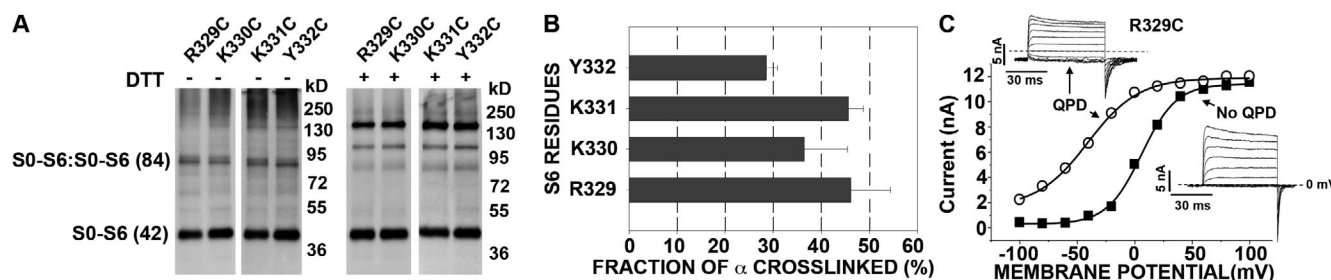
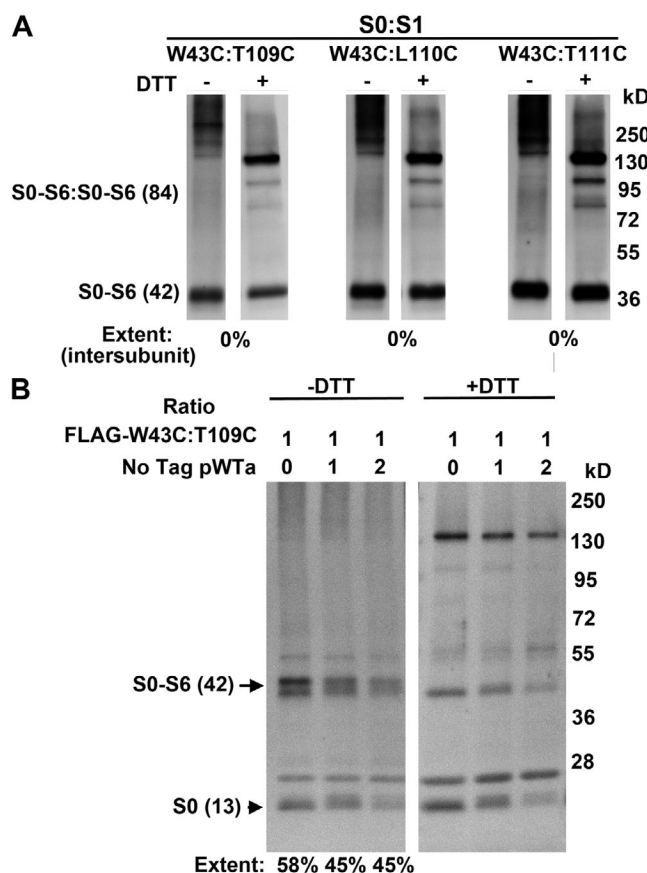
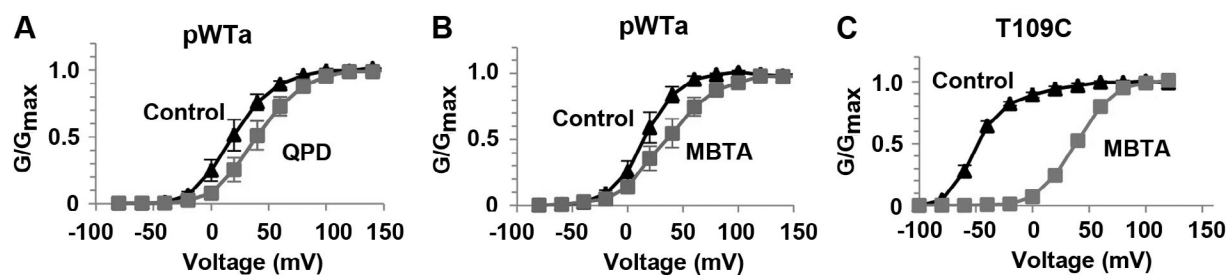


Liu et al., <http://www.jgp.org/cgi/content/full/jgp.201411337/DC1>

**Figure S1.** S6 to S6 cross-linking. Single Cys were substituted in the intracellular flank of S6 in the background of pWTb. Cleavage at HRV-3C site 2 in the  $\alpha$  monomer yields a 42-kD N-terminal fragment, and cleavage of both  $\alpha$ 's in a dimer yields an 84-kD fragment. (A) Anti-HA immunoblots. (B) Mean ( $\pm$  SEM) extents of cross-linking. (C) I-V curve of R329C before QPD and after QPD in inside-out macropatch. (Inset) Series of depolarizing pulses from -100 to +100 mV from a holding potential of -120 mV.



**Figure S2.** Intrасubunit cross-linking between S0 and S1. Expression, surface biotinylation, protein extraction, SDS-PAGE, Western blotting, and detection with an anti-HA antibody were as described previously (Liu et al. 2008. *J. Gen. Physiol.* 131:537–548; Liu et al. 2008. *Proc. Natl. Acad. Sci. USA.* 105:10727–10732; Liu et al. 2010. *J. Gen. Physiol.* 135:449–459; Wu et al. 2009. *J. Neurosci.* 29:8321–8328; Wu et al. 2013. *J. Gen. Physiol.* 141:105–117). The conditions of induction of disulfide bond formation with QPD are described in Materials and methods of the main text. (A) Anti-HA immunoblots showing QPD-induced cross-linking between indicated Cys in S0 and S1 in an  $\alpha$  background with only HRV site 2. (B) Anti-HA immunoblots showing QPD-induced cross-linking between indicated Cys in S0 and S1. We coexpressed different ratios of two  $\alpha$  constructs: the double-Cys-substituted mutant W43C-T109C in the background of both HRV sites (pWTc) and a pWTa construct without an HA-tag.



**Figure S3.** Effects on  $V_{50}$  of QPD and MBTA on pWTa and T109C. Normalized G-V curves of untreated (control) and after treatment either with 40  $\mu\text{M}$  QPD or 100  $\mu\text{M}$  MBTA for 5–7 min. Recordings were from inside-out macropatches. Mean  $\pm$  SEM. (A and B) pWTa. (C) T109C. See Fig. 5 C in the main text for effect of QPD on T109C.