

MLC1 is a plasma membrane protein mainly expressed in astrocytes. Genetic mutations in this protein can lead to megalencephalic leukoencephalopathy with subcortical cysts (MLC). However, the exact function and structure of the MLC1 protein, as well as the pathological mechanism, remain poorly understood. Our previous studies revealed that heterologously expressed wild-type MLC1 primarily localizes at the plasma membrane and induces significant changes in cellular morphology and motility. In contrast, pathogenic mutations such as S280L and P92S do not produce these effects. Additionally, we have determined the transmembrane topology and oligomeric assembly of MLC1 using systematic immunofluorescence staining, chemical crosslinking, negative-staining electron microscopy, and single-molecule photobleaching techniques.

To gain further insight into the structure and function of MLC1, we have attempted to characterize the structure and cellular function of MLC1. Preliminary cryo-EM data suggested that the MLC1 protein indeed forms a trimeric assembly. However, the current resolution is not sufficient to assign the side chains due to the orientation bias of MLC1 proteins on the EM grid. We are continuing efforts for creating various orientations of the MLC1 protein to resolve the cryo-EM structure. Interestingly, we found that wild-type and pathogenic mutants have mutually exclusive phosphorylation patterns. A wild-type mimicking mutation in the S280L mutant restored plasma membrane targeting and induced morphological change. Currently, we are screening candidate kinases responsible for these distinct phosphorylation patterns.

## Megalencephalic Leukoencephalopathy with subcortical Cysts

- Autosomal recessive
- Macrocephaly, ataxia, spasticity, seizures, delay in motor development and mild intellectual disability
- Incidence: One in 500,000<sup>UK</sup> ~ 1,000,000<sup>Japan</sup>

| Clinical phenotype | Name  | Phenotype MIM # | % of patient                                   | Type of mutation             | Target of mutation |
|--------------------|-------|-----------------|------------------------------------------------|------------------------------|--------------------|
| Classic MLC        | MLC1  | 604004          | ~75                                            | biallelic loss-of-function   | MLC1               |
|                    | MLC2A | 613925          | ~7                                             | biallelic loss-of-function   | GLIALCAM           |
|                    | MLC3  | 620447          | only in 3 unrelated patients                   | dominant mutations in GPRC5B | GPRC5B             |
| Remitting MLC      | MLC2B | 613926          | ~16                                            | dominant-negative mutations  | GLIALCAM           |
|                    | MLC4  | 620448          | one recessive mutation limited to two siblings | recessive mutation           | AQP4               |

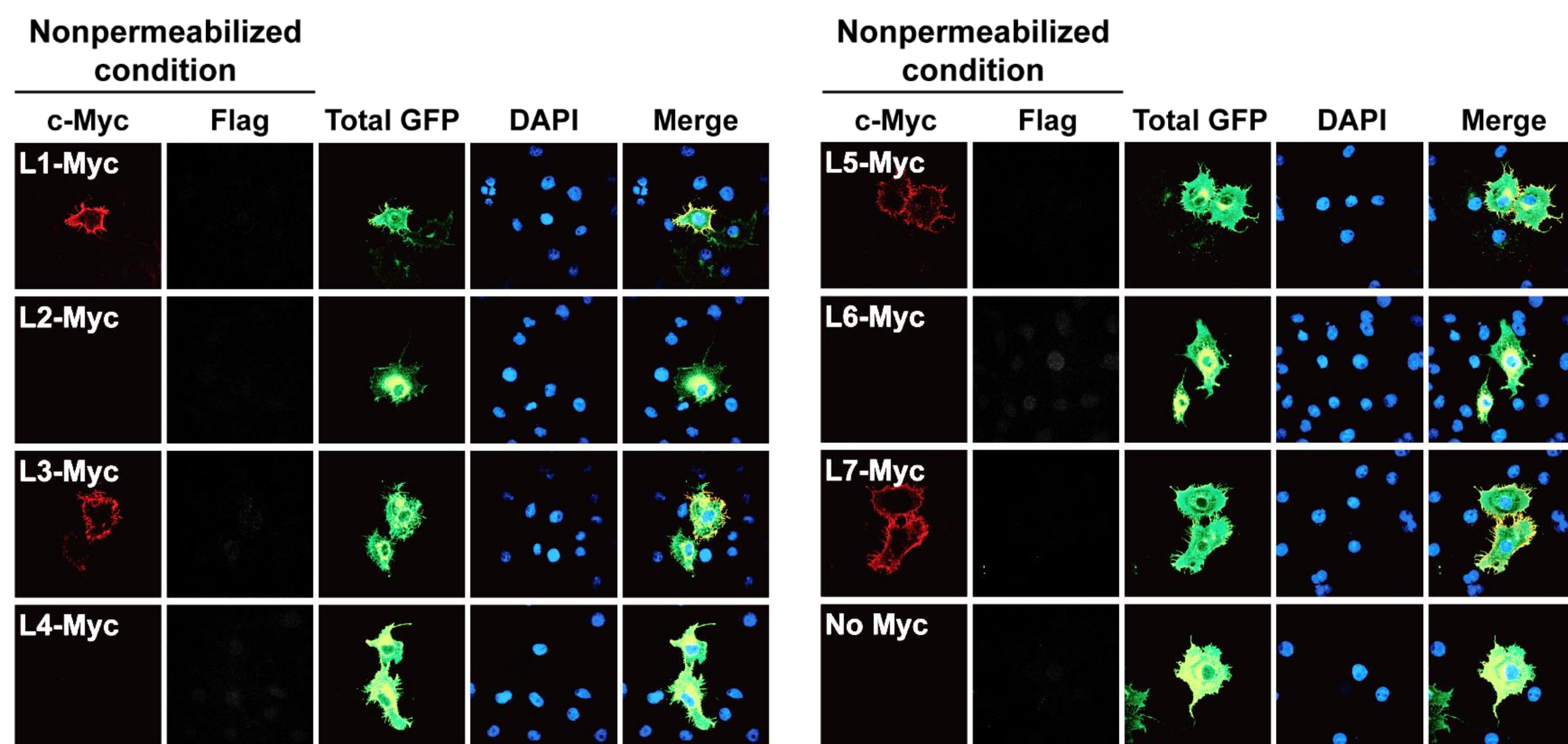
<sup>1</sup>Mutations in noncoding elements (intronic deletion and mutations upstream of the transcription start site) → diminish MLC1 expression; pathological impact is still uncertain

<sup>2</sup>Splicing variants identified in one patient led to decreased MLC1 levels and an eventually remitting phenotype

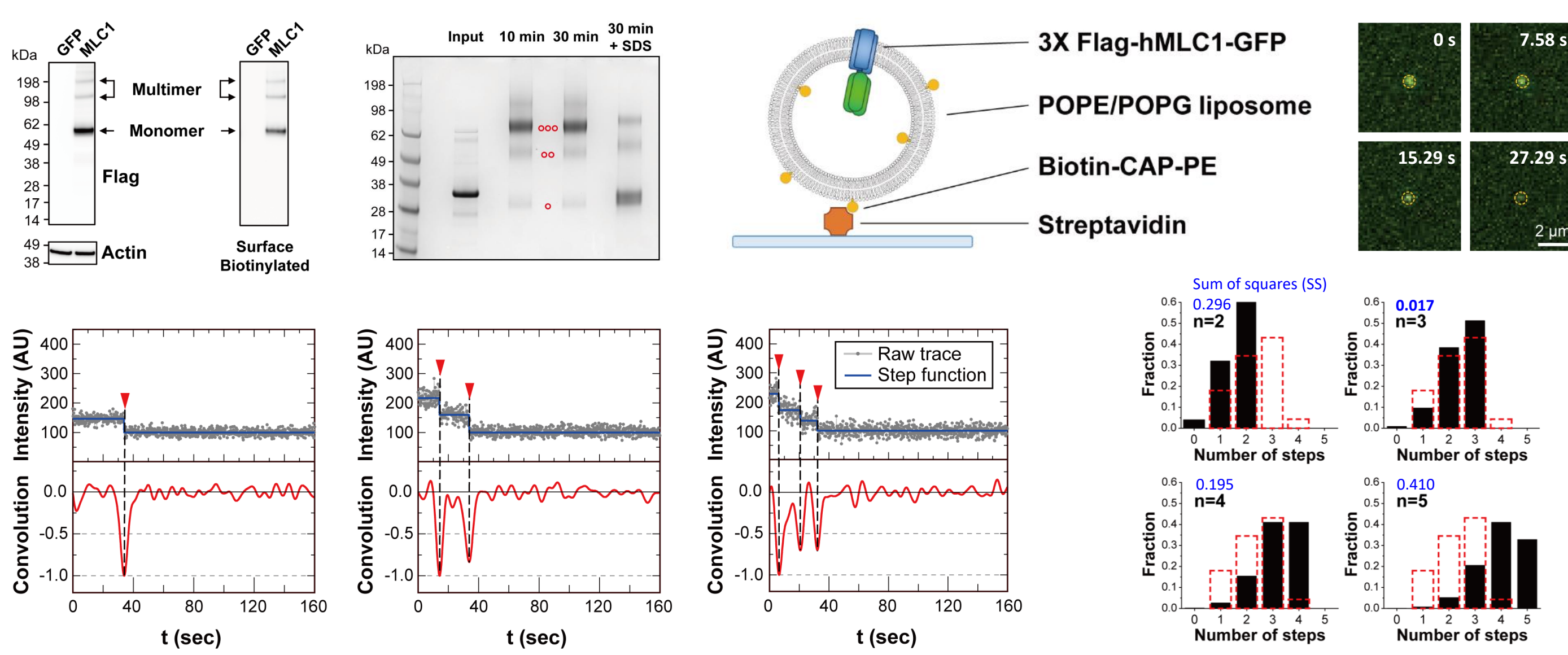
Brao A et al., *Mol Ther*, 2025

## Figure 1. Characterization of the MLC1 protein.

### A. Determination of the membrane topology.

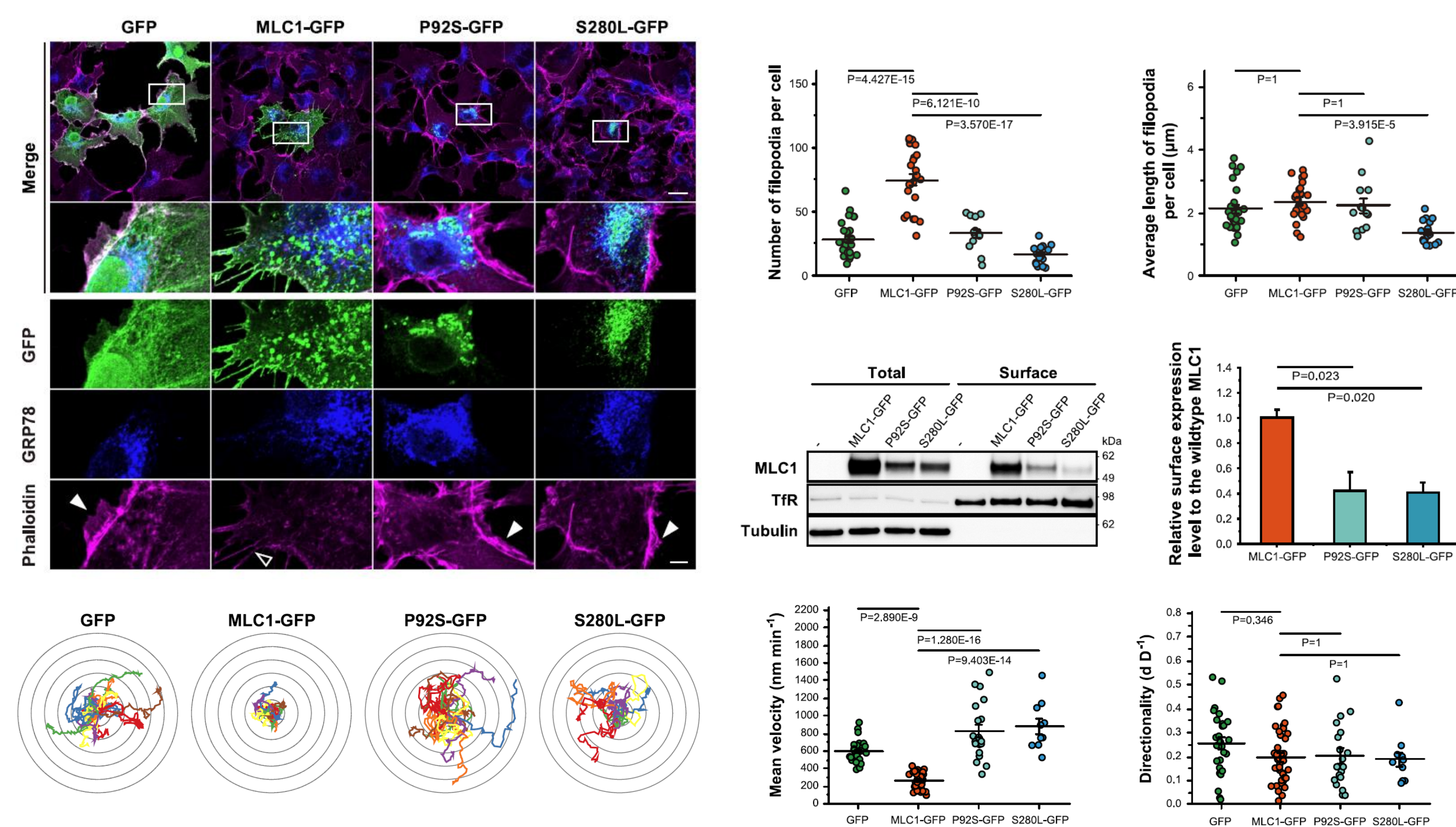


### B. Counting the number of the protomers.



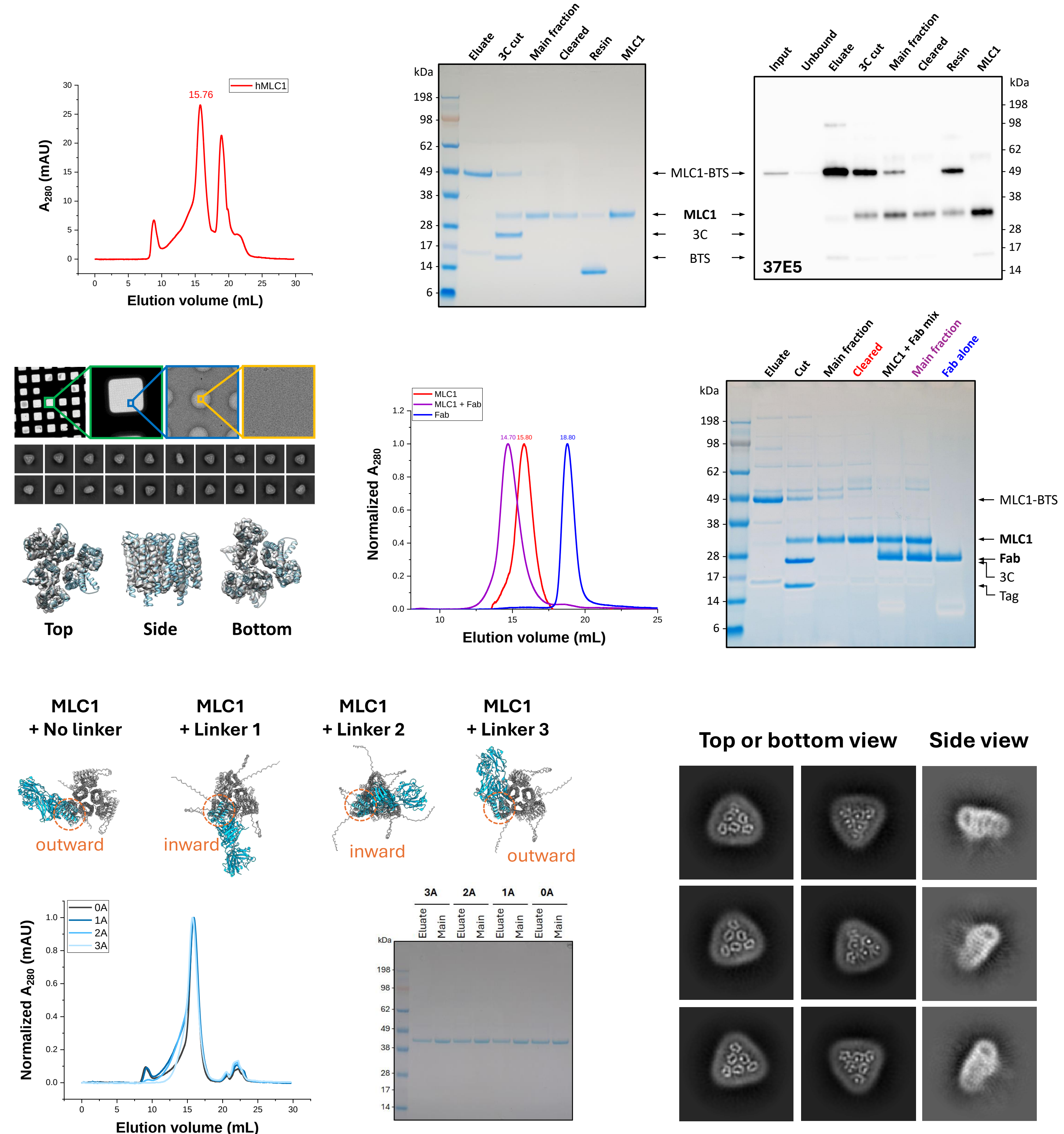
Hwang J et al., *Open Biol*, 2021

## Figure 3. MLC1 regulates cellular morphology and motility.



Hwang J et al., *Mol Brain*, 2019

## Figure 2. Structural analysis of the MLC1 protein.



## Figure 4. Phosphorylation-dependent subcellular localization of MLC1.

