Advancing stem cell research

The use of antibodies to characterize cells

Introduction

Stem cells are defined by two core properties: their ability to self-renew and their ability to be induced to differentiate into a variety of organ-specific cell types. Their regeneration potential, coupled with significant advances in cell biology methods to cultivate and differentiate them, has spawned extensive research into the applicability of stem cells to address unmet needs in medicine. These advances have resulted in several stem cell–based therapies currently in clinical trials [1].

Stem cell scientists pay close attention to the characteristics of their cell populations. Their properties are important since they provide information about the types of cells in culture and whether the differentiated cells are of the lineage of interest. Antibodies are often the go-to reagents for characterization. Antibodies that recognize specific biological markers are used to assess both stemness and the differentiated properties of the cell populations, and are used for a variety of applications like imaging, cell sorting, and fluorescence microscopy.

Given their importance and wide use in stem cell research, there is a critical need for extensively tested and specific antibodies for stem cell markers. However, antibodies can be notorious for their poor specificity, performance, and reproducibility. This “antibody reproducibility crisis” has prompted several research groups to insist on the need for meticulous antibody testing [2,3].

At Thermo Fisher Scientific, antibodies are tested using a two-part validation* approach, which includes functional testing in relevant applications and specificity testing to ensure that the antibody only recognizes the intended target [4]. Target biology is used to guide the testing of antibodies in appropriate cell systems and relevant applications. In addition, stem cell antibodies are tested in stem cell differentiation experiments identical to those a scientist would perform [5]. In this application note, we discuss multiple stem cell antibodies and how they are validated.

* The use or any variation of the word “validation” refers only to research use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. It does not ensure that the product(s) was validated for clinical or diagnostic uses.

Pluripotency markers

Pluripotency is defined as the potential to generate all the cell types of somatic lineages plus the germline. Two common sources of pluripotent stem cells (PSCs) are embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). While ESCs are derived from the inner cell mass of blastocyst-stage embryos, iPSCs are developed by reprogramming somatic cells. ESCs are of great medical interest because their properties enable them to serve as a versatile source for disease modeling and regenerative medicine. Because of the ease of availability of the starting material and lack of ethical concerns compared to cells of embryonic origin, iPSCs have great potential for use in research and therapy. Reprogramming somatic cells to iPSCs requires the overexpression of key factors that are abundantly expressed in ESCs. These proteins (Oct3/4, Sox2, Klf4, and c-Myc) are called the Yamanaka factors, named after the scientist who discovered them [6]. Most stem cell scientists today use the expression of the Yamanaka factors as a positive test for pluripotency of iPSCs. Thermo Fisher Scientific offers antibodies that are extensively validated and specific for these factors. c-Myc, a transcription factor expressed in ESCs and iPSCs, is also abundantly expressed across a wide variety of cell lines. Consistent with this biology, Invitrogen™ c-Myc antibody (Cat. No. 700648) detects a single band with the expected molecular size in western blot (WB) analysis across different cell lines tested (Figure 1A). Immunocytochemical (ICC) analysis using this antibody shows the expected nuclear localization (Figure 1B). Another transcription factor, Nanog, is expressed in NTERA-2 cells but not in HeLa cells (Figure 1C, 1D). This relative expression is detected by Invitrogen™ Nanog antibodies in ICC (Cat. No. PA1097, Figure 1C) and WB (Cat. No. 14-5768-82, Figure 1E). The specificity of Invitrogen™ Oct4 antibody (Cat. No. 701756) is demonstrated by siRNA-mediated knockdown of Oct4 in WB (Figure 1F). This antibody also performs well in chromatin immunoprecipitation (ChIP), which is a relevant application (Figure 1G).

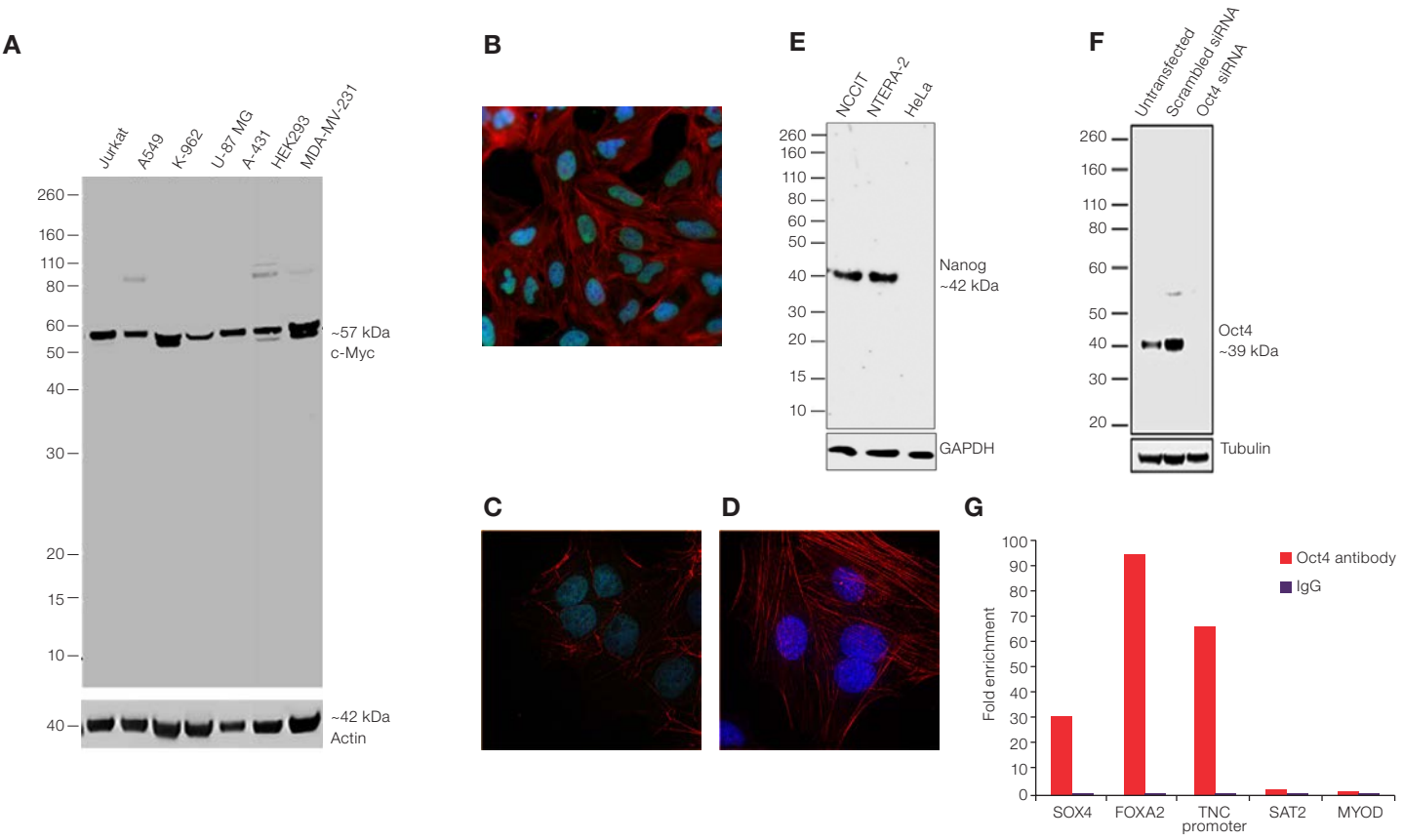

Figure 1. Antibodies against pluripotency markers. Functional validation of c-Myc antibody in WB **(A)** and ICC **(B)**. Differential expression of Nanog detected in ICC by Nanog antibody in NTERA-2 **(C)** and HeLa cells **(D)**. **(E)** Differential expression of Nanog detected in WB by Nanog antibody in NTERA-2 and HeLa cells. **(F)** Specificity of Oct4 antibody in WB demonstrated by siRNA-mediated knockdown. **(G)** ChIP using Oct4 antibody. In panels B, C, and D, antigens are labeled green, nuclei are labeled blue, and phalloidin is labeled red.

Lineage markers

iPSCs have the ability to differentiate into derivatives of the three embryonic germ layers: mesoderm, endoderm, and ectoderm. Markers expressed in each of these lineages are used to identify and confirm these populations. Commonly used endoderm markers include SOX17, FOXA2, OTX2, CXCR4, and GATA4. The homeoprotein encoded by the *OTX2* gene acts as a transcription factor and plays a role in brain, craniofacial, and sensory organ development. During brain regionalization, it is required for controlling anteroposterior and dorsoventral patterning of the midbrain. Invitrogen™ OTX2 antibody (Cat. No. 701948) is functional in ICC and detects the expected nuclear signal in NTERA-2 cells (Figure 2A). OTX2 is absent in iPSCs but upregulated upon neuroepithelial differentiation, and this relative expression pattern is detected by the OTX2 antibody (Figure 2B, 2C). FOXA2, also known as hepatocyte nuclear factor, is a transcriptional activator that plays a role in liver development by activating liver-specific genes such as albumin and transthyretin. FOXA2 is upregulated in definitive endoderm compared to iPSCs, as confirmed by the signal detected by Invitrogen™ FOXA2 antibody (Cat. No. 701698) in ICC (Figure 2E, 2F). Due to its role as a transcription factor, this antibody was also tested and is functional in ChIP (Figure 2D). Optineurin, a Golgi complex–associated protein, is involved in membrane vesicle trafficking, autophagy, signal transduction, and Golgi ribbon formation. Although present in most human and mouse tissues, mutation of this protein has been recently reported as a causative factor in glaucoma and ALS. Invitrogen™ optineurin antibody (Cat. No. 702766) functions both in WB and ICC (Figure 2G, 2H). Specificity is shown in WB using CRISPR-Cas9 knockout (Figure 2G). The specific expression in iPSCs differentiated into retinal ganglial cells is also demonstrated (Figure 2H).

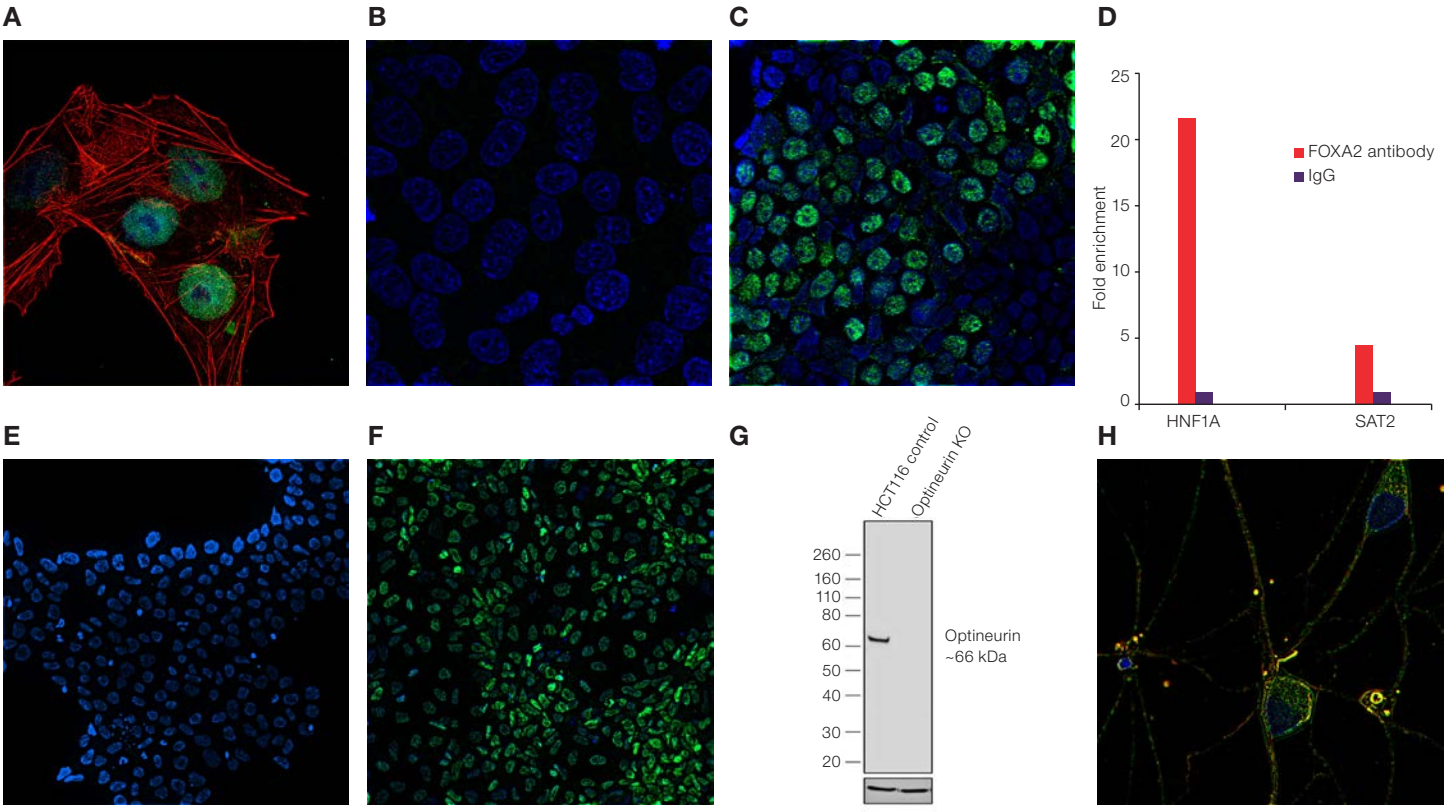

Figure 2. Antibodies against markers of differentiation in lineages derived from iPSCs. (A) Functional validation of OTX2 antibody in ICC. OTX2 is absent in iPSCs **(B)** but present upon differentiation to neuroepithelial cells **(C)**. **(D)** ChIP using FOXA2 antibody. FOXA2 is absent in iPSCs **(E)** but present upon differentiation into definitive endoderm **(F)**. Specificity of optineurin antibody demonstrated through CRISPR-Cas9 knockout (KO) **(G)** and expression of optineurin in retinal ganglion cells derived from iPSCs **(H)**. In all of the fluorescence images, antigens are labeled green, nuclei are labeled blue, and phalloidin is labeled red.

MSC differentiation markers

Mesenchymal stem cells (MSCs), also known as multipotent stromal cells or mesenchymal progenitor cells (MPCs), are fibroblasts of nonhematopoietic lineage that have considerable potential in a wide range of tissue engineering and regenerative medicine applications due to their multilineage differentiation potential, immunomodulatory activity, and secretion of paracrine factors. MSCs are a well-characterized adult stem cell type originally isolated and characterized from bone marrow (BM), but subsequently shown to exist in almost all postnatal tissues. In the presence of the right soluble factors, MSCs can differentiate into adipocytes, osteocytes, and chondrocytes. Characterization of these differentiations is particularly challenging as many of the transcription factors are transiently expressed during differentiation [7]. Figure 3 shows representative data for antibodies against markers with roles in trilineage differentiation. RUNX2 is a key transcription factor for differentiation of MSCs to the osteoblast lineage. RUNX2 is controlled by several upstream effectors like SOX9, MSX2, and other signaling molecules. RUNX2 exhibits a temporally controlled expression during osteoblast differentiation, as demonstrated by the signal detected by Invitrogen™ RUNX2 antibody (Cat. No. 702489) in ICC, specifically in osteoprogenitors (Figure 3A–C). The antibody that recognizes adiponectin (Cat. No. PA1054), an adipocyte marker, is functional in WB and has been tested using conditioned media derived from 3T3-L1 and NIH3T3 cells as well as adipocytes differentiated from bone marrow MSCs (Figure 3D). Another antibody (Cat. No. 710179) detects a signal in adipocytes differentiated from MSCs (Figure 3E) but not in MSC controls (Figure 3F). SOX9 is a crucial transcription factor for primary commitment to the chondroblast lineage. The specificity of Invitrogen™ SOX9 antibody (Cat. No. 702016) in WB has been demonstrated by siRNA-mediated gene knockdown (Figure 3G). SOX9 is also an intestinal crypt transcription factor, and ICC of MSCs differentiated into intestinal organoids shows a SOX9 signal in the crypts (Figure 3H).

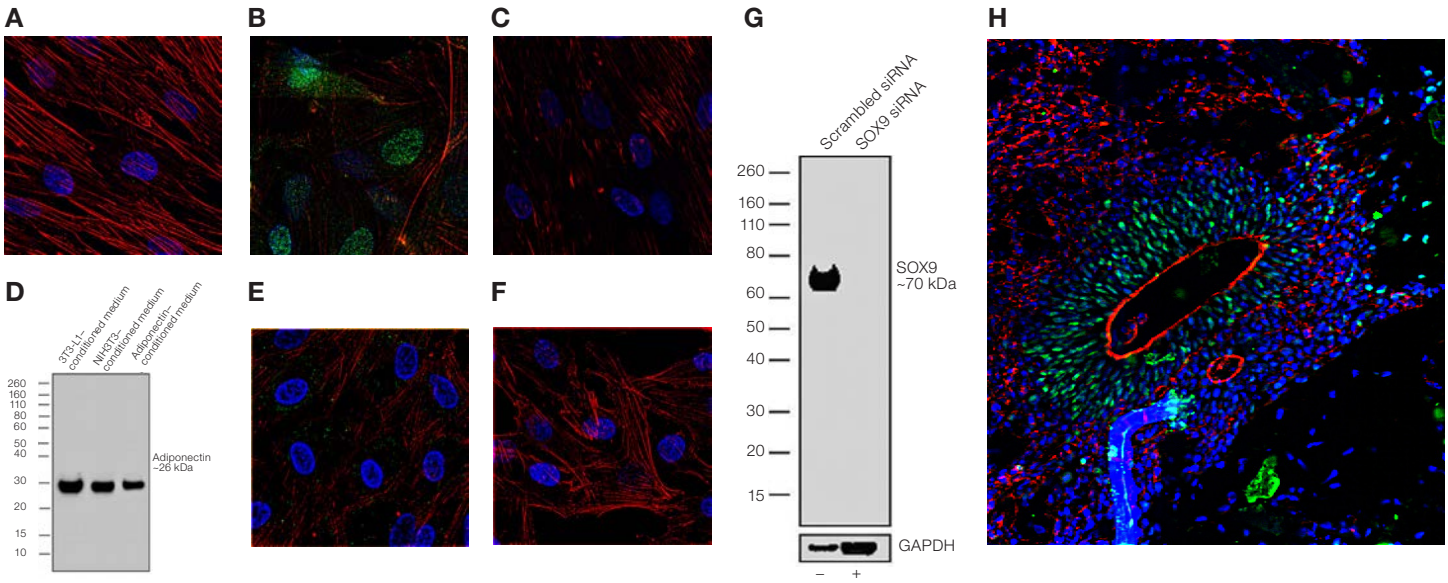

Figure 3. Antibodies against markers of MSC-derived differentiation. (A–C) Temporal expression of RUNX2 during osteoblast differentiation from MSCs. Functional validation of Invitrogen™ adiponectin antibody in WB **(D)** and in ICC in differentiated 3T3-L1 cells **(E)** compared to control **(F)**. **(G)** Specificity of the SOX9 antibody in WB using siRNA. **(H)** SOX9 staining of intestinal organoid differentiated from MSCs. In all of the fluorescence images, antigens are labeled green, nuclei are labeled blue, and phalloidin is labeled red.

NSC differentiation markers

Neural stem cells (NSCs) are a more specialized type of adult stem cells that give rise to specific neural cell types such as neurons and glial cells. NSCs reside in a distinct niche, such as the subventricular zone lining the lateral ventricles in the adult mammalian brain. Major promising sources of neural stem cells are human ESCs and iPSCs that enable scientists to efficiently differentiate the cells to specific types of neurons. Characterizing these differentiated cells is difficult using a single marker. Hence, it is crucial to have the right antibodies against multiple markers, to characterize each cell type. Nestin is one of the common markers used to characterize NSCs. This protein is transiently expressed in adult NSCs, and its expression starts to cease upon cell differentiation. Nestin is used as the marker for identifying NSCs in both embryonic and adult brain. Invitrogen™ nestin antibody (Cat. No. MA1110) has been tested in ICC and specifically detects a signal in neural rosettes but not in control cells (Figure 4A, 4B). Beta-III tubulin is a microtubulin expressed exclusively in mature neurons. Invitrogen™ beta-III tubulin antibody (Cat. No. 32-2600) detects a signal in neurons differentiated from NSCs in ICC (Figure 4C). Another beta-III tubulin antibody (Cat. No. PA5-25655) functions in WB and detects a differential signal in SH-SY5Y, a neuronal cell line, but not in HepG2, which is a liver cell line (Figure 4D). Glial fibrillary acidic protein (GFAP) is an intermediate filament protein that gives mechanical strength to astrocytes. GFAP-positive progenitors can generate specific types of neural cells during neurogenesis. Invitrogen™ GFAP antibody (Cat. No. PA518598) detects a signal in WB that is enriched in mouse and rat brains compared to ovary or liver (Figure 4E). Another GFAP antibody (Cat. No. MA512023) detects a signal in ICC in astrocytes that are differentiated from NSCs (Figure 4F).

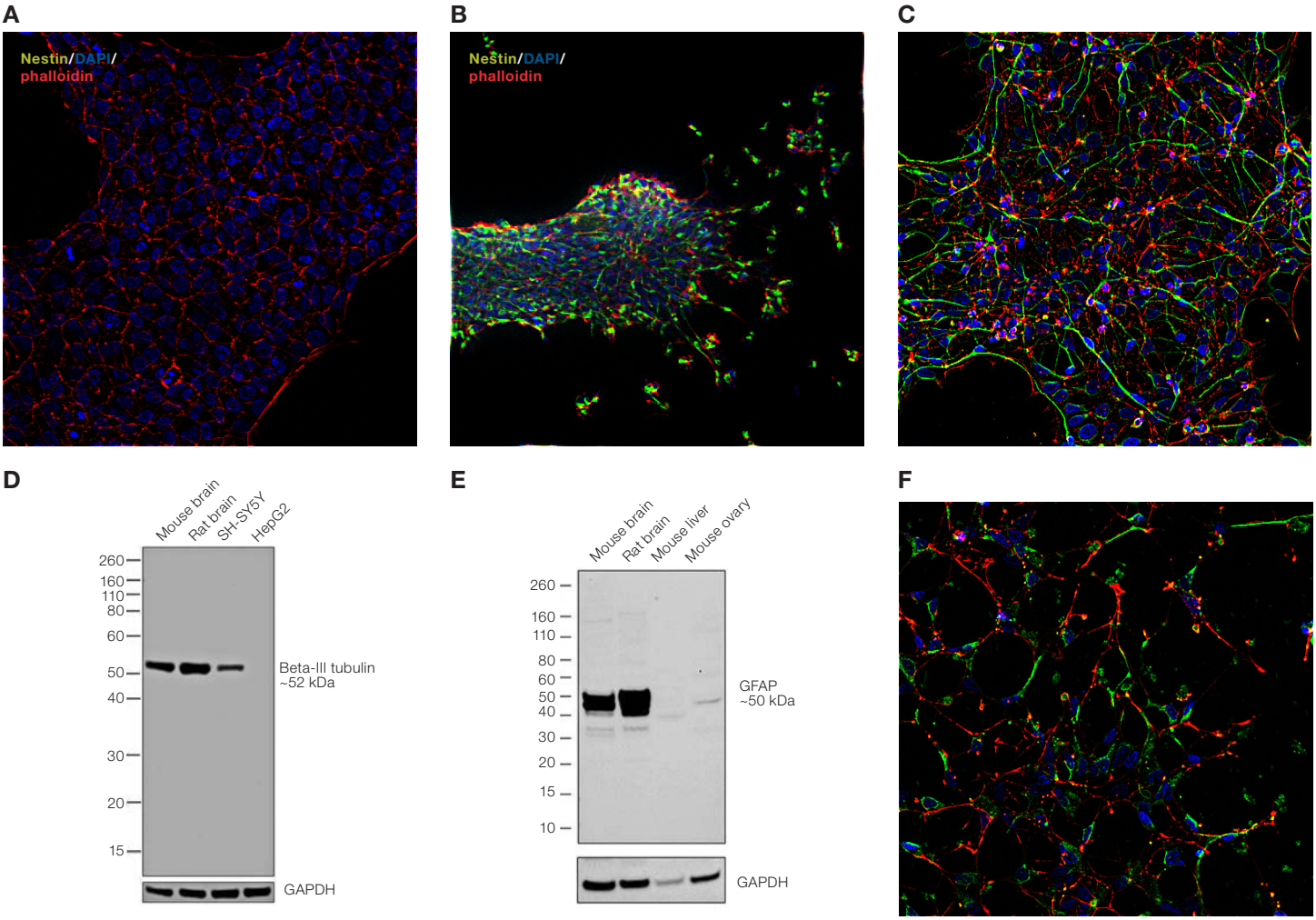

Figure 4. Antibodies against NSC markers and NSC-derived differentiation. Differential expression of nestin in control **(A)** compared to neuronal rosettes **(B)**. **(C)** Invitrogen beta-III tubulin antibody detects signal in mature neurons derived from NSCs. **(D)** Differential expression of beta-III tubulin in cells as detected by WB. **(E)** WB with GFAP antibody in rodent tissue. **(F)** GFAP antibody produces an ICC signal in differentiated astrocytes. In all of the fluorescence images, antigens are labeled green, nuclei are labeled blue, and phalloidin is labeled red.

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Conclusion

Advances in stem cell research are critically dependent on specific antibodies that work in relevant applications. Using a holistic understanding of the biology of the target, stem cell antibodies from Thermo Fisher Scientific are validated across applications and a variety of specificity tests to demonstrate that these antibodies can serve as ideal reagents to help advance research and regenerative medicine. Learn more at **thermofisher.com/antibodyvalidation**.

References

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Ordering information

Product	Cat. No.
Pluripotency markers	
c-Myc Recombinant Rabbit Monoclonal Antibody	700648
Nanog Polyclonal Antibody	PA1-097
eBioscience™ Nanog Monoclonal Antibody	14-5768-82
Oct4 Recombinant Rabbit Monoclonal Antibody	701756
Lineage markers	
OTX2 Recombinant Rabbit Monoclonal Antibody	701948
FOXA2 Recombinant Rabbit Monoclonal Antibody	701698
Optineurin Recombinant Rabbit Monoclonal Antibody	702766
MSC differentiation markers	
RUNX2 Recombinant Rabbit Monoclonal Antibody	702489
Adiponectin Polyclonal Antibody	PA1-054
Adiponectin Recombinant Polyclonal Antibody	710179
SOX9 Recombinant Rabbit Monoclonal Antibody	702016
NSC differentiation markers	
Nestin Monoclonal Antibody	MA1110
Beta Tubulin Monoclonal Antibody	32-2600
Beta-III Tubulin Polyclonal Antibody	PA5-25655
GFAP Polyclonal Antibody	PA5-18598
GFAP Monoclonal Antibody	MA5-12023

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