

## Kyei et al. Supplemental figures and movies

**Supplemental Fig. S1. Rab14 is expressed in RAW 264.7 macrophages.** (A) RT-PCR analysis of Rab14 expression in macrophages. Hprt (hypoxanthine guanine phosphoribosyl transferase) was a positive control. Rab27a, expressed in cytotoxic T lymphocytes (CTL) but not in RAW264.7 cells, was used as a negative control. (B) Western blot of RAW 264.7 cytosol shows Rab14 as a 24 kDa band. (C-E) Confocal images of cells transfected with EGFP-Rab14WT and immunostained with Rab14 antibody. (F) RAW264.7 cells (preincubated for 30 min with or without monoclonal anti-CD11b antibody) were synchronously infected for 10 min with Texas Red labeled or unlabeled BCG at a multiplicity of infection of 10:1. Cells were washed and macrophage associated bacteria plated on 7H11 plates and colonies counted after 2 weeks. The efficiency of BCG-macrophage association is expressed as a percentage of BCG input taken up by or adherent to macrophage. Bars, means  $\pm$  S.E.M (3 independent experiments),  $^{\dagger}p > 0.05$ , ANOVA.

**Supplemental Fig. S2. Knockdown of Rab14 with individual Rab14-specific siRNAs and effects on mycobacterial phagosome maturation.** (A) Western blot showing Rab14 knockdown efficiencies with 4 different siRNA duplexes:

- (i) sense: CAACUACUCUUACAUCUUUUU  
antisense: 5'-pAAAGAUGUAAGAGUAGUUGUU
- (ii) sense: ACAGAGAGAUGUUACCUAUU  
antisense: 5'-pAUAGGUAACAUCUCUCUGUUU
- (iii) sense: GAGGACGGCUAACCAGUGAUU  
antisense: 5'-pUCACUGGUUAGCCGUCCUCUU
- (iv) sense: GAAUAAAGCAGACUUGGAUU  
5'-pUCCAAGUCUGCUUUUUUUUCUU

(B-C) RAW 264.7 cells were transfected with Rab14 siRNA duplex (i) and infected with Texas Red labeled live BCG. Phagosome maturation was scored as colocalization between BCG (red) and CD63 (blue) and quantified (C). Bars, means  $\pm$  S.E.M (3 independent experiments); n, number of phagosomes counted per condition.  $^{**}p < 0.001$  between Rab14 siRNA duplex (i) and scrambled siRNA control.

**Supplemental Fig. S3. Rab14 colocalizes with early/recycling endosomal markers in macrophages.** (A) RAW 264.7 macrophages were transfected with EGFP-Rab14WT and immunostained for EEA1, syntaxin 13 and cellubrevin. Arrows indicate profiles with colocalization. (B) RAW 264.7 macrophages showing immunostained endogenous EEA1 and Rab14. (C) Mouse bone marrow macrophages were immunostained for endogenous EEA1 and Rab14.

**Supplemental Fig. S4**

**Overexpression of Rab14 does not change intracellular CD63 distribution.**

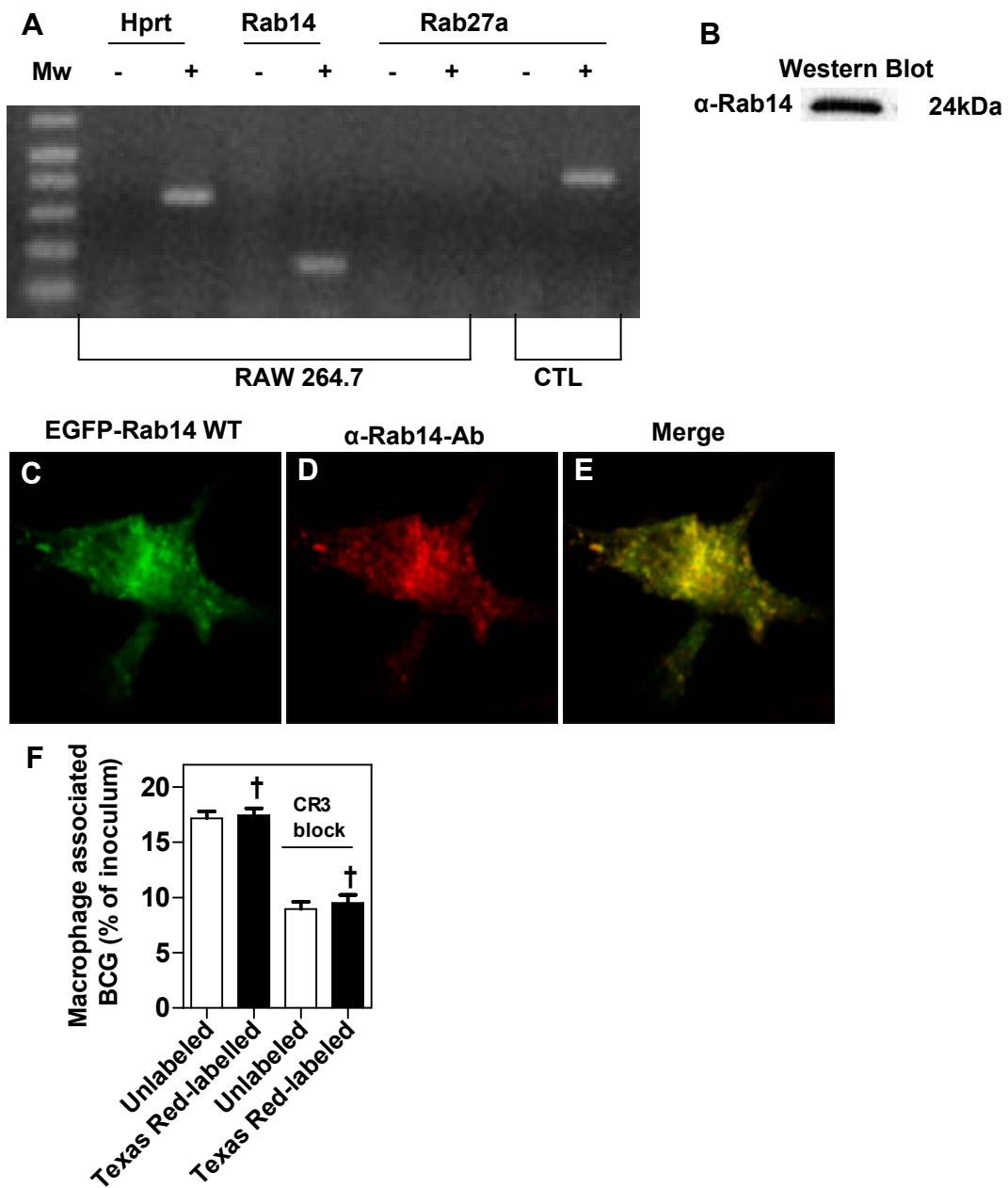
HeLa cells were transfected with the indicated Rab14 constructs for 24 h and immunostained for CD63.

**Supplemental Movie 1**

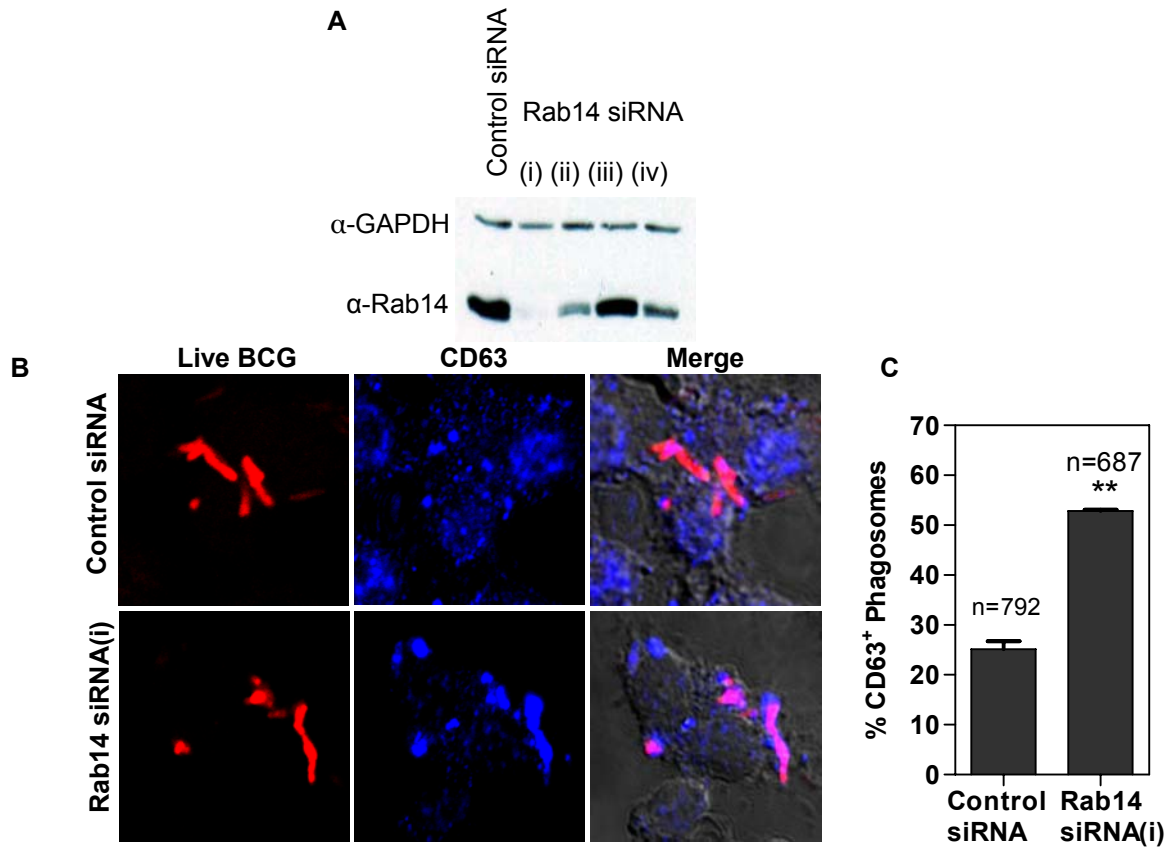
**EGFP-Rab14WT dynamics on live BCG phagosome.**

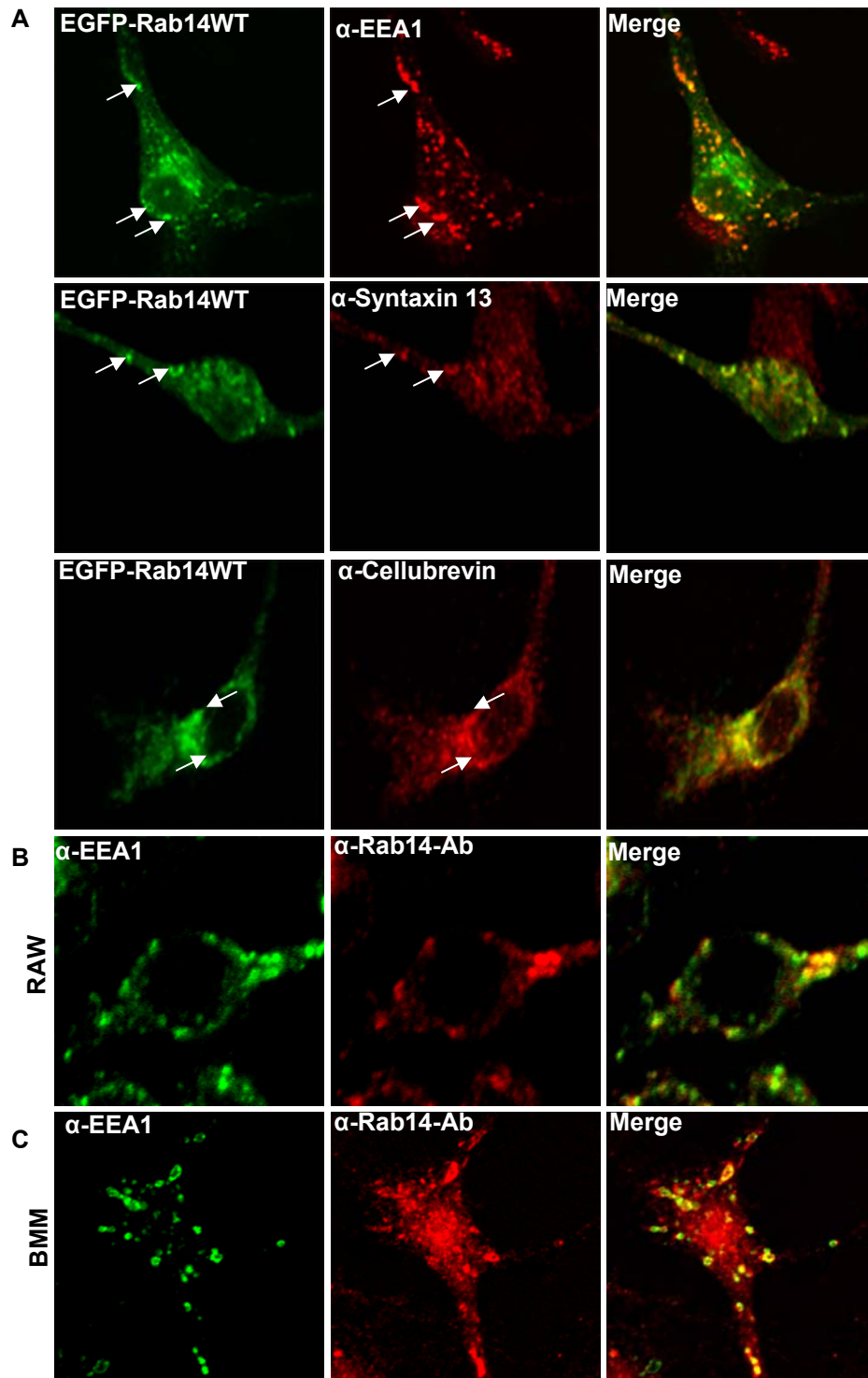
**Supplemental Movie 2**

**EGFP-Rab14WT dynamics on dead BCG phagosome**

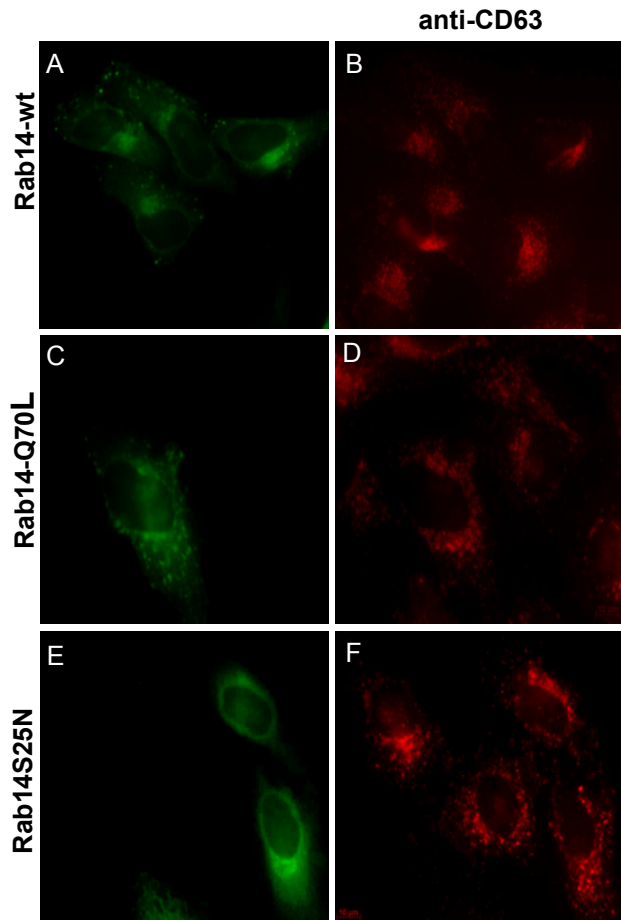


Supplemental Fig. S1





Supplemental Fig. S3



Supplemental Fig. S4