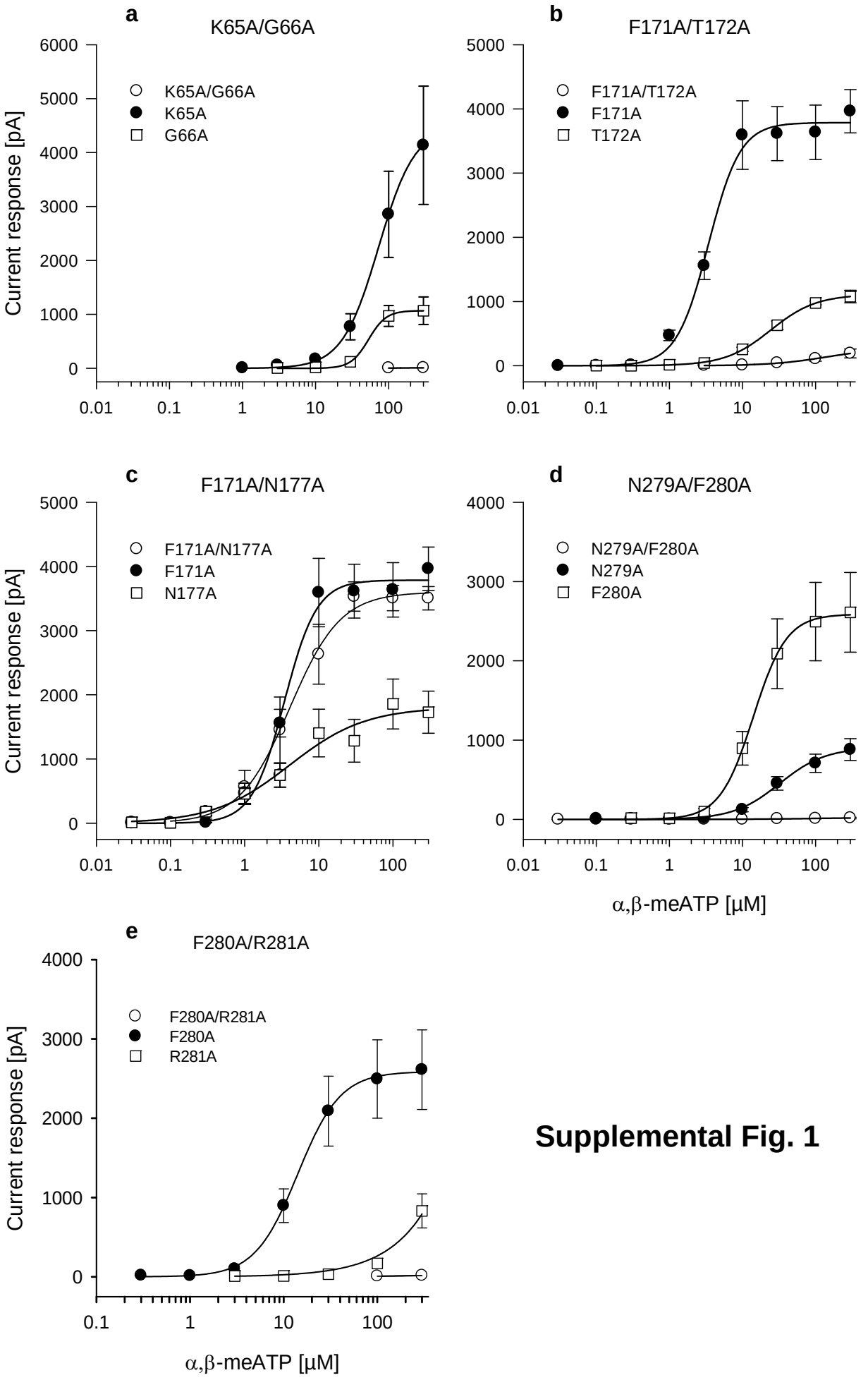
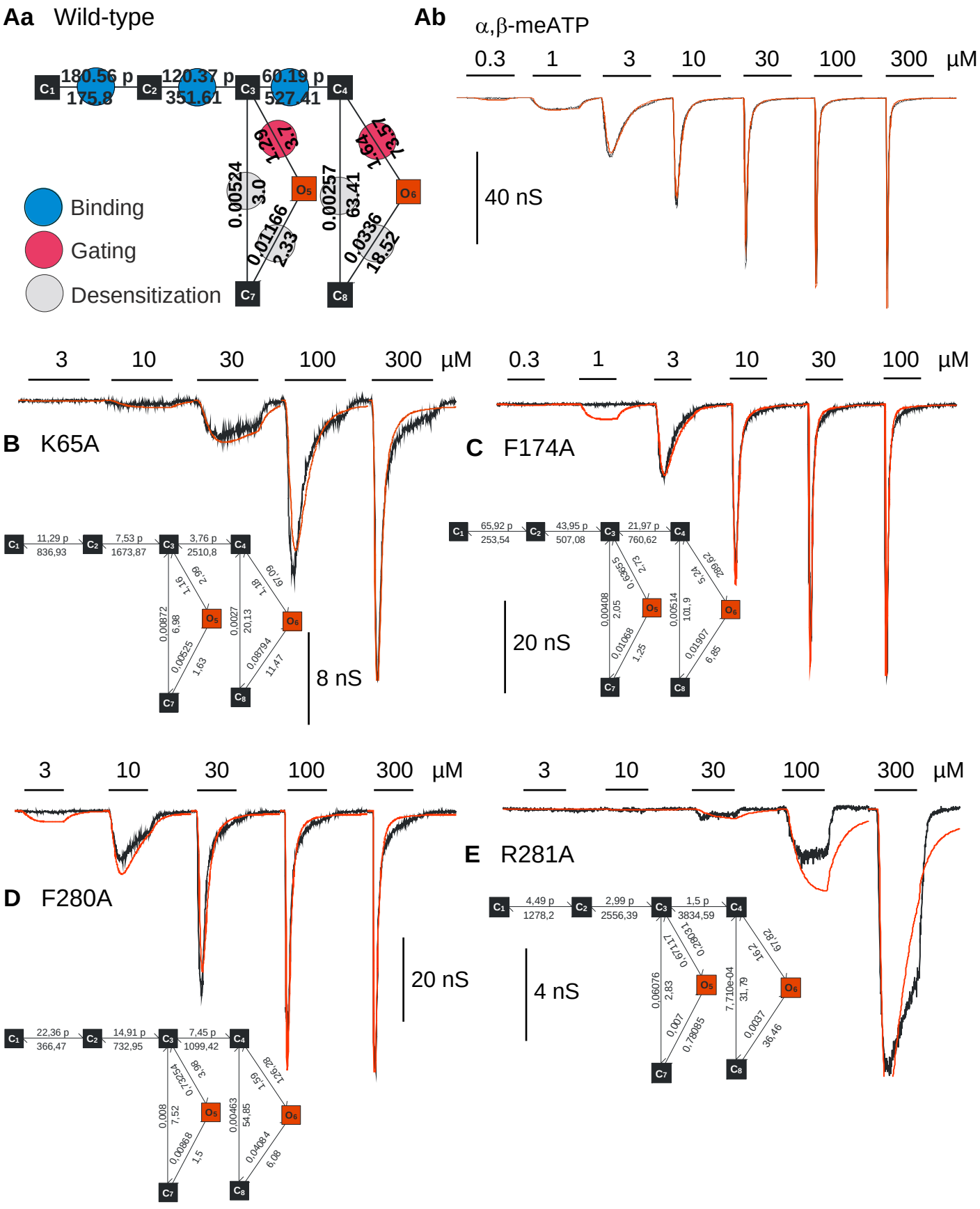
Supplemental Fig. 1. Current responses to α,β-meATP in HEK293 cells transfected with single and double binding site mutants of hP2X3 receptors. Whole-cell patch-clamp recordings were made as described in Fig. *1Ba*. Concentration-response curves for α,β-meATP were constructed on hP2X3 mutants, where two adjacent AAs in NBS1-4 were replaced both separately (data taken from Fig. *2*) and simultaneously with Ala. The double mutants K65/G66 (*a*) F171A/T172A (*b*), N279A/F280A (*d*) and F280A/R281A (*e*) were all insensitive to α,β-meATP, although the single mutants did react to the agonist, albeit to a lower extent than the WT hP2X3 receptor. By contrast, the additional replacement of a non-adjacent AA residue in the F171A mutant by Ala (F171A/N177A; *c*) did not interfere with the agonist effect. Each symbol indicates the mean±S.E.M. of 7-11 cells.



Supplemental Fig. 1

Supplemental Fig. 2. α,β-meATP-induced currents recorded in HEK 293 cells transfected with the wild-type hP2X3 receptor and its binding-site mutants were computed according to a simplified model of Karoly et al. (2008). The rate constants characterizing transitions between the unbound, closed-state receptor (C_1), and the receptor binding one (C_2), two (C_3) or three (C_4) agonist molecules, as well as the two open-state receptors (O_5, O_6) and the two desensitized receptors (C_7, C_8) are shown for the WT $P2X_3$ (*Aa*) and its mutants (*Ab*, insets of *B*-*F*). By using these on- and off-rate constants, the shape and amplitude of the α,β-meATP (0.3-300 μM)-induced currents were computer modelled; the quality of the fits can be judged by the close parallelism of the black lines (original tracings) and the orange lines (computed current traces) calculated with the indicated rate constants for binding (C_1-C_4), gating (C_3-O_5; C_4-O_6), and desensitization (C_3-C_7 and O_5-C_7; C_4-C_8 and O_6-C_8). Note changes in the respective constants for the mutants in comparison with the WT receptor (all kinetic constants marked by *p* had the dimension of mM^{-1} s^{-1}, whereas the non-marked ones had the dimension of s^{-1}. Horizontal lines indicate the agonist superfusion time (2 s). Representative fits for individual data points out a total of 4-7 similar experiments are shown in each panel.



Supplemental Fig. 2