

PIRIN POLYPEPTIDE AND IMMUNE MODULATION

FIELD OF INVENTION

- 5 The present invention relates to the polypeptide HP or a polynucleotide sequence encoding polypeptide HP or a host cell comprising said polynucleotide sequence or a host cell comprising an expression vector comprising said polynucleotide sequence for various therapeutic and nutritional uses.

10 BACKGROUND

- Bacteroides thetaiotaomicron* has potent anti-inflammatory effects *in vitro* and *in vivo* (Kelly et al. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol. 2004 Jan;5(1):104-12). It modulates molecular signalling pathways of NF- κ B (Kelly et al, Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol. 2004 Jan;5(1):104-12). In particular, it stops binding of the active component (RelA) of NF- κ B to key genes in the nucleus, thereby preventing the activation of pro-inflammatory pathways (Kelly et al, Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol. 2004 Jan;5(1):104-12).

- The full genome of *B. thetaiotaomicron* was sequenced and annotated by the Gordon Group (Washington University School of Medicine, USA) in 2003 [Xu et al, A genomic view of the human-*Bacteroides thetaiotaomicron* symbiosis. Science. 2003 Mar 28;299(5615):2074-6].

STATEMENTS OF INVENTION

- 30 Surprisingly, the present inventors found that HP (a hypothetical protein; gene ID 1075517; gene symbol BT_0187; accession number AA075294) identified from the genome of *Bacteroides thetaiotaomicron* (VPI5482), a pirin-related protein; deposited as AAO75294.1, which reduces inflammation in cells..

35

The present invention provides polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for use in the treatment and/or prevention of a disorder in a subject; wherein said disorder is an inflammatory disorder and/or an autoimmune disorder; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

In another aspect, the present invention provides polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for use in modulating the inflammation of a cell, a tissue or an organ in a subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

In a further aspect, the present invention provides polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for use in improving intestine barrier integrity in a subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5

or variants, homologues, fragments or derivatives thereof.

The present invention provides, in another aspect, polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said
5 polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for use in modifying the bacterial composition in a tissue or organ to provide a more beneficial microbiota. For example, the invention may be of use in reducing the level of one or more types of lactose fermenting bacteria (such as *E. coli*) in a tissue or an organ in a subject and/or reducing the
10 level of one or more types of non-lactose fermenting bacteria in a tissue or an organ in a subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or
15 variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

The present invention provides, in a further aspect, polypeptide HP, or a
20 polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for use in maintaining the length of the large intestine and/or small intestine of a subject, (e.g. said subject has IBD); wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO
25 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues,
30 fragments or derivatives thereof.

In a further aspect, the present invention provides polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising
35 said polynucleotide sequence, for use in reducing disruption to the intestine (such as

the large intestine) of a subject, (e.g. said subject has IBD); wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to
 5 SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

10 In another aspect, the present invention provides polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for use in regulating the expression of one or more pro-inflammatory genes and/or one or more barrier integrity genes in a cell or cells
 15 of a subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said
 20 polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

The present invention provides, in another aspect, polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said
 25 polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for use in regulating the expression in a cell or cells of a subject of one or more genes selected from the group consisting of regenerating islet-derived 3 beta gene (Reg3b), resistin-like gamma resistin like beta gene (Retnlg|Retnlb), sucrase-isomaltase (alpha-glucosidase) gene (Si), defensin
 30 alpha 24 gene (Defa24), hydroxysteroid 11-beta dehydrogenase 2 gene (Hsd11b2), hydroxysteroid (17-beta) dehydrogenase 2 gene (Hsd17b2), resistin-Like Molecule-beta (RELMb), and nuclear receptor 1D1 thyroid hormone receptor alpha gene (Nr1d1|Thra); (e.g. said subject has IBD); wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues,
 35 fragments or derivatives thereof; and wherein said polynucleotide sequence

encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

In a further aspect, the present invention provides polypeptide HP or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for use in reducing the activation of pro-inflammatory pathways in a cell or cells of a subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

The present invention provides, in a further aspect, polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for use in reducing the activity and/or expression of NF- κ B in a cell or cells (such as epithelial cells, epidermal cells, neuronal cells, and/or pancreatic cells) of a subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

The present invention provides, in another aspect, polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said

polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for use in improving alimentary canal health in a subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

10

The present invention provides, in a further aspect, a pharmaceutical composition comprising polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, and a pharmaceutically acceptable excipient, carrier or diluent; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

In a further aspect, the present invention provides a nutritional supplement comprising polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, and a nutritional acceptable excipient, carrier or diluent; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

In another aspect, the present invention provides a feedstuff, food product, dietary supplement, or food additive comprising polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

The present invention provides in a further aspect a process for producing a pharmaceutical composition according to the present invention, said process comprising admixing polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence with a pharmaceutically acceptable excipient, carrier or diluent; optionally said polypeptide or polynucleotide sequence or host cell is encapsulated in said process; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

In a further aspect, the present invention provides a process for producing a nutritional supplement according to the present invention, said process comprising admixing polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence with a nutritionally acceptable excipient, carrier or diluent; optionally said polypeptide or polynucleotide is encapsulated in said process; wherein said polypeptide has at least 75% identity

to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein
5 said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

In another aspect, the present invention provides a process for producing a feedstuff, food product, dietary supplement, or food additive according to the present
10 invention, said process comprising admixing polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence with a feedstuff, food product, dietary supplement, food additive or ingredient thereof; optionally said polypeptide or polynucleotide is
15 encapsulated in said process; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein
20 said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

The present invention provides, in another aspect, a method for treating and/or preventing a disorder in a subject, wherein said method comprises administering to
25 the subject polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence; wherein said polypeptide or polynucleotide sequence or host cell treats and/or prevents a disorder in the subject, wherein said disorder is an inflammatory disorder and/or an autoimmune disorder; wherein said polypeptide has at least 75% identity to SEQ ID
30 NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein
35 said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO

3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

The present invention provides, in a further aspect, a method for modulating the inflammation of a tissue or an organ in a subject wherein said method comprises
5 administering to the subject polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence; wherein said polypeptide, polynucleotide sequence or host cell modulates the inflammation of a tissue or an organ in the subject; wherein said polypeptide has at
10 least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to
15 SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

In another aspect, the present invention provides a method for improving intestine barrier integrity in a subject wherein said method comprises administering to the
20 subject polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence; wherein said polypeptide or polynucleotide sequence or host cell improves intestine barrier integrity in the subject; wherein said polypeptide has at least 75% identity to SEQ ID
25 NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO
30 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

The present invention provides, in another aspect, a method for reducing the level of one or more types of lactose fermenting bacteria (such as *E. coli*) in a tissue or an organ in a subject and/or reducing the level of one or more types of non-lactose
35 fermenting bacteria in a tissue or an organ in a subject, wherein said method

comprises administering to the subject polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence; wherein said polypeptide, polynucleotide sequence or
5 host cell reduces the level of one or more types of lactose fermenting bacteria (such as *E. coli*) in a tissue or an organ in the subject and/or reduces the level of one or more types of non-lactose fermenting bacteria in a tissue or an organ in the subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and
10 wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

15

The present invention provides, in a further aspect, a method for maintaining the length of the large intestine and/or small intestine of a subject wherein said method comprises administering to the subject polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said
20 polynucleotide sequence; (e.g. said subject has IBD); wherein said polypeptide or polynucleotide sequence or host cell maintains the length of the large intestine and/or small intestine of the subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence
25 encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives
30 thereof.

In a further aspect, the present invention provides a method for reducing disruption to the intestine (e.g. the large intestine) of a subject wherein said method comprises administering to the subject polypeptide HP, or a polynucleotide sequence encoding
35 polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host

cell comprising an expression vector comprising said polynucleotide sequence; (e.g. said subject has IBD); wherein said polypeptide or polynucleotide sequence or host cell reduces disruption to the intestine (e.g. large intestine) of the subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

The present invention provides, in another aspect, a method for regulating the expression of one or more pro-inflammatory or anti-inflammatory genes in a cell or cells of a subject wherein said method comprises administering to the subject polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence; wherein said polypeptide or polynucleotide sequence or host cell regulates the expression of one or more pro-inflammatory genes and/or anti-inflammatory genes in a cell or cells of the subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

In another aspect, the present invention provides a method for regulating the expression of one or more genes in a cell or cells of a subject wherein said method comprises administering to the subject polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, to said subject; wherein said polypeptide or polynucleotide sequence or host cell regulates the expression of one or more genes in a cell or cells of the subject; wherein the one or more genes are selected from the group

consisting of regenerating islet-derived 3 beta gene (Reg3b), resistin-like gamma resistin like beta gene (Retnlg|Retnlb), sucrase-isomaltase (alpha-glucosidase) gene (Si), defensin alpha 24 gene (Defa24), hydroxysteroid 11-beta dehydrogenase 2 gene (Hsd11b2), hydroxysteroid (17-beta) dehydrogenase 2 gene (Hsd17b2),
 5 resistin-Like Molecule-beta (RELMb), and nuclear receptor 1D1 thyroid hormone receptor alpha gene (Nr1d1|Thra); (e.g. said subject has IBD); wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to
 10 SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

15 The present invention provides, in a further aspect, a method for reducing the activation of pro-inflammatory pathways in a cell or cells of a subject wherein said method comprises administering to the subject polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said
 20 polynucleotide sequence; wherein said polypeptide or polynucleotide sequence or host cell reduces the activation of pro-inflammatory pathways in a cell or cells of the subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least
 25 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

30 In a further aspect, the present invention provides a method for reducing the activity and/or expression of NF- κ B in a cell or cells (such as epithelial cells, epidermal cells, neuronal cells, and/or pancreatic cells) of a subject wherein said method comprises administering to the subject polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host
 35 cell comprising an expression vector comprising said polynucleotide sequence;

wherein said polypeptide or polynucleotide or host cell reduces the activity and/or expression of NF- κ B in a cell or cells (such as epithelial cells, epidermal cells, neuronal cells, and/or pancreatic cells) of the subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

In another aspect, the present invention provides a method for improving alimentary canal health in a subject wherein said method comprises administering to the subject polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence; wherein said polypeptide or polynucleotide sequence or host cell improves alimentary canal health in the subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

The present invention provides, in a further aspect, use of polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for the manufacture of a medicament for the treatment and/or prevention of a disorder in a subject, wherein said disorder is an inflammatory disorder and/or an autoimmune disorder; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives

thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

- 5 The present invention provides, in another aspect, use of polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for the manufacture of a medicament for modulating the inflammation of a tissue or an organ in a subject; wherein said polypeptide has
10 at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to
15 SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

- In another aspect, the present invention provides use of polypeptide HP or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said
20 polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for the manufacture of a medicament for improving intestine barrier integrity in a subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence
25 encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

30

- The present invention provides, in a further aspect, use of polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for the manufacture of a medicament for maintaining
35 the length of the large intestine and/or small intestine of a subject; (e.g. said subject

has IBD); wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants,
5 homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

In a further aspect, the present invention provides use of a polypeptide HP, or a
10 polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for the manufacture of a medicament for modifying the bacterial composition in a tissue or organ to provide a beneficial microbiota, preferably, for use in reducing the level of one or more types of lactose fermenting
15 bacteria (such as *E. coli*) in a tissue or an organ in a subject and/or reducing the level of one or more types of non-lactose fermenting bacteria in a tissue or an organ in a subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which
20 has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

In another aspect, the present invention provides use of polypeptide HP, or a
25 polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for the manufacture of a medicament for reducing disruption to the intestine (e.g. large intestine) of a subject; (e.g. said subject has
30 IBD); wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide
35 sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5

or variants, homologues, fragments or derivatives thereof.

The present invention provides, in a further aspect, use of polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said
5 polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for the manufacture of a medicament for regulating the expression of one or more pro-inflammatory genes in a cell or cells of a subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or
10 SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

15

The present invention provides, in another aspect, use of polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for the manufacture of a medicament for regulating
20 the expression in a cell or cells of a subject of one or more genes selected from the group consisting of regenerating islet-derived 3 beta gene (Reg3b), resistin-like gamma resistin like beta gene (Retnl γ |Retnl β), sucrase-isomaltase (alpha-glucosidase) gene (Si), defensin alpha 24 gene (Defa24), hydroxysteroid 11-beta dehydrogenase 2 gene (Hsd11b2), hydroxysteroid (17-beta) dehydrogenase 2 gene
25 (Hsd17b2), resistin-Like Molecule-beta (RELM β), and nuclear receptor 1D1 thyroid hormone receptor alpha gene (Nr1d1|Thra); (e.g. said subject has IBD); wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to
30 SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

35 In another aspect, the present invention provides use of polypeptide HP, or a

polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for the manufacture of a medicament for reducing the activation of pro-inflammatory pathways in a cell or cells of a subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

In a further aspect, the present invention provides use of a polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for the manufacture of a medicament for reducing the activity and/or expression of NF- κ B in a cell or cells (such as epithelial cells, epidermal cells, neuronal cells, and/or pancreatic cells) of a subject; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof.

The present invention provides, in another aspect, use of a polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, for the manufacture of a medicament for improving alimentary canal health in a subject.

FIGURES

The invention is described with reference to the accompanying figures, wherein:

Figure 1A shows an alignment of the polynucleotide sequences encoding HP (SEQ ID NO 1), *E. coli* optimised HP (Rec 1 HP – SEQ ID NO 3) and *L. lactis* optimised HP (Rec 2 HP – SEQ ID NO 5). HP is deposited with GenBank under accession number: AAO75294.1 and is described in GenBank as possible Pirin family protein
5 [Bacteroides thetaiotaomicron VPI-5482].

Figure 1B shows an alignment of the polypeptide sequences HP (SEQ ID NO 2), *E. coli* optimised HP (Rec 1 HP – SEQ ID NO 4) and *L. lactis* optimised HP (Rec 2 HP – SEQ ID NO 6). HP is deposited with GenBank under accession number:
10 AAO75294.1 and is described in GenBank as possible Pirin family protein [Bacteroides thetaiotaomicron VPI-5482].

Figure 2 shows the change in weight, water and food intakes by rats given Dextran Sodium Sulphate (DSS) in water with (DSS/HP) or without (DSS) co-treatment with
15 hypothetical protein (HP).

Figure 3 shows the length of the colon and small intestine in rats given Dextran Sodium Sulphate in water with (DSS/HP) or without (DSS) co-treatment with
20 hypothetical protein (HP).

Figure 4 shows the numbers of lactose-fermenting (predominantly *E. coli*) and non-lactose-fermenting bacteria in tissues from rats given Dextran Sodium Sulphate (DSS) in water with (DSS/HP) or without (DSS) co-treatment with hypothetical protein (HP). [When Log10 = 1.0, no bacteria were detected].
25

Figure 5 shows the morphology of the ascending and descending colon from rats given Dextran Sodium Sulphate in water with (DSS/HP) or without (DSS) co-treatment with hypothetical protein (HP).

30 Figure 6 shows the mean histopathology scores and histopathology scores as percentage fields of view with pathology of grades 0-3 for the ascending colon from rats given Dextran Sodium Sulphate in water with (DSS/HP) or without (DSS) co-treatment with hypothetical protein (HP).

35 Figure 7 shows the heatmap of 377 differentially expressed genes in tissue from rats

given Dextran Sodium Sulphate in water with or without co-treatment with hypothetical protein (HP).

Figure 8 shows the expression of inflammation-associated genes (Realtime PCR) in ascending colons from rats given Dextran Sodium Sulphate in water with (DSS/HP) or without (DSS) co-treatment with hypothetical protein (HP).

DETAILED DESCRIPTION

10 HP

Without wishing to be bound by theory, the polypeptide HP of the present invention is a pirin-related protein.

15 Polypeptide HP has at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identity to the polypeptide sequence shown as SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof.

One example of polypeptide HP of the present invention is SEQ ID NO 2 deposited with GenBank as AAO75294.1; *Bacteroides thetaiotaomicron* comprising SEQ ID NO 1 can be found deposited as DSM2079 [E50(VPI5482), VPI5482] at DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH). The polypeptide sequence SEQ ID NO 2 has the following sequence:

```

25      10      20      30      40      50      60
MKKVIDRASS RGYFNHGWLK THHTFSFANY YNPERIHFGA LRVLNDDSDV PSMGFDTHPH

      70      80      90      100     110     120
KNMEVISIPL KGYLRHGDSV QNTKTITPGD IQVMSTGSGI YHSEYNDSKE EQLEFLQIWV

30      130     140     150     160     170     180
FPRIENTKPE YNNFDIRPLL KPNELSLFIS PNGKTPASIK QDAWFSGMDF DTERTIEYCM

      190     200     210     220     230
HQEGNGAYLF VIEGEISVAD EHLAKRDGIG IWDTKSFSIR ATKGTKLLVM EVPM

```

AAO75294.1 is described as being a possible Pirin family protein. AAO75294.1 was identified from *Bacteroides thetaiotaomicron* VPI-5482.

The polypeptides sequences deposited in GenBank as AAO76683.1 and
 5 CDE80552.1 are examples of polypeptides having at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6.

The polypeptide sequence of AAO76683.1 is as follows:

```

      10      20      30      40      50      60
10  MKKVIHKADT RGHSQYDWLD SYHTFSFDEY FSDSRINFGA LRVLNDDKVA PGEGFQTHPH

      70      80      90     100     110     120
    KNMEIISIPL KGHLQHGD SK KNSRIITVGE IQTMSAGTGI FHSEVNASPV EPVEFLQIWI

15      130     140     150     160     170     180
    MPRERNTHPV YKDFSIKELE RPNELAVIVS PDGSTPASLL QDTWFSIGKV EAGKKLGYHL

      190     200     210     220     230
    HQSHGGVYIF LIEGEIVVDG EVLKRRDGMG VYDTKSFELE TLKDSHILLI EVPM
  
```

20 The polypeptide sequence of AAO76683.1 is also referred to as SEQ ID NO 7 herein. AAO76683.1 is described in GenBank as being a putative Pirin family protein. AAO76683.1 was isolated from *Bacteroides thetaiotaomicron* VPI-5482. [E50(VPI5482), VPI5482]. *Bacteroides thetaiotaomicron* comprising SEQ ID NO 7
 25 Mikroorganismen und Zellkulturen GmbH).

The polypeptide sequence of CDE80552.1 is as follows:

```

      10      20      30      40      50      60
30  MKKVIHKADT RGHSQYDWLD SYHTFSFDEY FSDSRINFGA LRVLNDDKVA PGEGFQTHPH

      70      80      90     100     110     120
    KNMEIISIPL KGHLQHGD SK KNSRIITVGE IQTMSAGTGI FHSEVNASPV EPVEFLQIWI

35      130     140     150     160     170     180
    MPRERNTHPV YKDFSIKELE RPNELAVIVS PDGSTPASLL QDTWFSIGKV EAGKKLGYHL

      190     200     210     220     230
    HQSHGGVYIF LIEGEIVVDG EVLKRRDGMG VYDTKSFELE TLKDSHILLI EVPM
  
```

The polypeptide sequence of CDE80552.1 is also referred to as SEQ ID NO 8 herein. CDE80552.1 is described in GenBank as being a putative Pirin family protein. CDE80552.1 was isolated from *Bacteroides thetaiotaomicron* CAG:40.

- 5 The terms “polypeptide HP”, “HP polypeptide” and “HP” are used interchangeably herein.

In one embodiment, the polypeptide HP is the polypeptide shown as SEQ ID NO 2.

- 10 In another embodiment, the polypeptide HP is the polypeptide shown as SEQ ID NO 4.

In further embodiment, the polypeptide HP is the polypeptide shown as SEQ ID NO 6.

15

HP polypeptides can be derived from certain microorganisms. In one aspect, the HP polypeptide is derived from an anaerobic, gram negative bacterium which can live in the alimentary canal. In a further aspect, the HP polypeptide is derived from a *Bacteroides* spp such as a *Bacteroides thetaiotaomicron*.

20

- Examples of a polynucleotide sequence encoding polypeptide HP include the polynucleotide sequences shown as SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5; polynucleotide sequences encoding the polypeptide shown as SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6; polynucleotides sequences having at least 75%, 80%, 25 85%, 90%, 95%, 97%, 98%, or 99% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or variants, homologues, fragments or derivatives thereof; polynucleotides sequences encoding a polypeptide having at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity to the polypeptide shown as SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or variants, homologues, fragments or derivatives thereof; and 30 polynucleotide sequences encoding SEQ ID NO 7 or SEQ ID NO 8.

SEQ ID NOs 1, 3 and 5 are shown in Figure 1A.

SEQ ID NO 1 has the following sequence:

Atgaaaaaagtaatcgacagagcttcatcaagaggctattttaatcatggctggctcaaaacccaccacacattcag
 ttttgtaactattacaatccggaagaatccatttcggagccttgcgagtgctgaatgatgacagtgtagacccgtcg
 atgggatttgatactcatccacataaaaatatggaagtaatttcattccgttgaaagggtatctgagacatggcgaca
 gtgtacaaaatacgaaaacgattactcccgggtatatccaagtgatgagtagggcagtggtatctatcatagtgagt
 5 ataacgacagcaaggaagaacaattggaattcctgcaaatatgggtattccccgaatcgagaatacgaaacccg
 aatataacaatttcgatatacgtccgctgctgaaaccgaacgagttatctctgttcatttcaccgaacggcaagacacc
 ggctccatcaaacaggatgcctgggtctctatgggagacttcgatacggaaagaaccatcgaatattgtatgcatca
 ggaaggtaacggagcttatctgtttgtatagaaggagagatcagcgtggccgatgaacatctggccaaacgtgac
 ggcacggaatatgggataccaaaagcttctctatccgtgctactaaagggaccaaactctggtaatggaagtaccc
 10 atgtaa

SEQ ID NO 1 encodes SEQ ID NO 2 which is deposited with GenBank under
 accession number AAO75294.1.

15 The polynucleotide sequence of SEQ ID NO 1 was codon optimised for expression
 in *E. coli*. This codon optimised sequence is shown as SEQ ID NO 3. This
 sequence may also be referred herein as "Rec 1 HP" or "recombinant 1 HP".

SEQ ID NO 3 has the following sequence:

20 ggtaccatgaaaaaagtgattgatcgtgagcagccgtggctattttaacatggctggctgaaaacccatcatacc
 ttagcttcggaactattataatccggaacgcattcattttggcgcgctgctgtgctgaacgatgatagcgtggatccg
 agcatgggctttgatacccatccgcacaaaaacatggaagtattgacattccgctgaaaggctatctgcgtcatggc
 gatagcgtgcagaacacaaaaaccattacccgggtgatattcaggtgatgagcaccggcagcggcatttatcata
 gcgaatacaacgatagcaaagaagaacagctggaatttctgcagatttgggtgttccgcgtattgaaaacacaaaa
 25 ccggaataaacaactttgatattcgccgctgctgaaaccgaacgaactgagcctgtttattagcccgaacggcaa
 aaccccggcgagcattaaacaggatgcgtgggttagcatgggcgattttgataccgaacgcaccattgaatattgcat
 gcatcaggaaggcaacggcgctacctgtttgtattgaaggcgaaattagcgtggcggtgaacatctggccaaa
 cgtgatggcattggcatttgggataccaaaagcttcagcattcgtgaccaaaggcaccaaactgctggtgatgga
 agtgccgatgtaataagagctc

30

The polypeptide sequence encoded by SEQ ID NO 3 is shown as SEQ ID NO 4.
 SEQ ID NO 4 has the following sequence:

GTMKKVIDRASSRGYFNHGWLKTHHTFSFANYYNPERIHFGALRVLNDDSDPSM
 GFDTHPHKNMEVISIPLKGYLRHGDSVQNTKTITPGDIQVMSTGSGIYHSEYNDSK
 35 EEQLEFLQIWWFPRIENTKPEYNNFDIRPLLKPNELSLFISPNGKTPASIKQDAWFSM

GDFDERTIEYCMHQEGNGAYLFVIEGEISVADEHLAKRDGIGIWDTKSFSIRATKG
TKLLVMEVPM EL

The polynucleotide sequence of SEQ ID NO 1 was codon optimised for expression
5 in *Lactococcus lactis*. This codon optimised sequence is shown as SEQ ID NO 5.
This sequence may also be referred herein as "Rec 2 HP" or "recombinant 2 HP".

SEQ ID NO 5 has the following sequence:

ggtaccatgaaaaaagttattgatcgcttcacgcgtggatatttaaatcatggatggcttaaaactcatcacattta
10 gtttgccaattattataatccagaacgtattcatttgggtgctcttcgtgttctaatgatgattcagttgatccatcaatggga
tttgatacatccacataaaaaataggaagttattcaattccactaaaggatatcttcgtcatggtgattcagttcaaa
atacaaaaacaattacacctggagatattcaagttatgtctacaggatcaggaatttatcattcagaatataatgattca
aaagaagaacaactgaatttctcaaatttgggtcttccacgtattgaaaatacaaaaccagaatataataatttga
cattcgctccacttcttaaaccaaatgaactttcacttttatctcaccaaatggaaaaacaccagcttcaattaaacaag
15 atgcttggttttcaatgggagatttgatacagaacgtacaattgaatattgtatgcatcaagaaggtaacggcgcttatc
ttttgtattgaaggtgaaatttcagttgctgatgaacatcttgctaaacgtgatggaattggaatttgggatacaaaatca
tttcaattcgtgctacaaaagggtacaaaacttctgttatggaagtccaatgtaataagagctc

The polypeptide sequence encoded by SEQ ID NO 5 is shown as SEQ ID NO 6.
20 SEQ ID NO 6 has the following sequence:

GTMKKVIDRASSRGYFNHGWLKTHHTFSFANYYNPERIHFGALRVLNDDSDPSM
GFDTHPHKNMEVISIPLKGYLRHGDSVQNTKTITPGDIQVMSTGSGIYHSEYNDSK
EEQLEFLQIWVFPRIENTKPEYNNFDIRPLLKPNELSLFISPNGKTPASIKQDAWFSM
GDFDERTIEYCMHQEGNGAYLFVIEGEISVADEHLAKRDGIGIWDTKSFSIRATKG
25 TKLLVMEVPM EL

In one embodiment, the polynucleotide sequence encoding polypeptide HP has at
least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity to the polynucleotide
sequence shown as SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5 or to variants,
30 homologues, fragments or derivatives thereof.

In one embodiment, the polynucleotide sequence encoding polypeptide HP encodes
a polypeptide shown as SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or a
polypeptide having at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity
35 to the polypeptide shown as SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 or to

variants, homologues, fragments or derivatives thereof.

In one embodiment, the polypeptide HP is a truncated HP polypeptide. For example, the truncated polypeptide comprises at least 20, 30, 40, 50, 75, 100, 125,
5 150, 175 or 200 amino acids of polypeptide shown as SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6.

In one embodiment, the polynucleotide sequence encoding the polypeptide HP encodes a truncated HP polypeptide.

10

In one embodiment, the polypeptide HP is a fusion polypeptide. For example, the polypeptide is fused to glutathione S-transferase (GST).

Host cell

15

In one aspect, a host cell as described herein comprises a polynucleotide sequence encoding polypeptide HP.

In another aspect, a host cell as described herein comprises an expression vector
20 comprising a polynucleotide sequence encoding polypeptide HP.

In a further aspect, a host cell as described herein has been transformed with a nucleotide sequence that causes the host cell to overexpress HP. For example, a promoter is inserted into the genome of a host cell which enables the host cell to
25 overexpress a polynucleotide sequence HP (such as an endogenous polynucleotide sequence) - i.e. the promoter is capable of overexpressing the polynucleotide sequence encoding HP.

As used herein, the term "overexpress" in the phrase "a nucleotide sequence that causes the host cell to overexpress HP" and "promoter capable of overexpressing the polynucleotide sequence encoding HP" refers to an increase in expression from
30 zero to a level of expression or going from a lower level of expression to a higher level of expression (e.g. upregulation) when the transformed host cell is compared to the equivalent host cell prior to transformation.

35

In one embodiment, the level of mRNA encoding HP in a transformed host cell which overexpresses HP is increased (i.e. upregulated) such that the level of mRNA is at least 10%, 20%, 30%, 40% or 50% higher in a transformed host cell when compared to the equivalent host cell prior to transformation.

5

Examples of host cells overexpressing HP include: (i) host cells transformed with an expression vector encoding HP (prior to transformation said host cell was not capable of expressing HP); and (ii) host cells transformed to upregulate the expression of an endogenous HP (prior to transformation said host cell was capable of expressing said HP for a given set of culture conditions but after transformation said host cell is capable of expressing said HP at a higher level, in the same culture conditions).

The polynucleotide sequence encoding polypeptide HP may be codon optimised for the host cell. For instance, the polypeptide sequence may be codon optimised for expression in *E. coli* (such as SEQ ID NO 3) or the polynucleotide sequence may be codon optimised for expression in *Lactococcus lactis* (such as SEQ ID NO 5).

The term "host cell" - in relation to the present invention - includes any cell that comprises either the polynucleotide sequence encoding HP as described herein or an expression vector comprising said polynucleotide sequence as described herein. The host cell may be used in the recombinant production of a protein having the specific properties as defined herein. The host cell may contain a heterologous polynucleotide sequence coding for HP or may be a cell expressing its natural HP polynucleotide. For example, the host cell may be from *Bacteroides* spp such as *Bacteroides thetaiotaomicron*.

The term "host cell" as used herein may be interchangeably used with "host organism" and "host microorganism".

30

Thus, there is provided host cells transformed or transfected with a polynucleotide sequence encoding HP as described herein or an expression vector comprising said polynucleotide sequence as described herein.

The term "transfected cell" or "transfected host cell" as used herein means a host

cell transfected so that it comprises a polynucleotide sequence encoding HP as described herein or an expression vector comprising said polynucleotide sequence as described herein. In addition or alternatively, the host cell has been transformed with a nucleotide sequence that causes the host cell to overexpress a polynucleotide sequence encoding HP. For example, a promoter is inserted into the genome of a host cell which enables the host cell to overexpress an endogenous poly nucleotide sequence encoding HP.

The term "transformed cell" or "transformed host cell" as used herein means a host cell having a modified genetic structure.

The term "host cell" includes any cell which a vector is capable of transfecting or transducing.

Host cells will be chosen to be compatible with the vector and may for example be prokaryotic (for example bacterial), fungal, yeast or plant cells.

Host cells comprising polynucleotide sequences encoding polypeptide HP or an expression vector comprising said polynucleotide sequence may be used to express the polypeptide HP under *in vitro*, *in vivo* and *ex vivo* conditions.

In one embodiment, the host cell is a microorganism, such as a bacterium. Typically, the microorganism which inhabits the alimentary canal, or a section of the alimentary canal. Examples of suitable bacterial host cells are gram positive or gram negative bacterial species. For instance, the host cell may be selected from the group consisting of *Bacteroides* spp (such as *Bacteroides thetaiotaomicron*), *E. coli*, *Lactococcus* spp (such as *L. lactis*), *Lactobacillus* spp, *Bifidobacterium* spp, and *Streptococcus* spp (such as *Streptococcus thermophilus*).

In one embodiment, the host cell comprises an exogenous polynucleotide sequence encoding HP.

In another embodiment, the host cell comprises an endogenous polynucleotide sequence encoding HP. For example, the endogenous polynucleotide sequence under the control of a non-native promoter (such as a constitutive promoter). In a

further example, the host cell comprises multiple copies of the endogenous polynucleotide sequence.

5 The term "host cell" does not cover native nucleotide coding sequences in their natural environment when they are under the control of their native promoter which is also in its natural environment. An example of a host cell which has a native nucleotide sequence which is not in its natural environment is a *Bacteroides thetaiotaomicron* VPI-5482 comprising SEQ ID NO 1 in which SEQ ID NO 1 under the control of a non-native promoter (such as a constitutive promoter). In another example, a *Bacteroides*
10 *thetaitotaomicron* VPI-5482 comprising SEQ ID NO 1 has multiple copies of SEQ ID NO 1.

Depending on the nature of the nucleotide sequence, and/or the desirability for further processing of the expressed protein, eukaryotic hosts such as yeasts or
15 other fungi or insect cells (such as insect Sf9 cells) may be used. In general, yeast cells are preferred over fungal cells because they are easier to manipulate. However, some proteins are either poorly secreted from the yeast cell, or in some cases are not processed properly (e.g. hyperglycosylation in yeast). In these instances, a different fungal host organism should be selected.

20 The use of suitable host cells - such as yeast, fungal and plant host cells - may provide for post-translational modifications (e.g. myristoylation, glycosylation, truncation, lipidation and tyrosine, serine or threonine phosphorylation) as may be needed to confer optimal biological activity on the polypeptide described herein.

25 Host cells may be cultured under suitable conditions which allow expression of the polypeptide.

30 In some embodiments, the polypeptide can be extracted from host cells by a variety of techniques known in the art, including enzymatic, chemical and/or osmotic lysis and physical disruption. The polypeptide may be purified and isolated in a manner known *per se*.

35

Transformation of host cells

Teachings on the transformation of prokaryotic hosts is well documented in the art, for example see Sambrook *et al* (Molecular Cloning: A Laboratory Manual, 2nd edition, 1989, Cold Spring Harbor Laboratory Press). If a prokaryotic host is used
5 then the nucleotide sequence may need to be suitably modified before transformation - such as by removal of introns.

Filamentous fungi cells may be transformed using various methods known in the art – such as a process involving protoplast formation and transformation of the
10 protoplasts followed by regeneration of the cell wall in a manner known. The use of *Aspergillus* as a host microorganism is described in EP 0 238 023.

Another host organism can be a plant. A review of the general techniques used for transforming plants may be found in articles by Potrykus (*Annu Rev Plant Physiol Plant Mol Biol* [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27). Further teachings on plant transformation may be found in
15 EP-A-0449375.

20 General teachings on the transformation of fungi, yeasts and plants are presented in following sections.

Transformed fungus

25 A host cell may be a fungus - such as a mould. Examples of suitable such hosts include any member belonging to the genera *Thermomyces*, *Acremonium*, *Aspergillus*, *Penicillium*, *Mucor*, *Neurospora*, *Trichoderma* and the like.

In one embodiment, the host cell may be a filamentous fungus.

30

Transforming filamentous fungi is discussed in US-A-5741665 which states that standard techniques for transformation of filamentous fungi and culturing the fungi are well known in the art. An extensive review of techniques as applied to *N. crassa* is found, for example in Davis and de Serres, *Methods Enzymol* (1971) 17A:
35 79-143.

Further teachings which may also be utilised in transforming filamentous fungi are reviewed in US-A-5674707.

- 5 In addition, gene expression in filamentous fungi is taught in in Punt *et al.* (2002) Trends Biotechnol 2002 May;20(5):200-6, Archer & Peberdy Crit Rev Biotechnol (1997) 17(4):273-306.

The present description encompasses the production of transgenic filamentous fungi
10 according to the present description prepared by use of these standard techniques.

In one aspect, the host organism can be of the genus *Aspergillus*, such as *Aspergillus niger*.

- 15 A transgenic *Aspergillus* according to the present invention can also be prepared by following, for example, the teachings of Turner G. 1994 (Vectors for genetic manipulation. In: Martinelli S.D., Kinghorn J.R.(Editors) *Aspergillus*: 50 years on. Progress in industrial microbiology vol 29. Elsevier Amsterdam 1994. pp. 641-666).

20 **Transformed yeast**

In another embodiment, the transgenic organism can be a yeast.

- A review of the principles of heterologous gene expression in yeast are provided in, for
25 example, *Methods Mol Biol* (1995), 49:341-54, and *Curr Opin Biotechnol* (1997) Oct;8(5):554-60

In this regard, yeast – such as the species *Saccharomyces cerevisi* or *Pichia pastoris* (see FEMS Microbiol Rev (2000 24(1):45-66), may be used as a vehicle for
30 heterologous gene expression.

- A review of the principles of heterologous gene expression in *Saccharomyces cerevisiae* and secretion of gene products is given by E Hinchcliffe E Kenny (1993, "Yeast as a vehicle for the expression of heterologous genes", *Yeasts*, Vol 5, Anthony H Rose and J Stuart Harrison, eds, 2nd edition, Academic Press Ltd.).
35

For the transformation of yeast, several transformation protocols have been developed. For example, a transgenic *Saccharomyces* according to the present invention can be prepared by following the teachings of Hinnen *et al.*, (1978, *Proceedings of the National Academy of Sciences of the USA* **75**, 1929); Beggs, J D (1978, *Nature*, London, **275**, 104); and Ito, H *et al* (1983, *J Bacteriology* 153, 163-168).

The transformed yeast cells may be selected using various selective markers – such as auxotrophic markers dominant antibiotic resistance markers.

10

Transformed plants/plant cells

A host cell suitable for the present invention may be a plant. In this respect, the basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted genetic material. A review of the general techniques may be found in articles by Potrykus (*Annu Rev Plant Physiol Plant Mol Biol* [1991] **42**:205-225) and Christou (*Agro-Food-Industry Hi-Tech* March/April 1994 17-27).

20 Direct infection of plant tissues by *Agrobacterium* is a simple technique which has been widely employed and which is described in Butcher D.N. *et al.*, (1980), *Tissue Culture Methods for Plant Pathologists*, eds.: D.S. Ingrams and J.P. Helgeson, 203-208.

Other techniques for transforming plants include ballistic transformation, the silicon whisker carbide technique (see Frame BR, Drayton PR, Bagnaall SV, Lewnau CJ, Bullock WP, Wilson HM, Dunwell JM, Thompson JA & Wang K (1994) Production of fertile transgenic maize plants by silicon carbide whisker-mediated transformation, *The Plant Journal* **6**: 941-948) and viral transformation techniques (e.g. see Meyer P, Heidmann I & Niedenhof I (1992) The use of cassava mosaic virus as a vector system for plants, *Gene* **110**: 213-217).

30

Further teachings on plant transformation may be found in EP-A-0449375.

Plant cells may be grown and maintained in accordance with well-known tissue culturing methods such as by culturing the cells in a suitable culture medium supplied

35

with the necessary growth factors such as amino acids, plant hormones, vitamins, etc.

In a further aspect, the present description relates to a vector system which carries a nucleotide sequence or construct according to the present description and which is capable of introducing the nucleotide sequence or construct into the genome of an organism, such as a plant. The vector system may comprise one vector, but it may comprise two vectors. In the case of two vectors, the vector system is normally referred to as a binary vector system. Binary vector systems are described in further detail in Gynheung An *et al.*, (1980), Binary Vectors, *Plant Molecular Biology Manual* A3, 1-19.

One extensively employed system for transformation of plant cells uses the Ti plasmid from *Agrobacterium tumefaciens* or a Ri plasmid from *Agrobacterium rhizogenes* An *et al.*, (1986), *Plant Physiol.* 81, 301-305 and Butcher D.N. *et al.*, (1980), *Tissue Culture Methods for Plant Pathologists*, eds.: D.S. Ingrams and J.P. Helgeson, 203-208. After each introduction method of the desired promoter or construct or nucleotide sequence according to the present invention in the plants, the presence and/or insertion of further DNA sequences may be necessary. If, for example, for the transformation the Ti- or Ri-plasmid of the plant cells is used, at least the right boundary and often however the right and the left boundary of the Ti- and Ri-plasmid T-DNA, as flanking areas of the introduced genes, can be connected. The use of T-DNA for the transformation of plant cells has been intensively studied and is described in EP-A-120516; Hoekema, in: The Binary Plant Vector System Offset-drukkerij Kanters B.B., Alblasterdam, 1985, Chapter V; Fraley, *et al.*, *Crit. Rev. Plant Sci.*, 4:1-46; and An *et al.*, *EMBO J.* (1985) 4:277-284.

Culturing and production

Host cells transformed with the nucleotide sequence described herein may be cultured under conditions conducive to the production of the encoded polypeptide and which facilitate recovery of the polypeptide from the cells and/or culture medium.

The medium used to cultivate the cells may be any conventional medium suitable for growing the host cell in question and obtaining expression of the polypeptide.

The protein produced by a recombinant cell may be displayed on the surface of the cell.

- 5 The protein may be secreted from the host cells and may conveniently be recovered from the culture medium using well-known procedures.

Secretion

- 10 Often, it is desirable for the protein to be secreted from the expression host cell into the culture medium from where the protein may be more easily recovered. According to the present description, the secretion leader sequence may be selected on the basis of the desired expression host. Hybrid signal sequences may also be used with the context of the present invention.

15

Typical examples of heterologous secretion leader sequences are those originating from the fungal amyloglucosidase (AG) gene (*glaA* - both 18 and 24 amino acid versions e.g. from *Aspergillus*), the α -factor gene (yeasts e.g. *Saccharomyces*, *Kluyveromyces* and *Hansenula*) or the α -amylase gene (*Bacillus*).

20

By way of example, the secretion of heterologous proteins in *E. coli* is reviewed in Methods Enzymol (1990) 182:132-43.

Expression vectors

25

The term "expression vector" means a construct capable of *in vivo*, *ex vivo* or *in vitro* expression.

- 30 The term "construct" - which is synonymous with terms such as "conjugate", "cassette" and "hybrid" - includes a nucleotide sequence as described herein which optionally may be directly or indirectly attached to a promoter. An example of an indirect attachment is the provision of a suitable spacer group such as an intron sequence, such as the Sh1-intron or the ADH intron, intermediate the promoter and the nucleotide
35 sequence of the present invention. The same is true for the term "fused" in relation to

the present invention which includes direct or indirect attachment. In some cases, the terms do not cover the natural combination of the nucleotide sequence coding for the protein ordinarily associated with the wild type gene promoter and when they are both in their natural environment.

5

The construct may even contain or express a marker, which allows for the selection of the genetic construct.

For some applications, the construct comprises at least the nucleotide sequence described herein operably linked to a promoter.

10

The nucleotide sequence of the present description may be present in a vector in which the nucleotide sequence is operably linked to regulatory sequences capable of providing for the expression of the nucleotide sequence by a suitable host cell.

15

In some embodiments, the polynucleotide sequence encoding polypeptide HP of an expression vector may be codon optimised for the host cell which will be or has been transformed or transfected with the polynucleotide sequence.

20 The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A regulatory sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under condition compatible with the control sequences. For example, a promoter is operably linked to a coding
25 sequence if it controls the transcription of the coding sequence.

The term "regulatory sequences" includes promoters and enhancers and other expression regulation signals.

30 The term "promoter" is used in the normal sense of the art, e.g. an RNA polymerase binding site. The promoter may be heterologous or homologous to the nucleotide sequence.

Enhanced expression of the nucleotide sequence described herein may also be
35 achieved by the selection of heterologous regulatory regions, e.g. promoter,

secretion leader and terminator regions.

In one embodiment, the nucleotide sequence as described herein is operably linked to at least a promoter.

5

Other promoters may even be used to direct expression of the polypeptide described herein.

Examples of suitable promoters for directing the transcription of the nucleotide sequence in a bacterial, fungal or yeast host are well known in the art.

10

The promoter can additionally include features to ensure or to increase expression in a suitable host. For example, the features can be conserved regions such as a Pribnow Box or a TATA box.

15

Once transformed into a suitable host, the vector may replicate and function independently of the host genome, or may, in some instances, be incorporated into the genome into the genome of a suitable host cell. In some instances, the term "incorporated" covers stable incorporation into the genome.

20

The vectors for use in the present invention may be transformed into a suitable host cell as described herein to provide for expression of a polypeptide of the present description.

25 The vector may be a plasmid, a phage particle, or simply a potential genomic insert.

The choice of vector e.g. a plasmid, cosmid, or phage vector will often depend on the host cell into which it is to be introduced.

30 The vectors for use in the present invention may contain one or more selectable marker genes- such as a gene, which confers antibiotic resistance e.g. ampicillin, kanamycin, chloramphenicol or tetracyclin resistance. Alternatively, the selection may be accomplished by co-transformation (as described in WO91/17243).

35 Vectors may be used *in vitro*, for example for the production of RNA or used to

transfect, transform, transduce or infect a host cell.

The vector may further comprise a nucleotide sequence enabling the vector to replicate in the host cell in question. Examples of such sequences are the origins of replication of plasmids pUC19, pACYC177, pUB110, pE194, pAMB1 and pIJ702.

In one embodiment, an expression vector comprises one or more polynucleotide sequences according to the present invention. The polynucleotide sequence may be heterologous or homologous to a host cell transformed or transfected with the expression vector.

Disorders

Polypeptide HP or a polynucleotide sequence encoding polypeptide HP or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence may be used for the treatment and/or prevention of a disorder in a subject, wherein said disorder is an inflammatory disorder and/or an autoimmune disorder. The disorder may also be of the CNS, including autism.

In one embodiment, the disorder affects the alimentary canal, a section of the alimentary canal, the liver, liver cells, epithelial cells, epidermal cells, neuronal cells, the pancreas, and/or pancreatic cells (such as the islets of Langerhans), kidneys, spleen, lungs and heart and/or cells thereof.

Examples of sections (i.e. parts) of the alimentary canal include the mouth, the oesophagus, the stomach and the intestine (such as the small intestine (e.g. the duodenum, the jejunum and the ileum) and/or the large intestine (e.g. the caecum, ascending colon, transverse colon, descending colon, and sigmoid colon)).

Examples of epithelial cells include intestinal, oral, lung, nasal, vaginal epithelial cells.

In one embodiment, the disorder is selected from the group consisting of inflammatory bowel disorder (IBD), colitis, rheumatoid arthritis, psoriasis, multiple

sclerosis, type I diabetes, coeliac disease, atopic dermatitis, rhinitis, irritable bowel syndrome (IBS), ulcerative colitis, pouchitis, Crohn's disease, functional dyspepsia, atopic diseases, necrotising enterocolitis, non alcoholic fatty liver disease, gastrointestinal infection, Lupus, nephritis/glomerulonephritis, asthma, COPD,
 5 myococarditis and combinations thereof.

In one aspect, the disorder affects the intestine.

In one aspect, the disorder is an inflammatory disorder. For example, the disorder is
 10 an inflammatory bowel disorder (IBD) such as Crohn's disease.

In one aspect, the disorder is an autoimmune disorder. For example, the autoimmune disorder is selected from the group consisting of ulcerative colitis, pouchitis, rheumatoid arthritis, psoriasis, multiple sclerosis, type I diabetes, allergies
 15 (including coeliac disease), atopic dermatitis, rhinitis, Lupus, nephritis/glomerulonephritis, asthma, COPD and myococarditis.

Subject

20 In one embodiment, the subject is a monogastric animal.

Examples of monogastric animals include poultry, humans, rats, pigs, dogs, cats, horses and rabbits.

25 In another embodiment, the subject is a mammal such as a monogastric mammal.

Examples of monogastric mammals include omnivores (such as humans, rats, and pigs), carnivores (such as dogs and cats), and herbivores (such as horses and rabbits).

30

In one embodiment, the subject is a human.

In one aspect, the subject has a disorder is selected from the group consisting of inflammatory bowel disorder (IBD), colitis, rheumatoid arthritis, psoriasis, multiple
 35 sclerosis, type I diabetes, coeliac disease, atopic dermatitis, rhinitis, irritable bowel

syndrome (IBS), ulcerative colitis, pouchitis, Crohn's disease, functional dyspepsia, atopic diseases, necrotising enterocolitis, non alcoholic fatty liver disease, gastrointestinal infection Lupus, nephritis/glomerulonephritis, asthma, COPD, myocarditis and combinations thereof. For example, the subject has IBD.

5

Modulation/regulation

The terms "modulation" and "regulation" may be used interchangeably herein.

10 In one embodiment polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, is used to modulate the inflammation of a cell, a tissue or an organ in a subject.

15 In one embodiment, the term "modulation" refers to an increase and/or induction and/or promotion and/or activation. In an alternative embodiment, the term "modulation" refers to a decrease and/or reduction and/or inhibition.

In one embodiment, the term "regulation" refers to an upregulation. In an alternative
20 embodiment, the term "regulation" refers to a downregulation.

In one embodiment, the polypeptide HP or the polynucleotide sequence encoding polypeptide HP or the host cell as described herein reduces the inflammation of a cell, a tissue or an organ. For example, inflammation of the alimentary canal, a
25 section (i.e. part) of the alimentary canal (such as the intestine), the liver, liver cells, epithelial cells, epidermal cells, neuronal cells, the pancreas, and/or pancreatic cells (such as the islets of Langerhans), kidneys, spleen, lungs and heart and/or cells thereof is reduced.

30 In one example, inflammation of the alimentary canal or part thereof (such as the intestine) is reduced.

In another example, inflammation by epithelial cells of the tissue or the organ is reduced.

35

The term "inflammation" as used herein refers to one or more of the following: redness, swelling, pain, tenderness, heat, and disturbed function of a cell, a tissue or organ due to an inflammatory process triggered by over-reaction of the immune system.

5

In one embodiment, the numbers of cells which are inflamed in a subject is at least 10%, 20%, 30%, 40% or 50% lower after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the numbers of cells which are inflamed in a subject before the polypeptide HP or the polynucleotide sequence encoding HP or a host cell as described herein is administered to the subject.

In one embodiment, the amount of a tissue or organ which is inflamed in a subject is at least 10%, 20%, 30%, 40% or 50% lower after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the amount of tissue or organ which is inflamed in a subject before the polypeptide HP or the polynucleotide sequence encoding HP or a host cell as described herein is administered to the subject.

20 In one embodiment, the polypeptide HP or the polynucleotide sequence encoding polypeptide HP or the host cells as described herein reduces the inflammation by epithelial cells of the tissue or the organ.

For example, the epithelial cells are epithelial cells of the alimentary canal or part thereof (such as the intestine).

Without wishing to be bound by theory, the polypeptide HP or the polynucleotide sequence encoding polypeptide HP or the host cell as described herein increases the production of T cells (such as regulatory T cells which may also be referred to as Tregs) in a subject. This increase in Treg numbers may combat the effects of other effector T cells (also referred to as Teffs), such as Th1, Th17 and Th2 which drive inflammation, autoimmunity and allergic/atopic conditions. In Crohn's disease and ulcerative colitis the Teff/Treg cell balance is lost.

35 In one embodiment, the production of T cells in a subject is increased such that

there are at least 10%, 20%, 30%, 40% or 50% more T cells, or greater than 100% more T cells after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the number of T cells in the subject before the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein is administered to the subject.

Intestine barrier integrity

In one embodiment, the polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, is used to improve intestine barrier integrity in a subject.

The term “improving intestine barrier integrity” as used herein refers to a reduction in the numbers and/or types of microorganisms which spread from the intestine into other cells in a subject after administration of the polypeptide or polynucleotide or host cells as described herein when compared to the numbers and/or types of microorganisms which spread from the intestine into other cells in a subject before administration of the polypeptide or polynucleotide or host cell as described herein.

In one embodiment, the numbers of microorganisms which spread from the intestine into other cells in a subject are at least 10%, 20%, 30%, 40% or 50% lower after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the numbers of microorganisms which spread from the intestine into other cells in a subject before the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein is administered to the subject.

In one embodiment, there are at least 5%, 10%, 15% or 20% fewer types of microorganisms which spread from the intestine into other cells in a subject after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the types of microorganisms which spread from the intestine into other cells in a subject before the polypeptide HP or the polynucleotide sequence encoding HP or a host cell as described herein is administered to the subject.

Levels of bacteria

In one embodiment polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host
5 cell comprising an expression vector comprising said polynucleotide sequence, is used to modify the bacterial composition in a tissue or organ to provide a more beneficial microbiota. For example, the invention can be used to reduce the level of one or more types of lactose fermenting bacteria (such as *E. coli*) in a tissue or an organ in a subject and/or reduce the level of one or more types of non-lactose
10 fermenting bacteria in a tissue or an organ in a subject.

The term “reduce the level of one or more types of lactose fermenting bacteria” as used herein refers to a reduction in the numbers of lactose fermenting bacteria in a tissue or organ in a subject after administration of the polypeptide or polynucleotide
15 or host cell as described herein when compared to the numbers of lactose fermenting bacteria in a tissue or organ in a subject before administration of the polypeptide or polynucleotide or host cell as described herein.

In one embodiment, the numbers of lactose fermenting bacteria in a tissue or organ
20 in a subject are at least 10%, 20%, 30%, 40% or 50% lower after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the numbers of lactose fermenting bacteria in a tissue or organ in a subject before the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein is administered to the subject.

25

Examples of lactose fermenting bacteria include *E. coli*, *Enterobacter* and *Klebsiella*.

The term “reduce the level of one or more types of non-lactose fermenting bacteria” as used herein refers to a reduction in the numbers of non-lactose fermenting
30 bacteria in a tissue or organ in a subject after administration of the polypeptide or polynucleotide or host cells when compared to the numbers of non-lactose fermenting bacteria in a tissue or organ in a subject before administration of the polypeptide or polynucleotide or host cell as described herein.

35 In one embodiment, the numbers of non-lactose fermenting bacteria in a tissue or

organ in a subject are at least 10%, 20%, 30%, 40% or 50% lower after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the numbers of non-lactose fermenting bacteria in a tissue or organ in a subject before the polypeptide HP or the polynucleotide sequence
5 encoding HP or the host cell as described herein is administered to the subject.

Examples of non-lactose fermenting bacteria include as *Salmonella*, *Proteus species*, *Pseudomonas aeruginosa* and *Shigella*.

10 In one embodiment the tissue or organ is selected from the group consisting of mesenteric lymph nodes, liver, pancreas, spleen and combinations thereof.

Regulating appetite and/or weight

15

In one embodiment, polypeptide HP or a polynucleotide sequence encoding said polypeptide or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence is used to regulate the appetite (e.g. food intake) in a subject (such as a subject with IBD).

20

As used herein, the term “regulate appetite” or “regulating appetite” refers to the ability to modulate (e.g. increase or decrease) the desire for a subject to eat food.

In one embodiment, the term “regulate” or “regulation” refers to an increase in
25 appetite (e.g. food intake). In an alternative embodiment, the term “regulate” or “regulation” refers to a decrease in appetite (e.g. food intake).

For example, the polypeptide or polynucleotide or host cell as described herein maintains or stimulates the appetite in the subject.

30

In one embodiment, polypeptide HP or a polynucleotide sequence encoding said polypeptide or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence is used to regulate the weight of a subject (such as a subject with IBD).

35

In one embodiment, the term “regulate” or “regulation” refers to an increase in weight. In an alternative embodiment, the term “regulate” or “regulation” refers to a decrease in weight.

- 5 For example, the polypeptide or polynucleotide or host cell as described herein maintains the weight of a subject or increases the weight of a subject.

Without wishing to be bound by theory, the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein exerts a stimulatory
10 effect on the appetite of a subject by downregulating the expression of genes associated with the suppression of appetite (such as genes encoding satiety hormones). *Agt*, *Cartpt*, *Cck*, *Cxcl12* and *Gcg* are examples of genes associated with regulating appetite and the downregulation of one or more of these genes is associated with the suppression of appetite.

15

Cholecystokinin (Cck) and glucagon (Gcg) are examples of satiety hormones.

In one aspect, the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein stimulates the appetite in the subject such that the
20 subject consumes at least 5%, 10%, or 15% more food after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the subject before the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein is administered to the subject. In addition, or alternatively, the polypeptide HP or the polynucleotide sequence encoding HP or the
25 host cell as described herein stimulates the appetite in the subject such that after 1 month from first administration of the polypeptide or polynucleotide or host cell as described herein the weight of the subject is at least 2%, 5%, or 10% higher when compared to the subject before the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein is administered to the subject.

30

In one embodiment, the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein reduces the level of cholecystokinin (Cck) and/or glucagon (Gcg) in the blood of a subject.

35 In one aspect, the polypeptide HP or the polynucleotide sequence encoding HP or

the host cell as described herein reduces the level of cholecystokinin (Cck) and/or glucagon (Gcg) in the blood of a subject by at least 5%, 10%, 15% or 20% after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the subject before the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein is administered to the subject.

In one embodiment, the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein downregulates the expression of the gene encoding cholecystokinin (Cck) and/or the expression of the gene encoding glucagon (Gcg) in a cell or cells of a subject.

In one aspect, the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein decreases the expression of the gene encoding cholecystokinin (Cck) such that the expression level (e.g. mRNA level) is at least 5%, 10%, 15% or 20% lower after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the expression level in the subject before the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein is administered to the subject.

In one aspect, the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein decreases the expression of the gene encoding glucagon (Gcg) such that the expression level (e.g. mRNA level) is at least 5%, 10%, 15% or 20% lower after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the expression level in the subject before the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein is administered to the subject.

Maintaining the length of part of the intestine

In one embodiment polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, is used to maintain the length of part of the intestine (such as the large intestine and/or small intestine) of a subject.

Examples of sections (i.e. parts) of the intestine include the small intestine (e.g. the duodenum, the jejunum and the ileum) and/or the large intestine (e.g. the caecum, ascending colon, transverse colon, descending colon, and sigmoid colon).

5

The term “maintains the length” as used herein refers to there being no or only a small change in the length of part of the intestine after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the length of that part of the intestine before administration of the polypeptide or polynucleotide or host cell as described herein.

10

In one embodiment, the polypeptide or polynucleotide sequence or host cell as described herein prevents a reduction in the length of large intestine. In addition or alternatively, the polypeptide or polynucleotide sequence or host cell as described herein prevents an increase in the length of the small intestine.

15

In one embodiment, a small change in the length of the large intestine of a subject is a reduction in length of less than 5%, 2% or 1% after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the length of the large intestine in a subject before the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein is administered to the subject.

20

In one embodiment, a small change in the length of the small intestine of a subject is an increase in length of less than 1%, 2%, 5%, 7% or 10% after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the length of the small intestine in a subject before the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein is administered to the subject.

25

30

Intestine disruption

In one embodiment, polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, is

35

used to reduce disruption to the intestine (e.g. large intestine) of a subject (such as a subject with IBD).

5 The term “disruption to the intestine of a subject” as used herein refers to an affect on the integrity of the mucosal epithelium and/or an affect on the number of goblet cells in the epithelium and/or an affect on the number of immune cells infiltrating the lamina propria.

10 In one embodiment, the polypeptide or polynucleotide sequence or host cell of the description reduces or prevents disruption to the integrity of the mucosal epithelium and/or reduces or prevents a reduction in the number of goblet cells in the epithelium and/or reduces or prevents the infiltration of immune cells into the lamina propria.

15 In one embodiment, a reduction in disruption to the integrity of the mucosal epithelium is a reduction of at least 5%, 10%, 15% or 20% in the numbers of bacteria crossing from the intestinal lumen into intestinal cells after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the numbers of bacteria crossing from the intestinal lumen into intestinal cells in a
20 subject before the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein is administered to the subject.

In one embodiment, an increase in the number of goblet cells in the epithelium is an increase of at least 2%, 5%, 10%, 15% or 20% in the numbers of goblet cells in the
25 epithelium of a subject after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the number goblet cells in the epithelium of a subject before the polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein is administered to the subject.

30 In one embodiment, the reduction in the infiltration of immune cells into the lamina propria is such that over a fixed time period (such as 24 hours) there is a reduction of at least 5%, 10%, 15%, 20% or 30% in the numbers of immune cells (e.g. T cells) crossing into lamina propria after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the numbers of immune cells
35 (e.g. T cells) crossing from the into lamina propria cells in a subject before the

polypeptide HP or the polynucleotide sequence encoding HP or the host cell as described herein is administered to the subject.

Pro-inflammatory genes and barrier integrity genes

5

In one embodiment, polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, is used to regulate the expression of one or more pro-inflammatory genes and/or anti-inflammatory genes and/or one or more barrier integrity genes in a cell or cells of a subject.

In one embodiment, the term “regulate” refers to an upregulation in the expression of one or more pro-inflammatory genes or anti-inflammatory genes. In an alternative embodiment, the term “regulate” refers to a downregulation in the expression of one or more pro-inflammatory genes or anti-inflammatory genes.

In one embodiment, the polypeptide HP or the polynucleotide sequence encoding polypeptide HP or the host cell as described herein downregulates the expression of one or more pro-inflammatory genes in a cell or cells of a subject.

The term “pro-inflammatory gene” as used herein refers to a gene which, when expressed, promotes inflammation. Examples of pro-inflammatory genes include genes encoding but not limited to IL1- β , IL4, IL5, IL6, IL8, IL12, IL13, IL17, IL21, IL22, IL23, IL27, IFN γ , CCL2, CCL3, CCL5, CCL20, CXCL5, CXCL10, CXCL12, CXCL13, and TNF- α .

In one embodiment, the pro-inflammatory gene is selected from the group consisting of IL6, CXCL10, and TNF- α .

30

In one embodiment, the expression level (e.g. mRNA level) of one or more pro-inflammatory genes is decreased (i.e. downregulated) such that the level is at least 10%, 20%, 30%, 40% or 50% lower after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the level in the subject before polypeptide HP or the polynucleotide sequence encoding polypeptide

HP or the host cell as described herein is administered to the subject.

The term “barrier integrity genes” as used herein refers to a gene which, when expressed, has a role in the function of the barrier of the intestine such as the repair of the barrier and the prevention of microorganisms crossing the barrier. Examples of barrier integrity genes include genes encoding Retnlg|Retnlb, Si, Defa24, Hsd11b2, Hsd17b2, and Nr1d1|Thra.

In one embodiment, the term “regulate” refers to an upregulation in the expression of one or more barrier integrity genes. In an alternative embodiment, the term “regulate” refers to a downregulation in the expression of one or more barrier integrity genes.

In one embodiment, the polypeptide HP or the polynucleotide sequence encoding polypeptide HP or the host cell as described herein upregulates the expression of barrier integrity genes in a cell or cells of a subject

In one embodiment, the barrier integrity gene is selected from the group consisting of Retnlg|Retnlb, Si, Defa24, Hsd11b2, Hsd17b2, and Nr1d1|Thra.

In one embodiment, the expression level (e.g. mRNA level) of one or more barrier integrity genes is increased (i.e. upregulated) such that the level is at least 10%, 20%, 30%, 40% or 50% higher after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the level in the subject before polypeptide HP or the polynucleotide sequence encoding polypeptide HP or the host cell as described herein is administered to the subject.

In one embodiment, the polypeptide HP or the polynucleotide sequence encoding polypeptide HP or the host cell as described herein upregulates the expression of anti-inflammatory genes.

Regulating gene expression

In one embodiment, the polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host

cell comprising an expression vector comprising said polynucleotide sequence, is used in regulating the expression in a cell or cells of a subject (such as a subject with IBD) of one or more genes selected from the group consisting of regenerating islet-derived 3 beta gene (Reg3b), resistin-like gamma resistin like beta gene (Retnlg|Retnlb), sucrase-isomaltase (alpha-glucosidase) gene (Si), defensin alpha 24 gene (Defa24), hydroxysteroid 11-beta dehydrogenase 2 gene (Hsd11b2), hydroxysteroid (17-beta) dehydrogenase 2 gene (Hsd17b2), resistin-Like Molecule-beta (RELMb), and nuclear receptor 1D1 thyroid hormone receptor alpha gene (Nr1d1|Thra).

10

The terms "Reg", "Reg3" and "Reg3b" as used herein are interchangeable.

The terms "Hsd", "Hsd17b2" or "Hsd17b2" as used herein are interchangeable.

15 In one embodiment, the term "regulate" refers to an upregulation in the expression of the genes. In an alternative embodiment, the term "regulate" refers to a downregulation in the expression of the genes.

The present invention is useful in regulating the expression of pro-inflammatory genes and/or barrier integrity genes.

20

For the avoidance of doubt, pro-inflammatory genes include IL- β , IL4, IL5, IL6, IL8, IL12, IL13, IL17, IL21, IL22, IL23, IL27, IFN α , CCL2, CCL3, CCL5, CCL20, CXCL5, CXCL10, CXCL12, CXCL13 and TNF- α .

25

For the avoidance of doubt, barrier integrity genes include Retnlg|Retnlb, Si, Defa24, Hsd11b2, Hsd17b2, and Nrd1\Thra.

In one embodiment, the polypeptide or polynucleotide sequence or host cell as described herein decreases the expression of one or more genes selected from the group consisting of regenerating islet-derived 3 beta gene (Reg3b); resistin-like gamma resistin like beta gene (Retnlg|Retnlb); resistin-Like Molecule-beta (RELMb), sucrase-isomaltase (alpha-glucosidase) gene (Si); and defensin alpha 24 gene (Defa24). For example, the expression level (e.g. mRNA level) of one or more genes selected from the group is decreased (i.e. downregulated) such that the level

35

is at least 10%, 20%, 30%, 40% or 50% lower after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the level in the subject before polypeptide HP or the polynucleotide sequence encoding polypeptide HP or the host cell as described herein is administered to the subject.

5

In one embodiment, the polypeptide or polynucleotide sequence or host cell as described herein increases the expression of one or more genes selected from the group consisting of hydroxysteroid 11-beta dehydrogenase 2 gene (Hsd11b2); hydroxysteroid (17-beta) dehydrogenase 2 gene (Hsd17b2); and nuclear receptor 1D1 thyroid hormone receptor alpha gene (Nr1d1|Thra). For example, the expression level (e.g. mRNA level) of one or more genes selected from the group is increased (i.e. upregulated) such that the level is at least 10%, 20%, 30%, 40% or 50% higher after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the level in the subject before polypeptide HP or the polynucleotide sequence encoding polypeptide HP or the host cell as described herein is administered to the subject.

Proinflammatory pathways

In one embodiment, polypeptide HP or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, is used to reduce the activation of pro-inflammatory pathways in a cell or cells of a subject.

25

The reduction in the activation of pro-inflammatory pathways can be determined by determining the inflammation in a subject.

Inflammation in a subject can be determined by determining the levels of pro-inflammatory cytokines and chemokines in tissue, serum and/or faecal samples in a subject before, and after, the polypeptide or polynucleotide or host cell as described herein is administered to the subject. For example, the levels of one or more of the following can be monitored: IL-1, IL-4, IL5, IL6, IL-8, IL-12, IL-13, IL-17, IL-21, IL-22, IL23, $TNF\alpha$, $IFN\gamma$, CXCL1, CXCL10, CCL20 serum and faecal calprotectin, SA1009/SA1008 calcium binding proteins, and Type 1 interferons, CD markers such

as CD163, CD14, inflammatory transcription factors such as NF- κ B, STAT, and MAPkinases, c-reactive protein (CRP), erythrocyte sedimentation rate (ESR), complement proteins, serum albumin, histological evaluation of target tissues and organs, disease activity indices.

5

In one embodiment, polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, is used to reduce the activity and/or expression of NF- κ B in a cell or cells (such as epithelial cells, epidermal cells, neuronal cells, liver, spleen, kidney, lung, heart and/or pancreatic cells) of a subject.

For example, the activity of NF- κ B is decreased such that the activity of NF- κ B is at least 10%, 20%, 30%, 40% or 50% lower after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the level in the subject before the polypeptide HP or the polynucleotide sequence encoding polypeptide HP or the host cell as described herein is administered to the subject.

For example, the expression level (e.g. mRNA) of NF- κ B is decreased (i.e. downregulated) such that the level is at least 10%, 20%, 30%, 40% or 50% lower after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the level in the subject before the polypeptide HP or the polynucleotide sequence encoding polypeptide HP or the host cell as described herein is administered to the subject.

25

Alimentary canal

Parts of the alimentary canal include the mouth, the oesophagus, the stomach and the intestine (such as the small intestine (e.g. the duodenum, the jejunum and the ileum) and/or the large intestine (e.g. the caecum, ascending colon, transverse colon, descending colon, and sigmoid colon)).

Herein, the term "large intestine" may be used interchangeably with the term "colon".

In one embodiment, polypeptide HP, or a polynucleotide sequence encoding

polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, is used for improving alimentary canal health in a subject.

- 5 The term “improving alimentary canal health” as used herein refers to reducing the level of inflammation in the alimentary canal or part thereof and/or improving intestinal microbiota.

10 In one embodiment, the level of inflammation in the alimentary canal is at least 10%, 20%, 30%, 40% or 50% lower after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the level of inflammation in the alimentary canal of a subject before the polypeptide or polynucleotide or host cell as described herein is administered to the subject.

- 15 In one embodiment, polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, is used for improving intestinal microbiota in a subject.

- 20 The term “intestinal microbiota” as used herein refers to microorganisms that live in the digestive tract of the host animals. These microorganisms perform a wide variety of metabolic, structural, protective and other beneficiary functions.

25 As used herein, the term “improving intestinal microbiota” refers to increasing the number and/or type of desirable microorganisms present in the intestine of a subject (e.g. the host), and/or increasing the activity of said desirable microorganisms in terms of their metabolic, structural, protective and other beneficiary functions. The term “improving intestinal microbiota” may also refer to decreasing the number and/or type of undesirable microorganisms present in the intestine of a subject (e.g.
30 the host), and/or decreasing the activity of said undesirable microorganisms in terms of their metabolic, structural, protective and other beneficiary functions.

Microorganisms which are desirable in the intestine of a host are those microorganisms which have a protective and beneficiary function. Firmicutes and/or
35 Bacteroidetes bacteria are examples of desirable microorganisms in the intestine of

a host.

Microorganisms which are undesirable in the intestine of a host are those microorganisms which can interfere with the metabolic, structural, protective and other beneficiary functions of desirable microorganisms in the intestine have a protective and beneficiary function. In addition or alternatively, undesirable microorganisms are those which cause, for example, inflammation and/or diarrhoea. *E. coli* (ETEC, EPEC, EIEC, EHEC and/or EAEC) is an example of an undesirable microorganism in the intestine of a host.

10

For example, the numbers (i.e. levels) of Firmicutes and/or Bacteroidetes bacteria are increased and the numbers of *E. coli* are reduced; such an improvement in intestinal microbiota may occur in subjects with inflammatory bowel disease (IBD) once the polypeptide HP or the polynucleotide sequence encoding polypeptide HP or a host cell comprising said polynucleotide sequence or a host cell comprising an expression vector comprising said polynucleotide sequence has been administered to the subject.

In one embodiment, the number of desirable microorganisms (such as Firmicutes and/or Bacteroidetes bacteria) present in the intestine of a subject (e.g. the host), is increased such that the number of microorganisms is at least 10%, 20%, 30%, 40% or 50% higher, or greater than 100% higher after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the level in the subject before the polypeptide or polynucleotide or host cell as described herein is administered to the subject. In addition, or alternatively, the types of desirable microorganisms (such as Clostridium cluster XIVa bacteria) present in the intestine of a subject (e.g. the host), are increased such that there are at least 2%, 5%, 10%, or 15% more types of microorganisms after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the types in the subject before the polypeptide or polynucleotide or host cell as described herein is administered to the subject.

In one embodiment, the protein of the invention modifies the bacterial composition in the intestine of a subject to provide a beneficial microbiota. For example, the number of undesirable microorganisms (such as *E. coli* (ETEC, EPEC, EIEC, EHEC

and/or EAEC)) present in the intestine of a subject (e.g. the host), is decreased such that the number of microorganisms is at least 10%, 20%, 30%, 40% or 50% lower after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the level in the subject before the polypeptide or polynucleotide or host cell as described herein is administered to the subject. In addition, or alternatively, the types of undesirable microorganisms (such as *E. coli*) present in the intestine of a subject (e.g. the host), are decreased such that there are at least 1%, 2%, 5%, or 10%, fewer types of undesirable microorganisms after administration of the polypeptide or polynucleotide or host cell as described herein when compared to the types in the subject before the polypeptide or polynucleotide or host cell as described herein is administered to the subject.

Encapsulation

In one embodiment, the polynucleotide sequence encoding polypeptide HP or a host cell comprising said polynucleotide sequence or a host cell comprising an expression vector comprising said polynucleotide sequence is encapsulated.

In a further embodiment, a pharmaceutical composition comprising the polynucleotide sequence encoding polypeptide HP or a host cell comprising said polynucleotide sequence or a host cell comprising an expression vector comprising said polynucleotide sequence is encapsulated.

In another embodiment, a nutritional supplement comprising the polynucleotide sequence encoding polypeptide HP or a host cell comprising said polynucleotide sequence or a host cell comprising an expression vector comprising said polynucleotide sequence encoding said polypeptide is encapsulated.

In a further embodiment, a feedstuff, food product, dietary supplement, or food additive as described herein is encapsulated.

The term “encapsulated” as used herein refers to a means for protecting the polypeptide or polynucleotide or host cell as described herein from an incompatible environment by physical separation so that it can be delivered to the target site (e.g. the intestine) without degradation or significant degradation in order that the

polypeptide or polynucleotide or host cell can have an effect on the target site. An example is an enteric coated capsule or an enterically-resistant capsule.

5 Even when the objective of the encapsulation is the isolation of the polypeptide or polynucleotide or host cell from its surroundings, the protective coating or shell must be ruptured at the time of desired action. The rupturing of the protective coating or shell is typically brought about through the application of chemical and physical stimuli such as pressure, enzyme attack, chemical reaction and physical disintegration.

10

For example, encapsulation ensures that the polypeptide or polynucleotide or host cell can be ingested so that the polypeptide or polynucleotide or host cell can be delivered to the target site (e.g. the intestine) in an amount which is effective to produce an effect at the target site.

15

Pharmaceutical composition

20 In one embodiment, a pharmaceutical composition comprises polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, and optionally a pharmaceutically acceptable excipient, carrier or diluent.

25 The pharmaceutical composition may be any pharmaceutical composition. In one aspect, the pharmaceutical composition is to be administered orally, enterally or rectally. For example, the composition may be an edible composition. "Edible" means a material that is approved for human or animal consumption.

30 The pharmaceutical compositions may be for human or animal usage in human and veterinary medicine.

Examples of such suitable excipients for the various different forms of pharmaceutical compositions described herein may be found in the "Handbook of Pharmaceutical Excipients, 2nd Edition, (1994), Edited by A Wade and PJ Weller.

35

Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985).

- 5 Examples of suitable carriers include lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol and the like.

Examples of suitable diluents include one or more of: water, ethanol, glycerol, propylene glycol and glycerin, and combinations thereof.

10

The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as, or in addition to, the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s).

15

Examples of suitable binders include starch, gelatin, natural sugars such as glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweeteners, natural and synthetic gums, such as acacia, tragacanth or sodium alginate, carboxymethyl cellulose and polyethylene glycol.

20

Examples of suitable lubricants include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like.

- 25 Preservatives, stabilizers, dyes and even flavouring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

- 30 In one aspect, the polypeptide or polynucleotide sequence or host cell of the pharmaceutical composition is encapsulated.

In another aspect, the polypeptide of the pharmaceutical composition is a recombinant polypeptide.

35

In a further aspect, the polynucleotide sequence of the pharmaceutical composition encodes a recombinant polypeptide.

5 In another aspect, the host cell of the pharmaceutical composition produces or is capable of producing a recombinant polypeptide.

In a further aspect, an expression vector comprises said polynucleotide sequence of the pharmaceutical composition.

10 The pharmaceutical may be in the form of a solution or as a solid – depending on the use and/or the mode of application and/or the mode of administration.

As used herein, the term "medicament" encompasses medicaments for both human and animal usage in human and veterinary medicine. In addition, the term
15 "medicament" as used herein means any substance, which provides a therapeutic and/or beneficial effect. The term "medicament" as used herein is not necessarily limited to substances, which need Marketing Approval, but may include substances which, can be used in cosmetics, nutraceuticals, food (including feeds and beverages for example), probiotic cultures, nutritional supplements and natural
20 remedies. In addition, the term "medicament" as used herein encompasses a product designed for incorporation in animal feed, for example livestock feed and/or pet food.

Nutritional supplements

25

Nutritionally acceptable carriers, diluents and excipients include those suitable for human or animal consumption and that are used as standard in the food industry. Typical nutritionally acceptable carriers, diluents and excipients will be familiar to the skilled person in the art.

30

In one embodiment, a nutritional supplement comprises polypeptide HP, or a polynucleotide sequence encoding polypeptide HP, or a host cell comprising said polynucleotide sequence, or a host cell comprising an expression vector comprising said polynucleotide sequence, and a nutritional acceptable excipient, carrier or
35 diluent.

In one example, the polypeptide or polynucleotide sequence or host cell of the nutritional supplement is encapsulated.

- 5 In another example, the polypeptide of the nutritional supplement is a recombinant polypeptide.

In a further aspect, the polynucleotide sequence of the nutritional supplement encodes a recombinant polypeptide.

10

In another aspect, the host cell of the nutritional supplement produces or is capable of producing a recombinant polypeptide.

- 15 In a further example, the polynucleotide of the nutritional supplement is comprised in an expression vector.

Feedstuff/products

- 20 A further aspect of the invention relates to feedstuffs, food products, dietary supplements and food additives comprising polypeptide HP or a polynucleotide sequence encoding polypeptide HP or a host cell comprising said polynucleotide sequence or a host cell comprising an expression vector comprising said polynucleotide sequence.

- 25 The terms "feedstuff", "food product" "food additive" and "dietary supplement" as used herein are intended to cover all consumable products that can be solid, jellied or liquid.

- 30 The term "food product" is used in a broad sense – and covers food for humans as well as food for animals (i.e. a feed). In one aspect, the food product is for human consumption. Examples of food products include dairy products (such as milk, cheese, beverages comprising whey protein, milk drinks, lactic acid bacteria drinks, yoghurt, drinking yoghurt), bakery products, beverages and beverage powders.

- 35 The "feedstuff", "food product" "food additive" and "dietary supplement" may be in

the form of a solution or as a solid – depending on the use and/or the mode of application and/or the mode of administration.

As used herein the term “dietary supplement” includes a formulation which is or can be added to a food product or feedstuff as a nutritional supplement. The term “dietary supplement” as used here also refers to formulations which can be used at low levels in a wide variety of products that require gelling, texturising, stabilising, suspending, film-forming and structuring, retention of juiciness and improved mouthfeel, without adding viscosity.

10

Suitable food products may include, for example, functional food products, food compositions, pet food, livestock feed, health foods, feedstuffs and the like. In one aspect, the food product is a health food.

15 As used herein, the term "functional food product" means food that is capable of providing not only a nutritional effect, but is also capable of delivering a further beneficial effect to the consumer. Accordingly, functional foods are ordinary foods that have components or ingredients (such as those described herein) incorporated into them that impart to the food a specific functional - e.g. medical or physiological
20 benefit - other than a purely nutritional effect.

Examples of specific food products that are applicable to the present invention include milk-based products, ready to eat desserts, powders for re-constitution with, e.g., milk or water, chocolate milk drinks, malt drinks, ready-to-eat dishes, instant
25 dishes or drinks for humans or food compositions representing a complete or a partial diet intended for pets or livestock.

In one aspect, the feedstuff, food product, dietary supplement or food additive according to the present invention are intended for humans, pets or livestock such
30 as monogastric animals. The feedstuff, food product, dietary supplement or food additive may be intended for animals selected from the group consisting of dogs, cats, pigs, horses, or poultry. In a further embodiment, the food product, dietary supplement or food additive is intended for adult species, in particular human adults.

35 The term "milk-based product" as used herein means any liquid or semi-solid milk or

whey based product having a varying fat content. The milk-based product can be, e.g., cow's milk, goat's milk, sheep's milk, skimmed milk, whole milk, milk recombined from powdered milk and whey without any processing, or a processed product, such as yoghurt, curdled milk, curd, sour milk, sour whole milk, butter milk
5 and other sour milk products. Another important group includes milk beverages, such as whey beverages, fermented milks, condensed milks, infant or baby milks; flavoured milks, ice cream; milk-containing food such as sweets.

The feedstuffs, food products, dietary supplements or food additives of the present
10 invention may be - or may be added to - food supplements, also referred to herein as dietary or nutritional supplements or food additives.

The feedstuffs, food products, dietary supplements or food additives according to the invention may also be used in animal nutrition (e.g. in pig nutrition), particularly
15 in the early-weaned period and growing fattening period. The feedstuffs, food products, dietary supplements or food additives are expected to enhance immune function reduce and prevent infectious diseases, beneficially alter the microbiota composition, and improve growth and performance of animals, for example, through increased feed conversion efficiency.

20 In one embodiment the feedstuff, food product, dietary supplement, or food additive is encapsulated.

In one embodiment, the polypeptide of the feedstuff, food product, dietary
25 supplement, or food additive is a recombinant polypeptide.

In one example, the polynucleotide of the feedstuff, food product, dietary
supplement, or food additive is comprised in an expression vector.

30

Administration

The pharmaceutical compositions, the nutritional supplements, feedstuffs, food
35 products, dietary supplements or food additives of the present invention may be

adapted for oral, rectal, vaginal, parenteral, intramuscular, intraperitoneal, intraarterial, intrathecal, intrabronchial, subcutaneous, intradermal, intravenous, nasal, buccal or sublingual routes of administration.

5 In one aspect, the pharmaceutical compositions, the nutritional supplements, feedstuffs, food products, dietary supplements or food additives of the present invention are adapted for oral, rectal, vaginal, parenteral, nasal, buccal or sublingual routes of administration.

10 In a further aspect, the pharmaceutical compositions, the nutritional supplements, feedstuffs, food products, dietary supplements or food additives of the present invention are adapted for oral administration.

For oral administration, particular use is made of compressed tablets, pills, tablets,
15 gellules, drops, and capsules.

Other forms of administration comprise solutions or emulsions which may be injected intravenously, intraarterially, intrathecally, subcutaneously, intradermally, intraperitoneally or intramuscularly, and which are prepared from sterile or
20 sterilisable solutions. The pharmaceutical compositions of the present invention may also be in form of suppositories, pessaries, suspensions, emulsions, lotions, ointments, creams, gels, sprays, solutions or dusting powders.

An alternative means of transdermal administration is by use of a skin patch. For
25 example, the active ingredient can be incorporated into a cream consisting of an aqueous emulsion of polyethylene glycols or liquid paraffin. In another example, the active ingredient can also be incorporated into an ointment consisting of a white wax or white soft paraffin base together with such stabilisers and preservatives as may be required.

30 Pharmaceutical compositions, the nutritional supplements, feedstuffs, food products, dietary supplements or food additives may be formulated in unit dosage form, i.e., in the form of discrete portions containing a unit dose, or a multiple or sub-unit of a unit dose.

35

Dosage

A person of ordinary skill in the art can easily determine an appropriate dose of polypeptide HP or a polynucleotide sequence or a host cell as described herein to
5 administer to a subject without undue experimentation. Typically, a physician will determine the actual dosage which will be most suitable for an individual patient and it will depend on a variety of factors including the activity of the specific bacterial strain employed, the metabolic stability and length of action of that strain, the age, body weight, general health, sex, diet, mode and time of administration, rate of
10 excretion, drug combination, the severity of the particular condition, and the individual undergoing therapy. The dosages disclosed herein are exemplary of the average case. There can of course be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

15 Combinations

In one aspect, polypeptide HP or a polynucleotide sequence encoding polypeptide HP or a host cell comprising said polynucleotide sequence or a host cell comprising an expression vector comprising said polynucleotide sequence are administered in
20 combination with one or more other active agents. In such cases, polypeptide HP or a polynucleotide sequence encoding polypeptide HP or a host cell comprising said polynucleotide sequence or a host cell comprising an expression vector comprising said polynucleotide sequence may be administered consecutively, simultaneously or sequentially with the one or more other active agents.

25

For instance, at least two of the polypeptide HP, the polynucleotide sequence and the host cell as described herein are administered to the subject.

For example, one type of host cell according the present invention (e.g. a *L. lactis*
30 transformed with a polynucleotide sequence encoding HP) may be combined with another type of host cell according to the present invention (e.g. a *Lactobacillus* spp transformed with a polynucleotide sequence encoding HP).

In another example, one type of host cell according the present invention (e.g. a *L.*
35 *lactis* transformed with a polynucleotide sequence encoding HP) may be combined

with another microorganism such as *Bacteroides* spp (such as *Bacteroides thetaiotaomicron*), *Lactococcus* spp (such as *L. lactis*), *Lactobacillus* spp, *Bifidobacterium* spp, and *Streptococcus* spp (such as *Streptococcus thermophilus*).

5 Polynucleotide sequence

The scope of the present description encompasses polynucleotide sequences encoding HP polypeptides.

10 The term "nucleotide sequence" as used herein refers to an oligonucleotide sequence or polynucleotide sequence, and variant, homologues, fragments and derivatives thereof (such as portions thereof). The nucleotide sequence may be of genomic or synthetic or recombinant origin, which may be double-stranded or single-stranded whether representing the sense or anti-sense strand.

15

The term "nucleotide sequence" in relation to the present description includes genomic DNA, cDNA, synthetic DNA, and RNA. In one embodiment it means cDNA sequence.

In one embodiment, the nucleotide sequence when relating to and when encompassed
20 by the *per se* scope of the present description does not include the native nucleotide sequence when in its natural environment and when it is linked to its naturally associated sequence(s) that is/are also in its/their natural environment. For ease of reference, herein this embodiment is called the "non-native nucleotide sequence". In this regard, the term "native nucleotide sequence" means an entire nucleotide
25 sequence that is in its native environment and when operatively linked to an entire promoter with which it is naturally associated, which promoter is also in its native environment. However, the amino acid sequence encompassed by scope the present description can be isolated and/or purified post expression of a nucleotide sequence in its native organism. In one embodiment, however, the amino acid sequence
30 encompassed by scope of the present description may be expressed by a nucleotide sequence in its native organism but wherein the nucleotide sequence is not under the control of the promoter with which it is naturally associated within that organism.

Typically, the nucleotide sequence encompassed by the scope of the present
35 description is prepared using recombinant DNA techniques (i.e. recombinant DNA).

However, in an alternative embodiment of the invention, the nucleotide sequence could be synthesised, in whole or in part, using chemical methods well known in the art (see Caruthers MH *et al.*, (1980) *Nuc Acids Res Symp Ser* 215-23 and Horn T *et al.*, (1980) *Nuc Acids Res Symp Ser* 225-232).

5

The polynucleotide encompassed in the present description may be used in conjunction with other polynucleotide sequences. Thus the present description also covers a combination of polynucleotide sequences wherein the combination comprises the polynucleotide sequence encoding HP and another polynucleotide sequence, which may be another polynucleotide sequence encoding HP.

10

Preparation of the nucleotide sequence

A nucleotide sequence encoding either a peptide of the present description may be identified and/or isolated and/or purified from any cell or organism producing said peptide. Various methods are well known within the art for the identification and/or isolation and/or purification of nucleotide sequences. By way of example, DNA amplification techniques to prepare more of a sequence may be used once a suitable sequence has been identified and/or isolated and/or purified.

15

20

By way of further example, a genomic DNA and/or cDNA library may be constructed using chromosomal DNA or messenger RNA from the organism producing the peptide. If the amino acid sequence is known, labelled oligonucleotide probes may be synthesised and used to identify clones from the genomic library prepared from the organism. Alternatively, a labelled oligonucleotide probe containing sequences homologous to a similar known gene could be used to identify clones. In the latter case, hybridisation and washing conditions of lower stringency are used.

25

Alternatively, clones comprising the peptides of the present description could be identified by inserting fragments of genomic DNA into an expression vector, such as a plasmid, transforming bacteria with the resulting genomic DNA library, and then plating the transformed bacteria onto agar plates containing a substrate for the peptide thereby allowing clones expressing the peptide to be identified.

30

In a yet further alternative, the nucleotide sequence encoding the peptide may be

35

prepared synthetically by established standard methods, e.g. the phosphoroamidite method described by Beucage S.L. *et al.*, (1981) *Tetrahedron Letters* **22**, p 1859-1869, or the method described by Matthes *et al.*, (1984) *EMBO J.* **3**, p 801-805. In the phosphoroamidite method, oligonucleotides are synthesised, e.g. in an automatic DNA synthesiser, purified, annealed, ligated and cloned in appropriate vectors.

The nucleotide sequence may be of mixed genomic and synthetic origin, mixed synthetic and cDNA origin, or mixed genomic and cDNA origin, prepared by ligating fragments of synthetic, genomic or cDNA origin (as appropriate) in accordance with standard techniques. Each ligated fragment corresponds to various parts of the entire nucleotide sequence. The DNA sequence may also be prepared by polymerase chain reaction (PCR) using specific primers, for instance as described in US 4,683,202 or in Saiki R K *et al.*, (*Science* (1988) **239**, pp 487-491).

Amino acid sequences

The scope of the present description also encompasses HP polypeptides as defined herein.

The amino acid sequence may be prepared/isolated from a suitable source, or it may be made synthetically or it may be prepared by use of recombinant DNA techniques.

The polypeptide encompassed in the present description may be used in conjunction with other peptides. Thus the present description also covers a combination of peptides wherein the combination comprises the polypeptide HP and another peptide, which may be another HP polypeptide.

The amino acid sequence when relating to and when encompassed by the *per se* scope of the present invention is not a native peptide. In this regard, the term "native peptide" means an entire peptide that is in its native environment and when it has been expressed by its native nucleotide sequence.

Recombinant polypeptide

In one aspect the polypeptide sequence for use in the present invention is a recombinant sequence – i.e. a sequence that has been prepared using recombinant DNA techniques (such as the expression of the polypeptide using a host cell
5 comprising an expression vector encoding the polypeptide).

These recombinant DNA techniques are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature, for example, J. Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory*
10 *Manual*, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press.

Fusion proteins

The polypeptide sequence for use according to the present invention may be
15 produced as a fusion protein, for example to aid in extraction and purification. Examples of fusion protein partners include glutathione-S-transferase (GST), 6xHis, GAL4 (DNA binding and/or transcriptional activation domains) and (β-galactosidase). It may also be convenient to include a proteolytic cleavage site between the fusion protein partner and the protein sequence of interest to allow
20 removal of fusion protein sequences.

Typically, the fusion protein will not hinder the activity of the protein sequence.

Gene fusion expression systems in *E. coli* have been reviewed in *Curr Opin*
25 *Biotechnol* (1995) 6(5):501-6.

In another embodiment of the description, the polypeptide sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for agents capable of affecting the substance activity, it may be
30 useful to encode a chimeric substance expressing a heterologous epitope that is recognised by a commercially available antibody.

Sequence identity or sequence homology

The terms "polypeptide", "polypeptide sequence", "peptide", "protein" and "amino acid sequence" are used interchangeably herein.

5 The terms "polynucleotide sequence" and "nucleotide sequence" are used interchangeably herein.

The present invention also encompasses the use of sequences having a degree of sequence identity or sequence homology with amino acid sequence(s) of a polypeptide described herein (e.g. variants, homologues and derivatives) or of any
10 nucleotide sequence encoding such a polypeptide (hereinafter referred to as a "homologous sequence(s)"). Here, the term "homologue" means an entity having a certain homology with the subject amino acid sequences and the subject nucleotide sequences. Here, the term "homology" can be equated with "identity".

15 In the present context, a homologous sequence is taken to include an amino acid or a nucleotide sequence which may be at least 50, 60, 70, 75, 80, 85 or 90% identical, in some embodiments at least 95, 96, 97, 98 or 99% identical to the subject sequence. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of
20 the present invention it is preferred to express homology in terms of sequence identity.

In some embodiments, a homologous sequence is taken to include an amino acid sequence or nucleotide sequence which has one or several additions, deletions
25 and/or substitutions compared with the subject sequence.

In some embodiments, the present invention relates to the use of a protein whose amino acid sequence is represented herein or a protein derived from this (parent) protein by substitution, deletion or addition of one or several amino acids, such as 2,
30 3, 4, 5, 6, 7, 8, 9 amino acids, or more amino acids, such as 10 or more than 10 amino acids in the amino acid sequence of the parent protein and having the activity of the parent protein.

In some embodiments, the present invention relates to the use of a nucleic acid
35 sequence (or gene) encoding a protein whose amino acid sequence is represented

herein or encoding a protein derived from this (parent) protein by substitution, deletion or addition of one or several amino acids, such as 2, 3, 4, 5, 6, 7, 8, 9 amino acids, or more amino acids, such as 10 or more than 10 amino acids in the amino acid sequence of the parent protein and having the activity of the parent
5 protein.

In the present context, a homologous sequence is taken to include a nucleotide sequence which may be at least 50, 60, 70, 75, 85 or 90% identical, in some embodiments at least 95, 96, 97, 98 or 99% identical to a nucleotide sequence
10 encoding a polypeptide described herein (the subject sequence). Typically, the homologues will comprise the same or equivalent sequences that code for the domain(s) etc. as the subject sequence. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express
15 homology in terms of sequence identity.

The homologous amino acid sequence and/or nucleotide sequence may provide and/or encode a polypeptide which retains the functional activity and/or enhances the activity of the polypeptide.
20

In some aspects, an amino acid sequence as described herein has at least 50, 60, 70, 75, 80, 85 or 90% identity, in some embodiments at least 95, 96, 97, 98 or 99% identity to the subject sequence.

25 In some aspects, a nucleotide sequence as described herein has at least 50, 60, 70, 75, 80, 85 or 90% identity, in some embodiments at least 95, 96, 97, 98 or 99% identity to the subject sequence.

Homology comparisons can be conducted by eye, or more usually, with the aid of
30 readily available sequence comparison programs. These commercially available computer programs can calculate % homology between two or more sequences.

% homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly
35 compared with the corresponding amino acid in the other sequence, one residue at

a time. This is called an “ungapped” alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into
5 consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible
10 insertions and deletions without penalizing unduly the overall homology score. This is achieved by inserting “gaps” in the sequence alignment to try to maximize local homology.

However, these more complex methods assign “gap penalties” to each gap that
15 occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible - reflecting higher relatedness between the two compared sequences - will achieve a higher score than one with many gaps. “Affine gap costs” are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the
20 gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimized alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. Typically the default values are used when using such software for sequence comparisons.

25 Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the Vector NTI (Invitrogen Corp.). Examples of software that can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel *et al* 1999 Short Protocols in Molecular
30 Biology, 4th Ed - Chapter 18), BLAST 2 (see FEMS Microbiol Lett 1999 174(2): 247-50; FEMS Microbiol Lett 1999 177(1): 187-8 and tatiana@ncbi.nlm.nih.gov), FASTA (Altschul *et al* 1990 J. Mol. Biol. 403-410) and AlignX for example. At least BLAST, BLAST 2 and FASTA are available for offline and online searching (see Ausubel *et al* 1999, pages 7-58 to 7-60).

35

Although the final % homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST suite of programs. Vector NTI programs generally use either the public default values or a custom symbol comparison table if supplied (see user manual for further details). For some applications, it is preferred to use the default values for the Vector NTI package.

10

Alternatively, percentage homologies may be calculated using the multiple alignment feature in Vector NTI (Invitrogen Corp.), based on an algorithm, analogous to CLUSTAL (Higgins DG & Sharp PM (1988), Gene 73(1), 237-244).

15 Once the software has produced an optimal alignment, it is possible to calculate % homology, for example % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

Should Gap Penalties be used when determining sequence identity, then the following parameters can be used for pairwise alignment for example:

20

FOR BLAST	
GAP OPEN	0
GAP EXTENSION	0

FOR CLUSTAL	DNA	PROTEIN	
WORD SIZE	2	1	K triple
GAP PENALTY	15	10	
GAP EXTENSION	6.66	0.1	

In one embodiment, CLUSTAL may be used with the gap penalty and gap extension set as defined above.

25

In one embodiment, the degree of identity with regard to a nucleotide sequence is determined over at least 20 contiguous nucleotides, for example over at least 30 contiguous nucleotides, for example over at least 40 contiguous nucleotides, for example over at least 50 contiguous nucleotides, for example over at least 60 contiguous nucleotides, for example over at least 100 contiguous nucleotides, for

30

example over at least 200 contiguous nucleotides, for example over at least 300 contiguous nucleotides.

In one embodiment, the degree of identity with regard to a nucleotide sequence may
5 be determined over the whole sequence.

The sequences may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent substance. Deliberate amino acid substitutions may be made on the basis of
10 similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the secondary binding activity of the substance is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar
15 hydrophilicity values include leucine, isoleucine, valine, glycine, alanine, asparagine, glutamine, serine, threonine, phenylalanine, and tyrosine.

Conservative substitutions may be made, for example according to the Table below. Amino acids in the same block in the second column and preferably in the same line
20 in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar – uncharged	C S T M
		N Q
	Polar – charged	D E
		K R
AROMATIC		H F W Y

The present invention also encompasses homologous substitution (substitution and replacement are both used herein to mean the interchange of an existing amino acid
25 residue, with an alternative residue) that may occur i.e. like-for-like substitution such as basic for basic, acidic for acidic, polar for polar etc. Non-homologous substitution may also occur i.e. from one class of residue to another or alternatively involving the inclusion of unnatural amino acids such as ornithine (hereinafter referred to as Z), diaminobutyric acid ornithine (hereinafter referred to as B), norleucine ornithine
30 (hereinafter referred to as O), pyriylalanine, thienylalanine, naphthylalanine and

phenylglycine.

Replacements may also be made by unnatural amino acids include; α^* and α -disubstituted* amino acids, N-alkyl amino acids*, lactic acid*, halide
 5 derivatives of natural amino acids such as trifluorotyrosine*, p-Cl-phenylalanine*, p-Br-phenylalanine*, p-I-phenylalanine*, L-allyl-glycine*, β -alanine*, L- α -amino butyric acid*, L- γ -amino butyric acid*, L- α -amino isobutyric acid*, L- ϵ -amino caproic acid[#], 7-amino heptanoic acid*, L-methionine sulfone^{#*}, L-norleucine*, L-norvaline*, p-nitro-L-phenylalanine*, L-hydroxyproline[#], L-thioprolin*, methyl derivatives of
 10 phenylalanine (Phe) such as 4-methyl-Phe*, pentamethyl-Phe*, L-Phe (4-amino)[#], L-Tyr (methyl)*, L-Phe (4-isopropyl)*, L-Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxyl acid)*, L-diaminopropionic acid[#] and L-Phe (4-benzyl)*. The notation * has been utilised for the purpose of the discussion above (relating to homologous or non-homologous substitution), to indicate the hydrophobic nature of the derivative
 15 whereas # has been utilised to indicate the hydrophilic nature of the derivative, #* indicates amphipathic characteristics.

Variant amino acid sequences may include suitable spacer groups that may be inserted between any two amino acid residues of the sequence including alkyl
 20 groups such as methyl, ethyl or propyl groups in addition to amino acid spacers such as glycine or β -alanine residues. A further form of variation, involves the presence of one or more amino acid residues in peptoid form, will be well understood by those skilled in the art. For the avoidance of doubt, "the peptoid form" is used to refer to variant amino acid residues wherein the α -carbon
 25 substituent group is on the residue's nitrogen atom rather than the α -carbon. Processes for preparing peptides in the peptoid form are known in the art, for example Simon RJ *et al.*, *PNAS* (1992) **89**(20), 9367-9371 and Horwell DC, *Trends Biotechnol.* (1995) **13**(4), 132-134.

30 The nucleotide sequences for use in the present invention may include within them synthetic or modified nucleotides. A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones and/or the addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. For the purposes of the present invention, it is
 35 to be understood that the nucleotide sequences described herein may be modified

by any method available in the art. Such modifications may be carried out in order to enhance the *in vivo* activity or life span of nucleotide sequences of the present invention.

- 5 The present invention also encompasses the use of nucleotide sequences that are complementary to the sequences presented herein, or any derivative or fragment thereof. If the sequence is complementary to a fragment thereof then that sequence can be used as a probe to identify similar coding sequences in other organisms etc.
- 10 Polynucleotides which are not 100% homologous to the sequences of the present invention but fall within the scope of the invention can be obtained in a number of ways. Other variants of the sequences described herein may be obtained for example by probing DNA libraries made from a range of individuals, for example individuals from different populations. In addition, other homologues may be obtained and such
- 15 homologues and fragments thereof in general will be capable of selectively hybridising to the sequences shown in the sequence listing herein. Such sequences may be obtained by probing cDNA libraries made from or genomic DNA libraries from other animal species, and probing such libraries with probes comprising all or part of any one of the sequences in the attached sequence listings under conditions of medium to high
- 20 stringency. Similar considerations apply to obtaining species homologues and allelic variants of the polypeptide or nucleotide sequences of the invention.

Variants and strain/species homologues may also be obtained using degenerate PCR which will use primers designed to target sequences within the variants and

25 homologues encoding conserved amino acid sequences within the sequences of the present invention. Conserved sequences can be predicted, for example, by aligning the amino acid sequences from several variants/homologues. Sequence alignments can be performed using computer software known in the art. For example the GCG Wisconsin PileUp program is widely used.

30

The primers used in degenerate PCR will contain one or more degenerate positions and will be used at stringency conditions lower than those used for cloning sequences with single sequence primers against known sequences.

- 35 Alternatively, such polynucleotides may be obtained by site directed mutagenesis of

characterised sequences. This may be useful where for example silent codon sequence changes are required to optimise codon preferences for a particular host cell in which the polynucleotide sequences are being expressed. Other sequence changes may be desired in order to introduce restriction enzyme recognition sites, or to alter the property or function of the polypeptides encoded by the polynucleotides.

Polynucleotides (nucleotide sequences) of the invention may be used to produce a primer, e.g. a PCR primer, a primer for an alternative amplification reaction, a probe e.g. labelled with a revealing label by conventional means using radioactive or non-radioactive labels, or the polynucleotides may be cloned into vectors. Such primers, probes and other fragments will be at least 15, preferably at least 20, for example at least 25, 30 or 40 nucleotides in length, and are also encompassed by the term polynucleotides of the invention as used herein.

Polynucleotides such as DNA polynucleotides and probes according to the invention may be produced recombinantly, synthetically, or by any means available to those of skill in the art. They may also be cloned by standard techniques.

In general, primers will be produced by synthetic means, involving a stepwise manufacture of the desired nucleic acid sequence one nucleotide at a time. Techniques for accomplishing this using automated techniques are readily available in the art.

Longer polynucleotides will generally be produced using recombinant means, for example using a PCR (polymerase chain reaction) cloning techniques. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable cloning vector.

Recombinant polynucleotide sequence

In one aspect the polynucleotide sequence for use in the present invention is a recombinant polypeptide sequence – i.e. a sequence that has been prepared using recombinant DNA techniques (such as the expression of the polypeptide using a host cell comprising an expression vector encoding the polypeptide). Examples of

recombinant polynucleotide sequences include codon optimised sequences and polynucleotide sequences encoding fusion polypeptide.

5 These recombinant DNA techniques are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature, for example, J. Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press.

10 Synthetic

In one aspect the sequence for use in the present invention is a synthetic sequence – i.e. a sequence that has been prepared by *in vitro* chemical or enzymatic synthesis. It includes, but is not limited to, sequences made with optimal codon usage for host organisms - such as the methylotrophic yeasts *Pichia* and
15 *Hansenula*.

The present invention is further described by way of the following non-limiting examples.

20 EXAMPLES

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA and immunology, which are within the capabilities of a person of ordinary skill
25 in the art. Such techniques are explained in the literature. See, for example, J. Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press; Ausubel, F. M. *et al.* (1995 and periodic supplements; *Current Protocols in Molecular Biology*, ch. 9, 13, and 16, John Wiley & Sons, New York, N.Y.); B. Roe, J. Crabtree, and A.
30 Kahn, 1996, *DNA Isolation and Sequencing: Essential Techniques*, John Wiley & Sons; J. M. Polak and James O'D. McGee, 1990, *In Situ Hybridization: Principles and Practice*, Oxford University Press; M. J. Gait (Editor), 1984, *Oligonucleotide Synthesis: A Practical Approach*, Irl Press; D. M. J. Lilley and J. E. Dahlberg, 1992, *Methods of Enzymology: DNA Structure Part A: Synthesis and Physical Analysis of*
35 *DNA Methods in Enzymology*, Academic Press; and E. M. Shevach and W. Strober,

1992 and periodic supplements, *Current Protocols in Immunology*, John Wiley & Sons, New York, NY. Each of these general texts is herein incorporated by reference.

- 5 *Bacteroides thetaiotaomicron* HP (BT0187, a pirin-related protein) was shown in an NF- κ B luciferase reporter assay, to greatly reduce NF- κ B activity stimulated in epithelial cells in culture by flagellin-, PMA- or IL-1.

For large scale production, HP (referred to as HP in Figures 1A and 1B with Figure 10 1A showing the polynucleotide sequence and Figure 1B showing the polypeptide sequence) was expressed in *E. coli* or *L. lactis*.

The sequence was codon optimised for expression in (i) *E. coli* (referred to as Rec 1 HP in Figure 1B) and (ii) *L. lactis* (referred to as Rec 2 HP in Figure 1B).

15

Isolated recombinant HP was tested *in vitro* and encapsulated.

The efficacy of the encapsulated product was evaluated in a rat model of inflammatory bowel disease (Dextran Sodium Sulphate [DSS]-induced colitis).

20

Example 1 – the effect of HP on inflammatory bowel disease

Rat study: Hooded-Lister rats (Rowett strain; 6 month-old; ~ 480g) were reared, housed and managed under standard high quality conditions within the 25 Bioresources of the Rowett Institute of Nutrition and Health. They had free access to sterile distilled water containing Dextran sodium sulphate (MP Biomedicals UK, Cambridge; DSS; 36000-50000 mol. wt.) for 7 days [days 1-5, 40g DSS/l and days 6-7, 20g DSS/l]. Half of the DSS-treated rats were dosed daily (day 1-7) with HP protein and the remaining six DSS-treated rats were dosed daily (day 1-7) with 30 placebo. Untreated controls had free access to sterile distilled water but were not dosed. Untreated controls had access to sterile distilled water. All rats had free access to high quality rodent chow. Food intake, water intake and body weight were measured daily.

The rats were euthanased (isoflurane overdose and exsanguination) and dissected on day 8. The total length of the colon was measured and a piece of ascending colon 3-6 cm from the caecal/colon junction was collected in OCT or fixed in neutral buffered formalin, 2-3 cm from the caecal/colon junction was placed in RNAlater and a piece 0-2 cm from the caecal/colon junction was snap frozen. A piece of descending colon 3-6 cm from the rectum was collected in OCT or fixed in neutral buffered formalin, 2-3 cm from the rectum was placed in RNAlater and a piece 0-2 cm from the rectum was snap frozen. The small intestine was measured, a piece of ileal tissue 5-7 cm from the ileocaecal junction was collected in OCT or fixed in neutral buffered formalin, 7-9 cm from the ileocaecal junction was collected for microbiology, 9-10 cm from the ileocaecal junction was placed in RNAlater and a piece 9-17 cm from the ileocaecal junction was snap frozen. Transverse colon was collected for microbiology as were mesenteric lymph nodes, liver and spleen. Lactose-fermenting and non-lactose fermenting bacteria in tissues were evaluated using MacConkey no 3 agar.

Fixed colon samples were embedded in 8100 Technovit resin. 4µm sections were cut and stained with hematoxylin and eosin. Whole transverse cross-sectional areas were imaged and digitised using a Zeiss Axioskop microscope connected to a QImaging camera controlled by ImageProPlus software. These were examined in a blinded manner by 2 independent individuals and the severity of intestinal damage was graded based on the method of Berg et al. (1996) and data expressed as percentage of fields of view with pathology of 0 [no pathology] through to grade 3 [major pathology].

The rats treated with Dextran Sodium Sulphate (DSS) and rats treated with both DSS and HP (DSS/HP) had comparable water intake and so, the intake of DSS was also same between the treatment groups. At the same time, it was observed that the food intake by DSS rats was at a slightly lower rate than that of DSS/HP treated rats and controls. The DSS treated rats also tended to lose weight, while at the same time, the DSS/HP and control rats maintained their weight (Figure 2).

Rat colon length was reduced due to intake of DSS, a reported feature of DSS colitis. However, this change in the colon was prevented when rats were also treated with HP (Figure 3). In contrast, small intestine length was increased with

intake of DSS but unaltered in rats given DSS/HP (Figure 3).

Mesenteric lymph nodes, liver and spleen of rats given DSS were sub-clinically infected with lactose (predominantly *E. coli*) -fermenting and non-lactose-fermenting bacteria (Figure 4). This was not evident with DSS/HP. Spread of bacteria to these systemic tissues is likely to be result of loss of gut barrier integrity due to damage caused by DSS. HP appeared to prevent this loss of gut barrier integrity.

Histological analysis of ascending and descending colon was carried out (Figure 5 & 6). The severity of intestinal damage was graded based on the method of Berg et al. (1996) and data expressed as percentage of fields of view with pathology of 0 [no pathology] through to grade 3 [major pathology] or as mean histopathology score. Disruption to the tissue caused by DSS was moderate and patchy, with varying degrees of damage (from little or none to severe) localised throughout the tissue sections. Overall, the integrity of the mucosal epithelium was impaired, there was a reduced number of goblet cells in the epithelium and infiltration of immune cells into the lamina propria. In contrast, overall disruption to the colon caused by DSS was greatly reduced by co-treatment of rats with HP. It was therefore protective in the DSS-induced colitis model.

Affymetrix analysis

Principal component analysis (PCA) was performed on the microarray data to separate the samples in a 3-dimensional way. Control and DSS/HP clustered together, and DSS were separate from this cluster. This PCA therefore indicated that DSS transcriptome profile was very different from the control and DSS/HP profiles, which were very similar. In turn, this indicated that HP was effective in treating the inflammation, as these animals seemed similar to healthy animals.

ANOVA with unequal variance (Welch), $P < 0.05$, asymptotic, all against single condition, Tukey HSD post-hoc was carried out to get a list of differentially expressed genes. This generated a table of 377 genes with differential expression (Table 1 and Table 3).

Table1. Genes with differential expression between DSS and Control and DSS/HP

and control in rats given Dextran Sodium Sulphate in water with or without co-treatment with hypothetical protein (HP).

Gene description	Gene symbol	Fold change		p-value
		DSS vs Control	DSS/HP vs Control	
Regenerating islet-derived 3 beta	Reg3b	11.400	2.107	0.016
Resistin-like gamma resistin like beta	Retnlg Retnlb	3.957	1.556	0.020
Sucrase-isomaltase (alpha-glucosidase)	Si	3.903	1.347	0.040
Defensin, alpha, 24	Defa24	3.045	1.552	0.026
Hydroxysteroid 11-beta dehydrogenase 2	Hsd11b2	-2.002	1.001	0.041
Hydroxysteroid (17-beta) dehydrogenase 2	Hsd17b2	-2.530	-1.603	0.040
Nuclear receptor subfamily 3, group C, member 2	Nr3c2	-1.447	-1.092	0.009

5

Gene description	GO_biological_process (up to first 10)
Regenerating islet-derived 3 beta	GO:0006953 acute-phase response; GO:0006954 inflammatory response
Resistin-like gamma resistin like beta	
Sucrase-isomaltase (alpha-glucosidase)	GO:0005975 carbohydrate metabolic process; GO:0007568 aging; GO:0007584 response to nutrient; GO:0008152 metabolic process; GO:0009744 response to sucrose stimulus; GO:0009750 response to fructose stimulus; GO:0032868 response to insulin stimulus; GO:0033189 response to vitamin A; GO:0042594 response to starvation; GO:0051384 response to glucocorticoid stimulus
Defensin, alpha, 24	GO:0006952 defense response; GO:0042742 defense response to bacterium
Hydroxysteroid 11-beta dehydrogenase 2	GO:0001666 response to hypoxia; GO:0002017 regulation of blood volume by renal aldosterone; GO:0006950 response to stress; GO:0007565 female pregnancy; GO:0008152 metabolic process; GO:0008211 glucocorticoid metabolic process; GO:0032094 response to food; GO:0032868 response to insulin stimulus; GO:0042493 response to drug; GO:0048545 response to steroid hormone stimulus

Hydroxysteroid (17-beta) dehydrogenase 2	GO:0006694 steroid biosynthetic process; GO:0032526 response to retinoic acid; GO:0055114
Nuclear receptor subfamily 1, group D, member 1 thyroid hormone receptor alpha	GO:0006355 regulation of transcription, DNA-dependent; GO:0007623 circadian rhythm; GO:0001502 cartilage condensation; GO:0001503 ossification; GO:0001822 kidney development; GO:0001889 liver development; GO:0002155 regulation of thyroid hormone mediated signaling pathway; GO:0006950 response to stress; GO:0007420 brain development; GO:0007611 learning or memory

A heatmap was created of the subset of 377 genes (Fig 7). This heatmap showed clear clustering of the DSS animals away from the Control and DSS/HP animals, which themselves also clustered separately, albeit not as distant from each other.

- 5 The overall colour pattern of control and DSS/HP was very similar, while the DSS animals mostly showed inverse fold changes for this gene subset to the other two groups.

- 10 Seven of these genes showed comparatively high fold changes compared to controls. Of these seven genes, 4 were up regulated and the other 3 were down regulated with respect to control (Table 1). These fold changes were generally higher for the DSS animals than for the DSS/HP animals. The most affected genes were regenerating islet-derived 3 beta (Reg3b), resistin-like gamma |resistin like beta (Retnlg|Retnlb), sucrase-isomaltase (alpha-glucosidase) (Si) and defensin alpha 24 (Defa24), which were up regulated and hydroxysteroid 11-beta dehydrogenase 2 (Hsd11b2), hydroxysteroid (17-beta) dehydrogenase 2 (Hsd17b2), and nuclear receptor 1D1| thyroid hormone receptor alpha (Nr1d1|Thra), which were down regulated with respect to the control (Table 1).

20 Realtime PCR

Expression of inflammation-associated genes in the ascending colon was generally lower in rats treated with DSS and HP than in tissue from rats treated with DSS alone (Figure 8; Table 2). *Reg3* and *RELMb* expression were in particular greatly reduced as a result of treatment with HP.

25

Table 2. Statistical analysis of inflammation-associated genes (Realtime PCR) in ascending colons from rats given Dextran Sodium Sulphate in water with or without

co-treatment with hypothetical protein (HP).

DSS vs Control			DSS/HP vs Control		DSS vs DSS/HP	
	Fold change	p-value	Fold change	p-value	Fold change	p-value
RELM-b	6.61	0.03	1.2	0.17	5.49	0.04
Reg3	61.25	0.04	4.04	0.24	15.15	0.01
Defa24	23.08	0.01	6.37	0.01	3.62	0.22
CXCL10	1.1	0.91	1.78	0.48	-1.63	0.39
TNF	-1.16	0.9	2.19	0.54	-2.55	0.11
Hsd	-3.17	0.01	-2.28	0.01	-1.39	0.36
IL6	3.2	0.43	1.35	0.81	2.37	0.11

Summary

5

Hypothetical protein ameliorated moderate DSS-induced colitis. This protection was, in part, linked with reduced expression of pro-inflammatory markers in the gut tissue.

Table 3 details all genes with differential expression between DSS and Control and DSS/HP and control in rats given Dextran Sodium Sulphate in water with or without co-treatment with hypothetical protein (HP).

Gene description	Gene symbol	Fold change		p-value	GO biological process (up to first 10)
		DSS vs Control	DSS/HP vs Control		
Regenerating islet-derived 3 beta	Reg3b	11.400	2.107	0.016	GO:0006953 acute-phase response; GO:0006954 inflammatory response
Resistin-like gamma resistin like beta	Retnlg Retnlb	3.957	1.556	0.020	
Sucrase-isomaltase (alpha-glucosidase)	Si	3.903	1.347	0.040	GO:0005975 carbohydrate metabolic process; GO:0007568 aging; GO:0007584 response to nutrient; GO:0008152 metabolic process; GO:0009744 response to sucrose stimulus; GO:0009750 response to fructose stimulus; GO:0032868 response to insulin stimulus; GO:0033189 response to vitamin A; GO:0042594 response to starvation; GO:0051384 response to glucocorticoid stimulus
Defensin, alpha, 24	Defa24	3.045	1.552	0.026	GO:0006952 defense response; GO:0042742 defense response to bacterium
Matrix Gla protein	Mgp	2.223	1.176	0.048	GO:0001503 ossification; GO:0006461 protein complex assembly; GO:0007275 multicellular organismal development ; GO:0007584 response to nutrient; GO:0009612 response to mechanical stimulus; GO:0009725 response to hormone stimulus; GO:0030154 cell differentiation; GO:0030324 lung development; GO:0030500 regulation of bone mineralization; GO:0042221 response to chemical stimulus
Phospholipase A2, group IIA (platelets, synovial fluid)	Pla2g2a	2.006	1.262	0.027	GO:0006644 phospholipid metabolic process; GO:0008285 negative regulation of cell proliferation; GO:0016042 lipid catabolic process; GO:0035019 somatic stem cell maintenance; GO:0042127 regulation of cell proliferation; GO:0046473 phosphatidic acid metabolic process; GO:0050678 regulation of epithelial cell proliferation; GO:0050680 negative regulation of epithelial cell proliferation
Grenlin 1, cysteine knot superfamily, homolog (Xenopus laevis)	Grem1	1.982	1.338	0.010	GO:0001658 branching involved in ureteric bud morphogenesis; GO:0002689 negative regulation of leukocyte chemotaxis; GO:0006915 apoptosis; GO:0007267 cell-cell signaling; GO:0009887 organ morphogenesis; GO:0009954 proximal/distal pattern formation; GO:0010717 regulation of epithelial to mesenchymal transition; GO:0030308 negative regulation of cell growth; GO:0030326 embryonic limb morphogenesis; GO:0030514 negative regulation of BMP signaling pathway
Ribosomal protein L10A similar to ribosomal protein L10a	Rpl10a RGD1559639 RGD1566137	1.958	1.086	0.033	GO:0006396 RNA processing; GO:0006412 translation; GO:0006414 translational elongation
Hydroxysteroid 11-beta dehydrogenase 1	Hsd11b1	1.907	1.233	0.000	GO:0006278 RNA-dependent DNA replication; GO:0006694 steroid biosynthetic process; GO:0006704 glucocorticoid biosynthetic process; GO:0006713 glucocorticoid catabolic process; GO:0008152 metabolic process; GO:0030324 lung development; GO:0043456 regulation of pentose-phosphate shunt
Carbamoyl-phosphate synthetase 1	Cps1	1.858	1.245	0.020	GO:0000050 urea cycle; GO:0005980 glycogen catabolic process; GO:0006541 glutamine metabolic process; GO:0006807 nitrogen compound metabolic process; GO:0014075 response to amine stimulus; GO:0019433 triglyceride catabolic process; GO:0032496 response to lipopolysaccharide; GO:0033762 response to glucagon stimulus; GO:0034201 response to

Paraoxonase 3	Pon3	1.816	1.358		0.033	oleic acid; GO:0042493 response to drug GO:0019439 aromatic compound catabolic process; GO:0046395 carboxylic acid catabolic process
Ornithine carbamoyltransferase	Otc	1.816	1.190		0.032	GO:0000050 urea cycle; GO:0006526 arginine biosynthetic process; GO:0006591 ornithine metabolic process; GO:0008652 cellular amino acid biosynthetic process; GO:0051259 protein oligomerization; GO:0055081 anion homeostasis
Leukocyte immunoglobulin-like receptor, subfamily B, member 4	Lilrb4	1.769	1.185		0.013	
Cadherin 19, type 2	Cdh19	1.732	1.328		0.001	GO:0007155 cell adhesion; GO:0007156 homophilic cell adhesion
Complement factor H	Cfh	1.723	1.509		0.031	GO:0006956 complement activation; GO:0030449 regulation of complement activation
Vomerol nasal 1 receptor, E14	V1re14	1.715	1.369		0.004	GO:0007186 G-protein coupled receptor protein signaling pathway
Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4	Slc25a4	1.701	1.228		0.010	GO:0015866 ADP transport; GO:0015867 ATP transport; GO:0051935 glutamate uptake involved in synaptic transmission ; GO:0055085 transmembrane transport; GO:0060547 negative regulation of necrotic cell death
Immediate early response 3	Ier3	1.692	1.227		0.037	
ST3 beta-galactoside alpha-2,3-sialyltransferase 4	ST3gal4	1.666	-1.069		0.047	GO:0006486 protein amino acid glycosylation
Fc fragment of IgG, low affinity IIa, receptor (CD32) Fc fragment of IgG, low affinity IIb, receptor (CD32) Fc gamma receptor II beta Low affinity immunoglobulin gamma Fc region receptor III-like	Fcgr2a Fcgr2b LOC498276 LOC100362543	1.652	1.150		0.013	GO:0001788 antibody-dependent cellular cytotoxicity ; GO:0001798 positive regulation of type IIa hypersensitivity; GO:0001805 positive regulation of type III hypersensitivity; GO:0001812 positive regulation of type I hypersensitivity; GO:0001820 serotonin secretion ; GO:0006910 phagocytosis, recognition; GO:0006911 phagocytosis, engulfment; GO:0007166 cell surface receptor linked signaling pathway ; GO:0021675 nerve development; GO:0030593 neutrophil chemotaxis
Eukaryotic translation initiation factor 3, subunit E	Eif3e	1.647	1.287		0.025	GO:0000184 nuclear-transcribed mRNA catabolic process, nonsense-mediated decay; GO:0006413 translational initiation
Family with sequence similarity 96, member A	Fam96a	1.641	1.277		0.014	GO:0008150 biological process
Peroxisomal membrane protein 3	Pxmp3	1.631	1.093		0.007	GO:0001764 neuron migration; GO:0006699 bile acid biosynthetic process; GO:0007031 peroxisome organization; GO:0007399 nervous system development; GO:0008150 biological process; GO:0042632 cholesterol homeostasis; GO:0045540 regulation of cholesterol biosynthetic process; GO:0001764 neuron migration; GO:0006699 bile acid biosynthetic process; GO:0007031 peroxisome organization
Fibroblast growth factor 15	Fgf15	1.618	1.045		0.005	GO:0001755 neural crest cell migration; GO:0007507 heart development; GO:0008284 positive regulation of cell proliferation; GO:0008543 fibroblast growth factor receptor signaling pathway; GO:0046326 positive regulation of glucose import; GO:0046330 positive regulation of JNK cascade; GO:0070374 positive regulation of ERK1 and ERK2 cascade; GO:0070858 negative regulation of bile acid biosynthetic process
Phospholamban	Pln	1.616	1.194		0.021	GO:0002026 regulation of the force of heart contraction; GO:0006816 calcium ion transport // non-traceable author statement; GO:0006874 cellular calcium ion homeostasis; GO:0045822 negative regulation of heart contraction; GO:0048738 cardiac muscle tissue development; GO:0051924 regulation of calcium ion transport
Suppressor of cytokine signaling 3	Socs3	1.613	1.157		0.007	GO:0001558 regulation of cell growth; GO:0001666 response to hypoxia; GO:0001932 regulation of protein amino acid phosphorylation; GO:0007165 signal transduction; GO:0007243 intracellular protein kinase cascade; GO:0007259 JAK-STAT cascade; GO:0007568 aging; GO:0009408 response to heat; GO:0009617 response to bacterium;

PQ loop repeat containing 3	Pqlc3	1.608	1.073			GO:0009725 response to hormone stimulus
Midkine	Mdk	1.600	1.032		0.037	GO:0000087 M phase of mitotic cell cycle; GO:0007275 multicellular organismal development; GO:0009611 response to wounding; GO:0009725 response to hormone stimulus; GO:0016477 cell migration; GO:0030154 cell differentiation; GO:0030325 adrenal gland development; GO:0042493 response to drug; GO:0051384 response to glucocorticoid stimulus; GO:0051781 positive regulation of cell division
MOB1, Mps One Binder kinase activator-like 3 (yeast)	Mobkl3	1.593	1.310		0.047	GO:0006810 transport
Nuclear factor, interleukin 3 regulated	Nfil3	1.589	1.579		0.041	GO:0006355 regulation of transcription, DNA-dependent; GO:0048511 rhythmic process
Alpha-2u globulin PGCL1 alpha-2u-globulin (L type) alpha-2u globulin PGCL2 alpha2u globulin alpha-2u globulin PGCL3 alpha 2U globulin	LOC259246 LOC298116 LOC29898109 LOC298111 LOC259244 LOC366380	1.586	-1.100		0.021	GO:0006810 transport
Tp53rk binding protein	Trpkb	1.580	1.187		0.018	
Similar to protein C33A12.3	RGD1359508	1.574	1.141		0.013	
V-ral simian leukemia viral oncogene homolog A (ras related)	Rala	1.563	1.175		0.005	GO:0000910 cytokinesis; GO:0007165 signal transduction; GO:0007264 small GTPase mediated signal transduction; GO:0007265 Ras protein signal transduction; GO:0017157 regulation of exocytosis ; GO:0031532 actin cytoskeleton reorganization; GO:0051491 positive regulation of filopodium assembly; GO:0051665 membrane raft localization
Hematopoietic prostaglandin D synthase	Hpgds	1.539	1.220		0.009	GO:0001516 prostaglandin biosynthetic process; GO:0006633 fatty acid biosynthetic process; GO:0006693 prostaglandin metabolic process
Forkhead box E3	Foxe3	1.519	1.131		0.024	GO:0001654 eye development; GO:0006350 transcription; GO:0006355 regulation of transcription, DNA-dependent; GO:0006366 transcription from RNA polymerase II promoter; GO:0008150 biological process; GO:0045449 regulation of transcription; GO:0048468 cell development; GO:0050679 positive regulation of epithelial cell proliferation
Complement component 1, s subcomponent	C1s	1.513	1.170		0.006	GO:0006508 proteolysis; GO:0006958 complement activation, classical pathway; GO:0010001 glial cell differentiation; GO:0045087 innate immune response; GO:0051591 response to cAMP
Reticulocalbin 2, EF-hand calcium binding domain	Ren2	1.512	1.223		0.035	
Solute carrier family 7 (cationic amino acid transporter, y+ system), member 9	Slc7a9	1.500	1.047		0.046	GO:0006865 amino acid transport; GO:0055085 transmembrane transport; GO:0015804 neutral amino acid transport
Butyrylcholinesterase	Bche	1.488	1.042		0.019	GO:0007584 response to nutrient; GO:0007612 learning; GO:0019695 choline metabolic process; GO:0042493 response to drug; GO:0043279 response to alkaloid; GO:0050805 negative regulation of synaptic transmission; GO:0051384 response to glucocorticoid stimulus; GO:0051593 response to folic acid
SFT2 domain containing 1	Sft2d1	1.487	1.125		0.023	GO:0015031 protein transport; GO:0016192 vesicle-mediated transport
TCF3 (E2A) fusion partner	Tfpt	1.475	1.165		0.002	GO:0006917 induction of apoptosis
UDP-Gal:betaGlcNAc beta galactosyltransferase, polypeptide 4	B4galt4	1.466	1.188		0.008	GO:0005975 carbohydrate metabolic process

Somatostatin	Sst	1.460	1.139	0.020	GO:0001101 response to acid; GO:0006972 hyperosmotic response; GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0009408 response to heat; GO:0010243 response to organic nitrogen; GO:0030334 regulation of cell migration; GO:0042493 response to drug; GO:0043200 response to amino acid stimulus; GO:0048545 response to steroid hormone stimulus
Lin-7 homolog C (C. elegans)	Lin7c	1.459	1.195	0.005	GO:0006887 exocytosis; GO:0007269 neurotransmitter secretion
Glycoprotein (transmembrane) nmb	Gpnm	1.452	1.100	0.036	GO:0001649 osteoblast differentiation; GO:0007155 cell adhesion; GO:0030282 bone mineralization
Coiled-coil-helix-coiled-coil-helix domain containing 4 similar to coiled-coil-helix-coiled-coil-helix domain containing 4	Chchd4 LOC685505	1.449	1.121	0.033	GO:0015031 protein transport; GO:0055085 transmembrane transport
Olfactory receptor 63	Olr63	1.445	1.147	0.029	GO:0007165 signal transduction; GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0050911 detection of chemical stimulus involved in sensory perception of smell
Proline-rich acidic protein 1	Prp1	1.444	1.055	0.035	
Immunoglobulin superfamily, member 6	Igsf6	1.443	1.108	0.045	
Ly49 inhibitory receptor 9 hypothetical protein LOC497796 killer cell lectin-like receptor, subfamily A, member 17 similar to immunoreceptor Ly49s3	Ly49 LOC497796 Klra17 RGD1561306	1.440	1.026	0.034	
Allograft inflammatory factor 1	Aif1	1.431	1.107	0.010	GO:0001934 positive regulation of protein amino acid phosphorylation; GO:0010629 negative regulation of gene expression; GO:0014739 positive regulation of muscle hyperplasia; GO:0030335 positive regulation of cell migration; GO:0031668 cellular response to extracellular stimulus; GO:0032870 cellular response to hormone stimulus; GO:0034097 response to cytokine stimulus; GO:0042116 macrophage activation; GO:0043066 negative regulation of apoptosis; GO:0045429 positive regulation of nitric oxide biosynthetic process
Legumin	Lgmn	1.427	1.268	0.003	GO:0006508 proteolysis; GO:0040015 negative regulation of multicellular organism growth
Brain expressed X-linked 2 brain expressed gene 1 brain expressed gene 4	Bex2 Bex1 Bex4	1.420	1.194	0.043	GO:0006915 apoptosis; GO:0007049 cell cycle; GO:0002052 positive regulation of neuroblast proliferation; GO:0007275 multicellular organismal development; GO:0007399 nervous system development; GO:0030154 cell differentiation; GO:0045665 negative regulation of neuron differentiation; GO:0048011 nerve growth factor receptor signaling pathway
Tumor suppressor candidate 3	Tusc3	1.419	1.145	0.049	GO:0045454 cell redox homeostasis
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d-like 1	Atp5h Atp5h1	1.415	1.072	0.036	GO:0006811 ion transport; GO:0015986 ATP synthesis coupled proton transport; GO:0015992 proton transport; GO:0046034 ATP metabolic process
C-type lectin domain family 10, member A	Clec10a	1.413	-1.051	0.034	
Sodium channel, voltage-gated, type VII, alpha	Scn7a	1.412	1.274	0.017	GO:0006811 ion transport; GO:0006814 sodium ion transport; GO:0055085 transmembrane transport
Galactokinase 2	Galk2	1.409	1.182	0.000	GO:0007204 elevation of cytosolic calcium ion concentration
Necdin homolog (mouse)	Ndn	1.402	1.174	0.004	GO:0006012 galactose metabolic process; GO:0008152 metabolic process; GO:0046835 carbohydrate phosphorylation
					GO:0001764 neuron migration; GO:0006355 regulation of transcription, DNA-dependent; GO:0007409 axonogenesis; GO:0007413 axonal fasciculation; GO:0007417 central nervous system development; GO:0007585 respiratory gaseous exchange; GO:0008347 glial cell

BUD31 homolog (S. cerevisiae) pentatricopeptide repeat domain 1	Bud31 Pcd1	1.401	1.098			migration; GO:0019233 sensory perception of pain; GO:0048011 nerve growth factor receptor signaling pathway; GO:0048666 neuron development
Proenkephalin	Penk	1.394	1.073		0.047	
Musculoskeletal, embryonic nuclear protein 1	Mustn1	1.394	1.074		0.031	GO:0001662 behavioral fear response; GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0007218 neuropeptide signaling pathway; GO:0007610 behavior; GO:0019233 sensory perception of pain
EGF-like module containing, mucin-like, hormone receptor-like sequence 4	Emr4	1.380	1.175		0.025	GO:0030326 embryonic limb morphogenesis; GO:0042060 wound healing; GO:0042246 tissue regeneration
Testis specific X-linked gene	Tsx	1.378	1.245		0.000	GO:0007218 neuropeptide signaling pathway
Leceithin-retinol (phosphatidylcholine-retinol-O-acyltransferase)	Lrat	1.369	1.058		0.004	GO:0006776 vitamin A metabolic process; GO:0007601 visual perception; GO:0009790 embryonic development; GO:0042572 retinol metabolic process ; GO:0050896 response to stimulus
Integrin, alpha 1	Irga1	1.366	1.089		0.013	GO:0000187 activation of MAPK activity; GO:0006936 muscle contraction; GO:0007155 cell adhesion; GO:0007229 integrin-mediated signaling pathway; GO:0030593 neutrophil chemotaxis; GO:0042311 vasodilation; GO:0043525 positive regulation of neuron apoptosis; GO:0045123 cellular extravasation; GO:0048812 neuron projection morphogenesis; GO:0060326 cell chemotaxis
Stathmin-like 3	Stmn3	1.355	1.164		0.005	GO:0007019 microtubule depolymerization; GO:0031122 cytoplasmic microtubule organization; GO:0031175 neuron projection development; GO:0032314 regulation of Rac GTPase activity; GO:0035021 negative regulation of Rac protein signal transduction; GO:0051493 regulation of cytoskeleton organization
Family with sequence similarity 12, member B (epitidymal)	Fam12b	1.344	1.134		0.003	
Mitochondrial ribosomal protein L13	Mrpl13	1.344	1.089		0.022	GO:0006412 translation
Similar to RIKEN cDNA 1700023M03	RGD1305457	1.344	1.078		0.000	
Growth differentiation factor 9	Gdf9	1.343	1.019		0.029	GO:0001555 oocyte growth; GO:0030308 negative regulation of cell growth
Olfactory receptor 826 olfactory receptor 825 olfactory receptor 829	Olr826 Olr825 Olr829	1.334	1.264		0.001	GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0050911 detection of chemical stimulus involved in sensory perception of smell; GO:0007165 signal transduction
Oxidized low density lipoprotein (lectin-like) receptor 1	Olr1	1.333	1.081		0.022	GO:0006954 inflammatory response; GO:0006955 immune response; GO:0007155 cell adhesion; GO:0007159 leukocyte cell-cell adhesion; GO:0008219 cell death; GO:0042157 lipoprotein metabolic process; GO:0042542 response to hydrogen peroxide
Heat shock protein alpha 2	Hspa2	1.325	1.044		0.023	GO:0006950 response to stress; GO:0007275 multicellular organismal development; GO:0007283 spermatogenesis; GO:0030154 cell differentiation
Membrane-spanning 4-domains, subfamily A, member 2 (Fc fragment of IgE, high affinity I, receptor for; beta polypeptide)	Ms4a2	1.321	1.129		0.031	GO:0006954 inflammatory response; GO:0007165 signal transduction; GO:0007166 cell surface receptor linked signaling pathway; GO:0007202 activation of phospholipase C activity; GO:0007205 activation of protein kinase C activity by G-protein coupled receptor protein signaling pathway; GO:0043306 positive regulation of mast cell degranulation; GO:0050663 cytokine secretion; GO:0051279 regulation of release of sequestered calcium ion into cytosol
Mesenchyme homeobox 2	Meox2	1.320	1.087		0.013	GO:0001525 angiogenesis; GO:0001757 somite specification; GO:0006355 regulation of transcription, DNA-dependent; GO:0007275 multicellular organismal development;

Angiopoietin-like 3	Angptl3	1.319	1.166	0.002	GO:0007519 skeletal muscle tissue development; GO:0060021 palate development; GO:0060173 limb development
Phosphotriesterase related G protein-coupled receptor 119	Pter Gpr119	1.318 1.317	1.091 1.164	0.008 0.036	GO:0006071 glycerol metabolic process; GO:0006631 fatty acid metabolic process; GO:0006644 phospholipid metabolic process; GO:0007160 cell-matrix adhesion; GO:0007165 signal transduction; GO:0008203 cholesterol metabolic process; GO:0009395 phospholipid catabolic process; GO:0009725 response to hormone stimulus; GO:0010519 negative regulation of phospholipase activity; GO:0019915 lipid storage
Similar to 14-3-3 protein sigma stratifin	LOC298795 Sfin	1.309	1.084	0.036	GO:0009056 catabolic process GO:0007165 signal transduction ; GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0030073 insulin secretion
Serpine1 mRNA binding protein 1 Granzyme F	Serbp1 Gzmf	1.307 1.300	1.136 1.043	0.041 0.027	GO:0045767 regulation of anti-apoptosis GO:0006508 proteolysis
Tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	Tfpi	1.298	1.198	0.000	GO:0007596 blood coagulation; GO:0007598 blood coagulation, extrinsic pathway
Fibroblast growth factor 3	Fgf3	1.294	1.138	0.036	GO:0001759 induction of an organ; GO:0008284 positive regulation of cell proliferation; GO:0008543 fibroblast growth factor receptor signaling pathway; GO:0048538 thymus development
Secreted and transmembrane 1A Ubiquitin-conjugating enzyme	Sectm1a RGD69425	1.292 1.288	1.096 1.059	0.003 0.040	GO:0043123 positive regulation of I-kappaB kinase/NF-kappaB cascade GO:0008150 biological process; GO:0043687 post-translational protein modification; GO:0051246 regulation of protein metabolic process
Neuropeptide W	Npw	1.287	1.009	0.029	GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0007218 neuropeptide signaling pathway; GO:0007631 feeding behavior
Olfactory receptor 1075	LOC362526 Olf1075	1.282 1.279	1.026 -1.179	0.014 0.027	GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0050911 detection of chemical stimulus involved in sensory perception of smell
Myelin protein zero-like 1 Keratin 18	Mpzl1 Krt18	1.278 1.276	1.269 1.124	0.001 0.048	GO:0006915 apoptosis; GO:0008150 biological process; GO:0033209 tumor necrosis factor-mediated signaling pathway; GO:0043000 Golgi to plasma membrane CFTR protein transport; GO:0043066 negative regulation of apoptosis
Choline phosphotransferase 1	Chpt1	1.273	1.198	0.033	GO:0006656 phosphatidylcholine biosynthetic process; GO:0006663 platelet activating factor biosynthetic process; GO:0008654 phospholipid biosynthetic process
Neurogenic differentiation 1	Neurod1	1.271	1.152	0.020	GO:0003326 pancreatic A cell fate commitment; GO:0003329 pancreatic PP cell fate commitment; GO:0006355 regulation of transcription, DNA-dependent; GO:0007263 nitric oxide mediated signal transduction; GO:0007275 multicellular organismal development; GO:0007399 nervous system development; GO:0009749 response to glucose stimulus; GO:0009952 anterior/posterior pattern formation; GO:0021549 cerebellum development; GO:0030073 insulin secretion

Fibroblast growth factor 2	Fgf2	1.264	1.032	0.042	GO:0000186 activation of MAPKK activity; GO:0000189 nuclear translocation of MAPK; GO:0001525 angiogenesis; GO:0001658 branching involved in ureteric bud morphogenesis; GO:0001759 induction of an organ; GO:0001934 positive regulation of protein amino acid phosphorylation; GO:0002042 cell migration involved in sprouting angiogenesis ; GO:0006355 regulation of transcription, DNA-dependent; GO:0006700 C21-steroid hormone biosynthetic process; GO:0006915 apoptosis
Fin bud initiation factor homolog (zebrafish)	Fibin	1.264	1.014	0.001	
Olfactory receptor 7	Olr7	1.261	1.228	0.011	GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0050911 detection of chemical stimulus involved in sensory perception of smell
Sterol O-acyltransferase 2	Soat2	1.256	-1.037	0.016	GO:0007584 response to nutrient; GO:0008202 steroid metabolic process; GO:0008203 cholesterol metabolic process; GO:0033344 cholesterol efflux; GO:0034379 very-low-density lipoprotein particle assembly; GO:0034435 cholesterol esterification
Neurexin 1	Nrxn1	1.256	1.071	0.022	GO:0007268 synaptic transmission; GO:0007269 neurotransmitter secretion; GO:0007416 synapse assembly; GO:0051290 protein heterotrimerization
MARCKS-like 1	Mareksl1	1.251	1.104	0.033	GO:0008284 positive regulation of cell proliferation; GO:0016192 vesicle-mediated transport
Calcium/calmodulin-dependent protein kinase II inhibitor 1	Camk2n1	1.251	1.151	0.024	GO:0007268 synaptic transmission
Armadillo repeat containing, X-linked 1	Armcx1	1.247	1.042	0.003	
Protocadherin beta 2	Pcdhb2	1.244	1.090	0.037	GO:0007155 cell adhesion; GO:0007156 homophilic cell adhesion
ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (Hu antigen B)	Elavl2	1.242	-1.022	0.041	
DNA-damage regulated autophagy modulator 2 similar to CG4025-PA	Dram2 LOC689412	1.239	1.088	0.013	GO:0006915 apoptosis; GO:0006917 induction of apoptosis
Proteolipid protein 1	Plp1	1.238	1.183	0.019	GO:0007229 integrin-mediated signaling pathway; GO:0008366 axon ensheathment; GO:0010001 glial cell differentiation; GO:0022010 myelination in the central nervous system; GO:0042552 myelination; GO:0042759 long-chain fatty acid biosynthetic process; GO:0048469 cell maturation
Aspartoacylase	Aspa	1.237	1.012	0.036	GO:0008152 metabolic process; GO:0022010 myelination in the central nervous system; GO:0048714 positive regulation of oligodendrocyte differentiation
First gene upstream of Nf5d3	LOC362863	1.232	1.198	0.037	
PRKC, apoptosis, WT1, regulator	Pawr	1.232	1.140	0.049	GO:0006915 apoptosis; GO:0030889 negative regulation of B cell proliferation; GO:0042094 interleukin-2 biosynthetic process; GO:0042130 negative regulation of T cell proliferation; GO:0042986 positive regulation of amyloid precursor protein biosynthetic process; GO:0045449 regulation of transcription; GO:0050860 negative regulation of T cell receptor signaling pathway
Glutamate receptor, ionotropic, AMPA4	Gria4	1.231	1.181	0.036	GO:0007268 synaptic transmission
Fumarylacetoacetate hydrolase domain containing 1	Fahd1	1.229	1.152	0.039	GO:0008152 metabolic process
McKusick-Kaufman syndrome	Mkks	1.225	1.178	0.010	GO:0007286 spermatid development; GO:0007608 sensory perception of smell; GO:0008150 biological process; GO:0009296 flagellum assembly; GO:0021756 striatum development; GO:0021766 hippocampus development; GO:0021987 cerebral cortex development; GO:0035058 sensory cilium assembly; GO:0035176 social behavior; GO:0042384 cilium assembly

Protein kinase inhibitor, gamma	Pkig	1.223	1.226	0.017	GO:0000122 negative regulation of transcription from RNA polymerase II promoter; GO:0006469 negative regulation of protein kinase activity; GO:0007165 signal transduction; GO:0042308 negative regulation of protein import into nucleus
Cholecystokinin B receptor	Cckbr	1.222	1.103	0.036	GO:0001821 histamine secretion; GO:0002209 behavioral defense response; GO:0006915 apoptosis; GO:0007165 signal transduction; GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0007204 elevation of cytosolic calcium ion concentration; GO:0007586 digestion; GO:0008284 positive regulation of cell proliferation; GO:0032230 positive regulation of synaptic transmission, GABAergic; GO:0032868 response to insulin stimulus
RAS-like family 11 member B	Rasl11b	1.221	1.156	0.028	GO:0007165 signal transduction; GO:0007264 small GTPase mediated signal transduction
Galanin receptor 1	Galr1	1.219	1.117	0.047	GO:0007165 signal transduction; GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0007189 activation of adenylate cyclase activity by G-protein signaling pathway
Potassium voltage gated channel, Shab-related subfamily, member 1	Kenb1	1.217	1.040	0.038	GO:0006811 ion transport; GO:0006813 potassium ion transport; GO:0051259 protein oligomerization; GO:0055085 transmembrane transport
Protein disulfide isomerase family A, member 4	Pdia4	1.216	1.159	0.037	GO:0045454 cell redox homeostasis
Myosin, light polypeptide 1	Myf11	1.215	1.128	0.024	GO:0060048 cardiac muscle contraction
Persephin	Pspn	1.213	1.255	0.006	GO:0001658 branching involved in ureteric bud morphogenesis
Tumor necrosis factor (ligand) superfamily, member 13	Tnfrsf13	1.212	1.228	0.001	GO:0002426 immunoglobulin production in mucosal tissue; GO:0002636 positive regulation of germinal center formation; GO:0006955 immune response; GO:0008150 biological process; GO:0008284 positive regulation of cell proliferation; GO:0016064 immunoglobulin mediated immune response; GO:0048298 positive regulation of isotype switching to IgA isotypes; GO:0050776 regulation of immune response
ADP-ribosylation factor interacting protein 1	Arfp1	1.211	1.039	0.027	GO:0006886 intracellular protein transport; GO:0050708 regulation of protein secretion
Zinc finger, MYND-type containing 19	Zmynd19	1.211	1.043	0.035	
Centrosomal protein 70kDa	Cep70	1.210	1.158	0.024	
Ribosomal L24 domain containing 1	Rsl24d1	1.210	1.139	0.035	GO:0006412 translation; GO:0042254 ribosome biogenesis
Ring finger protein 133	Rnf133	1.209	1.157	0.018	GO:0051865 protein autoubiquitination
Plasma glutamate carboxypeptidase	Pgcp	1.205	1.147	0.044	GO:0006508 proteolysis; GO:0042246 tissue regeneration
DnaI (Hsp40) homolog, subfamily A, member 1	Dnaj1	1.199	1.320	0.014	GO:0006457 protein folding; GO:0007283 spermatogenesis; GO:0009408 response to heat; GO:0030317 sperm motility; GO:0030521 androgen receptor signaling pathway; GO:0042769 DNA damage response, detection of DNA damage
Transmembrane and coiled-coil domains 1	Tmco1	1.198	1.057	0.011	GO:0008150 biological process
Arrestin, beta 1	Arrb1	1.196	1.204	0.002	GO:0000187 activation of MAPK activity; GO:0002031 G-protein coupled receptor internalization; GO:0002032 desensitization of G-protein coupled receptor protein signaling pathway by arrestin; GO:0006366 transcription from RNA polymerase II promoter; GO:0006892 post-Golgi vesicle-mediated transport; GO:0006897 endocytosis; GO:0007165 signal transduction; GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0007188 G-protein signaling, coupled to cAMP nucleotide second messenger // ; GO:0007600 sensory perception
Dystonia 1	Dyt1	1.193	1.043	0.002	GO:0006457 protein folding; GO:0006979 response to oxidative stress; GO:0051085 chaperone mediated protein folding requiring cofactor
Latrophilin 3	Lphn3	1.191	1.172	0.025	GO:0007218 neuropeptide signaling pathway; GO:0007420 brain development
FK506 binding protein 14	Fkbp14	1.190	1.332	0.022	GO:0006457 protein folding

Nitric oxide synthase 2, inducible	Nos2	1.188	1.001	0.021	GO:0001542 ovulation from ovarian follicle; GO:0001666 response to hypoxia; GO:0001935 endothelial cell proliferation; GO:0001974 blood vessel remodeling; GO:0006527 arginine catabolic process; GO:0006801 superoxide metabolic process; GO:0006809 nitric oxide biosynthetic process; GO:0007165 signal transduction; GO:0007199 G-protein signaling, coupled to cGMP nucleotide second messenger; GO:0007243 intracellular protein kinase cascade
Olfactory receptor 1105	Olr1105	1.187	-1.050	0.026	GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0050911 detection of chemical stimulus involved in sensory perception of smell
Heat shock protein beta 2	Hspb2	1.186	-1.117	0.041	GO:0007525 somatic muscle development; GO:0009408 response to heat
Uncoupling protein 1 (mitochondrial, proton carrier)	Ucp1	1.185	1.100	0.009	GO:0006091 generation of precursor metabolites and energy; GO:0006839 mitochondrial transport; GO:0015992 proton transport; GO:0032870 cellular response to hormone stimulus; GO:0044253 positive regulation of multicellular organismal metabolic process; GO:0048545 response to steroid hormone stimulus; GO:0050873 brown fat cell differentiation; GO:0055085 transmembrane transport
Ankyrin repeat and SOCS box-containing 2	Asb2	1.182	1.117	0.037	
WD repeat domain 31	Wdr31	1.182	1.041	0.036	
Neurexophilin 4	Nxph4	1.180	-1.046	0.047	
Keratin 82	Krt82	1.178	1.063	0.043	
Feline leukemia virus subgroup C cellular receptor family, member 2	Ftver2	1.177	-1.071	0.028	GO:0055085 transmembrane transport
Alpha-2u globulin PGCL4 major urinary protein 4 alpha-2u globulin PGCL3 alpha-2u globulin PGCL1 alpha2u globulin alpha-2u globulin PGCL2 alpha 2U globulin	Obp3 Mup4 LOC259244 LOC259246 LOC298111 LOC298109 LOC366380	1.176	-1.185	0.030	GO:0006810 transport
Reelin	Reln	1.176	1.055	0.037	GO:0000904 cell morphogenesis involved in differentiation; GO:0001764 neuron migration; GO:0007411 axon guidance; GO:0007417 central nervous system development; GO:0007420 brain development; GO:0007626 locomotory behavior; GO:0010001 glial cell differentiation; GO:0018108 peptidyl-tyrosine phosphorylation; GO:0021511 spinal cord patterning; GO:0021800 cerebral cortex tangential migration
Vesicle-associated membrane protein 7	Vamp7	1.175	1.049	0.048	GO:0006888 ER to Golgi vesicle-mediated transport; GO:0006906 vesicle fusion; GO:0006911 phagocytosis, engulfment; GO:0008333 endosome to lysosome transport; GO:0015031 protein transport; GO:0016044 cellular membrane organization; GO:0016192 vesicle-mediated transport; GO:0017156 calcium ion-dependent exocytosis; GO:0043308 eosinophil degranulation; GO:0043312 neutrophil degranulation
Plasminogen	Plg	1.175	-1.022	0.037	GO:0006915 apoptosis; GO:0006917 induction of apoptosis; GO:0007596 blood coagulation; GO:0042246 tissue regeneration; GO:0045445 myoblast differentiation; GO:0046716 muscle cell homeostasis; GO:0048771 tissue remodeling; GO:0051603 proteolysis involved in cellular protein catabolic process; GO:0051918 negative regulation of fibrinolysis; GO:0051919 positive regulation of fibrinolysis
Family with sequence similarity 131, member B	Fam131b	1.173	1.085	0.020	
Cholinergic receptor, nicotinic, beta 2 (neuronal)	Chnb2	1.169	1.112	0.004	GO:0001508 regulation of action potential; GO:0001661 conditioned taste aversion; GO:0001666 response to hypoxia; GO:0006811 ion transport; GO:0006816 calcium ion transport; GO:0006939 smooth muscle contraction; GO:0007165 signal transduction;

						GO:0007271 synaptic transmission, cholinergic; GO:0007601 visual perception; GO:0007605 sensory perception of sound
Dynein light chain LC8-type 1	Dynll1	1.169	1.153	0.033		GO:0006809 nitric oxide biosynthetic process; GO:0007017 microtubule-based process; GO:0008633 activation of pro-apoptotic gene products; GO:0042133 neurotransmitter metabolic process; GO:0042326 negative regulation of phosphorylation
Kinesin family member 27	Kif27	1.167	1.078	0.005		GO:0007018 microtubule-based movement
LOC362793	RGD1307315	1.165	1.033	0.021		
Unc-50 homolog (C. elegans)	Unc50	1.163	1.035	0.044		GO:0007166 cell surface receptor linked signaling pathway; GO:0015031 protein transport
Sonic hedgehog	Shh	1.163	1.202	0.010		GO:0001525 angiogenesis; GO:0001569 patterning of blood vessels; GO:0001570 vasculogenesis; GO:0001656 metanephros development; GO:0001658 branching involved in ureteric bud morphogenesis; GO:0001666 response to hypoxia; GO:0001708 cell fate specification; GO:0001755 neural crest cell migration; GO:0001822 kidney development; GO:0001841 neural tube formation
Histidine decarboxylase	Hdc	1.162	1.041	0.044		GO:0001692 histamine metabolic process; GO:0006519 cellular amino acid and derivative metabolic process; GO:0006547 histidine metabolic process; GO:0006548 histidine catabolic process; GO:0019752 carboxylic acid metabolic process; GO:0042423 catecholamine biosynthetic process
Olfactory receptor 1593	Olr1593	1.162	1.083	0.037		GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0050911 detection of chemical stimulus involved in sensory perception of smell
Zinc finger protein 354A	Zfp354a	1.160	1.051	0.007		GO:0000122 negative regulation of transcription from RNA polymerase II promoter; GO:0001666 response to hypoxia; GO:0001822 kidney development; GO:0006355 regulation of transcription, DNA-dependent; GO:0007275 multicellular organismal development; GO:0007576 nucleolar fragmentation; GO:0051593 response to folic acid
Tumor protein p63	Tp63	1.157	1.048	0.026		GO:0000122 negative regulation of transcription from RNA polymerase II promoter; GO:0001302 replicative cell aging; GO:0001501 skeletal system development; GO:0001736 establishment of planar polarity; GO:0001738 morphogenesis of a polarized epithelium; GO:0001942 hair follicle development; GO:0002053 positive regulation of mesenchymal cell proliferation; GO:0002064 epithelial cell development; GO:0006915 apoptosis; GO:0006916 anti-apoptosis
Histone cluster 1, H1t	Hist1h1t	1.155	1.034	0.015		GO:0006334 nucleosome assembly; GO:0007275 multicellular organismal development; GO:0007283 spermatogenesis; GO:0007339 binding of sperm to zona pellucida; GO:0030154 cell differentiation; GO:0030317 sperm motility
Disabled homolog 1 (Drosophila)	Dab1	1.154	-1.073	0.018		GO:0001764 neuron migration; GO:0007162 negative regulation of cell adhesion; GO:0007264 small GTPase mediated signal transduction; GO:0007275 multicellular organismal development; GO:0007399 nervous system development; GO:0007420 brain development; GO:0021589 cerebellum structural organization; GO:0021795 cerebral cortex cell migration; GO:0021799 cerebral cortex radially oriented cell migration; GO:0021813 cell-cell adhesion involved in neuronal-glia interactions involved in cerebral cortex radial glia guided migration
Leucine rich repeat containing 66	Lrrc66	1.154	1.139	0.045		GO:0007165 signal transduction; GO:0045941 positive regulation of transcription; GO:0045944 positive regulation of transcription from RNA polymerase II promoter; GO:0045944 positive regulation of transcription from RNA polymerase II promoter
Neuronal PAS domain protein 4	Npas4	1.154	1.095	0.015		

N-acetylneuraminic acid phosphatase	Nanp	1.154	1.165	0.042	GO:0005975 carbohydrate metabolic process; GO:0008152 metabolic process; GO:0046380 N-acetylneuraminic biosynthetic process
MAD2L1 binding protein	Mad2l1bp	1.152	1.111	0.004	GO:0007093 mitotic cell cycle checkpoint; GO:0007096 regulation of exit from mitosis
Neurexin 5	NMS	1.152	-1.015	0.045	GO:0006940 regulation of smooth muscle contraction; GO:0007218 neuropeptide signaling pathway; GO:0045475 locomotor rhythm
Transmembrane protease, serine 8 (intestinal)	Trpsr8	1.149	1.203	0.035	GO:0006508 proteolysis; GO:0006811 ion transport; GO:0006814 sodium ion transport
Myoglobin	Mb	1.149	1.036	0.015	GO:0001666 response to hypoxia; GO:0006810 transport; GO:0007507 heart development; GO:0009725 response to hormone stimulus; GO:0015671 oxygen transport; GO:0031444 slow-twitch skeletal muscle fiber contraction; GO:0042542 response to hydrogen peroxide; GO:0043353 enucleate erythrocyte differentiation; GO:0050873 brown fat cell differentiation
Similar to RIKEN cDNA 1500031L02	RGD621352	1.145	1.411	0.006	GO:0055114 oxidation reduction
Dehydrogenase/reductase (SDR family) member 7	Dhrs7	1.145	-1.013	0.046	
Nuclear pore associated protein	Npap60	1.143	-1.073	0.033	GO:0001841 neural tube formation; GO:0015031 protein transport; GO:0046907 intracellular transport; GO:0051028 mRNA transport; GO:0055085 transmembrane transport
Olfactory receptor 484	Olr484	1.141	1.355	0.016	GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0050911 detection of chemical stimulus involved in sensory perception of smell
Torsin family 3, member A	Tor3a	1.140	1.088	0.020	GO:0051085 chaperone mediated protein folding requiring cofactor
Similar to Protein C20orf103 precursor	RGD1306991	1.139	-1.041	0.041	
Similar to RAN protein	RGD1306195	1.138	1.112	0.014	GO:0006886 intracellular protein transport; GO:0006913 nucleocytoplasmic transport; GO:0007165 signal transduction
Natriuretic peptide receptor A/guanylate cyclase A (atrioatriuretic peptide receptor A)	Npr1	1.138	1.050	0.034	GO:0006182 cGMP biosynthetic process; GO:0006468 protein amino acid phosphorylation; GO:0007166 cell surface receptor linked signaling pathway; GO:0007168 receptor guanylyl cyclase signaling pathway; GO:0008217 regulation of blood pressure; GO:0030828 positive regulation of cGMP biosynthetic process; GO:0042417 dopamine metabolic process
Olfactory receptor 174	Olr174	1.138	1.294	0.040	GO:0007186 G-protein coupled receptor protein signaling pathway ; GO:0050911 detection of chemical stimulus involved in sensory perception of smell
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, assembly factor 3	Ndufa3	1.131	-1.084	0.042	GO:0032981 mitochondrial respiratory chain complex I assembly I assembly
Interleukin 6 signal transducer	Il6st	1.128	1.061	0.001	GO:0005977 glycogen metabolic process; GO:0006642 triglyceride mobilization; GO:0007165 signal transduction; GO:0007259 JAK-STAT cascade; GO:0007584 response to nutrient; GO:0008284 positive regulation of cell proliferation; GO:0008593 regulation of Notch signaling pathway; GO:0014911 positive regulation of smooth muscle cell migration; GO:0019221 cytokine-mediated signaling pathway; GO:0030307 positive regulation of cell growth
Synuclein, alpha (non A4 component of amyloid precursor)	SncA	1.127	1.096	0.005	GO:0001774 microglial cell activation; GO:0001921 positive regulation of receptor recycling; GO:0001956 positive regulation of neurotransmitter secretion; GO:0001963 synaptic transmission, dopaminergic; GO:0006631 fatty acid metabolic process; GO:0006638 neutral lipid metabolic process; GO:0006644 phospholipid metabolic process; GO:0006916 anti-apoptosis; GO:0007006 mitochondrial membrane organization; GO:0008344 adult locomotory behavior
Interferon regulatory factor 3	Irf3	1.127	1.166	0.011	GO:0006355 regulation of transcription, DNA-dependent; GO:0007249 I-kappaB kinase/NF-kappaB cascade; GO:0009617 response to bacterium; GO:0031663 lipopolysaccharide-

							mediated signaling pathway; GO:0032496 response to lipopolysaccharide; GO:0043330 response to exogenous dsRNA; GO:0045351 type I interferon biosynthetic process
Asialoglycoprotein receptor 1	Asgr1	1.121	1.051		0.028		GO:0006897 endocytosis; GO:0031668 cellular response to extracellular stimulus
Heat shock 105kDa/110kDa protein 1	Hsp1	1.120	1.358		0.036		GO:0006950 response to stress; GO:0051085 chaperone mediated protein folding requiring cofactor
Actin, gamma 2, smooth muscle, enteric	Actg2	1.117	1.070		0.008		GO:0006936 muscle contraction
Similar to putative protein, with at least 9 transmembrane domains, of eukaryotic origin (43.9 kD) (2G415)	RGD1309228	1.111	1.099		0.007		
Transient receptor potential cation channel, subfamily V, member 2	Trpv2	1.111	1.060		0.018		GO:0006811 ion transport; GO:0006816 calcium ion transport; GO:0009266 response to temperature stimulus; GO:0009408 response to heat; GO:0055085 transmembrane transport
Glutathione S-transferase, theta 2	Gstt2	1.108	1.123		0.033		GO:0006749 glutathione metabolic process
Adenosine A3 receptor	Adora3	1.099	-1.150		0.048		GO:0001973 adenosine receptor signaling pathway; GO:0002553 histamine secretion by mast cell; GO:0002687 positive regulation of leukocyte migration; GO:0007165 signal transduction; GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0014061 regulation of norepinephrine secretion; GO:0014068 positive regulation of phosphoinositide 3-kinase cascade; GO:0043306 positive regulation of mast cell degranulation; GO:0050729 positive regulation of inflammatory response; GO:0050850 positive regulation of calcium-mediated signaling
Sarcoplipin	Sln	1.087	1.355		0.038		GO:0051924 regulation of calcium ion transport
N-myc downstream regulated gene 4	Ndr4	1.087	1.105		0.010		
Gypsy retrotransposon integrase 1	Gin1	1.086	1.054		0.025		GO:0015074 DNA integration
ATP-binding cassette, sub-family G (WHITE), member 3-like 1 ATP-binding cassette, sub-family G (WHITE), member 3-like 2 similar to ATP-binding cassette, sub-family G (WHITE), member 3	Abcg3l Abcg3l 2 RGDI564709 LOC360997	1.084	1.064		0.043		
Oxytocin, prepropeptide arginine vasopressin	Oxt Avp	1.084	-1.112		0.013		GO:0001696 gastric acid secretion; GO:0001975 response to amphetamine; GO:0002027 regulation of heart rate; GO:0002125 maternal aggressive behavior; GO:0003077 negative regulation of diuresis; GO:0003079 positive regulation of natriuresis; GO:0006950 response to stress; GO:0007204 elevation of cytosolic calcium ion concentration; GO:0007507 heart development; GO:0007565 female pregnancy
Thimet oligopeptidase 1	Thop1	1.080	-1.013		0.049		GO:0006508 proteolysis; GO:0006518 peptide metabolic process; GO:0007243 intracellular protein kinase cascade
cAMP responsive element binding protein 3	Creb3	1.078	1.137		0.021		GO:0006355 regulation of transcription, DNA-dependent
Peroxisomal biogenesis factor 12	Pex12	1.071	1.149		0.001		GO:0007031 peroxisome organization; GO:0015031 protein transport; GO:0016558 protein import into peroxisome matrix
Endothelin receptor type A endothelin-1 receptor-like	Ednra LOC100366209	1.071	1.130		0.009		GO:0001569 patterning of blood vessels; GO:0001666 response to hypoxia; GO:0001701 in utero embryonic development; GO:0001934 positive regulation of protein amino acid phosphorylation; GO:0007165 signal transduction; GO:0007204 elevation of cytosolic calcium ion concentration; GO:0007205 activation of protein kinase C activity by G-protein coupled receptor protein signaling pathway; GO:0007507 heart development; GO:0007585 respiratory gaseous exchange; GO:0008217 regulation of blood pressure
	Mk1	1.068	-1.152		0.038		GO:0030036 actin cytoskeleton organization

Protein geranylgeranyltransferase type I, beta subunit	Pggt1b	1.062	1.162	0.039	GO:0008284 positive regulation of cell proliferation; GO:0018348 protein amino acid geranylgeranylation; GO:0034097 response to cytokine stimulus; GO:0045787 positive regulation of cell cycle; GO:0051774 negative regulation of nitric-oxide synthase 2 biosynthetic process; GO:0051789 response to protein stimulus
Olfactory receptor 1356	Olr1356	1.061	1.323	0.021	GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0050911 detection of chemical stimulus involved in sensory perception of smell
Immunity-related GTPase family, cinema 1	Irgc1	1.060	-1.124	0.017	
Distal-less homeobox 5	Dlx5	1.050	-1.140	0.008	GO:0001649 osteoblast differentiation; GO:0001958 endochondral ossification; GO:0006355 regulation of transcription, DNA-dependent; GO:0007275 multicellular organismal development; GO:0007399 nervous system development; GO:0007409 axonogenesis; GO:0007411 axon guidance; GO:0008283 cell proliferation; GO:0030326 embryonic limb morphogenesis; GO:0030855 epithelial cell differentiation
REL-T-like 2 FCH and double SH3 domains 1	Rel12 Fchsd1	1.047	-1.088	0.041	GO:0010811 positive regulation of cell-substrate adhesion
Membrane magnesium transporter 2	Mmg12	1.047	1.171	0.025	GO:0006810 transport; GO:0006824 cobalt ion transport; GO:0006825 copper ion transport; GO:0006828 manganese ion transport; GO:0015674 di-, tri-valent inorganic cation transport; GO:0015675 nickel ion transport; GO:0015693 magnesium ion transport
STEAP family member 3	Steap3	1.046	1.177	0.004	GO:0006811 ion transport; GO:0006826 iron ion transport; GO:0006915 apoptosis; GO:0006917 induction of apoptosis; GO:0007049 cell cycle; GO:0009306 protein secretion; GO:0055114 oxidation reduction
Sialidase 2 (cytosolic sialidase)	Neu2	1.030	1.134	0.023	GO:0008152 metabolic process; GO:0010831 positive regulation of myotube differentiation ; GO:0045471 response to ethanol; GO:0045663 positive regulation of myoblast differentiation
Spermatogenesis associated 6	Spata6	1.028	1.057	0.045	GO:0007275 multicellular organismal development; GO:0007283 spermatogenesis; GO:0030154 cell differentiation
Fibroblast growth factor 20	Fgf20	1.027	-1.096	0.044	GO:0008284 positive regulation of cell proliferation; GO:0008543 fibroblast growth factor receptor signaling pathway; GO:0016049 cell growth; GO:0030154 cell differentiation; GO:0060113 inner ear receptor cell differentiation
Cholinergic receptor, nicotinic, alpha 9	Chma9	1.022	-1.124	0.006	GO:0006812 cation transport; GO:0006816 calcium ion transport; GO:0007204 elevation of cytosolic calcium ion concentration; GO:0007605 sensory perception of sound; GO:0042472 inner ear morphogenesis; GO:0050910 detection of mechanical stimulus involved in sensory perception of sound
Coagulation factor II (thrombin) receptor	F2r	1.005	1.345	0.007	GO:0000186 activation of MAPKK activity; GO:0002248 connective tissue replacement during inflammatory response; GO:0006919 activation of caspase activity; GO:0006954 inflammatory response; GO:0007165 signal transduction; GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0007205 activation of protein kinase C activity by G-protein coupled receptor protein signaling pathway; GO:0007260 tyrosine phosphorylation of STAT protein; GO:0007262 STAT protein nuclear translocation; GO:0007529 establishment of synaptic specificity at neuromuscular junction
Potassium channel, subfamily K, member 1	Kenk1	1.004	1.241	0.015	GO:0006811 ion transport; GO:0006813 potassium ion transport; GO:0035094 response to nicotine
Ameloblastin	Ambn	-1.000	-1.046	0.025	
Adhesion molecule with Ig like domain 3	Amigo3	-1.023	1.147	0.022	GO:0007155 cell adhesion; GO:0007157 heterophilic cell-cell adhesion; GO:0007399 nervous system development
Ankyrin repeat and SOCS box-containing 6	Asb6	-1.028	-1.072	0.015	

ATPase, H transporting, lysosomal V1 subunit B2	Atp6v1b2	-1.039	1.046	0.011	GO:0006811 ion transport; GO:0007035 vacuolar acidification; GO:0015986 ATP synthesis coupled proton transport; GO:0015992 proton transport; GO:0030641 regulation of cellular pH; GO:0046034 ATP metabolic process
Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 10	Mllt10	-1.041	-1.001	0.023	GO:0008150 biological_process
Phosphorylase kinase, beta	Phkb	-1.042	1.078	0.009	GO:0005976 polysaccharide metabolic process; GO:0005977 glycogen metabolic process
Olfactory receptor 1409	Olr1409	-1.059	-1.105	0.038	GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0050911 detection of chemical stimulus involved in sensory perception of smell
MIF4G domain containing	Mif4gd	-1.063	-1.109	0.002	GO:0006417 regulation of translation; GO:0016070 RNA metabolic process
Olfactory receptor 44	Olr44	-1.073	-1.122	0.039	GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0050911 detection of chemical stimulus involved in sensory perception of smell
Sulfatase modifying factor 2	Sumf2	-1.077	-1.088	0.034	
WD repeat domain 45	Wdr45	-1.078	1.120	0.049	
Syndecan binding protein	Sdcbp	-1.080	1.170	0.008	GO:0007265 Ras protein signal transduction
Bernardinelli-Seip congenital lipodystrophy 2 homolog (human)	Bsel2	-1.082	-1.006	0.047	GO:0008150 biological_process
Dehydrogenase/reductase (SDR family) member 1	Dhrs1	-1.083	1.116	0.006	GO:0055114 oxidation reduction
Polymerase (DNA directed), gamma	Polg	-1.089	-1.102	0.029	GO:0006264 mitochondrial DNA replication; GO:0006287 base-excision repair; gap-filling; GO:0007568 aging
Protein O-fucosyltransferase 1	Pofut1	-1.091	1.038	0.026	GO:0001525 angiogenesis; GO:0001756 somitogenesis; GO:0005975 carbohydrate metabolic process; GO:0006004 fucose metabolic process; GO:0006493 protein amino acid O-linked glycosylation; GO:0007219 Notch signaling pathway; GO:0007399 nervous system development; GO:0007507 heart development; GO:0008150 biological_process
Outer dense fiber of sperm tails 2	Odf2	-1.098	-1.005	0.044	GO:0007286 spermatid development
Nuclear prelamin A recognition factor-like	Narf1	-1.099	-1.006	0.017	GO:0001666 response to hypoxia; GO:0016226 iron-sulfur cluster assembly; GO:0032364 oxygen homeostasis; GO:0045449 regulation of transcription
Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	Slc6a4	-1.105	-1.065	0.038	GO:0001504 neurotransmitter uptake; GO:0006837 serotonin transport; GO:0007626 locomotory behavior; GO:0009636 response to toxin; GO:0015844 monoamine transport; GO:0021794 thalamus development; GO:0051610 serotonin uptake
Acyl-Coenzyme A oxidase 3, pristanoyl	Acox3	-1.106	-1.081	0.001	GO:0006629 lipid metabolic process; GO:0006631 fatty acid metabolic process; GO:0006635 fatty acid beta-oxidation; GO:0033540 fatty acid beta-oxidation using acyl-CoA oxidase; GO:0055114 oxidation reduction
Shroom family member 2	Shroom2	-1.115	1.191	0.035	GO:0000902 cell morphogenesis; GO:0007275 multicellular organismal development; GO:0016477 cell migration; GO:0030835 negative regulation of actin filament depolymerization; GO:0032438 melanosome organization; GO:0045217 cell-cell junction maintenance; GO:0051017 actin filament bundle assembly
Similar to RIKEN cDNA A730011L01 gene	LOC498029	-1.115	-1.077	0.023	GO:0006281 DNA repair
Neuropeptide FF receptor 1	Npffr1	-1.117	-1.111	0.024	GO:0007165 signal transduction; GO:0007186 G-protein coupled receptor protein signaling pathway
Kelch-like 36 (Drosophila)	Klhl36	-1.119	-1.021	0.014	
Phosphatidylinositol glycan anchor biosynthesis, class S	Pigs	-1.128	1.007	0.033	GO:0006506 GPI anchor biosynthetic process; GO:0016255 attachment of GPI anchor to protein
PDZ and LIM domain 1	Pdlim1	-1.131	1.111	0.049	GO:0001666 response to hypoxia; GO:0045449 regulation of transcription

Alpha-1-B glycoprotein WD repeat domain 7	Albg Wdr7	-1.132 -1.133	-1.048 -1.041	0.005 0.017	
Protein O-linked mannose beta1,2-N- acetylglucosaminyltransferase	Pomgnt1	-1.135	-1.056	0.012	GO:0006487 protein amino acid N-linked glycosylation; GO:0006493 protein amino acid O-linked glycosylation; GO:0008150 biological process
Fibroblast growth factor 11	Fgf11	-1.135	-1.162	0.016	
DEAD (Asp-Glu-Ala-Asp) box polypeptide 24	Ddx24	-1.139	1.006	0.027	
Heterogeneous nuclear ribonucleoprotein H1	Hnrph1	-1.143	1.100	0.040	GO:0006396 RNA processing
Rho-related BTB domain containing 2	Rhobtb2	-1.145	-1.024	0.013	GO:0007264 small GTPase mediated signal transduction
Ring finger protein 135	Rnf135	-1.147	-1.045	0.013	GO:0006270 DNA-dependent DNA replication initiation; GO:0007264 small GTPase mediated signal transduction; GO:0047497 mitochondrion transport along microtubule
Vaccinia related kinase 3	Vrk3	-1.150	1.011	0.046	GO:0006468 protein amino acid phosphorylation; GO:0032516 positive regulation of phosphoprotein phosphatase activity; GO:0070373 negative regulation of ERK1 and ERK2 cascade
Cardiotrophin-like cytokine factor 1	Cicf1	-1.150	-1.144	0.029	GO:0007166 cell surface receptor linked signaling pathway; GO:0007259 JAK-STAT cascade; GO:0030183 B cell differentiation
Prostate tumor overexpressed 1	Prov1	-1.154	-1.014	0.050	GO:0045449 regulation of transcription
Mitogen activated protein kinase kinase kinase 1	Map3k1	-1.158	-1.106	0.008	GO:000165 MAPKKK cascade; GO:0000186 activation of MAPKK activity; GO:0000209 protein polyubiquitination; GO:0006468 protein amino acid phosphorylation; GO:0006970 response to osmotic stress; GO:0007179 transforming growth factor beta receptor signaling pathway; GO:0007249 I-kappaB kinase/NF-kappaB cascade; GO:0007254 JNK cascade; GO:0007256 activation of JNKK activity; GO:0007257 activation of JUN kinase activity
Calcium homeostasis endoplasmic reticulum protein similar to 1700030K09Rik protein	Cherp RGD 31 1847	-1.160	-1.040	0.018	GO:0006396 RNA processing; GO:0006874 cellular calcium ion homeostasis; GO:0008285 negative regulation of cell proliferation
Progesterin and adipoQ receptor family member III	Paqr3	-1.161	-1.063	0.016	
Glioma tumor suppressor candidate region gene 2	Gltscr2	-1.162	-1.123	0.012	
Poly(rC) binding protein 2	Pebp2	-1.162	-1.015	0.014	GO:0008380 RNA splicing
THO complex 5	Thoc5	-1.163	-1.025	0.036	GO:0006397 mRNA processing; GO:0006406 mRNA export from nucleus; GO:0006810 transport; GO:0008150 biological process; GO:0008380 RNA splicing; GO:0030154 cell differentiation; GO:0045650 negative regulation of macrophage differentiation; GO:0046784 intronless viral mRNA export from host nucleus; GO:0051028 mRNA transport
Glycine-, glutamate-, thienylcyclohexylpiperidine-binding protein	LOC246295	-1.165	1.035	0.045	
Family with sequence similarity 113, member A	Fam113a	-1.171	1.022	0.049	
Leucine rich repeat (in FLII) interacting protein 1	Lrrrip1	-1.175	1.000	0.048	GO:0045449 regulation of transcription
AlkB, alkylation repair homolog 3 (E. coli)	Alkbh3	-1.177	-1.138	0.001	GO:0006281 DNA repair; GO:0055114 oxidation reduction
Amyloid beta (A4) precursor protein-binding, family B, member 3	Apbh3	-1.181	1.016	0.018	
Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	Cdkn2b	-1.185	-1.104	0.017	GO:0000079 regulation of cyclin-dependent protein kinase activity; GO:0000086 G2/M transition of mitotic cell cycle; GO:0007050 cell cycle arrest; GO:0008285 negative regulation of cell proliferation; GO:0014070 response to organic cyclic substance; GO:0030219

						megakaryocyte differentiation; GO:0030511 positive regulation of transforming growth factor beta receptor signaling pathway; GO:0030858 positive regulation of epithelial cell differentiation; GO:0031575 G1/S transition checkpoint; GO:0031668 cellular response to extracellular stimulus
CD2-associated protein	Cd2ap	-1.189	-1.089	0.039		GO:0016337 cell-cell adhesion; GO:0016477 cell migration; GO:0043161 proteasomal ubiquitin-dependent protein catabolic process; GO:0048259 // regulation of receptor-mediated endocytosis
ATPase, Na ⁺ /K ⁺ transporting, beta 1 polypeptide	Atp1b1	-1.192	-1.020	0.036		GO:0001666 response to hypoxia; GO:0006754 ATP biosynthetic process; GO:0006811 ion transport; GO:0006813 potassium ion transport; GO:0006814 sodium ion transport; GO:0030001 metal ion transport
Ring finger protein 114	Rnf114	-1.193	1.005	0.050		GO:0007275 multicellular organismal development; GO:0007283 spermatogenesis; GO:0030154 cell differentiation
Inositol polyphosphate phosphatase-like 1	Inpp1l	-1.198	-1.022	0.046		GO:0006006 glucose metabolic process; GO:0007420 brain development; GO:0008156 negative regulation of DNA replication; GO:0008285 negative regulation of cell proliferation; GO:0009791 post-embryonic development; GO:0010629 negative regulation of gene expression; GO:0010642 negative regulation of platelet-derived growth factor receptor signaling pathway; GO:0010977 negative regulation of neuron projection development; GO:0032868 response to insulin stimulus; GO:0032957 inositol trisphosphate metabolic process
TAO kinase 2	Taok2	-1.200	-1.058	0.010		GO:0000186 activation of MAPKK activity; GO:0006468 protein amino acid phosphorylation; GO:0006950 response to stress; GO:0008360 regulation of cell shape; GO:0030036 actin cytoskeleton organization; GO:0046330 positive regulation of JNK cascade; GO:0048041 focal adhesion assembly; GO:0000186 activation of MAPKK activity
Dynamin 2	Dnm2	-1.200	-1.085	0.036		GO:0006892 post-Golgi vesicle-mediated transport; GO:0006897 endocytosis; GO:0006898 receptor-mediated endocytosis; GO:0016044 cellular membrane organization; GO:0031623 receptor internalization; GO:0033572 transferrin transport
Calcium channel, voltage-dependent, beta 3 subunit	Cacnb3	-1.200	1.009	0.029		GO:0006811 ion transport; GO:0006816 calcium ion transport; GO:0050852 T cell receptor signaling pathway
Similar to chromosome 1 open reading frame 172	RGD1303271	-1.201	-1.049	0.048		
Pre-B-cell leukemia homeobox 2	Pbx2	-1.202	-1.007	0.019		GO:0006355 regulation of transcription, DNA-dependent; GO:0009954 proximal/distal pattern formation; GO:0030326 embryonic limb morphogenesis; GO:0045944 positive regulation of transcription from RNA polymerase II promoter
SCY1-like 1 (S. cerevisiae) latent transforming growth factor beta binding protein 3	Scyl1 Ltbp3	-1.204	1.032	0.034		GO:0006468 protein amino acid phosphorylation; GO:0006890 retrograde vesicle-mediated transport, Golgi to ER; GO:0016192 vesicle-mediated transport
Olfactory receptor 1765	Olr1765	-1.206	-1.436	0.003		GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0050911 detection of chemical stimulus involved in sensory perception of smell
Transmembrane BAX inhibitor motif containing 6	Tbim6	-1.209	-1.048	0.006		GO:0007283 spermatogenesis; GO:0030324 lung development; GO:0043066 negative regulation of apoptosis
Solute carrier organic anion transporter family, member 2b1	Slo2b1	-1.210	1.027	0.030		GO:0001889 liver development; GO:0006811 ion transport; GO:0015721 bile acid and bile salt transport; GO:0071718 sodium-independent icosanoid transport
Trace amine-associated receptor 8c	Taar8c	-1.210	1.130	0.011		GO:0007165 signal transduction; GO:0007186 G-protein coupled receptor protein signaling pathway

Transmembrane protein, adipocyte associated 1	Tpr1	-1.211	-1.019	0.033	GO:0042493 response to drug
DtGeorge syndrome critical region gene 2	Dger2	-1.211	-1.046	0.036	GO:000278 mitotic cell cycle; GO:0006468 protein amino acid phosphorylation; GO:0032436 positive regulation of proteasomal ubiquitin-dependent protein catabolic process; GO:0032922 circadian regulation of gene expression; GO:0042752 regulation of circadian rhythm; GO:0006090 pyruvate metabolic process; GO:0015711 organic anion transport; GO:0055085 transmembrane transport
Casein kinase 1, delta solute carrier family 16, member 3 (monocarboxylic acid transporter 4)	Csnk1d Slc16a3	-1.211	-1.042	0.021	GO:000278 mitotic cell cycle; GO:0006468 protein amino acid phosphorylation; GO:0032436 positive regulation of proteasomal ubiquitin-dependent protein catabolic process; GO:0032922 circadian regulation of gene expression; GO:0042752 regulation of circadian rhythm; GO:0006090 pyruvate metabolic process; GO:0015711 organic anion transport; GO:0055085 transmembrane transport
Scaffold attachment factor B	Safb	-1.219	-1.003	0.016	GO:0006355 regulation of transcription, DNA-dependent ; GO:0030520 estrogen receptor signaling pathway; GO:0040007 growth; GO:0042445 hormone metabolic process; GO:0050684 regulation of mRNA processing
MAP/microtubule affinity-regulating kinase 2	Mark2	-1.219	-1.025	0.044	GO:0006468 protein amino acid phosphorylation; GO:0006979 response to oxidative stress; GO:0007243 intracellular protein kinase cascade; GO:0007275 multicellular organismal development; GO:0030154 cell differentiation; GO:0045197 establishment or maintenance of epithelial cell apical/basal polarity
Era (G-protein)-like 1 (E. coli)	Era1	-1.219	1.006	0.040	GO:0002474 antigen processing and presentation of peptide antigen via MHC class I; GO:0006955 immune response; GO:0019882 antigen processing and presentation
RT1 class I, locus CE12 RT1 class I, locus CE14	RT1-CE12/RT1-CE14	-1.220	-1.156	0.002	GO:0006955 immune response; GO:0019882 antigen processing and presentation
Serine incorporator 3	Serinc3	-1.220	1.055	0.021	GO:0006658 phosphatidylserine metabolic process; GO:0006665 sphingolipid metabolic process; GO:0006917 induction of apoptosis; GO:0013825 L-serine transport; GO:0051347 positive regulation of transferase activity
Cancer susceptibility candidate 3	Casc3	-1.225	-1.024	0.048	GO:0000184 nuclear-transcribed mRNA catabolic process, nonsense-mediated decay; GO:0006397 mRNA processing; GO:0006417 regulation of translation; GO:0006810 transport; GO:0006950 response to stress; GO:0008298 intracellular mRNA localization; GO:0008380 RNA splicing; GO:0051028 mRNA transport
DAB2 interacting protein	Dab2ip	-1.233	-1.016	0.038	GO:0007165 signal transduction; GO:0051056 regulation of small GTPase mediated signal transduction
Gamma-glutamyl transferase 6	Ggt6	-1.233	-1.024	0.018	GO:0006750 glutathione biosynthetic process
Fatty acid desaturase 1	Fads1	-1.233	-1.192	0.021	GO:0006636 unsaturated fatty acid biosynthetic process; GO:0006810 transport; GO:0006950 response to stress; GO:0007568 aging; GO:0007584 response to nutrient; GO:0009267 cellular response to starvation; GO:0009744 response to sucrose stimulus; GO:0010033 response to organic substance; GO:0014070 response to organic cyclic substance; GO:0019369 arachidonic acid metabolic process
PTK2B protein tyrosine kinase 2 beta	Plk2b	-1.233	-1.101	0.009	GO:0000165 MAPKK cascade; GO:0000302 response to reactive oxygen species; GO:0001525 angiogenesis; GO:0001556 oocyte maturation; GO:0001666 response to hypoxia; GO:0006468 protein amino acid phosphorylation; GO:0006800 oxygen and reactive oxygen species metabolic process; GO:0006950 response to stress; GO:0006970 response to osmotic stress; GO:0007015 actin filament organization
RAD23 homolog A (S. cerevisiae)	Rad23a	-1.234	-1.089	0.008	GO:0006289 nucleotide-excision repair; GO:0006974 response to DNA damage stimulus; GO:0043161 proteasomal ubiquitin-dependent protein catabolic process
GDP dissociation inhibitor 1	Gdi1	-1.235	-1.013	0.025	GO:0007264 small GTPase mediated signal transduction; GO:0015031 protein transport; GO:0043087 regulation of GTPase activity

Solute carrier family 15, member 4	Slc15a4	-1.237	-1.100	0.029	GO:0006857 oligopeptide transport; GO:0015031 protein transport; GO:0015817 histidine transport
DALR anticodon binding domain containing 3	Dalrd3	-1.242	1.008	0.026	GO:0006420 arginyl-tRNA aminoacylation
Axin 1	Axin1	-1.244	-1.077	0.006	GO:0007275 multicellular organismal development; GO:0007605 sensory perception of sound; GO:0010800 positive regulation of peptidyl-threonine phosphorylation; GO:0016055 Wnt receptor signaling pathway; GO:0030163 protein catabolic process; GO:0030178 negative regulation of Wnt receptor signaling pathway; GO:0031398 positive regulation of protein ubiquitination; GO:0033138 positive regulation of peptidyl-serine phosphorylation; GO:0046330 positive regulation of JNK cascade; GO:0060070 Wnt receptor signaling pathway through beta-catenin
ATG7 autophagy related 7 homolog (S. cerevisiae)	Atg7	-1.247	-1.107	0.010	GO:0001889 liver development; GO:0006497 protein amino acid lipidation; GO:0006520 cellular amino acid metabolic process; GO:0006914 autophagy; GO:0006996 organelle organization; GO:0007628 adult walking behavior; GO:0008152 metabolic process; GO:0009791 post-embryonic development; GO:0015031 protein transport; GO:0016044 cellular membrane organization
General transcription factor II I	Gtf2i	-1.247	1.033	0.046	GO:0009790 embryonic development; GO:0051481 reduction of cytosolic calcium ion concentration
Phosphatidylinositol glycan anchor biosynthesis, class V	Pigv	-1.253	1.010	0.005	GO:0006506 GPI anchor biosynthetic process; GO:0016254 preassembly of GPI anchor in ER membrane
Anterior pharynx defective 1 homolog A (C. elegans)	Aph1a	-1.255	-1.029	0.046	GO:0001656 metanephros development; GO:0006509 membrane protein ectodomain proteolysis; GO:0006915 apoptosis; GO:0007220 Notch receptor processing; GO:0008624 induction of apoptosis by extracellular signals; GO:0016485 protein processing; GO:0031293 membrane protein intracellular domain proteolysis; GO:0042987 amyloid precursor protein catabolic process; GO:0043085 positive regulation of catalytic activity
Purinergic receptor P2X, ligand-gated ion channel 4	P2rx4	-1.260	-1.135	0.017	GO:0002028 regulation of sodium ion transport; GO:0006809 nitric oxide biosynthetic process; GO:0006810 transport; GO:0006811 ion transport; GO:0006816 calcium ion transport; GO:0007165 signal transduction; GO:0008217 regulation of blood pressure; GO:0010524 positive regulation of calcium ion transport into cytosol; GO:0010614 negative regulation of cardiac muscle hypertrophy; GO:0019228 regulation of action potential in neuron
Secretory carrier membrane protein 2	Scamp2	-1.262	1.071	0.018	GO:0006886 intracellular protein transport; GO:0006897 endocytosis; GO:0015031 protein transport
Bel2-like 1	Bel2l1	-1.264	-1.205	0.024	GO:0001541 ovarian follicle development; GO:0001666 response to hypoxia; GO:0001701 in utero embryonic development; GO:0001836 release of cytochrome c from mitochondria; GO:0006915 apoptosis; GO:0006916 anti-apoptosis; GO:0006950 response to stress; GO:0006979 response to oxidative stress; GO:0007281 germ cell development; GO:0007283 spermatogenesis
Olfactory receptor 1614	Olr1614	-1.267	-1.411	0.004	GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0050911 detection of chemical stimulus involved in sensory perception of smell
LUC7-like (S. cerevisiae)	Luc7l	-1.270	1.061	0.043	
Telomerase associated protein 1	Tep1	-1.271	-1.012	0.016	GO:0000722 telomere maintenance via recombination; GO:0000722 telomere maintenance via recombination; GO:0007004 telomere maintenance via telomerase
Serine/threonine kinase 39, STE20/SPS1 homolog	Stk39	-1.272	-1.061	0.030	GO:0006468 protein amino acid phosphorylation; GO:0043268 positive regulation of

(yeast)						potassium ion transport
CREB binding protein	Crebbp	-1.272	-1.102	0.002		GO:0006355 regulation of transcription, DNA-dependent; GO:0008283 cell proliferation; GO:0016573 histone acetylation; GO:0018076 N-terminal peptidyl-lysine acetylation; GO:0030718 germ-line stem cell maintenance; GO:0033261 regulation of S phase; GO:0045449 regulation of transcription; GO:0045893 positive regulation of transcription, DNA-dependent; GO:0045941 positive regulation of transcription; GO:0045944 positive regulation of transcription from RNA polymerase II promoter
Serine/threonine kinase 40	Slk40	-1.272	-1.073	0.016		GO:0006468 protein amino acid phosphorylation
Protein kinase N1	Pkn1	-1.274	-1.095	0.035		GO:0006468 protein amino acid phosphorylation; GO:0006972 hyperosmotic response; GO:0007165 signal transduction
Ligase III, DNA, ATP-dependent	Lig3	-1.276	-1.089	0.035		GO:0006260 DNA replication; GO:0006281 DNA repair; GO:0006310 DNA recombination; GO:0033151 V(D)J recombination
Post-GPI attachment to proteins 2	Pgap2	-1.277	1.012	0.026		GO:0006916 anti-apoptosis; GO:0006974 response to DNA damage stimulus; GO:0042770 DNA damage response, signal transduction
Glucagon	Geg	-1.280	-1.140	0.021		GO:0006109 regulation of carbohydrate metabolic process; GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0007188 G-protein signaling, coupled to cAMP nucleotide second messenger; GO:0009755 hormone-mediated signaling pathway; GO:0019216 regulation of lipid metabolic process; GO:0019538 protein metabolic process; GO:0032099 negative regulation of appetite; GO:0050796 regulation of insulin secretion
CDC42 small effector 1	Cdc42se1	-1.280	-1.105	0.010		GO:0006909 phagocytosis; GO:0008360 regulation of cell shape
Solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 3	Slc25a3	-1.282	1.010	0.025		GO:0055085 transmembrane transport
Ubiquitin-associated protein 1	Ubap1	-1.284	-1.090	0.006		
ATP citrate lyase	Acy	-1.292	-1.138	0.025		GO:0006084 acetyl-CoA metabolic process; GO:0006085 acetyl-CoA biosynthetic process; GO:0006101 citrate metabolic process; GO:0006629 lipid metabolic process; GO:0006633 fatty acid biosynthetic process; GO:0008152 metabolic process; GO:0044262 cellular carbohydrate metabolic process
Potassium channel, subfamily K, member 6 CWC15 spliceosome-associated protein homolog (S. cerevisiae) family with sequence similarity 35, member A	Kenk6 Cwc15 fam35a	-1.294	1.142	0.019		GO:0006811 ion transport ; GO:0006813 potassium ion transport; GO:0000398 nuclear mRNA splicing, via spliceosome; GO:0008150 biological process; GO:0008380 RNA splicing
Inositol (myo)-1(or 4)-monophosphatase 2	Inpa2	-1.294	-1.024	0.018		GO:0008150 biological process; GO:0046855 inositol phosphate dephosphorylation
Transmembrane protein 171	Tmem171	-1.296	1.129	0.022		
Zinc finger, DHHC-type containing 23	Zdhc23	-1.301	-1.025	0.002		
A kinase (PRK.A) anchor protein 1	Akap1	-1.301	1.061	0.022		GO:0010614 negative regulation of cardiac muscle hypertrophy; GO:0010738 regulation of protein kinase A signaling cascade; GO:0032869 cellular response to insulin stimulus; GO:0035308 negative regulation of protein amino acid dephosphorylation; GO:0051534 negative regulation of NFAT protein import into nucleus; GO:0070887 cellular response to chemical stimulus
Shisa homolog 5 (Xenopus laevis)	Shisa5	-1.302	1.022	0.010		GO:0006915 apoptosis; GO:0006917 induction of apoptosis; GO:0043123 positive regulation of l-kappaB kinase
Phosphatidic acid phosphatase type 2c	Ppap2c	-1.306	-1.145	0.048		

Nuclear RNA export factor 1	Nxf1	-1.307	1.174	0.026	GO:0006405 RNA export from nucleus; GO:0006406 mRNA export from nucleus; GO:0006810 transport; GO:0016973 poly(A)+ mRNA export from nucleus; GO:0051028 mRNA transport
Fas-activated serine/threonine kinase	Fasfk	-1.308	-1.082	0.001	GO:0006915 apoptosis
Ring finger protein 160	Rnf160	-1.317	1.091	0.042	
PCTAIRE protein kinase 1	Ptk1	-1.318	-1.126	0.029	GO:0006468 protein amino acid phosphorylation
Prickle homolog 3 (Drosophila)	Prickle3	-1.326	-1.096	0.042	
Solute carrier family 9 (sodium/hydrogen exchanger), member 3	Slc9a3	-1.333	1.233	0.025	GO:0002028 regulation of sodium ion transport; GO:0006812 cation transport; GO:0006814 sodium ion transport; GO:0006885 regulation of pH; GO:0006898 receptor-mediated endocytosis; GO:0007623 circadian rhythm; GO:0051384 response to glucocorticoid stimulus; GO:0055085 transmembrane transport
CDP-diacylglycerol synthase 1	Cds1	-1.339	-1.064	0.014	GO:0008654 phospholipid biosynthetic process
Trinucleotide repeat containing 6B	Tnrc6b	-1.342	-1.089	0.040	
Cytochrome P450, family 2, subfamily d, polypeptide 4 cytochrome P450, family 2, subfamily d, polypeptide 5	Cyp2d4(Cyp2d5)	-1.347	-1.127	0.001	GO:0008202 steroid metabolic process; GO:0009804 coumarin metabolic process; GO:0009820 alkaloid metabolic process; GO:0009822 alkaloid catabolic process; GO:0009892 negative regulation of metabolic process; GO:0010033 response to organic substance; GO:0016098 monoterpenoid metabolic process; GO:0017144 drug metabolic process; GO:0019369 arachidonic acid metabolic process; GO:0033076 isoquinoline alkaloid metabolic process
Hypothetical protein MGC:72616	RGD735175	-1.347	-1.059	0.021	
Basic helix-loop-helix family, member e40	Bhlhe40	-1.347	-1.158	0.022	GO:0007399 nervous system development; GO:0007623 circadian rhythm; GO:0009416 response to light stimulus; GO:0009649 entrainment of circadian clock; GO:0045892 negative regulation of transcription, DNA-dependent; GO:0048168 regulation of neuronal synaptic plasticity
Phosphatidylinositol 4-kinase, catalytic, alpha	P14ka	-1.348	-1.089	0.009	GO:0046854 phosphoinositide phosphorylation; GO:0048015 phosphoinositide-mediated signaling
Similar to 2310044H10Rik protein	MGC93975	-1.351	-1.168	0.001	
CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2	Cds2	-1.354	1.015	0.031	GO:0008654 phospholipid biosynthetic process
Prolyl endopeptidase-like	Prepl	-1.354	1.006	0.045	GO:0006508 proteolysis
B-cell translocation gene 1, anti-proliferative	Btg1	-1.360	-1.130	0.043	GO:0006479 protein amino acid methylation; GO:0006979 response to oxidative stress; GO:0007283 spermatogenesis; GO:0007286 spermatid development; GO:0008285 negative regulation of cell proliferation; GO:0042981 regulation of apoptosis; GO:0043434 response to peptide hormone stimulus; GO:0045603 positive regulation of endothelial cell differentiation; GO:0045663 positive regulation of myoblast differentiation; GO:0045766 positive regulation of angiogenesis
Claudin 3	Cldn3	-1.373	-1.030	0.031	GO:0001666 response to hypoxia; GO:0016338 calcium-independent cell-cell adhesion
Serine/threonine kinase 38	Stk38	-1.375	-1.058	0.006	GO:0006464 protein modification process; GO:0006468 protein amino acid phosphorylation; GO:0007243 intracellular protein kinase cascade; GO:0008150 biological process
Mucin and cadherin like	Mucdh1	-1.375	-1.064	0.009	GO:0007155 cell adhesion
Thyroid hormone receptor interactor 10	Tripl0	-1.377	-1.103	0.011	GO:0006897 endocytosis; GO:0042538 hyperosmotic salinity response
Solute carrier family 44, member 1	Slc44a1	-1.381	-1.043	0.010	GO:0015871 choline transport
Mitofusin 2	Mfn2	-1.382	-1.103	0.034	GO:0001825 blastocyst formation; GO:0006626 protein targeting to mitochondrion;

						GO:0007006 mitochondrial membrane organization; GO:0007050 cell cycle arrest; GO:0008053 mitochondrial fusion; GO:0008285 negative regulation of cell proliferation; GO:0046580 negative regulation of Ras protein signal transduction; GO:0048593 camera-type eye morphogenesis; GO:0048662 negative regulation of smooth muscle cell proliferation; GO:0051646 mitochondrion localization
Solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1	Slc9a3r1	-1.388	-1.041	0.037		GO:0016055 Wnt receptor signaling pathway; GO:0030643 cellular phosphate ion homeostasis
ATP-binding cassette, sub-family B (MDR/TAP), member 6	Abcb6	-1.390	1.002	0.008		GO:0006810 transport; GO:0055085 transmembrane transport
Spermatogenesis associated 2	Spta2	-1.390	-1.165	0.039		
Sphingomyelin synthase 2	Sgms2	-1.398	-1.072	0.022		GO:0006629 lipid metabolic process; GO:0006665 sphingolipid metabolic process; GO:0006686 sphingomyelin biosynthetic process
Ras homolog gene family, member T2	Rhot2	-1.404	1.018	0.049		GO:0006915 apoptosis; GO:0007264 small GTPase mediated signal transduction; GO:0019725 cellular homeostasis; GO:0047497 mitochondrion transport along microtubule
V-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	ErbB2	-1.417	-1.065	0.047		GO:0001889 liver development; GO:0007165 signal transduction; GO:0007166 cell surface receptor linked signaling pathway; GO:0007169 transmembrane receptor protein tyrosine kinase signaling pathway; GO:0007399 nervous system development; GO:0007417 central nervous system development; GO:0007422 peripheral nervous system development; GO:0007507 heart development; GO:0007519 skeletal muscle tissue development; GO:0007528 neuromuscular junction development
Integrin alpha FG-GAP repeat containing 3	Itfg3	-1.420	-1.003	0.039		
Endothelin 2	Edn2	-1.424	-1.091	0.001		GO:0001516 prostaglandin biosynthetic process; GO:0001543 ovarian follicle rupture; GO:0002690 positive regulation of leukocyte chemotaxis; GO:0003100 regulation of systemic arterial blood pressure by endothelin; GO:0007204 elevation of cytosolic calcium ion concentration; GO:0007205 activation of protein kinase C activity by G-protein coupled receptor protein signaling pathway; GO:0008217 regulation of blood pressure; GO:0008284 positive regulation of cell proliferation; GO:0010460 positive regulation of heart rate; GO:0014824 artery smooth muscle
Ring finger protein 10	Rnf10	-1.440	-1.069	0.013		GO:0008150 biological process; GO:0045941 positive regulation of transcription
Aldolase A, fructose-bisphosphate	Aldoa	-1.442	1.024	0.016		GO:0001666 response to hypoxia; GO:0006000 fructose metabolic process; GO:0006096 glycolysis; GO:0006754 ATP biosynthetic process; GO:0006941 striated muscle contraction; GO:0008152 metabolic process; GO:0008360 regulation of cell shape; GO:0009408 response to heat; GO:0030388 fructose 1,6-bisphosphate metabolic process; GO:0032496 response to lipopolysaccharide
Adenylate cyclase 6	Adcy6	-1.442	-1.031	0.036		GO:0006171 cAMP biosynthetic process; GO:0007193 inhibition of adenylate cyclase activity by G-protein signaling pathway ; GO:0009755 hormone-mediated signaling pathway; GO:0034199 activation of protein kinase A activity
UDP-glucose ceramide glucosyltransferase	Ugeg	-1.443	-1.257	0.001		GO:0006665 sphingolipid metabolic process; GO:0008610 lipid biosynthetic process
Nuclear receptor subfamily 3, group C, member 2	Nr3c2	-1.447	-1.092	0.009		GO:0006883 cellular sodium ion homeostasis; GO:0007588 excretion; GO:0031959 mineralocorticoid receptor signaling pathway; GO:0042127 regulation of cell proliferation
Inositol polyphosphate-5-phosphatase J	Inpp5j	-1.455	-1.112	0.023		GO:0010977 negative regulation of neuron projection development; GO:0031115 negative regulation of microtubule polymerization; GO:0033137 negative regulation of peptidyl-serine phosphorylation
cytokine-like nuclear factor n-pac	N-pac	-1.456	-1.106	0.032		GO:0006098 pentose-phosphate shunt; GO:0055114 oxidation reduction

Glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2)	Got2	-1.458	-1.135	0.017	GO:0006103 2-oxoglutarate metabolic process; GO:0006107 oxaloacetate metabolic process; GO:0006520 cellular amino acid metabolic process; GO:0006531 aspartate metabolic process; GO:0006532 aspartate biosynthetic process; GO:0006533 aspartate catabolic process; GO:0006536 glutamate metabolic process; GO:0009058 biosynthetic process; GO:0015908 fatty acid transport; GO:0019550 glutamate catabolic process to aspartate
MAX interactor 1	Mxi1	-1.466	-1.047	0.018	GO:0001222 negative regulation of transcription from RNA polymerase II promoter; GO:0006355 regulation of transcription, DNA-dependent
Vacuolar protein sorting 52 homolog (S. cerevisiae)	Vps52	-1.478	-1.160	0.025	GO:0015031 protein transport
Adenosylhomocysteinase	Ahey	-1.487	-1.135	0.008	GO:0001666 response to hypoxia; GO:0002439 chronic inflammatory response to antigenic stimulus; GO:0006730 one-carbon metabolic process; GO:0007584 response to nutrient; GO:0008152 metabolic process; GO:0019510 S-adenosylhomocysteine catabolic process; GO:0042745 circadian sleep/wake cycle
Meprin 1 alpha	Mep1a	-1.502	-1.200	0.037	GO:0006508 proteolysis
Myosin IE	Myo1e	-1.516	-1.229	0.004	GO:0001570 vasculogenesis; GO:0001701 in utero embryonic development; GO:0001822 kidney development; GO:0006807 nitrogen compound metabolic process; GO:0030097 hemopoiesis; GO:0035166 post-embryonic hemopoiesis; GO:0048008 platelet-derived growth factor receptor signaling pathway
Lymphocyte antigen 6 complex, locus E	Ly6e	-1.541	-1.316	0.001	GO:0001701 in utero embryonic development; GO:0030325 adrenal gland development; GO:0035265 organ growth; GO:0042415 norepinephrine metabolic process; GO:0048242 epinephrine secretion; GO:0055010 ventricular cardiac muscle tissue morphogenesis
Aldehyde dehydrogenase 1 family, member A1	Aldh1a1	-1.553	1.170	0.023	GO:0001822 kidney development; GO:0001889 liver development; GO:0002072 optic cup morphogenesis involved in camera-type eye development; GO:0006979 response to oxidative stress; GO:0007494 midgut development; GO:0014070 response to organic cyclic substance; GO:0032355 response to estradiol stimulus; GO:0032526 response to retinoic acid; GO:0042493 response to drug; GO:0042572 retinol metabolic process
Olfactory receptor 434	Olr434	-1.560	-1.382	0.000	GO:0007186 G-protein coupled receptor protein signaling pathway
Olfactory receptor 1607	Olr1607	-1.578	-1.370	0.043	GO:0007186 G-protein coupled receptor protein signaling pathway; GO:0050911 detection of chemical stimulus involved in sensory perception of smell
Serine peptidase inhibitor, Kunitz type 1	Spint1	-1.611	-1.093	0.011	GO:0001763 morphogenesis of a branching structure; GO:0001892 embryonic placenta development; GO:0030198 extracellular matrix organization; GO:0060670 branching involved in embryonic placenta morphogenesis; GO:0060674 placenta blood vessel development
D site of albumin promoter (albumin D-box) binding protein	Dbp	-1.647	-1.918	0.000	GO:0006355 regulation of transcription, DNA-dependent; GO:0042127 regulation of cell proliferation; GO:0048511 rhythmic process
Solute carrier family 5 (sodium-dependent vitamin transporter), member 6	Slc5a6	-1.650	-1.222	0.021	GO:0006811 ion transport; GO:0006814 sodium ion transport; GO:0015878 biotin transport; GO:0015887 pantothenate transmembrane transport; GO:0055085 transmembrane transport
Multiple inositol polyphosphate histidine phosphatase 1	Mimpp1	-1.658	-1.225	0.010	GO:0048015 phosphoinositide-mediated signaling
Tsukushin	Tsku	-1.682	1.012	0.014	
Solute carrier family 26, member 3	Slc26a3	-1.724	-1.112	0.034	GO:0006820 anion transport; GO:0008272 sulfate transport; GO:0055085 transmembrane transport
Cystathionase (cystathionine gamma-lyase)	Cth	-1.725	-1.172	0.046	GO:0006520 cellular amino acid metabolic process; GO:0006749 glutathione metabolic process; GO:0008285 negative regulation of cell proliferation; GO:0008652 cellular amino acid biosynthetic process; GO:0018272 protein-pyridoxal-5-phosphate linkage via peptidyl-N6-pyridoxal phosphate-L-lysine; GO:0019344 cysteine biosynthetic process; GO:0019346

Peripheral myelin protein 22	Pmp22	-1.955	-1.443	0.042	transsulfuration // traceable author statement; GO:0030308 negative regulation of cell growth; GO:0050667 homocysteine metabolic process; GO:0051289 protein homotetramerization
Hydroxysteroid 11-beta dehydrogenase 2	Hsd11b2	-2.002	1.001	0.041	GO:0007049 cell cycle; GO:0007050 cell cycle arrest; GO:0008285 negative regulation of cell proliferation; GO:0030154 cell differentiation; GO:0032288 myelin assembly; GO:0042552 myelination
Hydroxysteroid (17-beta) dehydrogenase 2	Hsd17b2	-2.530	-1.603	0.040	GO:0001666 response to hypoxia; GO:0002017 regulation of blood volume by renal aldosterone; GO:0006950 response to stress; GO:0007565 female pregnancy; GO:0008152 metabolic process; GO:0008211 glucocorticoid metabolic process; GO:0032094 response to food; GO:0032868 response to insulin stimulus; GO:0042493 response to drug; GO:0048545 response to steroid hormone stimulus
Nuclear receptor subfamily 1, group D, member 1 thyroid hormone receptor alpha	Nr1d1 Thra	-2.844	-2.072	0.018	GO:0006694 steroid biosynthetic process; GO:0032526 response to retinoic acid; GO:0055114 GO:0006355 regulation of transcription, DNA-dependent; GO:0007623 circadian rhythm; GO:0001502 cartilage condensation; GO:0001503 ossification; GO:0001822 kidney development; GO:0001889 liver development; GO:0002155 regulation of thyroid hormone mediated signaling pathway; GO:0006950 response to stress; GO:0007420 brain development; GO:0007611 learning or memory

References

- Kelly D, Campbell JI, King TP, Grant G, Jansson EA, Coutts AG, Pettersson S, Conway S. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol. 2004 Jan;5(1):104-12.
- Xu J, Bjursell MK, Himrod J, Deng S, Carmichael LK, Chiang HC, Hooper LV, Gordon JI. A genomic view of the human-Bacteroides thetaiotaomicron symbiosis. Science. 2003 Mar 28;299(5615):2074-6.
- Wendler WM, Kremmer E, Förster R, Winnacker EL. Identification of pirin, a novel highly conserved nuclear protein. J Biol Chem. 1997 Mar 28;272(13):8482-9.
- Berg, D.J., Davidson, N., Kühn, R., Müller, W., Menon, S., Holland, G., Thompson-Snipes, L., Leach, M.W., Rennick, D. Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4+ Th1-like responses (1996) Journal of Clinical Investigation, 98 (4), 1010-1020.
- All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in biochemistry and molecular biology or related fields are intended to be within the scope of the following claims.

30

CLAIMS

1. A pharmaceutical composition comprising polypeptide HP or a polynucleotide sequence encoding polypeptide HP and a pharmaceutically acceptable excipient, carrier or diluent; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5.
2. The pharmaceutical composition according to claim 1, wherein said polypeptide or polynucleotide sequence is encapsulated.
3. The pharmaceutical composition according to claim 1 or 2, wherein said polypeptide is a recombinant polypeptide.
4. The pharmaceutical composition according to claim 1 or 2, wherein an expression vector comprises said polynucleotide sequence.
5. A process for producing a pharmaceutical composition according to any one of claims 1 to 4, said process comprising admixing said polypeptide or polynucleotide sequence with a pharmaceutically acceptable excipient, carrier or diluent; optionally said polypeptide or polynucleotide sequence is encapsulated in said process; wherein said polypeptide has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6; and wherein said polynucleotide sequence encodes a polypeptide which has at least 75% identity to SEQ ID NO 2, SEQ ID NO 4 or SEQ ID NO 6 and/or wherein said polynucleotide sequence has at least 75% identity to SEQ ID NO 1, SEQ ID NO 3 or SEQ ID NO 5.
6. Use of polypeptide HP or a polynucleotide sequence encoding polypeptide HP in the manufacture of a pharmaceutical composition according to any one of claims 1-4 for the treatment and/or prevention of a disorder, wherein the disorder to be treated and/or prevented is an inflammatory disorder and/or wherein the disorder to be treated is an autoimmune disorder.
7. The use of claim 6, wherein the disorder affects the alimentary canal, a section of the alimentary canal, the liver, liver cells, epithelial cells, epidermal cells, neuronal cells, kidneys, spleen, lungs, heart, the pancreas, and/or pancreatic cells.
8. The use of claim 6, wherein the disorder is selected from the group consisting of inflammatory bowel disorder (IBD), colitis, rheumatoid arthritis, psoriasis, multiple sclerosis, type I diabetes, coeliac disease, atopic dermatitis, rhinitis, irritable bowel syndrome (IBS), ulcerative colitis, pouchitis, functional dyspepsia, atopic diseases, necrotising enterocolitis, non-alcoholic fatty liver disease, gastrointestinal infection, Lupus, nephritis/glomerulonephritis, asthma, COPD, myocarditis and combinations thereof.
9. The use of claim 6, wherein the disorder is an inflammatory bowel disorder (IBD).
10. The use of any one of claims 6-9, wherein the treatment and/or prevention comprises reducing the inflammation of a cell, a tissue or an organ in a subject.

11. The use of claim 10, wherein the cell, tissue or organ is the alimentary canal, a section of the alimentary canal, the liver, liver cells, epithelial cells, epidermal cells, neuronal cells, kidneys, spleen, lungs, heart, the pancreas, and/or pancreatic cells.
12. The use of claim 10 or claim 11, wherein the treatment and/or prevention comprises reducing the inflammation by epithelial cells of the tissue or the organ.
13. The use of any one of claims 6-9, wherein the treatment and/or prevention comprises improving intestine barrier integrity in a subject.
14. The use of any one of claims 6-9, wherein the treatment and/or prevention comprises modifying the bacterial composition in a tissue or organ to provide a beneficial microbiota, preferably wherein the treatment and/or prevention comprises reducing the level of one or more types of lactose fermenting bacteria in a tissue or an organ in a subject and/or reducing the level of one or more types of non-lactose fermenting bacteria in a tissue or an organ in a subject, optionally wherein said tissue or organ is selected from the group consisting of mesenteric lymph nodes, liver, pancreas, spleen, kidneys, heart, lung and combinations thereof.
15. The use of any one of claims 6-9, wherein the treatment and/or prevention comprises maintaining the length of the large intestine and/or small intestine of a subject, such as preventing a reduction in the length of large intestine and/or preventing an increase in the length of the small intestine.
16. The use of any one of claims 6-9, wherein the treatment and/or prevention comprises reducing disruption to the intestine of a subject, such as reducing or preventing disruption to the integrity of the mucosal epithelium and/or reducing or preventing a reduction in the number of goblet cells in the epithelium and/or reducing or preventing the infiltration of immune cells into the lamina propria.
17. The use of any one of claims 6-9, wherein the treatment and/or prevention comprises regulating the expression of one or more pro-inflammatory genes and/or one or more barrier integrity genes in a cell or cells of a subject.
18. The use of any one of claims 6-9, wherein the treatment and/or prevention comprises regulating the expression in a cell or cells of a subject of one or more genes selected from the group consisting of regenerating islet-derived 3 beta gene (Reg3b), resistin-like gamma resistin like beta gene (Retnlg|Retnlb) sucrase-isomaltase (alphaglucohydrolase) gene (Si), defensin alpha 24 gene (Defa24), hydroxysteroid 11-beta dehydrogenase 2 gene (Hsd11b2), hydroxysteroid (17-beta) dehydrogenase 2 gene (Hsd17b2), resistin-Like Molecule-beta (RELMb), and nuclear receptor 1D1 thyroid hormone receptor alpha gene (Nr1d1|Thra), such as decreasing the expression of one or more genes selected from the group consisting of regenerating islet-derived 3 beta gene (Reg3b); resistin-like gamma resistin like beta gene (Retnlg|Retnlb); resistin-Like Molecule-beta (RELMb), sucrase-isomaltase (alpha-glucosidase) gene (Si); and defensin alpha 24 gene (Defa24), or increasing the expression of one or more genes selected from the group consisting of hydroxysteroid 11-beta dehydrogenase 2 gene (Hsd11b2); hydroxysteroid (17-beta) dehydrogenase 2 gene (Hsd17b2); and nuclear receptor 1D1 thyroid hormone receptor alpha gene (Nr1d1|Thra).
19. The use of any one of claims 6-9, wherein the treatment and/or prevention comprises reducing the activation of pro-inflammatory pathways in a cell or cells of a subject.

20. The use of any one of claims 6-9, wherein the treatment and/or prevention comprises reducing the activity and/or expression of NF- κ B in a cell or cells of a subject.
21. The use of any one of claims 6-9, wherein the treatment and/or prevention comprises improving alimentary canal health in a subject.