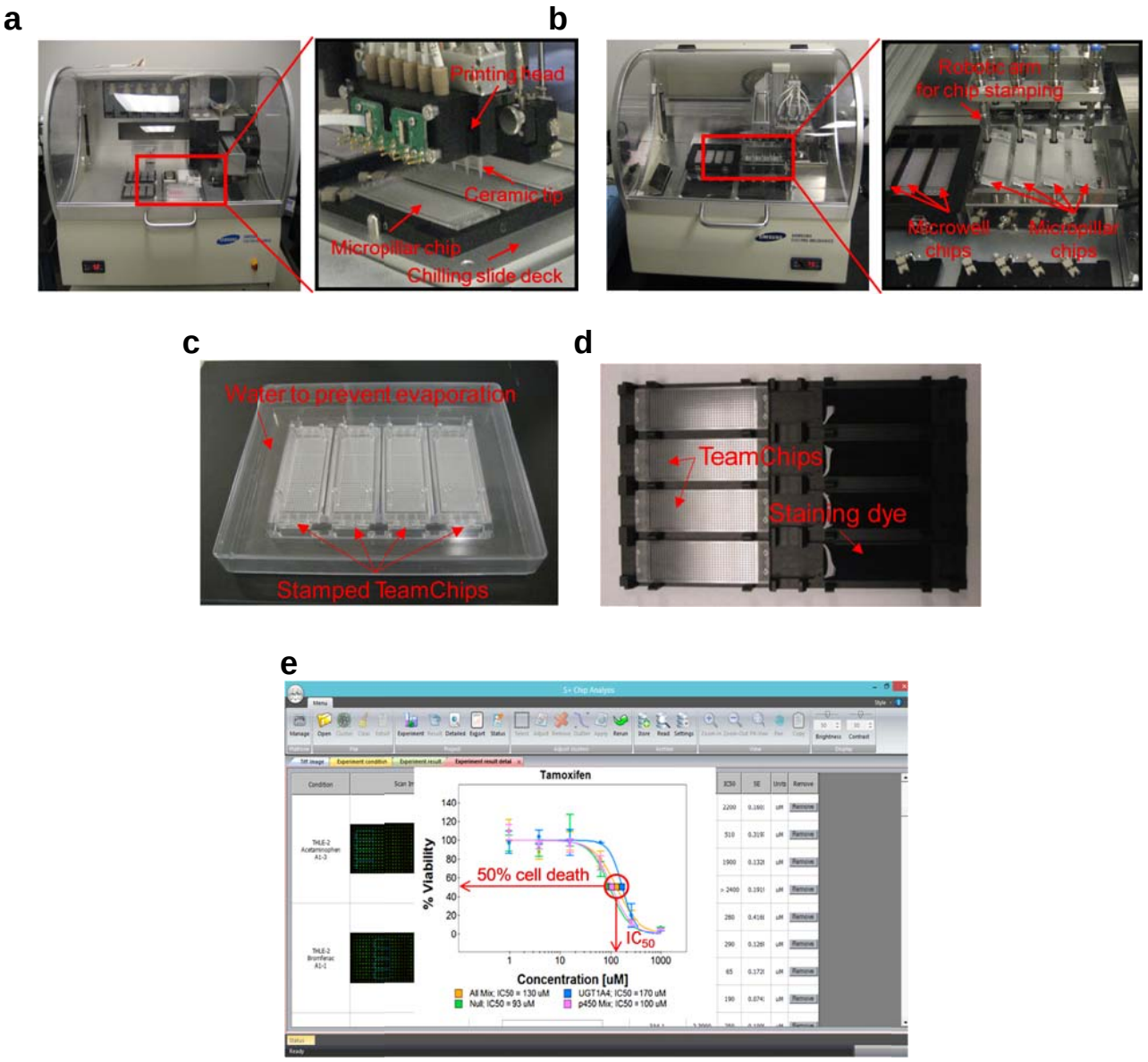
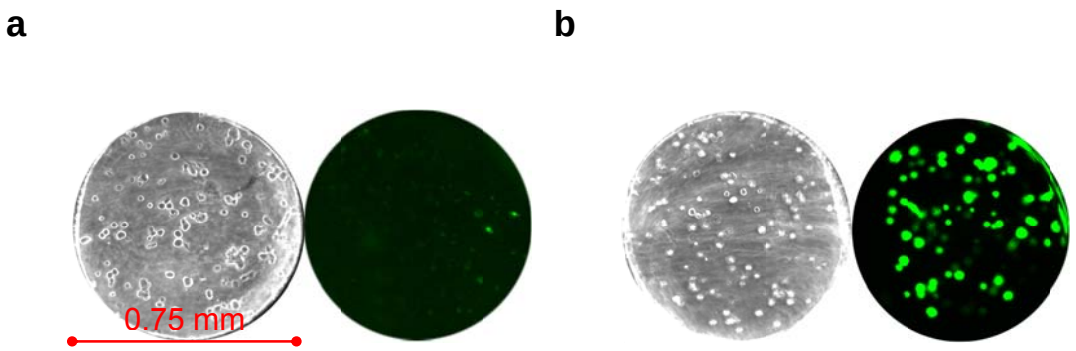
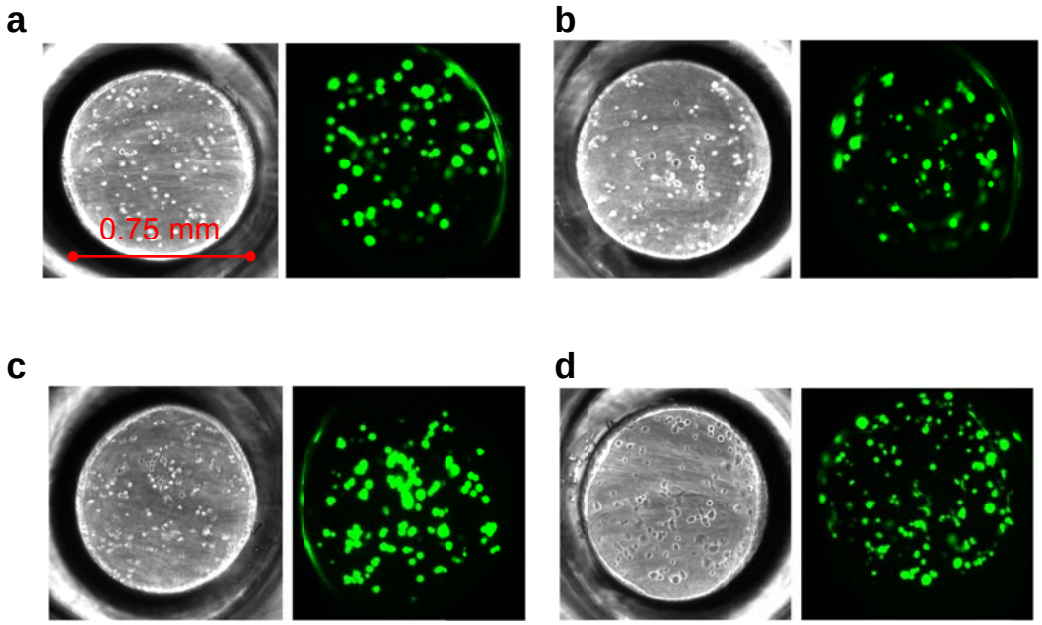
Supplementary Information



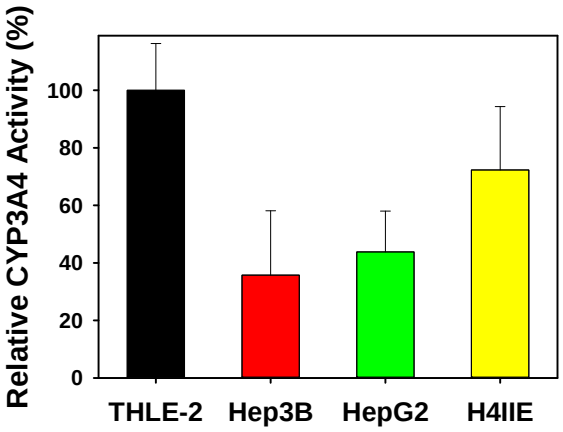
Supplementary Figure 1. The TeamChip platform and associated instruments, devices, and data analysis software. Photographs of (**a**) a microarray spotter with 6 tips for dispensing biological samples in solution, (**b**) a chip-handling system for stamping the micropillar chips onto the microwell chip or separating them from each other, (**c**) a gas-permeable incubation chamber for cell cultures, (**d**) a staining plate for uniform cell staining, and (**e**) data analysis software (S^+ Chip Analysis from SEMCO, Suwon, South Korea).



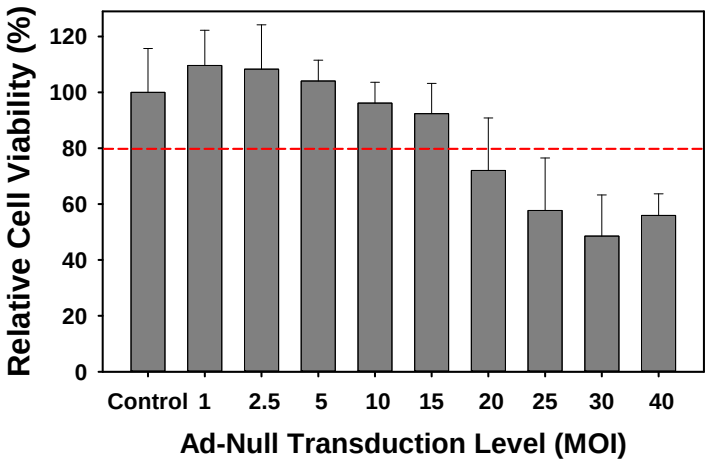
Supplementary Figure 2. Microscopic images of the THLE-2 cell line, derived from primary normal liver epithelial cells, on the micropillar chip infected with adenovirus carrying a gene for GFP (Ad-GFP) at 20 MOI (left: bright field image; right: fluorescent image). (**a**) THLE-2 cells encapsulated in alginate and (**b**) THLE-2 cells encapsulated in matrigel. Green dots indicate THLE-2 cells expressing GFP after treatment with Ad-GFP. The transduction efficiency of THLE-2 cells in alginate spots was significantly lower than in matrigel, presumably due to interaction of the virus particles with the alginate matrix. This experiment was performed to evaluate cell encapsulation matrices that allow high adenoviral gene transduction. The scale bar indicates the diameter of the micropillar with THLE-2 cells.



Supplementary Figure 3. Microscopic images of human cells encapsulated in matrigel on the micropillar chip infected with Ad-GFP (left: bright field image; right: fluorescent image). (**a**) THLE-2, (**b**) HepG2, (**c**) Hep3B, and (**d**) Beas-2B cells. Green dots indicate the cells expressing GFP after treatment with Ad-GFP. The transduction efficiency was nearly 100% for THLE-2, HepG2, Hep3B, and Beas-2B cell lines at a relatively low MOI of 20. The images used in Supplementary Figure 2 panel B and Figure 3 panel A are identical for ease of visualization. The scale bar indicates the diameter of the micropillar with human cells.

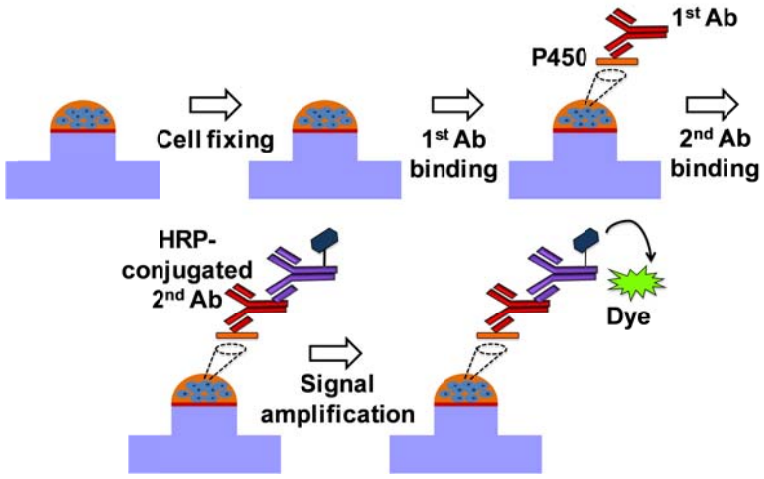


Supplementary Figure 4. The relative activity of CYP3A4 expressed in different liver cell lines by recombinant adenovirus carrying a gene for CYP3A4 (Ad-CYP3A4). To establish an optimal liver cell line for adenoviral gene expression for drug metabolizing enzymes, THLE-2, HepG2, Hep3B, and H4IIE cell lines were infected with Ad-CYP3A4 at 10 MOI in 96-well plates and the activity of expressed CYP3A4 in the liver cell lines was measured with P450 Glo^{TM} assay kit from Promega. CYP3A4 was selected as a model enzyme because it is one of the most important drug metabolizing enzymes among other CYP450 isoforms. THLE-2 cells demonstrated the highest expression level of CYP3A4 among the four liver cell lines tested, and hence was the cell line focused on in this study. Error bars represent standard deviations (n=3).



Supplementary Figure 5. Cell viability of THLE-2 cells encapsulated in matrigel on the micropillar chip transduced with adenovirus carrying CMV promoter alone (Ad-Null). Adenovirus is known to be toxic to mammalian cells at high titers (MOI)[1]. This experiment was performed to establish suitable concentrations of adenovirus that can be used for metabolizing enzyme expression on the chip. At MOI > 20, THLE-2 cells encapsulated in matrigel had reduced viability, presumably due to basal toxicity (less than 80% viable). Thus, the MOI was limited to less than 20 for this study. Error bars represent standard deviations (n=24).

a



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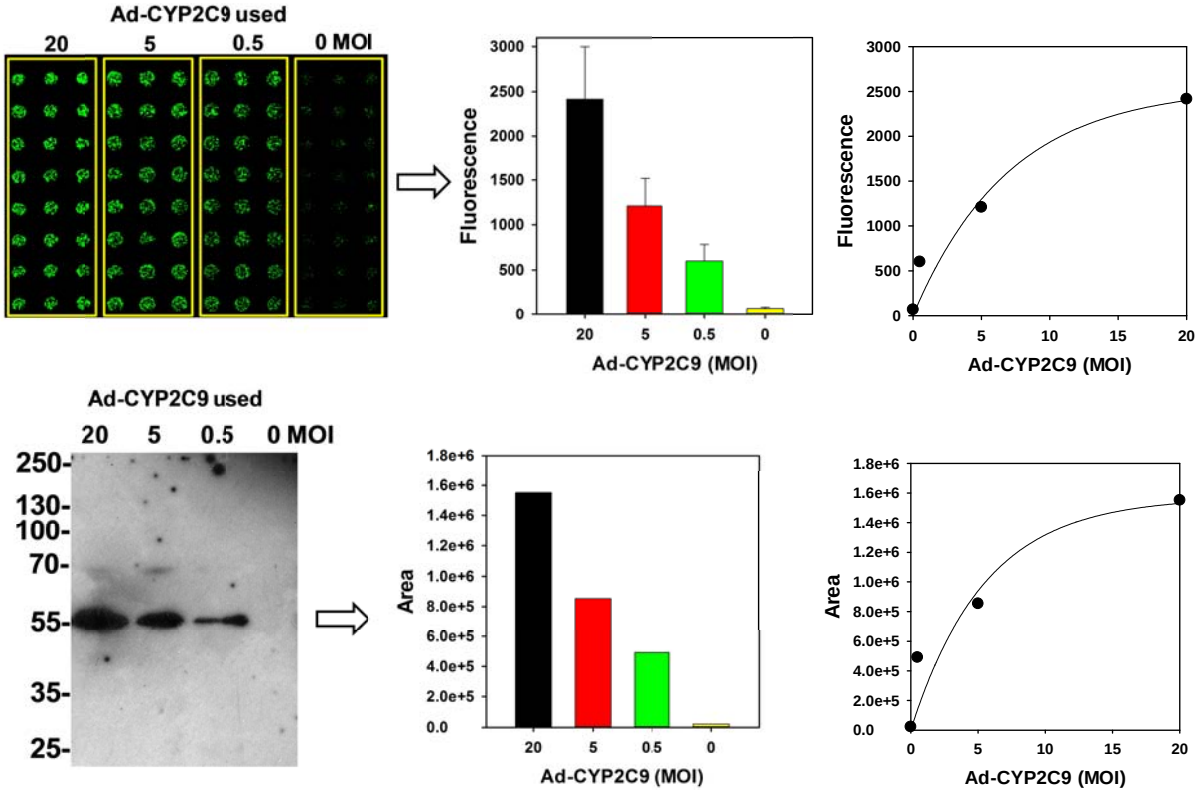
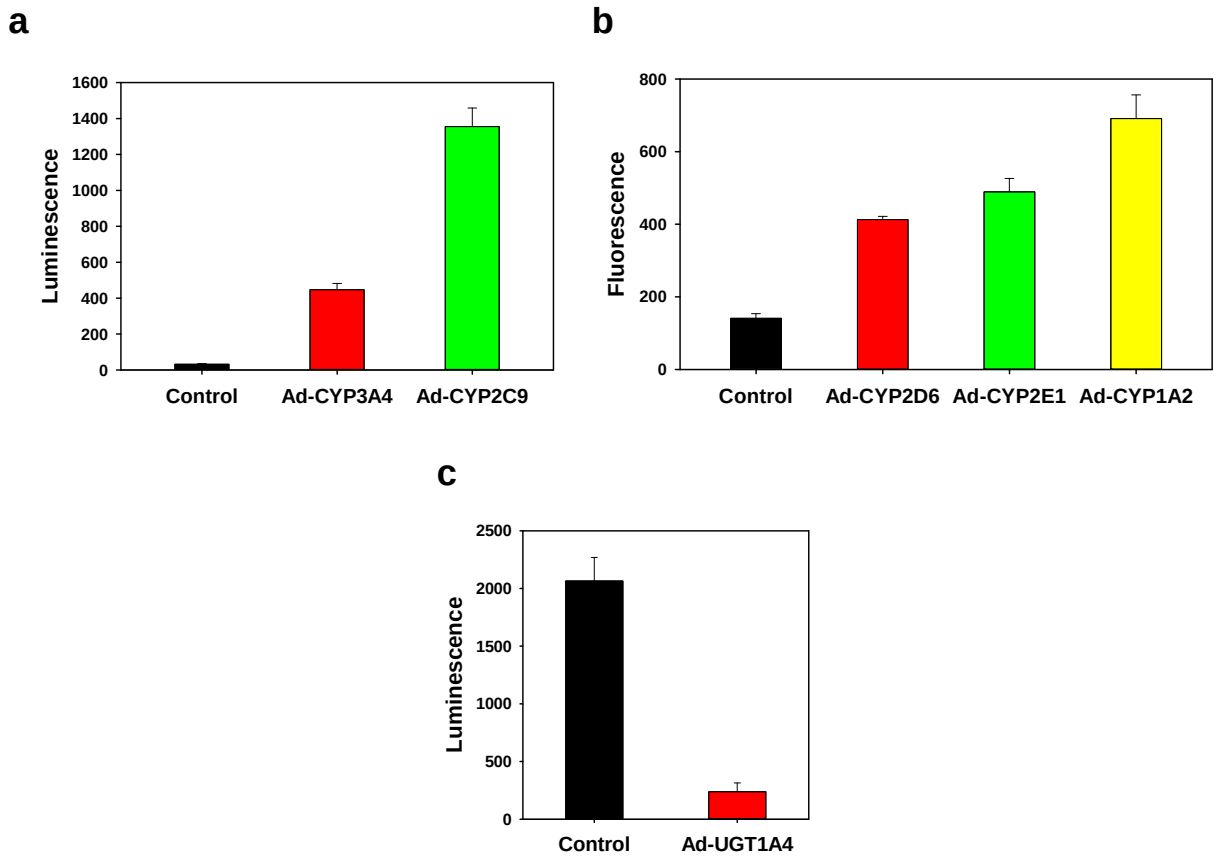
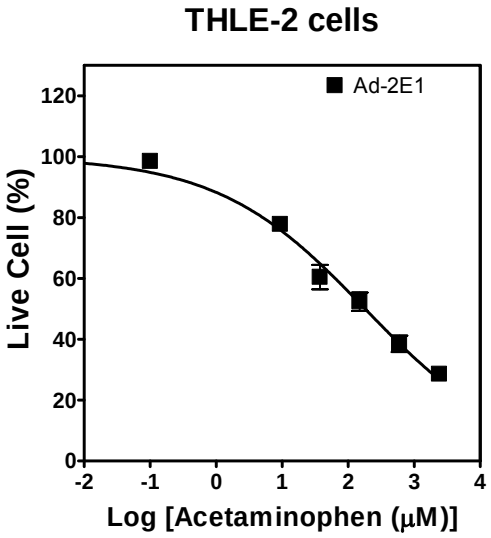


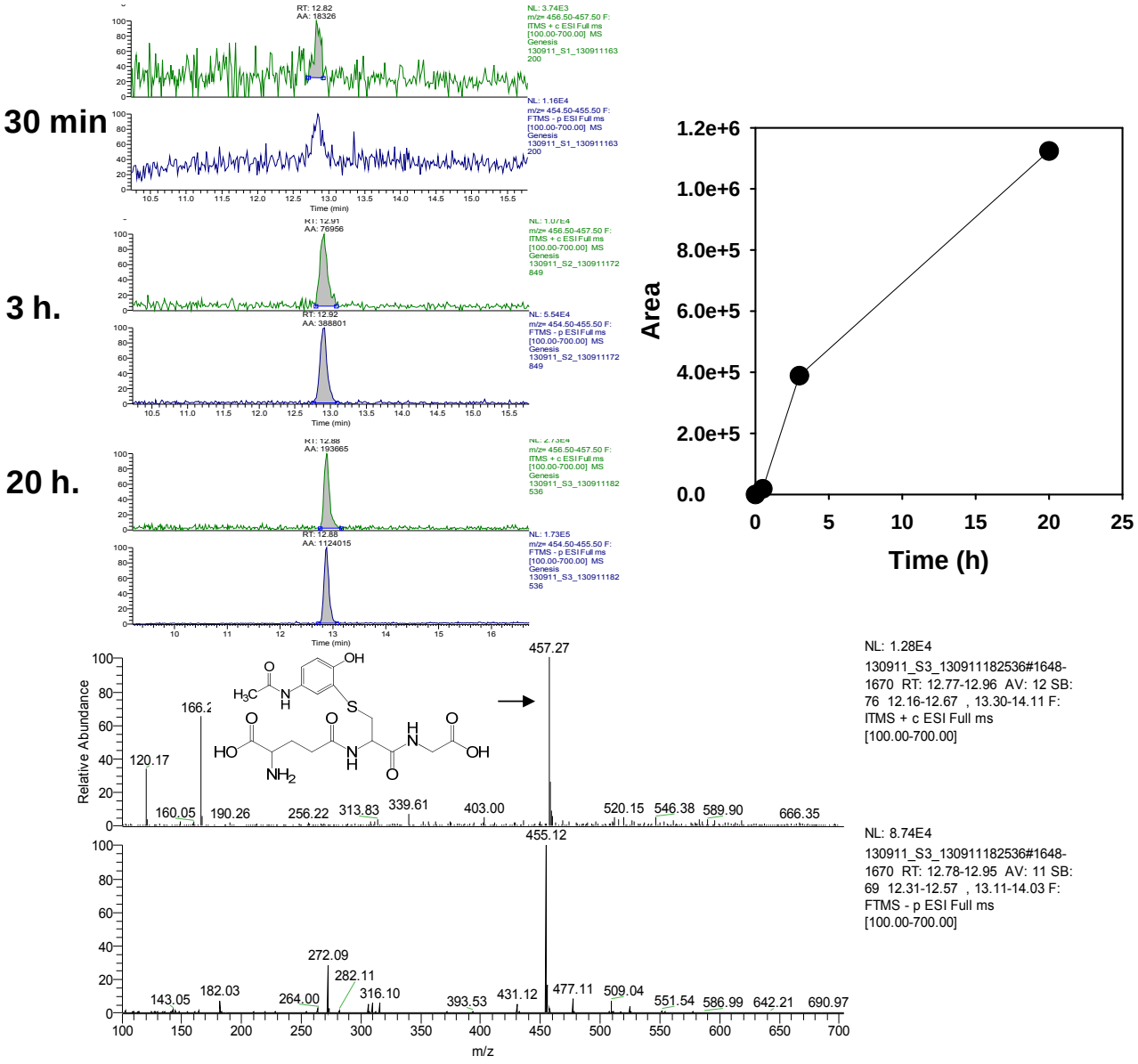
Supplementary Figure 6. Controlled expression of CYP2C9 in THLE-2 cells on the chip by using different concentrations of recombinant adenovirus carrying a gene for CYP2C9 (Ad-CYP2C9). (**a**) Schematic of in-cell immunofluorescence assay to measure CYP2C9 expression on the chip[2] and (**b**) scanned image from in-cell immunofluorescence assay of THLE-2 cells expressing CYP2C9 on the chip (top) and Western blot image of THLE-2 cell monolayers expressing CYP2C9 at different MOI of Ad-CYP2C9 (bottom). Error bars represent standard deviations (n=24).



Supplementary Figure 7. Activities of human drug-metabolizing enzymes expressed in THLE-2 cells after transduction with 10 MOI of recombinant adenoviruses (Ad-CYP3A4, Ad-CYP2C9, Ad-CYP2D6, Ad-CYP2E1, Ad-CYP1A2, and Ad-UGT1A4) in 96-well plates. (**a**) Activities of CYP3A4 and CYP2C9 expressed in THLE-2 cells measured with 50 μM of Luciferin-PFBE (P450 Glo™ assay kit from Promega) in BEGM media;.(**b**) activities of CYP2D6, CYP2E1, and CYP1A2 expressed in THLE-2 cells measured with 50 μM of Vivid® EOMCC (Invitrogen) in BEGM media; and (**c**) activity of UGT1A4 expressed in THLE-2 cells determined with 30 μM of proluciferin UGT substrate (UGT-Glo™ assay kit from Promega) in BEGM media. The luminescence signal is inversely proportional to UGT activity because the enzymatic product, glucuronidated luciferin, does not generate light. Error bars represent standard deviations (n=3).

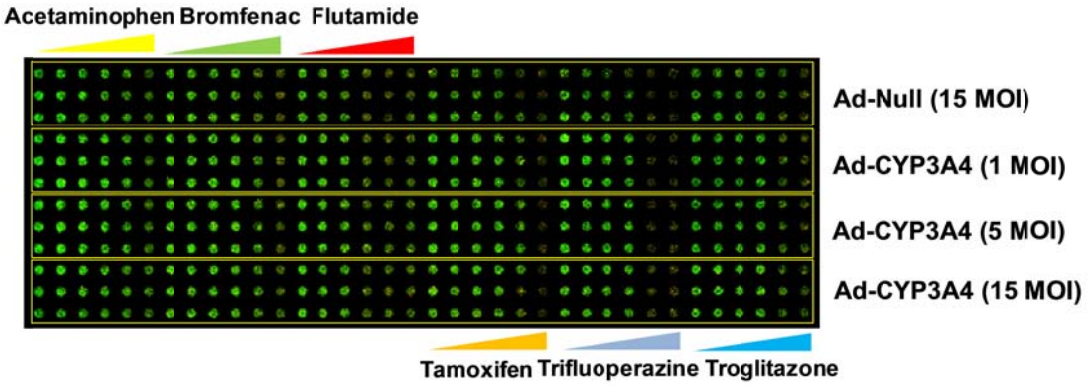


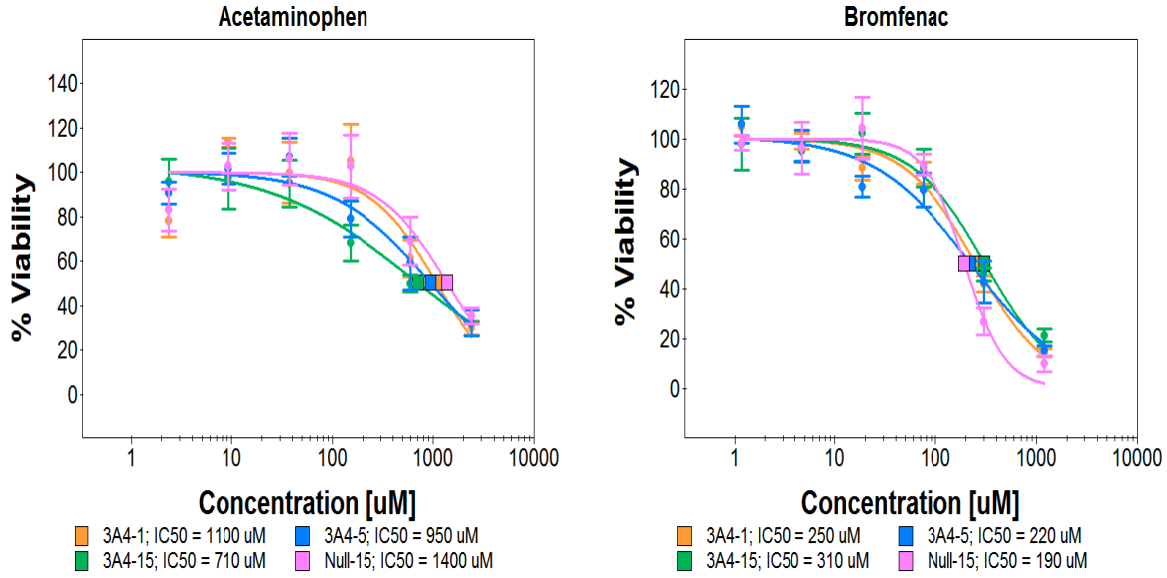
Supplementary Figure 8. Dose response of acetaminophen in THLE-2 cells expressing CYP2E1. The calculated IC_{50} is 180 ± 50 μM. Errors are reported as average deviations (n=3).

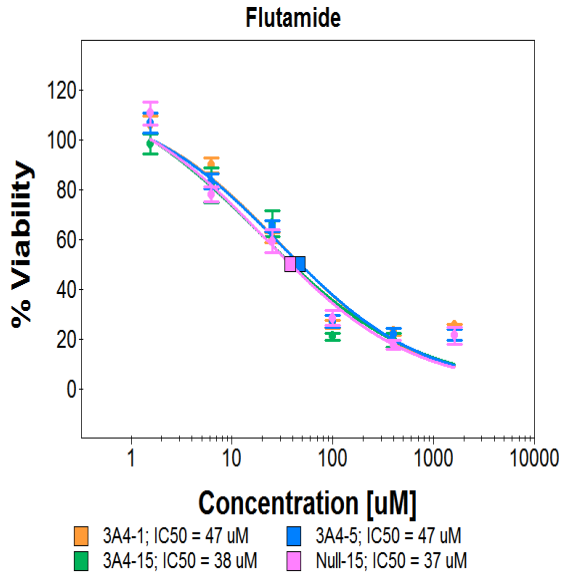


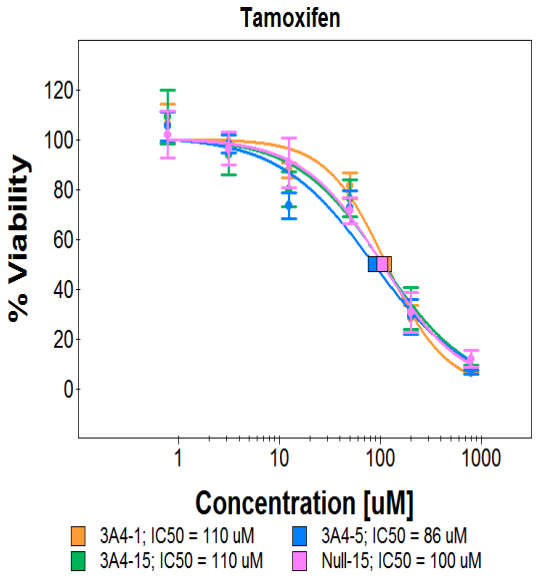
Supplementary Figure 9. LC-MS analysis of acetaminophen-glutathione conjugate (APAP-GSH) formation. APAP-GSH was formed from NAPQI, which is the CYP3A4-generated metabolite of acetaminophen. CYP3A4 was expressed in one million THLE-2 cells transduced by 15 MOI of Ad-CYP3A4. The reaction mixture contained 1 mM acetaminophen, 10 mM glutathione, and NADP regeneration system (Invitrogen) in 50 mM potassium phosphate buffer (pH 7.4).

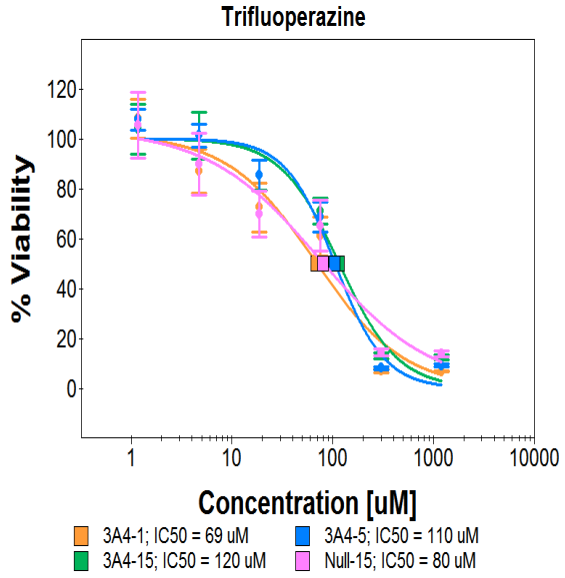
a

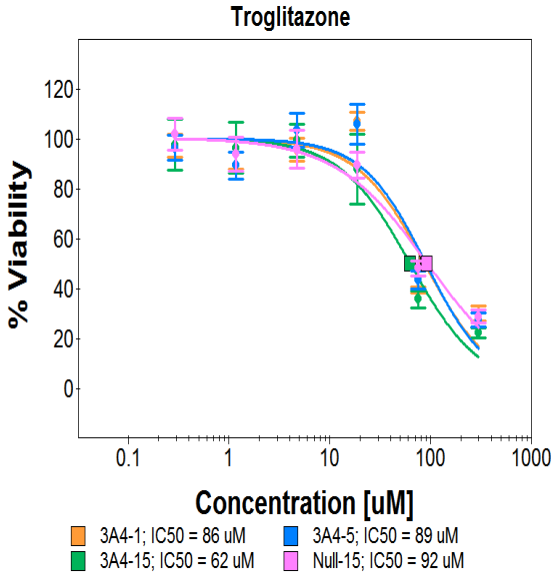

Acetaminophen Bromfenac Flutamide
Ad-Null (15 MOI)
Ad-CYP3A4 (1 MOI)
Ad-CYP3A4 (5 MOI)
Ad-CYP3A4 (15 MOI)
Tamoxifen Trifluoperazine Troglitazone


Acetaminophen
% Viability
Concentration [uM]
3A4-1; IC50 = 1100 uM
3A4-5; IC50 = 950 uM
3A4-15; IC50 = 710 uM
Null-15; IC50 = 1400 uM
Bromfenac
% Viability
Concentration [uM]
3A4-1; IC50 = 250 uM
3A4-5; IC50 = 220 uM
3A4-15; IC50 = 310 uM
Null-15; IC50 = 190 uM

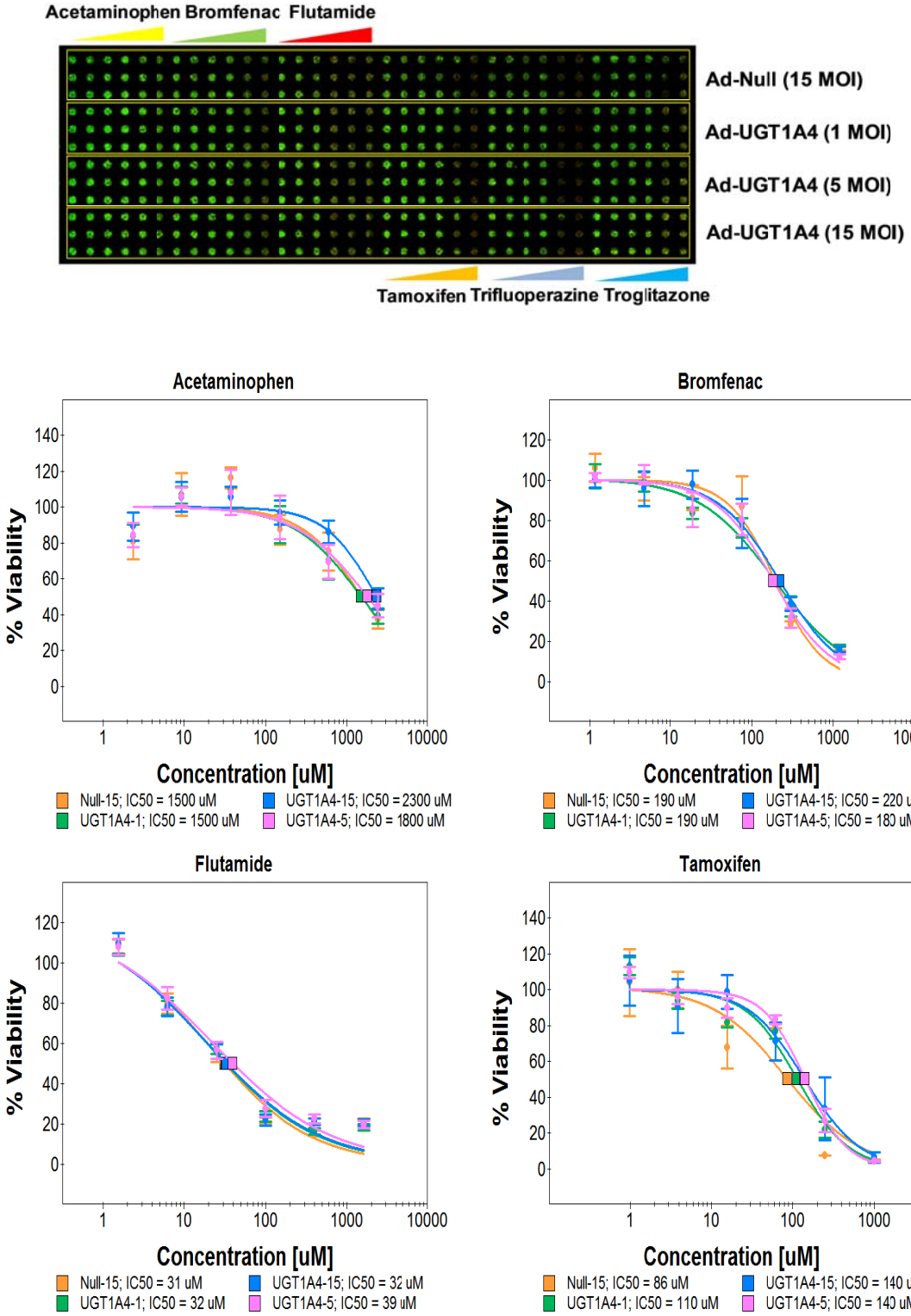

Flutamide
% Viability
120
100
80
60
40
20
0
1
10
100
1000
10000
Concentration [uM]
3A4-1; IC50 = 47 uM
3A4-5; IC50 = 47 uM
3A4-15; IC50 = 38 uM
Null-15; IC50 = 37 uM

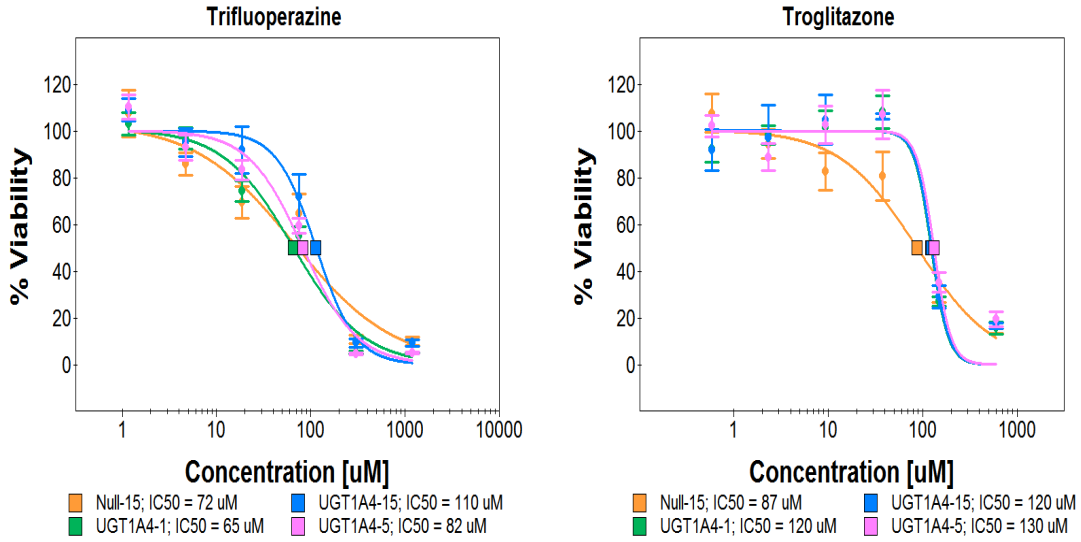

Tamoxifen
% Viability
120
100
80
60
40
20
0
1
10
100
1000
Concentration [uM]
3A4-1; IC50 = 110 uM
3A4-5; IC50 = 86 uM
3A4-15; IC50 = 110 uM
Null-15; IC50 = 100 uM


Trifluoperazine
% Viability
120
100
80
60
40
20
0
1
10
100
1000
10000
Concentration [uM]
3A4-1; IC50 = 69 uM
3A4-5; IC50 = 110 uM
3A4-15; IC50 = 120 uM
Null-15; IC50 = 80 uM

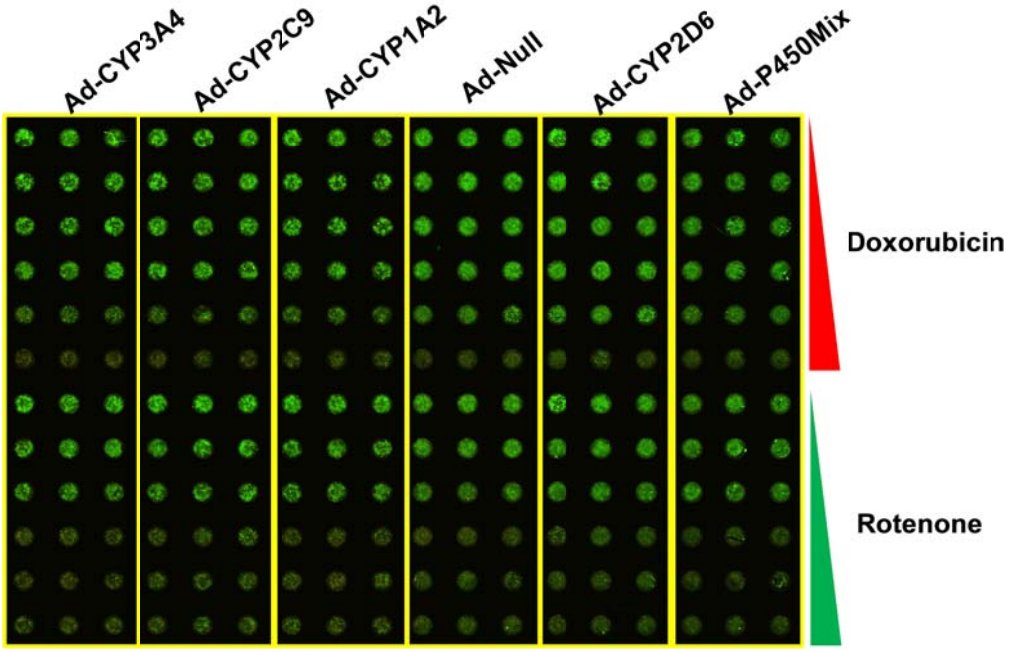

Troglitazone
% Viability
120
100
80
60
40
20
0
0.1
1
10
100
1000
Concentration [uM]
3A4-1; IC50 = 86 uM
3A4-5; IC50 = 89 uM
3A4-15; IC50 = 62 uM
Null-15; IC50 = 92 uM

b

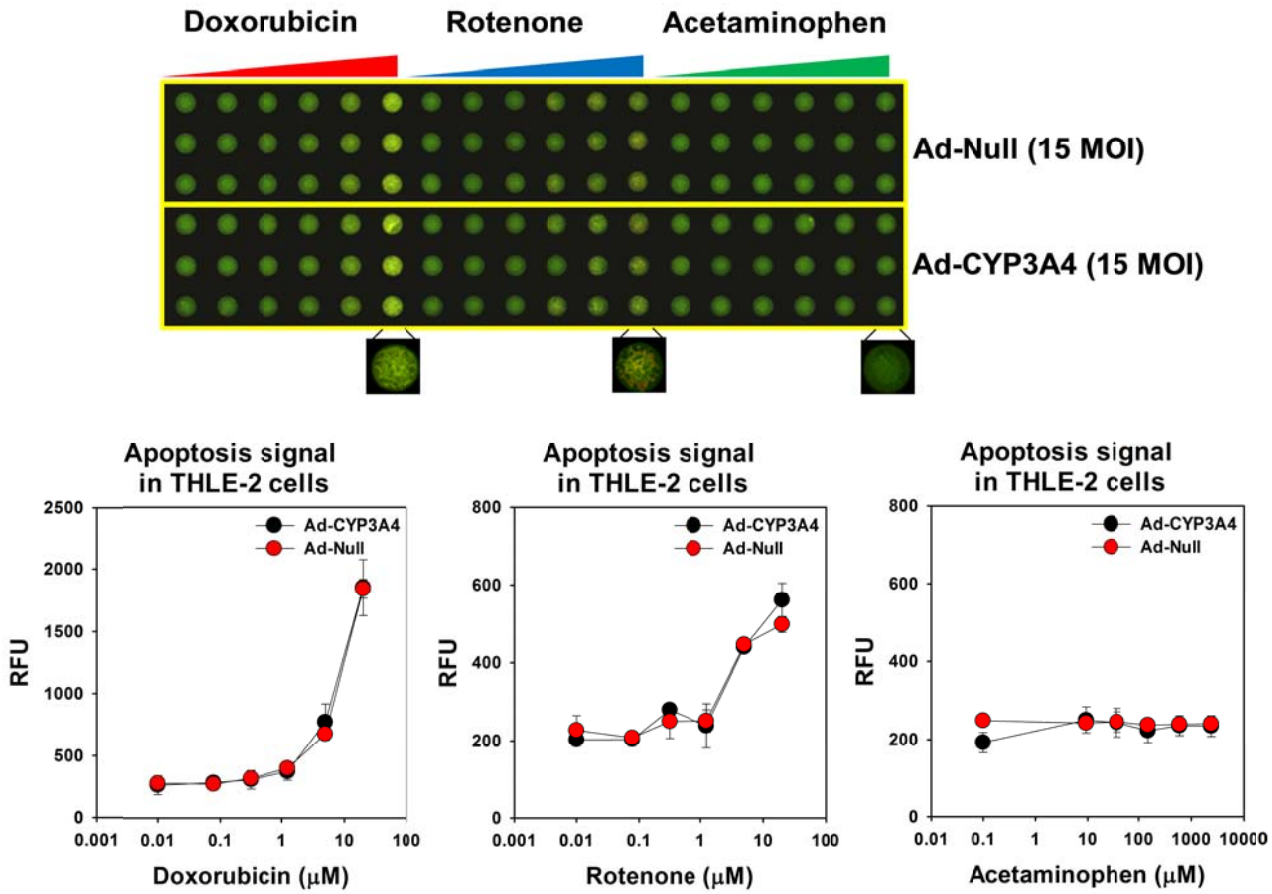

Acetaminophen Bromfenac Flutamide
Ad-Null (15 MOI)
Ad-UGT1A4 (1 MOI)
Ad-UGT1A4 (5 MOI)
Ad-UGT1A4 (15 MOI)
Tamoxifen Trifluoperazine Troglitazone
Acetaminophen
% Viability
Concentration [uM]
Null-15; IC50 = 1500 uM
UGT1A4-15; IC50 = 2300 uM
UGT1A4-1; IC50 = 1500 uM
UGT1A4-5; IC50 = 1800 uM
Bromfenac
% Viability
Concentration [uM]
Null-15; IC50 = 190 uM
UGT1A4-15; IC50 = 220 uM
UGT1A4-1; IC50 = 190 uM
UGT1A4-5; IC50 = 180 uM
Flutamide
% Viability
Concentration [uM]
Null-15; IC50 = 31 uM
UGT1A4-15; IC50 = 32 uM
UGT1A4-1; IC50 = 32 uM
UGT1A4-5; IC50 = 39 uM
Tamoxifen
% Viability
Concentration [uM]
Null-15; IC50 = 86 uM
UGT1A4-15; IC50 = 140 uM
UGT1A4-1; IC50 = 110 uM
UGT1A4-5; IC50 = 140 uM



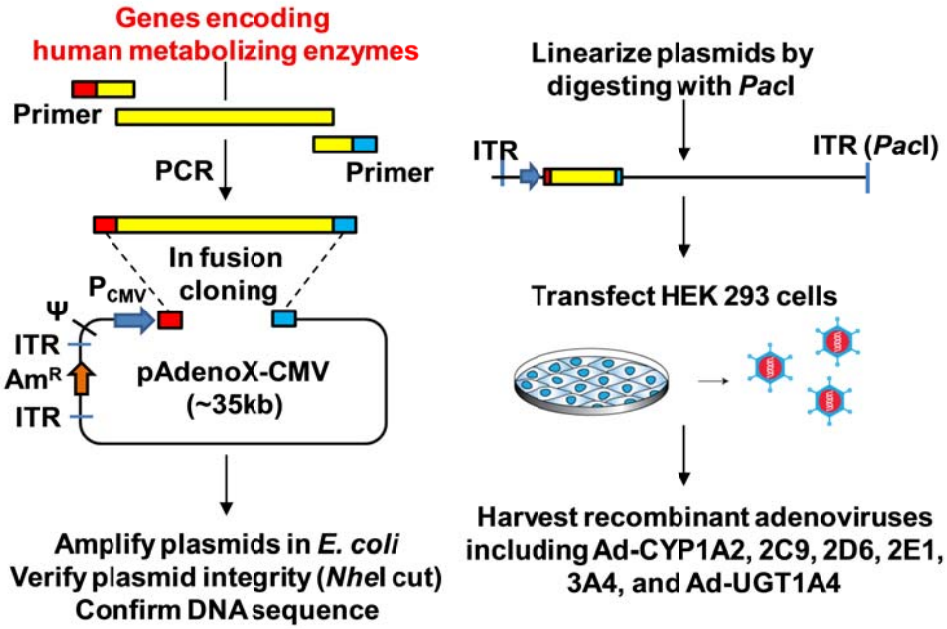
Supplementary Figure 10. Effects of human drug metabolizing enzymes expressed in THLE-2 cells on the toxicity of model compounds. (**a**) Toxicity of six test compounds against THLE-2 cells expressing different levels of CYP3A4 on the chip: (top) scanned image of the chip containing THLE-2 cells exposed to recombinant adenovirus carrying a gene for CYP3A4 (Ad-CYP3A4) and varying doses of the six compounds and (bottom) dose response curves for the compounds. Different levels of CYP3A4 were expressed in THLE-2 cells on the chip by exposing Ad-CYP3A4 at 1, 5, and 15 MOI for 24 h. Adenovirus carrying CMV promoter alone (Ad-Null, no drug metabolizing enzyme expressed) was used as a parent compound control. The CYP3A4-expressing THLE-2 cells were then incubated with six test compounds on the chip for 48 h. (**b**) Toxicity of six test compounds against THLE-2 cells expressing different levels of UGT1A4 on the chip: (top) the scanned image of the chip containing THLE-2 cells exposed to recombinant adenovirus carrying a gene for UGT1A4 (Ad-UGT1A4) and compounds and (bottom) dose response curves for the six compounds. Different levels of UGT1A4 were expressed in THLE-2 cells on the chip by transducing the cells with Ad-UGT1A4 at 1, 5, and 15 MOI for 24 h. Ad-Null was used as a control.



Supplementary Figure 11. Toxicity of doxorubicin and rotenone against THLE-2 cells expressing various CYP450 isoforms on the TeamChip. The scanned image of the chip was obtained after exposing THLE-2 cells to 15 MOI of a single Ad-CYP isoform or a mixture of Ad-CYP isoforms (P450 Mix, 3 MOI each) for 24 h and incubating with compounds for 48 h. Adenovirus carrying CMV promoter alone (Ad-Null, no drug metabolizing enzyme expressed) was used as a parent compound-alone control. The corresponding IC_{50} values are summarized in **Supplementary Table 5**.



Supplementary Figure 12. Scanned image of transduced THLE-2 cells (CYP3A4 and control) on the TeamChip after exposure to model compounds and staining with YO-PRO-1/propidium iodide (top) and quantitative analysis of apoptosis (bottom).



Supplementary Figure 13. Construction of recombinant adenoviruses carrying genes for human drug-metabolizing enzymes.

Supplementary Table 1. Comparison of CYP450 activity in THLE-2 cells transduced with recombinant adenoviruses and in human hepatocytes.

Enzyme	Reaction	CYP450 activity (pmoles/mg protein/minute)	
		Transduced THLE-2 cells[a]	Human hepatocytes[b]
CYP1A2	7-Ethoxyresorufin *O*-deethylation	4.9 ± 1.0*	2.3 ± 2.2
CYP2C9	Diclofenac 4'-hydroxylation	350 ± 40	148 ± 126
CYP3A4	Testosterone 6β-hydroxylation	~280[c]	92 ± 116

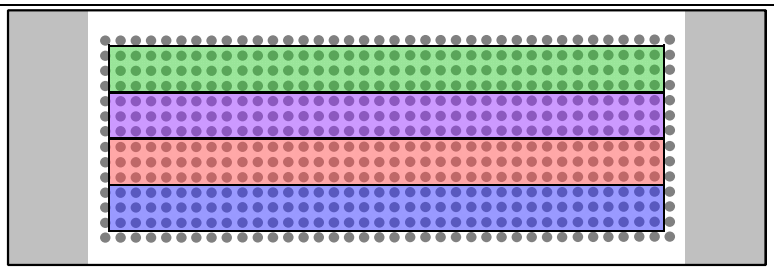
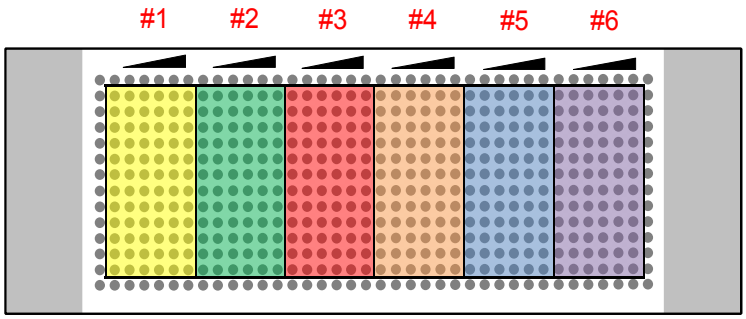
* ± values represent standard deviations (n=3).

[a] The CYP1A2 and CYP2C9 activities in THLE-2 cells were obtained after transducing with 10 MOI of Ad-CYP1A2 and Ad-CYP2C9 for 2 days. The CYP1A2 activity in transduced THLE-2 cells was determined by 7-*O*-deethylation of ethoxyresorufin to resorufin[3, 4]. Diclofenac 4'-hydroxylation activity for CYP2C9 was assayed according via HPLC.[5, 6]

[b] The reported enzyme activities in human hepatocytes were from Gomez-Lechon et al.[7]

[c] Testosterone 6β-hydroxylation activity reported for CYP3A4 in H4IIE cells was ~200 pmoles/mg proteins/minute after transducing with 10 MOI of Ad-CYP3A4 for 2 days.[8] Based on our relative CYP3A4 expression in H4IIE and THLE-2 cells for the same transduction conditions (shown in **Supplementary Figure 4**), we indirectly calculated testosterone 6β-hydroxylation activity in transduced THLE-2 cells.

Supplementary Table 2. The layout of the microwell chip containing four different recombinant adenoviruses to prepare the TeamChip for high-throughput gene transduction, and the additional microwell chip containing six compounds for metabolism-induced toxicity screening.

<table>
<tr><td rowspan="2">Recombinant adenoviruses</td><td>Four different sets of recombinant adenoviruses are printed transversely into four regions on the microwell chip.
Set 1: Ad-CYP2C9, Ad-CYP2D6, Ad-CYP3A4, and Ad-CYP1A2 at 15 MOI each
Set 2: Ad-Null at 15 MOI (no gene control), Ad-UGT1A4 at 15 MOI, a mixture of Ad-CYP1A2, Ad-CYP3A4, Ad-CYP2C9, Ad-CYP2D6, and Ad-CYP2E1 at 3 MOI each (“Ad-P450 Mix”), and a mixture of Ad-P450 Mix and Ad-UGT1A4 at 3 MOI each (“Ad-All Mix”)
Set 3: Ad-Null (no gene control), Ad-CYP3A4 at 1 MOI, Ad-CYP3A4 at 5 MOI, and Ad-CYP3A4 at 15 MOI
Set 4: Ad-Null, Ad-UGT1A4 at 1 MOI, Ad-UGT1A4 at 5 MOI, and Ad-UGT1A4 at 15 MOI</td></tr>
<tr><td>  </td></tr>
<tr><td rowspan="2">Test compounds</td><td>Six different compounds (acetaminophen, bromfenac, flutamide, tamoxifen, trifluoperazine, and troglitazone) are printed vertically into six blocks on the microwell chip (one compound with six increasing dosages in each block from left to right).</td></tr>
<tr><td> 
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</table>

Supplementary Table 3. IC_{50} values (μM) of acetaminophen determined for CYP3A4-expressiong THLE-2 cells in the presence of buthionine sulfoximine (BSO) or ketoconazole.

Test compounds	Drug metabolizing enzymes expressed in THLE-2 cells (15 MOI)	
	No enzyme	CYP3A4
Acetaminophen	> 1500	890 ± 80*
Acetaminophen + BSO (50 μM)	>1500	150 ± 40**
Acetaminophen + ketoconazole (5 μM)	>1500	>1500

- Errors are reported as average deviations (n=3).
- To determine statistically significant IC_{50} difference between no enzyme control and enzyme test conditions, ANOVA analysis was performed and the results were indicated as * for $P < 0.05$ and ** for $P < 0.01$. No indication means $P > 0.05$.

Supplementary Table 4. IC_{50} values (μM) of six test compounds obtained from the TeamChip.

(a)

Test compounds	Metabolic enzymes expressed in THLE-2 cells on the chip			
	No enzyme	CYP3A4 (1 MOI)	CYP3A4 (5 MOI)	CYP3A4 (15 MOI)
Acetaminophen	1400 ± 250	1100 ± 200	950 ± 160*	710 ± 76**
Bromfenac	190 ± 12	250 ± 31	220 ± 15	310 ± 21*
Flutamide	37 ± 9.7	47 ± 8.1	47 ± 12	38 ± 9.5
Tamoxifen	100 ± 9.3	110 ± 19	86 ± 34	110 ± 27
Trifluoperazine	80 ± 9.4	69 ± 18	110 ± 12	120 ± 8.4
Troglitazone	92 ± 6.4	86 ± 4.2	89 ± 7.3	62 ± 6.9*

(b)

Test compounds	Metabolic enzymes expressed in THLE-2 cells on the chip			
	No enzyme	UGT1A4 (1 MOI)	UGT1A4 (5 MOI)	UGT1A4 (15 MOI)
Acetaminophen	1500 ± 220	1500 ± 270	1800 ± 160	2300 ± 250*
Bromfenac	190 ± 5.1	190 ± 29	180 ± 40	220 ± 20
Flutamide	31 ± 6.6	32 ± 3.8	39 ± 11	32 ± 3.8
Tamoxifen	86 ± 7.3	110 ± 4.8	140 ± 8.8**	140 ± 8.6**
Trifluoperazine	72 ± 28	65 ± 15	82 ± 10	110 ± 9.7
Troglitazone	87 ± 12	120 ± 7.1*	130 ± 5.7 *	120 ± 11*

- Errors are reported as average deviations (n=4).
- To determine statistically significant IC_{50} difference between no enzyme control and enzyme test conditions, ANOVA analysis was performed and the results were indicated as * for $P < 0.05$ and ** for $P < 0.01$. No indication means $P > 0.05$.

Supplementary Table 5. IC_{50} values (μM) of doxorubicin and rotenone obtained for CYP450-expressing THLE-2 cells on the TeamChip.

Test compounds	Drug metabolizing enzymes expressed in THLE-2 cells on the chip					
	No enzyme	CYP1A2 (15 MOI)	CYP3A4 (15 MOI)	CYP2C9 (15 MOI)	CYP2D6 (15 MOI)	P450 Mix (each 3 MOI)
Doxorubicin	3.5 ± 1.0	3.9 ± 1.0	4.5 ± 0.5	4.1 ± 0.5	4.0 ± 1.0	4.4 ± 0.5
Rotenone	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1

- Errors are reported as average deviations (n=3).

Supplementary Table 6. Effects of combinatorial expression of human drug metabolizing enzymes on the toxicity of tamoxifen.

Test conditions	Recombinant adenovirus used (MOI)									Relative cell viability (%)
	Ad-Null	Ad-CYP1A2	Ad-CYP3A4	Ad-CYP2C9	Ad-CYP2D6	Ad-CYP2E1	Ad-UGT1A4	Ad-P450 Mix	Ad-All Mix	
1	15									19.6
2	10	5								31.0
3	10		5							21.4
4	10			5						19.8
5	10				5					17.7
6	10					5				25.4
7	10						5			35.5
8	10							5		24.9
9	10								5	13.3
10	5	5					5			62.7
11	5		5				5			68.3
12	5			5			5			47.5
13	5				5		5			31.3
14	5					5	5			32.3
15	5	5						5		46.1
16	5		5					5		41.2
17	5			5				5		40.0
18	5				5			5		30.6
19	5					5		5		29.8
20	5	5							5	18.7
21	5		5						5	17.9
22	5			5					5	21.0
23	5				5				5	29.7
24	5					5			5	29.8
25	5	10								27.7
26	5		10							19.3
27	5			10						26.1
28	5				10					13.0
29	5					10				9.2

30	5	5	5							19.1
31	5	5		5						21.3
32	5	5			5					16.6
33	5	5				5				20.2
34		10					5			100.0
35		5	5				5			91.3
36		5		5			5			65.1
37		5			5		5			60.8
38		5				5	5			41.9
39		10						5		58.9
40		5	5					5		68.9
41		5		5				5		52.8
42		5			5			5		54.2
43		5				5		5		52.8
44		10							5	22.1
45		5	5						5	17.6
46		5		5					5	24.0
47		5			5				5	22.9
48		5				5			5	22.3
49	5		5	5						22.8
50	5		5		5					18.7
51	5		5			5				23.1
52	5			5		5				19.8
53			10				5			78.8
54			5	5			5			50.8
55			5		5		5			36.1
56			5			5	5			30.8
57			10					5		54.8
58			5	5				5		42.5
59			5		5			5		34.8
60			5			5		5		32.7
61			10						5	17.9
62			5	5					5	23.2

63			5		5				5	30.3
64			5			5			5	20.6
65	5			5	5					22.1
66				10			5			33.9
67				5	5		5			20.3
68				5		5	5			19.1
69				10				5		22.0
70				5	5			5		15.2
71				5		5		5		13.5
72				10					5	34.2
73				5	5				5	31.3
74				5		5			5	26.3
75	5				5	5				15.4
76					10		5			11.9
77					5	5	5			10.9
78					10			5		15.9
79					5	5		5		13.6
80					10				5	37.2
81					5	5			5	34.4
82						10	5			17.5
83						10		5		11.4
84							10			5	11.8

The relative viability of multiple drug metabolizing enzyme-expressing THLE-2 cells exposed to 200 μM tamoxifen for 48 h was obtained by normalizing the fluorescence intensity of the THLE-2 cell spots with those of control THLE-2 cell spots incubated in the absence of a compound for 48 h.

Supplementary Table 7. Primer sequences of human drug metabolizing enzymes for the TeamChip.

Human drug metabolizing enzymes	Isoforms	Accession number	Oligonucleotide sequences used for PCR cloning	
Cytochromes P450 (CYP)	CYP1A2	Z00036	Forward	5'-GTAACTATAACGGTCATGGCATTGTCCCAGTCTGT-3'
			Reverse	5'-ATTACCTCTTTCTCCTCAATTGATGGAGAAGCGC-3'
	CYP2C9	AY341248	Forward	5'-GTAACTATAACGGTCATGGATTCTCTTGTGGTCCTTG-3'
			Reverse	5'-ATTACCTCTTTCTCCTCAGACAGGAATGAAGCACAG-3'
	CYP2D6	M20403	Forward	5'-GTAACTATAACGGTCATGGGGCTAGAAGCACTGG-3'
			Reverse	5'-ATTACCTCTTTCTCCCTAGCGGGGCACAGCACA-3'
	CYP2E1	J02625	Forward	5'-GTAACTATAACGGTCATGTCTGCCCTCGGAGTG-3'
			Reverse	5'-ATTACCTCTTTCTCCTCATGAGCGGGGAATG A C
	CYP3A4	D00003	Forward	5'-GTAACTATAACGGTCATGGCTCTCATCCCAGACTT-3'
			Reverse	5'-ATTACCTCTTTCTCCTCAGGCTCCACTTACGGTG-3'
UDP-glucuronosyl transferase	UGT1A4	M84128	Forward	5'-GTAACTATAACGGTCATGGCCAGAGGACTCCAG-3'
			Reverse	5'-ATTACCTCTTTCTCCTCAATGGGTCTTGGATTTGTG-3'

To effectively transduce liver cell lines on the chip platform and mimic a diverse human population, multiple drug metabolizing enzymes must be expressed. Therefore, we set out to construct a set of recombinant adenoviruses carrying genes for drug metabolizing enzymes by using the Adeno-X expression system from Clontech. Adenoviral gene delivery is a highly reliable method to introduce multiple genes into mammalian cells because of transiently high expression, no integration into the cellular genome, and the capability of infecting a wide variety of cell species, such as humans (skin, muscle, bone, nerve, and liver cells), pigs, rodent, mice, and rabbits[9].

Supplementary References

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