

**Table S1** Primary antibodies and opal information used in multiplex immunofluorescence staining

Panel	Primary antibody (Cat. No.)	Dilution (primary antibody)	Opal	Dilution (Opal)
Panel 1				
CK	ZSGB-BIO (ZM-0069)	1:200	Opal480	1:150
CD4	ABCAM (Ab133616)	1:200	Opal540	1:150
CD8	ABCAM (Ab93278)	1:400	Opal780	1:50
CD68	ABCAM (Ab213363)	1:500	Opal570	1:150
Panel 2				
CK	ZSGB-BIO (ZM-0069)	1:200	Opal480	1:150
CD31	ABCAM (Ab76533)	1:100	Opal570	1:150
$\alpha$ -SMA	ABCAM (Ab7817)	1:300	Opal520	1:150
HIF-1 $\alpha$	ABCAM (Ab51608)	1:100	Opal620	1:150
CD4	ABCAM (Ab133616)	1:200	Opal540	1:150

**Table S2** Relationship between CD4<sup>+</sup> T, CD8<sup>+</sup> T, and CD68<sup>+</sup> macrophage densities and treatment response in NSCLC patients overall and PD-L1 stratified

Density (/1,000 cells)	Non-response	Response	P
Overall patients			
CD4 <sup>+</sup> T (all)	46 (15, 84)	41 (33, 78)	0.76
CD8 <sup>+</sup> T (all)	16 (5, 25)	29 (9, 50)	0.10
CD68 <sup>+</sup> macrophage (all)	49 (30, 92)	45 (22, 68)	0.36
CD4 <sup>+</sup> T (epi)	0 (0, 3)	2 (0, 4)	0.12
CD8 <sup>+</sup> T (epi)	2 (0, 10)	9 (1, 28)	0.12
CD68 <sup>+</sup> macrophage (epi)	2 (0, 11)	3 (0, 8)	0.74
CD4 <sup>+</sup> T (str)	56 (15, 102)	66 (38, 100)	0.54
CD8 <sup>+</sup> T (str)	16 (6, 28)	38 (10, 61)	*
CD68 <sup>+</sup> macrophage (str)	53 (34, 107)	55 (31, 105)	0.85
TPS 1–49% subgroup			
CD4 <sup>+</sup> T (all)	42 (13, 64)	34 (20, 55)	0.96
CD8 <sup>+</sup> T (all)	14 (5, 20)	33 (17, 63)	*
CD68 <sup>+</sup> macrophage (all)	51 (30, 92)	45 (14, 52)	0.20
CD4 <sup>+</sup> T (epi)	0 (0, 3)	0 (0, 4)	0.71
CD8 <sup>+</sup> T (epi)	1 (0, 7)	15 (1, 41)	0.13
CD68 <sup>+</sup> macrophage (epi)	3 (1, 12)	3 (0, 7)	0.51
CD4 <sup>+</sup> T (str)	52 (14, 78)	48 (33, 67)	0.96
CD8 <sup>+</sup> T (str)	14 (6, 25)	37 (18, 50)	*
CD68 <sup>+</sup> macrophage (str)	53 (35, 105)	53 (19, 70)	0.30
TPS ≥50% subgroup			
CD4 <sup>+</sup> T (all)	73 (42, 102)	60 (36, 98)	0.79
CD8 <sup>+</sup> T (all)	23 (11, 43)	27 (8, 50)	0.71
CD68 <sup>+</sup> macrophage (all)	46 (27, 82)	45 (25, 73)	0.91
CD4 <sup>+</sup> T (epi)	0 (0, 0)	2 (0, 5)	0.07
CD8 <sup>+</sup> T (epi)	2 (0, 63)	7 (2, 21)	0.85
CD68 <sup>+</sup> macrophage (epi)	0 (0, 1)	2 (0, 8)	0.15
CD4 <sup>+</sup> T (str)	83 (49, 118)	81 (61, 112)	0.91
CD8 <sup>+</sup> T (str)	28 (14, 41)	45 (7, 76)	0.25
CD68 <sup>+</sup> macrophage (str)	52 (32, 95)	67 (36, 139)	0.63

Data are shown as median and quartiles. \*, P<0.05. PD-L1, programmed cell death-ligand 1; TPS, tumor proportion score; all, all compartment; epi, epithelial compartment; str, stromal compartment.

**Table S3** Impact of CD4<sup>+</sup> T and CD8<sup>+</sup> T combined analysis, CD4<sup>+</sup> T and CD68<sup>+</sup> macrophage combined analysis on neoadjuvant immunotherapy

Combined analysis	Non-response	Response	P
Overall			
CD4 <sup>+</sup> T & CD8 <sup>+</sup> T (all)			0.08
hihi	8 (36)	10 (39)	
hilo	5 (23)	4 (15)	
lohi	0 (0)	6 (23)	
lolo	9 (41)	6 (23)	
CD4 <sup>+</sup> T & CD68 <sup>+</sup> macrophage (all)			0.56
hihi	9 (41)	6 (23)	
hilo	4 (18)	8 (31)	
lohi	2 (9)	4 (15)	
lolo	7 (32)	8 (32)	
CD4 <sup>+</sup> T & CD8 <sup>+</sup> T (epi)			0.059
hihi	3 (14)	13 (50)	
hilo	4 (18)	3 (12)	
lohi	5 (23)	4 (15)	
lolo	10 (46)	6 (23)	
CD4 <sup>+</sup> T & CD68 <sup>+</sup> macrophage (epi)			0.17
hihi	6 (27)	10 (39)	
hilo	1 (4.5)	6 (23)	
lohi	5 (23)	3 (12)	
lolo	10 (46)	7 (27)	
CD4 <sup>+</sup> T & CD8 <sup>+</sup> T (str)			0.33
hihi	5 (23)	11 (42)	
hilo	4 (18)	4 (15)	
lohi	3 (14)	5 (19)	
lolo	10 (46)	6 (23)	
CD4 <sup>+</sup> T & CD68 <sup>+</sup> macrophage (str)			0.63
hihi	6 (27)	9 (35)	
hilo	3 (14)	6 (23)	
lohi	4 (18)	5 (19)	
lolo	9 (41)	6 (23)	
TPS 1–49% subgroup			
CD4 <sup>+</sup> T & CD8 <sup>+</sup> T (all)			*
hihi	4 (27)	4 (36)	
hilo	4 (27)	0 (0)	
lohi	0 (0)	4 (36)	
lolo	7 (47)	3 (27)	

Table S3 (continued)

Table S3 (continued)

Combined analysis	Non-response	Response	P
CD4 <sup>+</sup> T & CD68 <sup>+</sup> macrophage (all)			0.69
hihi	6 (40)	2 (18)	
hilo	2 (13)	2 (18)	
lohi	2 (13)	2 (18)	
lolo	5 (33)	5 (46)	
CD4 <sup>+</sup> T & CD8 <sup>+</sup> T (epi)			0.46
hihi	2 (13)	4 (36)	
hilo	4 (27)	1 (9)	
lohi	3 (20)	3 (27)	
lolo	6 (40)	3 (27)	
CD4 <sup>+</sup> T & CD68 <sup>+</sup> macrophage (epi)			>0.99
hihi	5 (33)	4 (36)	
hilo	1 (7)	1 (9)	
lohi	4 (27)	2 (18)	
lolo	5 (33)	4 (36)	
CD4 <sup>+</sup> T & CD8 <sup>+</sup> T (str)			0.36
hihi	2 (13)	3 (27)	
hilo	3 (20)	1 (9)	
lohi	2 (13)	4 (36)	
lolo	8 (53)	3 (27)	
CD4 <sup>+</sup> T & CD68 <sup>+</sup> macrophage (str)			0.58
hihi	4 (27)	2 (18)	
hilo	1 (7)	2 (18)	
lohi	3 (20)	4 (36)	
lolo	7 (47)	3 (27)	
TPS ≥50% subgroup			
CD4 <sup>+</sup> T & CD8 <sup>+</sup> T (all)			0.65
hihi	4 (57)	6 (43)	
hilo	1 (14)	4 (29)	
lohi	0 (0)	2 (14)	
lolo	2 (29)	2 (14)	
CD4 <sup>+</sup> T & CD68 <sup>+</sup> macrophage (all)			0.66
hihi	3 (43)	4 (29)	
hilo	2 (29)	6 (43)	
lohi	0 (0)	2 (14)	
lolo	2 (29)	2 (14)	

Table S3 (continued)

**Table S3** (continued)

Combined analysis	Non-response	Response	P
CD4 <sup>+</sup> T & CD8 <sup>+</sup> T (epi)			0.12
hihi	1 (14)	8 (57)	
hilo	0 (0)	2 (14)	
lohi	2 (29)	1 (7)	
lolo	4 (57)	3 (21)	
CD4 <sup>+</sup> T & CD68 <sup>+</sup> macrophage (epi)			0.08
hihi	1 (14)	5 (36)	
hilo	0 (0)	5 (36)	
lohi	1 (14)	1 (7)	
lolo	5 (71)	3 (21)	
CD4 <sup>+</sup> T & CD8 <sup>+</sup> T (str)			0.91
hihi	3 (43)	8 (57)	
hilo	1 (14)	3 (21)	
lohi	1 (14)	1 (7)	
lolo	2 (29)	2 (14)	
CD4 <sup>+</sup> T & CD68 <sup>+</sup> macrophage (str)			0.73
hihi	2 (29)	7 (50)	
hilo	2 (29)	4 (29)	
lohi	1 (14)	1 (7)	
lolo	2 (29)	2 (14)	

Data are shown as median (IQR). \*, P<0.05. hi, high; lo, low; all, all compartment; epi, epithelial compartment; str, stromal compartment.

**Table S4** The relationship between the nearest neighbor distance between CD4<sup>+</sup> T and CD8<sup>+</sup> T or CD68<sup>+</sup> macrophage and the efficacy of neoadjuvant immunotherapy in patients with non-small cell lung cancer

NND (μm)	Non-response	Response	P
Overall patients			
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (all)	47 (33, 86)	34 (22, 51)	*
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (all)	24 (20, 33)	29 (18, 39)	0.62
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (epi)	248 (124, 393)	110 (60, 173)	0.16
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (epi)	212 (71, 230)	145 (88, 166)	0.50
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (str)	49 (33, 100)	39 (26, 52)	0.052
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (str)	24 (20, 33)	28 (18, 39)	0.62
TPS 1–49% subgroup			
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (all)	69 (39, 101)	34 (24, 39)	**
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (all)	24 (20, 36)	31 (22, 44)	0.29
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (epi)	301 (101, 424)	58 (27, 102)	0.12
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (epi)	163 (50, 225)	72.50 (48, 114)	0.39
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (str)	69 (40, 125)	39 (27, 39)	*
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (str)	24 (20, 36)	28 (23, 44)	0.29
TPS ≥50% subgroup			
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (all)	33 (29, 36)	30 (20, 71)	0.86
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (all)	24 (20, 30)	21 (16, 34)	0.86
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (epi)	194 (194, 194)	123 (110, 182)	0.38
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (epi)	333 (333, 333)	148 (138, 167)	0.12
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (str)	33 (29, 36)	31 (20, 74)	0.93
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (str)	24 (20, 30)	21 (18, 34)	0.83

Data are shown as median and quartiles. \*, P<0.05; \*\*, P<0.01. NND, nearest neighbor distance; TPS, tumor proportion score; all, all compartment; epi, epithelial compartment; str, stromal compartment.

**Table S5** Analysis of the differences in CD8<sup>+</sup> T cell density and the nearest neighbor distance between CD4<sup>+</sup> T and CD8<sup>+</sup> T in patients with non-small cell lung cancer between PD-L1 high-expression and low-expression groups

Index	TPS 1–49% subgroup	TPS ≥50% subgroup	P
Density (/1000 cells)			
CD8 <sup>+</sup> T (all)	17 (6, 38)	23 (6, 49)	0.59
CD8 <sup>+</sup> T (epi)	4 (0, 21)	7 (0, 26)	0.67
CD8 <sup>+</sup> T (str)	21 (7, 42)	35 (7, 59)	0.28
NND (μm)			
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (all)	40 (30, 73)	32 (20, 71)	0.13
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (epi)	101 (45, 332)	146 (109, 209)	0.32
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (str)	42 (33, 72)	33 (20, 74)	0.10

Data are shown as median and quartiles. PD-L1, programmed cell death-ligand 1; NND, nearest neighbor distance. all, all compartment; epi, epithelial compartment; str, stromal compartment.

**Table S6** The relationship between the proximity between CD4<sup>+</sup> T and CD8<sup>+</sup> T or CD68<sup>+</sup> macrophage and the efficacy of neoadjuvant immunotherapy in patients with non-small cell lung cancer

Proximity	Non-response	Response	P
Overall patients			
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (all,10 μm)	7 (1, 18)	14 (5, 30)	0.29
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (all,10 μm)	33 (9, 58)	18 (7, 41)	0.25
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (epi,10 μm)	0 (0, 0)	0 (0, 0)	0.36
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (epi,10 μm)	0 (0, 0)	0 (0, 0)	0.80
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (str,10 μm)	7 (1, 18)	13 (5, 28)	0.30
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (str,10 μm)	33 (7, 57)	17 (7, 41)	0.30
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (all,20 μm)	22 (6, 57)	41 (10, 92)	0.46
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (all,20 μm)	57 (26, 123)	49 (23, 106)	0.61
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (epi,20 μm)	0 (0, 0)	0 (0, 0)	0.64
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (epi,20 μm)	0 (0, 0)	0 (0, 0)	0.52
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (str,20 μm)	20 (6, 56)	41 (8, 90)	0.50
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (str,20 μm)	56 (25, 121)	49 (23, 106)	0.61
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (all,30 μm)	40 (11, 85)	61 (15, 134)	0.62
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (all,30 μm)	74 (33, 172)	66 (29, 143)	0.73
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (epi,30 μm)	0 (0, 0)	0 (0, 0)	0.62
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (epi,30 μm)	0 (0, 0)	0 (0, 0)	0.52
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (str,30 μm)	37 (11, 82)	61 (13, 131)	0.63
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (str,30 μm)	71 (32, 168)	66 (28, 143)	0.72

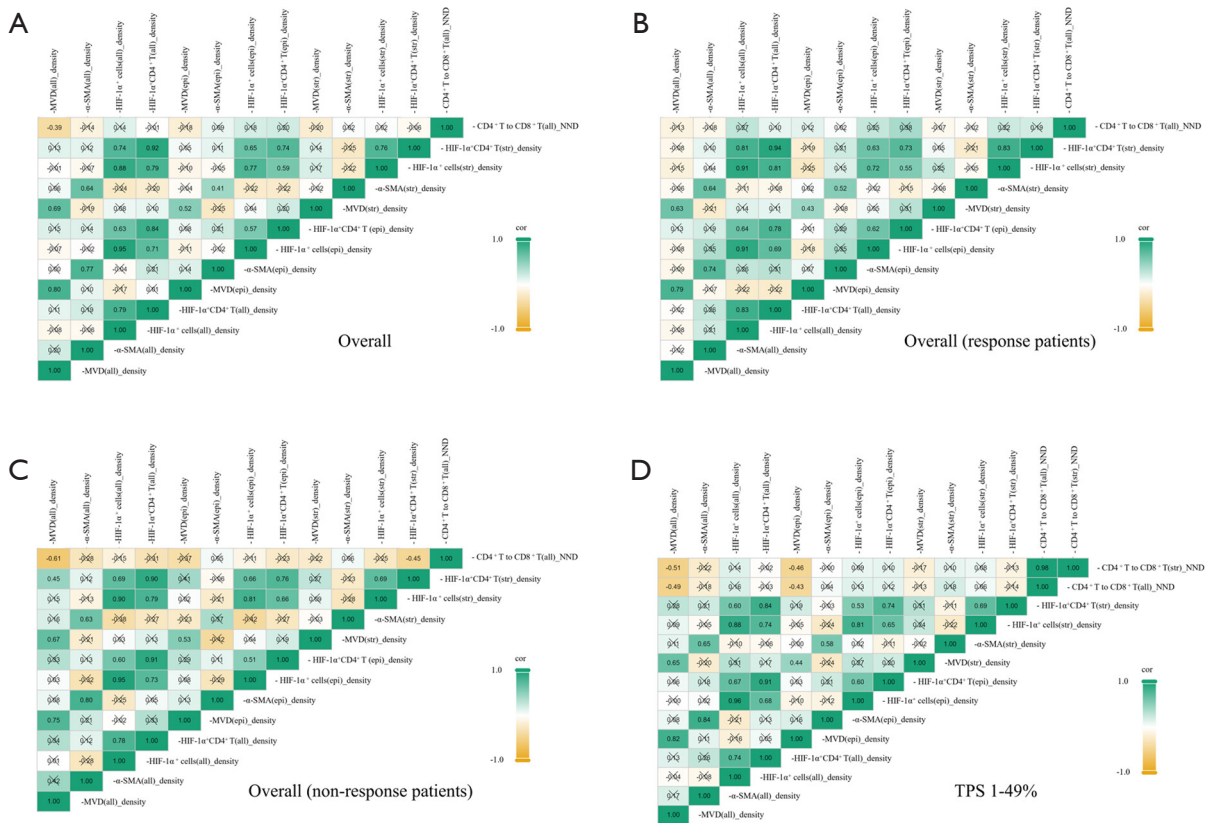
**Table S6** (continued)

**Table S6** (continued)

Proximity	Non-response	Response	P
TPS 1–49% subgroup			
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (all,10 μm)	4 (0, 15)	7 (3, 34)	0.24
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (all,10 μm)	24 (10, 54)	22 (3, 66)	0.74
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (epi,10 μm)	0 (0, 0)	0 (0, 0)	0.24
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (epi,10 μm)	0 (0, 0)	0 (0, 0)	0.87
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (str,10 μm)	4 (0, 15)	6 (3, 31)	0.24
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (str,10 μm)	24 (7, 54)	22 (3, 65)	0.74
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (all,20 μm)	10 (5, 52)	25 (8, 86)	0.44
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (all,20 μm)	39 (24, 116)	25 (8, 68)	0.30
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (epi,20 μm)	0 (0, 0)	0 (0, 0)	0.09
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (epi,20 μm)	0 (0, 0)	0 (0, 0)	0.97
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (str,20 μm)	10 (4, 50)	24 (6, 76)	0.48
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (str,20 μm)	39 (16, 116)	25 (8, 68)	0.34
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (all,30 μm)	14 (8, 76)	36 (10, 132)	0.66
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (all,30 μm)	56 (30, 148)	49 (11,51)	0.57
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (epi,30 μm)	0 (0, 0)	0 (0, 0)	0.09
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (epi,30 μm)	0 (0, 0)	0 (0, 0)	0.97
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (str,30 μm)	14 (7, 73)	35 (9, 124)	0.64
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (str,30 μm)	56 (22, 148)	49 (12, 98)	0.60
TPS ≥50% subgroup			
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (all,10 μm)	18 (8, 54)	13.50 (5, 27)	0.58
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (all,10 μm)	44 (23, 71)	16 (9, 27)	0.09
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (epi,10 μm)	0 (0, 0)	0 (0, 0)	NA
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (epi,10 μm)	0 (0, 0)	0 (0, 0)	NA
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (str,10 μm)	18 (8, 54)	13 (5, 26)	0.55
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (str,10 μm)	40 (23, 70)	16 (9, 27)	0.10
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (all,20 μm)	44 (23, 149)	41 (11, 90)	0.53
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (all,20 μm)	64 (41, 170)	63 (33, 102)	0.58
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (epi,20 μm)	0 (0, 0)	0 (0, 0)	0.16
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (epi,20 μm)	0 (0, 0)	0 (0, 0)	0.56
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (str,20 μm)	43 (23, 149)	41 (11, 88)	0.58
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (str,20 μm)	64 (41, 170)	62 (32, 102)	0.53
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (all,30 μm)	81 (40, 214)	61 (19, 120)	0.39
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (all,30 μm)	91 (62, 236)	87 (42, 139)	0.53
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (epi,30 μm)	0 (0, 0)	0 (0, 0)	0.16
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (epi,30 μm)	0 (0, 0)	0 (0, 0)	0.56
CD4 <sup>+</sup> T to CD8 <sup>+</sup> T (str,30 μm)	79 (40, 214)	61 (19, 117)	0.39
CD4 <sup>+</sup> T to CD68 <sup>+</sup> macrophage (str,30 μm)	91 (62, 236)	87 (39, 139)	0.50

Data are shown as median and quartiles. TPS, tumor proportion score; all, all compartment; epi, epithelial compartment; str, stromal compartment; NA, not applicable.





**Figure S1** The relationship between HIF-1 $\alpha$ , MVD, CAFs, and the nearest neighbor distance (NND) between CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells. (A) Overall patients. (B) Responders in overall patient cohort. (C) Non-responders in overall patient cohort. (D) PD-L1 low-expressing patients. HIF-1 $\alpha$ , hypoxia-inducible factor 1-alpha; MVD, microvessel density; CAF, cancer-associated fibroblast.