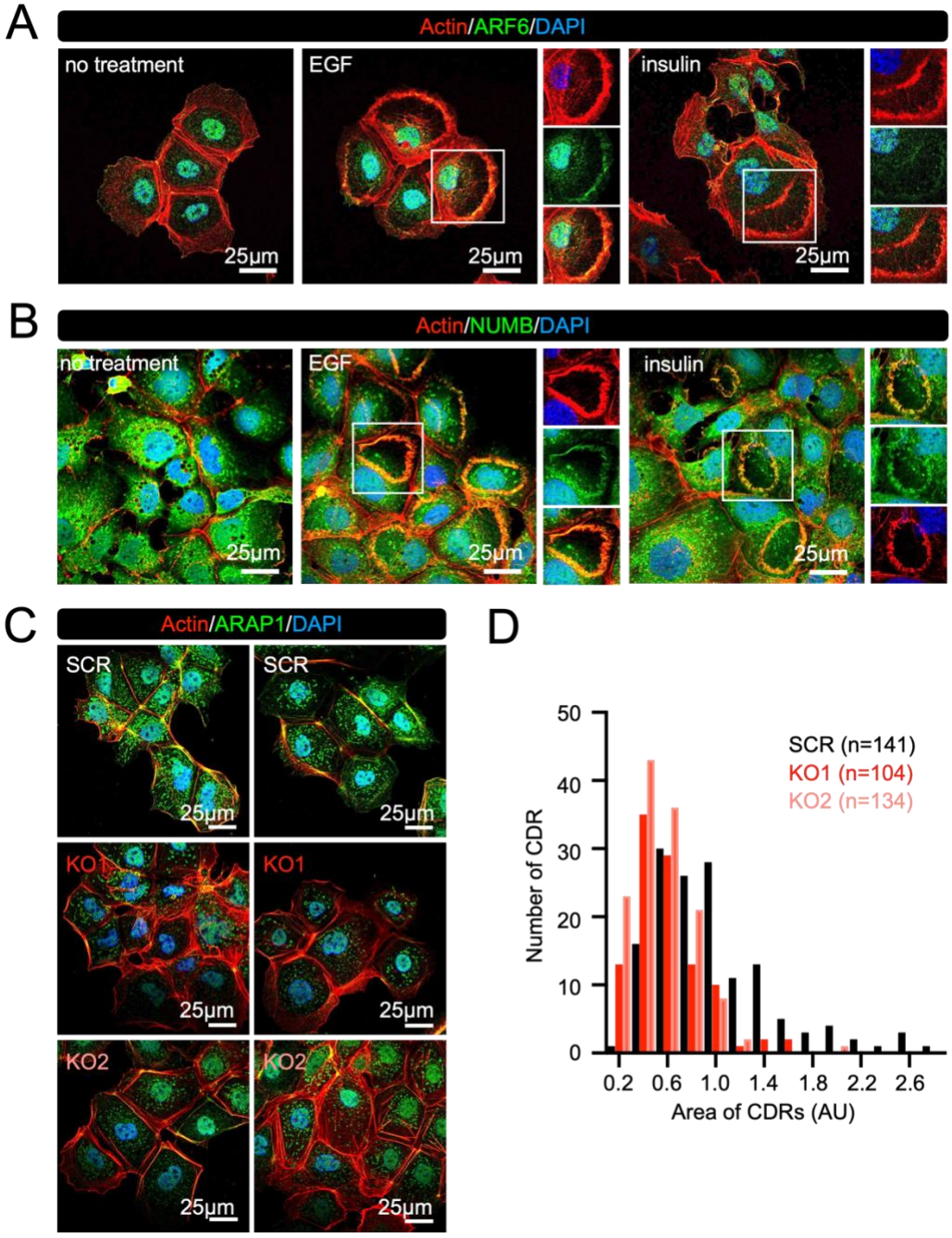
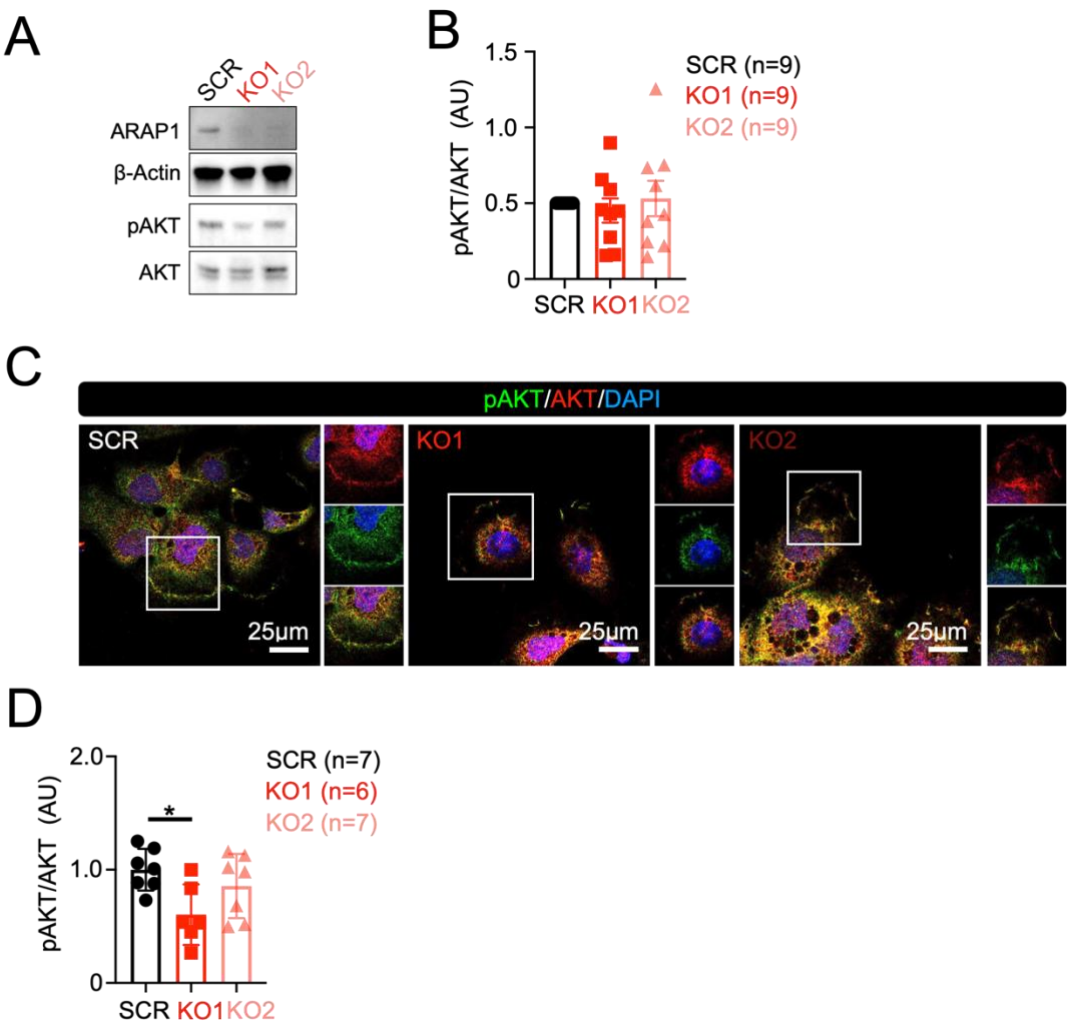
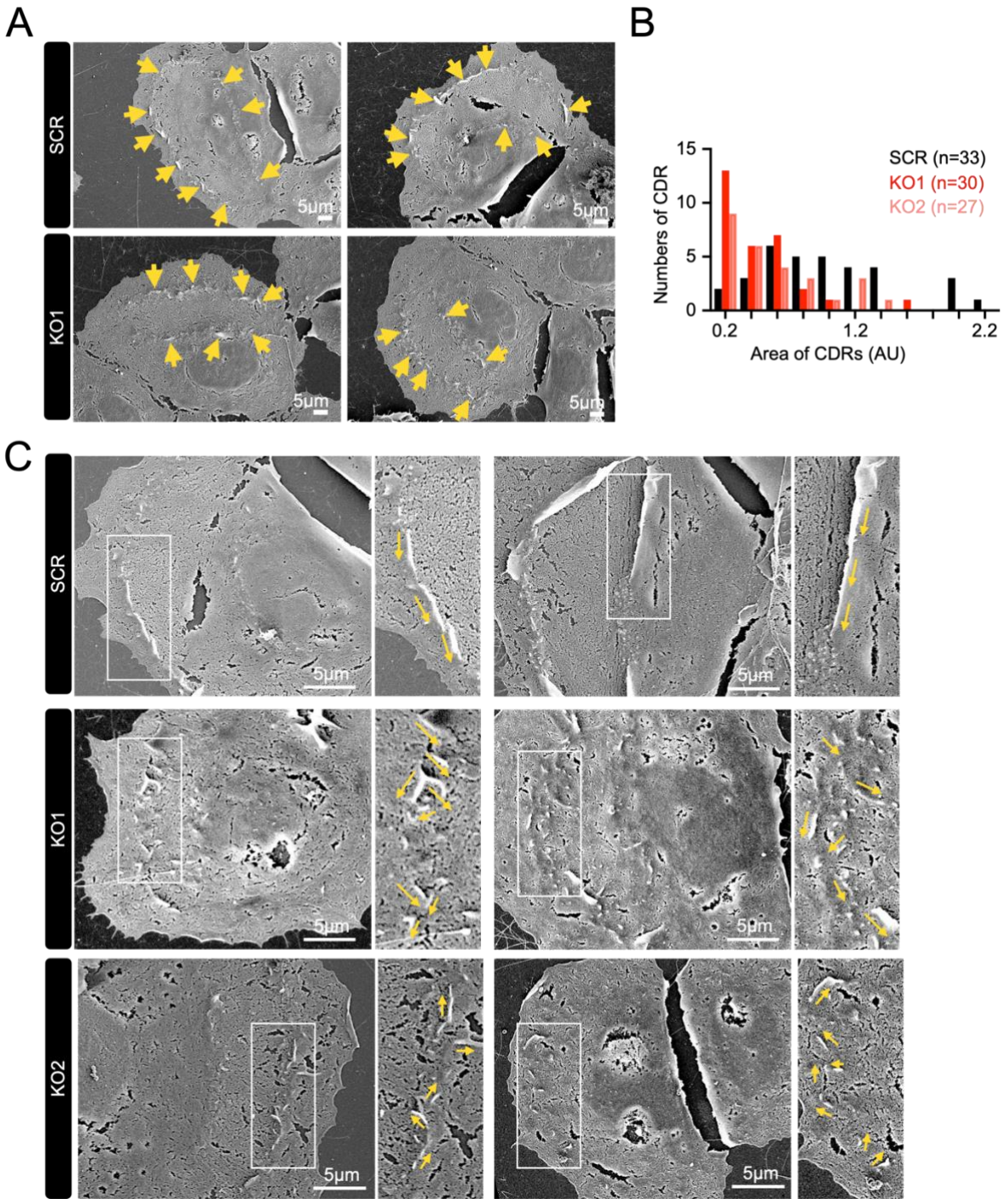


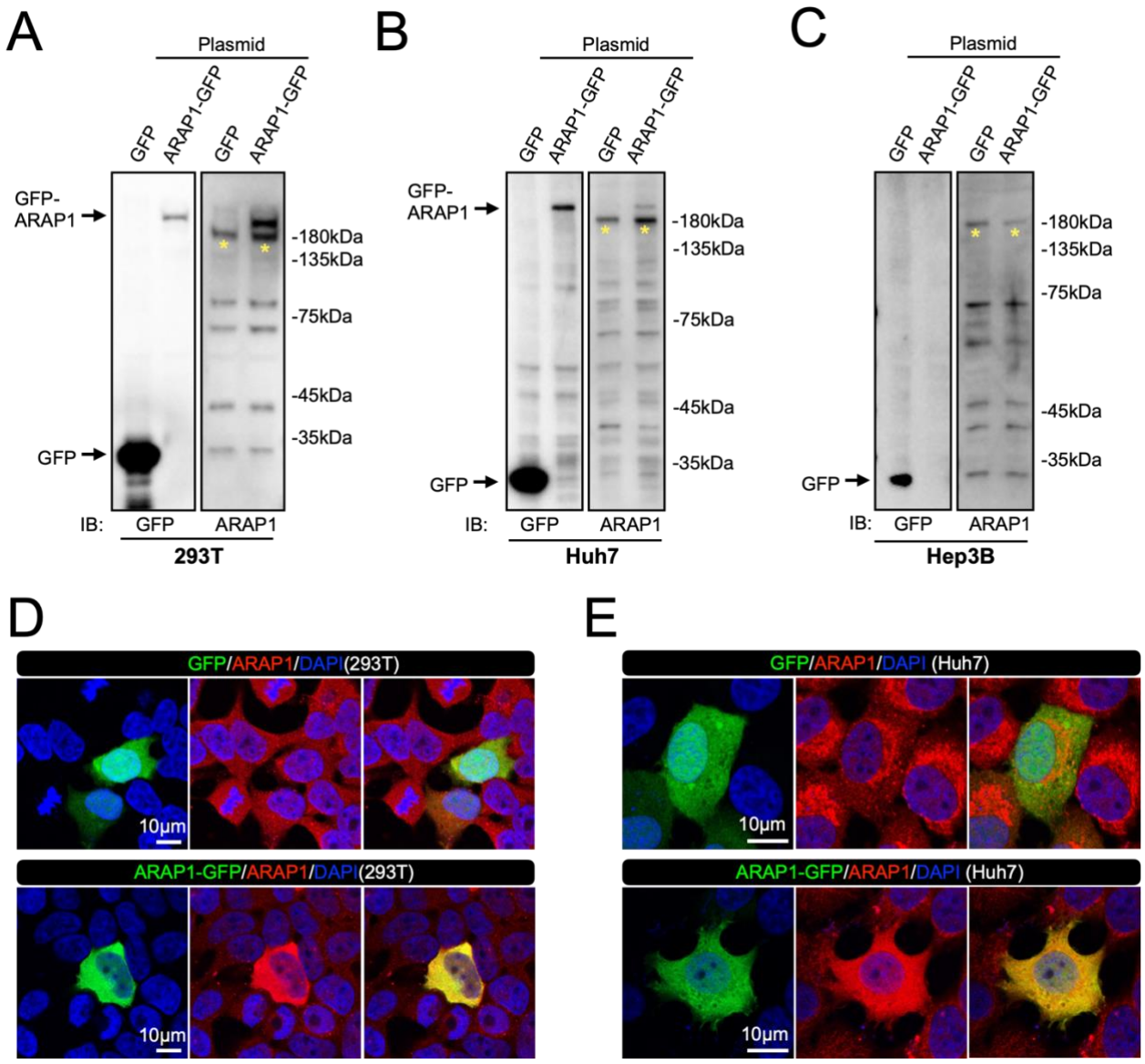
Supplementary Figure 1. Supplemental data for Figure 1. (**A and B**) Representative confocal images of Actin/ARF6 (**A**) and Actin/NUMB (**B**) with/without EGF and insulin. (**C**) Representative confocal images of Actin/ARAP1 in control (SCR) and knockout (KO1 and KO2) cells confirming the deletion of ARAP1. These images also verify that the ARAP1 antibody we used in this study is suitable for immunostaining. Two representative images are shown for each sample. (**D**) Distribution of circular dorsal ruffle size in control (SCR) and KO (KO1 and KO2) cells using the same image data set shown in Figure 1E.



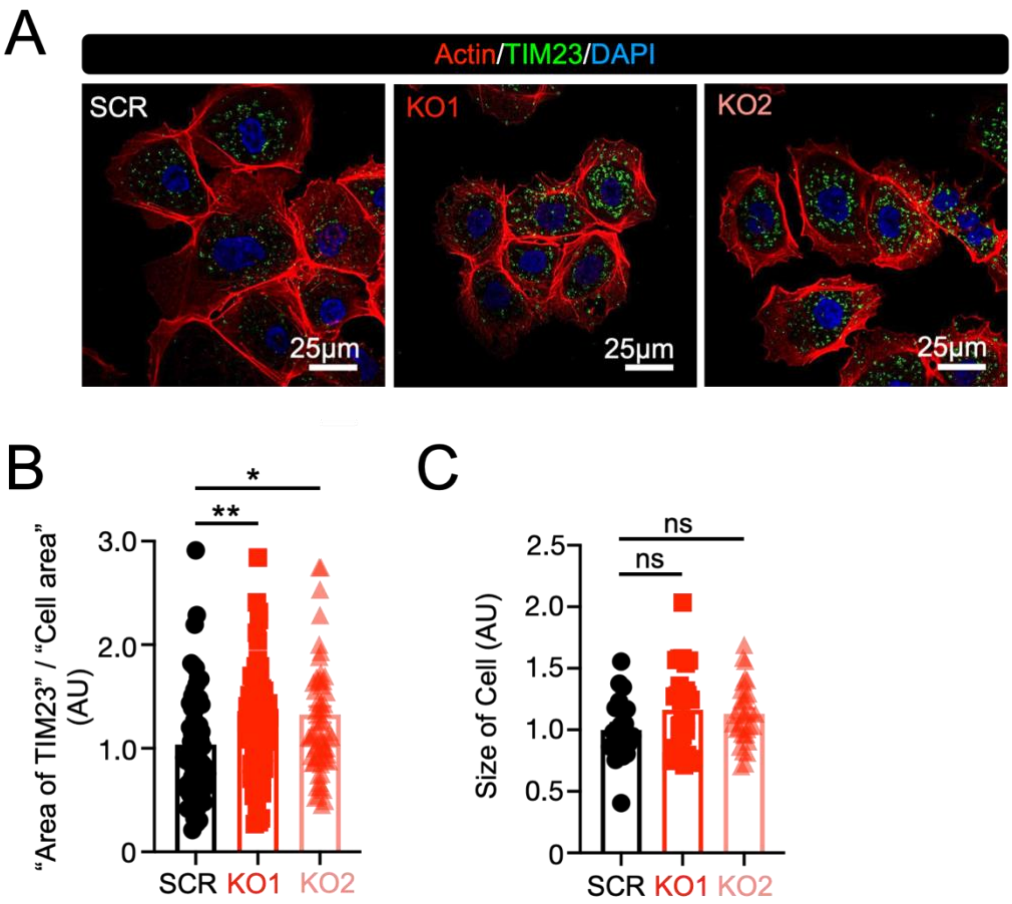
Supplementary Figure 2. Depletion of ARAP1 has a slight effect on EGF-stimulated AKT phosphorylation (pAKT). (**A-B**) Control and knockout cells were stimulated with EGF, and the signals were detected by western blot analysis for ratio calculations. Representative western blot images comparing pAKT and AKT in control (SCR) and knockout (KO1 and KO2) cells (**A**). Quantitative results for pAKT/ATK ratio values obtained from nine independent experiments (**B**). (**C**) Representative confocal images of pAKT/AKT staining of Hep3B cells following EGF stimulation. (**D**) Quantitative results obtained for the pAKT/AKT ratio in circular dorsal ruffles based on confocal microscopy observations. n = 7 (SCR), 6 (KO1), and 7 (KO2) cells from two independent experiments. *$p < 0.05$ (one-way ANOVA). AU: arbitrary unit.



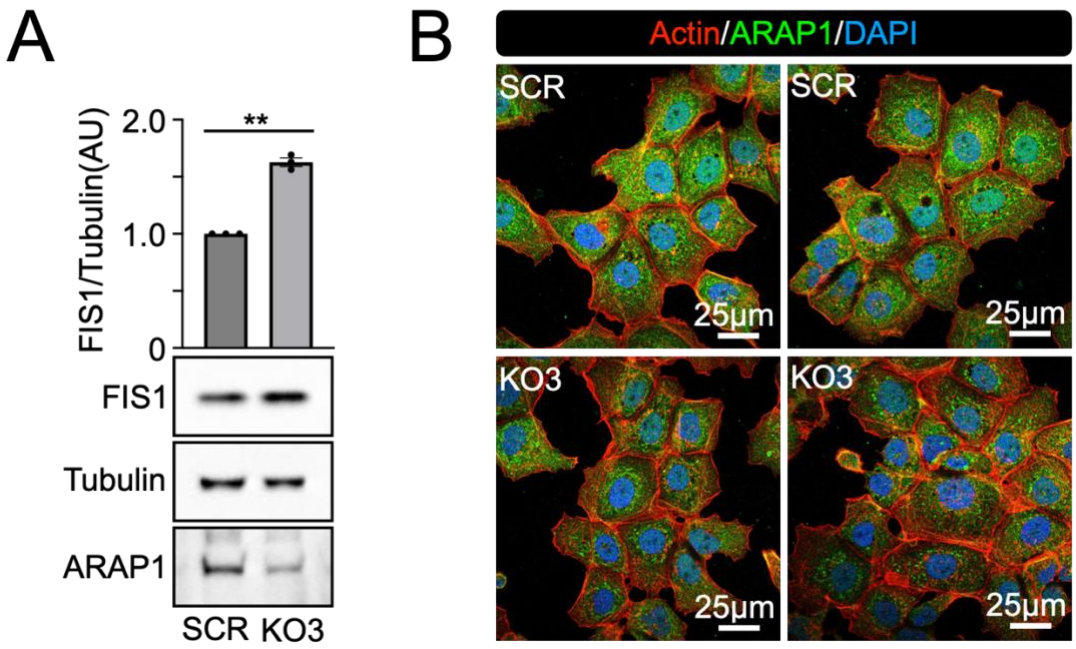
Supplementary Figure 3. Supplemental data for Figure 2. (**A**) Representative high-resolution scanning electron micrographs of control (SCR) and knockout (KO1) cells (as supplemental data for Figure 2A). Two representative images are shown for each sample. **(B)** Distribution of circular dorsal ruffle size using the same image data set used for Figure 2B. (**C**) Representative high-resolution scanning electron micrographs of control (SCR) and knockout (KO1 and KO2) cells (as supplemental data for Figure 2C). The orientation of vertical ruffles (arrows) in the KO cells was disrupted. Two representative images are shown for each sample.



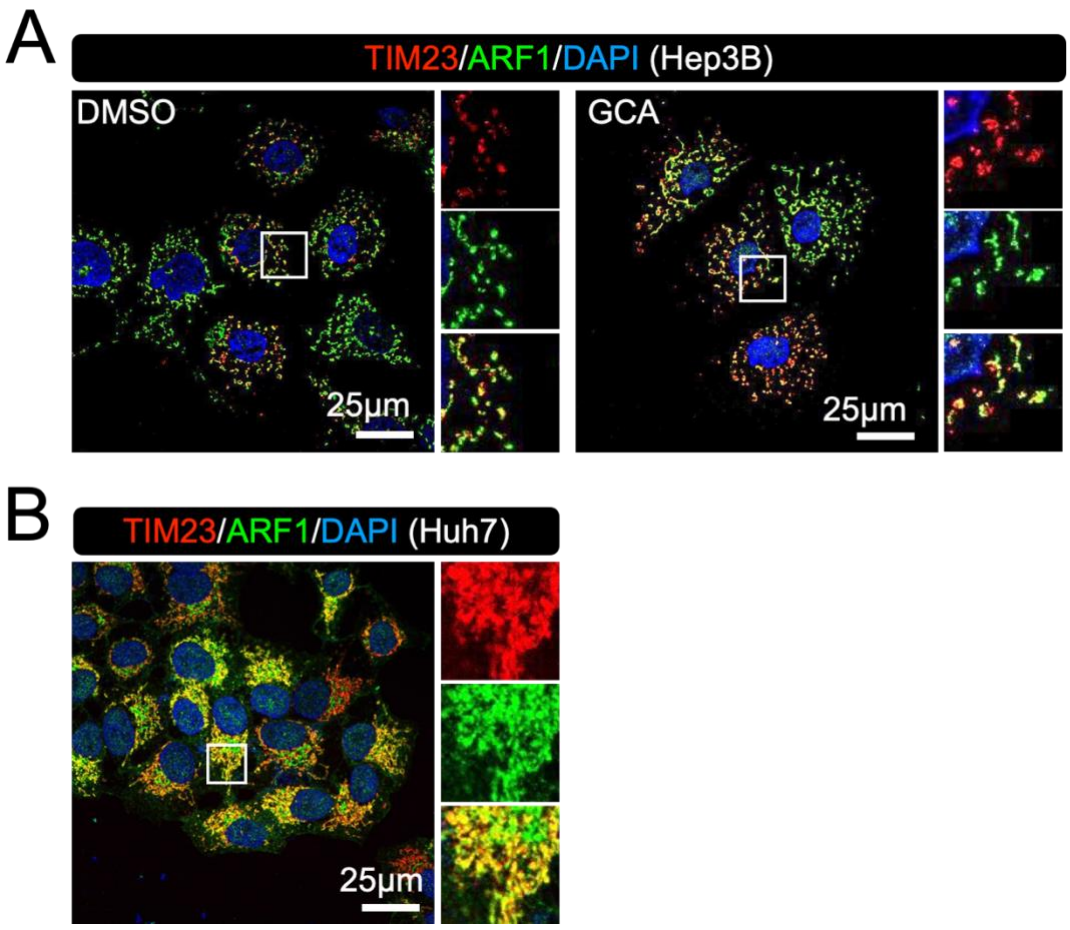
Supplementary Figure 4. Supplemental data showing the efficacy of ARAP1 antibody for IF staining. (A-C) Representative western blot results of 293T (**A**), Huh7 (**B**), and Hep3B (**C**) cells transfected by pEGFP-N1 or pGFP-ARAP1. GFP-ARAP1 was detected in 293T and Huh7 cells by both anti-GFP and -ARAP1 antibodies, but not in Hep3B cells. "*" indicates endogenous ARAP1. (**D-E**) Representative confocal images of 293T (**D**) and Huh7 (**E**) cells expressing GFP or GFP-ARAP1. Anti-ARAP1 antibody (red) identified GFP-ARAP1 but not GFP.



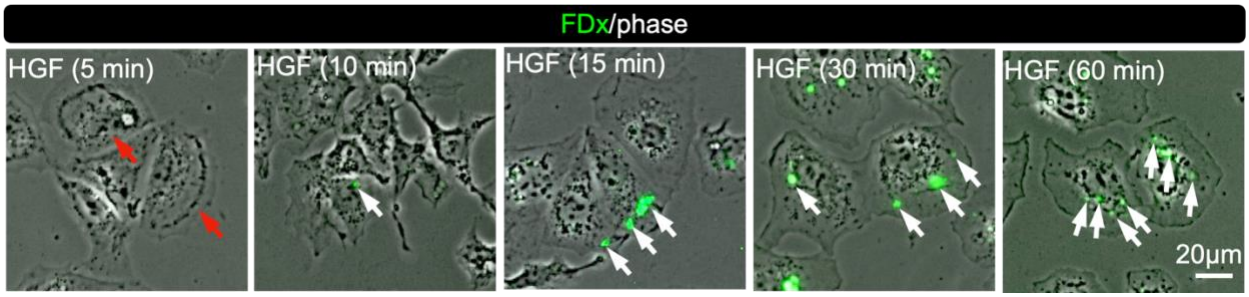
Supplementary Figure 5. Supplemental data for Figure 5A-C. The area of TIM23 distribution was expanded in the KO1 and KO2 cells compared with the SCR cells. (**A**) Representative confocal images of Actin/TIM23 in Hep3B cells. **(B-C)** The area of TIM23 distribution and the size of cells were measured using ImageJ software and the ratio of "area of TIM23"/"cell area" was calculated. The results revealed that the ratios for KO cells were higher than those for control cells (**B**), although the cell areas were the same **(C)**, indicating that the area of mitochondrial distribution was increased in response to the depletion of ARAP1. n = 64 (SCR), 74 (KO1), and 67 (KO2), from two independent experiments. $^{**}p < 0.01$; $^{*}p < 0.05$. ns: not significant (one-way ANOVA). AU: arbitrary unit.



Supplementary Figure 6. Supplemental data for Figure 5D. (**A**) ARAP1 KO (KO3) cells were generated to test if FIS1 expression is increased by the depletion of ARAP1 (as supplemental data for Figure 3I). Western blot analysis showed that FIS1 expression was increased in KO3 cells compared to the control (SCR). Three independent experiments were carried out for the quantification. $^{**}p < 0.01$ (two-tailed paired Student *t*-test). AU: arbitrary unit. (**B**) Representative confocal image showing that ARAP1 expression was decreased in KO3 cells. Two representative images are shown for each sample.



Supplementary Figure 7. Supplemental data for Figures 7. (**A**) Representative confocal images of TIM23/ARF1 in Hep3B cells without (DMSO)/with (GCA) the ARF1 inhibitor Golgicide A. (**B**) Representative confocal image of TIM23/ARF1 in the Huh7 cells.



Supplementary Figure 8. Supplemental data for Figures 8D. Image analysis identified circular dorsal ruffles CDRs (red arrows) and macropinosomes (MPs) identified using fluorescein isothiocyanate-labeled dextran with an average molecular weight of 70,000 (FDx70, green) (white arrows) in Hep3B cells following HGF stimulation.