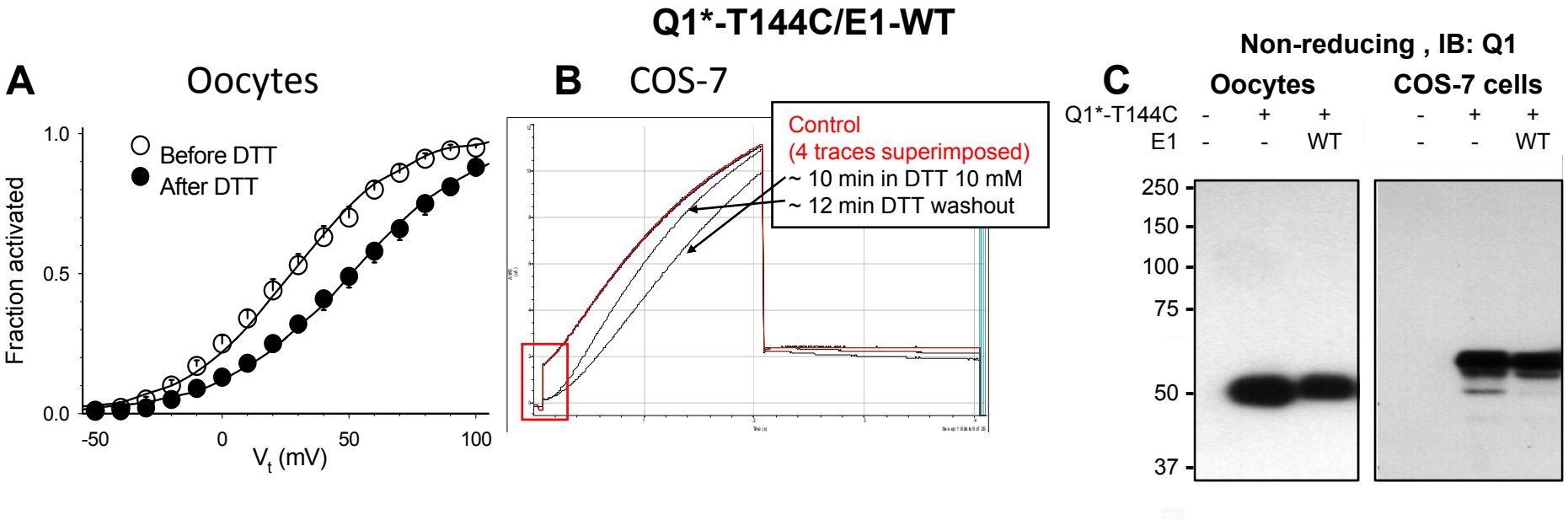

Fig. S1 (A) DTT treatment of Q1*-T144C/E1-WT expressed in oocytes shifts the activation curve in the positive direction. **(B)** DTT treatment of Q1*-T144C/E1-WT expressed in a COS-7 cell abolishes the constitutive current component, reflecting a positive shift in the voltage-dependence of activation similar to the oocyte data. **(C)** Oocytes and COS-7 cells expressing the cRNA(s) or cDNA(s) listed above ('-' no cRNA or no cDNA negative controls) are subjected to whole cell lysis followed by SDS-PAGE under non-reducing conditions. After blotting the proteins to membrane, the membrane is probed with C-20 pAb (oocytes) or V5 mAb (COS-7 cells) for Q1 detection. Only the Q1 monomer bands (~ 50 kDa in oocytes and 60 kDa in COS-7 cells) are detected for Q1*-T144C expressed alone or paired with E1-WT. There is no higher molecular weight Q1-positive band up to 250 kDa.

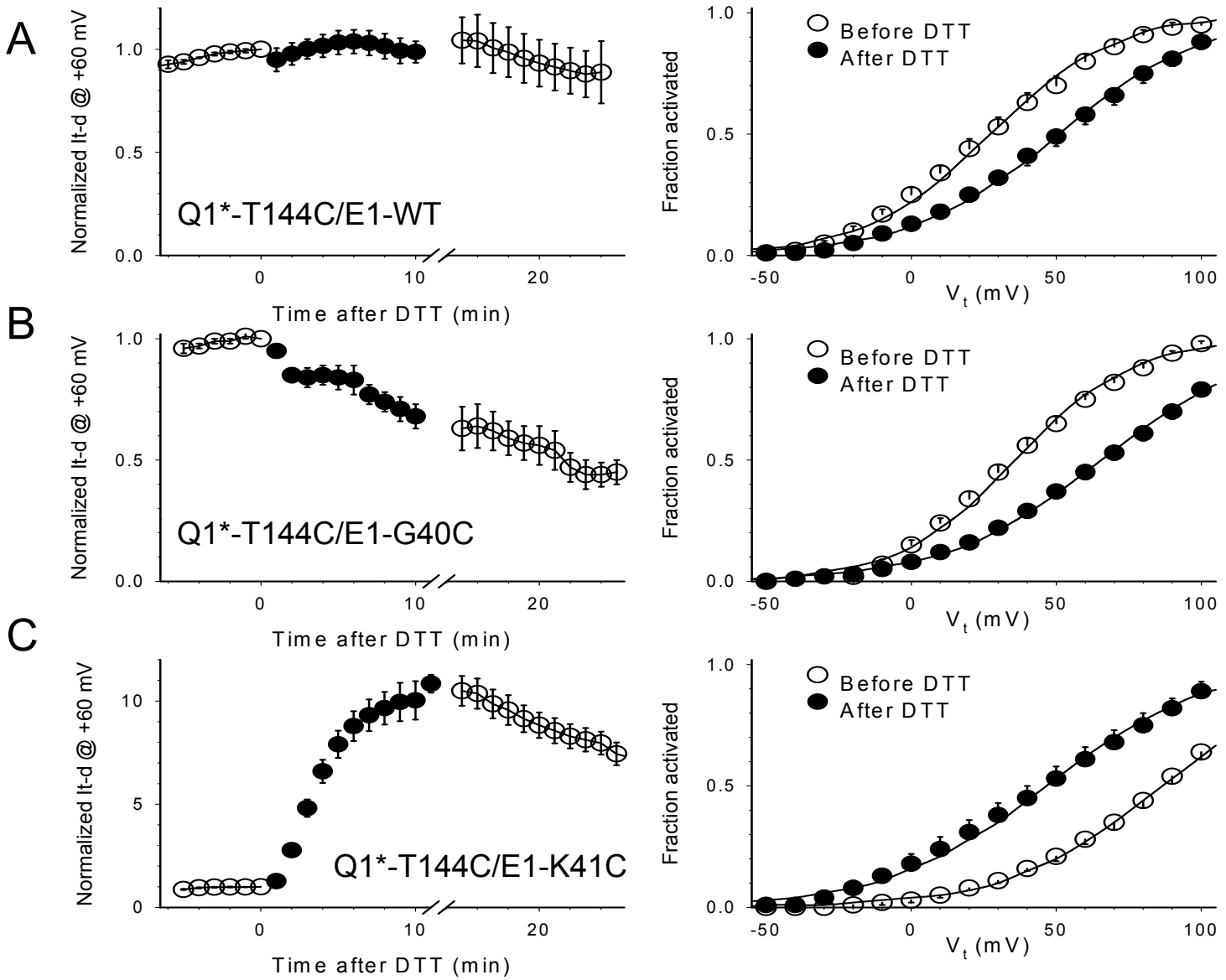

Fig. S2 *Left column*: time courses of changes in current amplitudes monitored by 2-s test pulses to +60 mV before, during, and after DTT (10 mM) exposure for the Q1*-T144C/E1 pairs specified in the insets. Closed circles: data points measured in DTT. *Right column*: activation curves before and after DTT treatment from the same sets of experiments. Data are pooled from 5 – 6 oocytes in each group and shown as mean±SE. **(A)** vs **(B)** Short exposure to DTT reveals that, relative to Q1*-T144C/E1-WT, Q1*-T144C/E1-G40C manifests more rapid decline in the current amplitude and more pronounced positive shift in the activation curve. **(C)** Short exposure of Q1*-T144C/E1-K41C to DTT, when the current amplitude reaches the peak of enhancement, reveals a prominent negative shift in the activation curve.

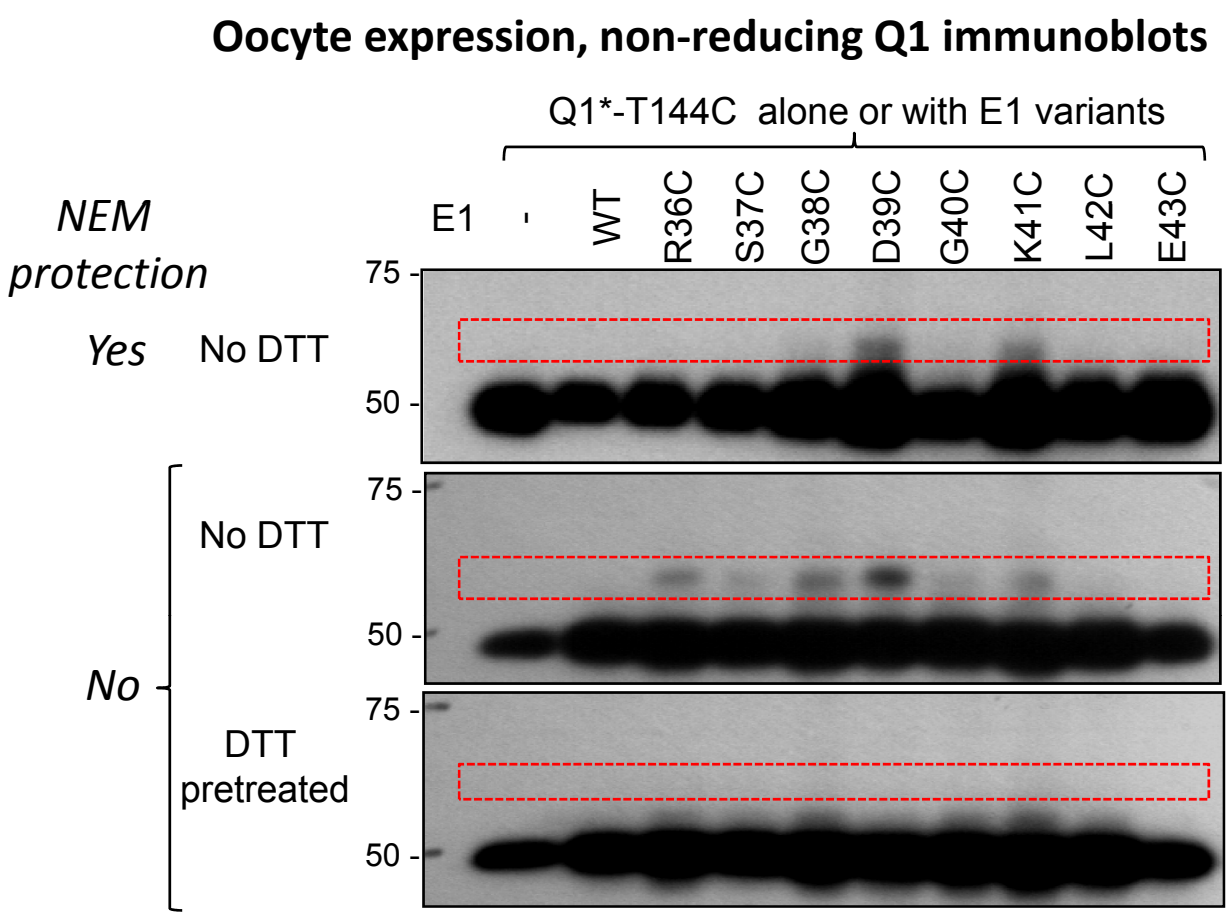

Fig. S3 Oocytes are injected with cRNA(s) listed above. Three days after cRNA injection, oocytes are subjected to whole cell lysis with (top) or without (middle & bottom) pretreatment with N-ethyl maleimide (NEM). In the latter group, each of the whole cell lysates is divided into 2 equal aliquots, one treated with DTT while the other serving as control. The whole cell lysates are run on non-reducing SDS-PAGE, and the membranes are probed for Q1 by C-20 pAb. The red dashed rectangles highlight Q1*/E1 disulfide bonded bands (middle), that are largely missing in NEM-preteated oocyte WCL (top) and abolished by DTT (bottom).

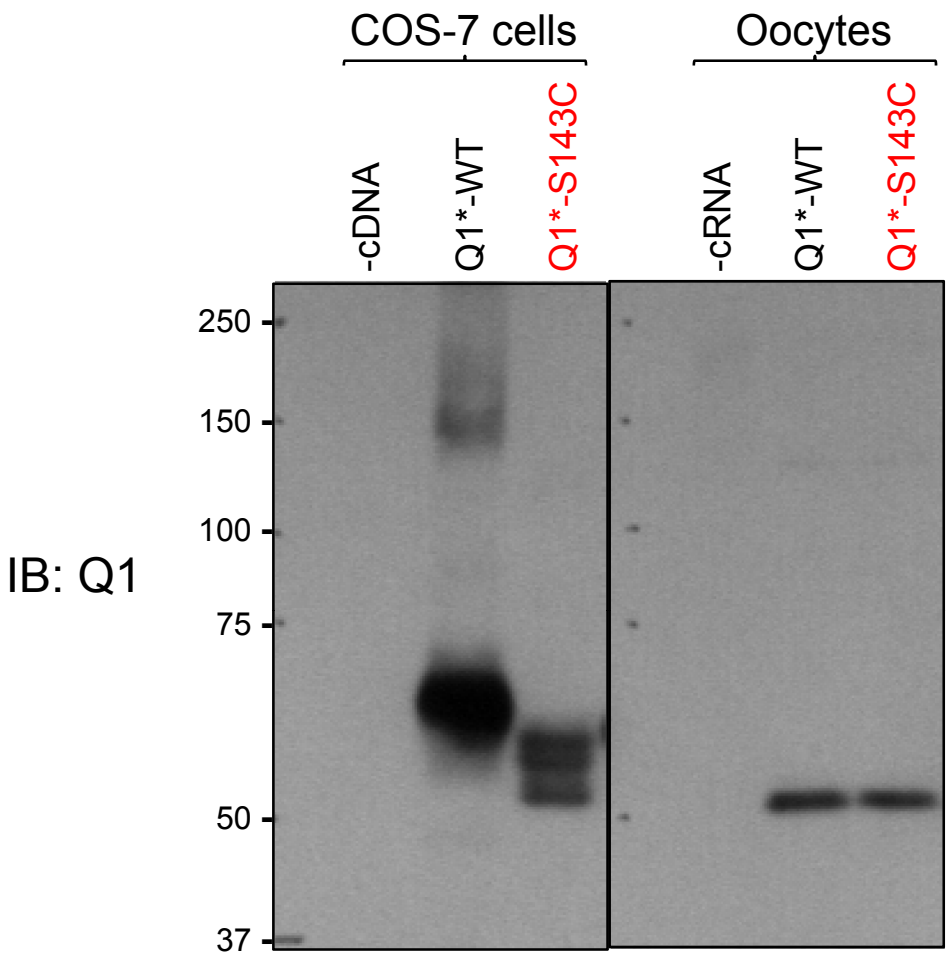

Fig. S4 In immunoblots of COS-7 cells Q1*-S143C migrates as doublet bands of < 60 kDa, significantly smaller than the size of Q1*-WT (left), while in immunoblots of oocytes both Q1*-S143C & Q1*-WT migrate as ~ 50 kDa bands. Since COS-7 cells can effectively N-glycosylate membrane proteins while oocytes cannot, the smaller Q1*-WT band size in oocytes likely reflects the low level of N-glycosylation. Since Q1*-WT is effectively expressed in oocytes, the lack of full N-glycosylation does not prevent the channel from reaching the cell surface. This is consistent with the observation that Q1*-S143C expressed in oocytes is functional (Fig. 12A in the main text). On the other hand, the small Q1*-S143C size in COS-7 immunoblots suggests that either the channels are not properly N-glycosylated (immature form, trapped inside cells) or are degraded. This is consistent with the lack of channel function in patch clamp experiments.