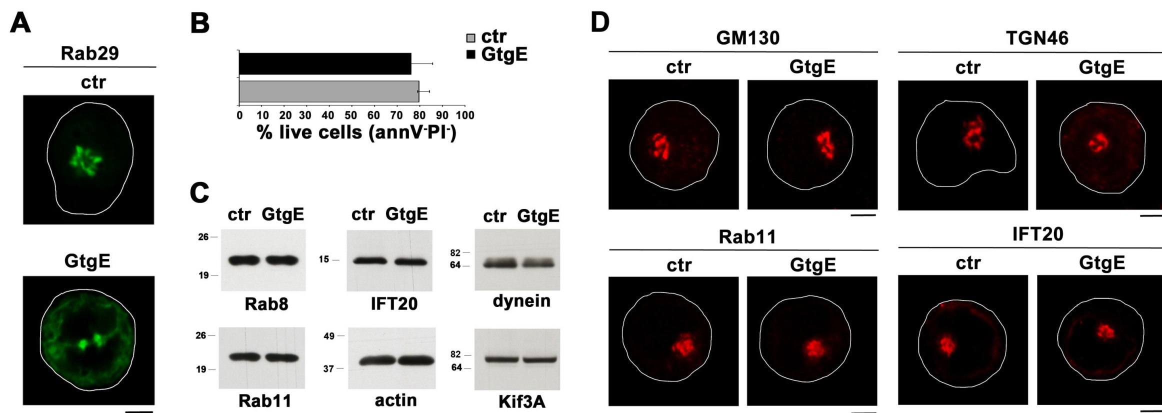

FIGURE S1

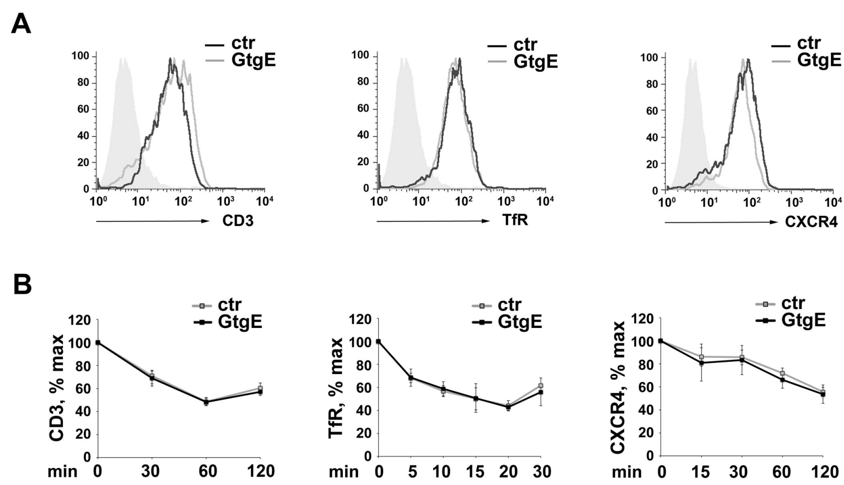

FIGURE S2

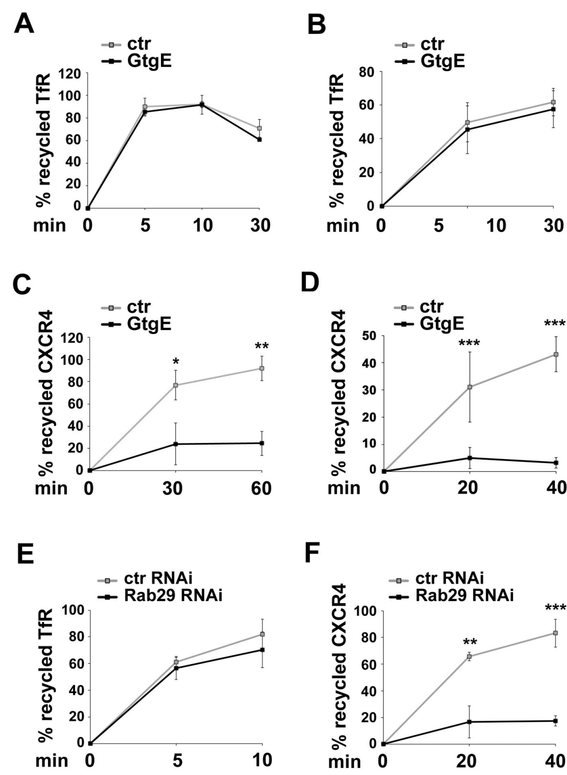

FIGURE S3

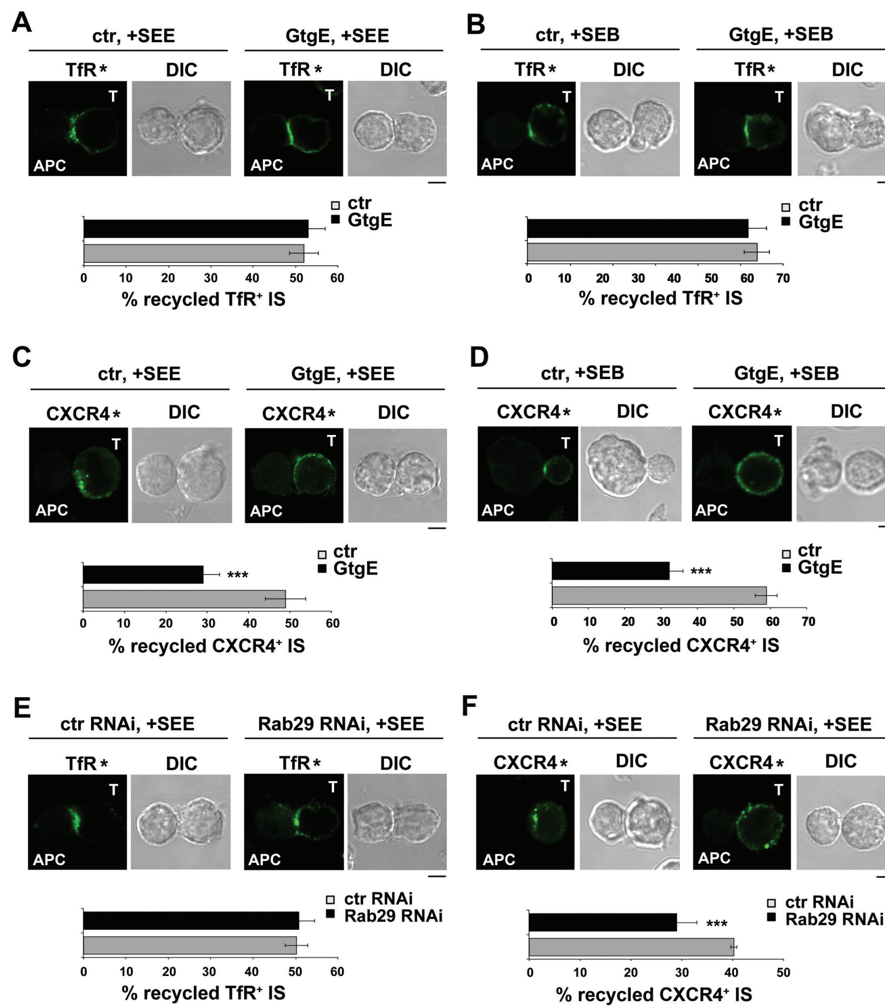

FIGURE S4

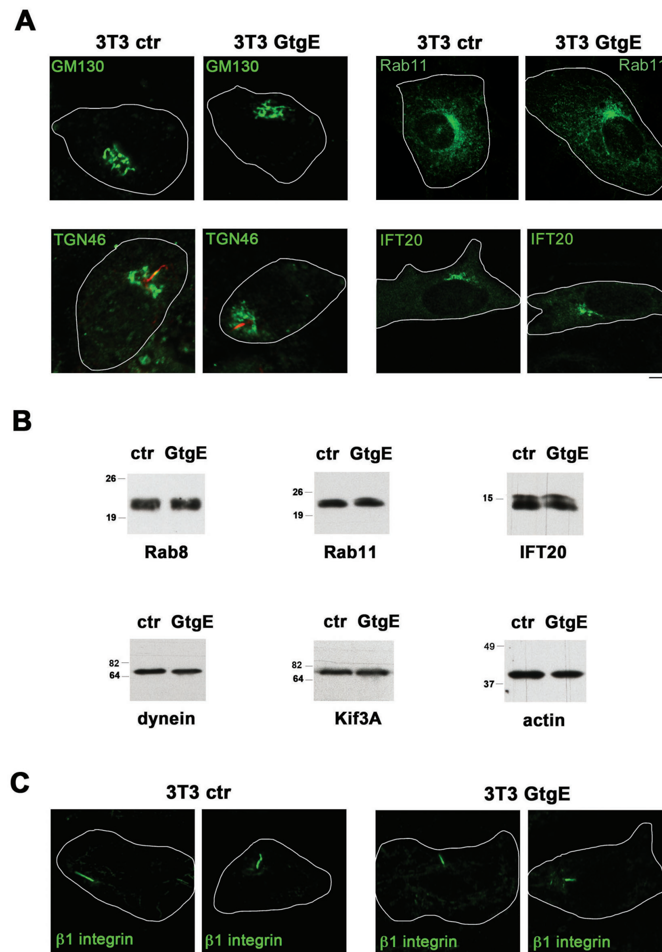

FIGURE S5

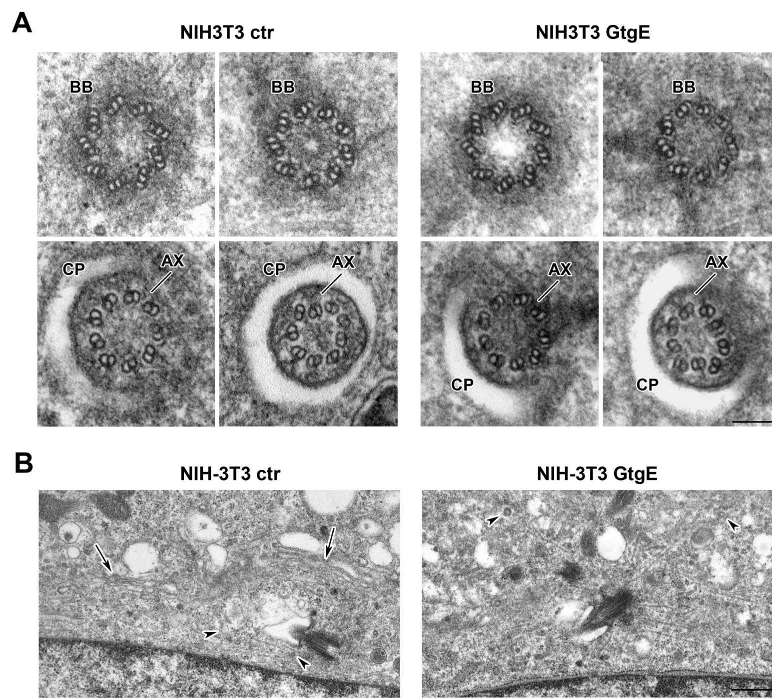

FIGURE S6

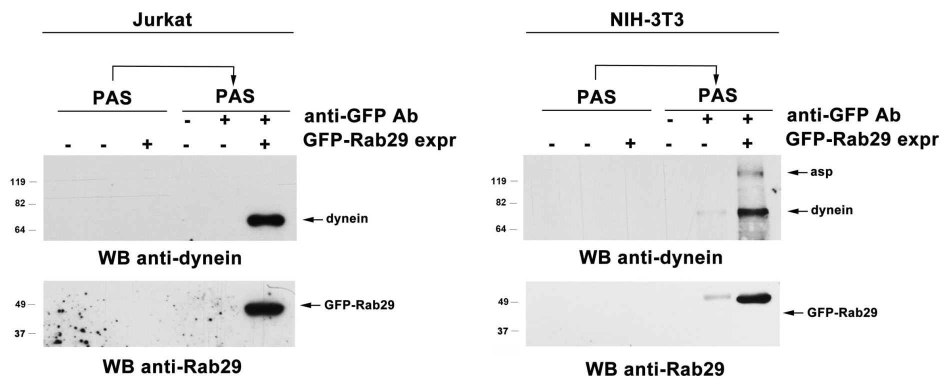

FIGURE S7

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Rab29 depletion by GtgE expression is not associated with off-target effects in T cells. A. Immunofluorescence analysis of Rab29 in control or GtgE-expressing Jurkat cells transiently transfected with a GFP-Rab29 expression construct. Median optical sections are shown. Size bar, 5 μm. **B.** Cell viability of control or GtgE-expressing Jurkat cells, measured by flow cytometric analysis of annexin-V/propidium iodide staining. Viable cells were identified as annV^{-}PI^{-} (n=3). **C.** Immunoblot analysis of Rab8, Rab11, IFT20, dynein and kinesin in lysates of control or GtgE-expressing Jurkat cells. Actin was used as loading control. **D.** Immunofluorescence analysis of GM130 (cis-Golgi), TGN46 (trans-Golgi network), Rab11 and IFT20 in control or GtgE-expressing Jurkat cells.

Figure S2. Rab29 depletion does not affect surface TCR, TfR or CXCR4 expression or internalization. A. FACS profile of the surface level of TCR, TfR or CXCR4 in control- and GtgE-expressing stable Jurkat transfectants. The FACS profile shown is representative of 3 independent experiments. **B**. Flow cytometric analysis of TCR, TfR or CXCR4 internalization in control- and GtgE-expressing stable Jurkat transfectants. The data, which for each time point refer to triplicate samples from 3 independent experiments, are presented as % of the receptor to the cell surface that had internalized after the 37°C shift at the indicated times. Error bars, SD.

Figure S3. Rab29 is required for CXCR4 but not TfR recycling in T cells. Flow cytometric analysis of TfR and CXCR4 recycling in control and GtgE-expressing Jurkat cells (**A,C**) or primary T cells (**B,D**), or control and Rab29KD Jurkat cells (**E,F**). The data, which for each time point refer to duplicate samples from 3 independent experiments, are presented as % of internalized receptors that have recycled to the cell surface (mean±SD). ***, $p<0.001$; **, $p<0.01$; *, $p<0.05$.

Figure S4. Rab29 is required for polarized recycling to the IS of CXCR4 but not TfR. Immunofluorescence analysis under non-permeabilizing conditions of recycled TfR (TfR*) and CXCR4 (CXCR4*) in conjugates of control or GtgE-expressing Jurkat cells (**A,C**) or primary T cells (**B,D**) and SEE/SEB-pulsed Raji cells, or of control or Rab29KD Jurkat cells and SEE-pulsed Raji cells (**E,F**). Median optical sections are shown. Size bar, 5 μm. Quantifications (%) of conjugates harboring recycled TfR or CXCR4 at the IS are shown on the right. At least 20 cells were analyzed in each experiment ($n \geq 3$). ***, $p<0.001$. The proportion of conjugates of control or GtgE-expressing Jurkat cells and SEE-pulsed Raji cells displaying IS polarization of early ($Rab5^+$) and recycling ($Rab11^+$, $Rab4^+$) endosomes was the following: $Rab5^+$ IS, 65.6±3.5% ctr vs 62.9±4.8% GtgE; $Rab11^+$ IS, 68.9±7.1% ctr vs 63.1±5.0% GtgE; GFP-$Rab4^+$ IS, 62.3±5.5% ctr vs 59.5±7.4% GtgE (at least 150 conjugates analyzed, n=3).

Figure S5. Rab29 depletion by GtgE expression is not associated with off-target effects in NIH3T3 cells. A. Immunofluorescence analysis of GM130 (cis-Golgi), TGN46 (trans-Golgi network) and IFT20 in control or GtgE-expressing NIH-3T3 cells. Cells stained for TGN46 (green) were co-stained for acetylated tubulin (red). **B.** Immunoblot analysis of Rab8, Rab11, IFT20, dynein and kinesin in lysates of control or GtgE-expressing NIH-3T3 cells. Actin was used as loading control. **C.** Immunofluorescence analysis of β1 integrin in control or GtgE-expressing NIH-3T3 cells. Size bar, 5 μm.

Figure S6. **Rab29 deficiency does not affect either the basal body or the ciliary axoneme but results in impaired vesicular trafficking to the primary cilium**. **A.** TEM analysis of the primary cilium in control and GtgE-expressing NIH-3T3 cells by cross sections, from the basal body along the primary cilium. AX, axoneme; BB, basal body; CP, ciliary pocket. Size bar, 100nm. **B**. TEM analysis of control and GtgE-expressing NIH3T3 cells in which the presence of vesicles both in the proximity of the periciliary membrane and in the cytosol is shown (examples indicated by arrowheads, the lowest of which in control cells is associated with a microtubule). Note the reduction in the presence of vesicles in the proximity of the periciliary membrane and the more

dispersed cytosolic distribution of vesicles in GtgE-expressing NIH3T3. Arrows indicate Golgi stacks. Size bar, 500 nm.

Figure S7. Immunoblot and immunoprecipitation controls. Immunoblot analysis with anti-Rab29 mAb of GFP-specific immunoprecipitates from lysates of Jurkat or NIH-3T3 cells transiently transfected with either empty vector or the same vector encoding GFP-Rab29. The preclearing controls (proteins that bound to Protein-A–Sepharose before the addition of primary antibody; PAS) were included. The migration of molecular mass markers is indicated. Total cell lysates were included in each gel to identify the migration of the proteins tested. The arrows indicate the migration of dynein and GFP-Rab29.