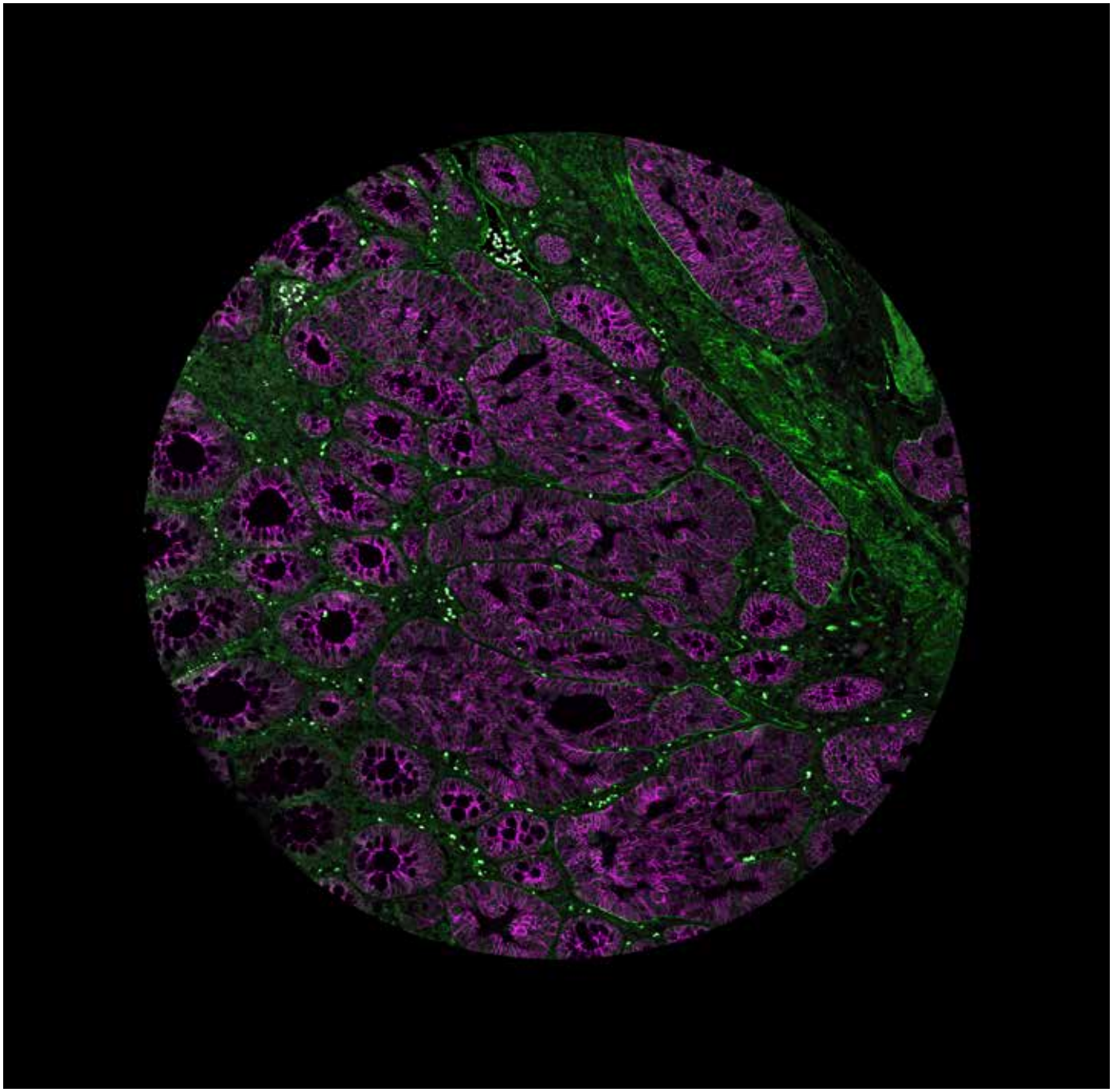

Epithelial to Mesenchymal Transition
MARKER PANEL

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ATLAS ANTIBODIES

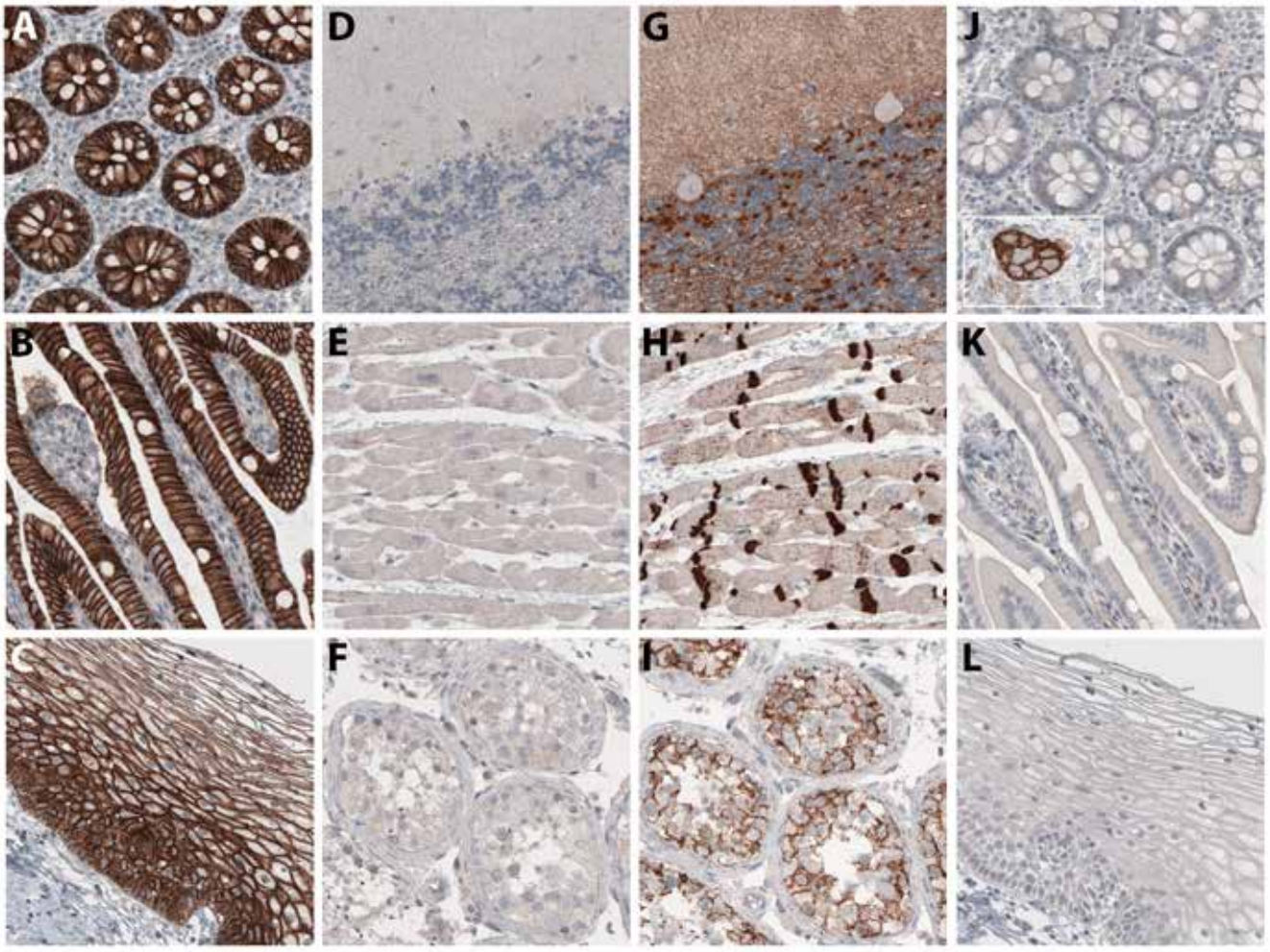

Figure 1.
E-cadherin (**A-F**) and N-cadherin (**G-L**) expression profiles in normal human tissues shown by IHC with the Anti-CDH1 antibody AMAb90863 and the Anti-CDH2 antibody AMAb91220. Note the differential expression of two cadherins in colon (**A, J**), duodenum (**B, K**), cervix (**C, L**), cerebellum (**D, G**), heart (**E, H**) and testis (**F, I**). Inset on J shows CDH2- immunoreactivity in the peripheral ganglion neurons in rectum.

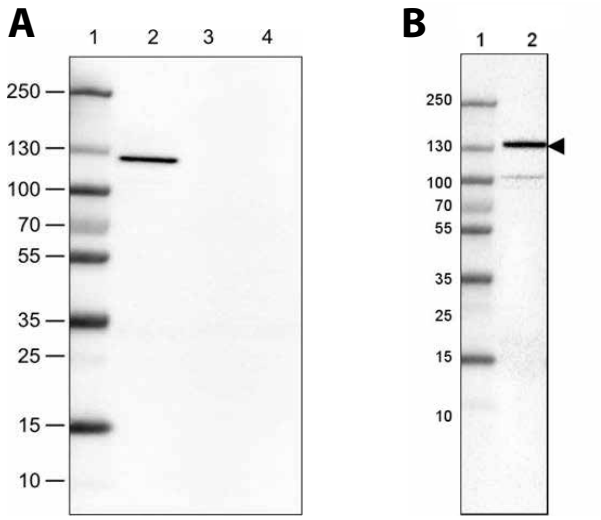

Figure 2.
Western blot using Anti-CDH1 antibody AMAb90863 (**A**) and Anti-CDH2 antibody AMAb91220 (**B**). **Blot A**: Lane 1: Marker [kDa], Lane 2: Human cell line MCF-7 (positive), Lane 3: Human cell line SK-BR-3 (negative), Lane 4: Human cell line HeLa (negative). **Blot B**: Lane 1: Marker [kDa], Lane 2 Human U-251 MG (positive).

Cover image:
Multiplexed IHC-IF staining of human colorectal cancer section showing membranous E-cadherin immunoreactivity in tumor cells (AMAb90865, magenta) and laminin gamma 1 positivity in basement membranes (AMAb91138, green).

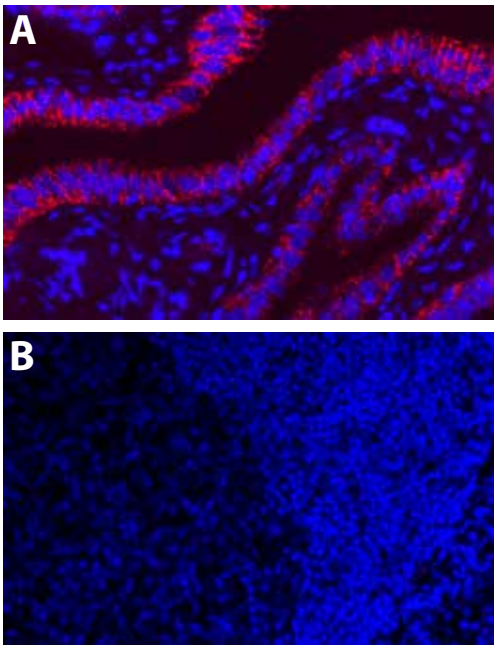

Figure 3.
E-cadherin – beta-catenin protein-protein interactions shown by *in situ* proximity ligation assay (PLA) in the epithelium of normal fallopian tube (**A**). The Anti-CDH1 mouse monoclonal antibody AMAb90865 and the Anti-CTNNB1 rabbit polyclonal antibody HPA029159 were used for the PLA reaction. Tonsil was used as negative control (**B**).

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The Epithelial to Mesenchymal Transition Marker Panel

Epithelial and mesenchymal cells are fundamentally different and represent the two main cell types in the body. Epithelial cells are polarised along the apical/basal axis and are tightly connected to each other as well as to underlying basement membrane by a number of cell junction proteins. In contrast, mesenchymal cells are adhered to the extracellular matrix and have enhanced migratory capacities.

Epithelial cells can transition into mesenchymal cells – a process known as epithelial-mesenchymal transition (EMT), which leads to loss of epithelial barrier functions and changes in cell adhesion and motility[1]. Normally, EMT occurs during development (embryogenesis), but it is also present in wound healing and cancer progression of epithelial tumors. In metastasis, tumor cells dissociate from the epithelial layer, penetrate through basement membrane into connective tissue and can then enter the vascular system for further dissemination and subsequent growth of distant metastases[2].

A number of factors drive and regulate the EMT process, including zinc finger proteins such as SNAI1, SNAI2, ZEB1 and ZNF703. These transcription factors down-regulate the expression of epithelial cell adhesion proteins such as E-cadherin, occludin, beta-catenin and claudin. In addition, they up-regulate expression of mesenchymal proteins, including N-cadherin, fibronectin, vimentin, S100A4 and others. Taken together, EMT leads to increase motility and invasiveness of cancer cells[1].

At Atlas Antibodies, we have developed a panel of monoclonal antibodies against the key EMT markers for cell junctions, cytoskeletal changes, transcription regulation and migration/motility.

The antibodies targeting selected EMT marker proteins are:

- IHC-validated in relevant normal and cancer human tissues
- WB-validated in positive and negative cell lines (when available)
- Available with different isotypes, allowing for multiplexing experiments
- Supplemented with information on antigens used for immunization and precise epitope sequence (when available)

The monoclonal antibodies within the panel have been developed using the same stringent conditions as for all PrecisA Monoclonals, ensuring a secured continuity and stable supply.

Key EMT Markers: Application Examples

E-cadherin (CDH1) is a calcium-dependent transmembrane protein forming adherence junctions between the epithelial cells. By forming a complex with beta-catenin (CTNNB1) this protein plays a key role in cellular adhesion in the epithelial tissues. E-cadherin suppresses tumor invasion and metastasis, while down-regulation of E-cadherin expression is a critical molecular feature of the EMT. Often, the loss of E-cadherin expression is associated with an increase in N-cadherin levels, so called 'cadherin switch'. The upregulation of N-cadherin expression in tumor cells induces increased motility, invasion and metastasis[3].

Figure 1 shows expression patterns of E-cadherin and N-cadherin in normal tissues. E-cadherin expression is detected in simple and stratified epithelial tissue, e.g. gastrointestinal tract (A, B) and cervix (C), while it is absent in brain (D), heart muscle (E) and testis (F) (visualised by the Anti-CDH1 monoclonal antibody AMAb90863).

On the contrary, N-cadherin (CDH2) expression is high in e.g. the nervous system (G and inset on J), heart muscle (H) and testis (I), while glandular epithelium of gastrointestinal tract (J, K) and squamous epithelium (L) show no protein expression (visualised by the Anti-CDH2 monoclonal antibody AMAb91220).

The specificity of the antibodies is further confirmed by Western Blot shown in Figure 2, using CDH1-positive (MCF7) and CDH1-negative (SK-BR-3 and HeLa) cell lines with the Anti-CDH1 antibody AMAb90863 (A) and the CDH2-positive U-251cell line with the Anti-CDH2 antibody AMAb91220 (B).

As mentioned above, the loss of beta-catenin (CTNNB1) from the E-cadherin-mediated cell-cell contacts is an important factor in the EMT process. Here, *in situ* proximity ligation assay (PLA)[4] was employed to show the CDH1-CTNNB1 interactions in the membrane of normal epithelial tissue of the fallopian tube using the Anti-CDH1 monoclonal antibody (AMAb90865) and Anti-CTNNB1 polyclonal antibody HPA029159 (Figure 3). Note the strong membranous positivity in the epithelial cells (A) and the absence of PLA signal in the underlying connective tissue (A) or lymphoid tissue of tonsil used as negative control (B).

During EMT, reprogramming of gene expression occurs in a highly regulated process. IHC images in Figure 4 demonstrate expression of some of the transcriptional factors involved in regulation of the EMT, including SNAI1 (AMAb91215, A), SIX1 (AMAb90544, B) and ZNF703 (AMAb90789, C) in various cancer tissues.

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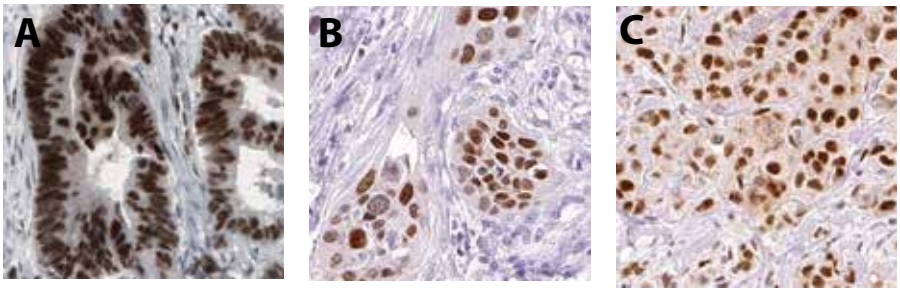

Figure 4.
Transcription factors involved in regulation of EMT. IHC images show nuclear immunoreactivity in tumor cells in (**A**) colorectal cancer (Anti-SNAI1 antibody AMAb91215), (**B**) cervical cancer (Anti-SIX1 antibody AMAb90544) and (**C**) breast cancer (Anti-ZNF703 AMAb90789).

Using Monoclonals of Defined Isotypes for Multiplexed Immunofluorescence

The EMT panel includes monoclonal antibodies with different isotypes, which allows for co-localization studies using immunofluorescence with isotype-specific secondary antibodies.

The images on Figure 5 show multiplexed staining of colorectal cancer tissue derived from two different patients using the Anti-CDH1 (A, E: AMAb90863, IgG1), Anti-CTNNB1 (B, F: AMAb91209, IgG2a) and LAMC1 (C, G: AMAb91138, IgG2b) monoclonal antibodies, respectively.

The tumor with higher degree of differentiation (indicated by preserved basement membrane, C) shows higher expression of E-cadherin (A) as compared to the tumor with lower differentiation grade (E). Also note the absence of LAMC1 immunoreactivity in the second tumor (G). Beta-catenin (CTNNB1) expression is preserved in both tumors (B, F). Panels D and H show overlay images for the two tumors.

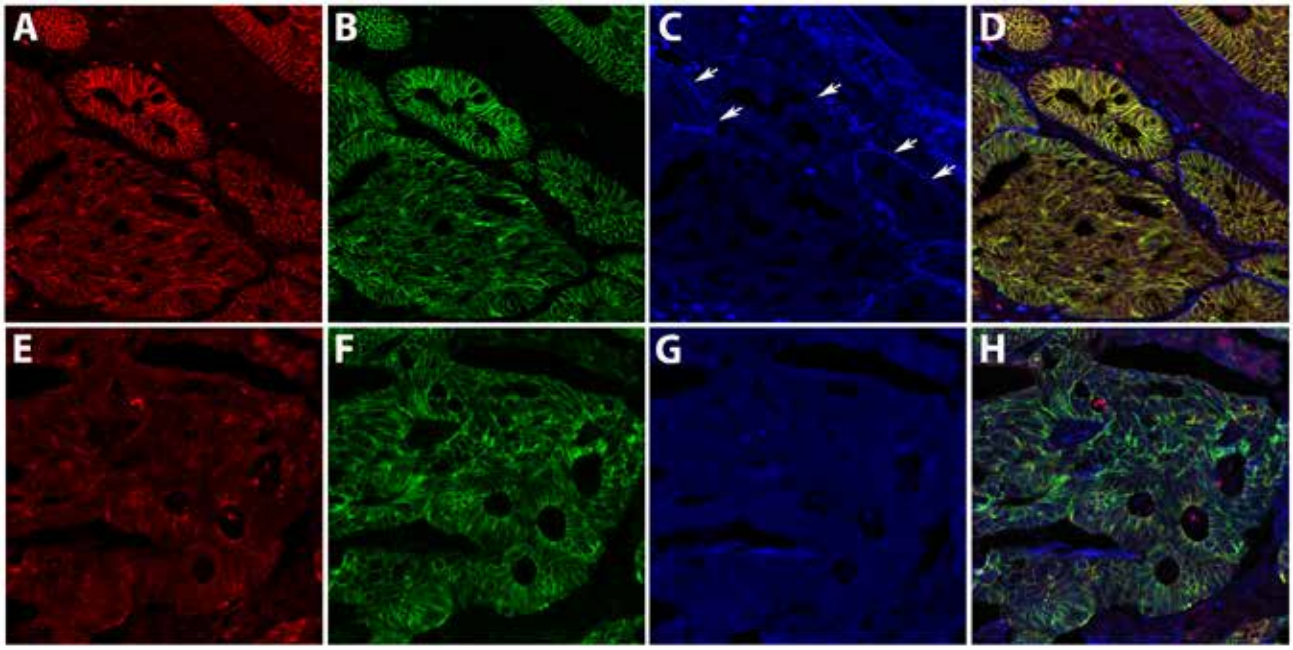

Figure 5.
Multiplexed IHC-IF staining of two colorectal tumors (**A-D** and **E-H**) showing E-cadherin (**A, E**), beta-catenin (**B, F**) and laminin-gamma 1 (**C, G**) immunoreactivity using primary antibodies of different isotypes: Anti-CDH1 AMAb90863, IgG1 (red), Anti-CTNNB1 AMAb91209, IgG2a (green) and Anti-LAMC1 (AMAb91138), IgG2b (blue). Arrowheads in C indicate basement membrane. Alexa Fluor® 647-, 594- and 488-labelled isotype-specific secondary antibodies (Life Technologies) were used for visualisation.

Marker	Product Name	Product Number	Validated Applications	Epitope	Isotype
Cell junctions	Anti-CDH1	AMAb90862	IHC*, WB*	NWTIQYNDPTQESII	IgG2b
Cell junctions	Anti-CDH1	AMAb90863	IHC*, WB*	APIPEPRTIF	IgG1
Cell junctions	Anti-CDH1	AMAb90865	IHC*, WB*	LKPKMALEVG	IgG2a
Cell junctions	Anti-OCLN	AMAb90889	IHC, WB	TSPVDDFRQPRYSSG	IgG2a
Cell junctions	Anti-OCLN	AMAb90890	IHC, WB	NDKRFYPESSYKSTP	IgG2a
Cell junctions	Anti-OCLN	AMAb90893	IHC, WB	RYSSGGNFETPSKRA	IgG1
Cell junctions	Anti-CTNNB1	AMAb91209	IHC, WB	TSQVLYEWEQGFSQS	IgG2a
Cell junctions	Anti-CTNNB1	AMAb91210	IHC, WB	TSQVLYEWEQGFSQS	IgG1
Cell junctions	Anti-CLDN1	AMAb91213	IHC, WB	KTTSYPTPRPYPKPA	IgG1
Cytoskeletal changes	Anti-VIM	AMAb90516	IHC, WB*	N.D.	IgG1
Cytoskeletal changes	Anti-S100A4	AMAb90596	IHC*, WB	KFKLNKSELKELLTR	IgG1
Cytoskeletal changes	Anti-S100A4	AMAb90598	IHC*, WB*, ICC-IF	CNEFFEGFPDKQPRKK	IgG2b
Cytoskeletal changes	Anti-S100A4	AMAb90599	IHC*, WB, ICC-IF	CNEFFEGFPD	IgG1
Transcription regulation	Anti-SNAI1	AMAb91215	IHC*	N.D.	IgG1
Transcription regulation	Anti-ZEB1	AMAb90510	IHC*, WB*, ICC-IF	N.D.	IgG1
Transcription regulation	Anti-SIX1	AMAb90544	IHC, WB*, ICC-IF	N.D.	IgG1
Transcription regulation	Anti-ZNF703	AMAb90789	IHC*, WB*	PGDKAGFRVP	IgG1
Transcription regulation	Anti-TP63	AMAb91224	IHC, WB	MQYLPQHTIETYRQQ	IgG1
Migration/Motility	Anti-CDH2	AMAb91220	IHC*, WB*	ENPYFAPNPK	IgG1
Migration/Motility	Anti-FN1	AMAb91223	IHC, WB	GRWKCDPVDQ	IgG1
Migration/Motility	Anti-MMP9	AMAb90804	IHC, WB	VPDLGRFQTF	IgG1
Migration/Motility	Anti-MMP9	AMAb90805	IHC, WB	RGESKSLGPALLLLQ	IgG1
Migration/Motility	Anti-MMP9	AMAb90806	IHC	RGESKSLGPALLLLQ	IgG2b

*Products with enhanced validation for indicated application

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