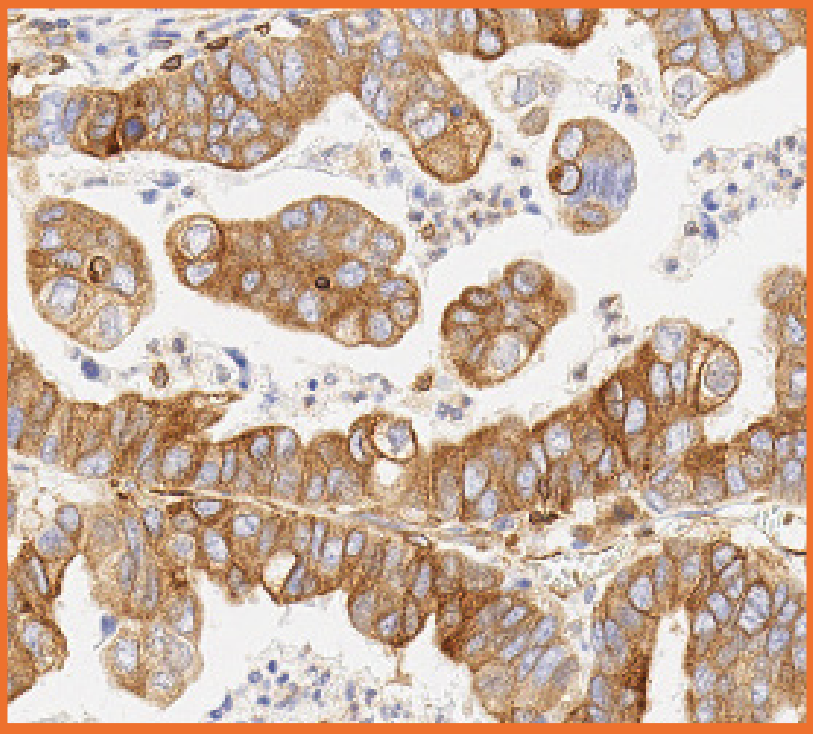
Enhanced validation data

Anti-STING recombinant antibody – ab239074



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Enhanced validation of Anti-STING recombinant antibody [EPR13130-55] – ab239074

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 & 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-STING recombinant monoclonal antibody (ab239074) in IHC in selected tissues and TMAs using a BOND™ RX Research Stainer (Leica®).

Target overview

HGNC symbol
STING1

Approved name
Stimulator of interferon response cGAMP interactor 1

Previous symbols:
TMEM173

Chromosomal location:
5q31.2

Function

- Facilitator of innate immune signaling that promotes the production of type I interferon (IFN-alpha and IFN-beta).
- Innate immune response is triggered in response to non-CpG double-stranded DNA from viruses and bacteria delivered to the cytoplasm.
- Able to activate both NF-kappa-B and IRF3 transcription pathways to induce expression of type I interferon and exert a potent anti-viral state following expression.
- May be involved in translocon function, the translocon possibly being able to influence the induction of type I interferons.
- May be involved in transduction of apoptotic signals via its association with the major histocompatibility complex class II (MHC-II).
- Mediates death signaling via activation of the extracellular signal-regulated kinase (ERK) pathway.

Tissue specificity

- Ubiquitously expressed.

Cellular localization

- Endoplasmic reticulum membrane.
- Mitochondrion outer membrane.
- Cell membrane.
- Cytoplasm > perinuclear region. In response to double-stranded DNA stimulation, relocalizes to perinuclear region, where the kinase TBK1 is recruited.

Target information above from: UniProt accession Q7Z7D3
The UniProt Consortium
The Universal Protein Resource (UniProt) in 2010
Nucleic Acids Res. 38:D142-D148 (2010)

Figure 1 shows STING protein levels in selected normal human tissues as determined by mass spectrometry[1].

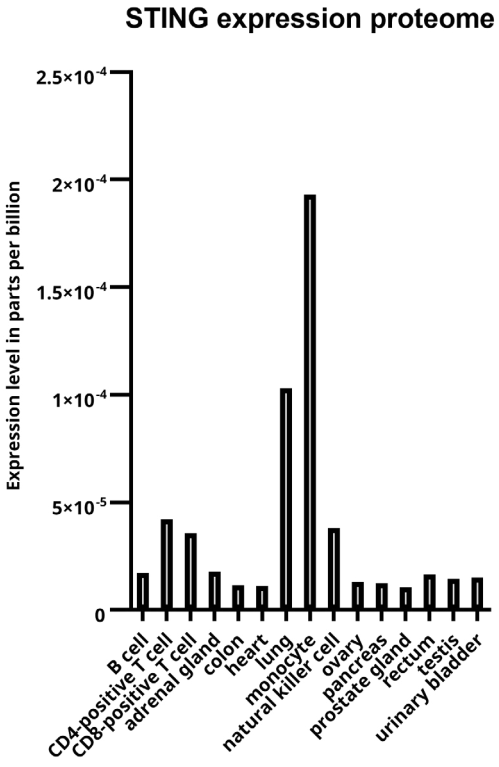


Figure 1. STING proteomic profile of 15 histologically normal human cell and tissue types. No detectable levels of STING were found in esophagus, frontal cortex, gall bladder, kidney, liver, retina and spinal cord.

Materials and methods

Human tissue	
Anatomic site	**Disease**
Tonsil	Normal
Lung	Normal

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

Human cancer tissue					
Tissue microarray (TMA)	**Cores**	**Cases**	**Normal/ Benign cases**	**Cancer cases**	**Source (#catalog number)**
Lung adenocarcinoma	102	102	5	97	Pantomics (#LUC1601)
Urinary bladder cancer	102	102	5	97	Pantomics (#BLC1021)
Pancreatic cancer	102	102	5	97	Pantomics (#PAC1021)
Colorectal cancer	102	102	5	97	Pantomics (#REC1021)
Stomach cancer	102	102	5	97	Pantomics (#STC1501)

Table 2. List of human cancer TMAs used in the enhanced validation.

Prestaining protocol		
Step	**Reagents**	**Pre-programmed protocol**
Dewax	Bond™ dewax solution (#AR922), alcohol, BOND wash solution (#AR9590)	Dewax
Antigen retrival	Bond™ epitope retrieval ER1 solution (#AR9961)	HIER with ER1 (pH 5.9–6.1), 20 min*, 100°C

*Except for lung tissue, which was incubated only for 10 minutes.

Table 3a. IHC prestaining protocol on BOND™ RX Research Stainer (Leica®).

Staining protocol			
Step	**Reagents**	**Number of washes**	**Incubation time, min**
Peroxide block	3-4% (v/v) Hydrogen peroxide	-	5
Wash	Bond™ wash solution	3x	0
Primary antibody	Recombinant Anti-STING antibody - ab239074 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 0.1µg/mL*	-	15
Wash	Bond™ wash solution	3x	0
Secondary detection	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 3b. 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. *Lung tissue, which was incubated with 0.05µg/mL of anti-STING antibody.

Percentage of IHC-positive tumor cells (A)	Intensity of IHC reaction (B)
0 = 0%	0 = no reaction
1 = <30%	1 = weak
2 = 30-60%	2 = moderate
3 = >60%	3 = strong
Final score = A x B (range 0-9)	

Table 4. IHC scoring method. A semi-quantitative IHC scoring method was used to determine the expression of STING in tumor cells (Table 4). This method assessed the extent of IHC staining using the proportion and intensity of stained tumor cells in TMA cores[2]. Results were analyzed on GraphPad Prism using box or scatter plots to show the distribution of scores. Incomplete cores or those with less than 50% tumor cells were excluded from the analysis.

STING expression in human normal tissues

STING was abundantly expressed in immune cells and bronchial epithelial cells (Figure 2).

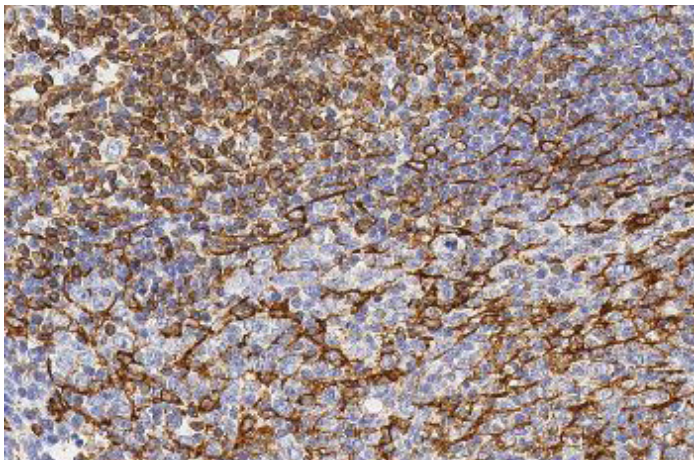
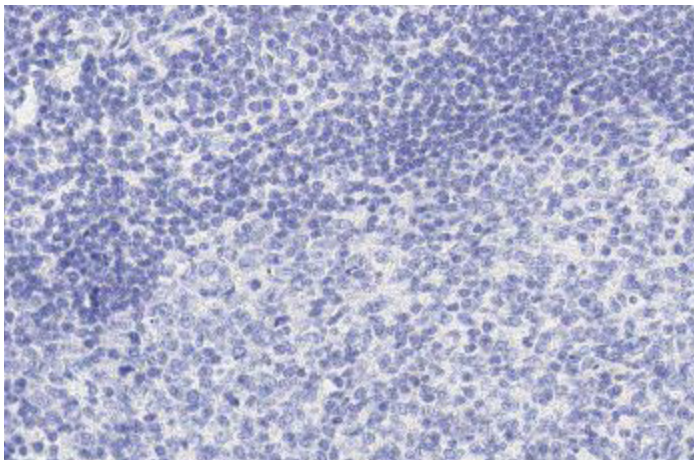
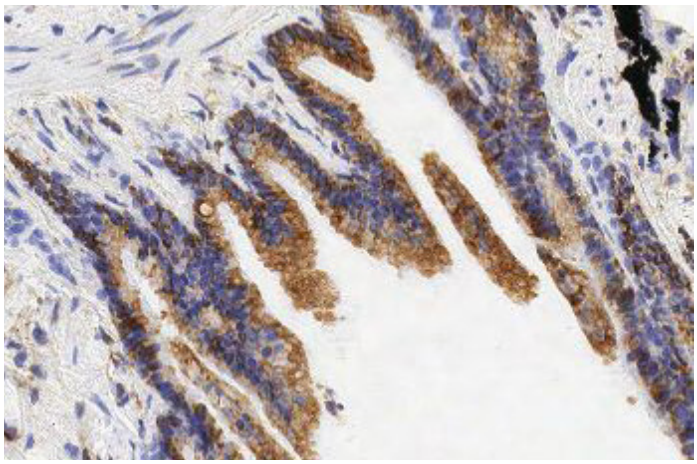
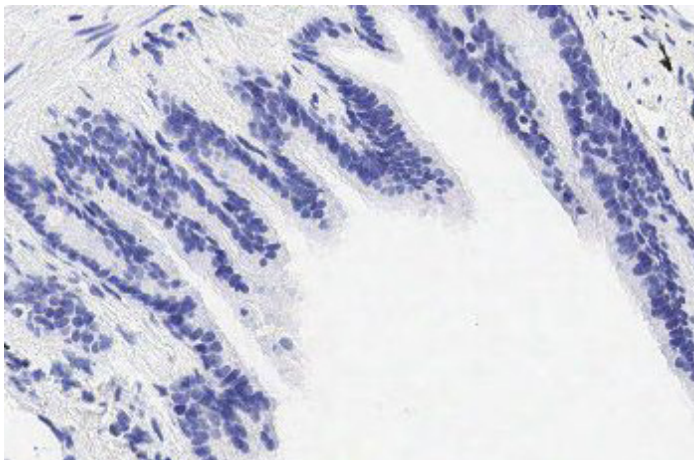
	STING	Isotype control
Tonsil		
Lung		

Figure 2. STING expression in human normal tissue. IHC staining of normal human tonsil and lung tissue using anti-STING antibody (ab239074) or rabbit IgG–isotype control antibody (ab172730). Positive staining in brown; hematoxylin counterstain in blue. Slides were scanned at 20x and images taken at 10x view on Aperio® ImageScope.

STING expression in cancer

STING expression varied in the analyzed cancer tissue microarrays (TMAs) (Figures 3-7), with stomach cancer TMA showing the lowest score and lung adenocarcinoma showing the highest expression. The staining intensity of cohorts of cancer subtypes was also evaluated separately (Figure 4 a-f).

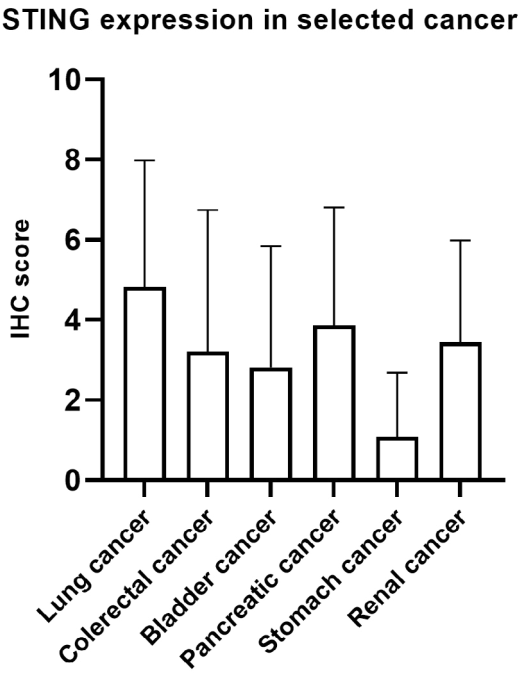


Figure 3. STING protein expression in a selection of cancer TMAs. The box plot (with SD) summarizes results from a semi-quantitative analysis of STING expression in TMA cores.

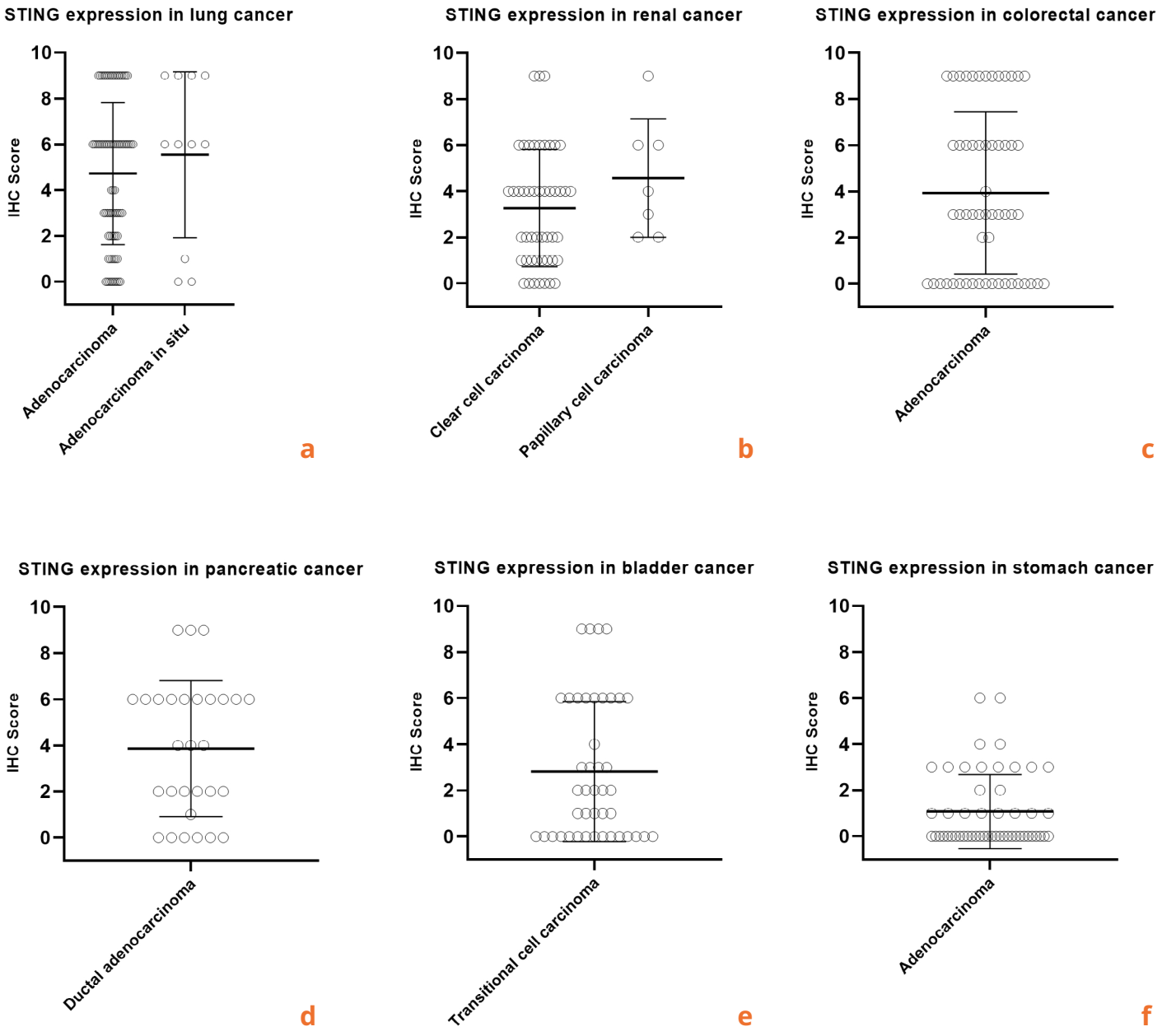
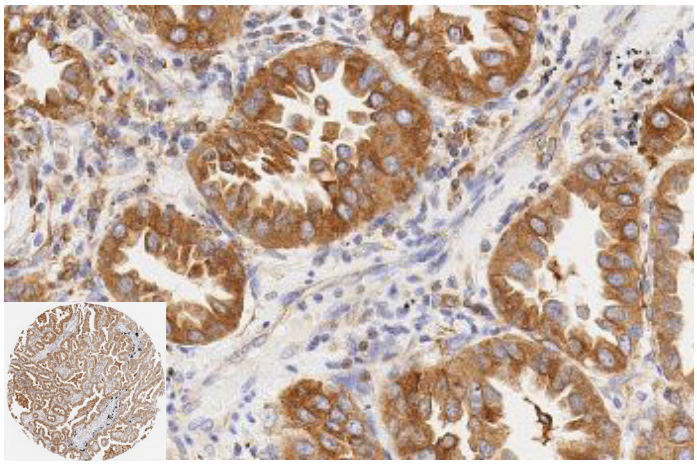


Figure 4. STING protein expression in of cohorts of cancer subtypes. The scatter plots (with SD) showing (a) STING IHC score in 75 TMA cores of lung adenocarcinoma and 11 TMA cores of adenocarcinoma *in situ* (b) STING IHC score in 57 TMA cores of colorectal adenocarcinoma (c) STING IHC in 43 TMA cores of bladder transitional cell carcinoma (d) STING IHC score in 29 TMA cores of pancreatic ductal adenocarcinoma (e) STING IHC score in 52 TMA cores of stomach adenocarcinoma (f) STING IHC score in 48 TMA cores of renal clear cell carcinoma adenocarcinoma and 7 TMA cores of papillary cell carcinoma.

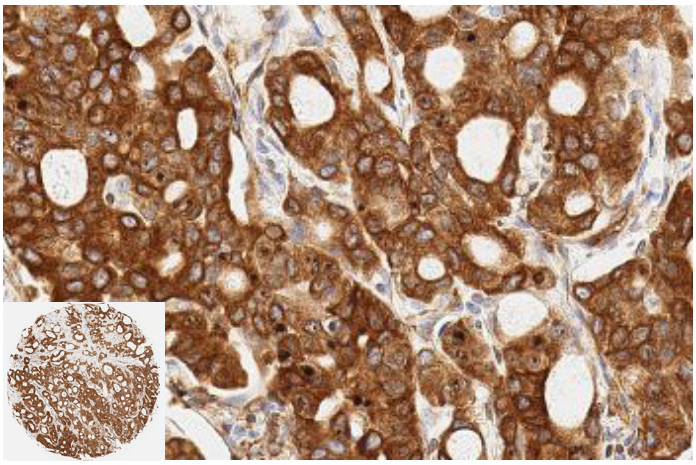
STING expression in lung cancer TMA

Below are the representative images of moderate to strong STING expression in lung adenocarcinoma (Figure 5).

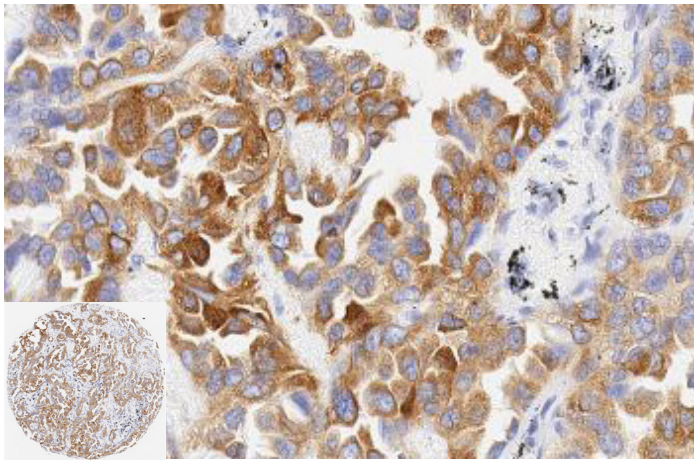
Lung adenocarcinoma
(moderate STING expression)



Lung adenocarcinoma
(strong STING expression)



Lung adenocarcinoma *in situ*
(moderate STING expression)



Lung adenocarcinoma *in situ*
(strong STING expression)

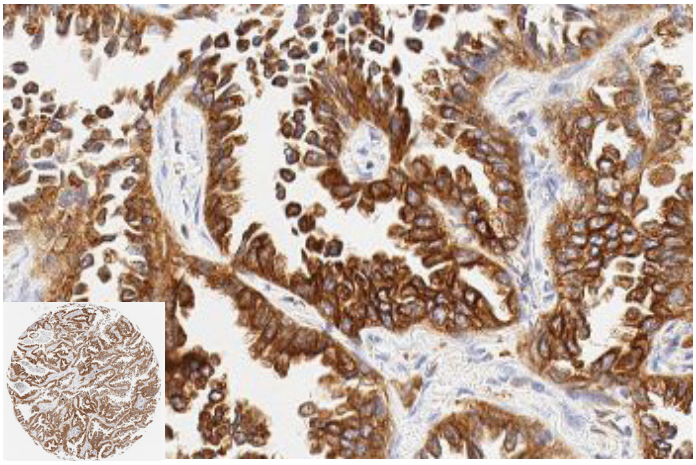


Figure 5. STING protein expression in lung cancer. These IHC images show medium or strong STING expression (brown) in adenocarcinoma cells in TMA cores. Positive staining in brown; hematoxylin counterstain in blue. Slides were scanned at 20x and magnified images taken at 10x view (whole core insets at 5x) on Aperio® ImageScope.

STING expression in colorectal cancer TMA

Below are the representative images of moderate to strong STING expression in colorectal adenocarcinoma (Figure 6).

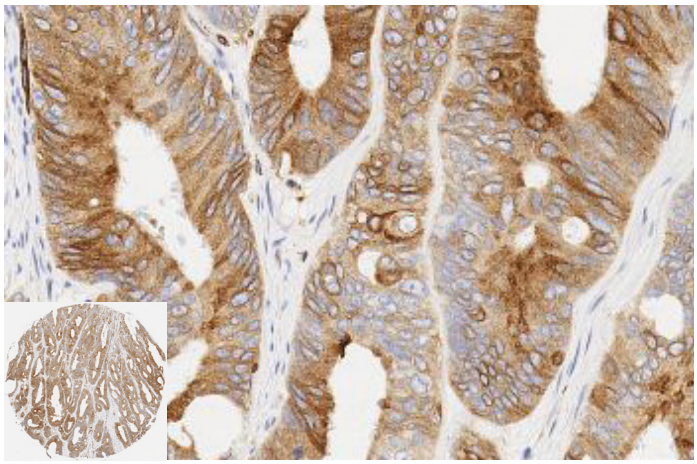
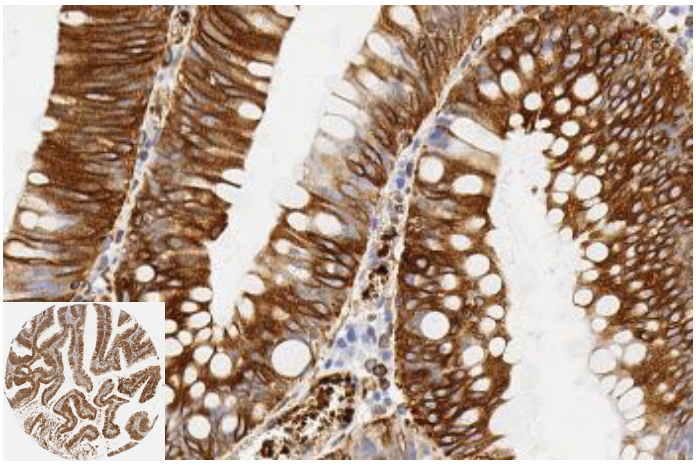
Colorectal adenocarcinoma (moderate expression)	**Colorectal adenocarcinoma** (strong expression)
	

Figure 6. STING protein expression in colorectal cancer. Images show medium or strong IHC staining (brown) of STING in adenocarcinoma cells in TMA cores. Positive staining in brown; hematoxylin counterstain in blue. Slides were scanned at 20x and magnified images taken at 10x view (whole core insets at 5x) on Aperio® ImageScope.

STING expression in pancreatic cancer TMA

Below are the representative images of moderate to strong STING expression in pancreatic ductal adenocarcinoma (Figure 7).

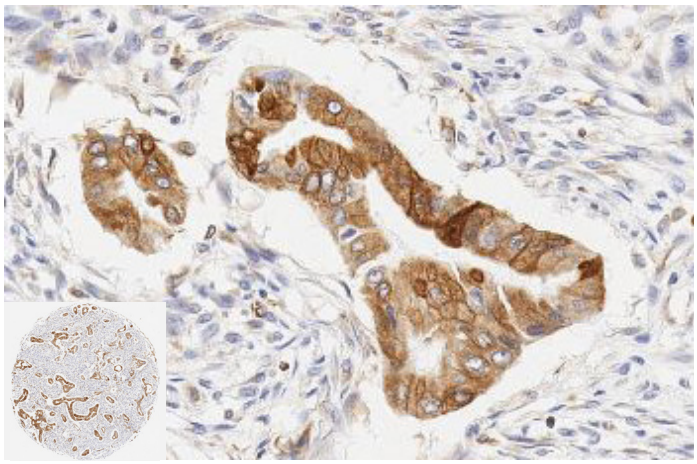
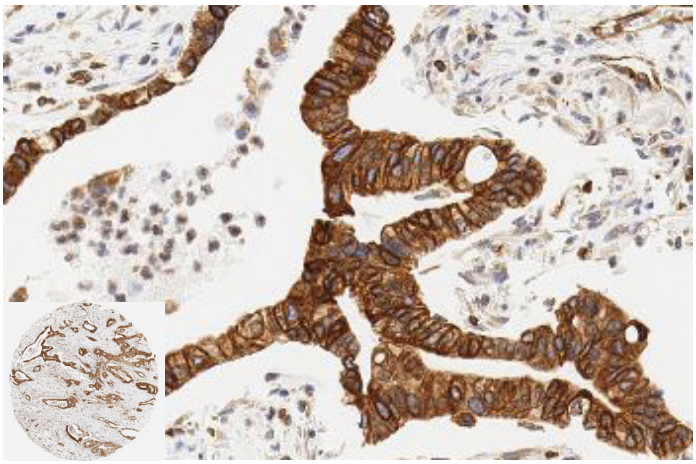
Pancreatic ductal adenocarcinoma (moderate expression)	**Pancreatic ductal adenocarcinoma** (strong expression)
	

Figure 7. STING protein expression in pancreatic cancer. These IHC images show medium or strong STING expression (brown) in adenocarcinoma cells in TMA cores. Positive staining in brown; hematoxylin counterstain in blue. Slides were scanned at 20x and magnified images taken at 10x view (whole core insets at 5x) on Aperio® ImageScope.

STING expression in urinary bladder cancer TMA

Below are the representative images of moderate to strong STING expression in transitional cell carcinoma (Figure 8).

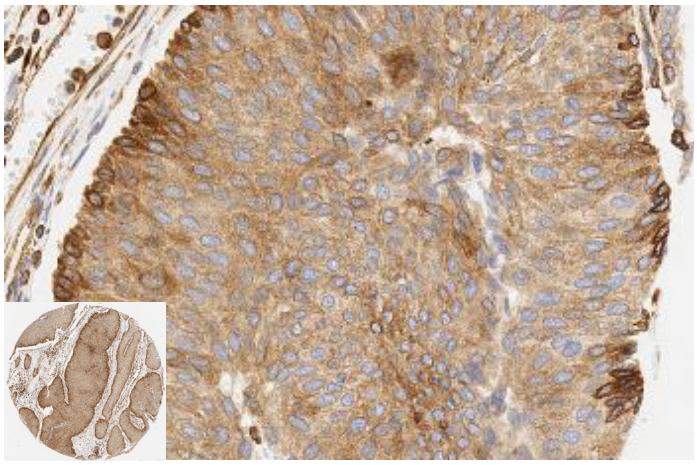
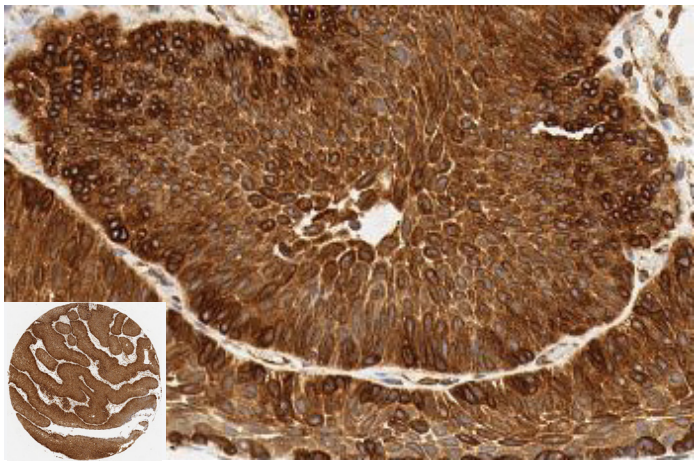
Transitional cell carcinoma (moderate expression)	**Transitional cell carcinoma** (strong expression)
	

Figure 8. STING protein expression in urinary bladder cancer. These IHC images show medium to strong STING expression (brown) in transitional carcinoma cells in TMA cores. Positive staining in brown; hematoxylin counterstain in blue. Slides were scanned at 20x and magnified images taken at 10x view (whole core insets at 5x) on Aperio® ImageScope.

STING expression in stomach cancer TMA

Below are the representative images of low to moderate STING expression in adenocarcinoma (Figure 9).

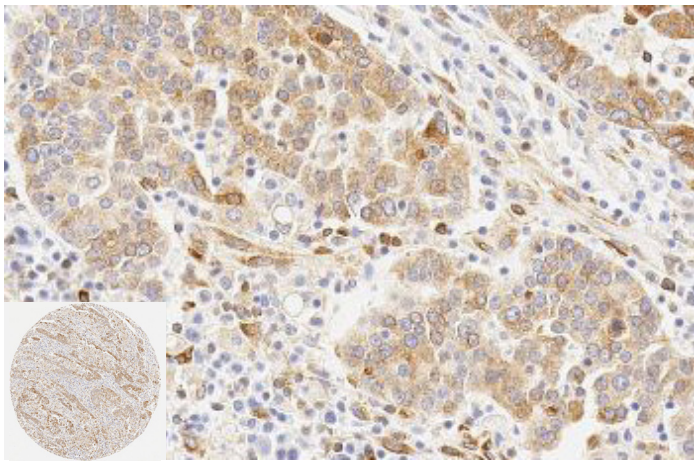
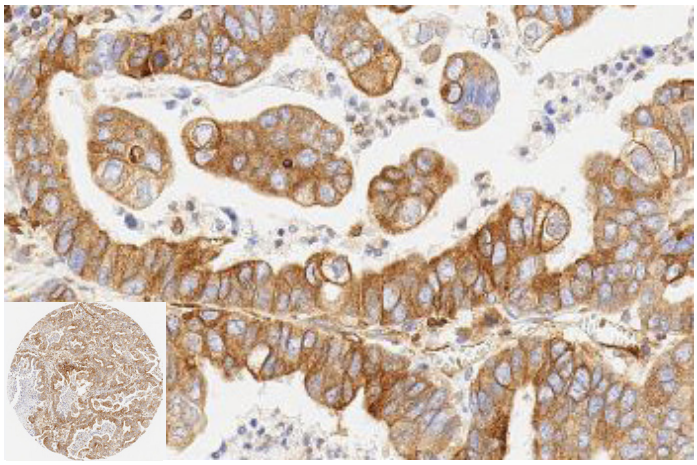
Adenocarcinoma (low expression)	**Adenocarcinoma** (moderate expression)
	

Figure 9. STING protein expression in stomach cancer. These IHC images show low to medium STING expression (brown) in adenocarcinoma cells in TMA cores. Positive staining in brown; hematoxylin counterstain in blue. Slides were scanned at 20x and magnified images taken at 10x view (whole core insets at 5x) on Aperio® ImageScope.

References

1. Kim MS, Pinto SM, Getnet D, Nirujogi RS, Manda SS, *et al.*. A draft map of the human proteome. *Nature*, **509**(7502), 575-81 (2014).

2. Ishikawa, H., Ma, Z., & Barber, G. N. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. *Nature*, **461**(7265), 788–792 (2009).

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