

Down-regulated and Commonly mutated *ALPK1* in Lung and Colorectal Cancers

Author names and affiliations

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Supplementary Data 1

| Variant Classification | Variant Type | dbSNP RS | Exon | ChromChange | AAChange |
|------------------------|--------------|-------------|--------|-------------|----------|
| Missense Mutation | SNP | rs147722416 | exon15 | c.G3692C | p.R1231P |
| Silent | SNP | novel | exon5 | c.G321T | p.A107A |
| Missense Mutation | SNP | novel | exon11 | c.C2654T | p.A885V |
| Missense Mutation | SNP | rs6533616 | exon6 | c.A523G | p.N175D |
| Silent | SNP | rs17044681 | exon11 | c.C2349T | p.S783S |
| Silent | SNP | novel | exon11 | c.T1389C | p.H463H |
| Missense Mutation | SNP | novel | exon3 | c.C86T | p.S29L |
| Missense Mutation | SNP | novel | exon11 | c.C2654T | p.A885V |
| Silent | SNP | novel | exon11 | c.C1125T | p.V375V |
| Missense Mutation | SNP | novel | exon5 | c.G449A | p.R150H |

Table S1. The current clinical cases of checking mutation sites in *ALPK1* and the collected data

from the TCGA database (<http://cancergenome.nih.gov/>) updated until July, 2015.

Supplementary Data 2

Large intestine

| AA Mutation | CDS Mutation | AA Mutation | CDS Mutation |
|--------------------|---------------------|--------------------|---------------------|
| p.V7A | c.20T>C | p.D756N | c.2266G>A |
| p.S29L | c.86C>T | p.A885V | c.2654C>T |
| p.K55K | c.165G>A | p.A885V | c.2654C>T |
| p.F61S | c.182T>C | p.L901I | c.2701C>A |
| p.A86T | c.256G>A | p.S938fs*14 | c.2812delT |
| p.A107A | c.321G>T | p.S957Y | c.2870C>A |
| p.A144S | c.430G>T | p.S957Y | c.2870C>A |
| p.R150H | c.449G>A | p.P962L | c.2885C>T |
| p.D253N | c.757G>A | p.W991R | c.2971T>C |
| p.V296M | c.886G>A | p.G1000R | c.2998G>A |
| p.G304C | c.910G>T | p.I1124M | c.3372A>G |
| p.V375V | c.1125C>T | p.K1125E | c.3373A>G |
| p.E438K | c.1312G>A | p.P1132S | c.3394C>T |
| p.H463H | c.1389T>C | p.K1140* | c.3418A>T |
| p.C467F | c.1400G>T | p.Y1166N | c.3496T>A |
| p.E496* | c.1486G>T | p.R1231P | c.3692G>C |

Table S2. The majority of mutation sites happened in the colorectal cancer, and the collected data

from the Cosmic database (<http://cancer.sanger.ac.uk/cosmic/>) updated until July. 2015.

Supplementary Data 3

Lung

| AA Mutation | CDS Mutation | AA Mutation | CDS Mutation |
|--------------------|---------------------|--------------------|---------------------|
| p.K58K | c.174G>A | p.P787P | c.2361A>T |
| p.A93A | c.279G>C | p.S832T | c.2494T>A |
| p.A107A | c.321G>T | p.S832Y | c.2495C>A |
| p.A109T | c.325G>A | p.C865Y | c.2594G>A |
| p.R153P | c.458G>C | p.R884M | c.2651G>T |
| p.M249I | c.747G>C | p.R884M | c.2651G>T |
| p.L267L | c.801G>T | p.S960L | c.2879C>T |
| p.A281G | c.842C>G | p.I969I | c.2907C>T |
| p.T292M | c.875C>T | p.I969M | c.2907C>G |
| p.C316S | c.947G>C | p.R973H | c.2918G>A |
| p.C316Y | c.947G>A | p.T1026M | c.3077C>T |
| p.E319* | c.955G>T | p.K1042* | c.3124A>T |
| p.L325L | c.975G>A | p.K1043I | c.3128A>T |
| p.H511Y | c.1531C>T | p.W1051C | c.3153G>T |
| p.L531L | c.1593C>G | p.Y1161C | c.3482A>G |
| p.E628D | c.1884G>T | p.M1086I | c.3258G>A |
| p.Q761Q | c.2283G>A | | |

Table S3. The majority of mutation sites happened in the lung cancer, and the collected data from the Cosmic database (<http://cancer.sanger.ac.uk/cosmic/>) updated until July. 2015.

Supplementary Data 4

| <i>Alpk1</i> exon | | Primer sequence (5'→3') | Amplicon size, bp |
|-------------------|---|------------------------------|-------------------|
| 1 | F | taccttcaccgaagcaattccta | 278 |
| | R | agtcaaacacttaagacaaataacttcc | |
| 2 | F | ttgatcttctgttcccttatccg | 267 |
| | R | tgtggaatgtttgcctctaaagtg | |
| 3 | F | tcttccctctctttgttccaca | 268 |
| | R | gtgcctcccatcagaacctc | |
| 4 | F | ttccttacctgaactctgaccttt | 238 |
| | R | gtctaaatgcctcacttggggata | |
| 5 | F | tattaatgaaaatgcctcccacgc | 315 |
| | R | tcaagtagctcgggagggaag | |
| 6 | F | cagttcctgctgcaatatatcagt | 265 |
| | R | aaagcttctcattcatcagtggc | |
| 7 | F | tgtgctcatgaagacttctgtga | 243 |
| | R | cacagtgaaacctgcagcc | |
| 8a | F | atactagctgttctccctgt | 150 |
| | R | aagaagcactagacaaaccacct | |
| 8b | F | agcagagttaatatgggcctcc | 211 |
| | R | actaactgtacctggccctatg | |
| 9 | F | ctttaggccatggatgagagcag | 205 |
| | R | ctgagaaagacagccactaacca | |
| 10 | F | agcaaaaccatttcatagcctgtt | 260 |
| | R | gccactgatgagacacaaagc | |
| 12 | F | ttcaggtgagctggcgtag | 305 |
| | R | agcaaggtagctcatcttaca | |
| 13 | F | accactttgcattttgtgtgtg | 333 |
| | R | tacagggacaacacacaaggat | |
| 14 | F | caaaccaagatttaccgaagtcca | 283 |
| | R | aattcccagtttcacatac | |
| 15 | F | gtgtgtgtgtgtgtgtatgtat | 319 |
| | R | gagaaccagatatttgcgagcatc | |
| 16 | F | tggattggtaatgtgacagacctt | 263 |
| | R | ctgtttccatgttgctttccttg | |

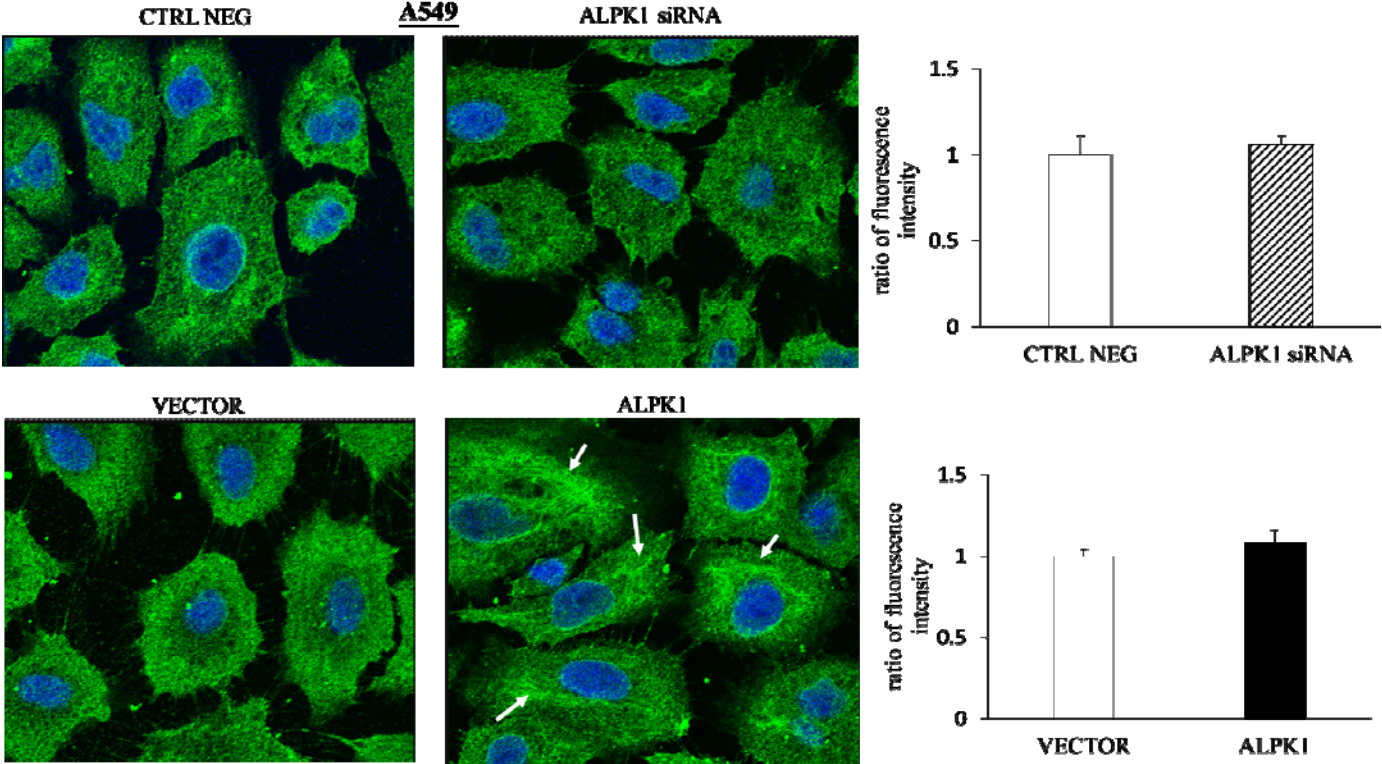
(Continued to the next page)

| <i>Alpk1</i> exon | | Primer sequence (5'→3') | Amplicon size, bp |
|-------------------|---|---------------------------|-------------------|
| 11a | F | gtcctgattcacttgcagtttca | 338 |
| | R | tgaaattgtacagcttccccattg | |
| 11b | F | tggaaaacaggagcttcacag | 292 |
| | R | ttatccaacctgcactcgaaact | |
| 11c | F | gaagttatgtctgtgattgccag | 324 |
| | R | ttcctctgaacattcttacctat | |
| 11d | F | cggtgtgtgaagtattgaaagtg | 354 |
| | R | aagcactggaagtctggctg | |
| 11e | F | cattctgatgcatttcgagtctcc | 308 |
| | R | tccaaattgtcattgttgggtcc | |
| 11f | F | gaaaagagcctggcaaagaacatc | 308 |
| | R | agaatcagaagaccaagaagcaga | |
| 11g | F | cctcataataccccaggcattttc | 327 |
| | R | taagggtgcatcttcagtgccttc | |
| 11h | F | agaagcctttgaataattgttgagt | 303 |
| | R | cagttgccctcctcatcca | |
| 11i | F | aattggcctgttcaaaatcctgac | 281 |
| | R | atgtttccaggctgatttcctcc | |
| 11j | F | gctctcatagactgtgcattctga | 334 |
| | R | tctagtctctgaaacagccaatca | |
| 11k | F | cattttccagtggttcttctgagg | 278 |
| | R | acctttcatgagctttaagtgatct | |

Table S4. The Primer sequences of *ALPK1* for HRM analysis.

Supplementary Data 5

(a)



(b)

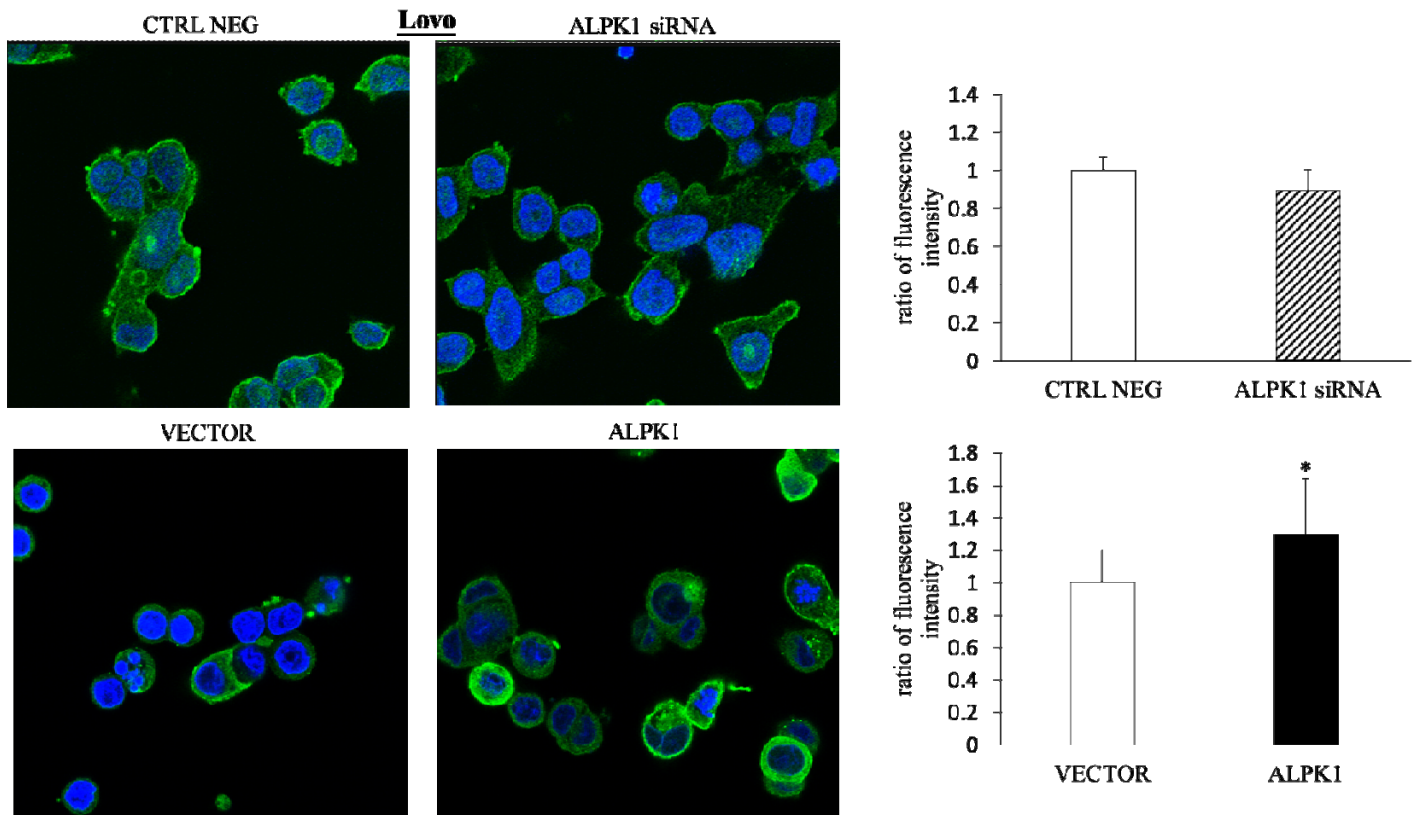


Figure S1.

ALPK1 caused various impacts on the actin distribution. The A549 (a) and Lovo (b) cancer cells were transfected with siRNA targeting *ALPK1* and *ALPK1* vector for 24h, after which the cells were stained for actin with Alexa Fluor 488 (green) and for the nucleus with DAPI (blue). The white arrows indicate the actin polymerization, and the confocal images (magnification, 1260 X) are shown in the panels on the left. Mean fluorescence intensities of approximately 40 cells per condition in four random fields were quantified using the ImageJ software program, and data were normalized to the pixel of CTRL NEG or VECTOR, respectively. Quantifications of actin staining are presented in the bar graphs on the right, and * $P < 0.05$ indicates a significant difference

between the two groups.