

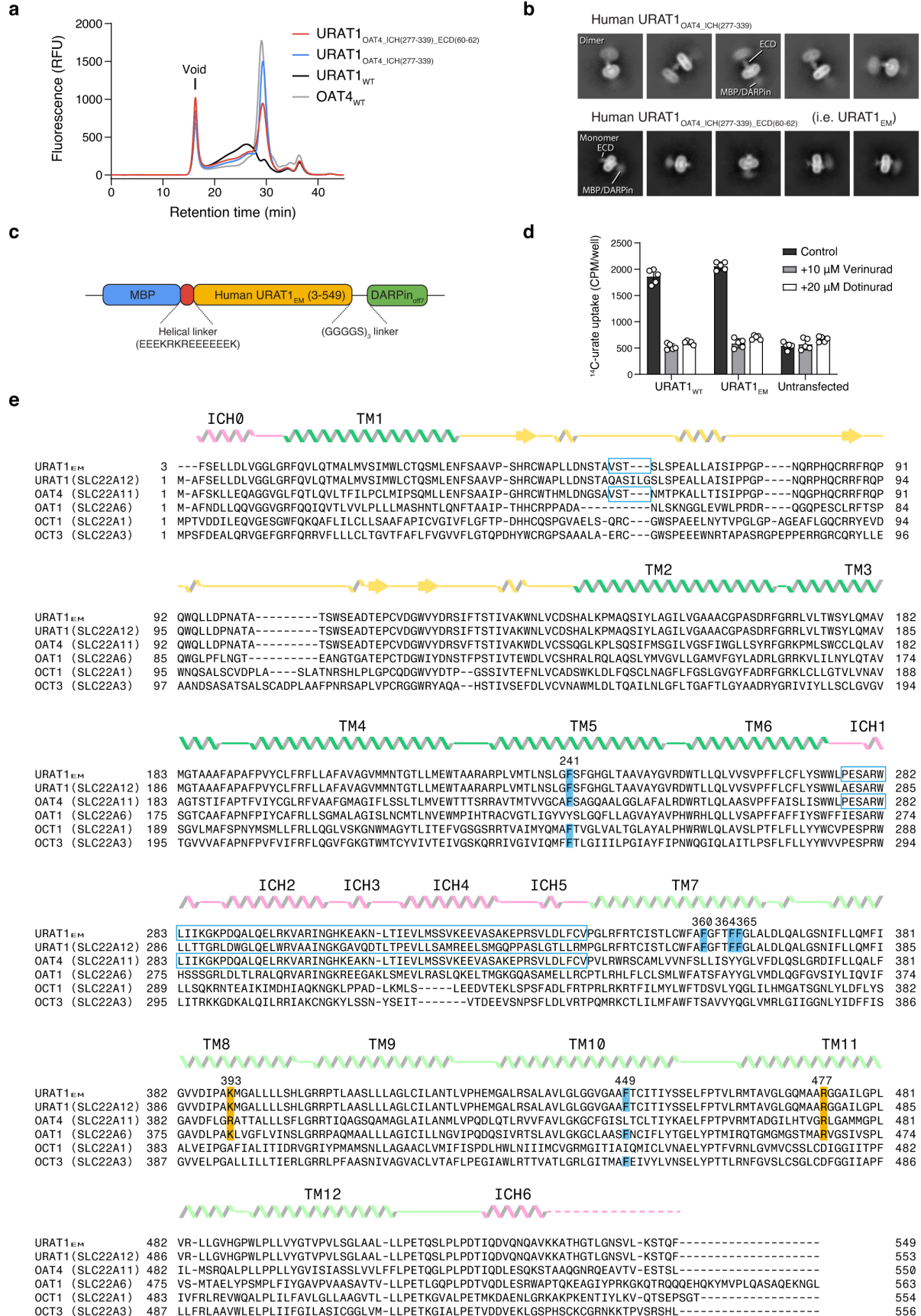


Fig. S1 Construct characterization and sequence alignment of URAT1

a Fluorescence size-exclusion chromatography of URAT1 and OAT4. **b** Representative 2D class averages of URAT1 constructs. **c** Schematic of the URAT1_{EM} construct. **d** ¹⁴C-urate uptake and drug inhibition of the wild-type transporter (URAT1_{WT}) and the cryo-EM construct (URAT1_{EM}). Uptake buffer: 20 mM HEPES pH 7.4, 125 mM sodium gluconate, 4.8 mM potassium gluconate, 1.2 mM monobasic potassium phosphate, 1.2 mM magnesium sulfate, 1.3 mM calcium gluconate, and 5.6 mM glucose. Graph shows individual data points, mean ± s.d.; n = 5 biological replicates. **e** Sequence alignment of the selected members from the SLC22 family. Blue shade, residues that form the phenylalanine cage; yellow shade, key gating residues that interacts with urate and anti-gout drugs. Blue box highlights the region of construct modification.