Figure S1. Deletion of TIP30, ACSL4, or Endo B1 Delays EGF-EGFR dissociation and inhibits the recruitment of Rab5a to endocytic vesicles

A-B, TIP30, ACSL4, or Endo B1 knockdown delays EGF-EGFR dissociation and inhibits the recruitment of Rab5a to endocytic vesicles. Localization of EGFR (red) and Rab5a (green) in control, TIP30 KD, ACSL4 KD and Endo B1 KD cells were monitored 120 min after $Alexa^{488}$-EGF (blue) internalization. Nucleus is stained by DAPI (gray). Typical images of cells in each group are shown. Boxed areas are magnified. Magenta represents red and blue overlap. Bars, 10 μm.
C, TIP30 deletion in primary hepatocytes leads to delayed EGF-EGFR dissociation and inhibits the recruitment of Rab5a to endocytic vesicles. Wild type and *Tip30*$^{-/-}$ primary hepatocytes were subjected to EGFR internalization analysis and were immunostained for EGFR (red) and Rab5a (green) 120 min after $Alexa^{488}$-EGF (blue) internalization. Bars, 10 μm.

Figure S2. Knockdown of TIP30 inhibits endosomal acidification.

A, Monitoring endosomal acidification in living cells. Control and TIP30 Knockdown cells were starved overnight and EGFR internalization analyses were performed by using Alex647-EGF (red) and pHrodo-EGF (green) that were mixed in a ratio of 3:7. Fluorescence from pHrodo indicates endosomal acidification. Images were acquired at the indicated time points. Bars, 5 μm.
B, Quantification of endosomal acidification. Alex647- and pHrodo-positive endosomes in 5 cells of each group were counted and percentage of pHrodo-positive endosomes was calculated. $^{**}P < 0.01$, relative to control cells; t test.

Figure S1

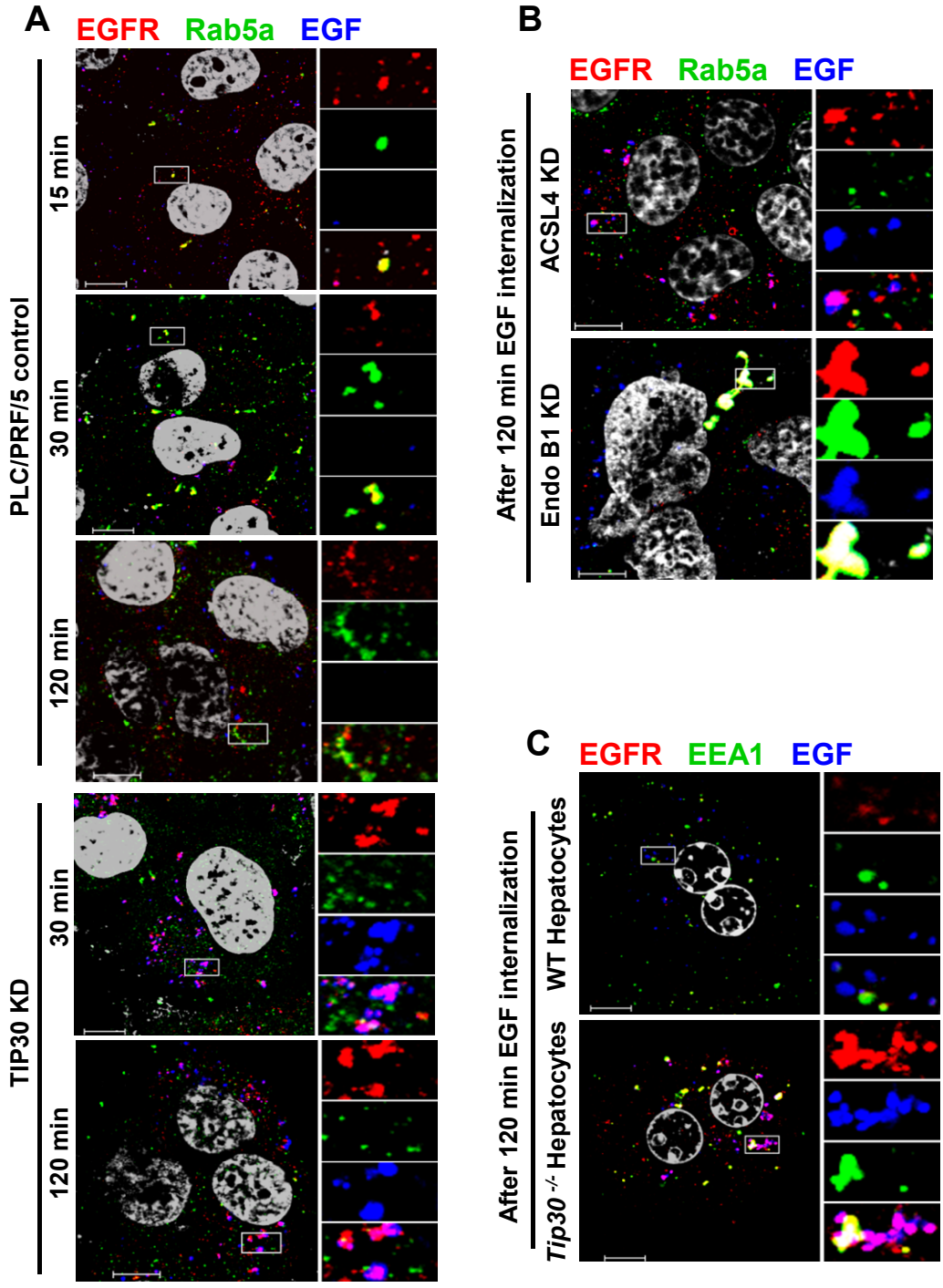
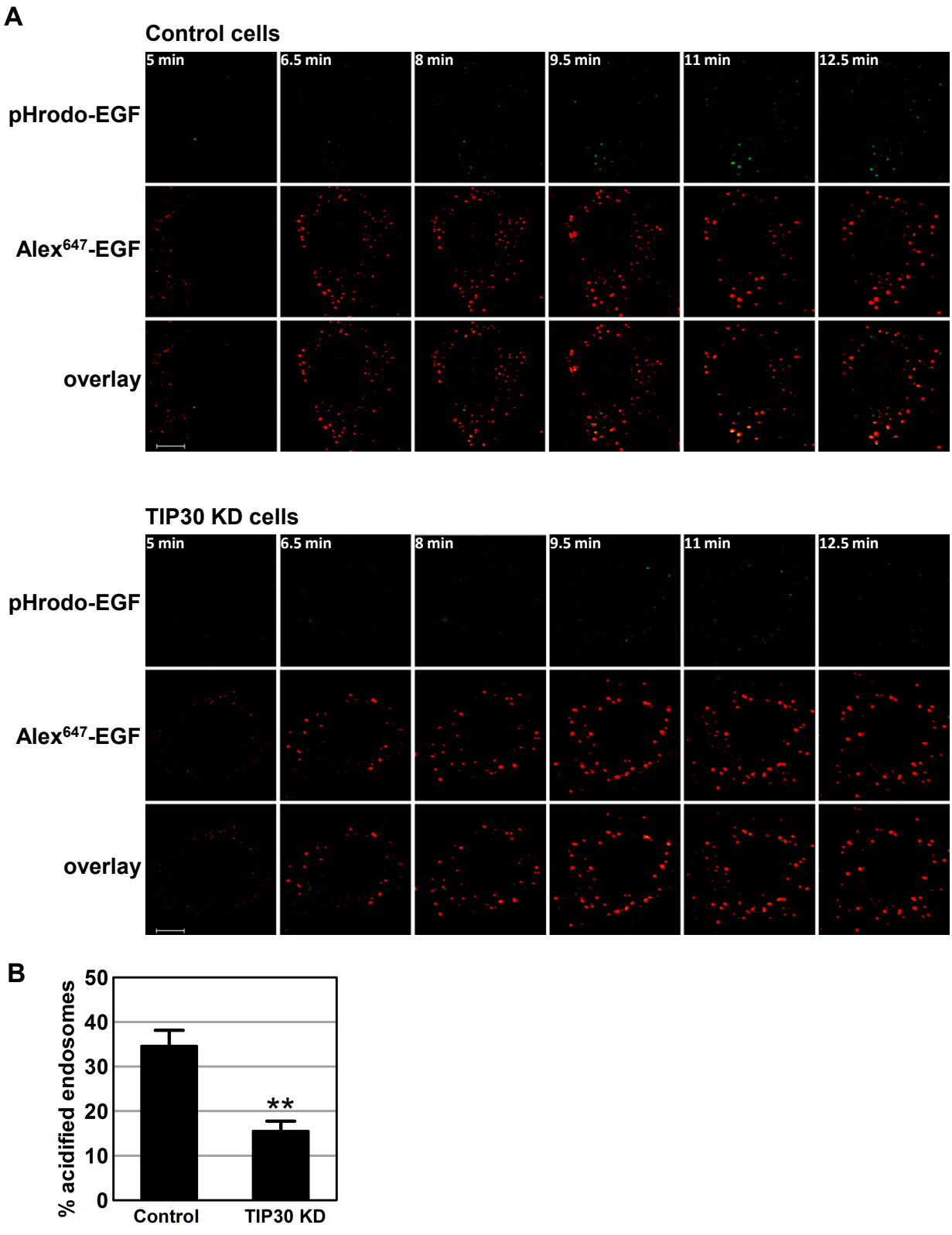

A
EGFR Rab5a EGF
PLC/PRF/5 control
15 min
30 min
120 min
TIP30 KD
30 min
120 min
B
EGFR Rab5a EGF
After 120 min EGF internalization
ACSL4 KD
Endo B1 KD
C
EGFR EEA1 EGF
After 120 min EGF internalization
WT Hepatocytes
Tip30 -/- Hepatocytes

Figure S2


A
Control cells
5 min
6.5 min
8 min
9.5 min
11 min
12.5 min
pHrodo-EGF
Alex647-EGF
overlay
TIP30 KD cells
5 min
6.5 min
8 min
9.5 min
11 min
12.5 min
pHrodo-EGF
Alex647-EGF
overlay
B
% acidified endosomes
50
40
30
20
10
0
**
Control
TIP30 KD