**Supplementary Information**

# Positively charged residues at the channel mouth boost single-file water flow

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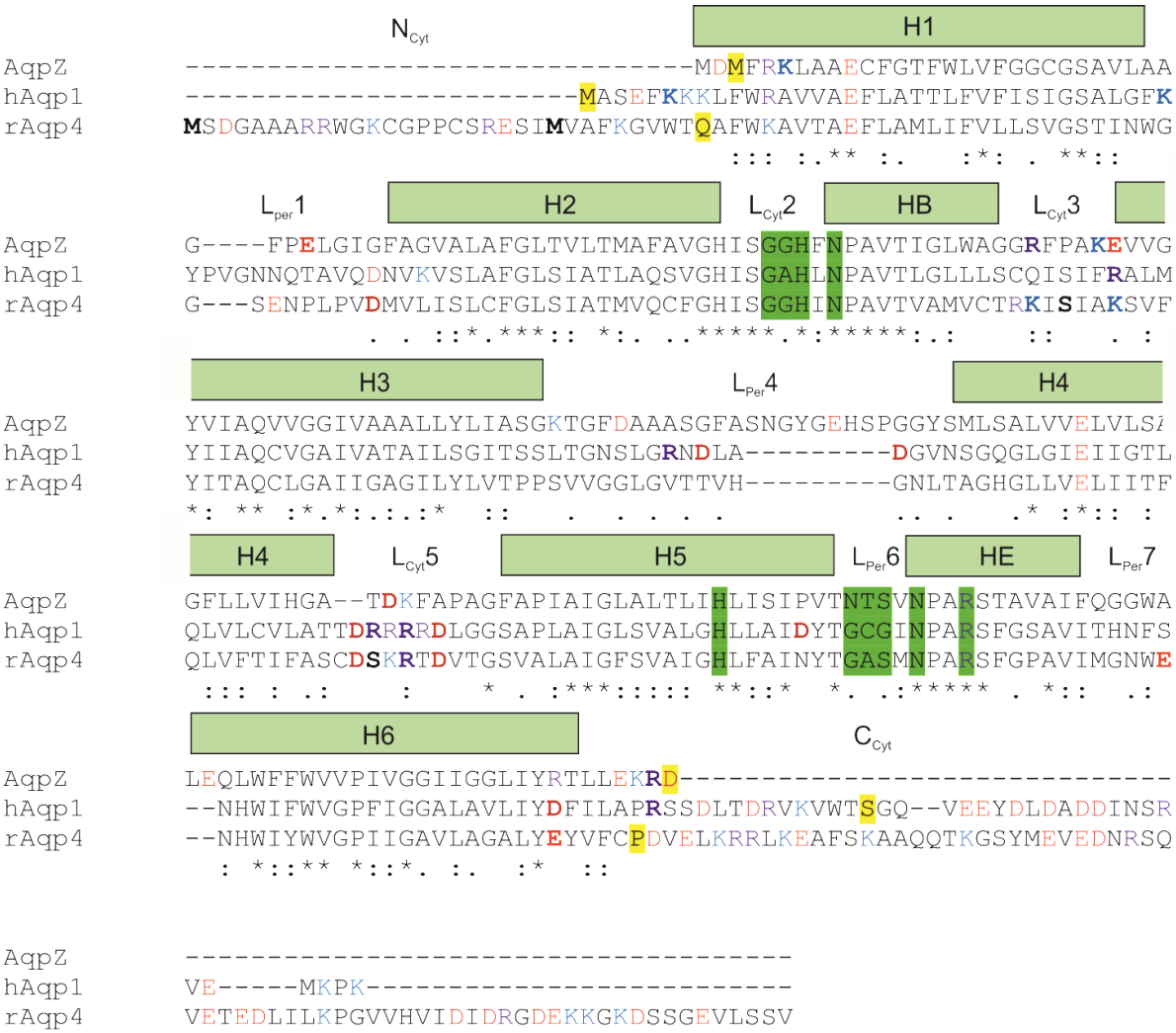


**Figure S1. Alignment of hAQP1, AQPZ and rAQP4.** The N- and C-termini are located both at the cytoplasmic side of the protein. Transmembrane helices H1 to H6, half-helices HB and HE as well as the location/orientation of loop L1 to L7 are indicated. Charged amino acid residues are labeled according to the color rules defined in Fig. 2. C- and N-terminal amino acids resolved in the respective structures are labeled in yellow. Potential H-bond forming residues located in the single-file pore region in AQP1, AQPZ and AQP4 are highlighted in green. M1, M23, Ser111 and Ser180 as

well as all charged amino acid residues which are located in the region of interest (Fig. 2) are depicted in bold letters. The multiple sequence alignment was performed with Clustal O(1.2.4).

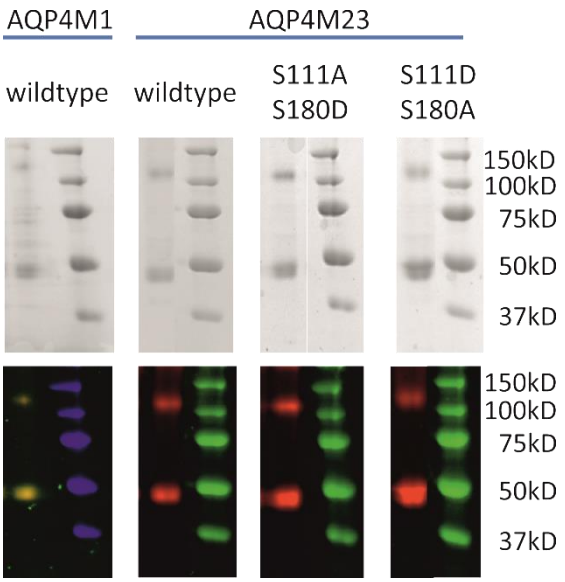


**Figure S2. SDS-PAGE gel of purified AQP4-eGFP fusion proteins.** Upper panel: Coomassie-stained gel showing purified mono- and oligomeric AQP4-eGFP at ~50kDa and ~120kDa, respectively. Lower panel: fluorescent imaging of AQP4-eGFP proteins.

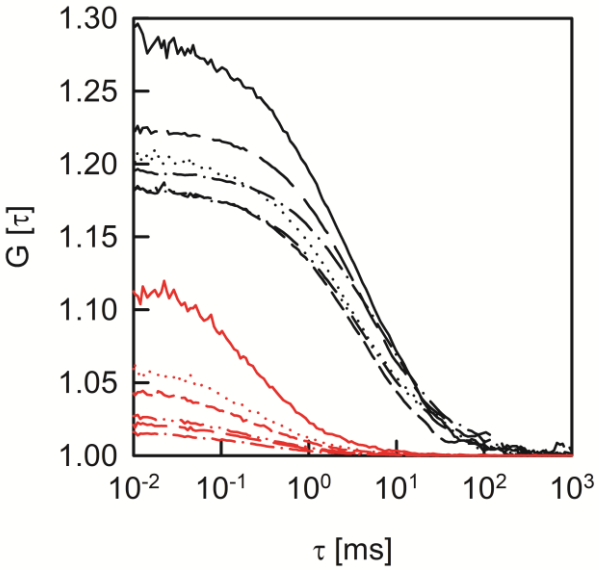


**Figure S3. Determination of reconstitution efficiency.** Representative FCS autocorrelation curves of an AQP4M23 reconstitution series as depicted in Fig. 3, A. PL (black lines) are dissolved in detergent (2% OG + 2% SDS) to form AQP4M23eGFP containing micelles (red lines). The ratio between AQP4 containing micelle and the number of PL serves to calculate the number of AQP4 units per PL.

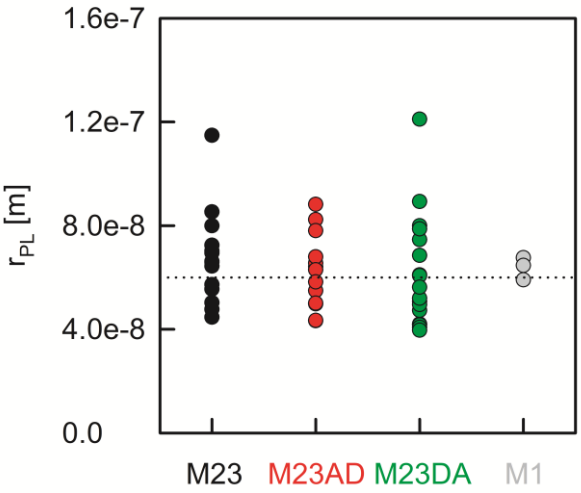


**Figure S4. Radius distribution of AQP4 containing PL's.** The black dotted line represents PL with a radius $r_{PL}$ of 60nm.

| hAQP1 | | AQPZ | | rAQP4 | |
|---|---|---|---|---|---|
| **K6** | **14.27** | **K6** | **9.26** | D100 | 11.30 |
| **K36** | **7.90** | E33 | 14.24 | **K140** | **9.08** |
| **R93** | **8.48** | **R77** | **8.64** | D142 | 13.26 |
| **R126** | **11.59** | **K81** | **14.22** | **K145** | **7.42** |
| D128 | 8.90 | E82 | 10.10 | D210 | 12.82 |
| D131 | 14.33 | D156 | 12.96 | D211 | 10.45 |
| D158 | 12.76 | **R232** | **14.30** | **R182** | **10.79** |
| **R159** | **9.14** | | | D215 | 9.06 |
| **R161** | **12.25** | | | E259 | 13.47 |
| D163 | 10.26 | | | E280 | 11.65 |
| D185 | 8.81 | | | | |
| D228 | 13.69 | | | | |
| **R234** | **12.79** | | | | |

**Table S1. Charged amino acids located within 15 Å to AQP's pore entrances and exits.** Distances in angstrom are measured in PyMol from the first water molecule next to a single-file water molecule at the periplasmic and cytoplasmic side of hAQP1, AQPZ, rAQP4 to charged amino acid residues. Positively charged amino acids are labeled in blue and negatively charged amino acids in red.