

Original file of repeated experiments

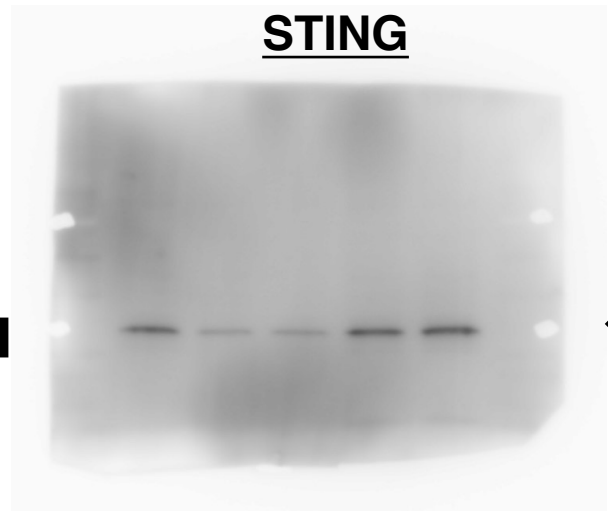
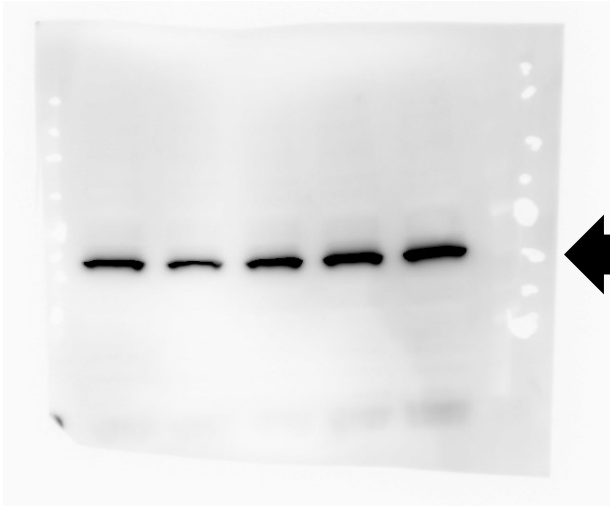
We have performed at least triplicate of all experiments to confirm our conclusions.

MM231

1st

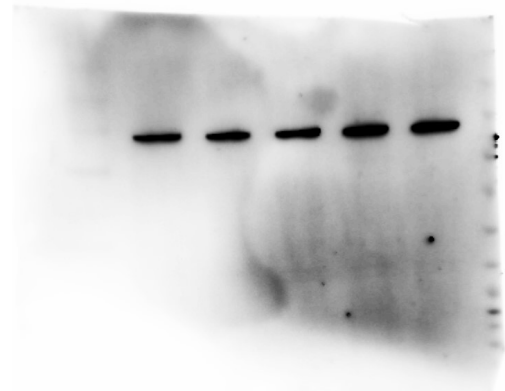
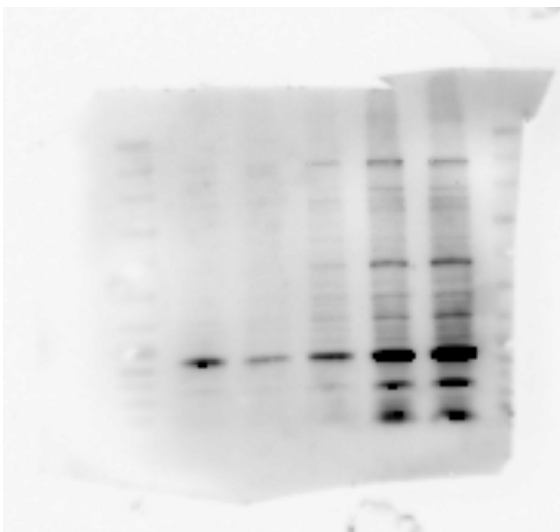
cGAS

STING



IFN β

Vinculin



Target band

Uncropped Gels and Blots images in MM231 were shown.

Figure 2f

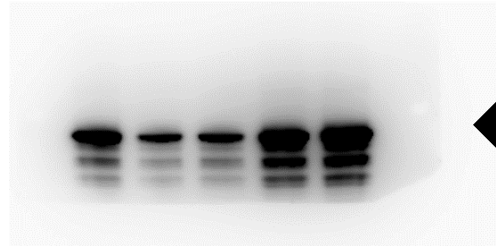
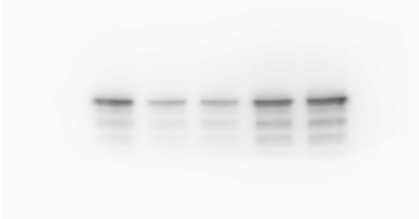
MM231

cGAS

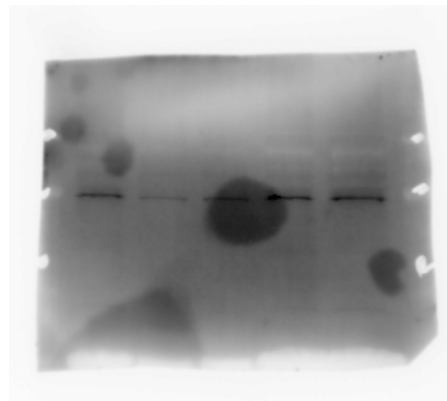
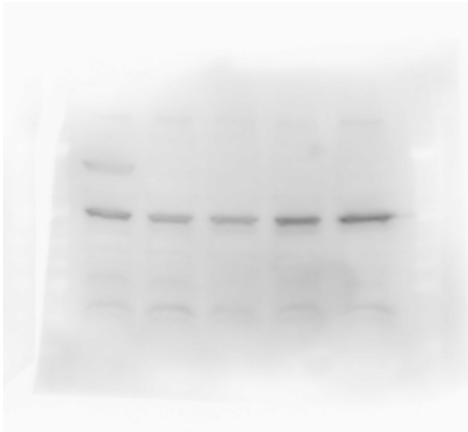
Short exposure

long exposure

2nd



3rd



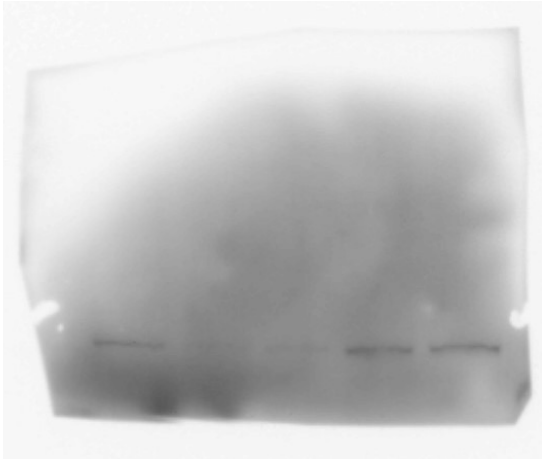
Uncropped Gels and Blots images in MM231 were shown.

Figure 2f

MM231

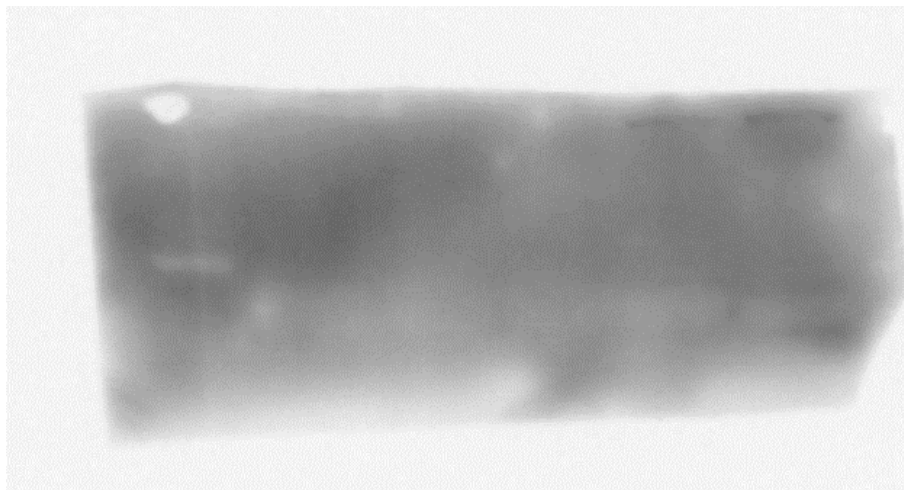
STING

2nd



← Target band

3rd



← Target band

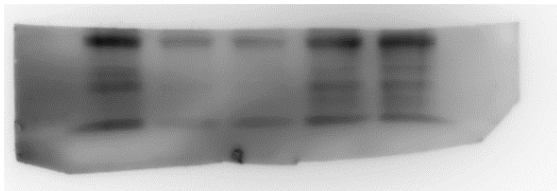
Uncropped Gels and Blots images in MM231 were shown.

Figure 2f

MM231

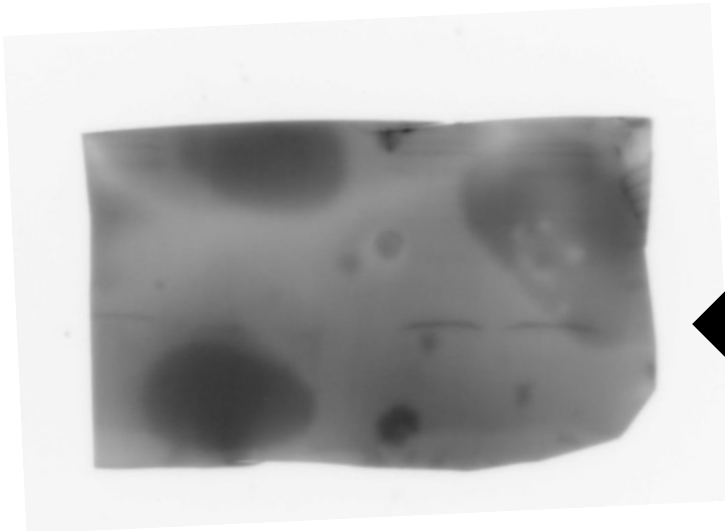
IFN β

2nd



**Target
band**

3rd



**Target
band**

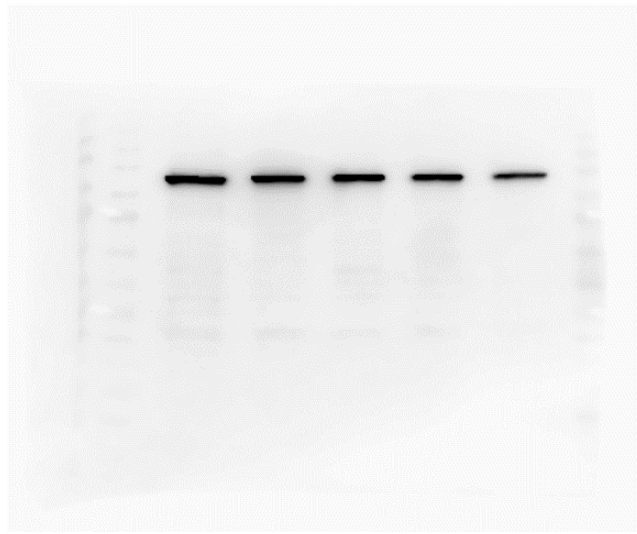
Uncropped Gels and Blots images in MM231 were shown.

Figure 2f

MM231

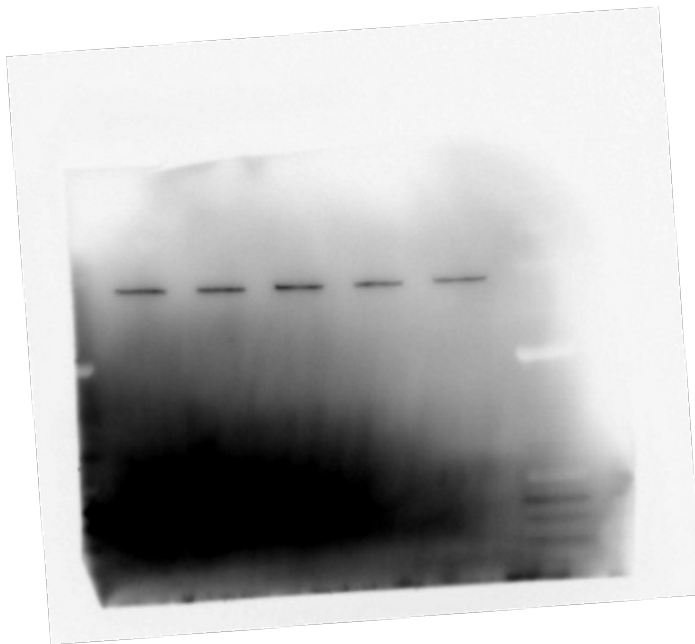
Vinculin

2nd



← Target band

3rd

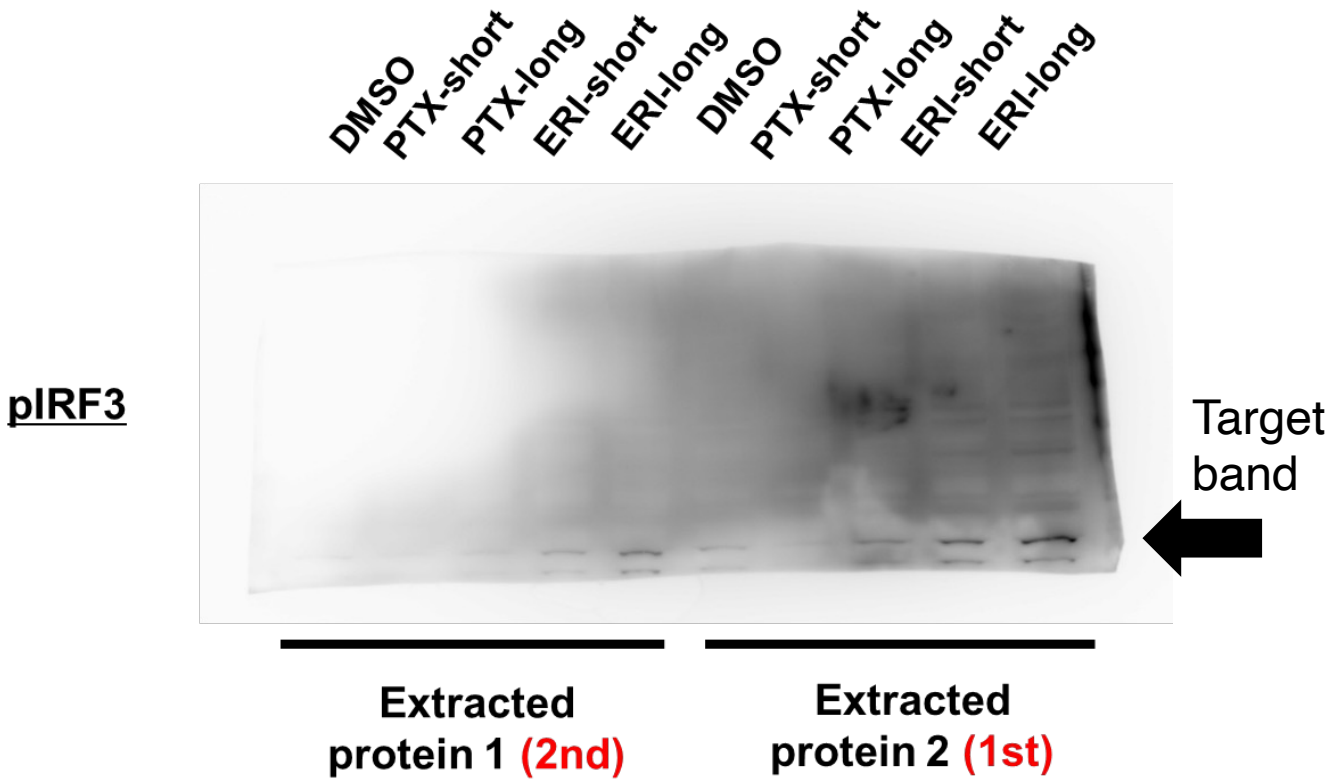


← Target band

Uncropped Gels and Blots images in MM231 were shown.

Figure 2f

MM231



Protein extraction was performed from MM231 cells twice independently and pIRF3 expression was evaluated. The 5 lanes on the left side are "extracted protein 1" and the 5 lanes on the right side are "extracted protein 2". The "extracted protein 2" was used as Figure2f.

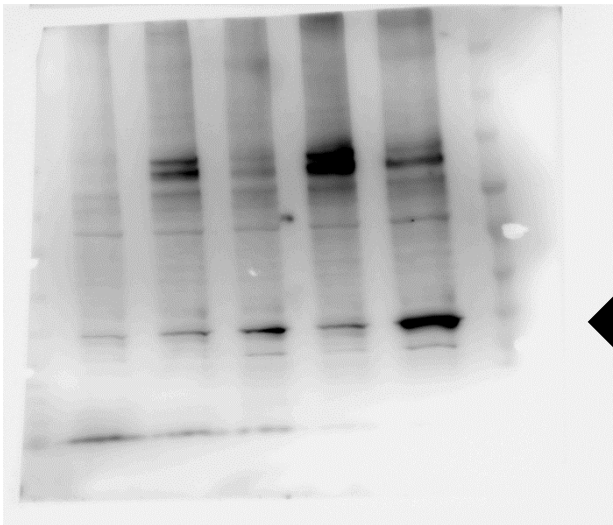
Uncropped Gels and Blots images in MM231 were shown.

Figure 2f

MM231

pIRF3

3rd



Target
band

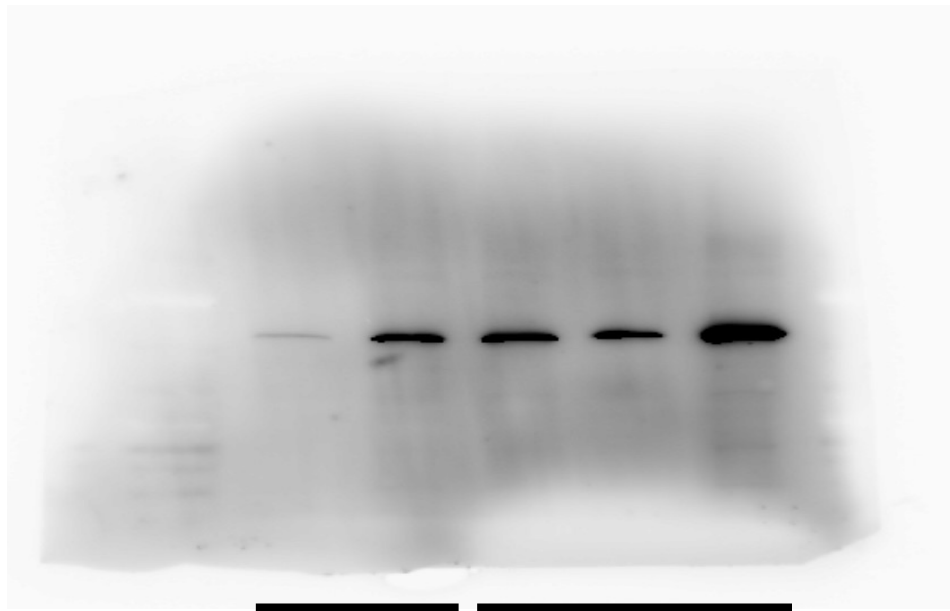
Uncropped Gels and Blots images in MM231 were shown.

Figure 2f

RPE1

cGAS (1st)

MM231-PTX-short
MM231-ERI-short
RPE-DMSO
RPE-PTX-short
RPE ERI-short



MM231

RPE1

MM231-PTX-short and MM231-ERI-short were also checked whether the bands were detected. Therefore, there are blots of MM231 at the left of the target bands.

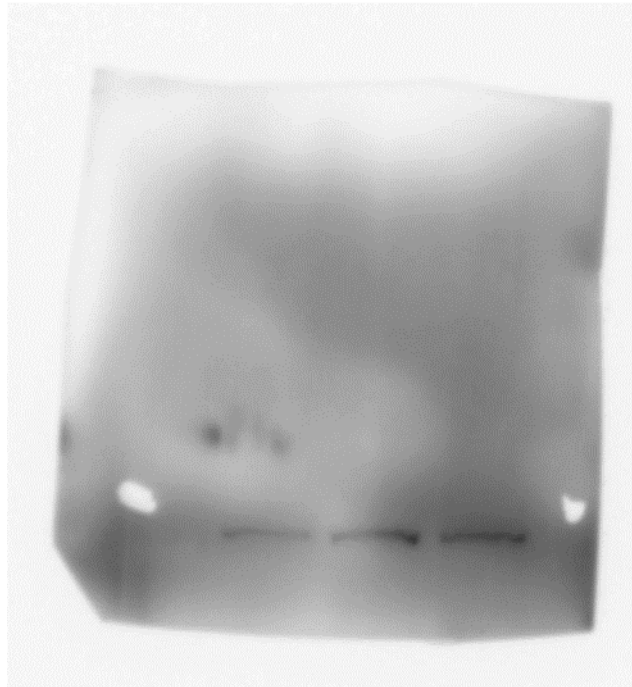
Uncropped Gels and Blots images in RPE1 were shown.

Figure 2f

RPE1

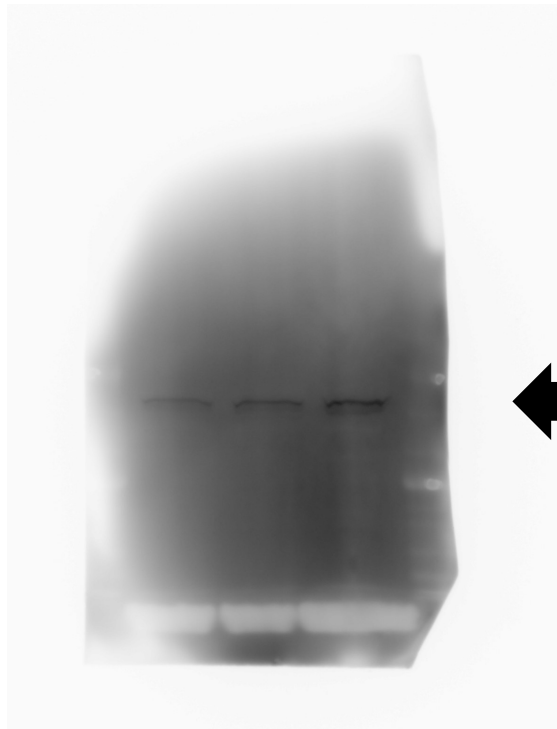
cGAS

2nd



← Target band

3rd



← Target band

Uncropped Gels and Blots images in RPE1 were shown.

Figure 2f

RPE1

STING (1st)

Short exposure

Long exposure



← Target band

Uncropped Gels and Blots images in RPE1 were shown.

Figure 2f

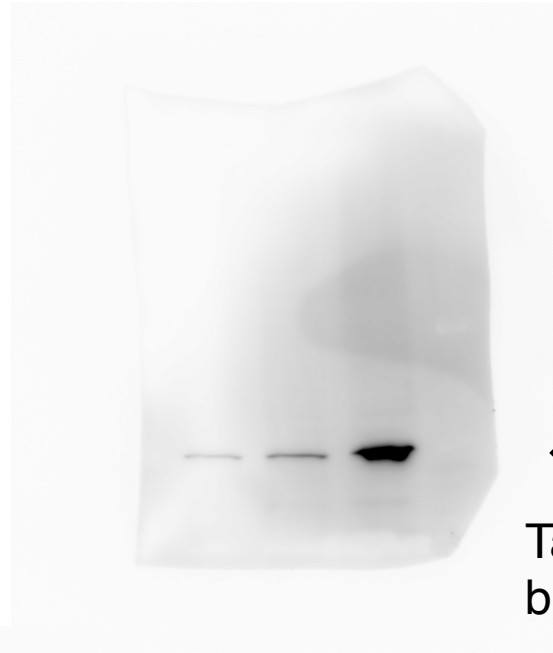
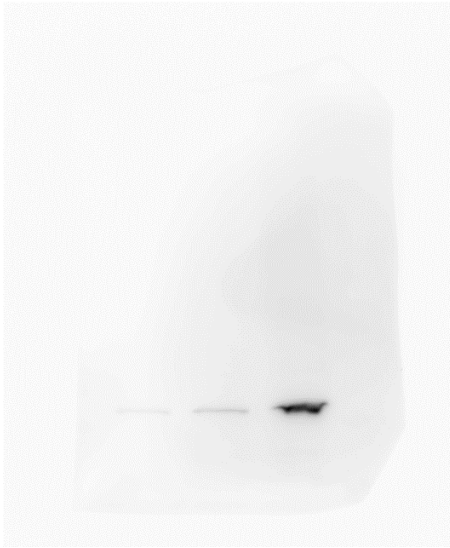
RPE1

STING

Short exposure

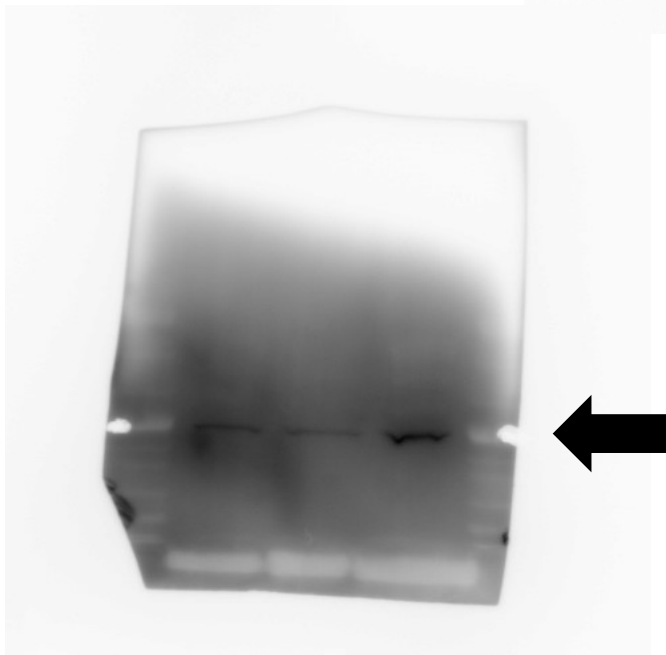
Long exposure

2nd



Target band

3rd

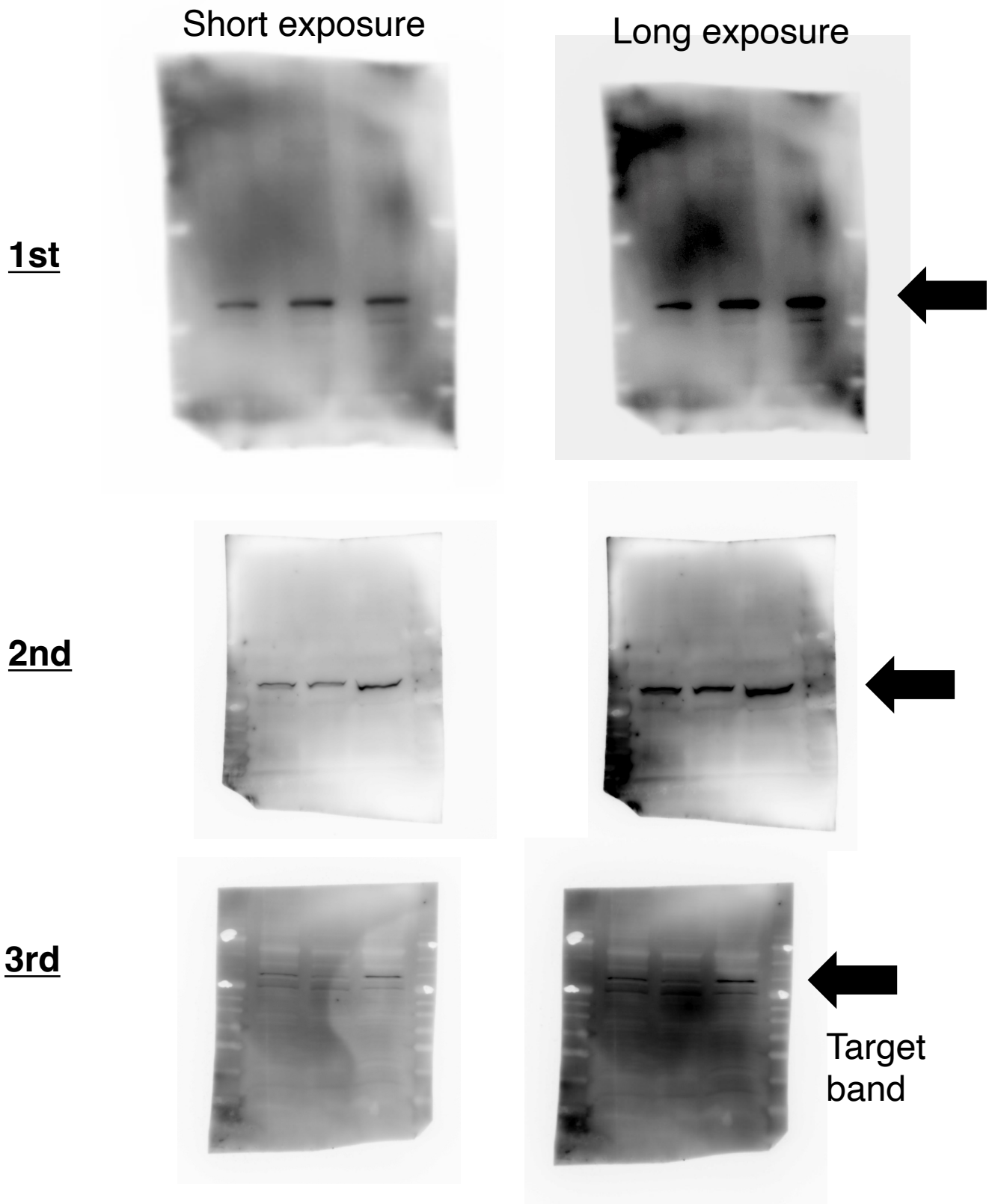


Uncropped Gels and Blots images in RPE1 were shown.

Figure 2f

RPE1

pIRF3

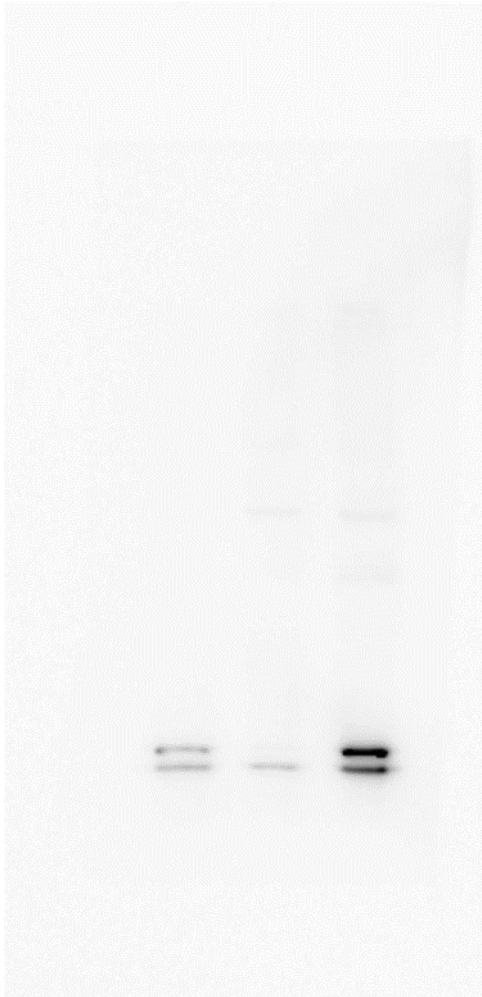


Uncropped Gels and Blots images in RPE1 were shown.

Figure 2f

RPE1

IFN β (1st)



← Target
band

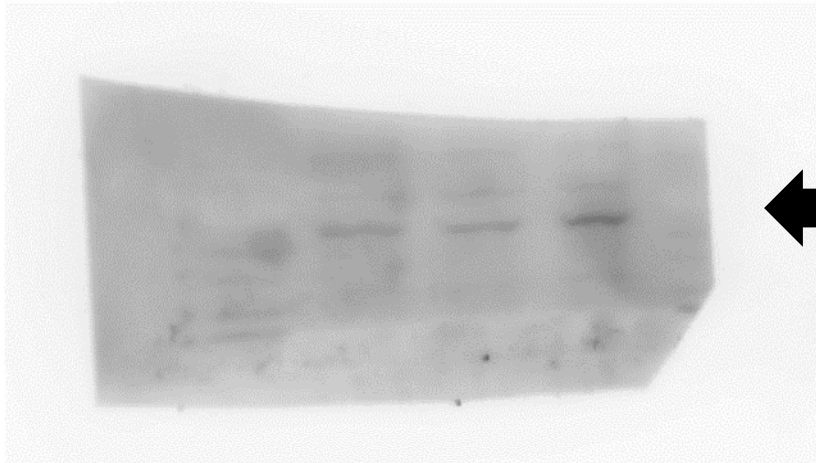
Uncropped Gels and Blots images in RPE1 were shown.

Figure 2f

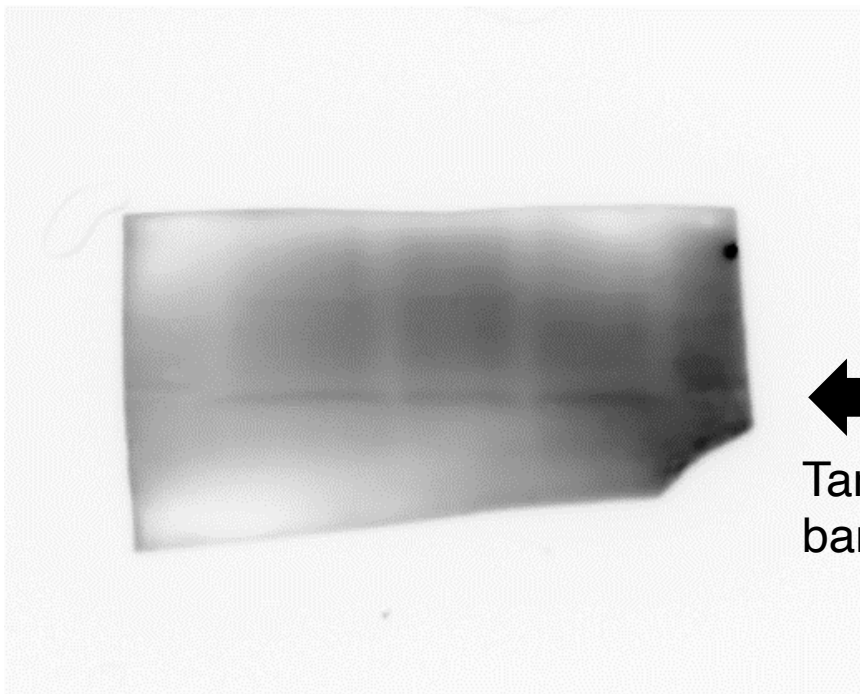
RPE1

IFN β

2nd



3rd



Target
band

Uncropped Gels and Blots images in RPE1 were shown.

Figure 2f

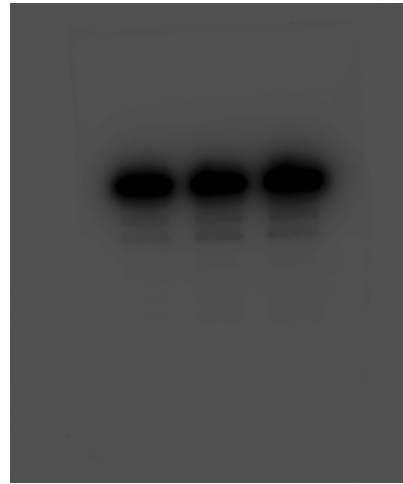
RPE1

Vinculin

Short exposure

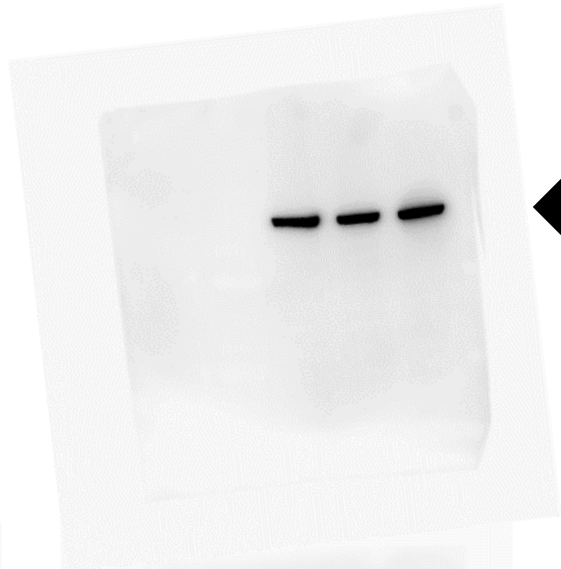
long exposure

1st



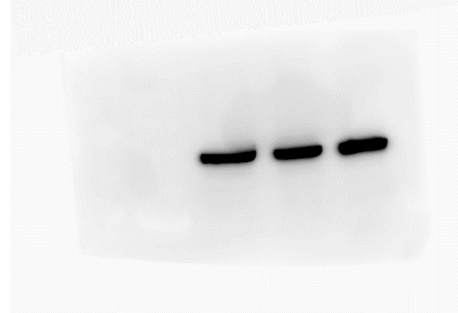
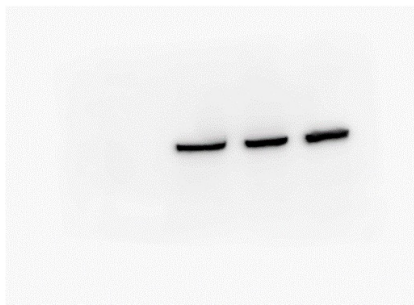
←
Target
band

2nd



←

3rd

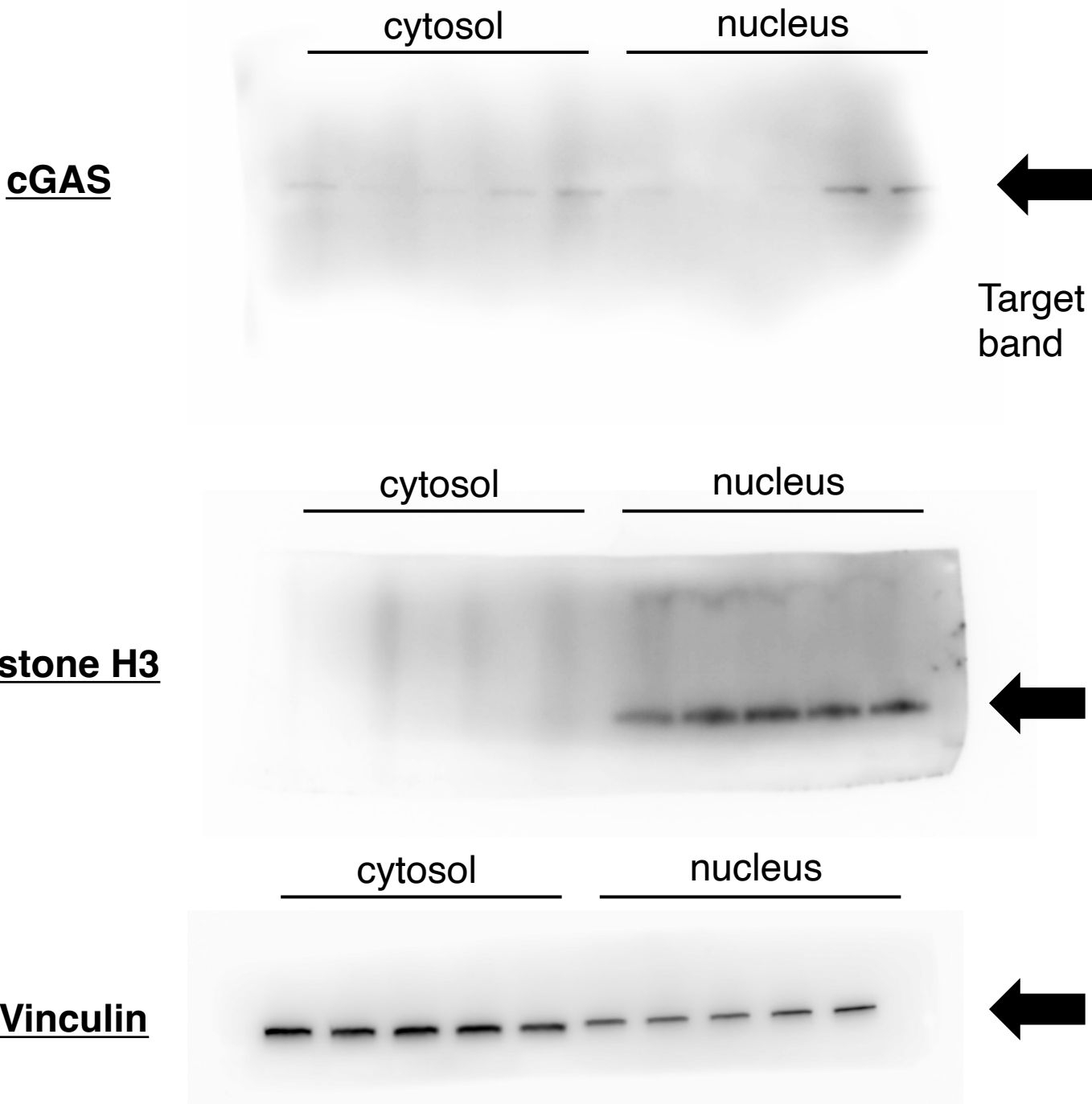


←

Uncropped Gels and Blots images in RPE1 were shown.

Figure 2f

1st

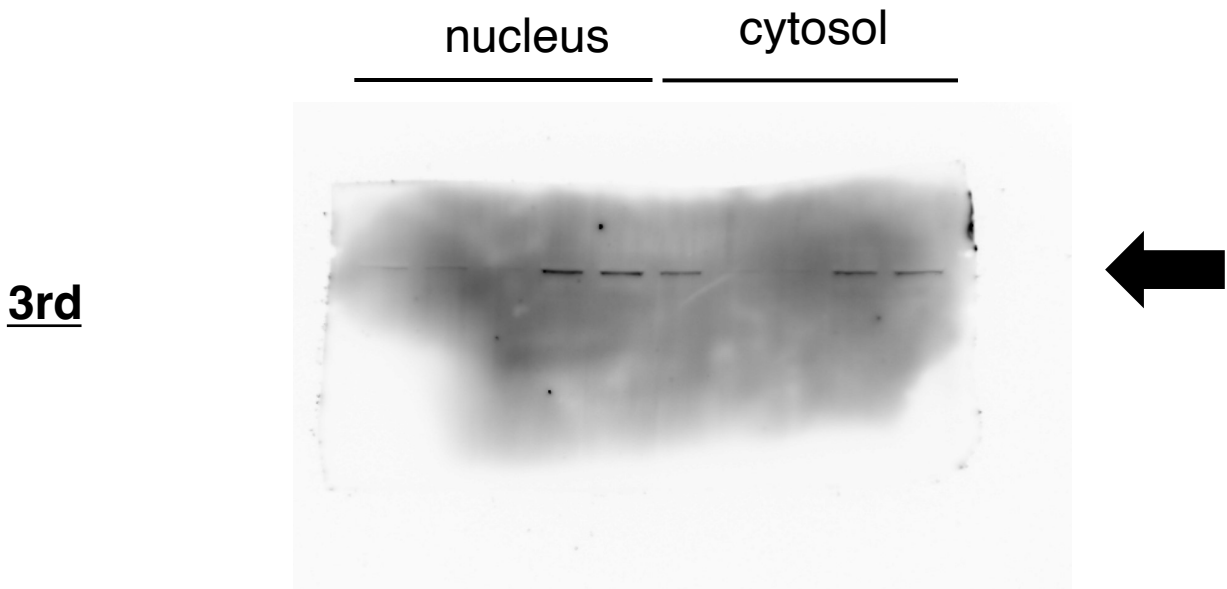
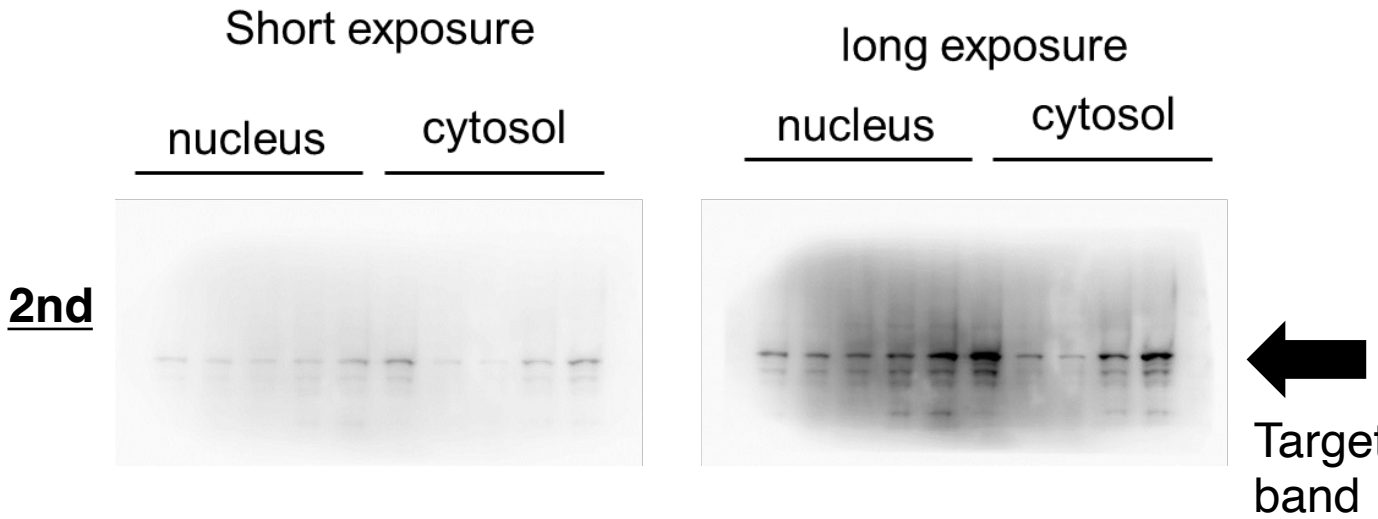


The order of the cytoplasmic and nuclear fractions is reversed in the 2nd and 3rd procedures.

Uncropped Gels and Blots images of Figure 2g were shown.

Figure 2g

cGAS

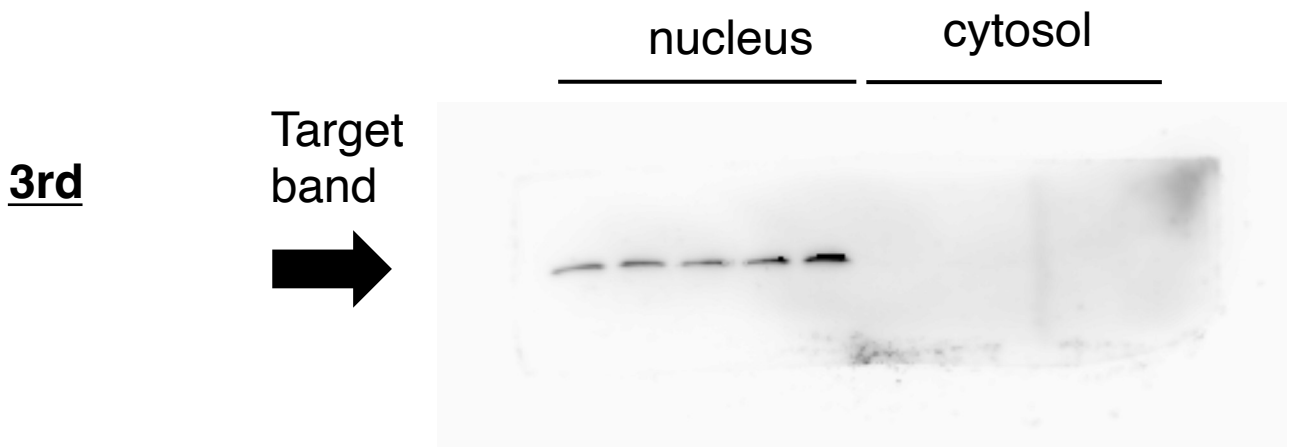
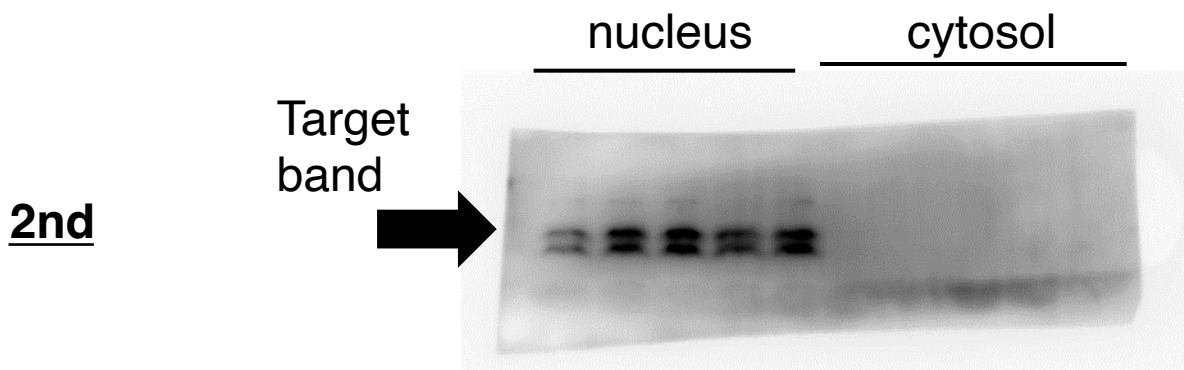


The order of the cytoplasmic and nuclear fractions is reversed in the 2nd and 3rd procedures.

Uncropped Gels and Blots images of Figure 2g were shown.

Figure 2g

Histone H3

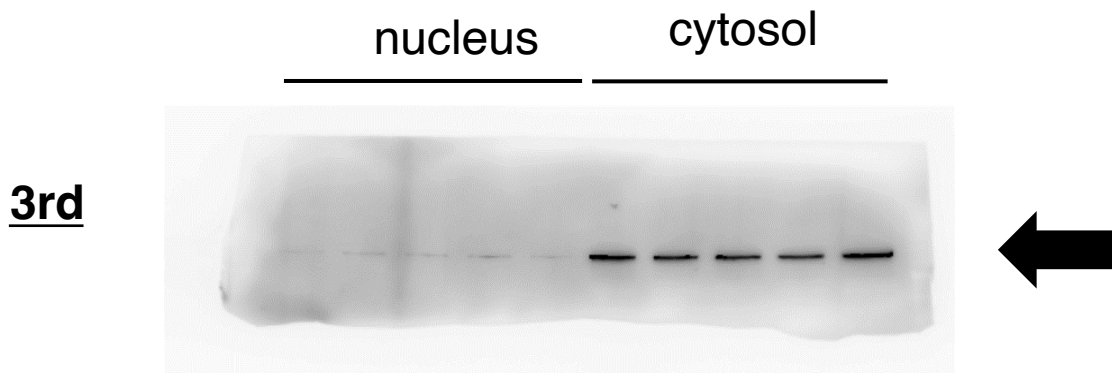
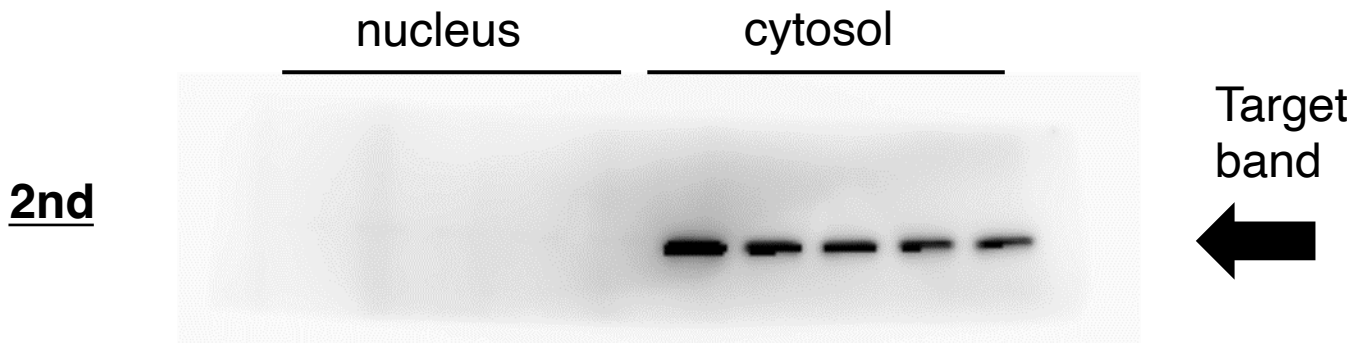


The order of the cytoplasmic and nuclear fractions is reversed in the 2nd and 3rd procedures.

Uncropped Gels and Blots images of Figure 2g were shown.

Figure 2g

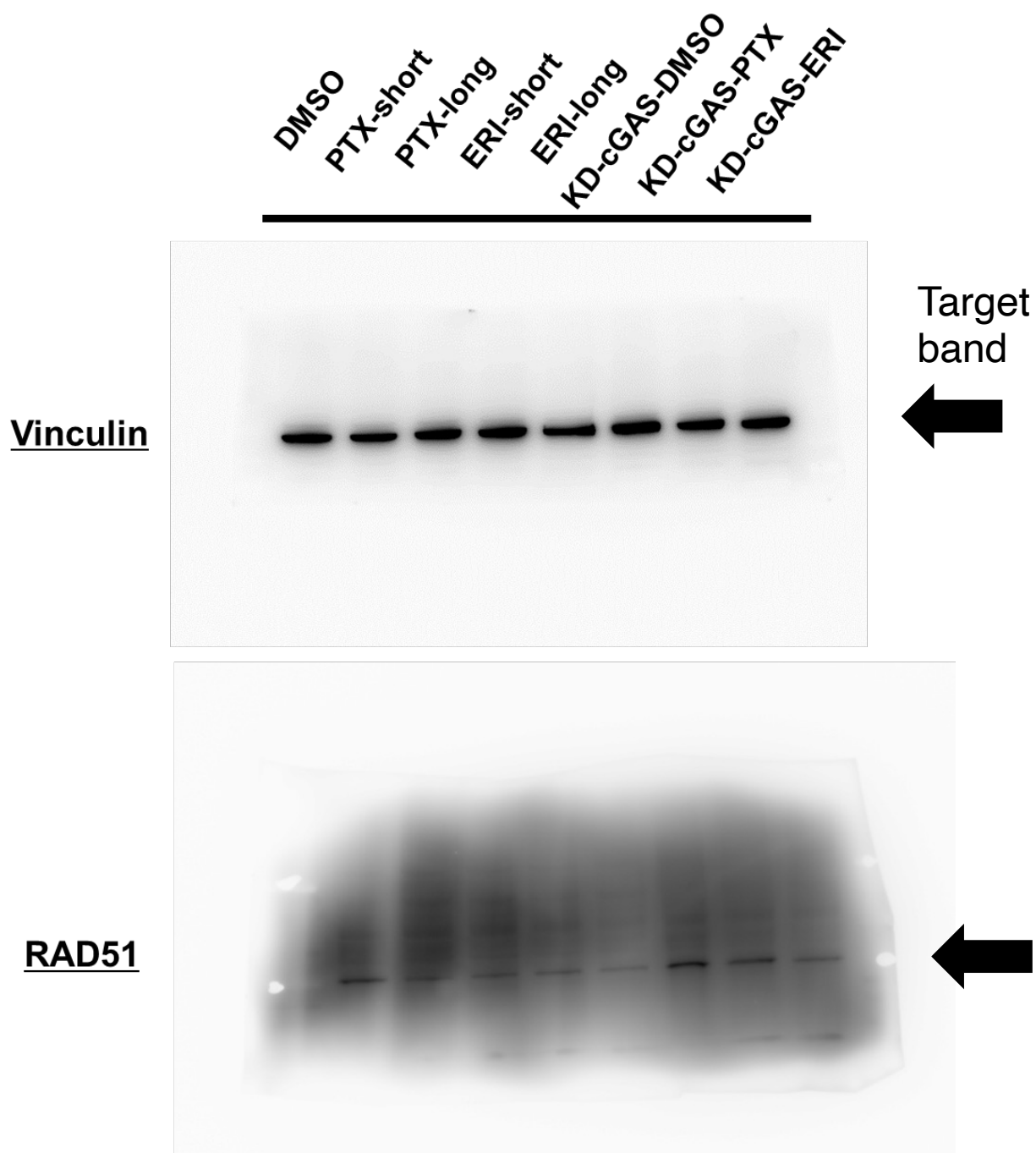
Vinculin



The order of the cytoplasmic and nuclear fractions is reversed in the 2nd and 3rd procedures.

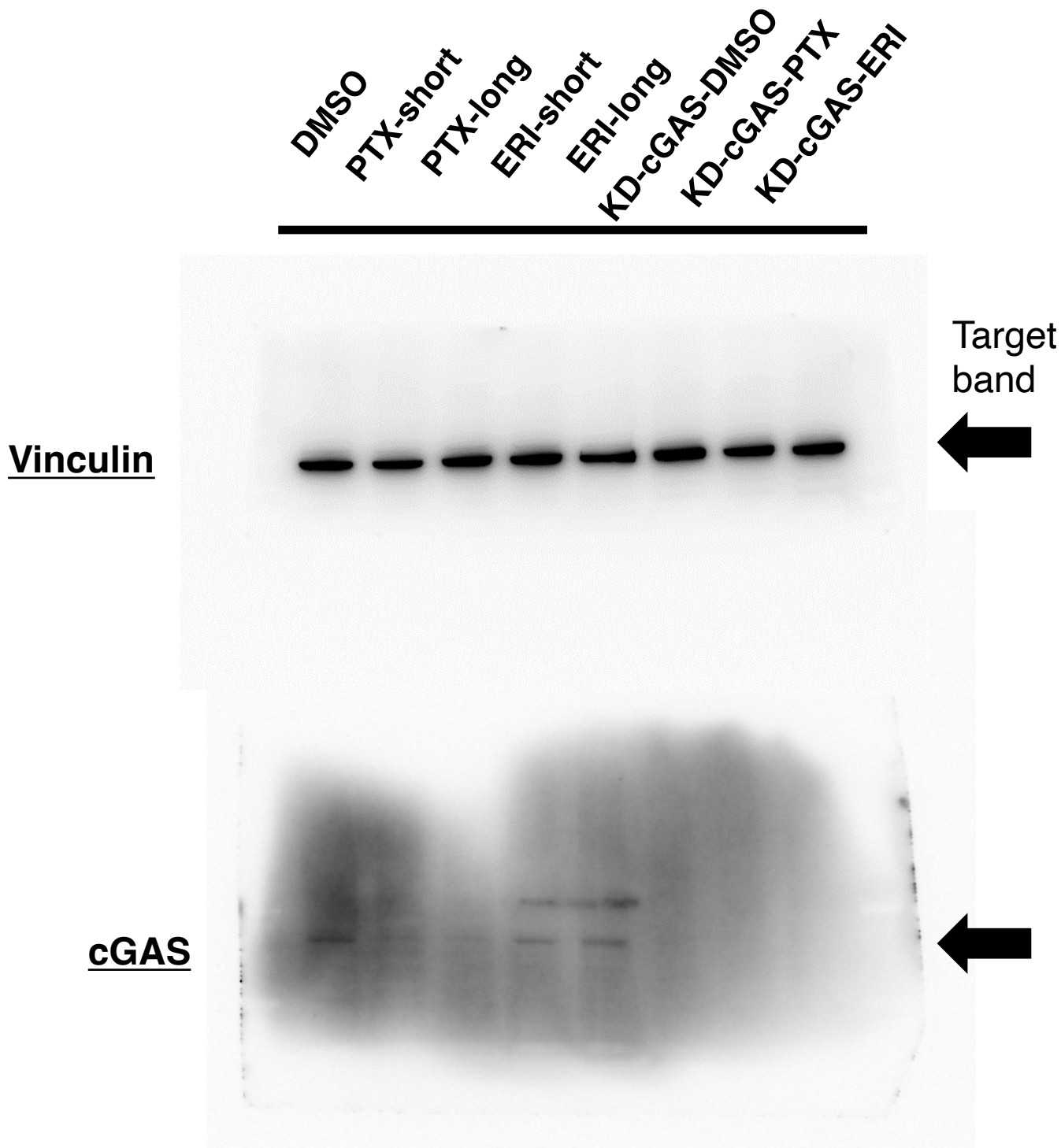
Uncropped Gels and Blots images of Figure 2g were shown.

Figure 2g



Uncropped Gels and Blots images of Figure 3c were shown.

Figure 3c

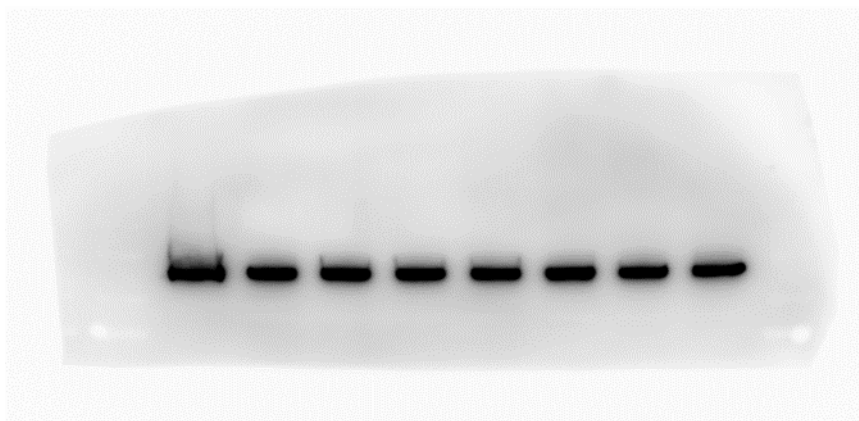


Uncropped Gels and Blots images of Figure 3c were shown.

Figure 3c

Vinculin

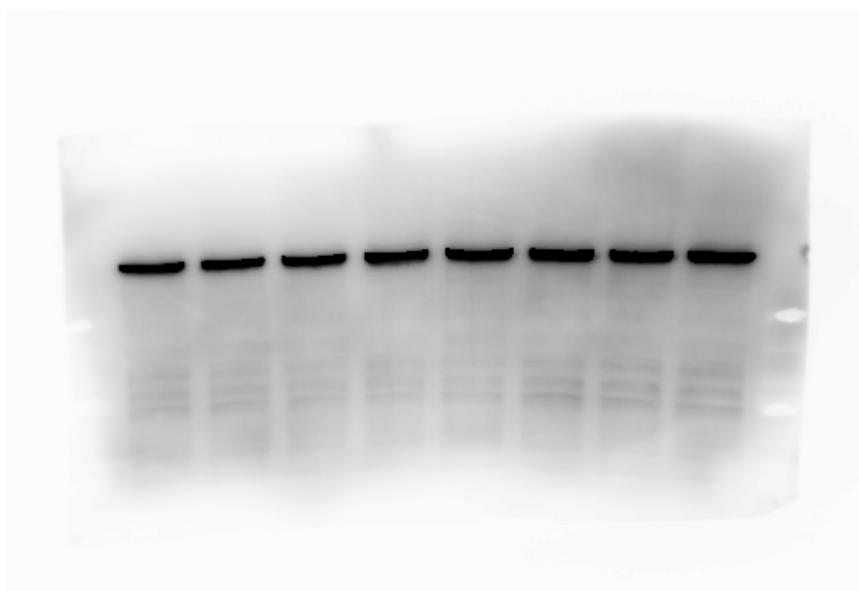
2nd



Target
band



3rd



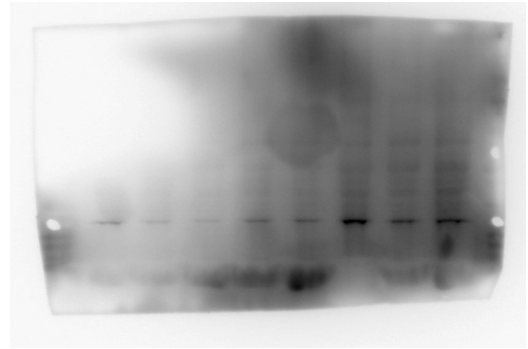
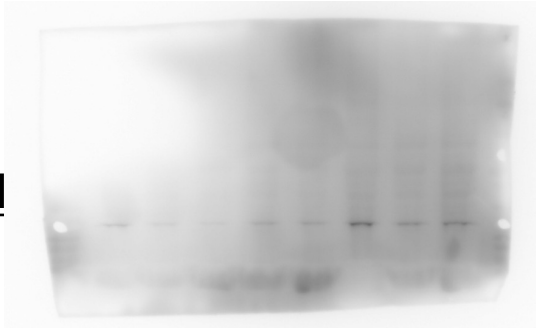
Uncropped Gels and Blots images of Figure 3c were shown.

RAD51

Short exposure

long exposure

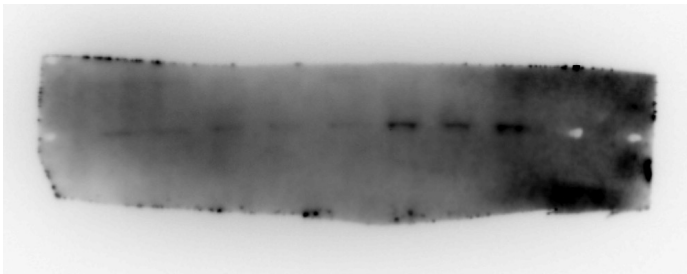
2nd



Target
band



3rd

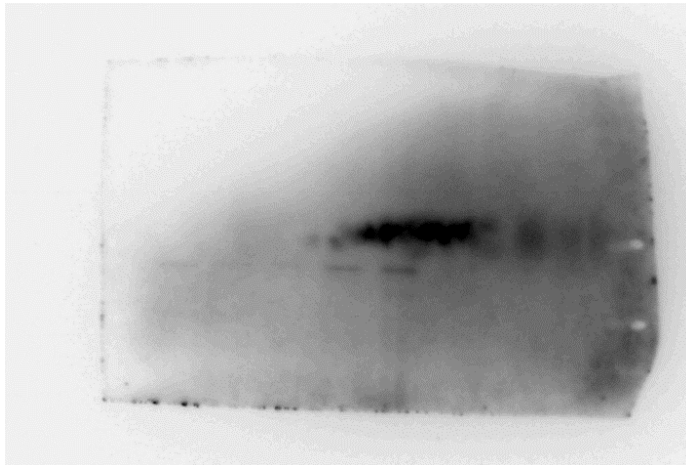


Uncropped Gels and Blots images of Figure 3c were shown.

Figure 3c

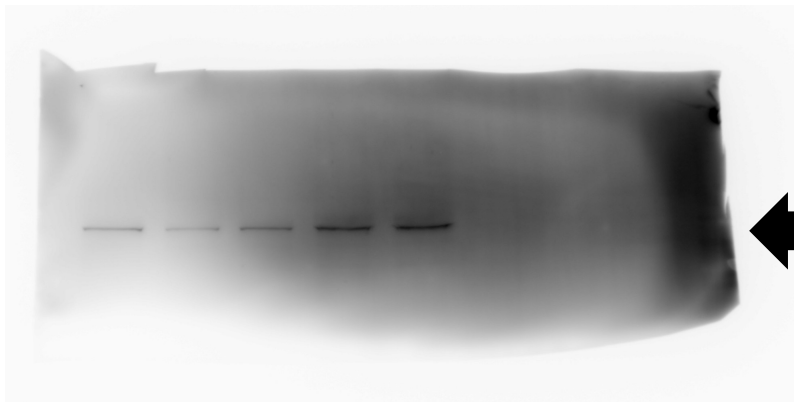
cGAS

2nd



← Target band

3rd



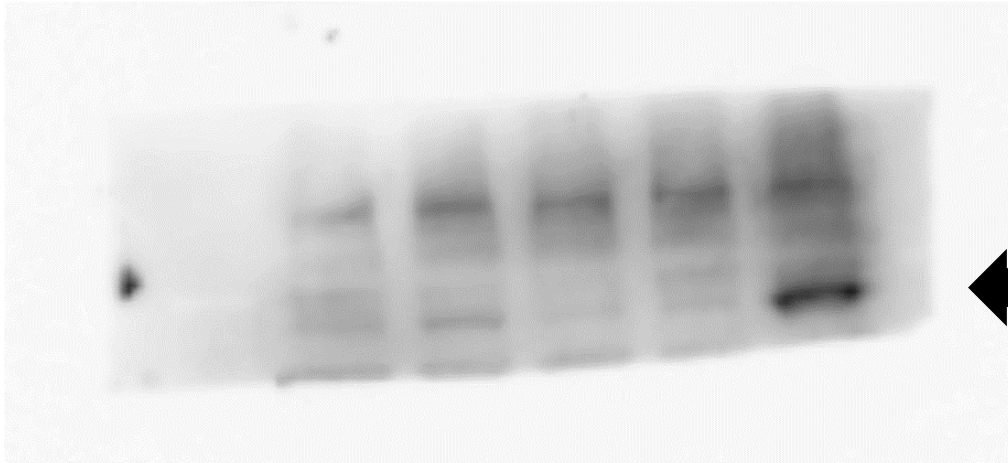
Uncropped Gels and Blots images of Figure 2g were shown.

Figure 3c

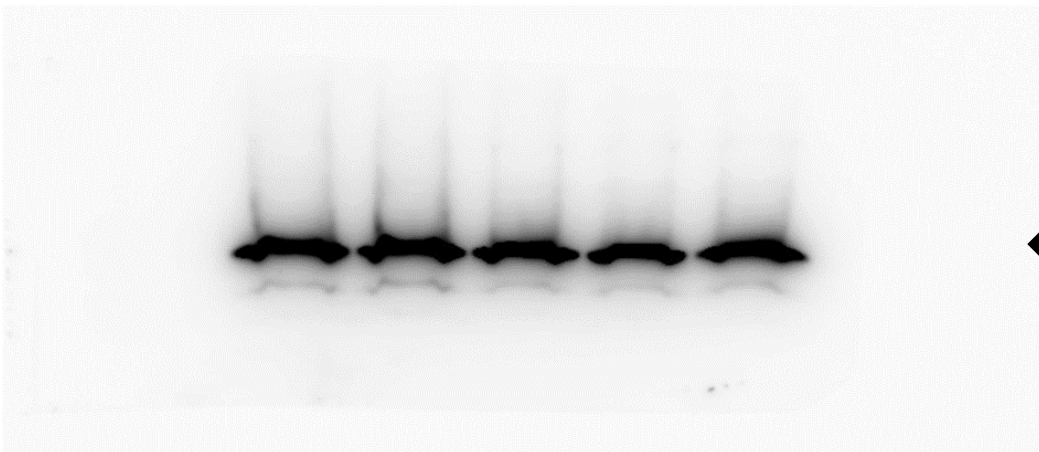
a

cGAS

1st



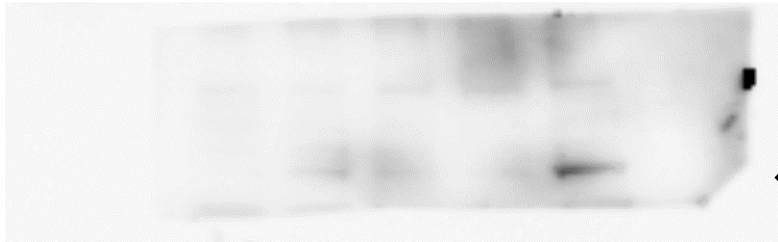
Histone H3



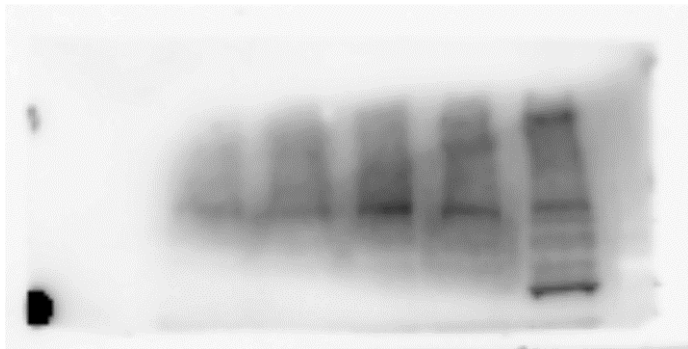
Uncropped Gels and Blots images of Supplementary Figure 2 were shown.

cGAS

2nd



3rd

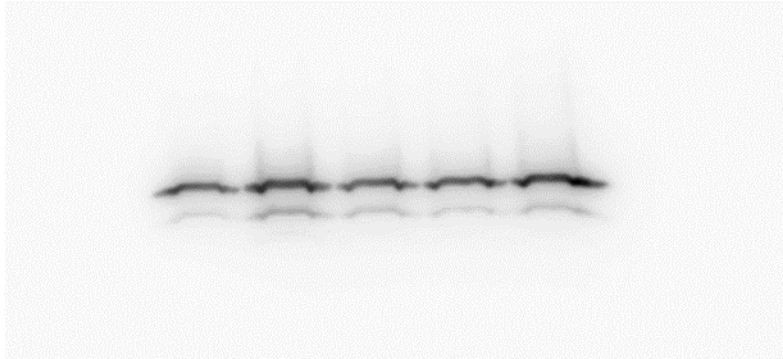


← Target band

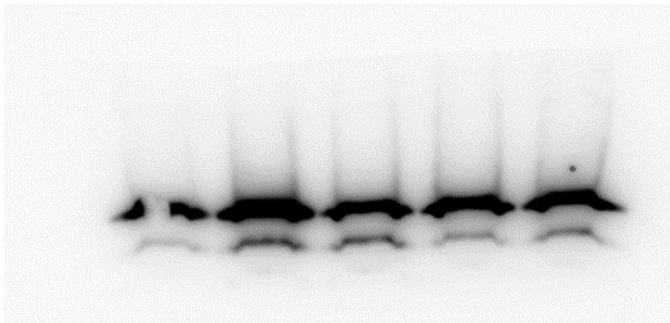
Uncropped Gels and Blots images of Supplementary Figure 2 were shown.

Histone H3

2nd



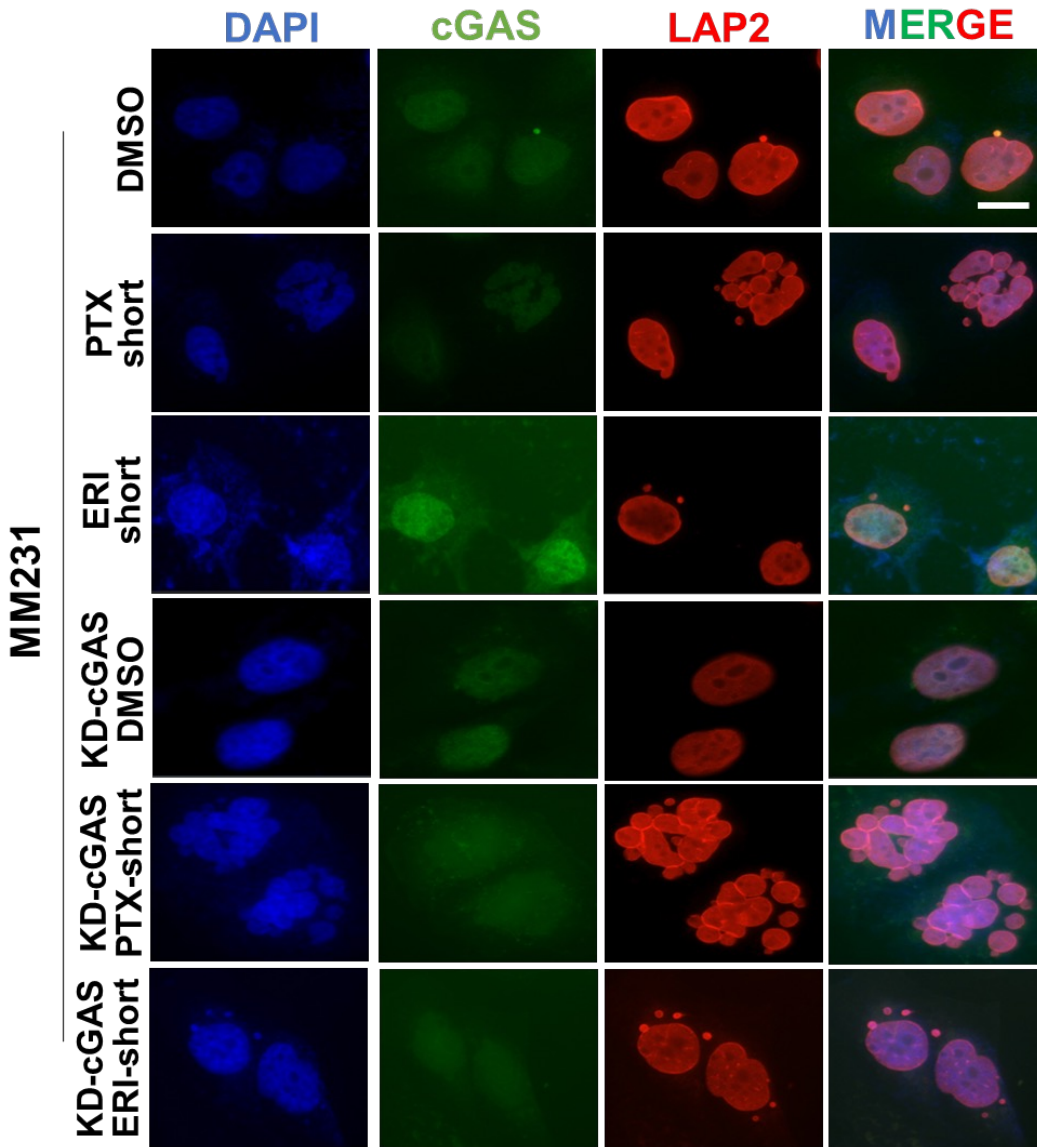
3rd



Uncropped Gels and Blots images of Supplementary Fig 2 were shown.

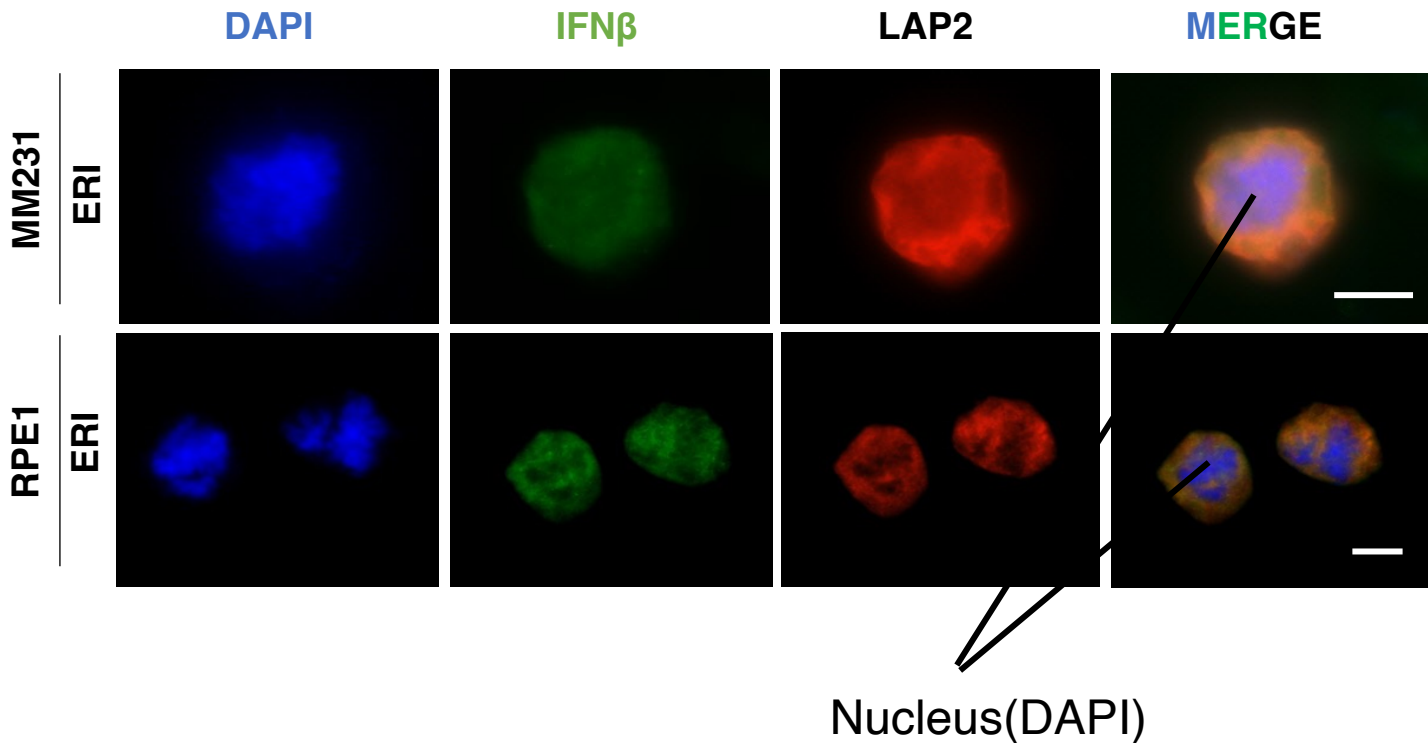
Supplemental figure regarding what reviewer pointed out

Supplementary Figure 2c



cGAS staining in Figure 2 seems to be a cGAS-specific finding because the staining of the cells seems to disappear when cGAS is knocked down as shown in **supplementary Fig.2c**.

Supplementary Figure

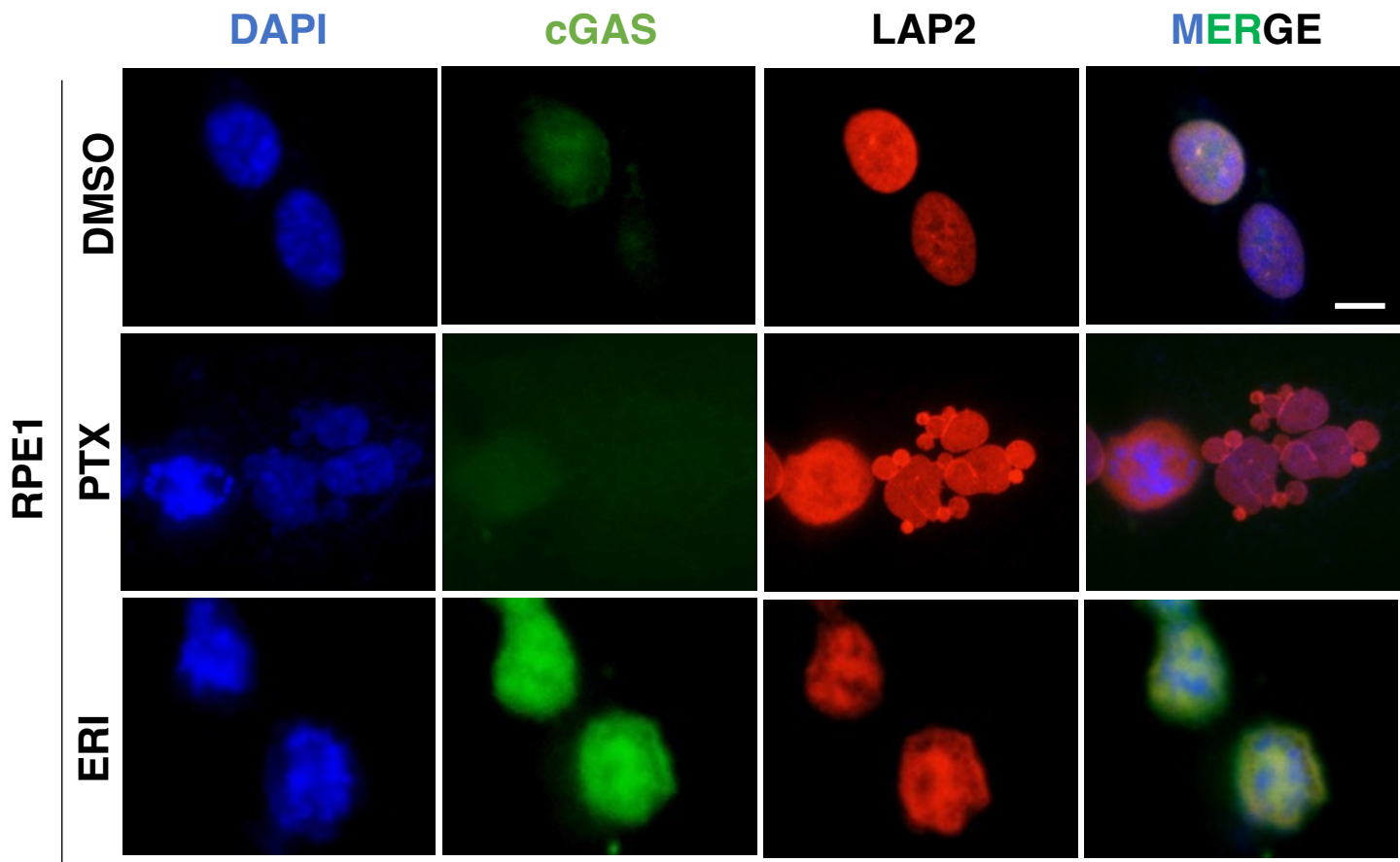


These are part of magnified images of the IFN β -stained cells in **Figure 2b and 2d**. IFN β was not stained in the nucleus but stained in the cytosol. The white bars are 200 μ m.

Additional information on figure 2b and 2d

Figure 2c

Figure 2c (with higher exposure)



An image gain-adjusted figure is attached after the images were taken (above).

We would like to change Figure 2c to these photos.

Figure 2c (before revise: lower exposure)

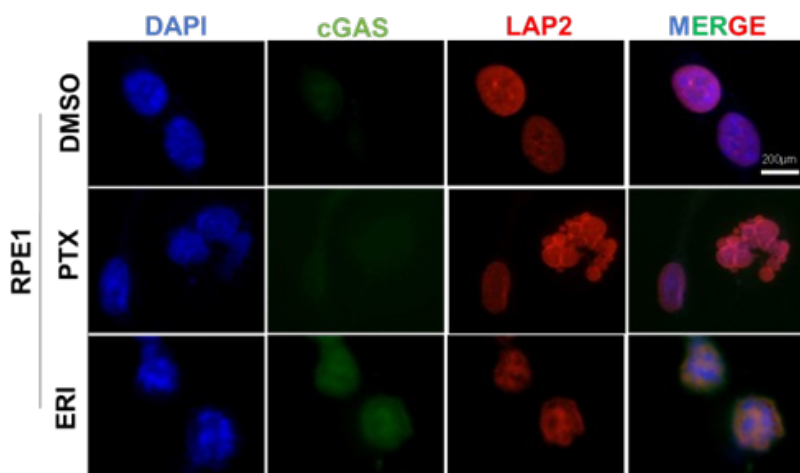
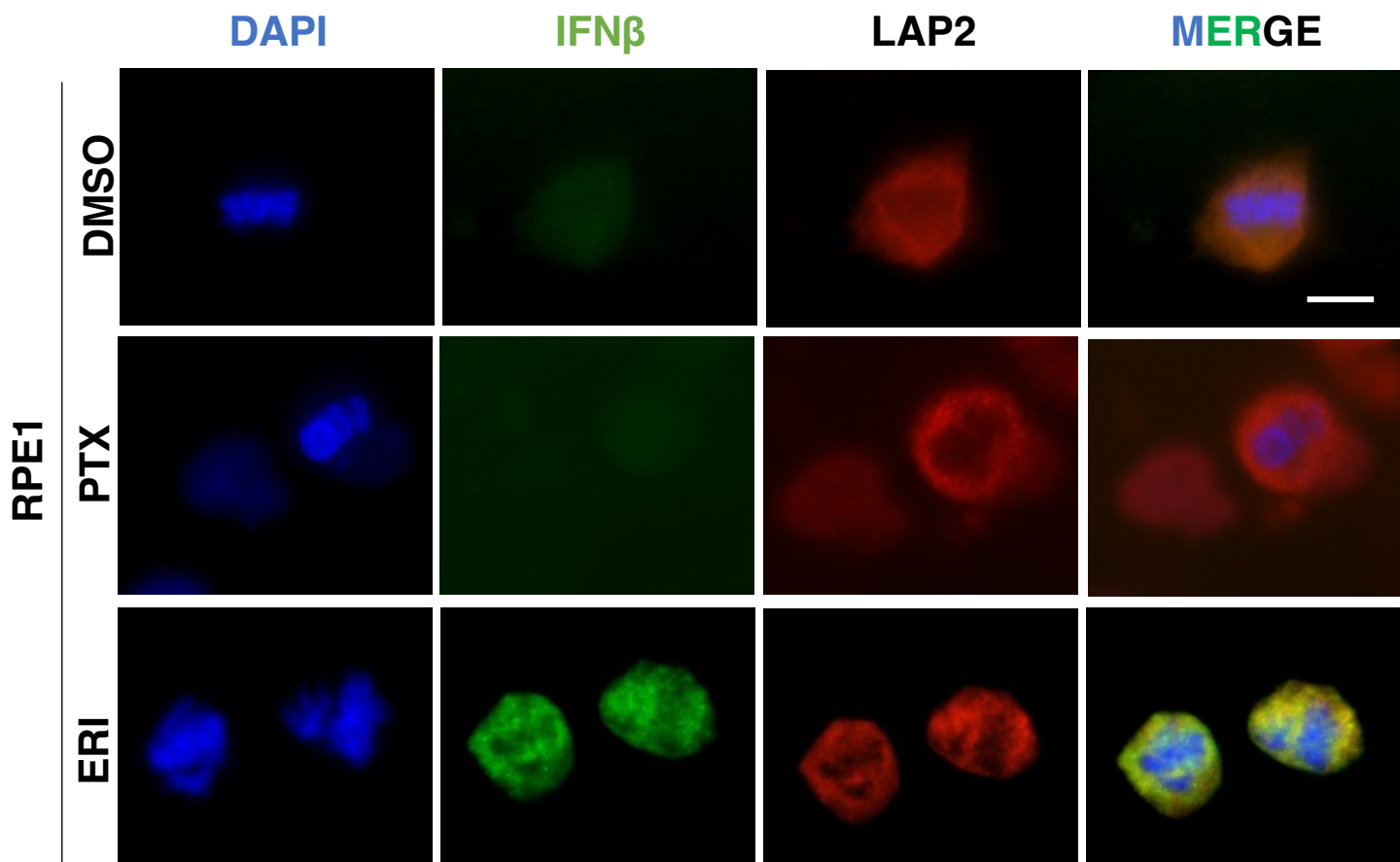


Figure 2d

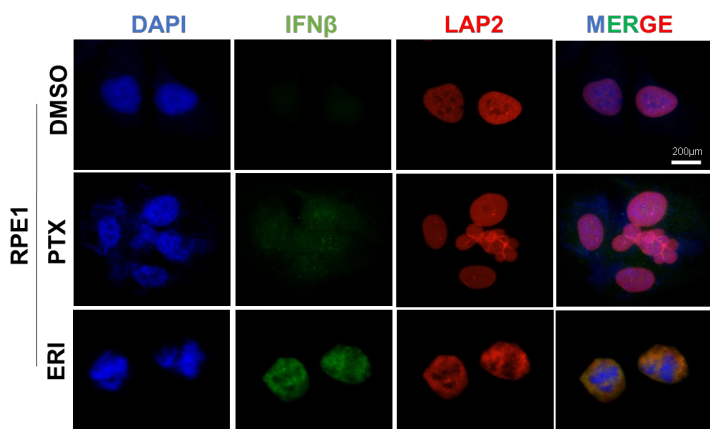
Figure 2d (with higher exposure)



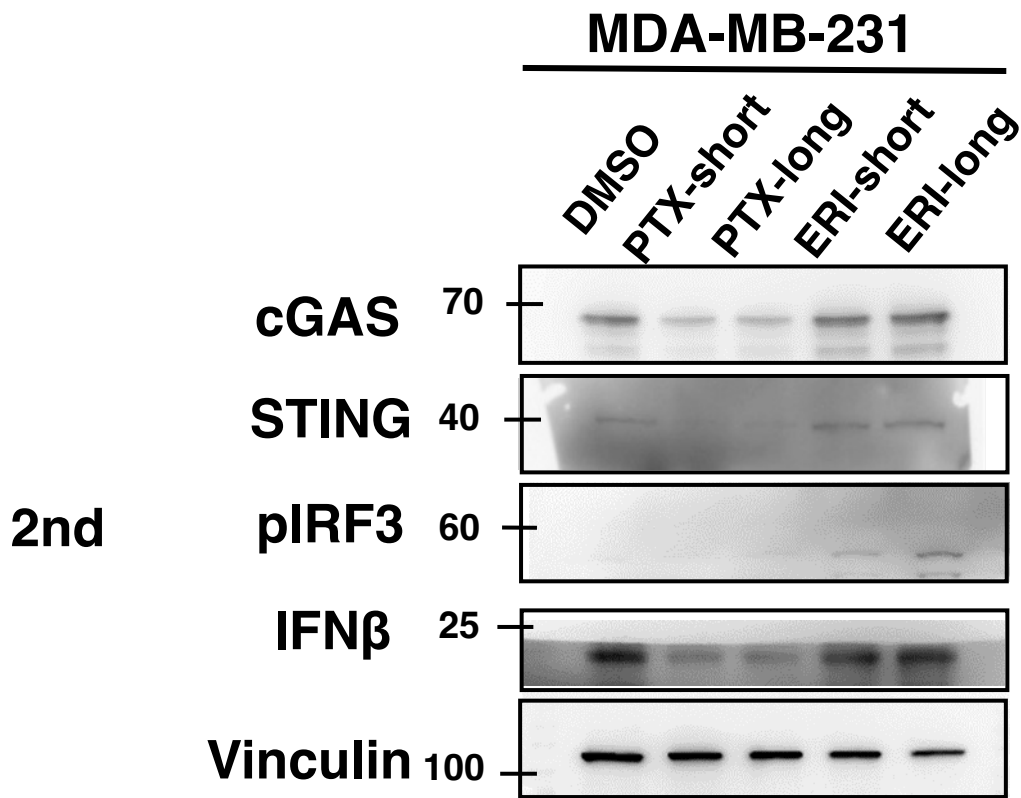
An image gain-adjusted figure is attached after the images were taken (above).

We would like to change Figure 2d to these photos.

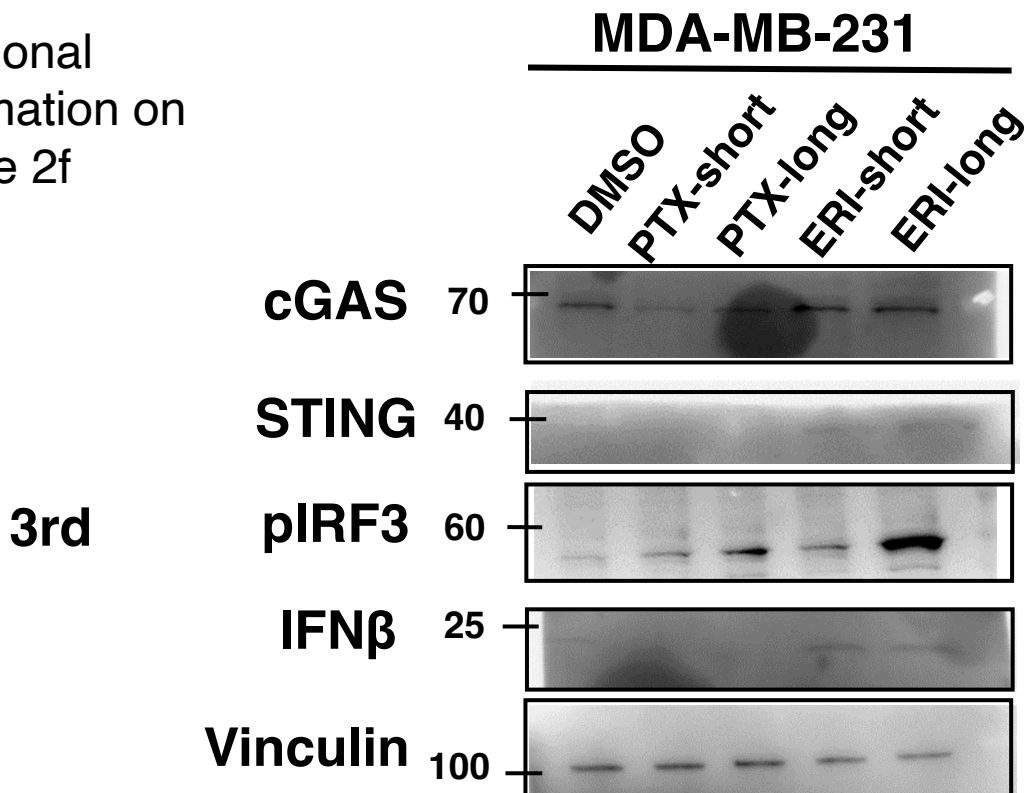
Figure 2d (before revise: lower exposure)



Supplementary Figure

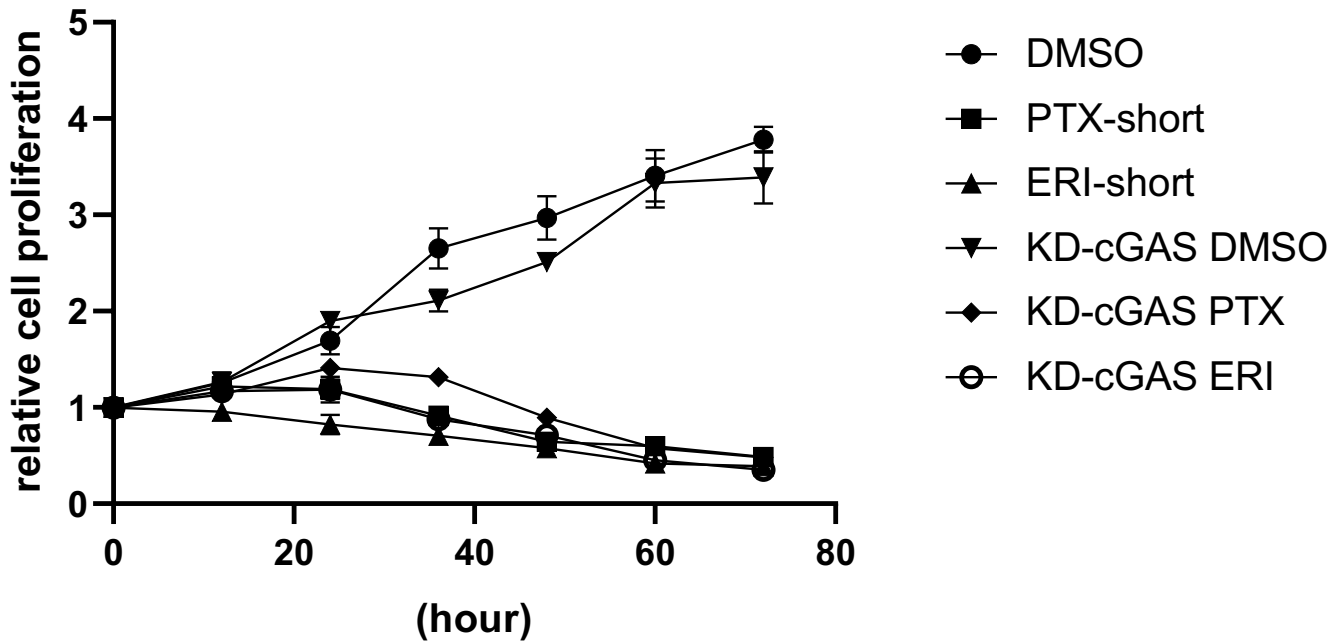


Additional
information on
Figure 2f



The results of other Western blotting in figure 2f were consistent with the conclusion that cGAS/STING/pIRF3/IFN β tended to be higher in the ERI long group than in the others.

Figure 3a



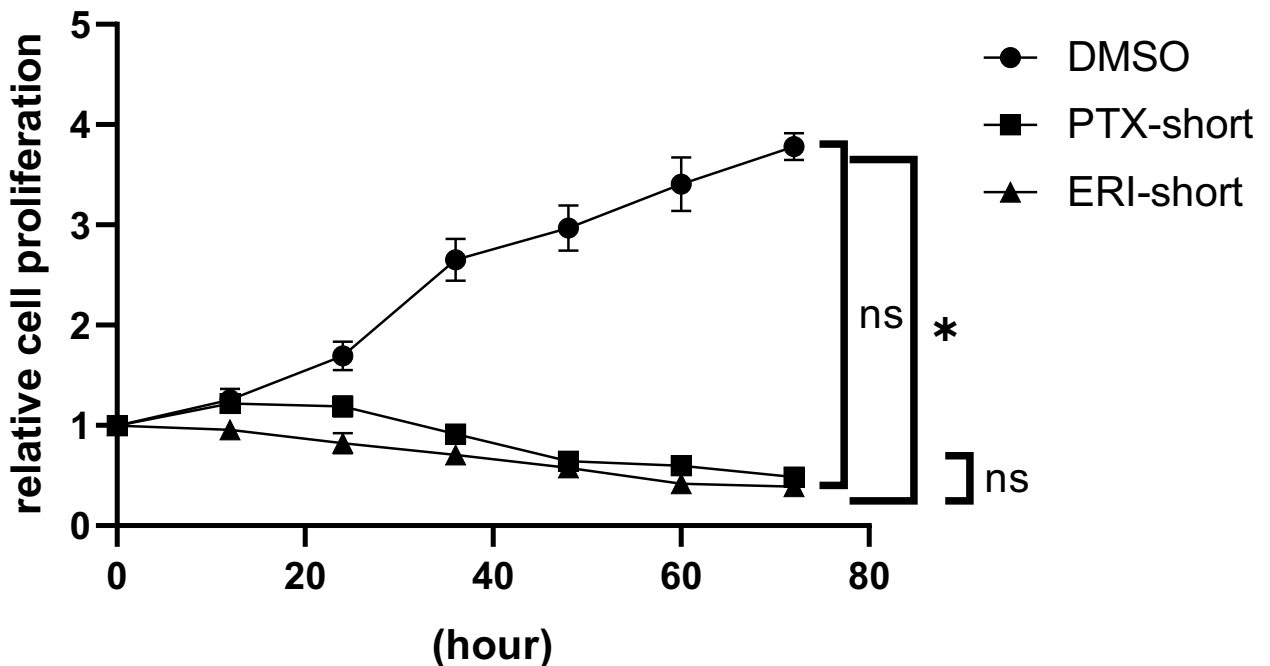
Proliferation at 0 hour was defined as 1, and the relative cell proliferation of above cells was blotted.

$$\text{Relative cell proliferation} = \frac{(\text{Mean OD value of cell A at time X})}{(\text{Mean OD value of DMSO at 0h})}$$

※OD: optical density

We would like to change Figure 3a to this graph.

Supplementary Figure



Statistical differences were evaluated by using one-way anova.

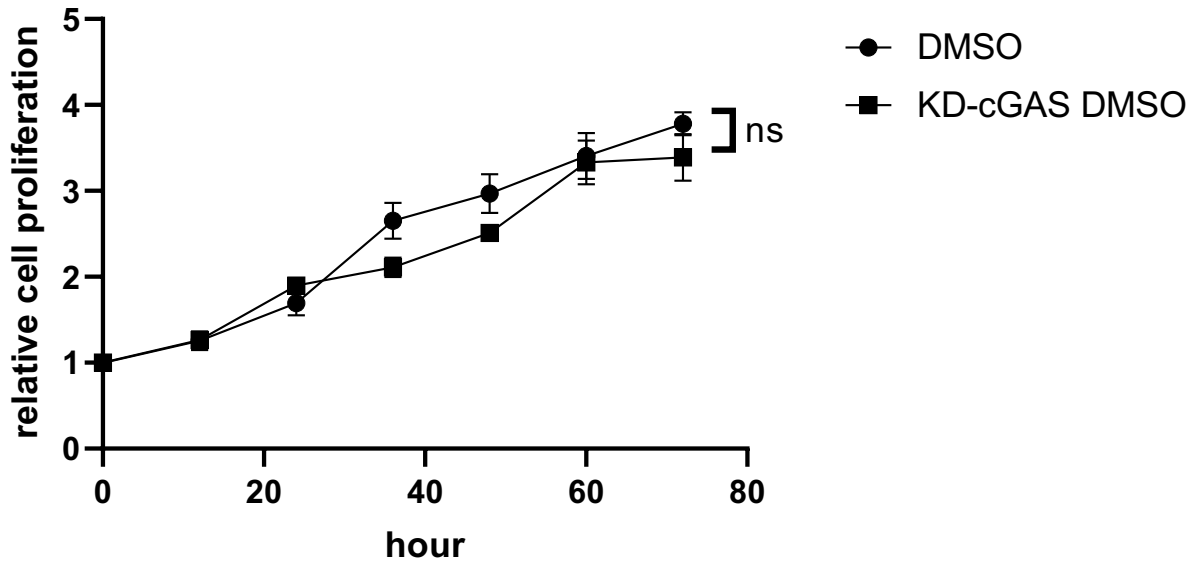
ERI-short significantly inhibited cell proliferation compared to DMSO (* $p=0.047$).

PTX-short inhibited cell proliferation compared to DMSO, but not significantly ($p=0.1142$).

There was no significant difference in the inhibition of cell proliferation between PTX-short and ERI-short ($p>0.9999$).

Additional information on Figure 3a

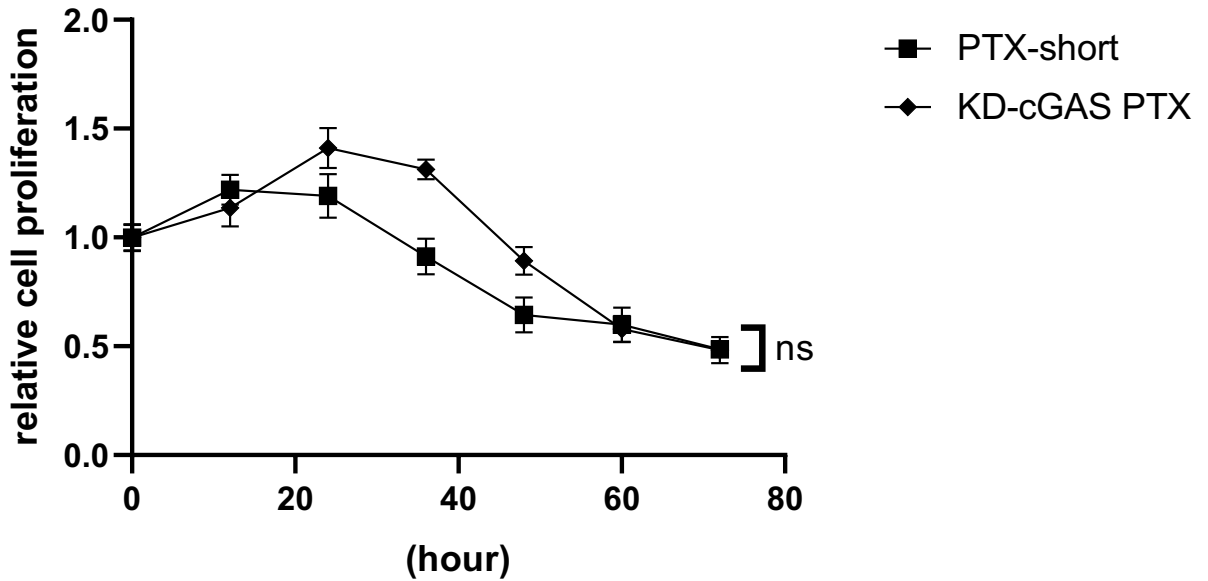
Supplementary Figure



There was no significant difference between DMSO and KD-cGAS-DMSO ($p > 0.9999$).

Additional information on Figure 3a

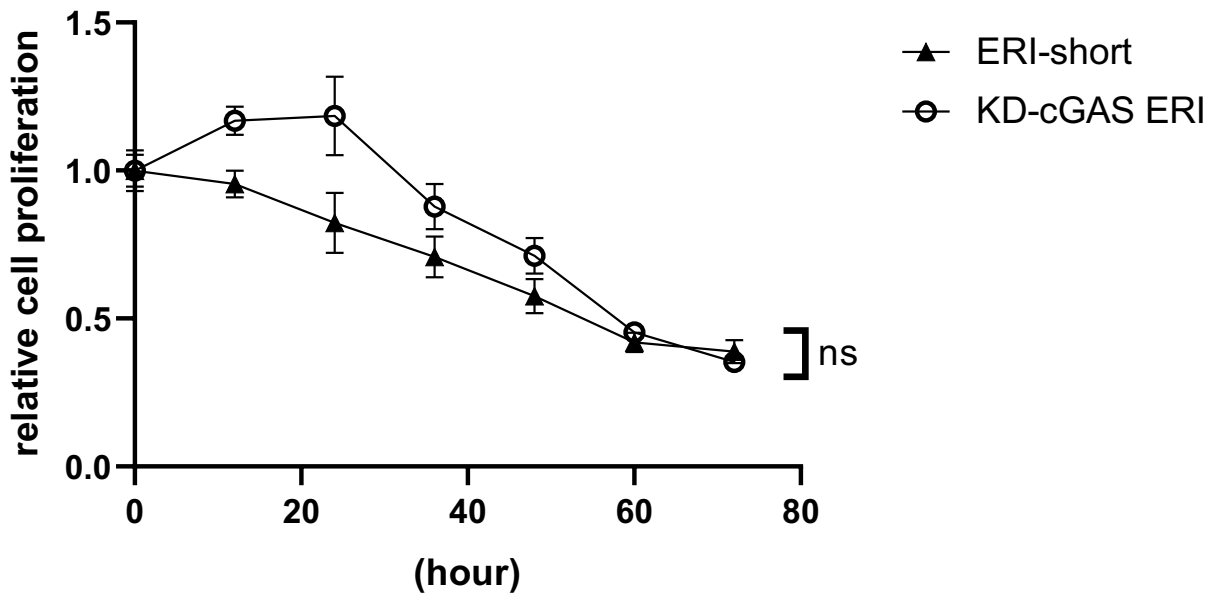
Supplementary Figure



There was no significant difference between PTX-short and KD-cGAS-PTX ($p > 0.9999$).

Additional information on Figure 3a

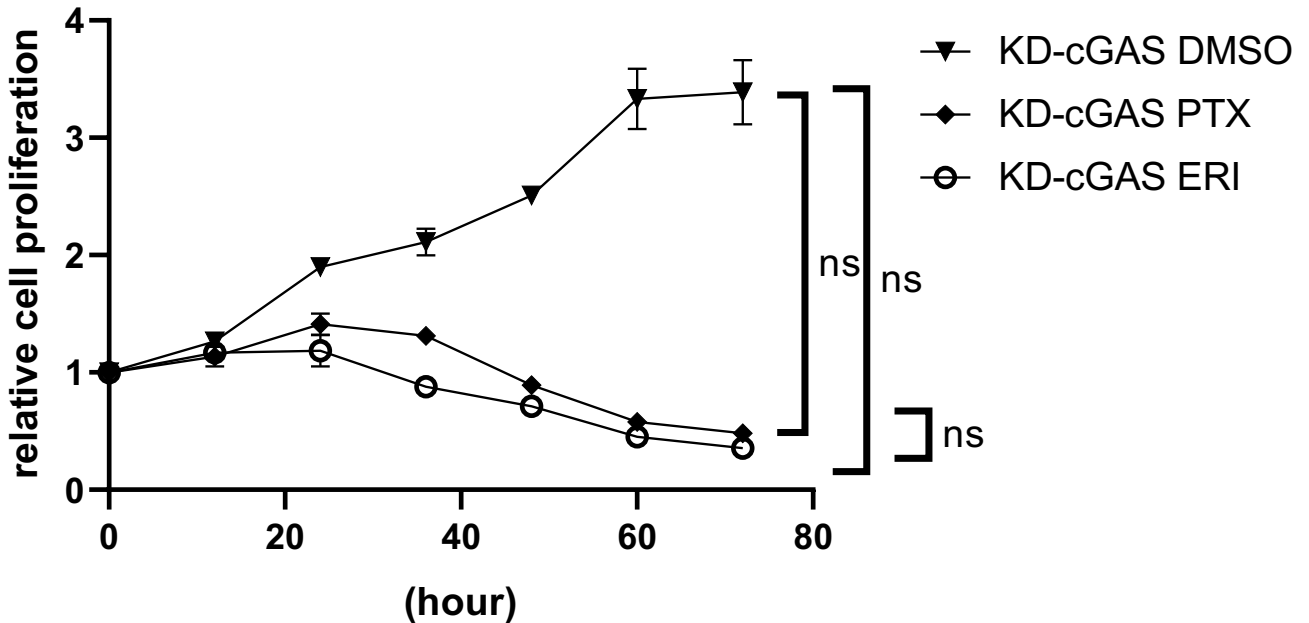
Supplementary Figure



There was no significant difference between ERI-short and KD-cGAS-ERI ($p > 0.9999$).

Additional information on Figure 3a

Supplementary Figure

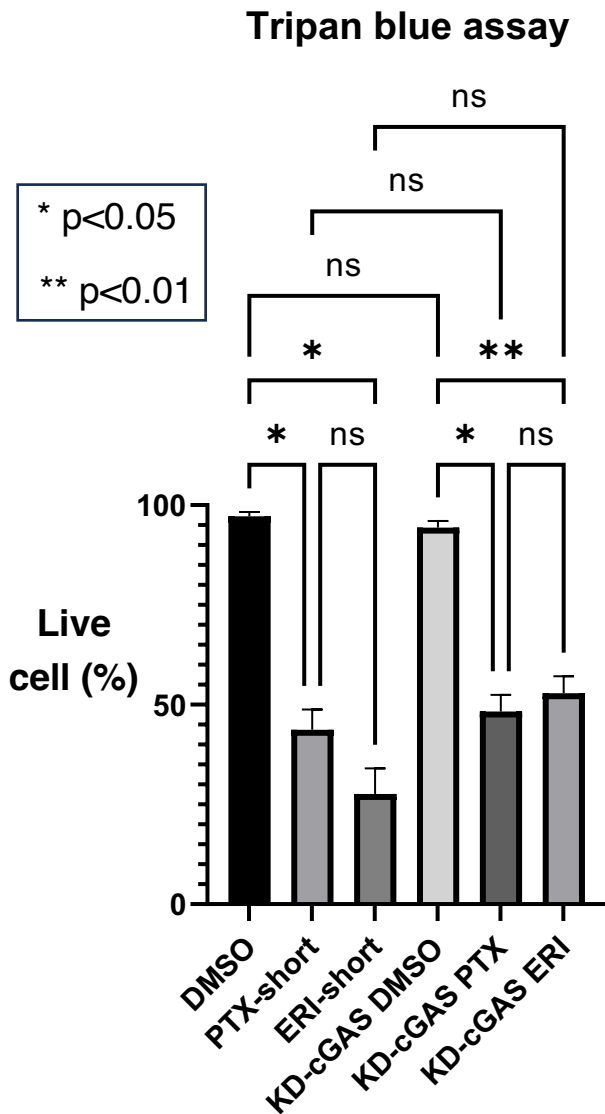


KD-cGAS ERI ($p=0.0526$) and KD-cGAS PTX ($p=0.4404$) inhibited cell proliferation compared to KD-cGAS DMSO, but not significantly.

There was no significant difference in the inhibition of cell proliferation between KD-cGAS PTX and KD-cGAS ERI ($p>0.9999$).

Additional information on Figure 3a

Figure 3b



The results of the statistical study are shown.

The percentage of viable cells was statistically compared by unpaired t test.

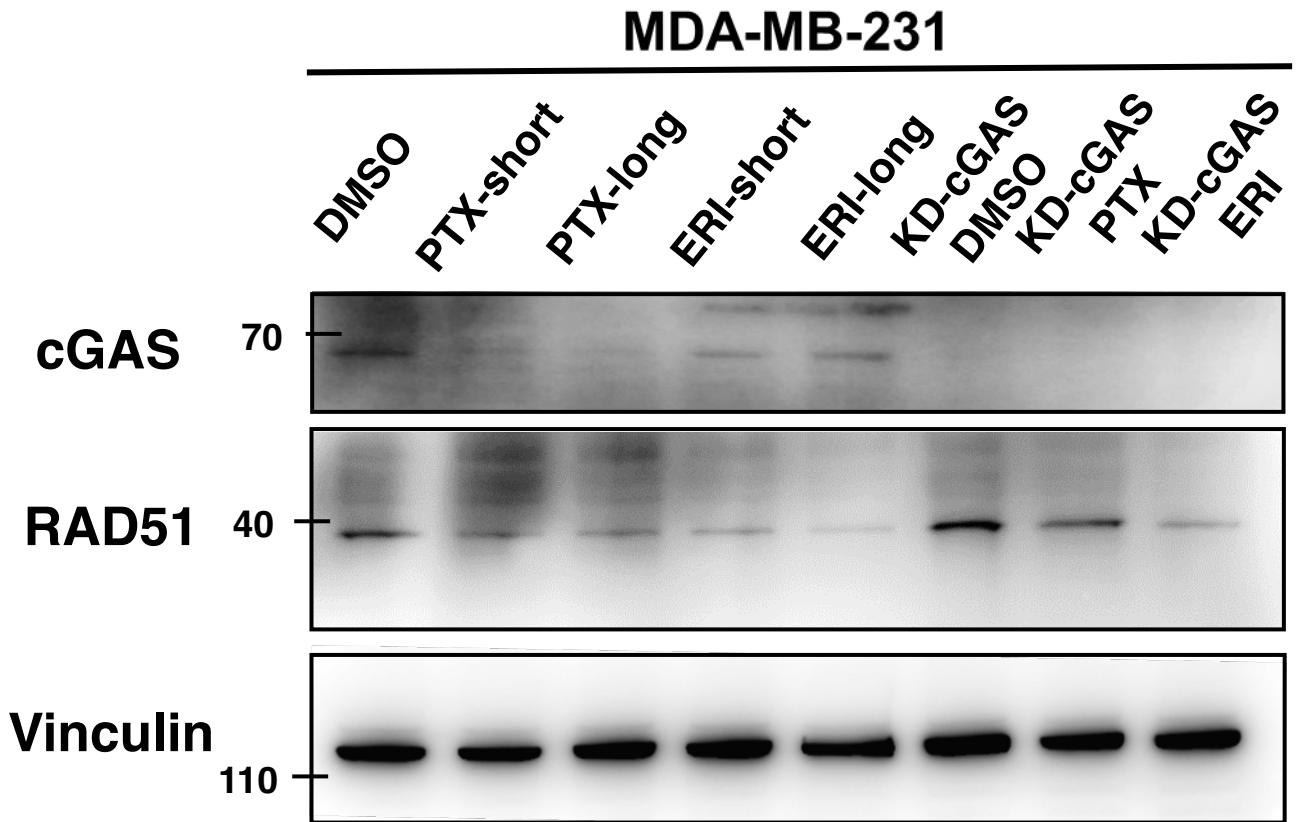
DMSO and KD-cGAS DMSO were not significantly different in the percentage of viable cells ($p=0.3967$).

PTX and KD-cGAS PTX were not significantly different in the percentage of viable cells ($p=0.5926$).

KD-cGAS ERI tended to have a higher percentage of viable cells than ERI short, but the difference was not significant ($p=0.0650$).

We would like to change Figure 3b to this graph.

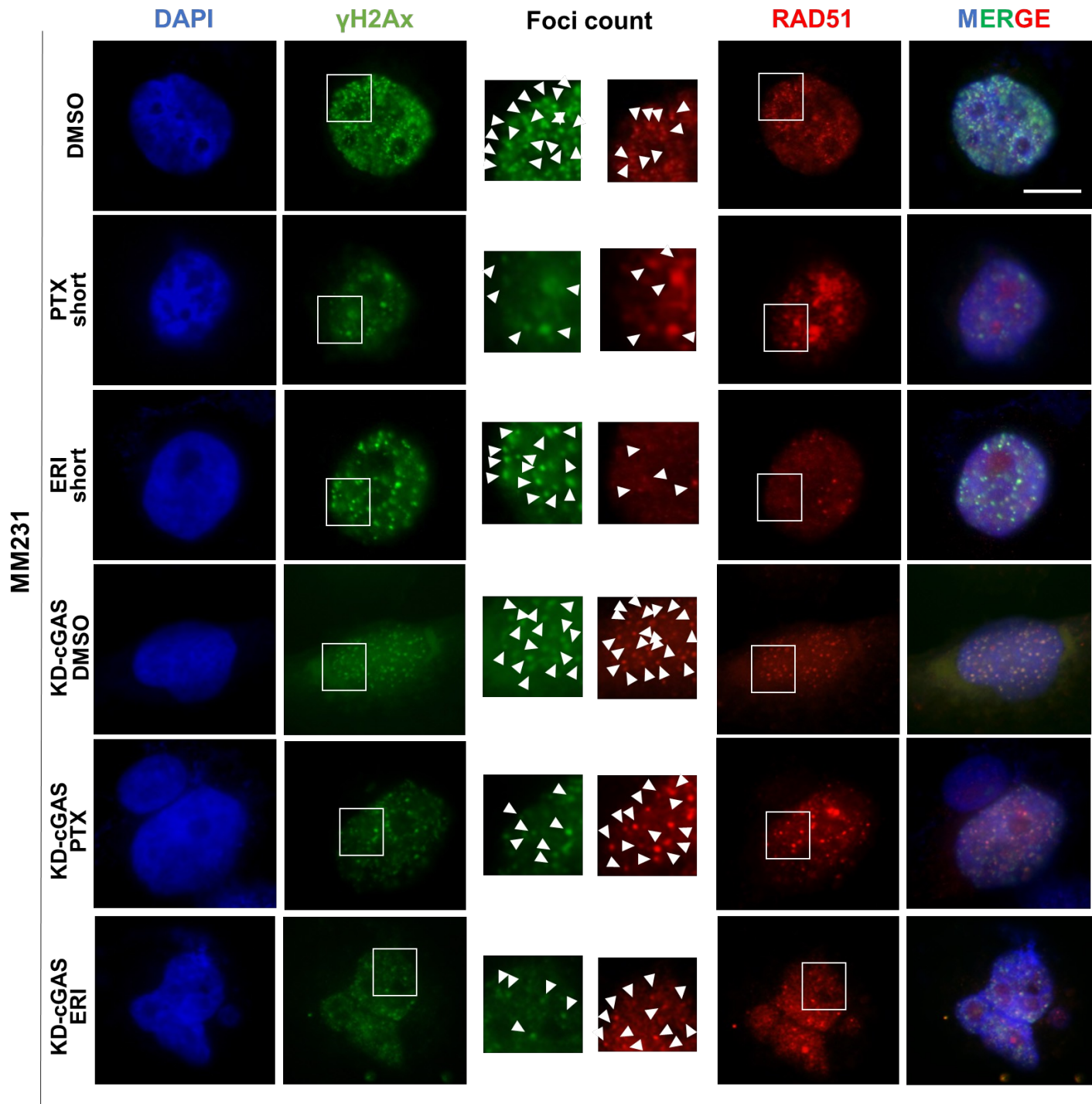
Figure 3c



The data shows that cGAS is no longer elevated by ERI or PTX treatment after knockdown of cGAS, and Rad51 is elevated at the same level as in the control in both cases.

We would like to change Figure 3c to this one.

Figure 3d



The photos in Figure 3d were not sufficient for evaluation of foci, especially since it was diffusely stained with RAD51.

We would like to replace Figure 3d to this one.

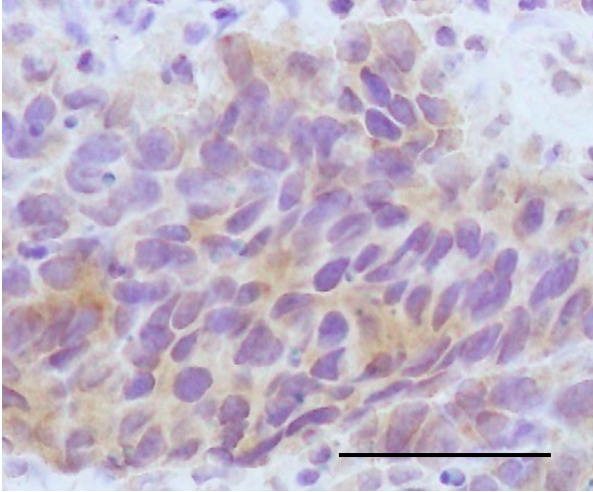
The white bar is 200 μ m.

White triangles point to foci.

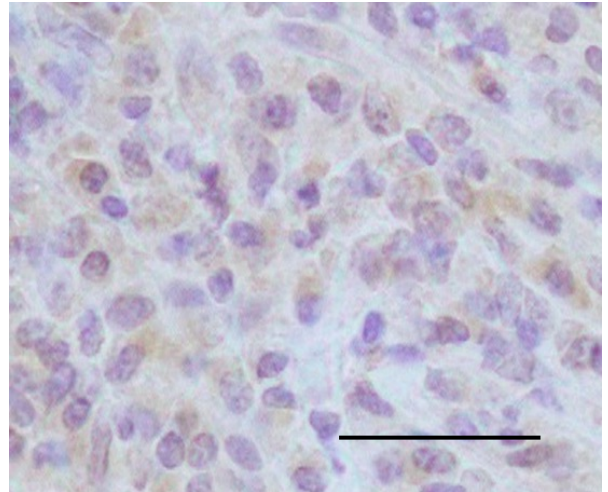
Supplementary Figure

(× 400)

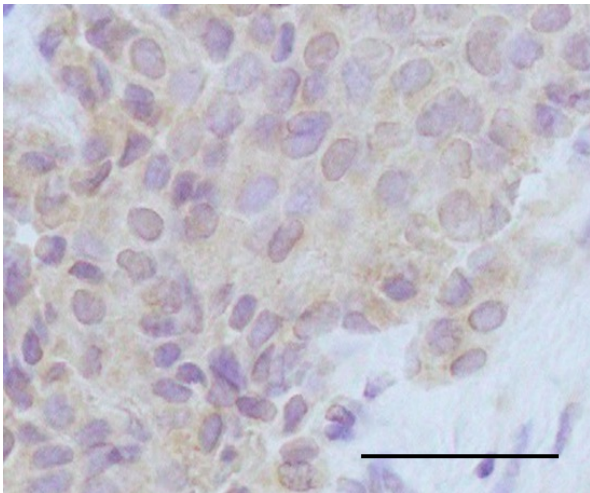
Case 1



Case 2



Case 3



There are three images with IFN β stained in the cytoplasm rather than the nucleus in IHC.

The black bars are 50 μ m.