

Investigating the Effect of Phosphorylation and Aggregation Capacity on Tau Turnover in *Drosophila* Models



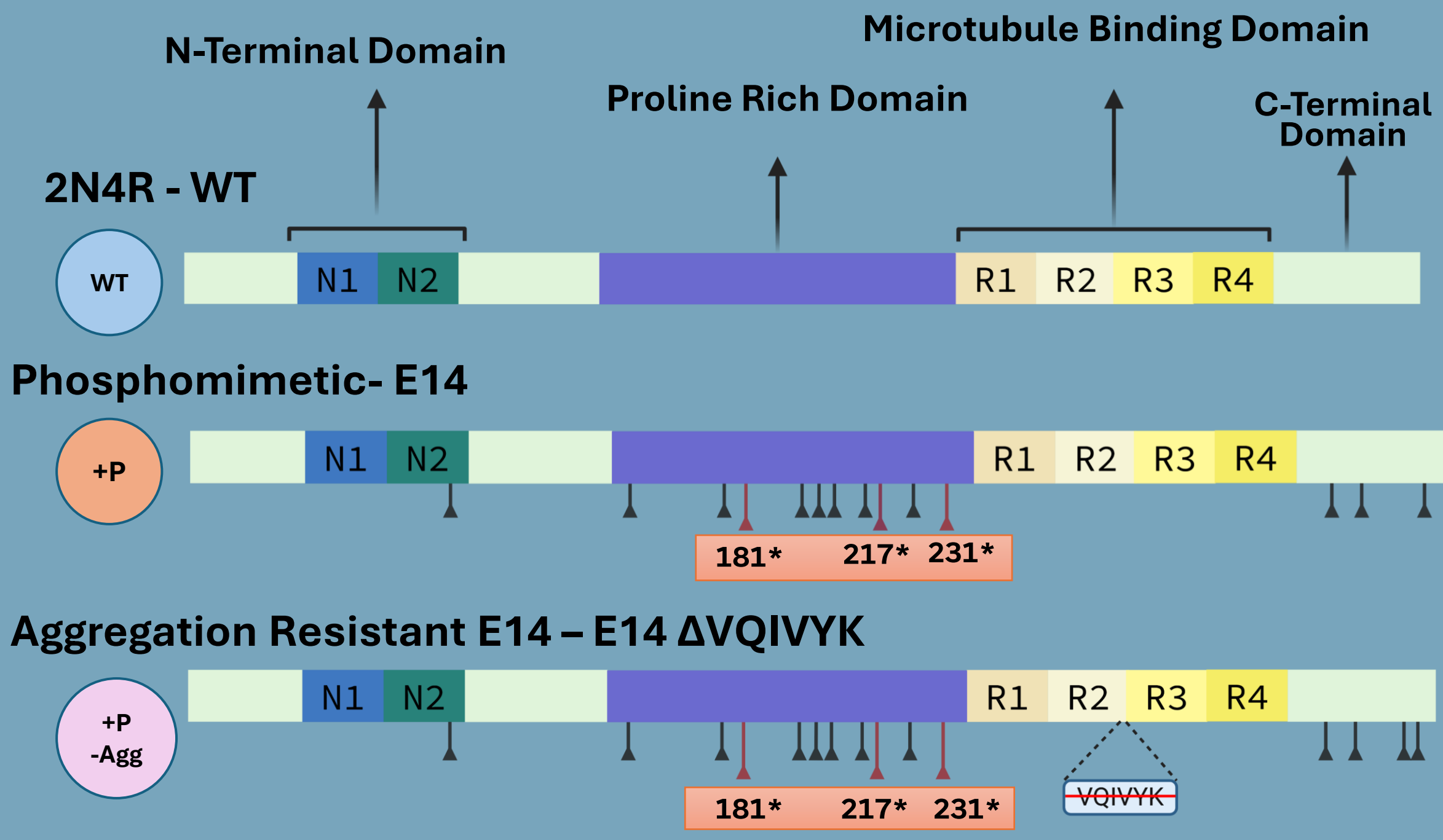
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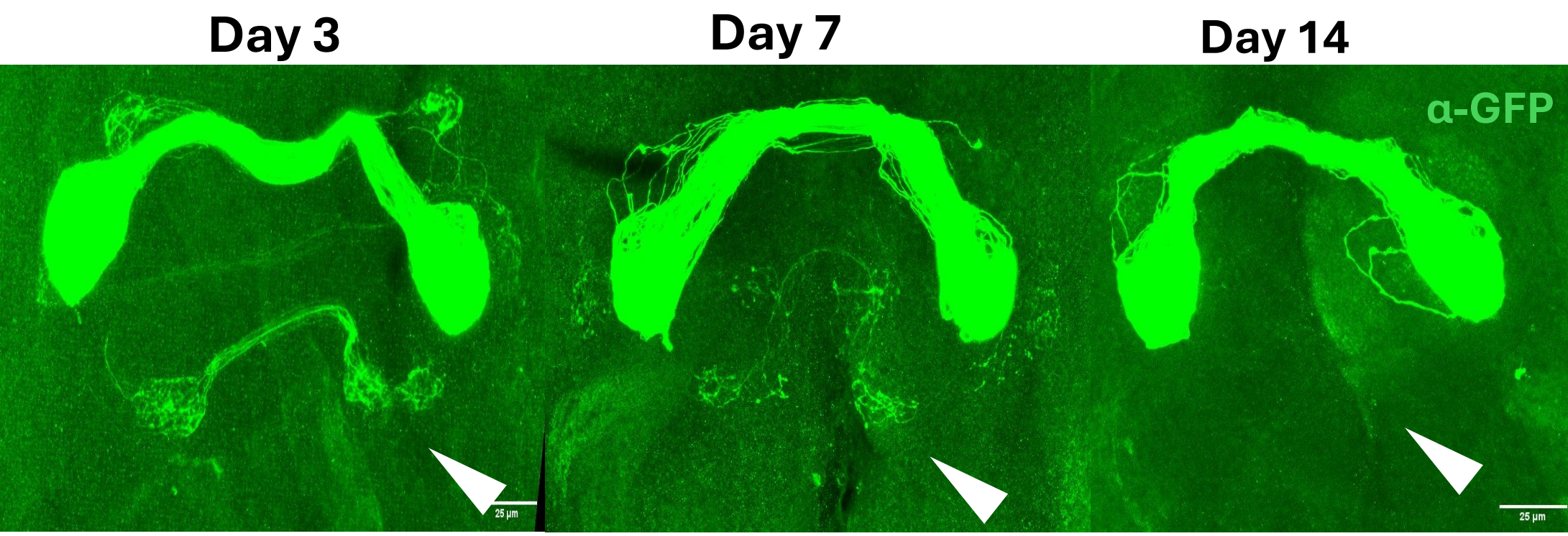
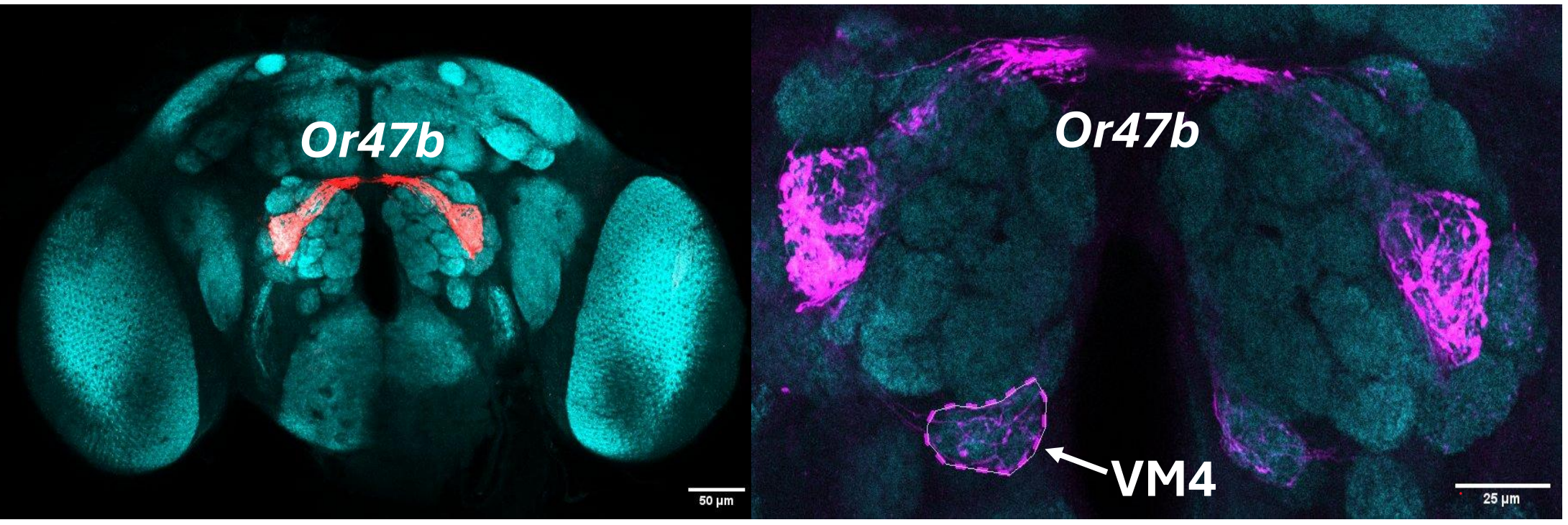
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1. Introduction – what is the physiological consequence of p181*, p217* and p231*?

- The accumulation of mis-folded tau is the pathological hallmark of multiple neurodegenerative disorders, including Alzheimer's disease. Inability to properly clear mis-folded tau results in its accumulation within neurons that promotes the cascade of aggregation and propagation and spreading of Tau pathology.
- Hyperphosphorylation of Tau results in its accumulation in the cytosol- prone to further modifications that evade lysosomal degradation and the different cellular mechanisms of clearance.
- AD-associated aggregates and neurofibrillary tangles have almost always been associated with hyperphosphorylated Tau (often referred to as pTau in the literature)- serving as an excellent biomarker for the prognosis.
- In this study, we aim to study the impact of hyperphosphorylation and aggregation on Tau clearance in vivo. We used the **modified Tau variant- E14**, that **chemically mimics the hyperphosphorylated protein** (phosphorylated in 14 disease-relevant sites, including T181, T217 and T231- widely known biomarkers). Additionally, we used the **E14 ΔVQIVYK** variant that still **mimics hyperphosphorylation but lacks the VQIVYK aggregation motif**.
- Utilising *Drosophila* transgenic lines expressing hTau variants, we were able to evaluate the resistance of hyperphosphorylated tau to turnover and the effect of inhibiting aggregation on its ability to be cleared.

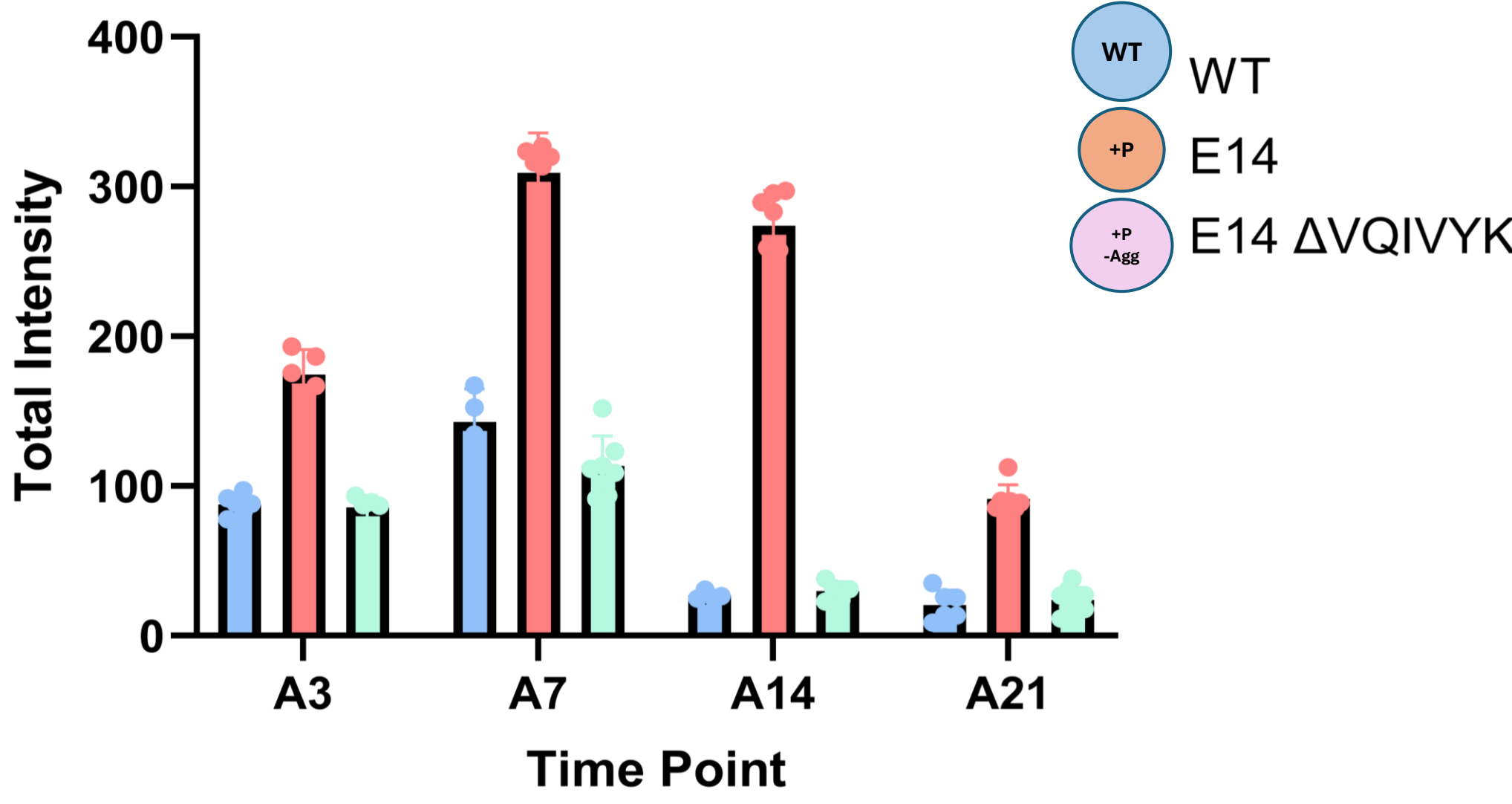
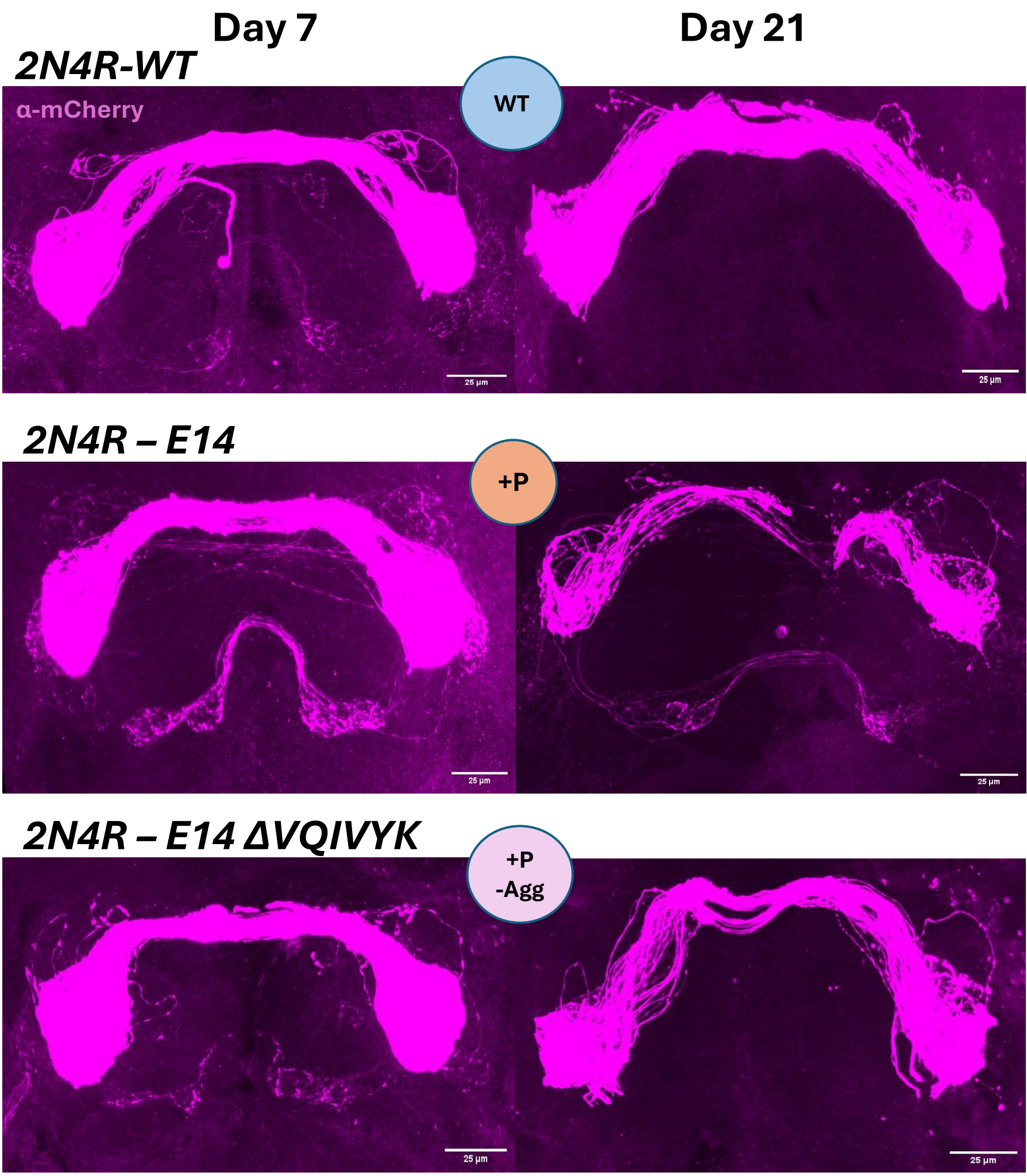


2. Phosphomimetic tau (including p217, p181 and p231) resists turnover but recovers in the absence of VQIVYK aggregation motif



Or47b Circuit expresses GFP for 14d; driver in region near VM4 is **on only** for 7d. This provides an effective **start-stop system** for protein expression. Absence of GFP signal at day 14 is **assumed** to be due to **turnover** of expressed GFP.

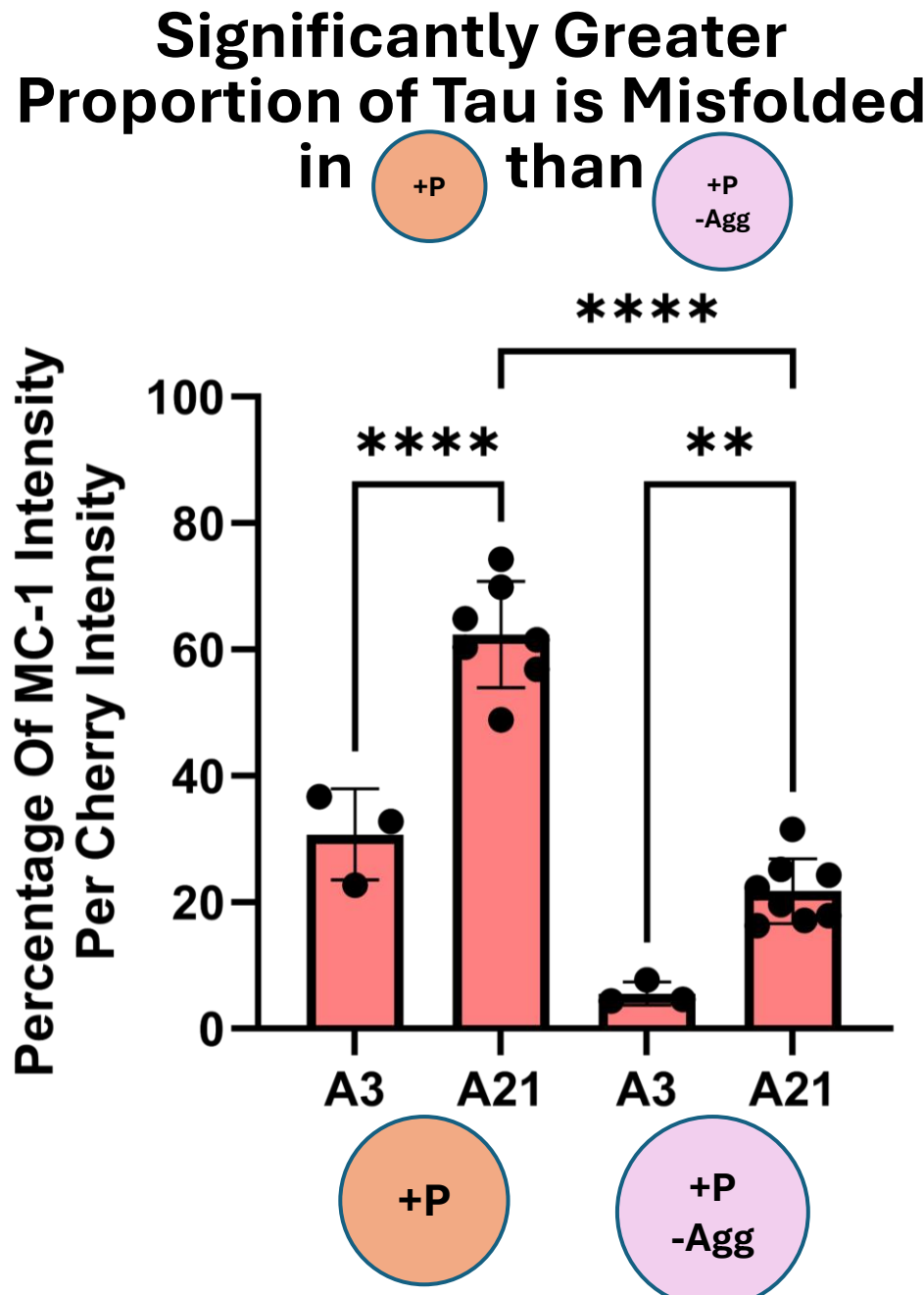
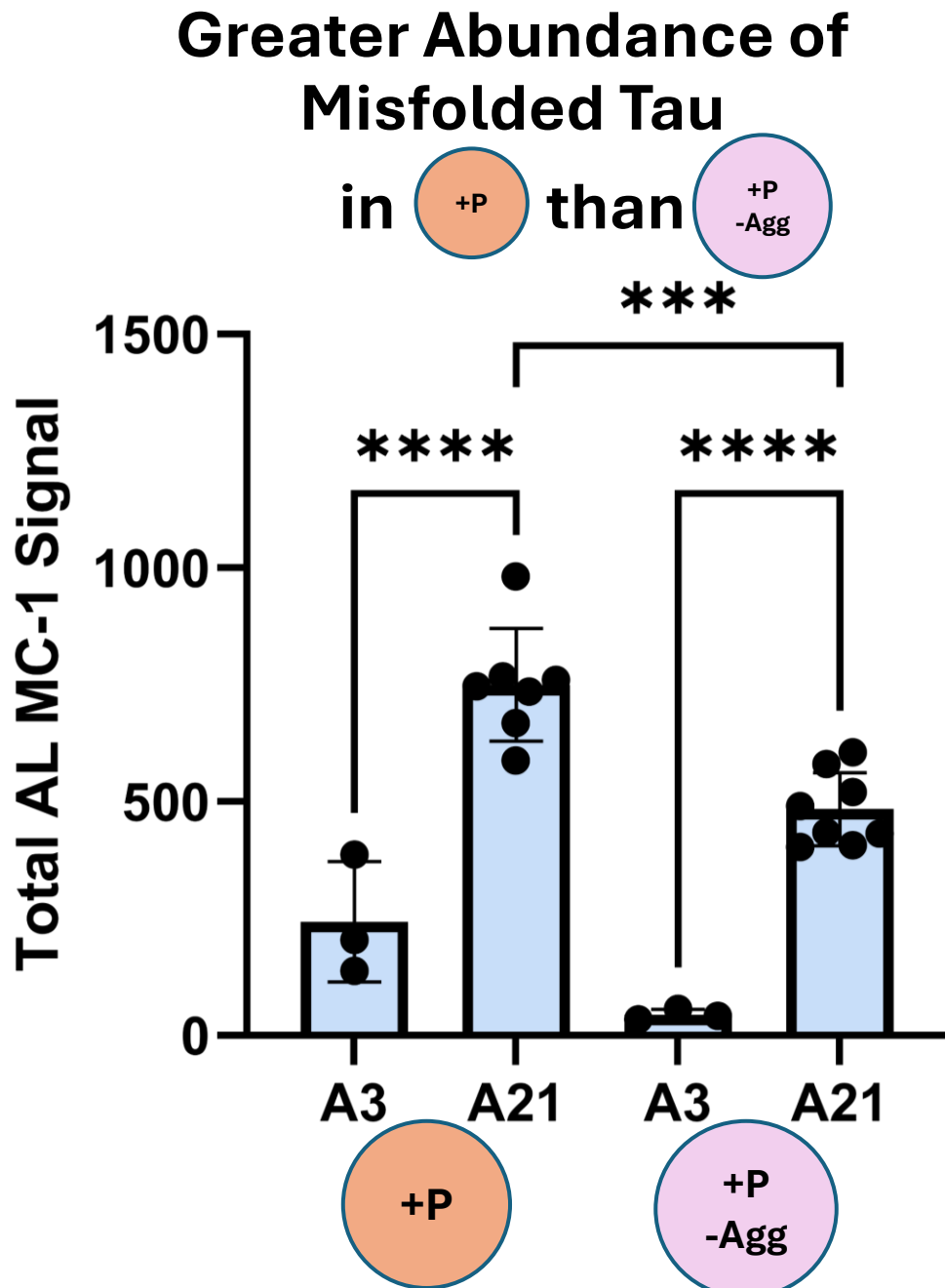
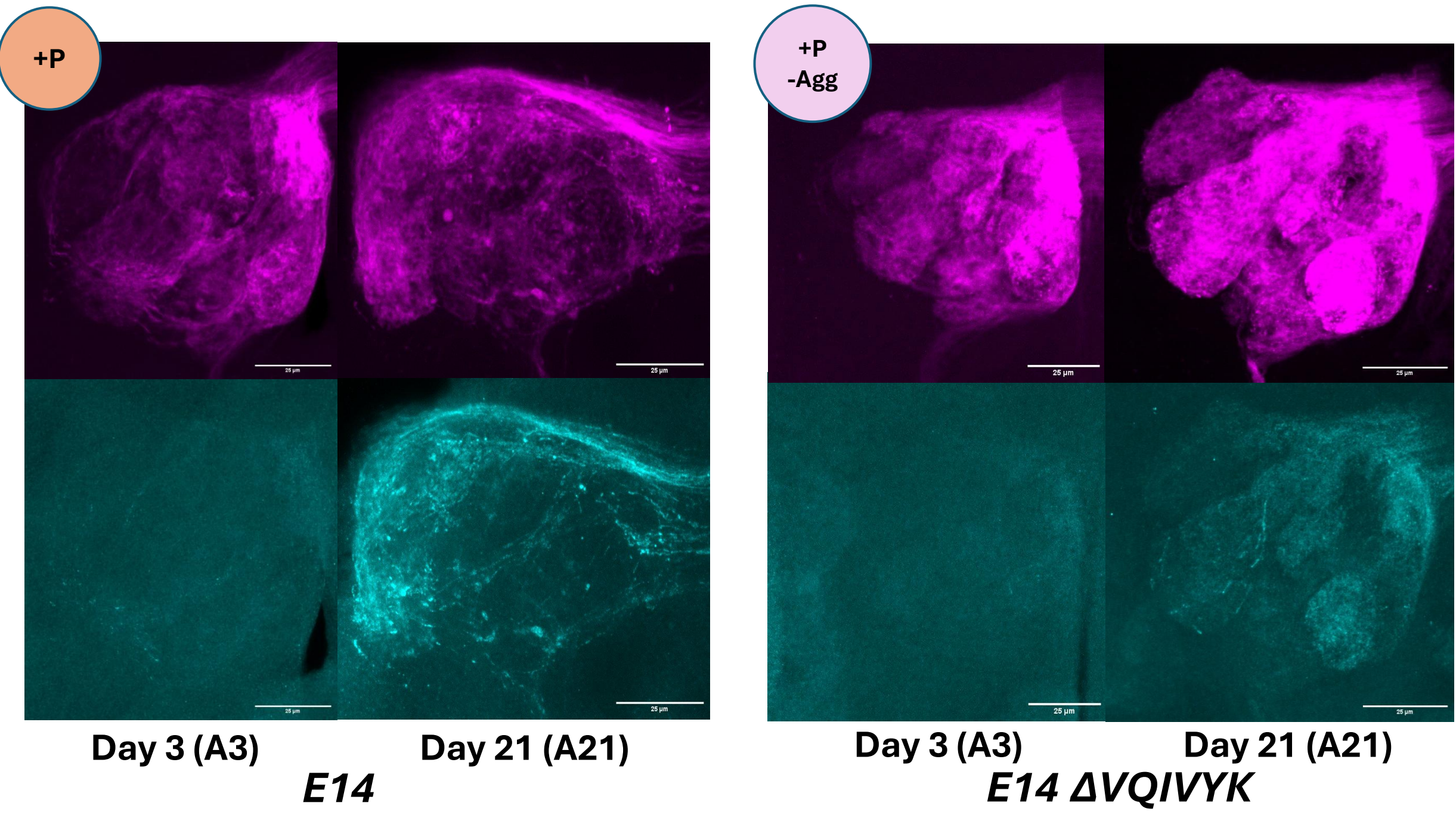
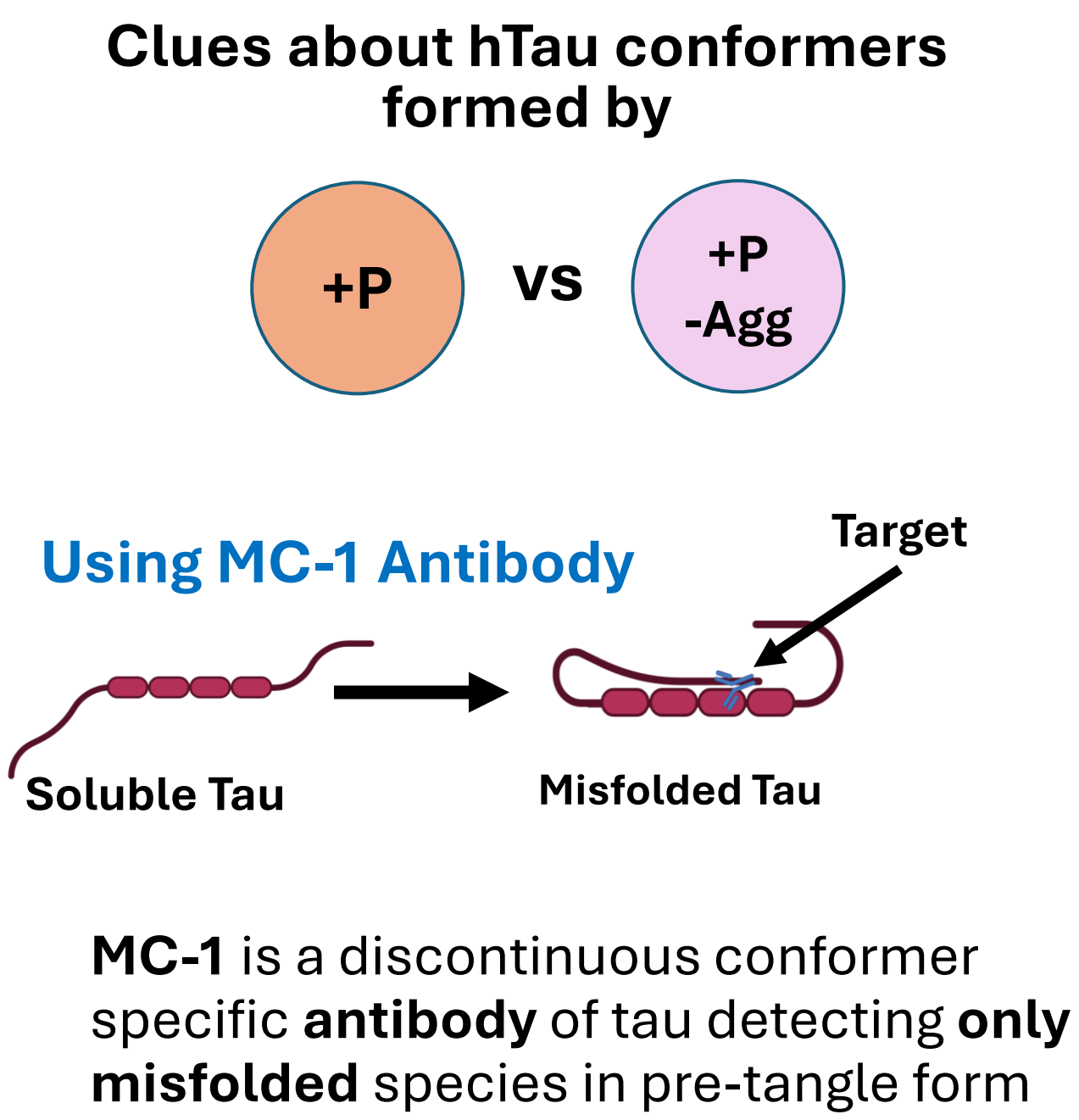
Are tau variants also turned over when driver switches off?



- WT tau is almost fully cleared by 2 weeks
- Phosphomimetic Tau (E14) is cleared much slower than WT, persisting beyond 3 weeks
- Removal of VQIVYK Aggregation motif returns E14 Tau's resistance against turnover to WT levels

Hyperphosphorylated Tau's **resistance to clearance** is **abolished** when its **aggregation capacity** is **removed**

3. Does the phospho-mimetic tau have a different conformation? Is this why it is resistant to turnover?



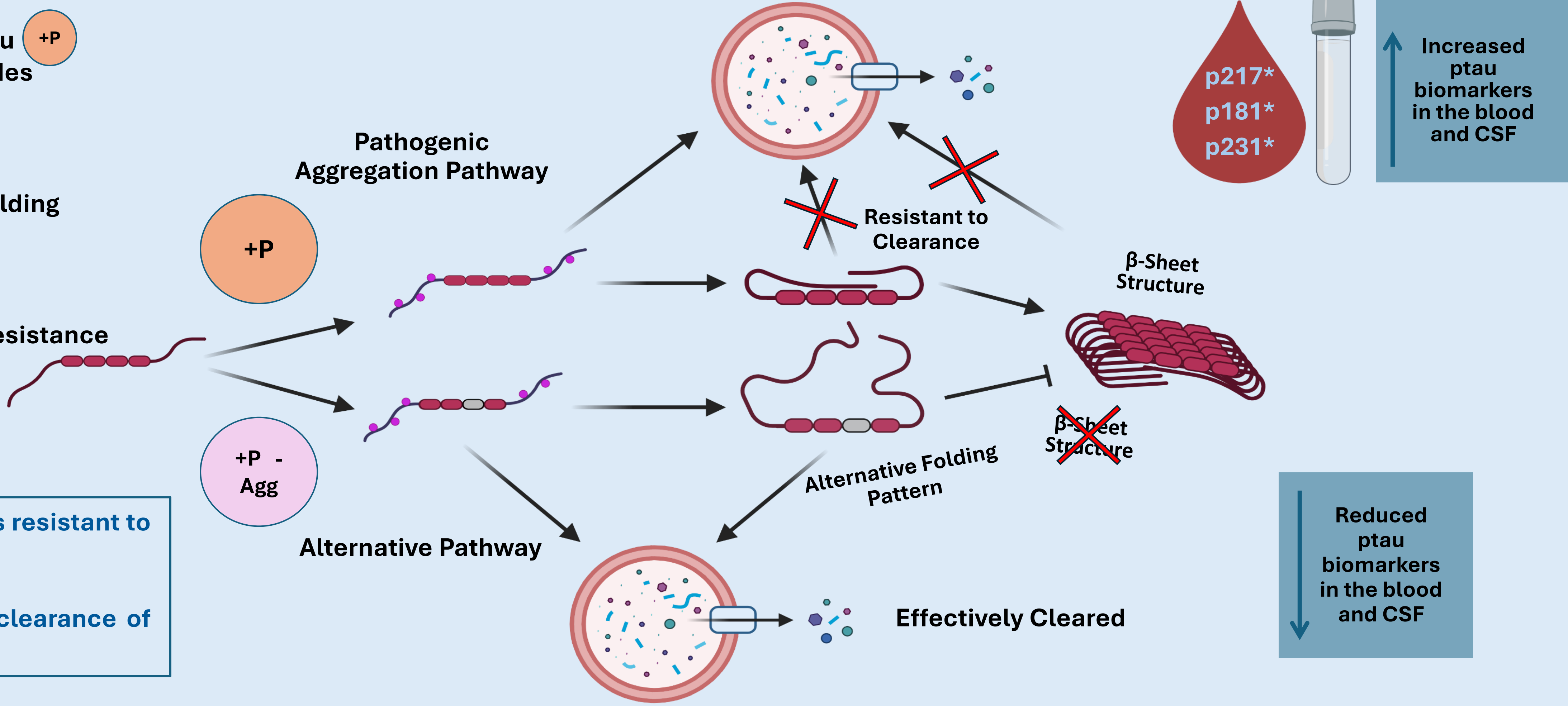
4. Delineating the effect of VQIVYK deletion in reversing the pathogenicity of hyperphosphorylated tau conformers

Our study, so far, suggests that the **phosphomimetic Tau** **acquires a misfolded pathogenic conformer** that **evades clearance and resists degradation**.

Deletion of VQIVYK motif in **+P - Agg** **reverts this misfolding phenotype** despite the hyperphosphorylation.

The resulting Tau conformers alleviate the turnover resistance upon hyperphosphorylation and are more visible to proteostasis surveillance.

Are pTau biomarkers picking up pathogenic Tau that is resistant to turnover?
Is VQIVYK domain a potential target for promoting clearance of pathological Tau?



FUTURE DIRECTIONS:

- Create transgenic lines mimicking phosphorylation at 181, 217 or 231 phospho-epitopes alone or in combination to assess impact on Tau turnover;
- Utilise *Drosophila*'s wide genetic toolbox to uncover cellular modulators of tau turnover