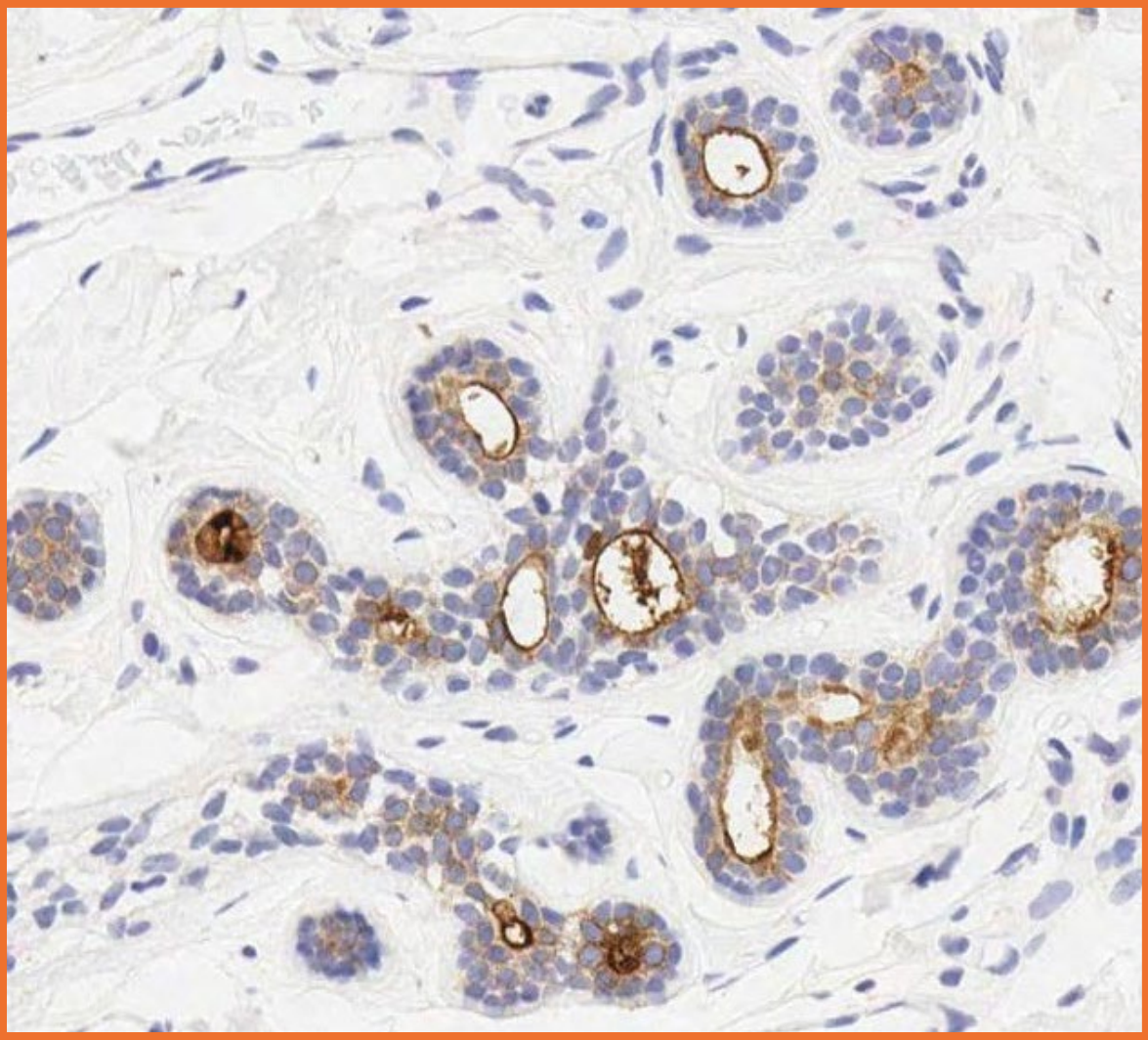
Enhanced validation data

Anti-B7H4 recombinant antibody – ab209242



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Enhanced validation of Anti-B7H4 recombinant antibody [EPR20236] – ab209242

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-B7H4 recombinant monoclonal antibody (ab209242) in IHC in selected tissues and TMAs using a BOND™ RX Research Stainer (Leica®).

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Target overview

HGNC symbol
VTCN1

Approved name
V-Set Domain Containing T Cell Activation Inhibitor 1

Alternative name
B7H4

Chromosomal location
1p13.1

Function

- Negatively regulates T cell-mediated immune response by inhibiting T cell activation, proliferation, cytokine production and development of cytotoxicity.
- When expressed on the cell surface of tumor macrophages, plays an important role, together with regulatory T-cells (Treg), in the suppression of tumor-associated antigen-specific T cell immunity.
- Involved in promoting epithelial cell transformation.

Tissue specificity

- Overexpressed in breast, ovarian, endometrial, renal cell (RCC) and non-small-cell lung cancers (NSCLC).
- Expressed on activated T- and B-cells, monocytes and dendritic cells, but not expressed in most normal tissues (at protein level).
- Widely expressed, including in kidney, liver, lung, ovary, placenta, spleen and testis.

Cellular localization

Cell membrane. Expressed at the cell surface. A soluble form has also been detected.

Target information above from: UniProt accession Q7Z7D3

The UniProt Consortium

The Universal Protein Resource (UniProt) in 2010

Nucleic Acids Res. 38:D142-D148 (2010)

Materials and methods

Human tissue	
Anatomic site	*Disease*
Breast	Normal
Kidney	Normal
Ovary	Normal
Endometrium	Normal

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

Human cancer tissues					
Tissue microarray (TMA)	*Cores*	*Cases*	*Normal/Benign cases*	*Cancer cases*	*Source (#catalog number)*
Breast cancer	102	102	5	97	Pantomics (#BRC1021)
Endometrial cancer	102	102	5	97	Pantomics (#EMC1021)
Ovary cancer	102	102	5	97	Pantomics (#OVC1021)
Kidney cancer	102	102	5	97	Pantomics (#KIC1021)

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

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

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

Step	Reagents	Number of washes	Time (minutes)
Peroxide block	3-4% (v/v) Hydrogen peroxide	-	5
Wash	Bond™ wash solution	3x	0
Primary antibody	Recombinant anti-B7H4 rabbit monoclonal [EPR20236] antibody – ab209242 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 0.8 µg/mL	-	15
Wash	Bond™ wash solution	3x	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 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.

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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)	

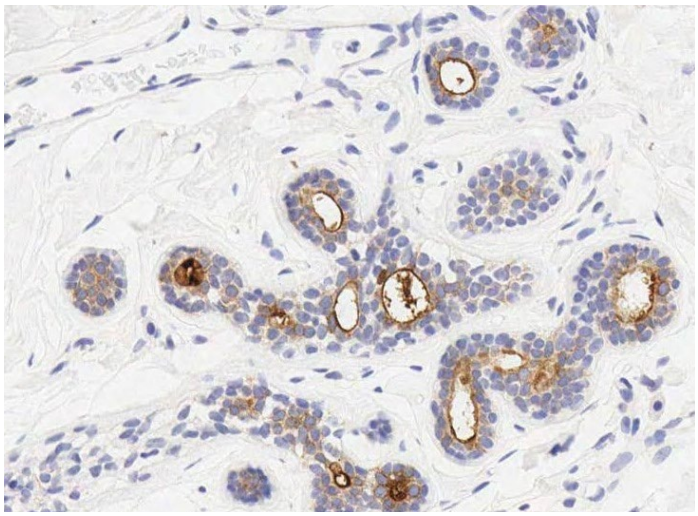
Table 4. IHC scoring method. A semi-quantitative IHC scoring method was used to determine the expression of B7H4 in tumor cells (Table 4). This method assessed the extent of IHC staining using the proportion and intensity of stained tumor cells in TMA cores[1]. Results were analyzed on GraphPad Prism using a box plot to show the distribution of scores. Incomplete cores or those with less than 50% tumor cells were excluded from the analysis.

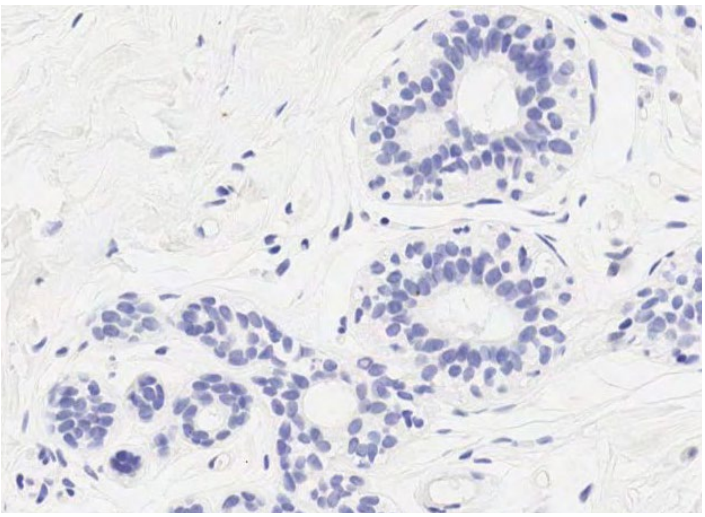
B7H4 expression in normal human tissues

B7H4 expression was detected in the apical luminal membrane of normal breast epithelium. Figure 1 shows low sporadic staining of distal convoluted renal tubules[2] and no staining in normal ovary and endometrium.

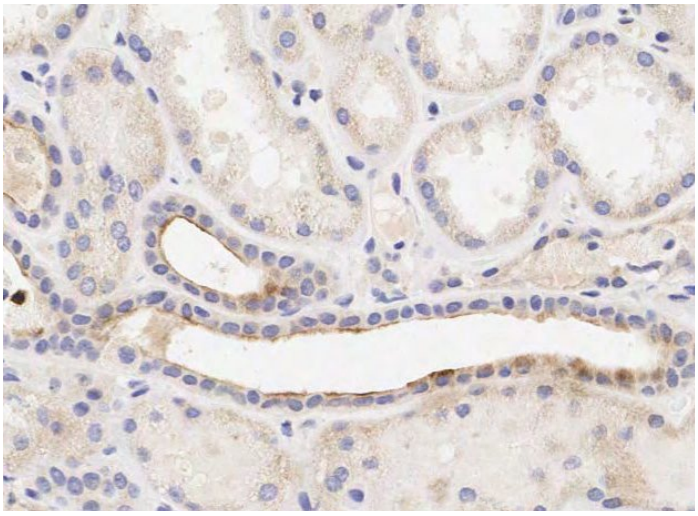
B7H4	Isotype control

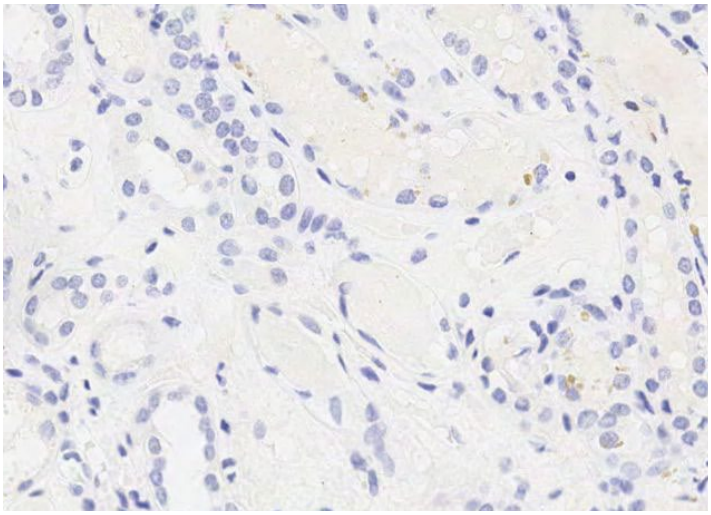
Breast





Kidney





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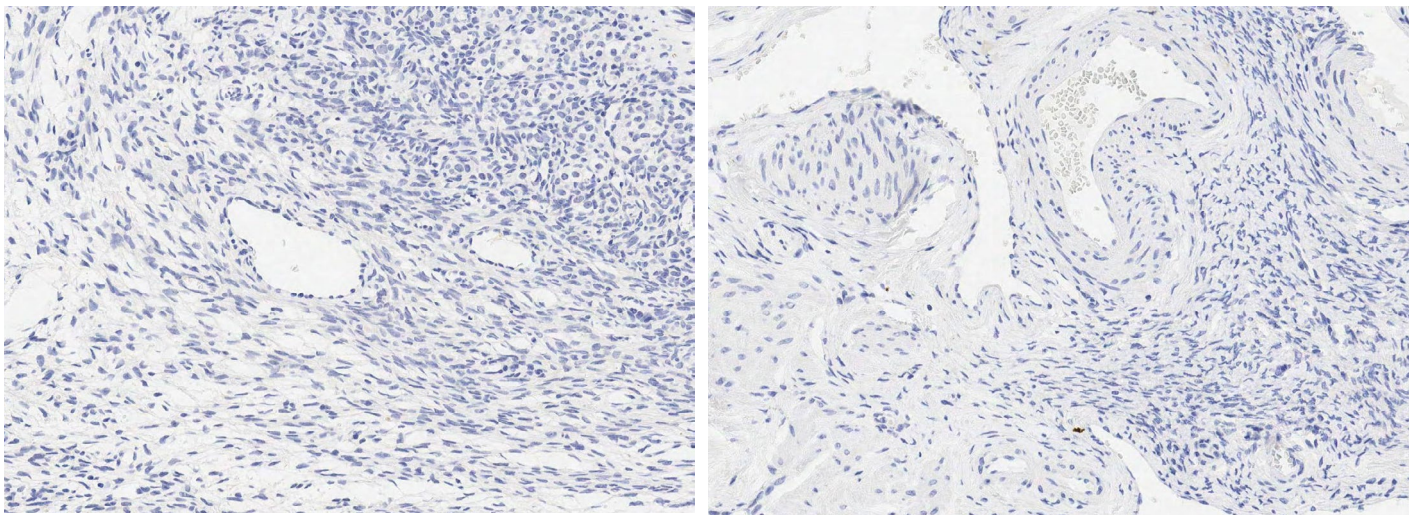
Ovary



Endometrium

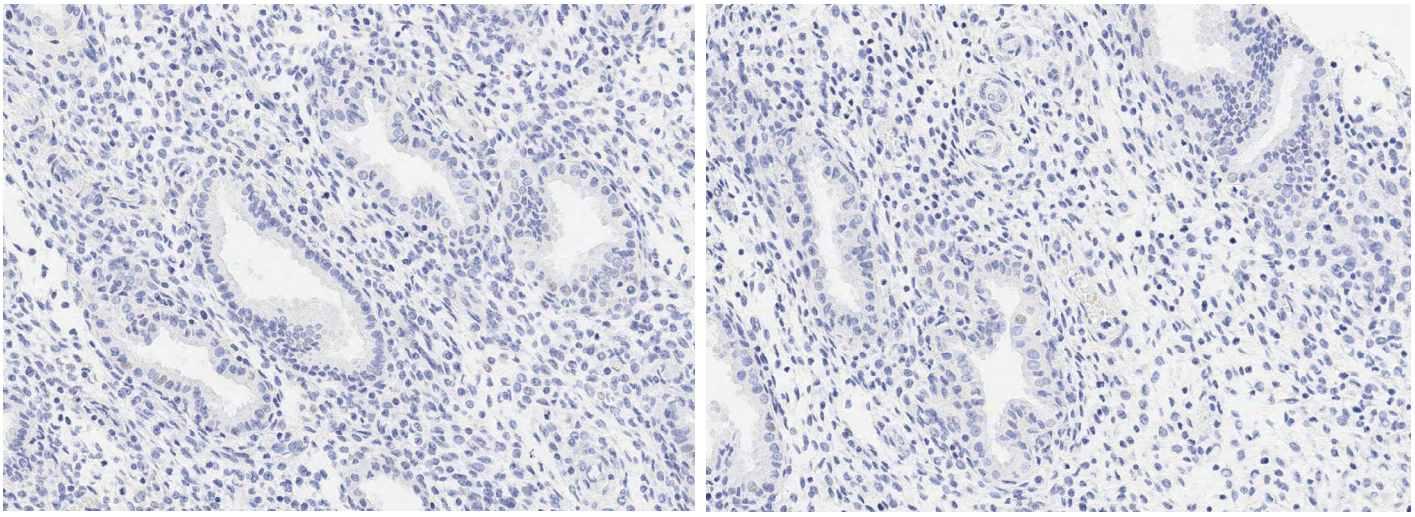
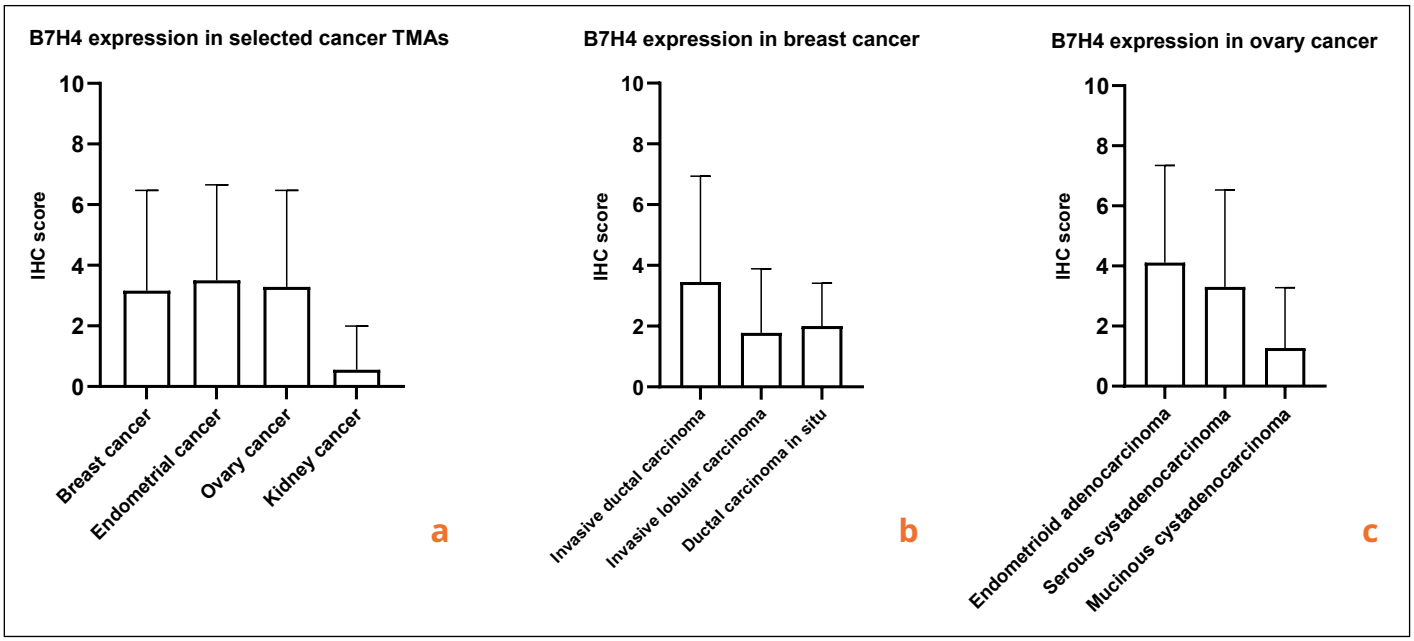


Figure 1. B7H4 expression in human normal tissue. IHC staining of normal human breast, kidney, ovary and endometrium tissue using anti-B7H4 (ab239074) or rabbit IgG–isotype control antibody (ab172730). Positive staining in brown; hematoxylin nuclear counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

B7H4 expression in cancer

B7H4 expression varied in the analyzed cancer tissue microarrays (TMAs) (Figure 2a), with breast, endometrial and ovary cancer TMAs having similar IHC scores and kidney cancer TMAs showing the lowest score. The staining intensity of cohorts of cancer subtypes was also evaluated separately (Figure 2 b-e).



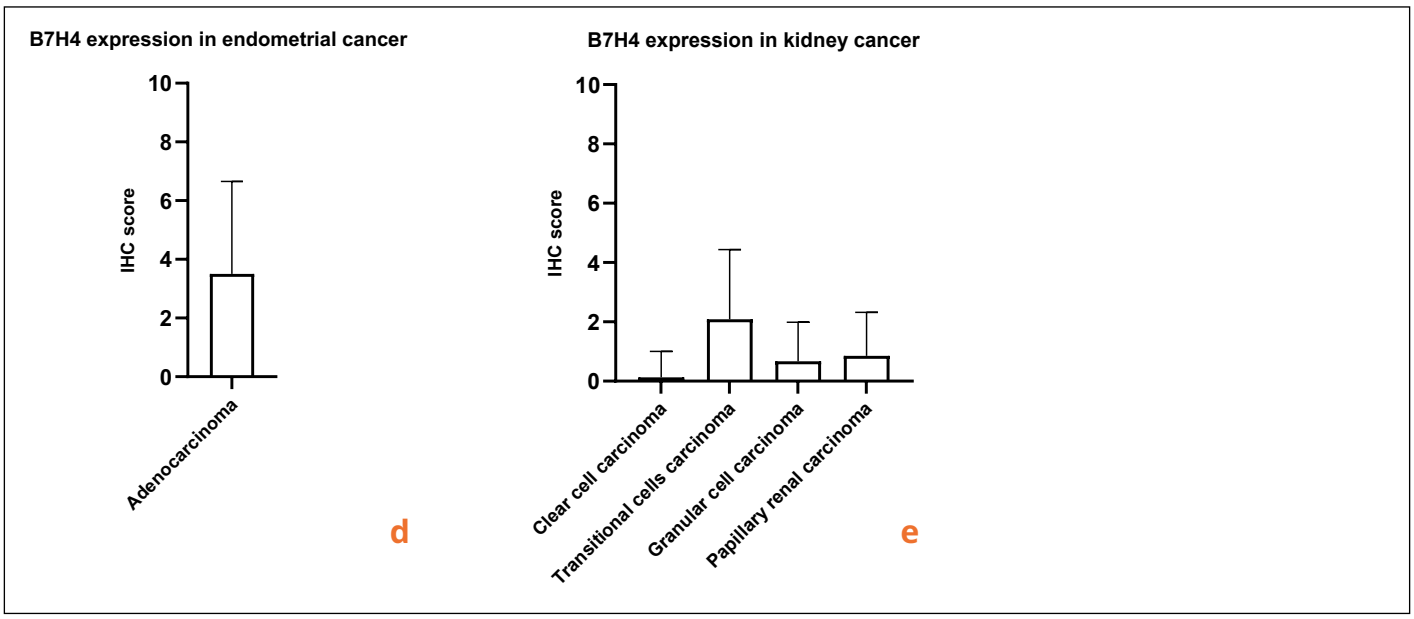


Figure 2. B7H4 protein expression in a selection of cancer TMAs. (a) The box plot (with SD) summarizes results from a semi-quantitative analysis of B7H4 expression in TMA cores. (b) B7H4 IHC score in 9 TMA cores of breast ductal carcinoma, 61 TMA cores of invasive ductal carcinoma, and 4 TMA cores of ductal carcinoma *in situ*. (c) B7H4 IHC score in 26 TMA cores of endometrioid adenocarcinoma, 43 TMA cores of serous cystadenocarcinoma, and 11 TMA cores of mucinous cystadenocarcinoma. (d) B7H4 IHC score in 73 TMA cores of endometrial adenocarcinoma. (e) B7H4 IHC score in 47 TMA cores of kidney clear cell carcinoma, 11 TMA cores of transitional cell carcinoma, 9 TMA cores of granular cell carcinoma, and 7 TMA cores of papillary renal cell carcinoma.

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B7H4 expression in breast cancer TMA

Below are the representative images of moderate to strong B7H4 expression in invasive ductal carcinoma (Figure 3).

Invasive ductal carcinoma (moderate B7H4 expression)	**Invasive ductal carcinoma** (strong B7H4 expression)

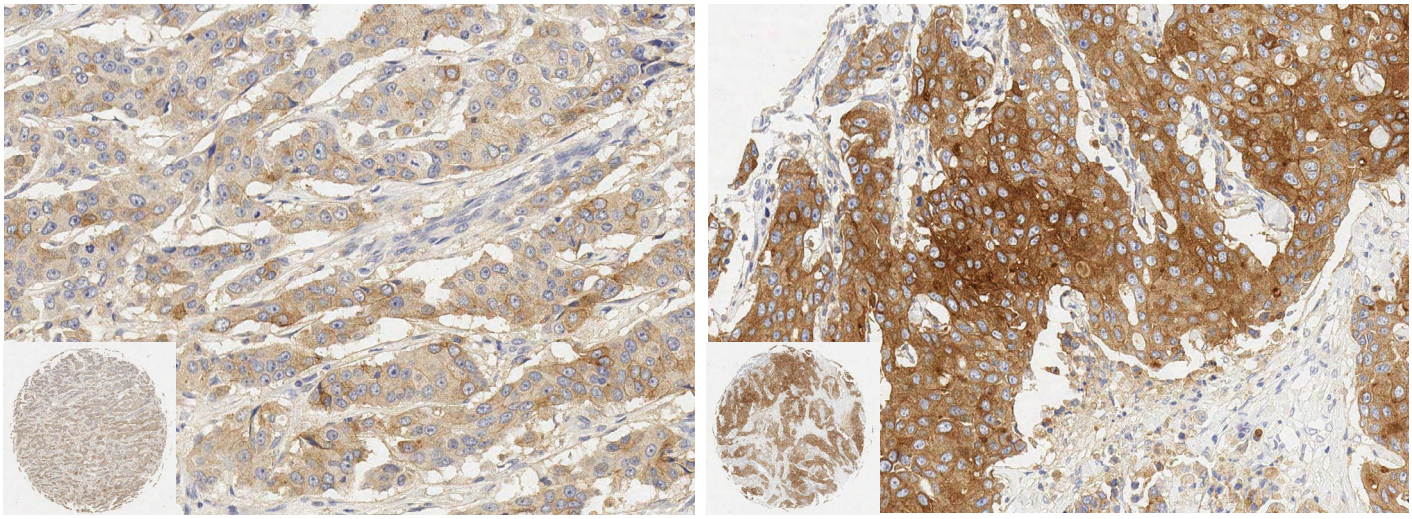


Figure 3. B7H4 expression in breast cancer. IHC images show moderate or strong B7H4 staining intensity in brown. Nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on the Aperio® AT2 and imaged at 20x (whole core insets at 5x) on Aperio® ImageScope.

B7H4 expression in ovary cancer TMA

Below are the representative images of moderate B7H4 expression in serum adenocarcinoma and strong B7H4 expression in cystadenocarcinoma (Figure 4).

Serum adenocarcinoma (moderate B7H4 expression)	**Cystadenocarcinoma** (strong B7H4 expression)

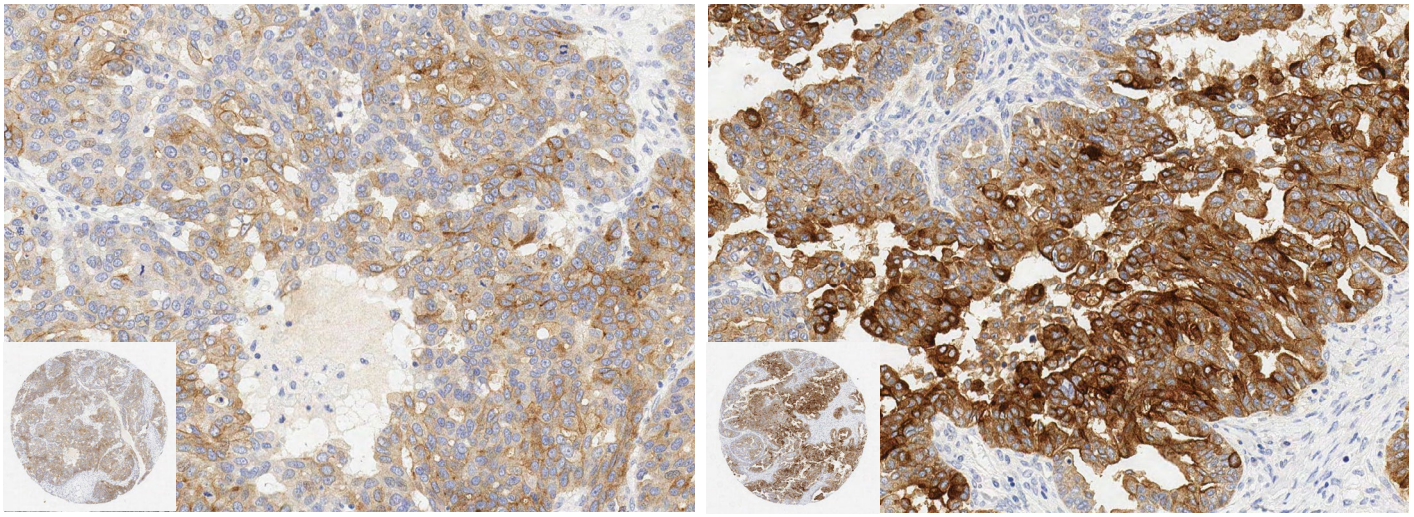


Figure 4. B7H4 expression in ovarian cancer. IHC images show moderate and strong B7H4 expression in brown. Hematoxylin nuclear counterstain in blue. Slides were scanned at 20x on the Aperio® AT2 and imaged at 20x (whole core insets at 5x) on Aperio® ImageScope.

B7H4 expression in endometrial cancer TMA

Below are the representative images of moderate to strong B7H4 expression in endometrial adenocarcinoma (Figure 5).

Endometrial adenocarcinoma (moderate B7H4 expression)	**Endometrial adenocarcinoma** (strong B7H4 expression)

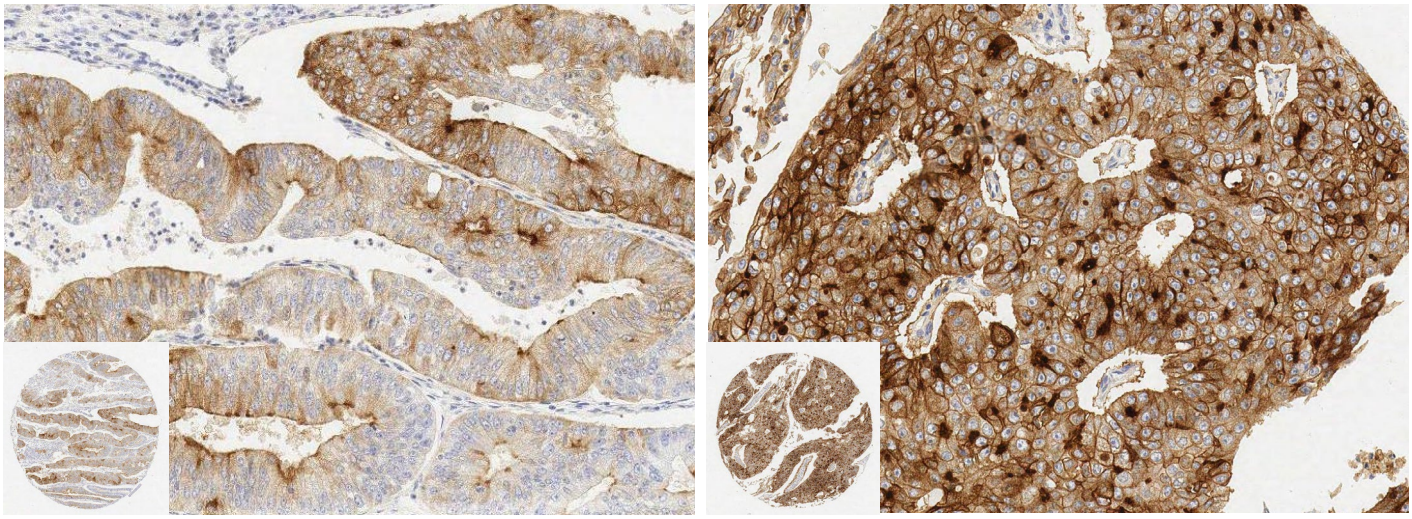


Figure 5. B7H4 expression in endometrial cancer. IHC images show moderate or strong B7H4 expression in brown. Nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on the Aperio® AT2 and imaged at 20x (whole core insets at 5x) on Aperio® ImageScope.

B7H4 expression in kidney cancer TMAs

B7H4 expression was detected in transitional cell carcinoma and absent from clear cell carcinoma. Below are the representative images of B7H4 expression in both tissue samples (Figure 6).

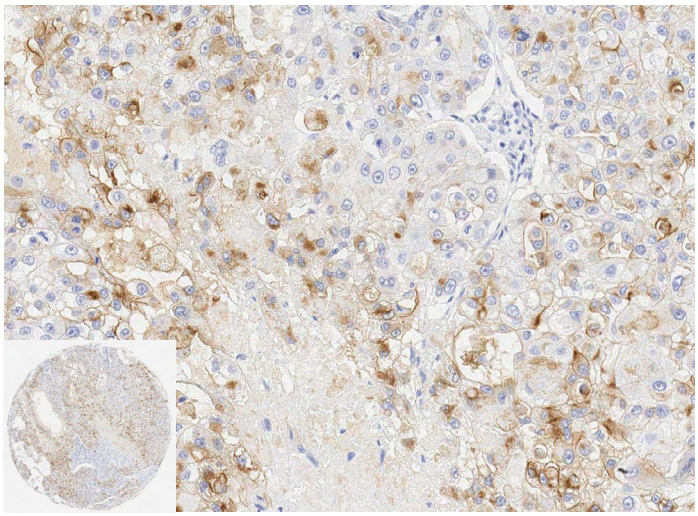
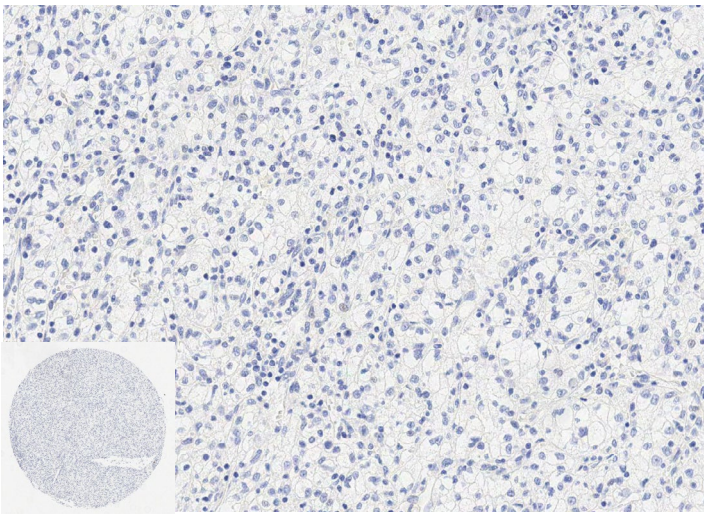
Transitional cell carcinoma (moderate B7H4 expression)	**Clear cell carcinoma** (no B7H4 expression)
	

Figure 6. B7H4 expression in kidney cancer. IHC mages show moderate or no B7H4 expression. Positive staining in brown; hematoxylin nuclear counterstain in blue. Slides were scanned on the Aperio® AT2 and imaged at 20x (whole core insets at 5x) on Aperio® ImageScope.

References

1. Fedchenko, N., & Reifenrath, J. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue - a review. *Diagnostic pathology*, **9**, 221 (2014).

2. Y Chen, C Yang, Z Xie, L Zou, Z Ruan, X Zhang, Y Tang, L Fei, Z Jia, Y Wu. Expression of the novel co-stimulatory molecule B7-H4 by renal tubular epithelial cells. *Kidney Int*, **70** (12):2092-9 (2006).

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