-1, Nucleic Acid Research, vol.17 (3), 1989
Andersson et al, High mobility group 1 protein (HMG-1) stimulates pro-inflammatory cytokine synthesis in human monocytes, Journal of Experimental Medicine, vol.192 (4) 2000

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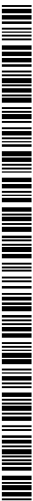


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(54) Title: USE OF HMGB POLYPEPTIDES FOR INCREASING IMMUNE RESPONSES

(57) Abstract: The present invention features polypeptides comprising an HMGB B box or a functional variant thereof that are useful for stimulating or increasing an immune response in an individual. Such polypeptides can be used in vaccine formulations and in cancer therapies.

USE OF HMGB POLYPEPTIDES FOR INCREASING IMMUNE RESPONSES

RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 60/427,848, filed November 20, 2002. The entire teachings of the above application
5 are incorporated herein by reference.

GOVERNMENT SUPPORT

The invention was supported, in whole or in part, by a grant RO1 GM 57226 from the National Institutes of Health. The Government has certain rights in the invention.

10 BACKGROUND OF THE INVENTION

The immune system functions to destroy or neutralize foreign matter. Immune responses protect against infection by microbes, including viruses, bacteria, fungi, and other parasites. In addition, the immune system functions to destroy the body's own cells that have become abnormal, for example, cancer cells, and cells
15 that are old and no longer useful to the body, such as erythrocytes.

Manipulation of the immune system is one way in which a therapeutic or protective immune response can be mounted, and a number of diseases can be treated through manipulation of the immune system. Current therapies for treating immunological disorders include anti-inflammatory agents, for example,
20 corticosteroids, cytotoxic agents, agents that modulate signaling events within the immune system, and antibodies.

There are many diseases in which the action of the immune system is inadequate. Therefore, there is a need for treatments of these diseases.

SUMMARY OF THE INVENTION

It has been found that HMGB polypeptides, as well as polypeptides comprising an HMGB B box or a functional variant thereof (collectively termed "HMGB B boxes") are useful for stimulating cytokine activity from cells administered such polypeptides. Thus, HMGB polypeptides and polypeptides comprising an HMGB B box can be used to increase an immune response in an individual and to treat a number of diseases for which an increased immune response is desired. Examples of conditions that can be treated using the reagents and methods as described herein include cancer and viral infections, including HIV/AIDS, allergic disease, and asthma. HMGB B boxes and functional variants described herein can also be used as part of a vaccine, in which an immune response is desired to prevent, ameliorate, or treat an infectious disease.

Accordingly, in one aspect, the invention features a pharmaceutical composition comprising an HMGB polypeptide or a functional fragment or variant thereof (collectively termed "HMGB polypeptides"), or an HMGB B box or a functional variant thereof (collectively termed "HMGB B boxes"), in an amount sufficient to treat a disease or condition in which an increase in an immune response in an individual administered the pharmaceutical composition is desired. In one embodiment, the pharmaceutical composition further comprises a vaccine.

In another aspect, the invention features an antibody attached to a polypeptide comprising an HMGB polypeptide or a functional fragment or variant thereof or an HMGB B box or a functional variant thereof. In one embodiment, the antibody is in a pharmaceutically acceptable carrier.

In another embodiment, the invention features a method of stimulating or increasing an immune response in an individual in need of immunostimulation, the method comprising administering to the individual a polypeptide comprising an HMGB polypeptide or a functional fragment or variant thereof or an HMGB B box

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or a functional variant thereof. In one embodiment, the individual is being treated for cancer. In another embodiment, the polypeptide is attached to an antibody specific to a target site in the individual in need of immunostimulation. In another embodiment, the polypeptide is co-administered with a vaccine. In another
 5 embodiment, the polypeptide is in a pharmaceutically acceptable carrier.

In another aspect, the invention features a method of treating cancer in an individual, the method comprising administering to the individual a therapeutically effective amount of a polypeptide comprising an HMGB polypeptide or a functional fragment or variant thereof or an HMGB B box or a functional variant thereof. In
 10 one embodiment, the individual is being treated for cancer. In another embodiment the polypeptide is attached to an antibody specific to a target site in the individual in need of immunostimulation. In another embodiment, the polypeptide is co-administered with a vaccine. In another embodiment, the polypeptide is in a pharmaceutically acceptable carrier.

15 BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic representation of HMGB1 mutants and their activity in TNF release (pg/ml).

FIG. 2A is a histogram showing the effect of 0 µg/ml, 0.01 µg/ml, 0.1 µg/ml, 1 µg/ml or 10 µg/ml of B box on TNF release (pg/ml) in RAW 264.7 cells.

20 FIG. 2B is a histogram showing the effect of 0 µg/ml, 0.01 µg/ml, 0.1 µg/ml, 1 µg/ml or 10 µg/ml of B box on IL-1β release (pg/ml) in RAW 264.7 cells.

FIG. 2C is a histogram showing the effect of 0 µg/ml, 0.01 µg/ml, 0.1 µg/ml, 1 µg/ml or 10 µg/ml of B box on IL-6 release (pg/ml) in RAW 264.7 cells.

FIG. 2D a scanned image of a blot of an RNase protection assay, showing
 25 the effect of B box (at 0 hours, 4 hours, 8 hours, or 24 hours after administration) or vector alone (at 4 hours after administration) on TNF mRNA expression in RAW 264.7 cells.

FIG. 2E is a histogram of the effect of HMGB1 B box on TNF protein release (pg/ml) from RAW 264.7 cells at 0 hours, 4 hours, 8 hours, 24 hours, 32 hours or 48
 30 hours after administration.

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FIG. 2F is a histogram of the effect of vector on TNF protein release (pg/ml) from RAW 264.7 cells at 0 hours, 4 hours, 8 hours, 24 hours, 32 hours or 48 hours after administration.

FIG. 3 is a schematic representation of HMG1 B box mutants and their activity in TNF release (pg/ml).

FIG. 4A is a scanned image of a hematoxylin and eosin stained kidney section obtained from an untreated mouse.

FIG. 4B is a scanned image of a hematoxylin and eosin stained kidney section obtained from a mouse administered HMG1 B box.

FIG. 4C is a scanned image of a hematoxylin and eosin stained myocardium section obtained from an untreated mouse.

FIG. 4D is a scanned image of a hematoxylin and eosin stained myocardium section obtained from a mouse administered HMG1 B box.

FIG. 4E is a scanned image of a hematoxylin and eosin stained lung section obtained from an untreated mouse.

FIG. 4F is a scanned image of a hematoxylin and eosin stained lung section obtained from a mouse administered HMG1 B box.

FIG. 4G is a scanned image of a hematoxylin and eosin stained liver section obtained from an untreated mouse.

FIG. 4H is a scanned image of a hematoxylin and eosin stained liver section obtained from a mouse administered HMG1 B box.

FIG. 4I is a scanned image of a hematoxylin and eosin stained liver section (high magnification) obtained from an untreated mouse.

FIG. 4J is a scanned image of a hematoxylin and eosin stained liver section (high magnification) obtained from a mouse administered HMG1 B box.

FIG. 5A is the amino acid sequence of a human HMG1 polypeptide (SEQ ID NO: 1).

FIG. 5B is the amino acid sequence of rat and mouse HMG1 (SEQ ID NO: 2).

FIG. 5C is the amino acid sequence of human HMG2 (SEQ ID NO: 3).

FIG. 5D is the amino acid sequence of a human, mouse, and rat HMG1 A box polypeptide (SEQ ID NO: 4).

FIG. 5E is the amino acid sequence of a human, mouse, and rat HMG1 B box polypeptide (SEQ ID NO: 5).

5 FIG. 5F is the nucleic acid sequence of a forward primer for human HMG1 (SEQ ID NO: 6).

FIG. 5G is the nucleic acid sequence of a reverse primer for human HMG1 (SEQ ID NO: 7).

10 FIG. 5H is the nucleic acid sequence of a forward primer for the carboxy terminus mutant of human HMG1 (SEQ ID NO: 8).

FIG. 5I is the nucleic acid sequence of a reverse primer for the carboxy terminus mutant of human HMG1 (SEQ ID NO: 9).

FIG. 5J is the nucleic acid sequence of a forward primer for the amino terminus plus B box mutant of human HMG1 (SEQ ID NO: 10).

15 FIG. 5K is the nucleic acid sequence of a reverse primer for the amino terminus plus B box mutant of human HMG1 (SEQ ID NO: 11).

FIG. 5L is the nucleic acid sequence of a forward primer for a B box mutant of human HMG1 (SEQ ID NO: 12).

20 FIG. 5M is the nucleic acid sequence of a reverse primer for a B box mutant of human HMG1 (SEQ ID NO: 13).

FIG. 5N is the nucleic acid sequence of a forward primer for the amino terminus plus A box mutant of human HMG1 (SEQ ID NO: 14).

FIG. 5O is the nucleic acid sequence of a reverse primer for the amino terminus plus A box mutant of human HMG1 (SEQ ID NO: 15).

25 FIG. 6 is a sequence alignment of HMG1 polypeptide sequence from rat (SEQ ID NO:2), mouse (SEQ ID NO:2), and human (SEQ ID NO: 18).

FIG. 7A is the nucleic acid sequence of HMG1L5 (formerly HMG1L10) (SEQ ID NO: 32) encoding an HMGB polypeptide.

30 FIG. 7B is the polypeptide sequence of HMG1L5 (formerly HMG1L10) (SEQ ID NO: 24) encoding an HMGB polypeptide.

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FIG. 7C is the nucleic acid sequence of HMG1L1 (SEQ ID NO: 33) encoding an HMGB polypeptide.

FIG. 7D is the polypeptide sequence of HMG1L1 (SEQ ID NO: 25) encoding an HMGB polypeptide.

5 FIG. 7E is the nucleic acid sequence of HMG1L4 (SEQ ID NO: 34) encoding an HMGB polypeptide.

FIG. 7F is the polypeptide sequence of HMG1L4 (SEQ ID NO: 26) encoding an HMGB polypeptide.

10 FIG. 7G is the nucleic acid sequence of the HMG polypeptide sequence of the BAC clone RP11-395A23 (SEQ ID NO: 35).

FIG. 7H is the polypeptide sequence of the HMG polypeptide sequence of the BAC clone RP11-395A23 (SEQ ID NO: 27) encoding an HMGB polypeptide.

FIG. 7I is the nucleic acid sequence of HMG1L9 (SEQ ID NO: 36) encoding an HMGB polypeptide.

15 FIG. 7J is the polypeptide sequence of HMG1L9 (SEQ ID NO: 28) encoding an HMGB polypeptide.

FIG. 7K is the nucleic acid sequence of LOC122441 (SEQ ID NO: 37) encoding an HMGB polypeptide.

20 FIG. 7L is the polypeptide sequence of LOC122441 (SEQ ID NO: 29) encoding an HMGB polypeptide.

FIG. 7M is the nucleic acid sequence of LOC139603 (SEQ ID NO: 38) encoding an HMGB polypeptide.

FIG. 7N is the polypeptide sequence of LOC139603 (SEQ ID NO: 30) encoding an HMGB polypeptide.

25 FIG. 7O is the nucleic acid sequence of HMG1L8 (SEQ ID NO: 39) encoding an HMGB polypeptide.

FIG. 7P is the polypeptide sequence of HMG1L8 (SEQ ID NO: 31) encoding an HMGB polypeptide.

DETAILED DESCRIPTION OF THE INVENTION

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The present invention features HMGB polypeptides and polypeptides comprising an HMGB B box or a functional variant thereof that are useful for stimulating or increasing an immune response in an individual. In one embodiment the polypeptide comprises or consists of a mammalian HMGB B box, for example, a human HMGB B box. Examples of an HMGB B boxes include polypeptides having the sequence of SEQ ID NO: 5, SEQ ID NO: 20, or SEQ ID NO: 45.

As used herein, an "HMGB polypeptide" or an "HMGB protein" is an isolated, substantially pure, or substantially pure and isolated polypeptide that has been separated from components that naturally accompany it or a recombinantly produced polypeptide having the same amino acid sequence, and increases inflammation, and/or increases release of a proinflammatory cytokine from a cell, and/or increases the activity of the inflammatory cytokine cascade. In one embodiment, the HMGB polypeptide has one of the above biological activities. In another embodiment, the HMGB polypeptide has two of the above biological activities. In a third embodiment, the HMGB polypeptide has all three of the above biological activities.

Preferably, the HMGB polypeptide is a mammalian HMGB polypeptide, for example, a human HMGB1 polypeptide. Examples of an HMGB polypeptide include a polypeptide comprising or consisting of the sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 18. Preferably, the HMGB polypeptide contains a B box DNA binding domain and/or an A box DNA binding domain, and/or an acidic carboxyl terminus as described herein. Other examples of HMGB polypeptides are described in GenBank Accession Numbers AAA64970, AAB08987, P07155, AAA20508, S29857, P09429, NP_002119, CAA31110, S02826, U00431, X67668, NP_005333, NM_016957, and J04179, the entire teachings of which are incorporated herein by reference. Additional examples of HMGB polypeptides include, but are not limited to mammalian HMG1 ((HMGB1) as described, for example, in GenBank Accession Number U51677), HMG2 ((HMGB2) as described, for example, in GenBank Accession Number M83665), HMG-2A ((HMGB3, HMG-4) as described, for example, in GenBank Accession Numbers NM_005342 and NP_005333), HMG14 (as described, for example, in

GenBank Accession Number P05114), HMG17 (as described, for example, in GenBank Accession Number X13546), HMGI (as described, for example, in GenBank Accession Number L17131), and HMGY (as described, for example, in GenBank Accession Number M23618); nonmammalian HMG T1 (as described, for example, in GenBank Accession Number X02666) and HMG T2 (as described, for example, in GenBank Accession Number L32859) (rainbow trout); HMG-X (as described, for example, in GenBank Accession Number D30765) (Xenopus); HMG D (as described, for example, in GenBank Accession Number X71138) and HMG Z (as described, for example, in GenBank Accession Number X71139) (Drosophila);

10 NHP10 protein (HMG protein homolog NHP 1) (as described, for example, in GenBank Accession Number Z48008) (yeast); non-histone chromosomal protein (as described, for example, in GenBank Accession Number O00479) (yeast); HMG 1/2 like protein (as described, for example, in GenBank Accession Number Z11540) (wheat, maize, soybean); upstream binding factor (UBF-1) (as described, for example, in GenBank Accession Number X53390); PMS1 protein homolog 1 (as described, for example, in GenBank Accession Number U13695); single-strand recognition protein (SSRP, structure-specific recognition protein) (as described, for example, in GenBank Accession Number M86737); the HMG homolog TDP-1 (as described, for example, in GenBank Accession Number M74017); mammalian

20 sex-determining region Y protein (SRY, testis-determining factor) (as described, for example, in GenBank Accession Number X53772); fungal proteins: mat-1 (as described, for example, in GenBank Accession Number AB009451), ste 11 (as described, for example, in GenBank Accession Number x53431) and Mc 1; SOX 14 (as described, for example, in GenBank Accession Number AF107043) (as well as

25 SOX 1 (as described, for example, in GenBank Accession Number Y13436), SOX 2 (as described, for example, in GenBank Accession Number Z31560), SOX 3 (as described, for example, in GenBank Accession Number X71135), SOX 6 (as described, for example, in GenBank Accession Number AF309034), SOX 8 (as described, for example, in GenBank Accession Number AF226675), SOX 10 (as described, for example, in GenBank Accession Number AJ001183), SOX 12 (as described, for example, in GenBank Accession Number X73039) and SOX 21 (as

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described, for example, in GenBank Accession Number AF107044)); lymphoid specific factor (LEF-1)(as described, for example, in GenBank Accession Number X58636); T-cell specific transcription factor (TCF-1)(as described, for example, in GenBank Accession Number X59869); MTT1 (as described, for example, in
 5 GenBank Accession Number M62810); and SP100-HMG nuclear autoantigen (as described, for example, in GenBank Accession Number U36501).

Other examples of HMGB proteins are polypeptides encoded by HMGB nucleic acid sequences having GenBank Accession Numbers NG_000897 (HMGL5 (formerly HMGIL10)) (and in particular by nucleotides 150-797 of NG_000897, as
 10 shown in FIGs. 7A and 7B); AF076674 (HMGIL1) (and in particular by nucleotides 1-633 of AF076674, as shown in FIGs. 7C and 7D; AF076676 (HMGIL4) (and in particular by nucleotides 1-564 of AF076676, as shown in FIGs. 7E and 7F); AC010149 (HMG sequence from BAC clone RP11-395A23) (and in particular by nucleotides 75503-76117 of AC010149), as shown in FIGs. 7G and 7H); AF165168
 15 (HMGIL9) (and in particular by nucleotides 729-968 of AF165168, as shown in FIGs. 7I and 7J); XM_063129 (LOC122441) (and in particular by nucleotides 319-558 of XM_063129, as shown in FIGs. 7K and 7L); XM_066789 (LOC139603) (and in particular by nucleotides 1-258 of XM_066789, as shown in FIGs. 7M and 7N); and AF165167 (HMGIL8) (and in particular by nucleotides 456-666 of
 20 AF165167, as shown in FIGs. 7O and 7P).

The HMGB polypeptides of the present invention also encompass sequence variants. Variants include a substantially homologous polypeptide encoded by the same genetic locus in an organism, i.e., an allelic variant, as well as other variants. Variants also encompass polypeptides derived from other genetic loci in an
 25 organism, but having substantial homology to a polypeptide encoded by an HMGB nucleic acid molecule, and complements and portions thereof, or having substantial homology to a polypeptide encoded by a nucleic acid molecule comprising the nucleotide sequence of an HMGB nucleic acid molecule. Examples of HMGB nucleic acid molecules are known in the art and can be derived from HMGB
 30 polypeptides as described herein. Variants also include polypeptides substantially homologous or identical to these polypeptides but derived from another organism,

i.e., an ortholog. Variants also include polypeptides that are substantially homologous or identical to these polypeptides that are produced by chemical synthesis. Variants also include polypeptides that are substantially homologous or identical to these polypeptides that are produced by recombinant methods.

- 5 Preferably, the HMGB polypeptide has at least 60%, more preferably, at least 70%, 75%, 80%, 85%, or 90%, and most preferably at least 95% sequence identity to a sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:18, as determined using the BLAST program and parameters described herein and one or more of the biological activities of an HMGB polypeptide (functional
10 variant).

- In other embodiments, the present invention is directed to an HMGB polypeptide fragment that has HMGB biological activity (functional fragment). By an "HMGB polypeptide fragment that has HMGB biological activity" or a "biologically active HMGB fragment" is meant a fragment of an HMGB polypeptide
15 that has the activity of an HMGB polypeptide. An example of such an HMGB polypeptide fragment is the HMGB B box, as described herein. Biologically active HMGB fragments can be generated using standard molecular biology techniques and assaying the function of the fragment by determining if the fragment, when administered to a cell increase release of a proinflammatory cytokine from the cell,
20 compared to a suitable control, for example, using methods described herein.

- As used herein, an "HMGB B box" also referred to herein as a "B box" is a substantially pure, or substantially pure and isolated polypeptide that has been separated from components that naturally accompany it, and consists of an amino acid sequence that is less than a full length HMGB polypeptide and has one or more
25 of the following biological activities: increasing inflammation, increasing release of a proinflammatory cytokine from a cell, and/or increasing the activity of the inflammatory cytokine cascade. In one embodiment, the HMGB B box polypeptide has one of the above biological activities. In another embodiment, the HMGB B box polypeptide has two of the above biological activities. In a third embodiment, the
30 HMGB B box polypeptide has all three of the above biological activities. Preferably, the HMGB B box has at least 25%, 30%, 40%, 50%, 60%, 70%, 80% or

90% of the biological activity of full length HMG. In another embodiment, the HMGB B box does not comprise an HMGB A box. In another embodiment, the HMGB B box is a fragment of an HMGB polypeptide (i.e., a polypeptide that is about 90%, 80%, 70%, 60%, 50%, 40%, 35%, 30%, 25%, or 20% the length of a full length HMG1 polypeptide). In another embodiment, the HMGB B box comprises or consists of the sequence of SEQ ID NO: 5, SEQ ID NO: 20, SEQ ID NO: 45, or the amino acid sequence in the corresponding region of an HMGB protein in a mammal, but is still less than the full length HMGB polypeptide. An HMGB B box polypeptide is also a recombinantly produced polypeptide having the same amino acid sequence as an HMGB B box polypeptide described above. Preferably, the HMGB B box is a mammalian HMGB B box, for example, a human HMGB1 B box. An HMGB B box often has no more than about 85 amino acids and no fewer than about 4 amino acids.

Examples of polypeptides having B box sequences within them include, but are not limited to HMGB polypeptides described herein. The B box sequences in such polypeptides can be determined and isolated using methods described herein, for example, by sequence comparisons to B boxes described herein and testing for B box biological activity. In particularly preferred embodiments, the B box comprises SEQ ID NO:5, SEQ ID NO:20, or SEQ ID NO:45, which are the sequences (three different lengths) of the human HMGB1 B box, or is a fragment of an HMGB B box that has B box biological activity. For example, a 20 amino acid sequence contained within SEQ ID NO:20 contributes to the function of the B box. This 20 amino acid B-box fragment has the following amino acid sequence: fkdnpakrl psafflcse (SEQ ID NO:23). Another example of an HMGB B box biologically active fragment consists of amino acids 1-20 of SEQ ID NO:5 (napkrppsaf flfcseyrpk; SEQ ID NO:16).

Examples of HMGB B box polypeptide sequences include the following sequences: FKDPNAPKRP PSAFFLCSE YRPKIKGEHP GLSIGDVAKK LGEMWNNTAA DDKQPYEKKA AKLKEKYEKD IAAY (human HMGB1; SEQ ID NO: 17); KKDPNAPKRP PSAFFLCSE HRPKIKSEHP GLSIGDTAKK LGEMWSEQSA KDKQPYEQKA AKLKEKYEKD IAAY (human HMGB2; SEQ

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ID NO: 40); FKDPNAPKRL PSAFFLFCSE YRPKIKGEHP GLSIGDVAKK
 LGEMWNNTAA DDKQPYEKKA AKLKEKEYEKD IAAY (HMG1L5 (formerly
 HMG1L10); SEQ ID NO: 41); FKDPNAPKRP PSAFFLFCSE YHPKIKGEHP
 GLSIGDVAKK LGEMWNNTAA DDKQPGEKKA AKLKEKEYEKD IAAY
 5 (HMG1L1; SEQ ID NO: 42); FKDSNAPKRP PSAFLLFCSE YCPKIKGEHP
 GLPISDVAKK LVEMWNNTFA DDKQLCEKKA AKLKEKEYEKD TATY
 (HMG1L4; SEQ ID NO: 43); FKDPNAPKRP PSAFFLFCSE YRPKIKGEHP
 GLSIGDVVKK LAGMWNNTAA ADKQFYEKKA AKLKEKEYEKD IAAY
 (HMG sequence from BAC clone RP11-359A23; SEQ ID NO: 44); and
 10 FKDPNAPKRP PSAFFLFCSE YRPKIKGEHP GLSIGDVAKK LGEMWNNTAA
 DDKQPYEKKA AKLKEKEYEKD IAAYRAKGKP DAAKKGVVKA EK (human
 HMGB1 box; SEQ ID NO: 45).

The HMGB B box polypeptides of the invention also encompasses sequence
 variants that are functional variants, and can be naturally-occurring or non-naturally-
 15 occurring. Functional variants include a substantially homologous polypeptide
 encoded by the same genetic locus in an organism, i.e., an allelic variant, as well as
 other variants. Functional variants also encompass polypeptides derived from other
 genetic loci in an organism, but having substantial homology to a polypeptide
 encoded by an HMGB nucleic acid molecule, and complements and portions thereof,
 20 or having substantial homology to a polypeptide encoded by a nucleic acid molecule
 comprising the nucleotide sequence of an HMGB B box nucleic acid molecule.
 Examples of HMGB B box nucleic acid molecules are known in the art and can be
 derived from HMGB B box polypeptides as described herein. Functional variants
 also include polypeptides substantially homologous or identical to these
 25 polypeptides but derived from another organism, i.e., an ortholog. Functional
 variants also include polypeptides that are substantially homologous or identical to
 these polypeptides that are produced by chemical synthesis. Functional variants also
 include polypeptides that are substantially homologous or identical to these
 polypeptides that are produced by recombinant methods:

30 Preferably, an HMGB B box polypeptide variant has at least 60%, more
 preferably, at least 70%, 75%, 80%, 85%, or 90%, and most preferably at least 95%

sequence identity to the sequence of an HMGB B box as described herein, for example, the sequence of SEQ ID NO: 5, SEQ ID NO: 20, or SEQ ID NO: 45, as determined using the BLAST program and parameters described herein. Preferably, the HMGB B box consists of the sequence of SEQ ID NO: 5, SEQ ID NO: 20, or
5 SEQ ID NO: 45, or the amino acid sequence in the corresponding region of an HMGB protein in a mammal, and has one or more of the biological activities of an HMGB B box, determined using methods described herein or other methods known in the art.

As used herein, two polypeptides (or a region of the polypeptides) are
10 substantially homologous or identical when the amino acid sequences are at least about 60%, 70%, 75%, 80%, 85%, 90% or 95% or more homologous or identical. The percent identity of two amino acid sequences (or two nucleic acid sequences) can be determined by aligning the sequences for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first sequence). The amino acids or
15 nucleotides at corresponding positions are then compared, and the percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions x 100). In certain embodiments, the length of the HMGB polypeptide or HMGB B box polypeptide aligned for comparison purposes is at least 30%, preferably, at least
20 40%, more preferably, at least 60%, and even more preferably, at least 70%, 80%, 90%, or 100% of the length of the reference sequence, for example, those sequence provided herein. The actual comparison of the two sequences can be accomplished by well-known methods, for example, using a mathematical algorithm. A preferred, non-limiting example of such a mathematical algorithm is described in Karlin *et al.*
25 (Proc. Natl. Acad. Sci. USA, 90:5873-5877, 1993). Such an algorithm is incorporated into the BLASTN and BLASTX programs (version 2.2) as described in Schaffer *et al.* (Nucleic Acids Res., 29:2994-3005, 2001). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., BLASTN) can be used. In one embodiment, the database searched is a non-
30 redundant (NR) database, and parameters for sequence comparison can be set at: no

filters; Expect value of 10; Word Size of 3; the Matrix is BLOSUM62; and Gap Costs have an Existence of 11 and an Extension of 1.

Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller,

- 5 CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0), which is part of the GCG (Accelrys, San Diego, CA) sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12 , and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are
10 known in the art and include ADVANCE and ADAM as described in Torellis and Robotti, Comput. Appl. Biosci., 10: 3-5, 1994; and FASTA described in Pearson and Lipman, Proc. Natl. Acad. Sci USA, 85: 2444-2448, 1988.

- In another embodiment, the percent identity between two amino acid sequences can be accomplished using the GAP program in the GCG software
15 package (Accelrys) using either a Blossom 63 matrix or a PAM250 matrix, and a gap weight of 12, 10, 8, 6, or 4 and a length weight of 2, 3, or 4. In yet another embodiment, the percent identity between two nucleic acid sequences can be accomplished using the GAP program in the GCG software package (Accelrys), using a gap weight of 50 and a length weight of 3.

- 20 HMGB polypeptides of functional fragments or variants thereof (collectively termed "HMGB polypeptides"), or polypeptides comprising an HMGB B box or a functional variant thereof (collectively termed "HMGB B boxes") can be used in pharmaceutical compositions to stimulate or increase an immune response. As used herein, by an "immune response" is meant a collective and coordinated response to
25 the introduction of a foreign substance in the body, by cells and molecules of the immune system. Cytokines play an important role in mediating immune responses. Thus molecules that stimulate cytokine activity are useful for developing and/or mediating immune responses.

- In one embodiment, the pharmaceutical composition comprises the HMGB B
30 box and a vaccine. The vaccine can be administered to a person in need of immunostimulation (i.e., a person who would benefit by mounting or increasing an

immune response to an antigen, a tumor cell or a tumor) in order to stimulate an immune response. Examples of vaccines include Hepatitis B, Diphtheria, Tetanus, Pertussis, Haemophilus influenzae Type B, Inactivated Polio, Measles, Mumps, Rubella, Varicella, Pneumococcal, Hepatitis A, Influenza, Japanese Encephalitis, Rotavirus, Yellow Fever, Trypanosoma cruzi, and Rabies. If desired, the pharmaceutical composition can further comprise an adjuvant. As used herein, an "adjuvant" is an immunologic reagent that increases an antigenic response. Examples of adjuvants for use in pharmaceuticals include immunostimulatory oligonucleotides, imidazoquinolines (e.g., imiquimod), monophosphoryl lipid A, and detoxified lipopolysaccharide (LPS), as described, for example, by O'Hagan et al. (Biomol. Eng. 18:69-85, 2001)). An example of an immunostimulatory oligonucleotide is an oligonucleotide having unmethylated CpG sequences.

In another example, the pharmaceutical composition comprises an HMGB polypeptide or functional fragment or variant thereof or an HMGB B box polypeptide or functional variant thereof attached to an antibody. The antibody specifically binds a polypeptide, preferably an epitope, or a target site (as determined, for example, by immunoassays, a technique well known in the art for assaying specific antibody-antigen binding) to deliver the HMGB B box polypeptide to the target site in order to stimulate or increase an immune response at the site where the antibody binds. Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, for example, anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above.

The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, and more specifically, molecules that contain an antigen binding site that specifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (for example, IgG, IgE, IgM, IgD, IgA and IgY), and of any class (for example, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of an immunoglobulin molecule.

In one embodiment, the antibodies are antigen-binding antibody fragments and include, without limitation, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. Antigen-binding antibody fragments, including single-chain
5 antibodies, can comprise the variable region(s) alone or in combination with the entirety or a portion of one or more of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and/or CH3 domains.

10 The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, donkey, sheep, rabbit, goat, guinea pig, hamster, horse, or chicken.

As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies produced by human B
15 cells, or isolated from human sera, human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and that do not express endogenous immunoglobulins, as described in U.S. Patent No. 5,939,598 by Kucherlapati et al., for example.

The antibodies of the present invention may be monospecific, bispecific,
20 trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. The term "epitope," as used herein, refers to a portion of a polypeptide which contacts an antigen-binding
25 site(s) of an antibody or T cell receptor.

The term "target site" as used herein, refers to a polypeptide that is recognized by an antibody and to which the antibody binds. The target site is preferably a site at which delivery or localization of an HMGB B box polypeptide or functional variant thereof is desired. The target site can be *in vivo* or *ex vivo*. The
30 target site can be, for example, a polypeptide localized on the surface of a cell or near (e.g., adjacent to) a cell to which delivery of an HMGB B box is desired. In one

embodiment the target site is a cancer target site, for example, a cancer cell or a site near a tumor, such that delivery of an HMGB polypeptide to the cancer cell or tumor occurs. In such a case, the antibody may be a tumor-associated antibody (i.e., an antibody that is preferentially or exclusively bound by a cancer cell or tumor).

- 5 In one embodiment, the antibody of the present invention, attached to an HMGB B box or functional variant thereof is a tumor-associated antibody that binds to a tumor-associated polypeptide, marker, or antigen at a cancer target site. Tumor-associated polypeptides or markers include, but are not limited to oncofetal antigens, placental antigens, oncogenic or tumor virus-associated antigens,
- 10 tissue-associated antigens, organ-associated antigens, ectopic hormones and normal antigens or variants thereof. A sub-unit of a tumor-associated marker can also be used to raise antibodies having higher tumor-specificity, e.g., the beta-subunit of human chorionic gonadotropin (HCG), which stimulates the production of antibodies having a greatly reduced cross-reactivity to non-tumor substances.
- 15 Suitable such marker substances to which specific antibodies may be raised and/or obtained which are useful in the present invention include, but are not limited to, alpha-fetoprotein (AFP), human chorionic gonadotropin (HCG) and/or its beta-subunit (HCG-beta), colon-specific antigen-p (CSAp), prostatic acid phosphatase, pancreatic oncofetal antigen, placental alkaline phosphatase, pregnancy
- 20 beta₂-globulin, parathormone, calcitonin, tissue polypeptide antigen, T-antigen, beta₂-microglobulin, mammary tumor-associated glycoproteins (MTGP), galactosyl transferase-II (GT-II), gp-52 viral-associated antigen, ovarian cystadenocarcinoma-associated antigen (OCAA), ovarian tumor-specific antigen (OCA), cervical cancer antigens (CA-58, CCA, TA-4), basic fetoprotein (BFP),
- 25 terminal deoxynucleotidyl transferase (TdT), cytoplasmic melanoma-associated antigens, human astrocytoma-associated antigen (HAAA), common glioma antigen (CGA), glioblastic antigen (GBA), glial fibrillary acidic protein (GFA), common meningioma antigen (CMA), ferritin, and tumor angiogenesis factor (TAF).

- Antibodies of the present invention may also be described or specified in
- 30 terms of their cross-reactivity. Antibodies used in the present invention may not display significant cross-reactivity, such that they do not bind any other analog,

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ortholog, or homolog of a polypeptide of the present invention. Alternatively, antibodies of the invention can bind polypeptides with at least about 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, or 50% identity (as calculated using methods known in the art) to a polypeptide at a target site.

5 Antibodies of the present invention can also be described or specified in terms of their binding affinity to a polypeptide at a target site. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and
10 10^{-15} M.

Antibodies used in the present invention can act as agonists or antagonists of a polypeptide at a target site. For example, the present invention includes antibodies which disrupt interactions with the polypeptides at the target site either partially or fully. The invention also includes antibodies that do not prevent binding, but
15 prevent activation or activity of the polypeptide. Activation or activity (for example, signaling) may be determined by techniques known in the art. Also included are antibodies that prevent both binding to and activity of a polypeptide at a target site. Likewise included are neutralizing antibodies.

The antibodies used in the invention include derivatives that do not prevent
20 the antibody from recognizing its epitope. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, for example, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, or proteolytic cleavage.

The antibodies used in the invention can be generated by any suitable method
25 known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, or the like, to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants can be used to increase the
30 immunological response, depending on the host species, and include, but are not limited to, Freund's adjuvant (complete and incomplete), mineral gels such as

aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques also known in the art, including hybridoma cell culture, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques as is known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 2nd ed. 1988). The term "monoclonal antibody" as used herein is not necessarily limited to antibodies produced through hybridoma technology, but also refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone.

Human antibodies are desirable for therapeutic treatment of human patients. These antibodies can be made by a variety of methods known in the art including phage display methods using antibody libraries derived from human immunoglobulin sequences. Human antibodies can also be produced using transgenic mice that are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. The transgenic mice are immunized with a selected antigen, for example, all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, for example, PCT publications WO 98/24893; WO 96/34096; WO 96/33735; and U.S.

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Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; and 5,939,598.

An HMGB polypeptide or HMGB B box polypeptide can be attached, coupled, or conjugated to an antibody using methods known to one of skill in the art.

- 5 In one embodiment, the polypeptide is covalently attached to the antibody. In another embodiment the polypeptide-antibody conjugate is produced using recombinant methods, and is generated as a fusion protein comprising the polypeptide and the antibody or an antigen binding fragment of an antibody. Alternatively, the polypeptide can be chemically crosslinked to the antibody. If
10 desired, spacers or linkers (for example, those available from Pierce Chemical Company) may be used to attached the polypeptide to the linker. Methods for attaching a polypeptide to an antibody are described, for example, by Jeanson et al. (J. Immunol Methods 111:261-270, 1988); and Zarling et al. (Int. J. Immunopharmacol. 13 Suppl 1:63-68-1991). Reactive groups that can be targeted
15 by coupling agents include primary amines, sulfhydryls, and carbonyls.

- The compositions of the invention can be administered alone or in combination with other therapeutic agents. Therapeutic agents that can be administered in combination with the compositions of the invention, include but are not limited to chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-
20 inflammatories, conventional immunotherapeutic agents, cytokines and/or growth factors. Combinations may be administered either concomitantly, for example, as an admixture, separately but simultaneously or concurrently; or sequentially.

- In another embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be
25 administered with the compositions of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU,
30 lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate);

hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephalen, chlorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids
5 and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, taxol, and etoposide).

In an additional embodiment, the compositions of the invention may be administered in combination with cytokines. Cytokines that may be administered
10 with the compositions of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha.

In additional embodiments, the compositions of the invention are administered in combination with other therapeutic or prophylactic regimens, such
15 as, for example, radiation therapy.

As described herein, the compositions comprising HMGB polypeptides or functional fragments or variants thereof or HMGB B box polypeptides or functional variants thereof can be formulated in a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier included with the polypeptide in these
20 compositions is chosen based on the expected route of administration of the composition in therapeutic applications. The route of administration of the composition depends on the condition to be treated. For example, intravenous injection may be preferred for treatment of a systemic disorder such as a leukemia or lymphoma, and oral administration may be preferred to treat a gastrointestinal
25 disorder such as a cancer of the gastrointestinal system, or an oral cancer. The route of administration and the dosage of the composition to be administered can be determined by the skilled artisan without undue experimentation in conjunction with standard dose-response studies. Relevant circumstances to be considered in making those determinations include the condition or conditions to be treated, the choice of
30 composition to be administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms. Thus, depending on the

condition, the composition can be administered orally, parenterally, intranasally, vaginally, rectally, lingually, sublingually, buccally, intrabuccally and transdermally to the patient.

Accordingly, compositions designed for oral, lingual, sublingual, buccal and
5 intrabuccal administration can be made without undue experimentation by means well known in the art, for example, with an inert diluent or with an edible carrier. The compositions may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the pharmaceutical compositions of the present invention may be incorporated with excipients and used in the form of
10 tablets, troches, capsules, elixirs, suspensions, syrups, wafers, chewing gums and the like.

Tablets, pills, capsules, troches and the like may also contain binders, recipients, disintegrating agent, lubricants, sweetening agents, and flavoring agents. Some examples of binders include microcrystalline cellulose, gum tragacanth or
15 gelatin. Examples of excipients include starch or lactose. Some examples of disintegrating agents include alginic acid, corn starch and the like. Examples of lubricants include magnesium stearate or potassium stearate. An example of a glidant is colloidal silicon dioxide. Some examples of sweetening agents include sucrose, saccharin and the like. Examples of flavoring agents include peppermint,
20 methyl salicylate, orange flavoring and the like. Materials used in preparing these various compositions should be pharmaceutically pure and non-toxic in the amounts used.

The compositions of the present invention can be administered parenterally such as, for example, by intravenous, intramuscular, intrathecal or subcutaneous
25 injection. Parenteral administration can be accomplished by incorporating the antibody compositions of the present invention into a solution or suspension. Such solutions or suspensions may also include sterile diluents such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents. Parenteral formulations may also include antibacterial agents
30 such as, for example, benzyl alcohol or methyl parabens, antioxidants such as, for example, ascorbic acid or sodium bisulfite and chelating agents such as EDTA.

Buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose may also be added. The parenteral preparation can be enclosed in ampules, disposable syringes or multiple dose vials made of glass or plastic.

5 Rectal administration includes administering the pharmaceutical compositions into the rectum or large intestine. This can be accomplished using suppositories or enemas. Suppository formulations can easily be made by methods known in the art. For example, suppository formulations can be prepared by heating glycerin to about 120°C, dissolving the antibody composition in the glycerin, mixing
10 the heated glycerin after which purified water may be added, and pouring the hot mixture into a suppository mold.

Transdermal administration includes percutaneous absorption of the composition through the skin. Transdermal formulations include patches, ointments, creams, gels, salves and the like.

15 The present invention includes nasally administering to the mammal a therapeutically effective amount of the composition. As used herein, nasally administering or nasal administration includes administering the composition to the mucous membranes of the nasal passage or nasal cavity of the patient. As used herein, pharmaceutical compositions for nasal administration of a composition
20 include therapeutically effective amounts of the agonist prepared by well-known methods to be administered, for example, as a nasal spray, nasal drop, suspension, gel, ointment, cream or powder. Administration of the composition may also take place using a nasal tampon or nasal sponge.

Administration of the pharmaceutical compositions of the invention the
25 pharmaceutical compositions of the invention can be administered to animals, for example, humans in an amount sufficient to mount an immune response for the treatment or prevention of a disease, for example a viral disease or a bacterial disease (e.g., through vaccination, or through anti-bacterial or anti-viral therapy), or to slow the proliferation of cancer cells or to kill them entirely, it would be clear to
30 those skilled in the art that the optimal schedule for administering such a pharmaceutical composition will vary based on the subject, the subjects height and

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weight and the severity of the disease. Ultimately, the use and schedule of administration of a pharmaceutical composition of the present invention will be decided by the treating physician, clinical protocols for determining dose range and scheduling are standard.

5 Example 1: Materials and Methods

Cloning of HMGB1 and Production of HMGB1 B Box Mutants

The following methods were used to prepare clones and mutants of human HMGB1. Recombinant full length human HMGB1 (651 base pairs; GenBank Accession Number U51677) was cloned by PCR amplification from a human brain

- 10 Quick-Clone cDNA preparation (Clontech, Palo Alto, CA) using the following primers; forward primer: 5' GATGGGCAAAGGAGATCCTAAG 3' (SEQ ID NO: 6) and reverse primer: 5' GCGGCCGCTTATTCATCATCATCATCTTC 3' (SEQ ID NO: 7). Human HMGB1 mutants were cloned and purified as follows. A truncated form of human HMGB1 was cloned by PCR amplification from a Human Brain
- 15 Quick-Clone cDNA preparation (Clontech, Palo Alto, CA). The primers used were (forward and reverse, respectively):

Carboxy terminus mutant (557 bp): 5' GATGGGCAAAGGAGATCCTAAG 3' (SEQ ID NO: 8) and 5' GCGGCCGC TCACTTGCTTTTTTCAGCCTTGAC 3' (SEQ ID NO: 9).

- 20 Amino terminus+B box mutant (486 bp): 5' GAGCATAAGAAGAAGCACCCA 3' (SEQ ID NO: 10) and 5' GCGGCCGC TCACTTGCTTTTTTCAGCCTTGAC 3' (SEQ ID NO: 11).

B box mutant (233 bp): 5' AAGTTCAAGGATCCCAATGCAAAG 3' (SEQ ID NO: 12) and 5' GCGGCCGCTCAATATGCAGCTATATCCTTTTC 3' (SEQ ID NO:

25 13).

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Amino terminus+A box mutant (261 bp): 5' GATGGGCAAAGGAGATCCTAAG 3' (SEQ ID NO: 13) and 5' TCACTTTTTTGTCTCCCTTTGGG 3' (SEQ ID NO: 14).

A stop codon was added to each mutant to ensure the accuracy of protein size. PCR products were subcloned into pCRII-TOPO vector EcoRI sites using the TA cloning method per manufacturer's instruction (Invitrogen, Carlsbad, CA). After amplification, the PCR product was digested with EcoRI and subcloned onto expression vector with a GST tag pGEX (Pharmacia); correct orientation and positive clones were confirmed by DNA sequencing on both strands. The recombinant plasmids were transformed into protease deficient *E. coli* strains BL21 or BL21(DE3)pLysS (Novagen, Madison, WI) and fusion protein expression was induced by isopropyl-D-thiogalactopyranoside (IPTG). Recombinant proteins were obtained using affinity purification with the glutathione Sepharose resin column (Pharmacia).

The HMGB mutants generated as described above have the following amino acid sequences:

Wild type HMGB1:

MGKGDPKKPTGKMSSYAFFVQTCREEHKKKHPDASVNFSEF
SKKCSERWKTMSAKEKGKFEDMAKADKARYEREMKTYIPPKGETKKKFKD
PNAPKRLPSAFFLFCSEYRPKIKGEHPGLSIGDVAKKLGEMWNNTAADDKQP
20 YEKKA AKLKEKYEKDIAAYRAKGKPDAAKKG VVKA ESKKKKEEEDEED
EEDEEEEEDEDEDEDEDDDE (SEQ ID NO: 18)

Carboxy terminus mutant:

MGKGDPKKPTGKMSSYAFFVQTCREEHKKKHPDAS
VNFSEFSKKCSERWKTMSAKEKGKFEDMAKADKARYEREMKTYIPPKGET
25 KKKFKDPNAPKRLPSAFFLFCSEYRPKIKGEHPGLSIGDVAKKLGEMWNNTA
ADDKQPYEKKA AKLKEKYEKDIAAYRAKGKPDAAKKG VVKA ESK (SEQ
ID NO: 19)

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B Box mutant:

FKDPNAPKRLPSAFFLFCSEYRPKIKGEHPGLSIGDVAKKLGEM
 WNNTAADDKQPYEKKAALKLEKEYEKDIAAY (SEQ ID NO: 20)

Amino terminus + A Box mutant:

- 5 MGKGDPPKPTGKMSSYAFFVQTCREEHKKK
 HPDASVNFSEFSKKCSERWKTMSAKEKGKFEDMAKADKARYEREMKTYT
 PKGET (SEQ ID NO: 21), wherein the A box consists of the sequence
 PTGKMSSYAFF
 VQTCREEHKKKHPDASVNFSEFSKKCSERWKTMSAKEKGKFEDMAKADK
 10 ARYEREMKTYIPPKGET (SEQ ID NO: 22)

- A polypeptide generated from a GST vector lacking HMGB1 protein was included as a control (containing a GST tag only). To inactivate the bacterial DNA that bound to the wild type HMGB1 and some of the mutants (carboxy terminus and B box), DNase I (Life Technologies), for carboxy terminus and B box mutants, or
 15 benzonase nuclease (Novagen, Madison, WI), for wild type HMGB1, was added at about 20 units/ml bacteria lysate. Degradation of DNA was verified by ethidium bromide staining of the agarose gel containing HMGB1 proteins before and after the treatment. The protein eluates were passed over a polymyxin B column (Pierce, Rockford, IL) to remove any contaminating LPS, and dialyzed extensively against
 20 phosphate buffered saline to remove excess reduced glutathione. The preparations were then lyophilized and redissolved in sterile water before use. LPS levels were less than 60 pg/μg protein for all the mutants and 300 pg/μg for wild type HMG-1 as measured by Limulus amoebocyte lysate assay (Bio Whittaker Inc., Walkersville, MD). The integrity of protein was verified by SDS-PAGE. Recombinant rat
 25 HMGB1 (Wang et al., Science 285: 248-251, 1999) was used in some experiments since it does not have degraded fragments as observed in purified human HMGB1.

Peptide Synthesis

Peptides were synthesized and HPLC purified at Utah State University Biotechnology Center (Logan, Utah) at 90% purity. Endotoxin was not detectable in the synthetic peptide preparations as measured by Limulus assay.

Cell Culture

- 5 Murine macrophage-like RAW 264.7 cells (American Type Culture Collection, Rockville, MD) were cultured in RPMI 1640 medium (Life Technologies, Grand Island NY) supplemented with 10% fetal bovine serum (Gemini, Catabasas, CA), penicillin and streptomycin (Life Technologies) and were used at 90% confluence in serum-free Opti-MEM I medium (Life Technologies, 10 Grand Island, NY). Polymyxin B (Sigma, St. Louis, MO) was routinely added at 100-1,000 units/ml to neutralize the activity of any contaminating LPS as previously described; polymyxin B alone did not influence cell viability assessed with trypan blue (Wang et al., supra). Polymyxin B was not used in experiments of synthetic peptide studies.

15 *Measurement of TNF Release From Cells*

- TNF release was measured by a standard murine fibroblast L929 (ATCC, American Type Culture Collection, Rockville, MD) cytotoxicity bioassay (Bianchi et al., Journal of Experimental Medicine 183:927-936, 1996) with the minimum detectable concentration of 30 pg/ml. Recombinant mouse TNF was obtained from 20 R&D system Inc., (Minneapolis, MN). Murine fibroblast L929 cells (ATCC) were cultured in DMEM (Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (Gemini, Catabasas, CA), penicillin (50 units/ml) and streptomycin (50 µg/ml) (Life Technologies) in a humidified incubator with 5% CO₂.

25 *Antibody Production*

- Polyclonal antibodies against HMGB1 B box were raised in rabbits (Cocalico Biologicals, Inc., Reamstown, PA) and assayed for titer by immunoblotting. IgG was purified from anti-HMGB1 antiserum using Protein A

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agarose according to manufacturer's instructions (Pierce, Rockford, IL). Anti-HMGB1 B box antibodies were affinity purified by using cyanogen bromide activated Sepharose beads (Cocalico Biological, Inc.). Non-immune rabbit IgG was purchased from Sigma (St. Louis, MO). Antibodies detected full length HMGB1 and B box in immunoassay, but did not cross react with TNF, IL-1 and IL-6.

Animal Experiments

TNF knock out mice were obtained from Amgen (Thousand Oaks, CA) and were on a B6x129 background. Age-matched wild-type B6x129 mice were used as control for the studies. Mice were bred in-house at the University of Florida specific pathogen-free transgenic mouse facility (Gainesville, FL) and were used at 6-8 weeks of age.

Male 6-8 week old Balb/c and C3H/HeJ mice were purchased from Harlan Sprague-Dawley (Indianapolis, IN) and were allowed to acclimate for 7 days before use in experiments. All animals were housed in the North Shore University Hospital Animal Facility under standard temperature, and a light and dark cycle.

D-galactosamine Sensitized Mice

The D-galactosamine-sensitized model has been described previously (Galanos et al., Proc Natl. Acad. Sci. USA 76: 5939-5943, 1979; and Lehmann et al., J. Exp. Med. 165: 657-663, 1997). Mice were injected intraperitoneally with 20 mg D-galactosamine-HCL (Sigma)/mouse (in 200 μ l PBS) and 0.1 or 1 mg of either HMGB1 B box or vector protein (in 200 μ l PBS). Mortality was recorded daily for up to 72 hours after injection; survivors were followed for 2 weeks, and no later deaths from B box toxicity were observed.

Statistical Analysis

Data are presented as mean \pm SEM unless otherwise stated. Differences between groups were determined by two-tailed Student's t-test, one-way ANOVA followed by the least significant difference test or 2 tailed Fisher's Exact Test.

Example 2: Mapping the HMGB1 Domains for Promotion of Cytokine Activity

HMGB1 has 2 folded DNA binding domains (A and B boxes) and a negatively charged acidic carboxyl tail. To elucidate the structural basis of HMGB1 cytokine activity, and to map the inflammatory protein domain, full length and truncated forms of HMGB1 were expressed by mutagenesis and the purified proteins were screened for stimulating activity in monocyte cultures (FIG. 1). Full length HMGB1, a mutant in which the carboxy terminus was deleted, a mutant containing only the B box, and a mutant containing only the A box were generated. These mutants of human HMGB1 were made by polymerase chain reaction (PCR) using specific primers as described herein, and the mutant proteins were expressed using a glutathione S-transferase (GST) gene fusion system (Pharmacia Biotech, Piscataway, NJ) in accordance with the manufacturer's instructions. Briefly, DNA fragments, made by PCR methods, were fused to GST fusion vectors and amplified in *E. coli*. The expressed HMGB1 protein and HMGB1 mutants were then isolated using GST affinity column.

The effect of the mutants on TNF release from Murine macrophage-like RAW 264.7 cells (ATCC) was carried out as follows. RAW 264.7 cells were cultured in RPMI 1640 medium (Life Technologies, Grand Island NY) supplemented with 10% fetal bovine serum (Gemini, Catabasas, CA), penicillin and streptomycin (Life Technologies). Polymyxin (Sigma, St. Louis, MO) was added at 100 units/ml to suppress the activity of any contaminating LPS. Cells were incubated with 1 µg/ml of full length (wild-type) HMGB1 and each HMGB1 mutant protein in Opti-MEM I medium for 8 hours, and conditioned supernatants (containing TNF which had been released from the cells) were collected and TNF released from the cells was measured by a standard murine fibroblast L929 (ATCC) cytotoxicity bioassay (Bianchi et al., supra) with the minimum detectable concentration of 30 pg/ml. Recombinant mouse TNF was obtained from R & D Systems Inc., (Minneapolis, MN) and used as control in these experiments. The results of this study are shown in FIG. 1. Data in FIG. 1 are all presented as mean ± SEM unless otherwise indicated. (N=6-10).

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As shown in FIG. 1, wild-type HMGB1 and carboxyl-truncated HMGB1 significantly stimulated TNF release by monocyte cultures (murine macrophage-like RAW 264.7 cells). The B box was a potent activator of monocyte TNF release. This stimulating effect of the B box was specific, because A box only weakly
5 activated TNF release.

Example 3: HMGB1 B Box Protein Promotes Cytokine Activity in a Dose Dependent Manner

To further examine the effect of HMGB1 B box on cytokine production, varying amounts of HMGB1 B box were evaluated for the effects on TNF, IL-1 β ,
10 and IL-6 production in murine macrophage-like RAW 264.7 cells. RAW 264.7 cells were stimulated with B box protein at 0-10 μ g/ml, as indicated in FIGS. 2A-2C for 8 hours. Conditioned media were harvested and measured for TNF, IL-1 β and IL-6 levels. TNF levels were measured as described herein, and IL-1 β and IL-6 levels were measured using the mouse IL-1 β and IL-6 enzyme-linked immunosorbent assay
15 (ELISA) kits (R&D System Inc., Minneapolis, MN) and N>5 for all experiments. The results of the studies are shown in FIGS. 2A-2C.

As shown in FIG. 2A, TNF release from RAW 264.7 cells increased with increased amounts of B box administered to the cells. As shown in FIG. 2B, addition of 1 μ g/ml or 10 μ g/ml of B box resulted in increased release of IL-1 β from
20 RAW 264.7 cells. In addition, as shown in FIG. 2C, IL-6 release from RAW 264.7 cells increased with increased amounts of B box administered to the cells.

The kinetics of B box-induced TNF release was also examined. TNF release and TNF mRNA expression was measured in RAW 264.7 cells induced by B box polypeptide or GST tag polypeptide only used as a control (vector) (10 μ g/ml) for 0
25 to 48 hours. Supernatants were analyzed for TNF protein levels by an L929 cytotoxicity assay (N=3-5) as described herein. For mRNA measurement, cells were plated in 100 mm plate and treated in Opti-MEM I medium containing B box polypeptide or the vector alone for 0, 4, 8, or 24 hours, as indicated in FIG. 2D. The vector only sample was assayed at the 4 hour time point. Cells were scraped off the
30 plate and total RNA was isolated by RNeasy method in accordance with the

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manufacturer's instructions (Tel-Test "B", Inc., Friendswood, TX). TNF (287 bp) was measured by RNase protection assay (Ambion, Austin, TX). Equal loading and the integrity of RNA was verified by ethidium bromide staining of the RNA sample on agarose-formaldehyde gel. The results of the RNase protection assay are shown in FIG. 2D. As shown in FIG. 2D, B box activation of monocytes occurred at the level of gene transcription, because TNF mRNA was increased significantly in monocytes exposed to B box protein (FIG. 2B). TNF mRNA expression was maximal at 4 hours and decreased at 8 and 24 hours. The vector only control (GST tag) showed no effect on TNF mRNA expression. A similar study was carried out measuring TNF protein released from RAW 264.7 cells 0, 4, 8, 24, 32 or 48 hours after administration of B box or vector only (GST tag), using the L929 cytotoxicity assay described herein. Compared to the control (medium only), B box treatment stimulated TNF protein expression (FIG. 2F) and vector alone (FIG. 2E) did not. Data are representative of three separate experiments. Together these data indicate that the HMGB1 B box domain has cytokine activity and is responsible for the cytokine stimulating activity of full length HMGB1.

In summary, the HMGB1 B box dose-dependently stimulated release of TNF, IL-1 β and IL-6 from monocyte cultures (FIGS. 2A-2C), in agreement with the inflammatory activity of full length HMGB1 (Andersson et al., J. Exp. Med. 192: 565-570, 2000). In addition, these studies indicate that maximum TNF protein release occurred within 8 hours (FIG. 2F). This delayed pattern of TNF release is similar to TNF release induced by HMGB1 itself, and is significantly later than the kinetics of TNF induced by LPS (Andersson et al., supra).

Example 4: The First 20 Amino Acids of the HMGB1 B Box Stimulate TNF Activity

The TNF-stimulating activity of the HMGB1 B box was further mapped. This study was carried out as follows. Fragments of the B box were generated using synthetic peptide protection techniques, as described herein. Five HMGB1 B box fragments (from SEQ ID NO: 20), containing amino acids 1-20, 16-25, 30-49, 45-64, or 60-74 of the HMGB1 B box were generated, as indicated in FIG. 3. RAW

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264.7 cells were treated with B box (1 μ g/ml) or a synthetic peptide fragment of the B box (10 μ g/ml), as indicated in FIG. 3 for 10 hours and TNF release in the supernatants was measured as described herein. Data shown are mean \pm SEM, (n=3 experiments, each done in duplicate and validated using 3 separate lots of synthetic peptides). As shown in FIG. 3, TNF-stimulating activity was retained by a synthetic peptide corresponding to amino acids 1-20 of the HMGB1 B box of SEQ ID NO: 20 (fkdpnapkrlpsafffcse; SEQ ID NO: 23). The TNF stimulating activity of the 1-20-mer was less potent than either the full length synthetic B box (1-74-mer), or full length HMGB1, but the stimulatory effects were specific because the synthetic 20-mers for amino acid fragments containing 16-25, 30-49, 45-64, or 60-74 of the HMGB1 B box did not induce TNF release. These results are direct evidence that the macrophage stimulating activity of the B box specifically maps to the first 20 amino acids of the HMGB B box domain of SEQ ID NO: 20). This B box fragment can be used in the same manner as a polypeptide encoding a full length B box polypeptide, for example, to stimulate release of a proinflammatory cytokine, or to treat a condition in a patient characterized by activation of an inflammatory cytokine cascade.

Example 5: HMGB1 B Box Protein is Toxic to D-galactosamine-sensitized Balb/c Mice

To investigate whether the HMGB1 B box has cytokine activity *in vivo*, we administered HMGB1 B box protein to unanesthetized Balb/c mice sensitized with D-galactosamine (D-gal), a model that is widely used to study cytokine toxicity (Galanos et al., supra). Briefly, mice (20-25 gram, male, Harlan Sprague-Dawley, Indianapolis, IN) were intraperitoneally injected with D-gal (20 mg) (Sigma) and B box (0.1 mg/ml/mouse or 1 mg/ml/mouse) or GST tag (vector; 0.1 mg/ml/mouse or 1 mg/ml/mouse), as indicated in Table 1. Survival of the mice was monitored up to 7 days to ensure no late death occurred. The results of this study are shown in Table 1.

Table 1: Toxicity of HMGB1 B box on D-galactosamine-sensitized Balb/c Mice

	Treatment	Alive/total
Control	-	10/10
Vector	0.1 mg/mouse	2/2
	1 mg/mouse	3/3
B box	0.1 mg/mouse	6/6
	1 mg/mouse	2/8*

5 P<0.01 versus vector alone as tested by Fisher's Exact Test

The results of this study showed that the HMGB1 B box was lethal to D-galactosamine-sensitized mice in a dose-dependent manner. In all instances in which death occurred, it occurred within 12 hours. Lethality was not observed in mice treated with comparable preparations of the purified GST vector protein devoid of B box.

Example 6: Histology of D-galactosamine-sensitized Balb/c Mice or C3H/HeJ Mice Administered HMGB1 B Box Protein

To further assess the lethality of the HMGB1 B box protein *in vivo* the HMGB1 B box was again administered to D-galactosamine-sensitized Balb/c mice. Mice (3 per group) received D-gal (20 mg/mouse) plus B box or vector (1 mg/mouse) intraperitoneally for 7 hours and were then sacrificed by decapitation. Blood was collected, and organs (liver, heart, kidney and lung) were harvested and fixed in 10% formaldehyde. Tissue sections were prepared with hematoxylin and eosin staining for histological evaluation (Criterion Inc., Vancouver, Canada). The results of these studies are shown in FIGS. 4A-4J, which are scanned images of hematoxylin and eosin stained kidney sections (FIG. 4A), myocardium sections (FIG. 4C), lung sections (FIG. 4F), and liver sections (FIGS. 4G and 4I) obtained from an untreated mouse and kidney sections (FIG. 4B), myocardium sections (FIG. 4D), lung sections (FIG. 4F), and liver sections (FIGS. 4H and 4J) obtained from mice treated with the HMGB1 B box. Compared to the control mice, B box

treatment caused no abnormality in kidneys (FIGS. 4A and 4B) and lungs (FIGS. 4E and 4F). The mice had some ischemic changes and loss of cross striation in myocardial fibers in the heart (FIGS. 4C and 4D as indicated by the arrow in FIG. 4D). Liver showed most of the damage by the B box as illustrated by active hepatitis (FIGS. 4G-4J). In FIG. 4J, hepatocyte dropouts are seen surrounded by accumulated polymorphonuclear leukocytes. The arrows in FIG. 4J point to the sites of polymorphonuclear accumulation (dotted) or apoptotic hepatocytes (solid). Administration of HMGB1 B box *in vivo* also stimulated significantly increased serum levels of IL-6 (315±93 vs. 20±7 pg/ml, B box vs. control, $p<0.05$) and IL-1 β (15±3 vs. 4±1 pg/ml, B box vs. control, $p<0.05$).

Administration of B box protein to C3H/HeJ mice (which do not respond to endotoxin) was also lethal, indicating that HMGB1 B box is lethal in the absence of LPS signal transduction. Hematoxylin and eosin stained sections of lung and kidney collected 8 hours after administration of B box revealed no abnormal morphologic changes. Examination of sections from the heart however, revealed evidence of ischemia with loss of cross striation associated with amorphous pink cytoplasm in myocardial fibers. Sections from liver showed mild acute inflammatory responses, with some hepatocyte dropout and apoptosis, and occasional polymorphonuclear leukocytes. These specific pathological changes were comparable to those observed after administration of full length HMGB1 and confirm that the B box alone can recapitulate the lethal pathological response to HMGB1 *in vivo*.

To address whether the TNF-stimulating activity of HMGB1 contributes to the mediation of lethality by B box, we measured lethality in TNF knock-out mice (TNF-KO, Nowak et al., Am. J. Physiol. Regul. Integr. Comp. Physiol. 278: R1202-R1209, 2000) and the wild-type controls (B6x129 strain) sensitized with D-galactosamine (20 mg/mouse) and exposed to B box (1 mg/mouse, injected intraperitoneally). The B box was highly lethal to the wild-type mice (6 dead out of nine exposed) but lethality was not observed in the TNF-KO mice treated with B box (0 dead out of 9 exposed, $p<0.05$ v. wild type). Together with the data from the RAW 264.7 macrophage cultures, described herein, these data now indicate that the B box of HMGB1 confers specific TNF-stimulating cytokine activity.

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While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

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Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

10

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of

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endeavour to which this specification relates.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A pharmaceutical composition comprising a polypeptide consisting of an HMGB B box, a biologically active fragment thereof, or a functional variant thereof, in an amount sufficient to treat a disease or condition by increasing an immune response in an individual administered said pharmaceutical composition.
2. The pharmaceutical composition of Claim 1, wherein said HMGB B box is mammalian.
3. The pharmaceutical composition of Claim 2, wherein said HMGB B box is human.
4. The pharmaceutical composition of Claim 3, wherein said polypeptide comprises an HMGB1 B box polypeptide.
5. The pharmaceutical composition of Claim 4, wherein said polypeptide consists of an HMGB1 B box polypeptide.
6. The pharmaceutical composition of Claim 1, further comprising a vaccine.
7. The pharmaceutical composition of Claim 6, further comprising an adjuvant.
8. The pharmaceutical composition of Claim 7, wherein said adjuvant is selected from the group consisting of one or more immunostimulatory oligonucleotides, an imidazoquinoline, monophosphoryl lipid A, and detoxified lipopolysaccharide.
9. The pharmaceutical composition of Claim 8, wherein said immunostimulatory oligonucleotides comprise unmethylated CpG sequences.
10. An antibody attached to a polypeptide comprising an HMGB B box or a functional variant thereof.

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11. The antibody of Claim 10, wherein said HMGB B box is mammalian.
12. The antibody of Claim 11, wherein said HMGB B box is human.
13. The antibody of Claim 12, wherein said polypeptide comprises an HMGB1 B box polypeptide.
14. The antibody of Claim 13, wherein said polypeptide consists of an HMGB1 B box polypeptide.
15. The antibody of Claim 10, wherein said antibody binds a tumor-associated polypeptide.
16. The antibody of Claim 10, wherein said antibody is in a pharmaceutically acceptable carrier.
17. A method of stimulating or increasing an immune response in an individual in need of immunostimulation, said method comprising administering to said individual a polypeptide consisting of an HMGB B box, a biologically active fragment thereof, or a functional variant thereof, in a amount sufficient to stimulate or increase said immune response.
18. The method of Claim 17, wherein said individual is being treated for cancer.
19. The method of Claim 17, wherein said HMGB B box is mammalian.
20. The method of Claim 19, wherein said HMGB B box is human.
21. The method of Claim 20, wherein said polypeptide comprises an HMGB1 B box.

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22. The method of Claim 21, wherein said polypeptide consists of an HMGB1 B box.
23. The method of Claim 17, wherein said polypeptide is co-administered with a vaccine.
24. The method of Claim 23, wherein said polypeptide is co-administered with a further adjuvant.
25. The method of Claim 24, wherein said adjuvant is selected from the group consisting of one or more immunostimulatory oligonucleotides, an imidazoquinoline, monophosphoryl lipid A, and detoxified lipopolysaccharide.
26. The method of Claim 25, wherein said immunostimulatory oligonucleotides comprise unmethylated CpG sequences.
27. The method of Claim 17, wherein said administration is systemic.
28. The method of Claim 17, wherein said administration is localized to a target site.
29. The method of Claim 17, wherein said polypeptide is attached to all antibody specific to a target site in the individual in need of immunostimulation.
30. The method of Claim 17, wherein said polypeptide is in a pharmaceutically acceptable carrier.
31. A method of treating cancer in an individual, said method comprising administering to said individual a therapeutically effective amount of a polypeptide consisting of an HMGB B box, a biologically active fragment thereof, or a functional variant thereof.
32. The method of Claim 31, wherein said HMGB B box is mammalian.

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33. The method of Claim 32, wherein said HMGB B box is human.
34. The method of Claim 33, wherein said polypeptide comprises an HMGB1 B box polypeptide.
35. The method of Claim 34, wherein said polypeptide consists of an HMGB1 B box polypeptide.
36. The method of Claim 31, wherein said polypeptide is co-administered with a vaccine.
37. The method of Claim 36, wherein said polypeptide is co-administered with a further adjuvant.
38. The method of Claim 37, wherein said adjuvant is selected from the group consisting of one or more immunostimulatory oligonucleotides, an imidazoquinoline, monophosphoryl lipid A, and detoxified lipopolysaccharide.
39. The method of Claim 38, wherein said immunostimulatory oligonucleotides comprise unmethylated CpG sequences.
40. The method of Claim 31, wherein said administration is systemic.
41. The method of Claim 31, wherein said administration is localized to a target site.
42. The method of Claim 41, wherein said target site is a tumor.
43. The method of Claim 31, wherein said polypeptide is attached to an antibody.
44. The method of Claim 43, wherein said antibody binds a tumor-associated

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polypeptide.

45. The method of Claim 31, wherein said polypeptide is in a pharmaceutically acceptable carrier.

46. A pharmaceutical composition according to any one of Claims 1 to 9 or an antibody according to any one of Claims 10 to 16 or a method according to any one of Claims 17 to 45 substantially as hereinbefore described with reference to the Figures and Examples.

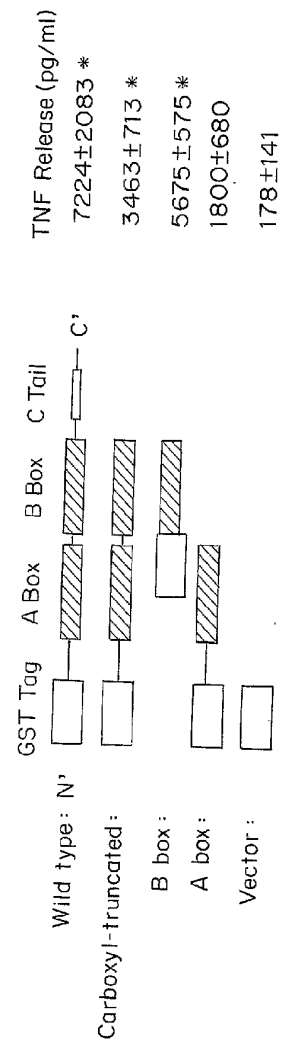


FIG. 1

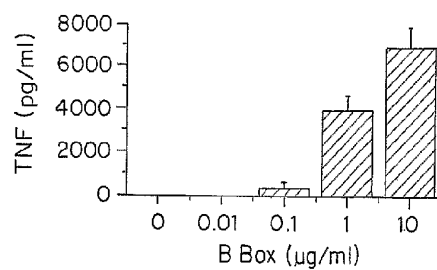


FIG. 2A

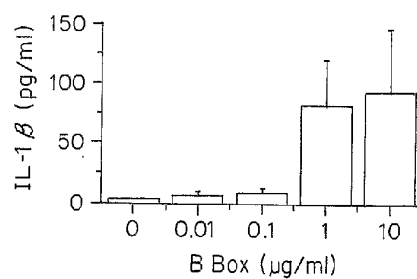


FIG. 2B

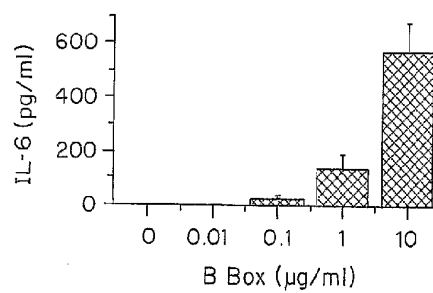


FIG. 2C

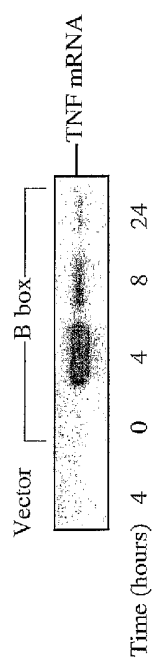


FIG. 2D

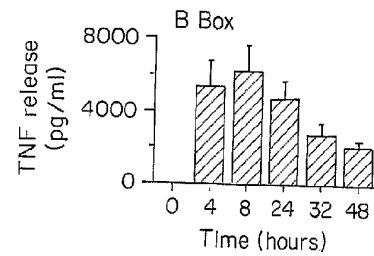


FIG. 2E

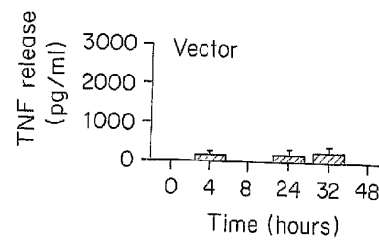


FIG. 2F

B box mutants	TNF release (pg/ml)
B box: 74 amino acids	5675±575
1-20	2100±756
16-35	100±10
30-49	120±75
45-64	100±36
60-74	100±20

FIG. 3

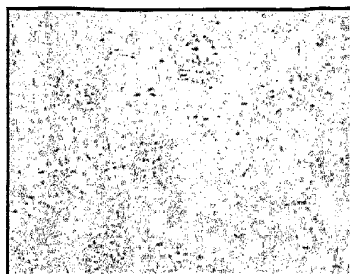


FIG. 4A

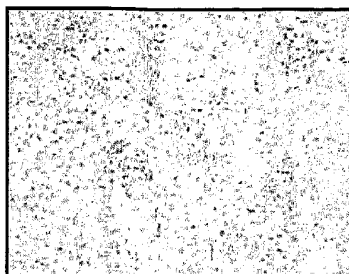


FIG. 4B

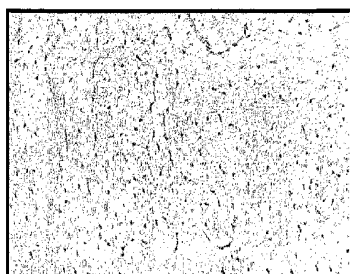


FIG. 4C

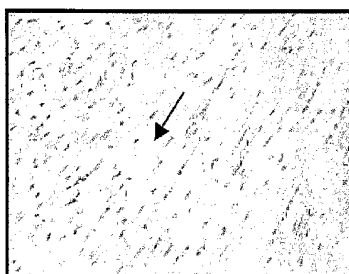


FIG. 4D



FIG. 4E

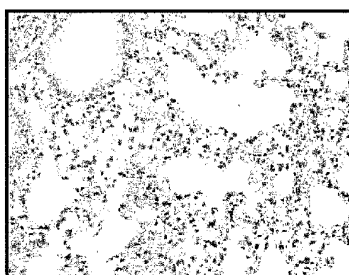


FIG. 4F

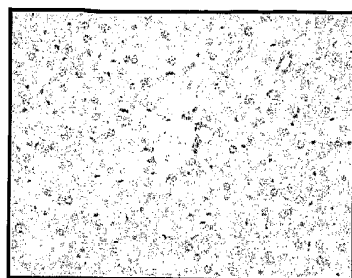


FIG. 4G

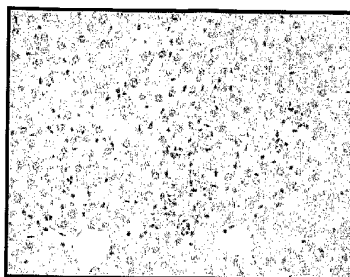


FIG. 4H

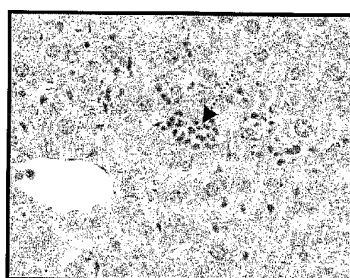


FIG. 4I

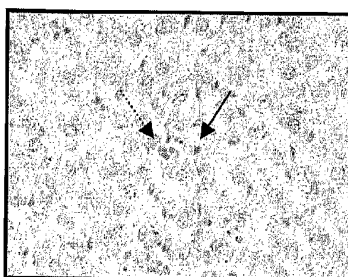


FIG. 4J

FIG. 5A

SEQ ID NO:1 - Human HMG1 amino acid sequence

1 mgkgdppkpr gkmssyaffv qtcreehkkk hpdasvnfse fskkcserwk tmsakekgkf
 61 edmakadkar yeremktyip pkgetkkkkf dpnapkrpps afflfcseyr pkikgehppl
 121 sigdvakklg emwnntaadd kqpyckkaak lkekyekdia ayrakgkpda akkgvvkaek
 181 skkkkeeedd eedeedeeee eedeedeeee dddde

FIG. 5B

SEQ ID NO:2 - Mouse and Rat HMG1 amino acid sequence

1 mgkgdppkpr gkmssyaffv qtcreehkkk hpdasvnfse fskkcserwk tmsakekgkf
 61 edmakadkar yeremktyip pkgetkkkkf dpnapkrpps afflfcseyr pkikgehppl
 121 sigdvakklg emwnntaadd kqpyckkaak lkekyekdia ayrakgkpda akkgvvkaek
 181 skkkkeeedd eedeedeeee eedeedeeee dddde

FIG. 5C

SEQ ID NO:3 - HUMAN HMG2 amino acid sequence

1 mgkgdppkpr gkmssyaffv qtcreehkkk hpdssvnfae fskkcserwk tmsakekskf
 61 edmakadkar ydremknyvp pkgdtkgkkk dpnapkrpps afflfcsehr pkiksehppl
 121 sigdtakklg emwseksakd kqpyeqkaak lkekyekdia ayrakgksea gkgggrptg
 181 skkknepcde eeeeeedeed eedeedeeee

FIG. 5D

SEQ ID NO:4 - Human, mouse and rat HMG1 A box protein sequence

1 pdasvnfsef skkcserwkt msakekgkfe dmakadkary eremktyipp kget

FIG. 5E

SEQ ID NO:5 - Human, mouse and rat HMG1 B box protein sequence

1 napkrppsaf flfcseyrpk ilgehpqisi gdvakklgem wnntaaddkq pyckkaaklk
 61 ekyekdiaa

FIG. 5F

SEQ ID NO:6 - forward PCR primer for human HMG1

gatgggcaaaggagatcctaag.

FIG. 5G

SEQ ID NO:7 - reverse PCR primer for human HMG1

gcggccgcttattcatcatcatcttc

FIG. 5H

SEQ ID NO:8 - forward PCR primer for -C mutant of human HMG1

gatgggcaaaggagatcctaag

FIG. 5I

SEQ ID NO:9 - reverse PCR primer for -C mutant of human HMG1
gcggccgctcactgtctttttcagccttgac

FIG. 5J

SEQ ID NO:10 - forward PCR primer for A+B boxes mutant of human HMG1
gagcataagaagaagcaccca

FIG. 5K

SEQ ID NO:11 - reverse PCR primer for A+B boxes mutant of human HMG1
gcggccgc tcaactgctttttcagccttgac

FIG. 5L

SEQ ID NO:12 - forward PCR primer for B box mutant of human HMG1
aagttcaaggatcccaatgcaag

FIG. 5M

SEQ ID NO:13 - reverse PCR primer for B box mutant of human HMG1
gcggccgctcaalagcagctatatccttttc

FIG. 5N

SEQ ID NO:14 - forward PCR primer for N⁷+A box mutant of human HMG1
gatgggcaaggagatcctaag

FIG. 5O

SEQ ID NO:15 - reverse PCR primer for N⁷-A box mutant of human HMG1
tcactttttgtctccccttggg

1 mgkgdpkkpr gkmssyaffv qcreehkkk hpdasvnfse fskkcserwk tmsakekgkf *rat* # P07155
 1 mgkgdpkkpr gkmssyaffv qcreehkkk hpdasvnfse fskkcserwk tmsakekgkf *mouse* #AAA20508
 1 mgkgdpkkpr gkmssyaffv qcreehkkk hpdasvnfse fskkcserwk tmsakekgkf *human* #AAA64970

A box

61 edmakadkar yeremktyip pkgetkkkfk dnapkrpps afffcseyr pkikgehppl *rat*
 61 edmakadkar yeremktyip pkgetkkkfk dnapkrpps afffcseyr pkikgehppl *mouse*
 61 edmakadkar yeremktyip pkgetkkkfk dnapkrpps afffcseyr pkikgehppl *human*

B box

121 sigdvakkdg emwnntaadd kqpyekkaak lkekyekdia ayrakgkpa akkgvvkaek *rat*
 121 sigdvakkdg emwnntaadd kqpyekkaak lkekyekdia ayrakgkpa akkgvvkaek *mouse*
 121 sigdvakkdg emwnntaadd kqpyekkaak lkekyekdia ayrakgkpa akkgvvkaek *human*

181 skkkkeeedd eedeeceeee eeeeda deee dddde *rat*
 181 skkkkeeedd eedeeceeee eeeeda deee dddde *mouse*
 181 skkkkeeedd eedeeceeee eedeeceede deee dddde *human*

FIG. 6

FIG. 7A**NG_000897 DNA** (bases 150-797)

```

ATGGGCAAAAG GAGATCCTAA GAAGCCGACA GGCAAAATGT CATCATATGC
ATTTTTTGTG CAAACTTGTC GGGAGGAGCA TAAGAAGAAG CACCCAGATG
CTTCAGTCAA CTTCTCAGAG TTTTCTAAGA AGTGCTCAGA GAGGTGGAAG
ACCATGTCTG CTAAAGAGAA AGGAAAATTT GAAGATATGG CAAAGGCGGA
CAAGGCCCGT TATGAAAGAG AAATGAAAAC CTATATCCCT CCCAAAGGGG
AGACAAAAAA GAAGTTCAAG GATCCCAATG CACCCAAGAG GCTTCCTTCG
GCCTTCTTCC TCTTCTGCTC TGAGTATCGC CCAAAAATCA AAGGAGAACA
TCCTGGCCTG TCATTGGTG ATGTTGCGAA GAAACTGGGA GAGATGTGGA
ATAACACTGC TGCAGATGAC AAGCAGCCTT ATGAAAAGAA GGCTGCGAAG
CTGAAGGAAA AATACGAAAA GGATATAGCT GCATATCGAG CTAAGGAAA
GCCTGATGCA GCAAAAAGG GAGTTGTCAA GGCTGAAAAA AGCAAGAAAA
AGAAGGAAGA GGAGGAAGAT GAGGAAGATG AAGAGGATGA GGAGGAGGAG
GAAGATGAAG AAGATGAAGA AGATGAAGAA GAAGATGATG ATGATGAA

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FIG. 7B**NG_000897 Protein**

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MGKGDPPKPT GKSSSYAFFV QTCREEHKKK HPDASVNFSE FSKKCSERWK
TMSAKEKGKF EDMAKADKAR YEREMKTYIP PKGETKKKFK DPNAPKRLPS
AFPLFCSEYR PKIKGEHPGL SIGDVAKKLG EMWNNTAADD KQPYEKKAOK
LKEKYEKIDIA AYRAKGKPDIA AKKGVVKAOK SKKKKEEED EEEEEDEEEE
EEEEDEEEE EDDDDDE

```

FIG. 7C**AF076674 DNA** (bases 1-633)

```

ATGGGCAAAAG GAGATCCTAA GAAGCCGAGA GGCAAAATGT CATCATATGC
ATTTTTTGTG CAAACTTGTC GGGAGGAGCA TAAGAAGAAG CACTCAGATG
CTTCAGTCAA CTTCTCAGAG TTTTCTAACA AGTGCTCAGA GAGGTGGAAG
ACCATGTCTG CTAAAGAGAA AGGAAAATTT GAGGATATGG CAAAGGCGGA
CAAGACCCAT TATGAAAGAC AAATGAAAAC CTATATCCCT CCCAAAGGGG
AGACAAAAAA GAAGTTCAAG GATCCCAATG CACCCAAGAG GCCTCCTTCG
GCCTTCTTCC TGTCTGCTC TGAGTATCAC CCAAAAATCA AAGGAGAACA
TCCTGGCCTG TCATTGGTG ATGTTGCGAA GAAACTGGGA GAGATGTGGA
ATAACACTGC TGCAGATGAC AAGCAGCCTG GTGAAAAGAA GGCTGCGAAG
CTGAAGGAAA AATACGAAAA GGATATTGCT GCATATCAAG CTAAGGAAA
GCCTGAGGCA GCAAAAAGG GAGTTGTCAA AGCTGAAAAA AGCAAGAAAA
AGAAGGAAGA GGAGGAAGAT GAGGAAGATG AAGAGGATGA GGAGGAGGAA
GATGAAGAAG ATGAAGAAGA TGATGATGAT GAA

```

FIG. 7D**AF076674 Protein**

```

MGKGDPPKPR GKSSSYAFFV QTCREEHKKK HSDASVNFSE FSNKCSERWK
TMSAKEKGKF EDMAKADKTH YERQMKTYIP PKGETKKKFK DPNAPKRPFS
AFPLFCSEYH PKIKGEHPGL SIGDVAKKLG EMWNNTAADD KQPGKKKAOK
LKEKYEKIDIA AYQAKGKPEA AKKGVVKAOK SKKKKEEED EEEEEDEEEE
DEEDEDDEDD E

```

FIG. 7E**AF076676 DNA** (bases 1-564)

ATGGGCAAAG GAGACCCCTAA GAAGCCGAGA GGCAAAATGT CATCATATGC
 ATTTTTTGTG CAAACTTGTC GGGAGGAGTG TAAGAAGAAG CACCCAGATG
 CTTCAGTCAA CTTCTCAGAG TTTTCTAAGA AGTGCTCAGA GAGGTGGAAG
 GCCATGTCTG CTAAAGATAA AGGAAAATTT GAAGATATGG CAAAGGTGGA
 CAAAGACCGT TATGAAAGAG AAATGAAAAC CTATATCCCT CCTAAAGGGG
 AGACAAAAAA GAAGTTCGAG GATTCCAATG CACCCAAGAG GCCTCCTTCG
 GCCTTTTTGC TGTCTGCTC TGAGTATTGC CCAAAAATCA AAGGAGAGCA
 TCCTGGCCTG CCTATTAGCG ATGTTGCAAA GAAACTGGTA GAGATGTGGA
 ATAACACTTT TGCAGATGAC AAGCAGCTTT GTGAAAAGAA GGCTGCAAAG
 CTGAAGGAAA AATACAAAAA GGATACAGCT ACATATCGAG CTAAAGGAAA
 GCCTGATGCA GCAAAAAAGG GAGTTGTCAA GGCTGAAAAA AGCAAGAAAA
 AGAAGGAAGA GGAG

FIG. 7F**AF076676 Protein**

MGKGDPPKKPR GKMSYAFFV QTCREECKKK HPDASVNFSE FSKKCSERWK
 AMSAKDKGKF EDMAKVDKDR YEREMKTYIP PKGETKKKFE DSNAPKRPPS
 AFLLFCSEYC PKIKGEHPGL PISDVAKKLV EMWNNTFADD KQLCEKKAAC
 LKEKYKKDTA TYRAKGKPKDA AKKGVVKAER SKKKKEEE

FIG. 7G**AC010149 DNA** (bases 75503-76117)

ATGGACAAAG CAGATCCTAA GAAGCTGAGA GGTGAAATGT TATCATATGC
 ATTTTTTGTG CAAACTTGTC AGGAGGAGCA TAAGAAGAAG AACCAGATG
 CTTCAGTCAA GTTCTCAGAG TTTTAAAGA AGTGCTCAGA GACATGGAAG
 ACCATTTTTG CTAAAGAGAA AGGAAAATTT GAAGATATGG CAAAGGCGGA
 CAAGGCCAT TATGAAAGAG AAATGAAAAC CTATATCCCT CCTAAAGGGG
 AGAAAAAAA GAAGTTCAAG GATCCCAATG CACCCAAGAG GCCTCCTTTG
 GCCTTTTTCC TGTCTGCTC TGAGTATCGC CCAAAAATCA AAGGAGAACA
 TCCTGGCCTG TCCATTGATG ATGTTGTGAA GAACTGGCA GGGATGTGGA
 ATAACACCGC TGCAGCTGAC AAGCAGTTTT ATGAAAAGAA GGCTGCAAAG
 CTGAAGGAAA AATACAAAAA GGATATTGCT GCATATCGAG CTAAAGGAAA
 GCCTAATTCA GCAAAAAAGA GAGTTGTCAA GGCTGAAAAA AGCAAGAAAA
 AGAAGGAAGA GGAAGAAGAT GAACAGGATG AACAAGAGGA GGAAAATGAA
 GAAGATGATG ATAAA

FIG. 7H**AC010149 Protein**

MDKADPKKLR GEMLSYAFFV QTCQEEHKKK NPDASVKFSE FLKKCSETWK
 TIFAKEKGKF EDMAKADKAH YEREMKTYIP PKGEKKKKFK DPNAPKRPPPL
 AFLLFCSEYR PKIKGEHPGL SIDDVVKKLA GMWNNTAAAD KQFYEKKAAC
 LKEKYKKDIA AYRAKGKPNs AKKRVVKAER SKKKKEEED EEDQEEENE
 EDDDK

FIG. 7I**AF165168 DNA** (bases 729-968)

ATGGGCAAG GAGATCCTAA GAAGCCGAGA GGCAAAATGT CATCATGTGC
ATTTTGTGTG CAAACTTGTT GGGAGGAGCA TAAGAAGCAG TACCCAGATG
CTTCAATCAA CTTCTCAGAG TTTTCTCAGA AGTGCCGAGA GACGTGGAAG
ACCACGATTG CTAAAGAGAA AGGAAAATTT GAAGATATGC CAAAGGCAGA
CAAGGCCCAT TATGAAAGAG AAATGAAAAC CTATATACCC

FIG. 7J**AF165168 Protein**

MGKGDPPKPR GKSSCAFFV QTCWEHHKQ YPDASINFSE FSQKCPETWK
TTIAKEKGKF EDMPKADKAH YEREMKTYIP

FIG. 7K**XM_063129 DNA** (bases 319-558)

AAACAGAGAG GCAAAATGCC ATCGTATGTA TTTTGTGTGC AAACCTTGTC
GGAGGAGCGT AAGAAGAAAC ACCCAGATGC TTCAGTCAAC TTCTCAGAGT
TTTCTAAGAA GTGCTTAGTG AGGGGGAAGA CCATGTCTGC TAAAGAGAAA
GGACAAATTG AAGCTATGGC AAGGGCAGAC AAGGCCCGTT ACGAAAGAGA
AATGAAAACA TATATCCCTC CTAAAGGGGA GACAAAAAAA

FIG. 7L**XM_063129 Protein**

KQRGKMPSYV FCVQTCPEER KKKHPDASVN FSEFSKKCLV RGKIMSAREK
GQFEAMARAD KARYEREMKT YIPPKGETKK

FIG. 7M**XM_066789 DNA** (bases 1-258)

ATGGGCAAAA GAGACCCCTAA GCAGCCAAGA GGCAAAATGT CATCATATGC
ATTTTGTGTG CAAACTGCTC AGGAGGAGCA CAAGAAGAAA CAACTAGATG
CTTCAGTCAG TTTCTCAGAG TTTTCTAAGA ACTGCTCAGA GAGGTGGAAG
ACCATGCTCG TTAAAGAGAA AGGAAAATTT GAAGACATGG CAAAGGCAGA
CAAGGCCCTGT TATGAAAGAG AAATGAAAAT ATATCCCTAC TTAAAGGGGA
GACAAAAA

FIG. 7N**XM_066789 Protein**

MGKRDPKQPR GKSSYAFFV QTAQEEHKKK QLDASVSFSE FSKNCSEKWK
TMSVKEKGKF EDMAKADKAC YEREMKIYPY LKGRQK

FIG. 7O**AF165167 DNA** (bases 456-666)

```
ATGGGC AAA GAGACCCTAA GAAGCCAAGA GAGAAAATGC CATCATATGC
ATTTTTGTG CAAACTTGTA GGGAGGCACA TAAGAACAAA CATCCAGATC
CTTCAGTCAA CTCCTCAGAG TTTTCTAAGA AGTGCTCAGA GAGGTGGAAG
ACCATGCCTA CTAAACAGAA AGGAAAATTC GAAGATATGG CAAAGGCAGA
CAGGGCCCAT A
```

FIG. 7P**AF165167 Protein**

```
MGKGD PKKPR EKMP SYAFFV QTCREAHKNK HPDASVNSSE FSKKCSERWK
TMPTK QKGKF EDMAKADRAH
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SEQUENCE LISTING

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IMMUNE RESPONSES

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<150> 60/427,848

<151> 2002-11-20

<160> 45

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 20          25          30
Asp Ala Ser Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg
 35          40          45
Trp Lys Thr Met Ser Ala Lys Glu Lys Gly Lys Phe Glu Asp Met Ala
 50          55          60
Lys Ala Asp Lys Ala Arg Tyr Glu Arg Glu Met Lys Thr Tyr Ile Pro
 65          70          75          80
Pro Lys Gly Glu Thr Lys Lys Lys Phe Lys Asp Pro Asn Ala Pro Lys
 85          90          95
Arg Pro Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu Tyr Arg Pro Lys
100          105          110
Ile Lys Gly Glu His Pro Gly Leu Ser Ile Gly Asp Val Ala Lys Lys
115          120          125
Leu Gly Glu Met Trp Asn Asn Thr Ala Ala Asp Asp Lys Gln Pro Tyr
130          135          140
Glu Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu Lys Asp Ile Ala
145          150          155          160
Ala Tyr Arg Ala Lys Gly Lys Pro Asp Ala Ala Lys Lys Gly Val Val
165          170          175
Lys Ala Glu Lys Ser Lys Lys Lys Lys Glu Glu Glu Glu Asp Glu Glu
180          185          190
Asp Glu Glu Asp Glu Glu Glu Glu Asp Glu Glu Asp Glu Asp Glu
195          200          205
Glu Glu Asp Asp Asp Asp Glu
210          215

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<210> 2

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<212> PRT

<213> Mus musculus

<400> 2

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Met Gly Lys Gly Asp Pro Lys Lys Pro Arg Gly Lys Met Ser Ser Tyr
 1          5          10          15
Ala Phe Phe Val Gln Thr Cys Arg Glu Glu His Lys Lys Lys His Pro

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      20      25      30
Asp Ala Ser Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg
      35      40      45
Trp Lys Thr Met Ser Ala Lys Glu Lys Gly Lys Phe Glu Asp Met Ala
      50      55      60
Lys Ala Asp Lys Ala Arg Tyr Glu Arg Glu Met Lys Thr Tyr Ile Pro
      65      70      75      80
Pro Lys Gly Glu Thr Lys Lys Lys Phe Lys Asp Pro Asn Ala Pro Lys
      85      90      95
Arg Pro Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu Tyr Arg Pro Lys
      100      105      110
Ile Lys Gly Glu His Pro Gly Leu Ser Ile Gly Asp Val Ala Lys Lys
      115      120      125
Leu Gly Glu Met Trp Asn Asn Thr Ala Ala Asp Asp Lys Gln Pro Tyr
      130      135      140
Glu Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu Lys Asp Ile Ala
      145      150      155      160
Ala Tyr Arg Ala Lys Gly Lys Pro Asp Ala Ala Lys Lys Gly Val Val
      165      170      175
Lys Ala Glu Lys Ser Lys Lys Lys Lys Glu Glu Glu Asp Asp Glu Glu
      180      185      190
Asp Glu Glu Asp Glu Glu Glu Glu Glu Glu Asp Glu Asp Glu
      195      200      205
Glu Glu Asp Asp Asp Glu
      210      215

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<212> PRT
<213> Homo sapiens

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<400> 3
Met Gly Lys Gly Asp Pro Asn Lys Pro Arg Gly Lys Met Ser Ser Tyr
      1      5      10      15
Ala Phe Phe Val Gln Thr Cys Arg Glu Glu His Lys Lys Lys His Pro
      20      25      30
Asp Ser Ser Val Asn Phe Ala Glu Phe Ser Lys Lys Cys Ser Glu Arg
      35      40      45
Trp Lys Thr Met Ser Ala Lys Glu Lys Ser Lys Phe Glu Asp Met Ala
      50      55      60
Lys Ser Asp Lys Ala Arg Tyr Asp Arg Glu Met Lys Asn Tyr Val Pro
      65      70      75      80
Pro Lys Gly Asp Lys Lys Gly Lys Lys Lys Asp Pro Asn Ala Pro Lys
      85      90      95
Arg Pro Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu His Arg Pro Lys
      100      105      110
Ile Lys Ser Glu His Pro Gly Leu Ser Ile Gly Asp Thr Ala Lys Lys
      115      120      125
Leu Gly Glu Met Trp Ser Glu Gln Ser Ala Lys Asp Lys Gln Pro Tyr
      130      135      140
Glu Gln Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu Lys Asp Ile Ala
      145      150      155      160
Ala Tyr Arg Ala Lys Gly Lys Ser Glu Ala Gly Lys Lys Gly Pro Gly
      165      170      175
Arg Pro Thr Gly Ser Lys Lys Lys Asn Glu Pro Glu Asp Glu Glu Glu
      180      185      190
Glu Glu Glu Glu Asp Glu Asp Glu Glu Glu Asp Glu Asp Glu
      195      200      205
Glu

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<210> 4
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 <213> Homo sapiens

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 Pro Asp Ala Ser Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu
 1 5 10 15
 Arg Trp Lys Thr Met Ser Ala Lys Glu Lys Gly Lys Phe Glu Asp Met
 20 25 30
 Ala Lys Ala Asp Lys Ala Arg Tyr Glu Arg Glu Met Lys Thr Tyr Ile
 35 40 45
 Pro Pro Lys Gly Glu Thr
 50

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 <211> 69
 <212> PRT
 <213> Homo sapiens

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 Asn Ala Pro Lys Arg Pro Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu
 1 5 10 15
 Tyr Arg Pro Lys Ile Lys Gly Glu His Pro Gly Leu Ser Ile Gly Asp
 20 25 30
 Val Ala Lys Lys Leu Gly Glu Met Trp Asn Asn Thr Ala Ala Asp Asp
 35 40 45
 Lys Gln Pro Tyr Glu Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu
 50 55 60
 Lys Asp Ile Ala Ala
 65

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 <213> Homo sapiens

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<210> 8
 <211> 22
 <212> DNA
 <213> Homo sapiens

<400> 8
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<210> 9
 <211> 32
 <212> DNA
 <213> Homo sapiens

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<212> DNA
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<400> 10
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<210> 11
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<212> DNA
<213> Homo sapiens

<400> 11
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<210> 12
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<213> Homo sapiens

<400> 12
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<210> 13
<211> 32
<212> DNA
<213> Homo sapiens

<400> 13
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<210> 14
<211> 22
<212> DNA
<213> Homo sapiens

<400> 14
gatgggcaaa ggagatccta ag          22

<210> 15
<211> 24
<212> DNA
<213> Homo sapiens

<400> 15
tcactttttt gtctccctt tggg          24

<210> 16
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<212> PRT
<213> Homo sapiens

<400> 16
Asn Ala Pro Lys Arg Pro Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu
 1           5           10           15
Tyr Arg Pro Lys
                20

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<210> 17
 <211> 74
 <212> PRT
 <213> Homo sapiens

<400> 17
 Phe Lys Asp Pro Asn Ala Pro Lys Arg Pro Pro Ser Ala Phe Phe Leu
 1 5 10 15
 Phe Cys Ser Glu Tyr Arg Pro Lys Ile Lys Gly Glu His Pro Gly Leu
 20 25 30
 Ser Ile Gly Asp Val Ala Lys Lys Leu Gly Glu Met Trp Asn Asn Thr
 35 40 45
 Ala Ala Asp Asp Lys Gln Pro Tyr Glu Lys Lys Ala Ala Lys Leu Lys
 50 55 60
 Glu Lys Tyr Glu Lys Asp Ile Ala Ala Tyr
 65 70

<210> 18
 <211> 216
 <212> PRT
 <213> Homo sapiens

<400> 18
 Met Gly Lys Gly Asp Pro Lys Lys Pro Thr Gly Lys Met Ser Ser Tyr
 1 5 10 15
 Ala Phe Phe Val Gln Thr Cys Arg Glu Glu His Lys Lys Lys His Pro
 20 25 30
 Asp Ala Ser Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg
 35 40 45
 Trp Lys Thr Met Ser Ala Lys Glu Lys Gly Lys Phe Glu Asp Met Ala
 50 55 60
 Lys Ala Asp Lys Ala Arg Tyr Glu Arg Glu Met Lys Thr Tyr Ile Pro
 65 70 75 80
 Pro Lys Gly Glu Thr Lys Lys Lys Phe Lys Asp Pro Asn Ala Pro Lys
 85 90 95
 Arg Leu Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu Tyr Arg Pro Lys
 100 105 110
 Ile Lys Gly Glu His Pro Gly Leu Ser Ile Gly Asp Val Ala Lys Lys
 115 120 125
 Leu Gly Glu Met Trp Asn Asn Thr Ala Ala Asp Asp Lys Gln Pro Tyr
 130 135 140
 Glu Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu Lys Asp Ile Ala
 145 150 155 160
 Ala Tyr Arg Ala Lys Gly Lys Pro Asp Ala Ala Lys Lys Gly Val Val
 165 170 175
 Lys Ala Glu Lys Ser Lys Lys Lys Lys Glu Glu Glu Asp Glu Glu
 180 185 190
 Asp Glu Glu Asp Glu Glu Glu Glu Asp Glu Glu Asp Glu Glu Asp
 195 200 205
 Glu Glu Glu Asp Asp Asp Glu
 210 215

<210> 19
 <211> 182
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 <213> Homo sapiens

<400> 19
 Met Gly Lys Gly Asp Pro Lys Lys Pro Thr Gly Lys Met Ser Ser Tyr
 1 5 10 15

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Ala Phe Phe Val Gln Thr Cys Arg Glu Glu His Lys Lys Lys His Pro
 20 25 30
 Asp Ala Ser Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg
 35 40 45
 Trp Lys Thr Met Ser Ala Lys Glu Lys Gly Lys Phe Glu Asp Met Ala
 50 55 60
 Lys Ala Asp Lys Ala Arg Tyr Glu Arg Glu Met Lys Thr Tyr Ile Pro
 65 70 75 80
 Pro Lys Gly Glu Thr Lys Lys Lys Phe Lys Asp Pro Asn Ala Pro Lys
 85 90 95
 Arg Leu Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu Tyr Arg Pro Lys
 100 105 110
 Ile Lys Gly Glu His Pro Gly Leu Ser Ile Gly Asp Val Ala Lys Lys
 115 120 125
 Leu Gly Glu Met Trp Asn Asn Thr Ala Ala Asp Asp Lys Gln Pro Tyr
 130 135 140
 Glu Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu Lys Asp Ile Ala
 145 150 155 160
 Ala Tyr Arg Ala Lys Gly Lys Pro Asp Ala Ala Lys Lys Gly Val Val
 165 170 175
 Lys Ala Glu Lys Ser Lys
 180

<210> 20
 <211> 74
 <212> PRT
 <213> Homo sapiens

<400> 20
 Phe Lys Asp Pro Asn Ala Pro Lys Arg Leu Pro Ser Ala Phe Phe Leu
 1 5 10 15
 Phe Cys Ser Glu Tyr Arg Pro Lys Ile Lys Gly Glu His Pro Gly Leu
 20 25 30
 Ser Ile Gly Asp Val Ala Lys Lys Leu Gly Glu Met Trp Asn Asn Thr
 35 40 45
 Ala Ala Asp Asp Lys Gln Pro Tyr Glu Lys Lys Ala Ala Lys Leu Lys
 50 55 60
 Glu Lys Tyr Glu Lys Asp Ile Ala Ala Tyr
 65 70

<210> 21
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 <212> PRT
 <213> Homo sapiens

<400> 21
 Met Gly Lys Gly Asp Pro Lys Lys Pro Thr Gly Lys Met Ser Ser Tyr
 1 5 10 15
 Ala Phe Phe Val Gln Thr Cys Arg Glu Glu His Lys Lys Lys His Pro
 20 25 30
 Asp Ala Ser Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg
 35 40 45
 Trp Lys Thr Met Ser Ala Lys Glu Lys Gly Lys Phe Glu Asp Met Ala
 50 55 60
 Lys Ala Asp Lys Ala Arg Tyr Glu Arg Glu Met Lys Thr Tyr Ile Pro
 65 70 75 80
 Pro Lys Gly Glu Thr
 85

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<210> 22
 <211> 77
 <212> PRT
 <213> Homo sapiens

<400> 22
 Pro Thr Gly Lys Met Ser Ser Tyr Ala Phe Phe Val Gln Thr Cys Arg
 1 5 10 15
 Glu Glu His Lys Lys Lys His Pro Asp Ala Ser Val Asn Phe Ser Glu
 20 25 30
 Phe Ser Lys Lys Cys Ser Glu Arg Trp Lys Thr Met Ser Ala Lys Glu
 35 40 45
 Lys Gly Lys Phe Glu Asp Met Ala Lys Ala Asp Lys Ala Arg Tyr Glu
 50 55 60
 Arg Glu Met Lys Thr Tyr Ile Pro Pro Lys Gly Glu Thr
 65 70 75

<210> 23
 <211> 20
 <212> PRT
 <213> Homo sapiens

<400> 23
 Phe Lys Asp Pro Asn Ala Pro Lys Arg Leu Pro Ser Ala Phe Phe Leu
 1 5 10 15
 Phe Cys Ser Glu
 20

<210> 24
 <211> 216
 <212> PRT
 <213> Homo sapiens

<400> 24
 Met Gly Lys Gly Asp Pro Lys Lys Pro Thr Gly Lys Met Ser Ser Tyr
 1 5 10 15
 Ala Phe Phe Val Gln Thr Cys Arg Glu Glu His Lys Lys Lys His Pro
 20 25 30
 Asp Ala Ser Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg
 35 40 45
 Trp Lys Thr Met Ser Ala Lys Glu Lys Gly Lys Phe Glu Asp Met Ala
 50 55 60
 Lys Ala Asp Lys Ala Arg Tyr Glu Arg Glu Met Lys Thr Tyr Ile Pro
 65 70 75 80
 Pro Lys Gly Glu Thr Lys Lys Lys Phe Lys Asp Pro Asn Ala Pro Lys
 85 90 95
 Arg Leu Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu Tyr Arg Pro Lys
 100 105 110
 Ile Lys Gly Glu His Pro Gly Leu Ser Ile Gly Asp Val Ala Lys Lys
 115 120 125
 Leu Gly Glu Met Trp Asn Asn Thr Ala Ala Asp Asp Lys Gln Pro Tyr
 130 135 140
 Glu Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu Lys Asp Ile Ala
 145 150 155 160
 Ala Tyr Arg Ala Lys Gly Lys Pro Asp Ala Ala Lys Lys Gly Val Val
 165 170 175
 Lys Ala Glu Lys Ser Lys Lys Lys Lys Glu Glu Glu Glu Asp Glu Glu
 180 185 190
 Asp Glu Glu Asp Glu Glu Glu Glu Asp Glu Glu Asp Glu Glu Asp
 195 200 205

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Glu Glu Glu Asp Asp Asp Asp Glu
210 215

<210> 25
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<212> PRT
<213> Homo sapiens

<400> 25
Met Gly Lys Gly Asp Pro Lys Lys Pro Arg Gly Lys Met Ser Ser Tyr
1 5 10 15
Ala Phe Phe Val Gln Thr Cys Arg Glu Glu His Lys Lys Lys His Ser
20 25 30
Asp Ala Ser Val Asn Phe Ser Glu Phe Ser Asn Lys Cys Ser Glu Arg
35 40 45
Trp Lys Thr Met Ser Ala Lys Glu Lys Gly Lys Phe Glu Asp Met Ala
50 55 60
Lys Ala Asp Lys Thr His Tyr Glu Arg Gln Met Lys Thr Tyr Ile Pro
65 70 75 80
Pro Lys Gly Glu Thr Lys Lys Lys Phe Lys Asp Pro Asn Ala Pro Lys
85 90 95
Arg Pro Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu Tyr His Pro Lys
100 105 110
Ile Lys Gly Glu His Pro Gly Leu Ser Ile Gly Asp Val Ala Lys Lys
115 120 125
Leu Gly Glu Met Trp Asn Asn Thr Ala Ala Asp Asp Lys Gln Pro Gly
130 135 140
Glu Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu Lys Asp Ile Ala
145 150 155 160
Ala Tyr Gln Ala Lys Gly Lys Pro Glu Ala Ala Lys Lys Gly Val Val
165 170 175
Lys Ala Glu Lys Ser Lys Lys Lys Lys Glu Glu Glu Glu Asp Glu Glu
180 185 190
Asp Glu Glu Asp Glu Glu Glu Glu Asp Glu Glu Asp Glu Glu Asp Asp
195 200 205
Asp Asp Glu
210

<210> 26
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<212> PRT
<213> Homo sapiens

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Met Gly Lys Gly Asp Pro Lys Lys Pro Arg Gly Lys Met Ser Ser Tyr
1 5 10 15
Ala Phe Phe Val Gln Thr Cys Arg Glu Cys Lys Lys Lys His Pro
20 25 30
Asp Ala Ser Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg
35 40 45
Trp Lys Ala Met Ser Ala Lys Asp Lys Gly Lys Phe Glu Asp Met Ala
50 55 60
Lys Val Asp Lys Asp Arg Tyr Glu Arg Glu Met Lys Thr Tyr Ile Pro
65 70 75 80
Pro Lys Gly Glu Thr Lys Lys Lys Phe Glu Asp Ser Asn Ala Pro Lys
85 90 95
Arg Pro Pro Ser Ala Phe Leu Leu Phe Cys Ser Glu Tyr Cys Pro Lys
100 105 110
Ile Lys Gly Glu His Pro Gly Leu Pro Ile Ser Asp Val Ala Lys Lys
115 120 125

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Leu Val Glu Met Trp Asn Asn Thr Phe Ala Asp Asp Lys Gln Leu Cys
 130 135 140
 Glu Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Lys Lys Asp Thr Ala
 145 150 155 160
 Thr Tyr Arg Ala Lys Gly Lys Pro Asp Ala Ala Lys Lys Gly Val Val
 165 170 175
 Lys Ala Glu Lys Ser Lys Lys Lys Lys Glu Glu Glu
 180 185

<210> 27
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<400> 27
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 1 5 10 15
 Ala Phe Phe Val Gln Thr Cys Gln Glu Glu His Lys Lys Lys Asn Pro
 20 25 30
 Asp Ala Ser Val Lys Phe Ser Glu Phe Leu Lys Lys Cys Ser Glu Thr
 35 40 45
 Trp Lys Thr Ile Phe Ala Lys Glu Lys Gly Lys Phe Glu Asp Met Ala
 50 55 60
 Lys Ala Asp Lys Ala His Tyr Glu Arg Glu Met Lys Thr Tyr Ile Pro
 65 70 75 80
 Pro Lys Gly Glu Lys Lys Lys Lys Phe Lys Asp Pro Asn Ala Pro Lys
 85 90 95
 Arg Pro Pro Leu Ala Phe Phe Leu Phe Cys Ser Glu Tyr Arg Pro Lys
 100 105 110
 Ile Lys Gly Glu His Pro Gly Leu Ser Ile Asp Asp Val Val Lys Lys
 115 120 125
 Leu Ala Gly Met Trp Asn Asn Thr Ala Ala Ala Asp Lys Gln Phe Tyr
 130 135 140
 Glu Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Lys Lys Asp Ile Ala
 145 150 155 160
 Ala Tyr Arg Ala Lys Gly Lys Pro Asn Ser Ala Lys Lys Arg Val Val
 165 170 175
 Lys Ala Glu Lys Ser Lys Lys Lys Lys Glu Glu Glu Asp Glu Glu
 180 185 190
 Asp Glu Gln Glu Glu Asn Glu Glu Asp Asp Asp Lys
 195 200 205

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<400> 28
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 1 5 10 15
 Ala Phe Phe Val Gln Thr Cys Trp Glu Glu His Lys Lys Gln Tyr Pro
 20 25 30
 Asp Ala Ser Ile Asn Phe Ser Glu Phe Ser Gln Lys Cys Pro Glu Thr
 35 40 45
 Trp Lys Thr Thr Ile Ala Lys Glu Lys Gly Lys Phe Glu Asp Met Pro
 50 55 60
 Lys Ala Asp Lys Ala His Tyr Glu Arg Glu Met Lys Thr Tyr Ile Pro
 65 70 75 80

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 <212> PRT
 <213> Homo sapiens

<400> 29
 Lys Gln Arg Gly Lys Met Pro Ser Tyr Val Phe Cys Val Gln Thr Cys
 1 5 10 15
 Pro Glu Glu Arg Lys Lys His Pro Asp Ala Ser Val Asn Phe Ser
 20 25 30
 Glu Phe Ser Lys Lys Cys Leu Val Arg Gly Lys Thr Met Ser Ala Lys
 35 40 45
 Glu Lys Gly Gln Phe Glu Ala Met Ala Arg Ala Asp Lys Ala Arg Tyr
 50 55 60
 Glu Arg Glu Met Lys Thr Tyr Ile Pro Pro Lys Gly Glu Thr Lys Lys
 65 70 75 80

<210> 30
 <211> 86
 <212> PRT
 <213> Homo sapiens

<400> 30
 Met Gly Lys Arg Asp Pro Lys Gln Pro Arg Gly Lys Met Ser Ser Tyr
 1 5 10 15
 Ala Phe Phe Val Gln Thr Ala Gln Glu Glu His Lys Lys Lys Gln Leu
 20 25 30
 Asp Ala Ser Val Ser Phe Ser Glu Phe Ser Lys Asn Cys Ser Glu Arg
 35 40 45
 Trp Lys Thr Met Ser Val Lys Glu Lys Gly Lys Phe Glu Asp Met Ala
 50 55 60
 Lys Ala Asp Lys Ala Cys Tyr Glu Arg Glu Met Lys Ile Tyr Pro Tyr
 65 70 75 80
 Leu Lys Gly Arg Gln Lys
 85

<210> 31
 <211> 70
 <212> PRT
 <213> Homo sapiens

<400> 31
 Met Gly Lys Gly Asp Pro Lys Lys Pro Arg Glu Lys Met Pro Ser Tyr
 1 5 10 15
 Ala Phe Phe Val Gln Thr Cys Arg Glu Ala His Lys Asn Lys His Pro
 20 25 30
 Asp Ala Ser Val Asn Ser Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg
 35 40 45
 Trp Lys Thr Met Pro Thr Lys Gln Lys Gly Lys Phe Glu Asp Met Ala
 50 55 60
 Lys Ala Asp Arg Ala His
 65 70

<210> 32
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 <212> DNA
 <213> Homo sapiens

<400> 32

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atggggcaaa gagatcctaa gaagccgaca ggcaaaatgt catcatatgc attttttgtg 60
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ttttctaaga agtgctcaga gaggtggaag accatgtctg ctaaagagaa agggaaatgt 180
gaagatatgg caaaggcgga caaggccogt tatgaaagag aaatgaaaac ctatatccct 240
cccaaaaggg agacaaaaaa gaagttcaag gatcccaatg caccagagag gcttcctctg 300
gcctttcttc tcttctgctc tgagtatcgc ccaaaaatca aaggagaaca tctgggctg 360
tccattgggtg atgttgcgaa gaaactggga gagatgtgga ataacactgc tgcagatgac 420
aagcagcctt atgaaaagaa ggctgcaag ctgaaggaaa aatacgaata ggatattgct 480
gcataatcag ctaaaggaaa gcctgatgca gcaaaaaagg gagttgtcaa ggctgaaaaa 540
agcaagaaaa agaaggaaag ggaggaagat gaggaagatg aagaggatga ggaggaggag 600
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<210> 33
 <211> 633
 <212> DNA
 <213> Homo sapiens

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<400> 33
atgggcaaa gagatcctaa gaagccgaga ggcaaaatgt catcatatgc attttttgtg 60
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ttttctaaga agtgctcaga gaggtggaag accatgtctg ctaaagagaa agggaaatgt 180
gaggatatgg caaaggcgga caagacccat tatgaaagac aaatgaaaac ctatatccct 240
cccaaaaggg agacaaaaaa gaagttcaag gatcccaatg caccagagag gcttcctctg 300
gcctttcttc tcttctgctc tgagtatcac ccaaaaatca aaggagaaca tctgggctg 360
tccattgggtg atgttgcgaa gaaactggga gagatgtgga ataacactgc tgcagatgac 420
aagcagcctg gtgaaaagaa ggctgcaag ctgaaggaaa aatacgaata ggatattgct 480
gcataatcag ctaaaggaaa gcctgatgca gcaaaaaagg gagttgtcaa agctgaaaaa 540
agcaagaaaa agaaggaaag ggaggaagat gaggaagatg aagaggatga ggaggaggaa 600
gatgaagaag atgaagaaga tgatgatgat gaa 633

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<210> 34
 <211> 564
 <212> DNA
 <213> Homo sapiens

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<400> 34
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ttttctaaga agtgctcaga gaggtggaag gccatgtctg ctaaagataa agggaaatgt 180
gaagatatgg caaagggtga caaagacogt tatgaaagag aaatgaaaac ctatatccct 240
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cctattagcg atgttgcaaa gaaactggta gagatgtgga ataacacttt tgcagatgac 420
aagcagcctt gtgaaaagaa ggctgcaag ctgaaggaaa aatacaaaaa ggatattgct 480
acataatcag ctaaaggaaa gcctgatgca gcaaaaaagg gagttgtcaa ggctgaaaaa 540
agcaagaaaa agaaggaaag ggag 564

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<210> 35
 <211> 615
 <212> DNA
 <213> Homo sapiens

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<400> 35
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caaaacttgtc agggaggagca taagaagaag aaccagatg cttcagtcac gttctcagag 120
tttttaaga agtgctcaga gacatggaag accatttttg ctaaagagaa agggaaatgt 180
gaagatatgg caaaggcgga caaggcccat tatgaaagag aaatgaaaac ctatatccct 240
cctaaagggg agaaaaaaa gaagttcaag gatcccaatg caccagagag gcttcctctg 300
gcctttcttc tcttctgctc tgagtatcgc ccaaaaatca aaggagaaca tctgggctg 360
tccattgatg atgttgtaaa gaaactggca gggatgtgga ataacacgc tgcagctgac 420
aagcagttt atgaaaagaa ggctgcaag ctgaaggaaa aatacaaaaa ggatattgct 480
gcataatcag ctaaaggaaa gcctaattca gcaaaaaaga gagttgtcaa ggctgaaaaa 540

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agcaagaaaa agaaggaaga ggaagaagat gaagaggatg aacaagagga ggaaatgaa 600
gaagatgatg ataaa 615

<210> 36
<211> 240
<212> DNA
<213> Homo sapiens

<400> 36
atgggcaaaag gagatcctaa gaagccgaga ggcaaaatgt catcatgtgc attttttgtg 60
caaaacttggt gggaggagca taagaagcag taccagatg cttcaatcaa ctctcagag 120
ttttctcaga agtgcccaga gacgtggaag accacgattg cttaaagagaa aggaaaattt 180
gaagatatgc caaaggcaga caaggcccat tatgaaagag aaatgaaaaa ctatalacc 240

<210> 37
<211> 240
<212> DNA
<213> Homo sapiens

<400> 37
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aagaagaaac acccagatgc ttcagtcaac ttctcagagt tttctaagaa gtgcttagtg 120
agggggaaga ccatgtctgc taaagagaaa ggacaatttg aagctatggc aagggcagac 180
aaggcccggt acgaaagaga aatgaaaaca tatatccctc cttaaaggga gacaaaaaa 240

<210> 38
<211> 258
<212> DNA
<213> Homo sapiens

<400> 38
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caaaactgtc aggaggagca caagaagaaa caactagatg cttcagtcag ttctcagag 120
ttttctaaga actgctcaga gaggtggaag accatgtctg ttaaagagaa aggaaaattt 180
gaagacatgg caaaggcaga caaggcctgt tatgaaagag aaatgaaat atatccctac 240
ttaaagggga gacaaaaa 258

<210> 39
<211> 211
<212> DNA
<213> Homo sapiens

<400> 39
atgggcaaaag gagaccctaa gaagccaaga gagaaaatgc catcatatgc attttttgtg 60
caaaacttgta gggaggcaca taagaacaaa catccagatg cttcagtcag ctctcagag 120
ttttctaaga agtgctcaga gaggtggaag accatgccta cttaacagaa aggaaaattc 180
gaagatatgg caaaggcaga caggcccat a 211

<210> 40
<211> 74
<212> PRT
<213> Homo sapiens

<400> 40
Lys Lys Asp Pro Asn Ala Pro Lys Arg Pro Pro Ser Ala Phe Phe Leu
1 5 10 15
Phe Cys Ser Glu His Arg Pro Lys Ile Lys Ser Glu His Pro Gly Leu
20 25 30
Ser Ile Gly Asp Thr Ala Lys Lys Leu Gly Glu Met Trp Ser Glu Gln
35 40 45

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Ser Ala Lys Asp Lys Gln Pro Tyr Glu Gln Lys Ala Ala Lys Leu Lys
 50 55 60
 Glu Lys Tyr Glu Lys Asp Ile Ala Ala Tyr
 65 70

<210> 41
 <211> 74
 <212> PRT
 <213> Homo sapiens

<400> 41
 Phe Lys Asp Pro Asn Ala Pro Lys Arg Leu Pro Ser Ala Phe Phe Leu
 1 5 10 15
 Phe Cys Ser Glu Tyr Arg Pro Lys Ile Lys Gly Glu His Pro Gly Leu
 20 25 30
 Ser Ile Gly Asp Val Ala Lys Lys Leu Gly Glu Met Trp Asn Asn Thr
 35 40 45
 Ala Ala Asp Asp Lys Gln Pro Tyr Glu Lys Lys Ala Ala Lys Leu Lys
 50 55 60
 Glu Lys Tyr Glu Lys Asp Ile Ala Ala Tyr
 65 70

<210> 42
 <211> 74
 <212> PRT
 <213> Homo sapiens

<400> 42
 Phe Lys Asp Pro Asn Ala Pro Lys Arg Pro Pro Ser Ala Phe Phe Leu
 1 5 10 15
 Phe Cys Ser Glu Tyr His Pro Lys Ile Lys Gly Glu His Pro Gly Leu
 20 25 30
 Ser Ile Gly Asp Val Ala Lys Lys Leu Gly Glu Met Trp Asn Asn Thr
 35 40 45
 Ala Ala Asp Asp Lys Gln Pro Gly Glu Lys Lys Ala Ala Lys Leu Lys
 50 55 60
 Glu Lys Tyr Glu Lys Asp Ile Ala Ala Tyr
 65 70

<210> 43
 <211> 74
 <212> PRT
 <213> Homo sapiens

<400> 43
 Phe Lys Asp Ser Asn Ala Pro Lys Arg Pro Pro Ser Ala Phe Leu Leu
 1 5 10 15
 Phe Cys Ser Glu Tyr Cys Pro Lys Ile Lys Gly Glu His Pro Gly Leu
 20 25 30
 Pro Ile Ser Asp Val Ala Lys Lys Leu Val Glu Met Trp Asn Asn Thr
 35 40 45
 Phe Ala Asp Asp Lys Gln Leu Cys Glu Lys Lys Ala Ala Lys Leu Lys
 50 55 60
 Glu Lys Tyr Lys Lys Asp Thr Ala Thr Tyr
 65 70

<210> 44
 <211> 74

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<212> PRT
 <213> Homo sapiens

<400> 44
 Phe Lys Asp Pro Asn Ala Pro Lys Arg Pro Pro Ser Ala Phe Phe Leu
 1 5 10 15
 Phe Cys Ser Glu Tyr Arg Pro Lys Ile Lys Gly Glu His Pro Gly Leu
 20 25 30
 Ser Ile Gly Asp Val Val Lys Lys Leu Ala Gly Met Trp Asn Asn Thr
 35 40 45
 Ala Ala Ala Asp Lys Gln Phe Tyr Glu Lys Lys Ala Ala Lys Leu Lys
 50 55 60
 Glu Lys Tyr Lys Lys Asp Ile Ala Ala Tyr
 65 70

<210> 45
 <211> 92
 <212> PRT
 <213> Homo sapiens

<400> 45
 Phe Lys Asp Pro Asn Ala Pro Lys Arg Pro Pro Ser Ala Phe Phe Leu
 1 5 10 15
 Phe Cys Ser Glu Tyr Arg Pro Lys Ile Lys Gly Glu His Pro Gly Leu
 20 25 30
 Ser Ile Gly Asp Val Ala Lys Lys Leu Gly Glu Met Trp Asn Asn Thr
 35 40 45
 Ala Ala Asp Asp Lys Gln Pro Tyr Glu Lys Lys Ala Ala Lys Leu Lys
 50 55 60
 Glu Lys Tyr Glu Lys Asp Ile Ala Ala Tyr Arg Ala Lys Gly Lys Pro
 65 70 75 80
 Asp Ala Ala Lys Lys Gly Val Val Lys Ala Glu Lys
 85 90