Dana-Farber Cancer Institute

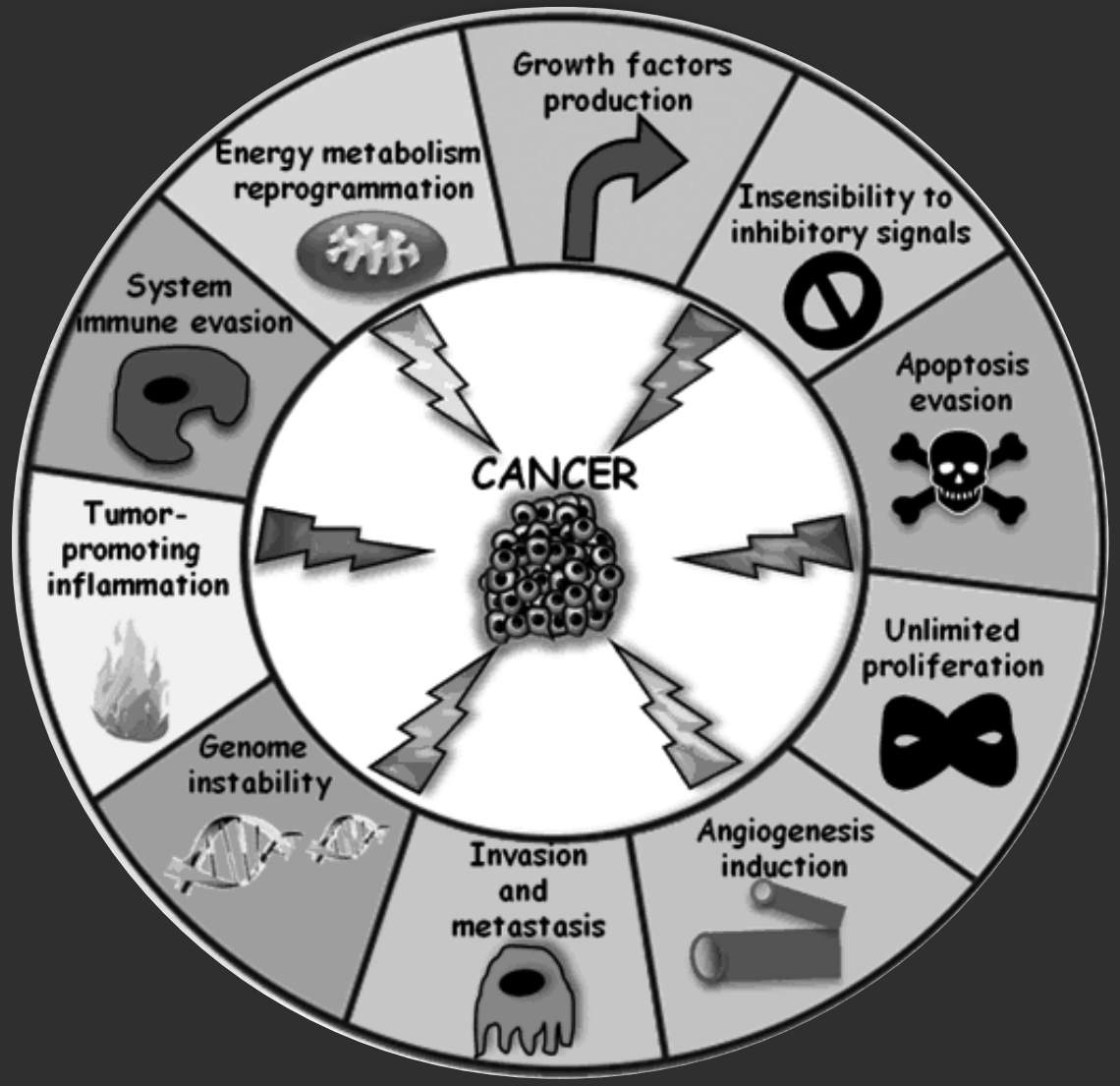
“Genome-Wide CRISPR *interference* Screen Identifies RNA Regulator of Lipogenesis (RROL) as a Leading LncRNA Dependency in Multiple Myeloma”

18th International Myeloma Workshop

Eugenio Morelli, M.D.

Munshi Lab

LncRNAs: the Dark that Matters



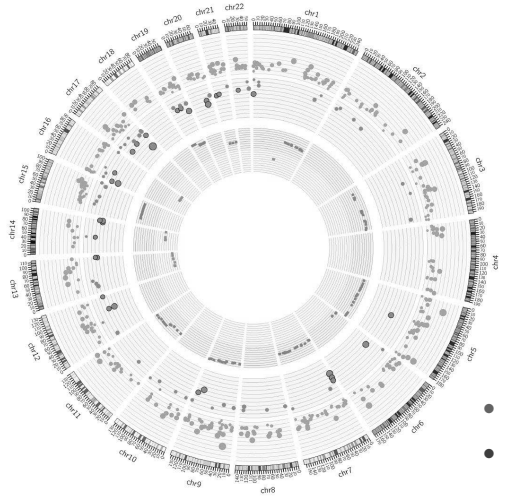
Disclosures

- *I do not have conflicts of interest.*

LncRNAs & Myeloma: a new source of genetic vulnerabilities?

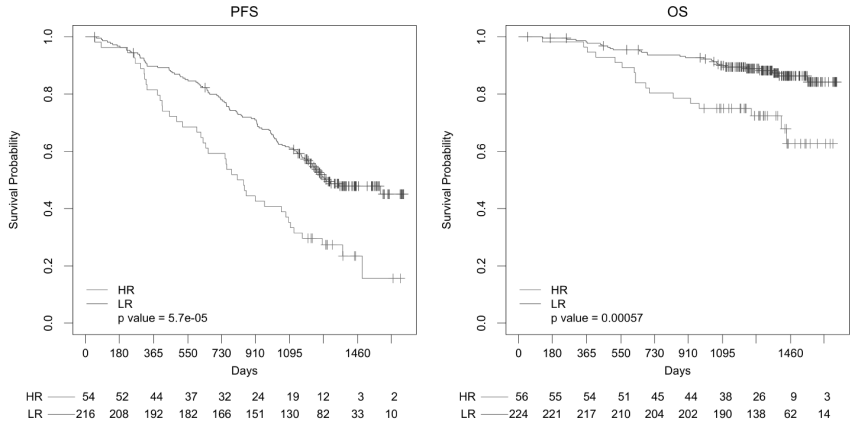
Rationale of the study

LncRNA are dysregulated in MM



- Up (n=474)
- Dn (n=395)

LncRNA signatures can predict the clinical outcome of MM patients



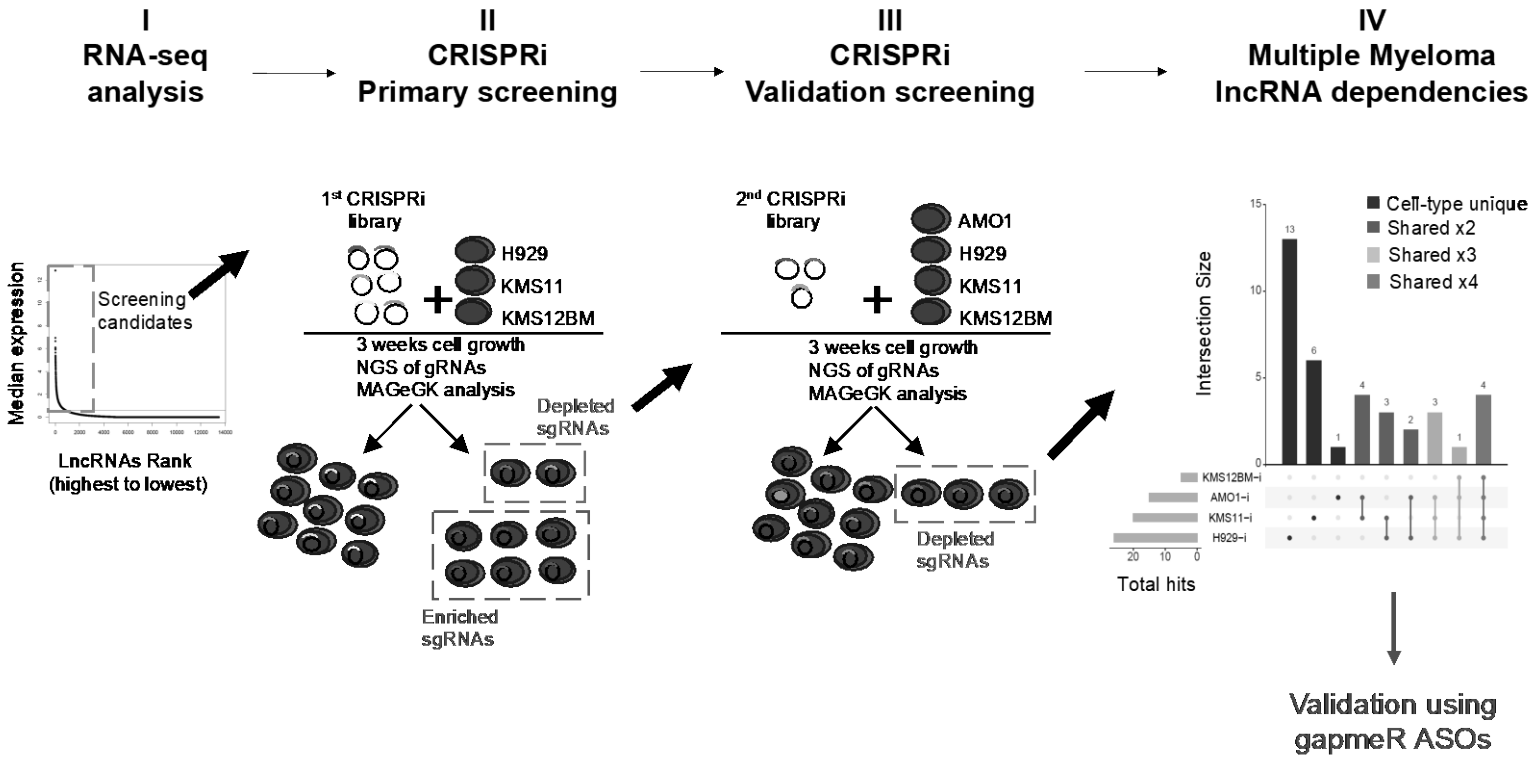
Samur M, Leukemia, 2018

To explore LncRNA Dependency in Multiple Myeloma

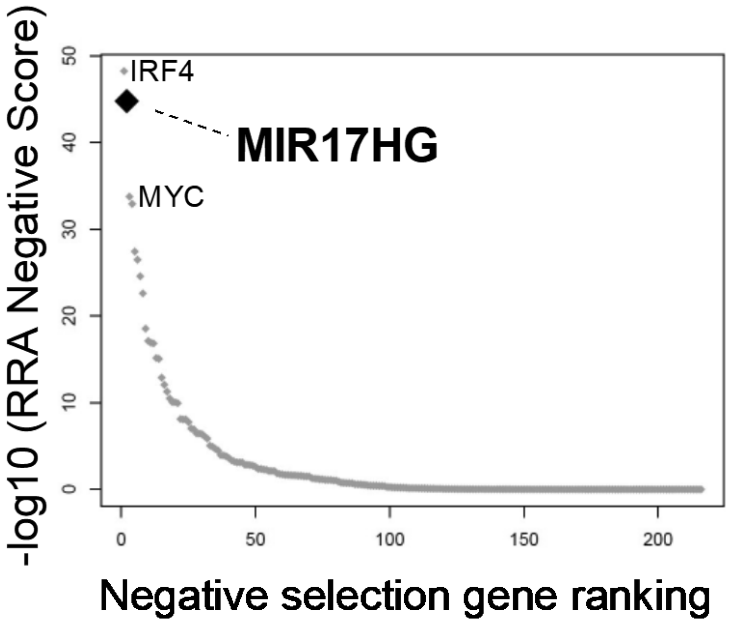
Systematic mapping of lncRNA dependencies → Molecular mechanisms & Regulatory functions → Therapeutic targeting

CRISPR *interference* (CRISPRi) viability screen identifies MIR17HG as a leading lncRNA dependency in MM

Screening Approach

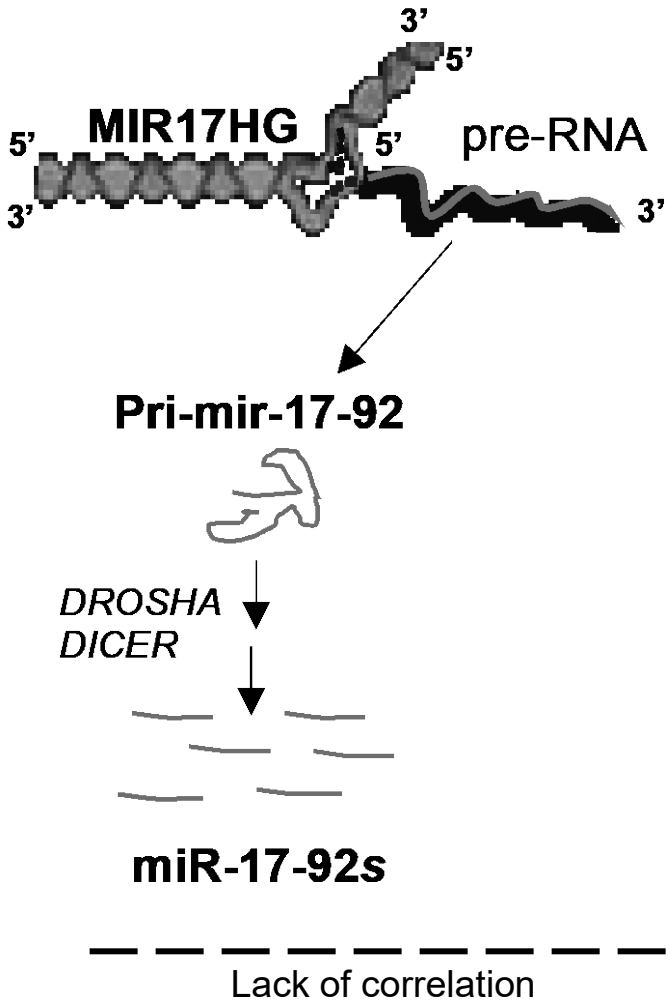


Ranked Analysis

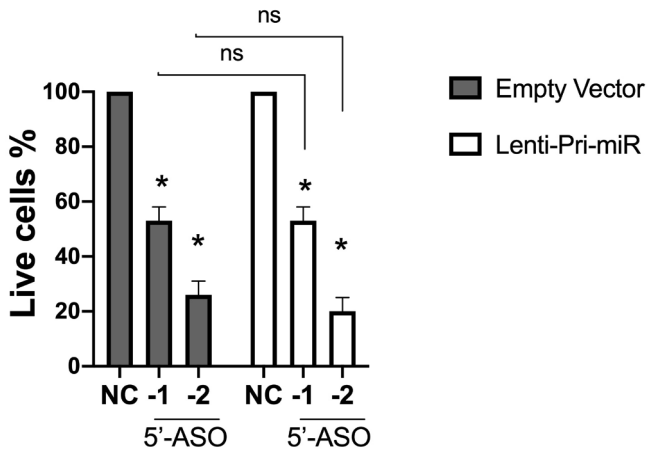


To Characterize MIR17HG Dependency in MM

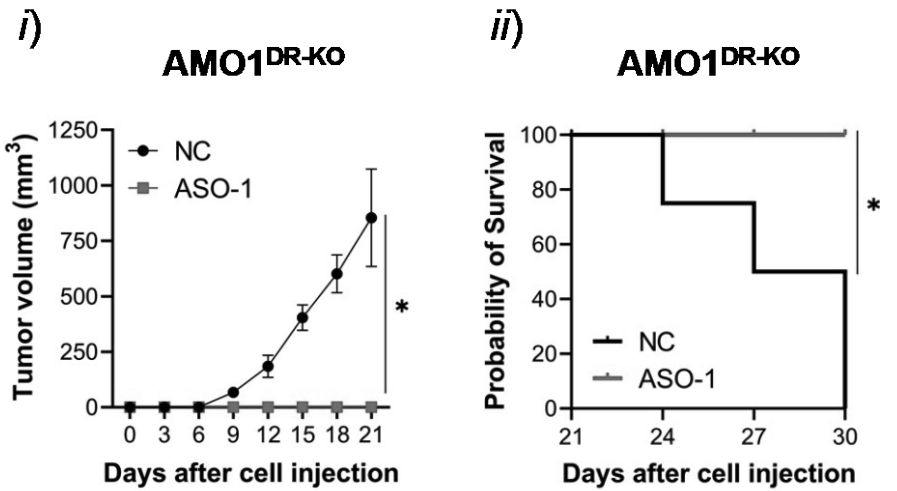
RNA Regulator of Lipogenesis (RROL) mediates MM dependency to MIR17HG



1. RROL depletion + Overexpression of pri-mir-17-92 = **NO RESCUE**

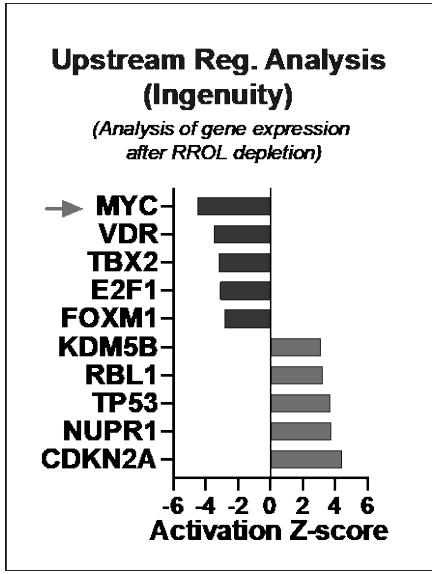


2. RROL depletion + Knockout of DROSHA = **NO RESCUE**



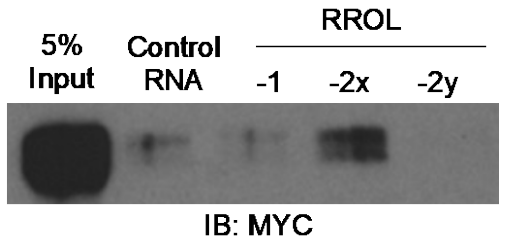
RROL promotes MYC transcriptional activity via direct RNA-protein interaction

RROL affects MYC transcriptional activity

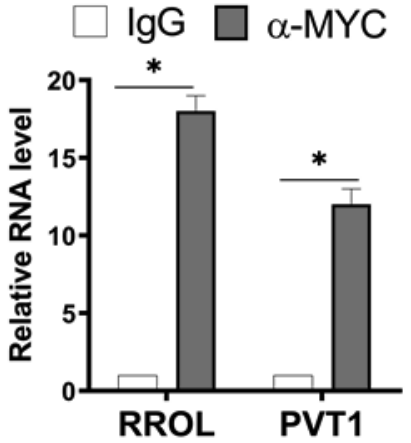


MIR17HG cooperates with MYC in tumorigenesis…
…via RROL?

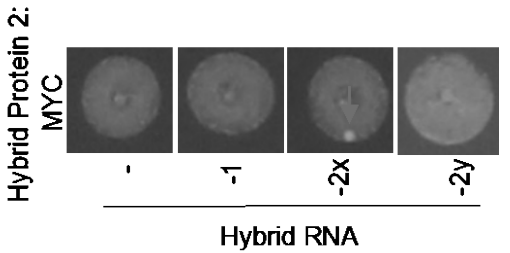
RNA Protein Pull-down

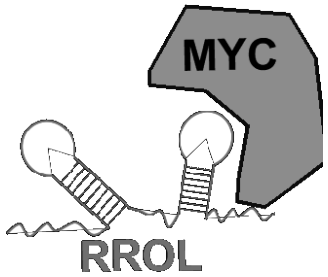


RNA Immunoprecipitation

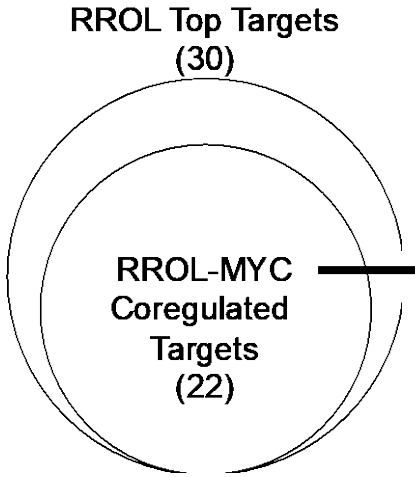


RNA Yeast-3-Hybrid



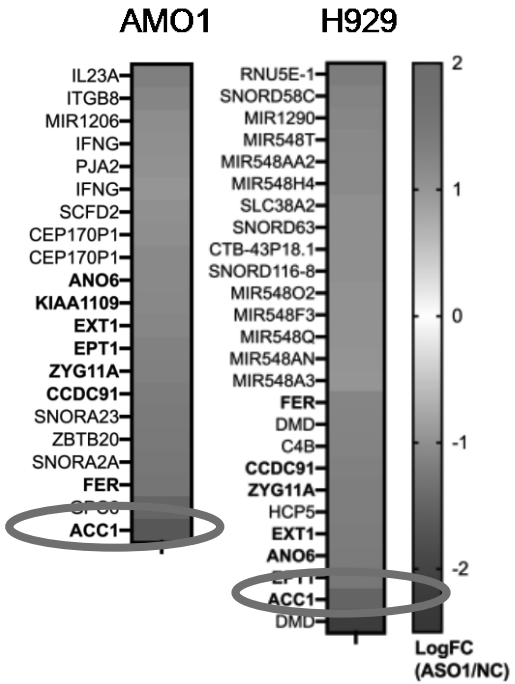


RROL provides chromatin scaffold for MYC occupancy at the ACC1 promoter

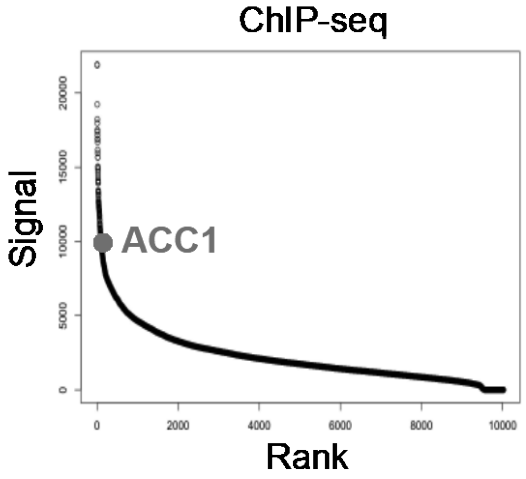


FOCUS on ACC1

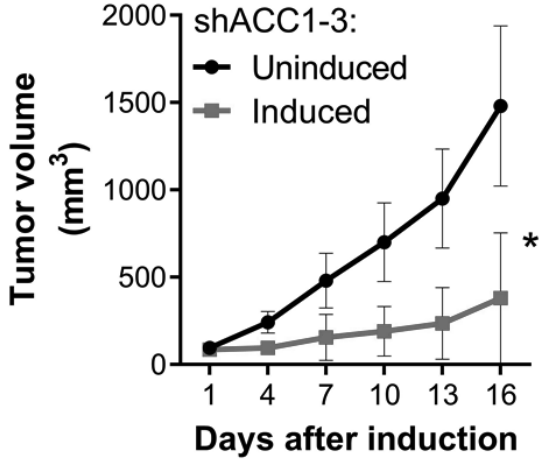
Top Target of RROL



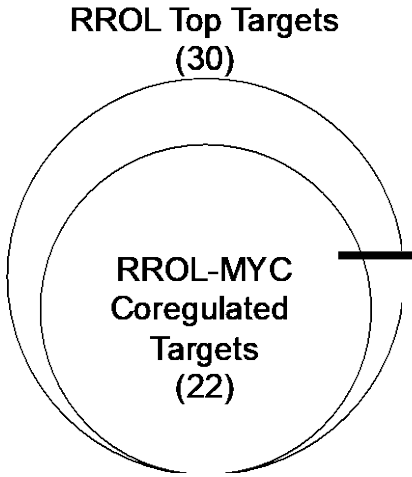
Top Target of MYC



Vulnerability in MM

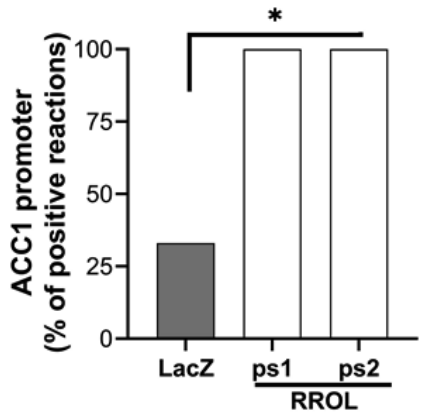


RROL provides chromatin scaffold for MYC occupancy at the ACC1 promoter

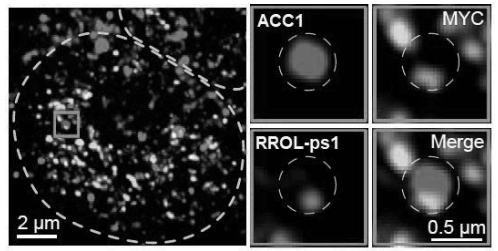


FOCUS on ACC1

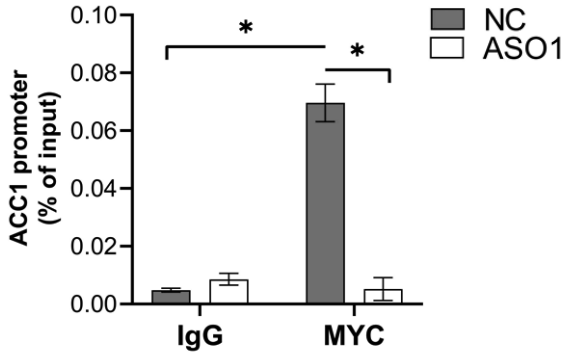
Chromatin Isolation by RNA Purification (ChIRP)

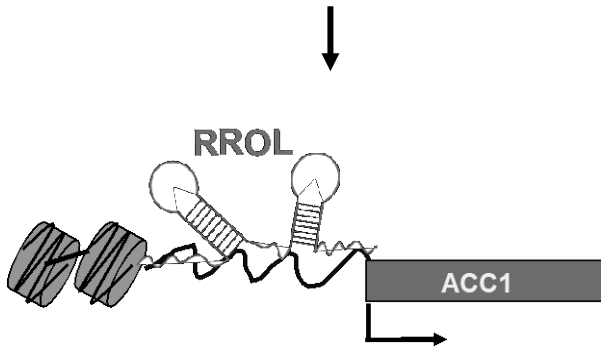


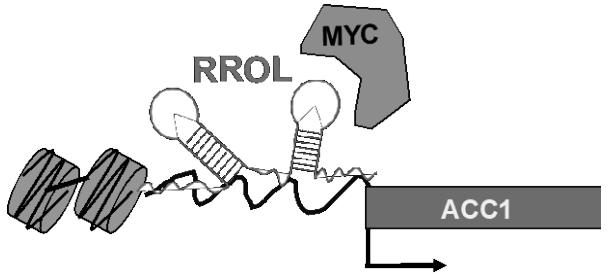
RNA FISH / IF



MYC ChIP-qPCR w/o RROL depletion



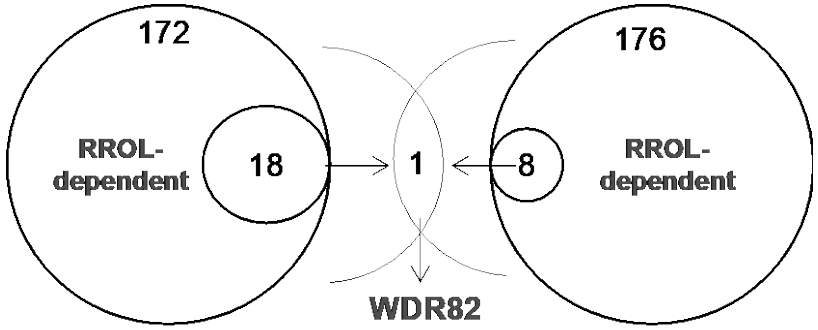




RROL assembles a MYC-WDR82 complex at the ACC1 promoter

Proximity-dependent biotin identification (BioID) w/o RROL depletion

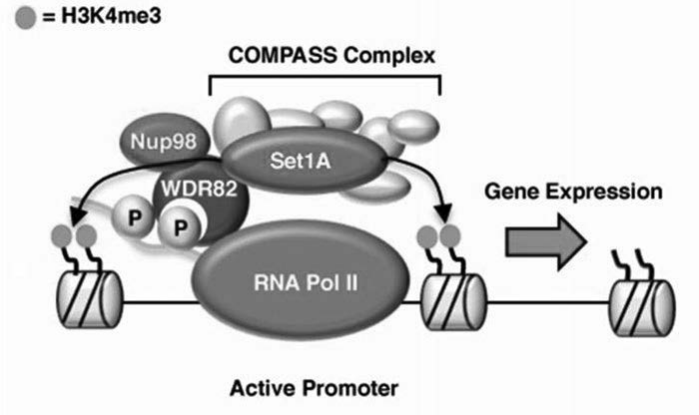
MYC Co-IP/MS w/o RROL depletion



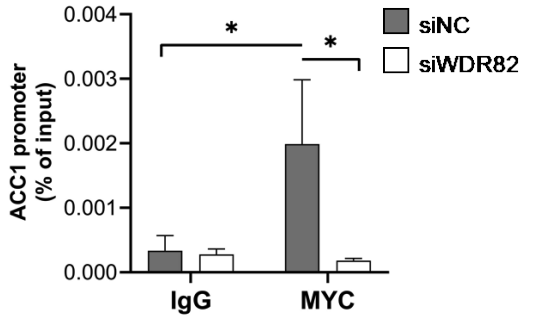
In vivo MYC-protein Interacting network

MM MYC-protein Interacting network

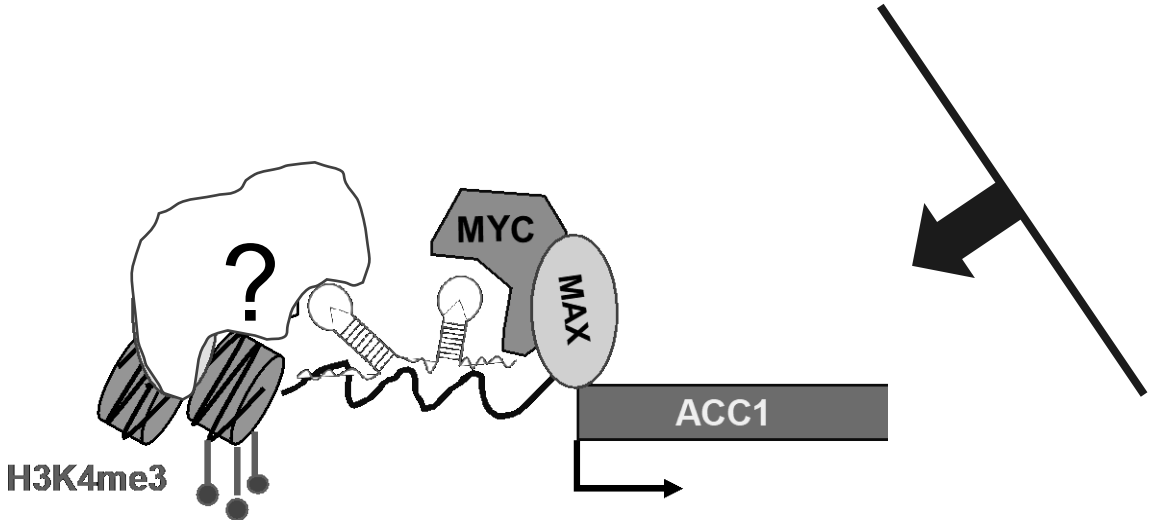
WDR82 promotes MYC occupancy at the ACC1 promoter



MYC ChIP-qPCR w/o WDR82 depletion



WDR82 occupy the ACC1 promoter in RROL-dependent manner



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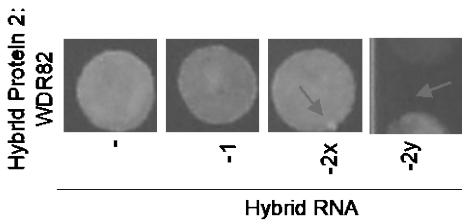
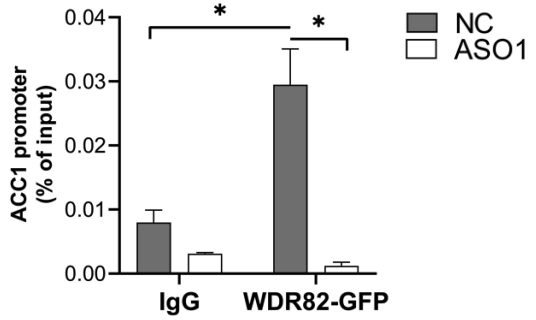
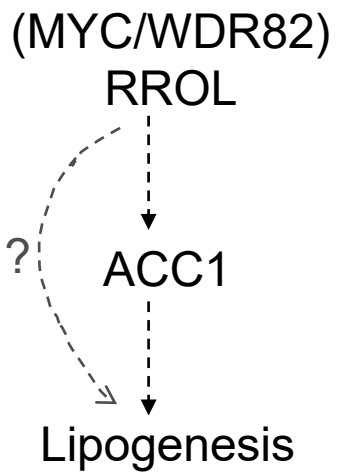
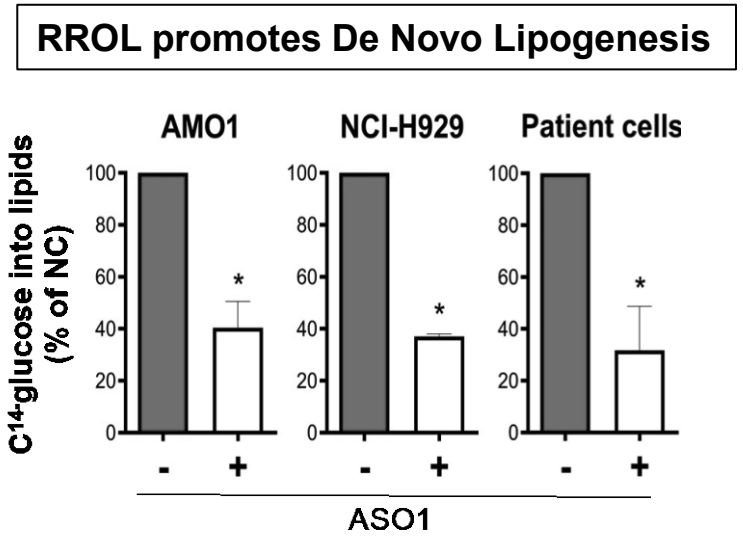


WDR82 ChIP-qPCR w/o RROL depletion



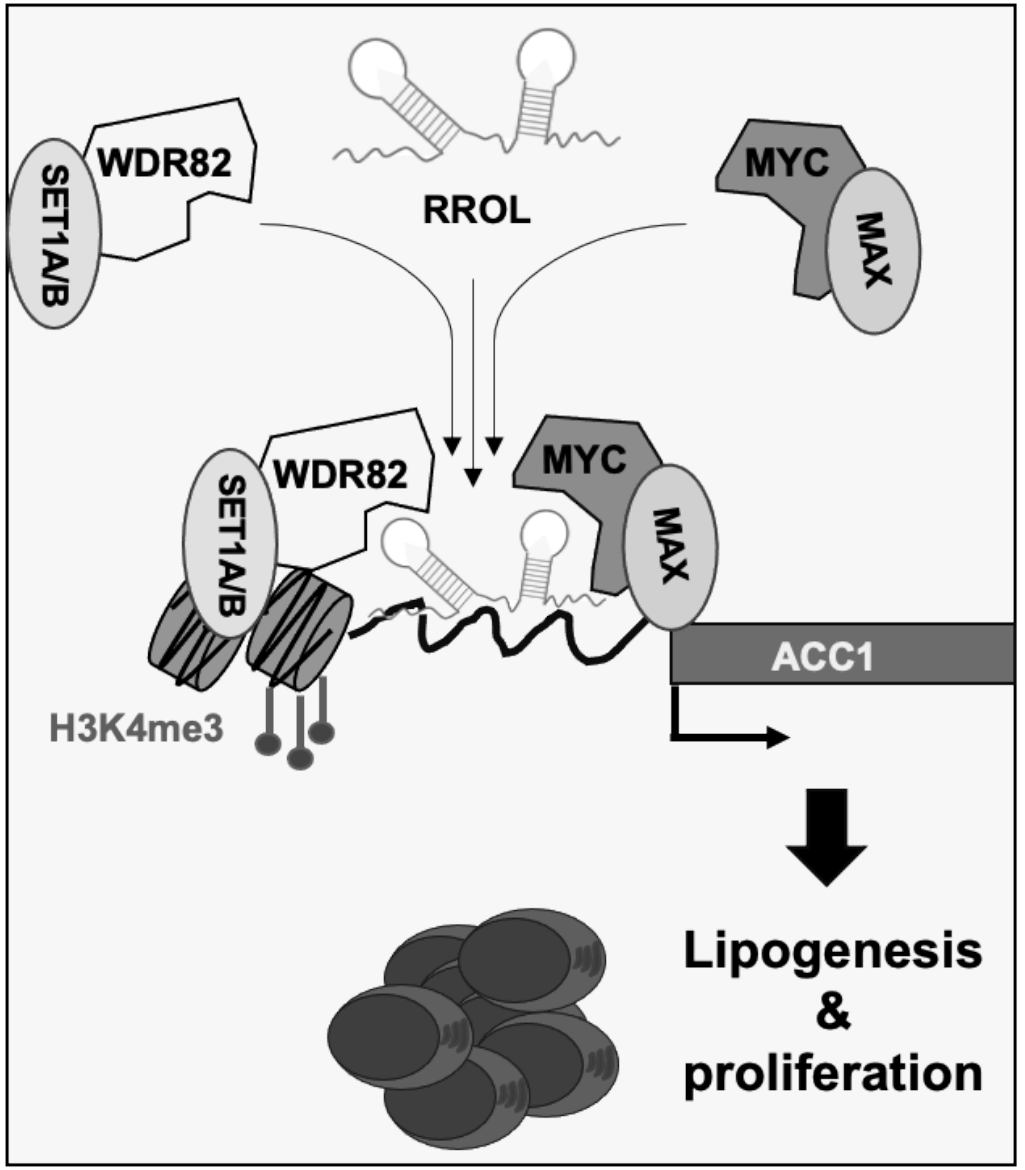
The RROL-ACC1 transcriptional axis promotes *de novo* lipogenesis in MM cells





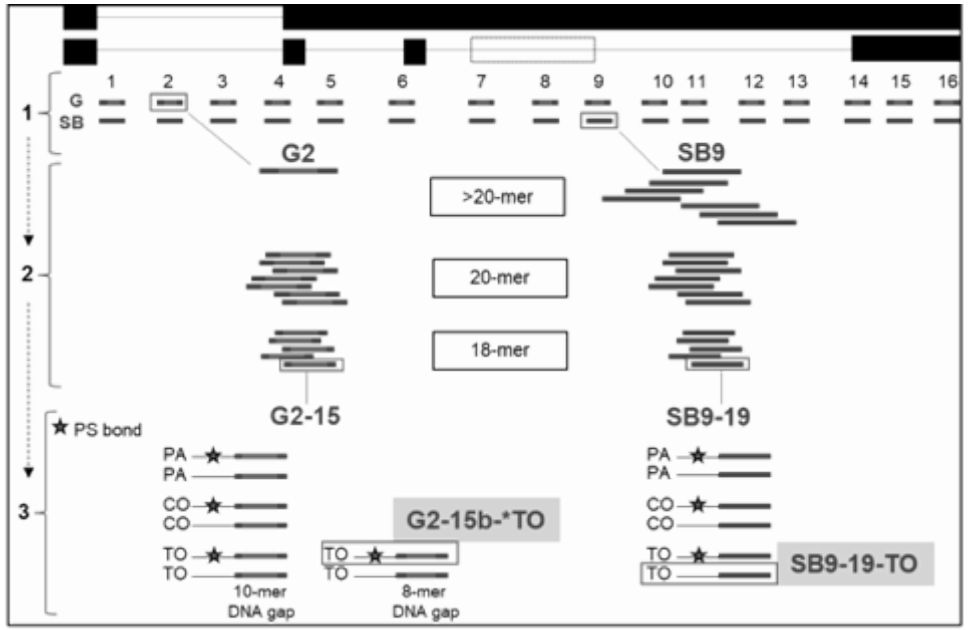
RNA Regulator of Lipogenesis (RROL)

Proposed model of RROL Dependency in MM



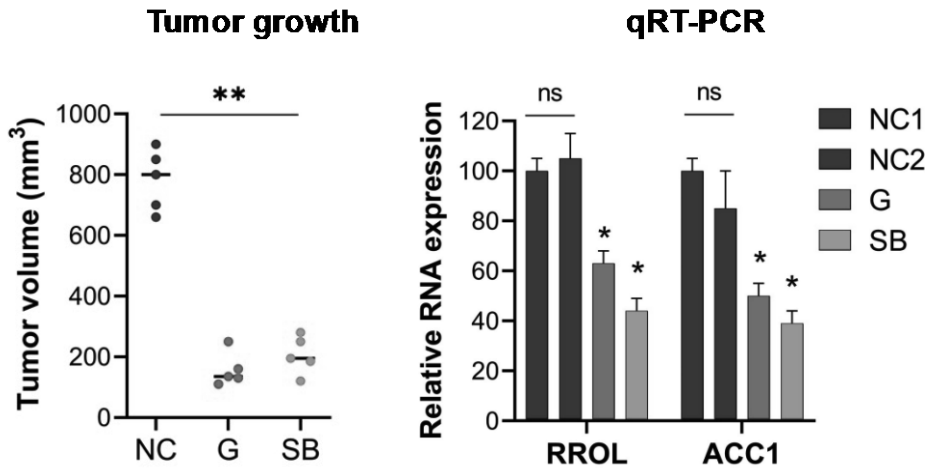
RROL is Therapeutically Actionable using Lipid-conjugated ASOs

Development of Therapeutic ASOs

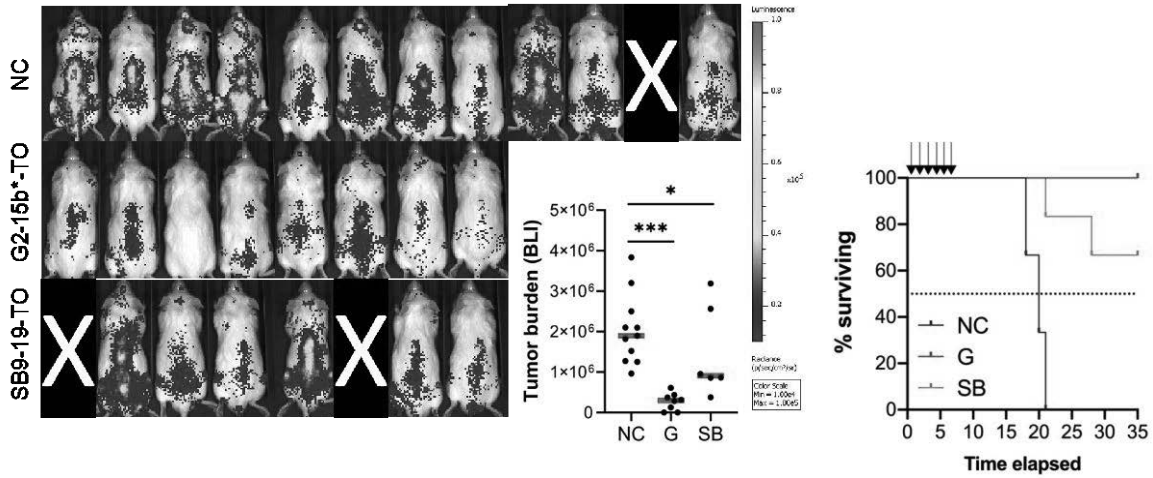


- Multistep ASO screen (>70 molecules)
- Derived 2 leading candidates
  - ❖ Steric-Blocker (SB)
  - ❖ Degrader or gapmeR (G)
- Lipid-conjugation for improved PK

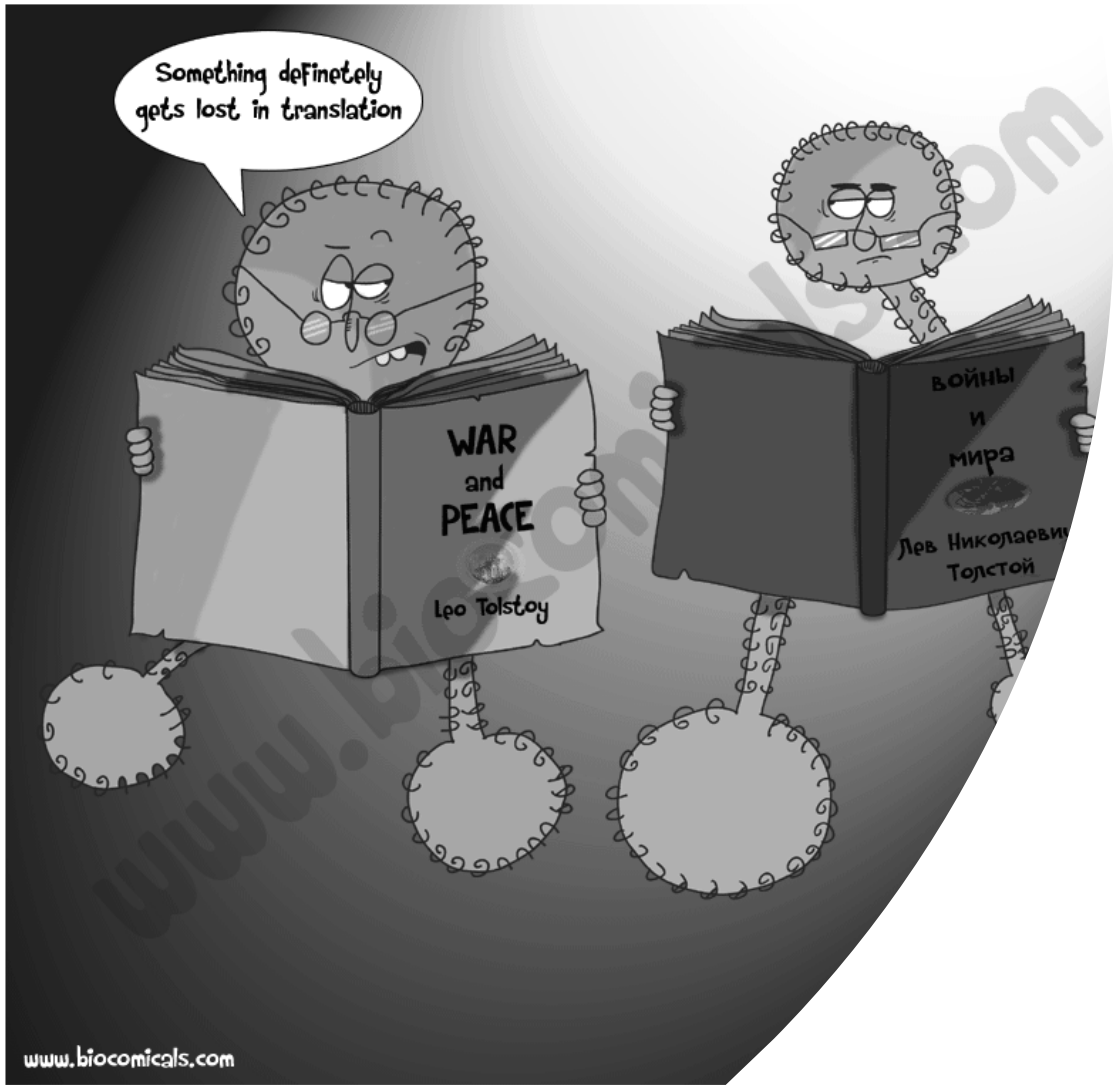
Subcutaneous MM xenograft



BLI-based MM xenograft



Take home message



- LncRNA are a relevant source of genetic vulnerabilities to be molecularly, functionally and therapeutically explored.
- RROL is a leading lncRNA dependency in MM.
- RROL is susceptible to therapeutic intervention.

Acknowledgements

Munshi Lab

N. Munshi
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M. Loda
C. Ribeiro

Lin Lab

C. Lin
W.D. Park

Young Lab

R. Young
J. Henninger

Tassone Lab

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N. Amodio
C. Federico
F. Scionti

Novina Lab

C. Novina
L. Wert Lamas

Penn Lab

L. Penn

Neri Lab

A. Neri
K. Todoerti

S. Gryaznov

All our patients!!!

Miracles for Myeloma

INTERNATIONAL MYELOMA FOUNDATION

DF/HCC DANA-FARBER / HARVARD CANCER CENTER

Specialized Program of Research Excellence (SPORE) in Multiple Myeloma

INTERNATIONAL MYELOMA SOCIETY IMS

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