Supplementary Information for

A Rab prenyl membrane-anchor allows effector recognition to be regulated by guanine nucleotide

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This PDF file includes:

Figs. S1 to S5

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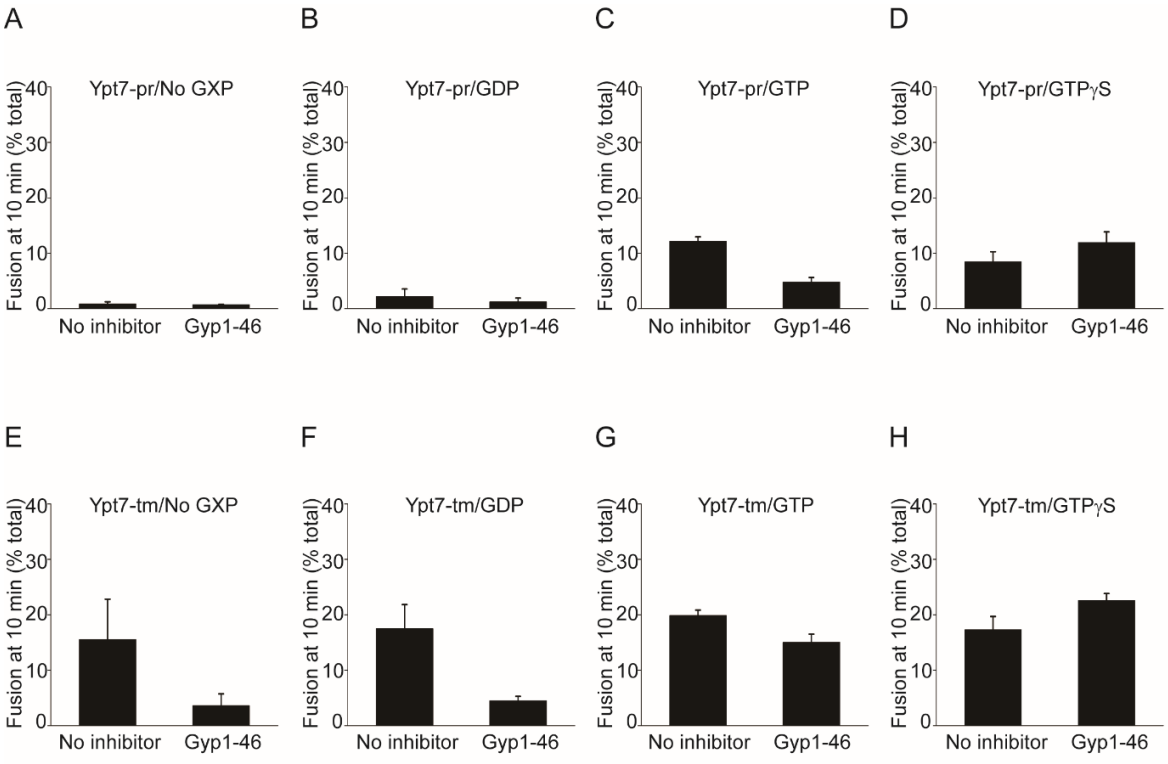

Fig. S1. The membrane anchor of Ypt7 regulates its response to bound guanine nucleotide. Fusion assays were performed as described in Fig. 1. The average and standard deviation of fusion at 10 min from three independent experiments is shown.

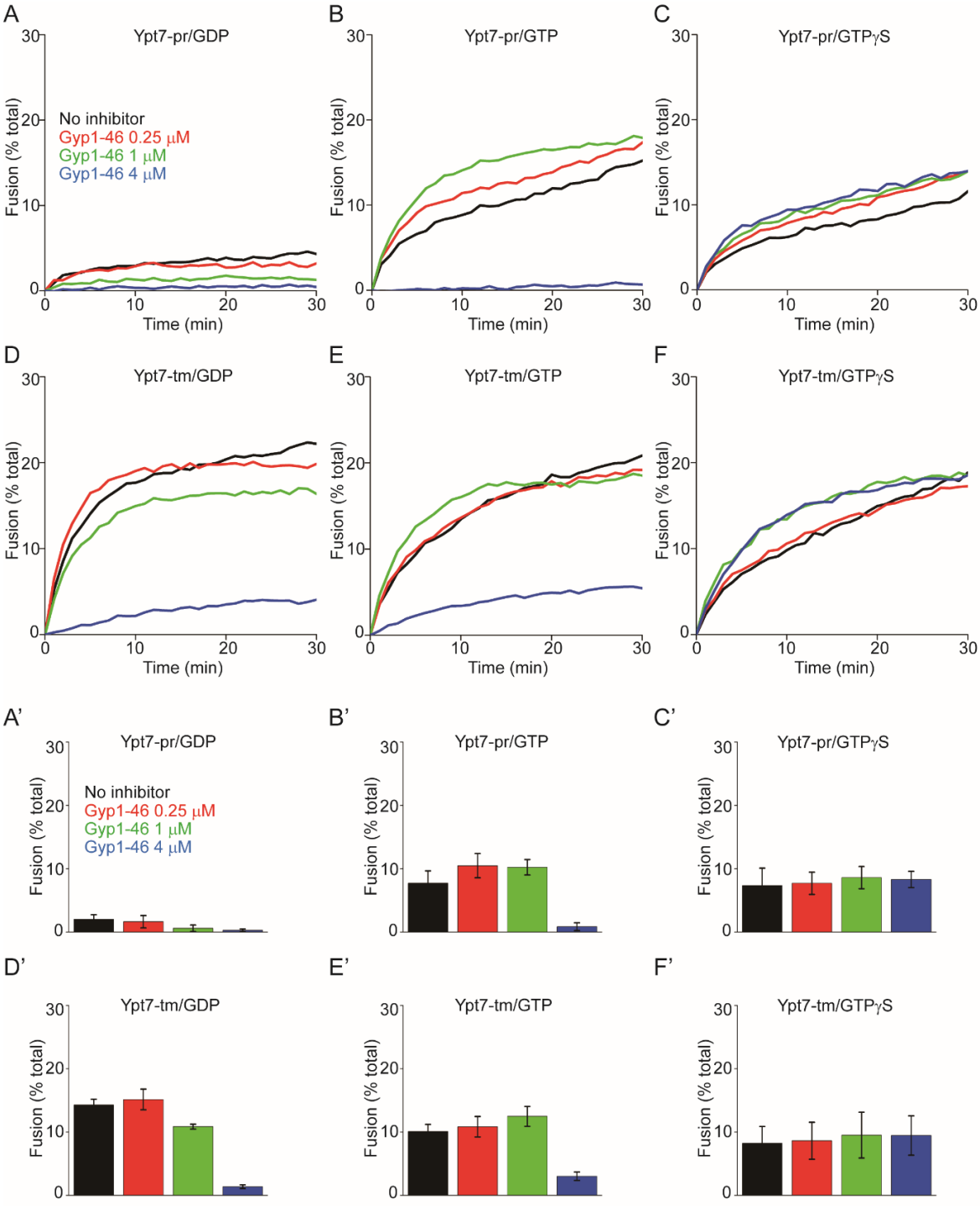

Fig. S2. The fusion of Ypt7-pr and Ypt7-tm proteoliposomes, charged with GDP or GTP, were inhibited by comparable levels of Gyp1-46.

Fusion reactions contained proteoliposomes bearing R- or QaQb- SNARE at 1:16000 SNARE to lipid molar ratio and a 1:8000 protein to lipid molar ratio of either Ypt7-pr (A-C) or Ypt7-tm (D-F). R- and QaQb- SNARE proteoliposomes were separately charged with either GDP, GTP or GTP γS as indicated, then preincubated with RB150 buffer or different concentrations of Gyp1-46; none (black line), 0.25 μM (red line), 1 μM (green line) or 4 μM (blue line). Content mixing was assayed in the presence of 50 nM HOPS and 100 nM Qc by measuring the FRET between luminal Cy5 and phycoerythrin for 30 min at 27˚C. Kinetic curves of content mixing assays in this figure are representative of n≥3 experiments.

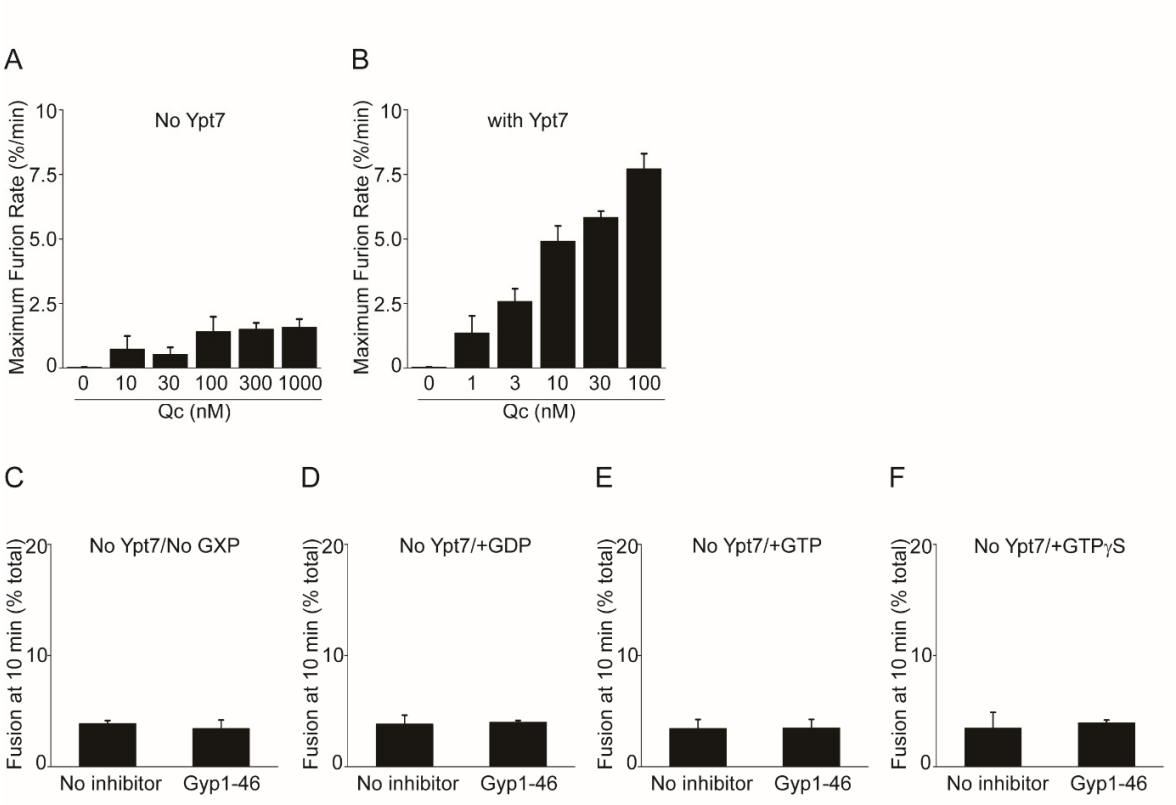

Fig. S3. Gyp1-46 and guanine nucleotide regulate fusion through their actions on Ypt7. Fusion assays were performed as described in Fig. 2. Average and standard deviations of maximum fusion rates are shown for the first 5 minutes (A, B) or 10 minutes (C-F) from three independent experiments. For the fusion without Qc, the first fusion value was subtracted from the last fusion value and divided by 30 minutes.

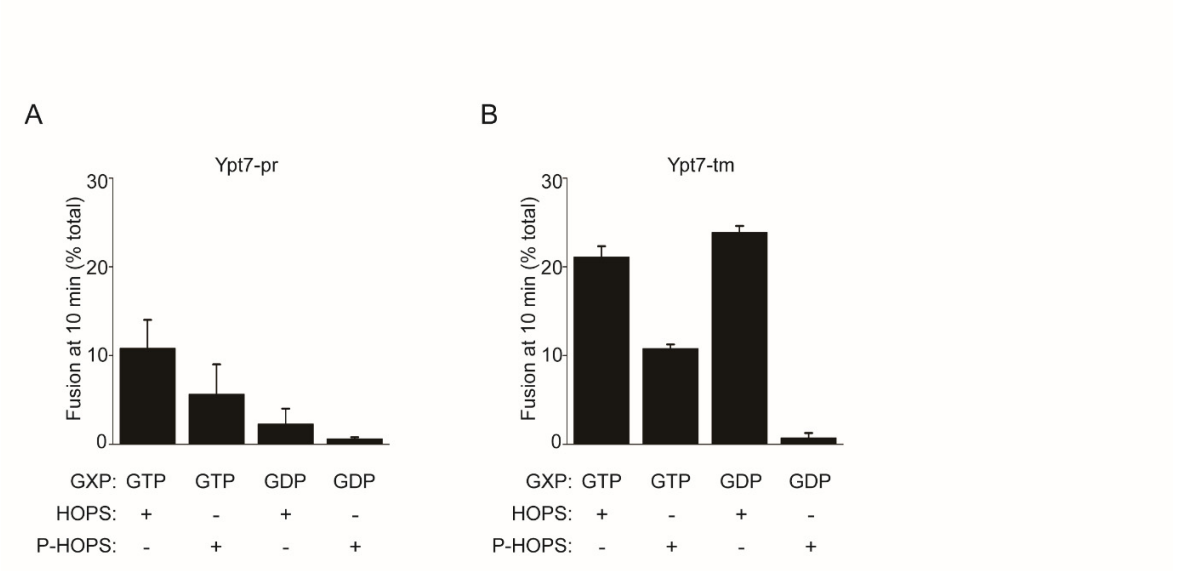

Fig. S4. Phosphorylated HOPS discriminates between GTP- and GDP-charged Ypt7-tm for proteoliposome fusion.

Fusion assays were performed as described in Fig. 4. R- and QaQb proteoliposomes bearing Ypt7-pr (A) or Ypt7-tm (B) were charged with either GDP or GTP, and fusion was assayed in the presence of 100 nM Qc, 50 nM HOPS and/or 50 nM P-HOPS. Average and standard deviations of fusion at 10 min from three independent experiments are shown.

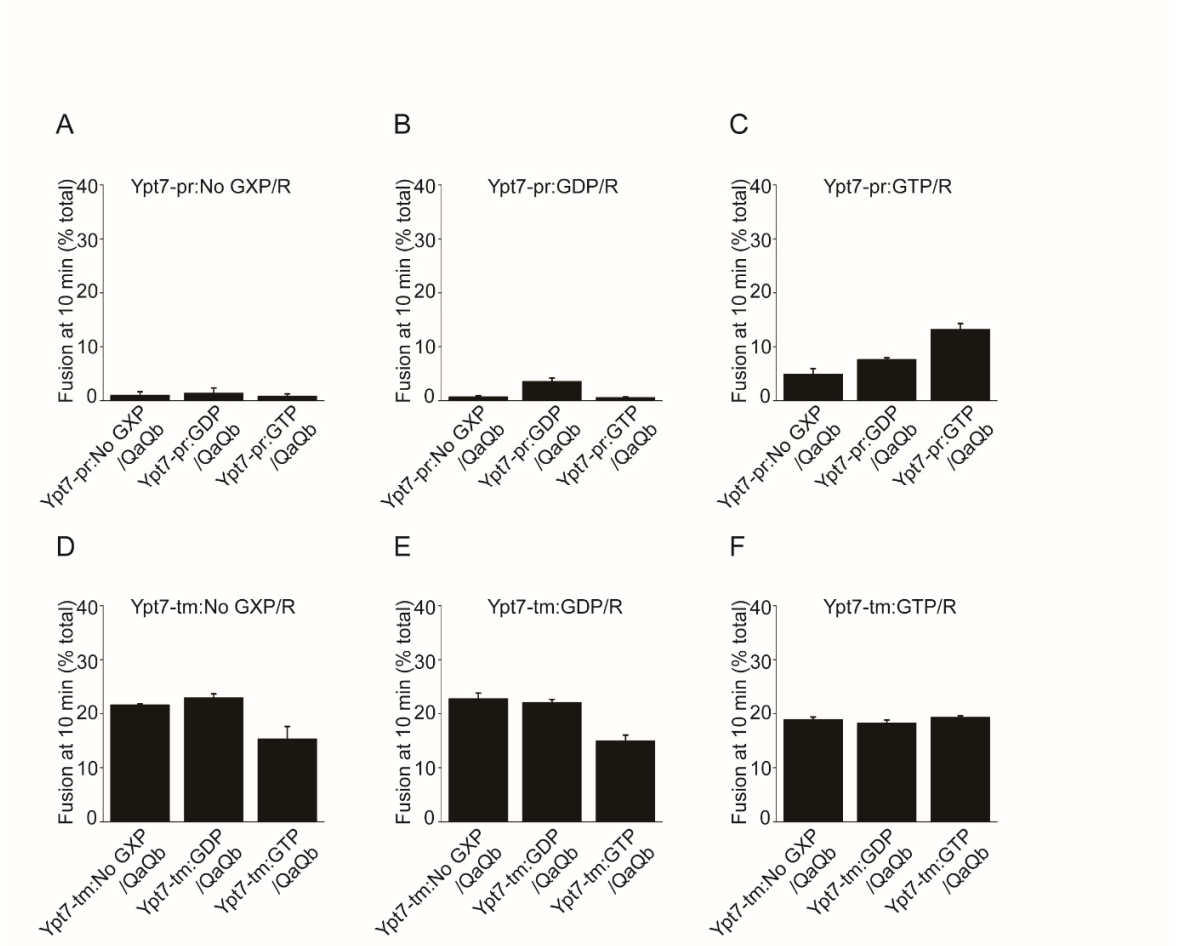

Fig. S5. With Ypt7-pr, GTP is required on the R-RPLs for optimal fusion with HOPS.

Fusion assays were performed as described in Fig. 5. Average and standard deviations of fusion at 10 min from three independent experiments are shown.