

8. Yokota T, Sakamoto N, Enomoto N, et al. Inhibition of intracellular hepatitis C virus replication by synthetic and vector-derived small interfering RNAs. *EMBO Rep* 2003; **4**: 602-8.
9. Lindenbach BD, Evans MJ, Syder AJ, et al. Complete replication of hepatitis C virus in cell culture. *Science* 2005; **309**: 623-6.
10. Wakita T, Pietschmann T, Kato T, et al. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat Med* 2005; **11**: 791-6.
11. Zhong J, Gastaminza P, Cheng G, et al. Robust hepatitis C virus infection in vitro. *Proc Natl Acad Sci U S A* 2005; **102**: 9294-9.
12. Hinrichsen H, Benhamou Y, Wedemeyer H, et al. Short-term antiviral efficacy of BILN 2061, a hepatitis C virus serine protease inhibitor, in hepatitis C genotype 1 patients. *Gastroenterology* 2004; **127**: 1347-55.
13. Lamarre D, Anderson PC, Bailey M, et al. An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus. *Nature* 2003; **426**: 186-9.
14. Reesink HW, Zeuzem S, Weegink CJ, et al. Rapid decline of viral RNA in hepatitis C patients treated with VX-950: a phase Ib, placebo-controlled, randomized study. *Gastroenterology* 2006; **131**: 997-1002.
15. Sarrazin C, Rouzier R, Wagner F, et al. SCH 503034, a novel hepatitis C virus

- protease inhibitor, plus pegylated interferon alpha-2b for genotype 1 nonresponders. *Gastroenterology* 2007; **132**: 1270-8
16. Amemiya F, Maekawa S, Itakura Y, et al. Targeting lipid metabolism in the treatment of hepatitis C. *J Infect Dis* in press.
17. Martell M, Gomez J, Esteban JI, et al. High-throughput real-time reverse transcription-PCR quantitation of hepatitis C virus RNA. *J Clin Microbiol* 1999; **37**:327-32.
18. Okada Y, Osada M, Kurata S, et al. p53 gene family p51(p63)-encoded, secondarytransactivator p51B(TAp63alpha) occurs without forming an immunoprecipitable complex with MDM2, but responds to genotoxic stress by accumulation. *Exp Cell Res* 2002; **276**:194-200.
19. Chou TC, Talaly P, A simple generalized equation for the analysis of multiple inhibitions of Michaelis-Menten kinetic systems. *J. Biol.Chem.* 1977; **252**: 6438-42.
20. Kitamura T, Koshino Y, Shibata F, et al. Retrovirus-mediated gene transfer and expression cloning: Powerful tools in functional genomics. *Exp Hematol* 2003; **31**:1007-1014.
21. Morita S, Kojima T, Kitamura T. Plat-E: an efficient and stable system for transient

- packaging of retroviruses. *Gene Ther* 2000; **7**:1063-6.
22. Blight KJ, Kolykhalov AA, Rice CM. Efficient initiation of HCV RNA replication in cell culture. *Science* 2000 **8**; **290**:1972-4.
23. Frese M, Pietschmann T, Moradpour D, et al. Interferon-alpha inhibits hepatitis C virus subgenomic RNA replication by an MxA-independent pathway. *J Gen Virol* 2001; **82**: 723-3.
24. Ho YS, Duh JS, Jeng JH, et al. Griseofulvin potentiates antitumorigenesis effects of nocodazole through induction of apoptosis and G2/M cell cycle arrest in human colorectal cancer cells. *Int J Cancer* 2001; **91**:393-401.
25. Bost AG, Venable D, Liu L, et al. Cytoskeletal requirements for hepatitis C virus (HCV) RNA synthesis in the HCV replicon cell culture system. *J Virol* 2003; **77**: 4401-8.
26. Honda M, Kaneko S, Matsushita E, et al. Cell cycle regulation of hepatitis C virus internal ribosomal entry site-directed translation. *Gastroenterology* 2000; **118**:152-62.
27. Venkatesan A, Sharma R, Dasgupta A. Cell cycle regulation of hepatitis C and encephalomyocarditis virus internal ribosome entry site-mediated translation in human embryonic kidney 293 cells. *Virus Res* 2003; **94**:85-95.
28. Nelson HB, Tang H. Effect of cell growth on hepatitis C virus (HCV) replication

- and a mechanism of cell confluence-based inhibition of HCV RNA and protein expression. *J Virol* 2006; **80**:1181-90.
29. Scholle F, Li K, Bodola F, Ikeda M, et al. Virus-host cell interactions during hepatitis C virus RNA replication: impact of polyprotein expression on the cellular transcriptome and cell cycle association with viral RNA synthesis. *J Virol* 2004; **78**:1513-24.
30. Panda D, Rathinasamy K, Santra MK, et al. Kinetic suppression of microtubule dynamic instability by griseofulvin: implications for its possible use in the treatment of cancer. *Proc Natl Acad Sci U S A* 2005; **102**:9878-883.

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 \pm standard deviation (SD). (B) Huh7/Rep-Feo cells were treated with various concentration of griseofulvin (2.5 to 40 μ M). 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 \pm 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 μ M and 40 μ M griseofulvin for 72 hours. Firefly luciferase assay was performed. Luciferase activity was normalized by the protein concentration. Error bars indicate means \pm 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 IFN α -2b (lane 2~4) and 20 (lane 5) or 80 μ M 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 μ M of griseofulvin.

Figure 7

The growth kinetics of griseofulvin treatment Huh7/Rep-Feo cells. The cells were cultured with 20 μ M griseofulvin, and cell viability was monitored by MTS assay at the times indicated. Error bars indicate means \pm 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 (EF1 α) 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 μ M 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 \pm 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

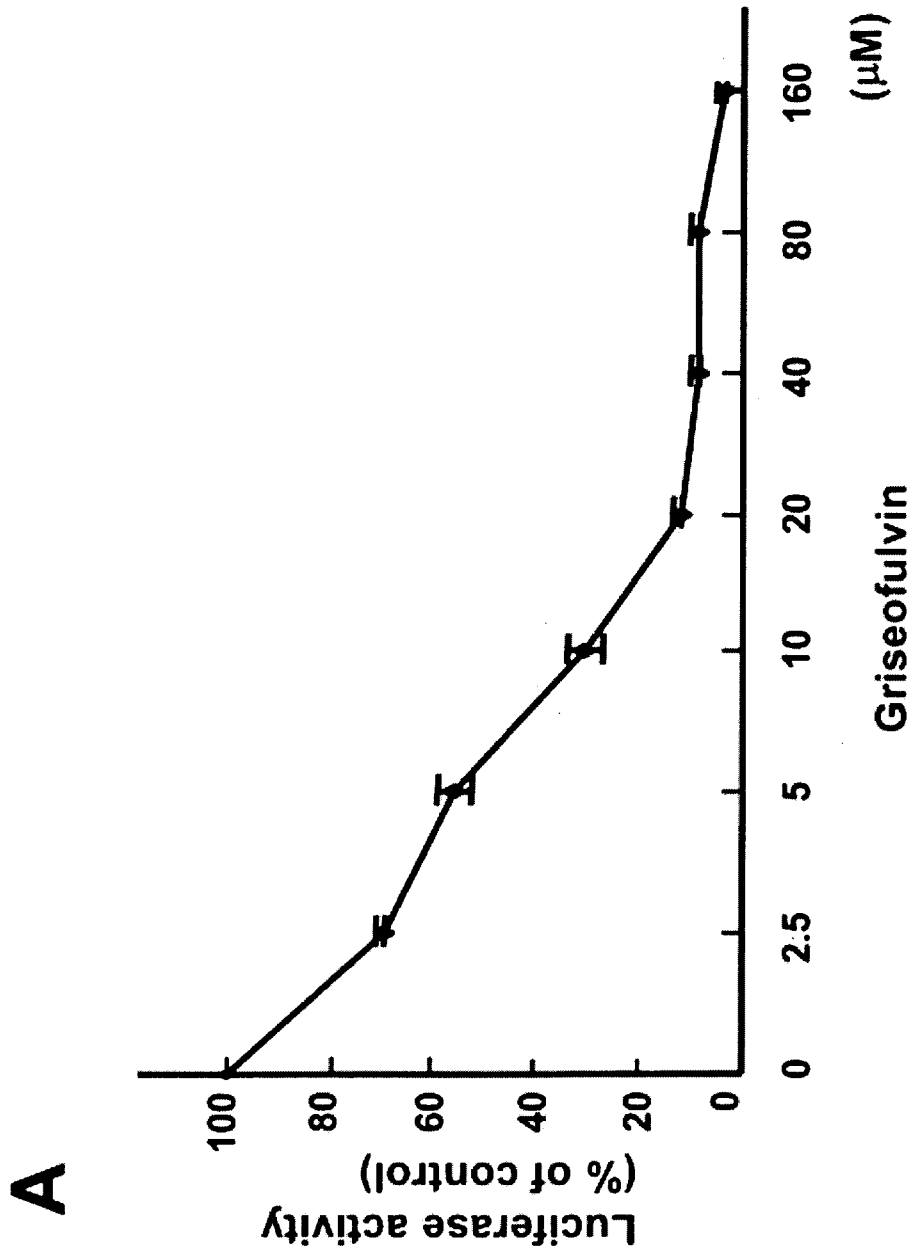


Fig.1

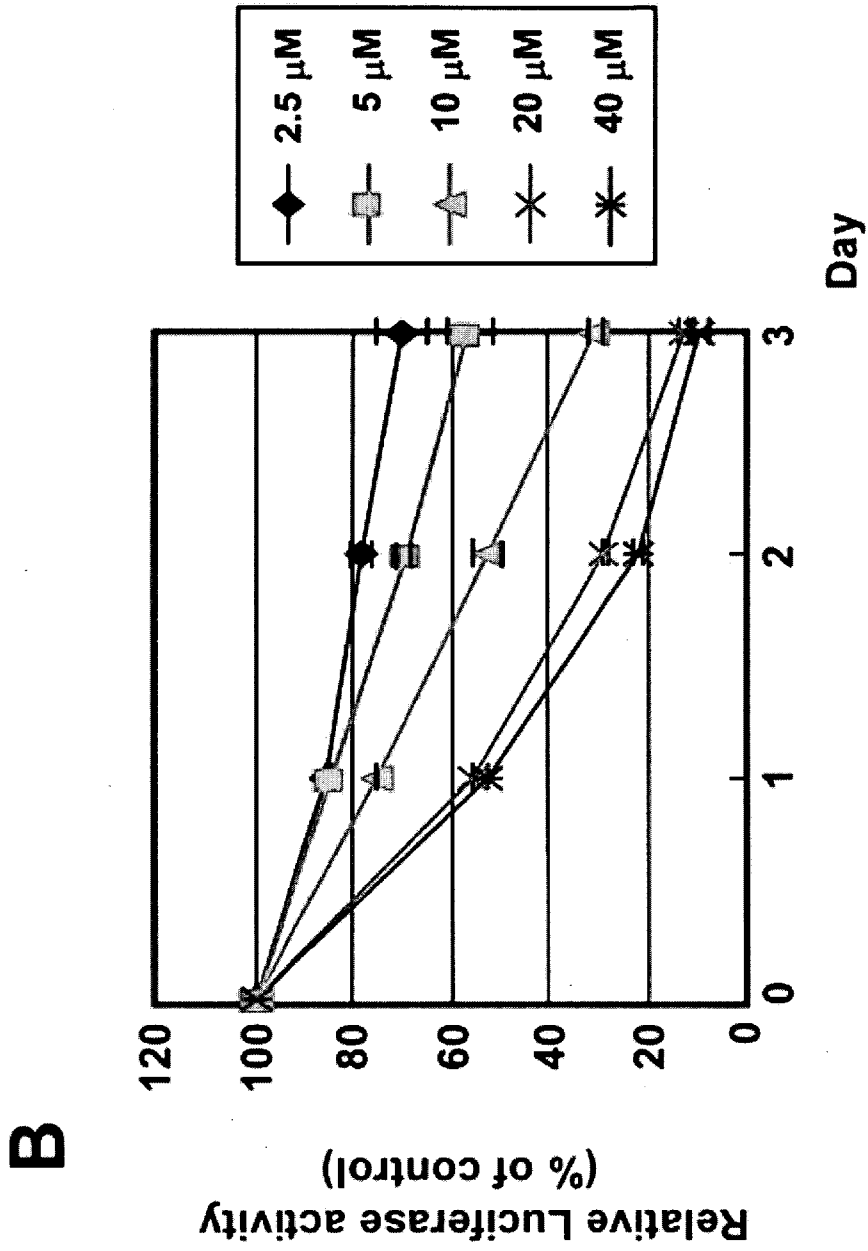


Fig.1

C

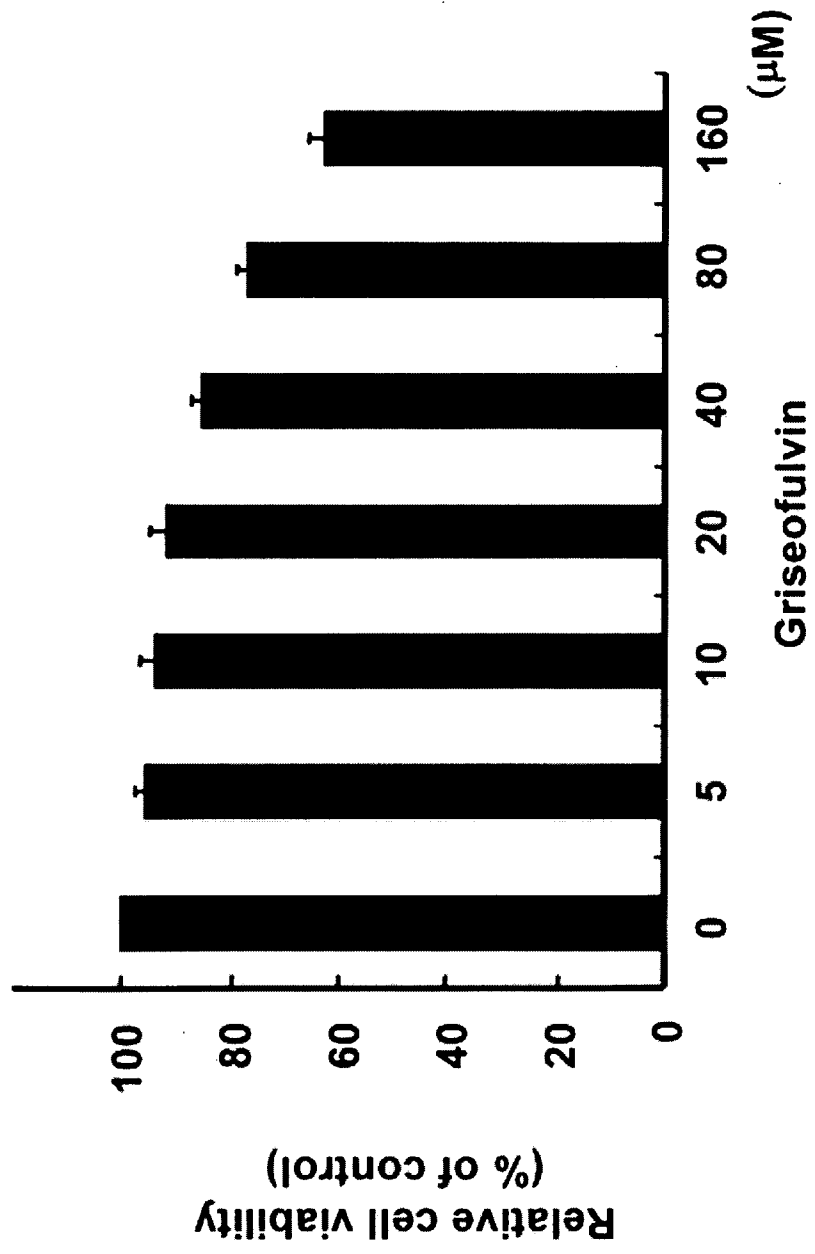


Fig.2

A

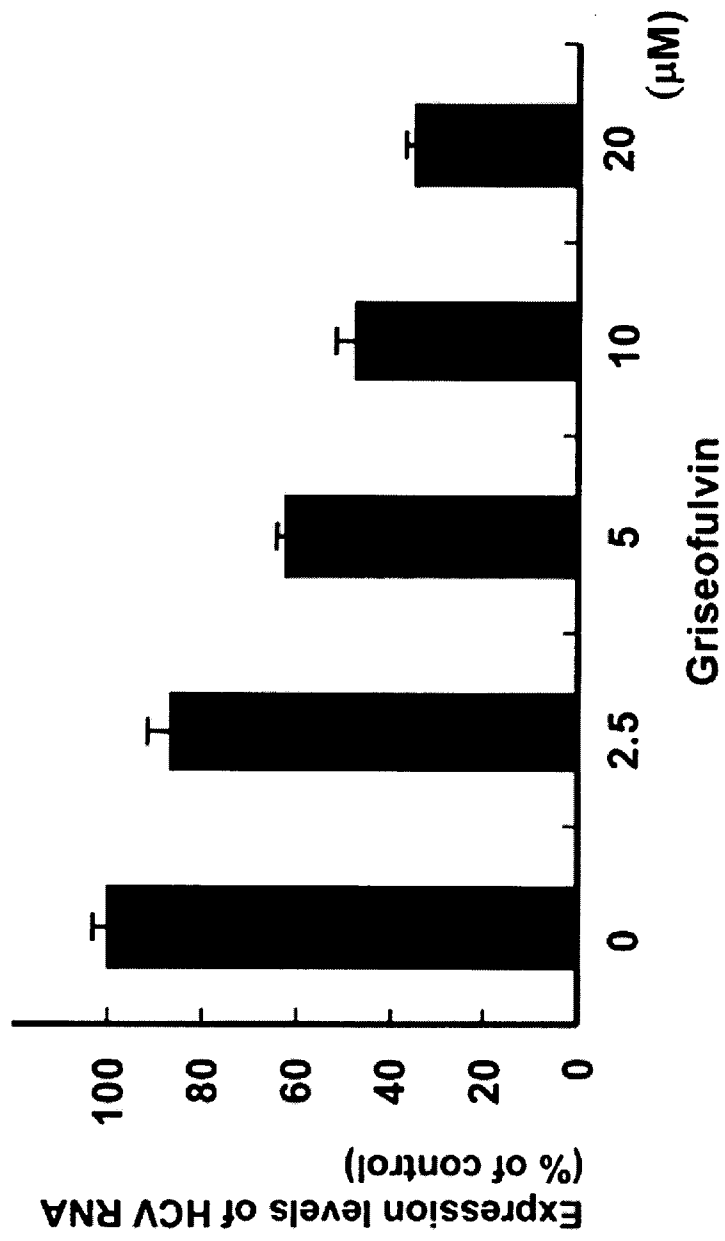


Fig.2

B

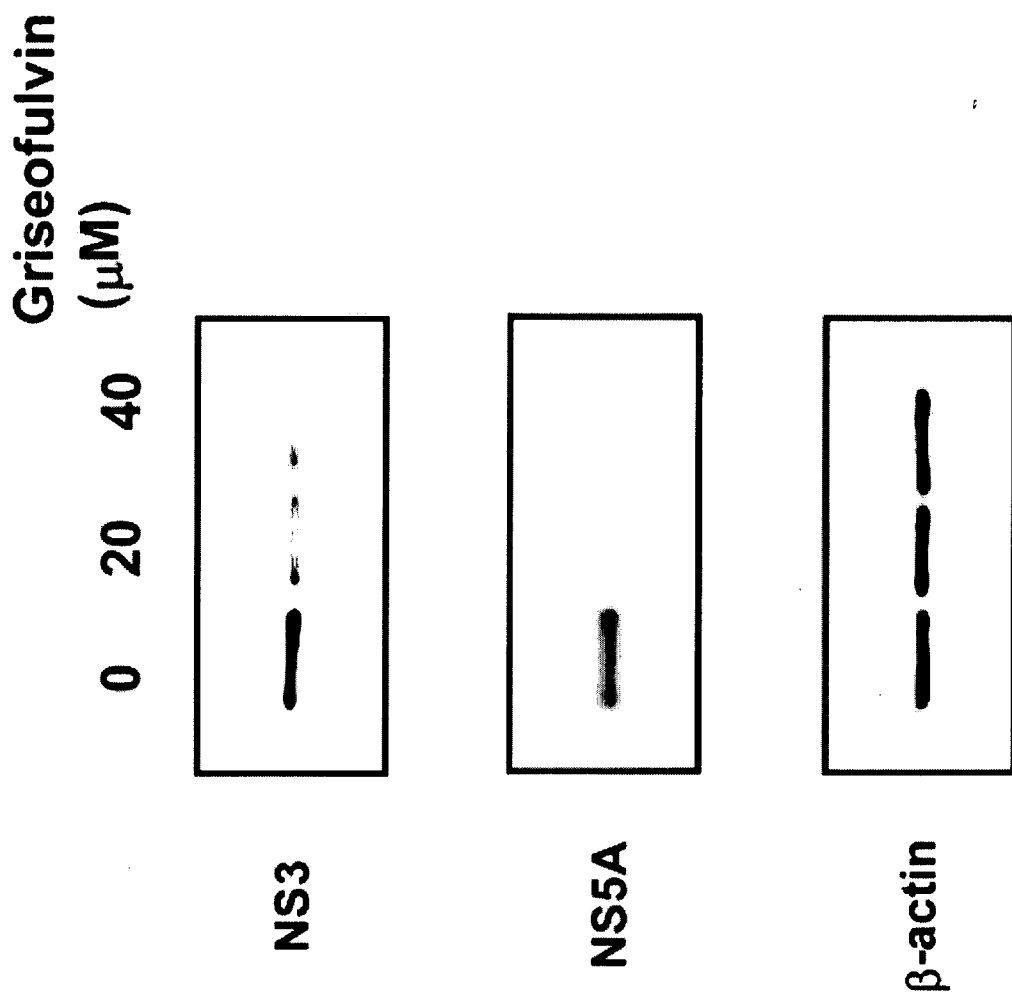


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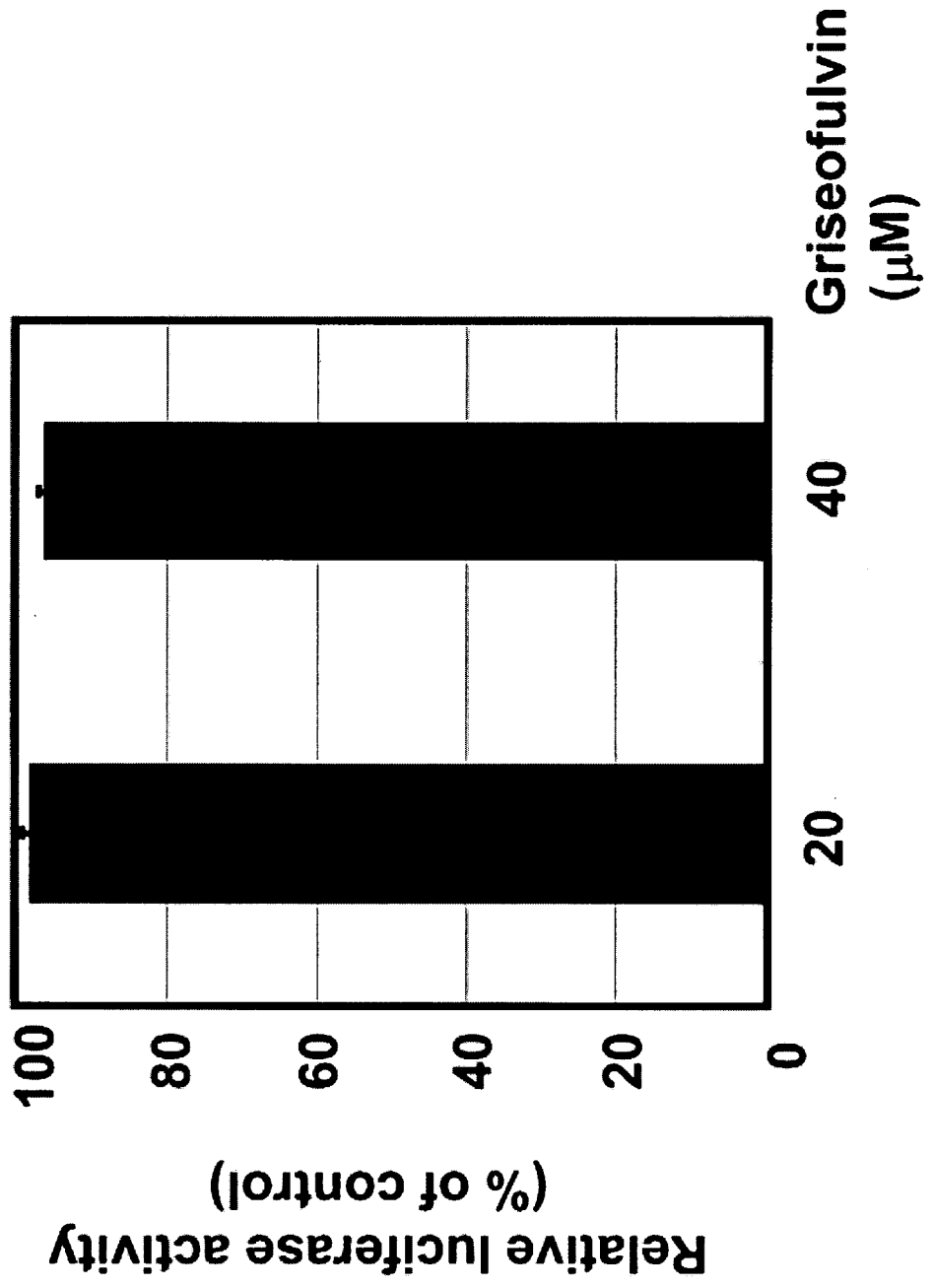


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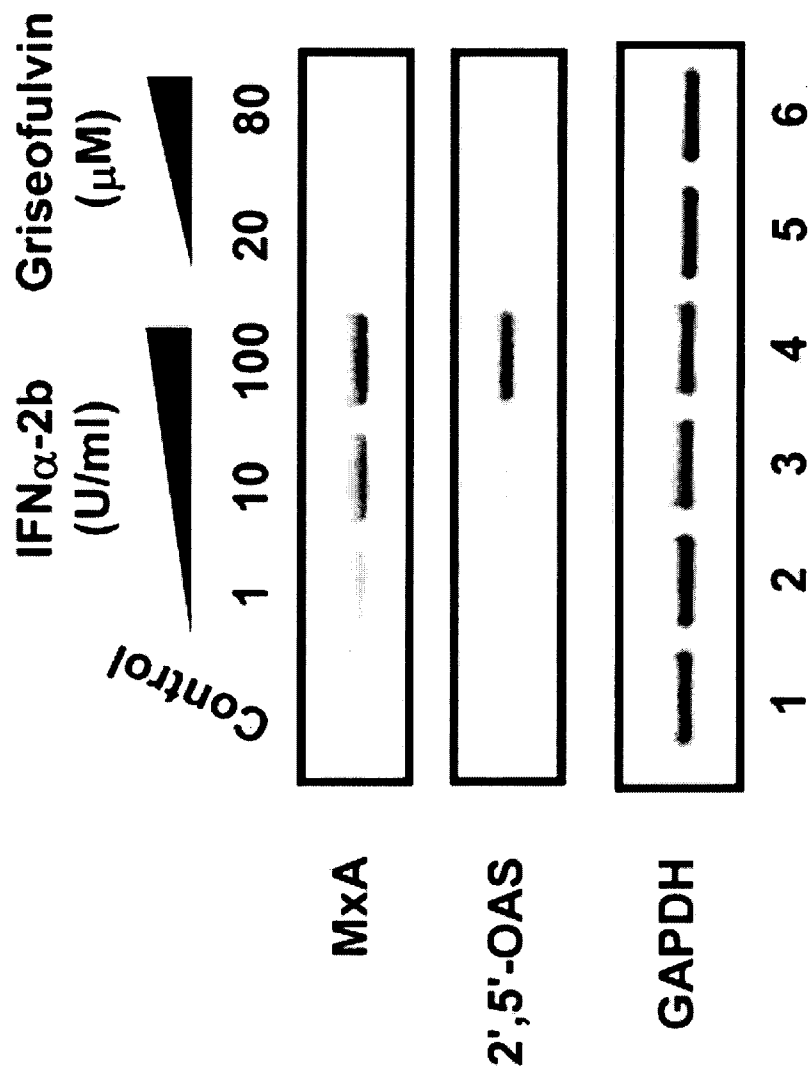


Fig.5

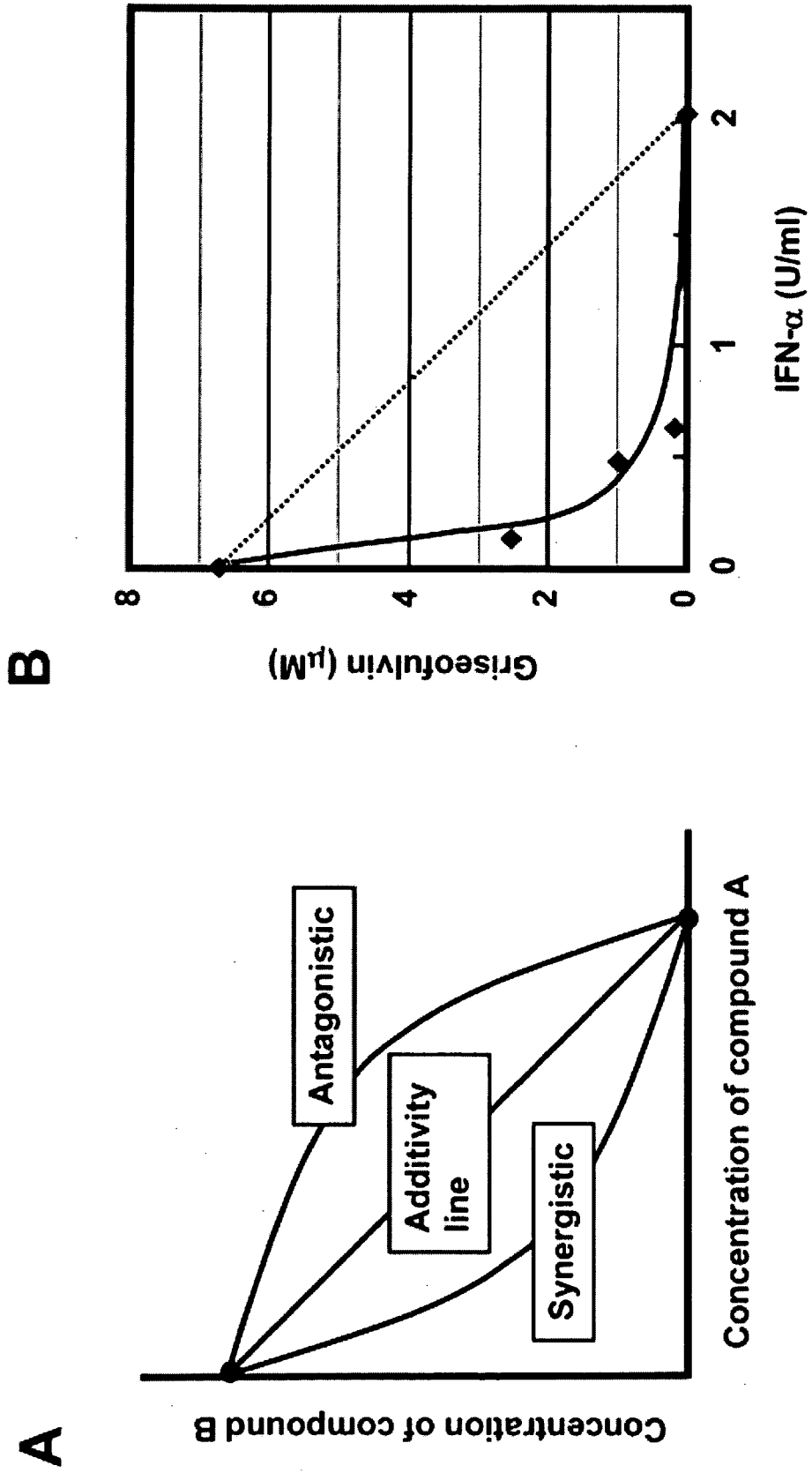


Fig.6

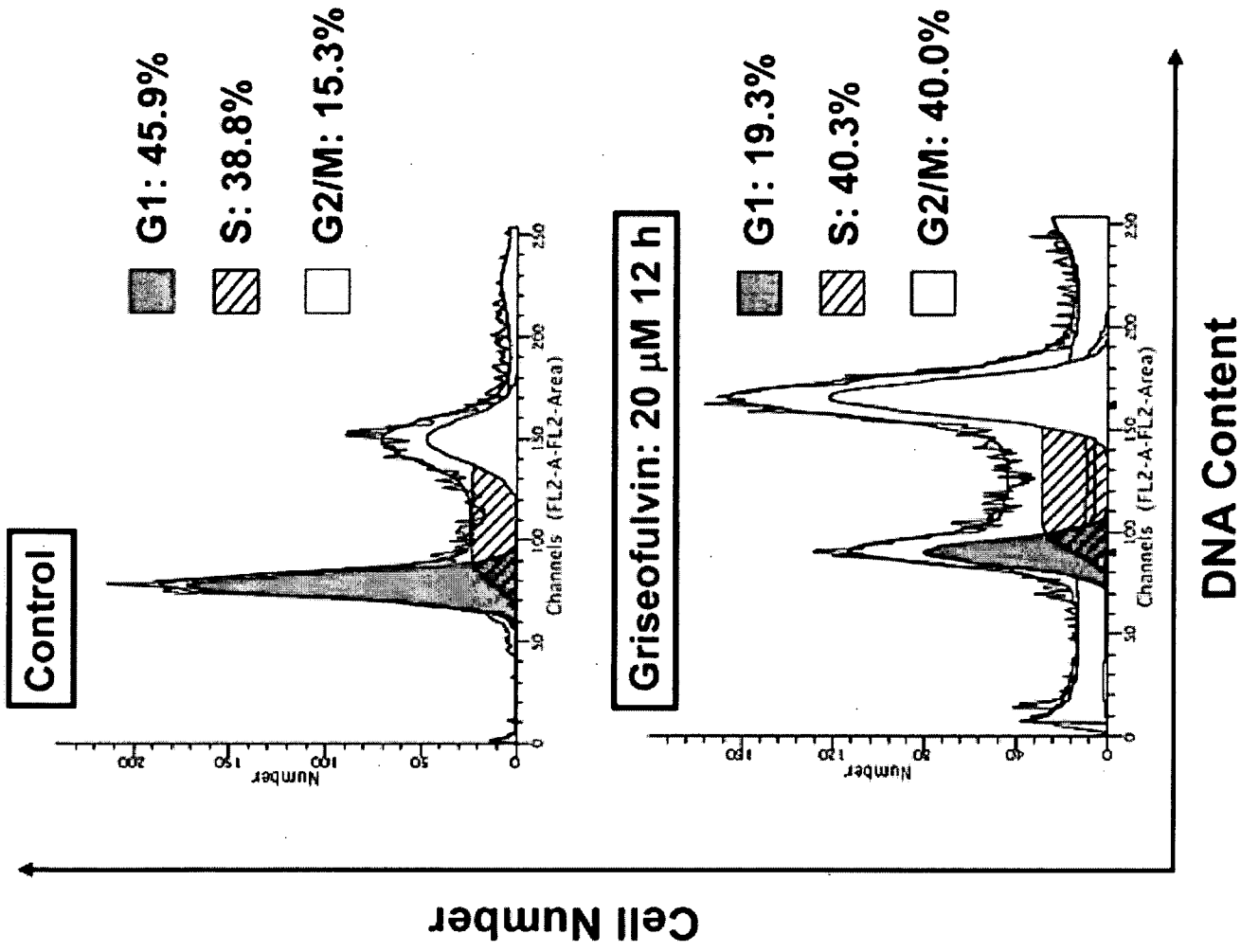


Fig.7

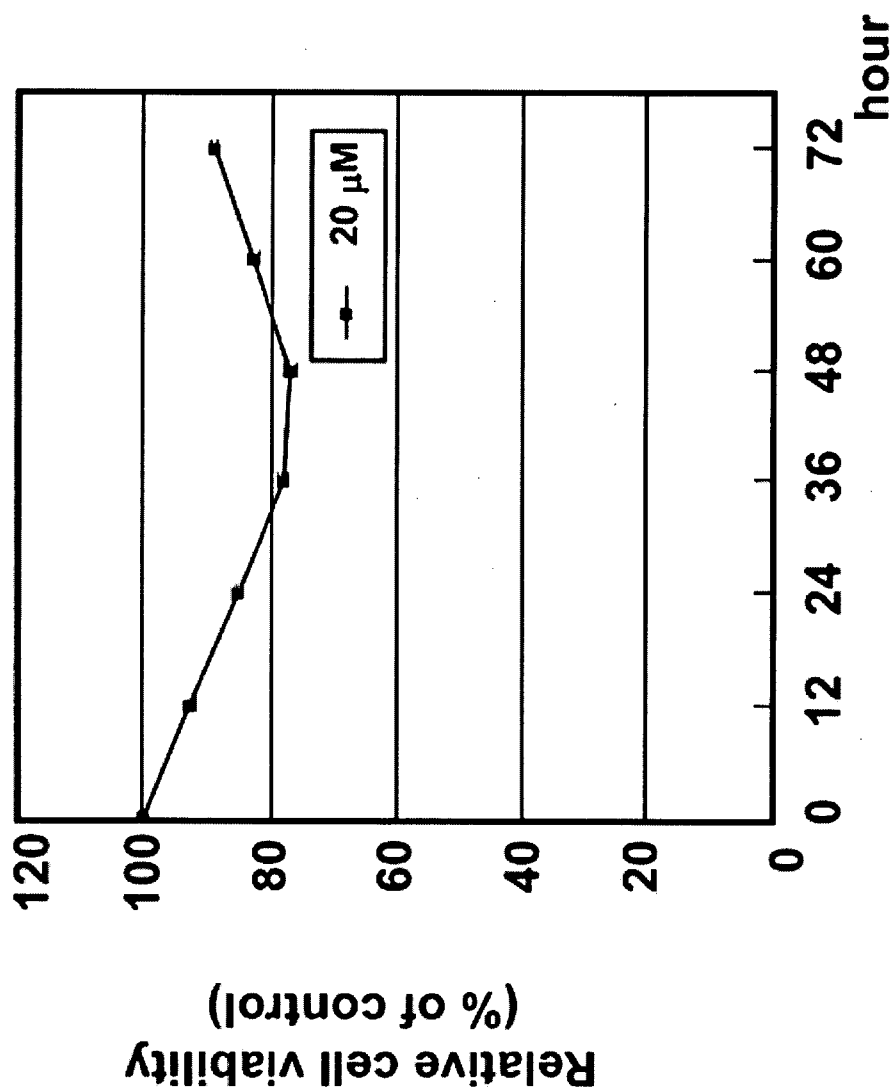


Fig.8

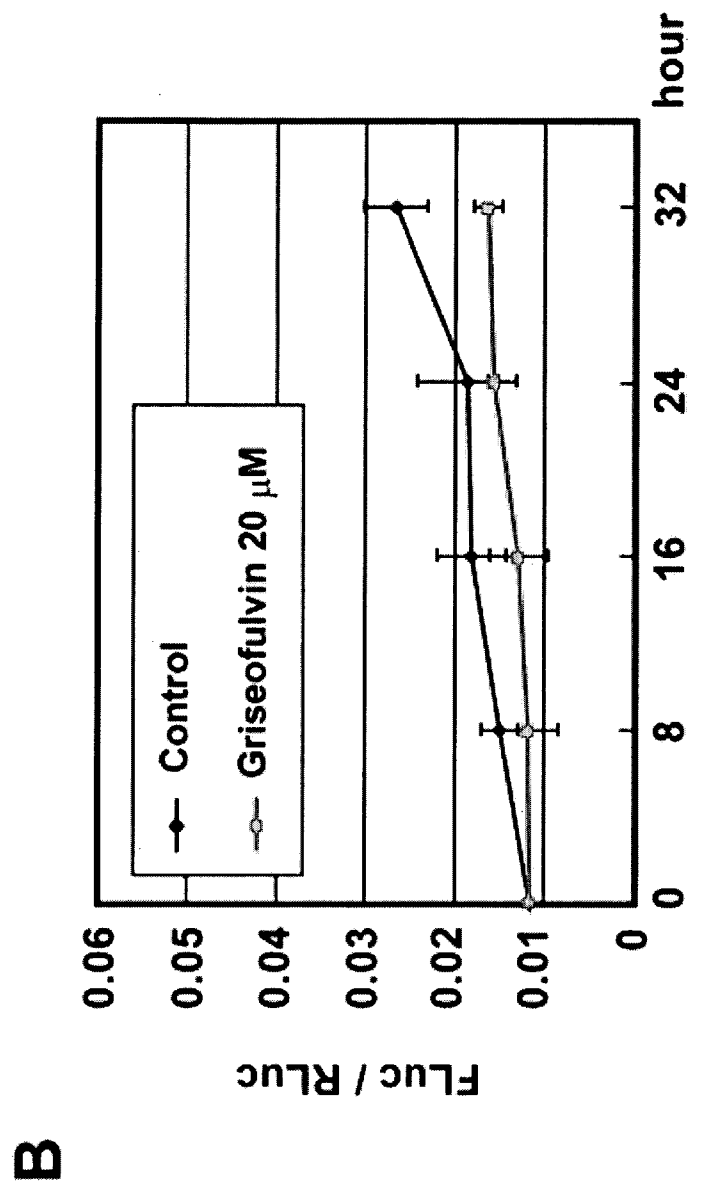
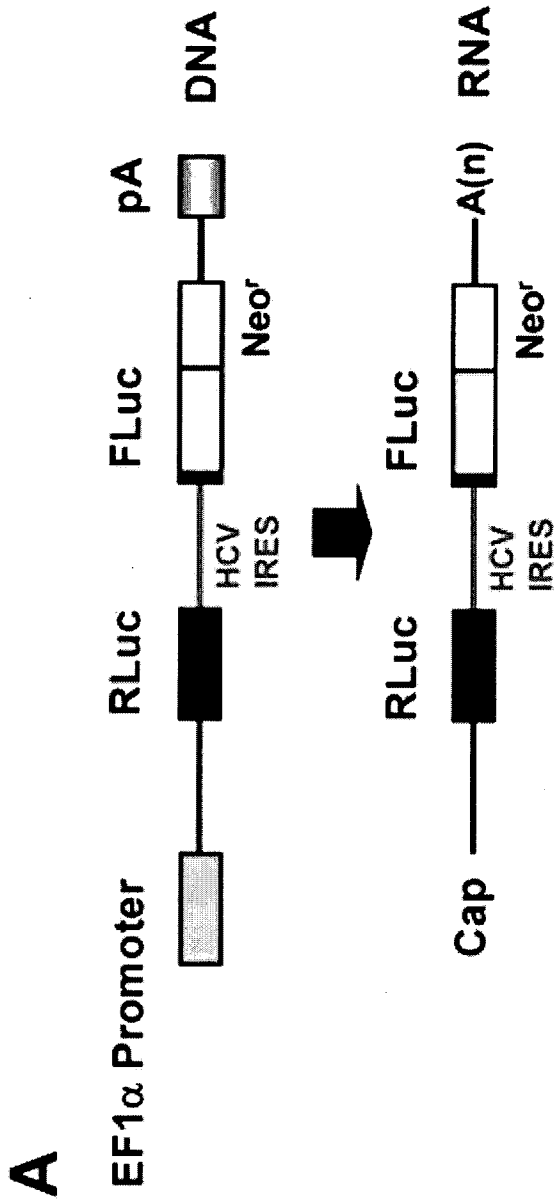


Fig.9

