

# Plasticity of PI4KIII $\alpha$ interactions at the plasma membrane

Jeeyun Chung, Fubito Nakatsu, Jeremy M Baskin & Pietro De Camilli\*

## Abstract

Plasma membrane PI4P is an important direct regulator of many processes that occur at the plasma membrane and also a biosynthetic precursor of PI(4,5)P<sub>2</sub> and its downstream metabolites. The majority of this PI4P pool is synthesized by an evolutionarily conserved complex, which has as its core the PI 4-kinase PI4KIII $\alpha$  (Stt4 in yeast) and also comprises TTC7 (Ypp1 in yeast) and the peripheral plasma membrane protein EFR3. While EFR3 has been implicated in the recruitment of PI4KIII $\alpha$  via TTC7, the plasma membrane protein Sfk1 was also shown to participate in this targeting and activity in yeast. Here, we identify a member of the TMEM150 family as a functional homologue of Sfk1 in mammalian cells and demonstrate a role for this protein in the homeostatic regulation of PI(4,5)P<sub>2</sub> at the plasma membrane. We also show that the presence of TMEM150A strongly reduces the association of TTC7 with the EFR3-PI4KIII $\alpha$  complex, without impairing the localization of PI4KIII $\alpha$  at the plasma membrane. Collectively our results suggest a plasticity of the molecular interactions that control PI4KIII $\alpha$  localization and function.

**Keywords** phospholipase C; PI4KA; Rolling blackout; Ypp1

**Subject Category** Membrane & Intracellular Transport

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## Introduction

Phosphoinositides are phospholipids derived from the phosphorylation of phosphatidylinositol at the 3-, 4- and 5-positions of the inositol ring [1,2]. The seven phosphoinositides are differentially localized on the cytosolic leaflets of cellular membranes, where they play a critical role in controlling interactions that occur at their surface. Via this mechanism, which helps generate a code of membrane identity, they regulate a multiplicity of cellular processes [1,2]. Among phosphoinositides, phosphatidylinositol 4-phosphate (PI4P) plays numerous fundamental roles in the Golgi complex, the endosomal system, and the plasma membrane [1–3].

PI4P is a biosynthetic precursor of PI(4,5)P<sub>2</sub> and thus an upstream metabolite in metabolic pathways leading to diacylglycerol (DAG), IP<sub>3</sub>, and PI(3,4,5)P<sub>3</sub> [4]. In addition, PI4P can contribute to the regulation of other membrane lipids, including sterols [5–8], phosphatidylserine [9], and sphingolipids [10]. Therefore, tight regulation of PI4P levels is important for a variety of cellular functions.

In mammals, four enzymes can phosphorylate phosphatidylinositol at the 4-position of the inositol ring to generate PI4P [2,3]. Of the four enzymes, PI 4-kinase type III $\alpha$  (PI4KIII $\alpha$ ; Stt4 in yeast) plays a major role in the generation of PI4P at the plasma membrane [11–14]. This enzyme is of fundamental physiological importance, as germline disruption of its gene in mice produces embryonic lethality, and its conditional disruption in mouse fibroblasts results in cell death [11,15,16]. Interest in this enzyme has been recently enhanced by its identification as a critical host factor for the replication of the hepatitis C virus in humans [17–19].

PI4KIII $\alpha$ /Stt4 localizes to the plasma membrane as part of an evolutionarily conserved complex comprising two adaptor proteins (each of them represented by two similar A and B isoforms), the peripheral membrane protein EFR3 and the scaffolding protein TTC7 (Ypp1 in yeast) [11,12,20]. Studies in yeast have identified an additional regulator of Stt4, termed Sfk1 (suppressor of four-kinase 1), a multipass transmembrane protein localized at the plasma membrane, whose overexpression partially rescues defects in a temperature-sensitive Stt4 mutant strain [21]. Sfk1 physically interacts with Stt4, and loss of Sfk1 compromises proper plasma membrane localization of Stt4 [21]. Despite the importance of Sfk1 in the regulation of Stt4 localization and function in yeast, no mammalian counterpart of Sfk1 has yet been reported, and major outstanding questions remain about how the PI4KIII $\alpha$  complex is regulated at the plasma membrane. Here, we identify TMEM150A as a mammalian homologue of yeast Sfk1. TMEM150A forms a complex at the plasma membrane with PI4KIII $\alpha$  and EFR3, but not TTC7, suggesting a plasticity of the protein interactions of PI4KIII $\alpha$ . We also find that levels of TMEM150A impact the rate of PI(4,5)P<sub>2</sub> re-synthesis following acute depletion of this lipid, possibly due to an impact of this protein on the pool of PI4P available for the synthesis of PI(4,5)P<sub>2</sub>.

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## Results and Discussion

### Putative identification of TMEM150A as a mammalian homologue of yeast Sfk1

BLAST searches for amino acid sequences in higher eukaryotes related to regions of Sfk1 highly conserved among various species of yeast and fungi yielded members of the mammalian TMEM150/FRAG1/DRAM family, whose defining structural feature is the presence of six predicted transmembrane regions (Fig 1A). DRAM (Damage-Regulated Autophagy Regulator) 1 and 2 were reported to be localized in lysosomal membranes and to function as p53-induced modulators of autophagy [22,23]. TMEM150A, TMEM150B, and TMEM150C remain uncharacterized. Thus, we explored whether TMEM150A proteins could be functional homologues of yeast Sfk1 in the control of the localization and function of PI4KIII $\alpha$ , the mammalian homologue of yeast Stt4 [21].

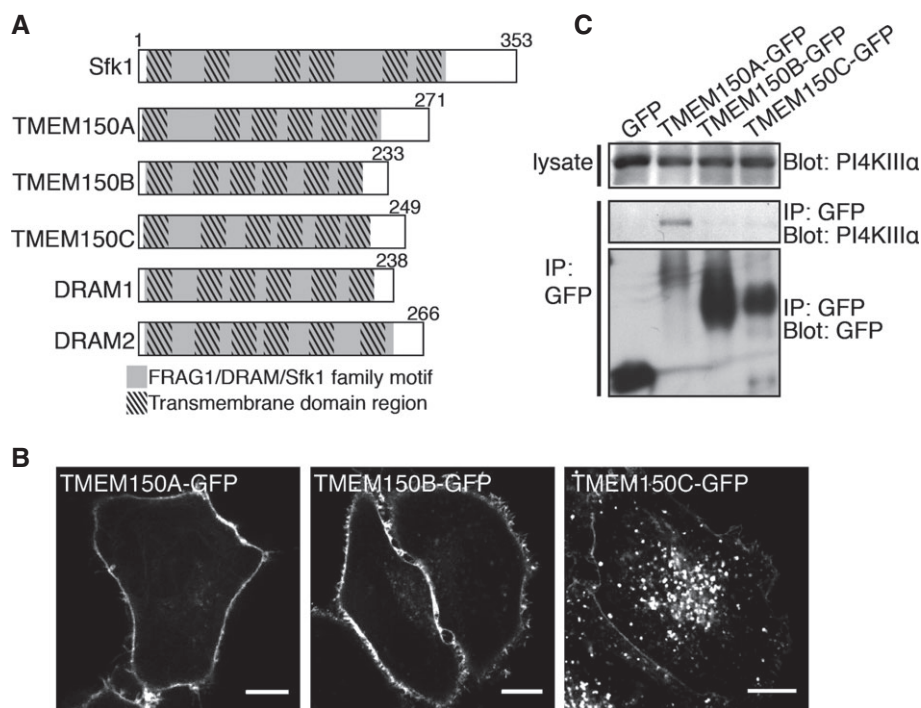
C-terminally GFP-tagged TMEM150A, TMEM150B, or TMEM150C were transiently expressed in HeLa cells. TMEM150A-GFP and TMEM150B-GFP were predominantly localized at the plasma membrane, and only a minor pool of these proteins was localized on intracellular structures. TMEM150C-GFP was localized at the plasma membrane, but a greater pool of this protein was localized on intracellular vesicular structures (Fig 1B) that co-localized with RFP-tagged LAMP1 (a lysosomal marker) (Supplementary Fig S1). Western blot analysis of anti-GFP immunoprecipitates

generated from these cells, and from cells expressing GFP alone as a control, revealed coprecipitation of PI4KIII $\alpha$  with TMEM150A, although not B or C (Fig 1C). Based on these results, we further explored the properties of TMEM150A and its potential function in PI4KIII $\alpha$  regulation.

### TMEM150A interacts with PI4KIII $\alpha$ via its C-terminal cytoplasmic region

To determine the topology of TMEM150A within the plasma membrane, live HeLa cells expressing TMEM150A-GFP were exposed to an anti-GFP antibody at 4°C without permeabilization by detergent. While intrinsic GFP fluorescence was observed, anti-GFP immunostaining did not give any signal without prior fixation and permeabilization (Fig 2A, upper panel), indicating that the C-terminal tail of TMEM150A is oriented toward the cytosol. By contrast, immunofluorescence analysis of a construct containing an HA tag in the middle of Loop 1 (termed HA-TMEM150A-GFP) produced fluorescence without any prior permeabilization, suggesting that Loop 1 is exposed to the extracellular medium (Fig 2A, bottom panel). Based on these results, we hypothesized that TMEM150A has the topology illustrated in Fig 2B, with a C-terminal cytosolic tail.

Next, we determined the region of TMEM150A responsible for the recovery of a pool of PI4KIII $\alpha$  in anti-TMEM150A-GFP immunoprecipitates. The C-tail is the major portion of TMEM150A protruding toward the cytosol. Thus, we focused on this region.

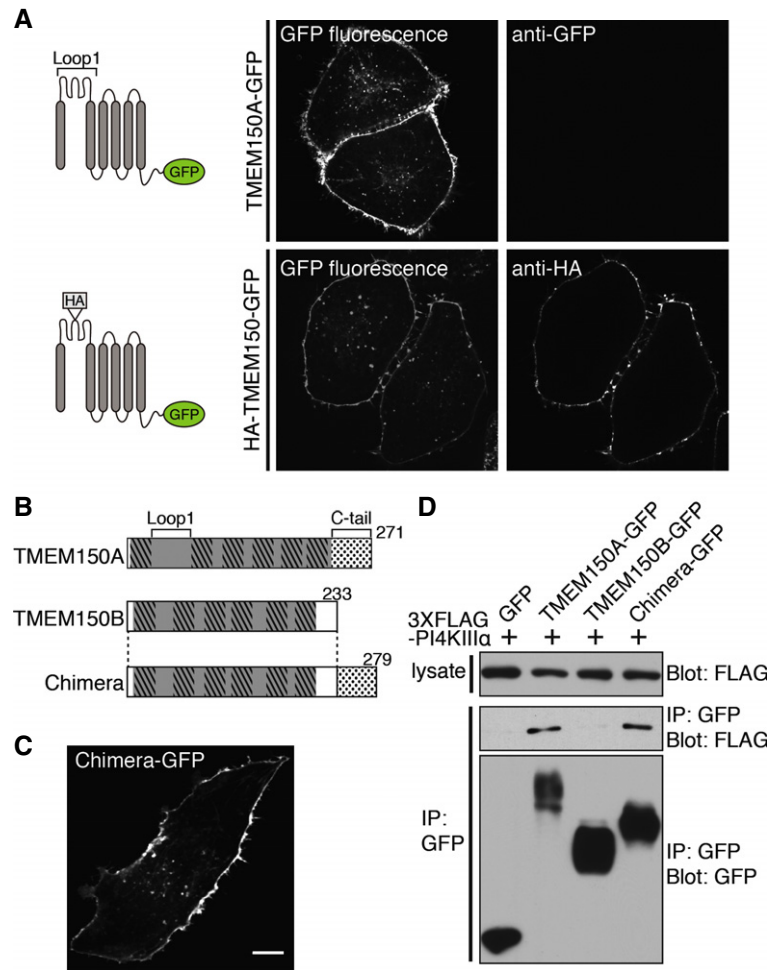


**Figure 1. Identification of a mammalian homologue of yeast Sfk1.**

A Domain cartoons of yeast Sfk1 and of its putative homologues in mammals. The light gray portions represent FRAG1/DRAM/Sfk1 family homology and the superimposed darker boxes represent transmembrane domain regions.

B Confocal imaging of live HeLa cells transfected with TMEM150A-GFP, TMEM150B-GFP, and TMEM150C-GFP as indicated. Scale bars: 20  $\mu$ m.

C Western blot analysis for GFP and PI4KIII $\alpha$  of anti-GFP immunoprecipitates from lysates of HeLa cells transfected with GFP or TMEM150A-GFP fusion proteins.



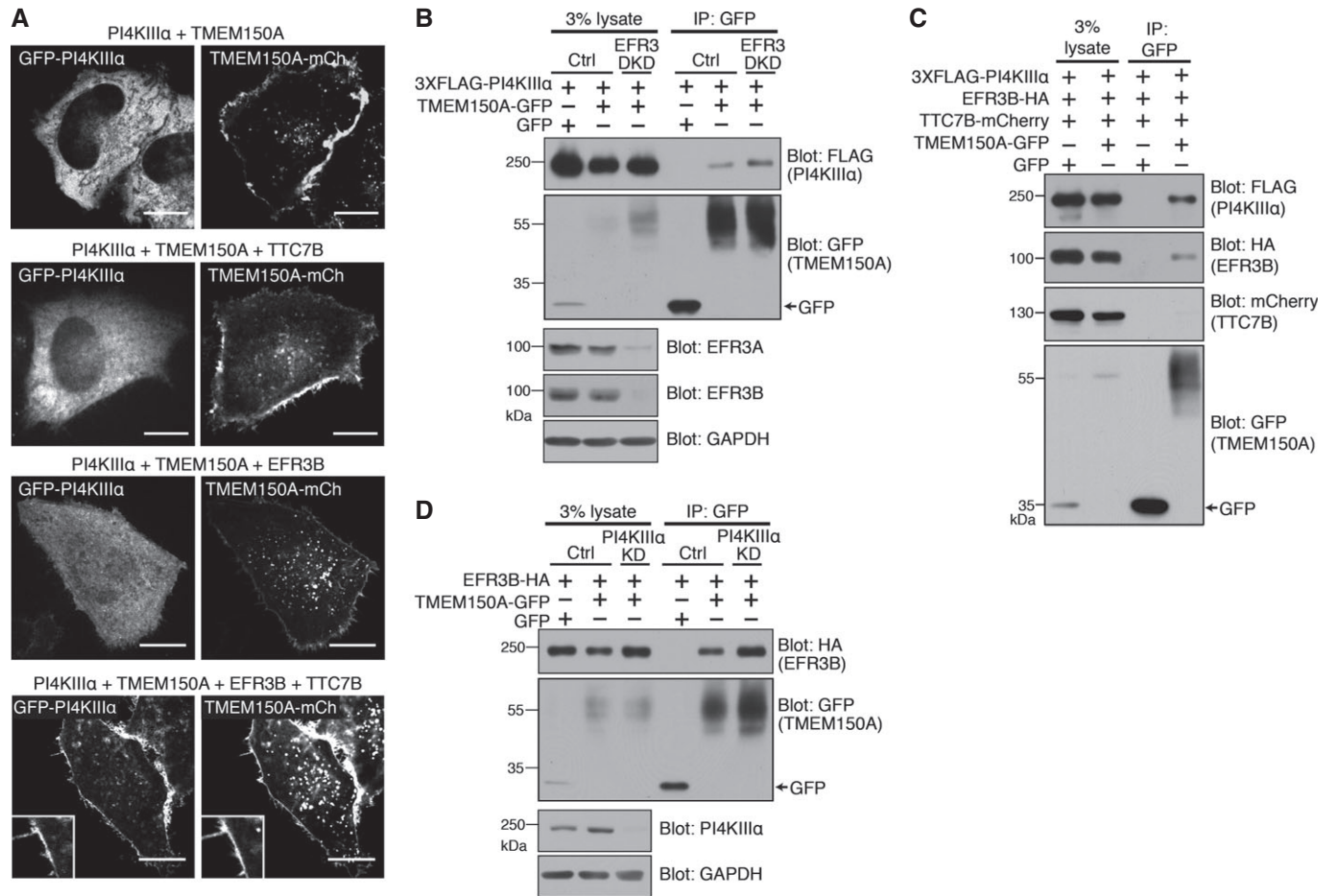
**Figure 2. TMEM150A interacts with the PI4KIII $\alpha$  complex via its C-terminal tail.**

- A Fluorescence of HeLa cells expressing TMEM150A-GFP (top) or HA-TMEM150A-GFP (bottom) (see cartoons at left) and incubated with anti-GFP (top) or anti-HA (bottom) antibodies without permeabilization before fixation. Left panels represent intrinsic GFP fluorescence, and right panels represent anti-GFP or anti-HA immunofluorescence.
- B Schematic diagram of TMEM150A, TMEM150B, and Chimera. Chimera was generated by fusing the C-terminal 40 amino acids of TMEM150A (dotted box) to the C-terminus of full-length TMEM150B.
- C Snapshot of confocal live imaging of HeLa cell expressing Chimera-GFP. Scale bar: 20  $\mu$ m.
- D Anti-FLAG and anti-GFP Western blots of immunoprecipitates generated from lysates of HeLa cells double-transfected with 3  $\times$  FLAG-PI4KIII $\alpha$  and either TMEM150A-GFP, TMEM150B-GFP, or Chimera-GFP.

Because TMEM150B-GFP does not coprecipitate PI4KIII $\alpha$ , we generated a chimeric protein (Chimera) in which the C-tail of TMEM150A was appended to the C-terminus of full-length TMEM150B (Fig 2B). This Chimera was targeted to the plasma membrane, similar to both TMEM150A-GFP and TMEM150B-GFP, indicating that its longer C-tail did not affect its subcellular targeting (Fig 2C). Anti-GFP immunoprecipitation experiments were then performed in cells coexpressing 3  $\times$  FLAG-PI4KIII $\alpha$  and either TMEM150A-GFP, TMEM150B-GFP, or Chimera-GFP. As shown in Fig 2D, anti-FLAG Western blotting revealed that PI4KIII $\alpha$  coprecipitated to the same extent with either TMEM150A or with Chimera, while it did not coprecipitate with TMEM150B. We conclude that the portion of TMEM150A required for the formation of a complex with PI4KIII $\alpha$  possibly indirectly is its cytosolic C-tail.

### TMEM150A alone is not sufficient to recruit PI4KIII $\alpha$ to the plasma membrane

We previously reported that PI4KIII $\alpha$  forms a complex with EFR3B and TTC7B at the plasma membrane [11] and that both EFR3B and TTC7B are required for PI4KIII $\alpha$  targeting to the plasma membrane (Supplementary Fig S2) [11,12,20]: EFR3 is the plasma membrane-anchored component [11,20], while PI4KIII $\alpha$  is recruited to EFR3 indirectly by TTC7 (see Fig 5F) [11,20]. In view of the recovery of a pool endogenous PI4KIII $\alpha$  in anti-TMEM150A-GFP immunoprecipitates, as described above, we explored whether overexpression of TMEM150A in HeLa cells could bypass the need for EFR3 and TTC7 in the plasma membrane recruitment of PI4KIII $\alpha$ . As shown by Fig 3A, this was not the case, as GFP-PI4KIII $\alpha$  had a diffuse cytosolic distribution in cells expressing



**Figure 3. Interplay between TMEM150A and components of the PI4KIII $\alpha$  complex.**

**A** Confocal imaging for GFP and mCherry fluorescence of live HeLa cells transfected with GFP-PI4KIII $\alpha$ , TMEM150A-mCherry, EFR3B-HA, and TTC7B-FLAG in various combinations. Scale bars: 20  $\mu$ m.

**B** Western blot analysis for the epitopes and proteins indicated, of starting lysates and anti-GFP immunoprecipitates generated from HeLa cells treated with control or EFR3 (EFR3A and EFR3B)-specific siRNAs 24 h prior to transfection of 3  $\times$  FLAG-PI4KIII $\alpha$  and TMEM150A-GFP as shown at the top.

**C** Western blot analysis for the epitopes and proteins indicated, of starting lysates and anti-GFP immunoprecipitates generated from HeLa cells transfected with 3  $\times$  FLAG-PI4KIII $\alpha$ , EFR3B-HA, TTC7B-mCherry, and TMEM150A-GFP as shown at the top.

**D** Western blot analysis for the epitopes and protein indicated, of starting lysates and anti-GFP immunoprecipitates generated from HeLa cells treated with control and PI4KIII $\alpha$ -specific siRNA 24 h prior to transfection of EFR3B-HA and TMEM150A-GFP as shown at the top.

TMEM150A-mCherry, which was localized in the plasma membrane. This discrepancy between the two localizations is consistent with the low recovery of PI4KIII $\alpha$  in anti-TMEM150A-GFP immunoprecipitates, in spite of the specificity of this recovery (Fig 3B). Co-overexpression of tagged PI4KIII $\alpha$ , EFR3B, TTC7B, and TMEM150A in different combinations revealed that plasma membrane targeting of PI4KIII $\alpha$  occurred only when both TTC7 and EFR3 were also co-overexpressed (Fig 3A). Thus, even in the presence of co-overexpressed TMEM150A, efficient plasma membrane targeting of GFP-PI4KIII $\alpha$  requires co-overexpression of both EFR3 and TTC7. However, while overexpression of EFR3 increases the recovery of GFP-PI4KIII $\alpha$  in anti-TMEM150A-GFP immunoprecipitates (see below in Fig 3C), a small pool of GFP-PI4KIII $\alpha$  is recovered in such immunoprecipitates also when only endogenous EFR3 is present or even after EFR3 (EFR3A and EFR3B) knockdown (Fig 3B). Conversely, siRNA-mediated knockdown of

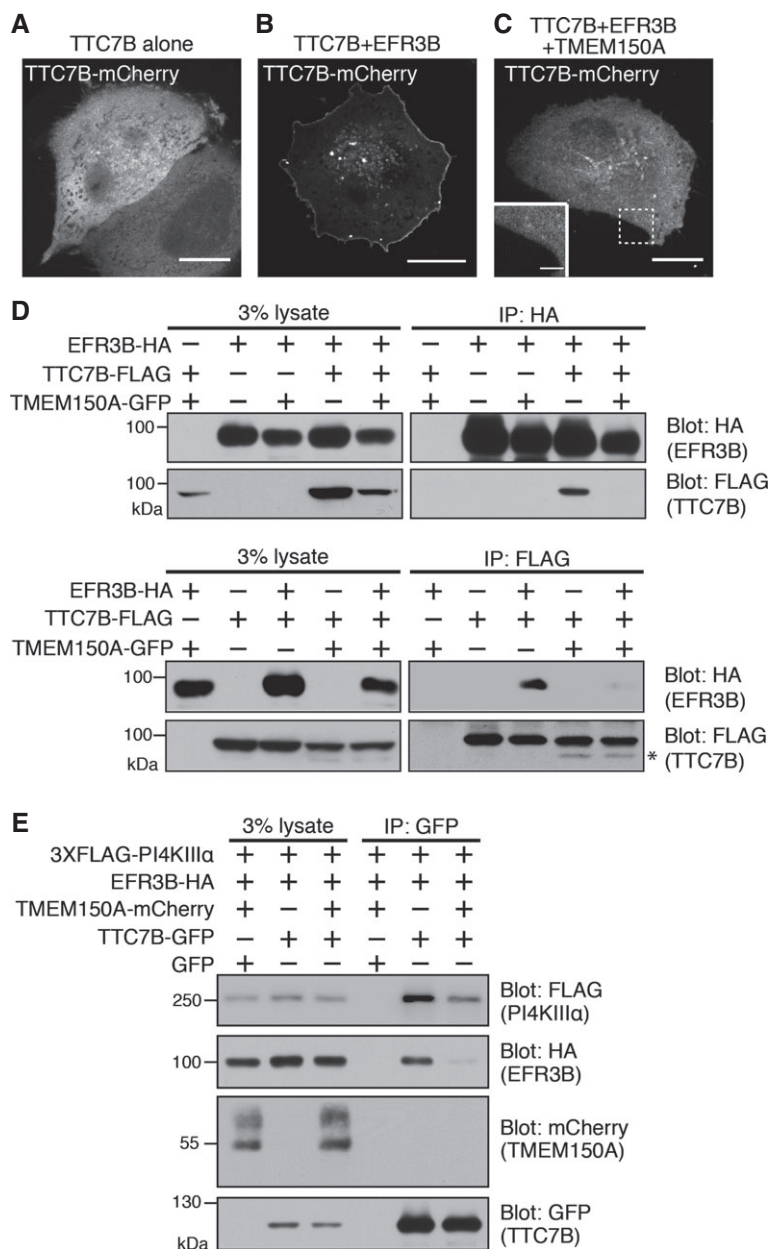
TMEM150A did not change the subcellular localization pattern of PI4KIII $\alpha$ , EFR3B, and TTC7B when each of these proteins was over-expressed alone or in various combinations (Supplementary Fig S3).

**Overexpression of TMEM150A affects the interactions of TTC7 with EFR3 and PI4KIII $\alpha$**

As our data support a link of TMEM150A to PI4KIII $\alpha$ , we explored whether TMEM150A interacts with EFR3 and TTC7 using biochemical experiments. HeLa cells were transiently transfected with 3  $\times$  FLAG-PI4KIII $\alpha$ , EFR3B-HA, TTC7B-mCherry, and either TMEM150A-GFP or GFP. Extracts prepared from these cells were then immunoprecipitated with anti-GFP antibodies. Western blotting of immunoprecipitates from TMEM150A-GFP-expressing cells, but not of those from GFP-expressing cells, revealed robust presence of both PI4KIII $\alpha$  and EFR3, but surprisingly, not of TTC7B (Fig 3C),

suggesting the occurrence of a complex between TMEM150A, PI4KIII $\alpha$ , and EFR3B that is distinct from the previously reported PI4KIII $\alpha$ /EFR3B/TTC7B complex [11,12,20]. Notably, when using cells expressing only TMEM150A-GFP and EFR3B-HA, a robust recovery of EFR3B-HA in TMEM150A-GFP immunoprecipitates, which was not affected by the siRNA-mediated knockdown of

PI4KIII $\alpha$ , was observed, revealing an interaction of these two plasma membrane-localized proteins (Fig 3D). Lack of dependence of the TMEM150A-EFR3B interaction on the presence of PI4KIII $\alpha$  was not unexpected because TTC7 is known to function as a bridge between PI4KIII $\alpha$  and EFR3 [11,20], and the PI4KIII $\alpha$ /EFR3B/TMEM150A-GFP complex does not include TTC7.



**Figure 4. The interaction of the EFR3-PI4KIII $\alpha$  complex with TMEM150A is mutually exclusive with the interaction with TTC7B.**

A–C Confocal imaging of live HeLa cells transfected with TTC7B-mCherry alone or with EFR3B-HA and TMEM150A-GFP as indicated. Only the fluorescence of TTC7B-mCherry is shown. Scale bars: 20  $\mu$ m.

D Anti-HA and anti-FLAG Western blots of starting lysates and immunoprecipitates generated from lysates of HeLa cells triple-transfected with EFR3B-HA, TTC7B-FLAG, and TMEM150A-GFP. Protein complexes were immunoprecipitated by anti-HA (top panels) or anti-FLAG (bottom panels) antibodies. The asterisk indicates fragments of TTC7B just below the full-length TTC7B bands.

E Western blot analysis for the epitopes and proteins indicated, of starting lysates and anti-GFP immunoprecipitates generated from HeLa cells transfected with 3  $\times$  FLAG-PI4KIII $\alpha$ , EFR3B-HA, TMEM150A-mCherry, and TTC7B-GFP as shown at the top.

We further explored the surprising absence of TTC7 in PI4KIII $\alpha$ -EFR3B-TMEM150A complexes, as TTC7 overexpression was needed to target efficiently PI4KIII $\alpha$  to the plasma membrane under all conditions. As previously described, TTC7B-mCherry has a diffuse cytosolic localization when expressed alone [11], but is recruited to the plasma membrane when co-expressed with EFR3, its direct and plasma membrane-localized interactor [11] (Fig 4A and B). However, the additional expression of TMEM150A-GFP in cells co-expressing TTC7B-mCherry and EFR3 abolished the plasma membrane localization of TTC7B (Fig 4C). Thus, the interaction of EFR3 with either TTC7 or with TMEM150A appears to be mutually exclusive. We corroborated this microscopic finding with biochemical experiments. In cells transfected with TMEM150A-GFP, EFR3B-HA, and TTC7B-FLAG, analysis of anti-EFR3B (anti-HA) and anti-TTC7B (anti-FLAG) immunoprecipitates revealed that TTC7B did not co-immunoprecipitate with EFR3B (Fig 4D, top panel, compare lanes 9 and 10). Vice versa, EFR3B did not coimmunoprecipitate with TTC7B (Fig 4D, bottom panel, compare lanes 8 and 10), if TMEM150A-GFP was also present. The appearance of a TTC7 fragment in cells overexpressing TMEM150A (Fig 4D, asterisk) may reflect an impact of TMEM150A on TTC7 stability.

We also assessed more directly if TTC7 is only present in complexes that exclude TMEM150A in HeLa cells overexpression expressing various combinations of the four proteins (PI4KIII $\alpha$ , EFR3, TMEM150A, and TTC7B), using TTC7-GFP as a bait in immunoprecipitation experiments. Both EFR3B and PI4KIII $\alpha$  were recovered in the immunoprecipitates in the absence of TMEM150A overexpression (Fig 4E, lane 5), but no EFR3B was recovered and the recovery of PI4KIII $\alpha$  was strongly reduced when TMEM150A was overexpressed (Fig 4E, lane 6).

Collectively, these results demonstrate that the interaction of EFR3 with TMEM150A is mutually exclusive with the interaction of TTC7 with EFR3 and that EFR3 and TMEM150A form a complex at the plasma membrane that does not include TTC7. Concerning PI4KIII $\alpha$ , its recruitment and concentration in the plasma membrane is critically dependent on TTC7 expression even if the bulk of TTC7 is cytosolic in the presence of TMEM150A. We hypothesize that a complex of PI4KIII $\alpha$  comprising TTC7 may represent an intermediate needed for the targeting/presentation of PI4KIII $\alpha$  to plasma membrane. At this membrane, the EFR3-TTC7 interaction is competed by the EFR3-TMEM150A interaction, which in turn leads to a stabilization of the plasma membrane localization of PI4KIII $\alpha$  even in the absence of TTC7, indicating that also the TTC7-PI4KIII $\alpha$  is lost.

#### TMEM150A affects the resynthesis of PI(4,5)P<sub>2</sub> at the plasma membrane following its acute depletion

If TMEM150A plays a role in anchoring PI4KIII $\alpha$  at the plasma membrane, one may expect its presence to have an impact of PI4P production in this membrane. In yeast, deletion of Sfk1 increases the severity of PI4P and PI(4,5)P<sub>2</sub> defects in a temperature-sensitive *STT4* mutant background [21]. To determine whether TMEM150A has a regulatory role in the metabolism of PI4P and its downstream metabolite PI(4,5)P<sub>2</sub> in mammalian cells, we first examined whether modulation of TMEM150A levels, via either overexpression or

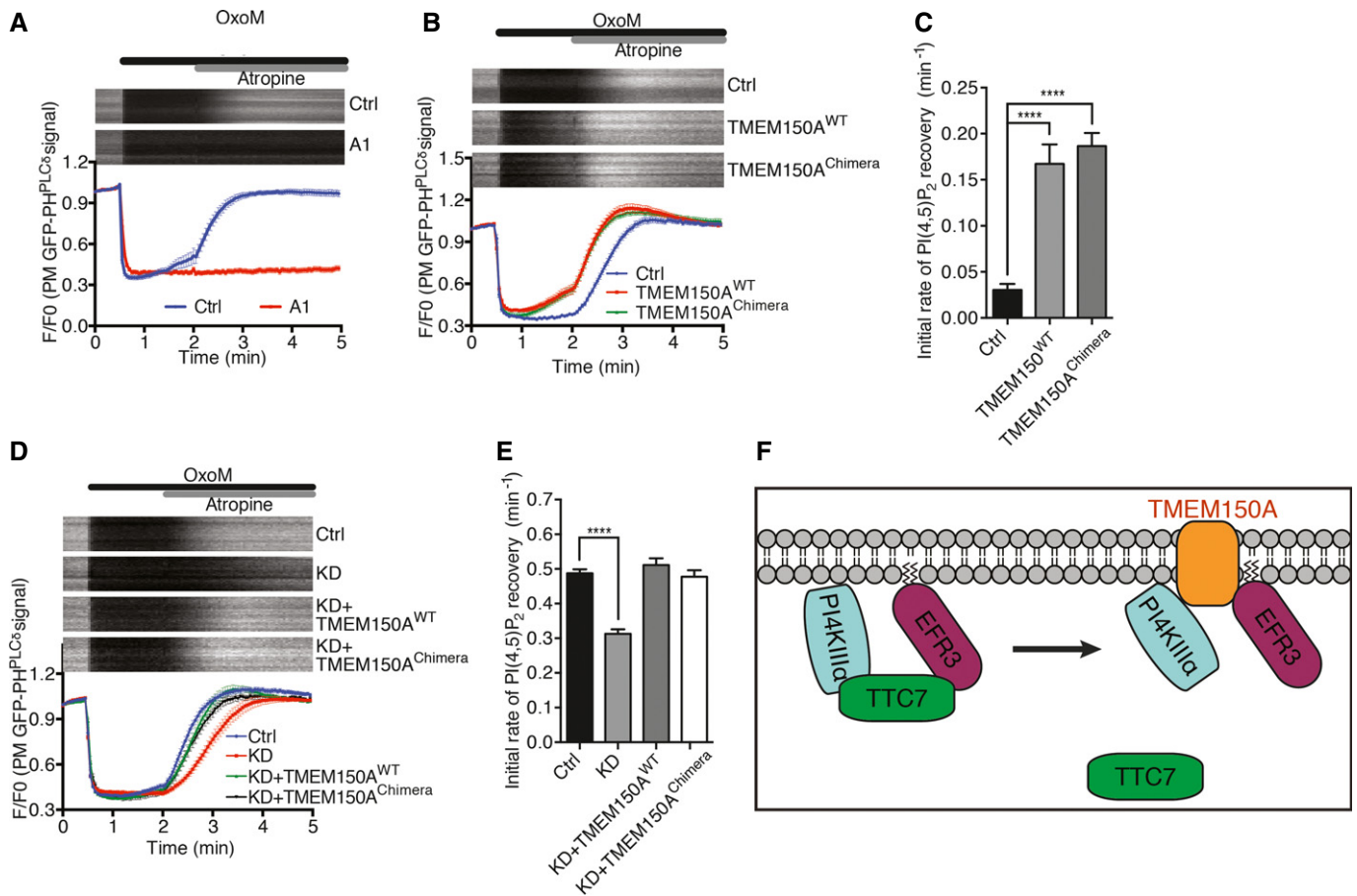
down-regulation through RNA interference, affects total levels of these phosphoinositides. As quantified by HPLC, no significant differences in the total levels of PI4P and PI(4,5)P<sub>2</sub> were observed in TMEM150A-overexpressing or knockdown cells (Supplementary Fig S4), although a modest decrease in the level of plasma membrane-associated PI4P, as detected by an immunofluorescence staining procedure [15,24,25], was observed in the TMEM150A knockdown cells (Supplementary Fig S5). This result prompted us to investigate whether an effect of TMEM150A overexpression or knockdown on phosphoinositide metabolism could be revealed by monitoring the response to an acute perturbation. To this aim, we examined the efficiency of PI(4,5)P<sub>2</sub> resynthesis after triggered PLC-mediated hydrolysis.

In cells transiently transfected with the M1 muscarinic acetylcholine receptor (M1R), stimulation with the agonist oxotremorine-M (Oxo-M) results in phospholipase C (PLC) activation, leading to the rapid and massive cleavage of plasma membrane PI(4,5)P<sub>2</sub> into IP<sub>3</sub> and DAG [26]. Subsequent application of the M1R antagonist atropine terminates PLC activation, allowing the cell to resynthesize PI(4,5)P<sub>2</sub> from PI4P at the plasma membrane. Importantly, it has been well established that PI(4,5)P<sub>2</sub> resynthesis under these conditions requires PI4P generation by PI4KIII $\alpha$  [13,15,25,27–29]. We used this model and monitored PI(4,5)P<sub>2</sub> changes in the plasma membrane by examining the fluorescence of GFP-PH<sub>PLC $\delta$</sub> , a genetically encoded PI(4,5)P<sub>2</sub> marker, using total internal reflection fluorescence (TIRF) microscopy [26,30] (Fig 5A).

In pilot experiments, we confirmed the requirement of PI4KIII $\alpha$  for PI(4,5)P<sub>2</sub> resynthesis, as this recovery was completely blocked by a very potent and highly specific PI4KIII $\alpha$  inhibitor, the compound A1 [15] (Fig 5A). Transient expression of TMEM150A accelerated PI(4,5)P<sub>2</sub> recovery (Fig 5B), with an initial rate of PI(4,5)P<sub>2</sub> recovery about 150% higher than that of control cells (Fig 5C). A similar effect was observed with the transient expression of the TMEM150 Chimera (Fig 5B), supporting the critical importance of the C-terminal region of TMEM150A, that is, its PI4KIII $\alpha$  binding region, in this effect. Conversely, knockdown of TMEM150A resulted in a reduction of the initial recovery rate of PI(4,5)P<sub>2</sub> levels, and the reduction was ‘rescued’ by co-expression of wild-type TMEM150A or the TMEM150 Chimera (Fig 5D and E).

#### Concluding remarks

Collectively, our study corroborates evidence for an important role of an integral membrane protein conserved from yeast to humans, Sfk1/TMEM150A, in the function of PI4KIII $\alpha$  to the plasma membrane. The occurrence of a complex comprising PI4KIII $\alpha$  and TTC7 that binds EFR3 is strongly supported by genetic, biochemical, and structural data [11,12,20]. Our present findings support a critical role of TTC7 together with EFR3 in the targeting of PI4KIII $\alpha$  to the plasma membrane. However, they also indicate that accumulation of TTC7 at the plasma membrane along with EFR3 and PI4KIII $\alpha$  is under the control of TMEM150A, as TMEM150A overexpression prevents such accumulation. We suggest the occurrence of two separate PI4KIII $\alpha$ -containing complexes: (1) a complex comprising PI4KIII $\alpha$ , EFR3, and TTC7, which is required for initial targeting of PI4KIII $\alpha$  to the plasma membrane and predominates in the absence of overexpressed TMEM150A; (2) another complex only in the plasma membrane that comprises PI4KIII $\alpha$ , EFR3, and TMEM150A,



**Figure 5. Levels of TMEM150A affect the rate of PI(4,5)P<sub>2</sub> resynthesis at the plasma membrane.**

- A** Time course of GFP fluorescence, as assessed by TIRF microscopy, from HeLa cells transfected with GFP-PH<sub>PLC $\beta$</sub>  and muscarinic receptor (M1R) and pretreated with compound A1 (100 nM) for 10 min. Oxo-M (10 mM) and atropine (50 mM) were added at the indicated times. Kymographs of representative cells (top) and normalized average traces (bottom;  $n \geq 10$ ) are shown. Quantitative data are represented as mean  $\pm$  SEM.
- B** Time course of GFP fluorescence as in (A) from HeLa cells expressing the indicated proteins together with GFP-PH<sub>PLC $\beta$</sub>  and M1R.
- C** Bar graph showing initial rate of PI(4,5)P<sub>2</sub> recovery (before atropine) from (B), represented as mean  $\pm$  SEM ( $n \geq 10$ ). Statistical significance was assessed by Student's *t*-test. \*\*\*\* $P < 0.0001$ .
- D** Time course of normalized GFP fluorescence, as in (A) from HeLa cells expressing the indicated proteins together with GFP-PH<sub>PLC $\beta$</sub>  and M1R, and in addition treated with control TMEM150A-specific siRNA 24 h prior to other transfection.
- E** Bar graph showing initial rate of PI(4,5)P<sub>2</sub> recovery (after atropine) from (D), represented as mean  $\pm$  SEM ( $n \geq 10$ ). Statistical significance was assessed by Student's *t*-test. \*\*\*\* $P < 0.0001$ .
- F** Schematic representation of PI4KIII $\alpha$  interactions at the plasma membrane. TTC7 interacts directly with both PI4KIII $\alpha$  and EFR3. The presence of TMEM150A in a complex comprising PI4KIII $\alpha$  is mutually exclusive to the presence in the complex of TTC7. The interactions of TMEM150A shown at the right were demonstrated biochemically and result in a positive regulation of PI4KIII $\alpha$  but may be indirect.

but that excludes TTC7 and predominates when TMEM150A is over-expressed. Additional work is required to test this hypothesis. It is also possible that levels of TMEM150A may control interactions of TTC7 indirectly. Collectively, however, our data clearly reveal a plasticity of the PI4KIII $\alpha$  complex. The mechanisms underlying this plasticity remain unknown.

Additionally, our data show that the presence of TMEM150A positively regulates PI4P production. As members of the Sfk1/TMEM150A comprise a highly conserved region containing six predicted transmembrane spans, they are unlikely to act simply as membrane-anchoring factors for Stt4/PI4KIII $\alpha$  and may affect PI4KIII $\alpha$  function both directly and indirectly. The expression of five TMEM150 family members with different localization and properties

in mammalian cells further supports this possibility. Addressing these properties and their potential impact on the dynamics of PI4P is an important priority for future work in this field.

## Materials and Methods

### Cell extracts, immunoprecipitation, and immunoblotting

For immunoprecipitation, cell lysates were incubated with Chromotek GFP-trap agarose beads (Allele Biotech), anti-HA affinity matrix (Roche), or anti-FLAG M2 affinity gel (Sigma-Aldrich), and the solubilized bead-bound material was processed for SDS-PAGE and

standard procedure of immunoblotting. Further details are described in the Supplementary Information.

### Antibody accessibility assay

Briefly, to detect cell surface epitopes, HeLa cells transfected with proper constructs were cooled to 4°C and incubated with primary antibodies without fixation at 4°C (non-permeabilized conditions). Cells were then fixed with 4% paraformaldehyde/PBS. Further details are described in the Supplementary Information.

### Immunostaining for the plasma membrane PI(4)P

Immunostaining of the plasma membrane PI(4)P was performed and quantified as described in the reference paper [16]. Further details are described in the Supplementary Information.

Other methods are described in the Supplementary Information.

**Supplementary information** for this article is available online: <http://embor.embopress.org>

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### Author contributions

JC, FN, JB, and PDC designed the experiments; JC performed the experiments; all authors contributed to writing the manuscript.

### Conflict of interest

The authors declare that they have no conflict of interest.

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