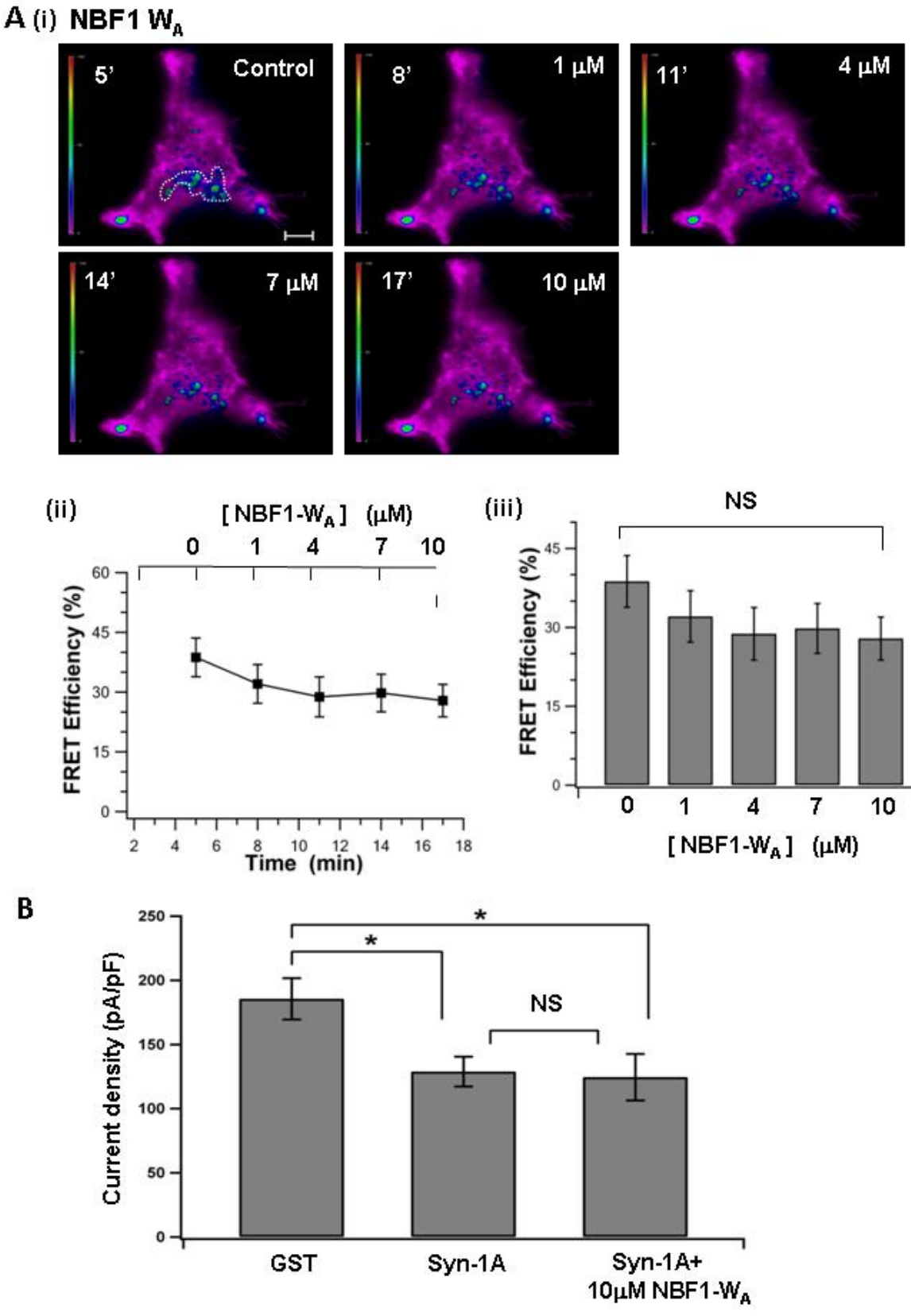
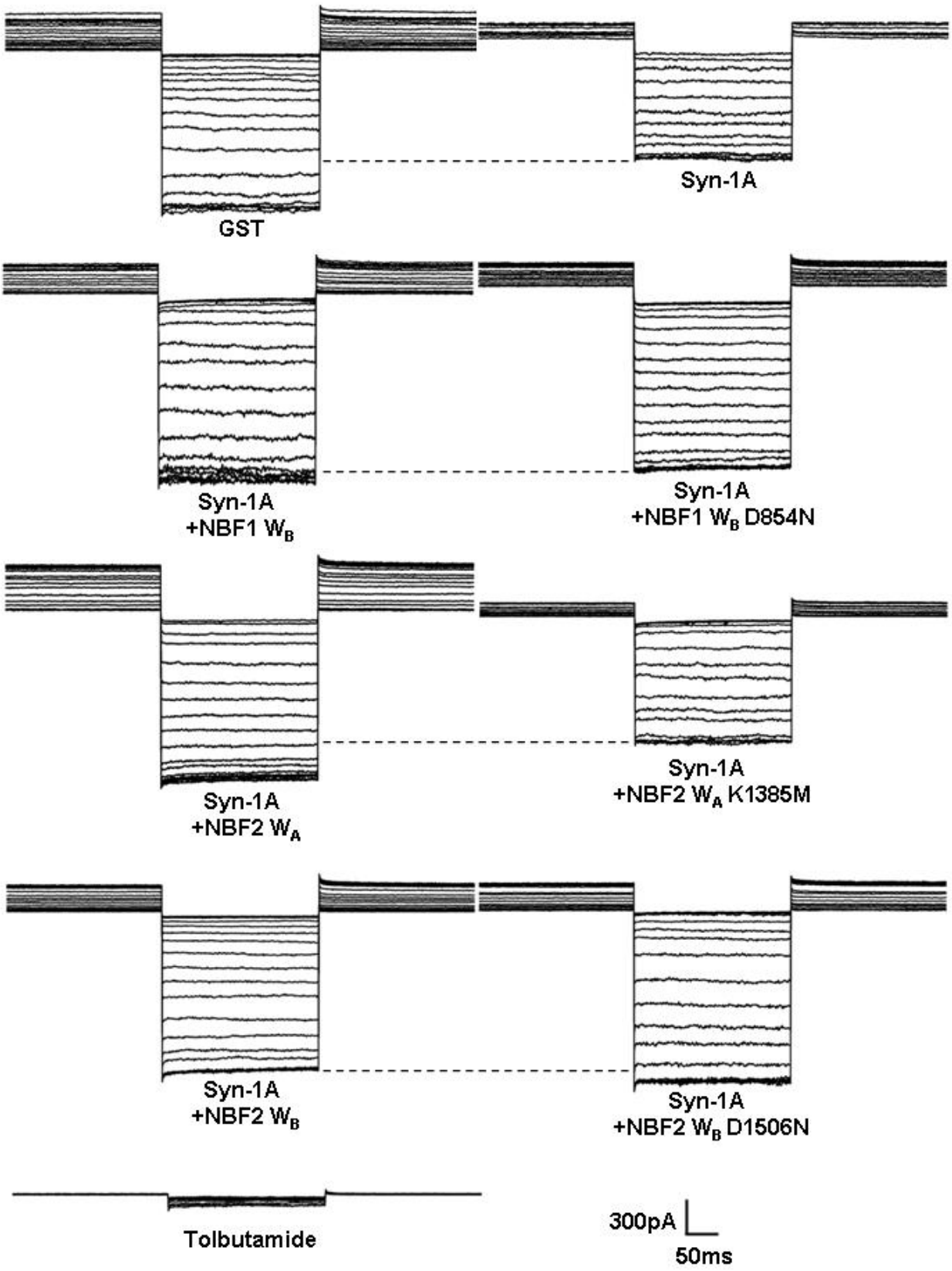
**Supplementary Figure 1. High concentration of NBF1 $W_A$ truncate does not affect Syn-1A-SUR1 interaction. A.** FRET study (as in Figure 4) shows that increasing concentrations of wt-NBF-1 $W_A$ truncate could not disrupt the FRET signal. *(i)* are representative recordings of FRET efficiency. Summaries of FRET efficiency, mean ± S.E.M., n=8 are shown as a graph *(ii)* and bar graph *(iii)*. **B.** Patch clamp study in INS-1 cells (as in Figure 2) showing that the highest concentration of wt NBF1 $W_A$ truncate (10 μM) used in *A* could not reverse Syn-1A inhibition of $K_{ATP}$ channel activity. Bar graph (means ± S.E.M.), n=4, * indicate $p < 0.05$.

**Supplementary Figure 2. Represetative examples of $K_{ATP}$ currents after dialysis with NBF1 or NBF2 Walker motif mutants.** Examples of whole cell currents in INS-1 cells after dialysis with indicated truncated wild type or Walker motif mutants of NBF1 and NBF2. Only NBF2-$W_A$ motif mutation NBF2-$W_A$ (K1385M) did not block Syn-1A inhibition of $K_{ATP}$ channels. $W_B$ motif mutants NBF1-$W_B$ (D854N) and NBF2-$W_B$ (D1506N), like their respective wild type proteins, blocked Syn-1A inhibition of $K_{ATP}$ currents. Also shown is tolbutamide (0.3 mM) inhibition of $K_{ATP}$ currents.

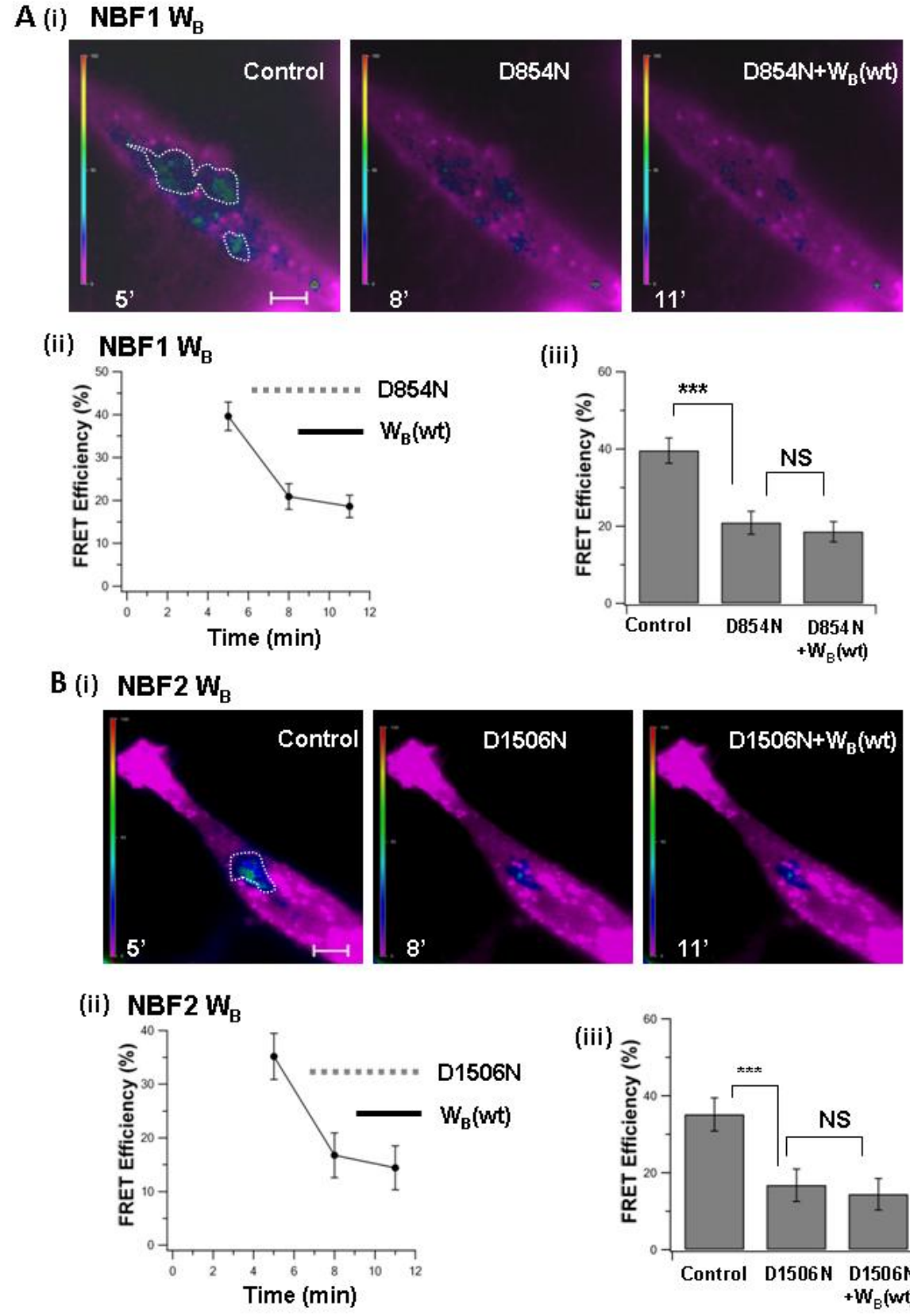
**Supplementary Figure 3. Both $W_B$ motif mutants NBF1-$W_B$ (D854N) (in A) and NBF2-$W_B$ (D1506N) (in B), like their respective wild type proteins, can disrupt Syn-1A interaction with SUR1 in live cells.** In **A** and **B**, (*i*) are the representative FRET recordings; scale bar indicates 5 μm and vertical scale bar indicates the FRET efficiency in pseudocolor. Summaries of data are shown in a graph (*ii*) and bar diagram (*iii*), mean ± S.E.M., N=7 for each. *** indicate $p < 0.001$. In both A and B, addition of the respective wild type proteins (1 μM) after the application of the $W_B$ motif mutant proteins (1 μM) did not further reduce FRET signals that were previously reduced by the $W_B$ motif mutants.




**Supplementary figure 1**




**Supplementary figure 2**




**Supplementary figure 3**