# KSBi-BIML 2026

Bioinformatics & Machine Learning(BIML) Workshop for Life Scientists

생명정보학 & 머신러닝 워크샵 (온라인)

# Bioinformatics and AI for microRNA

백대현 _ 서울대학교

KSBI KOREAN SOCIETY FOR BIOINFORMATICS | 한국생명정보학회

본 강의 자료는 한국생명정보학회가 주관하는 BIML 2026 워크샵을 목적으로
제작된 것으로 해당 목적 이외의 다른 용도로 사용할 수 없음을 분명하게 알립니다.

이를 다른 사람과 공유하거나 복제, 배포, 전송할 수 없으며 만약 이러한 사항을 위반할 경우
발생하는 **모든 법적 책임은 행위자 본인에게 있음**을 알립니다.

# KSBi-BIML 2026

## Bioinformatics & Machine Learning (BIML) Workshop for Life Scientists

한국생명정보학회가 주최하는 BIML-2026 동계 Bioinformatics &Machine Learning 교육 워크숍에 여러분을 초대합니다.

BIML 워크숍은 생명정보학 연구자들이 최신 AI바이오 분야의 인공지능 기반 분석 기술과 바이오 데이터 분석 기법을 이론과 실습을 통해 체계적으로 배울 수 있는 전문 교육 프로그램입니다. 2015년에 시작된 BIML 워크숍은 올해로 12년 차를 맞이하며, 국내 생명정보학 분야의 최초이자 최고 수준의 교육 프로그램으로 자리 잡았습니다. 이번 워크숍은 크게 인공지능바이오(AI바이오) 분야와 디지털바이오 분야, 두 분야로 구성됩니다.

AI바이오 분야에서는 생명정보 분석에 폭넓게 응용되고 있는 다양한 인공지능 기반 자료 모델링 기법을 다룰 예정입니다. 특히, 인공지능 심층학습을 활용한 단백질 구조 예측, 유전체 분석, 신약 개발에 대한 이론 및 실습 강의가 진행됩니다.

또한 디지털바이오 분야에서는 단일세포오믹스, 공간오믹스, 멀티오믹스, 메타오믹스에 대한 강의도 마련되어 있어, 연구자들의 분석 역량 강화에 실질적인 도움을 줄 것으로 기대됩니다.

또한 2024년부터 추가된 의료정보 자료 분석을 다루는 강의를 올해도 지속해서 운영하고자 합니다. 이는 최근 의료정보 자료 분석에 관한 연구 수요 증가를 반영한 것으로, 관련 연구를 수행하는 의과학자 및 의료정보 연구자들에게 유용한 지침을 제공할 것입니다.

또한, 올해도 생명정보학 기술의 다양화에 발맞춰 온라인 강좌를 대폭 확대했습니다. 올해는 무료 강좌 10개를 포함한 총 40개 이상의 강좌가 개설되며, 연구 주제에 맞는 강좌 추천과 강연료 할인 혜택도 제공합니다.

BIML-2026는 국내 주요 연구 중심 대학의 전임 교수 및 각 분야 최고 전문가들의 강의로 구성되어 있으며, 기초 이론부터 최신 연구 동향까지 아우르는 심도 있는 교육의 장이 될 것으로 확신합니다.

여러분의 많은 관심과 참여를 기대합니다!

2026년 2월

**한국생명정보학회장 류 성 호**

## 강의개요

# Bioinformatics and AI for microRNA

본 강의에서는 인간 microRNA의 생성 및 타겟팅에 대한 최신 생물정보학 연구 내용을 소개한다. 또한, 인공 지능(AI)을 활용한 microRNA 타겟팅 연구를 소개하고, AI가 어떻게 microRNA 타겟 발굴의 예측 정확도를 높일 수 있는지 고찰한다. 끝으로, 코로나 19를 일으킨 원인 바이러스인 SARS-CoV-2의 microRNA가 어떻게 host immune을 회피하는 지에 대한 최근 연구 결과에 대해 논의한다.

본 강의는 다음의 내용을 포함한다:

- 인간 microRNA 생성 기작
- 인간 microRNA 타겟팅 기작에 대한 최근 연구
- 인간 microRNA 타겟팅 연구를 위한 AI 기법
- SARS-CoV-2 microRNA

* 참고강의교재:
  강의자료에 첨부된 논문 2편(The regulatory impact of RNA-binding proteins on microRNA targeting, A high-resolution temporal atlas of the SARS-CoV-2 translatome and transcriptome)

* 교육생준비물: 노트북 (메모리 8GB 이상, 디스크 여유공간 30GB 이상)

* 강의 난이도: 초급

* 강의: 백대현 교수 (서울대학교 생명과학부)

# Curriculum Vitae

## Speaker Name: Daehyun Baek, Ph.D.


▶ **Personal Info**

| | |
|---|---|
| Name | Daehyun Baek |
| Title | Associate Professor |
| Affiliation | Seoul National University |

▶ **Contact Information**

| | |
|---|---|
| Address | Rm 423, Bldg 504, Seoul National University Seoul, South Korea, 08826 |
| Email | baek@snu.ac.kr |


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**Research Interest**

Artificial Intelligence (Deep Learning) for Biology and Medicine, Computational Biology and Bioinformatics, Noncoding Genome, Cancer Genomics

**Educational Experience**

| | |
|---|---|
| 1999 | B.S. in Electrical Engineering at KAIST (Minor in Biological Sciences) |
| 2007 | Ph.D. in Bioengineering at University of Washington (Advisor: Phil Green) |

**Professional Experience**

| | |
|---|---|
| 2007-2010 | Postdoctoral Fellow at Whitehead Institute / MIT / HHMI (Advisor: David Bartel) |
| 2010-Present | Assistant & Associate Professor of School of Biological Sciences at SNU |

**Selected Publications (5 maximum)**

1. D. Kim*, S. Kim*, J. Park*, H. R. Chang*, J. Chang*, J. Ahn*, ..., M.-S. Park#, Y. K. Kim#, and **D. Baek#**, A high-resolution temporal atlas of the SARS-CoV-2 translatome and transcriptome, *Nature Communications*, 2021 (**IF=14.92**)
2. S. Kim*, S. Kim*, H. R. Chang*, D. Kim*, ..., C. Shin#, and **D. Baek#**, The regulatory impact of RNA-binding proteins on microRNA targeting, *Nature Communications*, 2021 (**IF=14.92**)
3. D. Kim*, Y. M. Sung*, J. Park*, ..., and **D. Baek**, General Rules for Functional MicroRNA Targeting, *Nature Genetics*, 2016 (**cited 106 times, IF=38.33**)
4. D. Garcia*, D. **Baek*#**, ..., and D. Bartel#, Weak Seed-Pairing Stability and High Target-Site Abundance Decrease the Proficiency of lsy-6 and Other miRNAs, *Nature Structural and Molecular Biology*, 2011. (**cited 920 times, IF=15.37**)
5. **D. Baek***, J. Villen*, C. Shin*, ..., and D. Bartel, The Impact of MicroRNAs on Protein Output, *Nature*, 2008. (**cited 4,005 times, IF=49.96**)

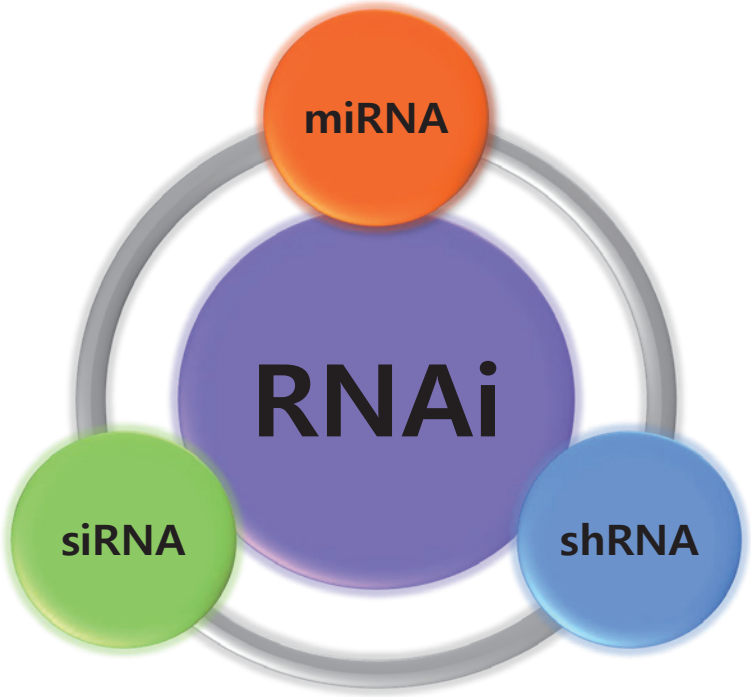
(*co-first authors, #co-corresponding authors)

# Bioinformatics and AI for MicroRNA

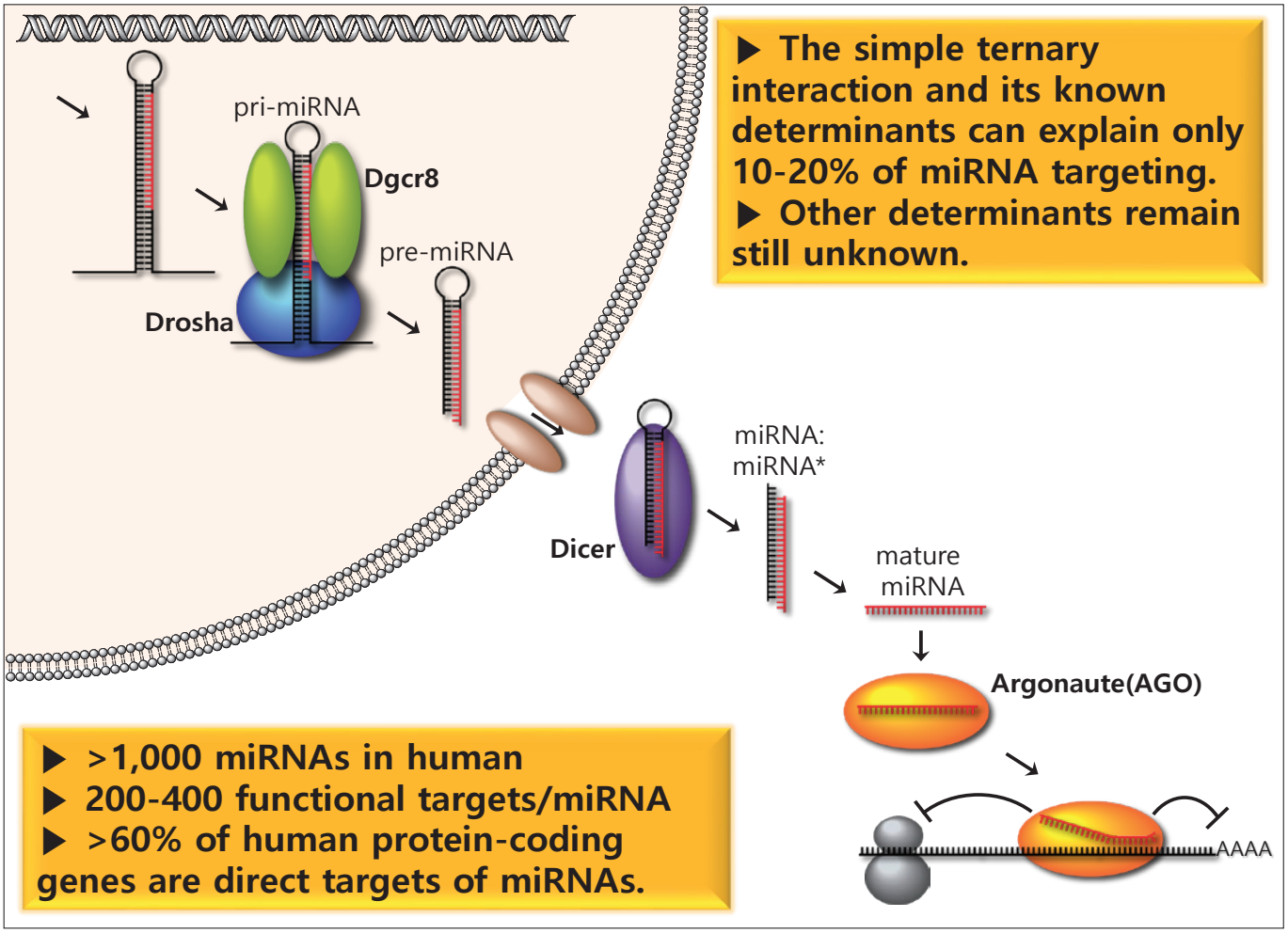
Daehyun Baek

School of Biological Sciences
Seoul National University

## RNA Interference



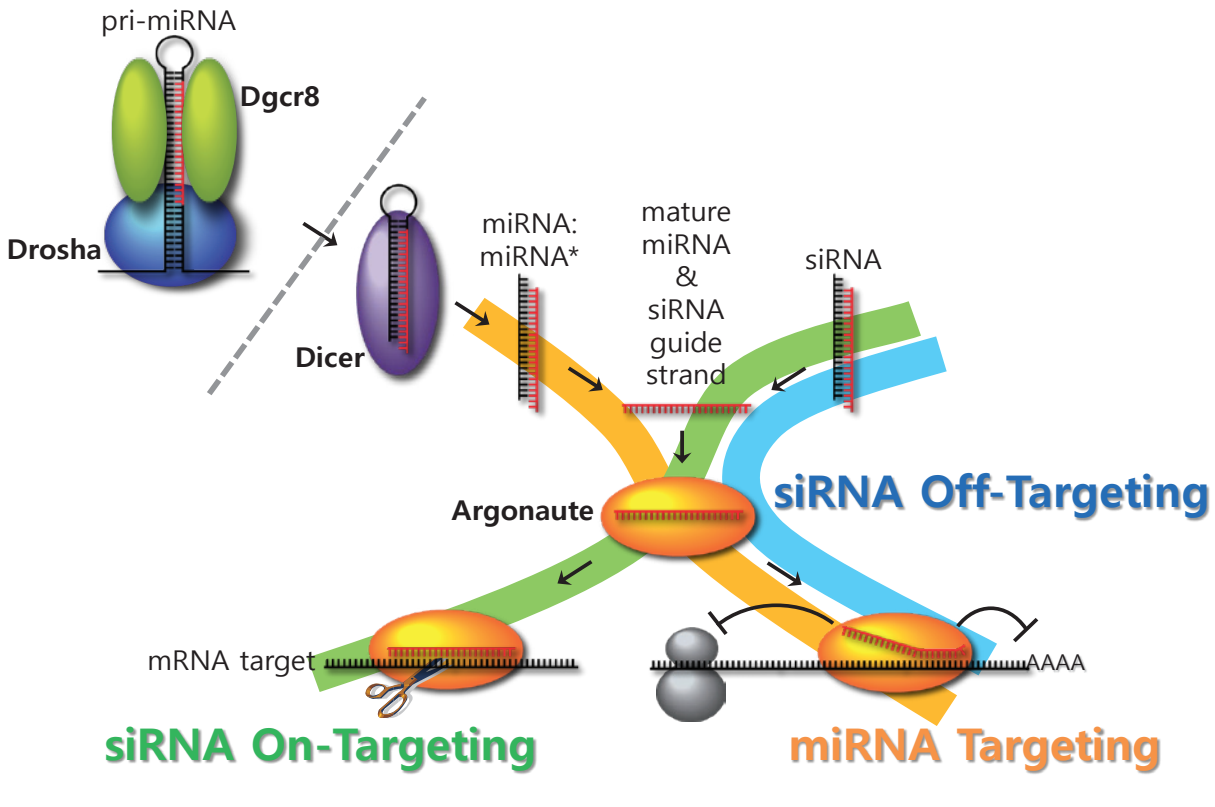

생성(biogenesis) 및 유전자 제어(targeting) 기작 규명 필요




▶ The simple ternary interaction and its known determinants can explain only 10-20% of miRNA targeting.
▶ Other determinants remain still unknown.

▶ >1,000 miRNAs in human
▶ 200-400 functional targets/miRNA
▶ >60% of human protein-coding genes are direct targets of miRNAs.

## RNAi Biogenesis and Targeting

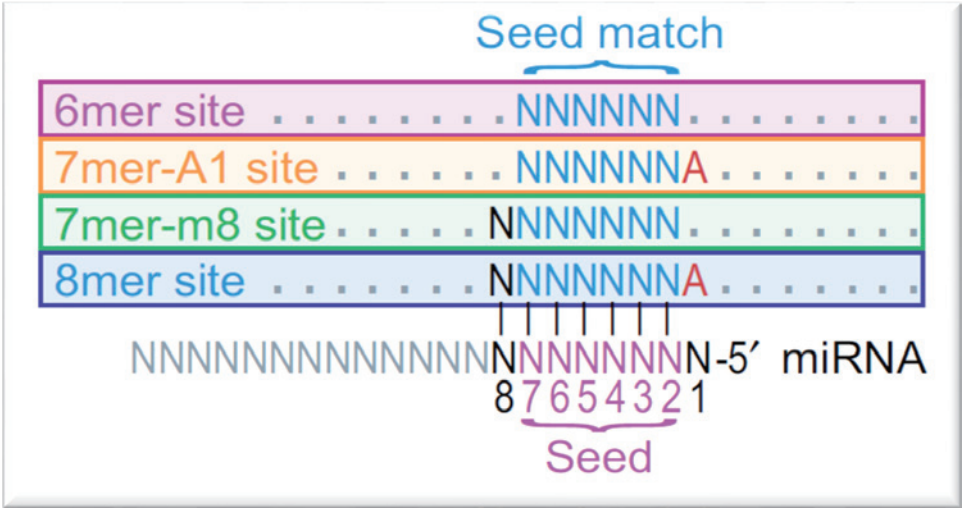


# General Rules for Functional MicroRNA Targeting

**Daehyun Baek**

**School of Biological Sciences**
**Seoul National University**

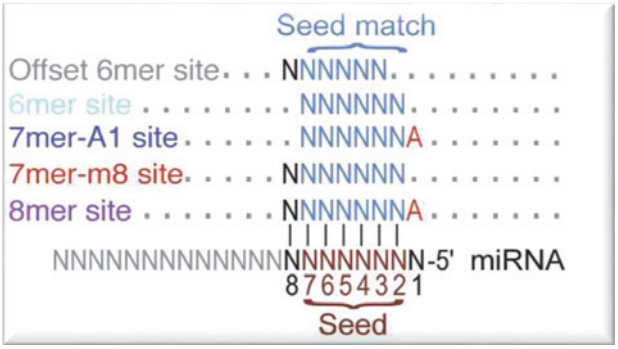
## Canonical Site Types(CSTs) of miRNA Targeting




(Baek *et al.*, Nature, 2008)

# Noncanonical Site Types(NSTs) of miRNA Targeting

## Offset 6mer




## Centered Site

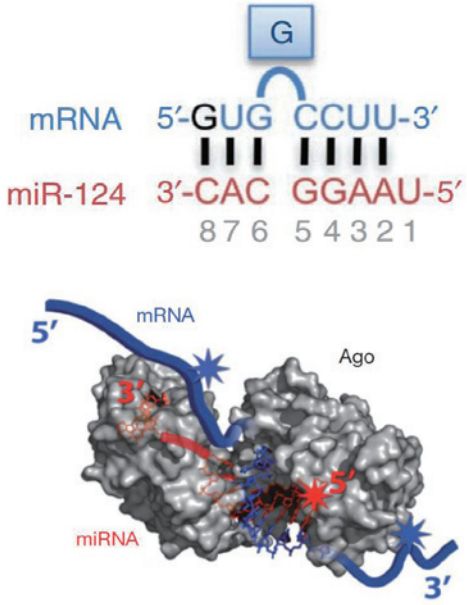
```
5′-nnnnnnnACAUGAAACUACnnn-3′  mRNA
          ||||||||||||
3′-GGGUUGUUGUACUUUGAUGGAU-5′  miRNA
```

(Friedman *et al.*, Genome Research, 2009)

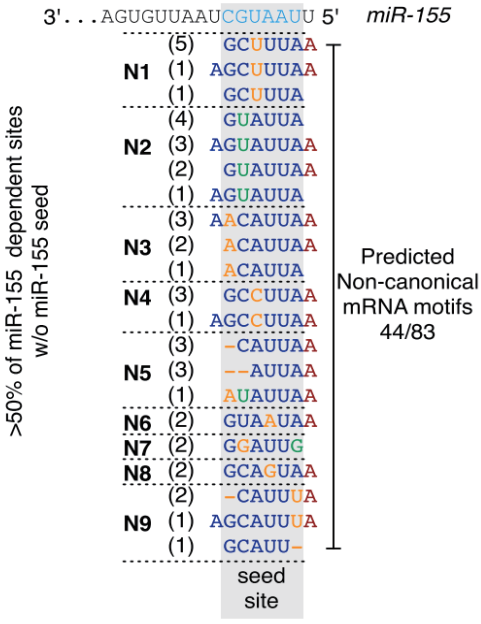
(Shin *et al.*, Molecular Cell, 2010)

# Noncanonical Site Types(NSTs) of miRNA Targeting

## Pivot Pairing




## Single Mismatch Sites




(Chi *et al.*, Nature, 2009)

(Chi *et al.*, NSMB, 2011)

(Loeb *et al.*, Molecular Cell, 2012)

# Limitations and Solutions

## Incomplete Searches

- Canonical target sites
- Offset 6-mer site
- Centered site

## Indirect Evidence

- AGO CLIPSeq-based analysis
- Based on limited number of miRNAs
- Pivot pairing site
- MIRZA sites

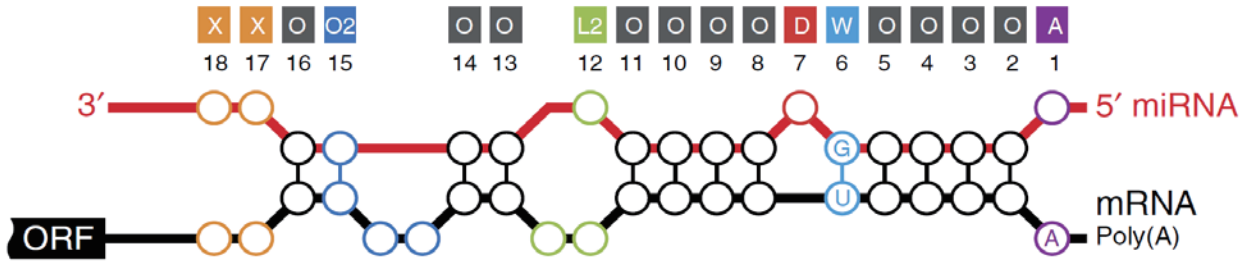
## Limitations

- No one has performed the comprehensive and systematic search for functional miRNA targeting rules.

## Solution: Extensive Bioinformatics Analysis

- Massive-scale search for functional, meaning those targets that elicit detectable mRNA repression, miRNA targets.
- **Goal: The most comprehensive discovery of miRNA targeting principles**

# Challenge: Complexity of miRNA-mRNA Interactions




O Match X Mismatch D Bulge on miRNA A Adenine at 1st mRNA position
B Other at 1st mRNA position W Wobble pairing O1–O9 Bulge on mRNA
L1–L9 Internal loop ø All but match . Does not matter

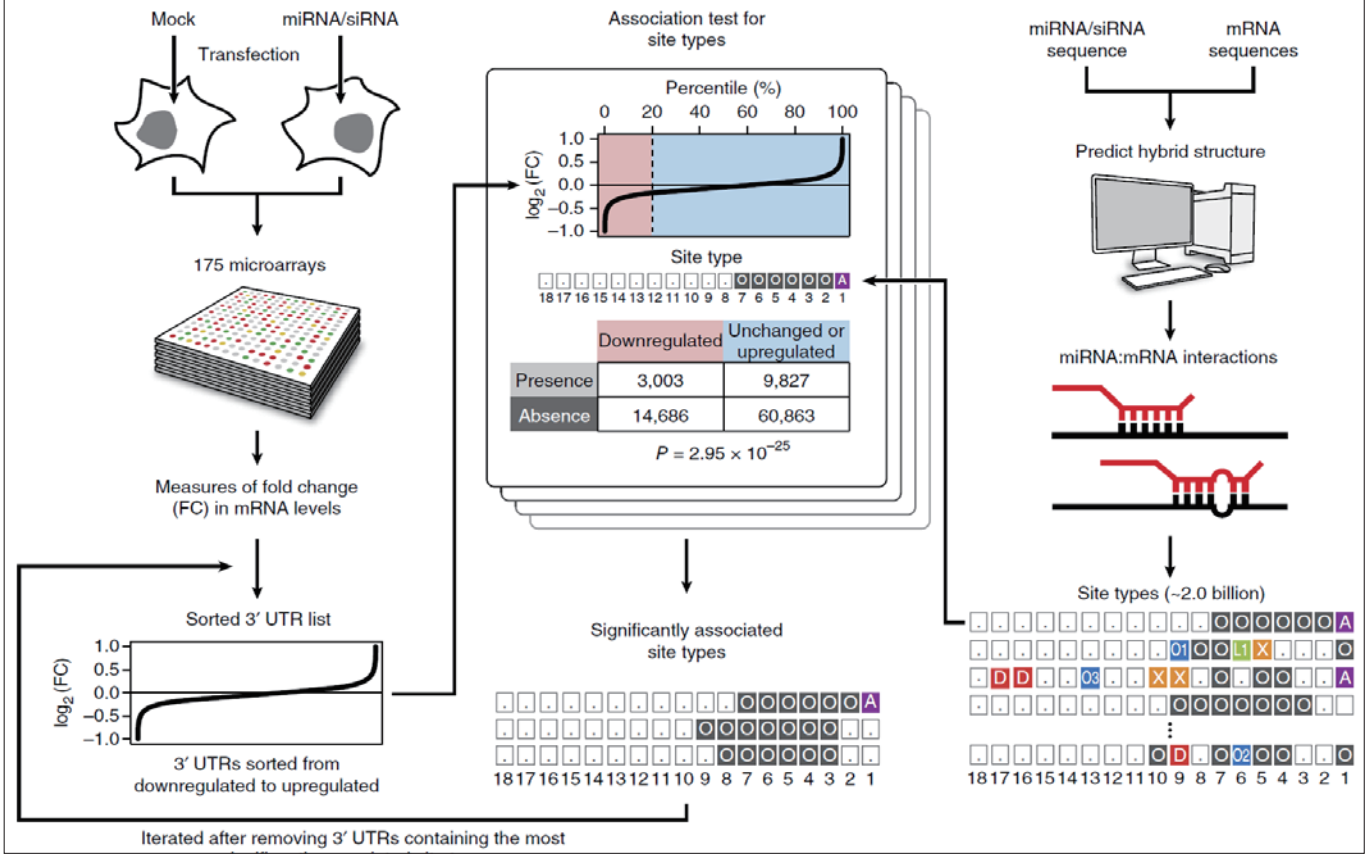
**The number of site types(STs) that can occur between human miRNAs and mRNAs with >8 targets: ~2 Billion**

# Comparison of Search Spaces

Previously evaluated <1 million STs

**>99.9% of the STs have remained unexamined.**

# Method Overview



# Solution

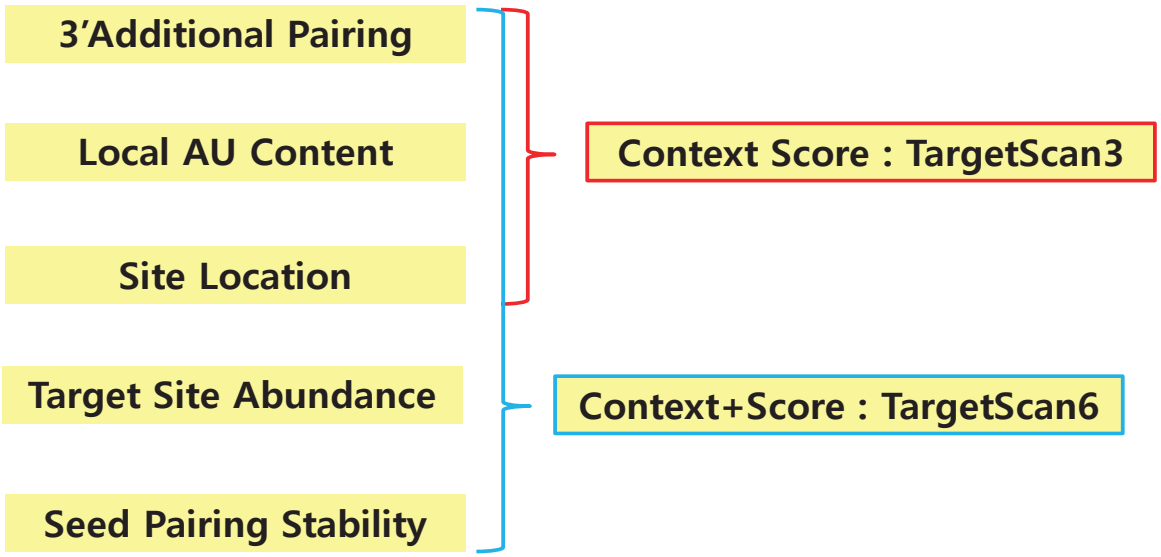
## High-Performance Hardware

- High-performance server system
- >1,500 CPU cores
- >1.2 PB of storage

## Optimized Software

- Implemented in C/C++
- Massive use of bit-operation
- Hash table based optimization
- Fast compression algorithm for data transfer

# Known Local Context of CSTs

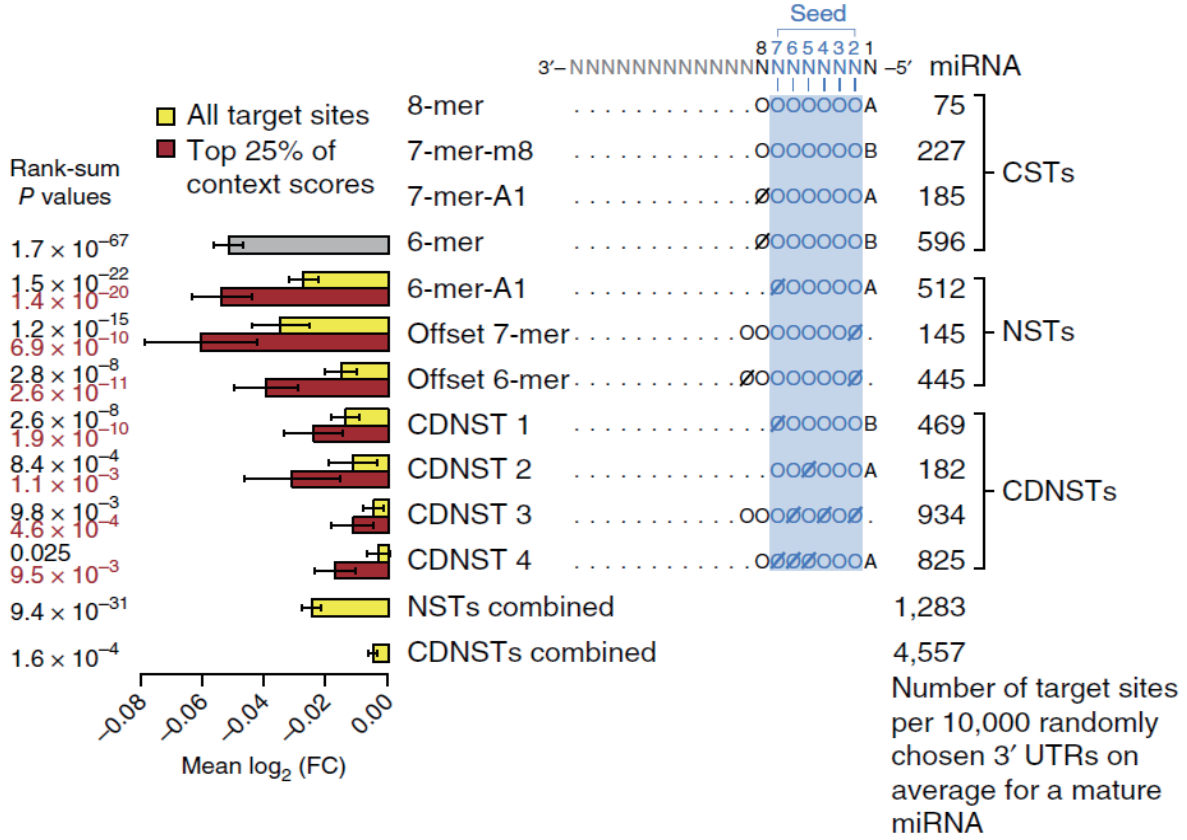



**5 known determinants for the overall proficiency of CSTs**

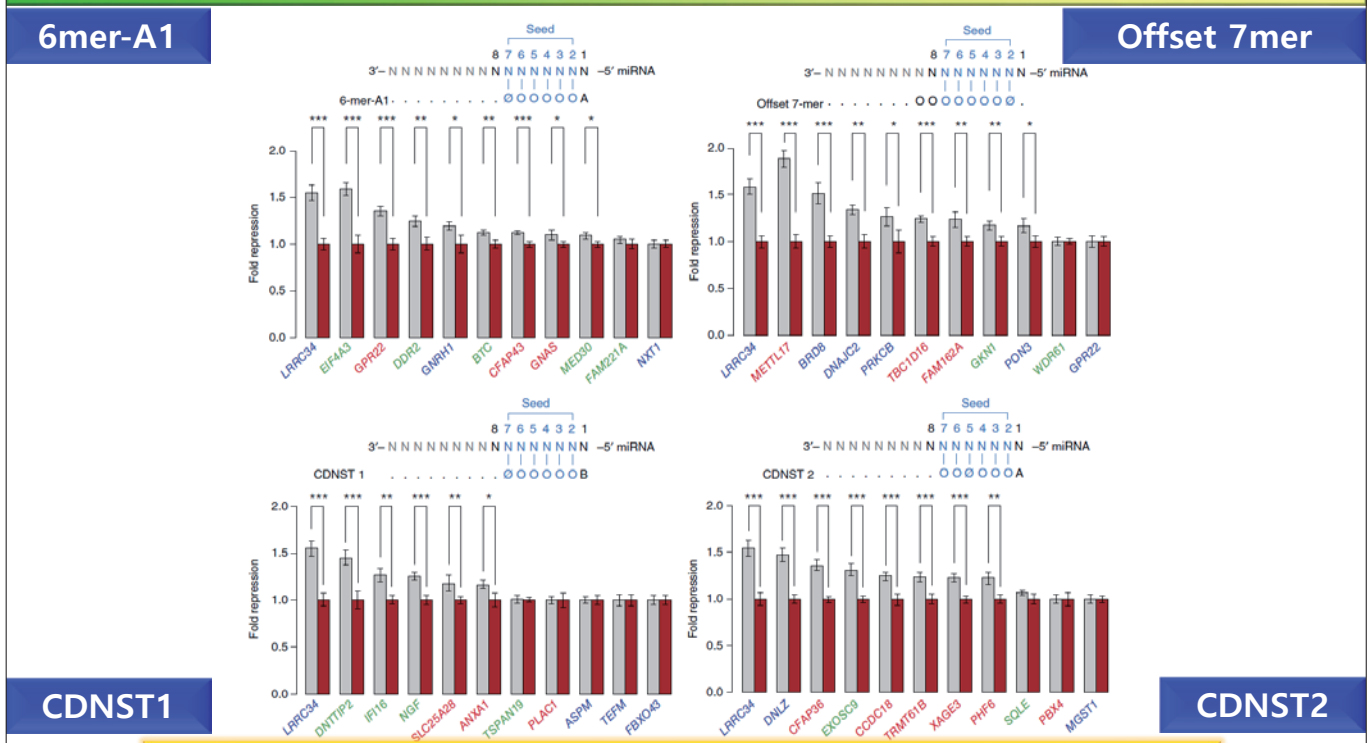
(Grimson *et al.*, Molecular Cell, 2007)

(Garcia and Baek *et al.*, NSMB, 2011)

# Discovery of Functional CDNSTs

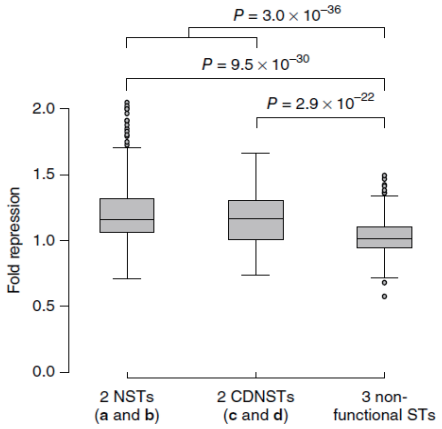


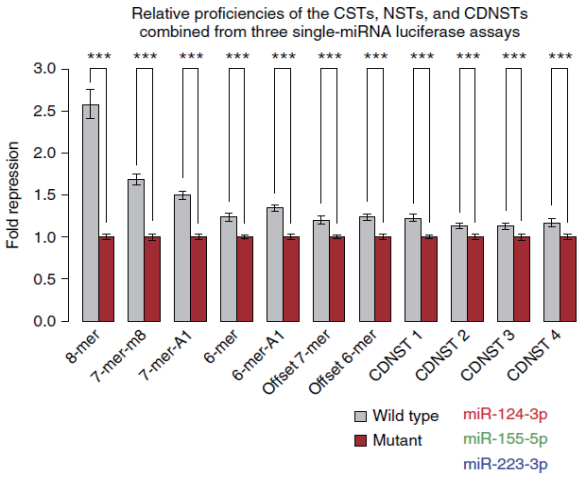

# Validation of NSTs and CDNSTs: Luciferase Assays




**70% of NST and CDNST targets were validated by luciferase assays.**

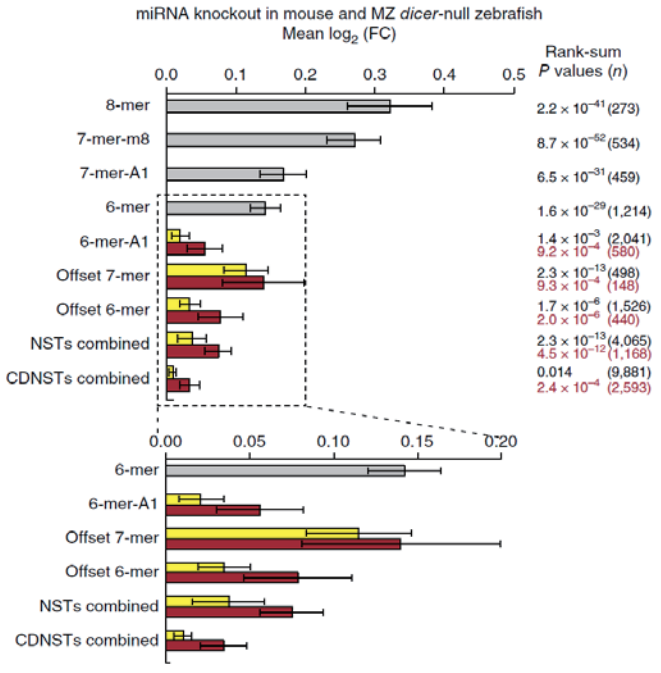
# Validation of NSTs and CDNSTs: Luciferase Assays

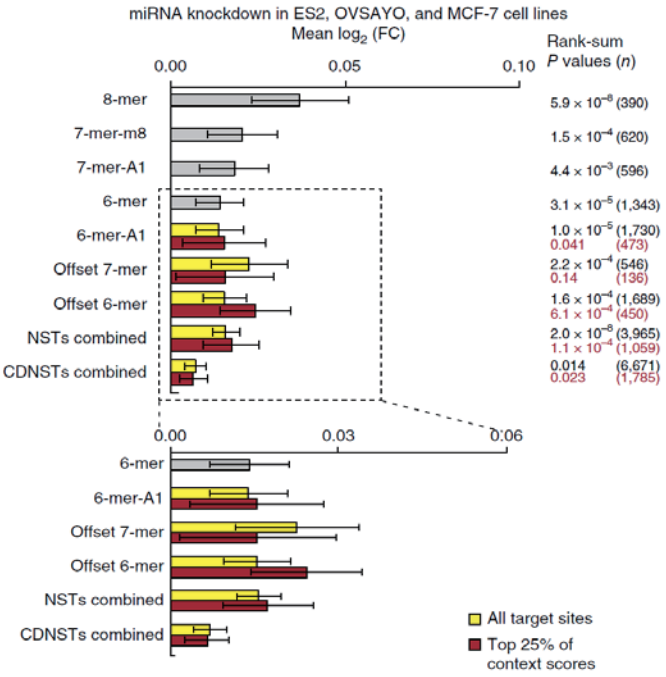


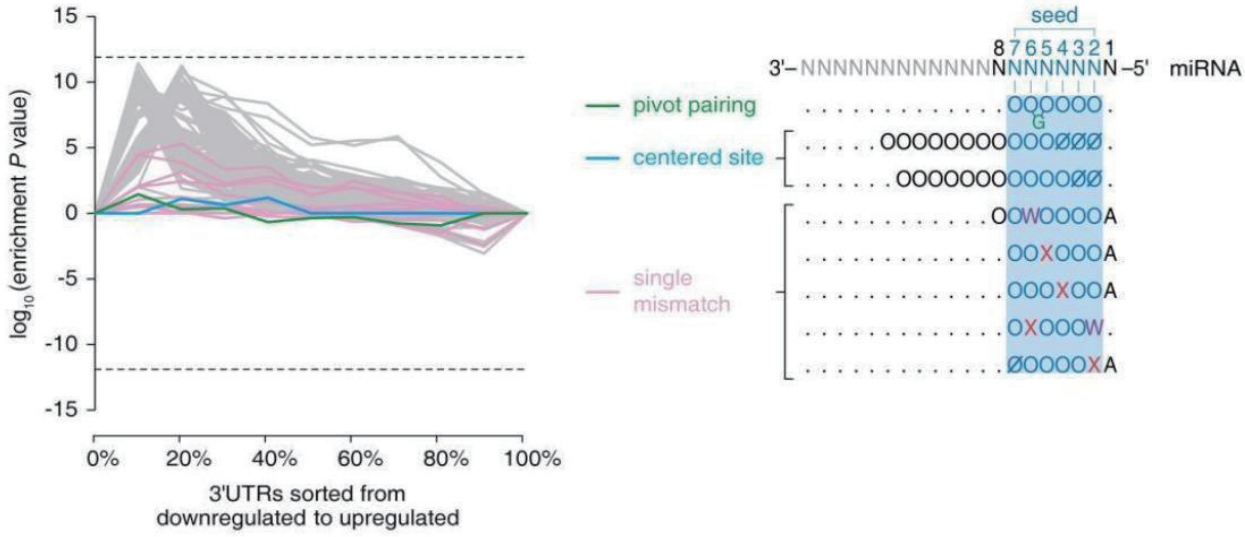

**70% of NST and CDNST targets were validated by luciferase assays.**

# Validation of NSTs and CDNSTs: miRNA KO/KD Data

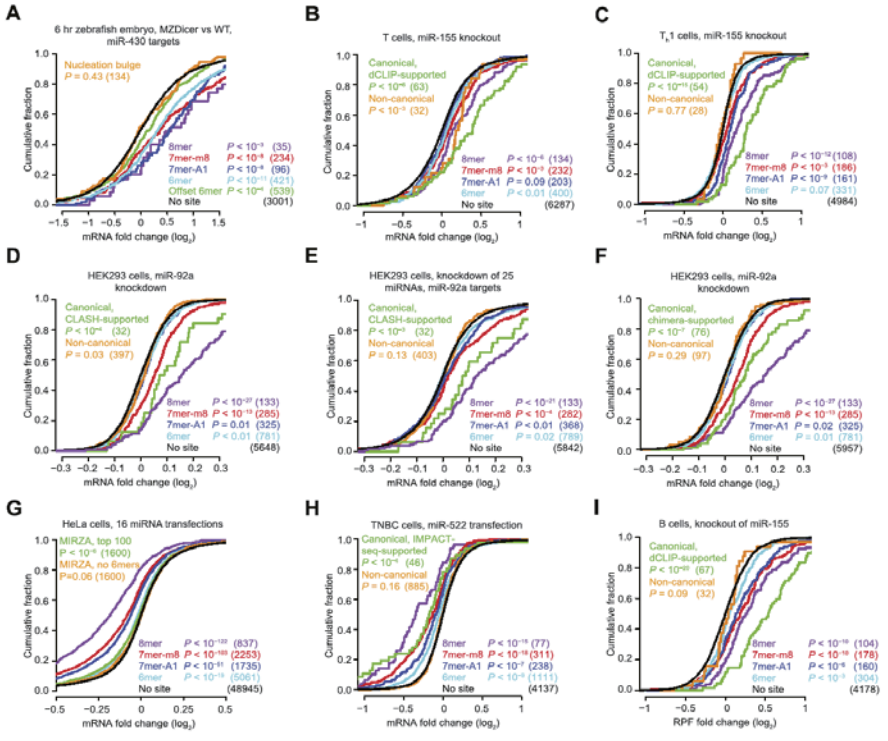


# Evaluation of Previously Reported NSTs



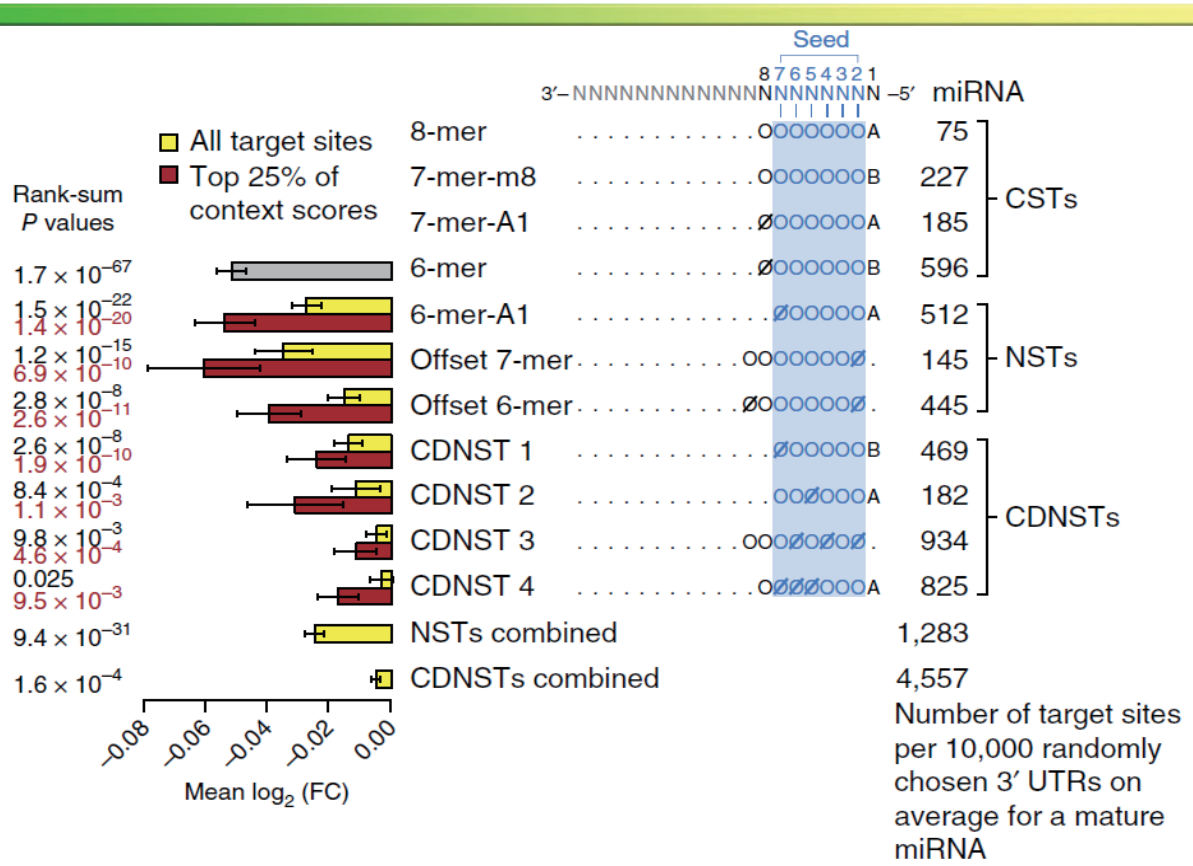

# Evaluation of Previously Reported NSTs



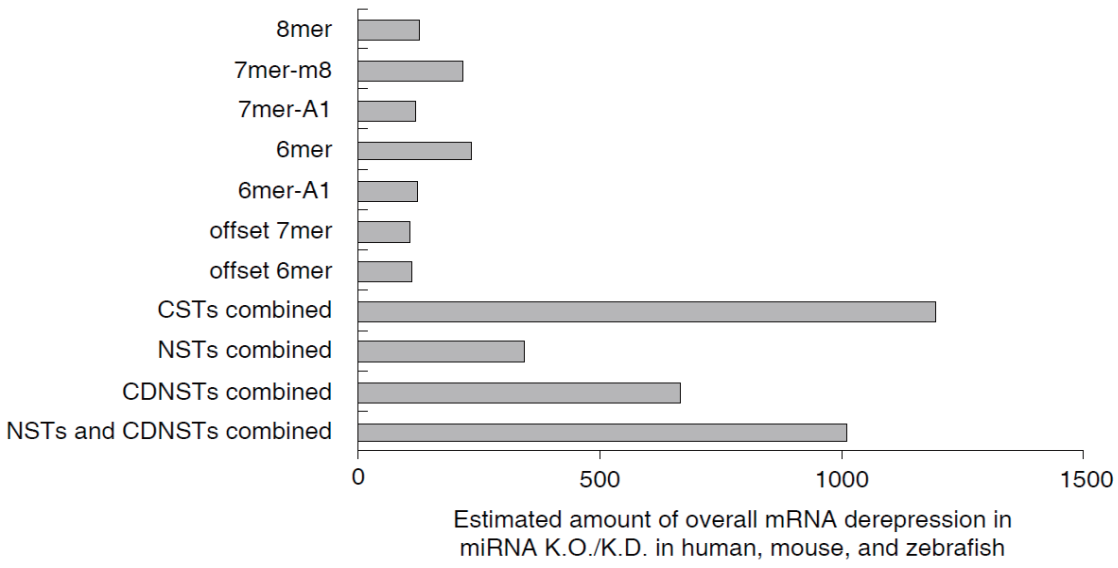

**Consistent with a recent work by the Bartel lab.**

(Agarwal *et al.*, eLife, 2015)

# Comprehensive View on Functional miRNA Targeting




# The Impact of Functional miRNA Targeting



## The Impact of Functional miRNA Targeting

| Site type name | Site type | Median PhyloP score[a] Site | Median PhyloP score[a] Control | Rank-sum *P* value |
|---|---|---|---|---|
| 8-mer | .....OOOOOOOA | 0.521 | 0.218 | $<1.0 \times 10^{-320}$[b] |
| 7-mer-m8 | .....OOOOOOOB | 0.241 | 0.172 | $<1.0 \times 10^{-320}$[b] |
| 7-mer-A1 | .....ØOOOOOOA | 0.291 | 0.221 | $<1.0 \times 10^{-320}$[b] |
| 6-mer | .....ØOOOOOOB | 0.193 | 0.175 | $6.3 \times 10^{-48}$ |
| 6-mer-A1 | .....ØOOOOOA | 0.281 | 0.265 | $1.9 \times 10^{-39}$ |
| Offset 7-mer | .....OOOOOOOØ. | 0.235 | 0.184 | $2.8 \times 10^{-257}$ |
| Offset 6-mer | .....ØOOOOOOØ. | 0.210 | 0.177 | $7.2 \times 10^{-120}$ |
| CDNST 1[c] | .....ØOOOOOB | 0.263 | 0.252 | $1.6 \times 10^{-12}$ |
| CDNST 2[c] | .....OOØOOOA | 0.387 | 0.361 | $3.5 \times 10^{-29}$ |
| CDNST 3[c] | .....OOOØOØOØ. | 0.189 | 0.172 | $9.0 \times 10^{-19}$ |
| CDNST 4[c] | .....OØØØOOOA | 0.275 | 0.291 | 1.0 |

## Conclusions

◈ **We have constructed a massive-scale bioinformatics pipeline that aims to systematically and comprehensively evaluate miRNA-target interactions.**

◈ **We discovered 7 NSTs and CDNSTs, many of which have not been reported previously.**

◈ **Luciferase assays and independent data analyses suggest that most of the newly discovered NSTs and CDNSTs may be functional.**

◈ **The miRNA-target interactions and their gene regulatory networks may be substantially more complex than currently perceived.**

nature genetics

# General rules for functional microRNA targeting

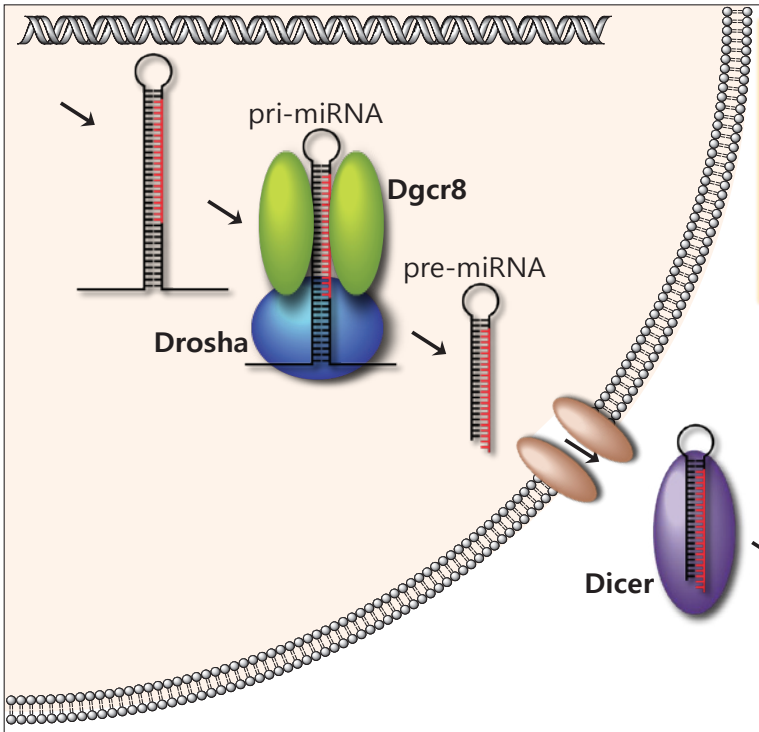
Doyeon Kim[1,2,4], You Me Sung[2,4], Jinman Park[1,2,4], Sukjun Kim[1,2], Jongkyu Kim[1,2], Junhee Park[2], Haeok Ha[2], Jung Yoon Bae[2], SoHui Kim[1,2] & Daehyun Baek[1–3]

**The functional rules for microRNA (miRNA) targeting remain controversial despite their biological importance because only a small fraction of distinct interactions, called site types, have been examined among an astronomical number of site types that can occur between miRNAs and their target mRNAs. To systematically discover functional site types and to evaluate the contradicting rules reported previously, we used large-scale transcriptome data and statistically examined whether each of approximately 2 billion site types is enriched in differentially downregulated mRNAs responding to overexpressed miRNAs. Accordingly, we identified seven non-canonical functional site types, most of which are novel, in addition to four canonical site types, while also removing numerous false positives reported by previous studies. Extensive experimental validation and significantly elevated 3′ UTR sequence conservation indicate that these non-canonical site types may have biologically relevant roles. Our expanded catalog of functional site types suggests that the gene regulatory network controlled by miRNAs may be far more complex than currently understood.**

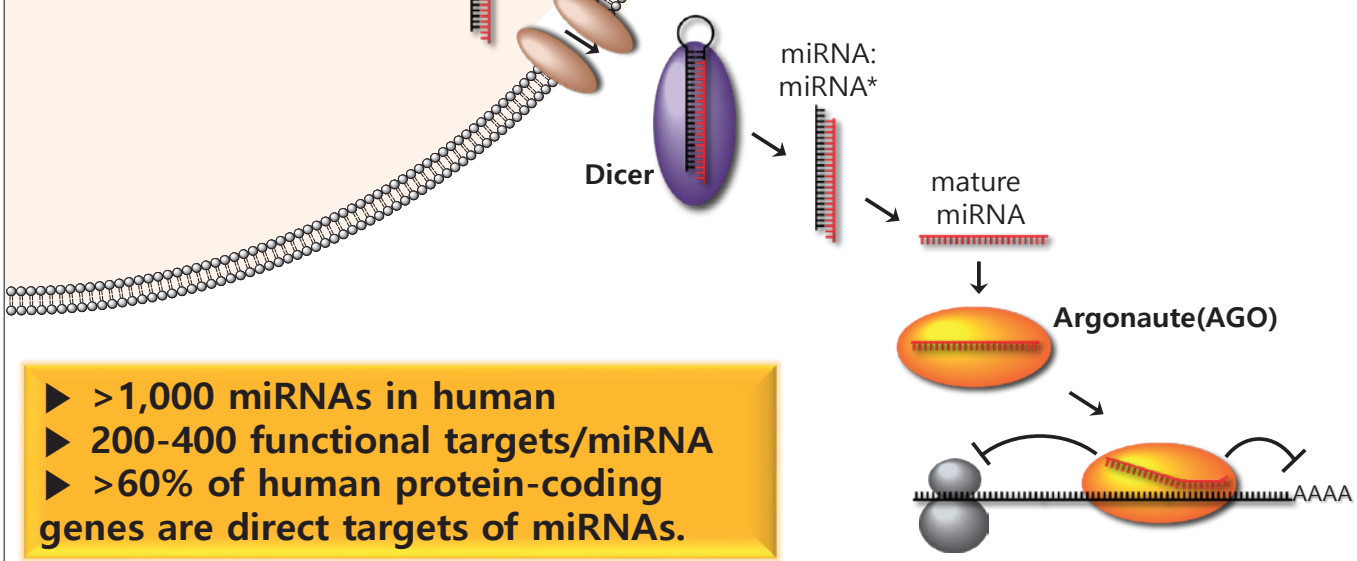
# Widespread Impact of RNA-Binding Proteins on MicroRNA Targeting

**Daehyun Baek**

**School of Biological Sciences**
**Seoul National University**

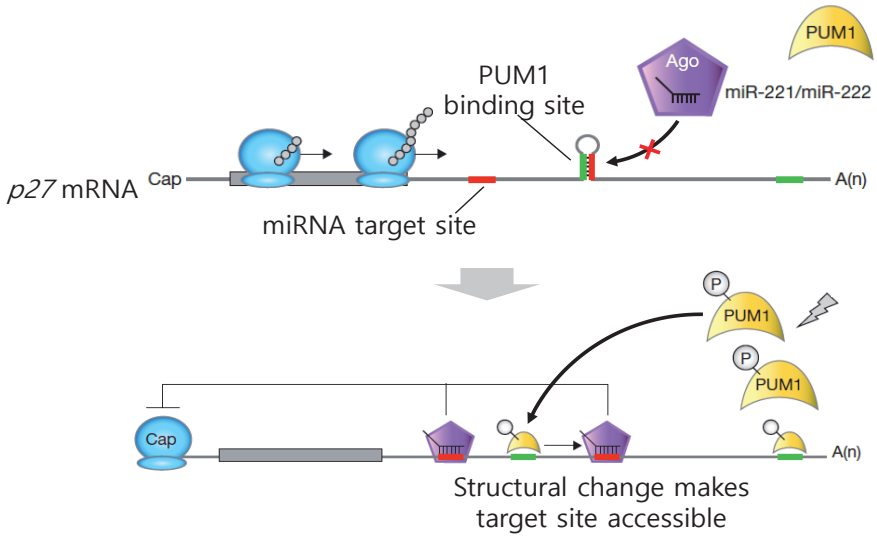



▶ The simple ternary interaction and its known determinants can explain only 10-20% of miRNA targeting.
▶ Other determinants remain still unknown.




▶ >1,000 miRNAs in human
▶ 200-400 functional targets/miRNA
▶ >60% of human protein-coding genes are direct targets of miRNAs.

## miRNA Targeting(MT)에 영향을 주는 RNA-결합 단백질(RBP)

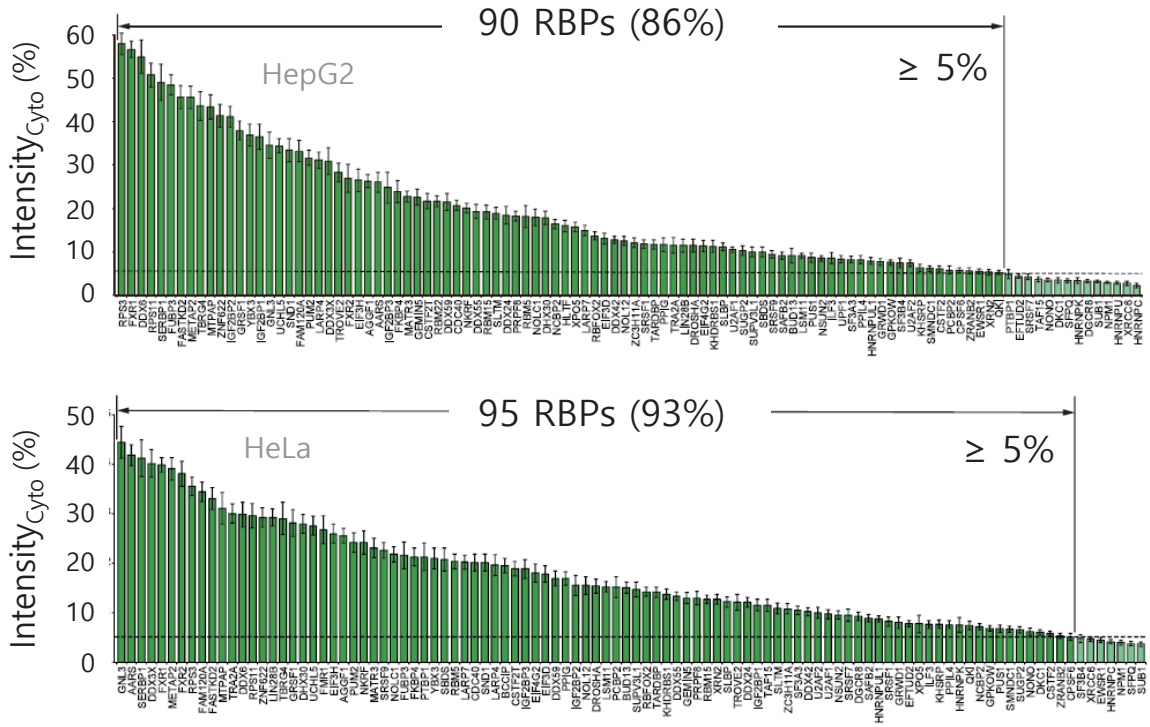



- MT 인헨서: Pumilio, PCBP2, FUS, and PTBP1
- MT 서프레서: Dnd1, RBM38, HuR, IGF2BP1, and PTBP1

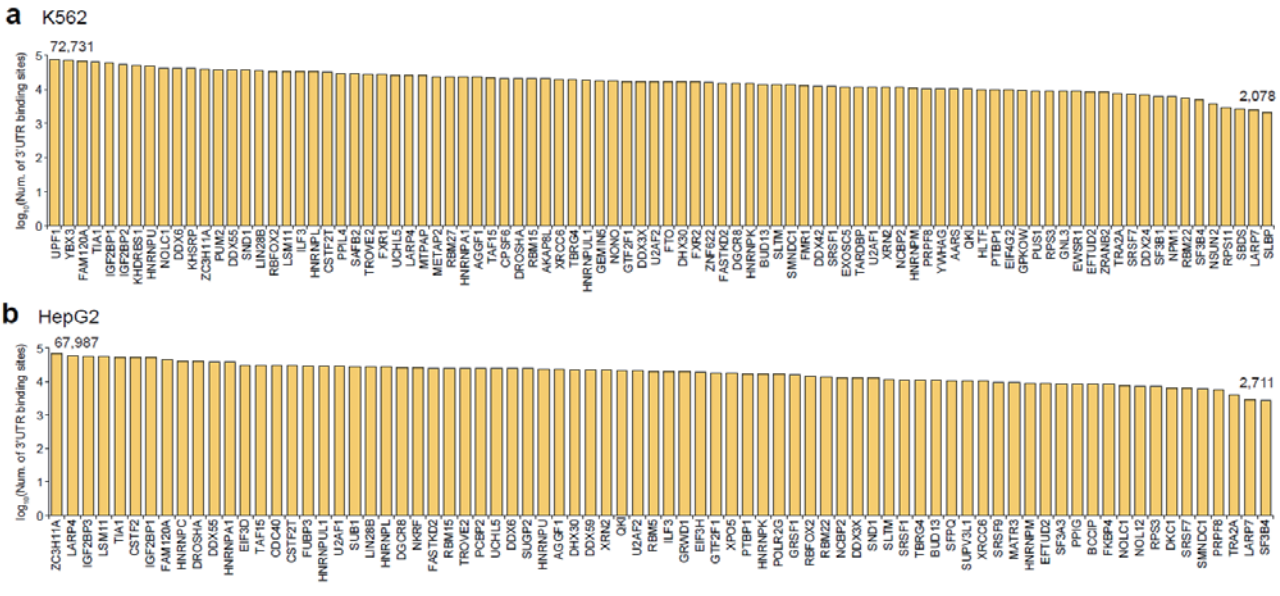
**>800 RBPs x 22,000 3'UTR RBP-결합 사이트 =
~17 million 개에 이르는 결합 중 극히 일부만 연구됨**

(Tribouleet *et al.*, 2010, Nature Cell Biology)

# Cytoplasmic Fraction of RBPs

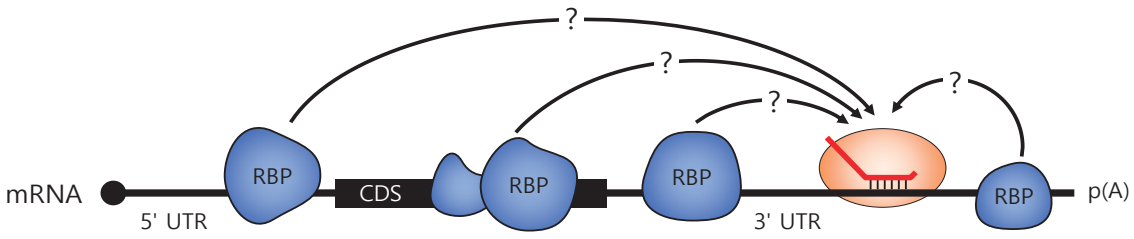



# Num of 3'UTR Binding Sites of Individual RBPs




**>2,000 Binding Sites of Each RBP Located in the 3' UTR**

# 핵심 가설: RBP가 MT 조절에 중요한 역할 수행

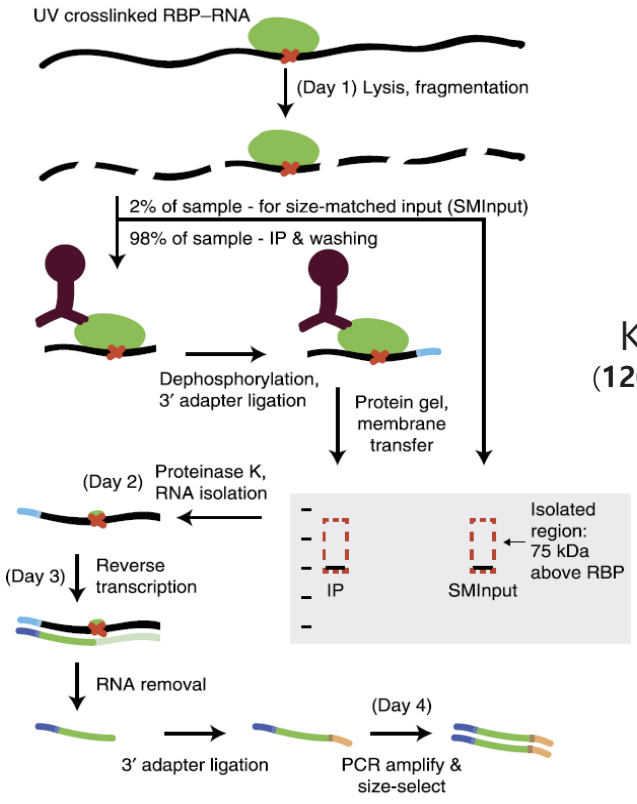


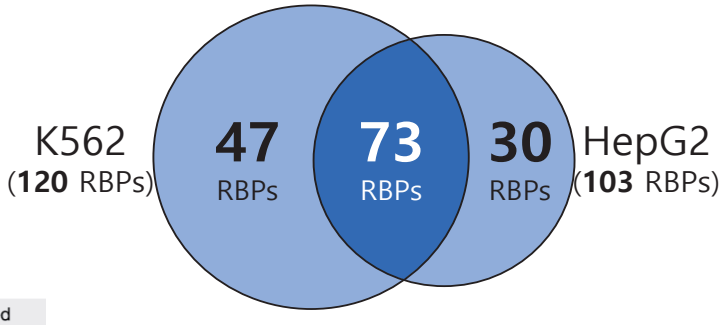

**What features** of RBPs affect miRNA targeting (MT) efficacy?

- The **distance** to the **5' end** or **3' end** of mRNA?
- The **distance** to the **CDS start** or **CDS end**?
- The **distance** to the **miRNA target site**?
- The **number** of RBP binding sites (RBSs)?
- The **density** of RBS?
- The **intensity** of RBP binding?

⋮

# ENCODE eCLIP-Seq 데이터(mRNA 상의 RBP-결합 위치 정보)

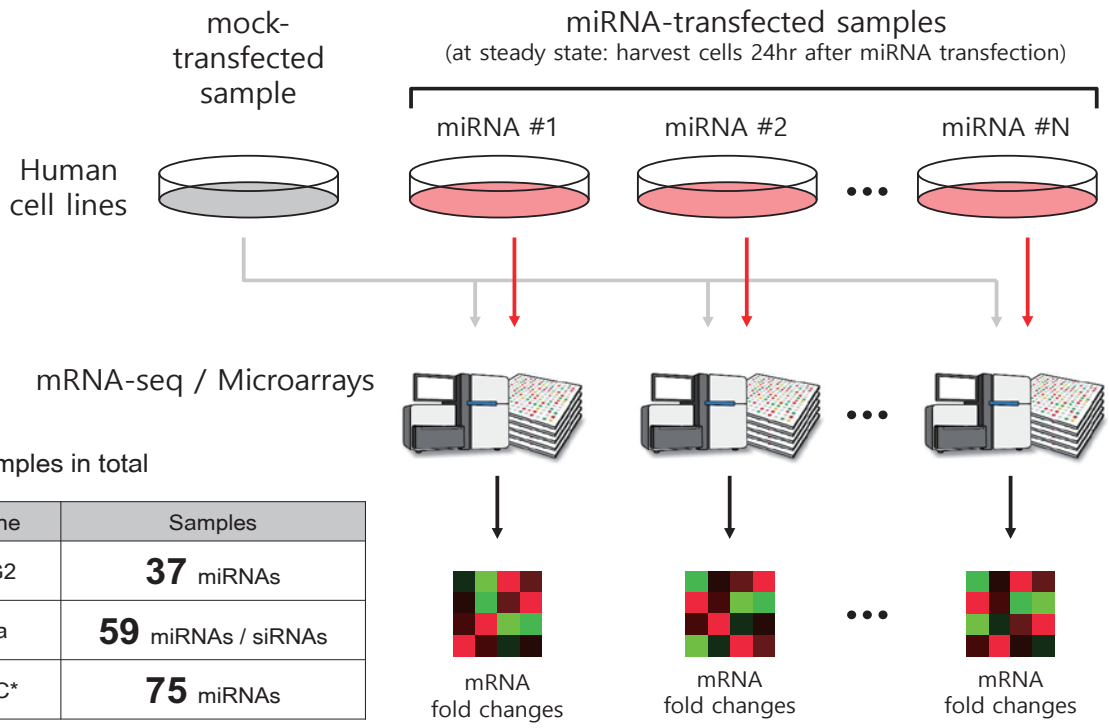



**150** RBPs

(Nostrand *et al.*, 2016, Nature Methods)

# 대규모 전사체 데이터를 통한 MT 효율 측정

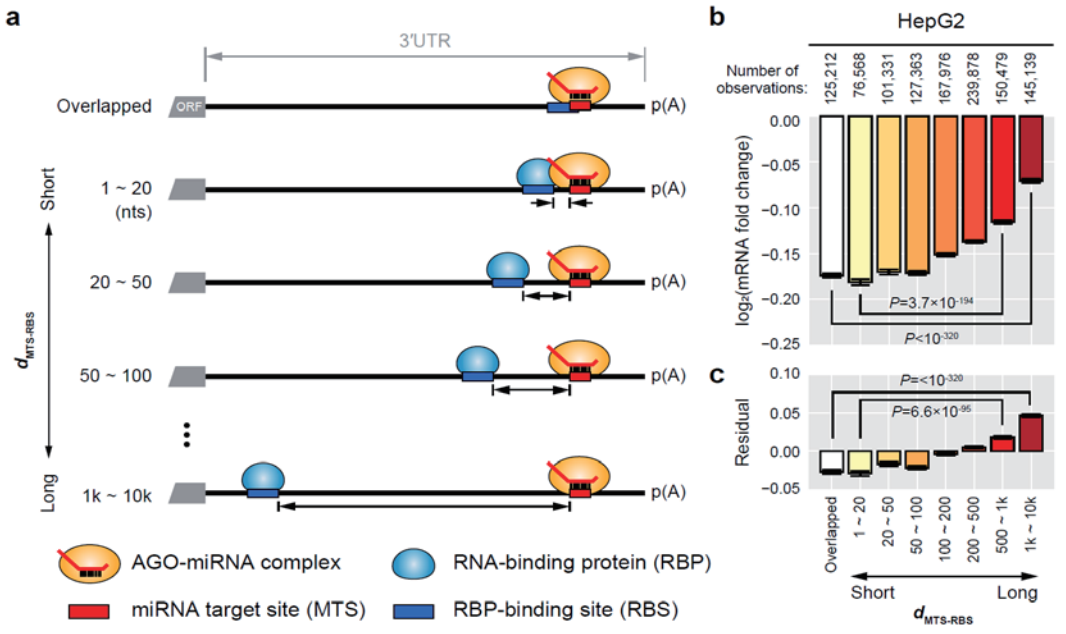



**171** samples in total

| Cell line | Samples |
|---|---|
| HepG2 | **37** miRNAs |
| HeLa | **59** miRNAs / siRNAs |
| OHCC* | **75** miRNAs |

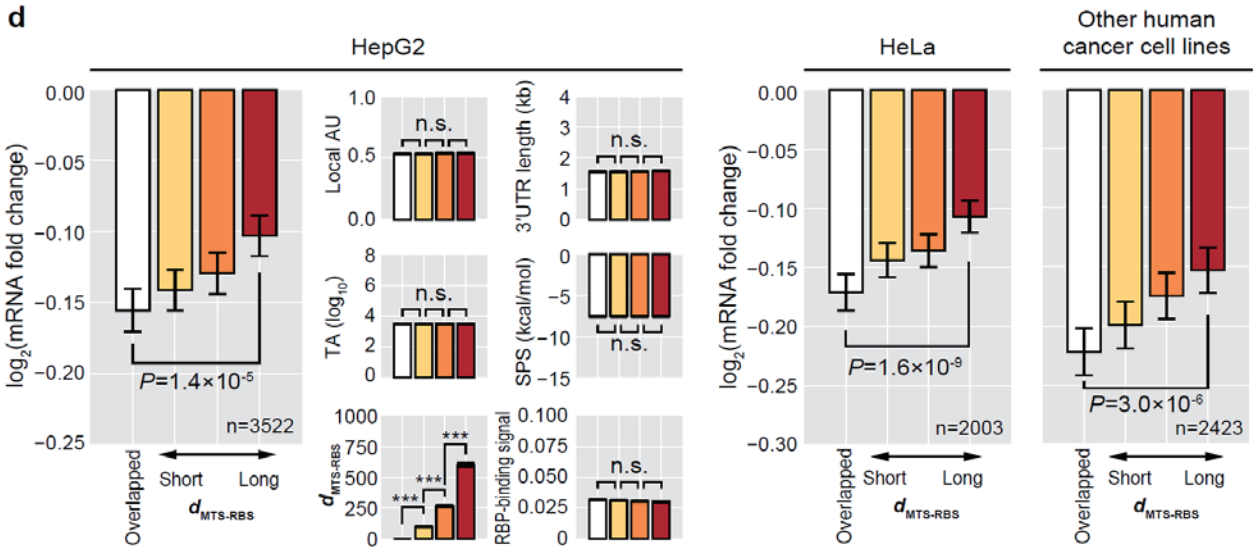
* OHCC: Other human cancer cell lines

# RBP-결합 위치에 따른 MT 효율의 영향



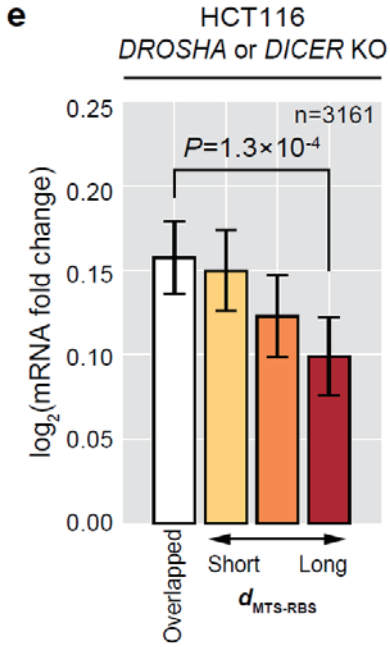

**RBP-결합 위치가 miRNA 타겟 사이트에 가까울수록 MT 효율이 강하게 증가함 ➔ RBP가 MT 인헨서로 동작**

# RBP는 강력한 MT 인헨서



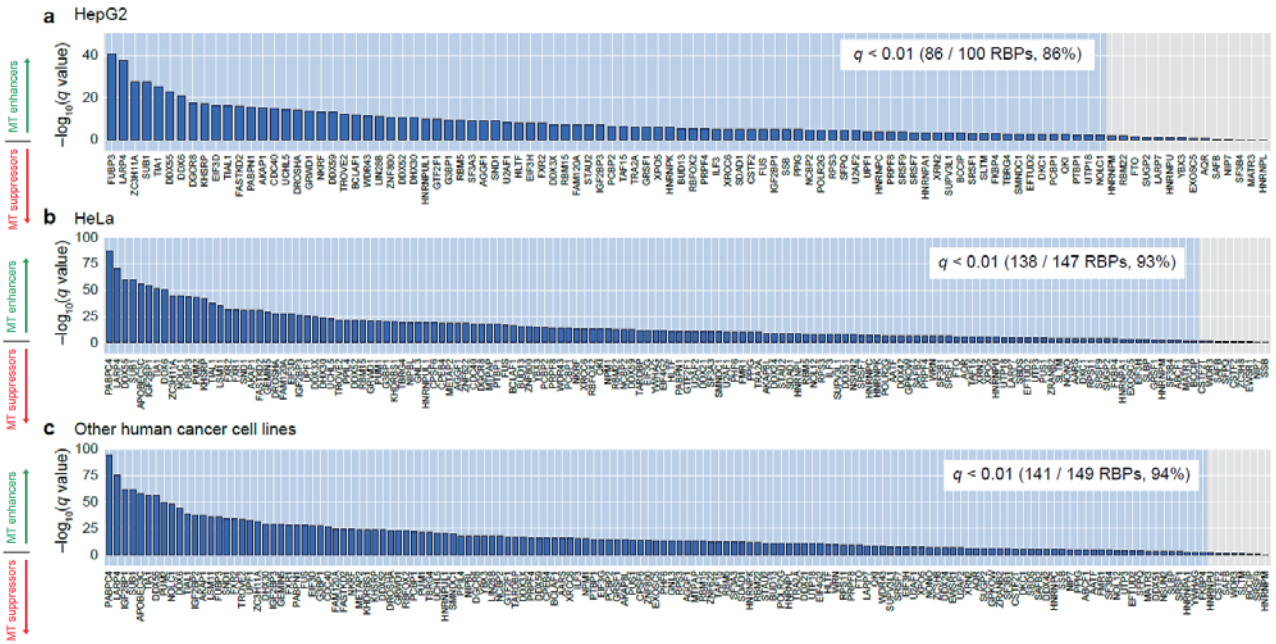

**서로 다른 여러가지 세포 조건에서 동일 현상 관찰됨**

# Endogenous 조건에서도 RBP는 강력한 MT 인헨서로 동작




**miRNA를 과발현시킨 조건뿐만 아니라**
**miRNA KO시킨 endogenous 조건에서도 동일 현상 관찰됨**

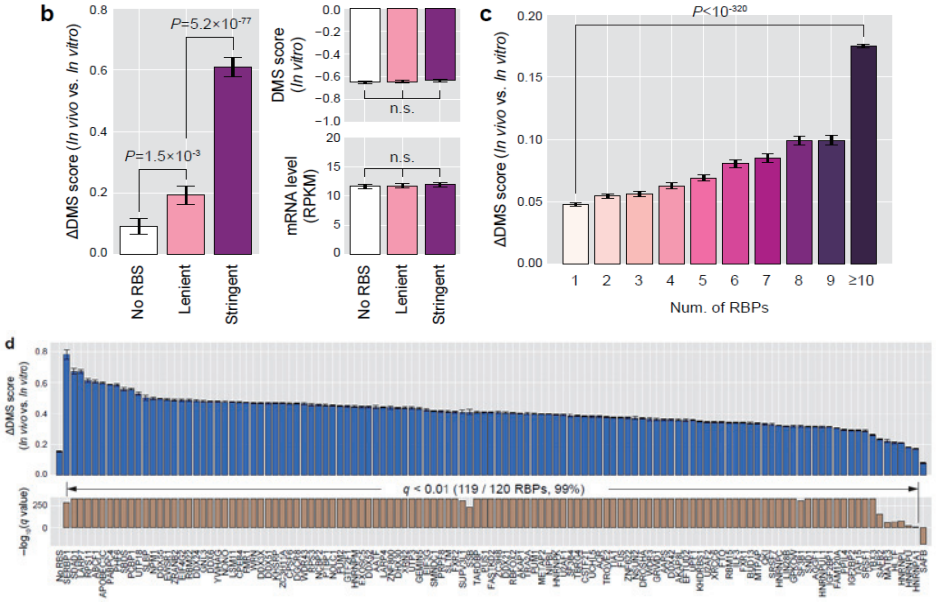
# 거의 모든 RBP들이 MT 인헨서로 동작




**RBP 개별 분석: ≥86% RBP들이 MT 인헨서로 동작하고**
**MT 서프레서로는 단 하나도 동작하지 않음**

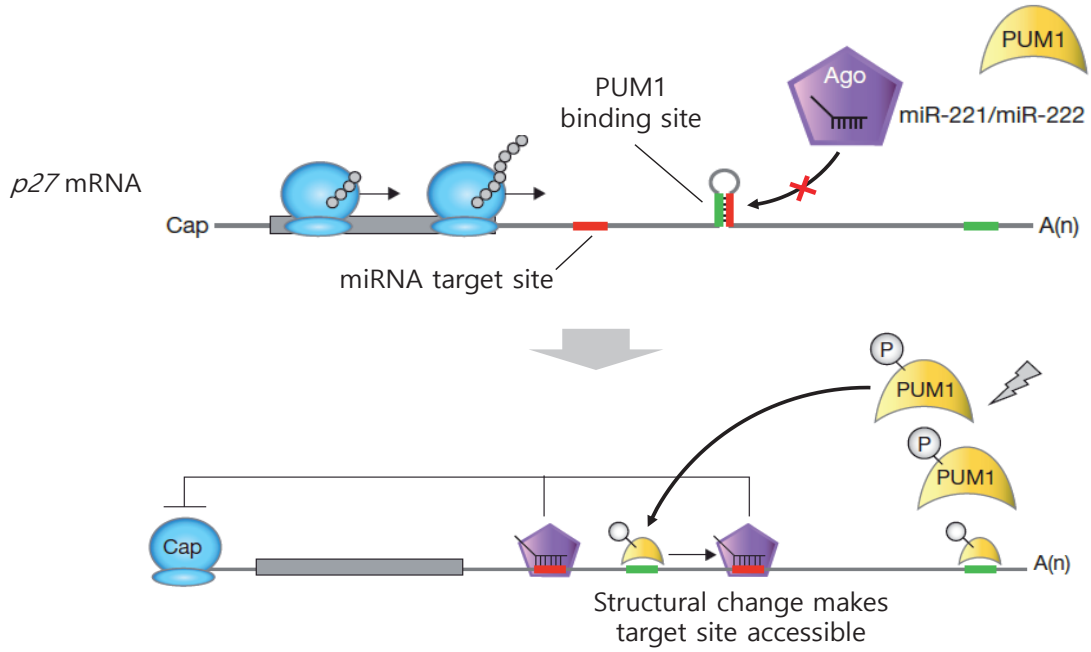
# 기작: RBP-결합이 mRNA의 Secondary Structure를 Open

- **DMS-seq detects unpaired nucleotides *in vivo*.**



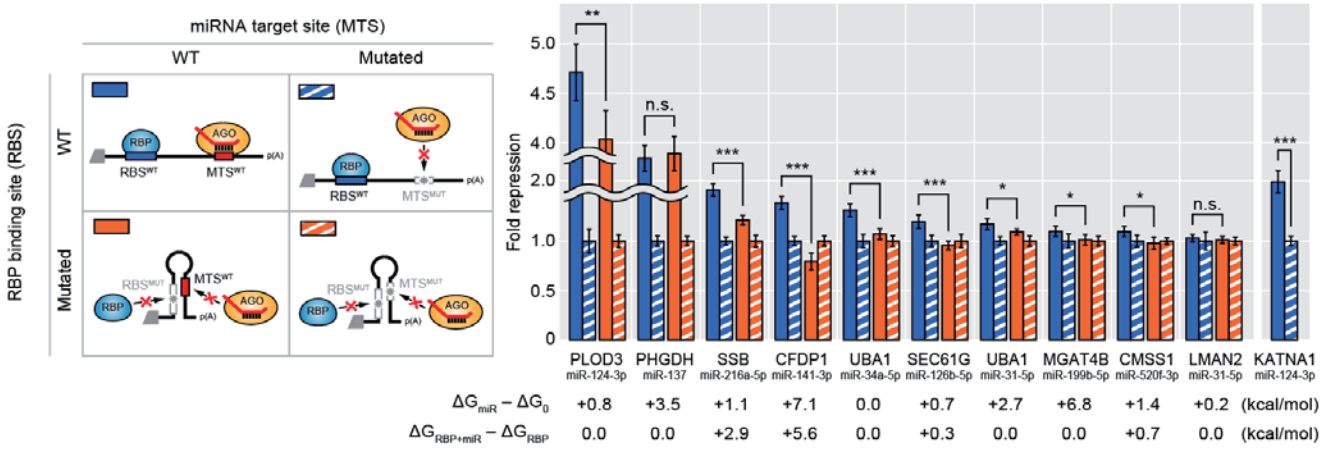

**PUM1에서처럼, RBP-결합이 mRNA의**
**secondary structure를 open하여 MT 효율을 증가**

# MT에 영향을 주는 RBP: PUM1



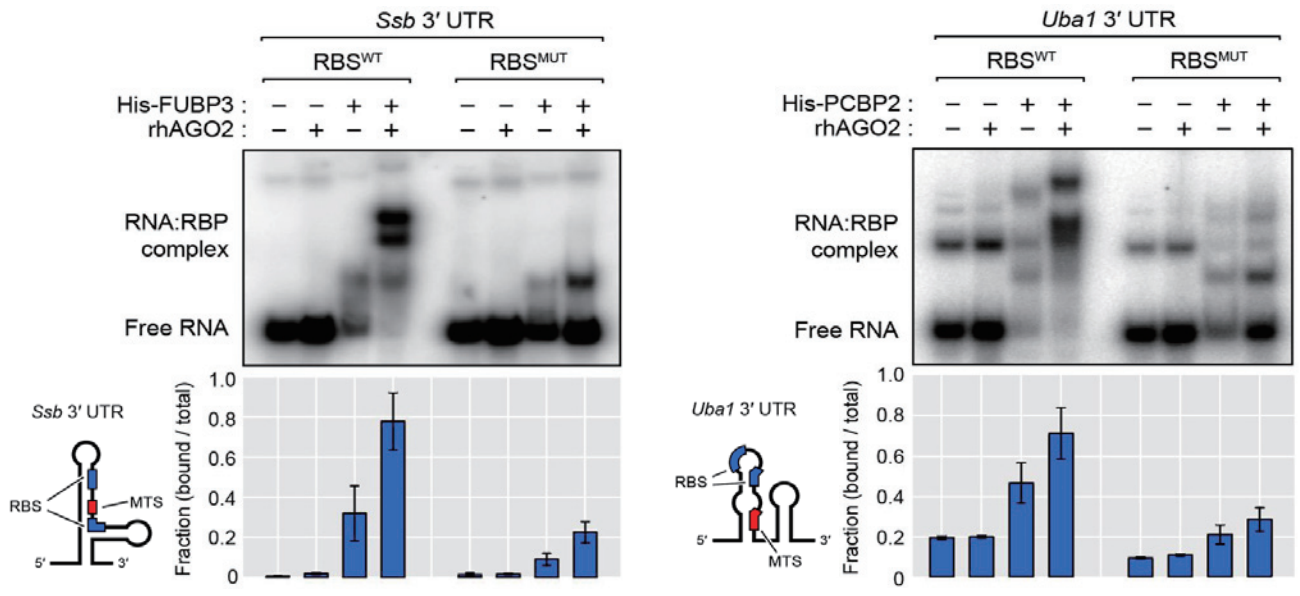

(Triboulet *et al.*, 2010, Nature Cell Biology)

# 실험적 검증: Luciferase Reporter Assay



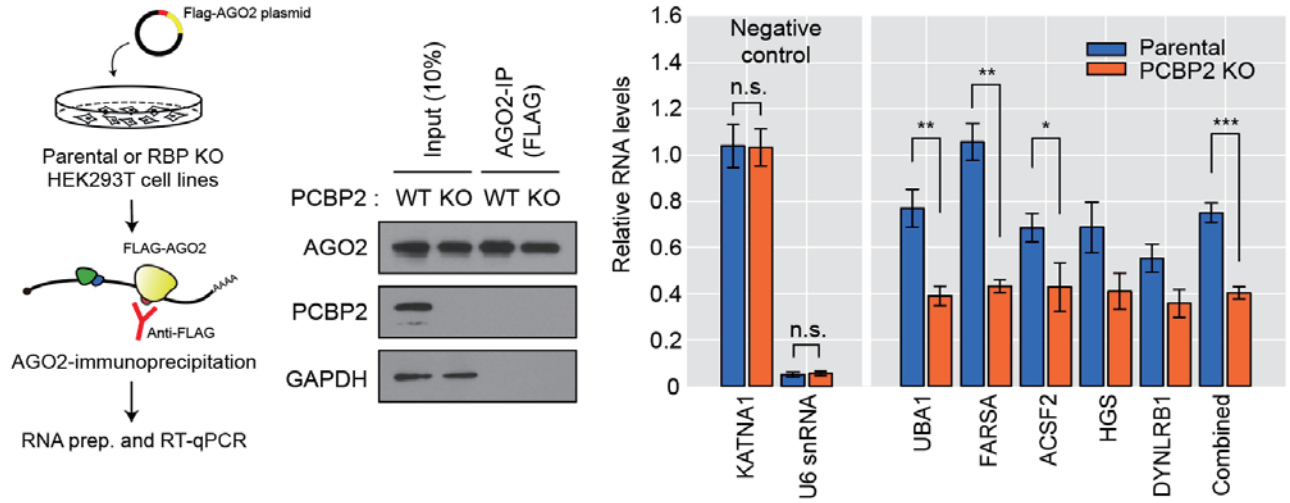

**80%의 3'UTR에서 RBP가 MT 효율을 직접적으로 제어**

# AGO Binding Changes *in vitro* - Gel Shift Assay (EMSA)



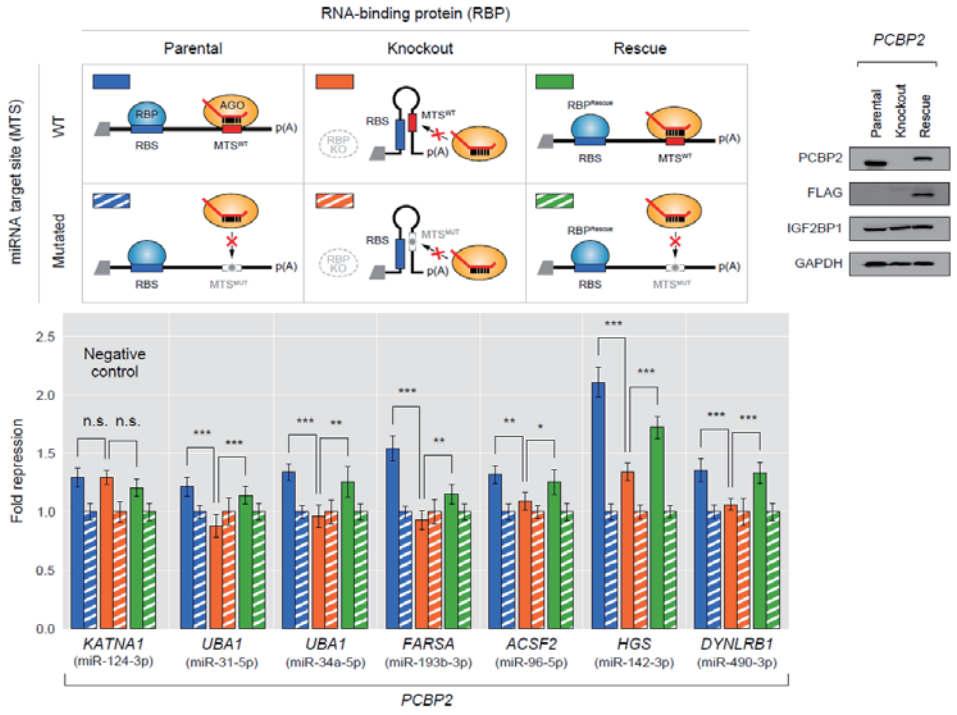

**The Disrupted RBS Reduces AGO Binding to the MTS.**

# AGO Binding Changes *in vivo* - RNA IP Experiment



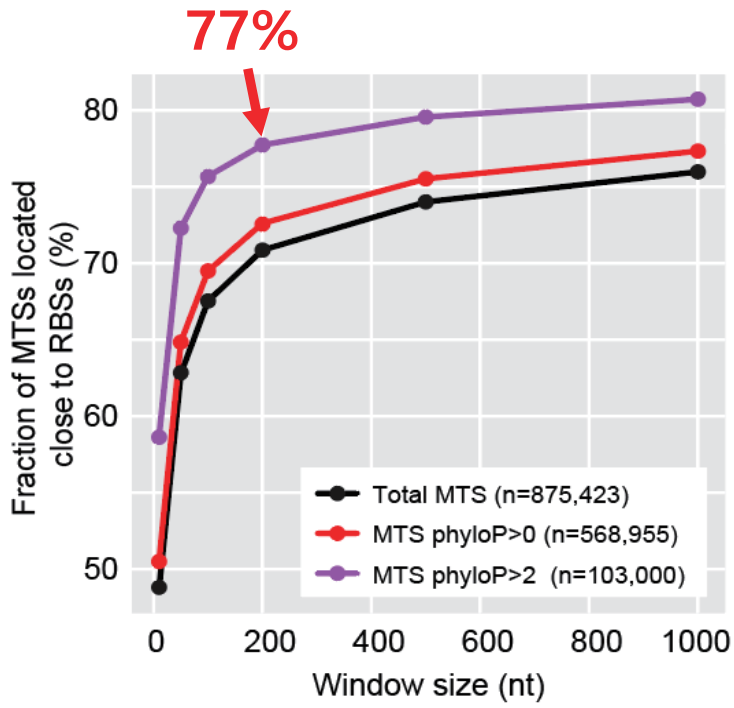

**The Absence of an RBP Reduces AGO Binding to the MTS *in vivo*.**

# Endogenous 조건에서의 실험적 검증: PCBP2 KO



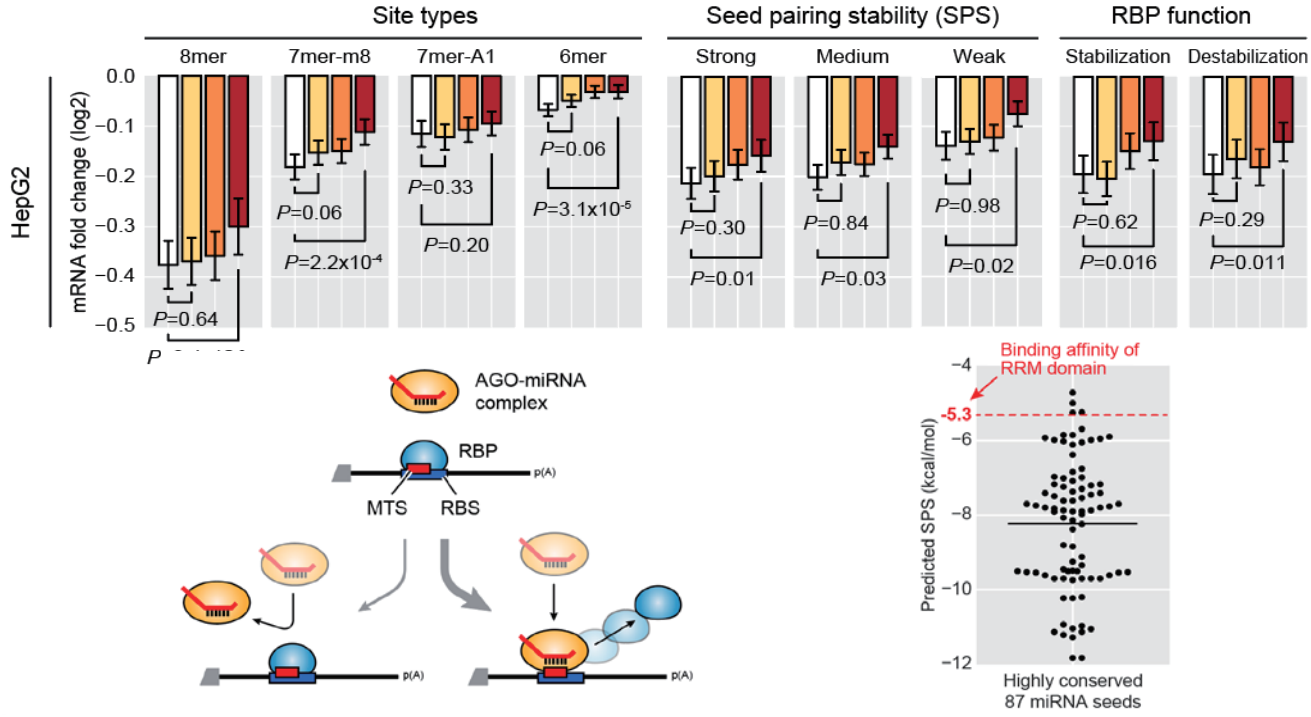

**RBP를 제거하자 MT 효율이 유의미하게 감소**

# Evolutionary Insight – Widespread Regulatory Impact



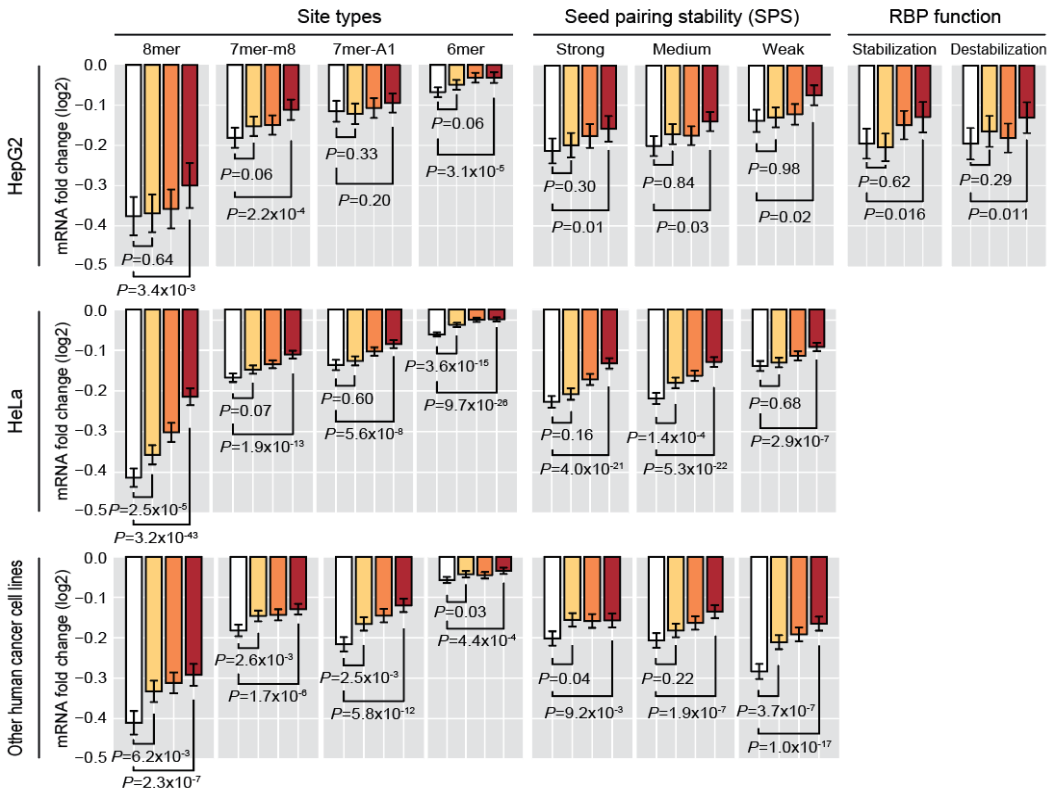

**>77% of Conserved Target Sites Contain 1≥ RBSs in Their Vicinities**

# Overlapping Sites: MTSs May Outcompete RBSs



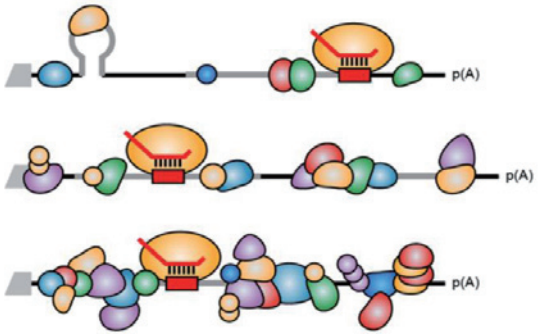

**For Numerous Cases, MTSs Consistently Outcompete the Overlapping RBSs**

# Overlapping Sites: MTSs May Outcompete RBSs



# Conclusions

- To gain a global insight into the regulatory impact of RBPs on MT, we have systematically evaluated the quantitative effect of 117 RBPs on MT efficacy.
- Most RBPs, if not all, significantly enhance MT, while no RBP detectably suppresses MT on a global scale.
- RBPs make the local secondary structure of the MTS more accessible to AGO and therefore enhance MT.
- MT should be understood in a context of hundreds of co-regulating RBPs rather than the currently accepted simplified model of a ternary interplay between AGO, miRNA, and mRNA target.
- Our study illuminates the previously unappreciated, widespread regulatory impact of RBPs on MT, unveiling the complex nature of the gene regulatory network governed by metazoan miRNAs.




nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-021-25078-5 OPEN

## The regulatory impact of RNA-binding proteins on microRNA targeting

Sukjun Kim[1,11], Soyoung Kim[2,11], Hee Ryung Chang[1,11], Doyeon Kim[1,11], Junehee Park[1], Narae Son[1], Joori Park[3,4], Minhyuk Yoon[2], Gwangung Chae[2], Young-Kook Kim[5], V. Narry Kim[1,6], Yoon Ki Kim[3,4], Jin-Wu Nam[7], Chanseok Shin[2,8,9✉] & Daehyun Baek[1,10✉]

Argonaute is the primary mediator of metazoan miRNA targeting (MT). Among the currently identified >1,500 human RNA-binding proteins (RBPs), there are only a handful of RBPs known to enhance MT and several others reported to suppress MT, leaving the global impact of RBPs on MT elusive. In this study, we have systematically analyzed transcriptome-wide binding sites for 150 human RBPs and evaluated the quantitative effect of individual RBPs on MT efficacy. In contrast to previous studies, we show that most RBPs significantly affect MT and that all of those MT-regulating RBPs function as MT enhancers rather than suppressors, by making the local secondary structure of the target site accessible to Argonaute. Our findings illuminate the unappreciated regulatory impact of human RBPs on MT, and as these RBPs may play key roles in the gene regulatory network governed by metazoan miRNAs, MT should be understood in the context of co-regulating RBPs.

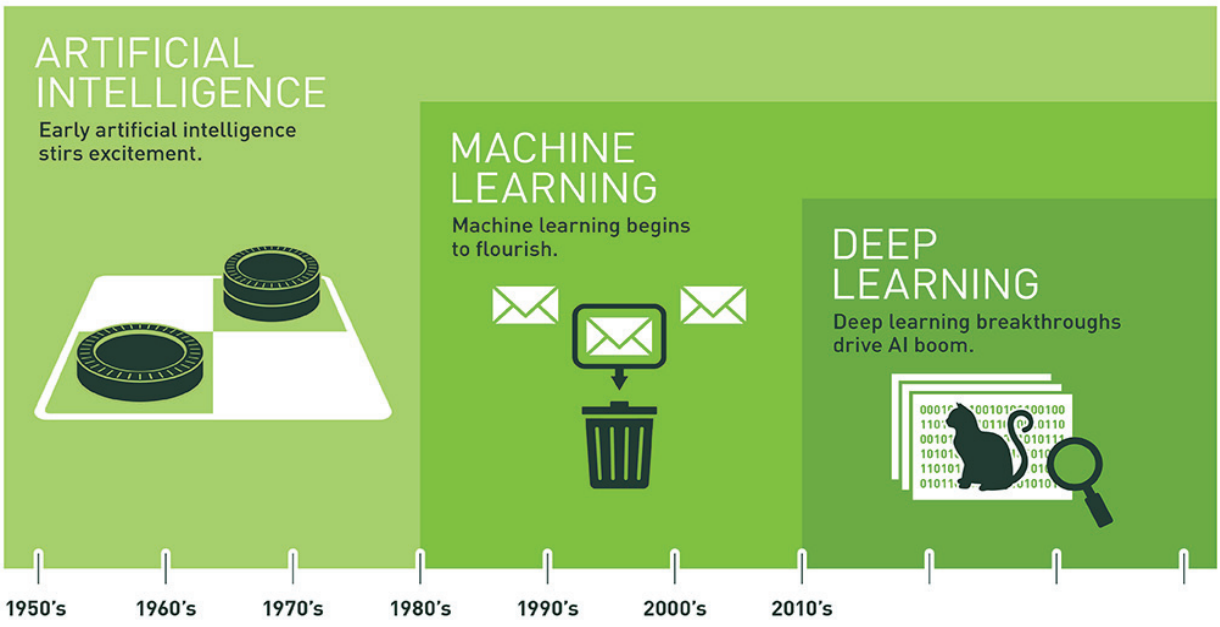
[1] School of Biological Sciences, Seoul National University, Seoul, Republic of Korea. [2] Department of Agricultural Biotechnology, Seoul National University, Seoul, Republic of Korea. [3] Creative Research Initiatives Center for Molecular Biology of Translation, Korea University, Seoul, Republic of Korea. [4] Division of Life Sciences, Korea University, Seoul, Republic of Korea. [5] Department of Biochemistry, Chonnam National University Medical School, Hwasun, Jeollanam-do, Republic of Korea. [6] Center for RNA Research, Institute for Basic Science, Seoul, Republic of Korea. [7] Department of Life Science, College of Natural Sciences, Hanyang University, Seoul, Republic of Korea. [8] Research Institute of Agriculture and Life Sciences, and Plant Genomics and Breeding Institute, Seoul National University, Seoul, Republic of Korea. [9] Research Center for Plant Plasticity, Seoul National University, Seoul, Republic of Korea. [10] Bioinformatics Institute, Seoul National University, Seoul, Republic of Korea. [11] These authors contributed equally: Sukjun Kim, Soyoung Kim, Hee Ryung Chang, Doyeon Kim. ✉email: cshin@snu.ac.kr; baek@snu.ac.kr

# AI Prediction for Functional MicroRNA Targeting


## Daehyun Baek

School of Biological Sciences
Seoul National University


## Artificial Intelligence vs. Deep Learning

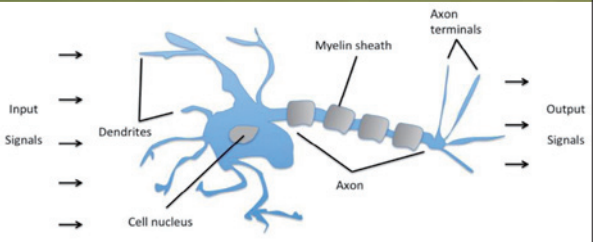


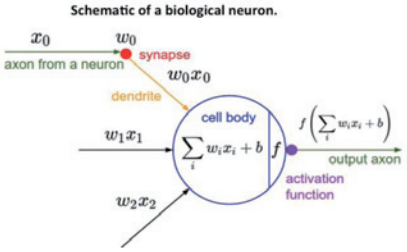

Since an early flush of optimism in the 1950s, smaller subsets of artificial intelligence – first machine learning, then deep learning, a subset of machine learning – have created ever larger disruptions.

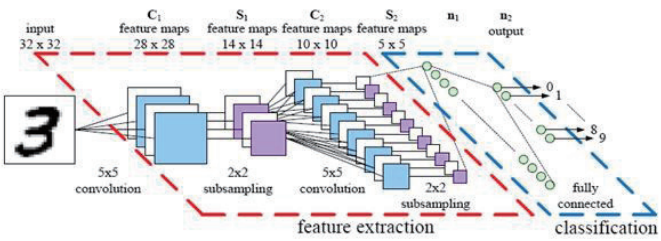
(blogs.nvidia.com)

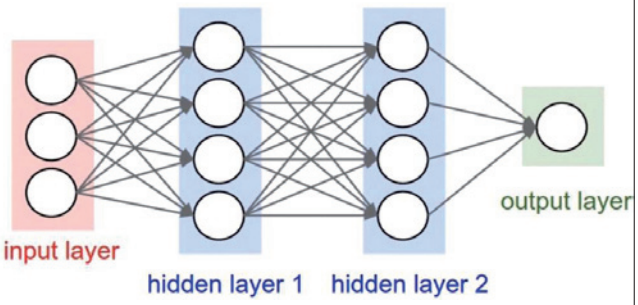
# Deep Learning 기반의 miRNA 타겟 예측

- Artificial neural network with multiple layer of simple but non-linear functions
- Good at high-dimensional, big data
- Convolutional neural network (CNN): Addition of feature extraction step for image and reduction of the number of model parameters



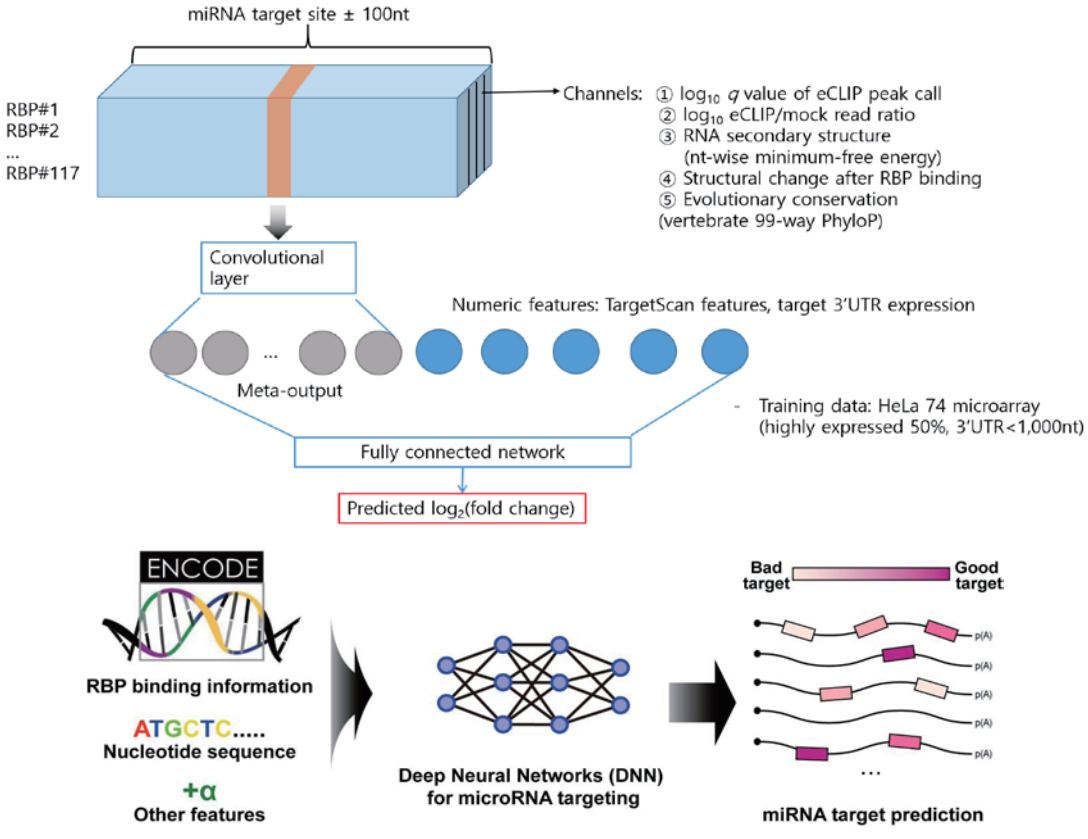



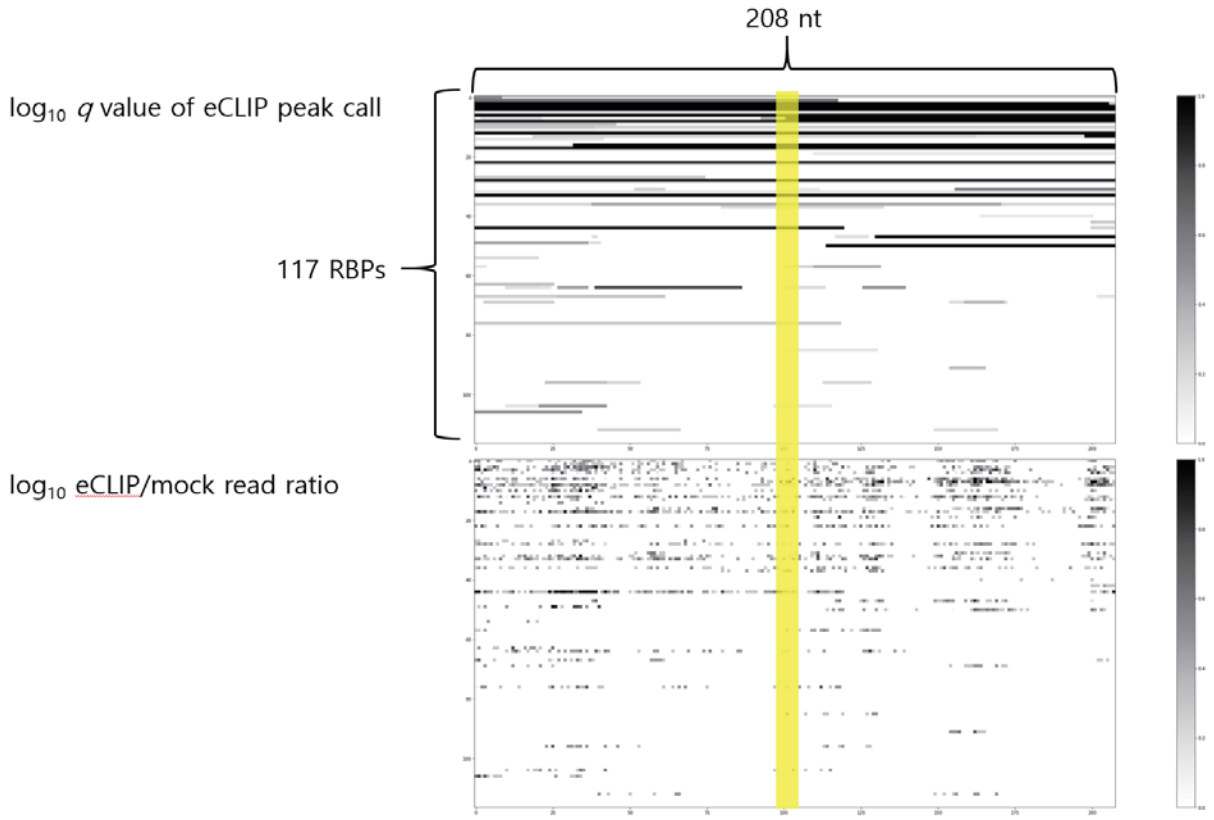




(www.cs231n.github.io)

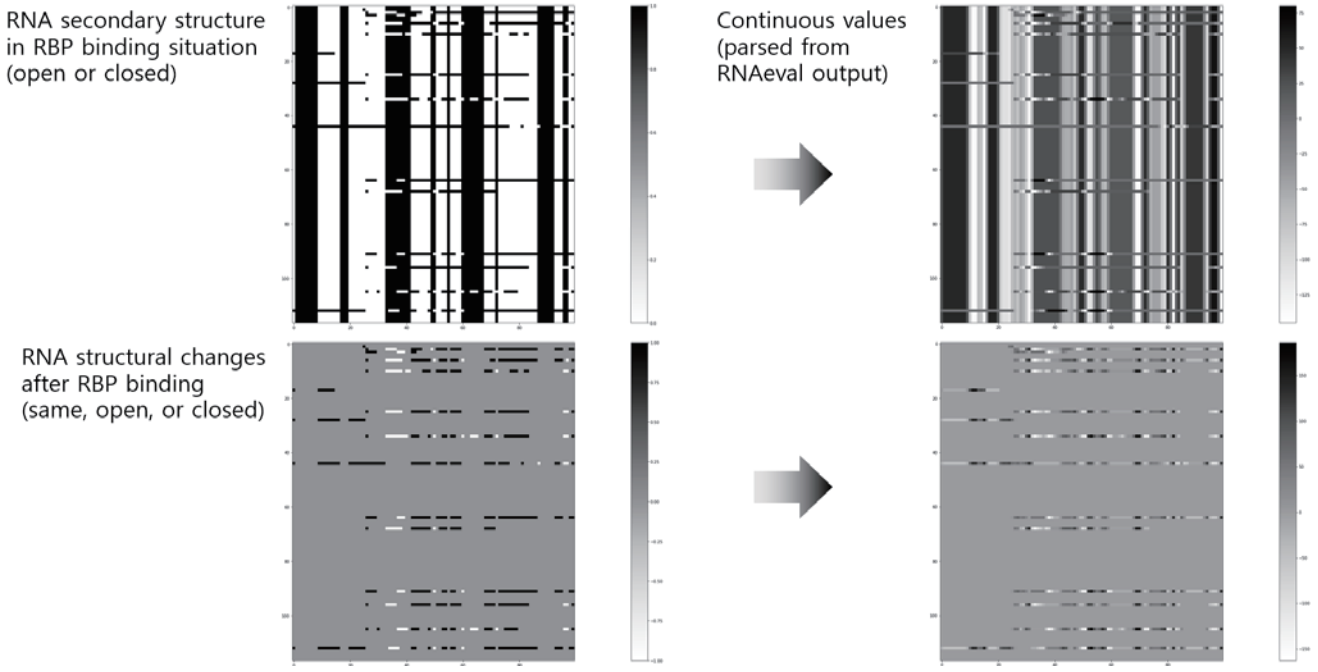
# Convolution Neural Network(CNN) 모델 for MT



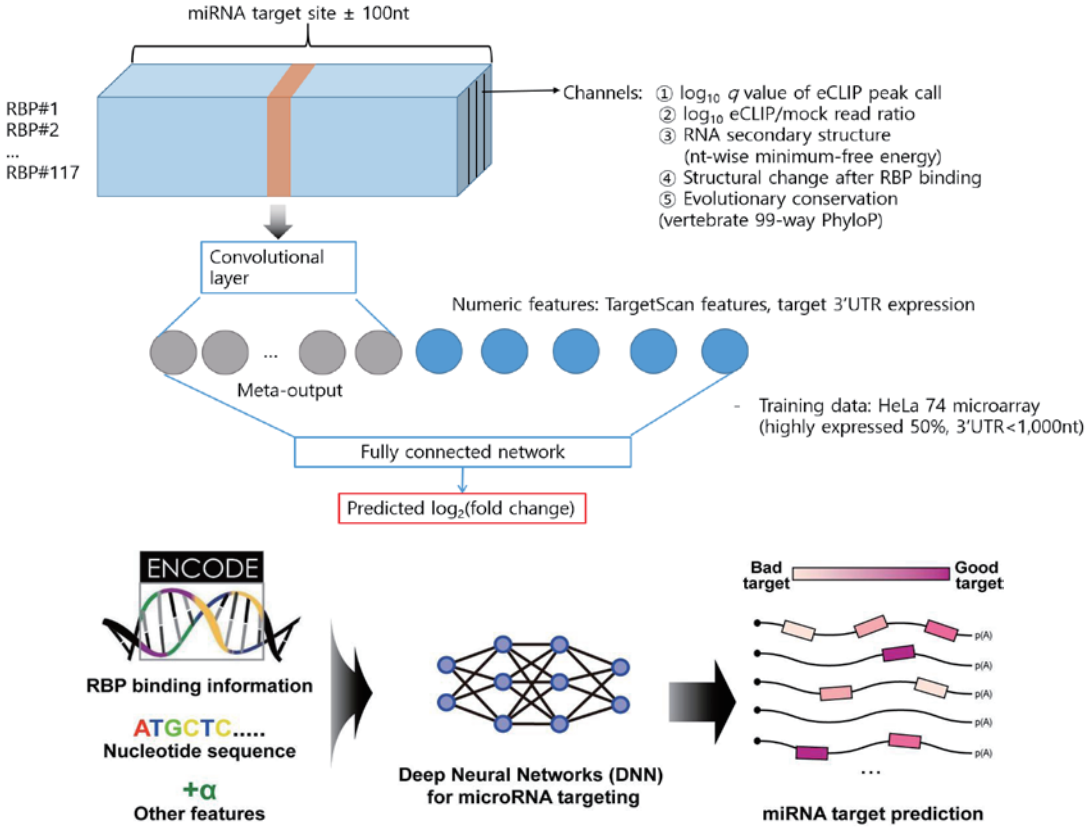
# CNN Features: RBP Binding Information




# CNN Features: RNA Secondary Structure

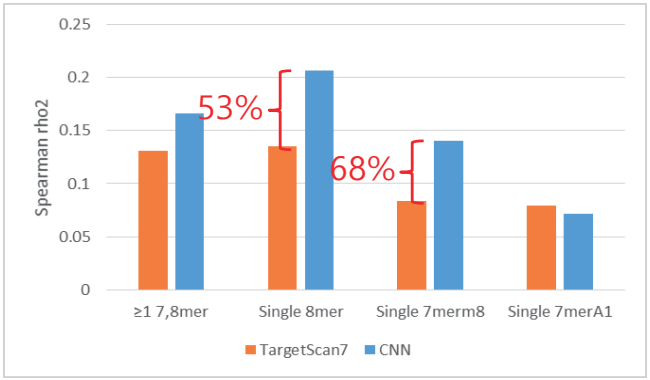


# Convolution Neural Network(CNN) 모델 for MT

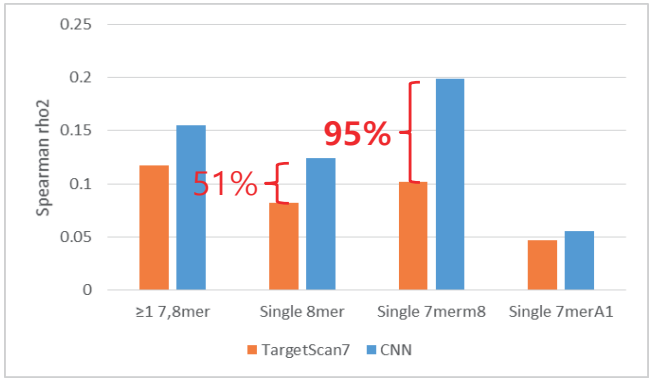



# Deep Learning 기반의 miRNA 타겟 예측

## HeLa




## HCT116




TargetScanHuman
Prediction of microRNA targets
Release 7.2: March 2018

**RBP-결합 정보를 활용하는 Deep Learning 적용 결과, miRNA 타겟 예측 정확도가 대폭 향상**

# A High-Resolution Temporal Atlas of the SARS-CoV-2 Translatome and Transcriptome

**Daehyun Baek**

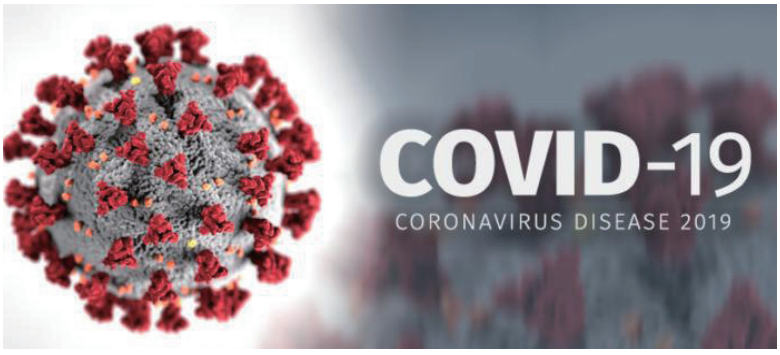
**School of Biological Sciences**
**Seoul National University**

## COVID-19

▹ **COVID-19 is caused by severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), which infected >34 million people resulting in >1 million deaths.**

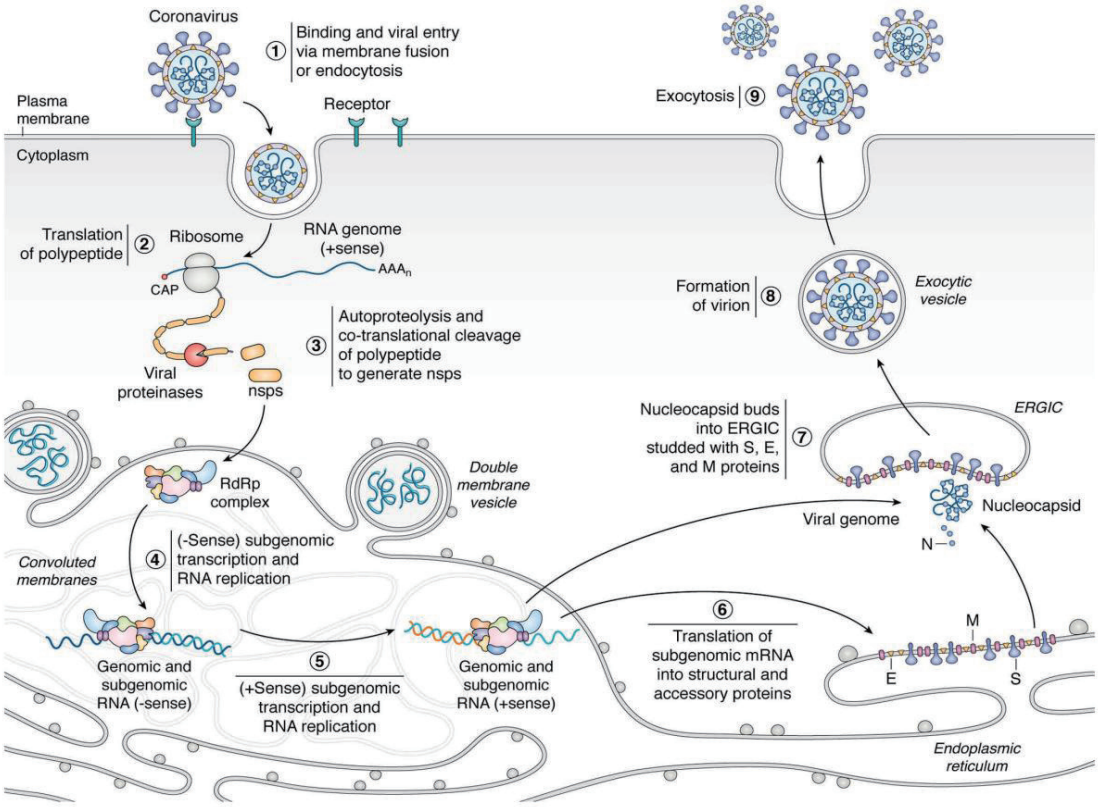
▹ **As the United Nations has recently declared, COVID-19 is not only a pandemic but also a substantial crisis deeply affecting the societies and economics on a global scale**

▹ **Although the SARS-CoV-2 transcriptome has been recently reported (Kim et al., 2020), temporal landscape of the SARS-CoV-2 translatome and its impact on the human genome remain unexplored.**



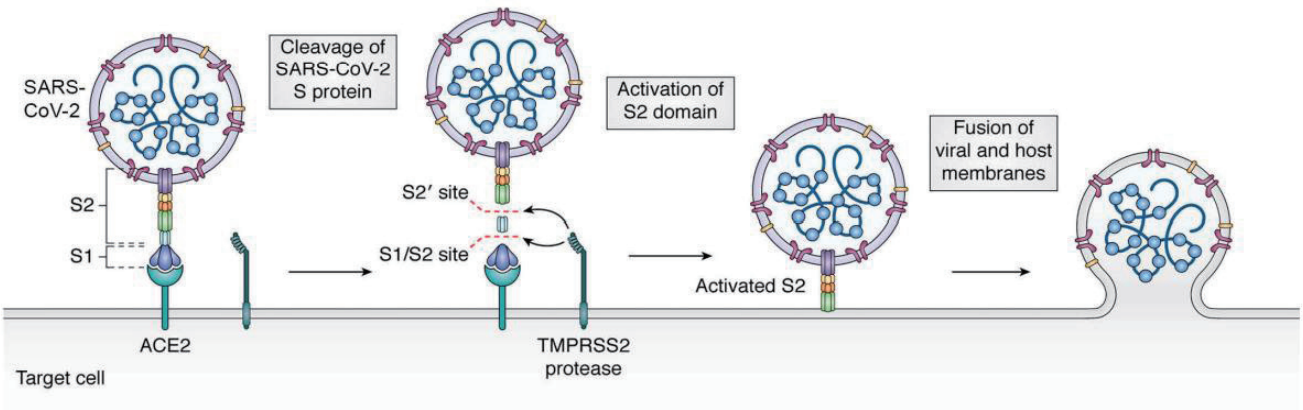

(www.fscluster.org)

# The Viral Life Cycle of Coronavirus



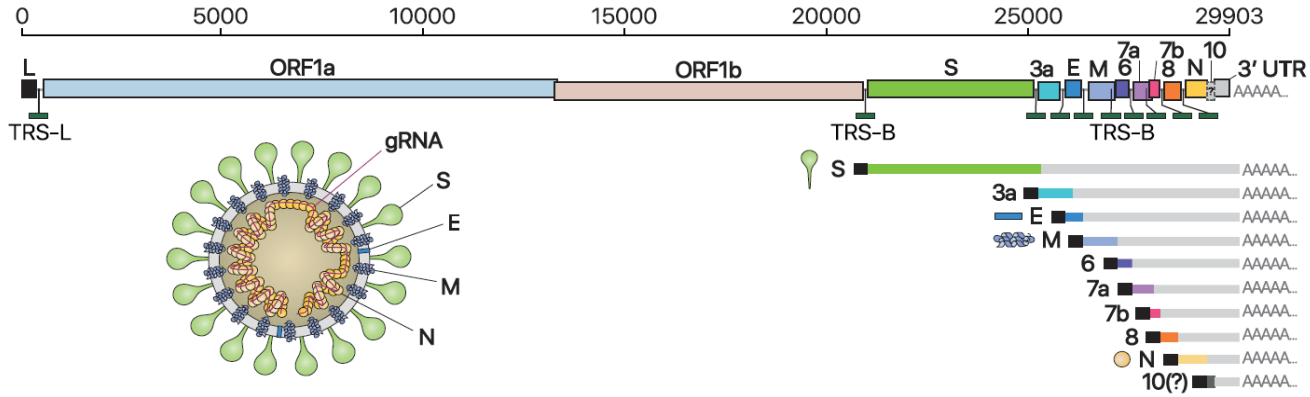

(Hartenian *et al.*, 2020)

# The Mechanism of SARS-CoV-2 Viral Entry



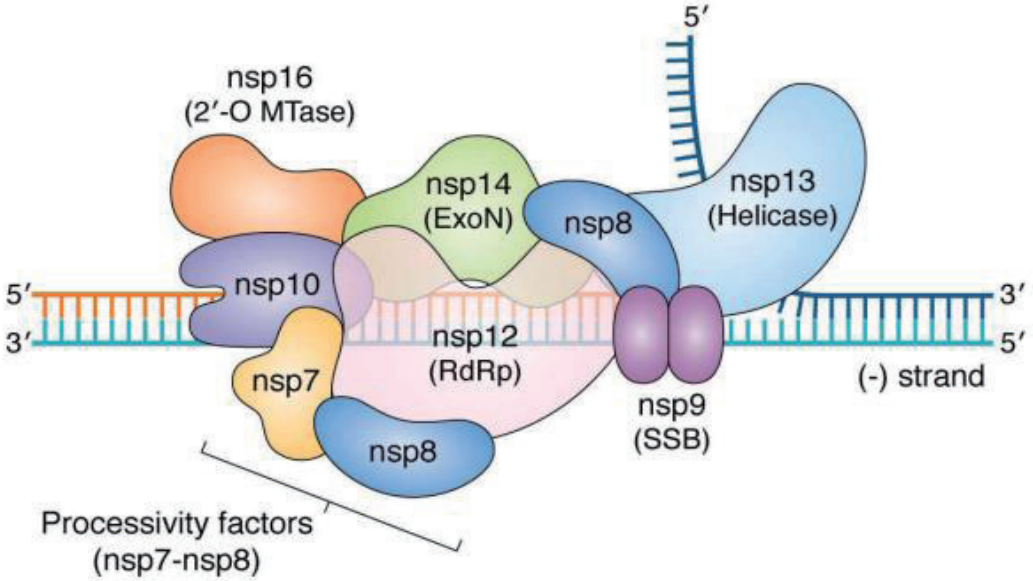

(Hartenian *et al.*, 2020)

# Genome Organization of SARS-CoV-2



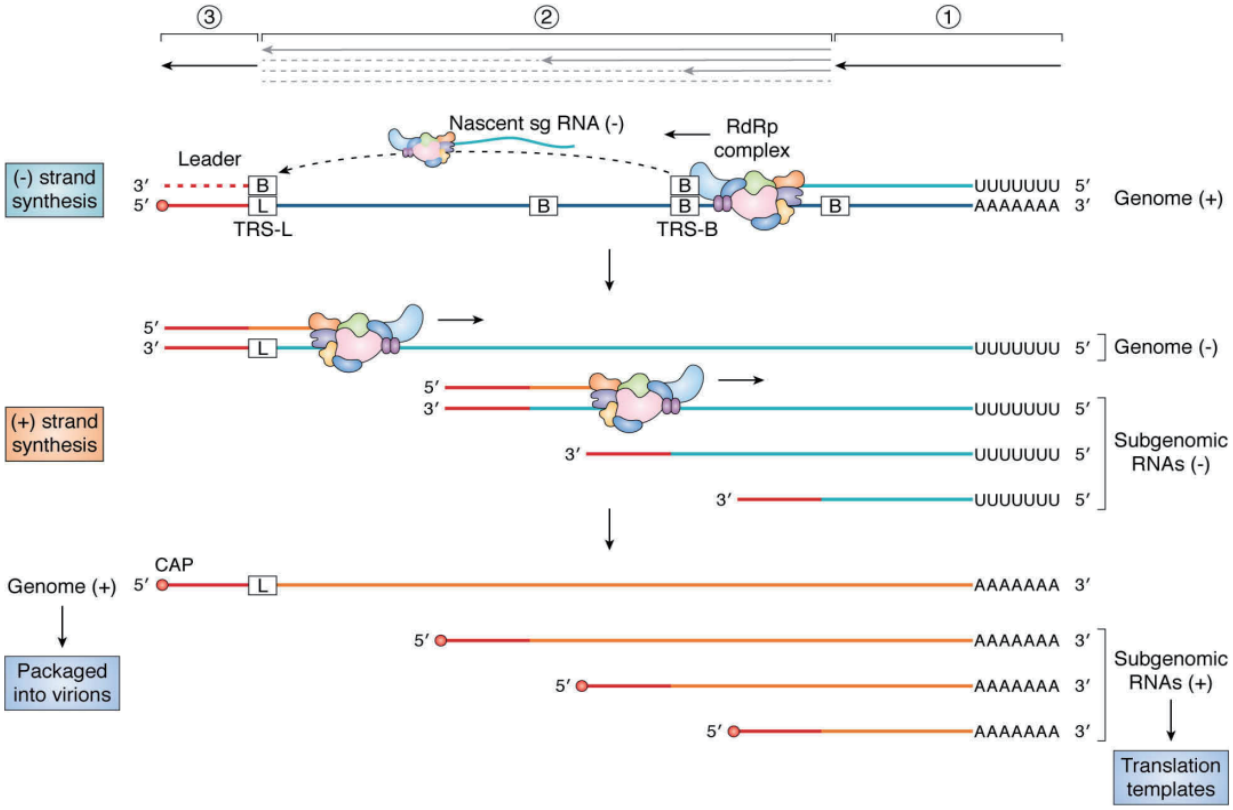

(Kim et al., Cell, 2020)

# Model of Putative Coronavirus Replisome



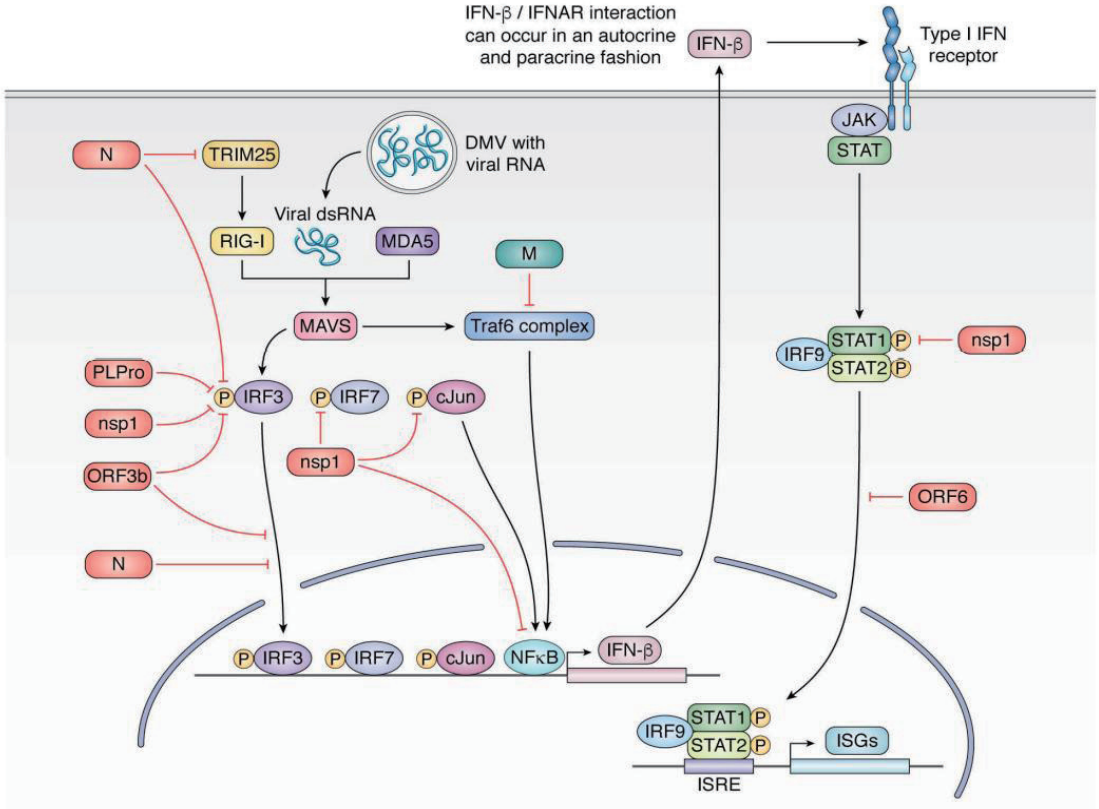

(Hartenian *et al.*, 2020)

# Discontinuous Transcription



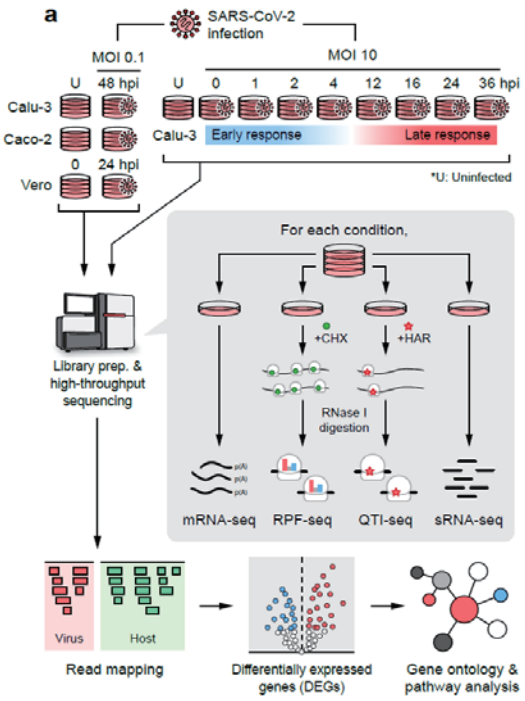

(Hartenian *et al.*, 2020)

# Innate Immune Antagonism by SARS-CoV



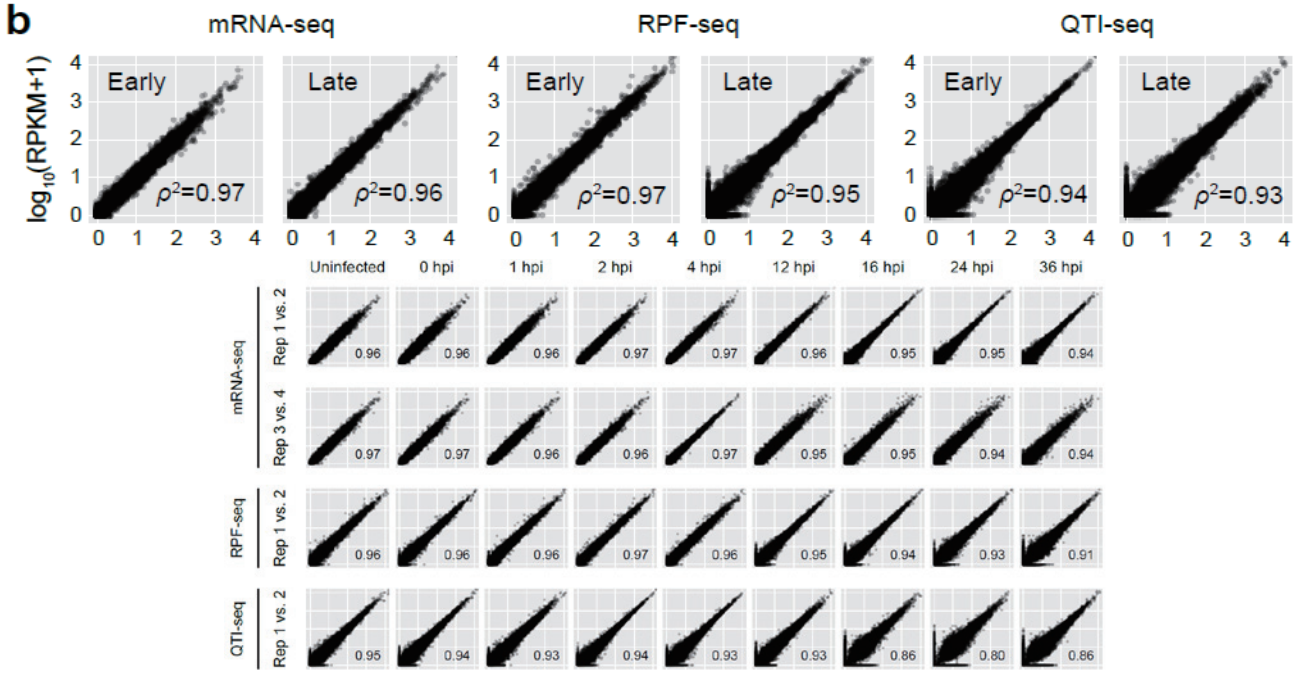

(Hartenian *et al.*, 2020)

# Experimental Design



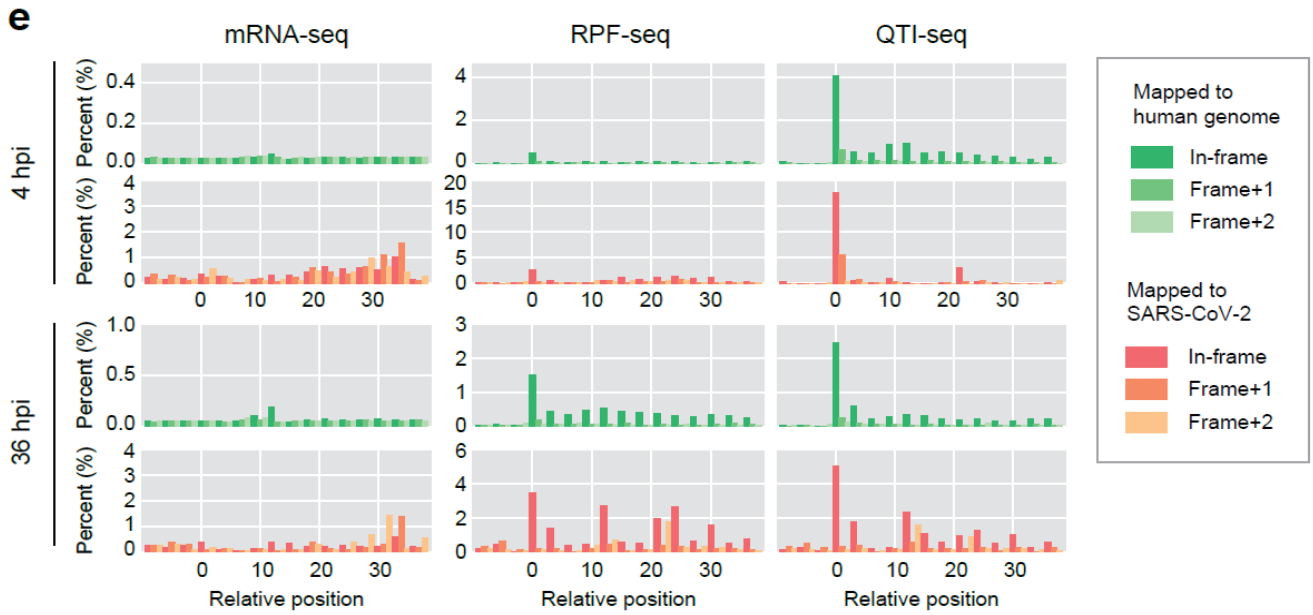

**Generation of massive-scale datasets of the SARS-CoV-2 translatome and transcriptome**

# Highly Reliable Data Quality



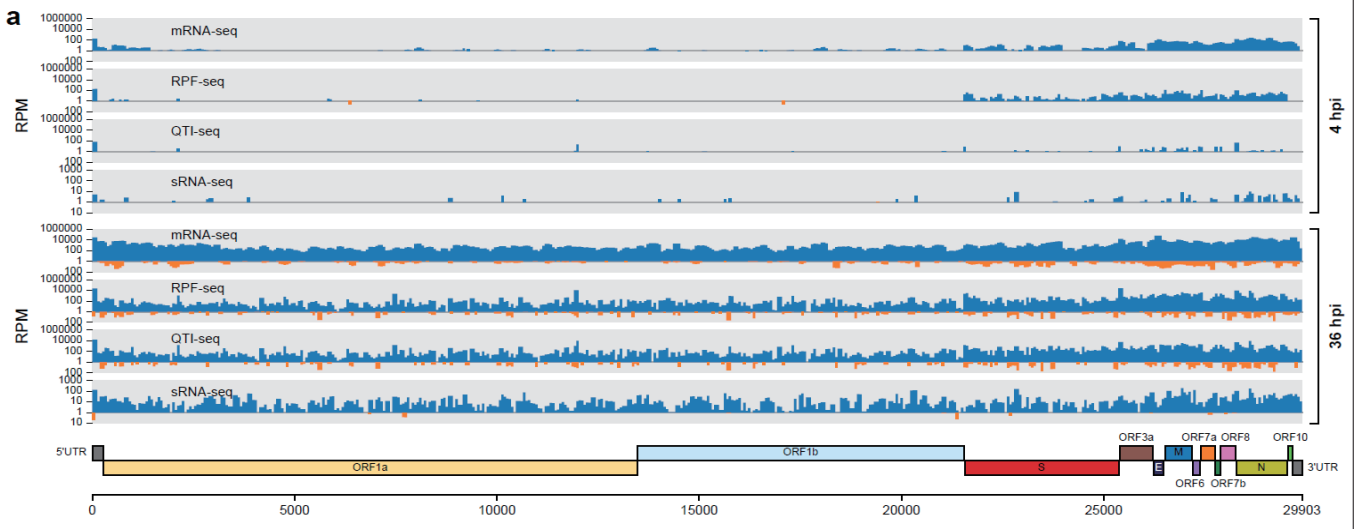

**Strong correlation between replicates indicates that our datasets are highly reliable.**

# Highly Reliable Data Quality



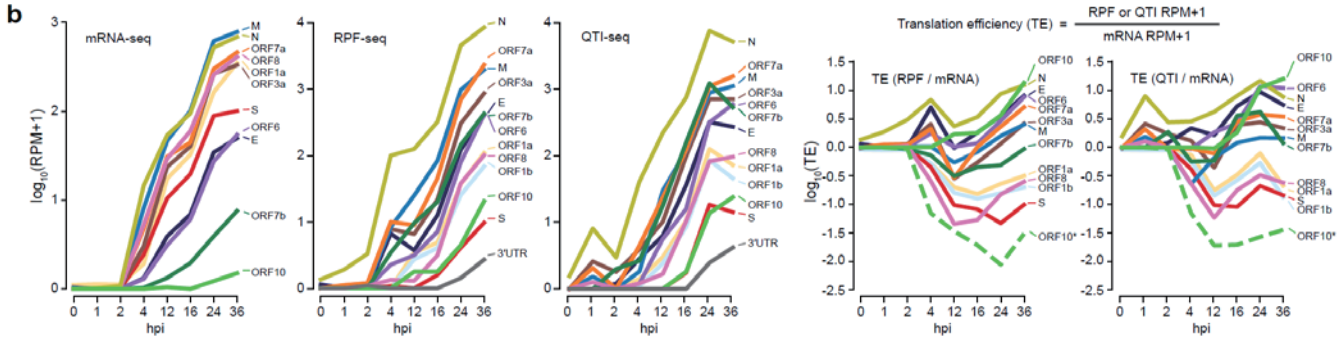

**Strong correlation between replicates indicates that our datasets are highly reliable.**

# A High-Resolution Temporal Atlas of the SARS-CoV-2 Translatome



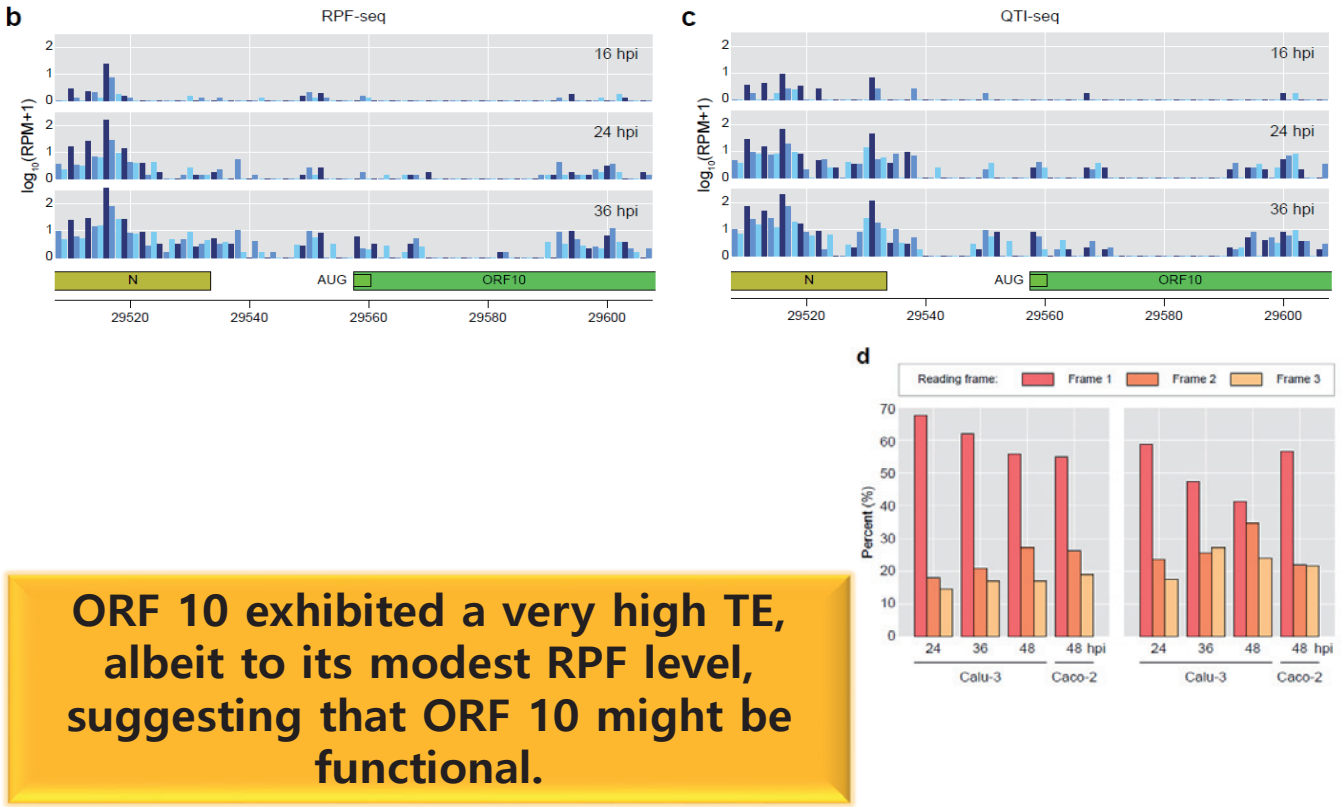

**Employing RPF-seq, QTI-seq, mRNA-seq, and sRNA-seq, a temporal atlas of SARS-CoV-2 translatome and transcriptome was constructed.**

# Temporal Expression of Individual SARS-CoV-2 Genes



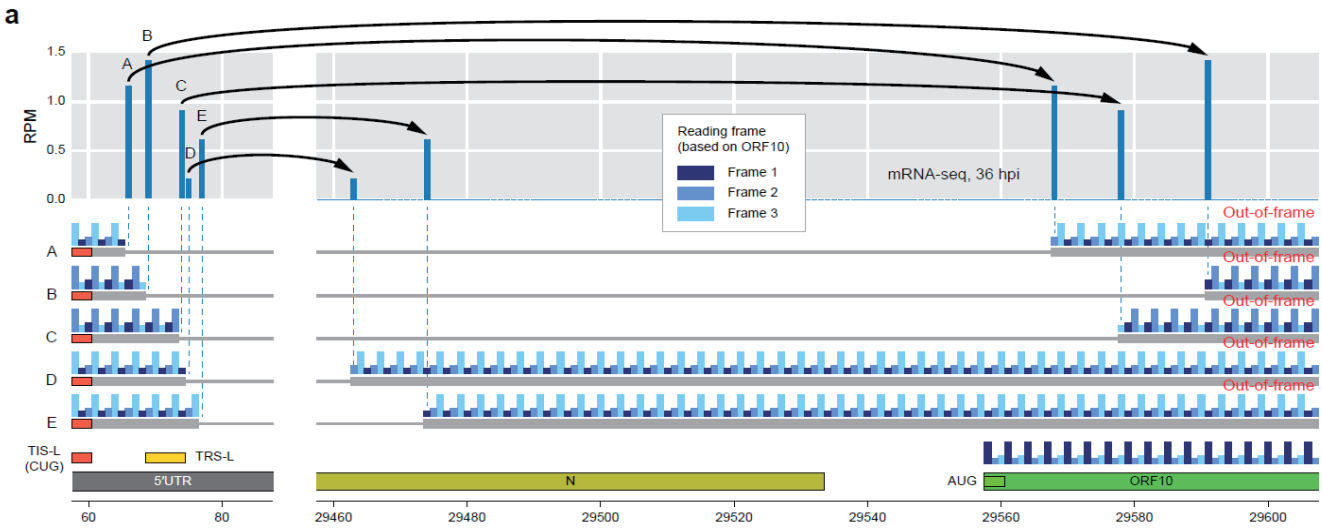

**The overall increment in expression level for all ORFs over time was observed on both mRNA and RPF levels.**

# ORF 10 May Be Functional



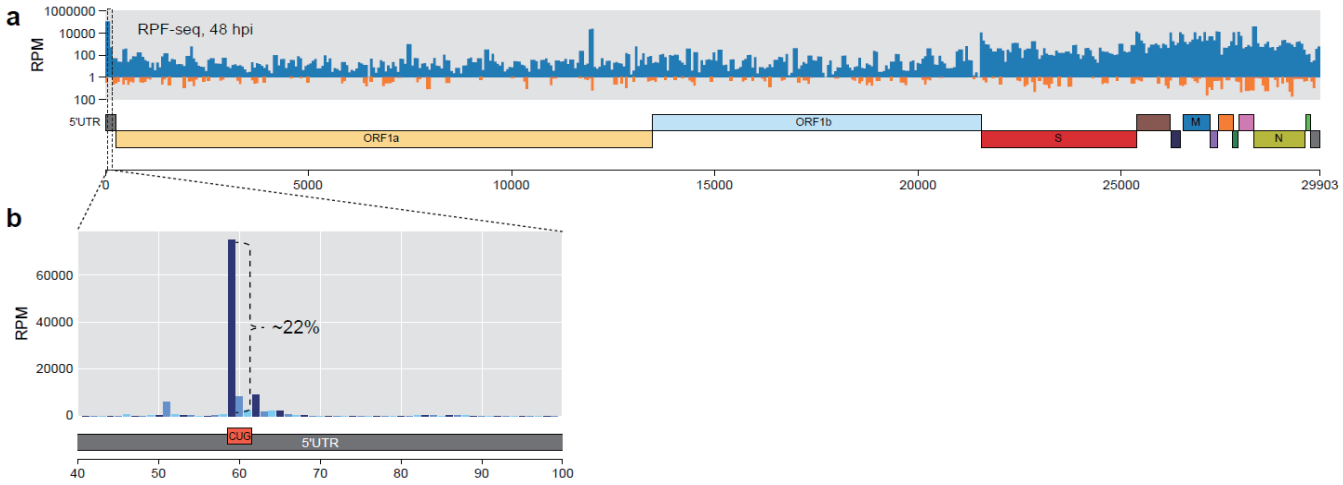

**ORF 10 exhibited a very high TE, albeit to its modest RPF level, suggesting that ORF 10 might be functional.**

# ORF 10 May Be Functional



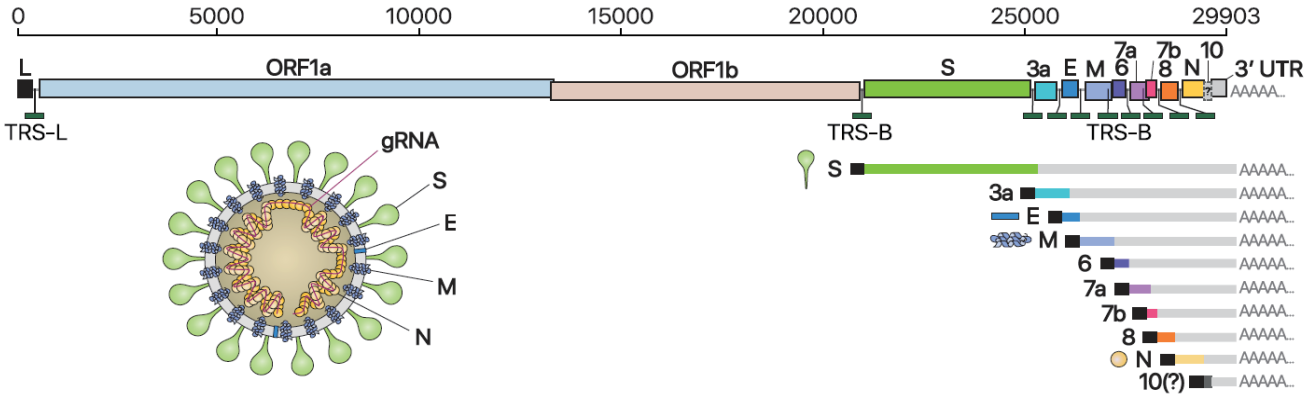

**Leaky scanning of ribosome in N sgRNA might lead to the translation of ORF 10.**

# Translation Initiation Site Located in the Leader (TIS-L)




**Substantial amount of the RPF-seq and QTI-seq reads were mapped on a CUG codon located in the leader sequence**

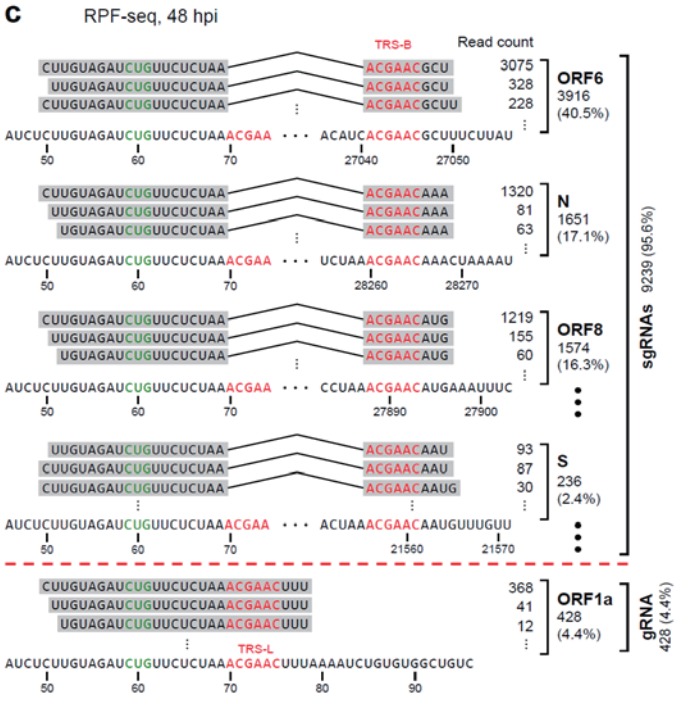
# Leader Sequence in SARS-CoV-2




**Leader sequence and TIS-L are included in all gRNAs and sgRNAs of SARS-CoV-2**

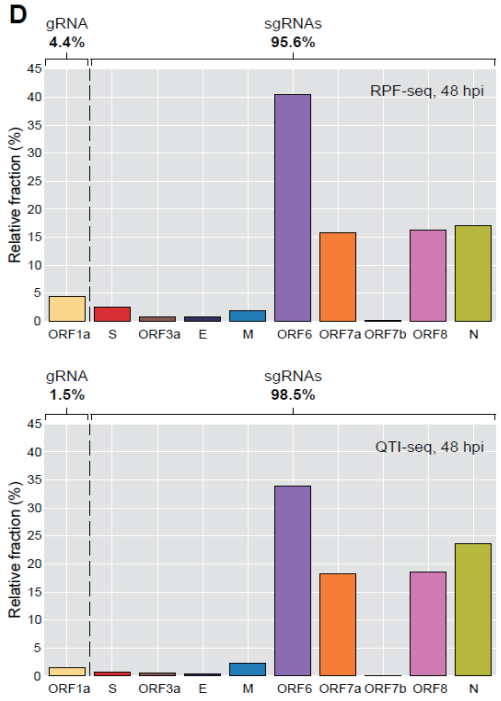
(Kim et al., Cell, 2020)

# TIS-L Reads Uniquely Mapped to the SARS-CoV-2 Genome



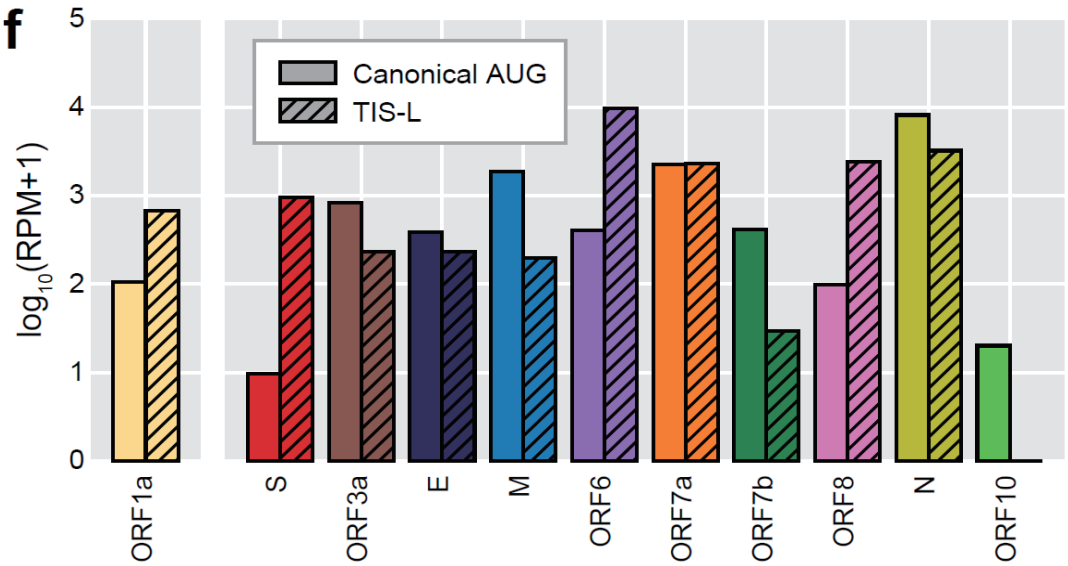

**Most RPF-seq and QTI-seq reads (>95%) were mapped to sgRNAs, while <5% of the reads were mapped to gRNA**

# TIS-L Reads Uniquely Mapped to the SARS-CoV-2 Genome



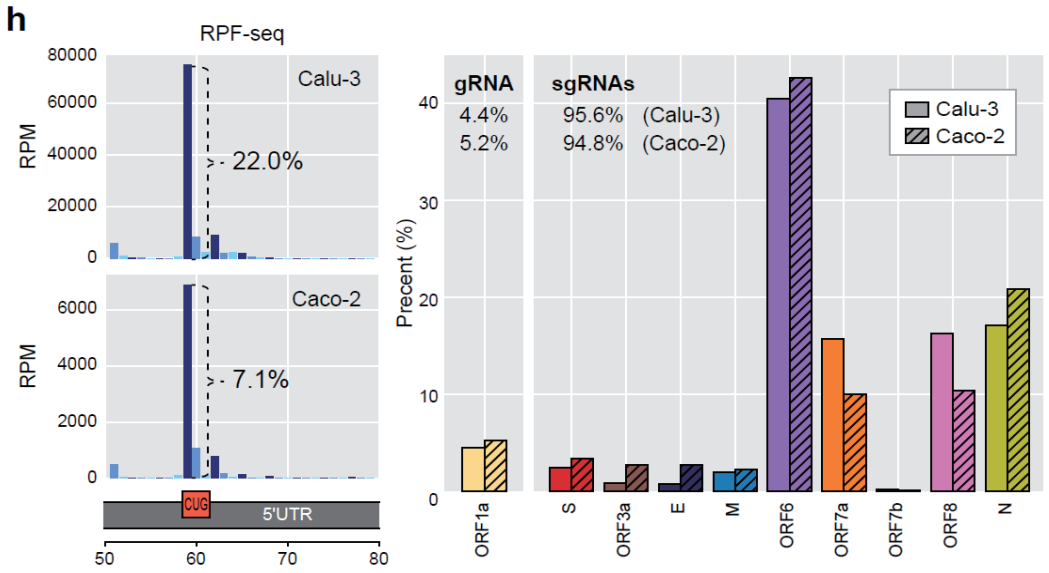

**The largest number of TIS-L reads mapped to ORF 6, followed by ORFs N, 8, 7a, 1a, and S.**

# Translation Initiation at Annotated AUGs vs. TIS-L



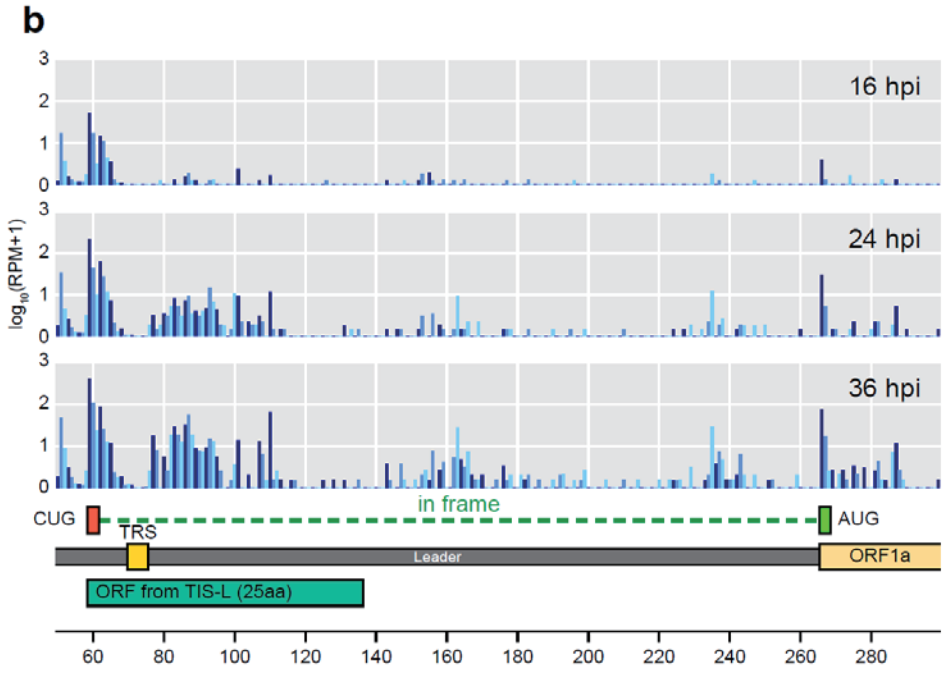

**Translation initiation of TIS-L was even higher than that of the annotated AUG for several ORFs including ORF S**

# Translation Initiation at Annotated AUGs vs. TIS-L



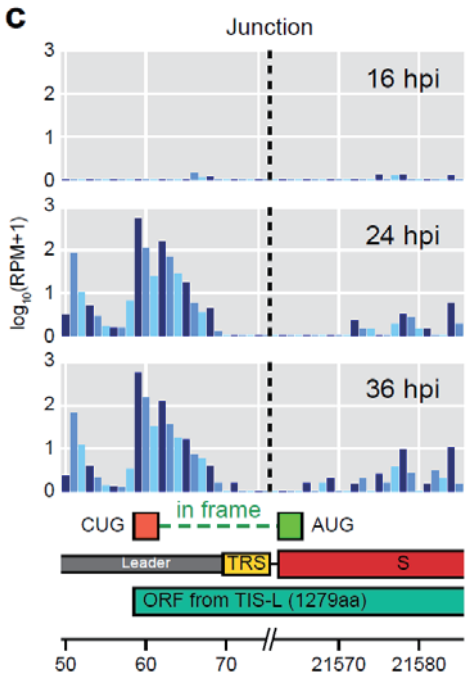

**Consistent with Calu-3, a considerable amount of reads were mapped to TIS-L in Caco-2.**

# TIS-L for ORF 1a



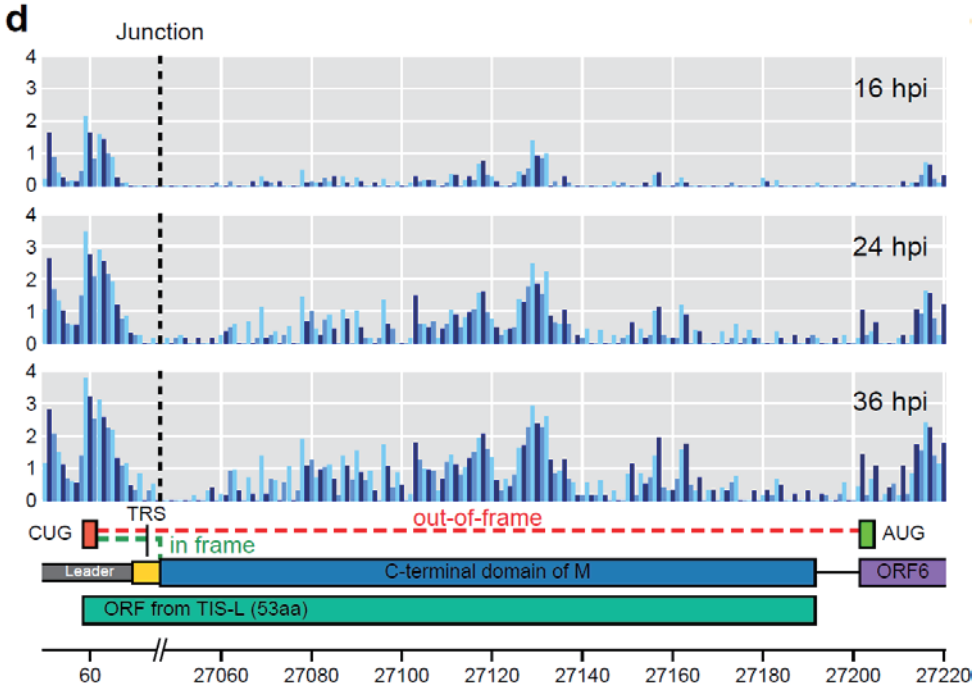

**For ORFs 1a and N, TIS-L is expected to create a short uORF that is not overlapping with the annotated ORF**

# TIS-L for ORF S



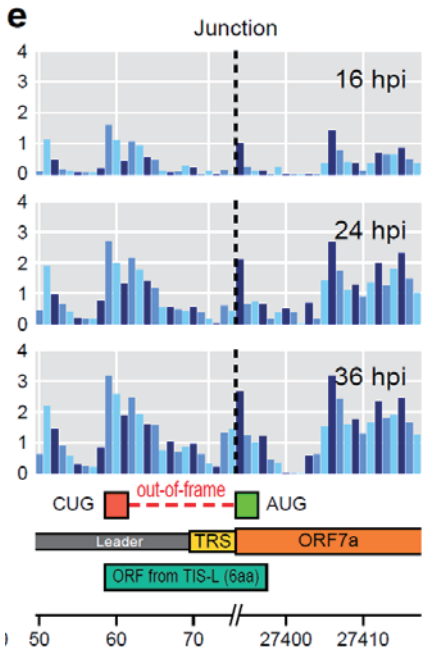

**TIS-L is in-frame with ORF S and thus expected to yield an extended ORF or to function as a translation enhancer.**

# TIS-L for ORF 6



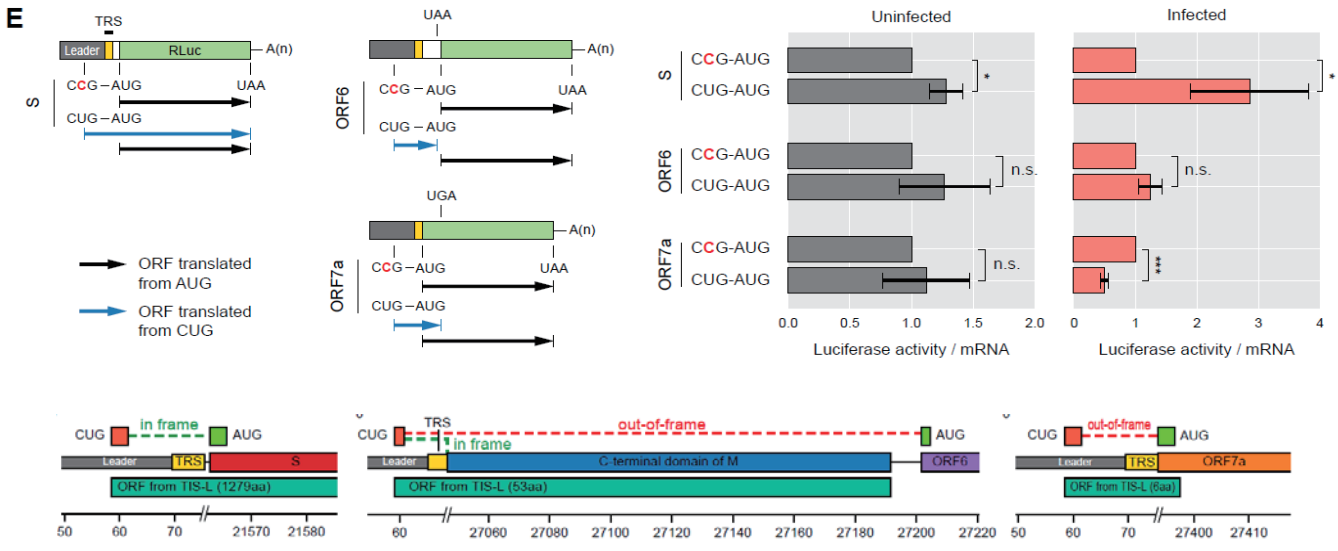

**TRS-B of ORF 6 is embedded in the middle of ORF M producing an uORF in-frame with the C-terminal region of ORF M**

# TIS-L for the Other ORFs (3a, E, M, 7a, 7b, and 8)



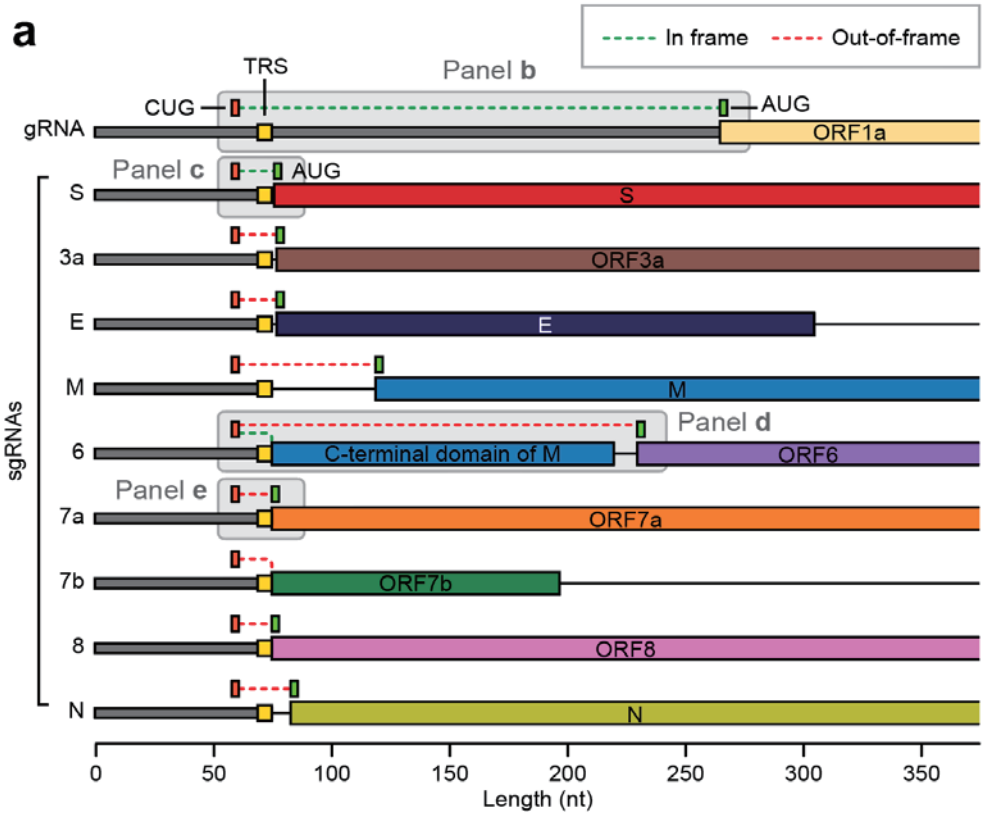

**Most uORFs derived from TIS-L overlap with annotated ORFs and are out of frame with them, likely functioning as a translation suppressor.**

# Experimental Validation by Luciferase Reporter Assay




**These results demonstrate that TIS-L has a substantial regulatory impact on most SARS-CoV-2 ORFs either positively or negatively.**

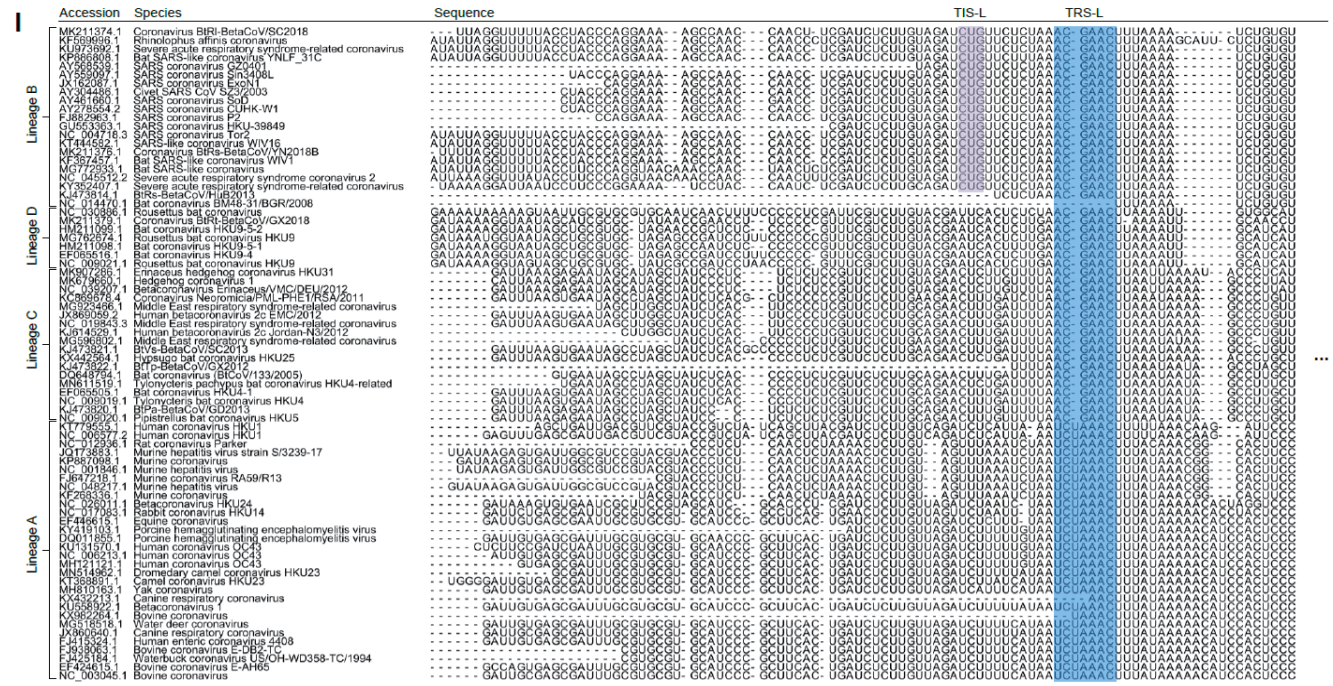
# The Impact of TIS-L on the SARS-CoV-2 Translatome




# Evolutionary Insight into TIS-L in Betacoronaviruses

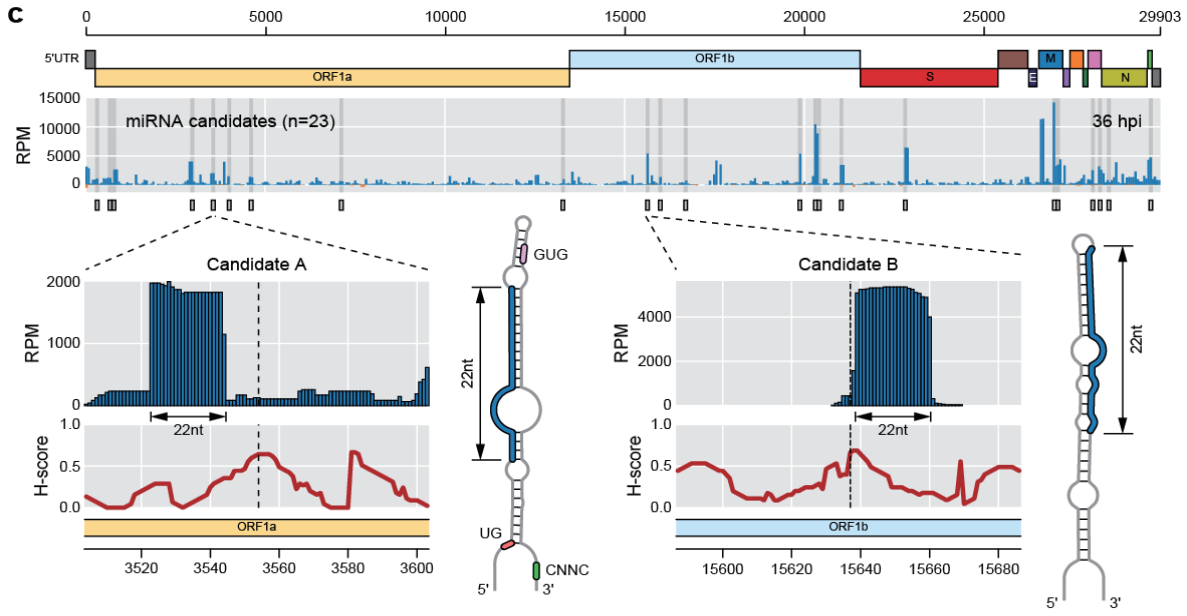

**TIS-L may bypass the reduced global translation of the host cells in response to viral infection.**

# Evolutionary Insight into TIS-L in Betacoronaviruses




**TIS-L is highly conserved in most of Lineage B viruses while absent in other lineages.**

# SARS-CoV-2 MicroRNAs

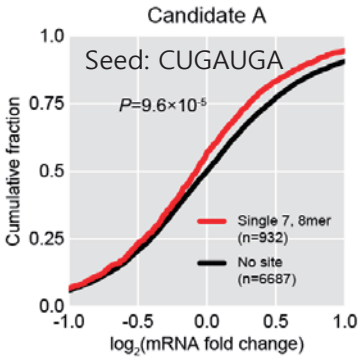


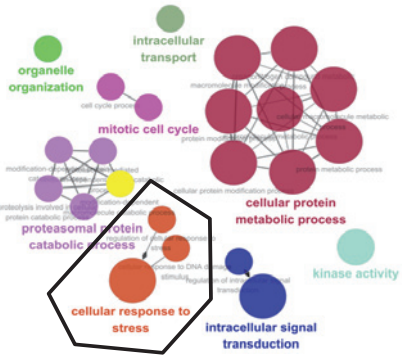

**Two miRNA candidates detected by small RNA-seq**

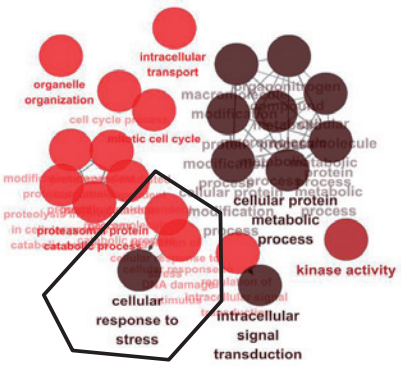
# SARS-CoV-2 MicroRNAs

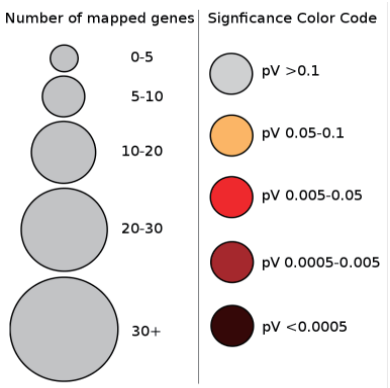
Color by groups

Color by significance




Target mRNAs with a single 7, 8mer site (n=932)
Associated GO terms ($P$ < 0.05) were displayed.

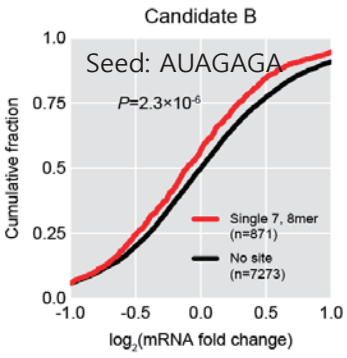
The most significant GO term:
**Cellular response to stress** ($P$=8.0x10$^{-11}$)

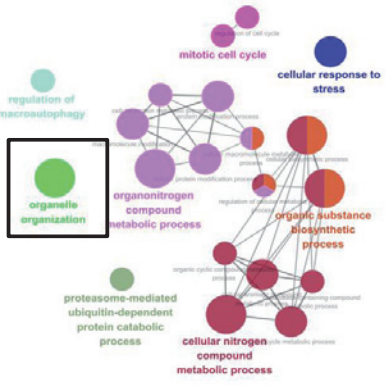
- 171 genes (7.7%) are associated

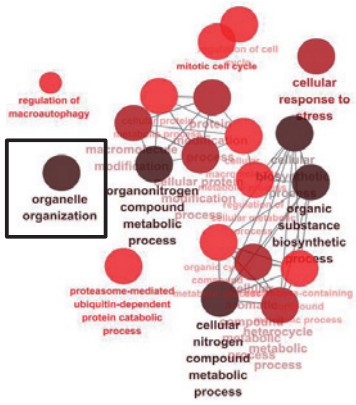
# SARS-CoV-2 MicroRNAs

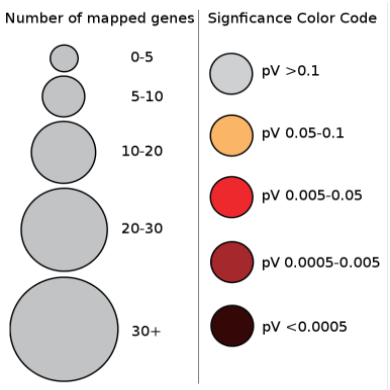
Color by groups

Color by significance



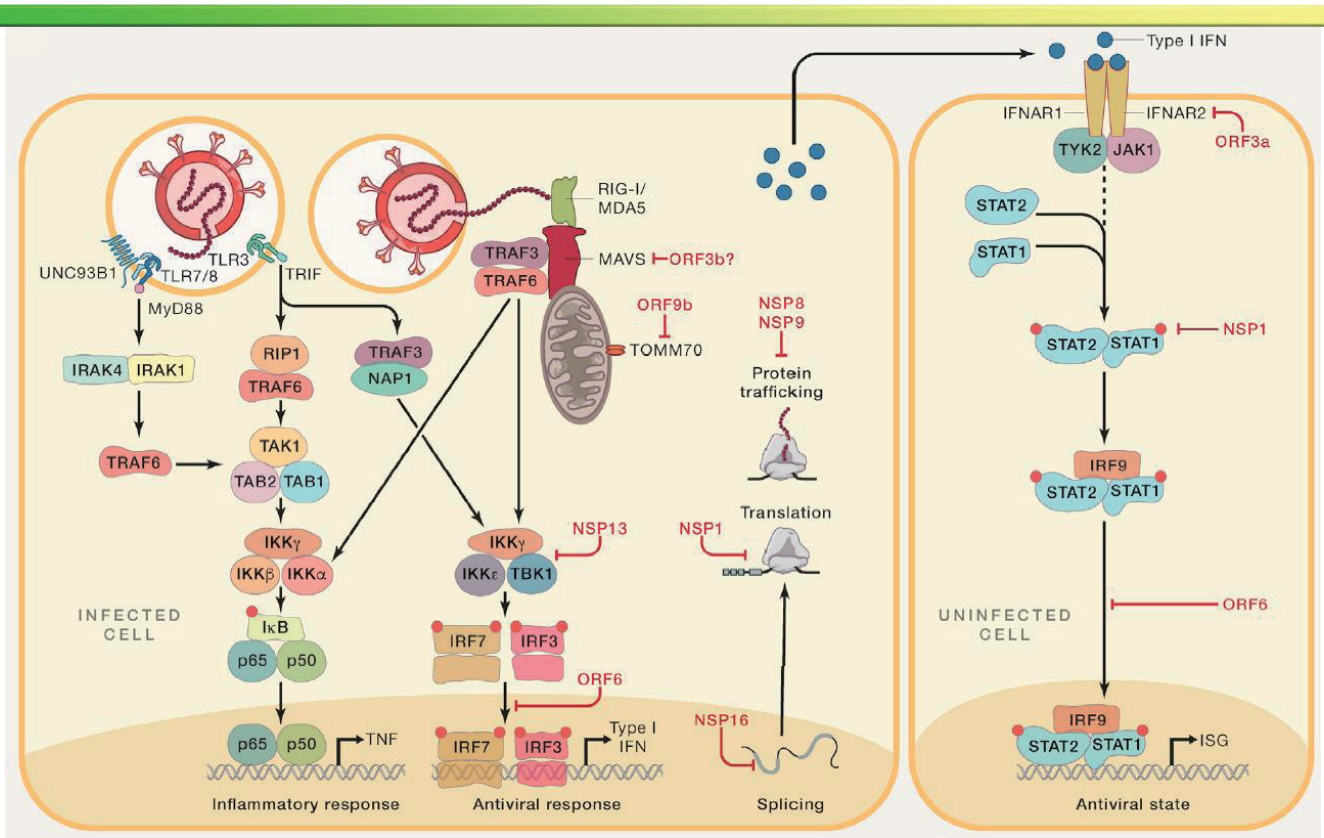




Target mRNAs with a single 7, 8mer site (n=871)
Associated GO terms ($P$ < 0.05) were displayed.

The most significant GO term:
**Organelle organization** ($P$=4.9x10$^{-8}$)

- 256 genes (6.1%) are associated

## SARS-CoV-2 MicroRNAs May Help Evade Human Immune Response




(Schultze and Aschenbrenner, 2021)

## Conclusions

▹ **We report the first high-resolution atlas of the translatome and transcriptome of SARS-CoV-2 for various time points after infecting human cells.**

▹ **Intriguingly, substantial amount of SARS-CoV-2 translation initiates at a novel translation initiation site (TIS) located in the leader sequence, that we termed TIS-L.**

▹ **Since TIS-L is included in all the genomic and subgenomic RNAs, the SARS-CoV-2 translatome may be regulated by a sophisticated interplay between TIS-L and downstream TISs.**

▹ **TIS-L functions as a strong translation enhancer for S protein, and as translation suppressors for most of the other proteins.**

▹ **Our global temporal atlas provides compelling insight into unique regulation of the SARS-CoV-2 translatome and helps comprehensively evaluate its impact on the human genome.**

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ARTICLE

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# A high-resolution temporal atlas of the SARS-CoV-2 translatome and transcriptome

Doyeon Kim[1,6], Sukjun Kim[1,6], Joori Park[2,3,6], Hee Ryung Chang[1,6], Jeeyoon Chang[2,3,6], Junhak Ahn[1,6], Heedo Park[4,6], Junehee Park[1], Narae Son[1], Gihyeon Kang[1], Jeonghun Kim[4], Kisoon Kim[4], Man-Seong Park[4✉], Yoon Ki Kim[2,3✉] & Daehyun Baek[1,5✉]

COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which infected >200 million people resulting in >4 million deaths. However, temporal landscape of the SARS-CoV-2 translatome and its impact on the human genome remain unexplored. Here, we report a high-resolution atlas of the translatome and transcriptome of SARS-CoV-2 for various time points after infecting human cells. Intriguingly, substantial amount of SARS-CoV-2 translation initiates at a novel translation initiation site (TIS) located in the leader sequence, termed TIS-L. Since TIS-L is included in all the genomic and sub-genomic RNAs, the SARS-CoV-2 translatome may be regulated by a sophisticated interplay between TIS-L and downstream TISs. TIS-L functions as a strong translation enhancer for ORF S, and as translation suppressors for most of the other ORFs. Our global temporal atlas provides compelling insight into unique regulation of the SARS-CoV-2 translatome and helps comprehensively evaluate its impact on the human genome.

[1] School of Biological Sciences, Seoul National University, Seoul, Republic of Korea. [2] Creative Research Initiatives Center for Molecular Biology of Translation, Korea University, Seoul, Republic of Korea. [3] Division of Life Sciences, Korea University, Seoul, Republic of Korea. [4] Department of Microbiology, Institute for Viral Diseases, College of Medicine, Korea University, Seoul, Republic of Korea. [5] Bioinformatics Institute, Seoul National University, Seoul, Republic of Korea. [6] These authors contributed equally: Doyeon Kim, Sukjun Kim, Joori Park, Hee Ryung Chang, Jeeyoon Chang, Junhak Ahn, Heedo Park. ✉email: manseong.park@gmail.com; yk-kim@korea.ac.kr; baek@snu.ac.kr



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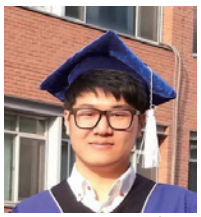
Hee Ryung Chang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Chanseok Shin


Jinwu Nam


Yoon Ki Kim


Sael Lee

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Ministry of Health and Welfare

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**Park lab:** Heedo Park and Jeonghun Kim

**Baek lab:** Doyeon Kim, Sukjun Kim, Hee Ryung Chang, Junhak Ahn, Junehee Park, Narae Son, and Gihyeon Kang

**Research Funding:**

Ministry of Science and ICT

Ministry of Health and Welfare