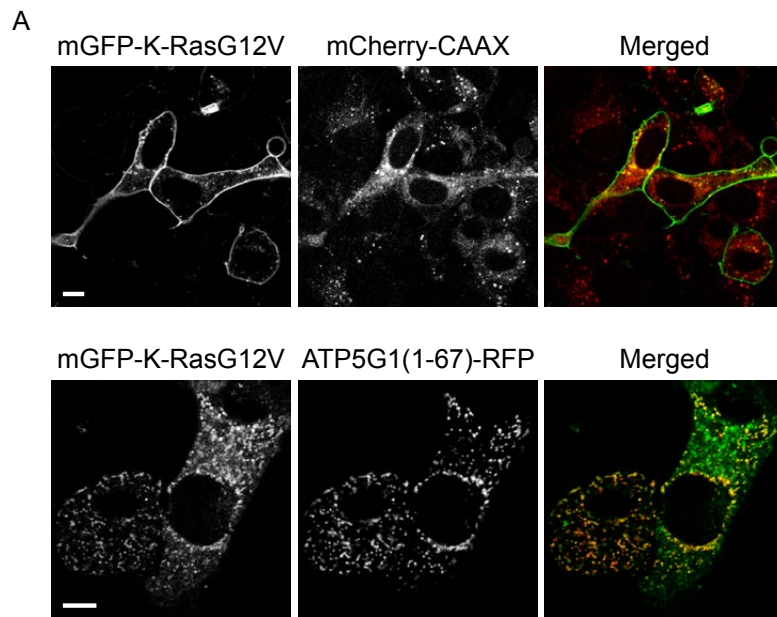


Figure S1

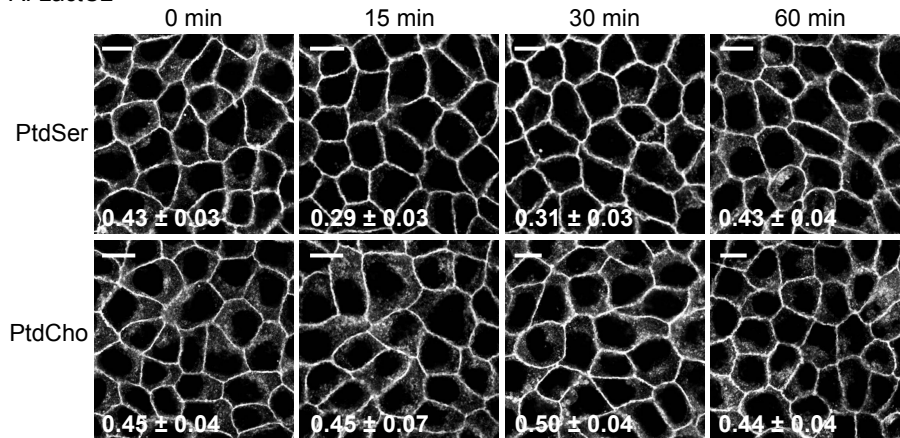


**Figure S1. ATP5G1(1-67)-RFP overexpression translocates K-RasG12V to mitochondria.**

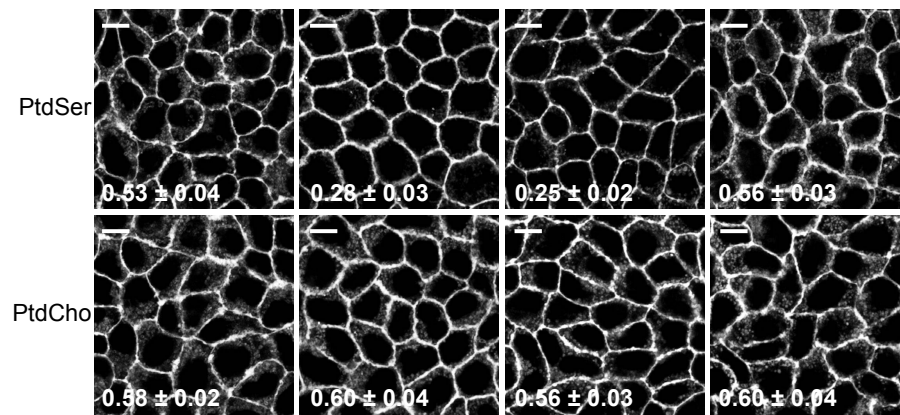
Baby hamster kidney cells co-expressing mGFP-K-RasG12V with mCherry-CAAX, an endomembrane marker or ATP5H1(1-67)-RFP, a mitochondrial marker were maintained in complete growth media (DMEM + 10% FBS + 2mL L-Glut) and fixed with 4% PFA and imaged by a confocal microscope. Scale bar 10 $\mu$ m.

Figure S2

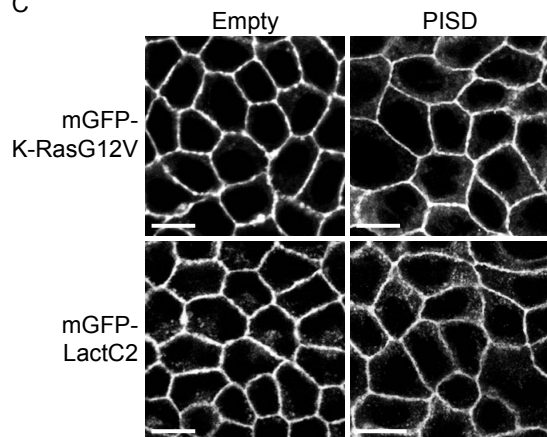
A. LactC2



B. K-RasG12V



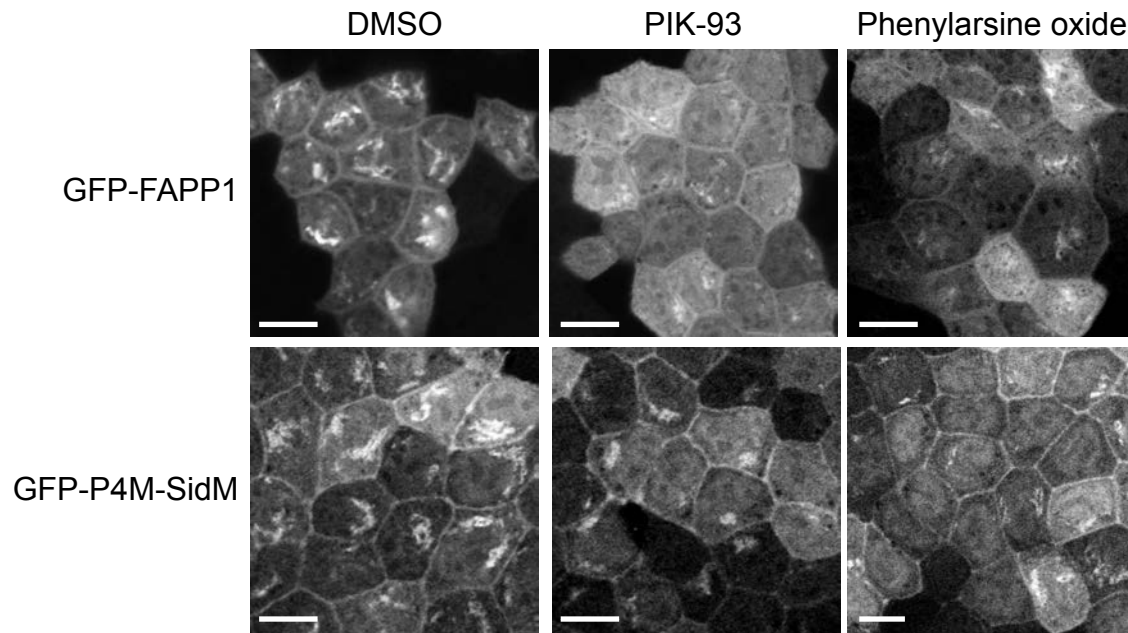
C



**Figure S2. PtdSer supplementation restores the PM localization of K-Ras and LactC2.** MDCK cells stably co-expressing ATP5G1(1-67)-RFP with mGFP-LactC2 (A) or -K-RasG12V (B) were supplemented with 10 $\mu$ M exogenous PtdSer or PtdCho in growth medium without glucose (DMDM without glucose/pyruvate + 10% FBS + 2mM L-glutamine + 1mM sodium pyruvate) and further incubated for indicated time points. Cells were fixed with 4% PFA and imaged by a confocal microscope. Inserted values represent a mean estimate  $\pm$  S.E.M of the fraction of ATP5G1(1-67)-RFP colocalized with mGFP-LactC2 or -K-RasG12V calculated by Manders coefficient from three

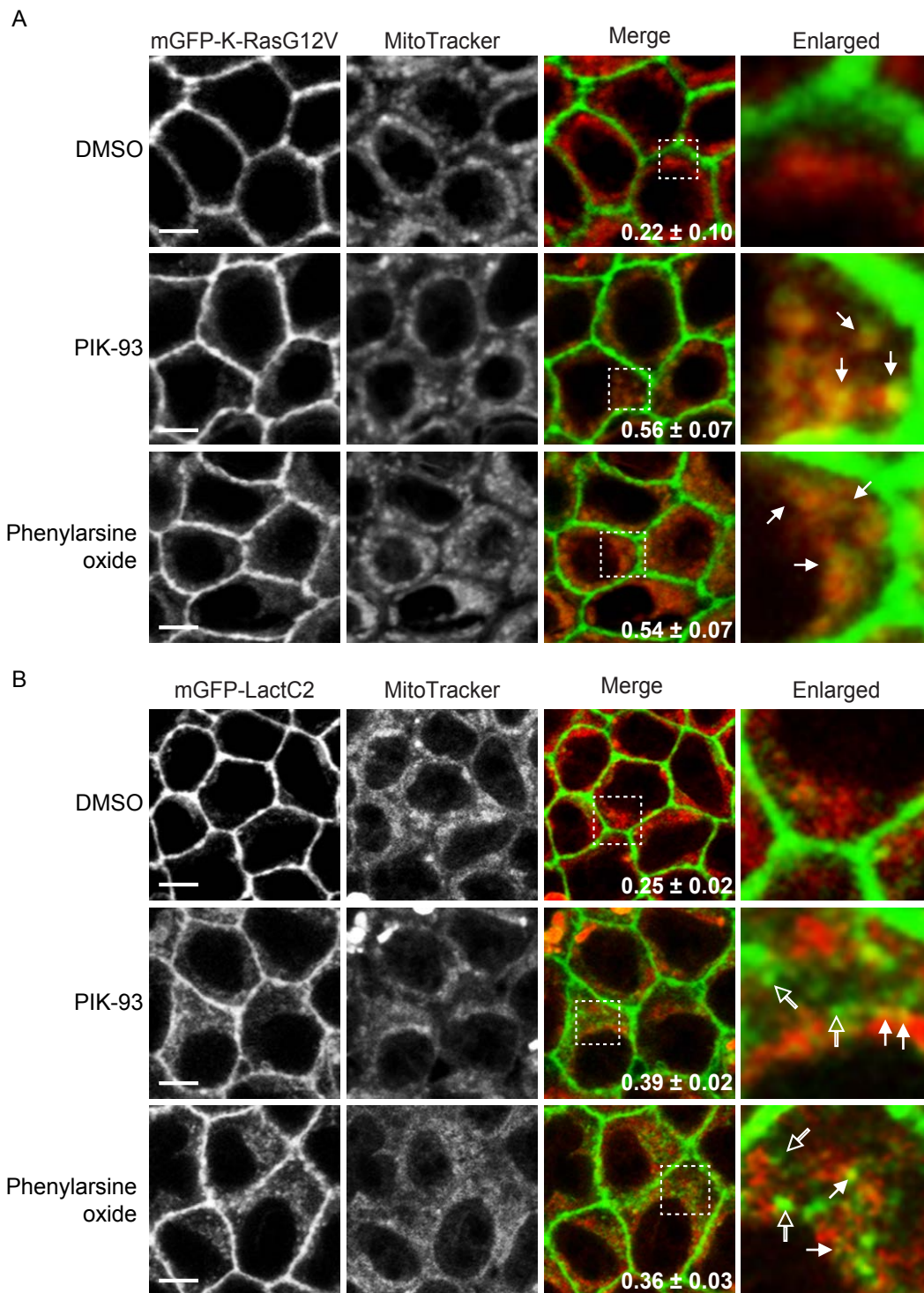
independent experiments. Shown are representative images of mGFP-LactC2 and -K-RasG12V. Scale bar 10 $\mu$ m. (C) MDCK cells stably expressing mGFP-K-RasG12V or -LactC2 were infected with lentivirus expressing PtdSer decarboxylase (PISD) and maintained in complete growth medium (DMEM + 10% FBS + 2mM L-glutamine). Cells were fixed with 4% PFA and imaged by a confocal microscope. Representative images are shown from three independent experiments. Scale bars 10 $\mu$ m.

Figure S3



**Figure S3. PI4K inhibitors reduce PI4P contents at the Golgi.** MDCK cells stably expressing mGFP-tagged PI4P markers, FAPP1-PH or P4M-SidM were treated with 10 $\mu$ M PIK-93 or 50nM phenylarsine oxide for 48h in complete growth medium (DMEM + 10% FBS + 2mM L-glut). Cells were fixed with 4% PFA and imaged by a confocal microscope. Scale bar 10 $\mu$ m.

Figure S4



**Figure S4. The Golgi-PI4K inhibition mislocalizes K-RasG12V and PtdSer to mitochondria and endomembrane, respectively.** MDCK cells stably expressing (A) mGFP-K-RasG12V or (B) -LactC2 were treated with vehicle (DMSO), 1 $\mu$ M PIK-93 or 50nM phenylarsine oxide for 48h, and further stained with 100nM MitoTracker Deep Red for 1h. Cells were fixed with 4% PFA and

imaged by a confocal microscope. Inserted values are a mean estimate  $\pm$  S.E.M of the fraction of MitoTracker colocalized with mGFP-K-RasG12V or -LactC2 that were calculated by Manders coefficient from three independent experiments. Selected regions indicated by the white squares are shown at a higher magnification. mGFP-K-RasG12V and -LactC2 colocalized or not colocalized with MitoTracker are indicated by closed and open arrowheads, respectively. Scale bar 10  $\mu$ m.

## Figure S5

### A. cDNA sequence

Empty ATGGGAGACATGGTGGTGGAACTGCCCCCTGAAGCCAACCTTCTGAGCCCACACTAGCCCTGCCAGGGA  
sgRNA2 ATGGGAGACATGGTGGTGGAACTGCCCCCTGAAGCCAACCTTCTGAGCCCACACTAGCCCTGCCAGGGA  
sgRNA6 ATGGGAGACATGGTGGTGGAACTGCCCCCTGAAGCCAACCTTCTGAGCCCACACTAGCCCTGCCAGGGA

Empty ATAATGGGGGTTCCCTTGCTAGGCGTCATCA-CAGAGGGGGTTGGGGAACGTGTCAGTGATTGACCGTGAGG  
sgRNA2 ATAATGGGGGTTCCCTTGCTAGGCGTCATCAACAGAGGGGGTTGGGGAACGTGTCAGTGATTGACCGTGAGG  
sgRNA6 ATAATGGGGGTTCCCTTGCTAGGCGTCATCA-CAGAGGGGGTTGGGGAACGTGTCAGTGATTGACCGTGAGG

Empty TGGCCCAGAAGGCCTGCCAGGAGGTGCTGGAGCAAGTCAAGCTTTTGCATGGAGGCGTGCCATATCTAG  
sgRNA2 TGGCCCANAAGGCCTGCCAGGAGGTGCTGGAGCAAGTCAAGCTTTTGCATGGAGGCGTGCCATATCTAG  
sgRNA6 TGGCCCAGAAGGCCTGCCAGGAGGTGCTGGAGCAAGTCAAGCTTTTGCATGGAGGCGTGCCATATCTAG

Empty CACAGACACCTCCCTGGAGCTGGTCAATGGGGATGTTCCGGACAGTGCTATCCGTTG-CCTGGATGATCCA  
sgRNA2 CACAGACACCTCCCTGGAGCTGGTCAATGGGGATGTTCCGGACAGTGCTATCCGTTG-CCTGGATGATCCA  
sgRNA6 CACAGACACCTCCCTGGAGCTGGTCAATGGGGATGTTCCGGACAGTGCTATCCGTTGCTCCTGGATGATCCA

Empty CCTACCCAGATAAGGGAGGAGGAAGATGAAATGGGGGCCACTGTGGCCTCGGGCACAGCCAAGGGAGCAAG  
sgRNA2 CCTACCCAGATAAGGGAGGAGGAAGATGAAATGGGGGCCACTGTGGCCTCGGGCACAGCCAAGGGAGCAAG  
sgRNA6 CCTACCCAGATAAGGGAGGAGGAAGATGAAATGGGGGCCACTGTGGCCTCGGGCACAGCCAAGGGAGCAAG

### B. Protein sequence

Empty MGDVVVEPAPLKPTSEPTTGLPGNNGSLLGVITEGVGELSVIDREVAQKACQEVLEQVKLLHGGVAISS  
sgRNA2 MGDVVVEPAPLKPTSEPTTGLPGNNGSLLGVINRGGWGTVSD-  
sgRNA6 MGDVVVEPAPLKPTSEPTTGLPGNNGSLLGVITEGVGELSVIDREVAQKACQEVLEQVKLLHGGVAISS

Empty TDTSLELVNGDVPDSAIRCLDDPPTQIREEEDEMGA TVASGTAKGARRRRQNSAKQSWLLRLEFESKLF D  
sgRNA2  
sgRNA6 TDTSLELVNGDVPDSAIRCPG-

**Figure S5. CRISPR/Cas9-mediated sgRNAs targeting canine PI4KB generates a frameshift mutation, resulting in premature stop codon.** Nucleotides in red are inserted nucleotide induced by CRISPR/Cas9 system.