nature portfolio

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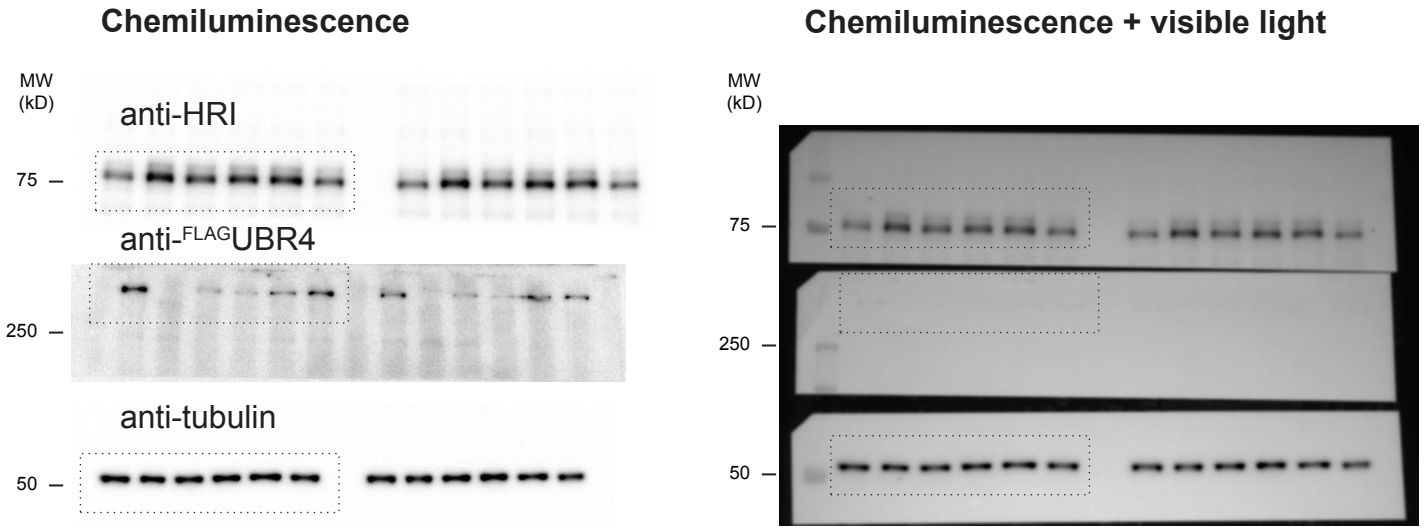
Supplementary information

Stress response silencing by an E3 ligase mutated in neurodegeneration

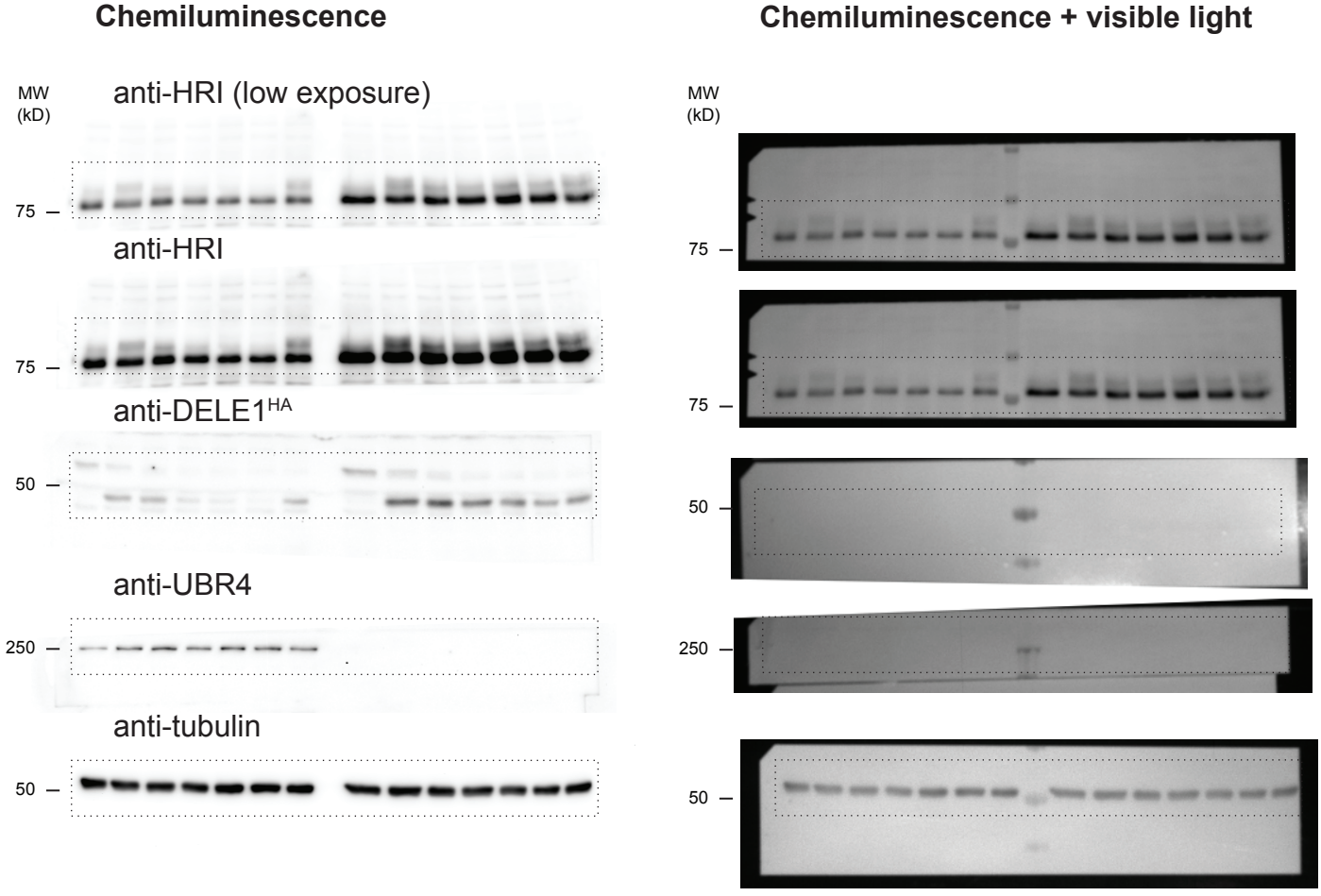
In the format provided by the authors and unedited

Figure 2d



The loading control tubulin was run on the same gel as ^{FLAG}UBR4 and HRI.

Figure 2e

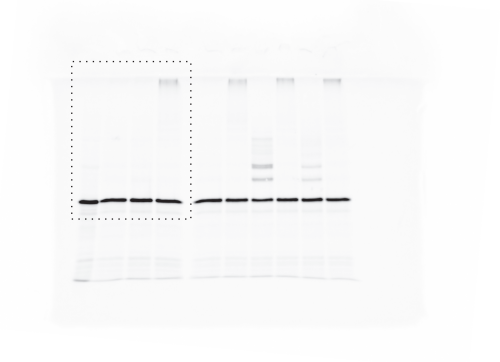


The loading control tubulin was obtained by stripping the DELE1^{HA} membrane, which was run on the same gel as UBR4.

Figure 2f

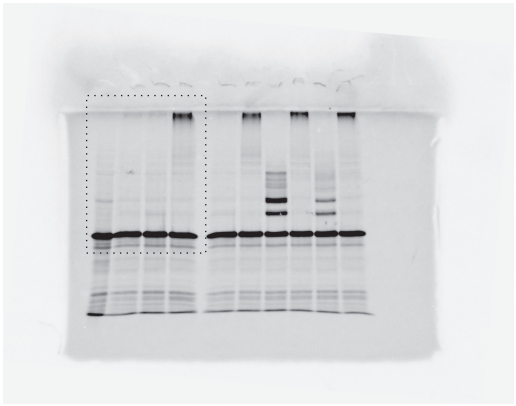
Raw files

HRI-sumo autoradiography



DELE1-sumo autoradiography

Contrasted raw files to discern typically low S^{35} autoradiography signal



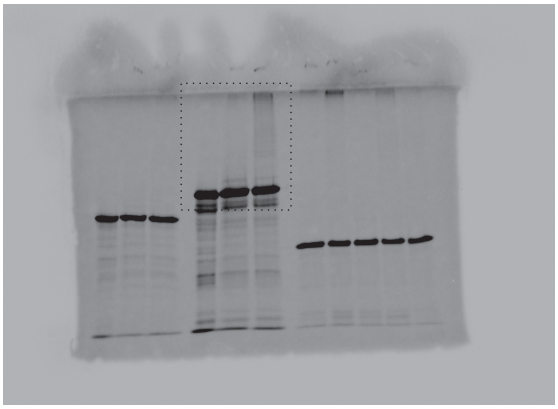
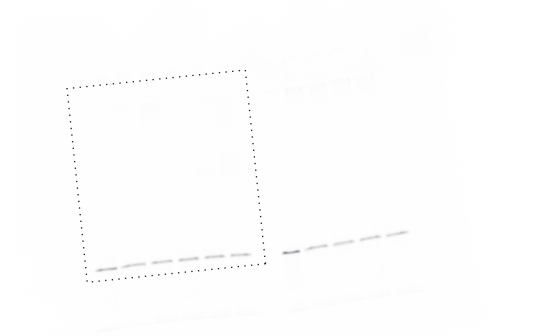


Figure 2g

HRI-sumo autoradiography



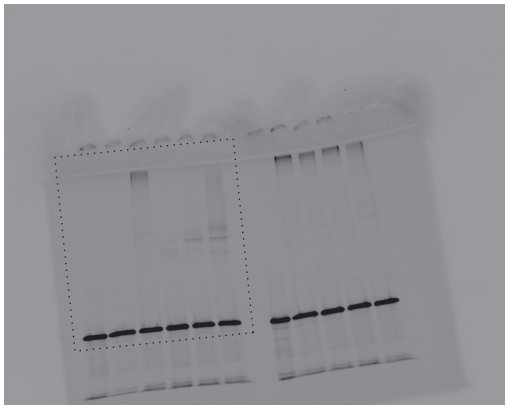
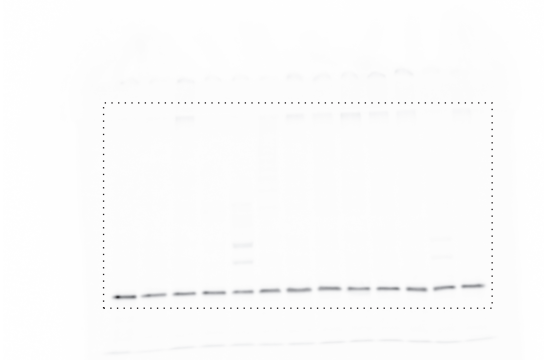


Figure 2h

HRI-sumo autoradiography



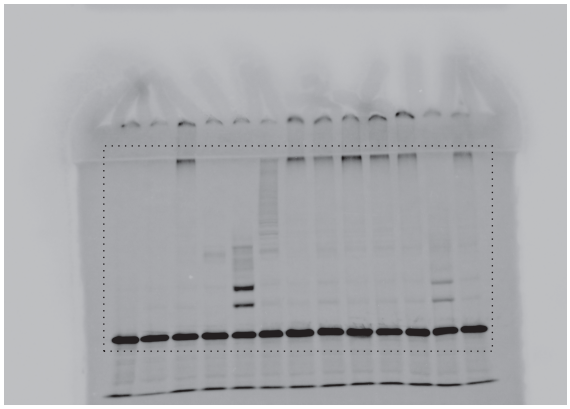
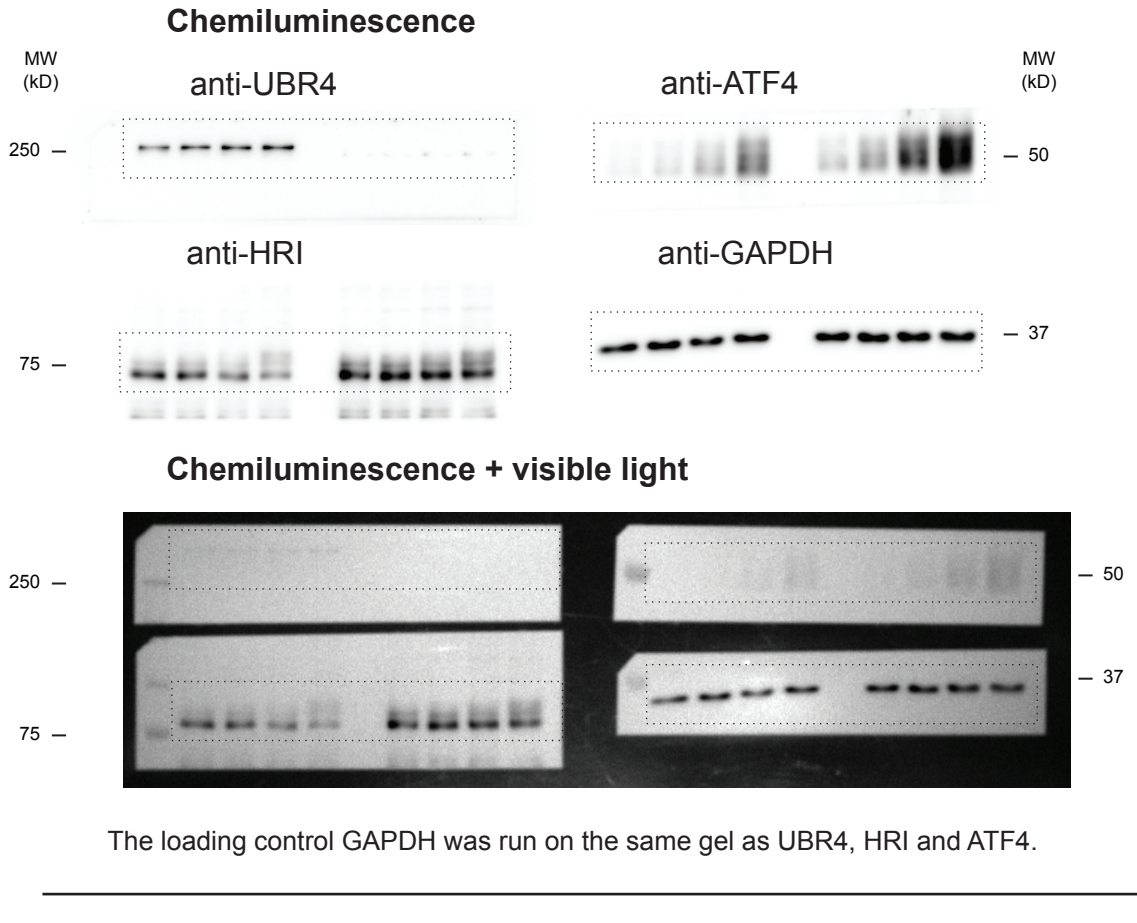
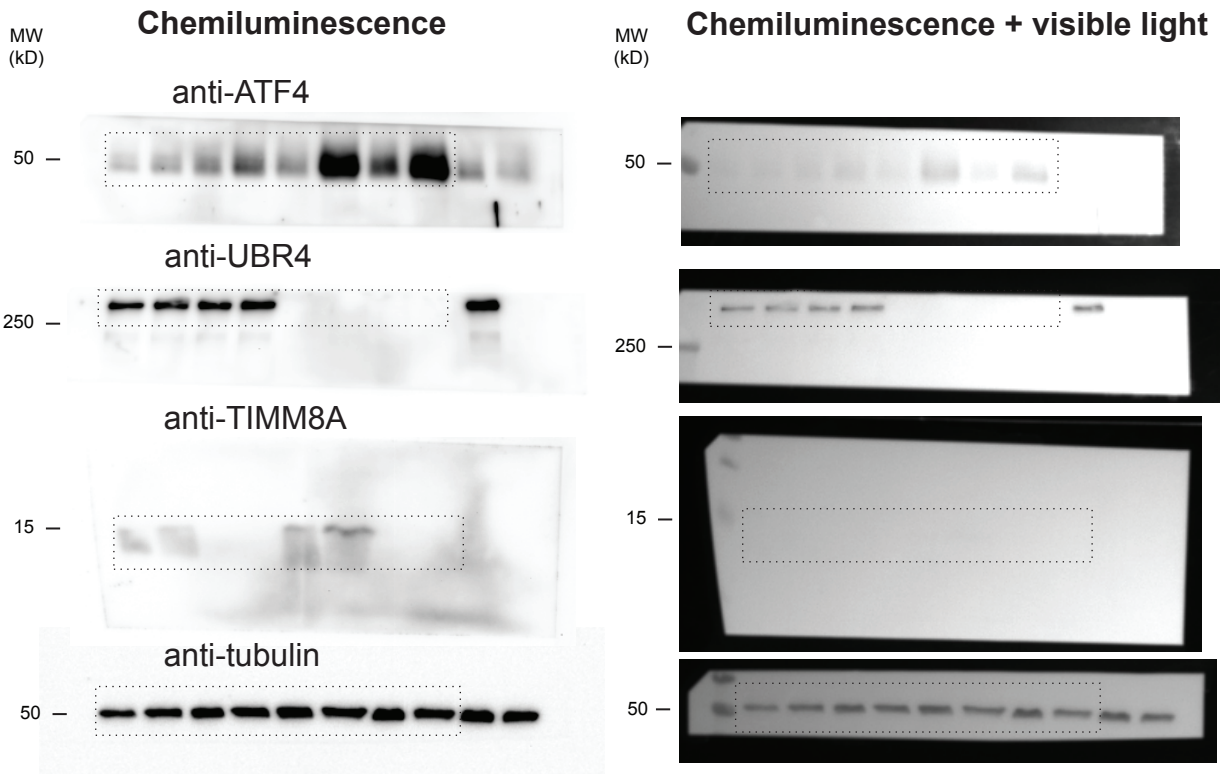


Figure 3b



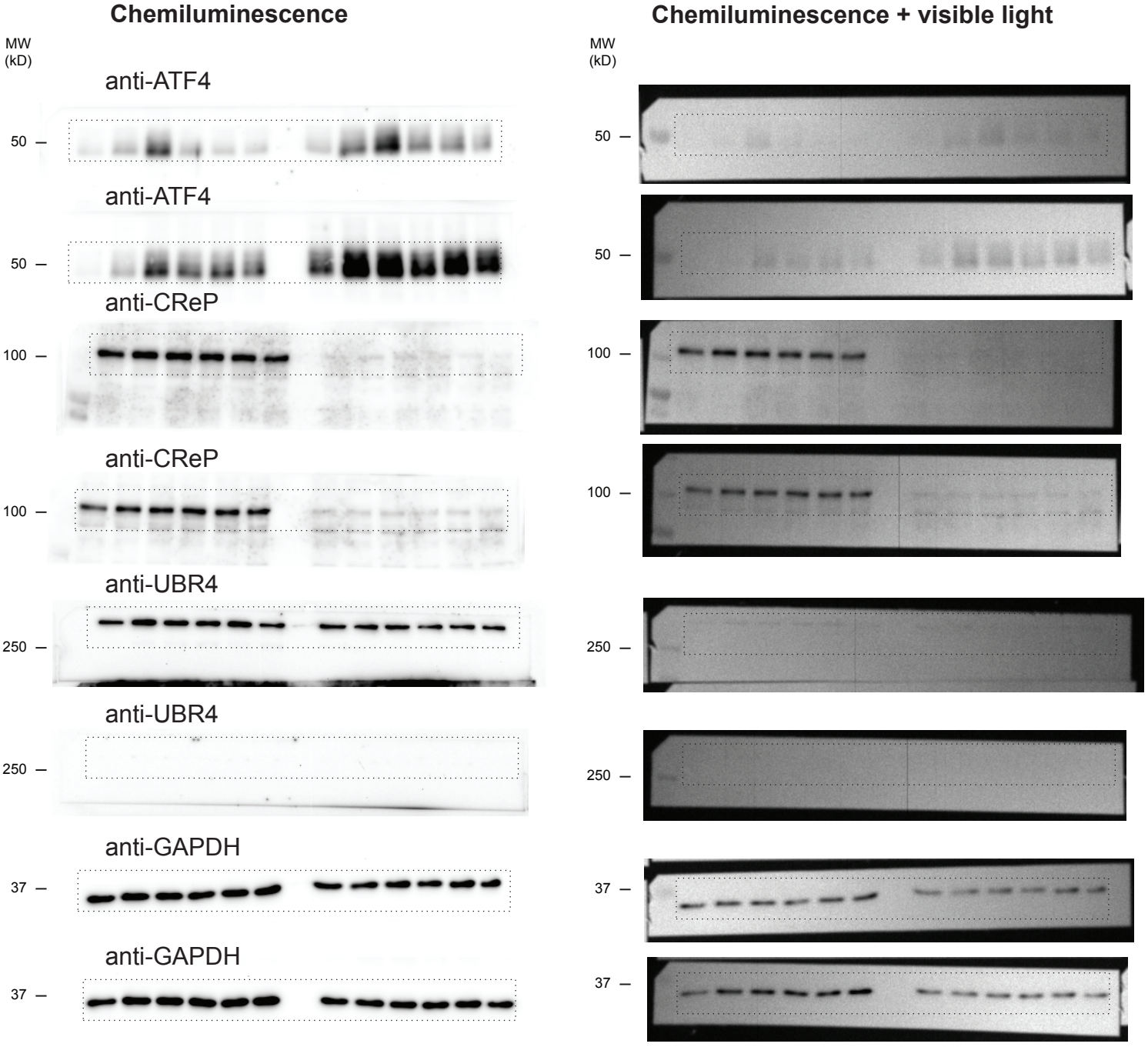
The loading control GAPDH was run on the same gel as UBR4, HRI and ATF4.

Figure 3c



The loading control tubulin was run on the same gel as TIMM8A.

Figure 3f

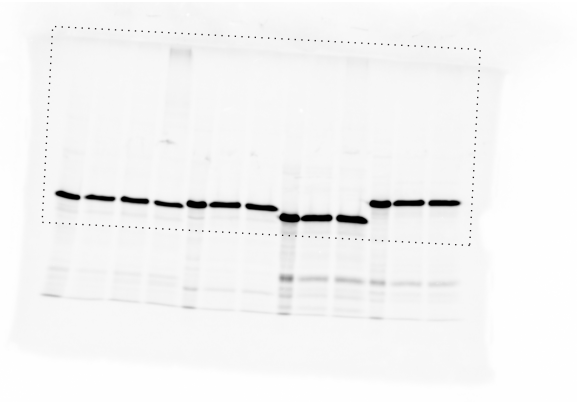


The loading controls GAPDH were run on the same gels as ATF4, CReP and UBR4 in wt and *ΔUBR4* respectively.

Figure 4d

Raw file

HRI-sumo autoradiography



Contrasted raw files to discern typically low S^{35} autoradiography signal

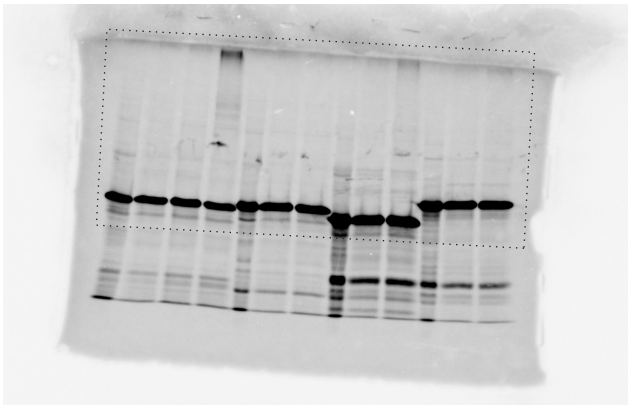


Figure 4e

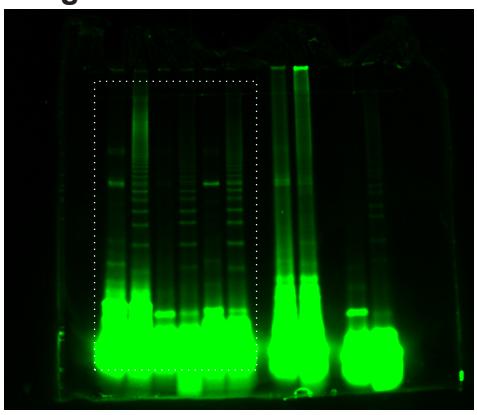


Figure 4g

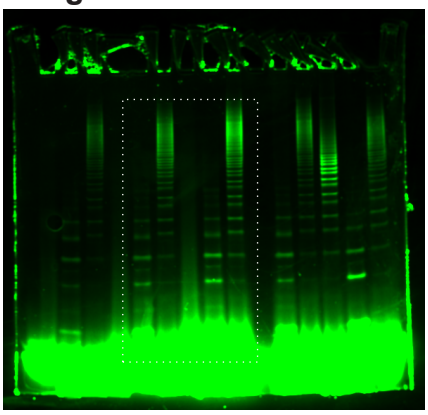


Figure 4h

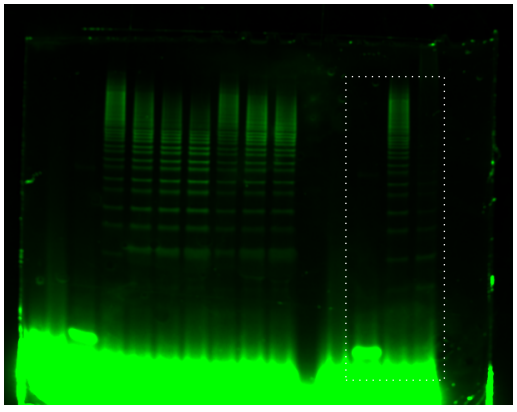


Figure 4i

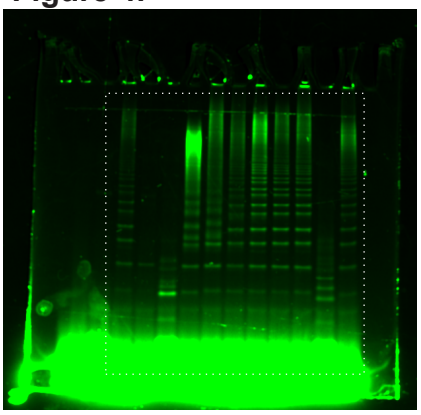
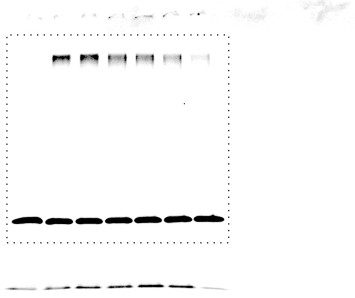


Figure 5d

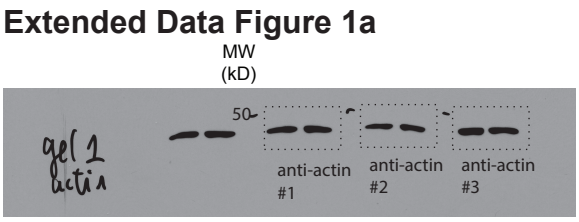
Raw file

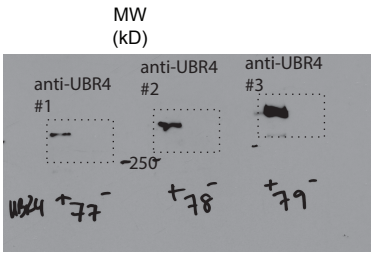
HRI-sumo autoradiography

Contrasted raw files to discern typically low S^{35} autoradiography signal



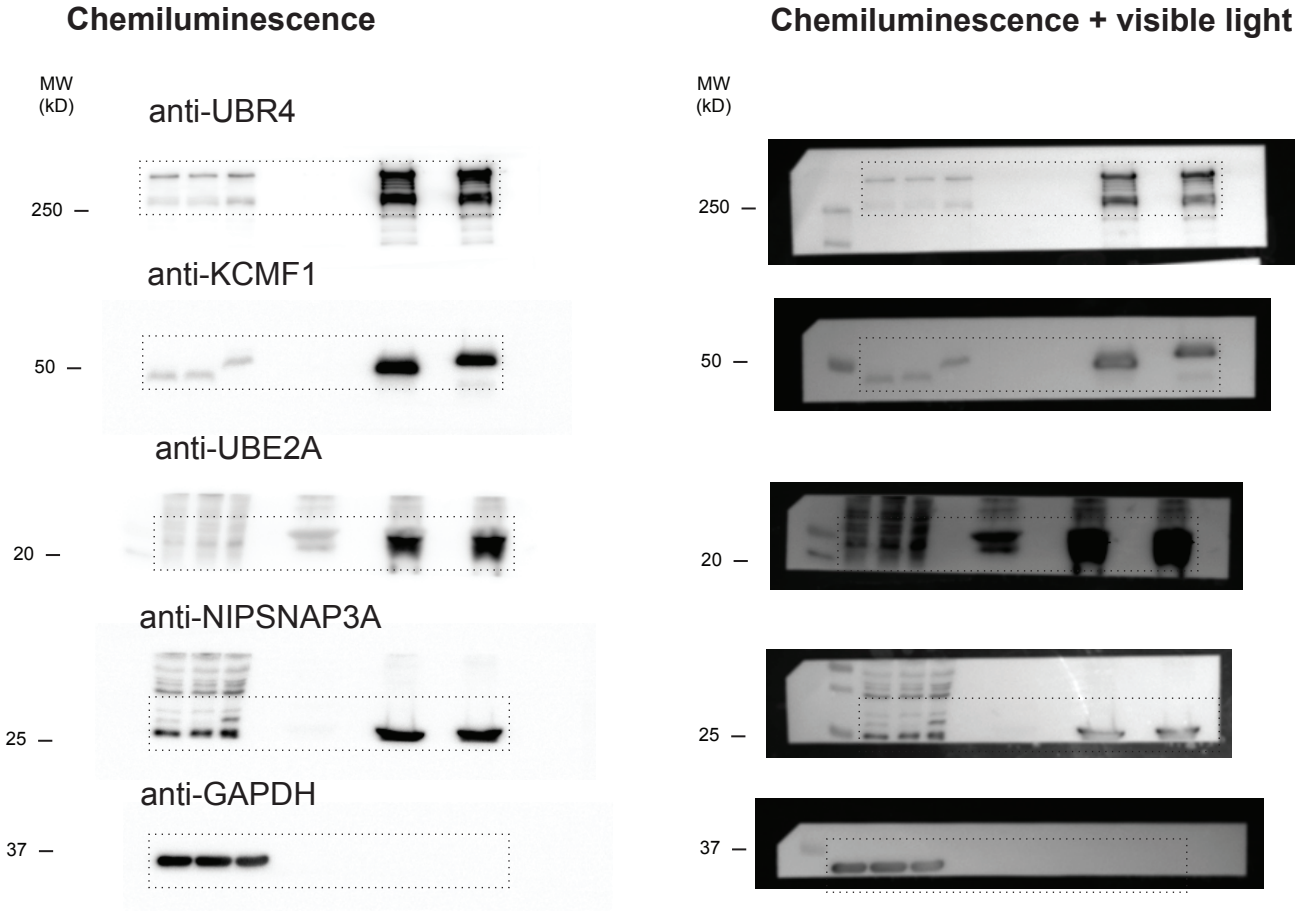
Extended Data Figure 1a





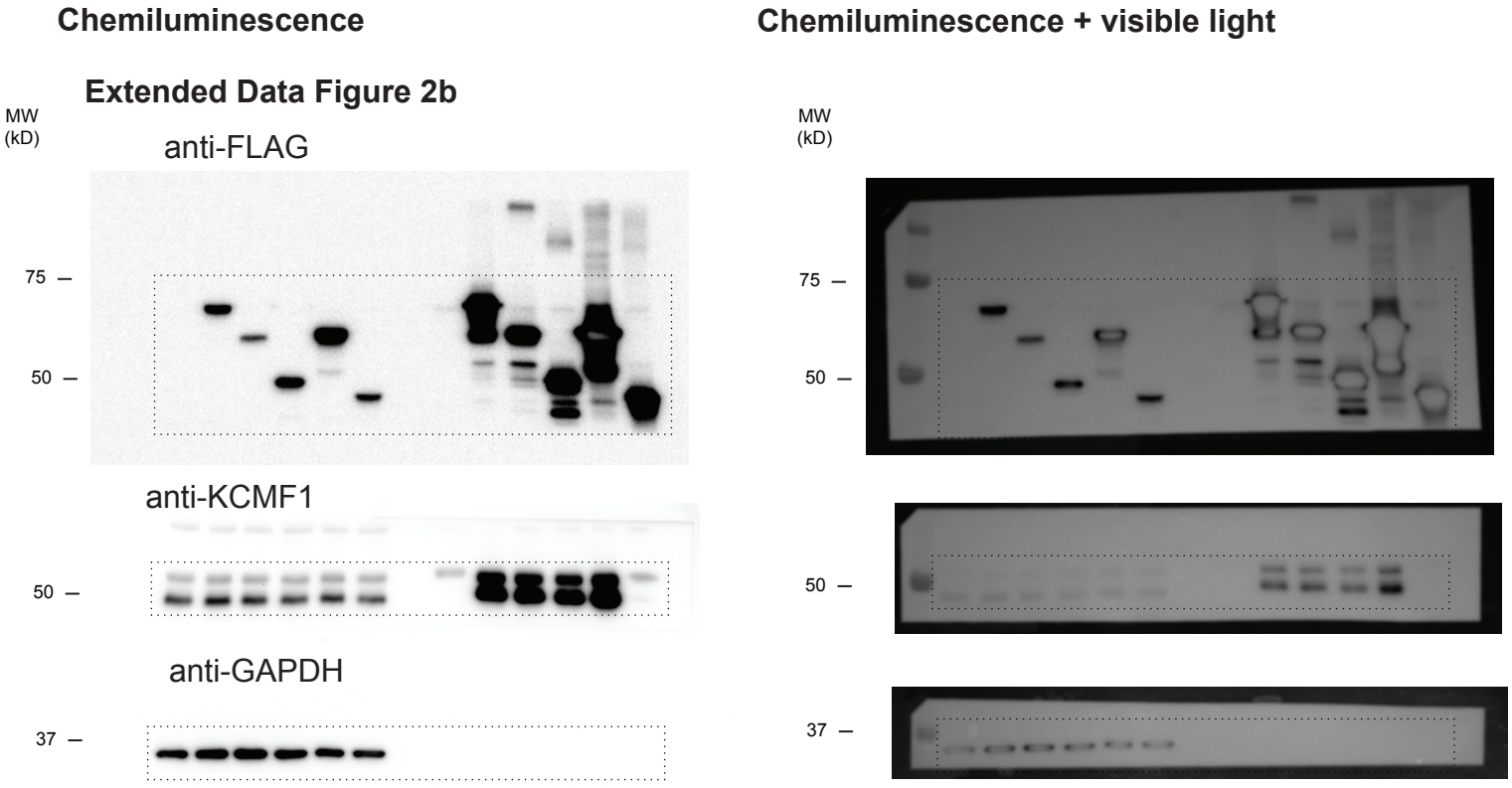
The loading control actin was blotted from the same gel as UBR4 samples.

Extended Data Figure 2a



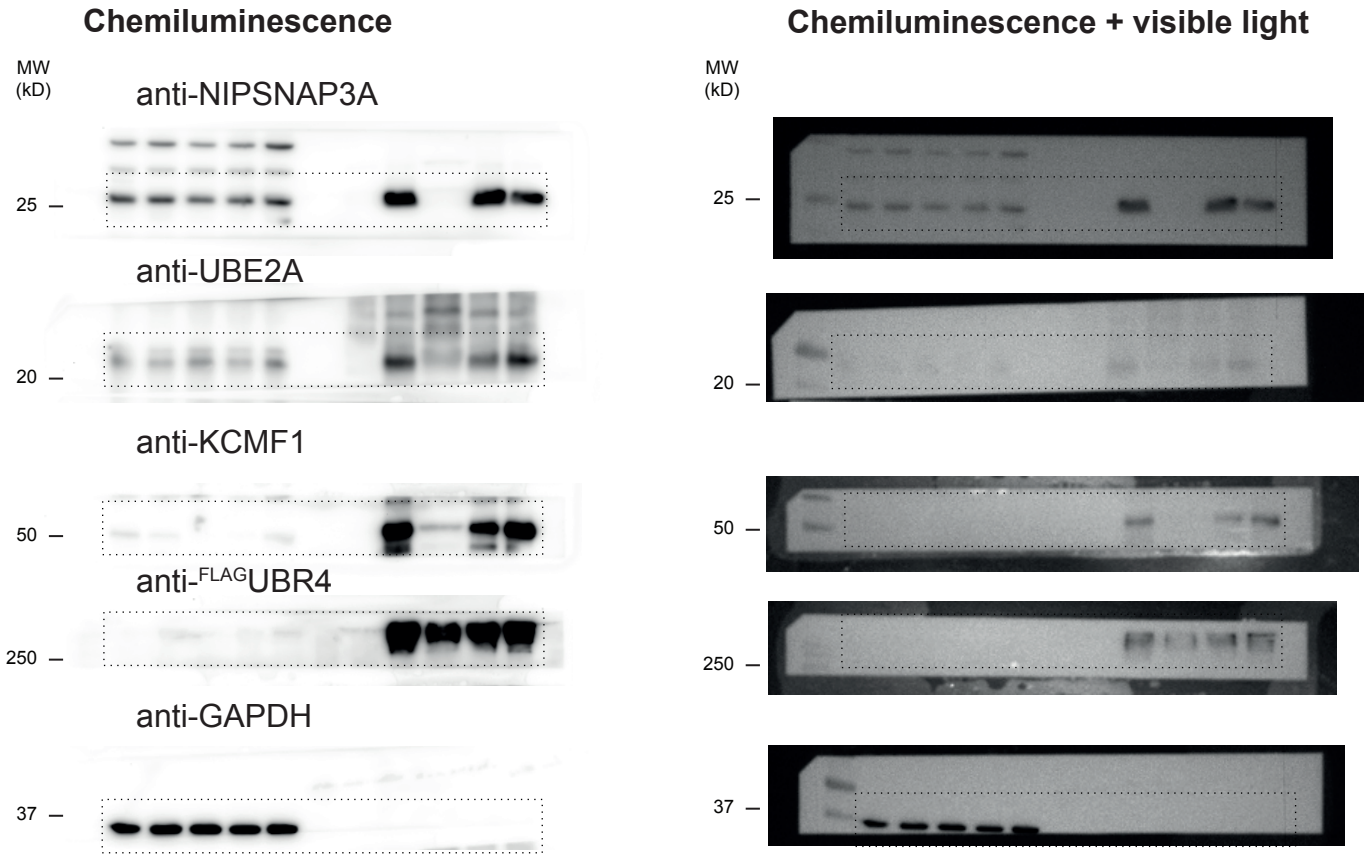
The loading control GAPDH was run on the same gel as UBR4 and KCMF1.

Extended Data Figure 2b



The loading control GAPDH was run on the same gel as KCMF1.

Extended Data Figure 2c



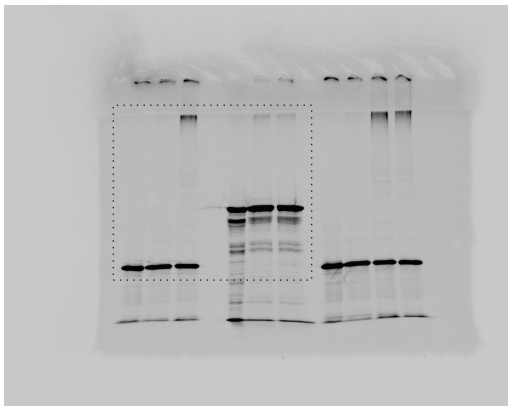
The loading control GAPDH was run on the same gel as UBE2A and ^{FLAG}UBR4.

Extended Data Figure 4a

Raw files

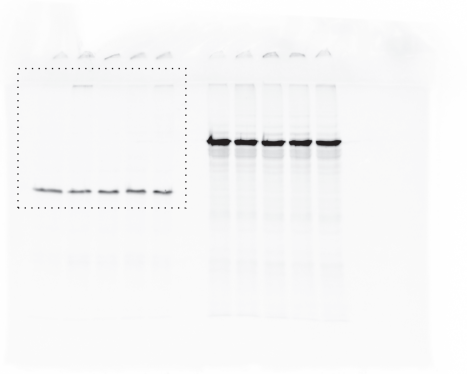
HRI-sumo/MBP-sumo autoradiography

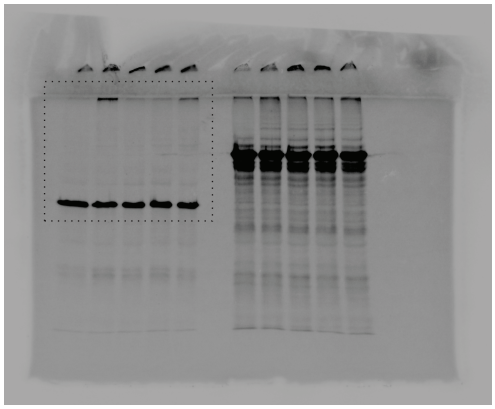
Contrasted raw files to discern typically low S^{35} autoradiography signal



Extended Data Figure 4b

HRI-sumo autoradiography

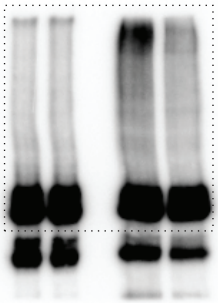




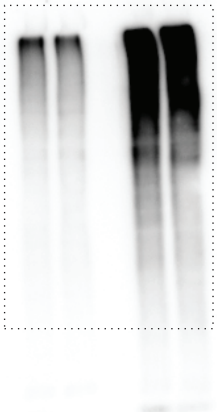
Extended Data Figure 4c

Chemiluminescence

Anti-FLAG

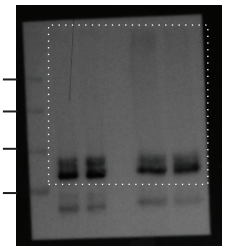


Anti-Ub



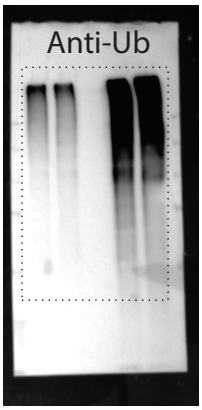
Chemiluminescence + visible light

Anti-FLAG



Ubiquitin and FLAG were run on different gels

Anti-Ub

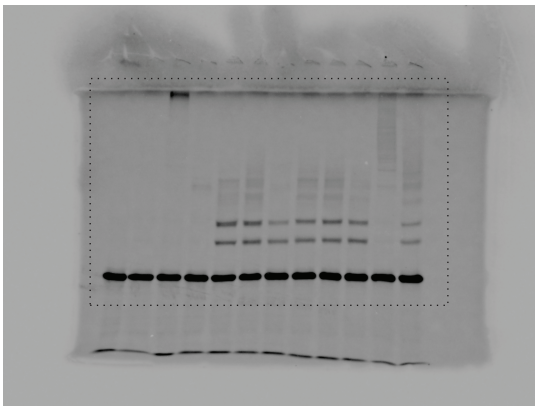


Extended Data Figure 4d

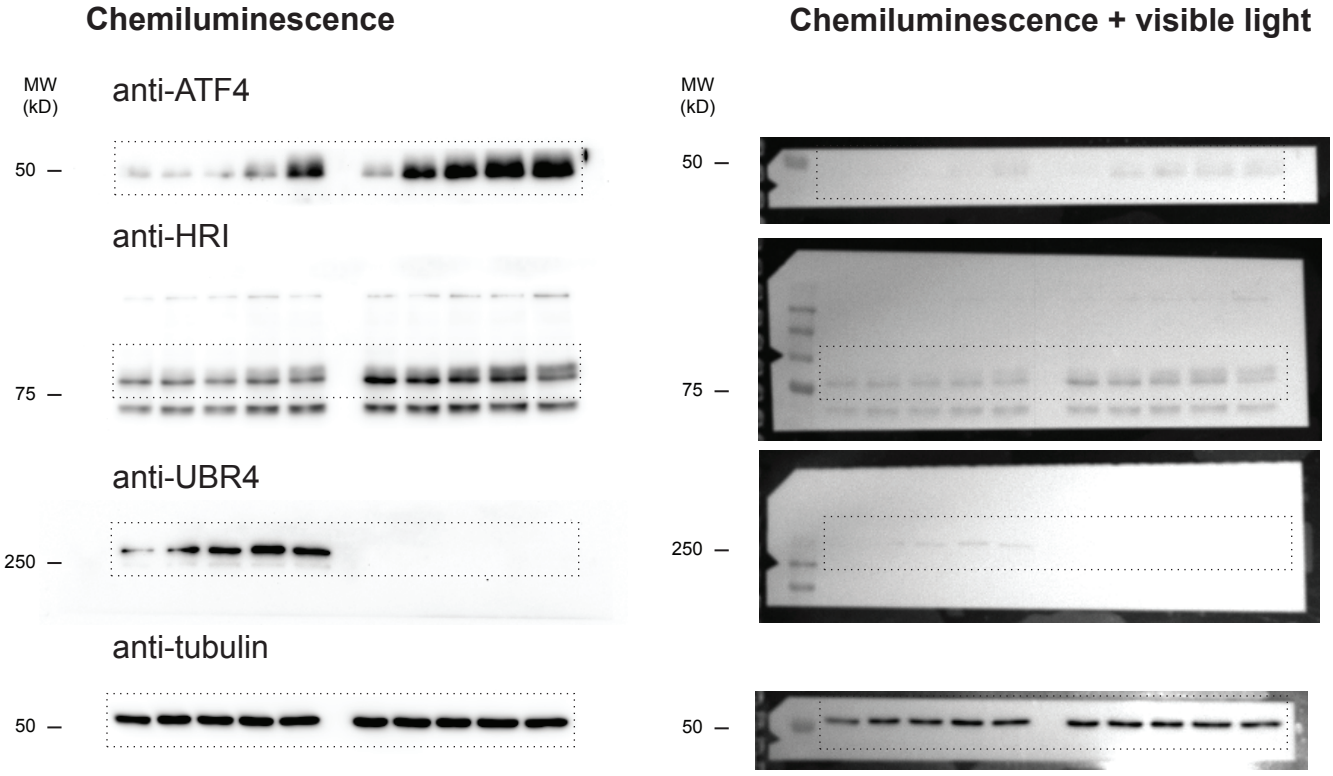
Raw file

HRI-sumo autoradiography

Contrasted raw file to discern typically low S^{35} autoradiography signal

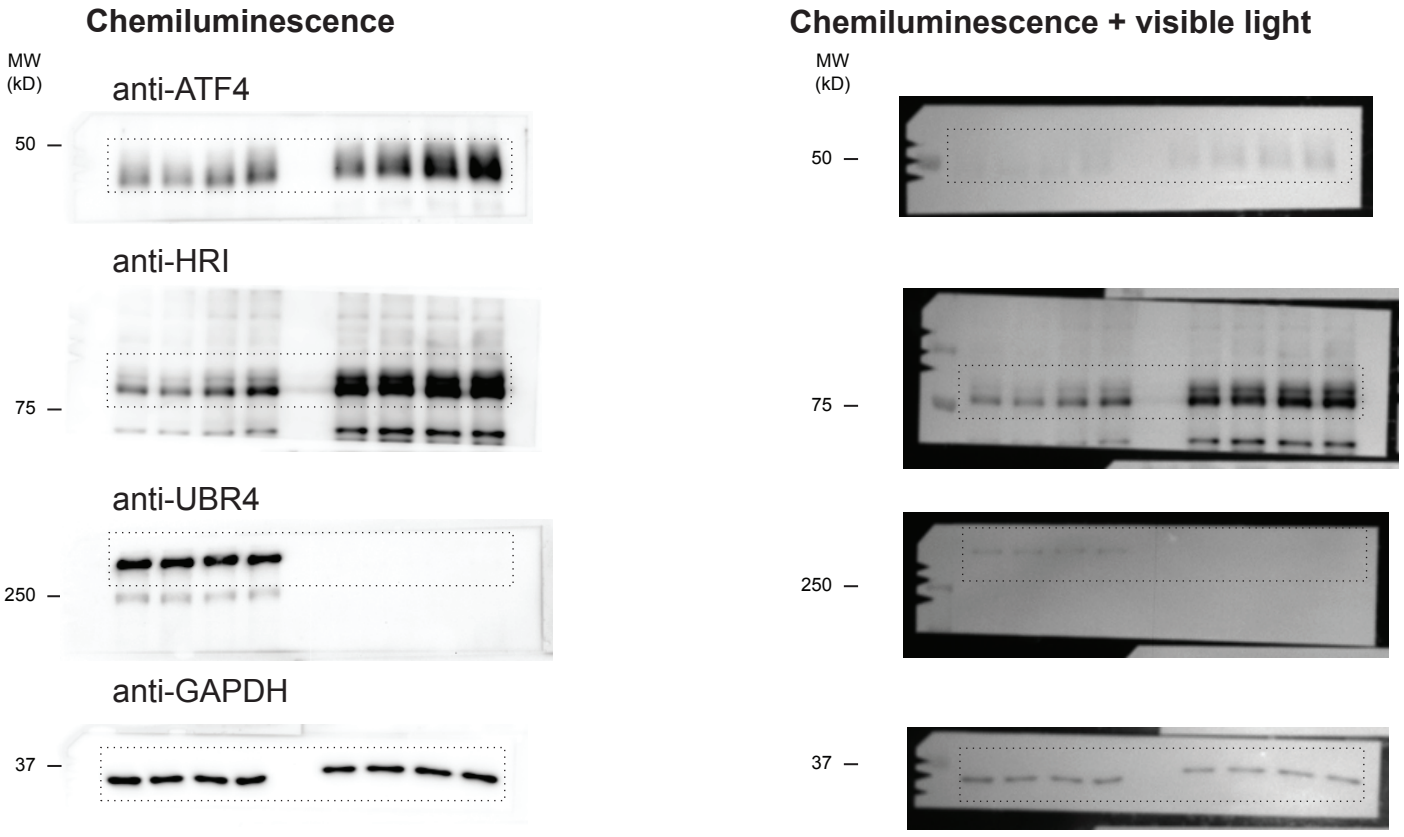


Extended Data Figure 5b



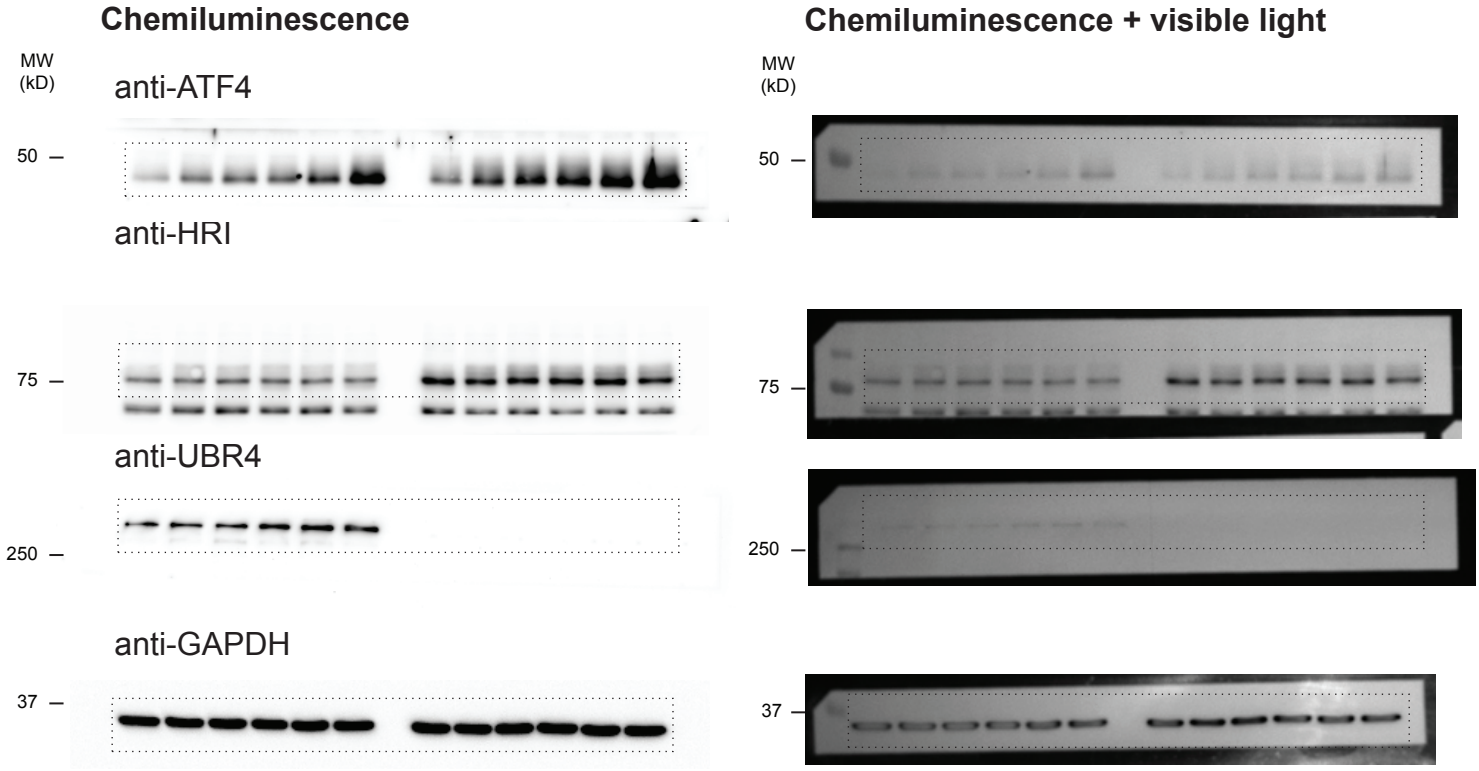
The loading control tubulin was run on the same gel as UBR4 .

Extended Data Figure 5c



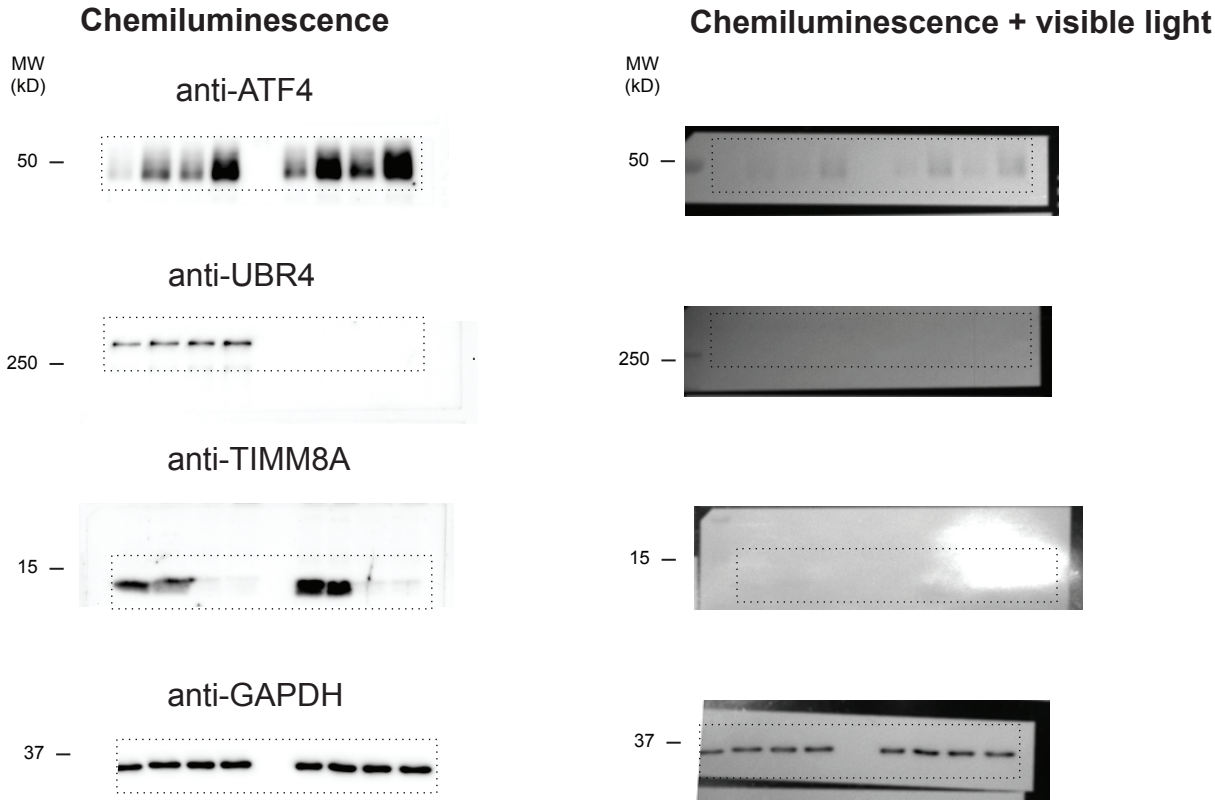
The loading control GAPDH was run on the same gel as ATF4, HRI and UBR4.

Extended Data Figure 5d



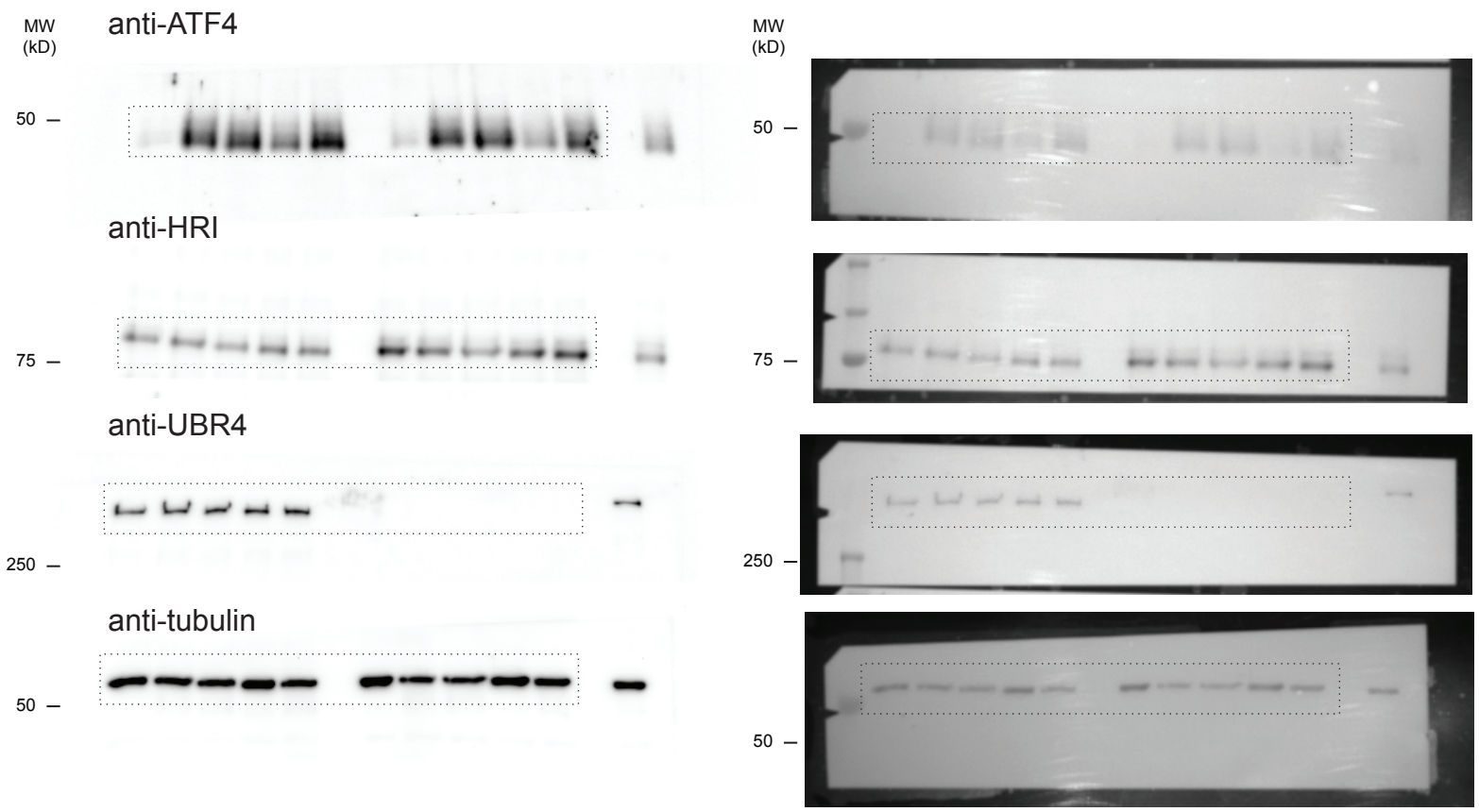
The loading control GAPDH was run on the same gel as ATF4, HRI and UBR4.

Extended Data Figure 5e



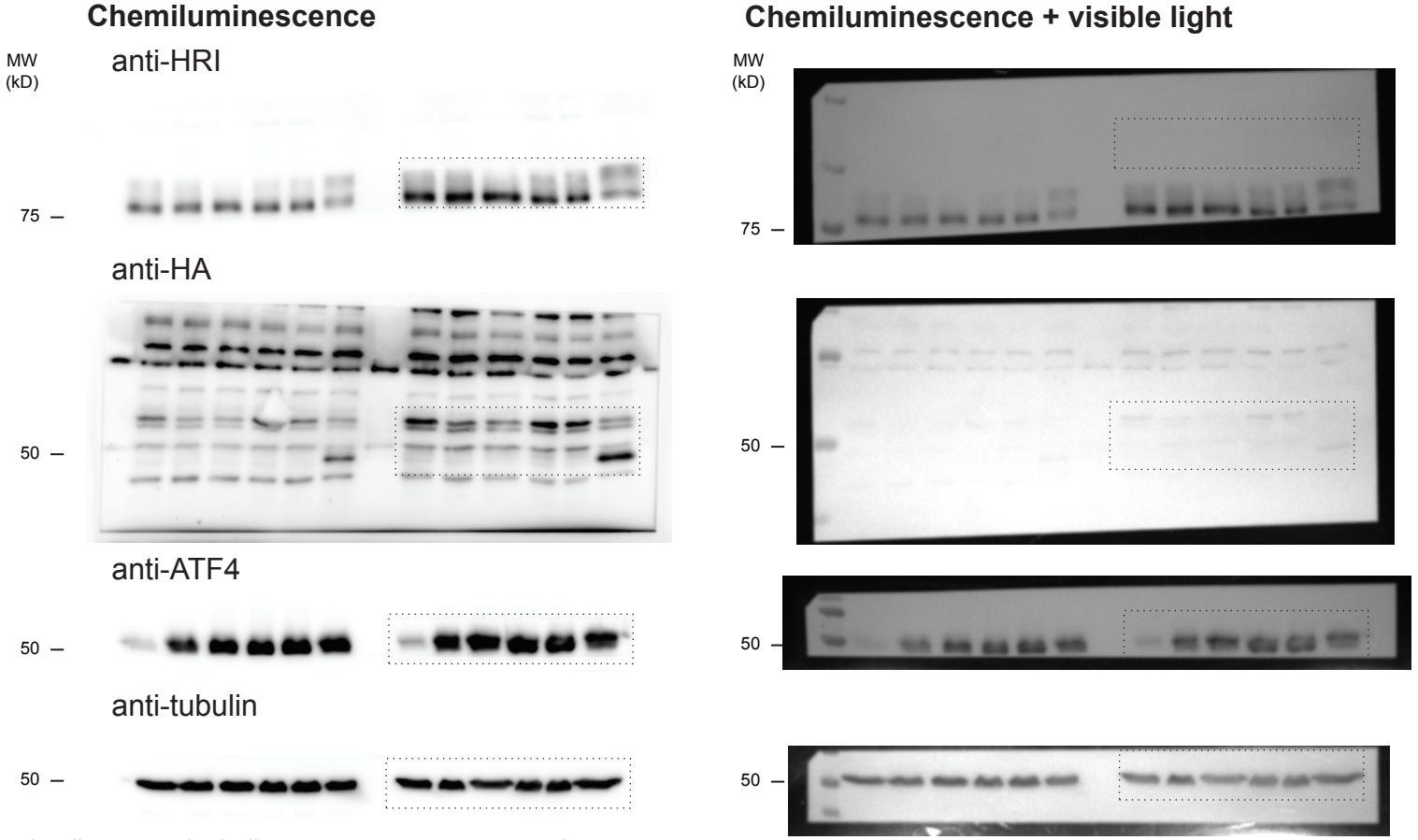
The loading control GAPDH was run on the same gel as ATF4 and UBR4.

Extended Data Figure 5h



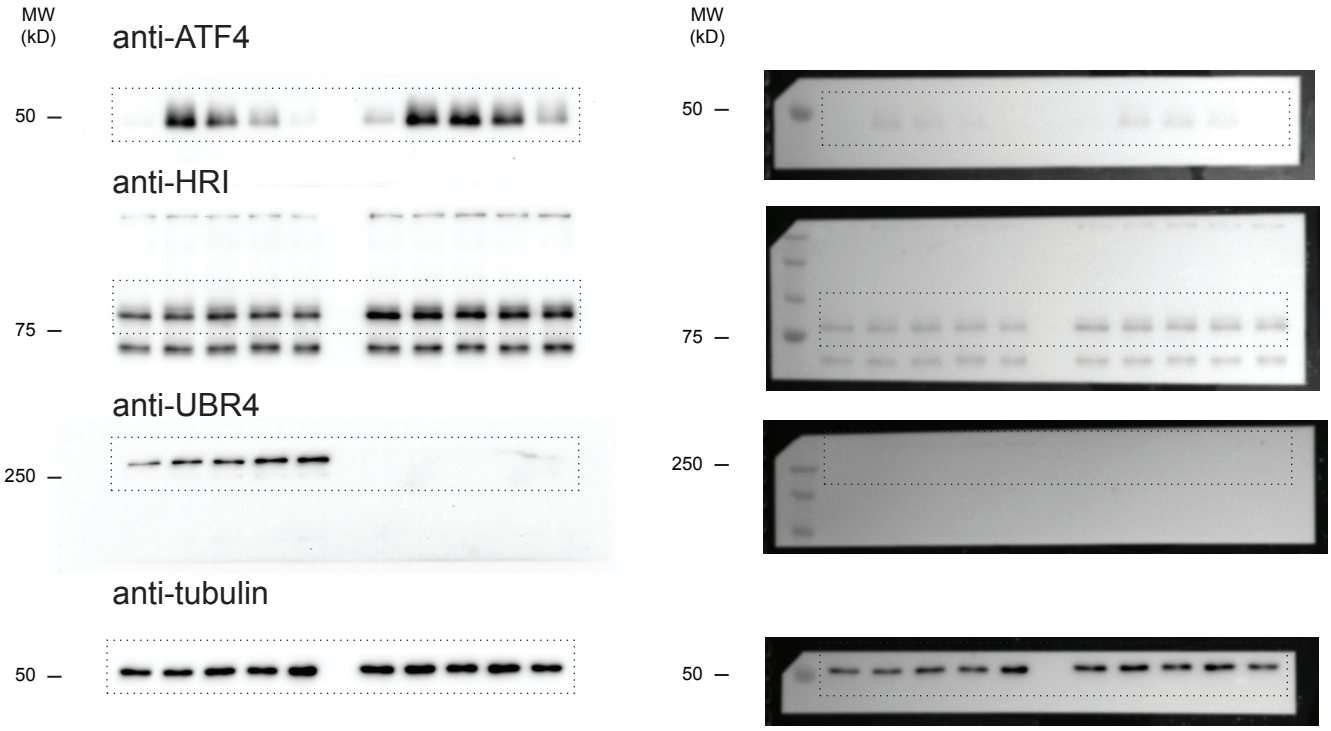
The loading control tubulin was obtained by stripping the ATF4 membrane, which was run on the same gel as HRI and UBR4.

Extended Data Figure 5i



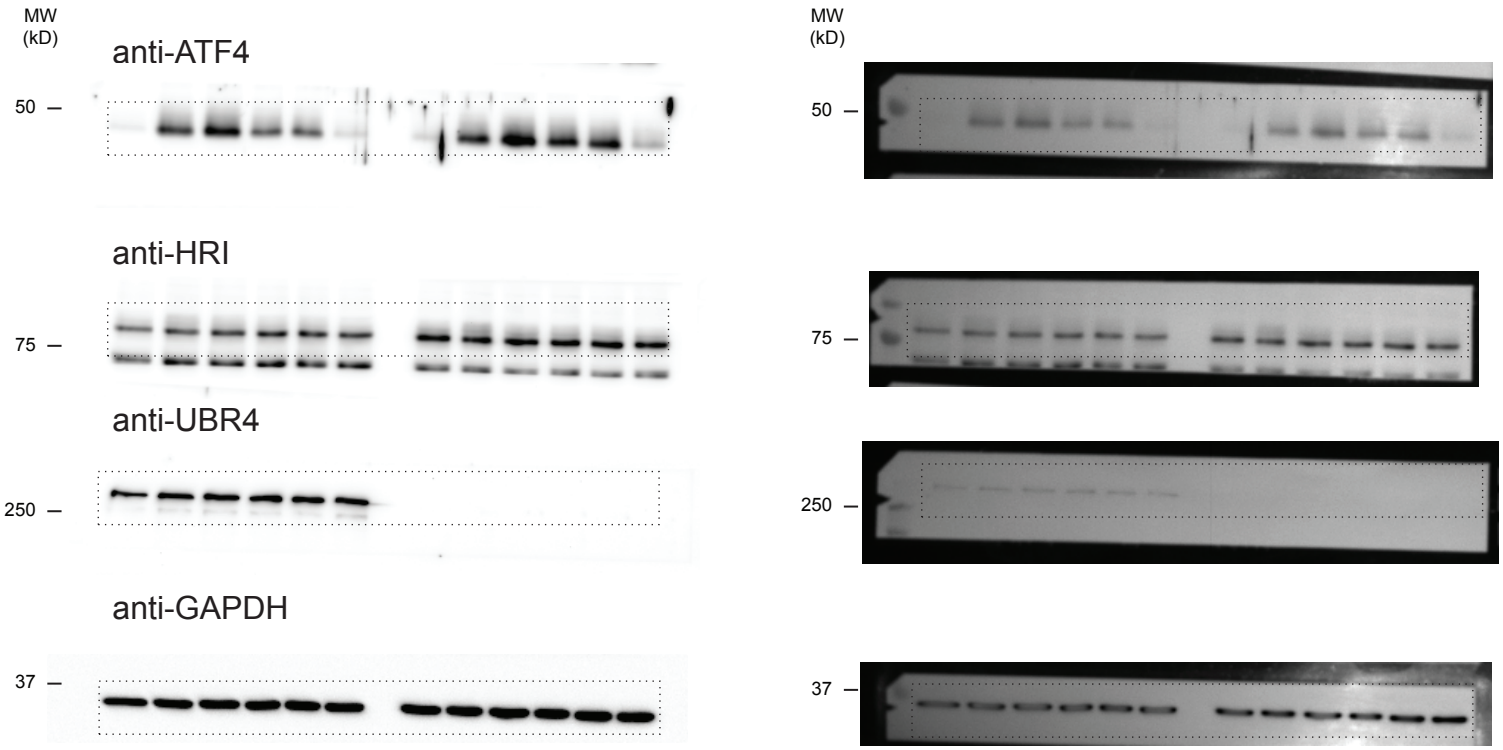
The loading control tubulin was run on a separate gel.

Extended Data Figure 6a



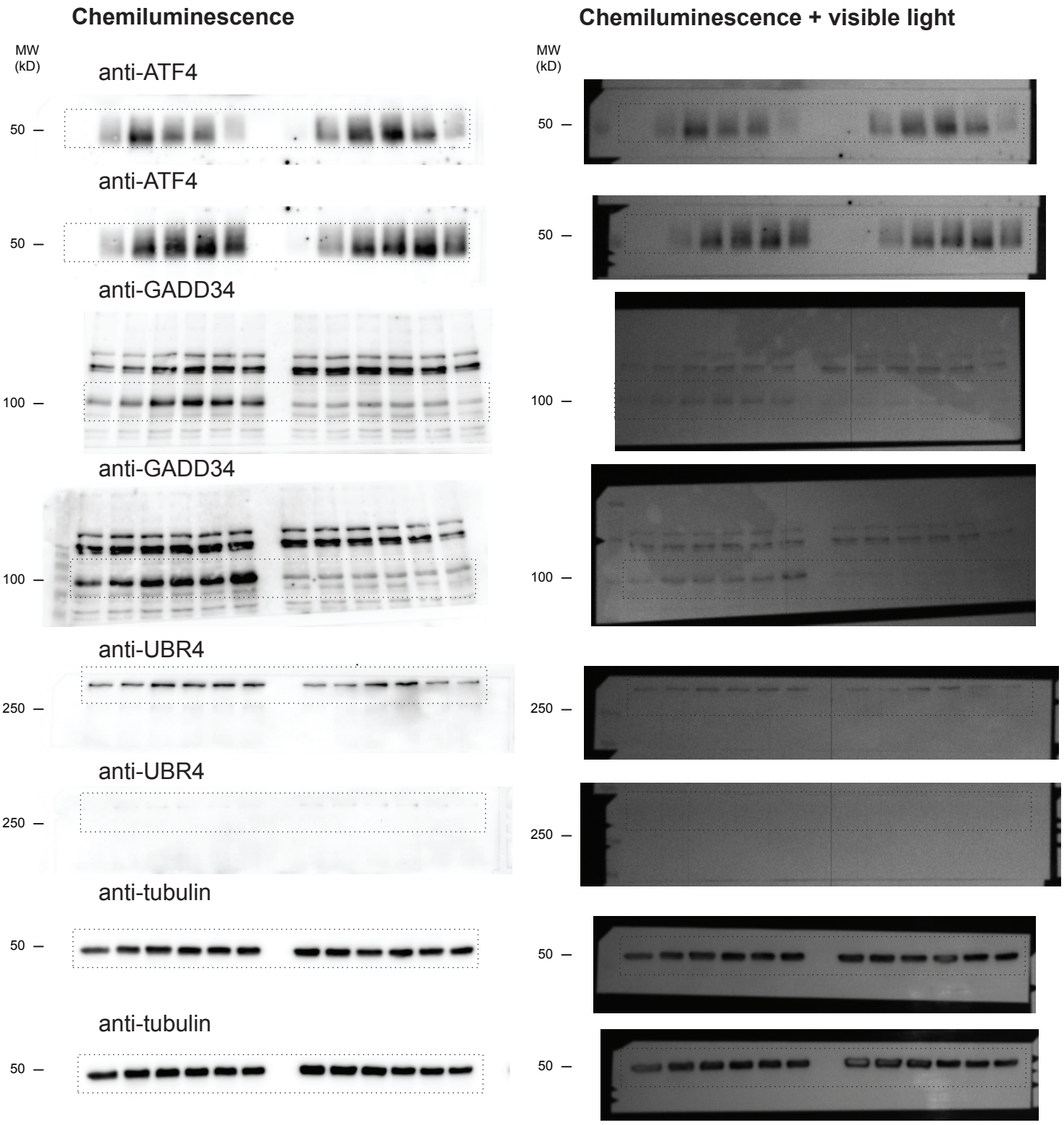
The loading control tubulin was run on the same gel as UBR4.

Extended Data Figure 6b



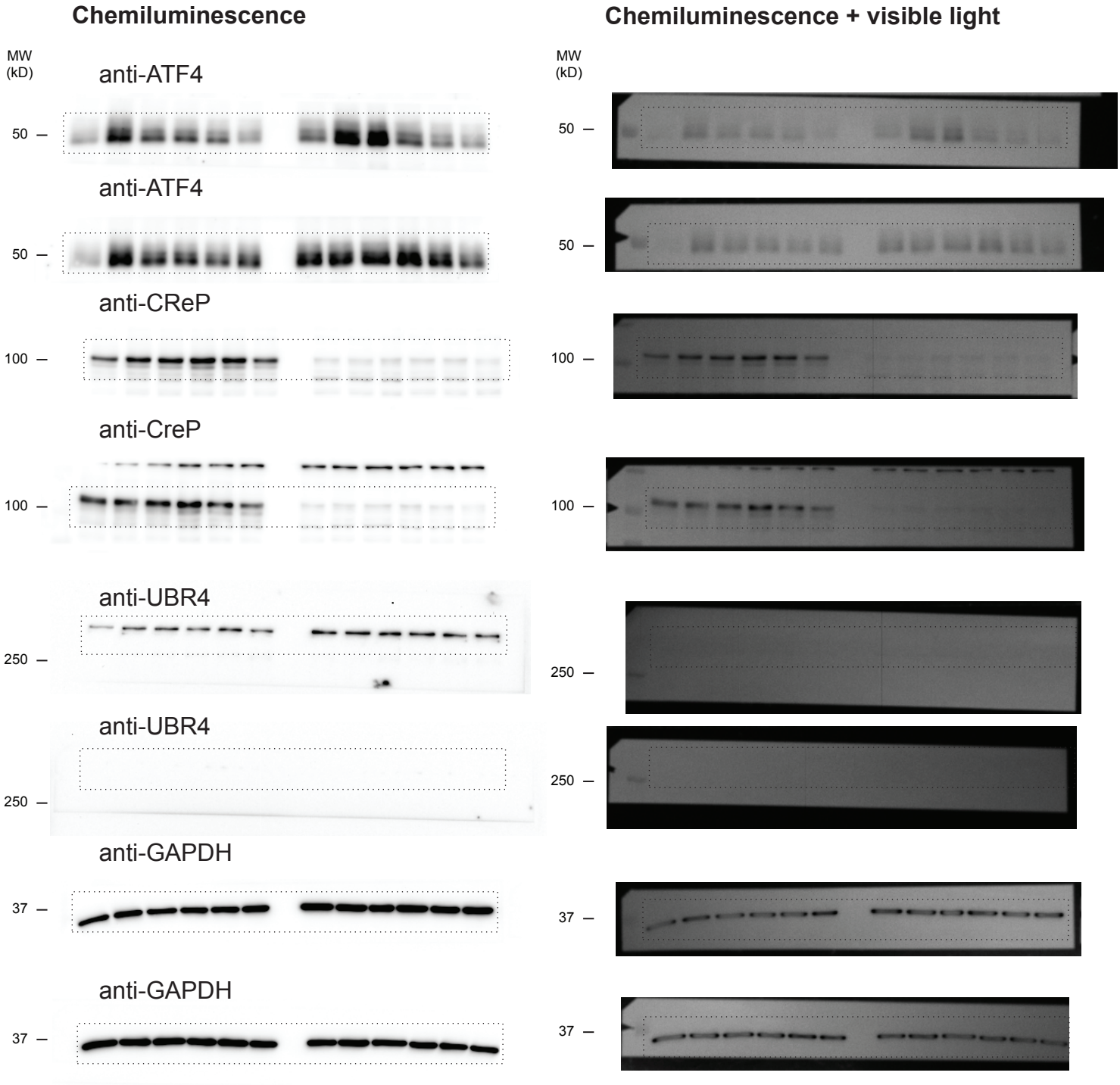
The loading control GAPDH was run on the same gel as ATF4, HRI and UBR4.

Extended Figure 6c



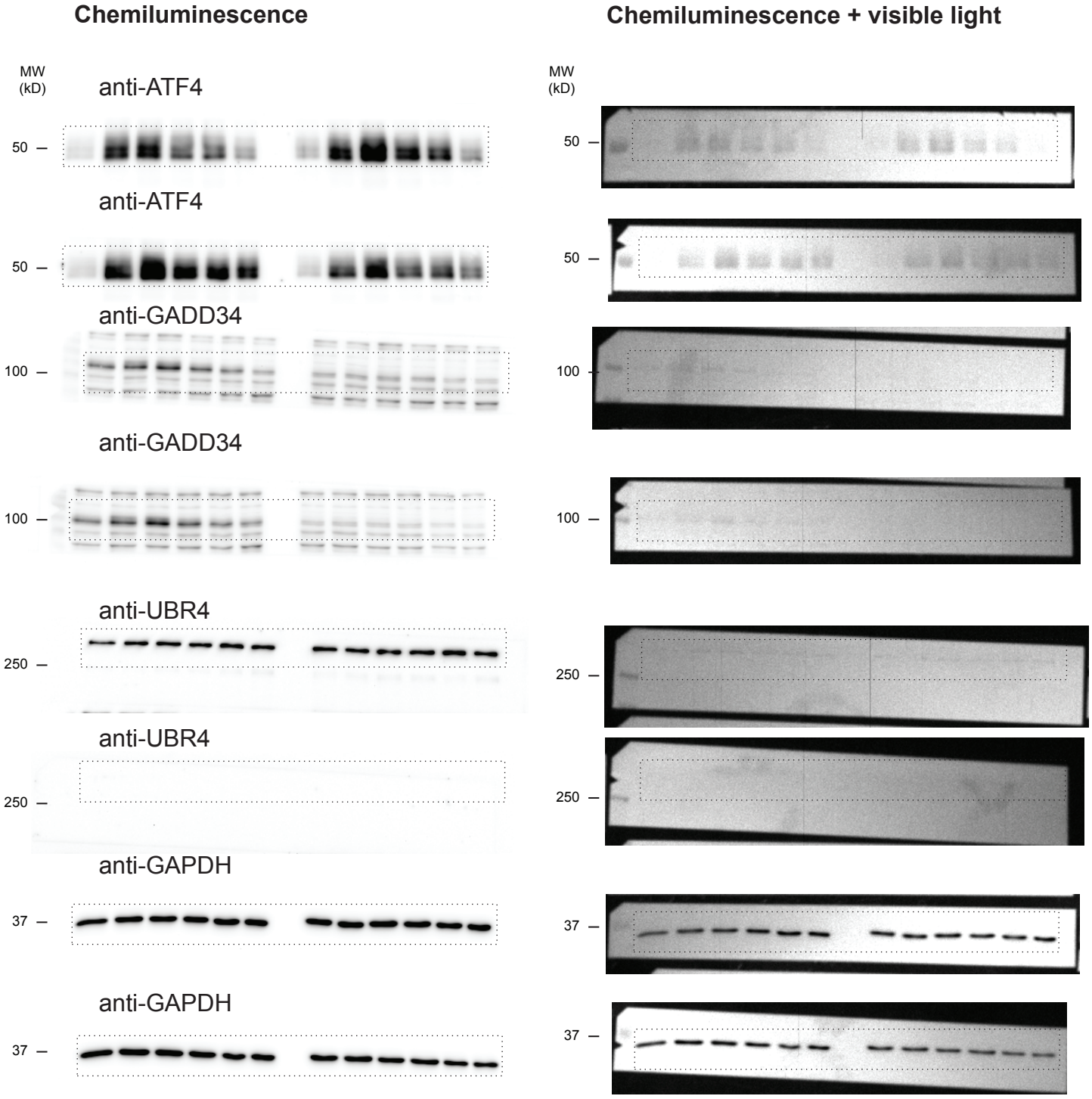
The loading controls tubulin were obtained by stripping the corresponding ATF4 membranes, which were run on the same gels as UBR4 in wt and *ΔUBR4* respectively.

Extended Data Figure 6d



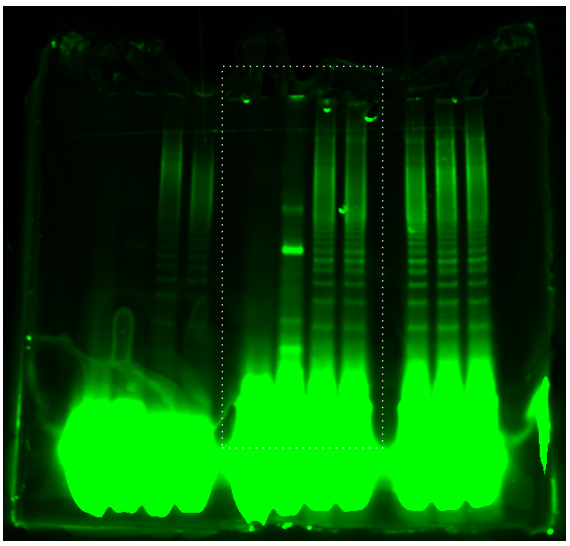
The loading controls GAPDH were run on the same gels as ATF4, CReP and UBR4 in wt and *ΔUBR4* respectively.

Extended Data Figure 6e



The loading controls GAPDH were run on the same gels as ATF4, GADD34 and UBR4 in wt and *ΔUBR4* respectively.

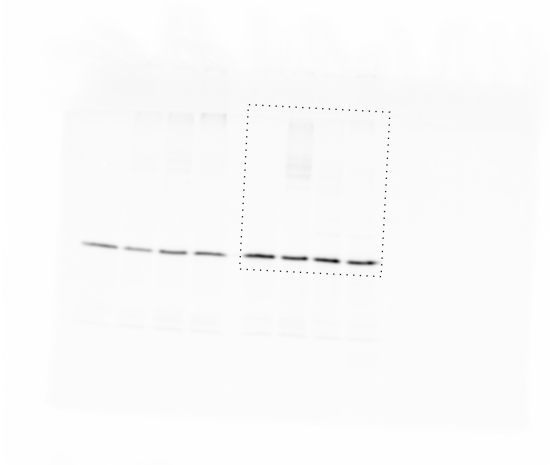
Extended Data Figure 7b



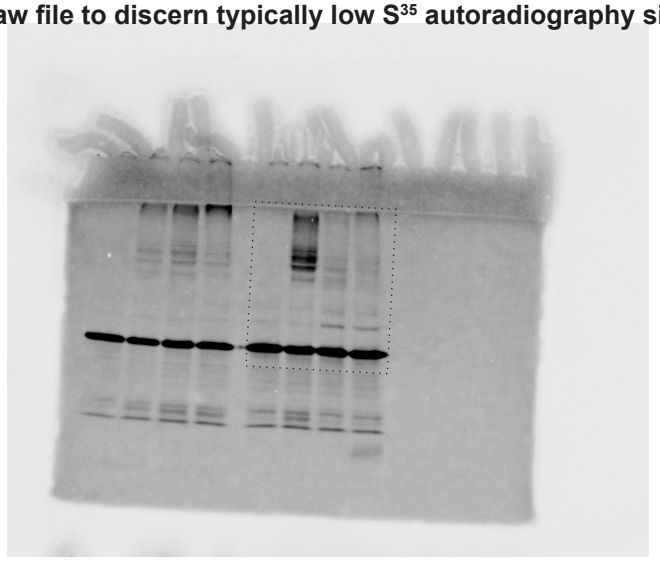
Extended Data Figure 7c

Raw file

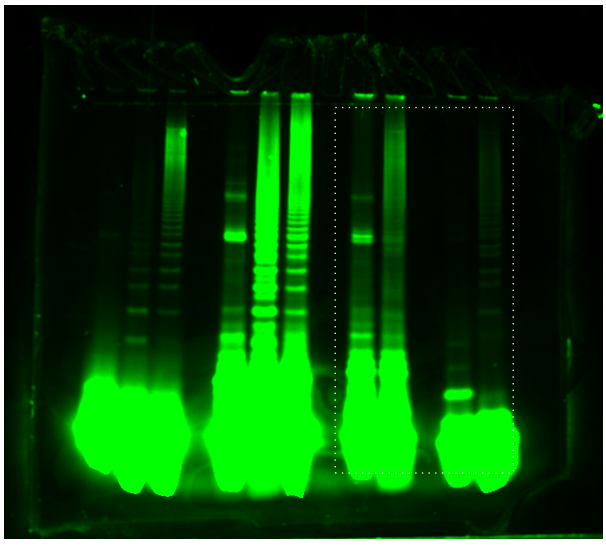
HRI-sumo autoradiography



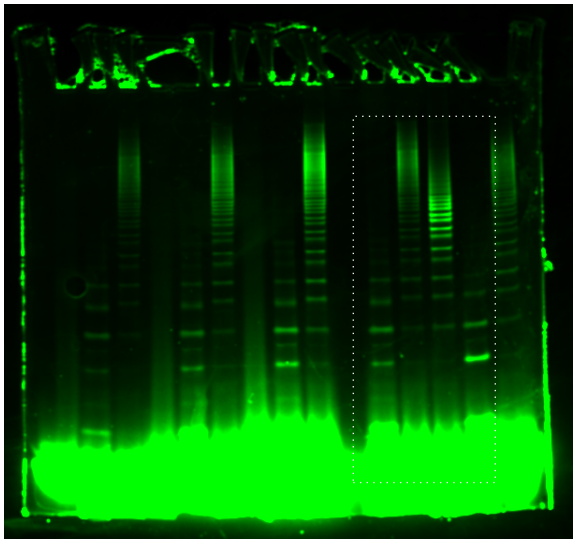
Contrasted raw file to discern typically low S^{35} autoradiography signal



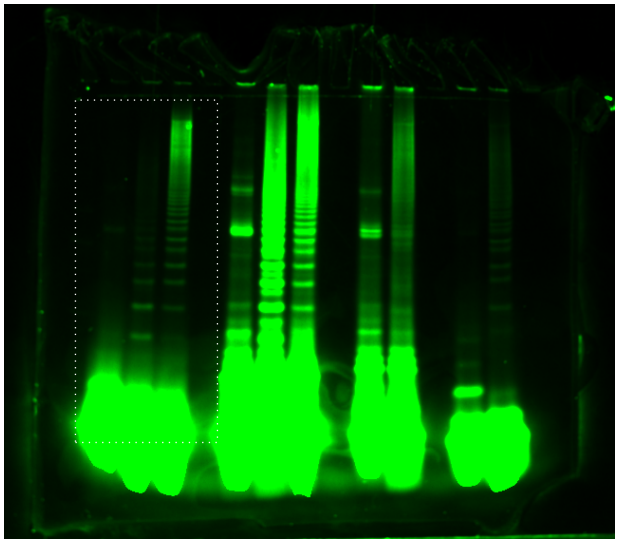
Extended Data Figure 7f



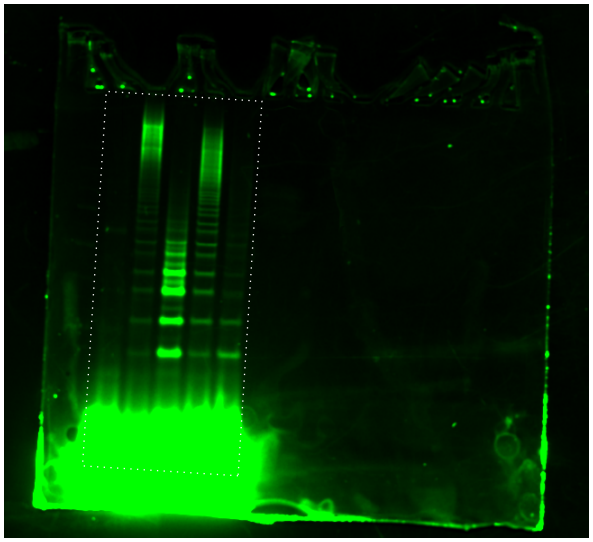
Extended Data Figure 8c



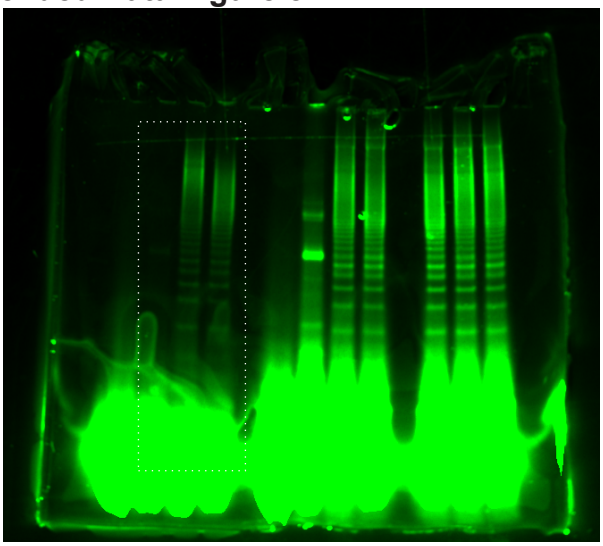
Extended Data Figure 8d



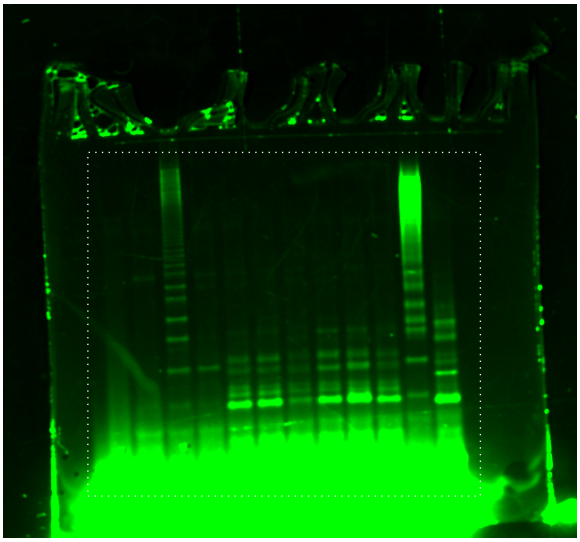
Extended Data Figure 8e



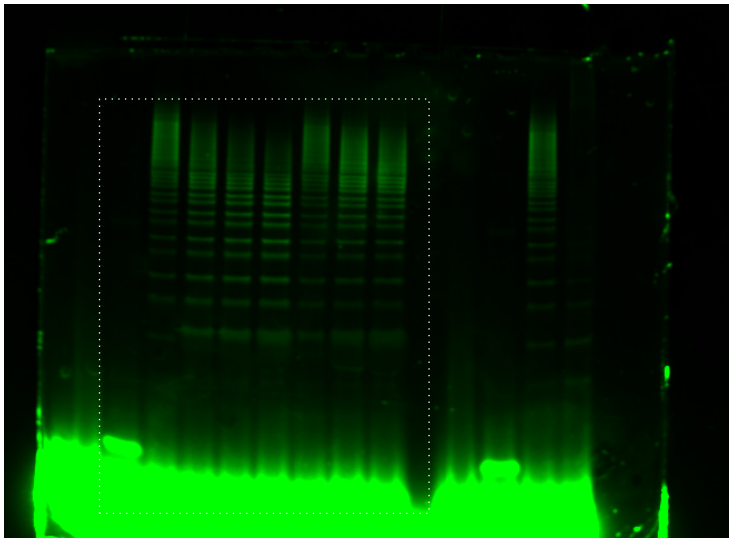
Extended Data Figure 8f



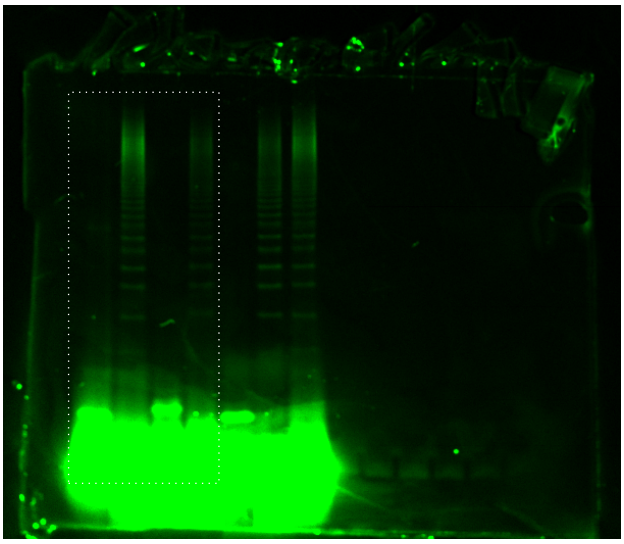
Extended Data Figure 8g



Extended Data Figure 8j

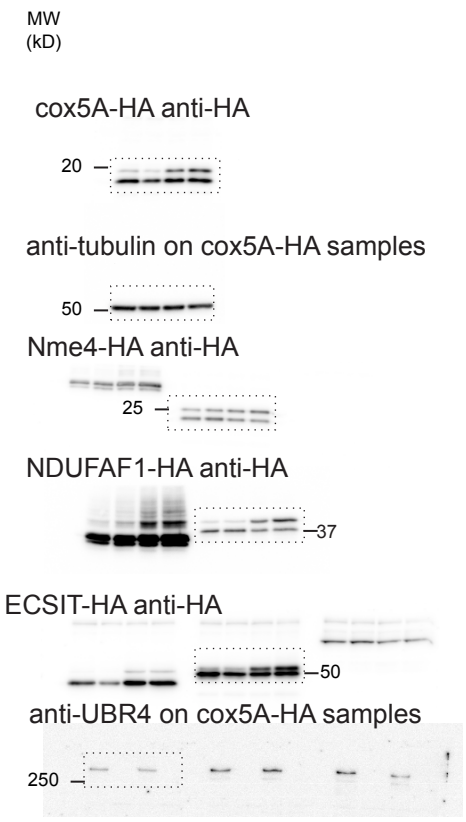


Extended Data Figure 8k

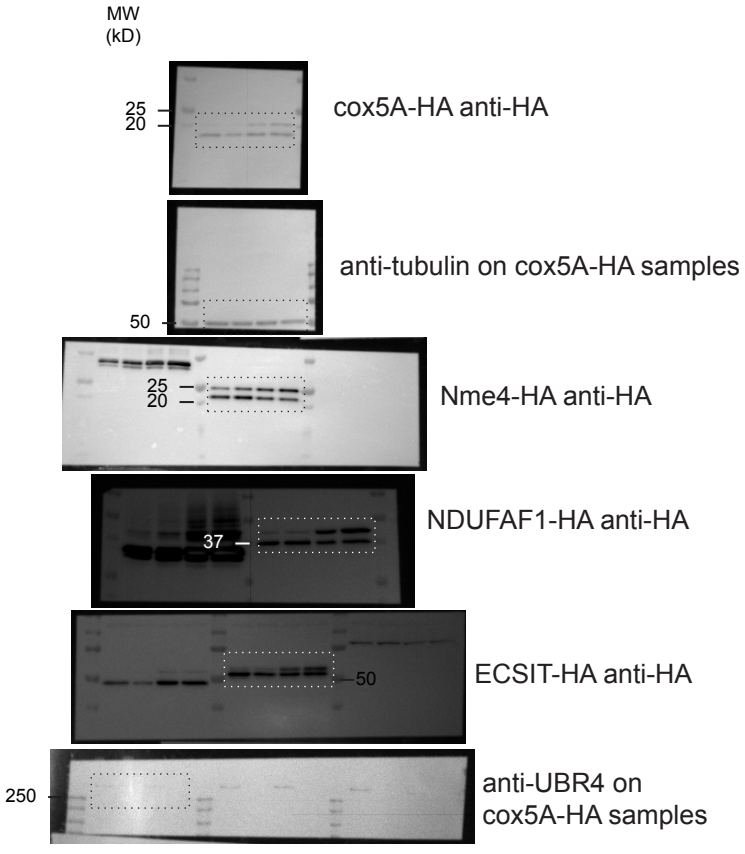


Extended Data Figure 8l

Chemiluminescence

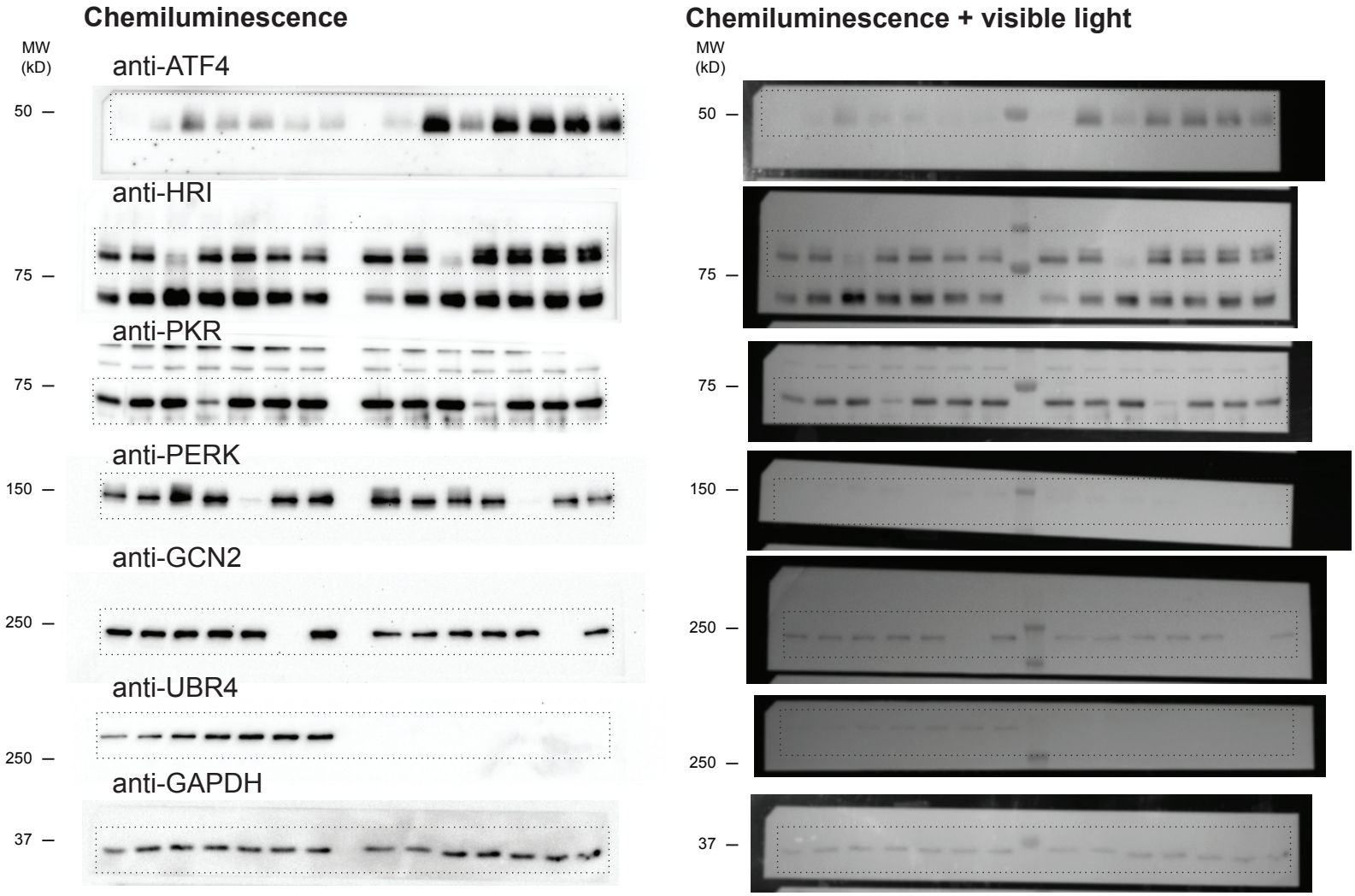


Chemiluminescence + visible light



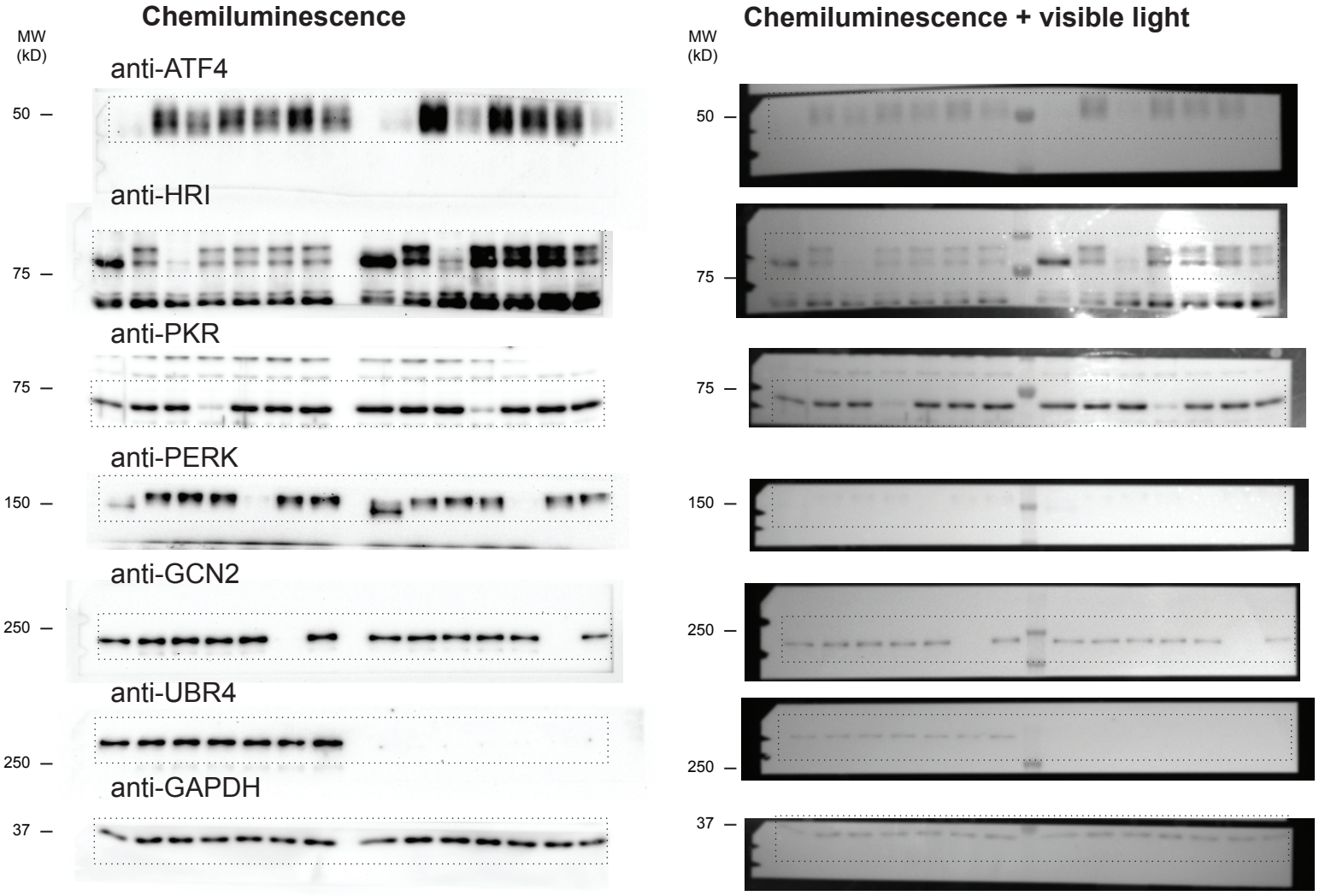
The loading control Tubulin was run on the same gel as cox5AHA.

Extended Data Figure 9a



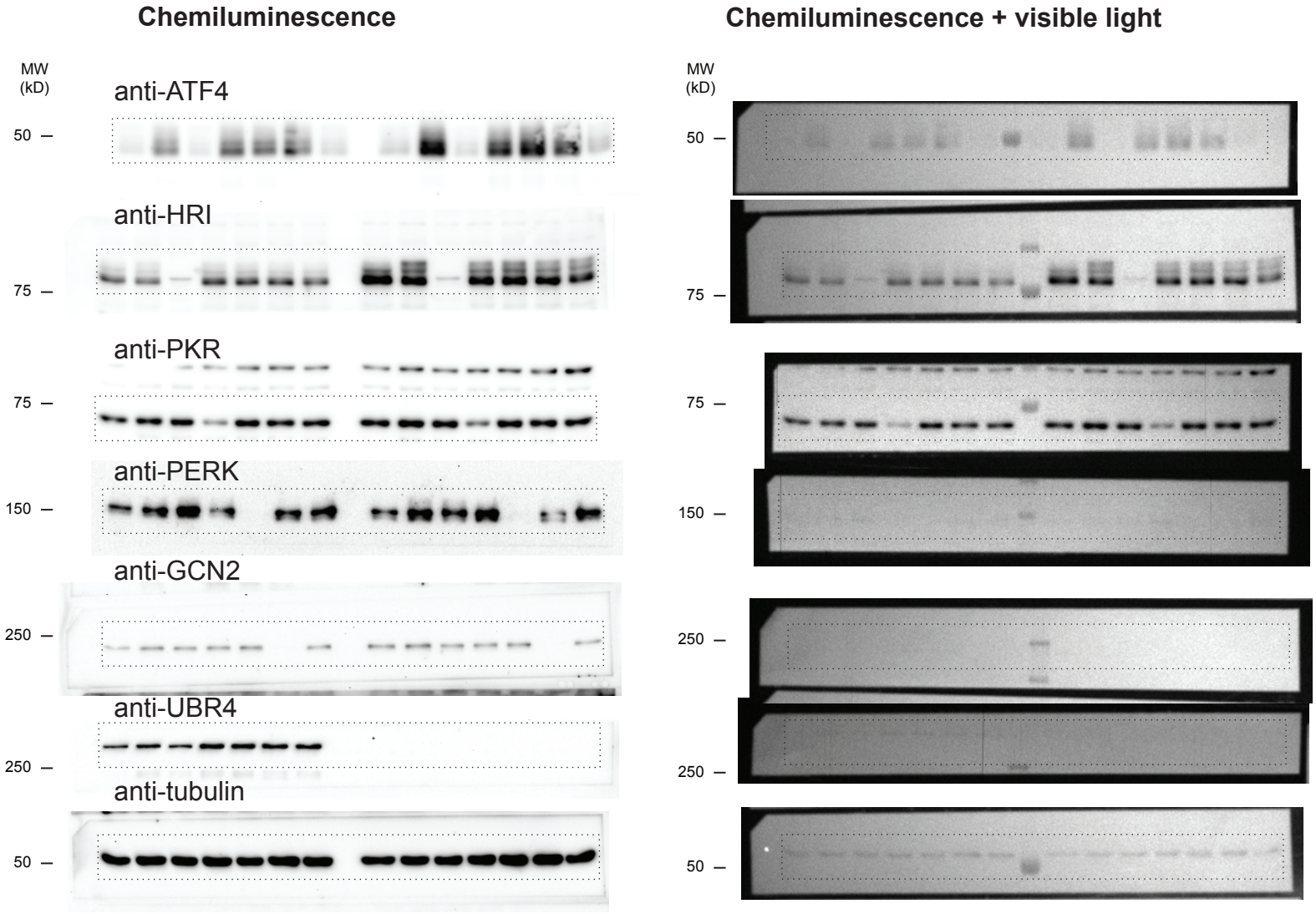
The loading control GAPDH was run on the same gel as PKR, PERK and UBR4.

Extended Data Figure 9b



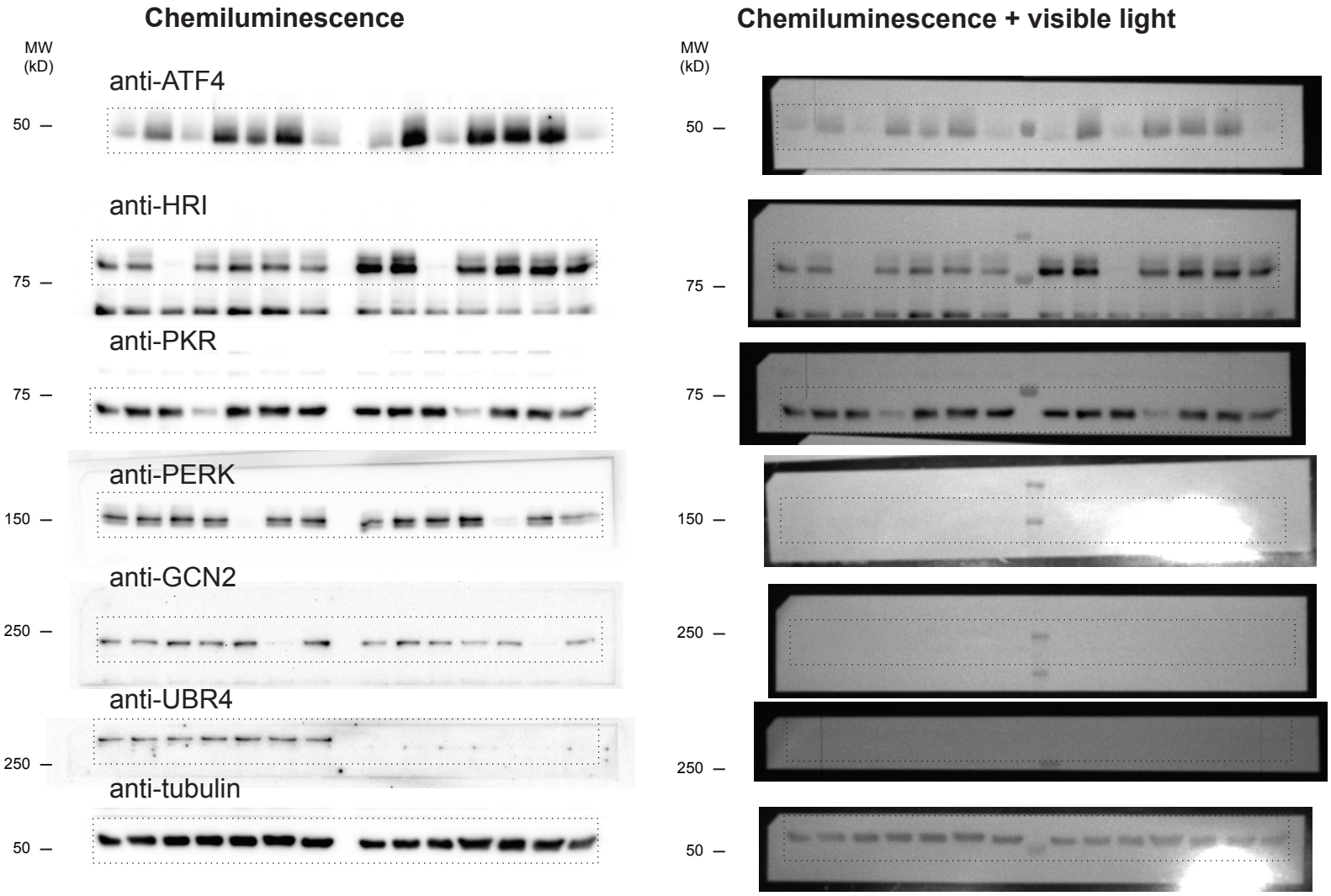
The loading control GAPDH was run on the same gel as PKR, PERK and UBR4.

Extended Data Figure 9c



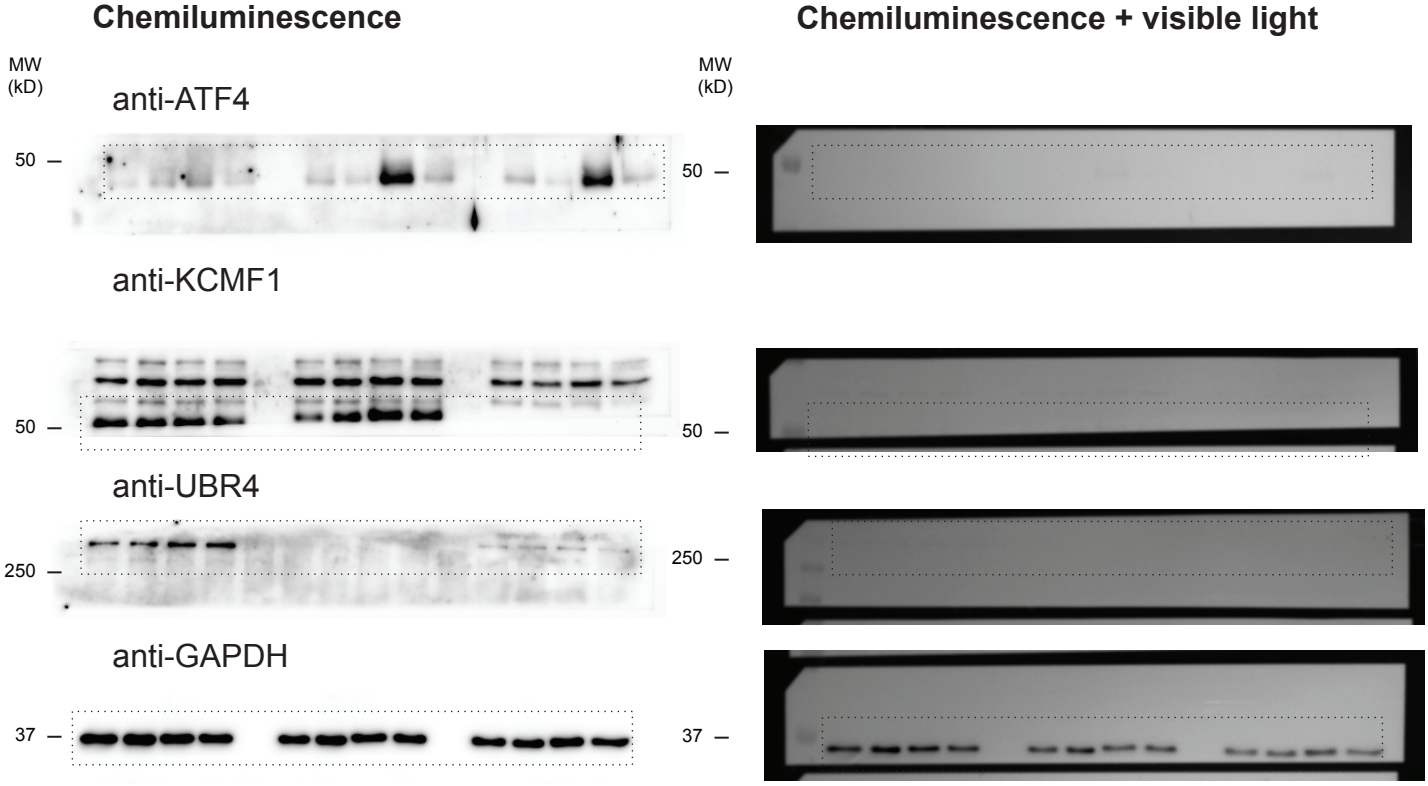
The loading control tubulin was run on the same gel as GCN2 and HRI.

Extended Data Figure 9d



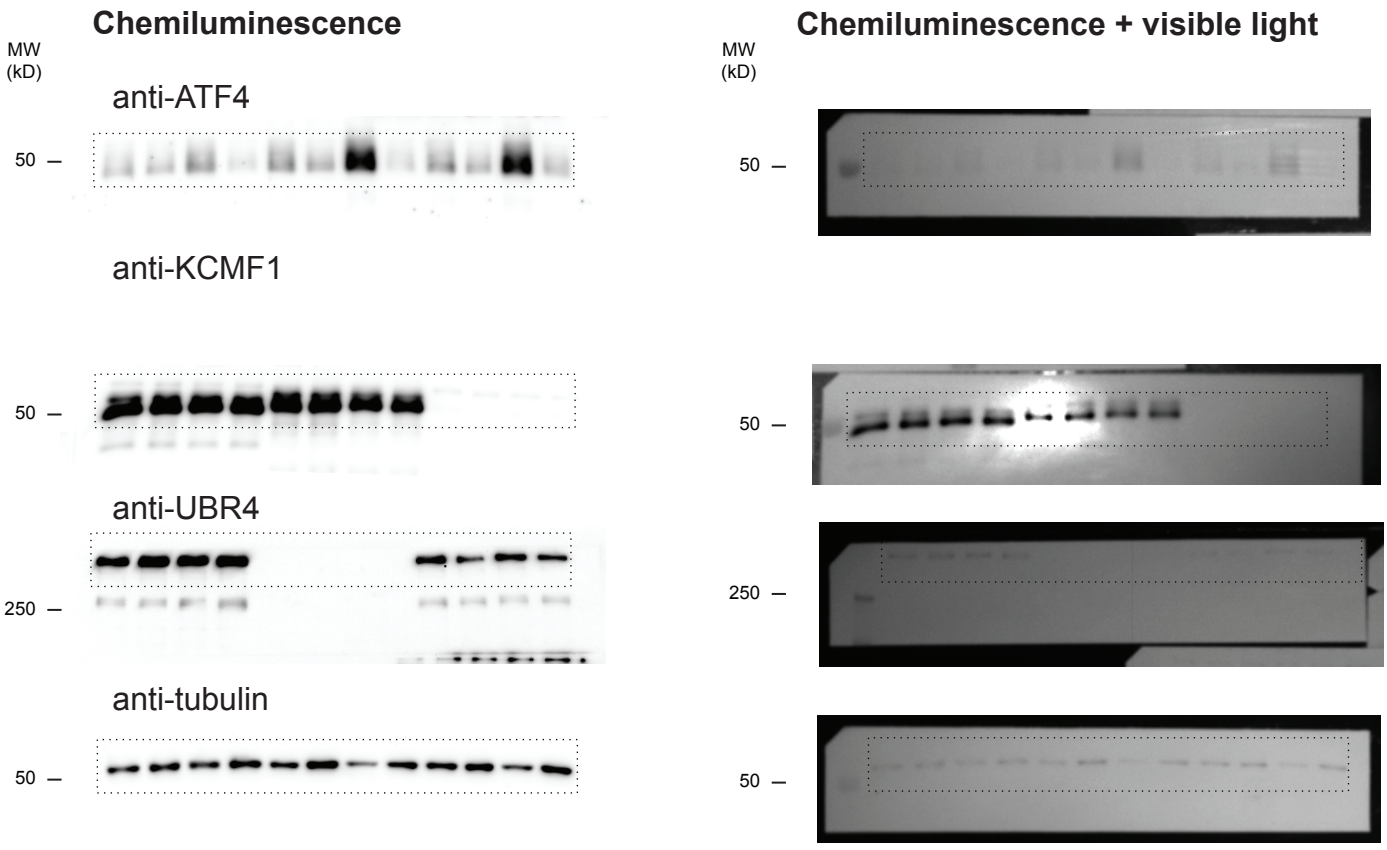
The loading control tubulin was run on the same gel as GCN2 and HRI.

Extended Data Figure 10a



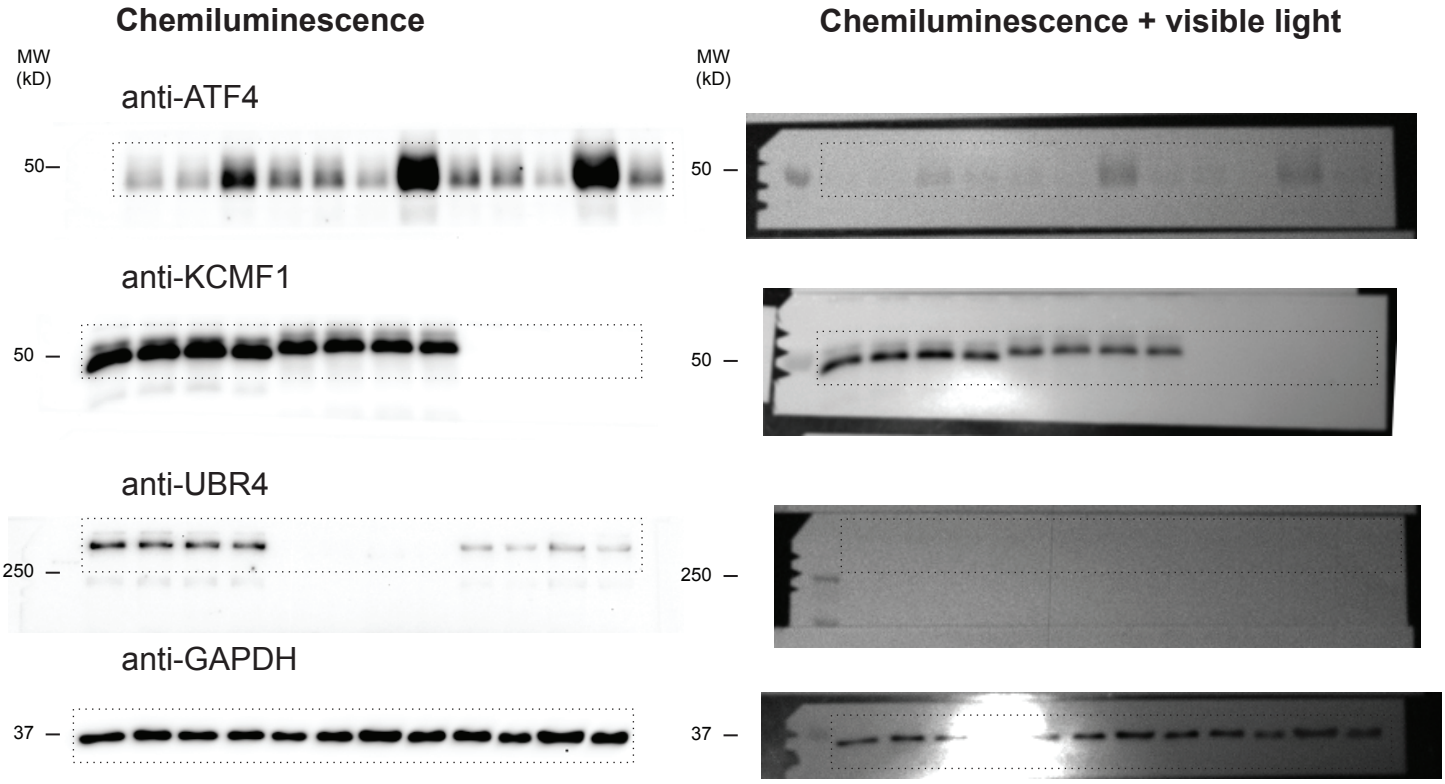
The loading control GAPDH was run on the same gel as UBR4 and KCMF1.

Extended Data Figure 10b



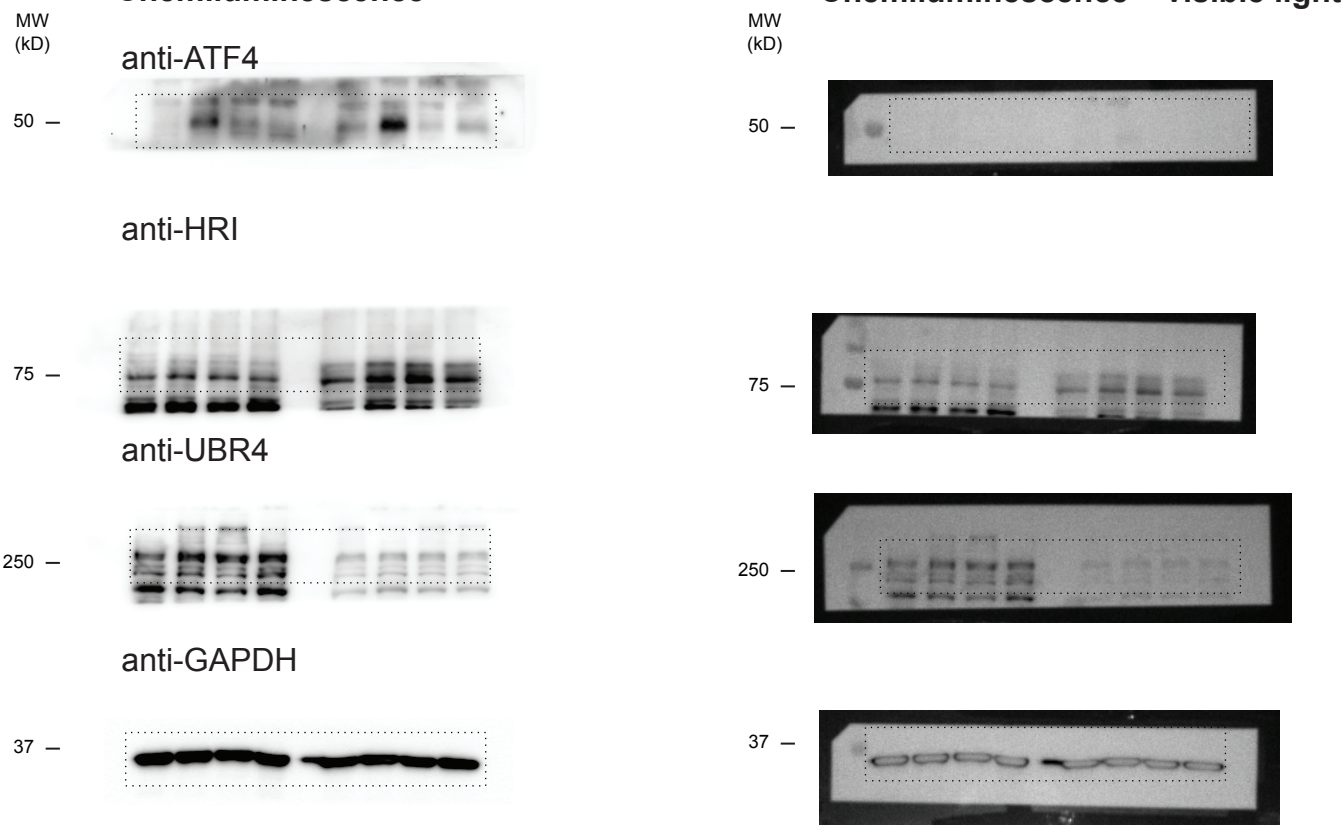
The loading control tubulin was obtained by stripping the ATF4 membrane, which was run on the same gel as UBR4.

Extended Data Figure 10c



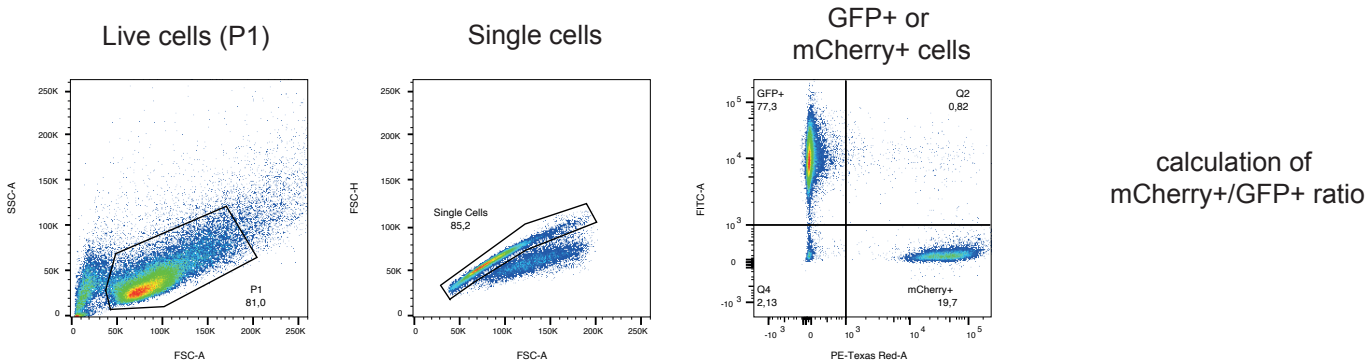
The loading control GAPDH was run on the same gel as UBR4 and ATF4.

Extended Data Figure 10e

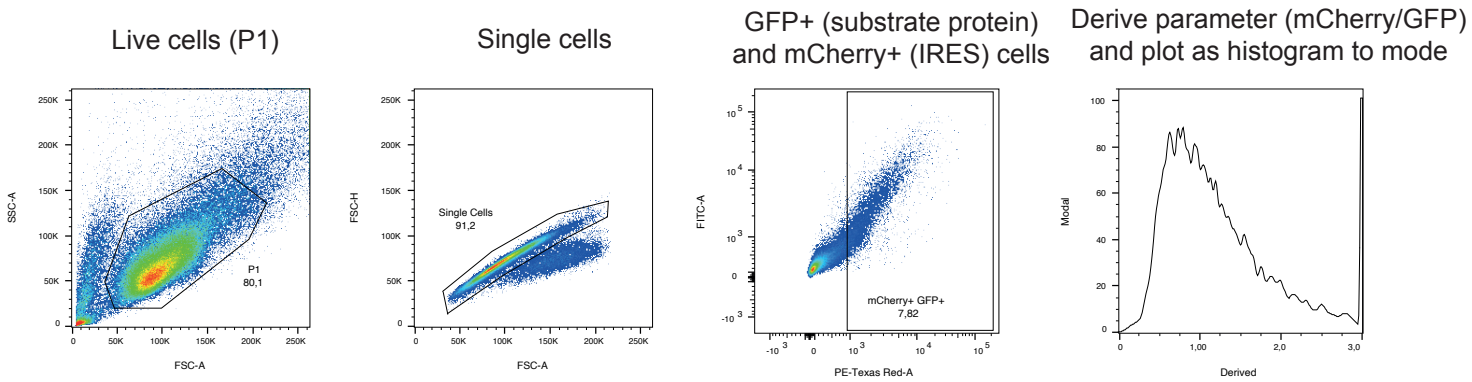


The loading control GAPDH was run on the same gel as UBR4, HRI and ATF4.

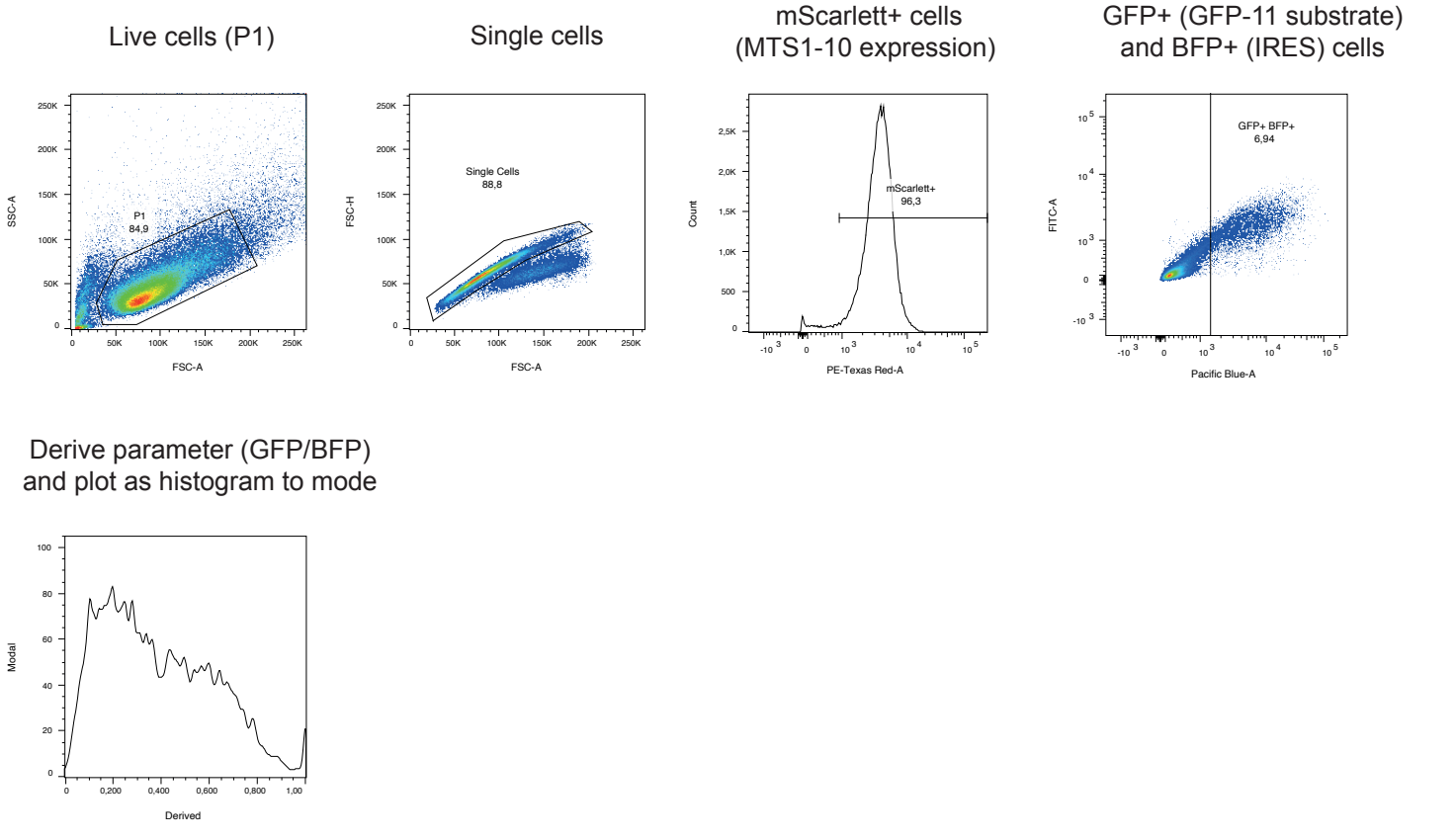
a Gating strategy for cell competition assays:



b Gating strategy for protein stability reporter assays:



c Gating strategy for mitochondrial protein import assays:



Supplementary Figure 2. Gating strategy for flow cytometry experiments. Representative plots shown for each type of flow cytometry analysis in this study. **a.** Gating strategy for cell competition assays as used in Fig. 1c, 1d, 1f, 5g, 5h and Extended Data Fig. 3e, 10f, 10g.
b. Gating strategy for protein stability reporter assays as used in Fig. 2b, 2c, 3a, 4a, 4c, 4f, 4j, 4k, 4l, 5a, 5e, 5f and Extended Data Fig. 3f, 3g, 3j, 3k, 4e, 5a, 5f, 5g, 6f, 6g, 7a, 7d, 7e, 7g, 8j, 8k, 8m, 8n, 8o, 8p.
c. Gating strategy for mitochondrial protein import assays as used in Fig. 2a, 5c and Extended Data Fig. 3a, 3b, 3c, 9e, 10d.