

## Supplementary Information for:

### Discrimination Between Alpha Synuclein Protein Variants with a Single Nanometer-Scale Pore

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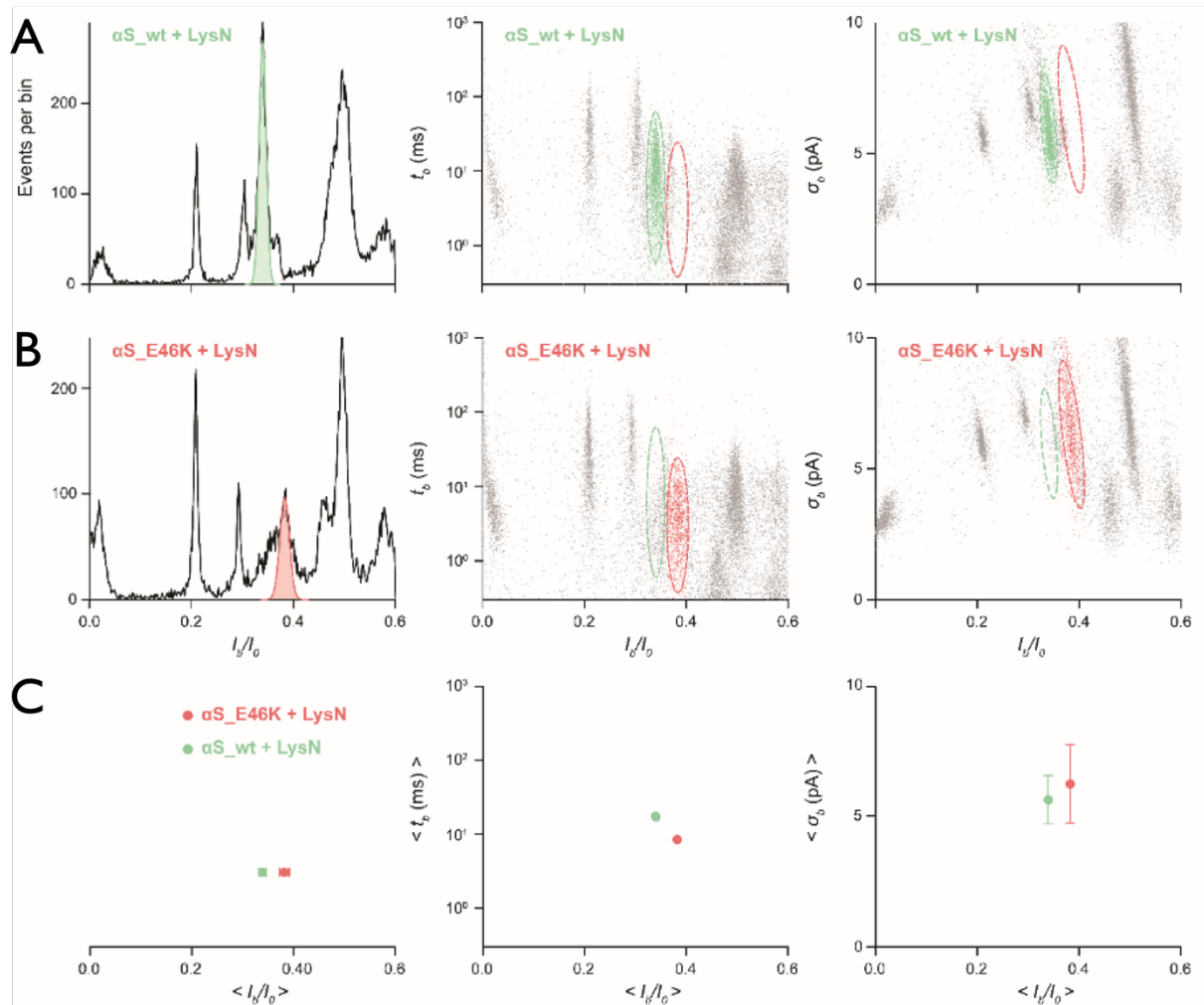
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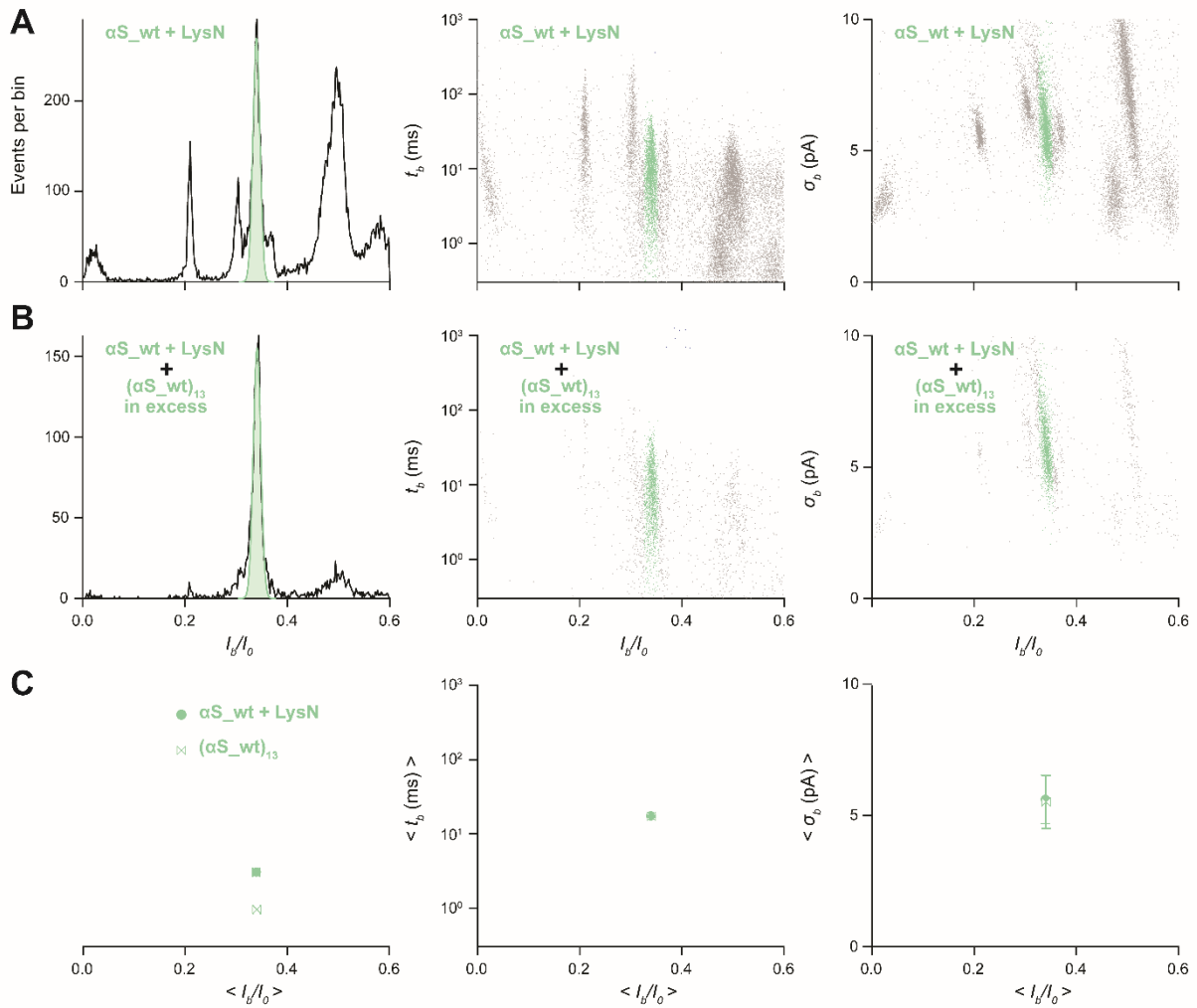
This *Supplementary Information* section provides additional data and analysis for polypeptides derived from LysN proteolysis of the wild type  $\alpha$  Synuclein protein and a mutated version of it (E46K).

### Discrimination between $\alpha$ S\_wt and $\alpha$ S\_E46K proteins

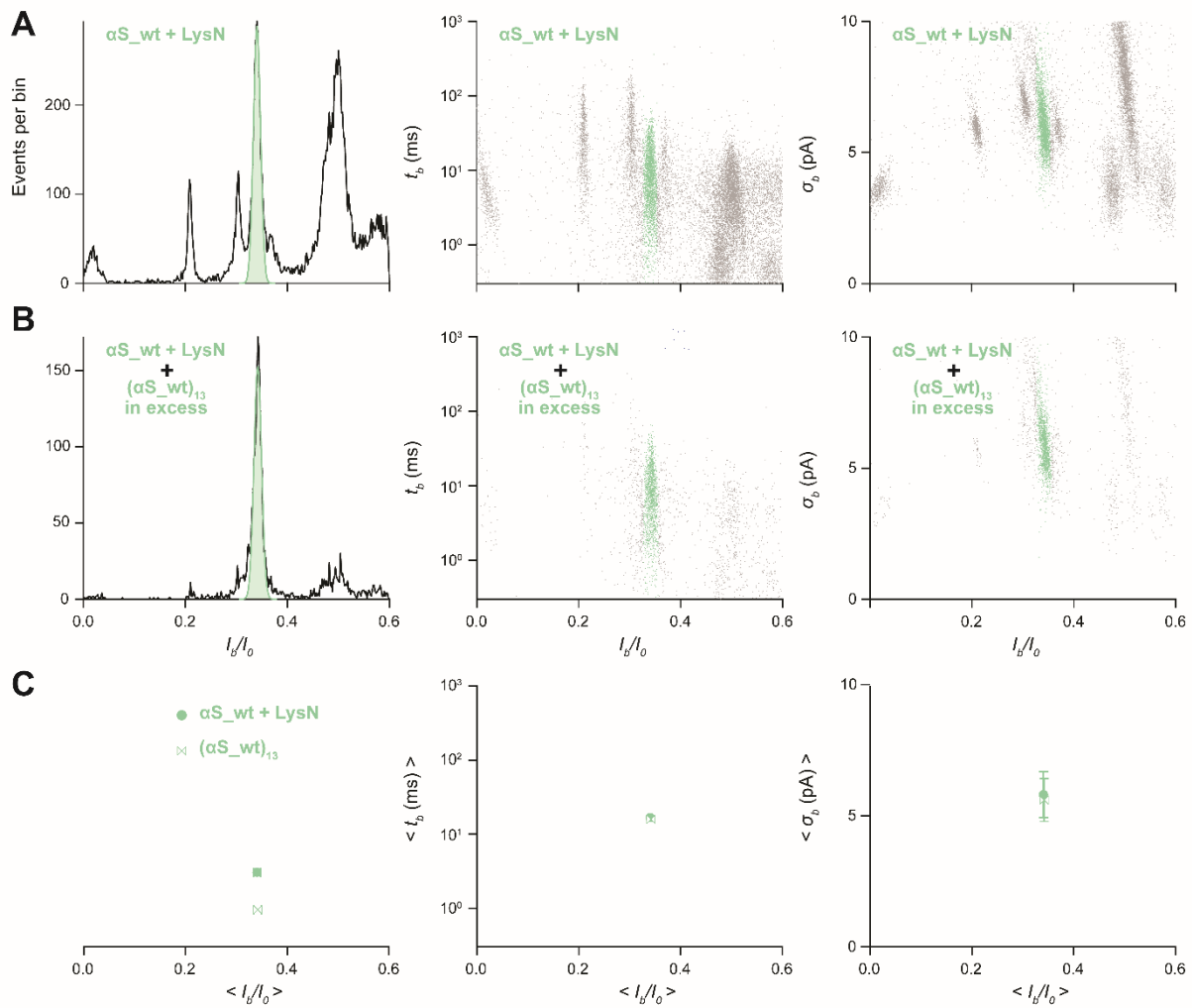


**Figure S1. AEL nanopore-based detection of LysN-induced polypeptide fragments from full length  $\alpha$ S\_wt or its E46K point mutant ( $\alpha$ S\_E46K).** Histograms of ionic current blockade values  $I_b/I_0$  (left), residence time-current blockade value event plots (center) and standard deviation-current blockade value event plots (right) for the complete digestion of **A)** full-length  $\alpha$ S\_wt or **B)** full-length  $\alpha$ S\_E46K. **C)** Plots for the mean values of the ionic current blockade depth ( $\langle I_b/I_0 \rangle$ ), residence time ( $\langle t_b \rangle$ ), and standard deviation of the current values ( $\langle \sigma_b \rangle$ ) of LysN derived polypeptide fragments from full length  $\alpha$ S\_wt (green) or  $\alpha$ S\_E46K (red).

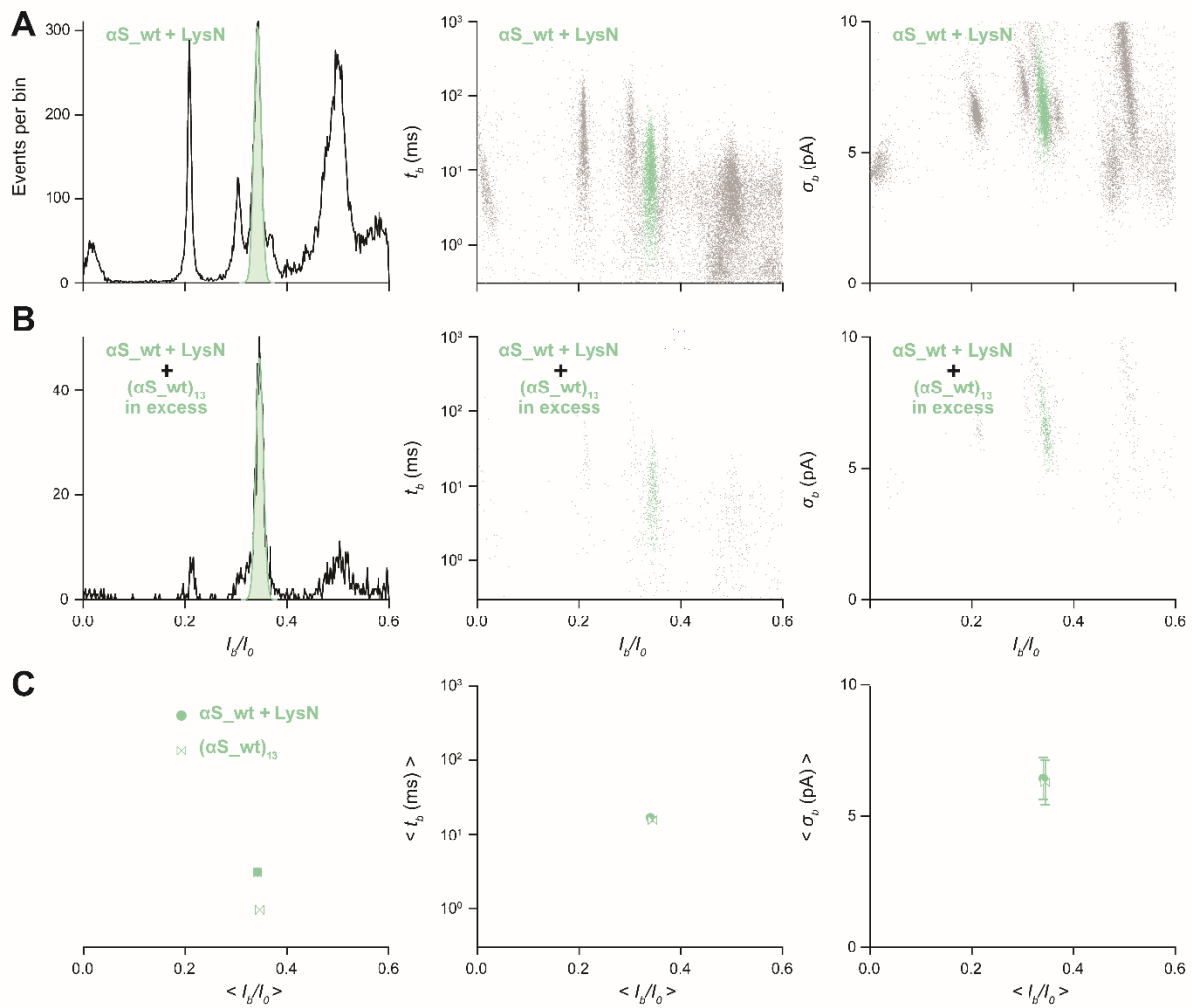
## Reproducibility of $\alpha$ S\_wt protein fragmentation and $(\alpha$ S\_wt)<sub>13</sub> identification



**Figure S2. AeL nanopore-based identification of the  $(\alpha$ S\_wt)<sub>13</sub> fragment from LysN proteolysis of full length wild-type  $\alpha$ -Synuclein, sample N°1.** Histograms of ionic current blockade values (*left*), residence time-current blockade value event plots (*center*) and standard deviation-current blockade value event plots (*right*) for **A**) the complete digestion of full-length  $\alpha$ S\_wt, and **B**) the complete digestion of full-length  $\alpha$ S\_wt plus an excess of synthetic  $(\alpha$ S\_wt)<sub>13</sub> polypeptide. **C**) Plots for the mean values of the ionic current blockade depth ( $\langle I_b/I_0 \rangle$ ), residence time ( $\langle t_b \rangle$ ), and standard deviation of the current values ( $\langle \sigma_b \rangle$ ) in the absence (*green filled circles*) and presence (*green empty hourglasses*) of excess synthetic  $(\alpha$ S\_wt)<sub>13</sub>.

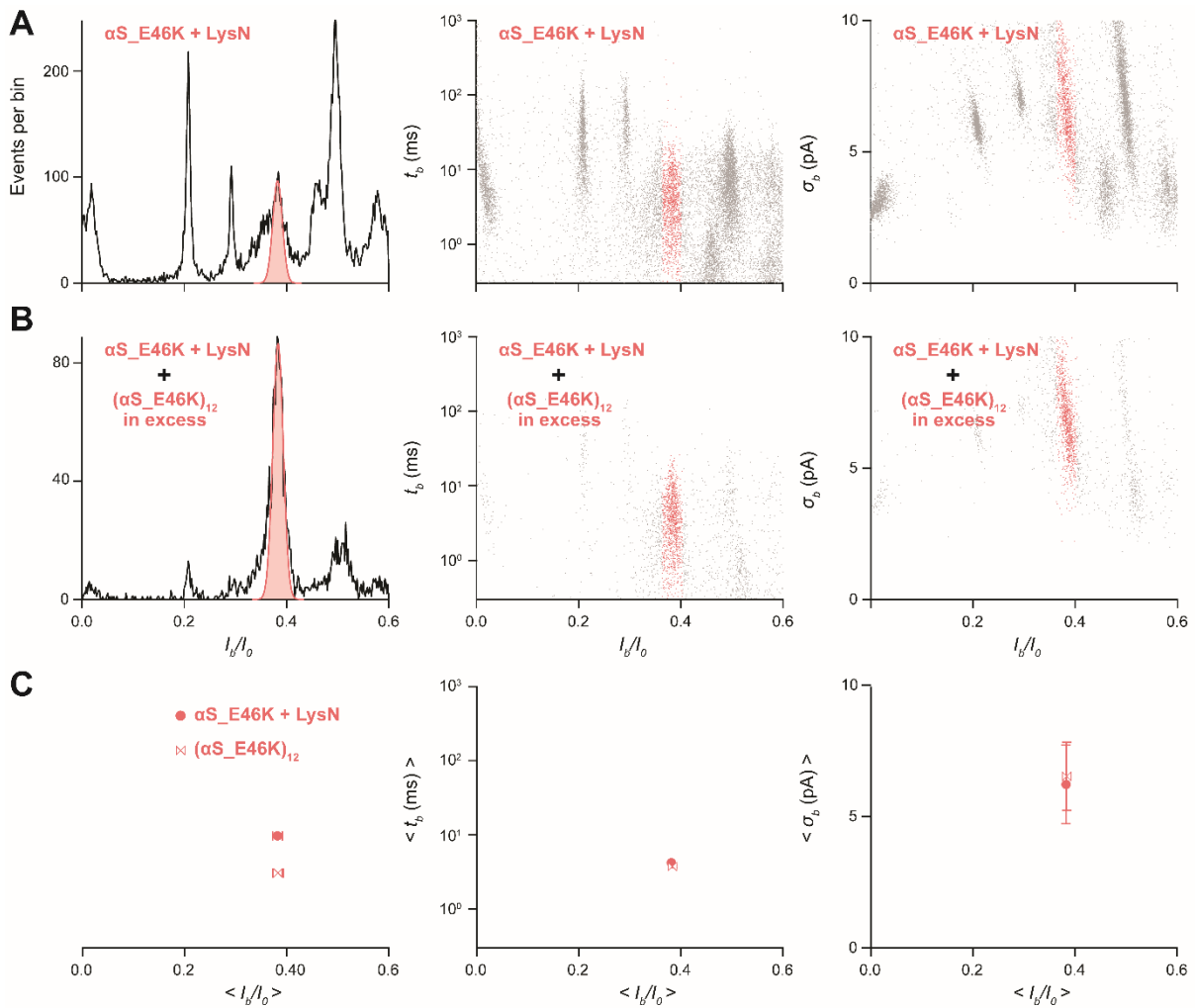


**Figure S3. AeL nanopore-based identification of the  $(\alpha S\_wt)_{13}$  fragment from LysN proteolysis of full length wild-type  $\alpha$ -Synuclein, sample N°2.** Histograms of ionic current blockade values (*left*), residence time-current blockade value event plots (*center*) and standard deviation-current blockade value event plots (*right*) for **A**) the complete digestion of full-length  $\alpha S\_wt$ , and **B**) the complete digestion of full-length  $\alpha S\_wt$  plus an excess of synthetic  $(\alpha S\_wt)_{13}$  polypeptide. **C**) Plots for the mean values of the ionic current blockade depth ( $\langle I_b/I_0 \rangle$ ), residence time ( $\langle t_b \rangle$ ), and standard deviation of the current values ( $\langle \sigma_b \rangle$ ) in the absence (*green filled circles*) and presence (*green empty hourglasses*) of excess synthetic  $(\alpha S\_wt)_{13}$ .

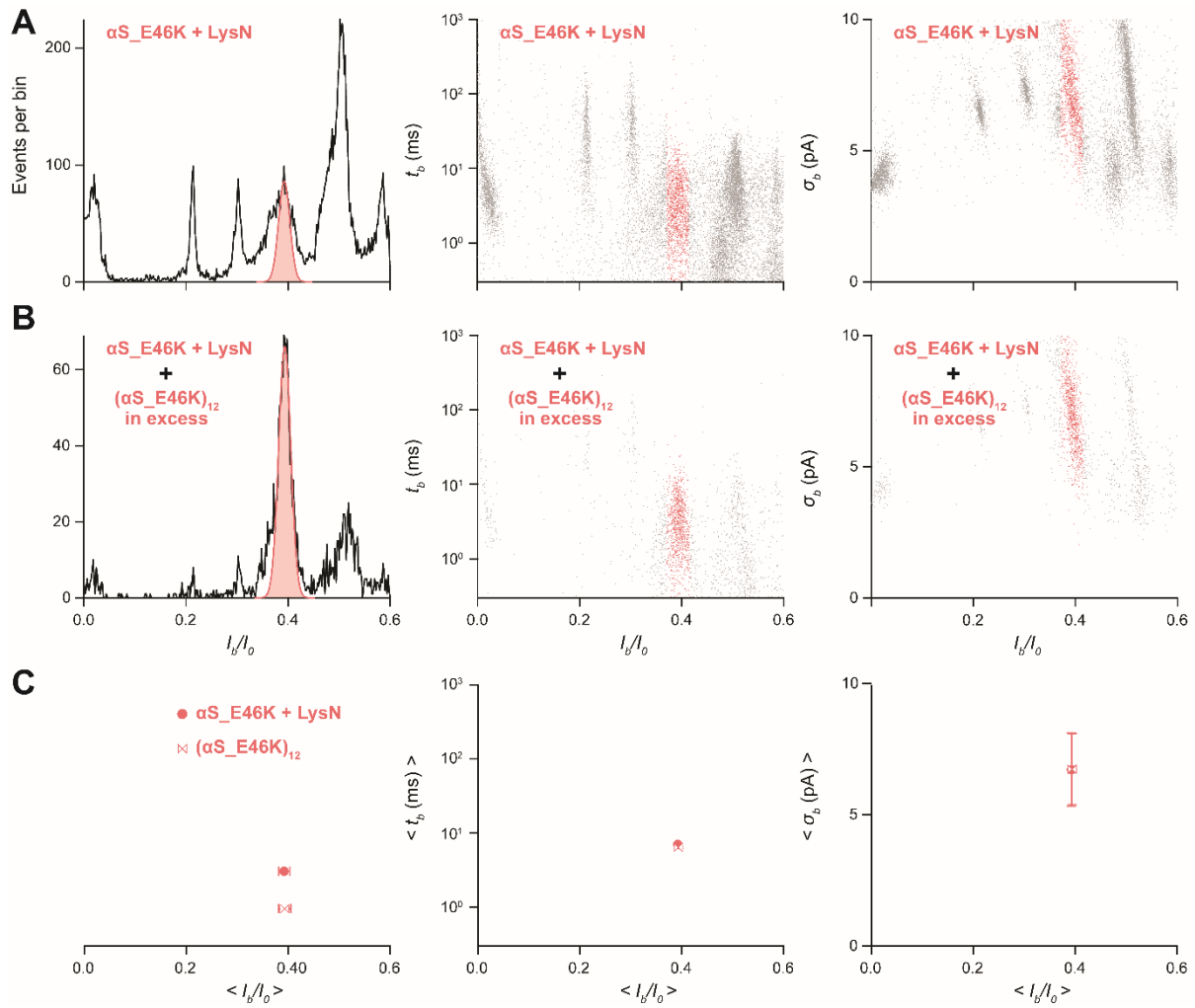


**Figure S4. AeL nanopore-based identification of the  $(\alpha\text{S\_wt})_{13}$  fragment from LysN proteolysis of full length wild-type  $\alpha$ -Synuclein, sample N°3.** Histograms of ionic current blockade values (*left*), residence time-current blockade value event plots (*center*) and standard deviation-current blockade value event plots (*right*) for **A**) the complete digestion of full-length  $\alpha\text{S\_wt}$ , and **B**) the complete digestion of full-length  $\alpha\text{S\_wt}$  plus an excess of synthetic  $(\alpha\text{S\_wt})_{13}$  polypeptide. **C**) Plots for the mean values of the ionic current blockade depth ( $\langle I_b/I_0 \rangle$ ), residence time ( $\langle t_b \rangle$ ), and standard deviation of the current values ( $\langle \sigma_b \rangle$ ) in the absence (*green filled circles*) and presence (*green empty hourglasses*) of excess synthetic  $(\alpha\text{S\_wt})_{13}$ .

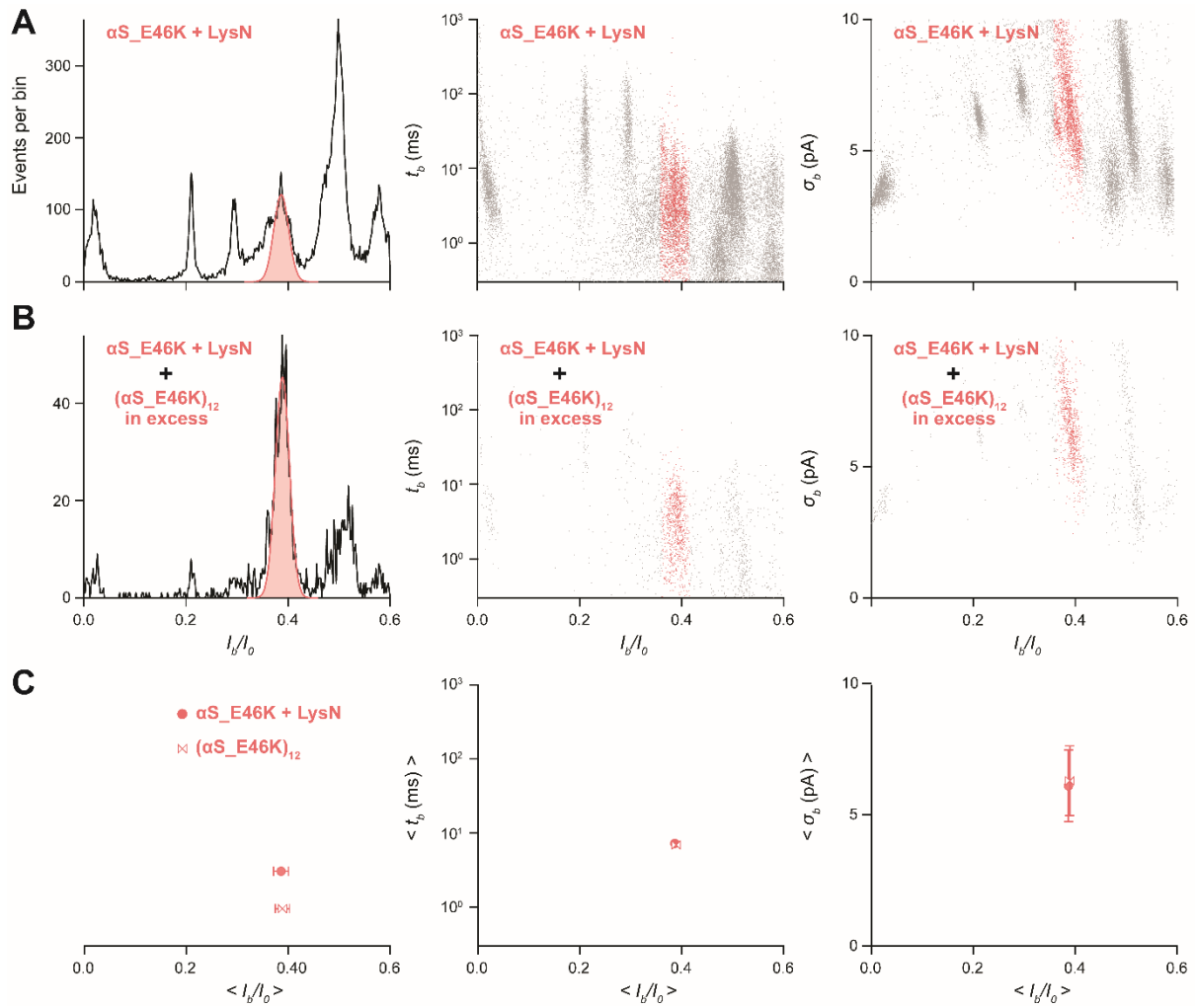
## Reproducibility of $\alpha$ S\_E46K protein fragmentation and $(\alpha$ S\_E46K)<sub>12</sub> identification



**Figure S5. AEL nanopore-based identification of the  $(\alpha$ S\_E46K)<sub>12</sub> fragment peak from LysN proteolysis of full length E46K  $\alpha$ -Synuclein point mutant, sample N°1.** Histograms of ionic current blockade values (*left*), residence time-current blockade value event plots (*center*) and standard deviation-current blockade value event plots (*right*) for **A**) the complete digestion of full-length E46K, or **B**) the complete digestion of full-length  $\alpha$ S\_E46K plus an excess of synthetic  $(\alpha$ S\_wt)<sub>13</sub> polypeptide. **C**) Plots for the mean values of the ionic current blockade depth ( $\langle I_b/I_0 \rangle$ ), residence time ( $\langle t_b \rangle$ ), and standard deviation of the current values ( $\langle \sigma_b \rangle$ ) in the absence (*red filled circles*) and presence (*red empty hourglasses*) of excess synthetic  $(\alpha$ S\_E46K)<sub>12</sub>.



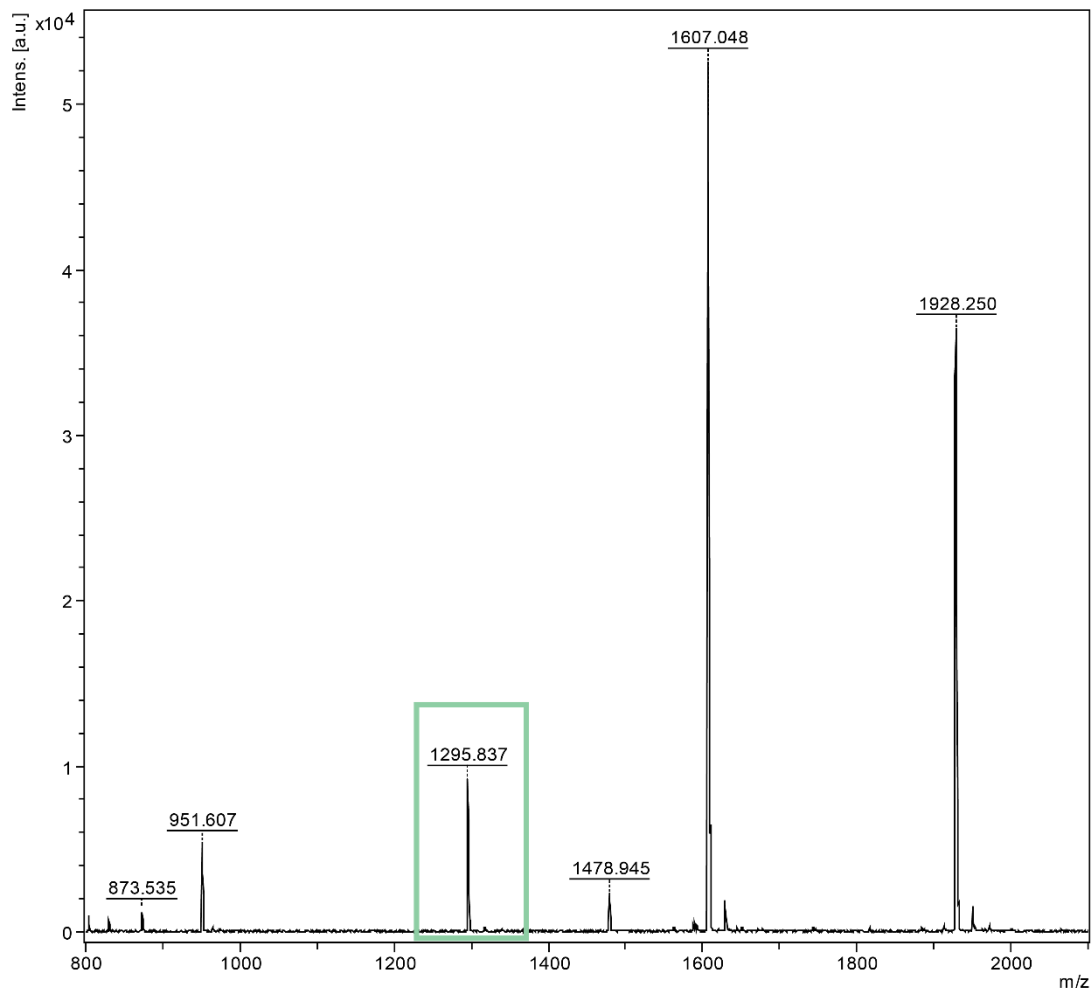
**Figure S6. AEL nanopore-based identification of the  $(\alpha\text{S\_E46K})_{12}$  fragment peak from LysN proteolysis of full length E46K  $\alpha$ -Synuclein point mutant, sample N°2.** Histograms of ionic current blockade values (*left*), residence time-current blockade value event plots (*center*) and standard deviation-current blockade value event plots (*right*) for **A**) the complete digestion of full-length E46K, or **B**) the complete digestion of full-length  $\alpha\text{S\_E46K}$  plus an excess of synthetic  $(\alpha\text{S\_wt})_{13}$  polypeptide. **C**) Plots for the mean values of the ionic current blockade depth ( $\langle I_b/I_0 \rangle$ ), residence time ( $\langle t_b \rangle$ ), and standard deviation of the current values ( $\langle \sigma_b \rangle$ ) in the absence (*red filled circles*) and presence (*red empty hourglasses*) of excess synthetic  $(\alpha\text{S\_E46K})_{12}$ .



**Figure S7. AEL nanopore-based identification of the  $(\alpha\text{S\_E46K})_{12}$  fragment peak from LysN proteolysis of full length E46K  $\alpha$ -Synuclein point mutant, sample N°3.** Histograms of ionic current blockade values (*left*), residence time-current blockade value event plots (*center*) and standard deviation-current blockade value event plots (*right*) for **A)** the complete digestion of full-length E46K, or **B)** the complete digestion of full-length  $\alpha\text{S\_E46K}$  plus an excess of synthetic  $(\alpha\text{S\_wt})_{13}$  polypeptide. **C)** Plots for the mean values of the ionic current blockade depth ( $\langle I_b/I_0 \rangle$ ), residence time ( $\langle t_b \rangle$ ), and standard deviation of the current values ( $\langle \sigma_b \rangle$ ) in the absence (*red filled circles*) and presence (*red empty hourglasses*) of excess synthetic  $(\alpha\text{S\_E46K})_{12}$ .

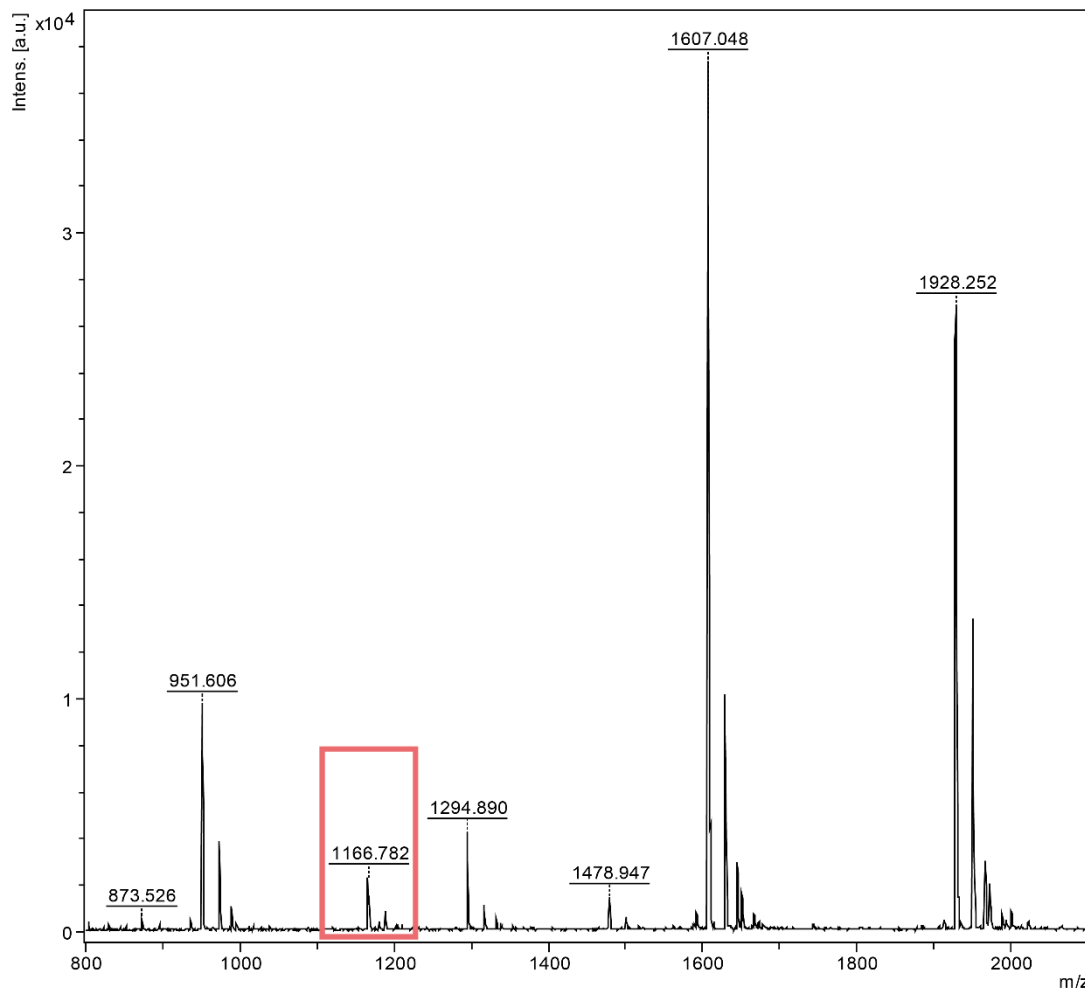


## MS analysis of $\alpha$ S<sub>wt</sub> protein fragmentation



**Figure S8. Mass spectrum of the wild-type  $\alpha$ -Synuclein digested by LysN protease.** Ion signal as a function of the mass-to-charge ratio (m/z) of the  $\alpha$ S<sub>wt</sub>. The mass-to-charge ratio that corresponds to  $(\alpha$ S<sub>wt</sub>)<sub>13</sub> polypeptide is overlaid in a green rectangle.

## MS analysis of $\alpha$ S\_E46K protein fragmentation



**Figure S9.** Mass spectrum of the E46K  $\alpha$ -Synuclein digested by LysN protease. Ion signal as a function of the mass-to-charge ratio (m/z) of the  $\alpha$ S\_E46K. The mass-to-charge ratio that corresponds to  $(\alpha$ S\_E46K)<sub>12</sub> polypeptide is overlaid in a red rectangle.

## Supplementary Note

**Figure 1B** in the main text showed that LysN proteolysis of full-length wild type  $\alpha$  Synuclein ( $\alpha$ S\_wt) or its E46K point mutant ( $\alpha$ S\_E46K) produced transient partial blockades of the aerolysin nanopore ionic current, as expected for polypeptides that reversibly partition, one at a time, in the pore. For each of those samples, the histograms of the partial blocked state ionic current relative to that of the unoccupied (i.e., fully open) pore differed principally by the presence of peaks at  $I_b/I_o = 0.34 \pm 0.01$  and  $0.38 \pm 0.01$ , for ( $\alpha$ S\_wt) and E46K respectively. Those results are reproduced here in **Figs. S1A (left) and S1B (left)**. The dwell time ( $t_b$ ) and current standard deviation  $\sigma_b$  versus  $I_b/I_o$  event plots (**Figs. S1A and S1B, center and right**) show that these measured parameters also differ between

the two samples. Finally, **Fig. 1C** shows that the mean values of the ionic current blockade depth ( $\langle I_b/I_0 \rangle$ , *left*), residence time ( $\langle t_b \rangle$ , *center*), and standard deviation of the current values ( $\langle \sigma_b \rangle$ , *right*) show quantitatively that the method can discriminate between the two subtly different proteins.

Given the proteolysis by LysN, the only difference between ( $\alpha S_{wt}$ ) and its E46K point mutant ( $\alpha S_{E46K}$ ) should be between the wild type protein's 13 amino acid fragment ( $(\alpha S_{wt})_{13}$ ) and ( $(\alpha S_{E46K})_{12}$ ) in the point mutant variant sample (**Tables 1 and 2, main text**). To test this, we challenged the LysN protease digested samples from each full-length protein with an excess of these respective synthetic polypeptides (i.e.,  $(\alpha S_{wt})_{13}$  or  $(\alpha S_{E46K})_{12}$ ) and the resultant histograms are shown in **Figs. S2 to S7** (A and B). These data, and the mean values of these measured parameters, which are shown in **Figs. S2C to S4C and S5C to S7C**, demonstrate that the observed peaks in **Fig. 1C** in the main paper and **Figs. S1A and S1B** are indeed attributable to the fragments  $(\alpha S_{wt})_{13}$  and  $(\alpha S_{E46K})_{12}$  for their respective full-length proteins.

Mass spectra for LysN-digested full-length  $\alpha S_{wt}$  and  $\alpha S_{E46K}$  proteins, are illustrated in **Figs. S8 and S9**, respectively. The  $(\alpha S_{wt})_{13}$  and  $(\alpha S_{E46K})_{12}$  polypeptide fragments that were discriminated by the nanopore (**Figs. S1 to S7**) are highlighted in the mass spectra plot by the green and red boxes.