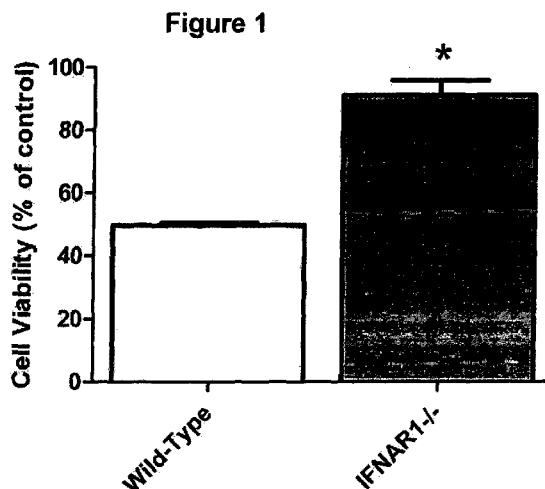




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(54) **Title:** METHODS AND COMPOSITIONS COMPRISING ANTAGONISTS OF TYPE 1 INTERFERON-MEDIATED SIGNALLING FOR REDUCING A NEUROINFLAMMATORY RESPONSE IN THE CENTRAL NERVOUS SYSTEM



(57) **Abstract:** The present disclosure relates generally to the prophylaxis and treatment of neuropathological inflammatory response in a subject. The present disclosure teaches the prophylaxis or treatment of a neuroinflammatory response following a neuropathological event or condition within the central nervous system pharmaceutical formulations comprising antagonists of Type 1 interferon-mediated signalling or antagonists of interferon alpha receptor 1 mediated signalling. Agents, medicaments and pharmaceutical compositions useful in the prophylaxis and treatment of neuropathological conditions and disorders are also enabled herein as are diagnostic assays and medical protocols.

**METHODS AND COMPOSITIONS COMPRISING ANTAGONISTS OF TYPE 1
INTERFERON-MEDIATED SIGNALLING FOR REDUCING A NEUROINFLAMMATORY
RESPONSE IN THE CENTRAL NERVOUS SYSTEM**

FILING DATA

- 5 [0001] This application is associated with Australian Patent Application No. 2010905239 filed 26 November 2010 and Australian Patent Application No. 2011902499, filed 24 June 2011, the entire contents of both of which, are incorporated herein by reference.

FIELD

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- [0002] The present disclosure relates generally to the prophylaxis and treatment of a neuropathological inflammatory response in a subject. The present disclosure teaches the prophylaxis or treatment of a neuroinflammatory response following a neuropathological event or condition within the central nervous system. Agents, medicaments and
15 pharmaceutical compositions useful in the prophylaxis and treatment of neuropathological conditions and disorders are also enabled herein as are diagnostic assays.

BACKGROUND

- 20 [0003] Bibliographic details of the publications referred to by author in this specification are collected alphabetically at the end of the description.

- [0004] Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common
25 general knowledge in any country.

- [0005] The immune system and the central nervous system (CNS) have long been considered separate systems with limited interaction. However, recent findings have shown support for a potential relationship between the two systems. This occurrence has been
30 most evident in situations of neural injury, specifically ischemia-reperfusion pathologies (Zierath *et al.*, *Neurocrit Care* 12(2):274-284, 2010). The widely accepted view was that the CNS is immune-privileged as the blood brain barrier (BBB) prevents communication

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with peripheral immune cells (such as macrophages and lymphocytes). This view is now being re-shaped with findings of CNS and immune system communication in neural injury without the disruption of the BBB (Kaur and Ling, *Curr Neurovasc Res* 5(1):71-81, 2008). The mechanism of this CNS neuroinflammation is unknown, thus posing a new approach
5 to investigate neuropathologies demonstrating distinct neuroinflammation.

[0006] Inflammation is a complex biological response initiated to remove a foreign pathogen from a given host. It is characterized by initial pathogen recognition, subsequent tissue oedema and multiple infiltrating cell types. Most notably, macrophages are recruited
10 to ingest and remove foreign matter. The pathogens responsible can be venoms, parasites, allergens and DNA/RNA fragments released from necrotic cells. Initially, this immune response is protective, removing the pathogen, but an exaggerated response can promote detrimental overcompensation. This results from excess differentiation resulting in hematopoietic cell infiltration and release of chemokines and cytokines. These cells adhere
15 to endothelium through intracellular adhesion molecule-1 (ICAM-1) and infiltrate the inflamed tissue (Caprio *et al.*, *Circ Res* 102(11):1359-1367, 2008). Although this is considered a peripheral event, this is also relevant to many neuropathologies including Alzheimer's disease (Akiyama *et al.*, *Neurobiol Aging* 21(3):383-421, 2000; Rogers *et al.*, *Glia* 40(2):260-269, 2002) which exhibit neuroinflammation within the CNS.

20

[0007] CNS ischemia-reperfusion injuries cause cell death and manifest as local tissue inflammation. Such conditions can arise following traumatic brain injury (TBI). TBI is the leading cause of death and disability in children and young adults. In the United States, approximately 50,000 individuals die of TBI per year (Summers *et al.*, *Mt Sinai J Med*
25 76:105-110, 2009). To date, there is no effective therapy to treat TBI. Head injuries have debilitating effects on sufferers, such as the development of motor and cognitive impairments and the development of other neurological disorders, including epilepsies, depression and dementias.

30 [0008] Brain injuries are highly heterogeneous; the type and extent of damage depends on each individual injury. Every injury, however, comprises a common primary and secondary component (Bramlett *et al.*, *J Cereb Blood Flow Metab* 24:133-150, 2004).

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Primary injury occurs immediately after the impact. The severity of this injury depends on the force of the impact. Secondary injury is a result of the complex morphological and biochemical changes in the brain following the impact, and can last from days to weeks.

- 5 [0009] Following a TBI, cerebral arteries often become damaged, which prevent perfusion to an area of the brain (ischemia). Lack of perfusion causes neuronal hypoxia and starvation. Some of the outcomes following primary injury include: excessive depolarisation, excitotoxicity, oxidative stress, DNA and mitochondrial damage, oedema, inflammation, disruption of BBB integrity, and consequently infiltration of peripheral
10 immune cells. These effects culminate in neuronal necrosis and apoptosis, and create a larger infarct size than caused by the primary injury alone.

- [0010] CNS ischemia-reperfusion injuries show characteristic macrophage and leukocyte recruitment resulting in production of various cytokines; however, the response is
15 primarily initiated by activated resident microglia (Banati *et al.*, *Glia* 7(1):111-118, 1993; Giulian and Vaca., *Stroke* 24(12 Suppl):184-190, 1993). Amongst others, mRNA and chaperone proteins released from necrotic cells, act as ligands for Toll-like receptors (TLRs) [Kariko *et al.*, *J Biol Chem* 279(13):12542-12550, 2004; Ohashi *et al.*, *J Immunol* 164(2):558-561, 2000]. Activated microglia secrete pro-inflammatory cytokines including
20 TNF α and interleukins (Block *et al.*, *Neuroreport* 11(5):963-967, 2000; Lambertsen *et al.*, *J Cereb Blood Flow Metab* 25(1):119-145, 2005) alongside chemo-attractants (Shohami *et al.*, *J Neurochem* 53:1541-1546, 1989). The CCL2/3 chemokines lead to the recruitment of macrophages (Cowell *et al.*, *Stroke* 33(3):795-801, 2002), further exacerbating the inflammatory response to the initial ischemia. Upon macrophage infiltration, a
25 characteriztic cytokine 'storm' develops, which contributes to neurodegeneration.

- [0011] The inflammatory component of a brain injury contributes to widespread cellular damage in the brain (Greve *et al.*, *Mt Sinai J Med* 76:97-104, 2009). Studies of gene expression changes between control and traumatized brain samples revealed that genes
30 playing a role in inflammation were up-regulated at an early stage after head injury (Crack *et al.*, *J Neural Transm* 116:1-12, 2009). The up-regulation of these genes suggests they play a major role in the secondary injury processes after ischemia-reperfusion injury.

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[0012] The Type 1 interferons (IFN α , IFN β and IFN ω) play a role in the production of pro-inflammatory cytokines and chemokines (Schindler *et al.*, *J Biol Chem* 282:20059-20063, 2007). Studies have shown an up-regulation in Type 1 interferon gene expression
5 as a result of microglial activation (Field *et al.*, *Brain Behav Immun* 24:996-1007, 2010). Interferons signal through interferon receptors, located widely on cells, including microglia and other immune cells (de Weerd *et al.*, *J Biol Chem* 282:20053-20057, 2007).

[0013] The Type 1 IFN receptor, the IFN α receptor (IFNAR), is composed of IFNAR1
10 and IFNAR2 subunits.

[0014] Upon binding of Type 1 IFN proteins, the IFNAR1 and IFNAR2 subunits dimerize, activating the receptor-bound kinases JAK1 and Tyk2 (Schindler *et al.*, 2007 *supra*). Activation of these proteins recruits Signal Transducers and Activators of Transcription
15 (STAT) proteins; and consequently, STATs are phosphorylated and activated by JAK1 and Tyk2. STAT1 and STAT2 dimerize and form a complex with an intracellular protein, Interferon Regulatory Factor 9 (IRF9) [Leung *et al.*, *Mol Cell Biol* 15:1312-1317, 1995]. This complex translocates to the nucleus, acting as a transcription factor, and up-regulates the expression of anti-viral and anti-proliferative proteins, including pro-inflammatory
20 cytokines (Lloyd *et al.*, *J Neuroinflammation* 5:28, 2008; Wei *et al.*, *J Neuroinflammation* 6:19, 2009).

[0015] The effects of certain treatments in reducing the neuroinflammatory cascade have been studied. For instance, one study investigated the effects of a small molecule inhibitor,
25 Minoxac, on the induction of pro-inflammatory cytokines after TBI (Lloyd *et al.*, 2008 *supra*). The exact mechanism of action of Minoxac is not known, but it is believed to reduce the overproduction of pro-inflammatory cytokines in microglia post-injury, which was seen in this study. In addition to this, in an animal model of epilepsy, administration of Minoxac prevented the chronic activation of microglia, which was linked to
30 neurodegeneration in the long term (Somera-Molina *et al.*, *Epilepsia* 48:1785-1800, 2007).

[0016] Another study looked at the synthetic tripeptide analog (Glypromate) [NNZ-2566]

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which was found to attenuate the increased expression in pro-inflammatory cytokine levels and attenuate neuropathological events following injury in a rat model of TBI (Wei *et al.*, 2009 *supra*). Glypromate is derived from Insulin-like Growth Factor (IGF-1) and is believed to induce repair of injured brain tissue. In this study, NNZ-2566 particularly
5 suppressed expression of IL-1 β (which is thought to play a significant role in secondary neuronal apoptosis), thus conferring a level of neuroprotection to the tissue.

[0017] These studies illustrate the relevance of blocking certain aspects of the inflammatory response after head injury. However, whilst studies of drug therapies which
10 targeted inflammation in TBI animal models have shown some benefit, when these therapies were put through clinical trials, they were not successful (Menon, *Crit Care Med* 37:5129-5135, 2009). This illustrates the complexity of this pathology.

[0018] Another complex pathology is Alzheimer's disease (AD) which is a particularly
15 devastating and progressive neurodegenerative disease characterized by widespread neuronal cell death and the loss of cognitive function culminating in dementia. It is a multifactorial disorder in which protein alteration, neuroimmune response, inflammation and altered neuron-glia signalling may all have roles in the progressive neurodegeneration. AD displays two pathological hallmarks, neurofibrillary tangles (NFTs) consisting of
20 hyperphosphorylated Tau, and extracellular senile plaques consisting of mostly aggregated 1-42 β -Amyloid (A β), however, the events leading to their generation remain unknown. A β 1-42 is generated from the improper cleavage of Amyloid Precursor Protein (APP) by Presenilin 1, resulting in improper folding of the peptide. The exact function of APP is unknown. Insoluble extracellular A β -containing senile plaques are largely
25 considered a long term accumulated by-product of APP cleavage and cannot be considered unique to AD as they are also found in healthy individuals. However, given oligomers of Ab impair synaptic plasticity (Selkoe, *Journal of Neuroimmune Pharmacology*:1-13, 2008), the intracellular domain of APP can induce neuroinflammation (Ghosal *et al*, *APP Intracellular Domain Impairs Adult Neurogenesis in Transgenic Mice by Inducing Neuroinflammation. PloS one* 5, e11866, 2010), and non-disease related protein oligomers
30 can induce tau hyperphosphorylation and neurodegeneration (Vieira *et al*, *Journal of neurochemistry* 103:736-748, 2007), it is likely that soluble oligomers of 1-42 A β play a

role in AD pathogenesis.

[0019] Two recognition sensors for β -Amyloid are currently known, the TLR4 receptor complex (Jin *et al.*, *J Neuroinflammation* 5:23, 2008, Walter *et al.*, *Cell Physiol Biochem* 20:947-956, 2007) and the NALP3 (NACHT, leucine-rich repeat and PYD containing 3) inflammasome (Halle *et al.*, *Nature immunology* 9:857-865, 2008). Following activation, NALP3 requires association of an apoptosis-associated speck-like protein containing a CARD (ASC) to activate caspase-1 for pro-IL-1 β cleavage and release of IL-1 β to surrounding cells.

10

[0020] Parkinson's disease (PD) is a chronic neurodegenerative motor function disorder, clinically characterized by resting tremor, rigidity, bradykinesia and aberrant control of movement (Jankovic and Stacy, *CNS drugs* 21(8):677-692, 2007). To date, no effective therapies have been developed to cure PD; however, therapeutic treatments focusing on the relief and management of symptoms are available. These include the administration of L-Dopa (Levodopa) in conjunction with Carbidopa, a monoamine oxidase inhibitor, in an attempt to increase dopamine levels in the brain, providing symptomatic relief. Unfortunately, these treatments do not alter the neurodegenerative cell loss seen in PD and therefore do not halt the progression of the disease.

20

[0021] The debilitating symptoms associated with PD have been primarily attributed to the loss of dopaminergic neurons in the substantia nigra pars compacta region of the midbrain (Mount *et al.*, *The Journal of Neuroscience* 27(12):3328, 2007). This neuronal loss results in a diminished capacity for monoamine release down striatal nerve terminals, leading to the development of abnormal motor control symptoms.

25

[0022] The etiology of the specific dopaminergic neurodegeneration remains unclear and not fully elucidated. Advances have been made in identifying causal factors that may be implicated in the initiation and progression of the disease. Evidence supports the involvement of oxidative stress, mitochondrial dysfunction, protein alteration and proapoptotic mechanisms, as all contributing in the neuronal degeneration in PD (Hirsch *et al.*, *Parkinsonism and related disorders* 11 (Suppl 1):S9, 2005).

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[0023] It is proposed that these identified factors may be directly involved in both the genetically linked (10% of cases), and the sporadic (90%) onset forms of PD. Gene mutations in DJ-1, PINK, PARK2 (parkin) and α -synuclein, all implicated in the ubiquitin-proteasome system (UPS), have been associated with autosomal dominant or autosomal recessive forms of PD. These genetic mutations can lead to protein alterations and their misfolding with a subsequent inability to be degraded via the UPS, leading to neuronal cell death (Bonifati, *Parkinsonism and related disorders* 13:S233, 2007).

[0024] While only a subset of PD cases can be attributed to genetic mutations, studies of these proteins have provided insight into pathways that may also be perturbed in sporadic forms of the disease. Furthermore, evidence has suggested that environmental toxins such as pesticides can act as a trigger, mediating their actions through the similar pathways, increasing the incidence of sporadic onset of PD.

[0025] There is, therefore, a need to design a therapy which can effectively attenuate secondary neuronal damage following a neurological event or condition such as AD, PD and other conditions within the CNS leading to a neuroinflammatory response.

SUMMARY

[0026] Certain neurological events and conditions lead to inflammation and to neuronal necrosis and apoptosis within the central nervous system (CNS). Such an inflammatory response is referred to herein as "neuroinflammation" which has pathological consequences. The present disclosure teaches a method for the treatment or prophylaxis of a neuropathological inflammatory response in the CNS and in particular the brain. The method enabled herein is predicated in part on targeting including antagonizing Type 1 interferon (IFN)-mediated signalling. The reduction or attenuation of a neuroinflammatory response reduces secondary neuronal damage and, in particular circumstances, reduces infarct size.

[0027] Accordingly, an aspect enabled herein is a method for reducing a neuroinflammatory response within the central nervous system (CNS) of a subject following a neurological event or condition, the method comprising administering to the subject an effective amount of an antagonist of Type 1 interferon (IFN)-mediated signalling, for a time and under conditions sufficient to prevent or attenuate neuroinflammation.

[0028] In an embodiment, the neuroinflammation is cerebral neuroinflammation resulting from acute neuronal injury such as traumatic brain injury (TBI) and trauma-induced paralysis, a neurodegenerative disease, drug or alcohol abuse, radiation or chemotherapy and/or starvation and/or any event or condition which leads to or has the potential to lead to ischemia-reperfusion injury in the brain. In another embodiment, the ischemia-reperfusion injury occurs following spinal cord trauma or disease. In another embodiment, the injury is a stroke. In another embodiment, the injury is caused by an infection by a pathogenic agent.

[0029] Examples of neurodegenerative conditions which can lead to ischemia-reperfusion injury and which can lead to neuroinflammation include Alzheimer's disease (AD), Parkinson's disease (PD) and other Parkinsonian conditions and syndromes, amyotrophic lateral sclerosis, Friedreich's ataxia, Huntington's disease, Lewy body disease, spinal

muscular atrophy, motor neuron disease, Alper's disease, Batten disease, Cerebro-oculo-facio-skeletal syndrome, corticobasal degeneration, Leigh's disease, Machads-Joseph disease, monomelic amyotrophy, multiple system atrophy, multiple sclerosis, neurodegeneration with brain iron accumulation, olivopontocerebellar atrophy, opsoclonus
5 myoclonus, paraneoplastic syndromes, prion diseases, progressive multifocal leukoencephalopathy and Aicardi-Coutieres syndrome as well as a range of demyelination diseases, oligodendrocyte cytotoxicity and spinal cord trauma. Other conditions include stroke and infection by a pathogenic agent.

10 [0030] In an embodiment, the neurological event or condition is a traumatic brain injury (TBI). Hence, another aspect enabled herein is a method for reducing a neuroinflammatory response within the central nervous system (CNS) of a subject following a neurological event or condition, the method comprising administering to the subject an effective amount of an antagonist of Type 1 interferon (IFN)-mediated
15 signalling, for a time and under conditions sufficient to prevent or attenuate neuroinflammation, wherein the neurological event or condition is TBI.

[0031] In another embodiment, the neurological event or condition is associated with the deposition of pathological forms of amyloid protein. Accordingly, taught herein is a
20 method for reducing a neuroinflammatory response within the central nervous system (CNS) of a subject following a neurological event or condition, the method comprising administering to the subject an effective amount of an antagonist of Type 1 interferon (IFN)-mediated signalling, for a time and under conditions sufficient to prevent or attenuate neuroinflammation, wherein the neurological event or condition is associated
25 with deposition of pathological forms of amyloid protein. Examples of such amyloid-associated pathologies include Alzheimer's disease (AD).

[0032] In yet another embodiment, the present disclosure teaches a method for reducing a neuroinflammatory response within the central nervous system (CNS) of a subject with
30 Parkinson's disease (PD) or other Parkinsonian condition or syndrome, the method comprising administering to the subject an effective amount of an antagonist of Type 1

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interferon (IFN)-mediated signalling, for a time and under conditions sufficient to prevent or attenuate neuroinflammation.

[0033] Another aspect enabled herein is a method for reducing a neuroinflammatory response within the central nervous system (CNS) of a subject following a neurological event or condition, the method comprising administering to the subject an effective amount of an antagonist of Type 1 interferon (IFN)-mediated signalling, for a time and under conditions sufficient to prevent or attenuate neuroinflammation, wherein the neurological event or condition is selected from AD and PD or other Parkinsonian condition or syndrome.

[0034] Another aspect enabled herein is a method for reducing a neuroinflammatory response within the central nervous system (CNS) of a subject following a neurological event or condition, the method comprising administering to the subject an effective amount of an antagonist of Type 1 interferon (IFN)-mediated signalling, for a time and under conditions sufficient to prevent or attenuate neuroinflammation, wherein the neurological event or condition is selected from stroke and infection by a pathogenic agent.

[0035] Yet a further aspect relates to ameliorating the signs and symptoms of cognitive impairment. Accordingly, the present disclosure describes a method for treating cognitive impairment in a human subject, the method comprising administering to the subject an effective amount of an antagonist of Type 1 interferon (IFN)-mediated signalling, for a time and under conditions sufficient to prevent or attenuate neuroinflammation.

[0036] Furthermore, taught herein is a method for treating cognitive impairment in a human subject, the method comprising administering to the subject an antagonist of Type 1 interferon-mediated signalling for a time and under conditions sufficient to ameliorate cognitive impairment or at least improve a subject's cognitive ability.

[0037] In an embodiment, the present disclosure teaches a method for reducing a neuroinflammatory response within the central nervous system (CNS) of a subject following a neurological event or condition, the method comprising administering to the

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subject an antagonist of interferon alpha receptor 1 (IFNAR1)-mediated signalling for a time and under conditions sufficient to prevent or ameliorate the symptoms of neuroinflammation.

- 5 [0038] Neuroprotective formulations comprising the Type 1 IFN-mediated signalling antagonist are also contemplated herein, as are diagnostic assays to screen for the level of Type 1 IFN-mediated signalling prior to or following a neuropathological event or condition. Aspects disclosed herein are also applicable for the development of trauma protocols for subjects who present with a TBI, AD, Parkinson's disease or other
10 Parkinsonian condition or syndrome, spinal cord injury or who exhibit symptoms of a neuropathological condition or disorder. Other conditions include stroke and infection in the CNS by a pathogenic agent.

[0039] The Type 1 IFN-mediated signalling antagonist may target a Type 1 IFN or its
15 receptor or a component thereof. A Type 1 IFN includes IFN α , IFN β and IFN ω . In an embodiment, the antagonist targets the IFN α receptor subunit (IFNAR1) and/or the ability for IFNAR1 to dimerize with IFNAR2. Combination therapy targeting a Type 1 IFN and IFNAR and optionally other components of the neuroinflammatory cascade are also taught herein. The type 1 IFN-mediated signalling antagonist may be used post or pre-
20 neurological event. Hence, it may be a therapeutic or a prophylactic.

[0040] The subject includes a higher order mammal including a human. A human subject may be of any age and includes a fetus.

- 25 [0041] Abbreviations used in the subject specification are defined in Table 2.

TABLE 1
Summary of sequence identifiers

1	Nucleotide sequence of the IFNAR1 gene
2	Nucleotide sequence of the IFNAR1 AttB1 subclone
3	Nucleotide sequence of the IFNAR1 AttB2 subclone
4	Nucleotide sequence of the IFNAR2 gene
5	Nucleotide sequence of the IFNAR2 AttB1 subclone
6	Nucleotide sequence of the IFNAR2 AttB2 subclone
7	Nucleotide sequence of the GADPH forward primer
8	Nucleotide sequence of the GADPH reverse primer
9	Nucleotide sequence of the Allalpha forward primer
10	Nucleotide sequence of the Allalpha reverse primer (1)
11	Nucleotide sequence of the Allalpha reverse primer (2)

TABLE 2
Abbreviations

18srRNA	18s ribosomal RNA
AD	Alzheimer's disease
ADC	Apparent Diffusion Coefficient
ANOVA	Analysis of Variance
APS	Ammonium Persulphate
BBB	Blood Brain Barrier
BSA	Bovine Serum Albumin
CCI	Controlled Cortical Impact
CNS	Central Nervous System
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
DWI	Diffusion Weighted Imaging
ECL	Enhanced Chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal Bovine Serum
H&E	Hematoxylin & Eosin
HEPES	4-(2-hydroxyethyl)-1-piperazineethane sulphonic acid
hIFN	human Type-1 Interferon
IFN	Interferon
IFNAR	Interferon Alpha Receptor
IFNAR1 ^{-/-}	IFNAR1 genetic knock-out
IFNAR2 ^{-/-}	IFNAR2 genetic knock-out
IL	Interleukin
IL-1 β	Interleukin-1 β
IL-6	Interleukin-6
IRF	Interferon Regulatory Factor
JAK	Janus Kinase

JAK-1	Janus Associated Kinase 1
KD	Knock down
KO	Knock out (^{-/-})
LB	Luria Broth
M17	human neuroblastoma M17 cell line
Map2	Neuronal marker indicating microtubule associated proteins
MAR1	Monoclonal antibody against IFNAR1
MCAO	Middle Cerebral Artery Occlusion
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	Magnetic Resonance Imaging
MTT	3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
NeuN	Neuronal nuclei
NMR	Nuclear Magnetic Resonance
OGD	Oxygen and Glucose Deprivation
6-OHDA	6-Hydroxydopamine (neurotoxic analog of dopamine)
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PD	Parkinson's disease
P-STAT	Phosphorylated-STAT
qRT-PCR	Quantitative Real Time Polymerase Chain Reaction
ROS	Reactive oxygen species
RT-PCR	Real-Time Polymerase Chain Reaction
SDS	Sodium Dodecyl Sulphate
SEM	Standard Error of the Means
SN	Substantia nigra
STAT	Signal Transducers and Activators of Transcription
TBI	Traumatic Brain Injury
TBS-T	Tris Buffered Saline-Tween 20
TE	Tris-EDTA
TEMED	N, N, N', N'-tetramethylethylenediamine

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TNF	Tumor Necrosis Factor
TNF α	Tumor Necrosis Factor Alpha
Tris	Tris(hydroxymethyl)aminomethane
TYK	Tyrosine Kinase
TYK-2	Tyrosine Kinase 2
WT	Wild type

BRIEF DESCRIPTION OF THE FIGURES

[0042] Some figures contain color representations or entities. Color photographs are available from the Patentee upon request or from an appropriate Patent Office. A fee may be imposed if obtained from a Patent Office.

[0043] **Figure 1** is a graphical representation showing isolated wild-type and IFNAR1^{-/-} C57B/6 neurons subjected to 4 hours oxygen and glucose deprivation (OGD) and 24 hours reperfusion. A subsequent MTT assay showed increased cell viability of IFNAR1^{-/-} neurons at 91.0±4.9% compared to wild-type with 49.8±0.8% (n=6, P<0.05, Student's t-test).

[0044] **Figure 2** is a graphical representation showing M17 cells subjected to 3 hours OGD and time-course reperfusion (0, 0.5, 2 and 24 hours), RT-PCR was then performed. IFNα mRNA levels, unlike IFNβ, were significantly elevated at 2 hours reperfusion (15.1±2.3 fold change) compared to control. IFNβ mRNA levels were only increased at 24 hours reperfusion (2.4±0.1 fold change) compared to control. (n=3, *P.05, One-way ANOVA, Dunnett's Post-hoc test.)

[0045] **Figures 3A through C** are graphical representations showing M17 cells subjected to 3 hours OGD and time-course reperfusion (0, 0.5, 2 and 24 hours), RT-PCR was then performed. **A)** Interleukin-6 (IL-6) mRNA levels were significantly increased at 0.5 hour (266.9±10.9 fold change) and 2 hours (194.6±39.4 fold change) reperfusion compared to control. **B)** Tumor Necrosis Factor α (TNF-α) mRNA levels were elevated at 0 hour (10.0±3.1 fold change) and 0.5 hour (10.1±2.8 fold change) as to control. **C)** Interleukin-1β (IL-1β) mRNA levels showed a significant increase at 24 hours reperfusion (4.6±1.2 fold change) compared to control. (n=3, *P<0.05, Dunnett's Post-hoc test.)

[0046] **Figure 4** is a graphical representation showing collated RT-PCR data for each cytokine mRNA levels post-3 hours OGD and time-course reperfusion (0, 0.5, 2 and 24 hours). OGD begins at -4 hours and subsequent reperfusion occurs from 0 hour. IFNα mRNA levels increases at 2 hours reperfusion before returning to basal levels. IFNβ and

IL-1 β mRNA levels only elevate at 24 hours reperfusion. TNF α mRNA levels show immediate increase at 0 and 0.5 hours reperfusion before returning to control levels. IL-6 mRNA levels are elevated at 0.5 and 2 hours reperfusion, and then return to basal levels.

- 5 [0047] **Figure 5** is a graphical representation showing M17 cells transfected with IFNAR1-GFP and IFNAR2-GFP overexpression plasmids and subjected to 3 hours OGD and 24 hours reperfusion. Curiously, M17-IFNAR1 cells demonstrated significant decrease in survival ($21.6 \pm 5.3\%$) compared to wild-type ($44.9 \pm 2.1\%$). Furthermore, M17-IFNAR2 cells showed no difference in survival ($39.8 \pm 5.7\%$) compared to wild-type. ($n=4$, * $P < 0.05$,
10 One-way ANOVA, Dunnett's Post-hoc test.)

[0048] **Figure 6** is a representation of the IFNAR1, AttB1 and AttB2 sequences used in Gateway (Registered Trademark) cloning.

- 15 [0049] **Figure 7** is a representation of the IFNAR2, AttB1 and AttB2 sequences used in Gateway (Registered Trademark) cloning.

- [0050] **Figure 8** is a graphical representation showing infarct volumes for Wild Type and IFNAR1^{-/-} mice 24 hours after TBI. Data represent Mean $\pm$ SEM for $n=5$ mice per
20 group, and * is indicative of a value of $P < 0.05$, as calculated using an unpaired Student's t-test with GraphPad Prism 5.0.

- [0051] **Figures 9A and B** are graphical representations showing mRNA expression of IFN α in wild-type and IFNAR1^{-/-}. **A)** Ipsilateral cortex and **B)** Contralateral cortex. The
25 control group represents sham-operated mice, and the other groups represent mice which were given TBI and had tissue collected 2, 4 and 24 hours later. Data represent Mean $\pm$ SEM for $n=3$ mice per group. * indicates a value of $P < 0.05$, as calculated using a one way ANOVA with a Bonferroni's post-hoc test to compare selected groups.

- 30 [0052] **Figures 10A and B** are a graphical representations showing mRNA expression of IFN β in wild type and IFNAR1^{-/-}. **A)** Ipsilateral cortex and **B)** Contralateral cortex. The control group represents sham-operated mice, and the other groups represent mice which

were given TBI and had tissue collected 2, 4 and 24 hours later. Data represent Mean $\pm$ SEM for n=3 mice per group. * indicates a value of $P < 0.05$, as calculated using a one way ANOVA with a Bonferroni's posthoc test to compare selected groups.

- 5 [0053] **Figure 11** is a graphical representation showing mRNA levels of IFN α and IFN β in post-mortem human brains. Time points indicate at what stage after TBI death occurred. Data are expressed as Mean $\pm$ SEM for n=8 $\pm$ 3 subjects per group. * indicates a value of $P < 0.05$, as calculated by a one way ANOVA with a Tukey's post-hoc analysis to compare all groups to each other.

10

- [0054] **Figures 12A and B** are graphical representations showing mRNA expression of TNF α in wild type and IFNAR1^{-/-}. **A)** Ipsilateral cortex and **B)** Contralateral cortex. The control group represents sham-operated mice, and the other groups represent mice which were given TBI and had tissue collected 2, 4 and 24 hours later. Data represent Mean $\pm$ SEM for n=3 mice per group. * indicates a value of $P < 0.05$, as calculated using a one way ANOVA with a Bonferroni's posthoc test to compare selected groups.

- [0055] **Figures 13A and B** are graphical representations showing mRNA expression of IL-10 in both wild type and IFNAR1^{-/-}. **A)** Ipsilateral cortex and **B)** Contralateral cortex. The control group represents sham-operated mice, and the other groups represent mice which were given TBI and had tissue collected 2, 4 and 24 hours later. Data represent Mean $\pm$ SEM for n=3 mice per group. * indicates a value of $P < 0.05$, as calculated using a one way ANOVA with a Bonferroni's post-hoc test to compare selected groups.

- 25 [0056] **Figure 14** is a representation of SYBRGreen nucleotide sequences.

- [0057] **Figures 15A through D** are graphical representations showing RT-PCR timecourse data showing relative fold increase in transcription levels at 4 hours, 8 hours, 24 hours, 48 hours and 96 hours time points after treatment with 10 μ M β -Amyloid. Relative transcription levels shown for **A)** Interferon alpha (IFN α); **B)** Interferon beta (IFN β); **C)** Interleukin 1-beta (IL-1 β); and **D)** Tumor Necrosis Factor alpha (TNF α).

- 30

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[0058] Figure 16 is a graphical representation of viability assay of wild-type and IFNAR^{-/-} neurons after treatment with 1-42 β -Amyloid. Mean viability is expressed as with respect to vehicle viability after treatments of 5 μ M, 10 μ M and 15 μ M β -Amyloid over 96 hours. Plotted values are mean & SEM. * p< 0.05, ** p< 0.01.

5

[0059] Figures 17A through C are photographic representations of fluorescent immunohistochemistry showing IFNAR1 localization on primary cultured mouse neurons. Panel A Map-2, Panel B NeuN Panel C-Merge. Note, Map2 is a neuronal marker indicating microtubule associated proteins.

10

[0060] Figures 18A and B are graphical and photographic representations of IFNAR^{-/-} (knock out; KO) mice (C57BL6) show reduced infarct size in a stroke model. The model comprises 2 hours of mid-cerebral artery occlusion followed by 24 hours of reperfusion. IFNAR^{-/-} mice show a reduced infarct size.

15

[0061] Figure 19 is a graphical representation showing that IFNAR1 mAb reduces infarct size in a middle cerebral artery occlusion (MCAO) stroke model. An amount of 0.5mg mAb was given iv 1 hour prior to stroke. The infarct volume was assessed 24 hours post-injury. The monoclonal Ab (mAb) is MAR-1.

20

[0062] Figure 20 is a graphical representation shown that IFNAR1 mAb reduces infarct size in a traumatic brain injury (TBI) model. An amount of 0.5mg mAb was given iv 30 minutes post-TBI induction. The infarct volume was assessed 24 hours post-injury.

25

[0063] Figure 21 is a graphical representation showing that IFNAR^{-/-} neurons are less susceptible to beta-amyloid [A β] (1-42) toxicity. Primary cultured mouse neurons from wt and IFNAR^{-/-} (KO) mice were exposed to a dose response curve of beta amyloid. The IFNAR^{-/-} neurons displayed less cell death at 96 hours after treatment.

30

[0064] Figures 22A and B are graphical representations showing elevated levels of type-1 IFNs in human AD brains. (A) qPCR shows a four-fold elevation of IFN-alpha and IFN-

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beta in post mortem brains from AD sufferers. (B) IFNAR1 and IFNAR2 receptor subunits remained unchanged.

5 [0065] Figure 23 is a graphical representation showing that IFNAR1^{-/-} (knock out) mice are protective against TBI in a stroke model.

[0066] Figures 24 A and B are graphical representations of levels of IFN α , IFN β , IFNAR1 and IFNAR2 in postmortum brains of Parkinson's disease subjects.

10 [0067] Figure 25 is a graphical representation of increasing levels of IFN α and IFN β following insult of M17 cells with AB₁₋₄₂ (*P<0.05m n=3).

[0068] Figure 26 is a graphical representation showing levels of murine IFN α (muIFN α) in an APP/PS1 transgenic mouse model.

15

[0069] Figures 27 A, B and C show Type 1 IFN mRNA transcript levels in M17 cells treated with 6-OHDA. M17 cells were treated with 50 μ M 6-OHDA for 0, 1, 2, 4, 8 and 24hours, before being harvested, RNA isolated and cDNA synthesized. QPCR analysis was performed using Taqman probes for IFN α , IFN β , and IFNAR1 to determine mRNA expression a) IFN α mRNA levels were increased up to 4 hours after treatment with 50 μ M 6-OHDA. A similar trend was seen with b) IFN β c) Human Interferon Receptor 1 subunit (hIFNAR1) mRNA levels were significantly decreased at 8 hours post treatment compared to control (all time-points are n=3 except 24hours n=2. *P<0.05, Dunnett's Post-hoc test).

25 [0070] Figures 28 A, B and C are graphical representations showing Type-1 IFN mRNA transcript levels in M17 cells treated with rotenone. M17 cells were treated for 24 hours with the indicated concentrations of rotenone before cell viability was assessed by MTT assay. A reduction in the percentage cell viability compared to a vehicle (DMSO) control was confirmed. Data expressed as mean $\pm$ S.E.M, n=4.

30

[0071] Figure 29 is a graphical representation showing the IFNAR1 knock down (KD) M17 cells are protected against rotenone-induced cell death. NC shRNA and IFNAR1 KD

cells were treated for 24 hours with the indicated concentrations of rotenone. M17 IFNAR1 KD cells demonstrated a significant increase in cell viability as assessed by MTT assay, compared to NC shRNA cells (n=6, *P<0.05, Two-way ANOVA, Bonferroni Post-hoc test).

5

[0072] Figures 30 A, B and C are graphical representations of type-I IFN mRNA transcript levels in M17 NC and IFNAR1-KD cells. NC and IFNAR1-KD cells were treated with 500nM rotenone for 0, 1, 2, 4, 8, and 24 hours, harvested and RNA extracted. QPCR was performed. Both A) IFN α and B) IFN β mRNA levels were increased 24 hours post treatment compared to vehicle C) hIFNAR1 mRNA levels in IFNAR1 KD cells showed a decreasing trend up to 8 hours post treatment, with no change in NC shRNA expression (n=2).

[0073] Figure 31 is a graphical representation showing the Type-1 IFN mRNA transcript levels are increased in Parkinson's disease (PD) patients. RNA was extracted from human controls (n=10) and PD (n=10) pre-frontal cortex and QPCR was performed on cDNA using Taqman probes. mRNA transcript levels for IFN α and IFN β were both increased compared to aged matched control (n=10, per group *P<0.05 One-way ANOVA, Dunnett's post-hoc test).

20

[0074] Figures 32 A and B are graphical representations showing mRNA expression levels in MPTP model. RNA was extracted from the substantia nigra of WT sham (n=10), WT treated MPTP (n=10) and IFNAR1^{-/-} treated MPTP (n=8). QPCR analysis shows mRNA transcript levels for A) IFN α are significantly decreased in the MPTP treated WT and IFNAR1^{-/-} mice compared to control. B) IFN β transcript levels show no significant change compared to control (*P<0.05 One-way ANOVA, Dunnett's post-hoc test).

25

[0075] Figures 33A and B are graphical representations of infarct volume in A) monoclonal antibody against IFNAR1 (MAR1) treatment versus vehicle after 60 minutes pre-TBI; and B) MAR1 treatment versus vehicle 30 minutes post-TBI.

30

DETAILED DESCRIPTION

[0076] Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or method step or group of elements or integers or method steps but not the exclusion of any other element or integer or method step or group of elements or integers or method steps.

[0077] Nucleotide and amino acid sequences are referred to by a sequence identifier number (SEQ ID NO). The SEQ ID NOs correspond numerically to the sequence identifiers <400>1 (SEQ ID NO:1), <400>2 (SEQ ID NO:2), etc. A summary of the sequence identifiers is provided in Table 1. A sequence listing is provided after the claims.

[0078] As used in the subject specification, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to "a neurodegenerative disease" includes a single disease, as well as two or more diseases; reference to "an antagonist" includes a single antagonist, as well as two or more antagonists; reference to "the disclosure" includes a single and multiple aspects described in the disclosure; and so forth. All aspects disclosed, described and/or claimed herein are encompassed by the term "invention". Such aspects are enabled across the width of the present invention.

[0079] Type 1 interferons (IFNs) are a super-family of pleiotropic cytokines that induce pro-inflammatory gene transcription via the classical JAK/STAT pathway. The Type 1 IFNs include IFN α , IFN β and IFN ω . Certain events or conditions within the central nervous system (CNS) have the capacity to induce a neuroinflammatory response having pathological consequences. Taught herein is the use of an antagonist of Type 1 IFN-mediated signalling within the CNS to attenuate an adverse neuropathological inflammatory response. This leads to a reduction in secondary neuronal damage including neuronal necrosis and apoptosis and can reduce infarct size.

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[0080] Reference herein to a "type 1 IFN-mediated signalling antagonist" means an antagonist of the activity of a Type 1 IFN, an inhibitor of Type 1 IFN gene expression or translation, an antagonist of a Type 1 IFN receptor activity, function or dimerization or a subunit thereof such as IFNAR1, an inhibitor of Type 2 IFN receptor gene expression and
5 an agent which blocks Type 1 IFN:receptor interaction or any agent which blocks, inhibits or otherwise reduces Type 1 IFN-mediated inflammatory signalling.

[0081] An aspect enabled herein is a method for reducing a neuroinflammatory response within the central nervous system (CNS) of a subject following a neurological event or
10 condition, the method comprising administering to the subject an effective amount of an antagonist of Type 1 interferon (IFN)-mediated signalling, for a time and under conditions sufficient to prevent or attenuate neuroinflammation.

[0082] In an embodiment, the neuroinflammation is cerebral neuroinflammation resulting
15 from acute neuronal injury such as traumatic brain injury and trauma-induced paralysis, a neurodegenerative disease, drug or alcohol abuse, radiation or chemotherapy and/or starvation and/or any event or condition which leads to or has the potential to lead to ischemia-reperfusion injury in the brain. Such other conditions include stroke and an infection within the CNS by a pathogenic agent. The event or condition may also occur in
20 other parts of the CNS including the spinal cord. Hence, spinal cord trauma or disease is also contemplated herein.

[0083] Examples of neurodegenerative conditions which can lead to ischemia-reperfusion injury and which can lead to neuroinflammation include Alzheimer's disease (AD),
25 Parkinson's disease (PD) and other Parkinsonian conditions and syndromes, amyotrophic lateral sclerosis, Friedreich's ataxia, Huntington's disease, Lewy body disease, spinal muscular atrophy, motor neuron disease, Alper's disease, Batten disease, Cerebro-oculo-facio-skeletal syndrome, corticobasal degeneration, Leigh's disease, Machads-Joseph disease, monomelic amyotrophy, multiple system atrophy, multiple sclerosis,
30 neurodegeneration with brain iron accumulation, olivopontocerebellar atrophy, opsoclonus myoclonus, paraneoplastic syndromes, prion diseases, progressive multifocal leukoencephalopathy, Aicardi-Coutieres syndrome as well as a range of demyelination

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diseases and oligodendrocyte cytotoxicity as well as spinal cord trauma. The term "Parkinson's disease" or "PD" includes other Parkinsonian condition or syndrome. Other conditions include stroke and infection of the CNS by a pathogenic agent.

5 [0084] In an embodiment, the neurological event or condition is traumatic brain injury (TBI). Accordingly, another aspect of the present disclosure contemplates a method for reducing a neuroinflammatory response within the central nervous system (CNS) of a subject following a neurological event or condition, the method comprising administering to the subject an effective amount of an antagonist of Type 1 interferon (IFN)-mediated
10 signalling, for a time and under conditions sufficient to prevent or attenuate neuroinflammation, wherein the neurological event or condition is traumatic brain injury.

[0085] In an embodiment, the neurological event or condition is associated with pathological forms of amyloid protein.

15

[0086] A pathological form of amyloid or A β includes oligomers of monomeric A β peptides linked *via* covalent bonds between inter-peptide tyrosyl residues (International Patent Application No. PCT/AU01/00786; Roher *et al*, *J. Biol Chem* 272(34):20631-20635, 1996; Cherny *et al*, *J. Biol. Chem.* 274:23223-24224, 1999).

20

[0087] Accordingly, another aspect enabled herein is a method for reducing a neuroinflammatory response within the central nervous system (CNS) of a subject following a neurological event or condition, the method comprising administering to the subject an effective amount of an antagonist of Type 1 interferon (IFN)-mediated
25 signalling, for a time and under conditions sufficient to prevent or attenuate neuroinflammation, wherein the neurological event or condition is associated with deposition of pathological forms of amyloid protein (A β).

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[0088] In an embodiment, the amyloid pathology is Alzheimer's disease (AD).

[0089] Hence, another aspect of the present disclosure contemplates a method for reducing a neuroinflammatory response within the central nervous system (CNS) of a subject

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following a neurological event or condition, the method comprising administering to the subject an effective amount of an antagonist of Type 1 interferon (IFN)-mediated signalling, for a time and under conditions sufficient to prevent or attenuate neuroinflammation, wherein the neurological event or condition is AD.

5

[0090] A related embodiment provides a method for reducing β -amyloid toxicity in a subject, the method comprising administering to the subject an effective amount of an antagonist of Type 1 interferon (IFN)-mediated signalling, for a time and under conditions sufficient to reduce or ameliorate the effects of β -amyloid toxicity. Reference to " β -amyloid" in this context means pathogenic forms of β -amyloid including A β (1-42) as well as aggregated and multimeric forms thereof.

[0091] In yet another embodiment, the present disclosure teaches a method for reducing a neuroinflammatory response within the central nervous system (CNS) of a subject with Parkinson's disease (PD) or other Parkinsonian condition or syndrome, the method comprising administering to the subject an effective amount of an antagonist of Type 1 interferon (IFN)-mediated signalling, for a time and under conditions sufficient to prevent or attenuate neuroinflammation.

[0092] The present disclosure teaches a method for reducing a neuroinflammatory response within the central nervous system (CNS) of a subject following a neurological event or condition, the method comprising administering to the subject an effective amount of an antagonist of Type 1 interferon (IFN)-mediated signalling, for a time and under conditions sufficient to prevent or attenuate neuroinflammation, wherein the neuroinflammatory response leads to cognitive impairment.

[0093] The amelioration of the signs and symptoms of cognitive impairment is also enabled herein. Accordingly, the present disclosure contemplates a method for treating cognitive impairment in a human subject, the method comprising administering to the subject an effective amount of an antagonist of Type 1 interferon (IFN)-mediated signalling, for a time and under conditions sufficient to prevent or attenuate neuroinflammation.

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[0094] Another embodiment provides a method for treating cognitive impairment in a human subject, the method comprising administering to said subject an antagonist of Type 1 interferon-mediated signalling for a time and under conditions sufficient to ameliorate cognitive impairment or at least improve a subject's cognitive ability. Reference to "cognitive impairment" includes from mild to severe as well as intermediate cognitive impairment.

[0095] The present disclosure teaches antagonists of Type 1 IFN-mediated signalling in the form of a compound, agent, chemical agent, pharmacologically active agent, medicament, active and drug. The terms "compound", "agent", "chemical agent", "pharmacologically active agent", "medicament", "active" and "drug" are used interchangeably herein to refer to the Type 1 IFN-mediated signalling antagonist which induces a desired pharmacological and/or physiological effect. The desired effect includes reducing Type 1 IFN-mediated signalling *via* the IFN or a receptor subunit such as IFNAR1. The desired physiological effect includes attenuation of a neuropathological inflammatory response including the potential for such an inflammatory response to reach a neuropathological level. The terms also encompass pharmaceutically acceptable and pharmacologically active ingredients of those active agents specifically mentioned herein including but not limited to salts, esters, amides, prodrugs, active metabolites, analogs, mimetics functional equivalents and the like. When the terms "compound", "agent", "chemical agent", "pharmacologically active agent", "medicament", "active", "drug" and "antagonist" are used, then it is to be understood that this includes the active agent *per se* as well as pharmaceutically acceptable, pharmacologically active salt, ester, amide, prodrug, metabolite and analogs thereof.

[0096] Reference to the antagonist in these terms includes combinations of two or more actives. A "combination" also includes multi-part such as a two-part composition where the agents are provided separately and given or dispensed separately or admixed together prior to dispensation. Examples include two separate agents wherein one agent down-regulates the activity or level of the Type 1 IFN and the other down-regulates the activity or level of a Type 1 IFN receptor subunit, and in particular IFNAR1. Agents when in

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combination with an IFN or IFNAR antagonist may also target a down-stream component of the Type 1 IFN-mediated signalling cascade.

[0097] The antagonists enabled herein provided systemically cross the blood brain barrier (BBB). This is possible due to the chemical nature of the agent or due to a permealization factor such as a co-factor fused to or formulated with the antagonist. Strategies for accessing the CNS are disclosed in Misra *et al.*, *J Pharm Sci* 6:252-273, 2003.

[0098] The "antagonist" includes a protein, polypeptide or peptide, small chemical molecule, antibody or derivative thereof including an immunoglobulin new antigen receptor (IgNAR) or a genetic molecule.

[0099] A "genetic molecule" is generally one which down-regulates expression of a gene encoding a Type 1 IFN or a Type 1 IFN receptor or a subunit thereof. In an embodiment, the targeted subunit is IFNAR1. The genetic molecule includes an antisense molecule directed to all or part of a gene or mRNA encoding the Type 1 IFN or a subunit portion of its receptor such as IFNAR1. In an embodiment, the antisense is from about 5 nucleotides in length to 15 to 80 nucleotides in length or from about 5 nucleotides in length to full length of the mRNA transcript or 5' or 3' regions thereof. Other genetic molecules include sense molecules such as single- or double-stranded RNAs, RNAi and siRNA molecules, short and long RNA duplexes, ribozymes, DNazymes, and any DNA or RNA or synthetic DNA or RNA agent which interferes with expression of the expression of the Type 1 IFN gene or of the gene encoding a receptor subunit such as IFNAR1. The genetic molecule may be naked or expressed by a viral or other vector or introduced as part of a formulation.

25

[00100] A number of viruses may be used as nucleic acid transfer vectors or as the basis for preparing nucleic acid transfer vectors to introduce a genetic agent to the CNS, including papovaviruses (e.g. SV40, Madzak *et al.*, *J Gen Virol* 73:1533-1536, 1992), adenovirus (Berkner, *Curr Top Microbiol Immunol* 158:39-66, 1992; Berkner *et al.*, *BioTechniques* 6:616-629, 1988; Gorziglia and Kapikian, *J Virol* 66:4407-4412, 1992; Quantin *et al.*, *Proc Natl Acad Sci USA* 89:2581-2584, 1992; Rosenfeld *et al.*, *Cell* 68:143-155, 1992; Wilkinson *et al.*, *Nucleic Acids Res* 20:233-2239, 1992; Stratford-Perricaudet *et*

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al, *Hum Gene Ther* 1:241-256, 1990; Schneider *et al*, *Nat Genetics* 18:180-183, 1998),
 vaccinia virus (Moss, *Curr Top Microbiol Immunol* 158: 5-38, 1992; Moss, *Proc Natl*
Acad Sci USA 93:11341-11348, 1996), adeno-associated virus (Muzyczka, *Curr Top*
Microbiol Immunol 158:97-129, 1992; Ohi *et al*, *Gene* 89:279-282, 1990; Russell and
 5 Hirata, *Nat Genetics* 18:323-328, 1998), herpesviruses including HSV and EBV
 (Margolskee, *Curr Top Microbiol Immunol* 158:67-95, 1992; Johnson *et al*, *J Virol*
 66:2952-2965, 1992; Fink *et al*, *Hum Gene Ther* 3:1-19, 1992; Breakefield and Geller, *Mol*
Neurobiol 1:339-371, 1987; Freese *et al*, *Biochem Pharmacol* 40:2189-2199, 1990; Fink *et*
al, *Ann Rev Neurosci* 19:265-287, 1996), lentiviruses (Naldini *et al*, *Science* 272:263-267,
 10 1996), Sindbis and Semliki Forest virus (Berglund *et al*, *Biotechnology* 11:916-920, 1993)
 and retroviruses of avian (Bandyopadhyay and Temin, *Mol Cell Biol* 4:749-754, 1984;
 Petropoulos *et al*, *J Virol* 66:3391-3397, 1992), murine (Miller, *Curr Top Microbiol*
Immunol 158:1-24, 1992; Miller *et al*, *Mol Cell Biol* 5:431-437, 1985; Sorge *et al*, *Mol*
Cell Biol 4:1730-1737, 1984; Mann and Baltimore, *J Virol* 54:401-407, 1985; Miller *et al*,
 15 *J Virol* 62:4337-4345, 1988) and human (Shimada *et al*, *J Clin Invest* 88:1043-1047, 1991;
 Helseth *et al*, *J Virol* 64:2416-2420, 1990; Page *et al*, *J Virol* 64:5270-5276, 1990;
 Buchsacher and Panganiban, *J Virol* 66:2731-2739, 1992) origin.

[0100] Non-viral nucleic acid transfer methods include chemical techniques including
 20 calcium phosphate co-precipitation, mechanical techniques, for example, microinjection,
 membrane fusion-mediated transfer *via* liposomes and direct DNA uptake and receptor-
 mediated DNA transfer. Viral-mediated nucleic acid transfer can be combined with direct
in vivo nucleic acid transfer using liposome delivery, allowing one to direct the viral
 vectors to particular cells. Alternatively, the retroviral vector producer cell line can be
 25 injected into particular tissue. Injection of producer cells would then provide a continuous
 source of vector particles.

[0101] An IgNAR (immunoglobulin new antigen receptor) is an antibody isotype found in
 cartilaginous marine animals (sharks and rays), which has evolved over hundreds of
 30 millions of years to be stably expressed in the potent urea environment of the blood stream
 (Greenberg *et al.*, *Nature* 374:168-173, 1995; Nuttall *et al.*, *Mol Immunol* 38:313-326,
 2001). The IgNAR response is antigen-driven in the shark, and both immune and naïve

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molecular libraries of IgNAR variable domains have been constructed and successfully screened for antigen-specific binding reagents (Greenberg *et al.*, 1995 *supra*; Nuttall *et al.*, 2001 *supra*). IgNAR's are bivalent, but target antigen through a single immunoglobulin variable domain (~14kDa) displaying two complementarity determining region (CDR) loops attached to varying numbers of constant domains (Nuttall *et al.*, *Eur J Biochem* 270:3543-3554, 2003; Roux *et al.*, *Proc Natl Acad Sci USA* 95:11804-11809, 1998). In contrast, traditional immunoglobulin (Ig) antibodies have a variable heavy (V_H) + variable light (V_L) domain format (~26kDa) and bind antigen through up to six CDRs (Chothia *et al.*, *Nature* 342:877-883, 1989; Padlan, *Mol Immunol* 31:169-217, 1994). The small size, and thermodynamic and chemical stability of IgNAR variable domains (V_{NARS}), offer distinct advantages over conventional antibodies. Furthermore, the small V_{NAR} size enables this unusual antibody domain access to cryptic antigenic epitopes through unusually long and variable CDR3 loops (Greenber *et al.*, 1995 *supra*; Ewert *et al.*, *Biochemistry* 41:3628-2636, 2002; Nuttall *et al.*, *Proteins* 55:187-197, 2004; Stanfield *et al.*, *Science* 305:1770-1773, 2004; Streltsov *et al.*, *Proc Natl Acad Sci USA* 101:12444-12449, 2004; Streltsov *et al.*, *Protein Sci* 14:2901-2909, 2005). IgNAR domains have been identified that recognize a variety of target antigens including: the apical membrane protein 1 (AMA-1) of *P. falciparum* (Nuttall *et al.*, 2004 *supra*); the Kgp protease from *Porphyromonas gingivalis* (Nuttall *et al.*, *FEBS Lett* 516:80-86, 2002); cholera toxin (Goldman *et al.*, *Anal Chem* 78:8245-8255, 2006); the Tom70 mitochondrial membrane spanning protein (Nuttall *et al.*, 2003 *supra*), and lysozyme (Streltsov *et al.*, 2004 *supra*).

[0102] The present disclosure teaches analogs and derivatives of a Type 1 IFN or an IFNAR such as IFNAR1 such as which include a modified side chain or which incorporate an unnatural amino acid and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or its analogs. This term also does not exclude modification of the glycosylation, acetylation and phosphorylation patterns. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid or polypeptides with substituted linkages. Such polypeptides may need to be able to enter the cell and/or cross the BBB or promote cerebral fluid half-life survival.

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[0103] Mimetics of the Type 1 IFN or IFNAR such as IFNAR1 are another useful group of agents to test for neuroprotective ability. The term is intended to refer to a substance which has some chemical similarity to the molecule it mimics and which acts as an antagonist. A peptide mimetic of a Type 1 IFN or IFNAR1, for example, may be a peptide-
5 containing molecule that mimics elements of protein secondary structure (Johnson *et al.*, *Peptide Turn Mimetics in Biotechnology and Pharmacy*, Pezzuto *et al* (Eds), Chapman and Hall, New York, 1993). The underlying rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions such as with a receptor or ligand. A peptide
10 mimetic, therefore, is designed to permit molecular interactions similar to the natural molecule but block signalling.

[0104] The designing of mimetics to a pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might
15 be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for a particular method of administration, e.g. peptides are unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal.

[0105] There are several steps commonly taken in the design of a mimetic from a compound having a given target property. First, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide, e.g. by substituting each residue in turn. Alanine scans of peptides, for example,
25 are commonly used to refine such peptide motifs. These parts or residues constituting the active region of the compound are known as its "pharmacophore".

[0106] Once the pharmacophore has been found, its structure is modeled according to its physical properties, e.g. stereochemistry, bonding, size and/or charge, using data from a
30 range of sources, e.g. spectroscopic techniques, x-ray diffraction data and NMR. Computational analysis, similarity mapping (which models the charge and/or volume of a

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pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modeling process.

5 [0107] In a variant of this approach, the three-dimensional structure of a receptor and ligand are modeled. This can be especially useful where the receptor and/or ligand change conformation on binding, allowing the model to take account of this in the design of the mimetic. Modeling can be used to generate agents which interact with the linear sequence or a three-dimensional configuration.

10 [0108] A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted onto it can conveniently be selected so that the mimetic is easy to synthesize, is likely to be pharmacologically acceptable, and does not degrade *in vivo*, while retaining the biological activity of the lead compound. Alternatively, where the mimetic is peptide-based, further
15 stability can be achieved by cyclizing the peptide, increasing its rigidity. The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Further optimization or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

20 [0109] Hence, the present disclosure teaches antagonists of Type 1 IFN-mediated signalling for use in mammals including higher order mammals such as humans. Non-human animals are contemplated such as in veterinary applications as well as for use in animal models. Reference to a mammal includes a mouse, rat, hamster, guinea pig, rabbit, pig, sheep, horse, goat, cow, camel and non-human primate (such as orangutan, gorilla,
25 marmoset and a macaque). In an embodiment, the subject is a human. Reference to a human includes a fetus, *in utero* as well as a human of any age.

[0110] The present disclosure teaches a method for reducing a neuroinflammatory response within the central nervous system (CNS) of a human subject following a
30 neurological event or condition, the method comprising administering to the subject an effective amount of an antagonist of Type 1 interferon (IFN)-mediated signalling, for a time and under conditions sufficient to prevent, reduce or attenuate neuroinflammation.

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[0111] In an embodiment, the present disclosure enables a method for reducing a neuroinflammatory response within the CNS of a subject following a neurological event or condition, the method comprising administering to the subject an antagonist of interferon
5 alpha receptor 1 (IFNAR1)-mediated signalling for a time and under conditions sufficient to prevent or ameliorate the symptoms of neuroinflammation.

[0112] The present disclosure further describes a method for reducing a neuroinflammatory response within the CNS of a human subject following a neurological
10 event or condition, the method comprising administering to the human subject an antagonist of interferon alpha receptor 1 (IFNAR1)-mediated signalling for a time and under conditions sufficient to prevent or ameliorate the symptoms of neuroinflammation.

[0113] These aspects enabled herein extend to ameliorating the effect of a TBI, stroke,
15 spinal cord trauma and/or a neurodegenerative disease. In an aspect, the methods herein are applicable to reducing secondary neuronal damage such as neuronal necrosis and apoptosis and infarct size. A neurodegenerative disease condition is one associated with deposition of pathological forms of A β , such as AD. The treatment of AD and other neurodegenerative diseases may further comprise behavioral modification protocols. Such
20 protocols include reducing the toxicity of pathological forms of A β .

[0114] The terms "effective amount" and "therapeutically effective amount" of an agent as used herein mean a sufficient amount of an agent (i.e. a Type 1 IFN-mediated signalling antagonist) to provide the desired therapeutic or physiological effect or outcome as
25 indicated above. Undesirable effects, e.g. side effects, are sometimes manifested along with the desired therapeutic effect; hence, a practitioner balances the potential benefits against the potential risks in determining what is an appropriate "effective amount". The exact amount required will vary from subject to subject, depending on the species, age and general condition of the subject, mode of administration and the like. Thus, it may not be
30 possible to specify an exact "effective amount". However, an appropriate "effective amount" in any individual case may be determined by one of ordinary skill in the art using only routine experimentation. In general, the amount is effective to attenuate a

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neuroinflammatory response within the CNS or to prevent or reduce the severity of a neuroinflammatory response developing or continuing. The prevention of a neuroinflammatory response developing is also useful in at risk subjects such as those with a genetic disposition or family history of neurodegenerative diseases including events such as stroke or subjects undergoing cancer therapy of the brain or surgery of the brain. The present disclosure teaches reducing, preventing or attenuating neuroinflammation anywhere in the CNS, including in the brain. The antagonist may be used as a therapeutic to treat a condition or as a preventative (i.e. prophylactically) to reduce the risk of neuroinflammation in anticipation of a neurological event.

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[0115] The antagonist may also be administered with a pharmaceutically acceptable carrier, excipient or diluent. By "pharmaceutically acceptable" carrier, excipient or diluent is meant a pharmaceutical vehicle comprised of a material that is not biologically or otherwise undesirable, i.e. the material may be administered to a subject along with the selected active agent without causing any or a substantial adverse reaction. Carriers may include excipients and other additives such as diluents, detergents, coloring agents, wetting or emulsifying agents, pH buffering agents, preservatives, and the like.

[0116] The antagonist may itself be a pharmaceutically acceptable form of a particular compound. By "pharmaceutically acceptable" means a salt, ester, amide, prodrug or derivative of a compound that is not biologically or otherwise undesirable.

[0117] The terms "treating" and "treatment" as used herein refer to reduction in severity and/or frequency of symptoms of the condition being treated, elimination of symptoms and/or underlying cause, prevention of the occurrence of symptoms of the condition and/or their underlying cause and improvement or remediation or amelioration of damage following a neurological event or condition leading to or having the potential to lead a neuroinflammatory response. In general terms, treatment may involve actively reversing a disease or ameliorating symptoms of, for example, a neuroinflammatory response including necrosis, apoptosis, senescence or arrest of cell growth, demyelination and/or axonal or neuronal degeneration. Amelioration of a downstream physiological, psychological or mental condition is also a useful indicator of treatment.

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[0118] "Treating" a subject, therefore, may involve prevention or reduction in extent of development of a condition or other adverse physiological or psychological event in a susceptible individual associated with a neuroinflammatory response as well as treatment
5 of a clinically symptomatic individual by ameliorating the symptoms of the neuroinflammatory response.

[0119] As indicated above, a "subject" as used herein refers to an animal, such as a mammal including a human who can benefit from the pharmaceutical agents and
10 formulations and methods of the present disclosure. A subject regardless of whether a human or non-human animal may be referred to as an individual, patient, animal, host or recipient. The compounds and methods enabled herein have particular applications in human medicine.

15 [0120] Taught herein is the use of neuroprotective agents to reduce a neuroinflammatory response or risk of such a response developing or the consequences of degenerative inflammatory processes such as immunodegenerative processes or conditions which induce cell cycle arrest, necrosis and/or apoptosis and/or contribute to infarct size.

20 [0121] In addition, the neuroprotective agent may reduce demyelination or promote or maintain myelination processes and/or prevent axonal or neuronal degeneration or promote axonal or neuronal repair.

[0122] The neuroprotective agent enabled herein is an agent which down-regulates the
25 extent or activity of Type 1 IFN-mediated signalling. Such an agent is referred to herein as a "Type 1 IFN signalling antagonist". This includes an antagonist of a Type 1 IFN or a portion of the Type 1 IFN receptor and in particular IFNAR. Reference to an "IFNAR" includes the IFNAR receptor or a subunit thereof such as IFNAR1. The present disclosure further teaches combinations of neuroprotective agents or neuroprotective formulations
30 comprising a Type 1 IFN signalling antagonist and another neuroprotective agent such as leukemia inhibitory factor (LIF) or ciliary neurotrophic factor (CNTF) or a homolog, derivative, analog or mimetic thereof.

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[0123] Hence, the present disclosure teaches a method for the treatment or prophylaxis of a neuropathological event, disease or condition in a subject, the method comprising administering to the subject an effective amount of a Type 1 IFN signalling antagonist for a time and under conditions sufficient to ameliorate adverse neurological inflammation or prevent or reduce its progression.

[0124] In an embodiment, a method is provided for the treatment or prophylaxis of a neuropathological event, disease or condition in a subject or at least delaying onset of symptoms thereof, the method comprising administering to the subject an effective amount of a Type 1 IFN alpha receptor (IFNAR1) antagonist for a time and under conditions sufficient to ameliorate adverse neurological inflammation or prevent or reduce its progression.

[0125] The neuropathological event, disease or condition includes traumatic brain injury (TBI) and trauma-induced paralysis, stroke, infection by a pathogen, a neurodegenerative disease including AD, drug or alcohol abuse, radiation or chemotherapy and/or starvation, spinal cord trauma or disease and/or any event or condition which leads to or has the potential to lead to ischemia-reperfusion injury in the brain or other parts of the CNS. A "pathogen" includes a virus, bacterium or other microorganism or other parasite.

[0126] Hence, as indicated above, the neuroprotective agent includes a Type 1 IFN antagonist or an IFNAR antagonist such as IFNAR1 antagonist. The antagonist act at the level of protein activity or function or gene expression including transcription, translation or processing.

[0127] In another aspect, the present disclosure teaches a method for the treatment or prophylaxis of a neuropathological event, disease or condition in a subject or delaying onset of symptoms thereof, the method comprising administering to the subject an effective amount of a neuroprotective formulation comprising a Type 1 IFN antagonist for a time and under conditions sufficient to reduce neurological inflammation or prevent or reduce its progression.

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[0128] Still another aspect taught herein to a method for the treatment of a neuropathological even, disease or condition in a subject or delaying onset of symptoms thereof, the method comprising administering to the subject an effective amount of a neuroprotective formulation comprising an IFNAR1 antagonist for a time and under conditions sufficient to reduce neurological inflammation, or prevent or reduce its progression.

[0129] As indicated above, the amount or time sufficient to treat the neurodegenerative disease or condition may be the amount or time required to ameliorate one or more symptoms of the neuropathological event, disease or condition. A symptom includes a psychological or mental symptom. Furthermore, the antagonism may be provided with a pharmaceutically acceptable carrier, excipient or diluent. The antagonist itself is considered pharmacologically acceptable.

[0130] The Type 1 IFN-mediated signalling antagonist may also be provided in combination with another neuroprotective agent such as LIF and/or CNTF or their homologs, derivatives, analogs or mimetics.

[0131] Hence, the present disclosure enables a method for the treatment or prophylaxis of a neuropathological event, disease or condition in a subject, the method comprising administering to the subject an effective amount of a neuroprotective formulation comprising a Type 1 IFN-mediated signalling antagonist and one or both of LIF and CNTF or a homolog, derivative, analog or mimetic thereof for a time and under conditions sufficient to reduce neurological inflammation or prevent or reduce its progression.

[0132] Also enabled herein are pharmaceutical compositions and formulations which include one or more of the neuroprotective agents hereinbefore described. The pharmaceutical compositions taught herein may be administered in a number of ways depending upon whether local or systemic treatment as desired including with means for the agent to cross the BBB. Administration includes intravenous, intra-arterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial

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administration, e.g., intrathecal or intraventricular, administration; or oral administration; or *via* a spinal tap. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Clearly, the formulation needs to enable the agent to cross the BBB. Hence, the agent itself may need to be modified.

5 Alternatively, the formulation may enable retrograde transport. The agents may also be specifically targeted to the brain or other parts of the CNS.

[0133] The pharmaceutical formulations described herein may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in

10 the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s).

[0134] The compositions described herein may be formulated into any of many possible dosage forms such as, but not limited to, injectable formulations, and tablets, capsules, gel

15 capsules and liquids.

[0135] Pharmaceutical compositions herein include, but are not limited to, solutions, emulsions, foams and liposome-containing formulations. The pharmaceutical compositions and formulations herein described may comprise one or more penetration

20 enhancers, carriers, excipients or other active or inactive ingredients.

[0136] Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. Emulsions may contain additional components in addition to the dispersed phases, and the active drug which may

25 be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Microemulsions are included as an embodiment taught herein.

[0137] Formulations include liposomal formulations. The term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers.

30 Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior that contains the composition to be delivered. Cationic liposomes are positively charged liposomes which are believed to interact with

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negatively charged DNA molecules to form a stable complex. Liposomes that are pH-sensitive or negatively-charged are believed to entrap DNA rather than complex with it. Both cationic and noncationic liposomes have been used to deliver DNA to cells.

5 [0138] Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome comprises one or more
10 glycolipids or is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety.

[0139] In an embodiment, various penetration enhancers may be employed to effect the efficient delivery of nucleic acids. In addition to aiding the diffusion of non-lipophilic
15 drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs. Penetration enhancers may be classified as belonging to one of five broad categories, i.e., surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants.

20 [0140] One of skill in the art will recognize that formulations are routinely designed according to their intended use, i.e. route of administration.

[0141] The formulation of therapeutic compositions and their subsequent administration (dosing) are within the skill of those in the art. Dosing is dependent on severity and
25 responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary
30 depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC₅₀s found to be effective *in vitro* and *in vivo* animal models. In general, dosage is from 0.01 µg to 100 g per kg of body weight, and may be given once or

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more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy
5 to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 μ g to 100 g per kg of body weight, once or more daily, to once every 20 years. Alternatively, the antagonists are provided in amounts of 1, 10, 100 or 1000 μ /ml or amounts inbetween.

10 [0142] The present disclosure teaches a neuroprotective formulation comprising a Type 1 IFN-mediated signalling antagonist and one or more pharmaceutically acceptable carriers and/or diluents.

[0143] In another embodiment, the present disclosure teaches a neuroprotective
15 formulation comprising a Type 1 IFN-mediated signalling antagonist and one or both of LIF and/or CNTF and one or more pharmaceutically acceptable carriers and/or diluents.

[0144] Diagnostic assays to assess the presence of a neuroinflammatory response such as following a neuropathological event, disease or condition are enabled herein. For example,
20 following acute neuronal injury or disease, the level of Type 1 IFN-mediated signalling may be determined such as *via* the level of a Type 1 IFN, its corresponding mRNA levels, activity of IFNAR1 or its corresponding mRNA levels or *via* a down-stream effector such as TNF α , IL-6 or a chemokine or other pro-inflammatory effector molecule.

25 [0145] Hence, a medical protocol is enabled herein to treat a subject which has or may experience a neuropathological event, disease or condition, the protocol including:

- (i) assessing physical trauma to the brain or other parts of the CNS;
- (ii) determining parameters of inflammation;
- (iii) administering an antagonist of Type 1 IFN-mediated signalling; and/or
30 (iv) monitoring patients including subjecting the patient to behavioral modification procedures. Inflammatory parameters may be detected by any means including HPLC, TLC, ELISA, RIA, immuno-fluorescent assay, Southern analysis,

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Western blot, Northern analysis, gel electrophoresis, nucleic acid expression and the like.

[0146] Another aspect of the present disclosure enables a medical protocol for treating acute neuronal injury in a subject, the protocol comprising administering to the subject, within from 1 to 120 minutes of the injury, a neuroprotective formulation comprising the antagonist of Type 1 interferon-mediated signalling. By "1 to 120" include 1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 or 120 minutes. In an embodiment, the time is 30 minutes.

[0147] Still another aspect taught herein the use of a Type I IFN-mediated signalling antagonist in the manufacture of a medicament in the treatment or prophylaxis of neuroinflammation associated with a neurological condition or event. Such events and conditions include TBI and other acute neuronal injuries as well as neurodegenerative diseases such as associated with deposition of pathological amyloid (e.g. AD). In an embodiment, stroke is excluded from a neurological condition. In another embodiment, it is included in reference to a neurological condition.

EXAMPLES

[0148] Aspects taught herein are further described by the following non-limiting Examples. In these Examples, materials and methods as outline below are employed.

Animals

[0149] Mice (eight-week-old male mice 23 ± 3 g) used were of a C57BL-6 strain with IFNAR1^{-/-} (knockout or KO) or IFNAR1^{+/+} (wild-type or wt) genotypes (Hwang *et al.*, *Proc Natl Acad Sci USA* 92(24):11284-11288, 1995).

Tissue Culture

Mixed hippocampal and cortical neurons

[0150] Pregnant female mice were killed by cervical dislocation when embryos were 14 days old. The embryos were extracted from the female uterus and their heads removed into a petridish of Solution 1 (30ml Krebs stock (36.25g NaCl, 2g KCl, 0.7g NaH₂PO₄·H₂O, 13g d-Glucose, 0.05g Phenol red, 20.7g HEPES (acid form) in 500ml H₂O, pH7.4), 270ml H₂O, 0.9g bovine serum albumin (BSA), 2.4ml 3.4% w/v MgSO₄, pH 7.4). The brains were removed, cerebral cortices were isolated and the meninges removed. The cortices were placed into a fresh petri dish of solution 1 and minced to a fine paste. The paste was transferred to a 50ml Falcon tube containing solution 2 (15ml sol 1, 1.5ml 10x trypsin (25mg trypsin in 10ml)) using a pasture pipette. The tube was incubated for 15-20 minutes at 37°C with occasional mixing by inversion. 12.5ml of Solution 4 (8.5ml Sol 1, 4ml Sol 3) was added to the digested tissue. The tissue was then centrifuged (1000rpm, 45 seconds), supernatant discarded and pellet resuspended in solution 3 (7.5ml Sol 1, 0.75ml 10x DNase/soybean trypsin inhibitor (SBTI) stock (8mg DNase, 52mg SBTI in 10ml), 75μL 3.4% w/v MgSO₄) by passing through a pasture pipette for 2 minutes, the solution was then transferred to a fresh 50ml Falcon tube. Remaining tissue was washed from the old tube with 2 pasture pipettes of solution 5 (6.25ml Sol 1, 50μL MgSO₄ stock, 7.5μL 1.2% w/v CaCl₂). Upon centrifugation (1000rpm, 5 minutes) and supernatant removal, the resulting pellet was resuspended in 10ml plating medium (Neurobasal media (Gibco) containing 10% v/v fetal bovine serum (FBS, Gibco)). Cells were then counted and diluted to achieve a final concentration of 1.12×10^5 cells/6cm dish. All tissue culture dishes were coated with poly-

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l-lysine before neurons were plated. Following 5 hours in plating medium, all medium was exchanged for neuronal culture medium (Neurobasal media containing 10ml B27 supplement (Gibco), 1.25ml 200mM l-glutamine and 0.5ml Gentamycin (Gibco) in 500ml), thereafter half medium changes were performed every 2-3 days with a final concentration of 20 μ M 5-chlorocytosine arabinoside (ARAC) added to prevent proliferation of murine embryonic fibroblasts.

Human neuroblastoma M17 cells (M17)

[0151] M17 cells (ATCC [Registered Trademark] number: CRL-2267 [Trademark]) were cultured in T75 flasks with culture medium (OptiMEM (Gibco), 5% v/v FBS, 0.5% w/v Penicillin-Streptomycin (Gibco)) at 37°C until 90% confluent. Cells were then plated at the densities listed in Table 3. For the Alzheimer's disease studies, M17 cells were housed in an incubator at 37°C and 5% CO₂ in OPTI-MEN (Invitrogen) in 10% v/v FBS and 1% w/v penicillin/streptomycin.

TABLE 3

Densities

Dish	Cell density
24-well plate	5 x 10 ⁴ cells/well
6-well plate	1 x 10 ⁵ cells/well
6cm dish	1 x 10 ⁵ cells/dish

[0152] Plated cultures were then incubated for 24 hours at 37°C. Following incubation cells were serum-starved to halt the cell cycle with culture medium (no FBS) for 24 hours at 37°C, cells were then prepared for treatment.

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Isolation of Murine Cortical Neurons

[0153] A 14 day pregnant mouse was killed by cervical dislocation and uterine horns were removed and placed in a 10cm dish containing working solution 1 (For 300ml: 30ml Krebs 10x stock, 270ml MilliQ, 0.9g BSA, 2.4ml 3.82% w/v MgSO_4). Fetuses were removed from placental sacks, decapitated and heads were placed in a new dish containing solution 1. Under a dissection microscope skull and meninges were removed and cortical tissue was placed in a dish and chopped finely with a scalpel. Flamed Pasteur pipettes were then used to separate tissue into tubes using 20ml solution 1 with 10% w/v trypsin added. Cells were then incubated for 15 minutes at 37°C with occasional shaking. 25ml of solution 1 with 3% w/v added 10X DNase (Sigma D-5025)/SBTI (Sigma T-9003) was then added and gently inverted until DNA strings appeared. Cell suspension was then spun for 1 minute (1000rpm, RT) and supernatant removed. Cell pellet was then resuspended in 8ml of solution 1 with 10% w/v DNase/STBI by 60 pasteur pipette passages and spun for 5 minutes at 1000rpm at RT. Following discarding of supernatant, cell pellet was resuspended in 10ml of Dulbecco's Modified Eagle Medium (Invitrogen) with 10% v/v FBS. The suspension was placed in a T-75 flask and allowed to incubate at 37°C for 1 hour to allow fibroblast adhesion. Cell media containing only neurons was then removed from the flask, cell density determined, and plated into appropriate poly-L-lysine coated plates and kept in an incubator (37°C and 5% v/v CO_2). Four hours following plating Neurons are given a full media change to DMEM with 10% v/v FBS and 0.5% w/v penicillin/streptomycin.

Tissue culture treatments***Oxygen Glucose Deprivation (OGD)***

[0154] To engender OGD, culture media was replaced with no glucose and FBS DMEM (0.5 standard culture media volume) and cells were introduced into an hypoxic chamber (1% v/v O_2 , 5% v/v CO_2 , 94% v/v N_2 and 60% v/v humidity) for 3 hours (M17 cells) or 4 hours (WT or $\text{IFNAR1}^{-/-}$ neurons). Cells were then removed from the chamber and reperfed with DMEM (high glucose, 0.5 standard culture media volume) for 0, 0.5, 2 or 24 hours. After this reperfusion event, cells were immediately utilized for MTT assay, RT-PCR or Western blot analysis.

IFN α /IFN β cytokine treatment

[0155] 6cm dishes of M17 cells were treated with human (hu) IFN α (huIFN α) and human IFN β (huIFN β) at 1, 10, 100 or 1000U/ml for a time course of 5, 10 or 30 minutes whilst incubated at 37°C. Post-treatment harvested for Western blot analysis.

5

SDS PAGE gel protein separation and western blotting

[0156] Cells (6cm dish) were scraped in 1ml PBS/plate and centrifuged at 5000x g for 5 minutes. The resultant cell pellet was resuspended in 100 μ L of lysis buffer (50mM Tris HCl, 150mM NaCl, 1% v/v Triton x-100, 1% v/v SDS, 1 tablet PhosphoSTOP (Registered Trademark) and protease inhibitor (Roche), pH 7.4) and lysed *via* sonication. 60 μ g of protein was added to 1:1 v/v reducing buffer (2.5ml 0.5M Tris, 2ml Glycerol, 4ml 10% w/v SDS, 1ml (3-mecaptoethanol, bromophenol blue, in 10ml) and heated to 99°C for 5 minutes. 10% w/v acrylamide SDS PAGE (17.85ml dH₂O, 11.25ml 1.5M Tris, 450 μ L 10% w/v SDS, 15ml 30% w/v Acrylamide/BIS (Bio-rad), 15 μ L N,N,N',N'tetramethylethylenediamine (TEMED, Bio-rad), 450 μ L 10% w/v ammonium persulphate (APS), per 5 gels) in running buffer (0.1%w/v SDS, 1.44% v/v glycine, 0.3% w/v Tris, pH 8.4) was used to separate the denatured protein. Samples were loaded on a stacking gel (3ml dH₂O, 1.25ml 0.5M Tris, 100 μ L 10% w/v SDS, 650 μ L Acrylamide/BIS, 7.5 μ L TEMED, 100 μ L APS) and run at 80-120V for 1.5 hours. Polyvinylidene fluoride (PVDF) membrane was presoaked in 100% v/v methanol for 5 minutes and washed in transfer buffer (20% v/v methanol in running buffer). Separated proteins were transferred from PAGE gel to PVDF membranes utilizing a semi-dry transfer unit (60mA/gel for 75 minutes). Membranes were blocked with 5% w/v skim milk powder in TBS-T (10mM Tris, 15mM NaCl, 0.01% v/v Tween-20) for 1 hour. Phospho-STAT-1, Phospho-STAT-3 (Cell Signalling) and α -tubulin (Millipore) antibodies were diluted 1:1000 in 5% w/v BSA in TBS-T and incubated on membranes at 4°C overnight. Membranes were then washed 3 times for 5 minutes with TBS-T and incubated with goat anti-rabbit or goat anti-mouse (for (3-tubulin) horseradish peroxidase (HRP) conjugated secondary antibodies, diluted 1:1000 in 5%w/v BSA in TBS-T, for 60 minutes at 25°C. HRP product was detected using an ECL advance western blotting detection kit (Amersham) After 4 washes of 5 minutes in TBS-T the ECL solution was applied to the membrane and visualized using the IQ350 imaging machine (GE Healthcare).

30

Real-Time Polymerase Chain Reaction (RT-PCR)***TRIzol (Registered Trademark) mRNA extraction***

[0157] Cells were plated at 2×10^5 cells/6cm dish and incubated overnight in OptiMEM (5% w/v FBS, 0.5% w/v Penicillin-Streptomycin). Post 3 hours OGD and 0, 0.5, 2 or 24 hours reperfusion media was removed and cells were washed in 1ml/6cm dish PBS. Cells were lysed in 1ml/6cm dish TRIzol (Registered Trademark) [Invitrogen], transferred to a 1.7ml microcentrifuge tube and incubated for 10 minutes at 25°C. 0.2ml/sample chloroform was added and after vigorous shaking was incubated at 25°C for 3 minutes. Samples were centrifuged (12,000x g, 15 minutes, 4°C) and resultant aqueous layer containing RNA was removed to fresh 1.7ml microcentrifuge tube. RNA was precipitated by adding 0.5ml/sample isopropanol and incubating at 25°C for 10 minutes. Following centrifugation (12,000x g, 10 minutes, 4°C), the supernatant was removed and resultant pellet was washed with 1ml/sample 75% v/v EtOH in DEPC treated dH₂O and centrifuged again (7,500x g, 5 minutes, 4°C). The RNA pellet was air-dried and redissolved in 30μL/sample RNAase free water. Sample RNA concentration was then analyzed by the nanodrop 1000 spectrophotometer (Thermo-scientific).

Reverse transcription of RNA to cDNA

[0158] RNA was reversed transcribed into cDNA using SuperScript (Registered Trademark) III kit (Invitrogen) according to manufacturer guidelines. Briefly, 1μg of sample RNA was converted to cDNA using reverse transcriptase under the following conditions: 25°C for 10 minutes, 50°C for 30 minutes then 85° for 5 minutes. Residual RNA was digested by the addition of RNase H to ensure purification of cDNA which was 1:10 in dH₂O for use in RT-PCR.

RT-PCR using Taqman (Registered Trademark) probes

[0159] Taqman probes (all reagents from Applied Biosystems) were used as RT-PCR primers, these included: Tumor Necrosis Factor α (TNFα, Hs00174128_m1), Interleukin-1β (IL-1β, Hs00174097_m1), Interleukin-6 (IL-6, Hs00985639_m1), Interferon-α1 (IFNα1, Hs00256882_sl), Interferon-β (IFNβ1, Hs01077958_sl) and 18s ribosomal RNA (18s rRNA, 4352930E). RT-PCR was performed in a 384-well plate with 4μL diluted

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cDNA, 0.5µL DEPC dH₂O, 0.5µL Taqman primer, 5µL Taqman Fast Universal PCR master mix (2x) per well. Each sample was repeated in triplicate and RT-PCR performed using the 7900ht Fast Real-Time PCR system (Applied Biosystems) under the following conditions: 50°C for 2 minutes, 94.5°C for 10 minutes, (97°C for 30 seconds, 59.7°C for 1 minute)x 40 repeats. Data were collated and fold change calculated using the AACt method.

Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)

[0160] Brains were dissected into ipsilateral and contralateral hemispheres 2, 4 and 24 hours after injury, or sham operation. Hemispheres were homogenized in 2 ml Trizol (Invitrogen), and placed at room temperature for 10 minutes. 0.2ml Chloroform (Chem Supply) per 1ml Trizol was added to the samples, and samples were centrifuged at 12000g for 15 minutes at 4°C to separate samples into phases. The colorless, aqueous phase of each sample, which contained RNA, was transferred into a new 1.7ml microcentrifuge tube. RNA was precipitated by adding 0.5ml Propan-2-ol (Chem Supply) per 1ml Trizol, and samples were again centrifuged at 12000g for 10 minutes at 4°C. The supernatant from the tubes was discarded, and the RNA pellet was washed with 75% v/v Ethanol (Chem Supply) in Diethyl pyrocarbonate (DEPC)-treated water (Sigma), vortexed and centrifuged at 7500g for 5 minutes at 4°C. The RNA pellet was air-dried and redissolved in RNase-free H₂O (Invitrogen). Concentration of the RNA samples was assessed using the NanoDrop 1000 Spectrophotometer (ThermoScientific).

[0161] 1 µg of RNA was transcribed per cDNA reaction using the SuperScript III kit (Invitrogen) according to these conditions: 10 minute incubation at 30°C, 30 minute incubation at 50°C, 5 minute incubation at 85°C, and finally, a 20 minutes incubation at 37°C with 1µl *E. coli* RNase H (from the same kit).

[0162] Samples were diluted 1:10 in RNase free H₂O. The following Taqman primers were obtained from Applied Biosciences: TNFα (ID: Mm00443258_m1), IFNβ (ID: Mm00439552_s1), IL-1β (ID: Mm01336189_m1) and 18S rRNA (ID: 4352930E). Taqman primers were incubated along with sample in a 384 well plate (MicroAmp, Singapore) in the following ratios: 0.5µl primer, 0.5µl H₂O, 5µl 2x Taqman Mix (Invitrogen) and 4µl diluted cDNA. SybrGreen primers were obtained from GeneWorks

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(sequences are listed in Figure 14): Allalpha forward, Allalpha Reverse 1, Allalpha Reverse 2, GADPH forward and GADPH reverse. SybrGreen primers were incubated in the following ratios: 2µl diluted cDNA, 5µl Fast SybrGreen Master Mix, 1µl Forward GADPH primer, 1µl Reverse GADPH primer and 1µl H₂O. The plate was incubated according to the following conditions and read using the 7900ht spectrophotometer (Applied Biosciences): 95°C for 20 minutes, 95°C for 3 minutes (40 repeats), 60°C for 30 minutes, 95°C for 15 minutes, 60°C for 15 minutes and 95°C for 15 minutes. Ct values were obtained for each sample, and relative transcript levels for each gene were calculated using the SSCT method.

10

MTT cell viability assay

[0163] M17 cells in 24-well plates were subjected to OGD and subsequent reperfusion (24 hours). Post-reperfusion 50µL/well MTT reagent (2mg/ml, Sigma) was added to media and incubated for 1 hour at 37°C. Culture medium was then removed and cells were solubilized with 200µL/well of dimethyl sulfoxide (DMSO) of which 100µL was transferred to a clean 96-well plate and absorbance read at 595nm.

Real Time PCR for Alzheimer's work

RNA Extraction

20 [0164] Cells were homogenized using TRIzol (Registered Trademark) [Invitrogen] as per manufacturers instructions. Briefly this involved incubating 1ml TRIzol (Registered Trademark) per 50-100µg tissue for 5 minutes at RT. Chloroform was added to induce phase separation and tubes were shaken vigorously. Cell suspension was spun at 12,000g (15 minutes, 4°C) and upper RNA-containing aqueous phase was placed in a new tube. RNA was then precipitated by adding isopropyl alcohol and incubated for 10 minutes at RT. RNA was then spun at 12,000g for 15 minutes and supernatant removed. RNA was then washed in DEPC ethanol, allowed to air dry and resolubilized in RNase-free H₂O. RNA concentrations were then determined using a Nanodrop 2000.

cDNA Preparation

30 [0165] Conversion of RNA to cDNA involved the use of a High Capacity RNA to cDNA kit (Applied Biosystems) as per manufacturers instructions. Briefly this involved loading

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1µg of RNA with 10µl 2X reaction mix with 1 µl RT enzyme mix and DEPC H₂O to 20µl. Tube was then heated at 35°C for 30 minutes, and 95°C for 5 minutes (termination step) and chilled on ice.

5 **RT PCR**

[0166] Relative expression levels of RNA were measured using the prepared cDNA in accordance with the TaqMan (Registered Trademark) [Applied Biosystems] primer preparation. This involves adding in triplicate 4µl cDNA with 5µl 2X TaqMan Mix, 0.5µl DEPC H₂O, and 0.5µl of primer of mRNA in question. PCR plate was then read on a
10 7900ht Fast Real-Time PCR System. Fold increase was determined by using the delta-delta method.

Gateway (Registered Trademark) cloning system

Ligation of subclone AttB1 and subclone Attb2 primers to mIFNAR1/mIFNAR2 genes

15 [0167] cDNA encoding mouse interferon receptor α 1 (mIFNAR1) and mouse interferon receptor α 2 (IFNAR2) were custom synthesized and inserted into vector puc57 by GeneScript (U.S.A.). Subclone forward primers containing AttB1 motif and subclone reverse primers containing AttB2 motif were designed and synthesized (Geneworks) for both mIFNAR1 and mIFNAR2 genes to use with the Gateway (Registered Trademark)
20 cloning system (Invitrogen). These primers were ligated to the mIFNAR1 and mIFNAR2 genes *via* PCR using GoTaq (Registered Trademark) polymerase (Promega). The nucleotide sequences are shown in Figures 6 and 7.

Polymerase chain reaction conditions

25 [0168] Briefly a 20µL sample reaction consisted of 4µL 5x GoTaq buffer, 1µL MgCl₂ (25mM), 0.4µL dNTPs (2mM), 0.2µL Subclone AttB1 primer (20µM), 0.2µL Subclone AttB2 primer (20µM), 0.1µL GoTaq polymerase (5U/µL), DEPC H₂O (to 20µL). The ligation reaction was performed under the conditions given in Table 4.

TABLE 4
Ligation conditions

Step	Temperature	Duration
Initial Denaturation	95°C	2 minutes
Denaturation	95°C	40 seconds
Annealing	60°C	40 seconds
Extension	72°C	110 seconds
Final Extension	72°C	5 minutes

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Purification of Att81-mIFNAR1/2 -Att82 PCR product

[0169] Samples were then electrophoresized on a 1% w/v agarose gel (1g agarose, 2μL Ethidium Bromide per 100ml TAE buffer (242g Tris Base, 57.1ml glacial acetic acid, 100ml 0.5M Ethylenediaminetetra-acetic acid (EDTA) per Liter)) and a 1.77kb
5 (mIFNAR1) or 1.54kb (mIFNAR2) was selectively cut from the entire gel. The gel bound AttB1-mIFNAR1/2-AttB2 ligation product was then purified from the PCR reaction using the QIAquick (Registered Trademark) PCR Purification Kit (QIAGEN) according to manufacturer's protocol. Briefly, the gel containing AttB1-mIFNAR1/2-AttB2 was redissolved and DNA was retained on a silicon filter whilst impurities were discarded.
10 Post-washing with ethanol buffer the purified DNA was then dissolved in elution buffer (10mM Tris-HCL, pH8.5).

The BP reaction

[0170] The Invitrogen Gateway (Registered Trademark) cloning system was used. The
15 purified AttB-mIFNAR1 and AttB-mIFNAR2 PCR products were translocated into vector pDONR201 (Invitrogen) using the BP Clonase (Trademark) II enzyme kit (Invitrogen), according to manufacturers guidelines. A 10μL reaction comprised of 0.6μL pDONR201 (289ng/μL), 1.8μL AttB-mIFNAR1 PCR product (33ng/μL) or 1.5μL AttB-mIFNAR2 PCR product (37ng/μL), 2μL BP Clonase (Trademark) II enzyme mix, TE buffer to 10μL.
20 The reaction was incubated for 24 hours at 25°C and subsequently terminated upon addition of Proteinase K (1μL, 2μg/μL).

DH5α bacterial transformation

[0171] The BP reaction product was transformed into DH5α competent *E. coli* cells
25 (Invitrogen) by adding 1μL BP reaction product to each DH5α aliquot (50μL/tube). Cells were then incubated on ice for 30 minutes and heat-shocked by incubation at 42°C for 30 seconds. 250μL/tube of S.O.C. medium (Invitrogen) was added and cells were shaken (37°C, 225rpm, 1 hour) before 20μL was spread evenly on a kanamycin (50μg/ml) selective agar plate (1% w/v Tryptone, 1% w/v NaCl, 0.5% w/v Yeast extract, 0.75% w/v
30 agar) and incubated for 16 hours at 37°C. A single bacterial colony was picked for colonies PCR to verify transformation success. The selected colony was grown in 10μL Luria Broth (LB, 1% w/v Tryptone, 1% w/v NaCl, 0.5% w/v Yeast Extract) of which 5μL was lysed at

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99°C for 5 minutes. PCR was performed using the conditions above to confirm successful mIFNAR1/2 insertion into pDONR201 and the subsequent DH5 α colony. The PCR products were then electrophoresized on a 1% w/v agarose gel and 1.77kb (for mIFNAR1) or 1.54kb (for mIFNAR2) bands were visualized using the IQ-350.

5

Isolation of pDONR201-mIFNAR1/2 plasmid

[0172] Once the selected colony was deemed positive for pDONR201-mIFNAR1/2 insertion, the remaining 5 μ L of culture (not used in PCR) was supplemented with a further 5ml kanamycin (50 μ g/ml) selective LB media and shaken for 16 hours (225rpm, 37°C).

10 Plasmid DNA was then isolated from the bacterial culture using QIAprep (Registered Trademark) Spin Miniprep kit (QIAGEN) as per manufacturer's protocol. Briefly, bacteria were lysed, excess protein precipitated and DNA retained in the filter of a spin column. After subsequent washing with an ethanol buffer, DNA was eluted from the filter column giving isolated pDONR201-mIFNAR1/2 plasmid. DNA yields were then measured using
15 the nanodrop 1000.

The LR reaction

[0173] The mIFNAR1/2 genes were removed from the pDONR201-mIFNAR1/2 plasmid and translocated into a pcDNA6.2/cEM-GFP destination vector using the LR Clonase
20 (Trademark) II kit (Invitrogen) as per manufacturers protocol. A 10 μ L reaction consisted of 1 μ L pDONR- mIFNAR1 (122.6ng/ μ L) or 1 μ L pDONR-mIFNAR2 (156.9ng/ μ L) entry clones, 2 μ L pcDNA6.2/cEM-GFP (75ng/ μ L) destination vector, 2 μ L LR Clonase (Tradeamrk), TE buffer to 10 μ L. The reaction was incubated for 24 hours at 25°C and subsequently terminated upon addition of Proteinase K (1 μ L, 2 μ g/ μ L). Reaction samples
25 were then transformed into DH5 α competent *E. coli* cells as per above. A colony was selected from an ampicillin (50mg/ml) agar plate and grown in 10 μ L LB media of which 5 μ L was lysed by heating (99°C, 5 minutes). PCR was then performed under the conditions described above and samples run on a 1% w/v agarose gel. Imaging the gel using the IQ-350 showed a 1.77kb mIFNAR1 specific or a 1.54kb mIFNAR2 specific band for
30 successful LR reaction and transformation samples. Positive colonies were then selected and shaken in 250ml ampicillin (50 μ g/ml) selective LB media for 16 hours (225rpm, 37°C).

Isolation of final pcDNA6.2/cEM-GFP-mIFNAR1/2 plasmids

[0174] Bacterial plasmid DNA was isolated using EndoFree (Registered Trademark) plasmid Maxi Kit (QIAGEN) following manufacturers protocol. Briefly, bacteria were
5 lysed, excess protein precipitated and DNA retained in the filter of a gravity flow column. After subsequent washing with an ethanol buffer DNA was eluted from the gravity flow column. The DNA was then precipitated using isopropanol, washed with endotoxin-free ethanol and resuspended in supplied TE buffer. DNA yields of pcDNA6.2/cEM-GFP-mIFNAR1/2 plasmids were then determined using the nanodrop 1000.

10

Statistical analysis

[0175] All numerical data are stated as mean $\pm$ SEM and analyzed using GraphPad Prism 5.0. RT-PCR and MTT assay data were analyzed using Student's t-test or one-way ANOVA and subsequent Dunnett's Post-hoc test where applicable. For all statistical tests
15 $P < 0.05$ was considered significant. For qRT-PCR data, a one way Analysis of Variance (ANOVA) was also performed followed by Bonferroni's post-hoc analysis, with a value of $P < 0.05$ considered statistically significant. Infarct volume values were analyzed using an unpaired Student's t-test, with a value of $P < 0.05$ considered statistically significant.

20 Animal model of Traumatic Brain Injury

[0176] Wild-type mice were injected intravenously with either monoclonal antibody (25mg/kg, Anti-mouse interferon α/β receptor [IFNAR1], Leinco Technologies Inc) or an Isotype control (25mg/kg, IgG Isotype control, Leinco Technologies Inc) 1h before the procedure. Mice were given a Controlled Cortical Impact (CCI), as described by Dixon *et al.*, *J Neurosci Methods* 39:253-262, 1991. Mice were anaesthetized for 5-10 seconds with
25 gaseous Isoflurane (1ml/ml, VCS, NSW, Aus), followed by an intra-peritoneal injection of Ketamine (100mg/kg, Parnell)/ Xylazine (10mg/kg, Parnell). A sagittal scalp incision was made to expose the underlying parietal bone and a 2 mm burr hole was drilled using a Dremel 10.8V drill with a 0.8mm tip (Dremel, Europe) into the skull above the right
30 parietal cortex, 1.5 mm posterior to bregma and 2.5 mm lateral to the midline. The rounded section of bone was removed to expose the underlying cortex. Mice were placed on a stereotaxic frame and a 1.5mm deep impact was made using a computer-controlled

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impactor into the brain on the exposed cortex. The following parameters were set onto the programme linked to the impactor (LinMot-Talk 1100): withdrawal of tip 20mm away from resting position at 1m/s, impact 21.5mm deep at 5m/s, interval of 100ms and withdrawal of tip 1.5mm towards resting position at 1m/s. Following impact, the removed
5 bone section was placed back onto the skull, with a small section of paraffin to close up the brain. The incision was closed up with a silk 5.0 metric suture (Syneture Tyco Healthcare). Mice were given Buprenorphine intra-peritoneally (0.6 mg/kg, Reckitt Benckiser Healthcare) and placed on a heat mat for postsurgical recovery. ARIB6 knockout mice (IFNAR1^{-/-}) were used for some TBIs, and compared to C56BL/6J mice. Sham controls
10 underwent anesthesia, scalp incision and one removal, but no injury, and then were stitched up, given analgesic and put on a heat blanket for recovery.

Preparation of serial sections for staining

[0177] Mice were transcardially perfused at various time points after injury (or sham
15 surgery) with 0.1% v/v heparinized Phosphate-Buffered Saline (Pfizer), followed by 4% v/v paraformaldehyde (Scharlab S.L.), and their brains dissected. Brains were cut by the Histology facility, University of Melbourne. Brain sections were cut into 10µm coronal sections starting at the rostral end, paraffin-embedded and mounted onto glass slides. Every 10th slide was stained with Haematoxylin and Eosin (H&E).

20

Infarct volume analysis

[0178] Infarct volume analysis was conducted on slides, which had been stained with H&E. Analysis was performed using Image J. Area of infarct was calculated by measuring around regions appearing less intensely stained for viable cells. The average area of infarct
25 per brain was then multiplied by the number of sections per slide by the thickness of each section (10µm) and the number of slides in the sequence to get the volume of infarct.

Immunohistochemistry

[0179] Paraffin-embedded sections were put in a 60°C oven for 20 minutes and taken through a series of Histolene (Lomb Scientific) incubations, followed by 100, 95 and 70% ethanol. Sections were incubated in Phosphate-Buffered Saline for a further 5 minutes.

5 Sections were blocked firstly for endogenous peroxidase activity with a Peroxidase blocking solution (DAKO), followed by a protein-blocking buffer (5% v/v whole Goat serum [Invitrogen] and Triton X-100 solution [Sigma] in 1x Phosphate Buffered Saline) for another hour. Where sections were used for immunofluorescence, only protein-blocking buffer was used. The primary antibody was diluted in an appropriate dilution

10 buffer (1% w/v BSA [Bovogen] in 1 x Phosphate Buffered Saline), and slides were incubated with the antibody overnight at 4°C. Primary antibodies used were: NeuN (Millipore) and Mac-1 (Monash University), [Flentjar *et al.*, *Exp Neurol* 177(1):9-20, 2002]). Sections were washed in PBS, and incubated in an appropriate secondary antibody. Fluorescent secondary antibodies (Alexa Fluor 594 anti-mouse and rabbit, Alexa Fluor 488

15 anti-mouse, rabbit and rat) were obtained from Invitrogen and the biotinylated secondary antibody (Anti-Mouse IgG, horse biotinylated) was obtained from Vector Laboratories. Biotinylated primary antibodies were visualized using the Vector Vecstain ABC kit (Vector Laboratories) using Diaminobenzidine (DAB [DAKO]) as a substrate.

20 Magnetic Resonance Imaging

[0180] Wild-type mice were intra-venously injected with IgG Isotype control (25mg/kg) or MAR1 antibody (25mg/kg) 1 hour before giving TBI, and imaged both 2 and 24 hours after TBI. Mice were initially anaesthetized with approximately 3% Isoflurane in a 1:1 mixture of medical grade air and oxygen. Anesthesia was maintained throughout scanning with

25 0.25-1.5% Isoflurane through a nosecone placed over the animal's snout and respiration was continuously monitored throughout the experiment with a pressure sensitive probe positioned over the animal's diaphragm. Anaesthetized animals were laid supinely on a purpose built animal holder and their head fixed into position with ear and bite bars. A surface receive coil was placed over the animals head and the cradle was inserted into a

30 transmit coil fixed inside a BGA12S gradient set for imaging with a 4.7 Tesla Bruker Biospec 47/30 scanner. The scanning protocol consisted of a 3-plane localizer sequence followed by multi-slice axial, coronal and sagittal scout images to ascertain the orientation

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and position of the brain. To calculate a T2 map, three T2-weighted images were acquired using a rapid acquisition, relaxation enhanced (RARE) sequence with the following imaging parameters: recovery time (TR) = 3000 ms, RARE factor = 8, field of view (FOV) = 12.8 x 12.8 mm², matrix size = 128 x 128, in-plane resolution = 100 x 100 μm², number of
 5 slices = 15, slice thickness = 0.6 mm, averages (NEX) = 16 and total scan time of 12 minutes. The effective echo times (TE_{eff}) were: 45 ms, 60 ms and 80 ms. Diffusion weighted images (DWI) were also acquired with the following parameters: TR = 3000 ms, TE = 60 ms, FOV = 12.8 x 12.8 mm², matrix size = 128 x 128, in-plane resolution = 100 x 100 μm², number of slices = 6, slice thickness = 0.6 mm, number of repetitions (NR) = 4 and total
 10 scan time of 12 minutes 48 seconds per repetition. A single b₀ and diffusion image were acquired with the following diffusion parameters: Δ = 7 ms, δ = 14 ms and b-value = 1500 s/mm². Images were analyzed for infarct volume using Matlab R2010b (Mathworks).

Preparation of Soluble β -Amyloid

15 [0181] In order to effectively aliquot smaller quantities for storage, β -Amyloid 1-42 peptide (Invitrogen) was solubilized in 500 μl 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) per 1 mg peptide weight and aliquoted 50 μl per tube. HFIP was then evaporated off using a speed vac for 20 minutes. Aliquots were then stored at -80°C until required. For experiments, aliquoted (~100 μg) β -Amyloid was dissolved in 130 μl of 20 mM NaOH and
 20 vortexed until properly dissolved. Tube was then placed in a sonicator water bath on ice for 15 minutes. Following sonication 455 μl of dH₂O and 65 μl of Dulbecco's PBS (Ca and Mg free) is added. Subsequent viability experiments use the same ratios of NaOH and PBS as a control vehicle. The concentration of the dissolved β -Amyloid was determined by measuring OD₂₁₄ using a spectrophotometer. An aliquot of 95 μl of water was added to a
 25 cuvette to blank the unit, and 5 μl of the peptide suspension was added to the cuvette and thoroughly mixed. The sample concentration was calculated based on the molar extinction coefficient of β -Amyloid, which is 75887 L/mole/cm. Thus, Sample concentration (Molar) of A β 42 = Abs₂₁₄ x Dilution Factor/75887.

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MTS Viability Assay

[0182] CellTiter 96 (Registered Trademark) AQueous MTS Reagent (Promega) was prepared according to manufacturer's instructions. Briefly, this involved 42mg of MTS Reagent powder dissolved in 21ml Dulbecco's PBS solution, pH to 6.0-6.5 and filter sterilized. For viability assays, 20 parts MTS solution was combined with 1 part phenazine methosulfate (PMS, Sigma) and 1/5 final well volume was added to cell wells in triplicate (i.e. 80µl MTS/PMS in 400µl well). MTS well preparation was then allowed to incubate for 2 hours at 37°C and absorbance was measured at 490nm. A Students T-Test was used to test statistical significance using Graphpad Prism 5 Software.

10

Western Blot Analysis

[0183] Protein was extracted by removal of media and scrapping cells into a tube using PBS. Cells were spun to a pellet at 5000g (5mins, 4°C). Supernatant was removed and cells were re-suspended in 150 µl Lysis Buffer (10uM Tris-HCl, 2% w/v SDS, Protease inhibitor, PMSI) and machinated using a probe sonicator.

15

[0184] Protein levels were detected using Western Blot Analysis. The 12% w/v resolving gel (8ml 30% w/v Acrylamide, 5ml 1.5M Tris, 200µl 10% w/v SDS, 6.68ml mQH₂O, 200µl 10% APS, 20µl TEMED) was prepared days in advance with the 5% w/v stacking gel (1.67ml 30% w/v Acrylamide, 2.5ml 0.5M Tris, 100µl 10% w/v SDS, 5.67ml mQH₂O, 100µl 10% w/v APS, 10µl TEMED) poured the day the gel was run. 50µg of protein was taken with an equal volume of 2XTris-glycine sample buffer with 5% v/v b-mercaptoethanol, boiled for 5 minutes at 95 degrees and loaded into the well. A ladder consisting of 2µl SeeBlue (Registered Trademark) Marker 2µl MagicMark (Trademark) [Invitrogen] and 6µl PBS was used. Gels were run at a voltage of 80mV until protein passed the stacking gel, when voltage was kept constant at 120mV. Protein was transferred from gel to membrane using a semi-dry apparatus at a constant current of 60mA per gel for 1 hour. Membranes were then blocked in 5% w/v milk powder (In TBST) for 1 hour. Primary antibodies (1/1000) were incubated at 4°C overnight on a roller. Membranes were rinsed three times with TBST and secondary antibodies (1/1000) were then incubated at RT in 5% Milk for 1 hour. Membrane were developed using ECL (Amersham) and imaged on an IQ350.

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Immunohistochemistry

[0185] Cells plated on glass cover slips were harvested for immunohistochemistry by removing cell media, rinsing three times with ice-cold PBS and fixing with 4% v/v para-
5 formaldehyde for 15 minutes at RT. Cells were then permeabilized using 0.2% v/v Triton X-100 in PBS at RT for 20 minutes and rinsed three times in PBS. CAS-Block (Trademark) [Invitrogen] was then used to block at RT for 1 hour. Cells were then incubated overnight with primary antibody (1/100) in CAS-Block (Trademark). Following primary antibody binding, cells were rinsed in PBS three times and incubated 2 hours with
10 the secondary antibody in CAS-Block (Trademark). Cells were then rinsed three times in PBS and mounted onto slides using Vectashield (Registered Trademark) mounting media with DAPI.

Materials and methods for Parkinson's disease work

15 Materials

[0186] Unless otherwise stated all chemicals and biological were acquired from Sigma-Aldrich. All H₂O refers to MilliQ dH₂O (Millipore).

Animals

20 [0187] Mice used were a C57BL-6 strain with IFNAR1^{-/-} (knockout) or IFNAR1^{+/+} (wild type) genotypes (Hwang *et al.*, *Supra* 1995) as described above.

Human neuroblastoma M17 cells (M17)

[0188] BE(2)-M17 (M17) human neuroblastoma cells were obtained from American Type
25 Culture Collection (ATCC) and were routinely cultured in T75 flasks in culture medium (OptiMEM (Gibco), 10% v/v FBS, 1% w/v Penicillin-Streptomycin (Gibco)) at 37°C/5%CO₂ until 90% confluent. Cells were plated out for experimental use at the following densities:

5x10⁴ cells/well (24-well plates) for cell viability assays

30 1x10⁵ cells/plate (6cm plates) for western blot or QPCR analysis.

Cells were incubated for 24 or 48 hours at 37°C/5% v/v CO₂ prior to treatments.

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Generation of M17 IFNAR1 KD cells

[0189] IFNAR1 (IFNAR1-KD) or negative control (NC) knockdown M17 cells were prepared as follows. M17 cells were transfected using FugeneHD (Roche) with HuSH
5 shRNA plasmids containing a IFNAR1 specific shRNA cassette or non-effective 29-mer scrambled shRNA cassette with a GFP tag (Origene). Clonal cell lines were generated using the selectable marker puromycin (0.5mg/mL) (GIBCO) and maintained in OptiMEM containing 10% v/v FBS and 1%w/v penicillin-streptomycin. Successful knockdown of IFNAR1 expression was confirmed by QPCR where IFNAR1-KD levels were compared to
10 NC M17cells.

6-OHDA and rotenone toxicity treatment

[0190] 24 well plates were treated with 6-OHDA and rotenone at the indicated concentrations for 24 hours before cell viability was assessed by MTT assay.

15

[0191] Dishes of 6cm were treated with 6-OHDA and rotenone (0.001% v/v DMSO vehicle) at the indicated concentrations over a time course period of 30min, 1, 2, 4, 8, 24 and 48 hours at 37 degrees/5% v/v CO₂. Cells were subsequently harvested for western blot or quantitative real time Polymerase Chain Reaction analysis (QPCR).

20

MTT cell viability assay

[0192] M17 cells were treated with neurotoxins in 24 well plates. Post treatment 50µl of 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reagent (2mg/ml, Sigma) was added to each well and incubated for an additional 3 hours at 37°C/5% v/v
25 CO₂. Culture medium was then removed and cells were solubilised with 200µl per well of dimethyl sulphoxide (DMSO). 180µl was transferred into a 96 well plate and absorbance read at 595nm using plate reader (Multiskan Ascent).

SDS PAGE gel protein separation and western blotting

30 [0193] Post-treatment, media was removed and 6cm dishes were rinsed in PBS before being harvested on ice in 1ml PBS and centrifuged at 5000x g for 5 minutes. The supernatant was removed and the pellet was resuspended in 60µl of protein lysis buffer

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(10mM Tris HCl pH 7.4, 2% w/v SDS, PhosphoSTOP® and protease inhibitor (Roche)), and lysed by sonication.

[0194] 50µg of protein was added to a 1:1 v/v reducing buffer (2x Tris-Glycine sample buffer (Invitrogen), 5% v/v β-mercaptoethanol) and heated at 95°C for 5 minutes. A 10% v/v acrylamide SDS PAGE gel was used to separate denatured proteins (per 2 gels, 7.90ml dH₂O, 5ml 1.5M Tris, 200µl 10% SDS, 6.70ml 30% w/v acrylamide/BIS (Bio-rad), 20µl N, N,N',N'- tetramethylethylenediamine (TEMED, Bio-rad), 200µl 10% ammonium persulphate (APS)), in running buffer (0.1%w/v SDS, 1.44% w/v glycine, 0.3% w/v Tris, pH 8.4). Samples were loaded on a stacking gel (5.62ml dH₂O, 2.5ml 0.5M Tris, 100µl 10% SDS, 1.67ml acrylamide /BIS, 10µl TEMED, 100µl APS) and run at 80-120V for 1-1.5 hours. Polyvinylidene fluoride (PVDF) membrane was pre soaked in 100% v/v methanol for 30seconds and rinsed in dH₂O for 2minutes before being placed in transfer buffer (20% v/v methanol in running buffer). The separated denatured proteins were transferred from PAGE gel to PVDF membrane by using a semi dry transfer unit (60mA/gel for 70minutes). Membranes were washed 3 x 10minutes with TBS-T and incubated with goat anti-rabbit (P-STAT3 or total-STAT3) or goat anti-mouse (β-tubulin) horseradish peroxidase (HRP) conjugated secondary antibodies (diluted 1:1000 in 5% w/v BSA in TBS-T), for 90 minutes at room temperature. Membranes were again washed 3 x 10minutes in TBS-T before detection using an ECL advance western blotting detection kit (Amersham) and visualisation with an IQ350 imaging machine (GE Healthcare).

Human Parkinson's disease (PD) samples

[0195] Human PD brain samples and their associated controls were obtained from the Australian Brain Bank. Samples were homogenized using a glass dounce homogeniser containing 1ml of Tris buffer solution (50mM Tris, 150mM NaCl, H₂O, 1ml 10% w/v Triton ½ protease tablet, 10 ml H₂O). The homogenate was collected and rotated for 90min at 4°C. Samples were then centrifuged at 2000xg for 10min. The supernatant was removed and transferred to eppendorf tubes for storage at -80°C until required.

Animal model of Parkinson's disease (PD)

[0196] This current study utilizes an established animal model of PD (Przedborski and Vila, *Ann NY Acad Sci* 991:198-198, 2003). Briefly, wild type and IFNAR1^{-/-} mice were
5 treated with MPTP hydrochloride (10mg/kg in 100µl saline) administered by intraperitoneal (i.p) injection, a total of 4 exposures over a 10 hour period. 21 days after treatment, mice were killed by CO₂ inhalation followed by cervical dislocation. The midbrain was removed and separated into two sections; the caudate putamen for assessment of dopamine levels and the substantia nigra for protein analysis by western blot
10 and mRNA transcript analysis by QPCR. The left hemisphere was kept for histological analysis of lesion size and for dopaminergic neuron quantitation.

Statistical analysis

[0197] All data is expressed as mean±S.E.M. QPCR and MTT assay data was analysed
15 using one or two-way ANOVA respectively, with subsequent Dunnett's or Bonferroni Post-hoc tests where appropriate. For all statistical tests P<0.05 was accepted as being statistically significant.

EXAMPLE 1***IFNAR1^{-/-} neurons demonstrate increased viability in response to OGD***

[0198] Previous data had demonstrated that Middle Cerebral Artery Occlusion (MCAO) surgery performed on IFNAR1^{-/-} and IFNAR2^{-/-} mice showed a decreased infarct size of IFNAR1^{-/-} ($24.9 \pm 7.1 \text{ mm}^3$), but not IFNAR2^{-/-} ($52.3 \pm 4.9 \text{ mm}^3$), mice compared to wild-type ($65.1 \pm 4.8 \text{ mm}^3$) (n=6-8, $P < 0.05$, One-way ANOVA, Dunnett's Post-hoc test, Figure 1). To support these findings primary cultures of IFNAR1^{-/-} and wild-type neurons were subjected to 4 hours OGD with 24 hours reperfusion and an MTT assay was performed. IFNAR1^{-/-} neurons showed a significant increase in cell viability ($91.0 \pm 4.9\%$) compared to wild-type ($49.8 \pm 0.8\%$) in response to OGD (n=6, $*P < 0.05$, students t-test, Figure 1).

EXAMPLE 2***IFN-dependent STAT phosphorylation***

[0199] IFN is known to induce STAT phosphorylation governed by the JAK-STAT pathway. To identify the optimal concentration of IFN required to induce STAT phosphorylation, M17 cells were treated with increasing concentrations of IFN α and IFN β (1, 10, 100 and 1000U/ml) for 15 minutes and harvested for Western blot analysis. 1000U/ml of IFN α induced the most prominent phosphorylation of STAT-1 in contrast to IFN β treatment which induced similar levels of STAT-1 phosphorylation across all concentrations.

EXAMPLE 3***IFN α preferentially induces P-STAT-1 not P-STAT-3, IFN β is ambiguous***

[0200] To investigate the STAT phosphorylation profile induced from IFN-dependant signalling, M17 cells were treated with 1000U/ml IFN α or IFN β (concentration selected from previous concentration-response Western blots) for a time-course of 5, 10 or 30 minutes. Western blot analysis of STAT-1 and STAT-3 phosphorylation was then conducted. IFN α treatment induced P-STAT-1 across all time-points with 10 minutes treatment showing the most prominent phosphorylation. IFN β treatment also induced P-STAT-1 across all time-points with noticeable phosphorylation at 10 and 30 minutes treatment. IFN β also stimulated STAT-3 phosphorylation at all time-points but the more robust responses came earlier than that of P-STAT-1 induction (5 and 10 minutes treatment). Interestingly IFN α treatment was causative of only minimal STAT-3 phosphorylation at 10 minutes compared to the robust P-STAT-1 induction (IFN α , 10 minutes).

EXAMPLE 4***M17 cells subjected to OGD show induction of P-STAT-1 but not P-STAT-3***

[0201] STATs initiate pro-inflammatory gene transcription leading to increased cytokine release. In the OGD environment, cells are exposed to an inflammation state which contributes to the persistent cell death. To analyze if STATs play a role in the signalling events responsible for OGD-dependant cell death, cells were exposed to 3 hours OGD and a time-course reperfusion period (0, 2 and 24 hours). OGD treatment induces P-STAT-1 across all reperfusion time-points (0, 2 and 24 hours) but is most prominent at 2 hours reperfusion. Interestingly, OGD treatment did not cause STAT-3 phosphorylation across any reperfusion time-points.

EXAMPLE 5***IFN α mRNA levels are elevated earlier in OGD reperfusion than IFN β mRNA levels***

[0202] Given the STAT-1 phosphorylation in the OGD response, the time-course of IFN mRNA transcript production was investigated. M17 cells were subjected to 3 hours OGD with a time-course reperfusion (0, 0.5, 2 and 24 hours) and cellular RNA was isolated. RNA was reverse transcribed to give cDNA and RT-PCR was performed. IFN α mRNA levels were significantly increased at 2 hours reperfusion (15.1 ± 2.3 fold change) before returning to basal levels by 24 hours. In contrast, IFN β mRNA levels were only increased after 24 hours reperfusion (2.4 ± 0.1 fold change) ($n=3$, $*P<0.05$, One-way ANOVA, Dunnett's Post-hoc test, Figure 2).

EXAMPLE 6***IL-6, TNF- α and IL-1 β cytokine mRNA levels show elevation post-OGD***

[0203] Apart from IFN α and IFN β mRNA common pro-inflammatory cytokines IL-6, TNF- α and IL-1 β were also analyzed *via* RT-PCR to confirm the inflammatory state created by OGD treatment. IL-6 mRNA levels were significantly increased at 0.5 and 2 hours reperfusion (266.9 ± 10.9 and 194.6 ± 39.4 fold change, respectively) before returning to basal levels by 24 hours reperfusion. TNF- α mRNA levels were significantly elevated at an earlier time-course of 0 and 0.5 hours reperfusion (10.0 ± 3.1 and 10.1 ± 2.8 fold change respectively) and returned to control levels by 2 hours reperfusion. IL-10 mRNA levels showed a later onset significant increase at 24 hours reperfusion (4.6 ± 1.2 fold change). ($n=3$, $*P<0.05$, One-way ANOVA, Dunnett's Post-hoc test, Figures 3A to C). Figure 4 shows a summary of collated RT-PCR data.

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EXAMPLE 7***Verification of mIFNAR1/2 gene insert within pcDNA6.2/cEM-GFP plasmid***

[0204] In order to investigate the effects of IFNAR1 and IFNAR2 subunit over-expression during OGD and subsequent reperfusion, the genes were inserted into the pcDNA6.2/cEM-GFP plasmid for subsequent cellular transfection. To verify both the IFNAR1 and IFNAR2 gene inserts in the plasmid, PCR using a forward plasmid-specific T7 and reverse gene-specific attB2 primers was used to amplify the gene product. A 1.77kb genomic band specific for the IFNAR1 gene insert was identified. A 1.54kb genomic band specific for the IFNAR2 gene insert was also observed.

EXAMPLE 8***Confirmation of mIFNAR1/2-GFP plasmid cellular transfection***

[0205] To evaluate the efficiency of M17 cell-line transfection with pcDNA6.2/cEM-GFP-MIFNAR1/MIFNAR2 constructs, fluorescence microscopy was used to detect GFP expression in the transient transfected M17 cell-line. Results show >50% transfection efficiency, with IFNAR1-GFP located in the cytoplasm and bound to the cell membrane of M17 cells.

EXAMPLE 9***Over-expression of IFNAR1 in OGD treated M17 cells is detrimental***

[0206] In order to evaluate the *in vitro* effect of IFNAR1 and IFNAR2 over-expression, M17 cells were subjected to 3 hours OGD and 24 hours reperfusion. MTT assay was then used to assess cell viability. Interestingly, M17-IFNAR1 cells showed decreased viability ($21.6 \pm 5.3\%$) in comparison to wild-type ($44.9 \pm 2.1\%$). Furthermore, M17-IFNAR2 cells showed no difference in cell viability ($39.8 \pm 5.7\%$) when compared to wild-type ($n=4$, $*P<0.05$, One-way ANOVA, Dunnett's Post-hoc test, Figure 5).

EXAMPLE 10***IFNAR1 is both membrane-bound and intra-nuclear in WT neurons***

[0207] Considering the difference in IFNAR1 and IFNAR2 over-expression in terms of cell viability IFNAR1 cellular trafficking was briefly investigated. Isolated CBL57/6 WT neurons were used for immunohistochemistry probed with mAb-IFNAR1. Fluorescence microscopy shows membrane-bound, but more interestingly, intra-nuclear localization of the IFNAR1 subunit.

EXAMPLE 11***Establishment of the Traumatic Brain Injury Model***

[0208] Traumatic Brain Injury (TBI) was induced using a computer-controlled impactor. To assess whether the injury followed impact parameters, the position of the impactor tip was traced using an oscilloscope, which gave exact coordinates of tip position at each time point (Actual position versus Demand position). This tracing showed that for the entirety of the injury delivery, the tip's actual position followed the desired position, giving a consistent injury.

EXAMPLE 12***H&E statining and NeuN Immunohistochemistry***

[0209] In order to establish whether or not each TBI was having an impact on neuronal tissue, mice were reperused 24 hours after injury, and brains were stored in 4% w/v PFA. Brains were cut by the Histology facility, University of Melbourne, into 10µm sections, rostral to caudal, and every 10th section was stained with H&E. Staining was illustrative of viable neuronal tissue. The infarct appears less intensely stained, and loss of tissue is apparent. Additionally, sections were stained with NeuN, which gives an indication of neuronal number within a particular brain section. Neurons were visible as dark brown, and the infarct area shows a decrease in neuronal numbers. Data demonstrated the contrast between damaged and intact tissue.

EXAMPLE 13***Magnetic Resonance Imaging***

[0210] Both procedures represent traditional methods used to assess neuronal damage following injury. In contrast, Magnetic Resonance Imaging (MRI) is a powerful approach used to analyze damage following TBI, as it allows an *in vivo* assessment of the progression of oedema and injury, done in real time. MRI scans were done on mice, 2 and then 24 hours after TBI. A T2-weighted scan of a wild-type mouse given TBI was conducted and imaged 2 and 24 hours later. T2 images show the diffusion of water inside tissue, and are thus ideal for visualization of tissue oedema. The development of the infarct and penumbra (surrounding area of damage) was shown, with the size of the infarct and penumbra increasing drastically after 24 hours (statistical analysis could not be performed on this as there were insufficient numbers for the 2 hour time point). Another imaging sequence used, Diffusion Weighted Imaging (DWI) allowed an assessment of the movement of water molecules within injured and uninjured tissue. This generated an Apparent Diffusion Coefficient (ADC) map, and using this sequence, brains were again measured for infarct volume. An ADC map of two sequential slices was taken of an injured brain, showing both infarct and penumbra size, in voxels. A T2 map was generated by T2-weighted Imaging of the same brain, showing again infarct and penumbra size. The two types of sequence give different values for infarct and penumbra size. To determine whether this difference was also observed through a more conventional way of measuring infarct volume (H&E sections measured through ImageJ), the infarct sizes, in voxels, of the three wild-type brains, which had been previously imaged at 24 h, were converted into mm³ (one voxel in this scanning sequence is equivalent to 100x100x600µm³). A comparison of all the methods was used to obtain infarct volumes (T2-weighted imaging, DWI and H&E infarct analysis). The mean infarct volumes obtained by DWI (1.50mm³ ± 0.22) and H&E analysis (2.56mm³ ± 0.13) are not statistically different (P>0.05, n=3), and both differ to the infarct volume obtained using T2-weighted imaging (5.65mm³ ± 0.48, P<0.05, n=3).

EXAMPLE 14***The role of Interferon signalling after neuronal injury*****5 Infarct Volume analysis**

[0211] To assess the effects of knocking out the IFNAR1 subunit after TBI, wild-type and IFNAR1^{-/-} mice were given TBI, perfused after 24 h, and had 10µm serial sections cut. H&E staining was used to measure infarct. Figure 8 shows the infarct volumes of injured wild-type and knockout mice. IFNAR1^{-/-} mice had a significantly smaller mean infarct size
 10 (0.84mm³ ± 0.23) compared to wild-type mice (2.72 mm³ ± 0.30) (n=3 per group, P<0.05).

Immune cell infiltration after TBI

[0212] To look at invading cells after TBI, 10µm serial sections were stained with Mac-1, an antibody that detects macrophages and activated microglia. Mac-1 staining is more
 15 intense in the ipsilateral cortex of wild-type mice compared to knockout mice, highlighting greater immune cell infiltration in wild-type mice given TBI. There is weak staining for Mac1 in the contralateral cortex of the wild-type and IFNAR1^{-/-} mouse.

Type 1 Interferons were differentially expressed following TBI

20 [0213] To study the effects of knocking out the IFNAR1 receptor subunit on gene expression, mice were given TBI and ipsilateral and contralateral hemispheres were collected for RNA extraction 2, 4 and 24 hours later. RNA was converted to cDNA, and RT-PCR was performed on each sample to calculate fold changes in mRNA levels. Expression of Type 1 IFN (IFNα and β) mRNA was analyzed. IFNα transcript levels in
 25 the ipsilateral cortex of the wild-type mice were increased by up to 5 fold in the 2 hours following TBI, and then reverted to basal levels 4 hours after TBI (Figure 9A). Comparatively, the contralateral cortex showed no change in IFNα expression following TBI (P>0.05, n=3) [Figure 9B]. IFNα expression was significantly higher in the ipsilateral cortex of wild-type mice 2 hours after TBI (3.52 ± 0.87 fold relative to control), compared
 30 to IFNAR1^{-/-} mice (0.66 ± 0.38 fold relative to control). There was no significant difference in IFNα expression between the contralateral cortices of wild-type and IFNAR1^{-/-} mice. IFNβ expression did not change significantly between wild-type and

knockout mice, except at 4 hours in the contralateral cortex of the wild-type mouse, whose IFN β mRNA levels were 5-10 times higher than in the knockout mouse ($P<0.05$, $n=3$) [Figures 10A and B]. Type 1 IFN expression was also studied in human subjects who had head injuries, and had died at various times after their injury (Figure 11). IFN β mRNA levels increased 8.9 fold compared to controls in subjects that had died 6 hours after TBI ($P<0.05$, $n=8$). IFN α mRNA levels decreased by 74% compared to controls in subjects that had died 3 hours after TBI ($P<0.05$, $n=7$).

Pro-inflammatory genes were differentially expressed after TBI

10 [0214] The expression of the pro-inflammatory genes, TNF α and IL-1 β after TBI was also studied in mice. TNF α was increased by up to 60 fold in wild-type mice, both 2 and 4 hours after TBI in the ipsilateral cortex (Figure 12A). In comparison, TNF α mRNA levels decreased by 95% compared to controls in IFNAR1 $^{-/-}$ mice 2, 4 and 24 hours after TBI in the ipsilateral cortex, which was a pronounced change in mRNA levels when compared to
15 wild-type mice. In the contralateral cortex, TNF α mRNA levels increased 20 fold 2 hours after TBI in wild-type mice, and decreased by 95% compared to controls in knockout mice (Figure 12B). In both ipsilateral and contralateral cortices, TNF α mRNA levels were significantly higher in wild-type mice compared to knockout mice ($P<0.05$, $n=3$). IL-1 β mRNA levels were significantly higher in wild-type mice compared to IFNAR1 $^{-/-}$ mice
20 ($P<0.05$, $n=3$) [Figure 13A]. IL-1 β mRNA levels increased by up to 15 fold in the ipsilateral cortex of wild-type mice 2, 4 and 24 hours after TBI compared to controls, whereas in knockout mice, mRNA levels decreased by 95% 2, 4 and 24 hours after TBI. In the contralateral cortex, the largest increase in IL-1 β was seen 4 hours following TBI in wild-type mice (an increase of 4 fold compared to controls), as compared to knockout mice,
25 whose levels were diminished by 100% ($P<0.05$, $n=3$) [Figure 13B].

EXAMPLE 15***Therapeutic targeting of the Interferon α receptor 1 (IFNAR1) subunit*****Infarct Volume Analysis**

5 [0215] In order to establish whether or not treatment with MAR1, a blocking monoclonal antibody targeting the IFNAR1 receptor, would be beneficial post-TBI, mice were treated with MAR1 or the IgG Isotype control 1 hour before TBI. Brains were perfused 24 hours after TBI, sectioned, and H&E stained. These sections were used to measure infarct volume using Image J. Infarct volumes of mice that were treated with either MAR1 or IgG

10 Isotype control before TBI were determined. Mice treated with MAR1 before TBI had a mean infarct volume of $1.26 \text{ mm}^3 \pm 0.38$, which was significantly lower than the mean infarct volume of IgG Isotype control-treated mice ($2.56 \text{ mm}^3 \pm 0.13$) ($n=3$ per group, $P<0.05$).

15 Invasion of peripheral immune cells

[0216] Mac-1 immunohistochemistry was used to identify invading cells from the periphery in MAR1 and IgG Isotype treated brains. Mac-1 positive cells were observed from both ipsilateral and contralateral cortices of mice given both treatments. Mac-1 staining is more intense in the ipsilateral cortex of IgG control-treated mice compared to

20 MAR1-treated mice, highlighting greater immune cell infiltration in IgG control-treated mice given TBI. Similarly, Mac-1 staining is more intense in the contralateral cortex of IgG control-treated mice compared to MAR1-treated mice.

EXAMPLE 16***IFN-mediated inflammatory response and Alzheimer's disease*****Overview**

- 5 [0217] The aim of this Example was to characterize IFN-mediated inflammatory response of A β neurons in isolation. In order to perform accurate analysis on this effect, it was necessary to characterize the incubation time required with A β to induce inflammatory response and cell death. Real-time PCR data were obtained to characterize the relative expression levels of various cytokines after A β treatment. Interferon Receptor knockout
10 (INFAR^{-/-}) mice were then used to visualize any changes in Tau morphology and quantify changes in viability.

Temporary Characterization of Cytokine Release

- [0218] M17 Treatment with 5 μ M A β showed marked increase in transcript levels of IFN α
15 and IFN β which became most pronounced at 96 hours (respectively). Similar increases were also observed in IL-1 β and TNF α transcript levels at 96 hours relative to baseline (respectively).

- [0219] Treatment with 10 μ M A β to M17 cells showed a similar trend, with relative
20 transcript expression levels of IFN α and subunit 1 of the human Interferon Receptor (hIFNR1) rising markedly at 96 hours (Figures 15A through 15D). Unexpectedly, changes in relative transcription of IL-1 β appears reduced in 10 μ M A β treatment and TNF α shows only a moderate increase. Given marked increases with 5 μ M A β treatment, this may be attributable to the small sample size being a poor representation of the true effect.

25

- [0220] From the PCR data, it was established that a 96 hours treatment protocol would stimulate appropriate inflammatory pathways given significant rises in both IFN α and IFN β occurred at 96 hours in an apparent dose-dependent manner.

Toxicity of β -Amyloid in IFNAR^{-/-} neurons

[0221] Murine wild-type and IFNAR^{-/-} neurons were treated with 5 μ M, 10 μ M and 15 μ M concentrations of A β in order to determine any differences in viability. IFNAR^{-/-} neurons show a dose-dependent resilience to treatment with A β compared to wild-type Neurons (Figure 16). Almost no toxicity is seen with a dose of 5 μ M A β in IFNAR^{-/-} neurons, whereas treatment with 10 μ M A β yields a statistically significant ($p = 0.0233$) attenuation of toxicity compared to wild-type. There was also a statistically significant difference ($p = 0.0064$) between IFNAR^{-/-} and WT neuronal viability in treatment with 15 μ M A β , where viability is comparable to the 0.2 μ M Staurosporine pro-apoptotic control (Bertrand *et al.*,
10 *Experimental cell research* 211:314-321, 1994).

Caspase 3 cleavage in A β treated IFNAR^{-/-} neurons

[0222] Cleaved Caspase 3 levels were examined in A β treated wild-type and IFNAR^{-/-} neurons as a measure of induced apoptosis. Caspase 3 is a downstream target of IFNAR induced Tyk2/STAT3 signalling and its cleavage thus expected to be reduced if not
15 attenuated in IFNAR^{-/-} neurons. Cleaved Caspase 3 levels appeared significantly diminished in IFNAR^{-/-} neurons as determined by western blot. Neurons labeled for Cleaved caspase 3 showed only minimal staining in A β treated IFNAR^{-/-} neurons while wild type neurons display cleaved caspase 3 labeling in the cell body often in close
20 proximity to nuclei.

Tau Morphology in A β Treated IFNAR^{-/-} neurons

[0223] IFNAR^{-/-} and wild-type neurons were labeled for total tau in an effort to visualize structural changes due to inflammation. Both IFNAR^{-/-} and wild-type neurons show
25 decreases in tau filaments compared to untreated control. In comparison to the untreated conditions, it appears only the more robust neuronal processes remain after A β treatment. There appears to be only a modest attenuation of tau loss in IFNAR^{-/-} neurons. Tau modification in IFNAR^{-/-} neurons is likely due to the activation of IFNAR independent inflammatory pathways such as the NALP3 inflammasome, resulting in caspase-1 induction
30 of IL-1 β mediated inflammation.

[0224] Interestingly, tau labeling reveals distinct differences in cellular morphology between wild-type and IFNAR^{-/-} neurons treated with staurosporine. Whether this is a direct result of immunochemical staining or if it represents an altered mechanism of induced apoptosis remains to be seen. In the IFNAR^{-/-} neuron it is possible that alterations in the normally IFN induced 2-5A oligoadenylate/RNase L pathway has modulated the apoptotic response as RNase L^{-/-} mice have shown resistance to staurosporine (Chawla -Sarkar *et al.*, *Apoptosis* 8:237-249, 2003).

10 Summary

[0225] The results show that Type 1 IFN signalling *via* its receptor is involved in A β -induced inflammatory response. An examination of the data shows distinct attenuation of cell death due to the removal of interferon-related inflammatory cascade in neurons.

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EXAMPLE 17

Role of Type-1 interferon signalling in stroke outcomes

[0226] To examine the role of Type-1 interferon signalling in stroke, IFNAR1^{-/-} mice underwent mid cerebral artery occlusion (MCAO) surgery. The mice demonstrated a decreased infarct size ($24.9 \pm 7.1 \text{ mm}^3$ n=8) compared to wild-type controls ($65.1 \pm 4.8 \text{ mm}^3$ n=8). Western blot and immunohistochemistry showed alterations in the Stat-1 and 3 phosphorylation profiles in the IFNAR1^{-/-}. Neuroprotection conferred by the absence of IFN signalling was confirmed in IFNAR-deficient primary cultures that were protected from cell death when exposed to oxygen glucose deprivation (OGD).

25

[0227] Co-culture experiments using IFNAR1^{-/-} glia and WT neurons and WT glia and IFNAR1^{-/-} neurons were carried out in the OGD model. IFNAR1^{-/-} neurons in the presence of WT glia no longer displayed a neuroprotective phenotype suggesting the glia are a major driver of the neuroinflammatory response. In an attempt to block IFNAR signalling *in vivo* a blocking monoclonal antibody targeting the IFNAR1 receptor (IFNAR1 mAb) was injected into WT mice *via* the tail vein (0.5mg) 30 minutes prior to MCAO. This resulted in a 60% decrease in infarct size when compared to the IgG control.

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[0228] Collectively these results indicate signalling through the IFNAR1 subunit is deleterious in stroke. Furthermore, data indicate that therapeutic agents targeting the IFNAR1 subunit are beneficial in reducing the severity of a neuro-inflammatory event following stroke and in doing so limit infarct size.

EXAMPLE 18

Role of Type-1 interferon in Alzheimer's disease (AD)

[0229] To test that Type-1 interferon signalling is involved in the exacerbation and progression of neuroinflammation in AD by A β (1-42), quantitative PCR (qPCR) was performed on both wild-type and IFNAR1^{-/-} primary neuronal cultures. IFN α and β were found to be dramatically up regulated 450- and 150-fold, respectively. Neurons lacking IFNAR1^{-/-} were protected against A β (1-42)-induced toxicity at the doses of 10 and 15 μ M (n=9, P< 0.05) indicating interferon signalling is involved in the cell death response elicited by the A β 1-42 peptide.

[0230] To further investigate the involvement and expression of Type-1 interferon in AD, ELISA was performed on brain homogenates from aged APP/PS1 brains (9 months). These mice displayed a statistically significant two-fold increase in IFN α levels compared to aged matched controls (n=4, P<0.05). Furthermore, qPCR performed on a pilot cohort of 9 AD brain samples (pre-frontal cortex brain region) from the Australian brain bank demonstrated IFN- α and IFN- β were up-regulated 3.9- and 4.4-fold, respectively in the AD cohort compared to controls.

25

[0231] This example shows that Type-1 interferons and their signalling is involved in the cytokine response and subsequent pathogenesis of AD. Hence, the regulation of Type-1 interferon signalling is a viable treatment strategy to slow disease progression.

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EXAMPLE 19***IFNAR1 localization***

[0232] Figures 17A through C show by fluorescent immunohistochemistry that IFNAR1 is
5 localized on primary cultured mouse neurons.

EXAMPLE 20***Effects on IFNAR1 mAb in a stroke model***

10 [0233] IFNAR^{-/-} (KO) C57 BL6 mice exhibited reduced infarct size in this stroke model. The infarct size was assessed 24 hours post injury in wild-type (IFNAR^{+/+}) and IFNAR^{-/-} C57 BL6 mice. The results are shown in Figure 18. Figure 19 shows that a mAb specific for IFNAR1 (IFNAR1 mAb) reduces infarct size when 0.5mg mAb is given iv 1 hour prior to the stroke. The infarct volume is assessed at 24 hours post-injury.

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EXAMPLE 21***Effects on IFNAR1 mAb in traumatic brain injury model***

[0234] The results presented in Figure 20 show that IFNAR1 mAb reduces infarct size in a
20 traumatic brain injury (TBI) model. An amount of 0.5mg mAb was given iv 30 minutes post-TBI induction and infarct volume was assessed 24 hours post-injury.

EXAMPLE 22***Amelioration of beta-amyloid (1-42) toxicity***

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[0235] IFNAR1^{-/-} neurons are less susceptible to beta-amyloid (A β) [1-42] toxicity. Primary cultured mouse neurons from wild-type and IFNAR1^{-/-} (KO) mice were exposed to a dose response curve of A β . The IFNAR1^{-/-} neurons displayed less cell death at 96 hours after treatment. The results are shown in Figure 21.

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EXAMPLE 23***Levels of Type-1 interferons in Alzheimer's disease (AD) brain tissue***

[0236] Figures 22A and B show that based on quantitative PCR (qPCR), there is a four-
5 fold elevation of IFN- α and IFN- β in post-mortem brains of AD sufferers. IFNAR1 and
IFNAR2 unchanged receptor subunits remained unchanged.

EXAMPLE 24***Role of Interferon α receptor 1 (IFNAR1) in traumatic brain injury (TBI)***

10 [0237] Figure 23 shows that infarct volume in knock out mice for IFNAR1 (IFNAR1^{-/-}) is
significantly reduced compared to in wild type mice or in IFNAR2 knock out mice
(IFNAR2^{-/-}). Hence, IFNAR1^{-/-} is protective against TBI.

EXAMPLE 24

15 ***Levels of type 1 interferon (IFN) in brain samples from Parkinson's disease (PD)
subjects***

[0238] Figures 24A and B show the levels of IFN α and IFN β in IFNAR1 and IFNAR2 in
post mortum brain tissue of subjects with Parkinson's disease (PD). Both IFN α and IFN β
were significantly elevated. The levels of the receptor components were not as
20 significantly elevated.

EXAMPLE 26***Transcript levels of IFN α and IFN β following insult with A β ₁₋₄₂***

[0239] Figure 25 shows that from 72 hours after M17 cells are insulted with 5 μ M A β ₁₋₄₂,
25 the level of transcription of genetic loci encoding IFN α and IFN β is significantly elevated
(*P<0.05, n=3).

[0240] This demonstrates the role of type 1 IFNs in Alzheimer's disease.

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EXAMPLE 27***Role of type 1 interferon (IFN) in Alzheimer's disease***

[0241] Figure 26 shows that genetically modified (transgenic; TG) mice producing modified forms of A β and which have a mutation in Presenilin 1 (PS1) [APP/PS1 TG mice] have significantly elevated forms of IFN α . This highlights the role of this IFN in Alzheimer's disease. In the same mouse model APP/PS1 TG mice had elevated levels of phosphorylated STAT-3, a down stream effector molecule from type 1 IFN stimulation.

EXAMPLE 28

10 ***6-OHDA and rotenone models of Parkinson's disease in M17 human neuroblastoma cells***

Effects of 6-OHDA on cell viability of M17 cells

[0242] To investigate the cytotoxic effect of 6-OHDA (6-hydroxydopamine) on M17 neuroblastoma cells, a concentration-response treatment over 24 hours was performed. The MTT assay was used to assess cell viability and confirmed that 6-OHDA (10 μ M-150 μ M) induced cell death in M17 cells compared to an untreated control.

6-OHDA induces STAT-3 phosphorylation in M17 cells

[0243] To identify if type-I IFN signalling through the JAK/STAT pathway is involved in the cell death response induced by 6-OHDA, M17 cells were treated with 25 μ M 6-OHDA for 0, 1, 2, 4, 8, 24 and 48 hours and analysed by western blot for STAT-3 phosphorylation (activation). Levels of P-STAT-3 (phosphorylated STAT-3) were elevated in M17 cells 48 hours post treatment, whilst total STAT-3 levels were unchanged.

25 **6-OHDA induced type-I IFN mRNA expression**

[0244] Given the STAT-3 phosphorylation response to 6-OHDA, a time course of type-I IFN mRNA transcript production was investigated in response to 6-OHDA by QPCR. M17 cells were treated with 50 μ M 6-OHDA and harvested at 0, 1, 2, 4, 8 and 24 hours. A trend towards increased IFN α and IFN β mRNA expression was evident up to 4 hours post 6-OHDA treatment, before returning to basal levels at 24 hours. Human Interferon receptor 1 (hIFNAR1) mRNA levels were unchanged compared to untreated control until 8 hours

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where there was significant 2 fold decrease in expression (n=3, *P<0.05, Dunnett's Post-hoc test, Figures 27A through C).

Effect of rotenone on M17 cell viability

- 5 [0245] To validate the use of rotenone as an *in vitro* model of PD in M17 cells, a rotenone concentration response treatment was initially performed and cell viability assessed. MTT assay confirmed concentrations as low as 10nM induced cell death after 24 hours compared to vehicle (DMSO). Considerable reduction in cell viability (<75%) occurs at concentrations $\geq 500\text{nM}$.

10

Rotenone activates STAT-3 signalling pathways

- [0246] To determine the potential involvement of the type-I IFN signalling cascade in the cellular response to rotenone, cells were treated for 0, 1, 2, 4, 8 and 24hours with $1\mu\text{M}$ rotenone and harvested for western blot analysis. Activation (phosphorylation) of STAT-3
15 was increased at 4, 8 and 24 hours post treatment (n=3, Figures 28A through C).

Rotenone induced type-I IFN mRNA expression

- [0247] To further determine the involvement of type-I IFNs in the cellular response to rotenone, M17 cells were treated with 500nM rotenone for 0, 1, 2, 4, 8 and 24 hours before
20 cells were harvested and RNA isolated. Both IFN α and IFN β transcript levels demonstrated a biphasic trend. Levels were elevated at 2 hours post treatment, declined at 4hours before increasing again at 8 and 24 hours. hIFNAR1 mRNA levels displayed no significant change compared to vehicle control.

25 Confirmation of IFNAR1 shRNA knock-down (KD) in M17 cells

- [0248] Human BE(2)M17 neuroblastoma (M17) cells stably expressing an IFNAR1 (IFNAR1-KD) or scrambled negative control (NC) shRNA vector were analysed by QPCR to confirm successful knock-down of IFNAR1 in this cell line. IFNAR1 expression was reduced 5 fold (80%) in IFNAR1-KD cells compared to the NC shRNA cells.

30

Comparison of NC shRNA to M17 parental cells.

[0249] To confirm that the transfection of a negative control (NC) shRNA vector had no effect on cell viability compared to M17 parental cells, a concentration response of both 6-OHDA (10-150 μ M) and rotenone (10-500nM) was performed. MTT assay confirmed no significant difference in cell viability after 24 hours treatment between the two cell types in response to either of these neurotoxins.

M17 IFNAR1-KD cells are less susceptible to 6-OHDA toxicity

[0250] To investigate the *in vitro* effects of reduced type-I-IFN signalling through IFNAR1 knock-down, M17 NC shRNA and IFNAR1-KD cells were treated with 10, 25, 50, 75, 100, 150 or 200 μ M 6-OHDA for 24 hours and cell viability determined by MTT assay. M17 IFNAR1-KD cells demonstrate increased cell viability (25-100 μ M) compared to NC shRNA cells (*P<0.05, Two-way ANOVA, Dunnett's Post-hoc test).

M17 IFNAR1-KD cells display decreased expression of phosphorylated-STAT-3

[0251] The effect of IFNAR1-KD on the type-I IFN driven JAK-STAT signalling cascade in response to 6-OHDA was examined by western blot analysis. NC shRNA and IFNAR1-KD M17 cells treated with 50 μ M 6-OHDA for 0, 1, 2, 4, 8, and 24 hours time course before being harvested and analysed by western blot for STAT-3 phosphorylation. Sustained STAT-3 phosphorylation was confirmed in the NC shRNA M17 cells after 4, 8 and 24 hours 6-OHDA treatment. The IFNAR1-KD M17 cells showed decreased phosphorylation at these time points compared to NC shRNA.

M17 IFNAR1 KD cells are less susceptible to rotenone-induced toxicity

[0252] The potential protective effects of reduced type-I IFN signalling in response to rotenone was analysed in NC shRNA and IFNAR1-KD M17 cells. Cells were treated with 10, 25, 50, 75, 100, 200 and 500nM rotenone for 24hours before cell viability was determined by MTT analysis. IFNAR1-KD M17 cells displayed increased cell viability at all concentrations compared to NC shRNA M17 cells in response to rotenone (n=6, *P<0.05, Two-way ANOVA, Bonferroni Post-hoc test, Figure 29).

Reduced STAT-3 phosphorylation in IFNAR1-KD cells following rotenone insult

[0253] The effect of rotenone on type-I IFN mediated JAK-STAT signalling was also investigated in NC and IFNAR1-KD cells. Both cell types were treated with 500nM rotenone and harvested at 0, 1, 2, 4, 8 and 24 hours post treatment for western blot analysis. Blots show that rotenone induced prominent phosphorylation of STAT-3 in the NC shRNA M17 cells at 8 hours post treatment, however this phosphorylation was diminished in IFNAR1-KD cells at the same time point.

Rotenone induces type-I IFN mRNA expression in NCshRNA and IFNAR1-KD cells

10 [0254] To further investigate the effect of type-I IFN involvement in the cellular response to rotenone, NC shRNA and IFNAR1-KD were treated with 500nM rotenone for 1, 2, 4, 8 and 24 hours before cells were harvested for QPCR analysis. Both IFN α and IFN β transcript levels in both the NC shRNA and IFNAR1-KD cells were elevated 24 hours post treatment compared to vehicle. hIFNAR1 mRNA levels were remained constant compared to vehicle (n=2, Figures 30A through C).

Type-I IFNs are elevated in post mortem human PD patients

[0255] In order to identify the expression levels of type-I IFNs in post mortem human PD brain samples, QPCR was performed. An increase in IFN α and a significant elevation in IFN β levels were observed compared to age matched controls (n=10, *P<0.05 One-way ANOVA, Dunnett's post-hoc test, Figure 31).

Analysis of STAT-3 phosphorylation in human PD brains

25 [0256] To identify the activation of the type-I IFN signalling pathway, human PD brain samples and matching controls were homogenised and analysed by western blot. No observable activation of STAT-3 was achieved when probed with an antibody for phospho-STAT-3. Subsequent re-probing for total-STAT-3 indicated a trend for decreased levels of total STAT-3.

30 MPTP mouse model

[0257] Wild type (WT) (n=10) and IFNAR^{-/-} mice (n=8) were injected with 4x 10mg/kg MPTP over 10 hours and brains analysed at a 21 day end point. The effect of MPTP on

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type-I IFN signalling was examined by western blot homogenates in the substantia nigra (SN) of both WT and IFNAR1^{-/-} mice. No detectable change in STAT-3 phosphorylation was observed in WT mice treated with MPTP. In addition, IFNAR1^{-/-} mice treated with MPTP also showed no activation of STAT-3.

5

Type-I IFN mRNA transcript levels in MPTP mouse model

[0258] The effect of MPTP on type-I IFN mRNA expression in the SN of mice was also investigated. RNA was extracted from untreated WT controls (n=10), as well as both MPTP WT (n=10) and IFNAR1^{-/-} (n=8) treated groups. QPCR analysis on IFN β mRNA
10 transcript levels showed no significant change compared to WT control, however IFN α expression showed a significant decrease (40%) in WT MPTP and IFNAR1^{-/-} MPTP treated groups compared with WT control (*P<0.05 One-way ANOVA, Dunnett's post-hoc test, Figures 32A and B).

15 Summary

[0259] This Example used the experimental toxin 6-OHDA and environmental neurotoxin rotenone in a human BE (2) M17 neuroblastoma (M17) cellular model. These toxins have been previously linked to PD pathogenesis and are widely used in *in-vitro* studies of PD pathogenesis (Bove *et al.*, *Neuro Rx* 2(3):484-494 2005; Greenamyre *et al.*, *Parkinsonism*
20 *Relat. Disord.* 9 Suppl 2: S59-64, 2003).

[0260] Western blot analysis confirmed that 25 μ M 6-OHDA increased phosphorylation (activation) of STAT-3 after 48 hours, indicative of activation of the JAK-STAT pathway. It is known that following activation, STATs dimerise and form a complex with Interferon
25 regulatory factors (IRFs), before nuclear translocation to transcriptionally up-regulate a number of genes including IFN α and IFN β (Takaoka and Yanai, *Cell Microbiol* 8(6):907-922, 2006). The up-regulation of type-I IFN expression in this positive feedback manner was confirmed by Q-PCR analysis with an increasing trend up to 4 hours post treatment. This finding is significant as unlike in the peripheral system where type-I IFN is well
30 characterized, the link between elevated levels of type-I IFNs and neuroinflammation in the CNS is still underrepresented. However, clear evidence can be found for IFN involvement in the CNS, with increased levels of type-II IFN- γ expression in the SN

resulting from elevated glial cell expression of major histocompatibility complex class II (MHC II) markers.

[0261] This study confirmed an activation of type-I IFN signalling in the presence of rotenone. Rotenone is PD associated environmental neurotoxin, known to exert its cytotoxic effects by leading to the production of ROS (Greenamyre *et al.*, 2003 *Supra*; Testa *et al.*, *Brain Res Mol Brain Res* 134(1):109-118, 2005). In the context of this study, the elevated ROS would further potentiate the neuroinflammatory response. Western blot analysis demonstrated rotenone induced STAT-3 phosphorylation 4 hours post treatment, consistent with activation of the JAK/STAT pathway. In addition, elevated levels of both IFN α and IFN β transcripts were identified by QPCR.

[0262] Collectively, these data indicate 6-OHDA and rotenone induced cell death, involves in part, the induction of a neuroinflammatory environment mediated by increased type-I IFNs. Indeed, knowledge of their neurotoxic mechanism of action supports such a rationale for the similar parallels found in these results. 6-OHDA is known to exert its cytotoxicity via uptake through catecholamine transporters on the cell surface, subsequently causing mitochondrial dysfunction and the production of ROS (Bove *et al.*, 2005, *Supra*). Rotenone also results in the generation of ROS, with the discovery of its mechanism of action, binding to complex I causing mitochondrial dysfunction. The ROS generated by these neurotoxins, leads to protein, lipid and DNA modifications and consequently widespread cellular death. In addition, these modifications also activate resident CNS microglia, leading to the secretion of pro-inflammatory cytokines such as TNF α , IL-1 β and IL-6 in response to a neurotoxic insult. Subsequently type-I interferon up-regulation may stimulate an initial release of IFN α and IFN β in a pro inflammatory response due to ROS formation from mitochondrial disruption. The activated IFNAR1 signalling cascade through phosphorylated STAT-3 leads to gene transcription with the autocrine production of IFN α and β . This then amplifies the pro-inflammatory response through increased type-I IFN signalling. This results in IFN stimulated gene factor 3 (ISGF3) formation and translocation to the nucleus, where it enhances pro apoptotic gene transcription. This further contributes to this deleterious neuroinflammatory environment.

[0263] The up-regulation of type-I-IFNs in M17 cells *in vitro* was also confirmed in post mortem human PD samples, with QPCR analysis showing significant increases in type-I IFN production compared to age matched controls. This increased expression of type-I IFN levels in both M17 and human PD brain samples highlighted their potential contribution to the neuroinflammatory response in PD. It is proposed herein that inhibition of type-I IFN signalling through inactivation of IFNAR-1 may exert protective effects in PD neurotoxic models.

[0264] To investigate this, IFNAR1 expression was knocked down by transfection of an IFNAR1 shRNA construct in M17 cells. QPCR confirmed the efficiency of the IFNAR1-knockdown (IFNAR1-KD), with an 80% reduction in IFNAR1 expression compared to stably expressing NC shRNA. Confirmation of the comparable responses between NC shRNA and M17 parental cells, validated its use as a control to IFNAR-1 KD cells.

[0265] MTT assays confirmed IFNAR1-KD cells were protected against both 6-OHDA and rotenone induced toxicity, with an increase in cell viability compared to NC cells. In addition, reduced type-I IFN signalling (decreased phosphorylation of STAT3) was identified in M17 IFNAR1 KD cells in both neurotoxic models. Reduced STAT-3 activation was observed.

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[0266] The MPTP mouse model was used to gain a greater understanding of the pro-inflammatory mediators in a complete CNS environment. Preliminary studies using this model involved pilot experiments of both wild type (WT) and IFNAR1^{-/-} mice with MPTP (4 doses of 10mg/kg) to induce a parkinsonian phenotype. QPCR analysis indicated type-I IFN mRNA transcript levels showed no change in IFN α but a down-regulation in IFN β compared to WT sham. Protein analysis indicated the presence of STAT-3.

[0267] In this study the use of the M17 cell line provided a reliable, highly reproducible model, which was able to give physiological relevance due to its human origin. However there are limitations in its use. Critically, the use of M17 cells in an acute setting to mimic a chronic disease is a limitation to overcome, as there is no effective technique available to replicate the prolonged neuroinflammatory state that is observed in PD. In addition, M17s

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are an oncogenic cell line which differ in morphology and physiology compared to those cell types that are directly associated with PD, such as neurons, glia and microglia.

[0268] The findings of this Example implicate type-I IFN signalling through IFNAR1 as contributing to a deleterious neuroinflammatory response and the exacerbation of cell death in our experimental models of PD. More specifically, IFN α and IFN β transcript levels were increased in both 6-OHDA and rotenone neurotoxicity models, inducing activation of STAT-3, possibly through IFNAR1 contributing to neuro-degeneration. Supporting these results is the finding that inhibition of the type-I IFN signalling cascade by IFNAR1 knockdown leads to reduced phosphorylation and activation of STAT-3 with a subsequent neuroprotective effect in response to PD associated neurotoxins. This confirms that signalling through the IFNAR1 receptor is a previously unrecognised contributor to the neurodegeneration in PD. This study identifies a target for therapeutic intervention to slow the exacerbation and progression of PD and other chronic neurodegenerative disorders.

EXAMPLE 29

Measurement of infarct volume

[0269] Figures 33A and B show the change in infarct volume (mm³) in mice given a monoclonal antibody to IFNAR^{-/-} (MAR1). Infarct volume (mm³) was significantly less in mice given MAR1 compared to mice given vehicle alone (no MAR1), 60 minutes prior to a traumatic brain injury (TBI) event (Figure 33A).

[0270] Figure 33B shows at 30 minutes post TBI event; the infarct volume remained significantly less. This further shows the utility of an IFNAR1 antagonist (e.g. an IFNAR1 mAb) to reduce an infarct prophylactically.

[0271] Those skilled in the art will appreciate that aspects described herein are susceptible to variations and modifications other than those specifically described. It is to be understood that these aspects include all such variations and modifications. The disclosure also includes all of the steps, features, compositions and compounds referred to or

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indicated in this specification, individually or collectively, and any and all combinations of any two or more of the steps or features.

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CLAIMS:

1. A method for reducing a neuroinflammatory response within the central nervous system of a subject following a neurological event or condition, said method comprising administering to said subject an effective amount of an antagonist of Type 1 interferon-mediated signalling for a time and under conditions sufficient to prevent or attenuate neuroinflammation.
2. The method of Claim 1 wherein the neuroinflammatory response is in the brain.
3. The method of Claim 2 wherein the neurological event or condition is acute neuronal injury.
4. The method of Claim 3 wherein the acute neuronal injury is selected from traumatic brain injury, neurodegenerative disease, drug abuse, irradiation or chemotherapy, starvation, spinal cord trauma and infection by a pathogenic agent.
5. The method of Claim 4 wherein the traumatic brain injury includes a severe head injury and trauma-induced paralysis.
6. The method of Claim 4 wherein the neurodegenerative disease is selected from Alzheimer's disease, Parkinson's disease and other Parkinsonian conditions and syndromes, amyotrophic lateral sclerosis, Friedreich's ataxia, Huntington's disease, Lewy body disease, spinal muscular atrophy, motor neuron disease, Alper's disease, Batten disease, Cerebro-oculo-facio-skeletal syndrome, corticobasal degeneration, Leigh's disease, Machads-Joseph disease, monomelic amyotrophy, multiple system atrophy, multiple sclerosis, neurodegeneration with brain iron accumulation, olivopontocerebellar atrophy, opsoclonus myoclonus, paraneoplastic syndromes, progressive multifocal leukoencephalopathy and Aicardi-Coutieres syndrome.

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7. The method of Claim 4 wherein the neurodegenerative disease is Alzheimer's disease or other conditions associated with deposition of pathological forms of amyloid.
8. The method of any one of Claims 1 to 7 wherein the antagonist targets a Type 1 interferon.
9. The method of Claim 8 wherein the Type 1 interferon is IFN α or IFN β .
10. The method of any one of Claims 1 to 7 wherein the antagonist targets the Type 1 interferon receptor or a component thereof.
11. The method of Claim 10 wherein the receptor component is Type 1 interferon alpha receptor 1 (IFNAR1).
12. The method of Claim 1 wherein the subject is a human.
13. The method of Claim 12 wherein the method reduces infarct size.
14. The method of Claim 1 wherein the antagonist is selected from a protein, small molecule, antibody including an immunoglobulin new antigen receptor (IgNAR) specific, for a Type 1 interferon or IFNAR1, a genetic molecule which down-regulates expression of a Type 1 interferon or IFNAR1, a modified Type 1 interferon and a soluble IFNAR1.
15. A method for a method for reducing a neuroinflammatory response within the central nervous system of a subject following a neurological event or condition, said method comprising administering to said subject an antagonist of interferon alpha receptor 1 (IFNAR1)-mediated signalling for a time and under conditions sufficient to prevent or ameliorate the symptoms of neuroinflammation.
16. The method of Claim 15 wherein the neuroinflammatory response is in the brain.

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17. The method of Claim 16 wherein the neurological event or condition is acute neuronal injury.
18. The method of Claim 14 wherein the acute neuronal injury is selected from traumatic brain injury, neurodegenerative disease, drug abuse, irradiation or chemotherapy and starvation, spinal cord trauma and infection by a pathogenic agent.
19. The method of Claim 18 wherein the traumatic brain injury includes a severe head injury and trauma-induced paralysis.
20. The method of Claim 18 wherein the neurodegenerative disease is selected from Alzheimer's disease, Parkinson's disease and other Parkinsonian conditions and syndromes, amyotrophic lateral sclerosis, Friedreich's ataxia, Huntington's disease, Lewy body disease, spinal muscular atrophy, motor neuron disease, Alper's disease, Batten disease, Cerebro-oculo-facio-skeletal syndrome, corticobasal degeneration, Leigh's disease, Machads-Joseph disease, monomelic amyotrophy, multiple system atrophy, multiple sclerosis, neurodegeneration with brain iron accumulation, olivopontocerebellar atrophy, opsoclonus myoclonus, paraneoplastic syndromes, progressive multifocal leukoencephalopathy and Aicardi-Coutieres syndrome.
21. The method of Claim 18 wherein the neurodegenerative disease is Alzheimer's disease or other conditions associated with deposition of pathological forms of amyloid.
22. The method of Claim 15 wherein the subject is a human.
23. The method of Claim 22 wherein the method reduces infarct size.

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24. The method of Claim 15 wherein the antagonist is selected from a protein, small molecule, antibody including an immunoglobulin new antigen receptor (IgNAR) specific for IFNAR1, a genetic molecule which down-regulates IFNAR1, a modified form of a Type 1 interferon and a soluble IFNAR1.

25. A neuroprotective formulation comprising an antagonist of Type 1 interferon-mediated signalling and one or more pharmaceutically acceptable carriers, diluents and/or excipients.

26. The neuroprotective formulation of Claim 25 wherein the antagonist is selected from a protein, small molecule, antibody including an immunoglobulin new antigen receptor (IgNAR) specific, for a Type 1 interferon or IFNAR1, a genetic molecule which down-regulates expression of a Type 1 interferon or IFNAR1, a modified Type 1 interferon and a soluble IFNAR1.

27. A medical protocol for treating acute neuronal injury in a subject, said protocol comprising administering to said subject, from 1 to 120 minutes of the injury, a neuroprotective formulation of Claim 25 or 26.

28. A method for treating cognitive impairment in a human subject, said method comprising administering to said subject an antagonist of Type 1 interferon-mediated signalling for a time and under conditions sufficient to ameliorate cognitive impairment or at least improve a subject's cognitive ability.

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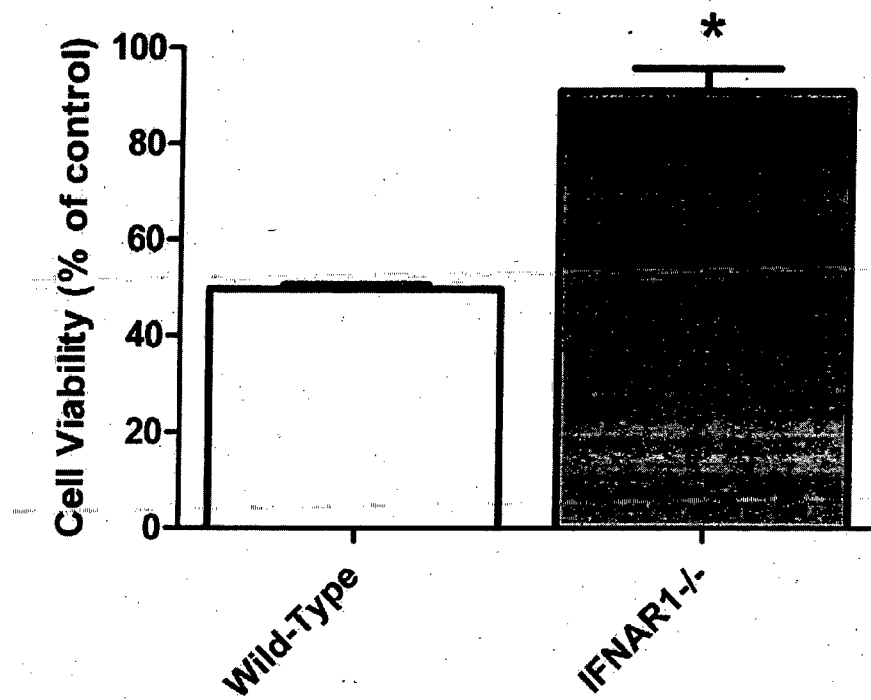


Figure 1

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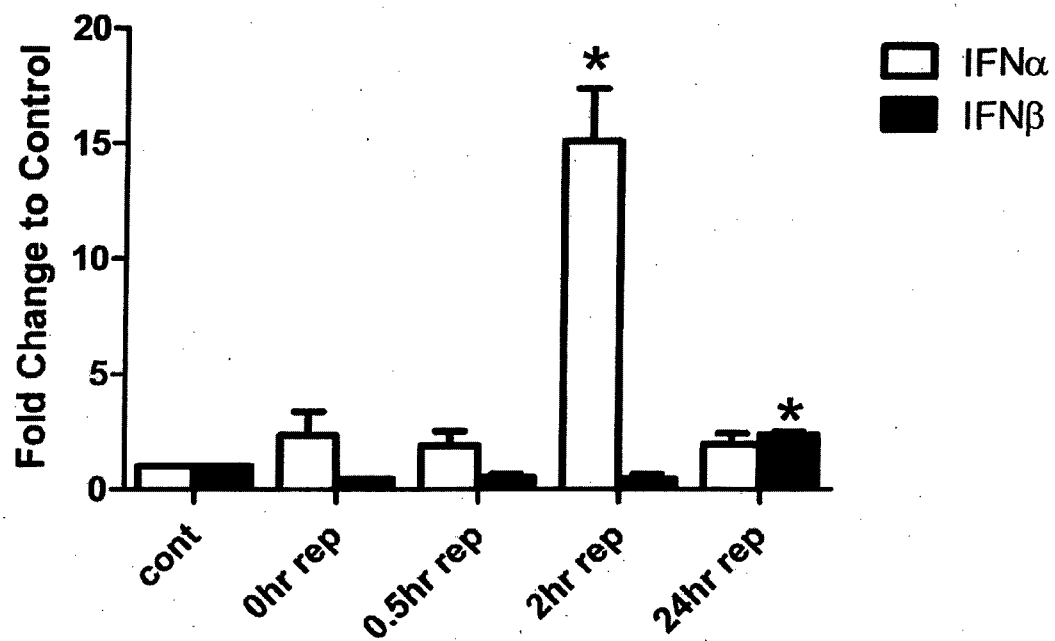
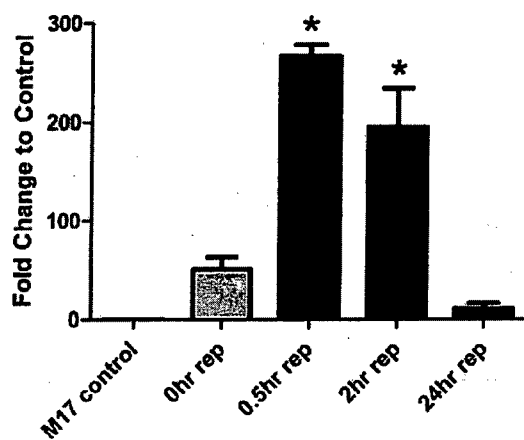
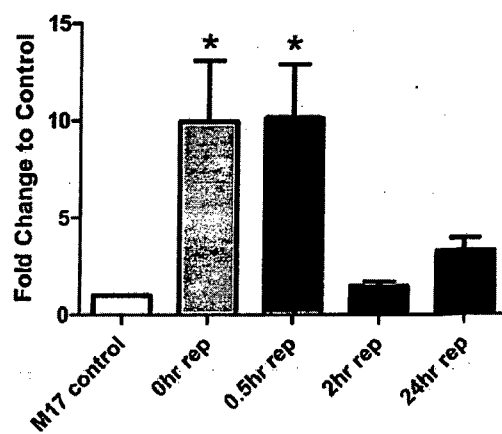
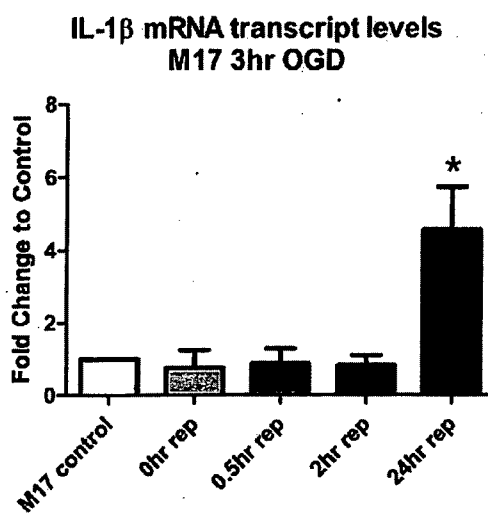


Figure 2

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A**B****C****Figures 3A to C**

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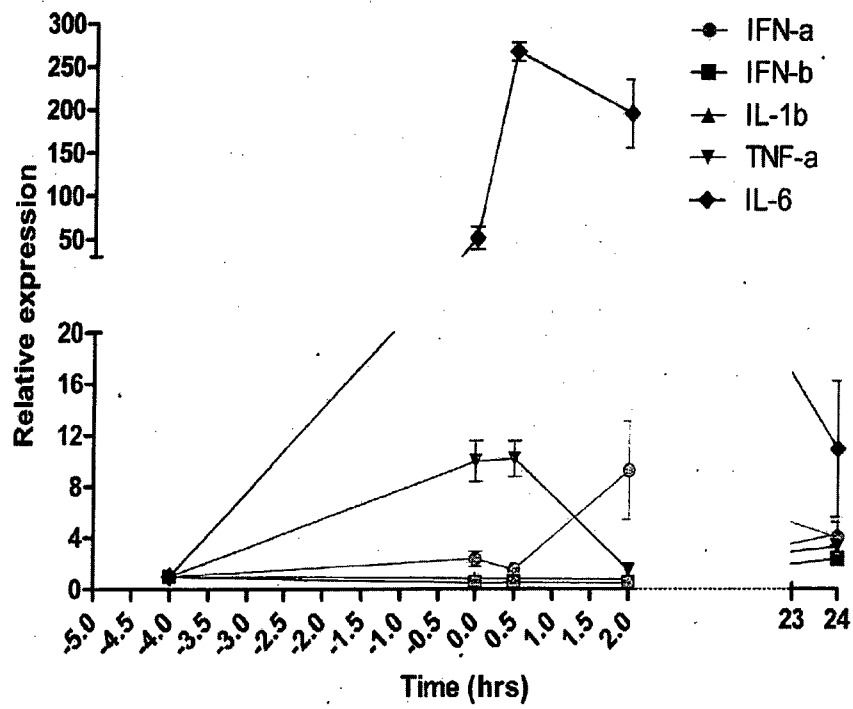


Figure 4

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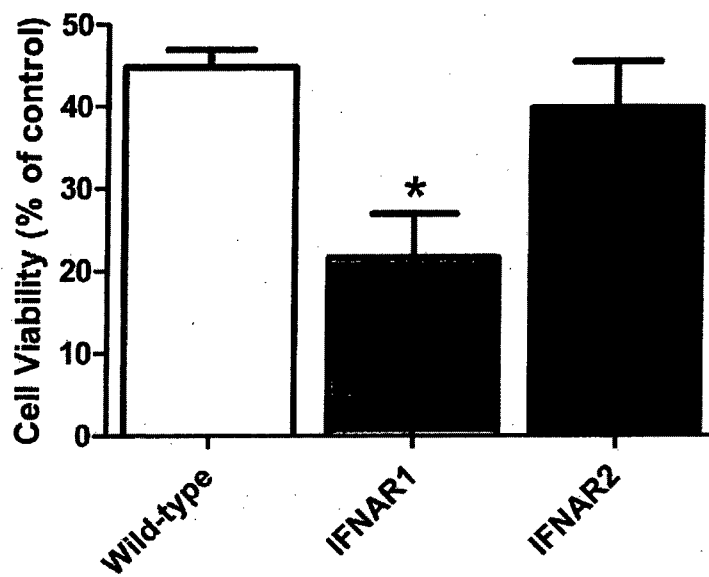


Figure 5

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Gene/Primer	Sequence 5' - 3'
IFNAR1 gene	ATGCTGGCCGTGGTGGGAGCTGCTGCCCTGGTGGTGGCTGGAGCTCCCTGGGTGC TGCCTTCTGCTGCCGGCGGAGAAAAACCTGAAGCCCCCTGAGAATATCGACGTGTACAT CATTGACGATAACTATACCCCTGAAAGGAGCTCCCACGGGGAGTCTATGGGCAGTGTGA CCTTCAGCGCCGAATACAGGACTAAGGACGAGGCCAAGTGGCTGAAAGTGCCCGAATG CCAGCATACCACAACCTACCAAGTGTGAGTTCAGCCTGCTGGATACTAACGTGTACATC AAAACCCAGTTTAGGGTGAGAGCTGAGGAAGGCAACTCCACCTCTAGTTGGAATGAAG TGGACCCCTTCATTCCCTTCTACACAGCTCACATGTCCCCACCCGAGGTGCGGCTGGAG GCTGAAGACAAGGCCATCCTGGTGCATATTTCTCCTCCAGGCCAGGATGGAAACATGT GGGCCCTGGAGAAGCCAAGCTTCTCCTATACCATCCGCATTTGGCAGAAAAGCTCCTC TGATAAGAAAACCATCAATTCCACATACTATGTGGAGAAGATTCCAGAACTGCTGCCC GAGACAACCTTACTGCCTGGAGGTGAAAGCCATCCACCCCTCTCTGAAGAAACATAGCA ACTATTCCACAGTGCAGTGTATTAGTACCACAGTGGCTAACAAGATGCCCGTGCCTGG GAATCTGCAGGTGGACGCCCAGGGCAAGTCTTACGTGCTGAAATGGGACTATATCGCT AGTGCCGATGTGCTGTTTAGGGCTCAGTGGCTGCCTGGCTACTCTAAGAGTAGCTCCGG ATCTAGAAGTGACAAGTGGAACCAATTCCCACTTGCGCCAACGTGCAGACTACCCAC TGCGTGTTCAGCCAGGATACCGTGTATACAGGCACTTTCTTTCTGCACGTGCAGGCTTC CGAGGGAAATCATACCTTTTCTGGAGTGAGGAAAAGTTTATCGACTCTCAGAAACAT ATTCTGCCCCCTCCACCCGTGATCACCGTGACAGCCATGAGCGATACTCTGCTGGTGTA CGTGAAGTGCCAGGACTCCACCTGTGATGGGCTGAATTATGAAATCATTCTTCTGGGAG AACACAAGCAATACTAAGATCTCCATGGAAAAAGATGGCCCCGAGTTTACCCTGAAGA ACCTGCAGCCTCTGACAGTGTACTGCGTGCAGGCTCGGGTGCTGTTCCGCGCCCTGCTG AACAAAACAAGCAATTTTCCGAAAAGCTGTGCGAGAAAAGTGGCTGGAAGCTTCT CCACTATCTGGATCATTACCGGACTGGGGTGGTGTCTTTAGCGTGATGGTGTGTAC GCCCTGCGGTCCGTGTGGAAGTATCTGTGCCACGTGTGCTTTCTCCACTGAAACCCCC TCGCTCCATCGACGAATTTCTTTCTGAGCCACCCAGTAAGAACCCTGGTGTGTGACAG CTGAGGAACACACTGAAAGGTGCTTCATCATTGAGAATACTGATACCGTGGCTGTGGA GGTGAAGCATGCCCCAGAGGAAGACCTGAGAAAATACTCTAGTCAGACATCTCAGGAT AGTGGCAACTATAGTAATGAGGAAGAGGAATCTGTGGGAACTGAGAGTGGGCAGGCT GTGCTGAGCAAGGCTCCTTGCGGCGGACCATGTAGCGTGCCATCCCTCCAGGCACCC TGGAAGACGGAACATGCTTCTGGGGAACGAGAAGTACCTGCAGAGCCCTGCTCTGAG AACAGAGCCAGCCCTGCTGTGCTGA
IFNAR1 AttB1 subclone	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATGCTGGCCGTGGTGGGAGCTGCT
IFNAR1 AttB2 Subclone	GGGGACCACTTTGTACAAGAAAGCTGGGTTGCACAGCAGGGCTGGCTCTGTTCTCAG

Figure 6

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Gene/ Primer	Sequence 5'-3'
IFNAR2 gene	ATGCGTTCTCGTTGTACCGTGAGTGCCGTTGGCCTGCTGTCGCTGTGTCTGGTCGT TAGTGCCCTCCCTGGAACCATTAACCCGTCAGCCTTTGACGGTTATCCGGATGAAC CGTGCACCATTAAACATCACGATTCGTAATAGCCGCCTGATTCTGTCTTGGGAACTG GAAAACAAAAGCGGTCCGCCGGCGAATTATACCCTGTGGTACACGGTCATGTCAA AAGACGAAAACCTGACCAAAGTGAAAAATTGCTCGGATACCACGAAAAGCTCTTG TGACGTGACGGATAAATGGCTGGAGGGTATGGAAAGTTATGTGGTTGCGATCGTT ATTGTCCATCGCGGCACCTGACCGTTTGCCGTTGTTCCGATTACATTGTCCCGGC CAATGCCCCGCTGGAACCGCCAGAATTTGAAATCGTGGGTTTCACCGATCACATT AATGTCACGATGGAATTTCCGCCGGTGACCTCAAAAATCATCCAGGAAAAAATGA AAACCACGCCGTTCTGTGATCAAAGAACAAATTTGGTGATAGCGTTTCGCAAAAAACA TGAACCGAAAGTCAACAATGTGACCGGCAACTTTACGTTCTGCTGCGTGATCTGC TGCCGAAAACCAATTATTGCGTCAGTCTGTACTTTGATGACGATCCGGCAATCAA ATCCCCGCTGAAATGTATTGTGCTGCAGCCGGGTCAAGAAAGTGGCCTGAGCGAA TCTGCTATCGTTGGTATTACCACGAGCTGCCTGGTCGTGATGGTGTTCTGTTTCTAC CATCGTGATGCTGAAACGCATCGGCTATATTTGTCTGAAAGATAATCTGCCGAAC GTTCTGAATTTTCGTCACTTCCTGACCTGGATTATCCCGGAACGCAGTCCGTCCGA AGCAATTGATCGTCTGGAATTATCCCGACCAACAAGAAAAAACGTCTGTGGAAT TATGACTACGAAGATGGTTTCAGACTCGGATGAAGAAGTGCCGACCGCTAGCGTTA CCGGCTATACGATGCATGGTCTGACGGGCAAACCGCTGCAGCAAACCTCTGATAC CAGCGCCTCTCCGGAAGACCCGCTGCACGAAGAAGATAGTGGCGCCGAAGAATCC GATGAAGCAGGTGCTGGTGCCGGTGACAGAACCGGAACTGCCGACCGAAGCCGGT GCGGGTCCGAGCGAAGATCCGACCGGTCCGTACGAACGTCGCAAATCAGTGCTGG AAGACTCGTTTCCGCGCGAAGATAACAGTAGCATGGATGAACCGGGCGATAACAT CATCTTCAACGTTAACCTGAACTCTGTTTTCTGCGTGCTCTGCATGACGAAGATG CGTCAGAAACCCTGTCGCTGGAAGAAGACACGATCCTGCTGGATGAAGGTCCGCA GCGTACCGAAAGCGACCTGCGCATTGCCGGCGGTGATCGTACCCAGCCGCCGCTG CCGAGTCTGCCGTCCCAAGACCTGTGGACCGAAGATGGCTCATCGGAAAAAAGTG ACACCAGTGACAGCGATGCGGACGTTGGCGATGGCTACATTATGCGTTGA
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Figure 7

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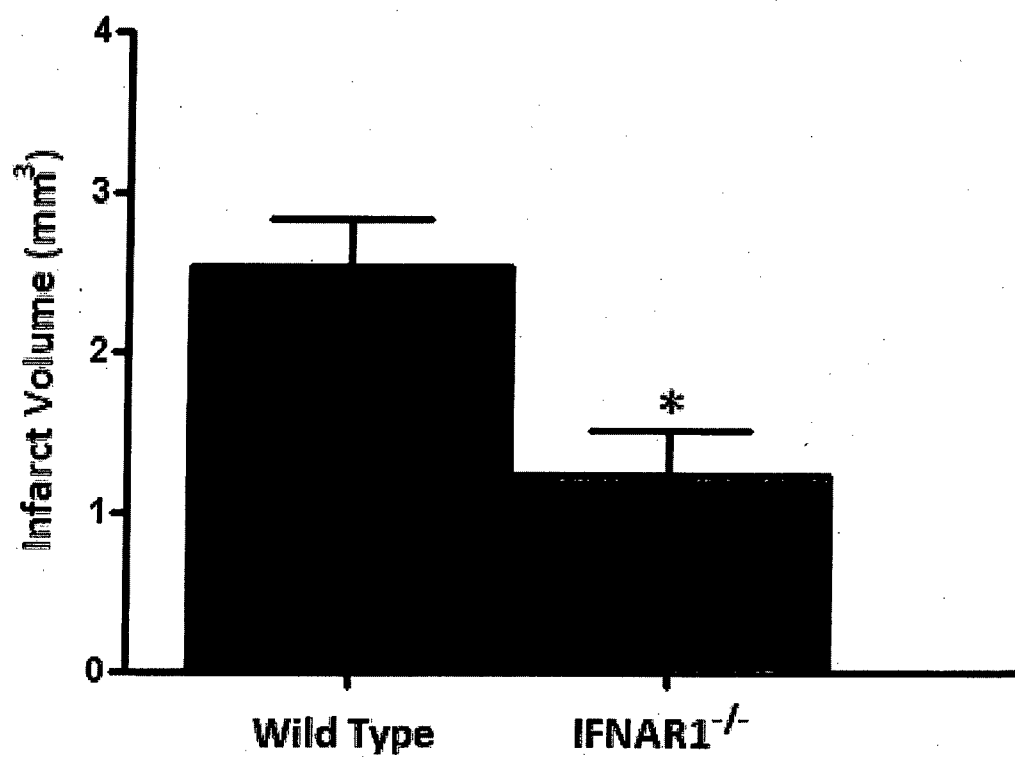
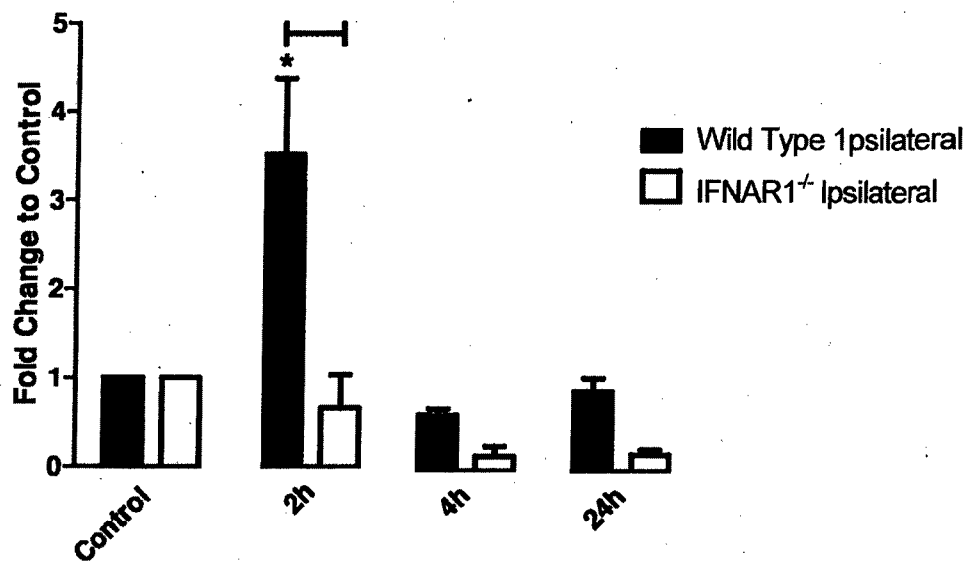
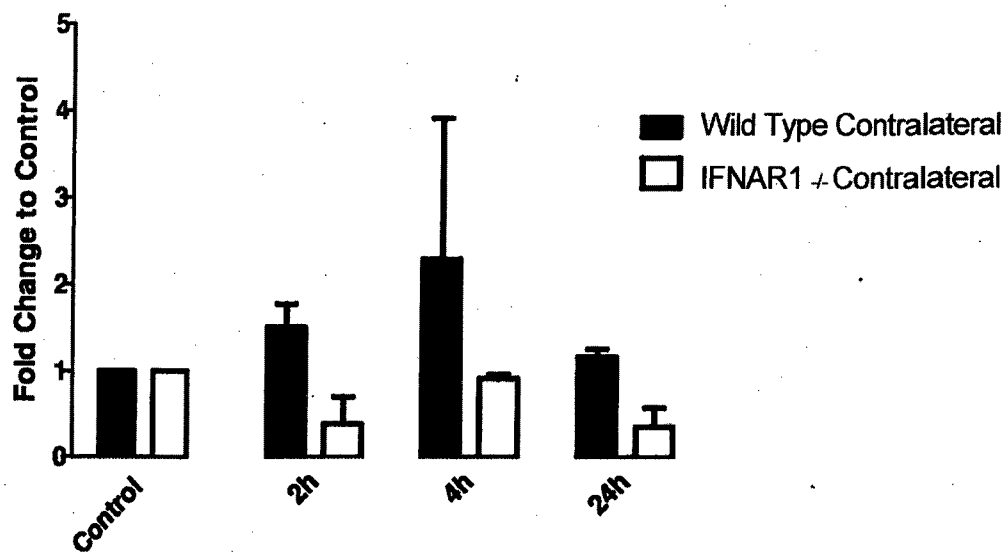


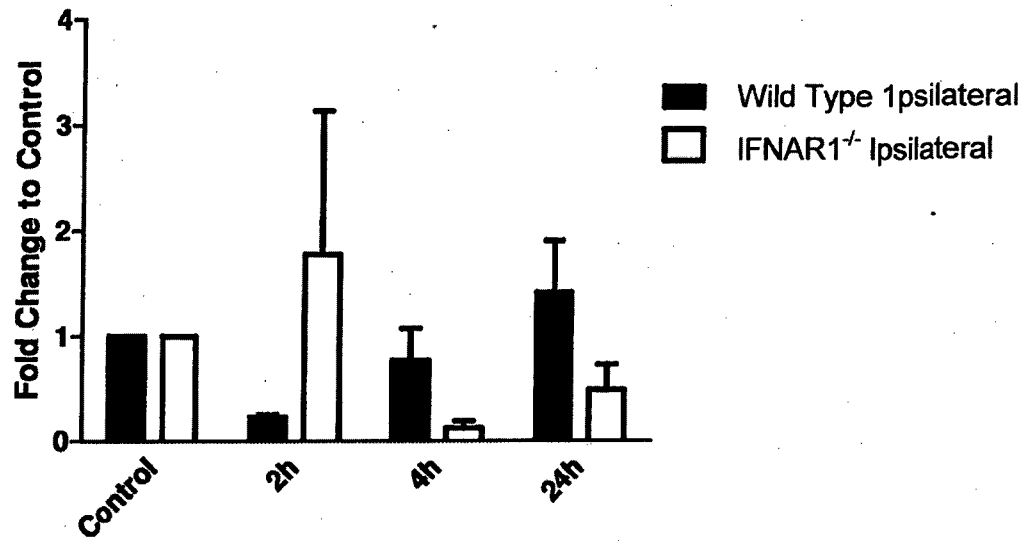
Figure 8

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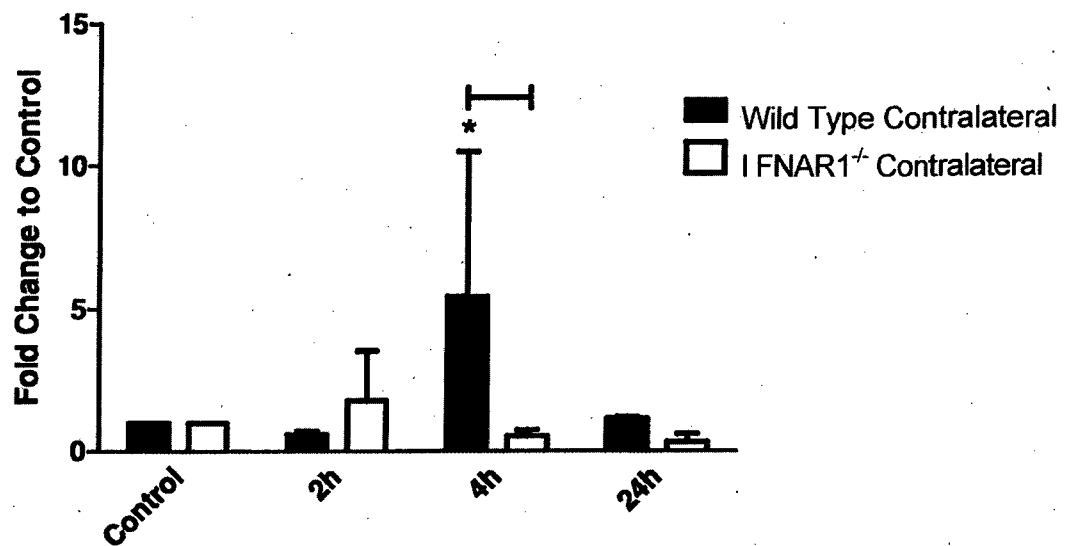
A**B****Figures 9A and B**

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A



B



Figures 10A and B

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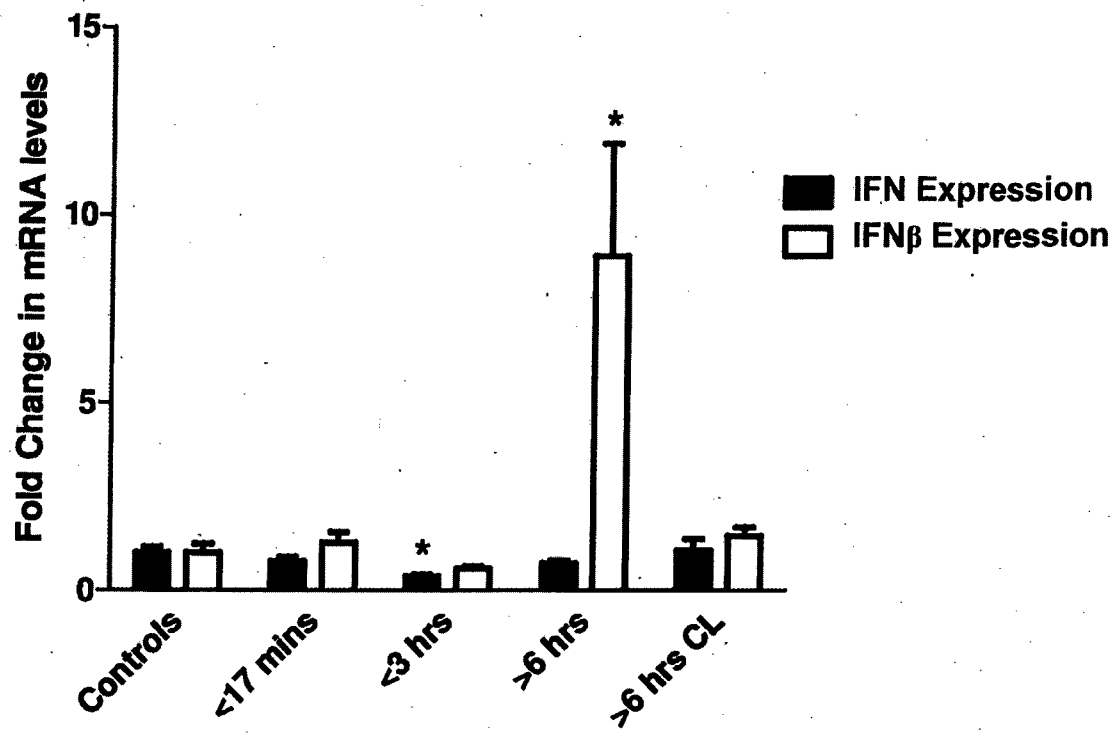
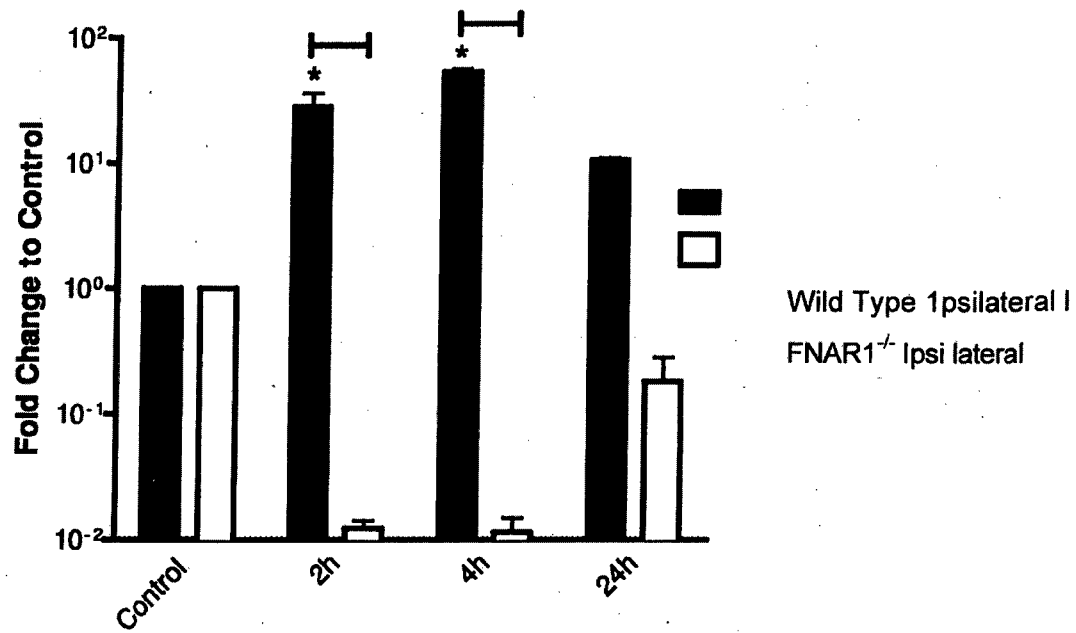


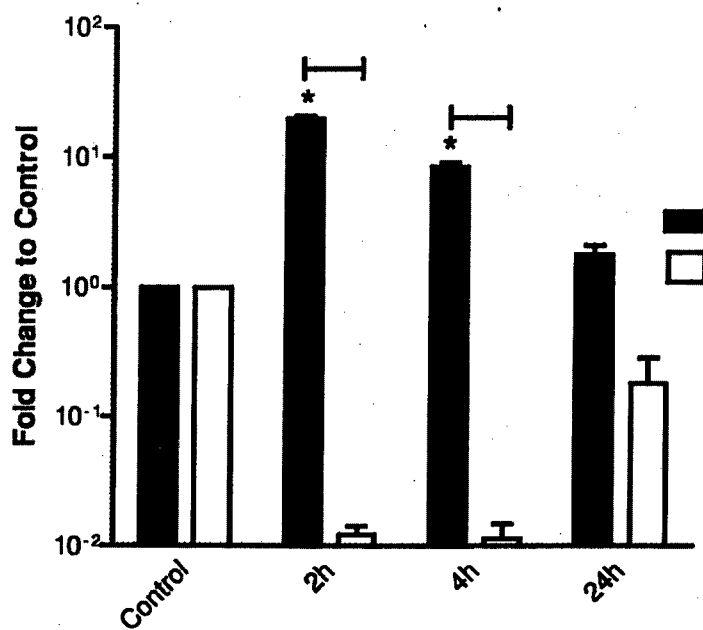
Figure 11

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A)

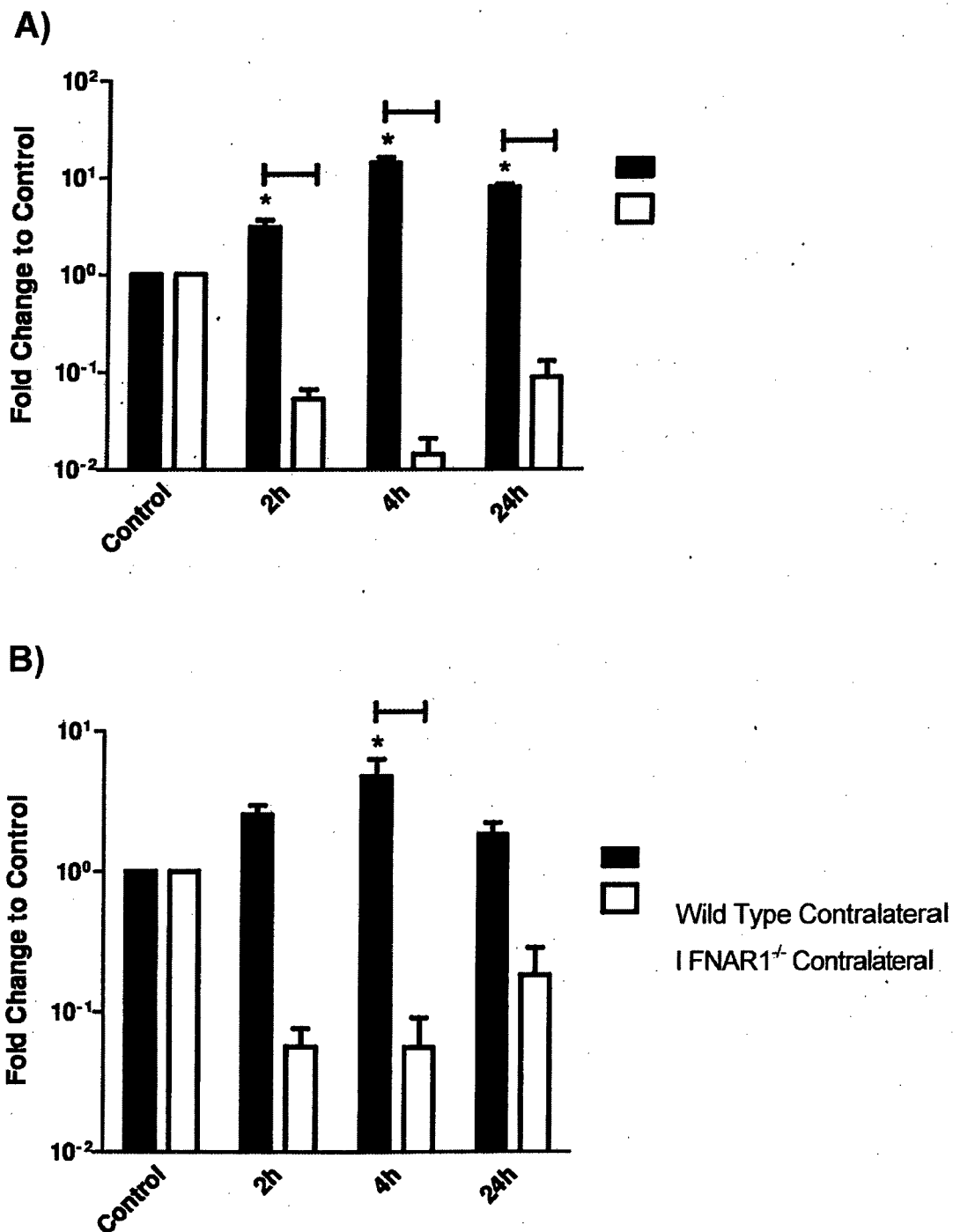


B)



Figures 12A and B

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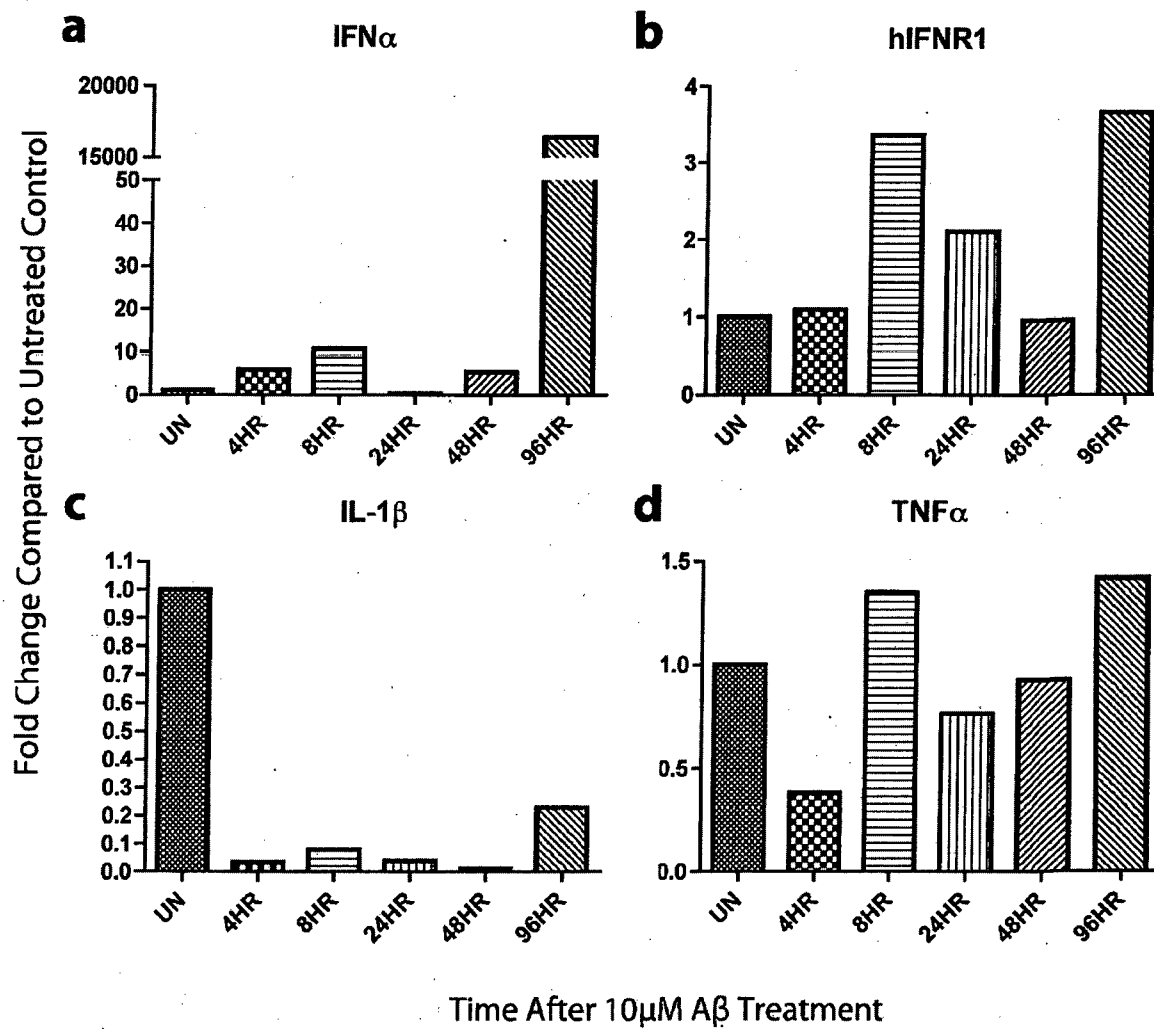
Figures 13A and B

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SYBRGreen Primer	Sequence (5'...3')
GADPH Forward Primer	ATCTTCTTGTGCAGTGCCAGC
GADPH Reverse Primer	ACTCCACGACATACTCAGCACC
All Alpha Forward Primer	GCAATCCTCCTAGACTCACTTCTGCA
All Alpha Reverse Primer (1)	TATAGTTCCTCACAGCCAGCAG
All Alpha Reverse Primer (2)	TATTTCTTCATAGCCAGCTG

Figure 14

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Figures 15A through D

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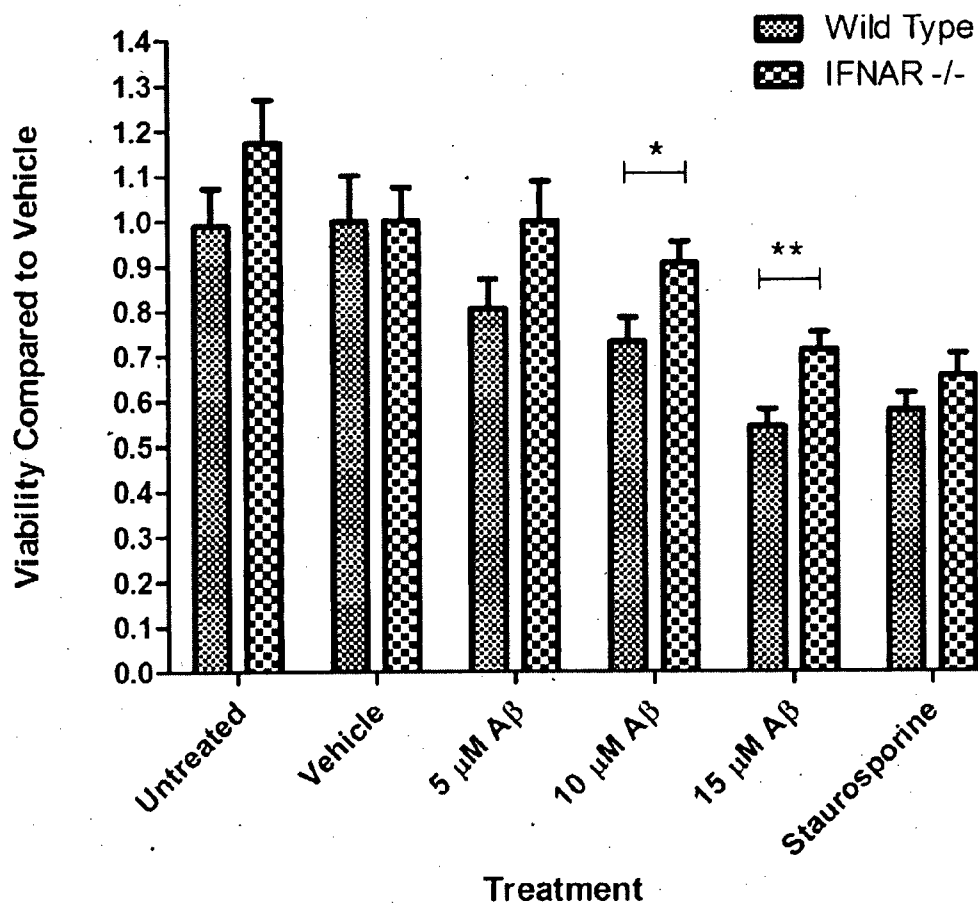


Figure 16

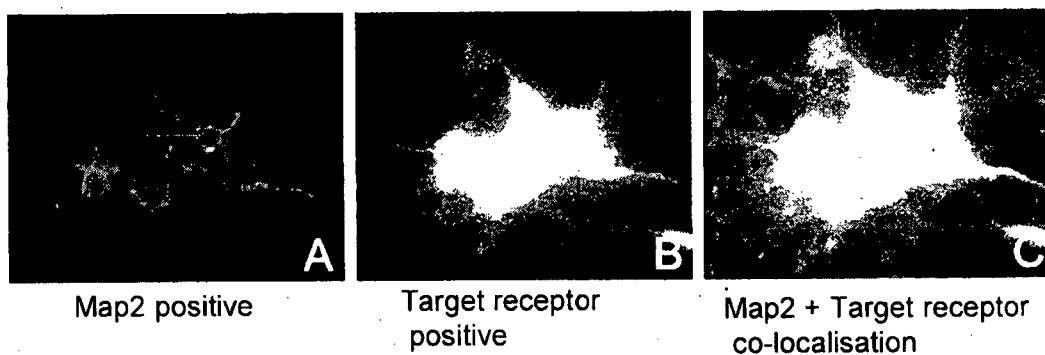
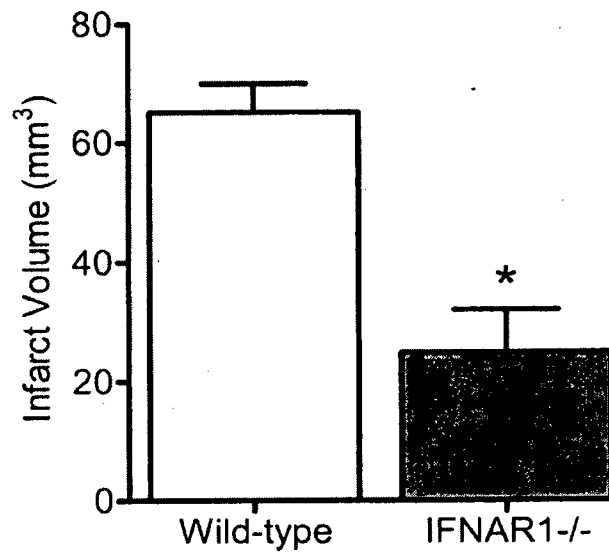


Figure 17

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(a)



(b)

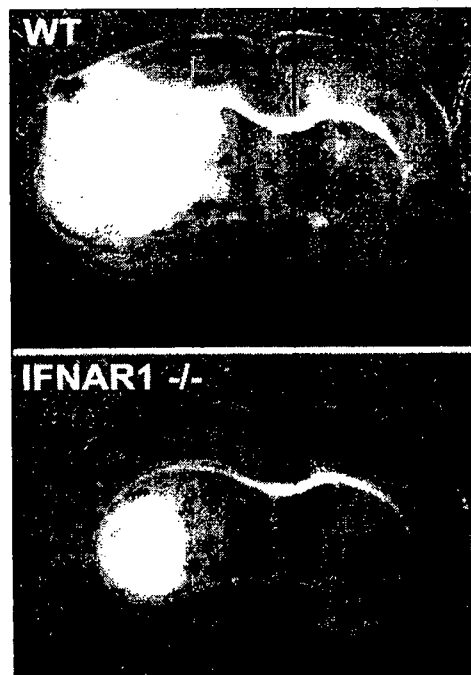


Figure 18

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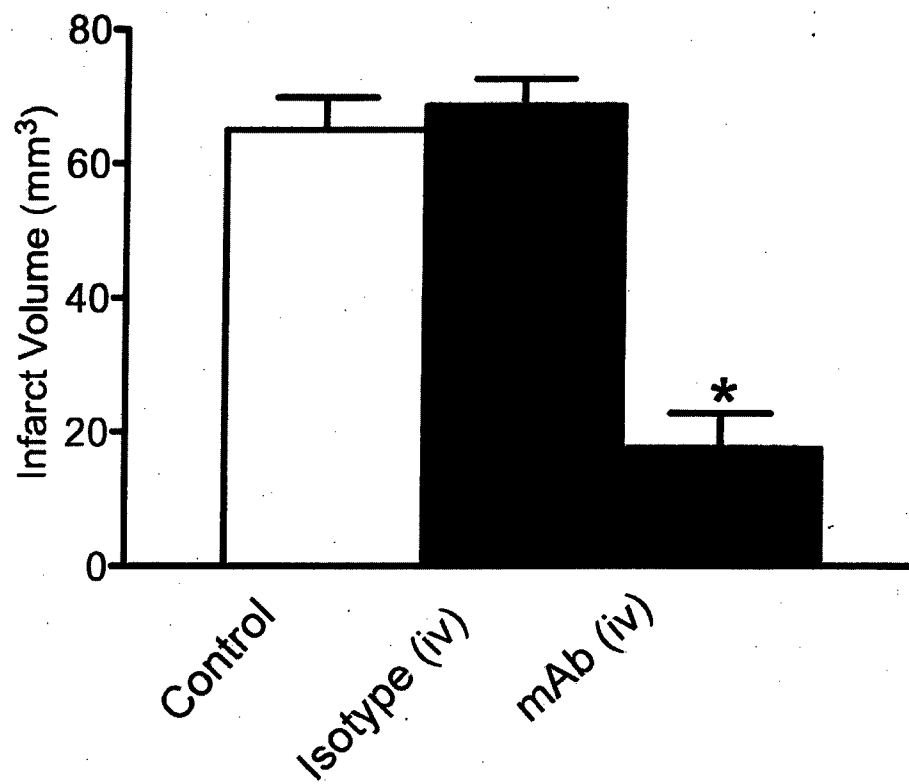


Figure 19

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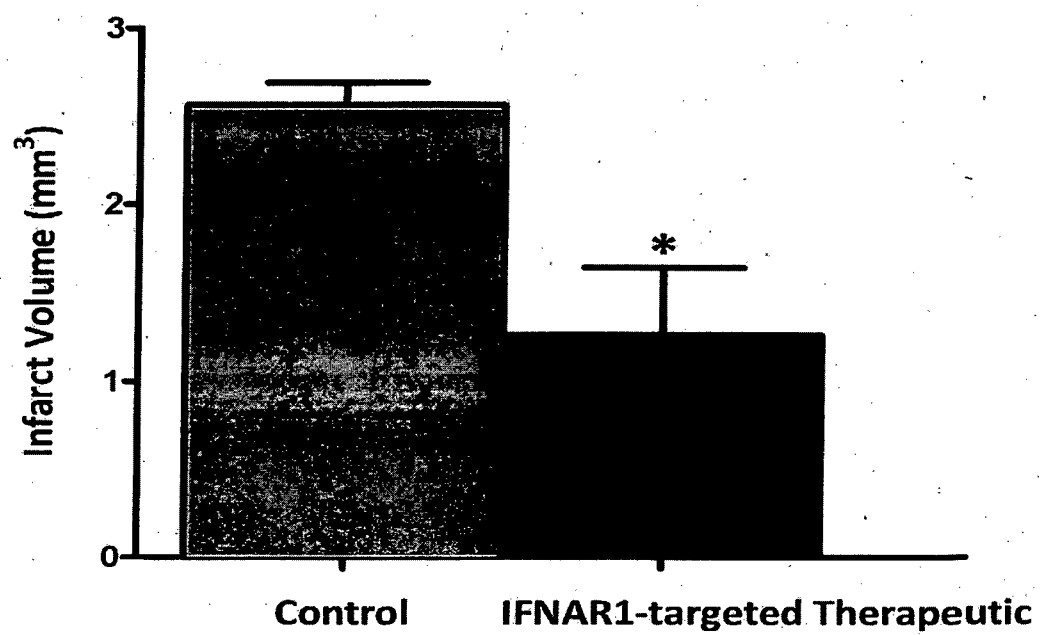


Figure 20

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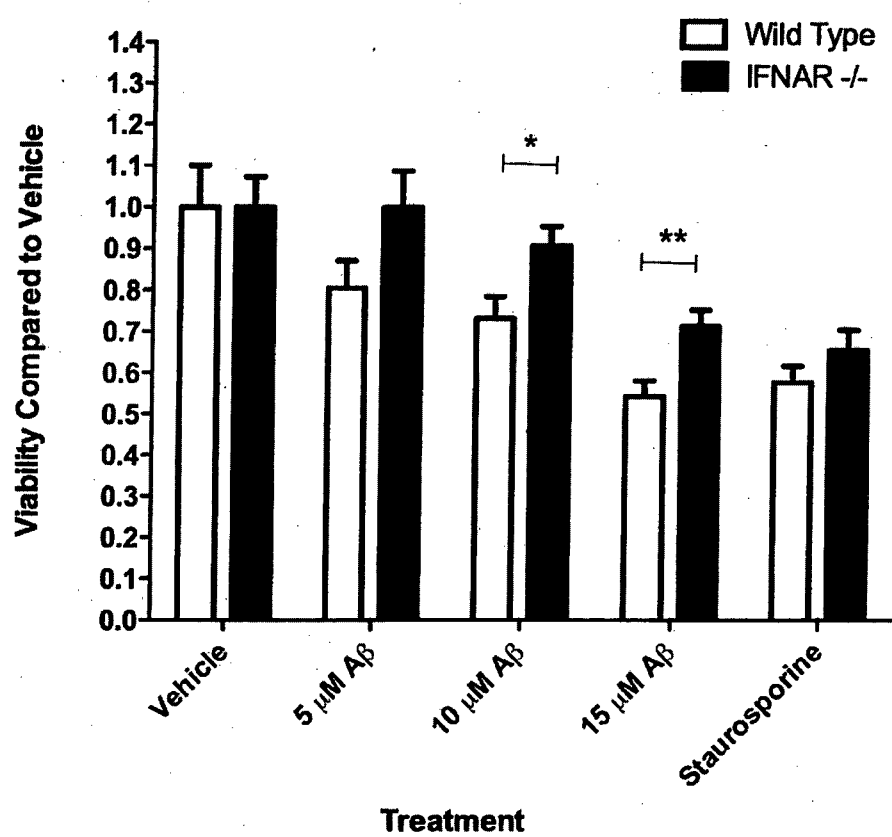


Figure 21

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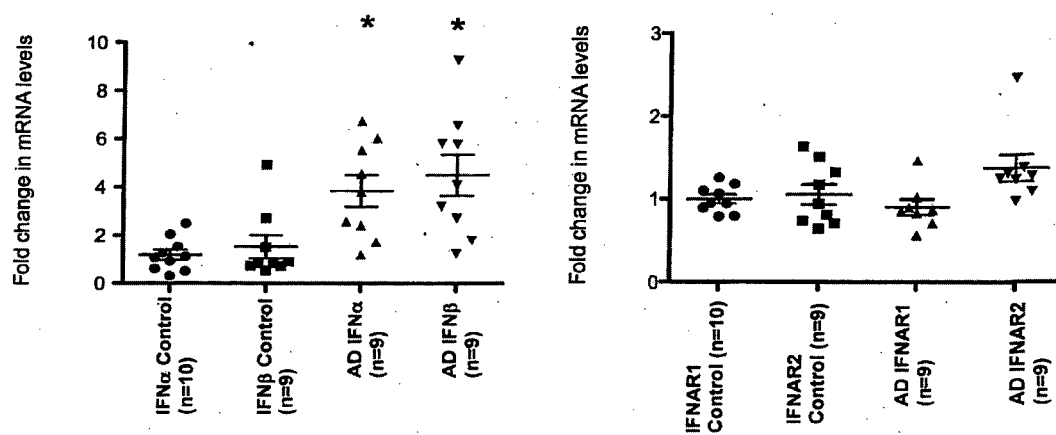


Figure 22

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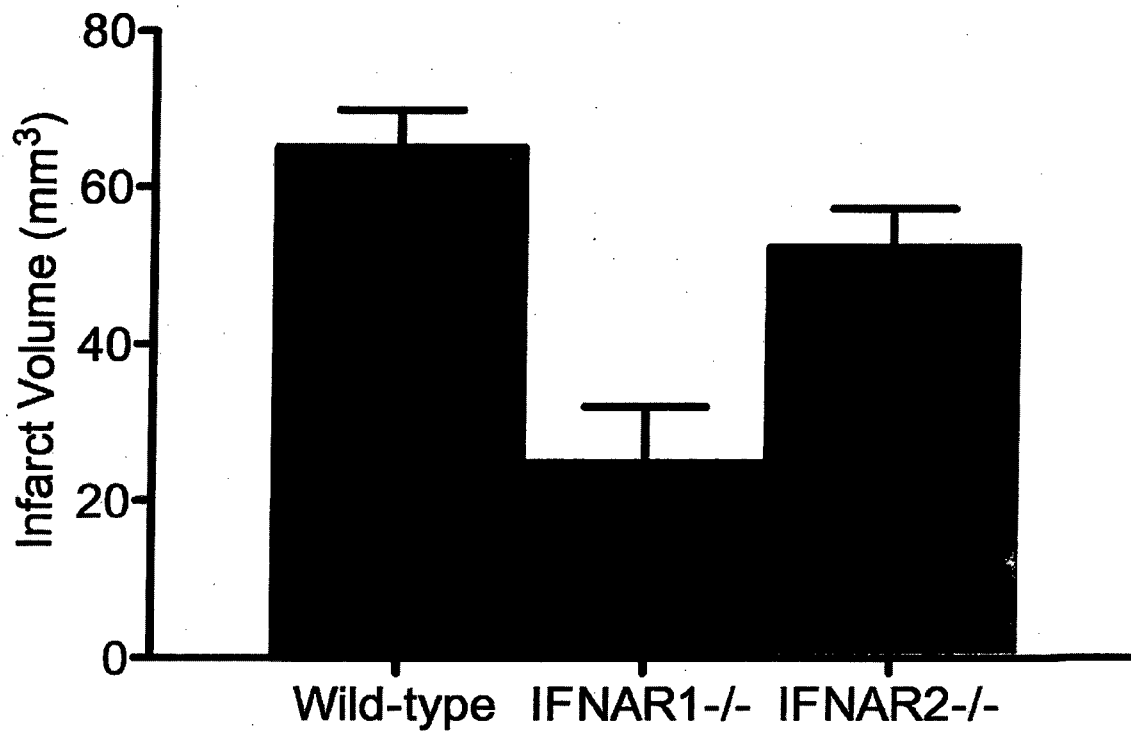
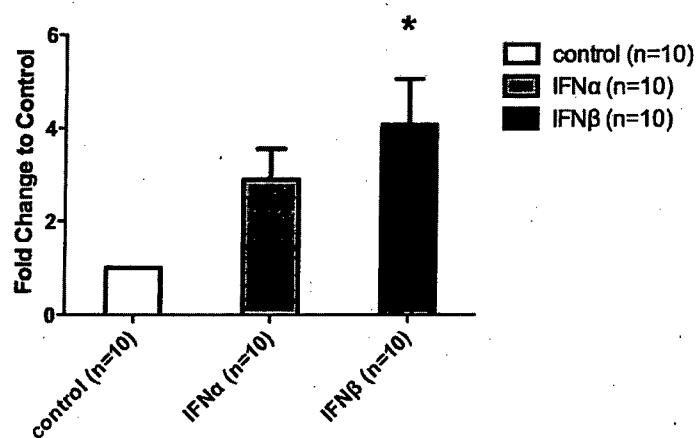


FIGURE 23

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A



B

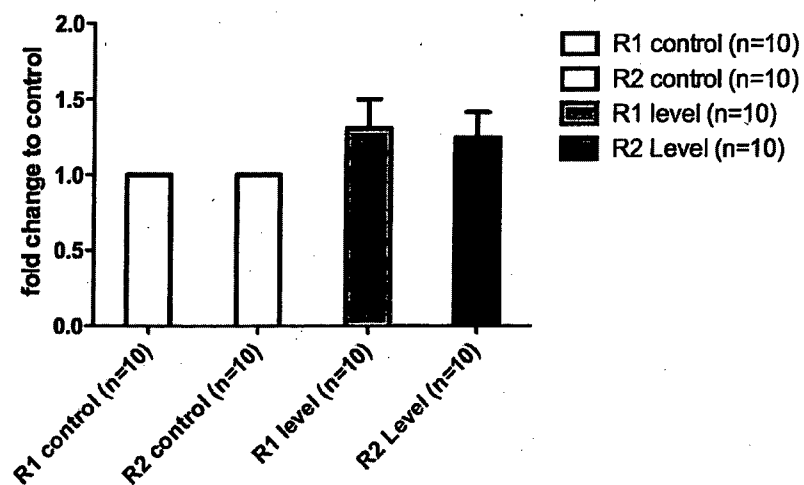


FIGURE 24

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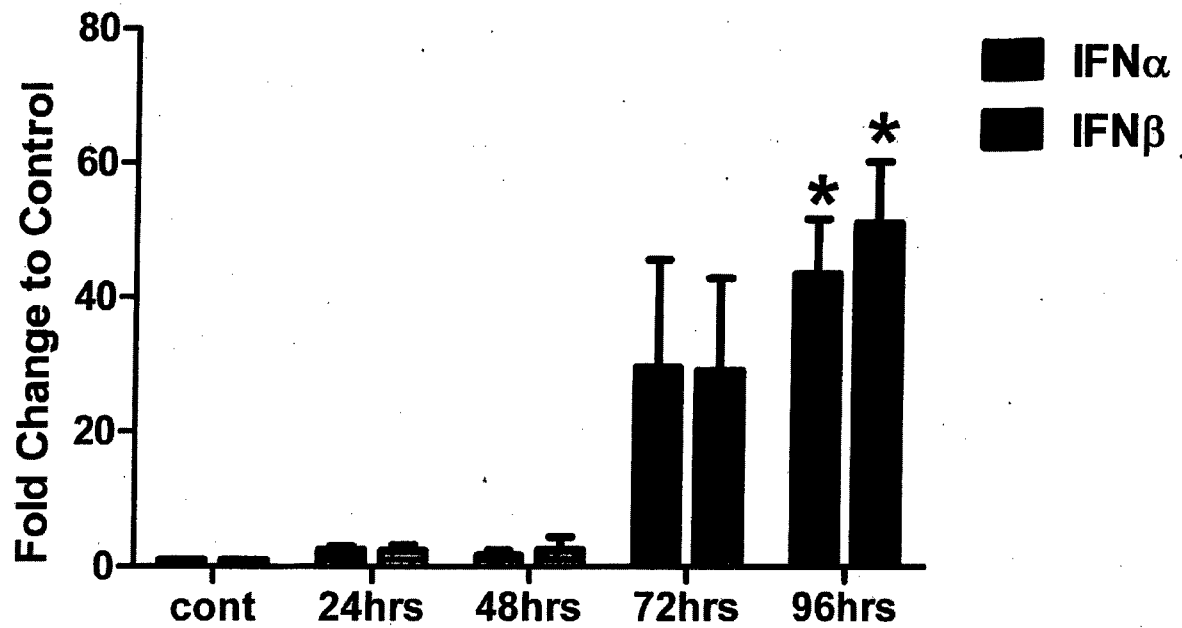


FIGURE 25

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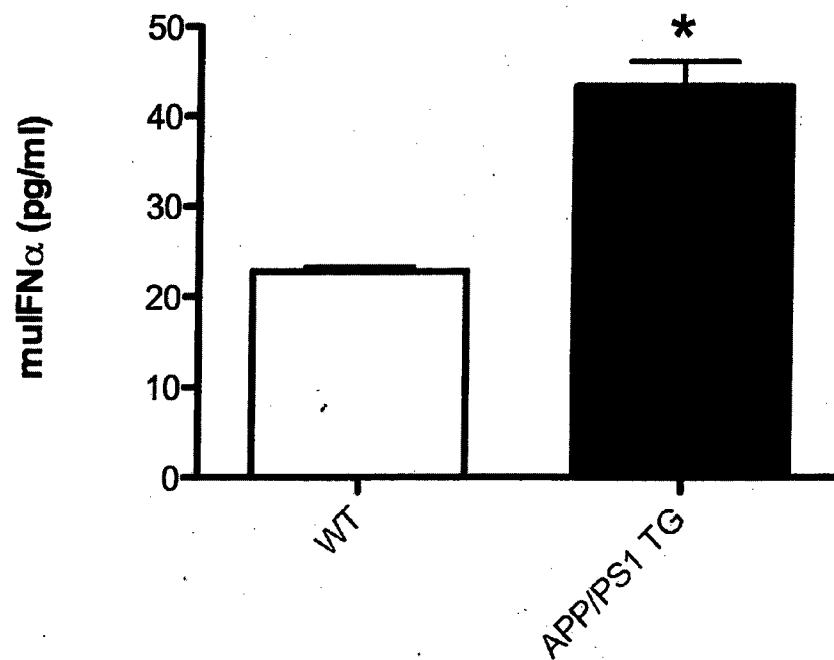
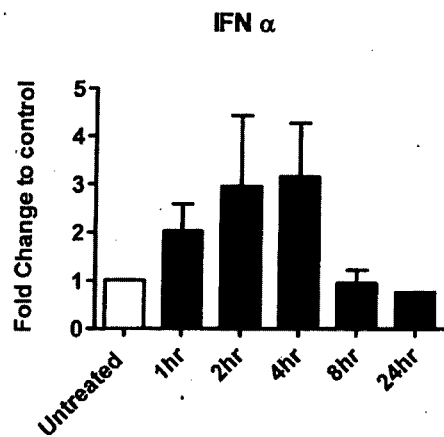


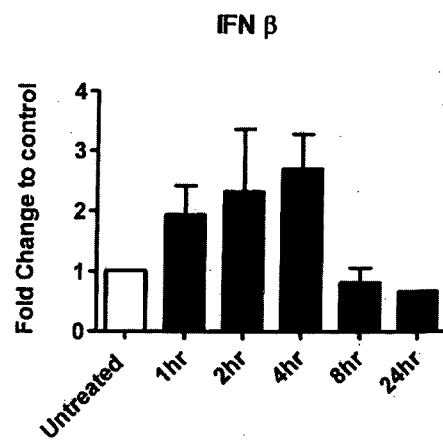
FIGURE 26

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a)



b)



c)

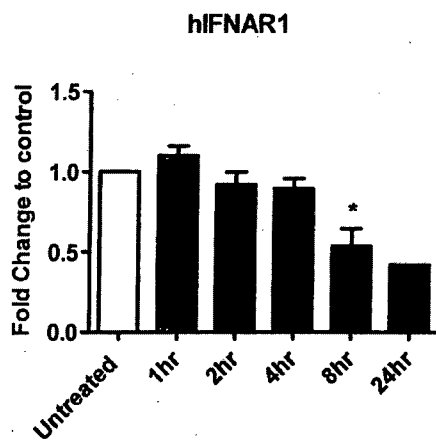


FIGURE 27

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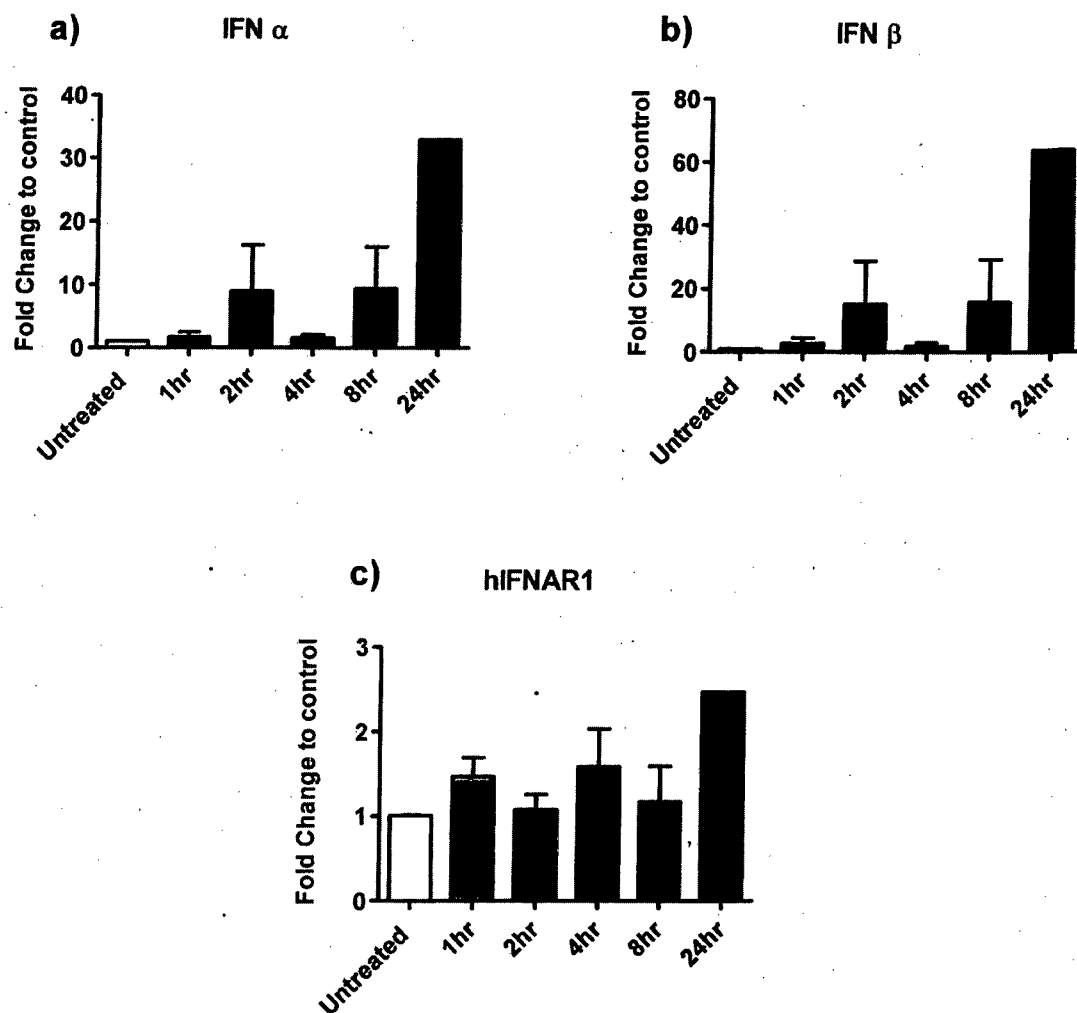


FIGURE 28

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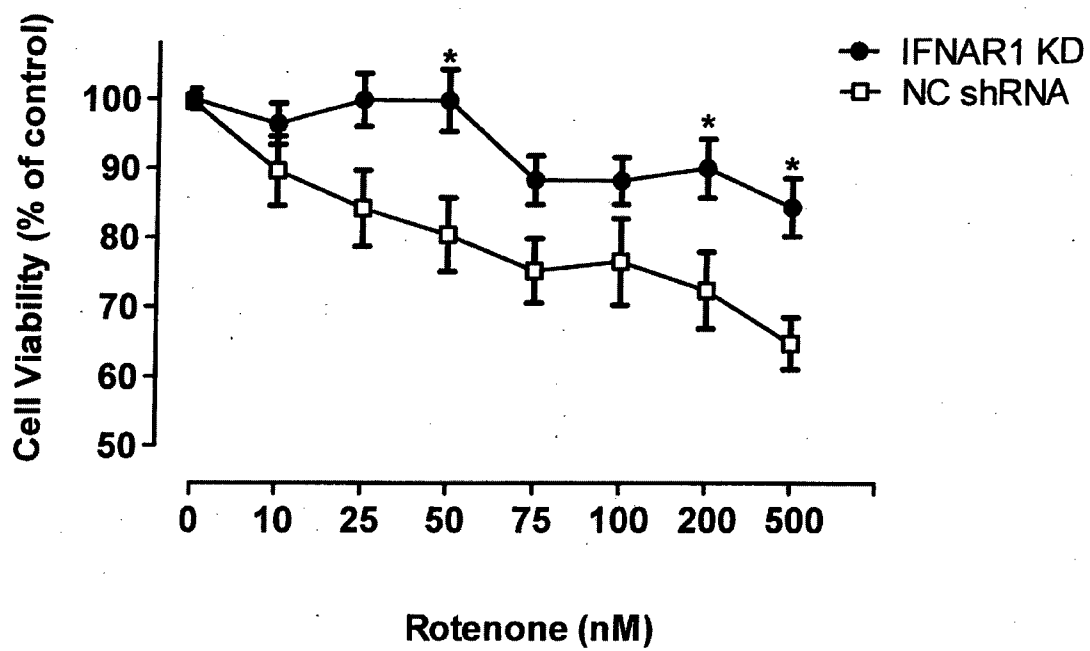


FIGURE 29

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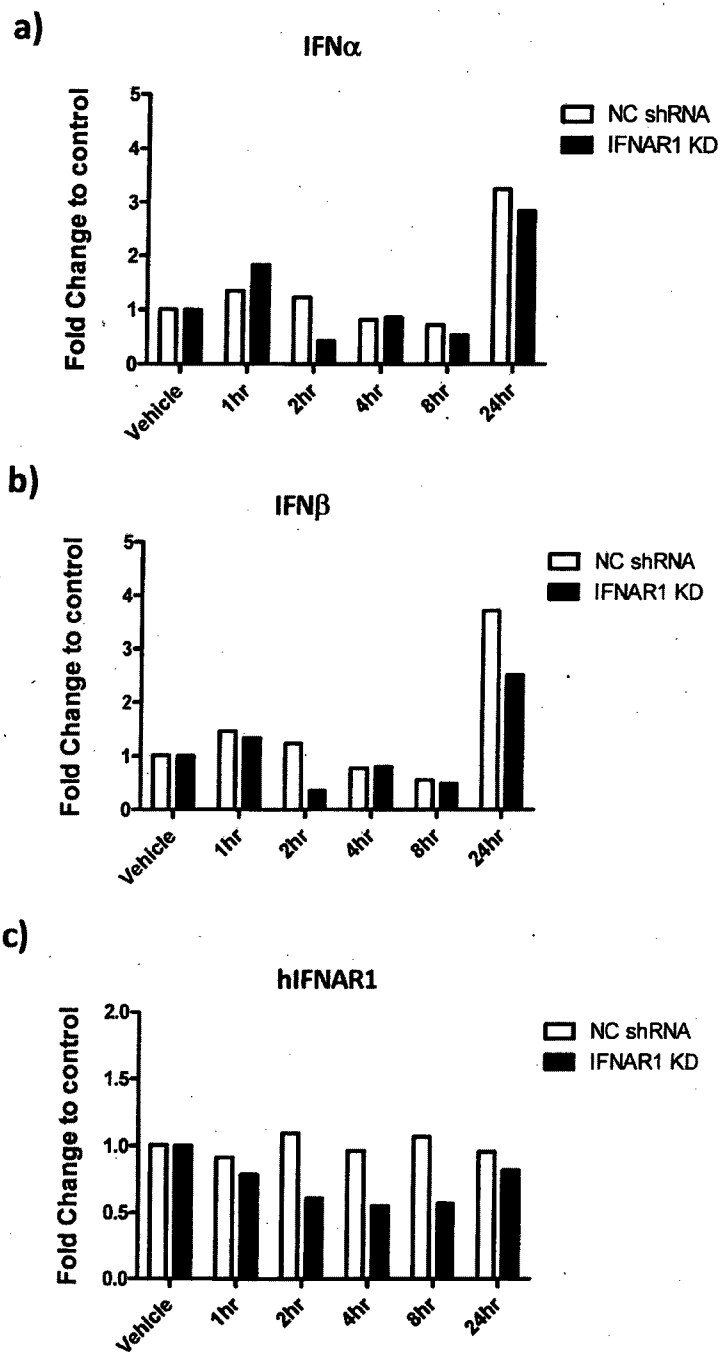


FIGURE 30

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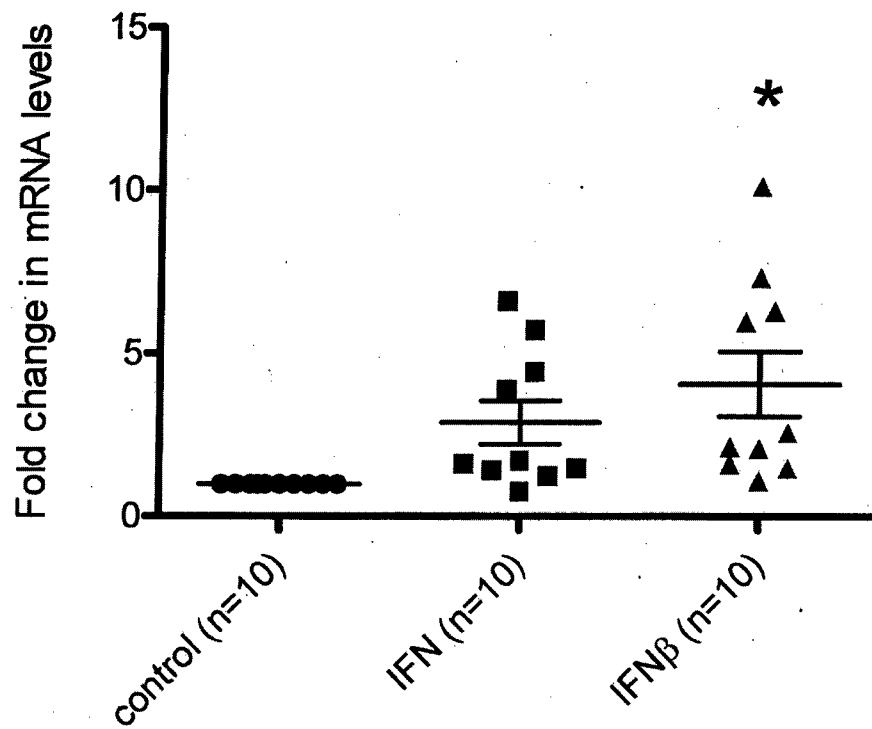


FIGURE 31

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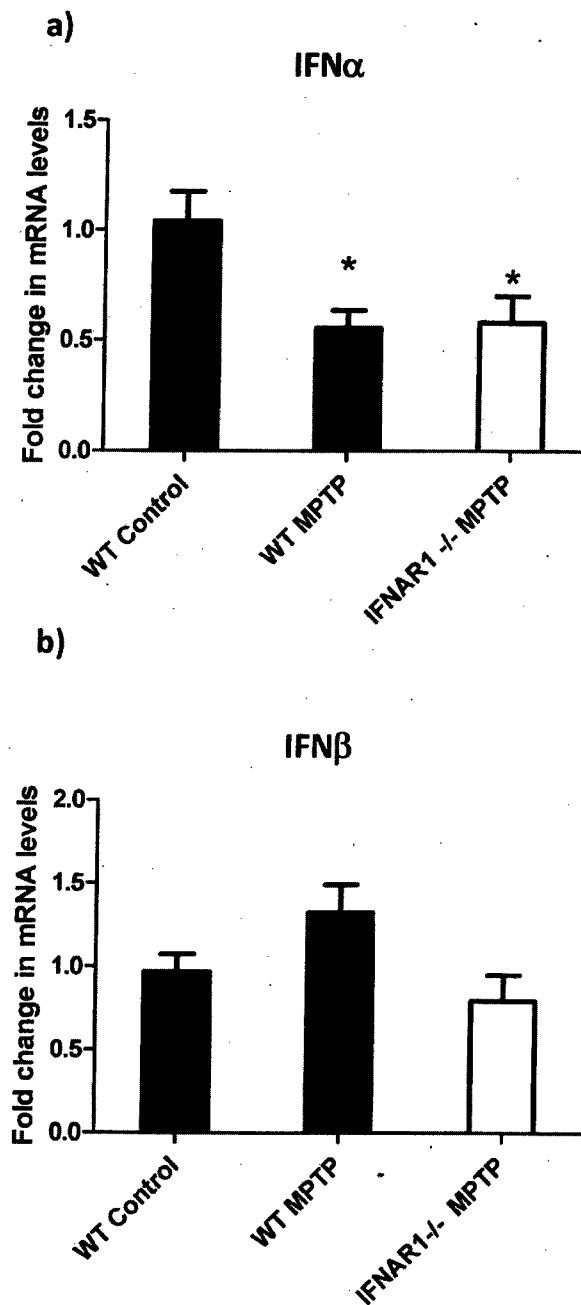


FIGURE 32

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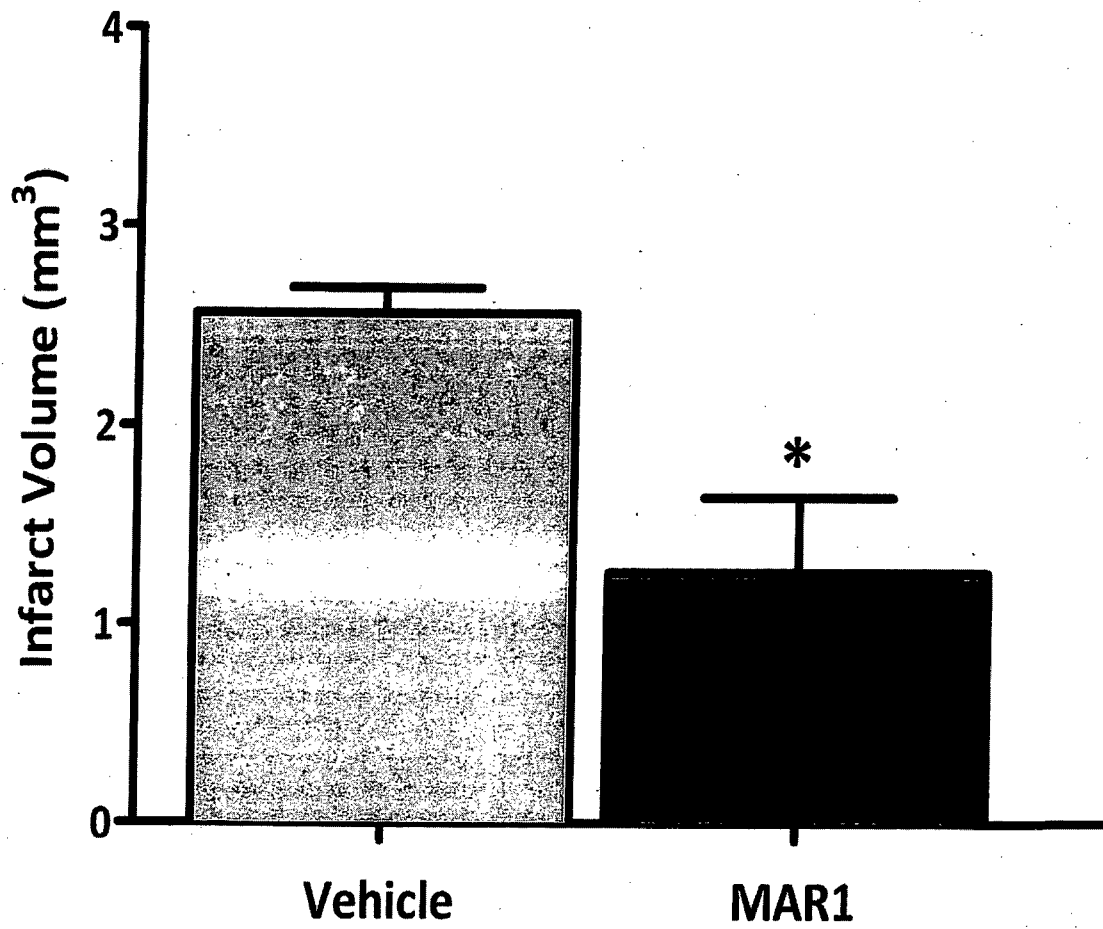


Figure 33A

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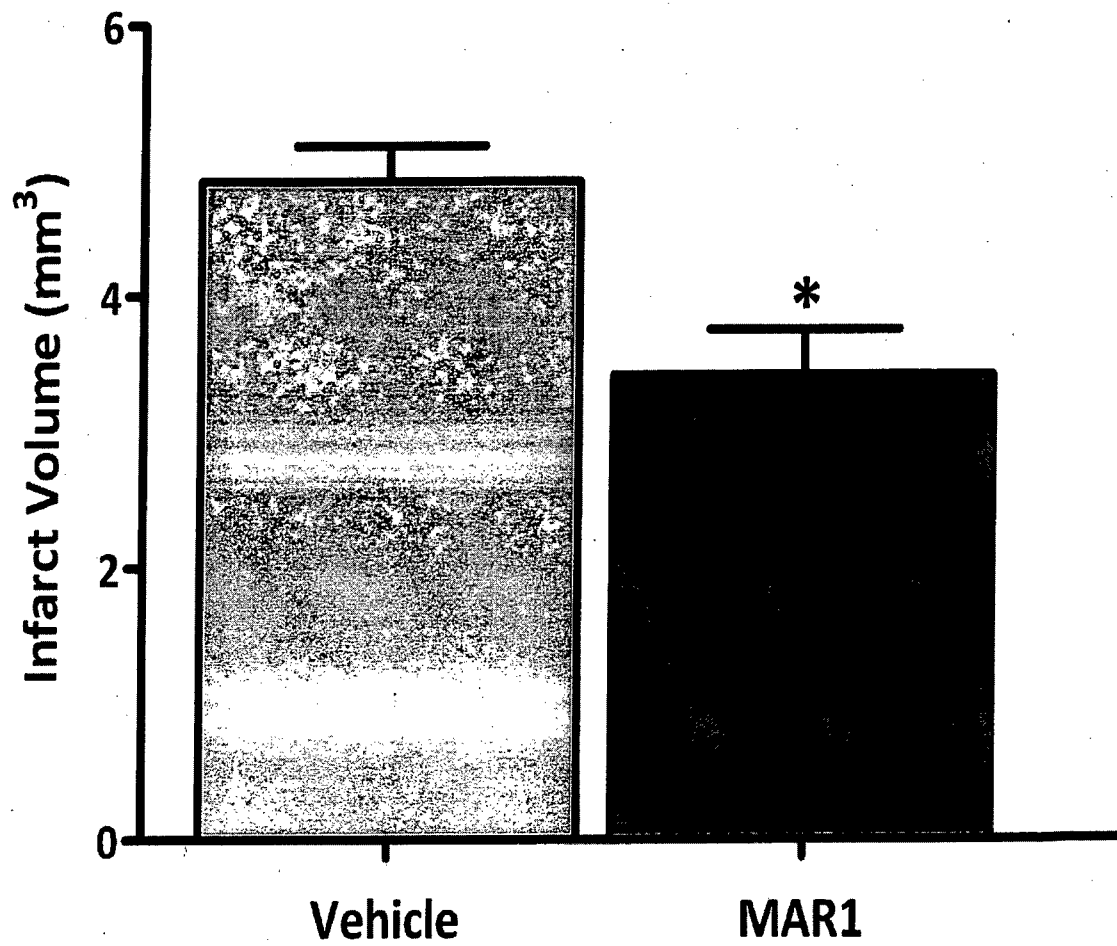


Figure 33B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2011/001533

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61P 25/00 (2006.01)

A61K 39/395 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DWPI, EPODOC, MEDLINE: type 1 interferon, type 1 interferon mediated signalling, interferon 1, interferon alpha, interferon beta, IFN, Roferon A, SCH 30500, RO 22-8181, Wellferon, Fiblaferon, interferon fibroblast, interferon leukocyte, interferon lymphoblast, IFNAR, antagonist, block, inhibit, neutralise, disrupt, reduce, attenuate, prevent, competitor, neuroinflammation, neuroinflammatory, nerve, neural, CNS, central nervous system, brain, inflammation, inflammatory, Alzheimer's, Parkinson's, arnyotrophic lateral sclerosis, Friedreich's, Huntington's, Lewy body, spinal muscular atrophy, motor neuron disease, Alper's, Batten, cerebro-oculo-facio-skeletal, corticobasal, Leigh's, Machads-Joseph, monomelic amyotrophy, multiple system atrophy, multiple sclerosis, MS, neurodegeneration, olivopontocerebellar, atrophy, opsoclonus myoclonus, paraneoplastic, progressive multifocal leukoencephalopathy, Aicardi-Coutieres, traumatic brain injury, drug abuse, irradiation, chemotherapy, starvation, spinal cord trauma, infection, pathogen, severe head injury, trauma induced paralysis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Sas, AR <i>et al</i> (2009) Interferon- α Causes Neuronal Dysfunction in Encephalitis. <i>The Journal of Neuroscience</i> 29: 3948-3955 Abstract; Page 3953-3954, final paragraph	1-9, 12-28 10-11
X Y	WO 2010/030671 A1 (UNIVERSITY OF MEDICINE AND DENTISTRY OF NEW JERSEY ET AL) 18 March 2010 Abstract; Page 21, lines 5-12; Claims 1, 14, 43, 44	25-26 10-11



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
19 December 2011

Date of mailing of the international search report
21/12/2011

Name and mailing address of the ISA/AU

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Authorized officer

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(ISO 9001 Quality Certified Service)
Telephone No : +61 2 6283 2656

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2011/001533**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:
 - a. (means)
☐ on paper
☐ in electronic form
 - b. (time)
☐ in the international application as filed
☐ together with the international application in electronic form
☐ subsequently to this Authority for the purposes of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:
There was a sequence listing originally filed but it was not used for the purposes of this search.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2011/001533

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/0018174 A1 (KIM, KJ ET AL) 23 January 2003 Abstract; Page 1, [0008-0010]; Page 20, [192-199]	25
X	WO 2001/054721 A1 (PHARMA PACIFIC PTY LTD ET AL) 2 August 2001 Abstract; Claims 1-9	25-26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2011/001533

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
WO	2010030671	US	2011224407		
US	2003018174	NONE			
WO	200154721	AU	28656/01	CA	2401086
		JP	2003531822	US	2003147889
				EP	1251873
				US	2006275298
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.					
END OF ANNEX					