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Supplemental Information

Mapping Physiological ADP-Ribosylation

Using Activated Ion Electron Transfer Dissociation

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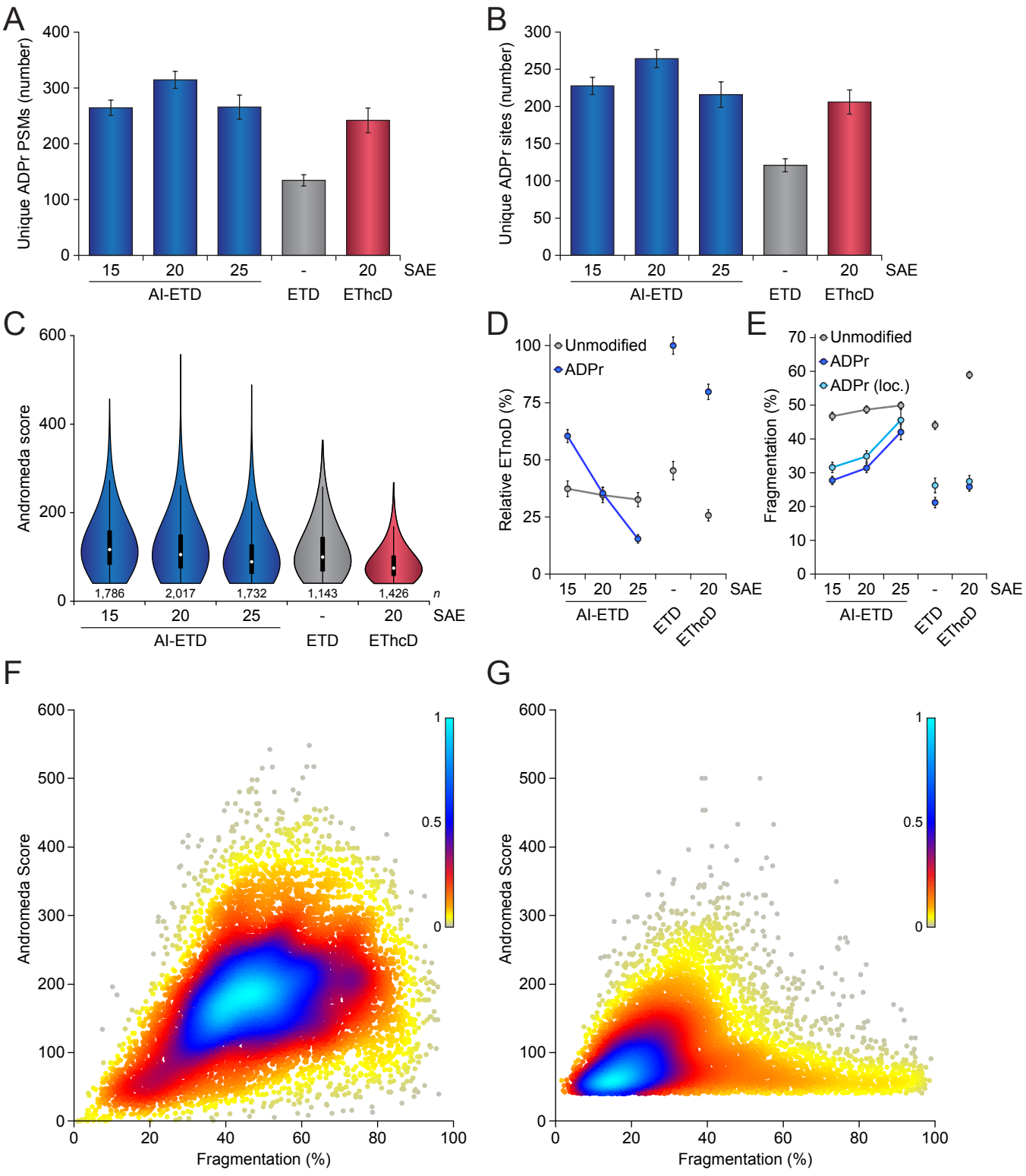
A
Unique ADPr PSMs (number)
400
300
200
100
0
15
20
25
-
20
SAE
AI-ETD
ETD
EThcD
B
Unique ADPr sites (number)
300
250
200
150
100
50
0
15
20
25
-
20
SAE
AI-ETD
ETD
EThcD
C
Andromeda score
600
400
200
0
1,786
2,017
1,732
1,143
1,426
n
15
20
25
-
20
SAE
AI-ETD
ETD
EThcD
D
Relative EToD (%)
100
75
50
25
0
Unmodified
ADPr
15
20
25
-
20
SAE
AI-ETD
ETD
EThcD
E
Fragmentation (%)
70
60
50
40
30
20
10
0
Unmodified
ADPr
ADPr (loc.)
15
20
25
-
20
SAE
AI-ETD
ETD
EThcD
F
Andromeda Score
600
500
400
300
200
100
0
1
0.5
0
0
20
40
60
80
100
Fragmentation (%)
G
Andromeda Score
600
500
400
300
200
100
0
1
0.5
0
0
20
40
60
80
100
Fragmentation (%)

FIGURE S1

Figure S1. Comparison of AI-ETD vs. ETD and EThcD for mapping ADP-ribosylation in Lys-C digested peptides, related to Figure 1. (A) Overview of the number of ADPr peptide-spectrum-matches (PSMs) identified and localized (>90% probability) for each dissociation method and Supplemental Activation Energy (SAE). Error bars represent SD, *n*=4 technical replicates. (B) As **A**, but displaying the number of ADPr sites identified. (C) As **A**, but displaying the spectral quality (in Andromeda Score) of all identified ADPr-modified peptides. Distribution of data points is visualized, line limits; 1.5× interquartile range (IQR), box limits; 3rd and 1st quartiles, white dot; mean. Number of data points (*n*) is visualized below the distributions. (D) Visualization of the average relative degree of non-dissociative electron transfer (ETnoD). Derived from all peptide-identified MS/MS spectra, and separately visualized for unmodified and ADP-ribosylated peptides. Error bars represent 5× SEM. (E) Visualization of the average degree of precursor fragmentation, calculated by dividing observed fragment ion peak intensity by the sum of non-ETD, ETnoD, and all fragment ion peak intensities. Derived from all peptide-identified MS/MS spectra, and separately visualized for unmodified, ADP-ribosylated, and localized ADP-ribosylated peptides. Error bars represent 5× SEM. (F) Spectral quality (in Andromeda Score) plotted against the average degree of precursor fragmentation, for unmodified peptides detected within ADPr samples. Coloring represents the relative density of dots in the plot, with higher values corresponding to higher density. (G) As **F**, but for non-localized ADP-ribosylated peptides.

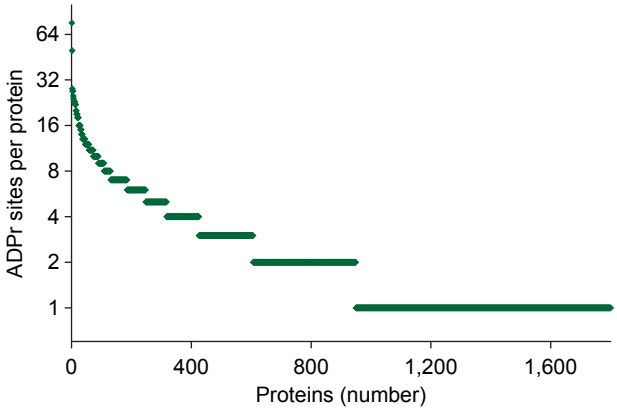
64
32
16
8
4
2
1
ADPr sites per protein
0
400
800
1,200
1,600
Proteins (number)

FIGURE S2

Figure S2. Distribution of ADPr sites across proteins, related to Figure 2. Visualization of the number of unique ADPr sites detected per protein, based on the benchmarking experiment.

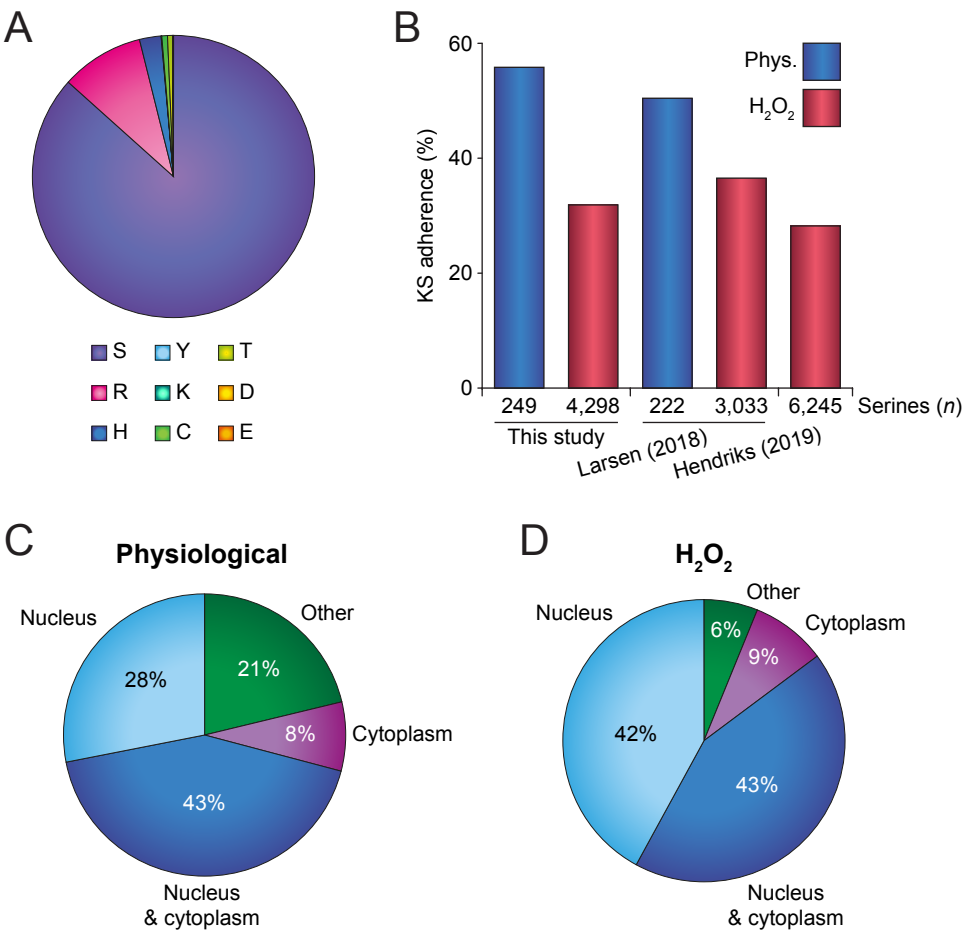
A
S
Y
T
R
K
D
H
C
E
B
60
40
20
0
KS adherence (%)
Phys.
H2O2
249
4,298
222
3,033
6,245
Serines (n)
This study
Larsen (2018)
Hendriks (2019)
C
Physiological
Nucleus
Other
28%
21%
8%
Cytoplasm
43%
Nucleus
& cytoplasm
D
H2O2
Nucleus
Other
6%
Cytoplasm
9%
42%
43%
Nucleus
& cytoplasm

FIGURE S3

Figure S3. KS motif adherence and subcellular localization, related to Figure 3. (A) Pie-chart overview of the amino acid residue intensity distribution of all physiological ADP-ribosylation sites identified in this study. (B) Overview of KS motif adherence of all serine ADPr sites identified under physiological conditions or in response to H_2O_2 treatment (benchmarking experiment), in this study or in two others (Hendriks et al., 2019; Larsen et al., 2018). KS motif modification is defined as an ADPr-modified serine with an N-terminal lysine residue. (C) Subcellular localization of ADPr target proteins identified under physiological conditions, with localization derived from Gene Ontology Cellular Compartments. (D) As **C**, but for ADPr target proteins identified in response to H_2O_2 treatment.