Cell Death & Differentiation
https://doi.org/10.1038/s41418-021-00763-6

ARTICLE

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Received: 5 September 2020 / Revised: 20 February 2021 / Accepted: 24 February 2021

Abstract

Introduction

[1–3]

[4–6]

[7]

Supplementary information

Materials and methods

Cell lines

3000 ( )

Immunofluorescence

20 . fi 3% 15 15 0.2% / -100 . 2% 30 1 1 fl fl (/ 63,). (5 8) 3 ( ).

Fluorescence recovery after photobleaching (FRAP)

2 42 (42, 42- 42- 120). fl 3 488 60%. fl 2.5 180 42- fi (7).

Immunoblotting

" " (10 7.5, 150 , 1% / -100, 0.1% 1%) fl 3/4 1 . ( ). 5 95 . 4%

4 1 (4.0).

Lung cancer tissue specimens

fi

Generation of stable cells

1 . 1- fi fi , 1, 2, 293 (fi) 1299 , 2 µz / fi 18- 1299 , 18- 293 / 2 2- 21 . 1299 , (2 µz /) fi 18- .

CRISPR/Cas9-mediated gene ablation

9 / 42 1299 22 . 42- (:// . /), : 42- #1, 42- #2, 9( )-2 (/ 458) 1299 48 , fl

96-
42
fi
fi .

Protein purification and in vitro phase separation assay

42-(742-1316)
1
-28
fi
-21
(
3) *Escherichia coli*.
0.4
37
12
fi
fi
fi
25
7.5
1
fi
42
1
(12.5
7.5
0.5
).
(
400, 2000,
4000)
25% (/)
0
600
fi
)
fi
(
/ 81,).

mRNA sequencing and alternative splicing (AS) analysis

1299
1299
42
/ 9
(
).
(
3
)
(
)
(
)
2000
(
18)
2.0.1.6 (
)
fl

Splicing assay via semi-quantitative RT-PCR

(
)
1
(
)
37
(2
)
(
)
fi
(25
).
2.
2%

Colony formation

6-
(750
)
6-
(2, 000
)
37
48
2-
fi
15
0.1%
15
/ (+
fi

Statistics

(
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t-
(
)
(
7).
3.6.2
. $P < 0.05$
fi

Results

Systematic survey reveals USP42 as a phase-separated DUB in the nucleus

2, 3, 23
71

USP42 C-terminal disordered region confers phase separation features

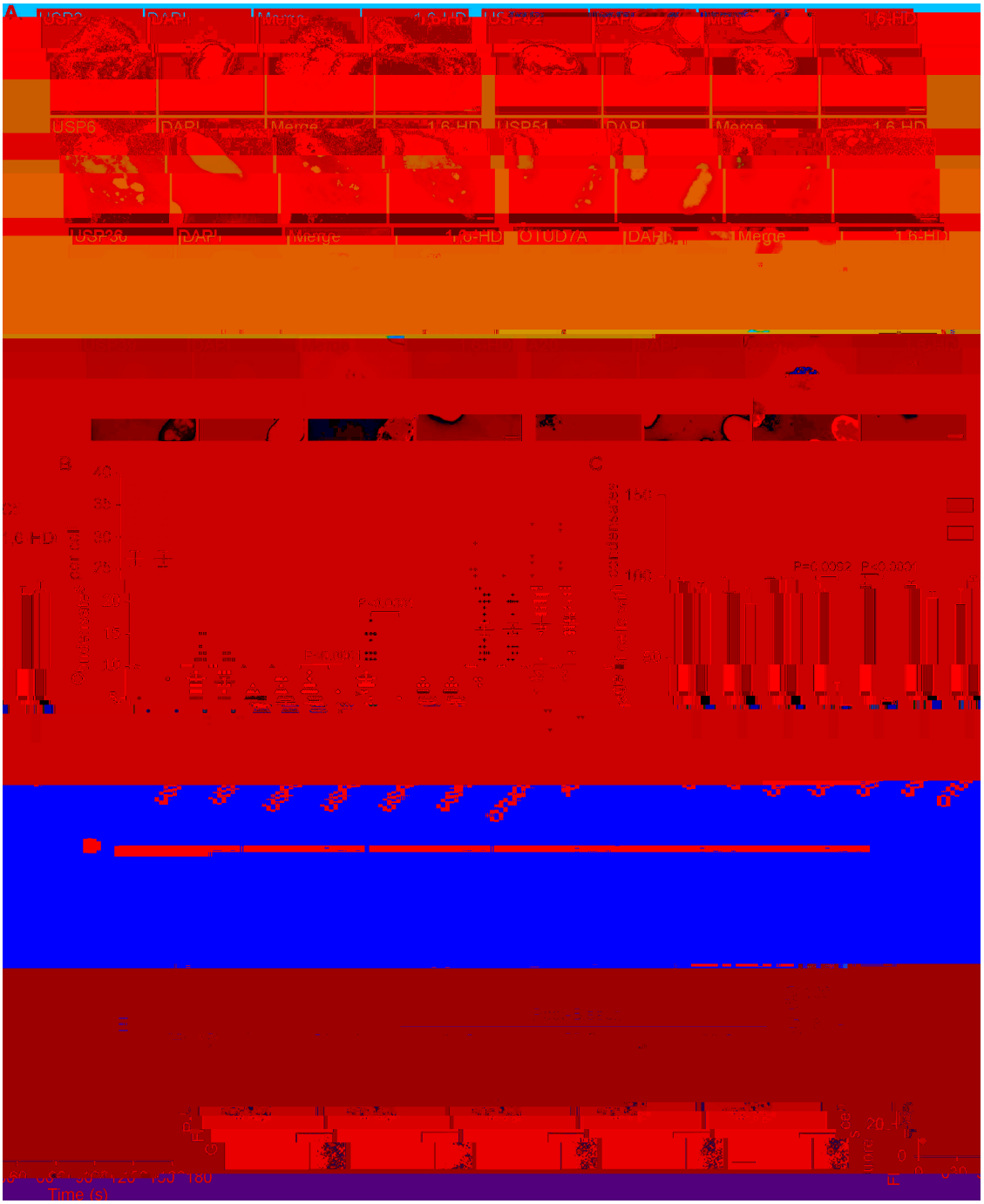
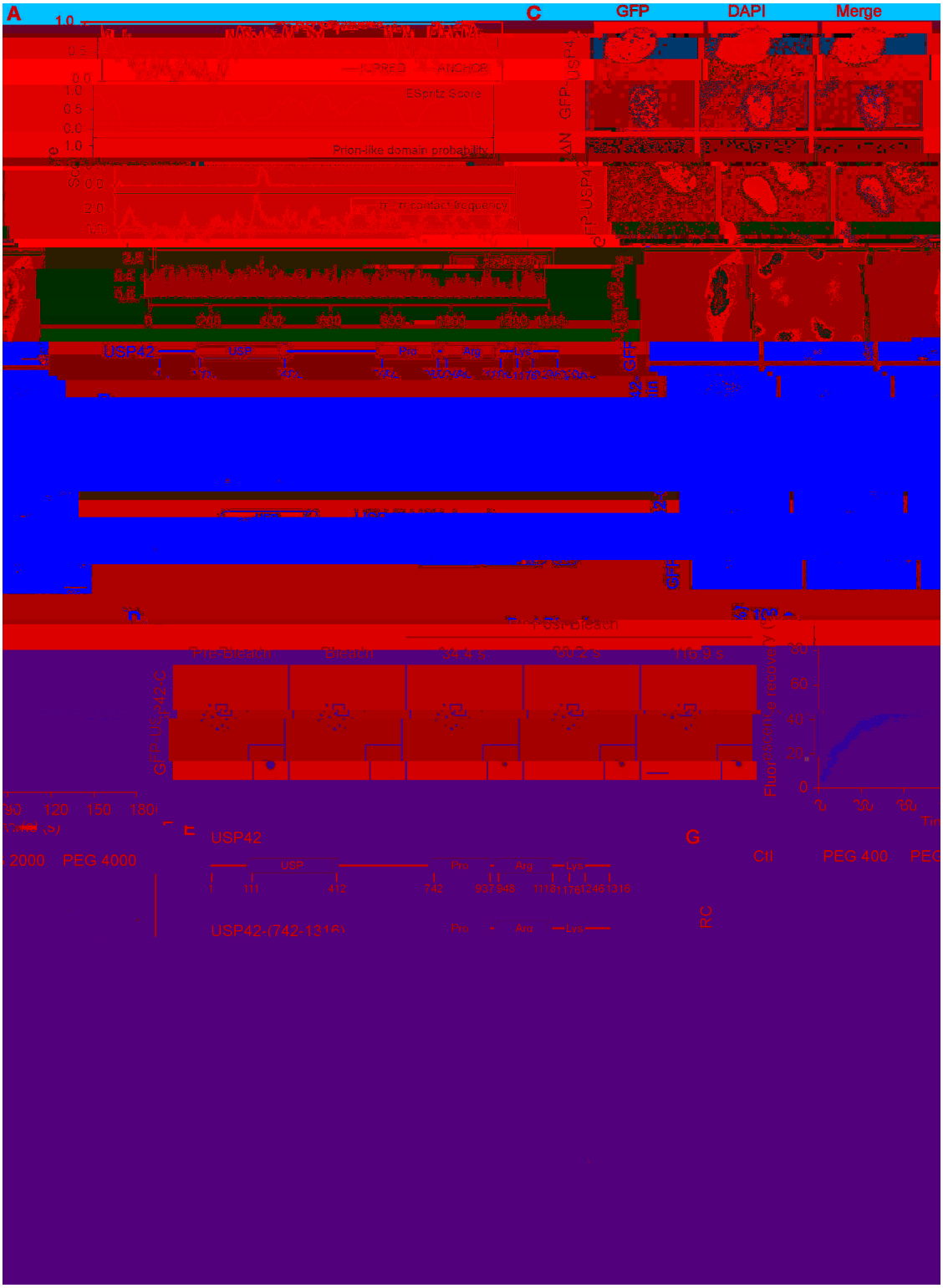


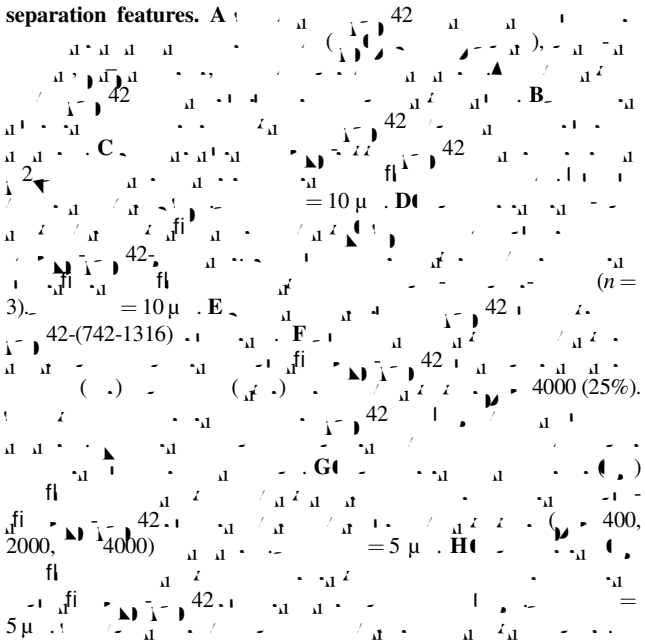
Fig. 1 Systematic survey identifies USP42 as a phase-separated DUB in the nucleus. A (3%, 7). = 10 μ . **B** 1,6- (**A**) **C** fi **D** 42 2 10- = 10 μ . **E** 2 fi 42. (*n* = 3). = 10 μ .

42 3, 36 . 2 (



35), 35- (. 3). 37, 38 . 42 35 39, 40 42 35 (. 4). 42 42

Fig. 2 USP42 C-terminal disordered region confers phase separation features. A



USP42 controls PLRG1 phase separation and integration into nuclear speckles

Fig. 3 Efficient nuclear speckle incorporation of USP42 relies on positively charged residues. A

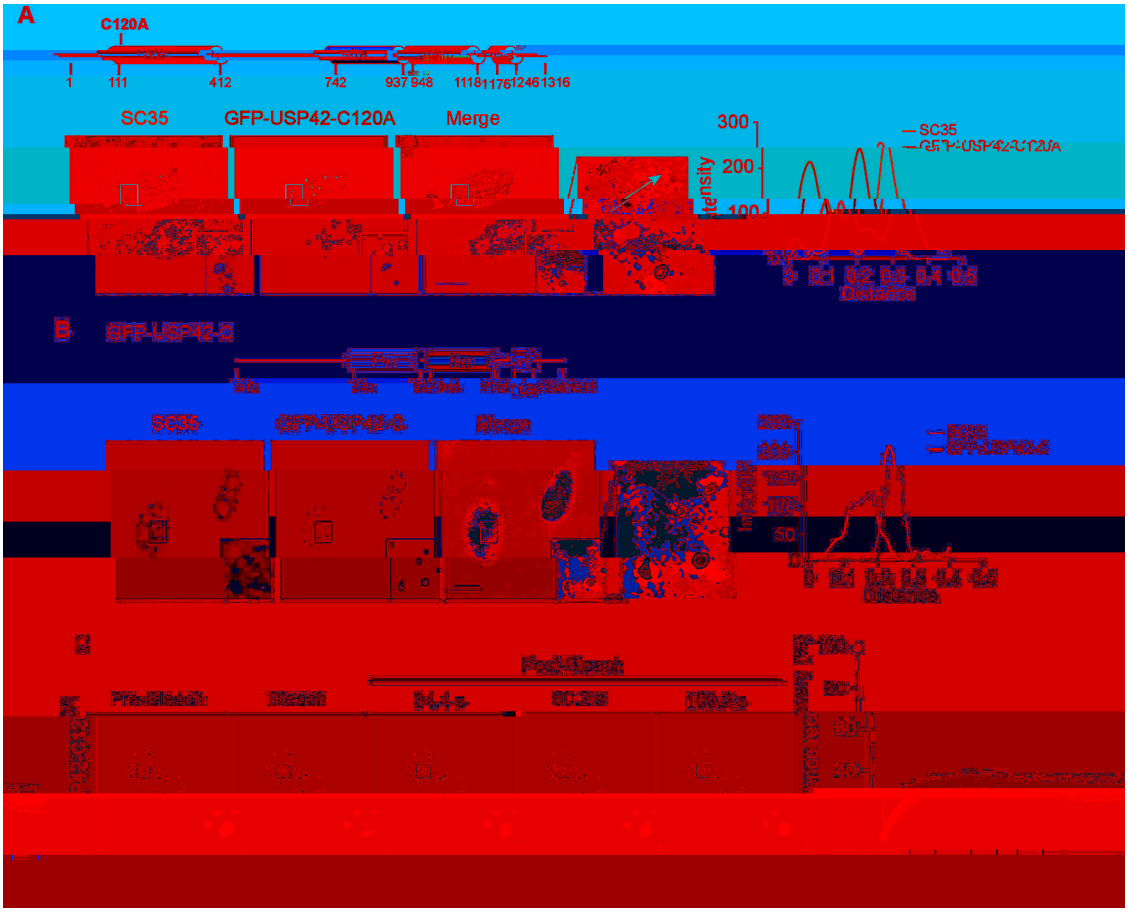


Fig. 4 USP42 DUB activity is required for its efficient nuclear speckle localization and phase separation. A, B 2 42- 120 ( ) 42- ( ) fi 35. = 10 μ . **C** 42- 120 2 fi (*n* = 3). = 10 μ .

(. 5). , fi 1 , 42 . ( ) , 1 fl (. 5). , “ ” “ ” 42 1, 3 .

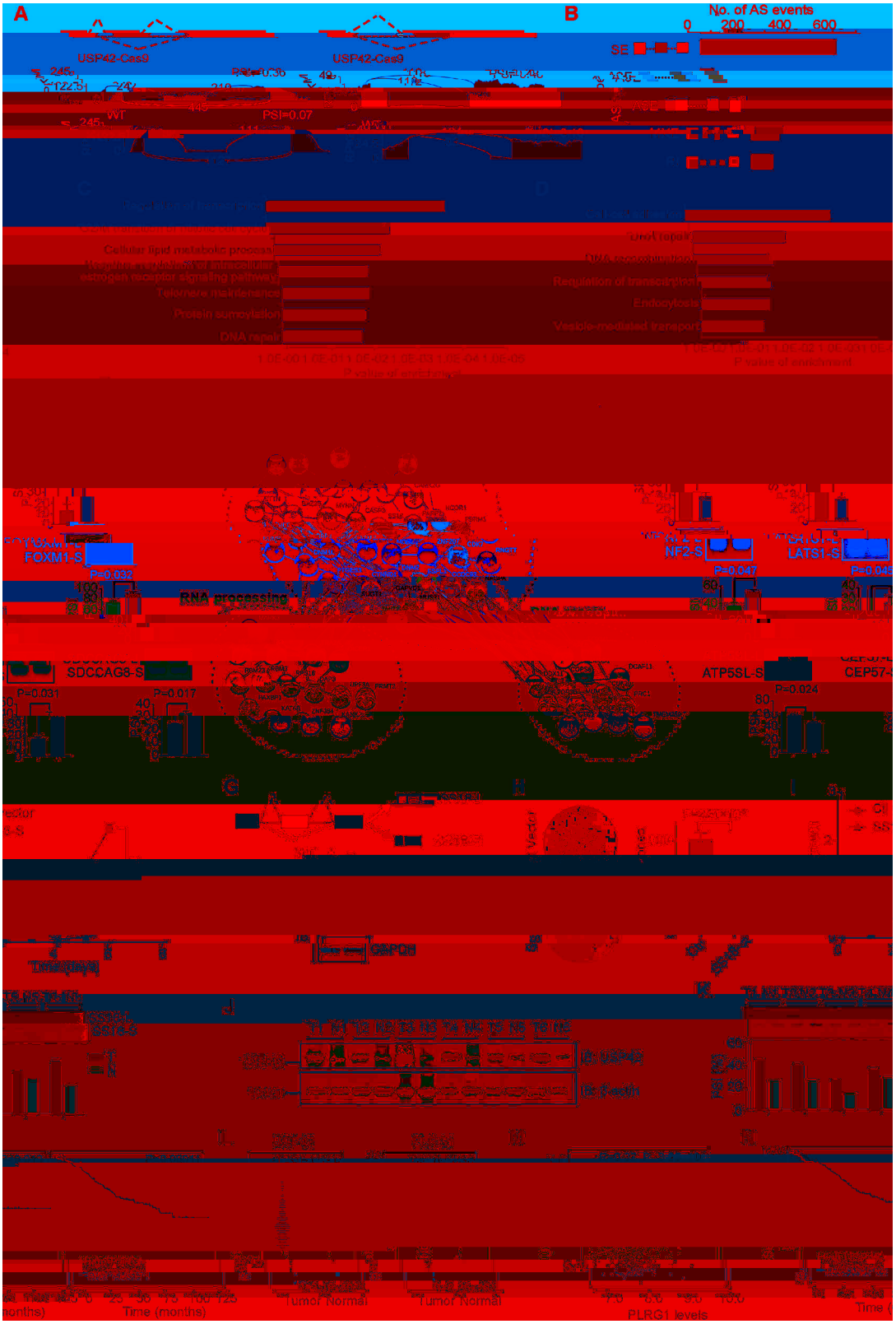
USP42 depletion hinders cell proliferation and alters nuclear speckle morphology

42 fi / 9 , 50%, (. 6). 42 53, 1299 53- fl 42 44 . 42 1 , (. 6 –). 42 fi 35 (. 6 ; . 6). 35 42- (. 6). 1 42- 1299 , 35 42 (. 6). 42 fi

Fig. 5 USP42 associates with and drives the phase separation of PLRG1. A 2 42 (120) 5 1 fl fi = 10 μ . **B** fl 42 1 2 42, (120) 1 fi = 10 μ . **C** 1 1 . **D** ( ) fl fi 1 (0.25 μ) 42 (15) 4000 (25%) (150) = 5 μ .

5). 35 42 fl fi 1 (42 fl . 5). fl (. 6 –).

USP42 influences diverse mRNA splicing events and is associated with the prognosis of non-small-cell lung cancer


A
B
No. of AS events
0
200
400
600
SE
USP42-Cas9
WT
PSI=0.07
RNA processing
FOXM1-S
NF2-S
LATS1-S
SDCCAG8-S
ATP5SL-S
P=0.031
P=0.017
P=0.024
P=0.047
P=0.032
Time (months)
Tumor
Normal
PLRG1 levels

◀ Fig. 7 USP42 modulates alternative splicing of cancer-related genes through regulating PLRG1. A 3′ 42. **B** fi 42. **C** *P* 42- **D** 42 1. *P* **E** 42- 1- **F** / 42- 9 1299 (*P* *t*-). **G** 18 42- 9 1299 42 **H** 1299 18= fi , *t* . *P* **I** 1299 18= 6 , 0. *P* *t* . **J** 42 ( ) ( ). β- **K** 18 **L** 42 1 *P* *t*- .. **M** 42 1 11969 31210 . $R = 0.37$, $P < 8.25 \times 10^{-8}$. **N** 42 1 11969 31210 ( ).

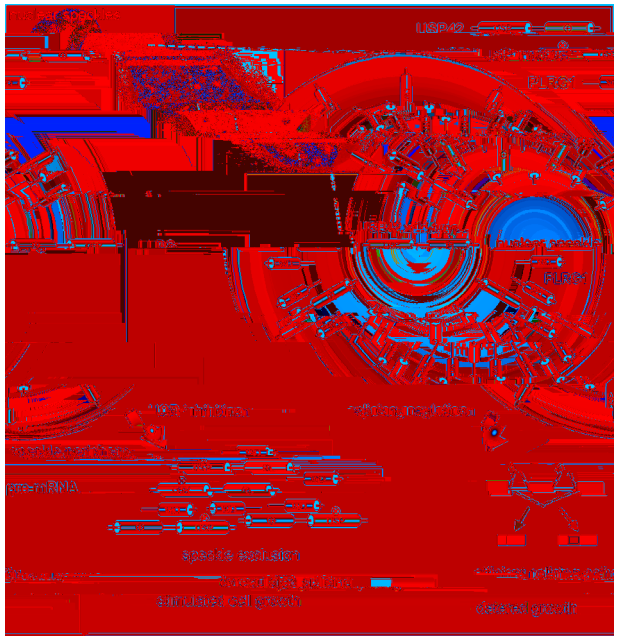


Fig. 8 A working model depicting PLRG1-mediated alternative splicing driven by USP42 phase separation. 42 42 fi 42, 42 42 1 1 fi

3, 50 . 7, 51 . fi 9, 10 . 42 42 (. 8). 42 42

42 , 42 42, 52 . 42 1

22. 9 . 2013;8:2281–308.
23. . 2019;37:1435–45.
24. 2013;93:1289–315.
25. 2014;1843:114–28.
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