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Supporting Information

Thermal Proteome Profiling Reveals Insight to Antiproliferative and Pro-Apoptotic Effects of Lagunamide A in the Modulation of DNA Damage Repair

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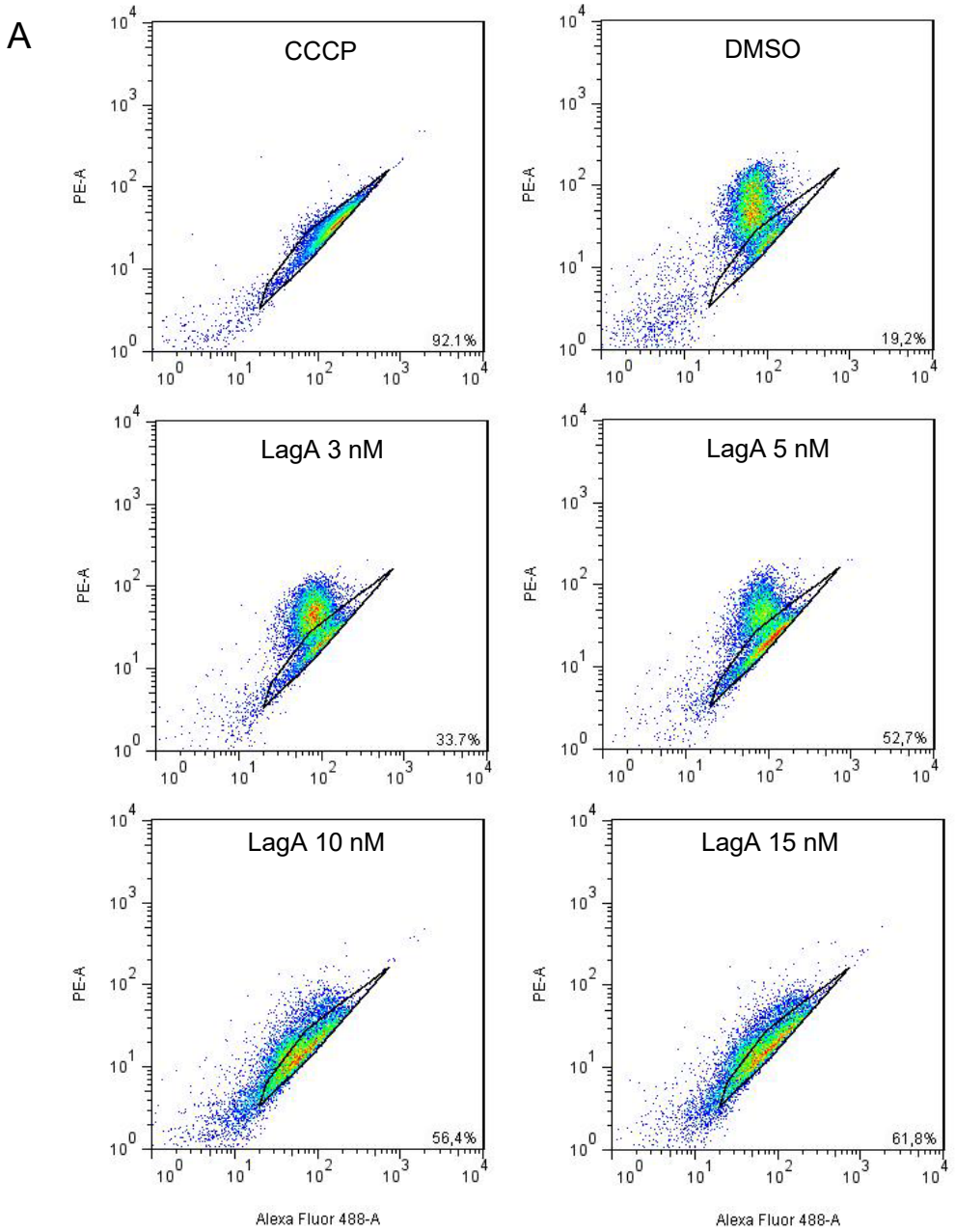
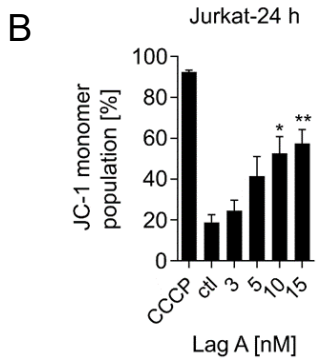
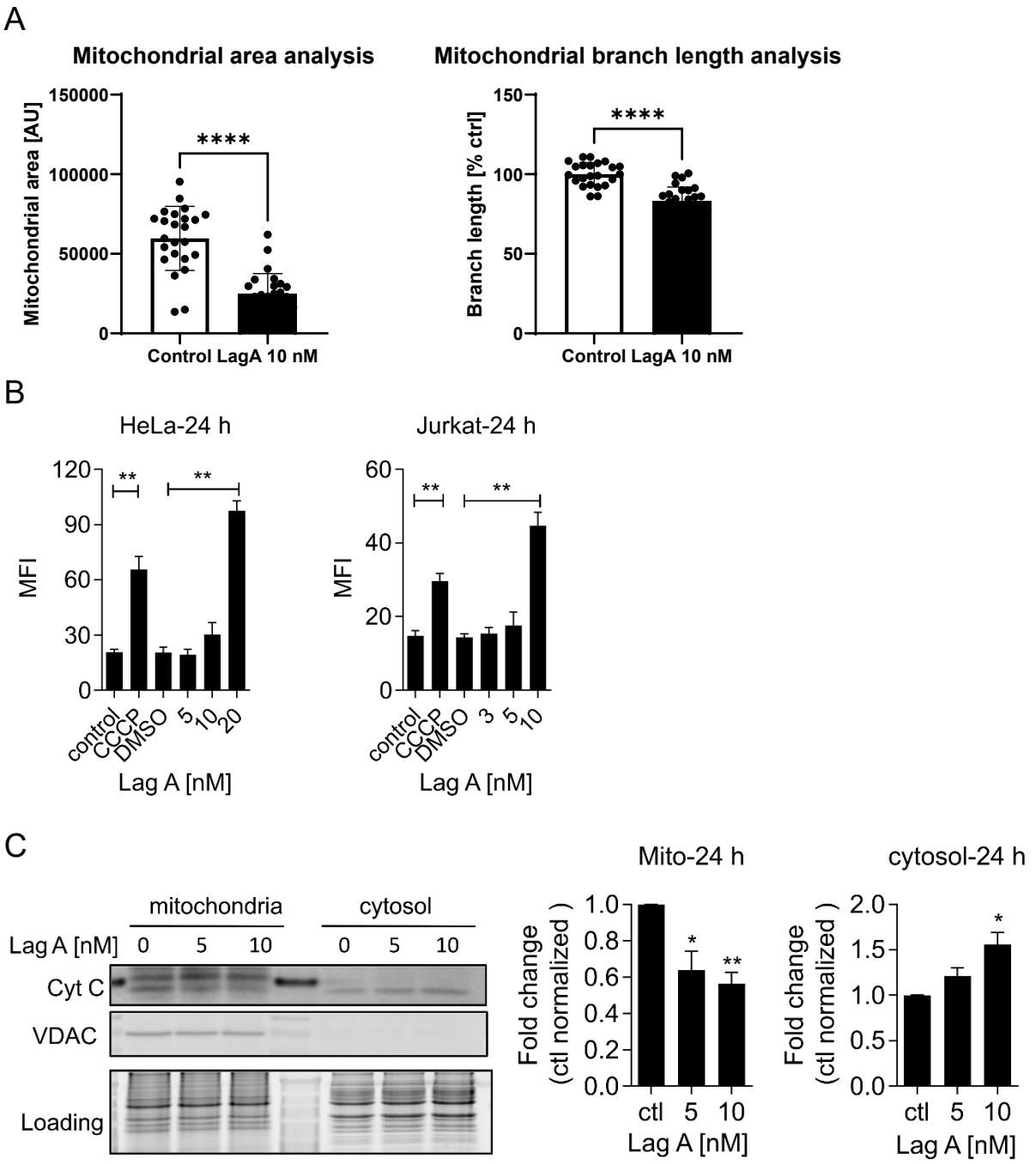
Supplementary Figure S1





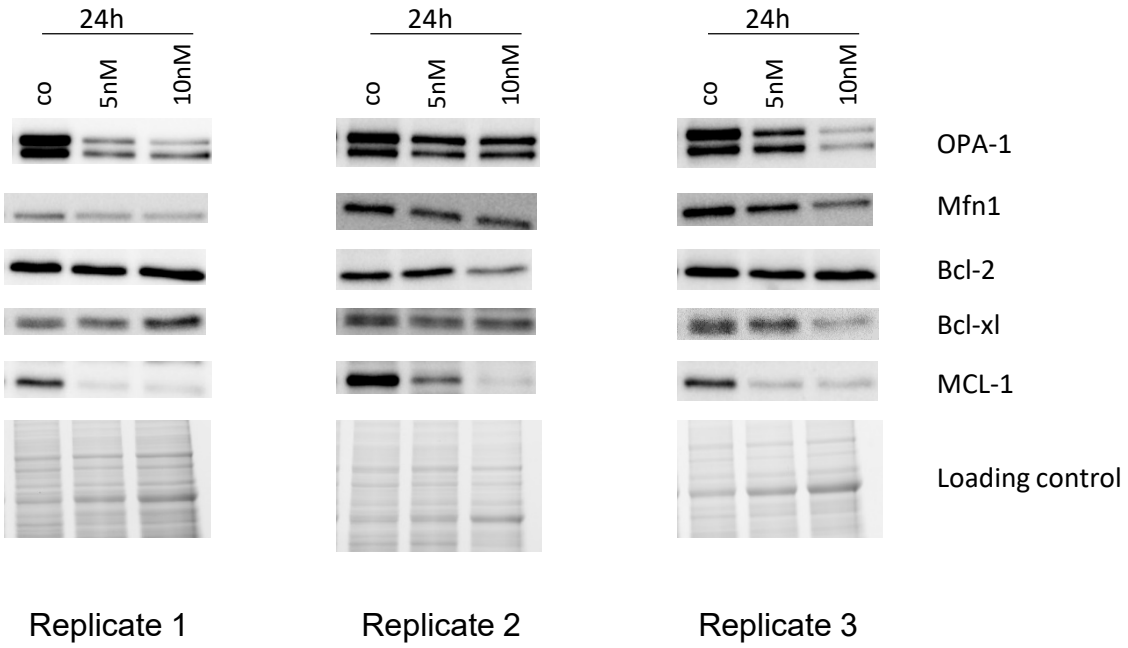
Supplementary Figure S1. Lag A impairs mitochondrial function. (A) Mitochondrial membrane potential of Jurkat cells treated with indicated concentrations of Lag A for 24 h was measured by JC-1 staining and FACS analysis. Representative Dot plots are shown. (B) Quantitative analysis (percentage of cells gated in panel A) of three independent replicates. Data are plotted as the means ± SEM, n=3. (one-way ANOVA, Dunnett's test, * $p<0.033$, ** $P<0.002$).

Supplementary Figure S2



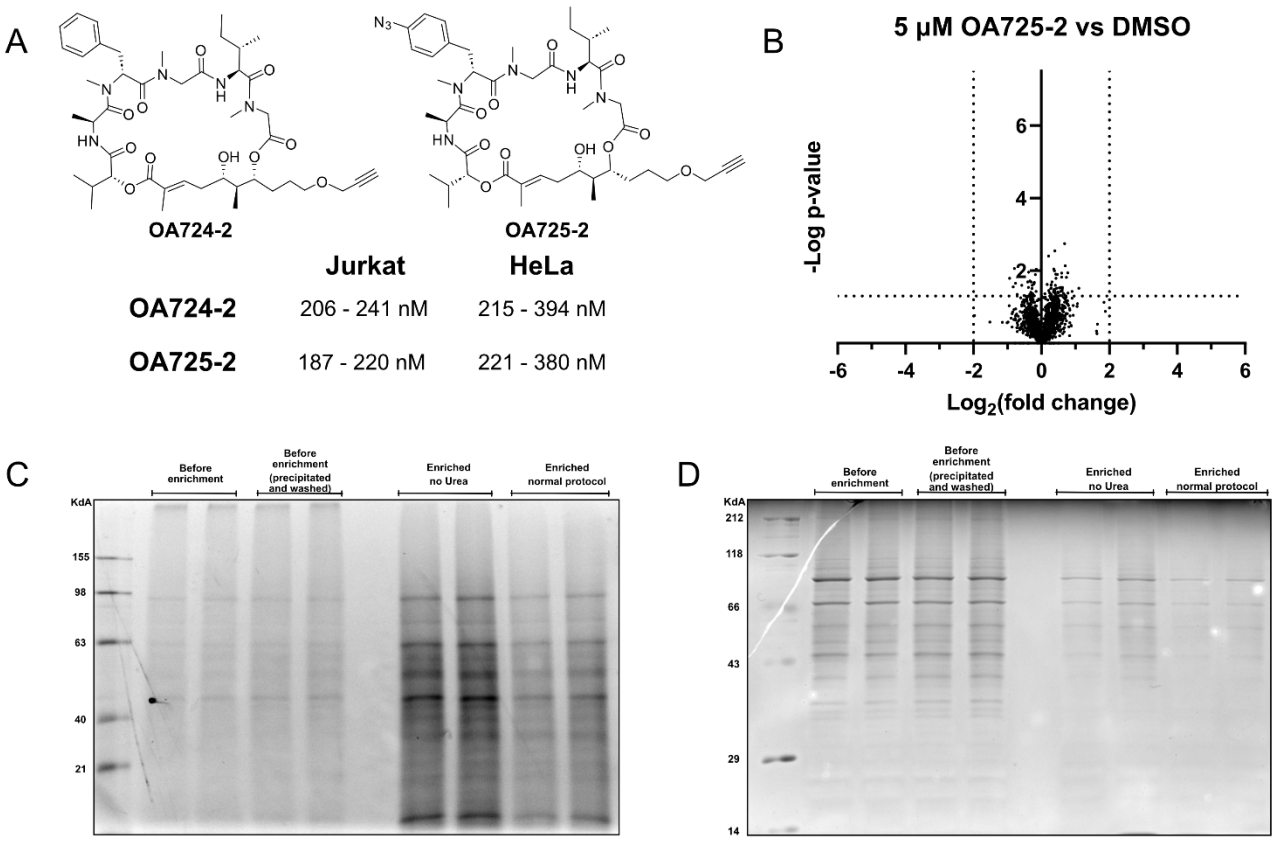
Supplementary Figure S2. Lag A impairs mitochondrial function. (A) Quantitative evaluation of the images of Figure 3 A and B in the main text. Left: Mitochondrial area of 40 mitochondria in the electron microcopy images, right: analysis of mean mitochondrial branch length (24 cells in the control group, 26 cells in the LagA treated group) in the confocal images. (B) Production of intercellular reactive oxygen species (ROS) in cells treated with indicated compounds for 24 h was detected by carboxy-H2DCFDA staining and FACS analysis. DMSO and CCCP were served as control and positive control respectively. Data are plotted as the means ± SEM, n=3. (one-way ANOVA, Dunnett's test,** P<0.002). (C) Cytochrome C release from mitochondria to cytosol was measured by sub-cellular fractionation of HeLa cells treated with Lag A for 24 h and western blot. VDAC serves as a marker of mitochondria. Bar graphs (C, right) indicate the expression level of cytochrome c in mitochondria and cytosol analyzed by ImageJ. Data are plotted as the means ± SEM, n=3. (one-way ANOVA, Dunnett's test, * p<0.033, ** P<0.002).

Supplementary Figure S3



Supplementary Figure S3. Lag A impairs mitochondrial function. Western blots of three independent replicates. OPA-1, Mfn1 and MCL-1 are consistently downregulated by treatment with Lag A for 24h. Images are related to Fig. 3 in the main text.

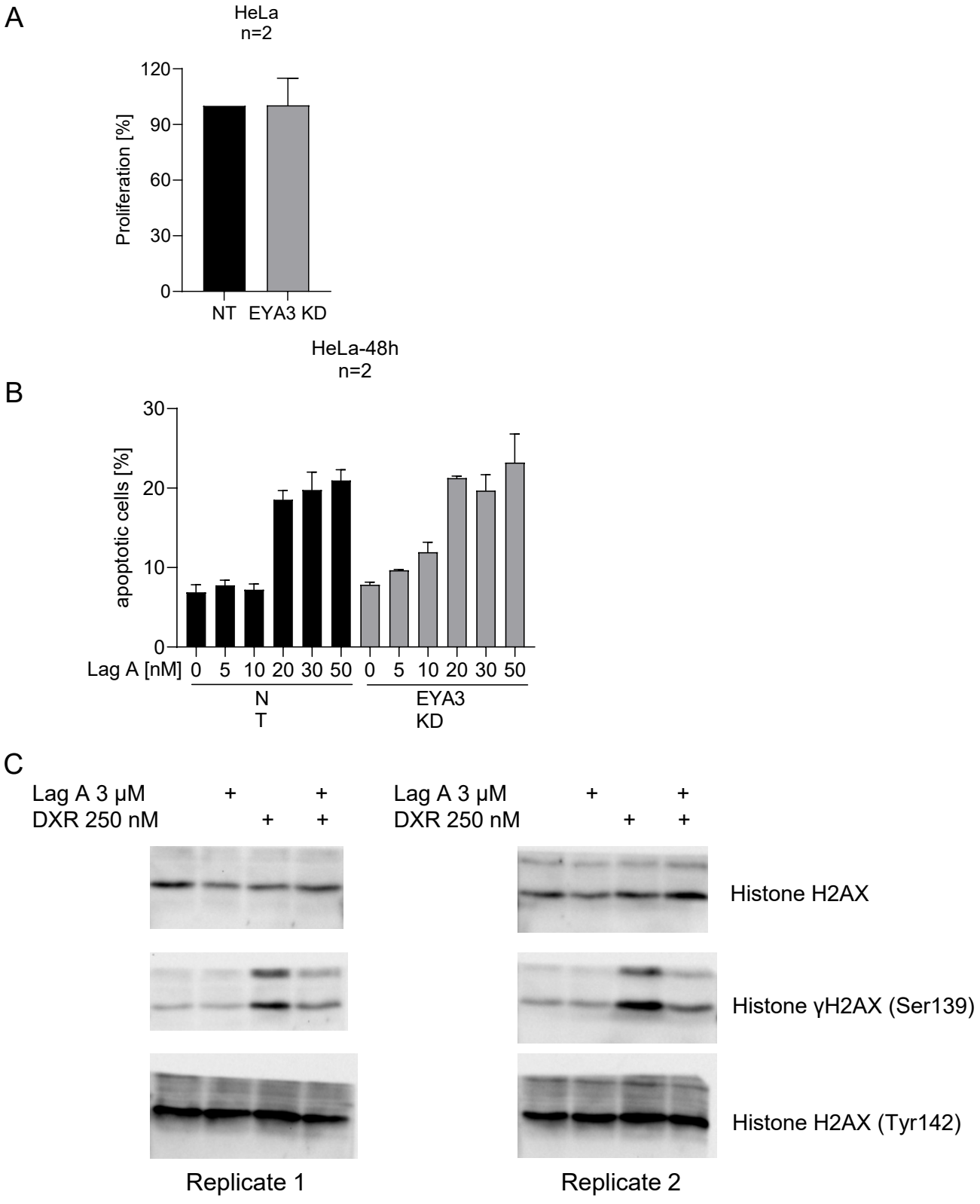
Supplementary Figure S4



Supplementary Figure S4. Affinity based protein profiling using Lag A photo-affinity probes did not identify any protein targets

(A) Structure of the photo-affinity probe OA-725-2 and its non-photo-affinity derivative OA-724-2. IC50 of relative metabolic activity measured using MTT assay are given for HeLa and Jurkat cells (95 % CV). (B) Volcano-plot of HeLa lysate treated with 5 µM OA725-2 vs. DMSO. Dotted lines indicate cut-off at $p < 0.05$ and a Log2(fold change) > 2. (C) Fluorescent imaging of SDS-PAGE gel to visualise labelled protiens. HeLa lysate was treated with 5 µM OA725-2 for 30 minutes, UV irradiated and clicked to a trifunctional linker bearing a biotin and rhodamine moiety. Samples were taken before and after enrichment. Experiment was conducted in duplicates and using different enrichment protocols. (D) Coomassie stained SDS-PAGE gel. The same gel as in (C) but stained with coomassie to visualise all proteins. Figure C and D indicate that the observed photo-crosslinking seems to be unspecific. One would expect the relative abundance of proteins to change after enrichment with avidin beads. This was not the case in this experiment as can be seen in the coomassie stained gel.

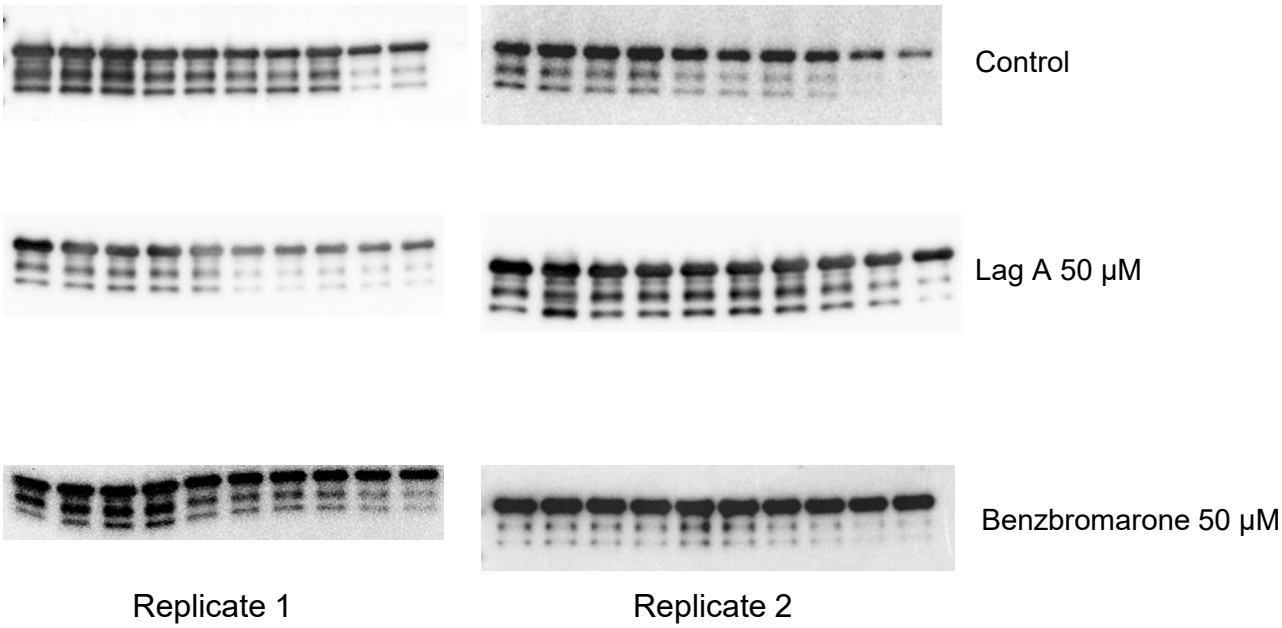
Supplementary Figure S5



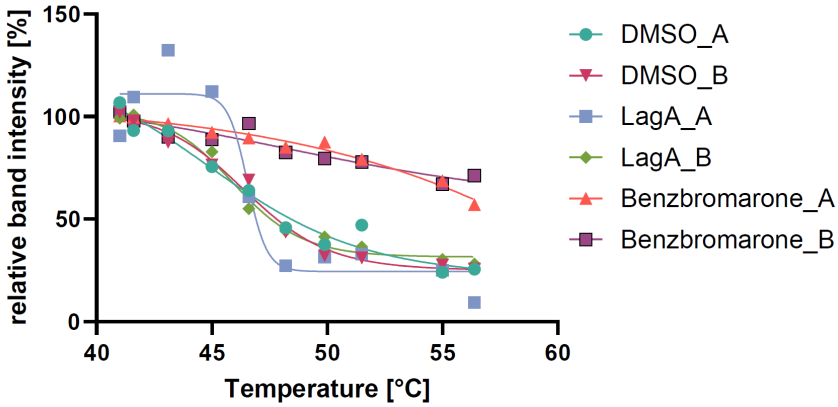
Supplementary Figure S5. (A) Knockdown of EYA3 does not influence proliferation. (B) Knockdown of EYA3 does not change the degree of apoptosos after treatment with Lag A alone. (C) Treatment with doxorubicine (DXR) causes phosphorylation of HsAX at Ser139. This effect is blocked by Lag A (middle panel), while total levels of H2AX remain constant (upper panel). Phosphorylation of H2AX at Tyr142 remains constant under all conditions (bottom panel).

Supplementary Figure S6

A



B



Supplementary Figure S6. (A) Thermal stability of isolated EYA under control conditions and after coincubation with 50 µM Lag A or the established EYA3 inhibitor benzbromarone. Western blots for EYA3 of two independent replicates are shown. (B) Densitometric analysis of the blots in (A). While benzbromarone clearly stabilize EYA3, Lag A has no such effect.

Supplementary Table S1

Bliss values (> 0 represents synergism)	DXR 1 µM	Synergistic effect
5 nM Lag A	5.76	Yes
10 nM Lag A	2.61	Yes

Supplementary Table 1: Evaluation of synergistiv effects of Lag A and DXR in HeLa cells by using the Bliss independence model.

Supplementary Table S2

Protein ID	Gene	ΔTm replicate 1 [°C]	ΔTm replicate 2 [°C]	adj. p-value replicate 1	adj. p-value replicate 2
O14777	NDC80	3.64	3.12	0.0224	0.1624
P16383	GCFC2	4.02	4.58	0.0649	0.0000
Q14683	SMC1A	4.62	3.62	0.0196	0.0024
Q99504	EYA3	4.28	4.00	0.0033	0.0003
P62888	RPL30	-5.30	-3.95	0.0057	0.0660
P62906	RPL10A	-4.19	-3.43	0.0353	0.0311
Q9H2W6	MRPL46	-6.22	-7.78	0.0003	0.0000

Supplementary Table 2: TPP hits that passed all significance filters