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# FIGURE LEGENDS

**Figure 1**

Inhibition of HCV replication in Huh7/Rep-Feo cells by griseofulvin. (A) Huh7/Rep-Feo cells were cultured with various concentrations of griseofulvin in the medium and luciferase assays were performed after 72 hours of culture. Luciferase assays were performed in triplicate. Error bars indicate means ± standard deviation (SD). (B) Huh7/Rep-Feo cells were treated with various concentration of griseofulvin (2.5 to 40 μM). Luciferase activity was measured at the time points indicated after exposure to griseofulvin. (C) MTS of Huh7/Rep-Feo cells cultured with the concentration of griseofulvin indicated.

**Figure 2**

The suppressive effect of griseofulvin for HCV replicon was confirmed by real-time RT-PCR and western blot analysis. (A) Incubation of Huh7/Rep-Feo cells with griseofulvin for 72 hours resulted in dose dependent antiviral effects. Real-time RT-PCR was performed on the extracted RNA. HCV RNA levels are shown as relative percentages of untreated control. Error bars indicate means ± SD. (B) Western blot

analyses of NS3 and NS5A protein expression were performed on protein extracts from cells that were treated for 72 hours with varying dose of griseofulvin. β-actin was used as a loading control.

**Figure 3**

No inhibition of firefly luciferase activity by griseofulvin. The pEF Fluc IN vector was stably transfected into Hun7 cells. The cells were cultured without (Control) and with 20 μM and 40 μM griseofulvin for 72 hours. Firefly luciferase assay was performed. Luciferase activity was normalized by the protein concentration. Error bars indicate means ± SD.

**Figure 4**

Griseofulvin did elicit an IFN response. Huh7/Rep-Feo cells were treated without (lane 1) or with 1, 10, 100 U/ml IFNα-2b (lane 2~4) and 20 (lane 5) or 80 μM griseofulvin (lane 6) for 72 hours. The messenger RNAs of MxA (upper panel), 2', 5'-oligoadenylate synthetase (2', 5'-OAS) (middle panel), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control (lower panel) were detected by RT-PCR analysis.

**Figure.5**

Effect of a combination of griseofulvin and IFN-α on intracellular HCV RNA replication. (A) A representative isobologram for analyzing interaction between two drugs. (B) Isobole plot of 50% inhibition of HCV RNA replication. Huh7/Rep-Feo cells were treated with griseofulvin in combination with IFN-α and a luciferase assay was performed after 72 hours of culture to obtain each isobole plot. The dotted line indicates an additive effect in the isobologram method used.

**Figure 6**

Griseofulvin induced G2/M phase arrest in Huh7/Rep-Feo cells. Flow cytometry analysis of DNA content of untreated Huh7/Rep-Feo cells (Control) and cells treated for 12 hours with 20 μM of griseofulvin.

**Figure 7**

The growth kinetics of griseofulvin treatment Huh7/Rep-Feo cells. The cells were cultured with 20μM griseofulvin, and cell viability was monitored by MTS assay at the times indicated. Error bars indicate means ± SD.

**Figure 8**

Griseofulvin does not influence HCV IRES-mediated translation. (A) Structure of the plasmid, pEF-Rluc-HCV IRES Feo. Transcription is initiated under the control of a composite Elongation factor 1 alpha (EF1α) promoter. The upstream cistron encodes *Renilla* luciferase (Rluc) and is translated by a cap-dependent mechanism in transfected cells, while the downstream cistron encodes a fusion (Feo) of the firefly luciferase (Fluc) and neomycin phosphotransferase ($Neo^r$) genes, translated under the control of the HCV IRES. (B) pEF-Rluc-HCV IRES Feo was stably transfected into Huh7 cells. The cells were treated without (Control) and with 20 μM of griseofulvin. Dual luciferase activities were measured at the indicated time points after exposure to griseofulvin. Values are displayed as ratios of Fluc to Rluc. Error bars indicate means ± SD.

**Figure 9**

Griseofulvin suppresses JFH-1 replication. Immunofluorescent staining of Huh 7.5.1/JFH-1 cells treated with various concentrations of griseofulvin. HCV NS3 protein is stained green and nuclei are stained with 7-AAD (red).

Fig.1

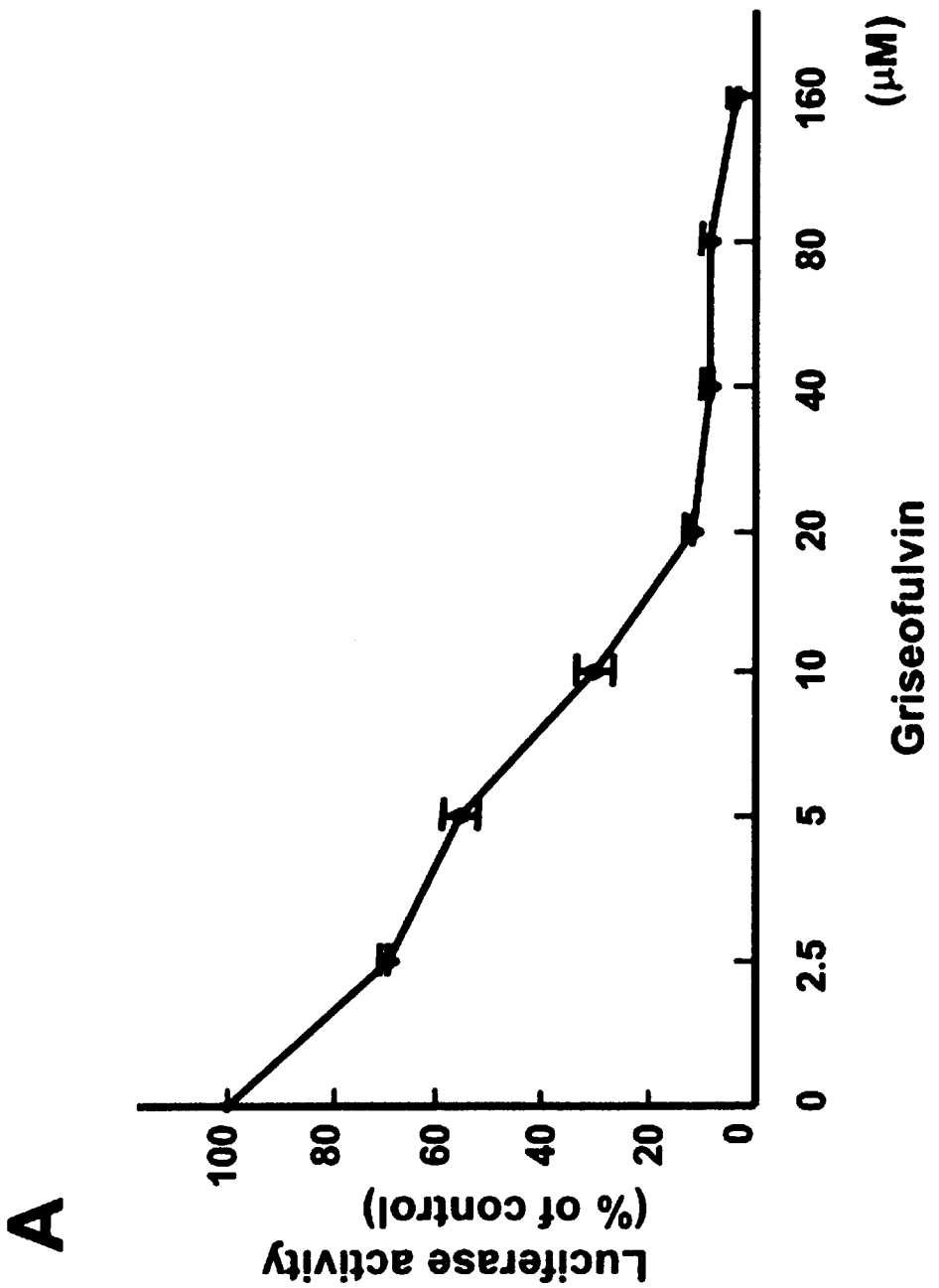

A
Luciferase activity
(% of control)
100
80
60
40
20
0
0
2.5
5
10
20
40
80
160
(μM)
Griseofulvin

Fig.1

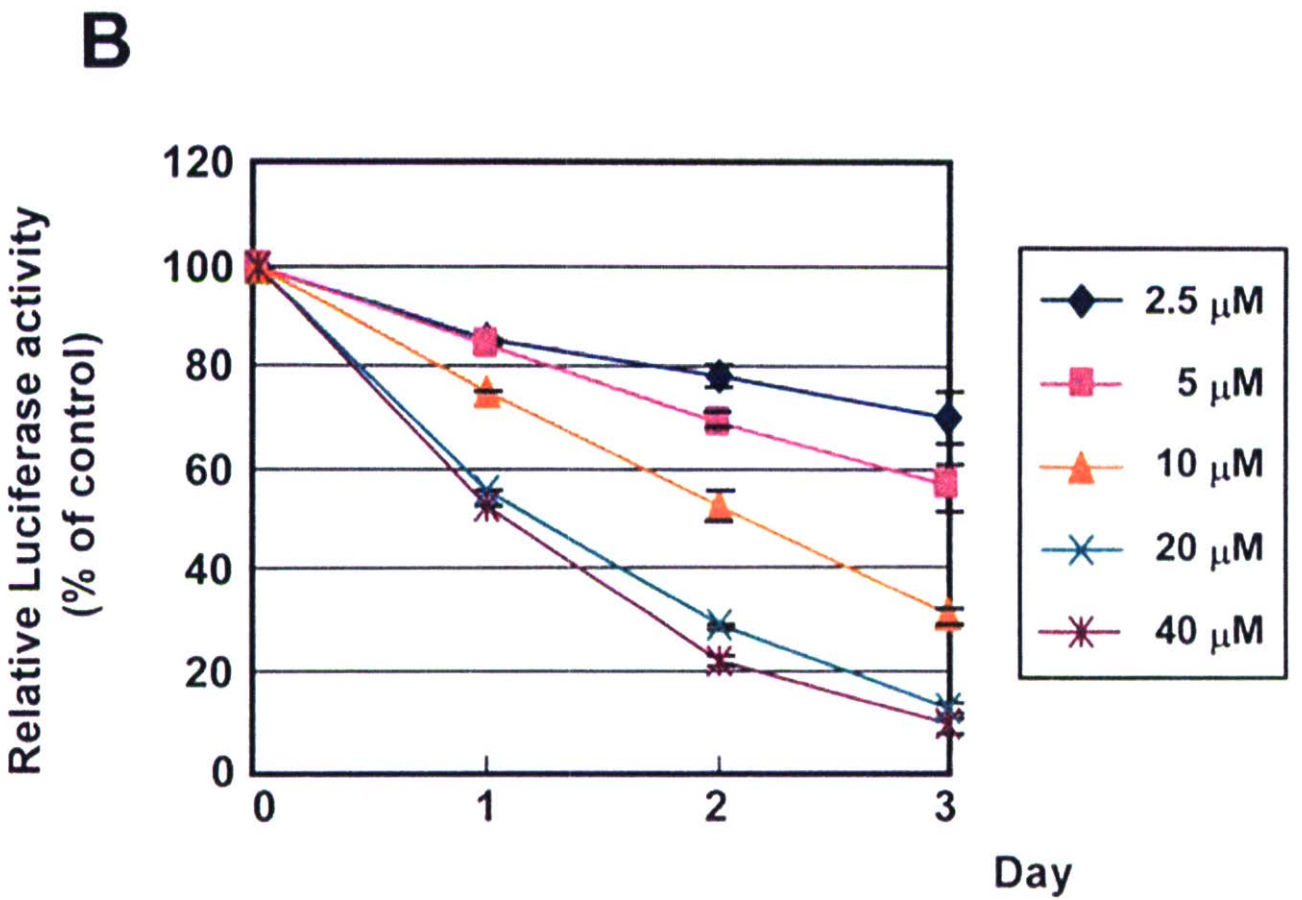

B
Relative Luciferase activity
(% of control)
120
100
80
60
40
20
0
0
1
2
3
Day
2.5 μM
5 μM
10 μM
20 μM
40 μM

Fig.1

C

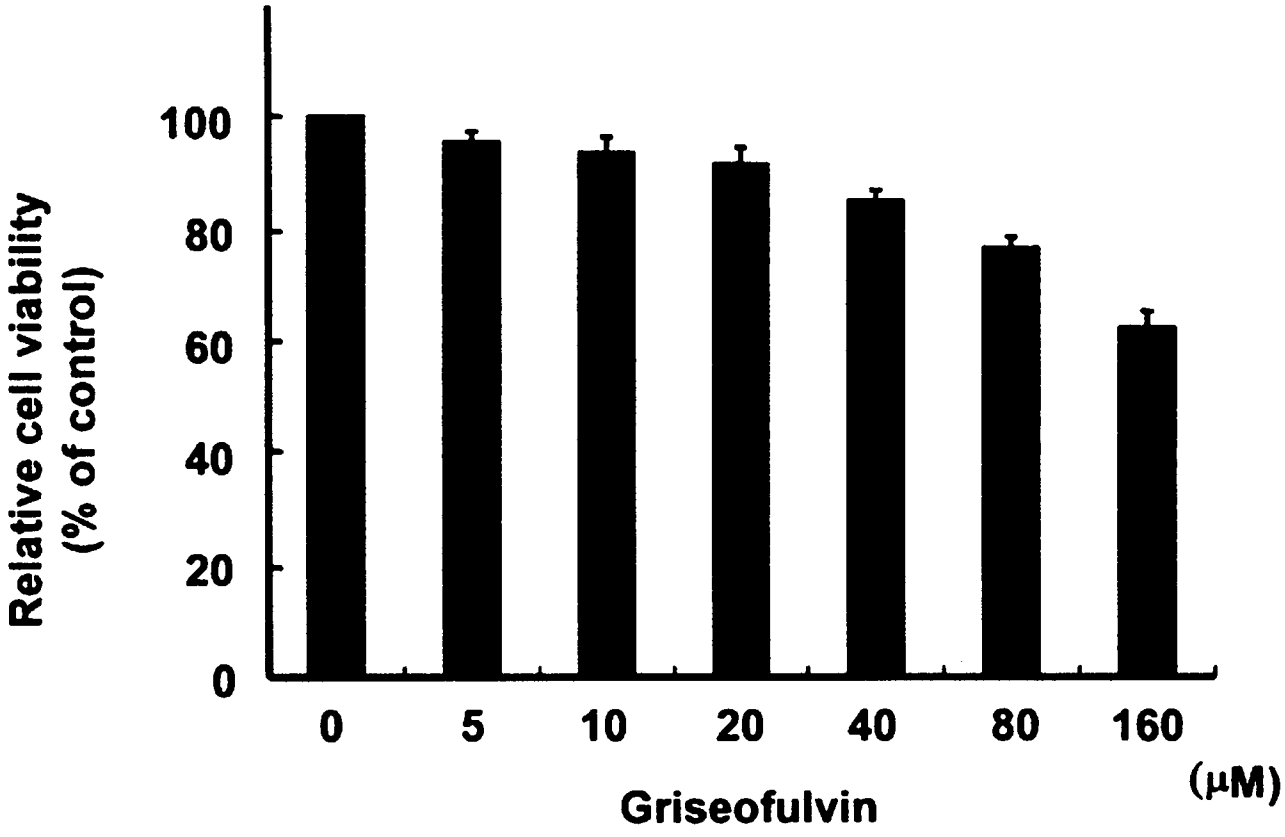

Relative cell viability
(% of control)
100
80
60
40
20
0
0
5
10
20
40
80
160
(μM)
Griseofulvin

Fig.2

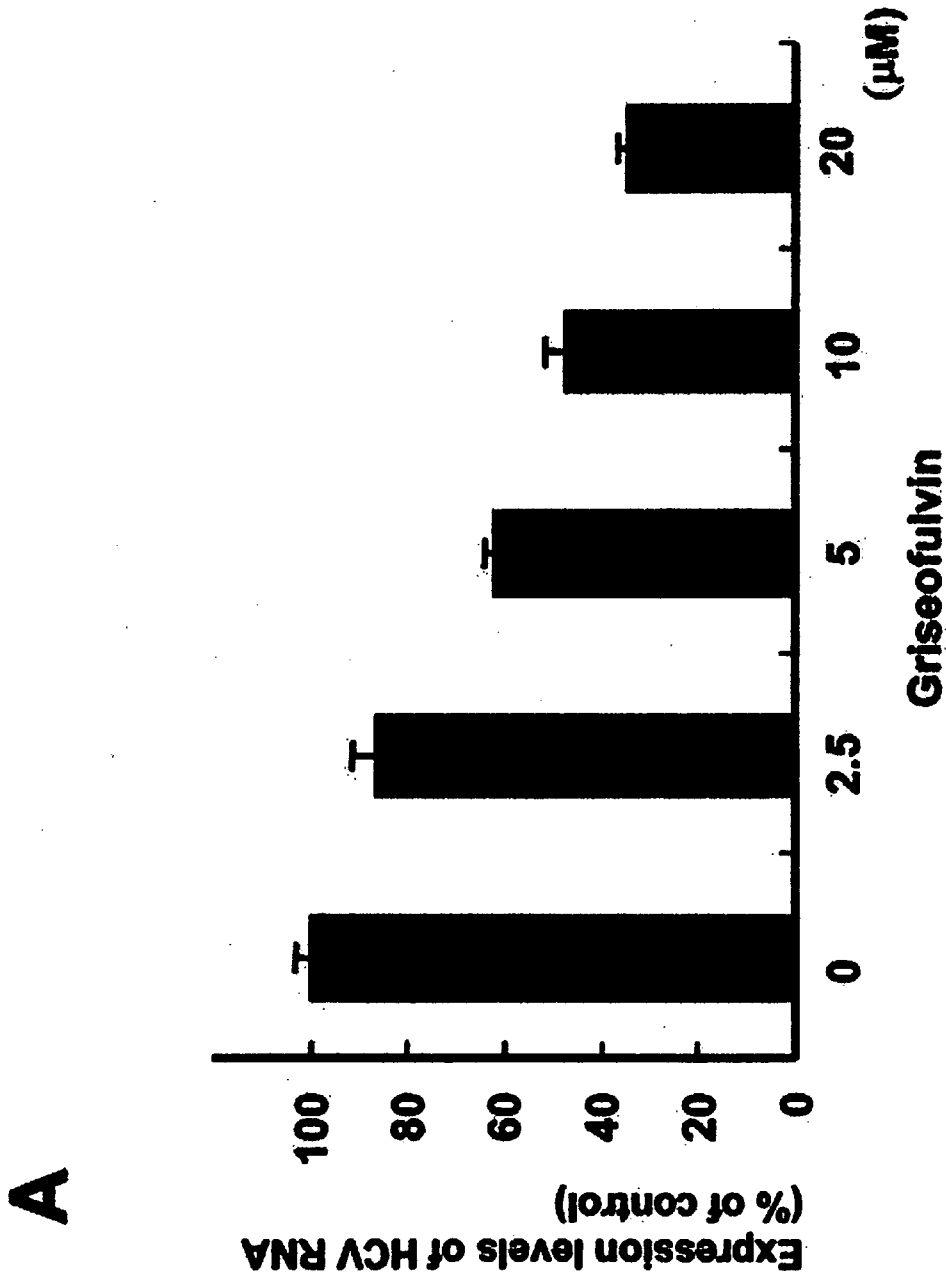

A
Expression levels of HCV RNA
(% of control)
100
80
60
40
20
0
0
2.5
5
10
20
(μM)
Griseofulvin

Fig.2

B

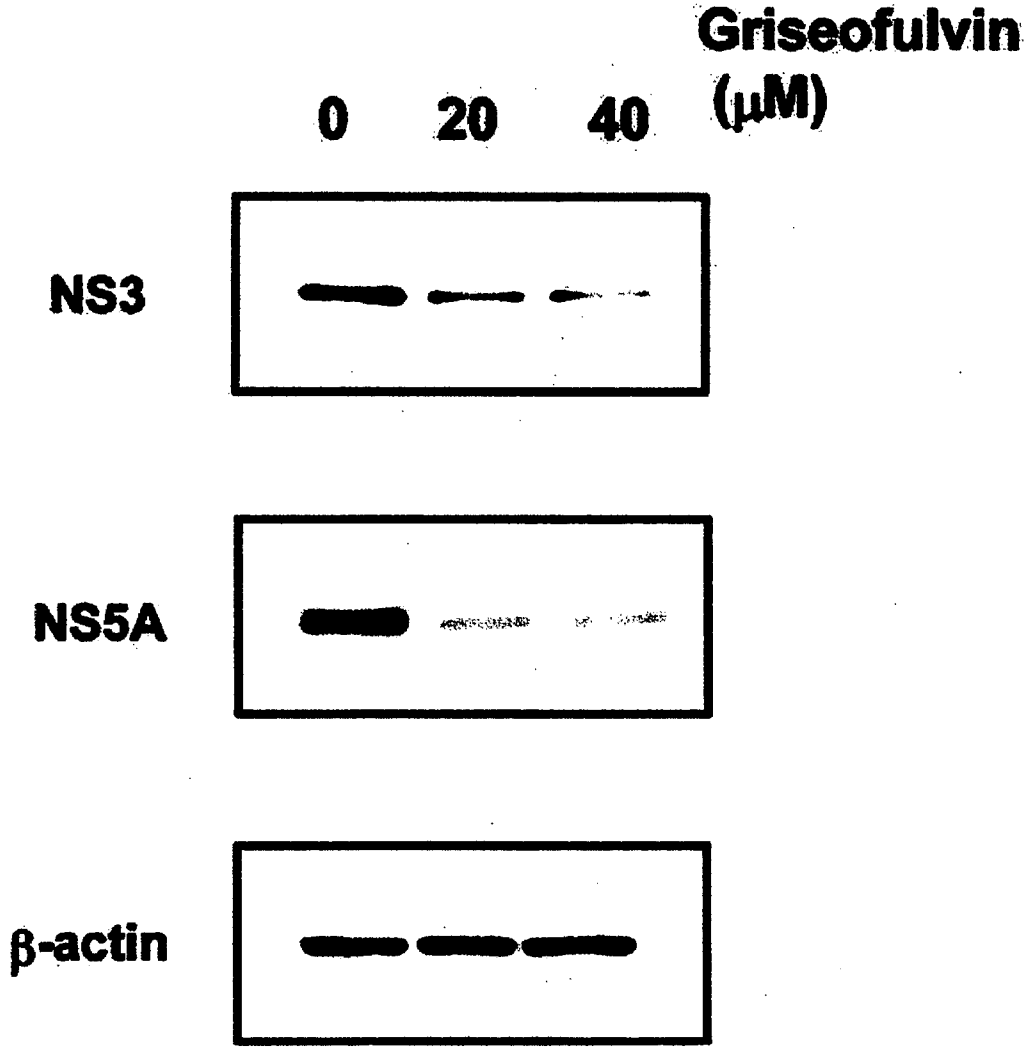


Fig.3

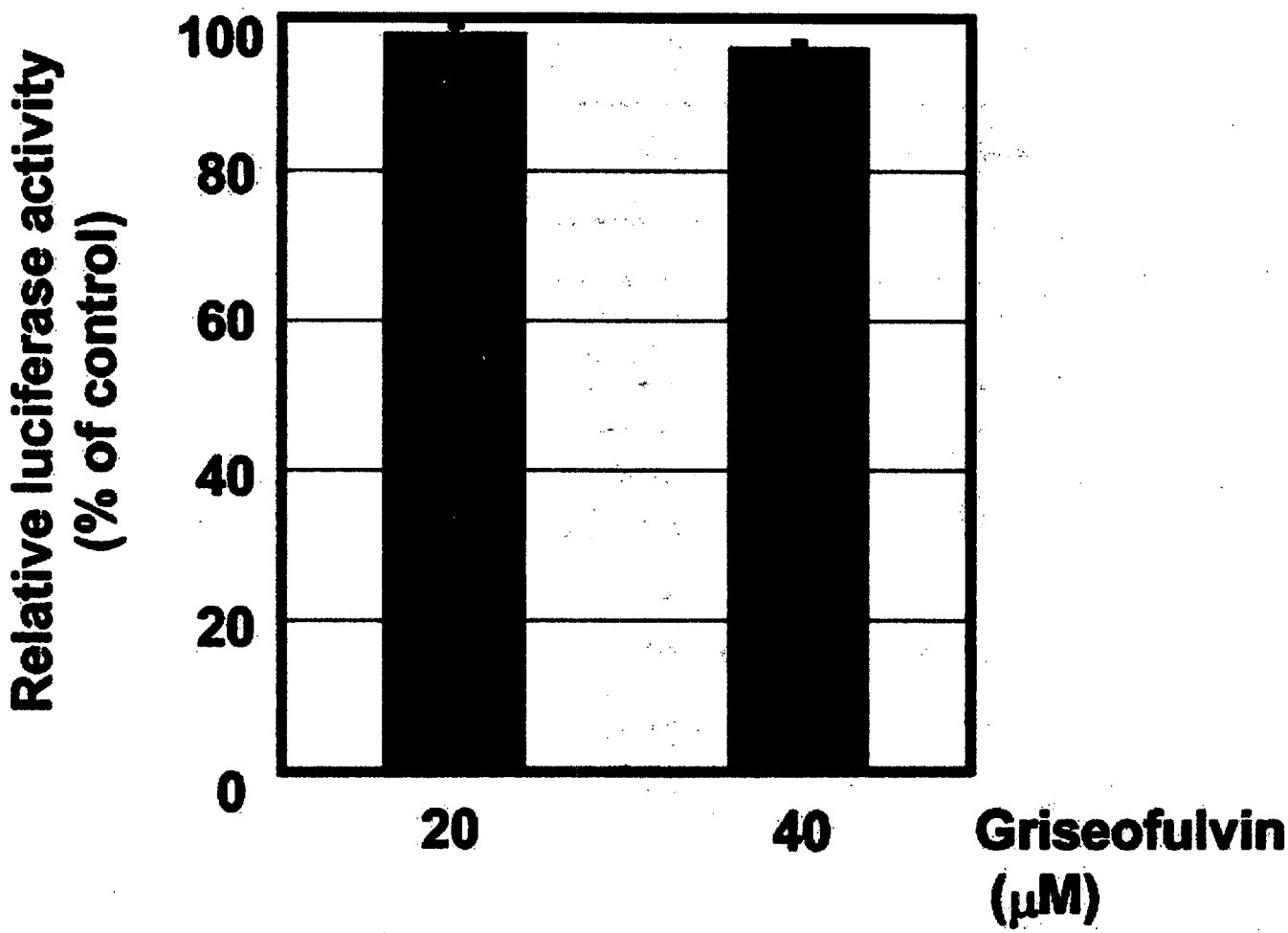

Relative luciferase activity
(% of control)
100
80
60
40
20
0
20
40
Griseofulvin
(μM)

Fig.4

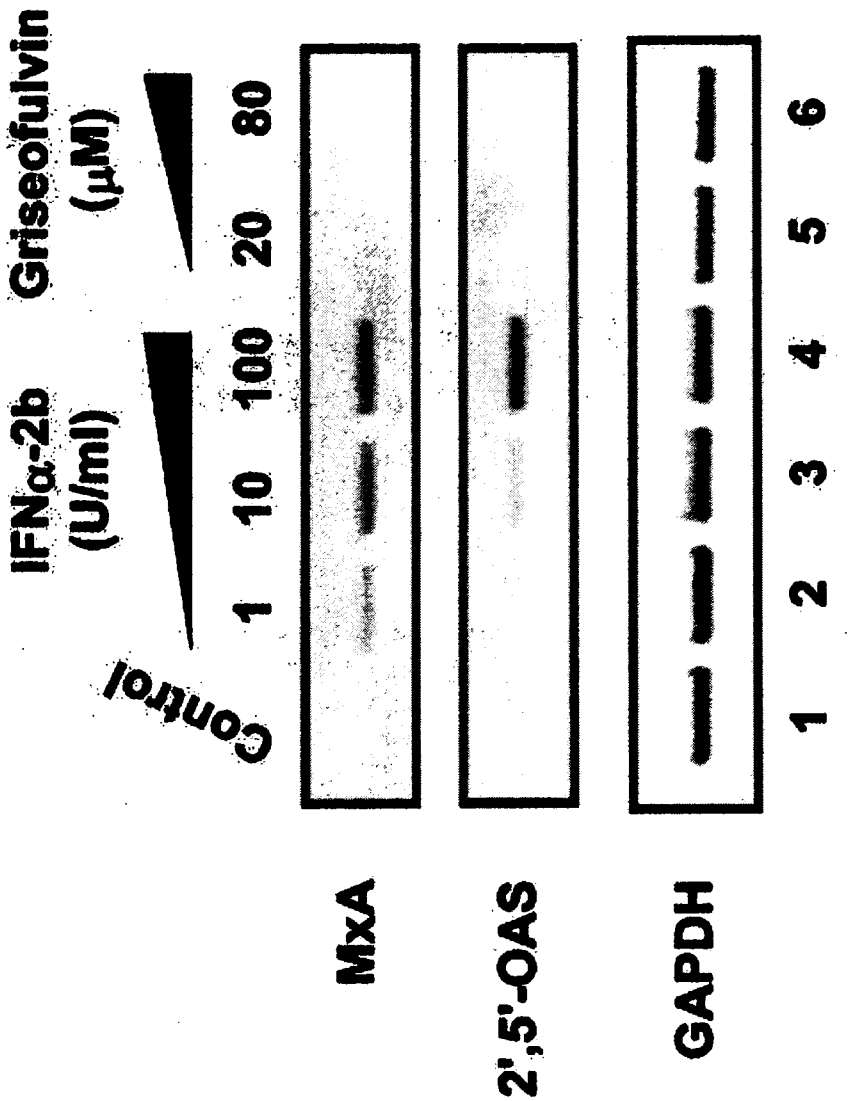

Control
IFNα-2b (U/ml)
1
10
100
Griseofulvin (μM)
20
80
MxA
2',5'-OAS
GAPDH
1
2
3
4
5
6

Fig.5

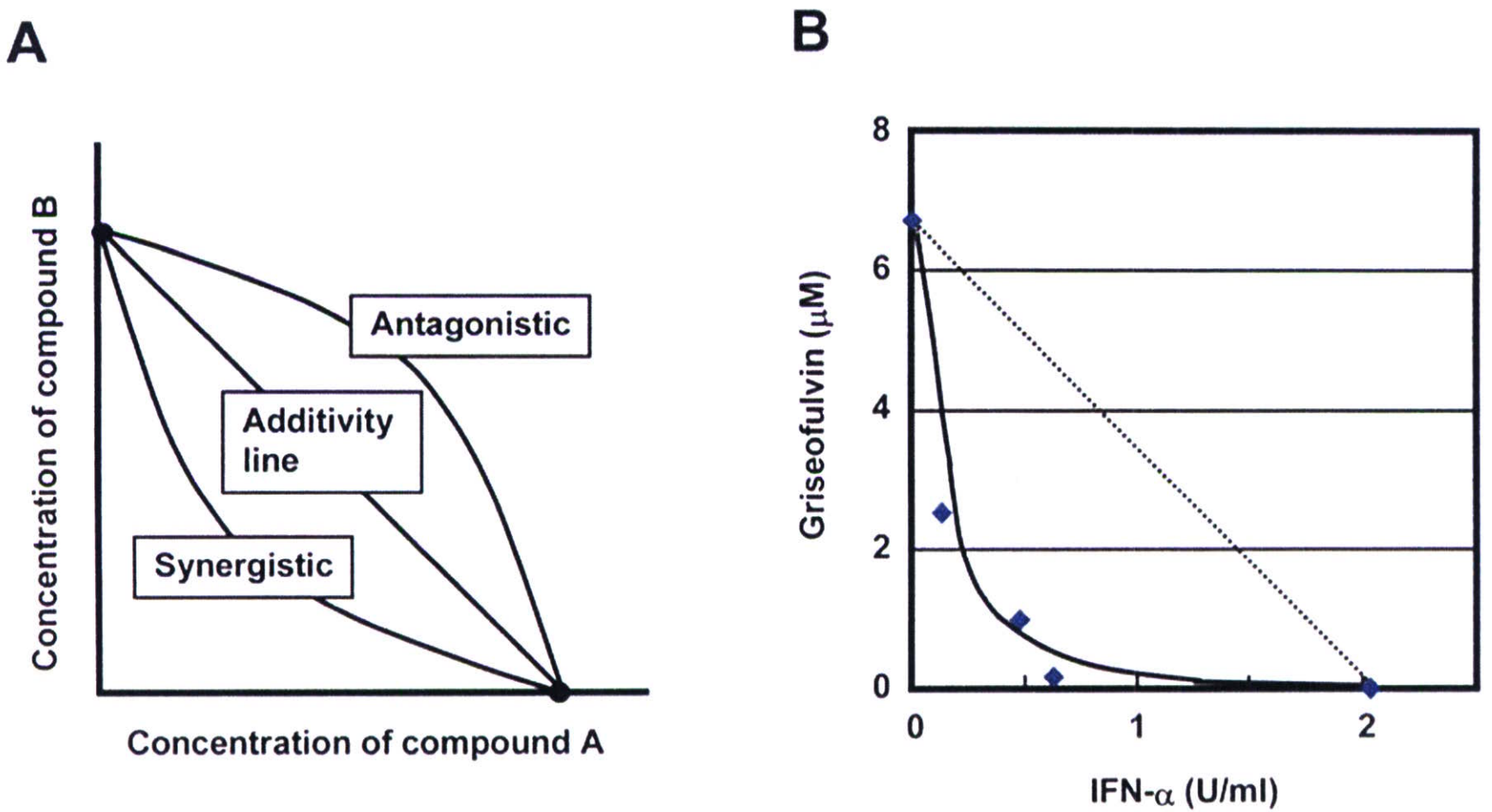
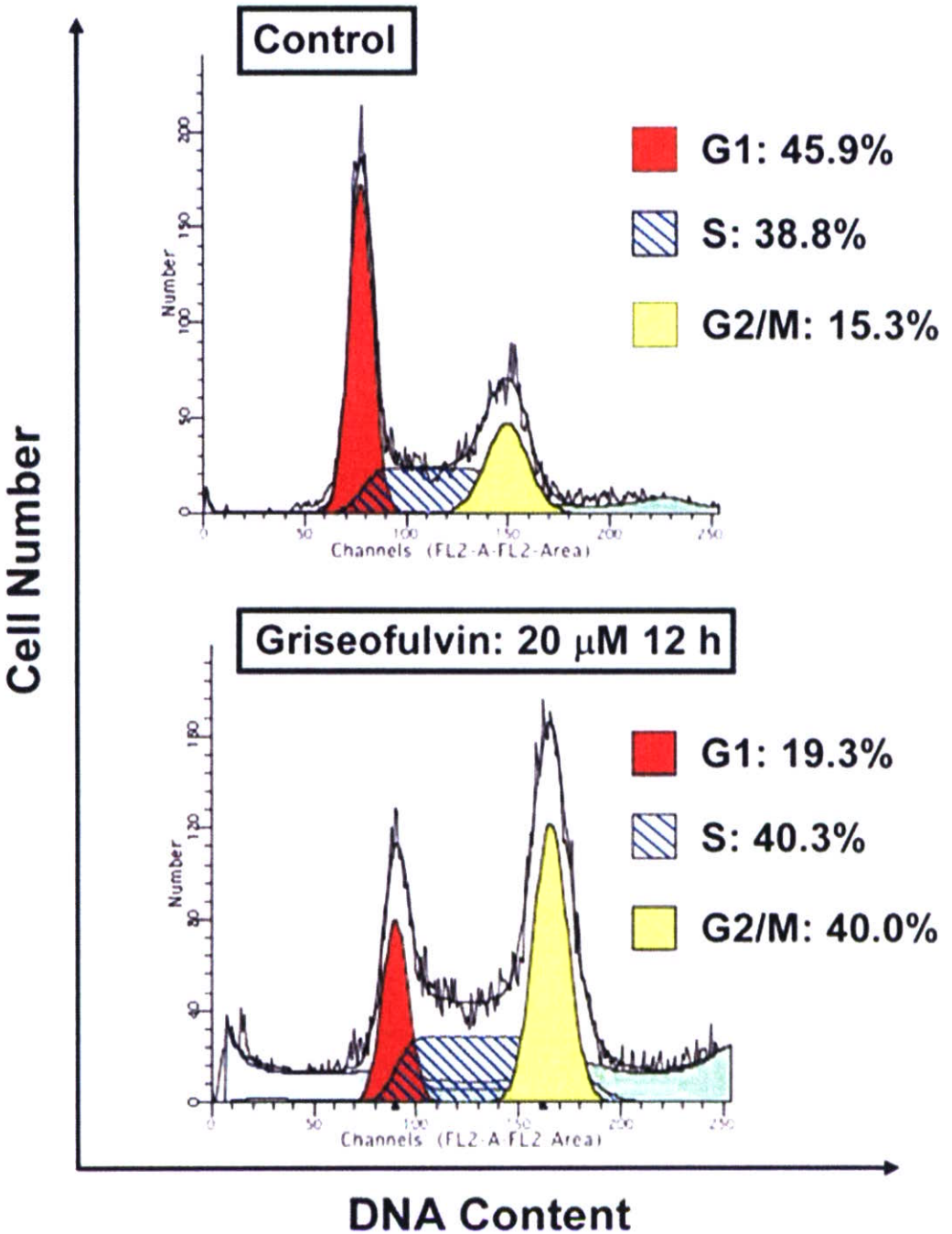
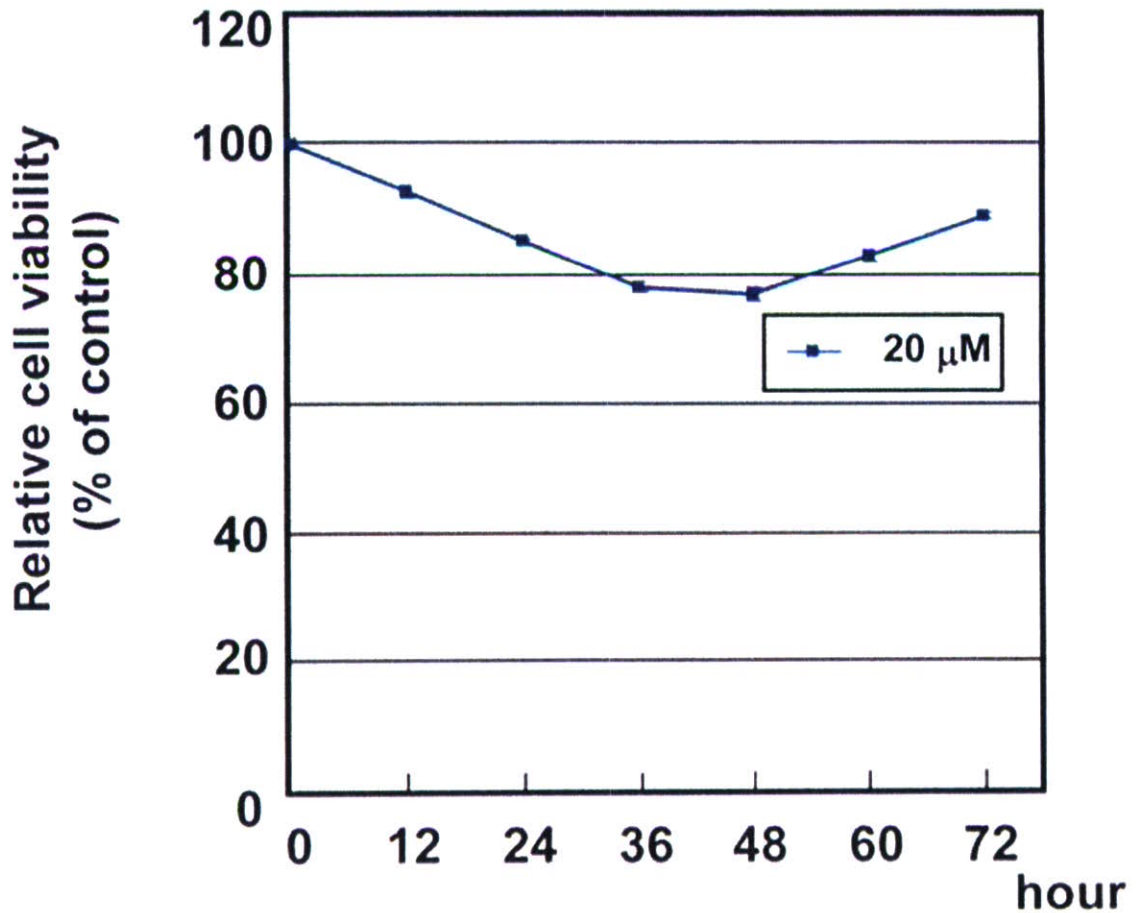

A
Concentration of compound B
Antagonistic
Additivity line
Synergistic
Concentration of compound A
B
Griseofulvin (μM)
8
6
4
2
0
0
1
2
IFN-α (U/ml)

Fig.6


Control
G1: 45.9%
S: 38.8%
G2/M: 15.3%
Number
Channels (FL2-A FL2-Area)
Griseofulvin: 20 μM 12 h
G1: 19.3%
S: 40.3%
G2/M: 40.0%
Number
Channels (FL2-A FL2-Area)
Cell Number
DNA Content

691

Fig.7


Relative cell viability
(% of control)
120
100
80
60
40
20
0
0
12
24
36
48
60
72
hour
20 μM

692

Fig.8

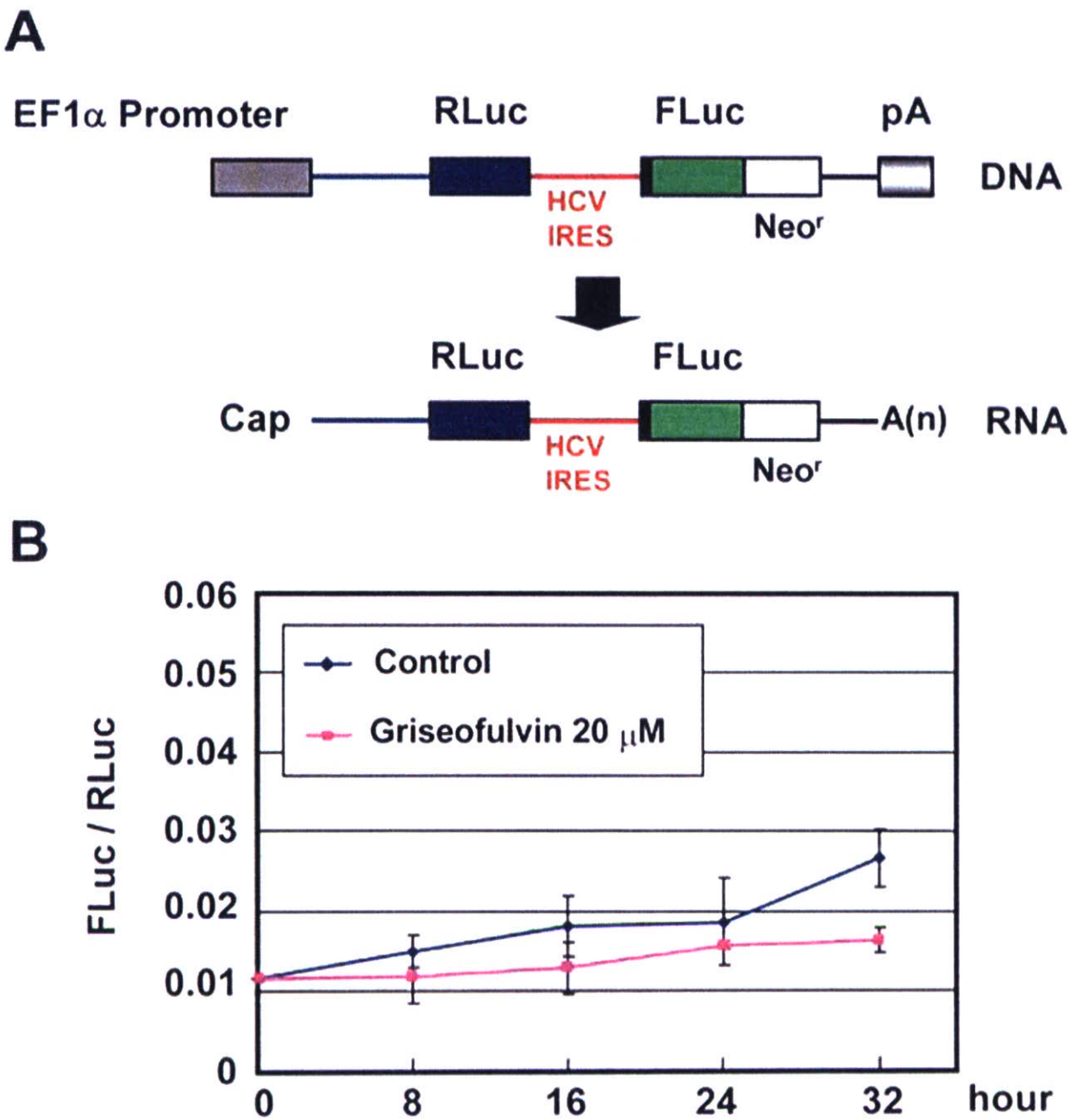
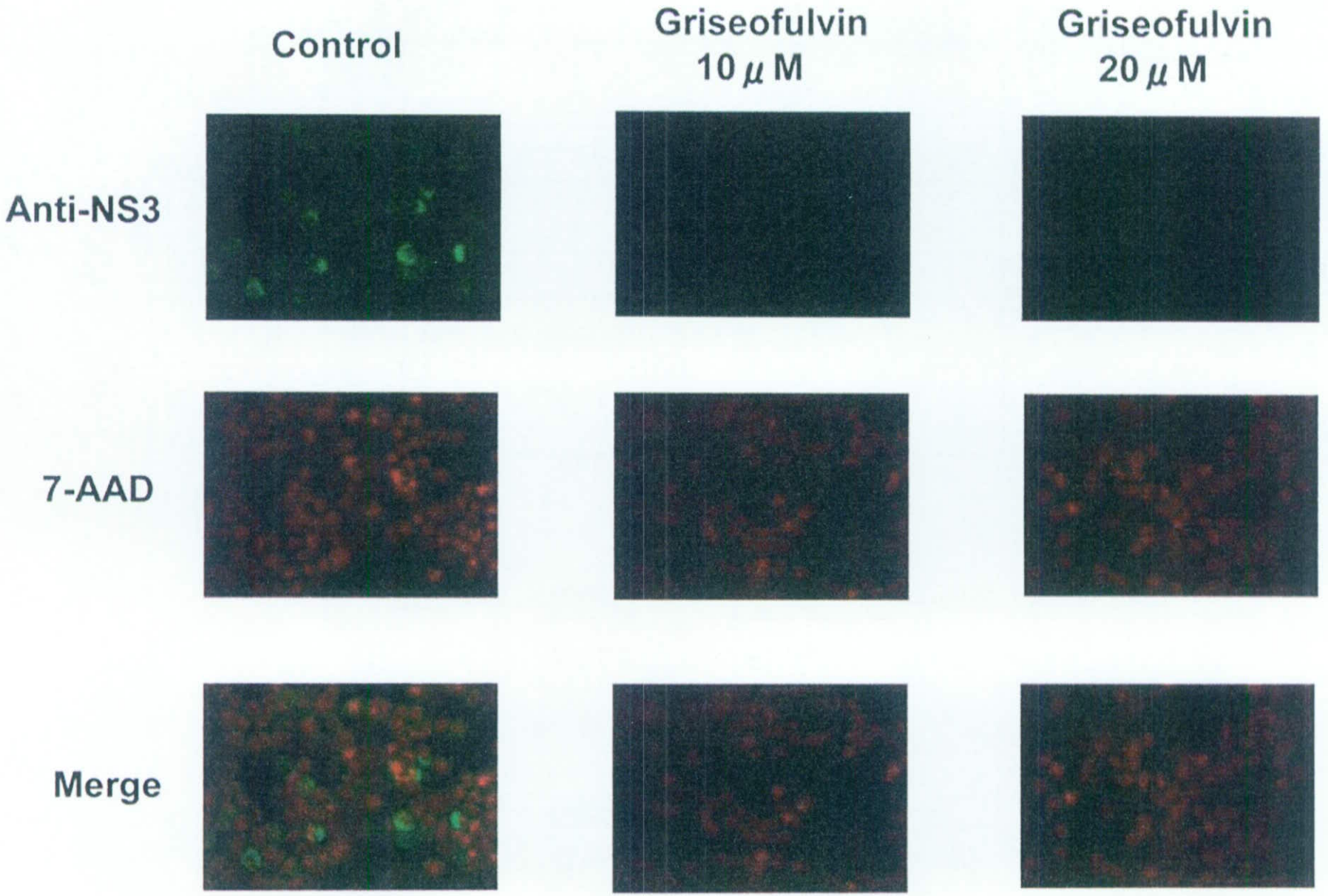

A
EF1α Promoter
RLuc
FLuc
pA
DNA
HCV
IRES
Neor
RLuc
FLuc
Cap
A(n)
RNA
HCV
IRES
Neor
B
FLuc / RLuc
0.06
0.05
0.04
0.03
0.02
0.01
0
Control
Griseofulvin 20 μM
0
8
16
24
32
hour

Fig.9


Control
Griseofulvin
10 μ M
Griseofulvin
20 μ M
Anti-NS3
7-AAD
Merge