Novel regulation of cell surface membrane proteins through selective autophagy

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Background/Objectives

- Cell surface membrane proteins (SMPs) mediate interaction with the environment and are a significant source of diagnostic markers and therapeutic targets; 66% of approved human medications target an SMP.
- Unraveling how SMP levels are regulated is critical for understanding cellular signal transduction mechanisms, which have immense clinical implications.
- Research implicate the endo-lysosomal system in SMP turnover, how SMPs are selectively recognized for internalization and degradation is still being determined.
- Autophagy recycles organelles and cytoplasmic proteins via different pathways. For selective regulation of cytosolic protein turnover, chaperone-mediated autophagy (CMA) targets proteins harboring a pentapeptide KFERQ motif (a biochemically KFERQ-related motif) for degradation.
- The KFERQ motif is recognized by the chaperone heat shock cognate71 kDa protein (Hsc70) that transports the KFERQ-containing protein to the lysosome. We found that 55% of SMPs contain a KFERQ-related motif in their cytoplasmic domain, making them potential selective targets for degradation through CMA.

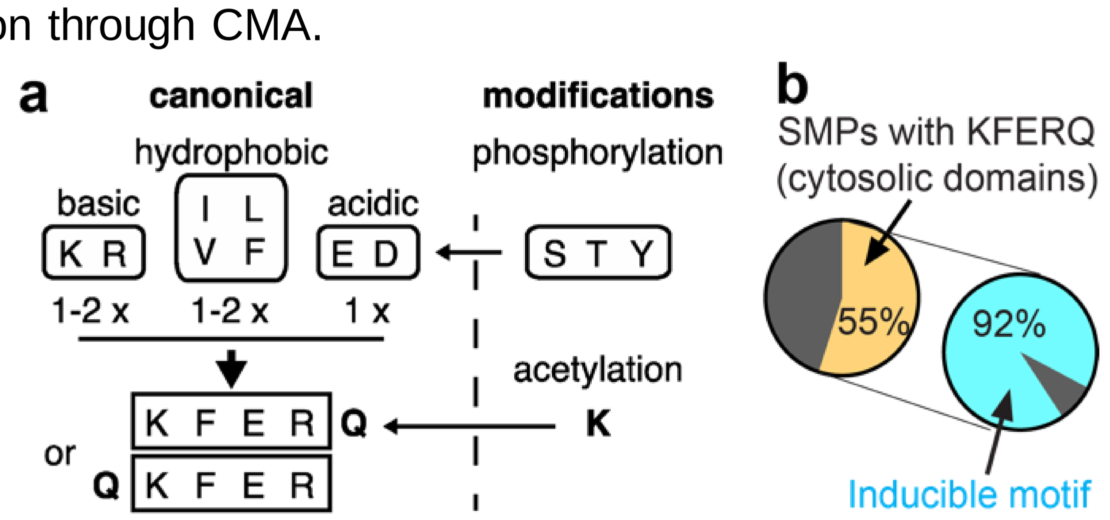

Figure 1. KFERQ motifs in cell surface membrane proteins. (a) A diagram of the rules of canonical, phosphorylation-, and acetylation-inducible KFERQ-related motifs (Kirchner et al. 2019). **(b)** The percentage of surface membrane proteins (SMPs) harboring KFERQ-related motifs in their cytoplasmic domains 92% of which are inducible by acetylation or phosphorylation.

Working Model

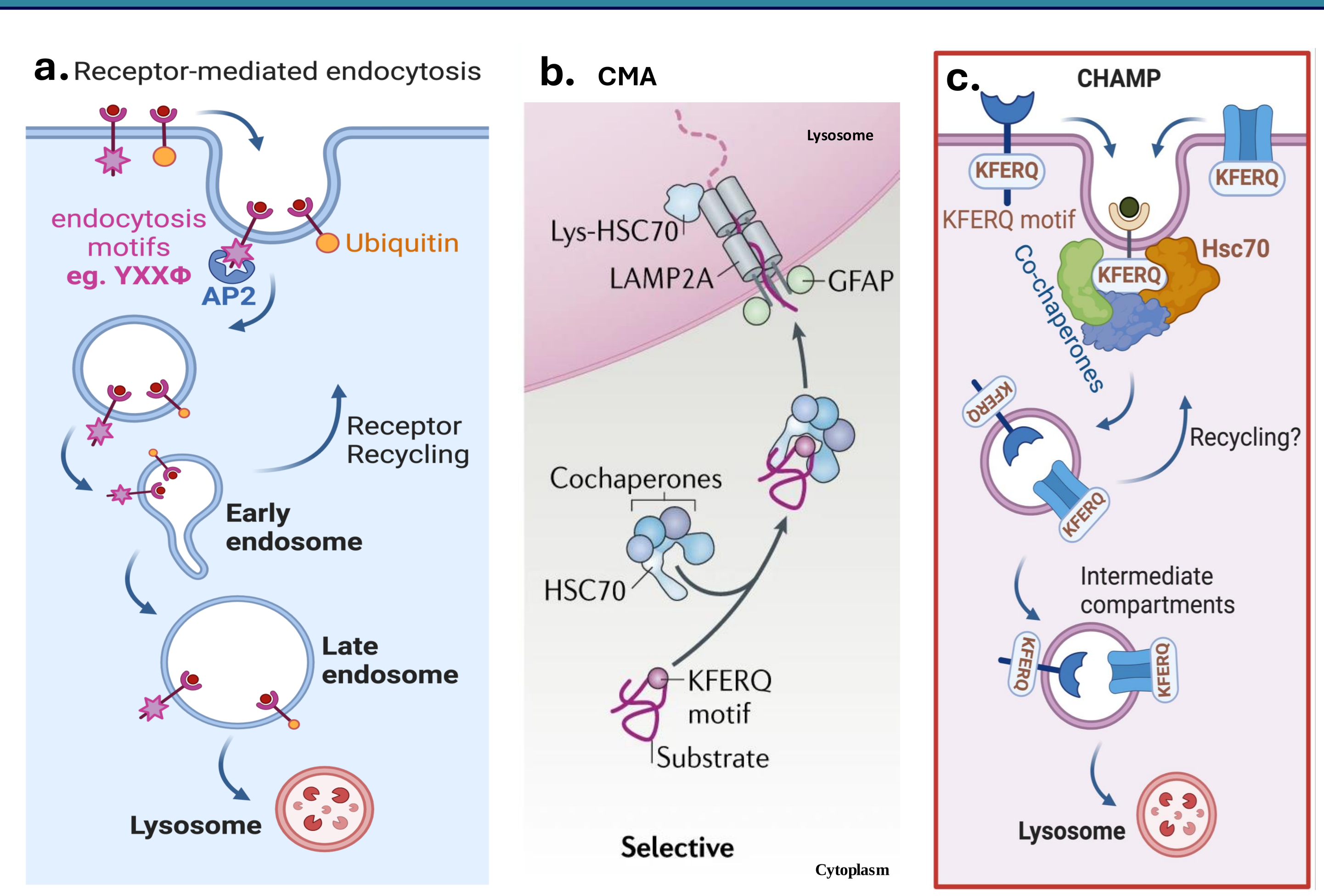

Figure 2. Our working model considering the literature. (a) Receptor-mediated endocytosis is a major regulator of SMPs for lysosomal degradation/recycling. Endocytosis motifs like YXXΦ, (D/E)XXXL(L/I), and LL motifs, or posttranslational modifications like ubiquitination serve as selective targeting signals for SMPs. **(b)** Chaperone-mediated autophagy (CMA) involves the selective degradation of KFERQ-like motif-bearing proteins delivered to the lysosomes via chaperone HSC70 and cochaperones, such as carboxyl terminus of HSC70-interacting protein (CHIP), heat shock protein 40 (HSP40) and HSP70–HSP90 organizing protein (HOP), and their internalization in lysosomes via the receptor lysosome-associated membrane protein type 2A (LAMP2A) (Kaushik and Cuervo 2018).**(c)** We propose that **ch**aperone-mediated **a**utophagy of surface **m**embrane **p**roteins (**CHAMP**), is a previously uncharacterized pathway that selectively targets the majority of SMPs for degradation. KFERQ-related motifs serve as selective targeting signals for SMP degradation by CHAMP.

Functions of SMPs with KFERQ motifs

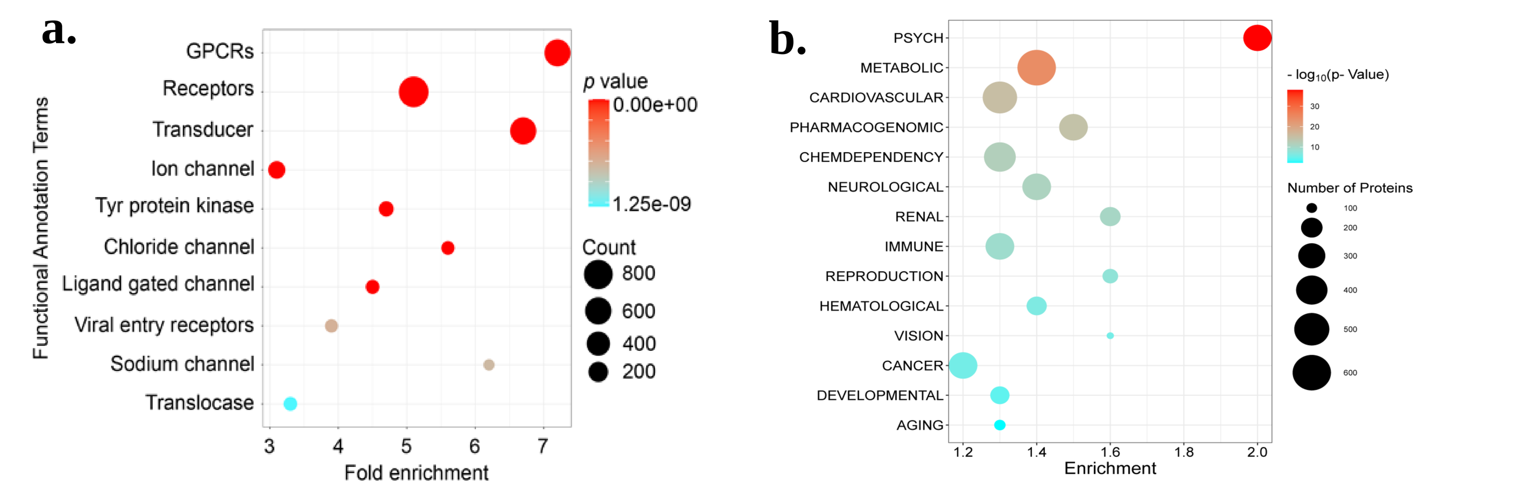

Figure 3. (a) Functional annotation analysis for molecular function of SMPs harboring KFERQ-like motif in their cytoplasmic domains. Count: number of SMPs in each functional annotation term. **(b)** Disease classes annotation analysis for SMPs harboring KFERQ-like motif in their cytoplasmic domains. Database for Annotation, Visualization and Integrated Discovery (DAVID).

CMA activation translocates SMPs to Lysosomes

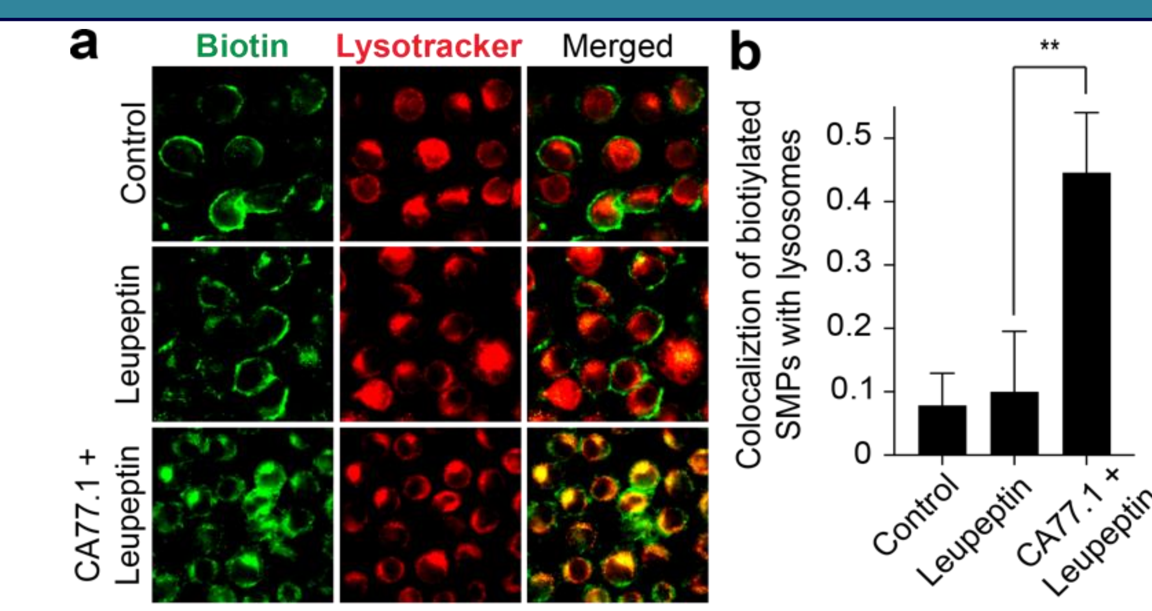

Figure 4. Chaperone-mediated autophagy (CMA) activation translocates SMPs to lysosomes. (a) We used cell surface biotinylating (Sulfo-NHS-lc-lc-biotin) to label SMPs with streptavidin-488 and lysotracker-red to label lysosomes. Selective CMA activation by CA77 for 1 hr. induced colocalization of SMPs with the lysosomes in Hela cells. Leupeptin is a protease inhibitor that blocks SMP degradation once localized to lysosomes. **(b)** Quantification of colocalization of SMPs with lysosomes expressed as Fisher-transformed Pearson coefficient. $n = 3$, mean +/- SD. ** $P = 0.0053$. One Way ANOVA, Tukey's post-hoc test.

Upregulation of SMPs in LAMP2 KO cells

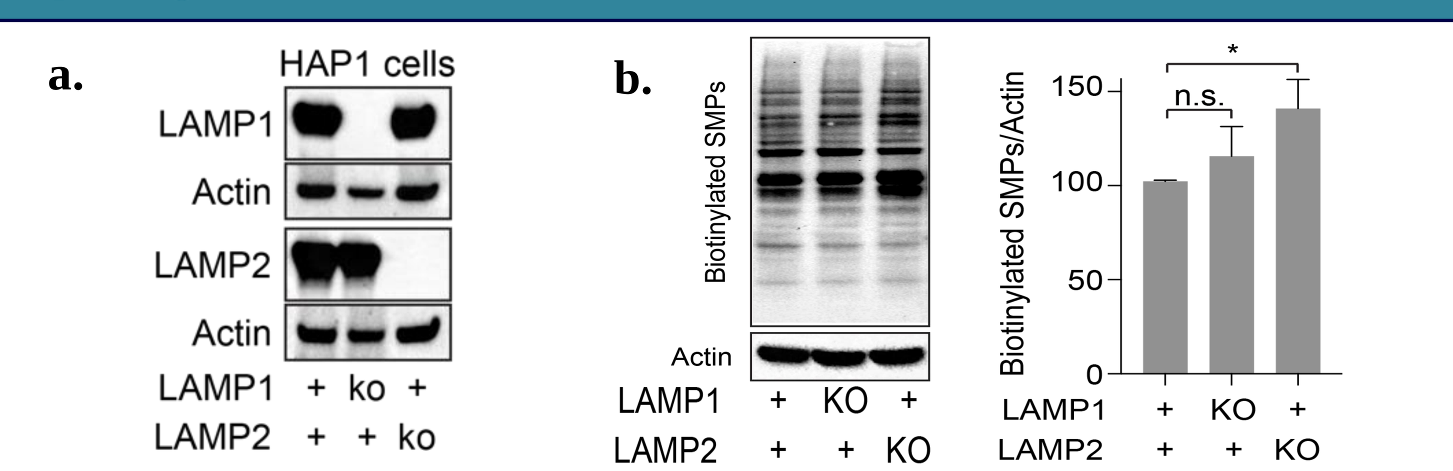

Figure 5. Upregulation of SMPs in LAMP2 KO cells (a) Western blot confirmation of LAMP1 & LAMP2 KO in HAP1 cells. **(b)** We used cell surface biotinylation (Sulfo-NHS-lc-lc-biotin) to label SMPs in WT, LAMP1 & LAMP2 KO HAP1 cells at baseline. Column graph shows quantification of biotinylated SMPs normalized to actin bands. $n = 3$, mean +/- SD. * $P = 0.046$. two tailed t test.

CMA induces Hsc70 binding to SMPs

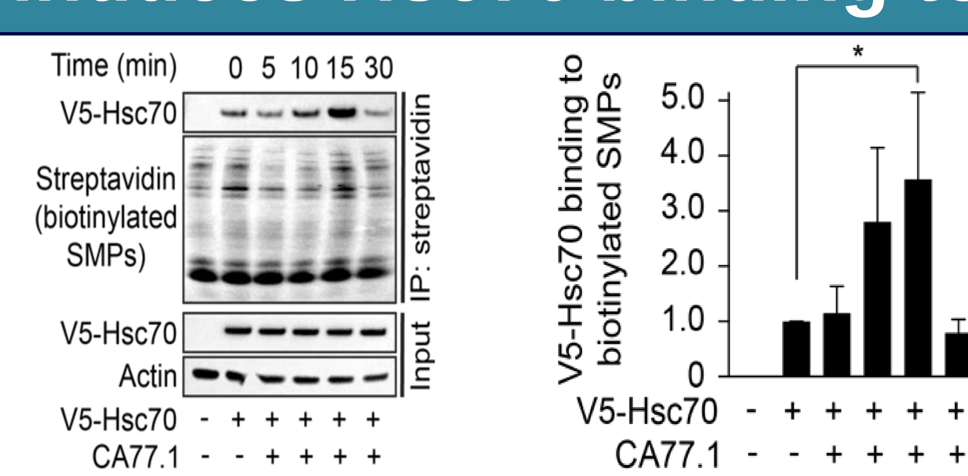

Figure 6. Activation of chaperone-mediated autophagy (CMA) induces binding of Hsc70 to SMPs. We used cell surface biotinylation (Sulfo-NHS-lc-lc-biotin) to label SMPs. Then we pulled down biotinylated SMPs with streptavidin agarose beads following vehicle or CMA activation treatment. Selective CMA activation by CA77 rapidly induces co-IP of V5-Hsc70 with biotinylated SMPs in Hela cells peaking at 15 minutes post-stimulation. Quantification of V5-Hsc70 pulled down by streptavidin normalized to total cell lysate actin is shown. $n = 3$, mean +/- SD. * $P = 0.033$. One Way ANOVA, Tukey's post-hoc test.

ACE2 harbors a functional KFERQ motif

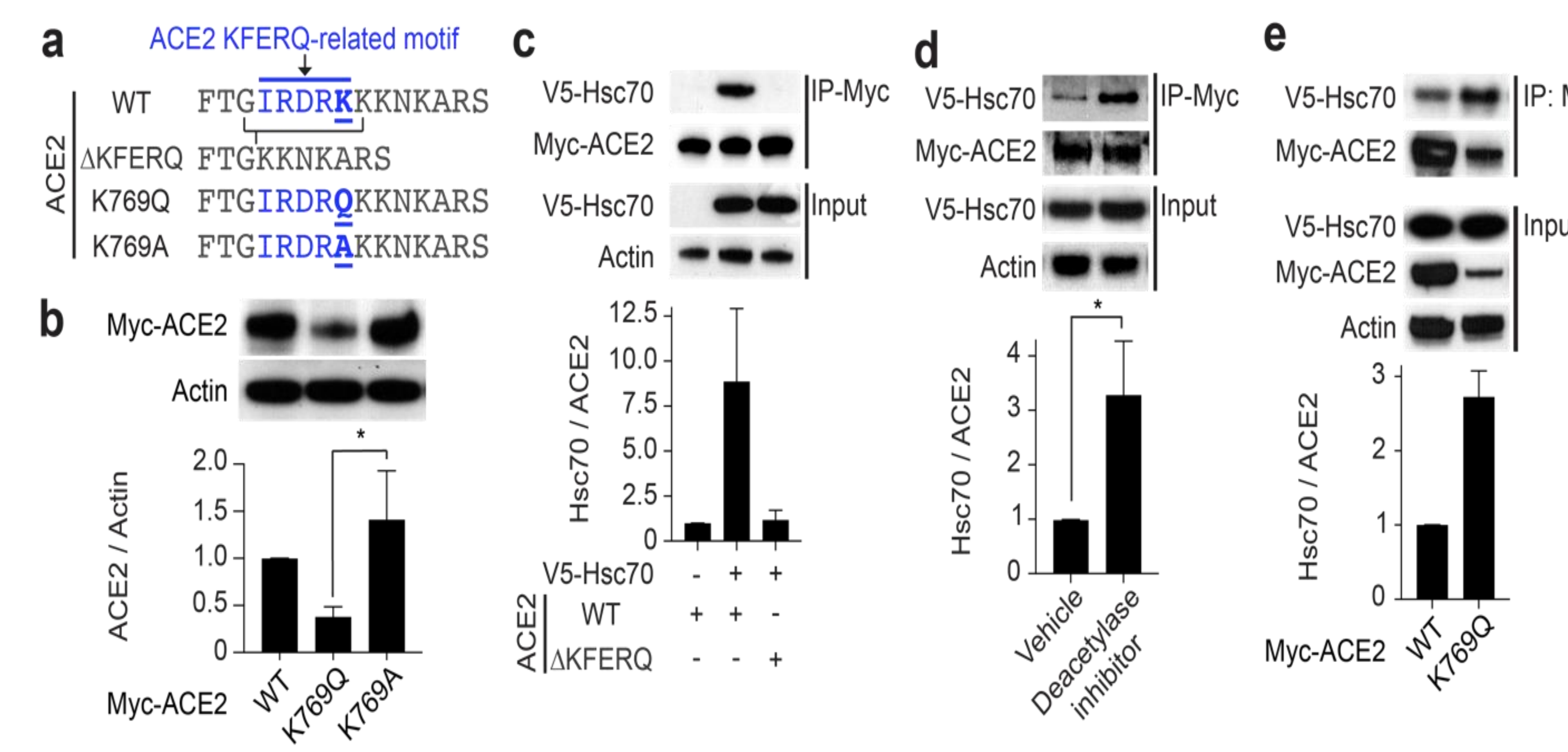

Figure 7. The KFERQ-related motif in ACE2 regulates its expression and binding to Hsc70 . (a) We identified an acetylation-dependent KFERQ-related motif in ACE2 cytoplasmic domain (IRDRK). We generated the indicated mutants. **(b)** Western blot of WT, and motif mutants; K769Q (constitutively active), and K769A (inactive motif) ACE2. Column graph shows quantification of myc-ACE2 normalized to actin bands. $n = 3$, mean +/- SD. * $P = 0.014$. One Way ANOVA, Tukey's post-hoc test. **(c)** The ACE2 KFERQ-related motif is required for binding to Hsc70. Western blot analysis of proteins pulled down with anti-myc IP and input controls. Column graph shows western blot quantification of V5-Hsc70 /myc-ACE2 bands. $n = 2$, Mean +/- SD. **(d)** Acetylation enhances the binding of Hsc70 to ACE2. Western blot of proteins pulled down with anti-myc-IP & input loading controls. Column graph shows quantification of V5-Hsc70/myc-ACE2 bands. $n = 3$, mean +/- SD is shown. * $P = 0.016$. Two tailed t test. Inhibition of deacetylation by treating cells with deacetylase inhibitors enhances the binding of Hsc70 to ACE2. **(e)** Constitutive activation of ACE2 KFERQ-related motif enhances its binding to Hsc70. Western blot of proteins after anti-myc-IP and input loading controls. Column graph shows quantification of V5-Hsc70/myc-ACE2 bands. $n = 2$, mean +/- SD.

Summary

- Bioinformatics analysis emphasize the importance of SMPs, containing a KFERQ-like motif, in multiple metabolic functions, and in different diseases.
- CMA activation is sufficient to translocate SMPs to the lysosomes. LAMP2 KO cells have significantly more SMPs suggesting that CMA is required for SMPs homeostasis.
- SMPs bind to Hsc70 during CMA activation.
- ACE2 harbors a functional acetylation-inducible cytosolic KFERQ motif that binds to Hsc70 when activated.
- These findings suggest that CMA activation is sufficient to target SMPs to lysosomes. This paves the way for exploring chaperone-mediated autophagy of surface membrane proteins (CHAMP) as a novel selective autophagy pathway that controls cell surface proteome homeostasis via the lysosome.

References

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