Tong et al., http://www.jgp.org/cgi/content/full/jgp.201511375/DC1

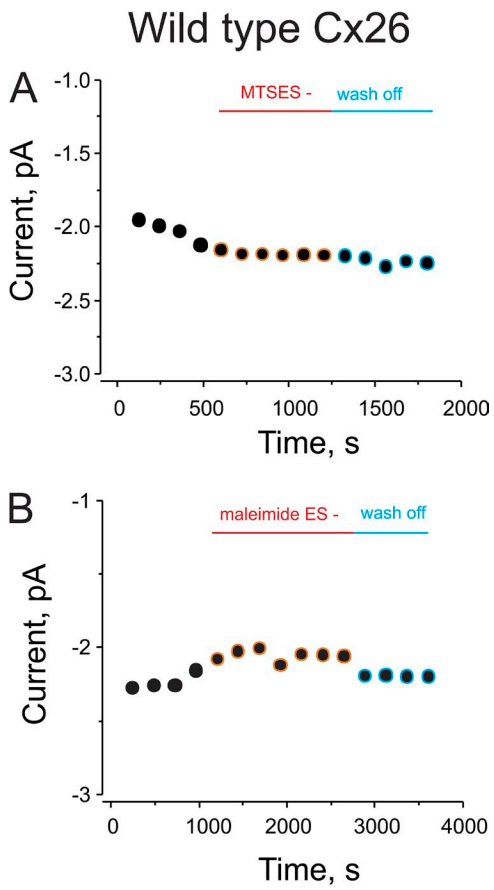

Figure S1. MTSES or maleimide ES does not significantly affect Cx26 wild-type hemichannel currents. (A) Time course of the maximal tail currents in the absence, the presence, and after wash off of MTSES from an oocyte expressing Cx26 hemichannels. (B) Time course of maximal tail currents in the absence, the presence, and after wash off of maleimide ES. The tail currents were measured after a depolarizing pulse from −80 to 0 mV in the presence of 0.25 mM of extracellular Ca^{2+}.

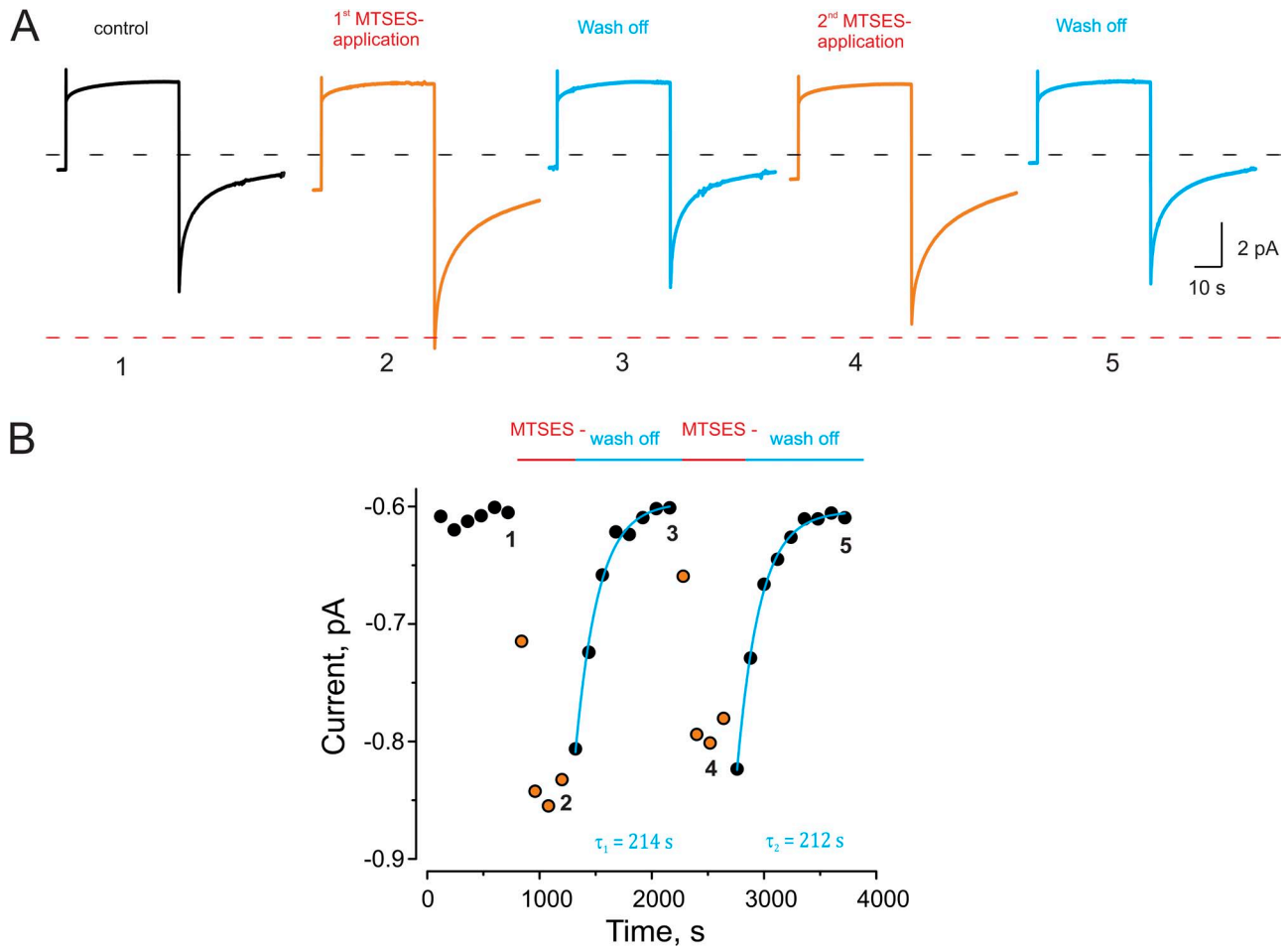

Figure S2. Repeated MTSES modification after washout. (A) Representative current traces from an oocyte expressing Cx26 D50C mutant hemichannels in response to a depolarizing pulse from −80 to 0 mV. Each trace corresponds to steady-state current traces before (black), in the presence (orange) of, or with washout (blue) of 500 µM MTSES. Dotted black line is the 0-pA current. Note that the second application of MTSES, after washout and recovery of the current, produced essentially the same effect as the first application (and that the recovery from the second application was the same as well). The peak tail currents from traces in A are represented by numbers in B.

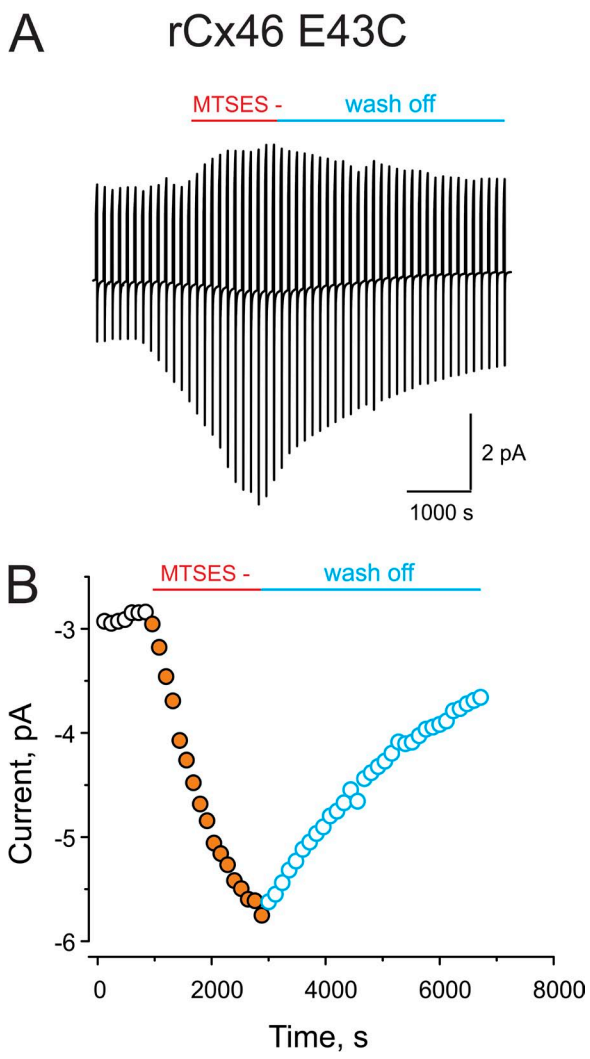

Figure S3. MTSES modification is reversible in Cx46 hemichannels containing a cysteine in the pore at position E43C. (A) Cumulative current traces in response to continuous depolarizing pulses from −80 to 0 mV. In this representation, capacitative current cannot be fully removed, yet it is possible to observe a slow increase in the currents after the addition of MTSES (which does not reach saturation). After wash of MTSES, there is a slow decrease in the currents, suggesting reversibility. (B) Time course of maximal tail currents in the absence, the presence, and after wash off of MTSES. As for Cx26 cysteine mutants, the tail currents were measured after a depolarizing pulse from −80 to 0 mV in the presence of 0.25 mM of extracellular Ca^{2+}.

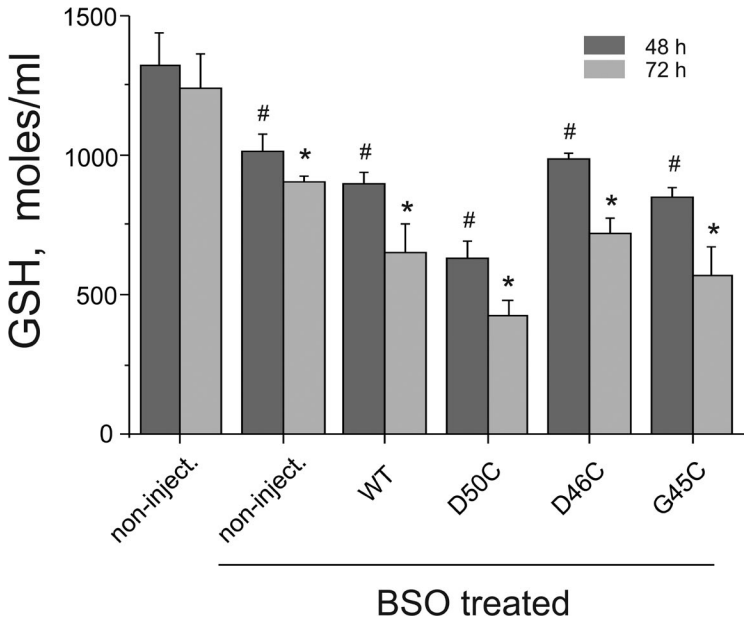

Figure S4. GSH levels are reduced after 48- or 72-h treatment with BSO. Noninjected oocytes or oocytes expressing Cx26, D50C, D46C, or G45C mutants were treated in the presence of 20 mM BSO for 48 h (dark gray bars) or 72 h (light gray bars) for comparison with noninjected oocytes without treatment. BSO significantly decreased the levels of GSH in all treated oocytes; however, the decrease was higher in oocytes expressing connexin mutants. BSO treatment was performed in low extracellular Ca^{2+} (0.25 mM). #, $P < 0.05$; *, $P < 0.01$ compared with noninjected oocytes without treatment at 48 and 72 h, respectively, using nonparametric one-way ANOVA with Tukey's post-hoc test. Error bars represent the mean ± SEM of at least three independent experiments.

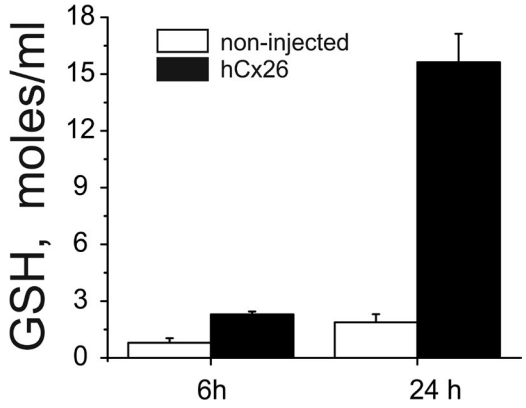

Figure S5. Extracellular GSH levels from noninjected oocytes or oocytes injected with the mRNA for hCx26. 30 oocytes expressing or not Cx26 hemichannels were incubated in Ringer's solution with 0.25 mM Ca^{2+} for 6 and 24 h. The extracellular medium was collected and total GSH levels measured as described in Materials and methods. Oocytes expressing hCx26 (filled bars) showed higher levels of extracellular GSH than those that were not injected (open bars). Error bars represent the mean ± SEM of three independent experiments.