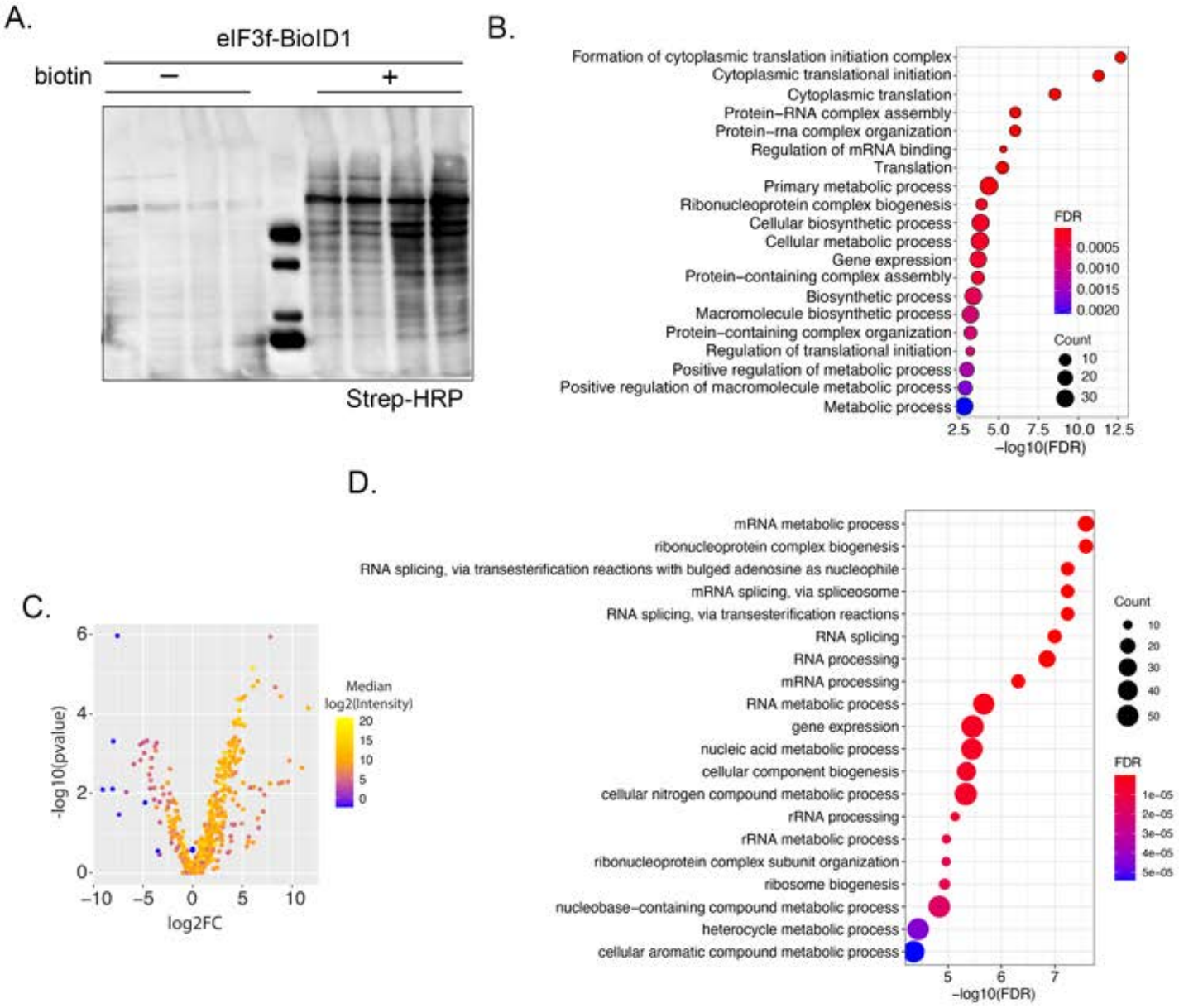
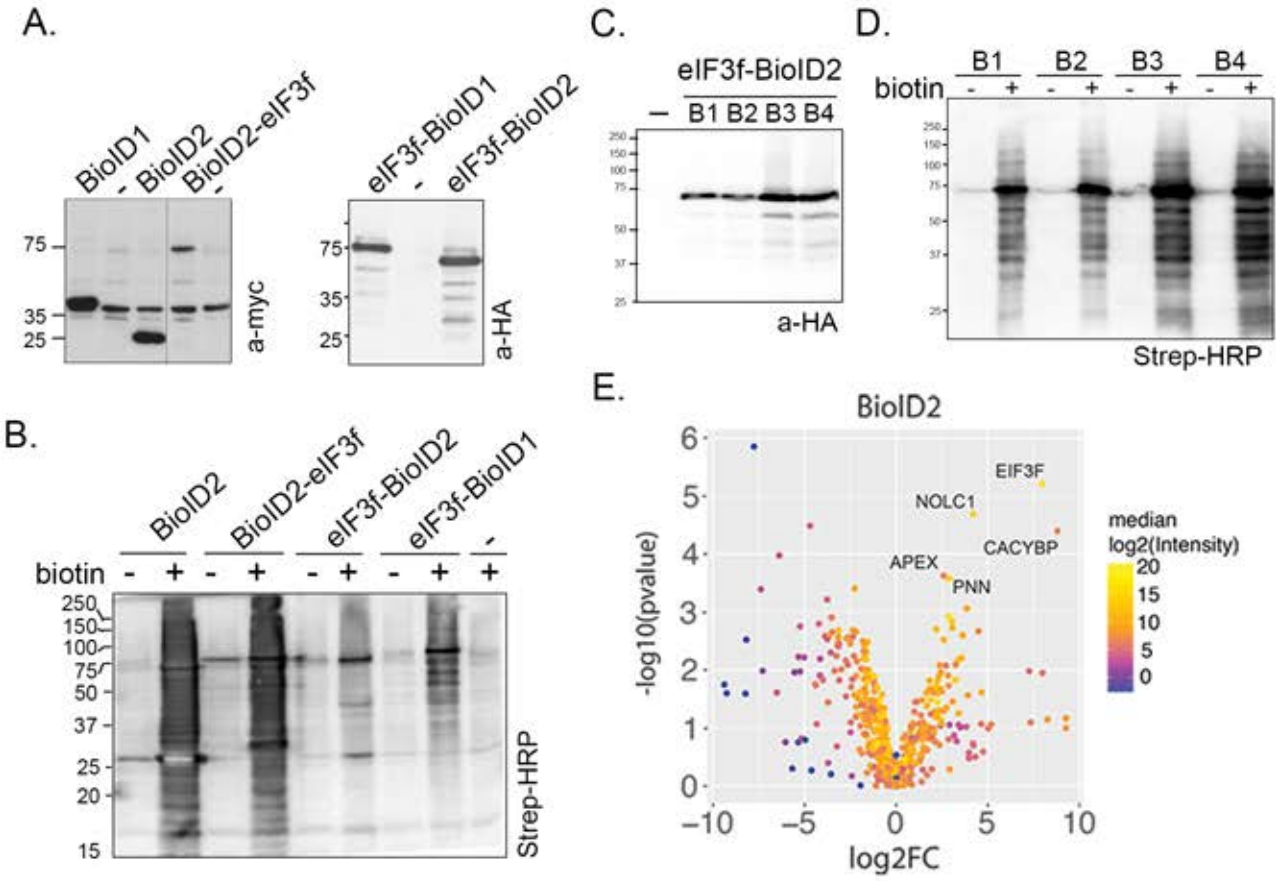
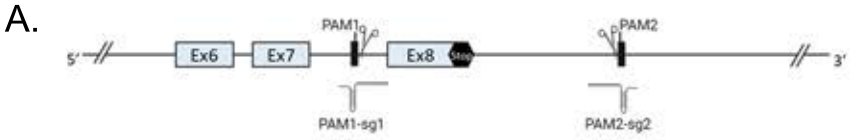


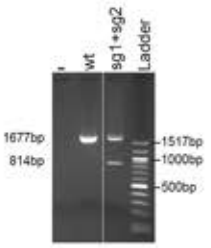
Supplementary Figure 1: Transient BioID1 biotinylation in HEK293 cells.
A. Streptavidin coupled HRP (abcam ≠ab7403) detection of biotin incorporation in lysates from HEK293 of overexpressing eIF3f-BioID1 and cultured 24hrs in presence or not (+/-) of 50µM biotin. The same lysates were further used for MS/LC coupled Streptavidin-dynabeads pull down experiment. Original blots are presented in Supplementary Figure 15. B. Functional annotation of proteins showing a significant enrichment after pulldown from HEK293 cell lysate expressing or not eIF3f_BioID1 in p resence of biotin. Only the top 20 pathways (based on FDR) are shown. C. Volcano plot distribution of the protein identified by MS/LC uponstreptavidin-dynabeads purification of biotinylated protein upon overexpression of BioID1 only proteinin HEK293 cells. D. Functional annotation of proteins showing a significant enrichment with BioID1 only protein. Only the top 20 pathways (based on FDR) are shown.

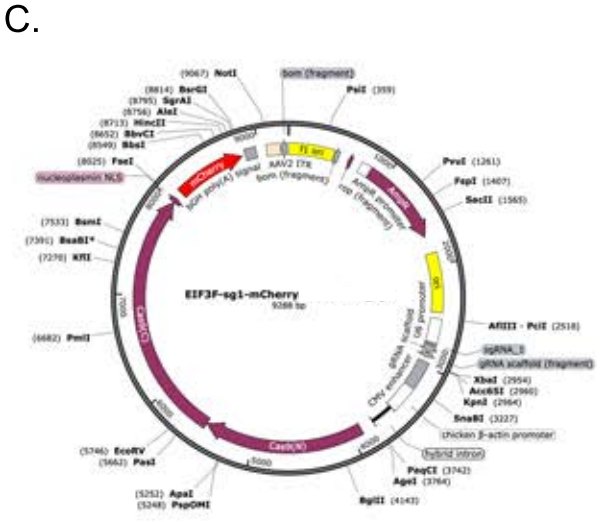


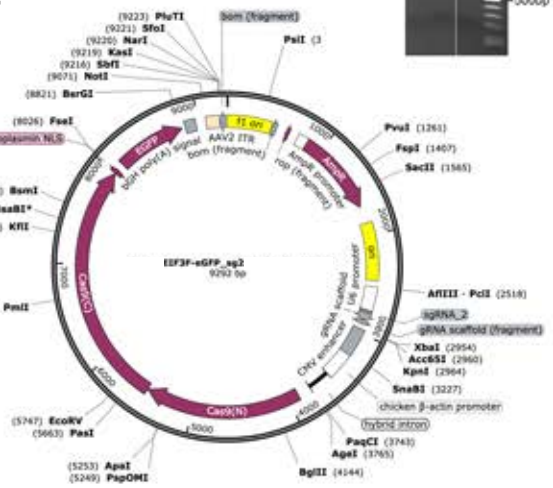
Supplementary Figure 2: BioID1 versus BioID2 C-terminal fusion chimera of eIF3f.

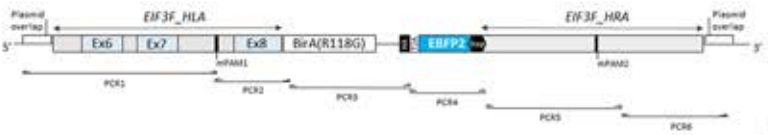
A.Transient expression in HEK293 cells of BioID1 and BioID2 fusion to the eIF3f protein (at C-ter or N-ter of eIF3f). Lysates obtained from triplicate transfections were separated on SDS gel and exogenously expressed protein detected with indicated antibody. Original blots are presented in Supplementary Figure 16. B. Cellular distribution of the different eIF3f-BioID1 ligase constructs expressed in A. C2C12 cells were transfected with the indicated constructs. N-terminally or C-terminally fused yellow fluorescent protein (YFP) to eIF3f protein was used as control. Original blots are presented in Supplementary Figure 17. C. Western blot detection of exogenously expressed eIF3f-BioID2 (B1 to B4) from HEK293 cell lysate used for MS/MS coupled Streptavidin-dynabeads pull down. Original blots are presented in Supplementary Figure 18. D. Streptavidin coupled HRP detection of biotin incorporation in lysates from HEK293 overexpressing BioID2 and cultured 24hrs in presence or not (+/-) of 50µM biotin. The same lysates were further used in E. We used the same protocol as previously described in figure 1. Original blots are presented in Supplementary Figure 19. E. Volcano plot distribution of the proteins identified by LC/MS upon Streptavidin-dynabeads purification of biotinylated proteins from transfected HEK cells (supplementary figure 1A) as described in A.

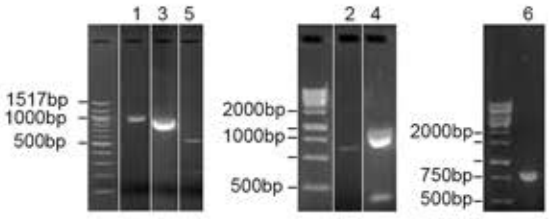














H.

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HA Tag

t2a

EBFP

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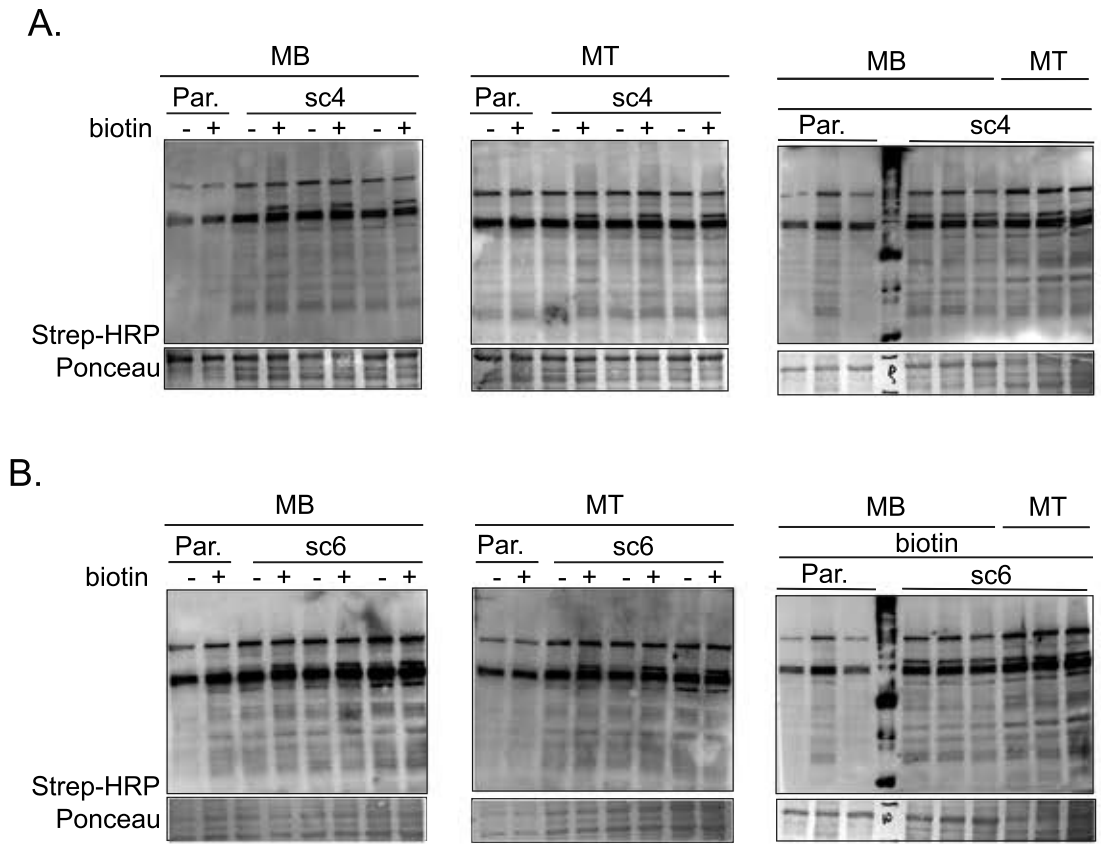
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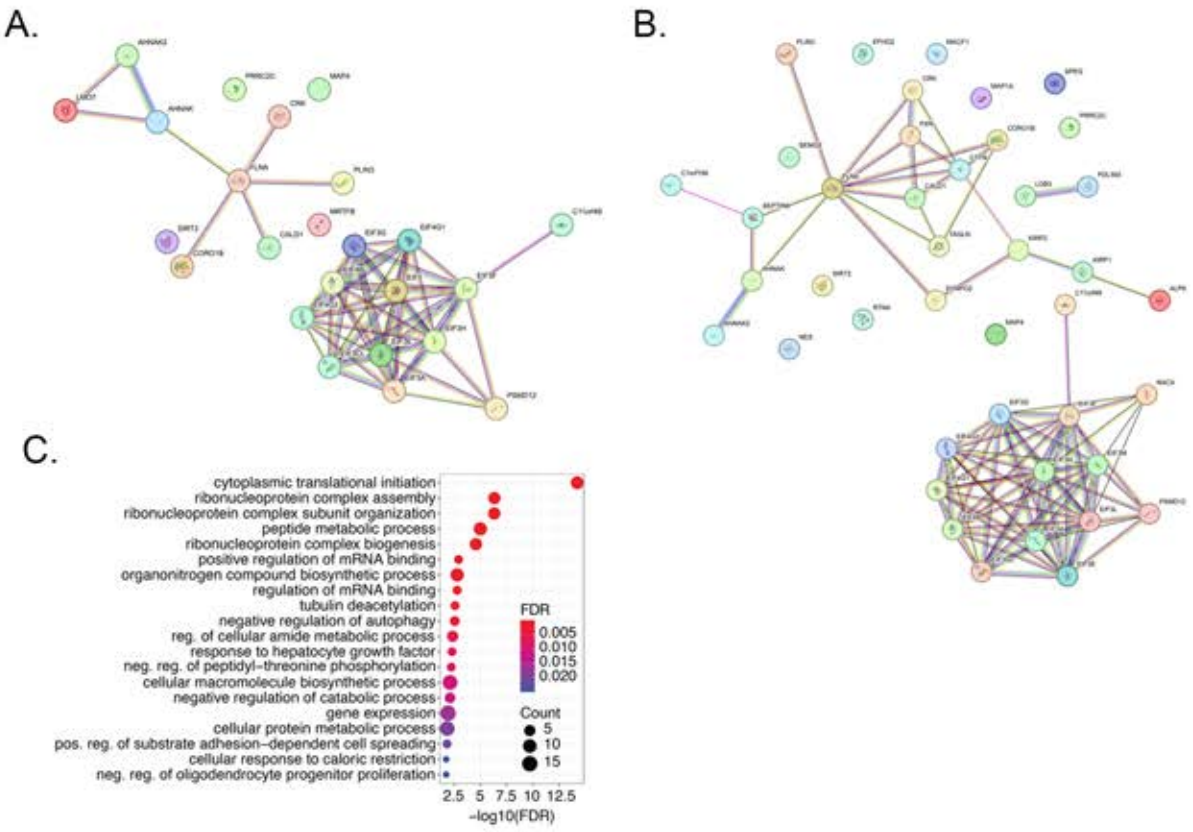
Supplementary Figure 3: Cloning strategy and sequencing of EIF3F-BioID1 chimera.

A. Schematic representation of EIF3F gene 3'end displaying the recombination sites for PAM1-sg1 and PAM2-sg2 on both 5' and 3' ends of Exon8. B. Resolution on agarose gel of the fragment cleavage from sg1 to sg2 sites (814 BP). C. Annotation map of the EIF3f-sg1-mCherry plasmid (created with SnapGene). D. Annotation map of the EIF3f-eGFP_sg2 plasmid (created with SnapGene). E. Schematic representation of the donor DNA displaing right (RHA) and left homology arms (LHA) flanking the exon8-BioID1 and HA tagged EBFP inserts F. The Gibson cloning strategy was used to assemble the 6 different PCR amplicons of donor DNA in pUC.19 plasmid backbone. The amplified fragments corresponding to PCR1 to 6 were resolved on agarose gel before purification and assembly. G. Annotation map of the Donor DNA plasmid (created with SnapGene). H. Sequencing of the integrated construct. mPAM: mutated protospacer-adjacent motif; sg: single guide RNA; EBFP2: enhanced blue fluorescent protein; T2A: self-cleaving 2A peptide.



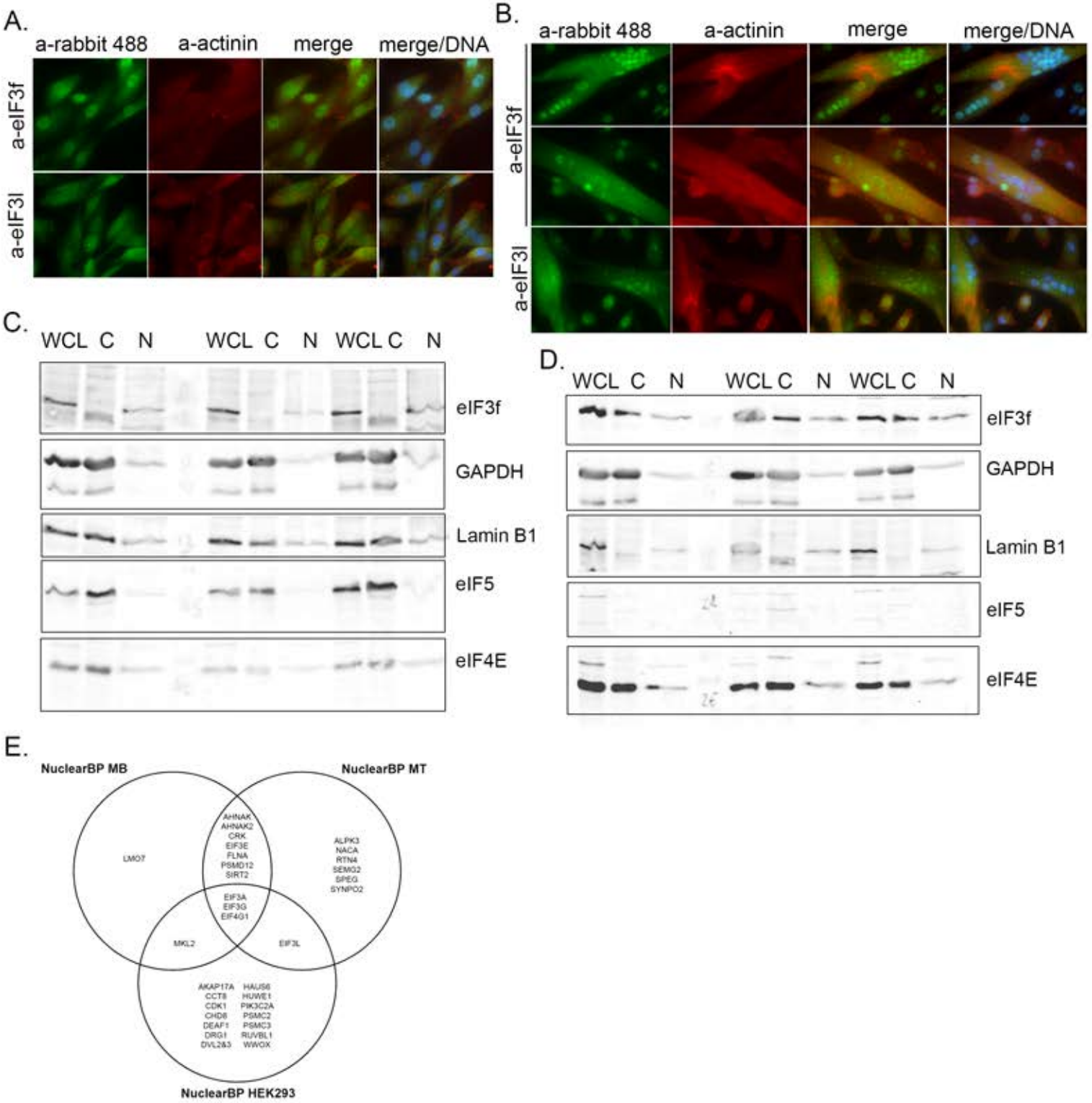
Supplementary Figure 4: Biotin incorporation in samples used for Streptavidin-dynabeads purification of eIF3f interactors.

A. Streptavidin coupled HRP detection of biotin incorporation form parental (Par.) and eIF3f-BioID1 knock-in sc4 lysates of MB or MT treated for 24hrs with 50μM biotin. The same lysates were further used for LC/MS coupled Streptavidin-dynabeads pull down experiment. Original blots are presented in Supplementary Figure 20. B. The same procedure as describe in A was conducted for sc6 lysate. Original blots are presented in Supplementary Figure 21.



Supplementary Figure 5: Interaction map and functional enrichment of the eIF3f interactors identified in MB and MT.

A. Interaction map of LC/MS significantly identified ($p<0.01$ and $log2FC>0$) proteins from streptavidin-dynabeads pull-down experiment from MB (sc4/6) cultured in presence of biotin versus parental cells. B. Same as A obtained from MT (sc4/6) cells. C. Functional enrichment analysis ($p<0.01$ and $log2FC>0$) of the 21 conserved proteins identified as eIF3f binding partners in MB and MT (shown in figure 3F and 3G).



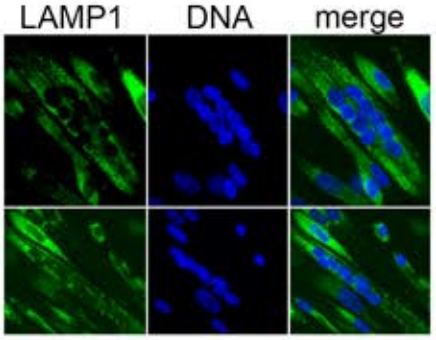
Supplementary Figure 6: Subcellular distribution of eIF3f protein and selected identified binding partners in MB and MT.

A. Immunodetection of eIF3 subunit F, and L (green) in parental myoblasts. Anti sarcomeric alpha-actinin-3 was visualised with Cy5 coupled anti mouse antibody and DNA was stained with Hoechst. B. The same immunodetection was conducted in myotubes. C. Whole cell lysate (WCL) or fractionated (C: cytoplasm and N: nuclear) lysates from parental MB were resolved on SDS/PAGE and probed with indicated antibodies. GAPDH and Lamin B1 were used respectively as cytoplasmic or nuclear markers. Signals from infrared (700nm) coupled secondary antibody were acquired with Odyssey (Li-Cor). Original blots are presented in Supplementary Figure 22. D. The same experiment as in C was conducted in sc4 MT. Original blots are presented in Supplementary Figure 23. E. Venn diagram of the nuclear binding partners (BP), according to https://www.genecards.org/nomenclature, identified for eIF3f-BioID1 chimaera in myoblasts, myotubes and HEK293 biotinylation experiments.

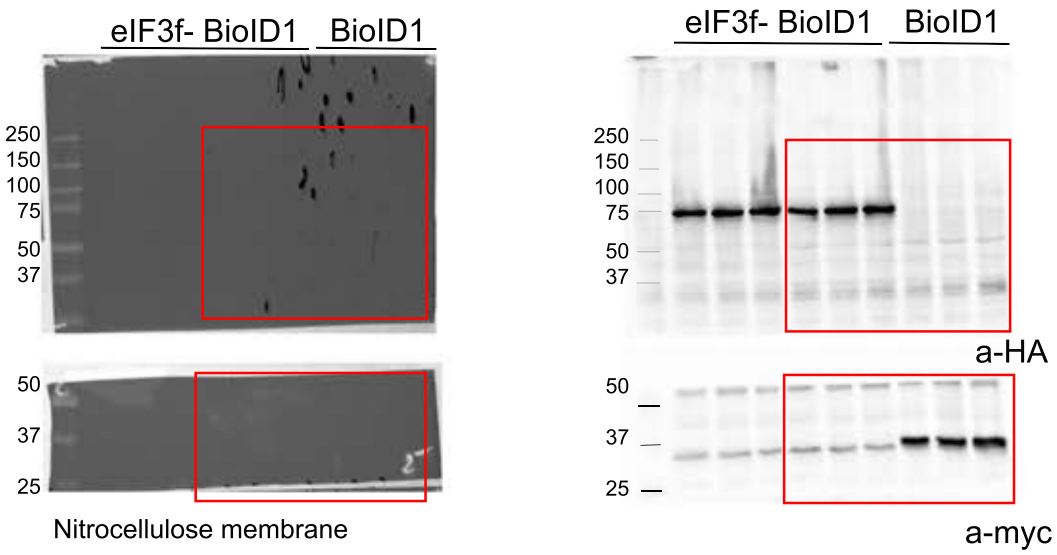
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sp|A4UGR9|XIRP2_HUMAN	5.2	4.84E-04
sp|P11055|MYH3_HUMAN	4.8	1.88E-03
sp|Q15772|SPEG_HUMAN	4.32	8.76E-04
sp|O00763|ACACB_HUMAN	3.92	8.34E-04
sp|Q702N8|XIRP1_HUMAN	3.86	6.95E-05
sp|P07686|HEXB_HUMAN	3.71	6.89E-04
sp|E9PAV3|NACAM_HUMAN	3.55	3.68E-04
sp|Q15050|RRS1_HUMAN	3.54	1.23E-03
sp|Q8WZ42|TITIN_HUMAN	3.54	7.88E-04
sp|Q9NQC3|RTN4_HUMAN	3.3	1.03E-03
sp|Q9NP64|NO40_HUMAN	3.2	3.39E-03
sp|Q9Y262|EIF3L_HUMAN	3.17	5.46E-04
sp|P13535|MYH8_HUMAN	2.98	2.88E-03
sp|P11182|ODB2_HUMAN	2.53	2.57E-03
sp|Q15361|TTF1_HUMAN	2.48	3.51E-03
sp|Q1ED39|KNOP1_HUMAN	2.17	2.87E-03
sp|P09651|ROA1_HUMAN	-1.73	3.55E-03
sp|Q9BR76|COR1B_HUMAN	-2.21	2.43E-03
sp|P16402|H13_HUMAN	-2.57	1.68E-03
sp|Q9H6J7|CK049_HUMAN	-2.69	8.59E-04
sp|Q5QNW6|H2B2F_HUMAN	-3.07	3.18E-03
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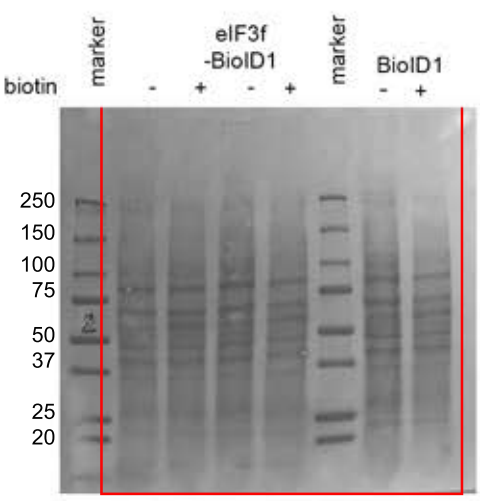
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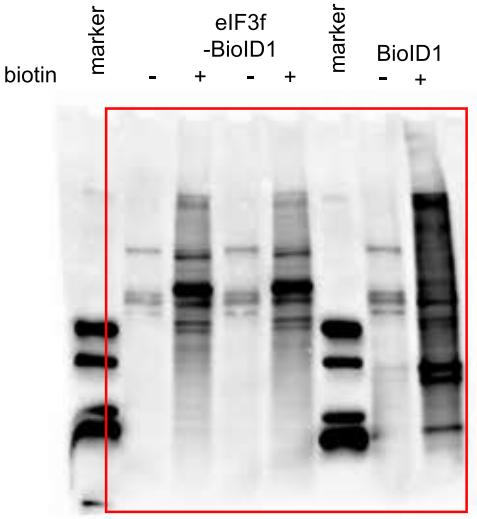
Supplementary Figure 7: "Core eIF3f-eIF3" interactors identified in MT and MB.
A. Table: list of interactors of the "core eIF3f-eIF3" identified in MT vs MB. Only proteins identified with high level of confidence are shown (min. med. int.>20; pVal<0.01). B. Immuno-detection of LAMP1 visualised with anti-rabbit Cy3 secondary antibody in parental differentiated (day 6) myotubes. DNA was stained with Hoechst.



Supplementary Figure 8
Uncropped western blot for Figure 1B.

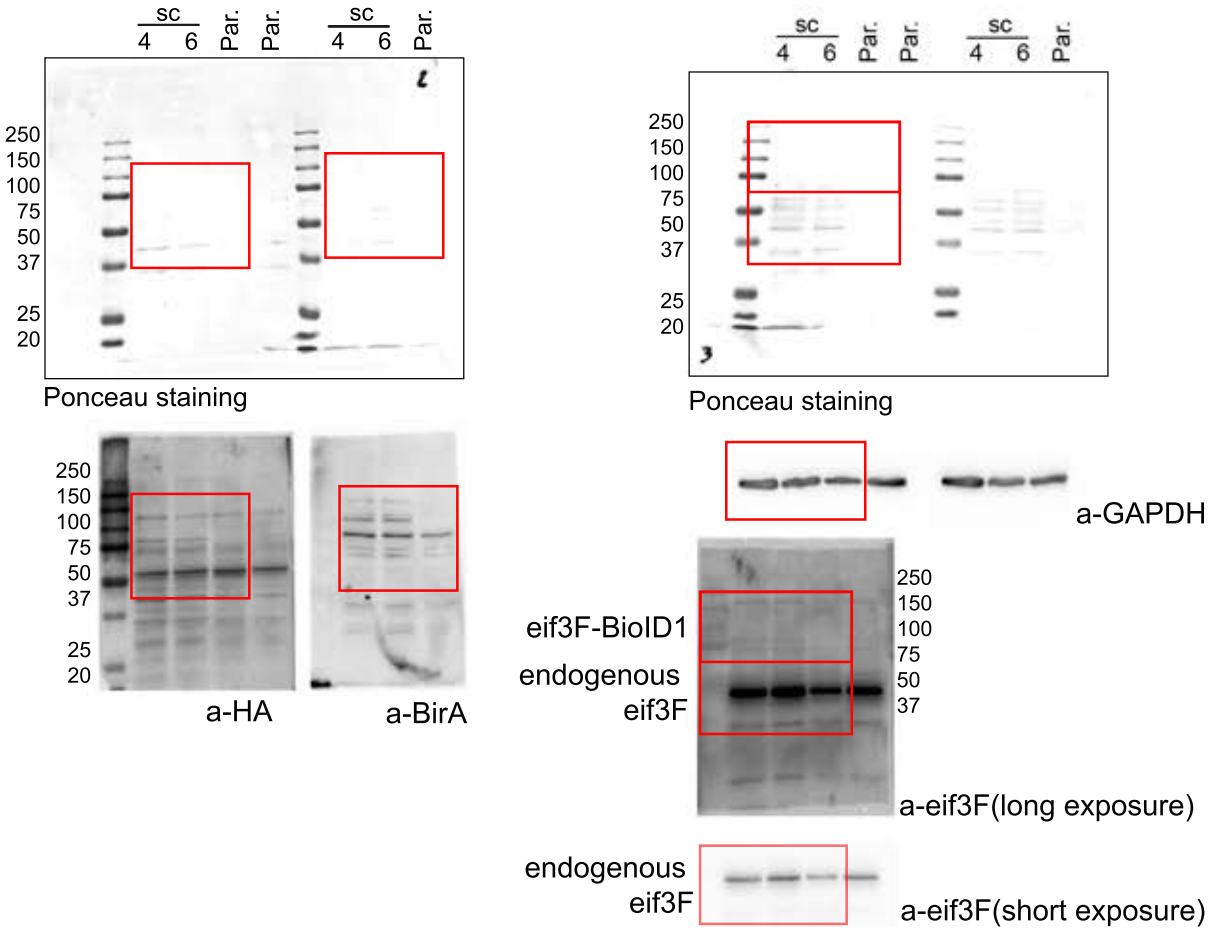


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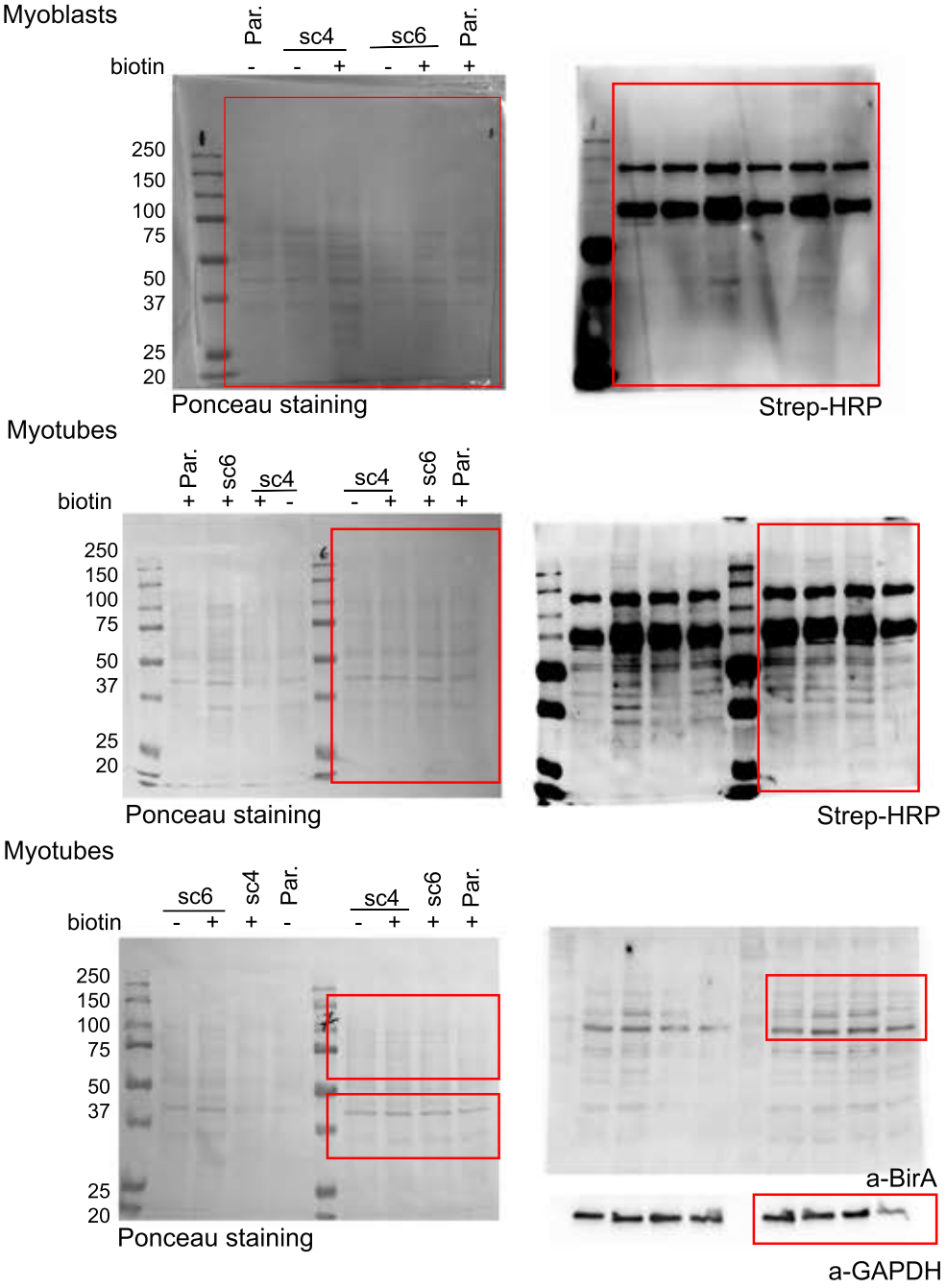


Strep-HRP

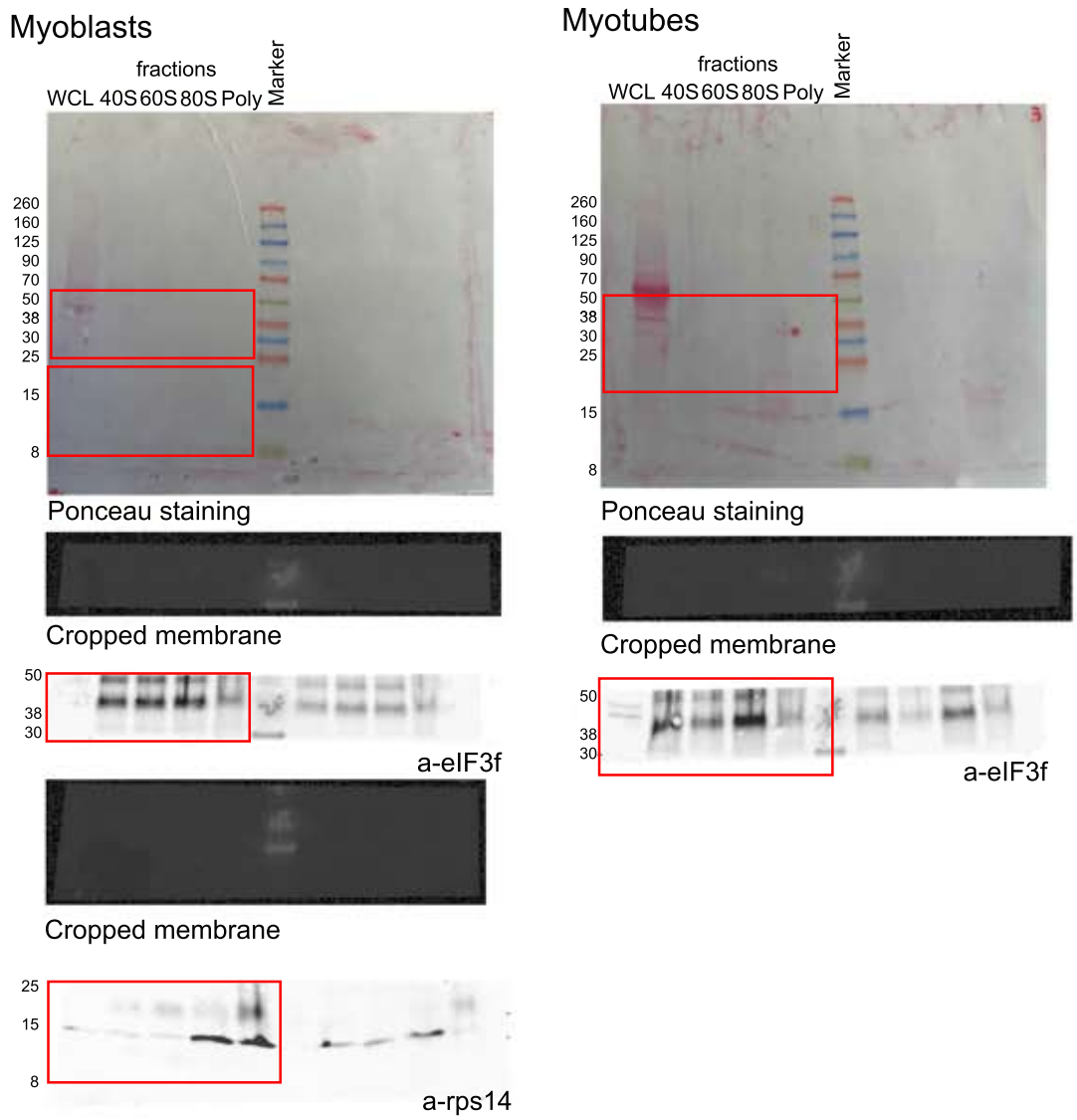
Supplementary Figure 9
Uncropped western blot for Figure 1C



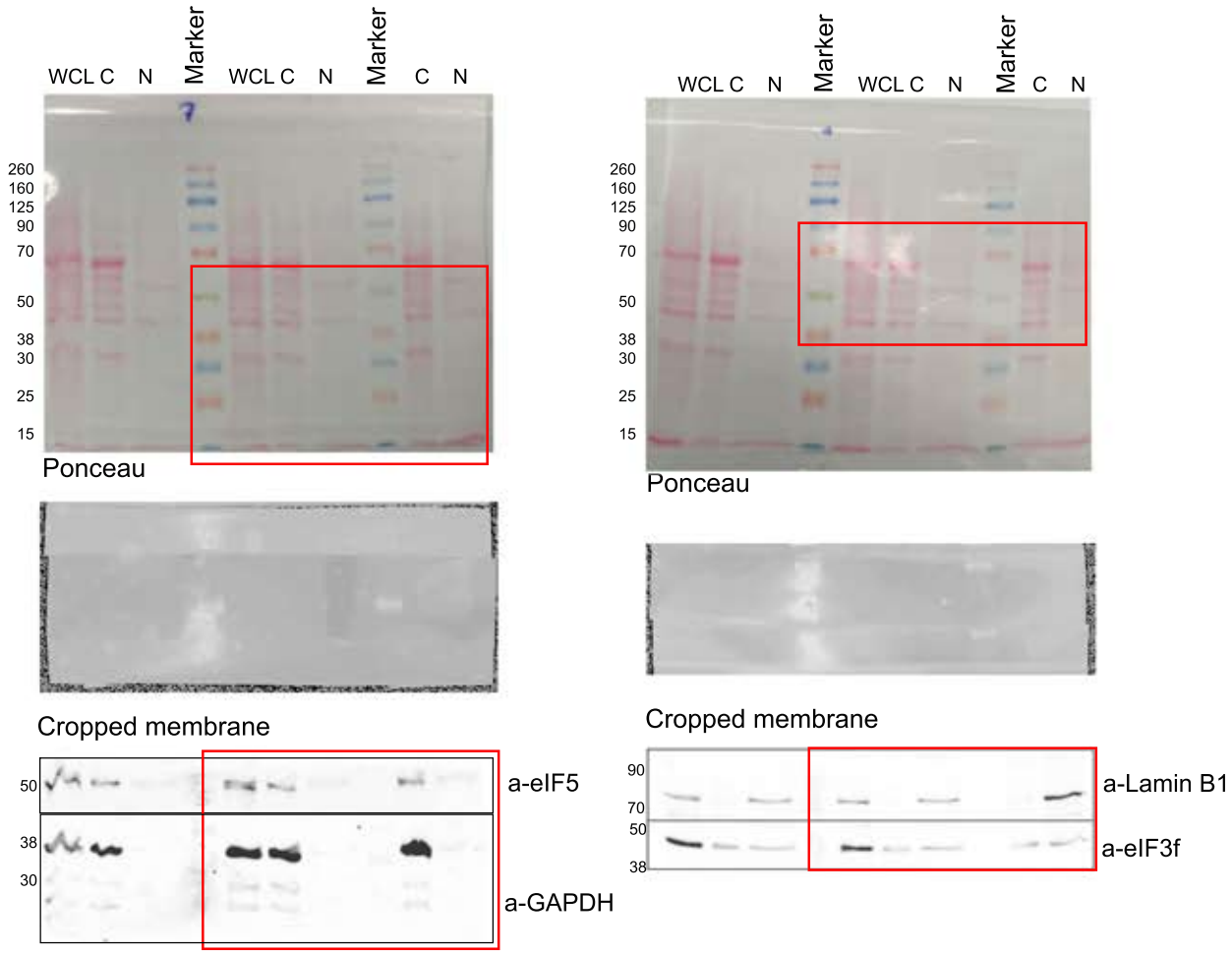
Supplementary Figure 10
Uncropped western blot for Figure 2D



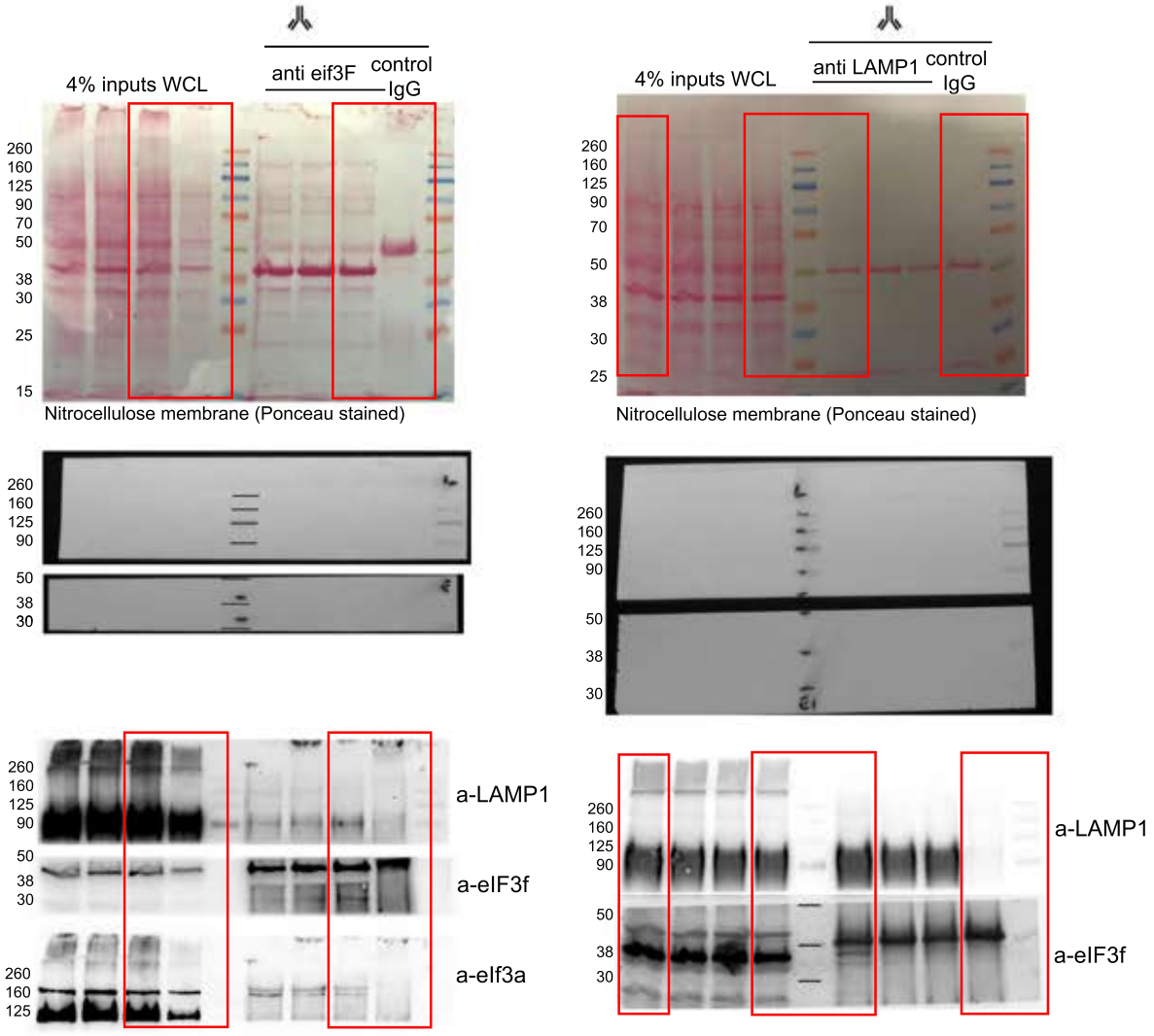
Supplementary Figure 11
Uncropped western blot for Figure 3A



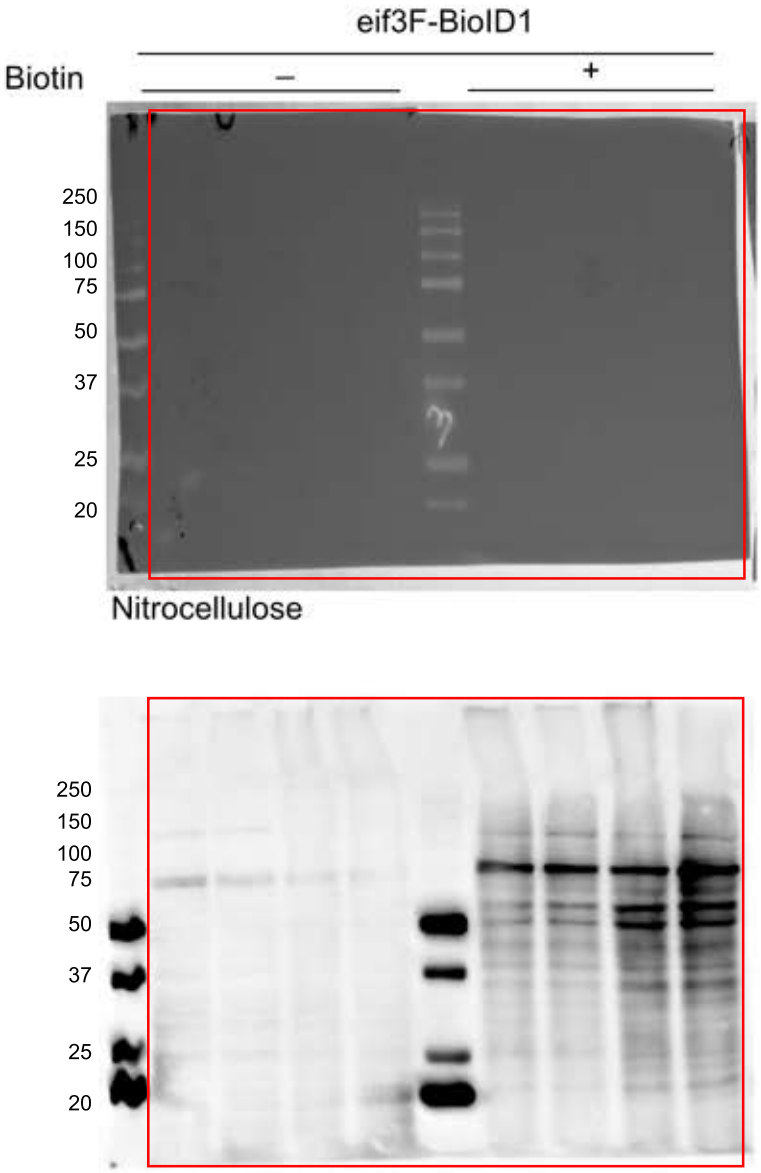
Supplementary Figure 12
Uncropped western blot for Figure 4B



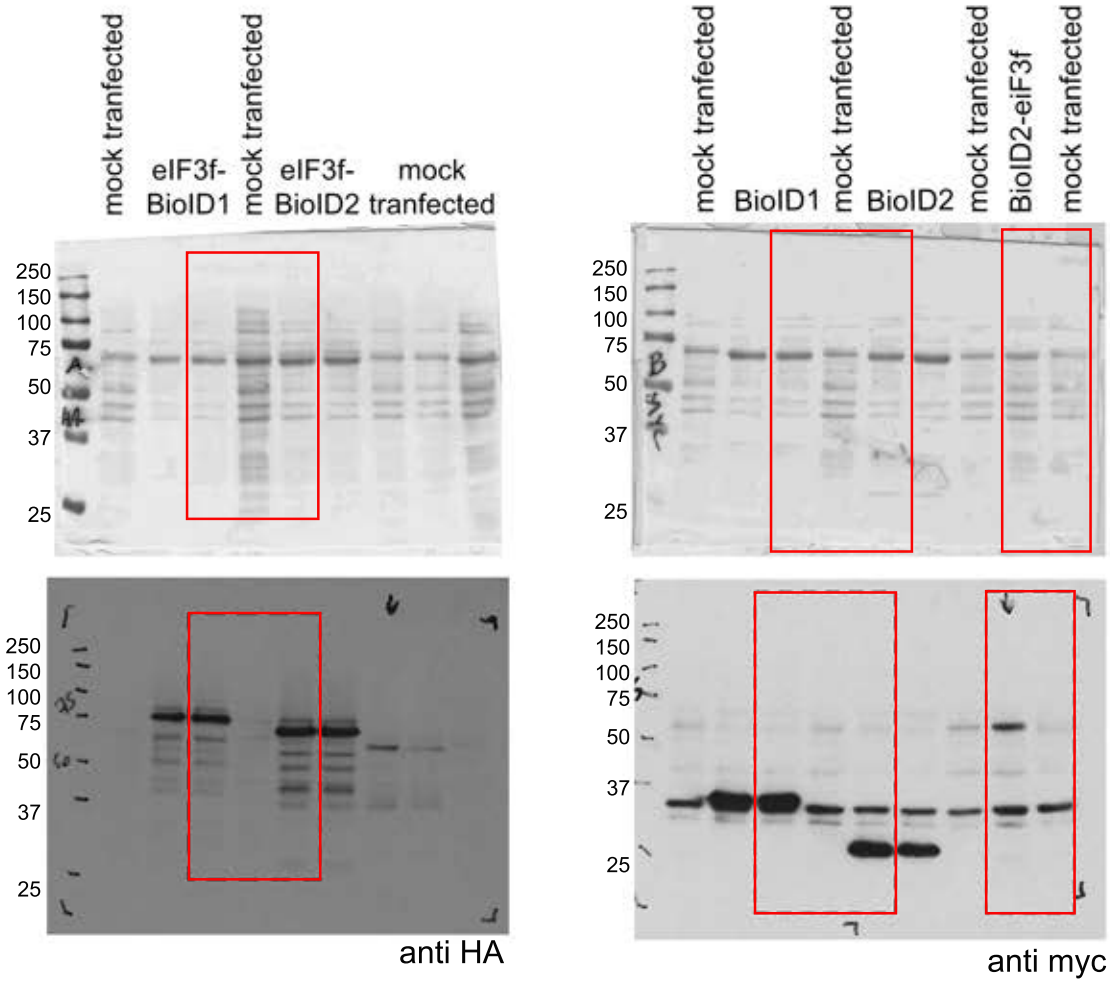
Supplementary Figure 13
Uncropped western blot for Figure 4D



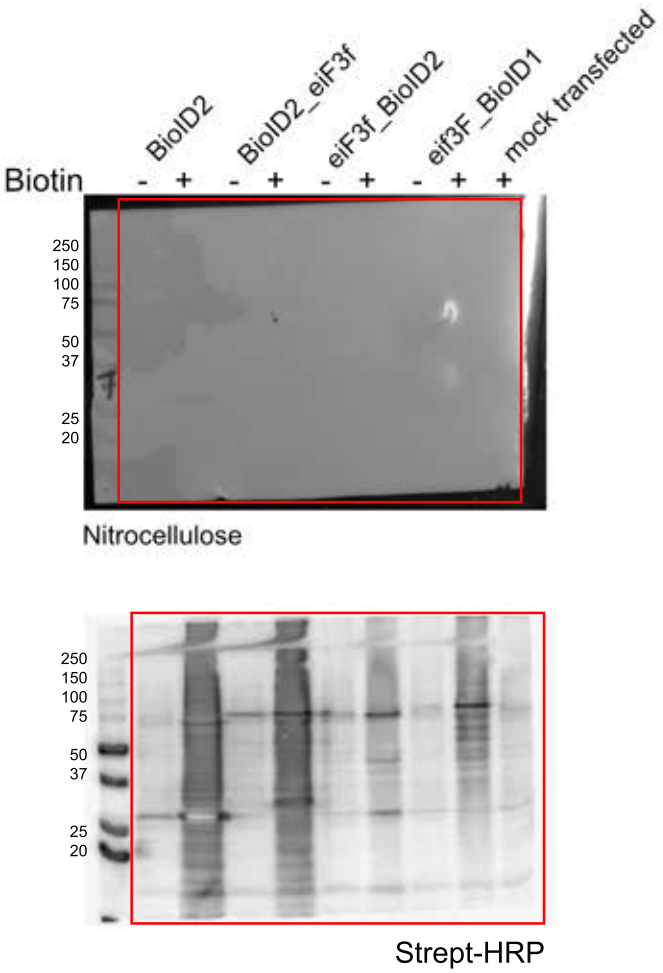
Supplementary Figure 14
Uncropped western blot for Figure 5B



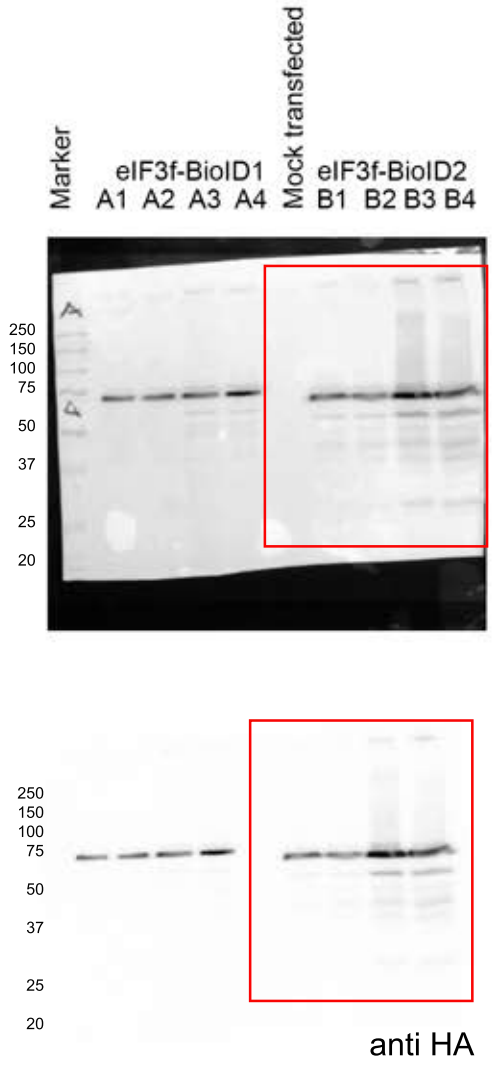
Supplementary Figure 15
Uncropped western blot for Supplementary Figure 1A



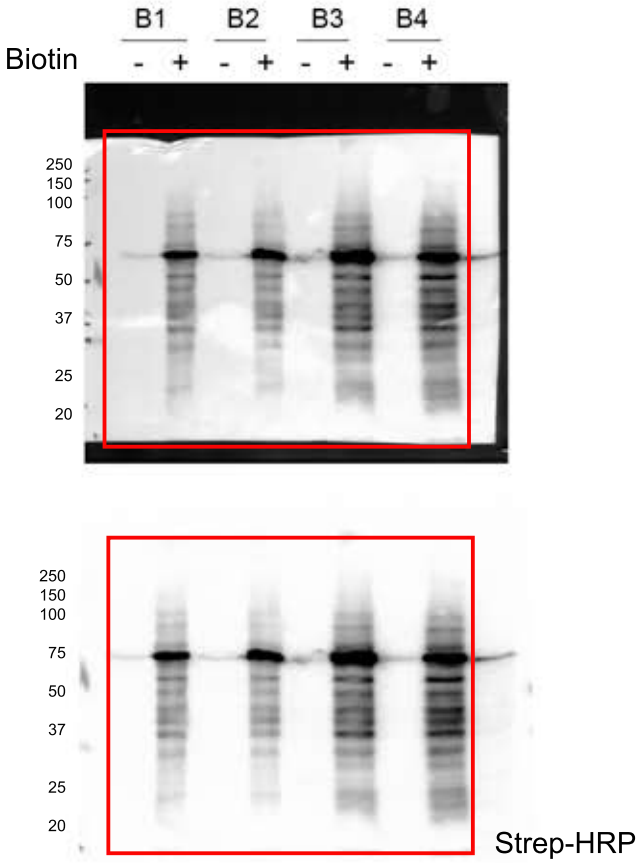
Supplementary Figure 16
Uncropped western blot for Supplementary Figure 2A



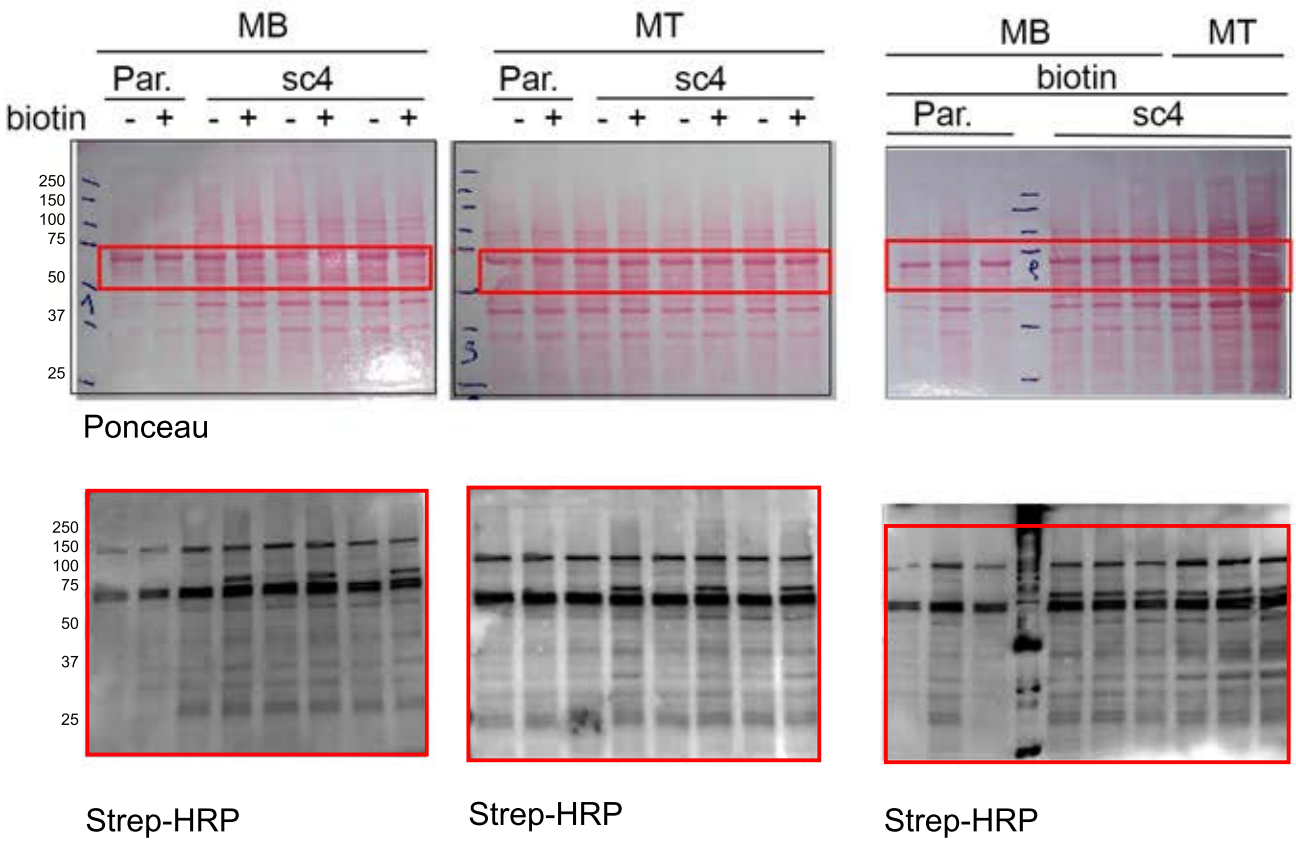
Supplementary Figure 17
Uncropped western blot for Supplementary Figure 2B



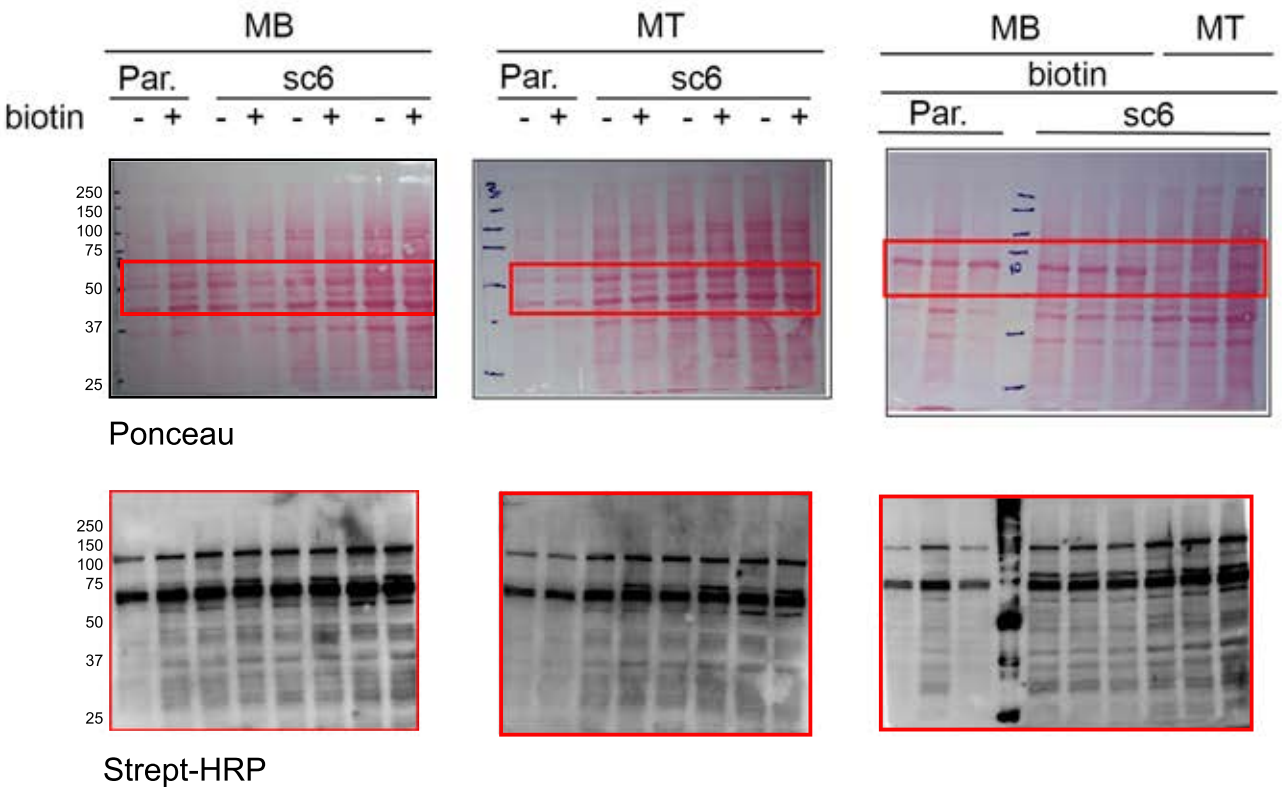
Supplementary Figure 18
Uncropped western blot for Supplementary Figure 2C



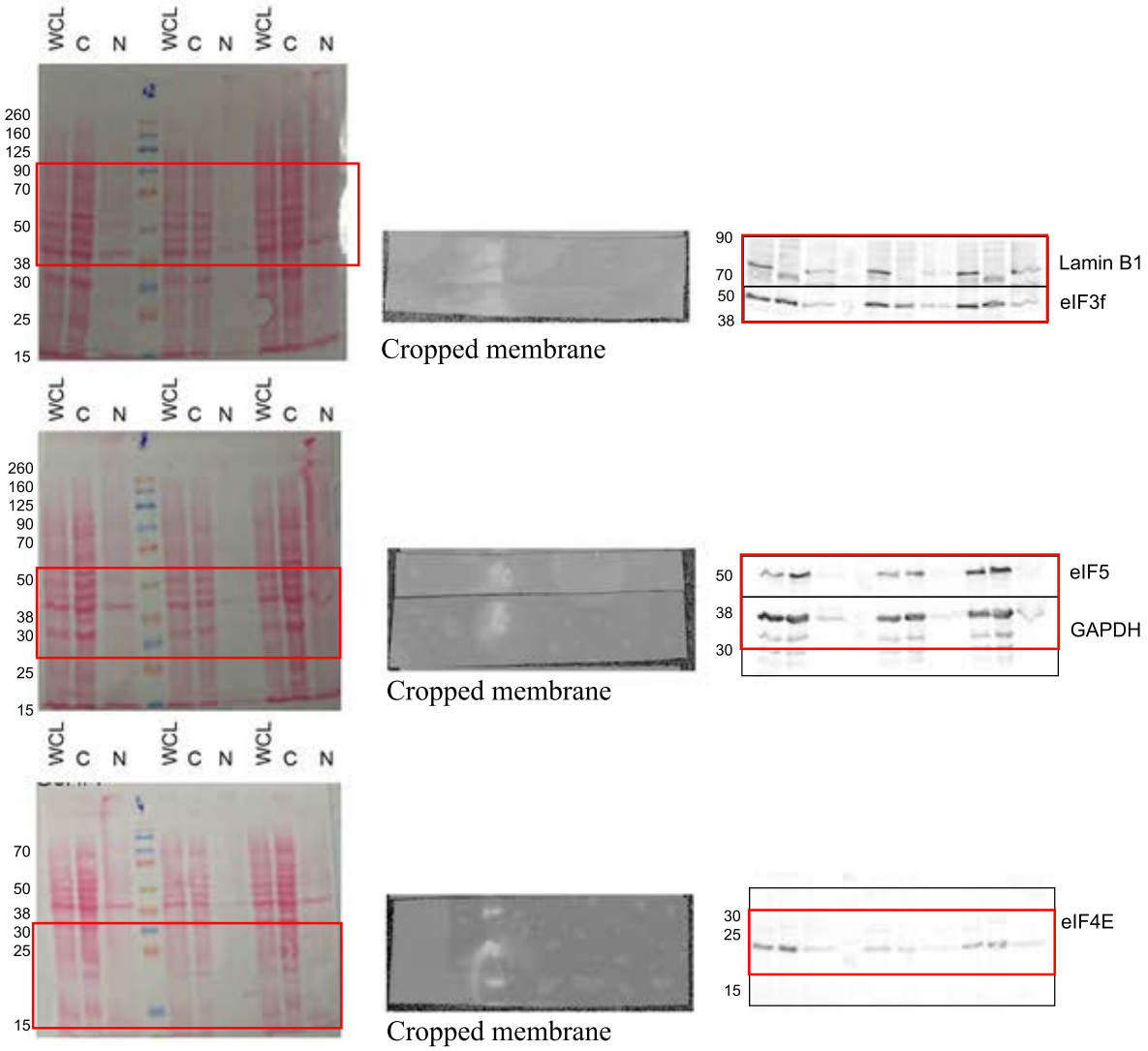
Supplementary Figure 19
Uncropped western blot for Supplementary Figure 2D



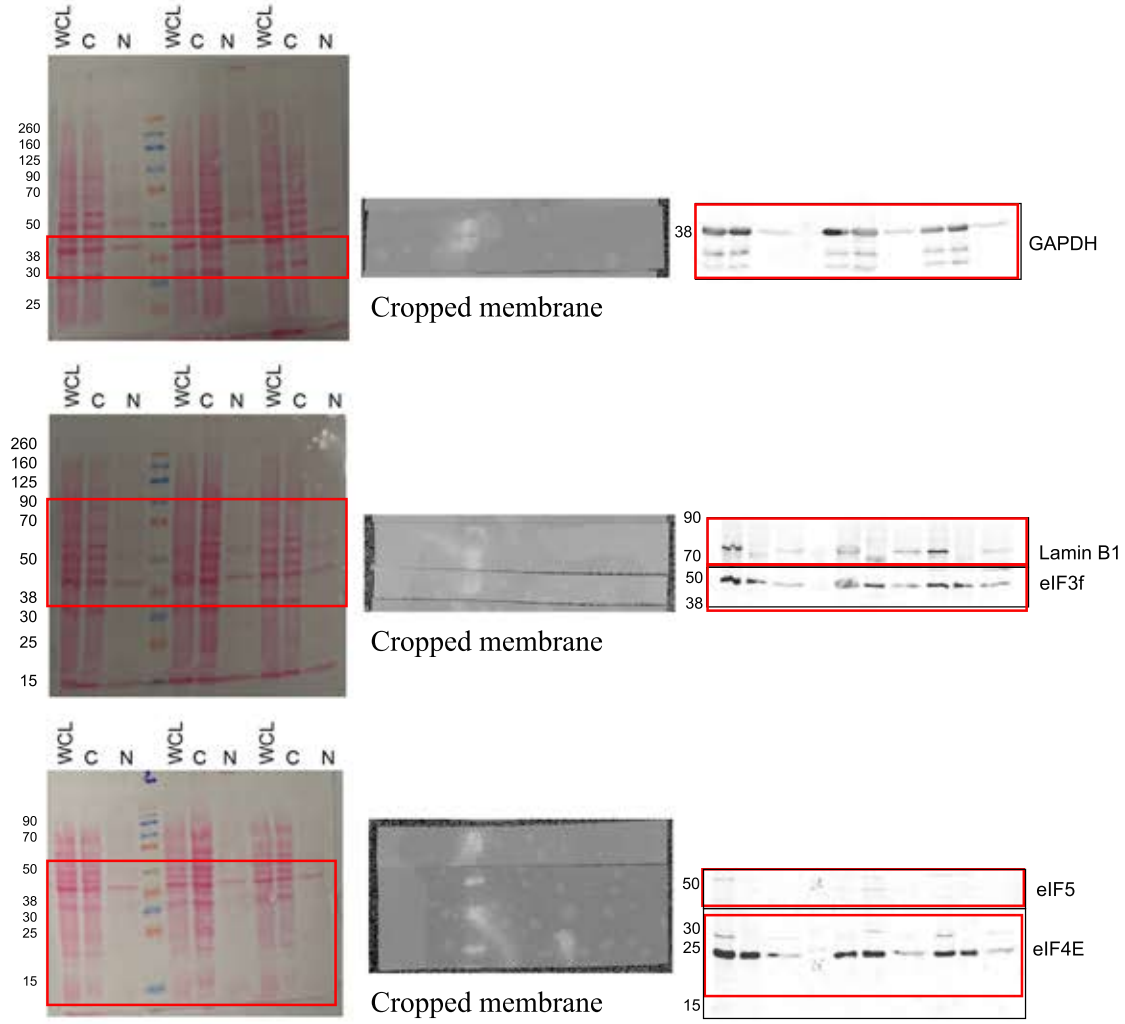
Supplementary Figure 20
Uncropped western blot for Supplementary Figure 4A



Supplementary Figure 21
Uncropped western blot for Supplementary Figure 4B

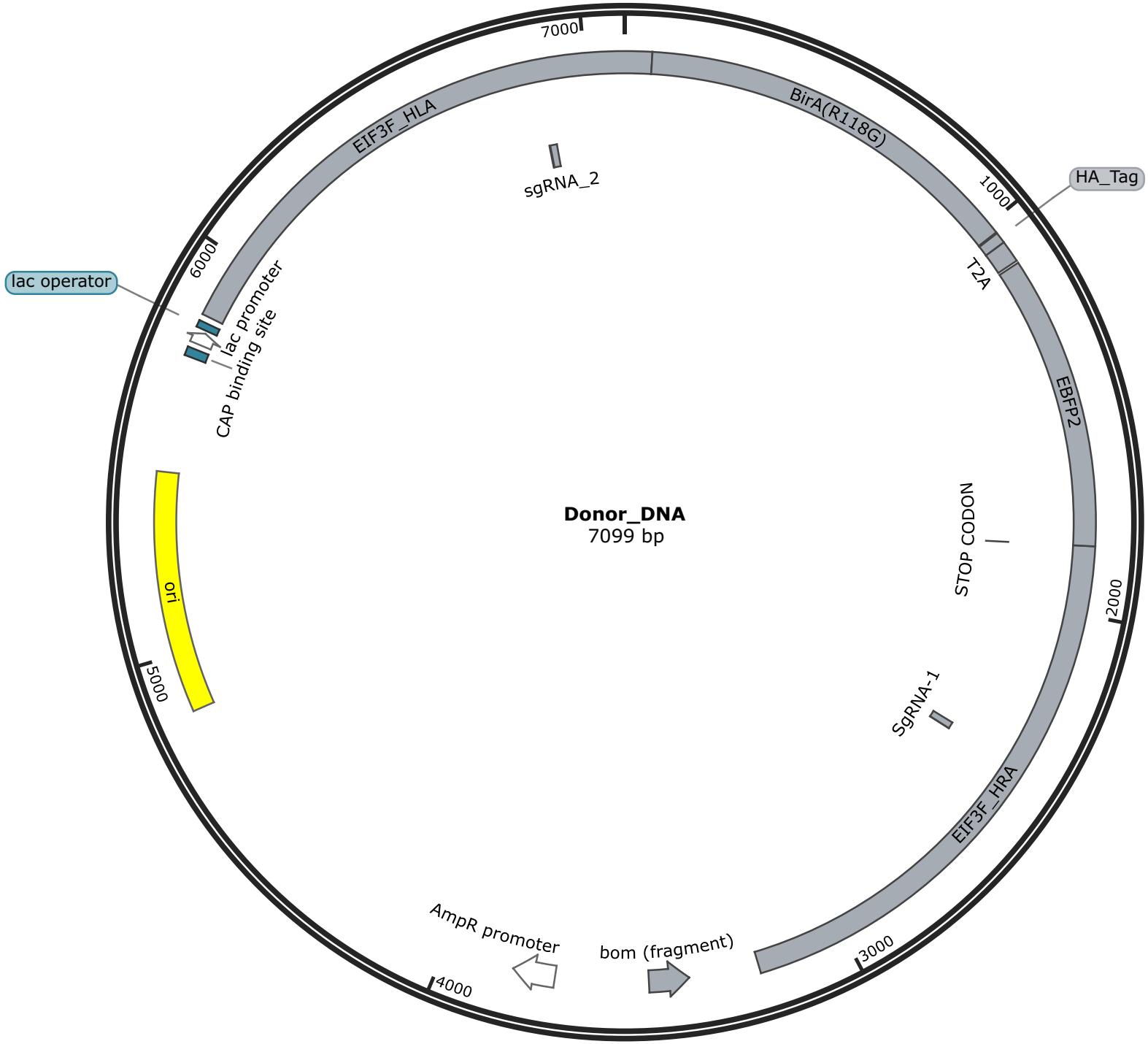


Supplementary Figure 22
Uncropped western blot for Supplementary Figure 6C



Supplementary Figure 23
Uncropped western blot for Supplementary Figure 6D

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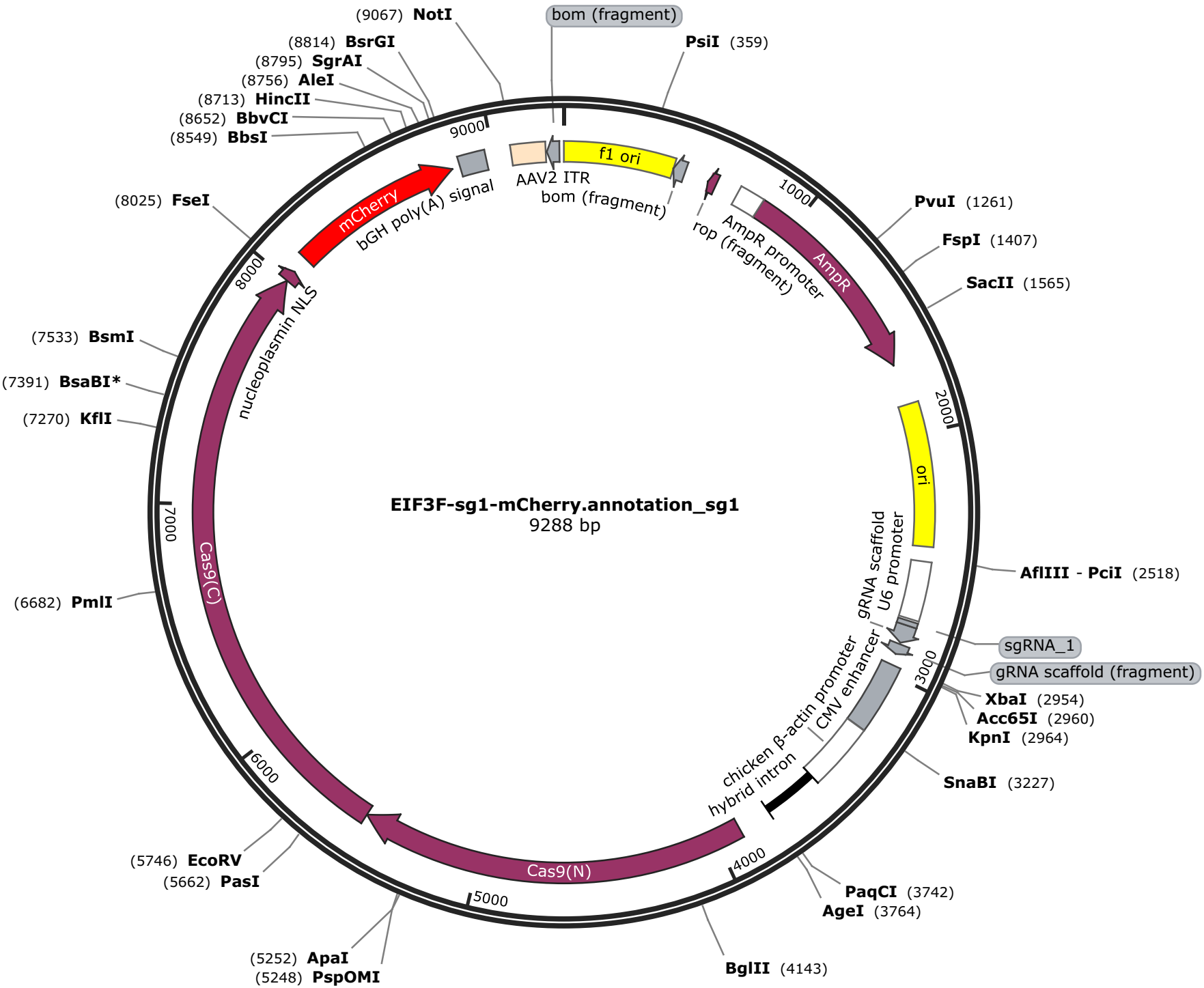
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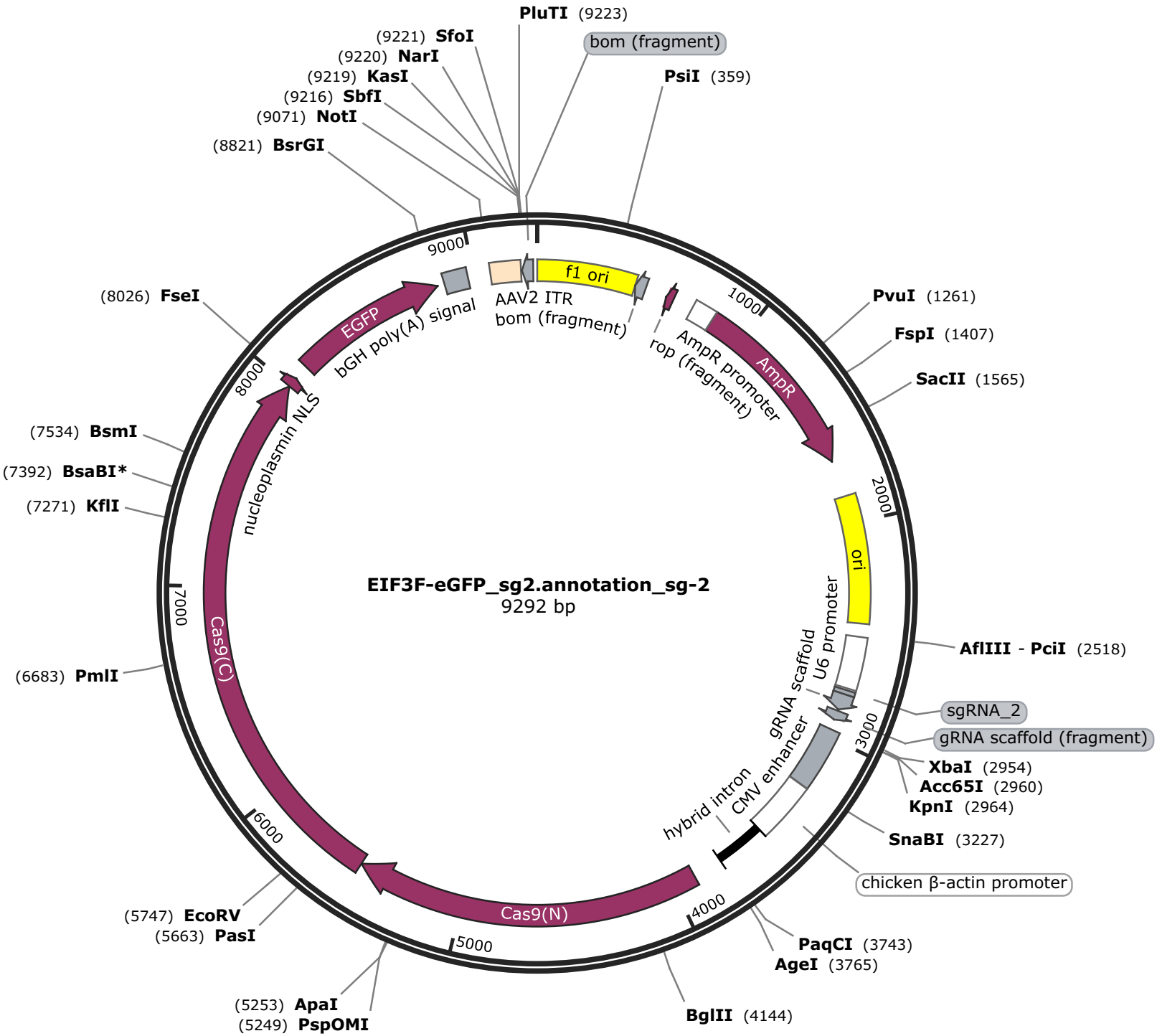
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