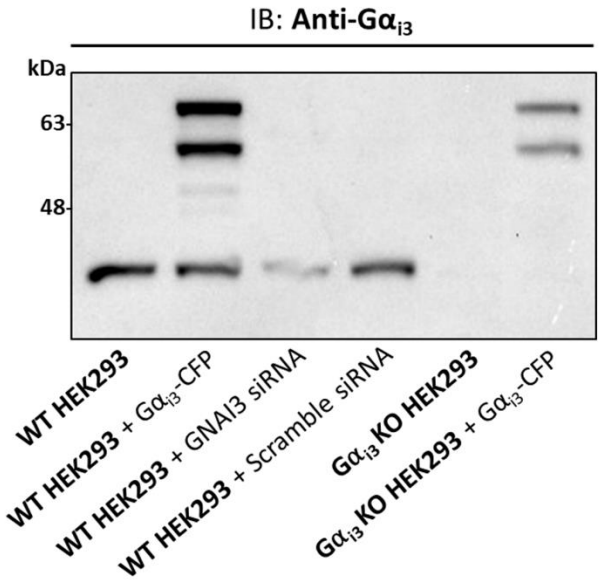

Fig. S1: profiling of Gαi3 protein expression in wild type and Gαi3-KO HEK293 cells. Western blot using (anti-Gαi3 antibody) performed on lysates of HEK293 cells. From the left lane to the right lane, HEK293 cells were untransfected, transfected with Gαi3-CFP, transfected with SiRNA against Gαi3, transfected with scramble SiRNA respectively. The two right-most lanes correspond to HEK293 cells in which Gαi3 was knocked out using CRISPR technique with Gαi3-CFP add-back in the last lane of the gel.

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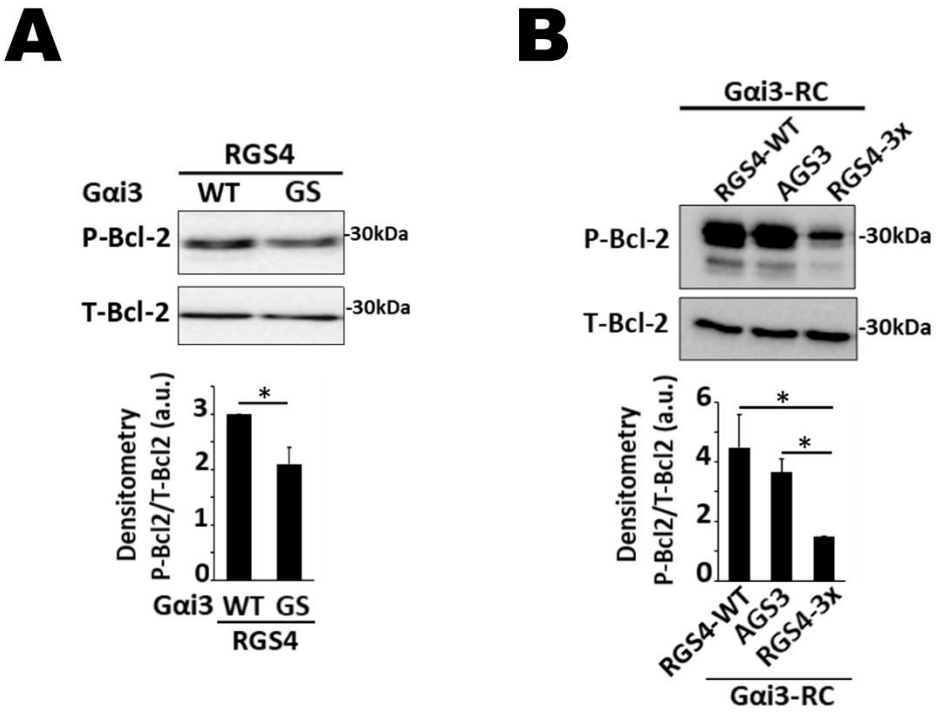

Fig. S2: Functional RGS4 or AGS3 co-expression with Gαi3-RC promotes increased levels of P-Bcl2. **A,** The *upper panel* shows Western blots for P-Bcl2 and T-Bcl2 following when cells expressing Gαi3-WT or Gαi3 (GS) are co-transfected with wild type RGS4. The *bottom panel* is the histogram showing the means of P-Bcl2/T-Bcl2 levels for the indicated constructs. **B**, The *upper panel* shows Western blots for P-Bcl2 and T-Bcl2 following when cells expressing Gαi3-RC and RGS4-WT, RGS4 (3x), a triple-mutant that inhibits Gαq but not Gi, and the GDI inhibitory protein AGS3. The *bottom panel* is the histogram showing the means of P-Bcl2/T-Bcl2 levels for the indicated constructs. In all panels error bars represent SEM (n=3). One-way ANOVA and Tukey's post hoc was used to determine *p<0.05 The western blots are representative to three independent experiments carried out on separate days.

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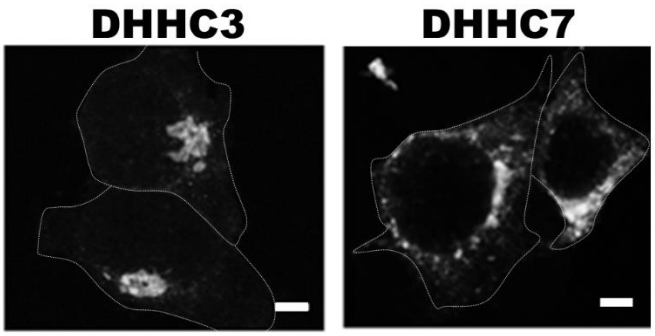

Fig. S3: Intracellular distribution of DHHC3 and DHHC7. Confocal microscopy images of HEK293 cells immunostained for HA tagged-DHHC3 and -DHHC7. *White bars* represent 1 µm. *White dashes* lines mark the outline of the cells.

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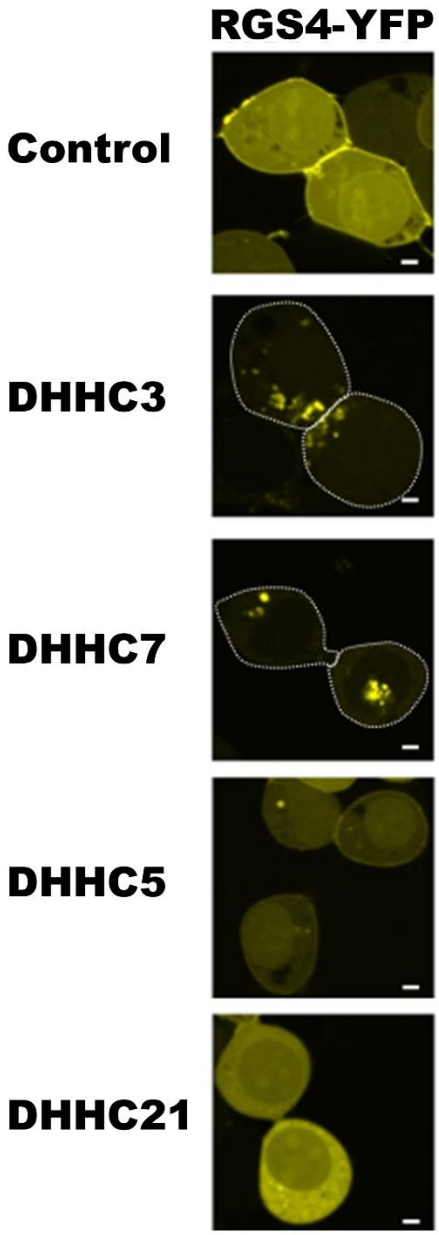

Fig. S4: Exogeneous expression of DHHC3 and DHHC7 impair the intracellular localization of RGS4-YFP. Confocal microscopy pictures of RGS4-YFP in function of the exogeneous expression of DHHC 3, 5, 7 and 21. RGS4-YFP was stably expressed in HEK cells. white bars are representative of 1 µm. *White dashes* lines mark the outline cells. Data are representative to at least three independent experiments carried out on separate days.

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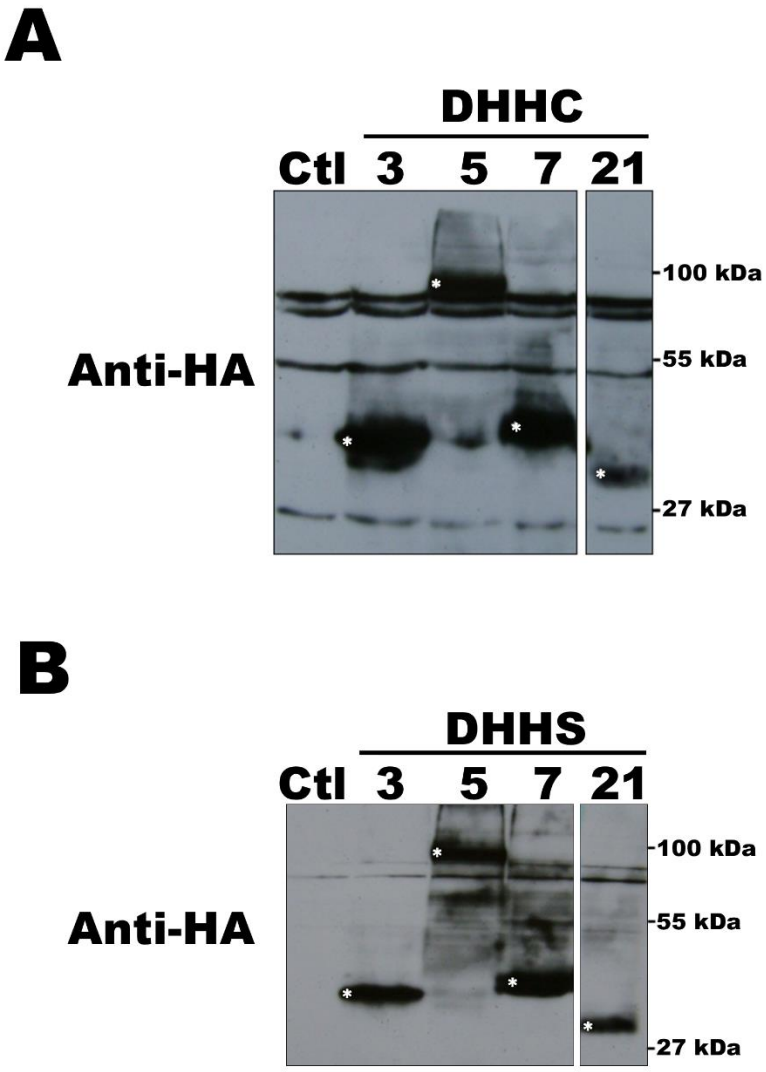

Fig. S5: Western blots showing the exogenous expression of the indicated HA-tagged DHHC clones in **A**, and DHHS clones in **B**. In both panels white stars have been added on the left side of the corresponding bands to indicate their position/size.

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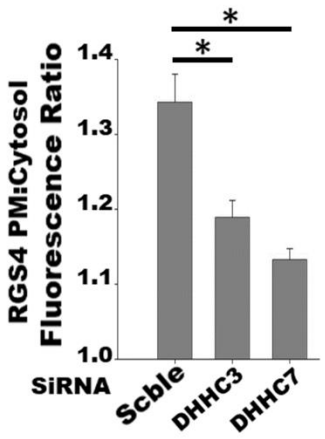

Fig. S6: Endogenous DHHC3 and DHHC7 palmitoyl-CoA transferase activity are important for RGS4 targeting to the plasma membrane. Histogram showing the means of plasma membrane to cytosol expression ratios of RGS4-YFP when either DHHC3 or DHHC7 mRNA expression was knocked down using SiRNA. The data are representative of three independent experiments carried out on separate days and error bars stand for SEM (n=3). One-way ANOVA and Tukey's post hoc were used to determine *p<0.05.

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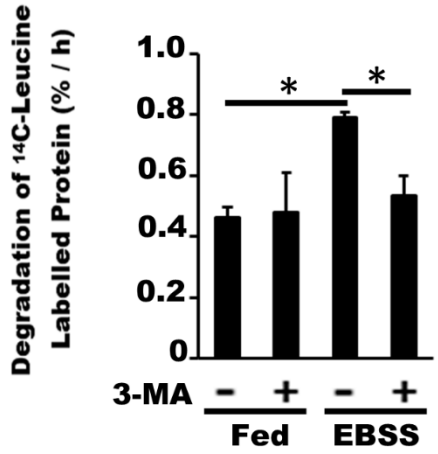

Fig. S7: Control assay to show the extent to which ^{14}C-leucine labelled protein degradation rates vary with nutrient availability in HEK293 cells and their dependence on VPS34 activity/autophagic flux. Histogram shows the means of degradation rates of *^{14}C* -leucine labelled long-lived proteins as a function of nutrient availability and VPS34 activity in HEK293 cells. Fed represents cells cultured in 10 % FBS DMEM/F-12 (v/v) medium, whereas EBSS represents cells cultured in a nutrient-deprived medium containing 0.2% of FBS and 0.2% of BSA. 3-MA is an inhibitor of VPS34 and autophagy flux. The data are representative of three independent experiments carried out on separate days. *Error bars* represent SEM (n=3) and one-way ANOVA and Tukey's post hoc was used to determine *p<0.05.

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Gene Name	Accession Number	Forward Primer 5' → 3'	Reverse Primer 5' → 3'
GAPDH	NM_002046.3	GAAGGTGAAGGTCGGAGTCA	GAAGATGGTGATGGGATTTC
18S	NR_003286	AGGAATTGACGGAAGGGCAC	GGACATCTAAGGGCATCACA
DHHC1	NM_013304	ACCTGCTCTGCTTCCACATT	GACTCGAGCTCCCTGTGAAC
DHHC2	NM_016353	TCTAGGTGATGGCTGCTCCT	AATGGCTTTGCAGGAAACTG
DHHC3	NM_016598.1	GGAACCATGTGGTTTATCCG	CACAATTCCGTTGATGATGC
DHHC4	NM_018106.3	ATTGATCTGTCTCTGCAGCC	CAGCTCTGGCATTCCTCTTC
DHHC5	NM_015457.2	GACAGCTTGAAGGAGCCAAC	AGCCACTGGATAGTGGATCG
DHHC6	NM_022494.1	CAGTTCACAGCTTGAAGGCA	TACCCATTTTGGCAAGGAAG
DHHC7	NM_017740	ACCTGAGAACCATGCTCACC	TGTAGATGACTTCCCCAGGC
DHHC8	NM_013373	AGGACTTTGACCACCACTGC	TGGTTCAGCACGTAGACCAG
DHHC9	NM_016032.2	CCTTGTCAGGAGGAGACAGC	GAACCACGGAGACCCAACT
DHHC11	NM_024786	AGATTCAGGAGCTCAGGTGC	CAGGAGGCTTTGCAAGGTAG
DHHC12	NM_032799	AATTCATCTCCTCACACCGC	GCCATCCACAGAAGAAGTGG
DHHC13	NM_019028.2	CAGTGCAGGAATCACAGCC	AGAGCTTCTCTTGCATTGGC
DHHC14	NM_024630.2	AGCCTGATCGACAGAAGAGG	TGAATGCACTGGTCTTGGTC
DHHC15	NM_144969	TGAGTCACAGAACCCACTGC	CCGTTTCCACAGCAAGAGAT
DHHC16	NM_032327.2	CTGTGCAGTTCTGTGGCACT	AAATACTCTGCCCTTGGCCT
DHHC17	NM_015336	ATGGTCAGATGGTCAGGAGC	TCCCAAAGGTTCACCATCAT
DHHC18	NM_032283	AGGGTTTCACACGTACCTCG	ATGGCTGTAGGGGTTGACAG
DHHC19	NM_001039617	GAATCTGCACCCTCCAATGT	GTGGAACTCCTGGAGAGCTG
DHHC20	NM_153251	CGTGGTCGTCTGGTCCTACT	AGCCACAAGGTAAACAACGG
DHHC21	NM_178566	GTGGTTCATTCCTTTCAGGC	AAAACCTGTAACGCATTGCC
DHHC22	NM_174976	CCTTGTCTCCACCCATGACT	CGTGAGGAGCCTAGAAATGC
DHHC23	NM_173570.3	CAATACCCACGGAGCACTTT	AATAAGCAGCACGTTGTCCC
DHHC24	NM_207340	ATCACCTTCCAGACCACAGC	GATGTTAAGGTCCAACCCGA

Table. S1: List of RT-qPCR primers used to measure DHHC protein mRNA expression levels in HEK293 cells.

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