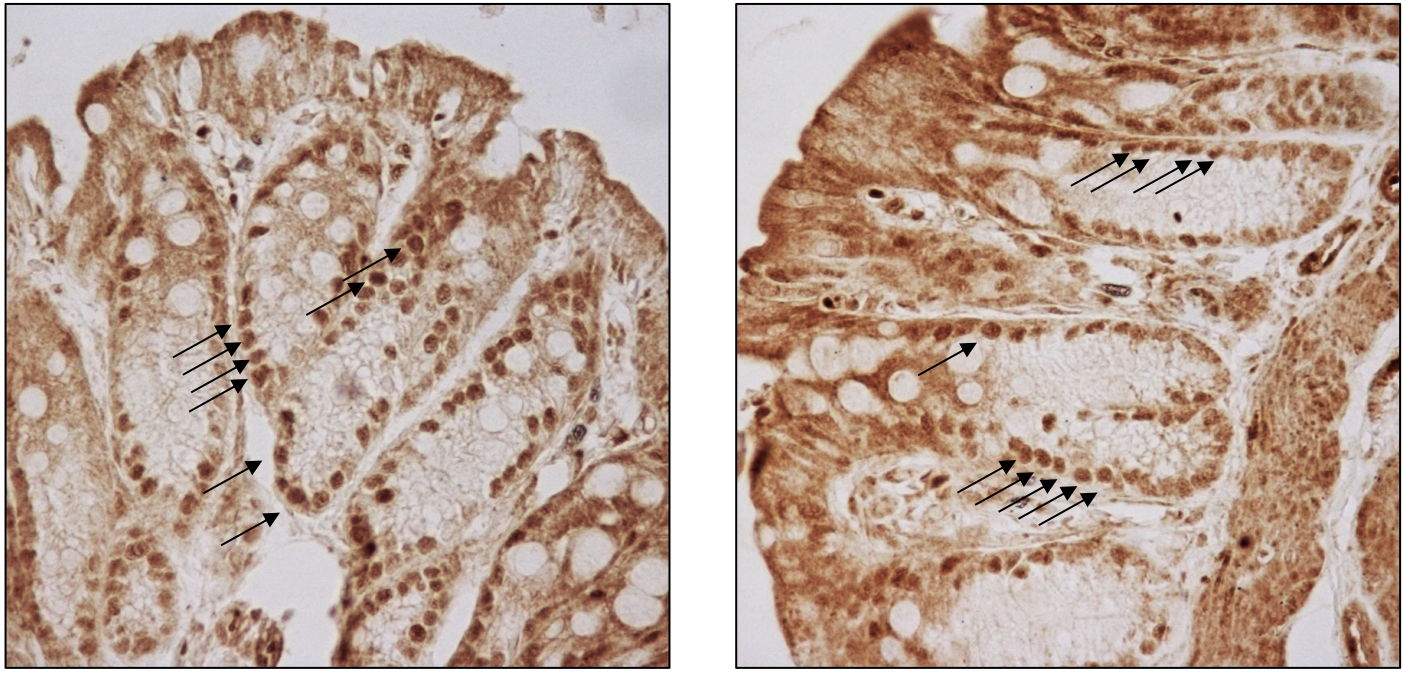
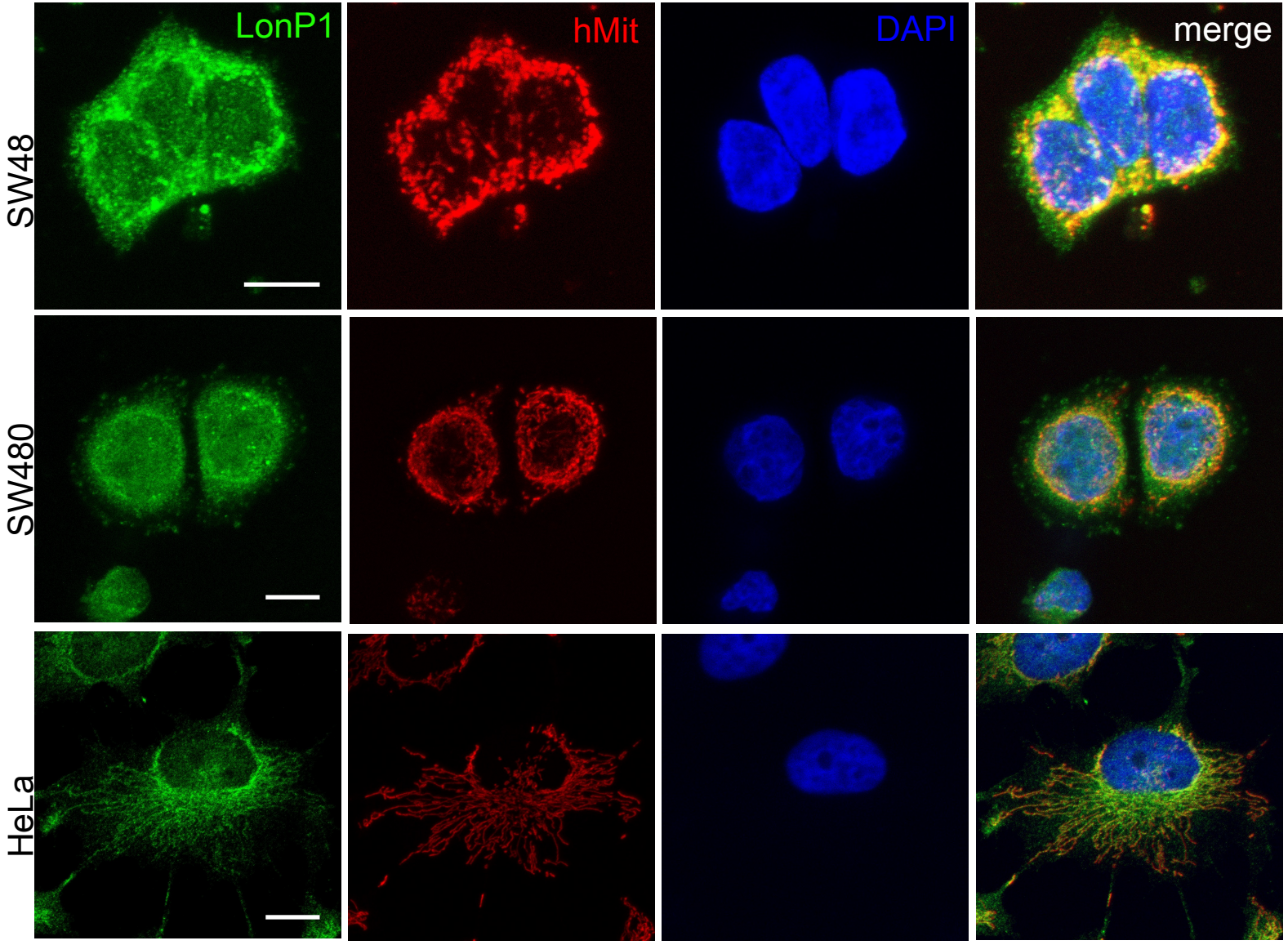
Supplementary material

“Evidence for mitochondrial Lonp1 expression in the nucleus”
Gibellini et al



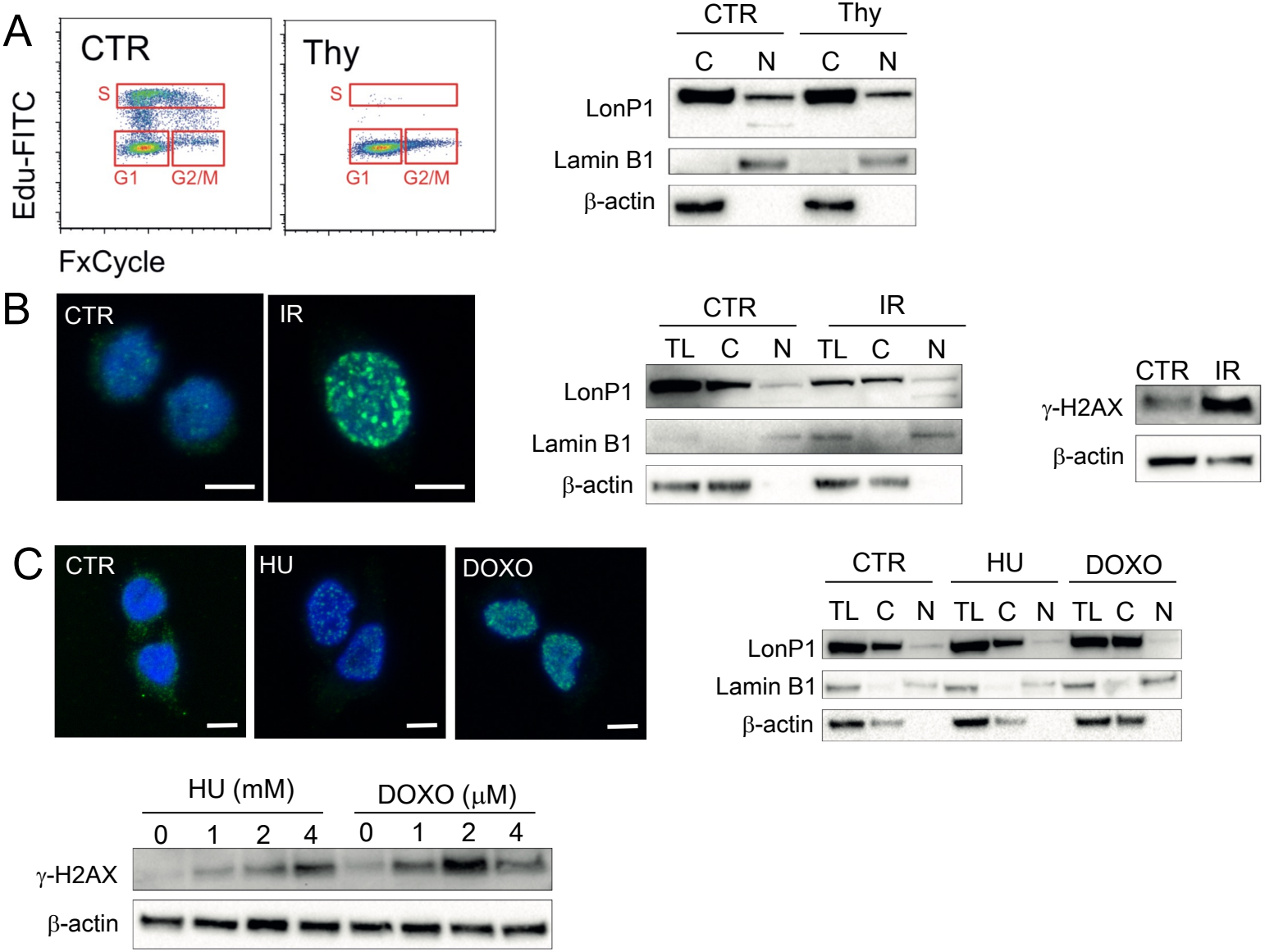
Supplementary Figure 1

Representative images of Lonp1 IHC on murine colon tissue, showing Lonp1 nuclear localization. Cells have been labelled with anti Lonp1 Ab from Sigma Aldrich (Cat. n. HPA002034) and revealed by an HRP-conjugated goat anti-rabbit Ab. Positive nuclei are indicated by arrows.

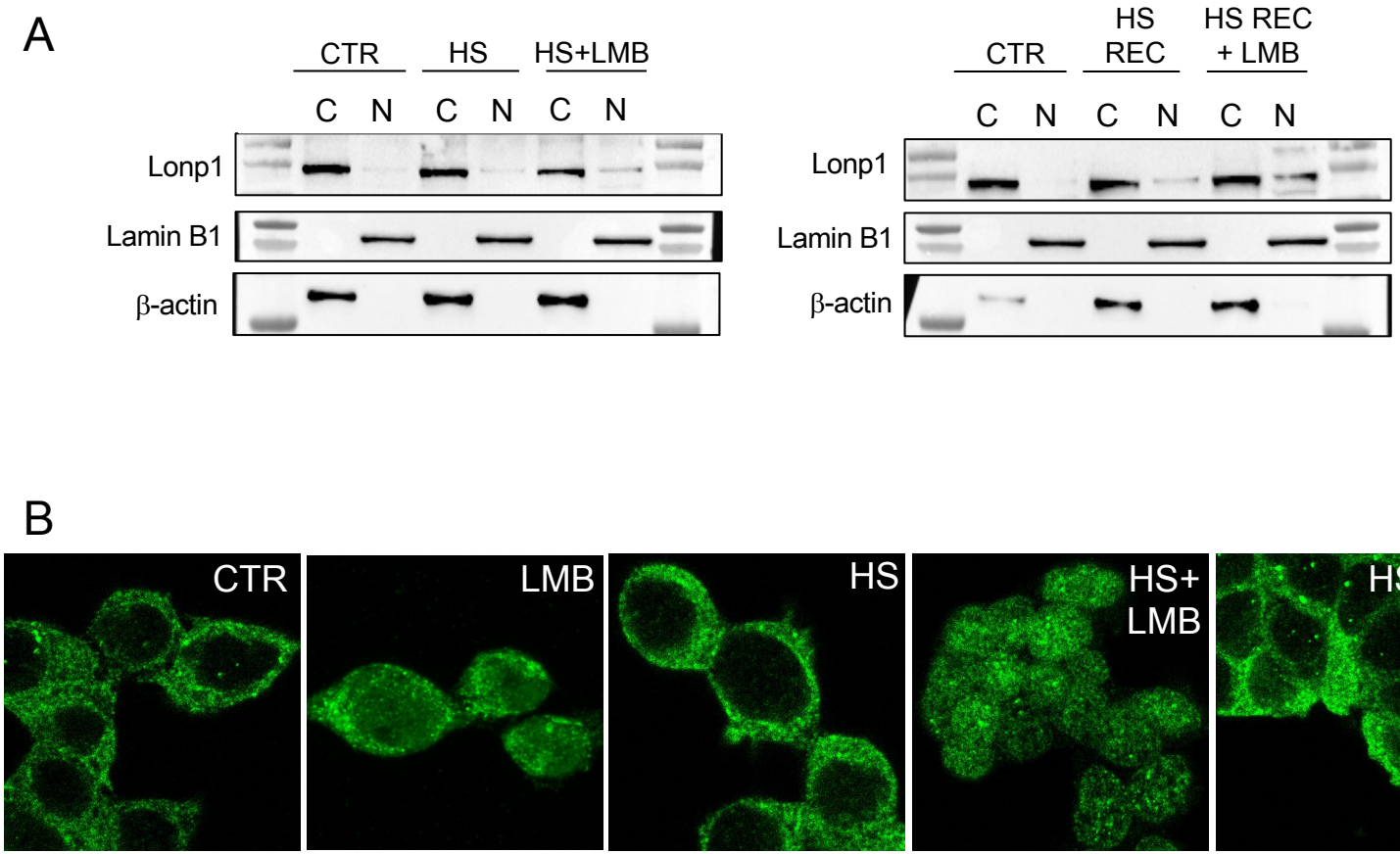


Supplementary Figure 2. Lonp1 localizes in the nucleus in SW48, SW480 and SW48 cells.

Representative confocal microscopy images of SW48, SW480 and HeLa cells after immunostaining with anti-Lonp1 and anti-human mitochondria (hMit) antibodies. Nuclei were counterstained with DAPI.

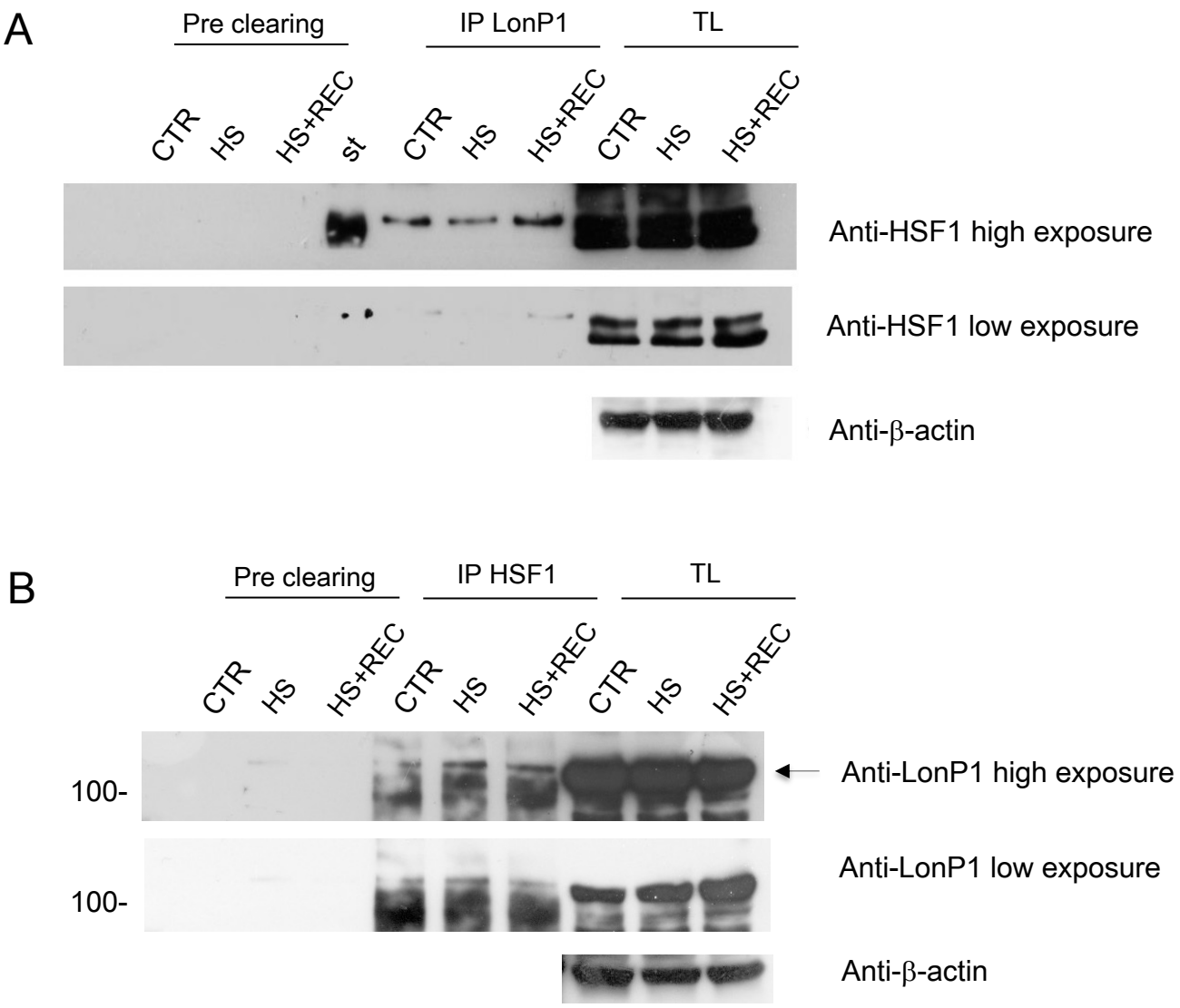


Supplementary Figure 3. DNA damage or cell cycle do not influence Lonp1 nuclear expression.
A. Cell cycle analysis of SW620 cells treated or not with thymidine, using 5-ethynyl-2´-deoxyuridine. Cell were treated with thymidine (Thy) to block cell cycle in the G1 phase. Representative immunoblot of cytosolic (C) and nuclear (N) fractions obtained from SW620 cells, treated or not with thymidine. B. Representative confocal microscopy images of SW620 control cells (CTR), and cells irradiated with 5 Gy (IR), immunostained with anti-γ-H2AX. Bars: 10 μm. Representative immunoblot reporting the expression of γ-H2AX in SW620 control cells, and cells irradiated with 5 Gy. C. Representative confocal microscopy images of SW620 control cells (CTR), and cells treated with hydroxyurea (HU) or doxorubicin (DOXO), immunostained with anti-γ-H2AX. Bars: 10 μm. Representative immunoblot of SW620 cells (CTRL), and cells treated with increasing concentrations of HU and DOXO. Right panel: Representative immunoblot of total lysate (TL), cytosolic (cyt) and nuclear (N) fractions obtained from SW620 treated or not with 4 mM HU or 2 μM DOXO.



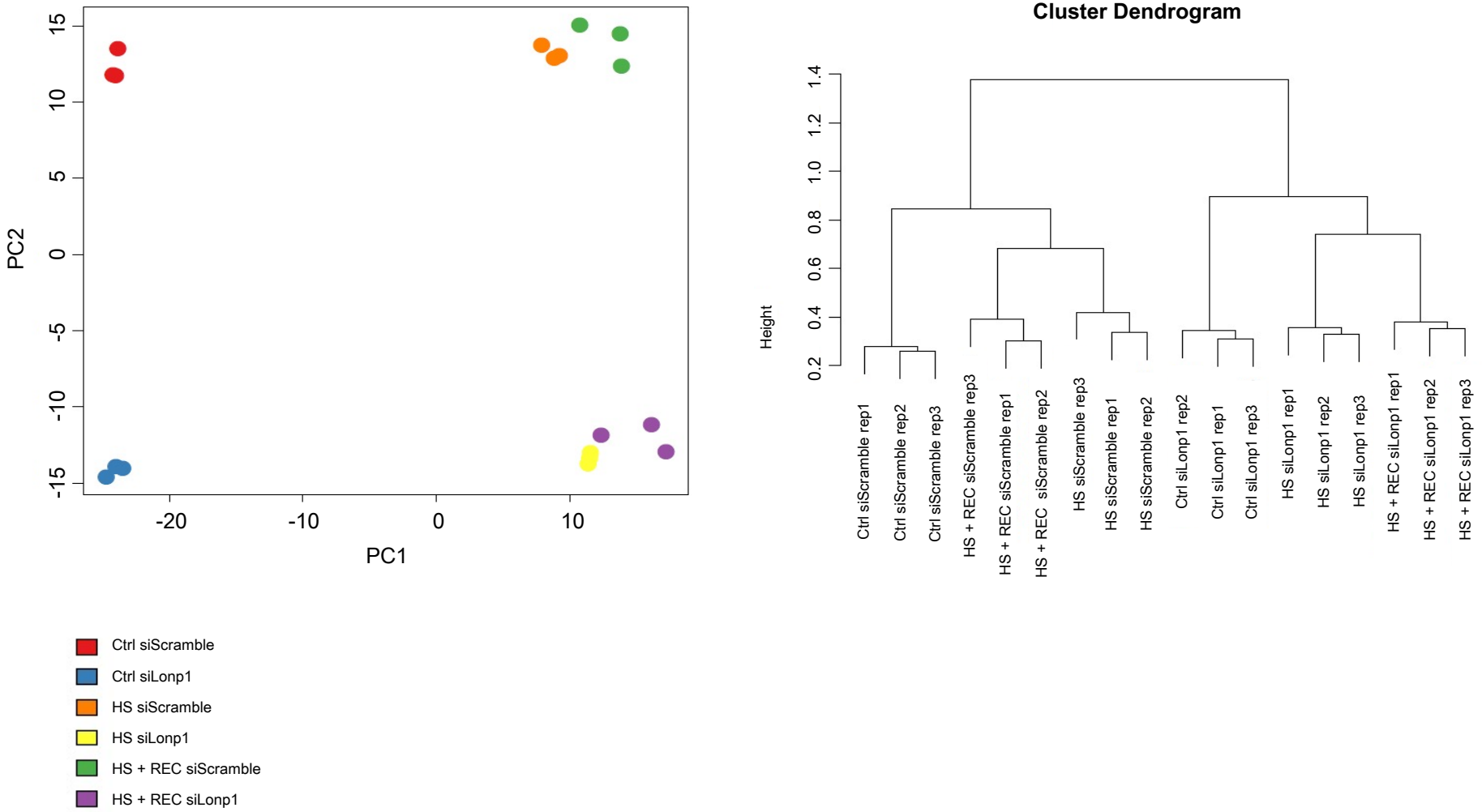
Supplementary Figure 4

A. Immunoblot showing Lonp1 expression in cytosolic (C) and nuclear (N) fractions from SW620 cells maintained at 37°C (CTR), at 42°C for 3 hrs to induce heat shock (HS) and kept at 42°C for 3 hrs and then left for 1 hour at 37°C to recover (HS+REC) in the presence or not of Leptomycin B (LMB) 50 nM. β-actin is the cytosolic fraction loading control and Lamin B1 is the nuclear fraction loading control. B. Confocal microscopy images of SW620 cells kept at 37°C (CTR), at 42°C for 3 hrs to induce heat shock (HS) and kept at 42°C for 3 hrs and then left for 1 hour at 37°C to recover (HS+REC) in the presence or not of Leptomycin B (LMB) 50 nM.



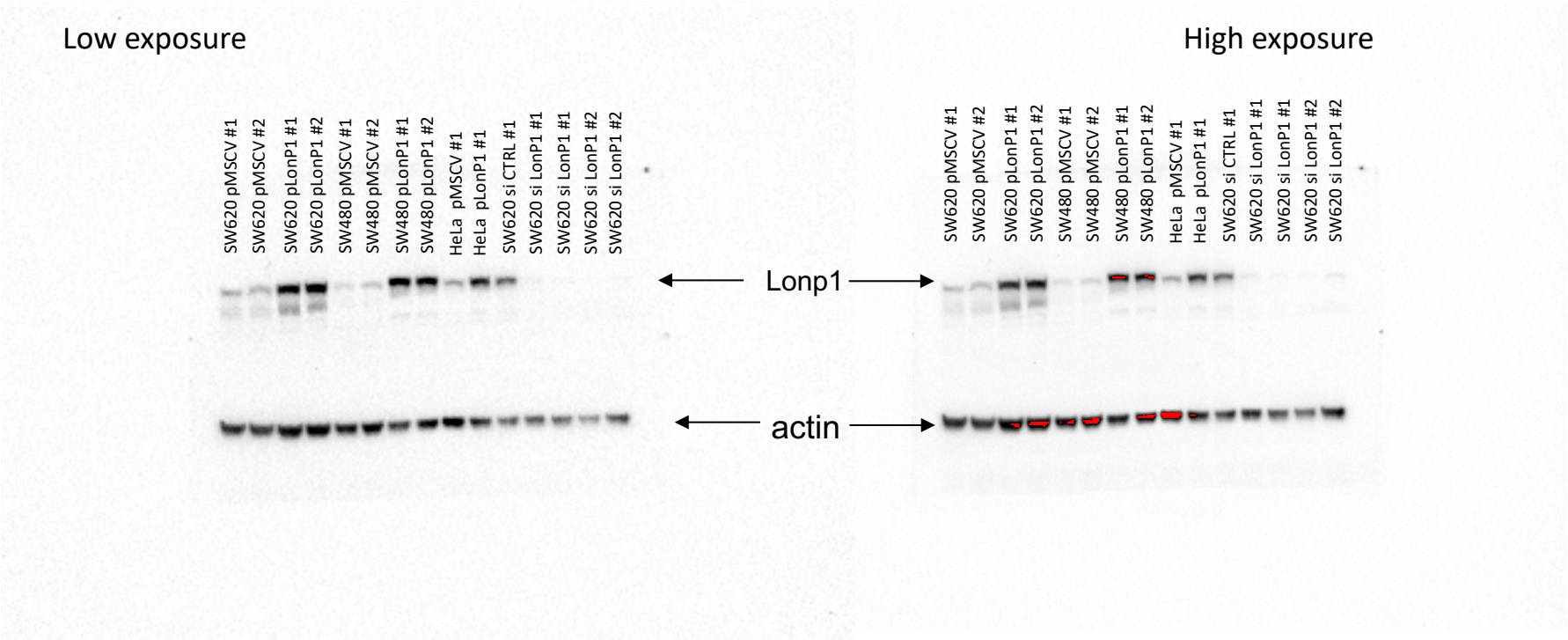
Supplementary Figure 5

A. Immunoprecipitation experiment showing the interaction between endogenous Lonp1 and HSF1 in SW620 cells after 3 hours of heat shock and recovery at 37°C to recover (HS+REC). Immunoprecipitation has been performed using anti-Lonp1 Ab (Sigma-Aldrich), and immunoblotted with anti-HSF1 Ab. Immunoblot on Pre cleared lysate and total lysate (TL) are also shown. B. Immunoprecipitation experiment showing the interaction between HSF-1 and endogenous Lonp1 in SW620 cells after heat-shock (HS) and after 3 hours of heat shock and recovery at 37°C to recover (HS+REC). Immunoprecipitation has been performed using and anti HSF-1 Ab and immunoblotted with anti-Lonp1 (Sigma Aldrich). Immunoblot on precleared lysate and total lysate (TL) are also shown. St=standard.



Supplementary Figure 6

Principal component analysis and unsupervised cluster analysis and of RNAseq data from SW620 cells undergoing heat shock for 1 hour, heat shock for 1h followed by 1h hour of recovery, and cells kept at 37°C, when Lonp1 was silenced or not. Ctrl siScramble: cells transfected with a scrambled siRNA, kept at 37°C. Ctrl siLonp1: cells transfected with siRNA for LONP1, kept at 37°C. HS siScramble: cells transfected with scrambled siRNA, kept at 42°C for 1 hour. HS_siLONP1: cells transfected with LONP1 siRNA, kept at 42°C for 1 hour. Hs +REC siScramble: cells transfected with scrambled siRNA, kept at 42°C for 1 hour and left at 37°C for 1 hour to recover. HS + Rec siLonp1: cells transfected with LONP1 siRNA, kept at 42°C for 1 hour and left at 37°C for 1 hour to recover.



Supplementary Figure 7

Specific recognition of human Lonp1 by anti human Lonp1 antibody used in this study. Human SW620, SW480 and HeLa cells stably overexpressing Lonp1 by retroviral transduction, or control parental cells transduced with the empty vector pMSCV were probed with anti Lonp1 Ab. Cell overexpressing Lonp1 (lane 3, 4, 7, 8,10) show higher signal in WB analysis, in comparison to their control cells (lanes 1,2, 5, 6,9) show. Conversely, cells where Lonp1 was silenced by using two specific, pre validated siRNAs, show a very low signal in WB analysis, if compared to a control, scramble siRNA. The image is reported with two different exposure, to make signal in silenced cells visible. Analysis is shown in duplicate for overexpression in SW620, and SW480 cells, and for silencing with two antiLonp1 siRNA, and once for overexpression in HeLa cells.

Fig 2B

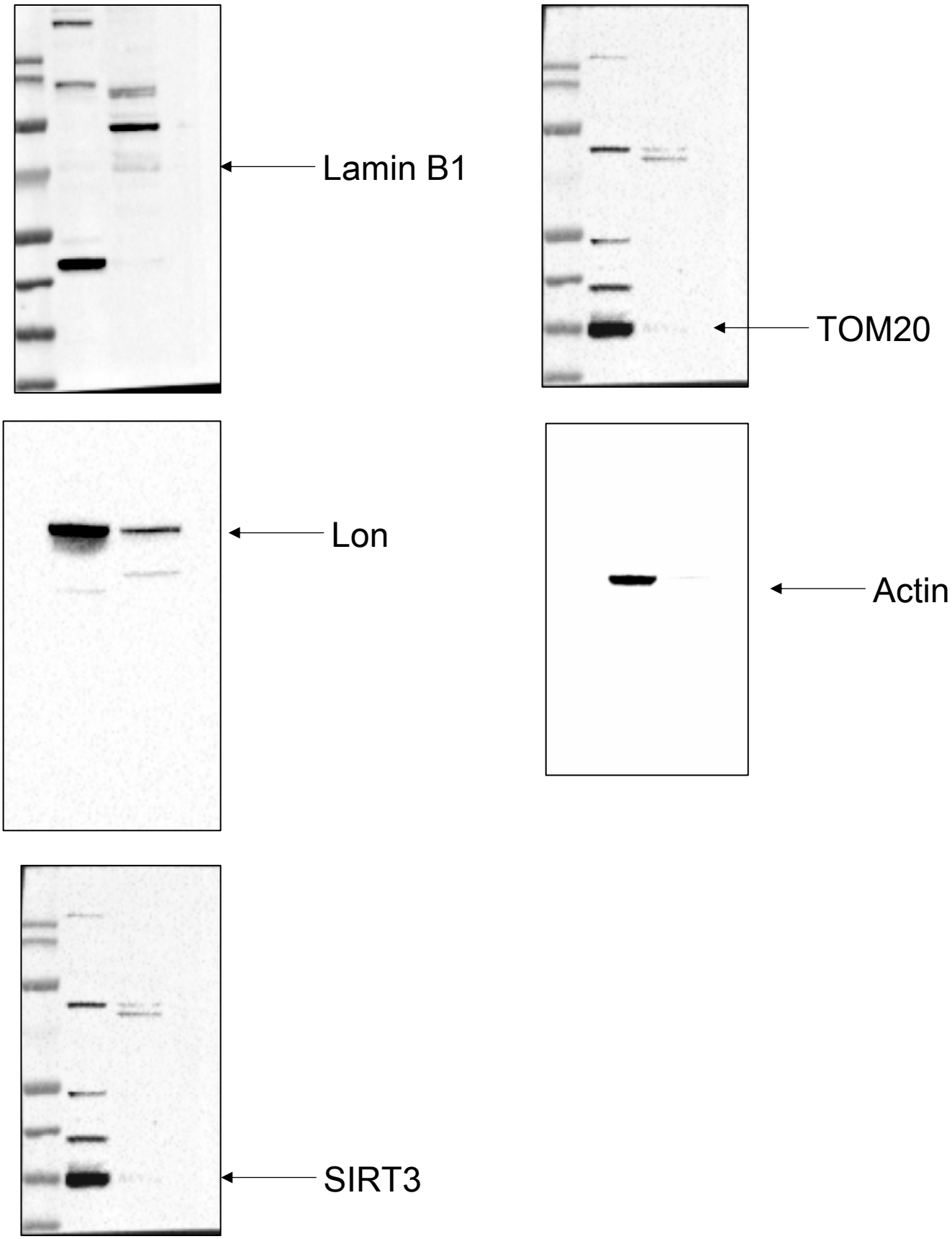


Fig 2B

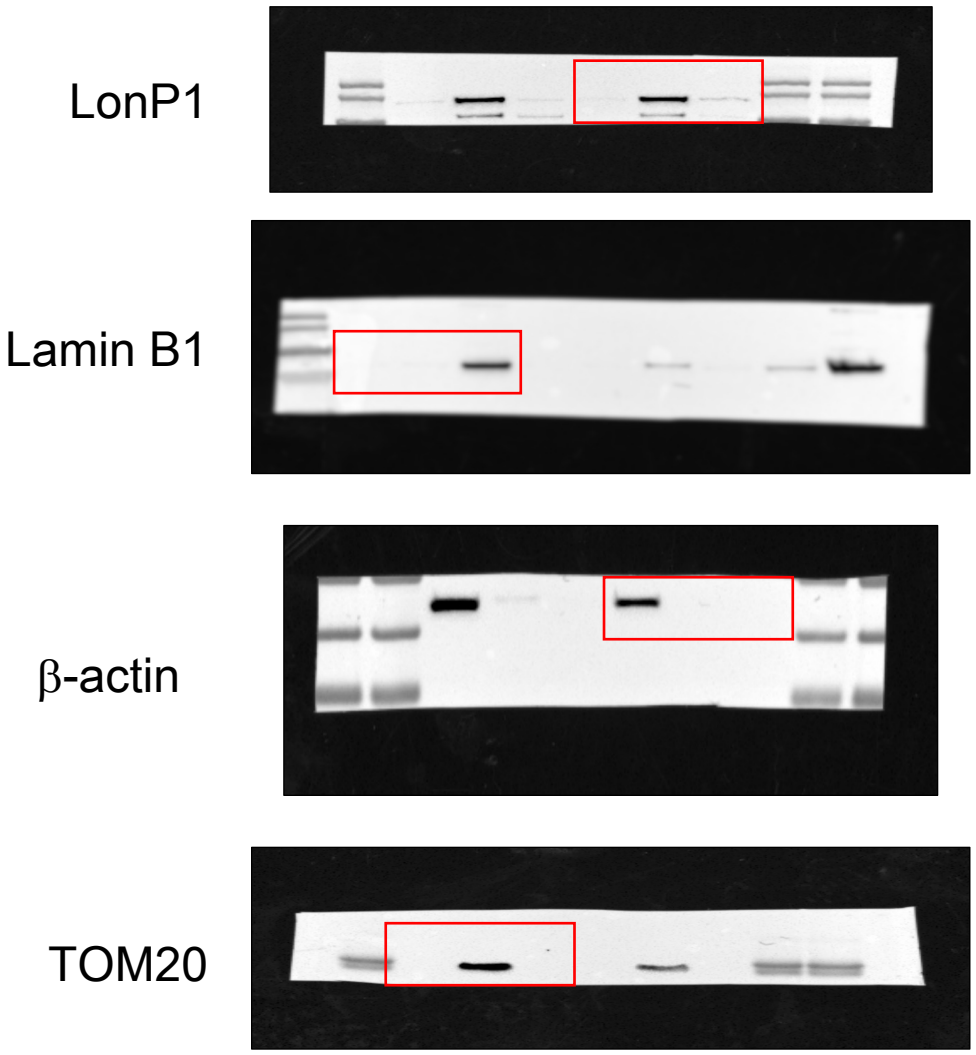
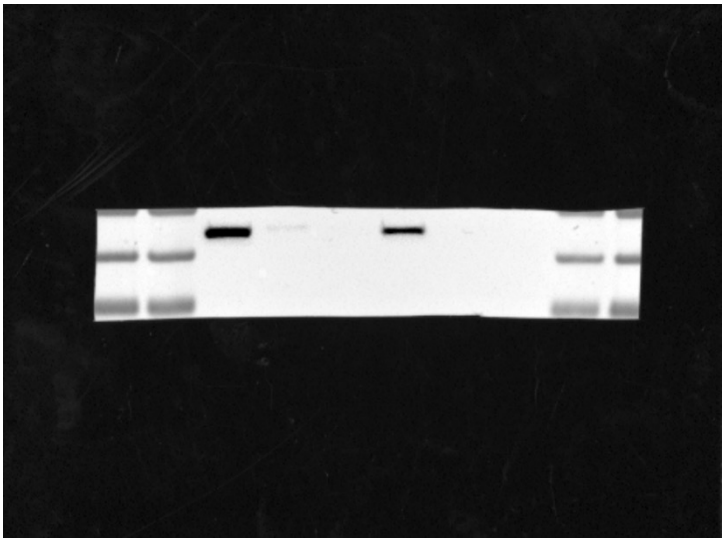


Figure 2 B different exposures of b-actin blot



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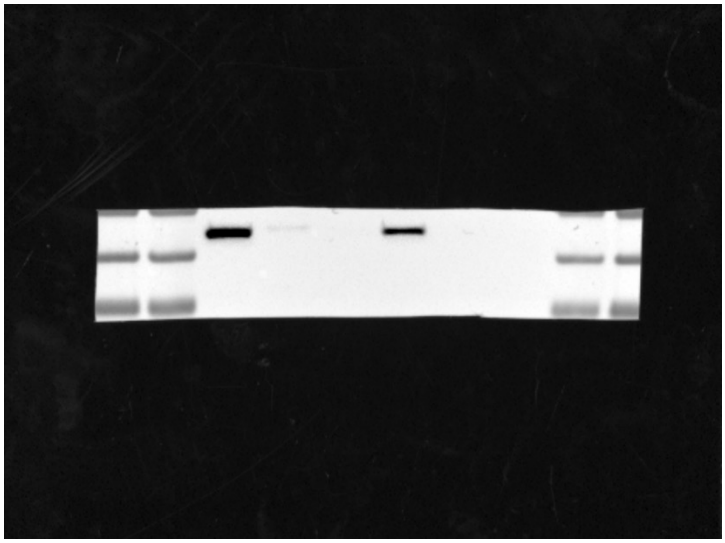

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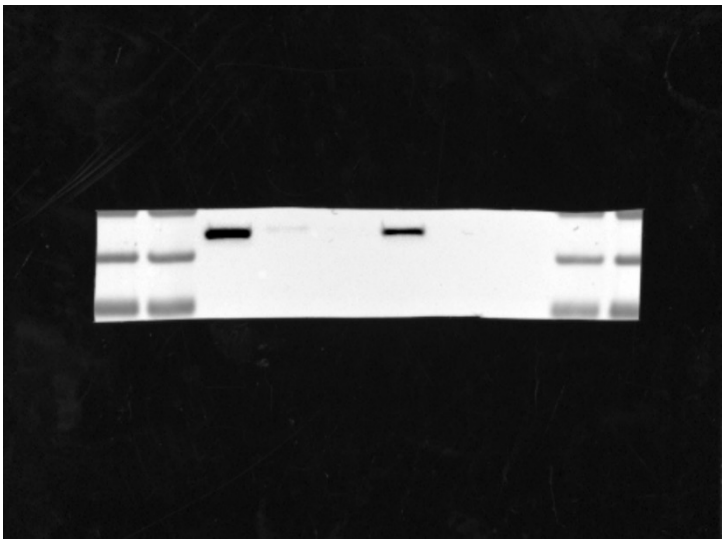

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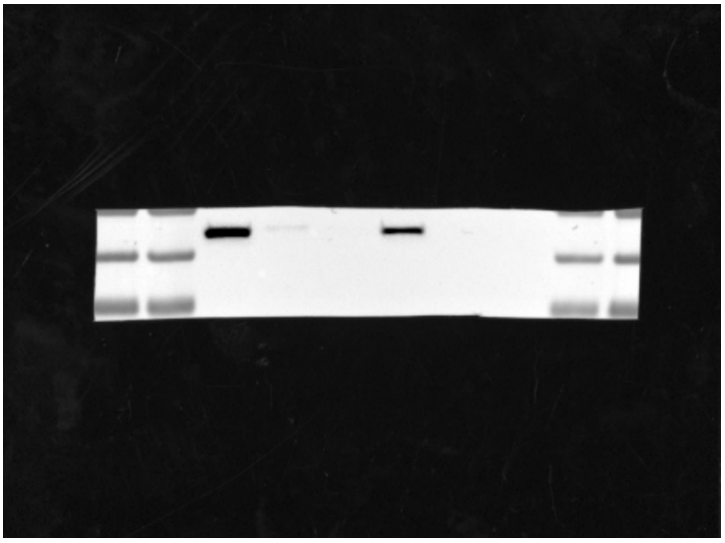
Figure 2 B different exposures of b-actin blot



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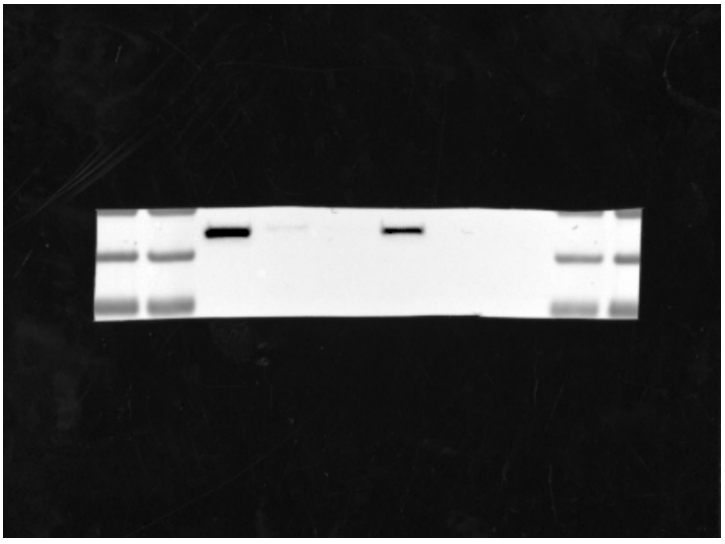

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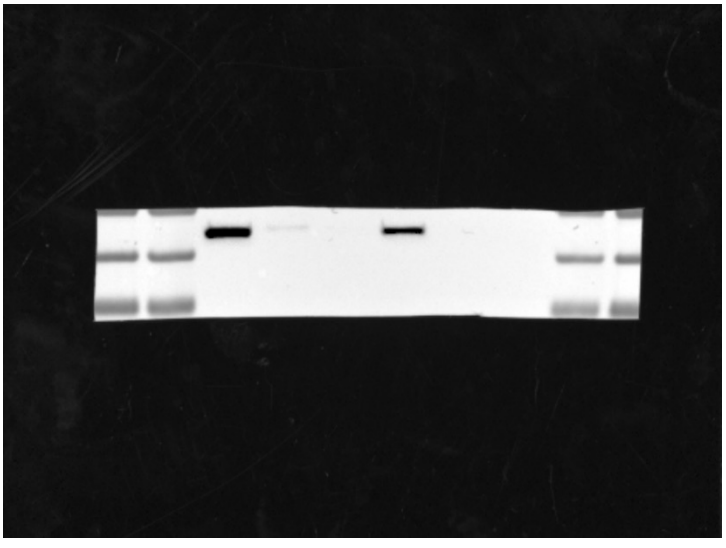

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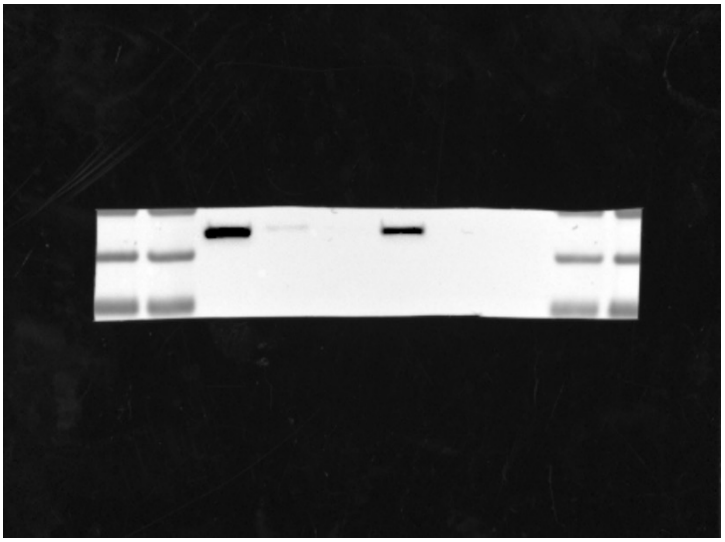

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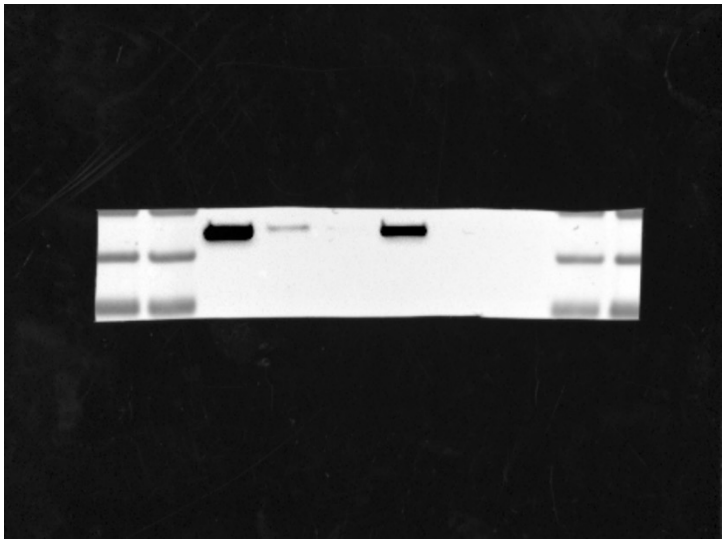

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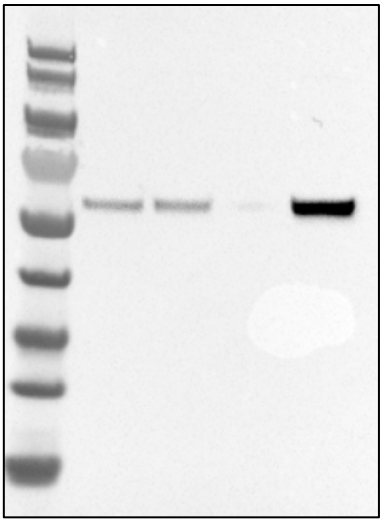
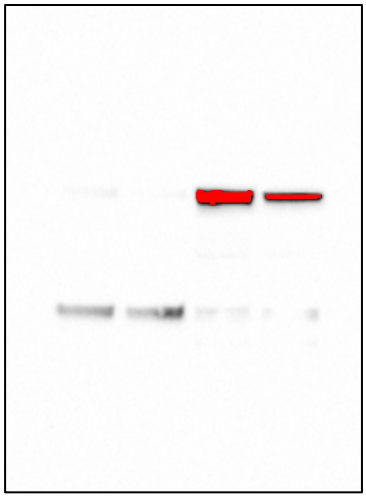
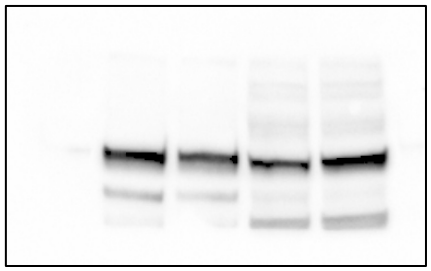
Fig 2E







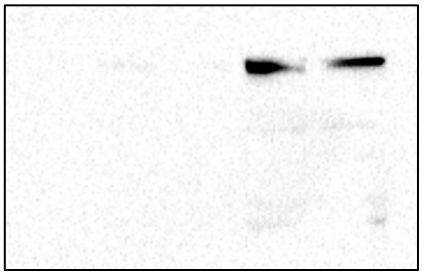


Figure 2 E actin different exposures

Figure 2 E actin different exposures

Fig 2G

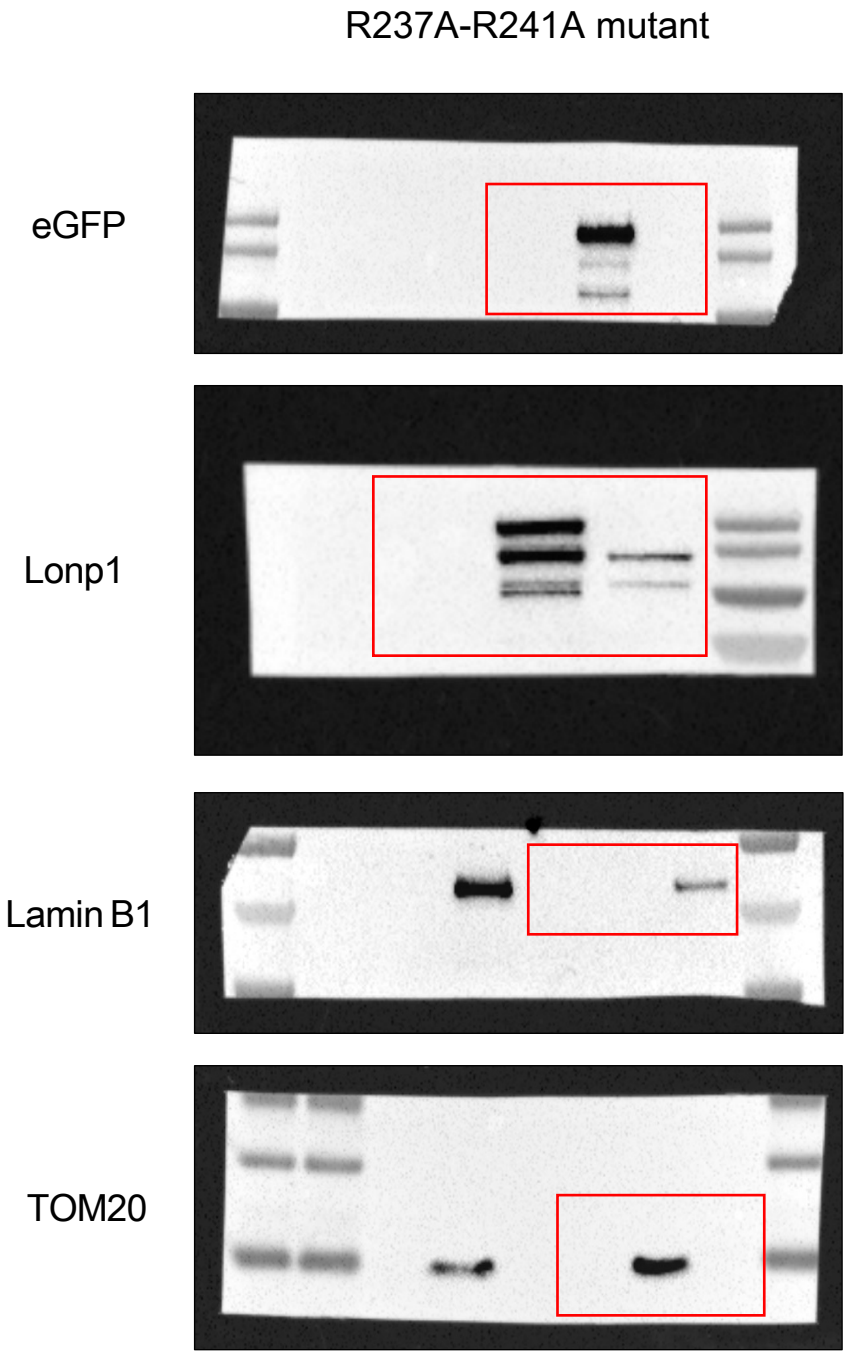


Fig. 3B

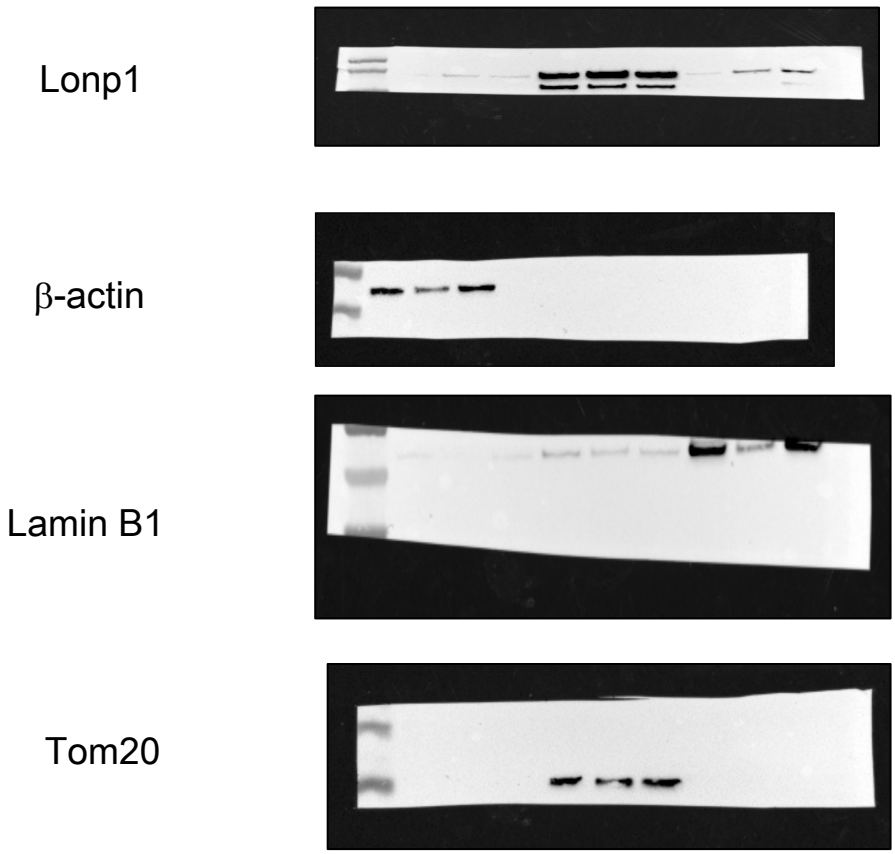
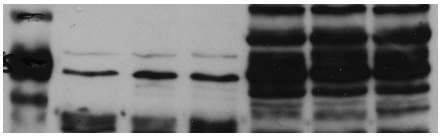


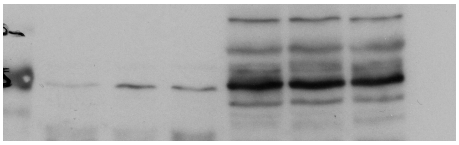
Fig 3D

IP eGFP

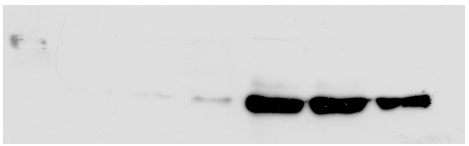
HSF1 IP



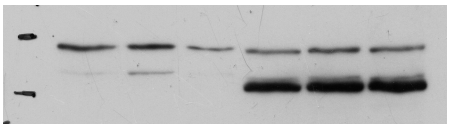
HSF1 TL



β-actin



Lonp1



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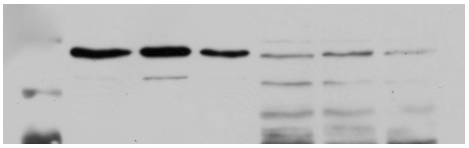


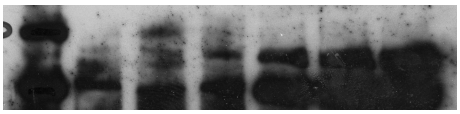
Fig 3D

IP HSF1

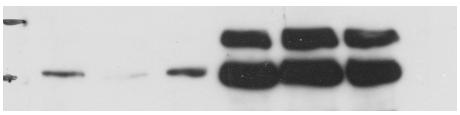
HSF1

β-actin

Lonp1 IP



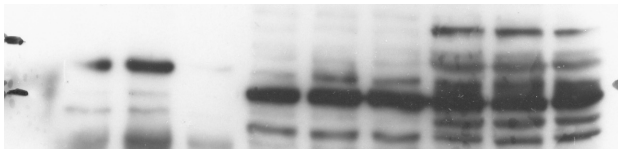
Lonp1 TL



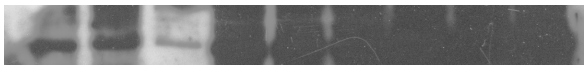
eGFP

Fig 3E

HSF1 NL



Lonp1 high exp



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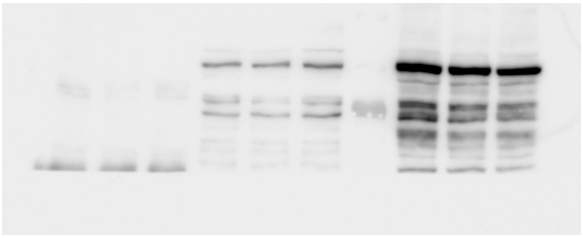
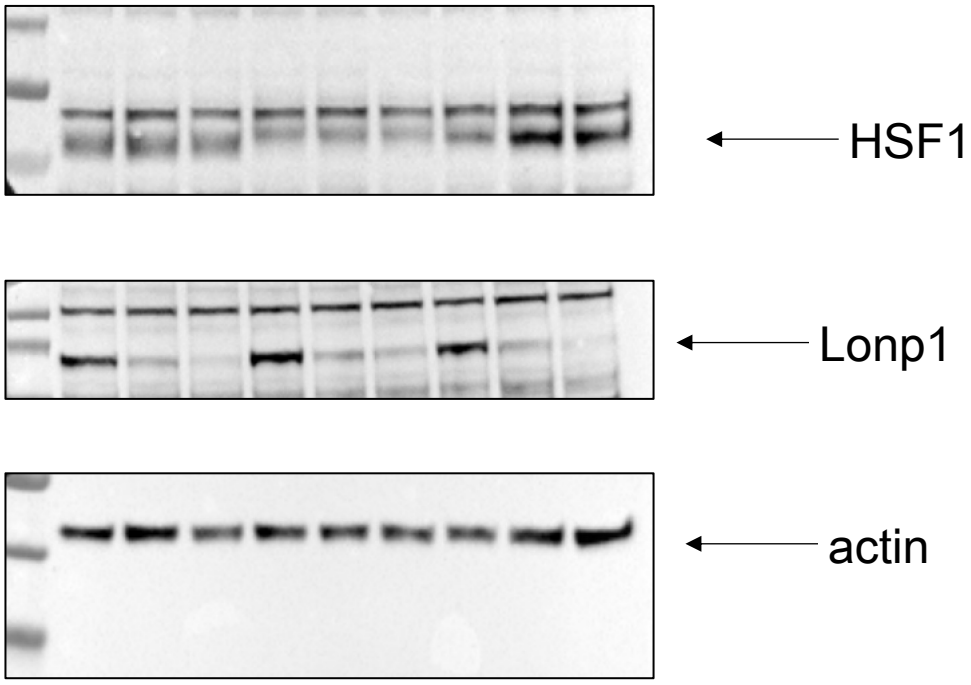
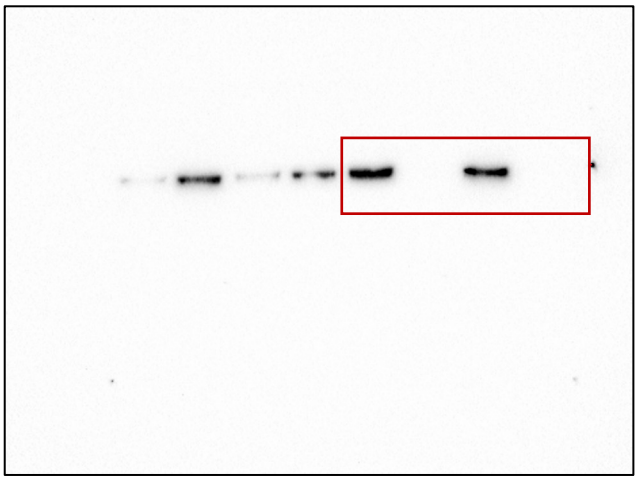


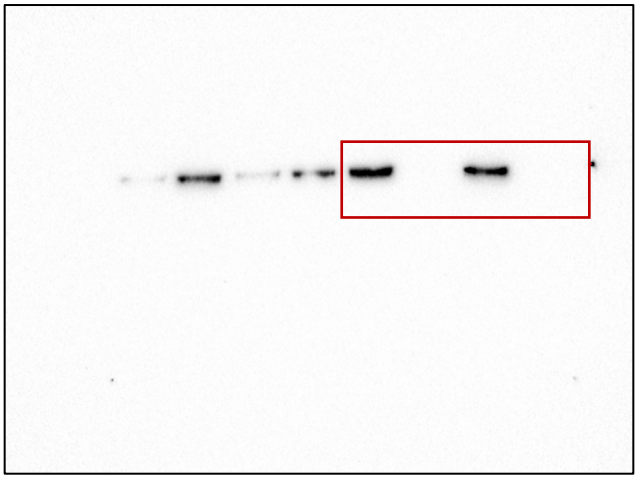
Figure 3F

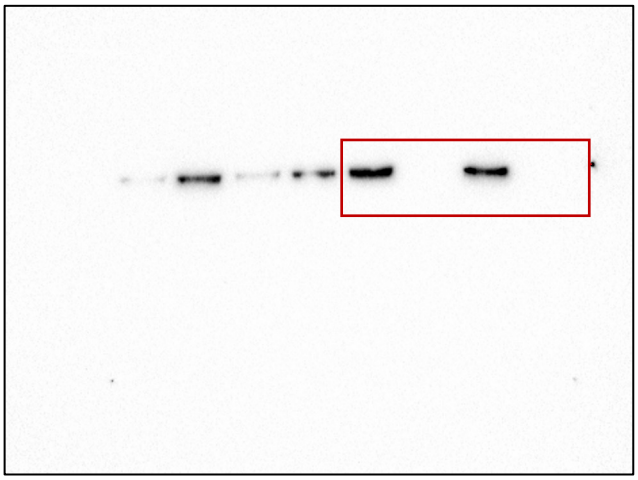


Supplementary Figure 3

Panel A Actin – different esposures

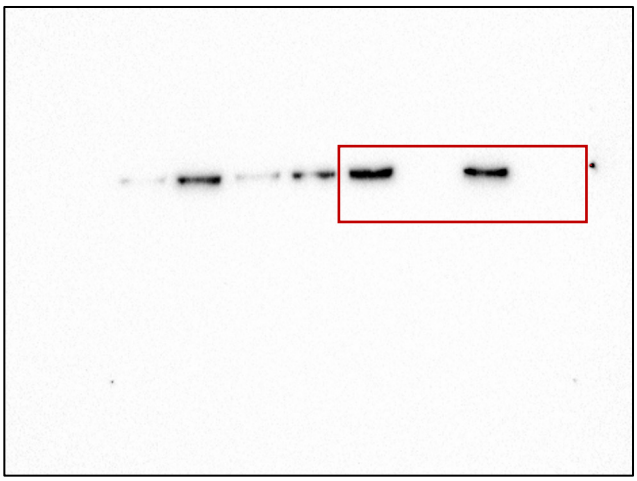


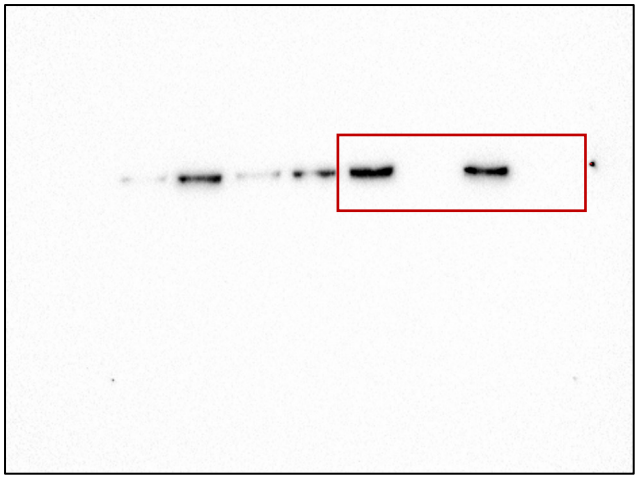


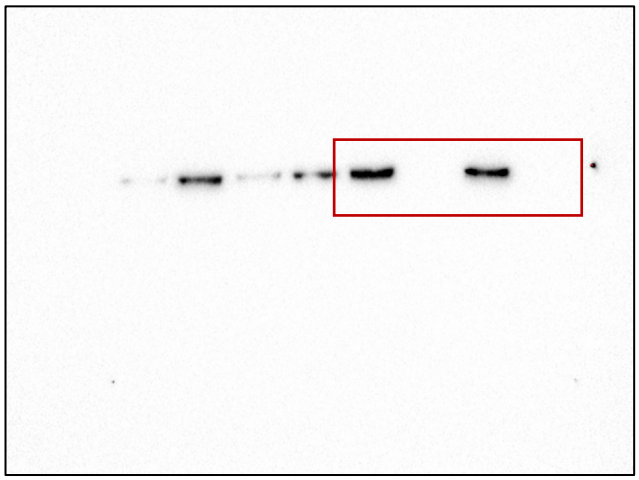


Supplementary Figure 3

Panel A Actin – different esposures

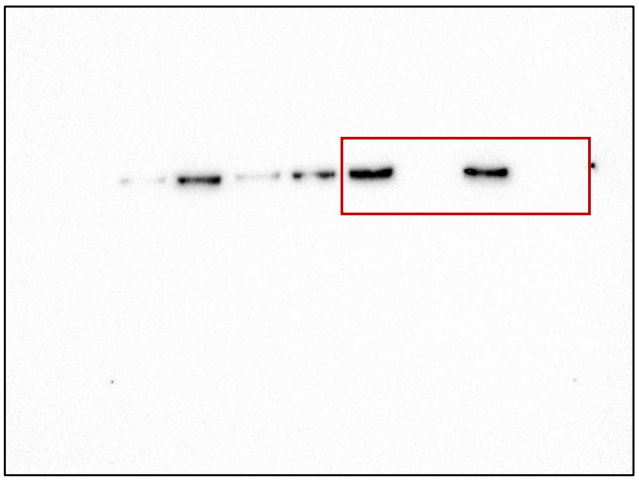


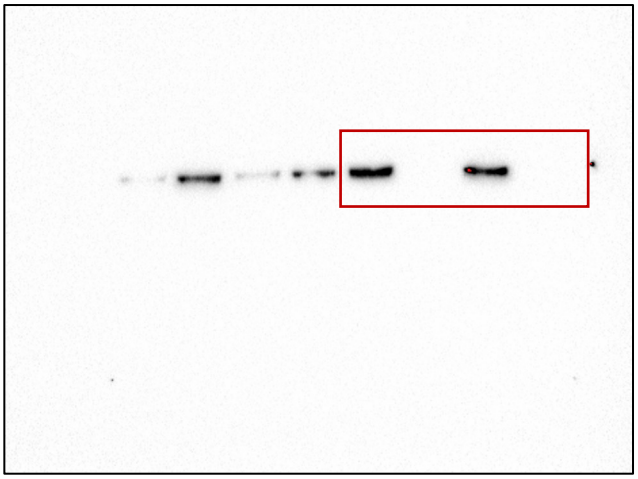




Supplementary Figure 3

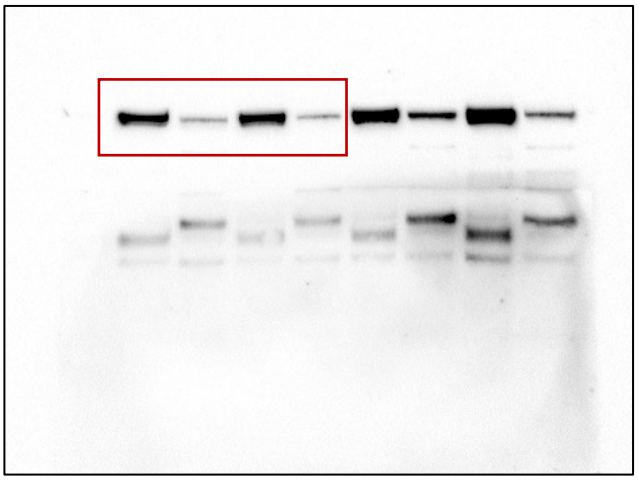
Panel A Actin – different esposures



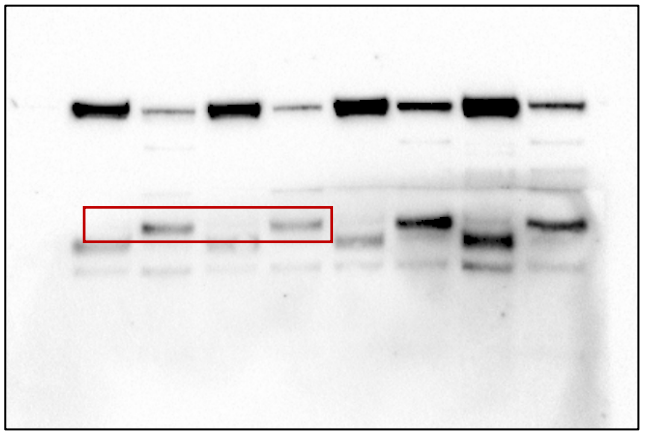


Supplementary Figure 3

Panel A Lonp1



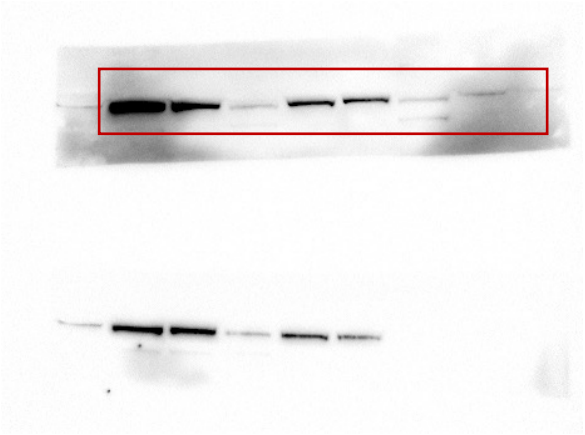
Panel A Lamin B1



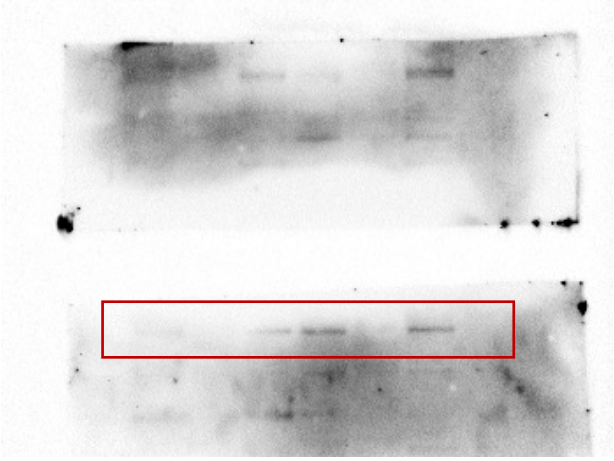
Supplementary Figure 3

Panel B Actin

Panel B Lonp1



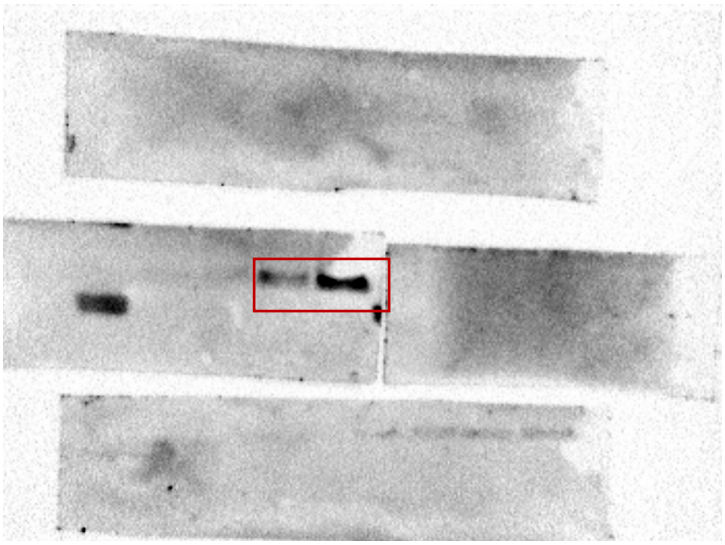
Panel B Lamin B1



Supplementary Figure 3

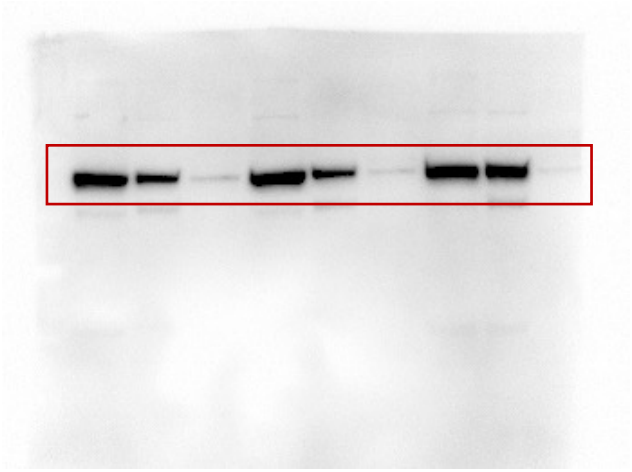
Panel B right blots - Actin

Panel B Right blots H2AX

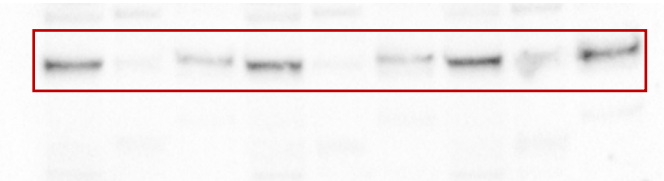


Supplementary Figure 3

Panel C Lonp1



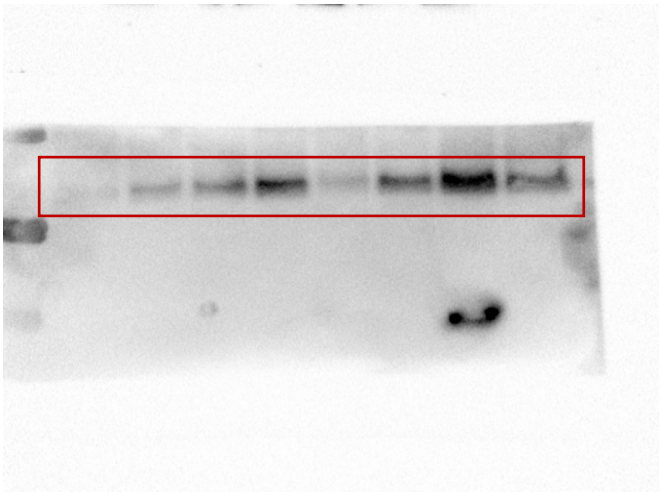
Panel C Lamin B1



Panel C actin

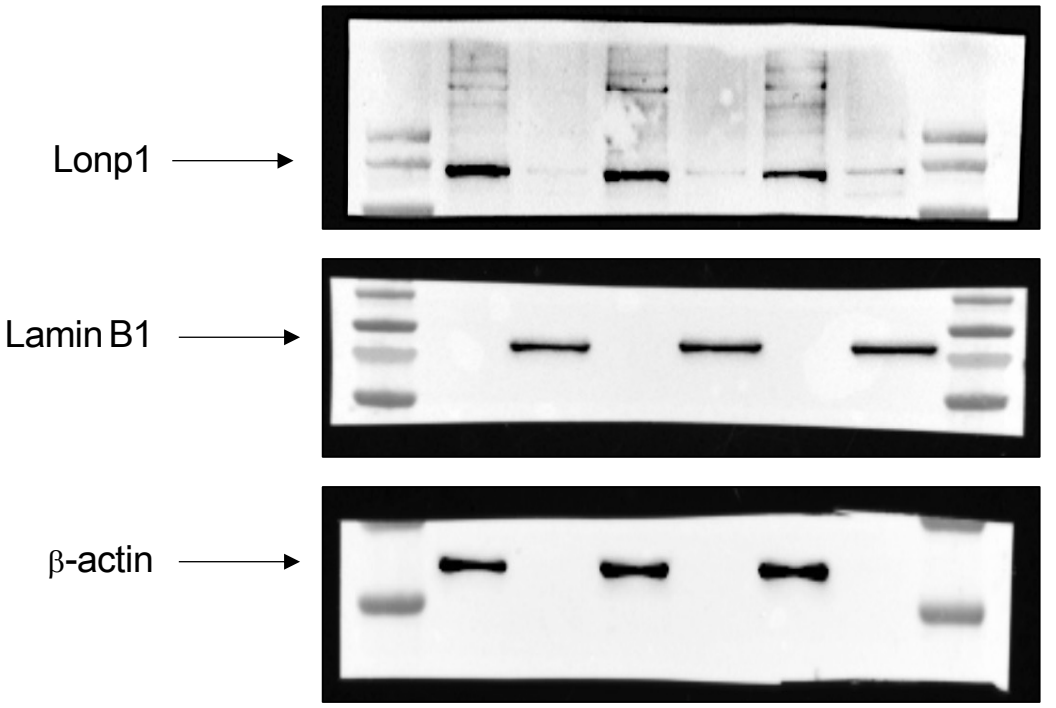
Supplementary Figure 3

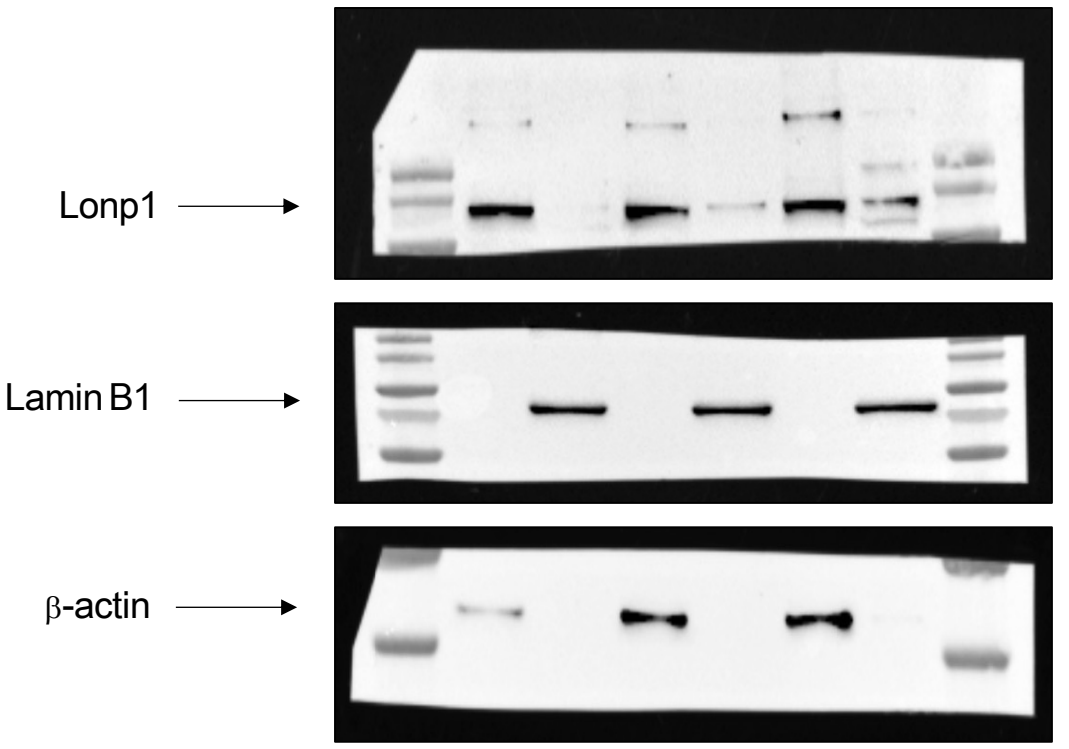
Panel C lower panel H2AX



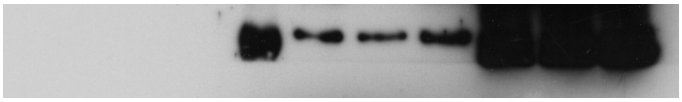
Panel C lower panel actin

Supplementary Figure 4

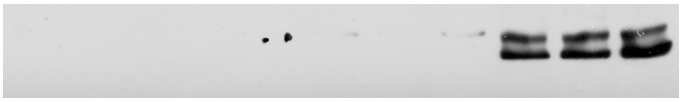




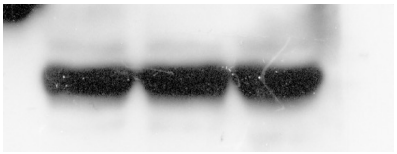
Supplementary Figure 5A



WB anti HSF-1 high exposure



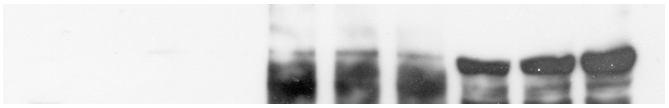
WB anti HSF-1 low exposure



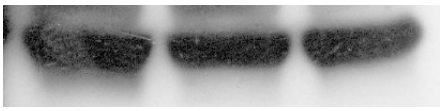
WB actin

Supplementary Figure 5B

WB anti Lonp1 high exposure



WB anti Lonp1low exposure



WB anti actin

Supplementary Figure 7