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Cancer Cell

lncRNA Epigenetic Landscape Analysis Identifies EPIC1 as an Oncogenic lncRNA that Interacts with MYC and Promotes Cell-Cycle Progression in Cancer

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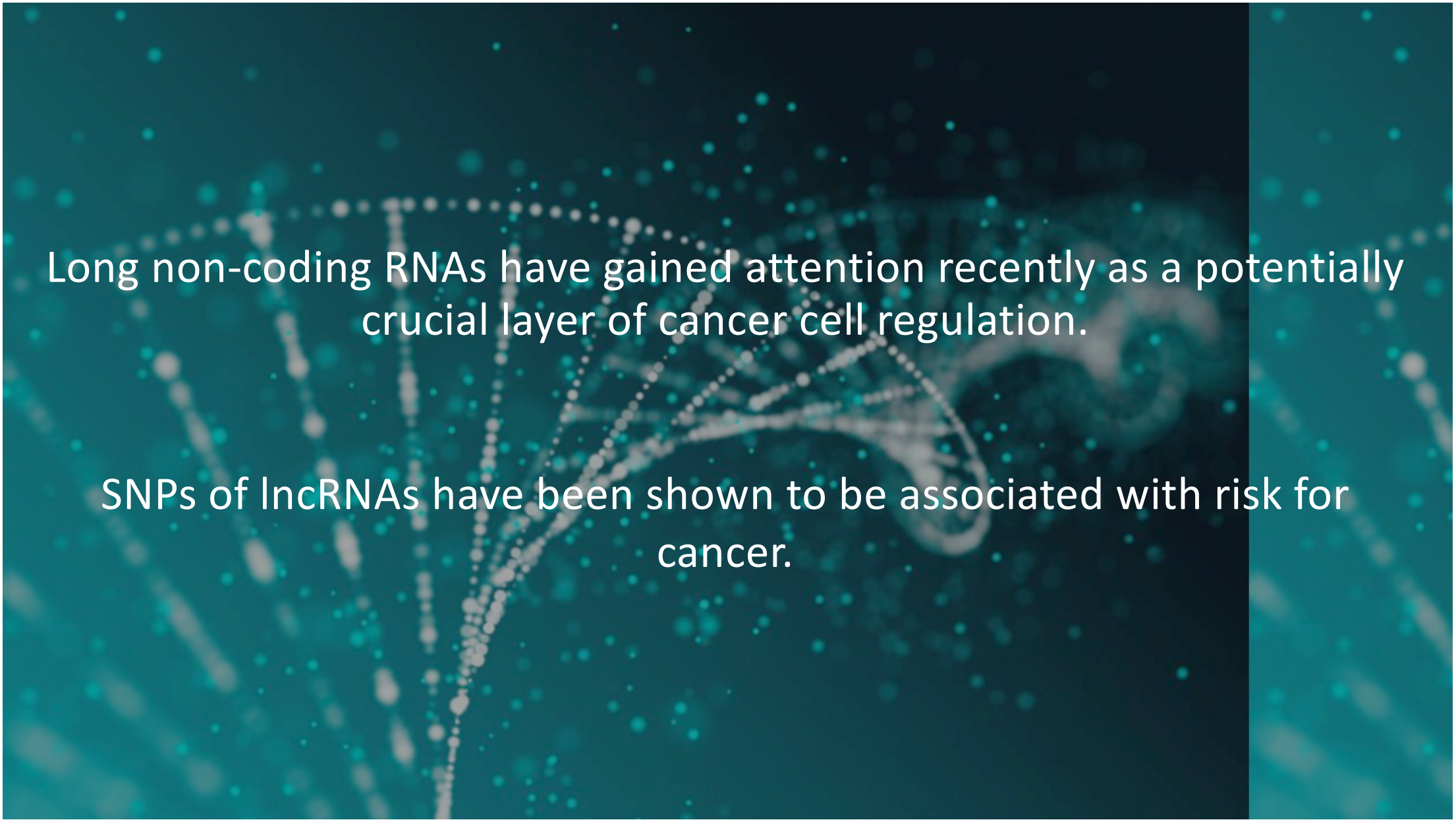
Introduction

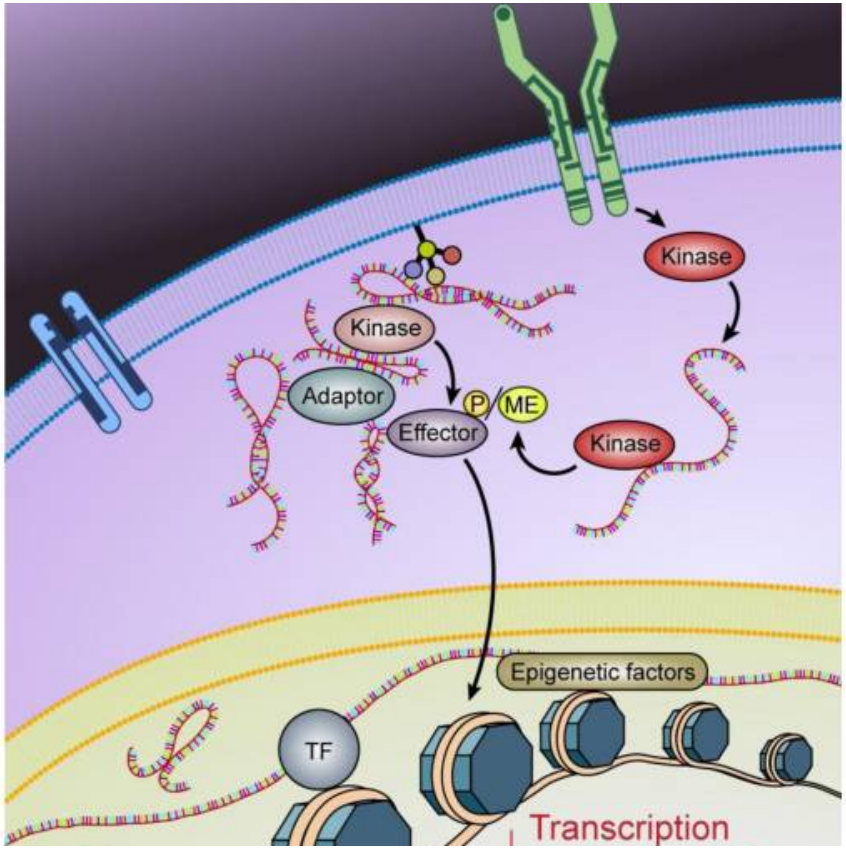
- Role and function of long non coding RNAs,
- Their contribute in Cancer progression,

lncRNA Epigenetic Landscape Analysis Identifies EPIC1 as an Oncogenic lncRNA that Interacts with MYC and Promotes Cell-Cycle Progression in Cancer

- DNA methylation alterations in lncRNA promoter in cancer
- Role of EPIC1 as oncogen
- Lay the foundation of identification of cancer-driving lncRNAs and develop cancer therapies


Long non-coding RNAs have gained attention recently as a potentially crucial layer of cancer cell regulation.
SNPs of lncRNAs have been shown to be associated with risk for cancer.

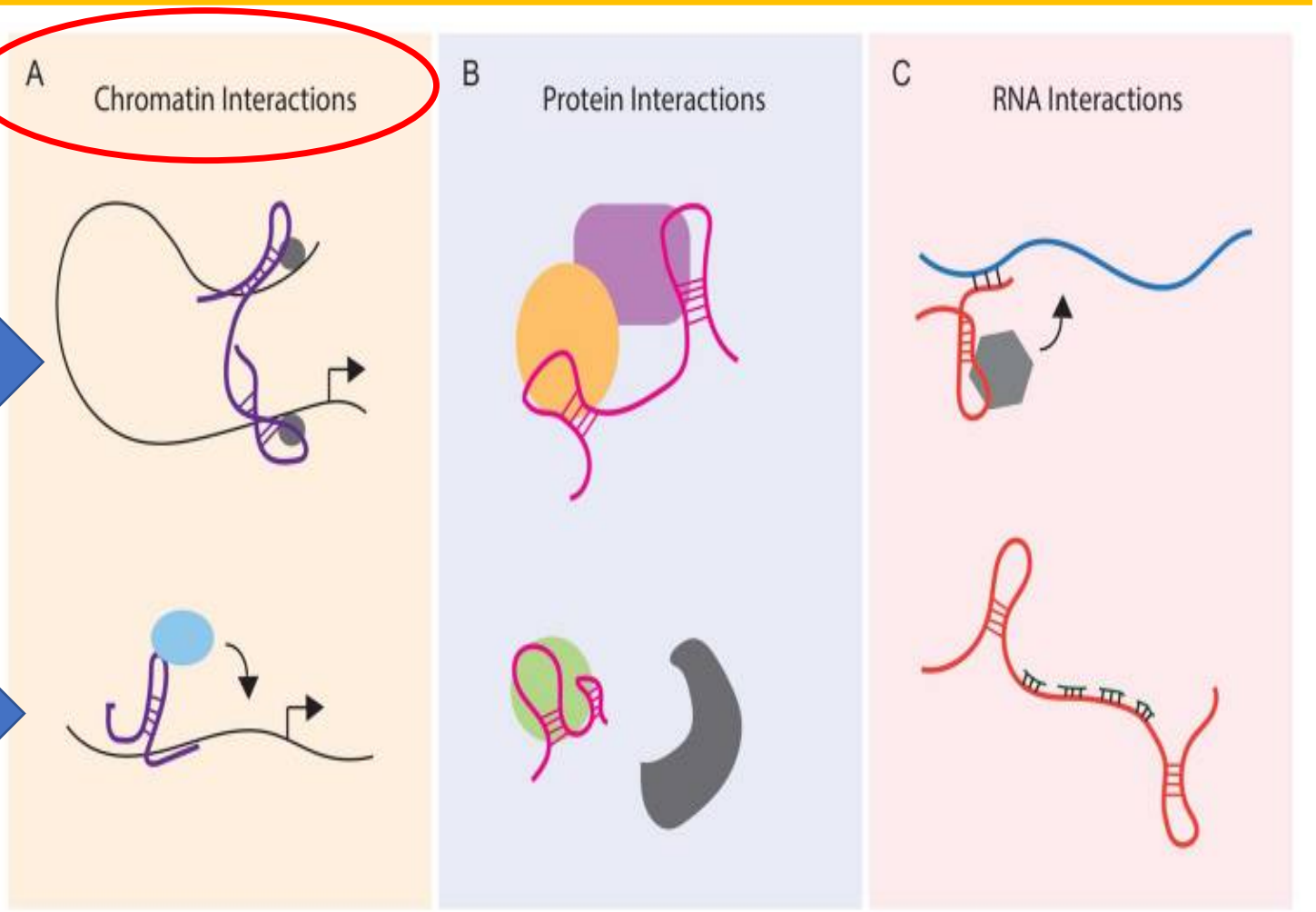
LncRNA derives from a number of sources



LncRNA mechanisms rely on interactions with cellular macromolecules

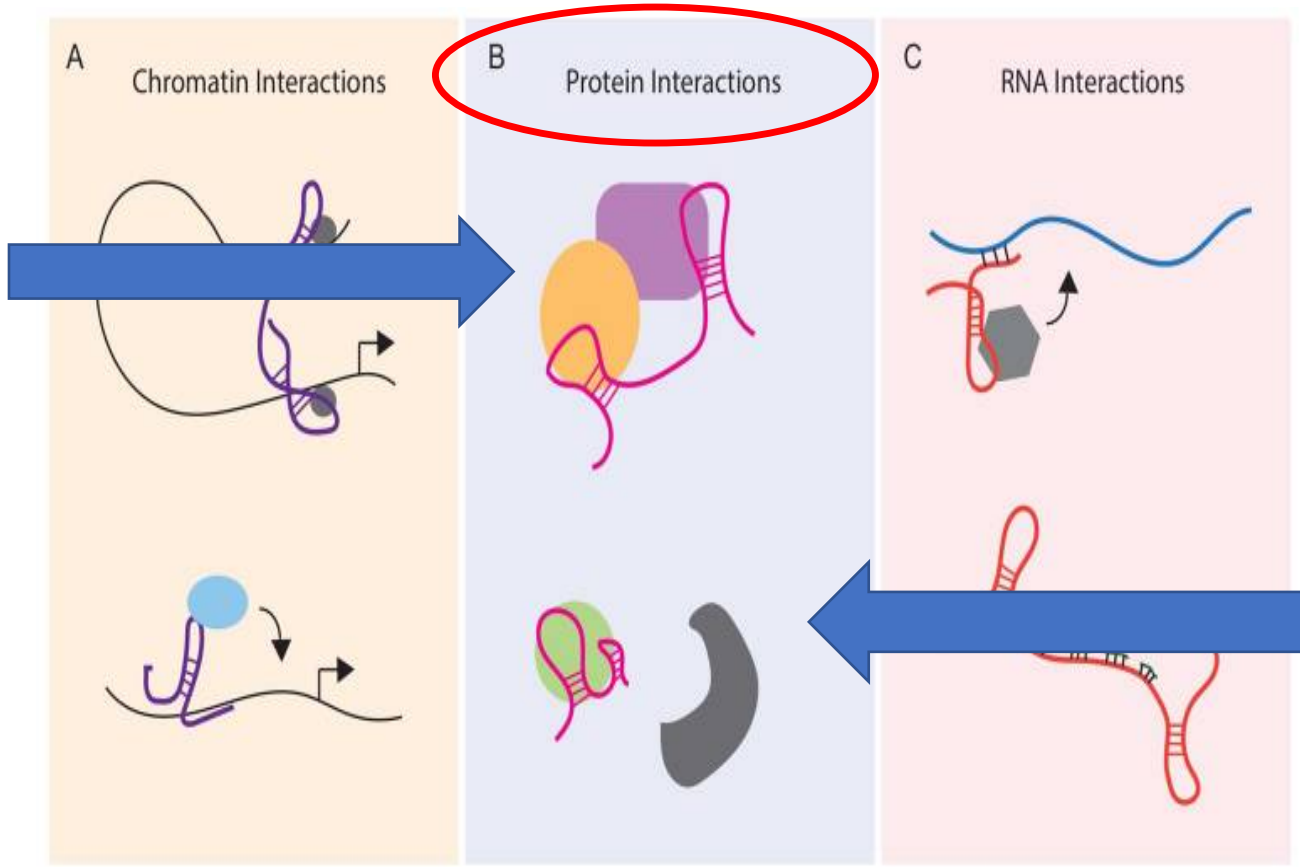
Chromatin bound lncRNAs can regulate gene expression by controlling local chromatin architecture

or directing the recruitment of regulatory molecules to specific loci



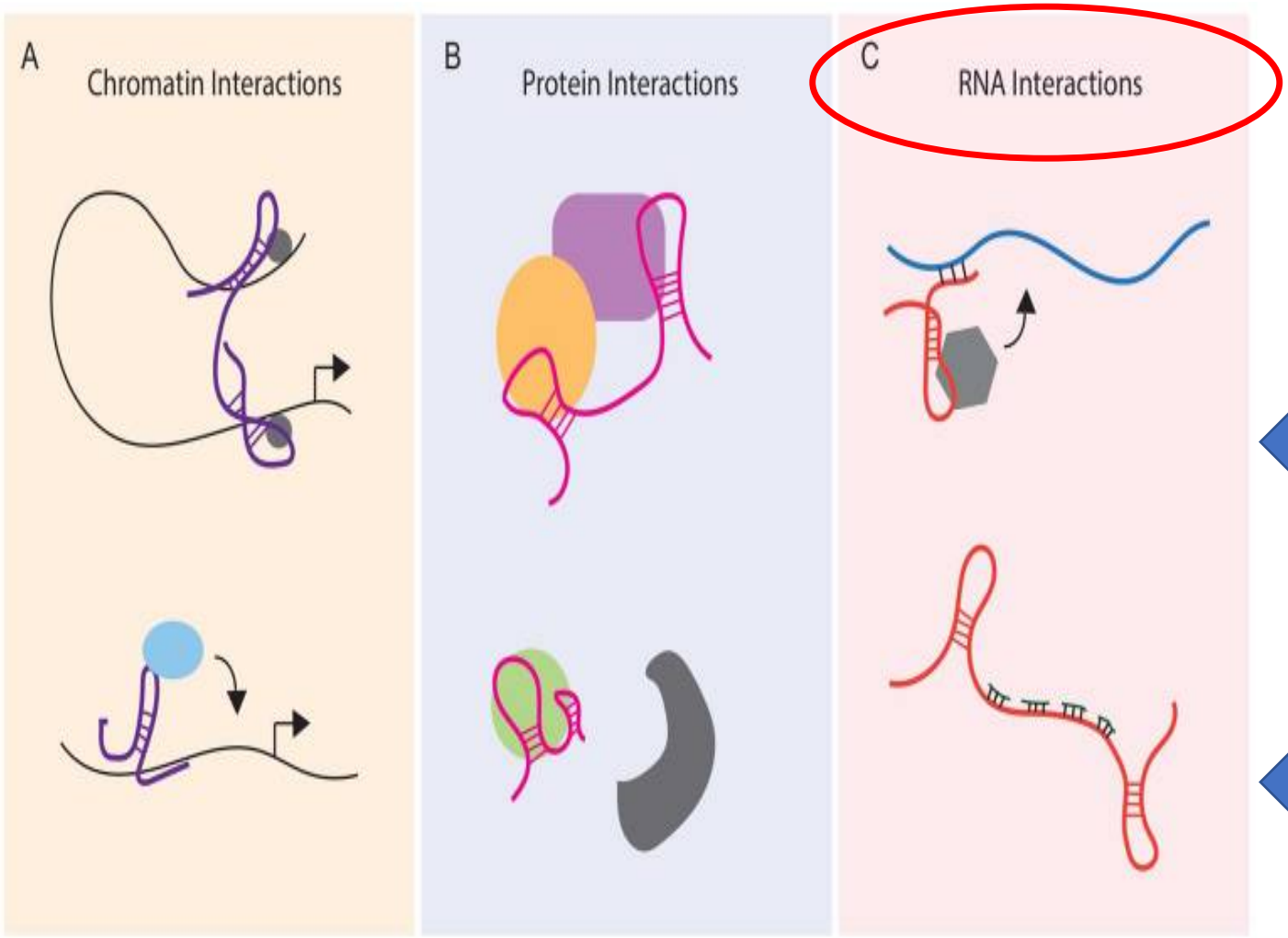
LncRNA mechanisms rely on interactions with cellular macromolecules

LncRNA interactions with multiple proteins can promote the assembly of protein complexes



or impair protein-protein interactions

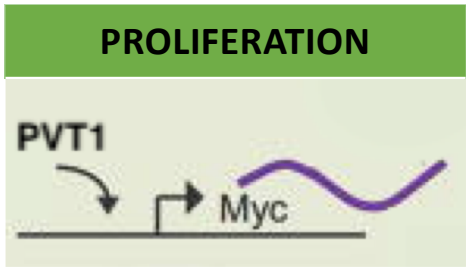
LncRNA mechanisms rely on interactions with cellular macromolecules



mRNA interactions with lncRNA can recruit protein machinery involved in multiple aspects of mRNA metabolism to affect splicing, mRNA stability, or translation

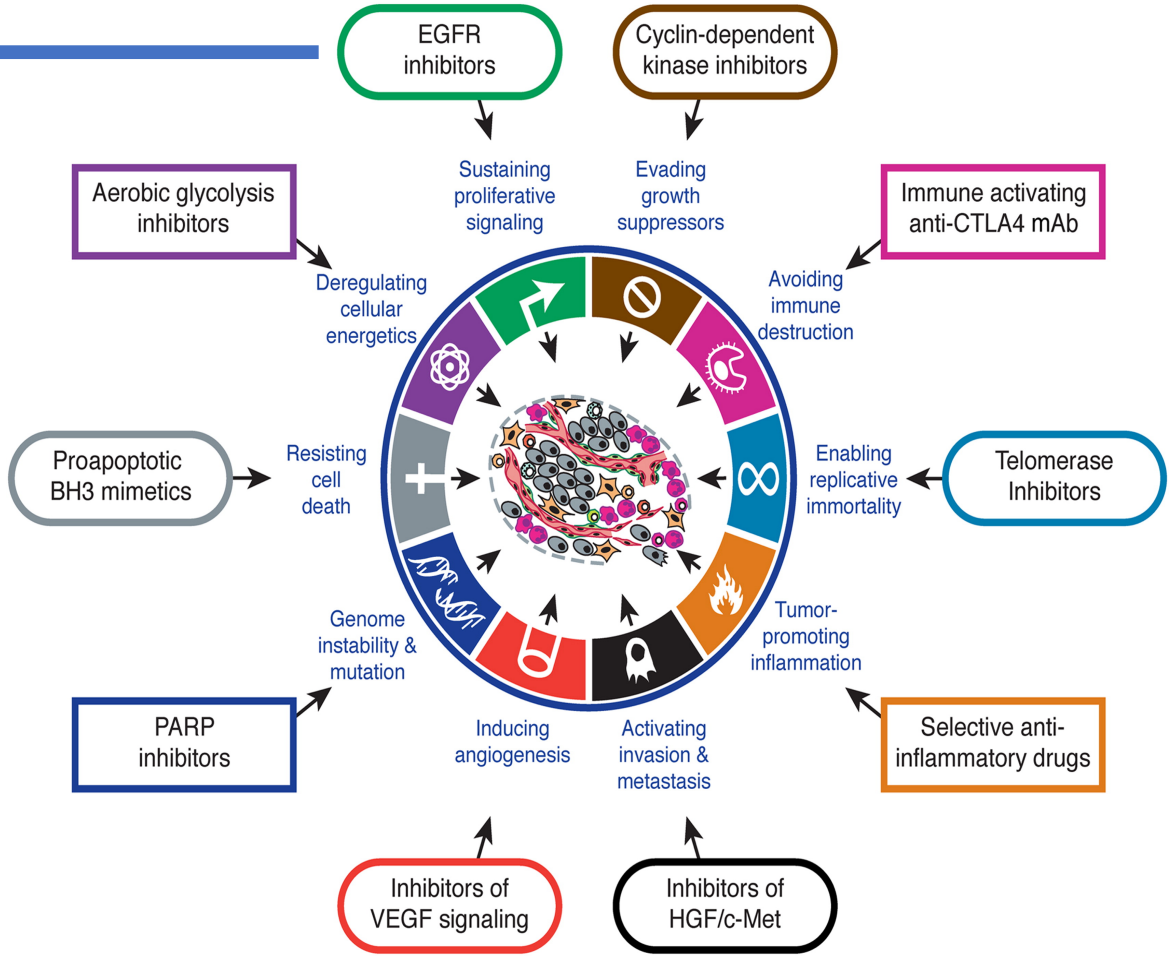
or sequester miRNA away from target mRNA.

LncRNA contribute to each of the six hallmarks of cancer.

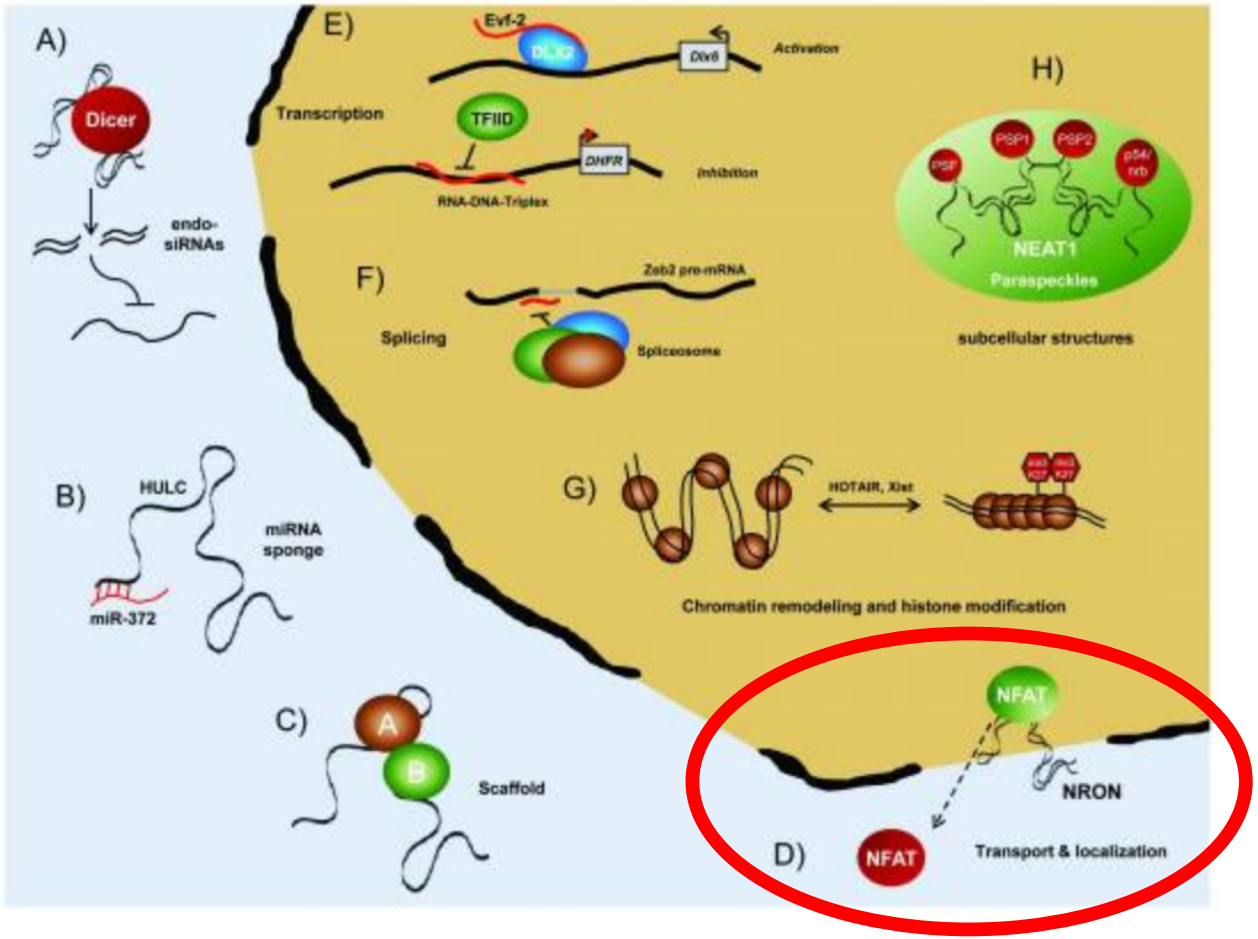


PVT1 as a therapeutic target in cancer:

- RNAi
- ASO
- Genome editing



Long ncRNAs: What, where and why?



Cancer Cell
Article

lncRNA Epigenetic Landscape Analysis Identifies *EPIC1* as an Oncogenic lncRNA that Interacts with MYC and Promotes Cell-Cycle Progression in Cancer

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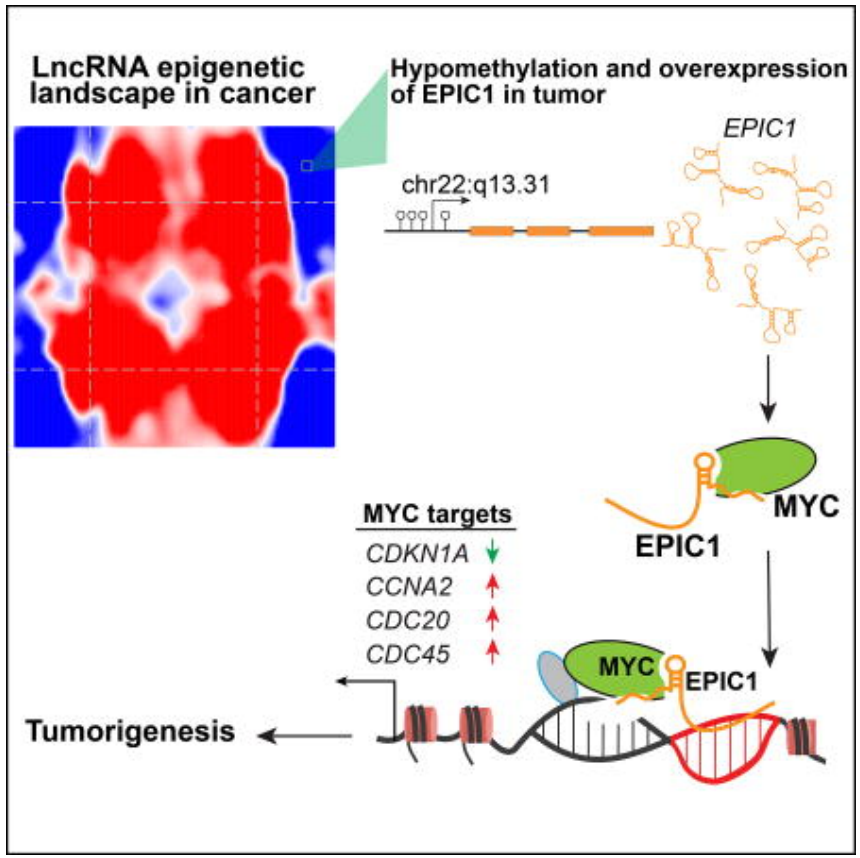
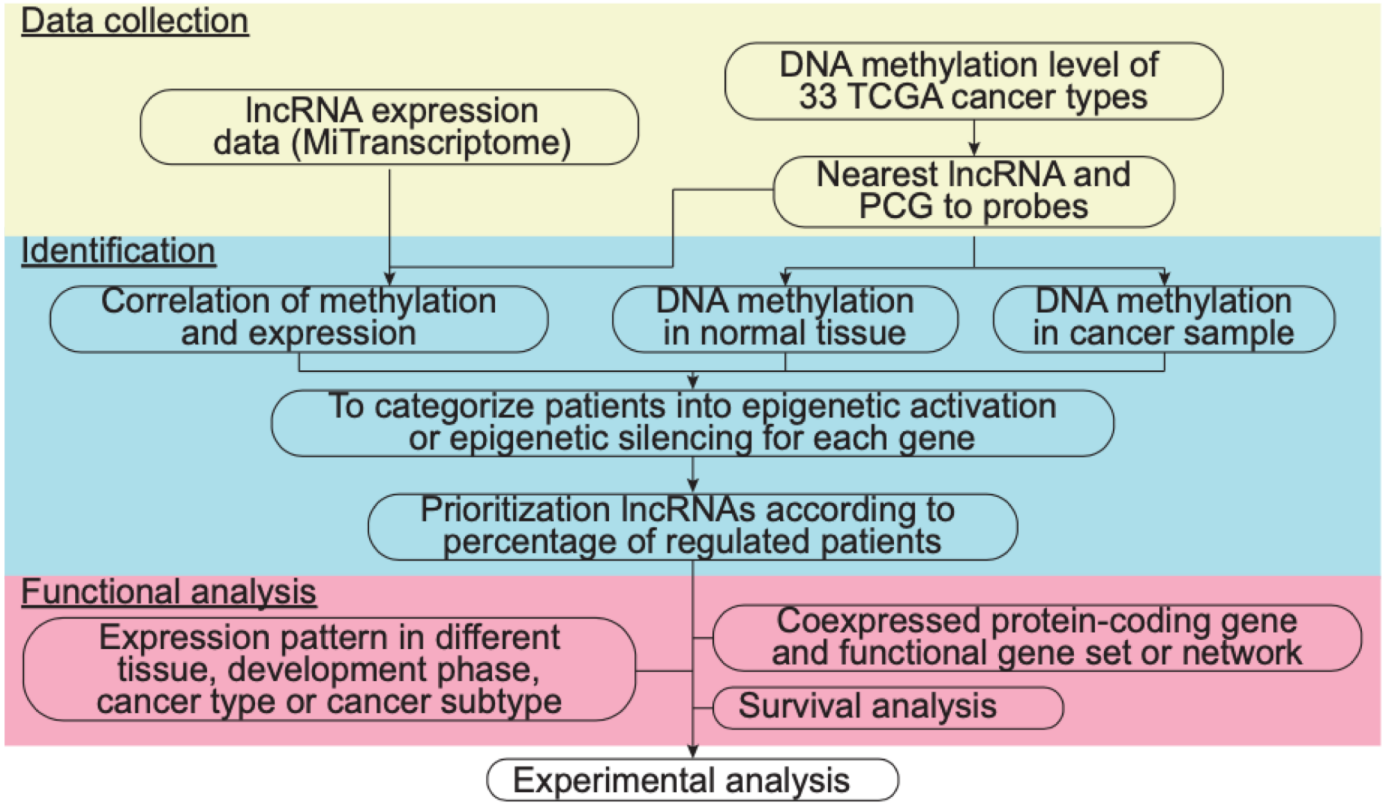
lncRNA Epigenetic Landscape Analysis Identifies EPIC1 as an Oncogenic lncRNA that Interacts with MYC and Promotes Cell-Cycle Progression in Cancer

- LncRNAs show a hypomethylation phenotype, in contrast to a CIMP phenotype in different type of cancer
- EPIC1 promotes **breast tumorigenesis** through regulating cancer cell-cycle progression
- EPIC1 directly interacts with MYC protein through EPIC1's 129–283 nt region
- EPIC1 regulates MYC targets by enhancing MYC occupancy on its target promoters



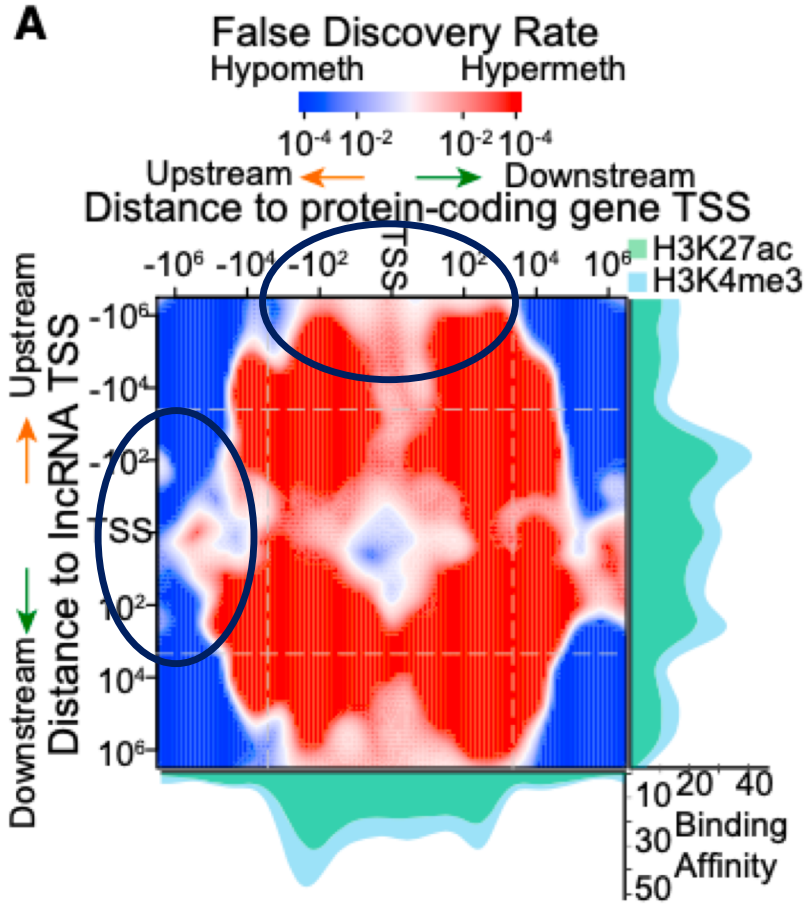
Results



		STEP
PHASE 1	Data collection to identified DNA methylation alteration in lncRNA promoter in cancer.	1 2
PHASE 2	Categorize Epigenetic activation or epigenetic silencing LncRNA. Role of EPIC1 as oncogene.	3 4 5
PHASE 3	Fuctional analysis of EPC1 and its interaction with trascription factor MYC.	6 7 8 9 10

PHASE 1

LncRNA Promoters Exhibit a Distinct Pattern of Epigenetic Alterations in Cancer Compared with PCG Promoters



1° STEP:

Aim: Interrogate lncRNA DNA methylation in cancer

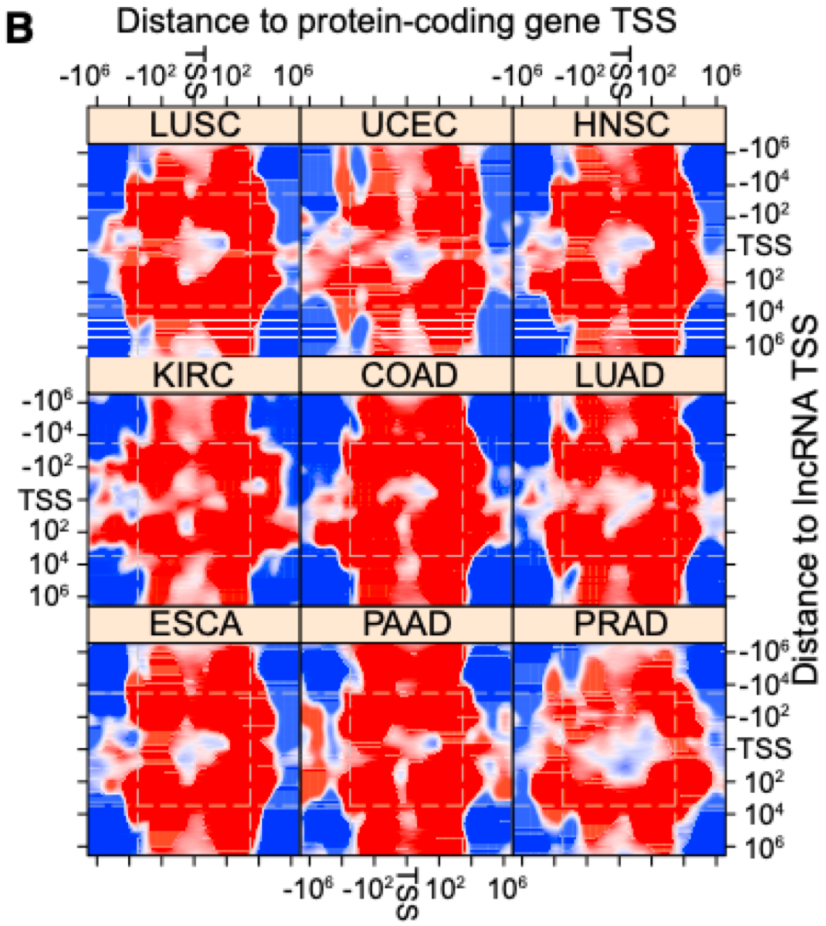
Method: computational pipeline to repurpose HM450 probes (Human Methylation 450) to lncRNA promoters.

- lncRNA promoters in breast cancer tissues: Both hypermethylated and hypomethylated
- PCG promoters: predominantly hypermethylated in breast cancer.

Conclusion:
the probes indeed represent the promoter methylation status of lncRNAs and PCGs

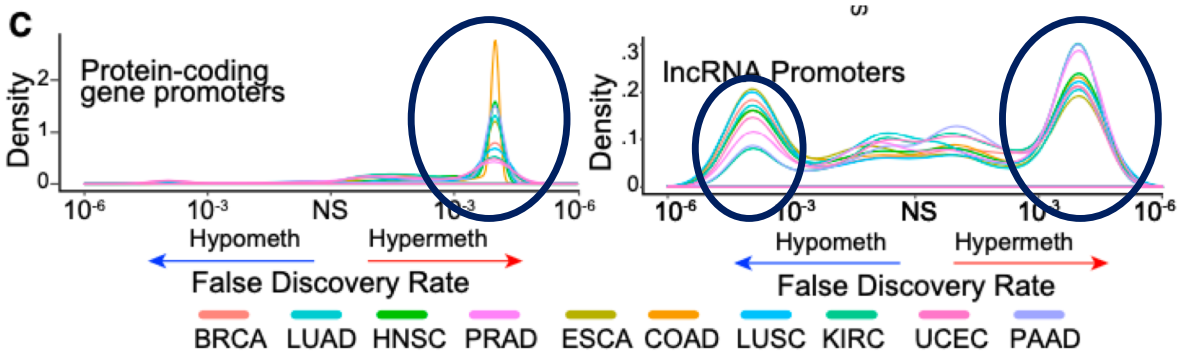
PHASE 1

LncRNA Promoters Exhibit a Distinct Pattern of Epigenetic Alterations in Cancer Compared with PCG Promoters



Intergenic lncRNAs that do not share promoters with PCGs are divided in:

- promoters hypomethylation,
- promoters hypermethylation.



Conclusion:
The lncRNA promoters were significantly hypomethylated in all cancer types

PHASE 1

Integrative Analysis Identified Recurrent Epigenetically Regulated lncRNAs in 20 Cancer Types

2° STEP;

Aim: Determine whether lncRNAs' expression is regulated by the DNA methylation changes at their promoters,

Method : Integration of lncRNA expression data from **MiTranscriptome**, focusing on 20 cancer types that have both DNA methylation and lncRNA expressione data

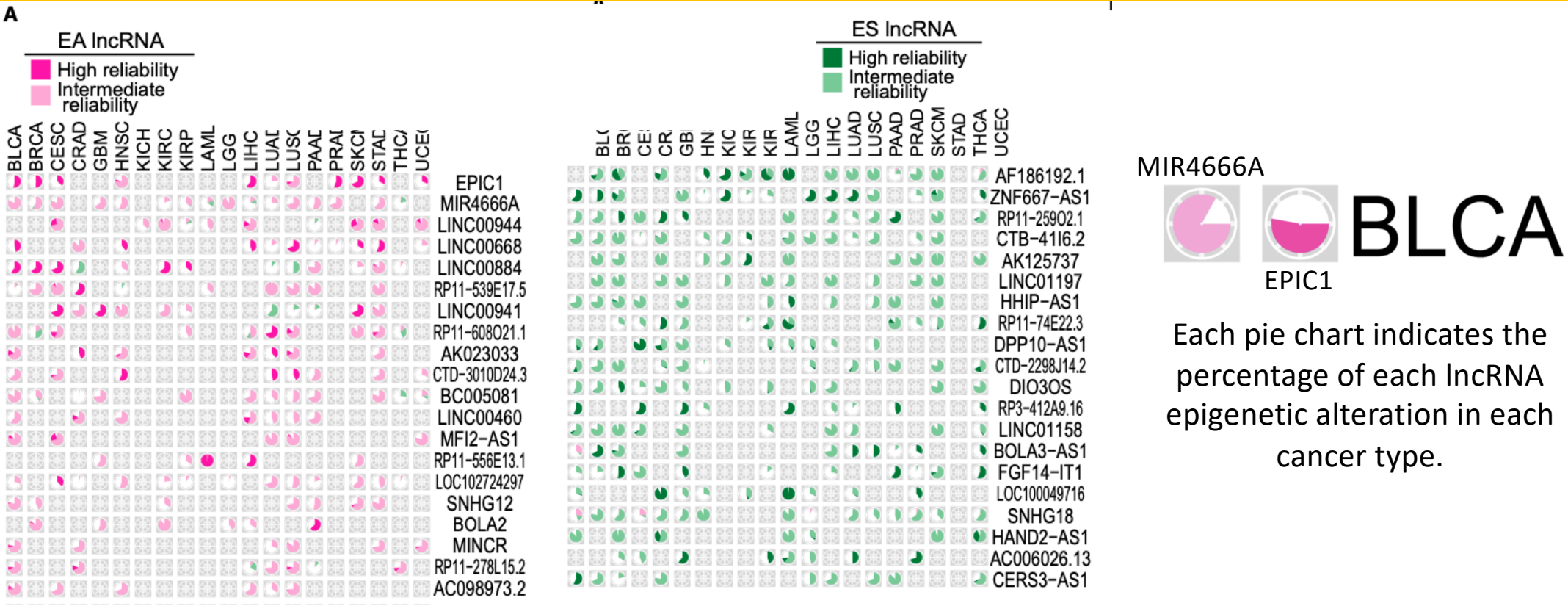
lncRNA

Epigenetic activation (EA)

Epigenetic silencing (ES)

PHASE 1

Integrative Analysis Identified Recurrent Epigenetically Regulated lncRNAs in 20 Cancer Types



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Each pie chart indicates the percentage of each lncRNA epigenetic alteration in each cancer type.

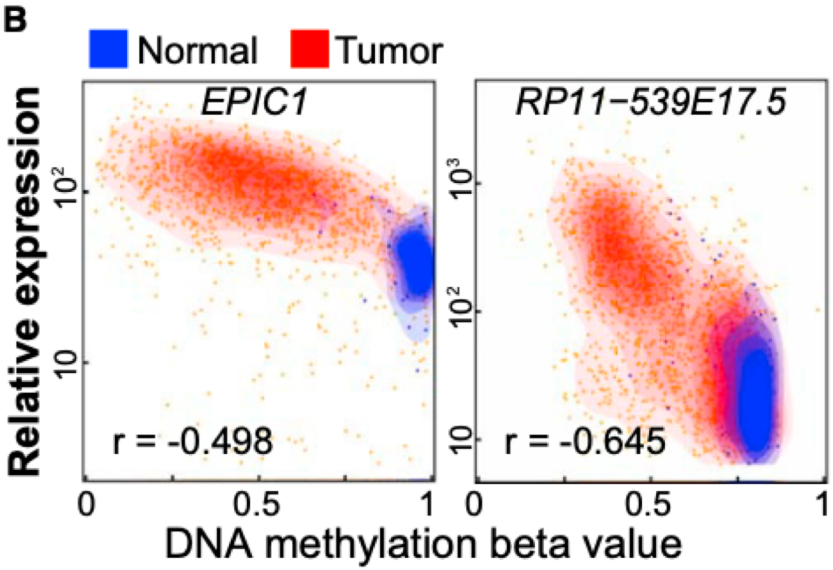
Conclusion:

This ‘‘on or off’’ expression pattern of EA lncRNAs potentiated them as promising diagnostic biomarkers.

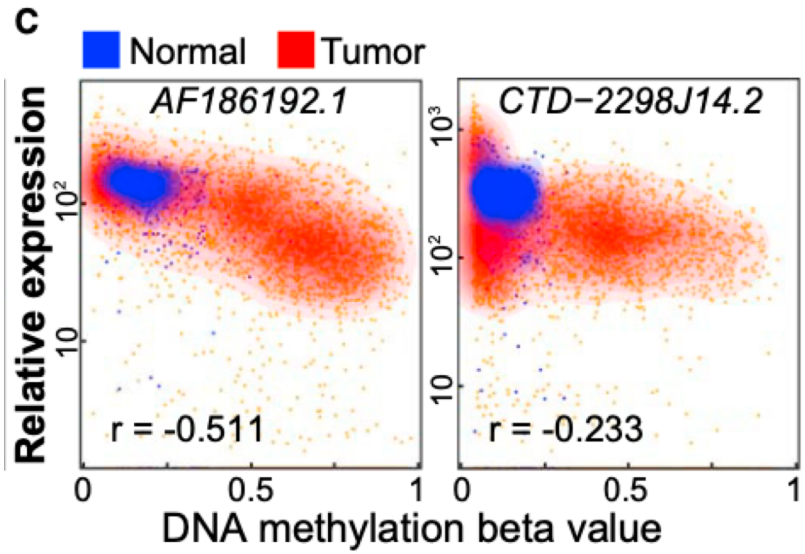
PHASE 1

Integrative Analysis Identified Recurrent Epigenetically Regulated lncRNAs in 20 Cancer Types

correlation of representative EA



correlation of representative ES



Conclusion:

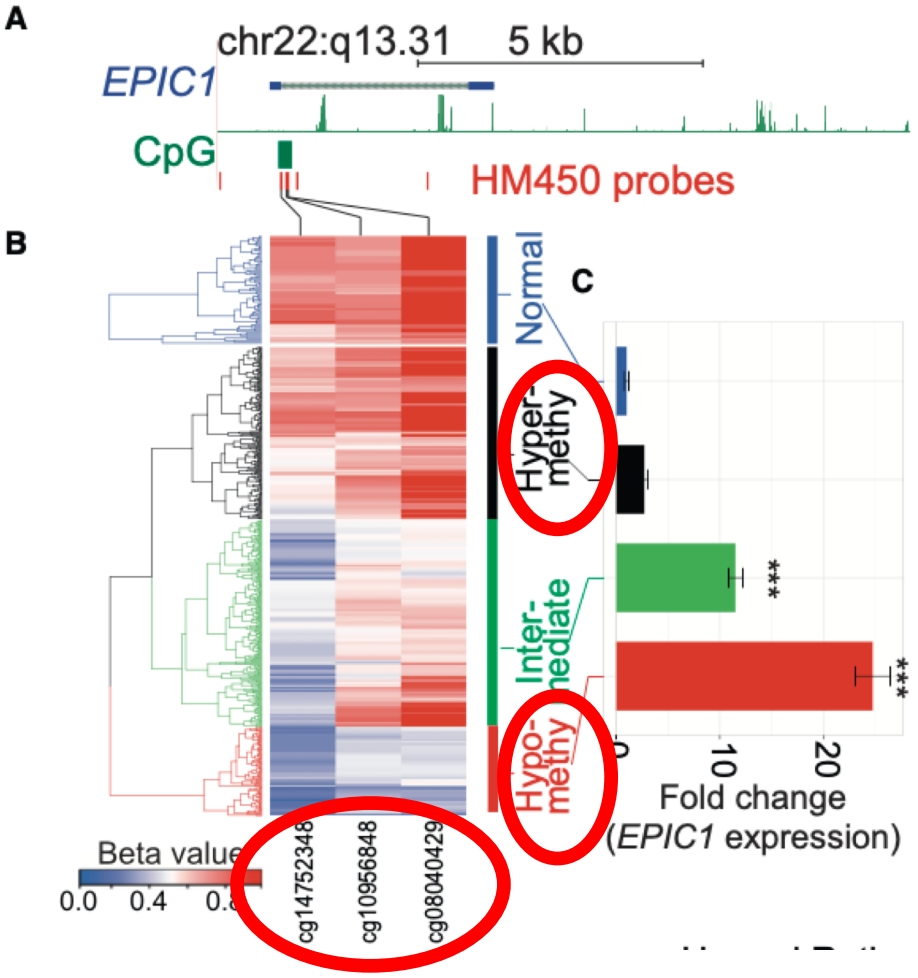
All the epigenetically regulated lncRNAs exhibited a significant negative correlation between their expression and promoter DNA methylation status

DO YOU REMEMBER THIS?

PHASE 2

WHAT IS EPIC1?

EP igenetically **I** nduced In **C** RNA**1**



- Intergenic lncRNA located on

- There are CpG islands within 164 bp downstream of this gene's transcription start site (TSS),

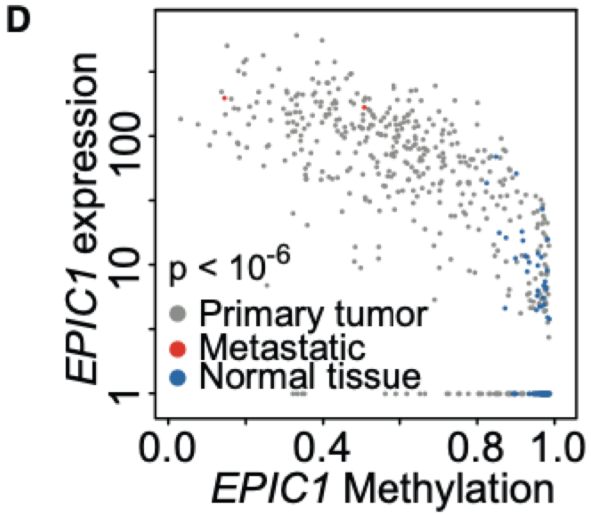
- This lncRNA is epigenetically activated in up to 90% of tumor samples across ten cancer types, including breast cancer,

PHASE 2

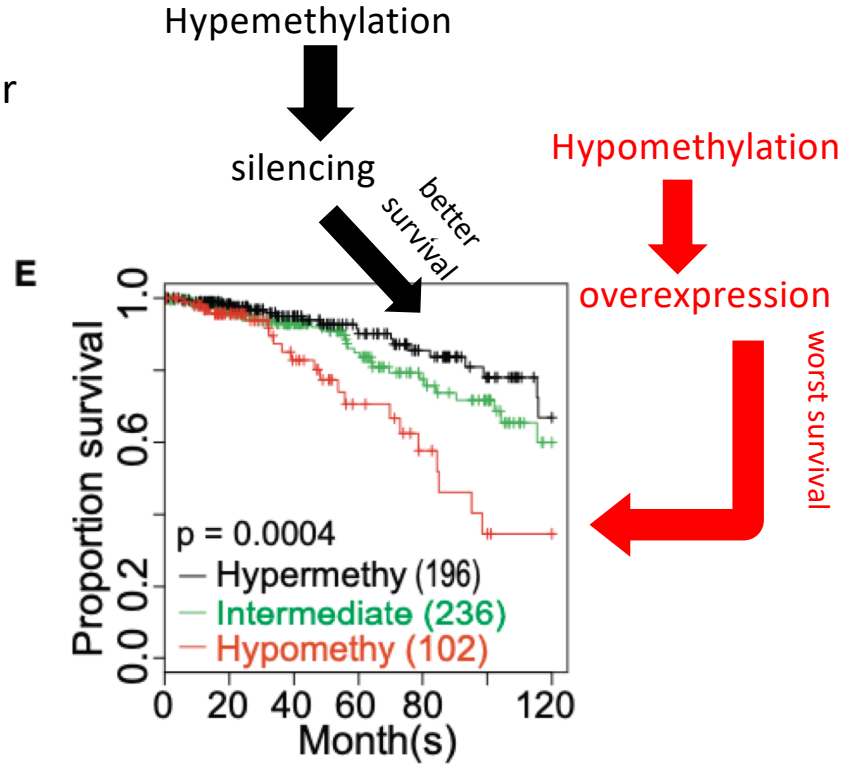
EPIC1 Is Epigenetically Activated and Correlated with Poor Survival in Breast Cancer

3° STEP

Determine if *EPIC1* expression is robustly associated with poor patient survival in breast cancer



Correlation of *EPIC1* expression with *EPIC1* DNA methylation status in breast cancer and normal tissues.



Conclusion:

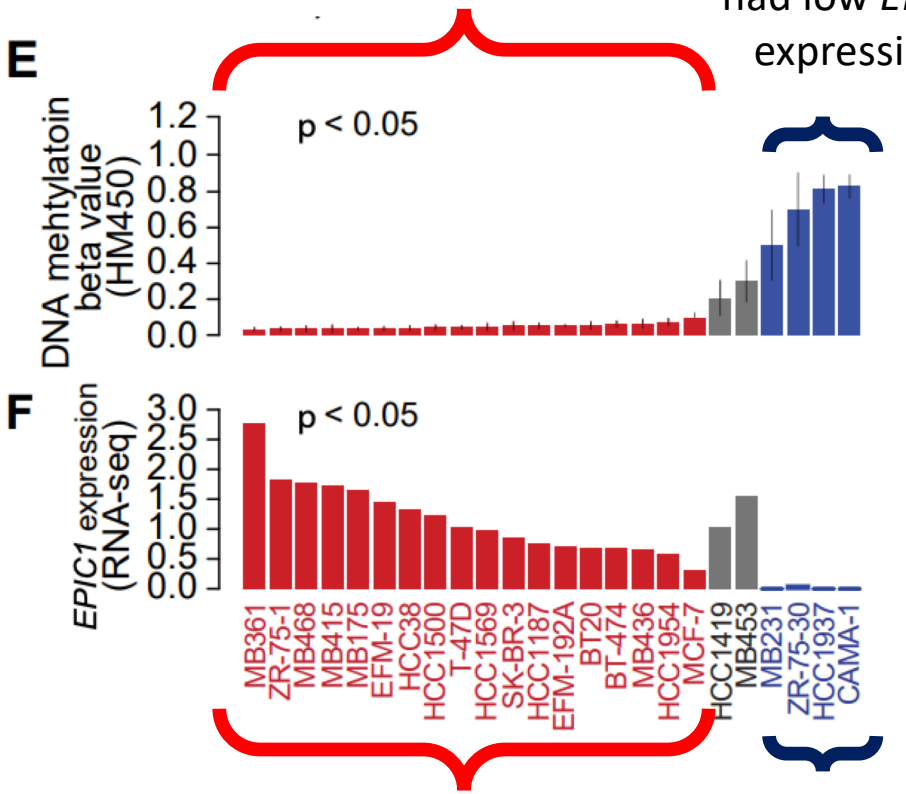
Patients whose tumors exhibit *EPIC1* hypomethylation and increased *EPIC1* expression have the worst survival.

PHASE 2

EPIC1 Is Epigenetically Activated and Correlated with Poor Survival in Breast Cancer

Epigenetic activation of *EPIC1*

promoter hypermethylation and had low *EPIC1* expression



4° STEP

Aim: Determing if *EPIC1* is directly regulated by DNA methylation

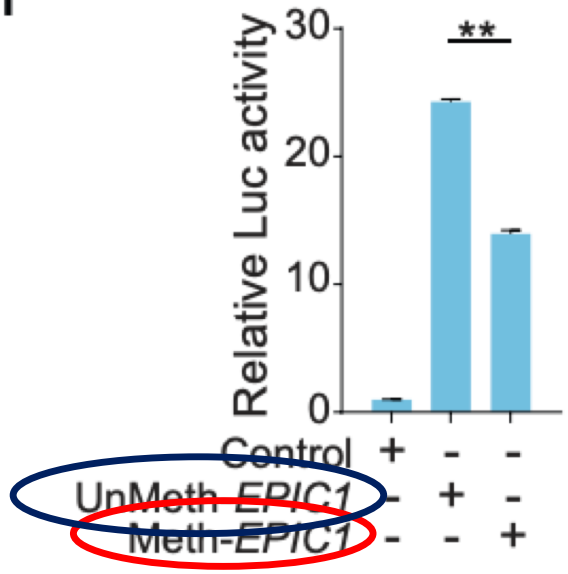
Method: Using RNA-seq and HMG450 DNA methylation data in the CCLE database

Conclusion:
significant negative correlation between endogenous *EPIC1* expression levels and its promoter methylation

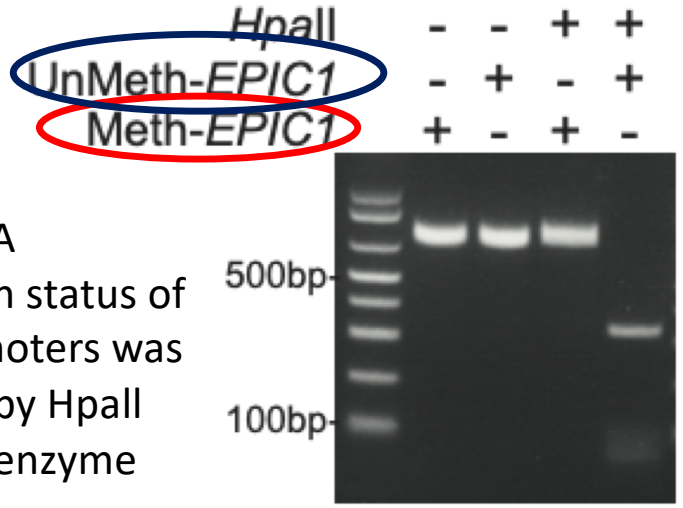
PHASE 2

EPIC1 Is Epigenetically Activated and Correlated with Poor Survival in Breast Cancer

I



Reporter assay of methylated and unmethylated *EPIC1* promoters



In vitro DNA methylation status of *EPIC1* promoters was confirmed by HpaII restriction enzyme

Concluson:

***EPIC1* is directly regulated by DNA methylation at the CpG islands in its promoter region.**

PHASE 2

EPIC1 Functions as a Potential Oncogenic lncRNA by Promoting Cell-Cycle Progression

F

>EPIC1 v1

AGTCCGCCATTGCAAACACGAAGCTCTTCCAGAAACGCCCTCACAGACACCCCGGAAGTCACGTACCCACTCTGTAGGTGCCCCGGGGCACAGGCAAGCG
GACGAGCCAGTTATCCCTCAGAGCTCCTGCTGCCTCGCCCGCTTTCTCTCGGAAACGTGAAGTGTGGCCTCAGCTGAAAGTGAGGTGGGCCTCATTCAAT
CAGTTGAATTCTTCAAGAGAGAAAAACTGAAGTCCCTTAGAAGGAAAGAGTTCTGCCTTCAGACTGTCTTTGAACTTAAGACTGTAGCGTCGACTCCTGC
CGGAATTTCCAGCCTGCTGGCCAGCTCTGCAGATTCACACTTGCCAGCCTCCACAATCGTGTGAGCCAATTCCTTAACTTCTCTTTCTCCGTGTATCCCT
TTGGTGCTGCCTCTCTGGGGAGCCCTGACTAATATGCATGCAGATGATACGGTGCCTGGCATTCTGAATACATGCACTAAATCCACCACTTTTCCCCATT
TATAGATTTGGATTAACACACTAACTTACTCATATCTGCAAGTATAAATAAAAAAAATTGCTGGTGC

>EPIC1 v2

AGTCCGCCATTGCAAACACGAAGCTCTTCCAGAAACGCCCTCACAGACACCCCGGAAGTCACGTACCCACTCTGTAGGTGCCCCGGGGCACAGGCAAGCG
GACGAGCCAGTTATCCCTCAGAGCTCCTGCTGCCTCGCCCGCTTTCTCTCGGAAACGTGAAGTGTGGCCTCAGCTGAAAGTGAGGTGGGCCTCATTCAAT
CAGTTGAATTCTTCAAGAGAGAAAAACTGAAGTCCCTTAGAAGGAAAGAGTTCTGCCTTCAGACTGTCTTTGAACTTAAGACTGTAGCGTCGACTCCTGC
CGGAATTTCCAGCCTGCTGGCCAGCTCTGCAGATTCACACTTGCCAGCCTCCACAATCTTCCTGGATTTGAAACTGAAGAAGCAAGCAATCTGGAAATGT
CAGTGGATGCACACAAAGAAACAACCGCAAAAGCCTGCTCGCTCTAGCCAAGGGACAAGAATAGGGGCAGTCCATCAAGACAGAATCCTTTTAAAAAATA
ACCACTCCACTCCAGCAATACCACAGAAGAATCTGGCTGTACCCCAGGTACATCAGCAAAGATAACCTTTACCTAGCAGTAAAGAGGTCCCCCTTACACT
GGGAGCCCTAGTGAAGAGCAGGGACTTTCACCCCCACTTAGCAGTGATGGGGCCCCACCCACCACAGTGCCAGCAGAGACCATGTGGGAGCCAGAATCCT
CATCCCTACCCAGCAGTAACAAGGAGCCCTCCTCACTGCGGGCATCAAGGGTGAGTGAGTGCAAAACCTGGGTGTCACTCGGAAGGGAAGAATGGTGTCT
CCTTCCTTCCCATCCCCTGCCAGAGTGATATCACTAGGAAAAAG

>EPIC1 v3

AGTCCGCCATTGCAAACACGAAGCTCTTCCAGAAACGCCCTCACAGACACCCCGGAAGTCACGTACCCACTCTGTAGGTGCCCCGGGGCACAGGCAAGCG
GACGAGCCAGTTATCCCTCAGAGCTCCTGCTGCCTCGCCCGCTTTCTCTCGGAAACGTGAAGTGTGGCCTCAGCTGAAAGTGAGGTGGGCCTCATTCAAT
CAGTTGAATTCTTCAAGAGAGAAAAACTGAAGTCCCTTAGAAGGAAAGAGTTCTGCCTTCAGACTGTCTTTGAACTTAAGACTGTAGCGTCGACTCCTGC
CGGAATTTCCAGCCTGCTGGCCAGCTCTGCAGATTCACACTTGCCAGCCTCCACAATCGCAGCTGAGGCGGAGGAACCCTAAGGGCTCATTGAGATCATG
GATTTGCCCTTCTATGCATTGATGGAGCACCTGCTGCCCACAGCGTCTGTATTTGGTGCTGGGATGCTGAGCCTCCTTCTTTATGAATTTTTAAAAGGAC
ACTGAGATCTTCAAACAGAGGCTGCCACTCTAAGCAAACAGATCCCGAGTCCTGGACTCTGAAGCTTGGGCCCAGTTCTCCTTTTCTCCGGGTTTCAGAT
CCCACTGTAAAGTGAGGGGGCCCTTCTGATTCAGGACCCGGGGAAGCCAGGGGCATGAGCATCGGTGCCTCTTCTCTATTTCAAGGACCCTTCTGGGTGT
AAAGTTCTCTGAGATGCCTTACATGGATTCCCACCACTGCAAGATAACCATCGTATGTAAAGTGTTATGACCAGCAGAGTGTAATTGAAGTGCATTCCAG
AGGGAAAGACAGCGGCTCAGATTCTATTGAAAGAAACATGACATAATGATACCACAGCAAAAGCCAATCTTGCTCCTTTTTA

5° STEP

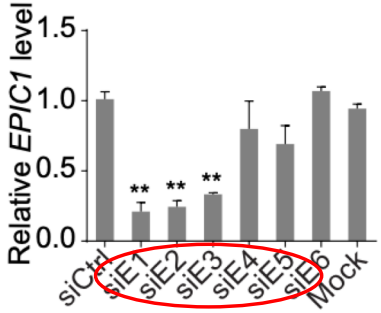
Aim: Evaluating the oncogenic role of *EPIC1* in cancer

Method: performing 5'-RACE and 3'-RACE cloning using total RNA MCF-7 cells

Three splice variants of *EPIC1* were cloned,
v1 (567 nt),
v2 (844 nt),
v3 (882 nt).

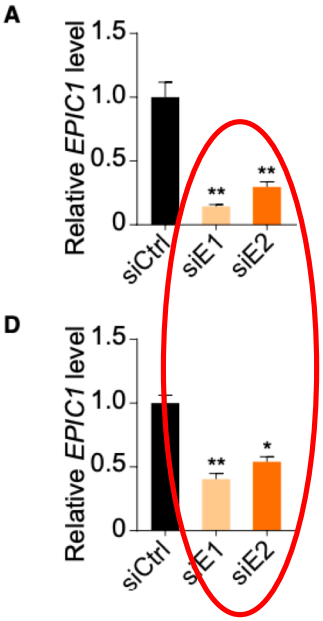
Designing 6 siRNAs in order to find someone that can readily knockdown *EPIC1* expression.

G

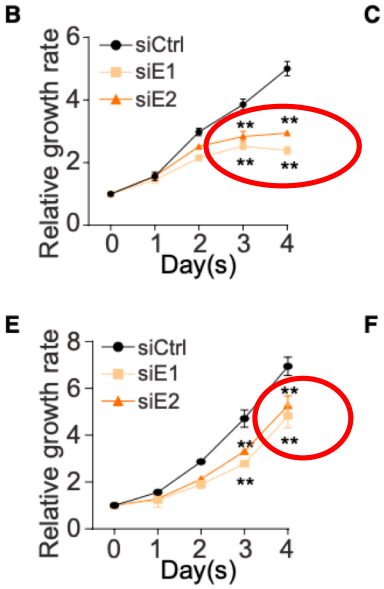


PHASE 2

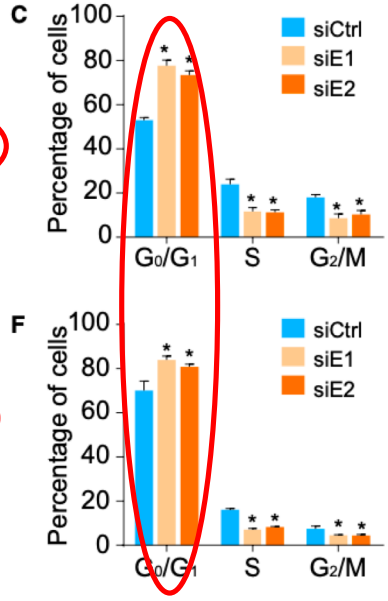
EPIC1 Functions as a Potential Oncogenic lncRNA by Promoting Cell-Cycle Progression



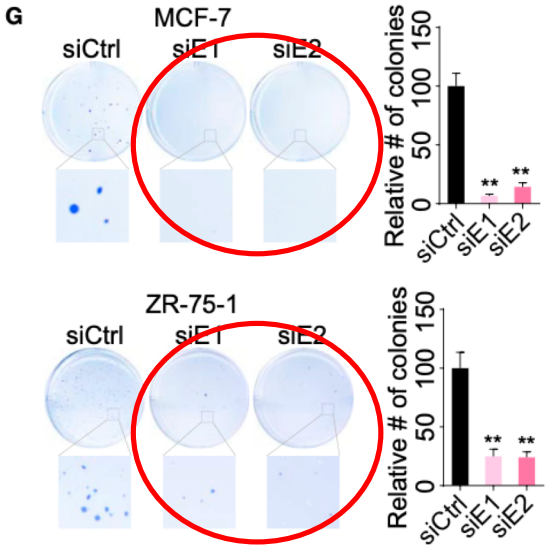
qRT-PCR analysis of EPC1



MTT assay



Cell cycle analys in cells treated with EPC1 siRNAs (siE1, siE2)



Anchorage indipendent colony formation assay of cells treated with EPC1 siRNAs

MCF-7

ZR-75-1

Conclusion:

***EPIC1* knockdown resulted in a decrease of cell proliferation in a time-dependent manner in cells MCF-7 and ZR-75-1 significantly inhibits the anchorage-independent growth of cancer cells. Moreover, resulted in G0/G1 arrest.**

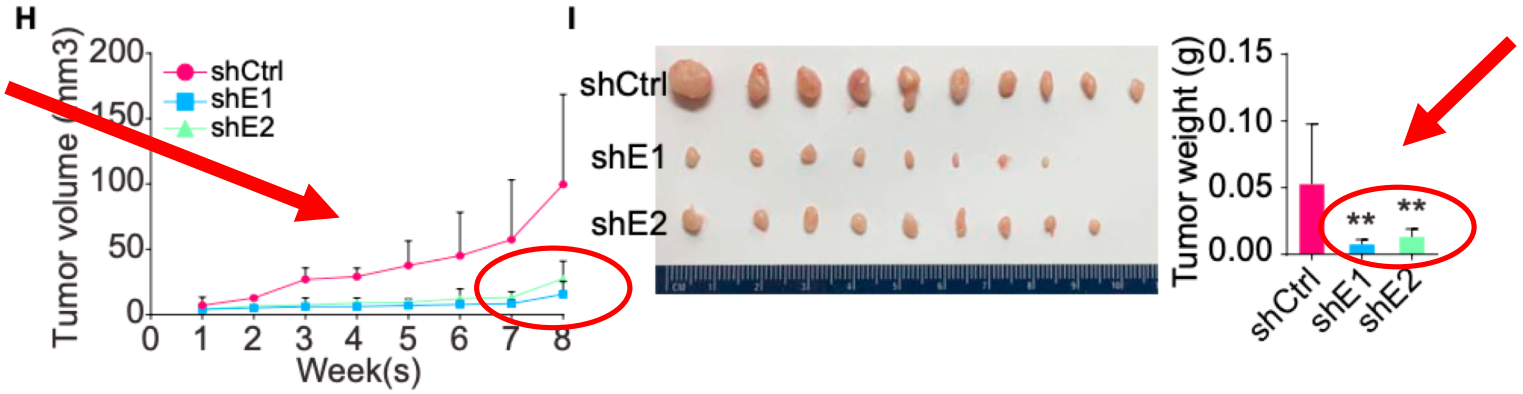
PHASE 2

EPIC1 Functions as a Potential Oncogenic lncRNA by Promoting Cell-Cycle Progression

Method: stabling *EPIC1* knockdown cells using lentiviral shRNAs.

significantly reduced cell proliferation

in vivo xenograft growth



Conclusion:

- **Oncogenic activity of *EPIC1 in vivo,***
- **Potential therapeutic target for breast cancer treatment.**

PHASE 3

EPIC1 Is a Nuclear lncRNA that Regulates MYC Targets

6° STEP

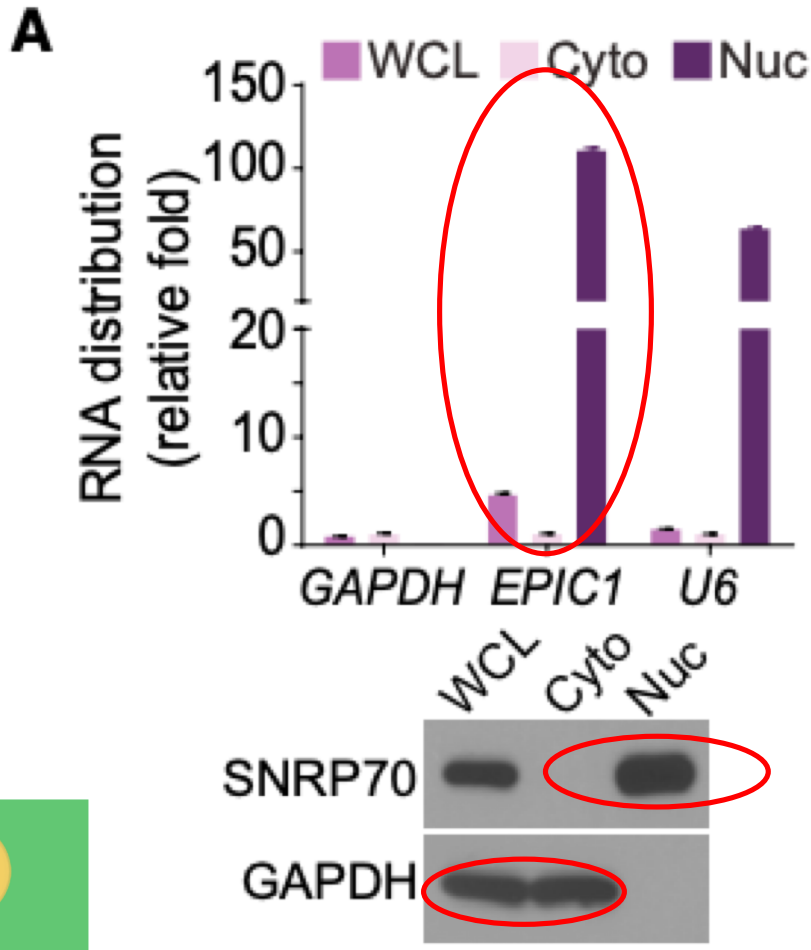
Aim: EPIC1 RNA is located in the nucleus

Methods:

- Cell fractionation PCR
- and subcellular RNA seq analysis

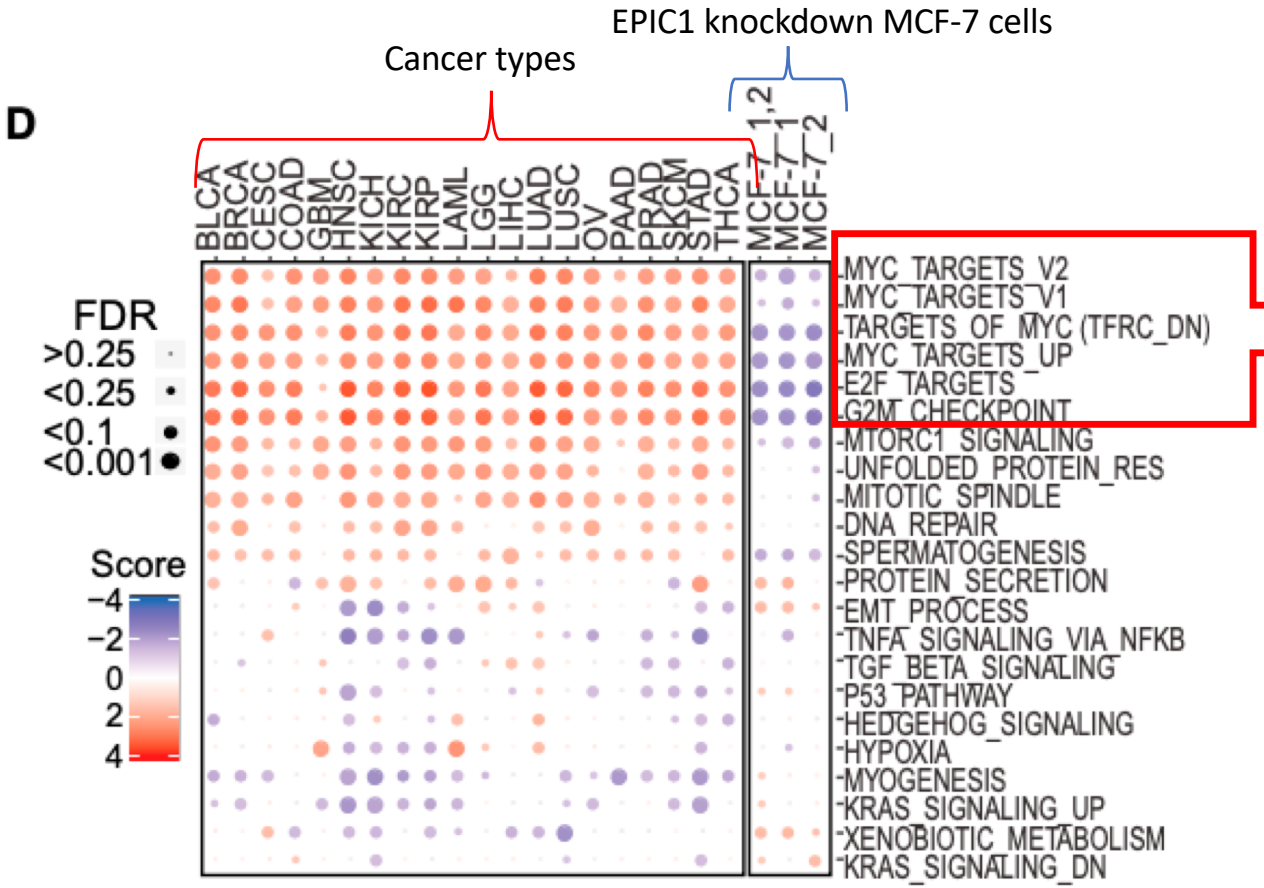
Conclusion:

EPIC1 might play a role in transcriptional regulation and chromatin interactions,



PHASE 3

EPIC1 Is a Nuclear lncRNA that Regulates MYC Targets



Methods: GSEA analysis

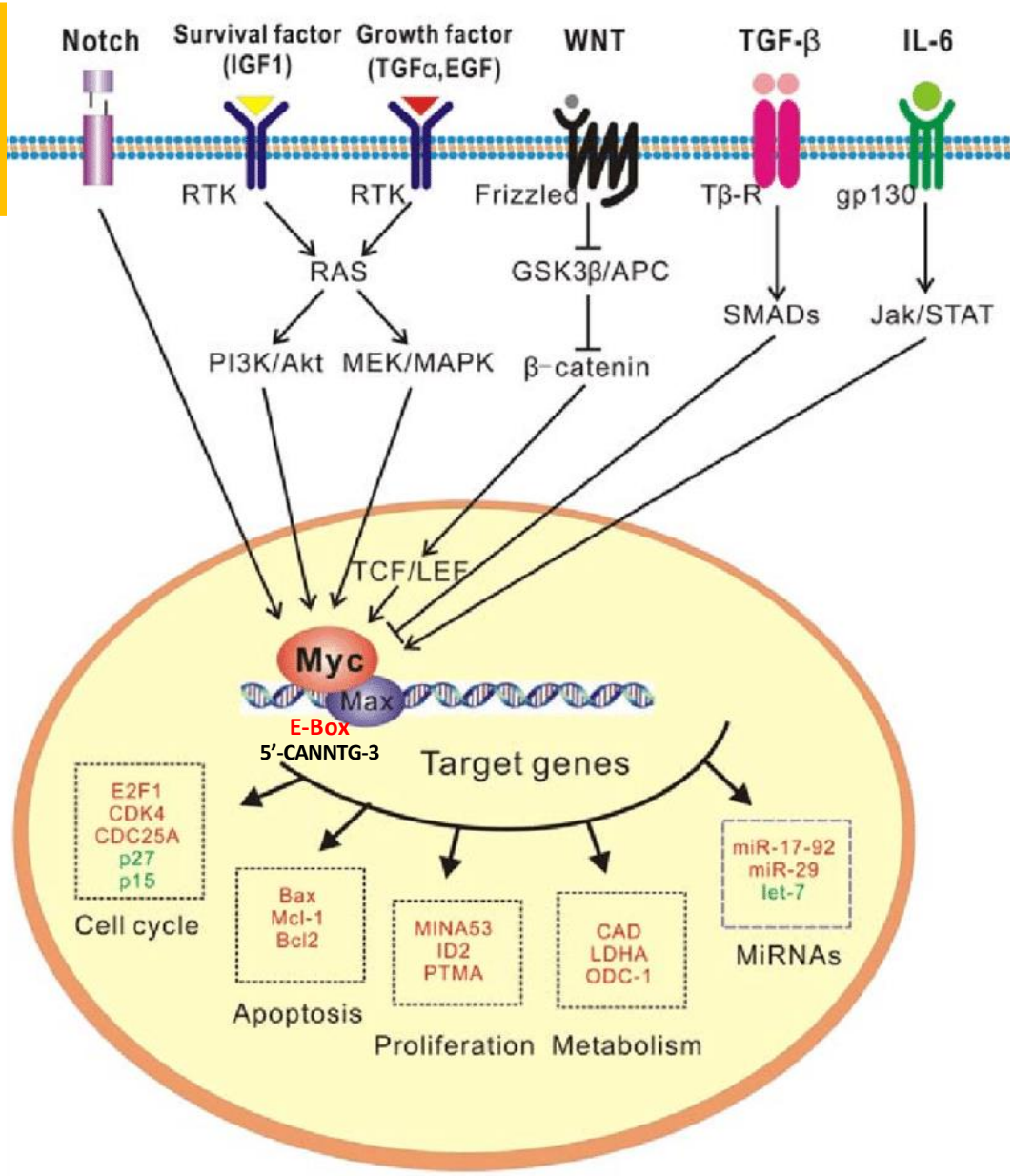
Conclusion:
Cell-cycle-related biological processes;
“MYC targets,”
“G2M checkpoint,”
“E2F targets”,

Enriched in the *EPIC1*-associated genes.

Just a little recap

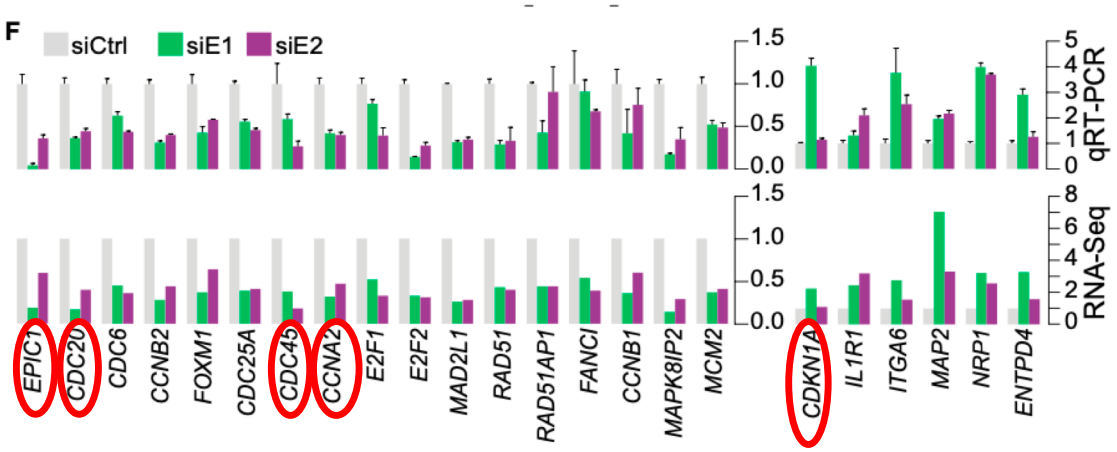
Myc

- In regulation cell growth and proliferation, the C-MYC protooncogene is depicted as a downsteam transduction pathway for receptor signaling that can cause positive or negative regulatory genes in C-MYC.
- The transcription factor C-MYC heterodimerizes with MAX and binds E-BOX (palindromic sequence 5’-CANNTG-3’) to regulate transcriptional proliferation of genes involved in cell growth and apoptosis.
- C-MYC is one of the most frequently mutated genes in tumors.



PHASE 3

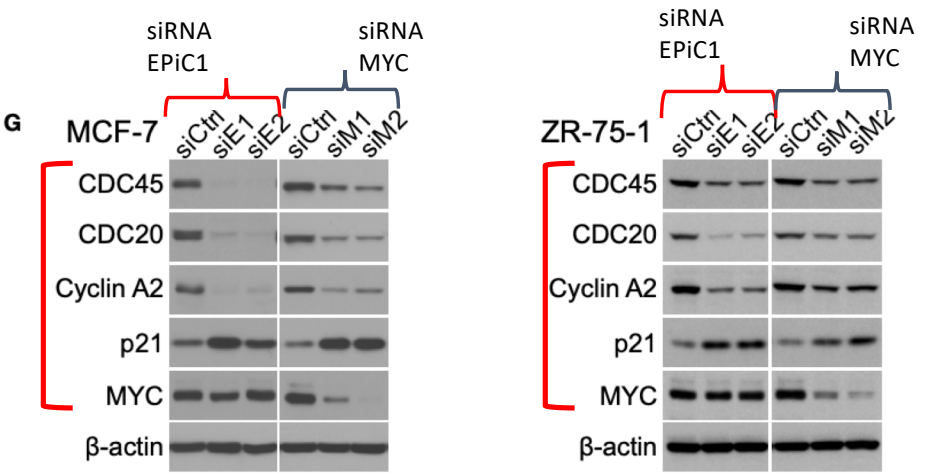
EPIC1 Is a Nuclear lncRNA that Regulates MYC Targets



EPIC1 knockdown have differentes conseguences;

- *CDC45*, *CDC20 CCNA2* downregulated,
- *CDKN1A* induced,
- cancer cells' arrest at G0/G1 phase.

MYC knockdown , in MCF-7 and ZR-75-1 cells, also led to a pattern of MYC target expression and cell growth comparable with *EPIC1* knockdown.

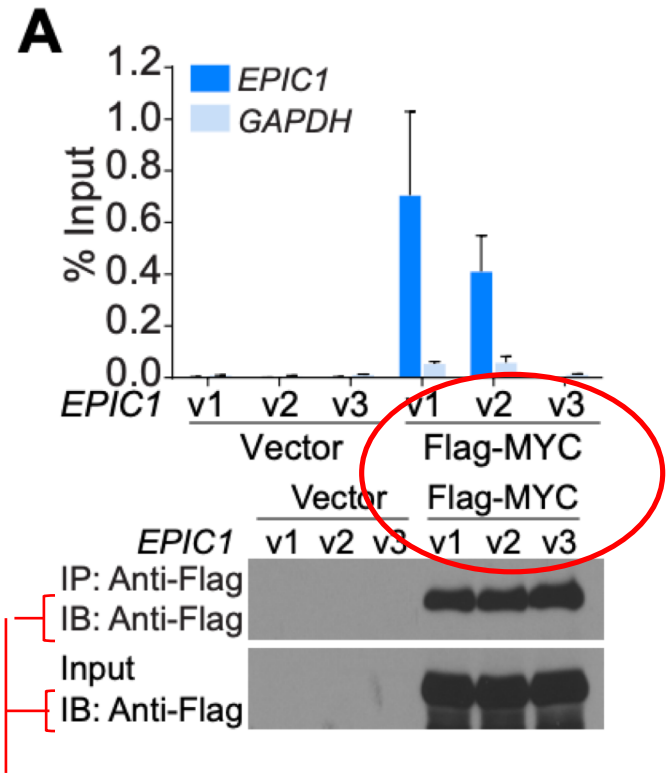


Conclusion:

The oncogenic role of *EPIC1* may be associated with MYC protein.

PHASE 3

EPIC1 Interacts with the 148–220 Amino Acid Region of MYC through Its 129–283 nt Sequence



IB=immunoblotting

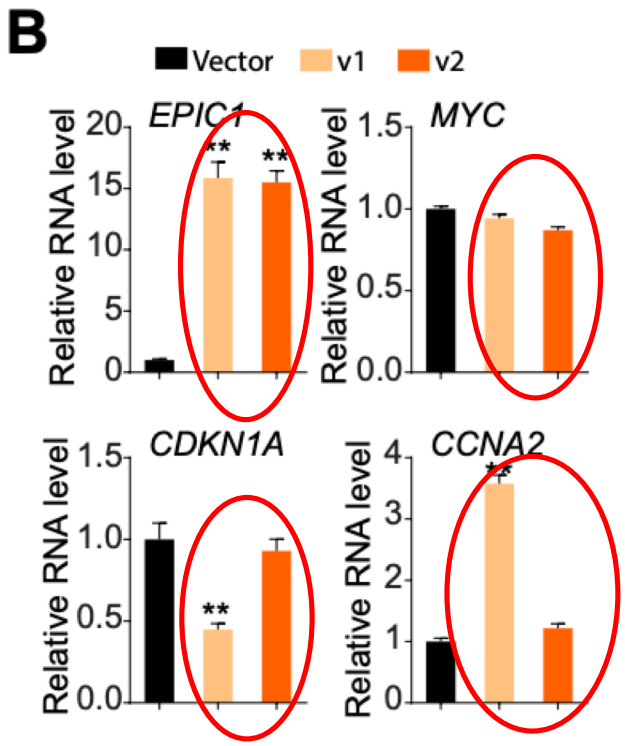
7° STEP

Aim: Interaction between *EPIC1* RNA and MYC protein,

Overexpressing v1, v2, v3 with Flag-tagged MYC protein,

Method: Performed RIP assay,

Conclusion:



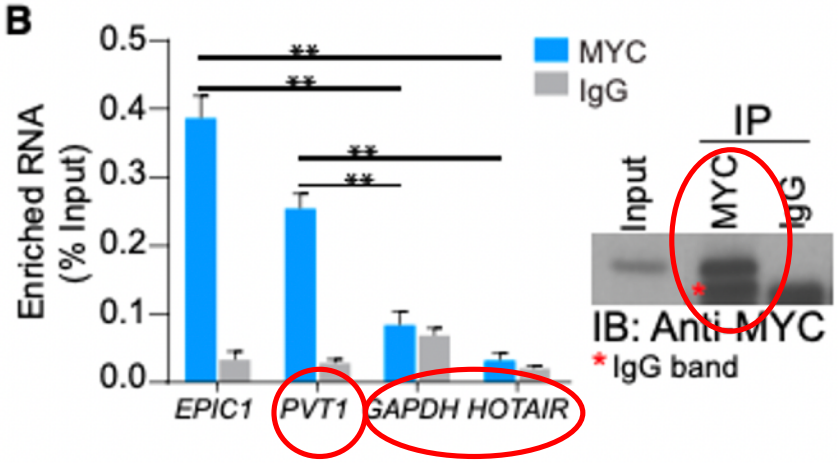
***EPIC1* isoforms v1 is the functional isoform of *EPIC1* gene in breast cancer.**

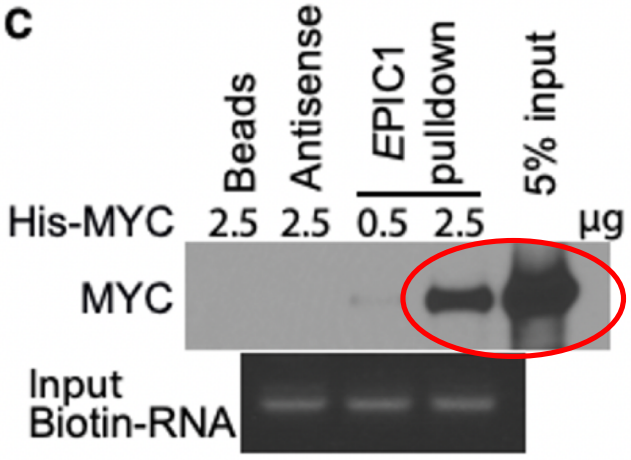
Method 1 : MYC RIP with cell lysates from MCF-7 cells

Conclusion 1:
confirm the interaction between endogenous EPIC1 and MYC protein

Method 2: in vitro binding assay using in-vitro-transcribed EPIC1 RNA and recombinant His- tagged MYC protein

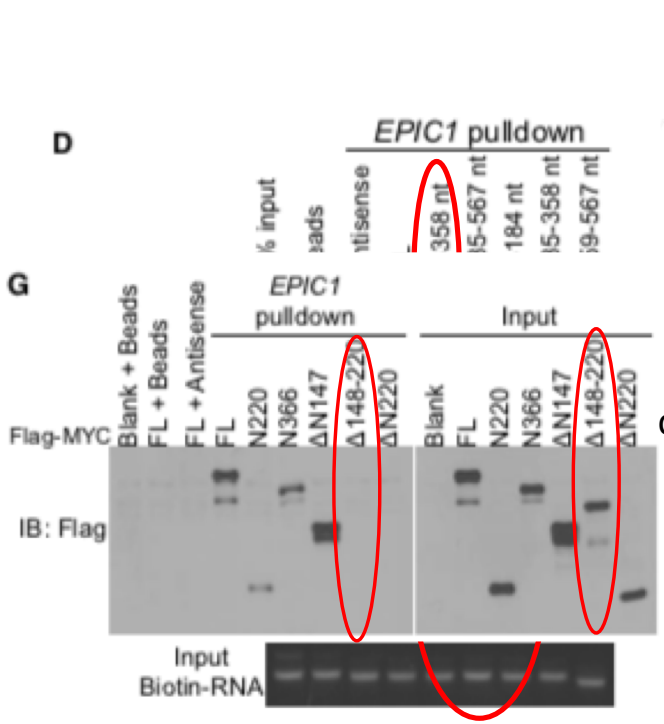
Conclusion 2:
EPIC1 binds directly to MYC protein





PHASE 3

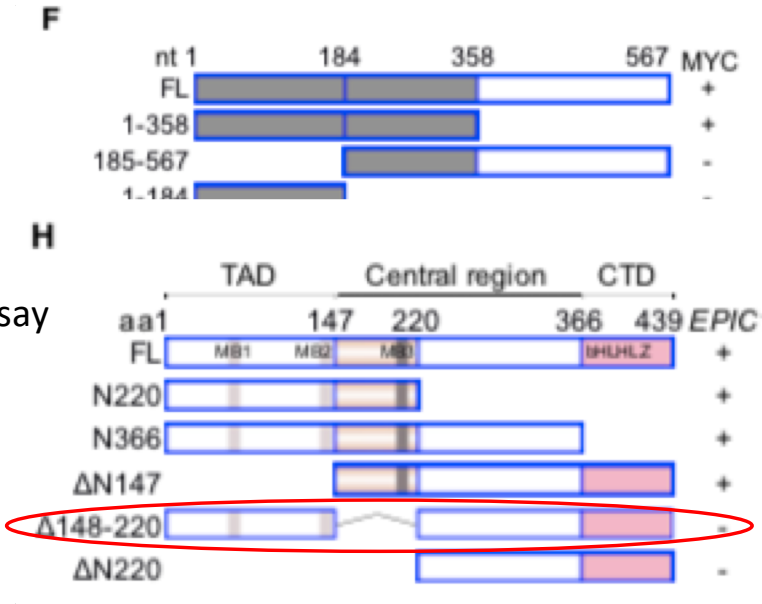
EPIC1 Interacts with the 148–220 Amino Acid Region of MYC through Its 129–283 nt Sequence



8° STEP

Detecting if EPIC1 binds directly MYC,

conducting an *in vitro* RNA pull-down assay using a series of truncated *EPIC1* fragments,



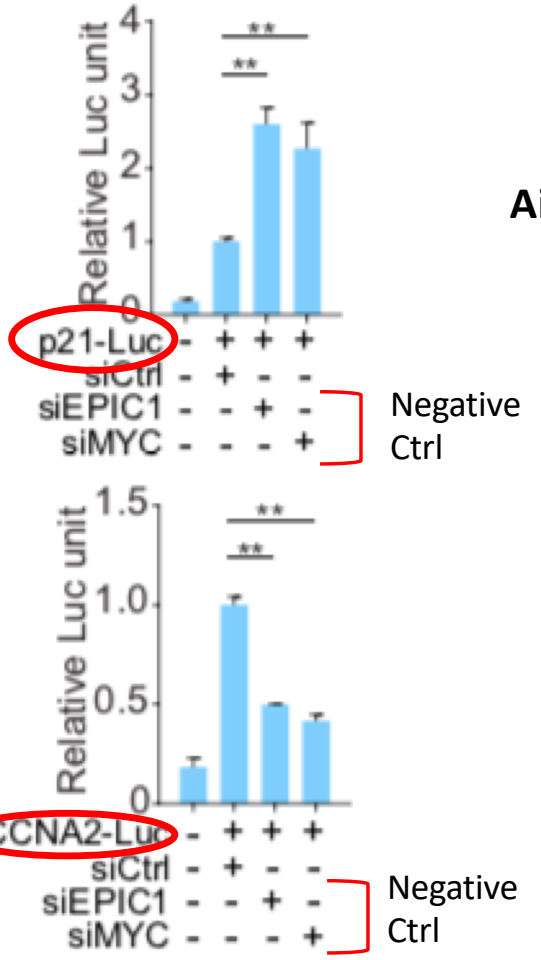
Conclusion:

- **Deletion of 129–283 nt abolished *EPIC1*'s interaction with MYC protein,**
- **Deletion of the 148–220 aa region of MYC protein abolished its interaction with *EPIC1*.**

PHASE 3

The oncogenic role of EPIC1 partially depends on its regulation of MYC occupancy on target promoters

A



9° STEP

Aim: Analyzing the effect of *EPIC1* on MYC target gene reporters (*p21* and *CCNA2* promoters) in MCF-7 cells,

Method: reporter assays

Conclusion:

***EPIC1* or *MYC* significantly regulates p21-Luc and CCNA2-Luc reporter luciferase activities,**

.

***EPIC1* may regulate the transcriptional activity of the MYC protein**

PHASE 3

The oncogenic role of EPIC1 partially depends on its regulation of MYC occupancy on target promoters

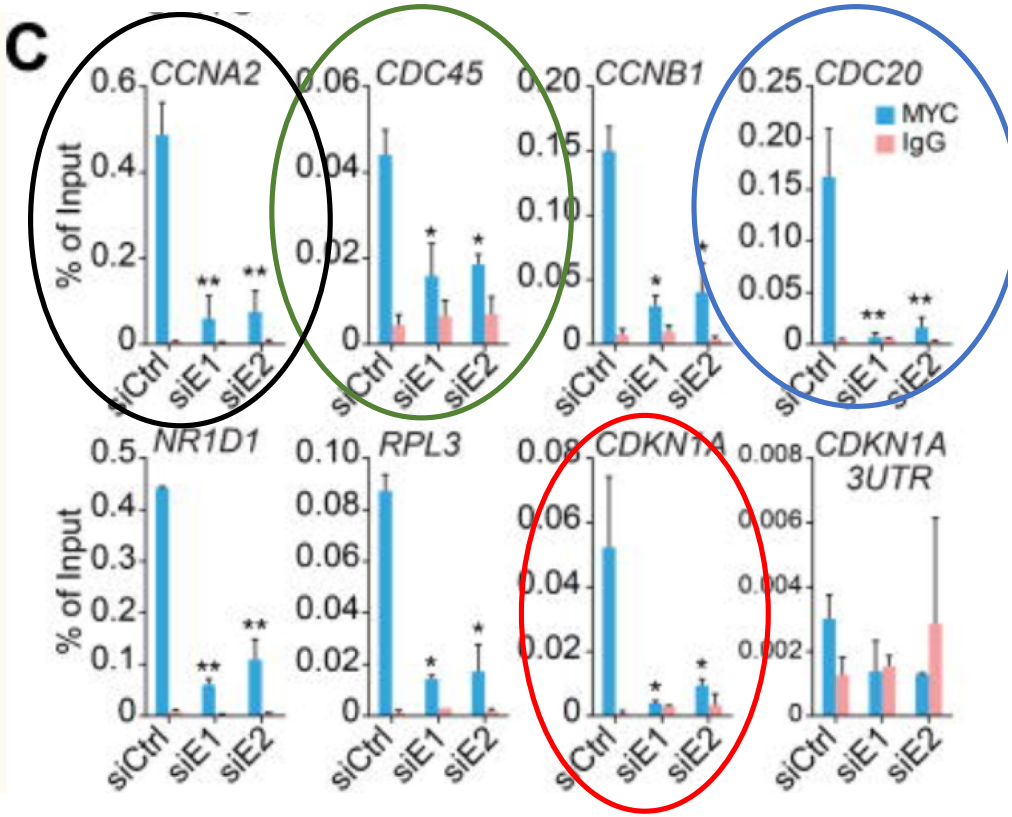
Method 1: integrated analysis on MYC ChIP-seq data and RNA-seq data of *EPIC1* knockdown MCF-7 cells,

Conclusion 1:
no correlation between global MYC binding affinity and differential expression, so, *EPIC1* regulate MYC's occupancy on a specific group of targets,

Method 2: ChIP-qPCR of *EPIC1* knockdown

Conclusion 2:
significantly reduction MYC's occupancies on the promoters of

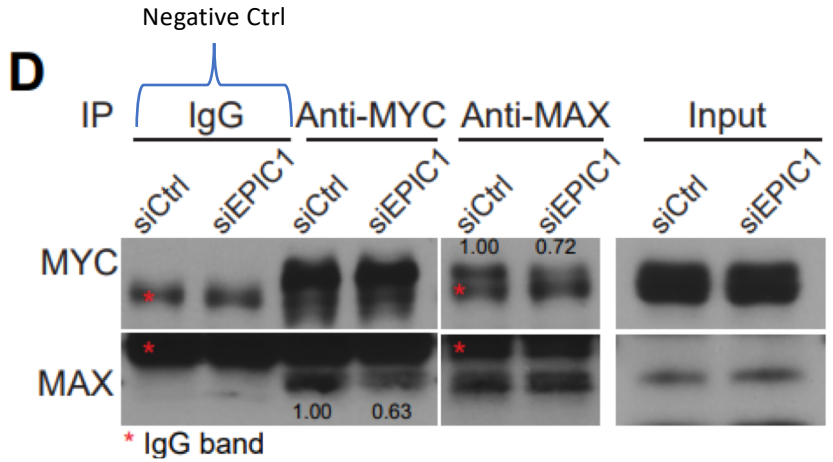
- *CDKN1A* (*p21*),
- *CCNA2*,
- *CDC20*,
- *CDC45*,



PHASE 3

The oncogenic role of EPIC1 partially depends on its regulation of MYC occupancy on target promoters

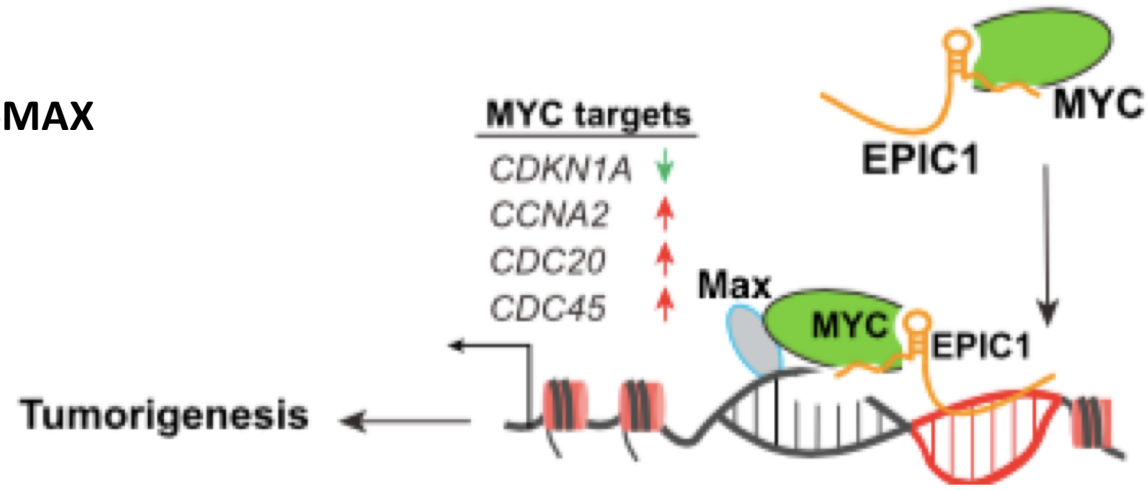
MYC binds to DNA and functions as a transcription factor by heterodimerization with another transcription factor, MAX



Method : MYC and MAX Co-IP assay in MCF-7 cells

Conclusion:

***EPIC1* knockdown reduce the formation of MYC-MAX complexes.**



PHASE 3

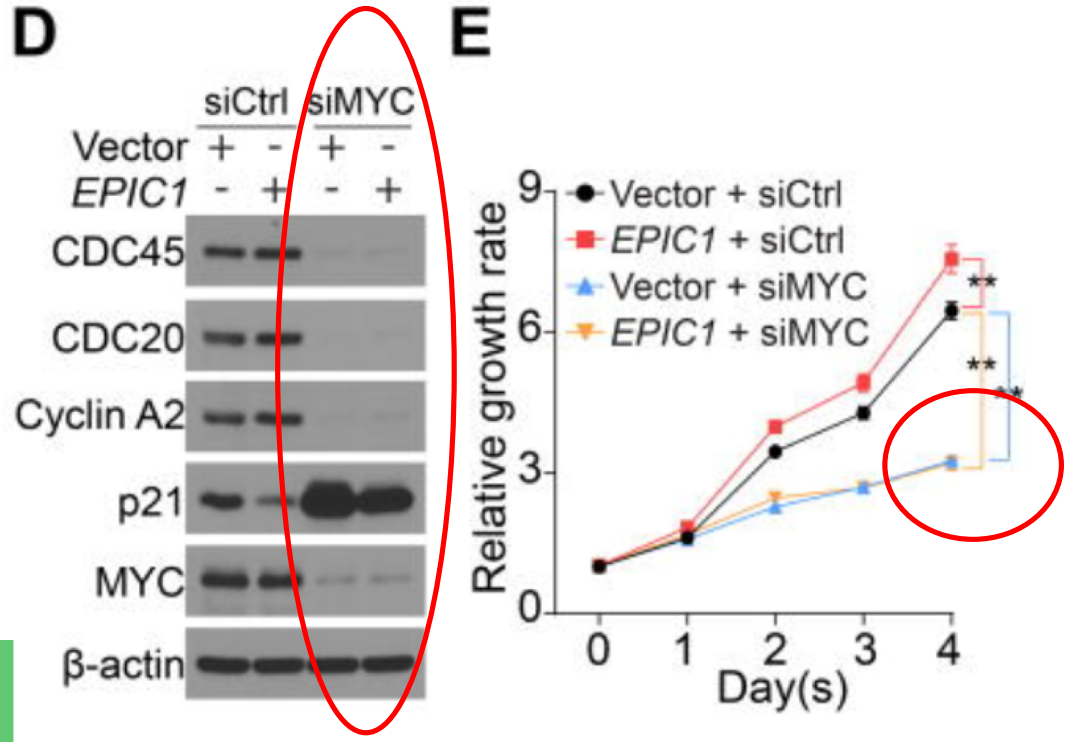
The oncogenic role of EPIC1 partially depends on its regulation of MYC occupancy on target promoters

STEP 10°

Aim: Determine the role of the *EPIC1*-MYC regulatory axis in cancer,

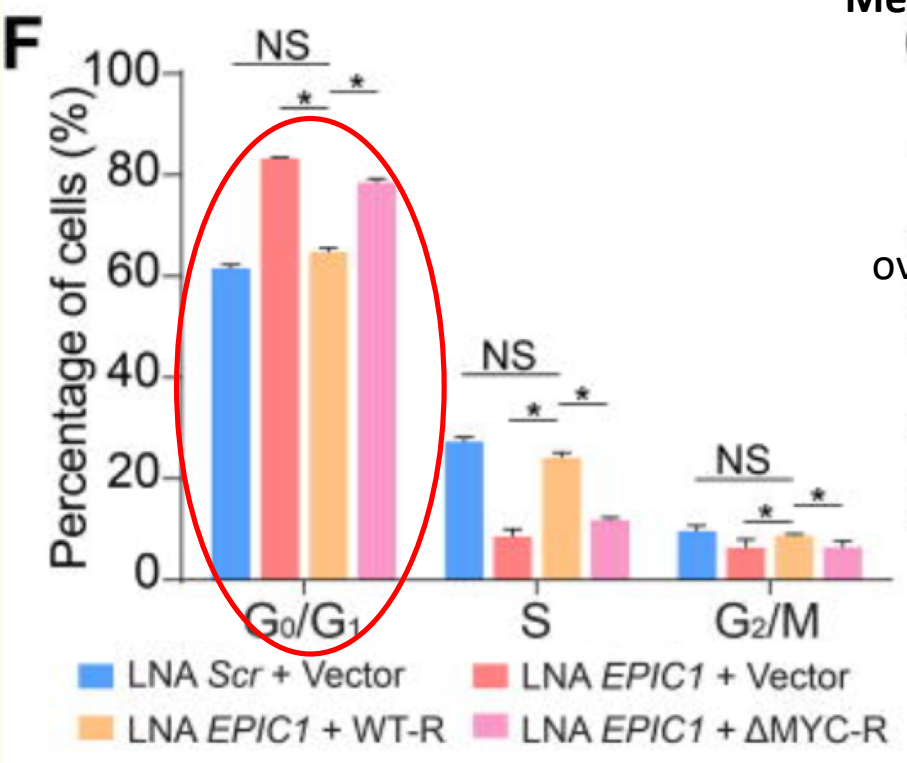
Method: MYC knockdown in *EPIC1* stably overexpressing MCF-7 cells

Conclusion:
***EPIC1* regulation of cell proliferation and MYC target expression were attenuated by MYC knockdown.**



PHASE 3

The oncogenic role of EPIC1 partially depends on its regulation of MYC occupancy on target promoters



Method: depleting the endogenous *EPIC1* expression using LNA in MCF-7 cells,

overexpression of LNA-resistant WT- *R-EPIC1* or deletion mutant of 129–283 nt MYC binding region;

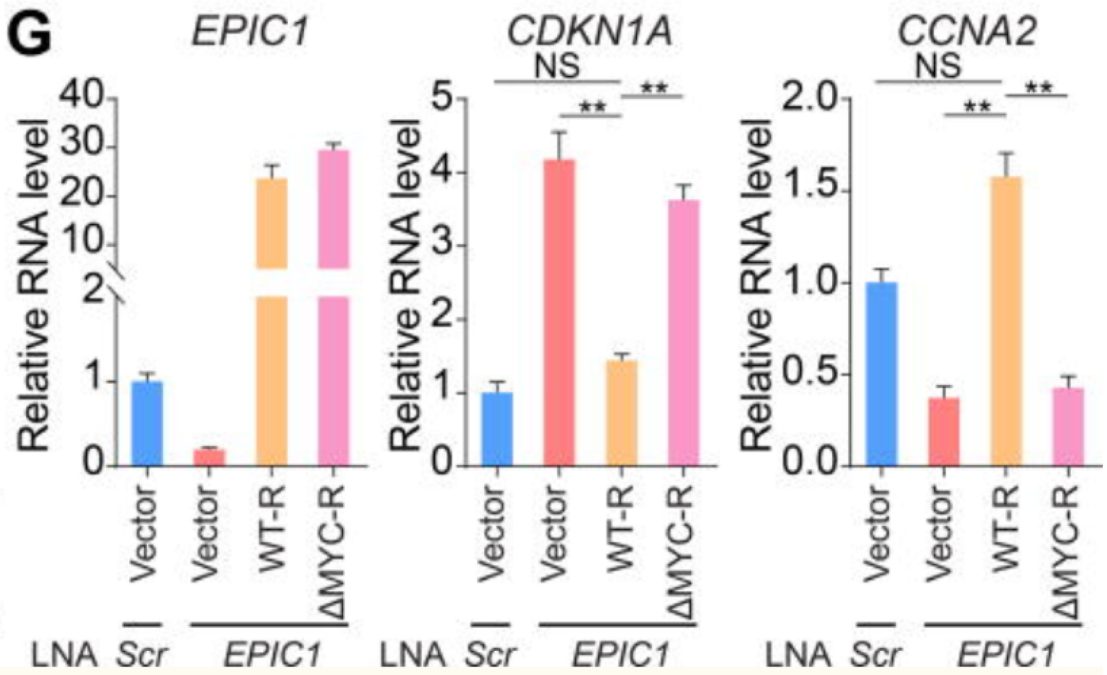
Conclusion:

- **LNA locked nucleic acid knockdown of *EPIC1* significantly caused G1 arrest of MCF-7 cells.**
- **LNA knockdown of *EPIC1* curtailed the expression of MYC target genes.**

Method: Reintroduction of wild-type *EPIC1*, but not Δ*MYC-EPIC1,* rescue the regulation of these genes,

Conclusion:

Oncogenic role of *EPIC1* in part dependent on its interaction with the MYC protein.



Conclusion

- Integrating HM450 microarray and RNA-seq data is a cost-effective strategy to research the DNA methylation regulation of lncRNA genes
- lncRNA
  methy
- EA lnc
  - in
    a
  - e
  - influence Myc occupancy on canonical MYC MAX binding site, but not the non canonical MYC binding site
  - may function as a ‘‘guide’’ RNA to facilitate MYC-MAX’s regulation on specific targets

TAKE-HOME MESSAGE

The establishment of a detailed knowledge base of the DNA methylation-altered lncRNAs in cancer will facilitate the identification of cancer-driving lncRNAs.
This can help to pave the way to develop cancer therapies.

THANK YOU FOR YOUR ATTENTION!

ANY QUESTIONS?

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