Garneau et al., http://www.jgp.org/cgi/content/full/jgp.201311097/DC1

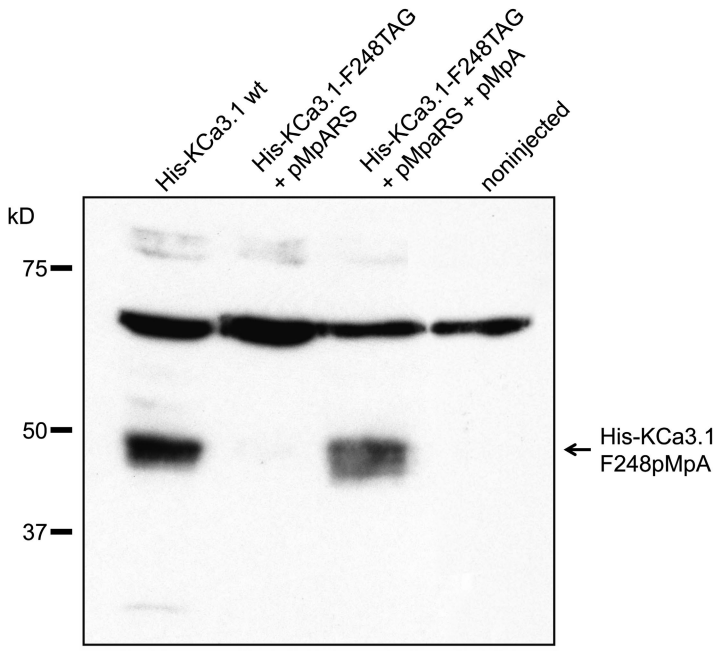

Figure S1. Amber suppression of *Bs*tRNA–*Ec*TyrRS. Western blot analysis of full-length expression of a His-KCa3.1 channel tagged with 6-His at position 132 of the channel: lane 1, His-KCa3.1 wild type; lane 2, His-KCa3.1-F248TAG mutant plus pU6-pMpa plasmid; lane 3, His-KCa3.1-F248TAG plus pU6-pMpa plasmid in oocytes injected with 5 mM of cytoplasmic pMpa; lane 4, noninjected oocytes. Full-length expression of His-KCa3.1-F248TAG is suppressed by *Ec*TyrRS together with *Bs*tRNA in the presence of pMpa. A 40-µg aliquot of the cell lysate for each reaction was analyzed with anti–His-HRP. A nonspecific protein of 70 kD also reacted with the anti–His-HRP antibody.

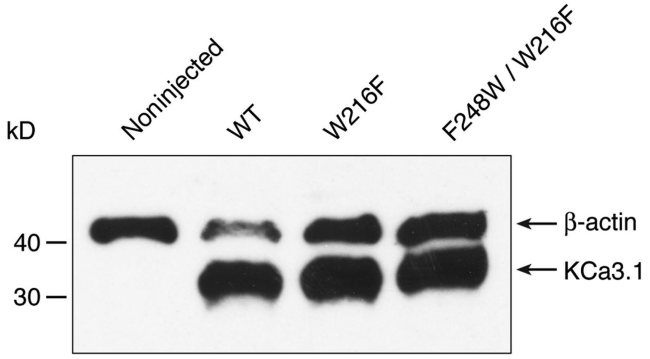

Figure S2. Western blot analysis confirming expression of W216F and F248W–W216F KCa3.1 mutants in *Xenopus* oocytes. Membranes from noninjected oocytes (lane 1), KCa3.1 wild type (lane 2), W216F KCa3.1 (lane 3), and F248W–W216F KCa3.1 (lane 4) were obtained and processed as described previously (Morales et al. 2013. *J. Gen. Physiol.* 142:37–60). A 10-µg aliquot of the cell lysate for each reaction was sequentially analyzed using rabbit anti-KCa3.1 (ab75956 1:2,000; Abcam) or mouse anti–β-actin antibodies (ab 1:10,000; Abcam), followed by hybridization with HRP-conjugated goat anti–rabbit or goat anti–mouse IgG at 1:25,000 dilutions (Jackson ImmunoResearch Laboratories) and detected using the ECL plus chemiluminescence kit (GE Healthcare). A ≈35-kD band was obtained for KCa3.1 wild-type and KCa3.1 mutants as well as for confirming channel expression in the absence of functional activity (F248W–W216F). 42 kD β-actin was taken as control.

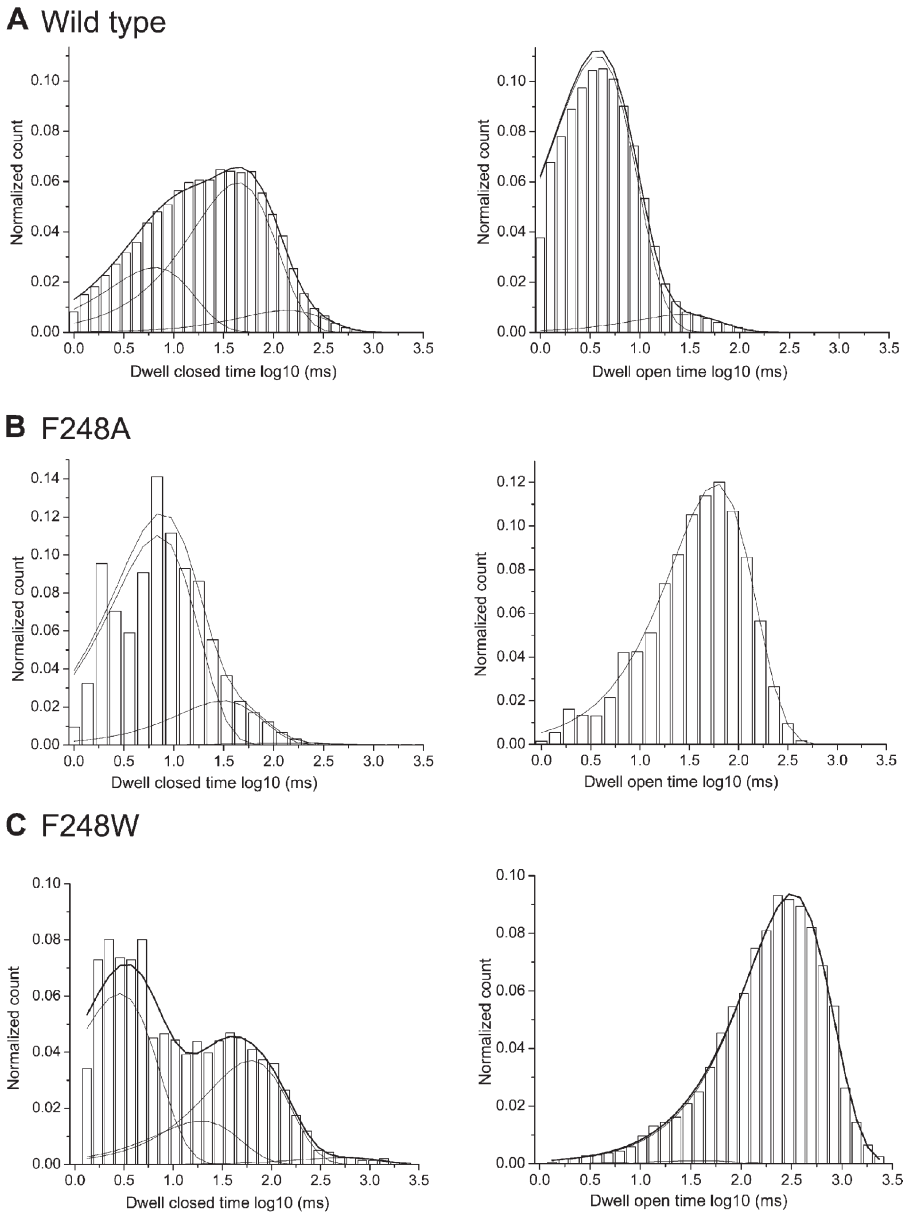

Figure S3. Open and closed dwell-time distributions computed for the wild-type (F248; A), F248A (B), and F248W (C) KCa3.1 mutant channels. Closed times could be fitted to a four closed-state distribution with respective values of 4.5 ms (1.7%), 6.8 ms (39%), 30 ms (53%), and 235 ms (6.3%) for the wild-type channel; 6 ms (79%), 26 ms (19%), 117 ms (1%), and 910 ms (1%) for the F248A mutant; and 3 ms (43%), 17 ms (15%), 54 ms (38%), and 475 ms (2%) for the F248W channel. Except for the F248A mutant, where the open state could be fitted to a single-state model with a mean value of 50 ms, the wild-type and F248W channels were best fitted by a two open-state distribution with respective values of 7 ms (89%) and 49 ms (11%) for the wild type channel, and 33 ms (1%) and 285 ms (99%) for the F248W mutant. Distributions were computed using the QUB package.