

Fig. S9 The Al binding site S<sub>Al1</sub>.

a, The extracellular view of the  $S_{All}$  site localized in the pore in the ALMT1<sub>Al/pH5</sub> structure. b, Al at the  $S_{All}$  site is coordinated by a pair of Met60. The Al density is shown at the contour level of 10  $\sigma$ . The map is sharpened with a B factor of -80 Å<sup>2</sup>. c, The  $S_{All}$  site in the map of ALMT1<sub>apo/pH5</sub>. The densities around the  $S_{All}$  site and Met60 are shown at the contour level of 4  $\sigma$ . No density is observed around the  $S_{All}$  site. d, Concentration-dependent extracellular Al activation of WT and mutant AtALMT1. Data are reported as means  $\pm$  s.e.m. of at least five independent biological replicates. Curves are least-square fits to a Hill equation with half-maximum activation concentration (EC<sub>50</sub>) values of 98.17  $\pm$  3.53, 97.12  $\pm$  5.13, and 91.12  $\pm$  1.24  $\mu$ M for WT, M60A, and M60L, respectively. e, The channel conductance of WT and mutant AtALMT1 measured at -180 mV. The two mutants show no significant (ns) differences in comparison with the WT. Also see Supplementary information, Fig. S2a, d. f, Sequence alignment of ALMT homologs that are directly activated by Al around Met60 (highlighted by the red box) in AtALMT1.