Supporting Information

Biological significance of *KRAS* mutant allele expression in ovarian endometriosis

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Table S1. The proportion of *KRAS* wild-type or p.G12V probe positive cells in cancer cell lines.

Cell line	mutation status of *KRAS*	RNA based in situ hybridization analysis			
		dapB	*PPIB*	*KRAS* wild-type	*KRAS* p.G12V
HT29	wild-type	0/840	881/881	835/854	0/739
		(0.0)	(100.0)	(97.7)	(0.0)
SW620	*KRAS* p.G12V homozygous	0/1340	1411/1411	0/1359	641/1701
		(0.0)	(100.0)	(0.0)	(37.2)
SKOV3	wild-type	0/716	741/741	695/711	0/1123
		(0.0)	(100.0)	(97.6)	(0.0)
SW626	*KRAS* p.G12V heterozygous	0/620	564/567	372/526	122/550
		(0.0)	(99.4)	(72.8)	(22.3)
OVCAR5	*KRAS* p.G12V homozygous	0/595	710/710	0/689	246/807
		(0.0)	(100.0)	(0.0)	(30.2)

positive cells / total counted cells(%)

Table S2. The proportion of *KRAS* wild-type or p.G12V probe positive cells positive cells in ovarian cancers.

Case	histology	*KRAS* p.G12V MAF (NGS)	RNA based in situ hybridization analysis			
			dapB	*PPIB*	*KRAS* wild-type	*KRAS* p.G12V
OV1	endometrioid	wild-type	4/1672	1648/1649	719/1714	0/1665
			(0.2)	(99.9)	(41.9)	(0.0)
OV2	clear	wild-type	3/1391	1310/1312	473/1311	0/1310
			(0.2)	(99.8)	(36.1)	(0.0)
OV3	clear	0.36	0/906	857/888	354/856	150/841
			(0.0)	(99.9)	(41.4)	(17.8)
OV4	clear	0.39	0/918	1215/1224	247/946	78/880
			(0.0)	(99.3)	(26.1)	(8.9)
OV5	clear	0.41	1/967	985/985	264/1103	85/1103
			(0.1)	(100.0)	(23.9)	(7.7)
OV6	endometrioid	0.44	0/1505	1498/1503	453/1620	59/1481
			(0)	(99.7)	(28.0)	(4.0)
OV7	endometrioid	0.63	1/1582	1583/1589	454/1597	190/1419
			(0.0)	(99.4)	(28.4)	(13.4)
OV8	clear	0.67	0/940	942/942	223/880	220/1064
			(0.0)	(100.0)	(25.3)	(20.7)
OV9	endometrioid	0.7	0/981	947/947	12/966	331/958
			(0.0)	(100.0)	(1.2)	(34.6)

positive cells / total counted cells(%)

Table S3. The proportion of *KRAS* wild-type or p.G12V probe positive cells in ovarian endometriosis.

Case	The way to collect samples	RNA based in situ hybridization analysis				Expected
		dapB	*PPIB*	*KRAS* wild-type	*KRAS* p.G12V	mutational status of *KRAS*
ENDO_1_Red	retrospective	0/578	753/753	4/904	106/751	*KRAS* p.G12V
		(0.0)	(100.0)	(0.4)	(14.1)	
ENDO_1_Yellow	retrospective	0/455	446/460	133/507	27/513	*KRAS* p.G12V
		(0.0)	(97.0)	(26.2)	(5.3)	
ENDO_1_Green	retrospective	0/161	143/151	20/131	0/161	no *KRAS* p.G12V
		(0.0)	(94.7)	(15.3)	(0.0)	
ENDO_2	retrospective	0/458	820/820	76/499	36/461	*KRAS* p.G12V
		(0.0)	(100.0)	(15.2)	(7.8)	
ENDO_3	retrospective	0/534	590/590	26/403	23/468	*KRAS* p.G12V
		(0.0)	(100.0)	(6.5)	(4.9)	
ENDO_4	retrospective	0/535	418/560	0/517	29/630	*KRAS* p.G12V
		(0.0)	(74.6)	(0.0)	(4.6)	
ENDO_5	retrospective	0/209	225/246	40/216	0/204	no *KRAS* p.G12V
		(0.0)	(91.5)	(18.5)	(0.0)	
ENDO_6	retrospective	0/355	370/408	15/348	0/367	no *KRAS* p.G12V
		(0.0)	(90.7)	(4.3)	(0.0)	
ENDO_7	prospective	0/501	491/501	143/492	30/736	*KRAS* p.G12V
		(0.0)	(98.0)	(29.1)	(4.1)	
ENDO_8	prospective	1/981	495/522	237/945	40/822	*KRAS* p.G12V
		(0.1)	(94.8)	(25.1)	(4.9)	
ENDO_9	prospective	1/999	206/210	101/973	79/878	*KRAS* p.G12V
		(0.1)	(98.1)	(10.4)	(9.0)	
ENDO_10	prospective	0/504	492/506	8/520	76/491	*KRAS* p.G12V
		(0.0)	(97.2)	(1.5)	(15.5)	
ENDO_11_Red	prospective	0/522	499/512	6/452	92/463	*KRAS* p.G12V
		(0.0)	(97.5)	(1.4)	(19.9)	
ENDO_11_Yello	prospective	0/167	206/210	40/161	21/163	*KRAS* p.G12V
		(0.0)	(98.1)	(24.8)	(12.9)	
ENDO_11_Gree	prospective	0/146	153/153	66/155	0/137	no *KRAS* p.G12V
		(0.0)	(100.0)	(42.6)	(0.0)	
ENDO_12	prospective	0/1210	1202/1202	618/1358	242/1163	*KRAS* p.G12V
		(0.0)	(100.0)	(45.5)	(20.8)	
ENDO_13	prospective	0/260	258/261	8/264	0/323	no *KRAS* p.G12V
		(0.0)	(98.9)	(3.0)	(0.0)	
ENDO_14	prospective	0/215	202/202	32/195	0/222	no *KRAS* p.G12V
		(0.0)	(100.0)	(16.4)	(0.0)	
ENDO_15	prospective	0/690	715/715	164/708	0/669	no *KRAS* p.G12V
		(0.0)	(100.0)	(23.2)	(0.0)	
ENDO_16	prospective	0/230	232/240	72/275	0/279	no *KRAS* p.G12V
		(0.0)	(96.7)	(26.2)	(0.0)	
ENDO_17	prospective	0/325	331/331	96/367	0/340	no *KRAS* p.G12V
		(0.0)	(100.0)	(26.2)	(0.0)	
ENDO_18	prospective	0/206	205/213	56/212	0/203	no *KRAS* p.G12V
		(0.0)	(96.2)	(26.4)	(0.0)	
ENDO_19	prospective	0/440	610/610	124/456	0/407	no *KRAS* p.G12V
		(0.0)	(100.0)	(27.2)	(0.0)	
ENDO_20	prospective	0/214	200/200	76/260	0/249	no *KRAS* p.G12V
		(0.0)	(100.0)	(29.2)	(0.0)	
ENDO_21	prospective	1/470	400/410	197/619	0/413	no *KRAS* p.G12V
		(0.2)	(97.6)	(31.8)	(0.0)	
ENDO_22	prospective	0/329	430/430	127/393	0/423	no *KRAS* p.G12V
		(0.0)	(100.0)	(32.3)	(0.0)	
ENDO_23	prospective	0/553	586/586	196/568	0/389	no *KRAS* p.G12V
		(0.0)	(100.0)	(34.5)	(0.0)	
ENDO_24	prospective	0/695	233/233	267/741	0/729	no *KRAS* p.G12V
		(0.0)	(100.0)	(36.0)	(0.0)	
ENDO_25	prospective	0/325	298/310	154/364	0/350	no *KRAS* p.G12V
		(0.0)	(96.1)	(42.3)	(0.0)	
ENDO_26	prospective	0/578	466/466	265/618	0/577	no *KRAS* p.G12V
		(0.0)	(100.0)	(42.9)	(0.0)	

positive cells / total counted cells(%)

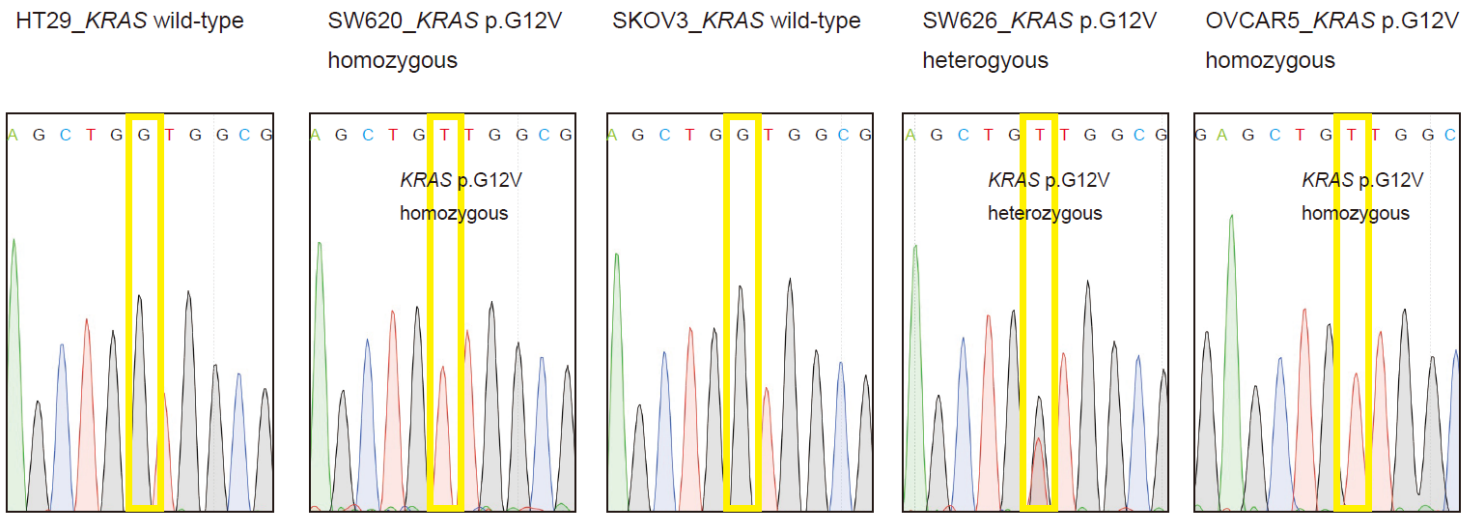


Figure S1. The chromatogram of Sanger sequencing for *KRAS* mutaion

KRAS mutation status was validated in cell blocks by Sanger sequencing.

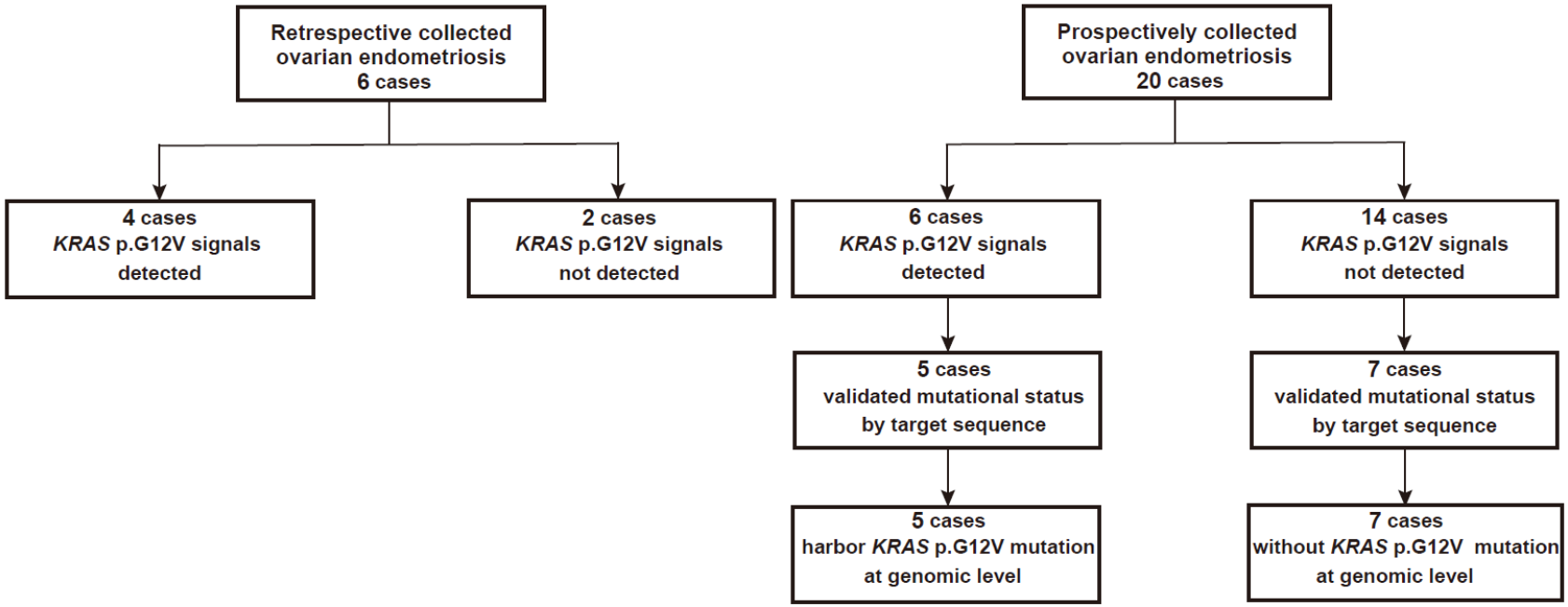


Figure S2. The analysis flowchart of ovarian endometriosis cases in this study.

Six retrospectively collected cases and 20 prospectively collected cases were recruited in this study.

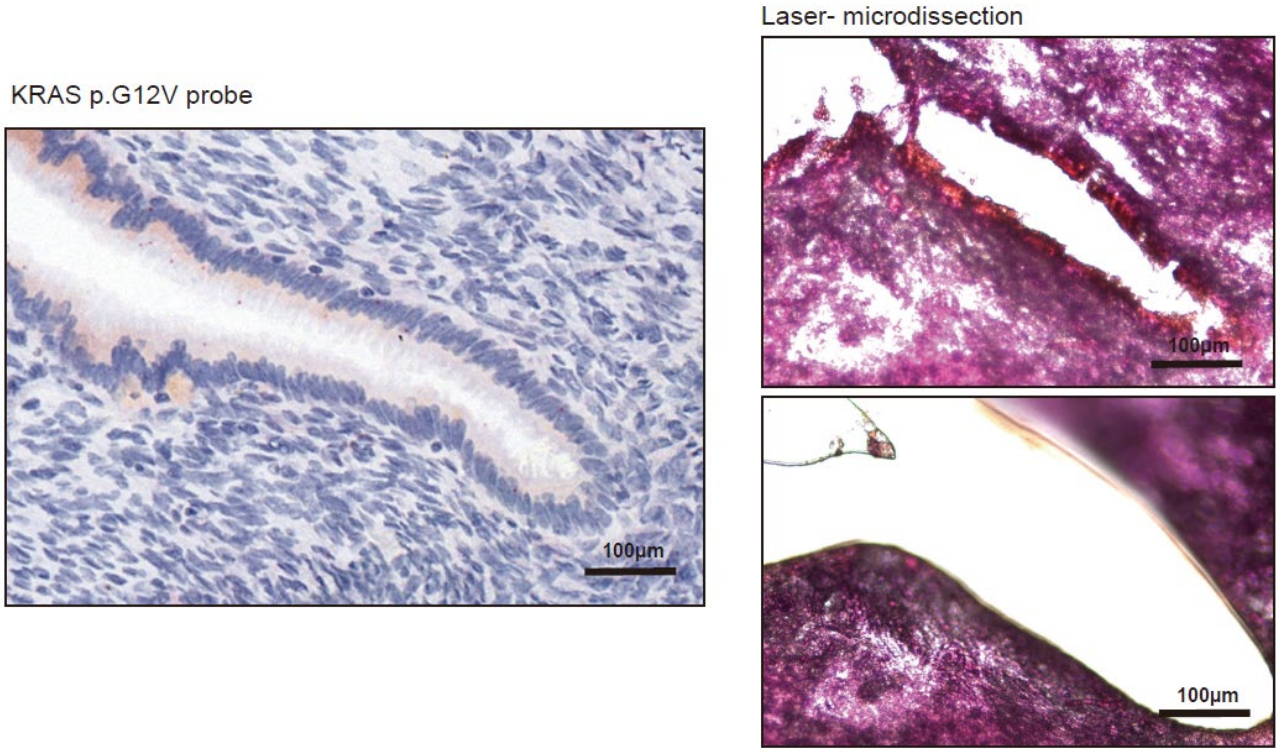


Figure S3. Laser microdissection of ovarian endometriotic epithelial cells for validation of mutational status.

To validate the *KRAS* mutational status in ovarian endometriosis, we performed laser microdissection of endometriotic epithelial cells and target gene sequencing for 12 available cases.

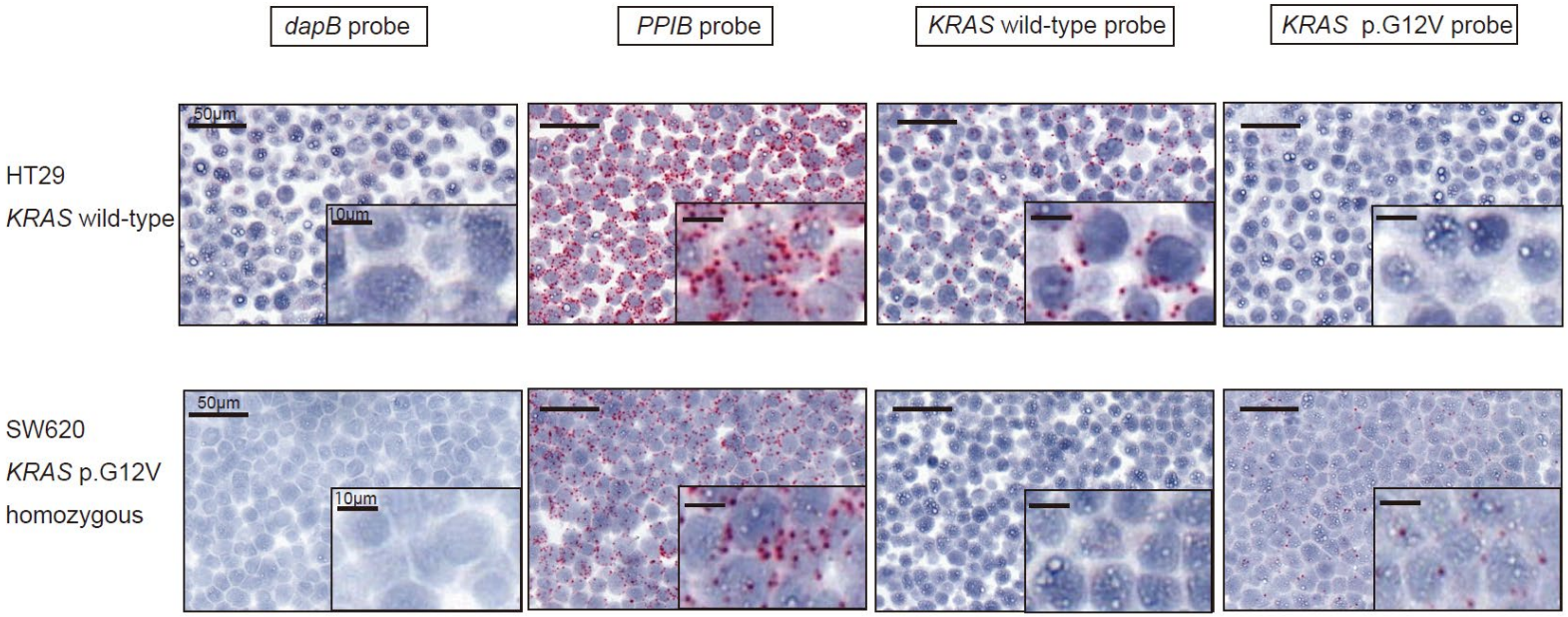


Figure S4. RNA-based in situ hybridization assay in colorectal cancer cell lines.

Representative images of validation of the *KRAS* p.G12V probe sets for colorectal cancer cell lines (a wild-type cell line and a homozygous mutant cell line) using a negative control probe (*dapB*), a positive control probe (*PPIB*), the wild-type probe and the mutant probe are shown. Probe binding is visualized as punctate red dots.

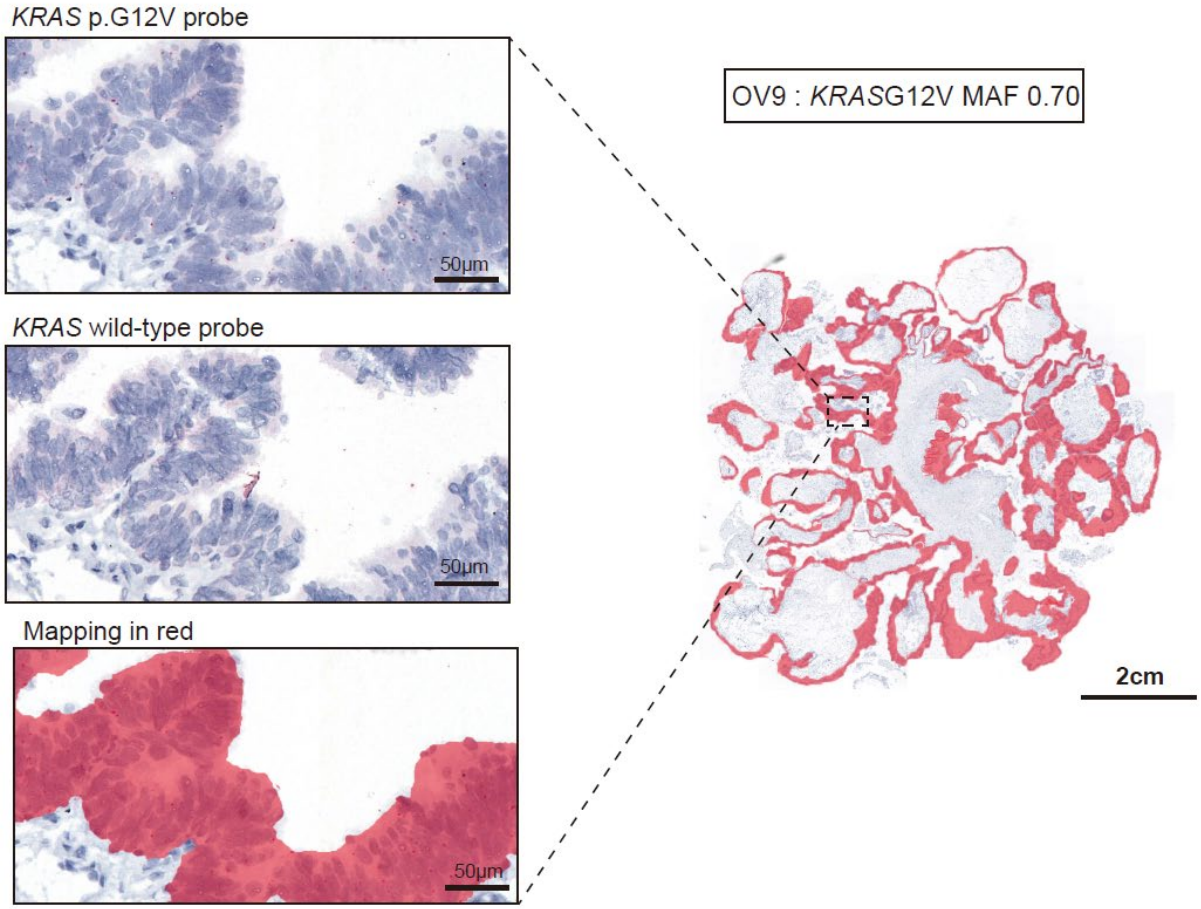


Figure S5. The spatial distribution of mutation signals in ovarian cancer.

Whole images of *KRAS* p.G12V and wild-type probe signals in one ovarian cancer sample (OV9) are displayed. The area of endometriotic epithelial cells with predominant mutational signals is mapped in red.

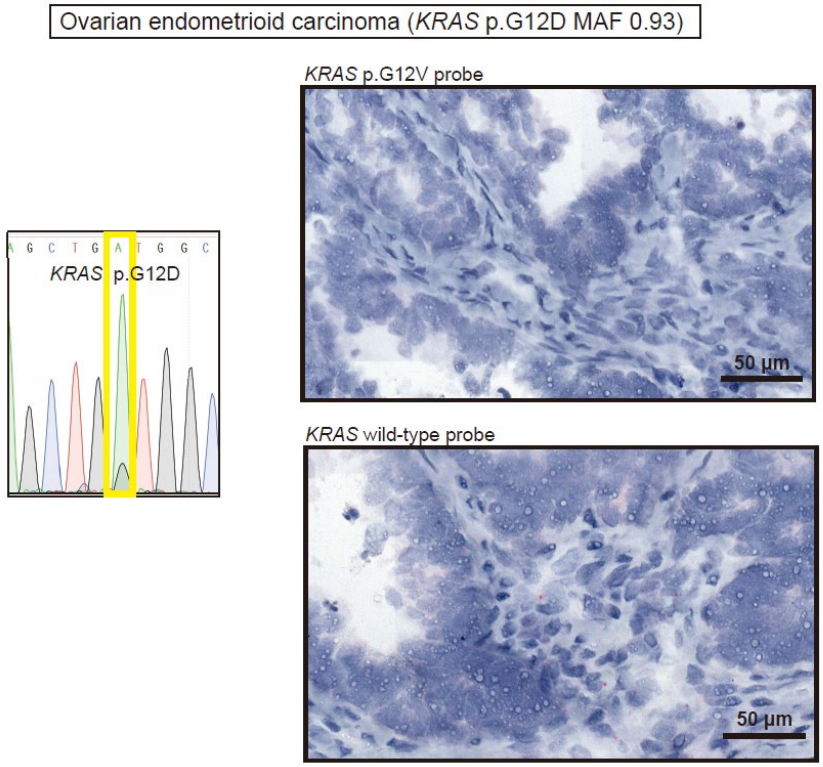


Figure S6. No cross-reactivity of the *KRAS* p.G12V probe with other *KRAS* mutations.
To confirm a lack of cross-reactivity of *KRAS* p.G12V probe with other *KRAS* mutations, we performed this assay for one ovarian cancer sample harboring the *KRAS* p.G12D mutation with a high MAF (0.93) but not the *KRAS* p.G12V mutation. No *KRAS* p.G12V mutation signal was observed in the *KRAS* p.G12D-mutated ovarian cancer sample.

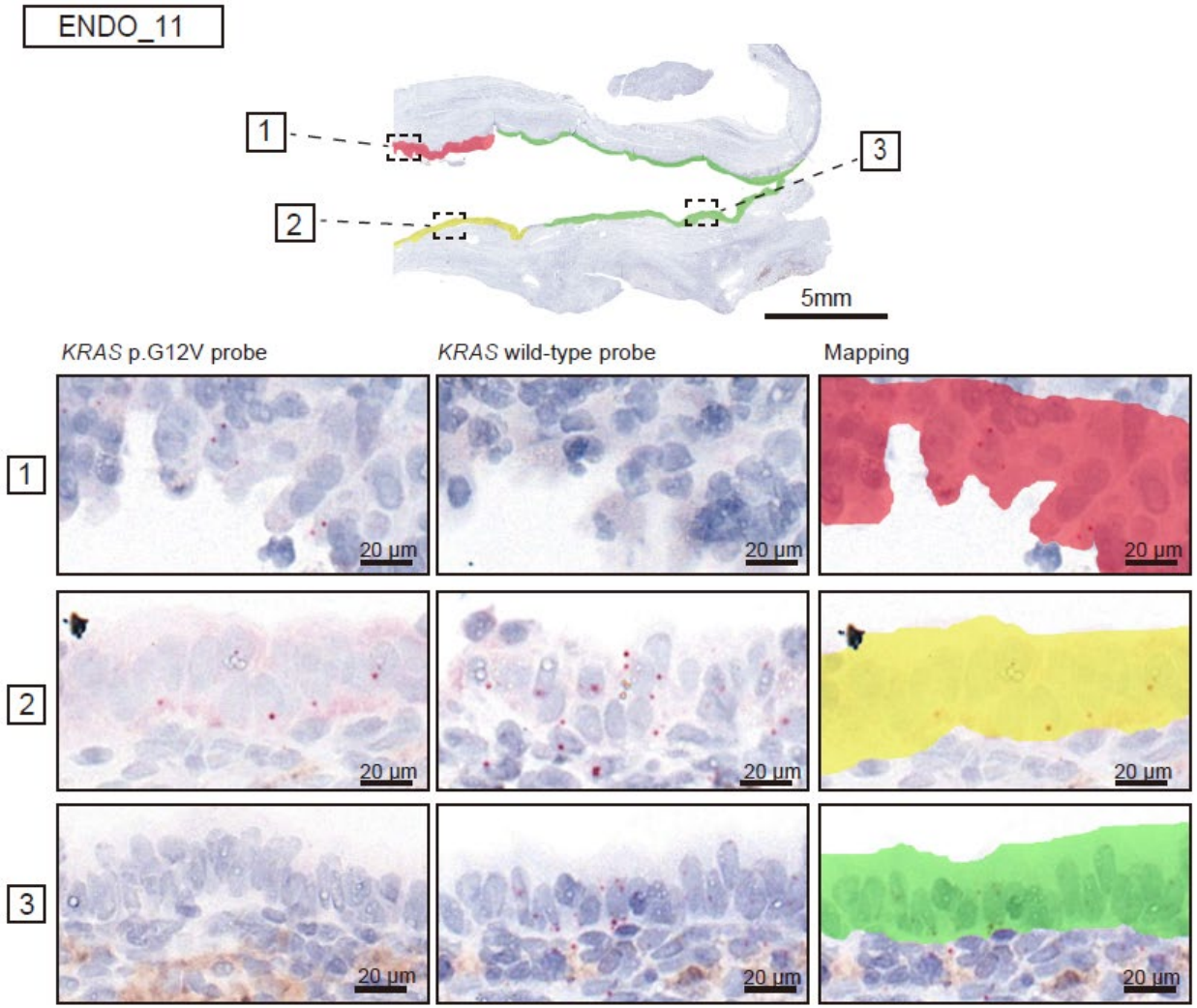


Figure S7. Intratumor heterogeneity of *KRAS* p.G12V mRNA expression and the topographical map in ENDO_11.

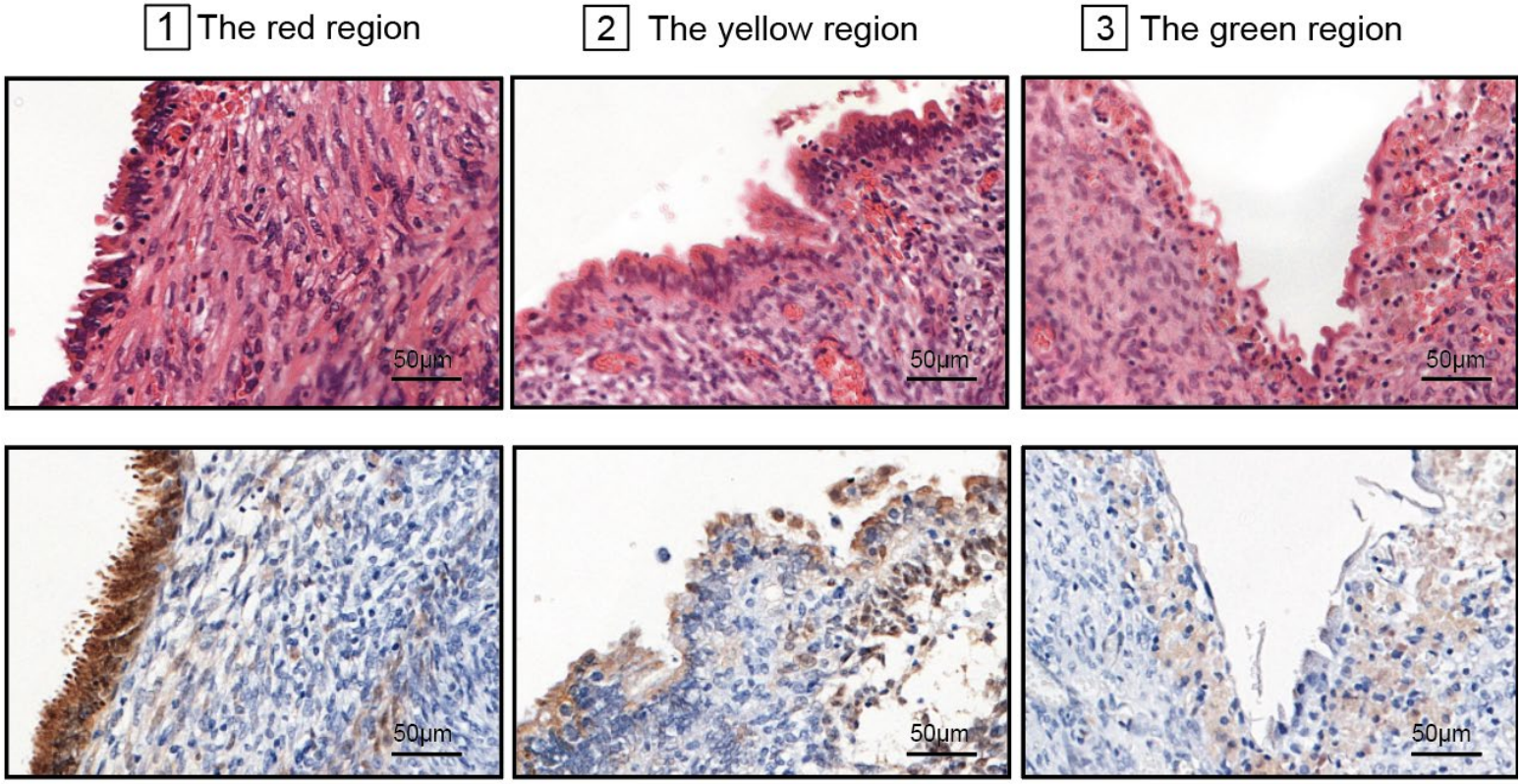


Figure S8. The result of immunohistochemical analysis for p-ERK expression in an ovarian endometriosis case (ENDO_1) with intratumor heterogeneity of *KRAS* p.G12V mRNA expression. Immunohistochemical analysis for p-ERK was performed on serial sections from in situ hybridization assay slides (ENDO_1). The staining intensity for p-ERK was strong in the red region, moderate in the yellow region, and low in the green region on the same slide.

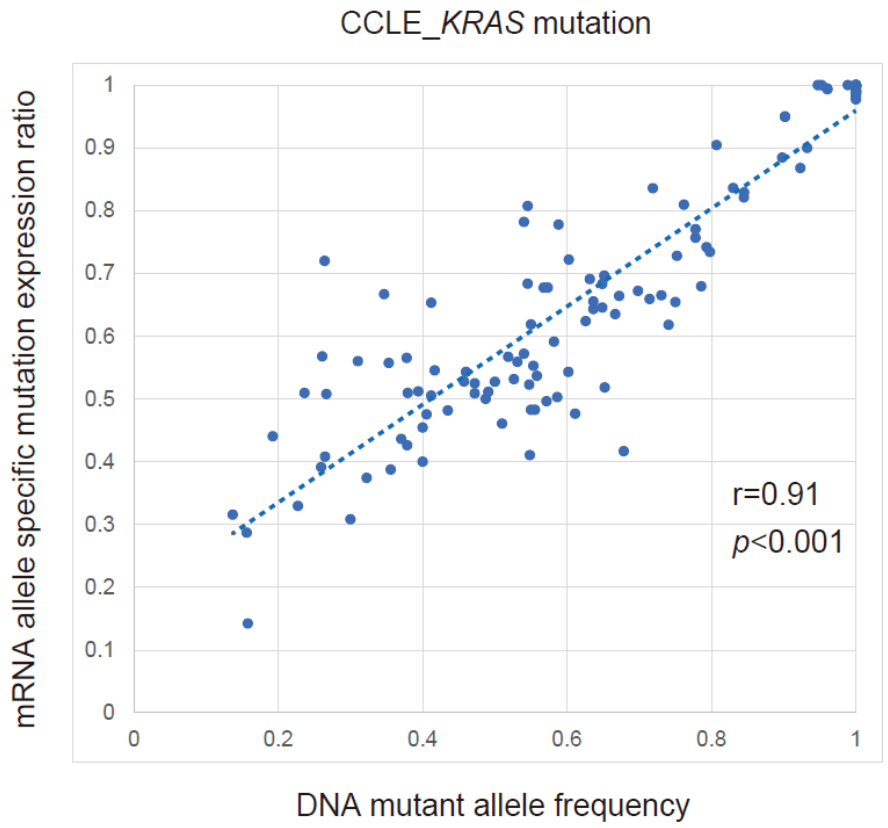
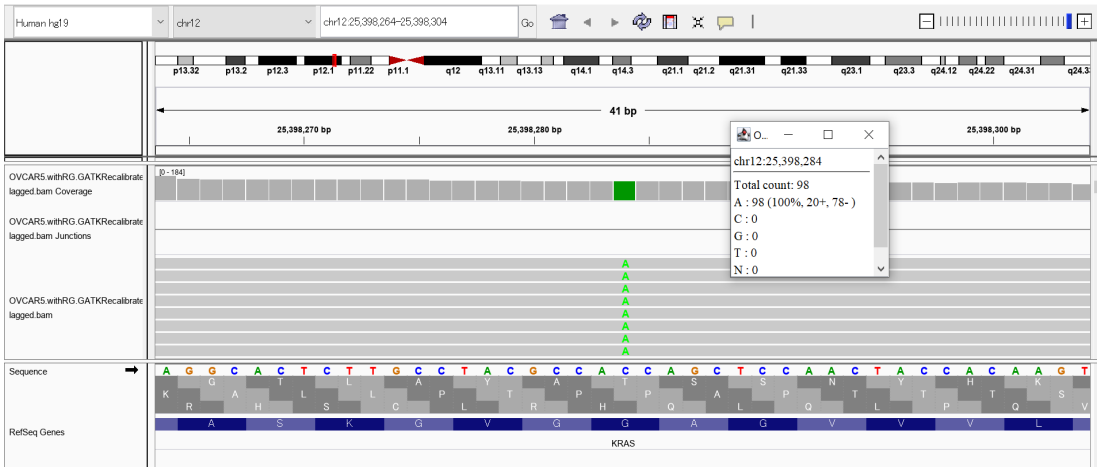


Figure S9. Correlation between *KRAS* mutant allele expression based on RNA sequencing and mutant allele frequency by DNA sequencing in human cancer cell lines.
Sequencing data of human cancer cell lines with *KRAS* mutations were downloaded from the Cancer Cell Line Encyclopedia (CCLE) portal. The mRNA allele-specific mutation expression ratio (the number of mutation reads divided by the total number of reads) correlated significantly positively with the mutant allele frequency.

A)



B)

	MAF (NGS)	*dapB*	*PPIB*	*KRAS* Wild-type	*KRAS* p.G12V	RNA_seq_ref (C)*	RNA_seq_alt (A)*
SKOV3	wild-type	0/716 (0)	741/741 (100)	695/711 (97.6)	0/1123 (0)	97	0
SW626	heterozygous	0/620 (0)	564/567 (99.4)	372/526 (72.8)	122/550 (22.3)	48	60
OVCAR5	homozygous	0/595 (0)	710/710 (100)	0/689 (0)	246/807 (30.2)	0	98
OV3	0.36	0/906 (0)	857/888 (99.9)	354/856 (41.4)	150/841 (17.8)	41	31
OV4	0.39	0/918 (0)	1215/1224 (99.3)	247/946 (26.1)	78/880 (8.9)	29	14
OV5	0.41	1/967 (0.1)	985/985 (100)	264/1103 (23.9)	85/1103 (7.7)	28	38
OV8	0.67	0/940 (0)	942/942 (100)	223/880 (25.3)	220/1064 (20.7)	5	17
OV9	0.7	0/981 (0)	947/947 (100)	1/966 (0.1)	331/958 (34.6)	3	32

* The number of reads

Figure S10. *KRAS* wild-type or mutant allele expression in cell lines and ovarian cancer samples was visualized by integrated genome viewers (IGV)

A) OVCAR5 which harbor *KRAS* p.G12V homozygous mutation has only mutant allele reads.
B) Wild-type or mutant allele expression at *KRAS* codon number 35 was confirmed in three ovarian cancer cell lines and five ovarian cancer.

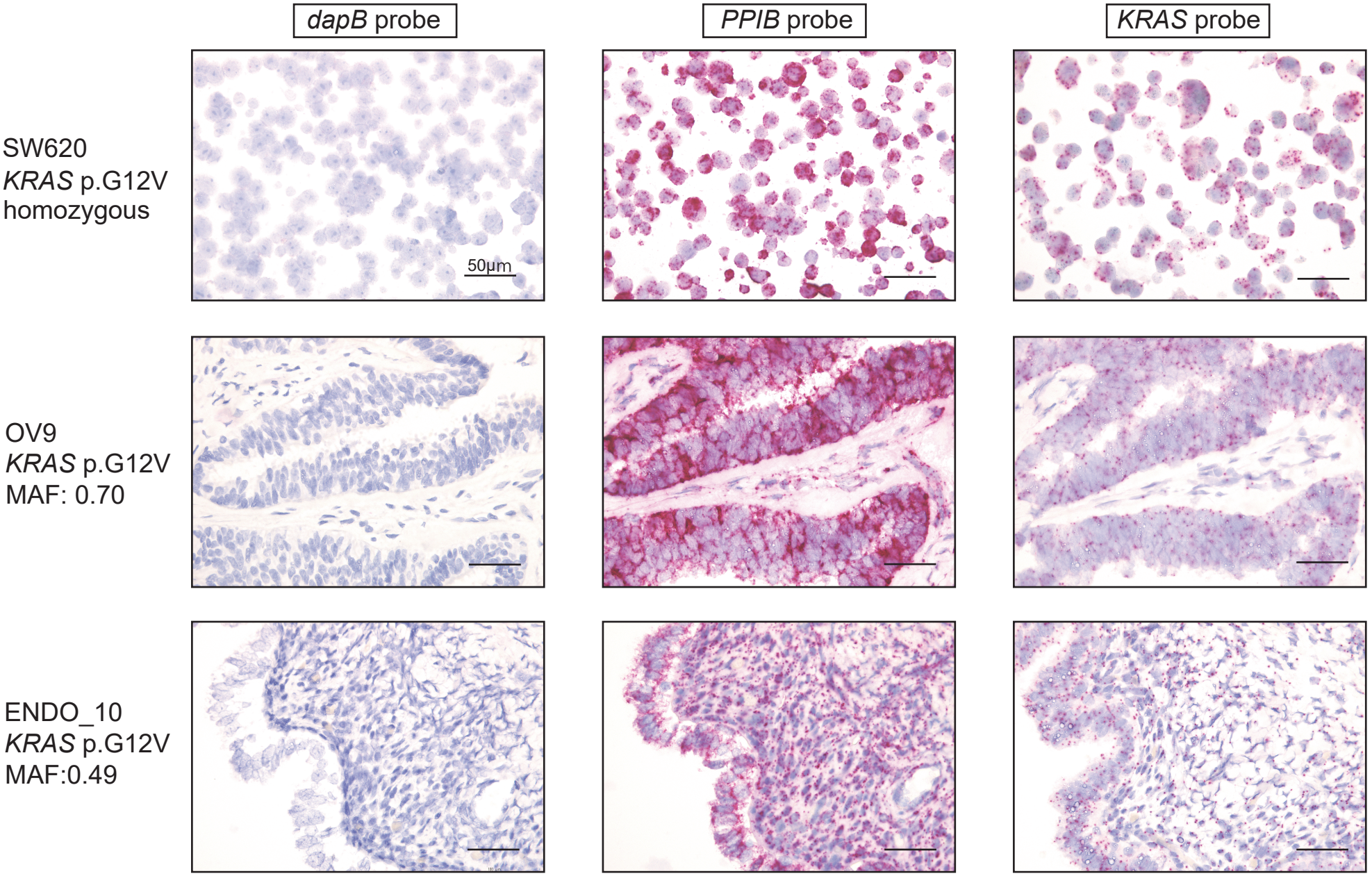
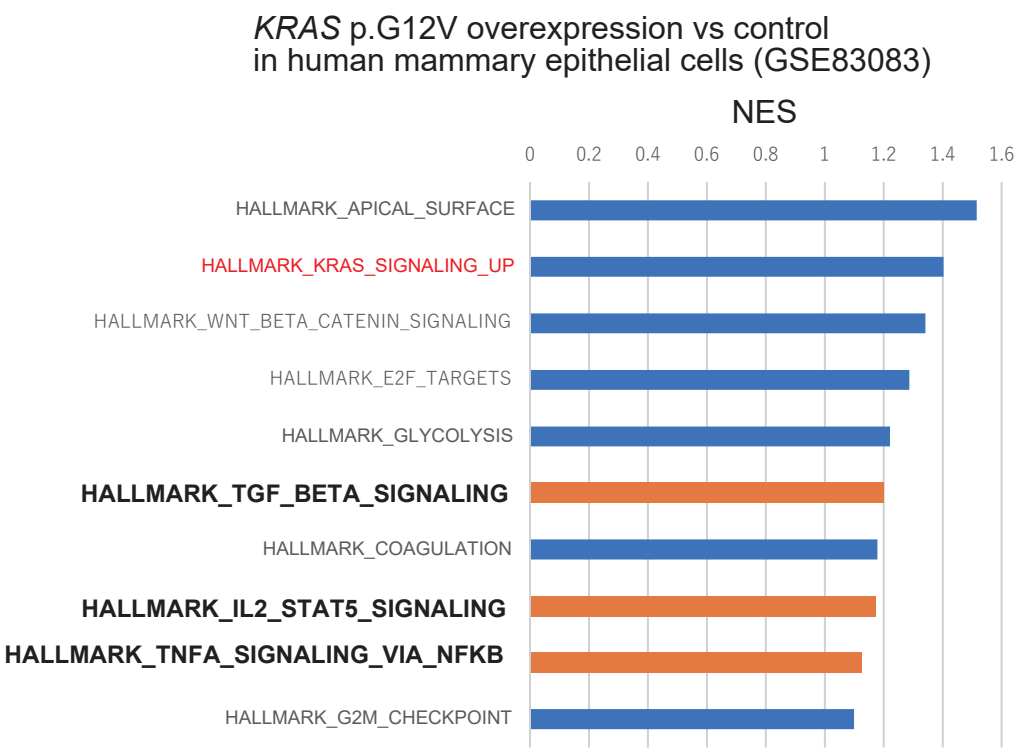
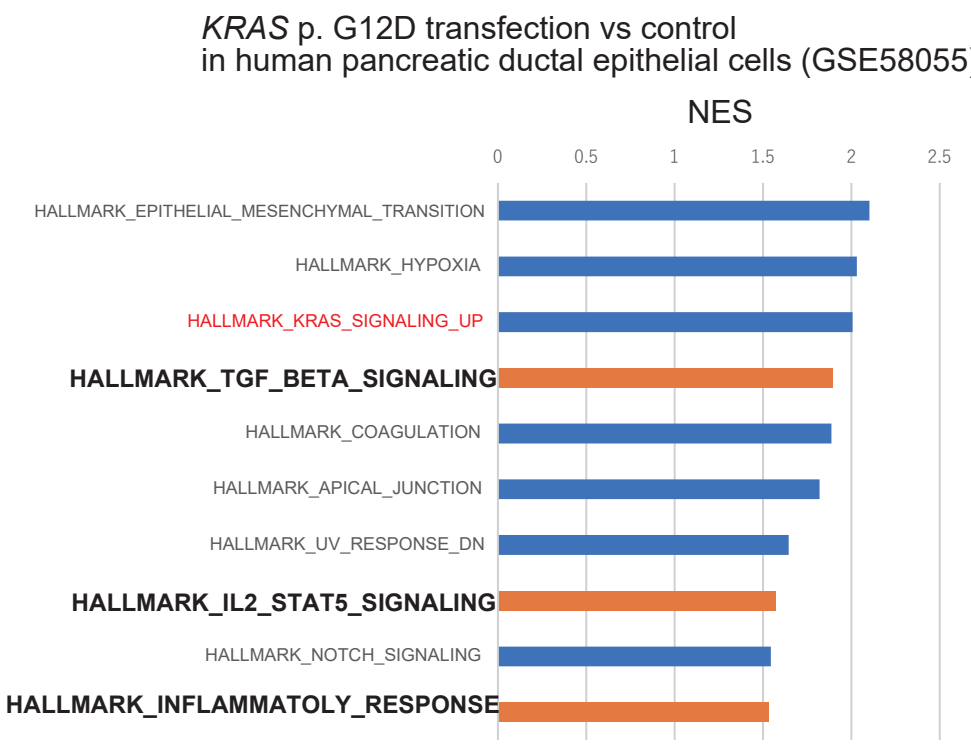


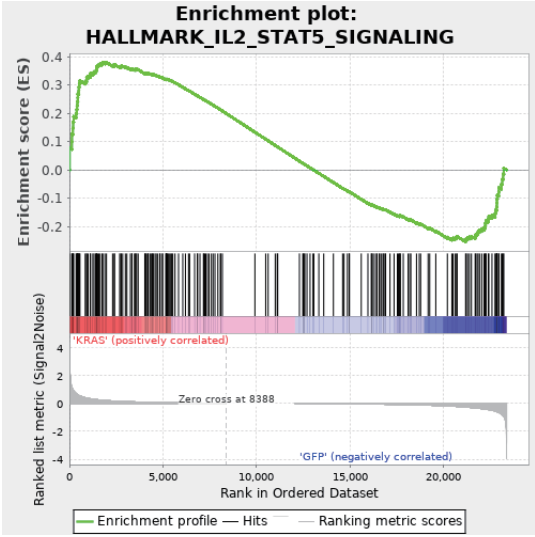
Figure S11. *KRAS* mRNA expression based on BaseScope™ assay was validated by RNAscope® assay in FFPE samples

Representative images of a validation of *KRAS* mRNA expression in SW620, OV9 and ENDO_10 using the negative control probe, the positive control probe and *KRAS* probe are shown. Probe binding is visualized as punctate red dots.





Enrichment plot: HALLMARK_IL2_STAT5_SIGNALING (GSE83083)



Enrichment plot: HALLMARK_IL2_STAT5_SIGNALING (GSE58055)

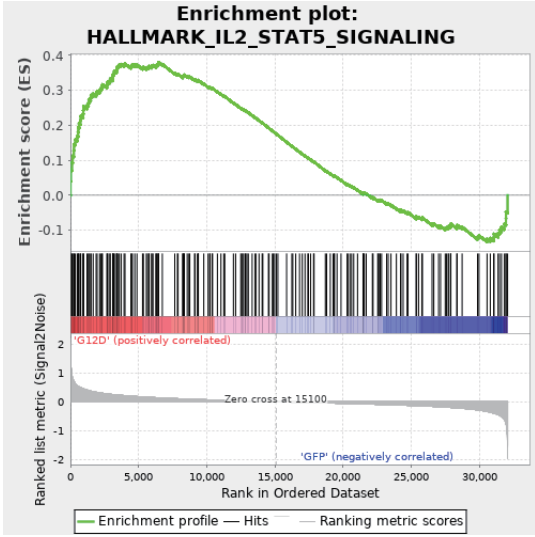


Figure S12. Top 10 pathways of differentially expressed genes enriched in *KRAS*-mutant overexpressed

Gene set enrichment analysis (GSEA) was performed by using publicly available data of *KRAS* p.G12V overexpressed human mammmary epithelial cells (GSEA83083) and *KRAS* p.G12D transfected human pancreatic ductal epithelial cells (GSE58055).