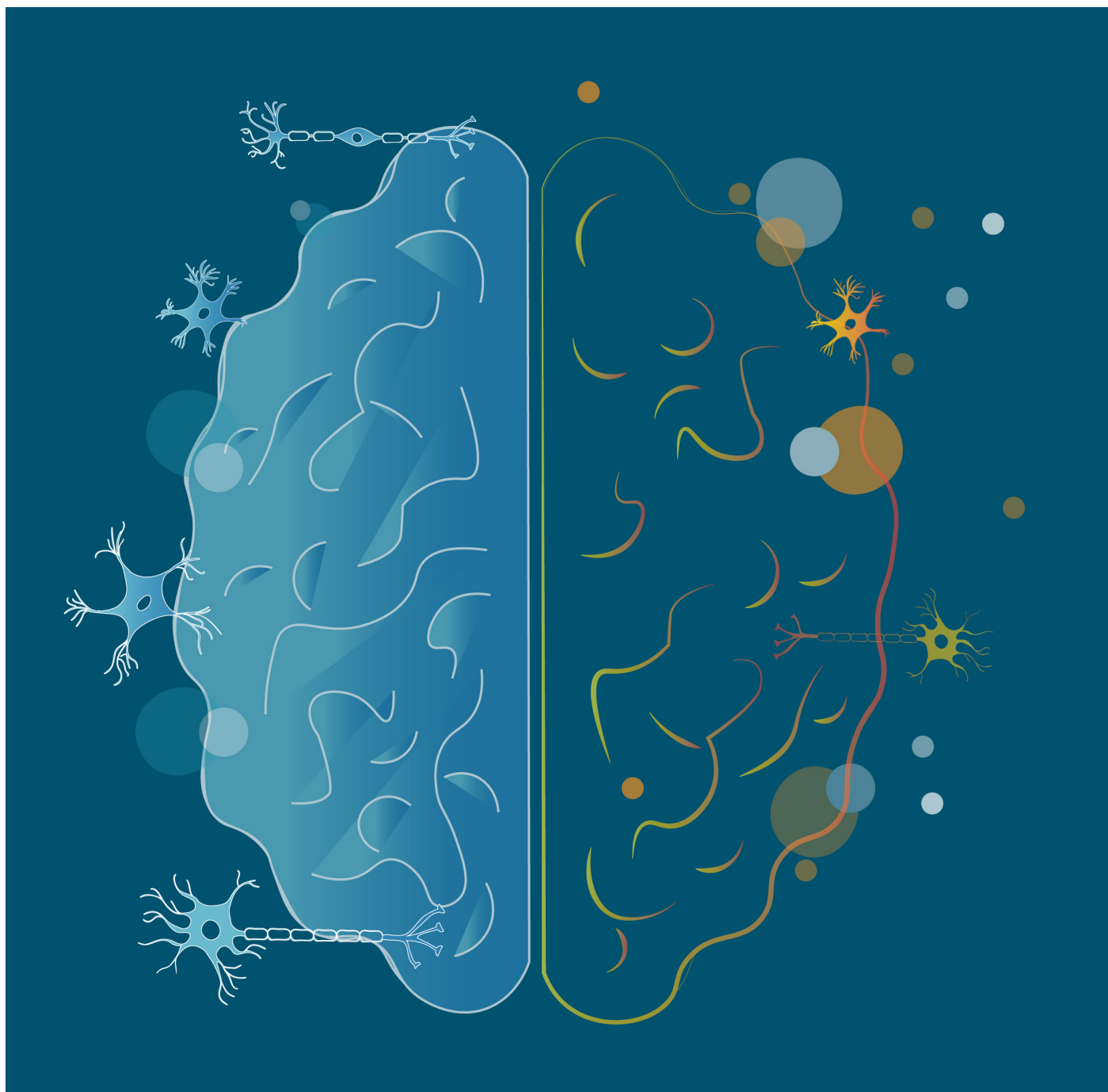




MAPPING THE BRAIN PROTEOME IN HEALTH AND DISEASE

 **ATLAS ANTIBODIES**

CONTENT

1. INTRODUCTION
2. ESTABLISHING A BASELINE: THE KEY TO UNDERSTANDING BRAIN FUNCTION AND PATHOLOGY
3. UNDERSTANDING BRAIN DISEASES: VARIATION FROM BASELINE
4. CELLULAR COMMUNICATION AND PROTEIN-PROTEIN INTERACTION
5. CONCLUSIONS
6. WHY ATLAS ANTIBODIES?

FIGURES & TABLES

- FIGURE 1. MAPPING THE BRAIN PROTEOME TO UNDERSTANDING BRAIN DISORDERS
FIGURE 2. BRAIN CELL TYPE IN HUMAN AND RODENT BRAIN
FIGURE 3. ASTROCYTES IN THE CEREBRAL CORTEX.
FIGURE 4. IHC AND ICC-IF STAINING ON HUMAN AND RODENT BRAIN
FIGURE 5. NEURON-SPECIFIC MARKERS
FIGURE 6. LAMINAR STRUCTURE OF CEREBRAL CORTEX
FIGURE 7. SOX PROTEINS EXPRESSION IN CORTICAL ASTROCYTES AND OLIGODENDROCYTES
FIGURE 8. CORTICAL INHIBITORY NEURONS
FIGURE 9. GLIAL MARKERS IN NEUROINFLAMMATION AND NEURODEGENERATIVE DISEASES
FIGURE 10. GLIAL MARKERS IN ALZHEIMER'S DISEASE
FIGURE 11. DEVELOPING MOUSE CORTEX
FIGURE 12. ADULT MOUSE CORTEX
FIGURE 13. GLIAL CELLS IN GLIOMAS
FIGURE 14. MOLBOOLEAN: DRD2-ADORA2A RECEPTORS INTERACTION IN RAT STRIATUM
FIGURE 15. MOLBOOLEAN: DRD2-ADORA2A RECEPTORS INTERACTION IN RAT STRIATAL NEURONS AND GLIA

- TABLE 1. COMMON MARKERS FOR MAJOR BRAIN CELL TYPES
TABLE 2. ANTIBODIES TARGETING ASTROCYTES
TABLE 3. ANTIBODIES TARGETING OLIGODENDROCYTES AND SCHWANN CELLS
TABLE 4. ANTIBODIES TARGETING MICROGLIA
TABLE 5. ANTIBODIES TARGETING NEURONS (ALL)
TABLE 6. NEURON-SPECIFIC ANTIBODIES MARKERS
TABLE 7. COMMON PROTEIN MARKERS ALTERED IN NEUROLOGICAL DISORDERS
TABLE 8. ANTIBODIES FOR NEURODEGENERATIVE DISEASES
TABLE 9. ANTIBODIES FOR NEUROINFLAMMATION
TABLE 10. ANTIBODIES FOR NEURODEVELOPMENTAL PROCESSES
TABLE 11. ANTIBODIES TARGETING GLIOMAS

1. INTRODUCTION

The brain is the most complex organ in the human body, with countless processes still shrouded in mystery. To tackle neurological diseases at their roots, it is crucial to understand the brain's molecular landscape, particularly its intricate signaling networks.

A critical step in this process is identifying the different brain cells and how they communicate and interact under healthy conditions. Establishing a baseline of normal cellular operations and communication provides the insight needed to recognize the changes or abnormalities that occur in neurological disorders. This foundational knowledge is the key to pinpointing the root causes of these disorders, ultimately leading to more effective interventions and treatments.

Spatial proteomics offers unprecedented insights into the complex architecture of the brain. Unlike traditional proteomic analyses that provide bulk protein measurements, spatial proteomics allows the visualization of specific proteins location within cells, tissues, and even subcellular compartments.

Central to spatial proteomics are primary antibodies that selectively bind to target proteins, enabling precise localization and visualization. Selective antibodies are essential for uncovering the biological underpinnings of brain conditions like gliomas, Alzheimer's and Parkinson's diseases, multiple sclerosis and epilepsy, and for developing the next generation of transformative treatments.

This white paper highlights the role of proteomics in unraveling the complexities of the brain, leveraging primary antibodies to pinpoint specific markers crucial for identifying various brain cell types and understanding their spatial organization within neural tissue. By mapping the distribution of proteins at subcellular resolutions, we will be able to answer fundamental questions about normal brain function and pathology.

2. ESTABLISHING A BASELINE: THE KEY TO UNDERSTANDING BRAIN FUNCTION AND PATHOLOGY

The human brain comprises various cell types, including neurons, astrocytes, oligodendrocytes, and microglia, with their exact numbers varying across different brain regions.

Approximately 86 billion neurons are present in the brain, while astrocytes, a type of glial cell, outnumber neurons and constitute 20-40% of all glial cells. Oligodendrocytes, also glial cells, are generally less abundant than astrocytes. Verkhatsky A, Butt AM (2013). "Numbers: how many glial cells are in the brain?". *Glial Physiology and Pathophysiology*. John Wiley and Sons. pp. 93–96.

Cell-type specific markers

Each brain cell type is defined by specific protein markers, which are crucial for their distinct roles and functions within neural tissue. Mapping these markers allows for precise spatial localization and interaction studies. This approach not only enables the accurate identification and characterization of each cell type but also provides a foundation for exploring their involvement in neurological disorders.

Neurons specific markers

Among the brain's cell types, neurons have distinct markers that vary depending on the brain region and specific neuronal subtype. These markers are not only critical for identifying different neuron types but also play a key role in understanding the functions of various brain regions and the underlying mechanisms of certain brain diseases. Organizing neuron-specific markers based on the types of neurons they are associated with requires careful interpretation, as some markers are expressed across multiple neuronal types or subtypes. Often, a combination of several markers is beneficial for defining a particular cell type.

By identifying and characterizing cell type-specific markers, we can establish a baseline of what constitutes a healthy brain. From this knowledge we can detect deviations associated with various neurological disorders. Primary antibodies are used to map neurons, astrocytes, oligodendrocytes, and microglia proteins within neural tissues with high precision in human and rodent tissues.

ASTROCYTES

anti-ADGRV1 (Cat.HPA067503)
anti-AQP9 (Cat.HPA074762)
anti-CD44 (Cat.HPA005785)
anti-EZR (Cat.AMAb90976)
anti-FGF2 (Cat.HPA065502)
anti-FYN (Cat.HPA023887)
anti-GFAP (Cat.AMAb91033)
anti-GLUL (Cat.AMAb91101)
anti-GPC5 (Cat.HPA040152)
anti-HPSE2 (Cat.HPA044603)
anti-PTPRZ1 (Cat.HPA015103)
anti-S100B (Cat.AMAb91038)
anti-SLC1A2 (Cat.HPA009172)
anti-SLC1A3 (Cat.HPA037467)

OLIGODENDROCYTES

anti-ALCAM (Cat.HPA010926)
anti-CNP (Cat.AMAb91069)
anti-COL1A2 (Cat.HPA059738)*
anti-COL9A1 (Cat.HPA074749)
anti-CRYAB (Cat.AMAb91661)
anti-CST3 (Cat.HPA013143)*
anti-GRIK2 (Cat.HPA014623)
anti-MBP (Cat.AMAb91063)
anti-OPALIN (Cat.AMAb91685)
anti-PDGFR (Cat.HPA004947)
anti-PLP1 (Cat.HPA004128)
anti-SOX6 (Cat.AMAb91383)
anti-STK32A (Cat.HPA040236)
anti-TLL1 (Cat.HPA060767)
anti-VCAN (Cat.HPA004726)
anti-VIM (Cat.HPA001762)*

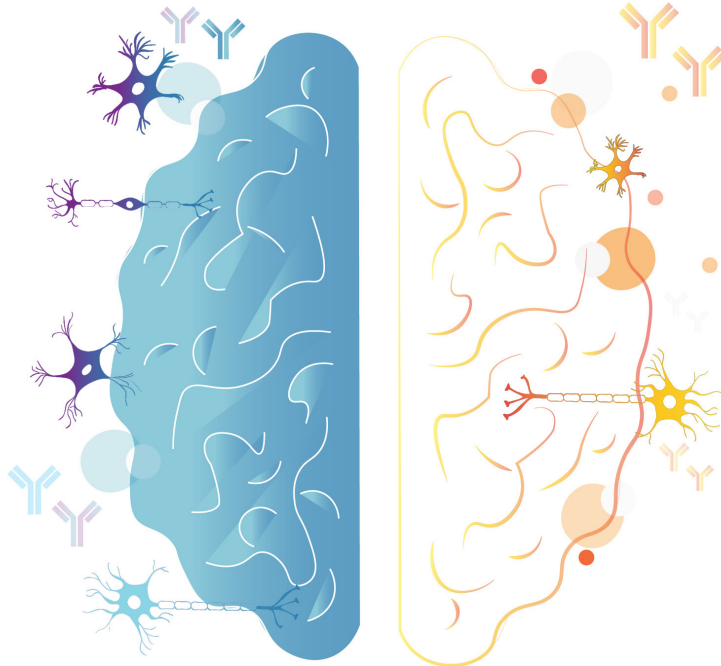
*Schwann cells

MICROGLIA

anti-AIF1 (Cat.AMAb91671)
anti-CD163 (Cat.AMAb91646)
anti-CUX1 (Cat.AMAb91352)
anti-HLA-DRA (Cat.AMAb91674)
anti-ITGAM (Cat.AMAb90911)
anti-PTPRC (Cat.AMAb90518)
anti-SPP1 (Cat.HPA027541)

NEURONS (ALL)

anti-MAP2 (Cat.HPA008273)
anti-NeuN (Cat.HPA070789)
anti-NEFM (Cat.AMAb91027)
anti-NSE (Cat.AMAb90556)
anti-SYP (Cat.HPA002858)
anti-TUBB3 (Cat.AMAb91394)



GLIOMAS

anti-EZH2 (Cat.AMAb91752)
anti-FGFR1 (Cat.HPA056402)
anti-FOXO3 (Cat.AMAb91872)
anti-GLI1 (Cat.AMAb91772)
anti-GFAP (Cat.AMAb91033)
anti-ID1 (Cat.AMAb91757)
anti-IL33 (Cat.AMAb91858)
anti-NF1 (Cat.AMAb91741)
anti-POSTN (Cat.AMAb91764)
anti-PTEN (Cat.AMAb91735)
anti-RUNX2 (Cat.HPA022040)
anti-SLC6A6 (Cat.HPA016488)
anti-ZEB2 (Cat.AMAb91862)

NEURODEGENERATION

anti-APOE (Cat.HPA065539)
anti-APP (Cat.HPA001462)
anti-CDK5 (Cat.HPA064535)
anti-CLU (Cat.HPA000572)
anti-CREB1 (Cat.HPA019150)
anti-GAPDH (Cat.AMAb91153)
anti-GRN (Cat.AMAb91385)
anti-GSK3B (Cat.HPA028017)
anti-HDAC6 (Cat.HPA026321)
anti-MAPT (Cat.HPA069524)
anti-NOS1 (Cat.HPA069509)
anti-NRGN (Cat.HPA038171)
anti-NTRK1 (Cat.HPA035799)
anti-PARK2 (Cat.HPA036012)
anti-PSEN1 (Cat.HPA030760)
anti-SNCA (Cat.HPA005459)

NEUROINFLAMMATION

anti-AGER (Cat.AMAb91635)
anti-GAP43 (Cat.AMAb91664)
anti-GZMB (Cat.AMAb91650)
anti-IL17RA (Cat.AMAb91617)
anti-ITGA4 (Cat.HPA074961)
anti-MBP (Cat.AMAb91063)
anti-MS4A1 (Cat.HPA014391)
anti-P2RX4 (Cat.HPA039494)
anti-P2RX7 (Cat.AMAb91714)
anti-PLP1 (Cat.AMAb91639)
anti-S100A8 (Cat.HPA024372)
anti-S100A9 (Cat.AMAb91690)
anti-SORT1 (Cat.AMAb91428)
anti-TCF7L2 (Cat.AMAb91716)
anti-TLR2 (Cat.AMAb91631)
anti-TREM1 (Cat.AMAb91459)
anti-TSPO (Cat.AMAb91854)

FIGURE 1. MAPPING THE BRAIN PROTEOME TO UNDERSTANDING BRAIN DISORDERS

The human brain consists of various cell types, including neurons, astrocytes, oligodendrocytes, and microglia, with their numbers varying across different brain regions. Both neurons and glial cells are fundamental to normal brain function but can play a key role in many brain disorders when altered. Primary antibodies are used to map proteins within distinct cell types with high precision in human and rodent neural tissues.

SIX KEY REASONS WHY MAPPING THE BRAIN PROTEOME IS CRUCIAL

1. Early Detection and Diagnosis

Variations in specific protein levels can indicate early stages of brain diseases before clinical symptoms become apparent.

Example: elevated levels of amyloid-beta and tau proteins are biomarkers for Alzheimer's disease, often detectable in cerebrospinal fluid or through imaging techniques before significant cognitive decline occurs. Early detection enables timely intervention, potentially slowing the disease progression.

2. Disease Characterization

Different brain diseases and conditions exhibit unique protein expression profiles.

Example: the accumulation of alpha-synuclein in Lewy bodies is a hallmark of Parkinson's disease, while mutations in IDH1 are commonly associated with gliomas. These specific protein alterations help differentiate between diseases, facilitating accurate diagnosis and appropriate treatment plans.

3. Monitoring Disease Progression

Tracking protein levels over time allows healthcare providers to monitor disease progression and treatment efficacy.

Example: in multiple sclerosis (MS) changes in myelin basic protein (MBP) levels can indicate the extent of demyelination and response to therapies. This ongoing assessment is vital for adjusting treatment strategies to achieve optimal outcomes.

4. Personalized Medicine

Variations in protein expression can inform personalized treatment approaches.

Example: the detection of MGMT promoter methylation in glioblastoma patients can predict responsiveness to alkylating agents, allowing for tailored chemotherapy regimens. Similarly, understanding individual protein expression profiles can help in selecting targeted therapies that are more likely to be effective.

5. Insight into Pathophysiology

Protein alterations provide insight into the underlying mechanisms of brain diseases.

Example: the role of inflammation in neurodegenerative diseases is highlighted by increased levels of glial fibrillary acidic protein (GFAP) and inflammatory cytokines.

Understanding these mechanisms can lead to the development of new therapeutic targets and strategies aimed at modulating these pathways to alter disease outcomes.

6. Non-Invasive Biomarker Development

Proteins that can be measured in accessible biological fluids (e.g., blood, cerebrospinal fluid) serve as non-invasive biomarkers for brain diseases.

Example: this is particularly valuable for conditions like Alzheimer's disease, where biomarkers such as amyloid-beta can be detected in the blood, providing a less invasive diagnostic option.

Mapping the brain proteome advances our understanding of the brain in both health and disease by providing detailed insights into the spatial distribution and interactions of cell-specific protein markers. It is crucial for uncovering the mechanisms underlying brain function and pathology. This approach allows for the detection of early signs of neurological disorders, monitoring of disease progression, and the development of personalized treatment strategies.

TABLE 1. COMMON MARKERS FOR MAJOR BRAIN CELL TYPES

Cell Type	Marker	Function
Astrocytes	GFAP	Key intermediate filament protein used for identifying astrocytes
	S100B	Protein involved in calcium signaling; marker for astrocytic activity and unipotent astrocytes.
	ALDH1L1	Enzyme involved in detoxifying aldehydes; specific marker for astrocytes
Oligodendrocytes	MBP	Major protein component of myelin sheath; crucial for myelin formation and stability
	OLIG2	Transcription factor important for development and maturation of oligodendrocytes
	CNPase	Enzyme involved in myelin formation and maintenance; marker for oligodendrocytes
Microglia	Iba1/AIF1	Protein involved in motility and phagocytic activity of microglia
	CD68	Glycoprotein highly expressed in microglia; associated with phagocytic activity
	TMEM119	Marker used to distinguish microglia from other macrophage-lineage cells
Neurons	NeuN/ RBFOX3	Reliable marker for identifying neuronal cells; present in the nuclei of neurons
	MAP2	Protein involved in stabilization of microtubules; marker for neuronal cell bodies and dendritic processes
	NSE	Enzyme highly expressed in neurons; marker for neuronal differentiation and activity

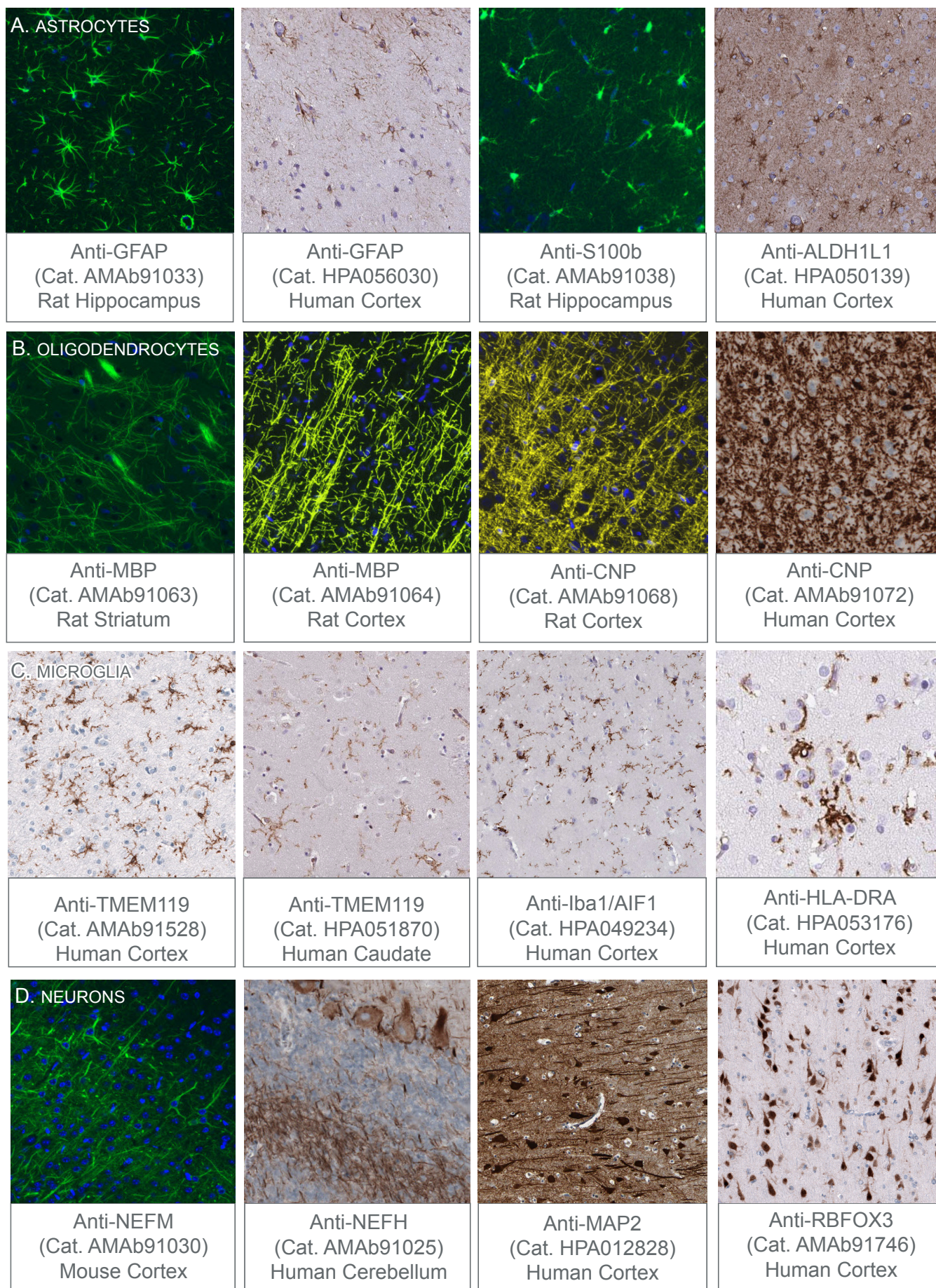


FIGURE 2. BRAIN CELL TYPE IN HUMAN AND RODENT BRAIN

Immunofluorescence and immunohistochemical staining of common protein markers specific to different brain cell types (i.e. astrocytes, oligodendrocytes, microglia, and neurons) in both human and rodent tissues using Atlas Antibodies' TripleA Polyclonals™ (HPAxxx) and PrecisA Monoclonals™ (AMAbxxx).

TABLE 2. ANTIBODIES TARGETING ASTROCYTES

Product Name	Product ID	Clonality / Isotype	Recommended Applications	PrEST Control Antigen
Anti-ADGRV1	HPA067503	Polyclonal	IHC, ICC-IF	APrEST92735
Anti-AQP9	HPA074762	Polyclonal	IHC*	APrEST94006
Anti-CD44	HPA005785	Polyclonal	IHC*, WB*, ICC-IF	APrEST83079
Anti-EZR	AMAb90976	Monoclonal, IgG1	IHC*, WB*, ICC-IF	APrEST85223
	HPA021616	Polyclonal	IHC*, WB*, ICC-IF	APrEST85223
Anti-FGF2	HPA065502	Polyclonal	IHC	APrEST94915
Anti-FYN	HPA023887	Polyclonal	IHC, WB*, ICC-IF	APrEST78892
Anti-GFAP	HPA056030	Polyclonal	IHC*, WB, ICC-IF	APrEST85954
	AMAb91033	Monoclonal, IgG1	IHC*, WB*	APrEST85954
	HPA063513	Polyclonal	IHC*	APrEST88570
Anti-GLUL	AMAb91101	Monoclonal, IgG1	IHC, WB*	APrEST70153
	AMAb91102	Monoclonal, IgG1	IHC, WB*	APrEST70153
	AMAb91103	Monoclonal, IgG2a	IHC, WB	APrEST70153
Anti-GPC5	HPA040152	Polyclonal	IHC*	APrEST88353
Anti-HPSE2	HPA044603	Polyclonal	IHC, WB	APrEST80360
Anti-PTPRZ1	HPA015103	Polyclonal	IHC*, WB	APrEST71554
Anti-S100B	AMAb91038	Monoclonal, IgG1	IHC*, WB	APrEST73328
	HPA015768	Polyclonal	IHC*, WB ICC-IF	APrEST73328
Anti-SLC1A2	HPA009172	Polyclonal	IHC*	APrEST71560
	HPA067499	Polyclonal	IHC*	APrEST94963
	HPA037468	Polyclonal	IHC*	APrEST79991
Anti-SLC1A3	HPA037467	Polyclonal	IHC*, ICC-IF	APrEST79992
Anti-SOX9	HPA001758	Polyclonal	IHC*, WB* ICC-IF	APrEST84775
	AMAb9795	Monoclonal, IgG2a	IHC*, WB* ICC-IF	APrEST84775

* Enhanced Validation

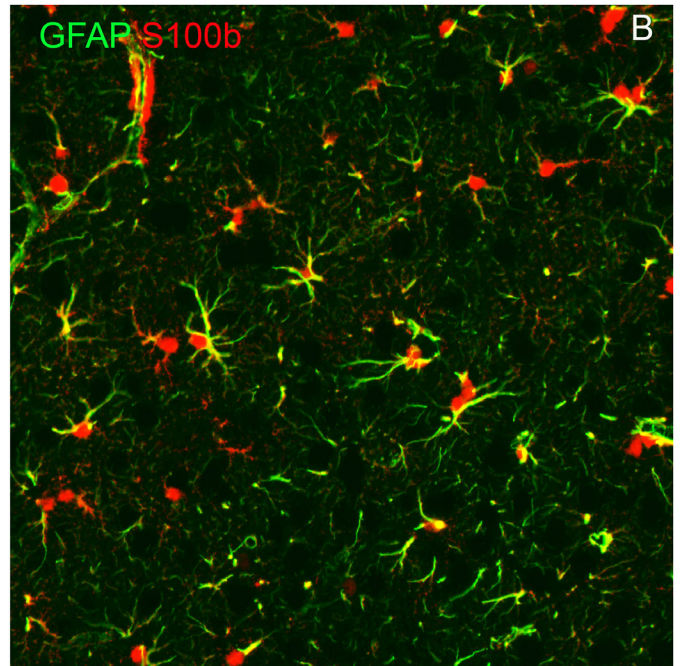
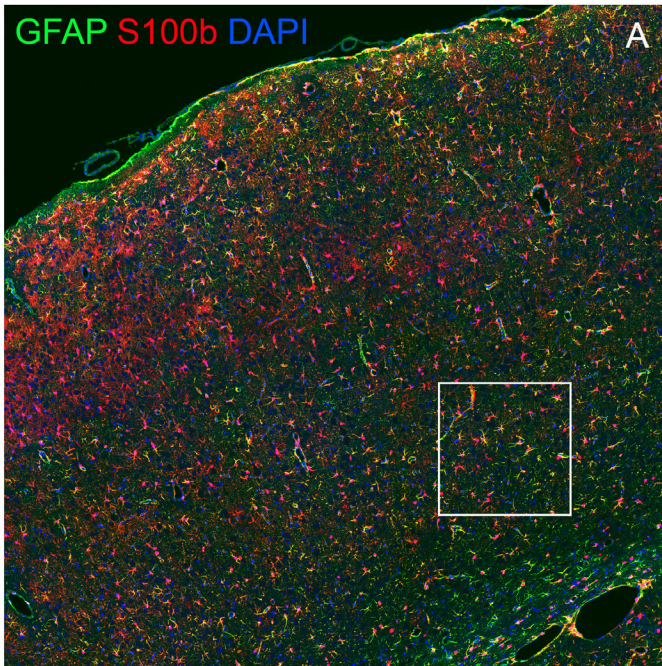


FIGURE 3. ASTROCYTES IN THE CEREBRAL CORTEX

Astrocytes represent the most numerous glial cell population in the cerebral cortex. Similar to neurons, astrocytes express both pan-astrocyte as well as layer-specific markers.

The following antibodies were used for immunostaining: Anti-GFAP (polyclonal HPA056030) and Anti-S100b (monoclonal AMAb91038). DAPI was used as nuclear counterstain.

(A) GFAP and S100b are the two most common markers used to identify the astrocytes (GFAP in green and S100b in red).

(B) The GFAP protein is mostly present in the processes, while S100b also stains the cell body of the astrocyte. S100b expression characterizes mature development stage in telencephalic GFAP-positive astrocytes, as S100b expression leads to loss of neural stem cell properties in the GFAP-positive cells.

TABLE 3. ANTIBODIES TARGETING OLIGODENDROCYTES & SCHWANN CELLS

Product Name	Product ID	Clonality / Isotype	Recommended Applications	PrEST Control Antigen
Anti-ALCAM	HPA010926	Polyclonal	IHC*	APrEST72229
Anti-CNP	AMAb91069	Monoclonal, IgG1	IHC, WB	-
	HPA023266	Polyclonal	IHC*, WB*, ICC-IF	APrEST75815
	HPA023280	Polyclonal	IHC*, WB*, ICC-IF	-
	HPA023278	Polyclonal	IHC*, WB*	APrEST75813
	HPA023338	Polyclonal	IHC*, WB*	APrEST75814
Anti-COL1A2 (#)	HPA059738	Polyclonal	ICC-IF	APrEST92030
Anti-COL9A1	HPA074749	Polyclonal	IHC	APrEST94417
Anti-CRYAB	HPA057100	Polyclonal	IHC*	APrEST88699
	AMAb91661	Monoclonal, IgG1	IHC*, WB, ICC-IF	-
	AMAb91662	Monoclonal, IgG2b	IHC*, WB	-
Anti-CST3 (#)	HPA013143	Polyclonal	IHC*, WB*, ICC-IF	APrEST71715
Anti-GRIK2	HPA014623	Polyclonal	IHC, WB	APrEST72943
Anti-MBP	AMAb91062	Monoclonal, IgG2a	IHC, WB, ICC-IF	APrEST78641
	AMAb91063	Monoclonal, IgG1	IHC, WB, ICC-IF	APrEST78641
	HPA064368	Polyclonal	WB, ICC-IF	APrEST90078
Anti-OPALIN	AMAb91686	Monoclonal, IgG2a	IHC*	APrEST72419
	AMAb91685	Monoclonal, IgG1	IHC*	APrEST72419
Anti-PDGFRα	HPA004947	Polyclonal	ICC-IF	APrEST93915
Anti-PLP1	HPA004128	Polyclonal	IHC*	APrEST74534
Anti-SOX6	HPA001923	Polyclonal	IHC*, ICC-IF	APrEST86388
	AMAb91383	Monoclonal, IgG2b	IHC*, ICC-IF	-
	HPA003908	Polyclonal	IHC*, WB*	APrEST86387
Anti-SOX10	HPA068898	Polyclonal	IHC*, ICC-IF	APrEST92827
	AMAb91297	Monoclonal, IgG1	IHC, ICC-IF	APrEST92827
Anti-STK32A	HPA040236	Polyclonal	IHC	APrEST80471
Anti-TLL1	HPA060767	Polyclonal	WB, ICC-IF	APrEST92104
Anti-VCAN	HPA004726	Polyclonal	IHC	APrEST86780
Anti-VIM (#)	HPA001762	Polyclonal	IHC*, WB*, ICC-IF	APrEST85020

* Enhanced Validation

TABLE 4. ANTIBODIES TARGETING MICROGLIA

Product Name	Product ID	Clonality / Isotype	Recommended Applications	PrEST Control Antigen
Anti-AIF1	AMAb91671	Monoclonal, IgG2b	IHC	
	AMAb91672	Monoclonal, IgG1	IHC	
	HPA062949	Polyclonal	ICC-IF	APrEST94239
	HPA049234	Polyclonal	IHC*	
Anti-CD163	AMAb91646	Monoclonal, IgG1	IHC*, WB	APrEST88760
	AMAb91648	Monoclonal, IgG1	IHC*	APrEST88760
Anti-HLA-DRA	AMAb91675	Monoclonal, IgG2b	IHC*, WB	
	AMAb91673	Monoclonal, IgG1	IHC*, WB	
	AMAb91674	Monoclonal, IgG2a	IHC*, WB	
	HPA050162	Polyclonal	IHC*	APrEST87550
	HPA053176	Polyclonal	IHC*	APrEST87637
Anti-ITGAM	AMAb90911	Monoclonal, IgG1	IHC*, WB	APrEST83070
Anti-PTPRC	HPA000440	Polyclonal	IHC*	APrEST79682
	AMAb90518	Monoclonal, IgG1	IHC*, WB	APrEST79682
Anti-SPP1	AMAb91653	Monoclonal, IgG1	IHC*	APrEST78076
	HPA027541	Polyclonal	IHC*, WB ICC-IF	APrEST78076

TABLE 5. ANTIBODIES TARGETING NEURONS (ALL)

Product Name	Product ID	Clonality / Isotype	Recommended Applications	PrEST Control Antigen
Anti-MAP2	HPA008273	Polyclonal	IHC*, ICC-IF	APrEST71546
Anti-NEFM (NF160)	AMAb91027	Monoclonal, IgG1	IHC, WB	APrEST76207
	AMAb91028	Monoclonal, IgG1	IHC, WB	APrEST76207
	AMAb91029	Monoclonal, IgG2a	IHC, WB	APrEST76207
	AMAb91030	Monoclonal, IgG2b	IHC, WB	APrEST76207
Anti-NEFH (NF200)	AMAb91025	Monoclonal, IgG1	IHC*, WB	APrEST87930
Anti-NEFL (NF68)	AMAb91314	Monoclonal, IgG1	IHC, WB, ICC-IF	APrEST88940
Anti-RBFOX3/NeuN	AMAb91746	Monoclonal, IgG2b	IHC*	
	AMAb91748	Monoclonal, IgG2b	IHC*	
	HPA030790	Polyclonal	IHC*, ICC-IF	APrEST75751
	HPA075862	Polyclonal	IHC, WB	APrEST95337
Anti-UCHL1 (PGP9.5)	AMAb91145	Monoclonal, IgG1	IHC, WB, ICC-IF	APrEST86224

TABLE 6. NEURON-SPECIFIC ANTIBODIES MARKERS

Product Name	Product ID	Clonality / Isotype	Recommended Applications	PrEST Control Antigen
Glutamatergic Neurons				
Anti-GRIA1	HPA035202	Polyclonal	IHC	APrEST78550
Anti-GRIA3	HPA058659	Polyclonal	IHC	APrEST87843
Anti-GRIA3	HPA073344	Polyclonal	IHC*	APrEST95169
Anti-GRIA4	HPA063282	Polyclonal	ICC-IF	APrEST95868
Anti-GRID2	HPA058538	Polyclonal	IHC	APrEST86144
Anti-GRIK1	HPA073879	Polyclonal	ICC-IF	APrEST95808
Anti-GRIK4	HPA074453	Polyclonal	IHC*	APrEST94001
Anti-GRIK5	HPA074001	Polyclonal	ICC-IF	APrEST94403
Anti-GRIN1	HPA067773	Polyclonal	IHC	APrEST94967
Anti-GRIN2B	HPA069762	Polyclonal	IHC	APrEST88269
Anti-SLC17A6	AMAb91086	Monoclonal, IgG1	IHC*	APrEST80507
Anti-SLC17A7	AMAb91041	Monoclonal, IgG2b	IHC*, WB	APrEST88047
	HPA063679	Polyclonal	IHC*	APrEST88047
GABAergic Neurons				
Anti-GAD1	AMAb91076	Monoclonal, IgG2a	IHC, WB	APrEST79051
	AMAb91078	Monoclonal, IgG1	IHC, WB	APrEST79051
	AMAb91079	Monoclonal, IgG2b	IHC, WB	APrEST79051
	HPA048871	Polyclonal	ICC-IF	APrEST94691
	HPA031949	Polyclonal	WB, ICC-IF	APrEST79051
Anti-GAD2	AMAb91048	Monoclonal, IgG1	IHC*, WB	APrEST80257
	HPA044637	Polyclonal	IHC	APrEST80257
Anti-GABBR1	HPA050483	Polyclonal	IHC	APrEST83906
Anti-GABBR2	HPA013820	Polyclonal	IHC*	APrEST72751
Anti-SLC6A1 (GAT1)	AMAb91043	Monoclonal, IgG1	IHC	APrEST83027
Dopaminergic Neurons				
Anti-TH	AMAb91112	Monoclonal, IgG1	IHC	APrEST87899
	HPA014010	Polyclonal	ICC-IF	APrEST94048
	HPA061003	Polyclonal	IHC*	APrEST87899
Anti-SLC6A3 (DAT)	AMAb91125	Monoclonal, IgG1	IHC	APrEST72519
	HPA012763	Polyclonal	IHC	APrEST94046

Product Name	Product ID	Clonality / Isotype	Recommended Applications	PrEST Control Antigen
Cholinergic Neurons				
Anti-CHAT	AMAb91129	Monoclonal, IgG1	IHC*	
	AMAb91130	Monoclonal, IgG2b	IHC*, ICC-IF	
	HPA048547	Polyclonal	IHC	APrEST86792
Anti-CHRNA5	HPA054381	Polyclonal	ICC-IF	APrEST78584
Noradrenergic Neurons				
Anti-DBH	HPA070789	Polyclonal	IHC*, WB, ICC-IF	APrEST90310
	HPA002130	Polyclonal	IHC*	APrEST84519
Anti-SLC6A2 (NET)	HPA076311	Polyclonal	IHC*	APrEST94017
	AMAb91116	Monoclonal, IgG1	IHC	APrEST86811
Serotonergic Neurons				
Anti-TPH2	AMAb91108	Monoclonal, IgG1	IHC	APrEST81951
Anti-SLC6A4 (5-HTT)	HPA074728	Polyclonal	ICC-IF	APrEST93243
Peptidergic Neurons				
Anti-CCK	HPA069515	Polyclonal	IHC*	APrEST91020
Anti-NPY	HPA044572	Polyclonal	IHC, ICC-IF	APrEST79629
	HPA056798	Polyclonal	IHC, ICC-IF	APrEST87772
Anti-SST	HPA019472	Polyclonal	IHC*	APrEST74709
Anti-VIP	HPA017324	Polyclonal	IHC	APrEST72428
	HPA072701	Polyclonal	ICC-IF	APrEST93097
Other Markers (Various Neuron Types)				
Anti-CALB1	HPA056734, AMAb92005 AMAb92006, AMAb92008	Often used for subpopulations of GABAergic and glutamatergic neurons.		
Anti-CALB2 (Calretinin)	AMAb91812, AMAb91813 AMAb91814	Marks specific interneuron subtypes.		
Anti-PVALB	HPA048536, AMAb92010 AMAb92011, AMAb92012	Common in a subtype of GABAergic interneurons.		
Anti-TBR1	HPA078644, HPA078657	Associated with glutamatergic neurons, particularly in cortical development.		
Anti-SLC18A2 (VMAT2)	HPA073224, AMAb91837 AMAb91838	Expressed in various monoaminergic neurons (dopaminergic, noradrenergic, serotonergic).		
Anti-SST	AMAb92013, AMAb92014	Expressed in a subset of GABAergic neurons and is known as a protein marker of inhibitory interneurons.		

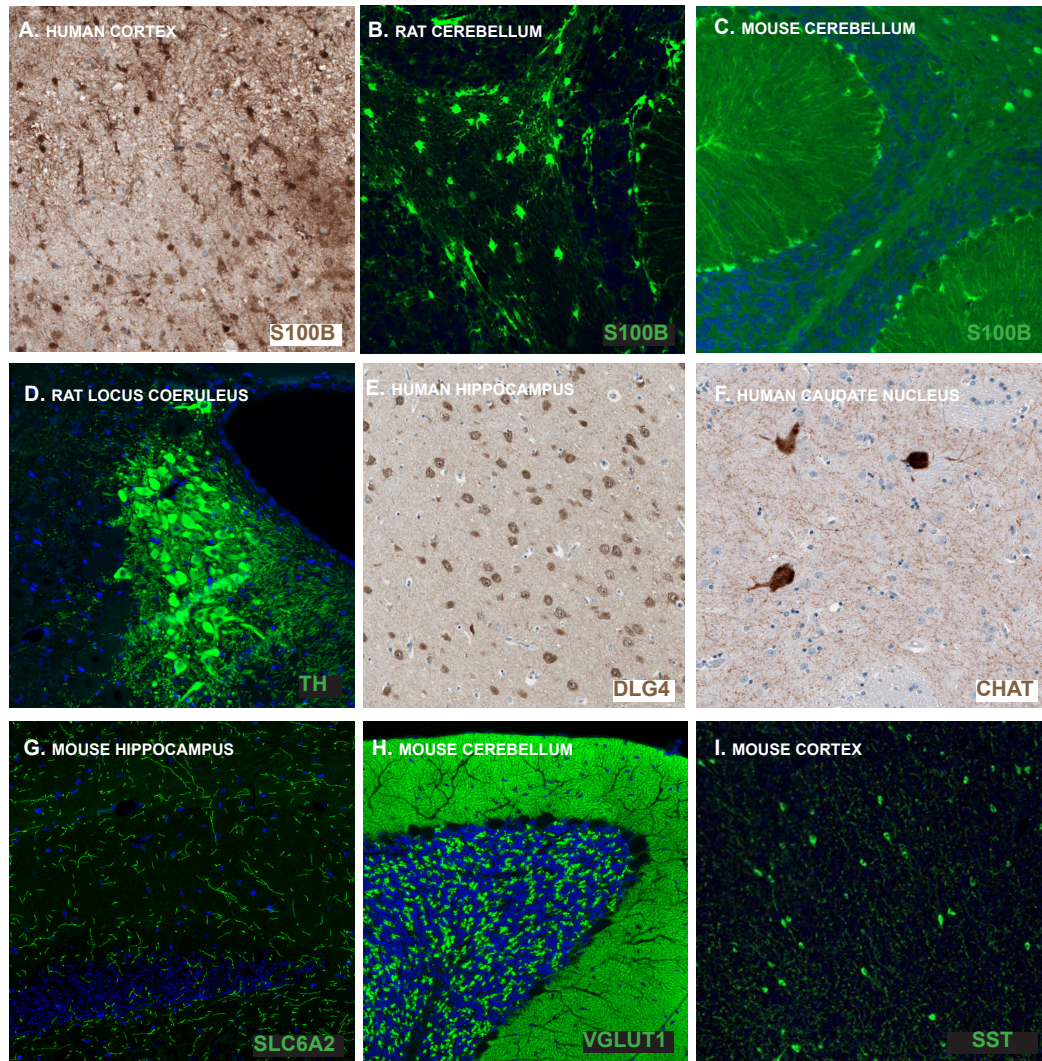


FIGURE 4.
IHC AND ICC-IF STAINING ON HUMAN AND RODENT BRAINS

(A) IHC staining of glial cells using the anti-S100B monoclonal antibody (IgG1, AMAb91038) on human cerebral cortex shows strong positivity in astrocytes.

(B,C) The immunofluorescence staining of rat (B) and mouse (C) cerebellum using the anti-S100B monoclonal antibody (AMAb91038) shows strong positivity in glial cells.

(D) Immunofluorescence staining of rat locus coeruleus using the monoclonal **anti-TH** antibody (IgG1, AMAb91112) shows strong positivity in noradrenergic neurons.

(E) IHC staining using the anti-DLG4 polyclonal antibody (HPA010122) shows high expression in human hippocampal neurons.

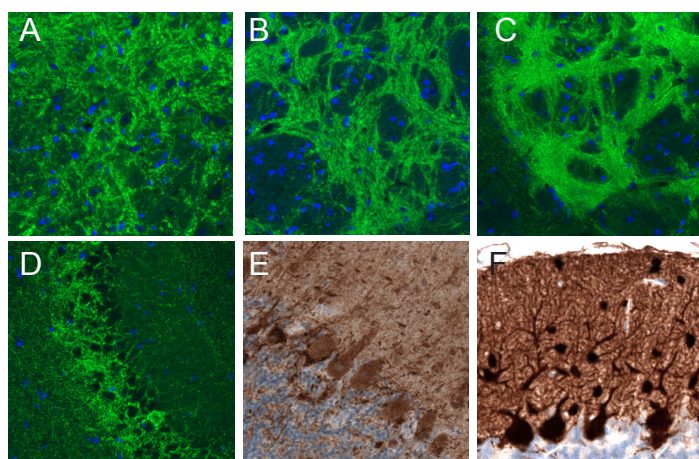
(F) IHC staining of human caudate nucleus using the anti-CHAT polyclonal antibody (HPA048547) shows strong cytoplasmic positivity in large cholinergic neurons.

(G) IHC staining of the mouse hippocampus using the anti-SLC6A2/NET monoclonal antibody (IgG1, AMAb91116) showing strong positivity in noradrenergic fibers.

(H) IHC staining of mouse cerebellum using the anti-VGLUT1 monoclonal antibody (IgG1, AMAb91041) showing strong positivity in glutamatergic fibers in the molecular and granular layers.

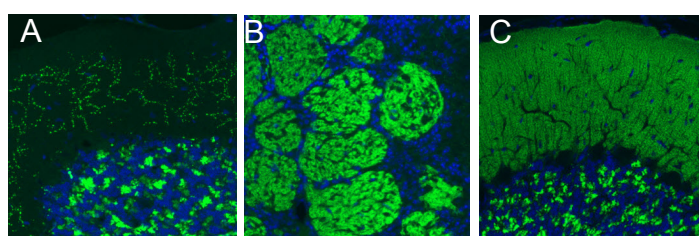
(I) IHC staining of mouse brain using the anti-SST polyclonal antibody (HPA019472) showing strong positivity in a subset of neurons in the cerebral cortex.

FIGURE 5. NEURON-SPECIFIC MARKERS



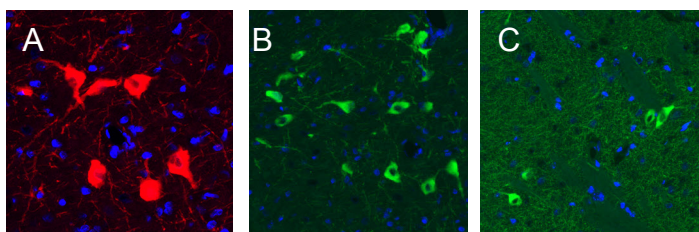
GABAergic neurons

- (A) GABAergic fibers in the rat basal forebrain. Anti-GAD2 (AMAb91048)
- (B) GABAergic fibers in the rat globus pallidus. Anti-VGAT (AMAb91043)
- (C) GABAergic fibers in the rat globus pallidus. Anti-GAD1 (AMAb91076)
- (D) GABAergic fibers in the mouse hippocampus. Anti-GAD1 (AMAb91078)
- (E) Purkinje cells in the human cerebellum. Anti-GAD1 (AMAb91079)
- (F) Purkinje cells and cells in the molecular layer of the human cerebellum. Anti-PVALB (AMAb92010)



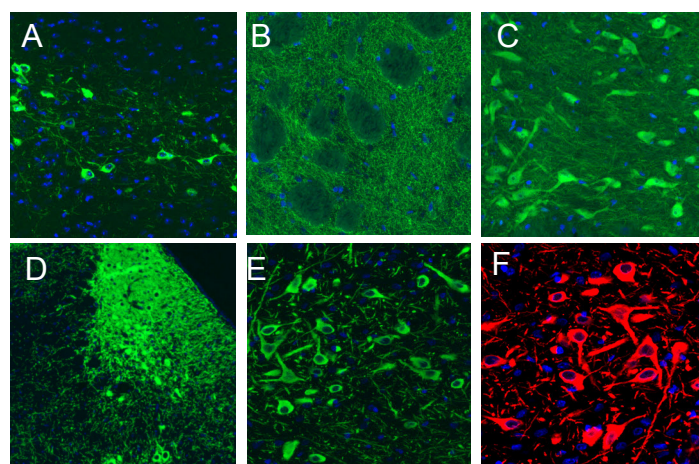
Glutamatergic neurons

- (A) Glutamatergic fibers in the rat cerebellum. Anti-VGluT2 (AMAb91081)
- (B) Glomeruli in the mouse olfactory bulb. Anti-VGluT2 (AMAb91086)
- (C) Glutamatergic processes in the rat cerebellum. Anti-VGluT1 (AMAb91041) -



Cholinergic neurons

- (A) Rat basal forebrain. Anti-CHAT (AMAb91129)
- (B) Rat basal forebrain. Anti-CHAT (AMAb91129)
- (C) Mouse striatum. Anti-CHAT (AMAb91130)



Monoaminergic neurons

- (A) Monoamine neurons in the mouse paraventricular nucleus. Anti-TH (AMAb91112)
- (B) Dopaminergic fibers in the rat striatum. Anti-DAT (AMAb91125)
- (C) Dopaminergic neurons in the rat substantia nigra. Anti-DDC (AMAb91089)
- (D) Noradrenaline neurons in the rat locus coeruleus. Anti-NET (AMAb91116)
- (E) Serotonergic neurons in the rat dorsal raphe. Anti-TPH2 (AMAb91108)
- (F) Serotonergic neurons in the rat dorsal raphe. TPH2 (AMAb91108)

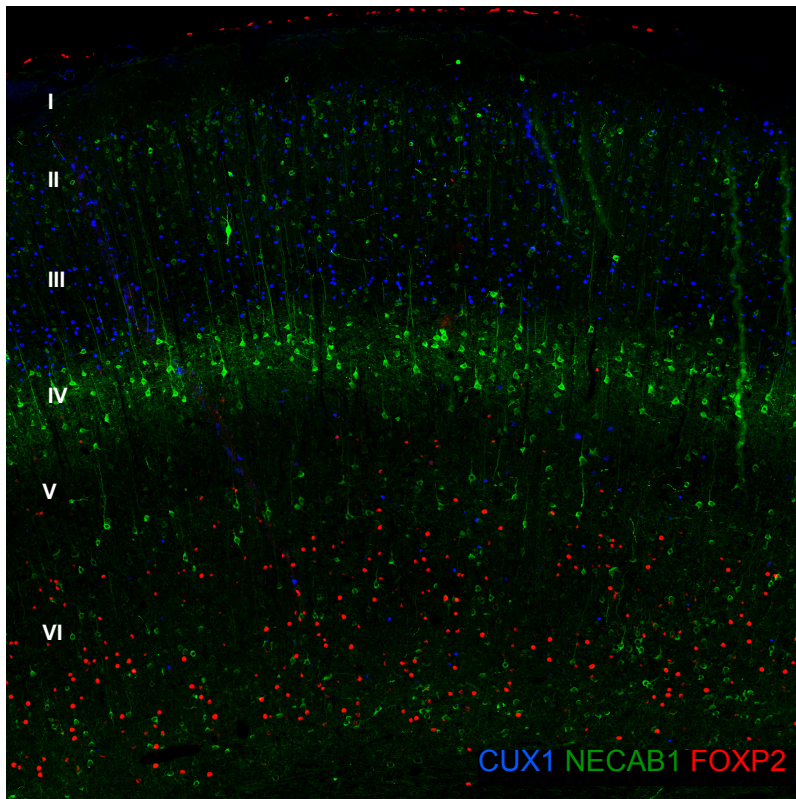


FIGURE 6. LAMINAR STRUCTURE OF THE CEREBRAL CORTEX

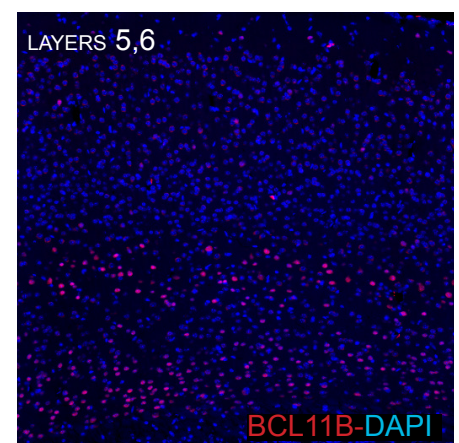
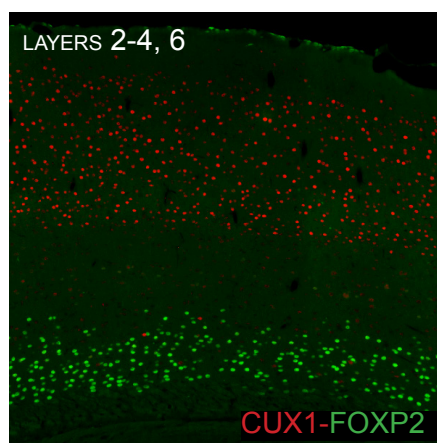
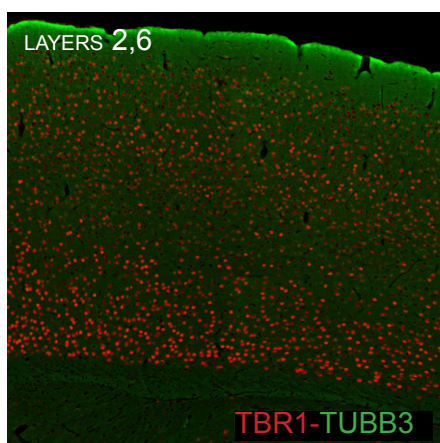
The cerebral cortex is organized into distinct layers, each with specific functions and cellular compositions.

A number of proteins show layer-specific expression and can be used as markers for the distinct cortical layers also across the species.

Thus, CUX1 is present in layers 2-3; BCL11B is expressed in layer 5, while FOXP2 is expressed in layer 6. These markers, combined with DAPI staining for nuclei, provide a detailed map of the cortical laminar structure.

Laminar structure of rat cerebral cortex.

Multiplexed immunofluorescence of the rat cerebral cortex using the Anti-CUX1 (HPA003277, blue), the Anti-NECAB1 (AMAb90800, green), and the Anti-FOXP2 (AMAb91362, red) antibodies. Note that CUX1 is mainly expressed in layers II-III, NECAB1 shows the most robust expression in layer IV neurons, while FOXP2 is primarily present in layer VI neurons.



Immunofluorescence staining with cortical layer markers in the adult mouse brain.

(Left) Cortical layers 2,6: the anti-TUBB3 monoclonal antibody (IgG1, AMAb91394) is visible in green; the anti-TBR1 polyclonal (HPA078644) is visible in red.

(Middle) Cortical layers 2-4 and 6: the anti-FOXP2 monoclonal antibody (IgG1, AMAb91361) is visible in green; the anti-CUX1 polyclonal (HPA003277) is visible in red.

(Right) Cortical layers 5-6: the anti-BCL11B (CTIP2) polyclonal antibody (HPA049117) is visible in red. Nuclei are stained in blue with DAPI.

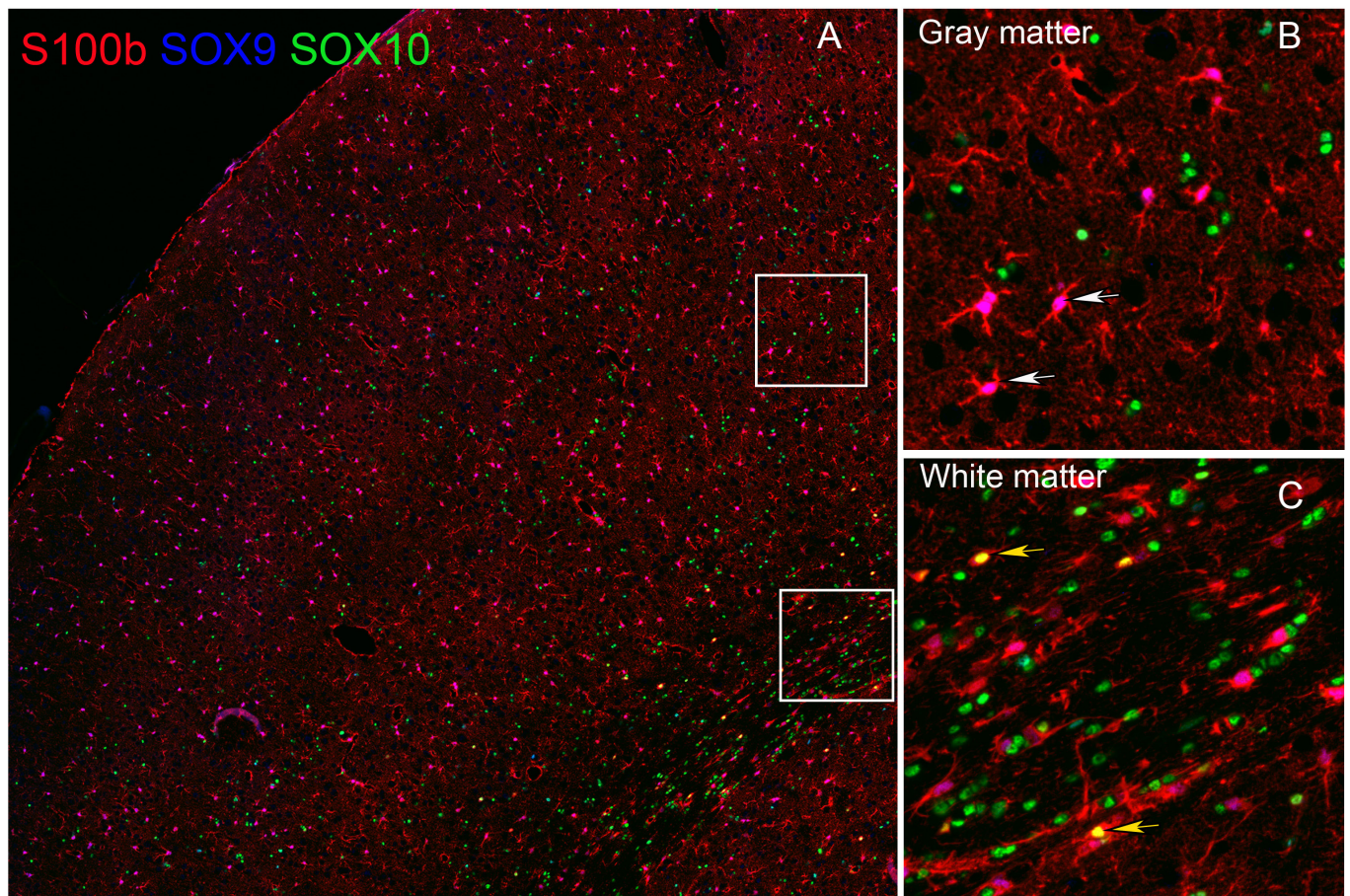


FIGURE 7. SOX PROTEINS EXPRESSION IN CORTICAL ASTROCYTES AND OLIGODENDROCYTES

Two distinct populations of glial cells can be identified by nuclear expression of SOX proteins: SOX9 marks astrocytes, while SOX10 is present in oligodendrocyte nuclei.

SOX9 is a marker for astrocytes, the most abundant glial cell type in the brain. Astrocytes play crucial roles in maintaining the extracellular environment, regulating blood flow, supporting synaptic function, and responding to injury. The expression of SOX9 in their nuclei is essential for the development and maintenance of astrocytic identity and function.

On the other hand, SOX10 is specifically expressed in the nuclei of oligodendrocytes, the glial cells responsible for the formation and maintenance of myelin sheaths around axons in the central nervous system. SOX10 is critical for the differentiation of oligodendrocyte precursor cells into mature oligodendrocytes and for the regulation of genes involved in myelination.

The distinct expression of SOX9 and SOX10 in astrocytes and oligodendrocytes, respectively, underscores their different roles in the brain's cellular

architecture and function, and allows researchers to identify and study these glial populations with precision.

(A) The image shows the distribution of glial cells in the rat cerebral cortex. SOX9 (blue) and SOX10 (green) were used in combination with astrocytic marker S100b (red).

(B) The majority of the S100b-expressing cells are astrocytes, as confirmed by nuclear SOX9 co-expression (indicated by white arrows on magenta nuclei, mostly present in the gray matter of the cerebral cortex).

(C) However, a smaller population of SOX10-positive cells, which are mostly found in the white matter, co-express S100b (indicated by yellow arrows on yellow nuclei) suggesting oligodendrocyte lineage. In fact, although S100b is mainly used as a mature astrocyte marker, it has been shown to be involved in differentiation/maturation of oligodendrocytes, as well as suggested as therapeutic target in multiple sclerosis, the disease characterized by neuroinflammation and demyelination.

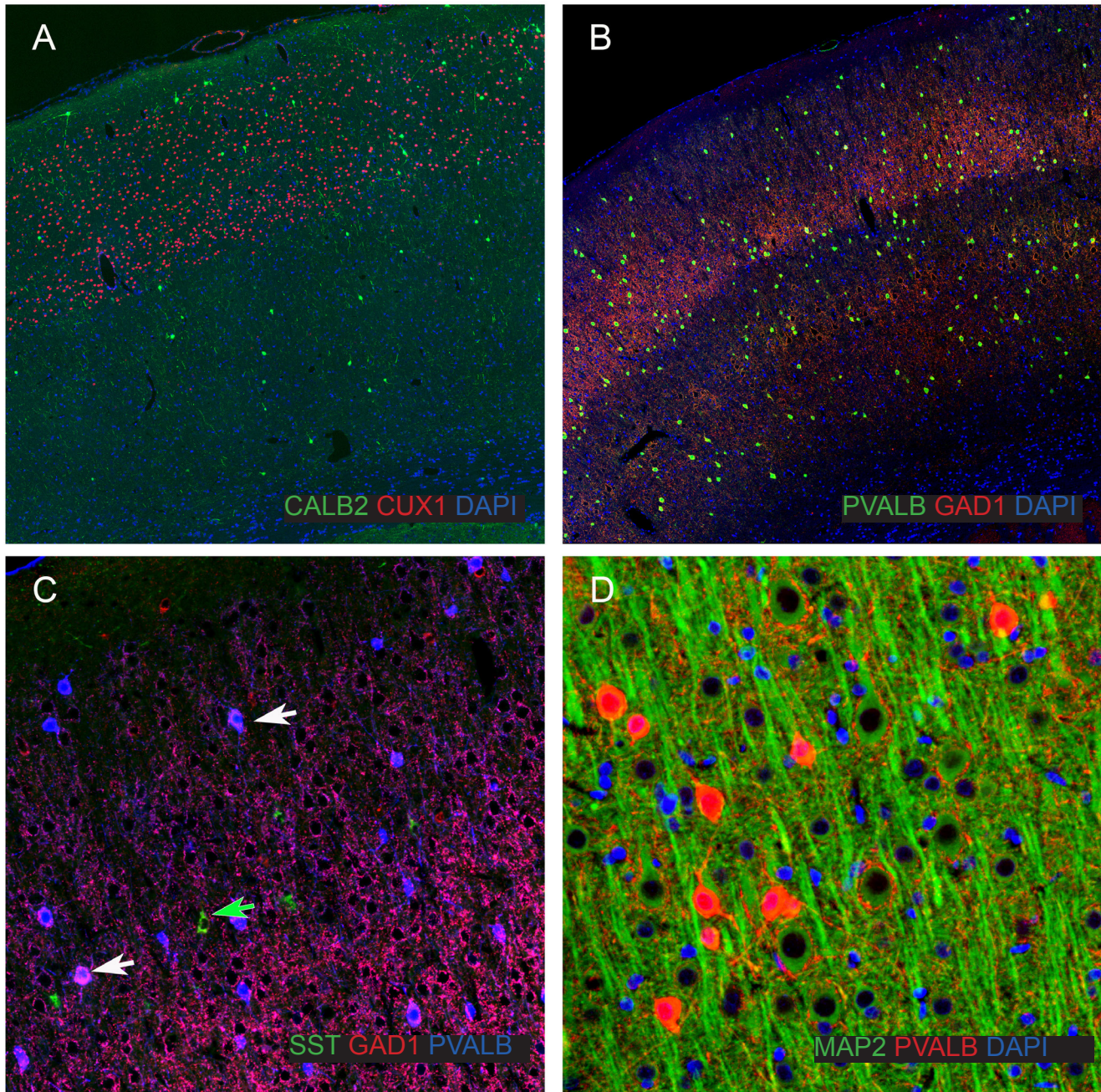


FIGURE 8. INHIBITORY CORTICAL NEURONS

Cerebral cortex is composed of various types of excitatory and inhibitory neurons and glial cells arranged in layers. The different classes of inhibitory neurons can be identified by the expression of various types of calcium binding proteins and neuropeptides. The immunofluorescence IHC images of rat cerebral cortex show distribution of different inhibitory interneurons, including CALB2 (A) and PVALB (B-D) positive neurons within cortical layers.

(A) The CALB2-positive interneurons (green) represent a smaller population of interneurons evenly distributed between upper and lower cortical layers (note the layers 2-4 indicated by expression of CUX1 (red)).

(B) The PVALB-positive neurons are the most numerous inhibitory interneurons in the cortex. Note co-expression with GABA (white arrows in C), while neuropeptide SST-positive neurons represent a different distinct population of interneurons (green arrow in C).

(D) The PVALB-positive neurons (red) make numerous synaptic contacts with pyramidal neuronal cell bodies, labelled here by MAP2 (green).

The following antibodies were used for immunostaining: Anti-CALB2 (monoclonal AMAb91813), Anti-CUX1 (polyclonal HPA003277), Anti-PVALB (monoclonal AMAb92011), Anti-GAD1 (monoclonal AMAb91079), Anti-SST (polyclonal HPA019472) Anti-MAP2 (polyclonal HPA012828). DAPI was used for nuclear counterstain.

3. UNDERSTANDING BRAIN DISEASES

VARIATION FROM BASELINE

Understanding baseline deviations and changes in protein levels in the brain aid in early detection, precise diagnosis, and monitoring disease progression. This approach not only enhances patient outcomes but also advances the development of targeted therapies and biomarkers, paving the way for better management of brain health. The following are examples of how brain proteins are altered in diseases compared to a healthy brain.

Astrocytes:

GFAP in neuroinflammation and gliomas

In healthy brain, GFAP is expressed at relatively low levels, mainly serving as a marker for mature astrocytes.

Under neuroinflammatory conditions, such as multiple sclerosis and traumatic brain injury, GFAP levels are significantly elevated. Specifically, the expression of GFAP increases (number of astrocytes and the intensity of their staining), indicating astrocyte hypertrophy and proliferation. This upregulation is part of the gliosis process, where astrocytes become reactive in response to central nervous system injury or disease, aiming to form a protective scar that isolates damaged tissue but can also contribute to inflammation and secondary injury.

Azzolini F, et al., Neuroinflammation Is Associated with GFAP and sTREM2 Levels in Multiple Sclerosis. Biomolecules. 2022 Jan 27;12(2):222.

In glioma, particularly in astrocytomas, GFAP expression is often altered. GFAP levels can be upregulated due to the reactive astrogliosis that occurs in response to the tumor environment. However, in higher-grade gliomas, such as glioblastoma, GFAP expression can become heterogeneous, with some tumor cells losing GFAP expression as they become more undifferentiated and aggressive. However, if this loss of GFAP is associated with a more invasive and malignant phenotype and a more aggressive state is still matter of debate.

Wilhelmsson U, Eliasson C, Bjerkvig R, Pekny M. Loss of GFAP expression in high-grade astrocytomas does not contribute to tumor development or progression. Oncogene. 2003 May 29;22(22):3407

Oligodendrocytes:

MBP in Multiple Sclerosis

In multiple sclerosis, the immune system mistakenly attacks the myelin sheath, the protective covering around nerve fibers in the central nervous system. This autoimmune response leads to the destruction of myelin, known as demyelination.

As a result, myelin basic protein (MBP), a major component of the myelin sheath, is released into the surrounding tissue and cerebrospinal fluid. MBP levels are often reduced in the areas where demyelination has occurred due to the loss of myelin integrity. Concurrently, elevated levels of MBP can be detected in the cerebrospinal fluid, reflecting ongoing myelin breakdown and serving as a biomarker for disease activity and severity in MS patients.

Lucchinetti C, et al., Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol. 2000 Jun;47(6):707-17.

Neurons:

β -Amyloid in Alzheimer's Disease and α -Synuclein in Parkinson's Disease

In a healthy brain, amyloid precursor protein (APP) typically expressed in neurons, is cleaved by α -secretase, which does not produce beta-amyloid, while only a small amount follows a pathway involving β -secretase and γ -secretase, leading to the production of beta-amyloid. This small amount of beta-amyloid is usually cleared efficiently.

However, in Alzheimer's disease and other neurodegenerative conditions, the processing of APP shifts towards increased production of beta-amyloid. This leads to the accumulation and aggregation of beta-amyloid peptides, particularly the A β 42 form, which forms plaques in the brain. These plaques, predominantly found in regions like the hippocampus and cerebral cortex disrupt cellular communication, induces inflammation, and contributes to neurodegeneration.

Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science. 2002 Jul 19;297(5580):353-6

α -Synuclein is primarily expressed in neurons, particularly in the presynaptic terminals. However, it can also be found at lower levels in other cell types, including glial cells such as astrocytes and oligodendrocytes, though its role in these cells is less well understood.

In Parkinson's disease, the protein α -synuclein misfolds and aggregates into insoluble fibrils, forming intracellular inclusions known as Lewy bodies. These Lewy bodies are predominantly found within neurons of the substantia nigra, a region critical for dopamine production. The aggregation of α -synuclein disrupts cellular processes, including mitochondrial function and proteasomal degradation, leading to neuronal cell death. In healthy brains, α -synuclein is present at much lower levels and does not form these pathological aggregates, thus maintaining normal neuronal function.

Spillantini MG, et al., Alpha-synuclein in Lewy bodies. Nature. 1997 Aug 28;388(6645):839-40

TABLE 7.
COMMON PROTEIN MARKERS ALTERED IN NEUROLOGICAL DISORDERS

Disease Category	Marker	Description
Neuroinflammation	GFAP	Marker for astrocytes, often upregulated during inflammation.
	IBA1	Microglial marker, indicative of microglial activation.
	CD68	Marker for activated microglia and macrophages.
	TNF- α	A pro-inflammatory cytokine often elevated in neuroinflammatory conditions.
	IL-1 β	Pro-inflammatory cytokine involved in the neuroinflammatory response.
	MHC Class II	Expressed on microglia and macrophages, associated with immune response activation.
Neurodegeneration - Alzheimer's Disease (AD)	β -Amyloid	Key protein involved in plaque formation in AD brains.
	Tau Protein	Hyperphosphorylated tau forms neurofibrillary tangles, a hallmark of AD.
	APP	Precursor molecule for β -amyloid; alterations in processing are involved in AD.
	BACE1	Enzyme involved in the generation of β -amyloid.
	PSEN1	Component of the γ -secretase complex involved in APP processing.
	APOE4	Genetic risk factor for AD, associated with amyloid deposition.
Neurodegeneration - Parkinson's Disease (PD)	α -Synuclein	Protein that forms Lewy bodies, characteristic of PD.
	Parkin	Protein involved in ubiquitin-proteasome pathways; mutations linked to familial PD.
	DJ-1	Protein associated with oxidative stress response; mutations linked to PD.
	LRRK2	Mutations are a common cause of familial PD.
	PINK1	Involved in mitochondrial function; associated with PD.
	TH	Rate-limiting enzyme in dopamine synthesis; used to identify dopaminergic neurons.
Multiple Sclerosis (MS)	MBP	Key protein in the myelin sheath, often targeted in MS.
	MOG	Myelin protein targeted by the immune system in MS.
	GFAP	Relevant in MS for indicating astrocytic response.
	CD3	T-cell marker reflecting the involvement of T-cells in MS pathology.
	IL-17	Cytokine produced by Th17 cells, involved in MS pathogenesis.
Gliomas	GFAP	Marker for astrocytic lineage, often used to identify astrocytomas.
	IDH1	Mutations common in lower-grade gliomas and secondary glioblastomas.
	MGMT	Promoter methylation status predictive of response to alkylating agents in glioblastoma.
	EGFR	Often amplified or mutated in glioblastoma.
	p53	Tumor suppressor protein; mutations are common in various gliomas.
	Ki-67	Proliferation marker used to gauge tumor growth rate.

TABLE 8.
ANTIBODIES FOR NEURODEGENERATIVE DISEASES

Product Name	Product ID	Clonality / Isotype	Recommended Applications	PrEST Control Antigen
Anti-APOE	HPA065539	Polyclonal	IHC*, WB, ICC-IF	APrEST88578
Anti-APP	HPA001462	Polyclonal	IHC*, WB	APrEST77493
Anti-CDK5	HPA064535	Polyclonal	IHC*, ICC-IF	APrEST92463
Anti-CLU	HPA000572	Polyclonal	IHC*	APrEST76162
Anti-CREB1	HPA019150	Polyclonal	IHC, WB, ICC-IF	APrEST86544
Anti-GAPDH	AMAb91153	Monoclonal, IgG2a	IHC, WB*, ICC-IF	APrEST81012
	HPA061280	Polyclonal	WB*, ICC-IF	APrEST89929
Anti-GRN	AMAb91384	Monoclonal, IgG1	IHC	APrEST86779
	AMAb91385	Monoclonal, IgG1	IHC, WB	APrEST86779
Anti-GSK3B	HPA028017	Polyclonal	IHC, WB*, ICC-IF	APrEST78016
Anti-HDAC6	HPA003714	Polyclonal	IHC	APrEST74347
	HPA026321	Polyclonal	IHC, ICC-IF	APrEST74348
Anti-MAPT	HPA069524	Polyclonal	IHC	APrEST88626
	HPA069570	Polyclonal	IHC*	APrEST88865
Anti-NOS1	HPA069509	Polyclonal	IHC	APrEST95018
Anti-NRGN	HPA038171	Polyclonal	IHC*	APrEST80662
Anti-NTRK1	HPA035799	Polyclonal	IHC*	APrEST87140
Anti-PARK2	HPA036012	Polyclonal	IHC, ICC-IF	APrEST94088
Anti-PARK7	HPA004190	Polyclonal	IHC, WB	APrEST86823
Anti-PSEN1	HPA030760	Polyclonal	IHC	APrEST70651
	HPA067496	Polyclonal	ICC-IF	APrEST92733
Anti-SNCA	HPA005459	Polyclonal	IHC*, WB*	APrEST86754

* Enhanced Validation

TABLE 9.
ANTIBODIES FOR NEUROINFLAMMATION

Product Name	Product ID	Clonality / Isotype	Recommended Applications	PrEST Control Antigen
Anti-AGER	AMAb91635	Monoclonal, IgG1	IHC*	
Anti-AGER	AMAb91634	Monoclonal, IgG2a	IHC*	
Anti-GAP43	AMAb91664	Monoclonal, IgG2b	IHC*	
Anti-GAP43	AMAb91665	Monoclonal, IgG1	IHC*	
Anti-GAP43	AMAb91666	Monoclonal, IgG2a	IHC*	
Anti-GAP43	HPA015600	Polyclonal	IHC*, ICC-IF	APrEST72530
Anti-GZMB	AMAb91650	Monoclonal, IgG1	IHC*	APrEST70654
Anti-IL17A	AMAb91615	Monoclonal, IgG1	IHC	
Anti-IL17RA	AMAb91617	Monoclonal, IgG2b	WB	
Anti-IL17RA	AMAb91619	Monoclonal, IgG1	WB	
Anti-ITGA4	HPA074961	Polyclonal	ICC-IF	APrEST92427
Anti-ITGA4	AMAb91699	Monoclonal, IgG2b	WB	
Anti-MS4A1	HPA014391	Polyclonal	IHC*, WB*	APrEST73137
Anti-P2RX4	HPA039494	Polyclonal	IHC*, WB, ICC-IF	APrEST81062
Anti-P2RX7	AMAb91714	Monoclonal, IgG2a	IHC, WB	
Anti-PLP1	AMAb91639	Monoclonal, IgG1	IHC*	APrEST74534
Anti-S100A8	HPA024372	Polyclonal	IHC*, WB, ICC-IF	APrEST70709
Anti-S100A8	AMAb91689	Monoclonal, IgG1	IHC*	APrEST70709
Anti-S100A9	AMAb91690	Monoclonal, IgG2a	IHC*	APrEST86252
Anti-SORT1	AMAb91428	Monoclonal, IgG1	IHC*, WB*	APrEST70149
Anti-TCF7L2	AMAb91716	Monoclonal, IgG1	IHC, WB	
Anti-TLR2	AMAb91631	Monoclonal, IgG1	WB	
Anti-TREM1	AMAb91459	Monoclonal, IgG1	WB*	
Anti-TSPO	AMAb91854	Monoclonal, IgG2a	IHC*, WB*, ICC-IF	
Anti-TSPO	AMAb91853	Monoclonal, IgG2b	IHC*, WB*, ICC-IF	

* Enhanced Validation

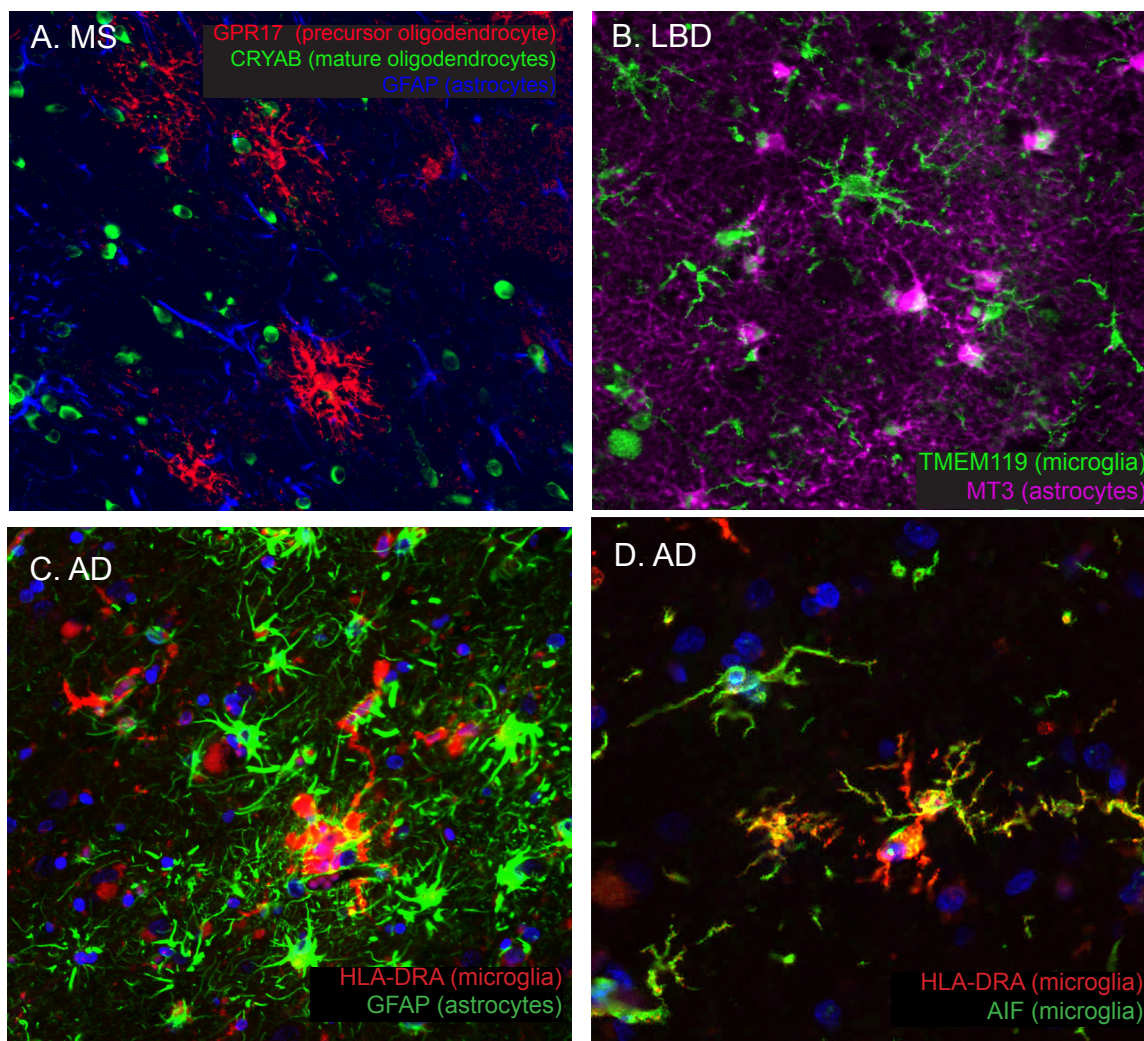


FIGURE 9.
GLIAL MARKERS IN NEUROINFLAMMATION AND NEURODEGENERATIVE DISEASES

(A) Multiple Sclerosis

Demyelination, the pathological process affecting myelinated nerve fibers and oligodendrocytes, is the primary pathological event in multiple sclerosis. The image shows the multiplexed immunofluorescence staining of rat brain (EAE, experimental autoimmune encephalomyelitis model of multiple sclerosis) using Anti-CRYAB antibody (AMAb91661, green) as marker for mature oligodendrocytes, Anti-GPR17 antibody (HPA029766, red) as marker for oligodendrocyte precursor cells, and anti-GFAP antibody (AMAb91033, blue) as marker for astrocytes.

(B) Lewy Body Dementia (LBD)

Lewy body dementia (LBD) is one of the most common neurodegenerative illnesses. The image shows a multiplexed immunofluorescence staining of astrocytes and microglia in the cerebral cortex of Lewy body dementia patient, using Anti-MT3 polyclonal antibody (HPA004011, magenta) as astrocyte marker and Anti-TMEM119 monoclonal antibody (AMAb91528, green) as microglia marker.

(C) Alzheimer's Disease

The interaction between astrocytes and reactive microglia is critical in the development of neuroinflammation and neurodegeneration. The image shows multiplexed immunofluorescence staining of human cerebral cortex from the Alzheimer's disease patient using Anti-GFAP antibody (AMAb91033, green) as astrocyte marker and Anti-HLA-DRA antibody (AMAb91674, red) as microglia marker. Nuclei counterstained by DAPI, in blue.

(D) Alzheimer's Disease

Multiplexed immunofluorescence staining of human cerebral cortex from the Alzheimer's disease patient using Anti-AIF antibody (HPA049234, green) and Anti-HLA-DRA antibody (AMAb91647, red) as different types of microglia markers. Nuclei are counterstained by DAPI, in blue.

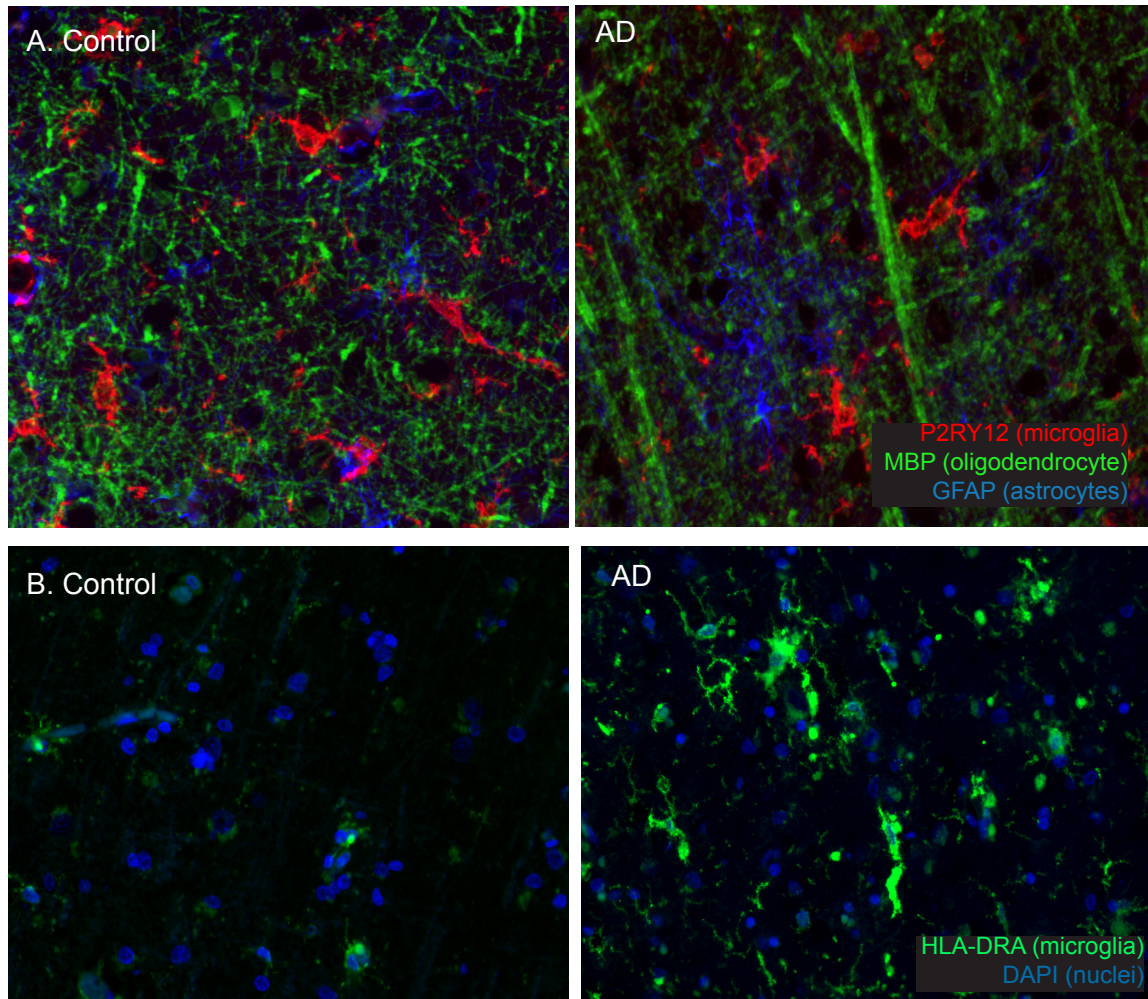


FIGURE 10. GLIAL MARKERS IN ALZHEIMER'S DISEASE

(A) Myelinated processes

Multiplexed ICC-IF staining of human cerebral cortex from control (left) and Alzheimer's disease patient (right). Microglial cells are visible in red (Anti-P2RY12, HPA014518), astrocytes in blue (Anti-GFAP, AMAb91033), and myelinated processes in green (Anti-MBP, AMAb91062).

(B) Activated microglia

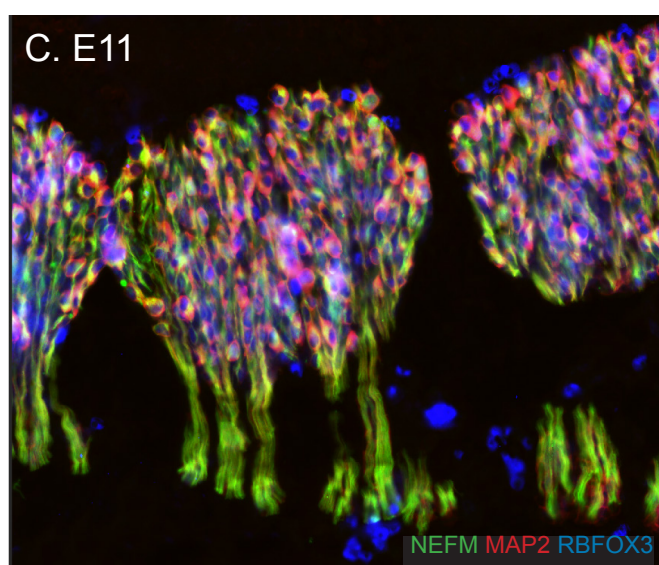
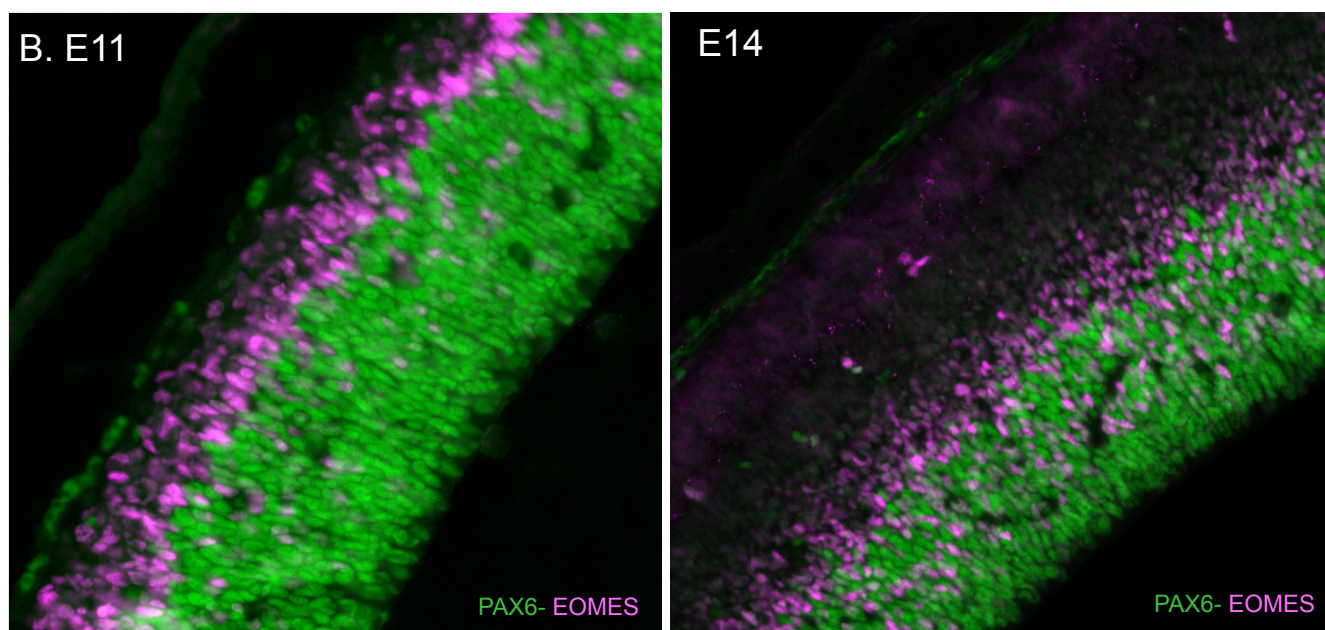
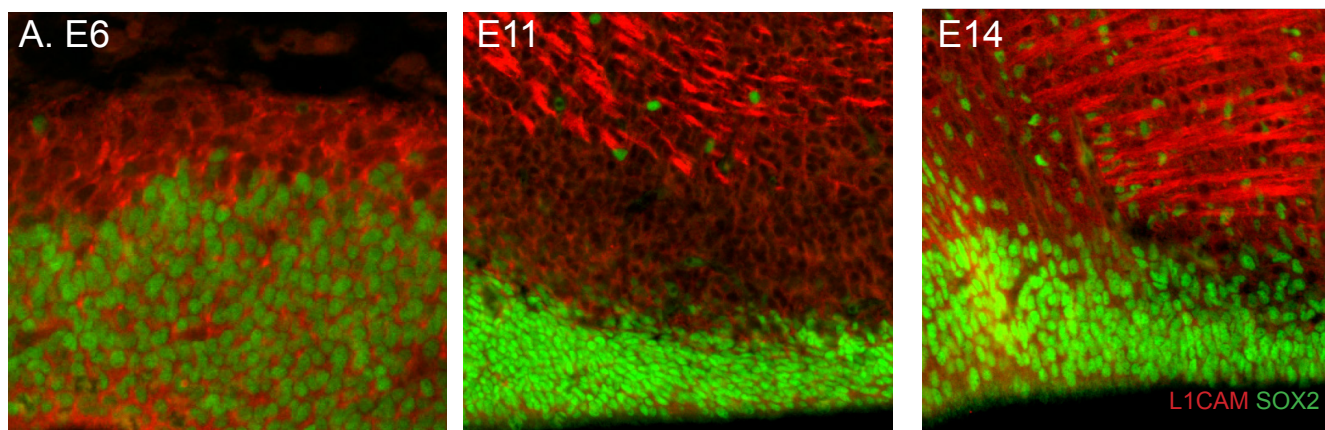
Immunofluorescence staining of human cerebral cortex from control (left) and Alzheimer's disease patient (right) using the Anti-HLA-DRA antibody (AMAb91674) showing a strong positivity in microglial cells (in green). Nuclei counterstained by DAPI, in blue.

TABLE 10.
ANTIBODIES FOR NEURODEVELOPMENTAL PROCESSES

Product Name	Product ID	Clonality / Isotype	Recommended Applications	PrEST Control Antigen
Anti-ATL3	HPA076616	Polyclonal	ICC-IF	APrEST94448
Anti-AUTS2	AMAb91455	Monoclonal, IgG1	IHC, ICC-IF	
Anti-BDNF	HPA056104	Polyclonal	WB, ICC-IF	APrEST89766
Anti-BMI1	HPA030472	Polyclonal	IHC, WB*	APrEST77904
Anti-DCX	HPA036121	Polyclonal	ICC-IF	APrEST90823
Anti-EOMES	HPA028896	Polyclonal	IHC	APrEST86712
Anti-FMR1	HPA050118	Polyclonal	IHC*, ICC-IF	APrEST87548
Anti-FMR1	HPA056084	Polyclonal	IHC*	APrEST87746
Anti-FOS	AMAb91417	Monoclonal, IgG1	WB	
Anti-FOXP2	HPA001679	Polyclonal	ICC-IF	APrEST77857
Anti-LMX1B	HPA073716	Polyclonal	ICC-IF	APrEST93163
Anti-NCAM1	AMAb91807	Monoclonal, IgG2b	IHC*	
Anti-NGFR	HPA004765	Polyclonal	IHC*, WB, ICC-IF	APrEST70014
Anti-NKX2-2	AMAb91708	Monoclonal, IgG1	IHC*, WB	APrEST84761
Anti-NR4A2	HPA000543	Polyclonal	IHC, ICC-IF	APrEST76146
Anti-NTRK2	HPA074873	Polyclonal	IHC*	APrEST95268
Anti-PCP2	HPA057428	Polyclonal	ICC-IF	APrEST91814
Anti-POU3F2	AMAb91406	Monoclonal, IgG1	IHC, WB*, ICC-IF	APrEST91088
Anti-POU3F2	HPA056261	Polyclonal	ICC-IF	APrEST91088
Anti-POU3F2	AMAb91407	Monoclonal, IgG1	IHC, ICC-IF	APrEST91088
Anti-PROM1	HPA004922	Polyclonal	IHC*, WB*	APrEST93420
Anti-RBFOX3	AMAb91748	Monoclonal, IgG2b	IHC*	
Anti-RELN	HPA077891	Polyclonal	ICC-IF	APrEST95491
Anti-SOX6	AMAb91382	Monoclonal, IgG1	WB	
Anti-SOX21	AMAb91311	Monoclonal, IgG2a	IHC, WB, ICC-IF	
Anti-SOX21	AMAb91309	Monoclonal, IgG1	IHC, WB	
Anti-SOX21	HPA064084	Polyclonal	ICC-IF	APrEST92430
Anti-SOX21	HPA048337	Polyclonal	IHC	APrEST92430

* Enhanced Validation

FIGURE 11. DEVELOPING MOUSE CORTEX



(C) ICC-IF staining of neurons in the developing dorsal root ganglia of the mouse embryo E11.

The **anti-NEFM** monoclonal antibody (IgG1, AMAb91027) is visible in green; the **anti-MAP2** polyclonal (HPA012828) is visible in red, and the **anti-RBFOX3** polyclonal (IgG2b, AMAb91748) in blue.

(A) (E6-E14) Neuroepithelial cells in mouse developing hindbrain.

Immunofluorescence of neuroepithelial cells in mouse embryos at E6 **(A)**, E11 **(B)** and E14 **(C)**. The anti-SOX2 monoclonal antibody (IgG1, AMAb91307) is visible in green; the anti-L1CAM monoclonal antibody (IgG2a, AMAb91829) is visible in red.

(B) (E11, E14) Immunofluorescence staining of intermediate progenitors in the developing mouse brain cerebral cortex.

Intermediate progenitors in the developing mouse cerebral cortex at E11 **(left)** and E14 **(right)**. The **anti-PAX6** monoclonal antibody (IgG1, AMAb91372) is visible in green; the **anti-EOMES** (TBR2) polyclonal antibody (HPA028896) is visible in purple.

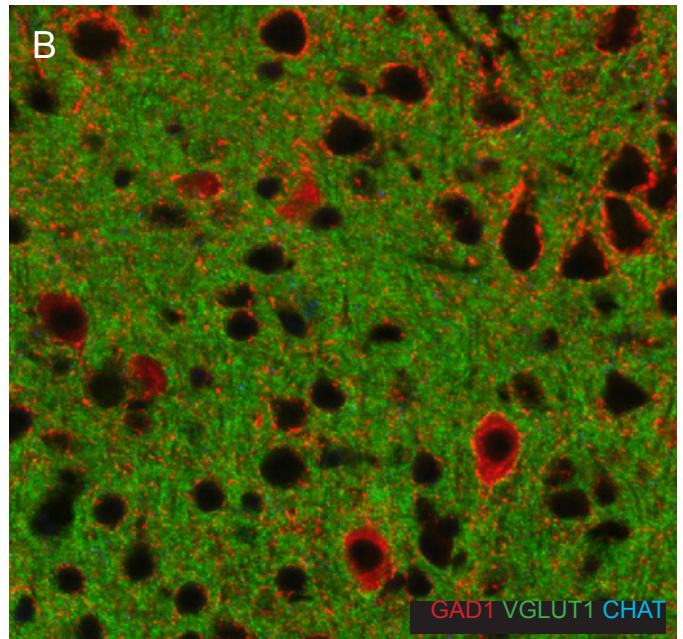
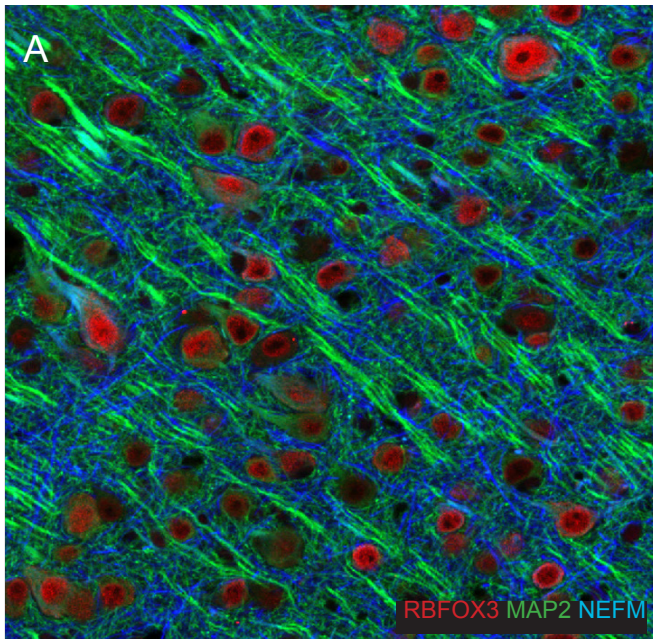


FIGURE 12. ADULT MOUSE CORTEX

ICC-IF staining of differentiated neurons in the adult mouse cerebral cortex.

(A) The anti-MAP2 polyclonal antibody (HPA012828) is visible in green; the anti-RBFOX3 monoclonal (IgG2b, AMAb91748) is visible in red, and the anti-NEFM monoclonal (IgG1, AMAb91027) in blue.

(B) The anti-VGluT1 monoclonal antibody (IgG2b, AMAb91041) is visible in green; the anti-GAD1 monoclonal (IgG2a, AMAb91076) is visible in red, and the anti-ChAT monoclonal (IgG1, AMAb91129) in blue.

TABLE 11.
ANTIBODIES TARGETING GLIOMAS

Product Name	Product ID	Isotype	Recommended Applications	Product Name	Product ID	Isotype	Recommended Applications
Anti-ADAM10	AMAb91898	IgG1	WB*, IHC*	Anti-LGR5	AMAb91887	IgG2a	WB, IHC*
	AMAb91899	IgG1	WB*, IHC*		AMAb91888	IgG1	IHC*
	AMAb91900	IgG2b	IHC*, ICC-IF	Anti-NF1	AMAb91741	IgG2b	ICC-IF
	AMAb91901	IgG2b	IHC*, ICC-IF		AMAb91745	IgG1	ICC-IF
Anti-ALDH1A3	AMAb91754	IgG2a	IHC*, WB, ICC-IF*	Anti-POSTN	AMAb91763	IgG2a	IHC*, ICC-IF
Anti-ATRX	AMAb90784	IgG1	IHC, WB*, ICC-IF		AMAb91764	IgG2a	IHC*, ICC-IF
Anti-CHI3L1	AMAb91777	IgG2b	IHC, WB	Anti-PROX1	AMAb91863	IgG1	WB, IHC
	AMAb91778	IgG1	IHC, WB		AMAb91865	IgG1	ICC-IF
Anti-EZH2	AMAb91749	IgG2b	IHC*, WB, ICC-IF	Anti-PTEN	AMAb91735	IgG1	IHC*, WB
	AMAb91750	IgG2a	IHC*, ICC-IF		AMAb91736	IgG2a	IHC*, WB
	AMAb91752	IgG2b	IHC*, WB, ICC-IF	Anti-RB-FOX3	AMAb91746	IgG2b	IHC*
Anti-FOXM1	AMAb91766	IgG1	IHC, ICC-IF		AMAb91748	IgG2b	IHC*
Anti-FOXO3	AMAb91872	IgG2a	WB, IHC	Anti-SALL4	AMAb91768	IgG1	IHC*, WB, ICC-IF
	AMAb91874	IgG2a	IHC		AMAb91769	IgG1	IHC*, WB, ICC-IF
Anti-GFAP	AMAb91033		IHC*, WB*		AMAb91770	IgG2a	ICC-IF
Anti-GLI1	AMAb91771	IgG1	WB, ICC-IF	Anti-WNT5A	AMAb91883	IgG2a	IHC
	AMAb91772	IgG1	IHC	Anti-YAP1	AMAb91878	IgG2b	IHC
	AMAb91773	IgG2b	IHC, ICC-IF	Anti-ZEB2	AMAb91862	IgG1	IHC*, WB, ICC-IF
Anti-ID1	AMAb91756	IgG1	IHC, ICC-IF				
	AMAb91757	IgG2a	IHC*, ICC-IF				
Anti-IDH1	AMAb90578	IgG2a	IHC, WB*, ICC-IF				
Anti-IGFBP2	AMAb91884	IgG1	WB, IHC*				
	AMAb91885	IgG2a	IHC*				
Anti-IL33	AMAb91858	IgG2b	IHC*, ICC-IF				

* Enhanced Validation

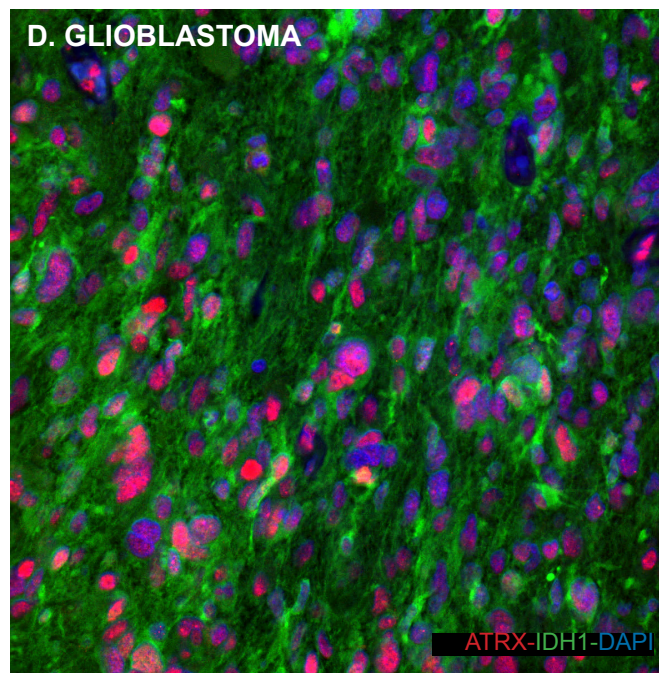
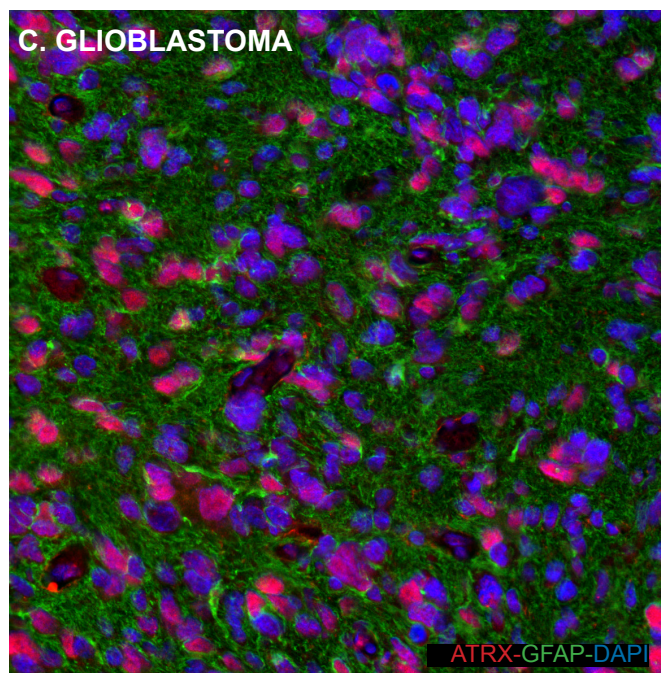
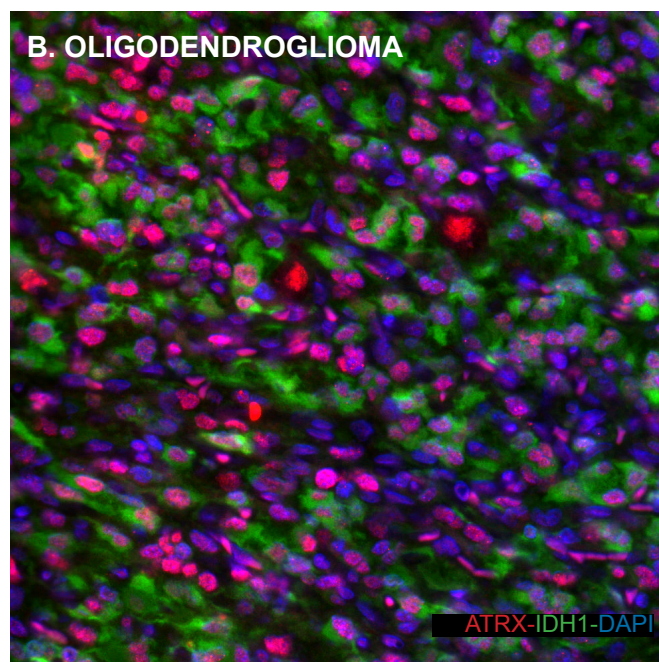
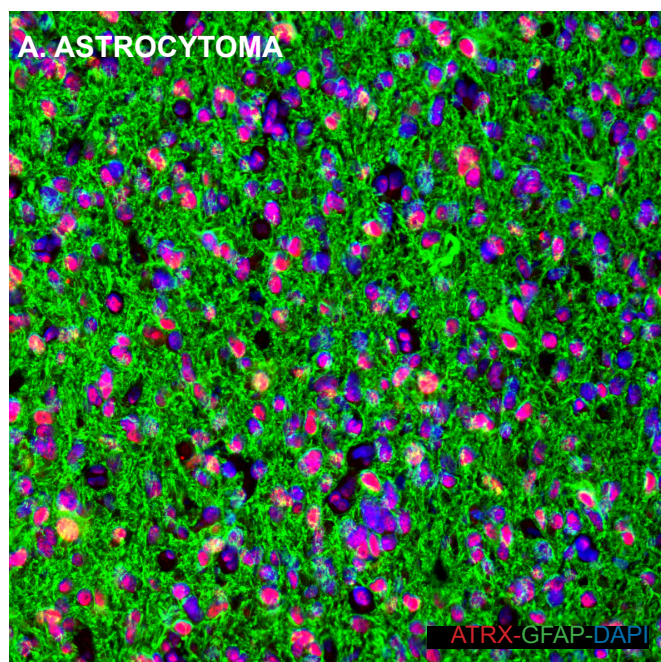


FIGURE 13. GLIAL CELLS IN GLIOMAS

(A) Astrocytoma: multiplexed IHC-IF staining of astrocytoma showing nuclear ATRX (anti-ATR-X monoclonal AMAb90784, red) and cytoplasmic GFAP (anti-GFAP monoclonal AMAb91033, green) immunoreactivity in tumor cells. Nuclei were counterstained with DAPI.

(B) Oligodendroglioma: Multiplexed IHC-IF staining of anaplastic oligodendroglioma showing ATRX (anti-ATR-X monoclonal AMAb90784, red) and cytoplasmic IDH1 (anti-IDH1 monoclonal AMAb90578 green) immunoreactivity in tumor cells. Nuclei were counterstained with DAPI.

(C) Glioblastoma: Multiplexed IHC-IF staining of glioblastoma multiforme showing nuclear ATRX (anti-ATR-X monoclonal AMAb90784, red) and cytoplasmic GFAP (anti-GFAP monoclonal AMAb91033, green) immunoreactivity in tumor cells. Nuclei were counterstained with DAPI.

(D) Glioblastoma: Multiplexed IHC-IF staining of glioblastoma multiforme showing nuclear ATRX (anti-ATR-X monoclonal AMAb90784, red) and cytoplasmic IDH1 (anti-IDH1 monoclonal AMAb90578 green) immunoreactivity in tumor cells. Nuclei were counterstained with DAPI.

4. CELLULAR COMMUNICATION AND PROTEIN-PROTEIN INTERACTION

Disruptions in protein interactions within the brain are increasingly recognized as critical factors in the development of various neurodevelopmental, neurological and psychiatric disorders, including Parkinson's disease, Alzheimer's disease, and schizophrenia.

Protein-protein interactions (PPIs) are fundamental to the proper functioning of cellular processes, as they regulate everything from signal transduction and metabolic pathways to synaptic transmission and structural integrity. When these interactions are altered, whether due to genetic mutations, environmental stressors, or pathological changes, normal cellular communication can break down, leading to disease.

In neurological disorders like Alzheimer's disease, the misfolding and aggregation of proteins such as amyloid-beta and tau disrupt PPIs within neurons, leading to synaptic dysfunction, neuronal death, and cognitive decline. Similarly, in Parkinson's disease, the accumulation of misfolded alpha-synuclein interferes with the normal interactions of proteins involved in dopamine signaling, contributing to motor dysfunction. These diseases also involve disruptions in glial cell function; for instance, altered interactions between astrocytic proteins and neuronal receptors can exacerbate neuroinflammation and contribute to neuronal damage.

Different cell types within the brain, including neurons, astrocytes, oligodendrocytes, and microglia, all rely on a delicate balance of PPIs to maintain brain homeostasis. In schizophrenia, for example, abnormalities in synaptic protein interactions, such as those involving NMDA receptors and associated signaling complexes, are thought to underlie some of the cognitive and behavioral symptoms of the disorder. Additionally, astrocytes and microglia, which are crucial for modulating the synaptic environment and immune responses in the brain, can become dysfunctional when their protein interactions are disrupted, leading to altered neurotransmitter levels, increased inflammation, and further neuronal dysfunction.

Investigating the molecular mechanisms behind these PPIs offers crucial insights into how communication breakdowns at the cellular and molecular levels contribute to the onset and progression of brain disorders.

By understanding these interactions more deeply, researchers can identify potential therapeutic targets to restore normal protein function, prevent disease progression, and ultimately develop more effective treatments for these complex neurological and psychiatric conditions.

Understanding brain disorders requires a thorough exploration of the complex communication networks between various brain cells.

Neurons, for example, transmit signals across synapses via neurotransmitters, facilitating cognitive processes, motor functions, and sensory perception.

Astrocytes, once thought to be a homogeneous group, are now recognized for their significant heterogeneity across and within brain regions. These cells, along with neurons, interact through intricate networks of proteins and receptors, with precise coordination being essential for normal brain function.

Disruptions in protein interactions can lead to neurological and psychiatric disorders, such as Parkinson's, Alzheimer's disease, and schizophrenia. Investigating the molecular mechanisms behind protein-protein interactions (PPIs) provides crucial insights into how communication breakdowns contribute to the onset and progression of these brain disorders.

MOLBOOLEAN: ADVANCED PROTEIN-PROTEIN INTERACTION (PPI) STUDIES

Identifying brain cell type-specific markers is crucial in understanding the molecular and cellular architecture of the brain. These markers distinguish between different types of neurons, glial cells, and other cell types, providing insights into the specific roles these cells play in brain function. However, knowing which proteins are present in different brain cell types is only one part of the puzzle, especially when it comes to understanding brain disorders.

Understanding protein-protein interactions, facilitated by methods like proximity ligation assays and the new MolBoolean™ technology (an advanced in situ proximity assay, Atlas Antibodies, Sweden), reveals how proteins communicate and influence each other's functions, aiding in dissecting the molecular underpinnings of neurological diseases. These approaches, when combined with specific primary antibodies, pinpoint critical interactions for neuronal function and dysfunction, offering insights that could lead to targeted therapeutic interventions in neurological disorders.

Malmqvist et al., (unpublished, 2024) employed MolBoolean™ to detect free and interacting protein fractions of dopamine D2 (DRD2) and adenosine A2A (ADORA2A) receptors in rat brain sections. A simultaneous immunofluorescent IHC (IHC-IF) analysis using the anti-GAD1 (AMAb91079, Atlas Antibodies) and anti-GFAP antibodies (AMAb91033, Atlas Antibodies) enabled the evaluation of MolBoolean results specifically in neurons and astrocytes. The findings revealed significant receptor interactions in the striatum, with a higher proportion of free proteins in the cerebral cortex. (Figure 8)

Similarly, in another recent study (Rivas-Santisteban et al., 2023) MolBoolean™ was employed to analyze the interaction between dopamine D2 (D2R) and adenosine A2A (A2AR) receptors in striatal medium spiny neurons, using rodent and non-human primate models of Parkinson's disease (PD). MolBoolean™ provided quantitative data on the proportions of individual receptors and receptor complexes under various experimental conditions, including heterologous HEK-293 cells, primary striatal neurons, rat 6-OHDA-PD model, and macaca MPTP-lesioned PD model.

MolBoolean™ enabled the researchers to determine the percentage of each individual receptor that were forming A2AR / D2R heterodimers, shedding more light on how A2AR mediate their antagonistic regulation on motor control in the brain.

These studies emphasize the critical role of PPIs in understanding brain functions and disorders. They also showcase MolBoolean's potential as a powerful tool in spatial proteomics, aiding in the dissection of the molecular underpinnings of neurological diseases.

In summary, while identifying brain cell type-specific markers is essential, it is not sufficient for understanding the full complexity of brain disorders. Protein-protein interaction studies provide the necessary context, revealing how these proteins work together within and across different cell types. This understanding is vital for uncovering the mechanisms underlying brain disorders and for developing effective therapeutic strategies.

Unlock the secrets of protein-protein interactions in cells and tissue with MolBoolean™

MolBoolean™ is a novel in situ proximity technology developed by Atlas Antibodies that enables the simultaneous detection of both free and interacting fractions for two protein targets.

- Complete spatial quantitative analysis of protein-protein interactions by simultaneous detection of free and interacting proteins.
- Accurate quantification by normalization of interaction data to total target protein levels.
- Biologically relevant data without the need for engineered protein expression.
- 1000-fold increased fluorescence signal by Rolling Circle Amplification, allowing detection and quantification of low abundant proteins.
- Adaptable to different research needs. Universal kit that can be used with the customer's choice of primary antibodies.
- Validated in both cells and tissue.

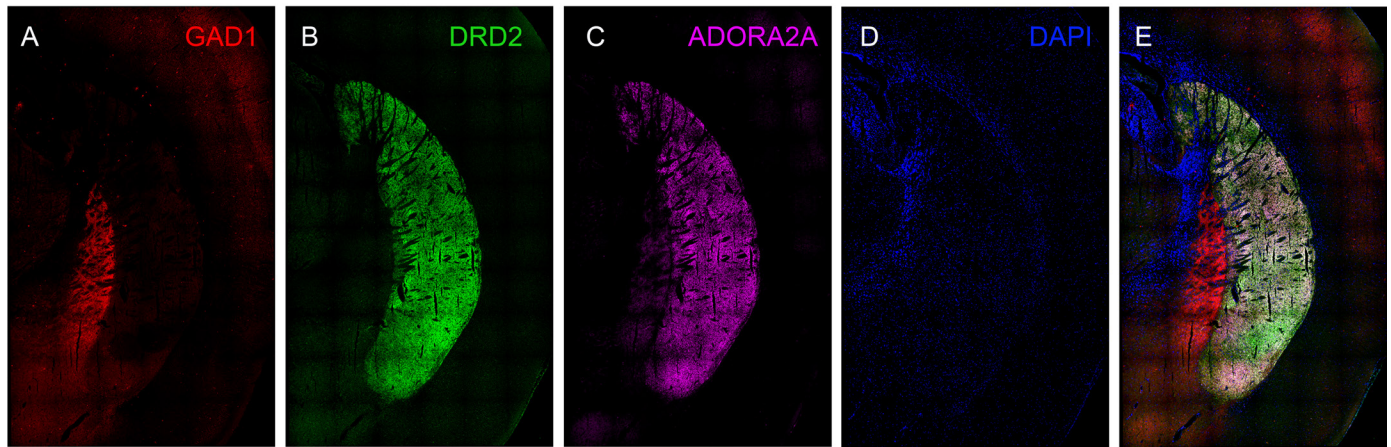


FIGURE 14.

MOLBOOLEAN: DRD2-ADORA2A RECEPTORS INTERACTION IN RAT STRIATUM

Low-power magnification image showing immunofluorescence analysis of DRD2 and ADORA2A receptor interactions in the rat brain.

(A) The GABAergic neurons and processed were visualized using the Anti-GAD1 antibody AMAb91079 (red) and indirect immunofluorescence,

(B) DRD2 expression was visualized using the Anti-DRD2 antibody HPA015691 followed by MolBoolean detection (green).

(C) ADORA2A expression was detected using the Anti-ADORA2A antibody Ab 05-717 (Merck/Millipore) followed by MolBoolean detection (magenta).

(D) Nuclei were counterstained by DAPI (blue).

(E) Interacting DRD2 and ADORA2A receptors are visualized as white signal on the overlay image.

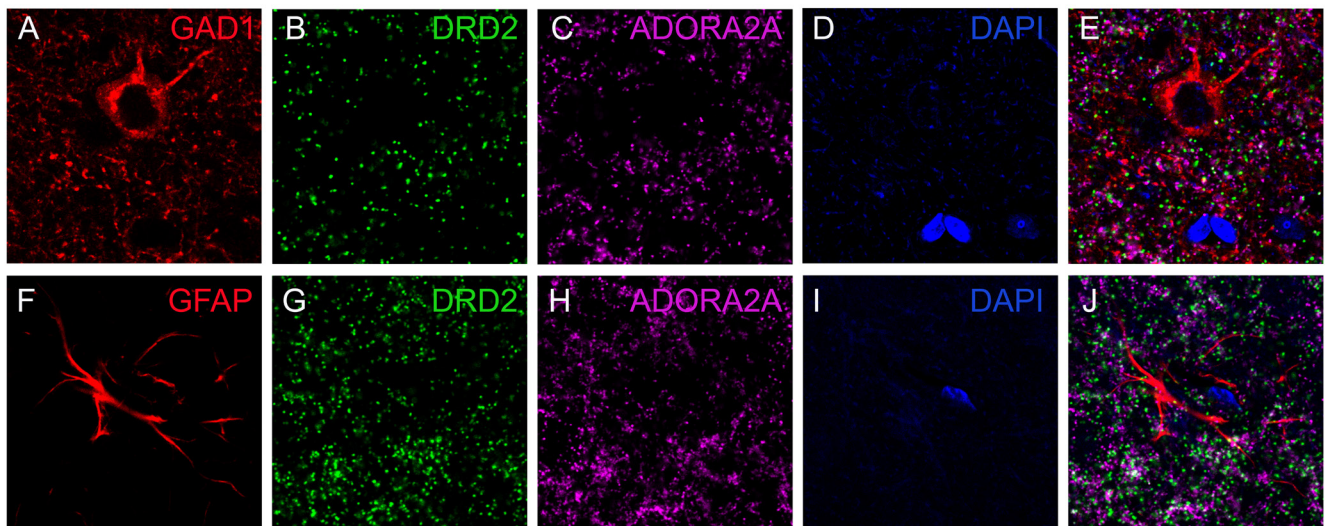


FIGURE 15.

MOLBOOLEAN: DRD2-ADORA2A RECEPTORS INTERACTION IN RAT STRIATAL NEURONS AND GLIA

High-power magnification images showing DRD2 and ADORA2A receptor interactions in cell subpopulations in the rat striatum, including GABAergic neuronal cell bodies and processes (**A-E**) and astrocytes (**F-J**). GABAergic neurons and astrocytes were detected using indirect immunofluorescence and the Anti-GAD1 antibody AMAb91079 (**A**, red) and the Anti-GFAP antibody AMAb91033 (**F**, red) respectively.

The interacting DRD2 and ADORA2A receptors are visible as white signal on **E** and **J**, while non-interacting receptors are seen as either green (DRD2) or magenta (ADORA2A) signal.

5. CONCLUSIONS

In summary, spatial proteomics offers valuable insights into the communication between neurons and neighboring brain cells, such as astrocytes and microglia.

By mapping the distribution of proteins at subcellular resolutions, we can answer fundamental questions about brain function and pathology.

Proteomic helps to gain insights in the complexities of the brain by leveraging primary antibodies to pinpoint specific markers crucial for identifying various brain cell types and understanding their spatial organization within neural tissue.

To fully grasp the diversity of these interactions, it is crucial to identify and use specific markers to accurately define distinct cell populations. This refined understanding will significantly advance our knowledge of normal brain function and the roles of various cell types in neurological disorders.

6. WHY ATLAS ANTIBODIES?

Atlas Antibodies is the manufacturer and provider of enhanced validated primary antibodies designed to selectively and specifically target the brain proteome.

Our antibodies (i.e. **PrecisA Monoclonals™** and **TripleA Polyclonals™**) and protein-protein interaction technology (i.e. **MolBoolean™**) enable researchers to identify specific brain cells and explore how they communicate, adapt, and malfunction to gain deeper insights into the brain's communication networks, revealing the molecular pathways that drive neurological disorders.

Atlas Antibodies products are designed to meet the highest standards of specificity and reliability, making them essential tools for neuroscience research. Each antibody is meticulously developed to ensure precise detection and quantification of key proteins involved in brain diseases, minimizing the risk of cross-reactivity and ensuring your results.

Founded by researchers from the Human Protein Atlas

The Human Protein Atlas was initiated in 2003 by Swedish researchers, headed by Professor Mathias Uhlen, and is funded by the Knut and Alice Wallenberg Foundation. It is a unique world-leading effort to create a complete map of human protein expression and localization in normal tissues, cancers, and cell lines. The project launched the first version of the entire human tissue proteome in 2016 as the Tissue Atlas, the subcellular proteome in 2017, and continues to add additional layers of knowledge exploring the human proteome.

Open access

One of the key advantages of Atlas Antibodies is our commitment to transparency and quality. We provide open access to epitope information, allowing researchers to fully understand the specific targets of our antibodies. This transparency ensures that you can select the right tools for your research with confidence.

Lot-to-lot reproducibility

Consistency is critical in scientific research, which is why we ensure lot-to-lot reproducibility across all our antibodies. This guarantees that your experiments can be reliably repeated, with consistent results every time, making your findings robust and credible.

Enhanced validation

Our antibodies undergo enhanced validation, going beyond standard testing protocols to ensure they perform as expected in various applications and conditions. This rigorous validation process is coupled with extensive data available on the Human Protein Atlas (HPA) portal, where you can access comprehensive information on antibody performance, expression patterns, and tissue specificity.

ATLAS ANTIBODIES' PRODUCTS AND TECHNOLOGIES

Triple A Polyclonals™

These antibodies originating from the Human Protein Atlas project are available under the brand Atlas Antibodies Advanced Polyclonals (Triple A Polyclonals™). The Triple A Polyclonals are since then been manufactured by Atlas Antibodies in our facilities in Sweden and distributed worldwide.

PrecisA Monoclonals™

Precise, accurate, and targeted monoclonal antibodies. In 2012, Atlas Antibodies started in-house development of mouse monoclonal antibodies against a selected number of targets. We have named them **PrecisA Monoclonals** to reflect the core values at Atlas Antibodies and our Swedish origin. We offer a growing portfolio of monoclonals for use in various applications and research areas. The Swedish word "precisa" stands for precise, accurate, and targeted. It is a perfect description of our mouse monoclonal antibodies.

PrEST Antigens™

In 2014, we expanded our product offering to include **PrEST Antigens™**, recombinant protein fragments used as immunogens for generating Triple A Polyclonals, and **PrecisA Monoclonals**. The **PrEST Antigens** are recombinant human Protein Epitope Signature Tags (**PrESTs**) consisting of 50-150 amino acids and are designed to have as low sequence identity as possible to other human proteins. The **PrEST Antigens** are provided as complements to our antibodies for control experiments.

MolBoolean™

In 2024 Atlas Antibodies released **MolBoolean™** a novel in situ proximity technology for protein-protein interaction studies. **MolBoolean™** mouse/rabbit is a kit for in situ protein proximity analysis in tissue and cells. The **MolBoolean™** assay utilizes a proprietary oligonucleotide setup that enables the simultaneous detection of both free and interacting (~40 nm proximity) fractions for two proteins of interest (protein A, protein B and interaction proteins AB).

VERY RELIABLE ANTIBODIES

Atlas Antibodies manufactures and provides over 22,000 highly validated monoclonal and polyclonal primary antibodies and control antigens targeting the majority of human proteins for tissue and cell analysis to explore and accelerate research in biology, pathology, and medicine. The portfolio covers different research areas such as neuroscience, cancer, cell biology, stem cell & development. All our products are rigorously evaluated for specificity, reproducibility, and performance and characterized for use in IHC, WB, and ICC-IF. Enhanced validation is applied as an extra level of security of antibody specificity in a defined context. Available in 25 µL and 100 µL unit sizes.

CREATED BY THE HUMAN PROTEIN ATLAS

With our roots in the Human Protein Atlas project, an integration of antibody-based imaging, proteomics, and transcriptomics, our antibodies are affinity-purified, reproducible, selective, and specific for their target proteins through our enhanced validation process. Our Triple A Polyclonals™ are developed within the Human Protein Atlas project.

VALIDATED BY ENHANCED VALIDATION

We take great care to validate our antibodies in IHC, WB, and ICC-IF. Our antibodies are validated in all major human tissues and organs and 20 cancer tissues. Over 500 staining images support each antibody. As an additional layer of security, we perform Enhanced Validation. By using 5 different enhanced validation methods, we validate our antibodies for each combination of protein, sample, and application. Discover our Triple A Polyclonals™ and PrecisA Monoclonals™ antibodies targeting the majority of human proteins in cells, tissues, and organs.

EVIDENCED BY SCIENCE

Made by researchers for researchers, our products are used all over the world and referenced in thousands of scientific peer-reviewed papers.

WE SUPPORT YOUR RESEARCH

Our scientific content and newsletter provide you with timely information about new product releases, research highlights, and much more. In addition, from our website, you can download informative white papers, protocols, guides, posters, infographics, roundups of recent research papers, and read blog posts and interviews.

HOW TO BUY OUR PRODUCTS

Our products are available worldwide. We deliver to all European destinations (excluding Russia), the USA, Canada, Australia, New Zealand, and Israel. In addition, we expand our offering through trusted partners worldwide. You can shop our entire catalog online or find your local supplier.

We are continuously updating our catalogs. Please refer to the online version for the latest updates of this document at atlasantibodies.com



Atlas Antibodies Advanced Polyclonals.

Triple A Polyclonals™ are rabbit polyclonal primary antibodies developed within the Human Protein Atlas project. IHC characterization data from 44 normal and 20 cancer tissues is available on the Human Protein Atlas portal.



Precise. Accurate. Targeted.

PrecisA Monoclonals™ are mouse monoclonal primary antibodies developed against a number of carefully selected targets. Clones are selected to recognize only unique non-overlapping epitopes and isotypes.

PrEST Antigens

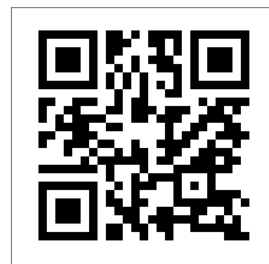
Recombinant protein fragments

PrEST Antigens™ are used as immunogens for the generation of Triple A Polyclonals and PrecisA Monoclonals.



Beyond Proximity Ligation

MolBoolean™ is a novel in situ proximity technology for protein-protein interaction studies. MolBoolean™ mouse/rabbit is a kit for in situ protein proximity analysis in tissue and cells. The MolBoolean™ assay utilizes a proprietary oligonucleotide setup that enables the simultaneous detection of both free and interacting fractions for two proteins.



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