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Antibodies and Methods for Immunohistochemistry of Extracellular Matrix Proteins

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Highlights

- Advice and literature on validation, characterization and use of antibodies in immunohistochemistry (IHC)
- Lists of ~200 tested and validated antibodies to ~100 ECM proteins
- Illustrative examples of the application of these antibodies and methods to FFPE tissue sections
- Compilation of online resources

Abstract

The diversity of extracellular matrix (ECM) proteins encoded in mammalian genomes and detected by proteomic analyses generates a need for well validated antibodies against these proteins. We present characterization of a large number of antibodies against ECM proteins, from both commercial and academic sources, together with discussion of methods and strategies for their effective use in immunohistochemistry and illustrations of their efficacy. These data should be of value to investigators seeking well validated antibodies to ECM proteins of interest and save significant time and money tracking down effective reagents.

Keywords

extracellular matrix antibodies, antibody validation, immunohistochemistry

Abbreviations

Ab, antibody; BL, basal lamina; CRC, colorectal cancer; ECM, extracellular matrix; FFPE, formaldehyde-fixed and paraffin-embedded; HIER, heat-induced epitope-retrieval; TMA, tissue microarray

Introduction

The extracellular matrix (ECM) is a fundamental component that surrounds or underlies most cells and tissues of metazoan organisms [1,2]. ECM provides both biophysical and biochemical signals important for cell proliferation, cell apoptosis, differentiation and migration [3,4]. Moreover, the ECM also serves as a depot for soluble growth factors and ECM-remodeling enzymes, whose release can be triggered by different physiological conditions [5,6]. These functions of ECM play crucial roles in embryonic development, homeostasis and in many diseases, such as fibroses, wound healing and scar formation, skeletal disorders and cancer [7-9].

Because of the importance of ECM, it is necessary to understand the composition and dynamics of ECM in various normal and disease states. This has been a challenge because of the insolubility of ECM but recent proteomic approaches have revealed in detail the complexity of the ECM *in vivo*, typically comprising at least 150-200 proteins in any tissue [e.g., 10-20]. These studies have provided lists of potential biomarkers for disease states such as tumors, metastases or fibroses. However, proteomic analyses are not practical for routine use as biomarkers for diagnostic or prognostic applications and there is a need for higher throughput methods to expand screens to larger numbers of samples than are feasible by proteomics. Furthermore, standard proteomic analyses do not provide information about distributions of the proteins detected. Both of these requirements can, in principle, be addressed by antibody-mediated detection methods. However, for many ECM proteins, validated antibodies are limited, although some do exist for well studied major ECM proteins, although by no means all. Genomic and proteomic studies have revealed that there are around 300 ECM proteins encoded in mammalian genomes plus many other proteins that are incorporated into or bound by ECM, such as ECM modulators (modifying enzymes, crosslinkers, proteases) and growth factors and cytokines; collectively the "matrisome" [21]. This complexity necessitates a much broader range of well validated anti-ECM antibodies than has been available.

Immunohistochemistry (IHC) is a widely used technique and can be performed on formaldehyde-fixed and paraffin-embedded (FFPE) tissues allowing access to archival material [22-24]. A key advantage of IHC is that it allows analysis not only of the anatomy of the tissue of interest but also visualization of the spatial distribution and expression of specific antigens or cellular components in a variety of tissue sections. However, the availability of well validated anti-ECM antibodies applicable to FFPE samples is even more limited. For all these reasons,

we have tested numerous antibodies to ECM proteins and developed reliable IHC staining protocols for use on FFPE tissue samples. Here we first provide general insights and recommendations for appropriate antibody characterization needed to set up robust validation and reproducible IHC staining protocols. We then summarize our results on the applicability of over 200 antibodies (most of them commercially available) to over 100 matrisome proteins. In Table 1 we list all antibodies which yielded positive results and in Supplementary Table 1 all antibodies which, in our experiments, either did not yield positive stains or gave uncertain staining results. We further show examples of data for ECM proteins of particular interest in order to illustrate some key aspects relevant to effective application of IHC to ECM proteins (Figures1-8). In addition, we provide a summary of antibodies that are more broadly used in tumor biology and histopathology to determine specific tissue distribution or subcellular localization (Supplementary Table 2). However, we wish to note that while we have tested antibody specificity at the IHC level by the criteria discussed below, we have only tested the molecular specificity of antibodies generated in our own laboratory and some others but not the majority of the commercial antibodies reported here. Finally, we provide useful online references which will assist antibody searching, IHC methodology and troubleshooting guidelines as well as tissue expression data of protein-coding genes in normal tissue and tumor samples from humans and, in some cases, mice (Supplementary Table 3).

Antibody reproducibility issues

Although antibodies are among the most frequently used tools in basic science research and in clinical assays, standardized methods for antibody validation across research applications are not universally applied. As a result, the quality and consistency of data generated through the use of antibodies can vary greatly and increasing concerns have been raised about reproducibility of scientific data using commercially available antibodies [25-30]. Indeed, the high failure rate of commercially available antibodies is concerning and it has been estimated that US$800 million are wasted annually on poorly performing antibodies and about US$350 million are lost in biomedical research because published results cannot be replicated, with underperforming antibodies being a contributor in many cases [31].

Antibody validation recommendations

Although the quality of diagnostic and research antibodies remains a problem [27, 32-34], several attempts to improve antibody validation have been made and very useful

recommendations have been published [e.g., 26, 30, 35-38]. Recently, the International Working Group for Antibody Validation (IWGAV) was established with the goal of improving the standardization and validation of antibodies used in common research applications [28]. This consortium proposed a set of standard guidelines that should help to ensure better antibody reproducibility.

Five conceptual recommendations for antibody validation are: (***1***) genetic strategies, such as testing the antisera against mutant tissues or cells known to lack the protein of interest (***2***) orthogonal strategies, using an antibody-independent method such as mass spectrometry to compare the expression of a target protein (***3***) independent antibody strategies, that is, use of two or more antibodies, ideally from different host species and with non-overlapping epitopes (***4***) testing against expressed recombinant proteins, and (***5***) immunocapture followed by mass spectrometry (see [28] for detailed information). At least one of these approaches and preferably more should be used as a minimum criterion for claiming that a particular antibody has been adequately validated for a specific application.

Additional methods for antibody validation include affinity measurements, epitope mapping, isotype or sequence determination and array-based specificity [28]. Another validation method commonly used for IHC applications is absorption [39], in which the antigen is pre-incubated with the antibody before the immunoassay. Although this method is useful to show target binding, it will not rule out cross-reactivity with proteins containing epitopes similar to those in the intended target. To validate an antibody for IHC, it must be shown to be specific and reproducible in the tissue context in which it will be used. Ideally, experiments using several independent antibodies (if possible raised in different species) to stain for the same molecule should be sought to show specificity, selectivity and reliability on FFPE tissue samples. This approach is more convincing and the data more reliable than using a single antibody [26]. Finally, it is recommended to repeat validation experiments for each new antibody vial or lot to ensure reproducibility.

Immunohistochemistry standardization

Immunohistochemistry (IHC) can be summarized in three major steps: (1) binding of the primary antibody to a specific antigen, (2) formation of an antibody-antigen complex following the addition of a secondary enzyme-conjugated detection antibody and finally, (3) a chromogenic enzyme substrate which leads to generation of a colored deposit at the site of the antibody-antigen complex. However, especially in the context of biomarker development on FFPE tissues

using IHC, standardization can be quite challenging. This is due to several factors known to affect antigenicity and influence subsequent staining procedures, including; varying fixation times, differences in fixative used as well as general handling variability throughout the labeling process. In this context, the need for improved standardization of IHC protocols has been addressed and recommendations and guidelines for IHC have been proposed to achieve reliable and consistent staining results [e.g., 25, 40, 41].

Antigen “recovery” or “retrieval”

Heat-induced epitope-retrieval (HIER) techniques, first developed by Shi et al. [42], are often employed to reveal epitopes that are otherwise difficult to detect following fixation in formalin and paraffin-embedding. Unfortunately, in practice, the application of IHC to routine processed FFPE sections has still proven to be challenging with significant inconsistency of results.

For this reason and to achieve the best staining results, each individual antibody presented here was tested following the recommendations and standardization guidelines for improved antibody validation and IHC optimization mentioned above. Following the proposed ‘test battery’ approach of Shi et al. [43], we screened the individual antibodies for the major variables involved in antigen retrieval. These include heating conditions (temperature and heating time), pH of the retrieval solution, and the retrieval buffer (for details see Supplementary Materials and Methods). Various pH values (pH 1, 6, and 9) of different buffer solutions (including acetate, citrate, phosphate and tris) and different temperatures (high, 120°C using decloaking chamber/pressure cooker or lower using a microwave) can be tested to find optimal staining results.

For most ECM-related antibodies tested, our observations favored HIER for 2 min at 120°C in a decloaking chamber (i.e., pressure cooker) using one of two different buffer conditions, i.e., 10 mM sodium-citrate (pH6.0) or 10 mM Tris (pH9.0) containing 0.05% Tween-20. The preferred condition and dilutions for each antibody tested as well as control tissues used are listed in Table 1. However, we emphasize that each antibody vial, whether in use or newly purchased, should be tested to obtain the best possible results for the tissue of interest. An antibody validated in one buffer system will not necessarily perform similarly in another and may vary depending on the sample used. Moreover, to assess reproducibility of IHC, validated antibody should be run on a variety of tissue samples, either as whole tissue sections or represented on a tissue microarray (TMA; [44]). For the majority of antibodies, we used a panel of previously reported FFPE mouse and human control tissue samples and tested different antibody

concentrations to obtain optimal staining results. This allows comparison of antibody localizations described in the literature with individual staining patterns obtained to assess the selectivity of each antibody as well as the reproducibility of localization data. Of most importance, every experiment should include a positive and negative control to assess performance of antibody and reagents used, ideally using a set of tissue samples with known variable expression levels of the protein of interest [45].

Validation of antibodies to extracellular matrix molecules

Using the established IHC protocols, we have tested numerous antibodies against ECM proteins and summarize our results on the applicability of over 200 antibodies to over 100 matrisome proteins on FFPE samples (see Table 1 for antibodies which yield positive results and Supplementary Table 1 for antibodies with negative or uncertain staining results). Again, we wish to note that we have tested specificity at the IHC level and we have not tested the molecular specificity of every antibody reported here. Readers should also refer to the results provided by the suppliers' datasheets and in the references cited. We would recommend that users particularly interested in specific ECM proteins should also test by molecular methods such as immnunoblotting, immunoprecipitation or absorption.

Finally, since ECM molecules are frequently cross-linked in complexes mediated by multiple domains specialized for protein interactions or specific ECM assembly forms, we have found protease treatment of FFPE sections to be very helpful in improving IHC detection of ECM proteins. We tested several methods for enzyme digestion in order to enhance the antigen exposure and staining results. Commonly used enzymes include trypsin, proteinase K, pronase, ficin, and pepsin [for review see 23]. Enzyme digestion may destroy some epitopes and increases the chance of damaged tissue morphology. However, in particular an additional enzyme digestion step using 0.2% pepsin in 0.2N HCl (Agilent) according to the manufacturers protocol prior to the immunostaining, improved the IHC results for several antibodies (see e.g., Figs. 3-5 and Table 1; see also Franciosi et al. [46]).

The Figures 1-8 which follow illustrate the application of the suggestions discussed above for a variety of ECM proteins and experimental situations and additional examples are included for reference in the Supplementary Material.

Figures and figure legends

Figure 1

Figure 1. Antibodies to individual laminin chains in colorectal cancer (CRC) liver metastasis

Images of representative CRC liver metastasis sections stained with hematoxylin and eosin (H&E, panel A) and the pan-laminin rabbit polyclonal antibody (Novus Biologicals, #NB300-144, panel B), reported to recognize Lm111 and Lm211. Several such polyclonal antibodies exist and stain most basal laminae (BL), as would be expected if they recognize 3-4 different laminin subunits, which among them occur in most laminins (see also Supplementary Figure Gallery pages 77-80). Such antibodies are valuable as markers of BL. However, in order to define precisely which of the 11 known laminin subunits (see [47] for laminin nomenclature) exist in any given BL, more specific reagents are necessary. We tested and validated ten monoclonal antibodies (mAb) and one rabbit polyclonal antibody to 10 of the subunits (see Table 1 and Supplementary Table 1) and representative examples are shown here (the others are shown in Supplementary Figure Gallery pages 81-91). Panels C and D show that the B1 and B2 subunits co-localize in the same BL (B3, found only in Lam332, does not, data not shown). Panels E-H illustrate the diversity of expression in different BL of the A4 and A5 subunits; A5 is found in the same BL as B1 and B2 in one metastasis (panel F, cf. C and D, see *) but not in a metastasis from a different patient (H), whereas A4 is found in the second patient but not the first (E, I). Clearly, full analyses of laminin distributions will require diverse monoclonal antibodies as well as molecular methods. Similar variations in distributions of other BL markers in CRC metastases are shown in Fig. 2.

Figure 2

Figure 2. Diversity of basal lamina composition in normal liver and CRC liver metastasis

IHC on sections of CRC liver metastases from two different patients exhibit different patterns of distribution of collagen IV (panels A, B) and perlecan (HSPG2, panels C, D), two canonical and well-studied basal laminae (BL) components found in healthy and diseased tissues [48,49]. One metastasis shows the typical pattern of BL staining of collagen IV in both the metastasis (T) and the normal liver (L, panel A). In addition, however, the tumor edge of the same metastasis (*) and the metastasis of patient #2 (panel B) show, in addition, widespread staining for collagen IV in the extracellular matrix of the metastasis. In the case of perlecan, it is often seen elsewhere than in BL (see Supplementary Figure Gallery pages 73-74) and that is true for both the metastases shown here, although the patterns of localization differ both between metastasis and liver (C) and between the two patients' metastases (C, D).

Figure 3

Figure 3. Use of pepsin treatment to enhance antibody detection of extracellular matrix proteins

As mentioned in the text, extracellular matrix proteins are highly complexed and crosslinked; this can impede access of antibodies to their epitopes and protease digestion can often help to reveal extracellular matrix antigens that are otherwise occluded [46]. Figure 3 shows this to be the case for several different extracellular matrix proteins. Representative images of mouse and human tissue sections stained for collagen IV (A, B), collagen III (C, D), collagen VII (E, F), LTBP1 (G, H), HMCN1 (I, J) and AGRN (K, L) are shown. In each case, treatment with pepsin reveals the presence of the extracellular matrix protein, which is not, or only weakly, detected using heat-induced epitope-retrieval (HIER) alone. Such protease treatments need to be used with care since over-digestion can destroy epitopes and can lead to damage to the tissue sections. Different protease conditions need to be tested to obtain optimal staining results for each antibody and tissue (see also text). It is worth noting that panels I and J show an example where pepsin treatment reveals hemicentin (HMCN1) staining of the extracellular matrix (J), while at the same time removing cell-associated signal (I).

Figure 4

Figure 4. Differential detection of agrin using antibodies to different epitopes

The large extracellular matrix protein agrin is associated with many basal laminae (BL) and is frequently upregulated in cancer. Antibodies to different epitopes of agrin have been reported to show differential distributions in kidney cryostat sections [e.g., 50,51]. However, IHC detection of agrin in formaldehyde-fixed and paraffin-embedded tissue sections has to date been challenging. As for the other extracellular matrix proteins described above, HIER combined with pepsin treatment greatly enhanced the staining efficacy of agrin antibodies (Fig. 3). However, even after pepsin treatment, anti-agrin antibodies to different epitopes (raised against N- and C-terminus of agrin) reveal diverse IHC staining patterns in several tissues (Fig. 4). Of particular note, although two different antibodies to agrin localize to all blood vessels in sections of mouse brain tissue (Fig. 4A and B), their staining pattern varies among a variety of tissues (Fig. 4C-H). Thus, while the BL of blood vessels (arrows in Fig. 4E, F), serving here as an internal positive control, are positive with both antibodies, the epithelial BLs appear negative for one antibody (panels E and G) while the other antibody clearly stains positive (F and H). These results underline the value of using more than one antibody to detect extracellular matrix proteins reliably.

Figure 5

Figure 5. Uses of species-specific antibodies to define the sources of extracellular matrix proteins

In xenograft models, it is of particular interest to determine which extracellular matrix proteins are made by the transplanted cells or tissues and which by the host. In addition to the potential use of *in situ* hybridization, species-specific antibody staining can provide valuable information. First, antibodies that distinguish cells of the two species involved can be used to distinguish transplant from host (A, B). Here, antibodies to human-specific anti-Lamin are used to mark orthotopic transplants of human CRC organoids (T) to mouse colon (A) and their resulting liver metastases (B, see also [52]). Other human-specific antibodies, including anti-human vimentin or anti-human mitochondria antibodies can similarly be used and we list several antibodies, useful for such analyses in Supplementary Table 2. Second, species-specific antibodies to specific extracellular matrix proteins can be used to define their source as shown for agrin in panels C-F. Panels C and D show staining with an antibody recognizing both mouse and human agrin, which stains agrin in the basal laminae (BL, arrowheads) of the human CRC organoid transplant (T) and also in the blood vessels of the surrounding normal mouse colon and liver tissue (arrows). In contrast, antibody specific for mouse agrin (E, F) recognizes only the host blood vessels but not the BL of the human CRC organoid transplant (T). Clearly in this context the majority of agrin is made by the transplanted organoids (boxes mark regions selected for inserts).

Figure 6

Figure 6. Insights from immunohistochemistry extend proteomic characterizations of tumor-related extracellular matrix proteins

Proteomic analyses have identified a set of extracellular matrix proteins predominantly expressed in human primary CRC tumors or metastases to liver and little or not at all in normal colon and liver samples [14]. Staining with antibodies to a number of these extracellular matrix proteins not only confirms their presence in CRC metastases to liver (Fig. 6A) but also provides additional information (Fig. 6B). Many core matrix proteins are detected in the extracellular matrix as expected (e.g., LTBP2, THBS2, HMCN1) but some, including surfactant protein D (SFTPD), are intracellular and others such as SERPINE2 and TIMP1 show distributions either in the extracellular matrix or intracellularly, or both (B). The proteomic definition of these proteins as extracellular matrix-associated matrisome proteins

relies on their insolubility, the IHC results show that is not always a reliable indicator that they are actually bound to the extracellular matrix.

Figure 7

Figure 7. Spatial distributions of extracellular matrix proteins

Although many matrix molecules are detected by proteomics in CRC metastases to liver, IHC shows that tumor-associated extracellular matrix proteins (e.g., LTBP2 as shown here) can be located in different parts of the metastases. LTBP2 can be widely distributed throughout the extracellular matrix of the metastasis (A, B), or only in the surrounding capsule (C, D) or in localized parts of another metastasis (E, F). These different distributions are likely to have different biological consequences.

Figure 8

Figure 8. Differential localization and expression patterns of extracellular matrix proteins during tumor progression

We also validated antibodies against proteins or specific splice variants of proteins often found during tumor development and metastasis. This figure shows two such proteins; tenascin C (B, E) and the EIIIB splice isoform of fibronectin (C, F) in human CRC-derived liver metastases (A-C) and in orthotopic CRC organoid transplants. Both proteins are clearly expressed in the tumor tissue (T) of the CRC liver metastases and at the invasive front (B, C) but are absent in the surrounding normal liver (L). Both proteins also show positive staining in the surrounding extracellular matrix of the CRC-derived organoids (T) and are present in the invading edges (inserts in D-F) of this CRC mouse model. Given the defined species specificity on human and mouse tissues for many antibodies (see Table 1), such antibodies can be used to study differential localizations in human tissue samples in comparison to available mouse models to decipher their potential involvement during tumor formation, progression and metastasis.

Useful links and resources

Since only limited numbers of antibodies to ECM proteins applied to FFPE samples have been described in the literature and we certainly do not cover all of them in our validation attempts, we have compiled a list of valuable online resources that will assist researchers in tracking down additional antibodies (Supplementary Table 3). Antibody product information and validation data can sometimes be difficult to decipher and, with hundreds of vendors to choose from, it becomes difficult to know when a search has been exhaustive. The summarized search engines will help to find and identify antibodies that could work for a particular application, and in some instances, compare antibodies from many different vendors. This saves valuable time otherwise spent visiting each vendor's website and allows extension of the search to novel suppliers. In addition, we provide useful online references relevant for proper antibody characterization, IHC standardization and methodology as well as troubleshooting guidelines (Supplementary Table 3).

Moreover, thanks to large open-access knowledge-based international efforts, such as the Human Protein Atlas (HPA, https://www.proteinatlas.org; [54-56]) or the GTEx consortium (https://www.gtexportal.org/, G.T. Consortium [57]) it is now possible to explore the expression of protein-coding genes in normal human tissues. In addition, the recently launched new open-access resource: Human Pathology Atlas as part of the HPA (www.proteinatlas.org/pathology), allows researchers to explore the possible prognostic value of all human protein-coding genes related to expression levels in different forms of human cancer, presenting Kaplan-Meier survival plots for all protein-coding genes in 17 different tumor types.

We also wish to highlight the Matrixome project, (http://dbarchive.biosciencedbc.jp/archive/matrixome/bm/home.html; [58,59]), which contains the mouse basement membrane bodymap, a database of body-wide localizations of basal lamina proteins in developing mouse embryos. Finally, the Matrisome project (http://matrisomeproject.mit.edu; [12,14]) provides, in addition to the methods and datasets available in current publications, valuable protocols and resources relevant to research on ECM proteins. In particular, the 2016 release of the ECM Atlas, an effort aimed at compiling mass spectrometry data from studies designed specifically to characterize the composition of the ECM of normal and diseased samples [14] is a useful resource to search for the distribution of ECM and ECM-associated molecules in a variety of tissues.

Conclusions

It is our hope that the guidance on reagents and methods and the illustrative examples of their application in studies of the ECM and its constituent and associated proteins will prove to be helpful to ECM scientists in their investigations. Not least, we hope that the data on well validated antibodies will save time and funds that would otherwise be expended finding appropriate antibodies for future investigations.

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Author Contributions

Conceived and designed the experiments: SR, ROH.

Performed the experiments: SR.

Analyzed the data: SR, ROH.

Wrote the paper: SR, ROH.

Disclosures

The authors declare that they have no competing financial interests.

Table 1 - Legend

Extracellular matrix-related antibodies

Antibodies to extracellular matrix proteins tested on formaldehyde-fixed and paraffin-embedded tissue samples using immunohistochemistry (IHC). This table provides an overview of all tested antibodies that showed positive staining signals. Presented are our IHC validation information and results, however, we have not validated the molecular specificity of every antibody (see text).

Matrisome division (CM, core matrisome; MA, matrisome-associated); matrisome category (COL, collagens; GP, ECM glycoproteins; PG, proteoglycans; REG, ECM regulators; SF, secreted factors; AP, ECM-affiliated proteins). Antibody host species (gt, goat; m, mouse; rb, rabbit; rt, rat; sh, sheep); mAb monoclonal antibody.

Heat-induced epitope retrieval (HIER) method used (0.05% Tween in Citric acid, pH6.0 or Tris, pH9.0) or protease treatment (Tris, pH9.0 + Pepsin treatment); for details see Supplementary Materials and Methods. Individual antibody dilutions recommended, the human and mouse reactivity as well as a positive control tissue, additional notes and references are listed. Additional abbreviations used: CRC, colorectal cancer; NT, not tested; PDAC, pancreatic ductal adenocarcinoma; TEB terminal end buds.

* Indicates the preferred HIER method applied for the individual antibody, however, other conditions also might work.

** Indicates the antibody dilution we have tested; however, each antibody vial should be tested for individual application and tissue since lot-specific variability can occur. For additional details on the individual antibodies see also the manufacturers datasheets.

‡ A representative image of the staining with the antibody is shown in the Supplementary Figure Gallery.

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Supplementary Materials and Methods

Human and mouse tissue samples

All human samples were kindly provided by collaborators from the Department of Pathology and the Cancer Center of the Massachusetts General Hospital (Boston, MA; V. Deshpande, G. Lauwers, M. Mino-Kenudson, K. Tanabe) as well as the Department of Medical Oncology of the Dana-Farber Cancer Institute (Boston, MA; S. Ogino). In addition, formaldehyde-fixed and paraffin-embedded (FFPE) human tissue blocks were purchased from Genvelop Life Science (Westborough, MA). Various mouse tissue samples were obtained from the animal facilities of the David H. Koch Institute for Integrative Cancer Research (Cambridge, MA) and processed for FFPE following standard procedures or snap-frozen and stored at -80°C until use.

Antibodies

All antibodies to extracellular matrix (ECM) and ECM-associated molecules used in this study are listed in Table 1 and Supplementary Table 1. In Table 1 we list all antibodies which yield positive staining results and in Supplementary Table 1 all antibodies which, in our experiments, either did not yield positive stains or gave uncertain staining results. Additional antibodies that are more broadly used in tumor biology and histopathology to determine specific tissue distribution or subcellular localization are listed in Supplementary Table 2.

Commercially available antibodies were from: Abcam (Cambridge, MA), ABclonal (Woburn, MA), Abgent (San Diego, CA), Agilent (Santa Clara, CA), Atlas Antibodies (Bromma, Sweden), Bethyl Laboratories (Montgomery, TX), BD Biosciences (San Jose, CA), Biocare Medical (Pecheco, CA), Biolegend (San Diego, CA), biorbyt (San Francisco, CA), Boster (Pleasanton, CA), Cell Signaling (Danvers, MA*)*, Developmental Studies Hybridoma Bank (DSHB, Iowa City, IA), Emfret Analytics (Eibelstadt, Germany), GeneTex (Irvine, CA), Leica Biosystems (Nussloch, Germany), LSBio (Seattle, WA), MilliporeSigma (Burlington, MA), Novus Biologicals (Littleton, CO), PROGEN Biotechnik (Heidelberg, Germany), Proteintech (Rosemont, IL), OriGene (Rockville, MD), R&D Systems (Minneapolis, MN), Rockland Immunochemicals (Limerick, PA), Santa Cruz Biotechnology (Santa Cruz, CA), Sigma-Aldrich (St. Louis, MO), Sino Biological (Wayne, PA), sdix (Newark, DE), Thermo Fisher Scientific (Fremont, CA),

Several well-established antibodies for validation on mouse and human FFPE samples were kindly provided by the following: rabbit anti-Agrn antibody (M.A. Ruegg, Biozentrum, University

of Basel, (Basel, Switzerland), rabbit anti-Agrn antibody (J.H. Miner, Washington University, St. Louis, MO), rabbit anti-LOXL2 antibodies (G. Neufeld, Technion, Israel Institute of Technology, Haifa, Israel), rabbit anti-COMP antibodies (Å. Oldberg Lund University, Lund, Sweden), rabbit anti-LTBP2 antibodies (M. Hyytiäinen, University of Helsinki, Helsinki, Finland) and several rabbit anti-SPP1 antibodies (D.R. Senger, Beth Israel Deaconess Medical Center, Boston, MA). Anti-FN monoclonal and polyclonal antibodies generated in the Hynes laboratory are also listed in Table 1.

Anti-fibronectin-EIIIB monoclonal antibody generation

Mice null for an alternatively spliced domain of fibronectin, FN-EIIIB domain (also known as ED-B), were immunized with recombinant EIIIB segment coupled to keyhole limpet hemocyanin (KLH). Lymphoblasts from seropositive mice were used to generate hybridomas, screened against recombinant FN fragments to identify clones selective for EIIIB and tested against cultured endothelial cells and mouse embryos (WT or EIIIB-null) by immunofluorescence microscopy (McMahon, A., unpublished).

Immunohistochemistry

All tissues used in this study were processed for FFPE following standard procedures. Consecutive sections (4-6 μm) were prepared using a Leica RM2255 rotary microtome (Leica Biosystems, Nussloch, Germany), dried at 60°C for 1h and stored at room temperature. The individual sections were dewaxed, rehydrated and either stained with hematoxylin and eosin (H&E) following standard procedures or treated with heat-induced antigen-retrieval (HIER) using a decloaking chamber (Biocare Medical, Pecheco, CA) prior to immunostaining. To achieve the best staining results, each individual antibody was tested following recommendations and guidelines for improved antibody validation and immunohistochemistry (IHC) optimization [e.g., 1-13].

We screened different antibody concentrations and adapted our protocols according to the 'test battery' approach of Shi et al. [1] to optimize the conditions for the major variables involved in antigen retrieval. These include the heating conditions, temperature and heating time, the pH of the retrieval solution, and the retrieval buffer. Various pH values (pH 1, 6, and 9) of different buffer solution (including acetate, citrate, phosphate, and tris) and different temperatures of super high 120°C (decloaking chamber, pressure cooker) or high 100°C (microwave, MW) and 98°C (MW) can be tested to find optimal results [see also 1,2,6,11].

For most ECM-related antibodies tested we found best results with heat-induced epitope-retrieval (HIER) at 120°C for 2 min using either of two buffer conditions (0.05% Tween in 10 mM sodium-citrate at pH6.0 or pH9.0). However, we also tested the UNI-TRIEVE (Innovex Biosciences, Richmond, CA) mild temperature retrieval solution formulated for non-boiling and gentle retrieval of antigens in 'fragile' FFPE tissue sections. UNI-TRIEVE solution is pH-independent and formulated to unmask antigens at 60°C-80°C using a water bath or a hot plate as the heating source. An incubation of 30 minutes yielded good tissue morphology and was sufficient to validate some antibodies including, e.g., BMP1, COMP, LOXL2, SPP1 (Supplementary Figure Gallery), on mouse and human cartilage tissue sections.

After the HIER step, the sections were cooled to room temperature in phosphate-buffered saline (PBS) for 10 min before further processing. To inactivate all endogenous peroxidase and alkaline phosphatase activity in the tissue, sections were subsequently pretreated using BLOXALL endogenous enzyme blocking solution (Vector Laboratories, Burlingame, CA) for 10 min. Prior to application of primary antibodies, incubation with normal serum to prevent non-specific binding of the antibodies to the tissue or to Fc receptors is recommended. Proteins such as BSA or casein may also be used as blocking solutions. In addition, the Mouse on Mouse (M.O.M.) basic kit (Vector Laboratories) can be applied to reduce the endogenous mouse Ig staining when using mouse primary antibodies on mouse tissue sections to yield cleaner staining results. Subsequently, the sections were incubated with the individual primary antibodies for 1h, followed by the secondary detection system according to the manufacturers' protocol.

Although the Avidin-Biotin Complex (ABC) method using 3,3'-diaminobenzidine (DAB) as substrate is widely used as a technique for IHC, we repeatedly noticed varying background staining intensities probably due to the fact that many of the antibodies are polyclonal antisera. To simplify and optimize the staining conditions and to increase the IHC sensitivity, we optimized a variety of secondary detection systems as well as enzyme substrates. Polymerized reporter enzyme staining systems have recently been developed which provide greater sensitivity than conventional antibody conjugates. This approach is based on a new method of attaching a micropolymer with a high density of active enzyme to a secondary antibody. This results in strong staining signal with low IHC background staining and reduced non-specific binding. We predominantly used the ImmPRESS polymer detection systems (Vector Laboratories), which is a ready-to-use, biotin-free detection system. Due to the one-step

incubation this approach also shortens the general assay time and we observed improved staining outcomes with high sensitivity and signal intensity and less non-specific background staining. For primary antibodies generated in sheep, horseradish-peroxidase-conjugated rabbit anti-sheep secondary antibodies (Thermo Fisher Scientific) were used instead of the ImmPRESS polymer detection system. Subsequently, the Vulcan Fast Red Chromogen Kit 2 (red staining; Biocare Medical, Concord, CA) or the DAB Quanto System (brown staining; Thermo Fisher Scientific) were applied as substrates. Hematoxylin was used as final counterstain.

To obtain consistent and reliable staining results on all tissues investigated, we adapted our protocols and used an automated staining system (LabVision Autostainer 360, Thermo Fisher Scientific) for most of the immunostainings presented. For image documentation the Leica Aperio AT2 slide scanner system was used. Appropriate positive and negative controls were performed for each batch of slides using a variety of mouse and human tissue sections. The preferred antigen retrieval conditions and dilutions for each antibody tested are listed in Table 1, Supplementary Tables 1 and 2.

Although we have not attempted quantification of the IHC staining we strongly recommend that all samples to be compared with one another should be processed using consistent staining reagents and conditions, ideally processed together whenever possible. There are available software programs for quantification of IHC staining but we have not tested them. We note that the large number of variables involved in IHC staining of FFPE tissues (often obtained from different sources using variable and often unknown fixation and embedding methods) can render quantification very challenging and rigorous control of fixation and staining conditions would be necessary to obtain reliable quantification.

Supplementary Table 1 - Legend

Additional extracellular matrix-related antibodies

Antibodies to extracellular matrix proteins tested on formaldehyde-fixed and paraffin-embedded tissue samples using immunohistochemistry (IHC), which, in our experiments, either did not yield positive stains or gave uncertain staining results. However, other antigen-retrieval or heat-induced epitope retrieval (HIER) approaches or tissue samples might yield appropriate staining results.

This table provides an overview of additional antibodies for which none of the HIER conditions (for details see Supplementary Materials and Methods) we tested showed a positive staining reactivity or we observed a 'non-specific' staining pattern. Each antibody was tested for a range of dilutions on a variety of tissues. However, since lot-specific variability can occur, other antibody samples should be tested for the individual application and tissue of interest. For additional details see also the antibody manufacturers datasheets.

Matrisome division (CM, core matrisome; MA, matrisome-associated); matrisome category (COL, collagens; GP, ECM glycoproteins; PG, proteoglycans; REG, ECM regulators; SF, secreted factors; AP, ECM-affiliated proteins). Antibody host species (gt, goat; m, mouse; rb, rabbit); rt, rat; mAb monoclonal antibody. Additional abbreviations used: NT not tested; DSHB, Developmental Studies Hybridoma Bank

Supplementary Table 2 - Legend

Reference antibodies

List of antibodies broadly used in tumor biology and histopathology to identify specific tissue distribution or subcellular localization on formaldehyde-fixed and paraffin-embedded tissue samples using immunohistochemistry.

This table provides an overview of all antibodies which reproducibly showed positive staining signals in our hands. Presented are our validation information and results, however, we have not tested each antibody for molecular specificity.

Indicated are the individual: protein name, gene symbol, antibody host species (gt, goat; m, mouse; rb, rabbit; rb mAb, rabbit monoclonal antibody; rt, rat), commercial antibody source and catalog number and heat-induced epitope retrieval (HIER) methods used (see also Supplementary Materials and Methods), recommended antibody dilutions, human and mouse reactivity as well as a positive control tissue and, if applicable, the targets of each antibody.

* Indicates the preferred HIER method applied for the individual antibody, however, other conditions also might work. For many antibodies both HIER conditions work alike.

** Indicates the antibody dilution we have tested; however, each antibody vial should be tested for individual application and tissue accordingly since lot specific variability can occur.

Supplementary Table 3 - Legend

Online resources

Lists of useful online resources relevant for antibody searching, antibody characterization, IHC standardization and methodology, troubleshooting guidelines as well as links to tissue expression data of protein-coding genes in normal and tumor tissues.

Supplementary Figure Gallery - Legend

Illustrative examples for observed staining patterns of many antibodies to extracellular matrix proteins. The images are alphabetically arranged by gene name and show the reactivity of the individual antibodies on representative human tissue samples, in some cases also for mouse tissues. In addition, the antibody host, supplier and catalog numbers are indicated. All images presented here are indicated in Table 1, which contains the individual staining procedure used for each antibody.

Supplementary Materials and Methods - References

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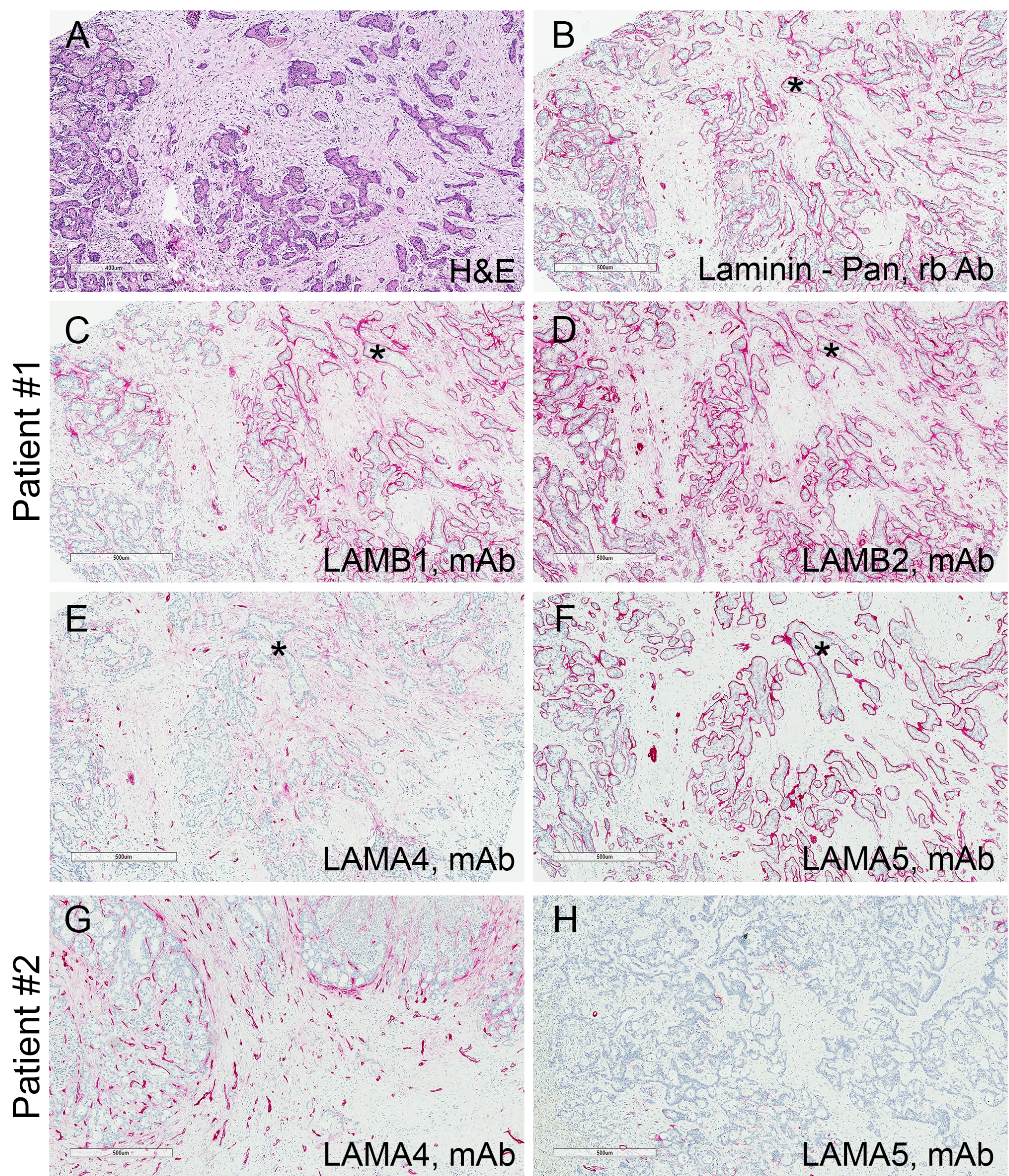


Figure 1

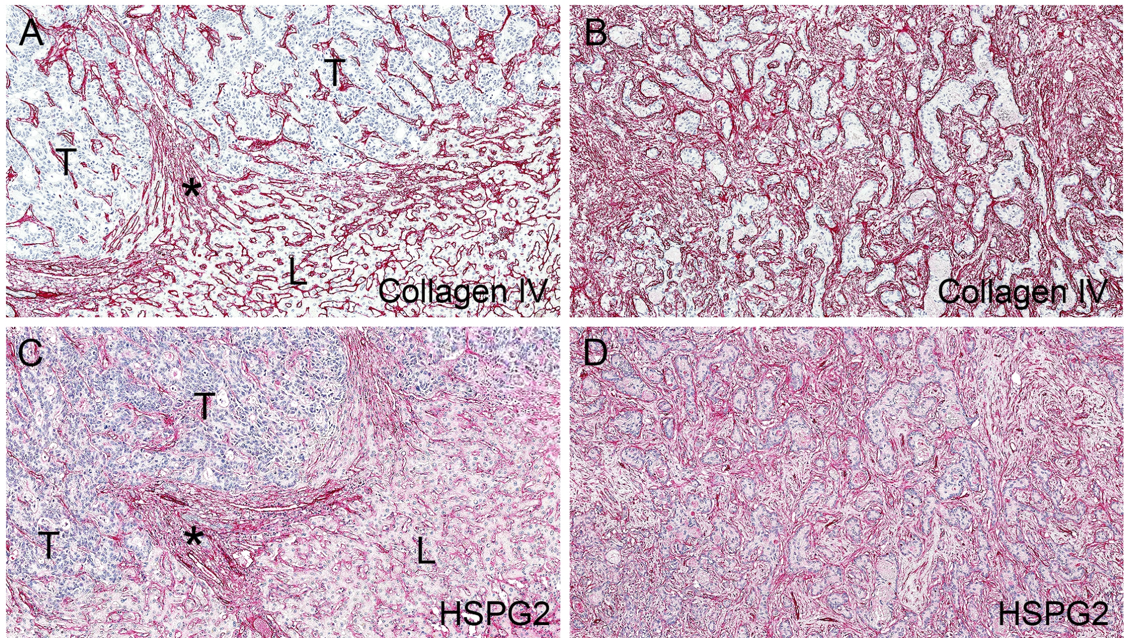
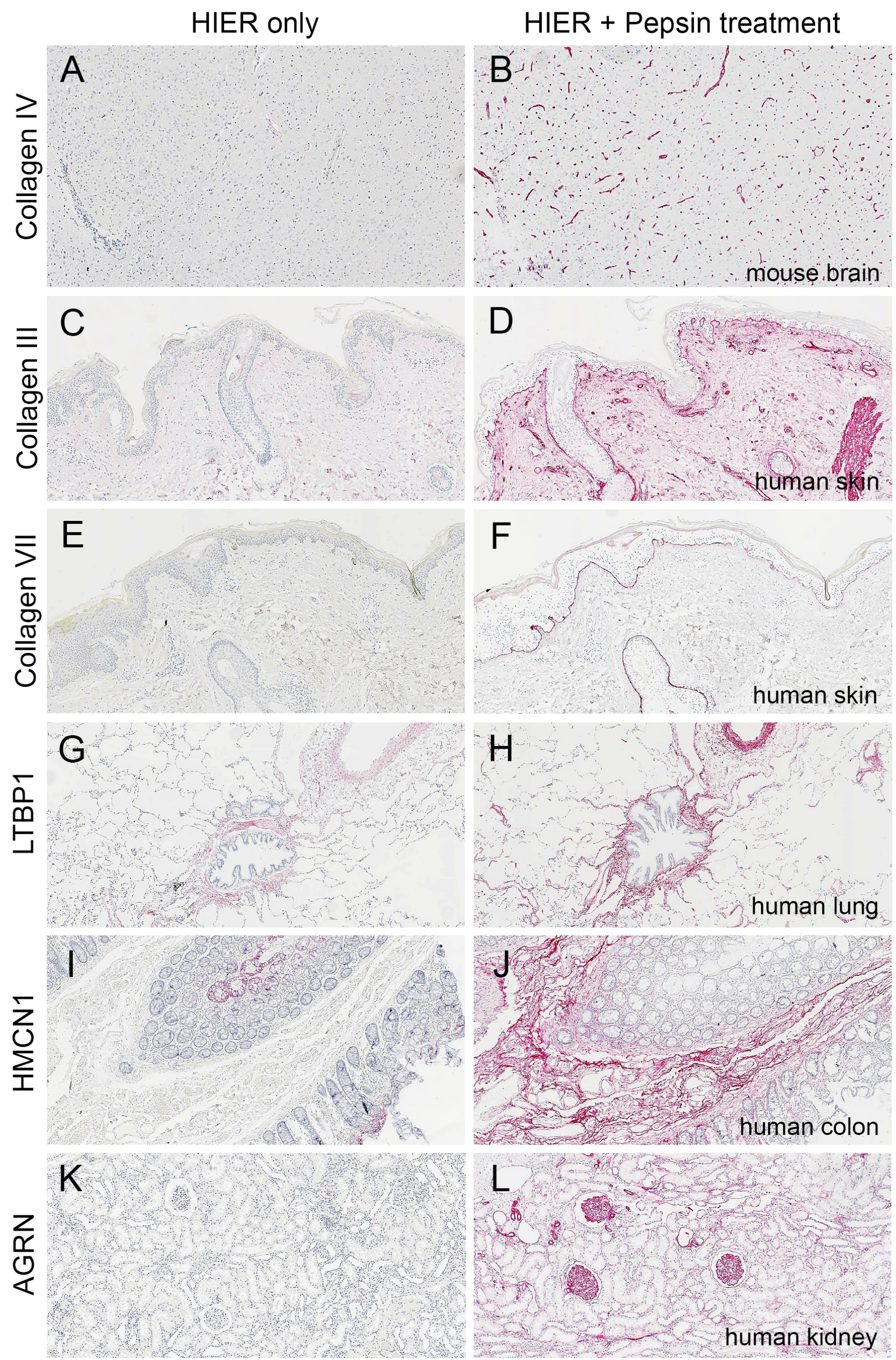


Figure 2



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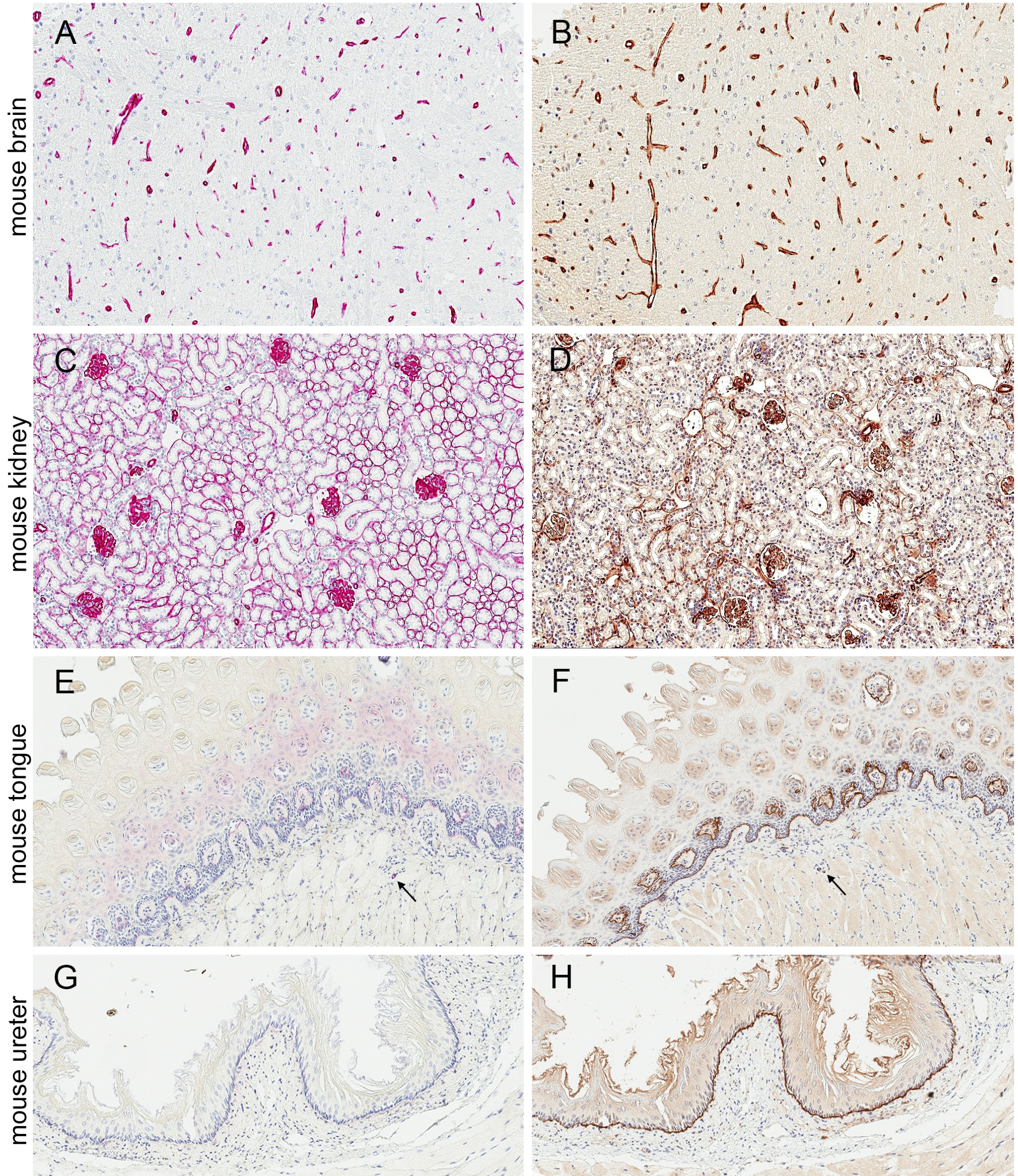

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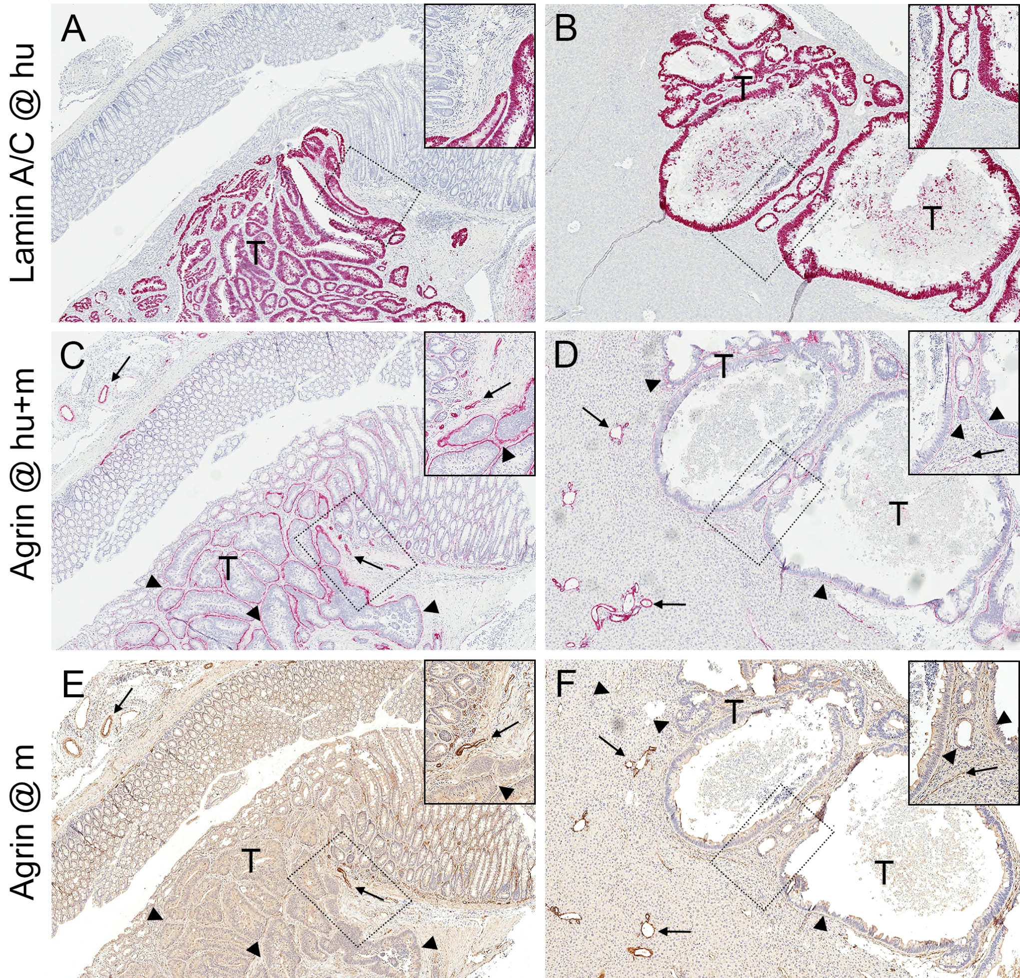
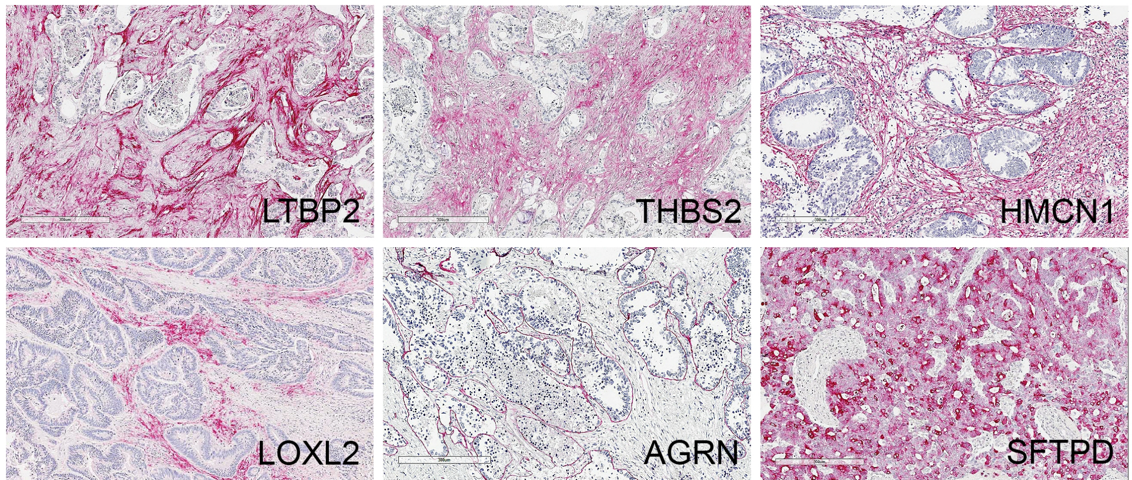


Figure 5



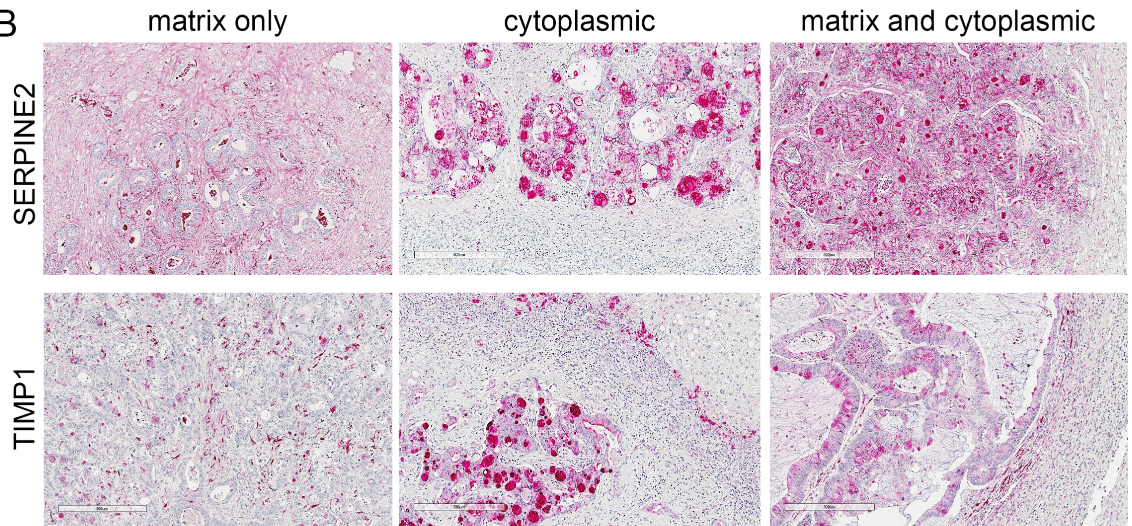


Figure 6

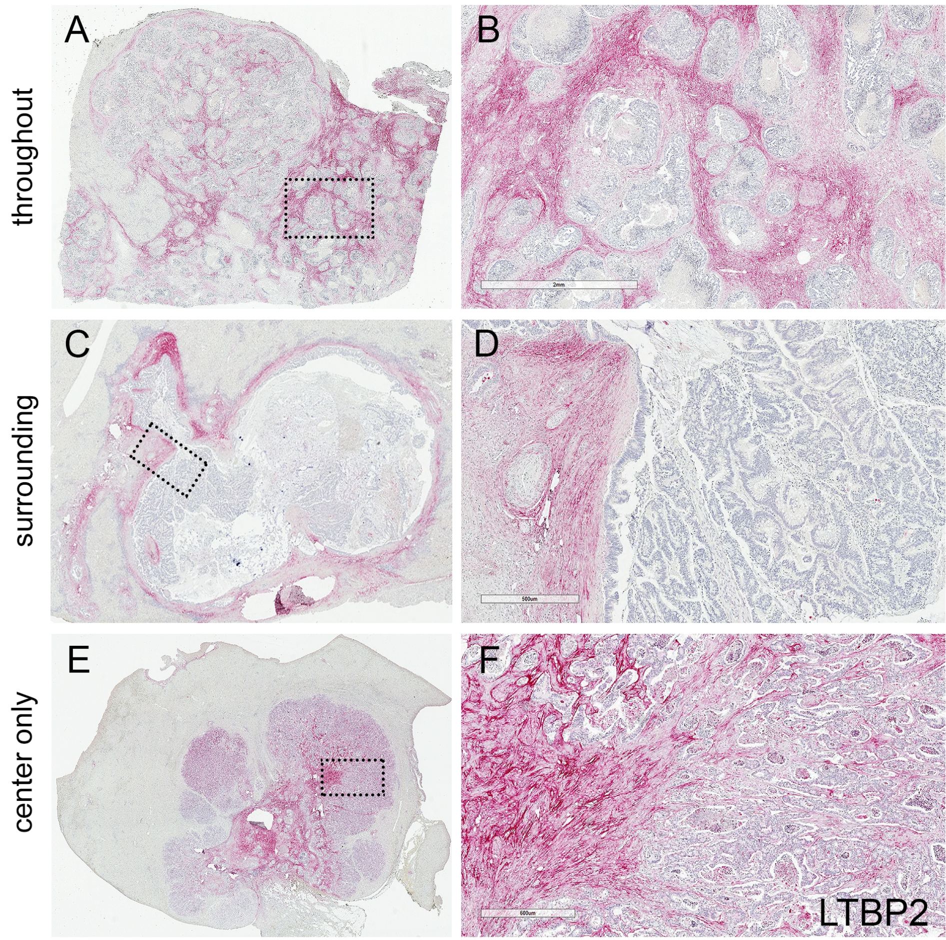


Figure 7

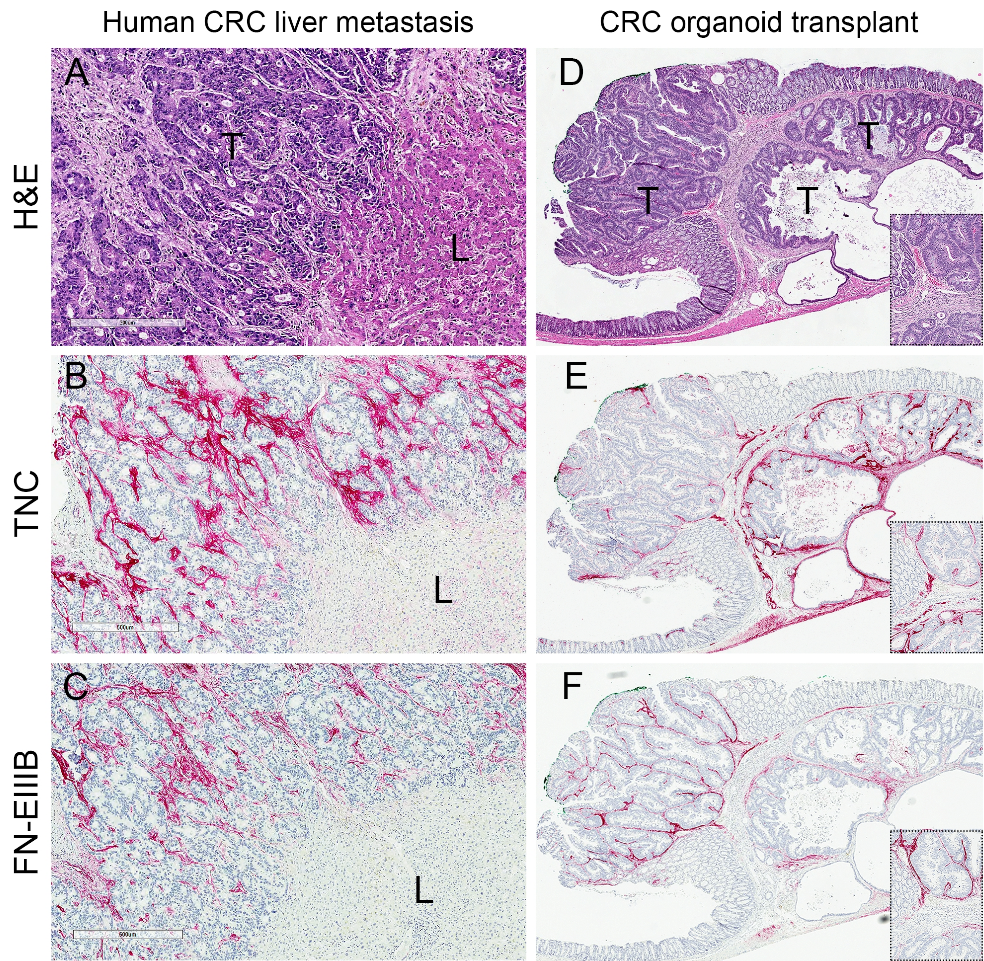


Figure 8

Table 1 - Extracellular matrix-related antibodies

Antibodies to extracellular matrix proteins tested on formaldehyde-fixed and paraffin-embedded tissue samples using immunohistochemistry (IHC). This table provides an overview of all tested antibodies that showed positive staining signals. Presented are our IHC validation information and results, however, we have not validated the molecular specificity of every antibody (see text).

Matrisome division (CM, core matrisome; MA, matrisome-associated); matrisome category (COL, collagens; GP, ECM glycoproteins; PG, proteoglycans; REG, ECM regulators; SF, secreted factors; AP, ECM-affiliated proteins). Antibody host species (gt, goat; m, mouse; rb, rabbit; rt, rat; sh, sheep); mAb monoclonal antibody.

Heat-induced epitope retrieval (HIER) method used (0.05% Tween in Citric acid, pH6.0 or Tris, pH9.0) or protease treatment (Tris, pH9.0 + Pepsin treatment); for details see Supplementary Materials and Methods. Individual antibody dilutions recommended, the human and mouse reactivity as well as a positive control tissue, additional notes and references are listed.

Additional abbreviations used: CRC, colorectal cancer; NT, not tested; PDAC, pancreatic ductal adenocarcinoma; TEB terminal end buds.

* Indicates the preferred HIER method applied for the individual antibody, however, other conditions also might work.

** Indicates the antibody dilution we have tested; however, each antibody vial should be tested for individual application and tissue since lot-specific variability can occur. For additional details on the individual antibodies see also the manufacturers datasheets.

‡ A representative image of the staining with the antibody is shown in the Supplementary Figure Gallery.

Protein name	Gene Symbol	Matrisome		Host	Company	Catalog number	HIER method *	Dilution **	Human reactivity	Mouse reactivity	Positive control tissue	Additional notes	Suppl. Figure Gallery page #
		Division	Category										
ADAM metallopeptidase 12	**ADAM12**	MA	REG	rb	Proteintech	14139-1-AP	Tris, pH9.0	1:200	YES ‡	YES	CRC, mouse TEB		2
ADAMTS metallopeptidase 17	**ADAMTS17**	MA	REG	m	Novus Biologicals	H00170691-M01	Tris, pH9.0	1:3000	YES ‡	YES - but background stain	CRC, mouse TEB		3
agrin	**AGRN**	CM	GP	rb	Novus Biologicals	NBP1-90209	Tris, pH9.0 + Pepsin	1:400	YES ‡	YES ‡	kidney, lung		4, 5
				rb	Abcam	ab85174	Tris, pH9.0 + Pepsin	1:1000-1:2000	YES ‡	YES ‡	kidney, lung		6, 7
				rb	Santa Cruz Biotechnology	sc-25528	Tris, pH9.0 + Pepsin	1:100	YES ‡	NO	kidney, lung		8
				rb	M.Ruegg	AS204	Tris, pH9.0 + Pepsin	1:2000-1:3000	occasional basement membrane stain	YES ‡	kidney, lung	works on frozen tissues / [ref.59]	9
				gt	R&D Systems	AF550	Tris, pH9.0 + Pepsin	1:250-1:500	occasional basement membrane stain	YES ‡	kidney, lung	works on frozen tissue sections	10
angiopoietin-like 4	**ANGPTL4**	MA	SF	rb	Abcam	ab196746	Tris, pH9.0	1:100	YES ‡	NT	placenta		11
annexin A5	**ANXA5**	MA	AP	rb, mAb	Abcam	ab108321	Tris, pH9.0	1:200-1:400	YES ‡	NO	colon, CRC		12
				rb	Abcam	ab14196	Tris, pH9.0	1:1000	YES	NT	colon, CRC		
				rb	Boster	PA1008	Tris, pH9.0	1:200	YES	NT	colon, CRC		
bone morphogenetic protein1	**BMP1**	MA	REG	rb	Abcam	ab118520	Tris, pH9.0	1:200	YES ‡	YES ‡	cartilage, ovary, mouse tail		13, 14
bone morphogenetic protein 2	**BMP2**	MA	SF	rb	Abcam	ab82511	Citric, pH6.0	1:250	YES ‡	NT	CRC		15
C1q and TNF related protein 5	**C1QTNF5**	MA	AP	rb	Abcam	ab36893	Tris, pH9.0	1:1500	YES ‡	NT	colon, pancreas		16
cartilage oligomeric matrix protein	**COMP**	CM	GP	rb	A.Oldberg	-	Tris, pH9.0	1:2000	YES	YES ‡	cartilage, CRC, PDAC	[ref.60]	17
				rb	Abcam	ab74524	Tris, pH9.0	1:250	YES ‡	YES ‡	cartilage, CRC, PDAC		18, 19
				rt	Abcam	ab11056	Tris, pH9.0	1:10	YES ‡	NO	cartilage, CRC, PDAC		20
CD109 molecule	**CD109**	MA	REG	rb	Sigma-Aldrich	HPA009292	Tris, pH9.0	1:100	YES ‡	NT	PDAC, skin		21
chemokine CXCL12/SDF1	**CXCL12**	MA	SF	m	R&D Systems	MAB350-100	Citric, pH6.0	1:250	YES - but weak	NT	placenta, mammary tissue		
				rb	Abcam	ab9797	Citric, pH6.0	1:200	YES ‡	YES	placenta, mammary tissue		22
chondroitin sulfate	**-**	MA	AP	m	Sigma-Aldrich	C8035	Citric, pH6.0	1:1000	YES ‡	YES - but weak	placenta		23
chondroitin sulfate proteoglycan 4 (NG2)	**CSPG4**	MA	AP	rb	MilliporeSigma	AB5320	Tris, pH9.0	1:50-1:100	YES ‡	NT	CRC		24
"collagen, type I Pan"	**-**	CM	COL	m	Abcam	ab88147	Tris, pH9.0	1:500	YES ‡	NO	intestine, liver, lung		25
				rb	Abcam	ab21286	Tris, pH9.0	1:2000	NT	YES ‡	intestine, liver, lung		26
				rb	MilliporeSigma	AB765P	Tris, pH9.0	1:100-1:250	NO	YES - but weak	mouse intestine or lung		
collagen, type I, α1	**COL1A1**	CM	COL	sh	R&D Systems	AF6220	Tris, pH9.0	1:75	YES ‡	YES - but weak	endometrium, PDAC		27
				rb	Rockland Immunochemica	600-401-D19	Tris, pH9.0 + Pepsin	1:250	YES	NO	endometrium, PDAC		
collagen, type III, α1	**COL3A1**	CM	COL	rb	Abcam	ab7778	Tris, pH9.0 + Pepsin	1:500-1:1000	YES ‡	NT	endometrium, skin		28

"collagen, type IV Pan"	**-**	CM	COL	rb	Abcam	ab6586	Tris, pH9.0 + Pepsin	1:2000-1:4000	YES ‡	YES ‡	most tissues		29, 30
"collagen, type VI Pan"	**-**	CM	COL	rb	MilliporeSigma	AB7821	Tris, pH9.0	1:2000-1:4000	YES ‡	YES ‡	ovary, kidney, intestine		31, 32
collagen, type VII, α1	**COL7A1**	CM	COL	rb	Abcam	ab93350	Tris, pH9.0 + Pepsin	1:250	YES ‡	NO	skin		33
collagen, type XIII, α1	**COL13A1**	CM	COL	rb	Novus Biologicals	NBP2-13854	Tris, pH9.0	1:250	YES ‡	YES ‡	mammary tissue, skin, tongue		34, 35
collagen, type XXII, α1	**COL22A1**	CM	COL	rb	Abcam	ab121846	Tris, pH9.0	1:200	NO	YES ‡	muscle-tendon junction		36
complement component C1qA	**C1QA**	MA	AP	rb, mAb	Abcam	ab189922	Tris, pH9.0	1:200-1:400	YES ‡	NT	CRC, kidney		37
CYR61 (cysteine-rich, angiogenic inducer, 61)	**CYR61**	CM	GP	rb	Novus Biologicals	NBP1-35742	Citric, pH6.0	1:50-1:100	YES ‡	NT	placental membrane		38
cystatin B	**CSTB**	MA	REG	rb	Abcam	ab53725	Tris, pH9.0	1:7500	YES ‡	YES	endometrium, placenta		39
decorin	**DCN**	CM	PG	rb	LSBio	LS-B8177	Tris, pH9.0	1:400	YES ‡	NT	kidney		40
dermatopontin	**DPT**	CM	GP	rb	ABclonal	A8643	Citric, pH6.0	1:1000-1:2000	YES ‡	NT	liver, placental cord		41
EGF-containing fibulin-like extracellular matrix protein 1	**EFEMP1**	CM	GP	rb	Thermo Fisher Scientific	PA5-29347	Tris, pH9.0	1:250	YES ‡	NT	lung, kidney		42
EGF-containing fibulin-like extracellular matrix protein 2	**EFEMP2**	CM	GP	rb, mAb	Abcam	ab125073	Tris, pH9.0	1:500	YES ‡	NO	colon, pancreas		43
elastin	**ELN**	CM	GP	m	Abcam	ab77804	Citric, pH6.0	1:250-1:500	YES ‡	NT	colon		44
				rb	Abcam	ab21610	Citric, pH6.0	1:250-1:500	YES	NT	colon		
elastin microfibril interfacer 1	**EMILIN1**	CM	GP	rb	Sigma-Aldrich	HPA002822	Tris, pH9.0	1:500-1:1000	YES ‡	NO	intestine, endometrium		45
endomucin	**EMCN**	MA	AP	rt	Thermo Fisher Scientific	14-5851	Tris, pH9.0	1:25	YES - but weak ‡	YES ‡	lung, kidney		46, 47
endostatin (collagen XVIII fragment)	**-**	CM	COL	gt	R&D Systems	AF1098	Tris, pH9.0 + Pepsin	1:300-1:500	YES ‡	NT	CRC, endometrium		48
fibromodulin	**FMOD**	CM	PG	rb	Abcam	ab81443	Citric, pH6.0	1:200	YES ‡	YES - but weak	CRC		49
fibronectin 1	**FN**	CM	GP	m	Hynes lab	JP-A1A2	Tris, pH9.0	1:750-1:2000	YES ‡	NT	placenta / CRC	[ref.61]	50
				m	Hynes lab	JP-A2C2	Tris, pH9.0	1:750	YES ‡	NT	placenta / CRC	[ref.61]	51
				rb	Abcam	ab2413	Tris, pH9.0	1:10000	YES ‡	YES ‡	placenta / CRC		52, 53
				rb	Hynes lab	HL-297.1	Tris, pH9.0	1:1000-1:2000	YES ‡	YES	placenta / CRC		54
				rb	Hynes lab	HL-R24	Tris, pH9.0	1:750	YES ‡	YES	placenta / CRC	[ref.62]	55
fibronectin 1 - EIIIA/ED1	**FN**	CM	GP	m	Abcam	ab6328	Tris, pH9.0	1:100	YES ‡	NO - unclear due to background stain	placenta / CRC		56
				m	Sigma-Aldrich	F6140	Tris, pH9.0	1:400	YES ‡	NT	placenta / CRC		57
				m	Hynes lab	JP-15.27	Tris, pH9.0	1:2000	YES ‡	NT	placenta / CRC	[refs.63,64]	58
				m	Hynes lab	JP-52.54	Tris, pH9.0	1:1500	YES ‡	NT	placenta / CRC	[refs.63,64]	59
				gt	Hynes lab	JP-G153	Tris, pH9.0	1:1500	YES ‡	YES	placenta / CRC	[ref.64]	60
fibronectin 1 - EIIIB/ED2	**FN**	CM	GP	m	Hynes lab	AM clone 3	Tris, pH9.0	1:400	YES ‡	YES ‡	placenta / CRC / mouse TEB		61, 62
fibronectin type III domain containing 1	**FNDC1**	CM	GP	rb	Abcam	ab122145	Tris, pH9.0	1:500	YES - but weak ‡	YES ‡	human CRC, mouse bone		63, 64
fibulin 1	**FBLN1**	CM	GP	m	Abcam	ab211536	Tris, pH9.0	1:50-1:75	YES - but weak ‡	NO	CRC, lung		65
fibulin 2	**FBLN2**	CM	GP	rb	Novus Biologicals	NBP1-33479	Tris, pH9.0	1:500	YES ‡	NO	lung		66
fibulin 5	**FBLN5**	CM	GP	rb, mAb	Abcam	ab109428	Tris, pH9.0	1:750	YES ‡	YES ‡	lung, intestine		67, 68
glypican 1	**GPC1**	MA	AP	m	MilliporeSigma	MAB2600	Tris, pH9.0	1:100	YES ‡	NO	CRC, PDAC		69
glypican 4	**GPC4**	MA	AP	rb	Abcam	ab118911	Tris, pH9.0	1:250	YES ‡	YES	kidney, pancreas		70
hemicentin 1	**HMCN1**	CM	GP	rb	MilliporeSigma	AB6066	Tris, pH9.0 + Pepsin	1:400	YES ‡	NT	colon, CRC		71

hemopexin	**HPX**	MA	AP	m	R&D Systems	MAB4490	Tris, pH9.0	1:1000-1:2000	YES ‡	NT	liver		72
				rb, mAb	Abcam	ab124935	Tris, pH9.0	1:200-1:500	YES	NT	liver		
heparan sulfate proteoglycan 2 (perlecan)	**HSPG2**	CM	PG	rb	Boster	PB9277	Tris, pH9.0	1:500-1:750	YES ‡	YES ‡	CRC, intestine, kidney		73, 74
hepatocyte growth factor	**HGF**	MA	SF	rb	Abcam	ab83760	Tris, pH9.0	1:100	YES ‡	NT	placenta		75
HtrA serine peptidase 1	**HTRA1**	MA	REG	rb	Abcam	ab38611	Tris, pH9.0	1:200-1:500	YES	NT	PDAC		
insulin-like growth factor binding protein, acid labile subunit	**IGFALS**	CM	GP	rb	Abcam	ab85222	Tris, pH9.0	1:50	YES	NT	CRC, liver		
interleukin 16 (lymphocyte chemoattractant factor)	**IL16**	MA	SF	rb	OriGene	TA327169	Tris, pH9.0	1:250-1:500	YES ‡	YES	mammary tissue, placenta		76
"laminin Pan"	**-**	CM	GP	rb	Novus Biologicals	NB300-144	Tris, pH9.0	1:500	YES ‡	YES ‡	intestine, pancreas, CRC		77, 78
				rb	Sigma-Aldrich	L9393	Tris, pH9.0	1:100-1:200	NT	YES ‡	intestine, pancreas, CRC		79
				rb	Abcam	ab11575	Tris, pH9.0	1:1000-1:2000	NT	YES ‡	intestine, pancreas, CRC		80
laminin, α1	**LAMA1**	CM	GP	m	Atlas Antibodies	AMAb91117	Citric, pH6.0	1:250	YES - but weak ‡	NO	CRC, PDAC		81
laminin, α2	**LAMA2**	CM	GP	m	Atlas Antibodies	AMAb91166	Tris, pH9.0	1:100	YES	NO	CRC, PDAC, placenta		
				m	Novus Biologicals	H00003908-M01	Tris, pH9.0	1:750-1:1000	YES ‡	NO	CRC, PDAC, placenta		82
laminin, α3	**LAMA3**	CM	GP	m	Atlas Antibodies	AMAb91123	Citric, pH6.0	1:250	YES ‡	NO	CRC, PDAC		83
laminin, α4	**LAMA4**	CM	GP	m	Atlas Antibodies	AMAb91133	Tris, pH9.0	1:250-1:500	YES ‡	NO	CRC, PDAC		84
laminin, α5	**LAMA5**	CM	GP	m	Atlas Antibodies	AMAb91124	Tris, pH9.0	1:250-1:500	YES ‡	NO	CRC, PDAC		85
laminin, β1	**LAMB1**	CM	GP	m	Atlas Antibodies	AMAb91092	Citric, pH6.0	1:100-1:200	YES ‡	NO	CRC, PDAC		86
laminin, β2	**LAMB2**	CM	GP	m	Atlas Antibodies	AMAb91096	Tris, pH9.0	1:1500	YES ‡	NO	CRC, PDAC		87
laminin, β3	**LAMB3**	CM	GP	m	Atlas Antibodies	AMAb91161	Tris, pH9.0	1:250	YES ‡	NO	CRC, placental mebrane		88
laminin, γ1	**LAMC1**	CM	GP	m	Atlas Antibodies	AMAb91137	Citric, pH6.0	1:100	YES ‡	NT	CRC, PDAC		89
laminin, γ2	**LAMC2**	CM	GP	rb	Novus Biologicals	NBP1-88576	Tris, pH9.0	1:500-1:1000	YES ‡	YES ‡	PDAC, intestine		90, 91
latent transforming growth factor β binding protein 1	**LTBP1**	CM	GP	m	R&D Systems	MAB388	Tris, pH9.0 + Pepsin	1:20	YES ‡	NT	lung		92
				rb	Novus Biologicals	NBP1-86501	Tris, pH9.0 + Pepsin	1:75	YES ‡	YES	lung, intestine, mouse TEB		93
latent transforming growth factor β binding protein 2	**LTBP2**	CM	GP	rb	Novus Biologicals	NBP1-88411	Tris, pH9.0	1:200	YES ‡	YES	lung, CRC		94
				rb	Abcam	ab121193	Tris, pH9.0	1:100-1:200	YES	NT	lung, CRC		
				rb	M.Hyytiäinen	-	Tris, pH9.0	1:500-1:1000	YES	NT	lung, CRC	[ref.65]	
latent transforming growth factor β binding protein 3	**LTBP3**	CM	GP	rb	Santa Cruz Biotechnology	sc-98276	Tris, pH9.0	1:200-1:500	YES ‡	NT	PDAC		95
lectin, galactoside-binding, soluble, 3	**LGALS3**	MA	AP	gt	R&D Systems	AF1154	Tris, pH9.0	1:1250	YES ‡	NT	colon, endometrium		96
lysyl oxidase-like 2	**LOXL2**	MA	REG	rb	Abcam	ab140833	Tris, pH9.0	1:200	YES ‡	YES ‡	cartilage / ovarian cancer	the indivudual antibodies show varying staining pattern	97, 98
				rb	G.Neufeld	9227 VIII	Tris, pH9.0	1:2000	YES ‡	NT	cartilage / ovarian cancer		99
				rb	Abcam	ab96233	Tris, pH9.0	1:250-1:500	YES	NT	cartilage / ovarian cancer		
				rb	Novus Biologicals	NBP1-32954	Tris, pH9.0	1:250-1:500	YES	NT	cartilage / ovarian cancer		
				rb, mAb	Abcam	ab179810	Citric, pH6.0	1:500-1:1000	YES - matrix and epithelial cells ‡	NT	cartilage / ovarian cancer		100
lysyl oxidase-like 4	**LOXL4**	MA	REG	rb	Abcam	ab88186	Tris, pH9.0	1:100	YES ‡	NO	CRC		101
matrix metallopeptidase 2	**MMP2**	MA	REG	rb	Abcam	ab110186	Citric, pH6.0	1:500	YES ‡	NT	CRC, placental membrane		102
				rb	Abcam	ab37150	Tris, pH9.0	1:2500	YES ‡	NT	CRC, placental membrane		103
matrix metallopeptidase 3 (stromelysin 1)	**MMP3**	MA	REG	rb, mAb	Abcam	ab52915	Citric, pH6.0	1:250-1:500	YES ‡	NT	CRC, placental membrane		104
matrix metallopeptidase 7 (matrilysin)	**MMP7**	MA	REG	rb, mAb	Abcam	ab205525	Tris, pH9.0	1:250-1:500	YES ‡	NO	CRC		105

matrix metallopeptidase 9	**MMP9**	MA	REG	rb	Abcam	ab38898	Tris, pH9.0	1:1000	YES ‡	YES	CRC, endometrium, intestine		106
matrix metallopeptidase 12	**MMP12**	MA	REG	rb	Abcam	ab137444	Citric, pH6.0	1:250	YES ‡	NT	CRC, endometrium		107
matrix metallopeptidase 14	**MMP14**	MA	REG	rb, mAb	Abcam	ab51074	Citric, pH6.0	1:500	YES ‡	NT	CRC, endometrium		108
milk fat globule-EGF factor 8 protein	**MFGE8**	CM	GP	m	R&D Systems	MAB27671	Citric, pH6.0	1:250-1:500	YES ‡	NT	endometrium, pancreas		109
mucin 1	**MUC1**	MA	AP	rb	Abcam	ab15481	Tris, pH9.0	1:400	YES ‡	YES ‡	lung		110, 111
mucin 2	**MUC2**	MA	AP	rb	Santa Cruz Biotechnology	sc-15334	Citric, pH6.0	1:200	YES ‡	NO	colon		112
mucin 16	**MUC16**	MA	AP	m	Agilent	M352001-2	Tris, pH9.0	1:50	YES ‡	NO	endometrium		113
				rb, mAb	Abcam	ab110640	Tris, pH9.0	1:250-1:500	YES ‡	NT	endometrium		114
NG2 (see chondroitin sulfate proteoglycan 4)	**CSPG4**	MA	AP										
nidogen 2	**NID2**	CM	GP	rb	Abcam	ab14513	Tris, pH9.0	1:2000-1:4000	NT	YES ‡	mouse mammary tissue, TEB		115
osteopontin (see SPP1)	**SPP1**	CM	GP										
periostin	**POSTN**	CM	GP	rb	Abcam	ab14041	Tris, pH9.0	1:500-1:1000	YES ‡	YES ‡	CRC, intestine		116, 117
perlecan (see heparan sulfate proteoglycan 2)	**HSPG2**	CM	PG										
peroxidasin	**PXDN**	CM	GP	rb	Novus Biologicals	NBP1-84316	Tris, pH9.0	1:200	NO	YES	cornea		
plexin A2	**PLXNA2**	MA	AP	rb	Abcam	ab39357	Tris, pH9.0	1:200	YES ‡	YES ‡	colon, kidney, PDAC		118, 119
procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3	**PLOD3**	MA	REG	rb	Proteintech	11027-1-AP	Tris, pH9.0	1:250	YES ‡	NT	PDAC		120
S100 calcium binding protein A4	**S100A4**	MA	SF	rb	MilliporeSigma	07-2274	Citric, pH6.0	1:250-1:500	NT	YES ‡	spleen		121
S100 calcium binding protein A10	**S100A10**	MA	SF	rb	Proteintech	11250-1-AP	Tris, pH9.0	1:200	YES ‡	NT	mammary tissue		122
S100 calcium binding protein A12	**S100A12**	MA	SF	rb	Novus Biologicals	NBP1-86694	Tris, pH9.0	1:1000-1:2000	YES - immune cells ‡	NT	bone marrow cells, placenta		123
S100 calcium binding protein A14	**S100A14**	MA	SF	rb	Novus Biologicals	NBP1-90000	Citric, pH6.0	1:500	YES ‡	NT	colon, CRC		124
S100 calcium binding protein A16	**S100A16**	MA	SF	rb	Abcam	ab130419	Tris, pH9.0	1:500	YES - few immune cells ‡	NT	endometrium, CRC, liver		125
S100 calcium binding protein B	**S100B**	MA	SF	rb, mAb	Abcam	ab52642	Tris, pH9.0	1:500	YES ‡	NT	mammary tissue		126
serpin peptidase inhibitor B1	**SERPINB1**	MA	REG	rb, mAb	Abcam	ab181084	Tris, pH9.0	1:2000	YES - immune cells ‡	NT	CRC		127
serpin peptidase inhibitor B5	**SERPINB5**	MA	REG	rb	OriGene	TA322980	Tris, pH9.0	1:1000	YES ‡	YES - but weak	endometrium, placenta, PDAC		128
serpin peptidase inhibitor E2	**SERPINE2**	MA	REG	m	OriGene	TA504611	Tris, pH9.0	1:250-1:500	YES ‡	NO	CRC, pancreas		129
				m	OriGene	TA504718	Tris, pH9.0	1:250-1:500	YES ‡	NO	CRC, pancreas		130
secreted protein, acidic, cysteine-rich (osteonectin)	**SPARC**	CM	GP	rb, mAb	Cell Signaling	8725	Citric, pH6.0	1:100	YES ‡	NT	cartilage, CRC, endometrium	the indivudual antibodies show varying staining pattern	131
				rb	Sino Biological	10929-RP02	Tris, pH9.0	1:500	YES ‡	NT	cartilage, CRC, endometrium		131
				gt	R&D Systems	AF942	Tris, pH9.0	1:200	NO	YES	cartilage, CRC, endometrium		
secreted phosphoprotein 1 (SPP1, osteopontin)	**SPP1**	CM	GP	rb	D.R.Senger	-	Tris, pH9.0	1:2000	YES ‡	YES	CRC, kidney, bone, cartilage	[ref.66]	132
				m	Abcam	ab166709	Tris, pH9.0	1:200	YES ‡	NT	CRC, kidney, bone, cartilage		133
				rb	Abcam	ab8448	Citric, pH6.0	1:1000-1:2000	YES ‡	NT	CRC, kidney, bone, cartilage		134
suppression of tumorigenicity 14	**ST14**	MA	REG	rb	Novus Biologicals	NBP1-56649	Tris, pH9.0	1:750	YES ‡	NT	colon, CRC		135
				rb	Bethyl Laboratories	Q9Y5Y6	Tris, pH9.0	1:100	YES ‡	NT	colon, CRC		136
surfactant protein D	**SFTPD**	MA	AP	m	Abcam	ab17781	Tris, pH9.0	1:400-1:500	YES ‡	NO	lung, gall bladder		137
sushi-repeat-containing protein, X-linked 2	**SRPX2**	CM	GP	rb	Abcam	ab91584	Tris, pH9.0	1:5000	YES ‡	YES	endometrium, placenta		138

syndecan 2	**SDC2**	MA	AP	rb	ABclonal	A1810	Tris, pH9.0	1:100	YES - but weak	NT	lung, liver		
tenascin C	**TNC**	CM	GP	rb, mAb	Abcam	ab108930	Tris, pH9.0	1:1500	YES ‡	YES ‡	CRC, placenta		139, 140
				rt	Sigma-Aldrich	T3413	Tris, pH9.0	1:250-1:500	NO	YES ‡	mouse tumor tissues	specific for mouse tissues	141
tenascin N	**TNN**	CM	GP	rb	Abcam	ab121887	Tris, pH9.0	1:250	YES ‡	NT	skin		142
tenascin X	**TNX**	CM	GP	sh	R&D Systems	AF6999	Tris, pH9.0	1:100	YES - but weak	NT	CRC, lung mesothelioma		
thrombin (coagulation factor II)	**F2**	MA	REG	m	Abcam	ab17199	Tris, pH9.0	1:100	YES	NT	liver		
				rb	Abcam	ab83981	Tris, pH9.0	1:500	YES	NT	liver		
thrombospondin 2	**THBS2**	CM	GP	m	BD Biosciences	611150	Tris, pH9.0	1:200-1:500	YES - matrix and epithelial cells	NT	CRC, PDAC		
				rb	OriGene	TA590658	Tris, pH9.0	1:200	YES ‡	NO	CRC, PDAC		143
				rb	sdix	2442.00.02	Tris, pH9.0	1:200	YES	NO	CRC, PDAC		
				rb	Abcam	ab112543	Tris, pH9.0	1:100-1:200	YES	NT	CRC, PDAC		
				rb	Thermo Fisher Scientific	PA5-50843	Tris, pH9.0	1:500	YES ‡	YES ‡	CRC, PDAC		144, 145
TIMP metallopeptidase inhibitor 1	**TIMP1**	MA	REG	m	Agilent	M729329-2	Citric, pH6.0	1:500	YES ‡	NO	pancreas, CRC		146
				rb, mAb	Abcam	ab211926	Tris, pH9.0	1:1000-1:1500	YES ‡	NO	pancreas, CRC		147
				rb	Abcam	ab216432	Tris, pH9.0	1:250	YES ‡	NT	pancreas, CRC		147
				rb	Boster	PA1385	Citric, pH6.0	1:2000	YES ‡	NO	pancreas, CRC		147
TIMP metallopeptidase inhibitor 2	**TIMP2**	MA	REG	m	OriGene	TA504043	Tris, pH9.0	1:100	YES - but weak	NT	pancreas		
"transforming growth factor, β Pan"	-	MA	SF	rb	Abcam	ab66043	Tris, pH9.0	1:250-1:500	YES ‡	YES ‡	CRC		148, 149
transforming growth factor, β-induced, 68kDa	**TGFBI**	CM	GP	rb	Sigma-Aldrich	HPA017019	Tris, pH9.0	1:250:1-500	YES ‡	NT	CRC, placenta		150, 151
"vascular endothelial growth factor Pan"	-	MA	SF	m	Thermo Fisher Scientific	MS-350-P0	Tris, pH9.0	1:250-1:500	NT	YES	spleen		
versican	**VCAN**	CM	PG	rb	MilliporeSigma	AB1033	Citric, pH6.0	1:250	NO	YES ‡	cartilage, mouse TEB	specific for mouse tissues	152
WNT1 inducible signaling pathway protein 2	**WISP2**	CM	GP	rb	Novus Biologicals	27260002	Citric, pH6.0	1:100	YES ‡	NT	CRC		153

Supplementary Table 1 - Additional extracellular matrix-related antibodies

Antibodies to extracellular matrix proteins tested on formaldehyde-fixed and paraffin-embedded tissue samples using immunohistochemistry (IHC), which, in our experiments, either did not yield positive stains or gave uncertain staining results. However, other antigen-retrieval or heat-induced epitope retrieval (HIER) approaches or tissue samples might yield appropriate staining results.

This table provides an overview of additional antibodies for which none of the HIER conditions (for details see Supplementary Materials and Methods) we tested showed a positive staining reactivity or we observed a 'non-specific' staining pattern. Each antibody was tested for a range of dilutions on a variety of tissues. However, since lot-specific variability can occur, other antibody samples should be tested for the individual application and tissue of interest. For additional details see also the antibody manufacturers datasheets.

Matrisome division (CM, core matrisome; MA, matrisome-associated); matrisome category (COL, collagens; GP, ECM glycoproteins; PG, proteoglycans; REG, ECM regulators; SF, secreted factors; AP, ECM-affiliated proteins). Antibody host species (gt, goat; m, mouse; rb, rabbit); rt, rat; mAb monoclonal antibody.

Additional abbreviations used: NT not tested; DSHB, Developmental Studies Hybridoma Bank

Protein name	Gene Symbol	Matrisome Division	Matrisome Category	Host	Company	Catalog number	Human reactivity	Mouse reactivity
agrin	**AGRN**	CM	GP	m	Santa Cruz Biotechnology	sc-374117	NO	NO
				m	DSHB - works on frozen tissue sections	6D2	NO	NO
				m	MilliporeSigma	MAB5204	NO	NO
				rb	J.Miner (made by T.Sasaki) - works on frozen tissue sections	C-ter LG1-3	NO	NO
				rb	Sigma-Aldrich	SAB4301032	NO	NO
				m	Abcam	ab12364	NO	NO
				rb	Thermo Fisher Scientific	PA5-37121	NO	NT
				gt	Santa Cruz Biotechnology	sc-6166	NO	NO
bone morphogenetic protein1	**BMP1**	MA	REG	m	OriGene	TA505813	NO	NT
connective tissue growth factor	**CTGF**	CM	GP	rb	Sigma-Aldrich	HPA031075	NO	NT
CYR61 (cysteine-rich, angiogenic inducer, 61)	**CYR61**	CM	GP	rb	Sigma-Aldrich	HPA029853	NO	NT
EGF-containing fibulin-like extracellular matrix protein 2	**EFEMP2**	CM	GP	m	MilliporeSigma	MAB2644	NO	NT
				rb	Novus Biologicals	NBP1-84724	NO	NO
elastin microfibril interfacer 3	**EMILIN3**	CM	GP	gt	Santa Cruz Biotechnology	sc-51359	NO	NT
fibroblast growth factor 2 (basic)	**FGF2**	MA	SF	m	BD Biosciences	610072	NO	NT
"fibrillin Pan"	**-**	CM	GP	m	MilliporeSigma	MAB2641	NO	NO
fibrillin 1	**FBN1**	CM	GP	rb	Sigma-Aldrich	SAB4500863	NO	NT
				rb	Abcam	ab53076	NO	NT

fibrillin 2	**FBN2**	CM	GP	rb	Novus Biologicals	NBP1-88169	NO	NO
glypican 1	**GPC1**	MA	AP	rb	Novus Biologicals	NBP1-33197	NO	NO
glypican 4	**GPC4**	MA	AP	rb, mAb	Abcam	ab168364	NO	NT
hemicentin 1	**HMCN1**	CM	GP	m	Proteintech	60033-1-Ig	NO	NO
				rb	Novus Biologicals	NBP2-30604	NO	NO
hemopexin	**HPX**	MA	AP	rb	GeneTex	GTX112519	NO	NO
				rb	Abcam	ab90947	NO	NO
heparan sulfate proteoglycan 2 (perlecan)	**HSPG2**	CM	PG	m	MilliporeSigma	MABT12	NO	NO
				rt	Novus Biologicals	NB600-583	NO	NO
host cell factor C1 (VP16-accessory protein)	**HCFC1**	MA	SF	rb	GeneTex	GTX114922	NO	NT
hyaluronan and proteoglycan link protein 1	**HAPLN1**	CM	PG	rb	Abcam	ab103455	NO	NT
"laminin Pan"	**-**	CM	GP	m	Sigma-Aldrich	L8271	NO	NT
laminin, β1	**LAMB1**	CM	GP	rt	Abcam	ab44941	NO	NO
latent transforming growth factor β binding protein 1	**LTBP1**	CM	GP	rb	Abgent	AP7780B	NO	NT
				rb	Abcam	ab78294	NO	NT
lectin, galactoside-binding, soluble, 3	**LGALS3**	MA	AP	rb	Sigma-Aldrich	HPA003162	NO	NT
lysyl oxidase-like 1	**LOXL1**	MA	REG	rb	Abcam	ab87748	NO	neg
lysyl oxidase-like 2	**LOXL2**	MA	REG	m	OriGene	TA807440	NO	NT
				rb	Abcam	ab133383	NO	NO
				rb	Santa Cruz Biotechnology	sc-66950	positive but different to others LOXL2 antibodies	
lysyl oxidase-like 3	**LOXL3**	MA	REG	rb	Abcam	ab122263	NO	NT
lysyl oxidase-like 4	**LOXL4**	MA	REG	m	Sigma-Aldrich	WH0084171M1	NO	NO

matrix-remodelling associated 5	**MXRA5**	CM	GP	rb	Sigma-Aldrich	HPA000508	NO	NO
				rb	Novus Biologicals	NBP1-84156	NO	NO
perlecan (see heparan sulfate proteoglycan 2)	**HSPG2**	CM	PG					
pleiotrophin	**PTN**	MA	SF	m	Novus Biologicals	H00005764-M01	NO	NT
				rb, mAb	Abcam	ab79411	NO	NT
plexin B2	**PLXNB2**	MA	AP	m	Thermo Fisher Scientific	MA5-24237	NO	NO
procollagen-lysine 1, 2-oxoglutarate 5-dioxygenase 1	**PLOD1**	MA	REG	rb	Abcam	ab171140	NO	NO
				rb	Novus Biologicals	NBP2-31885	NO	NT
S100 calcium binding protein A1	**S100A1**	MA	SF	rb	Proteintech	16027-1-AP	NO	NO
serpin peptidase inhibitor B1	**SERPINB1**	MA	REG	rb	GeneTex	GTX104522	NO	NT
serpin peptidase inhibitor E2	**SERPINE2**	MA	REG	rb, mAb	Abcam	ab134905	NO	NO
				rb	GeneTex	GTX124069	NO	NO
				rb	Abcam	ab154591	NO	NO
suppression of tumorigenicity 14	**ST14**	MA	REG	rb	GeneTex	GTX113557	NO	NT
				rb, mAb	MilliporeSigma	MABC115	NO	NO
"thrombospondin Pan"	**THBS**	CM	GP	m	Abcam	ab1823	NO	NO
thrombospondin 2	**THBS2**	CM	GP	m	Abcam	ab89805	NO	NO
				rb	Boster	A03253	NO	NO
TIMP metallopeptidase inhibitor 1	**TIMP1**	MA	REG	m	Thermo Fisher Scientific	MA5-13688	NO	NO
				rb	biorbyt	orb195994	NO	NO
				rb	OriGene	TA322880	NO	NO
				gt	R&D Systems	AF980	NT	NO
transforming growth factor, β1	**TGFB1**	MA	SF	m	R&D Systems	MAB240	NO	NT
transforming growth factor, β2	**TGFB2**	MA	SF	m	MilliporeSigma	MAB612	NO	NT

Supplementary Table 2 - Reference antibodies

List of antibodies broadly used in tumor biology and histopathology to identify specific tissue distribution or subcellular localization on formaldehyde-fixed and paraffin-embedded tissue samples using immunohistochemistry.

This table provides an overview of all antibodies which reproducibly showed positive staining signals in our hands. Presented are our validation information and results, however, we have not tested each antibody for molecular specificity.

Indicated are the individual: protein name, gene symbol, antibody host species (gt, goat; m, mouse; rb, rabbit; rb mAb, rabbit monoclonal antibody; rt, rat), commercial antibody source and catalog number and heat-induced epitope retrieval (HIER) methods used (see also Supplementary Materials and Methods), recommended antibody dilutions, human and mouse reactivity as well as a positive control tissue and, if applicable, the targets of each antibody.

* Indicates the preferred HIER method applied for the individual antibody, however, other conditions also might work. For many antibodies both HIER conditions work alike.

** Indicates the antibody dilution we have tested; however, each antibody vial should be tested for individual application and tissue accordingly since lot specific variability can occur.

Protein name	Gene symbol	Host	Company	Catalog number	HIER method *	Dilution **	Human reactivity	Mouse reactivity	Positive control tissue	Targets
BMI1 proto-oncogene	BMI1	rb	Bethyl Laboratories	IHC-00606	Citric, pH6.0	1:250-1:500	YES	YES	colon, lung	
carbonic anhydrase 9	CAIX	rb	Novus Biologicals	NB100-417	Tris, pH9.0	1:250-1:500	YES	NT	cancer tissue	hypoxia marker
catenin beta-1	CTNNB1	m	BD Biosciences	610154	Citric, pH6.0	1:500-1:1000	YES	YES	most tissues	cell-cell junctions
catenin beta-1	CTNNB1	rb, mAb	Abcam	ab32572	Tris, pH9.0	1:1000-2000	YES	YES	most tissues	cell-cell junctions
caudal type homeobox 2	CDX2	rb, mAb	Abcam	ab76541	Tris, pH9.0	1:500-1:1500	YES	YES	colon	gastrointestinal epithelial cell marker
CC-3, cleaved caspase-3	CASP3	rb	Cell Signaling	9661L	Citric, pH6.0	1:200-1:400	YES	YES	small intestine	marker for apoptotic cell death
CD3	CD3	rb, mAb	Abcam	ab16669	Citric, pH6.0	1:250-1:500	YES	YES	spleen	T-cells
CD3	CD3	rb	Abcam	ab5690	Citric, pH6.0	1:200	NT	YES	spleen	T-cells
CD4	CD4	m	Agilent	IS649	Tris, pH9.0	ready to use	YES	NO	spleen	T helper cells (also monocytes, macrophages, and dendritic cells)
CD4	CD4	rt	Thermo Fisher Scientific	14-9766-82	Citric, pH6.0	1:100	NT	YES	spleen	T helper cells (also monocytes, macrophages, and dendritic cells)
CD8	CD8	m	Agilent	IS623	Tris, pH9.0	ready to use	YES	NO	spleen	cytotoxic T cells (NK cells, for mouse samples also DCs)
CD8a	CD8a	rt	Thermo Fisher Scientific	14-0808-80	Tris, pH9.0	1:250-1:500	NT	YES	spleen	cytotoxic T cells (NK cells, for mouse samples also DCs)
CD31	PECAM1	rb	Abcam	ab28364	Citric, pH6.0	1:250-1:500	YES	YES	colon, lung	platelet and endothelial cells
CD34 molecule	CD34	rb, mAb	Abcam	ab81289	Citric, pH6.0	1:500-1:1000	YES	YES	colon, lung	hematopoietic stem and progenitor cells
CD45	PTPRC	m	Agilent	M070129-2	Citric, pH6.0	1:300	YES	NO	spleen	lymphocytes, myeloid cell
CD45-R, B220	PTPRC	rt	Thermo Fisher Scientific	14-0452-82	Citric, pH6.0	1:150	NT	YES	spleen	mainly B cell lineage, some activated T cells
chromogranin A	CHGA	m	Abcam	ab80787	Citric, pH6.0	1:750	YES	NO	colon, pancreas	neuro-endocrine cell marker
desmin	DES	m	Abcam	ab8470	Citric, pH6.0	1:100	YES	NT	muscle tissue	muscle cell marker
desmin	DES	m	PROGEN Biotechnik	10519	Citric, pH6.0	1:50-1:250	YES	YES	muscle tissue	muscle cell marker
desmin	DES	rb	Abcam	ab15200	Citric, pH6.0	1:500-1:1500	YES	YES	muscle tissue	muscle cell marker
dystroglycan alpha	DAG1	m	MilliporeSigma	05-298	Tris, pH9.0	1:50	YES	NT	placenta	
dystroglycan alpha	DAG1	m	Santa Cruz Biotechnology	sc-53987	Citric, pH6.0	1:25	YES	NT	placenta	
dystroglycan beta	DAG1	m	Leica Biosystems	NCL-b-DG	Citric, pH6.0	1:250-1:500	YES	NO	placenta	
E-cadherin	CDH1	m	BD Biosciences	610181	Citric, pH6.0	1:750-1:1000	YES	YES	epithelial tissues	epithelial cell junction marker
E-cadherin	CDH1	rb, mAb	Cell Signaling	3195S	Citric, pH6.0	1:250-1:500	YES	YES	epithelial tissues	epithelial cell junction marker
epidermal growth factor receptor	EGFR	rb, mAb	Abcam	ab52894	Citric, pH6.0	1:500	YES	YES	colon	

erb-b2 receptor tyrosine kinase	ERBB2	rb, mAb	Abcam	ab134182	Citric, pH6.0	1:250	YES	NT	mammary tissue, breast cancer	marker to test HER2 status
FERM domain containing 6	FRMD6	rb	Sigma-Aldrich	HPA001297	Tris, pH9.0	1:500	YES	NT	colon	
forkhead box P3	FOXP3	rb	Novus Biologicals	NB100-39002SS	Tris, pH9.0	1:1000:1:1500	NT	YES	spleen	regulatory T cells (Tregs)
glial fibrillary acidic protein	GFAP	rb	Agilent	Z0334	Tris, pH9.0	1:1000	YES	NT	brain, pancreas	cells and tumors of glial origin, astrocytes and ependymal cells
GPIb alpha, CD42b	GPIBA	m	Abcam	ab74449	Tris, pH9.0	ready to use	YES	NT	human spleen	platelets
GPIb alpha, CD42b	GPIBA	rt	Emfret Analytics	M042-0	Tris, pH9.0	1:500-1:750	NO	YES	mouse spleen	platelets
green fluorescent protein	GFP	gt	Rockland Immunochemicals	600-101-215	Citric, pH6.0	1:250-1:500	-	-	-	marker for GFP positive cells
high mobility group box 1	HMGB1	rb	Abcam	ab18256	Citric, pH6.0	1:5000	YES	YES	colon, lung	immunogenic cell death marker
human mitochondria marker	-	m	MilliporeSigma	MAB1273	Citric, pH6.0	1:300	YES	NO	most human tissues	human specific cell marker
integrin subunit alpha V	ITGAV	rb, mAb	Abcam	ab179475	Tris, pH9.0	1:500-1:1000	YES	YES	most tissues	
integrin subunit beta1	ITGB1	rb, mAb	Abcam	ab179471	Citric, pH6.0	1:1000-1:2500	YES	YES	most tissues	
IQ motif containing GTPase activating protein 1	IQGAP1	rb	Santa Cruz Biotechnology	sc-10792	Tris, pH9.0	1:500-1:1000	YES	YES	colon, lung	
keratin 5	KRT5	rb, mAb	Abcam	ab52635	Tris, pH9.0	1:1000	YES	NO	mammary tissue	basal epithelial cells
keratin 7	KRT7	m	Abcam	ab9021	Citric, pH6.0	1:500	YES	NT	liver	simple epithelia lining cavities of internal organs and gland ducts
keratin 14	KRT14	rb, mAb	Abcam	ab181595	Tris, pH9.0	1:2000	YES	NO	mammary tissue	basal epithelial cells
keratin 18	KRT18	m	PROGEN Biotechnik	61028	Tris, pH9.0	1:100	YES	NO	colon, liver	single layer epithelial tissues
keratin 20	KRT20	m	Abcam	ab854	Tris, pH9.0	1:50-1:100	YES	NO	colon	gastrointestinal cells, mature enterocytes, goblet cells, urothelial cells, Merkel cells
keratin 20	KRT20	rb, mAb	Abcam	ab76126	Tris, pH9.0	1:250	YES	NT	colon	gastrointestinal cells, mature enterocytes, goblet cells, urothelial cells, Merkel cells
"keratin Pan"	-	m	Abcam	ab27988	Citric, pH6.0	1:40	YES	NT	epithelial tissues	epithelial cell marker
"keratin Pan"	-	m	Abcam	ab8068	Citric, pH6.0	1:100	YES	NT	epithelial tissues	epithelial cell marker
"keratin Pan"	-	rb	Agilent	Z0622	Tris, pH9.0	1:2000-1:4000	YES	YES	epithelial tissues	epithelial cell marker
KI-67	MKI67	m	Agilent	M7240	Citric, pH6.0	1:300	YES	NO	colon	marker of proliferation
KI-67	MKI67	m	BD Biosciences	550609	Citric, pH6.0	1:250	YES	NO	colon	marker of proliferation
KI-67	MKI67	rb, mAb	Abcam	ab16667	Citric, pH6.0	1:250-1:500	YES	YES	colon	marker of proliferation
lamin A	LMNA	rb	Abcam	ab26300	Tris, pH9.0	1:200-1:500	YES	YES	most tissues	nuclear lamina
lamin A/C	LMNA	rb, mAb	Abcam	ab108595	Citric, pH6.0	1:2500	YES	NO	most tissues	nuclear lamina / human specific cell marker
LI Cadherin	CDH17	rb, mAb	Abcam	ab109190	Citric, pH6.0	1:400	YES	NT	colon, liver	
lymphocyte activating 3	LAG3	rb, mAb	Cell Signaling	15372	Citric, pH6.0	1:200	YES	NT	spleen	activated T cells, NK cells, B cells
LYVE1	LYVE1	rb	Abcam	ab14917	Tris, pH9.0	1:200	YES - weak stain	YES	colon, liver	lymphatic endothelial vessel
Ly6g	Ly6g	rt	Biolegend	127602	Tris, pH9.0	1:50	NT	YES	spleen	granulocytes and neutrophils
mutL homolog 1	MLH1	m	Sigma-Aldrich	G168-728	Citric, pH6.0	1:25-1:50	YES	NT	colon	
N-cadherin	CDH2	m	BD Biosciences	610920	Tris, pH9.0	1:250-1:500	YES	YES	placenta, liver	mesenchymal cell junction marker

NCAM1, neural cell adhesion molecule 1	NCAM1	m	OriGene	TA506208	Tris, pH9.0	1:500	YES	NT	brain, colon	neurons, glia and skeletal muscle cells
NF160, neurofilament medium	NEFM	m	Abcam	ab7794	Citric, pH6.0	1:500-1:1000	YES	NT	brain, colon	neuronal cells
NK1.1, killer cell lectin-like receptor B1	KLRB1	m	Thermo Fisher Scientific	MA1-70100	Citric, pH6.0	1:5000-1:10000	NT	YES	spleen	NK cells
p53	TP53	m	Abcam	M7001	Citric, pH6.0	1:50	YES	NO	cancer tissue	
PMS2, PMS1 homolog 2	PMS2	m	Biocare Medical	50-826-49	Citric, pH6.0	1:250	YES	NT	colon	
S6 ribosomal protein, phosphorylated, pS6	RPS6	rb, mAb	Cell Signaling	4858	Citric, pH6.0	1:250-1:500	YES	YES	colon, stomach	
SATB homeobox 2	SATB2	rb, mAb	Abcam	ab133328	Citric, pH6.0	1:250	YES	YES	colon	
SMAD family member 4	SMAD4	m	Santa Cruz Biotechnology	sc-7966	Citric, pH6.0	1:50	YES	NT	colon	
smoothelin	SMTN	rb	Santa Cruz Biotechnology	sc-28562	Tris, pH9.0	1:250	YES	NT	colon, endometrium	smooth muscle cells
smooth muscle actin (SMA)	ACTA2	m	Sigma-Aldrich	A2547	Tris, pH9.0	1:2500	YES	NT	colon, endometrium	smooth muscle cells
smooth muscle actin (SMA)	ACTA2	rb, mAb	Abcam	ab124964	Tris, pH9.0	1:2000-1:4000	YES	YES	colon, endometrium	smooth muscle cells
SRY (sex determining region Y)-box 9	SOX9	rb, mAb	Abcam	ab185230	Tris, pH9.0	1:1000-1:1500	YES	NT	colon	
TGFB receptor 1	TGFBR1	rb	Abcam	ab31013	Citric, pH6.0	1:200	YES	NT	placenta	
vimentin	VIM	m	PROGEN Biotechnik	61013	Tris, pH9.0	1:200	YES	NO	colon, lung	mesenchymal cell marker
vimentin	VIM	m	Leica Biosystems	NCL-L-VIM-V9	Citric, pH6.0	1:250-1:500	YES	NO	colon, lung	mesenchymal cell marker / human specific
vimentin	VIM	rb, mAb	Abcam	ab92547	Tris, pH9.0	1:2000-1:5000	YES	YES	human colon, human lung	mesenchymal cell marker
Yes associated protein 1	YAP1	rb, mAb	Cell Signaling	14074	Citric, pH6.0	1:200	YES	YES	cancer tissue	
ZEB1, zinc finger E-box binding homeobox 1	ZEB1	rb	Sigma-Aldrich	HPA027524	Tris, pH9.0	1:500	YES	NT	cancer tissue	epithelial-mesenchymal transition

Supplementary Table 3 - Online resources

Lists of useful online resources relevant for antibody searching, antibody characterization, IHC standardization and methodology, troubleshooting guidelines as well as links to tissue expression data of protein-coding genes in normal and tumor tissues.

Antibody search engines

http://www.antibodypedia.com
http://www.antibodyregistry.org
http://www.antibodyresource.com
http://www.biocompare.com
http://www.citeab.com
http://www.discover.nci.nih.gov/abminer
https://www.linscottsdirectory.com
http://www.pabmabs.com/wordpress
http://www.selectscience.net

Antibody validation, IHC standardization and methodology, troubleshooting

http://www.abcam.com/primary-antibodies/a-guide-to-antibody-validation
http://www.agilent.com/en-us/products/immunohistochemistry
https://atlasantibodies.com/#!/support/antibody-validation
http://www.histosearch.com
http://www.histosearch.com/histonet.html
http://www.ihcworld.com/index.htm
http://www.immunohistochemistry.us
https://ncifrederick.cancer.gov/rtp/lasp/phl/immuno/
http://www.novusbio.com/application/ihc
http://www.rndsystems.com/protocol-types/immunohistochemistry-ihc-protocols
http://www.vectorlabs.com/support/tutorials

Tissue expression databases

http://www.anatomyatlases.org
http://www.cancergenome.nih.gov
http://www.ctrgenpath.net/static/atlas/mousehistology/
http://dbarchive.biosciencedbc.jp/archive/matrixome/bm/home.html
http://www.encodeproject.org
http://www.gtexportal.org/
http://www.humancellatlas.org
http://www.humanproteomemap.org/index.php
https://jhubiostatistics.shinyapps.io/recount/
http://matrisomeproject.mit.edu
http://www.proteinatlas.org
http://www.proteinatlas.org/pathology
http://shogoin.stemcellinformatics.org