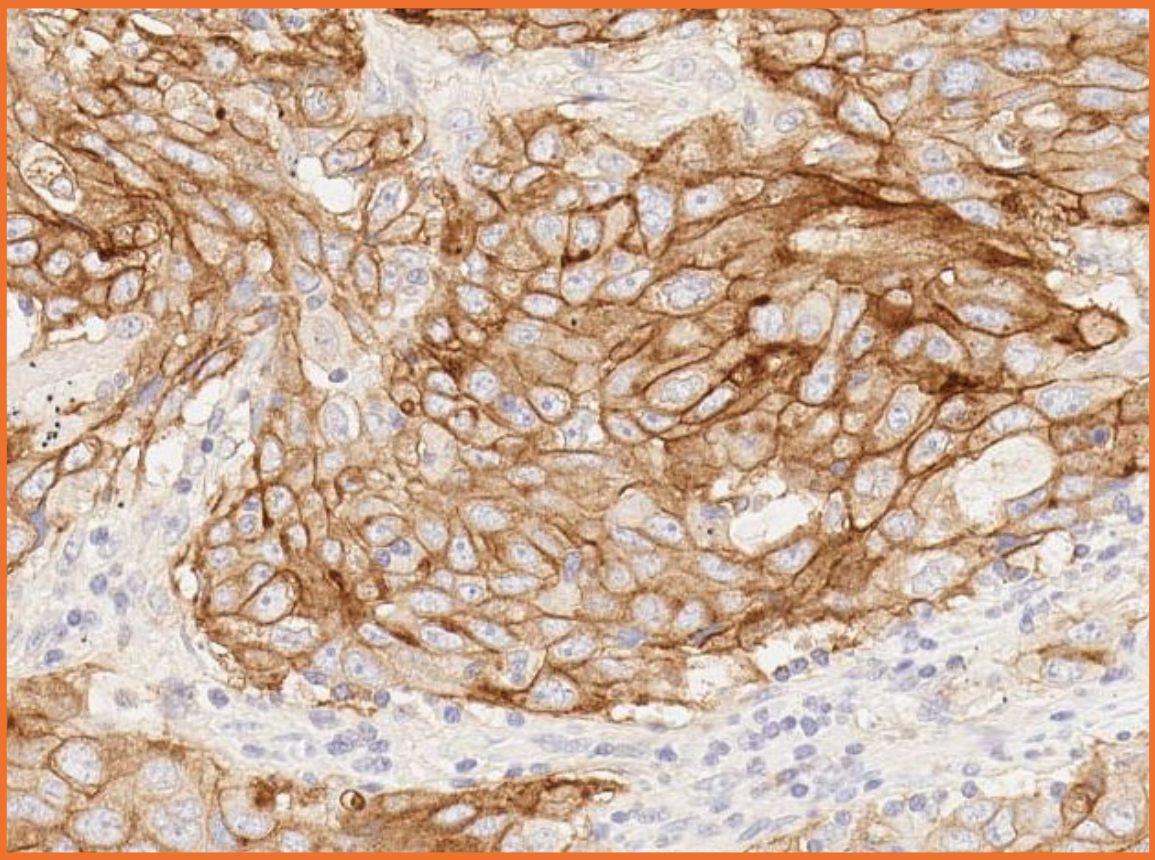
Enhanced validation data

Anti-CD73 recombinant antibody – ab133582



AB0341_v1.0_121824

progress happens together

abcam

Enhanced validation of Anti-CD73 recombinant antibody [EPR6114] – ab133582

Enhanced validation designed for your needs

We understand the challenge of finding the right antibody clone – highly specific and sensitive to your intended target – at early selection stages of your development program. To de-risk this clone selection process for you, we generated enhanced validation data for our best recombinant antibody clones to some of the most promising targets.

Our enhanced validation gives you an extra level of confidence in an antibody clone

— Provides additional data on the specificity and sensitivity of our recombinant antibodies in immunohistochemistry (IHC) and other relevant techniques

— Carried out in a custom manner, specific both to the target and the relevant research and clinical settings

— Builds upon our high-quality standard validation

Our framework for enhanced validation

— Our enhanced validation focuses on generating detailed IHC expression profiles for promising immuno-oncology targets in selected formalin-fixed paraffin-embedded (FFPE) human normal tissues and cancer tissue microarrays (TMAs)

— In this study, we demonstrate the sensitivity and specificity of anti-CD73 recombinant antibody ab133582 in IHC in selected tissues and TMAs using a BOND™ RX Research Stainer (Leica®)

— A quantitative analysis of CD73 expression was performed using the artificial intelligence (AI)-driven digital image analysis software Visiopharm® (Visiopharm A/S)

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Target overview

HGNC symbol
NT5E

Approved name
5’-nucleotidase ecto

Previous name
5’-nucleotidase ecto (CD73)

Chromosomal location
6q14.3

Function

- CD73 is a glycosylphosphatidylinositol (GPI)-anchored cell surface glycoprotein that functions as an enzyme in the purine metabolism pathway, converting extracellular ATP to adenosine [1,2]
- It is a critical regulator of cell homeostasis, stress responses, angiogenesis and disease mechanisms [2,3]
- It has key roles in tumor growth, immune suppression, inflammation and drug resistance and is an active therapeutic target [4-7]

Tissue specificity

- CD73 is widely expressed in different tissues and cell types, including but not limited to epithelia, endothelial, neurons, fibroblasts, myeloid and lymphoid cells [2,3]
- Upregulation of CD73 occurs in many tumors including rectal, breast, NSCLC and gastric cancer, and is generally associated with poor prognosis [4,5,7,8]

Cellular localization

- Cell membrane; lipid-anchor, GPI-anchor

Database links

Entrez Gene: 4907
OMIM®: 129190
Uniprot: P21589

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Materials and methods

Human tissues were selected based on the target's expression and its current relevance to ongoing research and clinical trials. Gene expression was further analyzed for oncology targets in cBioPortal for Cancer Genomics using the Cancer Genome Atlas (TCGA) PanCancer Atlas datasets [9-12].

Tissue microarray (TMA)	Cores	Cases	Normal/ Benign cases	Cancer cases	Source (#catalog number)
Multi-normal*	40	37	37	0	In-house TMA
Lung cancer	102	102	5	97	Pantomics (#LUC1021)
Breast cancer	102	102	5	97	Pantomics (#BRC1021)
Rectal cancer	102	102	5	97	Pantomics (#REC1021)

Table 1. List of human TMAs used in the enhanced validation. All tissues were sourced from Abcam-approved tissue suppliers.

*The multi-normal TMA consists of the following tissues from two donors: colon, cerebellum, small intestine mucosa, tonsil, stomach, testis, prostate, lung, skeletal muscle, breast, heart, skin, endometrium, spleen, pancreas, lymph node, kidney. Placenta and liver tissues originated from a single donor.

Step	Reagents	Method
Dewax	Bond™ dewax solution (AR922), alcohol, BOND wash solution (AR9590)	Dewax
Antigen retrieval	Bond™ epitope retrieval ER1 solution (AR9961)	HIER with ER1 (pH 6), 20 min, 100°C

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Enhanced validation data

Step	Reagents	Number of washes	Time (minutes)
Peroxide block	3-4% (v/v) Hydrogen peroxide	-	5
Wash	Bond™ wash solution	3x	0
Primary antibody	Anti-CD73 antibody [EPR6114]- ab133582 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 3 µg/mL	-	15
Wash	Bond™ wash solution	4x	0
Secondary antibody	Bond™ polymer refine detection (DS9800)	-	8
Wash	Bond™ wash solution	2x	4
	Deionized water	1x	0
Visualization	Mixed DAB refine (DS9800)	1x	0
	Mixed DAB refine (DS9800)	-	10
Wash	Deionized water	3x	0
Counterstain	Hematoxylin (DS9800)	-	5
Wash	Deionized water	1x	0
	Bond™ wash solution	1x	0
	Deionized water	1x	0

Table 2. IHC staining protocol on BOND™ RX Research Stainer (Leica®). The protocol used is the same as the default IHC protocol F on BOND™ RX Research Stainer (Leica®), apart from the standard post-primary step, which has been excluded from our protocol. All steps were performed at room temperature.

Staining intensity analysis

CD73 staining intensity analysis was performed using the artificial intelligence (AI)- driven digital image analysis software Visiopharm® (Version: 2023.09). TMA slides were de-arrayed, the tissue within each core was detected and artefact exclusion was performed using a trained AI model with DeepLabv3+architecture. Tissue was segmented into tumor and stroma by a trained AI model with DeepLabv3+architecture (Figure 1). Segmentation performance was assessed manually and adjusted where necessary. A defined exclusion criteria was applied to all TMAs.

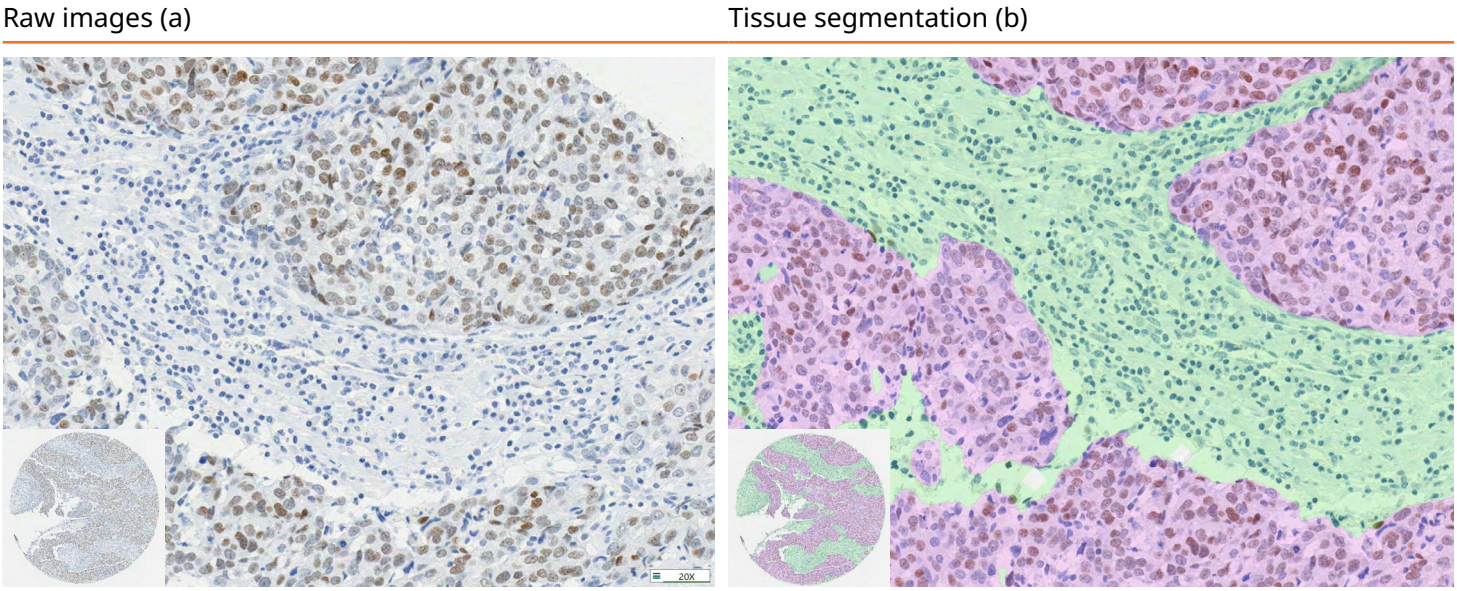


Figure 1. Digital tissue segmentation and cell detection. Whole TMA cores were segmented into tumor (pink overlay) and stroma (green overlay) compartments. AI driven digital image analysis was performed using Visiopharm® (Version: 2023.09).

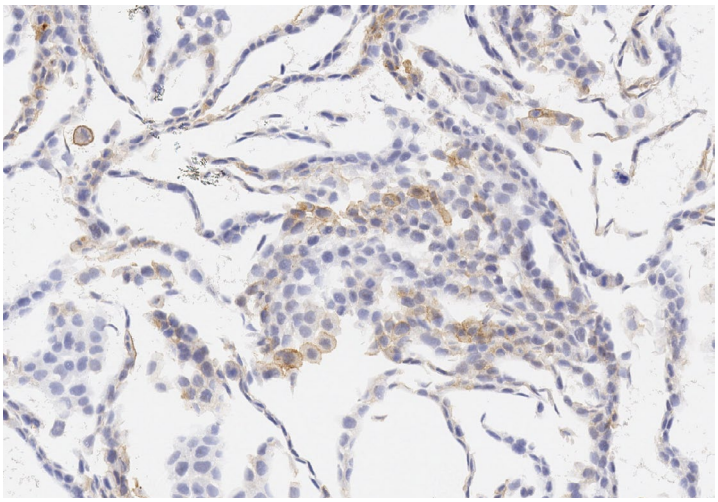
Mean DAB intensity was measured for tumor and stroma compartments, with an analytical range from 0 (+) to 250±2 (-), where a low mean DAB intensity score inversely corresponds to high expression. For graphical representation, the relative average DAB intensity was calculated using the formula (relative average DAB intensity = 250 - mean DAB intensity) and is represented in arbitrary units (AU). The graphical representation was generated using GraphPad Prism 9.

CD73 expression in FFPE cell pellets (BOND™ RX)

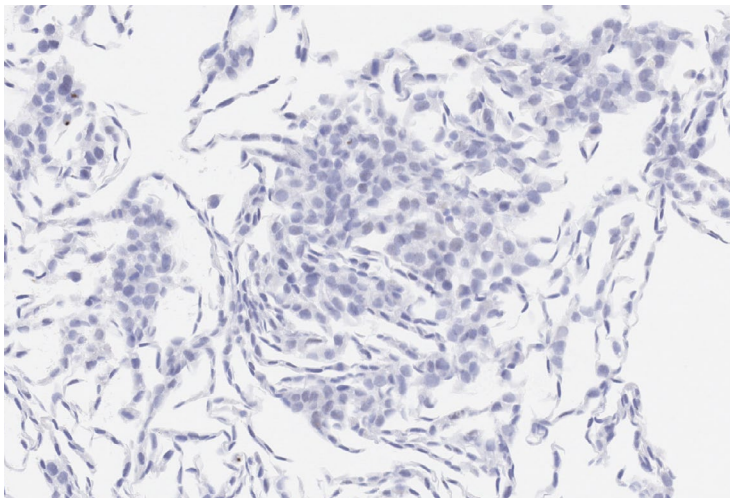
Below are the representative images of IHC staining in FFPE cell pellets using anti-CD73 antibody [EPR6114] (ab133582). Staining was detected in the NT5E wild-type cell line and absent in the knock-out cell line. As expected, a high level of expression was detected in U87-MG, a human glioblastoma cell line and absent from daudi, a human B lymphoblast cell line.

CD73

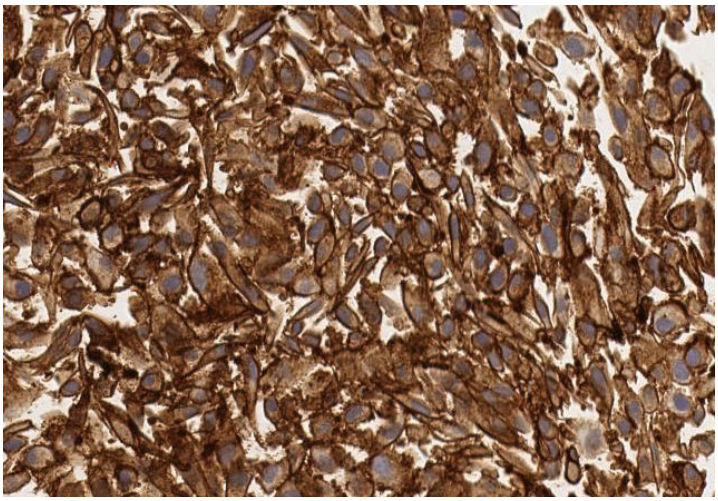
A431 NTSE +/+



A431 NTSE +/+



U87-MG



Daudi

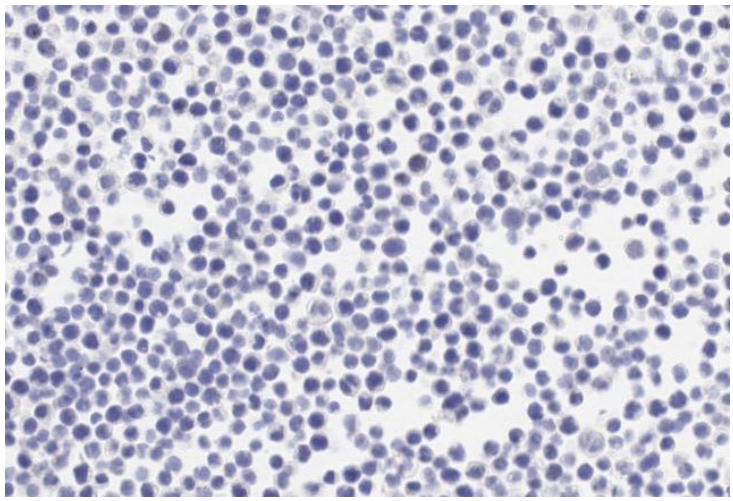


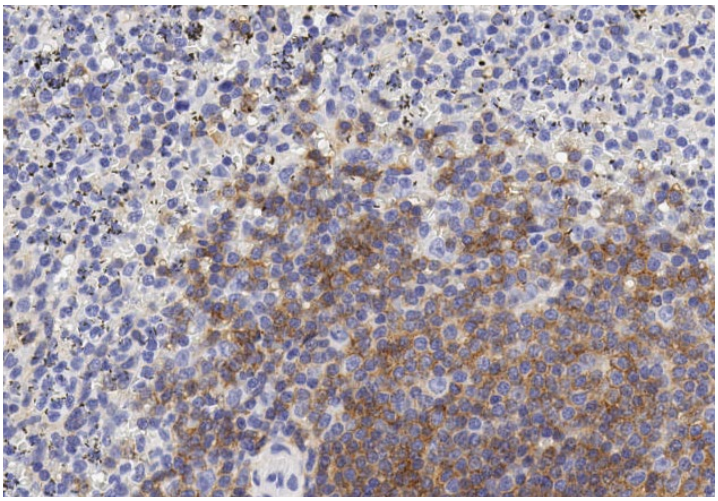
Figure 2. CD73 expression in human FFPE cell pellets. IHC staining of multi-normal human tissues using anti-CD73 antibody (ab133582) at a concentration of 5µg/ml. Positive staining in brown; nuclear hematoxylin counterstain in blue. No secondary controls were clean (data not shown). Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

CD73 expression in multi-normal TMA (DISCOVERY ULTRA)

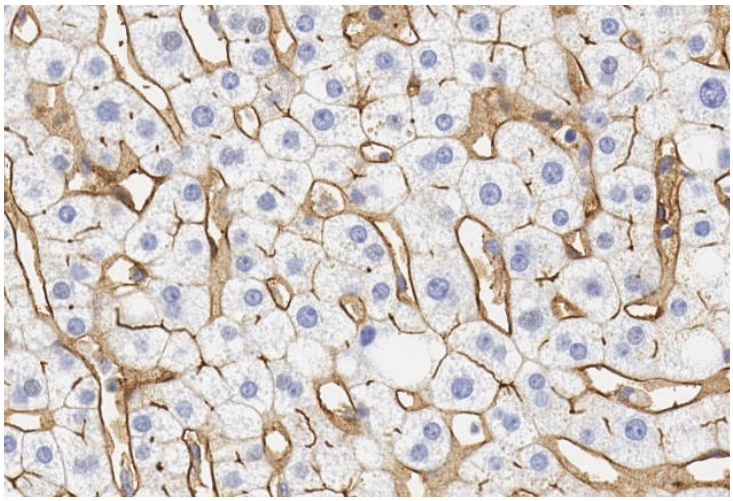
Below are the representative images of selected tissues from multi-normal TMA. CD73 was detected in the spleen, liver and pituitary gland and was absent in the lung.

CD73

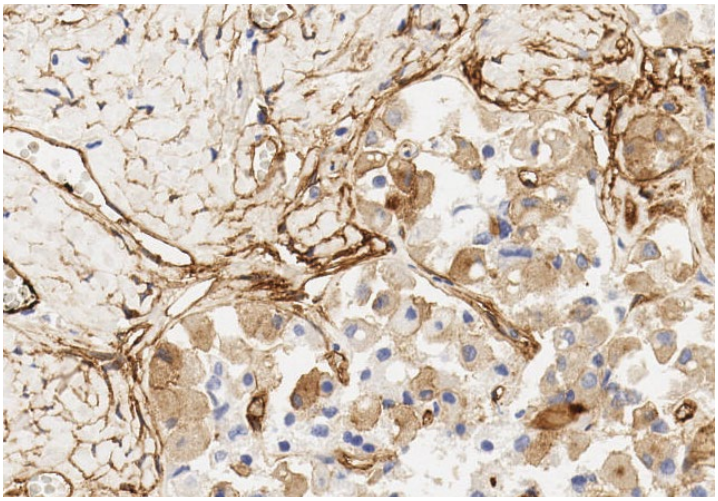
Spleen



Liver



Pituitary



Lung

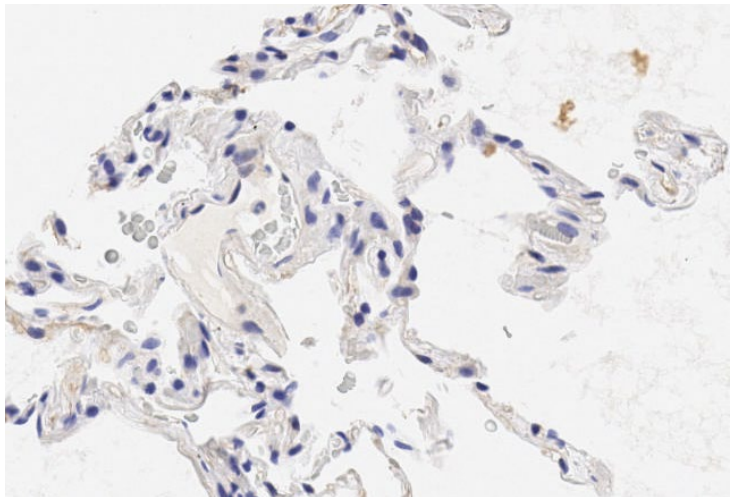


Figure 3. CD73 expression in normal tissue. IHC staining of multi-cancer human tissues using anti-CD73 (ab133582). Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

CD73 expression in cancer (BOND™ RX)

Cancer TMA cores were segmented into tumor and stroma components and CD73 staining intensity was evaluated (Figure a). The whole core (tumor and stroma) staining intensity of cohorts of cancer subtypes was also evaluated separately in scatter plots (with SD) (Figure b-d).

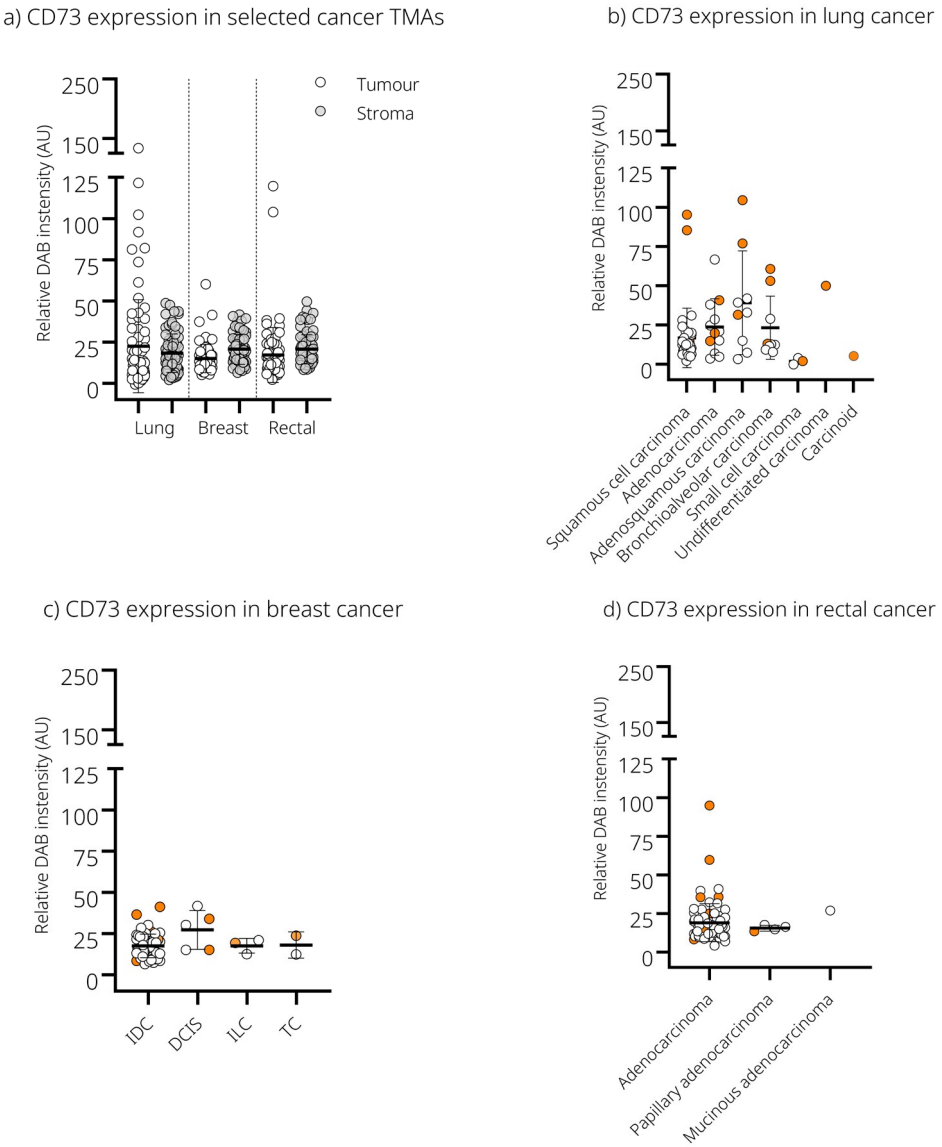


Figure 4. CD73 protein expression in a selection of cancer TMAs.
(a) The scatter plot (with SD) summarizes the relative average DAB intensity of CD73 expression in tumor and stroma components of TMA cores (maximum value 250).
(b) Relative average DAB intensity from 76 cores/cases of lung cancer; squamous cell carcinoma, grade 1-3 (37), adenocarcinoma, grade 1-3 (12), adenosquamous carcinoma (9), bronchioalveolar carcinoma (9), small cell carcinoma (3), undifferentiated carcinoma (1) carcinoid (1).
(c) Relative average DAB intensity from 67 cores/cases of breast cancer; invasive ductal carcinoma, grade 1-3 (IDC) (57), ductal carcinoma in situ (DCIS) (5), invasive lobular carcinoma (ILC) (3), tubular carcinoma (TC) (2), sarcoma (1).
(d) Relative average DAB intensity from 85 TMA cores/cases of rectal cancer; adenocarcinoma, grade 1-3 (80), papillary adenocarcinoma (4), mucinous adenocarcinoma (1).

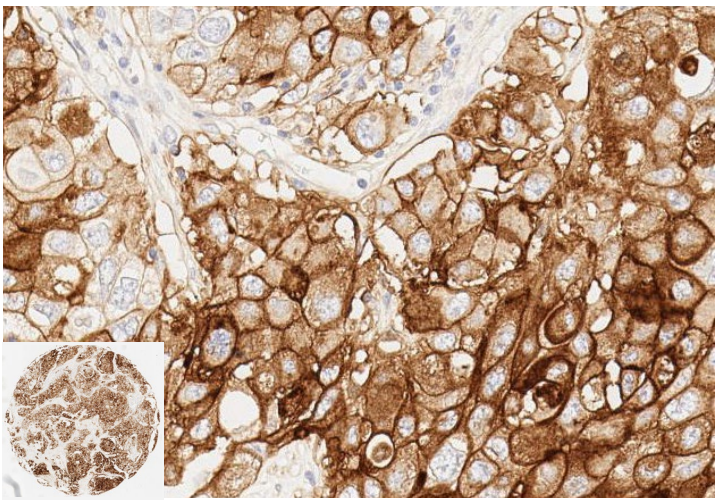
The IHC images corresponding to orange data points are shown in Figures 5-7.

CD73 expression in lung cancer TMA (BOND™ RX)

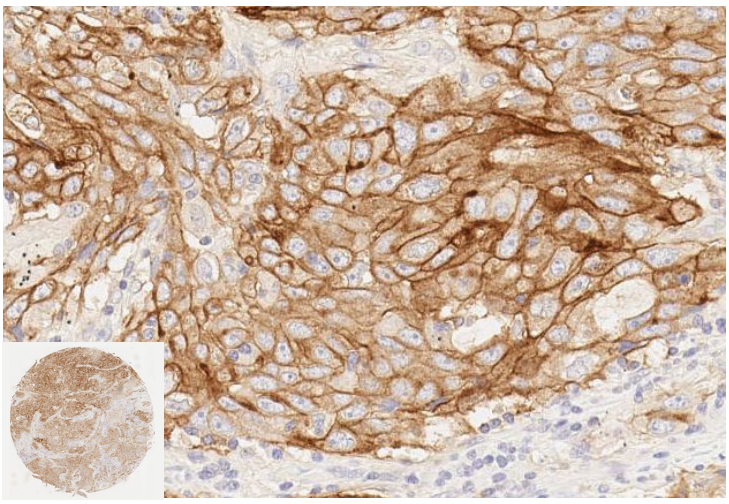
Below are the representative images of human lung cancer TMA showing strong to weak CD73 expression.

CD73

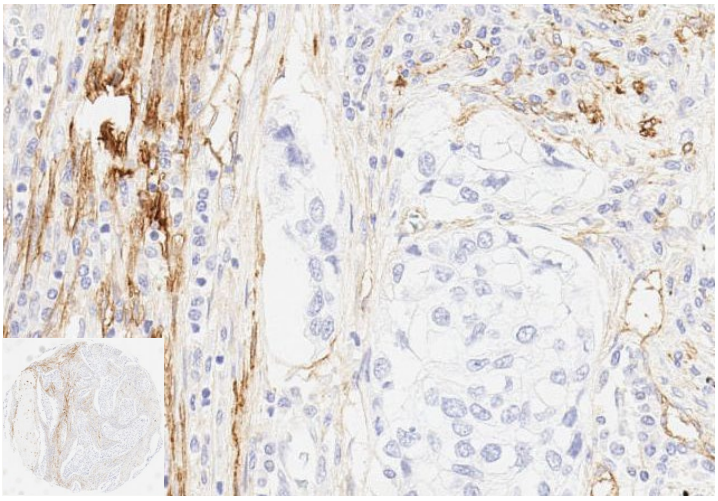
Squamous cell carcinoma (95.19)



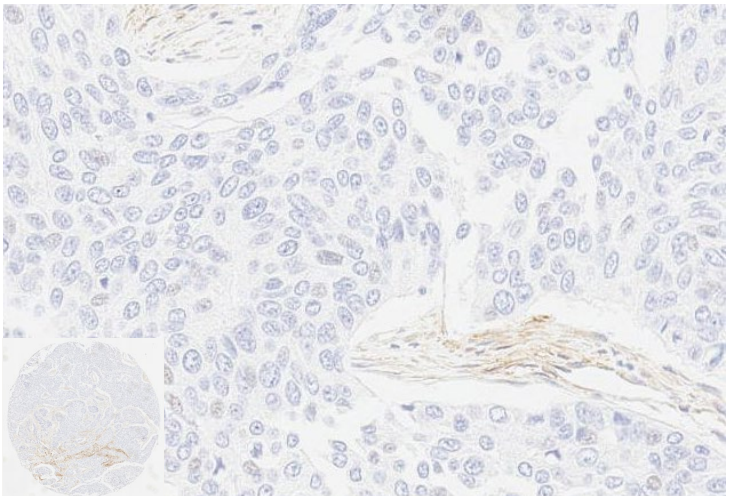
Squamous cell carcinoma (85.36)



Squamous cell carcinoma (14.45)

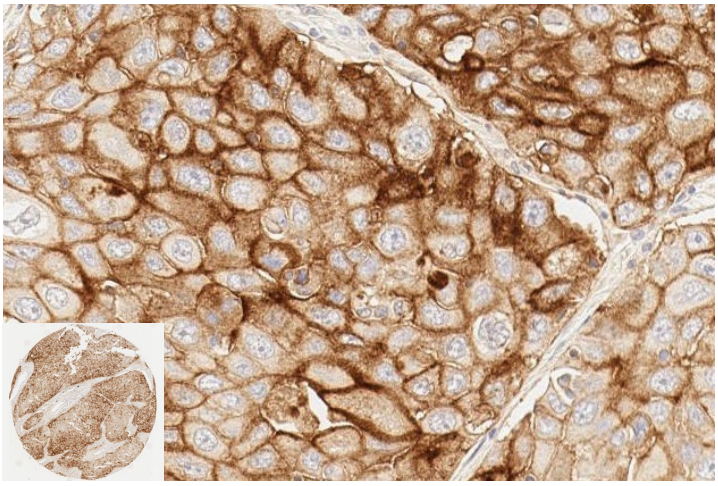


Squamous cell carcinoma (7.02)

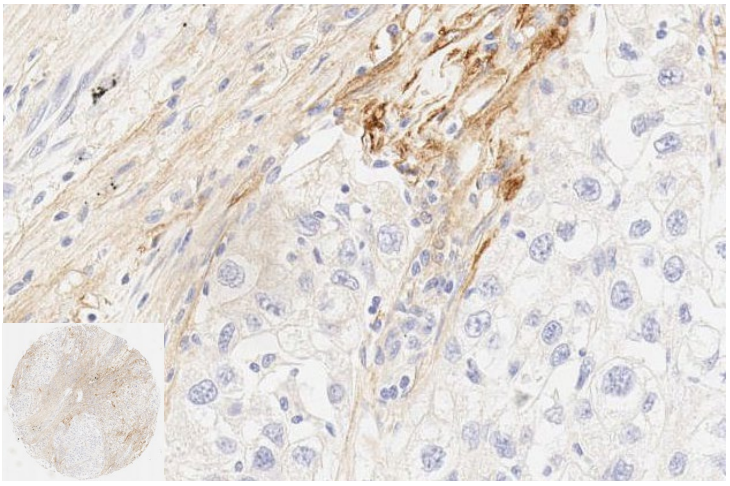


CD73

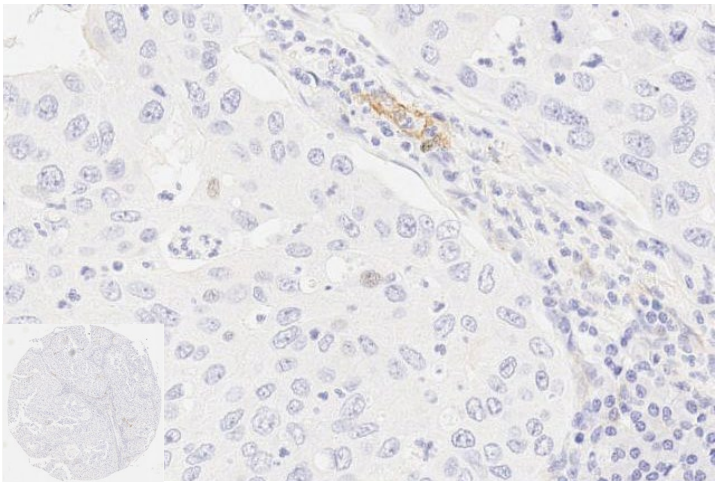
Adenosquamous carcinoma (104.54)



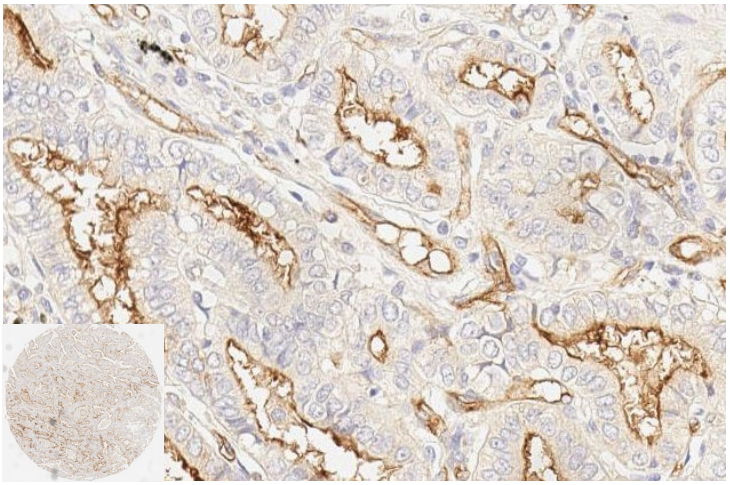
Adenosquamous carcinoma (31.40)



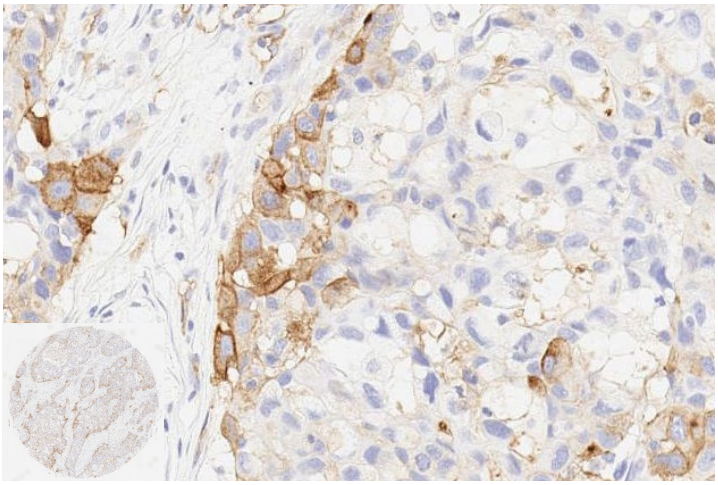
Adenosquamous carcinoma (7.3)



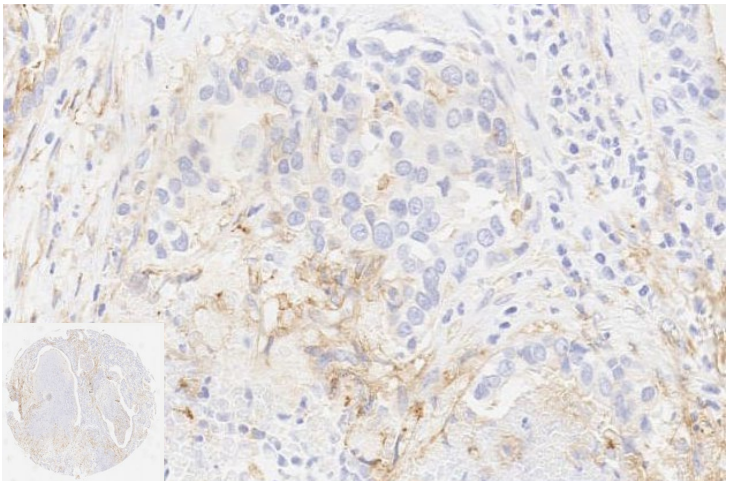
Adenocarcinoma (40.64)



Adenocarcinoma (19.72)

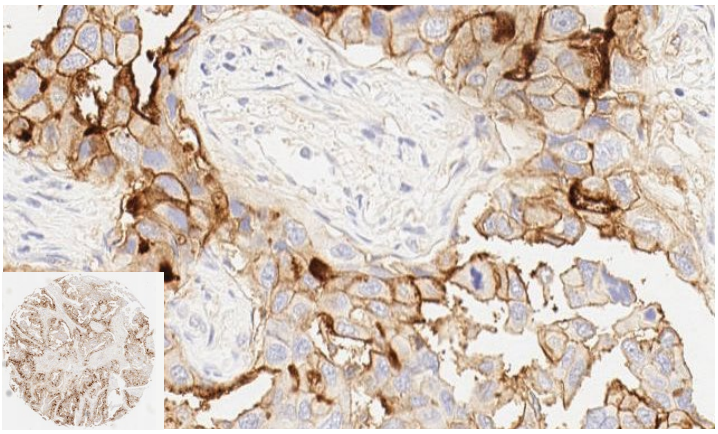


Adenocarcinoma (14.79)

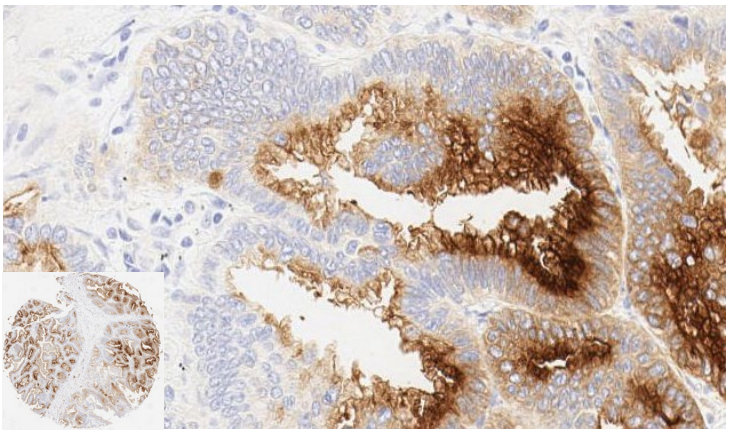


CD73

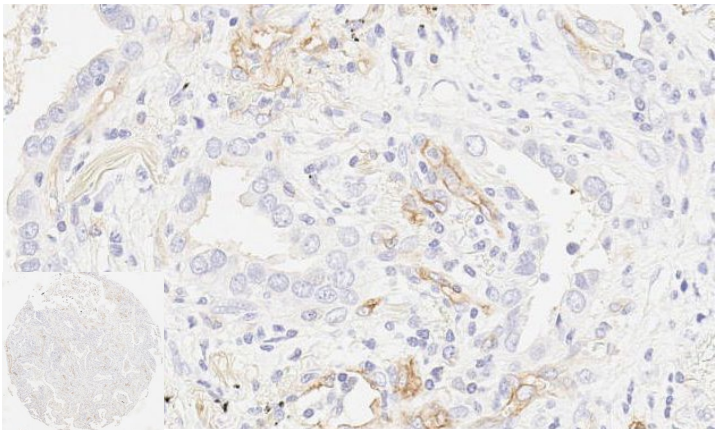
Bronchioalveolar carcinoma (60.72)



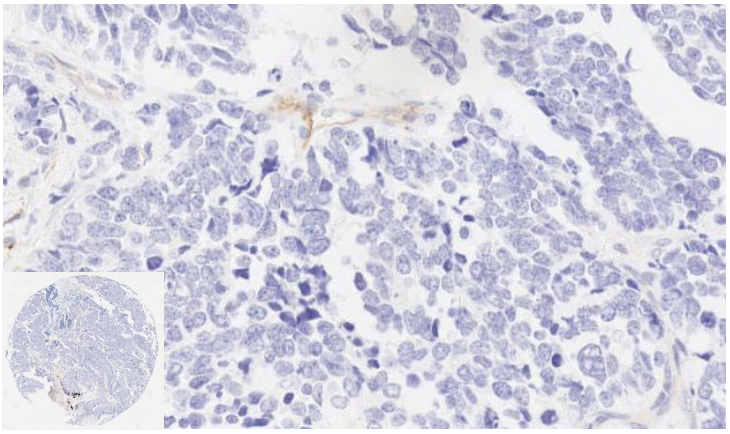
Bronchioalveolar carcinoma (53.11)



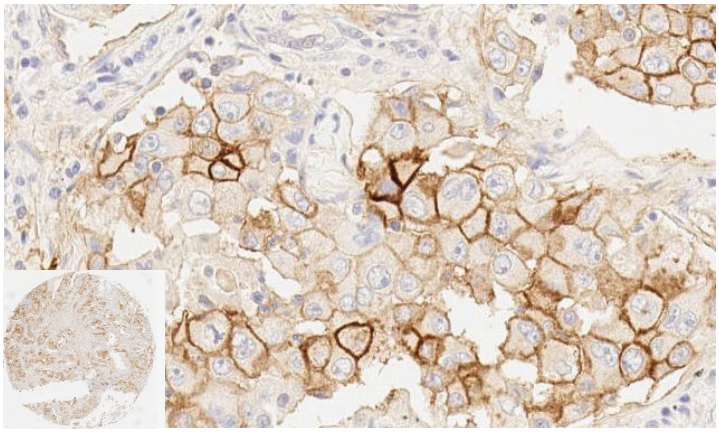
Bronchioalveolar carcinoma (12.88)



Small cell carcinoma (1.85)



Undifferentiated carcinoma, large cell (49.95)



Carcinoid (5.19)

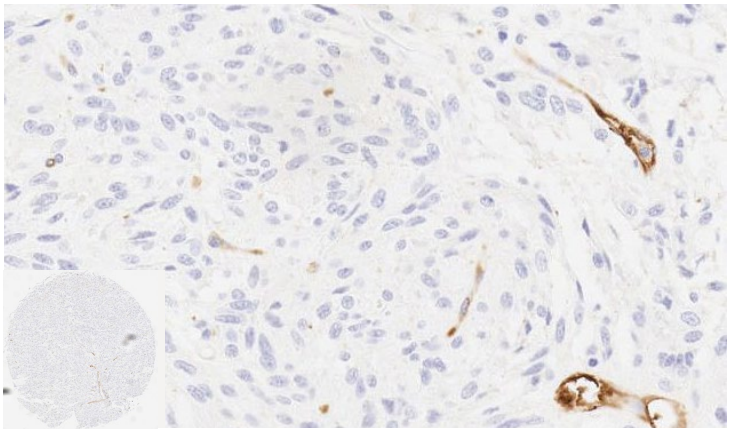


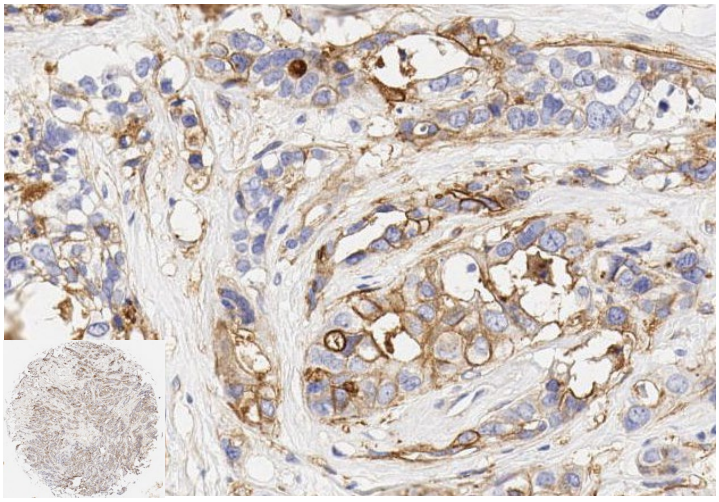
Figure 5. CD73 expression in lung cancer. IHC images showing CD73 staining with relative average DAB (a-h). Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x (whole core insets at 5x) on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

CD73 expression in breast cancer TMA (BOND™ RX)

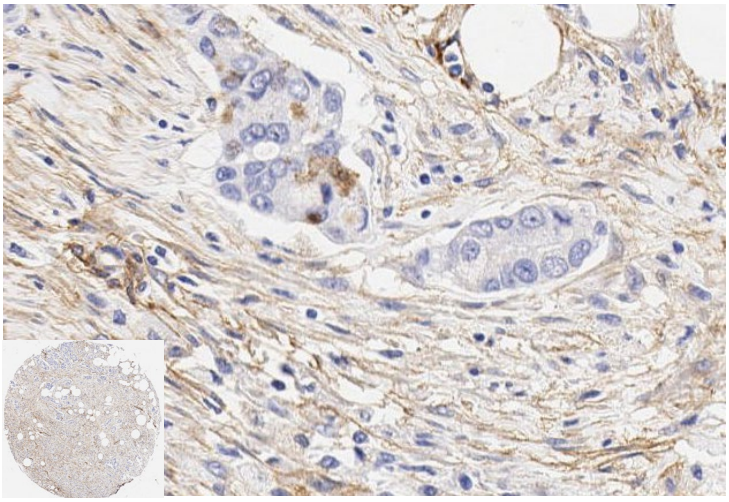
Below are the representative images of human breast cancer TMA showing strong to weak CD73 expression.

CD73

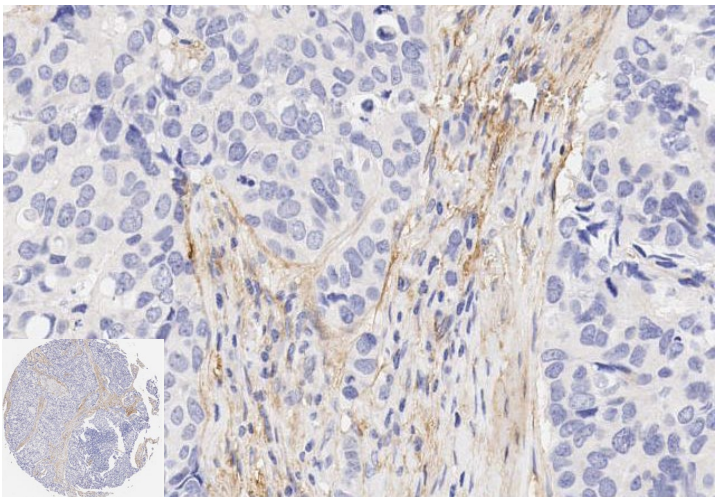
IDC (41.19)



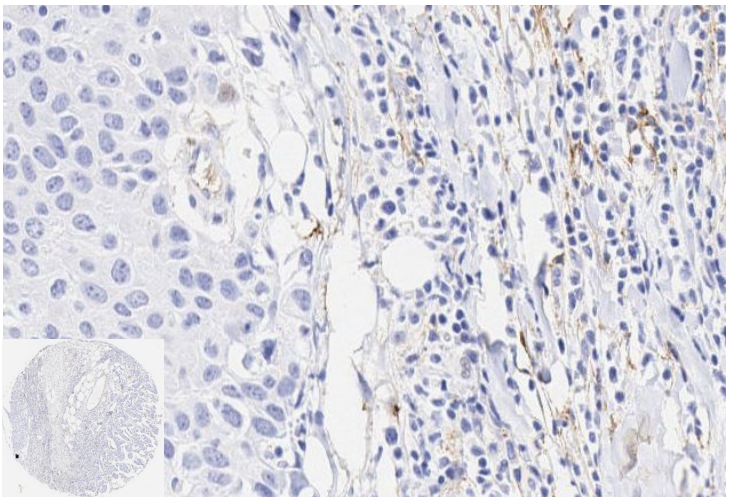
IDC (36.44)



IDC (21.27)

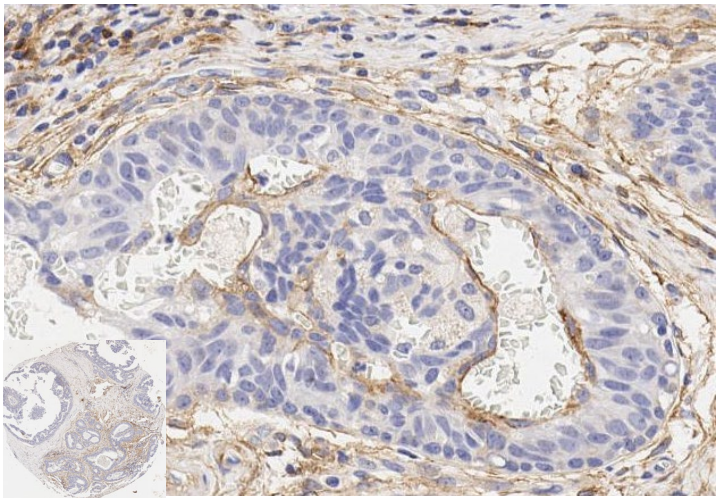


IDC (8)

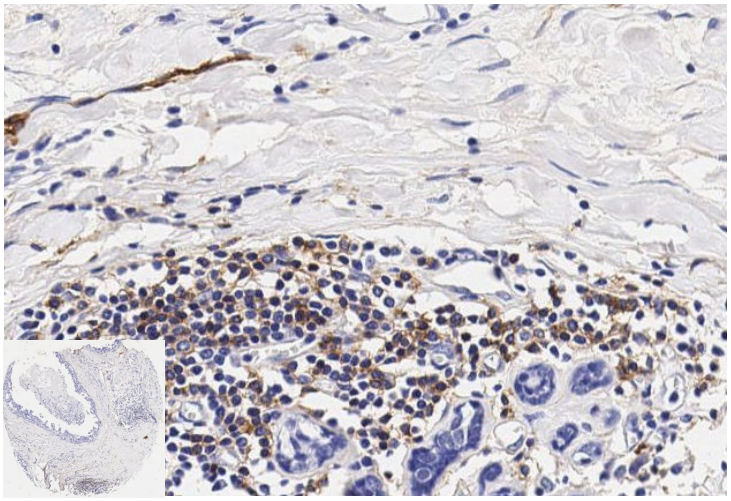


CD73

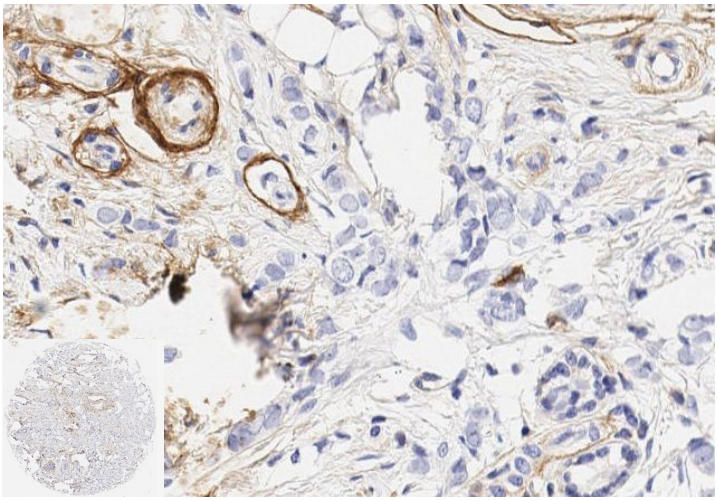
DCIS (33.95)



DCIS (15.07)



ILC (19.34)



TC (23.60)

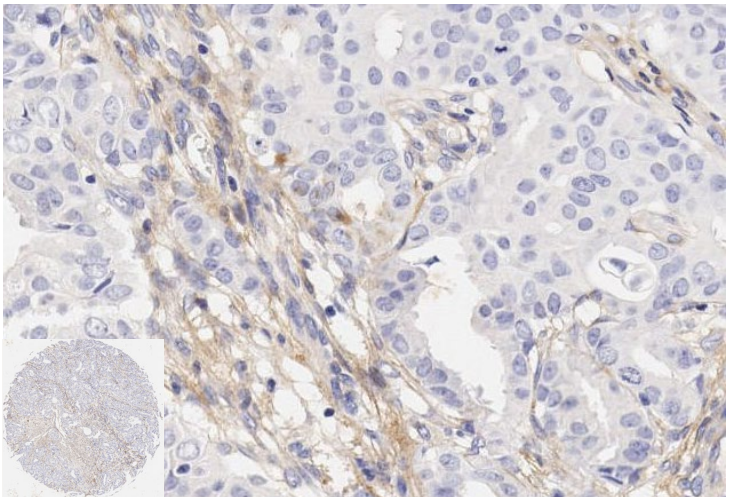


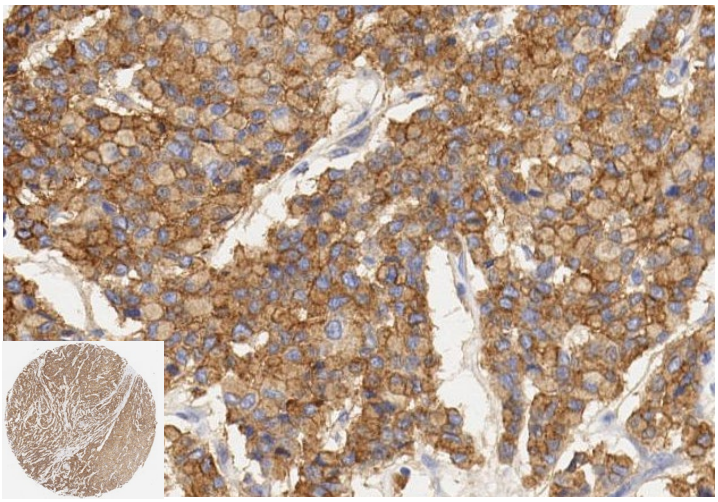
Figure 6. CD73 expression in breast cancer. IHC images showing CD73 staining with relative average DAB intensity score (a-h) Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x (whole core insets at 5x) on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

CD73 expression in rectal cancer TMA (BOND™ RX)

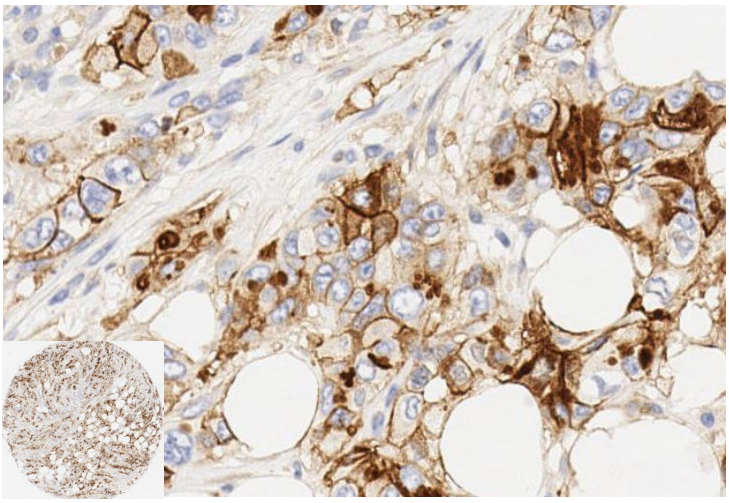
Below are the representative images of human rectal cancer TMA showing weak to strong CD73 expression.

CD73

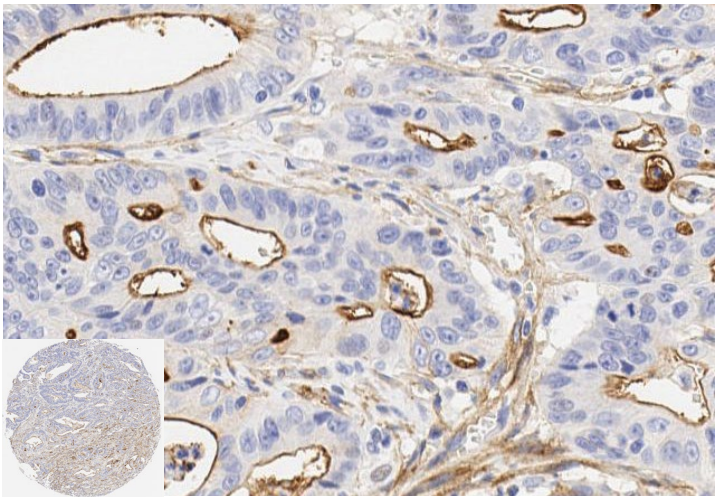
Adenocarcinoma (94.93)



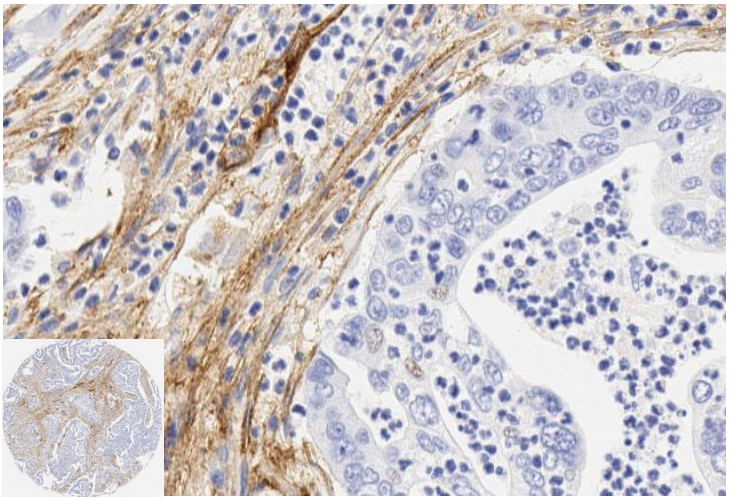
Adenocarcinoma (59.75)



Adenocarcinoma (35.69)

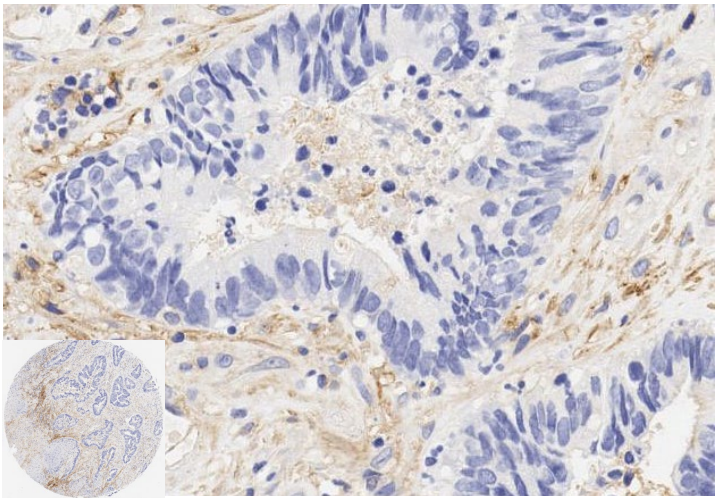


Adenocarcinoma (35.47)

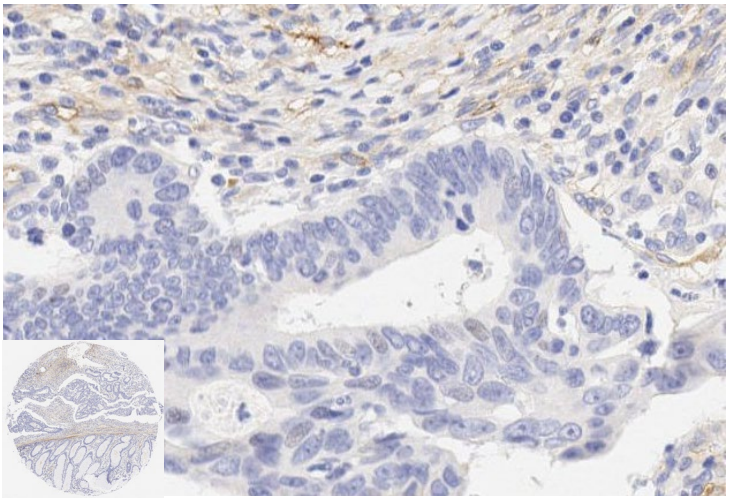


CD73

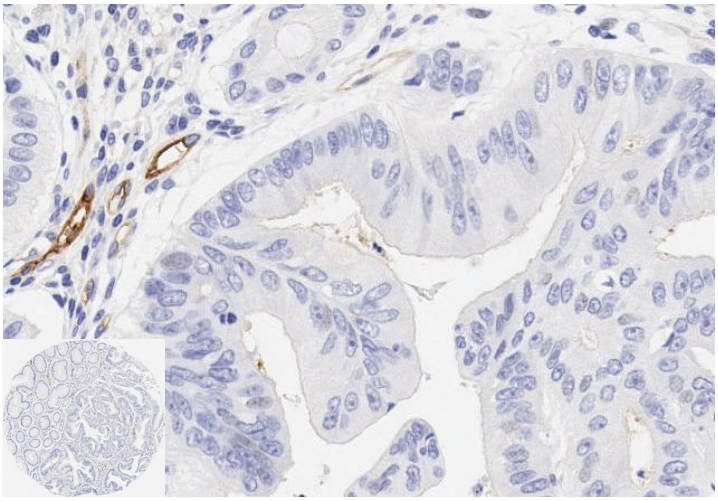
Adenocarcinoma (25.04)



Adenocarcinoma (15.50)



Adenocarcinoma (8.23)



Papillary adenocarcinoma (13.39)

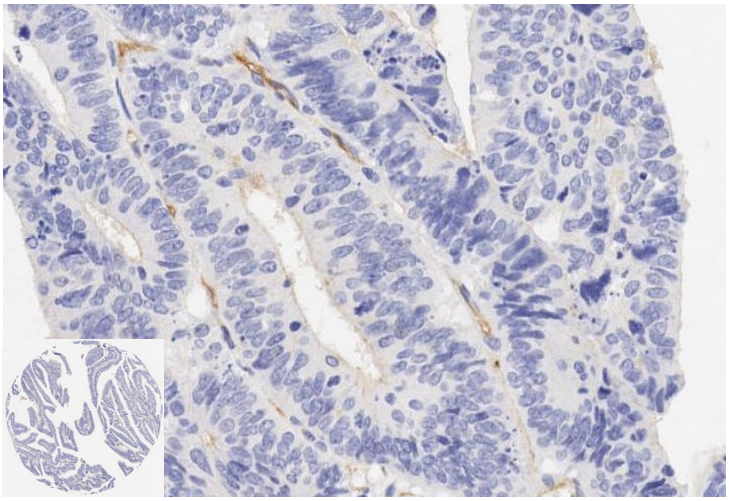


Figure 7. CD73 expression in rectal cancer. IHC images showing CD73 staining with relative average DAB (a-h). Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x (whole core insets at 5x) on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

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