

Supplementary Information

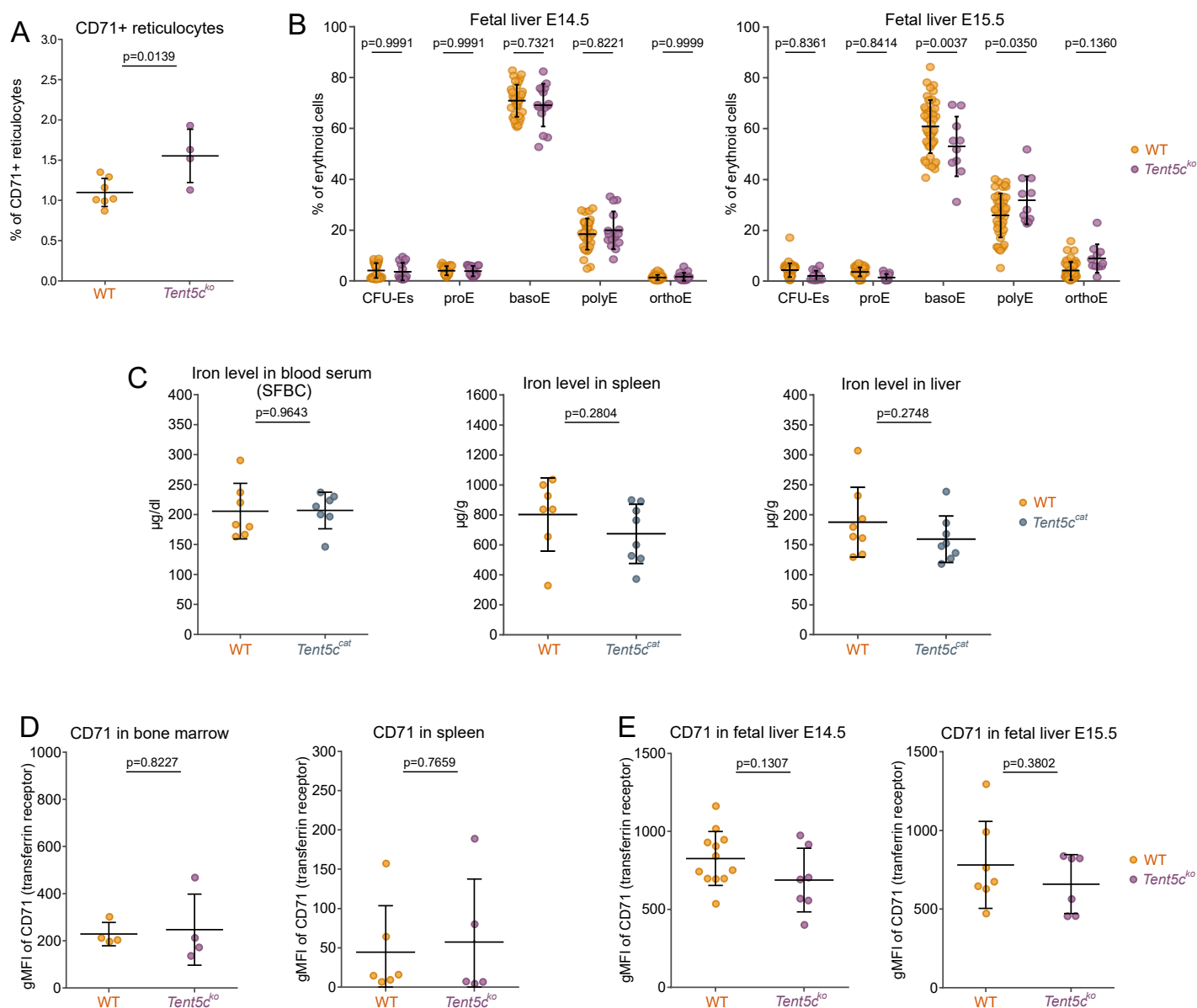
For the manuscript entitled

Efficient globin production during terminal erythropoiesis depends on the cooperative action of TENT5C poly(A) polymerase and LARP4B in mice

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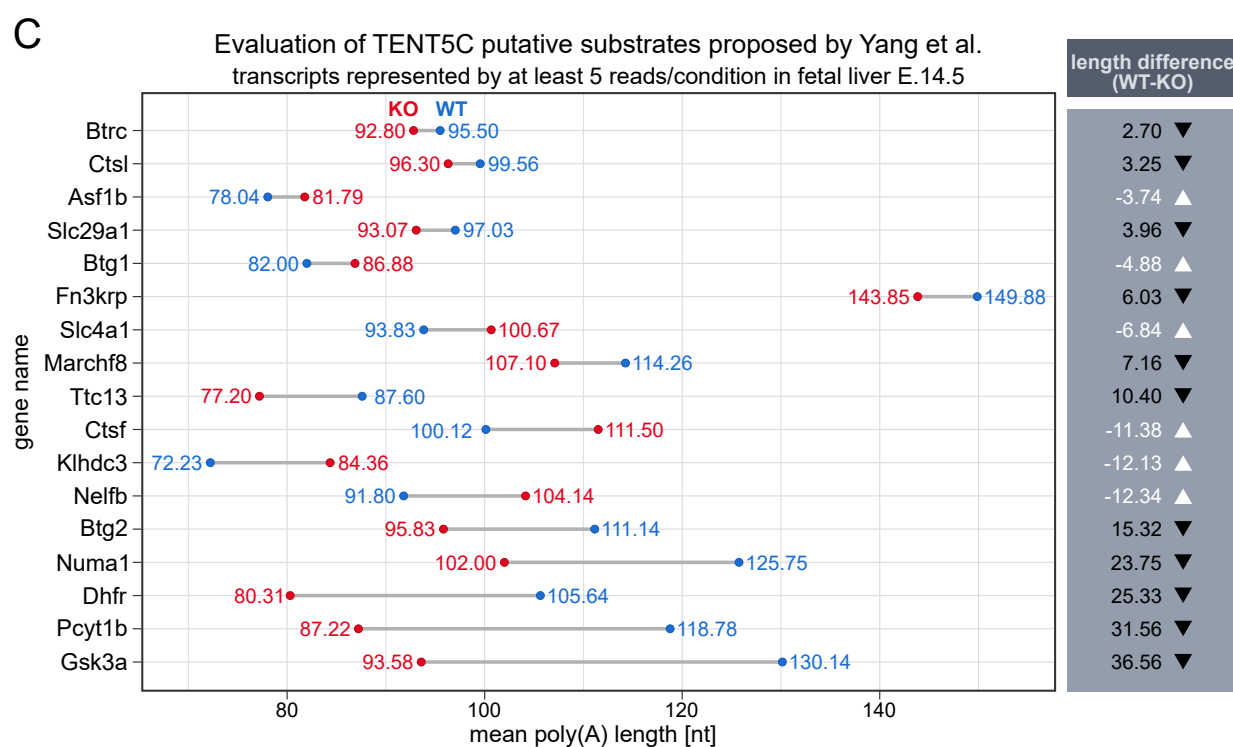
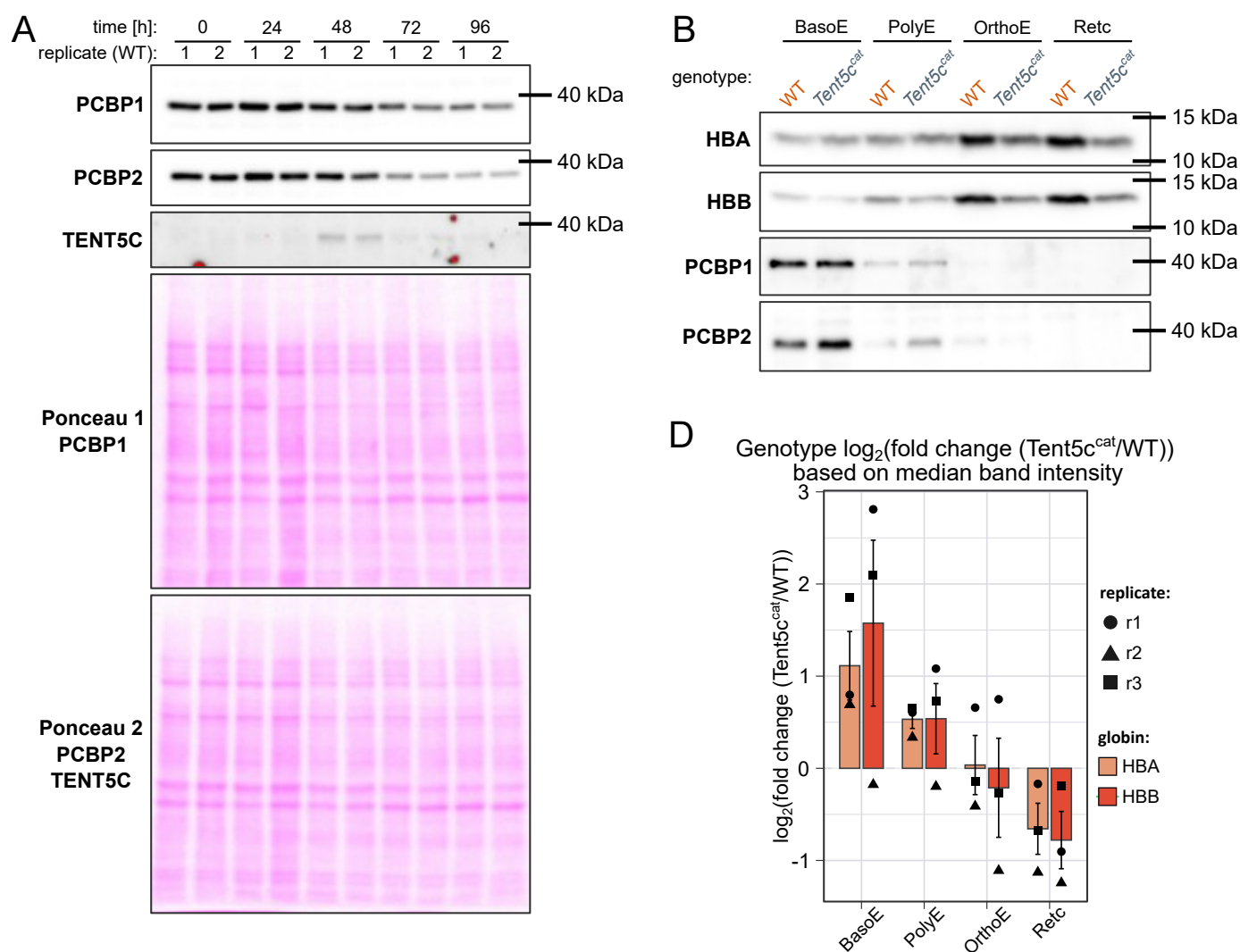
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Supplementary Figure 1. TENT5C-dependent anemic phenotype is not related to iron pathways

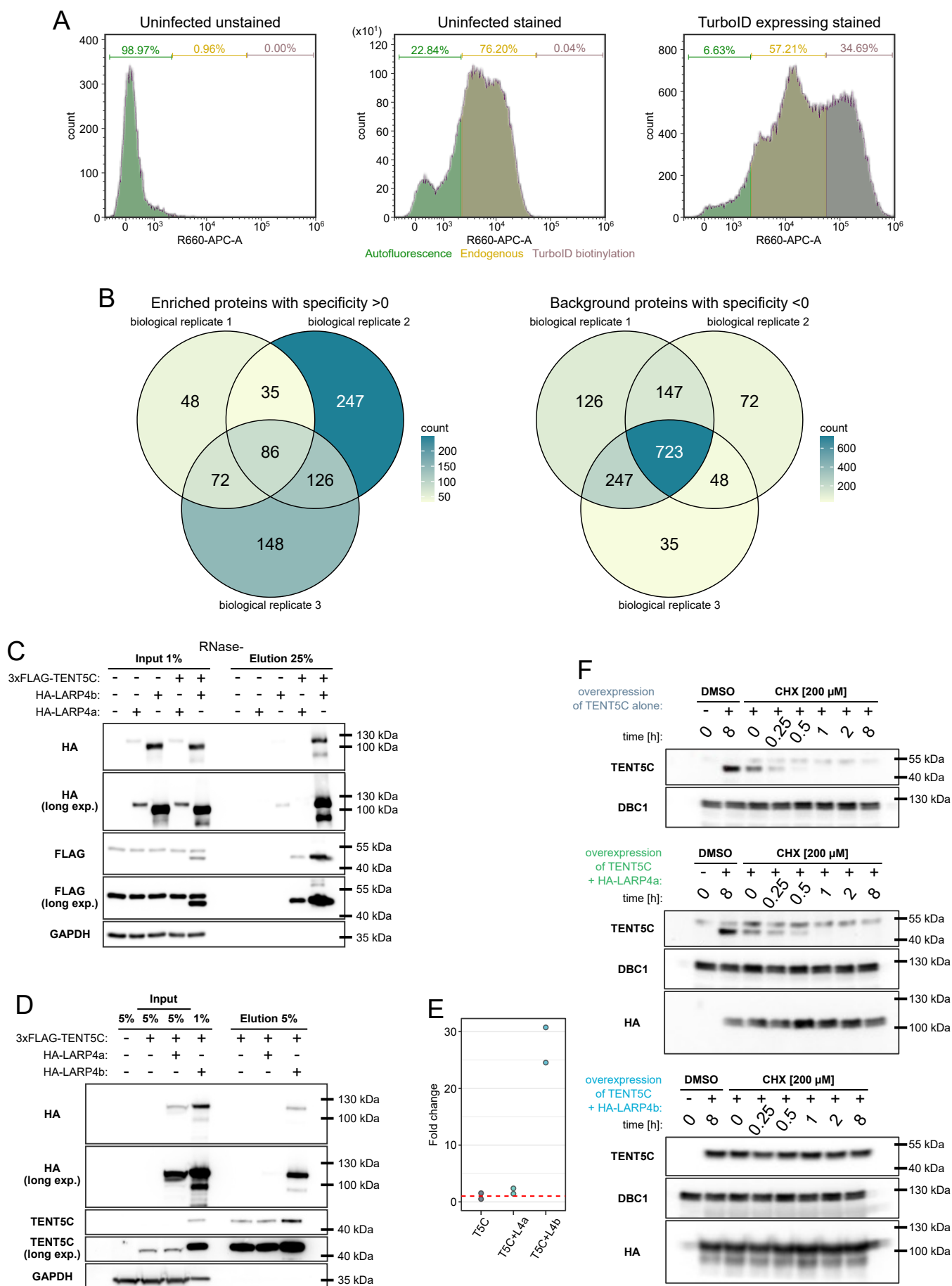
- (A) CD71+ reticulocytes (% of CD71+ reticulocytes) in peripheral blood of WT and *Tent5c*^{ko} mice (WT n=7, *Tent5c*^{ko} n=4).
- (B) Erythroblasts subpopulations in E14.5 and E15.5 fetal liver of WT and *Tent5c*^{ko} mice (WT n=33-46, *Tent5c*^{ko} n=11-15).
- (C) Iron levels in blood serum, spleen and liver of WT and *Tent5c*^{cat} mice (WT n=7-8, *Tent5c*^{cat} n=7-8).
- (D) CD71 expression (gMFI) in erythroid cells from bone marrow and spleen of WT and *Tent5c*^{ko} mice (WT n=4-6, *Tent5c*^{ko} n=4-6).
- (E) CD71 expression (gMFI) in erythroid cells from E14.5 and E15.5 fetal livers of WT and *Tent5c*^{ko} mice (WT n=7-12, *Tent5c*^{ko} n=6-7).

Data are presented as individual data points + mean ± SD (error bars), and significance was assessed by or two-tailed Student-t test (A, C-E) or two-way ANOVA.



Supplementary Figure 2. HBA, HBB, PCBP1, PCBP2 and TENT5C expression *ex vivo* and evaluation of putative TENT5C substrates suggested by Yang et al.

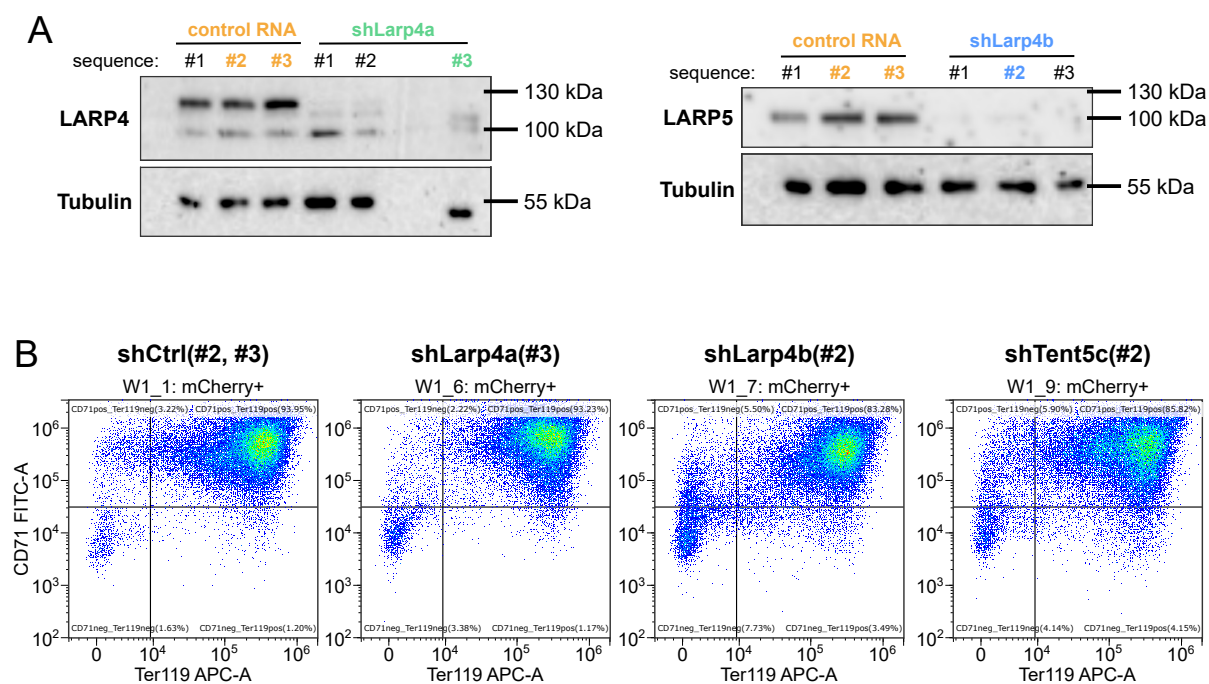
- (A) Western blot of PCBP1, PCBP2, and TENT5C over a time course (0–96 h) in *ex vivo* culture of fetal liver erythroblasts with two replicates per time point. Corresponding Ponceau S stained membranes are shown as loading controls.
- (B) Western Blot showing protein levels of HBA, HBB, PCBP1, and PCBP2 in wild-type (WT) and Tent5ccat erythroid cells at successive differentiation stages (BasoE, PolyE, OrthoE, Retc). Molecular weights are indicated by ticks corresponding to the respective ladder bands shown in Supplementary Figure 10. Data are representative of n=3 biological replicates.
- (C) Evaluation of putative TENT5C substrates proposed by Yang et al.: mean poly(A) tail length (WT, blue; KO, red) for transcripts represented by at least five reads per condition in E14.5 fetal-liver DRS (n=2). The right panel shows the WT–KO length difference (black downward-pointing arrow, shortening in KO; white upward-pointing arrow, lengthening in KO); genes are ordered by increasing absolute difference values.
- (D) Quantification of genotype-dependent changes in hemoglobin protein levels shown as $\log_2$ fold change based on median band intensity across differentiation stages. Individual biological replicates (n = 3) are represented by different symbols. Data are represented as mean $\pm$ SD (error bars).



Supplementary Figure 3. TurboID supplementary information

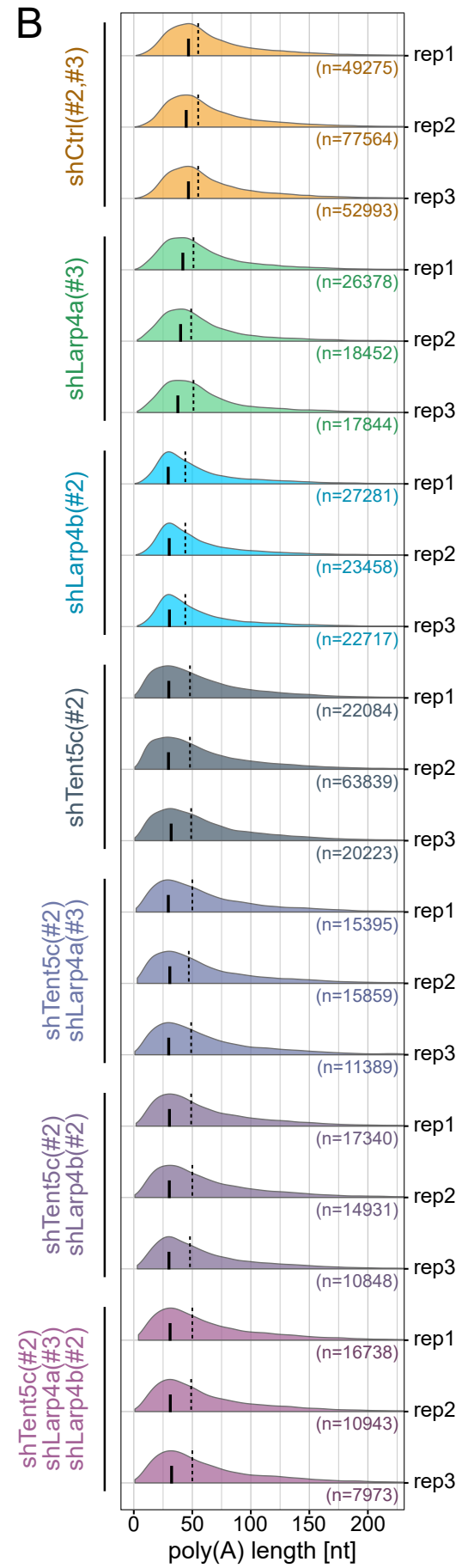
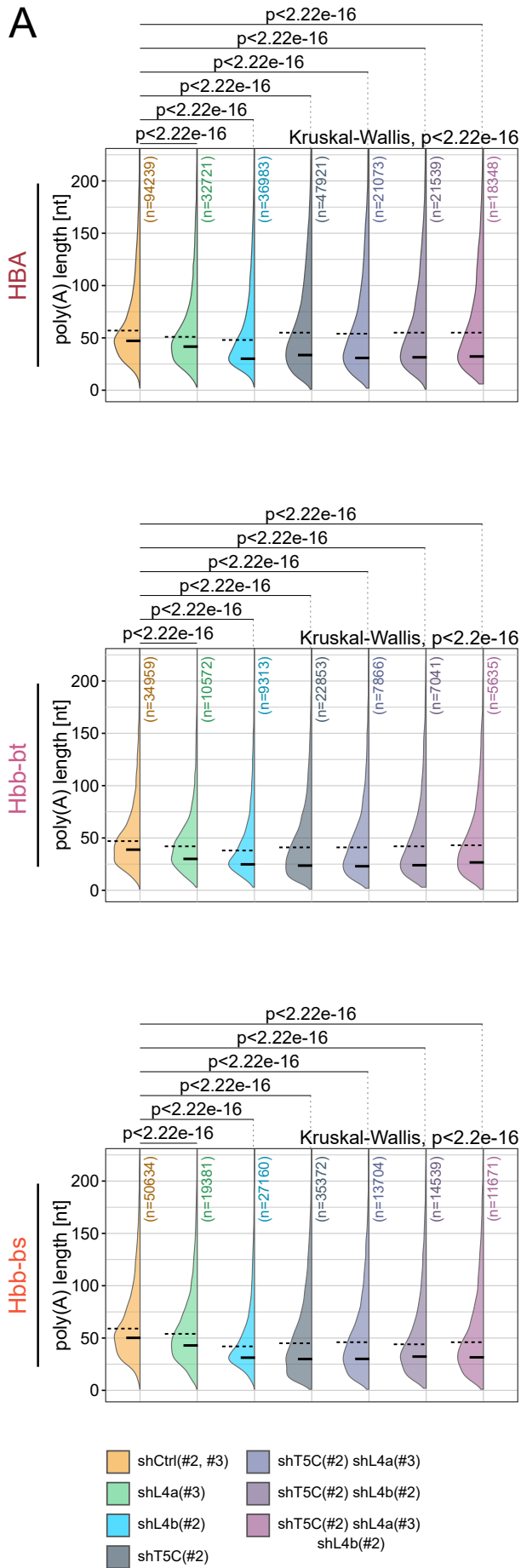
- (A) Flow-cytometry histograms (Streptavidin-AlexaFluor 647) representing intracellular staining of erythroblasts from TurboID experiment: uninfected unstained, uninfected stained, infected stained.
- (B) Venn diagrams of enriched (specificity > 0) and background (specificity < 0) proteins across 3 biological replicates of TurboID experiment.
- (C) Co-immunoprecipitation analysis of 3×FLAG-TENT5C with HA-LARP4A or HA-LARP4B in the absence of RNase (RNase-) in HEK293 cells. Input (1%) and elution (25%) fractions are shown. probed for HA, FLAG and GAPDH. Lane order (both fractions): untransfected control, HA-LARP4A, HA-LARP4B, 3×FLAG-TENT5C + HA-LARP4A, 3×FLAG-TENT5C + HA-LARP4B.
- (D) Co-immunoprecipitation of 3×FLAG-TENT5C with HA-LARP4A or HA-LARP4B in the presence of RNase (RNase+); input (5% and 1%) and elution (5%) fractions, probed for HA, TENT5C and GAPDH.
- (E) Quantification of immunoblot signal corresponding to 3xFLAG-TENT5C expression in HEK293T system (n = 2) with ectopic overexpression (normalized to DBC1). Fold-changes calculated relative to 3x-FLAG-TENT5C levels.
- (F) TENT5C immunoblots during a cycloheximide chase (CHX, 200 μM; DMSO control) in cells overexpressing TENT5C alone or together with HA-LARP4A or HA-LARP4B; DBC1 is the loading control.

In blot-containing panels (C, D, F), molecular weights are indicated by ticks corresponding to the respective ladder bands shown in Supplementary Figures 11-13, 16 and 17.



Supplementary Figure 4. Validation of LARP4A, LARP4B and TENT5C knockdown

- (A) Western blot confirming knockdown efficiency: LARP4A (LARP4; ~130 kDa) in control versus shLarp4a cells and LARP4B (LARP5; ~100 kDa) in control versus shLarp4b cells, with Tubulin as loading control. Three shRNA sequences (#1-#3) are shown per target; the sequences selected for downstream experiments are highlighted in bold and colour. Cells were FACS -sorted and equal numbers loaded per lane (25,000 cells). Molecular weights are indicated by ticks corresponding to the respective ladder bands shown in Supplementary Figure 19.
- (B) Representative flow cytometry dot-plots showing the major trajectory of erythroblast differentiation in cells expressing shRNA (control, shLarp4a, shLarp4b, shTent5c) with respect to CD71 (y-axis) and TER119 (x-axis).

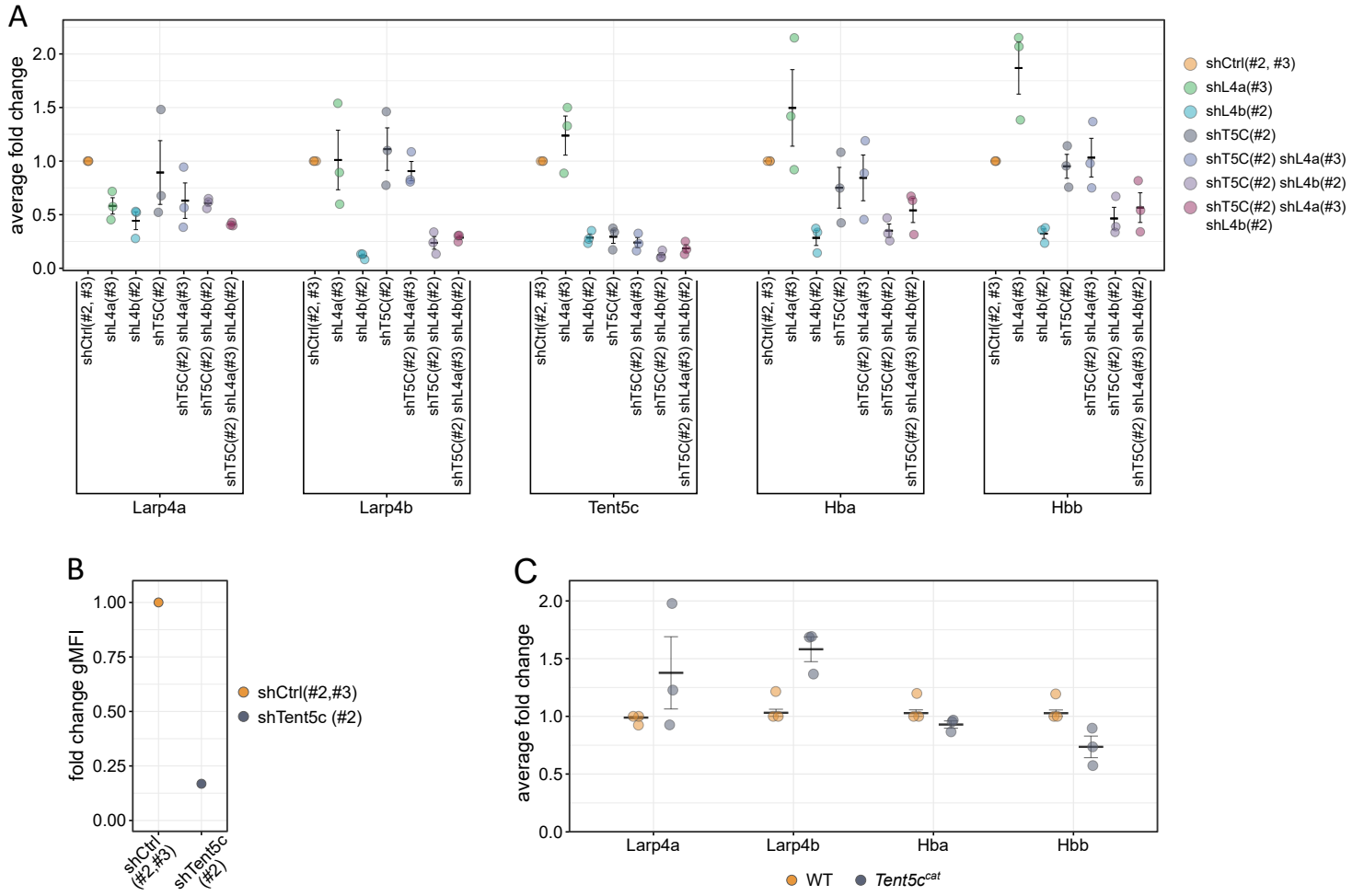


Supplementary Figure 5. Globin poly(A) tail length distribution pattern distributions upon LARP4A, LARP4B and/or TENT5C silencing.

Poly(A) tail-length distributions of globin transcripts in cells expressing shRNAs against a control, *Larp4a*, *Larp4b*, *Tent5c* and their combinations (n=3 biological replicates).

- (A) Poly(A) tail lengths distribution in globin transcripts in cells expressing shRNA (control, shLarp4a, Larp4b, Tent5c). Data grouped by condition and hemoglobin type.
- (B) Poly(A) tail lengths distribution in globin transcripts in cells expressing shRNA (control, shLarp4a, Larp4b, Tent5c). Data grouped by replicate.

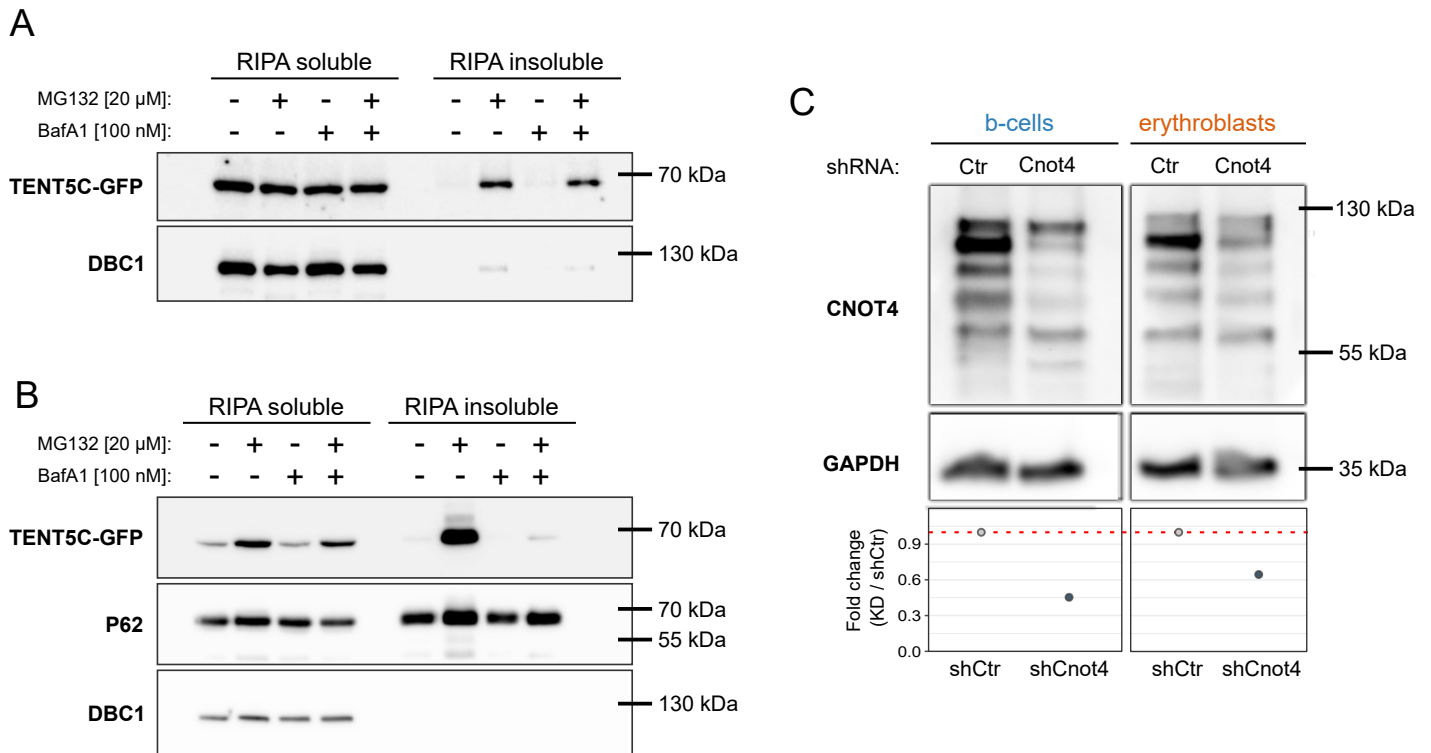
Read counts (n) are shown for each group within the plots and denote numbers of reads, distinct from the biological-replicate n. Significance was assessed by the Kruskal–Wallis test ($\alpha = 0.05$); pairwise comparisons are indicated ($p < 2.22 \times 10^{-16}$).



Supplementary Figure 6. Transcript-level effects of LARP4A/LARP4B and TENT5C perturbation.

- (A) RT-qPCR average fold change of *Larp4a*, *Larp4b*, *Tent5c*, *Hba* and *Hbb* across shRNA conditions (control and combinations), normalized to *Gapdh* and expressed relative to control (n=3).
- (B) Fold change in TENT5C-EGFP expression (gMFI) in shCtrl(#2,#3) versus shTent5c(#2) cells (n=1).
- (C) RT-qPCR average fold change of *Larp4a*, *Larp4b*, *Hba* and *Hbb* in WT versus *Tent5c^{cat}* bone-marrow erythroblasts (mixed population), normalized to *Gapdh* and expressed relative to WT (n=3).

Data in (A) and (C) are represented as mean $\pm$ SEM (error bars); n indicates biological replicates.

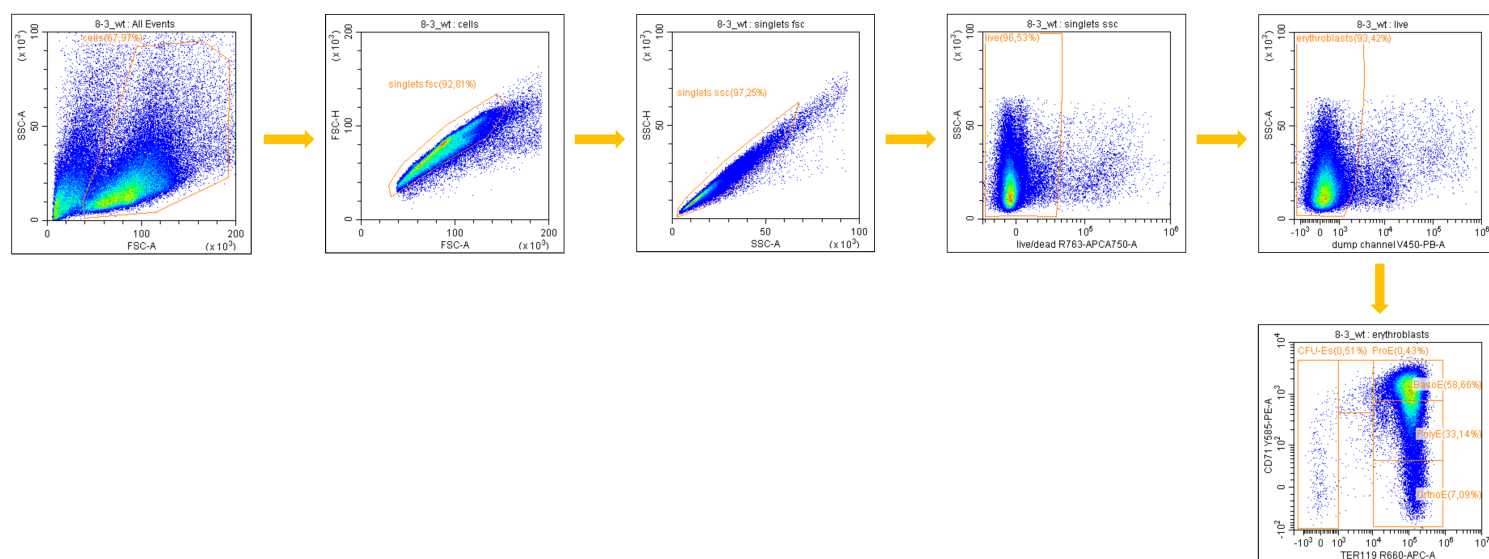


Supplementary Figure 7. TENT5C degradation pathways and CNOT4 silencing

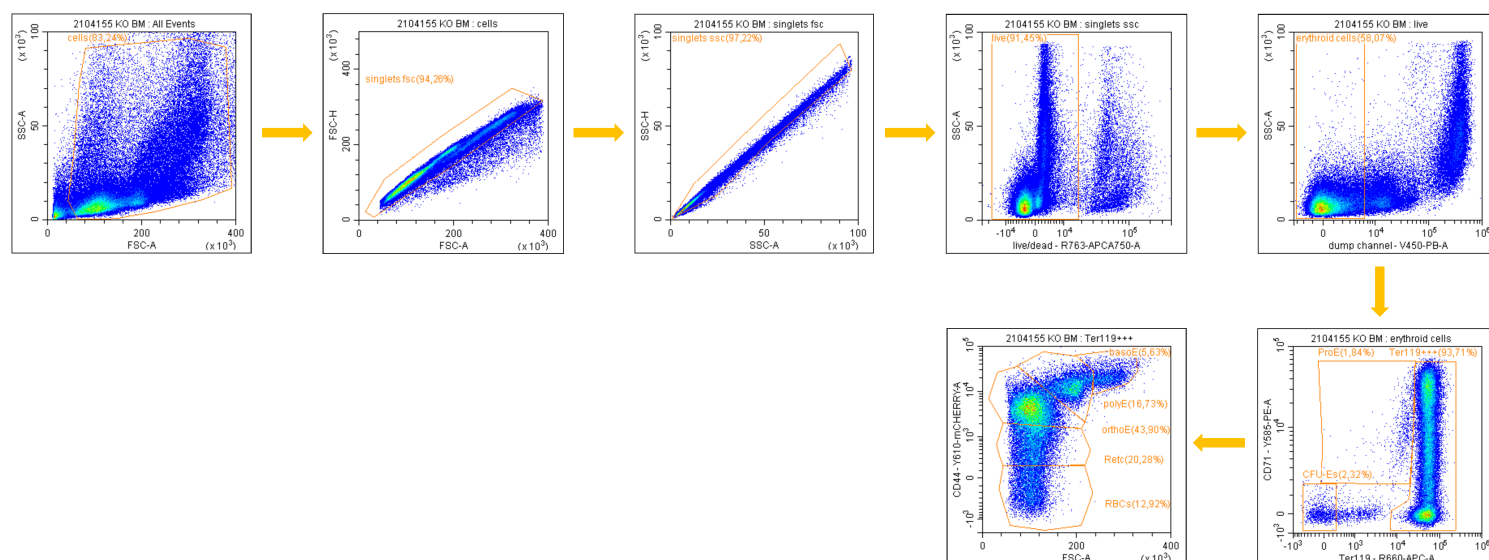
- (A) Western blot showing TENT5C-EGFP levels in RIPA-soluble and RIPA-insoluble fractions after inhibition of proteasomal (MG132, 20 μ M) and/or autophagic (BafA1, 100 nM) degradation, in B-cells; DBC1 is the loading control.
- (B) Western blot showing TENT5C-EGFP levels in RIPA-soluble and RIPA-insoluble fractions after inhibition of proteasomal (MG132, 20 μ M) and/or autophagic (BafA1, 100 nM) degradation, in erythroblasts; DBC1 is the loading control, and the autophagy receptor P62 is additionally probed.
- (C) Western blot showing CNOT4 knockdown efficiency in B-cells and erythroblasts expressing control or *Cnot4* shRNA (FACS-sorted, 25,000 cells per lane); GAPDH is the loading control, and the fold change (knockdown / control) is shown below each blot.

Blots are representative of independent experiments. Molecular weights are indicated by ticks corresponding to the respective ladder bands shown in Supplementary Figures 20-22.

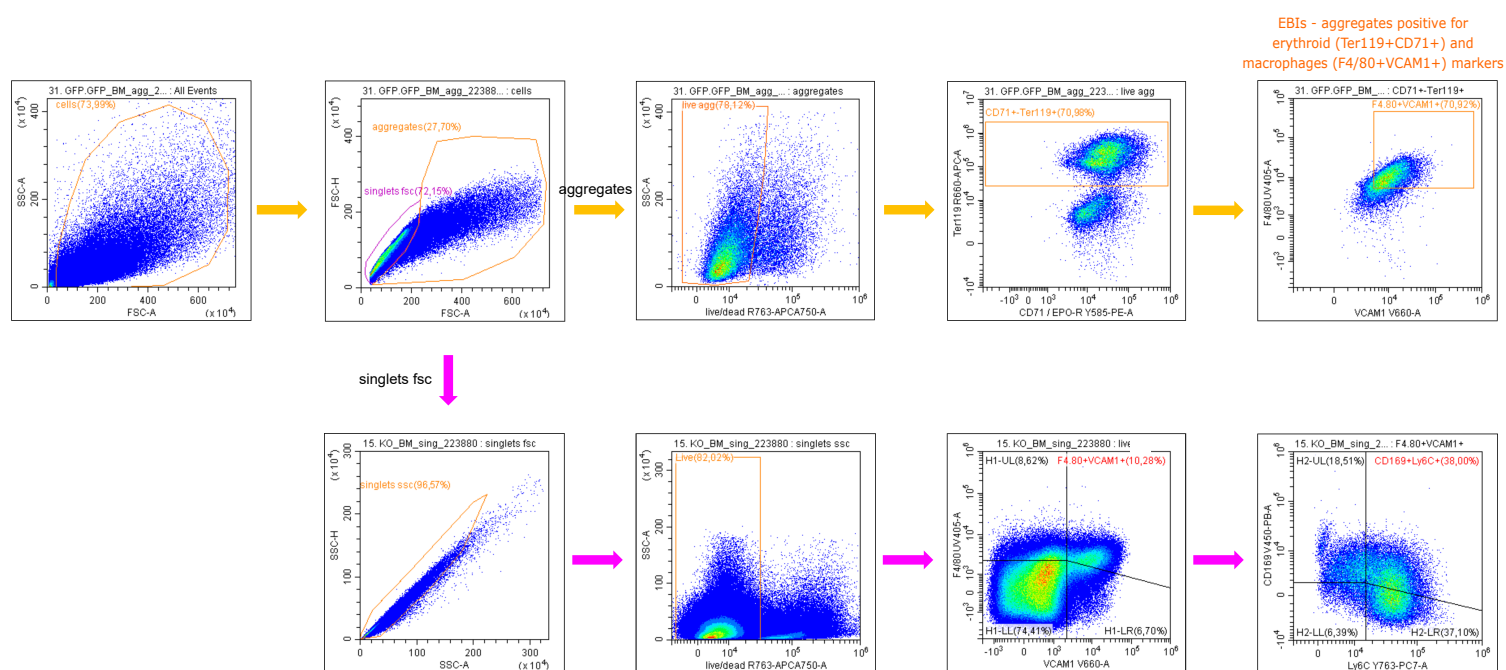
A gating strategy in fetal livers / ex vivo culture



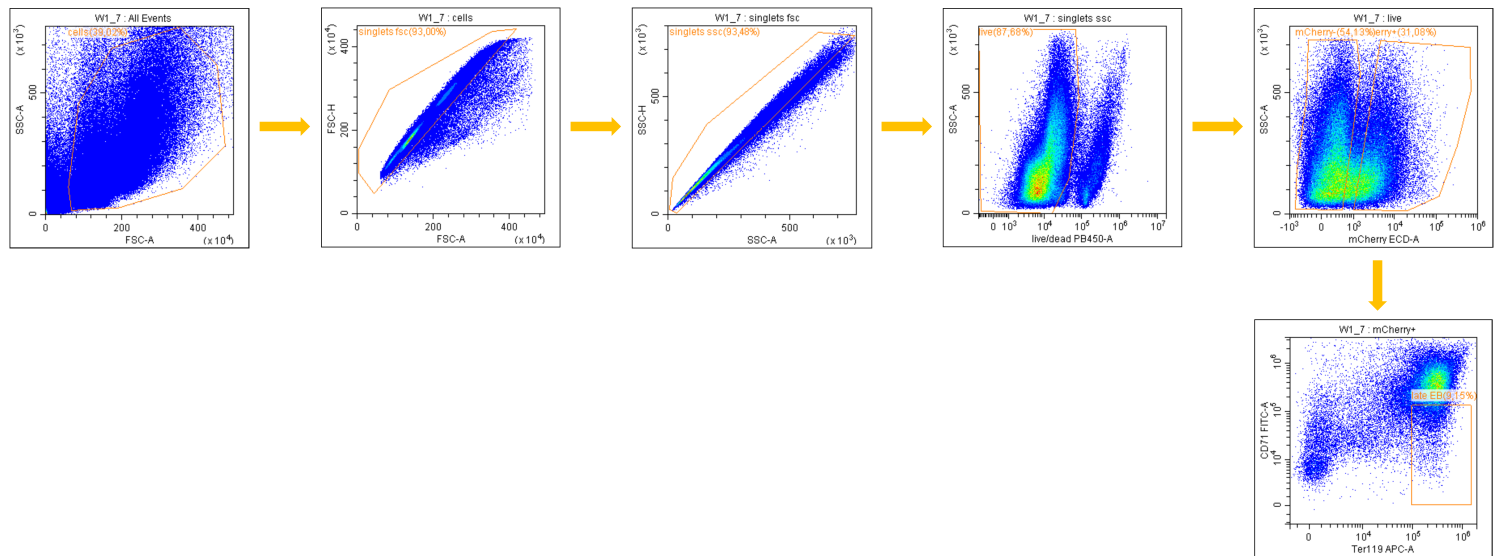
B gating strategy in adult bone marrow and spleens



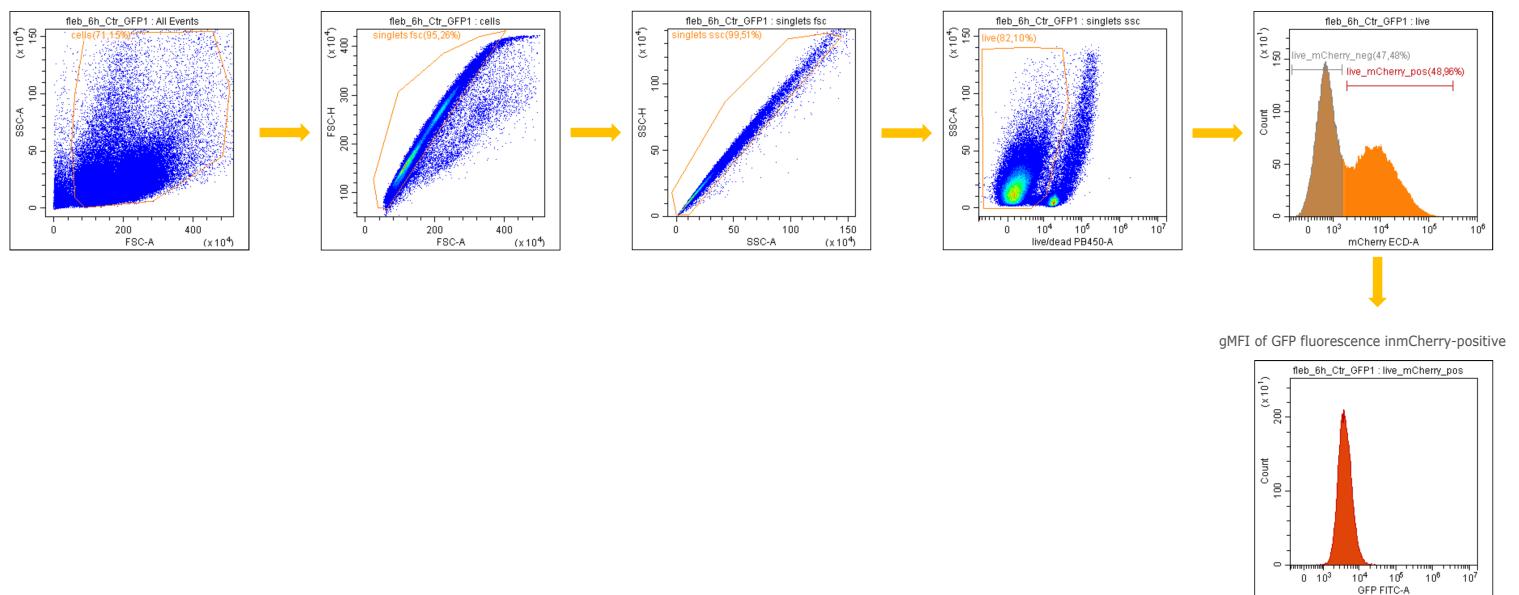
C gating strategy of bone marrow and spleen aggregates (EBIs)



D gating strategy for sorting late erythroblasts from *ex vivo* culture expressing shRNA



E gating strategy for cultured fetal liver erythroblasts transduced with shRNA and treated with CHX



Supplementary Figure S8. Gating strategies

- (A) Gating strategy used for cells isolated from fetal livers and *ex vivo* cultures.
- (B) Gating strategy used for cells isolated from adult bone marrow and spleen.
- (C) Gating strategy used for EBIs calculation and characterization of EBIs macrophages.
- (D) Gating strategy for sorting late erythroblasts from *ex vivo* culture transduced with shRNA against *Tent5c* and *Larps*.
- (E) Gating strategy used for cells isolated *ex vivo*, transduced with shRNA and treated with CHX.

A

Hemoglobins A (Gencode vM32)

Clustal format alignment by MAFFT (v7.511)

```

ENSMUST00000093209.4 | Hba-a1      GGGGAAGCACAAACCCAGCCCCAGAATCTCAGGGGCCCTAACAAAGTTTACTGGGTAGAGC
ENSMUST00000093207.4 | Hba-a2      -----

ENSMUST00000093209.4 | Hba-a1      AAGCACAAACCAAGCCAATGAGAACTGCTCCAAGGGCGTGTTCCACCCTGCCTGGAGGACAC
ENSMUST00000093207.4 | Hba-a2      -----

ENSMUST00000093209.4 | Hba-a1      GCCCTTGGAGGGCATATAAGTGCTACTTGCTGCAGGTCCAAGACACTTCTGATTCTGACA
ENSMUST00000093207.4 | Hba-a2      -----GACACTTCTGATTCTGACA
                                           *****

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ENSMUST00000093207.4 | Hba-a2      GACTCAGGAAGAAACCATGGTGCTCTCTGGGAAGACAAAAGCAACATCAAGGCTGCCTG
                                           *****

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ENSMUST00000093207.4 | Hba-a2      GGGGAAGATTGGTGGCCATGGTGCTGAATATGGAGCTGAAGCCCTGGAAAGGATGTTTG
                                           *****

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ENSMUST00000093207.4 | Hba-a2      TAGCTTCCCCACCACCAAGACCTACTTCCTCACTTTGATGTAAGCCACGGCTCTGCCCA
                                           *****

ENSMUST00000093209.4 | Hba-a1      GGTCAAGGGTCACGGCAAGAAGGTCGCCGATGCTCTGCGCAATGCTGCAGGCCACCTCGA
ENSMUST00000093207.4 | Hba-a2      GGTCAAGGGTCACGGCAAGAAGGTCGCCGATGCTCTGCGCAATGCTGCAGGCCACCTCGA
                                           *****

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                                           *****

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ENSMUST00000093207.4 | Hba-a2      TCCCGTCAACTTCAAGCTCCTGAGCCACTGCCTGCTGGTGACCTTGGCTAGCCACCACCC
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ENSMUST00000093209.4 | Hba-a1      TGCCGATTTACCCCCGCGGTGCATGCCTCTCTGGACAAATTCCTTGCCCTCTGTGAGCAC
ENSMUST00000093207.4 | Hba-a2      TGCCGATTTACCCCCGCGGTGCATGCCTCTCTGGATAAAATTCCTTGCCCTCTGTGAGCAC
                                           *****

ENSMUST00000093209.4 | Hba-a1      CGTGCTGACCTCCAAGTACCGTTAAGCTGCCTTCTGCGGGGCTTGCCCTCTGGCCATGCC
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```

B

Hemoglobins B (Gencode vM32)

Clustal format alignment by MAFFT (v7.511)

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ENSMUST00000098192.4|Hbb-bt AATCTGCTCAGAGAGGACAGAGTGGGCAGGAGCCAGCATTGGGTATATAAAGCTGAGCAG

ENSMUST00000023934.8|Hbb-bs -----CACATTTGCTTCTGACATAGTTGTGTTGACTCACAACCCAGAAAC
ENSMUST00000098192.4|Hbb-bt GGTCAATTGCTTCTTACGTTTGCTTCTGATTCTGTTGTGTTGACTTGCACCTCAGAAAC
                        .**.******. .*****.*****
                        .**.******. .*****.*****

ENSMUST00000023934.8|Hbb-bs AGACATCATGGTGACCTGACTGATGCTGAGAAAGCTGCTGTCTCTGGCCTGTGGGGAAA
ENSMUST00000098192.4|Hbb-bt AGACATCATGGTGACCTGACTGATGCTGAGAAAGCTGCTGTCTCTGGCCTGTGGGGAAA
*****

ENSMUST00000023934.8|Hbb-bs GGTGAACGCCGATGAAGTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTTGTCTACCCTTG
ENSMUST00000098192.4|Hbb-bt GGTGAACGCCGATGAAGTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTTGTCTACCCTTG
*****

ENSMUST00000023934.8|Hbb-bs GACCCAGCGGTACTTTGATAGCTTTGGAGACCTATCCTCTGCCTCTGCTATCATGGGTAA
ENSMUST00000098192.4|Hbb-bt GACCCAGCGGTACTTTGATAGCTTTGGAGACCTATCCTCTGCCTCTGCTATCATGGGTAA
*****

ENSMUST00000023934.8|Hbb-bs TGCCAAAGTGAAGGCCCATGGCAAGAAAGTGATAACTGCCTTTAACGATGGCCTGAATCA
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*****

ENSMUST00000023934.8|Hbb-bs CTTGGACAGCCTCAAGGGCACCTTTGCCAGCCTCAGTGAGCTCCACTGTGACAAGCTGCA
ENSMUST00000098192.4|Hbb-bt CTTGGACAGCCTCAAGGGCACCTTTGCCAGCCTCAGTGAGCTCCACTGTGACAAGCTGCA
*****

ENSMUST00000023934.8|Hbb-bs TGTGGATCCTGAGAACTTCAGGCTCCTGGGCAATATGATCGTGATTGTGCTGGGCCACCA
ENSMUST00000098192.4|Hbb-bt TGTGGATCCTGAGAACTTCAGGCTCCTGGGCAATATGATCGTGATTGTGCTGGGCCACCA
*****

ENSMUST00000023934.8|Hbb-bs CCTGGGCAAGGATTTACCCCCGCTGCACAGGCTGCCTTCCAGAAGGTGGTGGCTGGAGT
ENSMUST00000098192.4|Hbb-bt CCTGGGCAAGGATTTACCCCCGCTGCACAGGCTGCCTTCCAGAAGGTGGTGGCTGGAGT
*****

ENSMUST00000023934.8|Hbb-bs GGCCGCTGCCCTGGCTCACAAGTACCACTAAACCCCTTTCTCTGCTCTTGGCTGTGAACA
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***.*****.*****.*****.***.***.***
***.*****.*****.*****.***.***.***

ENSMUST00000023934.8|Hbb-bs ATGGTTAATTGTTCCCAAGAGAGCATCTGTGAGTTGTTGGCAAAATGATAAAGACATTTG
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* ***** **.* ** ***** * ***** **.* ***** **.* *****
* ***** **.* ** ***** * ***** **.* ***** **.* *****

ENSMUST00000023934.8|Hbb-bs AAAATCTGTCTTCTGACAAATAAAAGCATTTATT-TCACTGC--
ENSMUST00000098192.4|Hbb-bt GGCATCTAGCTTTTATCTAATAAATGATATTTACTGTCTATCTCAA
..****.***.*.******.***.***.*

```

Supplementary Figure 9. Sequence alignment of mouse hemoglobin transcripts.

(A) Clustal-format multiple sequence alignment of mouse hemoglobin A transcripts (*Hba-a1* and *Hba-a2*).

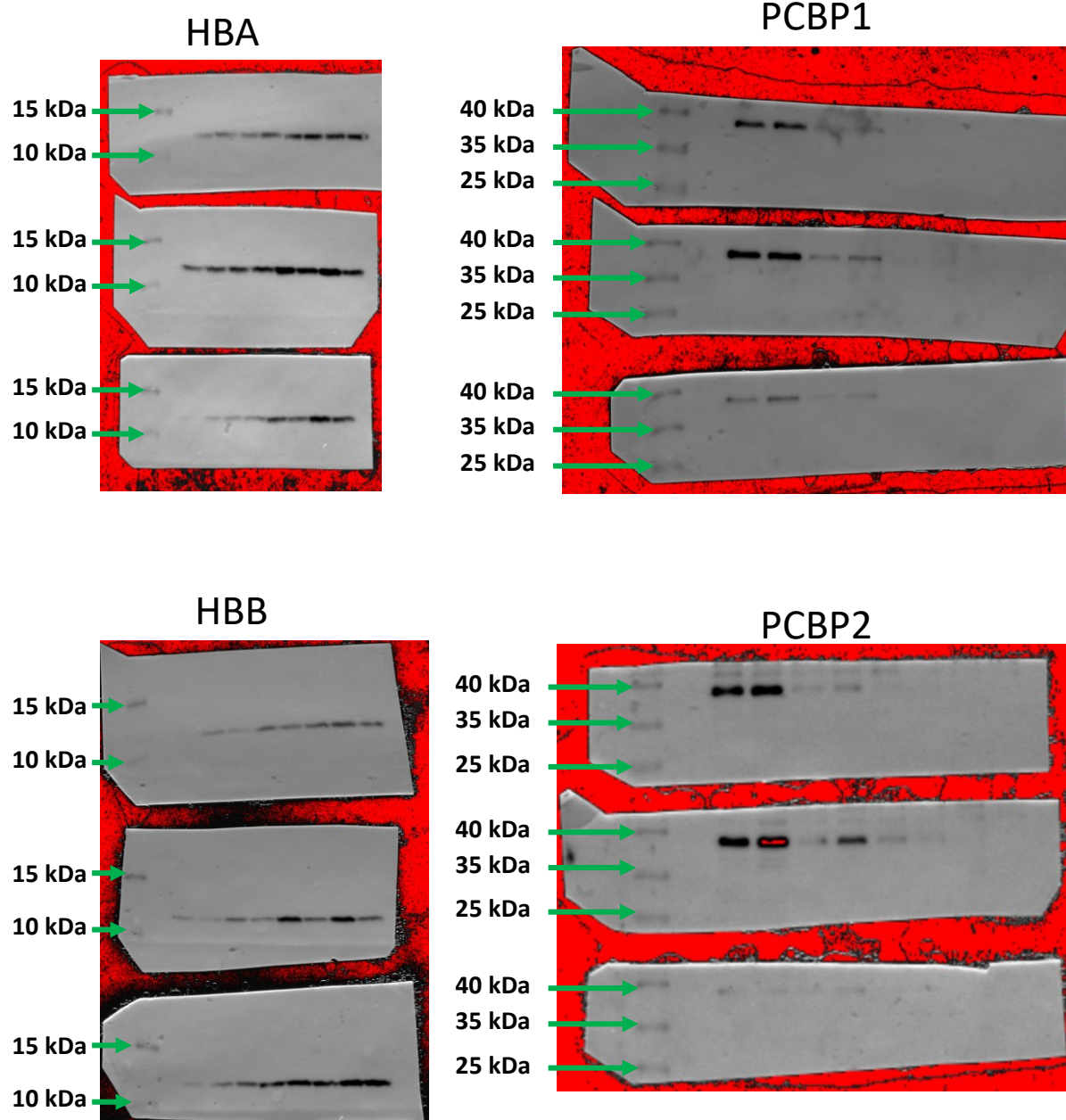
(B) Clustal-format multiple sequence alignment of mouse hemoglobin B transcripts (*Hbb-bs* and *Hbb-bt*).

Reference according to Gencode VM32, generated using MAFFT (v7.511).

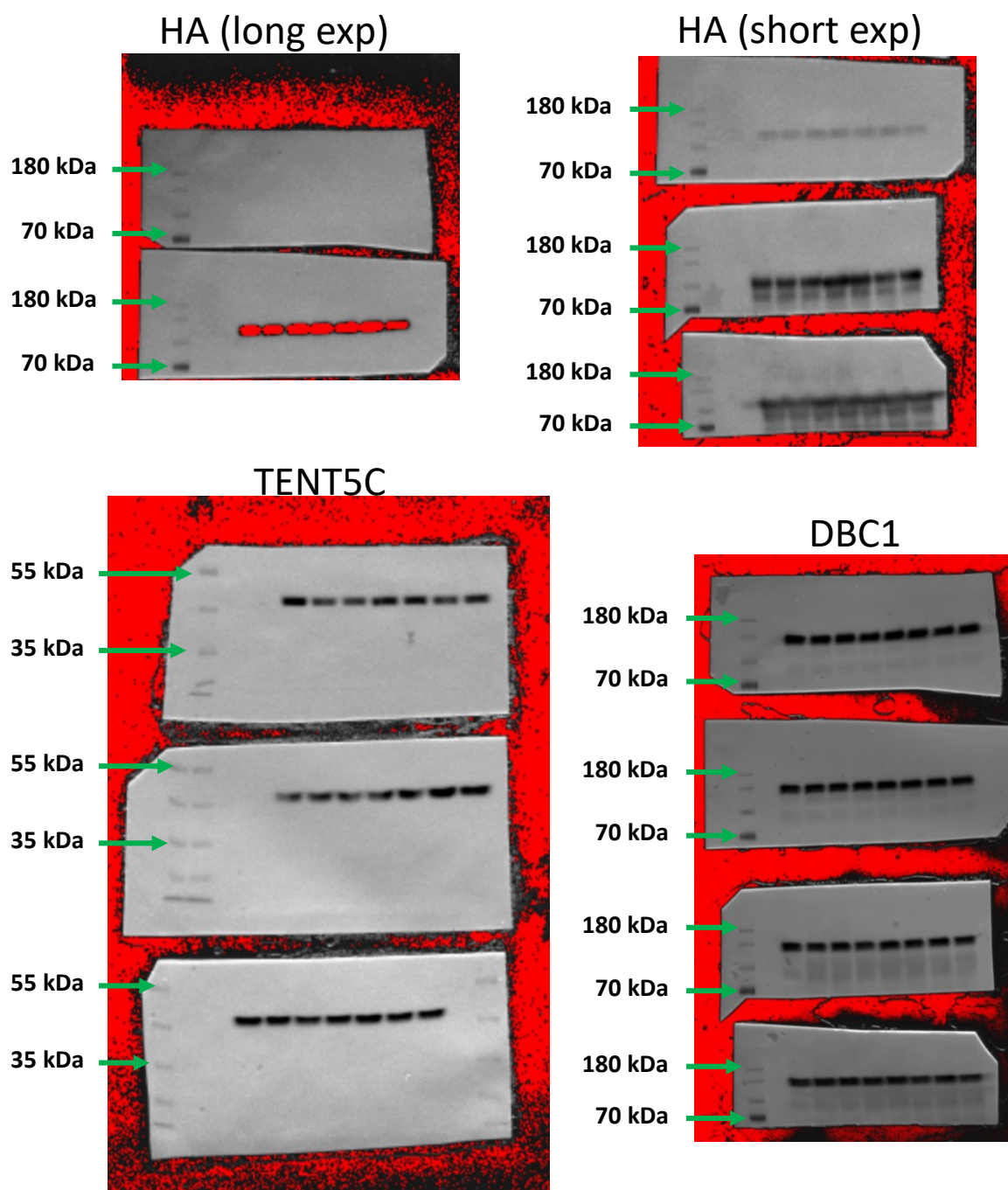
Uncropped Western Blots

Composite Western blot images combining chemiluminescence and visible-light channels were acquired using ChemiDoc MP (Bio-Rad) or iBright (Thermo Scientific) imaging systems.

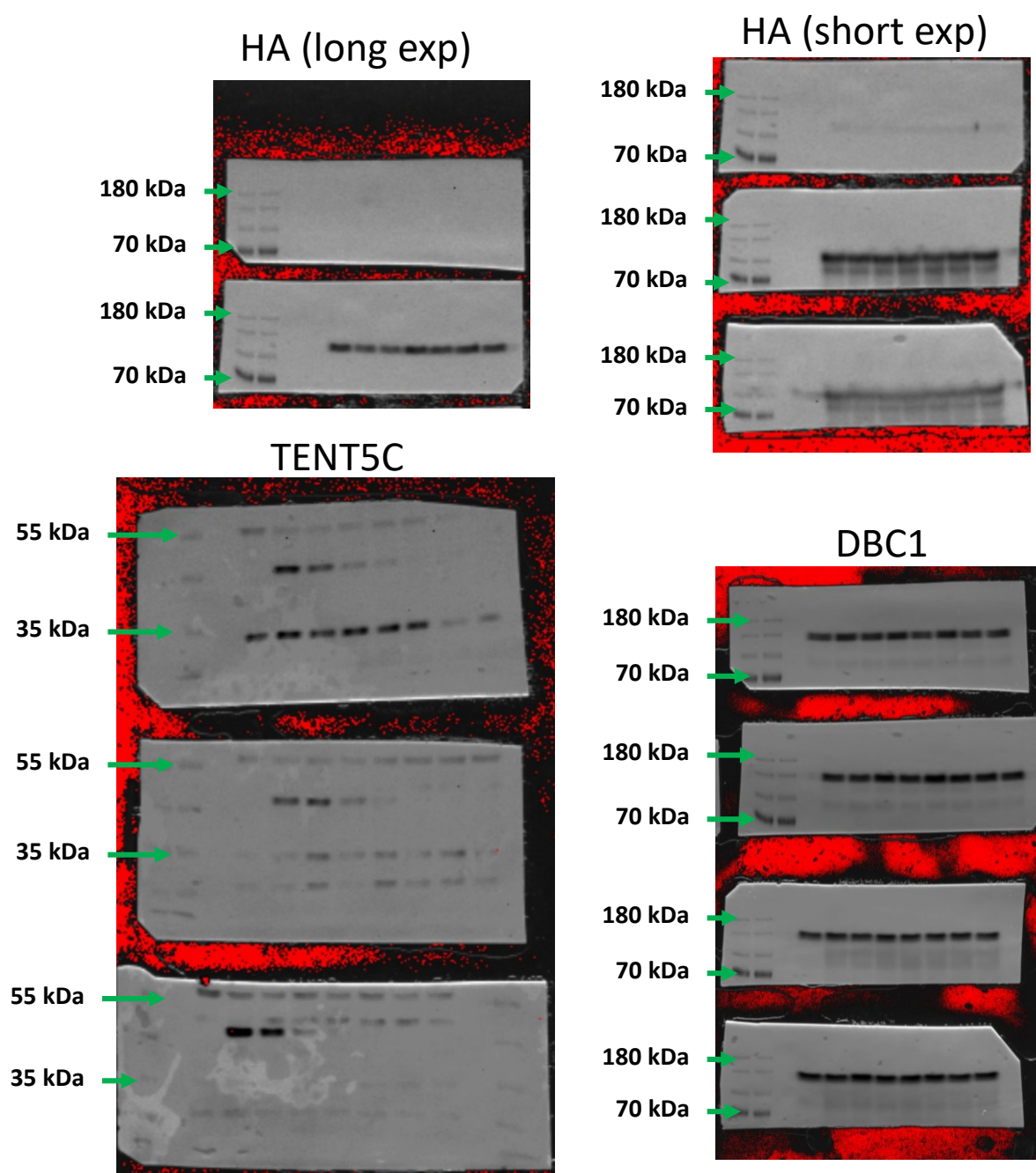
All blots include a Page Ruler Prestained Protein Ladder (Thermo Scientific, #26616). The corresponding molecular weights are 180, 130, 100, 70, 55, 40, 35, 25, 15, and 10 kDa.



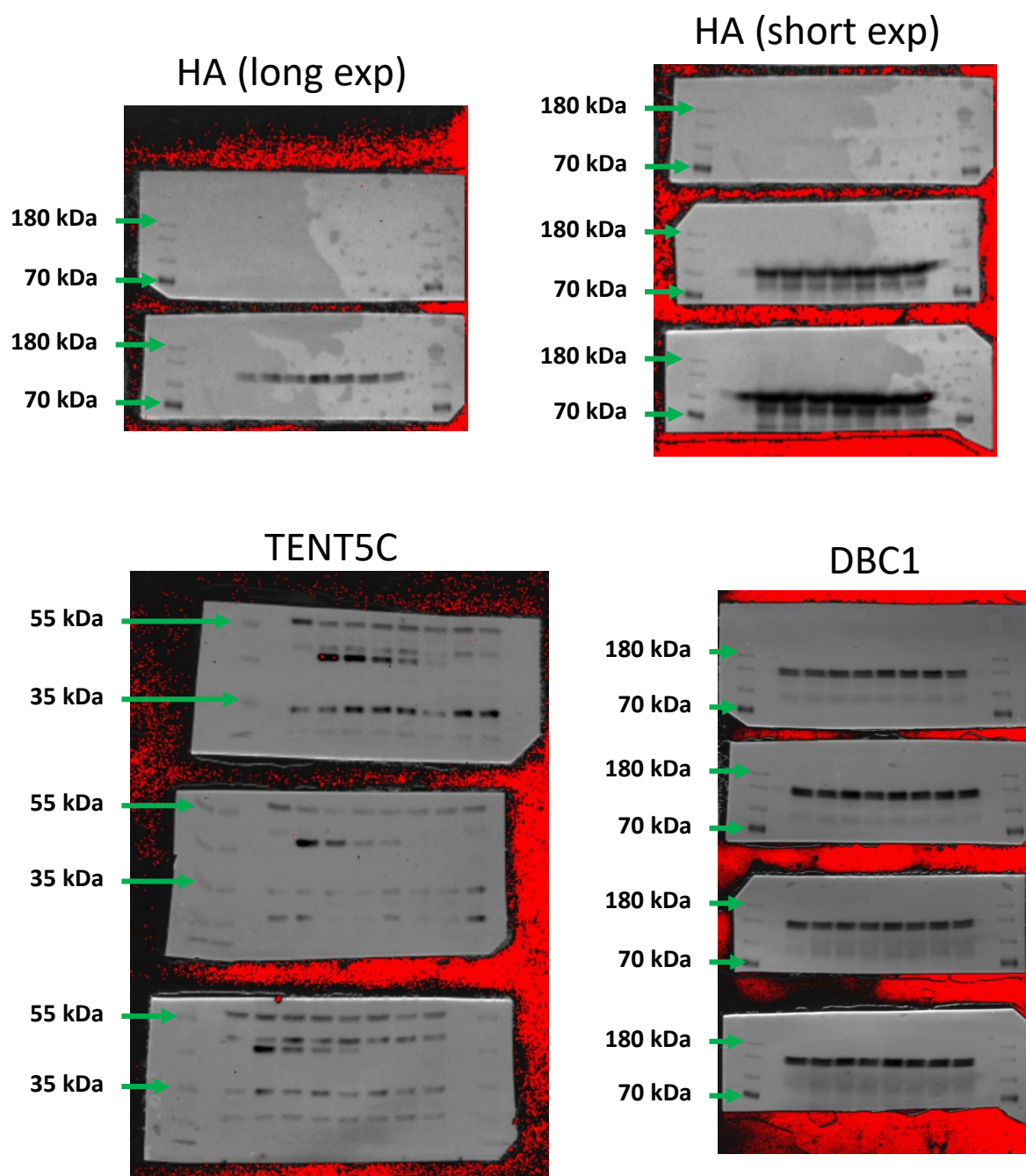
Supplementary Figure 10. Uncropped Western blots related to Figure 3F and Supplementary Figure 2B,D



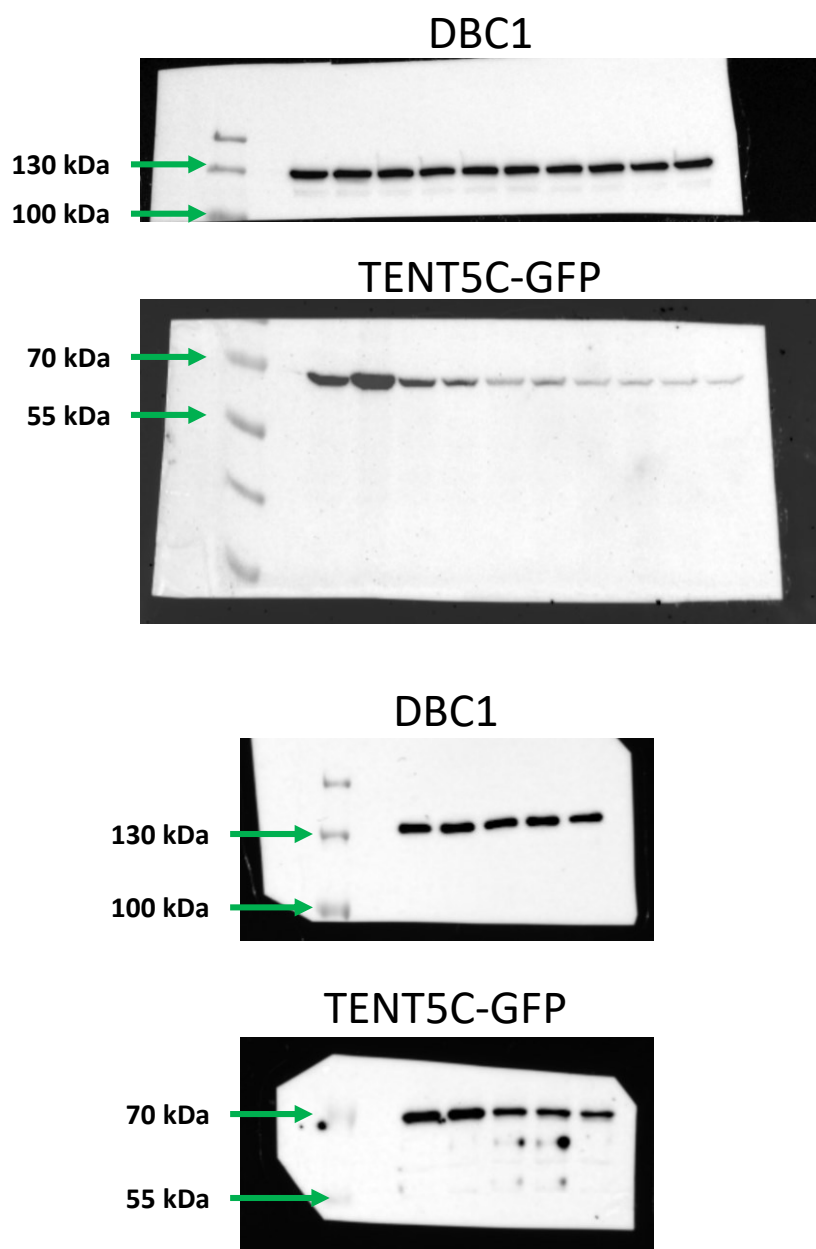
Supplementary Figure 11. Uncropped Western blots related to Figure 5D and Supplementary Figure 3F (part 1)



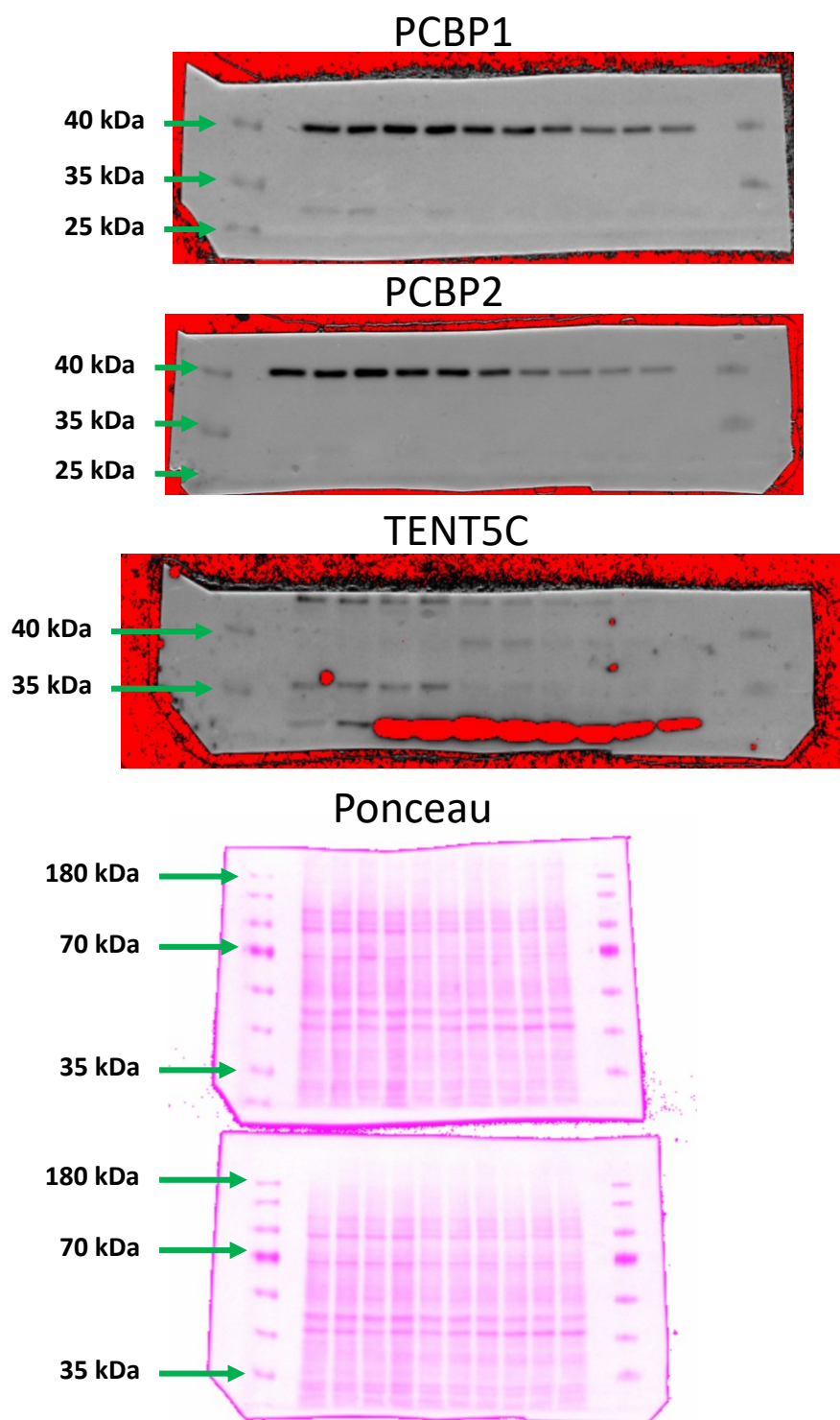
Supplementary Figure 12. Uncropped Western blots related to Figure 5D and Supplementary Figure 3F (part 2)



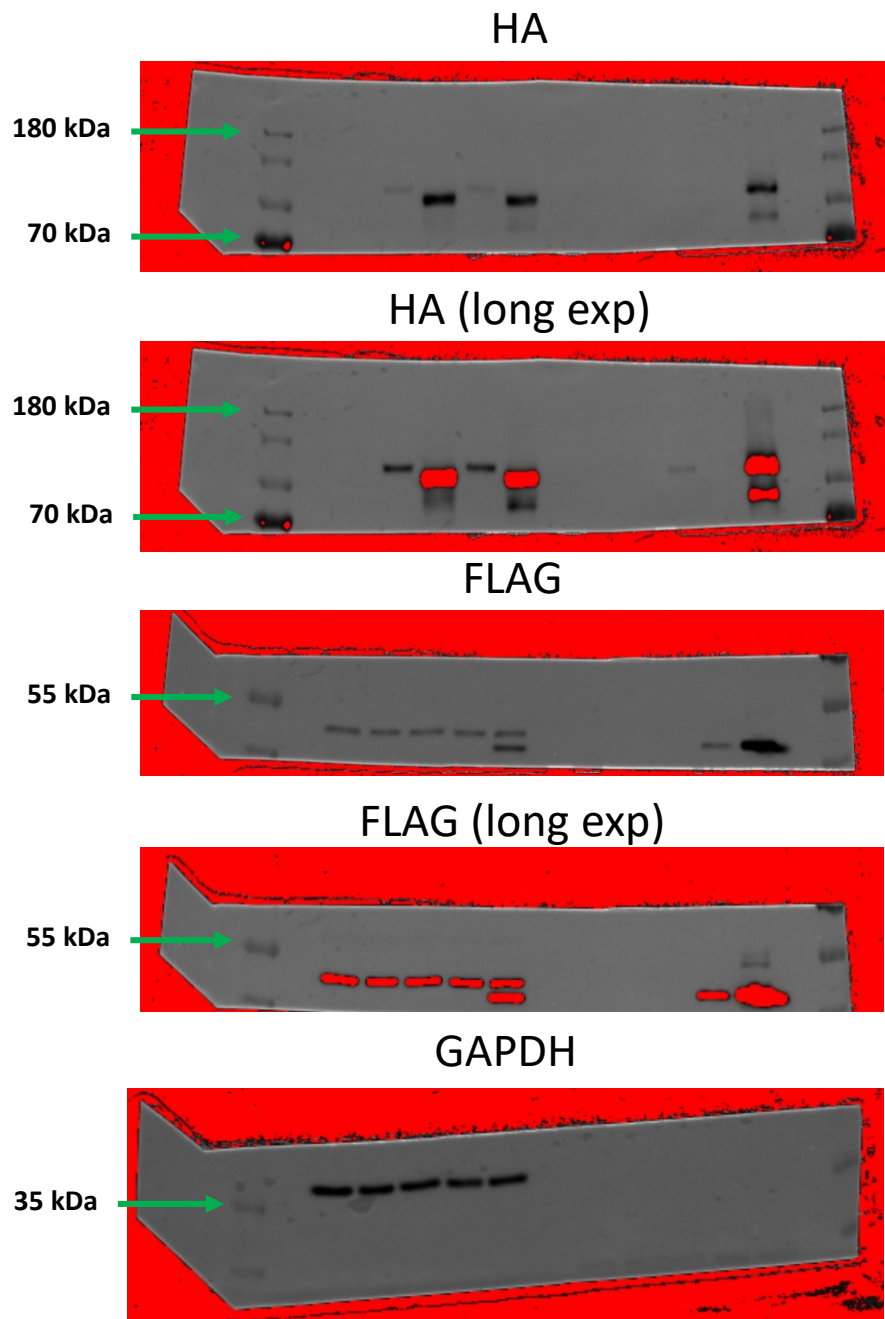
Supplementary Figure 13. Uncropped Western blots related to Figure 5D and Supplementary Figure 3F (part 3)



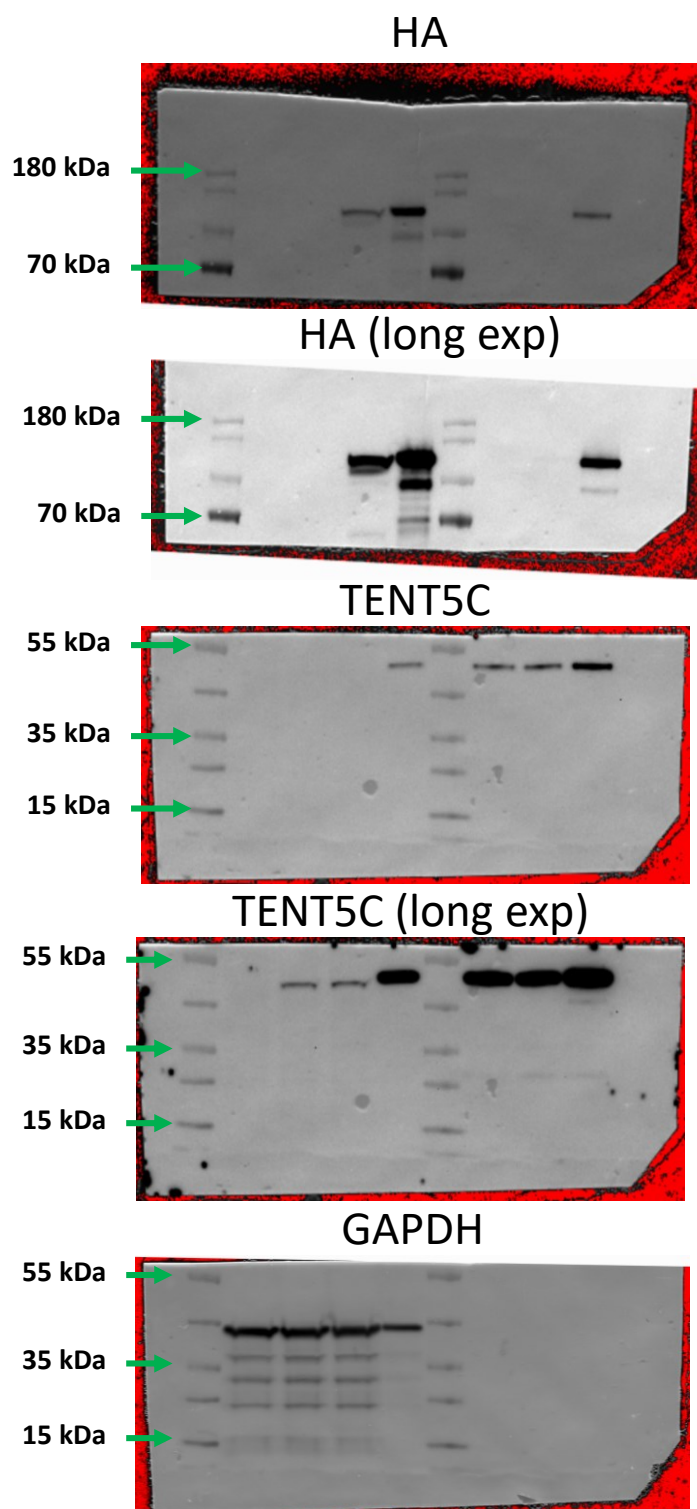
Supplementary Figure 14. Uncropped Western blots related to Figure 7A-C



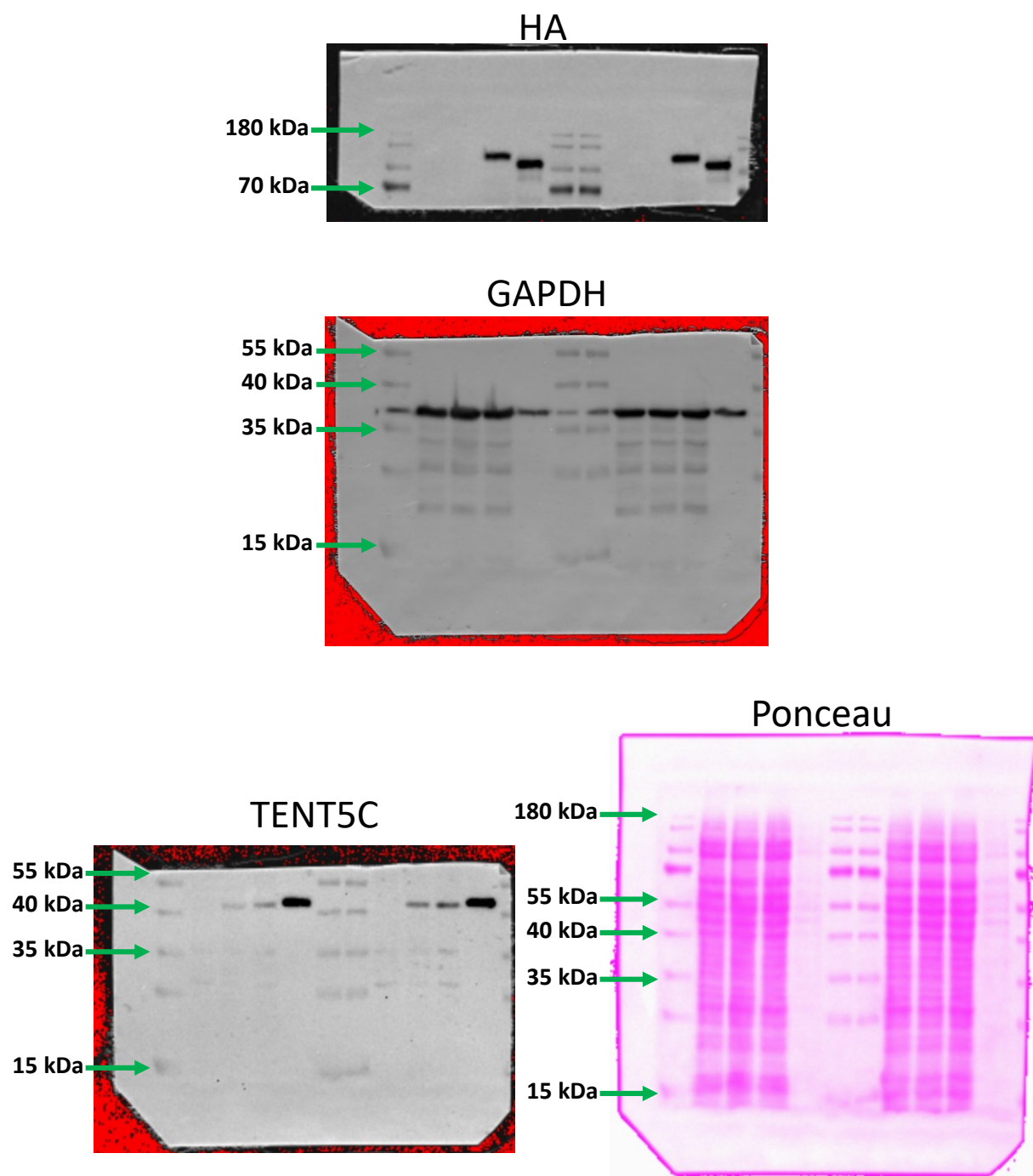
Supplementary Figure 15. Uncropped Western blots related to Supplementary Figure 2A



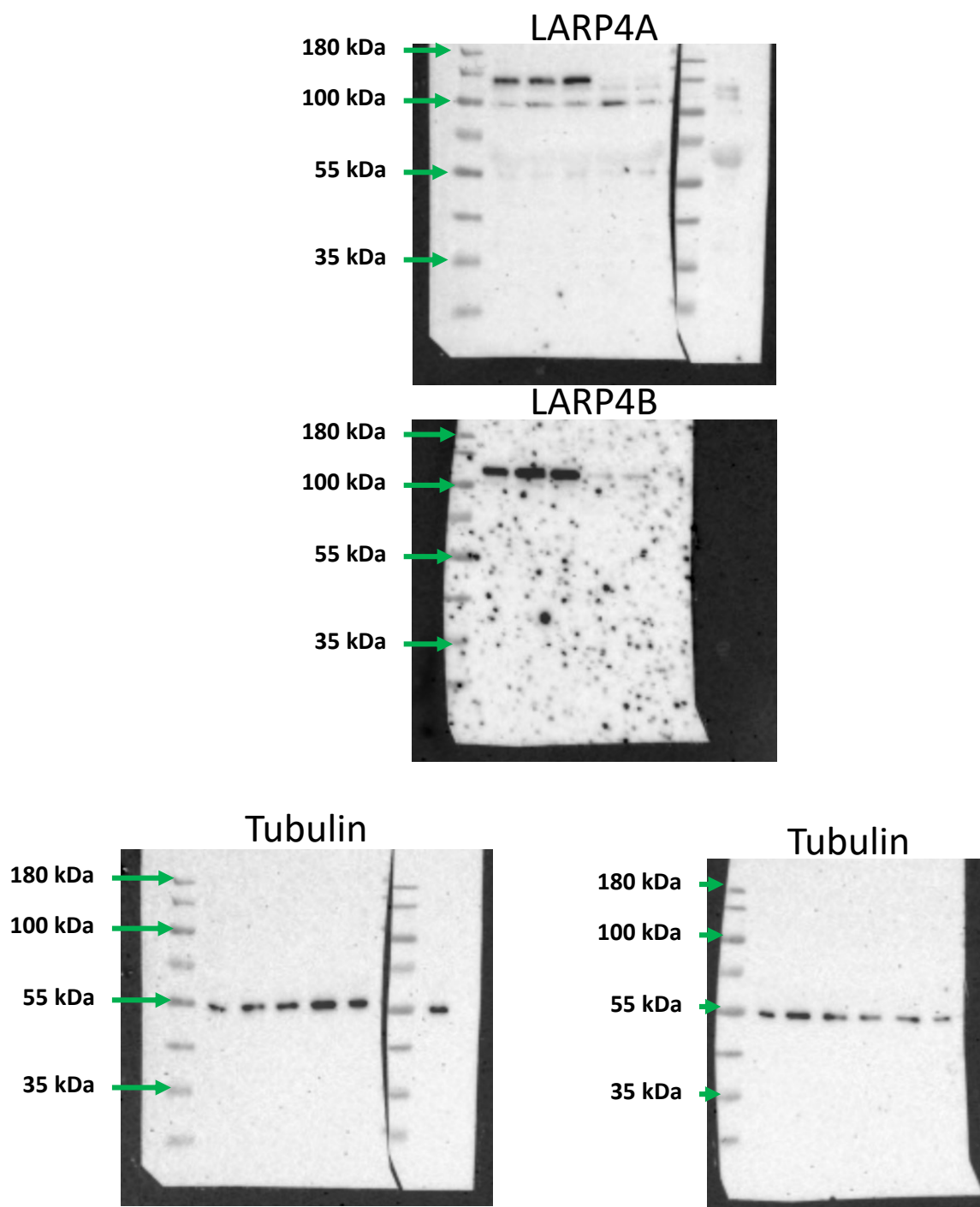
Supplementary Figure 16. Uncropped Western blots related to Supplementary Figure 3C



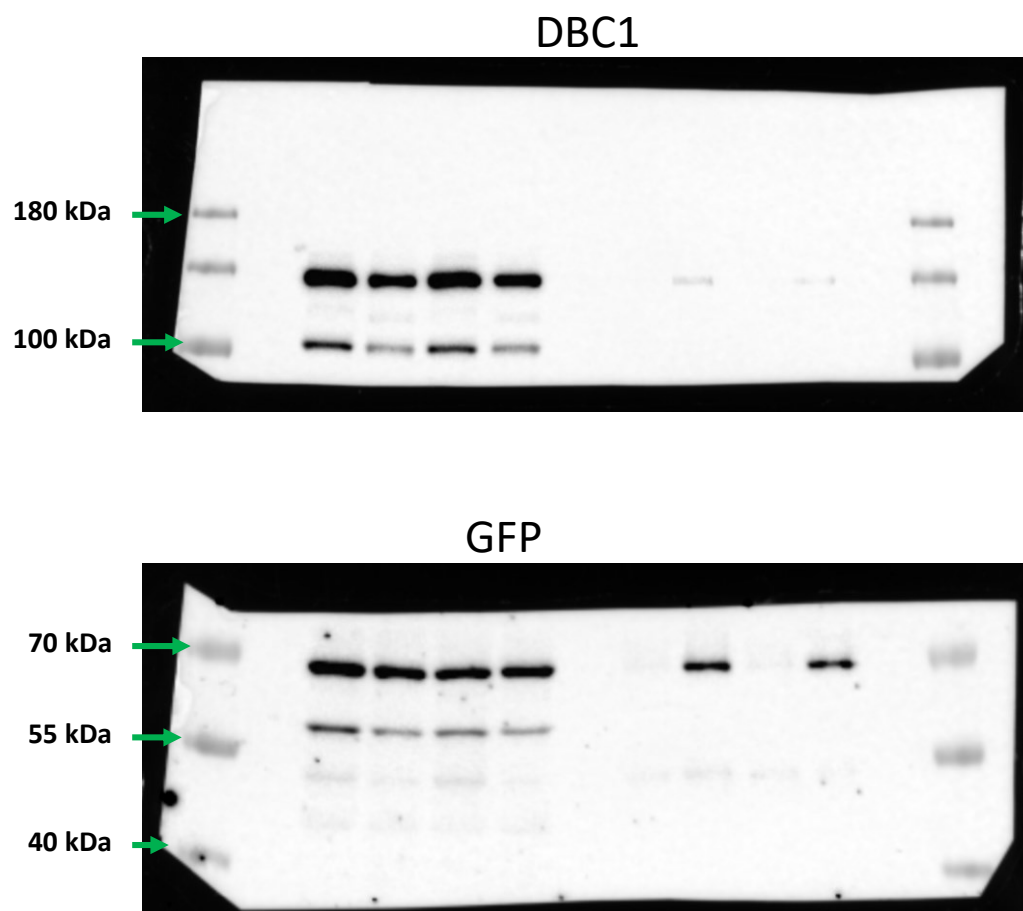
Supplementary Figure 17. Uncropped Western blots related to Supplementary Figure 3D



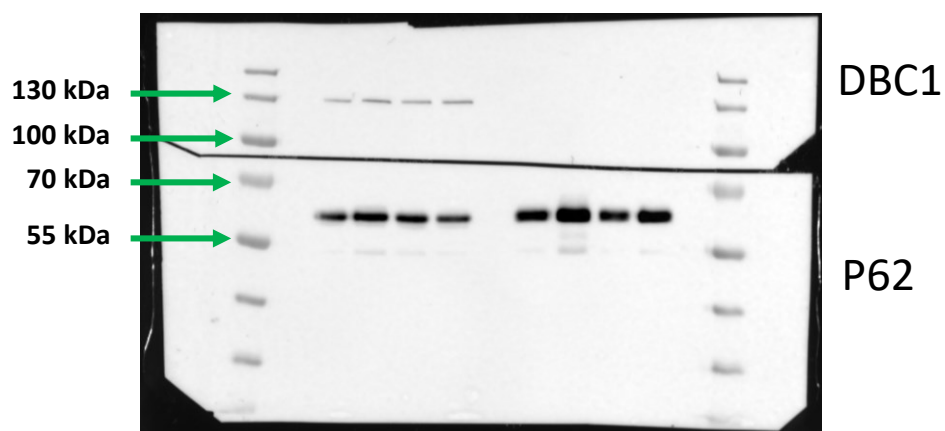
Supplementary Figure 18. Uncropped Western blots related to Supplementary Figure 3E



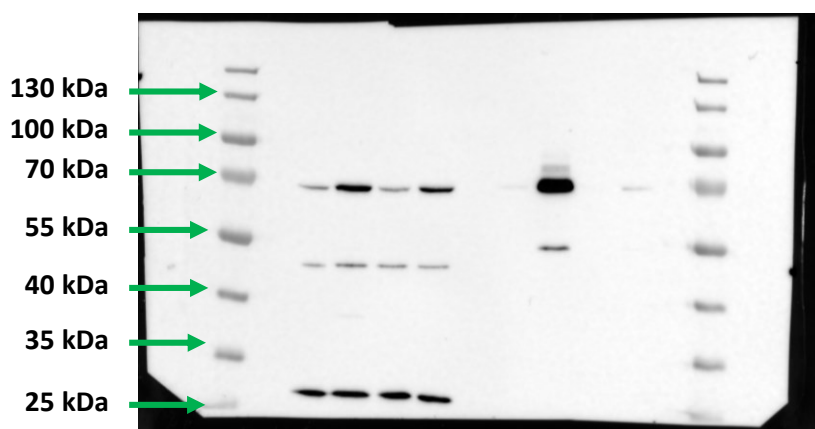
Supplementary Figure 19. Uncropped Western blots related to Supplementary Figure 4A



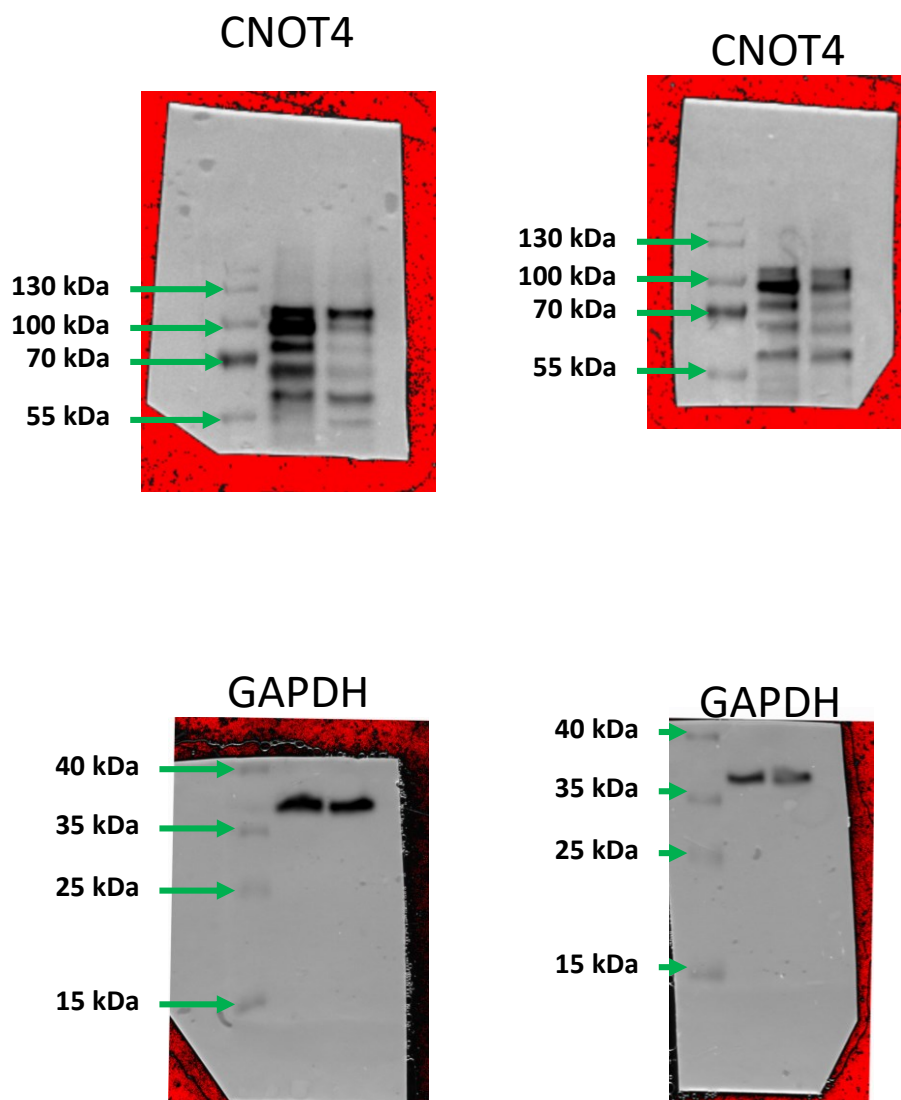
Supplementary Figure 20. Uncropped Western blots related to Supplementary Figure 7A



TENT5C-GFP



Supplementary Figure 21. Uncropped Western blots related to Supplementary Figure 7B



Supplementary Figure 22. Uncropped Western blots related to Supplementary Figure 7C