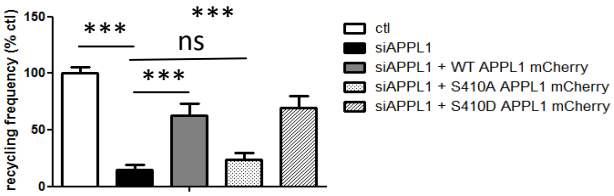
APPL1 integrates regulated GPCR sorting and signalling from the very early endosome

We have recently identified a novel endosomal compartment critical for the sorting and signalling of a subset of GPCRs including the luteinizing hormone receptor (LHR), we termed the very early endosome (VEE). This compartment is positive for the adaptor protein APPL1 (Adaptor Protein Containing PH Domain, PTB Domain And Leucine Zipper Motif 1) (1). APPL1 vesicles are known to act as signalling stations and APPL1 can interact with a number of proteins involved in endosomal trafficking (2). The aim of this study was to investigate the possible functional role of APPL1 on the post-endocytic sorting and signalling of LHR from the VEE.

To measure dynamic and rapid recycling of the LHR at the single event level, HEK293 cells stably expressing a pH sensitive-GFP tagged LHR (eclipLHR) were transfected with control or siRNA against APPL1 (siAPPL1). Four days after transfection cells were imaged live by total internal reflection fluorescence microscopy (TIR-FM) 5 min after addition of the ligand LH (10 nM). The number of recycling events (Fig. 1) were quantitated per cell during a 1 min movie acquisition (at least 16 cells per condition were imaged and analyzed). Cells lacking endogenous APPL1 exhibited a significant reduction in LHR recycling (mean $\pm$ SE of n=16 cells per condition; t-test, *** $p<0.001$) compared to control cells. To test the specificity of APPL1 function on LHR recycling from the VEE, GPCRs that endocytose to and recycle from the classic early endosome were tested for their ability to recycle when treated with siAPPL1 and appeared insensitive to loss of APPL1. As APPL1 is known to be regulated by PKA phosphorylation on Ser410 of APPL1 (3) and that Galphas/cAMP/PKA is the main LHR signalling pathway, we wanted to examine whether LHR signalling could drive its own recycling via APPL1. For this purpose we generated two APPL1 mutants, S410A- (phospho-deficient mutant) and S410D- (phospho-mimetic mutant), and tested them for their ability to rescue LHR recycling in a siAPPL1 background. As shown in Figure 1, both WT- and S410D-APPL1-mCherry were able to restore LHR recycling. On the contrary, no rescue was observed when S410A-APPL1 was used. The involvement of LH induced cAMP/PKA pathway in LHR recycling, was further confirmed by the ability of the PKA inhibitor KT5720 (15 min pre-treatment, 10 uM) to inhibit LHR recycling by 78.23$\pm$1.01%.



Our results suggest an unprecedented role of APPL1 in dictating the post-endocytic sorting of a GPCR. The mechanism regulating APPL1 mediated LHR recycling from VEEs seems to be regulated by PKA phosphorylation of APPL1 on S410 induced by LHR signal activation. These findings, together with our additional signalling studies, suggest an integrated system where LHR trafficking is driven by its own spatially controlled signalling at the level of the VEE.

(1) Jean-Alphonse F et al. (2014). J Biol Chem **289(7):**3960–77.
(2) Kalaidzidis I et al. (2015). J Cell Biol **211(1):**123–44.
(3) Erdemann KS et al. (2007). Dev Cell **13(3):**377–90.