Hallmarks of Neurodegeneration

Identifying Underlying Biological Processes

Cell Signaling TECHNOLOGY®

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Introduction

Neurodegenerative diseases are characterized by a loss of neuronal structure and function that leads to problems with movement (ataxia) or mental function (dementia). These changes occur due to genetic mutations or protein folding disorders that can accumulate with age. While pathophysiologies like amyloid plaques are well documented, many of the cellular processes that drive neurodegeneration have yet to be fully elucidated. Defects in these key processes may be shared among different neurodegenerative diseases, making it likely that new therapies targeting one process may alleviate the progression of many conditions.

We've put together a starter's guide on the cellular mechanisms that drive neurodegeneration in diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, and Huntington's disease. Wondering how to identify the many different cell types that make up the central nervous system (CNS)? You'll find a Cell Type Marker guide at the end of this book.

DRIVERS OF NEURODEGENERATION:

Protein Folding & Aggregation

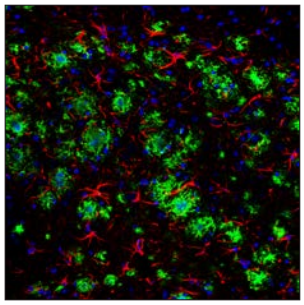
One hallmark of many neurodegenerative diseases is the accumulation of unfolded or misfolded proteins that lead to neurofibrillary tangles and plaques that cause neuronal cell cytotoxicity. There is increasing interest in the field to understand the mechanisms required for the production and processing of proteins known to form these aggregates, as these protein aggregates are associated with Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis. Some challenges to developing new therapies that target the protein aggregate formation include an incomplete knowledge of the mechanisms of action, as well as a lack of biomarkers to diagnose conditions early and to monitor disease progression and therapeutic response. Interested in investigating protein folding and aggregation?

Start with These Targets

β-amyloid

β-amyloid (Aβ) is a peptide that is the main component of the amyloid plaques observed in the brains of patients with Alzheimer's disease. The peptides are formed when the amyloid precursor protein (APP) is cleaved by β-secretase and γ-secretase.

β-Amyloid (D54D2) XP® Rabbit mAb #8243 – WB, IP, **IF-F**

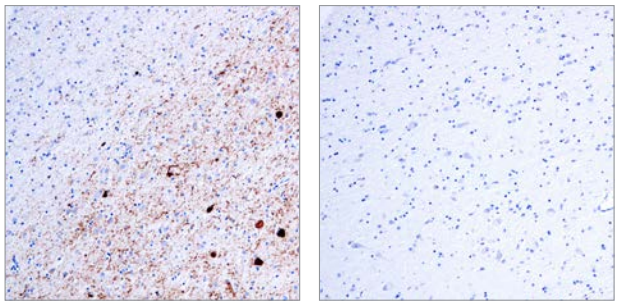


β-Amyloid (D54D2) XP® Rabbit mAb #8243: Confocal IF analysis of mouse subicular cortex from an amyloid mouse model of Alzheimer's disease using #8243 (green) and GFAP (GA5) Mouse mAb #3670 (red). Samples were mounted in ProLong® Gold Antifade Reagent with DAPI #8961 (blue).

Phospho-Tau (Thr205)

Under normal cellular conditions, tau promotes and stabilizes microtubule assembly, especially in axons. Phosphorylation of tau (Thr205) is well characterized in neurofibrillary tangles, with low levels in earlier stages and significantly higher levels in later stages of Alzheimer's disease.

Phospho-Tau (Thr205) (E7D3E) Rabbit mAb #49561 –
WB, IP, **IHC-P**, IF-F

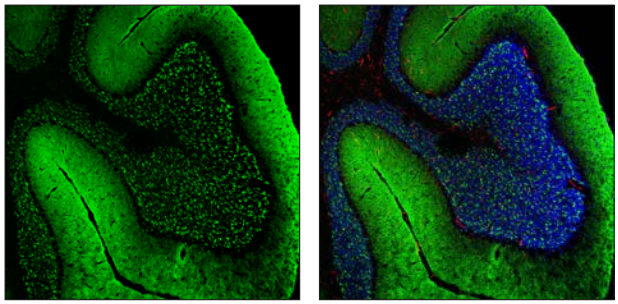


Phospho-Tau (Thr205) (E7D3E) Rabbit mAb #49561: IHC-P analysis of paraffin-embedded human Alzheimer's disease brain using #49561 in the presence of non-phospho-tau (Thr205) peptide (left) or phospho-tau (Thr205) peptide (right).

α/β-synuclein

α/β-synuclein is primarily localized to vesicles in presynaptic terminals. α-synuclein self-assembles into the toxic oligomers and amyloid fibrils that comprise Lewy bodies. β-synuclein inhibits α-synuclein aggregation and may serve a neuro-protective function.

α-Synuclein (E4U2F) XP® Rabbit mAb #51510 –
WB, IP, IHC-P, **IF-F**, IF-IC



α-Synuclein (E4U2F) XP® Rabbit mAb #51510 Confocal IF-F analysis of fixed frozen mouse cerebellum labeled with #51510 (left, green) and co-labeled with F4/80 (BM8.1) Rat mAb #71299 (right, red) and DAPI #4083 (right, blue).

DRIVERS OF NEURODEGENERATION: Neuroinflammation

Neuroinflammation is a condition observed in the central nervous system (CNS) in response to infection, toxic metabolites, traumatic injury, or autoimmunity. Immune cells, such as microglia, macrophages, and neuroepithelium-derived astrocytes, monitor synaptic homeostasis and facilitate the clearance of apoptotic cells in response to injury in the CNS to protect brain function. The immune system may play a significant role in shaping the brain during development and mediating damage, regeneration, and repair. These processes may be compromised in neurodegenerative diseases. Interested in investigating neuroinflammation?

Start with These Targets

Iba1/AIF-1

Iba1/AIF-1 is uniquely expressed in cells of monocytic lineage and is, therefore, widely used as a marker for microglia/macrophages in the brain and other tissue. Iba1/AIF-1 was originally cloned from activated macrophages in human atherosclerotic allogenic heart grafts undergoing chronic transplant rejection as well as from rat monocytes. Its function is not very well understood, but, as an F-actin-binding protein, Iba1/AIF-1 may function to remodel the actin cytoskeleton of microglia/macrophages.

Iba1/AIF-1 (E4O4W) XP® Rabbit mAb #17198 – WB, IP, IHC-Bond, IHC-P, IF-F, IF-IC, FC-FP

Iba1/AIF-1 (E5N4J) Mouse mAb (IHC Formulated) #58970 – IHC-Bond, IHC-P

TMEM119

TMEM119 is a cell-surface protein expressed exclusively by the microglia subset of myeloid and neural cells. TMEM119 is co-expressed with Iba1 in both ramified and amoeboid morphology microglia, and downregulated in disease-associated microglia. TMEM119 is important for identifying microglia in healthy tissue as well as distinguishing them from infiltrating macrophages and other cell types in neurodegenerative disease models.

TMEM119 (E3E10) Rabbit mAb #90840 – IF-F

TREM2

TREM2 is an immune receptor expressed on the cell surface of microglia, macrophages, osteoclasts, and immature dendritic cells. TREM2 forms a receptor-signaling complex with DAP12 to initiate cellular events like phagocytosis. TREM2 signaling is critical for the activation of microglia and may contribute to Alzheimer's disease pathogenesis by impairing microglia response, which leads to a buildup of β-amyloid.

TREM2 (D8I4C) Rabbit mAb #91068 – WB, IP, IF-IC

GPNMB

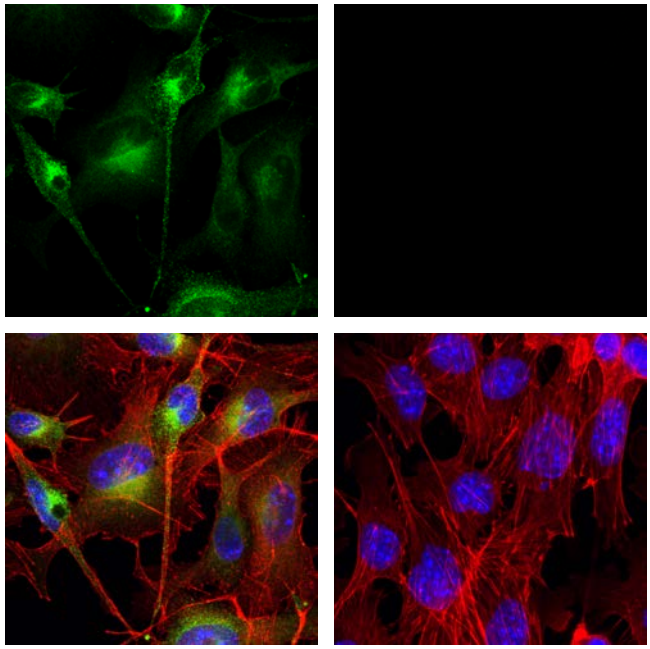
GPNMB is a type I transmembrane glycoprotein that serves as a marker of Alzheimer's disease. However, the precise role of GPNMB in neuroinflammation remains to be elucidated. GPNMB has been found to co-localize with the Iba1-positive microglia that cluster around amyloid plaques. Levels of GPNMB are also elevated in disease models that feature neuronal loss.

GPNMB (E7U1Z) Rabbit mAb #90205 – WB, IF-F, **IF-C**, FC-FP

iNOS

iNOS expression has been observed in brain glial cells and invading macrophages in response to injury. It is normally induced in an oxidative environment or in response to proinflammatory cytokines. iNOS has been linked with Alzheimer's disease and Parkinson's disease.

iNOS (D6B6S) Rabbit mAb #13120 – WB, IP, IF-IC, FC-FP



GPNMB (E7U1Z) Rabbit mAb #90205: Confocal IF analysis of B16-F10 cells (left, positive) or 4T1 cells (right, negative) using #90205 (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Samples were mounted in ProLong® Gold Antifade Reagent with DAPI #8961 (blue).

DRIVERS OF NEURODEGENERATION:

Altered Cell Signaling

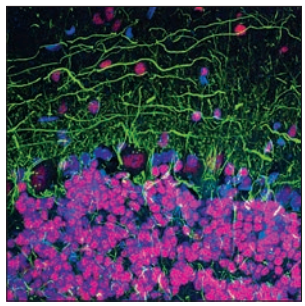
Abnormal cell-cell communication, for example, disrupted presynaptic input, as well as disrupted intracellular signaling, contribute to the pathogenesis of neurodegenerative disease. Understanding the signal transduction pathways that regulate gene expression will help explain disease initiation and progression, thereby informing efforts to develop therapeutic interventions. Interested in investigating altered cell signaling?

Start with These Targets

CREB

CREB signaling is a cellular transcription factor that plays an important role in the formation of memories. Perturbed signaling has been observed in the brains of Alzheimer's disease (AD) mouse models, suggesting CREB signaling may be disrupted in human AD brains as well. Disturbances in CREB function may also contribute to the development and progression of Huntington's disease.

CREB (48H2) Rabbit mAb #9197 –
WB, IP, IHC-P, **IF-F**, IF-IC, FC-FP, ChIP, C&R

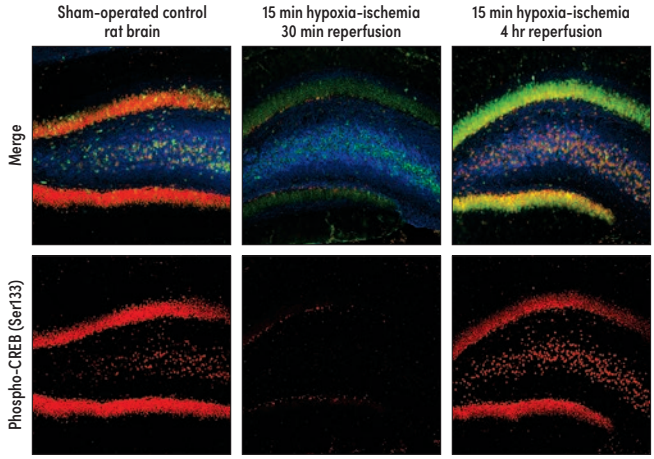


CREB (48H2) Rabbit mAb #9197: Confocal IF analysis of mouse cerebellum using #9197 (red) and Neurofilament-L (DA2) Mouse mAb #2835 (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

Phospho-CREB (Ser133)

CREB is a cellular transcription factor activated when phosphorylated on Ser133. phospho-CREB (Ser133) levels are reduced in the prefrontal cortex of patients with AD, indicating a dysfunction in CREB signaling. Reduced phospho-CREB levels in the peripheral blood mononuclear cells (PBMCs) of patients with AD correlate with phospho-CREB levels observed in postmortem AD brains, suggesting phospho-CREB expression in PBMCs may be a potential biomarker for disease progression.

Phospho-CREB (Ser133) (87G3) Rabbit mAb #9198 –
WB, IHC-P, **IF-F**, IF-IC, FC-FP, ChIP, ChIP-seq, C&R



Phospho-CREB (Ser133) (87G3) Rabbit mAb #9198: Confocal IF images of dentate gyrus labeled with #9198 (red), Neurofilament-L (DA2) Mouse mAb #2835 (blue) and Phospho-S6 Ribosomal Protein (Ser235/236) (2F9) Rabbit mAb (Alexa Fluor® 488 Conjugate) #4854. Sections were obtained from a sham-operated control rat (left) or rats subjected to 15 min of hypoxia-ischemia followed by 30 min (middle) or 4 h reperfusion (right).

GSK-3β

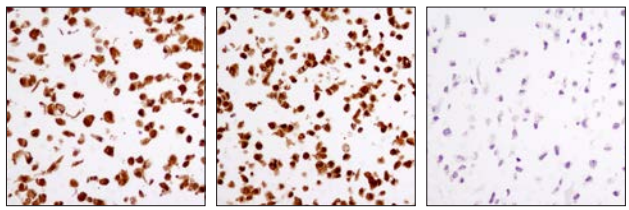
GSK-3β is known to interact with tau, β-amyloid, and α-synuclein and is implicated in the pathogenesis of AD and Parkinson's disease. It is one of the kinases responsible for tau hyperphosphorylation, resulting in neurofibrillary tangles. GSK-3β regulates several critical cellular events, such as axonal transport, microtubule dynamics, apoptosis, and inflammation, making GSK-3β a potential therapeutic target.

GSK-3β (D5C5Z) XP® Rabbit mAb #12456 – WB, IP, **IHC-P**, IF-IC, FC-FP

Phospho-GSK-3β (Ser9)

Phosphorylation of GSK-3β on Ser9 inactivates the protein, influencing its ability to regulate glycogen synthesis in response to insulin. In AD mouse models, GSK-3β Ser9 phosphorylation may also reduce APP processing by β-secretase, decreasing Aβ production.

Phospho-GSK-3β (Ser9) (D85E12) XP® Rabbit mAb #5558 –
WB, IP, IF-IC, FC-FP



GSK-3β (D5C5Z) XP® Rabbit mAb #12456: IHC analysis of paraffin-embedded MEF cell pellets, wild type (left), GSK-3α (-/-) (middle) and GSK-3β (-/-) (right) using #12456. (MEF wild type, GSK-3β (-/-), and GSK-3α (-/-) cells were kindly provided by Dr. Jim Woodgett, University of Toronto, Canada.)

DRIVERS OF NEURODEGENERATION:

Altered Epigenetics

Epigenetic regulation, including aberrant DNA methylation and histone modifications, have been linked to Alzheimer's disease, Huntington's disease, Parkinson's disease, multiple sclerosis, and amyotrophic lateral sclerosis. However, the exact effects on disease progression are unclear. Brain health is heavily reliant on epigenetic mechanisms, and loss of chromatin dynamics is observed in neurodegenerative diseases. Modifying the environment and targeting sites of potential risk for epigenetic changes are growing areas in the development of therapies against neurodegeneration. Interested in investigating altered epigenetics?

Start with These Targets

HDAC2

HDAC2 is a class I histone deacetylase that typically leads to gene repression. Deletion of HDAC2 in mouse Alzheimer's disease models results in improved cognition and decreased amyloid load. Increased HDAC2 expression has also been observed in Alzheimer's disease patients. HDAC2 is implicated in Huntington's disease and multiple sclerosis as well.

HDAC2 (D6S5P) Rabbit mAb #57156 – WB, IP, IF-IC, ChIP, **ChIP-seq**

HDAC6

HDAC6 is a class II histone deacetylase with increased expression in the cortex and hippocampus of patients with Alzheimer's disease. HDAC6 colocalizes with tau proteins and correlates with tau phosphorylation. Decreasing HDAC6 levels may result in improved cognition.

HDAC2 (DD2E5) Rabbit mAb #7558 – WB, IP, IHC-P, **IF-IC**, FC-FP

SirT1

SirT1 is a nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylase that regulates multiple cellular processes, including metabolism and aging. SirT1 deacetylates many protein substrates, including tau. SirT1 expression levels are decreased in the cortex of Alzheimer's patients, and reduced expression parallels the accumulation of insoluble tau protein. Increased acetylation of tau inhibits its normal function via impaired tau-microtuble interactions and promotes pathological tau aggregation.

SirT1 (1F3) Mouse mAb #8469 – WB, IP, IHC-P, IF-IC

MeCP2

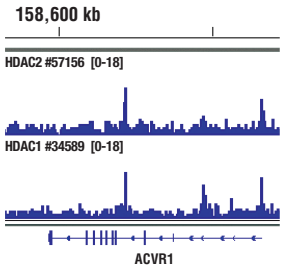
MeCP2 is highly expressed in neurons and is a critical transcriptional regulator of neuronal development and synaptic function. Mutations in MeCP2 are causative for various neurodevelopmental disorders. Mis-regulation results in reduced synaptic plasticity and impaired cognitive function. Increased expression of MeCP2 may contribute to Alzheimer's disease through increased repression of key target genes, increased tau accumulation, and increased neuronal degeneration through neuronal cell death.

MeCP2 (D4F3) XP® Rabbit mAb #3456 – WB, IP, IHC-P, IF-F, IF-IC, FC-FP

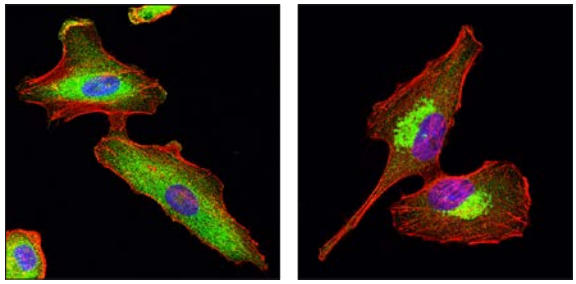
p300

p300 is a histone acetyltransferase that plays a role in the chromatin acetylation that is modulated in response to neuronal activity. Neuronal histone acetylation levels are lower in Alzheimer's disease mouse models. Activation of amyloid precursor protein (APP)-dependent signaling results in reduced histone acetyltransferase levels in primary neuronal cultures. p300 also plays a role in Huntington's disease and Parkinson's disease.

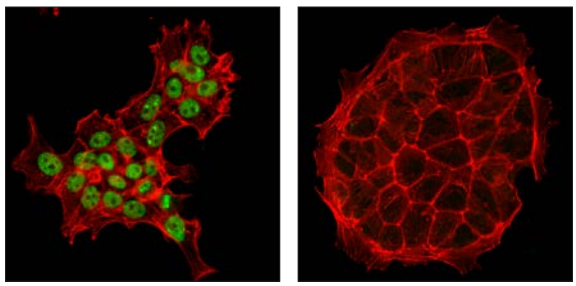
p300 (D8Z4E) Rabbit mAb #86377 – WB, IP, IHC-P, **IF-IC**



HDAC2 (D6S5P) Rabbit mAb #57156: Chromatin immunoprecipitations were performed with cross-linked chromatin from K-562 cells and [0-18] either #57156 or #34589, using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA libraries were prepared using DNA Library Prep Kit for Illumina® (ChIP-seq, CUT&RUN) #56795. HDAC2 and HDAC1 are known to have a similar binding pattern on chromatin. The figure shows binding of both HDAC2 and HDAC1 across the ACVR1 gene. For additional ChIP-seq tracks, please download the product data sheet.



HDAC6 (D2E5) Rabbit mAb #7558: Confocal IF analysis of A549 cells, untreated (left) or treated with MG132 (5 µM, 24 hr; right), using #7558 (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



p300 (D8Z4E) Rabbit mAb #86377: Confocal IF analysis of 293T cells (left, positive) and HCT-15 cells (right, negative) using #86377 (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red).

DRIVERS OF NEURODEGENERATION:

RNA-Binding Proteins

The misregulation of RNA-binding proteins (RBPs) has been linked to amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), and frontotemporal dementia (FTD). RBPs control the utilization of mRNA during cell stress through formation of stress granules (SGs). In response to cell stress, SGs accumulate in the cytoplasm and function to repress translation of mRNAs, later becoming resolved during recovery from stress. However, mutations in SG-associated RBPs lead to the pathological accumulation of protein aggregates which are readily apparent in neurodegenerative disorders. In addition, age-related changes in mRNA methylation and methylation-dependent RBPs have also been associated with neurodegeneration. While RBPs have been associated with multiple neurological disorders, their exact roles in disease progression are unclear. Nevertheless, RBPs are becoming attractive therapeutic targets for the treatment of neurodegeneration. Interested in investigating altered RBP function in neurodegenerative diseases?

Start with These Targets

TDP43

TDP-43 is an RNA-binding protein involved in transcriptional regulation and exon splicing. The pathological accumulation of TDP-43 in cytosolic SGs is observed in multiple neurodegenerative diseases, typically due to mutations that promote protein aggregation. TDP-43 is the major component of inclusion bodies, which are a hallmark of ALS. Accumulation of TDP-43 is also commonly found in the postmortem brain tissue of patients with Alzheimer's disease.

TDP43 (D9R3L) Rabbit mAb #89789 – WB, IHC-P, **IF-F**, IF-IC

FUS

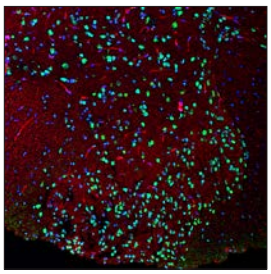
FUS is a DNA/RNA-binding protein that plays a role in transcriptional regulation, pre-mRNA splicing, and DNA damage response. Mutations in FUS are common in ALS and frontotemporal lobar degeneration (FTLD) patients, typically occurring within the protein's C-terminal nuclear localization sequence. These mutations cause mislocalization of the predominantly nuclear protein into the cytoplasm, and consequently promote the accumulation of FUS aggregates in cytosolic SGs.

FUS/TLS (E3O8I) Rabbit mAb #67840 – WB, IP, **IF-F**, IF-IC

G3BP1

G3BP1 is a core nucleating factor essential for the assembly of cytoplasmic SGs. Knockout of G3BP1 in mice results in late embryonic lethality due to widespread neuronal cell death. Elevated expression levels of G3BP1 have been observed in ALS and Alzheimer's disease patients.

G3BP1 (E9G1M) XP® Rabbit mAb #61559 – WB, IP, IHC-P, IF-IC



TDP43 (D9R3L) Rabbit mAb #89789: Confocal IF analysis of mouse ventral spinal cord using #89789 (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

METTL3

METTL3 is the enzyme responsible for m6A methylation along with heterodimer partner METTL14. METTL3 knockdown has been shown to affect genes relating to neurogenesis, cell cycle, and neuronal development. METTL3 itself has been implicated in Alzheimer's disease in a study showing that age-related m6A marks reduce the expression of key age-related genes. METTL3 and m6A levels also are reduced in human Alzheimer's brains. Overexpression of METTL3 rescues Aβ-induced synaptic loss. Interestingly, mouse models of Alzheimer's have shown increased expression of METTL3 and m6A, and decreased levels of the m6A eraser FTO.

METTL3 (E3F2A) Rabbit mAb #86132 – WB, IP, IHC-P, ChIP

YTHDF1

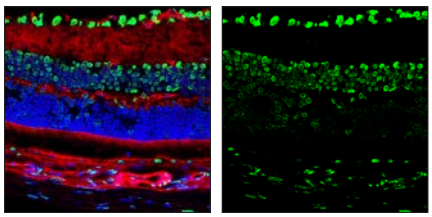
YTHDF1 is an m6A reader and has been shown to be key in learning and memory by promoting translation of genes such as CAMK2A, GRIN1, and GRIA1. It is preferentially expressed in the mouse hippocampus.

YTHDF1 (E8R5L) Rabbit mAb #43123 – WB, IP, IF-IC

YTHDF2

YTHDF2 knockouts are embryonic lethal at the late stages of embryo development. Depletion of YTHDF2 in the embryo results in impaired neuronal development, with reduced ability for self renewal and for neurons to form functional neurites. Furthermore, YTHDF2 deficiency inhibits the formation of glial cells. YTHDF proteins have also been shown to bind to polymethylated mRNAs and sequester them to neuronal RNA granules through liquid-liquid phase separation.

YTHDF2 (E2I2H) Rabbit mAb #71283 – WB, IP, IF-F, IF-IC



FUS/TLS (E3O8I) Rabbit mAb #67840: Confocal IF analysis of mouse retina using #67840 (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Sections were mounted in ProLong® Gold Antifade Reagent with DAPI #8961 (blue).

DRIVERS OF NEURODEGENERATION:

Altered Metabolism

The three main purposes of metabolism are the conversion of food to energy: the conversion of nutrients to proteins, carbohydrates, lipids, and nucleic acids, and the elimination of nitrogenous wastes. There is a strong correlation between metabolic changes and/or dysfunction with neurodegenerative diseases. In particular, abnormal glucose tolerance or insulin resistance are observed in many neurodegenerative conditions. It is not clear whether metabolic changes are a cause or a consequence. However, understanding the role altered metabolism plays in disease progression is important, because these changes are associated with all neurodegenerative diseases. Interested in investigating altered metabolism?

Start with These Targets

Phospho-Akt (Ser473)

AKT is a critical player in cellular processes, such as glucose metabolism, cell survival, cell growth, and migration. AKT is activated when phosphorylated on Ser473 in response to insulin to regulate glucose transport. AKT is associated with Alzheimer's disease; however, its actual role is not well understood. Its importance is recognized as it promotes tau hyperphosphorylation. AKT is also implicated in Parkinson's disease.

Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060 – WB, IP, IHC-P, **IF-IC**, FC-FP

Phospho-mTOR (Ser2448)

mTOR functions as an ATP and amino acid sensor that balances nutrient availability and cell growth. It is a part of the insulin and PI3K signaling pathway and is a core component of mTOR complexes. mTOR is implicated in neurogenerative diseases such as Huntington's disease and Alzheimer's disease.

Phospho-mTOR (Ser2448) (D9C2) XP® Rabbit mAb #5536 – WB, IP, **IF-IC**

Phospho-AMPKα (Thr172)

AMPKα is a central regulator of metabolism and is phosphorylated in response to low ATP levels. Activated AMPKα initiates downstream events to influence glucose and lipid metabolism. AMPKα dysregulation is associated with Alzheimer's disease and ALS.

Phospho-AMPKα (Thr172) (D4D6D) Rabbit mAb #50081 – WB, IP, **IHC-P**

Phospho-S6 Ribosomal Protein (Ser235/236)

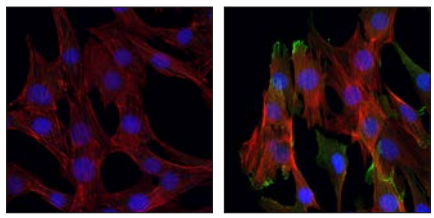
S6 ribosomal protein is commonly used as a marker for neuronal activity and a readout for mTORC1 activity. Phosphorylation of this ribosomal protein is altered in Huntington's disease and Alzheimer's disease.

Phospho-S6 Ribosomal Protein (Ser235/236) (E2R10) Mouse mAb #62016 – WB, IF-IC

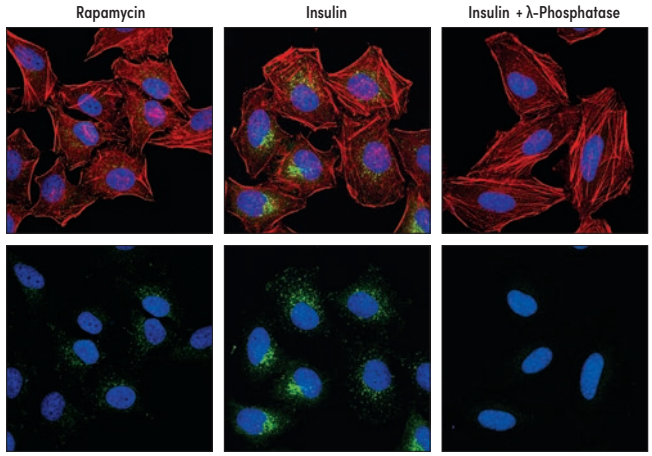
ApoE4

Apolipoproteins are transporters of lipids and cholesterol. ApoE4 is produced in the liver and brain and is linked to neuronal plasticity and synaptogenesis. People who carry the APOE4 allele are at higher risk of developing Alzheimer's disease, though the exact function of ApoE4 in Alzheimer's disease etiology remains unknown. The presence of the APOE4 allele also correlates with earlier onset of Parkinson's disease.

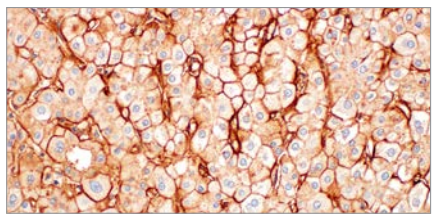
ApoE4 (E5M4L) Rabbit mAb #39327 - WB, **IHC-P**



Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060: Confocal IF analysis of C2C12 cells, LY294002-treated (left) or insulin-treated (right), using #4060 (green). Actin filaments have been labeled with Alexa Fluor® 555 Phalloidin #8953 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



Phospho-mTOR (Ser2448) (D9C2) XP® Rabbit mAb #5536: Confocal IF analysis of HeLa cells, rapamycin-treated (#9904, 10 nM, 2 hr, left), insulin-treated (50 nM, 6 min, middle) or insulin- and λ-phosphatase-treated (right), using #5536 (green). Actin filaments were labeled with DY-554 phalloidin. Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



ApoE4 (E5M4L) Rabbit mAb #39327: IHC analysis of paraffin-embedded human hepatocellular carcinoma using #39327.

DRIVERS OF NEURODEGENERATION:

Acquiring Cell Death

Mutations in cell death pathways, such as apoptosis, mitophagy, necroptosis, and autophagy, contribute to neuronal cell death and the progression of neurodegenerative diseases. Aberrant pro- and anti-apoptotic signaling, mitochondrial dysfunction, misregulation of autophagy or the unfolded protein response, and activation of the necrosome by stress and/or inflammation highlight just a few of the mechanisms by which neurons die or become diseased. Although many of these pathways are understood in non-neuronal cells, their mechanism of activation and dysregulation remains a mystery in neurons. Interested in investigating neurodegeneration-related cell death?

Start with These Targets

Cleaved PARP (ASP214)

PARP typically functions as a key player in the DNA repair pathway in response to oxidative stress. When cleaved by caspase-3 between Asp214 and Gly215, the N-terminal cleaved fragment inhibits DNA repair enzymes to push neurons toward apoptosis, making it a hallmark of apoptotic cells.

Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb #5625 – WB, IP, IHC-P, IF-IC, FC-FP

PINK1

PINK1 is a mitochondrial serine/threonine kinase involved in the normal function and integrity of mitochondria, as well as in reduction of cytochrome c release from mitochondria. PINK1 phosphorylates Parkin and promotes its translocation to mitochondria. Research studies have shown that mutations in PINK1 are linked to autosomal recessive early onset Parkinson's disease, and are associated with loss of protective function, mitochondrial dysfunction, aggregation of α-synuclein, as well as proteasome dysfunction.

PINK1 (D8G3) Rabbit mAb #6946 – WB, IP

SQSTM1/p62

Sequestosome 1, or SQSTM1/p62, is an autophagosome cargo protein that binds to protein aggregates to target them for selective autophagy. SQSTM1/p62 mutations lead to an increase in intracellular aggregation of α-synuclein, Huntingtin, tau protein, and β-amyloid to drive progression of Parkinson's disease, Huntington's disease, and Alzheimer's disease, respectively.

SQSTM1/p62 (D10E10) Rabbit mAb (IF Preferred) #7695 – IP, **IF-IC**

LC3A/B

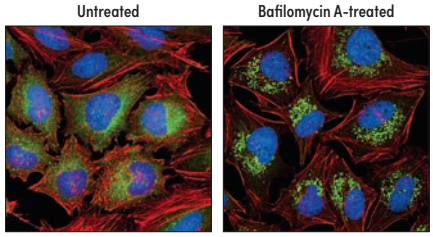
LC3A/B plays a critical role in autophagosome biogenesis and maturation and also functions as an adaptor protein to selectively recruit cargo to the autophagosome. An increase in LC3-positive microglia have been observed in tissues in Alzheimer's disease patients with TREM2 mutations, suggesting that disruptions in TREM2-dependent autophagy can contribute to Alzheimer's disease etiology.

LC3A/B (D3U4C) XP® Rabbit mAb #12741 – WB, IHC-P, IF-F, **IF-IC**, FC-FP

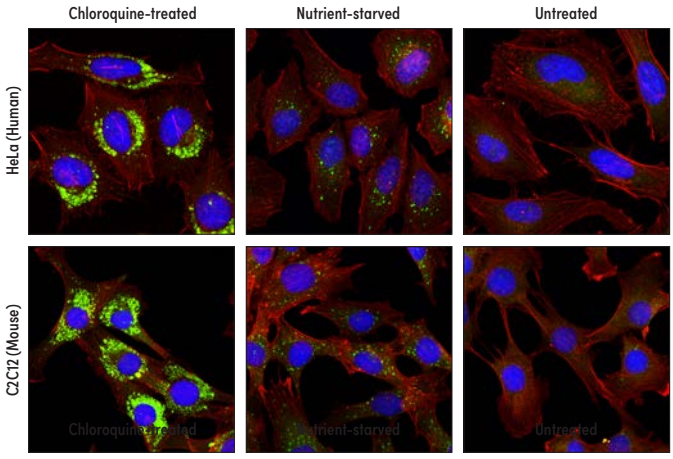
Phospho-RIP3 (Ser227)

Human Phospho-RIP3 (Ser227) phosphorylates MLKL1 to trigger TNF-induced necroptosis. This form of programmed cell death has been reported in multiple sclerosis and ALS.

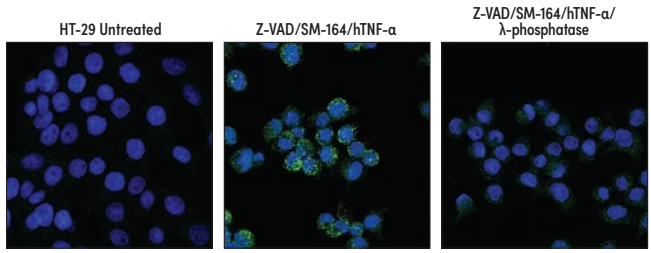
Phospho-RIP3 (Ser227) (D6W2T) Rabbit mAb #93654 – WB, **IF-IC**



SQSTM1/p62 (D10E10) Rabbit mAb (IF Preferred) #7695: Confocal IF analysis of HeLa cells, untreated (left) or treated with bafilomycin A (100 nM, 18 hr; right), using #7695 (green). Actin filaments were labeled with DY-554 phalloidin. Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



LC3A/B (D3U4C) XP® Rabbit mAb #12741: Confocal IF analysis of HeLa (upper) and C2C12 (lower) cells, chloroquine-treated (50 µM, overnight; left), nutrient-starved with EBSS (3 hr, middle) or untreated (right) using #12741 (green) and β-Actin (13E5) Rabbit mAb (Alexa Fluor® 555 Conjugate) #8046 (red). Blue pseudo-color = DRAQ5® #4084 (fluorescent DNA dye).



Phospho-RIP3 (Ser227) (D6W2T) Rabbit mAb #93654: Confocal IF analysis of HT-29 cells, untreated (left), pretreated with Z-VAD (20 µM, 30 min) followed by treatment with SM-164 (100 nM) and hTNF-α #8902 (20 ng/mL, 6 hr; middle), or pretreated with Z-VAD followed by treatment with SM-164 and hTNF-α and post-processed with λ-phosphatase (right), using #93654 (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

DRIVERS OF NEURODEGENERATION:

Acquiring Senescence

Cellular senescence is characterized by irreversible cell-cycle arrest in combination with a distinct secretory phenotype and expanded lysosomes in response to stress. Senescent cells accumulate in tissues during aging, and various markers of senescence are associated with neurodegenerative disease. p16 accumulates in senescent astrocytes and microglia in the context of tau pathology. p16 and p27 accumulate in senescent oligodendrocyte progenitor cells (OPCs) in the context of amyloid plaque pathology. Senolytic compounds are currently being investigated as promising therapeutics that may remove senescent cells and treat Alzheimer's disease. Interested in researching senescent cells?

Start with These Targets

p16 INK4A

p16 is a member of the INK4 family of cyclin-dependent kinase inhibitors that are responsible for arresting the cell cycle in the G1 phase. p16 is commonly used as a marker for senescent cells. Elevated expression of p16 has been observed in the neurons of Alzheimer's disease patients and may also play a role in the progression of multiple sclerosis.

p16 INK4A (E6N8P) Rabbit mAb #18769 – WB, IP, IF-IC

p21 Waf1/Cip1

p21 Waf1/Cip1, a CDK inhibitor, is a common marker of cellular senescence. It causes cell cycle arrest in response to stress-induced p53 to trigger senescence. p21 may be a critical mediator of cell cycle dysregulation in Alzheimer's disease.

p21 Waf1/Cip1 (12D1) Rabbit mAb #2947 – WB, IP, IHC-P, **IF-IC**, FC-FP

β-Galactosidase

Senescent cells can be identified by an increased level of lysosomal β-galactosidase activity. β-amyloid triggers senescence in *in vitro* models, driving expression of p16 INK4A and senescence-associated β-galactosidase. Increased β-galactosidase activity has also been observed in the cerebrospinal fluid (CSF) of Parkinson's disease patients.

Senescence β-Galactosidase Staining Kit #9860

TNF-α

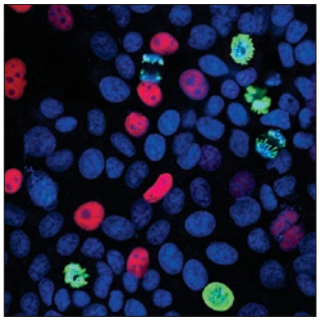
TNF-α is a cytokine whose dysregulation has been implicated in Alzheimer's disease and Parkinson's disease. Levels of TNF-α are increased in the brain tissue, CSF, and serum of Alzheimer's disease patients, potentially due to increased p38 MAPK activity. Aged *in vitro* rat microglia may acquire a senescent phenotype characterized by increased levels of IL-1β and TNF-α after treatment with β-amyloid oligomers. Elevated levels of TNF-α have also been observed in the CSF, serum, and dopaminergic regions of the striatum from patients with Parkinson's disease.

TNF-α (D2D4) XP® Rabbit mAb (Mouse Specific) #11948 – WB, IP, **IF-IC**, FC-FP

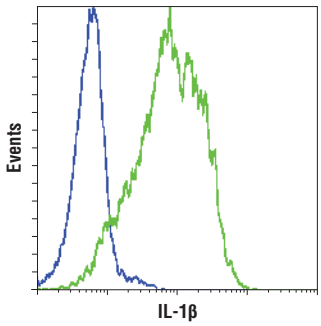
IL-1β

IL-1β is a pro-inflammatory cytokine that is secreted by senescent cells. It is elevated in Alzheimer's disease brain tissue, CSF, and serum, potentially due to increased p38MAPK activity. Aged *in vitro* rat microglia may acquire a senescent phenotype characterized by increased levels of IL-1β and TNF-α after treatment with beta-amyloid oligomers. Elevated levels of IL-1β have also been observed in the CSF, serum, and dopaminergic regions of the striatum from patients with Parkinson's disease.

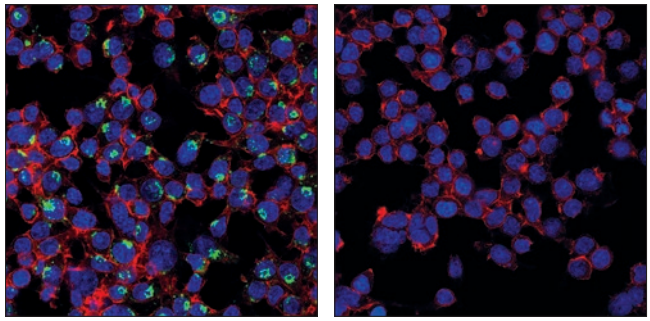
IL-1β (D3U3E) Rabbit mAb #12703 – WB, IF-IC, **FC-FP**



p21 Waf1/Cip1 (12D1) Rabbit mAb #2947: Confocal IF analysis of MCF7 cells using #2947 (red) and Phospho-Histone H3 (Ser10) (6G3) Mouse mAb #9706 (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



IL-1β (D3U3E) Rabbit mAb #12703: Flow cytometric analysis of THP-1 cells, untreated (blue) or LPS-treated (100 ng/ml, 3 hr; green), using #12703. Anti-rabbit IgG (H+L), $F(ab')_2$ Fragment (Alexa Fluor® 647 Conjugate) #4414 was used as a secondary antibody.



TNF-α (D2D4) XP® Rabbit mAb (Mouse Specific) #11948: Confocal IF analysis of Raw 264.7 cells, treated with LPS (100 ng/mL, 6 hr; left) or untreated (right), using #11948 (Rodent Specific) (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

CELL TYPE MARKERS:

Neurons & Astrocytes

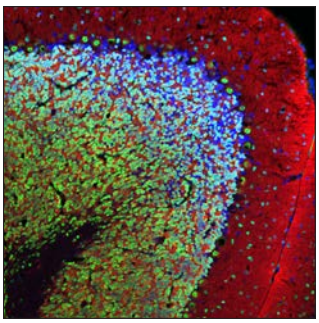
Neuronal Markers

Neurons are electrically excitable cells in the Central Nervous System (CNS) and Peripheral Nervous System (PNS) that send and receive electrical impulses and neurochemical signals to process information. Neurons drive activity in the brain, spinal cord, and peripheral sensory and motor systems. They are highly specialized to quickly transmit electrical signals and neurotransmitters across synapses. Although there is a wide variety of neuronal types and morphologies in the nervous system, signals are typically received on the cell body or dendrites and sent out along the axon. Neurons are often identified by observing the expression of cell-specific intracellular proteins, such as **NeuN**, **β3-Tubulin**, and **UCHL1**.

NeuN Neuronal Nuclei (NeuN) is a pre-mRNA alternative splicing regulator that was first identified in mammals by generating a monoclonal antibody that successfully targeted an antigen, which turned out to be Fox-3, in the nuclei of neurons. Antibodies to this protein are commonly used to label nuclei in the majority of neurons in vertebrates.

β3-Tubulin β3-tubulin (TUBB3) is one of six β-tubulin isoforms that comprise the major components of microtubules in neurons. It is highly expressed during fetal and postnatal development and plays a critical role in proper axon guidance, maturation, and maintenance. Antibodies against this cell marker stain the cytoskeleton of all neurons.

MAP2 Microtubule-associated protein 2 (MAP2) is a neuronal phosphoprotein that regulates the structure and stability of microtubules, neuronal morphogenesis, cytoskeleton dynamics, and organelle trafficking in axons and dendrites. Isoforms of MAP2 are expressed in the perikarya and dendrites of neurons, making antibodies that target MAP2 useful tools to highlight the dendritic projections of neurons.



NeuN (E4M5P) Mouse mAb #94403: Confocal IF analysis of mouse brain using NeuN (E4M5P) Mouse mAb (green) and Phospho-Tau (Thr205) (E7D3E) Rabbit mAb (Alexa Fluor® 647 Conjugate) #53001 (red).

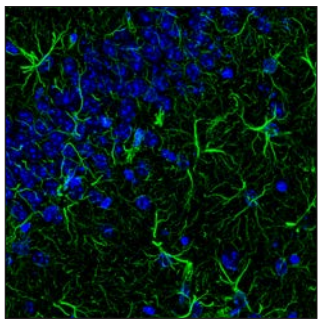
Astrocyte Markers

Astrocytes are the most prevalent type of glial cell in the CNS and are found within the brain and spinal cord. In a healthy nervous system, astrocytes play essential roles in development, regulation of blood flow (by supporting endothelial cells in the blood brain barrier), **synaptic transmission** and function, and energy and metabolism (by providing nutrients to neurons and synthesizing certain neurotransmitters). The loss or abnormal function of astrocytes is implicated in a wide variety of neurodegenerative disease processes. Chronic activation of astrocytes results in the formation of lesions similar to those observed in Alzheimer's Disease and Huntington's Disease. Some useful astrocyte markers include **GFAP** and **ALDH1L1**.

GFAP Glial fibrillary acidic protein (GFAP) is a class-III intermediate filament that makes up the cytoskeleton of astrocytes in the CNS. GFAP establishes and maintains astrocyte morphology and is important for mitosis as well as neuron-astrocyte communication. Antibodies that detect GFAP are often used to label astrocytes and reveal their distinctive morphologies in the brain.

ALDH1L1 Aldehyde dehydrogenase 1 family member L1 (ALDH1L1) is a key enzyme in folate metabolism and, as such, plays an important role in the regulation of cellular metabolism and proliferation. Downregulation of ALDH1L1 has been observed in tumors, leading to decreased suppression of cancer cell proliferation. Antibodies that target ALDH1L1 label the cytoplasm of astrocytes in the brain, effectively staining the cell body and processes of these cells.

S100B S100B is abundantly expressed in astrocytes. It is commonly used as a mature astrocytic marker in studies of the mammalian CNS development. S100B expression follows GFAP, and is selectively found in protoplasmic astrocytes, and immature and mature myelinating oligodendrocytes.



GFAP (E4L7M) XP® Rabbit mAb #80788: Confocal IF analysis of adult mouse hippocampus using #80788 (green). Samples were mounted in ProLong® Gold Antifade Reagent with DAPI #8961 (blue).

CELL TYPE MARKERS:

Oligodendrocytes

Oligodendrocytes are highly specialized glial cells that produce myelin, a lipid-rich substance that provides a protective sheath around axons and improves the conduction velocity of signals between neurons. They are characterized by the expression of the myelin family proteins: **Myelin Oligodendrocyte Glycoprotein (MOG)**, **Myelin-Associated Glycoprotein (MAG)**, and **Myelin Basic Protein (MBP)**. Oligodendrocyte progenitor cells exist in the brain to facilitate regeneration of cells due to injury. However, the breakdown of myelin and inability to regenerate fully myelinated oligodendrocytes is correlated with several neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS).

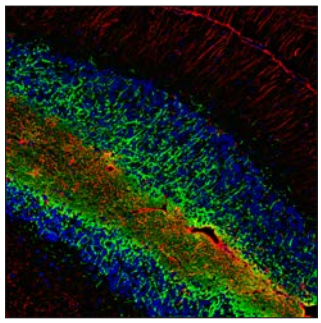
MBP Myelin basic protein (MBP) is one of the most abundant proteins in the CNS and plays an important role in the myelination of nerve cells. As a key component of myelin sheaths that surround axons, MBP contributes to the adhesion of the cytosolic membranes of compacted myelin. This is crucial to facilitate the conduction of neuronal impulses. MBP antibodies are often used to stain myelin sheaths of oligodendrocytes and Schwann cells in both thev CNS and PNS.

PLP1 Myelin proteolipid protein (PLP1) is the major membrane bound phospholipid protein that is enriched in oligodendrocytes of the CNS. It plays a crucial role in the formation and maintenance of the multilamellar structure of myelin. Antibodies against this cell marker stain the myelin of oligodendrocytes in the CNS.

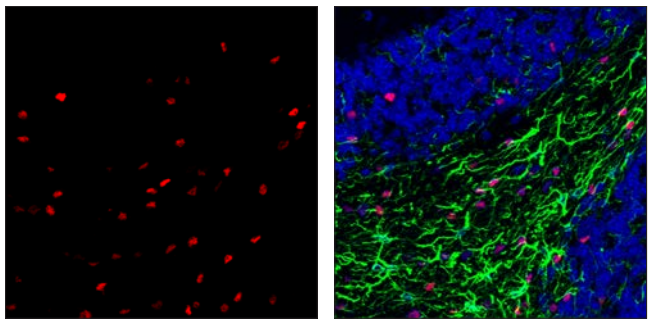
CNPase CNPase (2′, 3′-cyclic nucleotide 3′-phosphodiesterase) is an enzyme that catalyzes the hydrolysis of 2′, 3′-cyclic nucleotides. It plays an early role in oligodendrocyte differentiation and may help generate the compacted myelin that surrounds axons. CNPase is enriched in oligodendrocytes and Schwann cells and antibodies that target this enzyme are commonly used to label myelin in the CNS and PNS.

Olig2 Olig2 is a specific marker for primary and mature oligodendrocytes, which are the myelinating cells within the CNS. Oligodendroglial and myelin dysfunction have been linked to neurodegeneration and multiple system atrophy. Olig2 is also universally expressed in oligodendrogliomas, and is a commonly accepted marker for studying T-cell acute lymphoblastic leukaemia, neural crest differentiation, neural stem cells, and lineage-specificity.

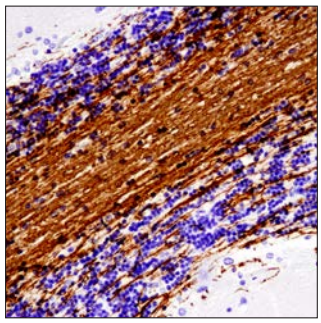
MOG Myelin-oligodendrocyte glycoprotein (MOG) is a type I membrane bound glycoprotein of the immunoglobulin superfamily that is enriched in the outer lamella of the myelin sheath. The myelin sheath is a multi-layered membrane structure composed of oligodendrocytes that increases the conduction velocity of axonal impulses. MOG's structure and expression during later stages of myelinogenesis suggests a role in myelin sheath maturation and maintenance. MOG may be an autoimmune target in CNS inflammatory diseases such as multiple sclerosis.



PLP1 (E9V1N) Rabbit mAb #28702: Confocal IF analysis of mouse cerebellum using #28702 (green) and GFAP (GA5) Mouse mAb #3670 (red). Samples were mounted in ProLong® Gold Antifade Reagent with DAPI #8961 (blue).



Olig2 (E6G6Q) XP® Rabbit mAb #65915: Confocal IF analysis of fixed frozen mouse cerebellum, labeled with #65915 (left, red) and co-labeled with GFAP (GA5) Mouse mAb (Alexa Fluor® 488 Conjugate) #3655 (right, green) and DAPI #4083 (right, blue).



MOG (E5K6T) XP® Rabbit mAb #96457: IHC analysis of paraffin-embedded human cerebellum using #96457 Rabbit mAb.

CELL TYPE MARKERS:

Microglia

Microglia are a type of glial cell present throughout the brain and spinal cord. As brain-resident macrophages, these cells play an important role as a first line of immune defense in the central nervous system (CNS). Activated microglia can act as antigen presenters and secrete cytokines to trigger further immune responses. With highly sensitive phagocytic processes that extend to multiple locations, they continuously detect and engulf damaged cells, infectious agents, debris, and plaques. Due to their multifaceted ability to monitor and clean up the CNS, these cells are critical for injury repair response and are implicated in neurodegenerative diseases. Microglia are also necessary during brain development as they are critical for dendritic pruning and, in a mature brain, they help maintain a homeostatic environment. Microglia are often identified by observing the expression of cell-specific intracellular proteins, such as **Iba1/AIF-1**, **CD11b**, and **TMEM119**.

Iba1/AIF-1

Iba1/AIF-1 is an evolutionarily conserved calcium-binding protein. As an F-actin-binding protein, Iba1/AIF-1 may remodel the actin cytoskeleton of microglia. Iba1/AIF-1 is uniquely expressed in cells of monocytic lineage and, therefore, antibodies targeting this protein are widely used to label microglia/macrophages in the brain and other tissue.

CD11b

The transmembrane protein Cluster of Differentiation Molecule 11b (CD11b)/Integrin alpha M (ITGAM) consists of α and β heterodimers that are expressed in cells of the innate immune system. Antibodies that detect CD11b/ITGAM are commonly used to identify cells of myeloid lineage, including neutrophils, monocytes, macrophages, and microglia.

TMEM119

TMEM119 is a transmembrane protein of unknown function that is specifically expressed in almost all microglia. This developmentally regulated protein is not expressed by macrophages or other immune cell types. Antibodies that detect TMEM119 act as specific cell surface markers to visualize microglia.

HS1

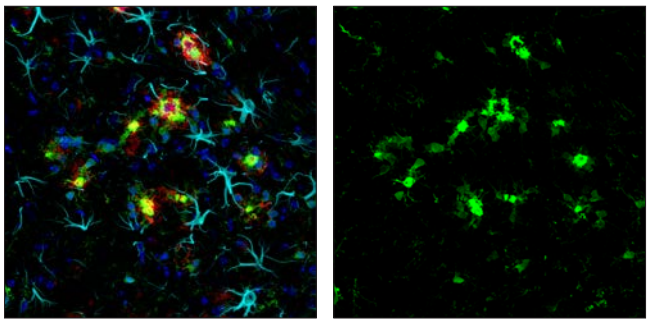
HS1 is a protein kinase substrate that is enriched in cells of hematopoietic origin. In the brain, HS1 is expressed in microglia and monocyte-derived macrophages. HS1 antibodies are useful tools to highlight the cytoplasm of microglia and macrophages.

ASC

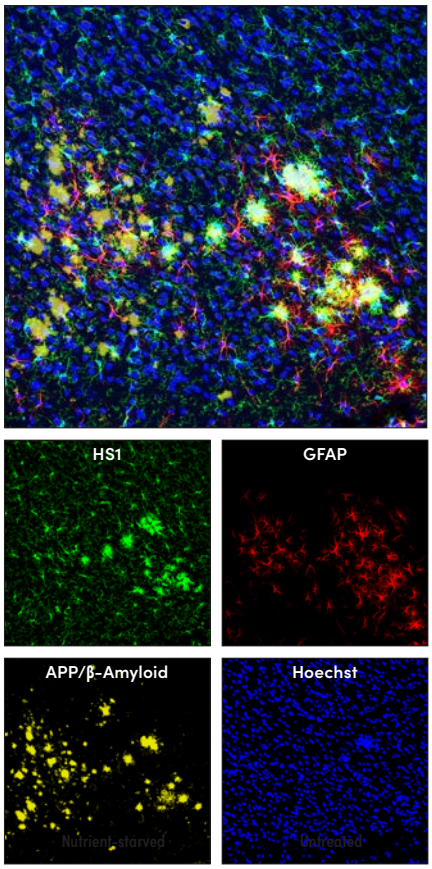
ASC/TMS1 is an adaptor protein, encoded by the PYCARD gene in humans, that is often expressed in the nucleus of monocytes, microglia, and macrophages. This pro-apoptotic protein is a key mediator of the inflammasome that is triggered in response to pathogen infection or tissue damage. During this process, it relocalizes to the perinuclear space, cytoplasm, and endoplasm, as well as the mitochondria, where it interacts with Bax to trigger cytochrome c release and cause subsequent apoptosis. ASC/TMS1 is also a crucial component of inflammatory signaling because it activates caspase-1 in response to pro-inflammatory signals.

Cathepsin D

Cathepsin D plays a role in neuronal degradation. It is the main protease that degrades β-amyloid and is known to degrade tau. Cathepsin D serves as a stage 2 TREM2-dependent marker of disease-associated microglia, as its expression is increased in mouse models of Alzheimer's disease, and it co-localizes with Iba1+ microglia surrounding β-amyloid plaques.

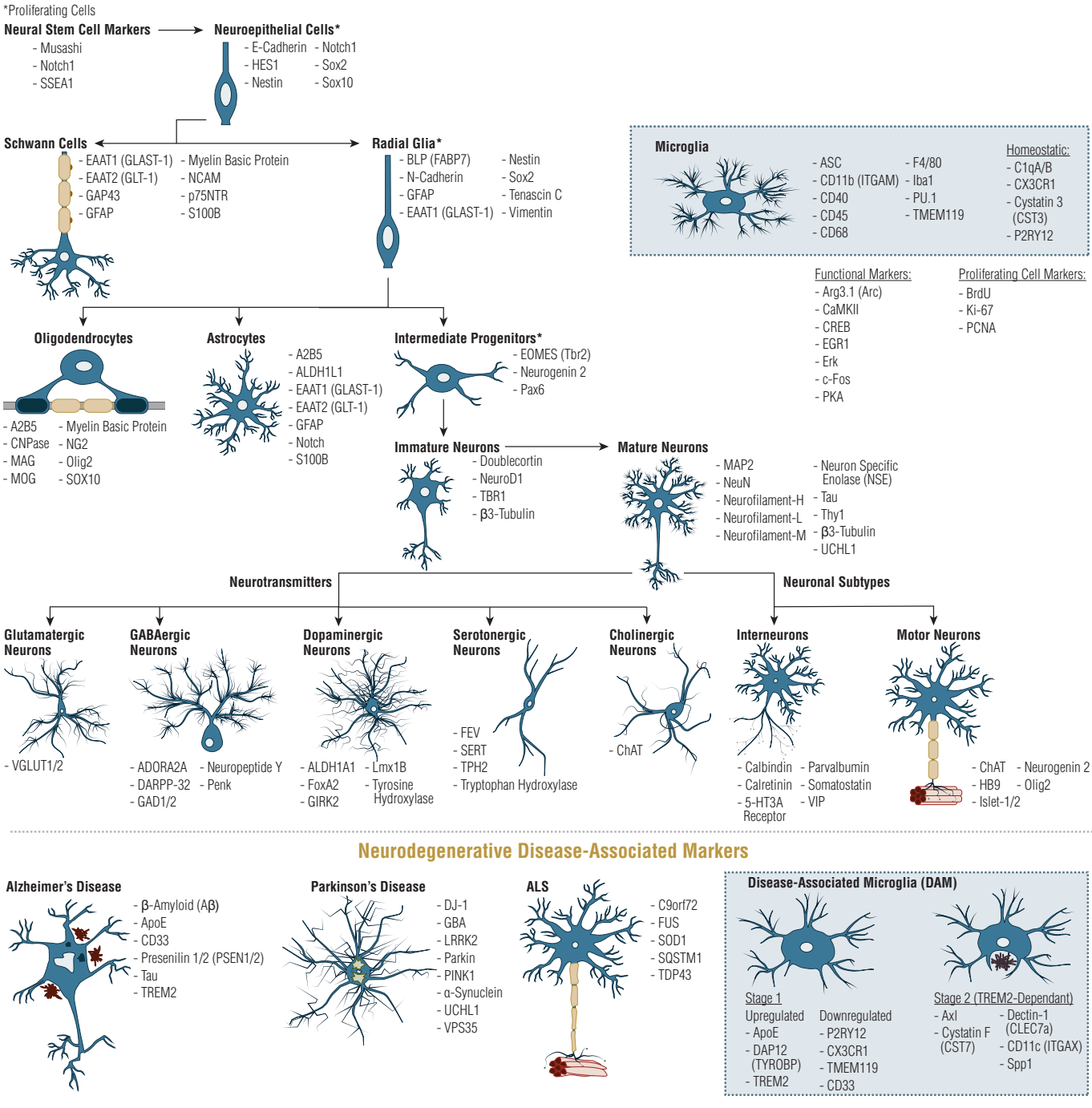


Iba1/AIF-1 (E4O4W) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) #20825: Confocal IF analysis of brain from an amyloid mouse model of Alzheimer's disease using #20825 (green), GFAP (GA5) Mouse mAb (Alexa Fluor® 555 Conjugate) #3656 (cyan pseudocolor), and β-Amyloid (D54D2) XP® Rabbit mAb (Alexa Fluor® 647 Conjugate) #42284 (red). Sections were mounted in ProLong® Gold Antifade Reagent with DAPI #8961 (blue).



HS1 (D5A9) XP® Rabbit mAb (Rodent Specific) #3892: Confocal IF analysis of mouse Tg2576 brain which overexpresses mutant human APP695. Sections were first labeled with #3892 (green) and APP/β-Amyloid (NAB228) Mouse mAb #2450 (yellow). After blocking free secondary binding sites with Mouse (G3A1) mAb IgG1 Isotype Control #5415, sections were incubated with GFAP (GA5) Mouse mAb (Alexa Fluor® 647 Conjugate) #3657 (red). Nuclei were labeled with Hoechst 33342 #4082 (blue).

Neuronal and Glial Cell Marker Atlas



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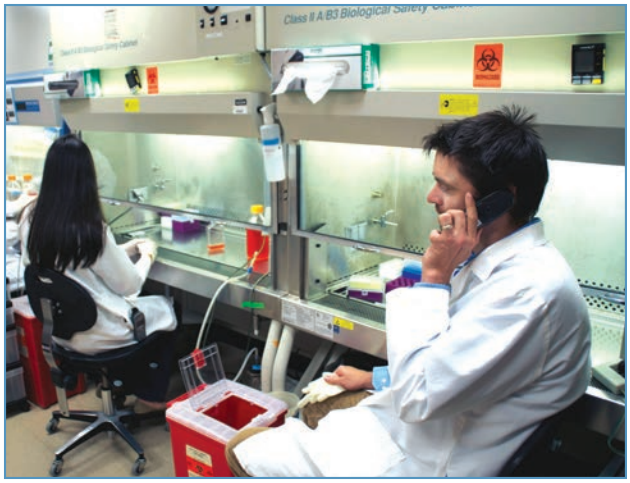

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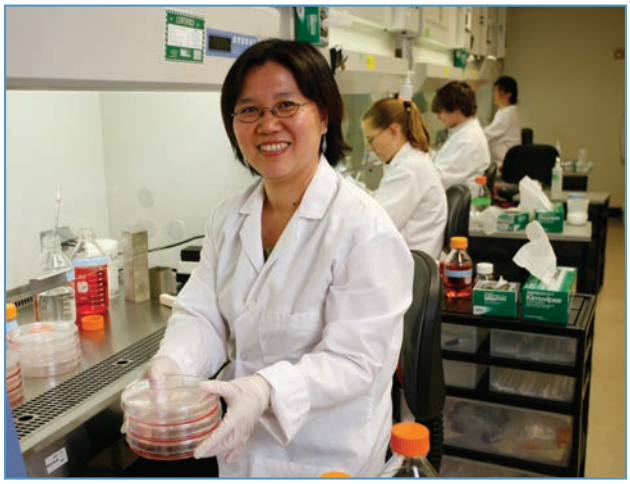

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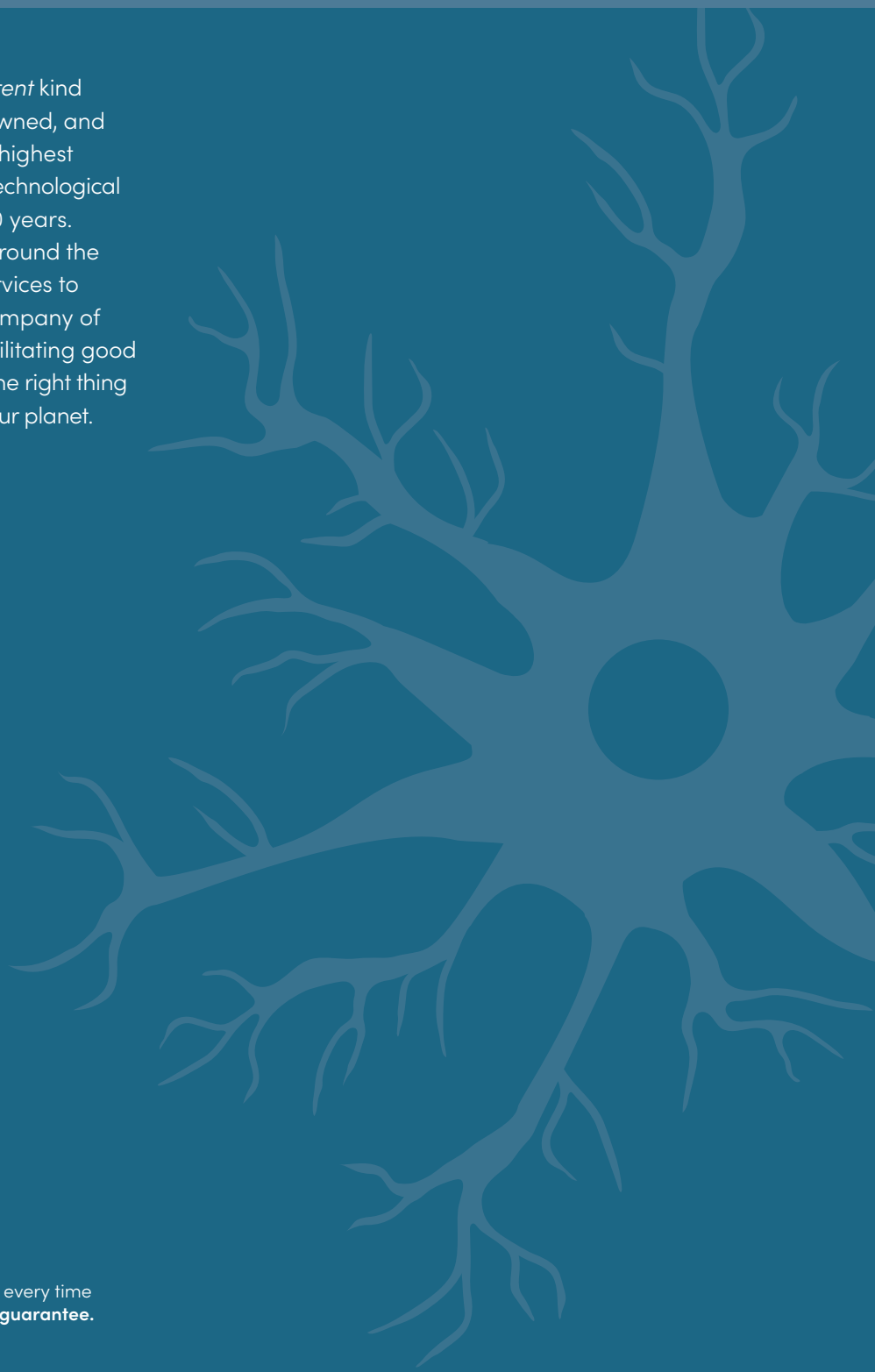

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