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The Science CRO

High throughput FISH screening identifies small molecules that modulate oncogenic lncRNA MALAT1 via GSK3B and hnRNPs

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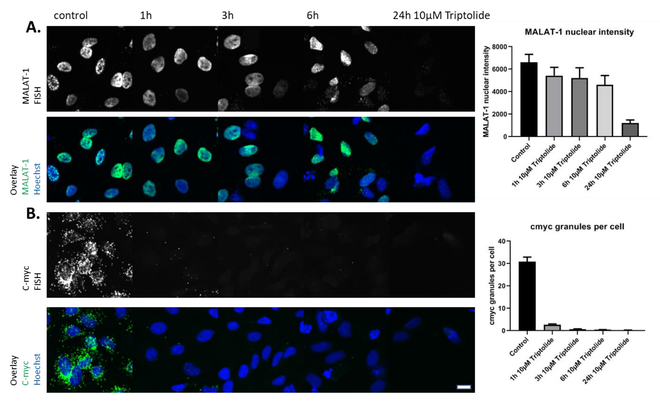
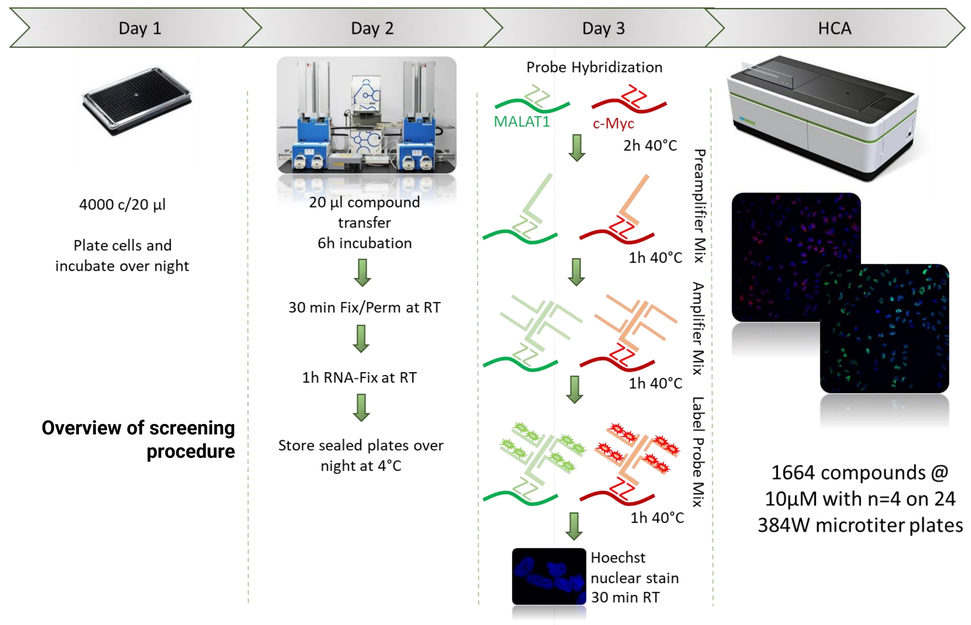
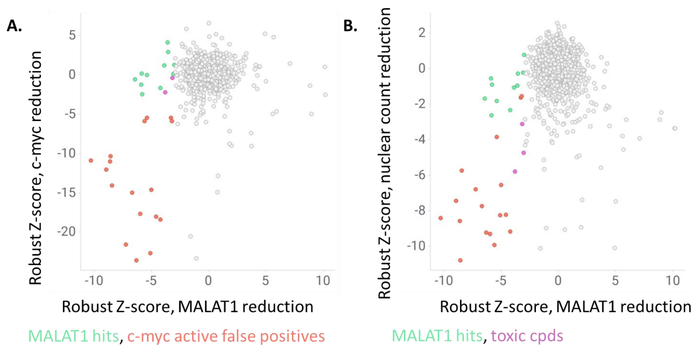
Setup of first reported HT-FISH for the identification of small molecules that modulate lncRNAs



A: Hela stained for MALAT1 lncRNA by fluorescence in-situ hybridization after treatment with transcription inhibitor Triptolide for various times. Nuclei are stained by Hoechst. B: Hela stained for MALAT1 lncRNA by fluorescence in-situ hybridization after treatment with transcription inhibitor Triptolide for various times. Nuclei are stained by Hoechst. Quantification of nuclear MALAT1 staining or c-myc granules per cell shown on the right. Bars show mean with SD. Scale bar ~10µm.



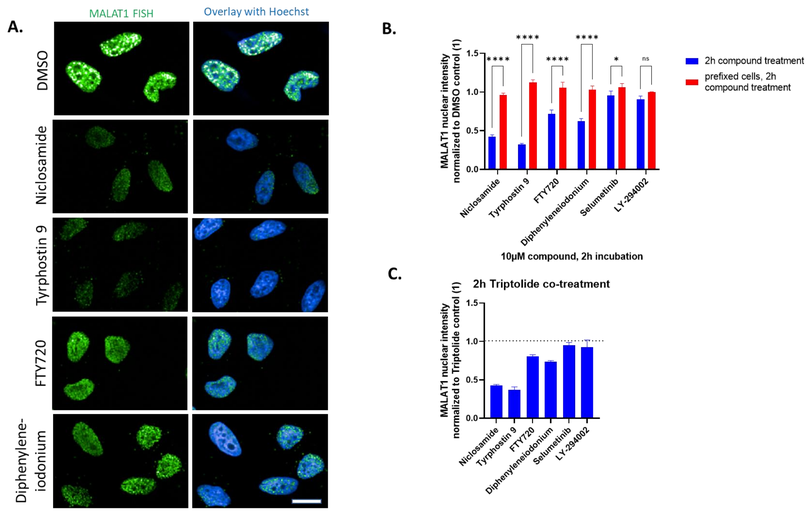
High-content based hit calling



Hits in green, false positives and toxic compounds identifed by additional readouts (A. reduction of c-myc mRNA and B. decrease in nuclear counts (e.g. toxicity)

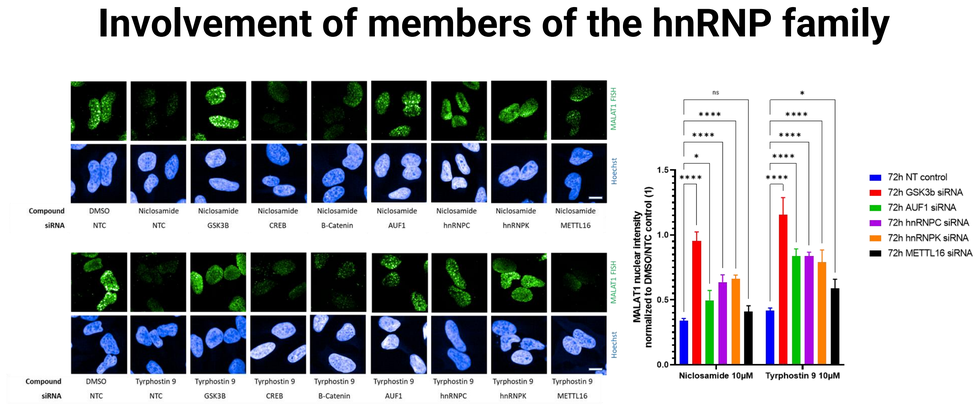
Mode of action studies show involvement of GSK3B and hnRNPs

Niclosamide and Tyrphostin reduce nuclear MALAT1 levels



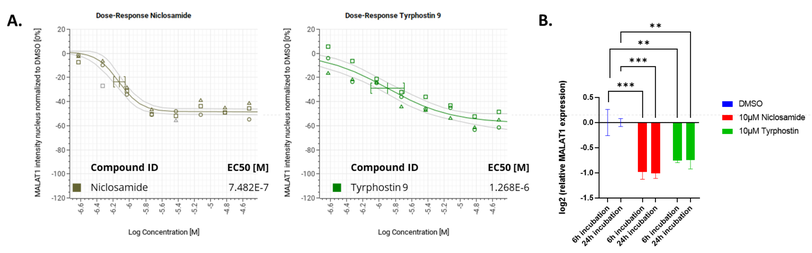
(A): Hela stained for MALAT1 lncRNA by fluorescence in-situ hybridization after treatment with DMSO control or HTS hits at 10 µM and 2 h incubation time. Nuclei are stained by Hoechst. Scale bar ~10 µm. (B): Quantification of nuclear MALAT1 staining compared with pre-fixed cells after 2 h compound addition. Highly significant effects compared to each pre-fixed control are detected for four of the compounds. Bars show mean with SD. **** p value < 0.0001; * p value < 0.1; NS = not significant. (C): Quantification of nuclear MALAT1 staining after co-treatment with 10 µM of the transcription inhibitor Triptolide and 10 µM of HTS hits. Data normalized to Triptolide/DMSO control (1). Bars show mean with SD.

Involvement of members of the hnRNP family



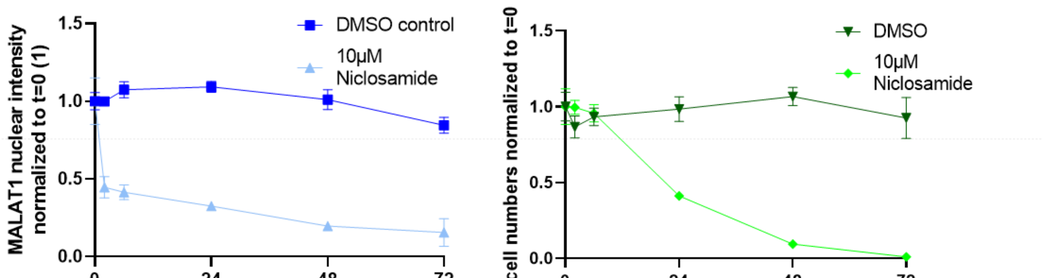
Hela stained for MALAT1 lncRNA by fluorescence in-situ hybridization after 72 h siRNA against the indicated target followed by 2 h treatment with DMSO control or HTS hits at 10 µM. Nuclei are stained by Hoechst. Scale bar ~10 µm. Quantification of nuclear MALAT1 staining after siRNA and compound treatment. GSK3B as well as to a lower extent hnRNPC and hnRNPK knockdown significantly prevent compound induced reduction of nuclear MALAT1 staining intensity. Bars show mean with SD. **** p value < 0.0001; * p value < 0.1; NS = not significant.

Hit characterization and validation in orthogonal assay



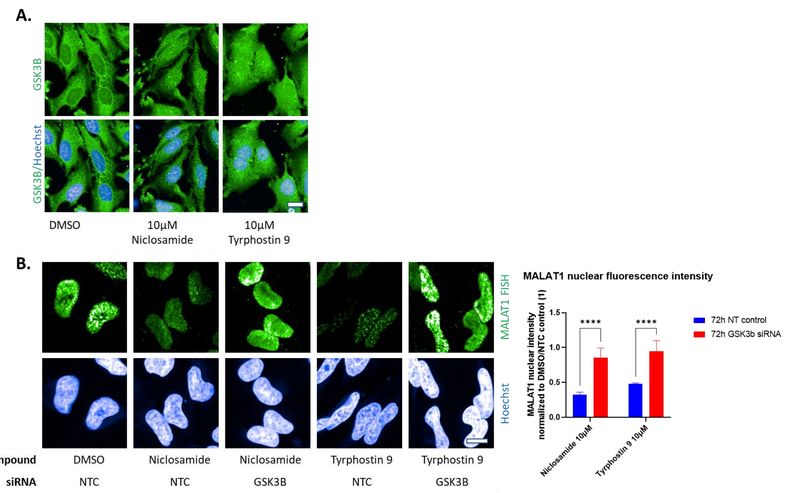
(A): EC50 determination of Niclosamide and Tyrphostin 9 for nuclear MALAT1 staining reduction after 2h incubation. Data normalized to DMSO control (0). (B): MALAT1 RNA expression levels measured by qRT-PCR, relative to DMSO control. GAPDH was used as normalization control. Mean +/− SEM (n = 6). *** p value < 0.001; ** p value < 0.01.

Effects on cancer cell proliferation



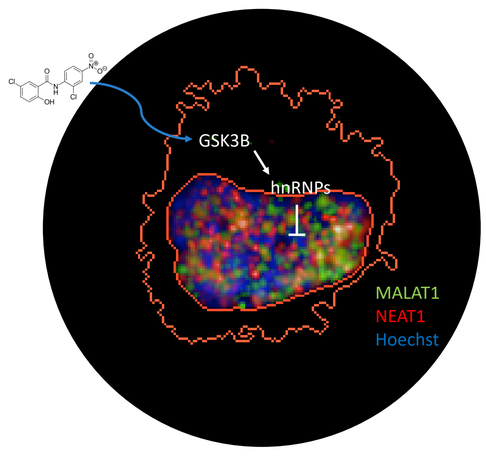
(A): Time course of MALAT1 nuclear intensity decrease and (B). effect on nuclear numbers. Hela cells were treated with DMSO or 10 µM Niclosamide and MALAT1 was stained by FISH. Nuclear MALAT1 levels and number of nuclei per well were determined. Mean of n = 4 per condition and timepoint with SD as error bars.

GSK3B shows relocalization to the nucleus after hit compound treatment and compound induced MALAT reduction depends on GSK3B



(A): Niclosamide and Tyrphostin 9 lead to nuclear translocation of GSK3B. Example pictures (6 h incubation) and quantification of GSK3B nuclear to cytoplasmic levels. Bars show mean with SD. **** p value < 0.0001, nuclei are stained by Hoechst, IF against GSK3B. One results from two independent experiments with similar outcomes shown. (B): Hela stained for MALAT1 lncRNA by fluorescence in-situ hybridization after 72 h siRNA against the indicated target followed by 2 h treatment with DMSO control or HTS hits at 10 µM. Nuclei are stained by Hoechst. Quantification of nuclear MALAT1 staining after siRNA and compound treatment. GSK3B knockdown significantly prevents compound induced reduction of nuclear MALAT1 staining intensity. Bars show mean with SD. **** p value < 0.0001. Scale bars ~10 µm.

Graphical abstract



- Niclosamid leads to nuclear translocation of GSK3B
- GSK3B potentially interacts with members of the hnRNP family of proteins to
- Modulate nuclear levels of the oncogenic lncRNA MALAT1

Identified compounds lead to GSK3B activation and modulation of MALAT1 and NEAT1 lncRNA levels in the nucleus involving hnRNPs