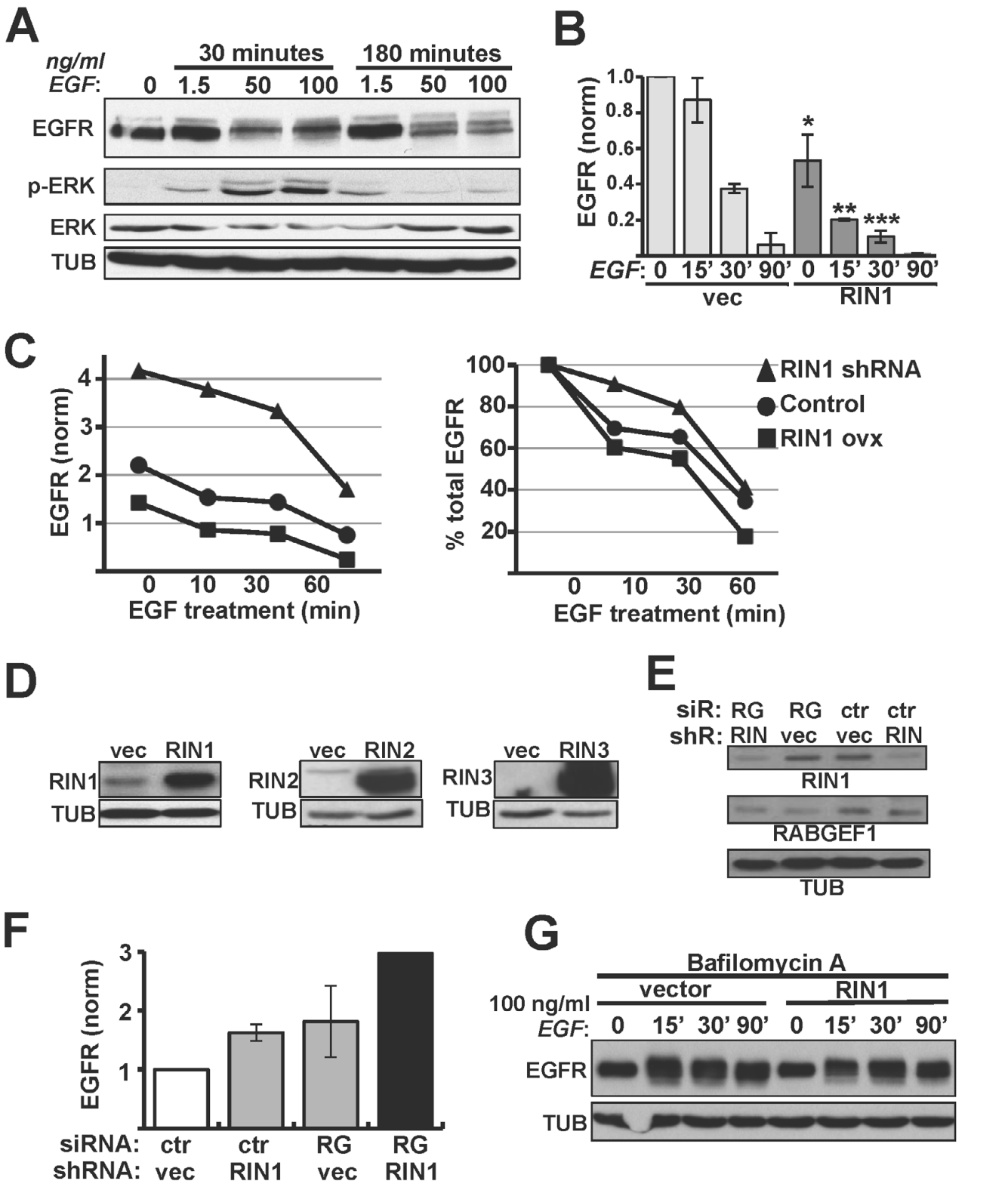

Fig. S1. EGF-induced down regulation of EGFR is controlled by RIN1. A. HeLa cells were stimulated with the indicated concentrations of EGF for the indicated times. Cell lysates were immunoblotted for EGFR, tubulin, total ERK1/2 and p-ERK1/2. **B.** EGFR quantification, normalized to tubulin, in HeLa cells transduced with vector or RIN1 following 100 ng/ml EGF treatment for the indicated times. Mean of three experiments ±SD (*p<0.05, **p<0.005, ***p<0.0005). **C.** Left: EGFR levels from Figure 1A, normalized to tubulin. Right: same data plotted as percent change in EGFR level. **D.** Vector transduced HeLa cells were immunoblotted for endogenous RIN1, RIN2 or RIN3. Cells transfected with RIN1, RIN2 or RIN3 were used as positive controls. **E.** Vector and RIN1-shRNA cells transfected with control or RABGEF1 (RG) siRNA, immunoblotted for RIN1, RABGEF1 or TUB. **F.** Quantification of EGFR levels, 30 minutes post 100 ng/ml EGF stimulation, relative to unstimulated cells, expressed as a fraction of control cells. RIN1 shRNA and RABGEF siRNA data are from three independent experiments. **G.** HeLa cells transduced with vector or RIN1 were pretreated with Bafilomycin A, stimulated with 100 ng/ml EGF for the indicated time, and lysates immunoblotted for EGFR. EGFR activation was confirmed by p-ERK immunoblot (data not shown).

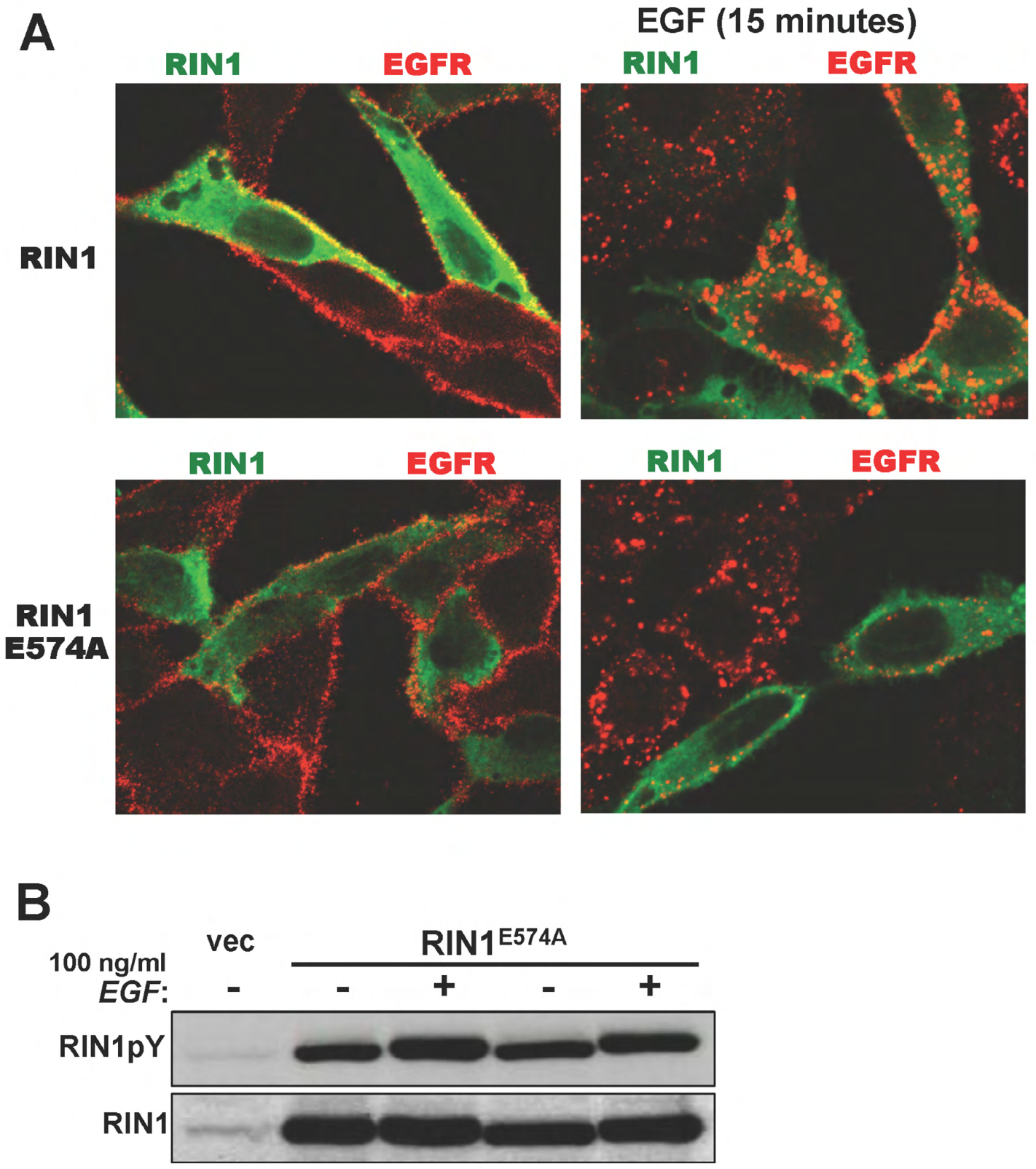

Fig. S2. RIN1→RAB5 signaling regulates early endosome size. **A.** HeLa cells stably transduced with RIN1 or RIN1^{E574A} were untreated or stimulated with 100 ng/ml EGF conjugated to AlexaFluor 647 (red) for 15 minutes, then stained for RIN1 (green). Untransduced cells serve as internal controls. **B.** HeLa cells transduced with vector, RIN1 or RIN1^{E574A} were untreated or stimulated with 100 ng/ml EGF for 15 minutes. Cell lysates were immunoblotted for pY-RIN1 and total RIN1. EGFR activation was confirmed by p-ERK immunoblot (data not shown).

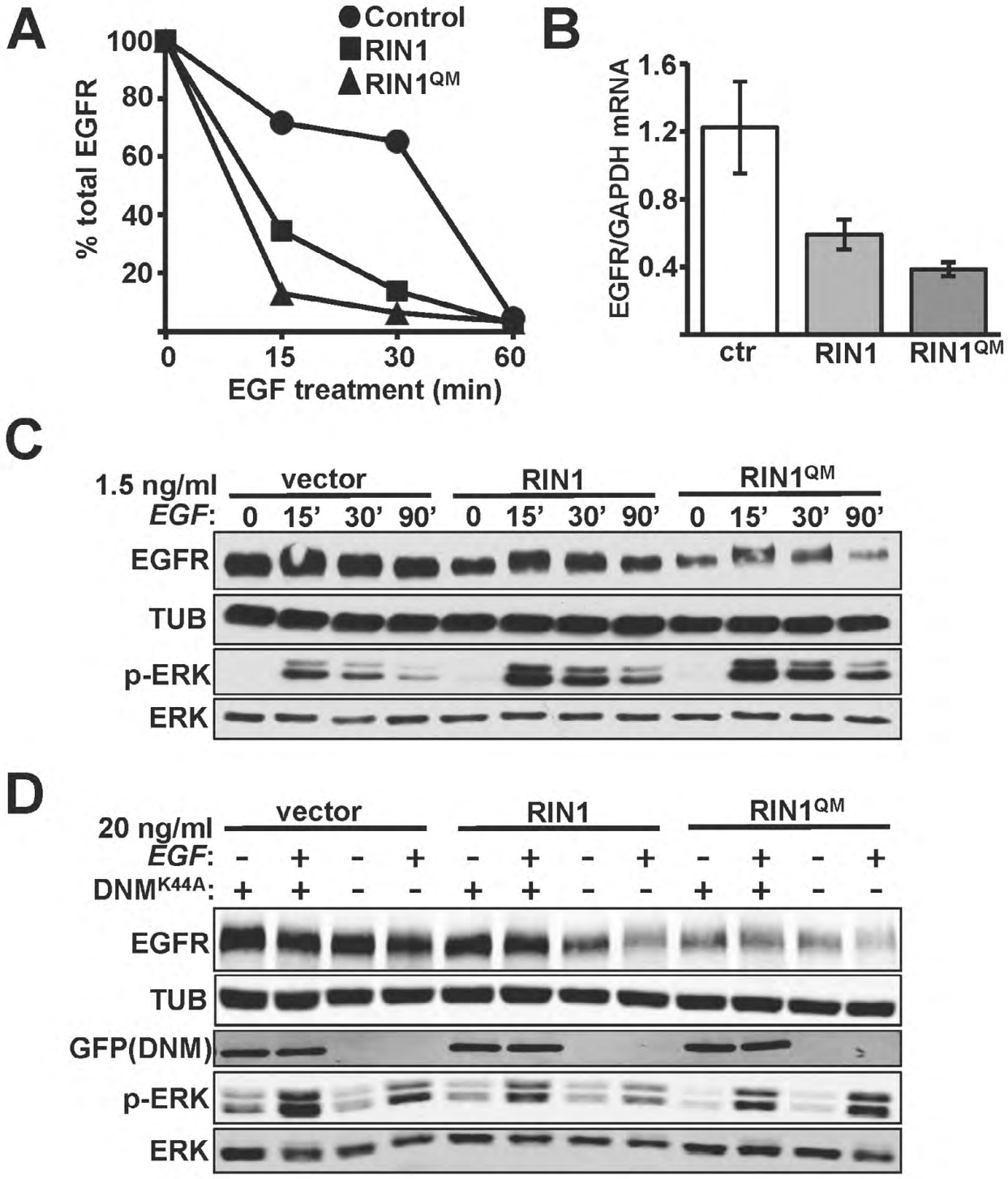

Fig. S3. RIN1^{QM} causes accelerated EGFR degradation. **A.** Quantification of EGFR degradation rate (Figure 3B data). **B.** EGFR mRNA levels normalized to GAPDH mRNA in vector, RIN1 or RIN1^{QM} HeLa cells. The data represent two independent qRT-PCR experiments, each performed in duplicate. **C.** HeLa cells transduced with vector, RIN1 or RIN1^{QM} were stimulated with 1.5 ng/ml EGF for the indicated times and lysates immunoblotted for EGFR, total ERK1/2 and p-ERK1/2. **D.** Vector, RIN1 or RIN1^{QM} HeLa cells were transfected with empty vector or dominant-negative dynamin (DNM^{K44A}). Cells were stimulated, or not, with 20 ng/ml EGF for 60 minutes and lysates immunoblotted for EGFR, TUB, total ERK1/2, p-ERK1/2 and GFP (dynamin).

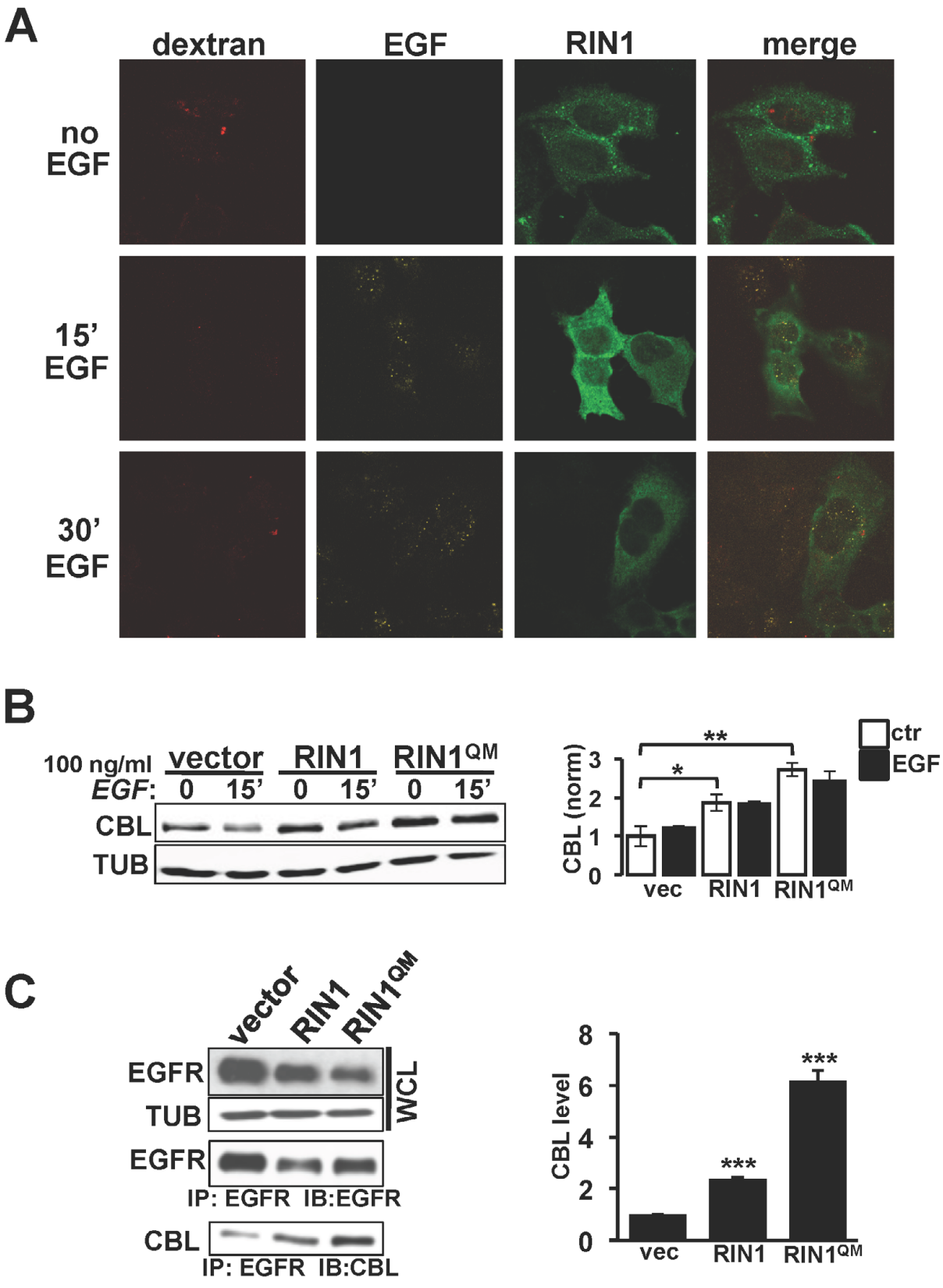

Fig. S4. RIN1^{QM} effects in HeLa cells. A. Control for Figure 5D. HeLa cells transduced with RIN1 were pulsed with 20 ng/ml EGF and 1mg/ml Dextran for 5 minutes, as with RIN1^{QM} cells in Figure 5D and chased in serum-free medium for the indicated time. Untransfected cells serve as internal controls. red=dextran; yellow=EGF, green=RIN1. **B.** Left: Transduced HeLa cells were stimulated, or not, with 100 ng/ml EGF for 15 min. Lysates were immunoblotted with anti-CBL or anti-TUB. Right: CBL levels normalized to TUB (mean of three experiments ± SD). EGFR activation was confirmed by p-ERK immunoblot (data not shown). **C.** Left: Transduced HeLa cells (as in B) were serum-starved overnight and lysates probed for EGFR or TUB. Anti-EGFR immunoprecipitates were blotted with anti-EGFR or anti-CBL. Right: Quantification of CBL associated with EGFR. Mean of three experiments ± SD. * $p<0.05$; ** $p<0.005$; *** $p<0.0005$.

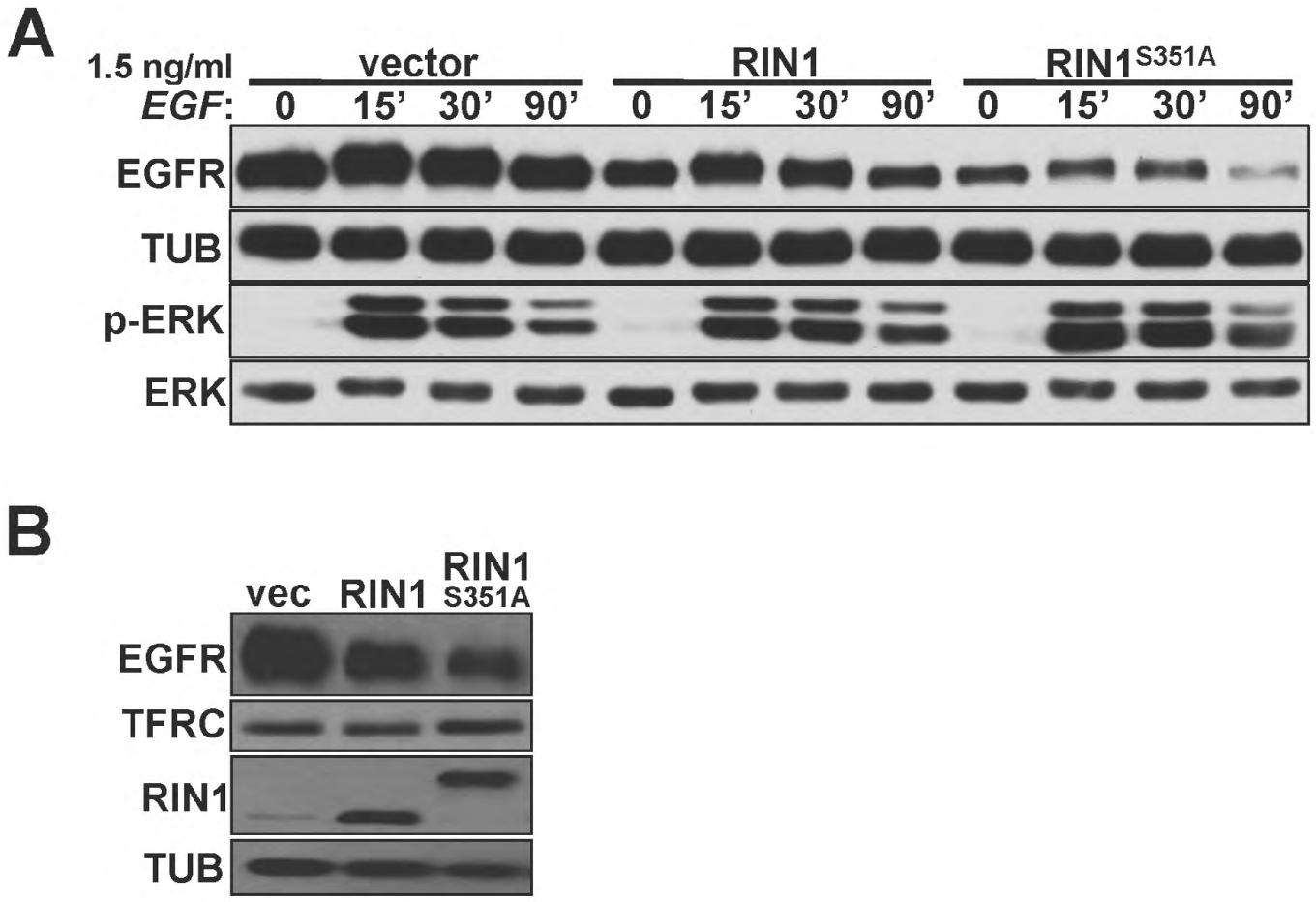

Fig. S5. Plasma membrane localization of RIN1 promotes EGFR degradation A. HeLa cells transduced with vec, RIN1 or RIN1^{S351A} were stimulated with 1.5 ng/ml EGF for the indicated times and lysates probed for EGFR, ERK1/2 and p-ERK1/2. **B.** Transduced HeLa cells were immunoblotted for EGFR, transferrin receptor (TFRC) and RIN1.

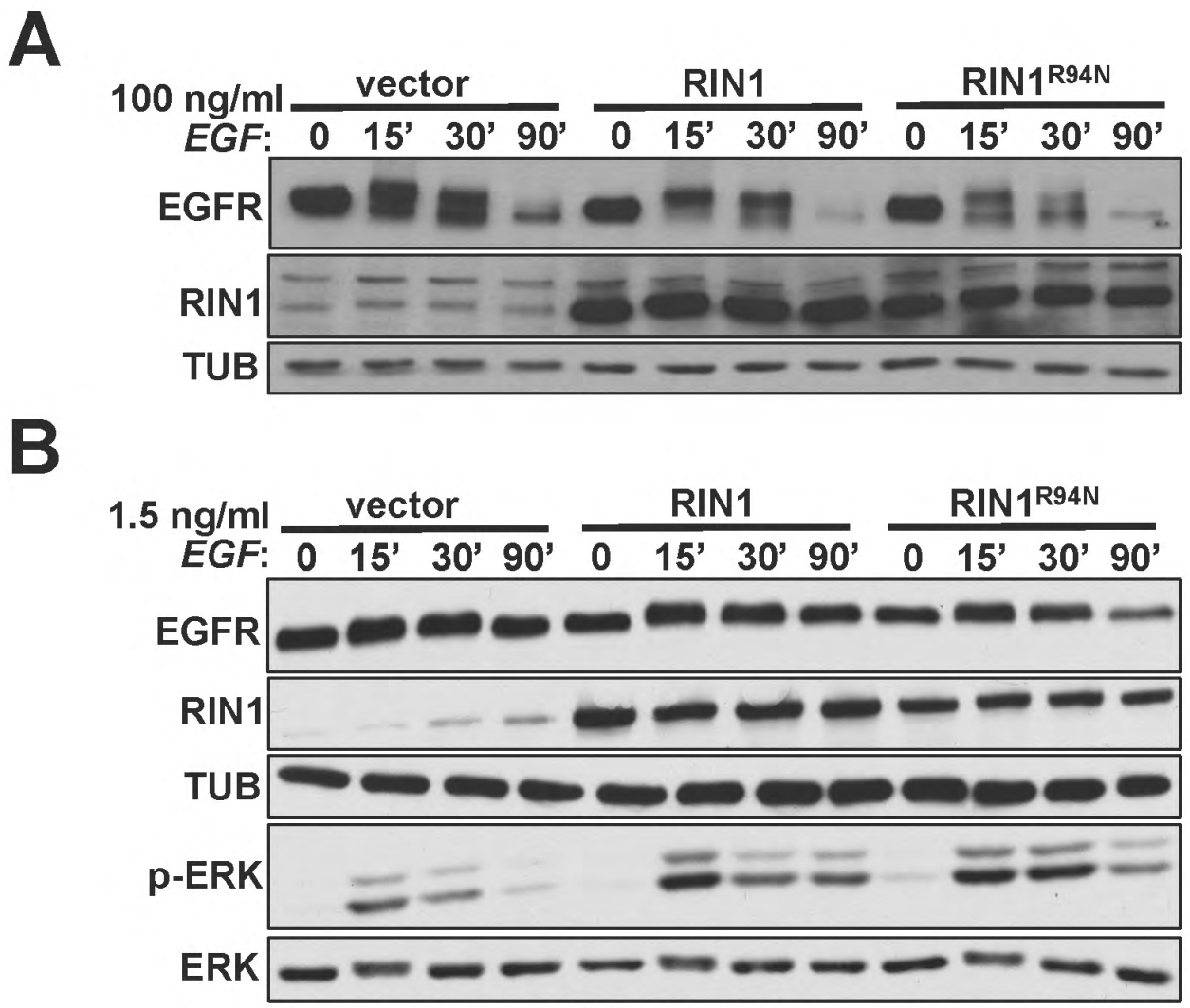

Fig. S6. RIN1^{R94N} does not alter EGFR signaling through p-ERK. A. Vector, RIN1 or RIN1^{R94N} HeLa cells were stimulated with 100 ng/ml EGF for the indicated time and lysates immunoblotted for EGFR, RIN1 or TUB. EGFR activation was confirmed by p-ERK immunoblot (data not shown). **B.** Vector, RIN1 or RIN1^{R94N} HeLa cells stimulated with 1.5 ng/ml EGF for the indicated time and lysates immunoblotted for EGFR, RIN1, TUB, total ERK1/2 or p-ERK1/2.

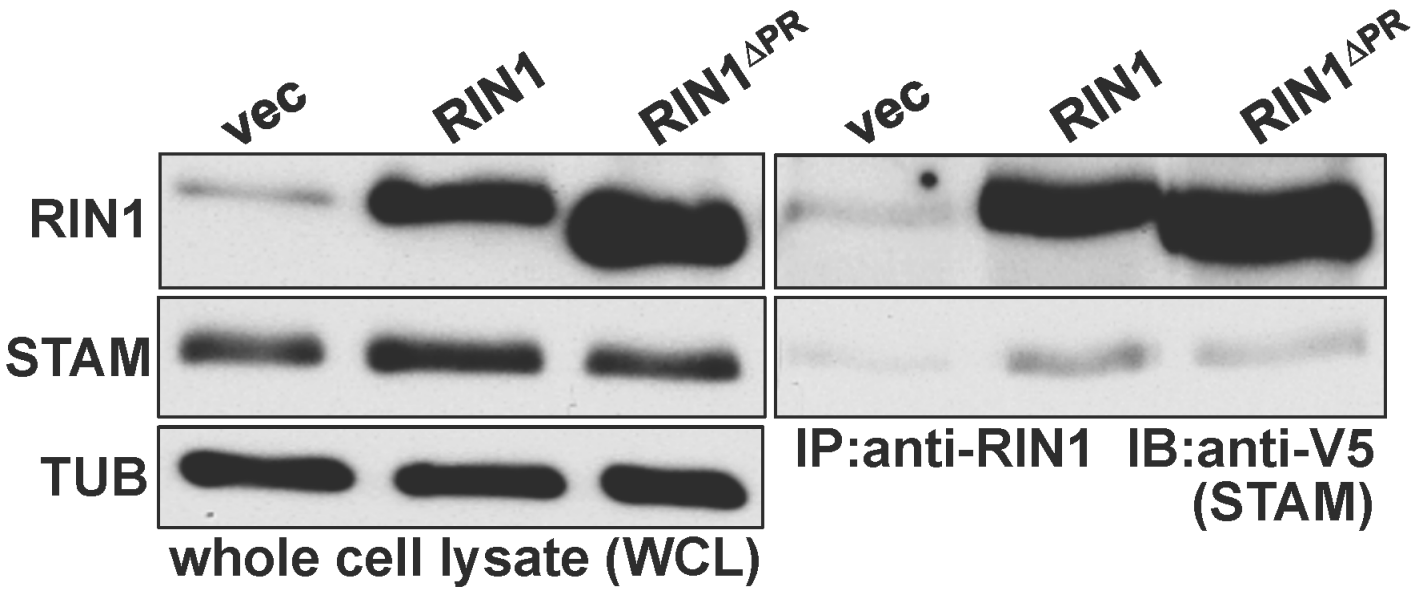

Fig. S7. ΔPR mutation reduces the RIN1::STAM interaction. HeLa cells were transduced with vector, RIN1 or RIN1$^{\Delta PR}$. Cell lysates and RIN1 immunoprecipitates were blotted for V5 (STAM) or RIN1.

Table S1. Characterization of RIN1 mutant proteins used in this work. The RIN1$^{\Delta PR}$ mutant used in these studies (construction described in the Methods section) is similar but not identical to a mutant described in Kong et al., 2007.

Mutant	Defect	Further characterization
RIN1^{E574A}	Reduced GEF activity(Galvis et al., 2009a, Galvis et al., 2009b, Hu et al., 2008) (can not activate RAB5)	Still binds to activated (GTP-bound) RAS (Hu et al., 2008) Still binds to activated (p-Tyr) EGFR (Hu et al., 2008) Still activates ABL tyrosine kinases (*this work*)
RIN1^{QM}	Reduced ABL interaction(Hu et al., 2005, Hu et al., 2008) (does not stimulate ABL)	Still binds activated (GTP-bound) RAS (Hu et al., 2008) Still binds to RAB5^{S34N} (GDP-bound) (Hu et al., 2008)
RIN1^{S351A}	Reduced 14-3-3 interaction(Doppler et al., 2005, Wang et al., 2002, Ziegler et al., 2011) (increased PM localization)	Still binds activated (GTP-bound) RAS (Wang et al., 2002) Still binds to activated (p-Tyr) EGFR (Hu et al., 2008)
RIN1 ^{R94N}	Reduced EGFR interaction(Barbieri et al., 2003, Hu et al., 2008) (SH2 cannot bind pTyr)	Still binds RAS and RAB5 (SH2 deletion) (Barbieri et al., 2003) Still binds to activated (p-Tyr) EGFR (Hu et al., 2008)
RIN1$^{\Delta PR}$	Reduced STAM binding (Kong et al., 2007) Reduced BIN1 binding (*this work*)	