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FOOTNOTE

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FIGURE LEGENDS

Fig. 1. CYP3A4 enzyme activity in various cell lines infected with AdCYP3A4. Cells were infected with AdCYP3A4 at MOI 10 for 48 hrs, and the testosterone 6β-hydroxylase activity was measured as described in materials and methods. Data are mean of two independent experiments.

Fig. 2. Cytotoxic effects of CYP3A4 expression on cell viability of HepG2 cells treated with various drugs. HepG2 cells were infected with AdCYP3A4 (●) or AdGFP (○) at MOI 10 for 48 hrs, and treated with 23 drugs for 24 hrs. Cell viability was measured by WST-8 assay. Each point represents mean ± SD (n = 3). $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ compared with the AdGFP group at each concentration of the drug.

Fig. 3. Expression level of (A) Nrf2 mRNA and (B) protein, and (C) testosterone 6β-hydroxylase activity in AdCYP3A4-infected HepG2 cells with siNrf2. After HepG2 cells were transfected with siNrf2 or siScramble at 10 nM followed by the infection with AdCYP3A4 or AdGFP at MOI 10 for 48 hrs, the expression level of Nrf2 mRNA and protein, and testosterone 6β-hydroxylase activity were measured as described in materials and methods. Each column represents mean ± SD (n = 3). $***P < 0.001$ compared with AdCYP3A4-infected cells with siScramble.

Fig. 4. Effects of the decreased level of Nrf2 on cell viability of CYP3A4-expressing HepG2 cells treated with the drugs. HepG2 cells were transfected with siNrf2 (▲) or siScramble (△) at 10 nM for 24 hrs following by the infection with AdCYP3A4 at MOI

10 for 48 hrs. After cells were treated with 6 drugs for 24 hrs, (A) WST-8 and (B) ATP assays were performed for evaluation of the cell viability. Each point represents mean ± SD (n = 3). $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared with AdCYP3A4-infected cells with siScramble in each concentration of drug.

Fig. 1.

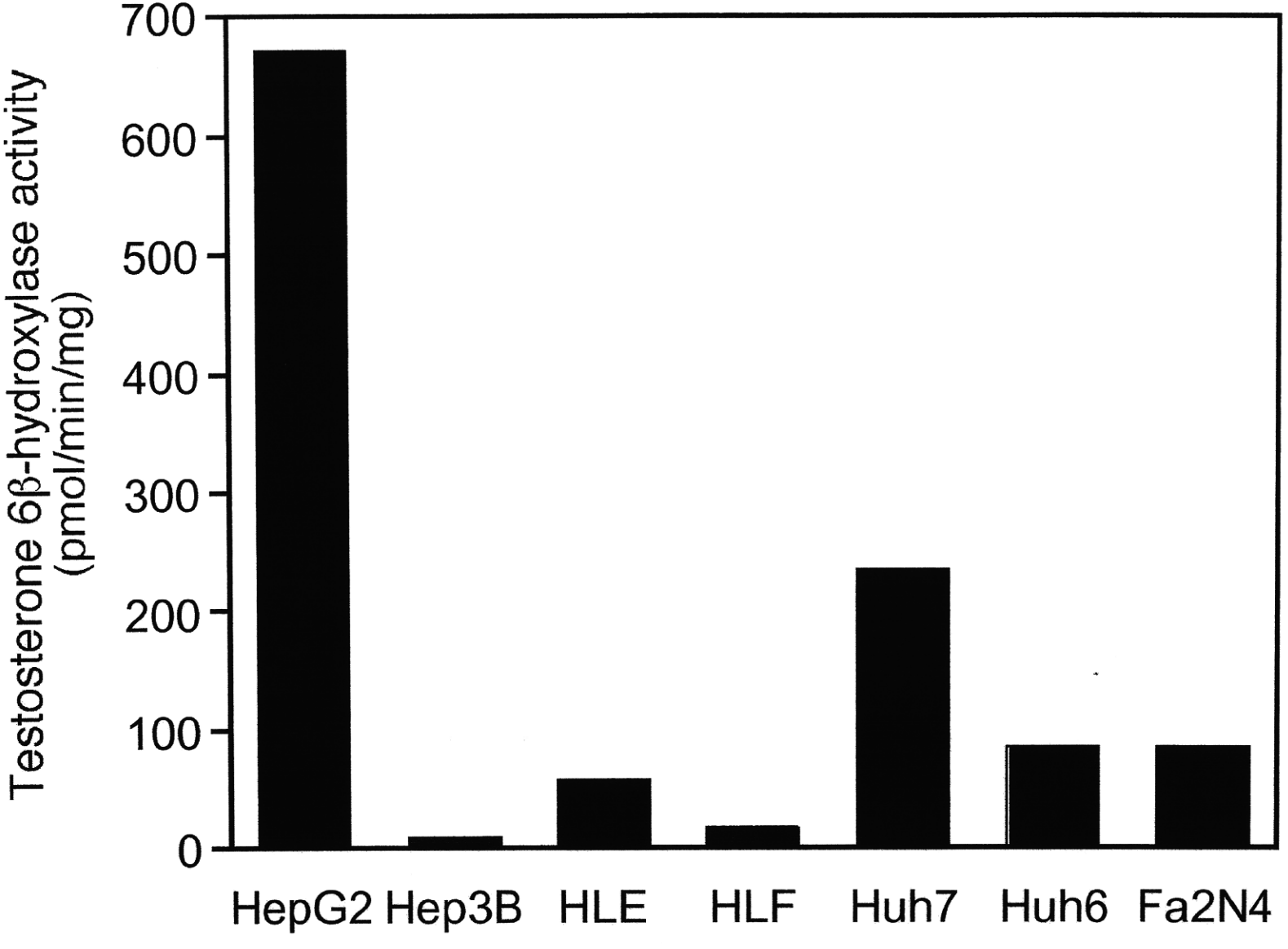

Testosterone 6β-hydroxylase activity
(pmol/min/mg)
700
600
500
400
300
200
100
0
HepG2
Hep3B
HLE
HLF
Huh7
Huh6
Fa2N4

Fig. 2.

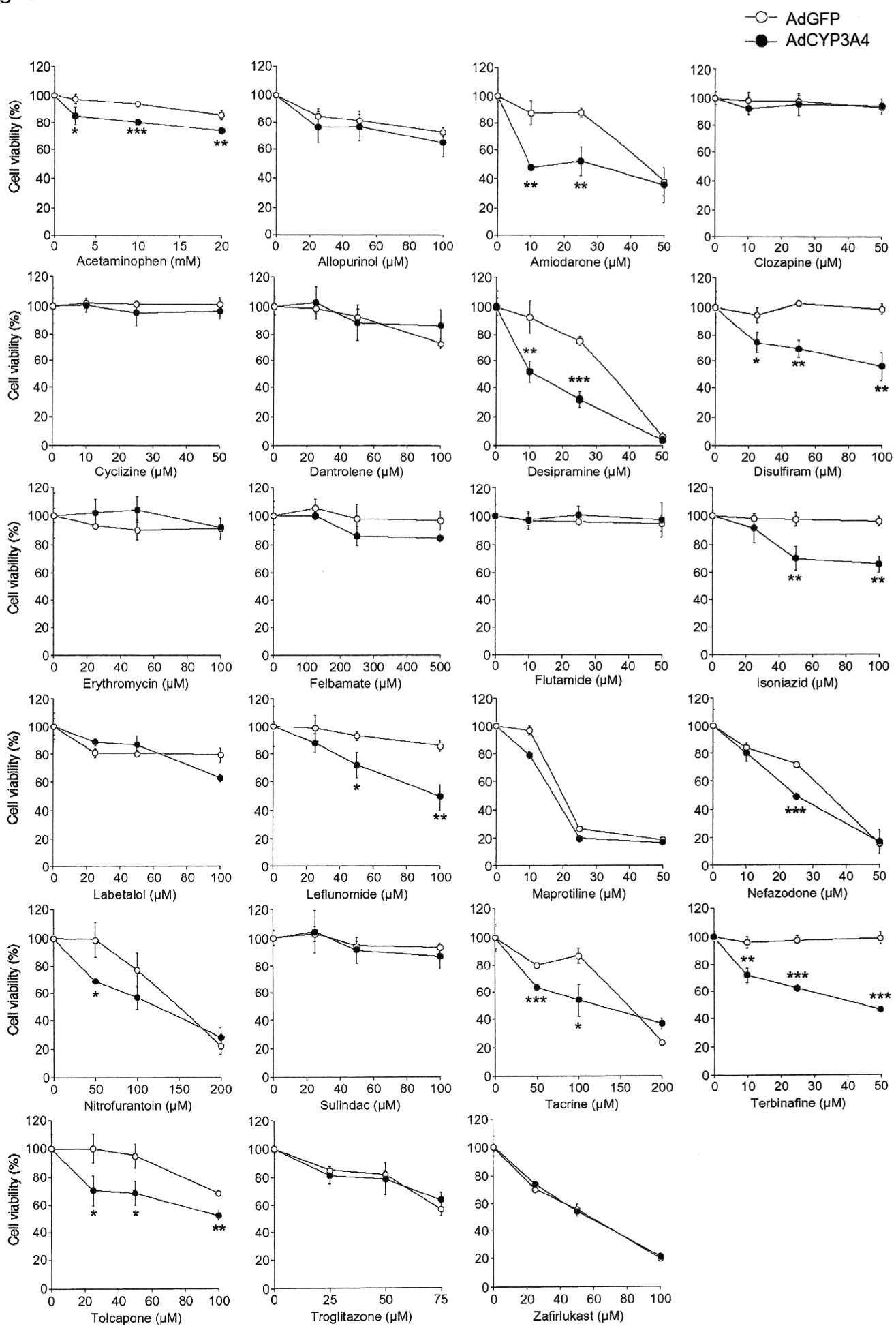
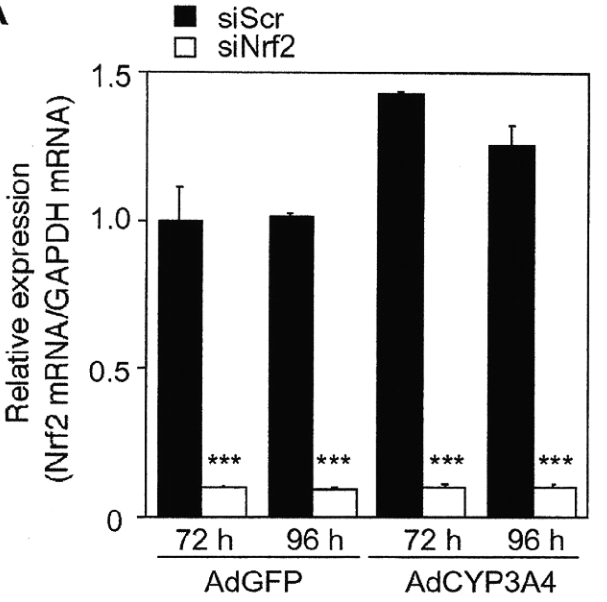
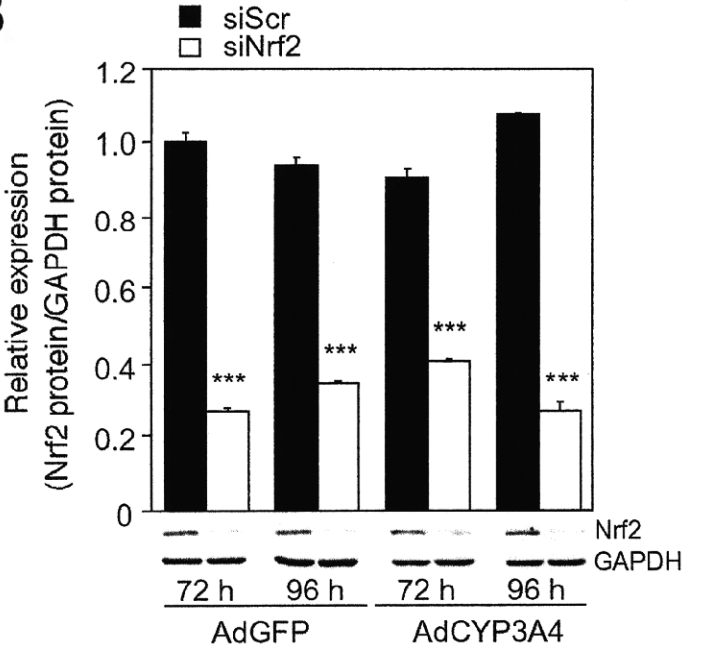

AdGFP
AdCYP3A4
Cell viability (%)
Acetaminophen (mM)
Allopurinol (μM)
Amiodarone (μM)
Clozapine (μM)
Cyclizine (μM)
Dantrolene (μM)
Desipramine (μM)
Disulfiram (μM)
Erythromycin (μM)
Felbamate (μM)
Flutamide (μM)
Isoniazid (μM)
Labetalol (μM)
Leflunomide (μM)
Maprotiline (μM)
Nefazodone (μM)
Nitrofurantoin (μM)
Sulindac (μM)
Tacrine (μM)
Terbinafine (μM)
Tolcapone (μM)
Troglitazone (μM)
Zafirlukast (μM)

Fig. 3

A


siScr
siNrf2
Relative expression
(Nrf2 mRNA/GAPDH mRNA)
1.5
1.0
0.5
0
***
72 h
96 h
72 h
96 h
AdGFP
AdCYP3A4

B


siScr
siNrf2
Relative expression
(Nrf2 protein/GAPDH protein)
1.2
1.0
0.8
0.6
0.4
0.2
0
***
Nrf2
GAPDH
72 h
96 h
72 h
96 h
AdGFP
AdCYP3A4

C

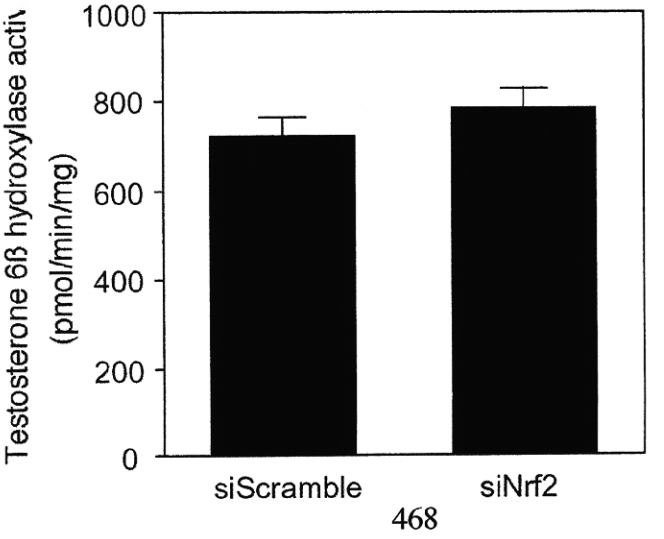
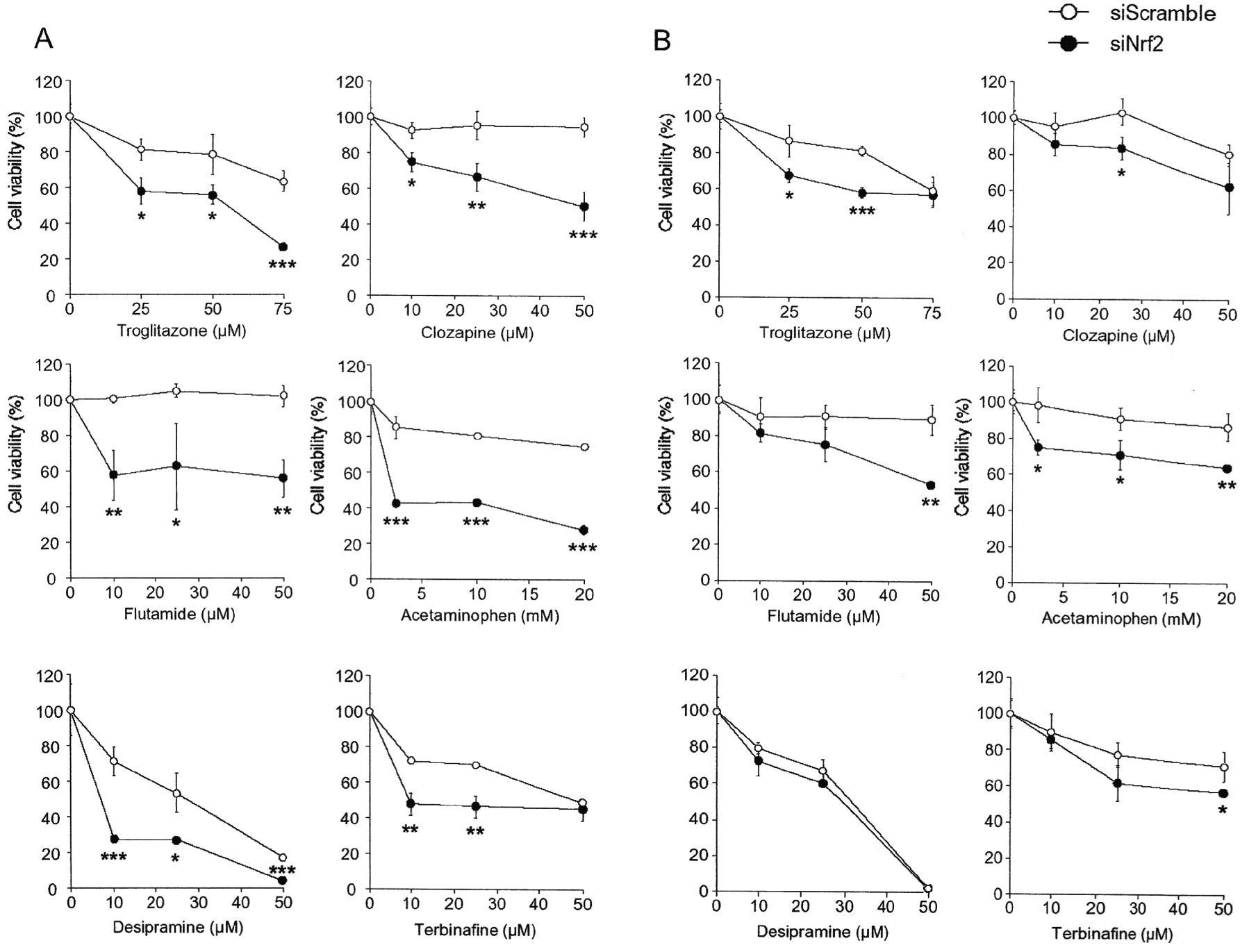

Testosterone 6ß hydroxylase activi
(pmol/min/mg)
1000
800
600
400
200
0
siScramble
siNrf2

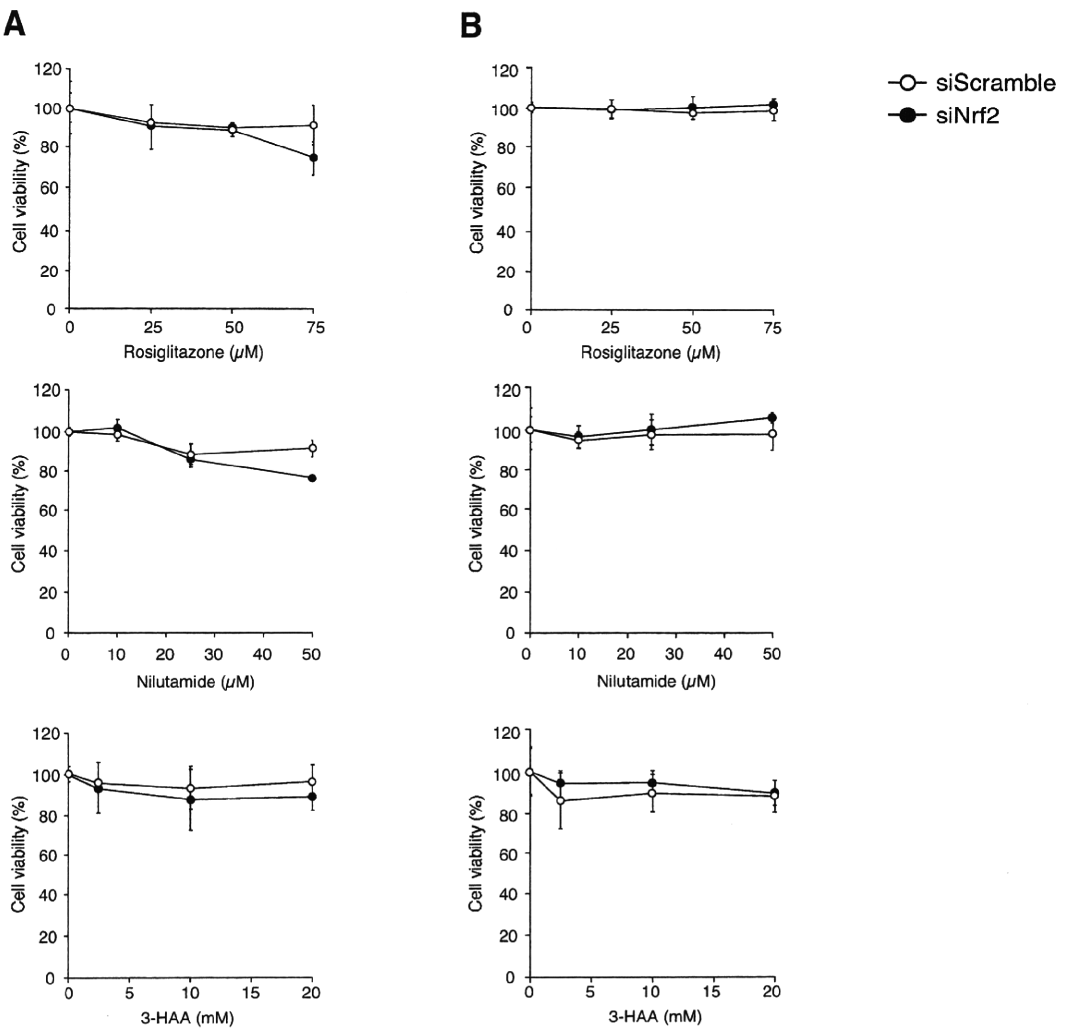
Fig. 4.


A
B
siScramble
siNrf2
Cell viability (%)
Troglitazone (μM)
Clozapine (μM)
Flutamide (μM)
Acetaminophen (mM)
Desipramine (μM)
Terbinafine (μM)

Development of A High-sensitive Cytotoxicity Assay System for CYP3A4-mediated Metabolic Activation

Hiroko Hosomi, Tatsuki Fukami, Atsushi Iwamura, Miki Nakajima, and Tsuyoshi Yokoi

Drug Metab Dispos



Supplement Fig. S1: Effects of CYP3A4 expression and the decreased level of Nrf2 on cell viability of HepG2 cells treated with rosiglitazone, nilutamide, and 3-hydroxyacetanilide. HepG2 cells were transfected with siNrf2 (closed triangle) and siScramble (open triangle) at 10 nM for 24 hrs following by the infection with AdCYP3 A4 at MOI 10 for 2 days. After cells were treated with drugs, (A) WST-8 and (B) ATP assays were performed for the evaluation of cell viability. Each data point represents mean ± SD (n = 3).