
Supplementary information

SARS-CoV-2 disrupts host epigenetic regulation via histone mimicry

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SARS-CoV-2 disrupts host epigenetic regulation via histone mimicry

Supplemental Information

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Table of Contents

Supplemental Figure and Table Legends. Pg. 3.

Supplemental Figure 1. Original western blot images. Pgs. 4-18.

Supplemental Figure 2. FACS gating strategy. Pg. 19.

Supplemental Table 1. Orf8 binding partners. Separate document.

Supplemental Table 2. Gene ontology analysis of gene expression changes following Orf8 expression. Separate document.

Supplemental Table 3. Histone PTM changes with Orf8 expression. Separate document.

Supplemental Table 4. All infection data. Separate document.

Supplemental Table 5. Gene ontology analysis of gene expression changes following SARS-CoV-2 infection of A549^{ACE} cells. Separate document.

Supplemental Table 6. Antibodies used in manuscript. Separate document.

Figure Legends

Supplemental Figure 1. Original western blot images. Full gel images for all western blot data. Corresponding figure panel is noted for each experiment. Orf8, H3, and all H3 modifications all run at the same size and thus in some instances controls such as unmodified H3 were run on separate gels (noted in figures).

Supplemental Figure 2. FACS gating strategy. Example of gating strategy and cell numbers isolated for all FACS experiments.

Supplemental Table 1. Orf8 binding partners. Full dataset of Orf8 binding partners identified through mass spectrometry. Note that methods used for Orf8 isolation for mass spectrometry differ for those used for identifying chromatin associated binding partners as shown in Extended Data Figure 4 which specifically enriched for Orf8 within the chromatin fraction.

Supplemental Table 2. Gene ontology analysis of gene expression changes following Orf8 expression. Gene ontology analysis from RNA-sequencing of Orf8 expressing HEK293T cells. Gene ontology significance based on clusterProfiler analysis with Benjamini-Hochberg adjusted p-values.

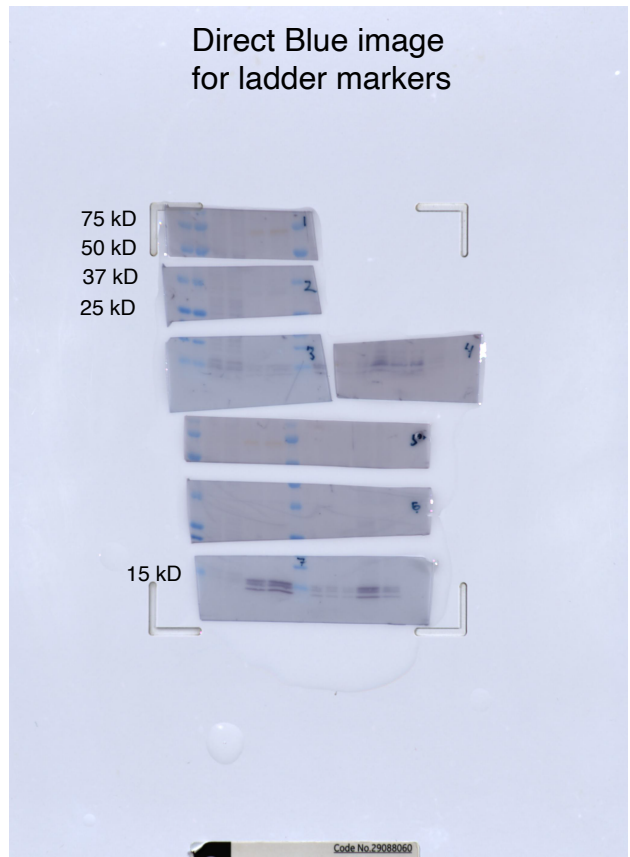
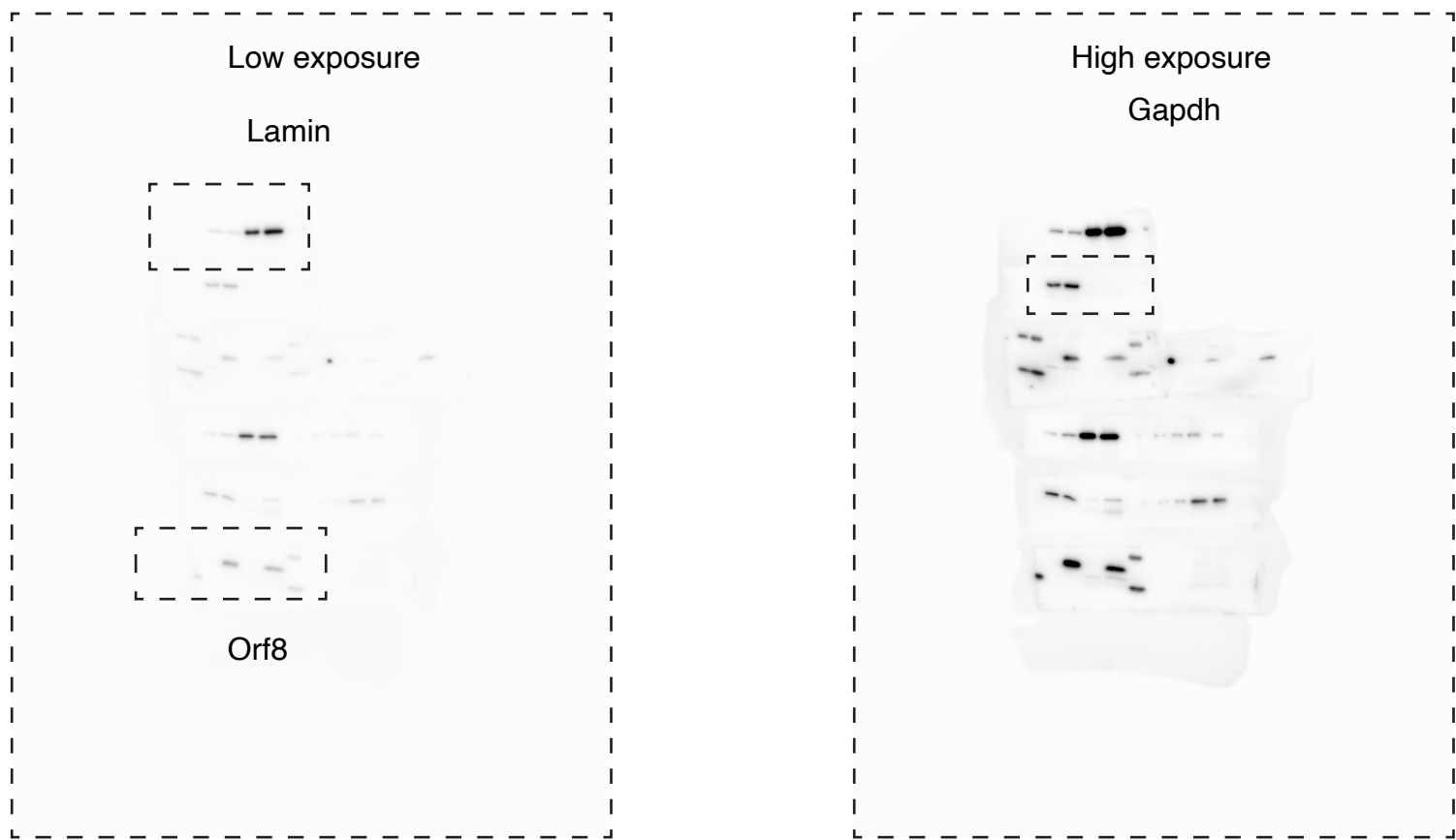
Supplemental Table 3. Histone PTM changes with Orf8 expression. Full dataset of histone post-translational modifications identified through mass spectrometry.

Supplemental Table 4. All infection data. All replicates of viral infection data including genome copy number and viral titer data for both A549^{ACE} cells and iAT2 cells.

Supplemental Table 5. Gene ontology analysis of gene expression changes following SARS-CoV-2 infection of A549^{ACE} cells. Gene ontology analysis from RNA-sequencing for A549^{ACE} cells following SARS-CoV-2 infection. Gene ontology significance based on clusterProfiler analysis with Benjamini-Hochberg adjusted p-values.

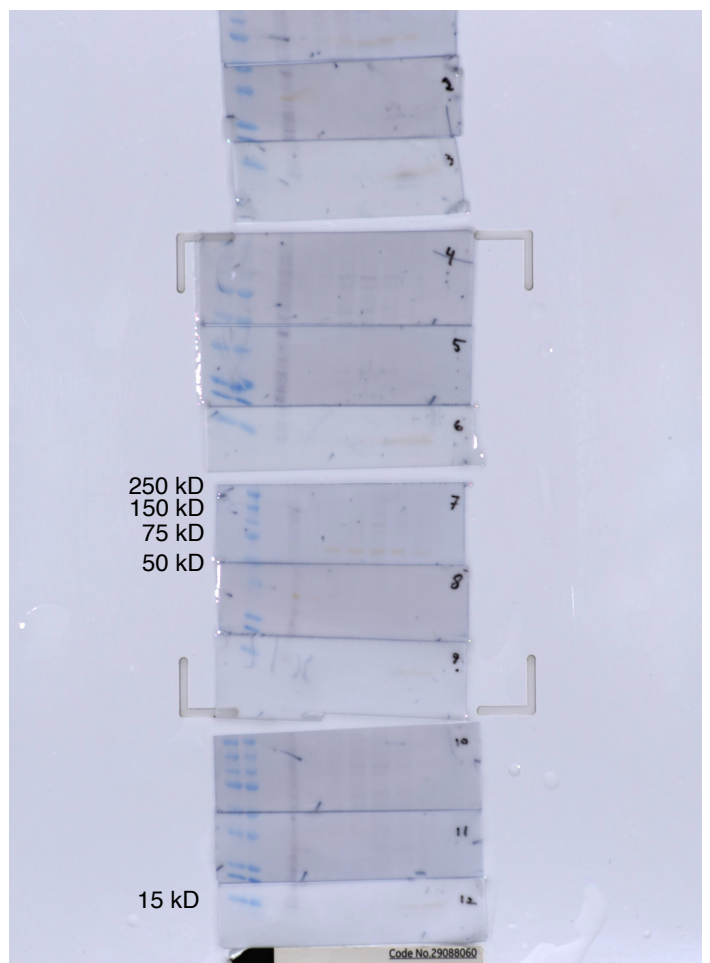
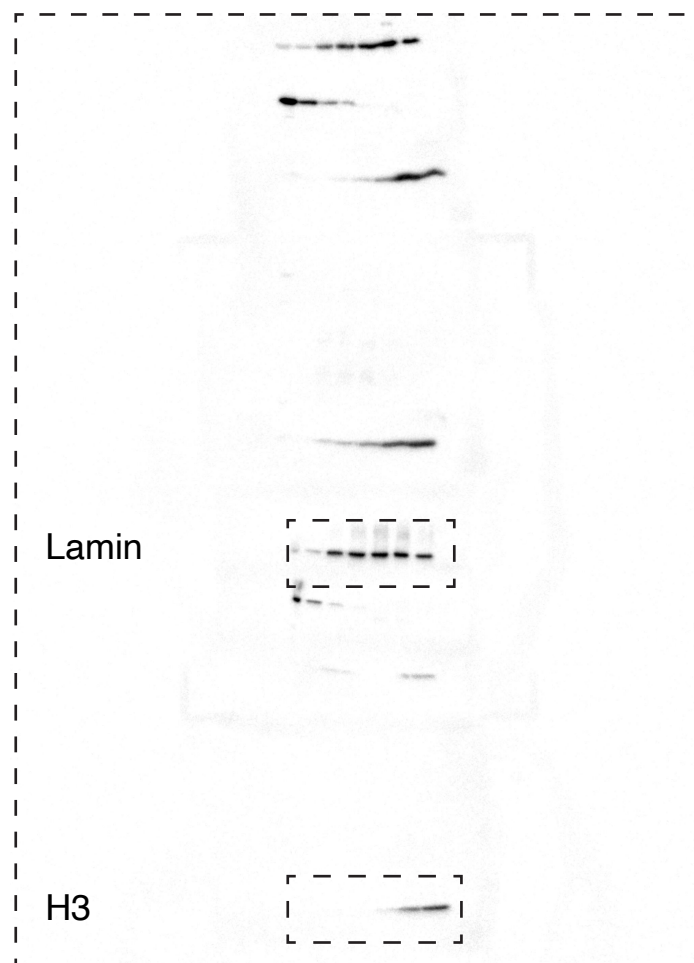
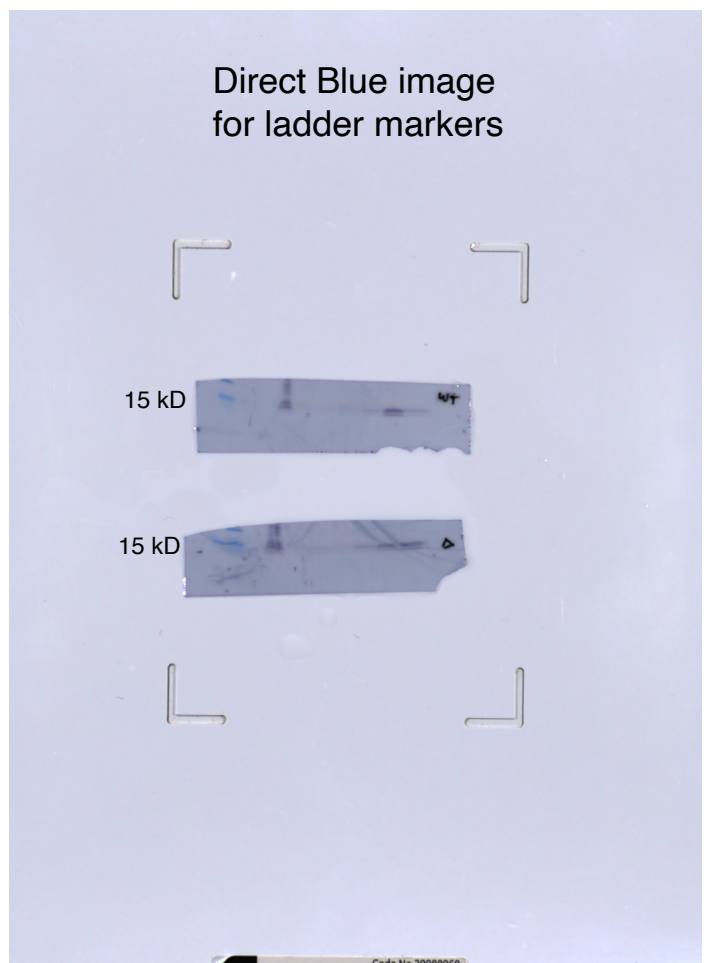
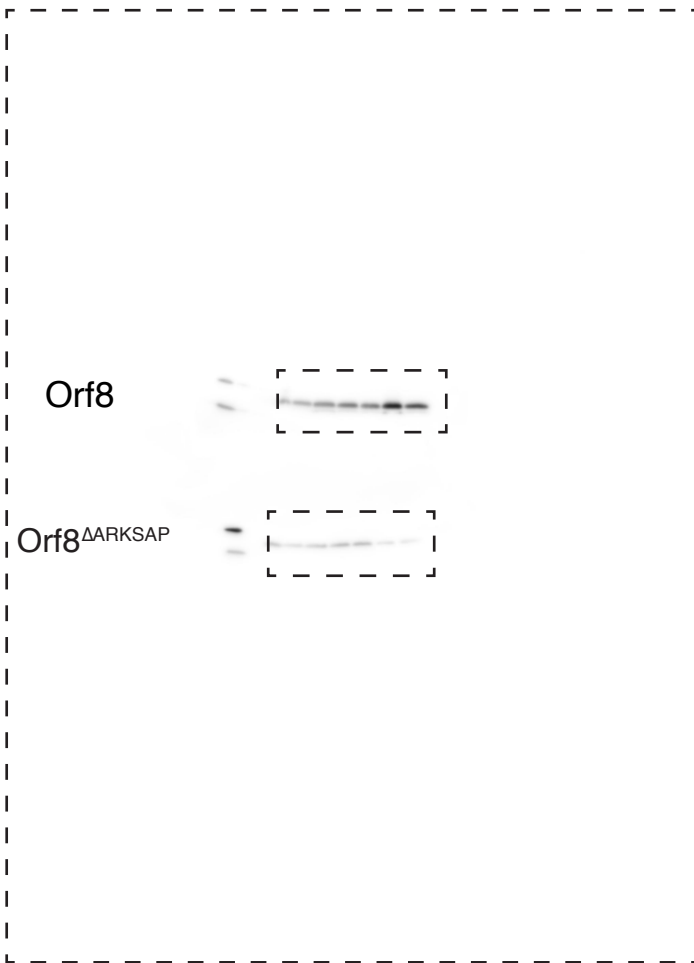
Supplemental Table 6. Antibodies used in manuscript. Information on all antibodies used. Assay abbreviations include: ICC = immunocytochemistry, WB = Western blot, IHC = immunohistochemistry.

Fig. S1a



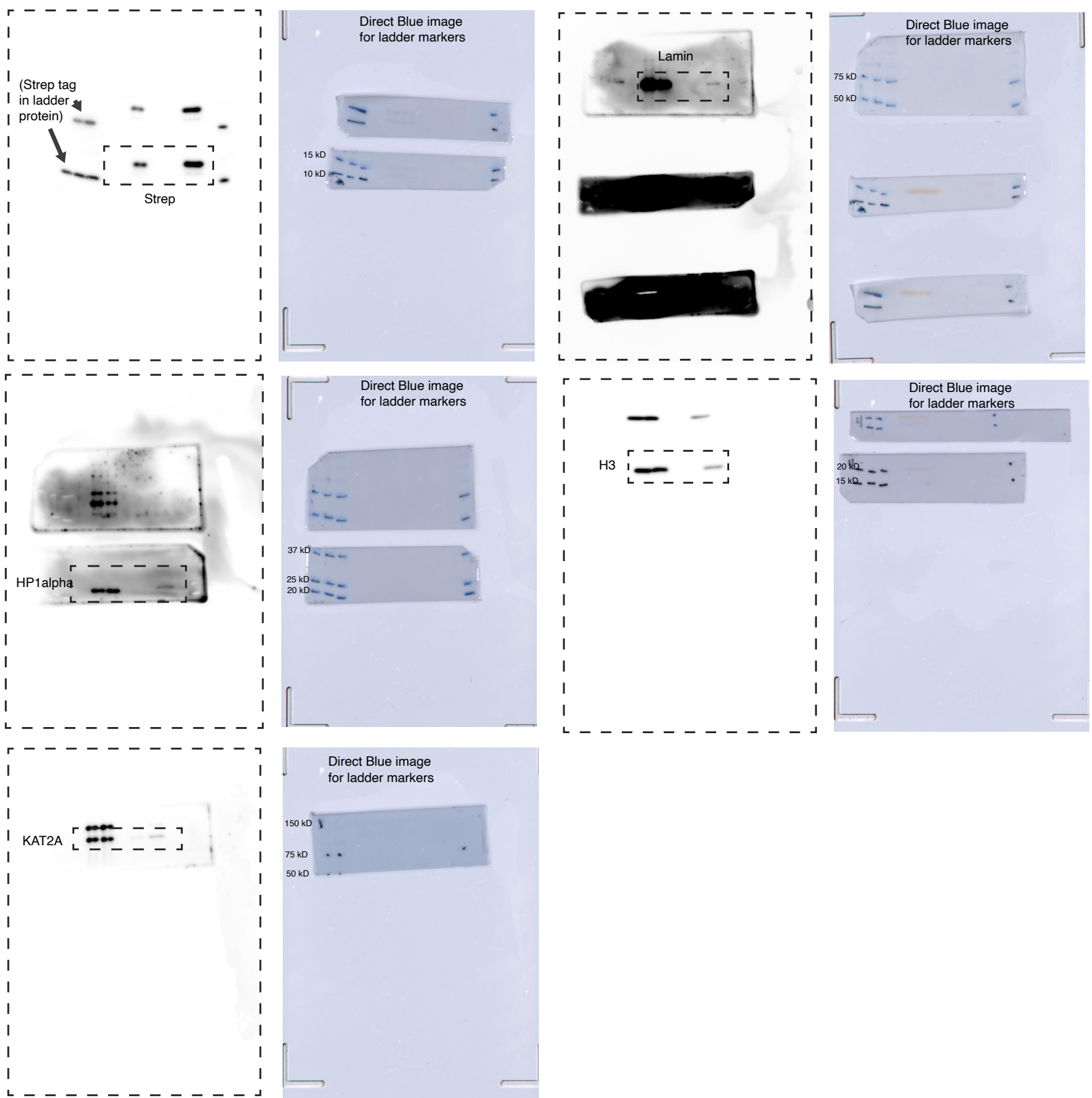
Supplemental Figure 1a. Nuclear Cytoplasmic Fractionation western blots from Extended Data Figure 2a. (Loading controls run on same gel)

Fig. S1b



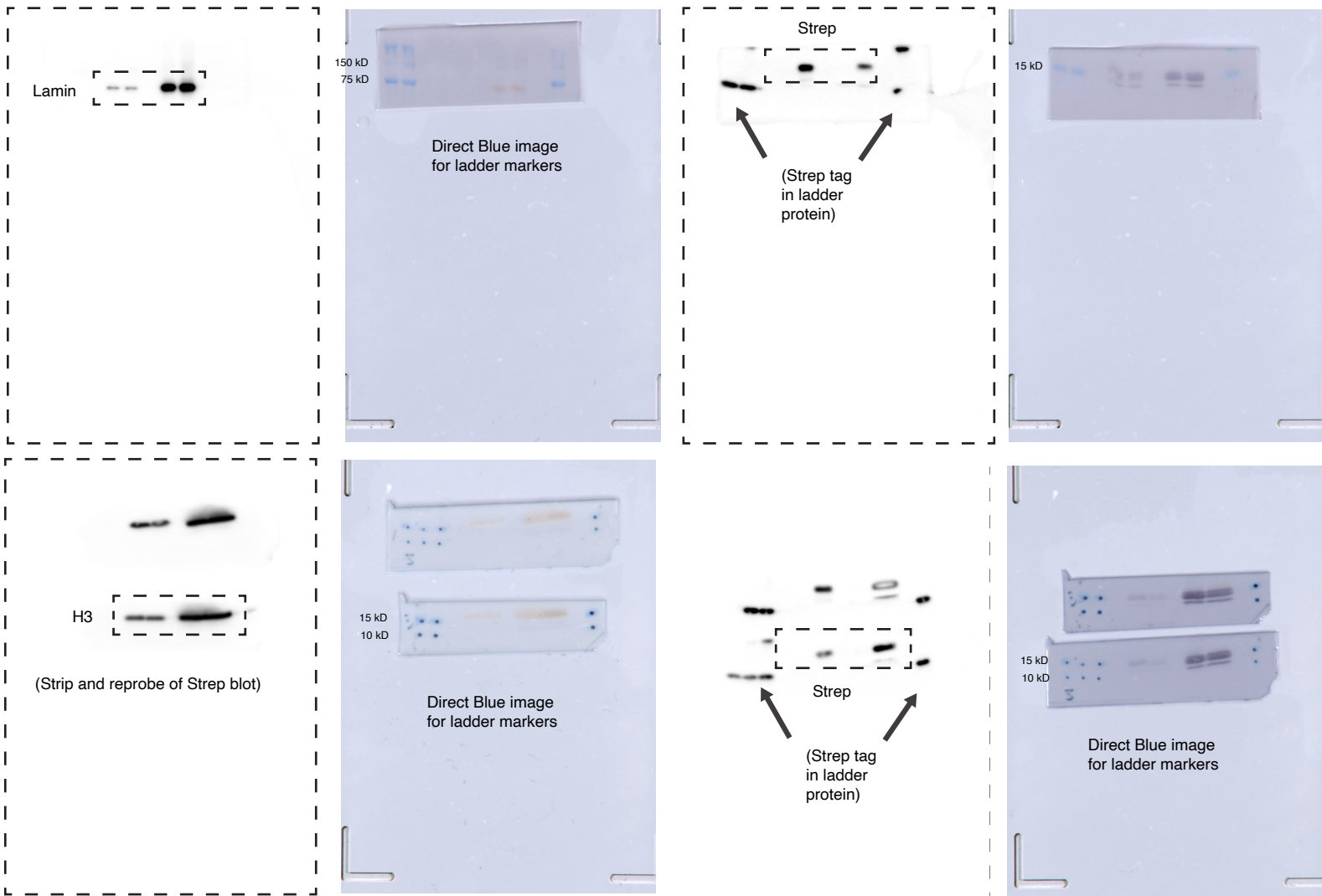
Supplemental Figure 1b Sequential Salt Extraction western blots from Figure 1c. 5
(Lamin controls run on same gels, H3 run on separate gels)

Fig. S1c



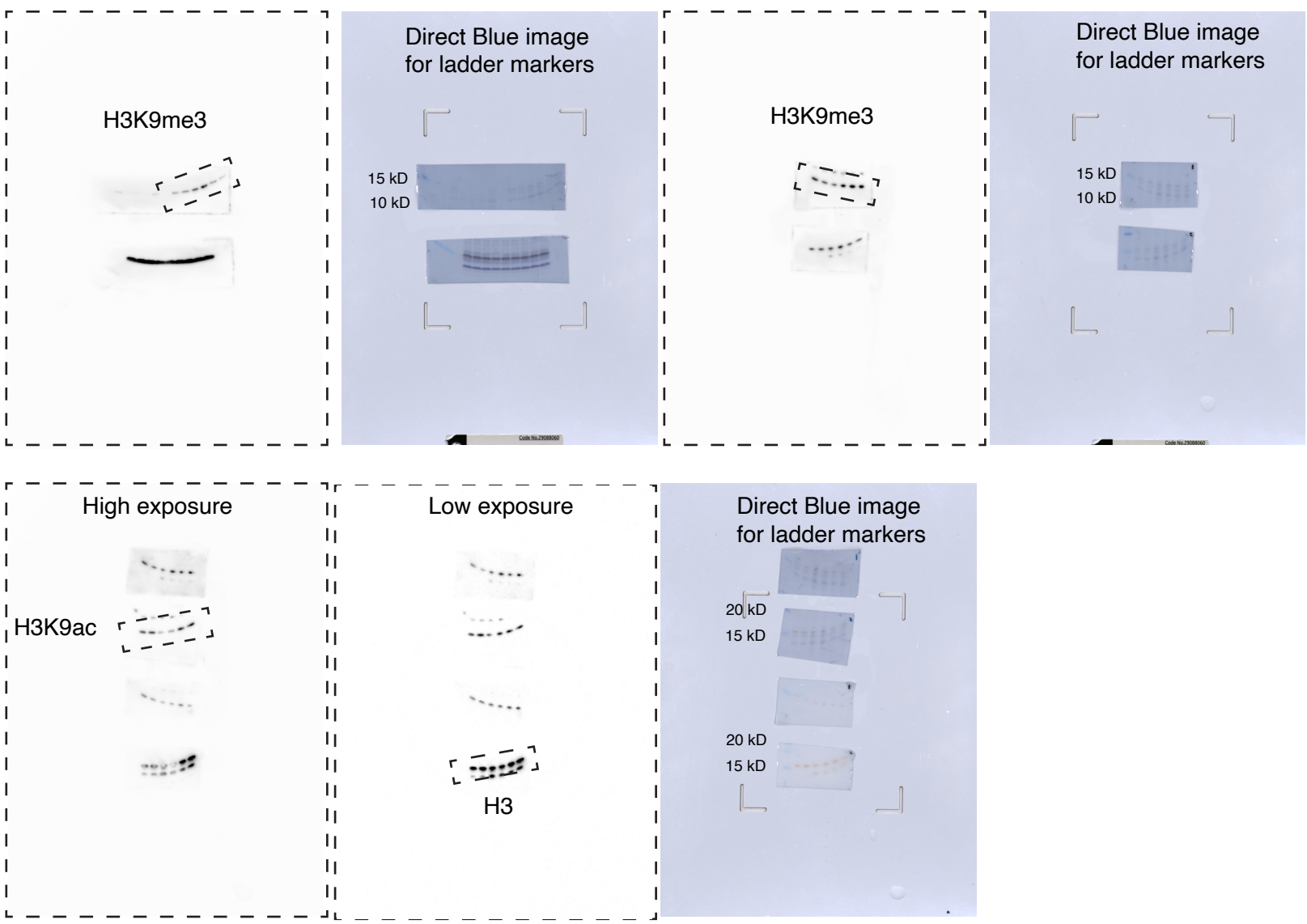
Supplemental Figure 1c. CoIP western blots from Extended Data Figure 3a

Fig. S1d



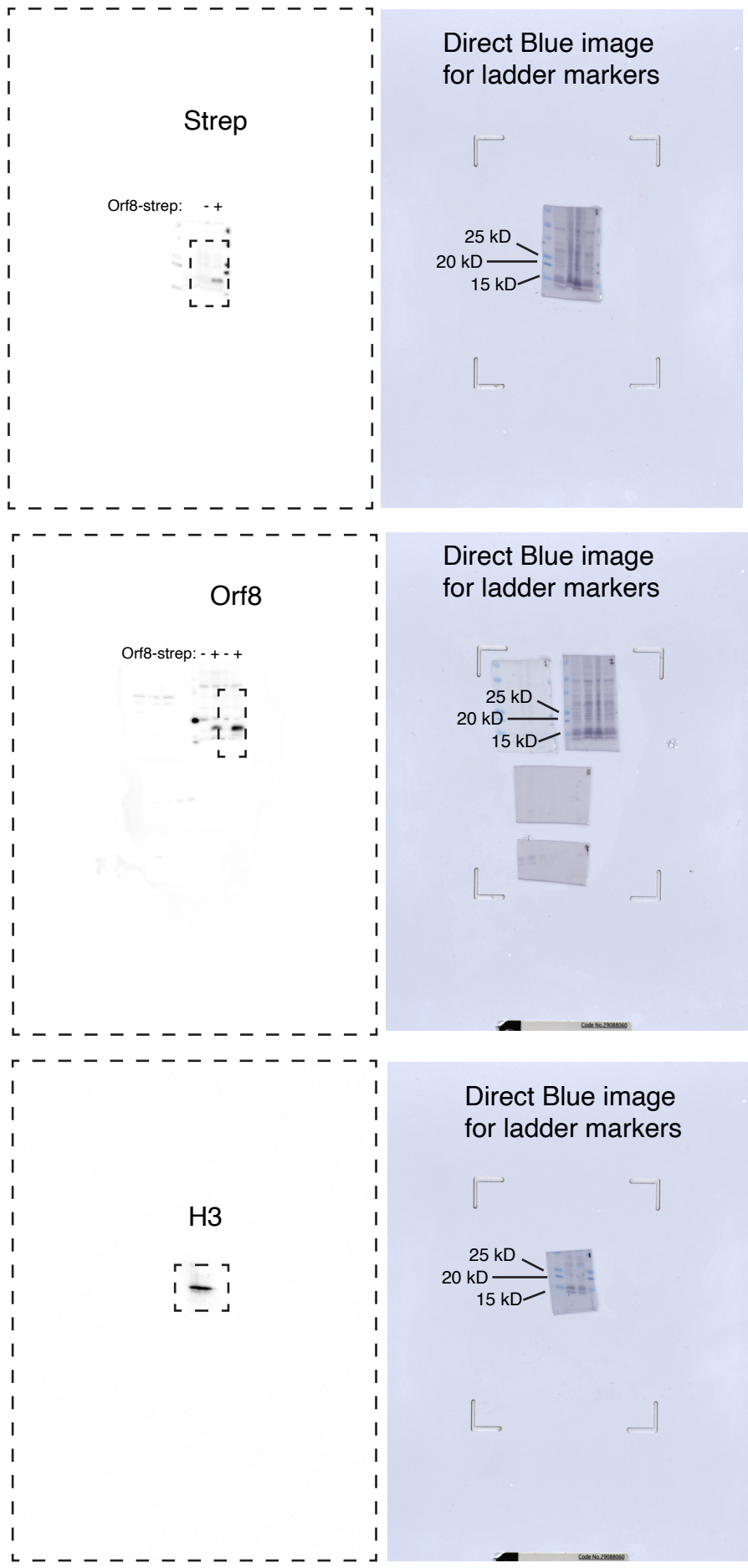
Supplemental Figure 1d. Reverse CoIP western blots from Extended Data Figure 3b

Fig. S1e



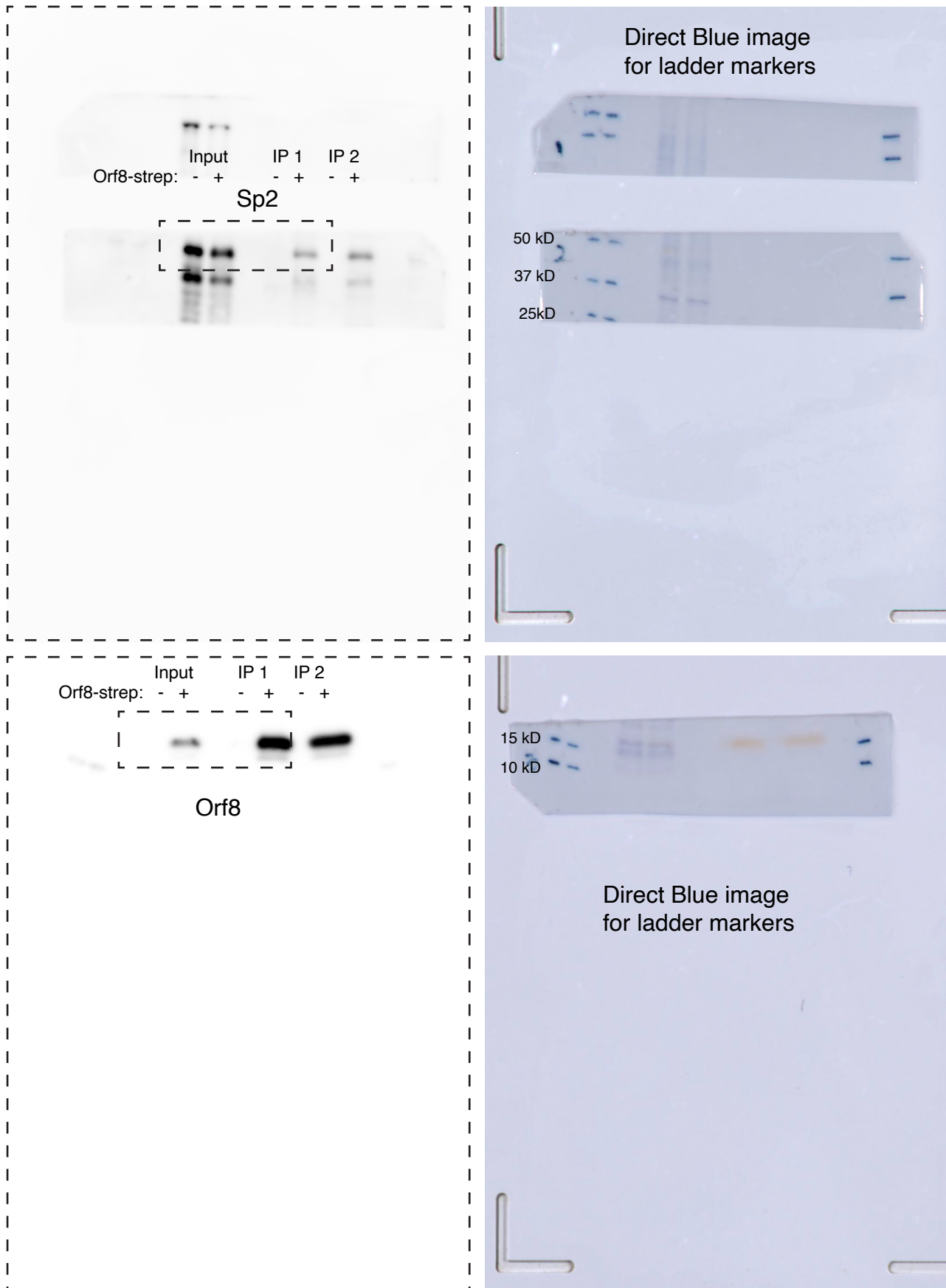
Supplemental Figure 1e. Histone PTM western blots from Figure 2h (H3 striped/reprobed from Histone H3 modification gel)

Fig. S1f



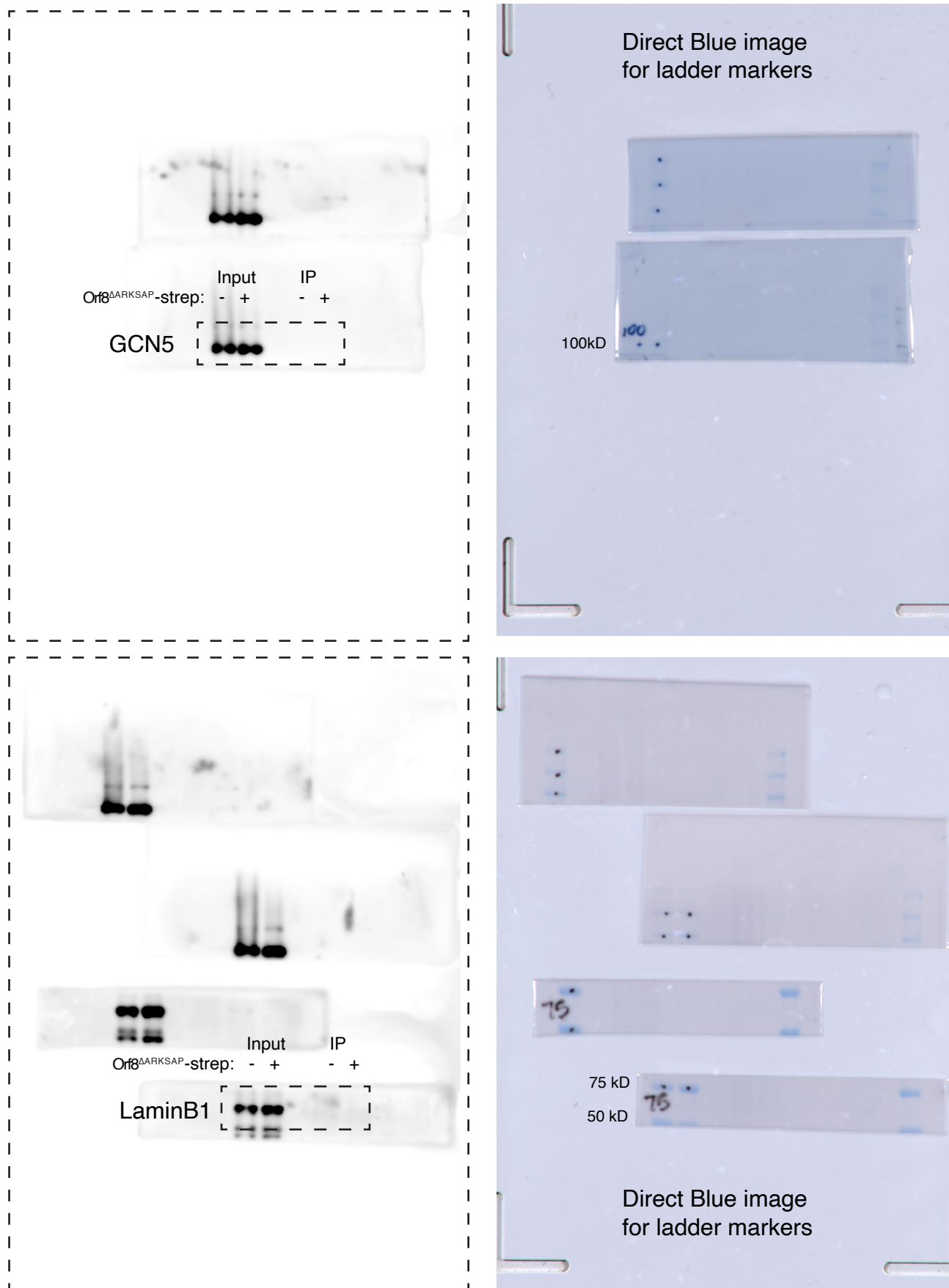
Supplemental Figure 1f. Orf8 antibody test western blots from Extended Data Figure 2c. (H3 and Orf8 run on separate gels)

Fig. S1g



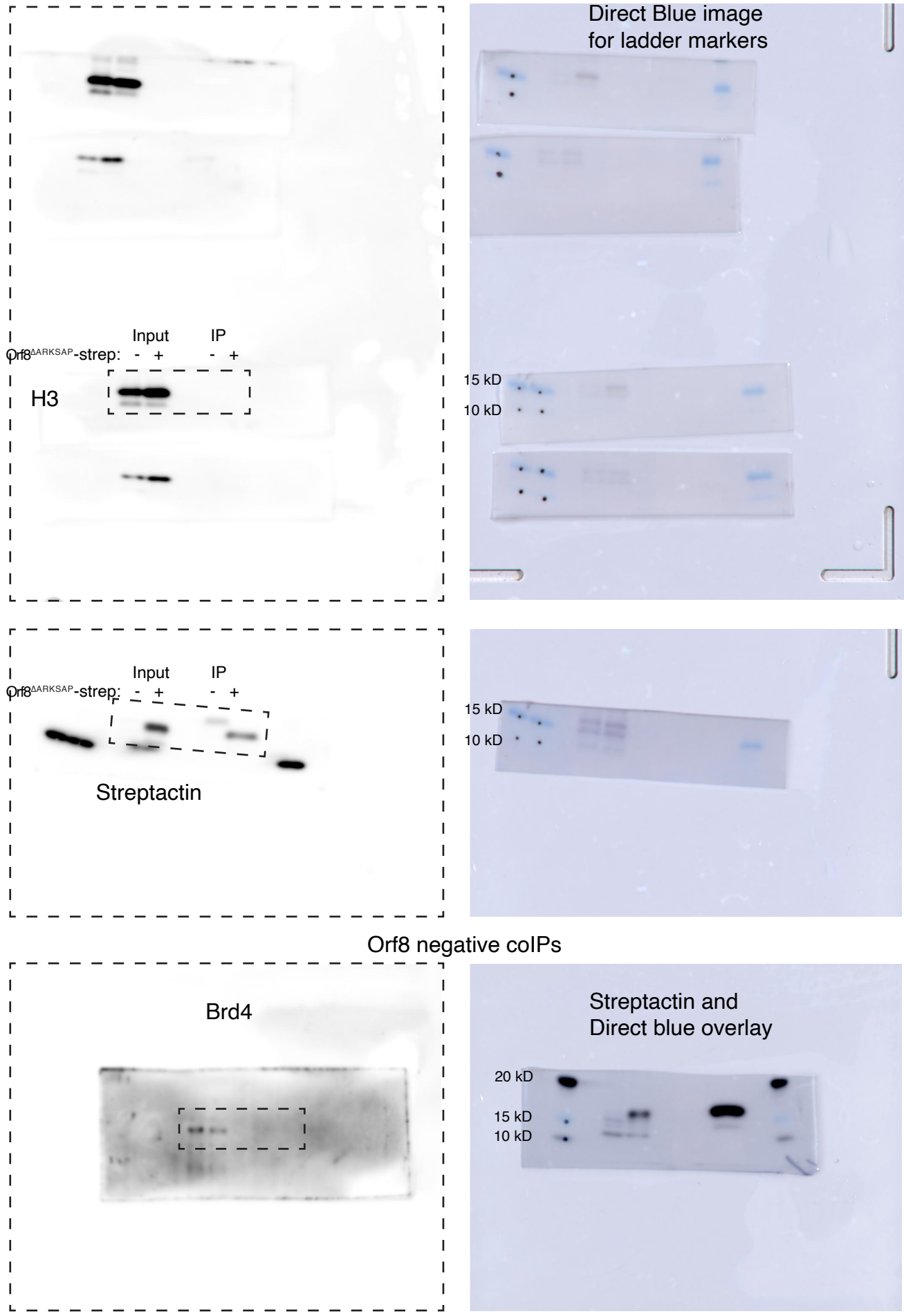
Supplemental Figure 1g. Orf8 CoIP western blots from Extended Data Figure 3f

Fig. S1h

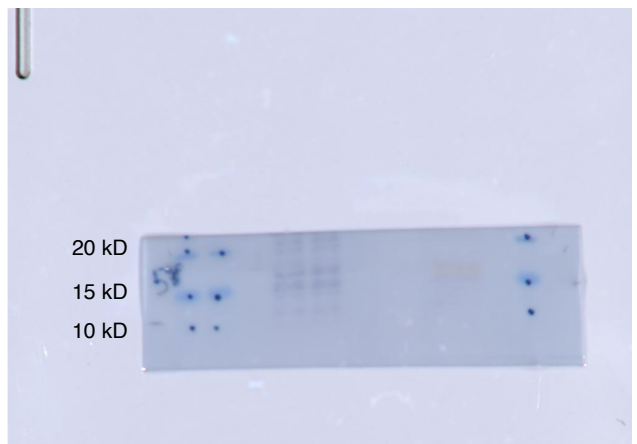
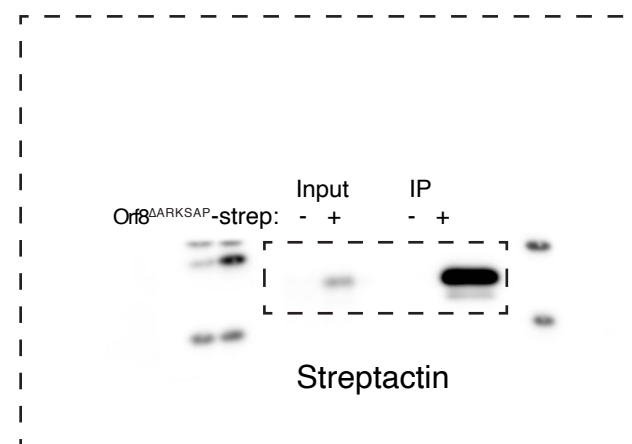
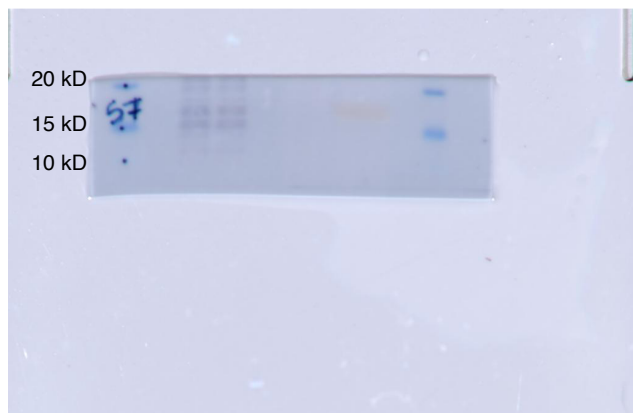
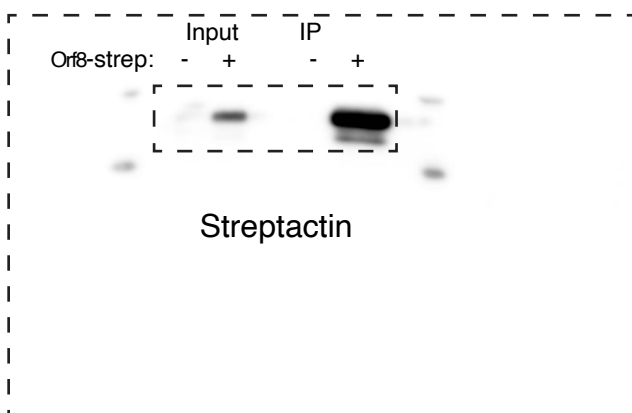
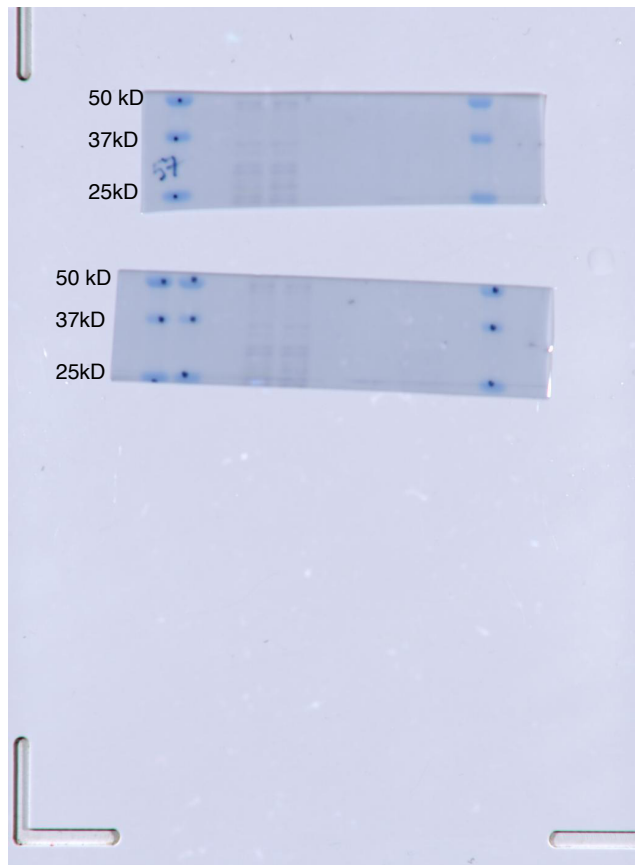
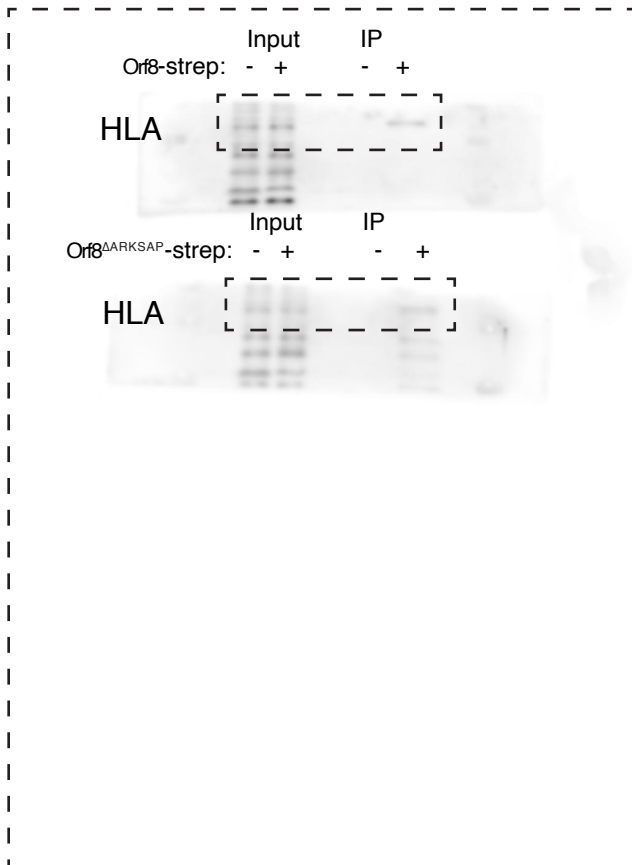


Supplemental Figure 1h. Orf8^{ΔARKSAP} CoIP western blots from Extended Data Figure 3c

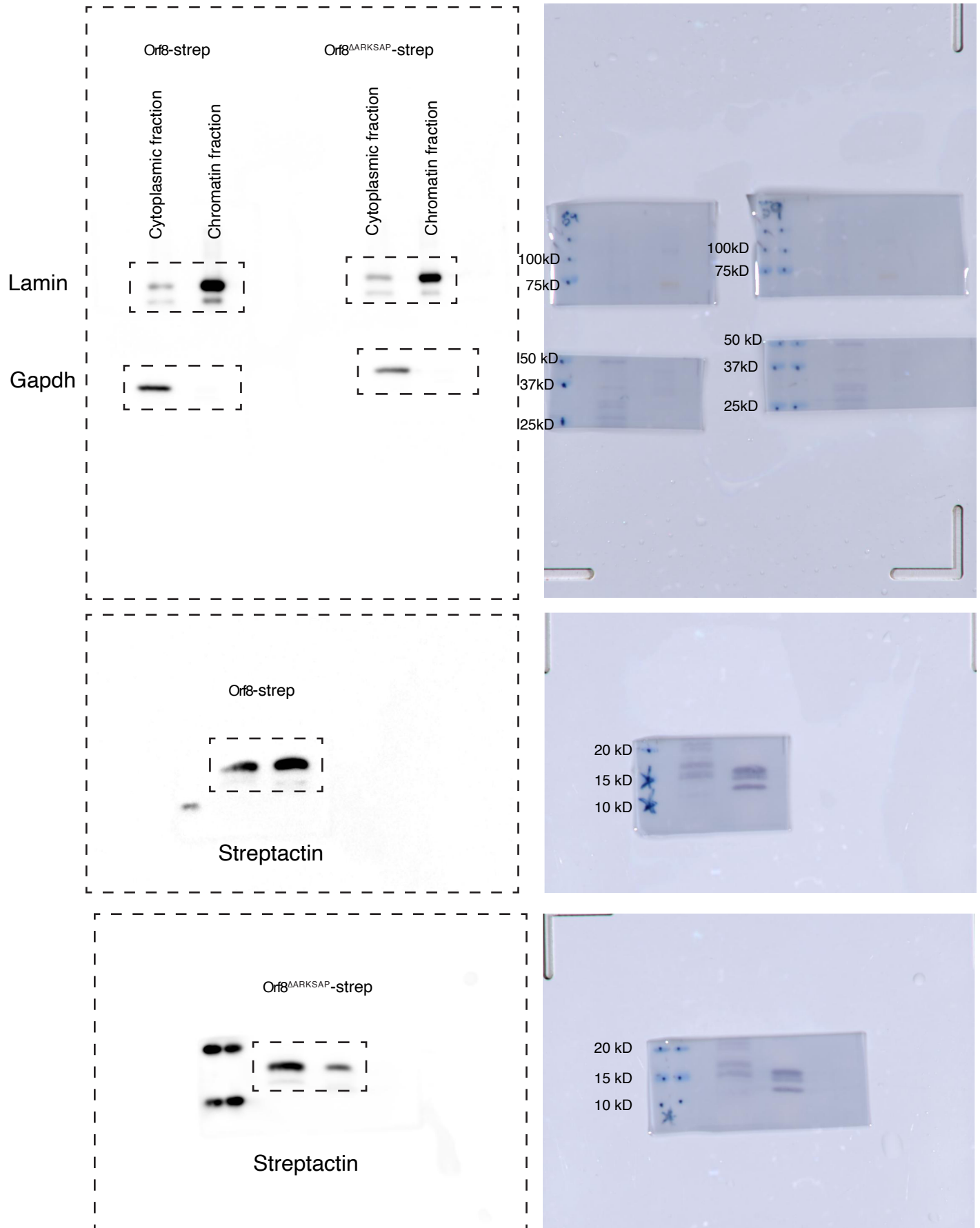
Fig. S1i



Supplemental Figure 1i. Orf8^{ΔARKSAP} and Orf8^{WT} CoIP western blots from Extended Data Figure 3c and 3e.

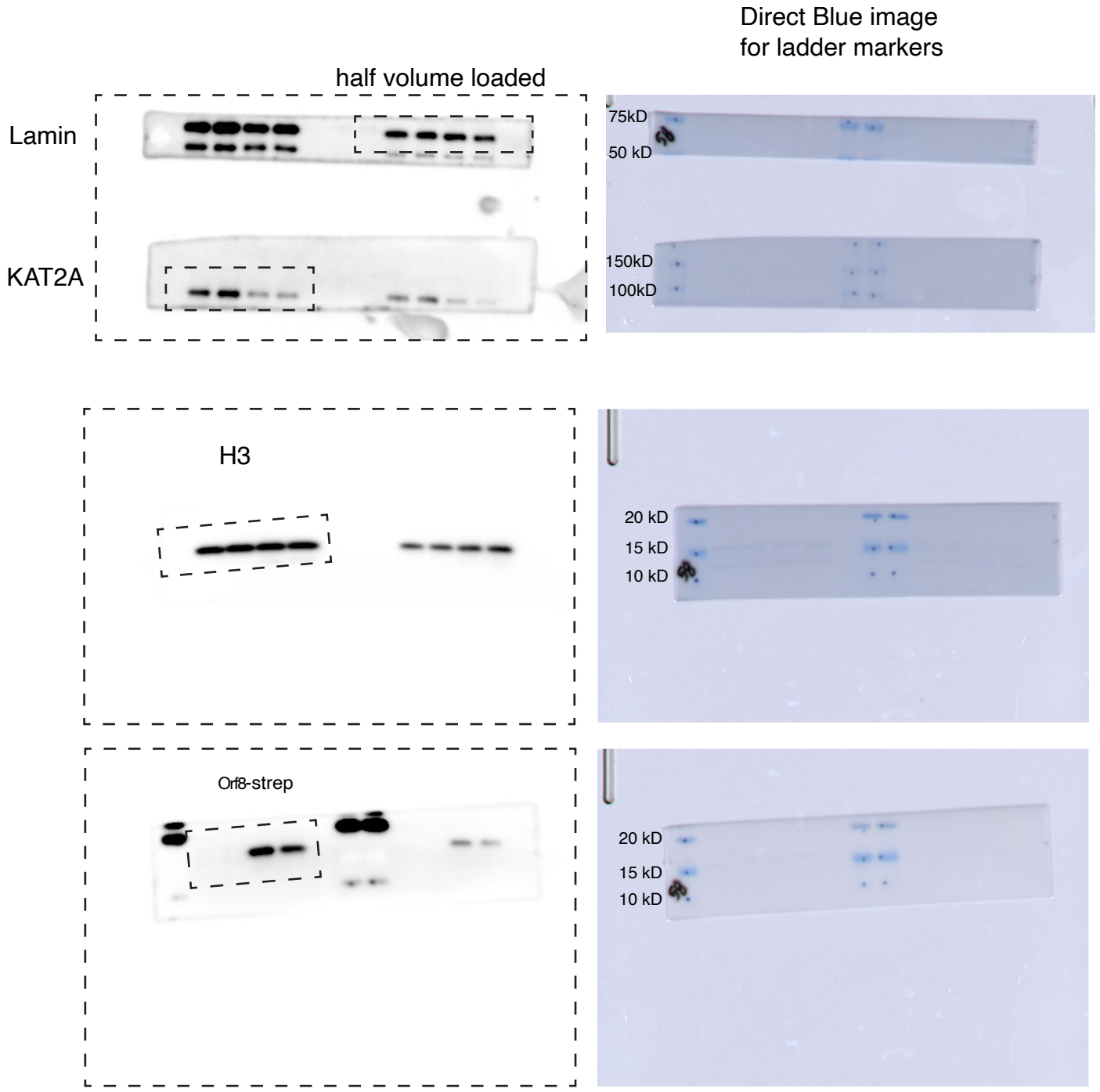


Supplemental Figure 1j. Orf8^{ΔARKSAP} and Orf8^{WT} CoIP western blots from Extended Data Figure 3c and 3d



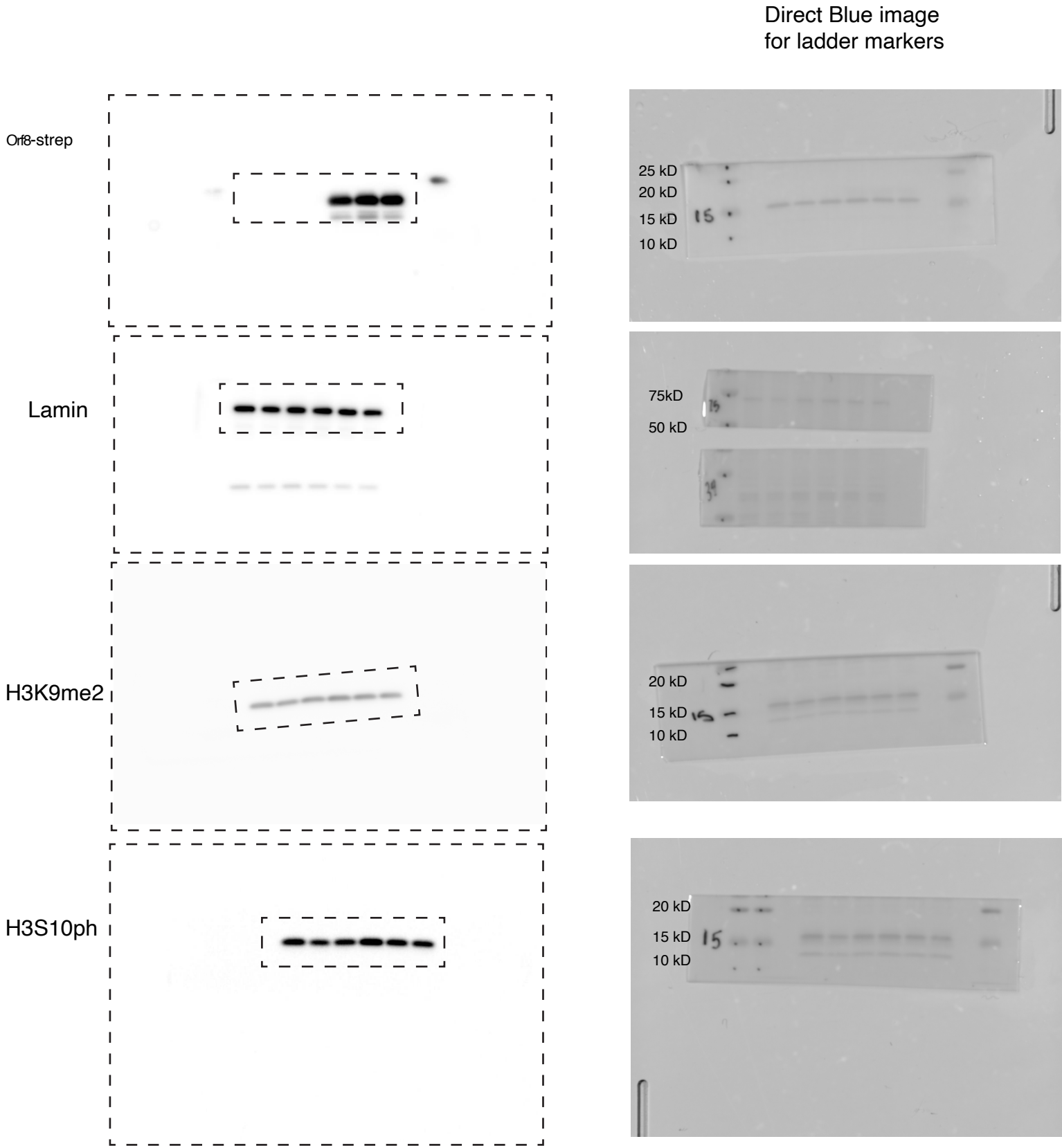
Supplemental Figure 1k. Cytoplasmic vs chromatin fractions from CHIP for Extended Data Figure 2g. (Loading controls run on same gel)

Fig. S1I



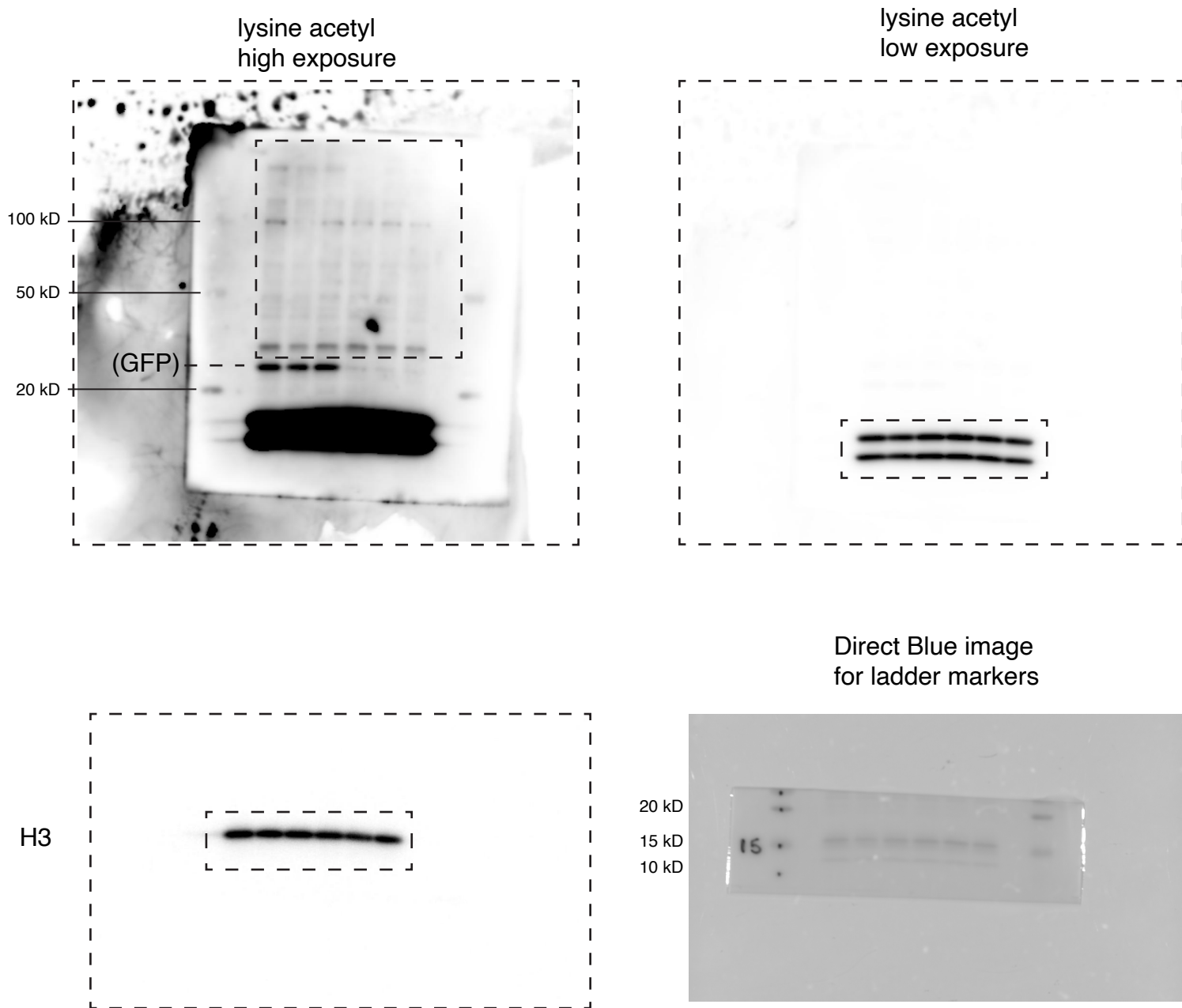
Supplemental Figure 1I. Orf8 effects on binding partners from Figure 1f. (Loading controls run on same gel, Orf8 blot stripped and reprobed for H3)

Fig. S1m



Supplemental Figure 1m. Orf8 effects on other histone PTMs from Extended Data Figure 4b. (Targets run on separate gels, Orf8 blot, stripped and reprobed for H3, shown in Supl. Fig. 1n)

Fig. S1n

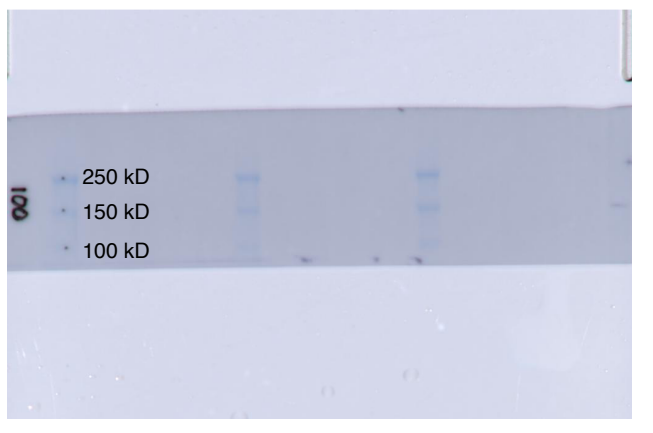
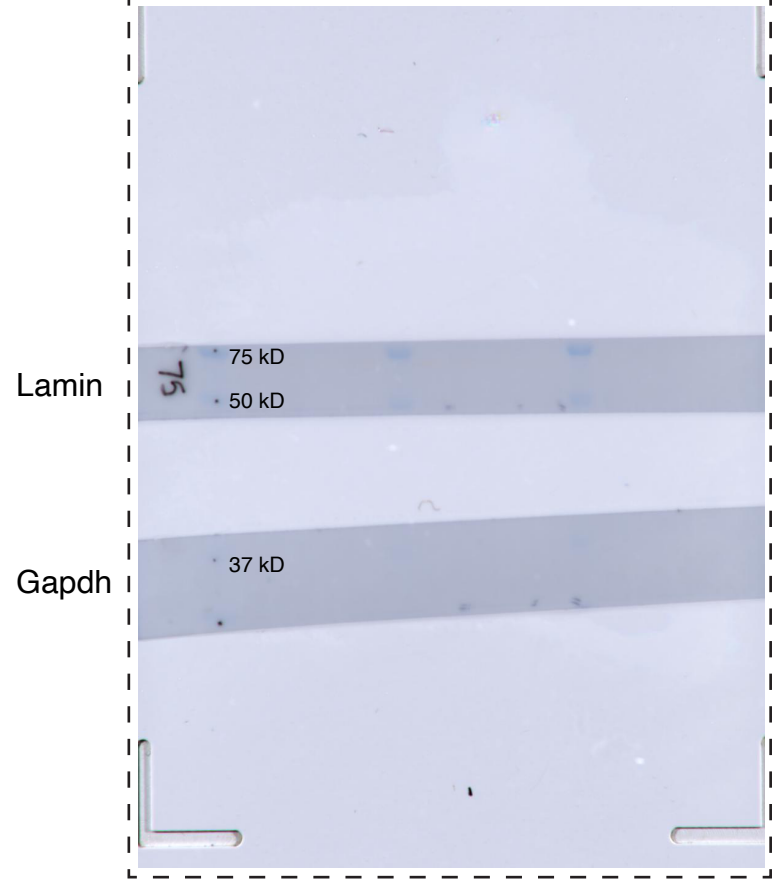
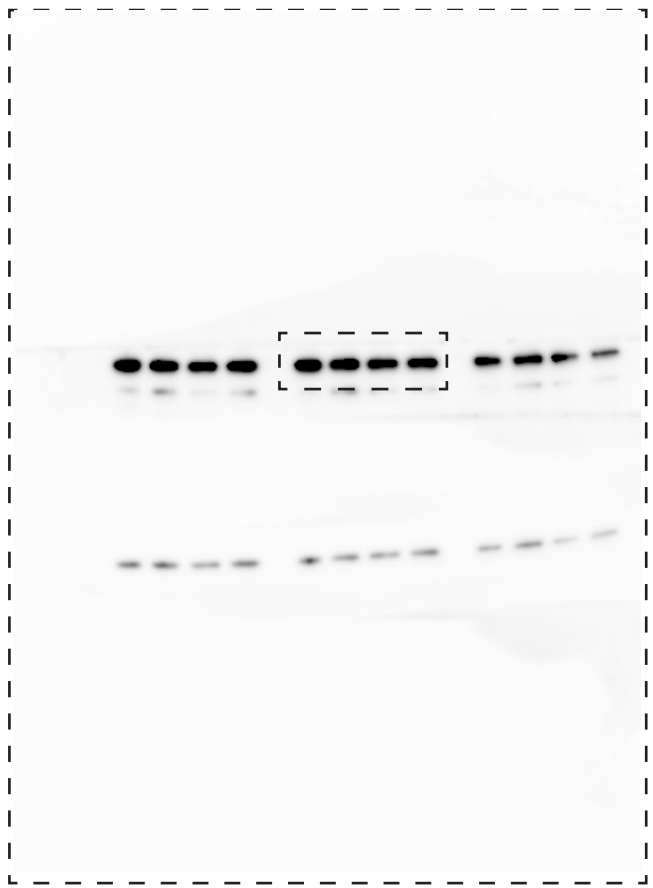
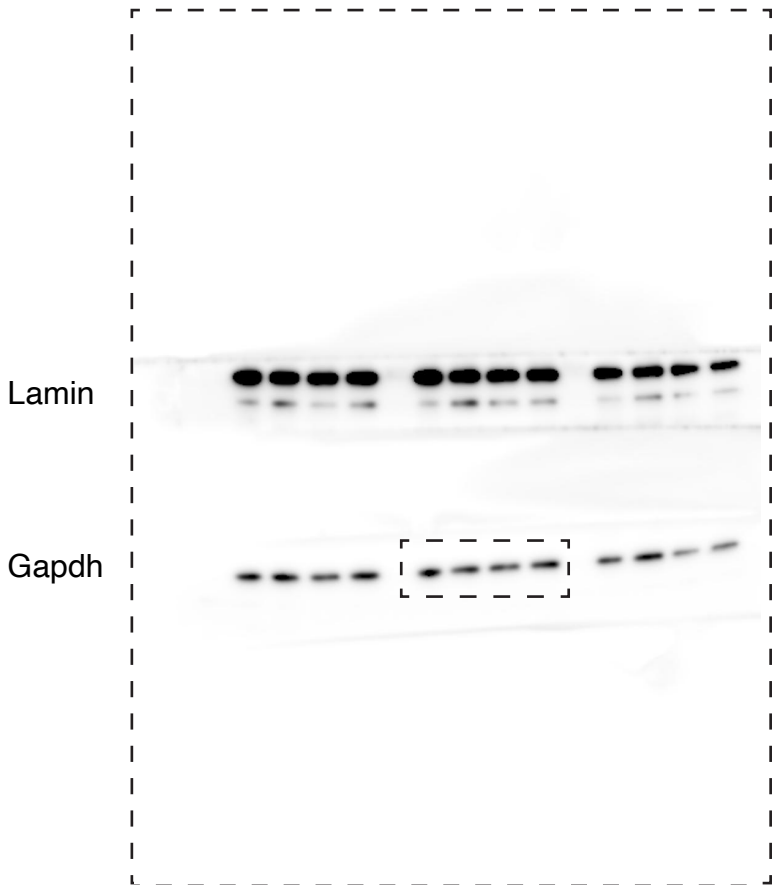


Supplemental Figure 1n. Orf8 effects on global acetylation from Extended Data Figure 4b. (H3 control run on separate blot from pan-acetyl, Orf8 blot shown in Sup. Fig. 1m stripped and reprobed for H3)

Fig. S1o

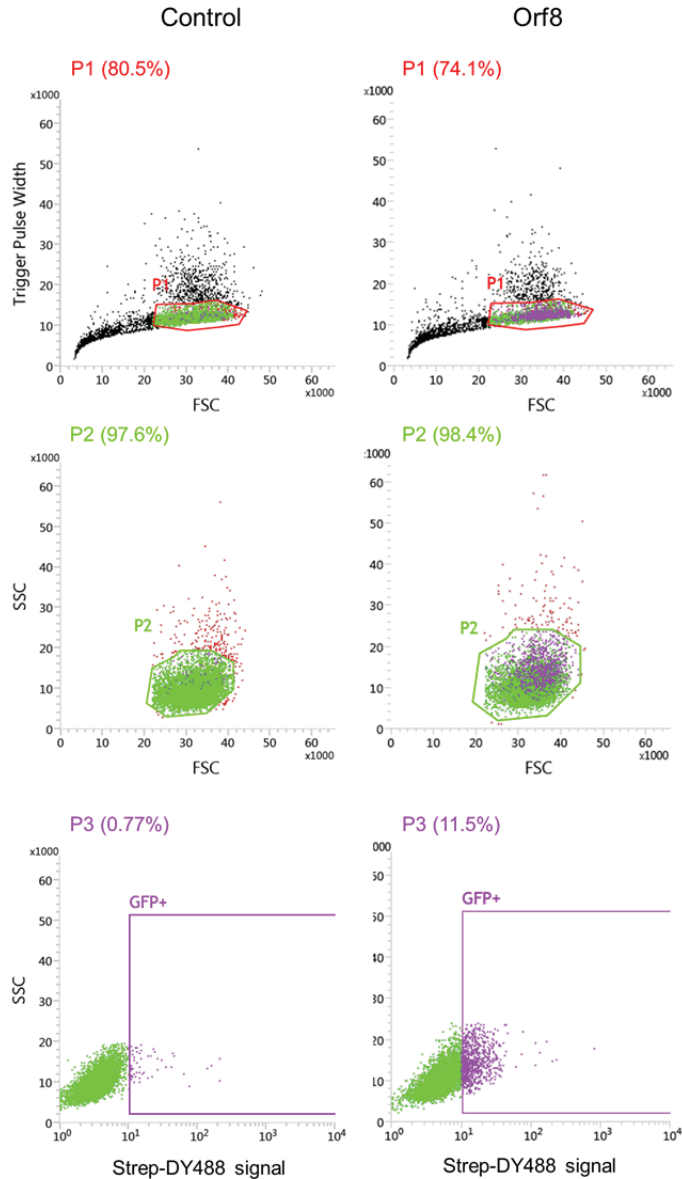
lamin/Gapdh
high exposure

Lamin/gapdh
low exposure



Supplemental Figure 1o. Effects of Infection on chromatin associated proteins from Figure 3f

Supplementary Figure 2



Cell numbers collected and used for experiments

| Experiment | Cells collected (Cells used for experiment if different from number collected) |
|----------------------------|--|
| <i>hPTM Mass Spec</i> | |
| GFP 1 | 1.00 x 10 ⁶ |
| GFP 2 | 1.00 x 10 ⁶ |
| GFP 3 | 1.60 x 10 ⁶ |
| GFP 4 | 1.00 x 10 ⁶ |
| Orf8 1 | 4.50 x 10 ⁵ |
| Orf8 2 | 5.00 x 10 ⁵ |
| Orf8 3 | 5.15 x 10 ⁵ |
| Orf8 4 | 5.00 x 10 ⁵ |
| <i>hPTM Western Blot</i> | |
| GFP 1 | 1.00 x 10 ⁶ |
| GFP 2 | 7.55 x 10 ⁵ |
| Orf8 1 | 1.00 x 10 ⁶ |
| Orf8 2 | 1.00 x 10 ⁶ |
| Orf8 ^Δ ARKSAP 1 | 1.00 x 10 ⁶ |
| Orf8 ^Δ ARKSAP 2 | 8.97 x 10 ⁵ |
| <i>ATAC</i> | |
| GFP 1 | 5.00 x 10 ⁴ |
| GFP 2 | 5.00 x 10 ⁴ |
| Orf8 1 | 5.00 x 10 ⁴ |
| Orf8 2 | 5.00 x 10 ⁴ |
| Orf8 ^Δ ARKSAP 1 | 5.00 x 10 ⁴ |
| Orf8 ^Δ ARKSAP 2 | 5.00 x 10 ⁴ |
| <i>RNA-Seq</i> | |
| GFP 1 | 1.00 x 10 ⁶ |
| GFP 2 | 7.86 x 10 ⁵ |
| Orf8 1 | 1.00 x 10 ⁶ |
| Orf8 2 | 6.50 x 10 ⁵ |
| Orf8 3 | 5.40 x 10 ⁵ |
| Orf8 ^Δ ARKSAP 1 | 8.98 x 10 ⁵ |
| Orf8 ^Δ ARKSAP 2 | 5.00 x 10 ⁵ |
| <i>H3K9ac</i> | |
| <i>CUT&TAG</i> | |
| GFP 1 | 1.00 x 10 ⁶ (1.20 x 10 ⁵) |
| GFP 2 | 1.00 x 10 ⁶ (1.20 x 10 ⁵) |
| Orf8 1 | 6.00 x 10 ⁵ (1.20 x 10 ⁵) |
| Orf8 2 | 4.28 x 10 ⁵ (1.20 x 10 ⁵) |
| Orf8 ^Δ ARKSAP 1 | 3.68 x 10 ⁵ (1.20 x 10 ⁵) |
| Orf8 ^Δ ARKSAP 2 | 4.74 x 10 ⁵ (1.20 x 10 ⁵) |

Supplemental Figure 2. FACS gating strategy.

Example of gating strategy and cell numbers isolated for all FACS experiments.