Supplementary figures to:

Cellular uptake of imatinib into leukemic cells is independent of human organic cation transporter 1 (OCT1)

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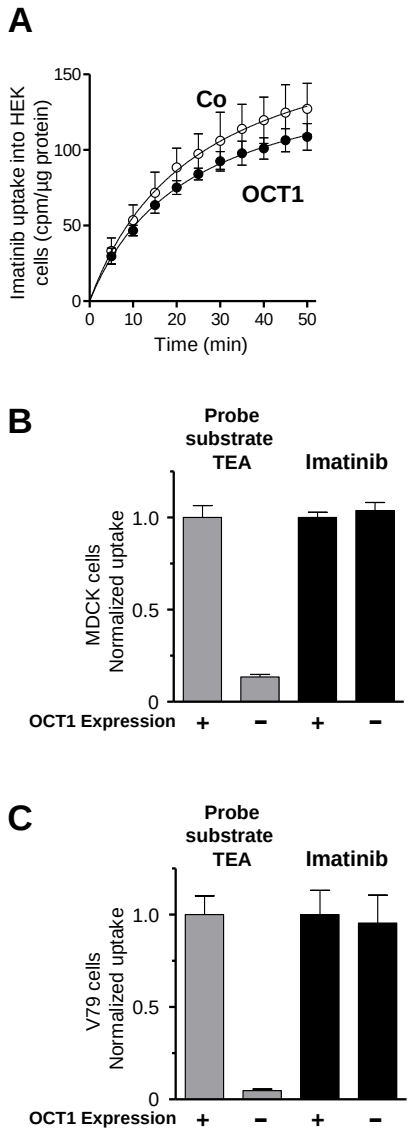
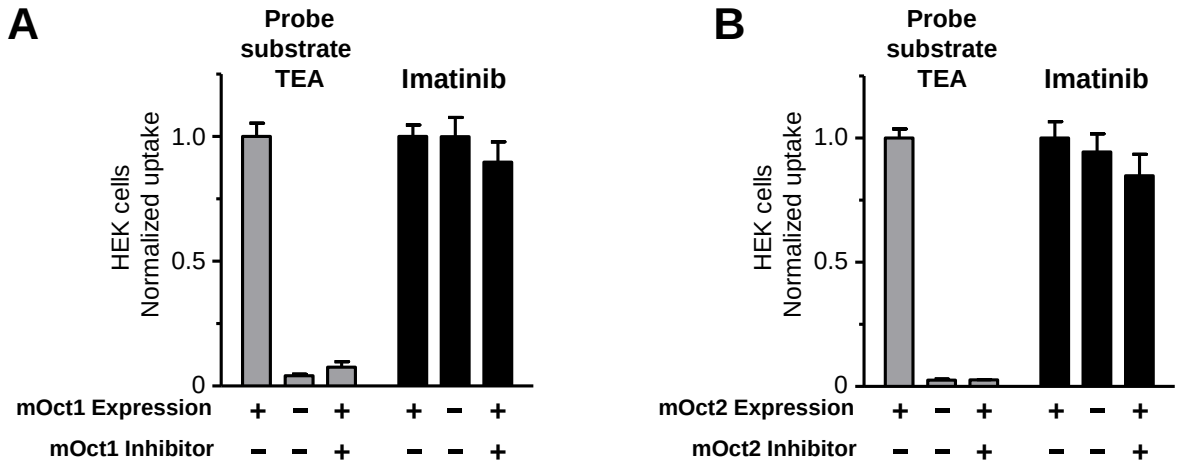
Supplementary Figure S1



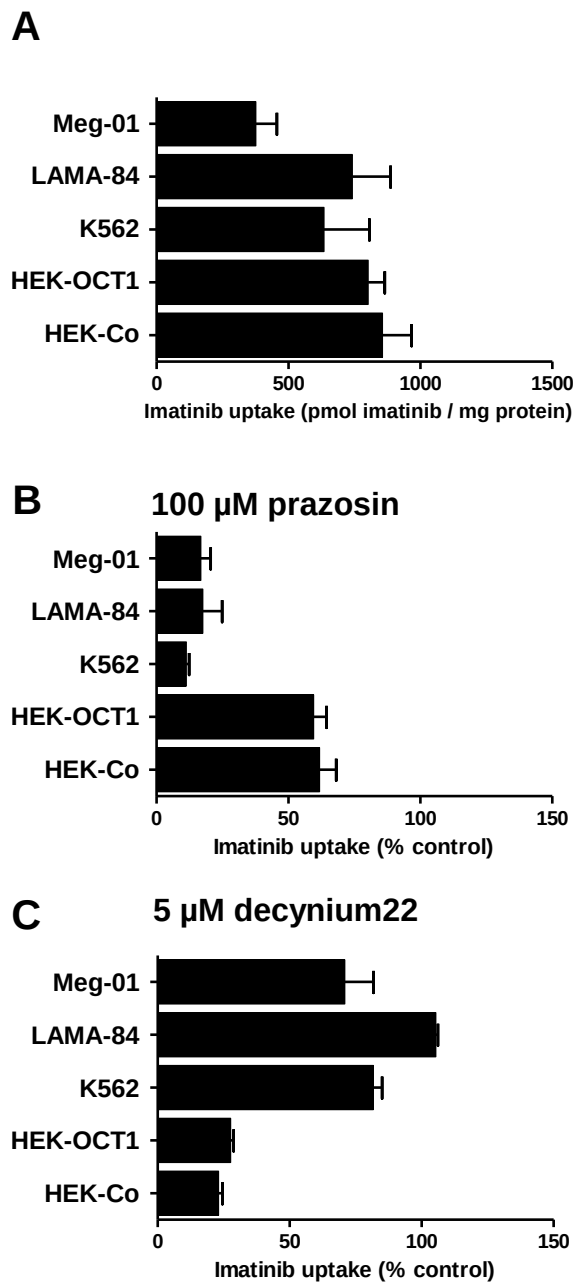
Uptake of imatinib and OCT1 probe substrate TEA into OCT1-expressing cells and vector-transfected control cells. (A) Time-dependent accumulation of [^{14}C]imatinib (10 µmol/L) into OCT1-expressing HEK cells and vector-transfected control cells (Co) was measured in real-time using the scintillation proximity assay (Lohmann et al., Anal Biochem 2007;366:117-25). Data are means ± SD of 3 determinations. B and C, uptake of probe substrate [^{14}C]TEA (50 µmol/L) or [^{14}C]imatinib (3 µmol/L) into OCT1-expressing MDCK cells or vector-transfected MDCK cells (no OCT1 expression) and into OCT1-expressing V79 cells or parental V79 cells (no OCT1 expression) was measured after 5 minutes. Data are means ± SE of 3 determinations.

Supplementary Figure S2



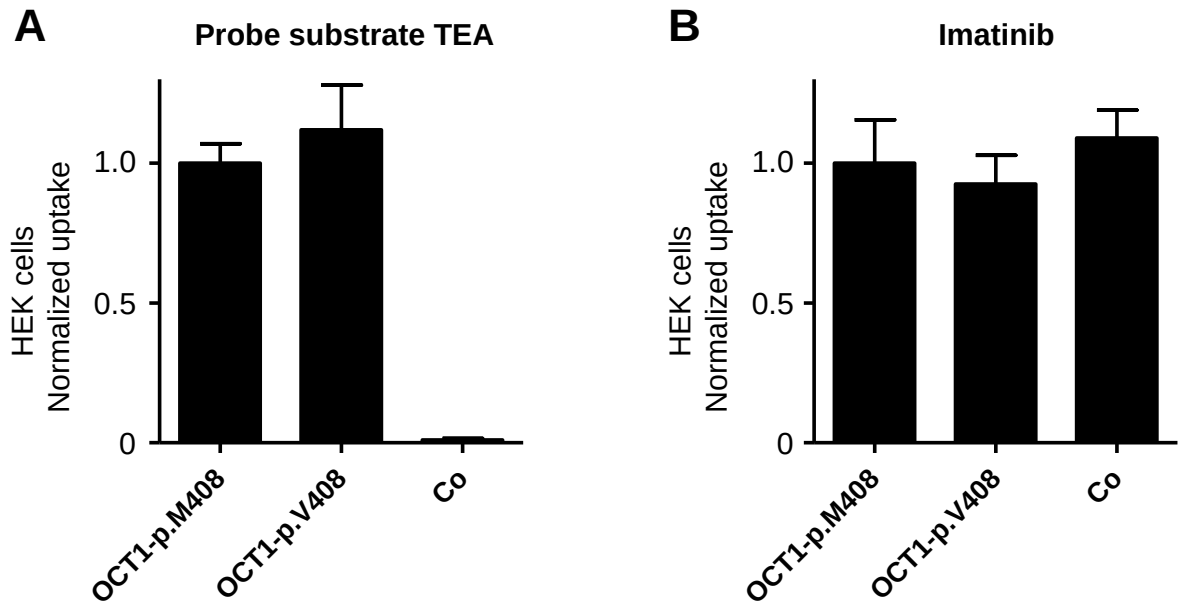
Cellular uptake of the OCT1 probe substrate TEA and imatinib into mOct1- (A) or mOct2-expressing HEK293 cells (B). Uptake of [^{14}C]TEA (100 µmol/l) or [^{3}H]imatinib (2 µmol/L) into mOct1- or mOct2-expressing HEK293cells or vector-transfected HEK293cells (no mOct1 or mOct2 expression) was measured after 5 minutes in the absence (-) or presence (+) of the OCT inhibitor decynium22 (5 µmol/L). Data are means ± SE of 3 determinations.

Supplementary Figure S3



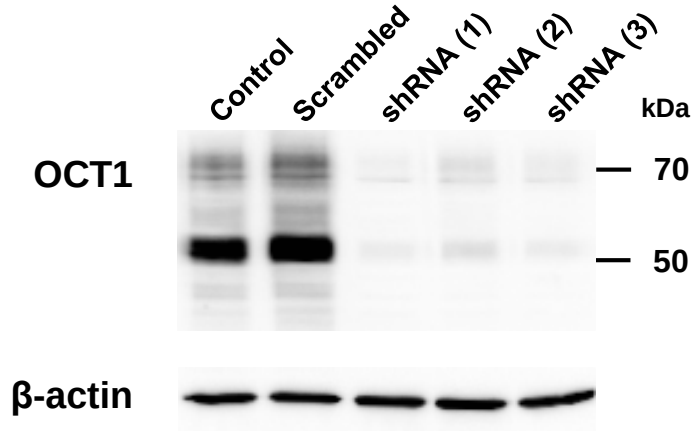
Cellular uptake of imatinib (2 µmol/L) into the CML cell lines Meg-01, LAMA-84 and K562 as well as into OCT1-expressing HEK cells and vector-transfected control cells (Co) was measured after an incubation period of 120 minutes. A, imatinib uptake in the absence of any inhibitor. B, inhibition of imatinib uptake in the presence of prazosin (100 µmol/L) or C, of decynium22 (5 µmol/L). Data are given as % of control in the absence of the respective inhibitor. Data are means ± SE of 3 determinations performed in triplicates.

Supplementary Figure S4



Cellular uptake of the OCT1 probe substrate TEA (A, 100 µmol/L; means ± SD of 3 determinations) and imatinib (B, 2 µmol/L; means ± SE of 2 determinations performed in triplicate) into HEK cells expressing variant p.V408 in comparison to HEK cells expressing reference sequence p.M408 and vector-transfected control cells (Co) after an incubation time of 10 minutes.

Supplementary Figure S5



Validation of knockdown of OCT1 protein expression by shRNA plasmids. According to the manufacturer's instructions (OriGene), HEK cells were transiently transfected with 1 µg of *OCT1/SLC22A1*-expressing plasmid (control) or with 1 µg of *OCT1/SLC22A1*-expressing plasmid together with 1 µg of scrambled shRNA plasmid or different OCT1-targeting shRNA plasmids (shRNA 1-3). After 72-h incubations, membrane fractions were prepared and immunoblotting was performed as described (Nies et al., Hepatology 2009; 50:1227-40). Membrane fractions (15 µg protein) were analyzed for OCT1 and β-actin expression by immunoblot analysis.