The integrator complex RNA hydrolase INTS11 is a key dependency and therapeutic target in 1p36-deleted cancers

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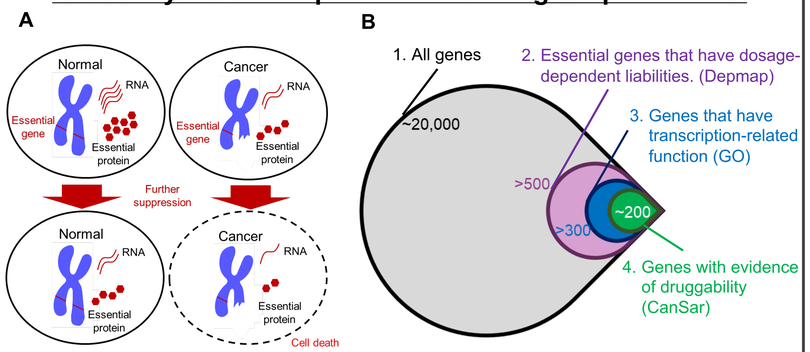
Abstract

- **There is a critical need to discover cancer drivers and dependencies as new drug targets. Synthetic lethality in cancer cells may be generated through single copy loss of an essential cellular regulator, creating a unique dosage dependency on the remaining target allele.**
- **We report that:**
  - **We Identified ~200 druggable, dosage-dependent transcriptional liabilities across several cancers that include key regulators of transcriptional initiation, elongation, splicing, and 3'-end processing.**
  - **The Integrator complex subunit INTS11 is a deep dependency in cancers with INTS11 copy number loss and low INTS11 expression.**
  - ***INTS11* copy-number loss frequently occurs as part of a 1p36-deletion, indicating that INTS11 might be a unique dependency in cancers that exhibit heterozygous deletion of this region (Δ1p36).**
  - **We validated INTS11 as a dosage-dependent cancer liability in Δ1p36 malignant glioma cells, highlighting this RNA hydrolase as a potential drug target for malignant glioma.**
  - **We screened a 69 compound metallo-β-lactamase-domain focused library for small molecules inhibiting INTS11 function and identified several compounds that inhibited INTS11-associated 3'-end processing.**

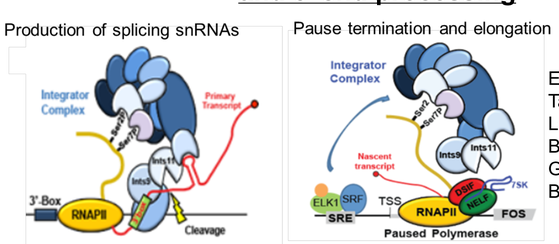
Identification of INTS11 as a dosage dependent cancer liability

Discovery of transcription-linked dosage dependencies



A) Model for genetic establishment of a cancer dosage dependency. Copy-number-linked dosage dependencies are otherwise termed CYCLOPS genes (Najhawan *et al.,* 2012). **B)** Discovery path to identify druggable transcriptional regulators that are dosage-dependent liabilities in cancer. Dosage dependency defined by shRNA vs expression.

Focus on INTS11: A Key regulator of pause termination and 3' end processing

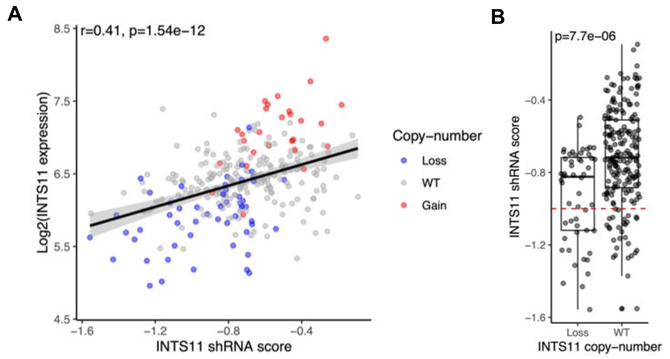


Elrod et al., 2019; Tatomer et al., 2019; Lai et al., 2015 Baillat and Wagner 2015; Gardini et al., 2014 Baillat et al., 2005

Model for integrator function in transcription. Integrator complex regulates the 3' end processing of splicing-associated snRNAs and eRNAs via metallo-β-lactamase-domain mediated cleavage by INTS11. RNA cleavage by INTS11 also cleaves promoter-proximal nascent RNAs to drive termination of paused Pol II.

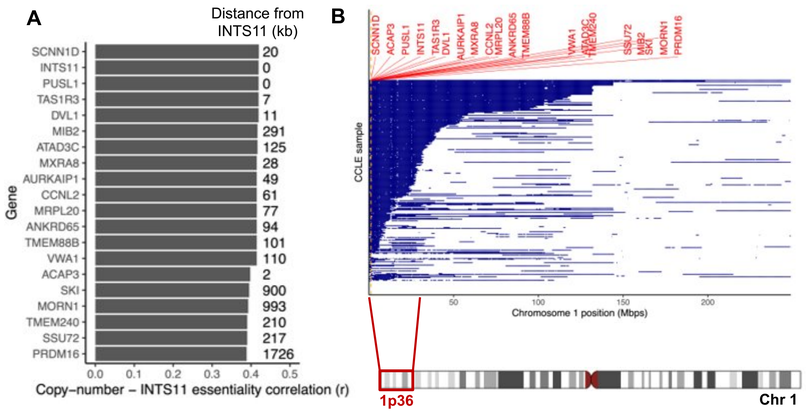
INTS11 copy-number loss frequently occurs as part of a 1p36-deletion

INTS11 is a dependency in cancer cell lines with low *INTS11* expression and *INTS11* copy-number loss



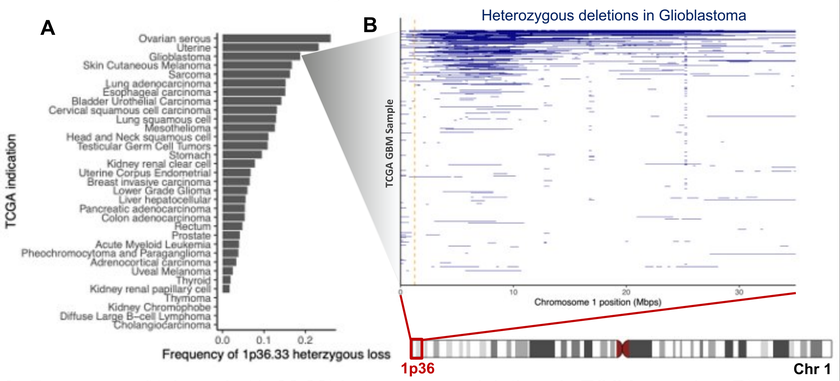
A) Sensitivity to *INTS11* knock-down (KD, as measured by DEMETER2 score via DepMap) is significantly correlated with *INTS11* expression level in CCLE cancer cell lines. **B)** CCLE cancer cell lines with *INTS11* copy-number loss are more sensitive to *INTS11* KD.

Genes whose copy-number loss is associated with sensitivity to INTS11 KD localize to 1p36 locus



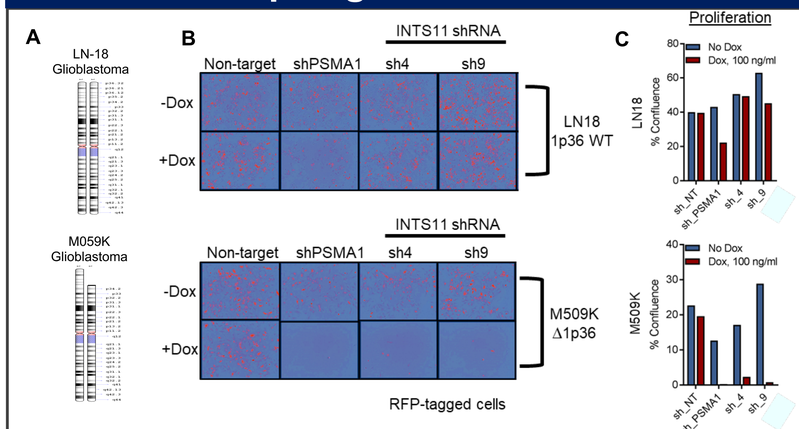
A) Top 20 genes whose copy-number loss is associated with *INTS11* shRNA KD (DEMETER2 score, DepMap) are located near the *INTS11* genomic locus. **B)** Heterozygous deletions (blue) on chromosome 1 in CCLE cell lines with *INTS11* deletions. *INTS11* locus is annotated with an orange dashed line.

INTS11 copy-number loss via 1p36 deletion occurs frequently in many human cancers



A) Frequency of telomeric (1p36.33) heterozygous deletions in TCGA samples. **B)** Heterozygous deletions (blue) on the telomeric end of chromosome 1p in TCGA glioblastoma samples. *INTS11* locus is annotated with an orange dashed line.

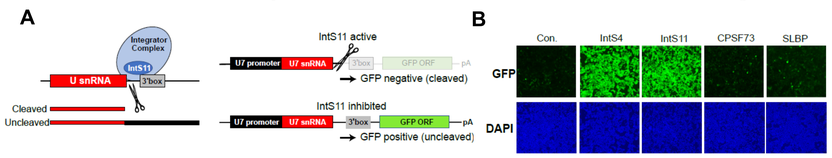
Validation of INTS11 as a dependency in Δ1p36 glioblastoma cells



Validation of INTS11 as a dependency in malignant glioma cell lines with 1p36-deletion. **A)** Δ1p36 deletion in M059K cells. **B)** RFP-signal detection in cells containing Dox-inducible shRNA for Non-targeting control, PSMA1 positive control, or shINTS11#4 or shINTS11#9. **C)** Quantification of cell confluence after 14 days Dox treatment.

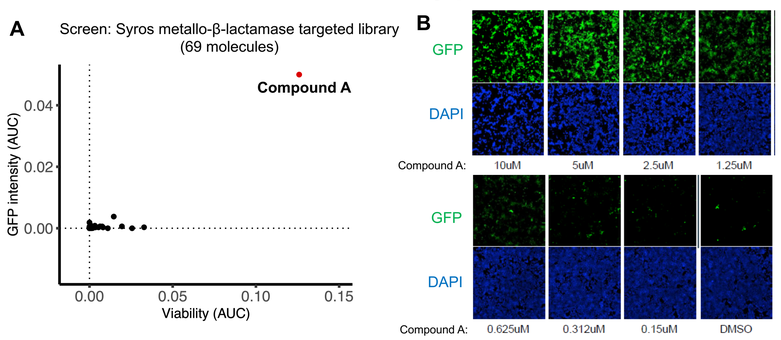
Cell-based assay to discover small molecule inhibitors of INTS11

A cell-based reporter screen to inform INTS11 activity



A) Schematic of a gain-of-function GFP-linked INTS11 reporter assay in HeLa cells. Active INTS11 facilitates cotranscriptional cleavage of the U7 snRNA 3' box. Upon inhibition of INTS11 function, readthrough and expression of GFP occurs. **B)** siRNA against INTS4 or INTS11 results in inhibition of INTS11 function and GFP signal gain.

Small molecule screen to identify putative INTS11 inhibitors



A) Summary GFP-signal data vs viability data for initial Syros metallo-β-lactamase (MBL) domain-targeted screen (69 molecules). **B)** Cell growth and GFP signal in response to Compound A at 72 hours post-treatment.

Conclusions

- **We have identified general transcriptional regulators that may be attractive drug targets in genetically defined tumors/patient populations.**
- **We identified INTS11 as a key druggable liability in malignant glioma with 1p36 deletions.**
- **We have identified a putative small molecule inhibitor of INTS11 for further study in malignant glioma and other cancers.**

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