Electronic Supplementary Material (ESI) for Metallomics.

Supporting Information

A recombinant platform to characterize the role of transmembrane protein hTMEM205 in Pt(II)-drug resistance and extrusion

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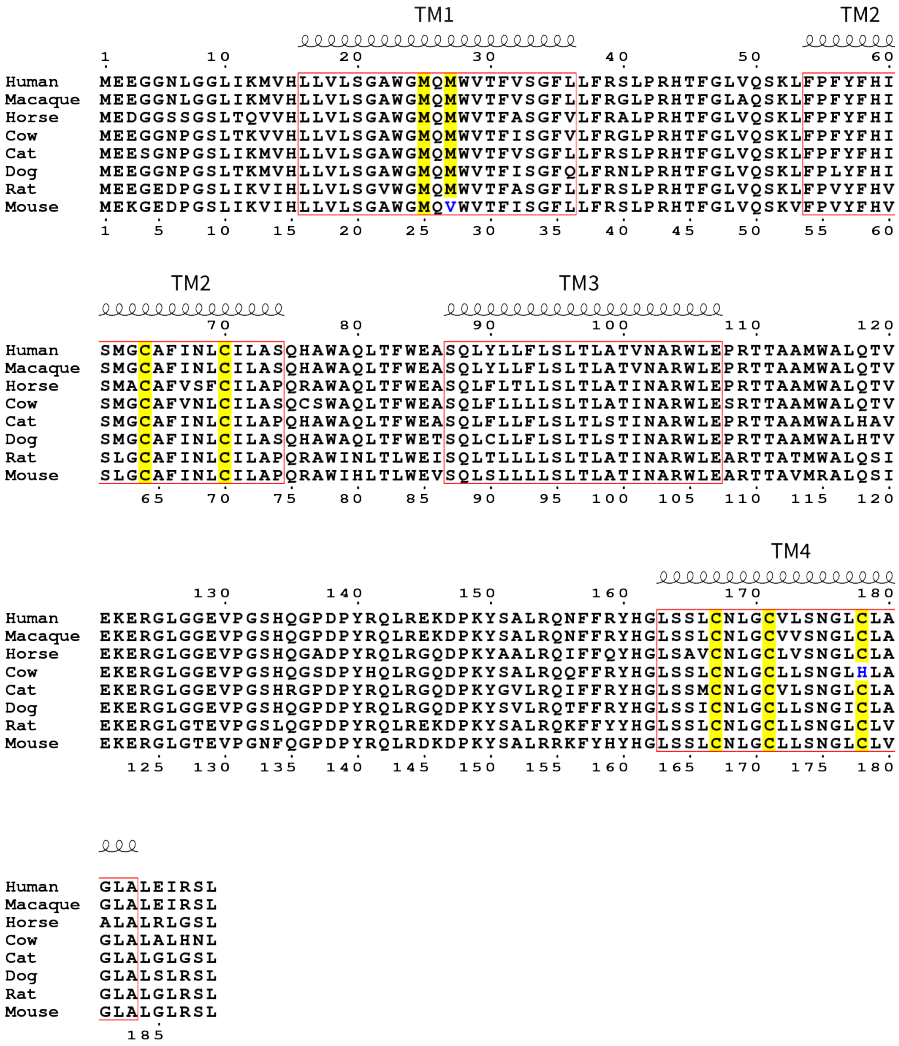
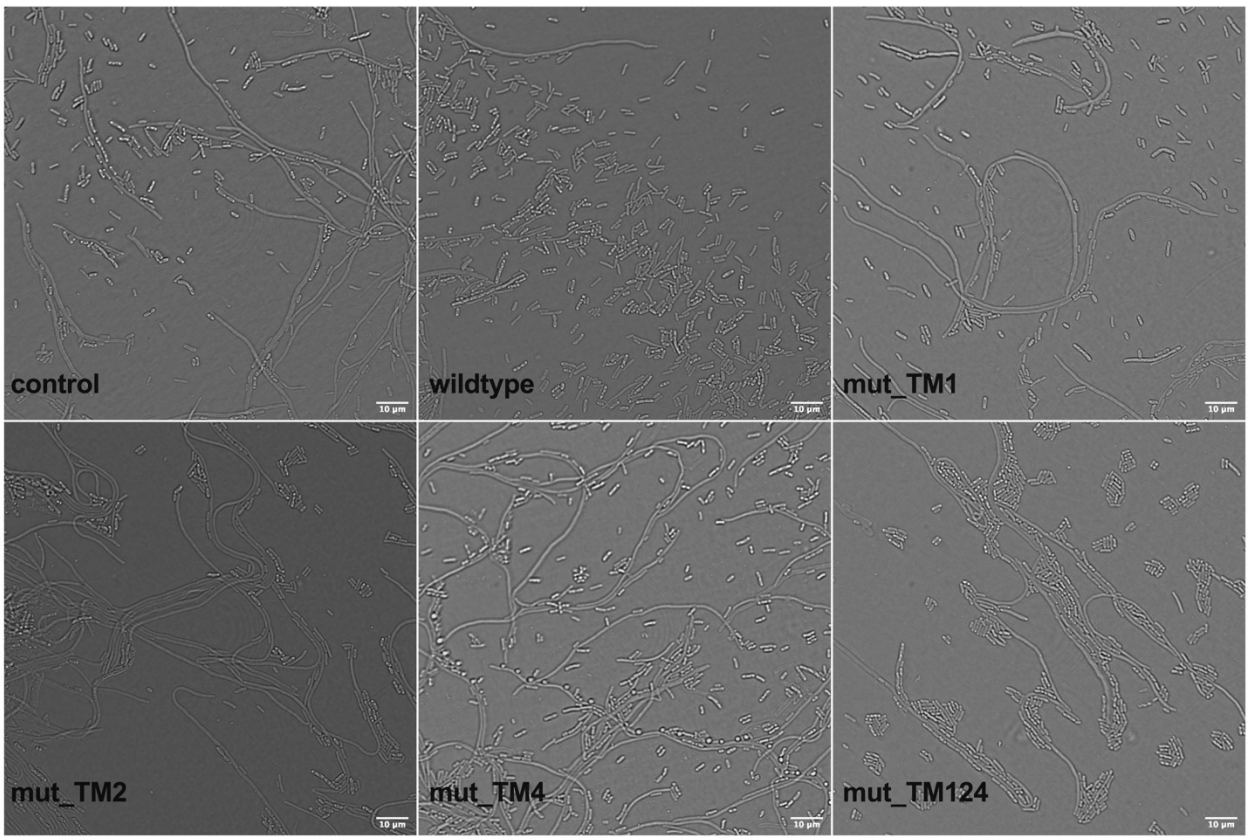
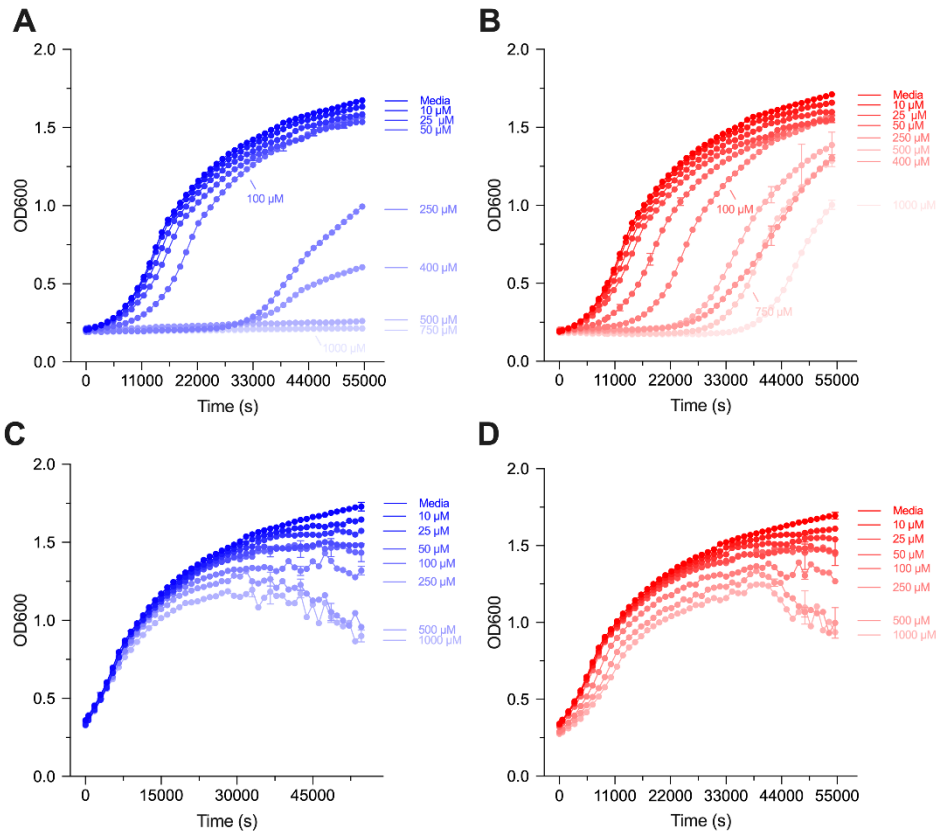
Supplementary Figures



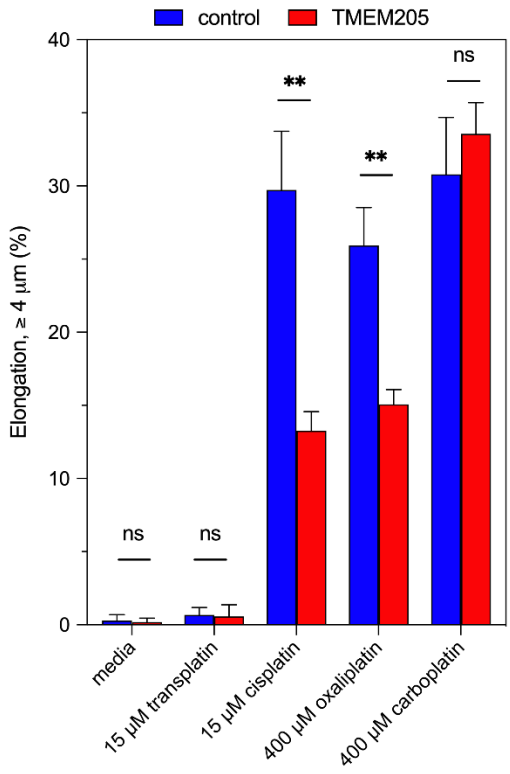
Supplementary Fig. 1. Sequence alignment of selected mammalian hTMEM205 orthologues. Uniprot accession numbers: Human (*Homo sapiens,* Q6UW68), Macaque (*Macaca mulatta,* H9YY46), Horse (*Equus caballus,* F7CLB0), Cow (*Bos taurus,* Q32L10), Cat (*Felis catus*, A0A337S1G6), Dog (*Canis Familaris*, E2RJL2), Rat (*Rattus norvegicus*, D3ZJZ0), Mouse (*Mus musculus*, Q91XE8). Transmembrane cysteine and methionine residues are highlighted in yellow while transmembrane helices obtained by topology predictions with TOPCONS are framed in a red box. The figure was generated using Espript 3.0 (http://espript.ibcp.fr/ESPript/ESPript/).



Supplementary Fig. 2. Representative raw confocal images of hTMEM205 *E. coli* cells and its mutants, after an 8 h exposure to 15 µM cisplatin (scale bar = 10 µm). Elongation analysis was performed on at least three biological replicates and at least two different image fields each.



Supplementary Fig. 3. Representative growth curves of control (blue) and hTMEM205 (red) *E. coli* cells in a spectrum of concentrations of oxaliplatin (A, B) and carboplatin (C, D). Data are mean ± s.d of three technical triplicates of one biological replicate. Three biological replicates were conducted, and analysis is reported in Fig. 3A and 3B.



Supplementary Fig. 4. Percent elongation of control vs. TMEM205 in different conditions. Four µm was assigned as the threshold for elongation. Data represents mean and s.d. of at least three biological replicates. Significance is expressed as $P > 0.05$ (ns) and $P < 0.01$ (**).

Supplementary Table

Mutant Name	Mutation positions	TM 1	TM 2	TM 4
Mut_TM1	M25A_M27A	**X**		
Mut_TM2	C64A_C70A		**X**	
Mut_TM4	C167A_C171A_C178A			**X**
Mut_TM124	M25A_M27A_ C64A_C70A_ C167A_C171A_C178A	**X**	**X**	**X**

Supplementary Table 1: hTMEM205 mutants in conserved cysteine and methionine transmembrane (TM) residues and corresponding location in TM helices according to the topology model.

Supplementary Video

Supplementary Video 1. Time-lapse of control and hTMEM205 *E. coli* cells growing in 50 μM cisplatin at 8 fps. Images were taken every 2 min for 5 h at 30 °C.