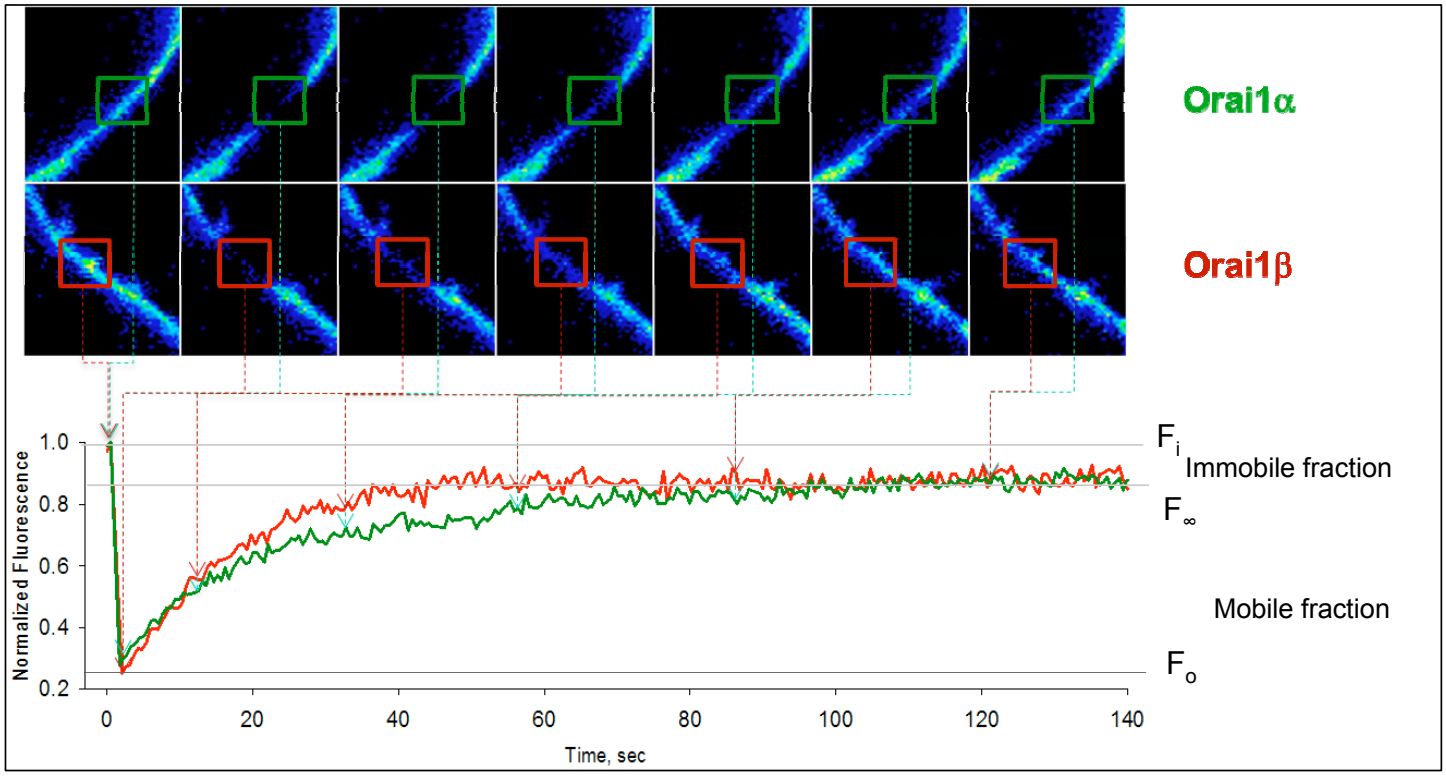

Fig. S1. Data for single FRAP rate determinations, as described in Methods. The difference between the fluorescence intensities at the asymptote (Fi) and that immediately following the bleach (Fo) were normalized to 1.0 at each curve before fitting with two exponential. Mobile fraction calculated as F∞- Fo.

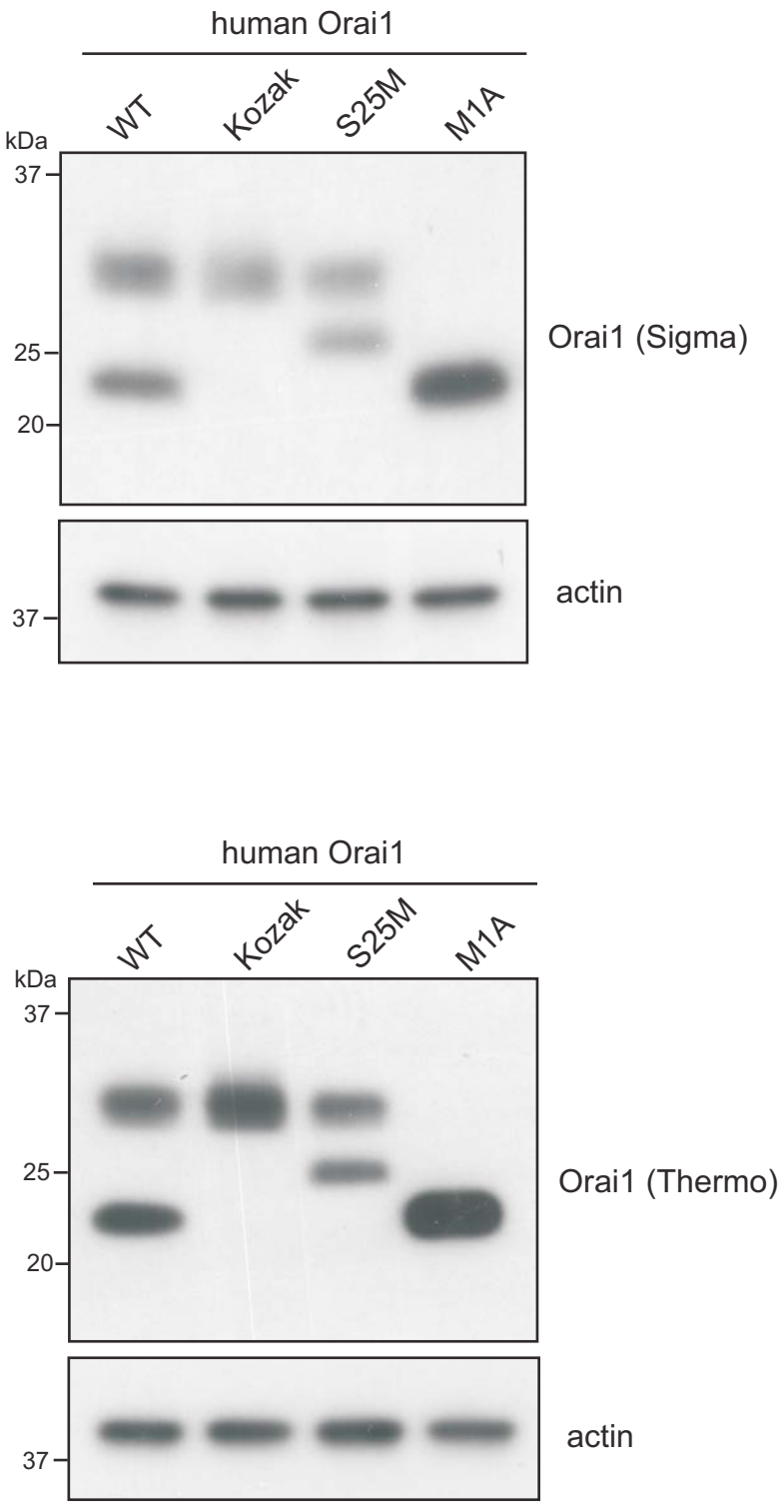

Fig. S2. Orai1 protein can be translated from an artificially introduced start codon after a leaky first start site. Western blot showing heterogeneously-expressed Orai1 proteins in HEK293 cells transfected with the cDNAs of WT with native 5'-UTR, WT with Kozak, S25M mutant and M1A mutant of Orai1. Cells were lysed in RIPA buffer. After overnight treatment with PNGaseF, samples were dissolved by 10% SDS-PAGE and analyzed by Western blotting using an anti-Orai1 antibody. Upper panel shows the blots probed with anti-Orai1 antibody purchased from Sigma whose epitope is ^{288}HRGDHPLTPGSHYA^{301} or one from Thermo whose epitope is ^{203}KKQPGQPRPTSKP ^{215} in human Orai1 amino acid sequence, respectively. Actin expression confirms that similar amounts of protein were loaded in each lane.

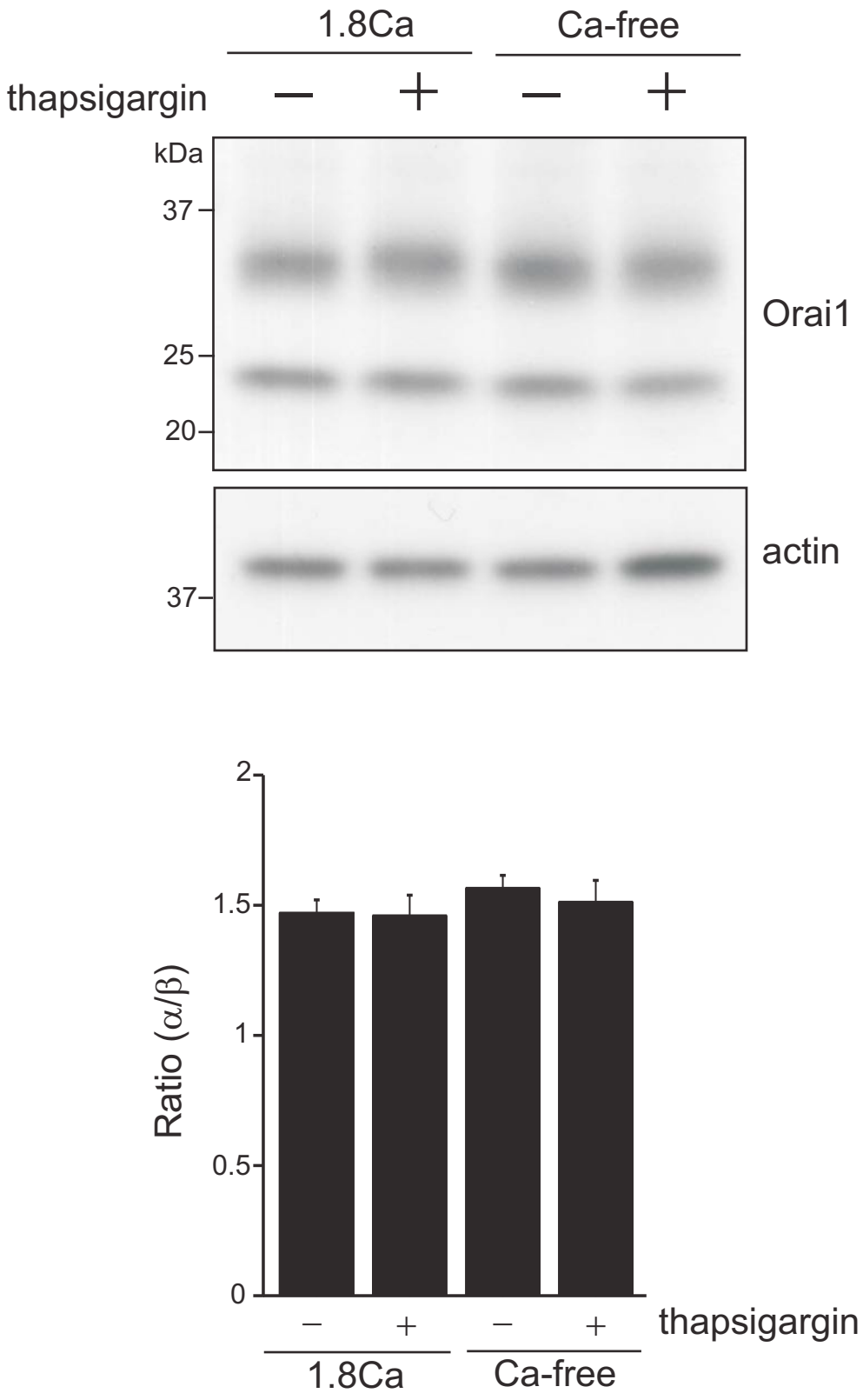

Fig. S3. Levels of Orai1 protein forms are not affected by store depletion in HEK293 cells. Western blot showing the expression of Orai1 protein in HEK293 cells treated with thapsigargin for one hour in the presence or absence of extracellular Ca^{2+}. Cells were lysed in RIPA buffer. After overnight treatment with PNGaseF, samples were dissolved by 10% SDS-PAGE and analyzed by Western blotting using an anti-Orai1 antibody. Actin expression confirms that similar amounts of protein were loaded in each lane. Representative blot from three independent experiments is shown. Lower panel shows the ration of Orai1α and Oraiβ quantified by densitometric analysis.