RALY participates in nerve trauma-induced nociceptive hypersensitivity through triggering eIF4G2 gene expression in primary sensory neurons

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Abstract

Background and Purpose: Peripheral nerve trauma-induced dysregulation of pain-associated genes in the primary sensory neurons of dorsal root ganglion (DRG) contributes to neuropathic pain genesis. RNA-binding proteins participate in gene transcription. We hypothesized that RALY, an RNA-binding protein, participated in nerve trauma-induced dysregulation of DRG pain-associated genes and nociceptive hypersensitivity. Methods and results: Immunohistochemistry staining showed that RALY was expressed exclusively in the nuclei of DRG neurons. Peripheral nerve trauma caused by chronic constriction injury (CCI) of unilateral sciatic nerve produced time-dependent increases in the levels of Raly mRNA and RALY protein in injured DRG. Blocking this increase through DRG microinjection of adeno-associated virus 5 (AAV5)-expressing Raly shRNA reduced the CCI-induced elevation in the amount of eukaryotic initiation factor 4 gamma 2 (eIF4G2) mRNA and eIF4G2 protein in injured DRG and mitigated the development and maintenance of CCI-induced nociceptive hypersensitivity, without altering basal (acute) response to noxious stimuli and locomotor activity. Mimicking DRG increased RALY through DRG microinjection of AAV5 expressing Raly mRNA upregulated the expression of eIF4G2 mRNA and eIF4G2 protein in the DRG and led to hypersensitive responses to noxious stimuli in the absence of nerve trauma. Mechanistically, CCI promoted the binding of RALY to the promoter of eIF4G2 gene and triggered its transcriptional activity. Conclusion and Implications: Our findings indicate that RALY participates in nerve trauma-induced nociceptive hypersensitivity likely through transcriptionally triggering eIF4G2 expression in the DRG. RALY may be a potential target in neuropathic pain management.

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Fig. 1

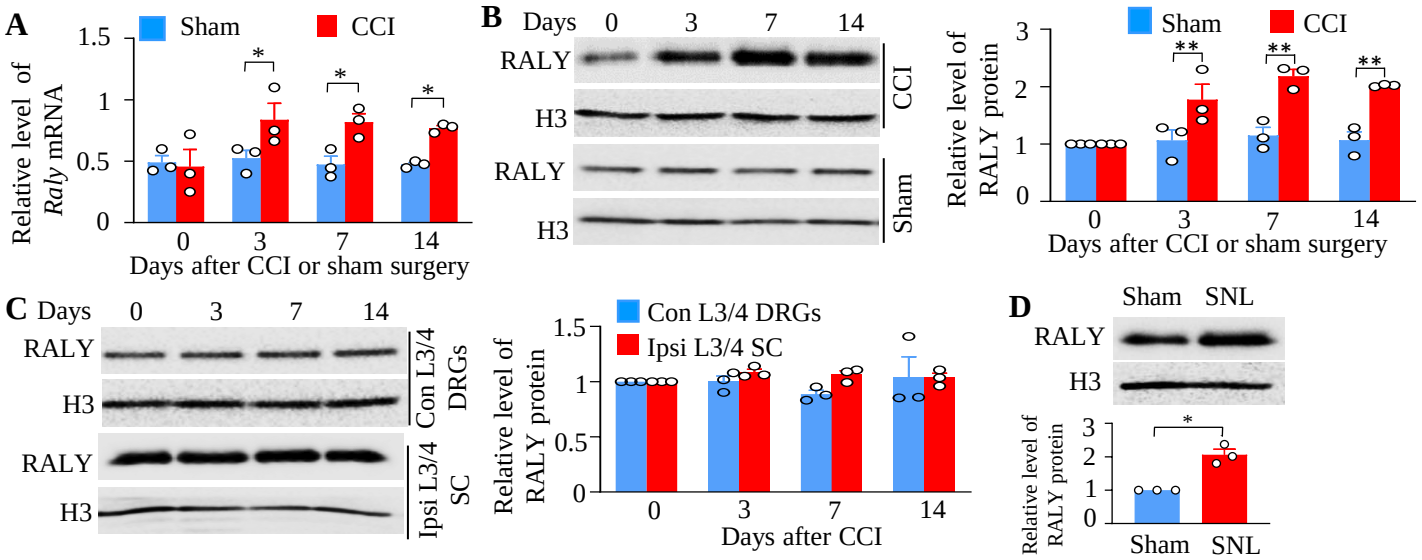

A
Sham
CCI
Relative level of Raly mRNA
1.5
1
0.5
0
*
*
*
0
3
7
14
Days after CCI or sham surgery
B
Days 0 3 7 14
RALY
H3
CCI
RALY
H3
Sham
Relative level of RALY protein
3
2
1
0
**
**
**
Sham
CCI
0
3
7
14
Days after CCI or sham surgery
C
Days 0 3 7 14
RALY
H3
Con L3/4 DRGs
RALY
H3
Ipsi L3/4 SC
Con L3/4 DRGs
Ipsi L3/4 SC
Relative level of RALY protein
1.5
1
0.5
0
0
3
7
14
Days after CCI
D
Sham SNL
RALY
H3
Relative level of RALY protein
3
2
1
0
*
Sham
SNL

Fig. 2

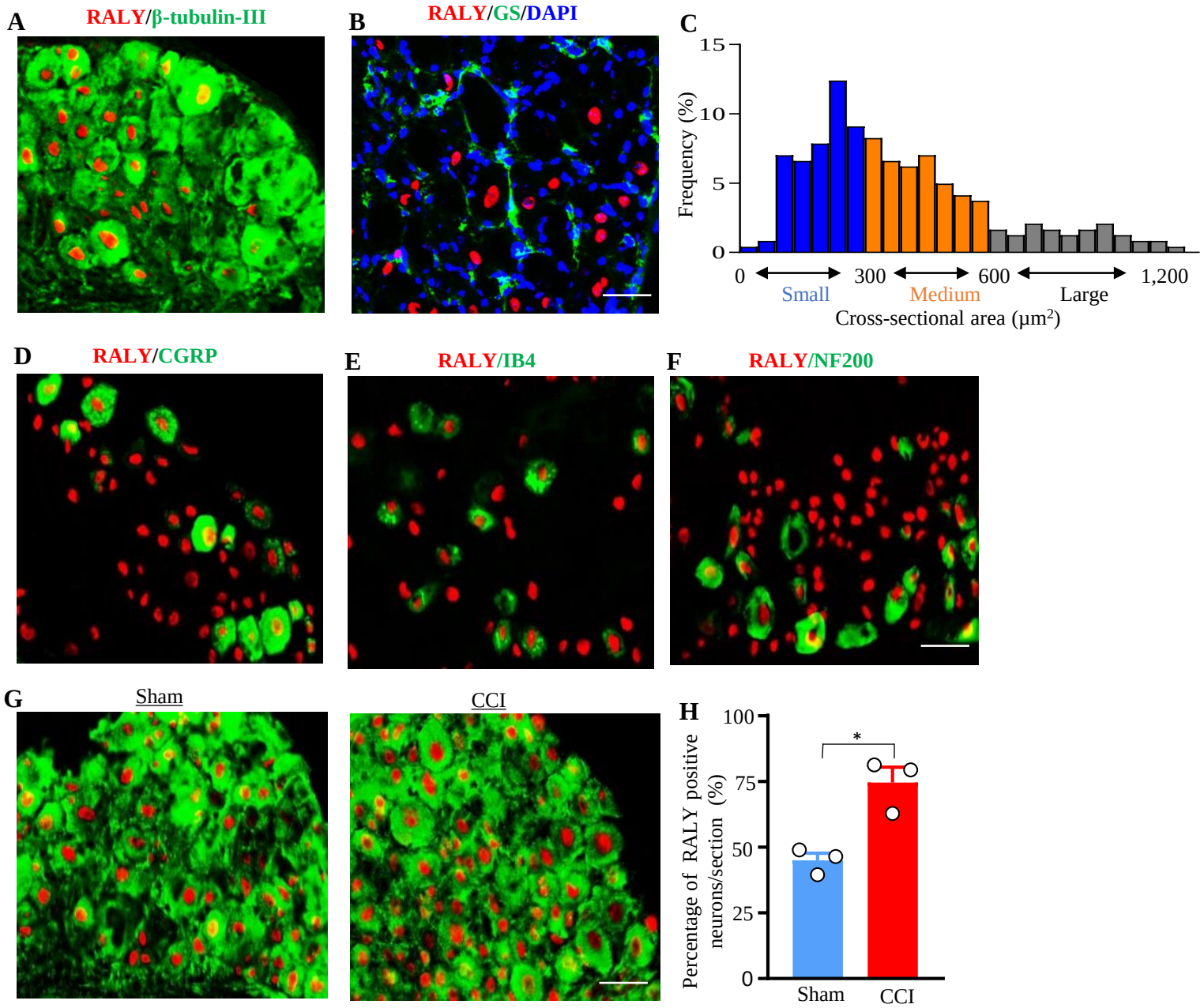

A
RALY/β-tubulin-III
B
RALY/GS/DAPI
C
Frequency (%)
15
10
5
0
0
300
600
1,200
Small
Medium
Large
Cross-sectional area (μm²)
D
RALY/CGRP
E
RALY/IB4
F
RALY/NF200
G
Sham
CCI
H
Percentage of RALY positive neurons/section (%)
100
75
50
25
0
*
Sham
CCI

Fig. 3

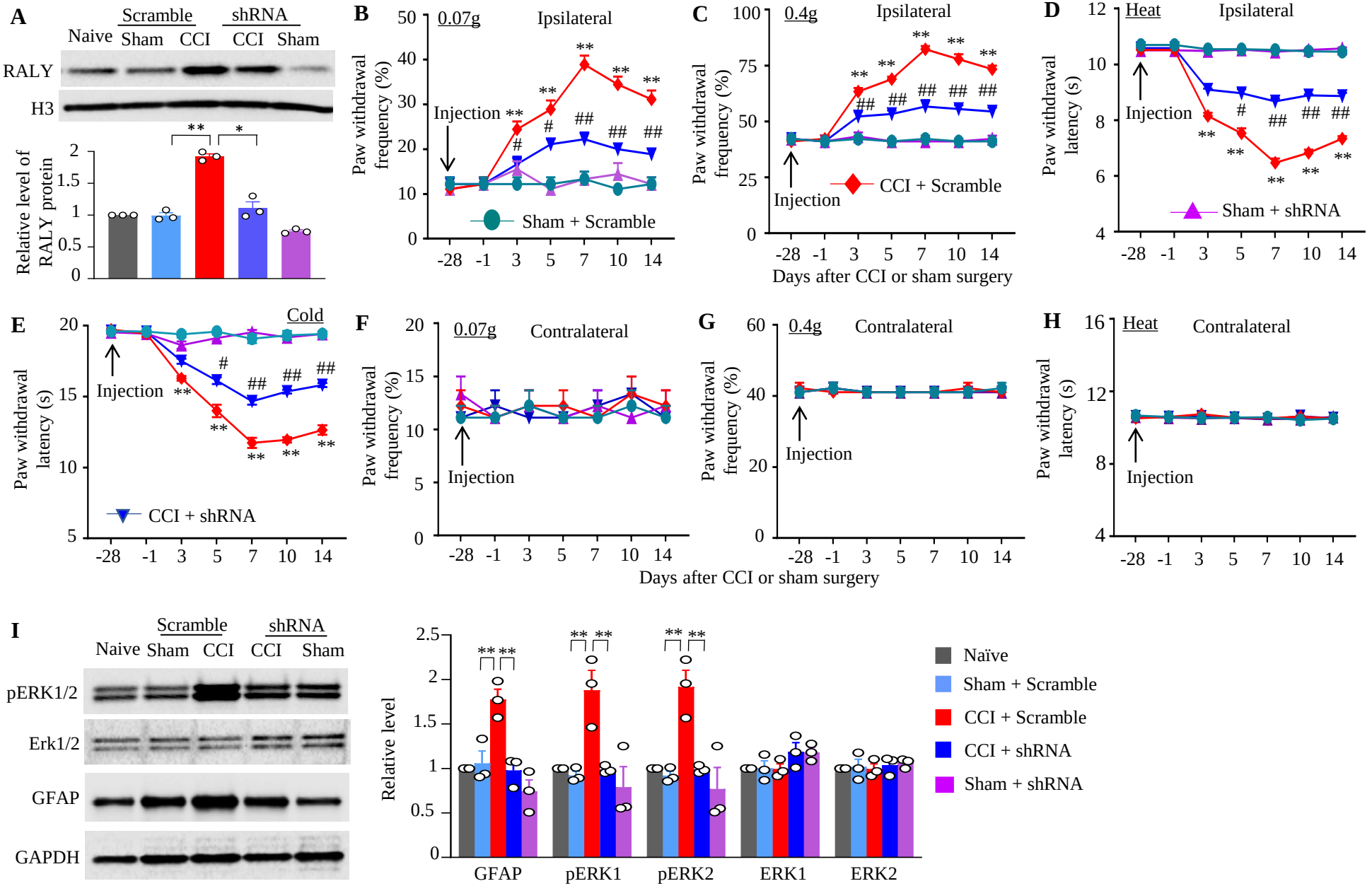

A
Scramble
shRNA
Naive
Sham
CCI
CCI
Sham
RALY
H3
Relative level of RALY protein
B
0.07g
Ipsilateral
Paw withdrawal frequency (%)
Injection
Sham + Scramble
C
0.4g
Ipsilateral
CCI + Scramble
Days after CCI or sham surgery
D
Heat
Ipsilateral
Paw withdrawal latency (s)
Sham + shRNA
E
Cold
CCI + shRNA
F
0.07g
Contralateral
G
0.4g
Contralateral
H
Heat
Contralateral
Days after CCI or sham surgery
I
pERK1/2
Erk1/2
GFAP
GAPDH
Relative level
GFAP
pERK1
pERK2
ERK1
ERK2
Naïve
Sham + Scramble
CCI + Scramble
CCI + shRNA
Sham + shRNA

Fig. 4

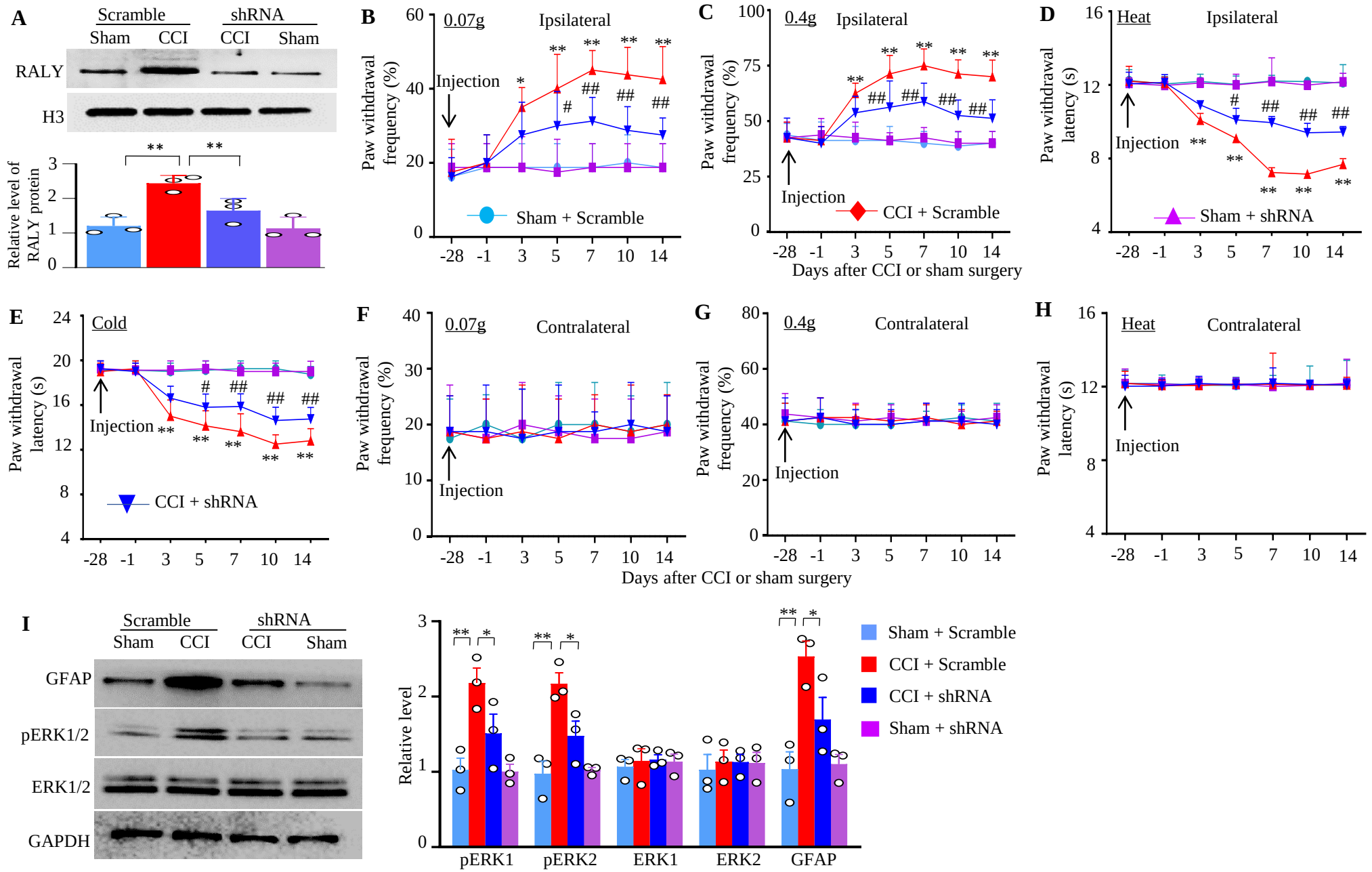

A
Scramble
shRNA
Sham
CCI
CCI
Sham
RALY
H3
Relative level of RALY protein
B
0.07g
Ipsilateral
Injection
Sham + Scramble
Paw withdrawal frequency (%)
C
0.4g
Ipsilateral
Injection
CCI + Scramble
Days after CCI or sham surgery
D
Heat
Ipsilateral
Injection
Sham + shRNA
Paw withdrawal latency (s)
E
Cold
Injection
CCI + shRNA
Paw withdrawal latency (s)
F
0.07g
Contralateral
Injection
Paw withdrawal frequency (%)
G
0.4g
Contralateral
Injection
Paw withdrawal frequency (%)
H
Heat
Contralateral
Injection
Paw withdrawal latency (s)
Days after CCI or sham surgery
I
Scramble
shRNA
Sham
CCI
CCI
Sham
GFAP
pERK1/2
ERK1/2
GAPDH
Relative level
pERK1
pERK2
ERK1
ERK2
GFAP
Sham + Scramble
CCI + Scramble
CCI + shRNA
Sham + shRNA

Fig. 5

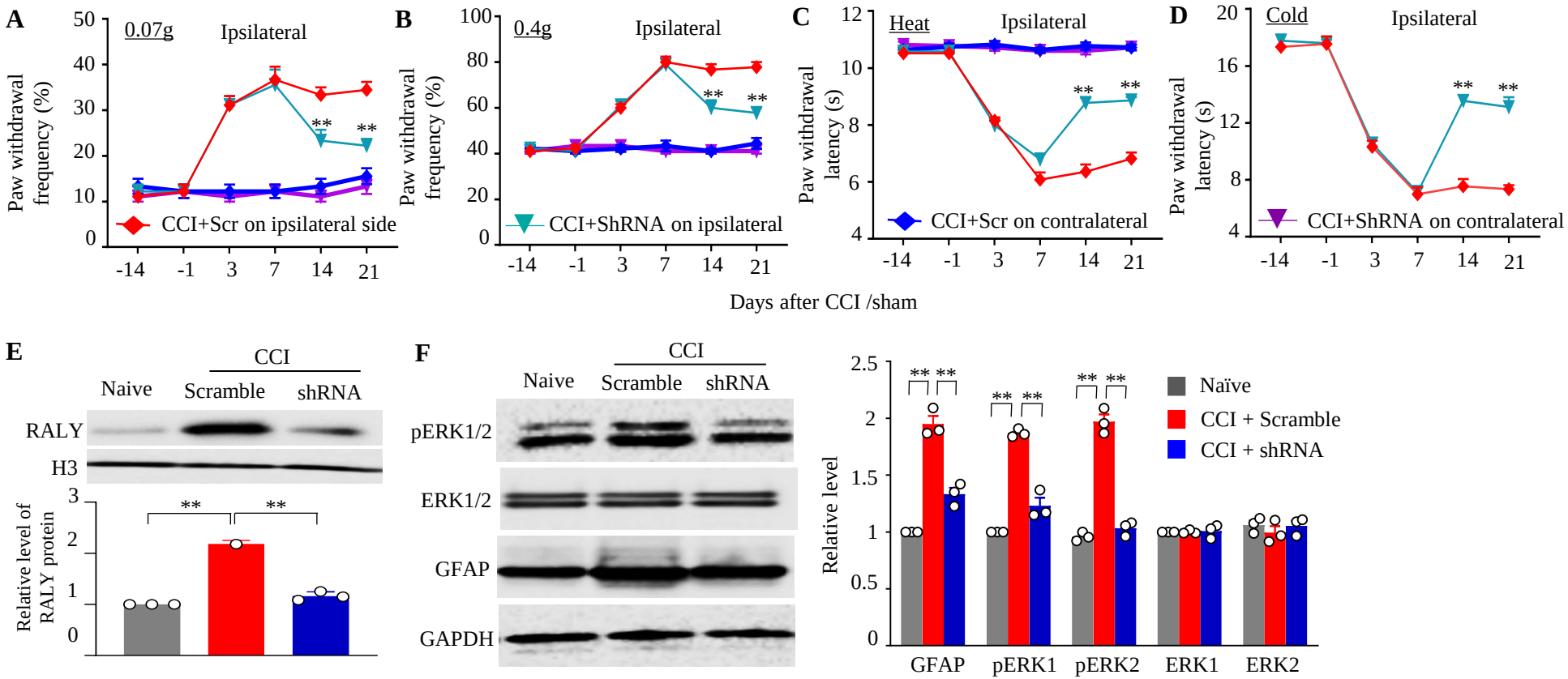

A
0.07g
Ipsilateral
Paw withdrawal frequency (%)
CCI+Scr on ipsilateral side
B
0.4g
Ipsilateral
Paw withdrawal frequency (%)
CCI+ShRNA on ipsilateral
C
Heat
Ipsilateral
Paw withdrawal latency (s)
CCI+Scr on contralateral
D
Cold
Ipsilateral
Paw withdrawal latency (s)
CCI+ShRNA on contralateral
Days after CCI /sham
E
CCI
Naive
Scramble
shRNA
RALY
H3
Relative level of RALY protein
F
CCI
Naive
Scramble
shRNA
pERK1/2
ERK1/2
GFAP
GAPDH
Relative level
Naïve
CCI + Scramble
CCI + shRNA
GFAP
pERK1
pERK2
ERK1
ERK2

Fig. 6

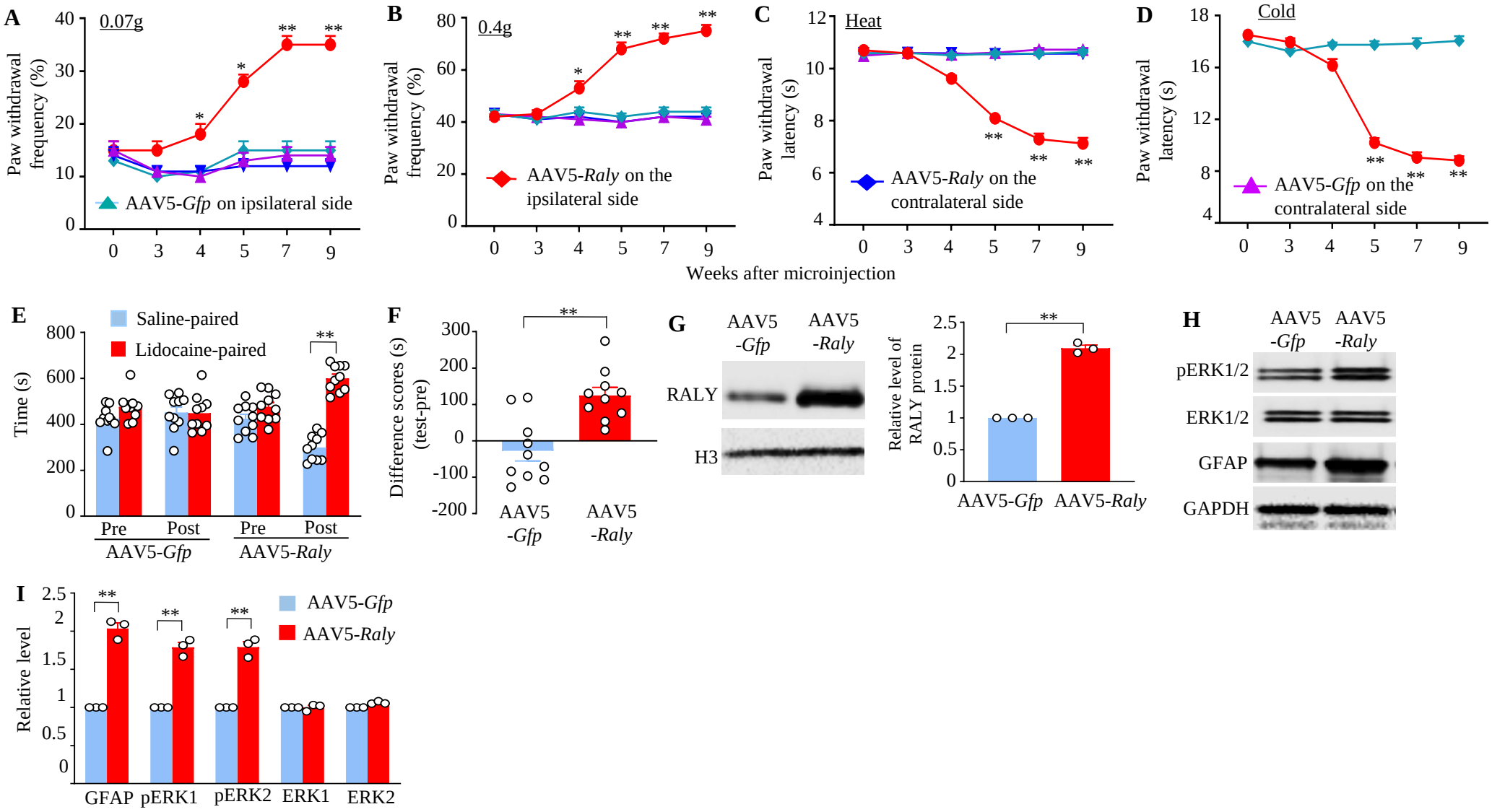

A
0.07g
Paw withdrawal frequency (%)
AAV5-Gfp on ipsilateral side
B
0.4g
Paw withdrawal frequency (%)
AAV5-Raly on the ipsilateral side
C
Heat
Paw withdrawal latency (s)
AAV5-Raly on the contralateral side
D
Cold
Paw withdrawal latency (s)
AAV5-Gfp on the contralateral side
Weeks after microinjection
E
Saline-paired
Lidocaine-paired
Time (s)
Pre
Post
AAV5-Gfp
AAV5-Raly
F
Difference scores (s) (test-pre)
AAV5 -Gfp
AAV5 -Raly
G
AAV5 -Gfp
AAV5 -Raly
RALY
H3
Relative level of RALY protein
H
pERK1/2
ERK1/2
GFAP
GAPDH
I
Relative level
AAV5-Gfp
AAV5-Raly
GFAP
pERK1
pERK2
ERK1
ERK2

Fig. 7

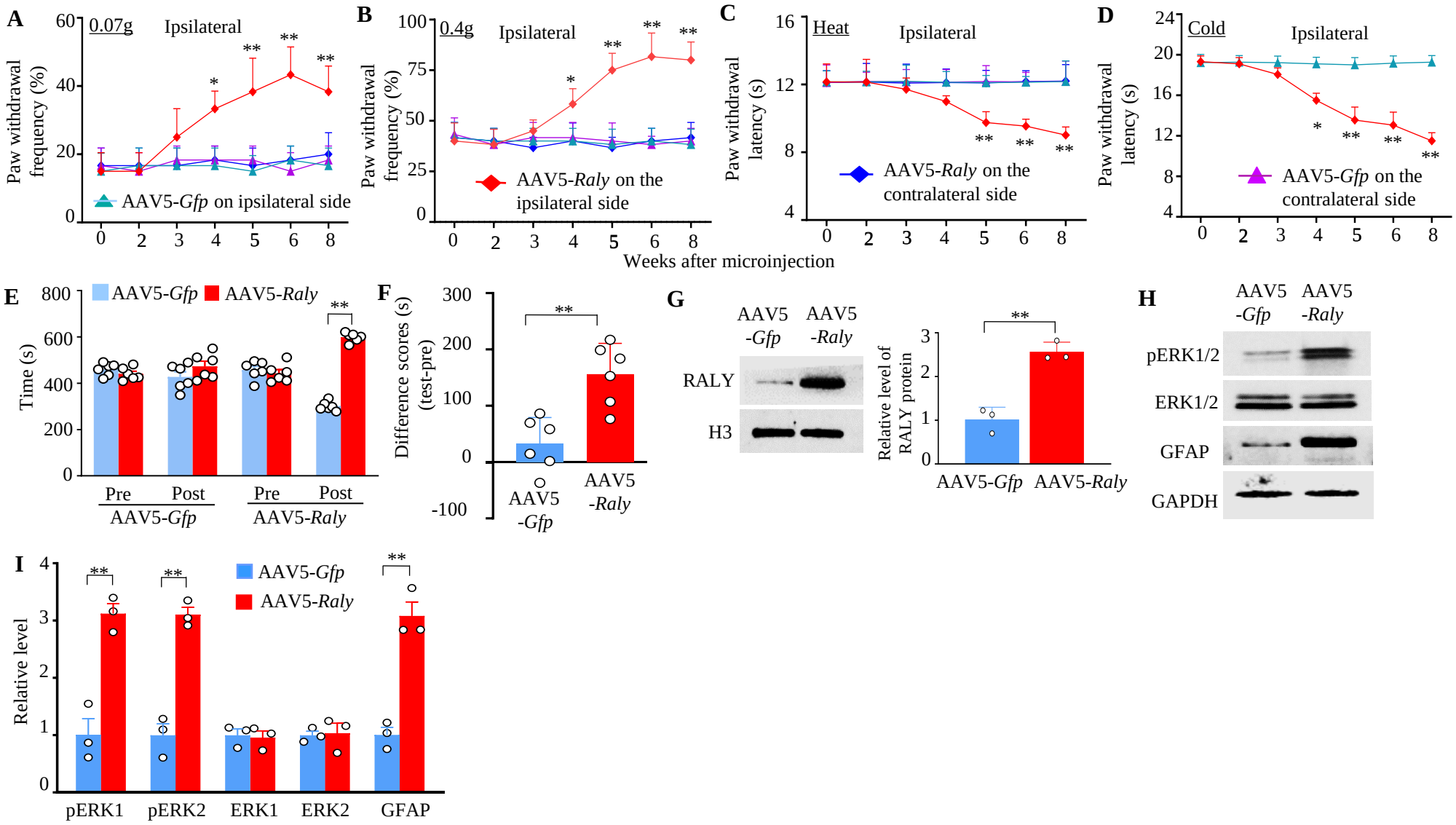
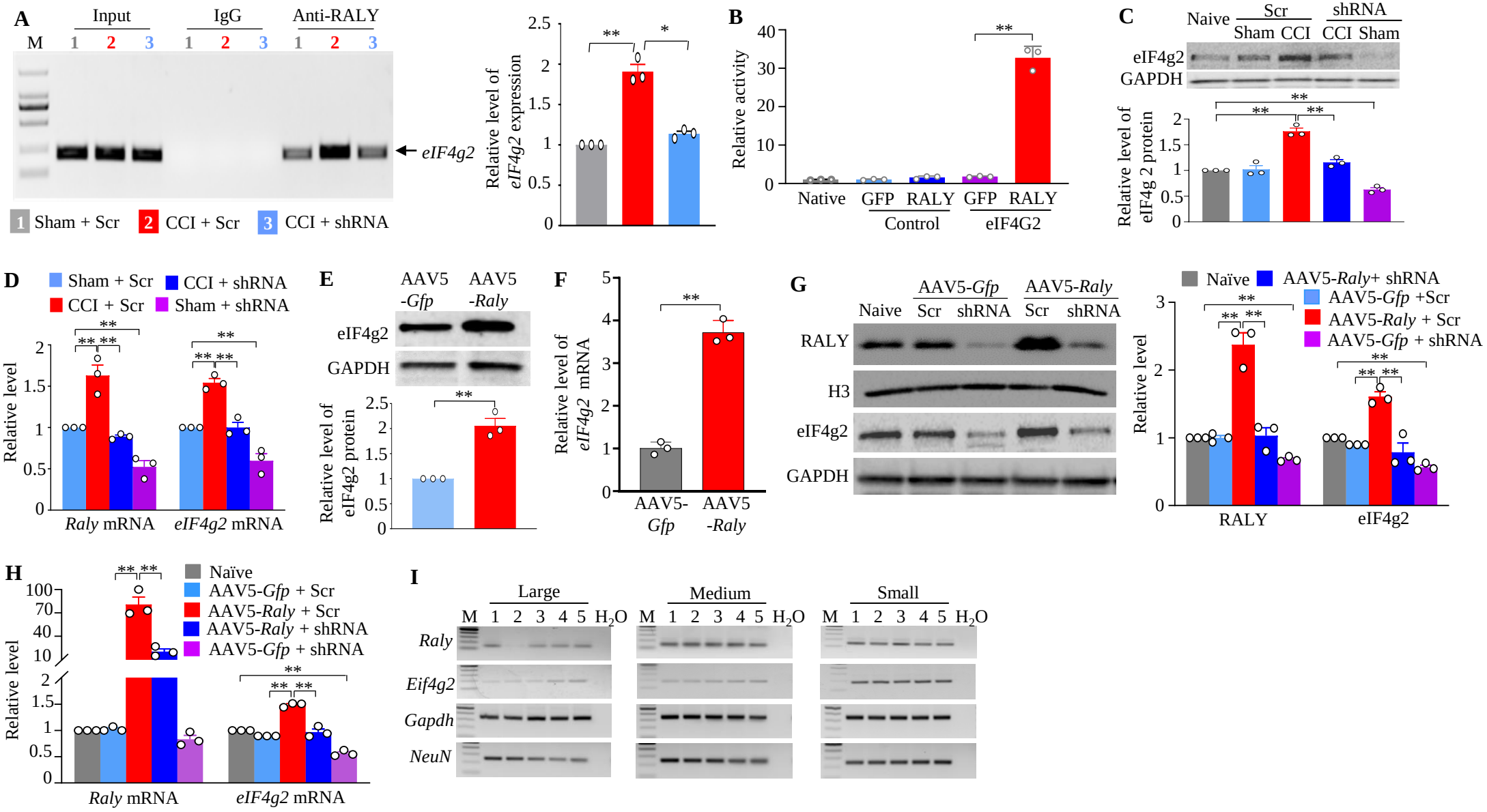

A
0.07g
Ipsilateral
Paw withdrawal frequency (%)
AAV5-Gfp on ipsilateral side
B
0.4g
Ipsilateral
Paw withdrawal frequency (%)
AAV5-Raly on the ipsilateral side
C
Heat
Ipsilateral
Paw withdrawal latency (s)
AAV5-Raly on the contralateral side
D
Cold
Ipsilateral
Paw withdrawal latency (s)
AAV5-Gfp on the contralateral side
Weeks after microinjection
E
AAV5-Gfp
AAV5-Raly
Time (s)
Pre
Post
Pre
Post
AAV5-Gfp
AAV5-Raly
F
Difference scores (s) (test-pre)
AAV5 -Gfp
AAV5 -Raly
G
AAV5 -Gfp
AAV5 -Raly
RALY
H3
Relative level of RALY protein
AAV5-Gfp
AAV5-Raly
H
AAV5 -Gfp
AAV5 -Raly
pERK1/2
ERK1/2
GFAP
GAPDH
I
AAV5-Gfp
AAV5-Raly
Relative level
pERK1
pERK2
ERK1
ERK2
GFAP

Fig. 8


A
Input
IgG
Anti-RALY
M 1 2 3 1 2 3 1 2 3
eIF4g2
1 Sham + Scr
2 CCI + Scr
3 CCI + shRNA
Relative level of eIF4g2 expression
B
Relative activity
Native
GFP RALY GFP RALY
Control
eIF4G2
C
Naive
Scr
Sham CCI
shRNA
CCI Sham
eIF4g2
GAPDH
Relative level of eIF4g 2 protein
D
Sham + Scr
CCI + shRNA
CCI + Scr
Sham + shRNA
Relative level
Raly mRNA
eIF4g2 mRNA
E
AAV5 -Gfp
AAV5 -Raly
eIF4g2
GAPDH
Relative level of eIF4g2 protein
F
Relative level of eIF4g2 mRNA
AAV5- Gfp
AAV5 -Raly
G
AAV5-Gfp
AAV5-Raly
Naive
Scr
shRNA
Scr
shRNA
RALY
H3
eIF4g2
GAPDH
Naïve
AAV5-Raly+ shRNA
AAV5-Gfp +Scr
AAV5-Raly + Scr
AAV5-Gfp + shRNA
Relative level
RALY
eIF4g2
H
Naïve
AAV5-Gfp + Scr
AAV5-Raly + Scr
AAV5-Raly + shRNA
AAV5-Gfp + shRNA
Relative level
Raly mRNA
eIF4g2 mRNA
I
Large
Medium
Small
M 1 2 3 4 5 H2O
Raly
Eif4g2
Gapdh
NeuN