nature cell biology

Supplementary information

https://doi.org/10.1038/s41556-024-01428-5

Tumour microenvironment programming by an RNA–RNA-binding protein complex creates a druggable vulnerability in IDH-wild-type glioblastoma

In the format provided by the authors and unedited

Supplementary Figure Legends

Supplementary Figure 1: Generation of candidate hits.

A) Mutation landscape of hotspot mutation in 286 glioma patients based on whole exome sequencing data from CGGA (http://www.cgga.org.cn/).

B) Volcano blot showing differential expressed genes (left panel for all genes) and lncRNAs(right panel for between IDH-wildtype group and IDH-mutant group (Cut-off criteria: p-value < 0.05 and |log2FC|≥2). The differentially expressed genes are in red (up-regulated) and in green (down-regulated), while the insignificantly changed genes are in grey. LOC105375914 was highlighted with a grey dot.

Supplementary Figure 2: Characterization of other RNAs in GBM.

A-E) qPCR analysis of **A)** *TERC*, **B)** *HOTAIR*, **C)** *MALAT1*, and **D)** *IGFBP2* expression in LN18 IDH-wildtype and IDH-mutant clones. p-values were calculated by two-tailed student's t-test method.

Supplementary Figure 3: Characterization of GBM patients using multi-omics.

A) Mutational profile of hotspot mutation in eight glioblastoma patient samples.

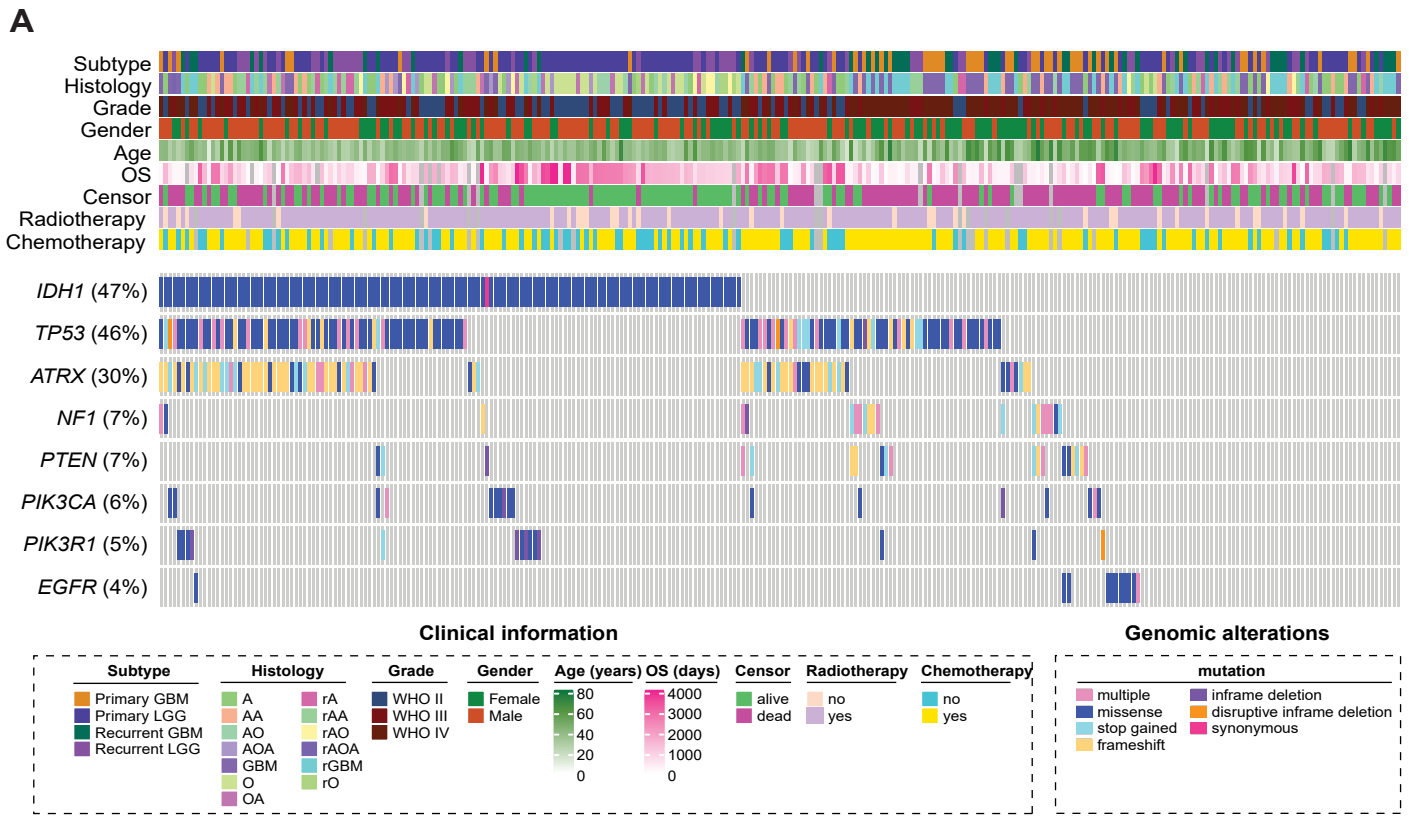
B) LOC expression level (FPKM) in 6 glioblastoma patients separated by group of low and high LOC expression.

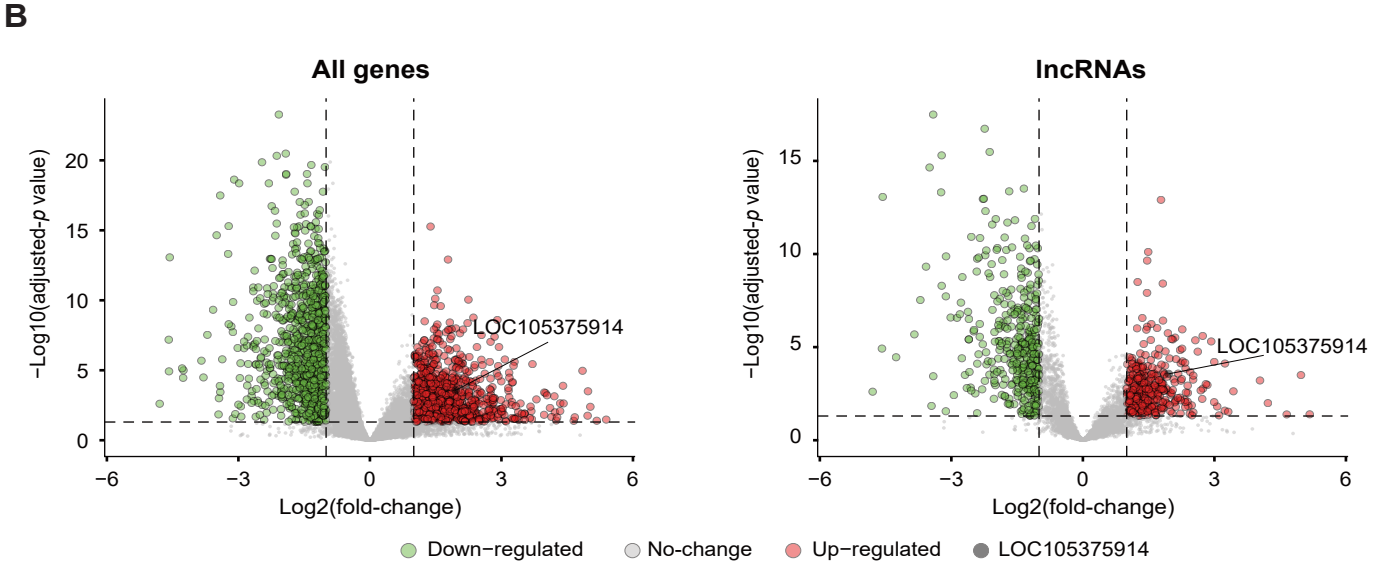
C) The CNV content of individual cells by using InferCNV. GAMs acted as reference cells. Copy numbers of chromosome7 and chromosome 10 were highlighted by red boxes.

Supplementary Figure 4: Model based on our study.

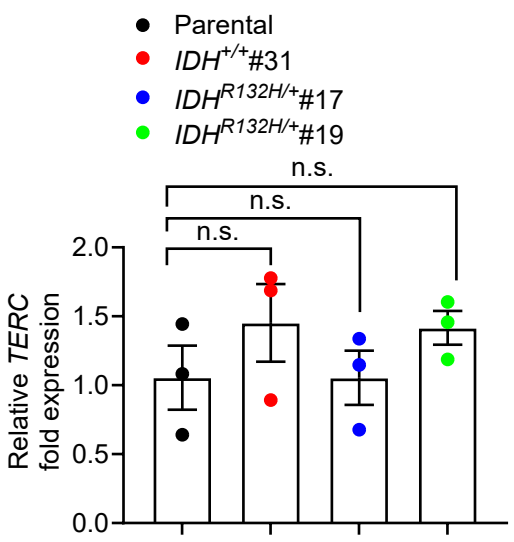
In this study, we described a novel mechanism by targeting RNA:RBP dependent GAM infiltration to impair tumor growth and improve survival in glioblastoma, IDH-wildtype. Mechanistically, in glioblastoma, IDH-wildtype, folded LOC can be unwinded by RNA helicase DHX15, unfolded LOC complexes with DHX15 ("active" LOC:DHX15 RNP) sequesters phosphatase Wip1 to activate NFκB (p65) and p38 dependent transcription factors (TFs), a rate limiting step where temporal and simultaneous activation of multiple transcription factors is required to achieve appropriate concentration of NFκB subunits to co-occupy the promoters of target gene which facilitates proper chromatin remodelling for precise spatiotemporal expression of targets like MIF1. p65 accumulation together with other TFs on the promoter region of MIF1 leads to augmented MIF1 expression and subsequent higher infiltration of GAMs in the TME via MIF1-CD74 axis. Subsequent activation of MIF1-CD74 axis also triggers GAMs derived TNFα production, which in turns boost LOC expression. In addition, some other tumor-promoting genes (TPGs) which drives oncogenesis and therapy resistance will be also activated by p65 and p38 dependent TFs to accelerate tumor formation and confer therapeutic resistance. Targeting LOC:DHX15 using small molecule inhibitor diminishes tumor growth and prolong survival by perturbing these dual forward feedback loops: 1) a cell autonomous feedback loop driven by "active" LOC:DHX15 RNP-NFκB and p38 signalling; 2) non-cell autonomous feedback loop (highlighted by red dashed box) maintained by a crosstalk between cancer cells and GAMs via MIF1:CD74-TNFα.

Sup-Fig.1

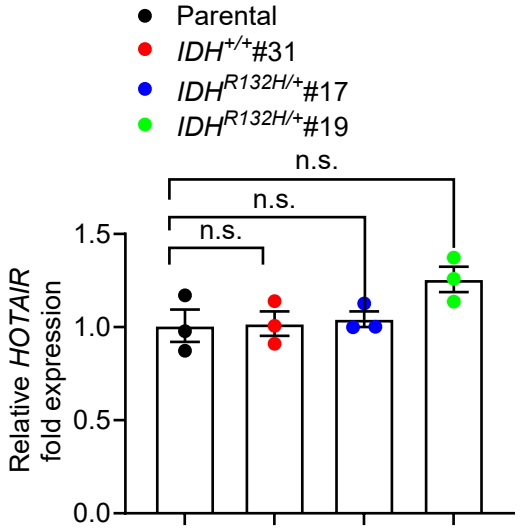

A
Subtype
Histology
Grade
Gender
Age
OS
Censor
Radiotherapy
Chemotherapy
IDH1 (47%)
TP53 (46%)
ATRX (30%)
NF1 (7%)
PTEN (7%)
PIK3CA (6%)
PIK3R1 (5%)
EGFR (4%)
Clinical information
Subtype
Primary GBM
Primary LGG
Recurrent GBM
Recurrent LGG
Histology
A
AA
AO
AOA
GBM
O
OA
rA
rAA
rAO
rAOA
rGBM
rO
Grade
WHO II
WHO III
WHO IV
Gender
Female
Male
Age (years)
80
60
40
20
0
OS (days)
4000
3000
2000
1000
0
Censor
alive
dead
Radiotherapy
no
yes
Chemotherapy
no
yes
Genomic alterations
mutation
multiple
missense
stop gained
frameshift
inframe deletion
disruptive inframe deletion
synonymous


B
All genes
lncRNAs
−Log10(adjusted-p value)
Log2(fold-change)
LOC105375914
Down−regulated
No-change
Up−regulated
LOC105375914

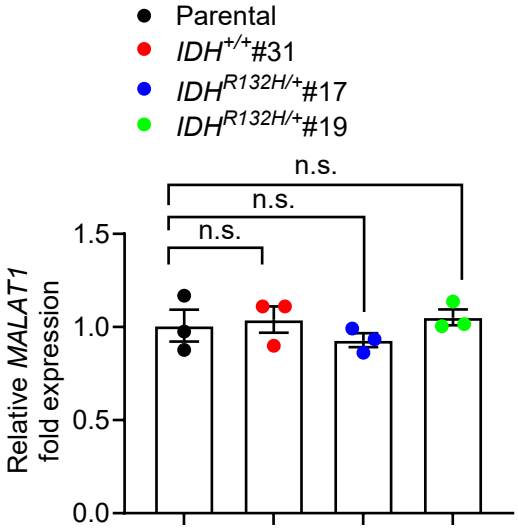
A


Parental
IDH+/+#31
IDHR132H/+#17
IDHR132H/+#19
n.s.
n.s.
n.s.
Relative TERC fold expression
2.0
1.5
1.0
0.5
0.0

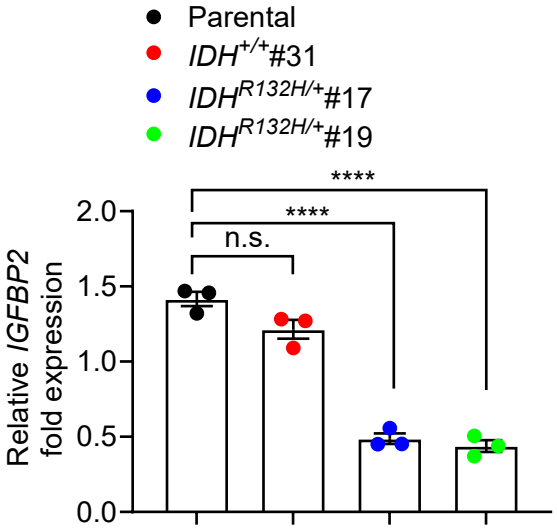
B

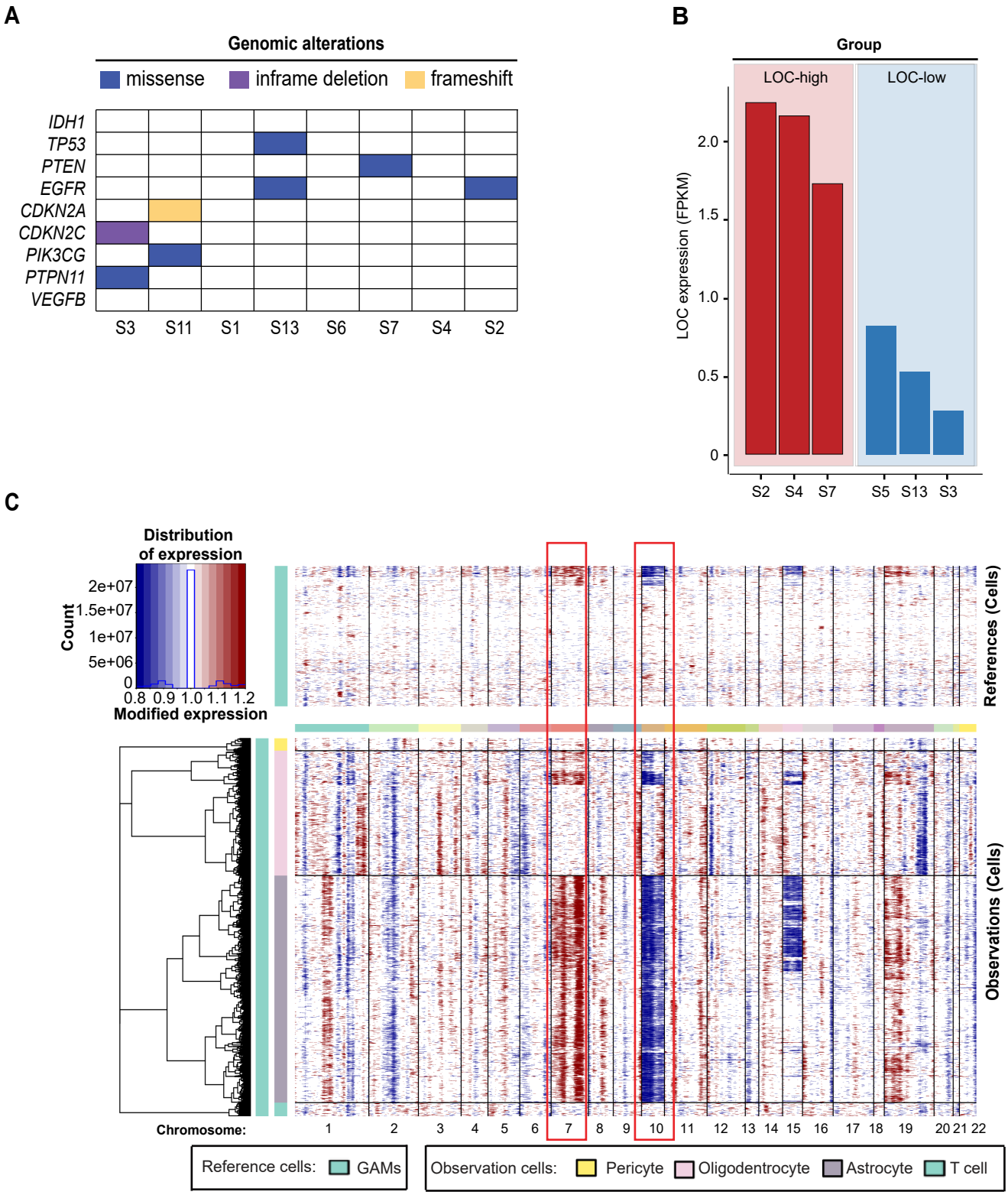

Parental
IDH+/+#31
IDHR132H/+#17
IDHR132H/+#19
n.s.
n.s.
n.s.
Relative HOTAIR fold expression
1.5
1.0
0.5
0.0

C

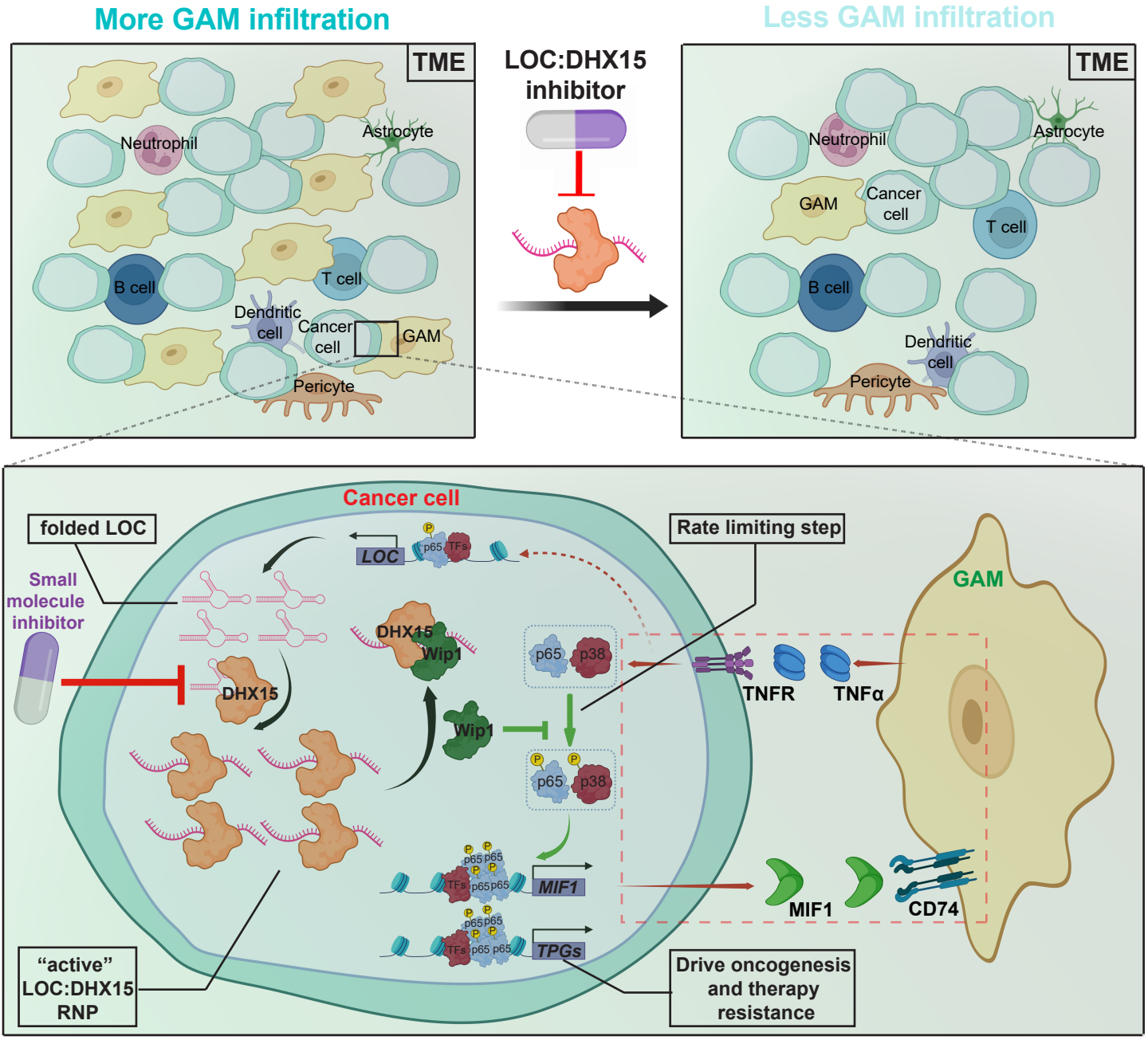

Parental
IDH+/+#31
IDHR132H/+#17
IDHR132H/+#19
n.s.
n.s.
n.s.
Relative MALAT1 fold expression
1.5
1.0
0.5
0.0

D


Parental
IDH+/+#31
IDHR132H/+#17
IDHR132H/+#19
****
****
n.s.
Relative IGFBP2 fold expression
2.0
1.5
1.0
0.5
0.0


A
Genomic alterations
missense
inframe deletion
frameshift
IDH1
TP53
PTEN
EGFR
CDKN2A
CDKN2C
PIK3CG
PTPN11
VEGFB
S3
S11
S1
S13
S6
S7
S4
S2
B
Group
LOC-high
LOC-low
LOC expression (FPKM)
2.0
1.5
1.0
0.5
0
S2
S4
S7
S5
S13
S3
C
Distribution of expression
Count
2e+07
1.5e+07
1e+07
5e+06
0
0.8 0.9 1.0 1.1 1.2
Modified expression
References (Cells)
Observations (Cells)
Chromosome:
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Reference cells: GAMs
Observation cells: Pericyte Oligodentrocyte Astrocyte T cell

Sup-Fig.4


More GAM infiltration
Less GAM infiltration
TME
Neutrophil
Astrocyte
B cell
T cell
Dendritic cell
Cancer cell
GAM
Pericyte
LOC:DHX15 inhibitor
TME
Neutrophil
Astrocyte
GAM
Cancer cell
T cell
B cell
Dendritic cell
Pericyte
Cancer cell
folded LOC
Small molecule inhibitor
DHX15
LOC
p65
TFs
DHX15
Wip1
Wip1
p65
p38
p65
p38
MIF1
TPGs
Rate limiting step
TNFR
TNFα
GAM
MIF1
CD74
"active" LOC:DHX15 RNP
Drive oncogenesis and therapy resistance