

Uncropped Blots

Glucocorticoid receptor dysfunction as a biomarker and target for bile acid therapy in SCA3/MJD

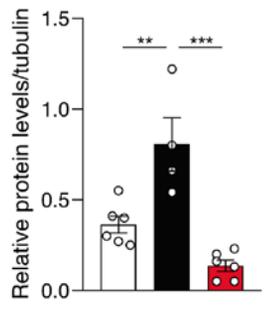
Sara Duarte-Silva^{1,2,§}, Jorge Diogo Da Silva^{1,2,3,4,§}, Daniela Monteiro-Fernandes^{1,2,§}, Marta Daniela Costa^{1,2}, Andreia Neves-Carvalho^{1,2}, Mafalda Raposo⁵, Carina Soares-Cunha^{1,2}, Joana S. Correia^{1,2}, Gonçalo Nogueira-Goncalves^{1,2}, Henrique S. Fernandes^{6,7}, Stephanie Oliveira^{1,2}, Ana Rita Ferreira-Fernandes^{1,2}, Fernando Rodrigues^{1,2}, Joana Pereira-Sousa^{1,2}, Daniela Vilasboas-Campos^{1,2}, Sara Guerreiro^{1,2}, Jonas Campos^{1,2}, , Liliana Meireles-Costa^{1,2}, Cecilia M. Rodrigues^{8,9}, Stephanie Cabantous¹⁰, Sergio F. Sousa^{6,7}, Manuela Lima^{5,11}, Andreia Teixeira-Castro^{1,2}, Patricia Maciel^{1,2,*}.

Important Notice:

One of the specificities of the design of our experiments that require Western Blots is that sample loading is made in sets of three (as we mostly have 3 experimental groups, WT, TG and TG TUDCA). However, the sample order is randomly chosen for each experiment. Therefore, each individual experiment has a different sample organization, which is indicated for each individual blot. Purple rectangles indicate the blot shown in the main/supplemental figures.

Figure 3B

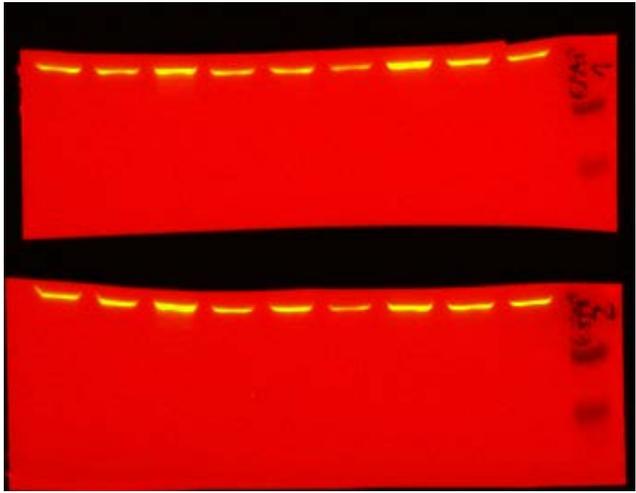
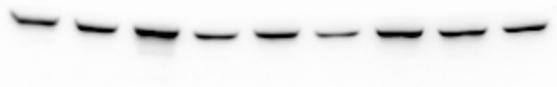
Set No. 1



Samples are organized in sets of three, from left to right, as indicated below.

TG	WT	TG TUDCA
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GFAP



Tubulin

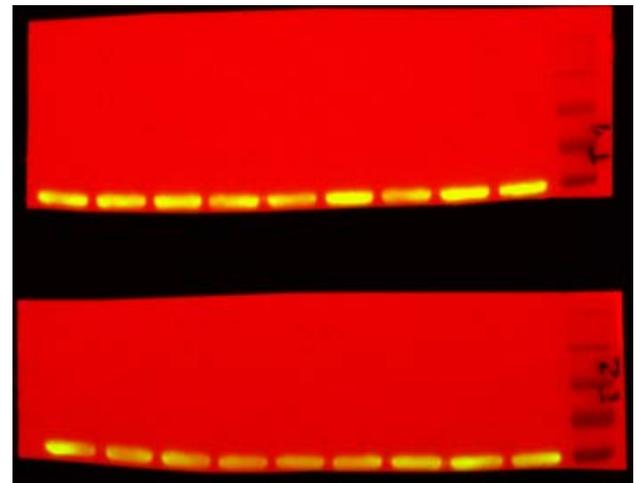
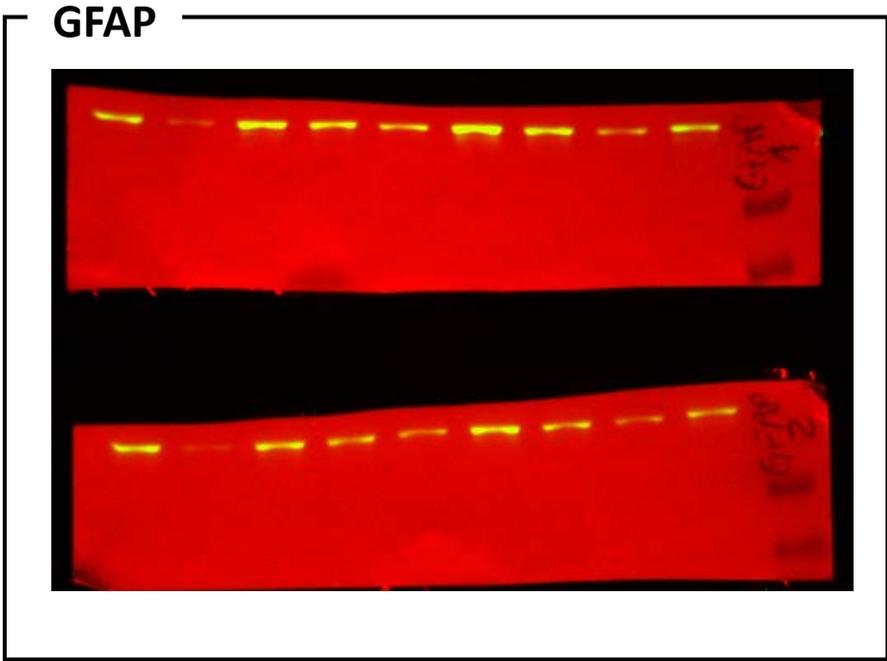


Figure 3B

WT	TG TUDCA	TG
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Set No. 2

Samples are organized in sets of three, from left to right, as indicated above.



Tubulin

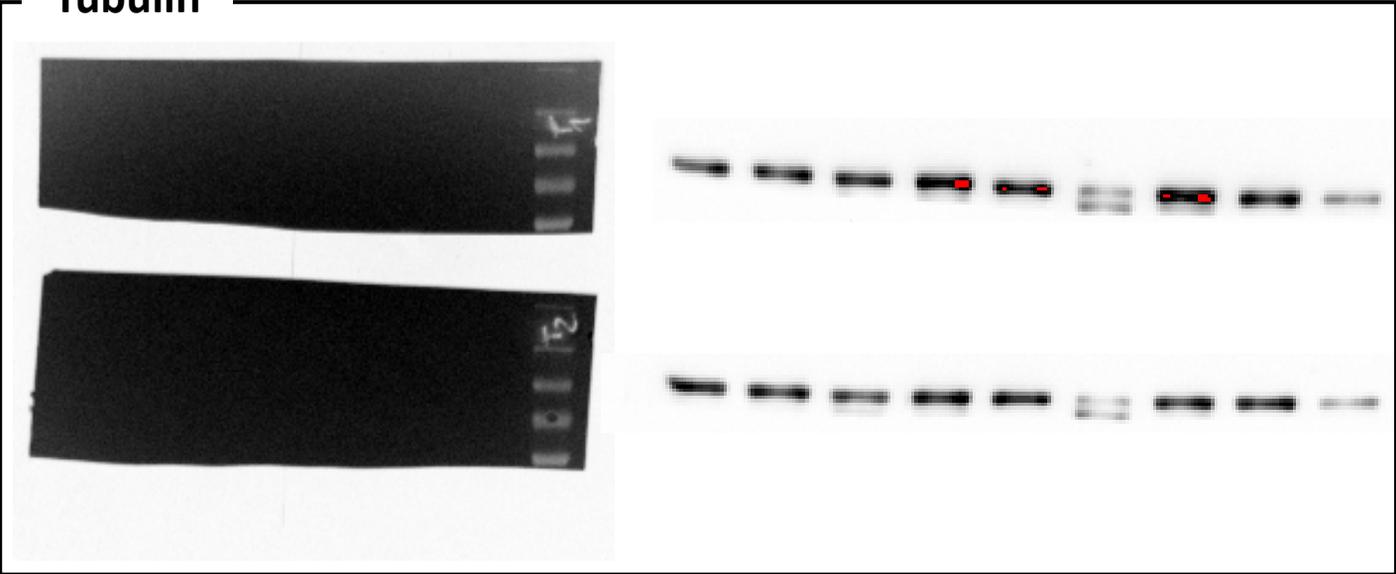
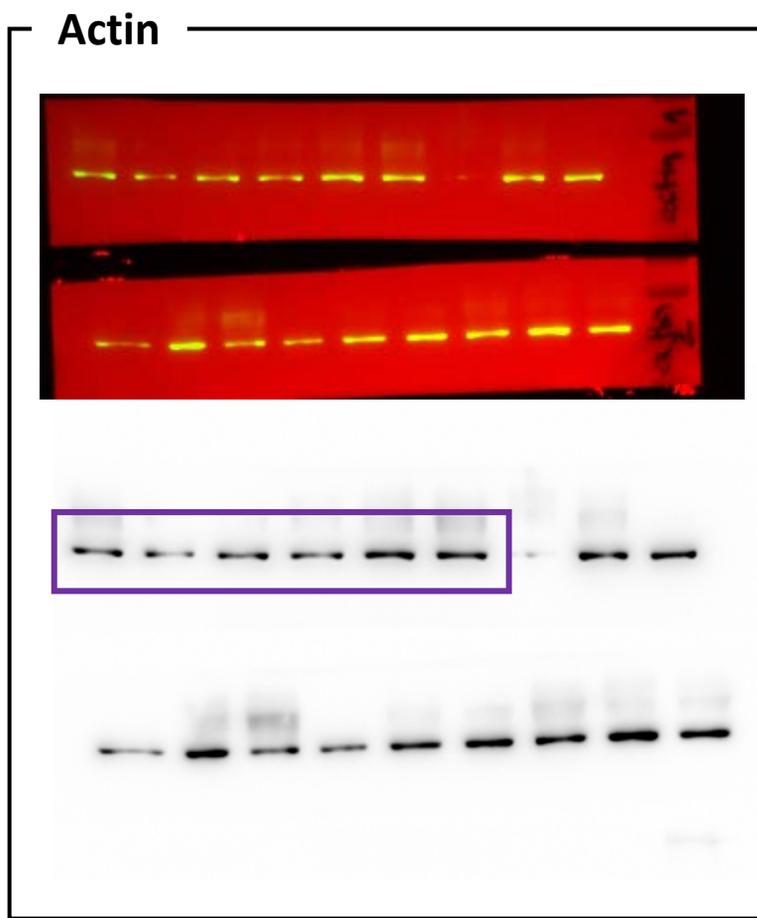
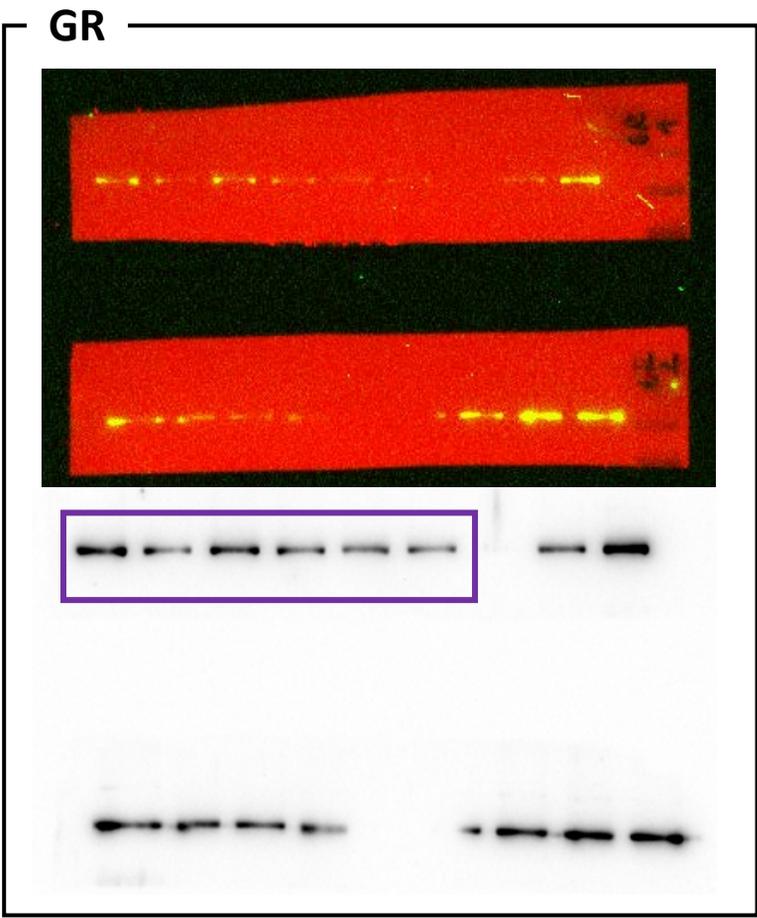
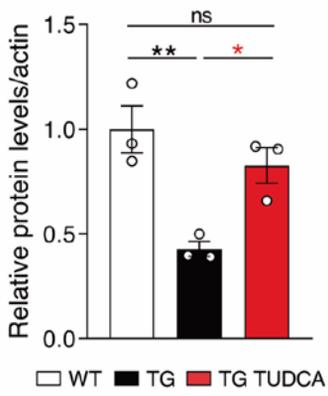


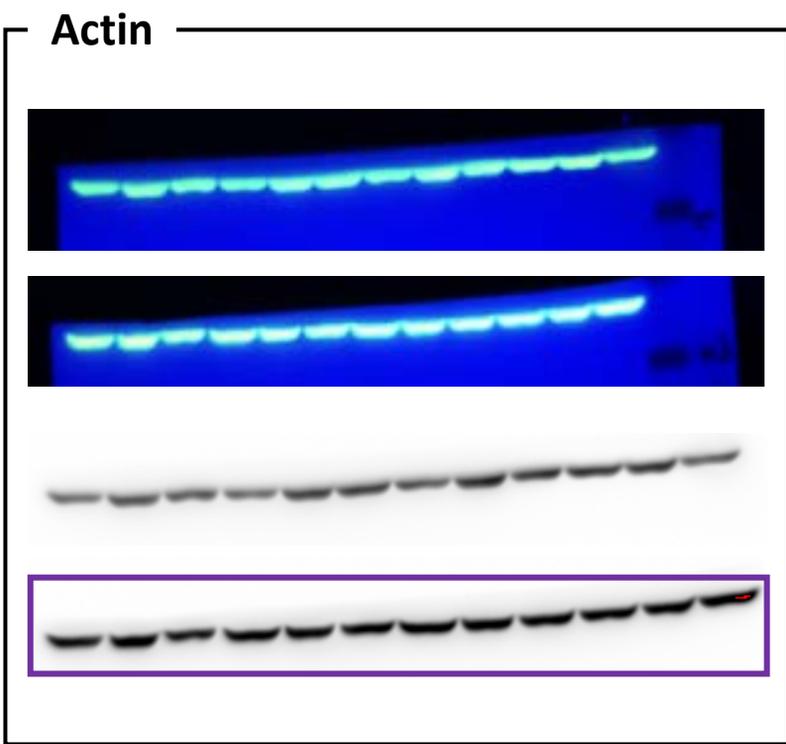
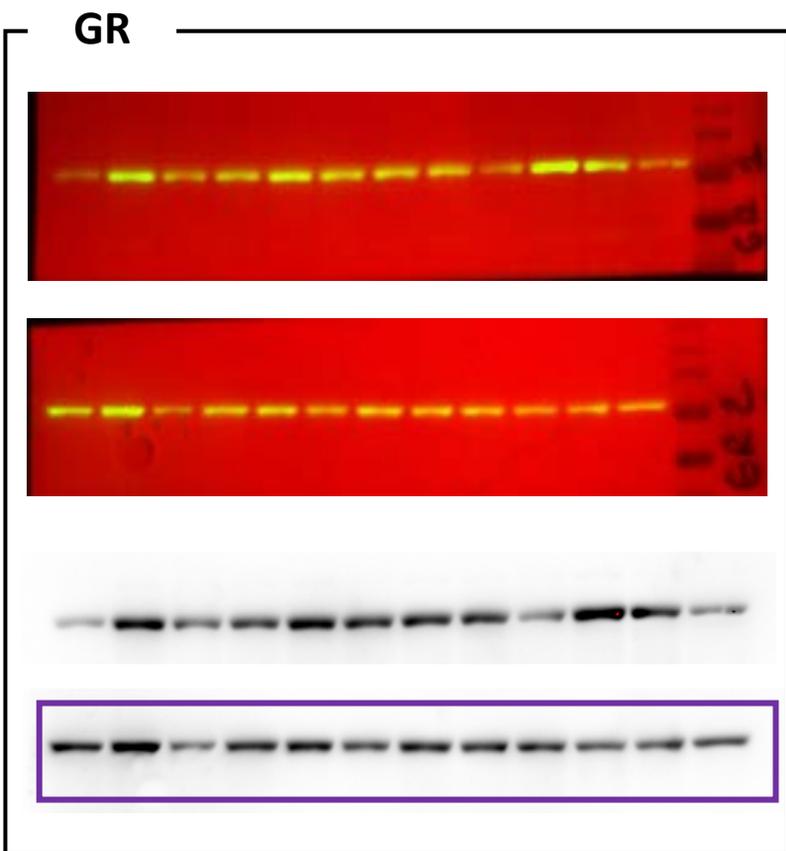
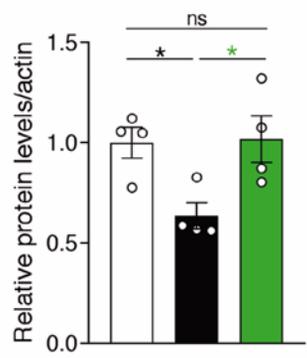
Figure 6C



WT	TG	TG TUDCA
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Samples are organized in sets of three, from left to right, as indicated above.

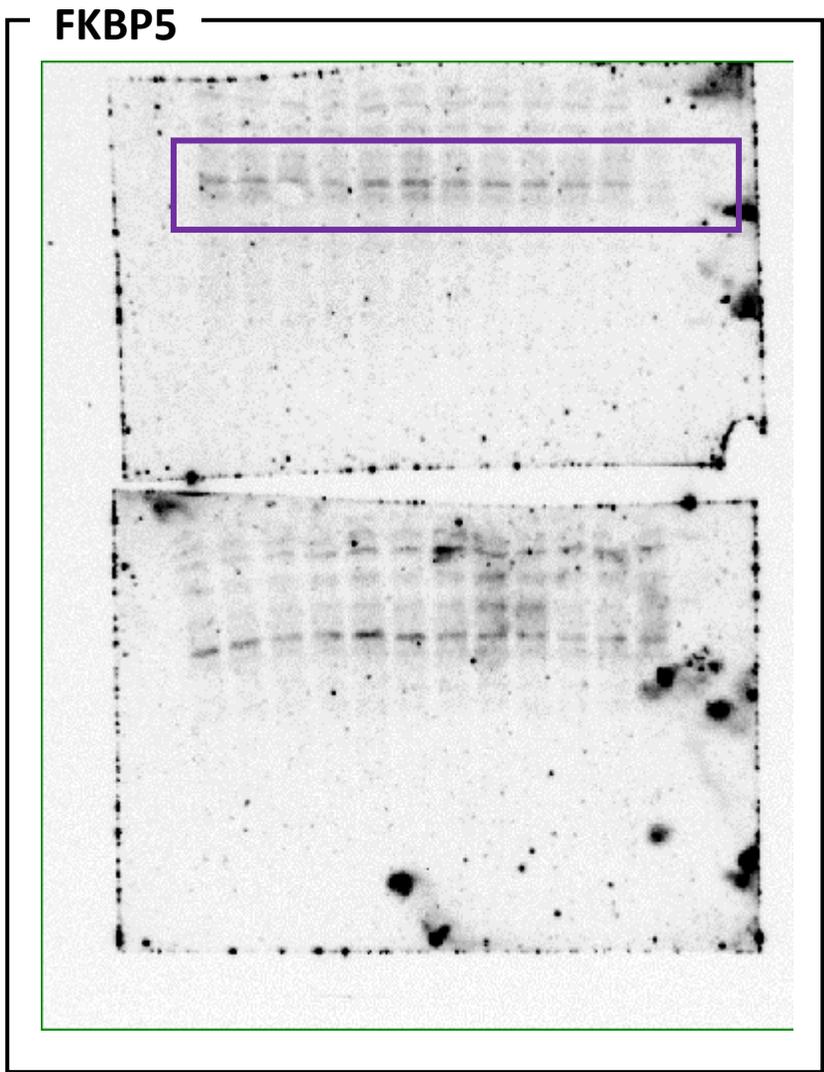
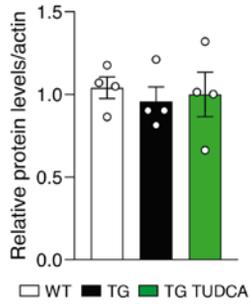
Figure 6D



WT	TG TUDCA	TG
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Samples are organized in sets of three, from left to right, as indicated above.

Figure 6E



TG	TG TUDCA	WT
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Samples are organized in sets of three, from left to right, as indicated above.

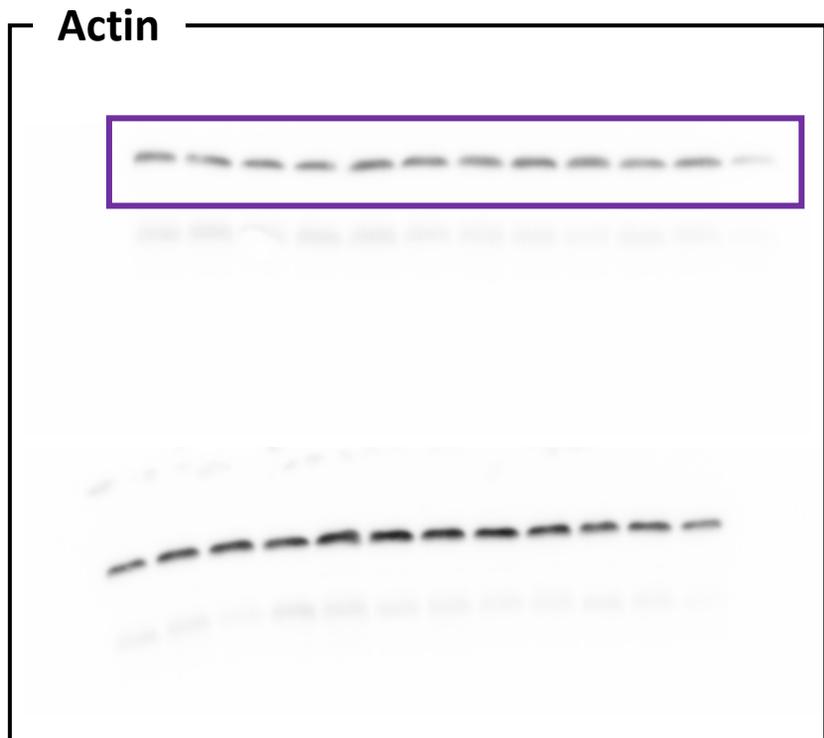
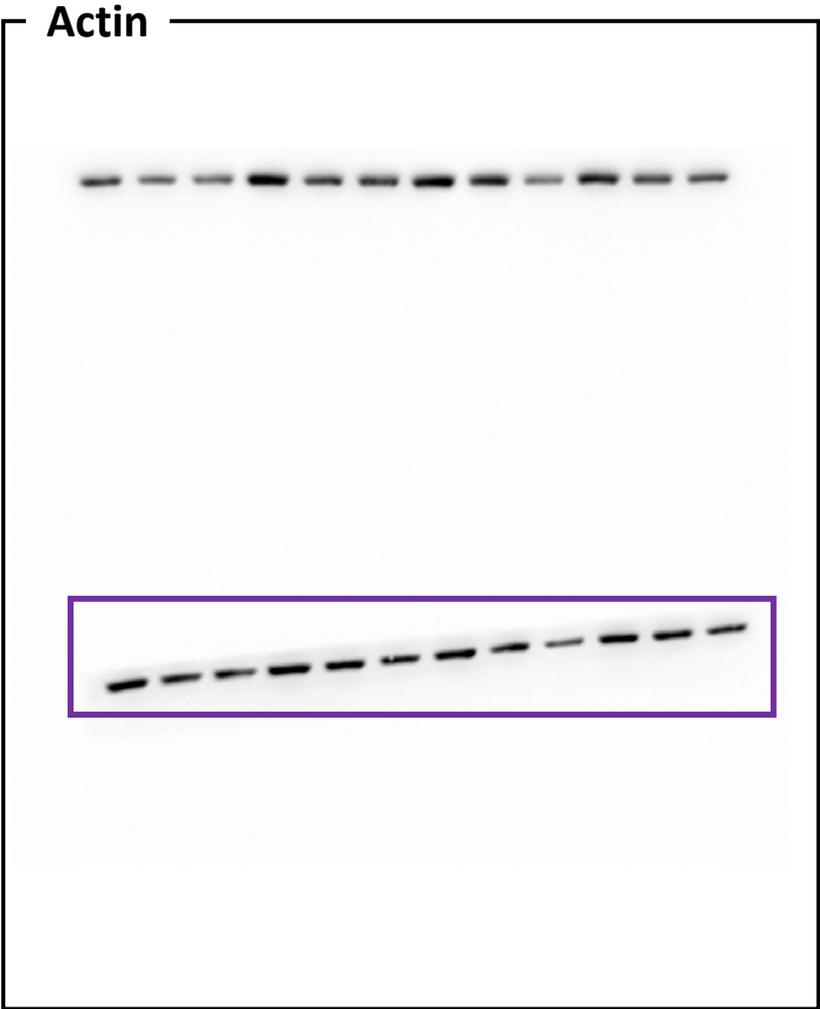
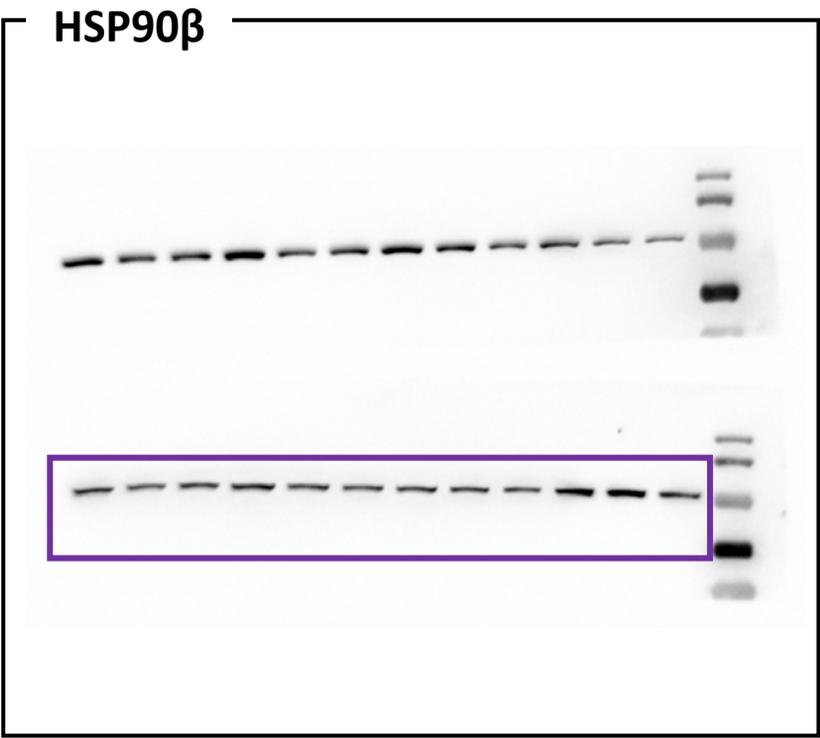
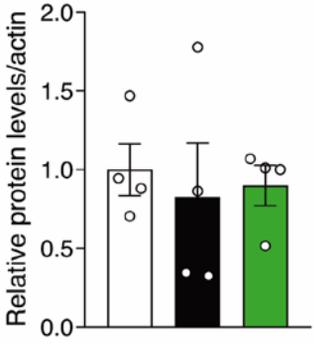


Figure 6F

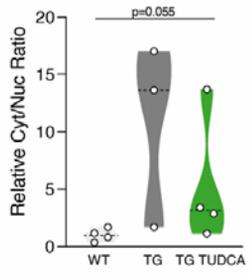


WT	TG	TG TUDCA
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Samples are organized in sets of three, from left to right, as indicated above.

Figure 6G

Set No. 1
(Single Group per Gel)



WT (1)

WT (2)

TG (1)

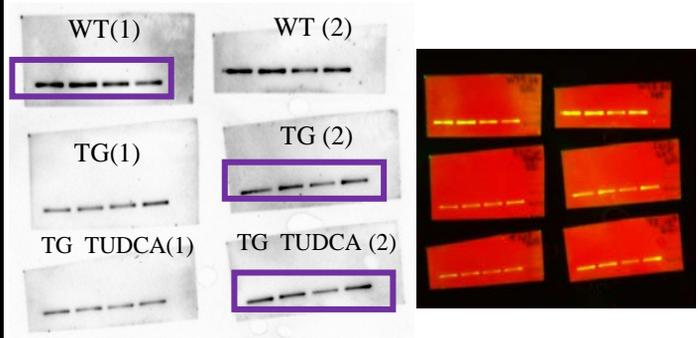
TG (2)

TG TUDCA (1)

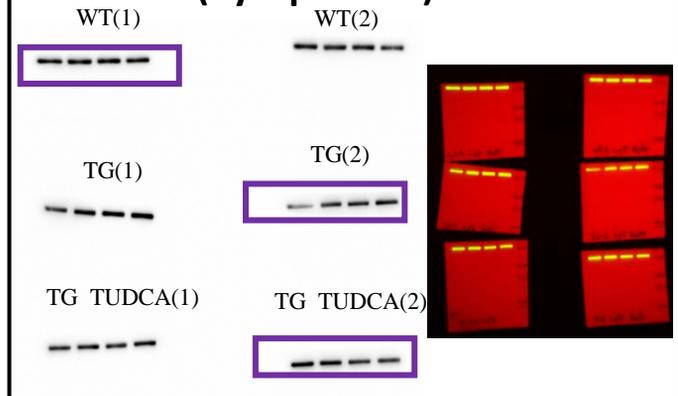
TG TUDCA (2)

Four biological replicates of each group are included in each gel

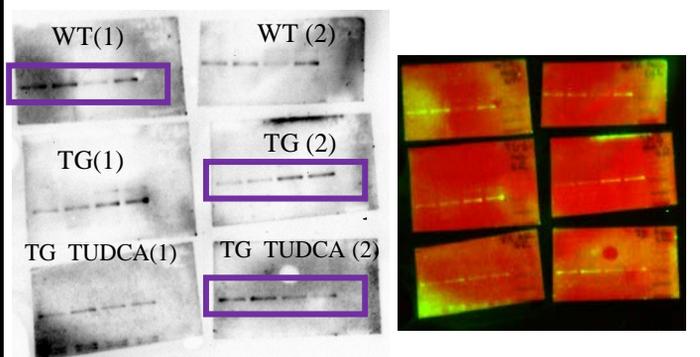
GR (Cytoplasmic)



Tubulin (Cytoplasmic)



GR (Nuclear)



H3 (Nuclear)

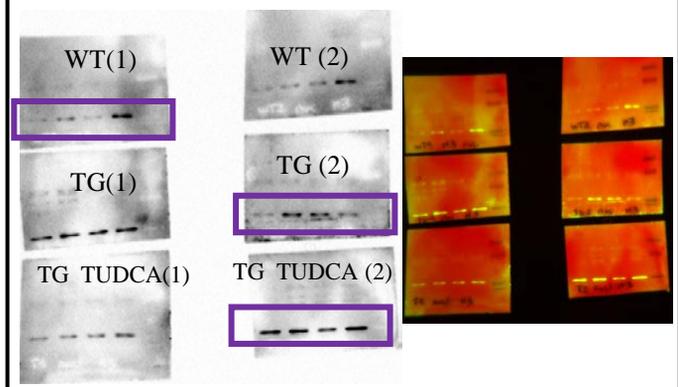
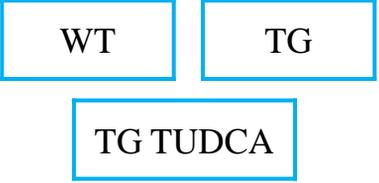
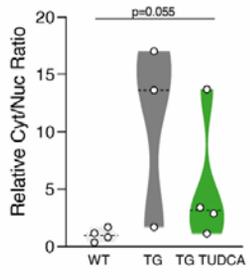


Figure 6G

Set No. 2
(Single Group per Gel)



Four biological replicates of each group are included in each gel

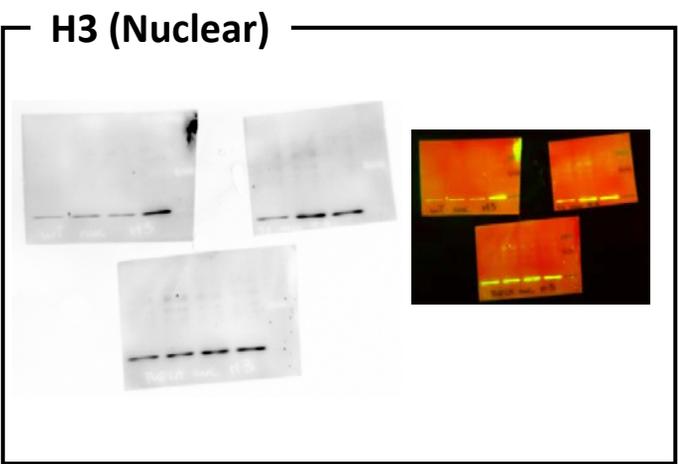
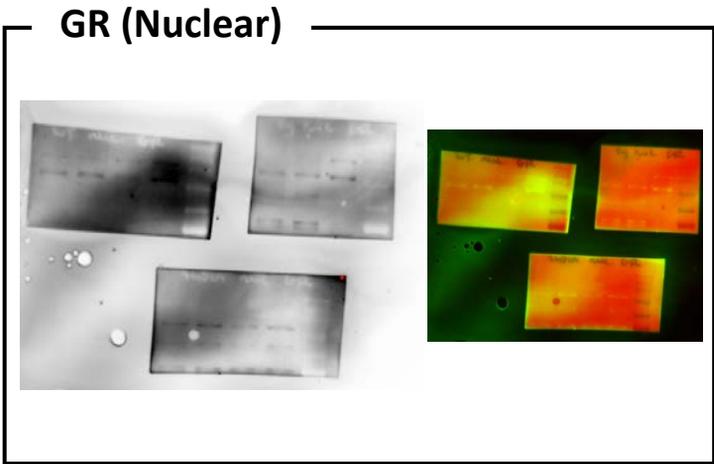
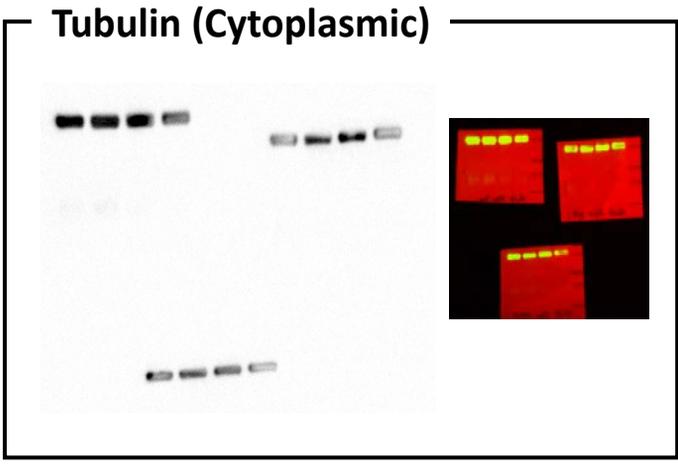
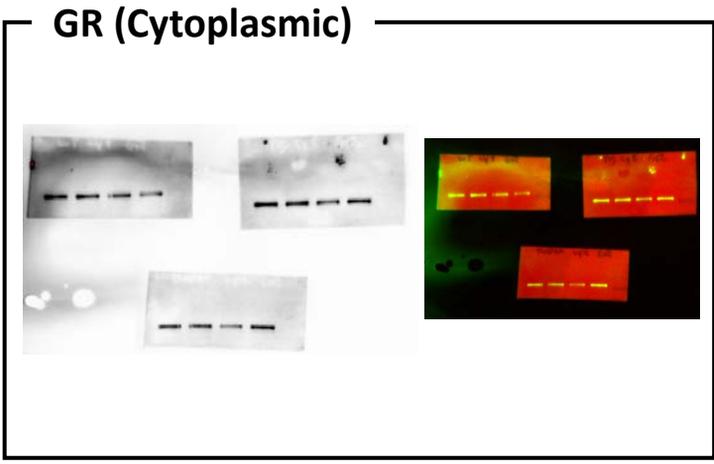
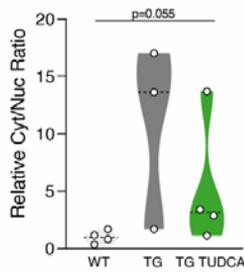


Figure 6G

Set No. 3

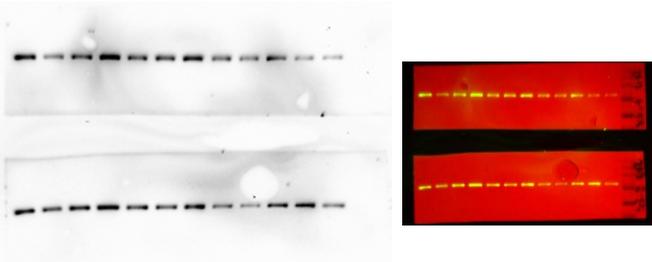
(All Groups per Gel)



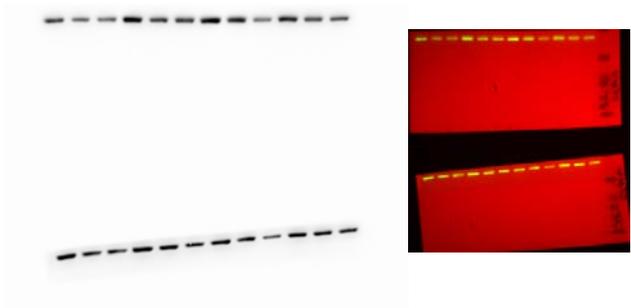
WT	TG	TG TUDCA
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Samples are organized in sets of three, from left to right, as indicated above.

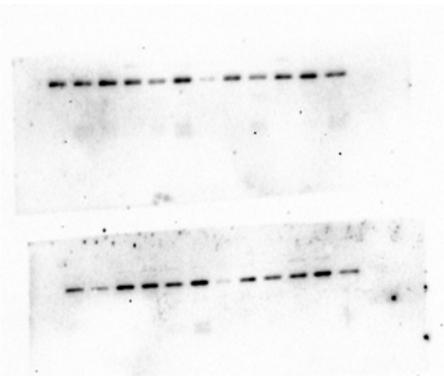
GR (Cytoplasmic)



Actin (Cytoplasmic)



GR (Nuclear)



H3 (Nuclear)

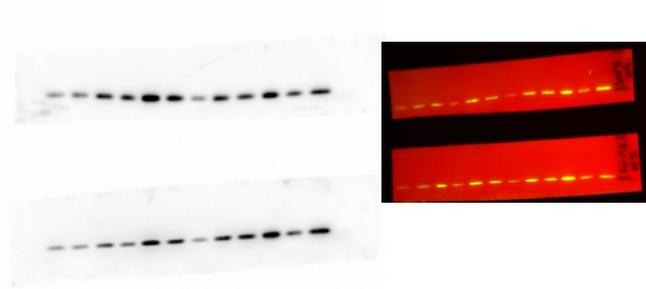
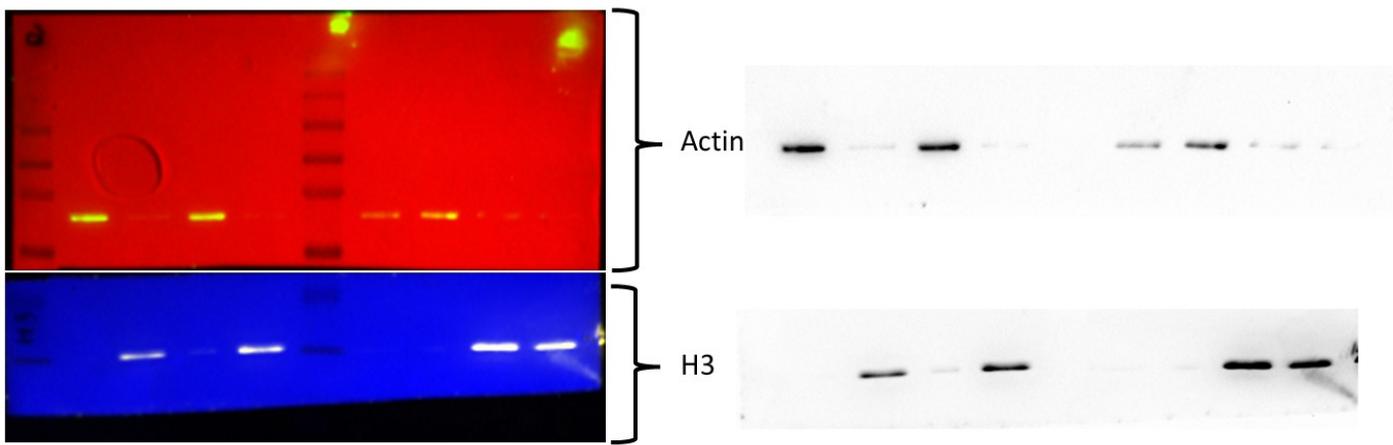


Figure 6G

Efficiency of the Cellular Fractionation Protocol

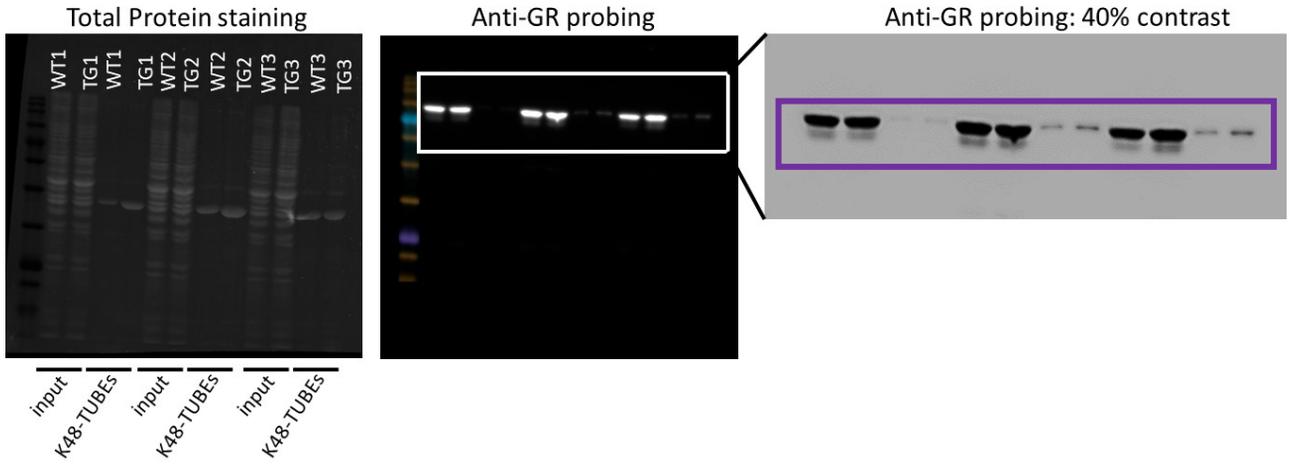
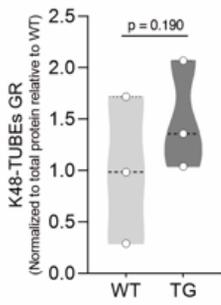
Immunoblotting of WT mouse brainstem after cellular fractionation of cytoplasm (showing the presence of actin and absence of H3) and nucleus (showing the presence of H3 and absence of actin)

Ladder, S3, SNF, S3, SNF, Ladder, S3, S3, SNF, SNF



S3 – cytoplasmic fraction
SNF – nuclear fraction

Figure 6H



Unbound fraction

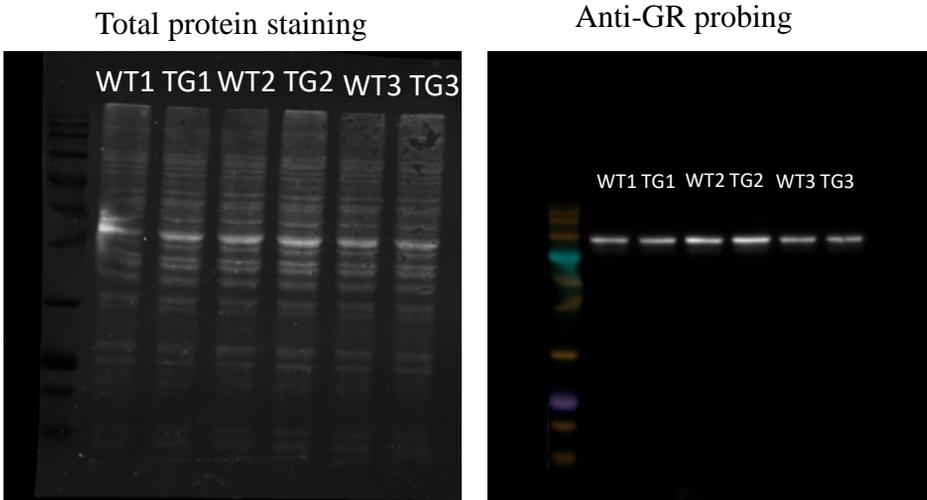
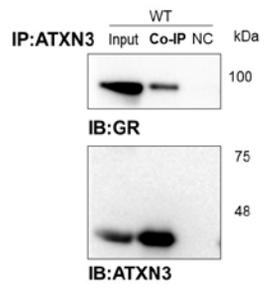


Figure 7A – co-IP



A 40% increase in image contrast was applied from the original blots

Co-immunoprecipitation of ATXN3; Immunoblot with GR

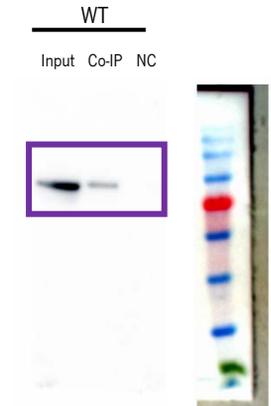
Replicate 1



Replicate 2

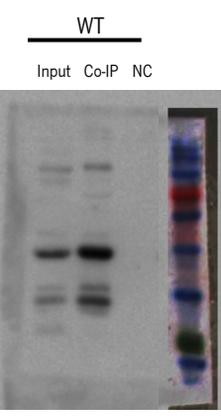


Replicate 3

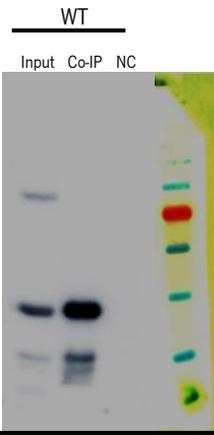


Co-immunoprecipitation of ATXN3 (rabbit anti-MJD1); Immunoblot with ATXN3 (mouse anti-ATXN3 1H9)

Replicate 1



Replicate 2

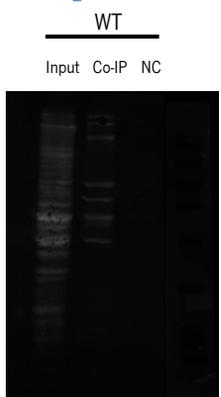


Replicate 3

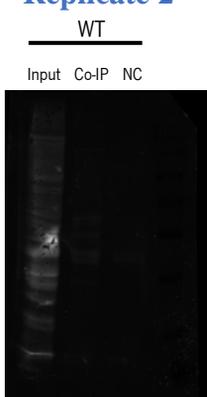


Total protein staining

Replicate 1



Replicate 2



Replicate 3

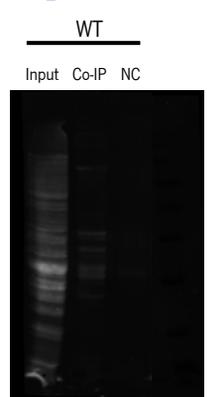
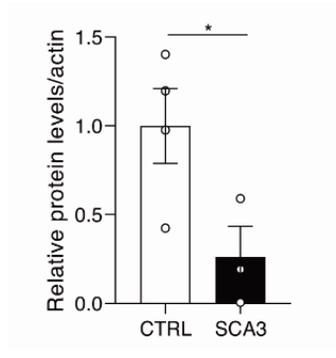
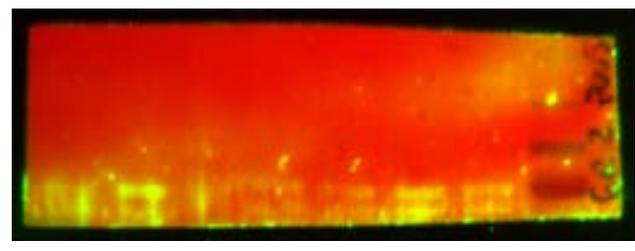
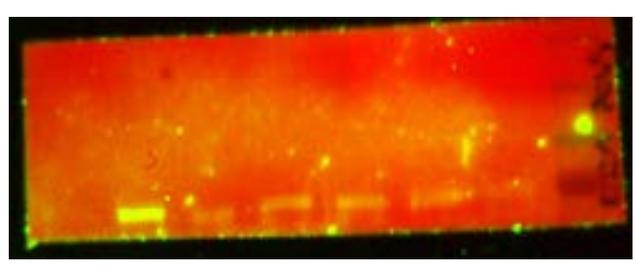
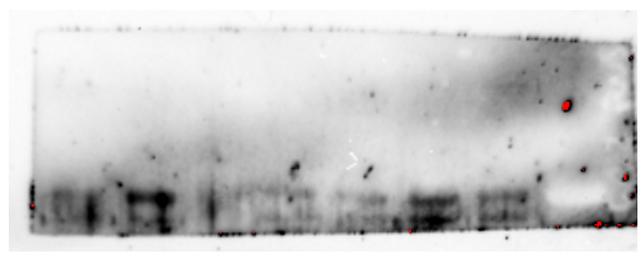
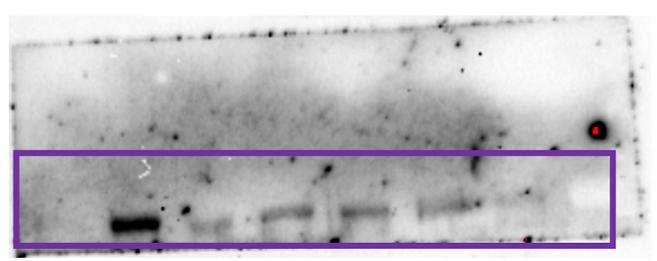


Figure 8A



SCA3 CTRL SCA3 CTRL SCA3 CTRL CTRL

GR



Actin

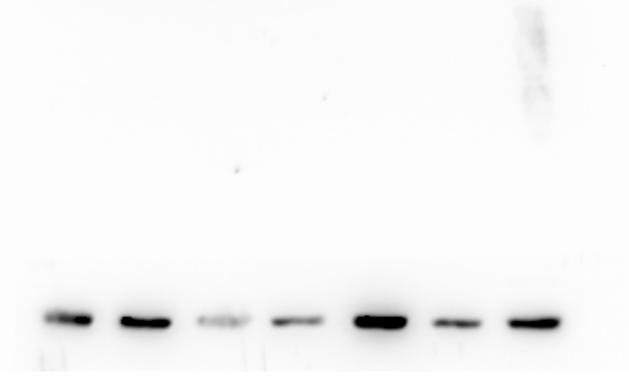
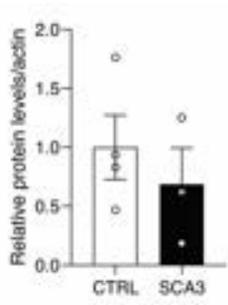
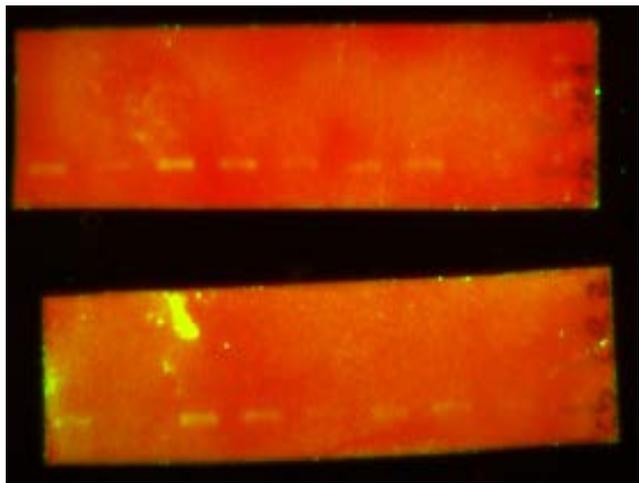
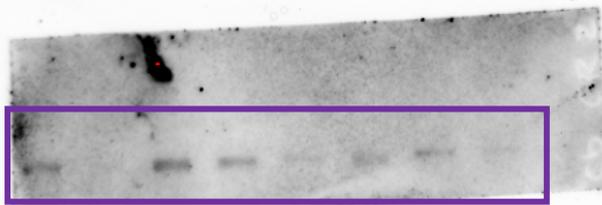
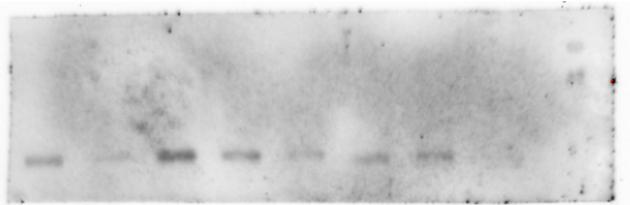


Figure 8B



CTRL SCA3 CTRL SCA3 CTRL SCA3 CTRL CTRL

GR



Actin

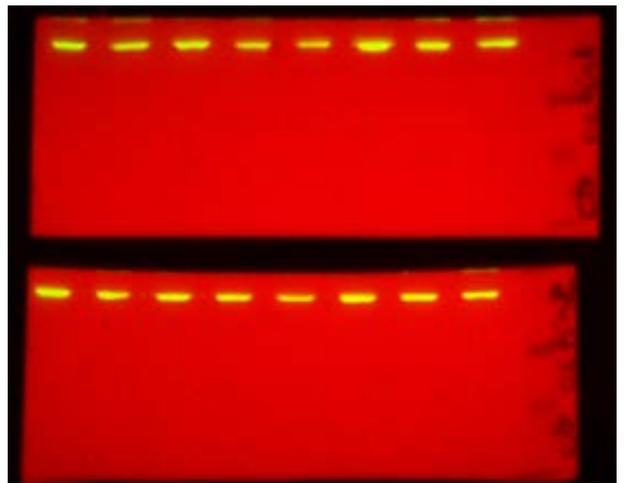
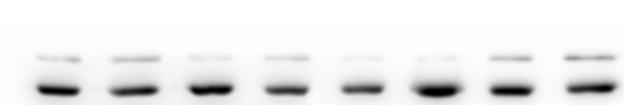
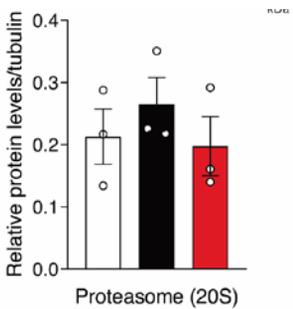


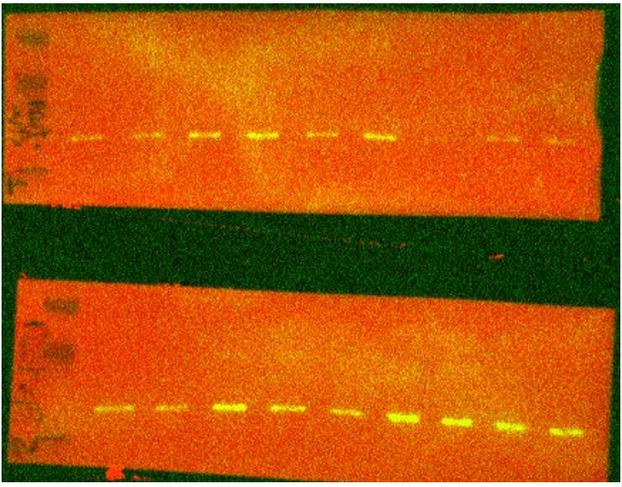
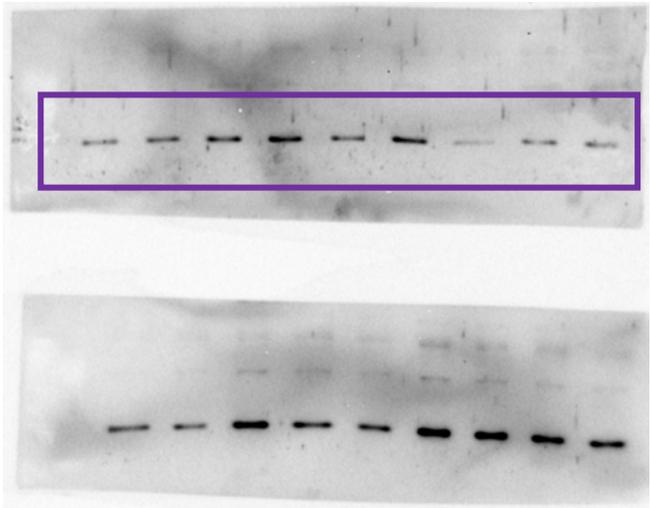
Figure S6E

WT	TG	TG TUDCA
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Samples are organized in sets of three, from left to right, as indicated above.



Proteasome (20S)



Tubulin

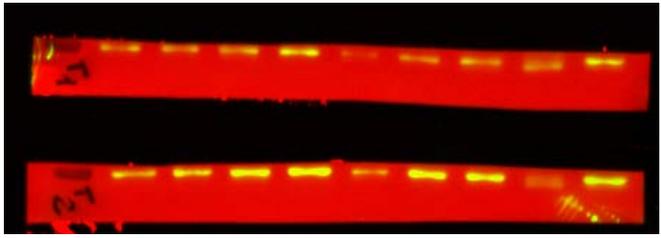
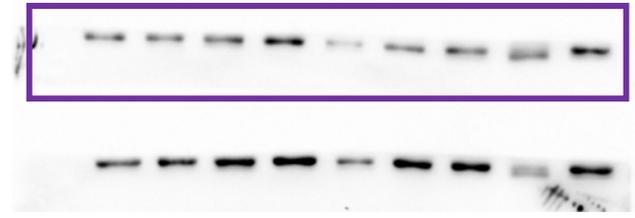
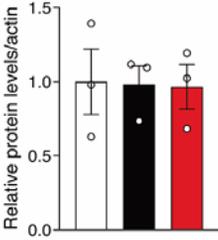


Figure S7B

WT	TG	TG TUDCA
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Samples are organized in sets of three, from left to right, as indicated above.



IBA-1

Actin

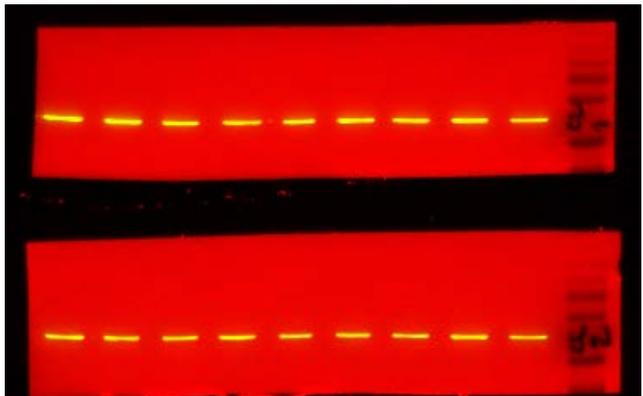
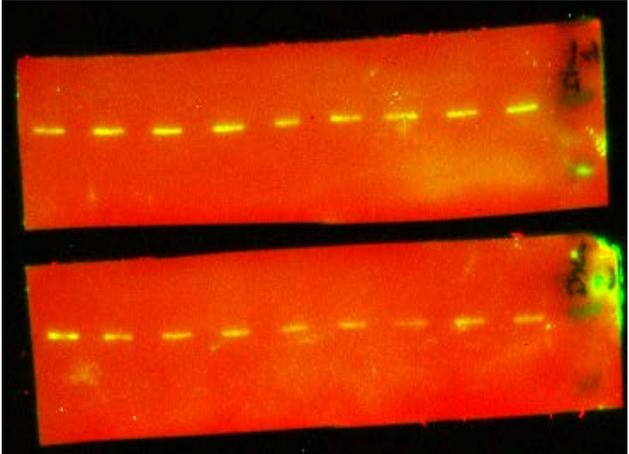
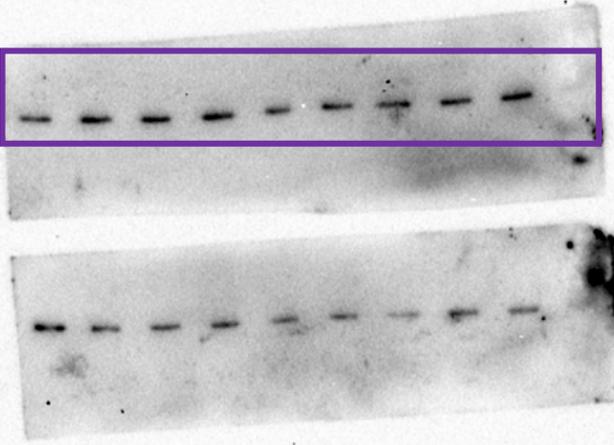


Figure S7C

WT TG TG TUDCA

Samples are organized in sets of three, from left to right, as indicated above.

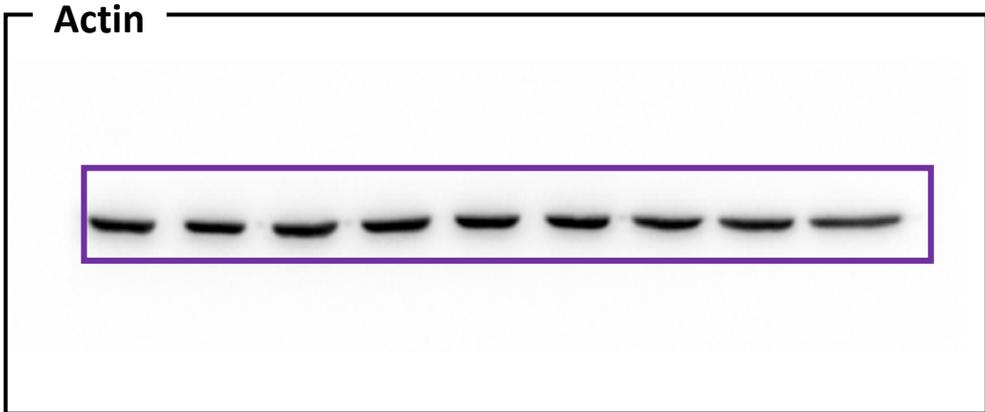
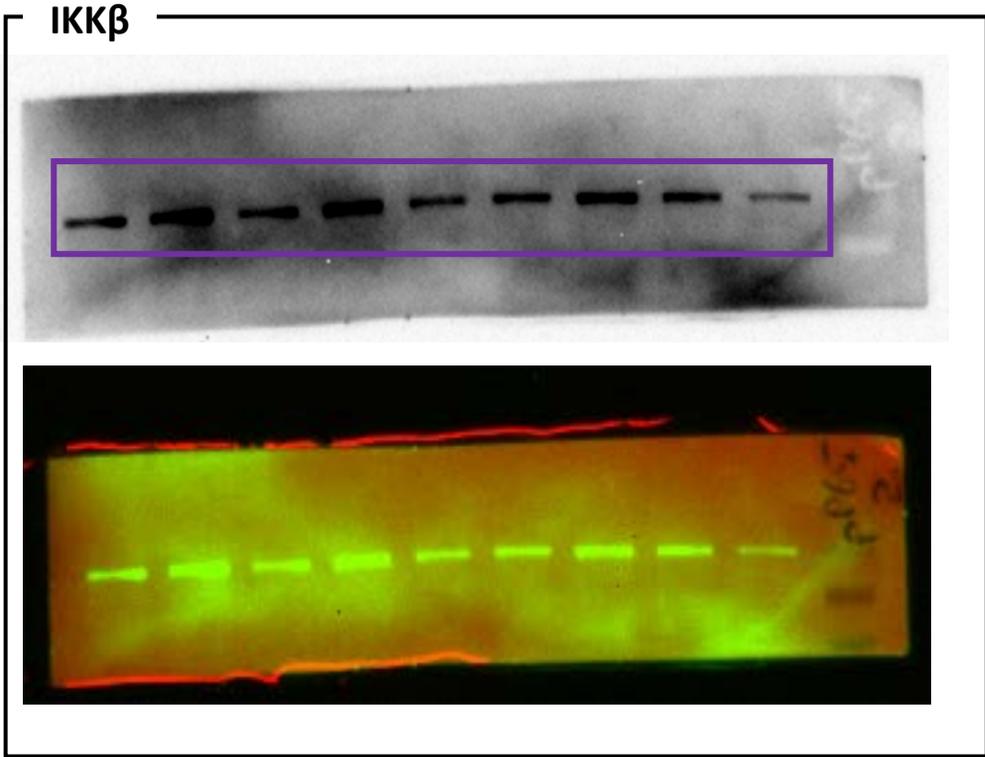
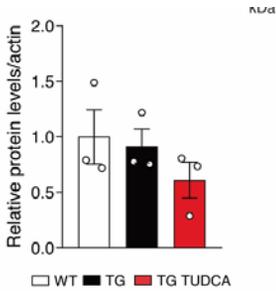


Figure S7D

WT	TG	TG TUDCA
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Samples are organized in sets of three, from left to right, as indicated above.

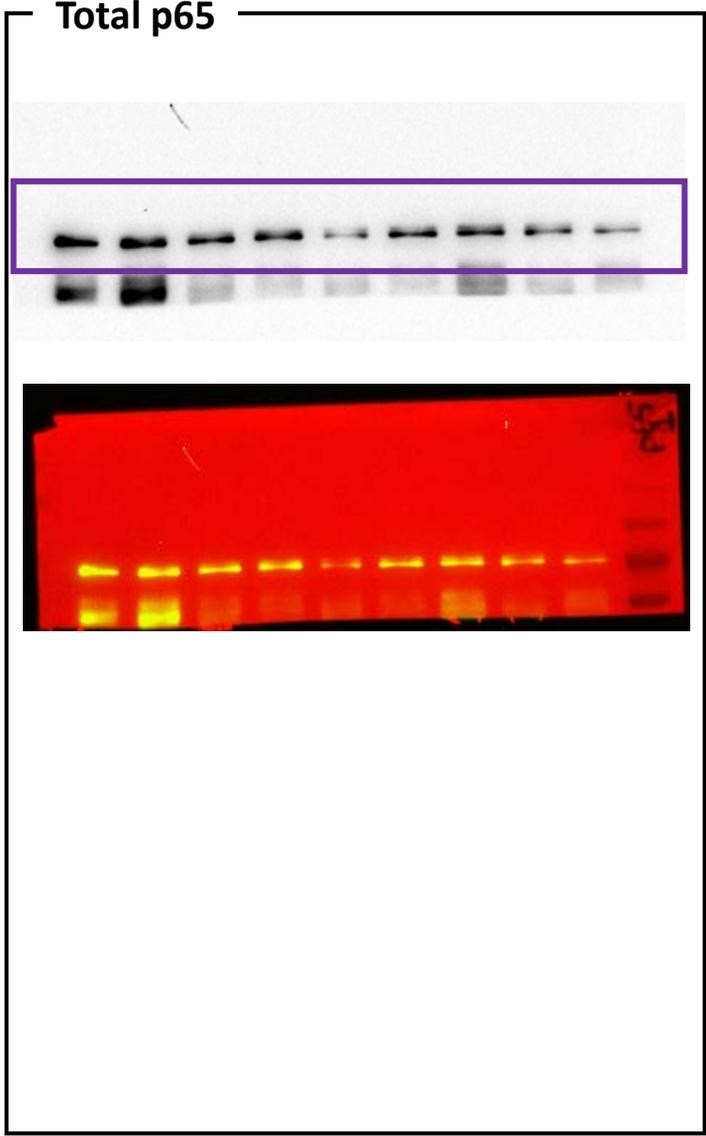
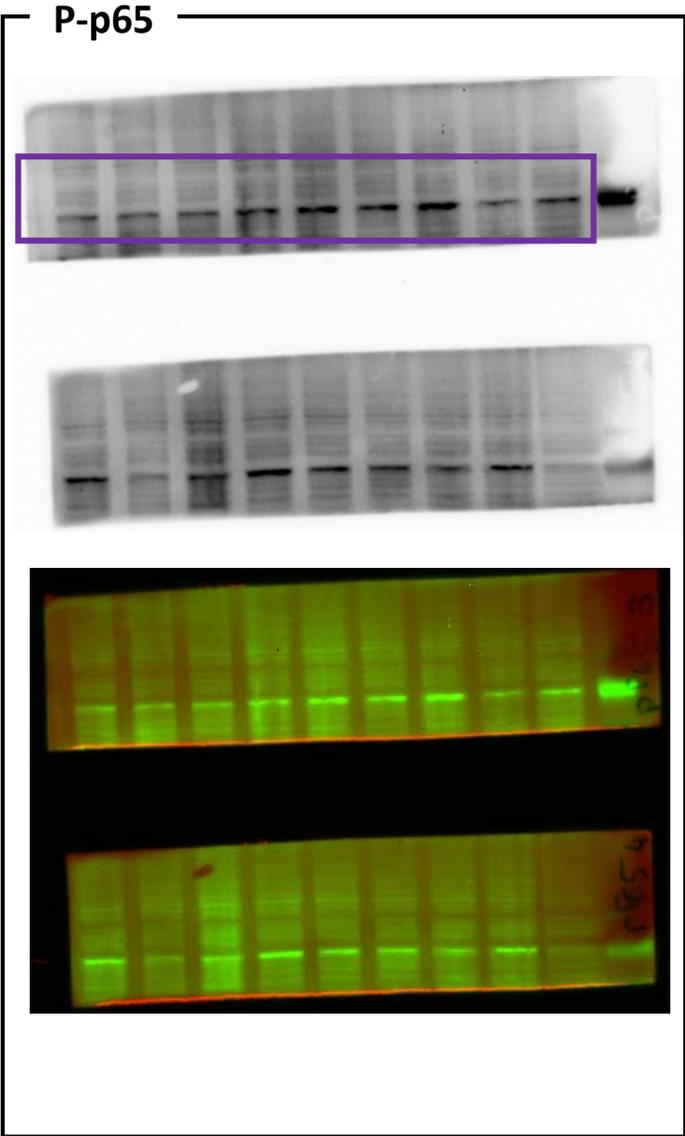
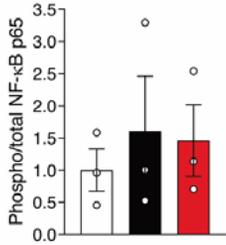


Figure S7E

WT	TG	TG TUDCA
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Samples are organized in sets of three, from left to right, as indicated above.

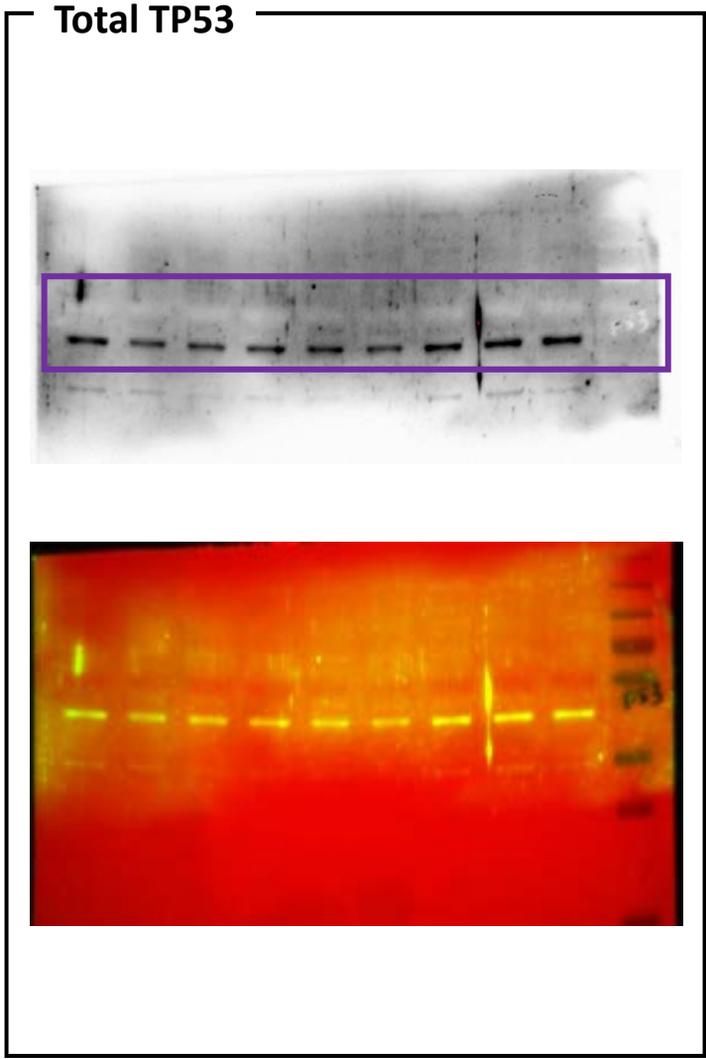
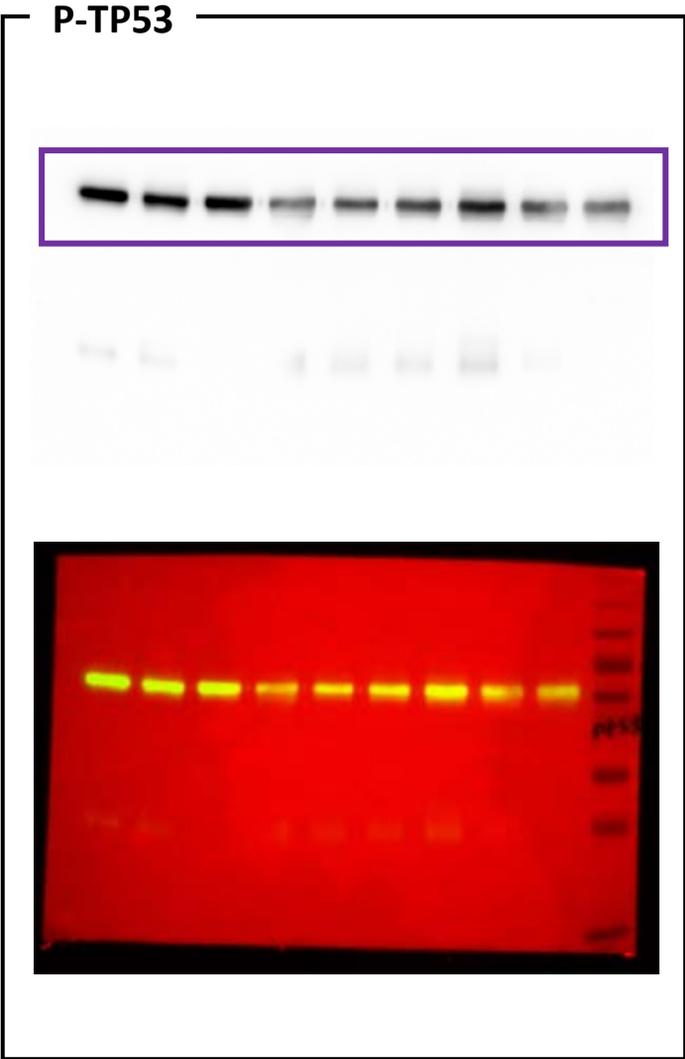
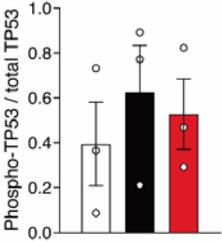
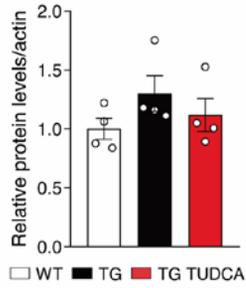


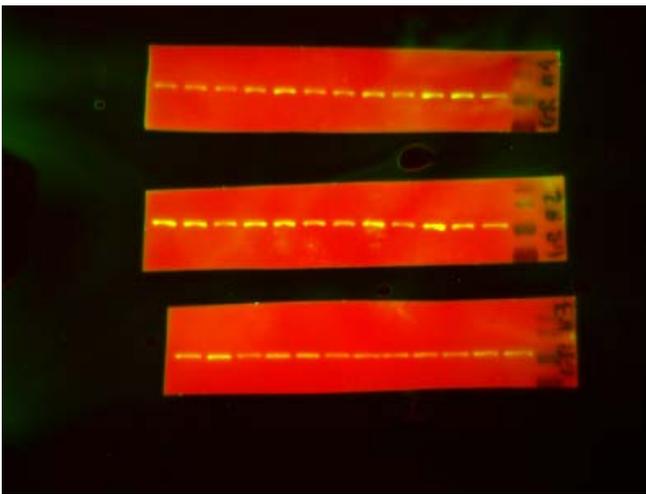
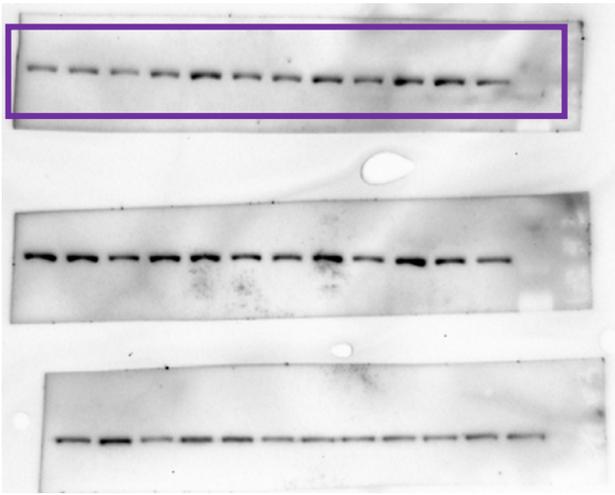
Figure S9B



WT	TG	TG TUDCA
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Samples are organized in sets of three, from left to right, as indicated above.

GR



Actin

