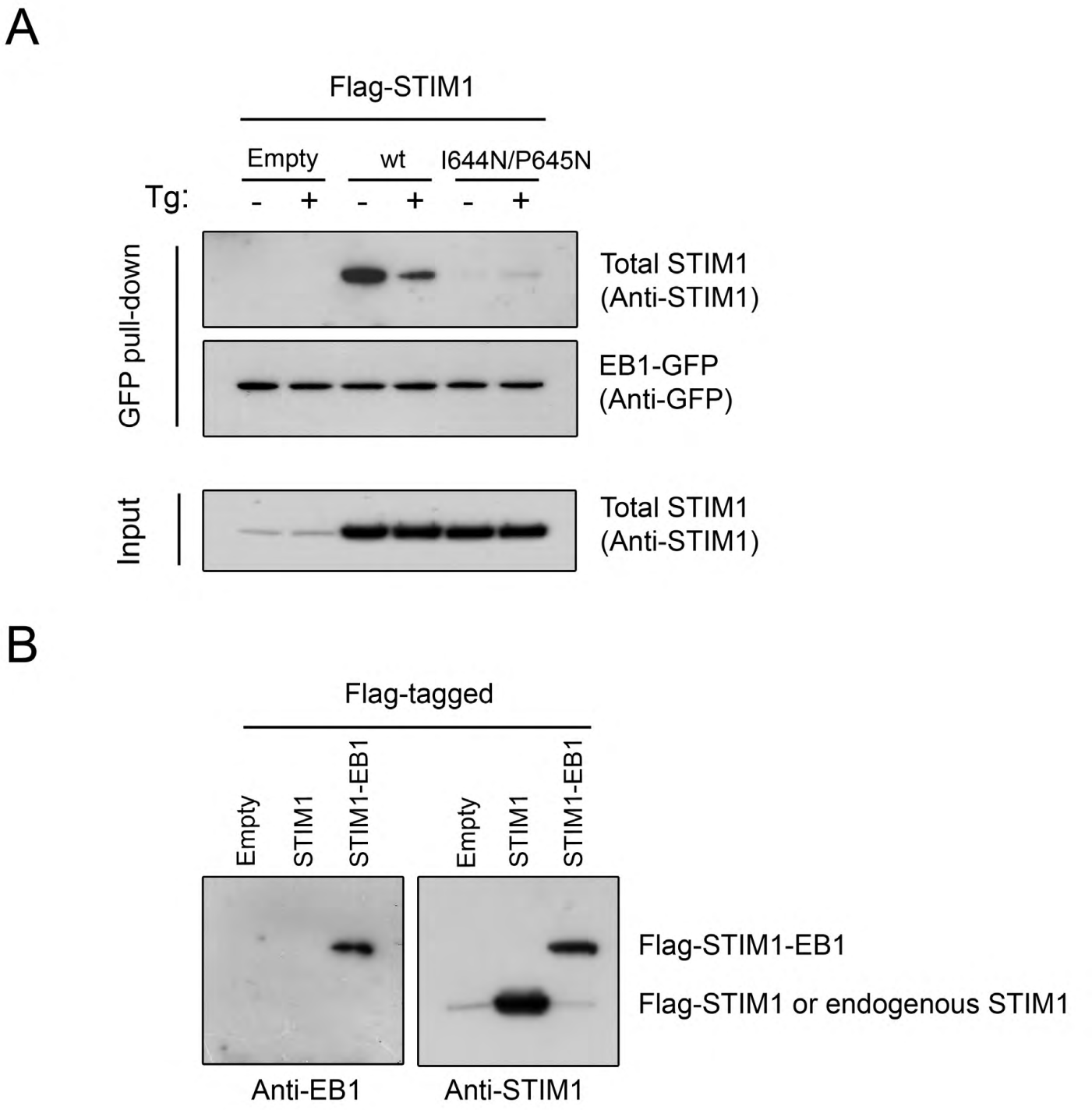

Fig. S1. Characterization of Flag-STIM1$^{I644N/P645N}$ and Flag-STIM1-EB1. (A) HEK293 cells stably transfected for the expression of Flag-peptide (empty), Flag-STIM1, or Flag-STIM1$^{I644N/P645N}$ were transfected for transient expression of EB1-GFP. Twenty four hours after transfection, cells were incubated in Ca^{2+}-containing HBSS (−Tg), or in Ca^{2+}-free HBSS with 1 μM thapsigargin for 10 min (+Tg). GFP-tagged EB1 from these cells was pulled-down with GFP-Trap and the level of STIM1 bound to EB1 was evaluated by immunoblot using an anti-STIM1 antibody (upper panel). The level of pulled-down EB1-GFP was evaluated with an anti-GFP antibody. Total amount of Flag-STIM1 in every experimental condition is shown (input). Note the lack of binding of STIM1$^{I644N/P645N}$ to EB1 demonstrating the constitutive dissociation of the complex in this experimental condition. (B) HEK293 cells stably transfected for the expression of Flag-peptide (empty), Flag-STIM1 or Flag-STIM1-EB1 were lysed to check the expression of these proteins by immunoblot. This analysis was performed with an anti-EB1 antibody (left). The membrane was then stripped and re-probed with an anti-STIM1 antibody (right).

Movie 1. Time-lapse epifluorescence microscopy shows proper STIM1-GFP multimerization under store depletion triggered with TBHQ in Ca^{2+}-free medium, and reversal of this process to non-multimerized state after refilling of Ca^{2+}-stores by washing with Ca^{2+}-containing HBSS.