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Supplemental Information

Validation and Invalidation of Chemical Probes for the Human *N*-myristoyltransferases

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Validation and invalidation of chemical probes for the human *N*-myristoyltransferases.

Supplementary Information

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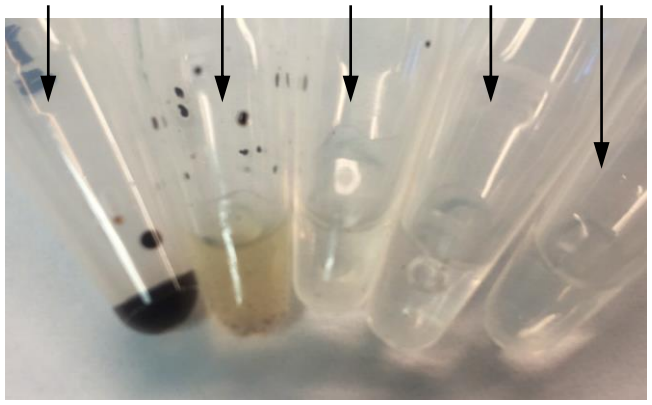
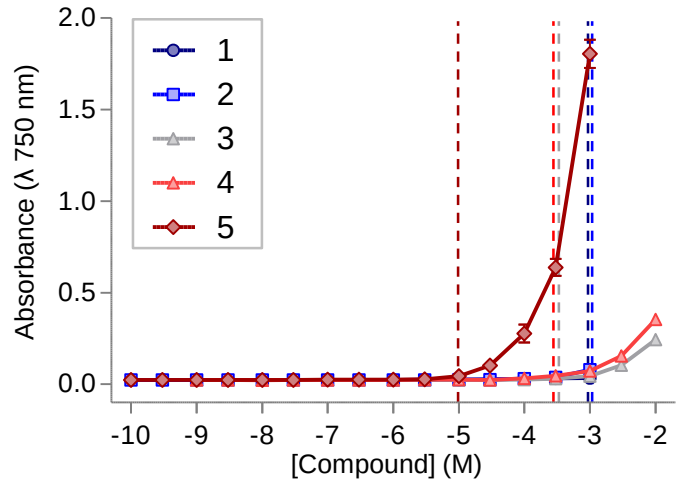
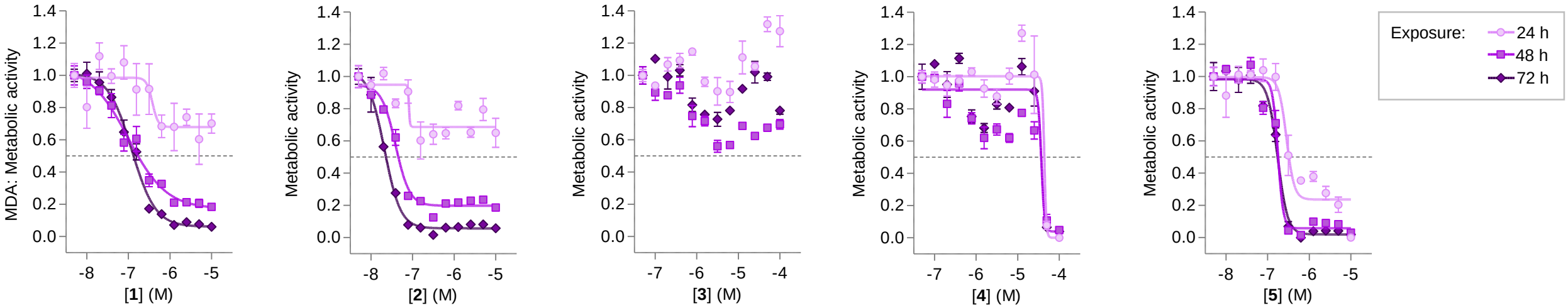
A


100% DMSO
10% (v/v) DMSO in buffer
100 mM
100 µM
10 µM
1 µM
0.1 µM

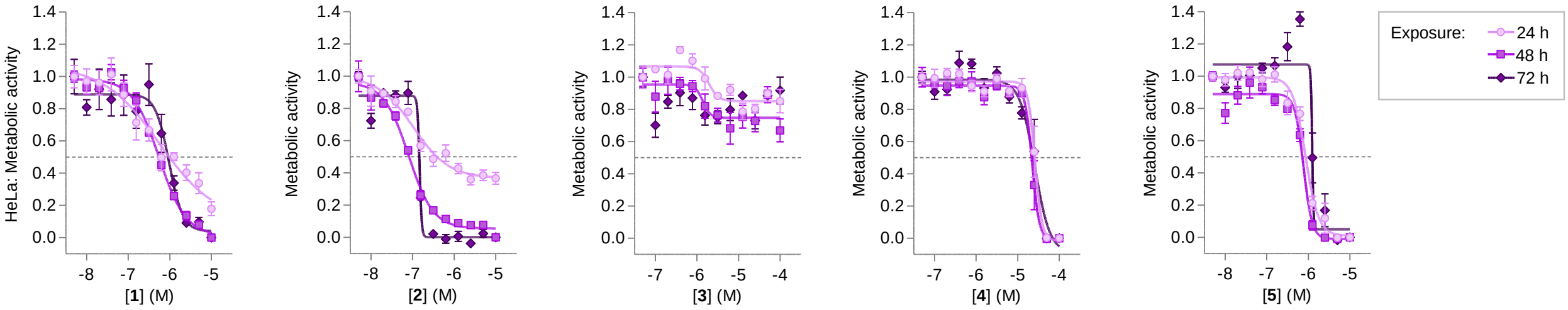
B


Absorbance (λ 750 nm)
[Compound] (M)
1
2
3
4
5

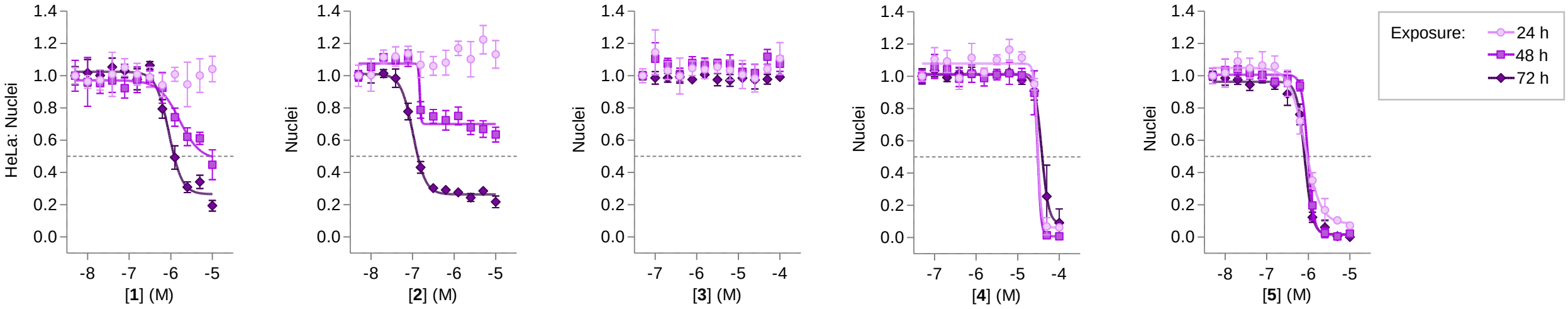
C


MDA: Metabolic activity
Metabolic activity
[1] (M)
[2] (M)
[3] (M)
[4] (M)
[5] (M)
Exposure:
24 h
48 h
72 h

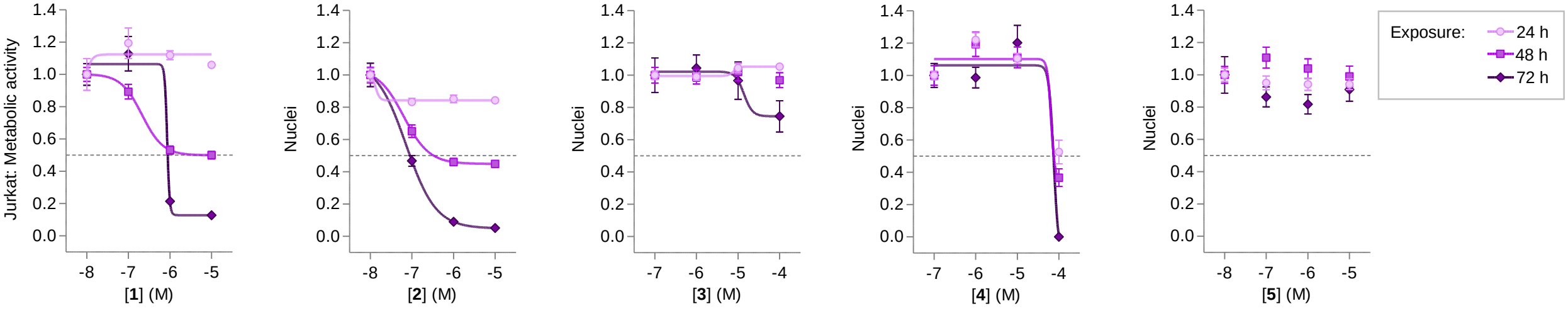
D


HeLa: Metabolic activity
Metabolic activity
[1] (M)
[2] (M)
[3] (M)
[4] (M)
[5] (M)
Exposure:
24 h
48 h
72 h

E


HeLa: Nuclei
Nuclei
[1] (M)
[2] (M)
[3] (M)
[4] (M)
[5] (M)
Exposure:
24 h
48 h
72 h

F


Jurkat: Metabolic activity
Nuclei
[1] (M)
[2] (M)
[3] (M)
[4] (M)
[5] (M)
Exposure:
24 h
48 h
72 h

Supplemental Figure 1. Effect of compounds 1-5 on recombinant protein and living cells.

Related to Figure 1.

(A) Image showing precipitation of **5** at various concentrations, within 5 min after dilution.

(B) Precipitation prevalence of compounds **1**-**5** in rNMT1 buffer, containing 0.1% (v/v) DMSO. Absorbance at 750 nm was determined after 15 min at room temperature. Arrows indicate prevalence of precipitates.

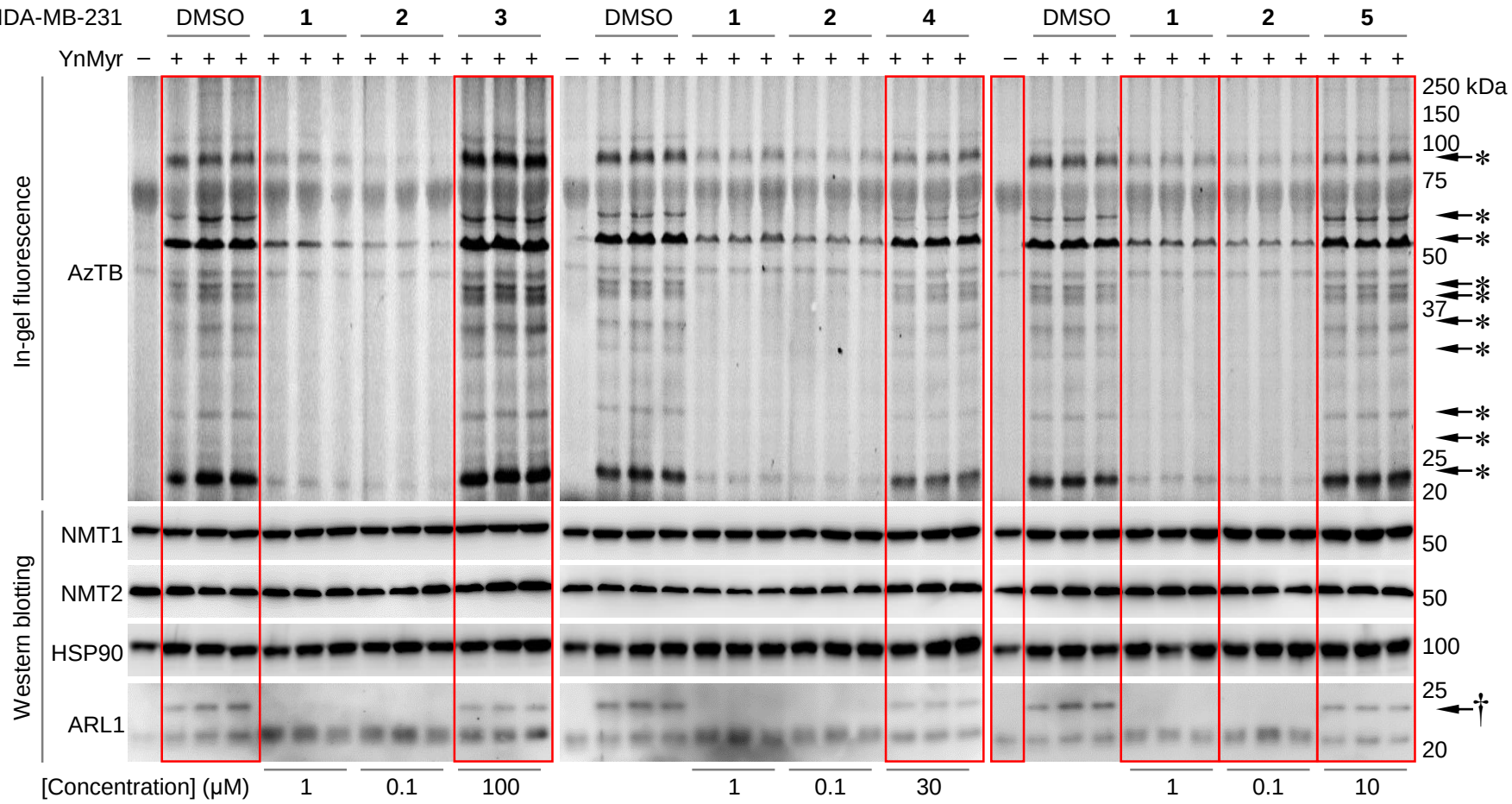
(C) Metabolic activity of MDA-MB-231 cells exposed to a range of **1**-**5** concentrations for 24, 48 and 72 h. Mean of quadruplicate measurements, ± SEM.

(D) Metabolic activity of HeLa cells exposed to a range of **1**-**5** concentrations for 24, 48 and 72 h. Mean of quadruplicate measurements, ± SEM.

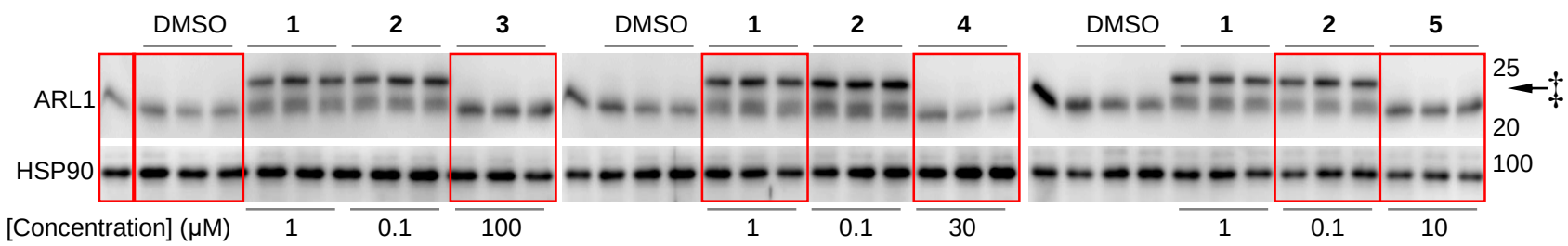
(E) Nuclei counts of HeLa cells after exposure to **1**-**5** for 24, 48 and 72 h. Mean of quadruplicate measurements, ± SEM.

(F) Metabolic activity of Jurkat cells exposed to a range of **1**-**5** concentrations for 24, 48 and 72 h. Mean of quadruplicate measurements, ± SEM.

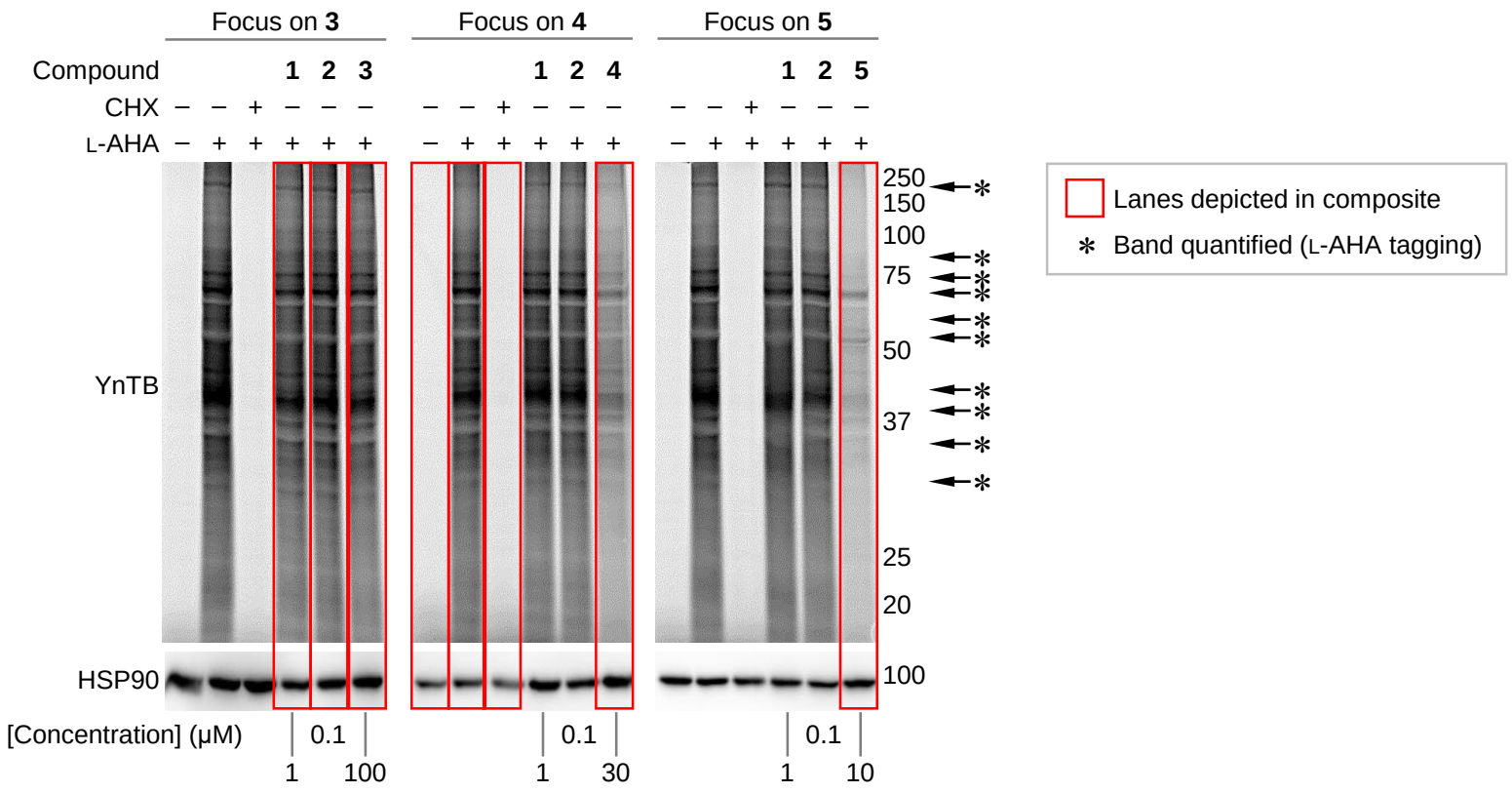
A


MDA-MB-231
DMSO 1 2 3 DMSO 1 2 4 DMSO 1 2 5
YnMyr
In-gel fluorescence
AzTB
Western blotting
NMT1
NMT2
HSP90
ARL1
250 kDa
150
100
75
50
37
25
20
[Concentration] (μM)
1 0.1 100 1 0.1 30 1 0.1 10
Lanes depicted in composite
Band quantified (YnMyr tagging)
Band quantifed (YnMyr-tagged ARL1)
Band quantifed (Sortase A-tagged ARL1)

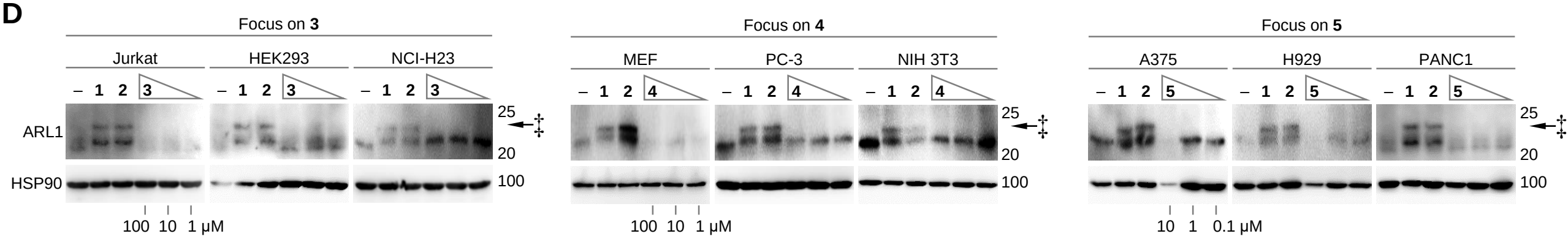
B


DMSO 1 2 3 DMSO 1 2 4 DMSO 1 2 5
ARL1
HSP90
25
20
100
[Concentration] (μM)
1 0.1 100 1 0.1 30 1 0.1 10

C


Focus on 3
Focus on 4
Focus on 5
Compound
CHX
L-AHA
YnTB
HSP90
250
150
100
75
50
37
25
20
[Concentration] (μM)
1 100 0.1 1 30 0.1 1 10
Lanes depicted in composite
Band quantified (L-AHA tagging)

D


Focus on 3
Focus on 4
Focus on 5
Jurkat
HEK293
NCI-H23
MEF
PC-3
NIH 3T3
A375
H929
PANC1
ARL1
HSP90
25
20
100
100 10 1 μM
100 10 1 μM
10 1 0.1 μM

Supplemental Figure 2. Effect of compounds 1-5 on MDA-MB-231 cells.

Related to Figures 2 and 4.

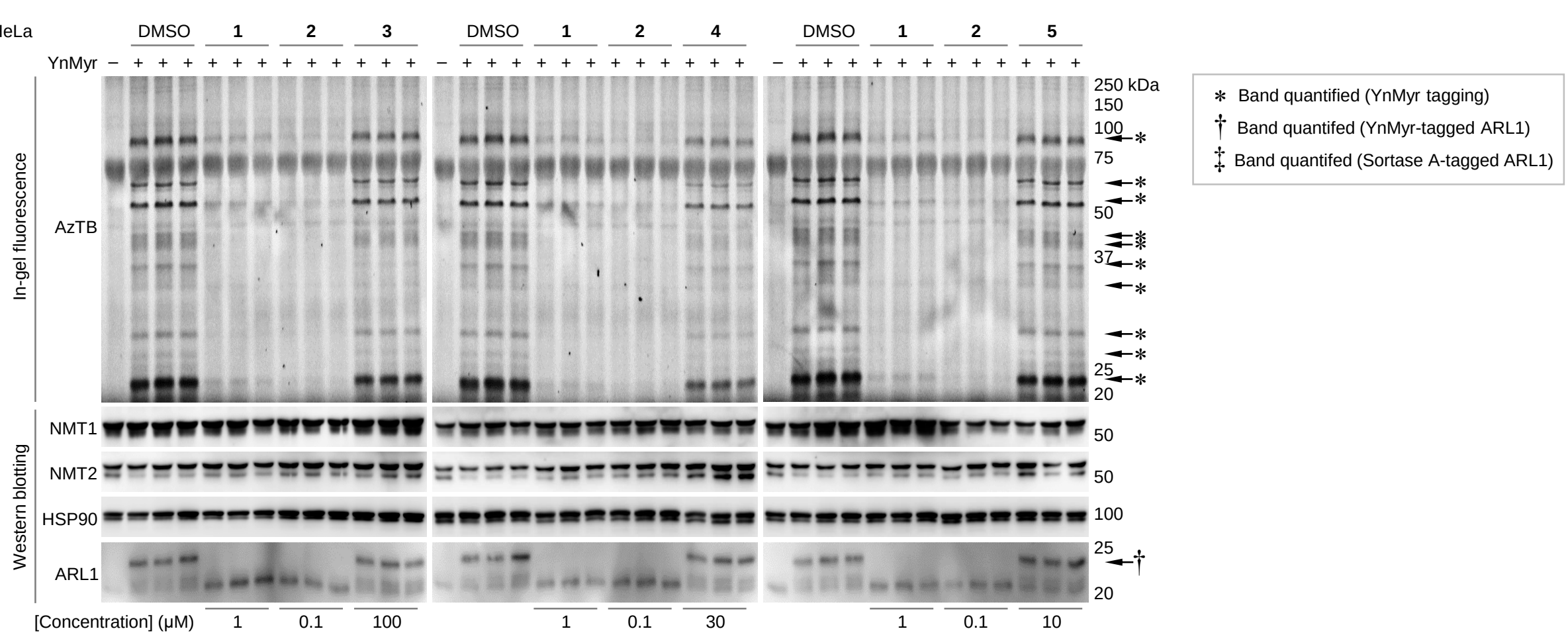
(A) Effects on *N*-myristoylation revealed by YnMyr-tagging in MDA-MB-231 cells. Left to right: cells exposed to DMSO (–), **1**, **2**, and per gel compared to **3**, **4** and **5**, in triplicate. Top to bottom: in-gel visualisation of YnMyr-tagged proteins; Westernblot (WB) detection of NMT1, NMT2, loading control HSP90 and NMT-substrate ARL1. Higher MW form of ARL1 (†) indicates NMT activity. Red squares depict regions selected for Fig 2B.

(B) Effects on *N*-myristoylation in MDA-MB-231 cells revealed by Sortase A-mediated peptide addition. Left to right: cells exposed to DMSO (–), **1**, **2**, and per gel compared to **3**, **4** and **5**, in triplicate. Top: WB detection of NMT-substrate ARL1. Higher MW form of ARL1 (‡) indicates NMT inhibition. Bottom: loading control HSP90. Red squares depict regions selected for Fig 2F.

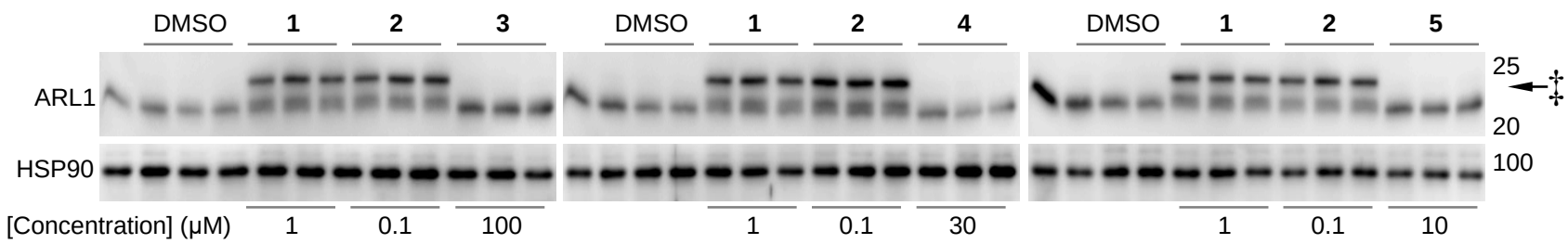
(C) Effects on *de novo* protein synthesis in MDA-MB-231 revealed by L-AHA incorporation. Left to right: cells exposed to DMSO (–), **1**, **2**, and per gel compared to **3**, **4** and **5**, in triplicate. Top: in-gel visualisation of L-AHA-tagged proteins. Below: loading control HSP90. Red squares depict regions selected for Fig 4B.

(D) Effects on *N*-myristoylation in a panel of cell lines revealed by Sortase A-mediated peptide addition. Left to right: cells exposed to DMSO (–), **1**, **2**, and compared to different concentrations of **3** (100, 10 and 1 µM), **4** (100, 10 and 1 µM) and **5** (10, 1 and 0.1 µM). Top: WB detection of NMT-substrate ARL1. Higher MW form of ARL1 (‡) indicates NMT inhibition. Bottom: loading control HSP90.

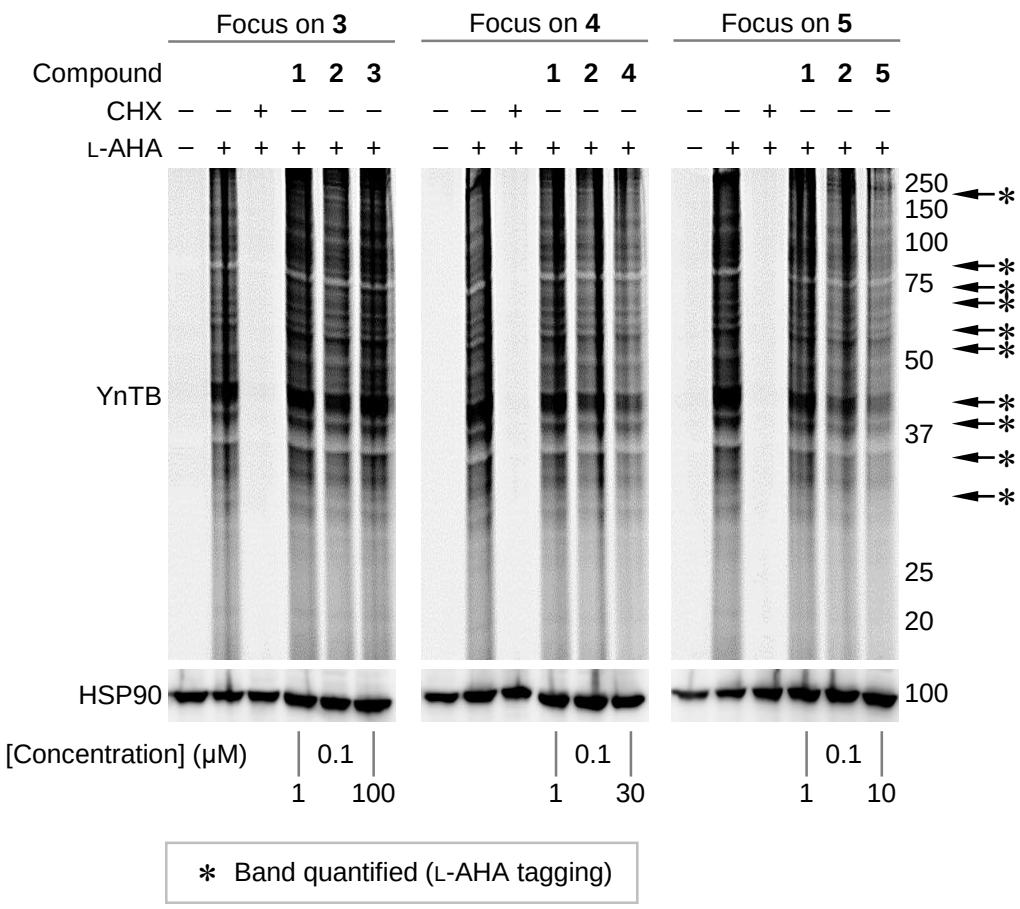
A


HeLa
DMSO 1 2 3
DMSO 1 2 4
DMSO 1 2 5
YnMyr
In-gel fluorescence
AzTB
Western blotting
NMT1
NMT2
HSP90
ARL1
250 kDa
150
100
75
50
37
25
20
[Concentration] (μM)
1 0.1 100
1 0.1 30
1 0.1 10
Band quantified (YnMyr tagging)
Band quantifed (YnMyr-tagged ARL1)
Band quantifed (Sortase A-tagged ARL1)

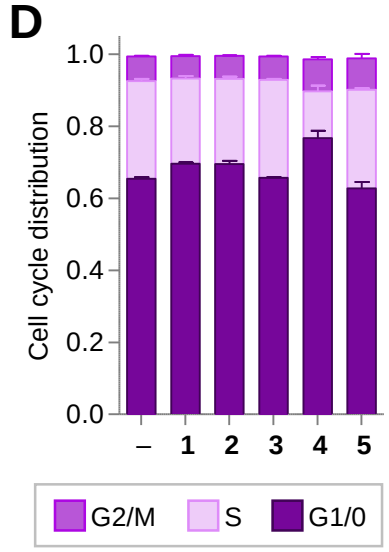
B


DMSO 1 2 3
DMSO 1 2 4
DMSO 1 2 5
ARL1
HSP90
25
20
100
[Concentration] (μM)
1 0.1 100
1 0.1 30
1 0.1 10

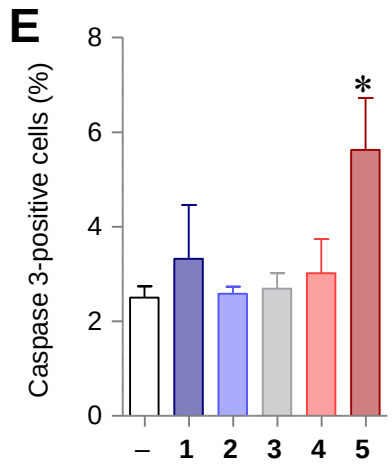
C


Focus on 3
Focus on 4
Focus on 5
Compound
CHX
L-AHA
YnTB
HSP90
250
150
100
75
50
37
25
20
[Concentration] (μM)
1 0.1 100
1 0.1 30
1 0.1 10
Band quantified (L-AHA tagging)

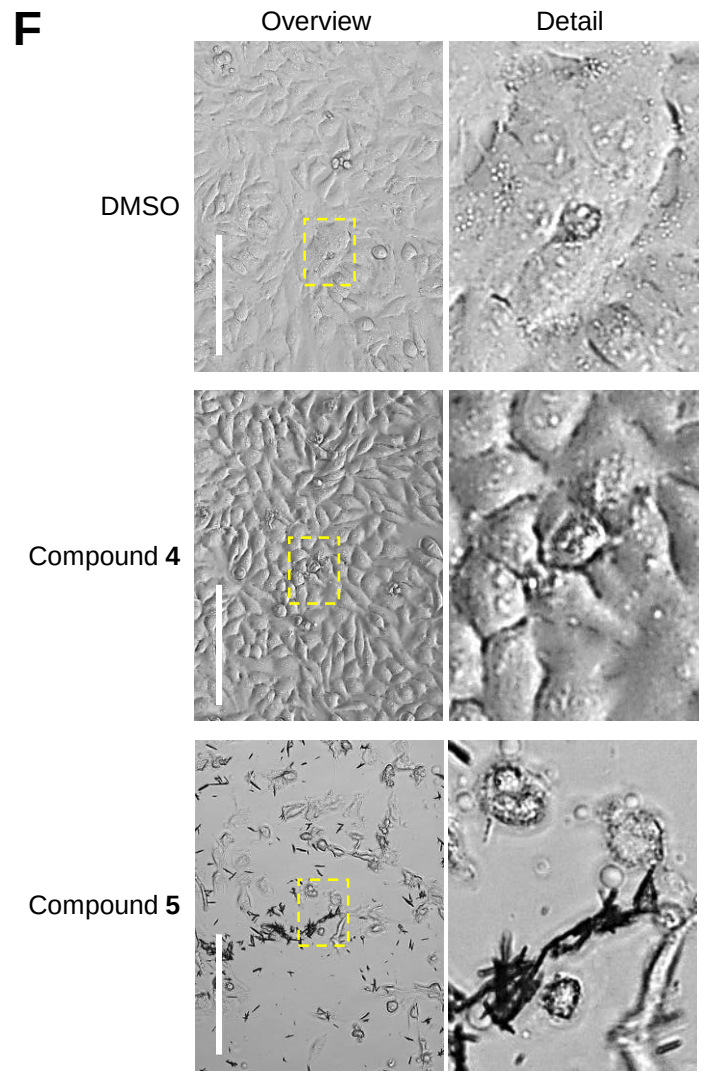
D


Cell cycle distribution
1.0
0.8
0.6
0.4
0.2
0.0
– 1 2 3 4 5
G2/M
S
G1/0

E


Caspase 3-positive cells (%)
8
6
4
2
0
– 1 2 3 4 5

F


Overview
Detail
DMSO
Compound 4
Compound 5

Supplemental Figure 3. Effect of compounds 1-5 on HeLa cells.

Related to Figures 2 and 4.

(A) Effects on *N*-myristoylation revealed by YnMyr-tagging in HeLa cells. Left to right: cells exposed to DMSO (–), **1**, **2**, and per gel compared to **3**, **4** and **5**, in triplicate. Top to bottom: in-gel visualisation of YnMyr-tagged proteins; Westernblot (WB) detection of NMT1, NMT2, loading control HSP90 and NMT-substrate ARL1. Higher MW form of ARL1 (†) indicates NMT activity.

(B) Effects on *N*-myristoylation in HeLa cells revealed by Sortase A-mediated peptide addition. Left to right: cells exposed to DMSO (–), **1**, **2**, and per gel compared to **3**, **4** and **5**, in triplicate. Top: WB detection of NMT-substrate ARL1. Higher MW form of ARL1 (‡) indicates NMT inhibition. Bottom: loading control HSP90.

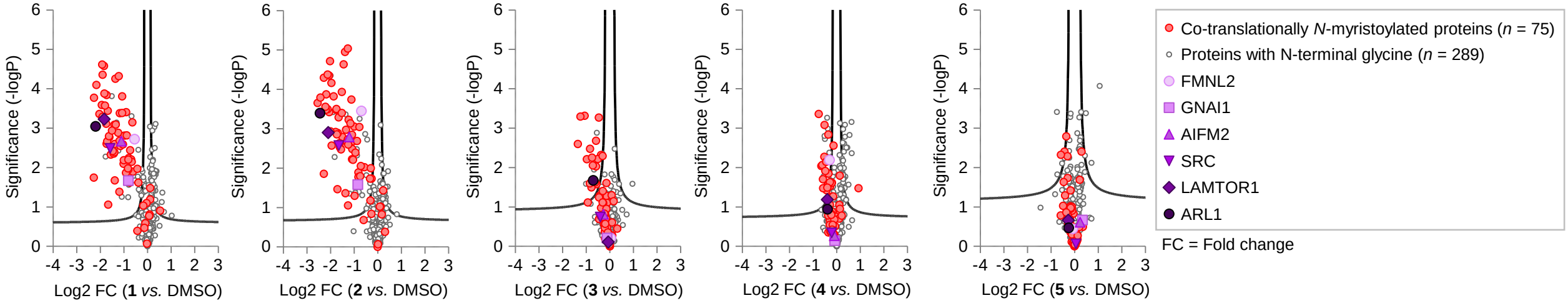
(C) Effects on *de novo* protein synthesis in HeLa revealed by L-AHA incorporation. Left to right: cells exposed to DMSO (–), **1**, **2**, and per gel compared to **3**, **4** and **5**, in triplicate. Top: in-gel visualisation of L-AHA-tagged proteins. Below: loading control HSP90.

(D) Cell cycle distribution of HeLa after exposure to **1**-**5** for 18 h. Cells were analysed for G2/M, S and G1/0 through DNA content and proliferation by FACS. For the gating strategy and quantifications, see Fig S5C and S5D, respectively.

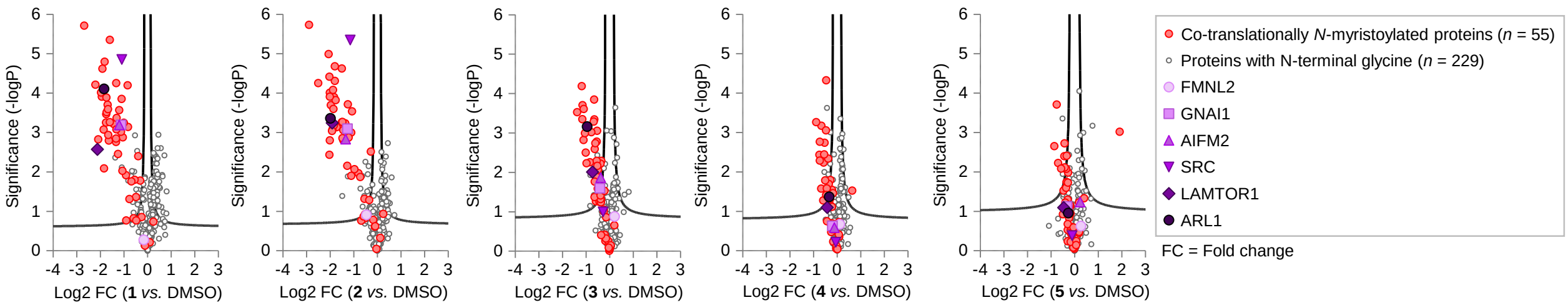
(E) Effect on apoptosis. In HeLa cells of (D), active Caspase-3 protein staining detected by FACS. For the gating strategy and quantifications, see Fig S5C and S5D, respectively.

(F) Brightfield micrographs depicting HeLa cells exposed to DMSO (top), **4** (middle) and **5** (bottom) for 18 h. Yellow square depicts the location of the detail area. Scale bar is 200 μm.

A MDA-MB-231



B HeLa



C HeLa

Supplemental Figure 4. Chemical proteomics on YnMyr-tagged proteins.

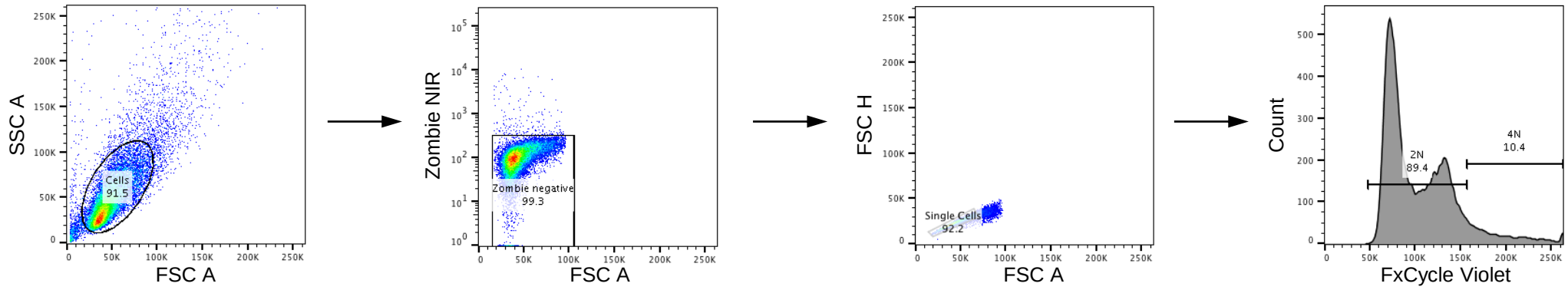
Related to Figure 3.

(A) Volcano plots for MDA-MB-231 showing the Log2 fold-change and significance of proteins identified after enrichment of YnMyr-tagged proteins and chemical proteomics. Depicted are co-translationally *N*-myristoylated proteins (red, $n = 75$) and proteins with N-terminal glycines (grey, $n = 289$). Left to right: Compound **1**-**5** versus DMSO. Responses of six known co-translationally *N*-myristoylated proteins are shown for each condition. A negative Log2 fold-change indicates less protein identified in cells with compound compared to cells treated with DMSO only. Black lines depict the significance threshold FDR (0.01).

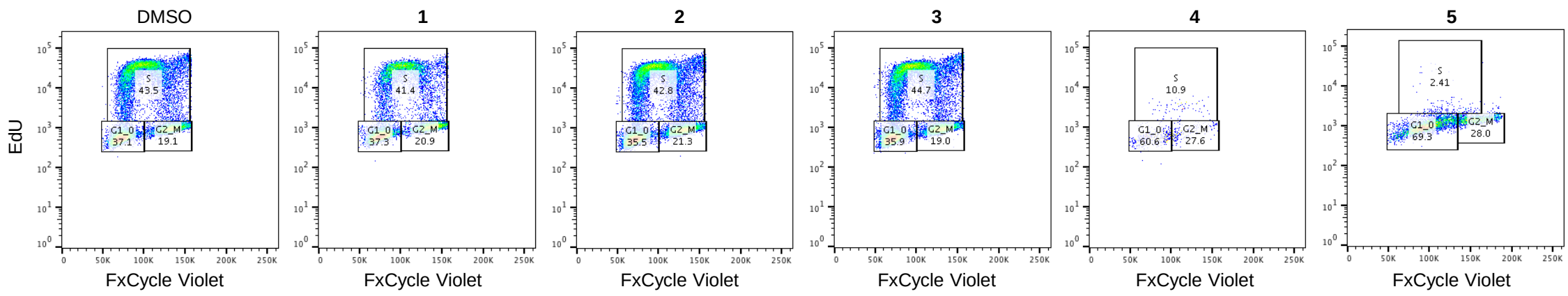
(B) Volcano plots for HeLa, as above. Co-translationally *N*-myristoylated proteins (red, $n = 55$) and proteins with N-terminal glycines (grey, $n = 229$).

(C) Hierarchical one-minus Pearson correlation clustering of the co-translationally *N*-myristoylated proteins quantified in HeLa ($n = 55$).

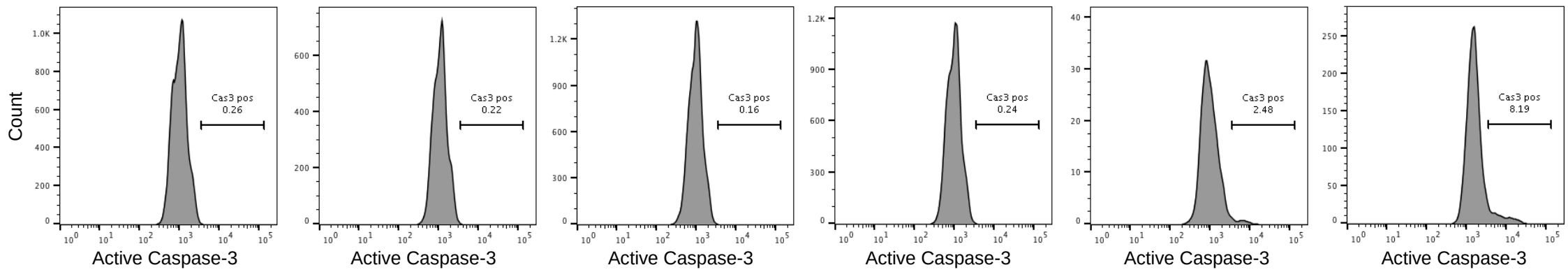
A MDA-MB-231
Gating strategy



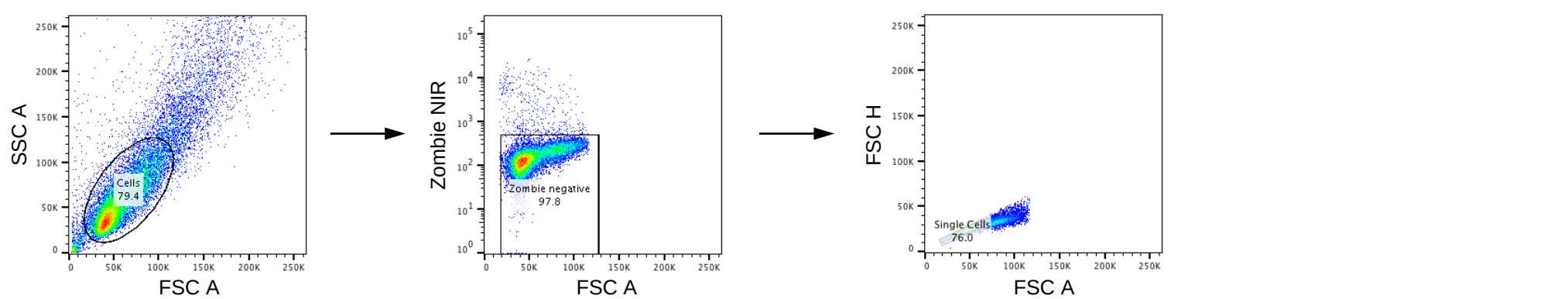
B MDA-MB-231
Cell cycle



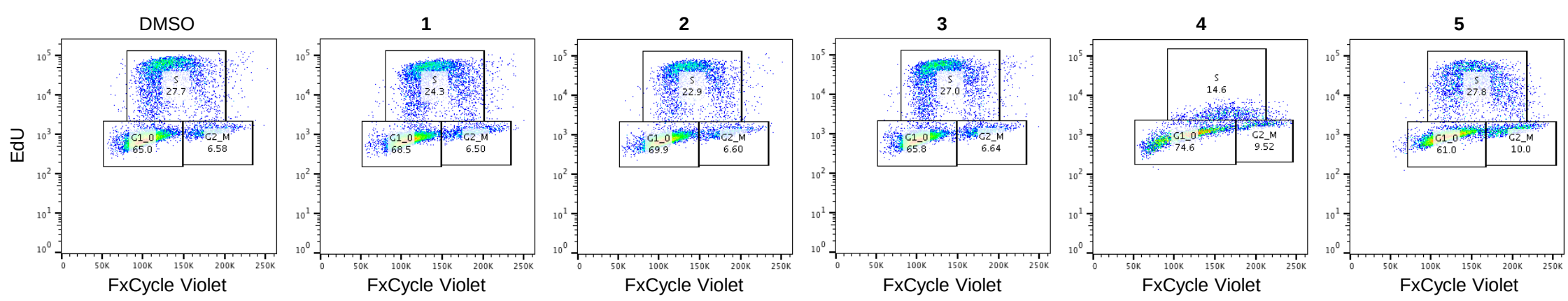
C MDA-MB-231
Apoptosis



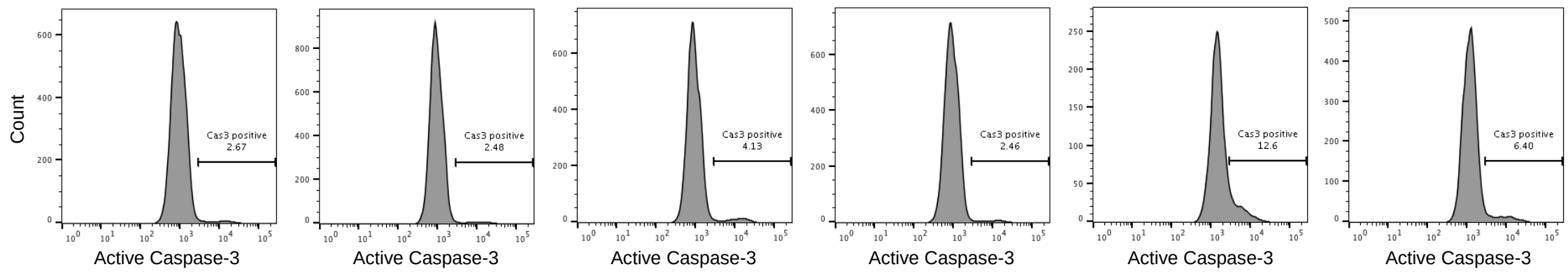
D HeLa
Gating strategy



E HeLa
Cell cycle



F HeLa
Apoptosis



Supplemental Figure 5. Effects on cell cycle and apoptosis in intact living cells.

Related to Figure 4.

(A) Gating strategy for MDA-MB-231 cells. Left to right: gating of cell size, gating of living cells (Zombie NIR negative), gating of single cells and gating of the cell population with 2n only.

(B) FACS plots showing DNA content (FxCycle Violet) staining compared to proliferation (EdU incorporation) in MDA-MB-231. Left to right: effects to cells exposed to DMSO, or **1**-**5** for 18 h.

(C) FACS plots showing active Caspase-3 protein staining detected by FACS. Left to right: effects to cells exposed to DMSO, or **1**-**5** for 18 h.

(D) Gating strategy for HeLa cells. Left to right: gating of cell size, gating of living cells (Zombie NIR negative), gating of single cells and gating of the cell population with 2n only.

(E) FACS plot showing DNA content (FxCycle Violet) staining compared to proliferation (EdU incorporation) in HeLa. Left to right: effects to cells exposed to DMSO, or **1**-**5** for 18 h.

(F) FACS plot showing active Caspase-3 protein staining detected by FACS. Left to right: effects to cells exposed to DMSO, or **1**-**5** for 18 h.