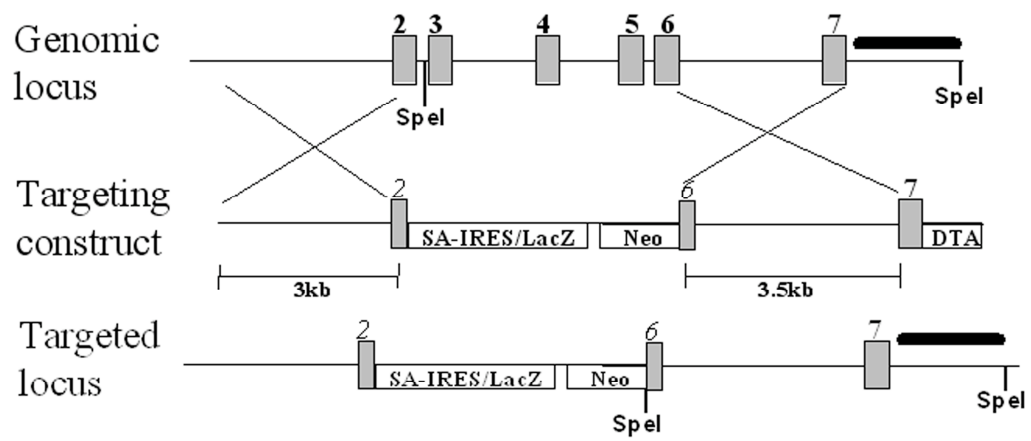
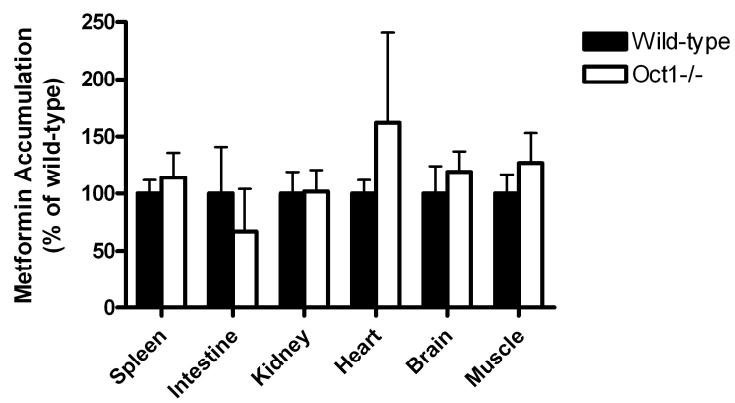


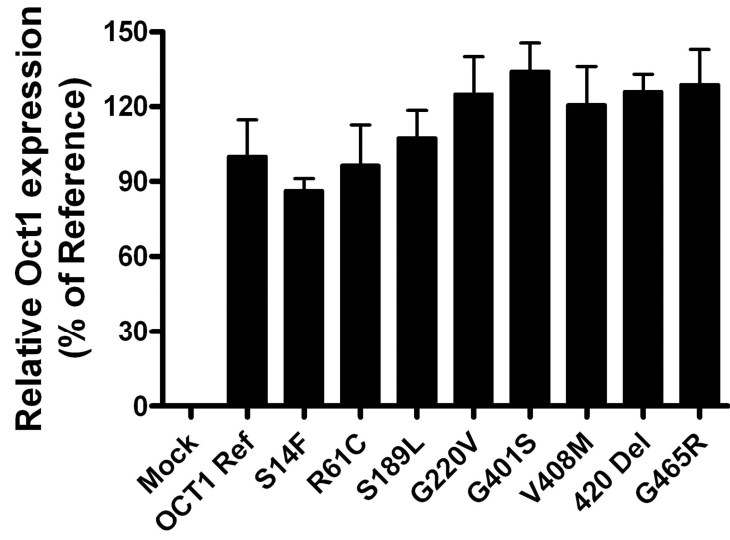
Supplemental Fig 1



Supplemental Fig2



Supplemental Fig3



Supplemental Fig4

SUPPLEMENTAL FIGURES AND TABLES

Figure 1 Time-dependent AMPK phosphorylation in HEK-OCT1 cells and mock cells. The cells were treated with metformin (250 μ M) for 0 – 5.0 hours. Cell extracts were detected with polyclonal antibodies against phospho-AMPK α (Thr172) (top) and β -actin (bottom) respectively.

Figure 2 Targeted disruption of the *Oct1* gene by homologous recombination. The exons 2 - 7 are shown. The strategy results in the complete deletion of exons 3-5 and partial deletion of exons 2 and 6. 5' and 3' homology arms (3kb and 3.5kb respectively) were cloned by proof-reading PCR and placed either side of the IRES-lacZ expression cassette and positive selection cassette to generate the targeting construct. The deleted region is replaced with an IRES-LacZ expression cassette and a positive selection cassette containing the neomycin phosphotransferase gene driven by the PGK promoter. Homologous recombination in neomycin resistant ES cells was confirmed by Southern blot of SpeI digested genomic DNA using a 3' external probe (black bar) which detects 8kb and 6kb bands at the wild-type and targeted locus respectively. Homologous recombination at the 5' end was confirmed in these ES cell clones by Southern blot and both ends were reconfirmed by PCR using primers external to the targeting construct.

Figure 3 Metformin accumulation in different tissues of *Oct1*^{+/+} and *Oct1*^{-/-} mice. The mice (n = 4 per group) were sacrificed 1 hour after the oral dose (15 mg/kg), and the tissues were removed immediately. The radioactivity in tissue homogenates was counted

and converted to mass amount. Data represent mean \pm SD. Liver is not shown here (See Figure 5B in the paper)

Figure 4 *OCT1* transcript levels in cell lines stably expressing human OCT1 and its variants as determined by RT-PCR. The total RNA amount of OCT1 reference and all the variants was similar with a remarkable increase over mock transfected cells. Data are expressed as mean \pm SD for samples analyzed in triplicate.

Figure 5 Phosphorylation of AMPK and ACC in HEK cells of OCT1-reference, R61C and 420del treated with different concentrations of metformin. The cells were treated with the indicated concentrations of metformin for one hour, washed with blank medium, and then incubated for five hours before harvest. Immunoblots were performed against phospho-ACC (Ser 79), phospho-AMPK α (Thr172), AMPK α , and β -actin respectively.

Table 1 Effect of metformin on glucose levels following oral glucose tolerance tests in healthy human volunteers with different *OCT1* genotypes.†

Genotype	Subject	Glucose AUC (min·mg/dL)		C _{30min} (mg/dL)		C _{max} (mg/dL)	
		w/o Met	w/ Met	w/o Met	w/ Met	w/o Met	w/ Met
OCT1- reference	1	21000	18700	130	97	141	121
	3	17700	15900	128	104	130	104
	4	22000	17400	119	112	140	112
	7	20000	20000	155	127	156	127
	9	20600	20900	180	106	180	156
	10	17900	17300	120	114	120	116
	12	19500	17700	150	116	150	116
	16	19400	18300	110	125	143	138
	Average	19800	18300 ^a	137	113 ^a	145	124 ^a
	SD	1500	1600	23.3	10.3	18.0	16.5
OCT1- Variant	2	19900	21200	162	129	162	138
	6	19700	20200	126	126	168	126
	8	21300	19900	122	115	157	126
	11	21400	23500	170	108	170	164
	14	25100	26400	164	128	207	168
	15	17500	19600	129	111	133	130
	17	20600	20300	170	150	170	150
	19	17400	17400	125	114	125	116

20	16400	20900	139	153	143	164
21	17500	21200	110	128	110	142
13	18700	22700	150	148	150	160
18	22400	23100	154	114	166	144
Average	19800	21400 ^{a,b}	143	127 ^{a,b}	155	144 ^b
SD	2500	2300	20.9	15.8	25.4	17.4

†The demographic characteristics and genotype for each volunteer are presented in Table 3. Glucose AUC: the area under the time-plasma glucose concentrations curve; C_{30min}: the plasma glucose concentration 30 minutes after glucose administration; C_{max}: the maximal plasma glucose concentration after glucose administration; w/o Met: without metformin treatment; w/ Met: with metformin treatment. ^a*P* < 0.05 vs. without metformin treatment (paired *Student's t*-test); ^b*P* < 0.05 vs. OCT1-reference (unpaired *Student's t*-test).

Table 2 The characteristics and *OCT1* polymorphisms of human healthy volunteers in the clinical study.

Subject No.	Polymorphism ^a	Body Weight (kg)	Height (cm)	Age (year)	Gender ^d
1	Reference	82.1	186	28	M
3	Reference	58.7	169	36	F
4	Reference	67	175	25	M
7	Reference	64.7	162	40	F
9	Reference	82.2	185	36	M
10	Reference	76.6	179	31	M
12	Reference	68.2	177	27	F
16	Reference	52.2	171	27	F
Subtotal		68.9 ± 10.8	176 ± 8.1	31.3 ± 5.4	4M, 4F
2	R61C	102	188	35	M
6	G401S	80	184	34	M
8	R61C ^b	97.1	186.3	27	M
11	R61C	60.9	163	34	F
13	420Del	67.9	173	35	F
14	G465R,420Del	66.3	160	26	F
15	G465R,420Del	74.2	159	26	F
17	R61C	75.1	186	27	M
18	G174S ^c ,420Del	79.1	168	40	F

19	G465R,420Del	62.2	162	32	F
20	G401S	69	183	25	M
21	G401S	55.4	157	19	F
Subtotal		74.1 ± 14.0	173 ± 12.3	30.0 ± 5.9	6M, 6F

^aIndividuals who carried any of the four polymorphisms, OCT1-R61C, OCT1-G401S, OCT1-420del and OCT1-G465R, are referred to as individuals with an OCT1-variant, and those who had the reference allele at all four positions are termed individuals with OCT1-reference alleles. Except subject 8, all other subjects were heterozygotes for the polymorphisms studied.

^bHomozygote for R61C.

^cFound in a volunteer after re-sequencing, and not determined on the cellular phenotype of OCT1 function (MPP⁺ and metformin uptake).

^dM: male; F: female

Table 3 Primers used in RT-PCR to detect OCT1, OCT2 and GAPDH.

Gene	Species	Primers (5'→ 3')
OCT1	Mouse	Sense: TTGGAGAGTTTGGCTGGTTC
		Anti-sense: CACCAGGAGGCAGAGCTTAC
	Rat	Sense: TTGGAGAGTTTGGCTGGTTC
		Anti-sense: CACCAAGAGACAGAGCTTAC
	Human	Sense: CTGTGTAGACCCCCTGGCTA
		Anti-sense: GTGTAGCCAGCCATCCAG
OCT2	Mouse	Sense: AACCTTCGTTCTGGACTT
		Anti-sense: GTTGACCAGGCAGACCATTT
	Rat	Sense: CTCAGCCAGTGCATGAGGTA
		Anti-sense: AAAGCGAAACACCAACATCC
	Human	Sense: CCTGGTATGTGCCAACTCCT
		Anti-sense: CACCAGGAGCCCAACTGTAT
GAPDH	Mouse	Sense: GGGTGTGAACCACGAGAAATATG
		Anti-sense: GAAGGCCATGCCAGTGAGC
	Rat	Sense: GGGTGTGAACCACGAGAAATATG
		Anti-sense: GAAGGCCATGCCAGTGAGC
	Human	Sense: AATCCCATCACCATCTTCCA
		Anti-sense: TGTGGTCATGAGTCCTTCCA