# Science Advances

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## Supplementary Materials for

### Peripheral myelin protein 22 alters membrane architecture

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**The PDF file includes:**

- Legends for movies S1 to S3
- fig. S1. Representative examples of objects observed in reconstitution experiments by negative stain.
- fig. S2. Examples of interperiod repeat distance measurements taken for MLAs and MLVs imaged with cryo-EM.
- fig. S3. MLV vesicles examined by cryo-ET.
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- table S1. Total counts, percentages, and SDs of WT PMP22 reconstitutions at the LPR of 1.0.
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- table S9. Total counts and normalized values from images of reconstitutions of WT and the *TrJ* mutant PMP22 construct.

**Other Supplementary Material for this manuscript includes the following:**
(available at advances.sciencemag.org/cgi/content/full/3/7/e1700220/DC1)

- movie S1 (.mov format). Cryo-ET of an MLA showing that it is composed of compressed wrapped vesicles (corresponding to Fig. 3B).
- movie S2 (.mov format). Cryo-ET of an MLA showing that it is composed of compressed wrapped vesicles (corresponding to Fig. 3C).
- movie S3 (.mov format). Cryo-ET of an MLV showing that it is composed of nested vesicles rather than compressed wrapped vesicles seen in MLAs (corresponding to fig. S3B).

**movie S1. Cryo-ET of an MLA showing that it is composed of compressed wrapped vesicles (corresponding to Fig. 3B).**

**movie S2. Cryo-ET of an MLA showing that it is composed of compressed wrapped vesicles (corresponding to Fig. 3C).**

**movie S3. Cryo-ET of an MLV showing that it is composed of nested vesicles rather than compressed wrapped vesicles seen in MLAs (corresponding to fig. S3B).**
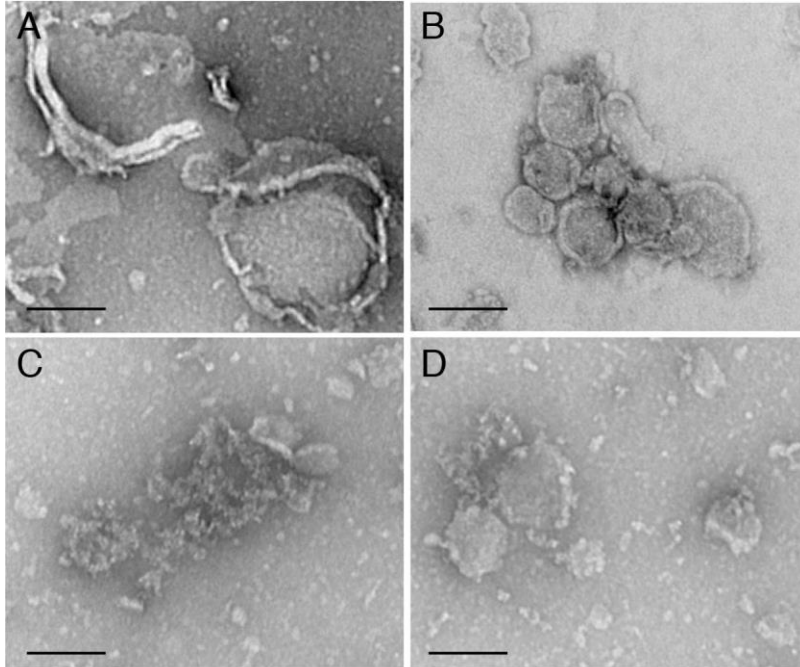
**fig. S1. Representative examples of objects observed in reconstitution experiments by negative stain.** (**A**) Example of disordered or "frayed" MLAs. (**B**) Example of clustered vesicles. (**C**) Example of aggregation. (**D**) Example of vesicles. Scale bars, 100 nm.
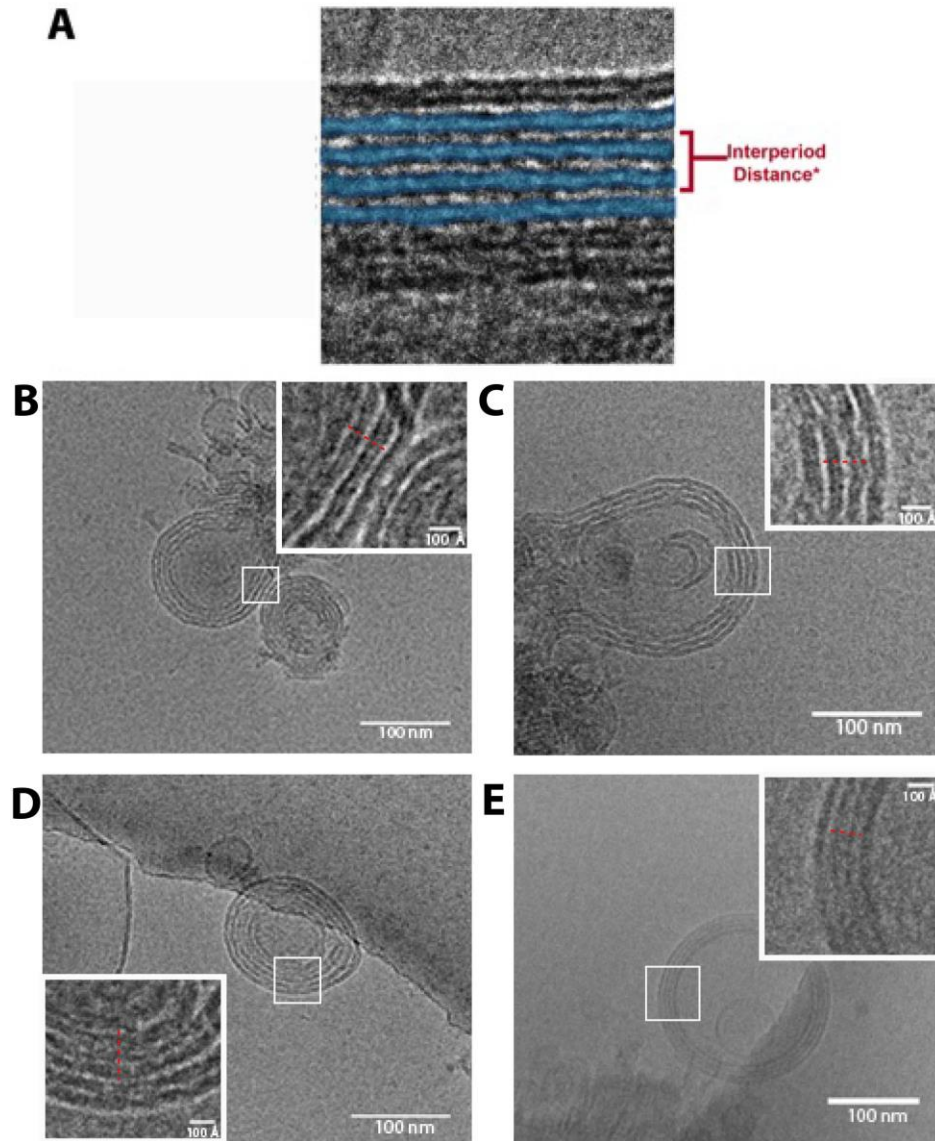
**fig. S2. Examples of interperiod repeat distance measurements taken for MLAs and MLVs imaged with cryo-EM.** (**A**) Cross section of bilayers with blue overlay indicating the delineation of the bilayer. The inteperiod repeat distance, analogous to that of myelin is shown in red. (**B**) and (**C**) Representative images of interperiod distances measured in MLAs. (**D**) and (**E**) Representative images of interperiod distances measured in MLVs. Inserts in (B), (C), (D), and (E) show a phase contrasted image of the white boxed area with the interperiod distance represented by a red line. Scale bars are as indicated.
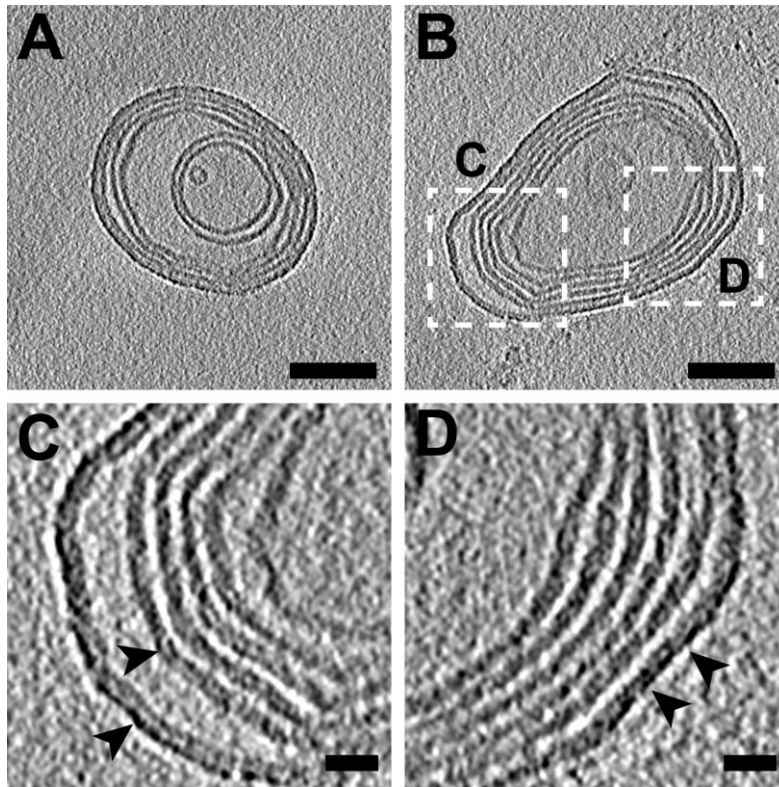
**fig. S3. MLV vesicles examined by cryo-ET.** (**A** to **B**) Two representative tomographic slices (0.588 nm) of MLV vesicles. (**C** to **D**) Zoom-in view (3X) of boxed regions from B showing detailed views of the lipid bilayer indicated by the arrowheads. Scale bars are 100 nm (A and B) and 20 nm (C and D).

**fig. S4. Schematic showing the organization of PMP22.** Sequence and topology diagram of PMP22. Blue residues are conserved residues from the claudin motif. The green residue is a conserved tryptophan in ECL2 in PMP22s. Cysteine residues are highlighted by a red ring.

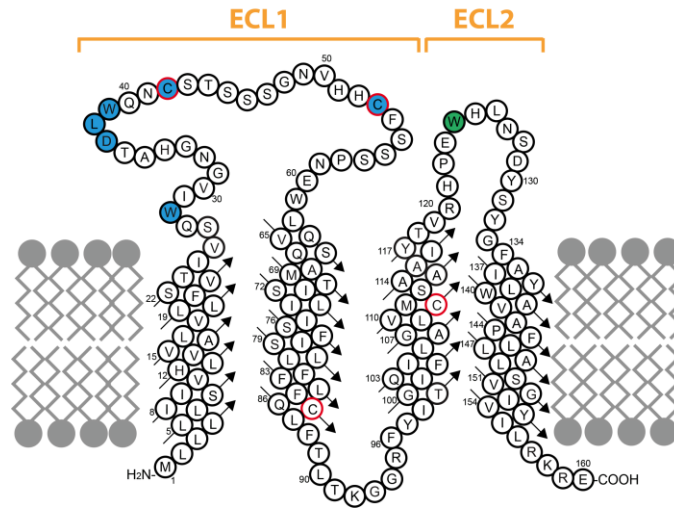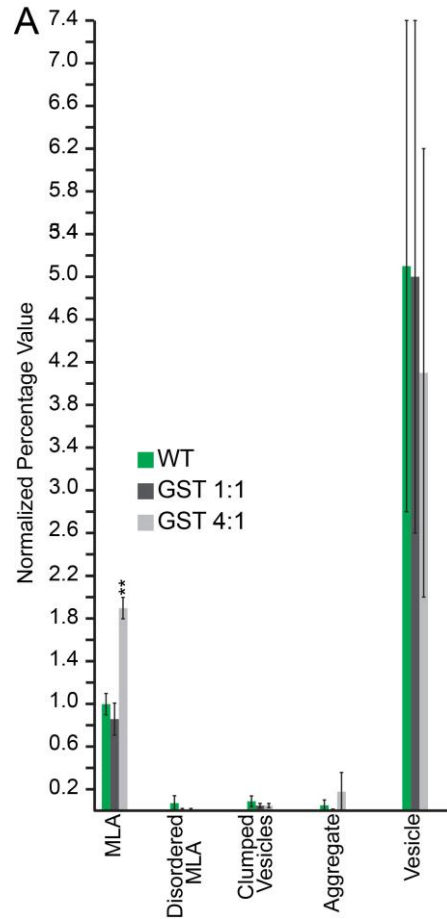**fig. S5. Addition of GST does not reduce MLA formation.** Quantification of the relative percentage of MLAs present in a series of negative stain EM images of PMP22 reconstitutions of WT PMP22 only and WT PMP22 incubated with GST. *All individual object counts were converted to percentage of total counts for a particular sample and were normalized to the percentage of total counts represented by MLAs in the WT PMP22 control. All values were normalized to the percentage of WT control MLAs, which was set to 1.0. Green = WT control, Black = GST + WT PMP22 (1:1 molar ratio), and Grey = GST peptide + WT PMP22 (4:1 molar ratio). Error bars represent S.E.M between biological replicates. ** = p<0.01.

**table S1. Total counts, percentages, and SDs of WT PMP22 reconstitutions at the LPR of 1.0.**

| | Experiment 1 | | Experiment 2 | | Experiment 3 | | Experiment 4 | | Experiment 5 | | Experiment 6 | | Weighted Average % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Raw counts | % | Raw counts | % | Raw counts | % | Raw counts | % | Raw counts | % | Raw counts | % | |
| MLA | 67 | 11.1 | 83 | 15.1 | 90 | 15.3 | 165 | 21.4 | 125 | 24.3 | 84 | 13.5 | 16.8 +/- 5.0 |
| Disordered MLA | 9 | 1.5 | 9 | 1.6 | 12 | 2.0 | 21 | 2.7 | 29 | 5.6 | 20 | 3.2 | 2.7 +/- 1.5 |
| Clumped vesicles | 23 | 3.8 | 15 | 2.7 | 5 | 0.9 | 7 | 0.9 | 21 | 4.1 | 28 | 4.5 | 2.7 +/- 1.6 |
| Sheet | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0.3 | 0.1 +/- 0.1 |
| Aggregation | 1 | 0.2 | 0 | 0 | 0 | 0 | 19 | 2.5 | 1 | 0.2 | 0 | 0 | 0.1 +/- 0.1 |
| Vesicle | 505 | 83.5 | 444 | 80.6 | 481 | 81.8 | 558 | 72.5 | 339 | 65.8 | 486 | 78.4 | 77. 1 +/- 6.7 |
| Total | 605 | 100 | 551 | 100 | 588 | 100 | 770 | 100 | 515 | 100 | 620 | 100 | 100 |

**table S2. Total counts and normalized values from images of PMP22 reconstitutions compared to protein-free and KCNQ1 potassium channel voltage sensor domain (Q1-VSD) controls.**

| | WT PMP22 | | Lipid Only Control | | multilamellar vesicles | | Q1-VSD | |
|---|---|---|---|---|---|---|---|---|
| | Raw counts | Normalized value^ | Raw counts | Normalized value^ | Raw counts | Normalized value^ | Raw counts | Normalized value^ |
| MLA | 67 | 1.0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Disordered MLA | 9 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Clumped vesicles | 23 | 0.3 | 42 | 0.6 | 3 | 0.05 | 122 | 1.9 |
| Sheet | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Aggregation | 1 | 0.01 | 0 | 0 | 0 | 0 | 26 | 0.41 |
| Vesicle | 505 | 7.5 | 614 | 8.5 | 589 | 9.0 | 422 | 6.7 |
| Total | 605 | | 656 | | 592 | | 570 | |

^Normalized values are normalized to MLA value from WT-control (WT PMP22 LPR 1.0).

**table S3. Total counts and normalized values from images of WT PMP22 reconstitutions at different LPRs.**

| | WT LPR 0.5 | | WT LPR 1.0 | | WT LPR 2.0 | | WT LPR 4.0 | | WT LPR 10.0 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Raw counts* | Normalized value^ | Raw counts* | Normalized value^ | Raw counts* | Normalized value^ | Raw counts* | Normalized value^ | Raw counts* | Normalized value^ |
| MLA | 54 | 0.39±0.13 | 257 | 1.0±0.1 | 93 | 0.37±0.12 | 18 | 0.06±0.02 | 1 | 0.01±0.01 |
| Disordered MLA | 133 | 0.65±0.22 | 21 | 0.04±0.04 | 24 | 0.11±0.04 | 51 | 0.22±0.07 | 5 | 0.01±0.01 |
| Clumped vesicles | 25 | 0.10±0.01 | 11 | 0.04±0 | 7 | 0.03±0.01 | 23 | 0.08±0.06 | 7 | 0.05±0.05 |
| Sheet | 0 | 0±0 | 0 | 0±0 | 0 | 0±0.0 | 0 | 0±0 | 0 | 0±0 |
| Aggregation | 9 | 0.04±0.02 | 19 | 0.04±0.04 | 35 | 0.19±0.15 | 18 | 0.08±0.06 | 10 | 0.04±0.03 |
| Vesicle | 506 | 2.9±0.2 | 818 | 3.0±0.2 | 785 | 3.5±0.2 | 804 | 3.7±1.4 | 852 | 4.0±0.2 |
| Total | 727 | | 1126 | | 944 | | 914 | | 875 | |

*Raw counts are pooled from three separate biological replicates.
^Normalized values are normalized to MLA value from WT-control (WT PMP22 LPR 1.0) in each biological replicate. +/- represents the S.E.M between replicates.

**table S4. Total counts and normalized values from images of reconstitutions of WT and Cys-less PMP22.**

| | WT PMP22 | | Cys-less PMP22 | |
|---|---|---|---|---|
| | Raw counts | Normalized value^ | Raw counts | Normalized value^ |
| MLA | 90 | 1.0 | 89 | 1.0 |
| Disordered MLA | 12 | 0.1 | 61 | 0.7 |
| Clumped vesicles | 5 | 0.06 | 11 | 0.1 |
| Sheet | 0 | 0 | 0 | 0 |
| Aggregation | 0 | 0 | 76 | 0.9 |
| Vesicle | 481 | 5.3 | 350 | 3.9 |
| Total | 588 | | 587 | |

^Normalized values are normalized to MLA value from WT-control (WT PMP22 LPR 1.0).

**table S5. Total counts and normalized values from images of PMP22 reconstitutions containing GST-ECL1 and GST-ECL2.**

| | WT-only control | | ECL1 1:1 ratio[#] | | ECL1 4:1 ratio[#] | | ECL2 1:1 ratio[#] | | ECL2 4:1 ratio[#] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Raw counts* | Normalized value^ | Raw counts* | Normalized value^ | Raw counts * | Normalized value^ | Raw counts* | Normalized value^ | Raw counts* | Normalized value^ |
| MLA | 220 | 1.0±0.1 | 158 | 0.99±0.25 | 105 | 0.59±0.15 | 62 | 0.26±0.10 | 5 | 0.04±0.03 |
| Disordered MLA | 30 | 0.14±0.07 | 12 | 0.09±0.07 | 41 | 0.22±0.03 | 33 | 0.10±.06 | 21 | 0.08±0.06 |
| Clumped vesicles | 32 | 0.13±0.05 | 28 | 0.15±0.04 | 44 | 0.21±0.08 | 18 | 0.07±0.01 | 23 | 0.14±0.07 |
| Sheet | 0 | 0±0 | 5 | 0.01±0.01 | 1 | 0±0 | 13 | 0.03±0.03 | 4 | 0.01±0.01 |
| Aggregation | 8 | 0.05±0.05 | 44 | 0.13±0.07 | 116 | 0.42±0.23 | 218 | 0.61±0.51 | 288 | 2.84±2.3 |
| Vesicle | 940 | 5.0±2.3 | 1015 | 5.0±2.2 | 629 | 4.9±2.5 | 845 | 5.3±2.6 | 468 | 3.3±0.3 |
| Total | 1230 | | 1262 | | 936 | | 1189 | | 809 | |

[#]molar ratio of GST-ECL1 or GST-ECL2 to PMP22
*Raw counts are pooled from three separate biological replicates.
^Normalized values are normalized to MLA value from WT-control (WT PMP22 LPR 1.0) in each biological replicate. +/- represent the S.E.M between replicates.

**table S6. Total counts and normalized values from images of PMP22 reconstitutions containing WT or GST.**

| | WT-only control | | GST 1:1 ratio[#] | | GST 4:1 ratio[#] | |
|---|---|---|---|---|---|---|
| | Raw counts* | Normalized value^ | Raw counts* | Normalized value^ | Raw counts* | Normalized value^ |
| MLA | 95 | 1±0.1 | 117 | 0.86±0.15 | 161 | 1.9±0.1 |
| Disordered MLA | 1 | 0.07±0.07 | 1 | 0.01±0.01 | 1 | 0.01±0.01 |
| Clumped vesicles | 11 | 0.09±0.05 | 7 | 0.05±0.02 | 4 | 0.05±0.02 |
| Sheet | 0 | 0±0 | 0 | 0±0 | 0 | 0±0 |
| Aggregation | 17 | 0.05±0.05 | 1 | 0.01±0.01 | 13 | 0.18±0.18 |
| Vesicle | 601 | 5.1±2.3 | 671 | 5.0±2.4 | 316 | 4.1±2.1 |
| Total | 715 | | 841 | | 495 | |

[#]molar ratio of GST to PMP22
*Raw counts are pooled from three separate biological replicates.
^Normalized values are normalized to MLA value from WT-control (WT PMP22 LPR 1.0) in each biological replicate. +/- represents S.E.M between replicates.

**table S7. Total counts and normalized values from images of reconstitutions containing only lipids and GST, GST-ECL1, or GST-ECL2.**

| | GST LPR 1.0 | | GST-ECL1 LPR 1.0 | | GST ECL2 LPR 1.0 | |
|---|---|---|---|---|---|---|
| | Raw counts | Normalized value^ | Raw counts | Normalized value^ | Raw counts | Normalized value^ |
| MLA | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| Disordered MLA | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| Clumped vesicles | 27 | 0.5 | 2 | 0.06 | 6 | 0.2 |
| Sheet | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| Aggregation | 12 | 0.3 | 3 | 0.09 | 3 | 0.1 |
| Vesicle | 195 | 3.2 | 128 | 3.7 | 93 | 3.5 |
| Total | 234 | | 133 | | 102 | |

^Normalized values are normalized to MLA value from WT-control (WT PMP22 LPR 1.0).

**table S8. Total counts and normalized values from images of reconstitutions of WT PMP22 compared to PMP22 constructs with mutations in conserved residues of ECL1 and ECL2.**

| | WT PMP22 | | PMP22 D37K | | PMP22 L38A | | PMP22 W39A | | PMP22 W124A | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Raw counts | Normalized value^ | Raw counts | Normalized value^ | Raw counts | Normalized value^ | Raw counts | Normalized value^ | Raw counts | Normalized value^ |
| MLA | 84 | 1.0 | 30 | 0.5 | 37 | 0.5 | 27 | 0.3 | 0 | 0 |
| Disordered MLA | 20 | 0.2 | 82 | 1.4 | 11 | 0.1 | 10 | 0.13 | 0 | 0 |
| Clumped vesicles | 28 | 0.3 | 9 | 0.2 | 3 | 0.04 | 19 | 0.2 | 3 | 0 |
| Sheet | 2 | 0.02 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0.2 |
| Aggregation | 0 | 0 | 5 | 0.08 | 22 | 0.3 | 1 | 0.01 | 484 | 7.1 |
| Vesicle | 486 | 5.8 | 316 | 5.3 | 517 | 6.5 | 522 | 6.7 | 1 | 0.01 |
| Total | 620 | | 442 | | 590 | | 579 | | 501 | |

^Normalized values are normalized to MLA value from WT-control (WT PMP22 LPR 1.0).

**table S9. Total counts and normalized values from images of reconstitutions of WT and the *TrJ* mutant PMP22 construct.**

| | WT | | L16P | |
|---|---|---|---|---|
| | Raw counts | Normalized value^ | Raw counts | Normalize value^ |
| MLA | 83 | 1.0 | 19 | 0.2 |
| Disordered MLA | 9 | 0.10 | 156 | 1.7 |
| Clumped vesicles | 15 | 0.2 | 13 | 0.1 |
| Sheet | 0 | 0 | 10 | 0.1 |
| Aggregation | 0 | 0 | 1 | 0.01 |
| Vesicle | 444 | 5.4 | 416 | 4.5 |
| Total | 551 | | 615 | |

^Normalized values are normalized to MLA value from WT-control (WT PMP22 LPR 1.0).