

Hepatic CYP3A4 enzyme compensatively maintains endogenous geranylgeranoic acid levels in *MAOB*-knockout human hepatoma cells.

Yuki Tabata^{1,2}, Yoshihiro Shidoji^{2*}

1. Department of Nutrition, Kiryu University, Gunma 379-2392, Japan; tabata-yu@kiryu-u.ac.jp (Y.T.), ORCID: 0000-0002-1117-5215

2. Molecular and Cellular Biology, Graduate School of Human Health Science, University of Nagasaki, Nagasaki 851-2195, Japan; shidoji@sun.ac.jp (Y.S.), ORCID: 0000-0002-2136-6752.

*Correspondence: shidoji@sun.ac.jp

Supplementary Table S1. The nucleotide sequences of each primer used for real-time RT-qPCR.

Genes	Primer	Sequence (5' – 3')
<i>CYP1A1</i>	F	CATCCCCACAGCACAACA
	R	CAGGGGTGAGAAACCGTTCA
<i>CYP1A2</i>	F	AGTCCAGGAGCACTATCAGG
	R	CCTGCTCCAAAGATGTCATT
<i>CYP1B1</i>	F	GCCACTATCACTGACATCTTCGG
	R	CACGACCTGATCCAATTCTGCC
<i>CYP2A6</i>	F	CTCATGAAGATCAGTGAGCGCTAT
	R	CTCCCCGTTGCTGAATACCA
<i>CYP2B6</i>	F	CCACCCTAACACCCATGACC
	R	CCCAGGTGTACCGTGAAGAC
<i>CYP2D6</i>	F	TTCCTGCCTTTCTCAGCAGG
	R	CCAGGAAAGCAAAGACACCA
<i>CYP3A4</i>	F	TATTTTGTCTACCATAAGGGCTTT
	R	AGCACAGGCTGTTGACCATC
<i>CYP3A5</i>	F	GTCTCTCTGTTTCCAAAAGATACC
	R	TGAAGAAGTCCTTGCGTGTC
<i>28S rRNA</i>	F	TTAGTGACGCGCATGAATGG
	R	TGTGGTTTCGCTGGATAGTAGGT

F: forward primer, R: reverse primer

CYP, cytochrome P450.

Supplementary Table S2. The condition of thermal cyclers for real-time RT-PCR.

	Temperature, Duration	Slope
Denature	95°C, 600 s	20°C / s
PCR (40 cycles)	95°C, 15 s	20°C / s
	60°C, 60 s	20°C / s
Melting	95°C, 0 s	20°C / s
	57°C, 15 s	20°C / s
	98°C, 0 s	-
Cooling	40°C, 30 s	20°C / s

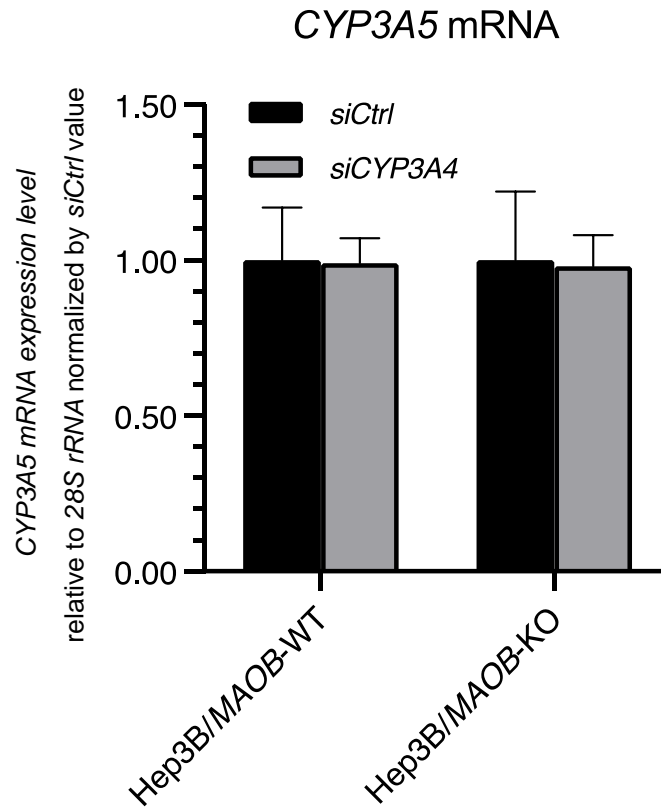
Supplementary Table S3. The condition of thermal cyclers for real-time RT-PCR of *28S rRNA*.

	Temperature, Duration	Slope
Denature	95°C, 600 s	4.4°C / s
PCR (40 cycles)	95°C, 10 s	4.4°C / s
	60°C, 10 s	2.2°C / s
	72°C, 3 s	4.4°C / s
Melting	95°C, 1 s	4.4°C / s
	65°C, 15 s	2.2°C / s
	95°C, 1 s	-
Cooling	40°C, 30 s	4.4°C / s

Supplementary Table S4. The sequences of each siRNA used for knockdown experiments.

		Sequence (5' – 3')
<i>siCYP3A4</i>	Sense A	CCAGAGACCUCAAAUUAUtt
	Antisense A	AGUAAUUUGAGGUCUCUGGtt
	Sense B	CCAAUGGACUGCAUAAAUtt
	Antisense B	UAUUUAUGCAGUCCAUUGGtt
	Sense C	CCAACUGUCUCGAUGCAAUtt
	Antisense C	AUUGCAUCGAGACAGUUGGtt

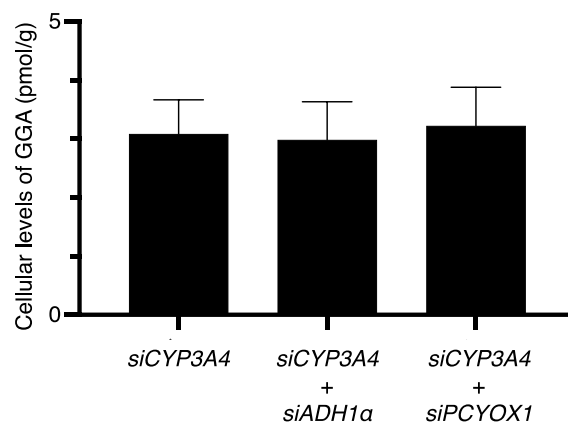
CYP, cytochrome P450.



Supplementary Figure S1.

The expression levels of *CYP3A5* mRNA in Hep3B/*MAOB*-WT cells and Hep3B/*MAOB*-KO cells treated with *CYP3A4* siRNA for 72 h are shown as relative expression level to *CYP3A5* mRNA in *siCtrl*-treated Hep3B/*MAOB*-WT cells.

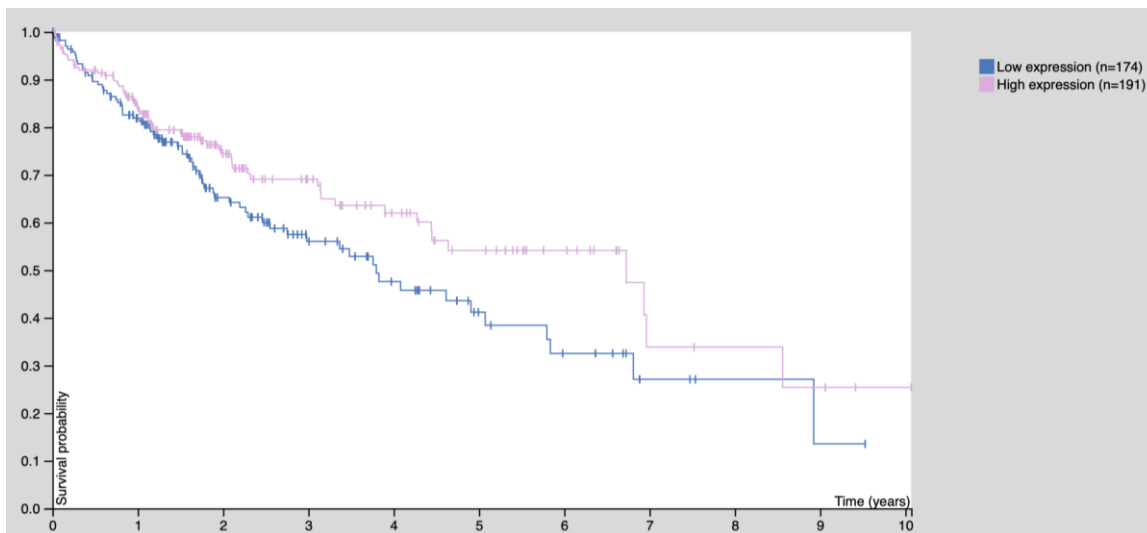
Each bar represents the mean \pm SEM (n = 3).



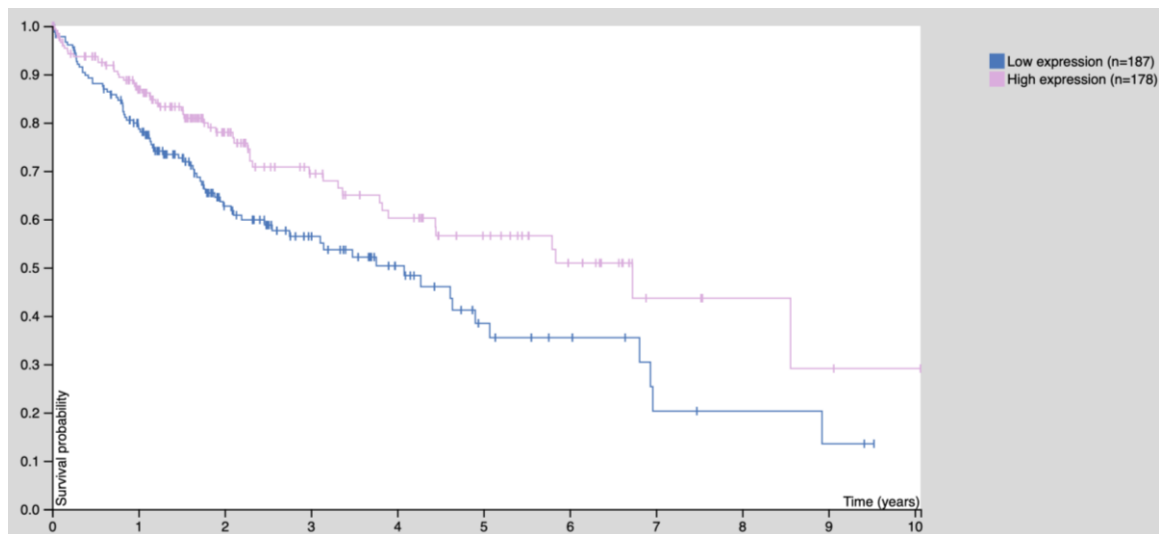
Supplementary Figure S2.

Cellular GGA levels in Hep3B/*MAOB*-KO cells treated with *CYP3A4* siRNA and *ADH1α* siRNA or *PCYOX1* siRNA. Each bar represents the mean \pm SEM ($n = 3$).

(A)



(B)



Supplementary Figure S3.

Prognosis of hepatoma patients between the high and low expression groups of the *MAOB* or *CYP3A4* gene shown by Kaplan-Meier plots.

Image credit: Human Protein Atlas, www.proteinatlas.org, ([32] Uhlén M et al, 2015).

Image available at the following URL:

<https://v21.proteinatlas.org/ENSG00000069535-MAOB/pathology/liver+cancer> (A) and

<https://v21.proteinatlas.org/ENSG00000160868-CYP3A4/pathology/liver+cancer> (B).