

High Dimensional Imaging Mass Cytometry Panel to Visualize the Tumor Immune Microenvironment Contexture

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Supplementary Table 1: IHC secondary antibodies

IHC secondary antibody	Provider	References
Peroxidase affinipure goat anti-mouse IgG (H+L)	Jackson	115-036-003
Peroxidase affinipure goat anti-rabbit IgG (H+L)	Jackson	111-035-003
Peroxidase affinipure goat anti-rat IgG (H+L)	Jackson	112-035-143
Peroxidase affinipure rabbit anti-goat IgG (H+L)	Jackson	305-035-003
Peroxidase goat anti-mouse IgM (H+L)	Southern biotech	1020-05

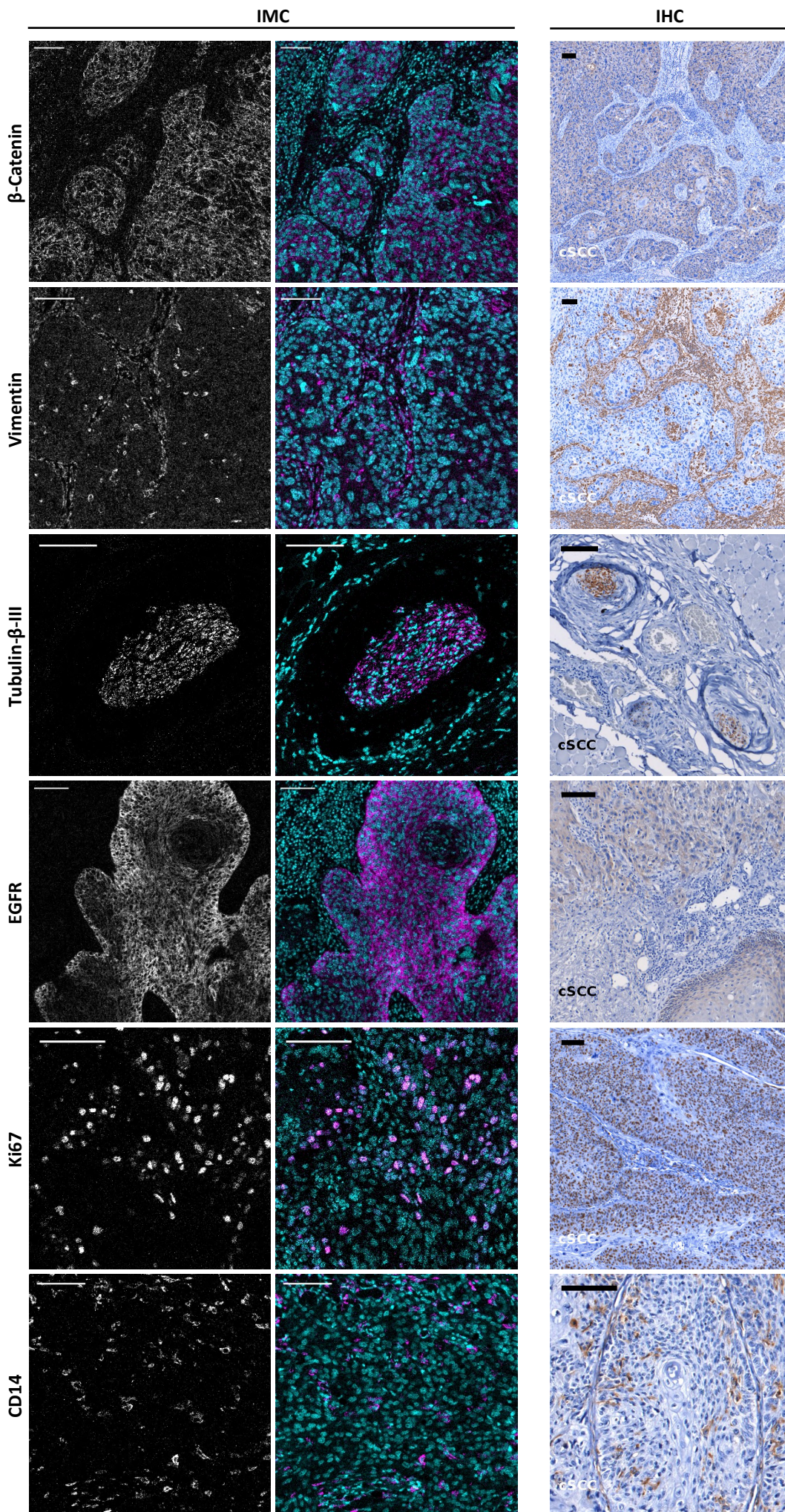
Supplementary Table 2: metal conjugation reagents

Metal	Provider	Reference
89Y	Sigma-Aldrich	204919-10G
139La	Sigma-Aldrich	203521-25G
141Pr	Fluidigm	201141A
142Nd	Fluidigm	201143A
143Nd	Fluidigm	201145A
144Nd	Fluidigm	201148A
145Nd	Fluidigm	201151A
146Nd	Fluidigm	201153A
147Sm	Fluidigm	201154A
148Nd	Fluidigm	201158A
149Sm	Fluidigm	201160A
150Nd	Fluidigm	201163A
151Eu	Fluidigm	201164A
152Sm	Fluidigm	201166A
153Eu	Fluidigm	201167A
154Sm	Fluidigm	201168A
155Gd	Fluidigm	201169A
156Gd	Fluidigm	201171A
158Gd	Fluidigm	201172A
159Tb	Fluidigm	201173A
160Gd	Fluidigm	201175A
161Dy	Fluidigm	201176A
162Dy	Fluidigm	201194A
163Dy	Fluidigm	201198A

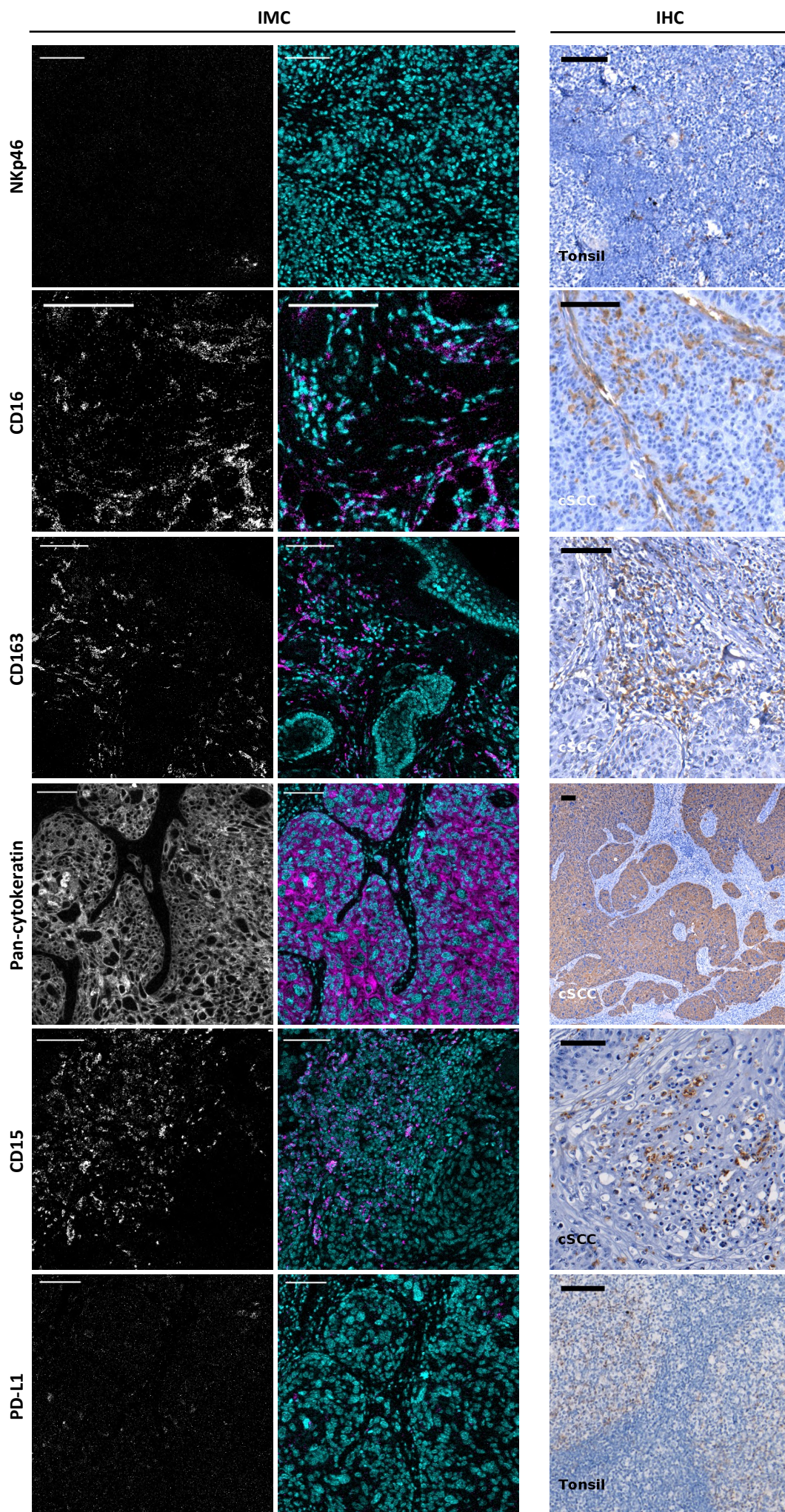
Supplementary Table 3: Targets of the 39-markers IMC panel:

Target				Metal		IHC performance	IMC performance
Marker	Clone	TiME cells/component	Abundance	Isotope	Intensity		
β-Catenin	E247	Epithelial - Tumor	+++	⁸⁹ Y	+	+++	++
Pan-cytokeratin	AE1-AE3	Epithelial - Tumor	++/+++	¹⁴⁸ Nd	++	+++	+++
EGFR	D38B1	Epithelial - Tumor	++	¹⁴² Nd	++	+++	++
Vimentin	EPR3776	Fibroblasts	+++	¹³⁹ La	+	+++	++
α-SMA	EPR5368	Pericytes - CAF	+++	¹⁹⁴ Pt	+	+++	+++
Podoplanin	D2-40	Lymphatic - tumor	+ / ++	¹⁶⁹ Tm	+++	+++	+++
Tubulin-β-III	TUJ1	Nerves	++	¹⁴¹ Pr	++	++	++
Pan-neurofilament	SMI-312	Nerves	+ / ++	¹⁶⁰ Gd	+++	++	++
NGFR	NGFR5	Nerves	+	¹⁵¹ Eu	+++	++	+
Tenascin C	BC-24	ECM	+ / ++	¹⁶³ Dy	+++	+++	+++
Fibronectin	F1	ECM	+ / ++	¹⁶⁸ Er	+++	+++	+++
CD45	D9M8I	Immune	+ / +++	¹⁵² Sm	+++	+++	+++
HLA-DR	YE2/36 HLK	Immune	+++	¹⁷⁴ Yb	++	+++	+++
CD16	EPR16784	Immune	++	¹⁴⁶ Nd	++	+++	++
CD14	EPR3653	Mono-derived	++	¹⁴⁴ Nd	++	+++	++
CD15	W6D3	Neutrophils	++	¹⁴⁹ Sm	++	+++	++
DC-SIGN	102E11-06	Dendritic	+	¹⁵³ Eu	+++	++	++
Langerin	929F3-01	Dendritic	+ / ++	¹⁶⁶ Er	+++	+++	+++
DC-LAMP	1010E1.01	Dendritic	++	¹⁷⁶ Yb	++	+++	+++
CD11c	EP1347Y	Phagocytes	+	¹⁵⁴ Sm	+++	++	++
CD163	EDHU-1	Macrophages	+	¹⁴⁷ Sm	++	+++	++
CD68	KP1	Macrophages	+ / +++	¹⁵⁹ Tb	+++	+++	+++
CD206	POLY	Macrophages	+	¹⁶⁴ Dy	+++	+++	+++
CD204	J5HTR3	Macrophages	++	¹⁷¹ Yb	+++	+++	+++
FOXP3	236A/E7	T	+	¹⁵⁵ Gd	+++	++	++
CD4	EPR6855	T	+	¹⁵⁶ Gd	+++	+++	++
CD8	D8A8Y	T	++	¹⁶² Dy	+++	+++	+++
CD3	POLY	T	+	¹⁷⁰ Er	+++	+++	++
CD20	H1 (FB1)	B	+	¹⁶¹ Dy	+++	+++	++
NKp46	195314	NK	+	¹⁴⁵ Nd	++	++	+
CD56	123A8	NK	+	¹⁵⁸ Gd	+++	++	+
PD-L1	E1L3N	Function	+	¹⁵⁰ Nd	+++	++	+
PD-1	EPR4877(2)	Function	+	¹⁶⁵ Ho	+++	++	+
TIM-3	D5D5R	Function	+	¹⁶⁷ Er	+++	++	++
TIGIT	BLR047F	Function	+	¹⁷² Yb	+++	++	+
Myeloperoxidase	POLY	Function	+++	¹⁷³ Yb	++	+++	+++
Granzyme B	EPR20129-217	Function	+++	¹⁷⁵ Lu	++	+++	+++
Ki67	8D5	Function	+++	¹⁴³ Nd	++	+++	+++
Active-Caspase-3	C92-605	Function	+++	¹⁹⁸ Pt	+	+++	+

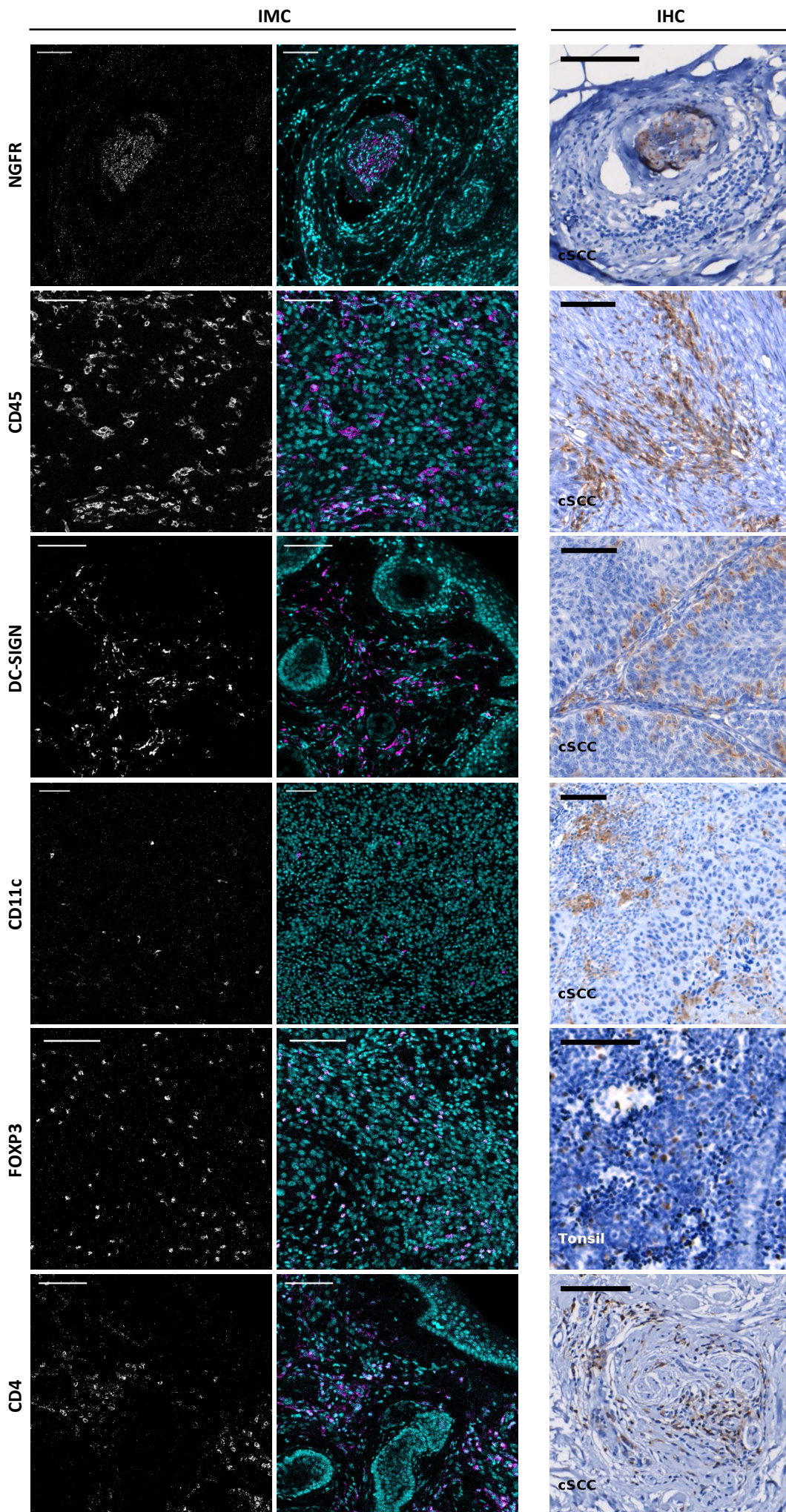
Classification of the antibodies included in the IMC panel based on the abundance of the targeted marker and intensity of the conjugated isotope (+: low, ++: mid, +++: high) and their staining performance in IHC and IMC (+: satisfactory, ++: good, +++: very good).



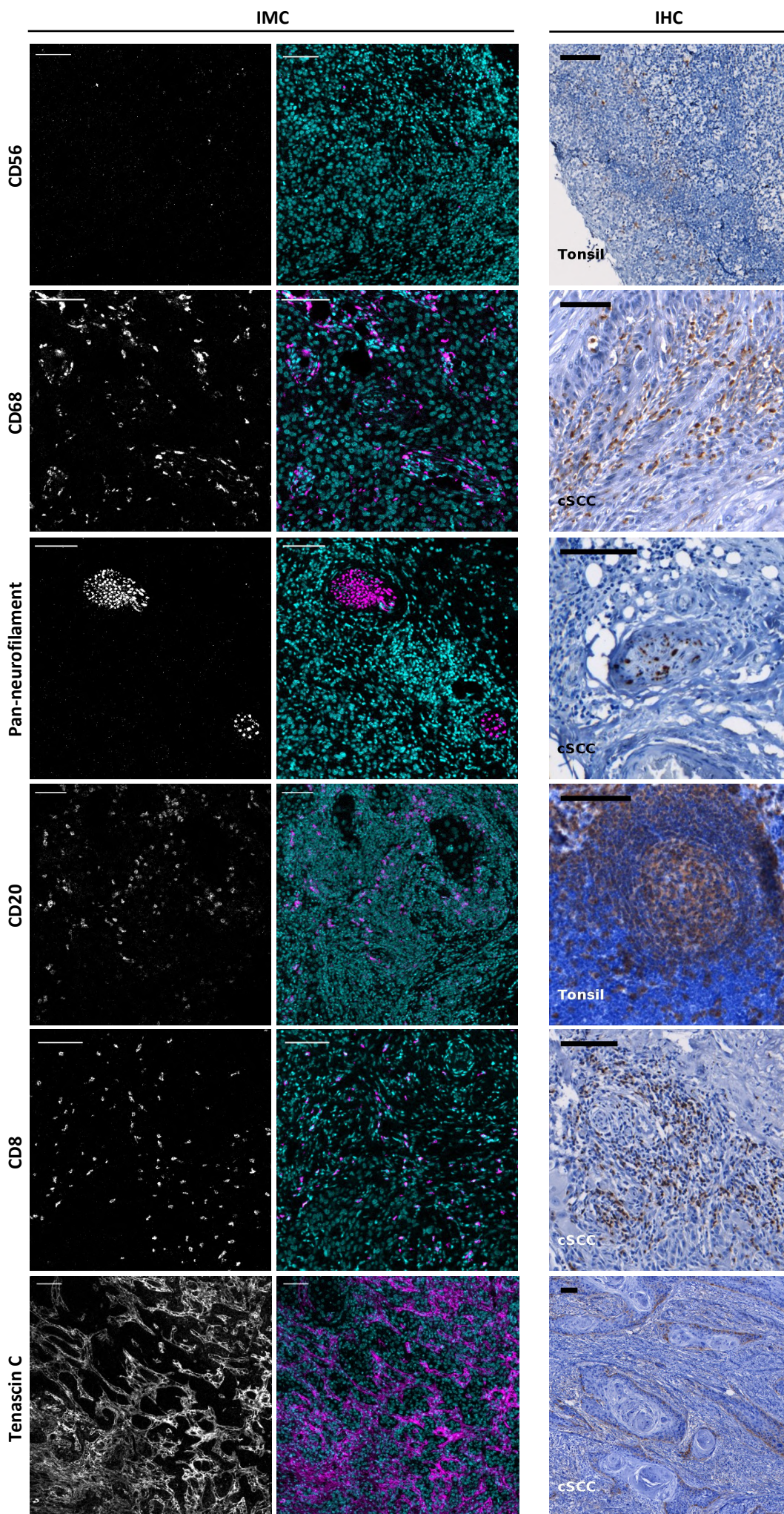
Supplementary Figure 1-A : Staining validation of the 39 antibodies used for IMC on cSCC sections and IHC on cSCC or tonsil sections. Single IMC antibody signal (left panels). IMC antibody (magenta) and nuclei (cyan) overlaid signals (middle panels). IHC antibody immunostaining (right panels). Scale bar = 100 μ m.



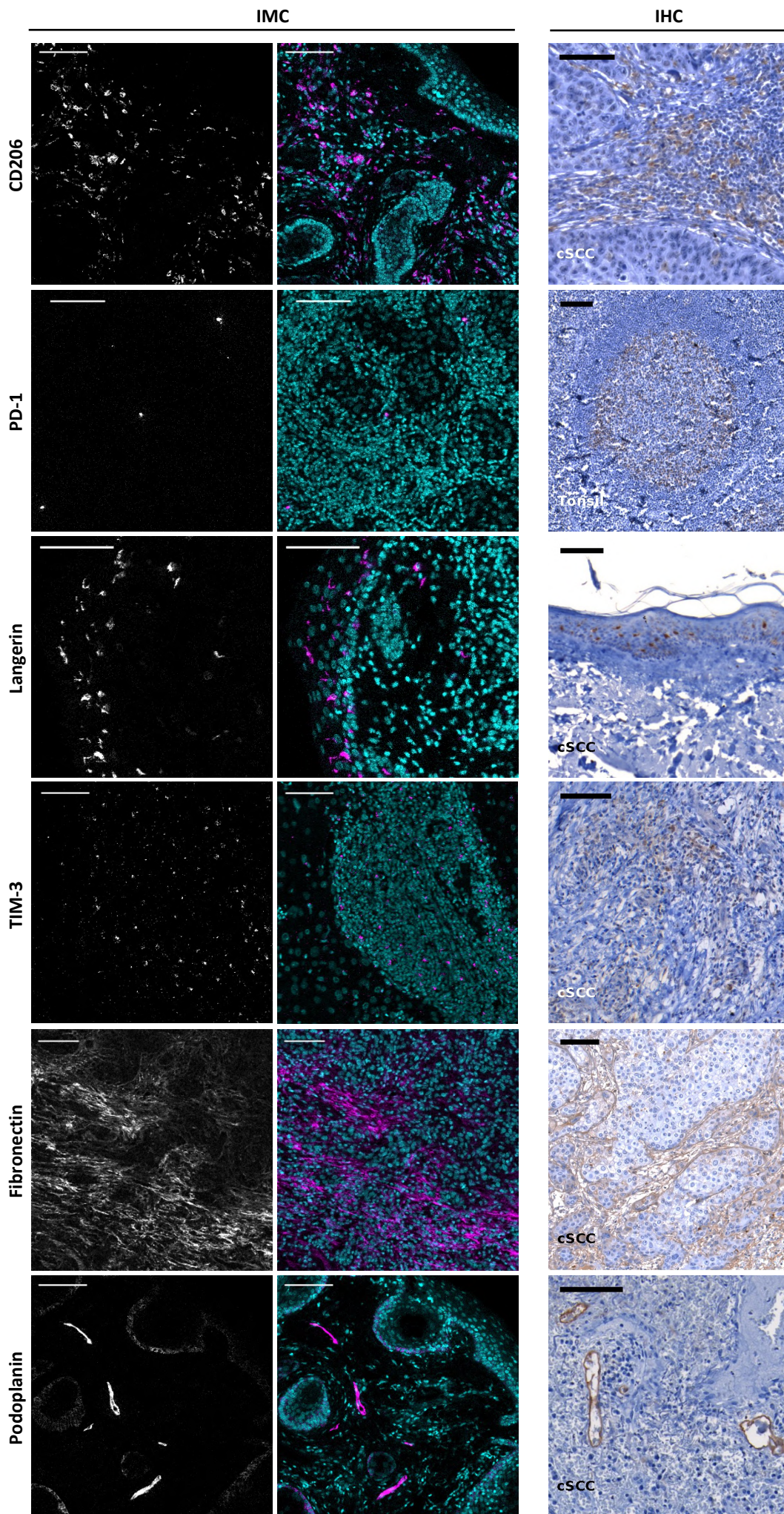
Supplementary Figure 1-B : Staining validation of the 39 antibodies used for IMC on cSCC sections and IHC on cSCC or tonsil sections. Single IMC antibody signal (left panels). IMC antibody (magenta) and nuclei (cyan) overlaid signals (middle panels). IHC antibody immunostaining (right panels). Scale bar = 100 μ m.



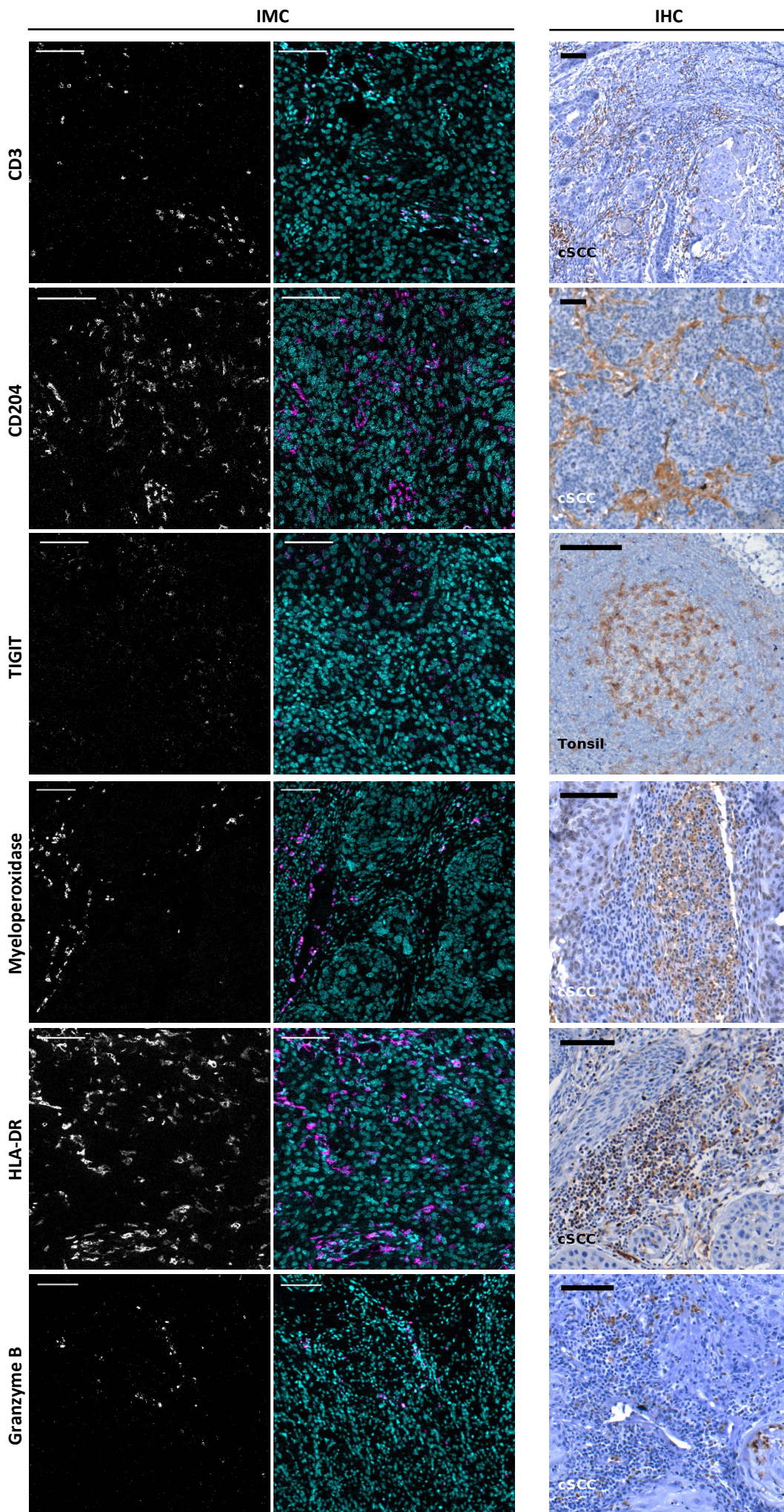
Supplementary Figure 1-C : Staining validation of the 39 antibodies used for IMC on cSCC sections and IHC on cSCC or tonsil sections. Single IMC antibody signal (left panels). IMC antibody (magenta) and nuclei (cyan) overlaid signals (middle panels). IHC antibody immunostaining (right panels). Scale bar = 100 μ m.



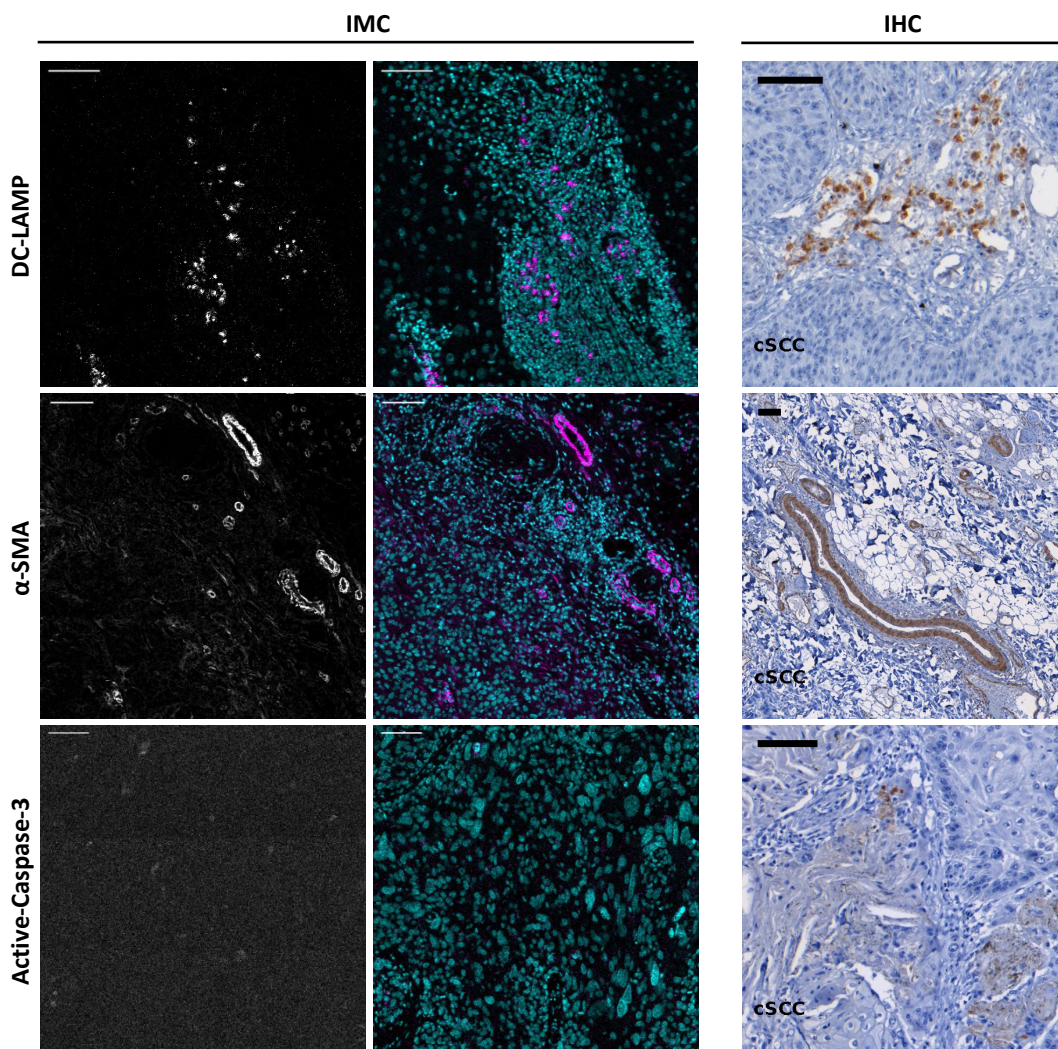
Supplementary Figure 1-D : Staining validation of the 39 antibodies used for IMC on cSCC sections and IHC on cSCC or tonsil sections. Single IMC antibody signal (left panels). IMC antibody (magenta) and nuclei (cyan) overlaid signals (middle panels). IHC antibody immunostaining (right panels). Scale bar = 100 μ m.



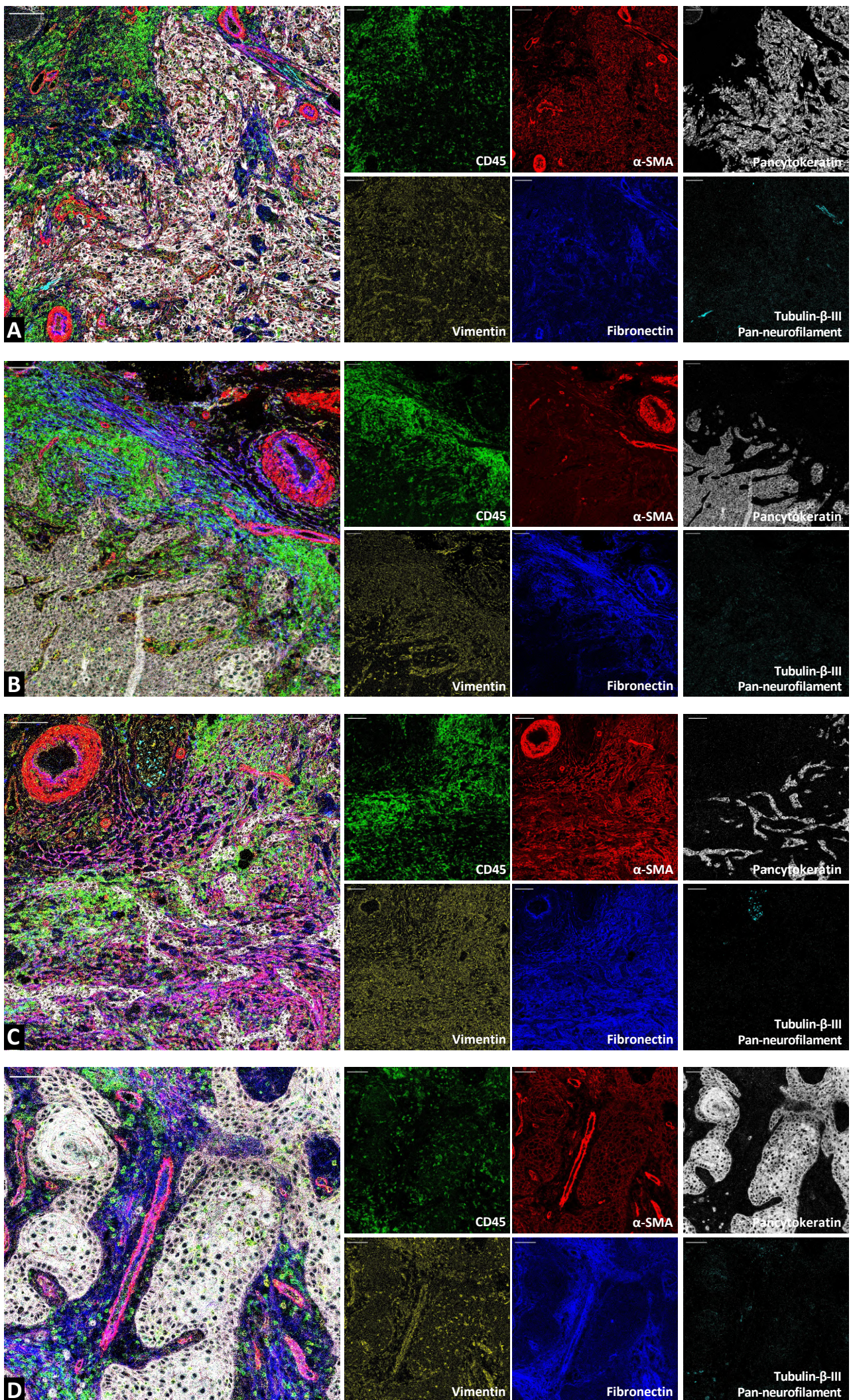
Supplementary Figure 1-E : Staining validation of the 39 antibodies used for IMC on cSCC sections and IHC on cSCC or tonsil sections. Single IMC antibody signal (left panels). IMC antibody (magenta) and nuclei (cyan) overlaid signals (middle panels). IHC antibody immunostaining (right panels). Scale bar = 100 μ m.



Supplementary Figure 1-F : Staining validation of the 39 antibodies used for IMC on cSCC sections and IHC on cSCC or tonsil sections. Single IMC antibody signal (left panels). IMC antibody (magenta) and nuclei (cyan) overlaid signals (middle panels). IHC antibody immunostaining (right panels). Scale bar = 100 μ m.



Supplementary Figure 1-G : Staining validation of the 39 antibodies used for IMC on cSCC sections and IHC on cSCC or tonsil sections. Single IMC antibody signal (left panels). IMC antibody (magenta) and nuclei (cyan) overlaid signals (middle panels). IHC antibody immunostaining (right panels). Scale bar = 100 μ m.



Supplementary Figure 2: Visualization by ICM of structural and cellular TiME components in a single region of four cSCC sections. Overlaid and single antibody signals representing immune cells (CD45), blood vessels and CAFs (αSMA), tumor cells (pan-cytokeratin), fibroblasts (vimentin), ECM (fibronectin) nerve fibers (pan-neurofilament and Tubulin-b-III), of the same region of four cSCC sections: cSCC-5 (A), cSCC-6 (B), cSCC-7 (C) and cSCC-8 (D). Scale bar = 100 μm.