IN VIVO PROXIMITY LABELING FOR THE DETECTION OF PROTEIN–PROTEIN AND PROTEIN–RNA INTERACTIONS

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1 | 293T-REx proliferation in presence of bio-ASA

293T-REx were plated at 50,000 cells/well in 6-well plates. Ten μM bio-ASA or vehicle control were added after 34 hours and the cells were harvested 2, 24, and 48 hours after treatment. Symbols indicate the mean of three biological replicates + s.d.

Figure S2 | Comparison of photoactivatable groups for IPL

(A) Chemical structure of 4 probes screened for IPL. First generation probes have a biotin (blue) linked by a spacer to a different photoactivatable group (red): I) psoralen, II) tetrafluorophenyl azide, III) benzophenone, IV) hydroxyphenyl azide. The distance of the photoactivatable group from biotin is indicated below each molecular structure.

(B) Test IPL using compounds II, III, and IV on 293T-REx cells expressing mSA–EZH2 or GAL4–EZH2 (negative control). Proteins were detected by SDS-PAGE and western blot for EZH2 before (left panel) and after pull-down with wild-type streptavidin-conjugated beads (right panel). The position on the blot of tagged (mSA and GAL4 have approximately the same mass) and endogenous EZH2 are indicated by arrows.

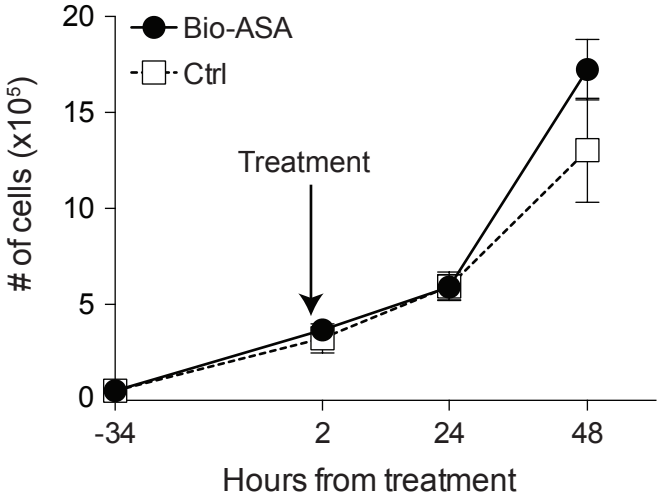
Figure S3 | Optimization of probe and detergent concentration for PRC2 IPL

(A) Titration of bio-ASA for IPL of cells expressing mSA–EZH2 or GAL4–EZH2 (negative control) induced (+dox) or not induced (-dox) to express the transgene. Specific biotinylation on mSA–EZH2 (top band) was monitored by streptavidin pull-down followed by EZH2 western blot. The amount of endogenous EZH2 (bottom band) recovered is an indication of the background levels of non-specific biotinylation.

(B) EZH2 IP in the presence of the indicated amounts of deoxycholate or sarkosyl detergents to test for their ability to disrupt EZH2–EED interactions.

Figure S4 | Self-labeling of mSA–SNRNP70

IPL was performed on cells expressing mSA–SNRNP70 and FH–SNRNP70 (negative control) to identify SNRNP70-associated RNAs (see **Fig. 4C–D**). After labeling a small portion of the cells was set aside for lysis and protein pull-down with streptavidin (SA) to verify specific labeling of mSA–SNRNP70 by western blot. As an additional specificity control, streptavidin pull-down was also carried out in presence of an excess of soluble biotin (lanes 4 and 6).



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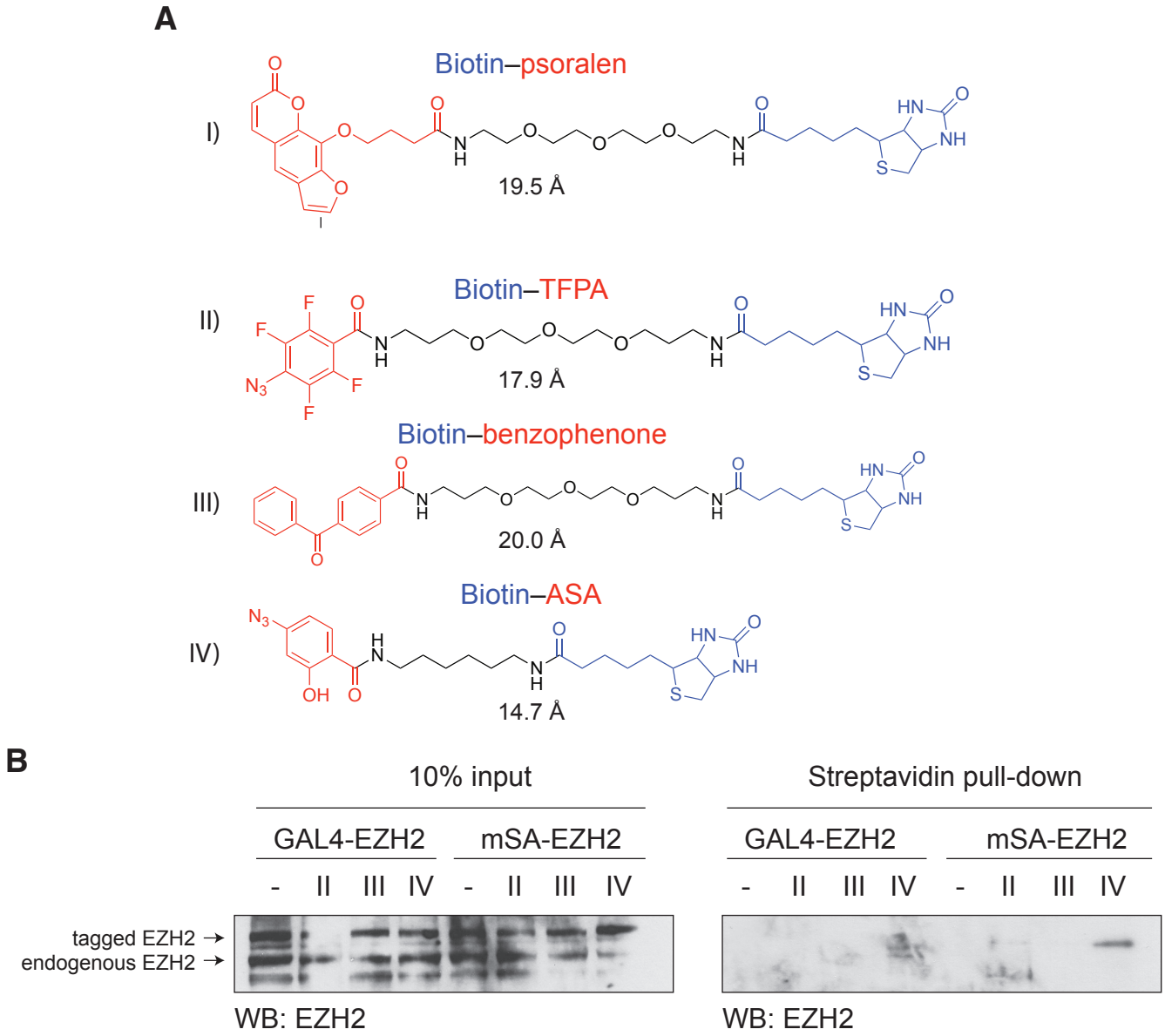

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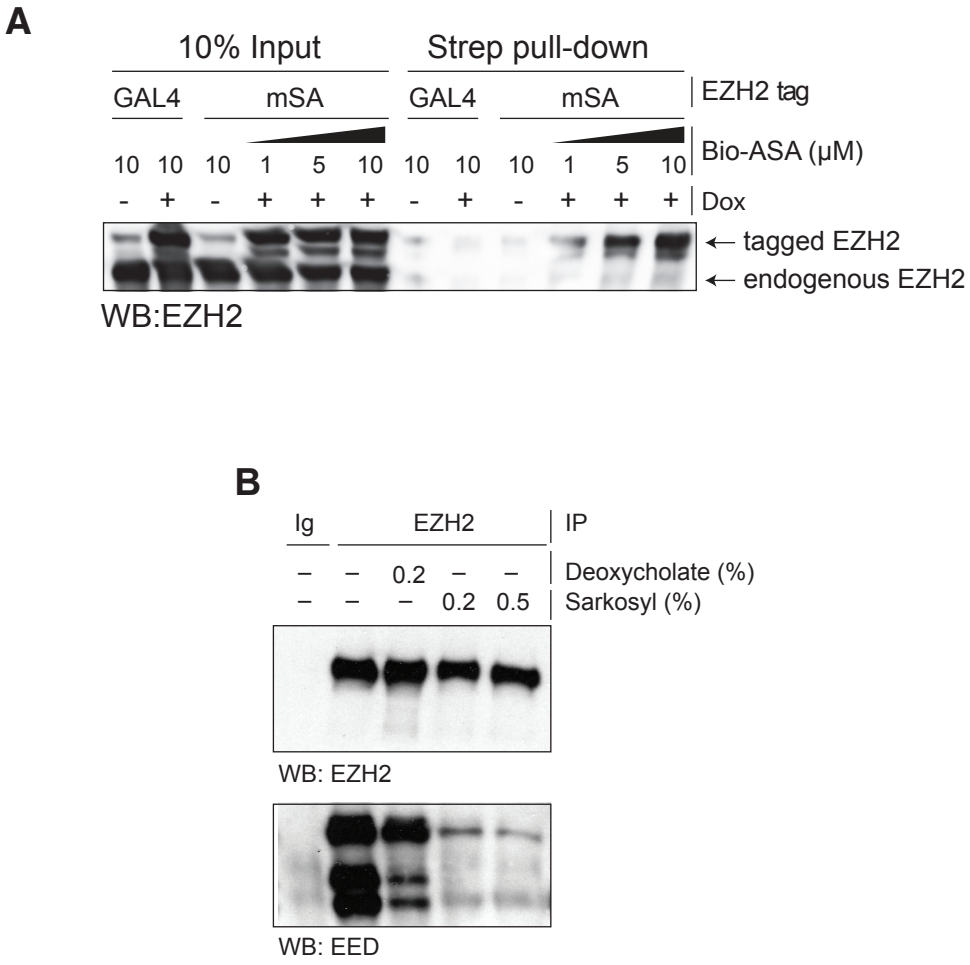

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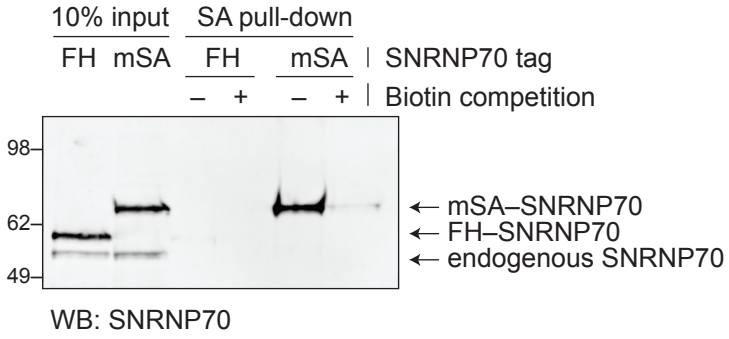

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