

Table S1 Antibodies used for immunohistochemistry and Western blot analysis

Antibody	Supplier, catalogue number and RRID	Epitope retrieval (IHC)	Dilution	Incubation time and temperature
Monoclonal mouse smooth muscle actin (clone 1A4)	Agilent (Santa Clara, California, USA) Cat# M0851, RRID:AB_2223500	Tris-EDTA (pH 9.0) [†]	1:1,000	20 min at RT
Monoclonal mouse anti-human CD68 (clone PG-M1)	Agilent (Santa Clara, California, USA) Cat# M0876, RRID:AB_2074844	Tris-EDTA (pH 9.0) [†]	1:100	20 min at RT
Polyclonal rabbit anti-collagen IV alpha 1	Novus Biologicals (Abingdon, UK) Cat# NB120-6586, RRID:AB_789360	Pepsin treatment for 30 min at 37 °C	1:75	1 hour at RT
Polyclonal rabbit anti-NHLRC2	Sigma-Aldrich (Steinheim, Germany) Cat# HPA038493, RRID:AB_10672519	Tris-EDTA (pH 9.0) [†]	1:500	o/n at +4°C
Polyclonal rabbit anti-NHLRC2	Novus biologicals (Abingdon, UK) Cat# NBP1-85019, RRID:AB_11057192	–	1:500	o/n at +4°C
Donkey anti-rabbit IRDye800CW	LI-COR Biosciences (Lincoln, NE, USA) Cat# 925-32213, RRID:AB_2715510	–	1:10,000	1 hour at RT

[†], Microwave heat treatment for 15 minutes. CD68, cluster of differentiation 68; EDTA, ethylenediaminetetraacetic acid; IHC, immunohistochemistry; NHLRC2, NHL repeat (named after *NCL-1*, *HT2A* and *LIN-41*)-containing protein 2; o/n, overnight; RRID, Research Resource Identifier; RT, room temperature.

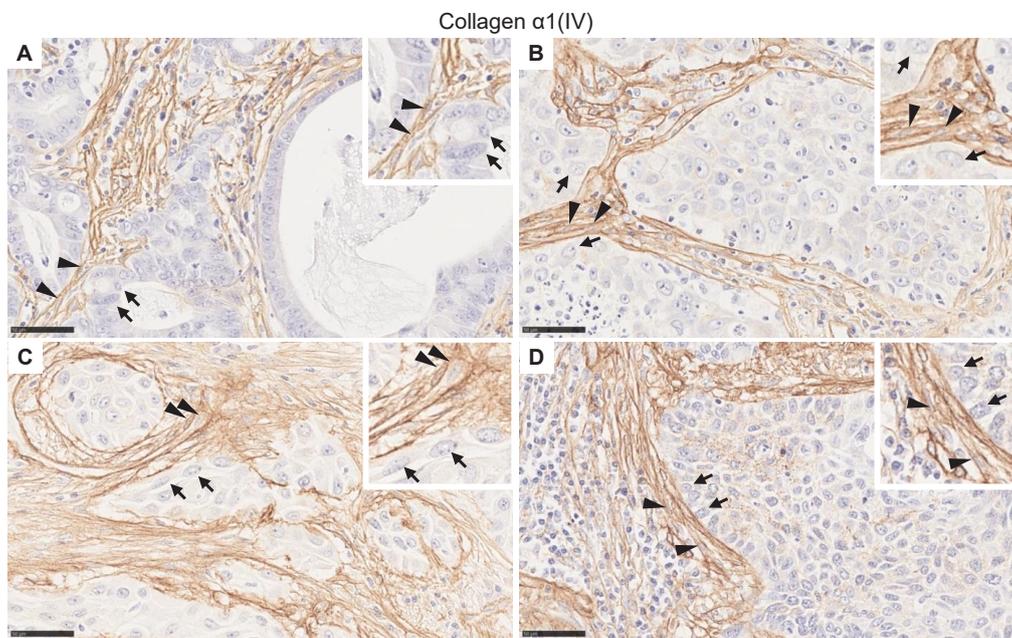


Figure S1 Immunohistochemical staining of collagen $\alpha 1(\text{IV})$ chain in lung adenocarcinoma and squamous cell carcinoma. Collagen $\alpha 1(\text{IV})$ was mainly expressed extracellularly within tumor stroma (arrowheads) while tumor cells (arrows) were negative or weakly positive. (A) Acinar adenocarcinoma (cribriform pattern). (B) Solid adenocarcinoma. (C) Non-keratinizing squamous cell carcinoma. (D) Basaloid squamous cell carcinoma. Scale bar 50 μm .

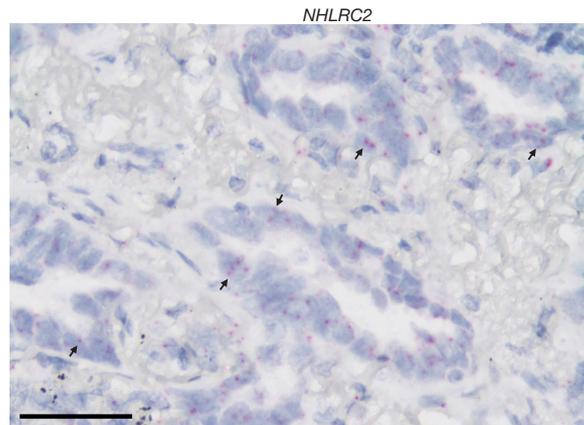


Figure S2 *NHLRC2* expression in lung adenocarcinoma by RNAscope *in situ* hybridization. *NHLRC2* expression is detected mainly in tumor cells (arrows). Scale bar 50 μ m. *NHLRC2*, NHL repeat (named after *NCL-1*, *HT2A* and *LIN-41*)-containing protein 2.

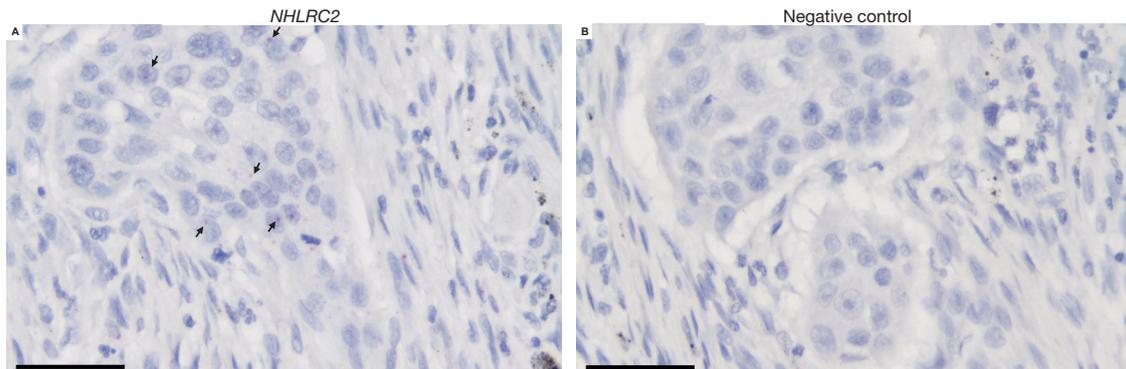


Figure S3 *NHLRC2* expression in lung squamous cell carcinoma by RNAscope *in situ* hybridization. (A) *NHLRC2* expression is observed mainly in tumor cells (arrows). (B) Negative control probe for bacterial gene *dapB* shows no signal. Scale bar 50 μ m. *dapB*, 4-hydroxy-tetrahydrodipicolinate reductase; *NHLRC2*, NHL repeat (named after *NCL-1*, *HT2A* and *LIN-41*)-containing protein 2.

Table S2 Histopathological tumor characteristics and immunohistochemical NHLRC2 expression determined by digital image analysis in lung adenocarcinomas and squamous cell carcinomas

Parameter	Adenocarcinoma, n=102			Squamous cell carcinoma, n=111		
	n	NHLRC2/tumor area, median (IQR)	P value	n	NHLRC2/tumor area, median (IQR)	P value
Atypia						
Mild/moderate	49	16.10 (11.52–20.19)	0.894	48	12.57 (6.85–16.98)	0.059
High	53	15.61 (11.02–22.09)		63	8.63 (6.77–13.67)	
Mitotic activity						
Low	19	13.90 (10.46–15.56)	0.042	0	–	–
Moderate/High	83	17.96 (11.54–23.20)		111	10.46 (6.85–15.65)	
Tumor necrosis						
Absent	12	13.59 (11.02–14.74)	0.149	6	5.47 (3.37–21.00)	0.272
Present	90	16.86 (11.49–22.73)		105	10.91 (7.06–15.53)	
Desmoplasia						
No/Mild	20	17.15 (13.10–22.50)	0.484	19	8.49 (4.94–16.62)	0.487
Strong	82	15.86 (11.02–22.00)		92	10.96 (7.09–15.56)	
Lymphovascular invasion						
Absent	30	14.90 (11.18–19.23)	0.617	29	9.84 (6.29–17.21)	0.849
Present	72	17.02 (11.31–22.61)		82	10.68 (6.96–15.34)	

IQR, interquartile range; n, number; NHLRC2, NHL repeat (named after *NCL-1*, *HT2A* and *LIN-41*)-containing protein 2.

Table S3 Clinical features and immunohistochemical NHLRC2 expression determined by digital image analysis in lung adenocarcinomas and squamous cell carcinomas

Parameter	Adenocarcinoma (n=102)			Squamous cell carcinoma (n=111)		
	n	NHLRC2/tumor area, median (IQR)	P-value	n	NHLRC2/tumor area, median (IQR)	P-value
Sex						
Male	67	16.10 (10.84–22.41)	0.669	97	10.41 (6.63–15.77)	0.748
Female	35	15.61 (12.80–21.79)		14	12.24 (8.78–15.34)	
Age						
<65	49	14.79 (9.88–20.52)	0.179	34	9.89 (6.05–16.32)	0.431
≥65	53	17.25 (13.26–22.92)		77	10.46 (7.27–15.52)	
Smoking status						
non-smoker	17	15.01 (11.55–19.57)	0.874	1	9.42	–
Ex/current smoker	81	16.10 (11.13–22.09)		107	11.01 (6.85–16.05)	
COPD						
No	54	16.38 (11.49–22.50)	0.440	55	11.01 (6.29–15.23)	0.788
Yes	34	14.00 (11.18–20.52)		54	9.37 (7.21–16.75)	
Stage						
IA-IB	55	15.61 (11.33–21.58)	0.852	46	11.96 (6.27–15.53)	0.869
IIA-IV	47	16.10 (11.33–22.29)		47	9.84 (6.85–16.26)	
FVC%						
<80	25	16.10 (11.55–22.00)	0.674	78	10.05 (7.06–15.53)	0.995
≥80	57	15.07 (11.49–21.81)		30	10.87 (6.74–15.77)	
FEV1%						
<80	43	14.84 (11.31–20.54)	0.877	31	9.84 (7.01–16.37)	0.862
≥80	43	15.54 (10.37–22.15)		71	11.29 (6.50–15.26)	
DLCO%						
<80	40	15.86 (11.07–21.90)	0.586	22	10.68 (7.08–15.97)	0.545
≥80	24	14.93 (12.40–22.39)		64	11.68 (8.21–15.77)	

COPD, chronic obstructive pulmonary disease; DLCO%, percent predicted diffuse capacity for carbon monoxide; FEV1%, percent predicted forced expiratory volume at 1 second; FVC%, percent predicted forced vital capacity; IQR, interquartile range; n, number; NHLRC2, NHL repeat (named after *NCL-1*, *HT2A* and *LIN-41*)-containing protein 2.

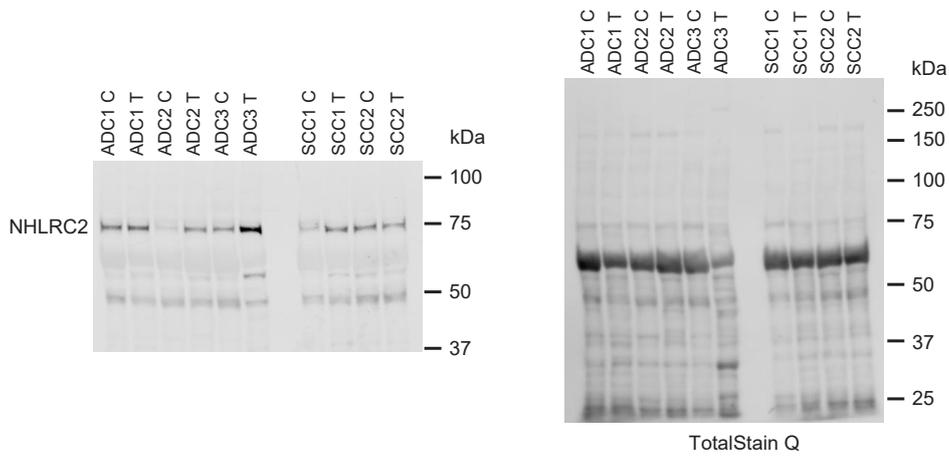


Figure S4 Immunoblot of NHL repeat (named after *NCL-1*, *HT2A* and *LIN-41*)-containing protein 2 (NHLRC2) expression and total protein stain (TotalStain Q) in lung tissue samples derived from adenocarcinoma (ADC) (n=3) and squamous cell carcinoma (SCC) (n=2) patients from tumor (T) and corresponding control (C) lung.

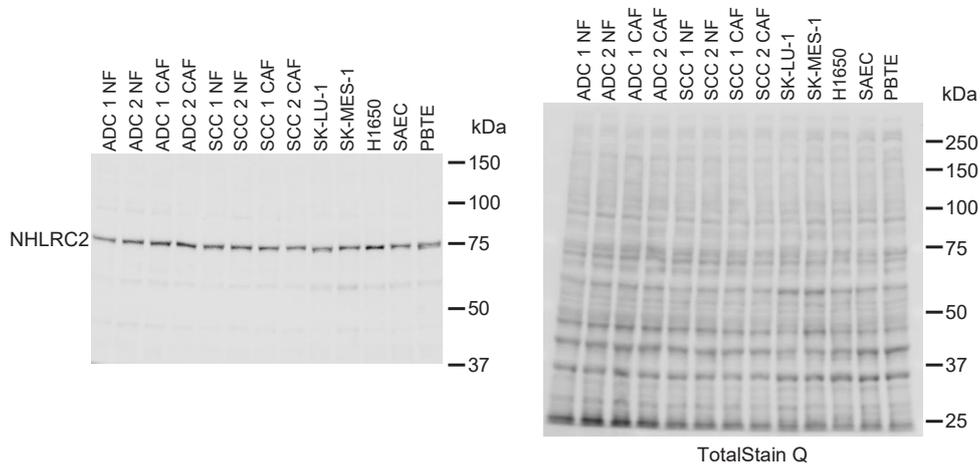


Figure S5 Immunoblot of NHL repeat (named after *NCL-1*, *HT2A* and *LIN-41*)-containing protein 2 (NHLRC2) expression and total protein stain (TotalStain Q) in stromal cells derived from adenocarcinoma (ADC) and squamous cell carcinoma (SCC) patients from tumor (cancer associated fibroblasts, CAF) and areas outside tumor (normal fibroblasts, NF), small airway epithelial cells (SAEC), primary bronchial/tracheal epithelial cells (PBTE), and lung cancer cells (SK-LU-1, SK-MES-1, H1650).