IDENTIFICATION OF KEY RESIDUES DETERMINING SPECIES DIFFERENCES IN INHIBITOR BINDING OF MICROSOMAL PROSTAGLANDIN E SYNTHASE-1

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SUPPLEMENTAL FIGURES

Figure S1. **Amino acids that differ between human and rat MPGES1 point mainly towards the membrane.** (A) Side view from the membrane plane (ER lumen on the top, cytosol on the bottom) and (B) top view from the luminal side of the endoplasmic reticulum on MPGES1. One of the subunits in the MPGES1 trimer is coloured in blue. Numbers of the transmembrane helices are indicated. Almost all residues that differ between human and rat MPGES1 point towards the phospholipid bilayer of the membrane. GSH is depicted in white as a U-shaped molecule, indicating the location of the putative active site.

Figure S2. **Rat and mouse MPGES1 share unique residues at the entrance to the active site and constitute an own subgroup in the phylogenetic tree.** (A) Amino acid sequences of human MPGES1 and available orthologous rodent enzymes were aligned using ClustalW. Sequences were obtained from http://www.ensembl.org, accession numbers: human (Homo sapiens), ENST00000340607; Guinea pig (Cavia porcellus), ENSCPOT00000011151, Kangaroo rat (Dipodomys ordii), ENSDORT00000003233; Mouse (Mus musculus), ENSMUST00000102852; Pika (Ochotona princeps), ENSOPRG00000014131; Rat (Rattus norvegicus), ENSRNOT00000008407; Squirrel (Spermophilus tridecemlineatus), ENSSTOT00000004897. Every 10^{th} residue is indicated by underlined residues. Based on the known structure of MPGES1 the positions of the four transmembrane helices were assigned. The consensus sequence denotes identical residues (*), strong conservation (:), weak conservation (.), and unconserved replacements (blank). Within the group of rodent sequences the three residues identified to be crucial for inhibitor binding are unique in rat and mouse MPGES1 and highlighted in the alignment with coloured background. All other residues that differ from the human sequence are highlighted with grey background. (B) Dendrogram showing the evolutionary relationship of the compared sequences of rodent and human MPGES1, generated by ClustalW. Rat and mouse MPGES1 cluster into an own group and are hence more distantly related to the human enzyme than other rodent orthologues.

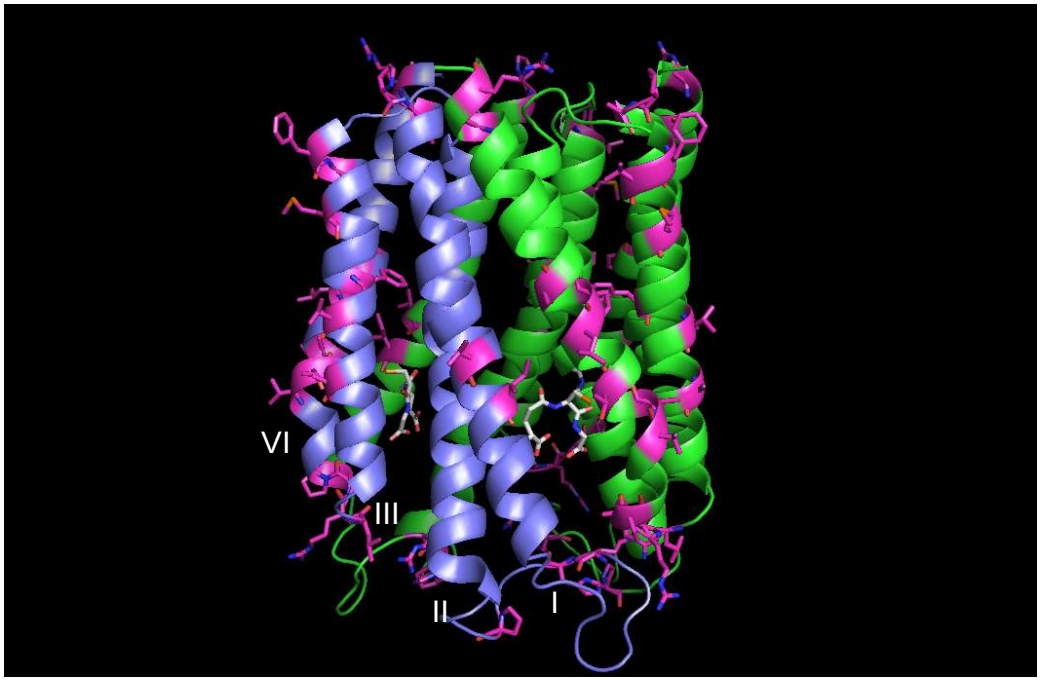
Figure S3. **The inhibitor binding site is conserved within the MAPEG superfamily.** (A) FLAP (PDB ID code 2Q7M) was co-crystallised with the inhibitor MK-591 (depicted as stick model with carbon atoms coloured in yellow) (1). Residues in TM1, TM2, TM4 and the second cytosolic loop were identified to make contact with MK-591. They are shown as stick models with carbon atoms coloured in blue and magenta. (B) We identified residues Thr-131, Leu-135, and Ala-138 (represented in stick model with carbon atoms coloured in magenta) to be important for inhibitor binding in MPGES1 (PDB ID code 3DWW). Residues Thr-131 and Ala-138 in MPGES1 correspond to residues Ile-113 and Leu-120, respectively, which are involved in binding of MK591 and coloured in magenta in (A). Note that TM4 in FLAP is much shorter than in MPGES1 and Ile-113 is thus situated in the second cytosolic loop of FLAP, whereas Thr-131 is situated in TM4 of MPGES1.

SUPPLEMENTAL REFERNCES

1. Ferguson, A. D., McKeever, B. M., Xu, S., Wisniewski, D., Miller, D. K., Yamin, T. T., Spencer, R. H., Chu, L., Ujjainwalla, F., Cunningham, B. R., Evans, J. F., Becker, J. W. (2007) *Science* **317**, 510-512.

Figure S1

A



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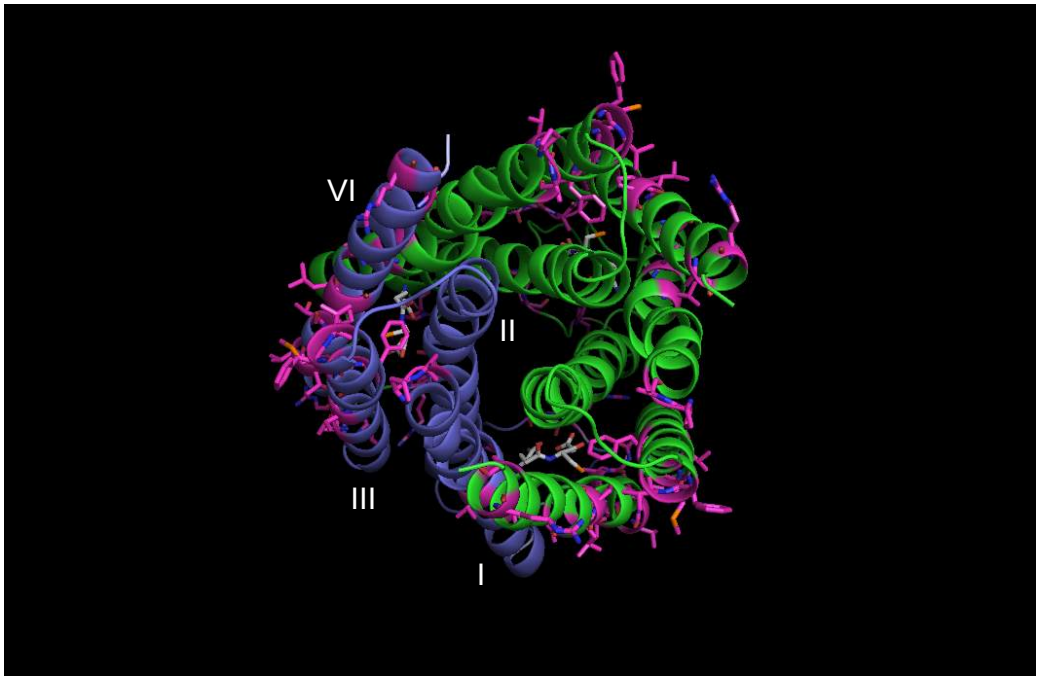
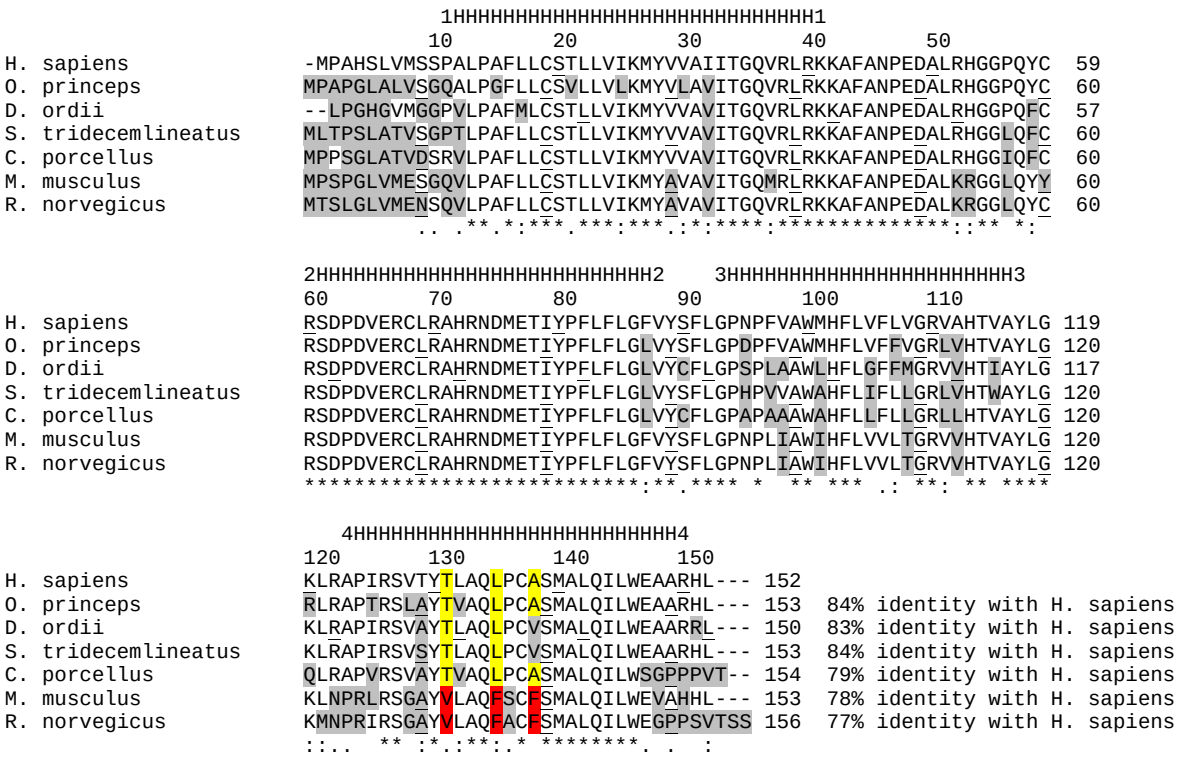


Figure S2

A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2HHHHHHHHHHHHHHHHHHHHHHHHHHH2
3HHHHHHHHHHHHHHHHHHHHHHH3
4HHHHHHHHHHHHHHHHHHHHHHHH4
H. sapiens
O. princeps
D. ordii
S. tridecemlineatus
C. porcellus
M. musculus
R. norvegicus
84% identity with H. sapiens
83% identity with H. sapiens
84% identity with H. sapiens
79% identity with H. sapiens
78% identity with H. sapiens
77% identity with H. sapiens

B

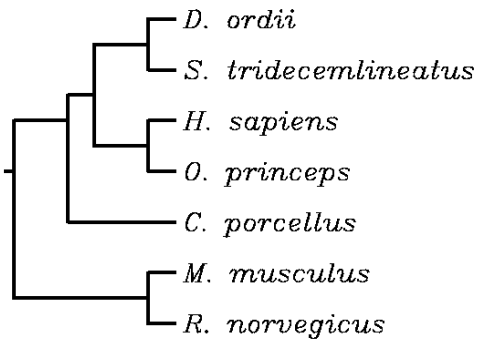
D. ordii
S. tridecemlineatus
H. sapiens
O. princeps
C. porcellus
M. musculus
R. norvegicus

Figure S3

A **B**