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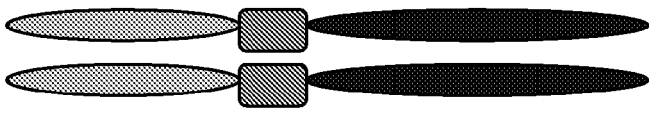

huApo A-1 linker huIgG Fc
(SSShinge-P238S-P331S CH2-CH3)

FIG. 2A

(57) Abstract: Compositions and methods relating to ApoA-1 fusion polypeptides are disclosed. The fusion polypeptides include a first polypeptide segment corresponding to an ApoA-1 polypeptide or ApoA-1 mimetic, and may also include a dimerizing domain such as, e.g., an Fc region, which is typically linked carboxyl-terminal to the first polypeptide segment via a flexible linker. In some embodiments, the fusion polypeptide further includes a second polypeptide segment located carboxyl-terminal to the first polypeptide segment and which confers a second biological activity (e.g., an RNase, paraoxonase, platelet-activating factor acetylhydrolase, cholesterol ester transfer protein, lecithin-cholesterol acyltransferase, or polypeptide that specifically binds to amyloid beta). Also disclosed are dimeric proteins comprising first and second ApoA-1 fusion polypeptides as disclosed herein. The fusion polypeptides and dimeric proteins are useful in methods for therapy.

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# APOA-1 FUSION POLYPEPTIDES AND RELATED COMPOSITIONS AND METHODS

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[1]** This application claims the benefit of U.S. Provisional Application No. 62/215,256, filed September 8, 2015, which is incorporated by reference herein in its entirety.

## REFERENCE TO SEQUENCE LISTING

**[2]** The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII Copy, created on August 31, 2016, is named "TRP_0110PC_20160831_Seq_Listing_ST25" and is 161,132 bytes in size.

## BACKGROUND OF THE INVENTION

Apolipoprotein A-I (ApoA-1) and High Density Lipoprotein (HDL)

**[3]** Cardiovascular disease is the leading cause of mortality in many nations, accounting for approximately 16.7 million deaths each year world-wide. The most common consequences of cardiovascular disease are myocardial infarction and stroke, which have a common underlying etiology of atherosclerosis.

**[4]** Epidemiological studies since the 1970's have shown that low levels of high density lipoprotein (HDL) is associated with increased risk for myocardial infarction. This has led to multiple approaches to new therapies targeting HDL (*see* Kingwell *et al.*, *Nature Reviews Drug Discovery* 13:445-64, 2014) and to a consensus view that the process of reverse cholesterol transport (RCT) is central to beneficial HDL activity rather than simply an increase in HDL without RCT. For example, in clinical trials so far, drugs that increase HDL by inhibition of RCT with inhibitors of cholesterol ester transfer protein (CETP) have not been efficacious. Further, it has more recently been realized that measuring levels of HDL is not sufficient to determine its function in patients because HDL is damaged by oxidation and glycation, including during chemotherapy and in patients with neurodegenerative disorders. *See* Keeney *et al.*, *Proteomics Clin Appl.* 7:109-122, 2013.

**[5]** Apolipoprotein A-1 (ApoA-1) is the principal protein component of HDL. Phillips, *Journal of Lipid Research* 54:2034-2048, 2013. Human ApoA-1 is a 243 amino acid protein, with a

series of eight 22-mer and two 11-mer amphipathic α-helices spanning residues 44-243. Lund-Katz and Phillips, *Subcell Biochem.* 51:183-227, 2010. The helices in the amino-terminal two-thirds of the molecule form a helix bundle structure, whereas the carboxyl-terminal region forms a separate, relatively disorganized domain important for lipid binding. The interaction of the C-terminal segment with lipids induces conformational changes in the ApoA-1 structure, increasing the α-helix content of the molecule and allowing subsequent opening of the N-terminal helix bundle. *See id.* The lipid affinity of ApoA-1 confers detergent-like properties, and it can solubilize phospholipids to form discoidal HDL particles containing a segment of phospholipid bilayer and two ApoA-1 molecules arranged in an anti-parallel, double-belt conformation around the edge of the disc. Phillips, *supra.* The conformational adaptability ApoA-1 also confers stability to HDL particles, including discoidal particles of different sizes as well as spherical HDL particles. *See id.* These characteristics allow ApoA-1 to partner with ABCA1 in mediating efflux of cellular phospholipid and cholesterol and the production of stable HDL particles. *See* Phillips, *supra*; Lund-Katz and Phillips, *supra.*

**[6]** Due to its important role in HDL particle formation and function, ApoA-1 has become the focus for several HDL-targeted therapeutic strategies. Drugs including niacin and fibrates that increase synthesis of ApoA-1, however, also decrease the concentration of VLDL and are thus not specific for HDL. Clinical trials of niacin were halted due to lack of efficacy, whereas fibrates that activate peroxisome proliferator activated receptors (PPARs) were found to cause a 10% reduction in major cardiovascular events ($p<0.05$) and a 13% reduction in coronary events ($p<0.0001$) in a meta-analysis. *See* Jun *et al.*, *Lancet* 375:1875, 2010. Because more effective therapies are needed, there are several other orally active drugs that increase ApoA-1 in preclinical development. *See* Kingwell *et al.*, *supra.*

**[7]** An alternative approach to increasing ApoA-1 is by direct injection of the purified protein. *See, e.g.*, Kingwell *et al.*, *Circulation* 128:1112, 2013. ApoA-1 has been purified from human plasma (reconstituted HDL) and tested in clinical trials. Recombinant ApoA-1 has also been expressed in both bacterial and mammalian expression systems and tested in clinical trials. These studies have shown that infusion of ApoA-1, reconstituted with phospholipids into pre-β HDL, causes reduction of plaque volume and improvement in plaque morphology as measured by intravascular ultrasound (IVUS) after small (47-60 patients) clinical trials. While promising, use of natural or recombinant ApoA-1 has several limitations, including a requirement for weekly administration due to a short ApoA-1 half-life and a high cost of manufacturing.

**[8]** Recombinant ApoA-1 Milano, a highly active ApoA-1 mutant, was expressed in bacterial cells and tested in clinical trials in patients with acute coronary syndromes (*see* Nissen *et al.*, *JAMA* 290:2292, 2003), where reduction in plaque volume was seen. While this study is considered the first to directly test and confirm the HDL hypothesis, ApoA-1 produced in bacterial systems has

not progressed due to low expression levels and high manufacturing costs. Recombinant ApoA-1 produced in mammalian cells has progressed further in clinical trials, including recently completed phase II studies. CER-001, in development by Cerenis Therapeutics, is a recombinant ApoA-1 produced by mammalian cells and formulated with specific lipids to form pre-β-like HDL particles. According to Cerenis, CER-001 met its primary end point of a reduction in carotid plaque volume measured by MRI in patients with familial hypercholesterolaemia in the MODE trial (NCT01412034). In the CHI-SQUARE trial (NCT01201837), Cerenis announced that CER-001 reduced plaque volume versus baseline in patients with acute coronary syndrome, but the reduction was not significant versus placebo.

**[9]** In another study in macaques, ApoA-1 Milano, reconstituted with lipids (POPC), was infused at relatively high doses (30, 100, and 300 mg/kg) given every second day for 21 infusions. Kempen *et al.*, *J. Lipid. Res.* 54:2341-2353, 2013. Drug infusion quickly decreased the endogenous cholesterol esterification rate, increased the formation of large ApoE-rich particles due to lack of LCAT activation, and caused a large increase in free cholesterol due to sustained stimulation of ABCA1-mediated efflux. *See id.* These results show that infusion of large amounts of reconstituted ApoA-1 Milano disrupt HDL metabolism by enhancing cholesterol efflux without the ability to process it through the normal metabolic pathways.

**[10]** While the prospects for HDL infusion therapy are very promising, there is a need for improved recombinant ApoA-1 molecules that overcome some of the limitations of current approaches. Several recombinant ApoA-1 fusion proteins have been produced, including ApoA-1 produced in bacteria with a His tag to simplify purification. *See, e.g.*, Prieto *et al.*, *Protein J.* 31:681-688, 2012; Ryan *et al.*, *Protein Expr. Purif.* 27:98-103, 2003. In another example, IFNα was attached to the amino terminus of ApoA-1 through a 3aa (Gly Ala Pro) linker. *See* Fioravanti *et al.*, *J. Immunol.* 188:3988-3992, 2012. The linker in this construct was created by the choice of restriction enzymes, and the fusion protein was tested by adenovirus delivery to target to the liver and reduce the toxicity of IFNα therapy. ApoA-1 has also been fused with an Fc domain (ApoA-1-Ig) and is available commercially from Creative Biomart (cat. No. APOA-1-33H) and from Life Technologies (Cat # 10686-HO2H-5). However, this ApoA-1-Ig molecule has very low functional activity (*see* Example 1).

**[11]** Additional recombinant ApoA-1 fusion proteins include anti-CD20 scFv-ApoA-1 (Crosby *et al.*, *Biochem. Cell Biol.* 10:1139/bcb, 2015), IL-15-ApoA-1 (Ochoa *et al.*, *Cancer Res.* 73:139-149, 2013), and a trimeric ApoA-1 fusion protein made by the addition of the trimerization domain of human tetranectin (Graversen *et al.*, *J. Cardiovascular Pharmacol.* 51:170-77, 2008). In these examples, the fusion was at the N-terminus of ApoA-1.

**[12]** The trimeric tetranectin-ApoA-1 (TN-ApoA-1) was effective in reverse cholesterol efflux and its half-life in mice was increased to 12 hours versus three hours for monomeric ApoA-1. *See* Gaversen *et al.*, *supra*. In an aggressive model of atherosclerosis (LDLR -/- mice fed a high-fat diet), trimeric TN-ApoA-1 slowed progression of lesions in the aortic roots. *See id.* Recent studies in nonhuman primates, however, showed that multiple infusions of lipidated TN-ApoA-1 were not well tolerated and resulted in high immunogenicity and lipid accumulation. *See* Regeness-Lechner *et al.*, *Toxilogical Sciences* 150:378-89, 2016. The trimer fusion protein was complexed with phospholipids and injected at concentrations of 100 mg/kg and 400 mg/kg every four days for three weeks, followed by a six week recovery period. After multiple infusions of lipidated TN-ApoA-1, clinical condition deteriorated and was accompanied by changes indicative of a progressive inflammatory response, increased levels of cytokines, C-reactive protein and vascular/perivascular infiltrates in multiple tissues. Rapid formation of antidrug antibodies occurred in all animals receiving lipidated TN-ApoA-1. *See id.* The accumulation of trimeric TN-ApoA-1 in tissues of the treated animals resembles fibril formation and deposition of ApoA-1 in patients who have mutations near the N-terminus. *See* Mizuguchi *et al.*, *J. Biol. Chem.* 290:20947-20959, 2015; Das *et al.*, *J. Mol. Biol.* 2015.10.029; Obici *et al.*, *Amyloid* 13:191-205, 2006.

**[13]** Current forms of ApoA-1 in clinical development require formulation with specific lipids into preβ-like HDL particles prior to infusion, because the half-life of ApoA-1 in the absence of lipids is very short. *See* Nanjee *et al*, *Arterioscler Thromb Vasc Biol* 16:1203-1214, 1996 (showing that lipid-free ApoA-1 has a half-life of only 2-2.3 hours after either bolus or slow infusion in humans). After lipid formulation, half-life increases to about 48 hours, so frequent (weekly) administration is still required.

**[14]** ApoA-1 therapy has also shown significant benefit in improving insulin sensitivity and glucose uptake (*see* Drew *et al.*, *Nature Reviews Endocrinology* 8:237, 2012), and may be useful in patients with diabetes and with NASH (non-alcoholic steatohepatitis). In addition, ApoA-1 binds amyloid-beta and prevents neurotoxicity in cultured hippocampal neuronal cells. *See* Koldamova *et al.*, *Biochemistry* 40:3553, 2001; Paula-Lima *et al.*, *Int. J. Biochem. Cell Biol.* 41:1361, 2009. Further, ApoA-1 polymorphisms are linked to risk for Alzheimer's disease and ApoA-1 is found at decreased levels in patients with neurodegenerative disorders. *See* Keeney *et al.*, *Proteomics Clin. Appl.* 7: 109-122, 2013).

**[15]** Efficacy of ApoA-1 therapy has also been demonstrated in animal models of cancer. One study examined the effect of ApoA-1 infusion on growth of tumors in mice. *See* Zamanian-Daryoush *et al.*, *J. Biol. Chem.* 288:21237-21252, 2013. Zamanian-Daryoush *et al.* found that ApoA-1 potently suppresses tumor growth and metastasis in multiple syngeneic tumor models, including B16F10L malignant melanoma and Lewis Lung carcinoma. The effect of ApoA-1 was due to

modulation of the immune response. Recruitment and expansion of myeloid-derived suppressor cells (MDSC) in the tumors was inhibited. There was also inhibition of tumor angiogenesis and the matrix-degrading protease MMP-9. In contrast, ApoA-1 therapy increased CD11b macrophages and increased amounts of IFNγ, IL-12b, and CXCL10, markers of a Th1 response supporting T cell activation. The authors showed that T cells were required for the potent suppressive effect of ApoA-1 on tumor growth, and ApoA-1 therapy caused a specific increase in $CD8^+$ T cells in the tumors. *See id.* While the results of Zamanian-Daryoush *et al.* are promising, the study used high doses of lipid-free ApoA-1 to achieve the observed effects (15 mg every second day per mouse), *see id.*, which was likely required because of the short half-life of ApoA-1.

**[16]** Another cancer study showed that ApoA-1 and mimetic peptides (L-4F, D-4F, L-5F) inhibit tumor development in a murine model of ovarian carcinoma. *See* Su *et al.*, *Proc. Natl. Acad. Sci. USA* 107:19997-20002, 2010. Su *et al.* found that ApoA-1 overexpression in transgenic mice, or peptide mimetic administration, reduced stimulatory phospholipids, implicating an additional mechanism for inhibition of tumor growth. *See id.*

**[17]** Studies have also suggested a role for ApoA-1 in the pathogenesis of multiple sclerosis (MS). In particular, ApoA-1 expression was shown to be lower in MS patients compared to healthy controls, and primary progressive MS patients had less plasma ApoA-1 than patients with other forms of MS. *See* Meyers *et al.*, *J. Neuroimmunol.* 277:176-185, 2014. Using experimental allergic encephalomyelitis (EAE) as a model for MS, mice deficient in ApoA-1 exhibited worse clinical disease and more neurodegeneration compared to wild-type animals. The authors suggest that agents that increase ApoA-1 levels are possible therapies for MS. *See id.* Another MS study found that the ApoA-1 promoter polymorphism A-allele, associated with elevated ApoA-1 levels, is correlated with improved cognitive performance in patients with MS; A-allele carriers displayed overall superior cognitive performance and had a three-fold decreased overall risk of cognitive impairment. *See* Koutsis *et al.*, *Mult. Scler.* 15:174-179, 2009.

Peptide Mimetics

**[18]** ApoA-1 mimetic peptides have shown efficacy in a number of animal models of disease and have properties that make them attractive as potential therapeutic agents. *See, e.g.*, Reddy *et al.*, *Curr. Opin. Lipidol.* 25:304-308, 2014 and White *et al.*, *J. Lipid. Res.* 55:2007-2021, 2014. Peptide 4F has been tested in high risk patients with coronary artery disease. Several ApoA-1 mimetic peptides that are resistant to oxidation have been described in the past several years. While these α-helical peptides show activity in animal models, they require daily dosing because of their short half-life. In addition, toxicity, including muscle toxicity and hypertriglyceridemia, have been seen in peptide-treated animals (these toxicities have been seen in mice treated with ApoA-1). Advances to reduce toxicity by sequence design and to reduce cost of peptide production were

described. *See, e.g.*, Bielicki, *Curr. Opin. Lipidol.* 27:40-46, 2016. Another approach has been to synthesize D-peptides, including the highly studied D-4F peptide. These have a longer half-life and can be given orally, but the high cost of manufacturing and accumulation of D-peptides in tissues may be preventing these peptides from moving past initial clinical testing.

RNase

**[19]** RNase has been studied as a therapy for cancer and autoimmune disease. For cancer therapy, both natural (onconase, frog RNase), and recombinant human RNase1 resistant to inhibition by cytoplasmic inhibitor (*see* US Patent No. 8,569,457) have been reported. In addition, targeting of RNase to tumor cells by conjugation of cytotoxic RNase (onconase) to anti-tumor antibodies has been reported. *See* Lui *et al.*, *Mol. Cancer* 13:1186, 2014; Newton *et al.*, *Blood* 97:528-535, 2001.

**[20]** RNase therapy has also been studied in a mouse model of cardiovascular disease. *See* Simsekyilmaz *et al.*, *Circulation* 129:598-606, 2014. They and others show that extracellular RNA accumulates at sites of vascular injury and that extracellular RNA causes production of inflammatory cytokines. *See* Fischer *et al.*, *Thromb. Haemost.* 108:730-741, 2012. RNase therapy reduced neointima formation in a mouse model of accelerated cardiovascular disease, reduced plaque macrophage content, and inhibited leukocyte recruitment to injured carotid arteries *in vivo*. *See* Simsekyilmaz *et al.*, *supra*.

**[21]** RNase therapy has also been studied in models of acute stroke, where it was found to reduce infarction size. *See* Walberer *et al.*, *Curr. Neurovasc. Res.* 6:12-19, 2009. Thus systemic treatment with RNase 1 rescued mice from arterial thrombotic occlusion to limit cerebral edema and to serve as a potent anti-inflammatory regimen *in vivo*. In these RNase therapy studies, the RNase was given by continuous infusion using osmotic minipumps implanted subcutaneously because the half-life of RNase 1 is very short.

**[22]** RNase therapy has also been studied in a mouse model of systemic lupus erythematosus (SLE). *See* Sun *et al.*, *J. Immunol.* 190:2536-2543, 2013. Overexpression of TLR7, an RNA sensor, causes a lupus-like disease with autoantibodies, kidney disease, and early mortality. Crossing these mice with mice that overexpress RNase A as a transgene resulted in progeny with increased survival, reduced lymphocyte activation, reduced kidney deposits of IgG and C3, and reduced hepatic inflammation and necrosis.

**[23]** Extracellular single stranded viral RNA caused widespread neurodegeneration after intrathecal administration to mice, and the neuronal damage was mediated by TLR7. *See* Lehmann *et al.*, *J. Immunol.* 189: 1448-58, 2012.

**[24]** RNase-Ig wherein human RNase 1 is fused to a mutated human IgG1 Fc domain comprising p238s and p331s mutations (*see* US Patent No. 8,937,157) is in clinical development by Resolve Therapeutics in patients with systemic lupus erythematosus (SLE).

Paraoxonase

**[25]** Human Paraoxonase 1 (PON1) is a lipolactonase with efficient esterase activity and capable of hydrolyzing organophosphates. PON1 prevents LDL and cell membrane oxidation and is considered to be atheroprotective. PON1 is exclusively associated with HDL and contributes to the antioxidative function of HDL. *See, e.g.*, Mackness *et al.*, *Gene* 567:12-21, 2015. Reductions in HDL-PON1 activity are present in a wide variety of inflammatory diseases where loss of PON1 activity leads to dysfunctional HDL which can promote inflammation and atherosclerosis. *See, e.g.*, Eren *et al.*, *Cholesterol.* 792090 doi 10.1155/2013/792090, 2013. PON1 activity is also decreased in patients with Alzheimer's disease and other dementias, suggesting a possible neuroprotective role of PON1. *See* Menini *et al.*, *Redox Rep.* 19:49-58, 2014.

**[26]** PON1 has shown protective activity in multiple animal models. Overexpression of human PON1 inhibited the development of atherosclerosis in mice with combined leptin and LDL receptor deficiency, a model of metabolic syndrome. *See* Mackness *et al.*, *Arterioscler. Thromb. Vasc. Biol.* 26:1545-50, 2006.

**[27]** In another study, injection of recombinant PON1 to mice prior to STZ-induced diabetes resulted in reduced incidence of diabetes and higher serum insulin levels. Addition of HDL simultaneously with PON1 had an additive effect on insulin secretion. *See* Koren-Gluser *et al.*, *Atherosclerosis* 219:510-518, 2011.

**[28]** In another study, a PON1 fusion protein containing a protein transduction domain (PTD) was used to transduce PON1 into cells and tissues. PON1 transduction protected microglial cells *in vitro* from oxidative stress-induced inflammatory responses and protected against dopaminergic neuronal cell death in a Parkinsons disease model. *See* Kim *et al.*, *Biomaterials* 64:45-56, 2015.

**[29]** In another study, recombinant PON1 was administered to mice where there was a significant reduction in cholesterol mass and an inhibition in the cholesterol biosynthesis rate, effects that could probably lead to attenuation of atherosclerosis. *See* Rosenblat *et al.*, *Biofactors* 37:462-467, 2011.

**[30]** In another study, mice were given recombinant adenovirus PON1 or PON3 and either was shown to protect against CCl(4)-induced liver injury. Overexpression of either human PON1 or human PON3 reduced hepatic oxidative stress and strengthened the antioxidant capabilities in the liver. *See* Peng *et al.*, *Toxicol. Lett.* 193:159-166, 2010.

**[31]** In another study, PON1 was fused to the C-terminus of an Fc domain, and expressed as a bispecific molecule using an antibody to human insulin receptor (HIR). This molecule, termed HIRMAb-PON1, was stable after expression in CHO cells, and was shown in Rhesus monkeys to have a high blood brain barrier permeation but was rapidly cleared by the liver. *See* Boado *et al.*, *Biotechnol. Bioeng.* 108: 186-196, 2011.

Platelet-activating factor acetylhydrolase

**[32]** Platelet-activating factor acetylhydrolase (PAF-AH) is an LDL and HDL-associated enzyme that hydrolyzes short chain acyl groups of phospholipids such as platelet-activating factor and oxidized phospholipids to reduce their inflammatory properties. *See* Watson *et al.*, *J. Clin. Invest.* 95:774-782, 1995; Stafforini, *Cardiovasc. Drugs Ther.* 23:73-83, 2009. Therapy with PAF-AH through adenovirus-mediated gene delivery has been reported to ameliorate proteinuria and glomerulosclerosis in a rat model. *See* Iso-O *et al.*, *Molecular Therapy* 13:118-126, 2006. PAF-AH also enhanced liver recovery after paracetamol intoxication in the rat, and PAF is associated with liver toxicity from high doses of acetaminophen. *See* Grypioti *et al.*, *Dig. Dis. Sci.* 52:2580-2590, 2007; Grypioti *et al.*, *Dig. Dis. Sci.* 53:1054-1062, 2008. A mutation in PAF-AH that causes a loss of function is present in 4% of Japanese, and PAF-AH was found to be an independent risk factor for cardiovascular disease and stroke in these individuals. *See* Blankenberg *et al.*, *J. Lipid Res.* 44:1381-1386, 2003. Recombinant PAF-AH was tested in phase III clinical trials in patients with acute respiratory distress syndrome (ARDS) and in patients with sepsis. *See* Karabina *et al.*, *Biochim. Biophys. Acta* 1761:1351-1358, 2006.

Cholesteryl ester transfer protein

**[33]** Cholesteryl ester transfer protein (CETP) transports cholesteryl ester from high-density lipoproteins (HDL) to low density and very low density lipoproteins (LDL and VLDL). Many CETP inhibitors have been developed and tested in clinical trials. Torecetrapib was the first CETP inhibitor to advance to late stage clinical trials, and showed a significant effect on plasma lipoprotein levels, raising antiatherogenic HDL cholesterol levels while lowering proatherogenic LDL cholesterol levels. Torecetrapib binds deeply within CETP and shifts the bound cholesteryl ester in the N-terminal pocket of the hydrophobic tunnel and displaces phospholipid from the pocket. *See* Liu *et al.*, *J. Biol. Chem.* 287:37321-37329, 2012. Initial hopes that CETP inhibitors would be useful for therapy of cardiovascular disease have not been fulfilled; four inhibitors have reached late stage clinical trials but have failed to show a reduction in cardiovascular events. *See* Kosmas *et al.*, *Clinical Medical Insights: Cardiology* 2016: 10 37-42 doi: 10.4137/CMC.S32667).

**[34]** Alternative views of CETP inhibitors and cardiovascular disease have emerged. *See, e.g.*, Miller, *F100Research* 3:124, 2014. There is mounting evidence for a protective role of CETP.

For example, multiple studies in man now show that cardiovascular disease is related inversely to CETP levels. In addition, CETP alleles that have reduced hepatic secretion are associated with increased risk of myocardial infarction. *See* Miller, *supra*. The original idea, that CETP inhibitors increase HDL cholesterol levels and would therefore be beneficial in reducing cardiovascular disease, may not be correct. It is likely that HDL cholesterol is beneficial because of its lipid transport function, and the CETP-mediated transfer of cholesteryl ester from HDL to LDL and VLDL is an important component of this function.

## SUMMARY OF THE INVENTION

**[35]** In one aspect, the present invention provides a fusion polypeptide comprising, from an amino-terminal position to a carboxyl-terminal position, ApoA1-L1-D, where ApoA1 is a first polypeptide segment having cholesterol efflux activity and which is selected from (i) a polypeptide comprising an amino acid sequence having at least 90% or at least 95% identity with amino acid residues 19-267, 25-267, or 1-267 of SEQ ID NO:2 and (ii) an ApoA-1 mimetic; L1 is a first polypeptide linker; and D is a dimerizing domain. In certain embodiments, L1 comprises at least two amino acid residues, at least three amino acid residues, or at least 16 amino acid residues. For example, in particular variations, L1 consists of from two to 60 amino acid residues, from three to 60 amino acid residues, from five to 40 amino acid residues, from 15 to 40 amino acid residues, or from 16 to 36 amino acid residues. In a more specific variation, L1 consists of 16 amino acid residues, 21 amino acid residues, 26 amino acid residues, 31 amino acid residues, or 36 amino acid residues; in some such embodiments, L1 has the amino acid sequence shown in residues 268-283 of SEQ ID NO:22, residues 268-288 of SEQ ID NO:26, residues 268-293 of SEQ ID NO:2, SEQ ID NO:54, or residues 268-303 of SEQ ID NO:24. In certain embodiments, the first polypeptide segment comprises the amino acid sequence shown in residues 19-267 or 25-267 of SEQ ID NO:2.

**[36]** In some embodiments of a fusion polypeptide as above, D is an immunoglobulin heavy chain constant region such as, for example, an immunoglobulin Fc region. In certain embodiments where the dimerizing domain is an immunoglobulin Fc region, the Fc region is a human Fc region such as, *e.g.*, a human Fc variant comprising one or more amino acid substitutions relative to the wild-type human sequence. Particularly suitable Fc regions include human γ1 and γ3 Fc regions. In some variations, the Fc region is a human γ1 Fc variant in which Eu residue C220 is replaced by serine; in some such embodiments Eu residues C226 and C229 are each replaced by serine, and/or Eu residue P238 is replaced by serine. In further variations comprising an Fc region as above, the Fc region is a human γ1 Fc variant in which Eu residue P331 is replaced by serine. Fc variants may include an amino acid substitution that reduces glycosylation relative to the wild-type human sequence; in some such embodiments, Eu residue N297 is replaced with another amino acid.

In further variations comprising an Fc region as above, the Fc region is an Fc variant comprising an amino acid substitution that increases or reduces binding affinity for an Fc receptor (*e.g.*, an amino acid substitution that increases or reduces binding affinity for at least one of FcγRI, FcγRII, and FcγRIII). In certain embodiments, an Fc variant includes an amino acid substitution that increases or reduces binding affinity for the neonatal Fc receptor (FcRn). Suitable Fc regions include (i) an Fc region comprising the amino acid sequence shown in residues 294-525 or 294-524 of SEQ ID NO:2 and (ii) an Fc region comprising the amino acid sequence shown in residues 294-525 or 294-524 of SEQ ID NO:13.

**[37]** In certain embodiments of a fusion polypeptide as above, the fusion polypeptide comprises an amino acid sequence having at least 90% or at least 95% identity with (i) residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:2, (ii) residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:13, (iii) residues 19-501, 19-500, 25-501, or 25-501 of SEQ ID NO:20, (iv) residues 19-515, 19-514, 25-515, or 25-514 of SEQ ID NO:22, (v) residues 19-520, 19-519, 25-520, or 25-519 of SEQ ID NO:26, or (vi) residues 19-535, 19-534, 25-535, or 25-534 of SEQ ID NO:24. In more specific variations, the fusion polypeptide comprises the amino acid sequence shown in (i) residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:2, (ii) residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:13, (iii) residues 19-501, 19-500, 25-501, or 25-501 of SEQ ID NO:20, (iv) residues 19-515, 19-514, 25-515, or 25-514 of SEQ ID NO:22, (v) residues 19-520, 19-519, 25-520, or 25-519 of SEQ ID NO:26, or (vi) residues 19-535, 19-534, 25-535, or 25-534 of SEQ ID NO:24.

**[38]** In some embodiments of a fusion polypeptide as above, the fusion polypeptide further includes a second polypeptide segment located carboxyl-terminal to the dimerizing domain. In particular variations, the second polypeptide segment is an RNase, a paraoxonase, a platelet-activating factor acetylhydrolase (PAF-AH), a cholesterol ester transfer protein (CETP), a lecithin-cholesterol acyltransferase (LCAT), or a polypeptide that specifically binds to amyloid beta (Aβ) such as, *e.g.*, an Aβ-specific scFv. A fusion polypeptide comprising a second polypeptide segment as above may be represented by the formula ApoA1-L1-D-L2-P (from an amino-terminal position to a carboxyl-terminal position), where ApoA1, L1, and D are each defined as above, where L2 is a second polypeptide linker and is optionally present, and where P is the second polypeptide segment. In some embodiments of a fusion polypeptide where L2 is present, L2 has the amino acid sequence shown in residues 526-541 of SEQ ID NO:4.

**[39]** In another aspect, the present invention provides a fusion polypeptide comprising a first polypeptide segment having cholesterol efflux activity and which is selected from (i) a polypeptide comprising an amino acid sequence having at least 90% or at least 95% identity with amino acid residues 19-267 or 25-267 of SEQ ID NO:2 and (ii) an ApoA-1 mimetic, and a second polypeptide segment located carboxyl-terminal to the first polypeptide segment, where the second

polypeptide segment is selected from an RNase, a paraoxonase, a platelet-activating factor acetylhydrolase (PAF-AH), a cholesterol ester transfer protein (CETP), a lecithin-cholesterol acyltransferase (LCAT), and a polypeptide that specifically binds to amyloid beta such as, *e.g.*, an Aβ-specific scFv. In some embodiments, the first polypeptide segment has the amino acid sequence shown in residues 19-267 or 25-267 of SEQ ID NO:2. In some variations, the fusion polypeptide further includes a linker polypeptide located carboxyl-terminal to the first polypeptide segment and amino-terminal to the second polypeptide segment. In some embodiments, the fusion polypeptide further includes a dimerizing domain.

**[40]** In some embodiments of a fusion polypeptide as above comprising an RNase as a second polypeptide segment, the RNase is human RNAse 1 or a functional variant or fragment thereof. In certain embodiments, the RNase has at least 90% or at least 95% identity with amino acid residues 542-675 of SEQ ID NO:4. In a specific variation, the RNase has the amino acid sequence shown in residues 542-675 of SEQ ID NO:4. In particular embodiments of a fusion polypeptide comprising an RNase and having the formula ApoA1-L1-D-L2-P as above, the fusion polypeptide comprises an amino acid sequence having at least 90% or at least 95% identity with (i) residues 19-675 or 25-675 of SEQ ID NO:4 or (ii) residues 19-675 or 25-675 of SEQ ID NO:14; in some such embodiments, the fusion polypeptide comprises the amino acid sequence shown in (i) residues 19-675or 25-675 of SEQ ID NO:4 or (ii) residues 19-675 or 25-675 of SEQ ID NO:14.

**[41]** In some embodiments of a fusion polypeptide as above comprising a paraoxonase as a second polypeptide segment, the paraoxonase is human paraoxonase 1 (PON1) or a functional variant thereof. In certain embodiments, the paraoxonase has at least 90% or at least 95% identity with amino acid residues 16-355 of SEQ ID NO:12, amino acid residues 16-355 of SEQ ID NO:42, or amino acid residues 16-355 of SEQ ID NO:44. In specific variations, the paraoxonase comprises the amino acid sequence shown in residues 16-355 of SEQ ID NO:12, residues 16-355 of SEQ ID NO:42, or residues 16-355 of SEQ ID NO:44. In particular embodiments of a fusion polypeptide comprising an paraoxonase and having the formula ApoA1-L1-D-L2-P as above, the fusion polypeptide comprises an amino acid sequence having at least 90% or at least 95% identity with (i) residues 19-883 or 25-883 of SEQ ID NO:28, (ii) residues 19-873 or 25-873 of SEQ ID NO:38, (iii) residues 19-883 or 25-883 of SEQ ID NO:46, or (iv) residues 19-883 or 25-883 of SEQ ID NO:48; in some such embodiments, the fusion polypeptide comprises the amino acid sequence shown in (i) residues 19-883 or 25-883 of SEQ ID NO:28, (ii) residues 19-873 or 25-873 of SEQ ID NO:38, (iii) residues 19-883 or 25-883 of SEQ ID NO:46, or (iv) residues 19-883 or 25-883 of SEQ ID NO:48.

**[42]** In some embodiments of a fusion polypeptide as above comprising a platelet-activating factor acetylhydrolase (PAF-AH) as a second polypeptide segment, the platelet-activating factor acetylhydrolase is a human PAF-AH or a functional variant thereof. In certain embodiments,

the platelet-activating factor acetylhydrolase has at least 90% or at least 95% identity with amino acid residues 22-441 of SEQ ID NO:32. In a specific variation, the paraoxonase comprises the amino acid sequence shown in residues 22-441 of SEQ ID NO:32. In particular embodiments of a fusion polypeptide comprising an platelet-activating factor acetylhydrolase and having the formula ApoA1-L1-D-L2-P as above, the fusion polypeptide comprises an amino acid sequence having at least 90% or at least 95% identity with residues 19-963 or 25-963 of SEQ ID NO:34; in some such embodiments, the fusion polypeptide comprises the amino acid sequence shown in residues 19-963 or 25-963 of SEQ ID NO:34.

**[43]** In some embodiments of a fusion polypeptide as above comprising a cholesterol ester transfer protein (CETP) as a second polypeptide segment, the cholesterol ester transfer protein is human CETP or a functional variant thereof. In certain embodiments, the cholesterol ester transfer protein has at least 90% or at least 95% identity with amino acid residues 18-493 of SEQ ID NO:30. In a specific variation, the cholesterol ester transfer protein comprises the amino acid sequence shown in residues 18-493 of SEQ ID NO:30. In particular embodiments of a fusion polypeptide comprising an platelet-activating factor acetylhydrolase and having the formula ApoA1-L1-D-L2-P as above, the fusion polypeptide comprises an amino acid sequence having at least 90% or at least 95% identity with residues 19-1019 or 25-1019 of SEQ ID NO:40; in some such embodiments, the fusion polypeptide comprises the amino acid sequence shown in residues 19-1019 or 25-1019 of SEQ ID NO:40.

**[44]** In certain embodiments of a fusion polypeptide as above, the fusion polypeptide is linked to a myeloperoxidase (MPO) inhibitor.

**[45]** In another aspect, the present invention provides a dimeric protein comprising a first fusion polypeptide and a second fusion polypeptide, where each of said first and second fusion polypeptides is a fusion polypeptide comprising a dimerizing domain, as described above.

**[46]** In another aspect, the present invention provides a polynucleotide encoding a fusion polypeptide as described above.

**[47]** In still another aspect, the present invention provides an expression vector comprising the following operably linked elements: a transcription promoter, a DNA segment encoding a fusion polypeptide as described above, and a transcription terminator. Also provided is a cultured cell into which has been introduced an expression vector as above, wherein the cell expresses the DNA segment.

**[48]** In another aspect, the present invention provides a method of making a fusion polypeptide. The method generally includes culturing a cell into which has been introduced an

expression vector as described above, where the cell expresses the DNA segment and the encoded fusion polypeptide is produced, and recovering the fusion polypeptide.

**[49]** In yet another aspect, the present invention provides a method of making a dimeric protein. The method generally includes culturing a cell into which has been introduced an expression vector as described above, where the cell expresses the DNA segment and the encoded fusion polypeptide is produced as a dimeric protein, and recovering the dimeric protein.

**[50]** In another aspect, the present invention provides a composition comprising a fusion polypeptide as described above and a pharmaceutically acceptable carrier.

**[51]** In another aspect, the present invention provides a composition comprising a dimeric protein as described above and a pharmaceutically acceptable carrier.

**[52]** In still another aspect, the present invention provides a method for treating a cardiovascular disease characterized by atherosclerosis. The method generally includes administering to a subject having the cardiovascular disease an effective amount of a fusion polypeptide or dimeric fusion protein as described above. In some embodiments, the cardiovascular disease is selected from the group consisting of coronary heart disease and stroke. In certain variations, the coronary heart disease is characterized by acute coronary syndrome.

**[53]** In another aspect, the present invention provides a method for treating a neurodegenerative disease. The method generally includes administering to a subject having the neurodegenerative disease an effective amount of a fusion polypeptide or dimeric fusion protein as described above. In some embodiments, the neurodegenerative disease is selected from the group consisting of Alzheimer's disease and multiple sclerosis. In certain embodiments, the neurodegenerative disease is characterized by dementia; in some such variations, the neurodegenerative disease is Alzheimer's disease.

**[54]** In another aspect, the present invention provides a method for treating a disease characterized by amyloid deposit. The method generally includes administering to a subject having the disease characterized by amyloid deposit an effective amount of a fusion polypeptide or dimeric fusion protein as described above. In some embodiments, the disease is Alzheimer's disease.

**[55]** In another aspect, the present invention provides a method for treating an autoimmune disease. The method generally includes administering to a subject having the autoimmune disease an effective amount of a fusion polypeptide or dimeric fusion protein as described above. In some embodiments, the autoimmune disease is selected from the group consisting of rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and type 1 diabetes.

**[56]** In yet another aspect, the present invention provides a method for treating an inflammatory disease. The method generally includes administering to a subject having the inflammatory disease an effective amount of a fusion polypeptide or dimeric fusion protein as described above. In some embodiments, the inflammatory disease is selected from the group consisting of rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, type 1 diabetes, type 2 diabetes, obesity, non-alcoholic steatohepatitis, coronary heart disease, and stroke. In other embodiments, the inflammatory disease is an inflammatory lung disease such as, for example, asthma, chronic obstructive pulmonary disease (COPD), bronchiectasis, idiopathic pulmonary fibrosis, hyperoxia, hypoxia, or acute respiratory distress syndrome.

**[57]** In still another aspect, the present invention provides a method for treating an infectious disease. The method generally includes administering to a subject having the infectious disease an effective amount of a fusion polypeptide or dimeric fusion protein as described above. In certain embodiments, the infectious disease is characterized by a bacterial infection; in some such embodiments, the bacterial infection is a *Pseudomonas aeruginosa* infection.

**[58]** In another aspect, the present invention provides a method for treating nephrotic syndrome (NS). The method generally includes administering to a subject having nephrotic syndrome an effective amount of a fusion polypeptide or dimeric fusion protein as described above. In specific variations, the subject's nephrotic syndrome is associated with a disease selected from the group consisting of a primary kidney disease (*e.g.*, minimal-change nephropathy, focal glomerulosclerosis, membranous nephropathy, or IgA nephropathy), amyloidosis, systemic lupus erythematosus, type 1 diabetes, and type 2 diabetes.

**[59]** In yet another aspect, the present invention provides a method for treating exposure to sulfur mustard gas or to an organophosphate. The method generally includes administering to a subject exposed to the sulfur mustard gas or to the organophosphate an effective amount of a fusion polypeptide or dimeric fusion protein as described above.

**[60]** In still another aspect, the present invention provides a method for treating cancer. The method generally includes administering to a subject having cancer an effective amount of a fusion polypeptide or dimeric fusion protein as described above. In some embodiments, the cancer is selected from the group consisting of malignant melanoma, renal cell carcinoma, non-small cell lung cancer, bladder cancer, and head and neck cancer. In certain variations, the cancer treatment is a combination therapy. In some combination therapy embodiments, the combination therapy includes a non-ApoA1-mediated immunomodulatory therapy such as, *e.g.*, an immunomodulatory therapy comprising an anti-PD-1/PD-L1 therapy, an anti-CTLA-4 therapy, or both. In other combination therapy embodiments, the combination therapy includes radiation therapy or chemotherapy. In some combination therapy embodiments, the combination therapy includes a targeted therapy; in some such

embodiments, the targeted therapy includes (i) a therapeutic monoclonal antibody targeting a specific cell-surface or extracellular antigen (*e.g.*, VEGF, EGFR, CTLA-4, PD-1, or PD-L1) or (ii) a small molecule targeting an intracellular protein such as, for example, an intracellular enzyme (*e.g.*, a proteasome, a tyrosine kinase, a cyclin-dependent kinase, serine/threonine-protein kinase B-Raf (BRAF), or a MEK kinase).

**[61]** The invention as claimed is further described in the following items 1 to 20:

1. A fusion polypeptide comprising, from an amino-terminal position to a carboxyl-terminal position, ApoA1-L1-D, wherein:

ApoA1 is a first polypeptide segment comprising an amino acid sequence having at least 90% or at least 95% identity with amino acid residues 19-267 or 25-267 of SEQ ID NO:2, wherein said first polypeptide segment has cholesterol efflux activity, optionally wherein said first polypeptide segment has the amino acid sequence shown in residues 19-267 or 25-267 of SEQ ID NO:2;

L1 is a first polypeptide linker comprising at least five amino acid residues; and

D is an immunoglobulin Fc region;

wherein the fusion polypeptide retains has increased ApoA1 cholesterol efflux activity as compared to either an ApoA1-Fc fusion polypeptide with a two amino acid linker or an ApoA1-Fc fusion polypeptide without a linker.

2. The fusion polypeptide of item 1, wherein L1 comprises at least 10 amino acid residues.

3. The fusion polypeptide of item 1 wherein L1 comprises at least 16 amino acid residues, consists of from 15 to 40 amino acid residues, or consists of from 16 to 36 amino acid residues,

optionally wherein L1 consists of 16 amino acid residues, 21 amino acid residues, 26 amino acid residues, 31 amino acid residues, or 36 amino acid residues,

optionally wherein L1 has the amino acid sequence shown in residues 268-283 of SEQ ID NO:22, residues 268-288 of SEQ ID NO:26, residues 268-293 of SEQ ID NO:2, SEQ ID NO:54, or residues 268-303 of SEQ ID NO:24.

4. The fusion polypeptide of any one of items 1 to 3, wherein the Fc region is a human Fc region,

optionally wherein the human Fc region is an Fc variant comprising one or more amino acid substitutions relative to the wild-type human sequence,

optionally wherein the Fc region is a human γ1 Fc region or a human γ3 Fc region.

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5. The fusion polypeptide of item 4, wherein the human Fc region is a human γ1 Fc variant in which Eu residue C220 is replaced by serine,

optionally wherein the Eu residue P331 is replaced by serine.

6. The fusion polypeptide of item 5, wherein Eu residues C226 and C229 are each replaced by serine,

optionally wherein Eu residue P238 is replaced by serine,

optionally wherein the Fc region has the amino acid sequence shown in (i) residues 294-525 or 294-524 of SEQ ID NO:2, or (ii) residues 294-525 or 294-524 of SEQ ID NO:13.

7. The fusion polypeptide of item 1, wherein the fusion polypeptide comprises an amino acid sequence having at least 90% or at least 95% identity with

(i) residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:2,

(ii) residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:13,

(iii) residues 19-515, 19-514, 25-515, or 25-514 of SEQ ID NO:22,

(iv) residues 19-520, 19-519, 25-520, or 25-519 of SEQ ID NO:26, or

(v) residues 19-535, 19-534, 25-535, or 25-534 of SEQ ID NO:24,

optionally wherein the fusion polypeptide comprises the amino acid sequence shown in any one of (i)-(v).

8. The fusion polypeptide of any one of items 1 to 7, further comprising a second polypeptide segment located carboxyl-terminal to the dimerizing domain, wherein the second polypeptide segment is selected from the group consisting of an RNase, a paraoxonase, a platelet-activating factor acetylhydrolase, and a cholesterol ester transfer protein,

optionally wherein the fusion polypeptide comprises, from an amino-terminal position to a carboxyl-terminal position, ApoA1-L1-D-L2-P, wherein ApoA1, L1, and D are as defined in item 1, L2 is a second polypeptide linker, wherein L2 is optionally present, and P is the second polypeptide segment.

9. The fusion polypeptide of item 8, wherein the second polypeptide segment is the RNase,

optionally wherein the RNase has at least 90% or at least 95% identity with amino acid residues 542-675 of SEQ ID NO:4,

optionally wherein the RNase has the amino acid sequence shown in residues 542-675 of SEQ ID NO:4.

10. The fusion polypeptide of item 9, wherein the fusion polypeptide comprises an amino acid sequence having at least 90% or at least 95% identity with

(i) residues 19-675 or 25-675 of SEQ ID NO:4 or

(ii) residues 19-675 or 25-675 of SEQ ID NO:14,

optionally wherein the fusion polypeptide comprises the amino acid sequence shown in (i) or (ii).

11. The fusion polypeptide of item 8, wherein the second polypeptide segment is the paraoxonase, optionally wherein the paraoxonase is human paraoxonase 1 (PON1) or a functional variant thereof,

optionally wherein the human PON1 or functional variant thereof has at least 90% or at least 95% identity with amino acid residues 16-355 of SEQ ID NO:12, amino acid residues 16-355 of SEQ ID NO:42, or amino acid residues 16-355 of SEQ ID NO:44,

optionally wherein the human PON1 or functional variant thereof has the amino acid sequence shown in residues 16-355 of SEQ ID NO:12, residues 16-355 of SEQ ID NO:42, or residues 16-355 of SEQ ID NO:44.

12. The fusion polypeptide of item 11, wherein the fusion polypeptide comprises an amino acid sequence having at least 90% or at least 95% identity with

(i) residues 19-883 or 25-883 of SEQ ID NO:28,

(ii) residues 19-873 or 25-873 of SEQ ID NO:38,

(iii) residues 19-883 or 25-883 of SEQ ID NO:46, or

(iv) residues 19-883 or 25-883 of SEQ ID NO:48,

optionally wherein the fusion polypeptide comprises the amino acid sequence shown in any one of (i)-(iv).

13. A dimeric protein comprising a first fusion polypeptide and a second fusion polypeptide, wherein each of said first and second fusion polypeptides is a fusion polypeptide as defined in any one of items 1 to 12.

14. A polynucleotide encoding the fusion polypeptide of any one of items 1 to 12.

15. An expression vector comprising the following operably linked elements:

a transcription promoter;

a DNA segment encoding the fusion polypeptide of any one of items 1 to 12; and

a transcription terminator.

16. A cultured cell into which has been introduced the expression vector of item 15, wherein the cell expresses the DNA segment.

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17. A method of making a fusion polypeptide, the method comprising:

culturing a cell into which has been introduced the expression vector of item 15, wherein the cell expresses the DNA segment and the encoded fusion polypeptide is produced; and

recovering the fusion polypeptide,

optionally wherein the encoded fusion polypeptide is produced and recovered as a dimeric protein.

18. A composition comprising:

a fusion polypeptide of any one of items 1 to 12 or a dimeric protein of item 13; and

a pharmaceutically acceptable carrier.

19. A method for treating a disease, the method comprising:

administering to a subject having the disease an effective amount of a fusion polypeptide of any one of items 1 to 12 or a dimeric fusion protein of item 13, wherein the disease is a cardiovascular disease characterized by atherosclerosis, optionally wherein the cardiovascular disease is selected from the group consisting of coronary heart disease and stroke, optionally wherein the coronary heart disease is characterized by acute coronary syndrome.

20. Use of the fusion polypeptide of any one of items 1 to 12 or a dimeric fusion protein of item 13 in the manufacture of a medicament for the treatment of a disease, wherein the disease is a cardiovascular disease characterized by atherosclerosis, optionally wherein the cardiovascular disease is selected from the group consisting of coronary heart disease and stroke, optionally wherein the coronary heart disease is characterized by acute coronary syndrome.

## DEFINITIONS

**[62]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art pertinent to the methods and compositions described. As used herein, the following terms and phrases have the meanings ascribed to them unless specified otherwise.

**[63]** The terms "a," "an," and "the" include plural referents, unless the context clearly indicates otherwise.

**[64]** A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides."

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**[65]** A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

**[66]** The terms "amino-terminal" (or "N-terminal") and "carboxyl-terminal" (or "C-terminal") are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide.

**[67]** The terms "polynucleotide" and "nucleic acid" are used synonymously herein and refer to a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural

sources, synthesized *in vitro*, or prepared from a combination of natural and synthetic molecules. Sizes of polynucleotides are expressed as base pairs (abbreviated "bp"), nucleotides ("nt"), or kilobases ("kb"). Where the context allows, the latter two terms may describe polynucleotides that are single-stranded or double-stranded. It will be recognized by those skilled in the art that the two strands of a double-stranded polynucleotide may differ slightly in length and that the ends thereof may be staggered as a result of enzymatic cleavage; thus all nucleotides within a double-stranded polynucleotide molecule may not be paired. Such unpaired ends will in general not exceed 20 nt in length.

**[68]** A "segment" is a portion of a larger molecule (*e.g.*, polynucleotide or polypeptide) having specified attributes. For example, a DNA segment encoding a specified polypeptide is a portion of a longer DNA molecule, such as a plasmid or plasmid fragment that, when read from the 5' to the 3' direction, encodes the sequence of amino acids of the specified polypeptide. Also, in the context of a fusion polypeptide in accordance with the present invention, a polypeptide segment "having cholesterol efflux activity" and "comprising an amino acid sequence having at least 90% or at least 95% identity with amino acid residue 19-267 or 25-267 of SEQ ID NO:2" is a portion of the longer polypeptide fusion molecule that, in addition to the specified polypeptide segment having cholesterol efflux activity, includes other polypeptide segments (*e.g.*, linker(s), dimerizing domain) as described herein.

**[69]** The term "expression vector" is used to denote a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription. Such additional segments include promoter and terminator sequences, and may also include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, etc. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

**[70]** The term "promoter" is used herein for its art-recognized meaning to denote a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

**[71]** A "secretory signal sequence" is a DNA sequence that encodes a polypeptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

**[72]** "Operably linked" means that two or more entities are joined together such that they function in concert for their intended purposes. When referring to DNA segments, the phrase

indicates, for example, that coding sequences are joined in the correct reading frame, and transcription initiates in the promoter and proceeds through the coding segment(s) to the terminator. When referring to polypeptides, "operably linked" includes both covalently (*e.g.*, by disulfide bonding) and non-covalently (*e.g.*, by hydrogen bonding, hydrophobic interactions, or salt-bridge interactions) linked sequences, wherein the desired function(s) of the sequences are retained.

**[73]** The term "recombinant" when used with reference, *e.g.*, to a cell, nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein, or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under-expressed or not expressed at all. By the term "recombinant nucleic acid" herein is meant nucleic acid, originally formed *in vitro*, in general, by the manipulation of nucleic acid using, *e.g.*, polymerases and endonucleases, in a form not normally found in nature. In this manner, operable linkage of different sequences is achieved. Thus an isolated nucleic acid, in a linear form, or an expression vector formed *in vitro* by ligating DNA molecules that are not normally joined, are both considered recombinant for the purposes disclosed herein. It is understood that once a recombinant nucleic acid is made and reintroduced into a host cell or organism, it will replicate non-recombinantly, *i.e.*, using the *in vivo* cellular machinery of the host cell rather than *in vitro* manipulations; however, such nucleic acids, once produced recombinantly, although subsequently replicated non-recombinantly, are still considered recombinant for the purposes disclosed herein. Similarly, a "recombinant protein" is a protein made using recombinant techniques, *i.e.*, through the expression of a recombinant nucleic acid as depicted above.

**[74]** The term "heterologous," when used with reference to portions of a nucleic acid, indicates that the nucleic acid comprises two or more subsequences that are not normally found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences, *e.g.*, from unrelated genes arranged to make a new functional nucleic acid, *e.g.*, a promoter from one source and a coding region from another source. Similarly, "heterologous," when used in reference to portions of a protein, indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (*e.g.*, two or segments of a fusion polypeptide).

**[75]** An "immunoglobulin" is a serum protein which functions as an antibody in a vertebrate organism. Five classes of "immunoglobulin," or antibody, protein (IgG, IgA, IgM, IgD, and IgE) have been identified in higher vertebrates. IgG comprises the major class; it normally exists as the second most abundant protein found in plasma. In humans, IgG consists of four subclasses, designated IgG1, IgG2, IgG3, and IgG4. The heavy chain constant regions of the IgG class are

identified with the Greek symbol γ. For example, immunoglobulins of the IgG1 subclass contain a γ1 heavy chain constant region. Each immunoglobulin heavy chain possesses a constant region that consists of constant region protein domains (CH1, hinge, CH2, and CH3) that are essentially invariant for a given subclass in a species. DNA sequences encoding human and non-human immunoglobulin chains are known in the art. *See, e.g.*, Ellison *et al.*, *DNA* 1:11-18, 1981; Ellison *et al.*, *Nuc. Acids Res.* 10:4071-4079, 1982; Kenten *et al.*, *Proc. Natl. Acad. Sci. USA* 79:6661-6665, 1982; Seno *et al.*, *Nuc. Acids Res.* 11:719-726, 1983; Riechmann *et al.*, *Nature* 332:323-327, 1988; Amster *et al.*, *Nuc. Acids Res.* 8:2055-2065, 1980; Rusconi and Kohler, *Nature* 314:330-334, 1985; Boss *et al.*, *Nuc. Acids Res.* 12:3791-3806, 1984; Bothwell *et al.*, *Nature* 298:380-382, 1982; van der Loo *et al.*, *Immunogenetics* 42:333-341, 1995; Karlin *et al.*, *J. Mol. Evol.* 22:195-208, 1985; Kindsvogel *et al.*, *DNA* 1:335-343, 1982; Breiner *et al.*, *Gene* 18:165-174, 1982; Kondo *et al.*, *Eur. J. Immunol.* 23:245-249, 1993; and GenBank Accession No. J00228. For a review of immunoglobulin structure and function, *see* Putnam, *The Plasma Proteins, Vol V*, Academic Press, Inc., 49-140, 1987; and Padlan, *Mol. Immunol.* 31:169-217, 1994.

**[76]** An "immunoglobulin hinge" is that portion of an immunoglobulin heavy chain connecting the CH1 and CH2 domains. The hinge region of human γ1 corresponds approximately to Eu residues 216-230.

**[77]** The terms "Fc fragment," "Fc region," or "Fc domain," as used herein, are synonymous and refer to the portion of an immunoglobulin that is responsible for binding to antibody receptors on cells and the C1q component of complement (in the absence of any amino acid changes, relative to the naturally occurring sequence, to remove such binding activity). Fc stands for "fragment crystalline," the fragment of an antibody that will readily form a protein crystal. Distinct protein fragments, which were originally described by proteolytic digestion, can define the overall general structure of an immunoglobulin protein. As originally defined in the literature, the Fc fragment consists of the disulfide-linked heavy chain hinge regions, CH2, and CH3 domains. As used herein, the term also refers to a single chain consisting of CH3, CH2, and at least a portion of the hinge sufficient to form a disulfide-linked dimer with a second such chain. As used herein, the term Fc region further includes variants of naturally occurring sequences, where the variants are capable of forming dimers and including such variants that have increased or decreased Fc receptor-binding or complement-binding activity.

**[78]** "Dimerizing domain," as used herein, refers to a polypeptide having affinity for a second polypeptide, such that the two polypeptides associate under physiological conditions to form a dimer. Typically, the second polypeptide is the same polypeptide, although in some variations the second polypeptide is different. The polypeptides may interact with each other through covalent and/or non-covalent association(s). Examples of dimerizing domains include an Fc region; a hinge

region; a CH3 domain; a CH4 domain; a CH1 or CL domain; a leucine zipper domain (*e.g.*, a jun/fos leucine zipper domain, *see, e.g.*, Kostelney *et al.*, *J. Immunol.*, 148:1547-1553, 1992; or a yeast GCN4 leucine zipper domain); an isoleucine zipper domain; a dimerizing region of a dimerizing cell-surface receptor (*e.g.*, interleukin-8 receptor (IL-8R); or an integrin heterodimer such as LFA-1 or GPIIIb/IIIa); a dimerizing region of a secreted, dimerizing ligand (*e.g.*, nerve growth factor (NGF), neurotrophin-3 (NT-3), interleukin-8 (IL-8), vascular endothelial growth factor (VEGF), or brain-derived neurotrophic factor (BDNF); *see, e.g.*, Arakawa *et al.*, *J. Biol. Chem.* 269:27833-27839, 1994, and Radziejewski *et al.*, *Biochem.* 32:1350, 1993); and a polypeptide comprising at least one cysteine residue (*e.g.*, from about one, two, or three to about ten cysteine residues) such that disulfide bond(s) can form between the polypeptide and a second polypeptide comprising at least one cysteine residue (hereinafter "a synthetic hinge"). A preferred dimerizing domain in accordance with the present invention is an Fc region.

**[79]** The term "dimer" or "dimeric protein" as used herein, refers to a multimer of two ("first" and "second") fusion polypeptides as disclosed herein linked together via a dimerizing domain. Unless the context clearly indicates otherwise, a "dimer" or "dimeric protein" includes reference to such dimerized first and second fusion polypeptides in the context of higher order multimers that may form in spherical HDL particles (*e.g.*, trimers), such as through an interaction of dimerized first and second fusion polypeptides with another ApoA-1 polypeptide that may be present (*e.g.*, through interaction with a naturally occurring, endogenous ApoA-1 protein). The term also includes reference to dimerized first and second fusion polypeptides in the context of higher order multimers that may be created by inclusion of an additional dimerizing domain in a first or second fusion polypeptide (*e.g.*, a first fusion polypeptide comprising an immunoglobulin light chain and a second fusion polypeptide comprising an immunoglobulin heavy chain can heterodimerize via the interaction between the CH1 and CL domains, and two such heterodimers may further dimerize via the Fc region of the immunoglobulin heavy chain, thereby forming a tetramer).

**[80]** The term "linker" or "polypeptide linker" is used herein to indicate two or more amino acids joined by peptide bond(s) and linking two discrete, separate polypeptide regions. The linker is typically designed to allow the separate polypeptide regions to perform their separate functions (such as, *e.g.*, where a dimerizing domain, linked to other polypeptide regions, associates with another, corresponding dimerization domain to form a dimer). The linker can be a portion of a native sequence, a variant thereof, or a synthetic sequence. Linkers are also referred to herein using the abbreviation "L." The use of a subscript (*e.g.*, "1" or "2") with "L" is used herein to differentiate among multiple linkers within a polypeptide chain, which linkers may be the same or different with respect to amino acid sequence.

**[81]** Unless the context clearly indicates otherwise, reference herein to "ApoA-1" is understood to include naturally occurring ApoA-1 polypeptides as well as functional variants, functional fragments, and mimetics thereof. "ApoA1," Apo A-1," "apoA-1," and "apo A-1" are used herein synonymously with "ApoA1."

**[82]** Unless the context clearly indicates otherwise, reference herein "RNase" (*e.g.*, "RNase 1"), "paraoxonase" (*e.g.*, "PON1"), "platelet-activating factor acetylhydrolase" ("PAF-AH"), "cholesterol ester transfer protein" ("CETP"), or "lecithin-cholesterol acyltransferase" ("LCAT") is understood to include naturally occurring polypeptides of any of the foregoing, as well as functional variants and functional fragments thereof.

**[83]** The term "allelic variant" is used herein to denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

**[84]** ApoA-1 fusion polypeptides of the present disclosure may be referred to herein by formulae such as, for example, "ApoA1-L1-D," "ApoA1-L1-D-L2-P," "ApoA1-L1-[Fc region]," "ApoA1-L1-D-L2-RNase," "ApoA1-L1-[Fc region]-L2-RNase1," "ApoA1-L1-D-L2-paraoxonase," or "ApoA1-L1-[Fc region]-L2-PON1." In each such case, unless the context clearly dictates otherwise, a term referring to a particular segment of a fusion polypeptide (*e.g.*, "ApoA1," "D" (for dimerizing domain), "L1" (for a first polypeptide linker), "Fc region," "RNase," "paraoxonase," *etc.*) is understood to have the meaning ascribed to such term herein and is inclusive of the various embodiments as described herein.

**[85]** The term "effective amount," in the context of treatment of a disease by administration of a soluble fusion polypeptide or dimeric protein to a subject as described herein, refers to an amount of such molecule that is sufficient to inhibit the occurrence or ameliorate one or more symptoms of the disease. For example, in the specific context of treatment of an autoimmune disease by administration of a dimeric ApoA1 fusion protein to a subject as described herein, the term "effective amount" refers to an amount of such molecule that is sufficient to modulate an autoimmune response in the subject so as to inhibit the occurrence or ameliorate one or more symptoms of the autoimmune disease. An effective amount of an agent is administered according to the methods of the present invention in an "effective regime." The term "effective regime" refers to a combination of amount of the agent being administered and dosage frequency adequate to accomplish treatment or prevention of the disease.

**[86]** The term "patient" or "subject," in the context of treating a disease or disorder as described herein, includes mammals such as, for example, humans and other primates. The term also includes domesticated animals such as, *e.g.*, cows, hogs, sheep, horses, dogs, and cats.

**[87]** The term "combination therapy" refers to a therapeutic regimen that involves the provision of at least two distinct therapies to achieve an indicated therapeutic effect. For example, a combination therapy may involve the administration of two or more chemically distinct active ingredients, or agents, for example, a soluble ApoA1 fusion polypeptide or dimeric protein according to the present invention and another agent such as, *e.g.*, another anti-inflammatory or immunomodulatory agent. Alternatively, a combination therapy may involve the administration of a soluble ApoA1 fusion polypeptide or dimeric protein according to the present invention, alone or in conjunction with another agent, as well as the delivery of another therapy (*e.g.*, radiation therapy). The distinct therapies constituting a combination therapy may be delivered, *e.g.*, as simultaneous, overlapping, or sequential dosing regimens. In the context of the administration of two or more chemically distinct agents, it is understood that the active ingredients may be administered as part of the same composition or as different compositions. When administered as separate compositions, the compositions comprising the different active ingredients may be administered at the same or different times, by the same or different routes, using the same or different dosing regimens, all as the particular context requires and as determined by the attending physician.

**[88]** The term "non-ApoA1-mediated immunomodulatory therapy," in the context of treating cancer, means an immunomodulatory therapy that does not specifically target ApoA-1 or ApoA-1-mediated signaling pathways.

**[89]** The term "targeted therapy," in the context of treating cancer, refers to a type of treatment that uses a therapeutic agent to identify and attack a specific type of cancer cell, typically with less harm to normal cells. In some embodiments, a targeted therapy blocks the action of an enzyme or other molecule involved in the growth and spread of cancer cells. In other embodiments, a targeted therapy either helps the immune system to attack cancer cells or delivers a toxic substance directly to cancer cells. In certain variations, a targeted therapy uses a small molecule drug or a monoclonal antibody as a therapeutic agent.

**[90]** Two amino acid sequences have "100% amino acid sequence identity" if the amino acid residues of the two amino acid sequences are the same when aligned for maximal correspondence. Sequence comparisons can be performed using standard software programs such as those included in the LASERGENE bioinformatics computing suite, which is produced by DNASTAR (Madison, Wisconsin). Other methods for comparing amino acid sequences by determining optimal alignment are well-known to those of skill in the art. (*See, e.g.*, Peruski and Peruski, *The Internet and the New Biology: Tools for Genomic and Molecular Research* (ASM Press,

Inc. 1997); Wu *et al.* (eds.), "Information Superhighway and Computer Databases of Nucleic Acids and Proteins," in *Methods in Gene Biotechnology* 123-151 (CRC Press, Inc. 1997); Bishop (ed.), *Guide to Human Genome Computing* (2nd ed., Academic Press, Inc. 1998).) Two amino acid sequences are considered to have "substantial sequence identity" if the two sequences have at least 80%, at least 90%, or at least 95% sequence identity relative to each other.

**[91]** Percent sequence identity is determined by conventional methods. *See, e.g.*, Altschul *et al., Bull. Math. Bio.* 48:603, 1986, and Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915, 1992. For example, two amino acid sequences can be aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "BLOSUM62" scoring matrix of Henikoff and Henikoff, *supra*, as shown in Table 1 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as: ([Total number of identical matches]/ [length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences])(100).

**Table 1: BLOSUM62 Scoring Matrix**

| | A | R | N | D | C | Q | E | G | H | I | L | K | M | F | P | S | T | W | Y | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 4 | | | | | | | | | | | | | | | | | | | |
| **R** | -1 | 5 | | | | | | | | | | | | | | | | | | |
| **N** | -2 | 0 | 6 | | | | | | | | | | | | | | | | | |
| **D** | -2 | -2 | 1 | 6 | | | | | | | | | | | | | | | | |
| **C** | 0 | -3 | -3 | -3 | 9 | | | | | | | | | | | | | | | |
| **Q** | -1 | 1 | 0 | 0 | -3 | 5 | | | | | | | | | | | | | | |
| **E** | -1 | 0 | 0 | 2 | -4 | 2 | 5 | | | | | | | | | | | | | |
| **G** | 0 | -2 | 0 | -1 | -3 | -2 | -2 | 6 | | | | | | | | | | | | |
| **H** | -2 | 0 | 1 | -1 | -3 | 0 | 0 | -2 | 8 | | | | | | | | | | | |
| **I** | -1 | -3 | -3 | -3 | -1 | -3 | -3 | -4 | -3 | 4 | | | | | | | | | | |
| **L** | -1 | -2 | -3 | -4 | -1 | -2 | -3 | -4 | -3 | 2 | 4 | | | | | | | | | |
| **K** | -1 | 2 | 0 | -1 | -3 | 1 | 1 | -2 | -1 | -3 | -2 | 5 | | | | | | | | |
| **M** | -1 | -1 | -2 | -3 | -1 | 0 | -2 | -3 | -2 | 1 | 2 | -1 | 5 | | | | | | | |
| **F** | -2 | -3 | -3 | -3 | -2 | -3 | -3 | -3 | -1 | 0 | 0 | -3 | 0 | 6 | | | | | | |
| **P** | -1 | -2 | -2 | -1 | -3 | -1 | -1 | -2 | -2 | -3 | -3 | -1 | -2 | -4 | 7 | | | | | |
| **S** | 1 | -1 | 1 | 0 | -1 | 0 | 0 | 0 | -1 | -2 | -2 | 0 | -1 | -2 | -1 | 4 | | | | |
| **T** | 0 | -1 | 0 | -1 | -1 | -1 | -1 | -2 | -2 | -1 | -1 | -1 | -1 | -2 | -1 | 1 | 5 | | | |
| **W** | -3 | -3 | -4 | -4 | -2 | -2 | -3 | -2 | -2 | -3 | -2 | -3 | -1 | 1 | -4 | -3 | -2 | 11 | | |
| **Y** | -2 | -2 | -2 | -3 | -2 | -1 | -2 | -3 | 2 | -1 | -1 | -2 | -1 | 3 | -3 | -2 | -2 | 2 | 7 | |
| **V** | 0 | -3 | -3 | -3 | -1 | -2 | -2 | -3 | -3 | 3 | 1 | -2 | 1 | -1 | -2 | -2 | 0 | -3 | -1 | 4 |

**[92]** Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA" similarity search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and a second amino acid sequence. The FASTA algorithm is described by Pearson and Lipman, *Proc. Nat'l Acad. Sci. USA* 85:2444, 1988, and by Pearson, *Meth. Enzymol.* 183:63, 1990. Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (*e.g.,* residues 19-267 or 25-267 of SEQ ID NO:2) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions, or deletions. The ten regions with the highest density of identities are then rescored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence and the ktup value), then the trimmed initial regions are examined to determine whether the regions can be joined to form an approximate alignment with gaps. Finally, the highest scoring

regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch-Sellers algorithm (Needleman and Wunsch, *J. Mol. Biol.* 48:444, 1970; Sellers, *SIAM J. Appl. Math.* 26:787, 1974), which allows for amino acid insertions and deletions. Illustrative parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, *Meth. Enzymol.* 183:63, 1990.

**[93]** When such a value is expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to ±10%.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[94]** FIG. 1 illustrates cholesterol efflux in BHK cell cultures ApoA-1 molecules and recombinant fusions thereof. ApoA-1-Fc fusion protein containing a 26 amino acid linker between ApoA-1 and the Fc region (ApoA-1(26)Fc) demonstrated increased cholesterol efflux as compared to either an ApoA-1-Fc fusion protein with a two amino acid linker (ApoA-1(2)Fc (Theripion)) or an ApoA-1-Fc fusion protein without a linker (ApoA-1(0)Fc (Sino Biol)) and had activity similar to wild-type human ApoA-1 (Control ApoA-1). ApoA-1 molecules were incubated for 2 hours with H3-cholesterol labeled BHK cells induced for ABCA1 expression. The Fc proteins were predicted to be dimers; however, the concentrations shown were calculated and normalized based on the mass of ApoA-1 per molecule.

**[95]** FIGS. 2A and 2B show schematic diagrams of certain embodiments of fusion proteins in accordance with the present disclosure, including component functional domains. FIG. 2A depicts a schematic representation of a human ApoA-1 joined at the carboxyl terminus, via a linker, to a human IgG Fc region (also referred to herein as a "THER fusion protein" or "THER molecule"). FIG. 2B depicts a schematic representation of a THER fusion protein further joined at the carboxyl terminus, via a linker, to an enzyme region (these fusions are also referred to herein a "Bifunctional Enzyme Lipid Transport" or "BELT" molecule; a BELT molecule may also be generally referred to herein as a THER fusion protein or molecule). The linker sequence and the domain present at the carboxyl terminus of the fusion protein varies depending on the construct.

**[96]** FIG. 3 shows a Western blot of culture supernatants (serum free) from transiently transfected 293T cells expressing five different THER molecules. Transfections and Western blot analysis were performed as described in Example 3, *infra*. From left to right: MOCK – mock transfection negative control; CD40IgG – CD40IgG DNA transfection positive control; THER0 – ApoA1-IgG fusion protein with a linker of two amino acids; THER2 – ApoA1-IgG fusion protein

with a linker of 16 amino acids; THER4 – ApoA1-IgG fusion protein with a linker of 26 amino acids; THER6 – ApoA1-IgG fusion protein with a linker of 36 amino acids; THER4RNA – ApoA1-IgG fusion protein with a linker of 36 amino acids, further linked via a second 18 amino acid linker to human RNase1.

**[97]** FIGS. 4A-4E show columnar graphs summarizing the initial screening of stable CHO clones expressing THER0 (FIG. 4A), THER2 (FIG. 4B), THER4 (FIG. 4C), THER6 (FIG. 4D), and THER4RNA2 (FIG. 4E) ApoA-1 fusion proteins and relative expression levels of the fusion proteins from 96 well culture supernatants (*see* Example 4, *infra*).

**[98]** FIGS. 5A-5C show results from analysis of a subset of THER clones that expressed higher levels of fusion protein, assessing their cell growth pattern (FIG. 5A), relative cell viability (FIG. 5B), and expression of fusion protein (FIG. 5C) after six and ten days of culture (*see* Example 4, *infra*).

**[99]** FIGS. 6A and 6B show nonreducing (FIG. 6A) and reducing (FIG. 6B) SDS-PAGE analysis of THER fusion proteins purified from CHO clone spent culture supernatants (*see* Example 4, *infra*).

**[100]** FIG. 7 shows Native PAGE gel analysis of the purified THER fusion proteins. Samples were prepared and BLUE Native PAGE gels were run and stained as described in Example 4, *infra*.

**[101]** FIG. 8 shows a graph summarizing the relative binding of the different fusion proteins in a sandwich ELISA, using an anti-IgG capture of fusion proteins and detection step with an HRP-conjugated anti-ApoA-1 antibody (*see* Example 5, *infra*).

**[102]** FIG. 9 shows results from a kinetic enzyme assay measuring the RNase activity present in samples of serial dilutions of purified ApoA1-IgG-RNase bispecific fusion protein (THER4RNA2). An RNASEALERT™ assay (IDT, Coralville, IA) was performed as described in Example 6, *infra*, using RNase A ("RNase") as a positive control and ApoA-1-lnk26-hIgG ("THER4") as a negative control. Each box displays the relative fluorescence units observed as a function of time during the course of a 45 minute assay, with a fixed concentration of a non-fluorescent RNA substrate that generates a fluorescent signal upon digestion of the RNA.

**[103]** FIG. 10 shows a subset of the data shown in FIG. 9, comparing the RNase enzyme activity at the 4 pmol/µl protein dilution.

**[104]** FIG. 11 shows the results of a BODIPY-cholesterol efflux assay using purified fusion proteins and differentiated human monocytic cell line, THP-1. Assays were performed in a 96 well plate format as described in Example 7, *infra*, and data are displayed as the mean efflux observed from 5 replicates, with baseline efflux (media alone) subtracted from all samples.

**[105]** FIG. 12 shows the results of a cholesterol efflux assay using the mouse monocyte-macrophage cell line J774 A.1 (ATCC, Manassas, VA). Both baseline and cAMP-stimulated efflux were assessed as described in Example 7, *infra*.

## DESCRIPTION OF THE INVENTION

### I. Overview

**[106]** The present invention provides compositions and methods relating to fusion polypeptides comprising a first polypeptide segment having cholesterol efflux activity and which is either an ApoA1 polypeptide or functional variant or fragment thereof or, alternatively, an ApoA-1 mimetic. In some aspects, the fusion polypeptide further includes a dimerizing domain with a peptide linker between the amino-terminal end of the dimerizing domain and the carboxyl-terminal end of the ApoA-1 polypeptide, variant, fragment, or mimetic, thereby allowing the fusion polypeptide to form stable dimers. In other, non-mutually exclusive aspects, the fusion polypeptides are bispecific constructs further comprising a second polypeptide segment carboxyl-terminal to the ApoA-1 polypeptide, variant, fragment, or mimetic and which confers a second biological activity. Exemplary second polypeptides include RNases, paraoxonases, platelet-activating factor acetylhydrolases (PAF-AHs), cholesterol ester transfer proteins (CETPs), lecithin-cholesterol acyltransferases (LCATs), and polypeptides that specifically bind to amyloid beta, any of which may be a naturally occurring protein or a functional variant or fragment thereof.

**[107]** The fusion molecules of the present invention can be used, for example, to increase reverse cholesterol transport in a subject and provide therapeutic benefit in the treatment of various diseases. ApoA-1, the major protein of HDL, has already shown beneficial activity in clinical trials in patients with acute coronary syndrome. The ApoA-1 fusion molecules of the present invention can be used to treat coronary heart disease, acute coronary syndrome, and other cardiovascular diseases characterized by atherosclerosis such as, *e.g.*, stroke. Fusion molecules of the present invention are also useful, for example, for the treatment of autoimmune diseases (*e.g.*, rheumatoid arthritis, systemic lupus erythematosus), inflammatory diseases, type 2 diabetes, obesity, and neurodegenerative diseases (*e.g.*, Alzheimer's disease). In some embodiments, fusion proteins of the present invention are used to replace defective ApoA-1 such as, for example, in the treatment of type 1 diabetes and dementia. In certain variations, fusion proteins as disclosed herein are used to treat multiple sclerosis (MS). ApoA-1 levels have been shown to be low in patients with MS, and ApoA-1 deficient mice have been shown to exhibit more neurodegeneration and worse disease in experimental allergic encephalomyelitis (EAE), a model for MS, than wild-type animals. *See* Meyers *et al.*, *J. Neuroimmunol.* 277: 176-185, 2014. Data further suggests a positive neuroprotective effect of ApoA-

1 on the central nervous system. *See* Gardner *et al.*, *Frontiers in Pharmacology*: 20 November 2015 doi: 10.3389/fphar.2015.00278.

**[108]** Several studies support the use of ApoA-1 therapy for autoimmune disease. For example, patients with systemic lupus erythematosus (SLE) have low HDL-cholesterol levels and the HDL that is present is often damaged by myeloperoxidase-mediated methionine oxidation and tyrosine chlorination of ApoA-1, resulting in loss of ABCA1-dependent cholesterol efflux activity. *See* Shao *et al.*, *J. Biol. Chem.* 281:9001-4, 2006; Hewing *et al.*, *Arterioscler. Thromb. Vasc. Biol.* 34:779-89, 2014. This promotes loss of anti-inflammatory properties and generation of pro-inflammatory HDL seen in patients with SLE. *See* Skaggs *et al.*, *Clin. Immunol.* 137:147-156, 2010; McMahon *et al.*, *Athritis Rheum.* 60:2428-2437, 2009. Autoantibodies to ApoA-1 are present in many patients with SLE, and SLE-disease activity assessed by SLEDAI and SLE disease related organ damage assessed by SLICC/ACR damage index are positively correlated with anti-ApoA-1 antibodies. *See* Batukla *et al.*, *Ann. NY Acad. Sci.* 1108:137-146, 2007; Ahmed *et al.*, *EXCLI Journal* 12:719-732, 2013. Further, increased ApoA-1 concentration attenuated autoimmunity and glomerulonephritis in lupus prone SLE 1,2,3 mice. *See* Black *et al.*, *J. Immunol.* 195:4685-4698, 2015.

**[109]** Cholesterol efflux capacity of HDL is also impaired in rheumatoid arthritis patients with high disease activity and is correlated with systemic inflammation and loss of HDL antioxidant activity. *See* Charles-Schoeman *et al.*, *Arthritis Rheum.* 60:2870-2879, 2009; Charles-Schoeman *et al.*, *Ann. Rheum. Dis.* 71:1157-1162, 2012. Treatment of arthritis in the Lewis rat by ApoA-1 and reconstituted HDL reduced acute and chronic joint inflammation, and decreased macrophage TLR2 expression and activation. *See* Wu *et al.*, *Arterioscler. Thromb. Basc. Biol.* 34:543-551, 2014. Therapy of collagen-induced arthritis in rats with ApoA-1 mimetic peptide D-4F in combination with pravastatin significantly reduced disease activity. *See* Charles-Schoeman *et al.*, *Clin. Immunol.* 127:234-244, 2008.

**[110]** Fusion molecules of the present invention may also be used in the treatment of infectious disease. During infection and endotoxemia, significant alterations in lipid metabolism and lipoprotein composition occur, including a reduction in ApoA-1 and changes in HDL composition and size. HDL can bind and neutralize Gram-negative LPS and Gram-positive lipoteichoic acid, promoting clearance of these inflammatory products. Pharmacological studies support the benefit of recombinant ApoA-1 during bacterial infection. *See, e.g.*, Pirillo *et al.*, *Handb Exp Pharmacol.* 224:483-508, 2015.

**[111]** Bifunctional ApoA-1 fusion molecules of the present invention that contain a paraoxonase (*e.g.*, PON1) are particularly useful for therapy of patients infected with *Pseudomonas aeruginosa*, a gram negative bacterium. This is particularly important for immunocompromised

patients, where infections with *P. aeruginosa* are common. *P. aeruginosa* secrete virulence factors and form biofilm in response to small signaling molecules called acyl-homoserine lactones in a concentration-dependent process called quorum sensing (QS). Paroxonase 1 degrades acyl-homoserine lactones and was shown to protect from lethality from *P. aeruginosa* in a transgenic in vivo model in *Drosophila melanogaster* where there are no endogenous PON homologs. *See* Estin *et al., Adv. Exp. Med. Biol.* 660:183-193, 2010.

**[112]** Fusion molecules of the present invention may also be used in the treatment of inflammatory disease. For example, ApoA-1 fusion polypeptides and dimeric proteins as described herein may alter the phenotype of neutrophils, macrophages, and/or antigen-presenting cells to reduce proinflammatory responses. Molecules of the present invention cause efflux of cholesterol from cell membranes, mediated by transporter molecules such as, *e.g.*, ABCA1. Efflux of cholesterol from antigen-presenting cells, including macrophages and dendritic cells, can inhibit proinflammatory responses mediated by these cells, resulting in reduced production of inflammatory cytokines. Studies support the benefit of ApoA-1 in mediating anti-inflammatory effects. For example, treatment with ApoA-1 was shown to inhibit the proinflammatory signaling in macrophages after stimulation of CD40 by altering the composition of lipid rafts. *See* Yin *et al., J. Atherosclerosis and Thrombosis* 19:923-36, 2012. ApoA-1 was also shown to cause a decrease in TRAF-6 recruitment to lipid rafts, and a decrease in activation of NF-kB. *See id.* Another study showed that treatment of human monocytes and macrophages with ApoA-1 or ApoA-1 mimetic 4F altered their response to LPS, resulting in decreased production of inflammatory cytokines MCP-1, MIP-1, RANTES, IL-6, and TNFα, but increased the production of IL-10. *See* Smythies *et al., Am. J. Physiol. Cell Physiol.* 298:C1538-48, 2010. doi:1152/ajpcell.00467.2009. Another study showed that treatment with ApoA-1 significantly decreased LPS-induced MCP-1 release from THP-1 cells, and inhibited expression of CD11b and VCAM-1. *See* Wang *et al., Cytokine* 49:194-2000, 2010. Thus ApoA-1 inhibits activation and adhesion of human monocytes and macrophages, and induces profound functional changes due to a differentiation to an anti-inflammatory phenotype.

**[113]** Inflammatory lung diseases are among inflammatory diseases that may be treated with ApoA-1 fusion molecules as described herein. Serum ApoA-1 was found to be positively correlated with FEV1 in patients with combined atopy and asthma, but not in atopic and nonatopic subjects without asthma. *See* Barochia *et al., Am. J. Respir. Crit. Care Med.* 191:990-1000, 2015. In another study, patients with idiopathic pulmonary fibrosis had low levels of ApoA-1 in bronchiolar lavage fluid compared to controls ($P<0.01$). *See* Kim *et al., Am. J. Respir. Crit. Care Med.* 182:633-642, 2010. Further, intranasal treatment with ApoA-1 in mice treated with bleomycin was very effective in reducing the number of inflammatory cells and collagen deposition in the lungs. *See id.*

**[114]** Obesity is another inflammatory disease amenable to treatment with ApoA-1 fusion molecules in accordance with the present invention. Evidence supports the use of ApoA-1 and HDL to combat obesity. *See, e.g.*, Mineo *et al.*, *Circ. Res.* 111:1079-1090, 2012. For example, overexpression of ApoA-1 or administration of the ApoA-1 mimetic peptide D-4F has been shown to decrease white adipose mass and insulin resistance and increase energy expenditure in mice fed a high-fat diet. Further, in *ob/ob* mice, the ApoA-1 mimetic L-4F was shown to lower adiposity and inflammation and improve glucose tolerance. *Id.*

**[115]** Yet another disorder that may be treated with ApoA-1 fusion molecules in accordance with the present invention is nephrotic syndrome (NS), which is associated with a higher risk for cardiovascular disease in patients. Urinary wastage of filterable HDL (*i.e.*, HDL3) and lipid-poor apo A1 is a common feature of patients with nephrotic syndrome. This is typically due to decreased re-uptake of these molecules via cubulin/megalin receptors in the renal proximal tubule. *See* Barth *et al.*, *Trends Cardiovasc. Med.* 11:26-31, 2001. ApoA-1 fusion molecules comprising an Fc region as described herein would bypass the need for reuptake in this usual manner, since the molecules are being recycled via FcRn due to the presence of the Fc domain.

**[116]** Fusion molecules as described herein may also be used for therapy of patients with cancer. It is expected that ApoA-1 fusion polypeptides and dimeric proteins of the present invention, while reducing proinflammatory responses, enhance activation and tumor infiltration of $CD8^+$ T-cells. Studies support the efficacy of ApoA-1 therapy in animal models of cancer and have shown that ApoA-1 therapy can cause a specific increase in $CD8^+$ T cells in tumors. *See, e.g.*, Zamanian-Daryoush *et al.*, *J. Biol. Chem.* 288:21237-21252, 2013. In some aspects, ApoA-1 fusion molecules of the present invention are useful in combination with one or more other anti-cancer therapies such as, for example, an anti-cancer immunotherapy.

**[117]** In certain aspects, the present invention provides a way to stabilize an active ApoA-1 dimer while also controlling the maturation from pre-beta particles to discoid particles and spherical particles by providing a flexible linker between a dimerizing domain (*e.g.*, an Fc domain) and the C-terminus of the ApoA-1 polypeptide, or functional variant, fragment, or mimetic thereof. A previous ApoA-1-Ig molecule not containing a linker exhibits low activity in cholesterol efflux assays compared to wild-type ApoA1. In contrast, dimerizing fusion polypeptides of the present invention retain ApoA-1 activity in cholesterol efflux assays and also allow for further improvements such as, *e.g.*, fusion of an RNase (*e.g.*, RNase 1) or other polypeptide segments C-terminal to the dimerizing domain. In certain preferred embodiments, the use of an Fc region as the dimerizing domain also allows for increased half-life of the dimer.

**[118]** While not intending to be bound by theory, it is believed that the length of the linker controls the ability of the stable ApoA-1 dimer to expand as it takes up cholesterol. The invention

provides ApoA-1 fusion molecules containing flexible linkers between the C-terminus of an ApoA-1 polypeptide, or variant, fragment, or mimetic thereof, and the N-terminus of a dimerizing domain such as, *e.g.*, an Fc domain. Linkers are of sufficient length to allow ApoA-1, or the functional variant, fragment, or mimetic thereof, to mediate cholesterol efflux from cells, an initial and critical step in Reverse Cholesterol Transport (RCT). Linkers are typically between 2 and 60 amino acids in length. It is believed that ApoA-1 fusion molecules with alternative linker lengths have distinct functional properties by controlling the maturation of the HDL particle by constraining the C-terminus of ApoA-1. HDL discoid particles of intermediate size may have improved atheroprotective properties, and may have improved CNS transport properties. The molecules of this invention may change the progress of HDL maturation at these intermediate discoid stages, thereby improving efficacy of the fusion proteins of the invention relative to wild type ApoA-1 proteins. The molecules of this invention are likely to affect the structure and composition of spherical HDL particles which are composed of trimeric ApoA-1 particles (*see* Silva *et al.*, *Natl. Acad. Sci. USA* 105:12176-12181, 2008). It is likely that molecules of this invention will interact with natural ApoA-1 in the formation of larger spherical HDL particles.

**[119]** In certain embodiments, the dimerizing domain is a immunoglobulin Fc region. ApoA-1-Fc fusion molecules of the present invention extend ApoA-1 half-life while retaining ApoA-1 reverse cholesterol efflux and eliminating the requirement for extensive lipid formulation. In addition, the presence of the Fc region allows purification using immobilized Protein A according to standard practices in and antibody and Fc fusion protein manufacturing.

**[120]** Structural studies of ApoA-1 (*see, e.g.*, Gogonea, *Frontiers Pharmacol.* 6:318, 2016) show that ApoA-1 assumes multiple conformations as it matures from lipid-free monomer to higher order forms. Recent data derived from small angle neutron scattering (SANS) show low resolution structures of ApoA-1 dimers in an open configuration around a lipid core, called the super double helix (DSH) model. Other structures from SANS studies show ApoA-1 in different open configurations depending on the composition of the lipid core; in these structures, the C-terminus of the ApoA-1 monomers are in different positions relative to each other. Similarly, spherical ApoA-1 particles that incorporate a third ApoA-1 monomer show the C-terminus of each monomer in a different position compared to the positions in dimeric discoid ApoA-1. *See, e.g.*, Gogonea, *supra*. The flexible linkers of the present disclosure are of sufficient length to allow ApoA-1 to assume these positions without conformational constraint.

**[121]** In certain embodiments, ApoA-1-[linker]-[dimerizing domain] molecules of the present invention include an additional polypeptide segment fused carboxyl-terminal to the dimerizing domain. Such variations allow for the creation of bispecific molecules with ApoA-1 functional activity and a second biological activity.

**[122]** In some aspects of the present invention, bispecific fusion molecules are provided comprising a (i) first polypeptide segment with reverse cholesterol transport activity and which is either an ApoA1 polypeptide or functional variant or fragment thereof or, alternatively, an ApoA1 mimetic and (ii) a second polypeptide segment carboxyl-terminal to the first polypeptide segment, wherein the second polypeptide segment is selected from an RNase, a paraoxonase, a platelet-activating factor acetylhydrolase (PAF-AH), a cholesterol ester transfer protein (CETP), a lecithin-cholesterol acyltransferase (LCAT), and a polypeptide that specifically binds to amyloid beta. Such second polypeptides may be a naturally occurring protein or a functional variant or fragment thereof. In some embodiments, a linker and dimerizing domain is included between the first and second polypeptides as summarized above. In alternative embodiments, the fusion polypeptide lacks a dimerizing domain.

**[123]** In some embodiments of the present ApoA-1 fusion molecules that lack an Fc region, the fusion molecule may be conjugated to PEG to provide extended half-life. Such variations may include bispecific molecules as described herein, such as, *e.g.*, fusion molecules comprising an RNase, a paraoxonase, a platelet-activating factor acetylhydrolase (PAF-AH), a cholesterol ester transfer protein (CETP), a lecithin-cholesterol acyltransferase (LCAT), or a polypeptide that specifically binds to amyloid beta.

**[124]** In some embodiments of a bispecific molecule as summarized above, the second polypeptide segment is an RNase. A preferred RNase is human RNase 1 or a functional variant or fragment thereof. In particular variations, the RNase retains its sensitivity to inhibition by cytoplasmic inhibitor and has very low toxicity to cells, but is highly active extracellularly. RNase has anti-inflammatory properties by digestion of inflammatory extracellular RNA and provides additional therapeutic benefit for treatment of various diseases, including cardiovascular diseases (*e.g.*, coronary artery disease, stroke), autoimmune diseases, inflammatory diseases, type 2 diabetes, infectious disease, and neurodegenerative diseases (*e.g.*, Alzheimer' disease).

**[125]** For example, a bispecific ApoA-1 fusion molecule comprising an RNase segment as described herein may be used, *e.g.*, for treatment of an inflammatory disease such as, for example, an inflammatory lung disease. One study has shown that TLR3, an RNA sensor, has a major role in the development of ARDS-like pathology in the absence of a viral pathogen. *See* Murray *et al.*, *Am. J. Respir. Crit. Care Med.* 178:1227-1237, 2008. Oxygen therapy is a major therapeutic intervention in ARDS, but contributes to further lung damage and susceptibility to viral infection. Oxygen therapy was a major stimulus for increased TLR3 expression and activation in cultured human epithelial cells, and absence or blockade of TLR3 protected mice from lung injury and inflammation after exposure to hyperoxic conditions. *See* Murray *et al.*, *supra*. Another study has shown that TLR3 activation by extracellular RNA occurs in response to acute hypoxia, and that therapy in mice with

RNaseA diminished lung inflammation after acute hypoxia. *See* Biswas *et al.*, *Eur. J. Immunol.* 45: 3158-3173, 2015. A bispecific ApoA-1 fusion molecule comprising an RNase segment as described herein may also be used, *e.g.*, for treatment of an autoimmune disease such as, for example, systemic lupus erythematosus (SLE). Studies show, for example, a role of RNA immune complexes and RNA receptors, including TLR7, in SLE disease pathogenesis, as well as a protective effect of RNase overexpression in mouse models of SLE. *See, e.g.*, Sun *et al.*, *J. Immunol.* 190:2536-2543, 2013.

**[126]** In other embodiments of a bispecific molecule as summarized above, the second polypeptide segment is a paraoxonase. A preferred paraoxonase is human paraoxonase 1 (PON1) or a functional variant or fragment thereof. Paraoxonase bispecific fusion molecules provide additional therapeutic benefit for the treatment of diseases amenable to ApoA-1-mediated therapy, including, for example, through its atheroprotective, antioxidant, anti-inflammatory, and/or neuroprotective properties. In some alternative embodiments, PON1 may attached to an ApoA-1 fusion molecule of the present invention through its natural, high affinity binding to ApoA-1, which binding is mediated by Tyr71 of PON1 (*see* Huang *et al.*, *J. Clin. Invest.* 123:3815-3828, 2013). Incubating an ApoA-1 fusion molecule with recombinant or natural PON1 prior to administration will be sufficient to "load" PON1 onto the ApoA-1 fusion molecule.

**[127]** A bispecific ApoA-1 fusion molecule comprising a paraoxonase segment as described herein may be used, *e.g.*, for treatment of an autoimmune disease or an inflammatory disease. For example, studies support use of a paraoxonase for treatment of autoimmune disease such as systemic lupus erythematosus (SLE). The autoantibody titer in many patients with systemic lupus erythematosus (SLE) is correlated with loss of activity of PON1 (*see* Batukla *et al.*, *Ann. NY Acad. Sci.* 1108:137-146, 2007), and SLE-disease activity assessed by SLEDAI and SLE disease related organ damage assessed by SLICC/ACR damage index are negatively correlated with PON1 activity (*see* Ahmed *et al.*, *EXCLI Journal* 12:719-732, 2013). PON1 activity is significantly reduced in patients with SLE, and is a risk factor for atherosclerosis. *See* Kiss *et al.*, *Ann. NY Acad. Sci.* 108:83-91, 2007. In addition, other studies support use of a paraoxonase for treatment of inflammatory disease such as inflammatory lung diseases. One study showed that patients with late lung diseases long after exposure to sulfur mustard gas (SM), including asthma, chronic obstructive pulmonary disease (COPD) and bronchiectasis, have significantly reduced levels of PON1 in bronchiolar lavage fluid ($p<0.0001$). *See* Golmanesh *et al.*, *Immunopharmacol. Immunotoxical.* 35:419-425, 2013. Another study showed that Iranian veterans exposed to SM twenty years ago still have significantly low serum levels of PON1 activity, and low PON1 was correlated with lung disease severity. *See* Taravati *et al.*, *Immunopharmacol. Immunotoxicol.* 34:706-713, 2012.

**[128]** Bispecific ApoA-1 fusion molecules comprising either an RNase segment or a paraoxonase segment as described herein may also be used, *e.g.*, for treatment of a neurological

disease. Such bispecific molecules are transported to the brain where they deliver a protective paraoxonase or RNase enzyme. For example, PON1 is protective in the brain because of its anti-oxidant properties, and RNase is protective by digesting extracellular RNA that promotes inflammation via stimulation of TLR7 and other RNA receptors. Exemplary neurological diseases amenable to treatment using an ApoA-1/paraoxonase or ApoA1/RNase bispecific molecule of the present invention include multiple sclerosis, Parkinson's disease, and Alzheimer's disease.

**[129]** Attachment of myeloperoxidase (MPO) inhibitors to ApoA-1 fusion molecules of the present invention may be particularly desirable as a way to protect ApoA-1 from inactivation due to oxidation mediated by MPO, and can also similarly protect paraoxonase from MPO-mediated oxidation and inactivation in the context of a bispecific fusion polypeptide comprising a paraoxonase such as PON1. Myeloperoxidase-mediated oxidation of ApoA-1 promotes crosslinking of ApoA-1, and may be implicated in the mechanism that leads to amyloid deposition in atherosclerotic plaques *in vivo*. *See* Chan *et al.*, *J. Biol. Chem.* 290: 10958-71, 2015. For a review of MPO inhibitors, *see* Malle *et al.*, *Br J Pharmacol.* 152: 838-854, 2007. The attachment of a MPO inhibitor to a molecule of the present invention can also localize the MPO inhibition to selectively protect ApoA-1 from oxidation while preserving MPO activity important in anti-microbial activity.

**[130]** In other embodiments of a bispecific molecule as summarized above, the second polypeptide segment is selected from a cholesterol ester transfer protein (CETP), and a lecithin-cholesterol acyltransferase (LCAT). CETP is involved in one of the major mechanisms by which HDL particles can deliver cholesterol to the liver during the process of reverse cholesterol transport (RCT), specifically, through unloading and transferring of cholesterol to LDL, which then transports cholesterol back to the liver via LDL receptors. This process of unloading requires CETP. By improving the initial part of the RCT pathway through the delivery of improved ApoA-1 molecules such as provided herein, then adding other RCT components can provide an attractive and potentially synergistic therapeutic approach. Providing more exogenous CETP in the form of a bispecific fusion molecule containing ApoA-1 can enhance CETP activity and overall reverse cholesterol transport.

**[131]** Bispecific fusions containing LCAT can provide an alternative means of enhancing endogenous CETP. Lecithin-cholesterol acyltransferase (LCAT) is an enzyme that is associated with HDL and converts free cholesterol to cholesteryl esters, which is then sequestered into the HDL particle and allows for its spherical shape formation. A human recombinant LCAT given to mice lacking LCAT significantly improved HDL-C levels, and when given to human ApoA-1 transgenic mice, the increase in HDL-C was eight-fold, suggesting synergy. *See* Rousset *et al.*, *J Pharmacol Exp Ther.* 335:140-8, 2010. A recombinant human LCAT fusion to Fc has been reported (*see* Spahr *et al.*, *Protein Sci.* 22:1739-53, 2013), and a bispecific molecule containing both ApoA-1 and LCAT may also improve RCT more efficiently than a mono-specific protein of either alone.

**[132]** In other embodiments of a bispecific molecule as summarized above, the second polypeptide segment is a polypeptide that specifically binds to amyloid beta (Aβ). In a specific variation, the second polypeptide is a Aβ-specific single chain antibody such as, for example, an Aβ-specific scFv. A scFv specific for amyloid beta peptide is described, for example, by Cattepoel *et al.*, *PLoS One* 6:e18296, 2011. In such embodiments, the Aβ-binding polypeptide is typically fused C-terminal to ApoA-1, or C-terminal to the dimerizing domain, if present. This bispecific fusion molecule has improved properties for therapy of patients with Alzheimer's disease.

II. Fusion Polypeptides and Dimeric Proteins

**[133]** Accordingly, in one aspect, the present invention provides a fusion polypeptide comprising, from an amino-terminal position to a carboxyl-terminal position, ApoA1-L1-D, where ApoA1 is a first polypeptide segment having cholesterol efflux activity and which is selected from (i) a naturally occurring ApoA-1 polypeptide or a functional variant or fragment thereof and (ii) an ApoA-1 mimetic; L1 is a first polypeptide linker; and D is a dimerizing domain. In some embodiments, the fusion polypeptide further includes a second polypeptide segment located carboxyl-terminal to the dimerizing domain. In particular variations, the second polypeptide segment is (a) a naturally occurring RNase, paraoxonase, platelet-activating factor acetylhydrolase (PAF-AH), cholesterol ester transfer protein (CETP), or lecithin-cholesterol acyltransferase (LCAT); (b) a functional variant or fragment of any of the naturally occurring proteins specified in (a); or (c) a polypeptide that specifically binds to amyloid beta (Aβ) such as, *e.g.*, an Aβ-specific scFv. Such a fusion polypeptide comprising a second polypeptide segment may be represented by the formula ApoA1-L1-D-L2-P (from an amino-terminal position to a carboxyl-terminal position), where ApoA1, L1, and D are each as previously defined, where L2 is a second polypeptide linker and is optionally present, and where P is the second polypeptide segment.

**[134]** In another aspect, the present invention provides a fusion polypeptide comprising a first polypeptide segment having cholesterol efflux activity and which is selected from (i) a naturally occurring ApoA-1 polypeptide or a functional variant or fragment thereof and (ii) an ApoA-1 mimetic, and a second polypeptide segment located carboxyl-terminal to the first polypeptide segment, where the second polypeptide segment is (a) a naturally occurring RNase, paraoxonase, platelet-activating factor acetylhydrolase (PAF-AH), cholesterol ester transfer protein (CETP), or lecithin-cholesterol acyltransferase (LCAT); (b) a functional variant or fragment of any of the naturally occurring proteins specified in (a); or (c) a polypeptide that specifically binds to amyloid beta (Aβ) such as, *e.g.*, an Aβ-specific scFv. In some variations, the fusion polypeptide further includes a linker polypeptide located carboxyl-terminal to the first polypeptide segment and amino-

terminal to the second polypeptide segment. In some embodiments, the fusion polypeptide further includes a dimerizing domain, which be located, for example, carboxyl-terminal to the first polypeptide segment and amino-terminal to the second polypeptide segment.

**[135]** Functional variants of a particular naturally occurring protein specified above can be readily identified using routine assays for assessing the variant for a relevant biological or biochemical activity corresponding to the natural protein. For example, in the case of ApoA-1, variants may be assayed for their ability to induce cholesterol efflux using known cholesterol efflux assays such as described herein. *See, e.g.*, Tang *et al.*, *J Lipid Res.* 47:107-14, 2006. In the case of RNase such as human RNase 1, variants may be assayed for their ability to digest single or double-stranded RNA is known assays to assess ribonuclease activity. *See, e.g.*, Libonati and Sorrentino, *Methods Enzymol.* 341234–248, 2001. Paraoxonase 1 (PON1) variants may be assayed for phosphotriesterase activity using diethyl p-nitrophenol phosphate (paraoxon) as a substrate, or for arylesterase activity using phenyl acetate as a substrate. *See, e.g.*, Graves and Scott, *Curr Chem Genomics* 2:51–61, 2008. Assays to assess relevant CETP and LCAT activities are also known. For example, assays for measuring LCAT and CETP enzyme activity are commercially available and include, *e.g.*, Cell Biolabs Cat. No. STA-615, Sigma-Aldrich Cat. No. MAK107, and Roar Biomedical Cat. No. RB-LCAT for LCAT, and Abcam Cat. No. ab65383 and Sigma-Aldrich Cat. No. MAK106 for CETP.

**[136]** In the case of Aβ-binding activity, polypeptides such as, *e.g.*, single chain antibodies may be assessed for binding activity using any of various known assays. For example, one assay system employs a commercially available biosensor instrument (BIAcore™, Pharmacia Biosensor, Piscataway, NJ), wherein a binding protein (*e.g.*, Aβ-binding candidate, such as an antibody) is immobilized onto the surface of a sensor chip, and a test sample containing a soluble antigen (*e.g.*, Aβ peptide) is passed through the cell. If the immobilized protein has affinity for the antigen, it will bind to the antigen, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding. Use of this instrument is disclosed, e.g., by Karlsson (*J. Immunol. Methods* 145:229-240, 1991) and Cunningham and Wells (*J. Mol. Biol.* 234:554-563, 1993). Aβ-binding polypeptides can also be used within other assay systems known in the art. Such systems include Scatchard analysis for determination of binding affinity (*see* Scatchard, *Ann. NY Acad. Sci.* 51: 660-672, 1949) and calorimetric assays (*see* Cunningham *et al., Science* 253:545-548, 1991; Cunningham *et al., Science* 254:821-825, 1991).

**[137]** Naturally occurring polypeptide segments for use in accordance with the present invention (*e.g.*, a naturally occurring ApoA-1 polypeptide, RNase, paraoxonase, or platelet-activating

factor acetylhydrolase) includes naturally occurring variants such as, for example, allelic variants and interspecies homologs consistent with the disclosure.

**[138]** Functional variants of a particular reference polypeptide (*e.g.*, a wild-type human ApoA-1) are generally characterized as having one or more amino acid substitutions, deletions or additions relative to the reference polypeptide. These changes are preferably of a minor nature, that is conservative amino acid substitutions (*see*, *e.g.*, Table 2, *infra*, which lists some exemplary conservative amino acid substitutions) and other substitutions that do not significantly affect the folding or activity of the protein or polypeptide; small deletions, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide, or a small extension that facilitates purification (an affinity tag), such as a poly-histidine tract, protein A (*Nilsson et al., EMBO J.* 4:1075, 1985; Nilsson *et al., Methods Enzymol.* 198:3, 1991), glutathione S transferase (Smith and Johnson, *Gene 67*:31, 1988), or other antigenic epitope or binding domain. (*See generally* Ford *et al., Protein Expression and Purification* 2:95-107, 1991.) DNAs encoding affinity tags are available from commercial suppliers (*e.g.*, Pharmacia Biotech, Piscataway, NJ). Conservative substitutions may also be selected from the following: 1) Alanine, Glycine; 2) Aspartate, Glutamate; 3) Asparagine, Glutamine; 4) Arginine, Lysine; 5) Isoleucine, Leucine, Methionine, Valine; 6) Phenylalanine, Tyrosine, Tryptophan; 7) Serine, Threonine; and 8) Cysteine, Methionine (*see, e.g.*, Creighton, *Proteins* (1984)).

**Table 2: Conservative amino acid substitutions**

| **Basic** | **Acidic** | **Polar** | **Hydrophobic** | **Aromatic** | **Small** |
|---|---|---|---|---|---|
| Arginine | Glutamate | Glutamine | Leucine | Phenylalanine | Glycine |
| Lysine | Aspartate | Asparagine | Isoleucine | Tryptophan | Alanine |
| Histidine | | | Valine | Tyrosine | Serine |
| | | | Methionine | | Threonine |
| | | | | | Methionine |

**[139]** Essential amino acids in a naturally occurring polypeptide can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* 244:1081-1085, 1989; Bass *et al., Proc. Natl. Acad. Sci. USA* 88:4498-4502, 1991). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity (*e.g.*, cholesterol efflux for ApoA-1 variants) to identify amino acid residues that are critical to the activity of the molecule. In addition, sites of relevant protein interactions can be determined by analysis of crystal structure as determined by such techniques as nuclear magnetic resonance, crystallography or

photoaffinity labeling. The identities of essential amino acids can also be inferred from analysis of homologies with related proteins (*e.g.*, species orthologs retaining the same protein function).

**[140]** Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer *Science* 241:53-57, 1988 or Bowie and Sauer *Proc. Natl. Acad. Sci. USA* 86:2152-2156, 1989. Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Another method that can be used is region-directed mutagenesis (Derbyshire *et al., Gene* 46:145, 1986; Ner *et al., DNA* 7:127, 1988).

**[141]** Variant nucleotide and polypeptide sequences can also be generated through DNA shuffling. (*See, e.g.*, Stemmer, *Nature* 370:389, 1994; Stemmer, *Proc. Nat'l Acad. Sci. USA* 91:10747, 1994; International Publication No. WO 97/20078.) Briefly, variant DNA molecules are generated by *in vitro* homologous recombination by random fragmentation of a parent DNA followed by reassembly using PCR, resulting in randomly introduced point mutations. This technique can be modified by using a family of parent DNA molecules, such as allelic variants or DNA molecules from different species, to introduce additional variability into the process. Selection or screening for the desired activity, followed by additional iterations of mutagenesis and assay provides for rapid "evolution" of sequences by selecting for desirable mutations while simultaneously selecting against detrimental changes.

**[142]** As previously discussed, a polypeptide fusion in accordance with the present invention can include a polypeptide segment corresponding to a "functional fragment" of a particular polypeptide. Routine deletion analyses of nucleic acid molecules can be performed to obtain functional fragments of a nucleic acid molecule encoding a given polypeptide. As an illustration, ApoA-1-encoding DNA molecules having the nucleotide sequence of residues 70-816 of SEQ ID NO:1 can be digested with *Bal*31 nuclease to obtain a series of nested deletions. The fragments are then inserted into expression vectors in proper reading frame, and the expressed polypeptides are isolated and tested for the ability to induce cholesterol efflux. One alternative to exonuclease digestion is to use oligonucleotide-directed mutagenesis to introduce deletions or stop codons to specify production of a desired fragment. Alternatively, particular fragments of a gene encoding a polypeptide can be synthesized using the polymerase chain reaction.

**[143]** Accordingly, using methods such as discussed above, one of ordinary skill in the art can prepare a variety of polypeptides that (i) are substantially identical to a reference polypeptide (*e.g.*, residues 19-267 or 25-267 of SEQ ID NO:2 for a human wild-type ApoA-1 polypeptide) and (ii) retains the desired functional properties of the reference polypeptide.

**[144]** Polypeptide segments used within the present invention (*e.g.*, polypeptide segments corresponding to ApoA-1, RNase, paraoxonase, platelet-activating factor acetylhydrolase, dimerizing domains such as, *e.g.*, Fc fragments) may be obtained from a variety of species. If the protein is to be used therapeutically in humans, it is preferred that human polypeptide sequences be employed. However, non-human sequences can be used, as can variant sequences. For other uses, including *in vitro* diagnostic uses and veterinary uses, polypeptide sequences from humans or non-human animals can be employed, although sequences from the same species as the patient may be preferred for *in vivo* veterinary use or for *in vitro* uses where species specificity of intermolecular reactions is present. Thus, polypeptide segments for use within the present invention can be, without limitation, human, non-human primate, rodent, canine, feline, equine, bovine, ovine, porcine, lagomorph, and avian polypeptides, as well as variants thereof.

**[145]** In certain embodiments, the first polypeptide segment is a human wild-type ApoA-1 polypeptide or a functional variant or fragment thereof. For example, in some embodiments, the first polypeptide segment comprises an amino acid sequence having at least 80% identity with amino acid residues 19-267 or 25-267 of SEQ ID NO:2. In more particular embodiments, the first polypeptide segment comprises an amino acid sequence having at least 85%, at least 90%, or at least 95% identity with amino acid residues 19-267 or 25-267 of SEQ ID NO:2. In yet other embodiments, the first polypeptide segment comprises an amino acid sequence having at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with amino acid residues 19-267 or 25-267 of SEQ ID NO:2. In specific variations, valine at the amino acid position corresponding to position 156 of mature human wild-type ApoA-1 is replaced by lysine, and/or arginine at the amino acid position corresponding to position 173 of mature human wild-type ApoA-1 is replaced by cysteine (also referred to herein, respectively, as V156K and R173C variants or mutations). Position 156 of the mature human wild-type ApoA-1 corresponds to amino acid position 180 of SEQ ID NO:2, and position 173 of mature human wild-type ApoA-1 corresponds to amino acid position 197 of SEQ ID NO:2. V156K and R173C mutations have improved activity and half-life in atherosclerotic mice compared to wild-type ApoA-1. *See* Cho *et al.*, *Exp Mol Med* 41:417, 2009.

**[146]** In other embodiments, the first polypeptide segment is an ApoA-1 mimetic such as, for example, the 4F peptide (*see* Song *et al.*, *Int. J. Biol. Sci.* 5:637-646, 2009). ApoA-1 mimetics are generally known in the art and are reviewed in Reddy *et al.*, *Curr. Opin. Lipidol.* 25: 304-308, 2014.

**[147]** In certain embodiments comprising a second polypeptide segment carboxyl-terminal to the first polypeptide segment (*e.g.*, carboxyl-terminal to a dimerizing domain), the second polypeptide segment is an RNase. In some embodiments, the RNase is a human RNAse 1 or a functional variant or fragment thereof. For example, in some embodiments, the second polypeptide segment comprises an amino acid sequence having at least 80% identity with amino acid residues

542-675 of SEQ ID NO:4. In more particular embodiments, the second polypeptide segment comprises an amino acid sequence having at least 85%, at least 90%, or at least 95% identity with amino acid residues 542-675 of SEQ ID NO:4. In yet other embodiments, the second polypeptide segment comprises an amino acid sequence having at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with amino acid residues 542-675 of SEQ ID NO:4.

**[148]** In other embodiments comprising a second polypeptide segment carboxyl-terminal to the first polypeptide segment (*e.g.*, carboxyl-terminal to a dimerizing domain), the second polypeptide segment is a paraoxonase. In some embodiments, the paraoxonase is a human paraoxonase 1 (PON1) or a functional variant or fragment thereof. For example, in some embodiments, the second polypeptide segment comprises an amino acid sequence having at least 80% identity with amino acid residues 16-355 of SEQ ID NO:12, amino acid residues 16-355 of SEQ ID NO:42, or amino acid residues 16-355 of SEQ ID NO:44. In more particular embodiments, the second polypeptide segment comprises an amino acid sequence having at least 85%, at least 90%, or at least 95% identity with amino acid residues 16-355 of SEQ ID NO:12, amino acid residues 16-355 of SEQ ID NO:42, or amino acid residues 16-355 of SEQ ID NO:44. In yet other embodiments, the second polypeptide segment comprises an amino acid sequence having at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with amino acid residues 16-355 of SEQ ID NO:12, amino acid residues 16-355 of SEQ ID NO:42, or amino acid residues 16-355 of SEQ ID NO:44.

**[149]** In yet other embodiments comprising a second polypeptide segment carboxyl-terminal to the first polypeptide segment (*e.g.*, carboxyl-terminal to a dimerizing domain), the second polypeptide segment is a platelet-activating factor acetylhydrolase (PAF-AH). In some embodiments, the platelet-activating factor acetylhydrolase is a human PAF-AH or a functional variant or fragment thereof. For example, in some embodiments, the second polypeptide segment comprises an amino acid sequence having at least 80% identity with amino acid residues 22-441 of SEQ ID NO:32. In more particular embodiments, the second polypeptide segment comprises an amino acid sequence having at least 85%, at least 90%, or at least 95% identity with amino acid residues 22-441 of SEQ ID NO:32. In yet other embodiments, the second polypeptide segment comprises an amino acid sequence having at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with amino acid residues 22-441 of SEQ ID NO:32.

**[150]** In still other embodiments comprising a second polypeptide segment carboxyl-terminal to the first polypeptide segment (*e.g.*, carboxyl-terminal to a dimerizing domain), the second polypeptide segment is a cholesterol ester transfer protein (CETP). In some embodiments, the cholesterol ester transfer protein is a human CETP or a functional variant or fragment thereof. For example, in some embodiments, the second polypeptide segment comprises an amino acid sequence having at least 80% identity with amino acid residues 18-493 of SEQ ID NO:30. In more particular

embodiments, the second polypeptide segment comprises an amino acid sequence having at least 85%, at least 90%, or at least 95% identity with amino acid residues 18-493 of SEQ ID NO:30. In yet other embodiments, the second polypeptide segment comprises an amino acid sequence having at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with amino acid residues 18-493 of SEQ ID NO:30.

**[151]** Polypeptide linkers for use in accordance with the present invention can be naturally-occurring, synthetic, or a combination of both. The linker joins two separate polypeptide regions (*e.g.*, a dimerizing domain and an ApoA-1 polypeptide) and maintains the linked polypeptide regions as separate and discrete domains of a longer polypeptide. The linker can allow the separate, discrete domains to cooperate yet maintain separate properties (*e.g.*, in the case of an Fc region dimerizing domain linked to an ApoA-1 polypeptide, Fc receptor (*e.g.*, FcRn) binding may be maintained for the Fc region, while functional properties of the ApoA-1 polypeptide (*e.g.*, lipid binding) will be maintained). For examples of the use of naturally occurring as well as artificial peptide linkers to connect heterologous polypeptides, *see, e.g.*, Hallewell *et al.*, *J. Biol. Chem.* 264, 5260-5268, 1989; Alfthan *et al.*, *Protein Eng.* 8, 725-731, 1995; Robinson and Sauer, *Biochemistry* 35, 109-116, 1996; Khandekar *et al.*, *J. Biol. Chem.* 272, 32190-32197, 1997; Fares *et al.*, *Endocrinology* 139, 2459-2464, 1998; Smallshaw *et al.*, *Protein Eng.* 12, 623-630, 1999; U.S. Patent No. 5,856,456.

**[152]** Typically, residues within the linker polypeptide are selected to provide an overall hydrophilic character and to be non-immunogenic and flexible. As used herein, a "flexible" linker is one that lacks a substantially stable higher-order conformation in solution, although regions of local stability are permissible. In general, small, polar, and hydrophilic residues are preferred, and bulky and hydrophobic residues are undesirable. Areas of local charge are to be avoided; if the linker polypeptide includes charged residues, they will ordinarily be positioned so as to provide a net neutral charge within a small region of the polypeptide. It is therefore preferred to place a charged residue adjacent to a residue of opposite charge. In general, preferred residues for inclusion within the linker polypeptide include Gly, Ser, Ala, Thr, Asn, and Gln; more preferred residues include Gly, Ser, Ala, and Thr; and the most preferred residues are Gly and Ser. In general, Phe, Tyr, Trp, Pro, Leu, Ile, Lys, and Arg residues will be avoided (unless present within an immunoglobulin hinge region of the linker), Pro residues due to their hydrophobicity and lack of flexibility, and Lys and Arg residues due to potential immunogenicity. The sequence of the linker will also be designed to avoid unwanted proteolysis.

**[153]** In certain embodiments, linker L1 comprises at least two or at least three amino acid residues (*e.g.*, at least five, at least 10, at least 16, at least 26, or at least 36 amino acid residues). In particular variations, L1 consists of from two to 60 amino acid residues, from three to 60 amino acid residues, from five to 40 amino acid residues, or from 15 to 40 amino acid residues. In other

variations, L1 consists of from two to 50, from two to 40, from two to 36, from two to 35, from two to 30, from two to 26, from three to 50, from three to 40, from three to 36, from three to 35, from three to 30, from three to 26, from five to 60, from five to 50, from five to 40, from five to 36, from five to 35, from five to 30, from five to 26, from 10 to 60, from 10 to 50, from 10 to 40, from 10 to 36, from 10 to 35, from 10 to 30, from 10 to 26, from 15 to 60, from 15 to 50, from 15 to 36, from 15 to 35, from 15 to 30, or from 15 to 26 amino acid residues. In other variations, L1 consists of from 16 to 60, from 16 to 50, from 16 to 40, or from 16 to 36 amino acid residues. In yet other variations, L1 consists of from 20 to 60, from 20 to 50, from 20 to 40, from 20 to 36, from 25 to 60, from 25 to 50, from 25 to 40, or from 25 to 36 amino acid residues. In still other variations, L1 consists of from 26 to 60, from 26 to 50, from 26 to 40, or from 26 to 36 amino acid residues. In more specific variations, L1 consists of 16 amino acid residues, 21 amino acid residues, 26 amino acid residues, 31 amino acid residues, or 36 amino acid residues. In some embodiments, L1 comprises or consists of the amino acid sequence shown in residues 268-293 of SEQ ID NO:2, residues 268-288 of SEQ ID NO:26, residues 268-283 of SEQ ID NO:22, SEQ ID NO:54, or residues 268-303 of SEQ ID NO:24.

**[154]** Exemplary L2 linkers comprise at least three amino acid residues and are typically up to 60 amino acid residues. In certain variations, L2 linkers have a range of sequence lengths as described above for L1. In a specific embodiment of a polypeptide comprising the formula ApoA1-L1-D-L2-P and where L2 is present and P is an RNase, L2 comprises or consists of the amino acid sequence shown in residues 526-541 of SEQ ID NO:4.

**[155]** In certain embodiments, polypeptide linkers comprise a plurality of glycine resides. For example, in some embodiments, a polypeptide linker (*e.g.*, L1) comprises a plurality of glycine residues and optionally at least one serine residue. In particular variations, a polypeptide linker (*e.g.*, L1) comprises the sequence Gly-Gly-Gly-Gly-Ser (SEQ ID NO:15), such as, *e.g.*, two or more tandem repeats of the amino acid sequence of SEQ ID NO:15. In some embodiments, a linker comprises the sequence $[Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser]_n$ ($[SEQ\ ID\ NO:15]_n$), where n is a positive integer such as, for example, an integer from 1 to 5, from 2 to 5, from 3 to 5, from 1 to 6, from 2 to 6, from 3 to 6, or from 4 to 6. In a specific variation of a polypeptide linker comprising the formula $[Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser]_n$, n is 4. In another specific variation of a polypeptide linker comprising the formula $[Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser]_n$, n is 3. In yet another specific variation of a polypeptide linker comprising the formula $[Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser]_n$, n is 5. In still another specific variation of a polypeptide linker comprising the formula $[Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser]_n$, n is 6. In certain embodiments, a polypeptide linker comprises a series of glycine and serine residues (*e.g.*, $[Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser]_n$, where n is defined as above) inserted between two other sequences of the polypeptide linker (*e.g.*, any of the polypeptide linker sequences described herein). In other embodiments, a polypeptide linker includes glycine and serine residues (*e.g.*, $[Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser]_n$, where n is defined as above) attached at one or both

ends of another sequence of the polypeptide linker (*e.g.*, any of the polypeptide linker sequences described herein). In one embodiment, a polypeptide linker comprises at least a portion of an upper hinge region (*e.g.*, derived from an IgG1, IgG2, IgG3, or IgG4 molecule), at least a portion of a middle hinge region (*e.g.*, derived from an IgG1, IgG2, IgG3, or IgG4 molecule) and a series of glycine and serine amino acid residues (*e.g.*, $[Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser]_n$, wherein n is defined as above).

**[156]** In another embodiment, a polypeptide linker comprises a non-naturally occurring immunoglobulin hinge region, *e.g.*, a hinge region that is not naturally found in an immunoglobulin and/or a hinge region that has been altered so that it differs in amino acid sequence from a naturally occurring immunoglobulin hinge region. In one embodiment, mutations can be made to a hinge region to make a polypeptide linker. In one embodiment, a polypeptide linker comprises a hinge domain that does not comprise a naturally occurring number of cysteines, *i.e.*, the polypeptide linker comprises either fewer cysteines or a greater number of cysteines than a naturally occurring hinge molecule.

**[157]** Various dimerization domains are suitable for use in accordance with the fusion polypeptides and dimeric fusion proteins as described herein. In certain embodiments, the dimerizing domain is an immunoglobulin heavy chain constant region, such as an Fc region. The Fc region may be a native sequence Fc region or a variant Fc region. In some embodiments, the Fc region lacks one or more effector functions (*e.g.*, one or both of ADCC and CDC effector functions).

**[158]** In some embodiments, the dimerizing domain is an Fc region of a human antibody with a mutation in the CH2 region so that the molecule is not glycosylated, including but not limited to N297 (EU numbering for human IgG heavy chain constant region) (corresponding to amino acid position 375 of SEQ ID NO:2). In another embodiment, the Fc region is human IgG1 (γ1) with the three cysteines of the hinge region (C220, C226, C229) each changed to serine, and the proline at position 238 of the CH2 domain changed to serine. In another preferred embodiment, the Fc region is human γ1 with N297 changed to any other amino acid. In another embodiment, the Fc region is human γ1 with one or more amino acid substitutions between Eu positions 292 and 300. In another embodiment, the Fc region is human γ1 with one or more amino acid additions or deletions at any position between residues 292 and 300. In another embodiment, the Fc region is human γ1 with an SCC hinge (*i.e.*, with cysteine C220 changed to serine and with a cysteine at each of Eu positions 226 and 229) or an SSS hinge (*i.e.*, each of the three cysteines at Eu positions 220, 226, and 229 changed to serine). In further embodiments, the Fc region is human γ1 with an SCC hinge and a P238 mutation. In another embodiment, the Fc domain is human γ1 with mutations that alter binding by Fc gamma receptors (I, II, III) without affecting FcRn binding important for half-life. In further embodiments, an Fc region is as disclosed in Ehrhardt and Cooper, *Curr. Top. Microbiol. Immunol.* 2010 Aug. 3 (Immunoregulatory Roles for Fc Receptor-Like Molecules); Davis *et al.*, *Ann. Rev.*

*Immunol.* 25:525-60, 2007 (Fc receptor-like molecules); or Swainson *et al.*, *J. Immunol.* 184:3639-47, 2010.

**[159]** In some embodiments of a fusion polypeptide comprising an Fc dimerizing domain, the Fc region comprises an amino acid substitution that alters the antigen-independent effector functions of the fusion protein. In some such embodiments, the Fc region includes an amino acid substitution that alters the circulating half-life of the resulting molecule. Such antibody derivatives exhibit either increased or decreased binding to FcRn when compared to antibodies lacking these substitutions and, therefore, have an increased or decreased half-life in serum, respectively. Fc variants with improved affinity for FcRn are anticipated to have longer serum half-lives, and such antibodies have useful applications in methods of treating mammals where long half-life of the administered antibody is desired. In contrast, Fc variants with decreased FcRn binding affinity are expected to have shorter half-lives, and such antibodies are also useful, for example, for administration to a mammal where a shortened circulation time may be advantageous, *e.g.*, where the starting antibody has toxic side effects when present in the circulation for prolonged periods. Fc variants with decreased FcRn binding affinity are also less likely to cross the placenta and, thus, are also useful in the treatment of diseases or disorders in pregnant women. In addition, other applications in which reduced FcRn binding affinity may be desired include those applications in which localization the brain, kidney, and/or liver is desired. In one exemplary embodiment, the antibodies of the invention exhibit reduced transport across the epithelium of kidney glomeruli from the vasculature. In another embodiment, the fusion proteins of the invention exhibit reduced transport across the blood brain barrier (BBB) from the brain, into the vascular space. In one embodiment, a fusion protein with altered FcRn binding comprises an Fc region having one or more amino acid substitutions within the "FcRn binding loop" of the Fc domain. Exemplary amino acid substitutions which altered FcRn binding activity are disclosed in International PCT Publication No. WO 05/047327, which is incorporated by reference herein.

**[160]** In other embodiments, a fusion polypeptide of the present invention comprises an Fc variant comprising an amino acid substitution which alters the antigen-dependent effector functions of the polypeptide, in particular ADCC or complement activation, *e.g.*, as compared to a wild type Fc region. In an exemplary embodiment, such fusion polypeptides exhibit altered binding to an Fc gamma receptor (FcγR, *e.g.*, CD16). Such fusion polypeptides exhibit either increased or decreased binding to FcγR when compared to wild-type polypeptides and, therefore, mediate enhanced or reduced effector function, respectively. Fc variants with improved affinity for FcγRs are anticipated to enhance effector function, and such fusion proteins have useful applications in methods of treating mammals where target molecule destruction is desired. In contrast, Fc variants with decreased FcγR binding affinity are expected to reduce effector function, and such fusion proteins are also useful, for

example, for treatment of conditions in which target cell destruction is undesirable, *e.g.*, where normal cells may express target molecules, or where chronic administration of the antibody might result in unwanted immune system activation. In one embodiment, the fusion polypeptide comprising an Fc region exhibits at least one altered antigen-dependent effector function selected from the group consisting of opsonization, phagocytosis, complement dependent cytotoxicity, antigen-dependent cellular cytotoxicity (ADCC), or effector cell modulation as compared to a polypeptide comprising a wild-type Fc region.

**[161]** In one embodiment, a fusion polypeptide comprising an Fc region exhibits altered binding to an activating FcγR (*e.g.*, FcγI, FcγIIa, or FcγRIIIa). In another embodiment, the fusion protein exhibits altered binding affinity to an inhibitory FcγR (*e.g.*, FcγRIIb). Exemplary amino acid substitutions which altered FcR or complement binding activity are disclosed in International PCT Publication No. WO 05/063815, which is incorporated by reference herein.

**[162]** A fusion polypeptide comprising an Fc region may also comprise an amino acid substitution that alters the glycosylation of the Fc region. For example, the Fc domain of the fusion protein may have a mutation leading to reduced glycosylation (*e.g.*, N- or O-linked glycosylation) or may comprise an altered glycoform of the wild-type Fc domain (*e.g.*, a low fucose or fucose-free glycan). In another embodiment, the molecule has an amino acid substitution near or within a glycosylation motif, for example, an N-linked glycosylation motif that contains the amino acid sequence NXT or NXS. Exemplary amino acid substitutions which reduce or alter glycosylation are disclosed in International PCT Publication No. WO 05/018572 and US Patent Application Publication No. 2007/0111281, which are incorporated by reference herein.

**[163]** It will be understood by those of skill in the art that various embodiments of Fc variants as described herein can be combined in the fusion polypeptides of the present invention, unless the context clearly indicates otherwise.

**[164]** In some embodiments, a dimerizing domain is an Fc region comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, or at least 95% identity with an amino acid sequence selected from sequence shown in (i) residues 294-525 or 294-524 of SEQ ID NO:2, or (ii) residues 294-525 or 294-524 of SEQ ID NO:13. In yet other embodiments, the Fc region comprises an amino acid sequence having at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence shown in (i) residues 294-525 or 294-524 of SEQ ID NO:2, or (ii) residues 294-525 or 294-524 of SEQ ID NO:13.

**[165]** In some embodiments of a fusion polypeptide comprising ApoA1-L1-D as described above, the fusion polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, or at least 95% identity with an amino acid sequence selected from sequence shown in (i)

residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:2, (ii) residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:13, (iii) residues 19-501, 19-500, 25-501, or 25-501 of SEQ ID NO:20, (iv) residues 19-515, 19-514, 25-515, or 25-514 of SEQ ID NO:22, (v) residues 19-520, 19-519, 25-520, or 25-519 of SEQ ID NO:26, or (vi) residues 19-535, 19-534, 25-535, or 25-534 of SEQ ID NO:24. In yet other embodiments, the fusion polypeptide comprises an amino acid sequence having at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence shown in (i) residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:2, (ii) residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:13, (iii) residues 19-501, 19-500, 25-501, or 25-501 of SEQ ID NO:20, (iv) residues 19-515, 19-514, 25-515, or 25-514 of SEQ ID NO:22, (v) residues 19-520, 19-519, 25-520, or 25-519 of SEQ ID NO:26, or (vi) residues 19-535, 19-534, 25-535, or 25-534 of SEQ ID NO:24.

**[166]** In some embodiments of a fusion polypeptide comprising ApoA1-L1-D-L2-P as described above and where P is an RNase, the fusion polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, or at least 95% identity with the amino acid sequence shown in (i) residues 19-675 or 25-675 of SEQ ID NO:4 or (ii) residues 19-675 or 25-675 of SEQ ID NO:14. In yet other embodiments, the fusion polypeptide comprises an amino acid sequence having at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence shown in (i) residues 19-675 or 25-675 of SEQ ID NO:4 or (ii) residues 19-675 or 25-675 of SEQ ID NO:14.

**[167]** In some embodiments of a fusion polypeptide comprising ApoA1-L1-D-L2-P as described above and where P is a paraoxonase, the fusion polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, or at least 95% identity with the amino acid sequence shown in (i) residues 19-883 or 25-883 of SEQ ID NO:28, (ii) residues 19-873 or 25-873 of SEQ ID NO:38, (iii) residues 19-883 or 25-883 of SEQ ID NO:46, or (iv) residues 19-883 or 25-883 of SEQ ID NO:48. In yet other embodiments, the fusion polypeptide comprises an amino acid sequence having at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence shown in (i) residues 19-883 or 25-883 of SEQ ID NO:28, (ii) residues 19-873 or 25-873 of SEQ ID NO:38, (iii) residues 19-883 or 25-883 of SEQ ID NO:46, or (iv) residues 19-883 or 25-883 of SEQ ID NO:48.

**[168]** In some embodiments of a fusion polypeptide comprising ApoA1-L1-D-L2-P as described above and where P is a platelet-activating factor acetylhydrolase (PAF-AH), the fusion polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, or at least 95% identity with the amino acid sequence shown in residues 19-963 or 25-963 of SEQ ID NO:34. In yet other embodiments, the fusion polypeptide comprises an amino acid sequence having

at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence shown in residues 19-963 or 25-963 of SEQ ID NO:34.

**[169]** In some embodiments of a fusion polypeptide comprising ApoA1-L1-D-L2-P as described above and where P is a cholesterol ester transfer protein (CETP), the fusion polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, or at least 95% identity with the amino acid sequence shown in residues 19-1019 or 25-1019 of SEQ ID NO:40. In yet other embodiments, the fusion polypeptide comprises an amino acid sequence having at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence shown in residues 19-1019 or 25-1019 of SEQ ID NO:40.

**[170]** The present invention also provides dimeric proteins comprising first and second polypeptide fusions as described above. Accordingly, in another aspect, the present invention provides a dimeric protein comprising a first fusion polypeptide and a second fusion polypeptide, where each of the first and second polypeptide fusions comprises, from an amino-terminal position to a carboxyl-terminal position, ApoA1-L1-D, where ApoA1 is a first polypeptide segment having cholesterol efflux activity and which is selected from (i) a naturally occurring ApoA-1 polypeptide or a functional variant or fragment thereof and (ii) an ApoA-1 mimetic; L1 is a first polypeptide linker; and D is a dimerizing domain. In some embodiments, each of the first and second fusion polypeptides further includes a second polypeptide segment located carboxyl-terminal to the dimerizing domain. In particular variations, the second polypeptide segment is (a) a naturally occurring RNase, paraoxonase, platelet-activating factor acetylhydrolase (PAF-AH), cholesterol ester transfer protein (CETP), or lecithin-cholesterol acyltransferase (LCAT); (b) a functional variant or fragment of any of the naturally occurring proteins specified in (a); or (c) a polypeptide that specifically binds to amyloid beta (Aβ) such as, *e.g.*, an Aβ-specific scFv. Such a fusion polypeptide comprising a second polypeptide segment may be represented by the formula ApoA1-L1-D-L2-P (from an amino-terminal position to a carboxyl-terminal position), where ApoA1, L1, and D are each as previously defined, where L2 is a second polypeptide linker and is optionally present, and where P is the second polypeptide segment.

**[171]** In another aspect, the present invention provides a dimeric protein comprising a first fusion polypeptide and a second fusion polypeptide, where each of the first and second fusion polypeptides comprises a first polypeptide segment, a second polypeptide segment, and a dimerizing domain, where the first polypeptide segment has cholesterol efflux activity and is selected from (i) a naturally occurring ApoA-1 polypeptide or a functional variant or fragment thereof and (ii) an ApoA-1 mimetic, and where the second polypeptide segment is located carboxyl-terminal to the first polypeptide segment and is (a) a naturally occurring RNase, paraoxonase, platelet-activating factor acetylhydrolase (PAF-AH), cholesterol ester transfer protein (CETP), or lecithin-cholesterol

acyltransferase (LCAT), (b) a functional variant or fragment of any of the naturally occurring proteins specified in (a), or (c) a polypeptide that specifically binds to amyloid beta (Aβ) such as, *e.g.*, an Aβ-specific scFv. In some embodiments, the dimerizing domain is located carboxyl-terminal to the first polypeptide segment and amino-terminal to the second polypeptide segment.

**[172]** In another aspect, the present invention provides (a) a first fusion polypeptide comprising an immunoglobulin heavy chain linked carboxyl-terminal to an ApoA-1 polypeptide or ApoA-1 mimetic and (b) a second fusion polypeptide comprising an immunoglobulin light chain linked carboxyl-terminal to the ApoA-1 polypeptide or ApoA-1 mimetic. The first and second fusion polypeptides can be co-expressed to create a stable tetramer composed of two double belt ApoA-1 dimers, wherein linkers between ApoA-1 and the heavy chain and between ApoA-1 and the light chain are of sufficient length to allow cholesterol efflux and reverse cholesterol transport.

**[173]** The fusion polypeptides of the present invention, including dimeric fusion proteins, can further be conjugated to an effector moiety. The effector moiety can be any number of molecules, including, *e.g.*, a labeling moiety such as a radioactive label or fluorescent label, a TLR ligand or binding domain, an enzyme, or a therapeutic moiety. In a particular embodiment, the effector moiety is a myeloperoxidase (MPO) inhibitor. MPO inhibitors are generally known (*see, e.g.*, Malle *et al.*, *Br J Pharmacol.* 152: 838-854, 2007) and may be readily conjugated to fusion polypeptides as described herein. Exemplary MPO inhibitors include inhibitors based on 3-alkylindole derivatives (*see* Soubhye *et al.*, *J Med Chem* 56:3943-58, 2013; describing studies of 3-alkylindole derivatives as selective and highly potent myeloperoxidase inhibitors, including a compound with high and selective inhibition of MPO (IC50 =18nM)); inhibitors based on 3-(aminoalkyl)-5-fluorindoles (*see* Soubhye *et al.*, *J Med Chem* 53: 8747-8759, 2010); inhibitors based on 2H-indazoles and 1H-indazolones (*see* Roth *et al.*, *Bioorg Med Chem* 22: 6422-6429, 2014; describing the evaluation 2H-indazoles and 1H-indazolones and the identification of compounds with IC50 values <1μM); and benzoic acid hydrazide-containing compounds (*see* Huang *et al.*, *Arch Biochem Biophys* 570: 14-22, 2015; showing inactivation of MPO by benzoic acid hydrazide-containing compounds, where the light chain subunit of MPO is freed from the larger heavy chain by cleavage of the ester bond)..

**[174]** In another embodiment, the fusion polypeptides of the present invention, including dimeric fusion proteins, are modified to extend half-life, such as, for example, by attaching at least one molecule to the fusion protein for extending serum half-life. Such molecules for attachment may include, *e.g.*, a polyethlyene glycol (PEG) group, serum albumin, transferrin, transferrin receptor or the transferrin-binding portion thereof, or a combination thereof. Methods for such modification are generally well-known in the art. As used herein, the word "attached" refers to a covalently or noncovalently conjugated substance. The conjugation may be by genetic engineering or by chemical means.

III. Materials and Methods for Making Polypeptide Fusions and Dimeric Proteins

**[175]** The present invention also provides polynucleotide molecules, including DNA and RNA molecules, that encode the fusion polypeptides disclosed above. The polynucleotides of the present invention include both single-stranded and double-stranded molecules. Polynucleotides encoding various segments of a fusion polypeptide (*e.g.*, a dimerizing domain such as an Fc fragment; ApoA1 and P polypeptide segments) can be generated and linked together to form a polynucleotide encoding a fusion polypeptide as described herein using known methods for recombinant manipulation of nucleic acids.

**[176]** DNA sequences encoding ApoA-1, RNases (*e.g.*, RNase 1), paraoxonases (*e.g.*, PON1), platelet-activating factor acetylhydrolase (PAF-AH), cholesterol ester transfer protein (CETP), and lecithin-cholesterol acyltransferase (LCAT) are known in the art. DNA sequences encoding various dimerizing domains (*e.g.*, immunoglobulin heavy chain constant regions such as Fc fragments) are also known. Polynucleotides encoding, *e.g.*, the variable regions of Aβ-binding antibodies, including scFvs, are also readily identifiable using techniques well-known in the art such as screening of recombinant antibody expression libraries (*e.g.*, phage display expression libraries). Additional DNA sequences encoding any of these polypeptides can be readily generated by those of ordinary skill in the art based on the genetic code. Counterpart RNA sequences can be generated by substitution of U for T. Those skilled in the art will readily recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among polynucleotide molecules encoding a given polypeptide. DNA and RNA encoding functional variants and fragments of such polypeptides can also be obtained using known recombinant methods to introduce variation into a polynucleotide sequence, followed by expression of the encoded polypeptide and determination of functional activity (*e.g.*, cholesterol efflux) using an appropriate screening assay.

**[177]** Methods for preparing DNA and RNA are well known in the art. For example, complementary DNA (cDNA) clones can be prepared from RNA that is isolated from a tissue or cell that produces large amounts of RNA encoding a polypeptide of interest. Total RNA can be prepared using guanidine HCl extraction followed by isolation by centrifugation in a CsCl gradient (Chirgwin et al., *Biochemistry* 18:52-94, 1979). Poly $(A)^+$ RNA is prepared from total RNA using the method of Aviv and Leder (*Proc. Natl. Acad. Sci. USA* 69:1408-1412, 1972). Complementary DNA is prepared from poly$(A)^+$ RNA using known methods. In the alternative, genomic DNA can be isolated. Methods for identifying and isolating cDNA and genomic clones are well known and within the level of ordinary skill in the art, and include the use of the sequences disclosed herein, or parts thereof, for probing or priming a library. Polynucleotides encoding polypeptides of interest are identified and isolated by, for example, hybridization or polymerase chain reaction ("PCR," Mullis, U.S. Patent

4,683,202). Expression libraries can be probed with antibodies to the polypeptide of interest, receptor fragments, or other specific binding partners.

**[178]** The polynucleotides of the present invention can also be prepared by automated synthesis. The production of short, double-stranded segments (60 to 80 bp) is technically straightforward and can be accomplished by synthesizing the complementary strands and then annealing them. Longer segments (typically >300 bp) are assembled in modular form from single-stranded fragments that are from 20 to 100 nucleotides in length. Automated synthesis of polynucleotides is within the level of ordinary skill in the art, and suitable equipment and reagents are available from commercial suppliers. *See generally* Glick and Pasternak, *Molecular Biotechnology, Principles & Applications of Recombinant DNA*, ASM Press, Washington, D.C., 1994; Itakura *et al.*, *Ann. Rev. Biochem.* 53:323-356, 1984; and Climie *et al.*, *Proc. Natl. Acad. Sci. USA* 87:633-637, 1990.

**[179]** In another aspect, materials and methods are provided for producing the polypeptide fusions of the present invention, including dimeric proteins comprising the fusion polypeptides. The fusion polypeptides can be produced in genetically engineered host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells (including cultured cells of multicellular organisms), particularly cultured mammalian cells. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, and Ausubel et al., eds., *Current Protocols in Molecular Biology*, Green and Wiley and Sons, NY, 1993.

**[180]** In general, a DNA sequence encoding a fusion polypeptide is operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator, within an expression vector. The vector will also commonly contain one or more selectable markers and one or more origins of replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers.

**[181]** To direct an ApoA-1 fusion polypeptide into the secretory pathway of a host cell, a secretory signal sequence is provided in the expression vector. The secretory signal sequence may be that of the native ApoA-1 polypeptide, or may be derived from another secreted protein (*e.g.*, t-PA; *see* U.S. Patent No. 5,641,655) or synthesized *de novo*. An engineered cleavage site may be included

at the junction between the secretory peptide and the remainder of the polypeptide fusion to optimize proteolytic processing in the host cell. The secretory signal sequence is operably linked to the DNA sequence encoding the polypeptide fusion, *i.e.*, the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide fusion into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain signal sequences may be positioned elsewhere in the DNA sequence of interest (*see, e.g.*, Welch *et al.*, U.S. Patent No. 5,037,743; Holland *et al.*, U.S. Patent No. 5,143,830). Secretory signal sequences suitable for use in accordance with the present invention include, for example, polynucleotides encoding amino acid residues 1-18 of SEQ ID NO:2.

**[182]** Expression of fusion polypeptides comprising a dimerizing domain, via a host cell secretory pathway, is expected to result in the production of dimeric proteins. Accordingly, in another aspect, the present invention provides dimeric proteins comprising first and second fusion polypeptides as described above (*e.g.*, a dimeric protein comprising a first fusion polypeptide and a second fusion polypeptide, where each of the first and second fusion polypeptides comprises, from an amino-terminal position to a carboxyl-terminal position, ApoA1-L1-D or ApoA1-L1-D-L2-P as described herein). Dimers may also be assembled *in vitro* upon incubation of component polypeptides under suitable conditions. In general, *in vitro* assembly will include incubating the protein mixture under denaturing and reducing conditions followed by refolding and reoxidation of the polypeptides to form dimers. Recovery and assembly of proteins expressed in bacterial cells is disclosed below.

**[183]** Cultured mammalian cells are suitable hosts for use within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler *et al.*, *Cell* 14:725, 1978; Corsaro and Pearson, *Somatic Cell Genetics* 7:603, 1981: Graham and Van der Eb, *Virology* 52:456, 1973), electroporation (Neumann *et al.*, *EMBO J.* 1:841-845, 1982), DEAE-dextran mediated transfection (Ausubel *et al.*, *supra*), and liposome-mediated transfection (Hawley-Nelson *et al.*, *Focus* 15:73, 1993; Ciccarone et al., *Focus* 15:80, 1993). The production of recombinant polypeptides in cultured mammalian cells is disclosed by, for example, Levinson *et al.*, U.S. Patent No. 4,713,339; Hagen *et al.*, U.S. Patent No. 4,784,950; Palmiter *et al.*, U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134. Suitable cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), 293 (ATCC No. CRL 1573; Graham et al., *J. Gen. Virol.* 36:59-72, 1977) and Chinese hamster ovary (*e.g.*, CHO-K1, ATCC No. CCL 61; CHO-DG44, Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4216-4220, 1980) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Manassas, Virginia. Strong transcription promoters can be used, such as

promoters from SV-40, cytomegalovirus, or myeloproliferative sarcoma virus. *See, e.g.*, U.S. Patent No. 4,956,288 and U.S. Patent Application Publication No. 20030103986. Other suitable promoters include those from metallothionein genes (U.S. Patents Nos. 4,579,821 and 4,601,978) and the adenovirus major late promoter. Expression vectors for use in mammalian cells include pZP-1, pZP-9, and pZMP21, which have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, VA USA under accession numbers 98669, 98668, and PTA-5266, respectively, and derivatives of these vectors.

**[184]** Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as "transfectants." Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." An exemplary selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems can also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. An exemplary amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (*e.g.*, hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used. Cell-surface markers and other phenotypic selection markers can be used to facilitate identification of transfected cells (*e.g.*, by fluorescence-activated cell sorting), and include, for example, CD8, CD4, nerve growth factor receptor, green fluorescent protein, and the like.

**[185]** Other higher eukaryotic cells can also be used as hosts, including insect cells, plant cells and avian cells. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar *et al.*, *J. Biosci. (Bangalore)* 11:47-58, 1987. Transformation of insect cells and production of foreign polypeptides therein is disclosed by Guarino *et al.*, U.S. Patent No. 5,162,222 and WIPO publication WO 94/06463.

**[186]** Insect cells can be infected with recombinant baculovirus, commonly derived from *Autographa californica* nuclear polyhedrosis virus (AcNPV). *See* King and Possee, *The Baculovirus Expression System: A Laboratory Guide*, Chapman & Hall, London; O'Reilly *et al.*, *Baculovirus Expression Vectors: A Laboratory Manual*, Oxford University Press., New York, 1994; and Richardson, Ed., *Baculovirus Expression Protocols. Methods in Molecular Biology*, Humana Press, Totowa, NJ, 1995. Recombinant baculovirus can also be produced through the use of a transposon-based system described by Luckow *et al.* (*J. Virol.* 67:4566-4579, 1993). This system, which utilizes transfer vectors, is commercially available in kit form (BAC-TO-BAC kit; Life Technologies,

Gaithersburg, MD). The transfer vector (*e.g.*, PFASTBAC1; Life Technologies) contains a Tn7 transposon to move the DNA encoding the protein of interest into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." *See* Hill-Perkins and Possee, *J. Gen. Virol.* 71:971-976, 1990; Bonning *et al.*, *J. Gen. Virol.* 75:1551-1556, 1994; and Chazenbalk and Rapoport, *J. Biol. Chem.* 270:1543-1549, 1995. Using techniques known in the art, a transfer vector encoding a polypeptide fusion is transformed into *E. coli* host cells, and the cells are screened for bacmids which contain an interrupted lacZ gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is isolated, using common techniques, and used to transfect *Spodoptera frugiperda* cells, such as Sf9 cells. Recombinant virus that expresses the polypeptide fusion is subsequently produced. Recombinant viral stocks are made by methods commonly used the art.

**[187]** For protein production, the recombinant virus is used to infect host cells, typically a cell line derived from the fall armyworm, *Spodoptera frugiperda* (*e.g.*, Sf9 or Sf21 cells) or *Trichoplusia ni* (*e.g.*, HIGH FIVE cells; Invitrogen, Carlsbad, CA). *See generally* Glick and Pasternak, *supra*. *See also* U.S. Patent No. 5,300,435. Serum-free media are used to grow and maintain the cells. Suitable media formulations are known in the art and can be obtained from commercial suppliers. The cells are grown up from an inoculation density of approximately 2-5 x $10^5$ cells to a density of 1-2 x $10^6$ cells, at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3. Procedures used are generally described in available laboratory manuals (*e.g.*, King and Possee, *supra*; O'Reilly *et al.*, *supra*.; Richardson, *supra*).

**[188]** Fungal cells, including yeast cells, can also be used within the present invention. Yeast species of particular interest in this regard include *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Pichia methanolica*. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki *et al.*, U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch *et al.*, U.S. Patent No. 5,037,743; and Murray *et al.*, U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (*e.g.*, leucine). An exemplary vector system for use in *Saccharomyces cerevisiae* is the *POT1* vector system disclosed by Kawasaki *et al.* (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (*see, e.g.*, Kawasaki, U.S. Patent No. 4,599,311; Kingsman *et al.*, U.S. Patent No. 4,615,974; and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. *See also* U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936; and 4,661,454. Transformation systems for other yeasts, including *Hansenula*

*polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago maydis, Pichia pastoris, Pichia methanolica, Pichia guillermondii*, and *Candida maltosa* are known in the art. *See, e.g.*, Gleeson *et al.*, *J. Gen. Microbiol.* 132:3459-3465, 1986; Cregg, U.S. Patent No. 4,882,279; and Raymond *et al.*, *Yeast* 14:11-23, 1998. *Aspergillus* cells may be utilized according to the methods of McKnight *et al.*, U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino *et al.*, U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533. Production of recombinant proteins in *Pichia methanolica* is disclosed in U.S. Patents Nos. 5,716,808; 5,736,383; 5,854,039; and 5,888,768.

**[189]** Prokaryotic host cells, including strains of the bacteria *Escherichia coli*, *Bacillus* and other genera are also useful host cells within the present invention. Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well-known in the art (*see, e.g.*, Sambrook *et al.*, *supra*). When expressing a fusion polypeptide in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine HCl or urea. The denatured polypeptide can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the alternative, the protein may be recovered from the cytoplasm in soluble form and isolated without the use of denaturants. The protein is recovered from the cell as an aqueous extract in, for example, phosphate buffered saline. To capture the protein of interest, the extract is applied directly to a chromatographic medium, such as an immobilized antibody or heparin-Sepharose column. Secreted polypeptides can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) and recovering the protein, thereby obviating the need for denaturation and refolding. *See, e.g.*, Lu *et al.*, *J. Immunol. Meth.* 267:213-226, 2002.

**[190]** Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell.

**[191]** Proteins of the present invention are purified by conventional protein purification methods, typically by a combination of chromatographic techniques. *See generally Affinity Chromatography: Principles & Methods*, Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988; and Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag, New York, 1994. Proteins comprising an immunoglobulin heavy chain polypeptide can be purified by affinity chromatography on immobilized protein A. Additional purification steps, such as gel filtration, can be used to obtain the desired level of purity or to provide for desalting, buffer exchange, and the like.

**[192]** For example, fractionation and/or conventional purification methods can be used to obtain fusion polypeptides and dimeric proteins of the present invention purified from recombinant host cells. In general, ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable chromatographic media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are suitable. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties.

**[193]** Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well-known and widely used in the art, and are available from commercial suppliers. Selection of a particular method for polypeptide isolation and purification is a matter of routine design and is determined in part by the properties of the chosen support. *See, e.g., Affinity Chromatography: Principles & Methods* (Pharmacia LKB Biotechnology 1988); and Doonan, *Protein Purification Protocols* (The Humana Press 1996).

**[194]** Additional variations in protein isolation and purification can be devised by those of skill in the art. For example, antibodies that specifically bind a fusion polypeptide or dimeric protein as described herein (*e.g.*, an antibody that specifically binds a polypeptide segment corresponding to ApoA-1) can be used to isolate large quantities of protein by immunoaffinity purification.

**[195]** The proteins of the present invention can also be isolated by exploitation of particular properties. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins, including those comprising polyhistidine tags. Briefly, a gel is first charged with divalent metal ions to form a chelate (Sulkowski, *Trends in Biochem.* 3:1, 1985). Histidine-rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography (*see, e.g.*, M. Deutscher, (ed.), *Meth. Enzymol.* 182:529, 1990). Within additional embodiments of the invention, a fusion of the polypeptide of interest and an affinity tag (*e.g.*, maltose-binding protein, an immunoglobulin domain) may be constructed to facilitate purification. Moreover, receptor- or ligand-binding properties of a fusion polypeptide or dimer thereof can be exploited for purification. For example, a fusion polypeptide comprising an Aβ-binding polypeptide segment may be isolated by using affinity chromatography wherein amyloid beta (Aβ) peptide is bound to a column and the fusion polypeptide is bound and subsequently eluted using standard chromatography methods.

**[196]** The polypeptides of the present invention are typically purified to at least about 80% purity, more typically to at least about 90% purity and preferably to at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% purity with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. The polypeptides of the present invention may also be purified to a pharmaceutically pure state, which is greater than 99.9% pure. In certain preparations, purified polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin.

## IV. Methods of Use and Pharmaceutical Compositions

**[197]** The fusion polypeptides and dimeric proteins of the present invention can be used to provide ApoA-1-mediated therapy for the treatment of various diseases or disorders. In some aspects relating to bispecific fusions further comprising a second polypeptide segment as described herein (*e.g.*, an RNase, paraoxonase, platelet-activating factor acetylhydrolase (PAF-AH), cholesterol ester transfer protein (CETP), or lecithin-cholesterol acyltransferase (LCAT)), the fusion polypeptides and dimeric proteins may further provide one or more additional biological activities for such treatment.

**[198]** In particular aspects, the present invention provides methods for treating a disease or disorder selected from a cardiovascular disease characterized by atherosclerosis, a neurodegenerative disease, a disease characterized by amyloid deposit, an autoimmune disease, an inflammatory disease,

an infectious disease, obesity, metabolic syndrome, nephrotic syndrome, burns, exposure to sulfur mustard gas, exposure to an organophosphate, and cancer. The methods generally include administering to a subject having the disease or disorder an effective amount of a fusion polypeptide or dimeric protein as described herein.

**[199]** Atherosclerotic cardiovascular diseases amenable to treatment in accordance with the present invention include, for example, coronary heart disease and stroke. In some variations of treatment of coronary heart disease, the coronary heart disease is characterized by acute coronary syndrome. In some embodiments, the atherosclerotic cardiovascular disease is selected from cerebral artery disease (*e.g.*, extracranial cerebral artery disease, intracranial cerebral artery disease), arteriosclerotic aortic disease, renal artery disease, mesenteric artery disease, and peripheral artery disease (*e.g.*, aortoiliac occlusive disease).

**[200]** Neurodegenerative diseases amenable to treatment in accordance with the present invention include, for example, neurodegenerative diseases characterized by amyloid deposit and/or dementia. An exemplary neurodegenerative disease characterized by amyloid deposit is Alzheimer's disease. Exemplary neurodegenerative diseases characterized by dementia include Alzheimer's disease, Parkinson's disease, Huntington's disease, and amylotrophic lateral sclerosis (ALS). In some embodiments, the neurodegenerative disease is an inflammatory disease such as, for example, a demyelinating inflammatory disease of the CNS (*e.g.*, multiple sclerosis (MS), including, for example, spino-optical MS, primary progressive MS (PPMS), and relapsing remitting MS (RRMS)).

**[201]** In some embodiments of a method for treating a neurodegenerative disease (*e.g.*, Alzheimer's disease or Parkinson's disease), a fusion molecule for the neurodegenerative disease treatment is a polypeptide having the structure ApoA1-L1-D-L2-RNase (*e.g.*, ApoA1-L1-[Fc region]-L2-RNase1) or ApoA1-L1-D-L2-paraoxonase (*e.g.*, ApoA1-L1-[Fc region]-L2-PON1), or a dimeric protein formed by dimerization of any of the foregoing fusion polypeptides; in some such embodiments, the fusion polypeptide comprises or consists of an amino acid sequence having at least 90%, at least 95%, or 100% identity with (i) amino acid residues 19-675 or 25-675 of SEQ ID NO:4, (ii) amino acid residues 19-657 or 25-675 of SEQ ID NO:14, (iii) amino acid residues 19-883 or 25-883 of SEQ ID NO:28, (iv) amino acid residues 19-873 or 25-873 of SEQ ID NO:38, (v) amino acid residues 19-883 or 25-883 of SEQ ID NO:46, or (vi) amino acid acid residues 19-883 or 25-883 of SEQ ID NO:48.

**[202]** Autoimmune diseases amenable to treatment in accordance with the present invention include, for example, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and type 1 diabetes. In other embodiments, the autoimmune disease is selected from coeliac disease, neuritis, polymyositis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis, vitiligo, Sjogren's syndrome, autoimmune pancreatitis, an inflammatory bowel disease (*e.g.*, Crohn's disease, ulcerative colitis),

active chronic hepatitis, glomerulonephritis, lupus nephritis, scleroderma, antiphospholipid syndrome, autoimmune vasculitis, sarcoidosis, autoimmune thyroid diseases, Hashimoto's thyroiditis, Graves disease, Wegener's granulomatosis, myasthenia gravis, Addison's disease, autoimmune uveoretinitis, pemphigus vulgaris, primary biliary cirrhosis, pernicious anemia, sympathetic opthalmia, uveitis, autoimmune hemolytic anemia, pulmonary fibrosis, chronic beryllium disease, and idiopathic pulmonary fibrosis. In some variations, the autoimmune disease is selected from rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus, lupus nephritis, scleroderma, psoriasis, Sjogren's syndrome, type 1 diabetes, antiphospholipid syndrome, and autoimmune vasculitis.

**[203]** In some embodiments, a fusion molecule for treatment of an autoimmune disease is a polypeptide having the structure ApoA1-L1-D (*e.g.*, ApoA1-L1-[Fc region]), ApoA1-L1-D-L2-RNase (*e.g.*, ApoA1-L1-[Fc region]-L2-RNase1), or ApoA1-L1-D-L2-paraoxonase (*e.g.*, ApoA1-L1-[Fc region]-L2-PON1), or a dimeric protein formed by dimerization of any of the foregoing fusion polypeptides; in some such embodiments, the fusion polypeptide comprises or consists of an amino acid sequence having at least 90%, at least 95%, or 100% identity with (i) amino acid residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:2, (ii) amino acid residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:13, (iii) amino acid residues 19-501, 19-500, 25-501, or 25-501 of SEQ ID NO:20, (iv) amino acid residues 19-515, 19-514, 25-515, or 25-514 of SEQ ID NO:22, (v) amino acid residues 19-535, 19-534, 25-535, or 25-534 of SEQ ID NO:24, (vi) amino acid residues 19-675 or 25-675 of SEQ ID NO:4, (vii) amino acid residues 19-657 or 25-675 of SEQ ID NO:14, (viii) amino acid residues 19-883 or 25-883 of SEQ ID NO:28, (ix) amino acid residues 19-873 or 25-873 of SEQ ID NO:38, (x) amino acid residues 19-883 or 25-883 of SEQ ID NO:46, or (xi) amino acid residues 19-883 or 25-883 of SEQ ID NO:48. In some particular variations of a method for treating rheumatoid arthritis (RA), a fusion molecule for the RA treatment is a polypeptide having the structure ApoA1-L1-D (*e.g.*, ApoA1-L1-[Fc region]), or a dimeric protein formed by dimerization of the foregoing fusion polypeptide; in some such embodiments, the fusion polypeptide comprises or consists of an amino acid sequence having at least 90%, at least 95%, or 100% identity with (i) amino acid residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:2, (ii) amino acid residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:13, (iii) amino acid residues 19-501, 19-500, 25-501, or 25-501 of SEQ ID NO:20, (iv) amino acid residues 19-515, 19-514, 25-515, or 25-514 of SEQ ID NO:22, or (v) amino acid residues 19-535, 19-534, 25-535, or 25-534 of SEQ ID NO:24. In some particular variations of a method for treating systemic lupus erythematosus (SLE), a fusion molecule for the SLE treatment is a polypeptide having the structure ApoA1-L1-D-L2-RNase (*e.g.*, ApoA1-L1-[Fc region]-L2-RNase1) or ApoA1-L1-D-L2-paraoxonase (*e.g.*, ApoA1-L1-[Fc region]-L2-PON1), or a dimeric protein formed by dimerization of any of the foregoing fusion polypeptides; in some such embodiments, the fusion polypeptide comprises or consists of an amino acid sequence having at least

90%, at least 95%, or 100% identity with (i) amino acid residues 19-675 or 25-675 of SEQ ID NO:4, (ii) amino acid residues 19-657 or 25-675 of SEQ ID NO:14, (iii) amino acid residues 19-883 or 25-883 of SEQ ID NO:28, (iv) amino acid residues 19-873 or 25-873 of SEQ ID NO:38, (v) amino acid residues 19-883 or 25-883 of SEQ ID NO:46, or (vi) amino acid residues 19-883 or 25-883 of SEQ ID NO:48. In some particular variations of a method for treating multiple sclerosis (MS), a fusion molecule for the MS treatment is a polypeptide having the structure ApoA1-L1-D-L2-paraoxonase (*e.g.*, ApoA1-L1-[Fc region]-L2-PON1), or a dimeric protein formed by dimerization of the foregoing fusion polypeptide; in some such embodiments, the fusion polypeptide comprises or consists of an amino acid sequence having at least 90%, at least 95%, or 100% identity with (i) amino acid residues 19-883 or 25-883 of SEQ ID NO:28, (ii) amino acid residues 19-873 or 25-873 of SEQ ID NO:38, (iii) amino acid residues 19-883 or 25-883 of SEQ ID NO:46, or (iv) amino acid residues 19-883 or 25-883 of SEQ ID NO:48.

**[204]** Inflammatory diseases amenable to treatment in accordance with the present invention include, for example, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, type 1 diabetes, type 2 diabetes, and obesity. In some embodiments, the inflammatory disease is an neurodegenerative inflammatory disease such as, for example, multiple sclerosis, Alzheimer's disease, or Parkinson's disease. In other embodiments, the inflammatory disease is an atherosclerotic disease (*e.g.*, coronary heart disease or stroke). In yet other variations, the inflammatory disease is selected from hepatitis (*e.g.*, non-alcoholic steatohepatitis), ankylosing spondylitis, arthritis (*e.g.*, osteoarthritis, rheumatoid arthritis (RA), psoriatic arthritis), Crohn's disease, ulcerative colitis, dermatitis, diverticulitis, fibromyalgia, irritable bowel syndrome (IBS), and nephritis. In other embodiments, the inflammatory disease is an inflammatory lung disease; in some such embodiments, the inflammatory lung disease is selected from asthma, chronic obstructive pulmonary disease (COPD), bronchiectasis, idiopathic pulmonary fibrosis, hyperoxia, hypoxia, and acute respiratory distress syndrome (ARDS). In some variations, a patient having the inflammatory lung disease is a patient that has been exposed to sulfur mustard gas (SM). In other variations, a patient having the inflammatory lung disease is a patient that has been exposed to an organophosphate, such as an insecticide or other neurotoxin.

**[205]** In some embodiments, a fusion molecule for treatment of an inflammatory disease (*e.g.*, an inflammatory lung disease) is a polypeptide having the structure ApoA1-L1-D (*e.g.*, ApoA1-L1-[Fc region]), ApoA1-L1-D-L2-RNase (*e.g.*, ApoA1-L1-[Fc region]-L2-RNase1), or ApoA1-L1-D-L2-paraoxonase (*e.g.*, ApoA1-L1-[Fc region]-L2-PON1), or a dimeric protein formed by dimerization of any of the foregoing fusion polypeptides; in some such embodiments, the fusion polypeptide comprises or consists of an amino acid sequence having at least 90%, at least 95%, or 100% identity with (i) amino acid residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:2, (ii) amino acid

residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:13, (iii) amino acid residues 19-501, 19-500, 25-501, or 25-501 of SEQ ID NO:20, (iv) amino acid residues 19-515, 19-514, 25-515, or 25-514 of SEQ ID NO:22, (v) amino acid residues 19-535, 19-534, 25-535, or 25-534 of SEQ ID NO:24, (vi) amino acid residues 19-675 or 25-675 of SEQ ID NO:4, (vii) amino acid residues 19-657 or 25-675 of SEQ ID NO:14, (viii) amino acid residues 19-883 or 25-883 of SEQ ID NO:28, (ix) amino acid residues 19-873 or 25-873 of SEQ ID NO:38, (x) amino acid residues 19-883 or 25-883 of SEQ ID NO:46, or (xi) amino acid residues 19-883 or 25-883 of SEQ ID NO:48. In some particular variations of a method for treating idiopathic pulmonary fibrosis, a fusion molecule for the idiopathic pulmonary fibrosis treatment is a polypeptide having the structure ApoA1-L1-D (*e.g.*, ApoA1-L1-[Fc region]), or a dimeric protein formed by dimerization of the foregoing fusion polypeptide; in some such embodiments, the fusion polypeptide comprises or consists of an amino acid sequence having at least 90%, at least 95%, or 100% identity with (i) amino acid residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:2, (ii) amino acid residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:13, (iii) amino acid residues 19-501, 19-500, 25-501, or 25-501 of SEQ ID NO:20, (iv) amino acid residues 19-515, 19-514, 25-515, or 25-514 of SEQ ID NO:22, or (v) amino acid residues 19-535, 19-534, 25-535, or 25-534 of SEQ ID NO:24. In some particular variations of a method for treating an inflammatory lung disease in a patient that has been exposed to sulfur mustard gas (SM) or an organophosphate, a fusion molecule for the treatment is a polypeptide having the structure ApoA1-L1-D-L2-paraoxonase (*e.g.*, ApoA1-L1-[Fc region]-L2-PON1), or a dimeric protein formed by dimerization of the foregoing fusion polypeptide; in some such embodiments, the fusion polypeptide comprises or consists of an amino acid sequence having at least 90%, at least 95%, or 100% identity with (i) amino acid residues 19-883 or 25-883 of SEQ ID NO:28, (ii) amino acid residues 19-873 or 25-873 of SEQ ID NO:38, (iii) amino acid residues 19-883 or 25-883 of SEQ ID NO:46, or (iv) amino acid residues 19-883 or 25-883 of SEQ ID NO:48. In some particular variations of a method for treating acute respiratory distress syndrome (ARDS), hypoxia, or hyperoxia, a fusion molecule for the treatment is a polypeptide having the structure ApoA1-L1-D-L2-RNase (*e.g.*, ApoA1-L1-[Fc region]-L2-RNase1), or a dimeric protein formed by dimerization of any of the foregoing fusion polypeptides; in some such embodiments, the fusion polypeptide comprises or consists of an amino acid sequence having at least 90%, at least 95%, or 100% identity with (i) amino acid residues 19-675 or 25-675 of SEQ ID NO:4, or (ii) amino acid residues 19-657 or 25-675 of SEQ ID NO:14. In certain embodiments, such ApoA1-L1-D-L2-RNase variations are used to treat premature infants that are treated with oxygen for an extended period of time.

**[206]** In some embodiments, a fusion molecule for treatment of exposure to sulfur mustard gas (SM) or exposure to an organophosphate is a polypeptide having the structure ApoA1-L1-D-L2-paraoxonase (*e.g.*, ApoA1-L1-[Fc region]-L2-PON1), or a dimeric protein formed by dimerization of the foregoing fusion polypeptide; in some such embodiments, the fusion polypeptide comprises or

consists of an amino acid sequence having at least 90%, at least 95%, or 100% identity with (i) amino acid residues 19-883 or 25-883 of SEQ ID NO:28, (ii) amino acid residues 19-873 or 25-873 of SEQ ID NO:38, (iii) amino acid residues 19-883 or 25-883 of SEQ ID NO:46, or (iv) amino acid residues 19-883 or 25-883 of SEQ ID NO:48.

**[207]** Infectious diseases amenable to treatment in accordance with the present invention include, for example, bacterial infections and parasitic infections. In some embodiments, the parasitic infection is a *Trypanosoma brucei* or *Leishmania* infection. In other embodiments, the bacterial infection is a *Pseudomonas aeruginosa* infection.

**[208]** In some embodiments of a method for treating a *Pseudomonas aeruginosa* infection, a fusion molecule for the *Pseudomonas aeruginosa* infection treatment is a polypeptide having the structure ApoA1-L1-D-L2-paraoxonase (*e.g.*, ApoA1-L1-[Fc region]-L2-PON1), or a dimeric protein formed by dimerization of the foregoing fusion polypeptide; in some such embodiments, the fusion polypeptide comprises or consists of an amino acid sequence having at least 90%, at least 95%, or 100% identity with (i) amino acid residues 19-883 or 25-883 of SEQ ID NO:28, (ii) amino acid residues 19-873 or 25-873 of SEQ ID NO:38, (iii) amino acid residues 19-883 or 25-883 of SEQ ID NO:46, or (iv) amino acid residues 19-883 or 25-883 of SEQ ID NO:48.

**[209]** In some embodiments, a fusion molecule for treatment of an infectious disease (*e.g.*, an inflammatory lung disease) is a polypeptide having the structure ApoA1-L1-D (*e.g.*, ApoA1-L1-[Fc region]), or a dimeric protein formed by dimerization of the foregoing fusion polypeptide; in some such embodiments, the fusion polypeptide comprises or consists of an amino acid sequence having at least 90%, at least 95%, or 100% identity with (i) amino acid residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:2, (ii) amino acid residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:13, (iii) amino acid residues 19-501, 19-500, 25-501, or 25-501 of SEQ ID NO:20, (iv) amino acid residues 19-515, 19-514, 25-515, or 25-514 of SEQ ID NO:22, or (v) amino acid residues 19-535, 19-534, 25-535, or 25-534 of SEQ ID NO:24.

**[210]** Cancers that may be treated in accordance with the present invention include, for example, the following: a cancer of the head and neck (*e.g.*, a cancer of the oral cavity, orophyarynx, nasopharynx, hypopharynx, nasal cavity or paranasal sinuses, larynx, lip, or salivary gland); a lung cancer (*e.g.*, non-small cell lung cancer, small cell carcinoma, or mesothelimia); a gastrointestinal tract cancer (*e.g.*, colorectal cancer, gastric cancer, esophageal cancer, or anal cancer); gastrointestinal stromal tumor (GIST); pancreatic adenocarcinoma; pancreatic acinar cell carcinoma; a cancer of the small intestine; a cancer of the liver or biliary tree (*e.g.*, liver cell adenoma, hepatocellular carcinoma, hemangiosarcoma, extrahepatic or intrahepatic cholangiosarcoma, cancer of the ampulla of vater, or gallbladder cancer); a breast cancer (*e.g.*, metastatic breast cancer or inflammatory breast cancer); a gynecologic cancer (*e.g.*, cervical cancer, ovarian cancer, fallopian tube cancer, peritoneal carcinoma,

vaginal cancer, vulvar cancer, gestational trophoblastic neoplasia, or uterine cancer, including endometrial cancer or uterine sarcoma); a cancer of the urinary tract (*e.g.*, prostate cancer; bladder cancer; penile cancer; urethral cancer, or kidney cancer such as, for example, renal cell carcinoma or transitional cell carcinoma, including renal pelvis and ureter); testicular cancer; a cancer of the central nervous system (CNS) such as an intracranial tumor (*e.g.*, astrocytoma, anaplastic astrocytoma, glioblastoma, oligodendroglioma, anaplastic oligodendroglioma, ependymoma, primary CNS lymphoma, medulloblastoma, germ cell tumor, pineal gland neoplasm, meningioma, pituitary tumor, tumor of the nerve sheath (*e.g.*, schwannoma), chordoma, craniopharyngioma, a chloroid plexus tumor (*e.g.*, chloroid plexus carcinoma); or other intracranial tumor of neuronal or glial origin) or a tumor of the spinal cord (*e.g.*, schwannoma, meningioma); an endocrine neoplasm (*e.g.*, thyroid cancer such as, for example, thyroid carcinoma, medullary cancer, or thyroid lymphoma; a pancreatic endocrine tumor such as, for example, an insulinoma or glucagonoma; an adrenal carcinoma such as, for example, pheochromocytoma; a carcinoid tumor; or a parathyroid carcinoma); a skin cancer (*e.g.*, squamous cell carcinoma; basal cell carcinoma; Kaposi's sarcoma; or a malignant melanoma such as, for example, an intraocular melanoma); a bone cancer (*e.g.*, a bone sarcoma such as, for example, osteosarcoma, osteochondroma, or Ewing's sarcoma); multiple myeloma; a chloroma; a soft tissue sarcoma (*e.g.*, a fibrous tumor or fibrohistiocytic tumor); a tumor of the smooth muscle or skeletal muscle; a blood or lymph vessel perivascular tumor (*e.g.*, Kaposi's sarcoma); a synovial tumor; a mesothelial tumor; a neural tumor; a paraganglionic tumor; an extraskeletal cartilaginous or osseous tumor; and a pluripotential mesenchymal tumor. In some such embodiments, an ApoA-1 fusion molecule as described herein is administered to a cancer patient as one of the distinct therapies of a combination therapy such as, for example, a combination therapy comprising a non-ApoA1-mediated immunomodulatory therapy (*e.g.*, a therapy comprising an immune checkpoint inhibitor), a radiation therapy, or a chemotherapy.

**[211]** In certain embodiments, a combination cancer therapy comprises an ApoA-1 fusion molecule as described herein and a targeted therapy such as, *e.g.*, a therapeutic monoclonal antibody targeting a specific cell-surface or extracellular antigen, or a small molecule targeting an intracellular protein (*e.g.*, an intracellular enzyme). Exemplary antibody targeted therapies include anti-VEGF (*e.g.*, bevacizumab), anti-EGFR (*e.g.*, cetuximab), anti-CTLA-4 (*e.g.*, ipilimumab), anti-PD-1 (*e.g.*, nivolumab), and anti-PD-L1 (*e.g.*, pembrolizumab). Exemplary small molecule targeted therapies include proteasome inhibitors (*e.g.*, bortezomib), tyrosine kinase inhibitors (*e.g.*, imatinib), cyclin-dependent kinase inhibitors (*e.g.*, seliciclib); BRAF inhibitors (*e.g.*, vemurafenib or dabrafenib); and MEK kinase inhibitors (*e.g.*, trametnib).

**[212]** In some cancer combination therapy variations comprising an immune checkpoint inhibitor, the combination therapy includes an anti-PD-1/PD-L1 therapy, an anti-CTLA-4 therapy, or

both. In certain aspects, ApoA-1 fusion molecules as described herein can increase the response rate to either anti-CTLA-4 or anti-PD-1/PD-L1 therapy, as well as the response rate to the combination of anti-CTLA-4 plus anti-PD-1/PD-L1 therapy. Fusion molecules of the invention may also be useful for reducing the toxicity associated with anti-CTLA-4, anti-PD-1/PD-L1, or the combination thereof.

**[213]** In certain variations, a cancer treated in accordance with the present invention is selected from malignant melanoma, renal cell carcinoma, non-small cell lung cancer, bladder cancer, and head and neck cancer. These cancers have shown responses to immune checkpoint inhibitors anti-PD-1/PD-L1 and anti-CTLA-4. *See* Grimaldi *et al.*, *Expert Opin. Biol. Ther.* 16:433-41, 2016; Gunturi *et al.*, *Curr. Treat. Options Oncol.* 15:137-46, 2014; Topalian *et al.*, *Nat. Rev. Cancer* 16:275-87, 2016. Thus, in some more specific variations, any of these cancers is treated with an ApoA-1 fusion molecule as described herein in combination with an anti-PD-1/PD-L1 therapy, an anti-CTLA-4 therapy, or both.

**[214]** In some embodiments, a fusion molecule for treatment of a cancer is a polypeptide having the structure ApoA1-L1-D (*e.g.*, ApoA1-L1-[Fc region]), ApoA1-L1-D-L2-RNase (*e.g.*, ApoA1-L1-[Fc region]-L2-RNase1), or ApoA1-L1-D-L2-paraoxonase (*e.g.*, ApoA1-L1-[Fc region]-L2-PON1), or a dimeric protein formed by dimerization of any of the foregoing fusion polypeptides; in some such embodiments, the fusion polypeptide comprises or consists of an amino acid sequence having at least 90%, at least 95%, or 100% identity with (i) amino acid residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:2, (ii) amino acid residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:13, (iii) amino acid residues 19-501, 19-500, 25-501, or 25-501 of SEQ ID NO:20, (iv) amino acid residues 19-515, 19-514, 25-515, or 25-514 of SEQ ID NO:22, (v) amino acid residues 19-535, 19-534, 25-535, or 25-534 of SEQ ID NO:24, (vi) amino acid residues 19-675 or 25-675 of SEQ ID NO:4, (vii) amino acid residues 19-657 or 25-675 of SEQ ID NO:14, (viii) amino acid residues 19-883 or 25-883 of SEQ ID NO:28, (ix) amino acid residues 19-873 or 25-873 of SEQ ID NO:38, (x) amino acid residues 19-883 or 25-883 of SEQ ID NO:46, or (xi) amino acid residues 19-883 or 25-883 of SEQ ID NO:48.

**[215]** For therapeutic use, a fusion polypeptide or dimeric protein as described herein is delivered in a manner consistent with conventional methodologies associated with management of the disease or disorder for which treatment is sought. In accordance with the disclosure herein, an effective amount of the fusion polypeptide or dimeric protein is administered to a subject in need of such treatment for a time and under conditions sufficient to prevent or treat the disease or disorder.

**[216]** Subjects for administration of fusion polypeptides or dimeric proteins as described herein include patients at high risk for developing a particular disease or disorder as well as patients presenting with an existing disease or disorder. In certain embodiments, the subject has been diagnosed as having the disease or disorder for which treatment is sought. Further, subjects can be

monitored during the course of treatment for any change in the disease or disorder (*e.g.*, for an increase or decrease in clinical symptoms of the disease or disorder). Also, in some variations, the subject does not suffer from another disease or disorder requiring treatment that involves administration of an ApoA-1 protein.

**[217]** In prophylactic applications, pharmaceutical compositions or medicants are administered to a patient susceptible to, or otherwise at risk of, a particular disease in an amount sufficient to eliminate or reduce the risk or delay the outset of the disease. In therapeutic applications, compositions or medicants are administered to a patient suspected of, or already suffering from such a disease in an amount sufficient to cure, or at least partially arrest, the symptoms of the disease and its complications. An amount adequate to accomplish this is referred to as a therapeutically or pharmaceutically effective dose or amount. In both prophylactic and therapeutic regimes, agents are usually administered in several dosages until a sufficient response (*e.g.*, atherosclerosis regression or stabilization of existing plaques in coronary heart disease) has been achieved. Typically, the response is monitored and repeated dosages are given if the desired response starts to fade.

**[218]** To identify subject patients for treatment according to the methods of the invention, accepted screening methods may be employed to determine risk factors associated with a specific disease or to determine the status of an existing disease identified in a subject. Such methods can include, for example, determining whether an individual has relatives who have been diagnosed with a particular disease. Screening methods can also include, for example, conventional work-ups to determine familial status for a particular disease known to have a heritable component. Toward this end, nucleotide probes can be routinely employed to identify individuals carrying genetic markers associated with a particular disease of interest. In addition, a wide variety of immunological methods are known in the art that are useful to identify markers for specific diseases. Screening may be implemented as indicated by known patient symptomology, age factors, related risk factors, *etc*. These methods allow the clinician to routinely select patients in need of the methods described herein for treatment. In accordance with these methods, treatment using a fusion polypeptide or dimeric protein of the present invention may be implemented as an independent treatment program or as a follow-up, adjunct, or coordinate treatment regimen to other treatments.

**[219]** For administration, a fusion polypeptide or dimeric protein in accordance with the present invention is formulated as a pharmaceutical composition. A pharmaceutical composition comprising a fusion polypeptide or dimeric protein as described herein can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic molecule is combined in a mixture with a pharmaceutically acceptable carrier. A composition is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other

suitable carriers are well-known to those in the art. *See, e.g.*, Gennaro (ed.), *Remington's Pharmaceutical Sciences* (Mack Publishing Company, 19th ed. 1995). Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, *etc*.

**[220]** A pharmaceutical composition comprising a fusion polypeptide or dimeric protein of the present invention is administered to a subject in an effective amount. The fusion polypeptide or dimeric protein may be administered to subjects by a variety of administration modes, including, for example, by intramuscular, subcutaneous, intravenous, intra-atrial, intra-articular, parenteral, intranasal, intrapulmonary, transdermal, intrapleural, intrathecal, and oral routes of administration. For prevention and treatment purposes, the fusion polypeptide or dimeric protein may be administered to a subject in a single bolus delivery, via continuous delivery (*e.g.*, continuous transdermal delivery) over an extended time period, or in a repeated administration protocol (*e.g.*, on an hourly, daily, or weekly basis).

**[221]** Determination of effective dosages in this context is typically based on animal model studies followed up by human clinical trials and is guided by determining effective dosages and administration protocols that significantly reduce the occurrence or severity of the subject disease or disorder in model subjects. Effective doses of the compositions of the present invention vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, whether treatment is prophylactic or therapeutic, as well as the specific activity of the composition itself and its ability to elicit the desired response in the individual. Usually, the patient is a human, but in some diseases, the patient can be a nonhuman mammal. Typically, dosage regimens are adjusted to provide an optimum therapeutic response, *i.e.*, to optimize safety and efficacy. Accordingly, a therapeutically or prophylactically effective amount is also one in which any undesired collateral effects are outweighed by beneficial effects (*e.g.*, in the case of treatment of atherosclerotic cardiovascular disease, where any undesired collateral effects are outweighted by any beneficial effects such as increase in HDL, atherosclerosis regression, and/or plaque stabilization). For administration of a fusion polypeptide or dimeric protein of the present invention, a dosage typically ranges from about 0.1 μg to 100 mg/kg or 1 μg/kg to about 50 mg/kg, and more usually 10 μg to 5 mg/kg of the subject's body weight. In more specific embodiments, an effective amount of the agent is between about 1 μg/kg and about 20 mg/kg, between about 10 μg/kg and about 10 mg/kg, or between about 0.1 mg/kg and about 5 mg/kg. Dosages within this range can be achieved by single or multiple administrations, including, *e.g.*, multiple administrations per day or daily, weekly, bi-weekly, or monthly administrations. For example, in certain variations, a regimen consists of an initial administration followed by multiple, subsequent administrations at weekly or bi-weekly intervals.

Another regimen consists of an initial administration followed by multiple, subsequent administrations at monthly or bi-monthly intervals. Alternatively, administrations can be on an irregular basis as indicated by monitoring of clinical symptoms of the disease or disorder and/or monitoring of disease biomarkers or other disease correlates (*e.g.*, HDL levels in the case of atherosclerotic cardiovascular disease).

**[222]** Particularly suitable animal models for evaluating efficacy of an ApoA-1 composition of the present invention for treatment of atherosclerosis include, for example, known mouse models that are deficient in the low density lipoprotein receptor (LDLR) or ApoE. LDLR deficient mice develop atherosclerotic plaques after eating a high fat diet for 12 weeks, and human ApoA-1 (reconstituted with lipids) is effective in reducing plaques in this model. ApoE deficient mice are also commonly used to study atherosclerosis, and human ApoA-1 (reconstituted with lipids) works rapidly in this model. Rabbits that are transgenic for hepatic lipase are another known atherosclerosis model for testing ApoA-1 compositions.

**[223]** One model of Alzheimer's disease uses overexpression of mutant amyloid-β precursor protein (APP) and presenilin 1 in mice. In these mice, overexpression of human ApoA-1 prevented memory and learning deficits. *See* Lewis *et al.*, *J Biol. Chem.* 285: 36958-36968, 2010.

**[224]** Also known is the collagen-induced arthritis (CIA) model for rheumatoid arthritis (RA). CIA shares similar immunological and pathological features with RA, making it an ideal model for evaluating efficacy of ApoA-1 compositions. *See, e.g.*, Charles-Schoeman *et al.*, *Clin Immunol.* 127:234-44, 2008 (describing studies showing efficacy of the ApoA-1 mimetic peptide, D-4F, in the CIA model). Another known model for RA is PG-polysaccharide (PG-PS)-induced arthritis in female Lewis rats. In these mice, administration of ApoA-1 protein or reconstituted HDLs reduced acute and chronic joint inflammation. Wu *et al.*, *Arterioscler Thromb Vasc Biol* 34:543-551, 2014.

**[225]** Animal models for multiple sclerosis (MS) include, for example, experimental allergic encephalomyelitis (EAE) models that rely on the induction of an autoimmune response in the CNS by immunization with a CNS antigen (also referred to as an "encephalitogen" in the context of EAE), which leads to inflammation, demyelination, and weakness. ApoA-1 deficient mice have been shown to exhibit more neurodegeneration and worse disease than wild-type animals in this model. *See* Meyers *et al.*, *J. Neuroimmunol.* 277: 176-185, 2014.

**[226]** Fusion molecules of the present invention can be evaluated for anti-tumor activity in animal tumor models such as, *e.g.*, B16 melanoma, a poorly immunogenic tumor. Multiple models of tumor immunotherapy have been studied. *See* Ngiow *et al.*, *Adv. Immunol.* 130:1-24, 2016. The B16 melanoma model has been studied extensively with checkpoint inhibitors anti-CTLA-4, anti-PD-1, and the combination thereof. Anti-CTLA-4 alone has a potent therapeutic effect in this model only

when combined with GM-CSF transduced tumor vaccine, or combined with anti-PD-1. *See* Weber, *Semin. Oncol.* 37:430-439, 2010; Ai *et al.*, *Cancer Immunol. Immunother.* 64:885-92, 2015; Haanen *et al.*, *Prog. Tumor Res.* 42:55-66, 2015. Efficacy of an ApoA-1 fusion molecule for treatment of malignant melanoma is shown, for example, by slowed tumor growth following administration to B16 melanoma mice that have formed palpable subcutaneous tumor nodules. Efficacy of an ApoA-1 fusion molecule can be evaluated in B16 melanoma mice either alone or, alternatively, in combination with another anti-cancer therapy (*e.g.*, anti-CTLA-4, with or without tumor vaccine or with or without anti-PD-1/PD-L1). For example, tumor rejection in B16 melanoma mice using a combination of an ApoA-1 fusion molecule as described herein and anti-CTLA-4, in the absence of tumor vaccine, demonstrates an enhanced response to anti-CTLA-4 using the ApoA-1 therapy. In exemplary studies to evaluate ApoA-1 fusion molecules comprising human protein sequences, which are functionally active in mice but are expected to be immunogenic in these models (and thereby likely to result in formation of neutralizing antibodies after 7-10 days), mice may be administered a fusion molecule of the present invention for a short period (for example, one week, administered in, *e.g.*, two doses of about 40mg/kg three days apart), and tumor growth then monitored, typically for two to three weeks after injection with the fusion molecule.

**[227]** Dosage of the pharmaceutical composition may be varied by the attending clinician to maintain a desired concentration at a target site. For example, if an intravenous mode of delivery is selected, local concentration of the agent in the bloodstream at the target tissue may be between about 1-50 nanomoles of the composition per liter, sometimes between about 1.0 nanomole per liter and 10, 15, or 25 nanomoles per liter depending on the subject's status and projected measured response. Higher or lower concentrations may be selected based on the mode of delivery, *e.g.*, trans-epidermal delivery versus delivery to a mucosal surface. Dosage should also be adjusted based on the release rate of the administered formulation, *e.g.*, nasal spray versus powder, sustained release oral or injected particles, transdermal formulations, *etc.* To achieve the same serum concentration level, for example, slow-release particles with a release rate of 5 nanomolar (under standard conditions) would be administered at about twice the dosage of particles with a release rate of 10 nanomolar.

**[228]** A pharmaceutical composition comprising a fusion polypeptide or dimeric protein as described herein can be furnished in liquid form, in an aerosol, or in solid form. Liquid forms, are illustrated by injectable solutions, aerosols, droplets, topological solutions and oral suspensions. Exemplary solid forms include capsules, tablets, and controlled-release forms. The latter form is illustrated by miniosmotic pumps and implants. *See, e.g.*, Bremer *et al., Pharm. Biotechnol.* 10:239, 1997; Ranade, "Implants in Drug Delivery," in *Drug Delivery Systems* 95-123 (Ranade and Hollinger, eds., CRC Press 1995); Bremer *et al.*, "Protein Delivery with Infusion Pumps," in *Protein Delivery: Physical Systems* 239-254 (Sanders and Hendren, eds., Plenum Press 1997); Yewey *et al.*, "Delivery

of Proteins from a Controlled Release Injectable Implant," in *Protein Delivery: Physical Systems* 93-117 (Sanders and Hendren, eds., Plenum Press 1997). Other solid forms include creams, pastes, other topological applications, and the like.

**[229]** Degradable polymer microspheres have been designed to maintain high systemic levels of therapeutic proteins. Microspheres are prepared from degradable polymers such as poly(lactide-co-glycolide) (PLG), polyanhydrides, poly (ortho esters), nonbiodegradable ethylvinyl acetate polymers, in which proteins are entrapped in the polymer. *See, e.g.*, Gombotz and Pettit, *Bioconjugate Chem.* 6:332, 1995; Ranade, "Role of Polymers in Drug Delivery," in *Drug Delivery Systems* 51-93 (Ranade and Hollinger, eds., CRC Press 1995); Roskos and Maskiewicz, "Degradable Controlled Release Systems Useful for Protein Delivery," in *Protein Delivery: Physical Systems* 45-92 (Sanders and Hendren, eds., Plenum Press 1997); Bartus *et al., Science* 281:1161, 1998; Putney and Burke, *Nature Biotechnology* 16:153, 1998; Putney, *Curr. Opin. Chem. Biol.* 2:548, 1998. Polyethylene glycol (PEG)-coated nanospheres can also provide carriers for intravenous administration of therapeutic proteins. *See, e.g.*, Gref *et al., Pharm. Biotechnol.* 10:167, 1997.

**[230]** Other dosage forms can be devised by those skilled in the art, as shown by, *e.g.*, Ansel and Popovich, *Pharmaceutical Dosage Forms and Drug Delivery Systems* (Lea & Febiger, 5th ed. 1990); Gennaro (ed.), *Remington's Pharmaceutical Sciences* (Mack Publishing Company, 19th ed. 1995), and Ranade and Hollinger, *Drug Delivery Systems* (CRC Press 1996).

**[231]** Pharmaceutical compositions as described herein may also be used in the context of combination therapy. The term "combination therapy" is used herein to denote that a subject is administered at least one therapeutically effective dose of a fusion polypeptide or dimeric protein as described herein and another therapeutic agent.

**[232]** Pharmaceutical compositions may be supplied as a kit comprising a container that comprises a fusion polypeptide or dimeric protein as described herein. A therapeutic molecule can be provided, for example, in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Alternatively, such a kit can include a dry-powder disperser, liquid aerosol generator, or nebulizer for administration of a therapeutic protein. Such a kit may further comprise written information on indications and usage of the pharmaceutical composition.

**[233]** The invention is further illustrated by the following non-limiting examples.

## Example 1

**[234]** *Molecule Design and Preparation:* Two ApoA-1-Fc cDNA constructs were designed, synthesized, expressed by transient transfection of COS7 cells, and the expressed proteins then purified by Protein A chromatography. One construct had the nucleotide sequence shown in SEQ ID NO:1 and encoded the fusion polypeptide of SEQ ID NO:2, and is also referred to herein as ApoA-1(26)Fc or THER4. This construct contained a DNA segment encoding a 26 amino acid linker (residues 268-293 of SEQ ID NO:2) between the C-terminal end of human ApoA-1 (residues 1-267 of SEQ ID NO:2) and a human γ1 Fc variant (residues 294-525 of SEQ ID NO:2). Upon expression in mammalian cells and cleavage of the secretory signal peptide (residues 1-18), and any potential cleavage of the propeptide (residues 19-24), this fusion polypeptide had a predicted amino acid sequence corresponding to residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:2 (the C-terminal lysine of the Fc region is known to be frequently cleaved in the production of Fc-containing proteins). The other construct contained ApoA-1 and Fc regions identical to those of the ApoA-1(26)Fc construct, but lacked a (gly4ser) linker between human ApoA-1 and the Fc regions; this construct is also referred to herein as ApoA-1(2)Fc (Theripion) or as THER0 (for no (gly4ser) repeat units). This construct does contain a two amino acid linker due to insertion of overlapping restriction sites between the ApoA-1 region and the hinge region of human IgG1.

**[235]** *Cholesterol efflux:* The cholesterol efflux activity of the ApoA-I fusion proteins were measured using an *in vitro* assay. *See* Tang et al., *J Lipid Res.* 47:107-14, 2006. *In vitro* cholesterol efflux assays were performed using radio-labelled cholesterol and BHK cells expressing a mifespristone-inducible human ABCA1. H3-cholesterol was added to growth media in order to label cellular cholesterol 24 hours prior to treatments, and ABCA1 is induced using 10nM mifepristone for 16-20 hours. Cholesterol efflux was measured by incubating cells with or without the fusion proteins for 2 hours at 37°C, chilled on ice, and medium and cells separated to measure radiolabeled cholesterol. Wild-type human ApoA-1 protein was used as a positive control. A commercially available ApoA-1-Fc protein, linked directly to Fc without any linker between the ApoA-1 and Fc regions (APOA1 Recombinant Human Protein, hIgG1-Fc tag; Sino Biological, Inc.), was also tested and is referred to herein as ApoA-1(0)Fc (Sino Biol). The results of this assay are shown in FIG. 1. Cholesterol efflux was increased in cultures containing ApoA-1-Fc with a 26 amino acid linker (ApoA-1(26)Fc), compared to either ApoA-1-Fc with a two amino acid linker (ApoA-1(2)Fc (Theripion)) or ApoA-1-Fc without a linker (ApoA-1(0)Fc (Sino Biol)). ApoA-1(26)Fc also had activity similar to wild-type human ApoA-1 (Control ApoA-1).

**Example 2: Generation of Fusion Constructs and Sequence Verification**

**[236]** Additional ApoA1 fusion constructs were designed and the fusion gene sequences were submitted to Blue Heron (Bothell, WA) for gene synthesis. A basic schematic diagramming the position of functional domains is shown in FIGS. 2A and 2B for the design of the ApoA1 fusion proteins. Fusion gene constructs inserted into pUC-based vectors isolated by restriction enzyme digestion, and fragments encoding the fusion genes were subcloned into the mammalian expression vector pDG. Briefly, *Hind*III+*Xba*I flanking restriction sites were used for removal of each expression gene from the vector, subfragments isolated by gel electrophoresis, DNA extracted using QIAquick purification columns, and eluted in 30 microliters EB buffer. Fragments were ligated into *Hind*III+*Xba*I digested pDG vector, and ligation reactions transformed into NEB 5-alpha, chemically competent bacteria. Clones were inoculated into 3 ml LB broth with 100 μg/ml ampicillin, grown at 37°C overnight with shaking at 200 rpm, and plasmid DNA prepared using the QIAGEN spin plasmid miniprep kits according to manufacturer's instructions. Sequencing primers were obtained from IDT Integrated DNA Technologies (Coralville, IA) and included the following:

pdgF-2: 5'-ggttttggcagtacatcaatgg-3' (SEQ ID NO:16);

pdgR-2: 5'-ctattgtcttcccaatcctccc-3' (SEQ ID NO:17);

higgras: 5'-accttgcacttgtactcctt-3' (SEQ ID NO:18).

**[237]** Plasmid DNA (800 ng) and sequencing primers (25 pmol, or 5 μl of a 5 pmol/μl stock) were mixed and submitted for DNA sequencing by GENEWIZ (South Plainfield, NJ). Chromatograms were then analyzed, sequences assembled into contigs, and sequence verified using Vector Nti Advance 11.5 software (Life Technologies, Grand Island, NY).

**Example 3: Expression of Fusion Proteins in a Transient HEK 293T Transfection System**

**[238]** This example illustrates transfection of plasmid constructs and expression of fusion proteins described herein in a mammalian transient transfection system. The Ig fusion gene fragments with correct sequence were inserted into the mammalian expression vector pDG, and DNA from positive clones was amplified using QIAGEN plasmid preparation kits (QIAGEN, Valencia, CA). Five different constructs were generated. These each included the native coding sequence of the human ApoA-1 gene (nucleotide sequence shown in SEQ ID NO:35, encoding the amino acid sequence shown in SEQ ID NO:36). Each sequence included the wild-type signal peptide (nucleotides 1-54 of SEQ ID NO:35, encoding amino acids 1-18 of SEQ ID NO:36) and propeptide sequences (nucleotides 55-72 of SEQ ID NO:35, encoding amino acids 19-24 of SEQ ID NO:36) for apolipoprotein A-1. The C-terminal Q (Gln) residues of the ApoA-1 sequence was linked via a

variable length linker segment to the human IgG1 hinge, CH2, and CH3 domains to create a single chain (ApoA-1)-lnk-human IgG1 Fc fusion gene/protein. The hinge sequence of the human IgG1 is mutated so that the three cysteines are substituted with serine residues, eliminating disulfide bond formation in this region or unpaired cysteines that might compromise proper folding of the fusion protein. The P238 and P331 residues of CH2 are also mutated to serines to eliminate effector functions such as ADCC and complement fixation. Each construct also included a linker sequence inserted between the carboxyl terminus of apolipoprotein A-1 (ending with the sequence ...TKKLNTQ (SEQ ID NO:35 residues 261-267) ) and the beginning of the hinge sequence of the human Fc (starting with the motif ...EPKSSDKT... (SEQ ID NO:2 residues 294-301). This linker sequence ranged from two amino acids (or four if the overlap with the flanking domains is included) to 36 amino acids in length, depending on the construct.

**[239]** The shortest linker included only two overlapping restriction sites (*Bgl*II and *Xho*I) with a linker length of six additional nucleotides or two additional non-native amino acids. The restriction sites were incorporated into the coding sequence of the molecule so that only two additional amino acids needed to be added to the amino acid sequence. The *Bgl*II site of the linker overlaps with the codon for the C-terminal glutamine of ApoA-1, and three of the nucleotides encoding the *Xho*I site form the codon for the first amino acid of the hinge (E-glutamic acid). The linker amino acid sequence (including the two overlapping amino acids) is shown in residues 267-270 of SEQ ID NO:20, which is encoded by nucleotides 816-825 of SEQ ID NO:19. The fusion gene and protein for this construct are identified as THER0 (since there are no (gly4ser) repeat units present) or apoA-1-lnk(2)hIgG. The nucleotide and amino acid sequences for THER0 are listed as SEQ ID NO:19 and SEQ ID NO:20. The figures use the THER0 nomenclature to specify this construct.

**[240]** The second construct included a linker that encodes two (gly4ser) sequences flanked by restriction sites (16 amino acid linker), and the fusion gene and protein for this construct are identified as THER2 (or apoA-1-lnk(16)-hIgG1 or apoA-1-(g4s)2-hIgG1). The nucleotide and amino acid sequences for THER2 are listed as SEQ ID NO:21 and SEQ ID NO:22, respectively. The (gly4ser)2 linker sequence is shown in residues 268-283 of SEQ ID NO:22, and the encoding nucleotide sequence for the (gly4ser)2 linker is shown in residues 817-864 of SEQ ID NO:21.

**[241]** The third construct included a linker that encodes four (gly4ser) sequences flanked by restriction sites (26 amino acid linker), and the fusion gene and protein for this construct is identified as THER4 (or apoA-1-(g4s)4-mthIgG or apoA-1-lnk(26)-mthIgG), where "4" in "THER4" refers to the number of (gly4ser) repeat units, and the number 26 refers to the total number of amino acids encoded in the non-native, introduced linker sequence. The nucleotide and amino acid sequences for THER4 are listed as SEQ ID NO:1 and SEQ ID NO:2, respectively. The (gly4ser)4 linker sequence is

shown in SEQ ID NO:50 (residues 268-293 of SEQ ID NO:2), and the encoding nucleotide sequence for the (gly4ser)4 linker is shown in SEQ ID NO:49 (residues 817-894 of SEQ ID NO:1).

**[242]** The fourth construct included a linker that encodes six (gly4ser) sequences flanked by restriction sites (36 amino acid linker), and the fusion gene and protein for this construct is identified as THER6 (or apoA-1-(g4s)6-mthIgG or apoA-1-lnk(36)-mthIgG). The nucleotide and amino acid sequences for THER6 are listed as SEQ ID NO:23 and SEQ ID NO:24, respectively. The (gly4ser)6 linker sequence is shown in SEQ ID NO:52 (residues 268-303 of SEQ ID NO:24), and the encoding nucleotide sequence for the (gly4ser)6 linker is shown in SEQ ID NO:51 (residues 817-924 of SEQ ID NO:23).

**[243]** The fifth construct included a linker that encodes four (gly4ser) sequences flanked by restriction sites (36 amino acid linker), but in addition, the construct included a second linker and an enzyme sequence at the carboxyl terminus of the IgG1 domain. The (gly4ser)4 linker sequence is as described above for THER4 (nucleotide and amino acid sequences shown in SEQ ID NO:49 and SEQ ID NO:50, respectively). The second linker is an 18 amino acid long sequence (VDGASSPVNVSSPSVQDI; amino acid residues 1-18 of SEQ ID NO:8, encoded by nucleotides 1-54 of SEQ ID NO:7) that includes an N-linked glycosylation site, followed by a sequence that encodes human RNase1 enzyme activity. The linker sequence is listed as the first 54 nucleotides of SEQ ID NO 7, or the first 18 amino acids of SEQ ID NO 8, followed by the RNase sequence. The ApoA-1-lnk-hIgG1 segment is fused to the NLG-RNase, and this construct is identified as THER4RNA2. The nucleotide and amino acid sequences are identified as SEQ ID NO:3 and SEQ ID NO:4, respectively.

**[244]** Miniprep DNA for each of the five constructs was prepared and the concentration checked by Nanodrop analysis.

**[245]** The day before transfection, approximately 1.2 x $10^6$ 293T cells were plated to 60 mm dishes. Mini-plasmid preparations (4.0 µg DNA for 60mm plates) were used for 293T transfections using the QIAGEN POLYFECT® reagent (Catalog # 301105/301107) and following the manufacturer's instructions. Culture supernatants were harvested 48-72 hours after transfection. For most transfections, media was changed to serum-free media 24 hours after transfection, and cultures incubated for a further 48 hours prior to harvest.

**[246]** Culture supernatants were used directly for further analysis. 7 µl of each serum-free culture supernatant from transiently transfected cells was loaded onto gels with a 4X dilution of 4X LDS sample buffer (Life Technologies, Grand Island, NY) added to each sample to give a final concentration of 1X LDS loading buffer. For reducing gels, sample reducing agent was added to 1/10 final volume. Samples were heated at 72°C for 10 minutes and loaded on NuPAGE® 4-12% Bis-Tris

gels (Life Technologies/ThermoFisher Scientific, Grand Island, NY). Gels were subjected to electrophoresis in 1X NuPAGE® MOPS SDS-PAGE running buffer (NP0001, Life Technologies/ThermoFisher) at 180 volts for 1.5 hours, and proteins transferred to nitrocellulose using the XCell II™ Blot Module (Catalog #EI002/EI9051, Life Technologies/ThermoFisher, Grand Island, NY) at 30 volts for 1 hour. Blots were blocked overnight at 4°C in PBS containing 5% nonfat milk. Blots were incubated with 1:250,000X dilution of horseradish peroxidase conjugated goat anti-human IgG (Jackson Immunoresearch, Catalog #109-036-098, Lot# 122301). Blots were washed three times for 30 minutes each in PBS/0.05% Tween 20, and were developed in ThermoScientific ECL reagent (Catalog #32106) for 1 minute. Blots were exposed to autoradiograph film for 30 seconds to 2 minutes, depending on the blot. FIG. 3 shows Western Blot analysis of culture supernatants from representative 293T transient transfections. Positive and negative controls (CD40IgG and mock transfection/no DNA, respectively) were included in each transfection series. Transfected samples are as indicated in FIG. 3; lanes from left to right are as follows: Lane #1 – mock transfection; Lane #2 – CD40IgG; Lane #3 – MW markers; Lane #4 – THER0; Lane #5 – THER2; Lane #6 – THER4; Lane #7 – THER6; Lane #8 – MW marker; Lane #9 – THER4RNA2.

**[247]** The THER0, THER2, THER4, and THER6 fusion proteins run at a position above the 50 kDa molecular weight marker. The predicted molecular weight for these fusion proteins should be approximately 55, 56, 56.6, and 57 kDa, respectively. The increasing linker length is evident by altered mobility for each fusion protein. The THER4RNA2 molecule is predicted to be approximately 73.2 kDa, while ApoA-1 is predicted to run at 28.6 kDa. The CD40IgG control is expected to run at approximately 55 kDa.

## Example 4: Expression of THERmthIgG and Multi-subunitIg Fusion Constructs and Fusion Proteins in Stable CHO Cell Lines

**[248]** This example illustrates expression of the different Ig fusion genes described herein in eukaryotic cell lines and characterization of the expressed fusion proteins by SDS-PAGE and by IgG sandwich ELISA.

*Transfection and selection of stable cell lines expressing fusion proteins*

**[249]** Stable production of the Ig fusion protein was achieved by electroporation of a selectable, amplifiable plasmid, pDG, containing the THER-mthIgG cDNAs (human apo A-1 forms separated from the hinge and Fc domain of human IgG1 by linkers of varying lengths) under the control of the CMV promoter, into Chinese Hamster Ovary (CHO) CHO DG44 cells.

**[250]** The pDG vector is a modified version of pcDNA3 encoding the DHFR selectable marker with an attenuated promoter to increase selection pressure for the plasmid. Plasmid DNA

(200 μg) was prepared using QIAGEN HISPEED® maxiprep kits, and purified plasmid was linearized at a unique *Asc*I site (New England Biolabs, Ipswich, MA Catalog # R0558), purified by phenol extraction (Sigma-Aldrich, St. Louis, MO), ethanol precipitated, washed, and resuspended in EX-CELL® 302 tissue culture media, (Catalog #14324, SAFC/Sigma Aldrich, St. Louis, MO). Salmon sperm DNA (Sigma-Aldrich, St. Louis, MO) was added as carrier DNA just prior to phenol extraction and ethanol precipitation. Plasmid and carrier DNA were coprecipitated, and the 400 μg was used to transfect $2x10^7$ CHO DG44 cells by electroporation.

**[251]** For transfection, CHO DG44 cells were grown to logarithmic phase in EX-CELL® 302 media (Catalog # 13424C, SAFC Biosciences, St. Louis, MO) containing glutamine (4 mM), pyruvate, recombinant insulin (1 μg/ml), penicillin-streptomycin, and 2xDMEM nonessential amino acids (all from Life Technologies, Grand Island, NY), hereafter referred to as "EX-CELL 302 complete" media. Media for untransfected cells and cells to be transfected also contained HT (diluted from a 100x solution of hypoxanthine and thymidine) (Invitrogen/Life Technologies, Grand Island, NY). Electroporations were performed at 280 volts, 950 microFarads, using a BioRad (Hercules, CA) GENEPULSER® electroporation unit with capacitance extender. Electroporation was performed in 0.4 cm gap sterile, disposable cuvettes. Electroporated cells were incubated for 5 minutes after electroporation prior to transfer of culture to non-selective EX-CELL 302 complete media in T75 flasks.

**[252]** Transfected cells were allowed to recover overnight in non-selective media prior to selective plating in 96 well flat bottom plates (Costar) at varying serial dilutions ranging from 250 cells/well (2500 cells/ml) to 2000 cells/well (20,000 cells/ml). Culture media for cell cloning was EX-CELL 302 complete media containing 50 nM methotrexate. Transfection plates were fed at five day intervals with 80 μl fresh media. After the first couple of feedings, media was removed and replaced with fresh media. Plates were monitored and individual wells with clones were fed until clonal outgrowth became close to confluent, after which clones were expanded into 24 well dishes containing 1 ml media. Aliquots of the culture supernatants from the original 96 well plate were harvested to a second 96 well plate prior to transfer and expansion of the cells in 24 well plates. This second plate was frozen until ELISA analysis to estimate IgG concentrations.

*Screening culture supernatants for production levels of recombinant fusion proteins*

**[253]** Once clonal outgrowth of initial transfectants was sufficient, serial dilutions of culture supernatants from master wells were thawed and the dilutions screened for expression of Ig fusion protein by use of an IgG sandwich ELISA. Briefly, NUNC MAXISORP® plates were coated overnight at 4°C with 2 μg/ml F(ab'2) goat anti-human IgG (Jackson Immunoresearch, West Grove, PA; Catalog # 109-006-098) in PBS. Plates were blocked in PBS/3% BSA, and serial dilutions of culture supernatants incubated at room temperature for 2-3 hours or overnight at 4°C. Plates were

washed three times in PBS/0.05 % Tween 20, and incubated with horseradish peroxidase conjugated F(ab'2)goat anti-human IgG (Jackson Immunoresearch, West Grove, PA, Catalog # 109-036-098) at 1:7500-1:10,000 in PBS/0.5% BSA, for 1-2 hours at room temperature. Plates were washed five times in PBS/0.05% Tween 20, and binding detected with SUREBLUE RESERVE™ TMB substrate (KPL Labs, Gaithersburg, MD; catalog #53-00-02). Reactions were stopped by addition of equal volume of 1N HCl, and absorbance per well on each plate was read at 450nM on a SYNERGY™ HT plate reader (Biotek, Winooski, VT). Concentrations were estimated by comparing the OD450 of the dilutions of culture supernatants to a standard curve generated using serial dilutions of a known standard, a protein A purified human IgG fusion protein with an Ig tail identical to that of the THER clones. Data was collected and analyzed using GEN5™ software (Biotek, Winooski, VT) and Microsoft Office EXCEL® spreadsheet software.

**[254]** The results of initial screening of the CHO transfectants are summarized in Table 3 and FIGS. 4A-4E and 5A-5C. Table 3 shows a summary of the number of clones screened, the range of expression levels observed from initial 96-well cultures, and the expression observed from initial T25 and/or 24 well spent cultures. FIGS. 4A-4E show a series of columnar graphs representing the production levels obtained from each CHO clone of a transfection series. The clones from the THER0, THER2, THER4, THER6, and THER4RNA2 transfections are displayed as a group in each of the five panels shown. Each clone was screened at least once by IgG sandwich ELISA to assess expression level of the fusion protein. FIGS. 5A-5C show three panels showing the results of 6 and 10 day assays of fusion protein expression from the CHO transfectants with the highest expression after initial screening. Six and ten day assays were performed by setting up 5 ml cultures at $1x10^5$ viable cells/ml ($5x10^5$ initial inoculum) in T25 flasks. The cultures were grown for six days after which a 1 ml aliquot was removed and live and dead cells counted. Cells were then centrifuged and the culture supernatants saved for further analysis by IgG sandwich ELISA and other analyses. The remainder of the cultures were incubated for a further four days until day 10, and the cells counted for cell number, viability, and a supernatant sample harvested for IgG sandwich ELISA. The results are tabulated in columnar form for each clone as shown in the graphs for cell number at day 6 and day 10, viability, and concentration of fusion protein.

**Table 3: Expression of ApoA1-IgG fusion proteins in stably transfected CHO DG44 cells**

| Construct | Clones Screened | 96 well sups expression range (µg/ml) | Spent T25 top producer (µg/ml) | 6/10 day assay on top clones (µg/ml) |
|---|---|---|---|---|
| THER0 | 45 | 0-46.5 | 135 | 60/230 |
| THER2 | 135 | 0-36 | 145 | 45/125 |
| THER4 | 237 | 0-76 | 165 | 70/200 |
| THER6 | 192 | 0-57 | 145 | 85/250 |
| THER4RNA2 | 50 | 0-45 | 90 | 50/118 |

**[255]** The clones with the highest production of the fusion protein were expanded into T25 and then T75 flasks to provide adequate numbers of cells for freezing and for scaling up production of the fusion protein. Production levels were further increased in cultures from the four best clones by progressive amplification in methotrexate-containing culture media. At each successive passage of cells, the EX-CELL 302 complete media contained an increased concentration of methotrexate, such that only the cells that amplified the DHFR plasmid could survive. Media for transfections under selective amplification contained varying levels of methotrexate (Sigma-Aldrich) as selective agent, ranging from 50 nM to 1 µM, depending on the degree of amplification achieved.

*Purification of fusion proteins from culture supernatants*

**[256]** Supernatants were collected from spent CHO cell cultures expressing the Apo A-1-lnk-mthIgG1 construct, filtered through 0.2 µm PES express filters (Nalgene, Rochester, N.Y.) and subjected to gravity flow affinity chromatography over a Protein A-agarose (IPA 300 crosslinked agarose, or IPA 400HC crosslinked agarose) column (Repligen, Waltham, Mass.). The column was conditioned with 0.1M citrate buffer, pH2.2, then supernatant adjusted to pH 8.0 with 0.5M $NHCO_3$, and loaded by gravity flow to allow binding of the fusion proteins. Columns were then washed with several column volumes column wash buffer (90mM Tris-Base, 150mM NaCl, 0.05% sodium azide, pH 8.7) or Dulbecco's modified PBS, pH 7.4 prior to elution. Bound protein was eluted using 0.1 M citrate buffer, pH 3.2. Fractions (0.8-0.9 ml) were collected into 0.2 ml 0.5M $NaCO_3$-$NaHCO_3$ buffer to neutralize each fraction. Protein concentration of aliquots (2 µl) from each fraction were determined at 280nM using a Nanodrop (Wilmington DE) microsample spectrophotometer, with blank determination using 0.1 M citrate buffer, pH 3.2, 0.5M $NaCO_3$ at a 10:1 v:v ratio. Fractions containing fusion protein were pooled, and buffer exchange performed by dialysis using Spectrum Laboratories G2 (Ranch Dominguez, CA, Catalog #G235057, Fisher Scientific catalog # 08-607-007) FLOAT-A-LYZER® units (MWCO 20kDa) against D-PBS (Hyclone, ThermoFisher Scientific, Dallas, TX), pH 7.4. Dialysis was performed in sterile, 2.2 liter Corning roller bottles at 4°C overnight.

**[257]** After dialysis, protein was filtered using 0.2 μM filter units, and aliquots tested for endotoxin contamination using PYROTELL® LAL gel clot system single test vials (STV) (Catalog # G2006, Associates of Cape Cod, East Falmouth, MA). The predicted OD 280 of a 1 mg/ml solution of the THER4 fusion protein was determined to be 1.19 (mature protein without either the signal peptide or the 6 amino acid propeptide) or 1.27 (including the 6 amino acid propeptide) using the protein analysis tools in the VECTOR NTI® Version 11.5 Software package (Informax, North Bethesda, MD) and the predicted cleavage site from the online ExPASy protein analysis tools. It is unclear whether the fusion protein secreted from the CHO cells is homogeneous with regard to complete cleavage of the propeptide from the recombinant molecules. The OD280 for each purified fusion protein was corrected using these tools.

*Reducing and Nonreducing SDS-PAGE Analysis of apo A-1 Ig Fusion Proteins*

**[258]** Purified fusion proteins were analyzed by electrophoresis on SDS-Polyacrylamide 4-12% Bis-Tris NuPAGE® gels (Life Technologies, Grand Island, NY). Fusion protein samples were heated at 72°C for 10 minutes in LDS sample buffer with and without reduction of disulfide bonds and applied to 4-12% BIS-Tris gels (Catalog #NP0301, LIFE Technologies, Grand Island, NY). Five micrograms of each purified protein was loaded on the gels. The proteins were visualized after electrophoresis by IMPERIAL™ protein staining (Pierce Imperial Protein Stain Reagent, Catalog #24615, ThermoFisher Scientific/Pierce, Rockford, IL), and destaining in distilled water. Molecular weight markers were included on the same gel (KALEIDOSCOPE™ Prestained Standards, Catalog #161-0324, Bio-Rad, Hercules, CA). The results from representative nonreducing and reducing gels are shown in FIGS. 6A and 6B, respectively. Lanes are as follows from left to right: Lane #1 – KALEIDOSCOPE prestained MW markers; Lane #2 – THER0; Lane #3 – THER2; Lane #4 – THER4; Lane #5 – THER6; Lane #6 – THER4RNA2; Lane #7 – KALEIDOSCOPE Prestained MW markers. Approximate molecular weights are indicated on the figures.

**[259]** Again, the linker length difference between the different fusion proteins is evident on both the reducing and nonreducing gels, with the THER0 protein running at just over 50kDa. The absence of hinge disulfides is evident by the similar mobility for each protein when electrophoresed under reducing or nonreducing conditions.

*Native Gel Electrophoresis of apo A-1 IgG Fusion Proteins*

**[260]** The protein A purified fusion proteins were subjected to native PAGE analysis. BLUE Native PAGE gels were run using 4-16% Bis-Tris NativePAGE™ gels (Life Technologies/ThermoFisher) with cathode and anode buffers prepared according to manufacturer's instructions. Samples (4.5 μg each fusion protein) were prepared without heating, using 4X sample buffer, without detergents. Gels were run for 30 minutes at 150 volts, followed by 1 hour at 180 volts,

and the final hour at 220 volts. Gels were washed in distilled water and incubated for two hours in IMPERIAL™ Protein stain. Gels were extensively destained overnight with repeated washes in distilled water to remove the blue dye present in the cathode buffer used for running the gels. FIG. 7 shows a representative native gel using these conditions. Molecular weight markers were GE Healthcare high molecular weight calibration markers, a mixture of six large, multicomponent proteins, resuspended in the loading buffer used for samples, again without added detergents. Samples were loaded as follows: Lane #1 – ORENCIA® (abatacept; CTLA4hIgG); Lane #2 – anti-mouse CD40 monoclonal antibody 1C10; Lane #3 – THER4RNA2; Lane #4 – GE Healthcare High MW calibration markers; Lane #5 – THER6; Lane #6 – THER4; Lane #7 – THER2; Lane #8 – THER0; Lane #9 – GE Healthcare high MW markers; Lane #10 – Athens Research Apo A-1.

**[261]** The native ApoA1-IgG fusion proteins run at an approximate molecular weight somewhere between the 140 and 233 kDa markers and with a similar mobility as ORENCIA® (abatacept), a CTLA4Ig fusion protein with the same human IgG1 Fc domain. The THER4RNase bispecific fusion protein did not stain well with the IMPERIAL stain possibly due to the highly basic composition of the RNase domain, but appears to migrate in a more diffuse pattern with the predominate visible band migrating between the 233 and 440 kDa standards.

## Example 5: Use of an IgG/Apo A-1 Sandwich ELISA to Assess Binding of THER Apo A-1 Fusion Proteins

**[262]** An antigen binding ELISA was performed to assess the ability of Ig fusion proteins, captured by immobilized anti-human IgG (Fc-specific) to bind to and be detected by a horseradish peroxidase conjugated antibody specific for human apolipoprotein A-1. High protein-binding, 96-well ELISA plates (NUNC MAXISORP® plates, ThermoFisher Scientific) were coated with 1.5 µg/ml goat anti-human IgG (Jackson Immunoresearch). Plates were blocked overnight at 4°C with PBS/3% BSA. Serial dilutions of each THER fusion protein, starting at 5 µg/ml, were incubated overnight at 4°C. The plate was washed three times and then incubated with horseradish peroxidase conjugated anti-human apolipoprotein A-1 (ThermoFisher Scientific, catalog # PAI-28965) diluted 1:1500. Plates were incubated at room temperature for 2 hours. Plates were washed four times, then SUREBLUE RESERVE™ TMB substrate (Catalog #: 53-00-02, KPL, Gaithersburg, MD) was added to the plate at 80µl/well. Development was stopped by addition of 80 µl/well 1N HCl. Samples were read at 450 nm using a SYNERGY™ HT Biotek plate reader (Biotek Instruments, Winooski, VT) and data analyzed using GEN5™ 2.0 software.

**[263]** FIG. 8 shows the results from a representative Apo A-1 binding ELISA. OD450 is plotted versus concentration of fusion protein. THER 0, 2, 4, 6, and THER4RNA2 fusion proteins all

exhibited similar dose-response curves, indicating that the molecules can each be captured by binding to the Ig tail and detected by binding of the Apo A-1 domain to the antibody targeted to human Apo A-1. Human apolipoprotein A-1 (Athens Research & Technology, catalog # 16-16-120101) was included as a control and was not captured by the anti-human Fc specific antibody. At higher concentrations, the molecule showed weak binding by the antibody targeted to Apo A-1, indicating that the Apo A-1 may have bound weakly to the plastic without capture by the anti-Fc antibody.

## Example 6: Expression and Testing of an RNase Bifunctional Enzyme Lipid Transport Fusion Molecule

**[264]** For the Apo A-1 IgG RNase fusion protein (THER4RNA2), RNase activity was assayed to determine whether fusion of the enzyme to the carboxyl end of the fusion construct interfered with ability of the molecule to digest RNA. FIGS. 9 and 10 show the results of an RNASEALERT™ assay (IDT, Coralville, IA) performed using the fluorescence and kinetic assay functions of the SYNERGY™ HT plate reader. RNASEALERT™ Substrate is a synthetic RNA oligonucleotide that has a fluorescein (R) on one end and a dark quencher (Q) on the other end. When intact, the substrate has little or no fluorescence, but when cleaved by an RNase, the substrate fluoresces green (490 nm excitation, 520 nm emission) and can be detected with the appropriately equipped fluorescence plate reader. A positive signal in this assay shows increasing fluorescence signal over time due to cleavage of the substrate by RNase present in the sample(s). Microplates were incubated with RNASEALERT substrate (a fixed concentration of 20 pmol/µl), 1X RNASEALERT buffer, and fusion protein or enzyme controls dilutions added to each well of a 96 well plate. Enzyme activity assays were performed in triplicate for each sample, and the kinetic assay allowed to proceed for 45 minutes, with successive readings every 60 seconds. The increasing fluorescence at each time point is displayed for each well as a trace of RFU/well as a function of time in FIG. 9. Serial dilutions of enzyme/fusion protein included 20 pmol/µl, 13.4 pmol/µl, 8.9 pmol/µl, 6 pmol/µl, 4 pmol/µl, 2.7 pmol/µl, 1.8 pmol/µl, and no enzyme. RNase A (Ambion/ThermoFisher, catalog #AM2270) was included as a positive control, and THER4 (apo A-1-lnk26-hIgG) was included as a negative control for comparing to the THER4RNA2 fusion protein. Overlays of the traces generated using the 4 pmol/µl enzyme are shown in FIG. 10. Two replicates of the RNaseA, THER4RNA2, and THER4, are shown. All enzymes are at 4 pmol/µl and the substrate is present at 20 pmol/µl.

## Example 7: Measurement of Cholesterol Efflux to Fusion Protein Acceptors

**[265]** Using two separate assays, THER0, THER2, THER4, THER6, and THER4RNA2 fusion proteins were assessed for their ability to act as acceptor molecules for reverse cholesterol

transport from pre-loaded monocyte/macrophage mammalian cell lines. The first assay used the human monocytic/macrophage cell line THP-1 and a fluorescently labeled derivative of cholesterol, BODIPY-cholesterol or TOPFLUOR-cholesterol (cholesterol compound with fluorescent boron dipyrromethene difluoride linked to sterol carbon-24) (Avanti Polar Lipids, Alabaster, AL). The THP-1 cells were grown in RPMI with 4mM glutamine, 10% FBS and maintained in mid-logarithmic growth prior to plating. The protocol was adapted from the procedures outlined in Sankaranararyanan *et al.* (*J. Lipid Res.* 52:2332-2340, 2011) and Zhang *et al.* (*ASSAY and Drug Development Technologies*: 136-146, 2011). Cells were harvested and plated to 96-well flat bottom tissue culture plates at $2x10^6$ cells/ml or $2x10^5$ cells/well in 100 µl RPMI media containing 33 ng/ml PMA. Cells were maintained in culture for 36-48 hours to allow for differentiation to occur prior to the assay. Culture media was aspirated and plates were washed in 1xPBS. Labeling media consisting of the following components (Phenol Red free RPMI with media supplements, 0.2% FBS, with ACAT inhibitor at 2 µg/ml, Sandoz 58-035 (Sigma-Aldrich, St. Louis MO), LXR agonist TO-901317 at 2.5 µM (Sigma-Aldrich, St. Louis, MO), 35 ng/ml PMA (Sigma-Aldrich, St. Louis, MO), and 1.25 mM methyl beta-cyclodextrin (Sigma-Aldrich, St. Louis, MO), 50 uM cholesterol (Sigma-Aldrich, St. Louis, MO0, and 25 µM TOPFLUOR cholesterol (Avanti Polar Lipids, Alabaster, AL) was added at a volume of 100 µl/well and incubated at 37C, 5% CO2 for 10-12 hours. Equilibration media, RPMI complete with 10% FBS, 33 ng/ml PMA (100 ul/well), was added to each well and incubated for 8 hours prior to incubation with acceptors. Labeling/equilibration media was aspirated from plates, and plates were washed twice with 200 µl/well PBS + 0.15% BSA. Efflux acceptor reagents were added to individual wells in efflux buffer and incubated for two hours prior to assay. Acceptors were added to efflux buffer at concentrations ranging from 100nM to 500nM, depending on the assay. Efflux buffer was phenol red-free RPMI with growth supplements and 0.15% BSA. Samples were run in sets of 6-12 per condition/acceptor, and a minimum of five replicates used for statistical analysis. APO A-1 was run as a positive control, and efflux media alone was used as the background negative control (baseline efflux). The efflux reaction was allowed to proceed for two hours, after which culture media was harvested to black, flat bottom 96-well plates (media reading). Cell lysates were prepared by addition of 100 ul 0.1 N NaOH to each well of the efflux plate, and incubation for 15 minutes on a plate shaker at 4°C. Cell lysates were transferred to black, 96-well plates (lysate reading), and fluorescence for media and lysate samples measured using a SYNERGY™ HT plate reader with excitation at 485 nm and emission at 528 nm. Efflux was calculated as the ratio of the fluorescence measurements: (media/(media+lysate) x 100). The specific efflux was calculated by subtracting the baseline readings of the samples with no acceptor present from the total efflux/sample for each tested acceptor. Data analysis was performed using GraphPad Prism v 4.0 Software (San Diego, CA). The assay results are shown in FIG. 11.

**[266]** The second assay used the mouse macrophage cell line J774A.1 (ATCC, Manassas, VA) to assess reverse cholesterol transport (RCT) using a radioactive derivative of cholesterol, [$^3$H]-cholesterol as described by Sankaranararyanan *et al.* (*J. Lipid Res.* 52:2332-2340, 2011) and Yancey *et al.* (*J. Lipid Res.* 45:337-346, 2004). Briefly, J774 cells ($3.5x10^5$ per well in 24 well plates) were incubated for 24 hours in 0.25 ml RPMI media supplemented with 5% FBS, ACAT inhibitor Sandoz 58-035 (2 μg/ml), and 4 μCi/ml of [$^3$H]-cholesterol. ACAT inhibitor was present at all times during the assay. Cells were equilibrated 16-24 hours in media with or without cAMP (0.3mM) prior to incubation with acceptors. The presence of cAMP upregulates the ABCA1 molecule. Labeled cells were washed in media containing 1% BSA, then acceptor molecules were added at 50, 100 and 200nM in MEM-HEPES media and incubated for 4 hours prior to measurements. All treatments were performed in triplicate. The [$^3$H] cholesterol in 100 μl of the media was then measured by liquid scintillation counting. The percentage efflux is based on the total [$^3$H]cholesterol present in the cells before the efflux incubation ($t_0$ sample). To measure the [$^3$H]cholesterol present in the cells, the cell lipids were extracted by incubating the cell monolayers overnight in isopropanol. After lipid extraction, the total [$^3$H]cholesterol present in the lipid extract was measured by liquid scintillation counting. Data analysis was performed using GraphPad Prism software 4.0 (San Diego, CA). The assay results are shown in FIG. 12.

## Example 8: Construction of a PON1 Bifunctional Enzyme Lipid Transport Fusion Molecule

**[267]** In addition to the apoA-1-IgG-RNase expression constructs described above, additional molecules which physically link the ApoA-1 phospholipid transport function to the active sites of other enzyme domains are constructed. One such molecule contains a segment corresponding to human paraoxanase 1 (PON1), with nucleotide and encoded amino acid sequences as shown in SEQ ID NO:11 and SEQ ID NO:12, respectively. This arylesterase enzyme is present in human serum exclusively associated with high density lipoprotein (HDL), and inhibits oxidation of low density lipoprotein molecules. This protection from oxidation also inhibits development of vascular and coronary diseases. The mature protein form of PON1 is unique in that it retains its amino terminal signal peptide after secretion (amino acid residues 1 to 15 of SEQ ID NO:12, encoded by nucleotide residues 1 to 45 of SEQ ID NO:11). Expression of a mutant form of PON1 with a cleavable amino terminus demonstrated that PON1 associates with lipoproteins through its amino terminus by binding to phospholipids directly rather than first binding to ApoA-1. *See* Sorenson *et al.*, *Arteriosclerosis, Thrombosis, and Vascular Biology* 19:2214-2225, 1999. Removal of the signal sequence was found to eliminate binding of the PON1 moiety to phospholipids, proteoliposomes, and serum lipoproteins. Additionally, in the absence of phospholipid, wild-type PON1 does not bind directly to ApoA-1. *See* Sorenson *et al.*, *supra*. These PON1 signal sequence mutants showed

reduced enzyme activity, possibly due to inability to bind the optimal phospholipid substrates. Nevertheless, a recombinant, active form of human PON1 has been expressed in bacteria that is missing this signal sequence. *See* Stevens *et al.*, *Proc. Natl. Acad. Sci. USA* 105:12780-12784, 2008. The presence of ApoA-1 does appear to stabilize arylesterase activity of the enzyme.

**[268]** Removal of the amino terminal signal sequence of PON1 (and thereby the phospholipid binding moiety) and substitution of this region with human apoA-1-lnk-IgG directly links the enzyme activity with the phospholipid binding domain of ApoA-1, stabilizing the arylesterase enzyme activity while providing the optimal substrates bound to the ApoA-1 domain. Such a molecule still traffics and is transported with the phospholipids bound by ApoA-1 and retains enzyme activity due to replacement of the signal sequence domain with an alternative phospholipid binding domain. In addition, a bifunctional molecule fusing these two domains exhibits improved expression and facilitates targeting of the PON1 activity to the choroid plexus through active binding of apo A-1. PON1 has been expressed at the carboxyl terminus of an insulin receptor targeted antibody (*see* Boado *et al.*, *Mol. Pharm.* 5:1037-1043, 2008; Boado *et al.*, *Biotechnology and Bioengineering* 108:186-196, 2011); however, the amino terminal signal peptide was included in this fusion protein. The fusion gene and protein described here provides a novel method of PON1 fusion protein expression, eliminating the requirement for the signal peptide by a direct physical coupling of the truncated enzyme to the apo A-1 domain, thereby preserving and stabilizing both the binding function and arylesterase activity of PON1.

**[269]** Sequences for the fusion gene and protein are shown in SEQ ID NO:27 and SEQ ID NO:28 for THER4PON1 (nucleotide and amino acid sequences, respectively) and in SEQ ID NO:37 and SEQ ID NO:38 for THER2PON1 (nucleotide and amino acid sequences, respectively). Similar fusion genes and proteins contain alternative linker forms of ApoA-1 fused to the hIgG1-linker-PON1 segment(s). The PON1 sequences within the THER4PON1 and THER2PON1 molecules correspond to the Q192 allele for human PON1.

**[270]** Alternative forms of PON1 are also used to construct bifunctional fusion molecules linking PON1 to Apo A-1. A sequence polymorphism that affects enzyme activity for different substrates is present at position 192 of the PON1 sequence. *See* Steven *et al.*, *supra*. The amino acid at this position may be glutamine (Q) or arginine (R) in humans, or a lysine (K) in rabbits. The arginine allele at position 192 has been reported to have a higher catalytic activity *in vitro* and *in vivo*. Similarly, the rabbit form of PON1 with a lysine at position 192 has been reported to have a more stable catalytic activity *in vitro* and *in vivo* (*see* Steven *et al.*, *supra*; Richter *et al.*, *Circulation Cardiovascular Genetics* 1:147-152, 2008). These alternative PON1 sequences are shown in SEQ ID NO:41 (nucleotide) and SEQ ID NO:42 (amino acid) for the PON1 Q192K form, and SEQ ID NO:43 (nucleotide) and SEQ ID NO:44 (amino acid) for the PON1 Q192R form. The fusion construct

between these alternate PON1 forms and the THER4 sequence (apoA-1(g4s)4hIgGNLG—...) is designated as THER4PON1 Q192K (nucleotide and amino acid sequences shown in SEQ ID NO:45 and SEQ ID NO:46, respectively) or THER4PON1 Q192R (nucleotide and amino acid sequences shown in SEQ ID NO:47 and SEQ ID NO:48, respectively), depending on the polymorphism present in the PON1 sequence at amino acid 192 of the PON1 sequence (or at amino acid 720 of the THER4PON1Q variants shown in SEQ ID NO:46 and SEQ ID NO:48). Similarly, a THER2 form of the fusion gene/protein is indicated as THER2PON1 Q192K or THER2PON1Q192R. For all of these fusion constructs, the PON1 amino terminal signal sequence (amino acids 1-15 of SEQ ID NO:12) is removed.

**[271]** Bispecific Enzyme Lipoprotein Transfer Proteins comprising PON1 are screened for arylesterase/PON1 activity using the nontoxic substrates 4-(chloromethyl)phenyl acetate (CMPA) and phenyl acetate (*see* Richter *et al.*, *supra*). These substrates are preferable for screening activity since the substrate and reaction product are relatively nontoxic compared to organophosphate pesticides. The CMPA substrate (Sigma-Aldrich, Inc. St Louis, MO) is incubated with serial dilutions of fusion protein and rates of CMPA hydrolysis assayed at 280 nm for 4 minutes at 25°C using ultraviolet transparent 96-well plates (Costar, Cambridge, Mass). Dilutions are run in triplicate or quadruplicate and substrate concentration fixed at 3mmol/L in 20mM Tris-HCl (pH 8.0), 1.0 mM $CaCl_2$. Similarly, arylesterase assays are performed on phenyl acetate as substrate. The rates of PA hydrolysis are measured at 270nm, for 4 minutes under both high and low salt conditions.

## Example 9: Construction of a PAFAH or CETP Bifunctional Enzyme Lipid Transport Fusion Molecule

**[272]** In addition to the apoA-1-IgG-RNase and apoA-1-IgG-PON1 expression constructs described above, additional molecules which physically link the ApoA-1 phospholipid transport function to the active sites of other enzyme domains are constructed.

**[273]** One such molecule contains a segment corresponding to human PAFAH (lipoprotein-associated phospholipase A2, human phospholipase A2 group VII, platelet activating factor acetyl hydrolase), with nucleotide and encoded amino acid sequences as shown in SEQ ID NO:31 and SEQ ID NO:32, respectively (*see also* GenBank accession number NM_005084 (transcript variant 1)). The PAFAH amino acid sequence is encoded by nucleotides 270 to 1592 of SEQ ID NO:31, with the STOP codon at nucleotides 1593 to 1595. The fusion gene and protein are designed fusing the PAFAH coding sequence at the carboxyl end of the human IgG with the N-linked glycosylation linker inserted between the two molecules. The THER4PAFAH nucleotide and encoded amino acid sequences are shown as SEQ ID NO:33 and SEQ ID NO:34, respectively. The PAFAH sequence

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without the 21 amino acid signal peptide (MVPPKLHVLFCLCGCLAVVYP; residues 1-21 of SEQ ID NO:32) is fused to the NLG linker at amino acid position 544 in SEQ ID NO 34.

**[274]** Another such molecule contains a segment corresponding to human CETP or cholesteryl ester transfer protein (CETP), transcript variant 1, with nucleotide and encoded amino acid sequences as shown in SEQ ID NO:29 and SEQ ID NO:30, respectively (*see also* GenBank accession number NM_000078). The CETP protein is encoded by nucleotides 58 to 1537 of SEQ ID NO:29. The fusion gene and protein are designed fusing the CETP coding sequence at the carboxyl end of the human IgG with the N-linked glycosylation linker inserted between the two molecules. The THER4CETP (or human apo A-1-(g4s)4-hIgG-NLG-CETP) nucleotide and encoded amino acid sequences are shown as SEQ ID NO:39 and SEQ ID NO:40, respectively. The nucleotides (57-107 of SEQ ID NO 29) encoding the signal peptide (amino acids 1-17 of SEQ ID NO 30) are removed in order to create the fusion gene between the NLG linker sequence and the CETP mature peptide. The fusion junction between these two protein domains is located at amino acid 544 of SEQ ID NO 40.

**[275]** From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entireties for all purposes.

**[276]** In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

**[277]** It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

CLAIMS

1. A fusion polypeptide comprising, from an amino-terminal position to a carboxyl-terminal position, ApoA1-L1-D, wherein:

ApoA1 is a first polypeptide segment comprising an amino acid sequence having at least 90% or at least 95% identity with amino acid residues 19-267 or 25-267 of SEQ ID NO:2, wherein said first polypeptide segment has cholesterol efflux activity, optionally wherein said first polypeptide segment has the amino acid sequence shown in residues 19-267 or 25-267 of SEQ ID NO:2;

L1 is a first polypeptide linker comprising at least five amino acid residues; and

D is an immunoglobulin Fc region;

wherein the fusion polypeptide has increased ApoA1 cholesterol efflux activity as compared to either an ApoA1-Fc fusion polypeptide with a two amino acid linker or an ApoA1-Fc fusion polypeptide without a linker.

2. The fusion polypeptide of claim 1, wherein L1 comprises at least 10 amino acid residues.

3. The fusion polypeptide of claim 1, wherein L1 comprises at least 16 amino acid residues, consists of from 15 to 40 amino acid residues, or consists of from 16 to 36 amino acid residues,

optionally wherein L1 consists of 16 amino acid residues, 21 amino acid residues, 26 amino acid residues, 31 amino acid residues, or 36 amino acid residues,

optionally wherein L1 has the amino acid sequence shown in residues 268-283 of SEQ ID NO:22, residues 268-288 of SEQ ID NO:26, residues 268-293 of SEQ ID NO:2, SEQ ID NO:54, or residues 268-303 of SEQ ID NO:24.

4. The fusion polypeptide of any one of claims 1 to 3, wherein the Fc region is a human Fc region,

optionally wherein the human Fc region is an Fc variant comprising one or more amino acid substitutions relative to the wild-type human sequence,

optionally wherein the Fc region is a human γ1 Fc region or a human γ3 Fc region.

5. The fusion polypeptide of claim 4, wherein the human Fc region is a human γ1 Fc variant in which Eu residue C220 is replaced by serine,

optionally wherein the Eu residue P331 is replaced by serine.

6. The fusion polypeptide of claim 5, wherein Eu residues C226 and C229 are each replaced by serine,

optionally wherein Eu residue P238 is replaced by serine,

optionally wherein the Fc region has the amino acid sequence shown in (i) residues 294-525 or 294-524 of SEQ ID NO:2, or (ii) residues 294-525 or 294-524 of SEQ ID NO:13.

7. The fusion polypeptide of claim 1, wherein the fusion polypeptide comprises an amino acid sequence having at least 90% or at least 95% identity with

(i) residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:2,

(ii) residues 19-525, 19-524, 25-525, or 25-524 of SEQ ID NO:13,

(iii) residues 19-515, 19-514, 25-515, or 25-514 of SEQ ID NO:22,

(iv) residues 19-520, 19-519, 25-520, or 25-519 of SEQ ID NO:26, or

(v) residues 19-535, 19-534, 25-535, or 25-534 of SEQ ID NO:24,

optionally wherein the fusion polypeptide comprises the amino acid sequence shown in any one of (i)-(v).

8.The fusion polypeptide of any one of claims 1 to 7, further comprising a second polypeptide segment located carboxyl-terminal to the dimerizing domain, wherein the second polypeptide segment is selected from the group consisting of an RNase, a paraoxonase, a platelet-activating factor acetylhydrolase, and a cholesterol ester transfer protein,

optionally wherein the fusion polypeptide comprises, from an amino-terminal position to a carboxyl-terminal position, ApoA1-L1-D-L2-P, wherein ApoA1, L1, and D are as defined in claim 1, L2 is a second polypeptide linker, wherein L2 is optionally present, and P is the second polypeptide segment.

9. The fusion polypeptide of claim 8, wherein the second polypeptide segment is the RNase,

optionally wherein the RNase has at least 90% or at least 95% identity with amino acid residues 542-675 of SEQ ID NO:4,

optionally wherein the RNase has the amino acid sequence shown in residues 542-675 of SEQ ID NO:4.

10. The fusion polypeptide of claim 9, wherein the fusion polypeptide comprises an amino acid sequence having at least 90% or at least 95% identity with

(i) residues 19-675 or 25-675 of SEQ ID NO:4 or

(ii) residues 19-675 or 25-675 of SEQ ID NO:14,

optionally wherein the fusion polypeptide comprises the amino acid sequence shown in (i) or (ii).

11. The fusion polypeptide of claim 8, wherein the second polypeptide segment is the paraoxonase, optionally wherein the paraoxonase is human paraoxonase 1 (PON1) or a functional variant thereof,

optionally wherein the human PON1 or functional variant thereof has at least 90% or at least 95% identity with amino acid residues 16-355 of SEQ ID NO:12, amino acid residues 16-355 of SEQ ID NO:42, or amino acid residues 16-355 of SEQ ID NO:44,

optionally wherein the human PON1 or functional variant thereof has the amino acid sequence shown in residues 16-355 of SEQ ID NO:12, residues 16-355 of SEQ ID NO:42, or residues 16-355 of SEQ ID NO:44.

12. The fusion polypeptide of claim 11, wherein the fusion polypeptide comprises an amino acid sequence having at least 90% or at least 95% identity with

(i) residues 19-883 or 25-883 of SEQ ID NO:28,

(ii) residues 19-873 or 25-873 of SEQ ID NO:38,

(iii) residues 19-883 or 25-883 of SEQ ID NO:46, or

(iv) residues 19-883 or 25-883 of SEQ ID NO:48,

optionally wherein the fusion polypeptide comprises the amino acid sequence shown in any one of (i)-(iv).

13. A dimeric protein comprising a first fusion polypeptide and a second fusion polypeptide, wherein each of said first and second fusion polypeptides is a fusion polypeptide as defined in any one of claims 1 to 12.

14. A polynucleotide encoding the fusion polypeptide of any one of claims 1 to 12.

15. An expression vector comprising the following operably linked elements:

a transcription promoter;

a DNA segment encoding the fusion polypeptide of any one of claims 1 to 12; and

a transcription terminator.

16. A cultured cell into which has been introduced the expression vector of claim 15, wherein the cell expresses the DNA segment.

17. A method of making a fusion polypeptide, the method comprising:

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culturing a cell into which has been introduced the expression vector of claim 15, wherein the cell expresses the DNA segment and the encoded fusion polypeptide is produced; and

recovering the fusion polypeptide,

optionally wherein the encoded fusion polypeptide is produced and recovered as a dimeric protein.

18. A composition comprising:

a fusion polypeptide of any one of claims 1 to 12 or a dimeric protein of claim 13; and

a pharmaceutically acceptable carrier.

19. A method for treating a disease, the method comprising:

administering to a subject having the disease an effective amount of a fusion polypeptide of any one of claims 1 to 12 or a dimeric fusion protein of claim 13, wherein the disease is a cardiovascular disease characterized by atherosclerosis, optionally wherein the cardiovascular disease is selected from the group consisting of coronary heart disease and stroke, optionally wherein the coronary heart disease is characterized by acute coronary syndrome.

20. Use of the fusion polypeptide of any one of claims 1 to 12 or a dimeric fusion protein of claim 13 in the manufacture of a medicament for the treatment of a disease, wherein the disease is a cardiovascular disease characterized by atherosclerosis, optionally wherein the cardiovascular disease is selected from the group consisting of coronary heart disease and stroke, optionally wherein the coronary heart disease is characterized by acute coronary syndrome.

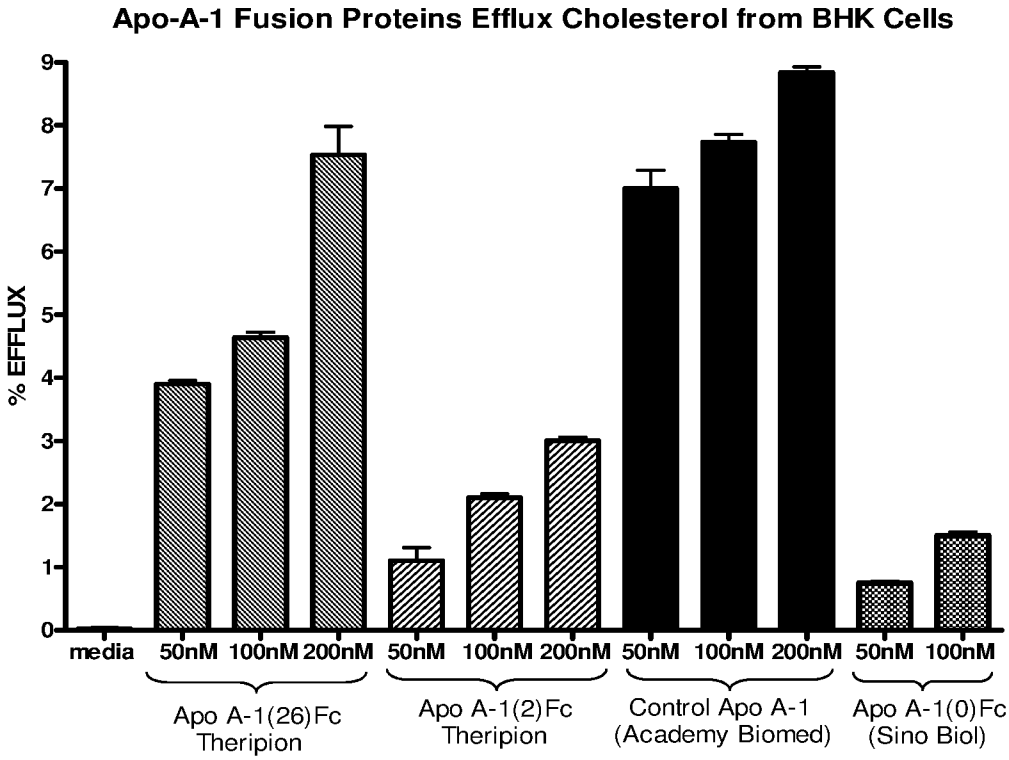
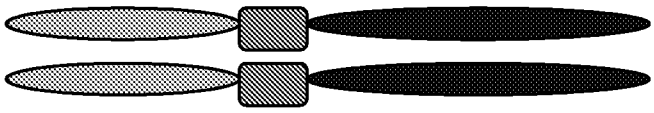

Apo-A-1 Fusion Proteins Efflux Cholesterol from BHK Cells
% EFFLUX
0
1
2
3
4
5
6
7
8
9
media
50nM
100nM
200nM
50nM
100nM
200nM
50nM
100nM
200nM
50nM
100nM
Apo A-1(26)Fc Theripion
Apo A-1(2)Fc Theripion
Control Apo A-1 (Academy Biomed)
Apo A-1(0)Fc (Sino Biol)

FIG. 1



huApo A-1 linker huIgG Fc
(SSShinge-P238S-P331S CH2-CH3)

FIG. 2A

huApo A-1 linker huIgG Fc linker enzyme

FIG. 2B

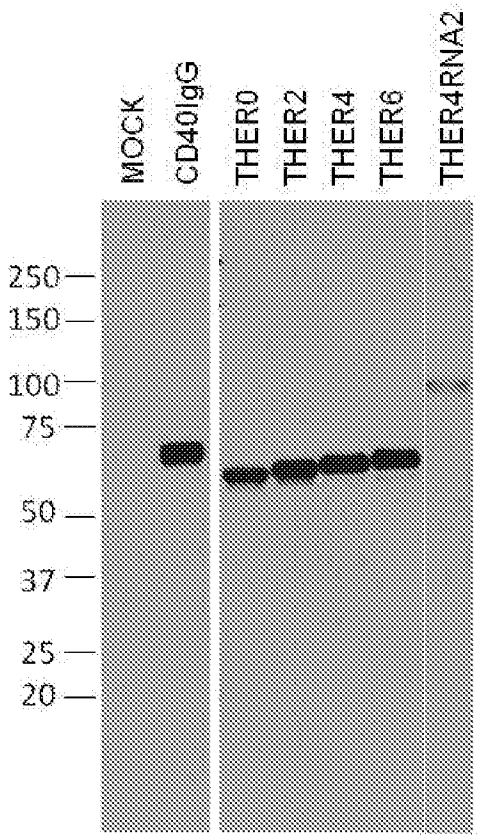

MOCK
CD40IgG
THER0
THER2
THER4
THER6
THER4RNA2
250
150
100
75
50
37
25
20

FIG. 3

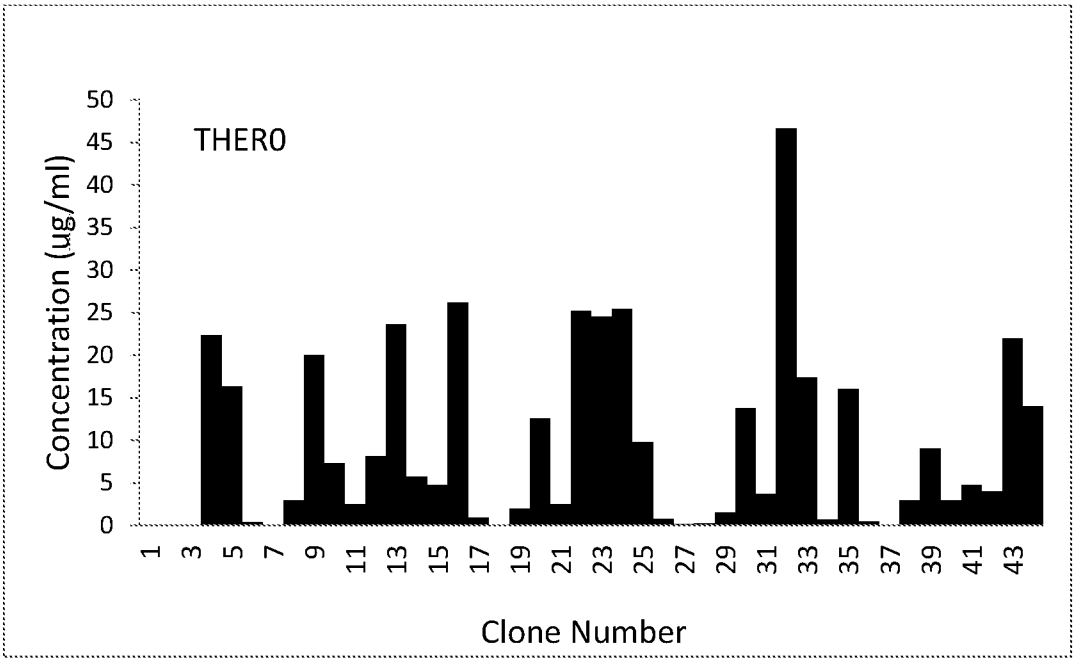

THER0
Concentration (ug/ml)
50
45
40
35
30
25
20
15
10
5
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43
Clone Number

FIG. 4A

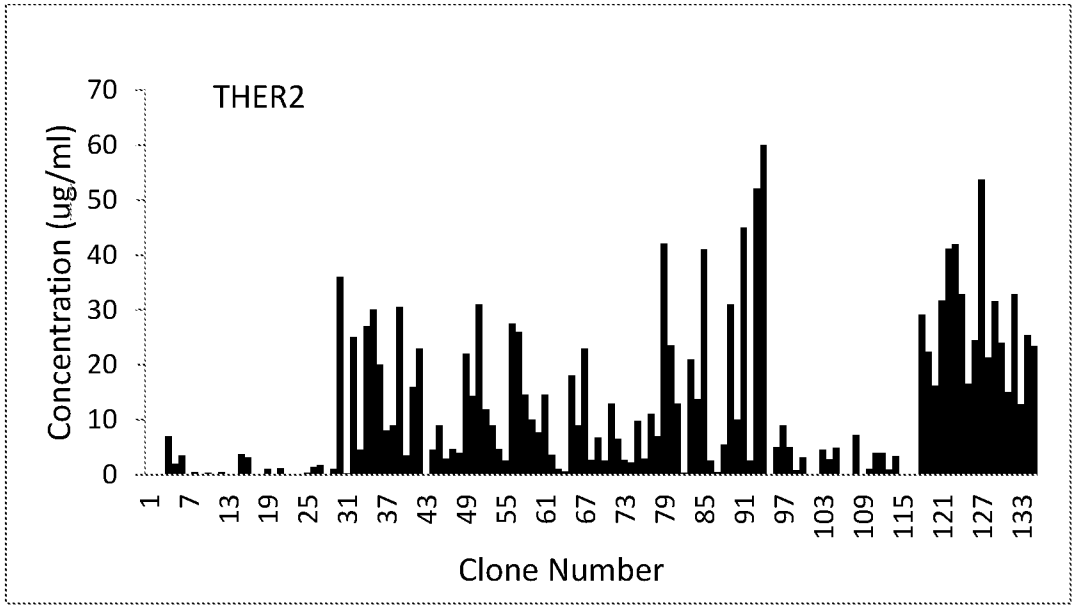

THER2
Concentration (ug/ml)
70
60
50
40
30
20
10
0
1 7 13 19 25 31 37 43 49 55 61 67 73 79 85 91 97 103 109 115 121 127 133
Clone Number

FIG. 4B

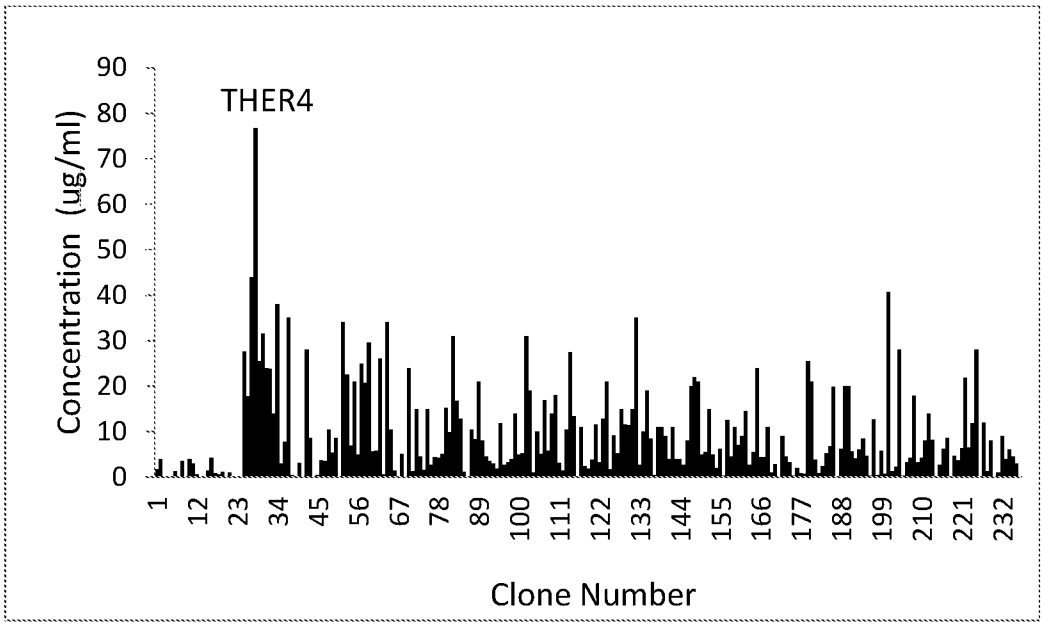

THER4
Concentration (ug/ml)
0
10
20
30
40
50
60
70
80
90
1
12
23
34
45
56
67
78
89
100
111
122
133
144
155
166
177
188
199
210
221
232
Clone Number

FIG. 4C

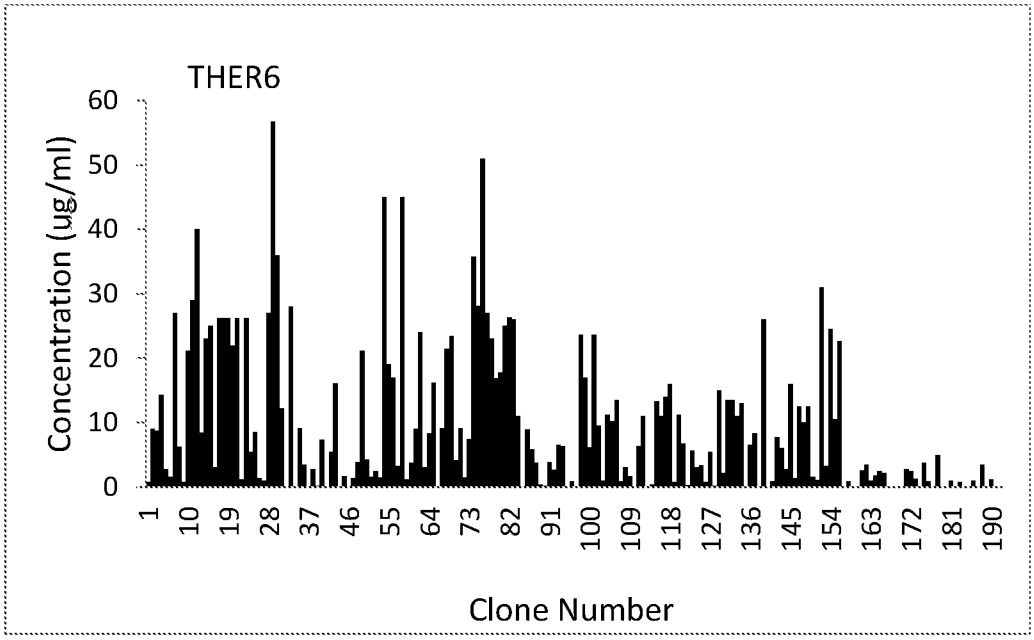

THER6
Concentration (ug/ml)
0
10
20
30
40
50
60
1
10
19
28
37
46
55
64
73
82
91
100
109
118
127
136
145
154
163
172
181
190
Clone Number

FIG. 4D

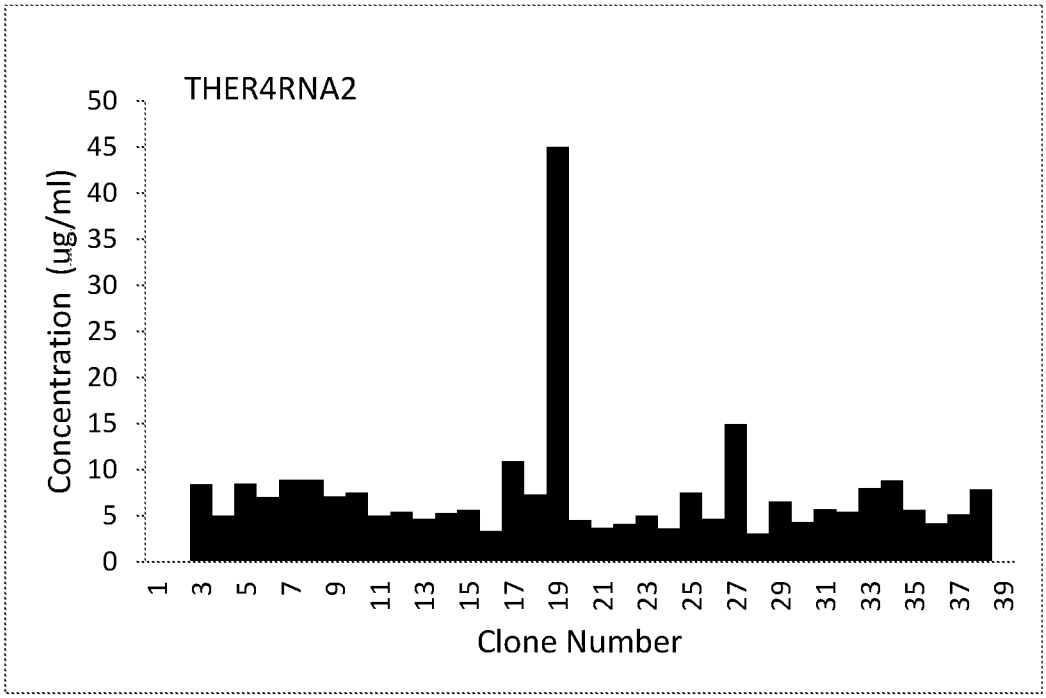

THER4RNA2
Concentration (µg/ml)
50
45
40
35
30
25
20
15
10
5
0
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
35
37
39
Clone Number

FIG. 4E

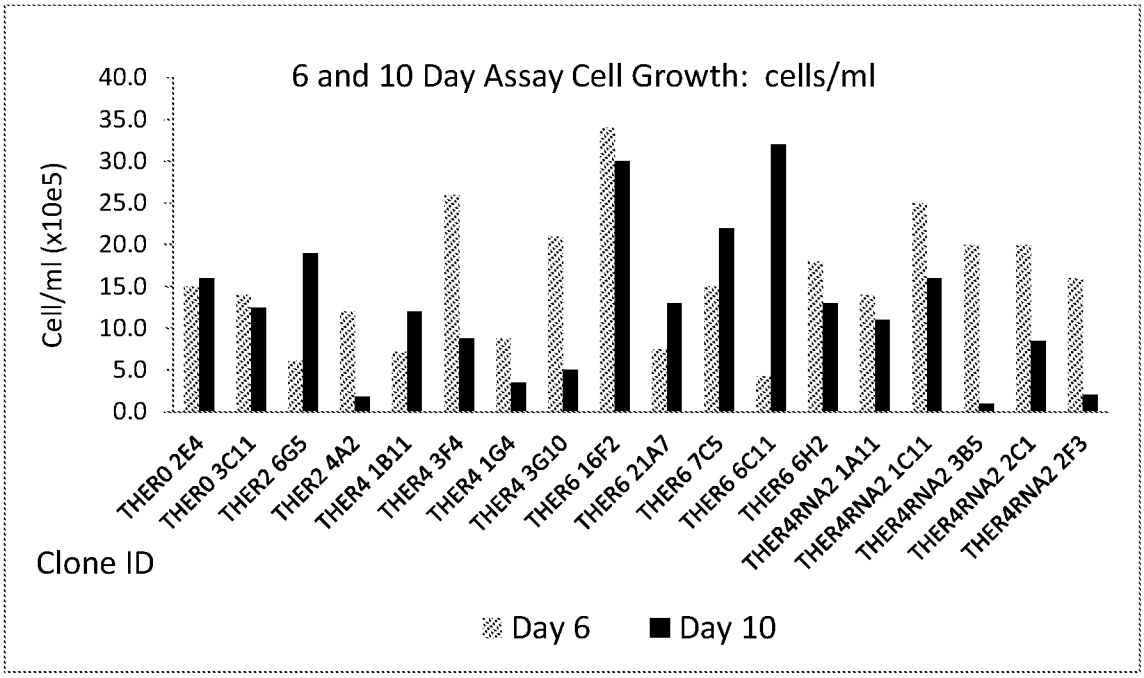

6 and 10 Day Assay Cell Growth: cells/ml
Cell/ml (x10e5)
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
THER0 2E4
THER0 3C11
THER2 6G5
THER2 4A2
THER4 1B11
THER4 3F4
THER4 1G4
THER4 3G10
THER6 16F2
THER6 21A7
THER6 7C5
THER6 6C11
THER6 6H2
THER4RNA2 1A11
THER4RNA2 1C11
THER4RNA2 3B5
THER4RNA2 2C1
THER4RNA2 2F3
Clone ID
Day 6
Day 10

FIG. 5A

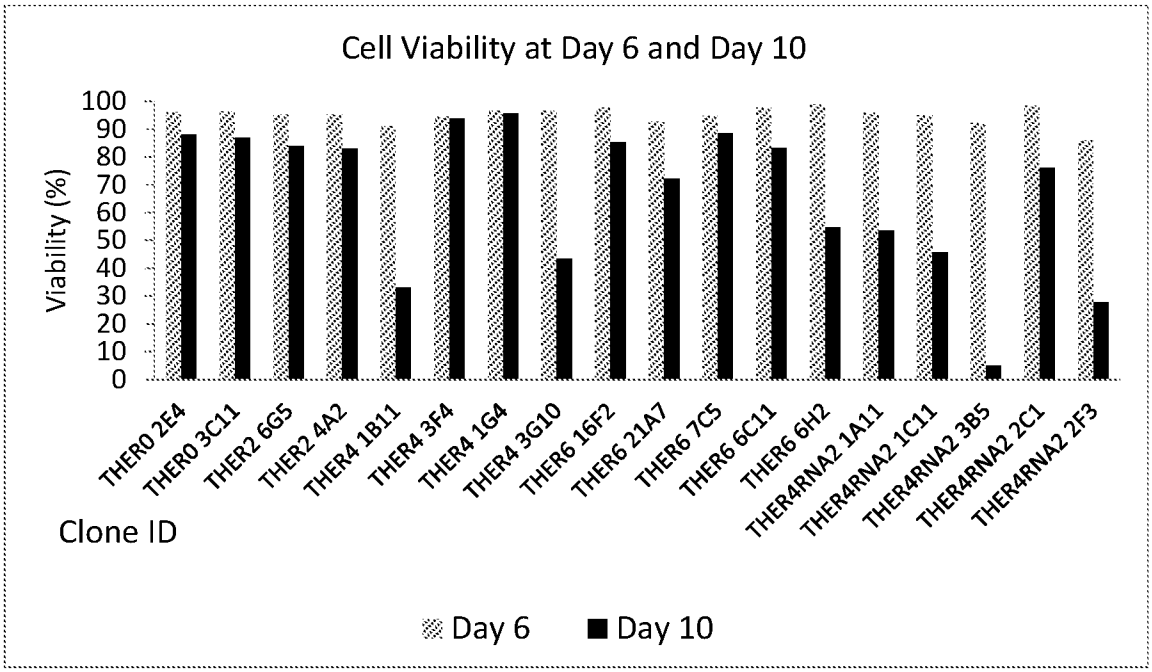

Cell Viability at Day 6 and Day 10
Viability (%)
100
90
80
70
60
50
40
30
20
10
0
THER0 2E4
THER0 3C11
THER2 6G5
THER2 4A2
THER4 1B11
THER4 3F4
THER4 1G4
THER4 3G10
THER6 16F2
THER6 21A7
THER6 7C5
THER6 6C11
THER6 6H2
THER4RNA2 1A11
THER4RNA2 1C11
THER4RNA2 3B5
THER4RNA2 2C1
THER4RNA2 2F3
Clone ID
Day 6
Day 10

FIG. 5B

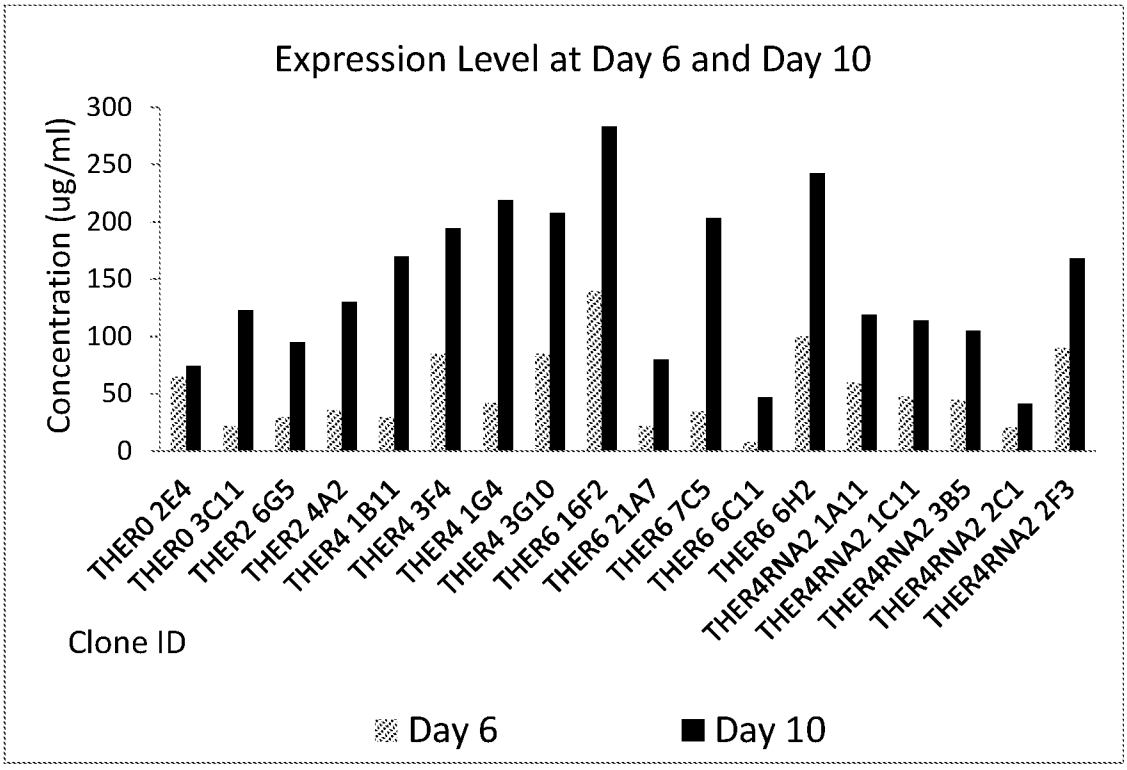

Expression Level at Day 6 and Day 10
Concentration (ug/ml)
300
250
200
150
100
50
0
THER0 2E4
THER0 3C11
THER2 6G5
THER2 4A2
THER4 1B11
THER4 3F4
THER4 1G4
THER4 3G10
THER6 16F2
THER6 21A7
THER6 7C5
THER6 6C11
THER6 6H2
THER4RNA2 1A11
THER4RNA2 1C11
THER4RNA2 3B5
THER4RNA2 2C1
THER4RNA2 2F3
Clone ID
Day 6
Day 10

FIG. 5C

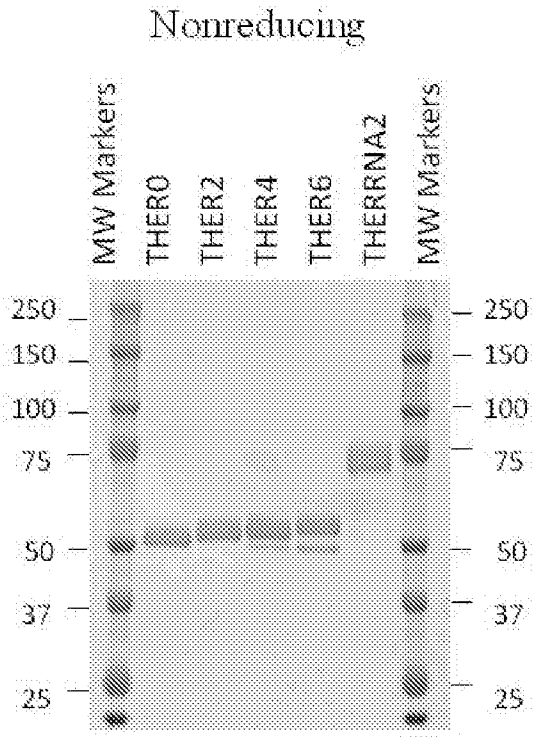

Nonreducing
MW Markers
THER0
THER2
THER4
THER6
THERRNA2
MW Markers
250
150
100
75
50
37
25

FIG. 6A

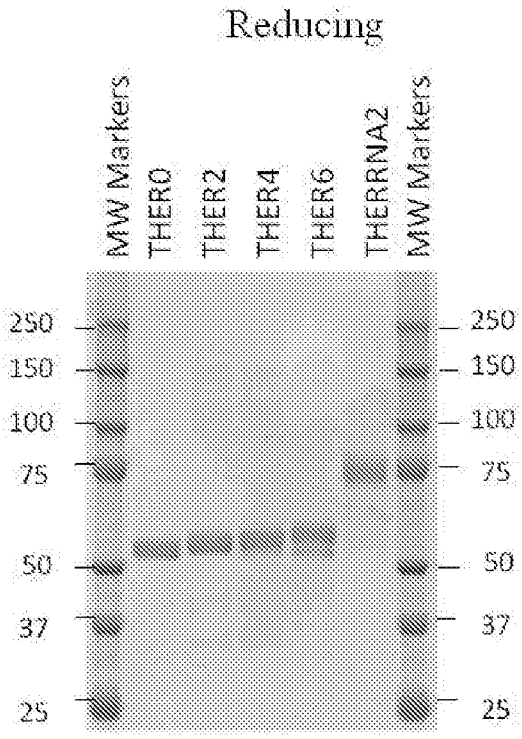

Reducing
MW Markers
THER0
THER2
THER4
THER6
THERRNA2
MW Markers
250
150
100
75
50
37
25

FIG. 6B

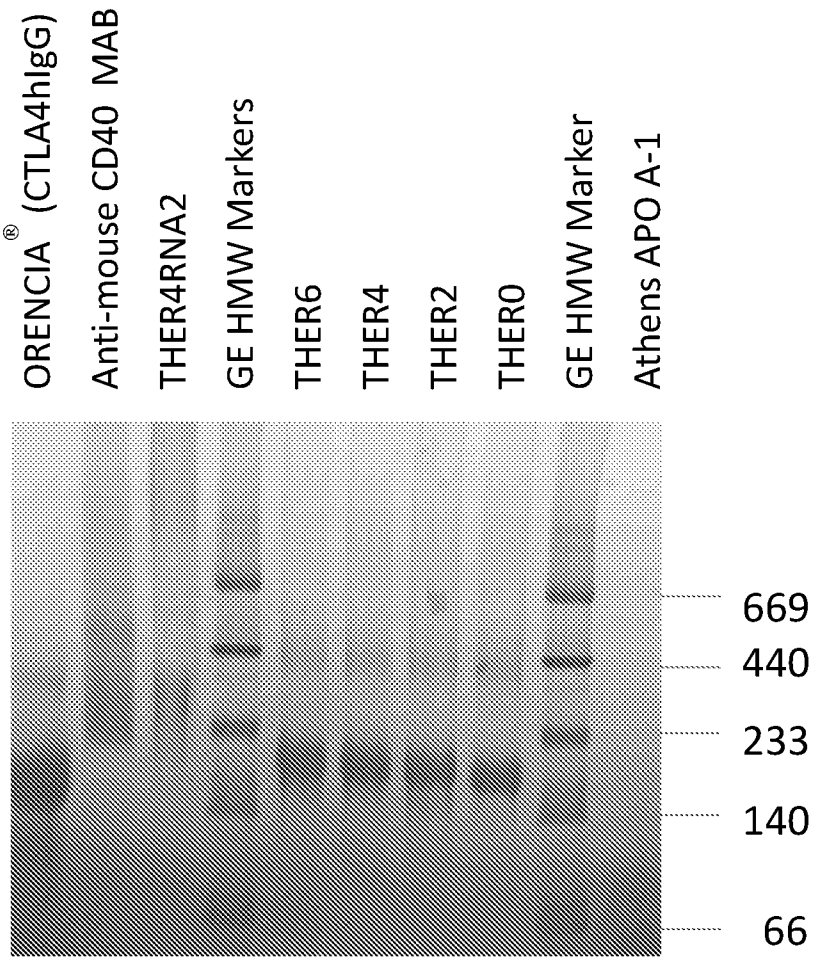

ORENCIA® (CTLA4hIgG)
Anti-mouse CD40 MAB
THER4RNA2
GE HMW Markers
THER6
THER4
THER2
THER0
GE HMW Marker
Athens APO A-1
669
440
233
140
66

FIG. 7

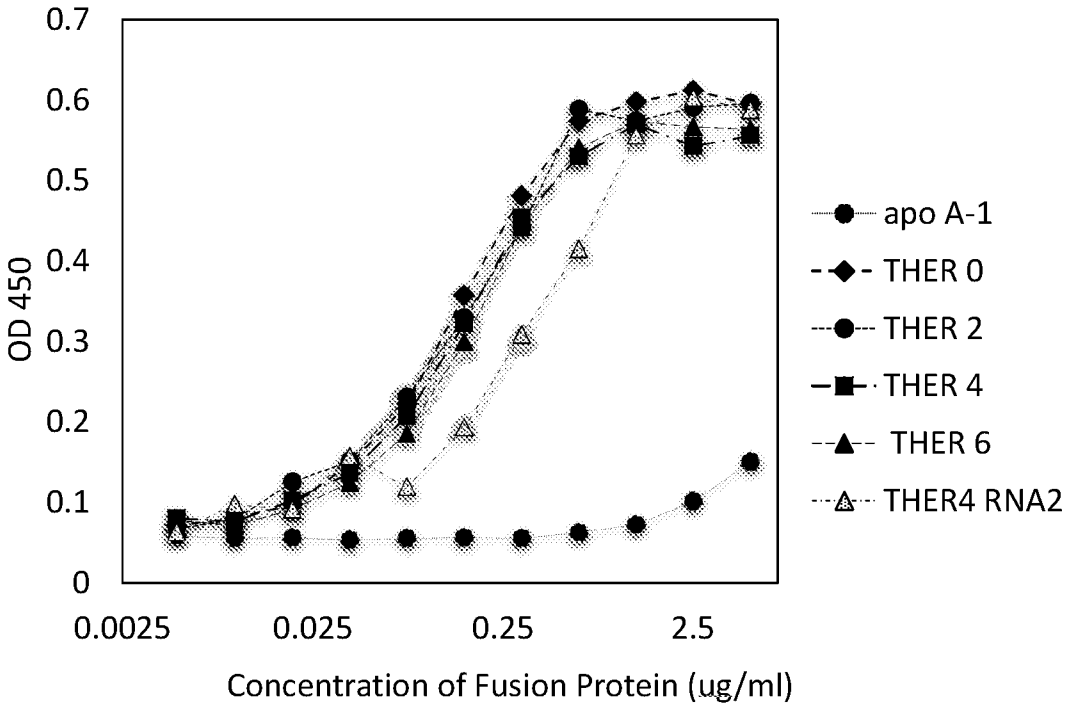

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
OD 450
0.0025
0.025
0.25
2.5
Concentration of Fusion Protein (ug/ml)
apo A-1
THER 0
THER 2
THER 4
THER 6
THER4 RNA2

FIG. 8

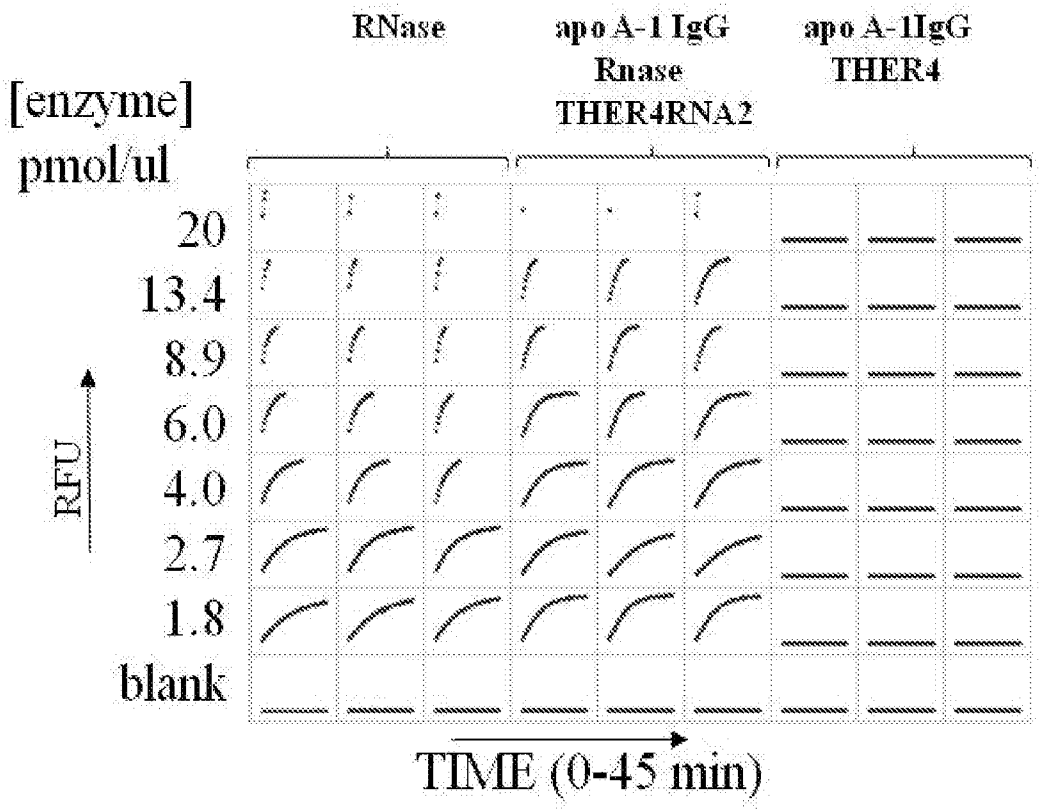

RNase
apo A-1 IgG
Rnase
THER4RNA2
apo A-1IgG
THER4
[enzyme]
pmol/ul
20
13.4
8.9
6.0
4.0
2.7
1.8
blank
RFU
TIME (0-45 min)

FIG. 9

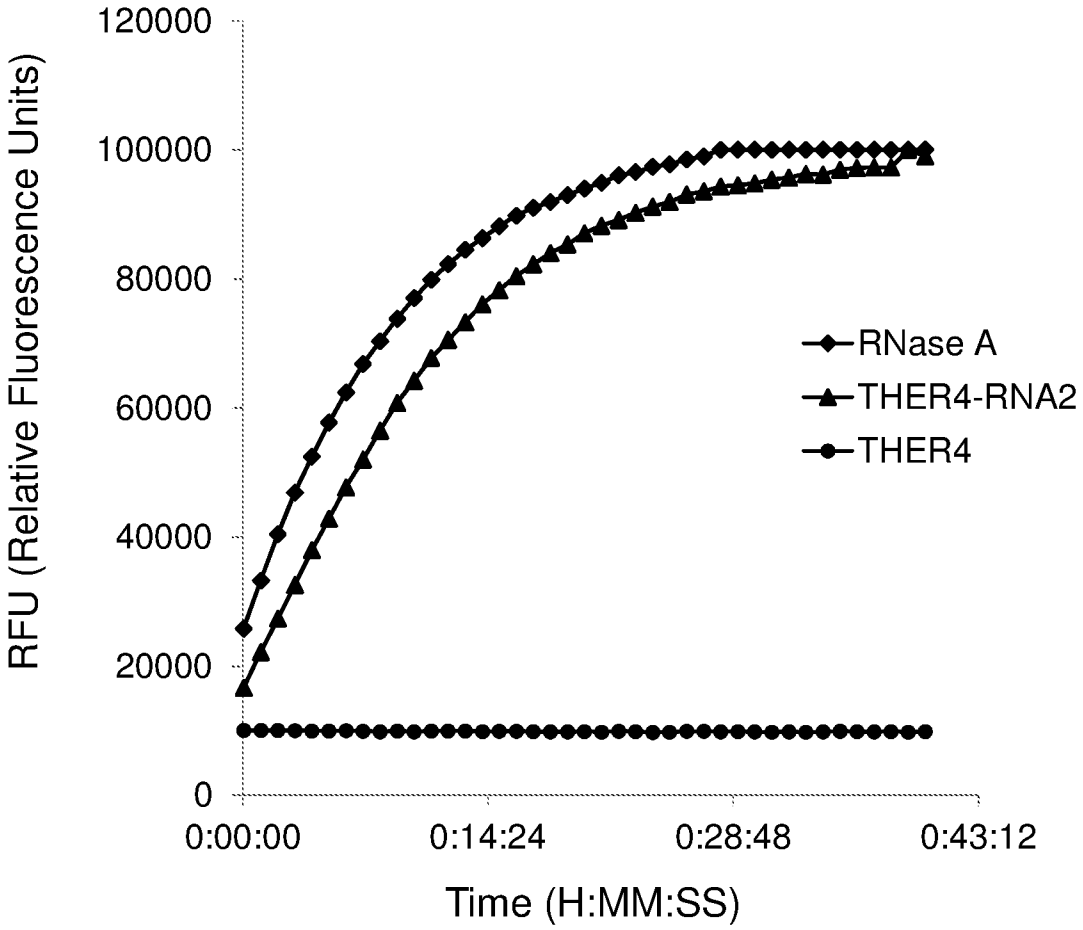

120000
100000
80000
60000
40000
20000
0
RFU (Relative Fluorescence Units)
0:00:00
0:14:24
0:28:48
0:43:12
Time (H:MM:SS)
RNase A
THER4-RNA2
THER4

FIG. 10

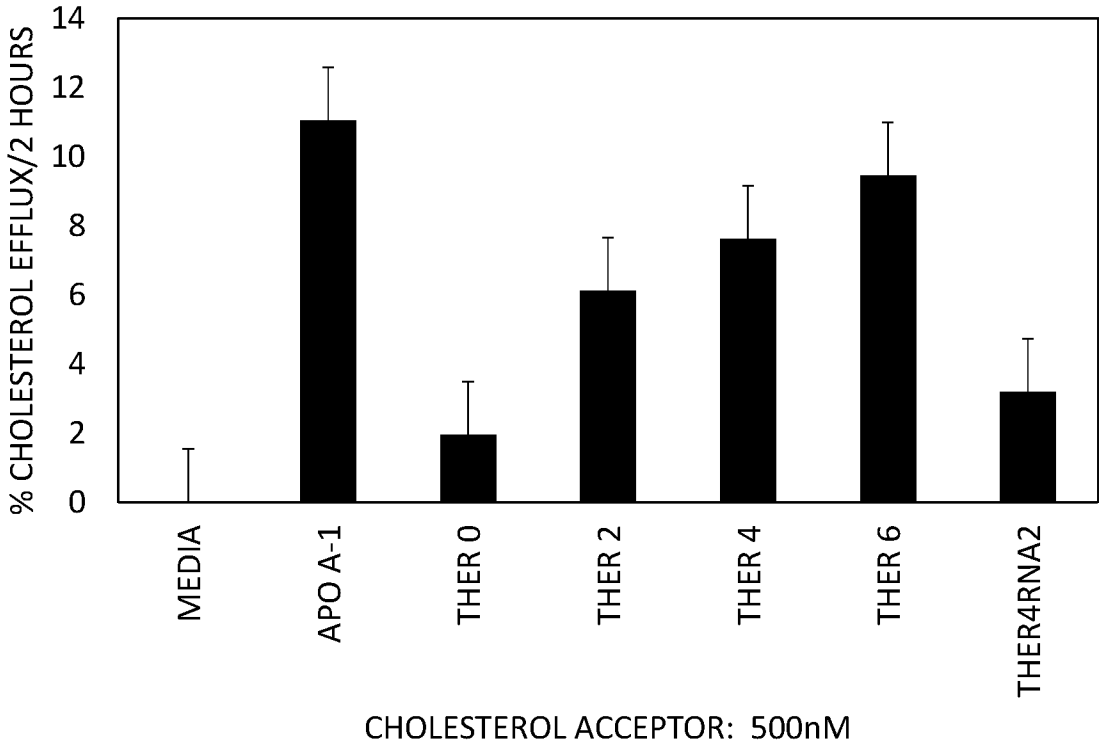

% CHOLESTEROL EFFLUX/2 HOURS
14
12
10
8
6
4
2
0
MEDIA
APO A-1
THER 0
THER 2
THER 4
THER 6
THER4RNA2
CHOLESTEROL ACCEPTOR: 500nM

FIG. 11

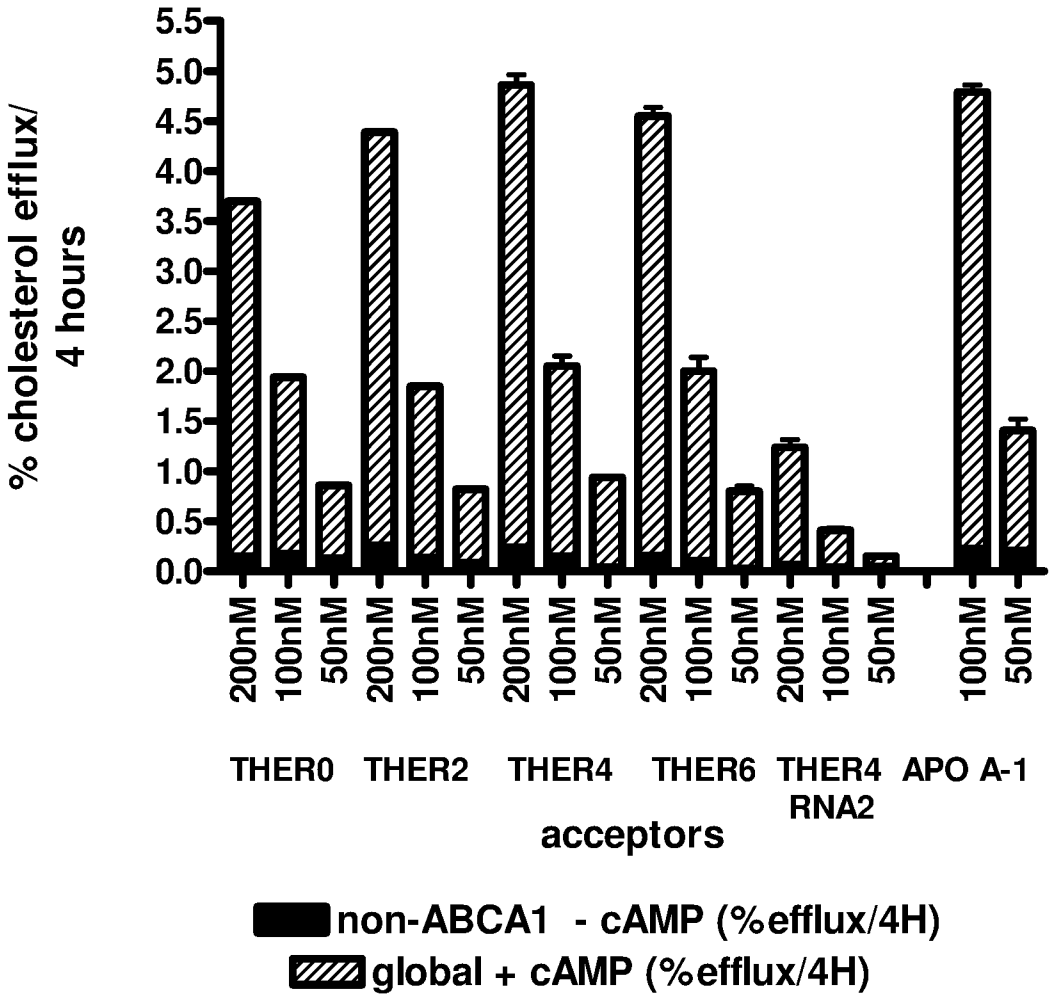

% cholesterol efflux/ 4 hours
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
200nM
100nM
50nM
THER0
THER2
THER4
THER6
THER4 RNA2
APO A-1
acceptors
non-ABCA1 - cAMP (%efflux/4H)
global + cAMP (%efflux/4H)

FIG. 12

SEQUENCE LISTING

<110> Theripion Inc.

<120> ApoA-1 Fusion Polypeptides and Related Compositions and Methods

<130> TRP-0110PC

<150> US 62/215,256
<151> 2015-09-08

<160> 54

<170> PatentIn version 3.5

<210> 1
<211> 1602
<212> DNA
<213> Artificial

<220>
<223> hWTAPO1-(g4s)4-mthIgG DNA

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agccaggctc ggcatttctg gcagcaagat gaaccccccc agagcccctg ggatcgagtg 120

aaggacctgg ccactgtgta cgtggatgtg ctcaaagaca gcggcagaga ctatgtgtcc 180

cagtttgaag gctccgcctt gggaaaacag ctaaacctaa agctccttga caactgggac 240

agcgtgacct ccaccttcag caagctgcgc gaacagctcg gccctgtgac ccaggagttc 300

tgggataacc tggaaaagga gacagagggc ctgaggcagg agatgagcaa ggatctggag 360

gaggtgaagg ccaaggtgca gccctacctg gacgacttcc agaagaagtg gcaggaggag 420

atggagctct accgccagaa ggtggagccg ctgcgcgcag agctccaaga gggcgcgcgc 480

cagaagctgc acgagctgca agagaagctg agcccactgg gcgaggagat gcgcgaccgc 540

gcgcgcgccc atgtggacgc gctgcgcacg catctggccc cctacagcga cgagctgcgc 600

cagcgcttgg ccgcgcgcct tgaggctctc aaggagaacg gcggcgccag actggccgag 660

taccacgcca aggccaccga gcatctgagc acgctcagcg agaaggccaa gcccgcgctc 720

gaggacctcc gccaaggcct gctgcccgtg ctggagagct tcaaggtcag cttcctgagc 780

gctctcgagg agtacactaa gaagctcaac acccaagatc tctccggagg aggtggctca 840

ggtggtggag gatctggagg aggtgggagt ggtggaggtg gttctaccgg tctcgagccc 900

aaatcttctg acaaaactca cacatctcca ccgtccccag cacctgaact cctgggagga 960

tcgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggacccct 1020

gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 1080

tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 1140

agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 1200

gagtacaagt gcaaggtctc caacaaagcc ctcccagcct ccatcgagaa aaccatctcc 1260

aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgcccccatc ccgggatgag 1320

ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 1380

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 1440

ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg 1500

cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 1560

cagaagagcc tctctctctc tccgggtaaa tgataatcta ga 1602

<210> 2
<211> 525
<212> PRT
<213> Artificial

<220>
<223> hWTAPO1-(g4s)4-mthIgG

<220>
<221> SIGNAL
<222> (1)..(18)

<400> 2

Met Lys Ala Ala Val Leu Thr Leu Ala Val Leu Phe Leu Thr Gly Ser
1 5 10 15

Gln Ala Arg His Phe Trp Gln Gln Asp Glu Pro Pro Gln Ser Pro Trp
20 25 30

Asp Arg Val Lys Asp Leu Ala Thr Val Tyr Val Asp Val Leu Lys Asp
35 40 45

Ser Gly Arg Asp Tyr Val Ser Gln Phe Glu Gly Ser Ala Leu Gly Lys
50 55 60

Gln Leu Asn Leu Lys Leu Leu Asp Asn Trp Asp Ser Val Thr Ser Thr
65 70 75 80

Phe Ser Lys Leu Arg Glu Gln Leu Gly Pro Val Thr Gln Glu Phe Trp
85 90 95

Asp Asn Leu Glu Lys Glu Thr Glu Gly Leu Arg Gln Glu Met Ser Lys
100 105 110

Asp Leu Glu Glu Val Lys Ala Lys Val Gln Pro Tyr Leu Asp Asp Phe
115 120 125

```
Gln Lys Lys Trp Gln Glu Glu Met Glu Leu Tyr Arg Gln Lys Val Glu
    130                 135                 140

Pro Leu Arg Ala Glu Leu Gln Glu Gly Ala Arg Gln Lys Leu His Glu
145                 150                 155                 160

Leu Gln Glu Lys Leu Ser Pro Leu Gly Glu Glu Met Arg Asp Arg Ala
                165                 170                 175

Arg Ala His Val Asp Ala Leu Arg Thr His Leu Ala Pro Tyr Ser Asp
            180                 185                 190

Glu Leu Arg Gln Arg Leu Ala Ala Arg Leu Glu Ala Leu Lys Glu Asn
        195                 200                 205

Gly Gly Ala Arg Leu Ala Glu Tyr His Ala Lys Ala Thr Glu His Leu
    210                 215                 220

Ser Thr Leu Ser Glu Lys Ala Lys Pro Ala Leu Glu Asp Leu Arg Gln
225                 230                 235                 240

Gly Leu Leu Pro Val Leu Glu Ser Phe Lys Val Ser Phe Leu Ser Ala
                245                 250                 255

Leu Glu Glu Tyr Thr Lys Lys Leu Asn Thr Gln Asp Leu Ser Gly Gly
            260                 265                 270

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
        275                 280                 285

Gly Ser Thr Gly Leu Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
    290                 295                 300

Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val Phe Leu
305                 310                 315                 320

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
                325                 330                 335

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
            340                 345                 350

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
        355                 360                 365

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
    370                 375                 380
```

```
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
385                 390                 395                 400

Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys
                405                 410                 415

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
            420                 425                 430

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
        435                 440                 445

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
    450                 455                 460

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
465                 470                 475                 480

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
                485                 490                 495

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
            500                 505                 510

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        515                 520                 525
```

<210> 3
<211> 2052
<212> DNA
<213> Artificial

<220>
<223> hWTAPO1-(g4s)4-mthIgG-hRNase1 DNA

<400> 3

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aaggacctgg ccactgtgta cgtggatgtg ctcaaagaca gcggcagaga ctatgtgtcc      180

cagtttgaag gctccgcctt gggaaaacag ctaaacctaa agctccttga caactgggac      240

agcgtgacct ccaccttcag caagctgcgc gaacagctcg gccctgtgac ccaggagttc      300

tgggataacc tggaaaagga gacagagggc ctgaggcagg agatgagcaa ggatctggag      360

gaggtgaagg ccaaggtgca gccctacctg gacgacttcc agaagaagtg gcaggaggag      420

atggagctct accgccagaa ggtggagccg ctgcgcgcag agctccaaga gggcgcgcgc      480

cagaagctgc acgagctgca agagaagctg agcccactgg gcgaggagat gcgcgaccgc      540
```

gcgcgcgccc atgtggacgc gctgcgcacg catctggccc cctacagcga cgagctgcgc 600

cagcgcttgg ccgcgcgcct tgaggctctc aaggagaacg gcggcgccag actggccgag 660

taccacgcca aggccaccga gcatctgagc acgctcagcg agaaggccaa gcccgcgctc 720

gaggacctcc gccaaggcct gctgcccgtg ctggagagct tcaaggtcag cttcctgagc 780

gctctcgagg agtacactaa gaagctcaac acccaagatc tctccggagg aggtggctca 840

ggtggtggag gatctggagg aggtgggagt ggtggaggtg gttctaccgg tctcgagccc 900

aaatcttctg acaaaactca cacatctcca ccgtccccag cacctgaact cctgggagga 960

tcgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggacccct 1020

gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 1080

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gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 1440

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cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 1560

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gagcccctgg tagatgtcca gaatgtctgt ttccaggaaa aggtcacctg caagaacggg 1860

cagggcaact gctacaagag caactccagc atgcacatca cagactgccg cctgacaaac 1920

ggctccaggt accccaactg tgcataccgg accagcccga aggagagaca catcattgtg 1980

gcctgtgaag ggagcccata tgtgccagtc cactttgatg cttctgtgga ggactctacc 2040

taataatcta ga 2052

<210> 4
<211> 675
<212> PRT
<213> Artificial

<220>
<223> hWTAPO1-(g4s)4-mthIgG-hRNase1

<400> 4

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Met Lys Ala Ala Val Leu Thr Leu Ala Val Leu Phe Leu Thr Gly Ser
1               5                  10                  15

Gln Ala Arg His Phe Trp Gln Gln Asp Glu Pro Pro Gln Ser Pro Trp
            20                  25                  30

Asp Arg Val Lys Asp Leu Ala Thr Val Tyr Val Asp Val Leu Lys Asp
        35                  40                  45

Ser Gly Arg Asp Tyr Val Ser Gln Phe Glu Gly Ser Ala Leu Gly Lys
    50                  55                  60

Gln Leu Asn Leu Lys Leu Leu Asp Asn Trp Asp Ser Val Thr Ser Thr
65                  70                  75                  80

Phe Ser Lys Leu Arg Glu Gln Leu Gly Pro Val Thr Gln Glu Phe Trp
                85                  90                  95

Asp Asn Leu Glu Lys Glu Thr Glu Gly Leu Arg Gln Glu Met Ser Lys
            100                 105                 110

Asp Leu Glu Glu Val Lys Ala Lys Val Gln Pro Tyr Leu Asp Asp Phe
        115                 120                 125

Gln Lys Lys Trp Gln Glu Glu Met Glu Leu Tyr Arg Gln Lys Val Glu
    130                 135                 140

Pro Leu Arg Ala Glu Leu Gln Glu Gly Ala Arg Gln Lys Leu His Glu
145                 150                 155                 160

Leu Gln Glu Lys Leu Ser Pro Leu Gly Glu Glu Met Arg Asp Arg Ala
                165                 170                 175

Arg Ala His Val Asp Ala Leu Arg Thr His Leu Ala Pro Tyr Ser Asp
            180                 185                 190

Glu Leu Arg Gln Arg Leu Ala Ala Arg Leu Glu Ala Leu Lys Glu Asn
        195                 200                 205

Gly Gly Ala Arg Leu Ala Glu Tyr His Ala Lys Ala Thr Glu His Leu
    210                 215                 220

Ser Thr Leu Ser Glu Lys Ala Lys Pro Ala Leu Glu Asp Leu Arg Gln
225                 230                 235                 240

Gly Leu Leu Pro Val Leu Glu Ser Phe Lys Val Ser Phe Leu Ser Ala
                245                 250                 255
```

```
Leu Glu Glu Tyr Thr Lys Lys Leu Asn Thr Gln Asp Leu Ser Gly Gly
            260                 265                 270

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
        275                 280                 285

Gly Ser Thr Gly Leu Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
    290                 295                 300

Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val Phe Leu
305                 310                 315                 320

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
                325                 330                 335

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
            340                 345                 350

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
        355                 360                 365

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
    370                 375                 380

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
385                 390                 395                 400

Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys
                405                 410                 415

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
            420                 425                 430

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
        435                 440                 445

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
    450                 455                 460

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
465                 470                 475                 480

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
                485                 490                 495

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
```

500 505 510

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Val Asp Gly
515 520 525

Ala Ser Ser Pro Val Asn Val Ser Ser Pro Ser Val Gln Asp Ile Pro
530 535 540

Ser Leu Gly Lys Glu Ser Arg Ala Lys Lys Phe Gln Arg Gln His Met
545 550 555 560

Asp Ser Asp Ser Ser Pro Ser Ser Ser Ser Thr Tyr Cys Asn Gln Met
565 570 575

Met Arg Arg Arg Asn Met Thr Gln Gly Arg Cys Lys Pro Val Asn Thr
580 585 590

Phe Val His Glu Pro Leu Val Asp Val Gln Asn Val Cys Phe Gln Glu
595 600 605

Lys Val Thr Cys Lys Asn Gly Gln Gly Asn Cys Tyr Lys Ser Asn Ser
610 615 620

Ser Met His Ile Thr Asp Cys Arg Leu Thr Asn Gly Ser Arg Tyr Pro
625 630 635 640

Asn Cys Ala Tyr Arg Thr Ser Pro Lys Glu Arg His Ile Ile Val Ala
645 650 655

Cys Glu Gly Ser Pro Tyr Val Pro Val His Phe Asp Ala Ser Val Glu
660 665 670

Asp Ser Thr
675

<210> 5
<211> 414
<212> DNA
<213> Homo sapiens

<400> 5
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tcagacagtt cccccagcag cagctccacc tactgtaacc aaatgatgag gcgccggaat 120

atgacacagg ggcggtgcaa accagtgaac acctttgtgc acgagcccct ggtagatgtc 180

cagaatgtct gtttccagga aaaggtcacc tgcaagaacg ggcagggcaa ctgctacaag 240

agcaactcca gcatgcacat cacagactgc cgcctgacaa acggctccag gtaccccaac 300

tgtgcatacc ggaccagccc gaaggagaga cacatcattg tggcctgtga agggagccca 360

tatgtgccag tccactttga tgcttctgtg gaggactcta cctaataatc taga 414

<210> 6
<211> 134
<212> PRT
<213> Homo sapiens

<400> 6

Asp Ile Pro Ser Leu Gly Lys Glu Ser Arg Ala Lys Lys Phe Gln Arg
1 5 10 15

Gln His Met Asp Ser Asp Ser Ser Pro Ser Ser Ser Ser Thr Tyr Cys
20 25 30

Asn Gln Met Met Arg Arg Arg Asn Met Thr Gln Gly Arg Cys Lys Pro
35 40 45

Val Asn Thr Phe Val His Glu Pro Leu Val Asp Val Gln Asn Val Cys
50 55 60

Phe Gln Glu Lys Val Thr Cys Lys Asn Gly Gln Gly Asn Cys Tyr Lys
65 70 75 80

Ser Asn Ser Ser Met His Ile Thr Asp Cys Arg Leu Thr Asn Gly Ser
85 90 95

Arg Tyr Pro Asn Cys Ala Tyr Arg Thr Ser Pro Lys Glu Arg His Ile
100 105 110

Ile Val Ala Cys Glu Gly Ser Pro Tyr Val Pro Val His Phe Asp Ala
115 120 125

Ser Val Glu Asp Ser Thr
130

<210> 7
<211> 462
<212> DNA
<213> Artificial

<220>
<223> 3-NLG-WThRNase1 DNA

<400> 7
gtcgacggag ctagcagccc cgtgaacgtg agcagcccca gcgtgcagga tatcccttcc 60

ctgggcaagg aatcccgggc caagaaattc cagcggcagc atatggactc agacagttcc 120

cccagcagca gctccaccta ctgtaaccaa atgatgaggc gccggaatat gacacagggg 180

cggtgcaaac cagtgaacac ctttgtgcac gagcccctgg tagatgtcca gaatgtctgt 240

ttccaggaaa aggtcacctg caagaacggg cagggcaact gctacaagag caactccagc 300

atgcacatca cagactgccg cctgacaaac ggctccaggt accccaactg tgcataccgg 360

accagcccga aggagagaca catcattgtg gcctgtgaag ggagcccata tgtgccagtc 420

cactttgatg cttctgtgga ggactctacc taataatcta ga 462

<210> 8
<211> 150
<212> PRT
<213> Artificial

<220>
<223> 3-NLG-WThRNase1

<400> 8

Val Asp Gly Ala Ser Ser Pro Val Asn Val Ser Ser Pro Ser Val Gln
1 5 10 15

Asp Ile Pro Ser Leu Gly Lys Glu Ser Arg Ala Lys Lys Phe Gln Arg
20 25 30

Gln His Met Asp Ser Asp Ser Ser Pro Ser Ser Ser Ser Thr Tyr Cys
35 40 45

Asn Gln Met Met Arg Arg Arg Asn Met Thr Gln Gly Arg Cys Lys Pro
50 55 60

Val Asn Thr Phe Val His Glu Pro Leu Val Asp Val Gln Asn Val Cys
65 70 75 80

Phe Gln Glu Lys Val Thr Cys Lys Asn Gly Gln Gly Asn Cys Tyr Lys
85 90 95

Ser Asn Ser Ser Met His Ile Thr Asp Cys Arg Leu Thr Asn Gly Ser
100 105 110

Arg Tyr Pro Asn Cys Ala Tyr Arg Thr Ser Pro Lys Glu Arg His Ile
115 120 125

Ile Val Ala Cys Glu Gly Ser Pro Tyr Val Pro Val His Phe Asp Ala
130 135 140

Ser Val Glu Asp Ser Thr
145 150

<210> 9
<211> 97

<212> DNA
<213> Artificial

<220>
<223> (g4s)5 linker DNA

<400> 9
agatctctcc ggaggaggtg gctcaggtgg tggaggatct ggaggaggtg gctcaggtgg 60

tggaggatct ggaggaggtg ggagtaccgg tctcgag 97

<210> 10
<211> 32
<212> PRT
<213> Artificial

<220>
<223> (g4s)5 linker

<400> 10

Asp Leu Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
1 5 10 15

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Thr Gly Leu Glu
20 25 30

<210> 11
<211> 1099
<212> DNA
<213> Homo sapiens

<400> 11
atggcgaagc tgattgcgct caccctcttg gggatgggac tggcactctt caggaaccac 60

cagtcttctt accaaacacg acttaatgct ctccgagagg tacaacccgt agaacttcct 120

aactgtaatt tagttaaagg aatcgaaact ggctctgaag acttggagat actgcctaat 180

ggactggctt tcattagctc tggattaaag tatcctggaa taaagagctt caaccccaac 240

agtcctggaa aaatacttct gatggacctg aatgaagaag atccaacagt gttggaattg 300

gggatcactg gaagtaaatt tgatgtatct tcatttaacc ctcatgggat tagcacattc 360

acagatgaag ataatgccat gtacctcctg gtggtgaacc atccagatgc caagtccaca 420

gtggagttgt ttaaatttca agaagaagaa aaatcgcttt tgcatctaaa aaccatcaga 480

cataaacttc tgcctaattt gaatgatatt gttgctgtgg gacctgagca cttttatggc 540

acaaatgatc actattttct tgacccctac ttacaatcct gggagatgta tttgggttta 600

gcgtggtcgt atgttgtcta ctatagtcca agtgaagttc gagtggtggc agaaggattt 660

gattttgcta atggaatcaa catttcaccc gatggcaagt atgtctatat agctgagttg 720

ctggctcata agattcatgt gtatgaaaag catgctaatt ggactttaac tccattgaag 780

tcccttgact ttaataccct cgtggataac atatctgtgg atcctgagac aggagacctt 840

tgggttggat gccatcccaa tggcatgaaa atcttcttct atgactcaga gaatcctcct 900

gcatcagagg tgcttcgaat ccagaacatt ctaacagaag aacctaaagt gacacaggtt 960

tatgcagaaa atggcacagt gttgcaaggc agtacagttg cctctgtgta caaagggaaa 1020

ctgctgattg gcacagtgtt tcacaaagct ctttactgtg agctctaaca gaccgatttg 1080

cacccatgcc atagaaact 1099

<210> 12
<211> 355
<212> PRT
<213> Homo sapiens

<220>
<221> SIGNAL
<222> (1)..(15)

<400> 12

Met Ala Lys Leu Ile Ala Leu Thr Leu Leu Gly Met Gly Leu Ala Leu
1 5 10 15

Phe Arg Asn His Gln Ser Ser Tyr Gln Thr Arg Leu Asn Ala Leu Arg
20 25 30

Glu Val Gln Pro Val Glu Leu Pro Asn Cys Asn Leu Val Lys Gly Ile
35 40 45

Glu Thr Gly Ser Glu Asp Leu Glu Ile Leu Pro Asn Gly Leu Ala Phe
50 55 60

Ile Ser Ser Gly Leu Lys Tyr Pro Gly Ile Lys Ser Phe Asn Pro Asn
65 70 75 80

Ser Pro Gly Lys Ile Leu Leu Met Asp Leu Asn Glu Glu Asp Pro Thr
85 90 95

Val Leu Glu Leu Gly Ile Thr Gly Ser Lys Phe Asp Val Ser Ser Phe
100 105 110

Asn Pro His Gly Ile Ser Thr Phe Thr Asp Glu Asp Asn Ala Met Tyr
115 120 125

Leu Leu Val Val Asn His Pro Asp Ala Lys Ser Thr Val Glu Leu Phe
130 135 140

Lys Phe Gln Glu Glu Glu Lys Ser Leu Leu His Leu Lys Thr Ile Arg
145 150 155 160

```
His Lys Leu Leu Pro Asn Leu Asn Asp Ile Val Ala Val Gly Pro Glu
                165                 170                 175

His Phe Tyr Gly Thr Asn Asp His Tyr Phe Leu Asp Pro Tyr Leu Gln
            180                 185                 190

Ser Trp Glu Met Tyr Leu Gly Leu Ala Trp Ser Tyr Val Val Tyr Tyr
        195                 200                 205

Ser Pro Ser Glu Val Arg Val Val Ala Glu Gly Phe Asp Phe Ala Asn
    210                 215                 220

Gly Ile Asn Ile Ser Pro Asp Gly Lys Tyr Val Tyr Ile Ala Glu Leu
225                 230                 235                 240

Leu Ala His Lys Ile His Val Tyr Glu Lys His Ala Asn Trp Thr Leu
                245                 250                 255

Thr Pro Leu Lys Ser Leu Asp Phe Asn Thr Leu Val Asp Asn Ile Ser
            260                 265                 270

Val Asp Pro Glu Thr Gly Asp Leu Trp Val Gly Cys His Pro Asn Gly
        275                 280                 285

Met Lys Ile Phe Phe Tyr Asp Ser Glu Asn Pro Pro Ala Ser Glu Val
    290                 295                 300

Leu Arg Ile Gln Asn Ile Leu Thr Glu Glu Pro Lys Val Thr Gln Val
305                 310                 315                 320

Tyr Ala Glu Asn Gly Thr Val Leu Gln Gly Ser Thr Val Ala Ser Val
                325                 330                 335

Tyr Lys Gly Lys Leu Leu Ile Gly Thr Val Phe His Lys Ala Leu Tyr
            340                 345                 350

Cys Glu Leu
        355
```

<210> 13
<211> 525
<212> PRT
<213> Artificial

<220>
<223> hWTAPO1-(g4s)4-mthIgG with proline at Eu position 331 in Fc region

<400> 13

```
Met Lys Ala Ala Val Leu Thr Leu Ala Val Leu Phe Leu Thr Gly Ser
1               5                  10                  15

Gln Ala Arg His Phe Trp Gln Gln Asp Glu Pro Pro Gln Ser Pro Trp
            20                  25                  30

Asp Arg Val Lys Asp Leu Ala Thr Val Tyr Val Asp Val Leu Lys Asp
        35                  40                  45

Ser Gly Arg Asp Tyr Val Ser Gln Phe Glu Gly Ser Ala Leu Gly Lys
    50                  55                  60

Gln Leu Asn Leu Lys Leu Leu Asp Asn Trp Asp Ser Val Thr Ser Thr
65                  70                  75                  80

Phe Ser Lys Leu Arg Glu Gln Leu Gly Pro Val Thr Gln Glu Phe Trp
                85                  90                  95

Asp Asn Leu Glu Lys Glu Thr Glu Gly Leu Arg Gln Glu Met Ser Lys
            100                 105                 110

Asp Leu Glu Glu Val Lys Ala Lys Val Gln Pro Tyr Leu Asp Asp Phe
        115                 120                 125

Gln Lys Lys Trp Gln Glu Glu Met Glu Leu Tyr Arg Gln Lys Val Glu
    130                 135                 140

Pro Leu Arg Ala Glu Leu Gln Glu Gly Ala Arg Gln Lys Leu His Glu
145                 150                 155                 160

Leu Gln Glu Lys Leu Ser Pro Leu Gly Glu Glu Met Arg Asp Arg Ala
                165                 170                 175

Arg Ala His Val Asp Ala Leu Arg Thr His Leu Ala Pro Tyr Ser Asp
            180                 185                 190

Glu Leu Arg Gln Arg Leu Ala Ala Arg Leu Glu Ala Leu Lys Glu Asn
        195                 200                 205

Gly Gly Ala Arg Leu Ala Glu Tyr His Ala Lys Ala Thr Glu His Leu
    210                 215                 220

Ser Thr Leu Ser Glu Lys Ala Lys Pro Ala Leu Glu Asp Leu Arg Gln
225                 230                 235                 240

Gly Leu Leu Pro Val Leu Glu Ser Phe Lys Val Ser Phe Leu Ser Ala
                245                 250                 255
```

```
Leu Glu Glu Tyr Thr Lys Lys Leu Asn Thr Gln Asp Leu Ser Gly Gly
            260                 265                 270

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
        275                 280                 285

Gly Ser Thr Gly Leu Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
    290                 295                 300

Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val Phe Leu
305                 310                 315                 320

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
                325                 330                 335

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
            340                 345                 350

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
        355                 360                 365

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
    370                 375                 380

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
385                 390                 395                 400

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
                405                 410                 415

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
            420                 425                 430

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
        435                 440                 445

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
    450                 455                 460

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
465                 470                 475                 480

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
                485                 490                 495

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
```

500 505 510

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
515 520 525

<210> 14
<211> 675
<212> PRT
<213> Artificial

<220>
<223> hWTAPO1-(g4s)4-mthIgG-hRNase1 with proline at Eu position 331 in Fc region

<400> 14

Met Lys Ala Ala Val Leu Thr Leu Ala Val Leu Phe Leu Thr Gly Ser
1 5 10 15

Gln Ala Arg His Phe Trp Gln Gln Asp Glu Pro Pro Gln Ser Pro Trp
20 25 30

Asp Arg Val Lys Asp Leu Ala Thr Val Tyr Val Asp Val Leu Lys Asp
35 40 45

Ser Gly Arg Asp Tyr Val Ser Gln Phe Glu Gly Ser Ala Leu Gly Lys
50 55 60

Gln Leu Asn Leu Lys Leu Leu Asp Asn Trp Asp Ser Val Thr Ser Thr
65 70 75 80

Phe Ser Lys Leu Arg Glu Gln Leu Gly Pro Val Thr Gln Glu Phe Trp
85 90 95

Asp Asn Leu Glu Lys Glu Thr Glu Gly Leu Arg Gln Glu Met Ser Lys
100 105 110

Asp Leu Glu Glu Val Lys Ala Lys Val Gln Pro Tyr Leu Asp Asp Phe
115 120 125

Gln Lys Lys Trp Gln Glu Glu Met Glu Leu Tyr Arg Gln Lys Val Glu
130 135 140

Pro Leu Arg Ala Glu Leu Gln Glu Gly Ala Arg Gln Lys Leu His Glu
145 150 155 160

Leu Gln Glu Lys Leu Ser Pro Leu Gly Glu Glu Met Arg Asp Arg Ala
165 170 175

Arg Ala His Val Asp Ala Leu Arg Thr His Leu Ala Pro Tyr Ser Asp

```
            180                 185                 190

Glu Leu Arg Gln Arg Leu Ala Ala Arg Leu Glu Ala Leu Lys Glu Asn
        195                 200                 205

Gly Gly Ala Arg Leu Ala Glu Tyr His Ala Lys Ala Thr Glu His Leu
    210                 215                 220

Ser Thr Leu Ser Glu Lys Ala Lys Pro Ala Leu Glu Asp Leu Arg Gln
225                 230                 235                 240

Gly Leu Leu Pro Val Leu Glu Ser Phe Lys Val Ser Phe Leu Ser Ala
                245                 250                 255

Leu Glu Glu Tyr Thr Lys Lys Leu Asn Thr Gln Asp Leu Ser Gly Gly
            260                 265                 270

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
        275                 280                 285

Gly Ser Thr Gly Leu Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
    290                 295                 300

Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val Phe Leu
305                 310                 315                 320

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
                325                 330                 335

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
            340                 345                 350

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
        355                 360                 365

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
    370                 375                 380

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
385                 390                 395                 400

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
                405                 410                 415

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
            420                 425                 430
```

```
Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
        435                 440                 445

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
    450                 455                 460

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
465                 470                 475                 480

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
                485                 490                 495

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
            500                 505                 510

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Val Asp Gly
        515                 520                 525

Ala Ser Ser Pro Val Asn Val Ser Ser Pro Ser Val Gln Asp Ile Pro
    530                 535                 540

Ser Leu Gly Lys Glu Ser Arg Ala Lys Lys Phe Gln Arg Gln His Met
545                 550                 555                 560

Asp Ser Asp Ser Ser Pro Ser Ser Ser Ser Thr Tyr Cys Asn Gln Met
                565                 570                 575

Met Arg Arg Arg Asn Met Thr Gln Gly Arg Cys Lys Pro Val Asn Thr
            580                 585                 590

Phe Val His Glu Pro Leu Val Asp Val Gln Asn Val Cys Phe Gln Glu
        595                 600                 605

Lys Val Thr Cys Lys Asn Gly Gln Gly Asn Cys Tyr Lys Ser Asn Ser
    610                 615                 620

Ser Met His Ile Thr Asp Cys Arg Leu Thr Asn Gly Ser Arg Tyr Pro
625                 630                 635                 640

Asn Cys Ala Tyr Arg Thr Ser Pro Lys Glu Arg His Ile Ile Val Ala
                645                 650                 655

Cys Glu Gly Ser Pro Tyr Val Pro Val His Phe Asp Ala Ser Val Glu
            660                 665                 670

Asp Ser Thr
        675
```

<210> 15
<211> 5
<212> PRT
<213> Artificial

<220>
<223> linker

<400> 15

Gly Gly Gly Gly Ser
1 5

<210> 16
<211> 22
<212> DNA
<213> Artificial

<220>
<223> sequencing primer

<400> 16
ggttttggca gtacatcaat gg 22

<210> 17
<211> 22
<212> DNA
<213> Artificial

<220>
<223> sequencing primer

<400> 17
ctattgtctt cccaatcctc cc 22

<210> 18
<211> 20
<212> DNA
<213> Artificial

<220>
<223> sequencing primer

<400> 18
accttgcact tgtactcctt 20

<210> 19
<211> 1531
<212> DNA
<213> Artificial

<220>
<223> THER0 DNA (apo A-1-(lnk2)-mthIgG; apo A-1-mthIgG)

<400> 19
gttaagcttg ccaccatgaa agctgcggtg ctgaccttgg ccgtgctctt cctgacgggg 60

agccaggctc ggcatttctg gcagcaagat gaaccccccc agagcccctg ggatcgagtg 120

aaggacctgg ccactgtgta cgtggatgtg ctcaaagaca gcggcagaga ctatgtgtcc 180

cagtttgaag gctccgcctt gggaaaacag ctaaacctaa agctccttga caactgggac 240

agcgtgacct ccaccttcag caagctgcgc gaacagctcg gccctgtgac ccaggagttc 300

tgggataacc tggaaaagga gacagagggc ctgaggcagg agatgagcaa ggatctggag 360

gaggtgaagg ccaaggtgca gccctacctg gacgacttcc agaagaagtg gcaggaggag 420

atggagctct accgccagaa ggtggagccg ctgcgcgcag agctccaaga gggcgcgcgc 480

cagaagctgc acgagctgca agagaagctg agcccactgg gcgaggagat gcgcgaccgc 540

gcgcgcgccc atgtggacgc gctgcgcacg catctggccc cctacagcga cgagctgcgc 600

cagcgcttgg ccgcgcgcct tgaggctctc aaggagaacg gcggcgccag actggccgag 660

taccacgcca aggccaccga gcatctgagc acgctcagcg agaaggccaa gcccgcgctc 720

gaggacctcc gccaaggcct gctgcccgtg ctggagagct tcaaggtcag cttcctgagc 780

gctctcgagg agtacactaa gaagctcaac acccaagatc tcgagcccaa atcttctgac 840

aaaactcaca catctccacc gtccccagca cctgaactcc tgggaggatc gtcagtcttc 900

ctcttccccc caaaacccaa ggacaccctc atgatctccc ggacccctga ggtcacatgc 960

gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc 1020

gtggaggtgc ataatgccaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt 1080

gtggtcagcg tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc 1140

aaggtctcca acaaagccct cccagcctcc atcgagaaaa ccatctccaa agccaaaggg 1200

cagccccgag aaccacaggt gtacaccctg cccccatccc gggatgagct gaccaagaac 1260

caggtcagcc tgacctgcct ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg 1320

gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac 1380

ggctccttct tcctctacag caagctcacc gtggacaaga gcaggtggca gcaggggaac 1440

gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc 1500

tctctctctc cgggtaaatg ataatctaga a 1531

<210> 20
<211> 501
<212> PRT
<213> Artificial

<220>
<223> THER0 polypeptide (apo A-1-(lnk2)-mthIgG; apo A-1-mthIgG)

<400> 20

Met Lys Ala Ala Val Leu Thr Leu Ala Val Leu Phe Leu Thr Gly Ser
1 5 10 15

```
Gln Ala Arg His Phe Trp Gln Gln Asp Glu Pro Pro Gln Ser Pro Trp
            20                  25                  30

Asp Arg Val Lys Asp Leu Ala Thr Val Tyr Val Asp Val Leu Lys Asp
        35                  40                  45

Ser Gly Arg Asp Tyr Val Ser Gln Phe Glu Gly Ser Ala Leu Gly Lys
    50                  55                  60

Gln Leu Asn Leu Lys Leu Leu Asp Asn Trp Asp Ser Val Thr Ser Thr
65                  70                  75                  80

Phe Ser Lys Leu Arg Glu Gln Leu Gly Pro Val Thr Gln Glu Phe Trp
                85                  90                  95

Asp Asn Leu Glu Lys Glu Thr Glu Gly Leu Arg Gln Glu Met Ser Lys
            100                 105                 110

Asp Leu Glu Glu Val Lys Ala Lys Val Gln Pro Tyr Leu Asp Asp Phe
        115                 120                 125

Gln Lys Lys Trp Gln Glu Glu Met Glu Leu Tyr Arg Gln Lys Val Glu
    130                 135                 140

Pro Leu Arg Ala Glu Leu Gln Glu Gly Ala Arg Gln Lys Leu His Glu
145                 150                 155                 160

Leu Gln Glu Lys Leu Ser Pro Leu Gly Glu Glu Met Arg Asp Arg Ala
                165                 170                 175

Arg Ala His Val Asp Ala Leu Arg Thr His Leu Ala Pro Tyr Ser Asp
            180                 185                 190

Glu Leu Arg Gln Arg Leu Ala Ala Arg Leu Glu Ala Leu Lys Glu Asn
        195                 200                 205

Gly Gly Ala Arg Leu Ala Glu Tyr His Ala Lys Ala Thr Glu His Leu
    210                 215                 220

Ser Thr Leu Ser Glu Lys Ala Lys Pro Ala Leu Glu Asp Leu Arg Gln
225                 230                 235                 240

Gly Leu Leu Pro Val Leu Glu Ser Phe Lys Val Ser Phe Leu Ser Ala
                245                 250                 255

Leu Glu Glu Tyr Thr Lys Lys Leu Asn Thr Gln Asp Leu Glu Pro Lys
            260                 265                 270
```

```
Ser Ser Asp Lys Thr His Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu
        275                 280                 285

Leu Gly Gly Ser Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
    290                 295                 300

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
305                 310                 315                 320

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
                325                 330                 335

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
            340                 345                 350

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
        355                 360                 365

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
    370                 375                 380

Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
385                 390                 395                 400

Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
                405                 410                 415

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
            420                 425                 430

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
        435                 440                 445

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
    450                 455                 460

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
465                 470                 475                 480

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
                485                 490                 495

Leu Ser Pro Gly Lys
            500
```

<210>  21

<211> 1573
<212> DNA
<213> Artificial

<220>
<223> THER2 DNA (apo A-1-(lnk16)-mthIgG; apo A-1-(g4s)2-mthIgG)

<400> 21
gttaagcttg ccaccatgaa agctgcggtg ctgaccttgg ccgtgctctt cctgacgggg 60

agccaggctc ggcatttctg gcagcaagat gaaccccccc agagcccctg ggatcgagtg 120

aaggacctgg ccactgtgta cgtggatgtg ctcaaagaca gcggcagaga ctatgtgtcc 180

cagtttgaag gctccgcctt gggaaaacag ctaaacctaa agctccttga caactgggac 240

agcgtgacct ccaccttcag caagctgcgc gaacagctcg gccctgtgac ccaggagttc 300

tgggataacc tggaaaagga gacagagggc ctgaggcagg agatgagcaa ggatctggag 360

gaggtgaagg ccaaggtgca gccctacctg gacgacttcc agaagaagtg gcaggaggag 420

atggagctct accgccagaa ggtggagccg ctgcgcgcag agctccaaga gggcgcgcgc 480

cagaagctgc acgagctgca agagaagctg agcccactgg gcgaggagat gcgcgaccgc 540

gcgcgcgccc atgtggacgc gctgcgcacg catctggccc cctacagcga cgagctgcgc 600

cagcgcttgg ccgcgcgcct tgaggctctc aaggagaacg gcggcgccag actggccgag 660

taccacgcca aggccaccga gcatctgagc acgctcagcg agaaggccaa gcccgcgctc 720

gaggacctcc gccaaggcct gctgcccgtg ctggagagct tcaaggtcag cttcctgagc 780

gctctcgagg agtacactaa gaagctcaac acccaagatc tctccggagg aggtggctca 840

ggaggaggtg ggagtaccgg tctcgagccc aaatcttctg acaaaactca cacatctcca 900

ccgtccccag cacctgaact cctgggagga tcgtcagtct tcctcttccc cccaaaaccc 960

aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtggtggt ggacgtgagc 1020

cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 1080

aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc 1140

gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc 1200

ctcccagcct ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag 1260

gtgtacaccc tgcccccatc ccgggatgag ctgaccaaga accaggtcag cctgacctgc 1320

ctggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 1380

gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt cttcctctac 1440

agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1500

atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctctctctc tccgggtaaa 1560

tgataatcta gaa 1573

<210> 22
<211> 515
<212> PRT
<213> Artificial

<220>
<223> THER2 polypeptide (apo A-1-(lnk16)-mthIgG; apo A-1-(g4s)2-mthIgG)

<400> 22

Met Lys Ala Ala Val Leu Thr Leu Ala Val Leu Phe Leu Thr Gly Ser
1 5 10 15

Gln Ala Arg His Phe Trp Gln Gln Asp Glu Pro Pro Gln Ser Pro Trp
20 25 30

Asp Arg Val Lys Asp Leu Ala Thr Val Tyr Val Asp Val Leu Lys Asp
35 40 45

Ser Gly Arg Asp Tyr Val Ser Gln Phe Glu Gly Ser Ala Leu Gly Lys
50 55 60

Gln Leu Asn Leu Lys Leu Leu Asp Asn Trp Asp Ser Val Thr Ser Thr
65 70 75 80

Phe Ser Lys Leu Arg Glu Gln Leu Gly Pro Val Thr Gln Glu Phe Trp
85 90 95

Asp Asn Leu Glu Lys Glu Thr Glu Gly Leu Arg Gln Glu Met Ser Lys
100 105 110

Asp Leu Glu Glu Val Lys Ala Lys Val Gln Pro Tyr Leu Asp Asp Phe
115 120 125

Gln Lys Lys Trp Gln Glu Glu Met Glu Leu Tyr Arg Gln Lys Val Glu
130 135 140

Pro Leu Arg Ala Glu Leu Gln Glu Gly Ala Arg Gln Lys Leu His Glu
145 150 155 160

Leu Gln Glu Lys Leu Ser Pro Leu Gly Glu Glu Met Arg Asp Arg Ala
165 170 175

Arg Ala His Val Asp Ala Leu Arg Thr His Leu Ala Pro Tyr Ser Asp
180 185 190

Glu Leu Arg Gln Arg Leu Ala Ala Arg Leu Glu Ala Leu Lys Glu Asn
195 200 205

Gly Gly Ala Arg Leu Ala Glu Tyr His Ala Lys Ala Thr Glu His Leu

210 215 220

Ser Thr Leu Ser Glu Lys Ala Lys Pro Ala Leu Glu Asp Leu Arg Gln
225 230 235 240

Gly Leu Leu Pro Val Leu Glu Ser Phe Lys Val Ser Phe Leu Ser Ala
245 250 255

Leu Glu Glu Tyr Thr Lys Lys Leu Asn Thr Gln Asp Leu Ser Gly Gly
260 265 270

Gly Gly Ser Gly Gly Gly Gly Ser Thr Gly Leu Glu Pro Lys Ser Ser
275 280 285

Asp Lys Thr His Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly
290 295 300

Gly Ser Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
305 310 315 320

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
325 330 335

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
340 345 350

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
355 360 365

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
370 375 380

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Ser Ile
385 390 395 400

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
405 410 415

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
420 425 430

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
435 440 445

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
450 455 460

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
465 470 475 480

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
485 490 495

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
500 505 510

Pro Gly Lys
515

<210> 23
<211> 1633
<212> DNA
<213> Artificial

<220>
<223> THER6 DNA (apo A-1-(lnk36)-mthIgG; apo A-1-(g4s)6-mthIgG)

<400> 23
gttaagcttg ccaccatgaa agctgcggtg ctgaccttgg ccgtgctctt cctgacgggg 60

agccaggctc ggcatttctg gcagcaagat gaaccccccc agagcccctg ggatcgagtg 120

aaggacctgg ccactgtgta cgtggatgtg ctcaaagaca gcggcagaga ctatgtgtcc 180

cagtttgaag gctccgcctt gggaaaacag ctaaacctaa agctccttga caactgggac 240

agcgtgacct ccaccttcag caagctgcgc gaacagctcg gccctgtgac ccaggagttc 300

tgggataacc tggaaaagga gacagagggc ctgaggcagg agatgagcaa ggatctggag 360

gaggtgaagg ccaaggtgca gccctacctg gacgacttcc agaagaagtg gcaggaggag 420

atggagctct accgccagaa ggtggagccg ctgcgcgcag agctccaaga gggcgcgcgc 480

cagaagctgc acgagctgca agagaagctg agcccactgg gcgaggagat gcgcgaccgc 540

gcgcgcgccc atgtggacgc gctgcgcacg catctggccc cctacagcga cgagctgcgc 600

cagcgcttgg ccgcgcgcct tgaggctctc aaggagaacg gcggcgccag actggccgag 660

taccacgcca aggccaccga gcatctgagc acgctcagcg agaaggccaa gcccgcgctc 720

gaggacctcc gccaaggcct gctgcccgtg ctggagagct tcaaggtcag cttcctgagc 780

gctctcgagg agtacactaa gaagctcaac acccaagatc tctccggagg aggtggctca 840

ggtggtggag gatctggagg aggtgggagt ggtggaggtg gttctggagg aggtggtagt 900

ggaggtggag gttctaccgg tctcgagccc aaatcttctg acaaaactca cacatctcca 960

ccgtccccag cacctgaact cctgggagga tcgtcagtct tcctcttccc cccaaaaccc 1020

aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtggtggt ggacgtgagc 1080

cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 1140

aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc 1200

gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc 1260

ctcccagcct ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag 1320

gtgtacaccc tgcccccatc ccgggatgag ctgaccaaga accaggtcag cctgacctgc 1380

ctggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 1440

gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt cttcctctac 1500

agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1560

atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctctctctc tccgggtaaa 1620

tgataatcta gaa 1633

<210> 24
<211> 535
<212> PRT
<213> Artificial

<220>
<223> THER6 polypeptide (apo A-1-(lnk36)-mthIgG; apo A-1-(g4s)6-mthIgG)

<400> 24

Met Lys Ala Ala Val Leu Thr Leu Ala Val Leu Phe Leu Thr Gly Ser
1 5 10 15

Gln Ala Arg His Phe Trp Gln Gln Asp Glu Pro Pro Gln Ser Pro Trp
20 25 30

Asp Arg Val Lys Asp Leu Ala Thr Val Tyr Val Asp Val Leu Lys Asp
35 40 45

Ser Gly Arg Asp Tyr Val Ser Gln Phe Glu Gly Ser Ala Leu Gly Lys
50 55 60

Gln Leu Asn Leu Lys Leu Leu Asp Asn Trp Asp Ser Val Thr Ser Thr
65 70 75 80

Phe Ser Lys Leu Arg Glu Gln Leu Gly Pro Val Thr Gln Glu Phe Trp
85 90 95

Asp Asn Leu Glu Lys Glu Thr Glu Gly Leu Arg Gln Glu Met Ser Lys
100 105 110

Asp Leu Glu Glu Val Lys Ala Lys Val Gln Pro Tyr Leu Asp Asp Phe
115 120 125

Gln Lys Lys Trp Gln Glu Glu Met Glu Leu Tyr Arg Gln Lys Val Glu
130 135 140

```
Pro Leu Arg Ala Glu Leu Gln Glu Gly Ala Arg Gln Lys Leu His Glu
145                 150                 155                 160

Leu Gln Glu Lys Leu Ser Pro Leu Gly Glu Glu Met Arg Asp Arg Ala
                165                 170                 175

Arg Ala His Val Asp Ala Leu Arg Thr His Leu Ala Pro Tyr Ser Asp
            180                 185                 190

Glu Leu Arg Gln Arg Leu Ala Ala Arg Leu Glu Ala Leu Lys Glu Asn
        195                 200                 205

Gly Gly Ala Arg Leu Ala Glu Tyr His Ala Lys Ala Thr Glu His Leu
    210                 215                 220

Ser Thr Leu Ser Glu Lys Ala Lys Pro Ala Leu Glu Asp Leu Arg Gln
225                 230                 235                 240

Gly Leu Leu Pro Val Leu Glu Ser Phe Lys Val Ser Phe Leu Ser Ala
                245                 250                 255

Leu Glu Glu Tyr Thr Lys Lys Leu Asn Thr Gln Asp Leu Ser Gly Gly
            260                 265                 270

Gly Gly Ser Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
        275                 280                 285

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Thr Gly Leu Glu
    290                 295                 300

Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro Pro Ser Pro Ala Pro
305                 310                 315                 320

Glu Leu Leu Gly Gly Ser Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
                325                 330                 335

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
            340                 345                 350

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
        355                 360                 365

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
    370                 375                 380

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
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385 390 395 400

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
405 410 415

Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
420 425 430

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
435 440 445

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
450 455 460

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
465 470 475 480

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
485 490 495

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
500 505 510

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
515 520 525

Leu Ser Leu Ser Pro Gly Lys
530 535

<210> 25
<211> 1588
<212> DNA
<213> Artificial

<220>
<223> THER3 DNA (apo A-1-(lnk21)-mthIgG; apo A-1-(g4s)3-mthIgG)

<400> 25
gttaagcttg ccaccatgaa agctgcggtg ctgaccttgg ccgtgctctt cctgacgggg 60

agccaggctc ggcatttctg gcagcaagat gaaccccccc agagcccctg ggatcgagtg 120

aaggacctgg ccactgtgta cgtggatgtg ctcaaagaca gcggcagaga ctatgtgtcc 180

cagtttgaag gctccgcctt gggaaaacag ctaaacctaa agctccttga caactgggac 240

agcgtgacct ccaccttcag caagctgcgc gaacagctcg gccctgtgac ccaggagttc 300

tgggataacc tggaaaagga gacagagggc ctgaggcagg agatgagcaa ggatctggag 360

gaggtgaagg ccaaggtgca gccctacctg gacgacttcc agaagaagtg gcaggaggag 420

atggagctct accgccagaa ggtggagccg ctgcgcgcag agctccaaga gggcgcgcgc 480

cagaagctgc acgagctgca agagaagctg agcccactgg gcgaggagat gcgcgaccgc 540

gcgcgcgccc atgtggacgc gctgcgcacg catctggccc cctacagcga cgagctgcgc 600

cagcgcttgg ccgcgcgcct tgaggctctc aaggagaacg gcggcgccag actggccgag 660

taccacgcca aggccaccga gcatctgagc acgctcagcg agaaggccaa gcccgcgctc 720

gaggacctcc gccaaggcct gctgcccgtg ctggagagct tcaaggtcag cttcctgagc 780

gctctcgagg agtacactaa gaagctcaac acccaagatc tctcaggtgg tggaggatct 840

ggaggaggtg ggagtggtgg aggtggttct accggtctcg agcccaaatc ttctgacaaa 900

actcacacat ctccaccgtc cccagcacct gaactcctgg gaggatcgtc agtcttcctc 960

ttcccccaa aacccaagga caccctcatg atctcccgga cccctgaggt cacatgcgtg 1020

gtggtggacg tgagccacga agaccctgag gtcaagttca actggtacgt ggacggcgtg 1080

gaggtgcata atgccaagac aaagccgcgg gaggagcagt acaacagcac gtaccgtgtg 1140

gtcagcgtcc tcaccgtcct gcaccaggac tggctgaatg gcaaggagta caagtgcaag 1200

gtctccaaca aagccctccc agcctccatc gagaaaacca tctccaaagc caaagggcag 1260

ccccgagaac cacaggtgta caccctgccc ccatcccggg atgagctgac caagaaccag 1320

gtcagcctga cctgcctggt caaaggcttc tatcccagcg acatcgccgt ggagtgggag 1380

agcaatgggc agccggagaa caactacaag accacgcctc ccgtgctgga ctccgacggc 1440

tccttcttcc tctacagcaa gctcaccgtg gacaagagca ggtggcagca ggggaacgtc 1500

ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctct 1560

ctctctccgg gtaaatgata atctagaa 1588

<210> 26
<211> 520
<212> PRT
<213> Artificial

<220>
<223> THER3 polypeptide (apo A-1-(lnk21)-mthIgG; apo A-1-(g4s)3-mthIgG)

<400> 26

Met Lys Ala Ala Val Leu Thr Leu Ala Val Leu Phe Leu Thr Gly Ser
1 5 10 15

Gln Ala Arg His Phe Trp Gln Gln Asp Glu Pro Pro Gln Ser Pro Trp
20 25 30

Asp Arg Val Lys Asp Leu Ala Thr Val Tyr Val Asp Val Leu Lys Asp
35 40 45

Ser Gly Arg Asp Tyr Val Ser Gln Phe Glu Gly Ser Ala Leu Gly Lys

50 55 60

Gln Leu Asn Leu Lys Leu Leu Asp Asn Trp Asp Ser Val Thr Ser Thr
65 70 75 80

Phe Ser Lys Leu Arg Glu Gln Leu Gly Pro Val Thr Gln Glu Phe Trp
85 90 95

Asp Asn Leu Glu Lys Glu Thr Glu Gly Leu Arg Gln Glu Met Ser Lys
100 105 110

Asp Leu Glu Glu Val Lys Ala Lys Val Gln Pro Tyr Leu Asp Asp Phe
115 120 125

Gln Lys Lys Trp Gln Glu Glu Met Glu Leu Tyr Arg Gln Lys Val Glu
130 135 140

Pro Leu Arg Ala Glu Leu Gln Glu Gly Ala Arg Gln Lys Leu His Glu
145 150 155 160

Leu Gln Glu Lys Leu Ser Pro Leu Gly Glu Glu Met Arg Asp Arg Ala
165 170 175

Arg Ala His Val Asp Ala Leu Arg Thr His Leu Ala Pro Tyr Ser Asp
180 185 190

Glu Leu Arg Gln Arg Leu Ala Ala Arg Leu Glu Ala Leu Lys Glu Asn
195 200 205

Gly Gly Ala Arg Leu Ala Glu Tyr His Ala Lys Ala Thr Glu His Leu
210 215 220

Ser Thr Leu Ser Glu Lys Ala Lys Pro Ala Leu Glu Asp Leu Arg Gln
225 230 235 240

Gly Leu Leu Pro Val Leu Glu Ser Phe Lys Val Ser Phe Leu Ser Ala
245 250 255

Leu Glu Glu Tyr Thr Lys Lys Leu Asn Thr Gln Asp Leu Ser Gly Gly
260 265 270

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Thr Gly Leu
275 280 285

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro Pro Ser Pro Ala
290 295 300

Pro Glu Leu Leu Gly Gly Ser Ser Val Phe Leu Phe Pro Pro Lys Pro
305 310 315 320

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
325 330 335

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
340 345 350

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
355 360 365

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
370 375 380

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
385 390 395 400

Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
405 410 415

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
420 425 430

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
435 440 445

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
450 455 460

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
465 470 475 480

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
485 490 495

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
500 505 510

Ser Leu Ser Leu Ser Pro Gly Lys
515 520

<210> 27
<211> 2677
<212> DNA
<213> Artificial

<220>
<223> THER4PON1 DNA

<400> 27
gttaagcttg ccaccatgaa agctgcggtg ctgaccttgg ccgtgctctt cctgacgggg 60

agccaggctc ggcatttctg gcagcaagat gaaccccccc agagcccctg ggatcgagtg 120

aaggacctgg ccactgtgta cgtggatgtg ctcaaagaca gcggcagaga ctatgtgtcc 180

cagtttgaag gctccgcctt gggaaaacag ctaaacctaa agctccttga caactgggac 240

agcgtgacct ccaccttcag caagctgcgc gaacagctcg gccctgtgac ccaggagttc 300

tgggataacc tggaaaagga gacagagggc ctgaggcagg agatgagcaa ggatctggag 360

gaggtgaagg ccaaggtgca gccctacctg gacgacttcc agaagaagtg gcaggaggag 420

atggagctct accgccagaa ggtggagccg ctgcgcgcag agctccaaga gggcgcgcgc 480

cagaagctgc acgagctgca agagaagctg agcccactgg gcgaggagat gcgcgaccgc 540

gcgcgcgccc atgtggacgc gctgcgcacg catctggccc cctacagcga cgagctgcgc 600

cagcgcttgg ccgcgcgcct tgaggctctc aaggagaacg gcggcgccag actggccgag 660

taccacgcca aggccaccga gcatctgagc acgctcagcg agaaggccaa gcccgcgctc 720

gaggacctcc gccaaggcct gctgcccgtg ctggagagct tcaaggtcag cttcctgagc 780

gctctcgagg agtacactaa gaagctcaac acccaagatc tctccggagg aggtggctca 840

ggtggtggag gatctggagg aggtgggagt ggtggaggtg gttctaccgg tctcgagccc 900

aaatcttctg acaaaactca cacatctcca ccgtccccag cacctgaact cctgggagga 960

tcgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggacccct 1020

gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 1080

tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 1140

agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 1200

gagtacaagt gcaaggtctc caacaaagcc ctcccagcct ccatcgagaa aaccatctcc 1260

aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgcccccatc ccgggatgag 1320

ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 1380

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 1440

ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg 1500

cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 1560

cagaagagcc tctctctctc tccgggtaaa gtcgacggag ctagcagccc cgtgaacgtg 1620

agcagcccca gcgtgcagga tatcctcttc aggaaccacc agtcttctta ccaaacacga 1680

cttaatgctc tccgagaggt acaacccgta gaacttccta actgtaattt agttaaagga 1740

atcgaaactg gctctgaaga cttggagata ctgcctaatg gactggcttt cattagctct 1800

ggattaaagt atcctggaat aaagagcttc aaccccaaca gtcctggaaa aatacttctg 1860

atggacctga atgaagaaga tccaacagtg ttggaattgg ggatcactgg aagtaaattt 1920

gatgtatctt catttaaccc tcatgggatt agcacattca cagatgaaga taatgccatg 1980

tacctcctgg tggtgaacca tccagatgcc aagtccacag tggagttgtt taaatttcaa 2040

gaagaagaaa aatcgctttt gcatctaaaa accatcagac ataaacttct gcctaatttg 2100

aatgatattg ttgctgtggg acctgagcac ttttatggca caaatgatca ctattttctt 2160

gacccctact tacaatcctg ggagatgtat ttgggtttag cgtggtcgta tgttgtctac 2220

tatagtccaa gtgaagttcg agtggtggca gaaggatttg attttgctaa tggaatcaac 2280

atttcacccg atggcaagta tgtctatata gctgagttgc tggctcataa gattcatgtg 2340

tatgaaaagc atgctaattg gactttaact ccattgaagt cccttgactt taataccctc 2400

gtggataaca tatctgtgga tcctgagaca ggagaccttt gggttggatg ccatcccaat 2460

ggcatgaaaa tcttcttcta tgactcagag aatcctcctg catcagaggt gcttcgaatc 2520

cagaacattc taacagaaga acctaaagtg acacaggttt atgcagaaaa tggcacagtg 2580

ttgcaaggca gtacagttgc ctctgtgtac aaagggaaac tgctgattgg cacagtgttt 2640

cacaaagctc tttactgtga gctctaataa tctagaa 2677

<210> 28
<211> 883
<212> PRT
<213> Artificial

<220>
<223> THER4PON1 polypeptide

<400> 28

Met Lys Ala Ala Val Leu Thr Leu Ala Val Leu Phe Leu Thr Gly Ser
1 5 10 15

Gln Ala Arg His Phe Trp Gln Gln Asp Glu Pro Pro Gln Ser Pro Trp
20 25 30

Asp Arg Val Lys Asp Leu Ala Thr Val Tyr Val Asp Val Leu Lys Asp
35 40 45

Ser Gly Arg Asp Tyr Val Ser Gln Phe Glu Gly Ser Ala Leu Gly Lys
50 55 60

Gln Leu Asn Leu Lys Leu Leu Asp Asn Trp Asp Ser Val Thr Ser Thr
65 70 75 80

Phe Ser Lys Leu Arg Glu Gln Leu Gly Pro Val Thr Gln Glu Phe Trp
85 90 95

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Asp Asn Leu Glu Lys Glu Thr Glu Gly Leu Arg Gln Glu Met Ser Lys
            100                 105                 110

Asp Leu Glu Glu Val Lys Ala Lys Val Gln Pro Tyr Leu Asp Asp Phe
        115                 120                 125

Gln Lys Lys Trp Gln Glu Glu Met Glu Leu Tyr Arg Gln Lys Val Glu
    130                 135                 140

Pro Leu Arg Ala Glu Leu Gln Glu Gly Ala Arg Gln Lys Leu His Glu
145                 150                 155                 160

Leu Gln Glu Lys Leu Ser Pro Leu Gly Glu Glu Met Arg Asp Arg Ala
                165                 170                 175

Arg Ala His Val Asp Ala Leu Arg Thr His Leu Ala Pro Tyr Ser Asp
            180                 185                 190

Glu Leu Arg Gln Arg Leu Ala Ala Arg Leu Glu Ala Leu Lys Glu Asn
        195                 200                 205

Gly Gly Ala Arg Leu Ala Glu Tyr His Ala Lys Ala Thr Glu His Leu
    210                 215                 220

Ser Thr Leu Ser Glu Lys Ala Lys Pro Ala Leu Glu Asp Leu Arg Gln
225                 230                 235                 240

Gly Leu Leu Pro Val Leu Glu Ser Phe Lys Val Ser Phe Leu Ser Ala
                245                 250                 255

Leu Glu Glu Tyr Thr Lys Lys Leu Asn Thr Gln Asp Leu Ser Gly Gly
            260                 265                 270

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
        275                 280                 285

Gly Ser Thr Gly Leu Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
    290                 295                 300

Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val Phe Leu
305                 310                 315                 320

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
                325                 330                 335

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
            340                 345                 350
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Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
        355                 360                 365

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
    370                 375                 380

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
385                 390                 395                 400

Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys
                405                 410                 415

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
            420                 425                 430

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
        435                 440                 445

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
    450                 455                 460

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
465                 470                 475                 480

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
                485                 490                 495

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
            500                 505                 510

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Val Asp Gly
        515                 520                 525

Ala Ser Ser Pro Val Asn Val Ser Ser Pro Ser Val Gln Asp Ile Leu
    530                 535                 540

Phe Arg Asn His Gln Ser Ser Tyr Gln Thr Arg Leu Asn Ala Leu Arg
545                 550                 555                 560

Glu Val Gln Pro Val Glu Leu Pro Asn Cys Asn Leu Val Lys Gly Ile
                565                 570                 575

Glu Thr Gly Ser Glu Asp Leu Glu Ile Leu Pro Asn Gly Leu Ala Phe
            580                 585                 590

Ile Ser Ser Gly Leu Lys Tyr Pro Gly Ile Lys Ser Phe Asn Pro Asn
```

595 600 605

Ser Pro Gly Lys Ile Leu Leu Met Asp Leu Asn Glu Glu Asp Pro Thr
610 615 620

Val Leu Glu Leu Gly Ile Thr Gly Ser Lys Phe Asp Val Ser Ser Phe
625 630 635 640

Asn Pro His Gly Ile Ser Thr Phe Thr Asp Glu Asp Asn Ala Met Tyr
645 650 655

Leu Leu Val Val Asn His Pro Asp Ala Lys Ser Thr Val Glu Leu Phe
660 665 670

Lys Phe Gln Glu Glu Glu Lys Ser Leu Leu His Leu Lys Thr Ile Arg
675 680 685

His Lys Leu Leu Pro Asn Leu Asn Asp Ile Val Ala Val Gly Pro Glu
690 695 700

His Phe Tyr Gly Thr Asn Asp His Tyr Phe Leu Asp Pro Tyr Leu Gln
705 710 715 720

Ser Trp Glu Met Tyr Leu Gly Leu Ala Trp Ser Tyr Val Val Tyr Tyr
725 730 735

Ser Pro Ser Glu Val Arg Val Val Ala Glu Gly Phe Asp Phe Ala Asn
740 745 750

Gly Ile Asn Ile Ser Pro Asp Gly Lys Tyr Val Tyr Ile Ala Glu Leu
755 760 765

Leu Ala His Lys Ile His Val Tyr Glu Lys His Ala Asn Trp Thr Leu
770 775 780

Thr Pro Leu Lys Ser Leu Asp Phe Asn Thr Leu Val Asp Asn Ile Ser
785 790 795 800

Val Asp Pro Glu Thr Gly Asp Leu Trp Val Gly Cys His Pro Asn Gly
805 810 815

Met Lys Ile Phe Phe Tyr Asp Ser Glu Asn Pro Pro Ala Ser Glu Val
820 825 830

Leu Arg Ile Gln Asn Ile Leu Thr Glu Glu Pro Lys Val Thr Gln Val
835 840 845

Tyr Ala Glu Asn Gly Thr Val Leu Gln Gly Ser Thr Val Ala Ser Val
850 855 860

Tyr Lys Gly Lys Leu Leu Ile Gly Thr Val Phe His Lys Ala Leu Tyr
865 870 875 880

Cys Glu Leu

<210> 29
<211> 1717
<212> DNA
<213> Homo sapiens

<400> 29
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ctggctgcca cagtcctgac cctggccctg ctgggcaatg cccatgcctg ctccaaaggc 120

acctcgcacg aggcaggcat cgtgtgccgc atcaccaagc ctgccctcct ggtgttgaac 180

cacgagactg ccaaggtgat ccagaccgcc ttccagcgag ccagctaccc agatatcacg 240

ggcgagaagg ccatgatgct ccttggccaa gtcaagtatg ggttgcacaa catccagatc 300

agccacttgt ccatcgccag cagccaggtg gagctggtgg aagccaagtc cattgatgtc 360

tccattcaga acgtgtctgt ggtcttcaag gggaccctga agtatggcta caccactgcc 420

tggtggctgg gtattgatca gtccattgac ttcgagatcg actctgccat tgacctccag 480

atcaacacac agctgacctg tgactctggt agagtgcgga ccgatgcccc tgactgctac 540

ctgtctttcc ataagctgct cctgcatctc caaggggagc gagagcctgg gtggatcaag 600

cagctgttca caaatttcat ctccttcacc ctgaagctgg tcctgaaggg acagatctgc 660

aaagagatca acgtcatctc taacatcatg gccgattttg tccagacaag ggctgccagc 720

atcctttcag atggagacat tggggtggac atttccctga caggtgatcc cgtcatcaca 780

gcctcctacc tggagtccca tcacaagggt catttcatct acaagaatgt ctcagaggac 840

ctccccctcc ccaccttctc gcccacactg ctgggggact cccgcatgct gtacttctgg 900

ttctctgagc gagtcttcca ctcgctggcc aaggtagctt tccaggatgg ccgcctcatg 960

ctcagcctga tgggagacga gttcaaggca gtgctggaga cctggggctt caacaccaac 1020

caggaaatct tccaagaggt tgtcggcggc ttccccagcc aggcccaagt caccgtccac 1080

tgcctcaaga tgcccaagat ctcctgccaa aacaagggag tcgtggtcaa ttcttcagtg 1140

atggtgaaat tcctctttcc acgcccagac cagcaacatt ctgtagctta cacatttgaa 1200

gaggatatcg tgactaccgt ccaggcctcc tattctaaga aaaagctctt cttaagcctc 1260

ttggatttcc agattacacc aaagactgtt tccaacttga ctgagagcag ctccgagtcc 1320

gtccagagct tcctgcagtc aatgatcacc gctgtgggca tccctgaggt catgtctcgg 1380

ctcgaggtag tgtttacagc cctcatgaac agcaaaggcg tgagcctctt cgacatcatc 1440

aaccctgaga ttatcactcg agatggcttc ctgctgctgc agatggactt tggcttccct 1500

gagcacctgc tggtggattt cctccagagc ttgagctaga agtctccaag gaggtcggga 1560

tggggcttgt agcagaaggc aagcaccagg ctcacagctg gaaccctggt gtctcctcca 1620

gcgtggtgga agttgggtta ggagtacgga gatggagatt ggctcccaac tcctccctat 1680

cctaaaggcc cactggcatt aaagtgctgt atccaag 1717

<210> 30
<211> 493
<212> PRT
<213> Homo sapiens

<400> 30

Met Leu Ala Ala Thr Val Leu Thr Leu Ala Leu Leu Gly Asn Ala His
1 5 10 15

Ala Cys Ser Lys Gly Thr Ser His Glu Ala Gly Ile Val Cys Arg Ile
20 25 30

Thr Lys Pro Ala Leu Leu Val Leu Asn His Glu Thr Ala Lys Val Ile
35 40 45

Gln Thr Ala Phe Gln Arg Ala Ser Tyr Pro Asp Ile Thr Gly Glu Lys
50 55 60

Ala Met Met Leu Leu Gly Gln Val Lys Tyr Gly Leu His Asn Ile Gln
65 70 75 80

Ile Ser His Leu Ser Ile Ala Ser Ser Gln Val Glu Leu Val Glu Ala
85 90 95

Lys Ser Ile Asp Val Ser Ile Gln Asn Val Ser Val Val Phe Lys Gly
100 105 110

Thr Leu Lys Tyr Gly Tyr Thr Thr Ala Trp Trp Leu Gly Ile Asp Gln
115 120 125

Ser Ile Asp Phe Glu Ile Asp Ser Ala Ile Asp Leu Gln Ile Asn Thr
130 135 140

Gln Leu Thr Cys Asp Ser Gly Arg Val Arg Thr Asp Ala Pro Asp Cys
145 150 155 160

Tyr Leu Ser Phe His Lys Leu Leu Leu His Leu Gln Gly Glu Arg Glu
165 170 175

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Pro Gly Trp Ile Lys Gln Leu Phe Thr Asn Phe Ile Ser Phe Thr Leu
            180                 185                 190

Lys Leu Val Leu Lys Gly Gln Ile Cys Lys Glu Ile Asn Val Ile Ser
        195                 200                 205

Asn Ile Met Ala Asp Phe Val Gln Thr Arg Ala Ala Ser Ile Leu Ser
    210                 215                 220

Asp Gly Asp Ile Gly Val Asp Ile Ser Leu Thr Gly Asp Pro Val Ile
225                 230                 235                 240

Thr Ala Ser Tyr Leu Glu Ser His His Lys Gly His Phe Ile Tyr Lys
                245                 250                 255

Asn Val Ser Glu Asp Leu Pro Leu Pro Thr Phe Ser Pro Thr Leu Leu
            260                 265                 270

Gly Asp Ser Arg Met Leu Tyr Phe Trp Phe Ser Glu Arg Val Phe His
        275                 280                 285

Ser Leu Ala Lys Val Ala Phe Gln Asp Gly Arg Leu Met Leu Ser Leu
    290                 295                 300

Met Gly Asp Glu Phe Lys Ala Val Leu Glu Thr Trp Gly Phe Asn Thr
305                 310                 315                 320

Asn Gln Glu Ile Phe Gln Glu Val Val Gly Gly Phe Pro Ser Gln Ala
                325                 330                 335

Gln Val Thr Val His Cys Leu Lys Met Pro Lys Ile Ser Cys Gln Asn
            340                 345                 350

Lys Gly Val Val Val Asn Ser Ser Val Met Val Lys Phe Leu Phe Pro
        355                 360                 365

Arg Pro Asp Gln Gln His Ser Val Ala Tyr Thr Phe Glu Glu Asp Ile
    370                 375                 380

Val Thr Thr Val Gln Ala Ser Tyr Ser Lys Lys Lys Leu Phe Leu Ser
385                 390                 395                 400

Leu Leu Asp Phe Gln Ile Thr Pro Lys Thr Val Ser Asn Leu Thr Glu
                405                 410                 415

Ser Ser Ser Glu Ser Val Gln Ser Phe Leu Gln Ser Met Ile Thr Ala
```

420 425 430

Val Gly Ile Pro Glu Val Met Ser Arg Leu Glu Val Val Phe Thr Ala
435 440 445

Leu Met Asn Ser Lys Gly Val Ser Leu Phe Asp Ile Ile Asn Pro Glu
450 455 460

Ile Ile Thr Arg Asp Gly Phe Leu Leu Leu Gln Met Asp Phe Gly Phe
465 470 475 480

Pro Glu His Leu Leu Val Asp Phe Leu Gln Ser Leu Ser
485 490

<210> 31
<211> 1880
<212> DNA
<213> Homo sapiens

<400> 31
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cgcccgccgc ctgccagagc tgctcggccc gcagccaggg ggacagcggc tggtcggagg 120

ctcgcagtgc tgtcggcgag aagcagtcgg gtttggagcg cttgggtcgc gttggtgcgc 180

ggtggaacgc gcccagggac cccagttccc gcgagcagct ccgcgccgcg cctgagagac 240

taagctgaaa ctgctgctca gctcccaaga tggtgccacc caaattgcat gtgcttttct 300

gcctctgcgg ctgcctggct gtggtttatc cttttgactg gcaatacata aatcctgttg 360

cccatatgaa atcatcagca tgggtcaaca aaatacaagt actgatggct gctgcaagct 420

ttggccaaac taaaatcccc cggggaaatg ggccttattc cgttggttgt acagacttaa 480

tgtttgatca cactaataag ggcaccttct tgcgtttata ttatccatcc caagataatg 540

atcgccttga cacccttttgg atcccaaata aagaatattt ttggggtctt agcaaatttc 600

ttggaacaca ctggcttatg ggcaacattt tgaggttact ctttggttca atgacaactc 660

ctgcaaactg gaattcccct ctgaggcctg gtgaaaaata tccacttgtt gttttttctc 720

atggtcttgg ggcattcagg acactttatt ctgctattgg cattgacctg gcatctcatg 780

ggtttatagt tgctgctgta gaacacagag atagatctgc atctgcaact tactatttca 840

aggaccaatc tgctgcagaa ataggggaca agtcttggct ctaccttaga accctgaaac 900

aagaggagga gacacatata cgaaatgagc aggtacggca aagagcaaaa gaatgttccc 960

aagctctcag tctgattctt gacattgatc atggaaagcc agtgaagaat gcattagatt 1020

taaagtttga tatggaacaa ctgaaggact ctattgatag ggaaaaaata gcagtaattg 1080

gacattcttt tggtggagca acggttattc agactcttag tgaagatcag agattcagat 1140

gtggtattgc cctggatgca tggatgtttc cactgggtga tgaagtatat tccagaattc 1200

ctcagcccct ctttttatc aactctgaat atttccaata tcctgctaat atcataaaaa 1260

tgaaaaaatg ctactcacct gataaagaaa gaaagatgat tacaatcagg ggttcagtcc 1320

accagaattt tgctgacttc acttttgcaa ctggcaaaat aattggacac atgctcaaat 1380

taaagggaga catagattca aatgtagcta ttgatcttag caacaaagct tcattagcat 1440

tcttacaaaa gcatttagga cttcataaag attttgatca gtgggactgc ttgattgaag 1500

gagatgatga gaatcttatt ccagggacca acattaacac aaccaatcaa cacatcatgt 1560

tacagaactc ttcaggaata gagaaataca attaggatta aaataggttt tttaaaagtc 1620

ttgtttcaaa actgtctaaa attatgtgtg tgtgtgtgtg tgtgtgtgtg tgtgtgagag 1680

agagagagag agagagagag agagagagag agaattttaa tgtattttcc caaaggactc 1740

atattttaaa atgtaggcta tactgtaatc gtgattgaag cttggactaa gaatttttc 1800

cctttagatg taaagaaaga atacagtata caatattcaa aaaaaaaaaa aaaaaaaaaa 1860

aaaaaaaaaa aaaaaaaaaa 1880

<210> 32
<211> 441
<212> PRT
<213> Homo sapiens

<400> 32

Met Val Pro Pro Lys Leu His Val Leu Phe Cys Leu Cys Gly Cys Leu
1 5 10 15

Ala Val Val Tyr Pro Phe Asp Trp Gln Tyr Ile Asn Pro Val Ala His
20 25 30

Met Lys Ser Ser Ala Trp Val Asn Lys Ile Gln Val Leu Met Ala Ala
35 40 45

Ala Ser Phe Gly Gln Thr Lys Ile Pro Arg Gly Asn Gly Pro Tyr Ser
50 55 60

Val Gly Cys Thr Asp Leu Met Phe Asp His Thr Asn Lys Gly Thr Phe
65 70 75 80

Leu Arg Leu Tyr Tyr Pro Ser Gln Asp Asn Asp Arg Leu Asp Thr Leu
85 90 95

Trp Ile Pro Asn Lys Glu Tyr Phe Trp Gly Leu Ser Lys Phe Leu Gly
100 105 110

Thr His Trp Leu Met Gly Asn Ile Leu Arg Leu Leu Phe Gly Ser Met

```
        115                 120                 125

Thr Thr Pro Ala Asn Trp Asn Ser Pro Leu Arg Pro Gly Glu Lys Tyr
    130                 135                 140

Pro Leu Val Val Phe Ser His Gly Leu Gly Ala Phe Arg Thr Leu Tyr
145                 150                 155                 160

Ser Ala Ile Gly Ile Asp Leu Ala Ser His Gly Phe Ile Val Ala Ala
                165                 170                 175

Val Glu His Arg Asp Arg Ser Ala Ser Ala Thr Tyr Tyr Phe Lys Asp
            180                 185                 190

Gln Ser Ala Ala Glu Ile Gly Asp Lys Ser Trp Leu Tyr Leu Arg Thr
        195                 200                 205

Leu Lys Gln Glu Glu Glu Thr His Ile Arg Asn Glu Gln Val Arg Gln
    210                 215                 220

Arg Ala Lys Glu Cys Ser Gln Ala Leu Ser Leu Ile Leu Asp Ile Asp
225                 230                 235                 240

His Gly Lys Pro Val Lys Asn Ala Leu Asp Leu Lys Phe Asp Met Glu
                245                 250                 255

Gln Leu Lys Asp Ser Ile Asp Arg Glu Lys Ile Ala Val Ile Gly His
            260                 265                 270

Ser Phe Gly Gly Ala Thr Val Ile Gln Thr Leu Ser Glu Asp Gln Arg
        275                 280                 285

Phe Arg Cys Gly Ile Ala Leu Asp Ala Trp Met Phe Pro Leu Gly Asp
    290                 295                 300

Glu Val Tyr Ser Arg Ile Pro Gln Pro Leu Phe Phe Ile Asn Ser Glu
305                 310                 315                 320

Tyr Phe Gln Tyr Pro Ala Asn Ile Ile Lys Met Lys Lys Cys Tyr Ser
                325                 330                 335

Pro Asp Lys Glu Arg Lys Met Ile Thr Ile Arg Gly Ser Val His Gln
            340                 345                 350

Asn Phe Ala Asp Phe Thr Phe Ala Thr Gly Lys Ile Ile Gly His Met
        355                 360                 365
```

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Leu Lys Leu Lys Gly Asp Ile Asp Ser Asn Val Ala Ile Asp Leu Ser
    370                 375                 380

Asn Lys Ala Ser Leu Ala Phe Leu Gln Lys His Leu Gly Leu His Lys
385                 390                 395                 400

Asp Phe Asp Gln Trp Asp Cys Leu Ile Glu Gly Asp Asp Glu Asn Leu
                405                 410                 415

Ile Pro Gly Thr Asn Ile Asn Thr Thr Asn Gln His Ile Met Leu Gln
            420                 425                 430

Asn Ser Ser Gly Ile Glu Lys Tyr Asn
        435                 440
```

<210> 33
<211> 2907
<212> DNA
<213> Artificial

<220>
<223> THER4PAFAH DNA

<400> 33

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gttaagcttg ccaccatgaa agctgcggtg ctgaccttgg ccgtgctctt cctgacgggg       60

agccaggctc ggcatttctg gcagcaagat gaaccccccc agagcccctg ggatcgagtg      120

aaggacctgg ccactgtgta cgtggatgtg ctcaaagaca gcggcagaga ctatgtgtcc      180

cagtttgaag gctccgcctt gggaaaacag ctaaacctaa agctccttga caactgggac      240

agcgtgacct ccaccttcag caagctgcgc gaacagctcg gccctgtgac ccaggagttc      300

tgggataacc tggaaaagga gacagagggc ctgaggcagg agatgagcaa ggatctggag      360

gaggtgaagg ccaaggtgca gccctacctg gacgacttcc agaagaagtg gcaggaggag      420

atggagctct accgccagaa ggtggagccg ctgcgcgcag agctccaaga gggcgcgcgc      480

cagaagctgc acgagctgca agagaagctg agcccactgg gcgaggagat gcgcgaccgc      540

gcgcgcgccc atgtggacgc gctgcgcacg catctggccc cctacagcga cgagctgcgc      600

cagcgcttgg ccgcgcgcct tgaggctctc aaggagaacg gcggcgccag actggccgag      660

taccacgcca aggccaccga gcatctgagc acgctcagcg agaaggccaa gcccgcgctc      720

gaggacctcc gccaaggcct gctgcccgtg ctggagagct tcaaggtcag cttcctgagc      780

gctctcgagg agtacactaa gaagctcaac acccaagatc tctccggagg aggtggctca      840

ggtggtggag gatctggagg aggtgggagt ggtggaggtg gttctaccgg tctcgagccc      900

aaatcttctg acaaaactca cacatctcca ccgtccccag cacctgaact cctgggagga      960

tcgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggacccct     1020
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gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 1080

tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 1140

agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 1200

gagtacaagt gcaaggtctc caacaaagcc ctcccagcct ccatcgagaa aaccatctcc 1260

aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgcccccatc ccgggatgag 1320

ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 1380

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 1440

ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg 1500

cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 1560

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aaatcatcag catgggtcaa caaaatacaa gtactgatgg ctgctgcaag ctttggccaa 1740

actaaaatcc cccggggaaa tgggccttat tccgttggtt gtacagactt aatgtttgat 1800

cacactaata agggcacctt cttgcgttta tattatccat cccaagataa tgatcgcctt 1860

gacacccttt ggatcccaaa taaagaatat ttttggggtc ttagcaaatt tcttggaaca 1920

cactggctta tgggcaacat tttgaggtta ctctttggtt caatgacaac tcctgcaaac 1980

tggaattccc ctctgaggcc tggtgaaaaa tatccacttg ttgtttttc tcatggtctt 2040

ggggcattca ggacacttta ttctgctatt ggcattgacc tggcatctca tgggtttata 2100

gttgctgctg tagaacacag agatagatct gcatctgcaa cttactattt caaggaccaa 2160

tctgctgcag aaatagggga caagtcttgg ctctacctta gaaccctgaa acaagaggag 2220

gagacacata tacgaaatga gcaggtacgg caaagagcaa aagaatgttc ccaagctctc 2280

agtctgattc ttgacattga tcatggaaag ccagtgaaga atgcattaga tttaaagttt 2340

gatatggaac aactgaagga ctctattgat agggaaaaaa tagcagtaat tggacattct 2400

tttggtggag caacggttat tcagactctt agtgaagatc agagattcag atgtggtatt 2460

gccctggatg catggatgtt tccactgggt gatgaagtat attccagaat tcctcagccc 2520

ctcttttta tcaactctga atatttccaa tatcctgcta atatcataaa aatgaaaaaa 2580

tgctactcac ctgataaaga aagaaagatg attacaatca ggggttcagt ccaccagaat 2640

tttgctgact tcacttttgc aactggcaaa ataattggac acatgctcaa attaaaggga 2700

gacatagatt caaatgtagc tattgatctt agcaacaaag cttcattagc attcttacaa 2760

aagcatttag gacttcataa agattttgat cagtgggact gcttgattga aggagatgat 2820

gagaatctta ttccagggac caacattaac acaaccaatc aacacatcat gttacagaac 2880

tcttcaggaa tagagaaata caattag 2907

<210> 34
<211> 963
<212> PRT
<213> Artificial

<220>
<223> THER4PAFAH polypeptide

<400> 34

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Met Lys Ala Ala Val Leu Thr Leu Ala Val Leu Phe Leu Thr Gly Ser
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Gln Ala Arg His Phe Trp Gln Gln Asp Glu Pro Pro Gln Ser Pro Trp
            20                  25                  30

Asp Arg Val Lys Asp Leu Ala Thr Val Tyr Val Asp Val Leu Lys Asp
        35                  40                  45

Ser Gly Arg Asp Tyr Val Ser Gln Phe Glu Gly Ser Ala Leu Gly Lys
    50                  55                  60

Gln Leu Asn Leu Lys Leu Leu Asp Asn Trp Asp Ser Val Thr Ser Thr
65                  70                  75                  80

Phe Ser Lys Leu Arg Glu Gln Leu Gly Pro Val Thr Gln Glu Phe Trp
                85                  90                  95

Asp Asn Leu Glu Lys Glu Thr Glu Gly Leu Arg Gln Glu Met Ser Lys
            100                 105                 110

Asp Leu Glu Glu Val Lys Ala Lys Val Gln Pro Tyr Leu Asp Asp Phe
        115                 120                 125

Gln Lys Lys Trp Gln Glu Glu Met Glu Leu Tyr Arg Gln Lys Val Glu
    130                 135                 140

Pro Leu Arg Ala Glu Leu Gln Glu Gly Ala Arg Gln Lys Leu His Glu
145                 150                 155                 160

Leu Gln Glu Lys Leu Ser Pro Leu Gly Glu Glu Met Arg Asp Arg Ala
                165                 170                 175

Arg Ala His Val Asp Ala Leu Arg Thr His Leu Ala Pro Tyr Ser Asp
            180                 185                 190

Glu Leu Arg Gln Arg Leu Ala Ala Arg Leu Glu Ala Leu Lys Glu Asn
        195                 200                 205
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Gly Gly Ala Arg Leu Ala Glu Tyr His Ala Lys Ala Thr Glu His Leu
    210                 215                 220

Ser Thr Leu Ser Glu Lys Ala Lys Pro Ala Leu Glu Asp Leu Arg Gln
225                 230                 235                 240

Gly Leu Leu Pro Val Leu Glu Ser Phe Lys Val Ser Phe Leu Ser Ala
                245                 250                 255

Leu Glu Glu Tyr Thr Lys Lys Leu Asn Thr Gln Asp Leu Ser Gly Gly
            260                 265                 270

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
        275                 280                 285

Gly Ser Thr Gly Leu Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
    290                 295                 300

Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val Phe Leu
305                 310                 315                 320

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
                325                 330                 335

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
            340                 345                 350

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
        355                 360                 365

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
    370                 375                 380

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
385                 390                 395                 400

Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys
                405                 410                 415

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
            420                 425                 430

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
        435                 440                 445

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
    450                 455                 460
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Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
465                 470                 475                 480

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
                485                 490                 495

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
            500                 505                 510

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Val Asp Gly
        515                 520                 525

Ala Ser Ser Pro Val Asn Val Ser Ser Pro Ser Val Gln Asp Ile Phe
    530                 535                 540

Asp Trp Gln Tyr Ile Asn Pro Val Ala His Met Lys Ser Ser Ala Trp
545                 550                 555                 560

Val Asn Lys Ile Gln Val Leu Met Ala Ala Ala Ser Phe Gly Gln Thr
                565                 570                 575

Lys Ile Pro Arg Gly Asn Gly Pro Tyr Ser Val Gly Cys Thr Asp Leu
            580                 585                 590

Met Phe Asp His Thr Asn Lys Gly Thr Phe Leu Arg Leu Tyr Tyr Pro
        595                 600                 605

Ser Gln Asp Asn Asp Arg Leu Asp Thr Leu Trp Ile Pro Asn Lys Glu
    610                 615                 620

Tyr Phe Trp Gly Leu Ser Lys Phe Leu Gly Thr His Trp Leu Met Gly
625                 630                 635                 640

Asn Ile Leu Arg Leu Leu Phe Gly Ser Met Thr Thr Pro Ala Asn Trp
                645                 650                 655

Asn Ser Pro Leu Arg Pro Gly Glu Lys Tyr Pro Leu Val Val Phe Ser
            660                 665                 670

His Gly Leu Gly Ala Phe Arg Thr Leu Tyr Ser Ala Ile Gly Ile Asp
        675                 680                 685

Leu Ala Ser His Gly Phe Ile Val Ala Ala Val Glu His Arg Asp Arg
    690                 695                 700

Ser Ala Ser Ala Thr Tyr Tyr Phe Lys Asp Gln Ser Ala Ala Glu Ile
```

705 710 715 720

Gly Asp Lys Ser Trp Leu Tyr Leu Arg Thr Leu Lys Gln Glu Glu Glu
725 730 735

Thr His Ile Arg Asn Glu Gln Val Arg Gln Arg Ala Lys Glu Cys Ser
740 745 750

Gln Ala Leu Ser Leu Ile Leu Asp Ile Asp His Gly Lys Pro Val Lys
755 760 765

Asn Ala Leu Asp Leu Lys Phe Asp Met Glu Gln Leu Lys Asp Ser Ile
770 775 780

Asp Arg Glu Lys Ile Ala Val Ile Gly His Ser Phe Gly Gly Ala Thr
785 790 795 800

Val Ile Gln Thr Leu Ser Glu Asp Gln Arg Phe Arg Cys Gly Ile Ala
805 810 815

Leu Asp Ala Trp Met Phe Pro Leu Gly Asp Glu Val Tyr Ser Arg Ile
820 825 830

Pro Gln Pro Leu Phe Phe Ile Asn Ser Glu Tyr Phe Gln Tyr Pro Ala
835 840 845

Asn Ile Ile Lys Met Lys Lys Cys Tyr Ser Pro Asp Lys Glu Arg Lys
850 855 860

Met Ile Thr Ile Arg Gly Ser Val His Gln Asn Phe Ala Asp Phe Thr
865 870 875 880

Phe Ala Thr Gly Lys Ile Ile Gly His Met Leu Lys Leu Lys Gly Asp
885 890 895

Ile Asp Ser Asn Val Ala Ile Asp Leu Ser Asn Lys Ala Ser Leu Ala
900 905 910

Phe Leu Gln Lys His Leu Gly Leu His Lys Asp Phe Asp Gln Trp Asp
915 920 925

Cys Leu Ile Glu Gly Asp Asp Glu Asn Leu Ile Pro Gly Thr Asn Ile
930 935 940

Asn Thr Thr Asn Gln His Ile Met Leu Gln Asn Ser Ser Gly Ile Glu
945 950 955 960

Lys Tyr Asn

<210> 35
<211> 801
<212> DNA
<213> Homo sapiens

<400> 35
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ttctggcagc aagatgaacc cccccagagc ccctgggatc gagtgaagga cctggccact 120

gtgtacgtgg atgtgctcaa agacagcggc agagactatg tgtcccagtt tgaaggctcc 180

gccttgggaa aacagctaaa cctaaagctc cttgacaact gggacagcgt gacctccacc 240

ttcagcaagc tgcgcgaaca gctcggccct gtgacccagg agttctggga taacctggaa 300

aaggagacag agggcctgag gcaggagatg agcaaggatc tggaggaggt gaaggccaag 360

gtgcagccct acctggacga cttccagaag aagtggcagg aggagatgga gctctaccgc 420

cagaaggtgg agccgctgcg cgcagagctc caagagggcg cgcgccagaa gctgcacgag 480

ctgcaagaga agctgagccc actgggcgag gagatgcgcg accgcgcgcg cgcccatgtg 540

gacgcgctgc gcacgcatct ggccccctac agcgacgagc tgcgccagcg cttggccgcg 600

cgccttgagg ctctcaagga gaacggcggc gccagactgg ccgagtacca cgccaaggcc 660

accgagcatc tgagcacgct cagcgagaag gccaagcccg cgctcgagga cctccgccaa 720

ggcctgctgc ccgtgctgga gagcttcaag gtcagcttcc tgagcgctct cgaggagtac 780

actaagaagc tcaacaccca a 801

<210> 36
<211> 267
<212> PRT
<213> Homo sapiens

<400> 36

Met Lys Ala Ala Val Leu Thr Leu Ala Val Leu Phe Leu Thr Gly Ser
1 5 10 15

Gln Ala Arg His Phe Trp Gln Gln Asp Glu Pro Pro Gln Ser Pro Trp
20 25 30

Asp Arg Val Lys Asp Leu Ala Thr Val Tyr Val Asp Val Leu Lys Asp
35 40 45

Ser Gly Arg Asp Tyr Val Ser Gln Phe Glu Gly Ser Ala Leu Gly Lys
50 55 60

Gln Leu Asn Leu Lys Leu Leu Asp Asn Trp Asp Ser Val Thr Ser Thr

65 70 75 80

Phe Ser Lys Leu Arg Glu Gln Leu Gly Pro Val Thr Gln Glu Phe Trp
85 90 95

Asp Asn Leu Glu Lys Glu Thr Glu Gly Leu Arg Gln Glu Met Ser Lys
100 105 110

Asp Leu Glu Glu Val Lys Ala Lys Val Gln Pro Tyr Leu Asp Asp Phe
115 120 125

Gln Lys Lys Trp Gln Glu Glu Met Glu Leu Tyr Arg Gln Lys Val Glu
130 135 140

Pro Leu Arg Ala Glu Leu Gln Glu Gly Ala Arg Gln Lys Leu His Glu
145 150 155 160

Leu Gln Glu Lys Leu Ser Pro Leu Gly Glu Glu Met Arg Asp Arg Ala
165 170 175

Arg Ala His Val Asp Ala Leu Arg Thr His Leu Ala Pro Tyr Ser Asp
180 185 190

Glu Leu Arg Gln Arg Leu Ala Ala Arg Leu Glu Ala Leu Lys Glu Asn
195 200 205

Gly Gly Ala Arg Leu Ala Glu Tyr His Ala Lys Ala Thr Glu His Leu
210 215 220

Ser Thr Leu Ser Glu Lys Ala Lys Pro Ala Leu Glu Asp Leu Arg Gln
225 230 235 240

Gly Leu Leu Pro Val Leu Glu Ser Phe Lys Val Ser Phe Leu Ser Ala
245 250 255

Leu Glu Glu Tyr Thr Lys Lys Leu Asn Thr Gln
260 265

<210> 37
<211> 2647
<212> DNA
<213> Artificial

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<223> THER2PON1 DNA

<400> 37
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agccaggctc ggcatttctg gcagcaagat gaaccccccc agagcccctg ggatcgagtg 120

aaggacctgg ccactgtgta cgtggatgtg ctcaaagaca gcggcagaga ctatgtgtcc 180

cagtttgaag gctccgcctt gggaaaacag ctaaacctaa agctccttga caactgggac 240

agcgtgacct ccaccttcag caagctgcgc gaacagctcg gccctgtgac ccaggagttc 300

tgggataacc tggaaaagga gacagagggc ctgaggcagg agatgagcaa ggatctggag 360

gaggtgaagg ccaaggtgca gccctacctg gacgacttcc agaagaagtg gcaggaggag 420

atggagctct accgccagaa ggtggagccg ctgcgcgcag agctccaaga gggcgcgcgc 480

cagaagctgc acgagctgca agagaagctg agcccactgg gcgaggagat gcgcgaccgc 540

gcgcgcgccc atgtggacgc gctgcgcacg catctggccc cctacagcga cgagctgcgc 600

cagcgcttgg ccgcgcgcct tgaggctctc aaggagaacg gcggcgccag actggccgag 660

taccacgcca aggccaccga gcatctgagc acgctcagcg agaaggccaa gcccgcgctc 720

gaggacctcc gccaaggcct gctgcccgtg ctggagagct tcaaggtcag cttcctgagc 780

gctctcgagg agtacactaa gaagctcaac acccaagatc tctccggagg aggtggctca 840

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ccgtccccag cacctgaact cctgggagga tcgtcagtct tcctcttccc cccaaaaccc 960

aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtggtggt ggacgtgagc 1020

cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 1080

aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc 1140

gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc 1200

ctcccagcct ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag 1260

gtgtacaccc tgcccccatc ccgggatgag ctgaccaaga accaggtcag cctgacctgc 1320

ctggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 1380

gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt cttcctctac 1440

agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1500

atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctctctctc tccgggtaaa 1560

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gaacttccta actgtaattt agttaaagga atcgaaactg gctctgaaga cttggagata 1740

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aagtccacag tggagttgtt taaatttcaa gaagaagaaa aatcgctttt gcatctaaaa 2040

accatcagac ataaacttct gcctaatttg aatgatattg ttgctgtggg acctgagcac 2100

ttttatggca caaatgatca ctattttctt gacccctact tacaatcctg ggagatgtat 2160

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gctgagttgc tggctcataa gattcatgtg tatgaaaagc atgctaattg gactttaact 2340

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aatcctcctg catcagaggt gcttcgaatc cagaacattc taacagaaga acctaaagtg 2520

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tctagaa 2647

<210> 38
<211> 873
<212> PRT
<213> Artificial

<220>
<223> THER2PON1 polypeptide

<400> 38

Met Lys Ala Ala Val Leu Thr Leu Ala Val Leu Phe Leu Thr Gly Ser
1 5 10 15

Gln Ala Arg His Phe Trp Gln Gln Asp Glu Pro Pro Gln Ser Pro Trp
20 25 30

Asp Arg Val Lys Asp Leu Ala Thr Val Tyr Val Asp Val Leu Lys Asp
35 40 45

Ser Gly Arg Asp Tyr Val Ser Gln Phe Glu Gly Ser Ala Leu Gly Lys
50 55 60

Gln Leu Asn Leu Lys Leu Leu Asp Asn Trp Asp Ser Val Thr Ser Thr
65 70 75 80

Phe Ser Lys Leu Arg Glu Gln Leu Gly Pro Val Thr Gln Glu Phe Trp
85 90 95

Asp Asn Leu Glu Lys Glu Thr Glu Gly Leu Arg Gln Glu Met Ser Lys
100 105 110

```
Asp Leu Glu Glu Val Lys Ala Lys Val Gln Pro Tyr Leu Asp Asp Phe
        115                 120                 125

Gln Lys Lys Trp Gln Glu Glu Met Glu Leu Tyr Arg Gln Lys Val Glu
    130                 135                 140

Pro Leu Arg Ala Glu Leu Gln Glu Gly Ala Arg Gln Lys Leu His Glu
145                 150                 155                 160

Leu Gln Glu Lys Leu Ser Pro Leu Gly Glu Glu Met Arg Asp Arg Ala
                165                 170                 175

Arg Ala His Val Asp Ala Leu Arg Thr His Leu Ala Pro Tyr Ser Asp
            180                 185                 190

Glu Leu Arg Gln Arg Leu Ala Ala Arg Leu Glu Ala Leu Lys Glu Asn
        195                 200                 205

Gly Gly Ala Arg Leu Ala Glu Tyr His Ala Lys Ala Thr Glu His Leu
    210                 215                 220

Ser Thr Leu Ser Glu Lys Ala Lys Pro Ala Leu Glu Asp Leu Arg Gln
225                 230                 235                 240

Gly Leu Leu Pro Val Leu Glu Ser Phe Lys Val Ser Phe Leu Ser Ala
                245                 250                 255

Leu Glu Glu Tyr Thr Lys Lys Leu Asn Thr Gln Asp Leu Ser Gly Gly
            260                 265                 270

Gly Gly Ser Gly Gly Gly Gly Ser Thr Gly Leu Glu Pro Lys Ser Ser
        275                 280                 285

Asp Lys Thr His Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly
    290                 295                 300

Gly Ser Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
305                 310                 315                 320

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
                325                 330                 335

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
            340                 345                 350

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
        355                 360                 365
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Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
    370                 375                 380

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Ser Ile
385                 390                 395                 400

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
                405                 410                 415

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
            420                 425                 430

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
        435                 440                 445

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
    450                 455                 460

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
465                 470                 475                 480

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
                485                 490                 495

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
            500                 505                 510

Pro Gly Lys Val Asp Gly Ala Ser Ser Pro Val Asn Val Ser Ser Pro
        515                 520                 525

Ser Val Gln Asp Ile Leu Phe Arg Asn His Gln Ser Ser Tyr Gln Thr
    530                 535                 540

Arg Leu Asn Ala Leu Arg Glu Val Gln Pro Val Glu Leu Pro Asn Cys
545                 550                 555                 560

Asn Leu Val Lys Gly Ile Glu Thr Gly Ser Glu Asp Leu Glu Ile Leu
                565                 570                 575

Pro Asn Gly Leu Ala Phe Ile Ser Ser Gly Leu Lys Tyr Pro Gly Ile
            580                 585                 590

Lys Ser Phe Asn Pro Asn Ser Pro Gly Lys Ile Leu Leu Met Asp Leu
        595                 600                 605

Asn Glu Glu Asp Pro Thr Val Leu Glu Leu Gly Ile Thr Gly Ser Lys
    610                 615                 620
```

```
Phe Asp Val Ser Ser Phe Asn Pro His Gly Ile Ser Thr Phe Thr Asp
625                 630                 635                 640

Glu Asp Asn Ala Met Tyr Leu Leu Val Val Asn His Pro Asp Ala Lys
                645                 650                 655

Ser Thr Val Glu Leu Phe Lys Phe Gln Glu Glu Glu Lys Ser Leu Leu
            660                 665                 670

His Leu Lys Thr Ile Arg His Lys Leu Leu Pro Asn Leu Asn Asp Ile
        675                 680                 685

Val Ala Val Gly Pro Glu His Phe Tyr Gly Thr Asn Asp His Tyr Phe
    690                 695                 700

Leu Asp Pro Tyr Leu Gln Ser Trp Glu Met Tyr Leu Gly Leu Ala Trp
705                 710                 715                 720

Ser Tyr Val Val Tyr Tyr Ser Pro Ser Glu Val Arg Val Val Ala Glu
                725                 730                 735

Gly Phe Asp Phe Ala Asn Gly Ile Asn Ile Ser Pro Asp Gly Lys Tyr
            740                 745                 750

Val Tyr Ile Ala Glu Leu Leu Ala His Lys Ile His Val Tyr Glu Lys
        755                 760                 765

His Ala Asn Trp Thr Leu Thr Pro Leu Lys Ser Leu Asp Phe Asn Thr
    770                 775                 780

Leu Val Asp Asn Ile Ser Val Asp Pro Glu Thr Gly Asp Leu Trp Val
785                 790                 795                 800

Gly Cys His Pro Asn Gly Met Lys Ile Phe Phe Tyr Asp Ser Glu Asn
                805                 810                 815

Pro Pro Ala Ser Glu Val Leu Arg Ile Gln Asn Ile Leu Thr Glu Glu
            820                 825                 830

Pro Lys Val Thr Gln Val Tyr Ala Glu Asn Gly Thr Val Leu Gln Gly
        835                 840                 845

Ser Thr Val Ala Ser Val Tyr Lys Gly Lys Leu Leu Ile Gly Thr Val
    850                 855                 860

Phe His Lys Ala Leu Tyr Cys Glu Leu
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865 870

<210> 39
<211> 3084
<212> DNA
<213> Artificial

<220>
<223> THER4CETP DNA

<400> 39
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agccaggctc ggcatttctg gcagcaagat gaaccccccc agagcccctg ggatcgagtg 120

aaggacctgg ccactgtgta cgtggatgtg ctcaaagaca gcggcagaga ctatgtgtcc 180

cagtttgaag gctccgcctt gggaaaacag ctaaacctaa agctccttga caactgggac 240

agcgtgacct ccaccttcag caagctgcgc gaacagctcg gccctgtgac ccaggagttc 300

tgggataacc tggaaaagga gacagagggc ctgaggcagg agatgagcaa ggatctggag 360

gaggtgaagg ccaaggtgca gccctacctg gacgacttcc agaagaagtg gcaggaggag 420

atggagctct accgccagaa ggtggagccg ctgcgcgcag agctccaaga gggcgcgcgc 480

cagaagctgc acgagctgca agagaagctg agcccactgg gcgaggagat gcgcgaccgc 540

gcgcgcgccc atgtggacgc gctgcgcacg catctggccc cctacagcga cgagctgcgc 600

cagcgcttgg ccgcgcgcct tgaggctctc aaggagaacg gcggcgccag actggccgag 660

taccacgcca aggccaccga gcatctgagc acgctcagcg agaaggccaa gcccgcgctc 720

gaggacctcc gccaaggcct gctgcccgtg ctggagagct tcaaggtcag cttcctgagc 780

gctctcgagg agtacactaa gaagctcaac acccaagatc tctccggagg aggtggctca 840

ggtggtggag gatctggagg aggtgggagt ggtggaggtg gttctaccgg tctcgagccc 900

aaatcttctg acaaaactca cacatctcca ccgtccccag cacctgaact cctgggagga 960

tcgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggacccct 1020

gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 1080

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agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 1200

gagtacaagt gcaaggtctc caacaaagcc ctcccagcct ccatcgagaa aaccatctcc 1260

aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgcccccatc ccgggatgag 1320

ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 1380

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 1440

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cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 1560

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caggtggagc tggtggaagc caagtccatt gatgtctcca ttcagaacgt gtctgtggtc 1920

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tgccaaaaca agggagtcgt ggtcaattct tcagtgatgg tgaaattcct ctttccacgc 2700

ccagaccagc aacattctgt agcttacaca tttgaagagg atatcgtgac taccgtccag 2760

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actgtttcca acttgactga gagcagctcc gagtccgtcc agagcttcct gcagtcaatg 2880

atcaccgctg tgggcatccc tgaggtcatg tctcggctcg aggtagtgtt tacagccctc 2940

atgaacagca aaggcgtgag cctcttcgac atcatcaacc ctgagattat cactcgagat 3000

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<210> 40
<211> 1019
<212> PRT
<213> Artificial

<220>
<223> THER4CETP polypeptide

<400> 40

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Met Lys Ala Ala Val Leu Thr Leu Ala Val Leu Phe Leu Thr Gly Ser
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Gln Ala Arg His Phe Trp Gln Gln Asp Glu Pro Pro Gln Ser Pro Trp
            20                  25                  30

Asp Arg Val Lys Asp Leu Ala Thr Val Tyr Val Asp Val Leu Lys Asp
        35                  40                  45

Ser Gly Arg Asp Tyr Val Ser Gln Phe Glu Gly Ser Ala Leu Gly Lys
    50                  55                  60

Gln Leu Asn Leu Lys Leu Leu Asp Asn Trp Asp Ser Val Thr Ser Thr
65                  70                  75                  80

Phe Ser Lys Leu Arg Glu Gln Leu Gly Pro Val Thr Gln Glu Phe Trp
                85                  90                  95

Asp Asn Leu Glu Lys Glu Thr Glu Gly Leu Arg Gln Glu Met Ser Lys
            100                 105                 110

Asp Leu Glu Glu Val Lys Ala Lys Val Gln Pro Tyr Leu Asp Asp Phe
        115                 120                 125

Gln Lys Lys Trp Gln Glu Glu Met Glu Leu Tyr Arg Gln Lys Val Glu
    130                 135                 140

Pro Leu Arg Ala Glu Leu Gln Glu Gly Ala Arg Gln Lys Leu His Glu
145                 150                 155                 160

Leu Gln Glu Lys Leu Ser Pro Leu Gly Glu Glu Met Arg Asp Arg Ala
                165                 170                 175

Arg Ala His Val Asp Ala Leu Arg Thr His Leu Ala Pro Tyr Ser Asp
            180                 185                 190

Glu Leu Arg Gln Arg Leu Ala Ala Arg Leu Glu Ala Leu Lys Glu Asn
        195                 200                 205

Gly Gly Ala Arg Leu Ala Glu Tyr His Ala Lys Ala Thr Glu His Leu
    210                 215                 220

Ser Thr Leu Ser Glu Lys Ala Lys Pro Ala Leu Glu Asp Leu Arg Gln
225                 230                 235                 240

Gly Leu Leu Pro Val Leu Glu Ser Phe Lys Val Ser Phe Leu Ser Ala
                245                 250                 255
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Leu Glu Glu Tyr Thr Lys Lys Leu Asn Thr Gln Asp Leu Ser Gly Gly
            260                 265                 270

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
        275                 280                 285

Gly Ser Thr Gly Leu Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
    290                 295                 300

Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val Phe Leu
305                 310                 315                 320

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
                325                 330                 335

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
            340                 345                 350

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
        355                 360                 365

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
    370                 375                 380

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
385                 390                 395                 400

Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys
                405                 410                 415

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
            420                 425                 430

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
        435                 440                 445

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
    450                 455                 460

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
465                 470                 475                 480

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
                485                 490                 495

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
```

500 505 510

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Val Asp Gly
515 520 525

Ala Ser Ser Pro Val Asn Val Ser Ser Pro Ser Val Gln Asp Ile Cys
530 535 540

Ser Lys Gly Thr Ser His Glu Ala Gly Ile Val Cys Arg Ile Thr Lys
545 550 555 560

Pro Ala Leu Leu Val Leu Asn His Glu Thr Ala Lys Val Ile Gln Thr
565 570 575

Ala Phe Gln Arg Ala Ser Tyr Pro Asp Ile Thr Gly Glu Lys Ala Met
580 585 590

Met Leu Leu Gly Gln Val Lys Tyr Gly Leu His Asn Ile Gln Ile Ser
595 600 605

His Leu Ser Ile Ala Ser Ser Gln Val Glu Leu Val Glu Ala Lys Ser
610 615 620

Ile Asp Val Ser Ile Gln Asn Val Ser Val Val Phe Lys Gly Thr Leu
625 630 635 640

Lys Tyr Gly Tyr Thr Thr Ala Trp Trp Leu Gly Ile Asp Gln Ser Ile
645 650 655

Asp Phe Glu Ile Asp Ser Ala Ile Asp Leu Gln Ile Asn Thr Gln Leu
660 665 670

Thr Cys Asp Ser Gly Arg Val Arg Thr Asp Ala Pro Asp Cys Tyr Leu
675 680 685

Ser Phe His Lys Leu Leu Leu His Leu Gln Gly Glu Arg Glu Pro Gly
690 695 700

Trp Ile Lys Gln Leu Phe Thr Asn Phe Ile Ser Phe Thr Leu Lys Leu
705 710 715 720

Val Leu Lys Gly Gln Ile Cys Lys Glu Ile Asn Val Ile Ser Asn Ile
725 730 735

Met Ala Asp Phe Val Gln Thr Arg Ala Ala Ser Ile Leu Ser Asp Gly
740 745 750

```
Asp Ile Gly Val Asp Ile Ser Leu Thr Gly Asp Pro Val Ile Thr Ala
        755                 760                 765

Ser Tyr Leu Glu Ser His His Lys Gly His Phe Ile Tyr Lys Asn Val
    770                 775                 780

Ser Glu Asp Leu Pro Leu Pro Thr Phe Ser Pro Thr Leu Leu Gly Asp
785                 790                 795                 800

Ser Arg Met Leu Tyr Phe Trp Phe Ser Glu Arg Val Phe His Ser Leu
                805                 810                 815

Ala Lys Val Ala Phe Gln Asp Gly Arg Leu Met Leu Ser Leu Met Gly
            820                 825                 830

Asp Glu Phe Lys Ala Val Leu Glu Thr Trp Gly Phe Asn Thr Asn Gln
        835                 840                 845

Glu Ile Phe Gln Glu Val Val Gly Gly Phe Pro Ser Gln Ala Gln Val
    850                 855                 860

Thr Val His Cys Leu Lys Met Pro Lys Ile Ser Cys Gln Asn Lys Gly
865                 870                 875                 880

Val Val Val Asn Ser Ser Val Met Val Lys Phe Leu Phe Pro Arg Pro
                885                 890                 895

Asp Gln Gln His Ser Val Ala Tyr Thr Phe Glu Glu Asp Ile Val Thr
            900                 905                 910

Thr Val Gln Ala Ser Tyr Ser Lys Lys Lys Leu Phe Leu Ser Leu Leu
        915                 920                 925

Asp Phe Gln Ile Thr Pro Lys Thr Val Ser Asn Leu Thr Glu Ser Ser
    930                 935                 940

Ser Glu Ser Val Gln Ser Phe Leu Gln Ser Met Ile Thr Ala Val Gly
945                 950                 955                 960

Ile Pro Glu Val Met Ser Arg Leu Glu Val Val Phe Thr Ala Leu Met
                965                 970                 975

Asn Ser Lys Gly Val Ser Leu Phe Asp Ile Ile Asn Pro Glu Ile Ile
            980                 985                 990

Thr Arg Asp Gly Phe Leu Leu Leu  Gln Met Asp Phe Gly  Phe Pro Glu
        995                 1000                1005
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His Leu Leu Val Asp Phe Leu Gln Ser Leu Ser
1010 1015

<210> 41
<211> 1068
<212> DNA
<213> Artificial

<220>
<223> human PON1 Q192K DNA

<400> 41
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cagtcttctt accaaacacg acttaatgct ctccgagagg tacaacccgt agaacttcct 120

aactgtaatt tagttaaagg aatcgaaact ggctctgaag acttggagat actgcctaat 180

ggactggctt tcattagctc tggattaaag tatcctggaa taaagagctt caaccccaac 240

agtcctggaa aaatacttct gatggacctg aatgaagaag atccaacagt gttggaattg 300

gggatcactg gaagtaaatt tgatgtatct tcatttaacc ctcatgggat tagcacattc 360

acagatgaag ataatgccat gtacctcctg gtggtgaacc atccagatgc caagtccaca 420

gtggagttgt ttaaatttca agaagaagaa aaatcgcttt tgcatctaaa aaccatcaga 480

cataaacttc tgcctaattt gaatgatatt gttgctgtgg gacctgagca cttttatggc 540

acaaatgatc actattttct tgacccctac ttaaaatcct gggagatgta tttgggttta 600

gcgtggtcgt atgttgtcta ctatagtcca agtgaagttc gagtggtggc agaaggattt 660

gattttgcta atggaatcaa catttcaccc gatggcaagt atgtctatat agctgagttg 720

ctggctcata agattcatgt gtatgaaaag catgctaatt ggactttaac tccattgaag 780

tcccttgact ttaataccct cgtggataac atatctgtgg atcctgagac aggagacctt 840

tgggttggat gccatcccaa tggcatgaaa atcttcttct atgactcaga gaatcctcct 900

gcatcagagg tgcttcgaat ccagaacatt ctaacagaag aacctaaagt gacacaggtt 960

tatgcagaaa atggcacagt gttgcaaggc agtacagttg cctctgtgta caaagggaaa 1020

ctgctgattg gcacagtgtt tcacaaagct ctttactgtg agctctaa 1068

<210> 42
<211> 355
<212> PRT
<213> Artificial

<220>
<223> human PON1 Q192K polypeptide

<400> 42

Met Ala Lys Leu Ile Ala Leu Thr Leu Leu Gly Met Gly Leu Ala Leu
1 5 10 15

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Phe Arg Asn His Gln Ser Ser Tyr Gln Thr Arg Leu Asn Ala Leu Arg
            20                  25                  30

Glu Val Gln Pro Val Glu Leu Pro Asn Cys Asn Leu Val Lys Gly Ile
        35                  40                  45

Glu Thr Gly Ser Glu Asp Leu Glu Ile Leu Pro Asn Gly Leu Ala Phe
    50                  55                  60

Ile Ser Ser Gly Leu Lys Tyr Pro Gly Ile Lys Ser Phe Asn Pro Asn
65                  70                  75                  80

Ser Pro Gly Lys Ile Leu Leu Met Asp Leu Asn Glu Glu Asp Pro Thr
                85                  90                  95

Val Leu Glu Leu Gly Ile Thr Gly Ser Lys Phe Asp Val Ser Ser Phe
            100                 105                 110

Asn Pro His Gly Ile Ser Thr Phe Thr Asp Glu Asp Asn Ala Met Tyr
        115                 120                 125

Leu Leu Val Val Asn His Pro Asp Ala Lys Ser Thr Val Glu Leu Phe
    130                 135                 140

Lys Phe Gln Glu Glu Glu Lys Ser Leu Leu His Leu Lys Thr Ile Arg
145                 150                 155                 160

His Lys Leu Leu Pro Asn Leu Asn Asp Ile Val Ala Val Gly Pro Glu
                165                 170                 175

His Phe Tyr Gly Thr Asn Asp His Tyr Phe Leu Asp Pro Tyr Leu Lys
            180                 185                 190

Ser Trp Glu Met Tyr Leu Gly Leu Ala Trp Ser Tyr Val Val Tyr Tyr
        195                 200                 205

Ser Pro Ser Glu Val Arg Val Val Ala Glu Gly Phe Asp Phe Ala Asn
    210                 215                 220

Gly Ile Asn Ile Ser Pro Asp Gly Lys Tyr Val Tyr Ile Ala Glu Leu
225                 230                 235                 240

Leu Ala His Lys Ile His Val Tyr Glu Lys His Ala Asn Trp Thr Leu
                245                 250                 255

Thr Pro Leu Lys Ser Leu Asp Phe Asn Thr Leu Val Asp Asn Ile Ser
```

260 265 270

Val Asp Pro Glu Thr Gly Asp Leu Trp Val Gly Cys His Pro Asn Gly
275 280 285

Met Lys Ile Phe Phe Tyr Asp Ser Glu Asn Pro Pro Ala Ser Glu Val
290 295 300

Leu Arg Ile Gln Asn Ile Leu Thr Glu Glu Pro Lys Val Thr Gln Val
305 310 315 320

Tyr Ala Glu Asn Gly Thr Val Leu Gln Gly Ser Thr Val Ala Ser Val
325 330 335

Tyr Lys Gly Lys Leu Leu Ile Gly Thr Val Phe His Lys Ala Leu Tyr
340 345 350

Cys Glu Leu
355

<210> 43
<211> 1065
<212> DNA
<213> Homo sapiens

<400> 43
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cagtcttctt accaaacacg acttaatgct ctccgagagg tacaacccgt agaacttcct 120

aactgtaatt tagttaaagg aatcgaaact ggctctgaag acttggagat actgcctaat 180

ggactggctt tcattagctc tggattaaag tatcctggaa taaagagctt caaccccaac 240

agtcctggaa aaatacttct gatggacctg aatgaagaag atccaacagt gttggaattg 300

gggatcactg gaagtaaatt tgatgtatct tcatttaacc ctcatgggat tagcacattc 360

acagatgaag ataatgccat gtacctcctg gtggtgaacc atccagatgc caagtccaca 420

gtggagttgt ttaaatttca agaagaagaa aaatcgcttt tgcatctaaa aaccatcaga 480

cataaacttc tgcctaattt gaatgatatt gttgctgtgg gacctgagca cttttatggc 540

acaaatgatc actattttct tgacccctac ttaagatcct gggagatgta tttgggttta 600

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tcccttgact ttaataccct cgtggataac atatctgtgg atcctgagac aggagacctt 840

tgggttggat gccatcccaa tggcatgaaa atcttcttct atgactcaga gaatcctcct 900

gcatcagagg tgcttcgaat ccagaacatt ctaacagaag aacctaaagt gacacaggtt 960

tatgcagaaa atggcacagt gttgcaaggc agtacagttg cctctgtgta caaagggaaa 1020

ctgctgattg gcacagtgtt tcacaaagct ctttactgtg agctc 1065

<210> 44
<211> 355
<212> PRT
<213> Homo sapiens

<400> 44

Met Ala Lys Leu Ile Ala Leu Thr Leu Leu Gly Met Gly Leu Ala Leu
1 5 10 15

Phe Arg Asn His Gln Ser Ser Tyr Gln Thr Arg Leu Asn Ala Leu Arg
20 25 30

Glu Val Gln Pro Val Glu Leu Pro Asn Cys Asn Leu Val Lys Gly Ile
35 40 45

Glu Thr Gly Ser Glu Asp Leu Glu Ile Leu Pro Asn Gly Leu Ala Phe
50 55 60

Ile Ser Ser Gly Leu Lys Tyr Pro Gly Ile Lys Ser Phe Asn Pro Asn
65 70 75 80

Ser Pro Gly Lys Ile Leu Leu Met Asp Leu Asn Glu Glu Asp Pro Thr
85 90 95

Val Leu Glu Leu Gly Ile Thr Gly Ser Lys Phe Asp Val Ser Ser Phe
100 105 110

Asn Pro His Gly Ile Ser Thr Phe Thr Asp Glu Asp Asn Ala Met Tyr
115 120 125

Leu Leu Val Val Asn His Pro Asp Ala Lys Ser Thr Val Glu Leu Phe
130 135 140

Lys Phe Gln Glu Glu Glu Lys Ser Leu Leu His Leu Lys Thr Ile Arg
145 150 155 160

His Lys Leu Leu Pro Asn Leu Asn Asp Ile Val Ala Val Gly Pro Glu
165 170 175

His Phe Tyr Gly Thr Asn Asp His Tyr Phe Leu Asp Pro Tyr Leu Arg
180 185 190

Ser Trp Glu Met Tyr Leu Gly Leu Ala Trp Ser Tyr Val Val Tyr Tyr

195 200 205

Ser Pro Ser Glu Val Arg Val Val Ala Glu Gly Phe Asp Phe Ala Asn
210 215 220

Gly Ile Asn Ile Ser Pro Asp Gly Lys Tyr Val Tyr Ile Ala Glu Leu
225 230 235 240

Leu Ala His Lys Ile His Val Tyr Glu Lys His Ala Asn Trp Thr Leu
245 250 255

Thr Pro Leu Lys Ser Leu Asp Phe Asn Thr Leu Val Asp Asn Ile Ser
260 265 270

Val Asp Pro Glu Thr Gly Asp Leu Trp Val Gly Cys His Pro Asn Gly
275 280 285

Met Lys Ile Phe Phe Tyr Asp Ser Glu Asn Pro Pro Ala Ser Glu Val
290 295 300

Leu Arg Ile Gln Asn Ile Leu Thr Glu Glu Pro Lys Val Thr Gln Val
305 310 315 320

Tyr Ala Glu Asn Gly Thr Val Leu Gln Gly Ser Thr Val Ala Ser Val
325 330 335

Tyr Lys Gly Lys Leu Leu Ile Gly Thr Val Phe His Lys Ala Leu Tyr
340 345 350

Cys Glu Leu
355

<210> 45
<211> 2677
<212> DNA
<213> Artificial

<220>
<223> THER4PON1 Q192K DNA

<400> 45
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aaggacctgg ccactgtgta cgtggatgtg ctcaaagaca gcggcagaga ctatgtgtcc 180

cagtttgaag gctccgcctt gggaaaacag ctaaacctaa agctccttga caactgggac 240

agcgtgacct ccaccttcag caagctgcgc gaacagctcg gccctgtgac ccaggagttc 300

tgggataacc tggaaaagga gacagagggc ctgaggcagg agatgagcaa ggatctggag 360

gaggtgaagg ccaaggtgca gccctacctg gacgacttcc agaagaagtg gcaggaggag 420
atggagctct accgccagaa ggtggagccg ctgcgcgcag agctccaaga gggcgcgcgc 480
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gaggacctcc gccaaggcct gctgcccgtg ctggagagct tcaaggtcag cttcctgagc 780
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cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 1560
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gatgtatctt catttaaccc tcatgggatt agcacattca cagatgaaga taatgccatg 1980
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ggcatgaaaa tcttcttcta tgactcagag aatcctcctg catcagaggt gcttcgaatc 2520

cagaacattc taacagaaga acctaaagtg acacaggttt atgcagaaaa tggcacagtg 2580

ttgcaaggca gtacagttgc ctctgtgtac aaagggaaac tgctgattgg cacagtgttt 2640

cacaaagctc tttactgtga gctctaataa tctagaa 2677

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<211> 883
<212> PRT
<213> Artificial

<220>
<223> THER4PON1 Q192K polypeptide

<400> 46

Met Lys Ala Ala Val Leu Thr Leu Ala Val Leu Phe Leu Thr Gly Ser
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Gln Ala Arg His Phe Trp Gln Gln Asp Glu Pro Pro Gln Ser Pro Trp
20 25 30

Asp Arg Val Lys Asp Leu Ala Thr Val Tyr Val Asp Val Leu Lys Asp
35 40 45

Ser Gly Arg Asp Tyr Val Ser Gln Phe Glu Gly Ser Ala Leu Gly Lys
50 55 60

Gln Leu Asn Leu Lys Leu Leu Asp Asn Trp Asp Ser Val Thr Ser Thr
65 70 75 80

Phe Ser Lys Leu Arg Glu Gln Leu Gly Pro Val Thr Gln Glu Phe Trp
85 90 95

Asp Asn Leu Glu Lys Glu Thr Glu Gly Leu Arg Gln Glu Met Ser Lys
100 105 110

Asp Leu Glu Glu Val Lys Ala Lys Val Gln Pro Tyr Leu Asp Asp Phe
115 120 125

Gln Lys Lys Trp Gln Glu Glu Met Glu Leu Tyr Arg Gln Lys Val Glu
130 135 140

```
Pro Leu Arg Ala Glu Leu Gln Glu Gly Ala Arg Gln Lys Leu His Glu
145                 150                 155                 160

Leu Gln Glu Lys Leu Ser Pro Leu Gly Glu Glu Met Arg Asp Arg Ala
                165                 170                 175

Arg Ala His Val Asp Ala Leu Arg Thr His Leu Ala Pro Tyr Ser Asp
            180                 185                 190

Glu Leu Arg Gln Arg Leu Ala Ala Arg Leu Glu Ala Leu Lys Glu Asn
        195                 200                 205

Gly Gly Ala Arg Leu Ala Glu Tyr His Ala Lys Ala Thr Glu His Leu
    210                 215                 220

Ser Thr Leu Ser Glu Lys Ala Lys Pro Ala Leu Glu Asp Leu Arg Gln
225                 230                 235                 240

Gly Leu Leu Pro Val Leu Glu Ser Phe Lys Val Ser Phe Leu Ser Ala
                245                 250                 255

Leu Glu Glu Tyr Thr Lys Lys Leu Asn Thr Gln Asp Leu Ser Gly Gly
            260                 265                 270

Gly Gly Ser Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
        275                 280                 285

Gly Ser Thr Gly Leu Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
    290                 295                 300

Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val Phe Leu
305                 310                 315                 320

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
                325                 330                 335

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
            340                 345                 350

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
        355                 360                 365

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
    370                 375                 380

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
385                 390                 395                 400
```

```
Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys
                405                 410                 415

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
            420                 425                 430

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
        435                 440                 445

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
    450                 455                 460

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
465                 470                 475                 480

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
                485                 490                 495

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
            500                 505                 510

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Val Asp Gly
        515                 520                 525

Ala Ser Ser Pro Val Asn Val Ser Ser Pro Ser Val Gln Asp Ile Leu
    530                 535                 540

Phe Arg Asn His Gln Ser Ser Tyr Gln Thr Arg Leu Asn Ala Leu Arg
545                 550                 555                 560

Glu Val Gln Pro Val Glu Leu Pro Asn Cys Asn Leu Val Lys Gly Ile
                565                 570                 575

Glu Thr Gly Ser Glu Asp Leu Glu Ile Leu Pro Asn Gly Leu Ala Phe
            580                 585                 590

Ile Ser Ser Gly Leu Lys Tyr Pro Gly Ile Lys Ser Phe Asn Pro Asn
        595                 600                 605

Ser Pro Gly Lys Ile Leu Leu Met Asp Leu Asn Glu Glu Asp Pro Thr
    610                 615                 620

Val Leu Glu Leu Gly Ile Thr Gly Ser Lys Phe Asp Val Ser Ser Phe
625                 630                 635                 640

Asn Pro His Gly Ile Ser Thr Phe Thr Asp Glu Asp Asn Ala Met Tyr
                645                 650                 655
```

```
Leu Leu Val Val Asn His Pro Asp Ala Lys Ser Thr Val Glu Leu Phe
            660                 665                 670

Lys Phe Gln Glu Glu Glu Lys Ser Leu Leu His Leu Lys Thr Ile Arg
        675                 680                 685

His Lys Leu Leu Pro Asn Leu Asn Asp Ile Val Ala Val Gly Pro Glu
    690                 695                 700

His Phe Tyr Gly Thr Asn Asp His Tyr Phe Leu Asp Pro Tyr Leu Lys
705                 710                 715                 720

Ser Trp Glu Met Tyr Leu Gly Leu Ala Trp Ser Tyr Val Val Tyr Tyr
                725                 730                 735

Ser Pro Ser Glu Val Arg Val Val Ala Glu Gly Phe Asp Phe Ala Asn
            740                 745                 750

Gly Ile Asn Ile Ser Pro Asp Gly Lys Tyr Val Tyr Ile Ala Glu Leu
        755                 760                 765

Leu Ala His Lys Ile His Val Tyr Glu Lys His Ala Asn Trp Thr Leu
    770                 775                 780

Thr Pro Leu Lys Ser Leu Asp Phe Asn Thr Leu Val Asp Asn Ile Ser
785                 790                 795                 800

Val Asp Pro Glu Thr Gly Asp Leu Trp Val Gly Cys His Pro Asn Gly
                805                 810                 815

Met Lys Ile Phe Phe Tyr Asp Ser Glu Asn Pro Pro Ala Ser Glu Val
            820                 825                 830

Leu Arg Ile Gln Asn Ile Leu Thr Glu Glu Pro Lys Val Thr Gln Val
        835                 840                 845

Tyr Ala Glu Asn Gly Thr Val Leu Gln Gly Ser Thr Val Ala Ser Val
    850                 855                 860

Tyr Lys Gly Lys Leu Leu Ile Gly Thr Val Phe His Lys Ala Leu Tyr
865                 870                 875                 880

Cys Glu Leu
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<210> 47

<211> 2677
<212> DNA
<213> Artificial

<220>
<223> THER4PON1 Q192R DNA

<400> 47
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aaggacctgg ccactgtgta cgtggatgtg ctcaaagaca gcggcagaga ctatgtgtcc 180

cagtttgaag gctccgcctt gggaaaacag ctaaacctaa agctccttga caactgggac 240

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tgggataacc tggaaaagga gacagagggc ctgaggcagg agatgagcaa ggatctggag 360

gaggtgaagg ccaaggtgca gccctacctg gacgacttcc agaagaagtg gcaggaggag 420

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gcgcgcgccc atgtggacgc gctgcgcacg catctggccc cctacagcga cgagctgcgc 600

cagcgcttgg ccgcgcgcct tgaggctctc aaggagaacg gcggcgccag actggccgag 660

taccacgcca aggccaccga gcatctgagc acgctcagcg agaaggccaa gcccgcgctc 720

gaggacctcc gccaaggcct gctgcccgtg ctggagagct tcaaggtcag cttcctgagc 780

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ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 1380

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ggattaaagt atcctggaat aaagagcttc aaccccaaca gtcctggaaa aatacttctg 1860

atggacctga atgaagaaga tccaacagtg ttggaattgg ggatcactgg aagtaaattt 1920

gatgtatctt catttaaccc tcatgggatt agcacattca cagatgaaga taatgccatg 1980

tacctcctgg tggtgaacca tccagatgcc aagtccacag tggagttgtt taaatttcaa 2040

gaagaagaaa aatcgctttt gcatctaaaa accatcagac ataaacttct gcctaatttg 2100

aatgatattg ttgctgtggg acctgagcac ttttatggca caaatgatca ctattttctt 2160

gacccctact taagatcctg ggagatgtat ttgggtttag cgtggtcgta tgttgtctac 2220

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cacaaagctc tttactgtga gctctaataa tctagaa 2677

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<211> 883
<212> PRT
<213> Artificial

<220>
<223> THER4PON1 Q192R polypeptide

<400> 48

Met Lys Ala Ala Val Leu Thr Leu Ala Val Leu Phe Leu Thr Gly Ser
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Gln Ala Arg His Phe Trp Gln Gln Asp Glu Pro Pro Gln Ser Pro Trp
20 25 30

Asp Arg Val Lys Asp Leu Ala Thr Val Tyr Val Asp Val Leu Lys Asp
35 40 45

Ser Gly Arg Asp Tyr Val Ser Gln Phe Glu Gly Ser Ala Leu Gly Lys
50 55 60

Gln Leu Asn Leu Lys Leu Leu Asp Asn Trp Asp Ser Val Thr Ser Thr

65 70 75 80

Phe Ser Lys Leu Arg Glu Gln Leu Gly Pro Val Thr Gln Glu Phe Trp
85 90 95

Asp Asn Leu Glu Lys Glu Thr Glu Gly Leu Arg Gln Glu Met Ser Lys
100 105 110

Asp Leu Glu Glu Val Lys Ala Lys Val Gln Pro Tyr Leu Asp Asp Phe
115 120 125

Gln Lys Lys Trp Gln Glu Glu Met Glu Leu Tyr Arg Gln Lys Val Glu
130 135 140

Pro Leu Arg Ala Glu Leu Gln Glu Gly Ala Arg Gln Lys Leu His Glu
145 150 155 160

Leu Gln Glu Lys Leu Ser Pro Leu Gly Glu Glu Met Arg Asp Arg Ala
165 170 175

Arg Ala His Val Asp Ala Leu Arg Thr His Leu Ala Pro Tyr Ser Asp
180 185 190

Glu Leu Arg Gln Arg Leu Ala Ala Arg Leu Glu Ala Leu Lys Glu Asn
195 200 205

Gly Gly Ala Arg Leu Ala Glu Tyr His Ala Lys Ala Thr Glu His Leu
210 215 220

Ser Thr Leu Ser Glu Lys Ala Lys Pro Ala Leu Glu Asp Leu Arg Gln
225 230 235 240

Gly Leu Leu Pro Val Leu Glu Ser Phe Lys Val Ser Phe Leu Ser Ala
245 250 255

Leu Glu Glu Tyr Thr Lys Lys Leu Asn Thr Gln Asp Leu Ser Gly Gly
260 265 270

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
275 280 285

Gly Ser Thr Gly Leu Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
290 295 300

Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val Phe Leu
305 310 315 320

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Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
                325                 330                 335

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
            340                 345                 350

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
        355                 360                 365

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
    370                 375                 380

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
385                 390                 395                 400

Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys
                405                 410                 415

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
            420                 425                 430

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
        435                 440                 445

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
    450                 455                 460

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
465                 470                 475                 480

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
                485                 490                 495

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
            500                 505                 510

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Val Asp Gly
        515                 520                 525

Ala Ser Ser Pro Val Asn Val Ser Ser Pro Ser Val Gln Asp Ile Leu
    530                 535                 540

Phe Arg Asn His Gln Ser Ser Tyr Gln Thr Arg Leu Asn Ala Leu Arg
545                 550                 555                 560

Glu Val Gln Pro Val Glu Leu Pro Asn Cys Asn Leu Val Lys Gly Ile
                565                 570                 575
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Glu Thr Gly Ser Glu Asp Leu Glu Ile Leu Pro Asn Gly Leu Ala Phe
            580                 585                 590

Ile Ser Ser Gly Leu Lys Tyr Pro Gly Ile Lys Ser Phe Asn Pro Asn
        595                 600                 605

Ser Pro Gly Lys Ile Leu Leu Met Asp Leu Asn Glu Glu Asp Pro Thr
    610                 615                 620

Val Leu Glu Leu Gly Ile Thr Gly Ser Lys Phe Asp Val Ser Ser Phe
625                 630                 635                 640

Asn Pro His Gly Ile Ser Thr Phe Thr Asp Glu Asp Asn Ala Met Tyr
                645                 650                 655

Leu Leu Val Val Asn His Pro Asp Ala Lys Ser Thr Val Glu Leu Phe
            660                 665                 670

Lys Phe Gln Glu Glu Glu Lys Ser Leu Leu His Leu Lys Thr Ile Arg
        675                 680                 685

His Lys Leu Leu Pro Asn Leu Asn Asp Ile Val Ala Val Gly Pro Glu
    690                 695                 700

His Phe Tyr Gly Thr Asn Asp His Tyr Phe Leu Asp Pro Tyr Leu Arg
705                 710                 715                 720

Ser Trp Glu Met Tyr Leu Gly Leu Ala Trp Ser Tyr Val Val Tyr Tyr
                725                 730                 735

Ser Pro Ser Glu Val Arg Val Val Ala Glu Gly Phe Asp Phe Ala Asn
            740                 745                 750

Gly Ile Asn Ile Ser Pro Asp Gly Lys Tyr Val Tyr Ile Ala Glu Leu
        755                 760                 765

Leu Ala His Lys Ile His Val Tyr Glu Lys His Ala Asn Trp Thr Leu
    770                 775                 780

Thr Pro Leu Lys Ser Leu Asp Phe Asn Thr Leu Val Asp Asn Ile Ser
785                 790                 795                 800

Val Asp Pro Glu Thr Gly Asp Leu Trp Val Gly Cys His Pro Asn Gly
                805                 810                 815

Met Lys Ile Phe Phe Tyr Asp Ser Glu Asn Pro Pro Ala Ser Glu Val
            820                 825                 830
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Leu Arg Ile Gln Asn Ile Leu Thr Glu Glu Pro Lys Val Thr Gln Val
835 840 845

Tyr Ala Glu Asn Gly Thr Val Leu Gln Gly Ser Thr Val Ala Ser Val
850 855 860

Tyr Lys Gly Lys Leu Leu Ile Gly Thr Val Phe His Lys Ala Leu Tyr
865 870 875 880

Cys Glu Leu

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<211> 82
<212> DNA
<213> Artificial

<220>
<223> (gly4ser)4 linker DNA

<400> 49
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aggtggttct accggtctcg ag 82

<210> 50
<211> 26
<212> PRT
<213> Artificial

<220>
<223> (gly4ser)4 linker

<400> 50

Asp Leu Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
1 5 10 15

Gly Ser Gly Gly Gly Gly Ser Thr Gly Leu
20 25

<210> 51
<211> 112
<212> DNA
<213> Artificial

<220>
<223> (gly4ser)6 linker DNA

<400> 51
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aggtggttct ggaggaggtg gtagtggagg tggaggttct accggtctcg ag 112

<210> 52
<211> 36
<212> PRT
<213> Artificial

<220>
<223> (gly4ser)6 linker

<400> 52

Asp Leu Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
1 5 10 15

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
20 25 30

Ser Thr Gly Leu
35

<210> 53
<211> 93
<212> DNA
<213> Artificial

<220>
<223> (gly4ser)5 linker DNA

<400> 53
gatctctccg gaggaggtgg ctcaggtggt ggaggatctg gaggaggtgg gagtggtgga 60

ggtggttctg gaggaggtgg tagtaccggt ctc 93

<210> 54
<211> 31
<212> PRT
<213> Artificial

<220>
<223> (gly4ser)5 linker

<400> 54

Asp Leu Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
1 5 10 15

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Thr Gly Leu
20 25 30