lncRNA MAGI2-AS3 suppresses castration-resistant prostate cancer proliferation and migration via the miR-106a-5p/RAB31 axis

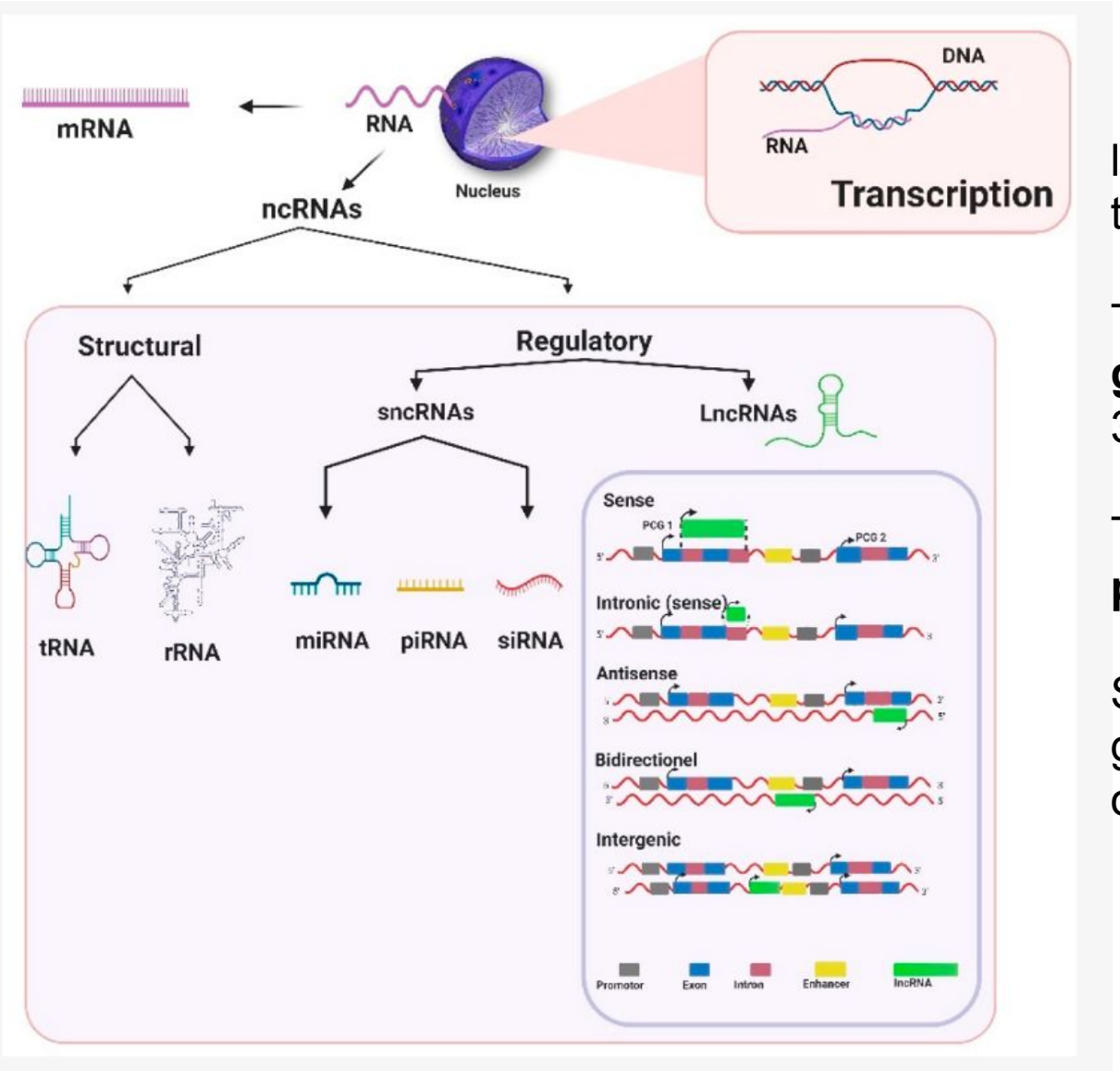
Nguyen Minh Hoang

Tran Ba Thien

10/07/2023

Contents

WHAT IS LONG NON-CODING RNAs (lncRNAs)?



Classification of non-coding RNAs (ncRNAs).

lncRNAs are defined as RNAs **longer than 200 nucleotides** that are **not translated** into functional **proteins.**

The resulting lncRNAs are often **capped by 7-methyl guanosine (m7G)** at their 5′ ends, **polyadenylated** at their 3′ ends and **spliced similarly to mRNAs.**

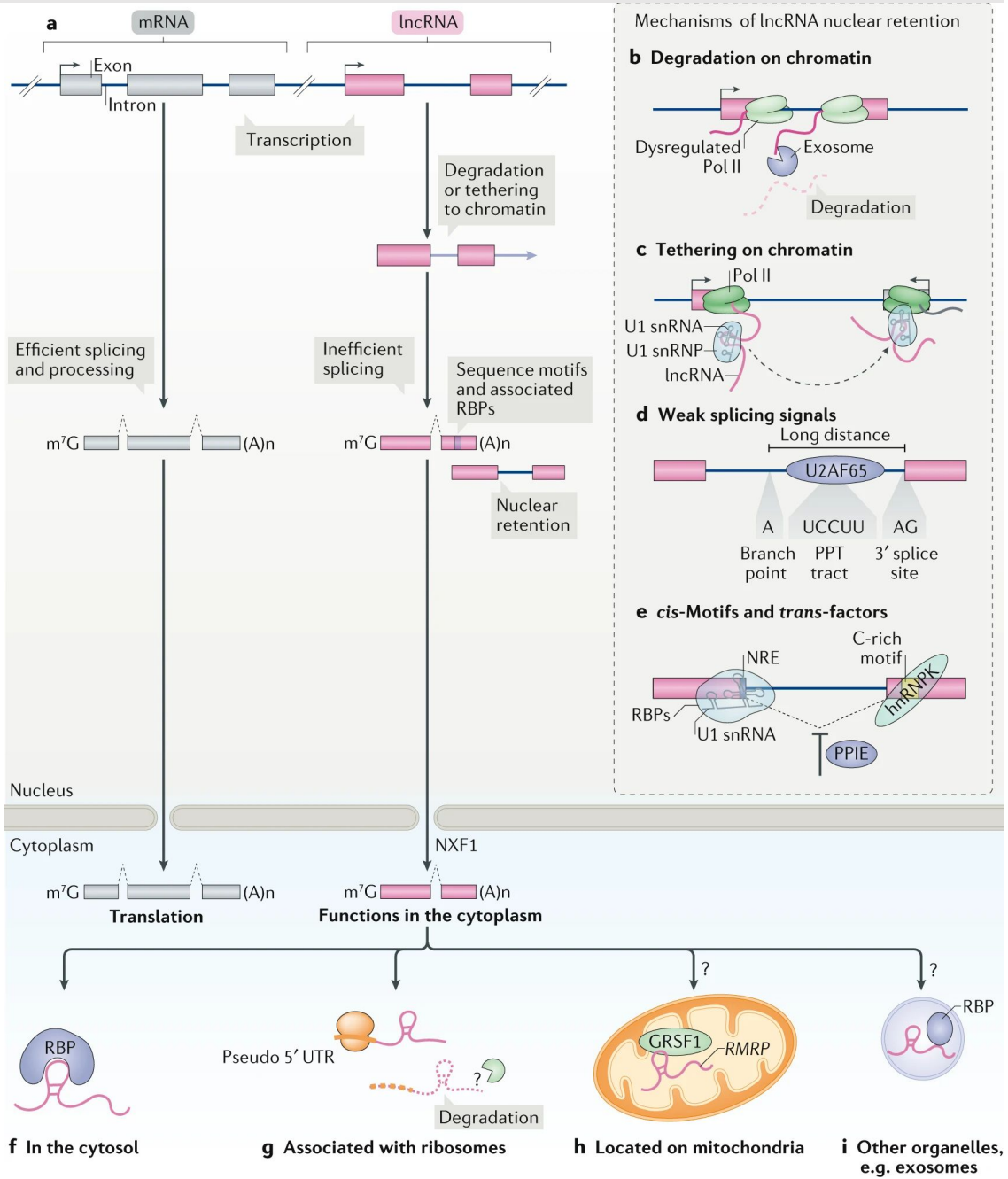
These mainly include lncRNAs transcribed by **RNA polymerase II (Pol II).**

Statistics from **Human GENCODE** suggest that the human genome contains more than **16,000 lncRNA genes,** but other estimates exceed **100,000 human lncRNAs.**

https://doi.org/10.3390/life11010056

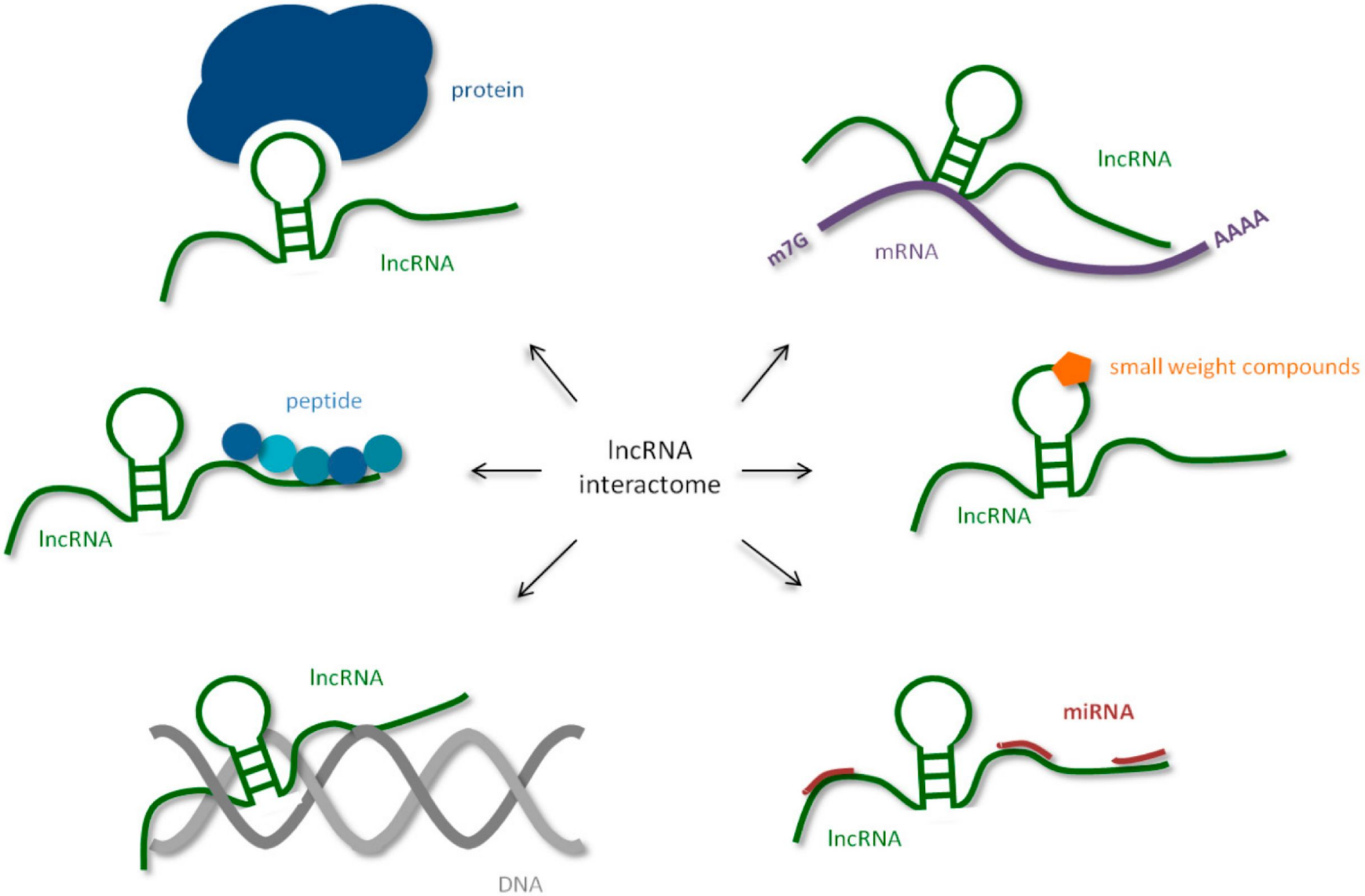
Biogenesis and cellular fates of long non-coding RNAs

- LncRNA is in the nucleus, whereas others are spliced and exported to the cytoplasm. A large fraction of lncRNAs are exported to the cytosol
- lncRNAs are spliced less efficiently than mRNA.



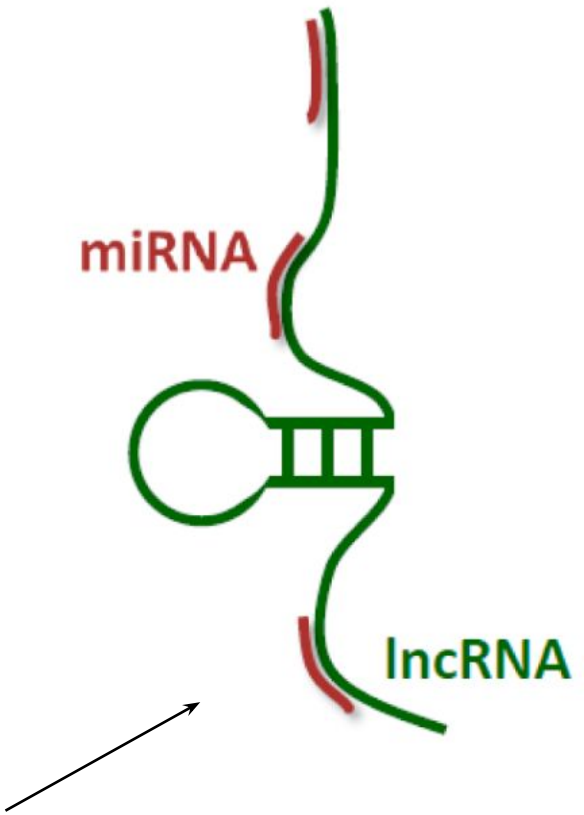
https://doi.org/10.1038/s41580-020-00315-9

Interaction of human lncRNA with cellular biomolecules.



https://www.mdpi.com/1422-0067/21/3/1027

Interactions of LncRNA with miRNAs, the ceRNA Hypothesis



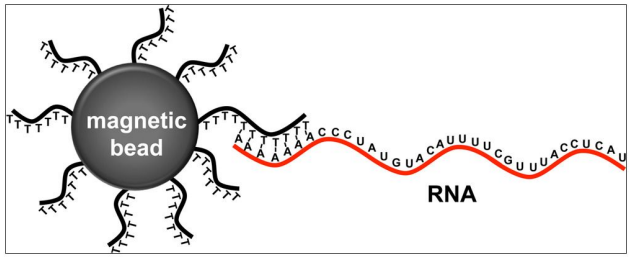
- ncRNAs and miRNAs influence each other -> ceRNA (a competitive, endogenous RNA)
- By sharing identical MREs and competing for common miRNAs, they change miRNA's activity, which results in modified mRNA translation
- lncRNA-associated miRNA have been conducted for many diseases, such as ovarian and prostate cancer, glioblastoma, thyroid carcinoma, as well as breast, lung, kidney and gut cancers

10.3390/ijms21031027

HOW TO DETECT OF LNCRNA

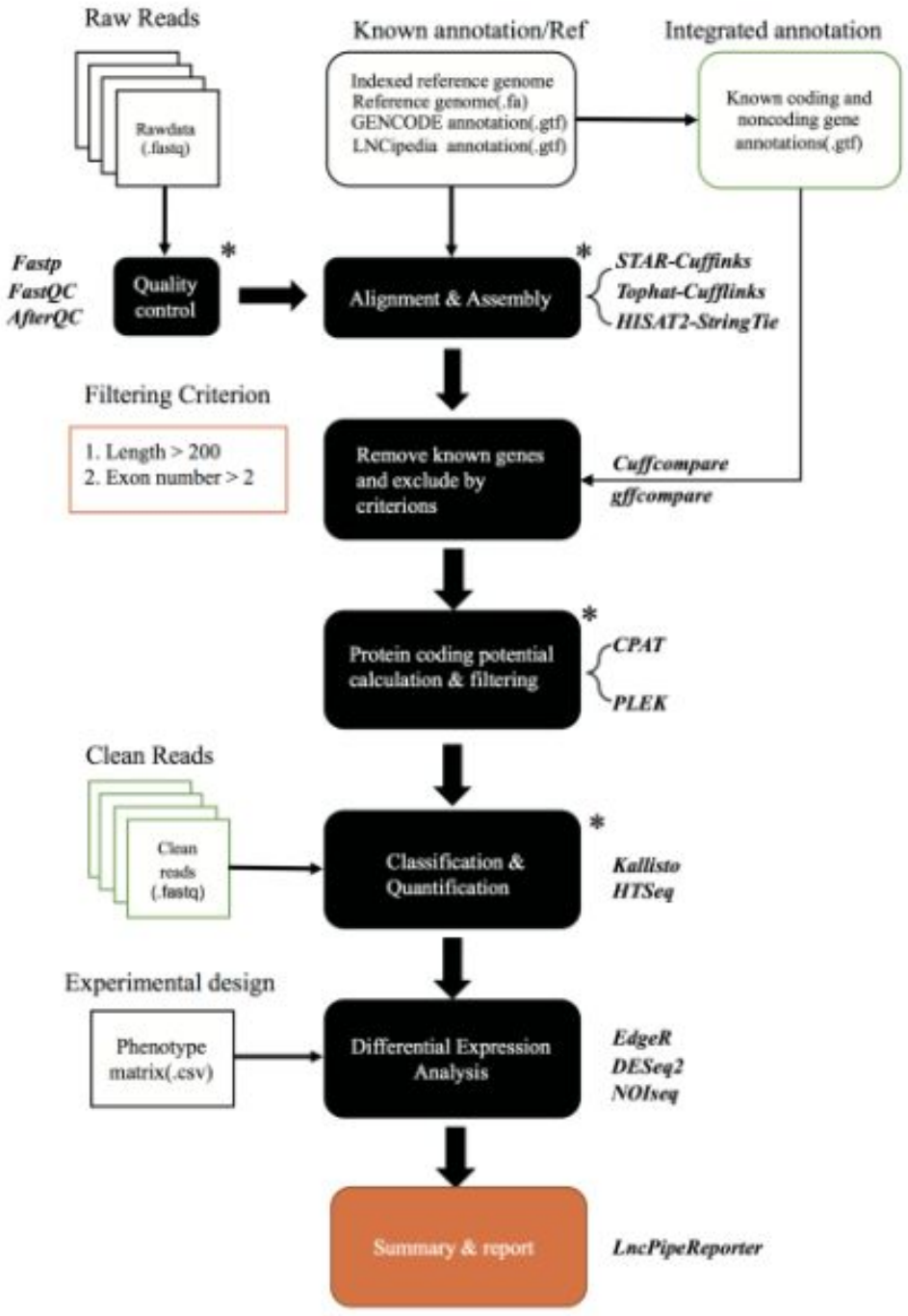
Detecting the expression of long non-coding RNAs (lncRNAs) can be accomplished using various experimental techniques.

- **RNA sequencing (RNA-seq)**
- **Microarray analysis**
- Reverse transcription quantitative polymerase chain reaction (RT-qPCR)
- Northern blotting:
- In situ hybridization (ISH)



10.3390/ht6030012

PIPELINE FOR ANALYSIS



https://nf-co.re/lncpipe

DATABASES RELATED TO LNCRNAS

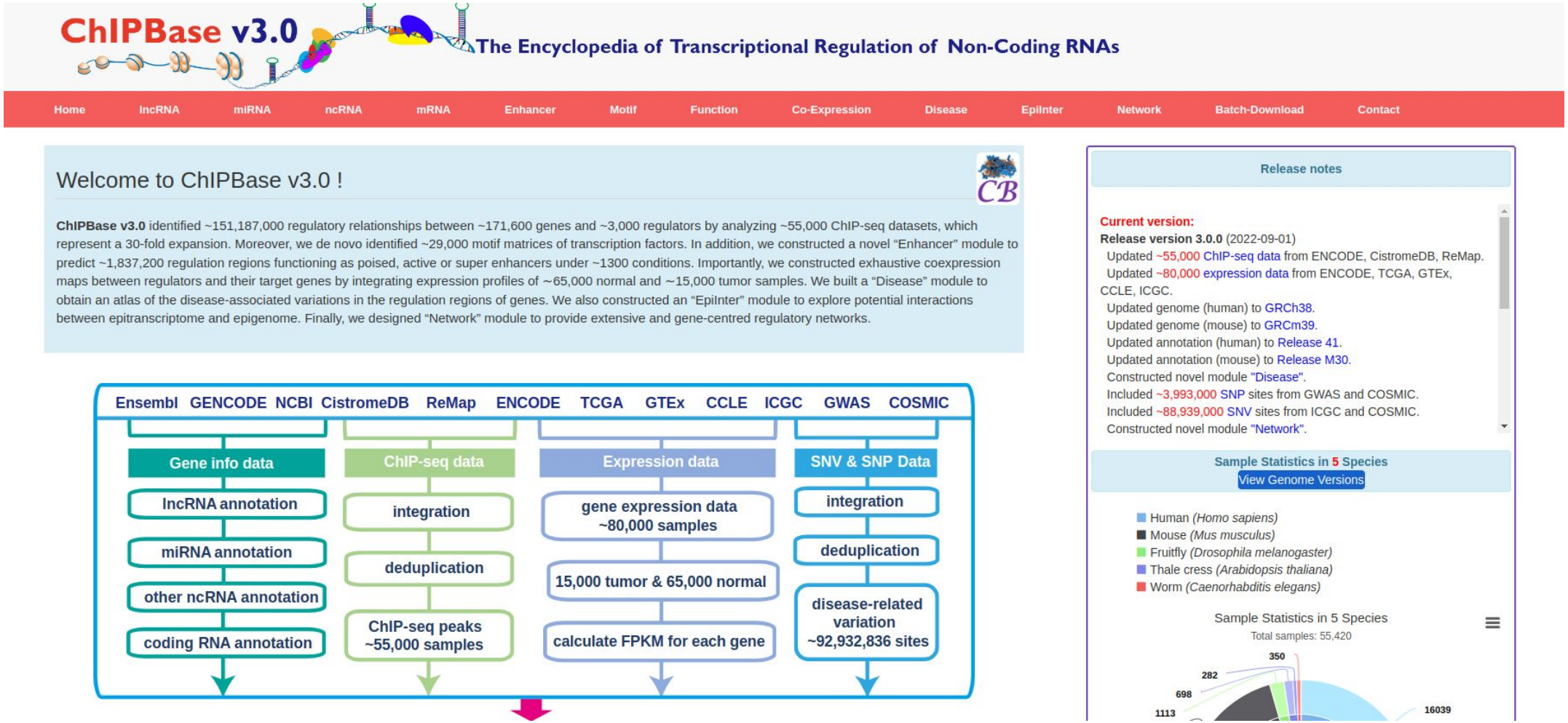
TABLE 1. lncRNA databases evaluated

Database	Description	Website	Reference
CHIPBase	Database for decoding the transcriptional regulation of long noncoding RNA and microRNA genes from ChIP-Seq data	deepbase.sysu.edu.cn/chipbase	Yang et al. (2013b)
DIANA-LncBase	Experimentally verified and computationally predicted microRNA targets on long noncoding RNAs	diana.imis.athena-innovation.gr	Paraskevopoulou et al. (2013)
LNCipedia	A database for annotated human lncRNA transcript sequences and structures	www.lncipedia.org	Volders et al. (2013)
lncRNAdb	Database providing comprehensive annotations of eukaryotic long noncoding RNAs	www.lncrnadb.org	Amaral et al. (2011)
lncRNADisease	Experimentally supported lncRNAs-disease associations	cmbi.bjmu.edu.cn/lncrnadisease	Chen et al. (2013)
lncRNome	Comprehensive database of long noncoding RNAs in humans	genome.igib.res.in/lncRNome	Bhartiya et al. (2013)
Noncode v3.0	Integrative annotation of long noncoding RNAs	noncode.org/NONCODERv3	Bu et al. (2012)
The Functional lncRNA Database	Database of lncRNAs manually extracted from the literature along with a parallel database containing all annotated protein-coding human RNAs	www.valadkhanlab.org	Niazi and Valadkhan (2012)

10.1261/rna.044040.113

DATABASES RELATED TO LNCRNAS

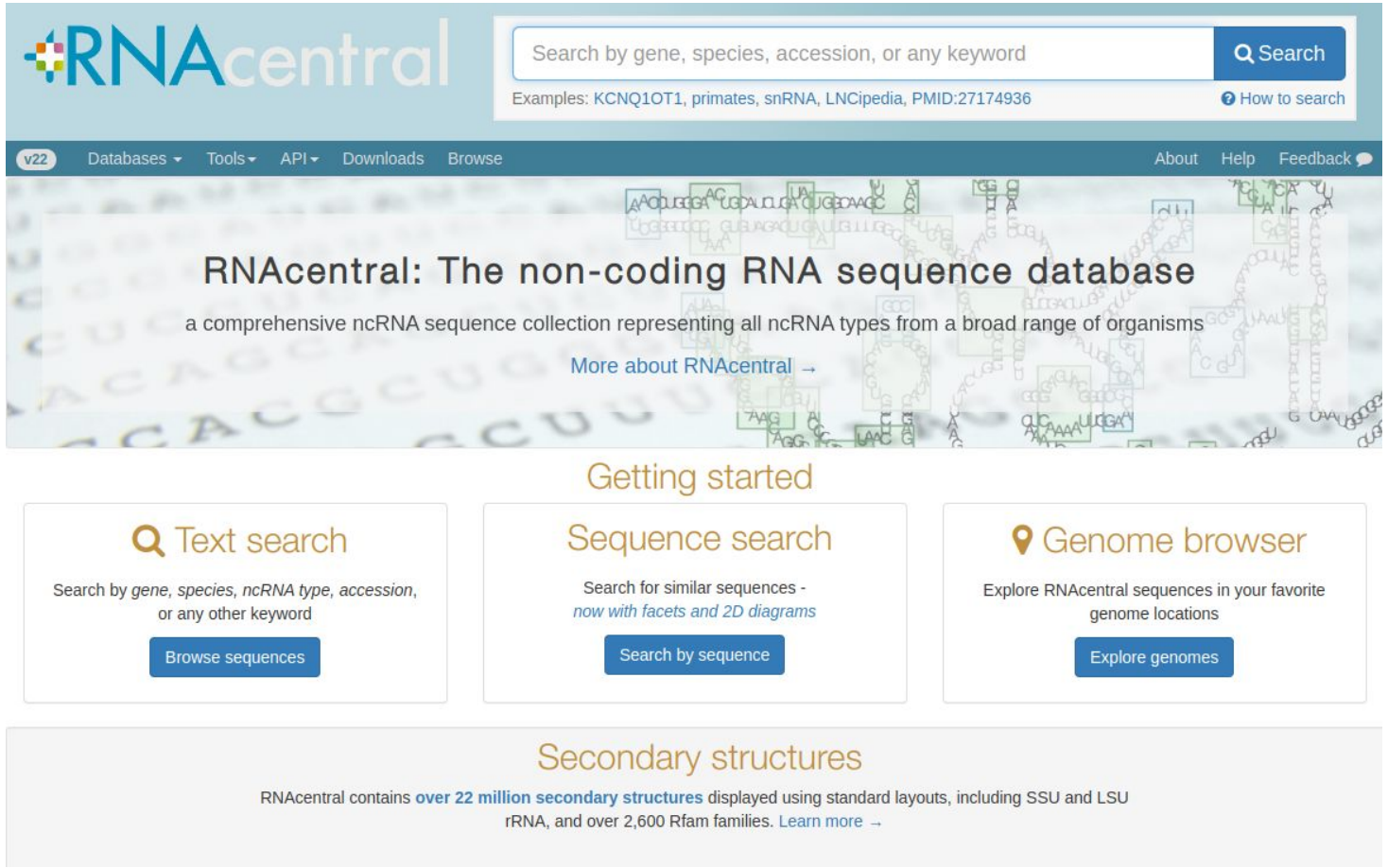
- ChIPBase



https://rnasysu.com/chipbase3/lncRNA.php

DATABASES RELATED TO LNCRNAS

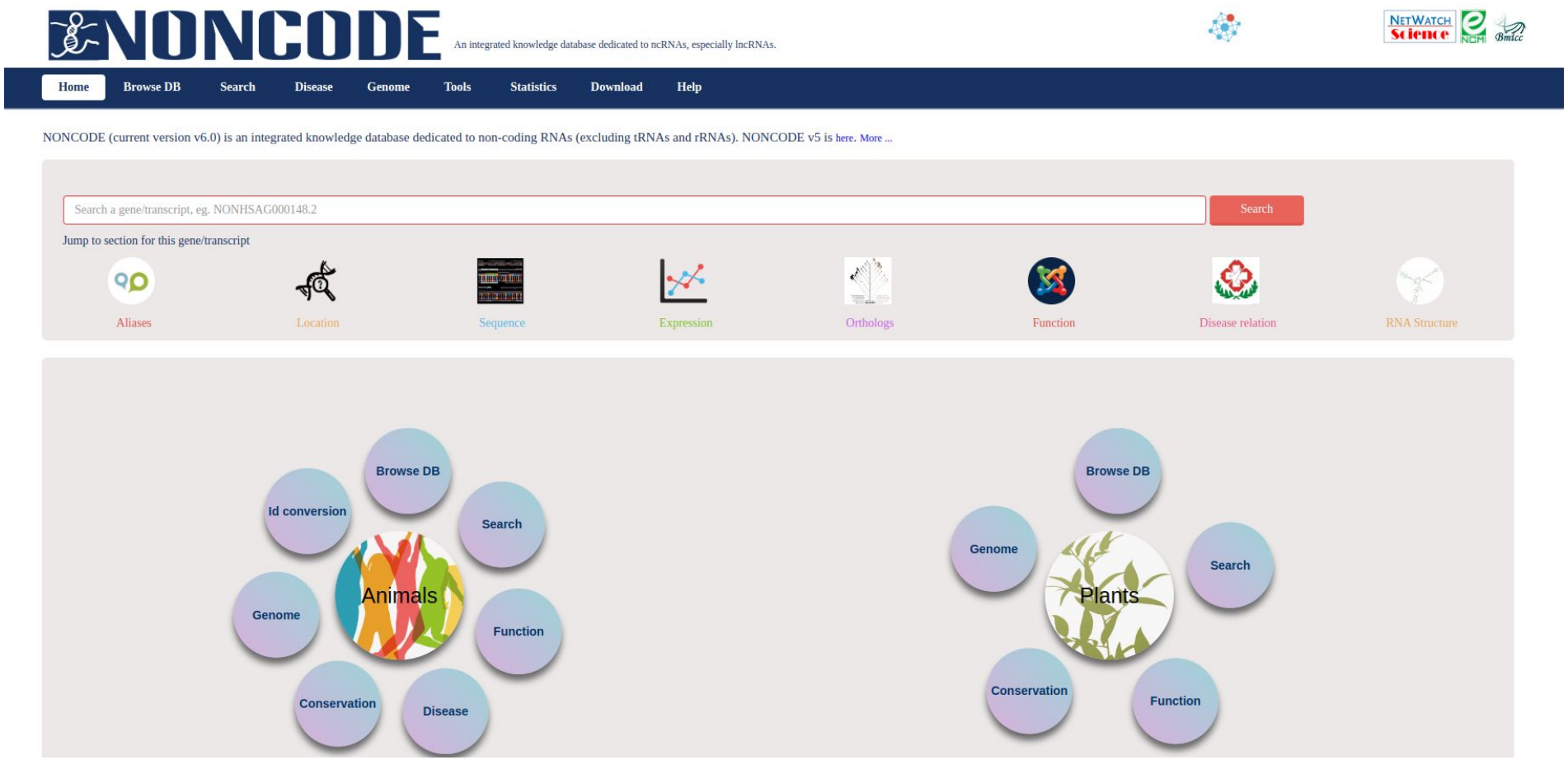
lncRNAdb



https://rnacentral.org/

DATABASES RELATED TO LNCRNAS

NONCODE



http://www.noncode.org/

DATABASES RELATED TO LNCRNAS

LncRNADisease

http://www.rnanut.net/lncrnadisease/

Example for analysis of lncRNA

Genomics 115 (2023) 110599

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

lncRNA MAGI2-AS3 suppresses castration-resistant prostate cancer proliferation and migration via the miR-106a-5p/RAB31 axis

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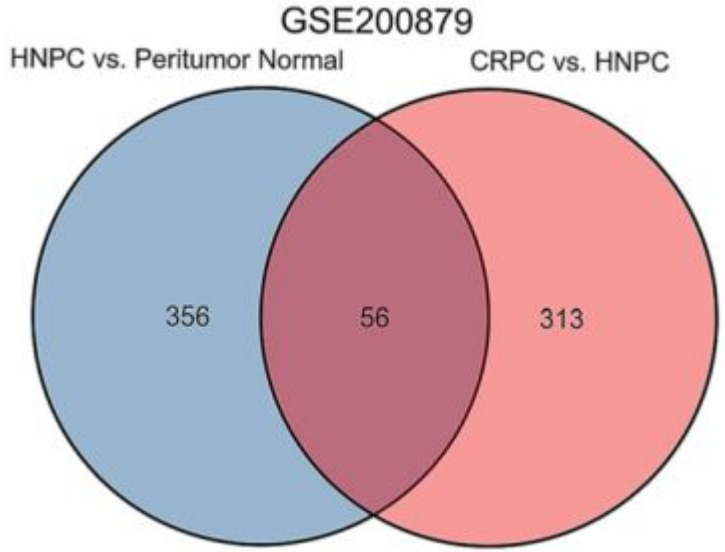
Volume 115, Issue 2, March 2023, 110599

INTRODUCTION

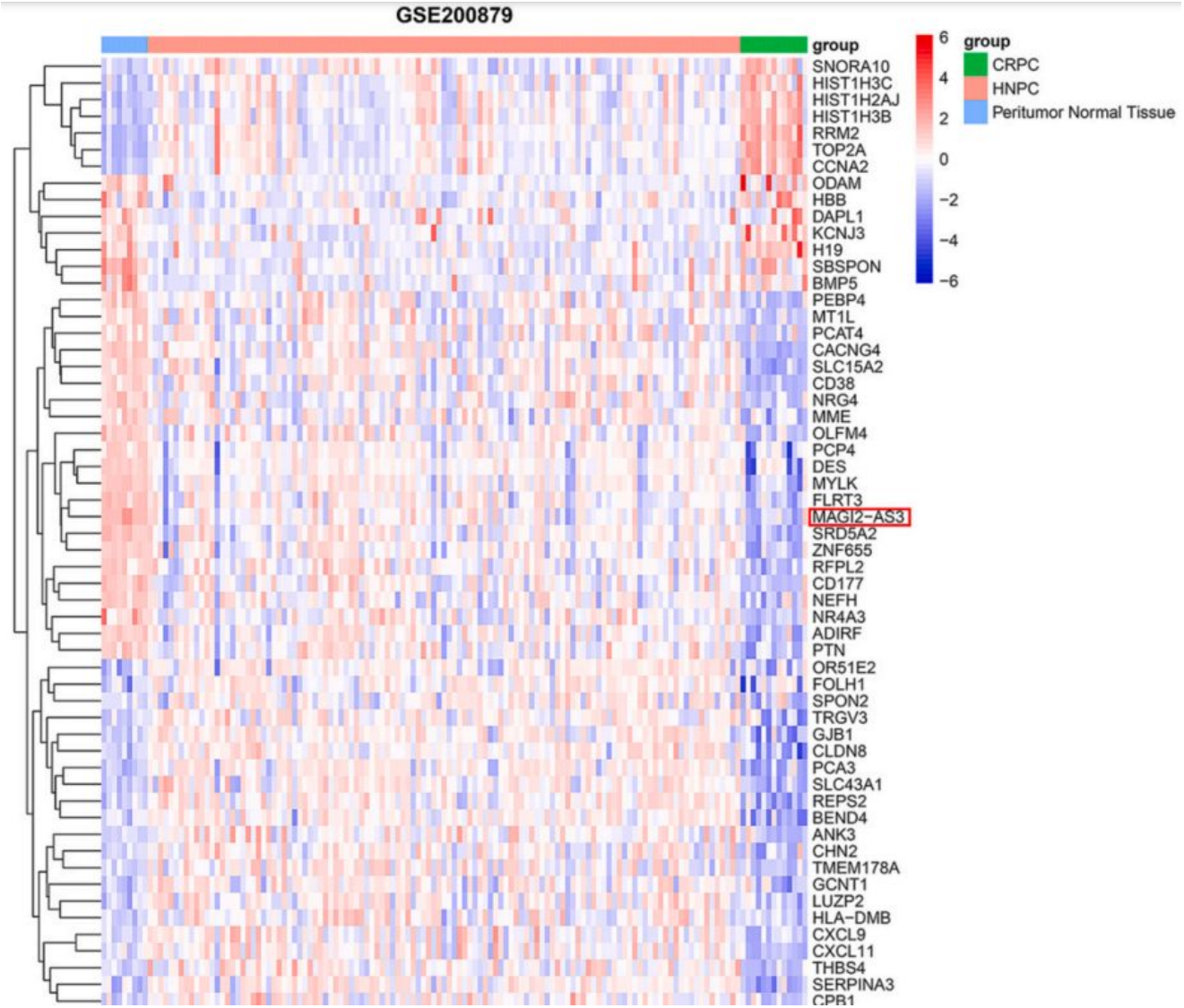
- **Prostate cancer (PCa)** is a **common malignant cancer** in elderly males in **Western countries**
- Despite remarkable progress in **early diagnosis and prevention, most cases of PCa** are diagnosed in the *advanced stages of metastasis and castration-resistant prostate cancer (CRPC) develops*, ultimately accounting for the second leading cause of **cancer-related death among men.**
- Several molecular mechanisms related to **castration resistance** have been identified. These mechanisms are mainly involved in **androgen receptor (AR) signal** transduction, including AR amplification, mutation, activation, coegulation, selective clipping, and aberrant post-translational modifications.
- Some **aberrantly expressed lncRNAs** have been identified in **PCa** clinical samples and cell lines, which may be ubiquitously involved in **multiple biological processes**, such as infarction,autophagy, apoptosis, cell senescence, resistance to chemotherapy, and cancer cell metastasis.

STEP 1: To screen for **abnormally expressed lncRNAs**, They selected **two GEO datasets** to identify the lncRNAs involved in **CRPC development**

Benign prostate hyperplasia (BPH) and adjacent normal tissues -> hormone-naïve PCa (HNPC) -> CRPC and metastatic PCa tissues



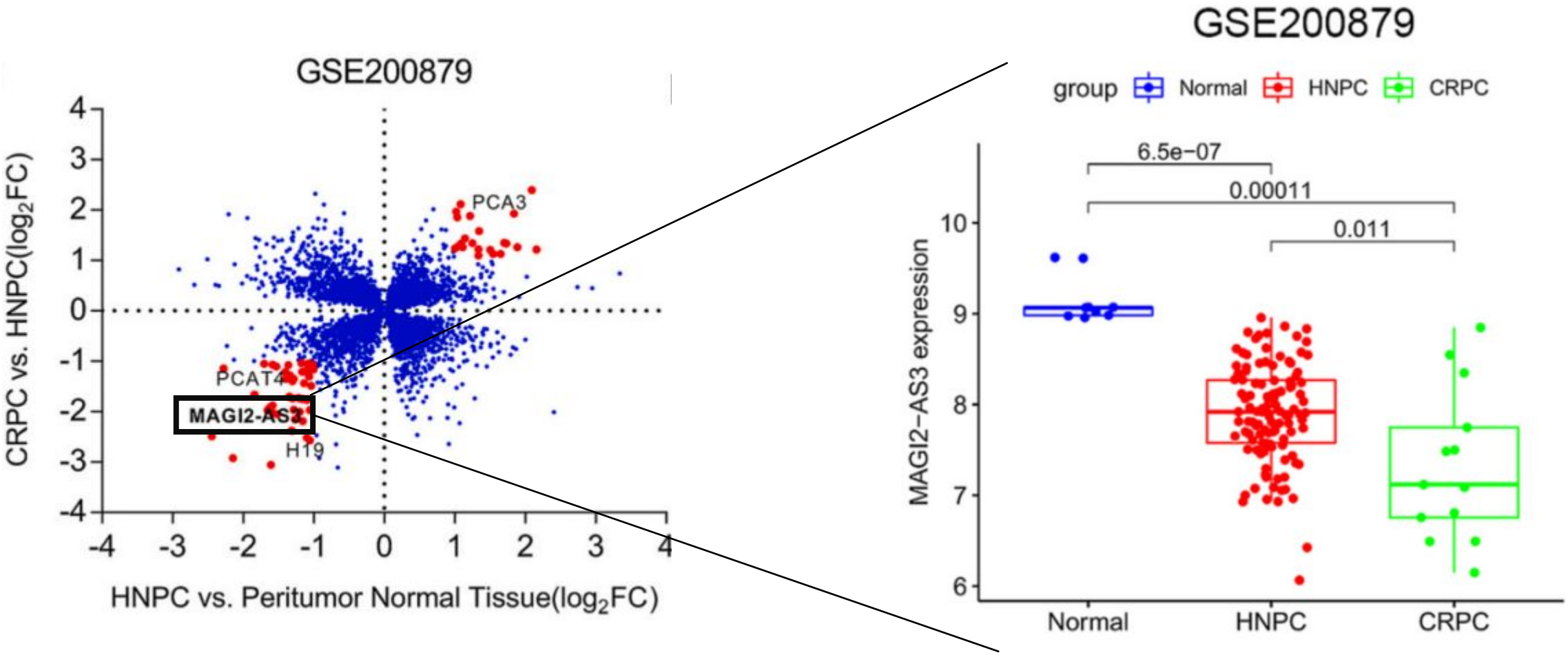
Venn Diagram depicting the common DEGs



Heatmap of all DEGs analyzed with GSE200879

STEP 1: To screen for **abnormally expressed lncRNAs**, They selected **two GEO datasets** to identify the lncRNAs involved in **CRPC development**

Benign prostate hyperplasia (BPH) and adjacent normal tissues -> hormone-naïve PCa (HNPC) -> CRPC and metastatic PCa tissues



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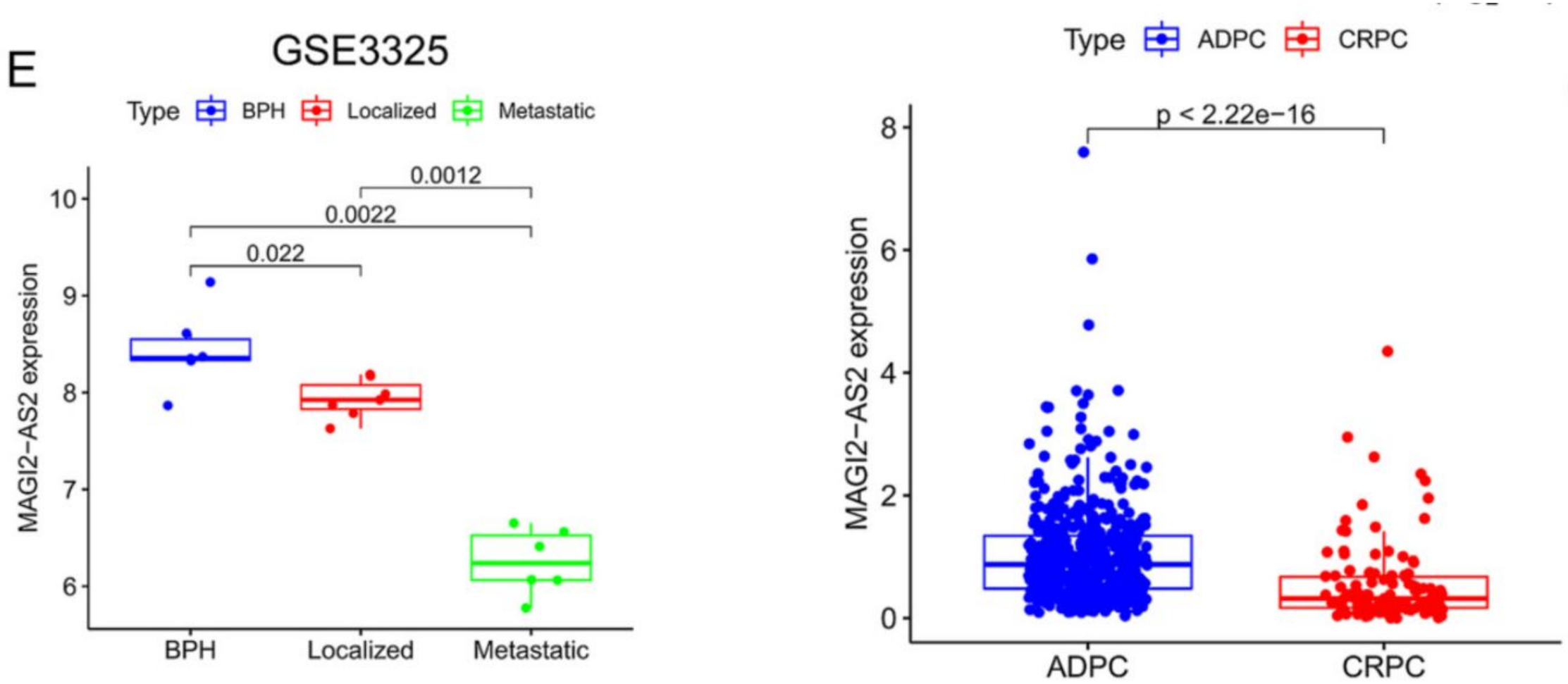
Benign prostate hyperplasia (BPH) and adjacent normal tissues -> hormone-naïve PCa (HNPC) -> CRPC and metastatic PCa tissues



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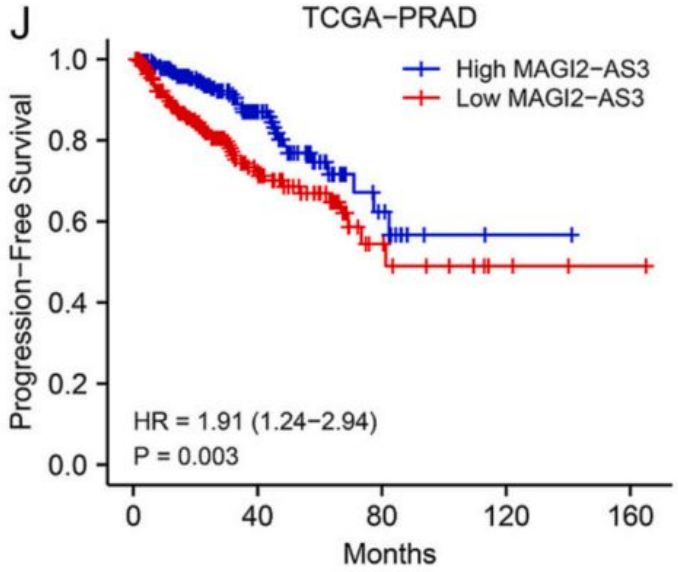
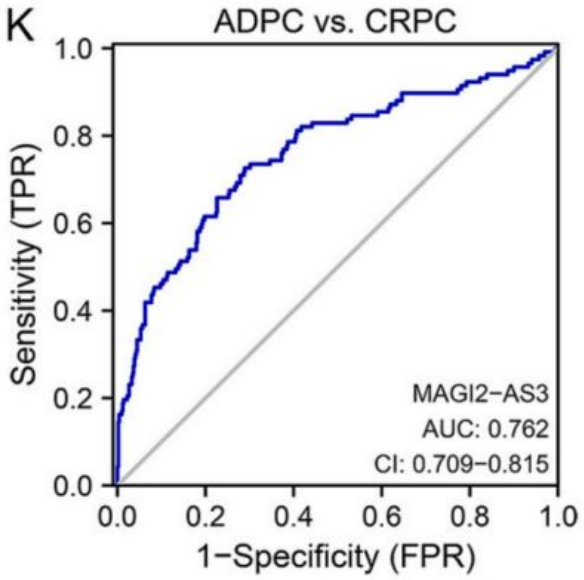
Benign prostate hyperplasia (BPH) and adjacent normal tissues -> hormone-naïve PCa (HNPC) -> CRPC and metastatic PCa tissues

Table 1
Association between MAGI2-AS3 and the clinicopathological features of prostate cancer patients in TCGA.

Variables	Cases	MAGI2-AS3 expression		
		Low (n = 177)	High (n = 177)	P-value
Age (year)				
<60	136	74(41.8%)	62(35.0%)	0.190
≥60	218	103(58.2%)	115(65.0%)	
Postoperative PSA (ng/mL)				
<10	341	170(96.0%)	171(96.6%)	0.777
≥10	13	7(4.0%)	6(3.4%)	
TNM stage				
I/II	123	54(30.5%)	69(39.0%)	0.094
III/IV	231	123(69.5%)	108(61.0%)	
Gleason score				
≤7 (3 + 4)	124	46 (26.0%)	78(44.1%)	<0.001*
≥7 (4 + 3)	230	131 (74.0%)	99 (55.9%)	
Lymph-node Metastases				
Negative	288	136 (76.8%)	152 (85.9%)	0.029*
Positive	66	41 (23.4%)	25 (14.1%)	

* Statistically significant (P<0.05).





RESULT STEP 1:

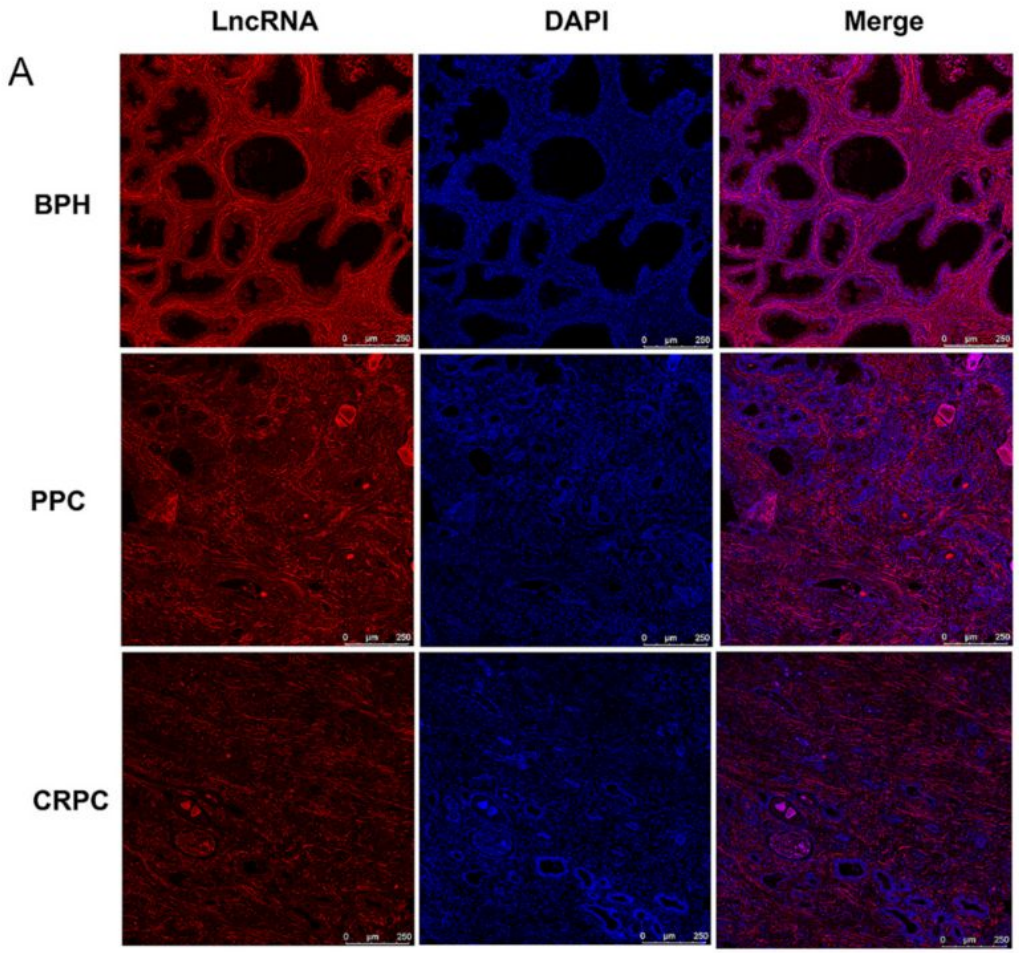
MAGI2-AS3 was abundant in benignprostate hyperplasia (BPH) and adjacent normal tissues, downregulated in localized or hormone-naïve PCa (HNPC), and more significantly downregulated in CRPC and metastatic PCa tissues

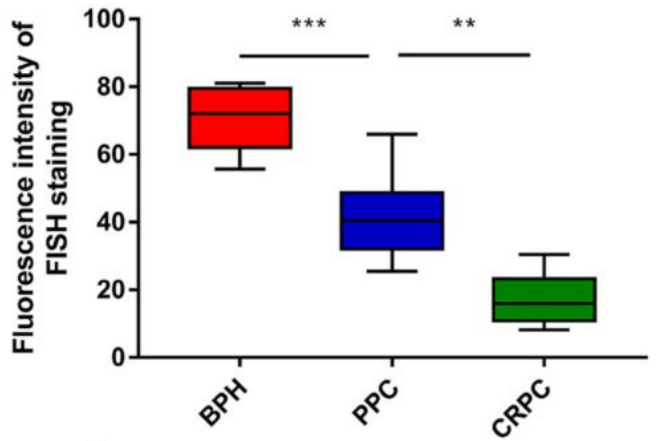
MAGI2-AS3 is **downregulated in CRPC** and can be a **prognostic biomarker** based on **RNA-sequencing analysis**

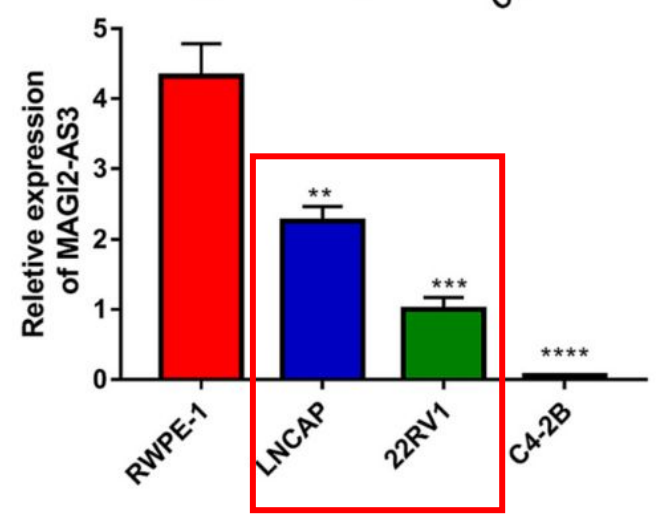
STEP 2: To determine the expression levels of MAGI2-AS3

***Tissue specimens**: 7 BPH, 35 PPC, and 6 CRPC*
***Cells lines**: LNCaP, 22RV1, C4–2B, and RWPE-1 cells*

Significantly decreased MAGI2- AS3 in CRPC **tissues** compared to primary prostate cancer (PPC) or BPH tissues.(AB).
q-PCR result for MAGI2-AS3 in prostate cell lines (C)

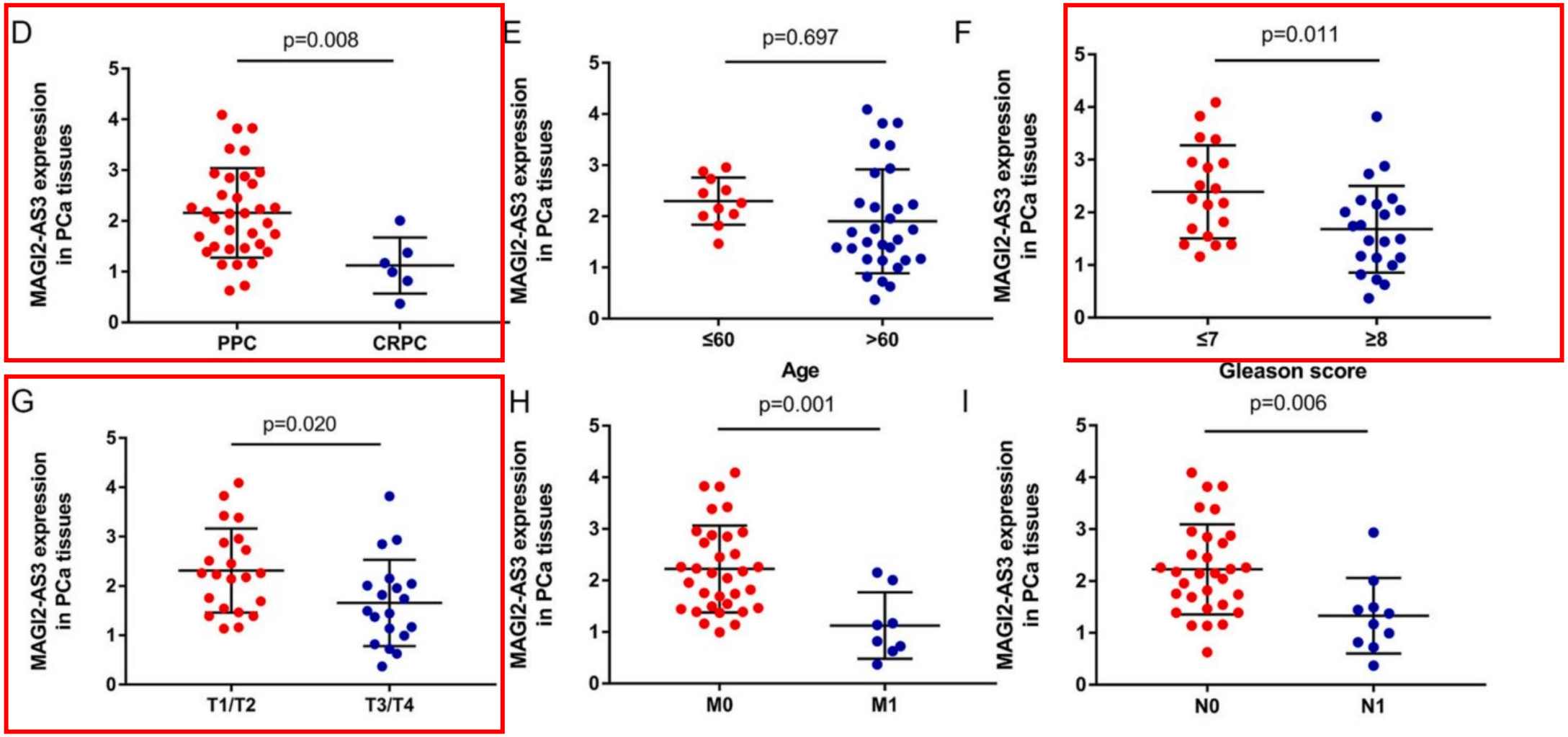






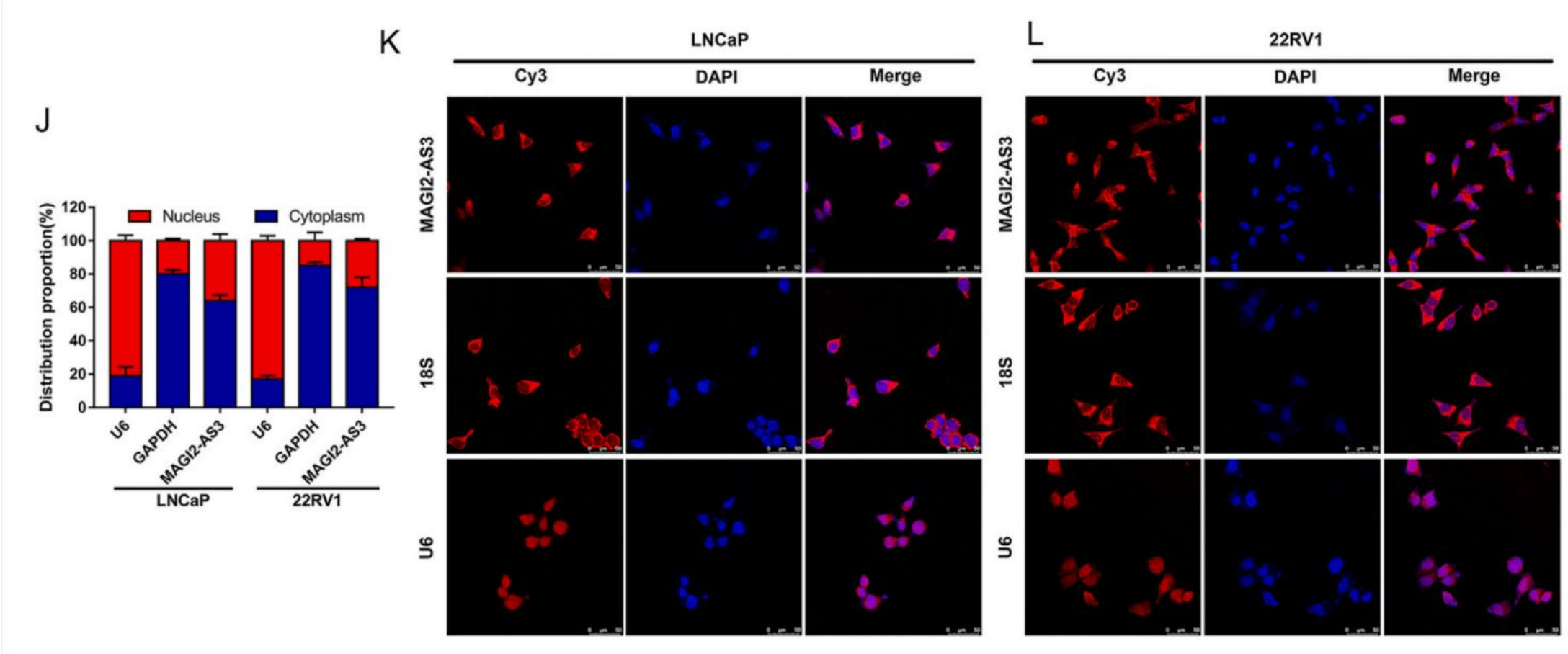
STEP 2: To determine the expression levels of MAGI2-AS3

The RT-PCR results confirmed that MAGI2-AS3 levels were negatively associated with PCa progression



STEP 2: To determine the expression levels of MAGI2-AS3

The nuclear and cytoplasmic distribution of MAGI2-AS3



STEP 2: To determine the expression levels of MAGI2-AS3

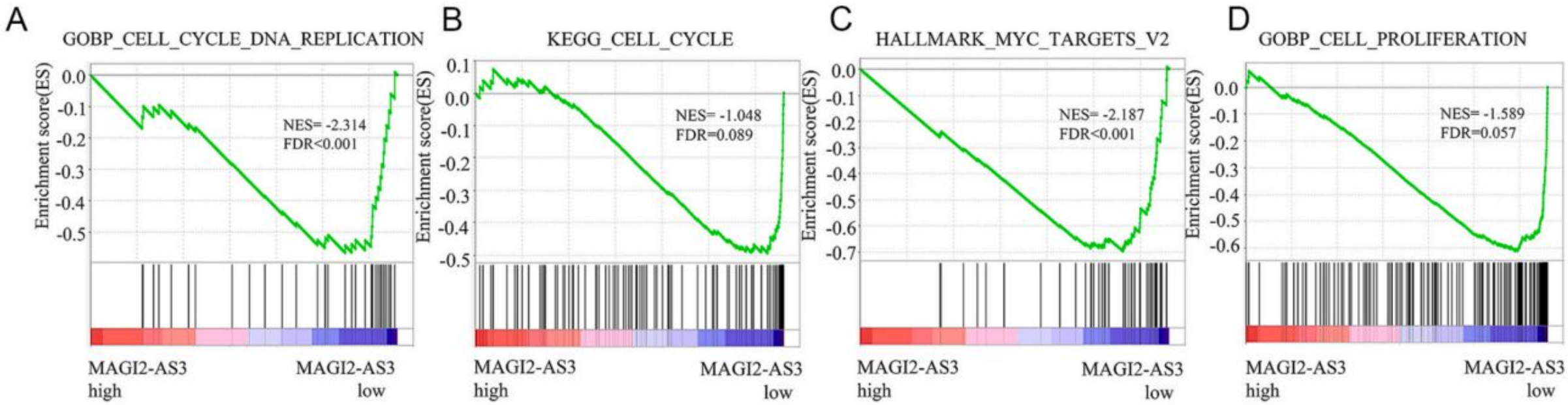
Significantly decreased MAGI2- AS3 in CRPC tissues compared to primary prostate cancer (PPC) or BPH tissues

MAGI2-AS3 levels were negatively associated with PCa progression

STEP 3: To determine the function of MAGI2-AS3 in CRPC

GSE200879 dataset: including 129 samples: 116 HNPC, 13 CRPC
Platform: **Expression profiling by array**

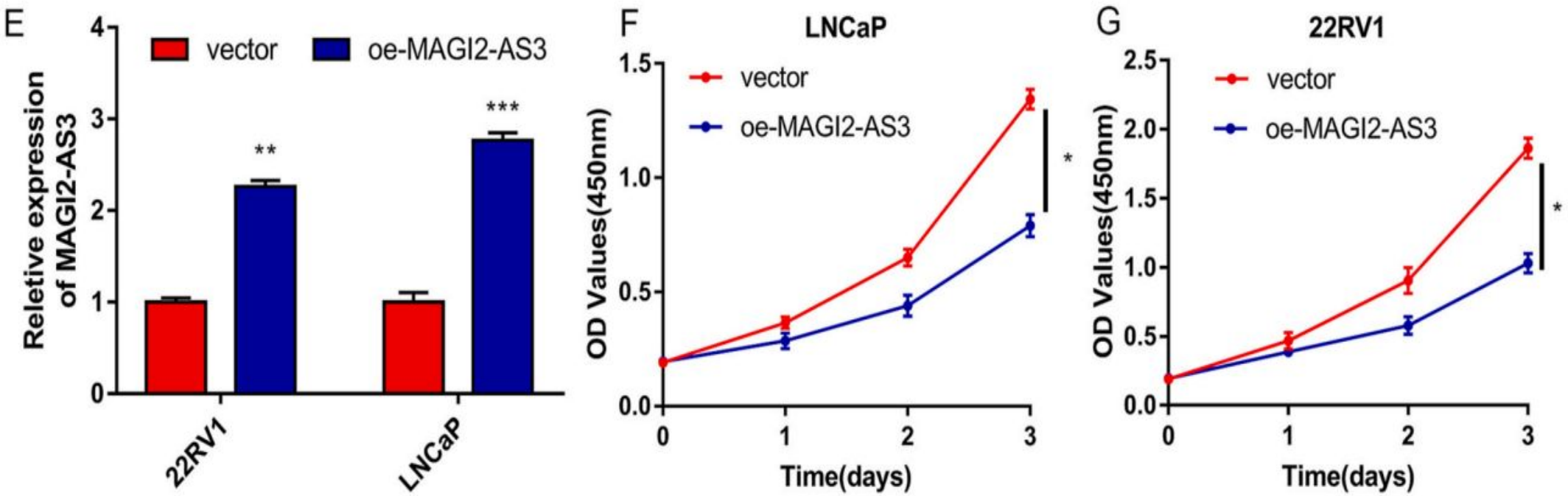
Cell cycle, cell cycle DNA replication, MYC targets, and cell proliferation were found to be enriched in the **low MAGI2-AS3 expression group.**



https://www.mdpi.com/1422-0067/23/9/4491

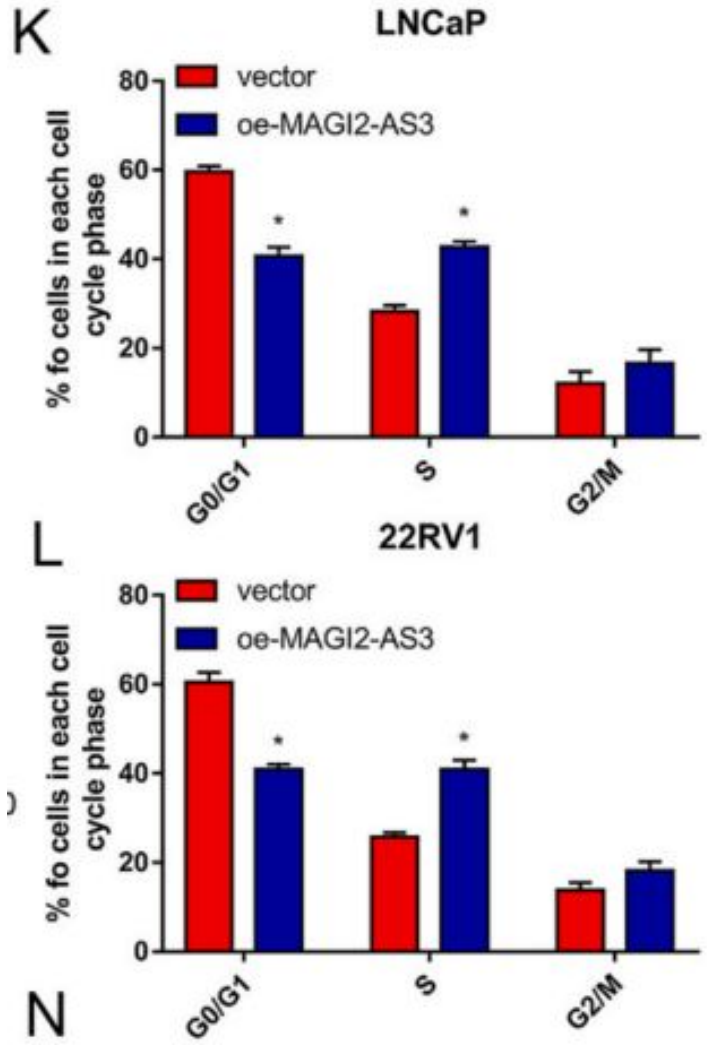
STEP 3: To determine the function of MAGI2-AS3 in CRPC

The full-length cDNA of MAGI2-AS3 (NCBI Reference sequence: NR_038345.1) was synthesized and cloned into the lentiviral vector pcDNA3.1(+) to generate **recombinant lentiviruses overexpressing MAGI2-AS3 (oeMAGI2-AS3**)



STEP 3: To determine the function of MAGI2-AS3 in CRPC

The cell cycle distribution of LNCaP and 22RV1 cells before and after MAGI2-AS3 knockdown or overexpression was detected using flow cytometry. The transition from the G1 phase to the S phase of the cell cycle



- The transition from the G1 phase to the S phase of the cell cycle was significantly blocked after MAGI2-AS3 silencing. In contrast, MAGI2-AS3 overexpression led to the opposite trend.

STEP 3: To determine the function of MAGI2-AS3 in CRPC

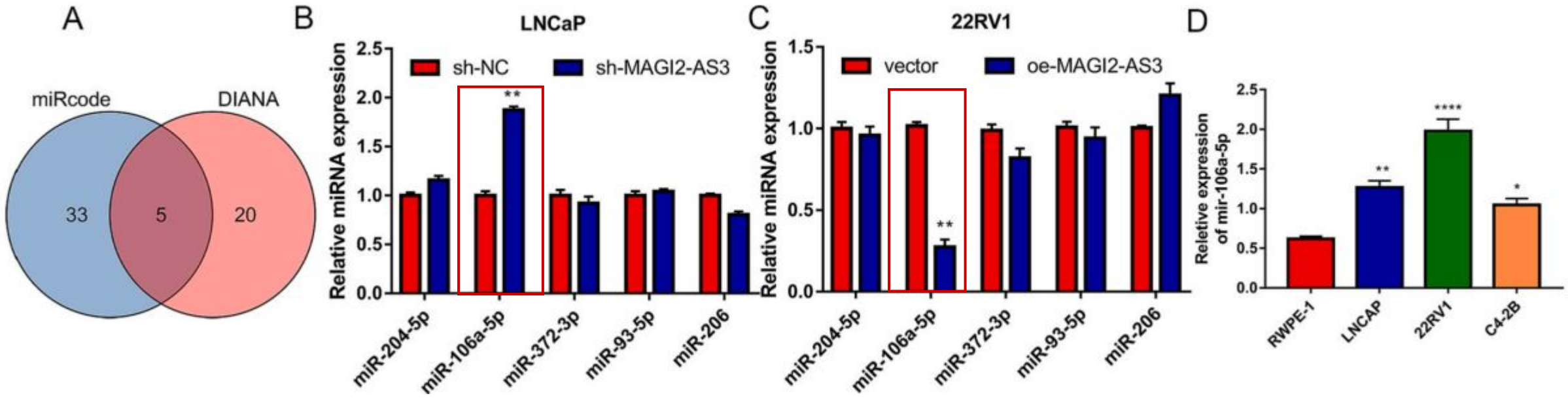
- **MAGI2-AS3 inhibits the proliferation of the PCa cell lines.**

STEP 4: HOW MAGI2-AS3 RESTRAINS PCa PROGRESSION

MAGI2-AS3 is **mainly present in the cytoplasm** of PCa cells, **suggesting** a **post-transcriptional role** in the malignant biological behaviorof tumors.

lncRNA + miRNA -> ceRNA

MAGI2-AS3 was overexpressed or knocked down in the PCa cells

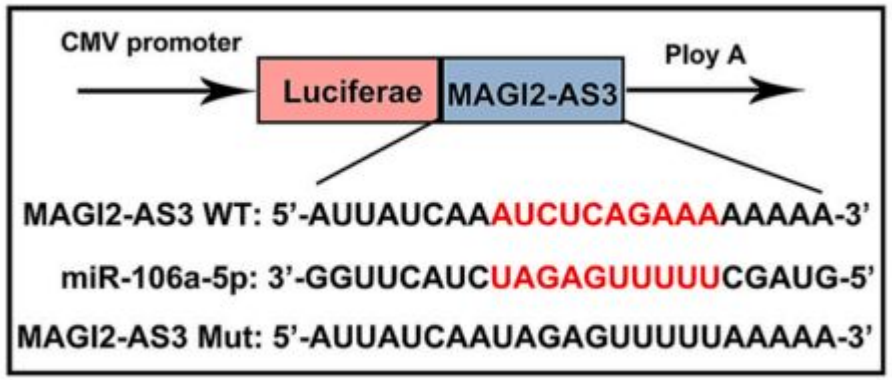


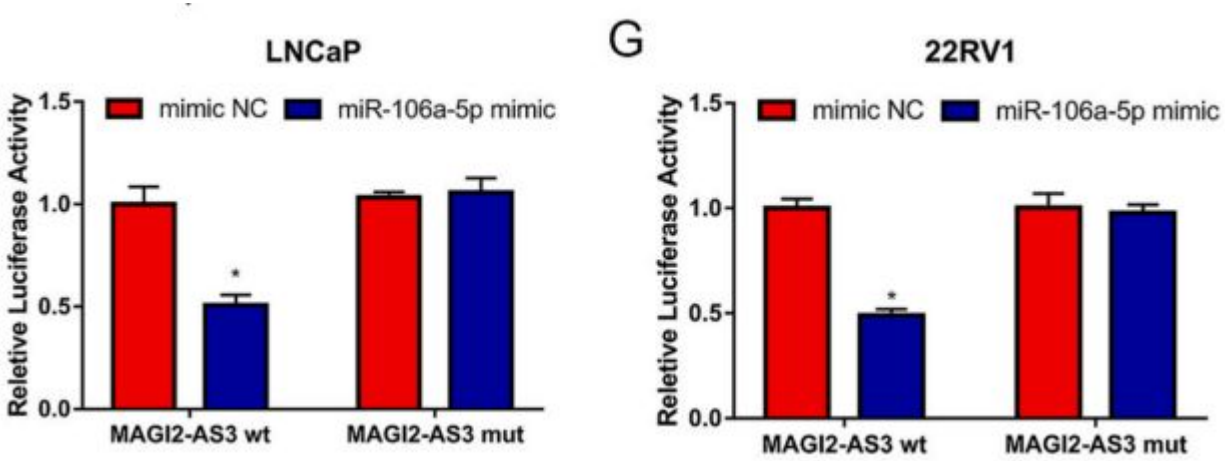
miR-106a-5p expression was significantly elevated or decreased in LNCaP and 22RV1 cells.

miR-106a-5p was upregulated in both LNCaP and 22RV1 cells compared to RWPE-1 cells.

STEP 4: HOW MAGI2-AS3 RESTRAINS PCa PROGRESSION

miR-106a-5p mimics **significantly weakened the luciferase activity** of vectors containing **MAGI2-AS3 wt** but c**ould not decrease** the luciferase activity of the **MAGI2-AS3 mut** vectors.

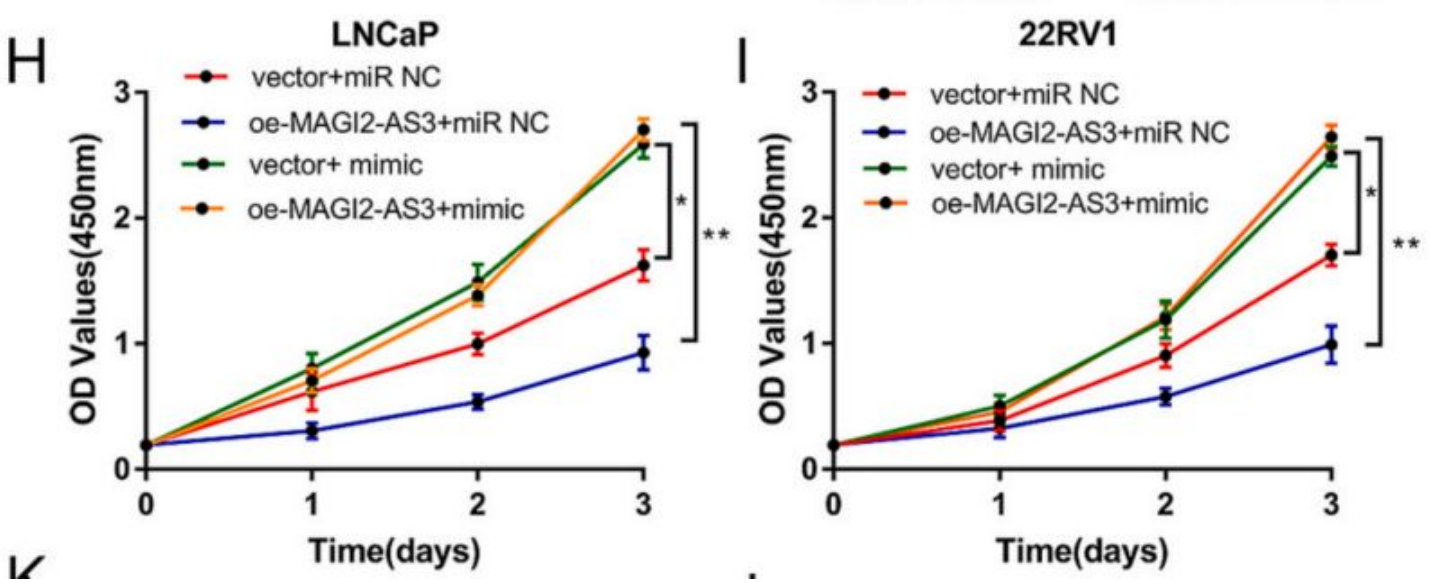


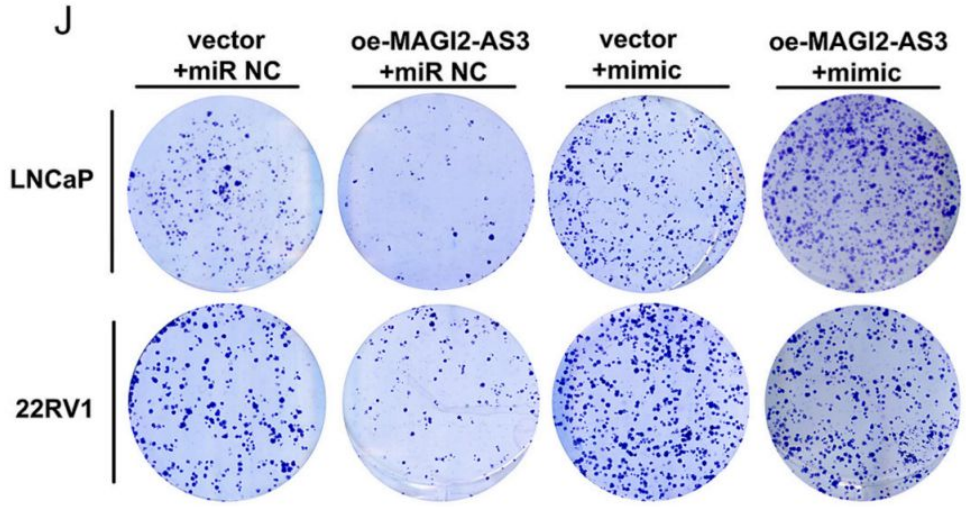


miR-106a-5p mimics significantly weakened the luciferase activity of vectors containing MAGI2-AS3 wt but could not decrease the luciferase activity of the MAGI2-AS3 mut vectors

STEP 4: HOW MAGI2-AS3 RESTRAINS PCa PROGRESSION

Investigate the biological role of miR-106a-5p





The decreased proliferation ability of cells upon overexpression of MAGI2-AS3 could be rescued by the miR-106a-5p mimic

STEP 4: HOW MAGI2-AS3 RESTRAINS PCa PROGRESSION

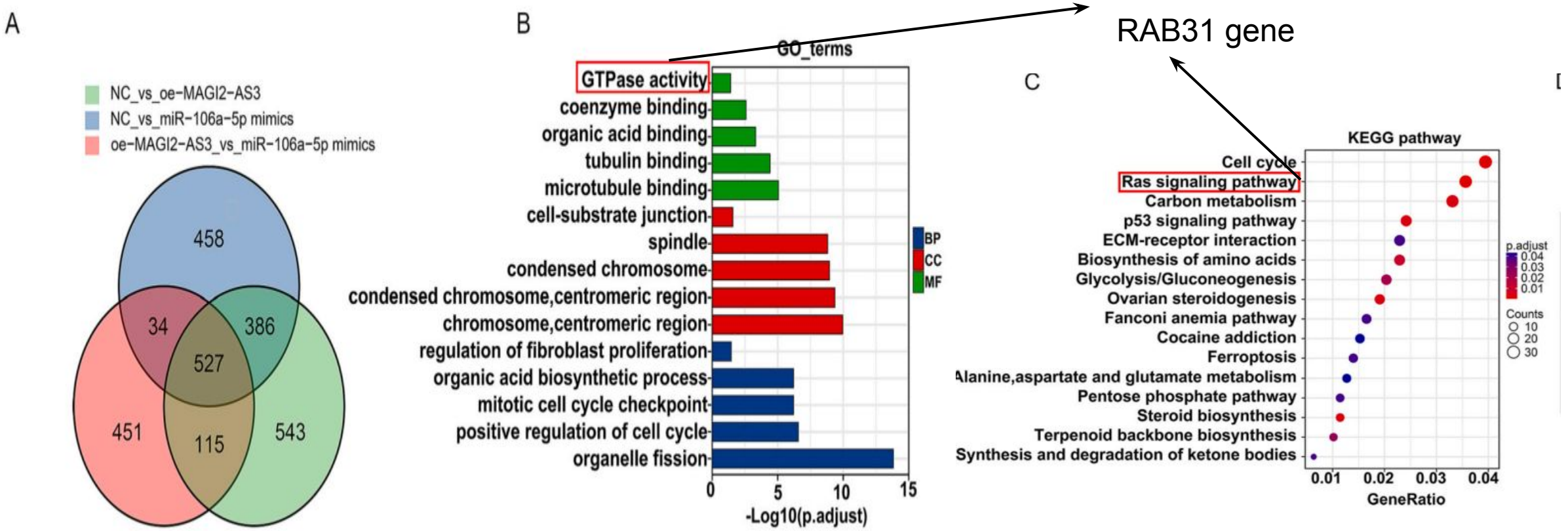
Overall, the above experiments demonstrated that MAGI2-AS3 interacts with miR-106a-5p to influence PCa via miR-106a-5p.

STEP 5: The downstream mechanisms that may be regulated by the MAGI2-AS3/miR-106a-5p axis to inhibit PCa progression

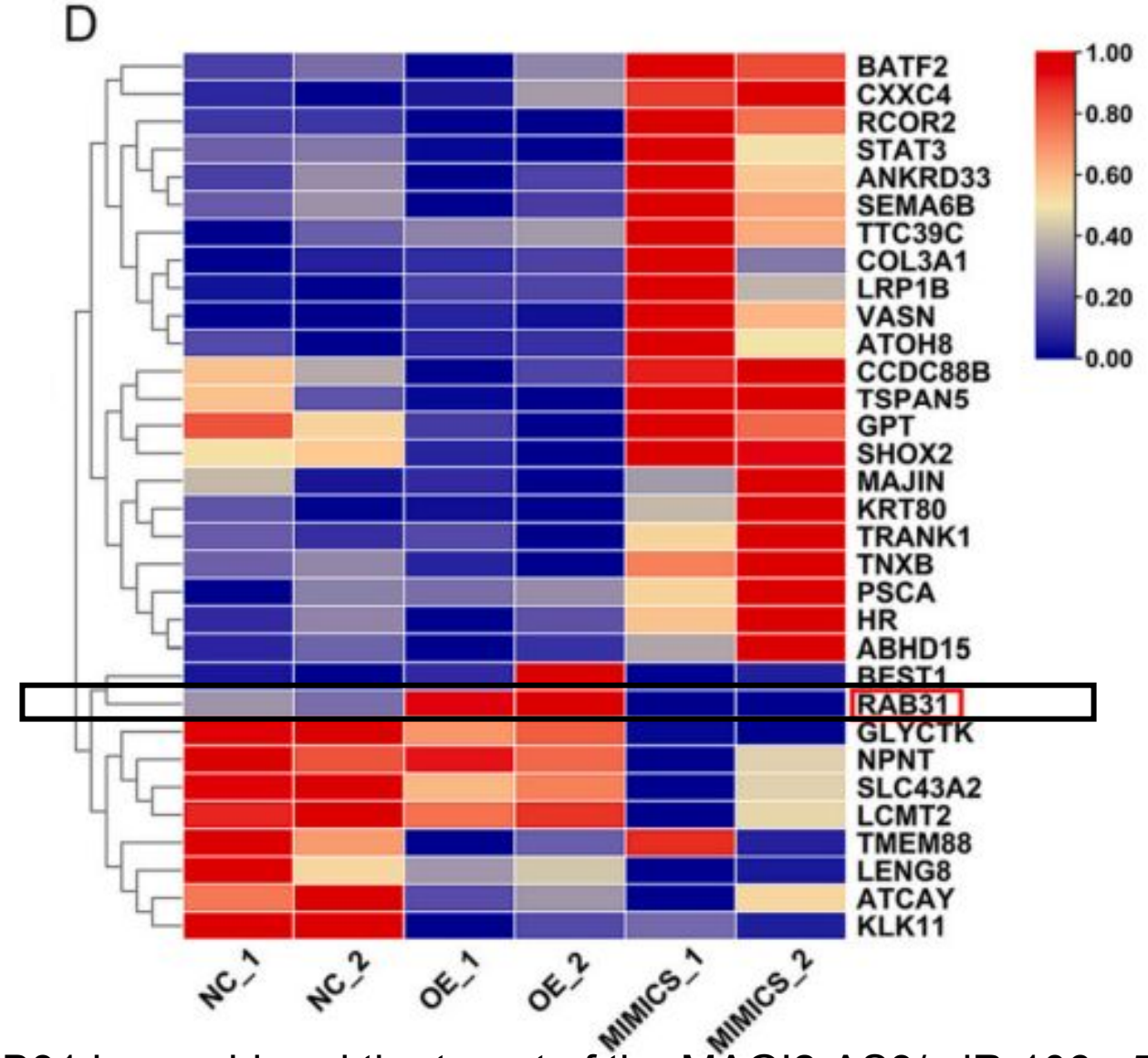
RNA-sequencing was conducted after the **overexpression of MAGI2-AS3 or miR-106a-5p in 22RV1 cells**

The expression levels of **527 genes** were found to be significantly altered.

The **527 genes** were enriched in different cancer-related signaling pathways, including **GTPase** activity and the Ras signaling pathway, both of which contain the **RAB31 gene**.



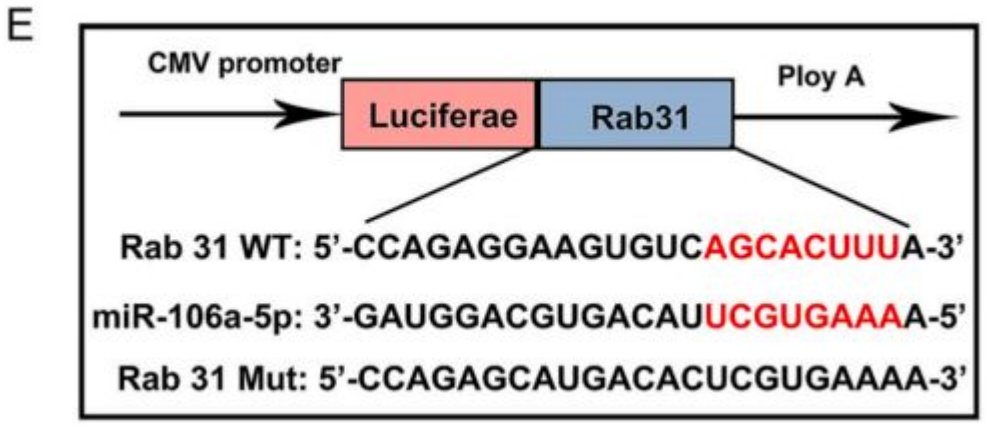
STEP 5: The downstream mechanisms that may be regulated by the MAGI2-AS3/miR-106a-5p axis to inhibit PCa progression



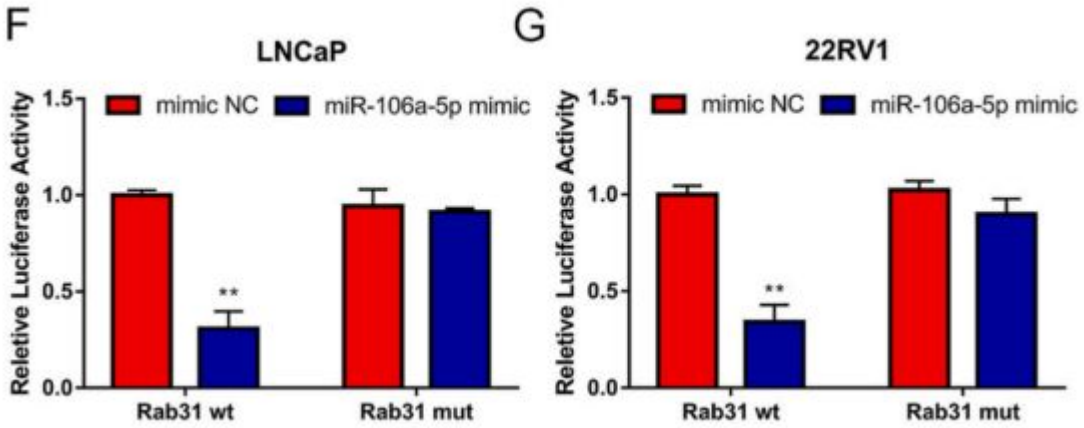
RAB31 is considered the target of the MAGI2-AS3/miR-106a-5p axis

STEP 5: The downstream mechanisms that may be regulated by the MAGI2-AS3/miR-106a-5p axis to inhibit PCa progression

The RAB31 3'UTR fragment, containing the wt or mut binding sites, was inserted into the vector and co-transfected into 22RV1 and LNCaP cells with the miR-106a-5p mimic or miR-NC mimic.



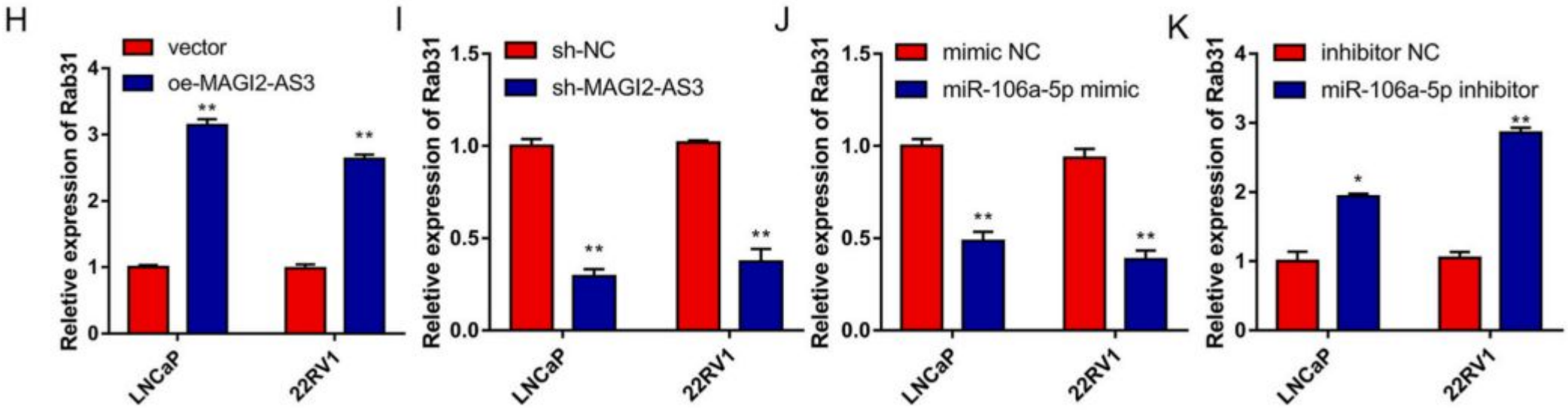
Potential binding sites between miR106a-5p and RAB31



The miR-106a-5p mimic, which contained the RAB31 wt binding site but not the mut-binding site, significantly weakened the luciferase activity

STEP 5: The downstream mechanisms that may be regulated by the MAGI2-AS3/miR-106a-5p axis to inhibit PCa progression

To further confirm that MAGI2-AS3 could regulate RAB31 expression through competitive endogenous binding with miR-106a-5p in PCa. RAB31 WAS expression in LNCaP and 22RV1 cells stably transfected with the miR-106a-5p mimic or inhibitor and MAGI2-AS3 silencing or overexpression vectors.



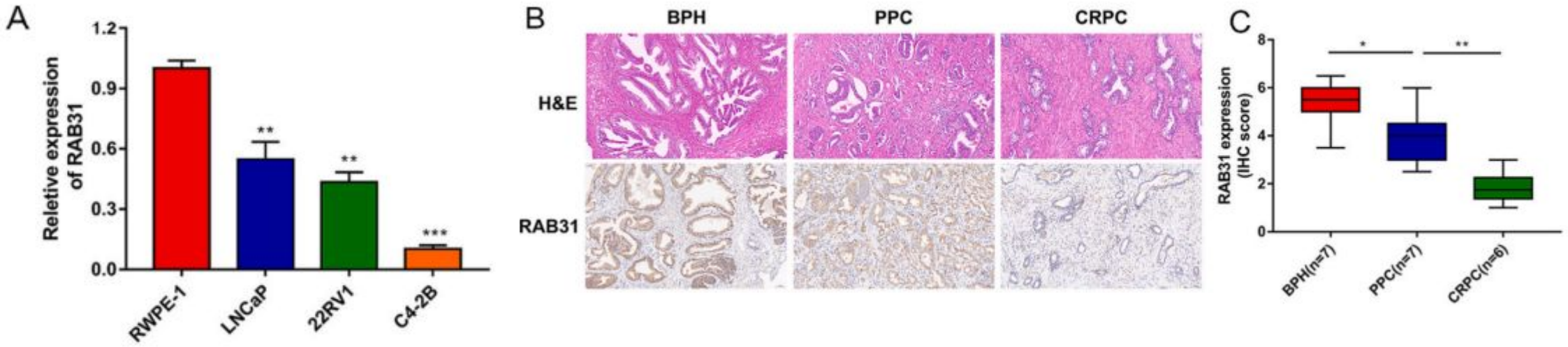
The RAB31 mRNA level was found to be markedly controlled by MAGI2-AS3 or miR-106a-5p

STEP 5: The downstream mechanisms that may be regulated by the MAGI2-AS3/miR-106a-5p axis to inhibit PCa progression

The RAB31 mRNA level was found to be markedly controlled by MAGI2-AS3 or miR-106a-5p. These results indicate that RAB31 is the downstream target gene of MAGI2-AS3.

STEP 6: The downstream mechanisms that may be regulated by the MAGI2-AS3/miR-106a-5p axis to inhibit PCa progression

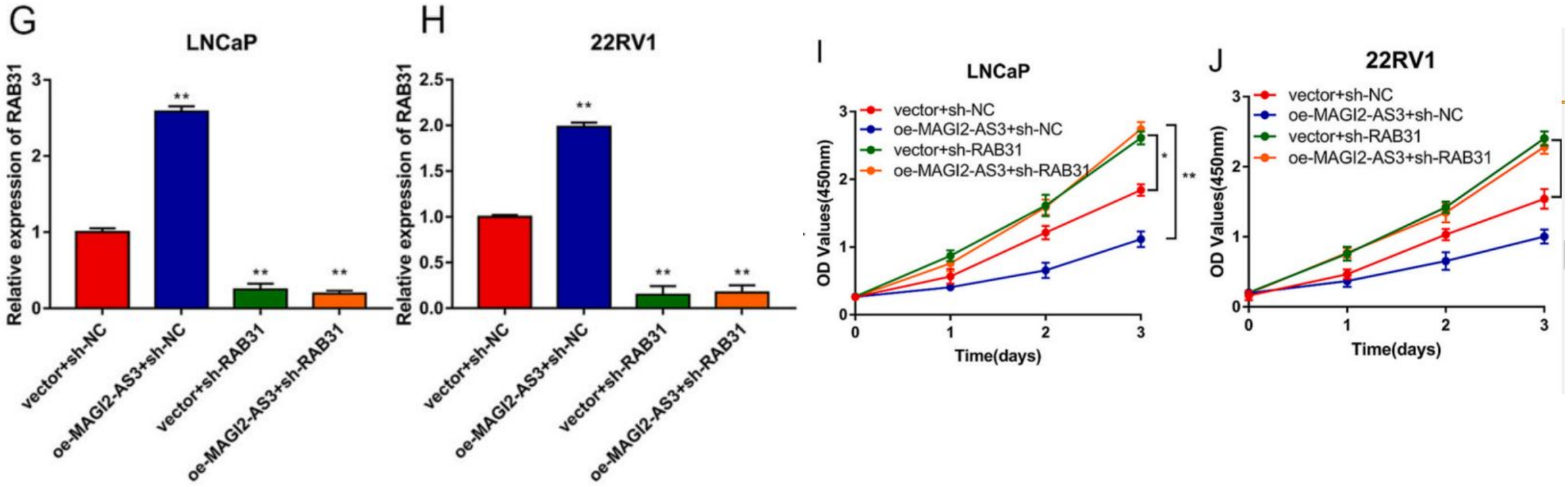
The **protein expression of RAB31** was identified in a broad spectrum of tumors and **decreased in PCa tissues compared with normal tissues** (two GEO datasets (GSE200879 and GSE3325) and two studies (TCGA-PRAD, SU2C/PCF Dream Team Study).
RAB31 is strongly associated with PCa progression



qRT-PCR revealed that RAB31 expression was suppressed in PCa cells
IHC showed that RAB31 was decreased in PCa tissues compared to BPH and more significantly decreased in CRPC samples than in PPC specimens

STEP 6: The downstream mechanisms that may be regulated by the MAGI2-AS3/miR-106a-5p axis to inhibit PCa progression

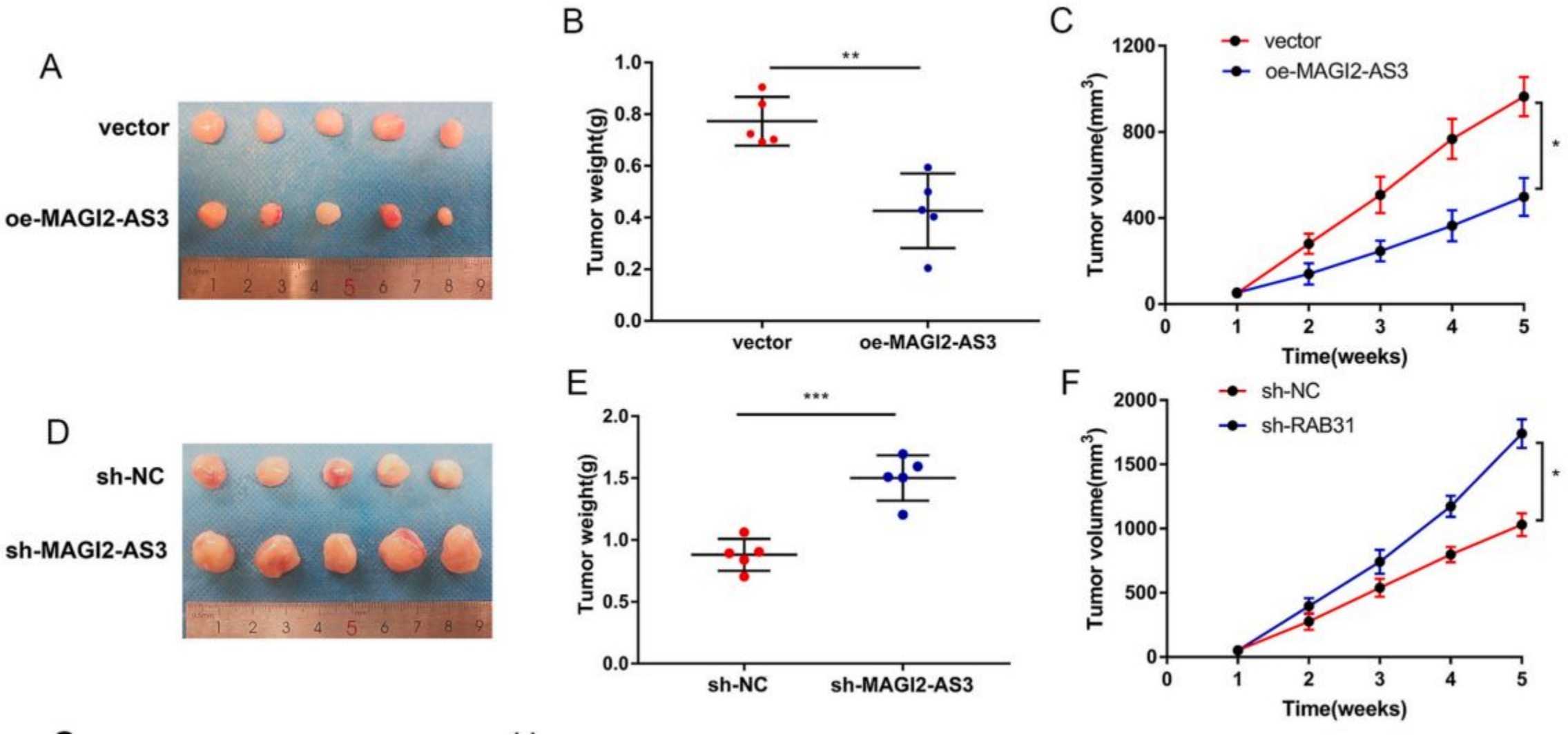
LNCaP and 22RV1 cells stably overexpressing MAGI2-AS3 were transfected with sh-RAB31 vector, and the RAB31 mRNA levels were measured after transfection.



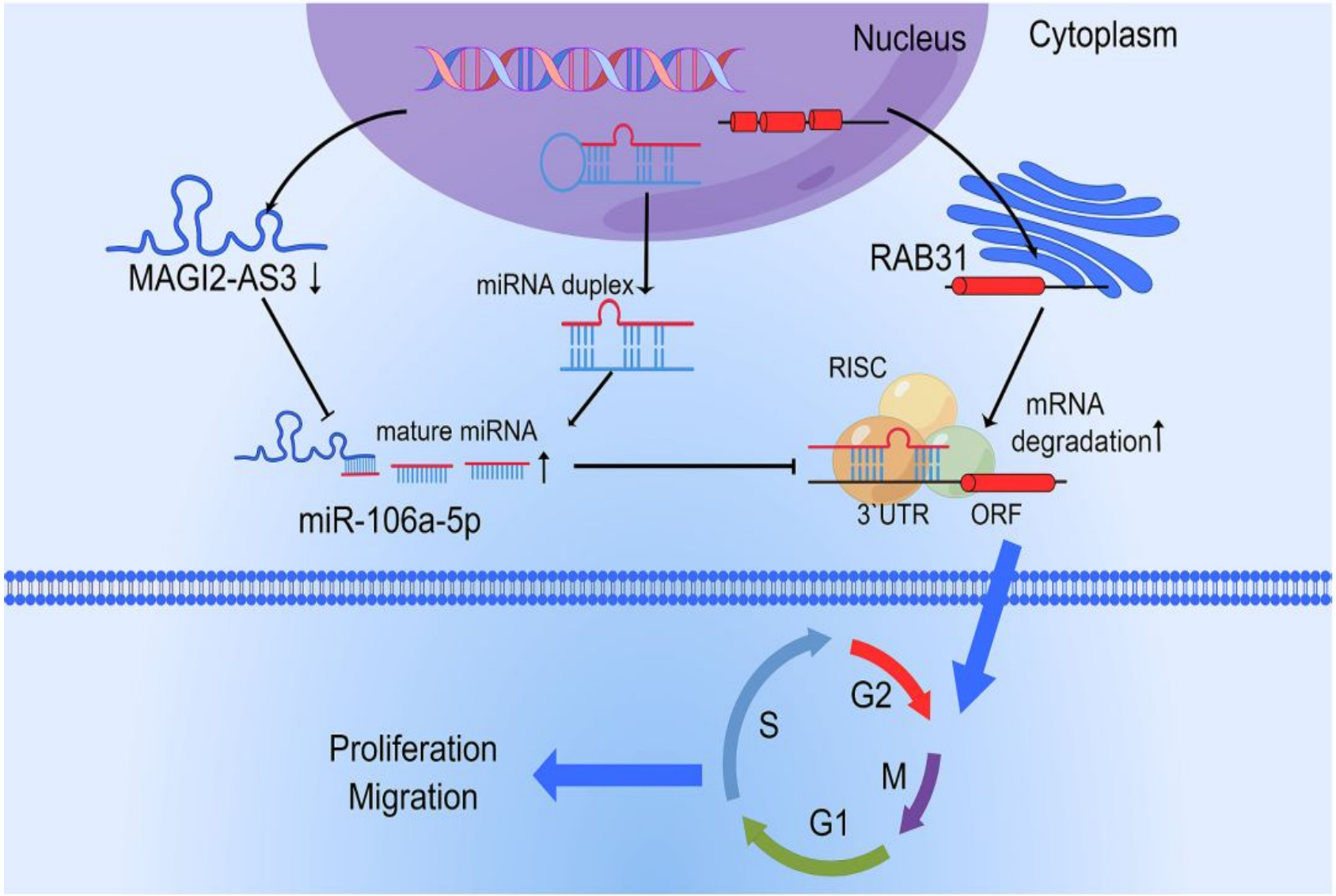
RAB31 knockdown improved the proliferation and migration of PCa cells induced by MAGI2-AS3

STEP 7: MAGI2-AS3 represses PCa progression in vivo

22RV1 cells stably transfected with MAGI2-AS3 or sh-MAGI2-AS3 and NC were implanted subcutaneously into nude mice to establish xenograft tumors.



Schematic representation of the results of this study.



Discussion

- Demonstrate the role of MAGI2-AS3 in the inhibition of CRPC progression.
- MAGI2-AS3 expression could negatively accommodate the migration and proliferation of PCa in vitro and in vivo.
- MAGI2-AS3 was identified to function as a tumor suppressor by interacting with miR-106a-5p to regulate the expression of RAB31 in CRPC.
- MAGI2-AS3 was identified to function as a tumor suppressor by interacting with miR-106a-5p to regulate the expression of RAB31 in CRPC
- mRNA level of RAB31 and found a remarkable association between lower rab31 mRNA level and poorer prognosis in PCa patients.
- GSEA also revealed that decreased RAB31 expression may influence the proliferation and cell cycle signaling pathways of CRPC, including the G2M checkpoint and MYC targets.