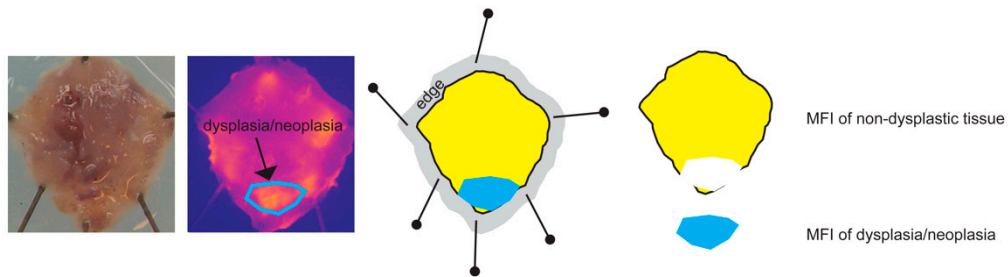


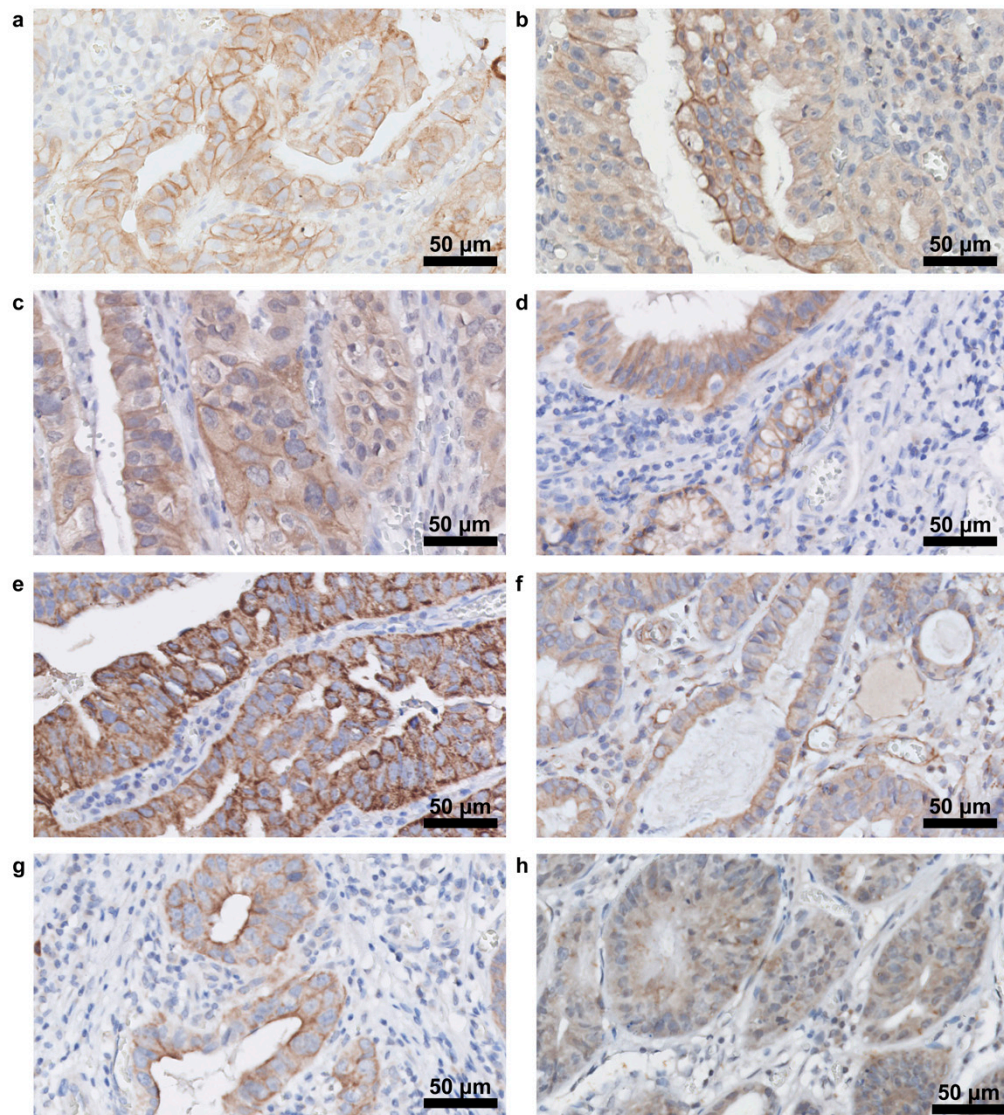
# Validation of Novel Molecular Imaging Targets Identified by Functional Genomic mRNA Profiling to Detect Dysplasia in Barrett's Esophagus

**Journal:** *Cancers*

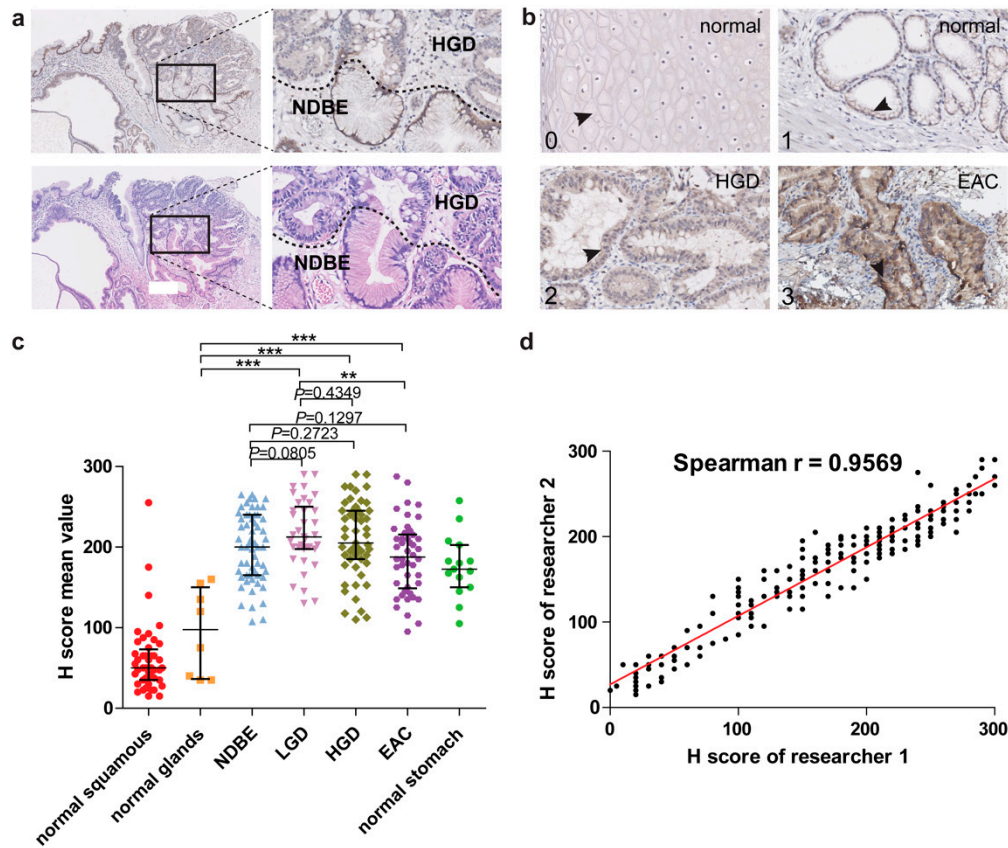
## Supplementary materials



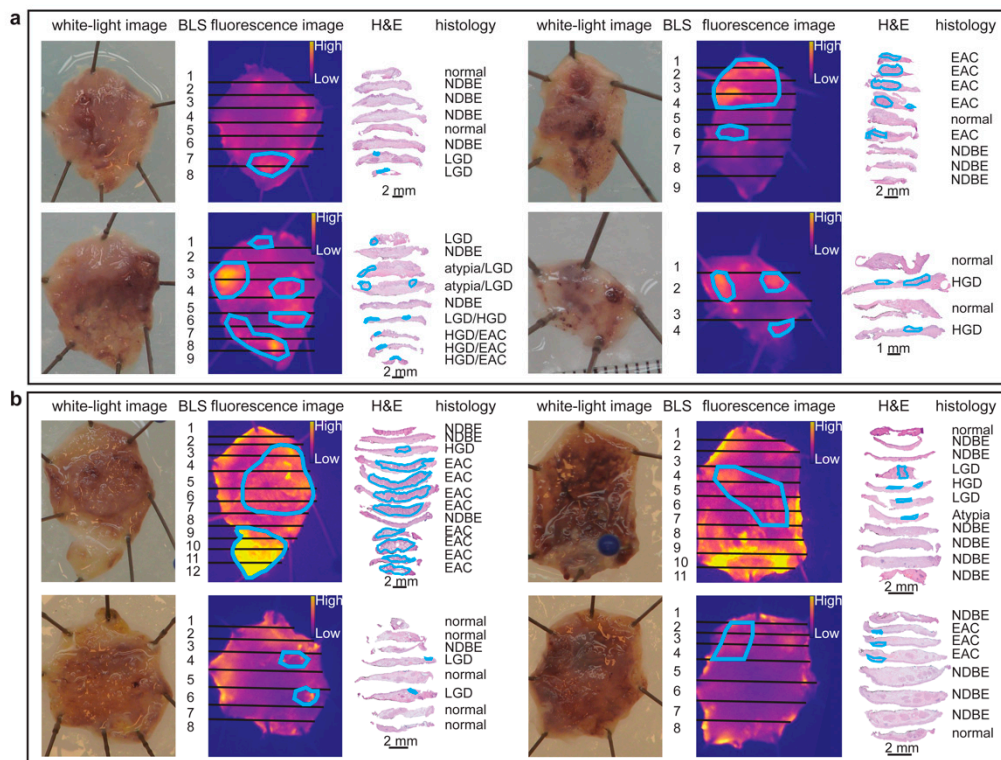
**Figure S1** The diagram for the calculation of mean fluorescence intensity correlated to histology on fresh specimens. The blue circle highlighted the dysplastic or neoplastic tissue after histology correlation of bread-loaf slices. The yellow color highlighted the non-dysplastic tissue. Mean fluorescence intensity (MFI) of different histology type tissue was then calculated in Image J according to the delineations. MFI: mean fluorescence intensity.



**Figure S2** The subcellular localization of the proteins. CDH11 (a) shows plasma membranous staining; ERBB3 (b) shows mainly cell membranous, sometimes cytoplasmic staining; TMPRSS4 (c), DDR1 (d), PKC $\iota$  (e), SPARC (f) and SULF1 (g) show mainly cytoplasmic, sometimes membranous staining; GREM1 (h) shows cytoplasmic staining.



**Figure S3** Immunohistochemistry results of Tmprss4. **(a)** The IHC images in low and high magnification, accompanied by H&E staining results in serial cutting slices and pathological delineation. **(b)** It explains how we define staining intensity of 0 to 3. **(c)** The comparison of mean H score among seven groups, including normal squamous epithelium, normal glands, NDBE, LGD, HGD, EAC, normal stomach. Two researchers performed H scoring separately and blindly. The H score mean value means the average value of H score of researcher 1 and researcher 2. **(d)** The correlation analysis of H scoring by researcher 1 and researcher 2, with Spearman correlation. \*\*  $P$  value  $< 0.001$ ; \*\*\*  $P$  value  $< 0.0001$ . EAC: esophageal adenocarcinoma; HGD: high grade dysplasia; LGD: low grade dysplasia; NDBE: non-dysplastic Barrett's esophagus.



**Figure S4** The histology correlation overview of SPARC-800CW and SULF1-800CW

**(a)** The fluorescence of SPARC-800CW after incubation and rinsing on endoscopic mucosal resection (EMR) specimens. **(b)** The fluorescence of SULF1-800CW after incubation and rinsing on EMR specimens. In the left is the white light image; in the middle is the fluorescence image; in the right are the H&E images of bread-loaf slices. The dysplastic BE tissue was delineated with blue circled lines. Due to the coagulation effect caused during in-vivo EMR procedures, the edge of the specimens showed high fluorescence. BLS: bread-loaf slices; EAC: esophageal adenocarcinoma; HGD: high grade dysplasia; LGD: low grade dysplasia; NDBE: non-dysplastic Barrett's esophagus; normal: normal tissue including normal squamous epithelium and normal stroma tissue.



**Table S1.** The characteristics of 25 patients included for immunohistochemical staining (in excel format)

**Table S2.** The utilized primary antibodies and optimized immunohistochemistry protocols of nine candidate genes.

Primary antigen	Mono /poly-clonal	Company	Concentration /Volume	Dilution	Antigen-retrieval
#9 GREM1	Polyclonal	invitrogen	1mg/ml	1:250	Tris/HCL
#11 SPARC	Monoclonal	ThermoFisher	2 mg/ml	1:1000	Tris/EDTA
#12 ENPEP	Monoclonal	abcam	100ul	1:50	Tris/EDTA & Tris/HCL
#15 SULF1	Polyclonal	invitrogen	100ul	1:100	Tris/HCL
#24 ERBB3	Monoclonal	OriGene	1mg/ml	1:50	EDTA
#26 PKC $\alpha$	Monoclonal	Santa Cruz	200ug/ml	1:250	Tris/HCL
#50 CDH11	Monoclonal	ThermoFisher	500ug/ml	1:100	Citrate (pH6.0)
#72 DDR1	Monoclonal	USBiological	100ul	1:500	EDTA
#111 TMPRSS4	Polyclonal	Proteintech	150ul	1:1000	Tris/EDTA

**Table S3.** The overview of all overexpressed genes in esophageal adenocarcinoma compared to Barrett's esophagus by FGmRNA profiling (in excel format).

**Table S4.** Top 500 overexpressing genes of FGmRNA profiling in esophageal adenocarcinoma compared to Barrett's esophagus.

1	MGP	51	CD52	101	MME	151	ATP11A
2	IGF2BP3	52	THEM6	102	RASSF7	152	IDS
3	ADAP1	53	MAPRE1	103	NSFL1C	153	PPIA
4	ICA1	54	MS4A6A	104	TMEM106B	154	ELMO3
5	ACOT8	55	STAU1	105	F8A1,F8A2,F8A3	155	ARFGAP1
6	IGFBP7	56	H2AFV	106	HSPD1	156	OSER1
7	GPR137B	57	BARX1	107	SMCHD1	157	TUFT1
8	TYROBP	58	SDC4	108	LAPTM5	158	CHMP3
9	GREM1	59	LTK	109	STAT6	159	AHCY
10	UBE2C	60	WIPI2	110	COBL	160	TMEM189
11	SPARC	61	CYP2B6	111	TMPRSS4	161	SCAMP3
12	ENPEP	62	SKAP2	112	BLCAP	162	SMG5
13	YWHAB	63	CD14	113	KLF12	163	DDX52
14	LTBP2	64	RAMP1	114	SLMO2	164	DDX27, SS18
15	SULF1	65	GPSM2	115	IFT52	165	ZNF12
16	GAST	66	MYBL2	116	EFEMP2	166	CEBPB
17	FSTL1	67	XPO1	117	ZMYM1	167	TNFRSF25
18	LAMA4	68	SNRPB	118	FRZB	168	MRPS18A
19	RAB40B	69	CD248	119	PRH1-PRR4	169	GATAD1
20	MTMR11	70	TCF7L2	120	CLIP2	170	C1R
21	EIF3B	71	MEG3	121	HDHD3	171	LBH
22	HS2ST1	72	DDR1	122	LAMB3	172	RALGAPB

23	FOXA2	73	AGT	123	TNFAIP6	173	LLGL2
24	ERBB3	74	CTSA	124	C6orf123	174	DPH3P1, TCFL5
25	COL5A2	75	VEGFA	125	LONRF3	175	EPS8L3
26	PRKCI	76	MTMR1	126	OSBPL3	176	PQBP1
27	DDAH2	77	AMIGO2	127	CDK8	177	WWP1
28	TNNC2	78	APOD	128	TTC22	178	NID2
29	DKK3	79	OGN	129	SPATA7	179	PSMB2
30	GLS	80	SORD	130	TRAC	180	CD2
31	FZD5	81	DPM1	131	HMGB1	181	TGIF2- C20orf24
32	CXCL13	82	CTSK	132	TMEM176A	182	SEPT7
33	COL9A1	83	ARHGAP12	133	PLEKHB1	183	PDS5B
34	BZW2	84	JUP	134	C1orf106	184	APOOL
35	ADD3	85	SERINC3	135	DDX27	185	VBP1
36	SMAD6	86	TAX1BP1	136	ADNP	186	PLXDC1
37	TTC30A	87	FKBP1A	137	AGR2	187	RAB25
38	PSMB4	88	ESD	138	MRPL13	188	SDS
39	C1orf27	89	SIGIRR	139	FNBP1L	189	TPP2
40	ELF3	90	EPN3	140	EPB41L1	190	PPIA
41	GFRA1	91	KCNK1	141	F2R	191	BGN
42	ATP9A	92	ACVR1B	142	DIRAS3	192	PTPRC
43	ZGPAT	93	OGT	143	BCAP31	193	EDN3
44	GPX2	94	ZNF239	144	RSU1	194	RPE65
45	PELI2	95	PSMA1	145	GPM6A	195	PLEKHA6
46	HLA-DPA1	96	ENOX2	146	PIK3C2B	196	VAPA
47	LSR	97	MTCH1	147	SOX9	197	COL4A1
48	COL1A2	98	RAD54B	148	KIF2C	198	OPCML
49	MMP1	99	FANCF	149	PROCR	199	DNAJB2
50	CDH11	100	HEATR1	150	CUL4A	200	SAP18
201	PSMA7	251	DDX47	301	SLC2A1	351	COA1
202	TAGLN2	252	ZNF75D	302	PSEN2	352	ANGPT1
203	PRKCA	253	ITGBL1	303	CTTN	353	STX16
204	CREBZF	254	ZAK	304	HSPE1	354	POFUT1
205	C1orf116	255	TGM2	305	LRRC32	355	POP1
206	MS4A4A	256	PRSS8	306	RPIA	356	HPRT1
207	TMEM185B	257	RAB22A	307	ERGIC3	357	GRB10
208	ITGA4	258	MXRA5	308	RNF128	358	NCALD
209	SH3BGRL	259	SLAMF1	309	ARFGEF2	359	WIPF2

210	DPY19L4	260	SP140L	310	FYB	360	ALG3
211	IL17RB	261	ZKSCAN1	311	FEZ1	361	POLD2
212	RCBTB1	262	SLC12A9	312	TRAPPC6A	362	ASPN
213	PCBP2	263	MAPK14	313	COL3A1	363	GGCX
214	ZMYM2	264	EIF2AK1	314	MOSPD2	364	SMAGP
215	TNFSF10	265	TMCO1	315	AAK1	365	ERBB2
216	ZNF32	266	AC104699.1	316	TPX2	366	AURKAPS1, RAB3GAP2
217	PCMTD2	267	MEST	317	PKP3	367	ARRB1
218	INTS1	268	OTTHUMG0000 0003319	318	NF1	368	HLA-DRB1, HLA-DRB3, HLA-DRB4
219	IKBKE	269	CBLC	319	BORA	369	RPL37A
220	PRPF6	270	ENTPD1	320	GABBR1, UBD	370	CYP4A11
221	SERPINA4	271	LZTS3	321	ITPKA	371	CYP2B6
222	IFITM3	272	HLA-DMB	322	ATR	372	CR1
223	DYNLRB1	273	RBP4	323	TAF2	373	PLEKHG6
224	UGGT2	274	YME1L1	324	TSPAN8	374	GORASP2
225	YTHDF1	275	TPP1	325	YWHAZ	375	VIL1
226	FOXH1	276	CLEC4A	326	LSM5	376	HOXA1
227	PSMA2	277	TNFSF11	327	GULP1	377	XPNPEP2
228	CTBP2	278	DIS3	328	CMPK1	378	ARHGEF4
229	CHCHD2	279	COL1A1	329	TP53TG1	379	RERGL
230	IL13RA1	280	KPNA3	330	GGCT	380	CCZ1, CCZ1B
231	RALYL	281	LCN2	331	MORC4	381	SCAMP5
232	SSR3	282	SNX13	332	SPON2	382	GAD1
233	NFKBIE	283	AURKA	333	AAR2	383	SLC9A8
234	PLAGL2	284	FAM49A	334	ISG20L2	384	NEK3
235	OVOL2	285	SPR	335	SH3GLB1	385	AP5Z1, MIR4656
236	DLGAP4	286	CXorf57	336	UBE2VIP9	386	TGIF2
237	ADIPOQ	287	GYPA	337	C3	387	THY1
238	KPNA4	288	EMD	338	TRAC	388	DBI
239	PGAP3	289	TMEM176B	339	PLAUR	389	DROSHA
240	GDNF	290	UFC1	340	RAD21	390	VCAM1
241	RAB31	291	RASSF2	341	TXNDC9	391	HSPA14
242	U2SURP	292	HDAC1	342	PPARGC1A	392	NCOA1
243	TTC17	293	PLOD3	343	SPSB1	393	NPL
244	PRSS2, PRSS3	294	HEBP2	344	MUC1	394	ZFP36L2
245	CR2	295	KDELR2	345	ZNF217	395	S100P

246	HNF1A	296	NQO1	346	AVL9	396	TBC1D4
247	AKAP11	297	CCT6A	347	ESM1	397	FOLH1, FOLH1B
248	AKR1C3	298	COMMD3-BMI1	348	PSMF1	398	ORC2
249	NR4A3	299	BAD	349	ABCB6	399	STAG2
250	MFAP5	300	PLA2G7	350	PRPS1	400	FGF12
401	RAB17	426	GTF3A	451	PTPRR	476	PDK2
402	GUCY1B3	427	LGALS8	452	MTERFD1	477	GATA4
403	PEX3	428	DICER1	453	RARA	478	YKT6
404	SLC5A5	429	TFEB	454	UBE2D4	479	CSNK2A1
405	ATP6V1C1	430	DNASE1	455	RAC1	480	C11orf49
406	GLI1	431	CSE1L	456	NDRG3	481	MICU2
407	FAM50A	432	DNPH1	457	EIF3F	482	MYOC
408	SSB	433	STIL	458	KCNMB3	483	PRR15L
409	FTSJ2	434	TRIM44	459	EFNA1	484	NCOA6
410	ZNF318	435	HLA-DPB1	460	PDZK1IP1	485	CST4
411	IGSF6	436	LAD1	461	AGO1	486	ZC3H3
412	PGK1	437	RPL39	462	PRRC2C	487	MCF2L-AS1
413	POR	438	CUX1	463	CKAP5	488	AKAP4
414	ANKEF1	439	RPS3	464	ARHGEF7	489	TCF4
415	SCNM1, TNFAIP8L2- SCNM1	440	EEF1D	465	EFR3A	490	FAR2
416	ESRP1	441	MYRF	466	UCKL1	491	WNT2
417	NELFCD	442	CFLAR	467	PPIH	492	SUN1
418	BAIAP2L2	443	IFITM2	468	CACYBP	493	KRT18
419	GCLC	444	HSP90AB1	469	NRP1	494	STK39
420	ATXN7L3B	445	FERMT1	470	AMMECR1	495	EIF6
421	PDE6D	446	TMPRSS2	471	MOSPD1	496	PYGM
422	HLA-DQB1	447	XPOT	472	INTS8	497	CNPY3
423	MCAM	448	KCNJ8	473	CHRD1	498	LGALS4
424	HMHA1	449	SNRNP200	474	WNT4	499	TOX3
425	NUDT15	450	TTK	475	CD1E	500	HIST1H2BD



**Table S5.** Database search overview of the top 150 overexpressing genes by FGmRNA profiling.

RANK	GENE	Location	Location (GeneCards)	Location (ICAN)	IHC esophagus	IHC GE	Carcinogenesis	Imaging
8	TYROBP	M	M	Cell surface, IC	None	None	Shabo I, et al. [33]	None
9	GREM1	S	EC	Cell surface	Laurila, et al. [34]		Karagiannis GS, et al. [35]	None
11	SPARC	IC,S	EC, N	Basement M, platelet alpha granule lumen, cell surface	Yamashita K, et al. [36] Miura JT, et al. [37]	Lussier C, et al. [38]	Nagaraju GP, et al. [39]	None
12	ENPEP	M	M, EC, lysosome	Apical part of cell, apical M, brush border	None	Yuan Y, et al. [40]	Aravalli RN, et al. [41]	None
15	SULF1	IC,S	M, ER, EC, golgi apparatus	Cell surface, M raft, Golgistack	None	Gopal G, et al. [42]	Lai JP, et al. [43]	Pospisil P, et al. [44]
24	ERBB3	IC,M,S	EC,M	M	Yoon HH, et al. [45] Paterson AL, et al. [46] Nishigaki H, et al. [47]	None	Mang article, genecards.org gives a good summary	Terwisschavan Scheltinga AG, et al. [48]
26	PRKCI	IC	Cytosol, EC, N, cytoskeleton	Microtubule, cytoskeleton protein complex, cell leading edge	None	Yang YL, et al. [49]	Yang YL, et al. [50]	None
31	FZD5	M	M	Bicellular tight junction, Golgi M, cell surface	None	None	Snow GE, et al. [51]	None
41	GFRA1	M	M, EC	Anchored component of M, extrinsic component of M	None	None	Liu Z, et al. [52]	None
46	HLA-DPA1	IC,M,S	ER, endosome, lysosome, M, golgi apparatus	ER to Golgi transport, vesicle membrane, cell surface	None	None	None	None
50	CDH11	IC, M, S	M, EC	Not assigned	None	Torres S, et al. [53]	Yao J, et al. [54]	None

51	CD52	M	M, EC	Anchored component of M	None	None	Involved in the leukemia's	None
54	MS4A6A	IC,M	M	Not assigned	None	None	None	None
58	SDC4	M	EC,M, golgi apparatus	Focal adhesion, cell surface	None	None	None	None
59	LTK	M,S	M	Not assigned	None	None	Roll JD, et al. [55]	None
63	CD14	S	M, EC, golgi apparatus, endosome	external side of membrane, receptor complex, membrane microdomain, anchored component of membrane, endosomal part, endocytic vesicle	It is expressed on monocytes. [56]		Companioni O, et al. [57]	None
64	RAMP1	M	M	Transporter complex	None	None	Logan M, et al. [58]	None
69	CD248	M	EC, M	proteinaceous extracellular matrix, collagen trimer	None	TEM1 is expressed in several types of mesenchymal cells. [59]	None	Cicone F, et al. [60]
72	DDR1	IC, M	M	Basolateral plasma membrane, apicolateral plasma membrane	Sugimoto K, et al. [61] Nemoto T, et al. [62]	Hur H, et al. [63]	Jin H, et al. [64]	None
77	AMIGO2	M	M, N	Not assigned	None	None	None	None
83	ARHGAP12	IC	M, cytosol	Side of membrane	None	None	None	None

84	JUP	IC, M, cell junctions	M, N, EC, cytoskeleton, cytosol	Desmosome, apicolateral plasma membrane, intermediate filament cytoskeleton, lateral plasma membrane, cell cell junction, intercalated disc	It shows down regulated expression in ESCC compared to normal esophagus. [65]		Fredericks E, et al. [66]	None
85	SERINC3	M	M	Autophagosome	None	None	Bossolasco M, et al. [67]	None
86	TAX1BP1	IC, M	cytosol	plasma membrane protein complex	None	None	None	None
87	FKBP1A	IC, M	M, ER, cytosol	chromosomal region	None	None	None	None
89	SIGIRR	IC, M	M	Not assigned	None	None	None	None
91	KCNK1	M	M	Apical part of cell, apical plasma membrane, plasma membrane protein complex, cluster of actin based cell projection, cell projection membrane, brush border, plasma membrane receptor complex, lateral plasma membrane	None	None	None	None
96	ENOX2	IC, M	M, EC, cytosol	Side of membrane, external side of plasma membrane, secretory vesicle	None	None	None	None

101	MME	IC, M	M, endosome, golgi apparatus	Not assigned	Lee KW, et al. [68]	None	Lee KW, et al. [68] reported that MME can facilitate tumorigenesis; HoweverCheng CY, et al. [69] showed controversial results.	None
108	LAPTM5	Cytosol, M	M, lysosome	MHC protein complex	None	None	Nuylan M, et al. [70]	None
111	TMPRSS4	IC, M	M	Apical plasma membrane	None	Huang A, et al. [71, 72]	Huang A, et al. [71]	Cohen AS, et al. [73]
132	TMEM176A	M, mitochondria	Not assigned	Plasma membrane protein complex	Down regulated expression in ESCC compared to adjacent normal esophagus tissue. [74]		None	None
140	EPB41L1	IC, M	Cytoskeleton, cytosol, M	Apical part of cell	None	None	None	None

141	F2R	IC, M	M, EC, endosome, golgi apparatus	Plasma membrane raft, neuromuscular junction, cell substrate junction	None	None	None	None
143	BCAP31	M, ER	M, ER, cytosol	Side of membrane, endoplasmic reticulum golgi intermediate compartment membrane, etc.	None	BCAP31 is localized in cytoplasm and ER membrane. [75]	Wang A, et al. [76]	None
145	GPM6A	IC, M	M	Cell body, axon part, postsynapse, neuron spine, axon, dendrite, site of polarized growth, axonal growth cone, filopodium, postsynaptic membrane	None	None	None	None
149	PROCR	IC, M	M, EC	Cell substrate junction, anchoring junction, plasma membrane protein complex	None	None	Wang Q, et al. [77]	Dou WT, et al. [78]

The top 150 genes with protein products predicted to be localized on plasma membrane were listed in the table. Abbreviations: EC: extracellular; GE: gastroesophageal; IC: intracellular; IHC: immunohistochemistry; M: membrane; N: nucleus; S: secreted.



**Table S6.** The anti-TMPRSS4 immunohistochemistry results in dysplastic Barrett's esophagus and non-dysplastic Barrett's esophagus.

Targets	H-score DBE			High (201-300)		Intermediate (101-200)		Negative-low (0-100)	
	n	Mean (SD)	Mean value	n	%	n	%	n	%
TMPRSS4	138	204 (46)	High	71	51.45	66	47.83	1	0.72
	H-score NDBE			High (201-300)		Intermediate (101-200)		Negative-low (0-100)	
	n	Mean (SD)	Mean value	n	%	n	%	n	%
TMPRSS4	59	200 (43)	Intermediate	29	49.15	30	50.85	0	0

Table demonstrating IHC results of five targets in DBE and NDBE. Mean intensities and standard deviation within different categories are shown using the H-score. DBE: dysplastic Barrett's esophagus; NDBE: non-dysplastic Barrett's esophagus; SD: standard deviation.