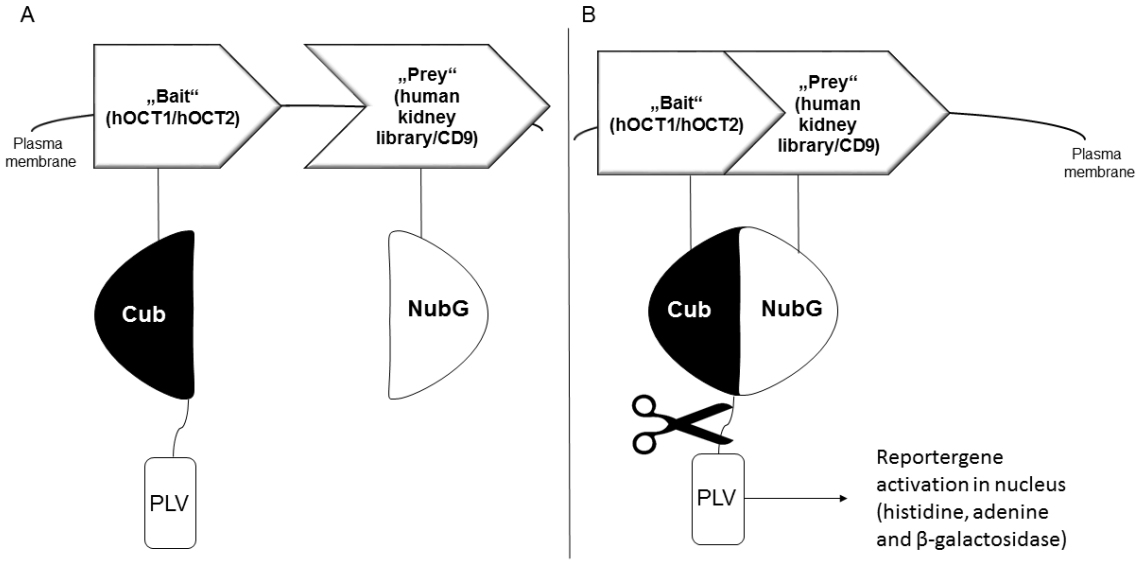
Supplemental materials

Table 1. Primers used in this study

Primer for cloning of His-tag/GFP-tag CD9 and GFP/CFP-tags for hOCT2 (5' to 3')	
CD9-Mlu-F	CTC TAA CGC GTA TGC CGG TCA AAG
CD9-NotI-R	GAA TTG CGG CCG CCT AGA CCA TC
CD9-Xho-F	GAA TTC CTC GAG ATG CCG GTC AAA G
CD9-PacI-R	CTC GCT TAA TTA ACT AGA CCA TCT CGC
hOCT2GFP-F	CTC AGA TCT CGA GCT ATG CCC ACC ACC GTG GAC GAT
hOCT2GFP-R	CGG GAT GGA TCC GTT CAA TGG AAT GTC TAG TTT
Primer for mbSUS (5' to 3')	
hOCT1-B1-F	ACA AGT TTG TAC AAA AAA GCA GGC TCT CCA ACC ACC ATG ATG CCC ACC GTG GAT GAC ATT
hOCT1-B2-R	TCC GCC ACC ACC AAC CAC TTT GTA CAA GAA AGC TGG GTA GGT GCC CGA GGG TTC TGA GGT
hOCT2-B1-F	ACA AGT TTG TAC AAA AAA GCA GGC TCT CCA ACC ACC ATG ATG CCC ACC ACC GTG GAC GAT
hOCT2-B2-R	TCC GCC ACC ACC AAC CAC TTT GTA CAA GAA AGC TGG GTA GTT CAA TGG AAT GTC TAG TTT
CD9-B1-F	ACA AGT TTG TAC AAA AAA GCA GGC TCT CCA ACC ACC ATG CCG GTC AAA GG
CD9-B2-R	TCC GCC ACC ACC AAC CAC TTT GTA CAA GAA AGC TGG GTA GAC CAT CTC GC
hOCT2-168 aa-B2-R	TCC GCC ACC ACC AAC CAC TTT GTA CAA GAA AGC TGG GTA GTA GCC GAT ACT CAT AGA GC
hOCT2-212 aa-B2-R	TCC GCC ACC ACC AAC CAC TTT GTA CAA GAA AGC TGG GTA AAG GTG AGA GCG GGA
hOCT2-266 aa-B2-R	TCC GCC ACC ACC AAC CAC TTT GTA CAA GAA AGC TGG GTA CTG CAA CCA CCT CCA GTG AGG
hOCT2-379 aa-B2-R	TCC GCC ACC ACC AAC CAC TTT GTA CAA GAA AGC TGG GTA ATC CAG GTA GAT ATT GTC

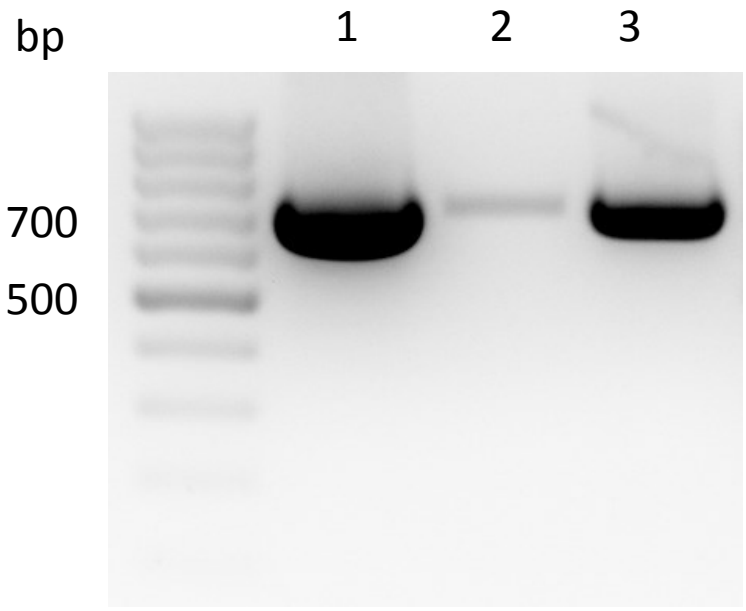
Supplemental materials – Figure 1



This figure describes the mating-based split-ubiquitin system (mbSUS).

The hOCT1 or the hOCT2 fused to the C-terminal end of ubiquitin (C_{ub}) represents the “bait”, while a kidney library or a potential interacting protein identified in the screening, in this example CD9, fused to the N-terminal end of ubiquitin (N_{ub}) represents the “prey” (panel A). While in this case the “bait” is a plasma membrane protein, the “prey” can be a plasma membrane or a cytosolic protein. The “bait” is contructed by the insertion of hOCT1 or hOCT2 into the pMetYCgate vector, which contains also an artificial transcription factor (protein-A-LexA-VP16 - PLV). Upon “bait-prey” interaction (panel B), a functional ubiquitin is reconstituted. The reconstituted ubiquitin molecule is recognized by ubiquitin-specific proteases (the scissors in panel B), which cleaves the PLV. The PLV migrates into the nucleus, where it activates the transcription of reporter genes, which allow the yeast to grow on media lacking certain essential amino acids (in this example histidine and adenine). Activation of lacZ gene transcription leads to the β-galactosidase synthesis. This enzyme converts the substrate X-gal into a blue compound, allowing its use as a further indicator of “bait-prey” interaction.

Supplementary materials Figure 2.



CD9 expression in human proximal tubules. PCR analysis of CD9 expression in human kidneys and in freshly isolated human proximal tubules. Pieces of normal human renal tissue surrounding the tumor were obtained from urologic patients subjected to tumor nephrectomy, after obtaining written consent from each patient, as approved by the ethics commission of the Universitätsklinikum Münster. Immediately after nephrectomy, kidney pieces were cut and collected into chilled HCO_3-free phosphate buffer. Proximal tubules were isolated from kidney samples according to a method, which we previously described [1]. Total RNA from human renal tissue or isolated proximal tubules were isolated using RNeasy-kit (Qiagen, Hilden, Germany). For cDNA synthesis, 2 µg total RNA was used with the SuperScript-III First-Strand Synthesis SuperMix (Invitrogen, Karlsruhe, Germany). PCR experiments with CD9 specific primers demonstrated significant expression of CD9 in a CD9 plasmid used as positive control (lane 1) and in whole human kidneys (lane 3). A tiny CD9 signal was also evident in isolated human proximal tubules (lane 2). The marker lines are also presented with bp = base pairs.

Reference List

1. Pietig, G.; Mehrens, T.; Hirsch, J. R.; et al. Properties and Regulation of Organic Cation Transport in Freshly Isolated Human Proximal Tubules. *J. Biol. Chem.* **2001,** *276*, 33741-33746.