Article

Effective RNA knockdown using CRISPR-Cas13a and molecular targeting of the *EML4-ALK* transcript in H3122 lung cancer cells

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Supplementary Material

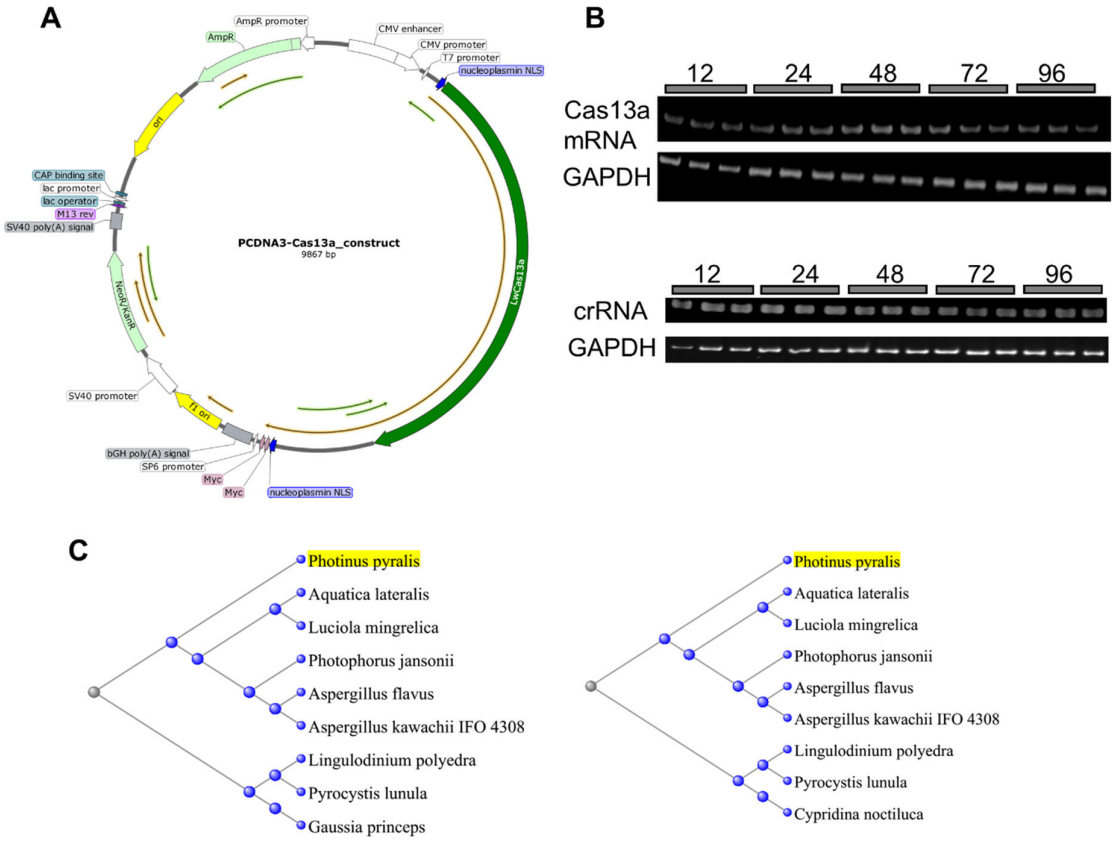


Figure S1. (**A**) Circular map of pcDNA3-Cas13a construct vector plasmid. (**B**) RT-PCR gel images of Cas13a and crRNA guide. (**C**) Phylogenic tree of Luciferin originated from various species. Identity of Firefly luciferase protein with gaussia and cypridinia is 29.03% and 27.45% respectively.

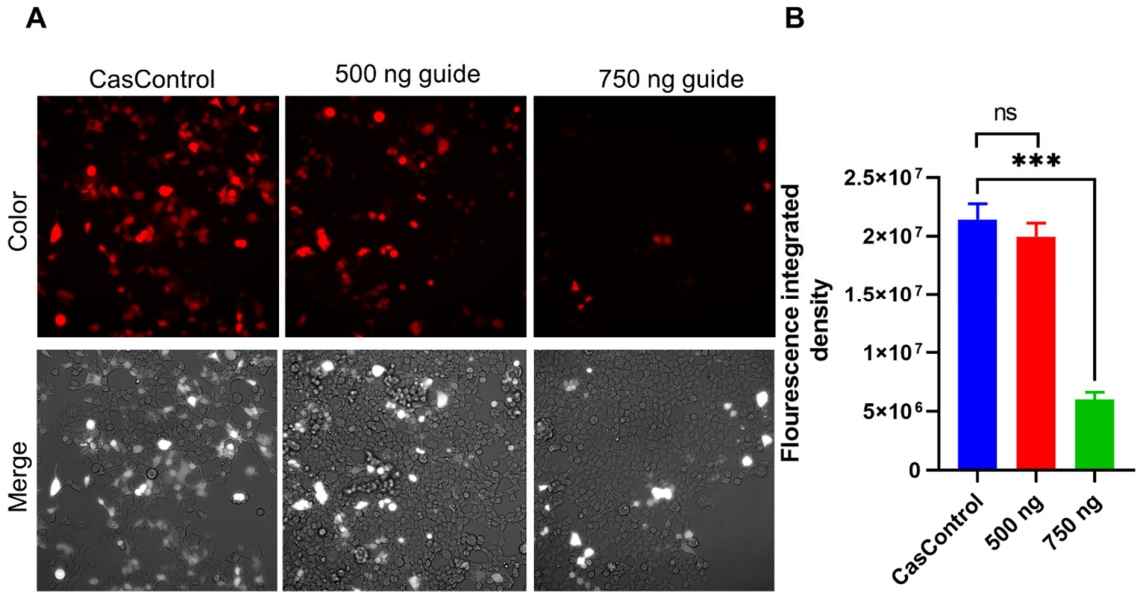


Figure 2. mCherry mRNA knockdown by Cas13a. (**A**) Fluorescence images (20×) of mCherry knockdown at two guide RNA concentrations; (**B**) Graphical representative of A, ***$p < 0.005$.

Supplementary Material

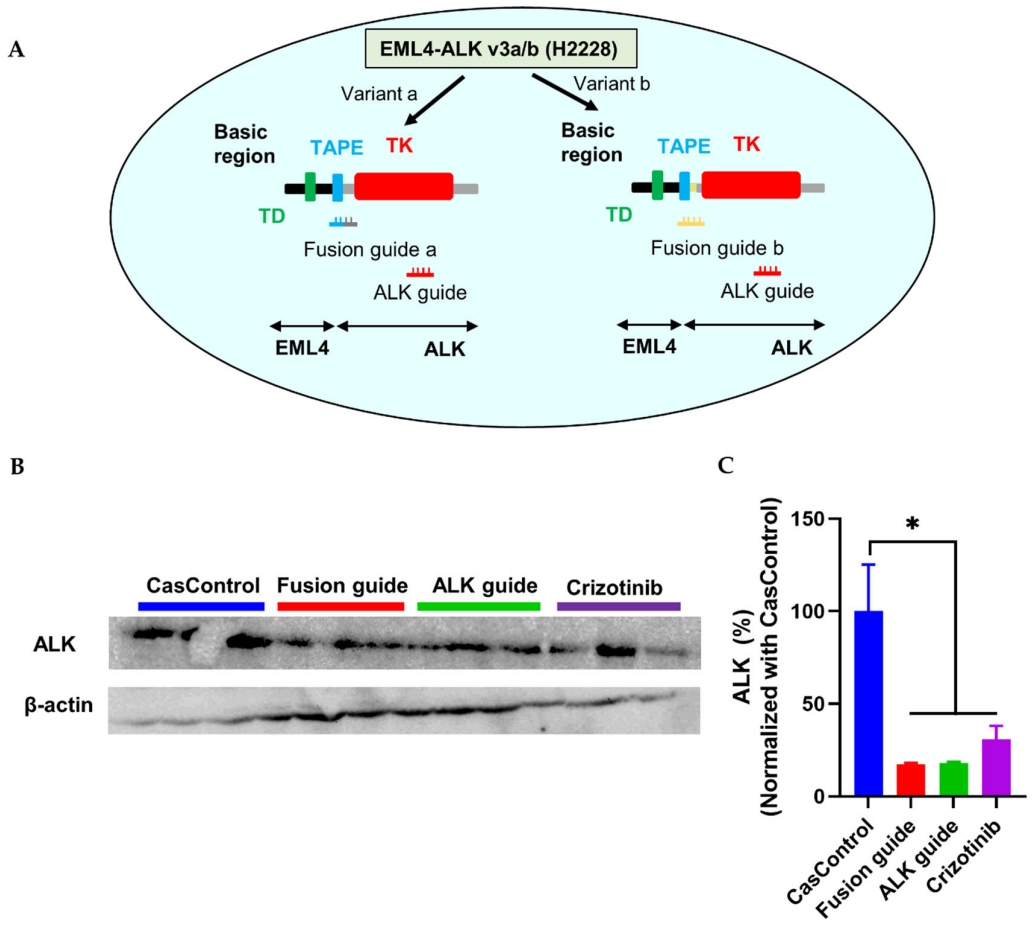


Figure S3. *EML4-ALK* knockdown using Cas13a in H2228 cells. (**A**) Graphical representation of *EML4-ALK* v3a/b oncofusion with the associated functional domain and guide RNA location in H2228 cells. Left side: TD, trimerisation domain; TAPE, tandem atypical propeller domain; HELP, hydrophobic motif in EML proteins; and TK, tyrosine kinase domain. Fusion guide a/b, *EML4-ALK* target guide at the fusion point of *EML4* and *ALK* in variant 3a/3b; ALK guide, *EML4-ALK* target guide in the TK domain. (**B**) Western blot analysis of ALK in H2228 cells treated with Cas13a and indicated guide RNAs or crizotinib (as a control drug) for 72 h. Total β-actin was used as a loading control. ALK protein was down-regulated over 80% in both indicated guide RNAs. (**C**) Graphical representative of **B,** $^{*}p < 0.05$.

Table 1. Oligo sequences used for PCR amplification reaction.

Target	Oligos (5′ to 3′)
	mCherry
mCherry_Fwd	cccggatccCACCATGGTGAGCAAGGGCGA
mCherry_Rev	ggggaattcTTACTTGTACAGCTCGTCCATGC
	pcDNA3-Cas13a
Cas13a_Fwd	gggctcgagTAGAGCGCTGCCACCATGAA
Cas13a_Rev	gggtctagattacatttcattcaagtcctcttcagaaatgagcttttgctccatttcattc aagtcctcttcagaaatgagcttttgctcggtaccTTTCTTCTTCTTAGCC TGTCCAGC
pcDNA3_Fwd	aggacttgaatgaaatgtaatctagAGGGCCCTATTCTATAGTGTCACC
pcDNA3_Rev	cttcatggtggcagcgctctactcgagCTCGAGCGGCCGCCAGTGTGAT

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Table S2. crRNA and guide RNA sequences

Target RNA name	cDNA of guide RNA (5′ to 3′)	Length (bp)	Reference
Non-targeting guide	TTTACAACGTCGTGACTGGGAAAACCCT	28	[16]
crRNA (or DR)	GATTTAGACTACCCCAAAAACGAAGGGGA CTAAAAC	36	[16]
Luc guide-1	CGAGAATCTCACGCAGGCAGTTCTATGAGG	30	custom
Luc guide-2	GTAATCCTGAAGGCTCCTCAGAAACAGCTC	30	custom
	Length guide		
Luc 12 guide	GTAATCCTGAAG	12	custom
Luc 18 guide	GTAATCCTGAAGGCTCCT	18	custom
Luc 24 guide	GTAATCCTGAAGGCTCCTCAGAAA	24	custom
Luc 28 guide	GTAATCCTGAAGGCTCCTCAGAAACAGC	28	custom
Luc 30 guide	GTAATCCTGAAGGCTCCTCAGAAACAGCTC	30	custom
Luc 40 guide	GTAATCCTGAAGGCTCCTCAGAAACAGCTC TTCTTCAAAT	40	custom
Luc 50 guide	GTAATCCTGAAGGCTCCTCAGAAACAGCTC TTCTTCAAA TCTATACATTA	50	custom
	Mismatch guide		
Luc WT	GTAATCCTGAAGGCTCCTCAGAAACAGC	28	custom
Luc M1	ATAATCCTGAAGGCTCCTCAGAAACAGC	28	custom
Luc M2	GCAATCCTGAAGGCTCCTCAGAAACAGC	28	custom
Luc M3	GTGATCCTGAAGGCTCCTCAGAAACAGC	28	custom
Luc M4	GTAATCCTGAAGGATCCTCAGAAACAGC	28	custom
Luc M5	GTAATCCTGAAGGCCCCTCAGAAACAGC	28	custom
Luc M6	GTAATCCTGAAGGCTACTCAGAAACAGC	28	custom
Luc M7	GTAATCCTGAAGGCTCCTCAGAAACGGC	28	custom
Luc M8	GTAATCCTGAAGGCTCCTCAGAAACAAC	28	custom
Luc M9	GTAATCCTGAAGGCTCCTCAGAAACAGA	28	custom
	mCherry		
mCherry guide	CGAAGTTCATCACGCGCTCCCACTTGAA	28	custom
	EML4-ALK guide		
ALK guide	CTGGCAGCAATGTCTCGGTGGATGAAGTGG	30	Custom
Fusion guide (H3122)	GCTTCCGGCGGTACACTTTAGGTCC	25	Custom
Fusion guide a (H2228)	TCCGGCGGTACACTTGGTTGATGATGAC	28	Custom
Fusion guide b (H2228	TTTTCGCGAGTTGACATTTTTGCTTGG	27	Custom

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Table S3. Primers sequences used for sanger sequencing

Target	Sequences (5′ to 3′)
PCDNA3-Cas13a	
T7_forward primer_seqF1	TAATACGACTCACTATAGGG
Cas13a_SeqF2	GAAGATCAACGAGAACAACG
Cas13a_SeqF3	AGGCCTTCCTGAGAAACAT
Cas13a_Seq4	AGTTCCTGAACAAGTTCGT
Cas13a_SeqF5	AAGTTCCTGGACTTCAACG
Cas13a_SeqF6	ACATCCGGAACTACATTGC
BGH-rev	TAGAAGGCACAGTCGAGG
pcDNA_SeqF1	AGAGGGCCCTATTCTATAGTG
pcDNA_SeqF2	GTGTGTCAGTTAGGGTGTG
pcDNA_SeqF3	ATACGCTTGATCCGGCTA
pcDNA_SeqF4	TCATAGCTGTTTCCTGTGTG
pcDNA_SeqF5	GATTAGCAGAGCGAGGTAT
pcDNA_SeqF6	AACGATCAAGGCGAGTTAC
pcDNA_SeqF7	GCTTGACCGACAATTGCAT
pCS2+crRNA-guide RNA	
Sp6_fwd	GTGCCTAATGGGAGGTCT

Table S4. Primers sequences used for RT-PCR and qRT-PCR

Target gene	Sequences (5′ to 3′)	Product length (bp)	Assay name
hGAPDH	Fwd: GACAGTCAGCCGCATCTTC Rev: GTTCACACCCATGACGAACAT	464	RNA stability; RT-PCR
	Fwd: GACAGTCAGCCGCATCTTC Rev: ACTCCGACCTTCACCTTCC	79	qRT-PCR
h18S rRNA	Fwd: GGCCCTGTAATTGGAATGAGTC Rev: CCAAGATCCAACTACGAGCTT	146	RNA stability; qRT-PCR
Cas13a_HEPN	Fwd: GGACAACAAGAACGAGATCGAG Rev: GCTGGGGGCGATATTCTTGAA	136	RNA stability;
crRNA-guideRNA	Fwd: ATTTAGACTACCCCAAAAACGAAGG Rev: CAGAGCTGTTTCTGAGGAGCC	70	qRT-PCR/RT-PCR
EML4	Fwd: CTGCTGCAAGTACTTCTGATGTTC Rev: CTTCAGAGATTGCAAGACGCCTC	132	Expression and Knockdown; qRT-PCR
ALK	Fwd: ACATTGCCTGTGGCTGTCAG Rev: ACTTAACTGGCAGCATGGCACA	189	

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Sanger sequences of pcDNA3-Cas13a construct

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Supplementary Material

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Supplementary Material

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