

Addiction Therapy-2014

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MiR-155 Modulates Morphine-induced Immunosuppression by Targeting SK3 Channel in Microglia



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Background

2

Morphine & Immunosuppression

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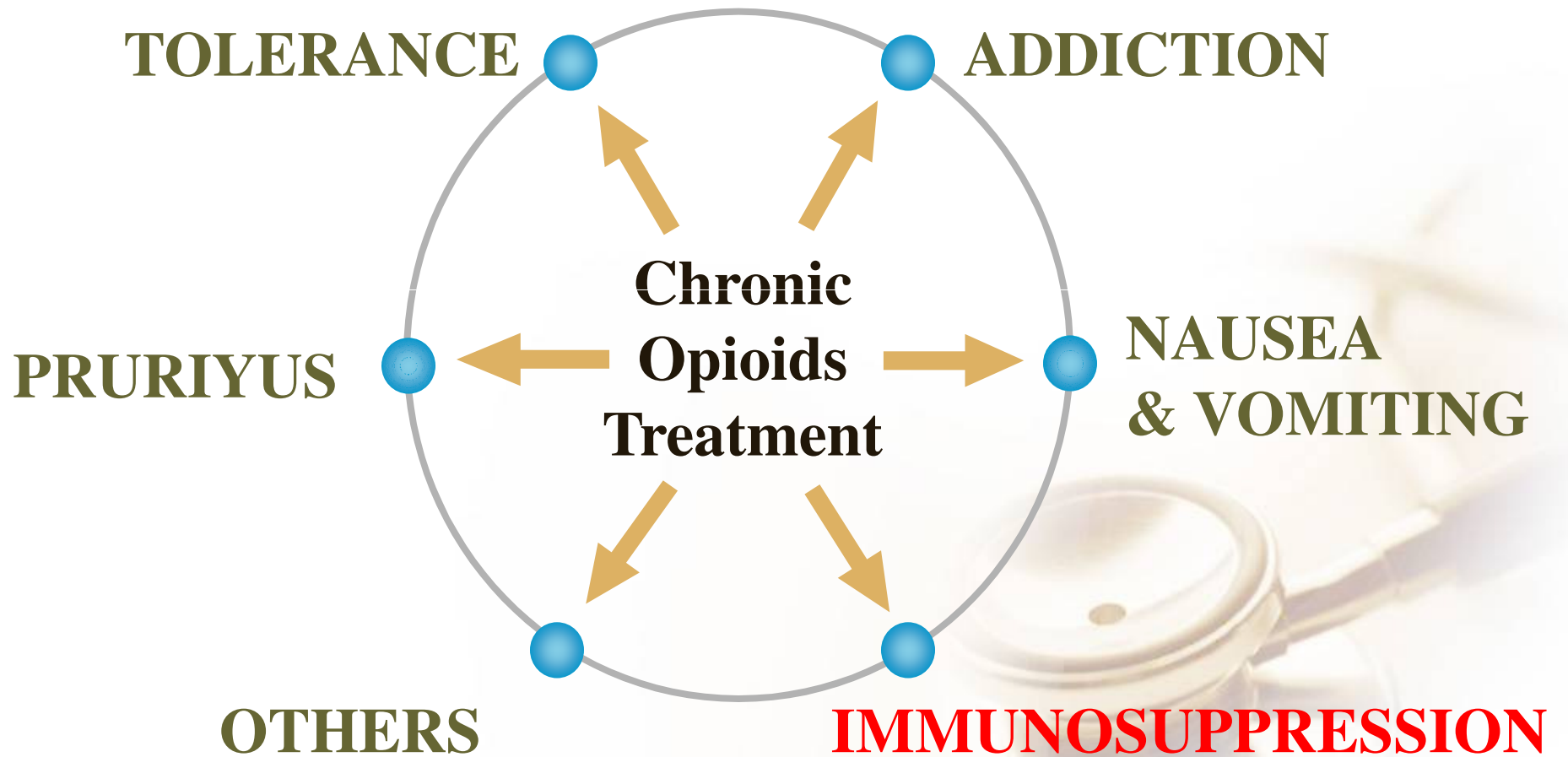
The Regulating Effects of MiR-155

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MiR-155 Targeting SK3 Channel



Background



Morphine-induced Immunosuppression

**Lymphocyte
Proliferation
& Apoptosis**

**NK Cell
Cytotoxicity**

**Cell Markers
Phenotype**

**Cytokines
Chemokine
Free radical**

...

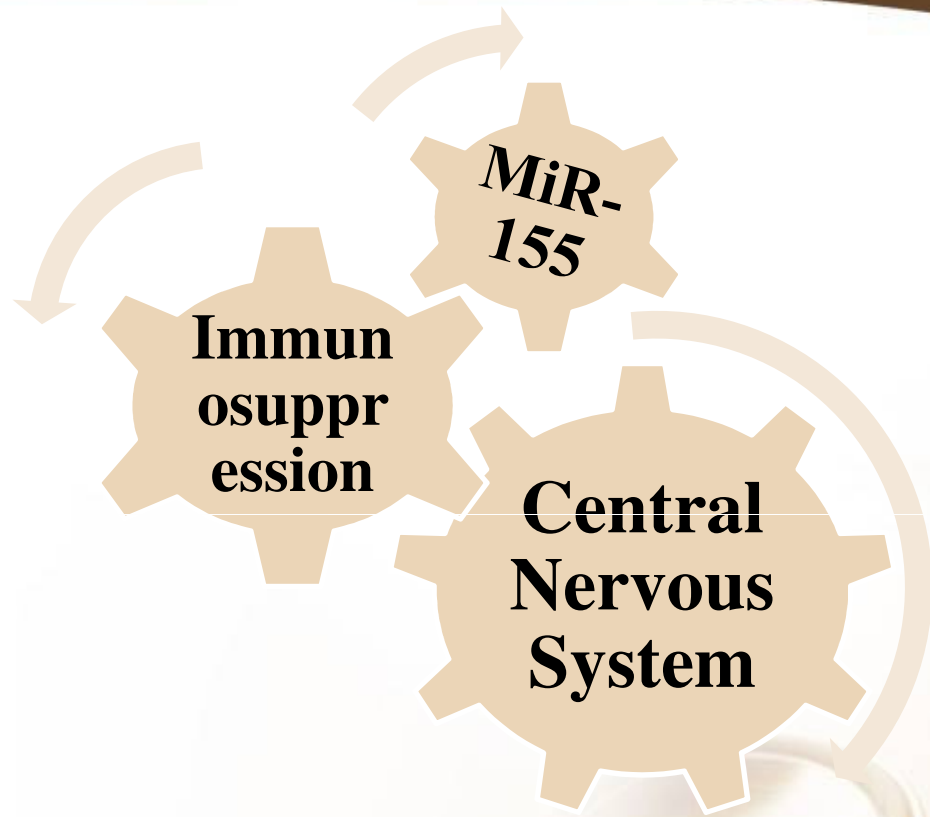
MicroRNA-155

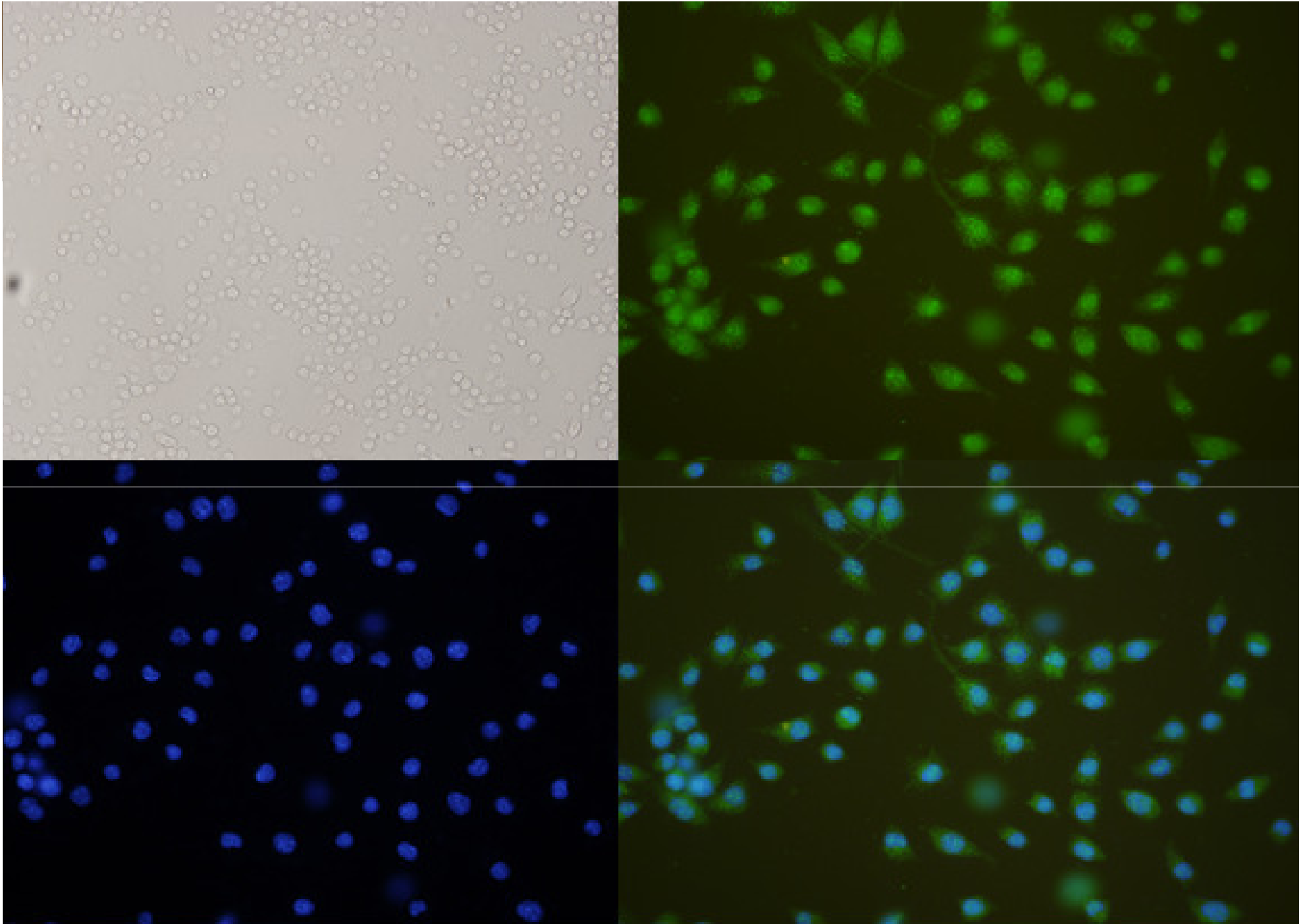
Tumorigenesis

- Proliferation ↑
- Invasion ↑
- Migration ↑
- Apoptosis ↓

Immune Response

- Inflammation ↑
- Lymphocytes' Proliferation & Differentiation ↑
- Virus infection ↑

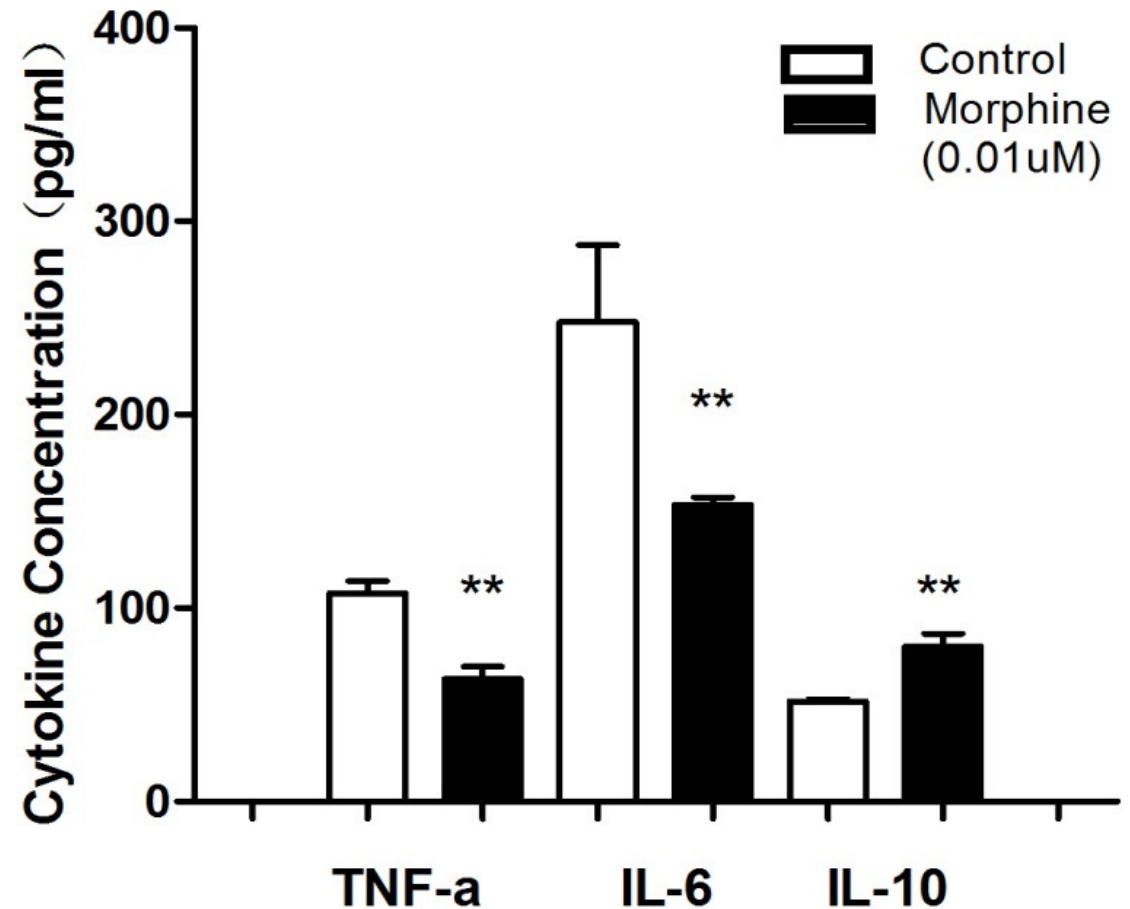




C57BL/6 mice primary microglia culture. Stained by Iba-1. Purity>98%

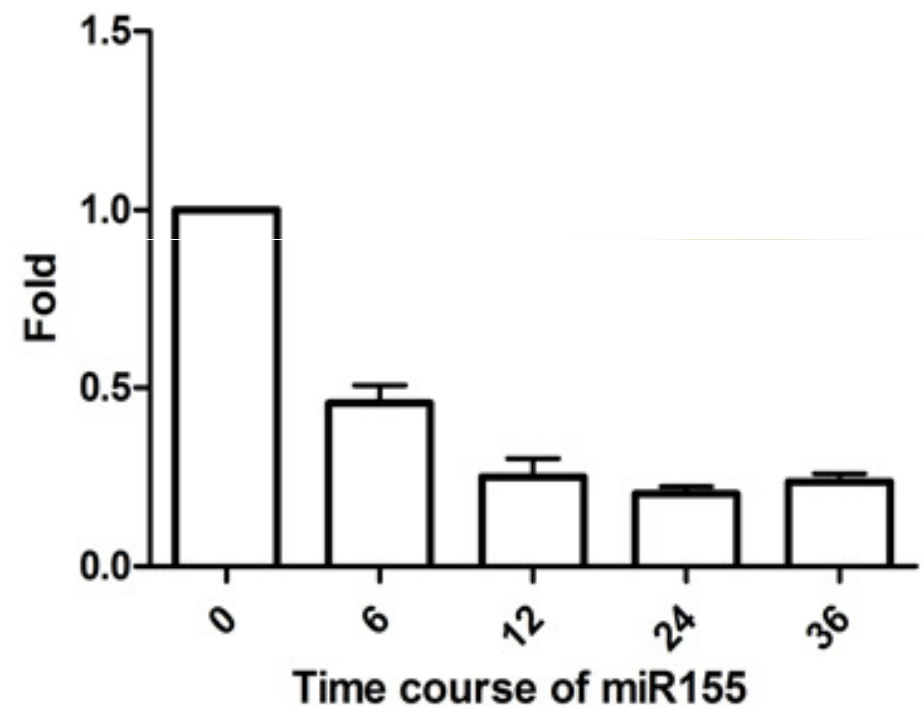
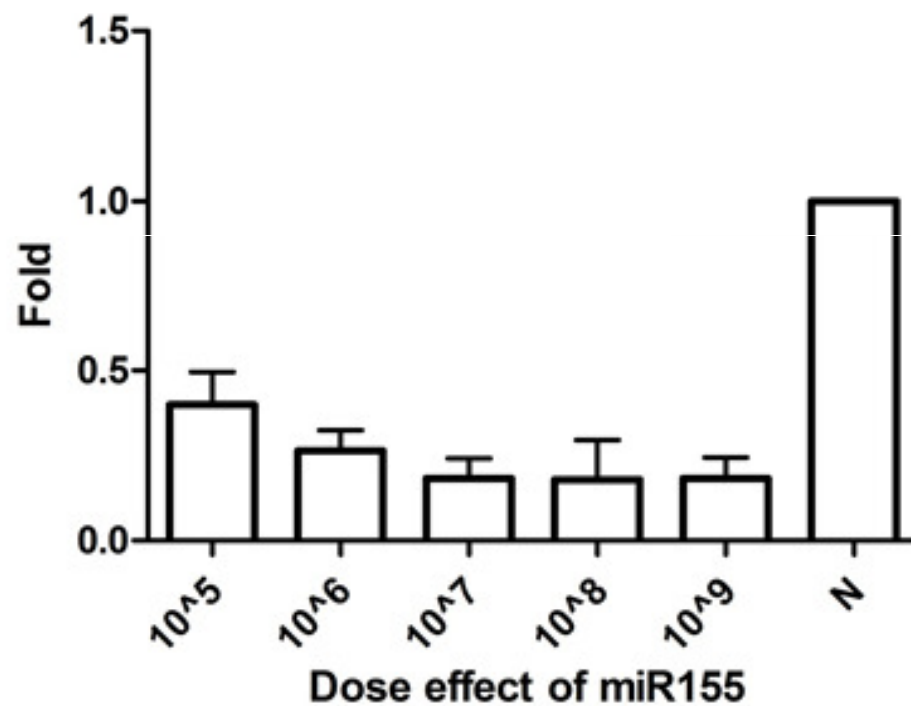
Morphine & Immunosuppression

Morphine induce immunosuppression in morphine-treated mice primary microglia(24h).



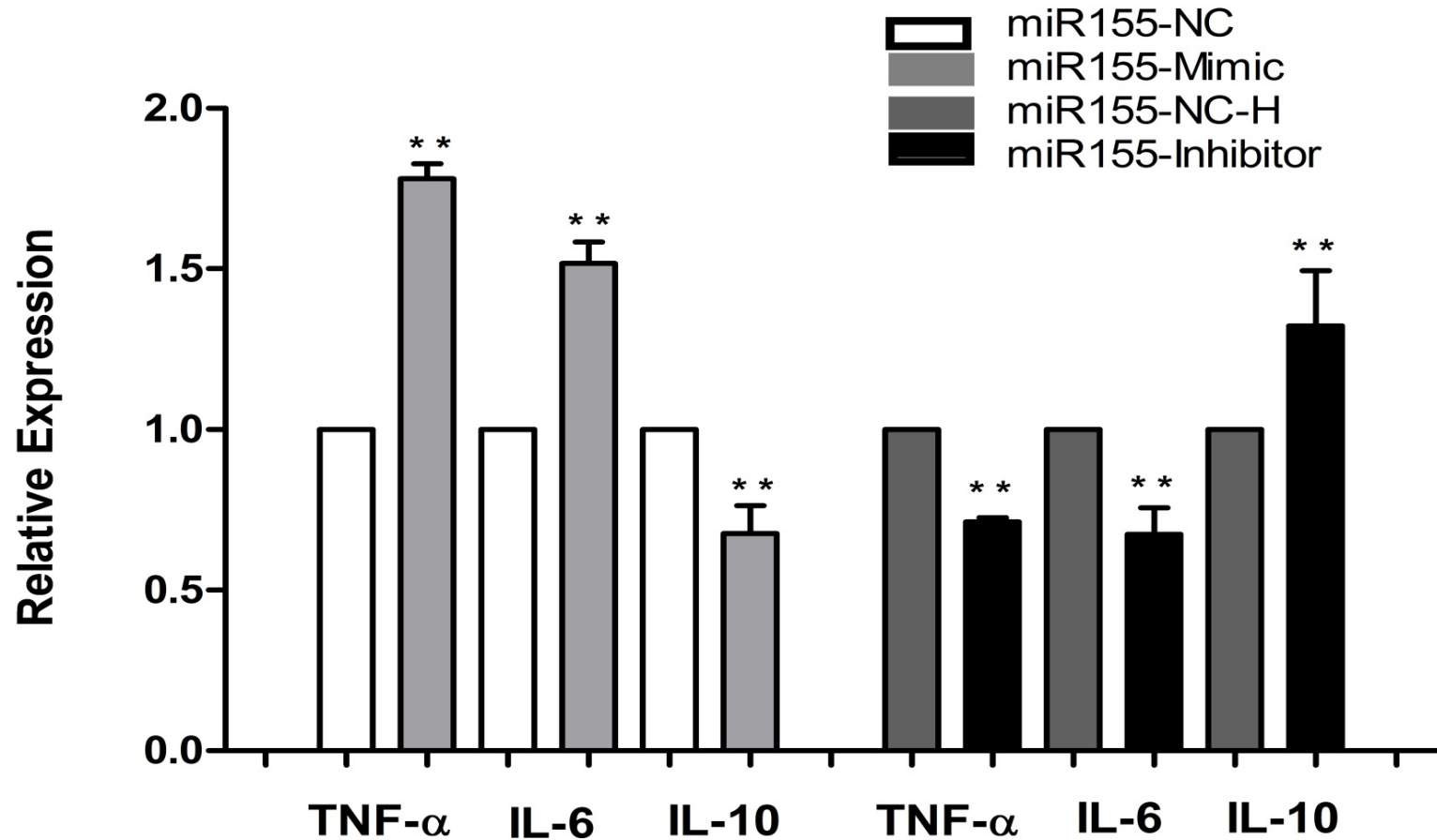
The Regulating Effects of MiR-155

MiR-155 was downregulated in morphine-treated primary microglia cells



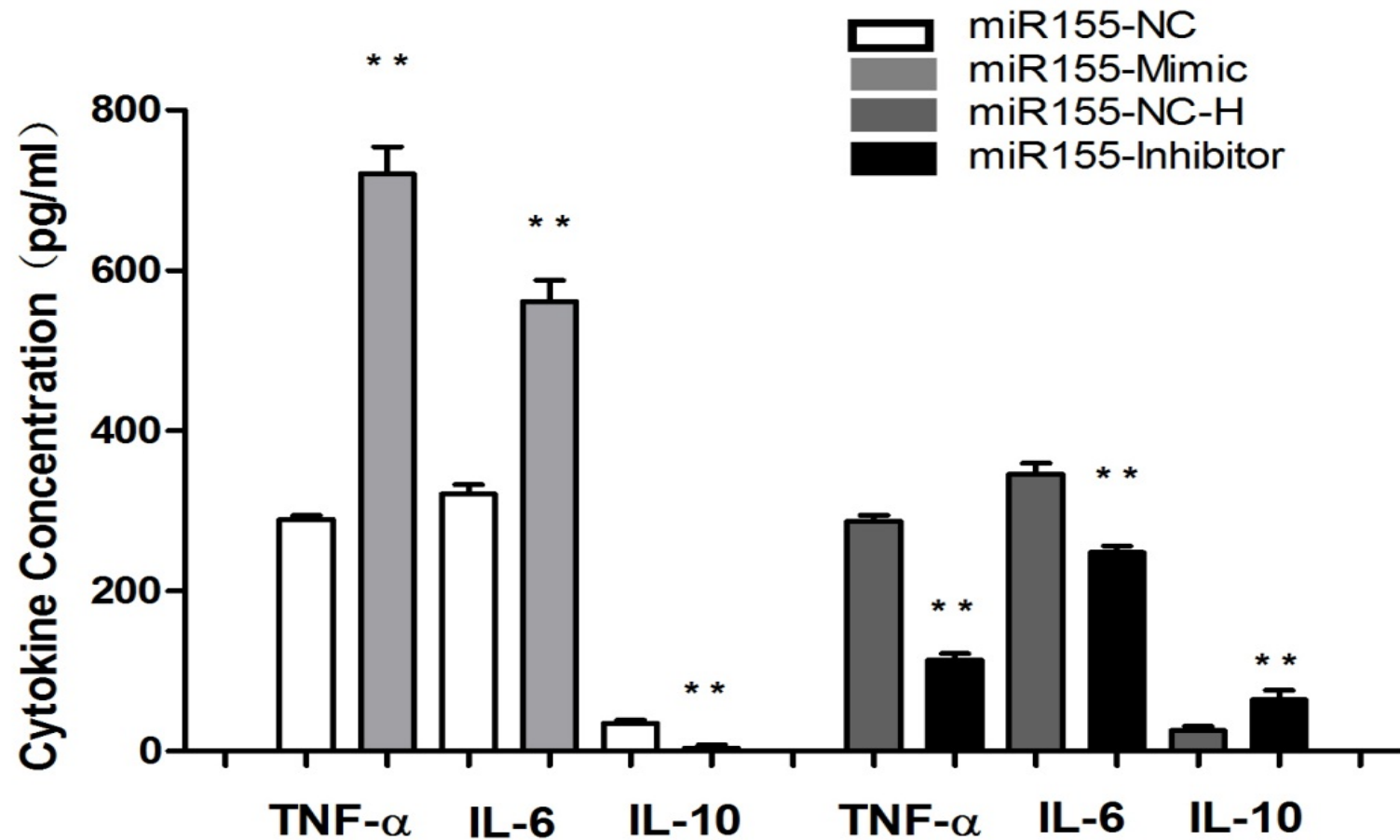
N=3 Student's t test $P < 0.05$

The Regulating Effects of MiR-155



mRNA levels of inflammatory cytokines in miR155 transfected primary microglia cells

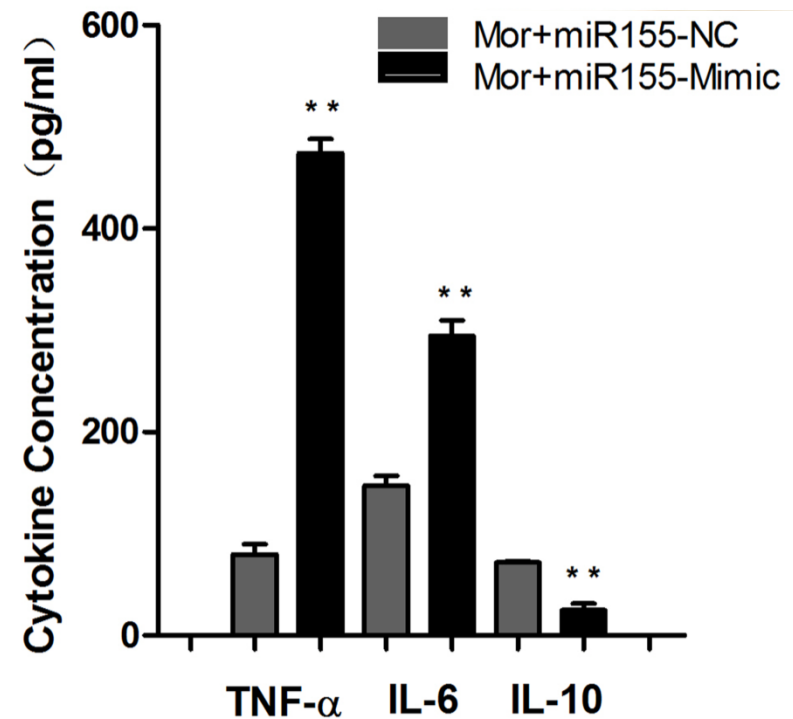
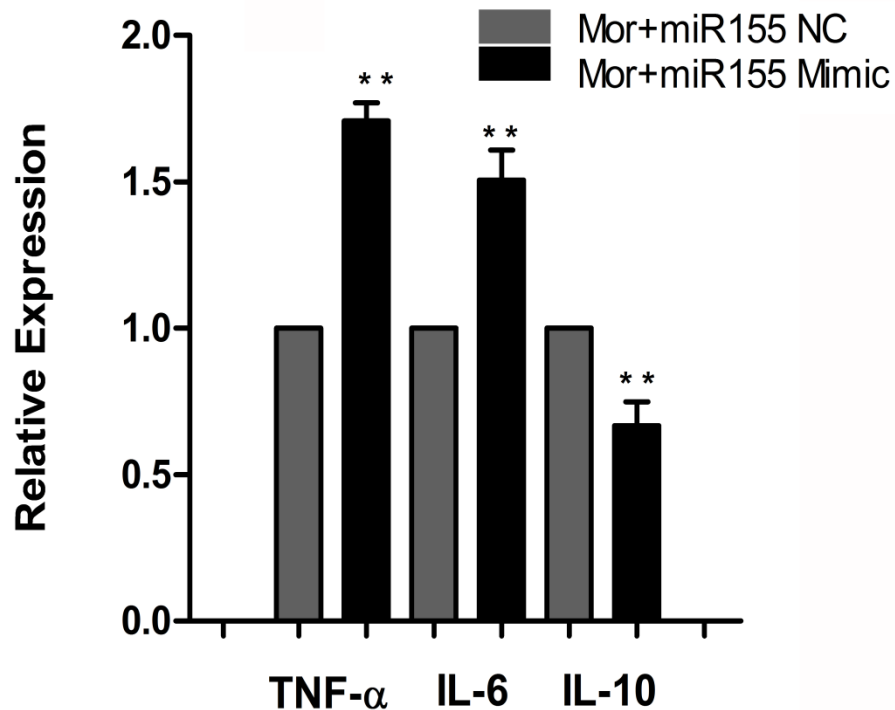
The Regulating Effects of MiR-155



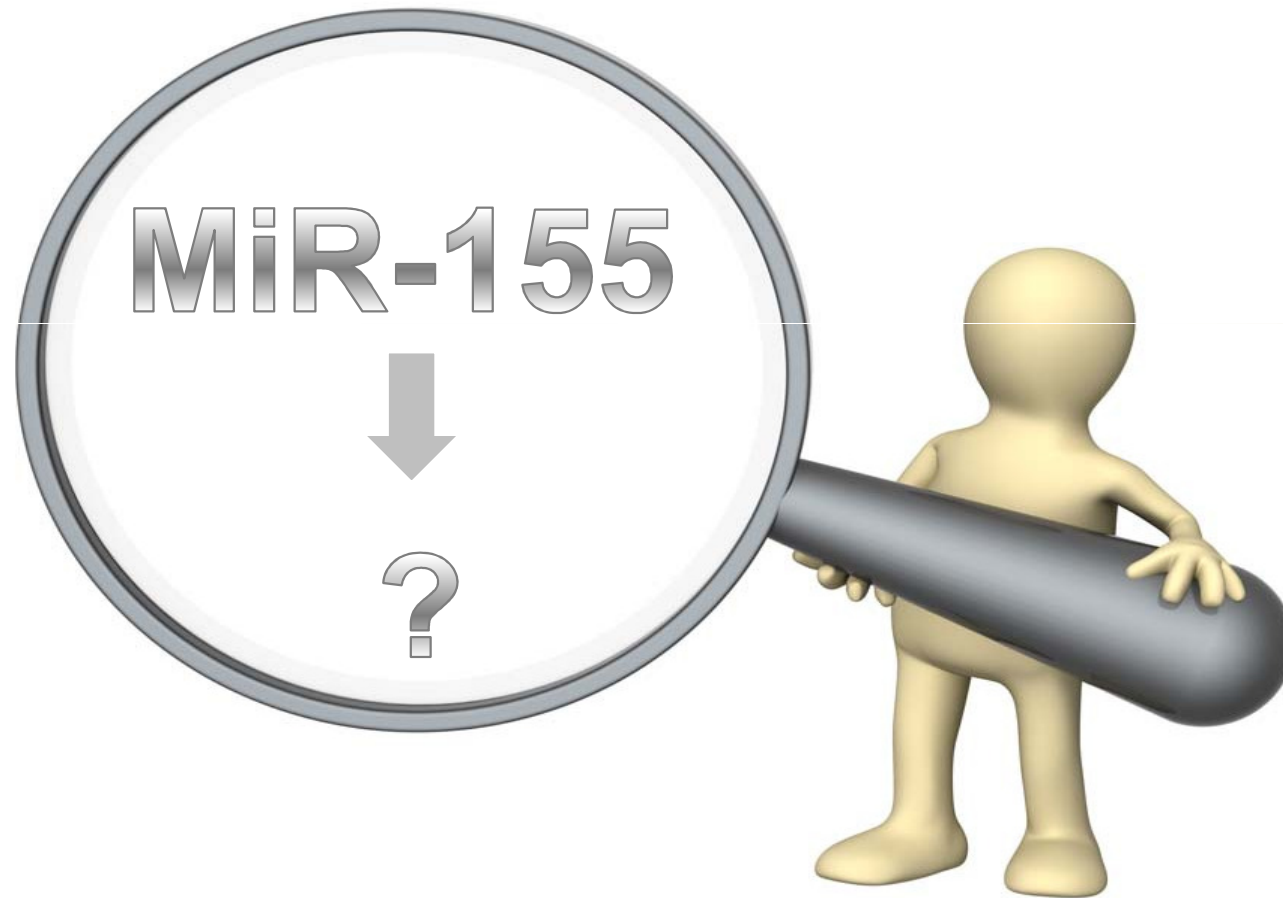
Extracellular levels of inflammatory cytokines in miR155 transfected primary microglia cells

The Regulating Effects of MiR-155

Upregulated miR155 reverse morphine-induced cytokines expressions in primary microglia.



The Regulating Effects of MiR-155



MiR-155 Targeting SK3 Channel

Bioinformatic analysis

microRNA Targets

microRNA.org - Targets and Expression

Predicted microRNA targets & target downregulation scores. Experimentally observed targets. August 2010 Release Last Update: 2010-11-01 [[release notes](#)]

[miRNA](#) [Target mRNA](#) [miRNA Expression](#) [Downloads](#) [FAQ](#)

miRBase Targets



TargetScan

The TargetScanMouse logo consists of a blue circular icon with a white 'X' and a red arrow pointing to the right. To the right of the icon, the text "TargetScanMouse" is written in blue and red, with "Prediction of microRNA targets" in smaller black text below it. To the right of this, "Release 6.2: June 2012" is written in blue. Below the logo is a horizontal bar with a red-to-orange gradient. At the bottom, the text "Search for predicted microRNA targets in mammals" is on the left, and "[Go to TargetScanHuman]" is on the right.

Search for predicted microRNA targets in mammals [Go to TargetScanHuman]

PicTar

PicTar WEB INTERFACE

Choose Species:	mouse
Choose Dataset:	target predictions for all mouse microRNAs based on conservation in mammals
microRNA ID:	mmu_miR_155

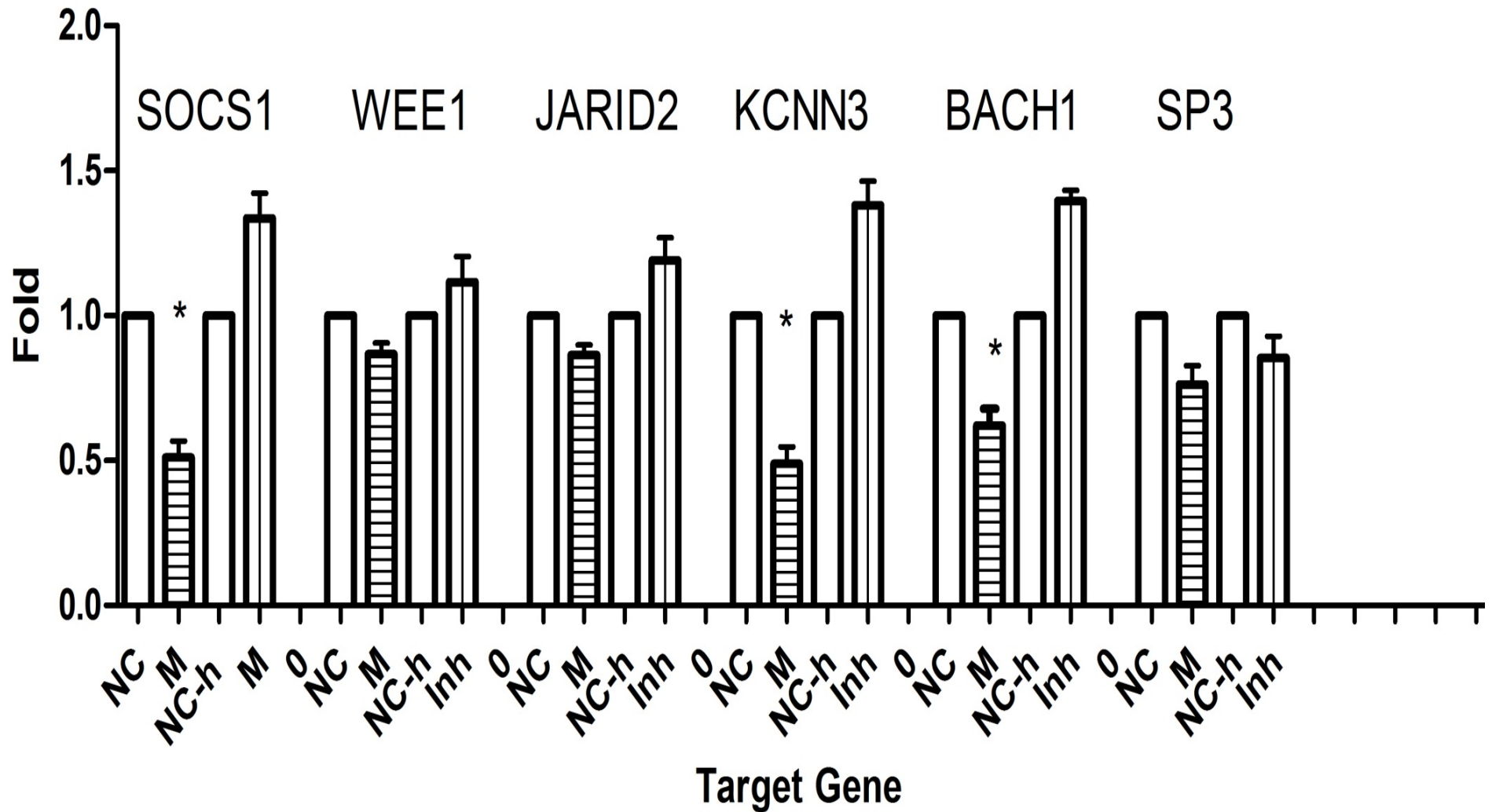
Click above for all microRNAs linked to Rfam

MiR-155 Targeting SK3 Channel

70 duplicated targets

	A	B	C	D	E	F
1	Target Gene	Gene ID	Official Full Name	Also known as	Function	Forward Primer (mouse)
2	SOCS1	12703		JAB; Cish1; Cish7; SSI-1; SOCS1		TCTATTGGGGACCCCTG
3	SPI1	20375	SFFV proviral integration 1		immunity	
4	Wee1	22390	WEE 1 homolog 1	Wee1A	inflammation; cancer	GCTGTGCTTGGACAGCA
5	SMARCA4	20586	SWI/SNF related, matrix associated	Brg1; SWI6	tumor suppressor; T helper differentiation and activation	
6	JARID2	16468		Jmj; jumori	CANCER	AGGAGACTGGAAGAGGC
7	RNF123	84585		Kpc1; BCOR	embryo development; immunity	
8	KCNN3	140493	potassium intermediate conductance	SK3; SKC	microglia activation	TGTTATGGTGATAGAGAC
9	BACH1	12013	BTB and CNC homology 1		apoptosis	CCGCAGCATCCATTTCA
10	Tspan14	52588	tetraspanin 14		apoptosis	
11	Sp3	20687	trans-acting transcription factor 3		cell survival and inflammation	CAGCTTGTCACAGTTTC
12	Ptpn2	19255	protein tyrosine phosphatase, non-receptor		immunity	
13	MGP	17313	matrix Gla protein		immune system development	
14	E2f2	242705	E2F transcription factor 2		immunity	
15	CYR61	16007	cysteine rich protein 61		immunity	
16	CLCN5	12728	chloride channel 5		immunity	
17	CEBPB	12608	CCAAT/enhancer binding protein beta	LAP; LIP; CRP2; NF-M; Nfil6; NF-IL6; IL-6DBP; C/EBPbeta		
18	Cdc73	214498	cell division cycle 73, Paf1/RNA polymerase II complex component			
19	Arid2	77044	AT rich interactive domain 2 (ARID, RFX-like)			
20	Apc	11789	adenomatous polyposis coli	CC1; Min; mAPC; AI047805; AU020952; AW124434		
21	AICDA	11628	activation-induced cytidine deaminase			

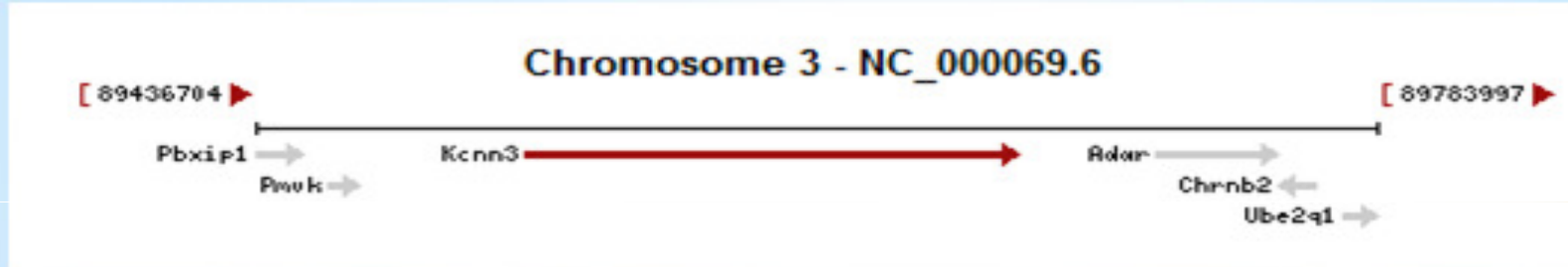
MiR-155 Targeting SK3 Channel



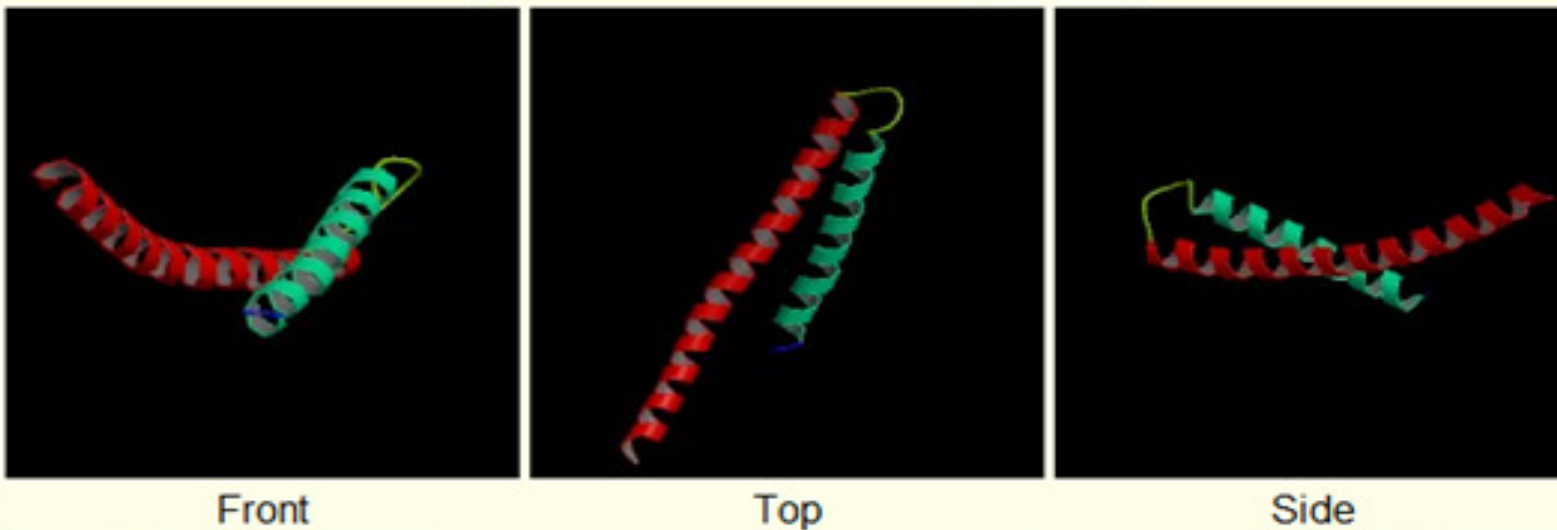
MiR-155 Targeting SK3 Channel

KCNN3

Potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3 (Kcnn3)

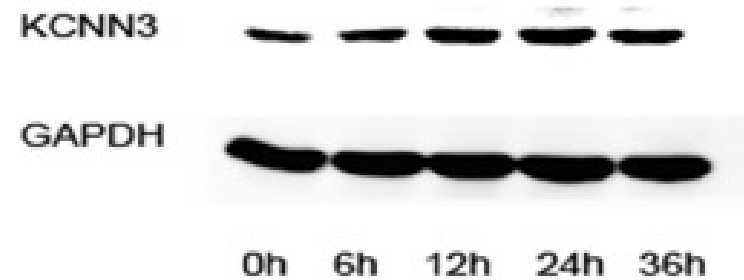
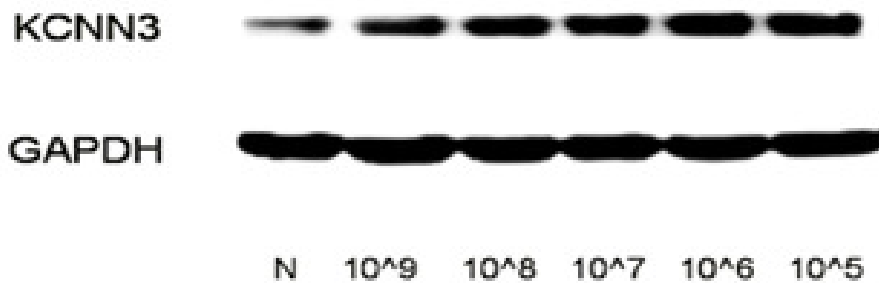
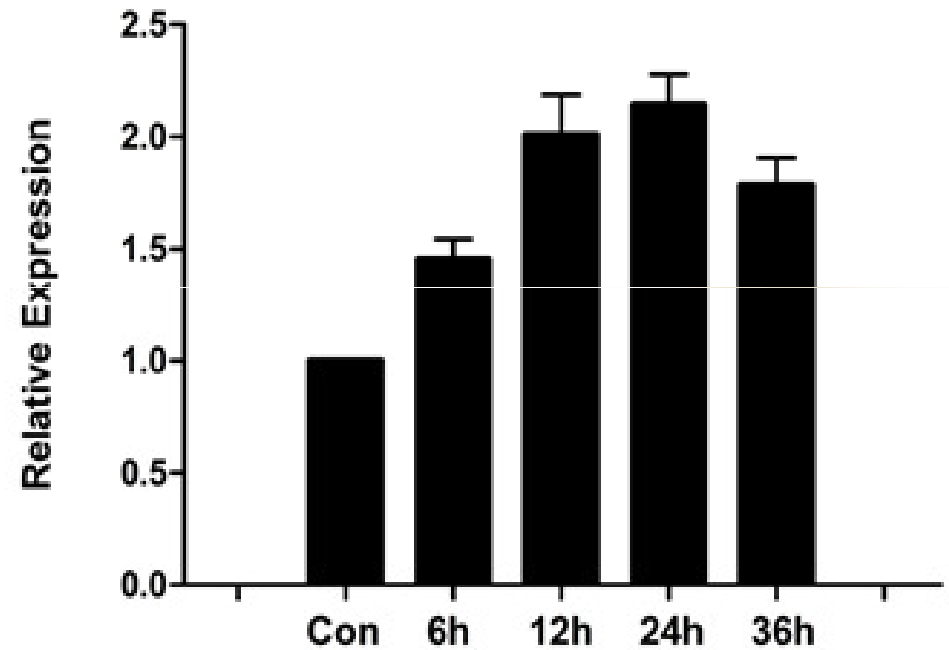
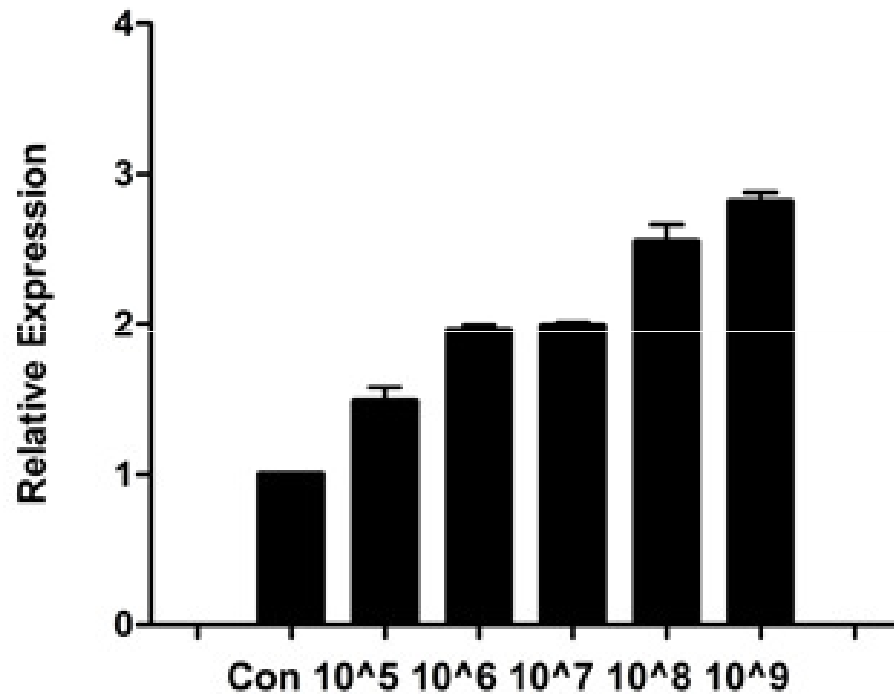


ModBase Predicted Comparative 3D Structure on [Q3UUY9](#)



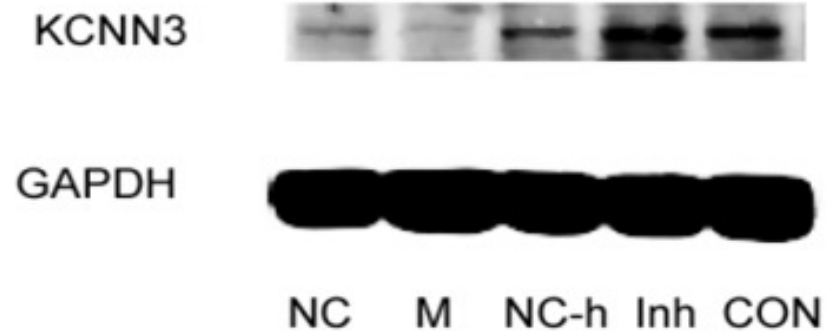
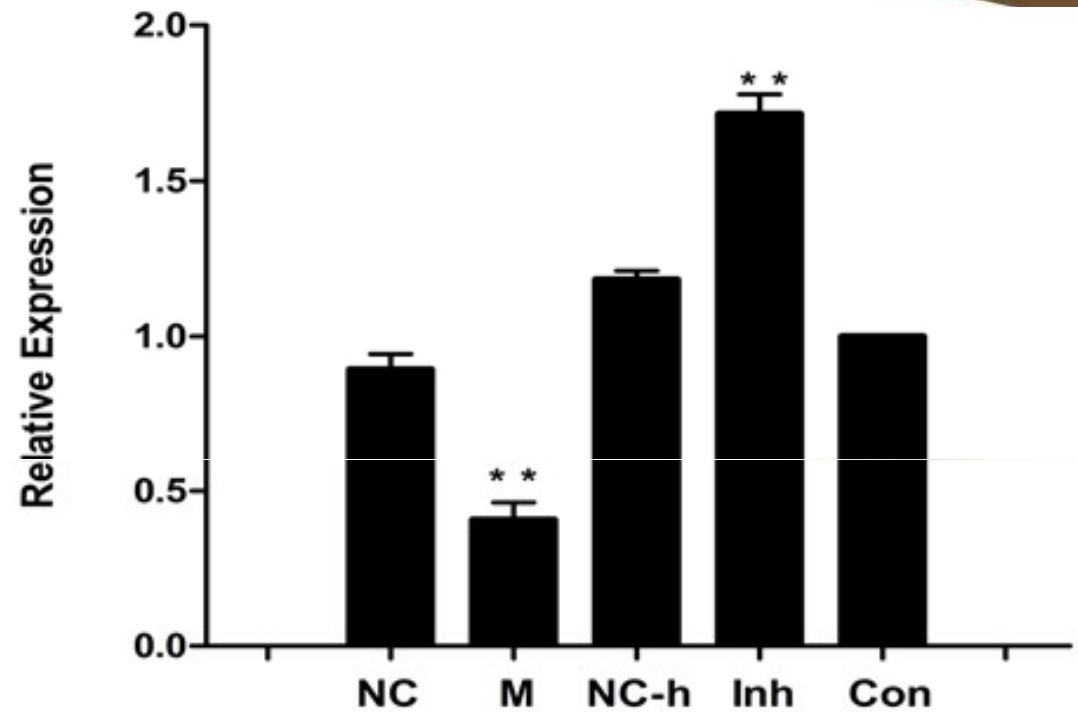
MiR-155 Targeting SK3 Channel

KCNN3 expressions in morphine-treated primary microglia.



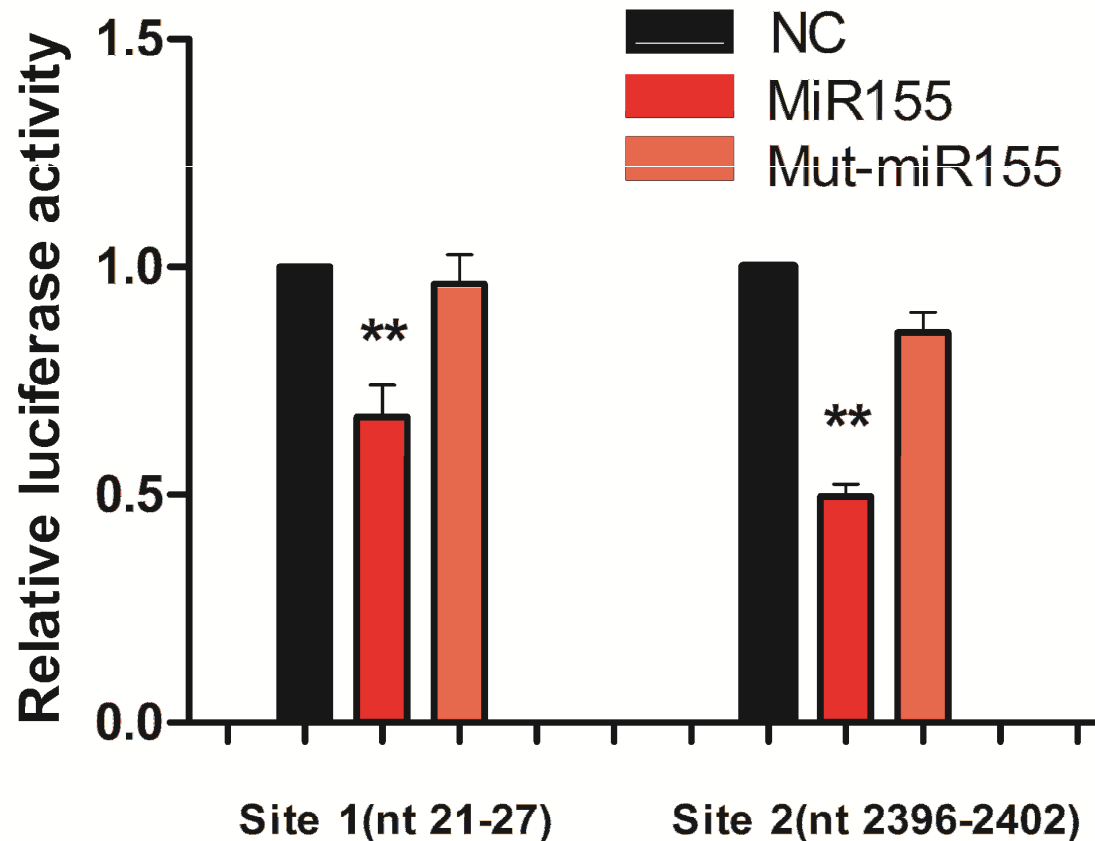
MiR-155 Targeting SK3 Channel

KCNN3 expression
was down-regulated
by miR-155.



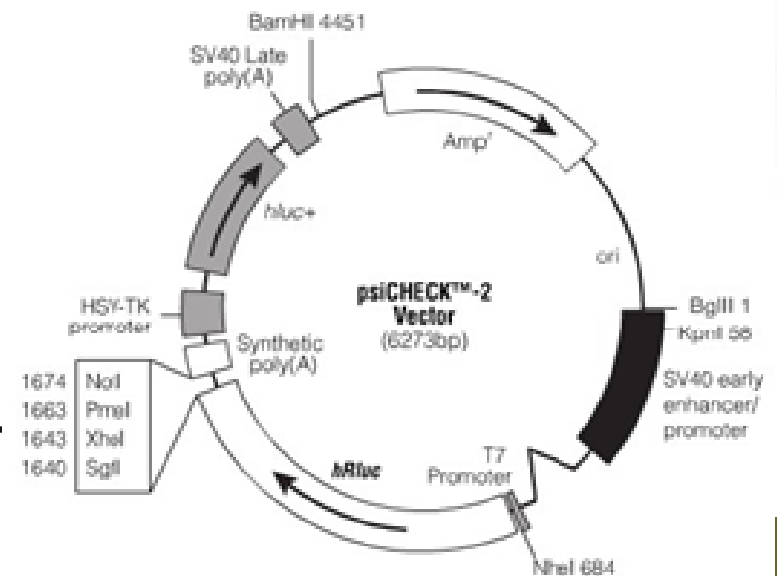
The Regulating Effects of MiR-155

MiR-155 target the 3'UTR of KCNN3



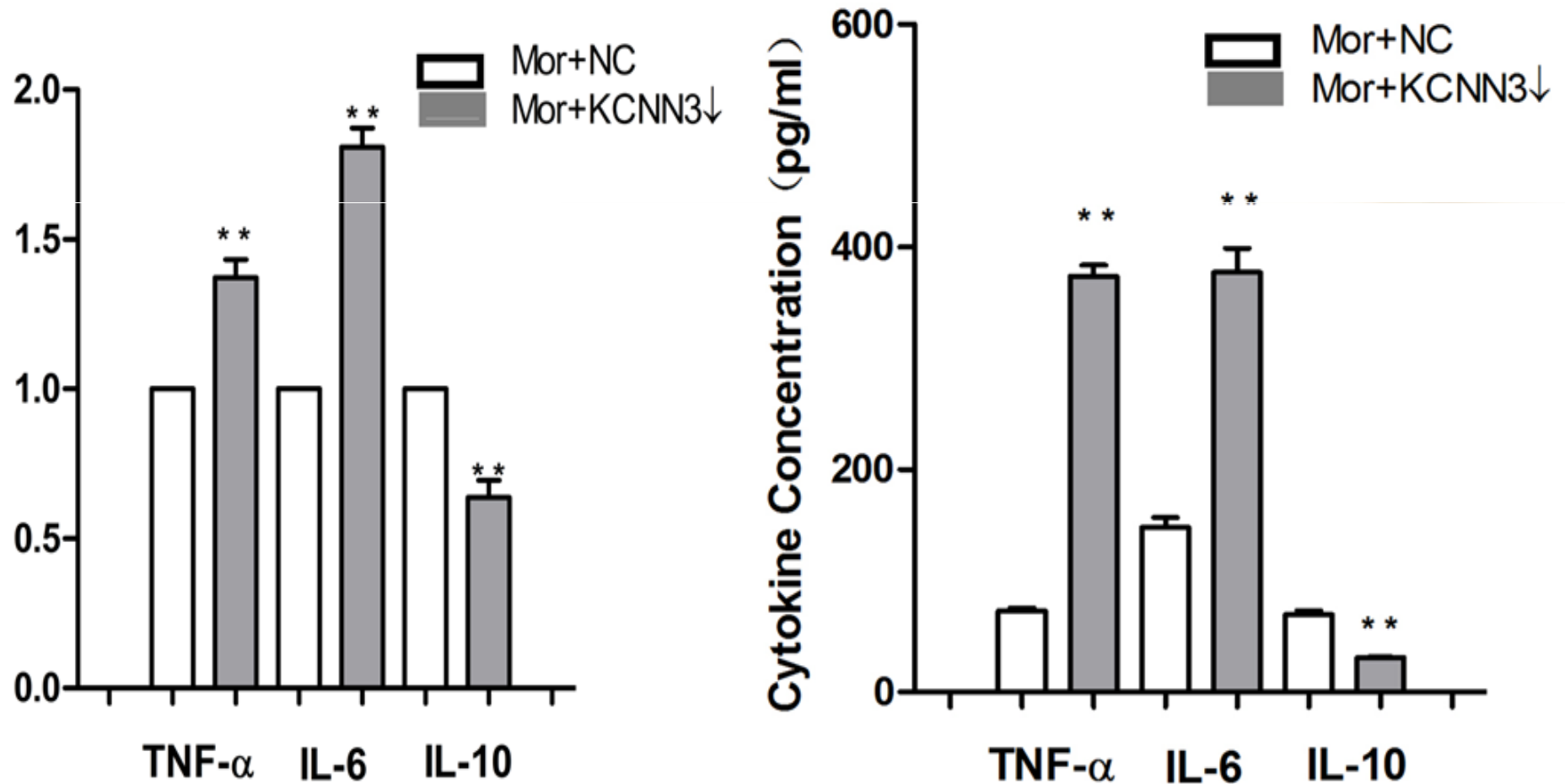
MiR-155 binding sites

	KCNN3 site1(nt21-27)	KCNN3 site2(nt2396-2402)
wt 3'UTR	...ACTCCAGAAGCATTACCC...	...GCACCACTAGCATTAGAGA...
iR-155 3'-5'	...GUGUAAUUCGUAUU...	...GUGUAAUUCGUAUU...
iR-155 3'-5'	...GUGUAAU <u>UCAUGAU</u>GUGUAAU <u>UCAUGAU</u> ...



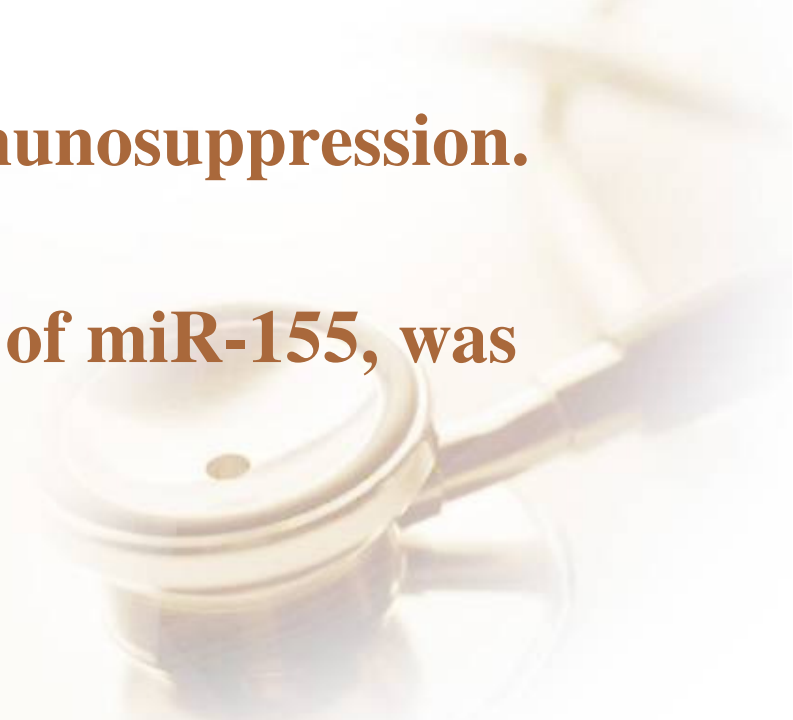
The Regulating Effects of MiR-155

Down-regulated KCNN3 reverse morphine-induced cytokines expressions in primary microglia.





Conclusions:

- **Our results demonstrated a reversal effect of miR-155 on morphine-induced immunosuppression.**
 - **SK3 channel, the direct target of miR-155, was involved in this process.**
- 

Acknowledgement





Thank You !



Meet the eminent gathering once again at

Addiction Therapy-2015

Florida, USA

August 3 - 5, 2015

Addiction Therapy – 2015 Website:

addictiontherapy.conferenceseries.com