Supplementary Data File

Title:

TDP-25 ROUTING TO AUTOPHAGY AND PROTEASOME AMELIORATES ITS AGGREGATION IN AMYOTROPHIC LATERAL SCLEROSIS TARGET CELLS.

Authors:

Maria Elena Cicardi [1], ***Riccardo Cristofani*** [1], ***Paola Rusmini*** [1], ***Marco Meroni*** [1], ***Veronica Ferrari***[1], ***Giulia Vezzoli***[1], ***Barbara Tedesco***[1], ***Margherita Piccolella*** [1], ***Elio Messi*** [1], ***Mariarita Galbiati***[1], ***Alessandra Boncoraglio***[1], ***Serena Carra*** [2], ***Valeria Crippa***[1], ***Angelo Poletti*** [1,3§].

Afflation

[1] Department of Excellence: Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB), Centro di Eccellenza sulle Malattie Neurodegenerative, Università degli Studi di Milano, Milano, (Italy).

[2] Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Università degli Studi di Modena e Reggio Emilia, and Center for Neuroscience and Neurotechnology, Modena, Italy

[3] Centro InterUniversitario sulle Malattie Neurodegenerative, Università degli Studi di Firenze, Genova, Roma Tor Vergata and Milano, Italy

[§] Corresponding authors: Angelo Poletti. Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB), Università degli Studi di Milano - Via Balzaretti 9, 20133 Milano (Italy). ph. +39-02-50318215; fax: +39-02-50318204_ e-mail: <angelo.poletti@unimi.it>

FIGURE S1

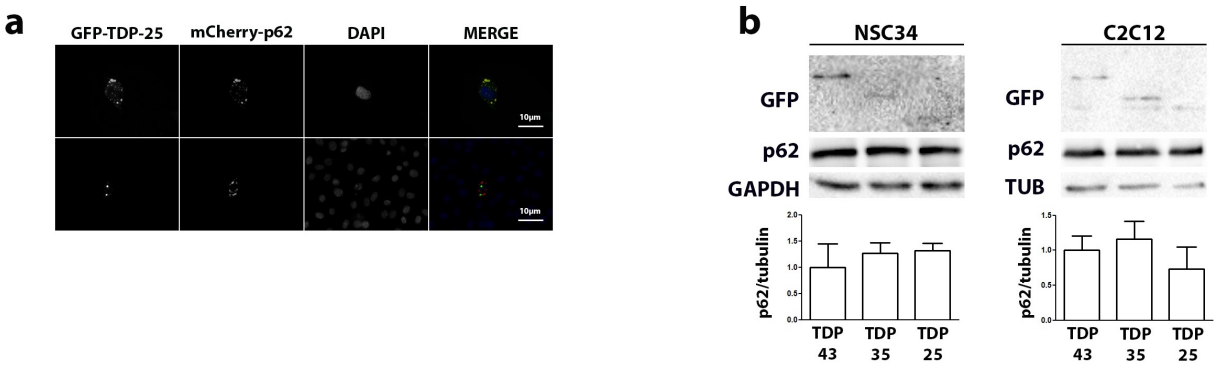

Figure S1: p62 localization and levels upon GFP-TDP-25 expression.
(a) Microscopy analysis. NSC34 and C2C12 were transfected with GFP-TDP-25 and mCherry-p62. Green: GFP-TDP-25. Red: mCherry-p62. Nuclei staining: DAPI. Image were acquired with 63X magnification.
(b) NSC34 and C2C12 were transfected with GFP-TDP-43, GFP-TDP35 and GFP-TDP-25. WB shows GFP-TDPs protein levels and p62 protein levels. Tubulin/GAPDH were used as loading control.
Graphics show p62 levels quantification normalized on tubulin.

FIGURE S2

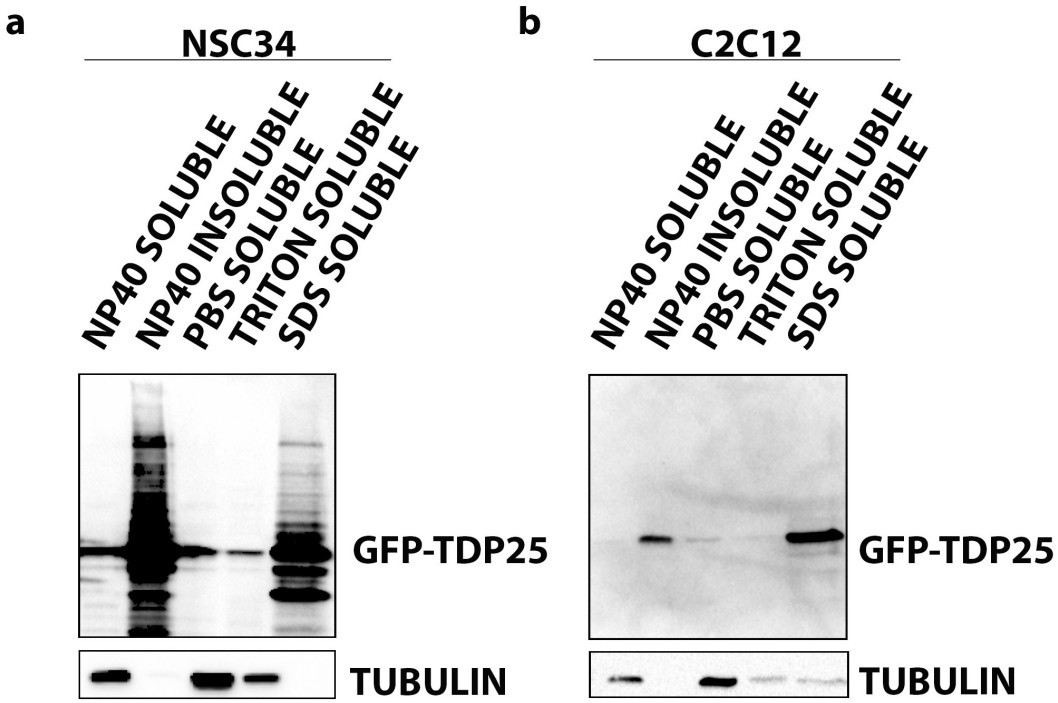

Figure S2: Comparison between fractionation and NP-40 extraction.
(a) Western Blot shows both extracts of fractionation and extracts from NP-40 extraction of NSC34 transiently overexpressing GFP-TDP-25.
(b) Western Blot shows both extracts of fractionation and extracts from NP-40 extraction of C2C12 transiently overexpressing GFP-TDP-25.

FIGURE S3

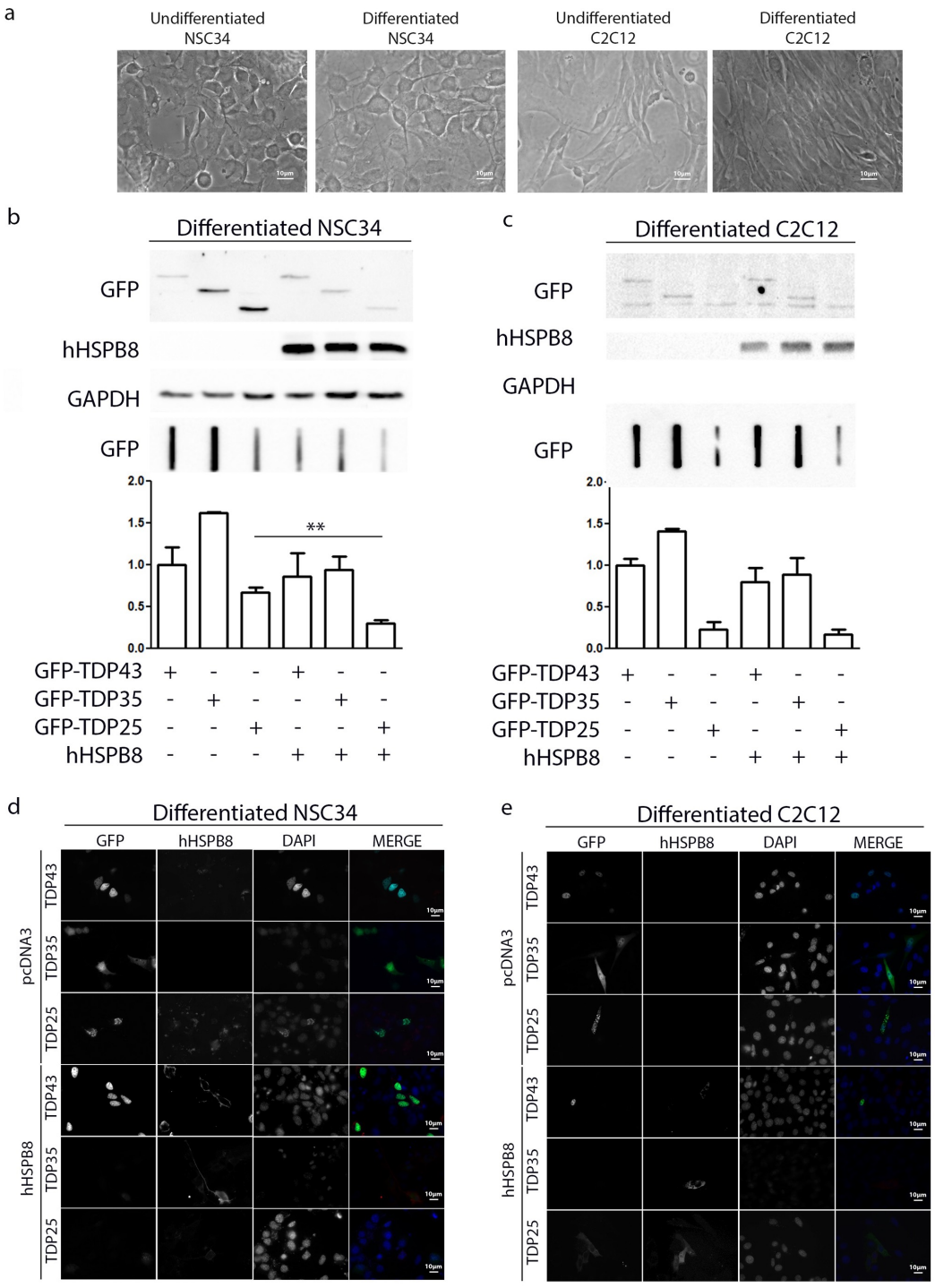

Figure S3: HspB8 pro-degradative activity in differentiated NSC34 and C2C12.
(a) bright field acquisition of undifferentiated and differentiated NSC34 and C2C12 cells. 32X magnification.
(b) Microscope analysis. Differentiated NSC34 transiently overexpressing GFP-TDP-43, GFP-TDP-35 and GFP-TDP-25, and co-transfected with pCI-HspB8 or pcDNA3 as control vector. 63X magnification. Green: GFP-TDPs; Red: hHspB8; nuclei staining: DAPI.
(c) Microscope analysis. Differentiated C2C12 transiently overexpressing GFP-TDP-43, GFP-TDP-35 and GFP-TDP-25, and co-transfected with pCI-HspB8 or pcDNA3 as control vector. 63X magnification. Green: GFP-TDPs; Red: hHspB8; nuclei staining: DAPI.
(d) Differentiated NSC34 transiently overexpressing GFP-TDP-43, GFP-TDP-35 and GFP-TDP-25. Upper inset shows PBS extracts WB analysis. Middle inset shows PBS extracts FRA analysis. Lower inset shows quantification of FRA analysis (* $p<0,05$). (e) Differentiated C2C12 transiently overexpressing GFP-TDP-43, GFP-TDP-35 and GFP-TDP-25. Upper inset shows PBS extracts WB analysis. Middle inset shows PBS extracts FRA analysis. Lower inset shows quantification of FRA analysis.

FIGURE S4

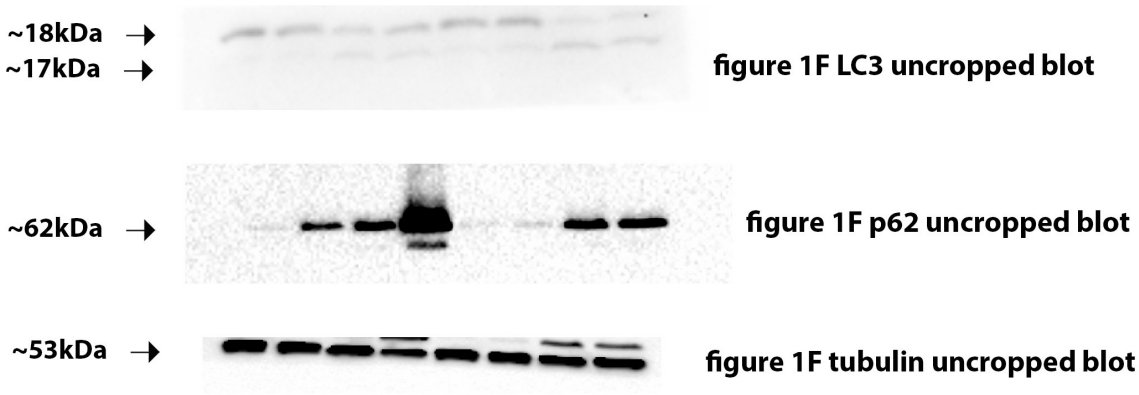

Figure S4: Uncropped Western Blot from Figure 1F.
Blots were halved in order to process the membrane against different antibodies, so the entire blots are not available.

FIGURE S5

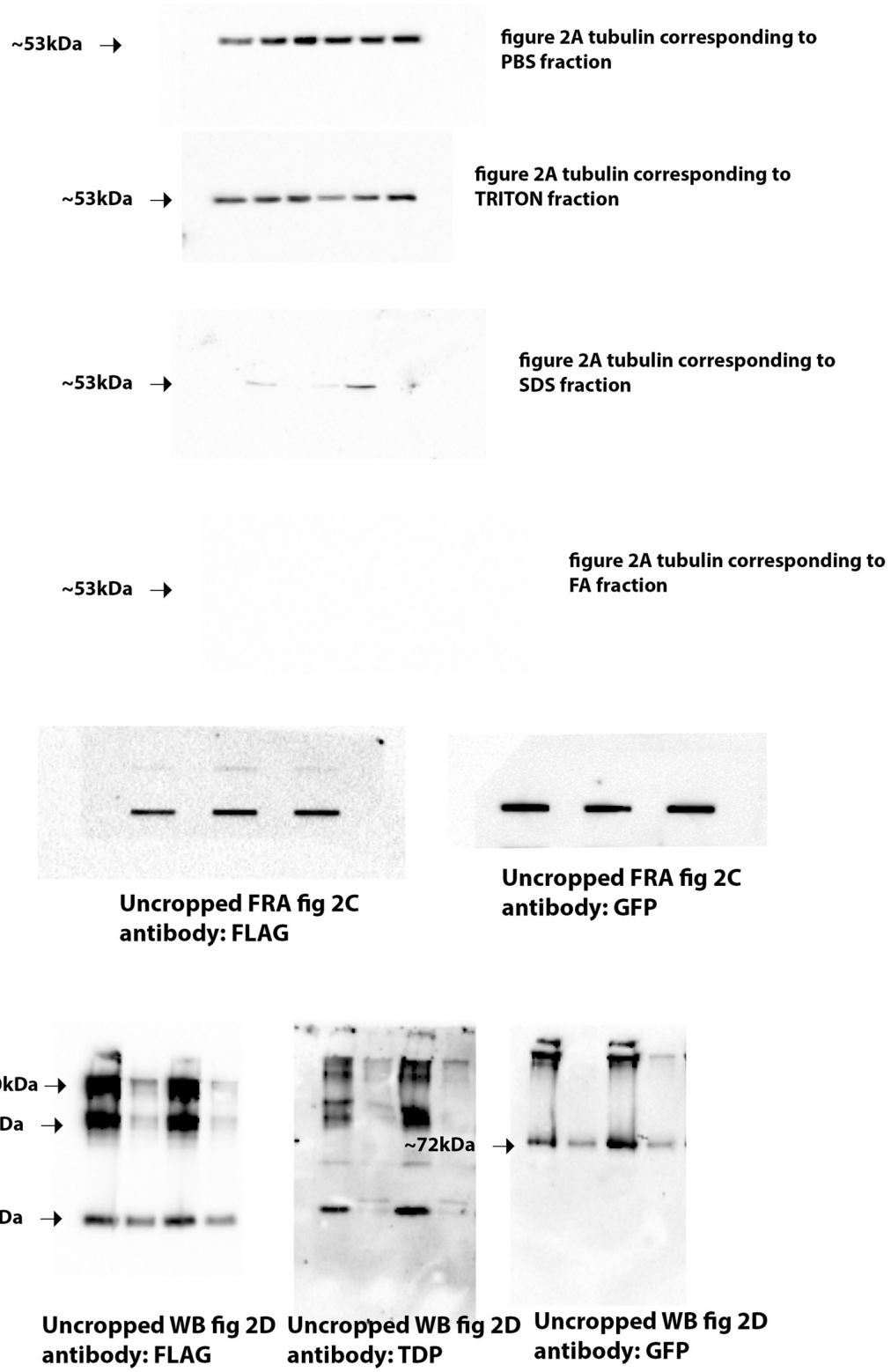

Figure S5: Uncropped Western Blot and Filter Retardation Assay from Figure 2 A, C, D.
Blots were halved in order to process the membrane against different antibodies, so the entire blots are not available.

FIGURE S6

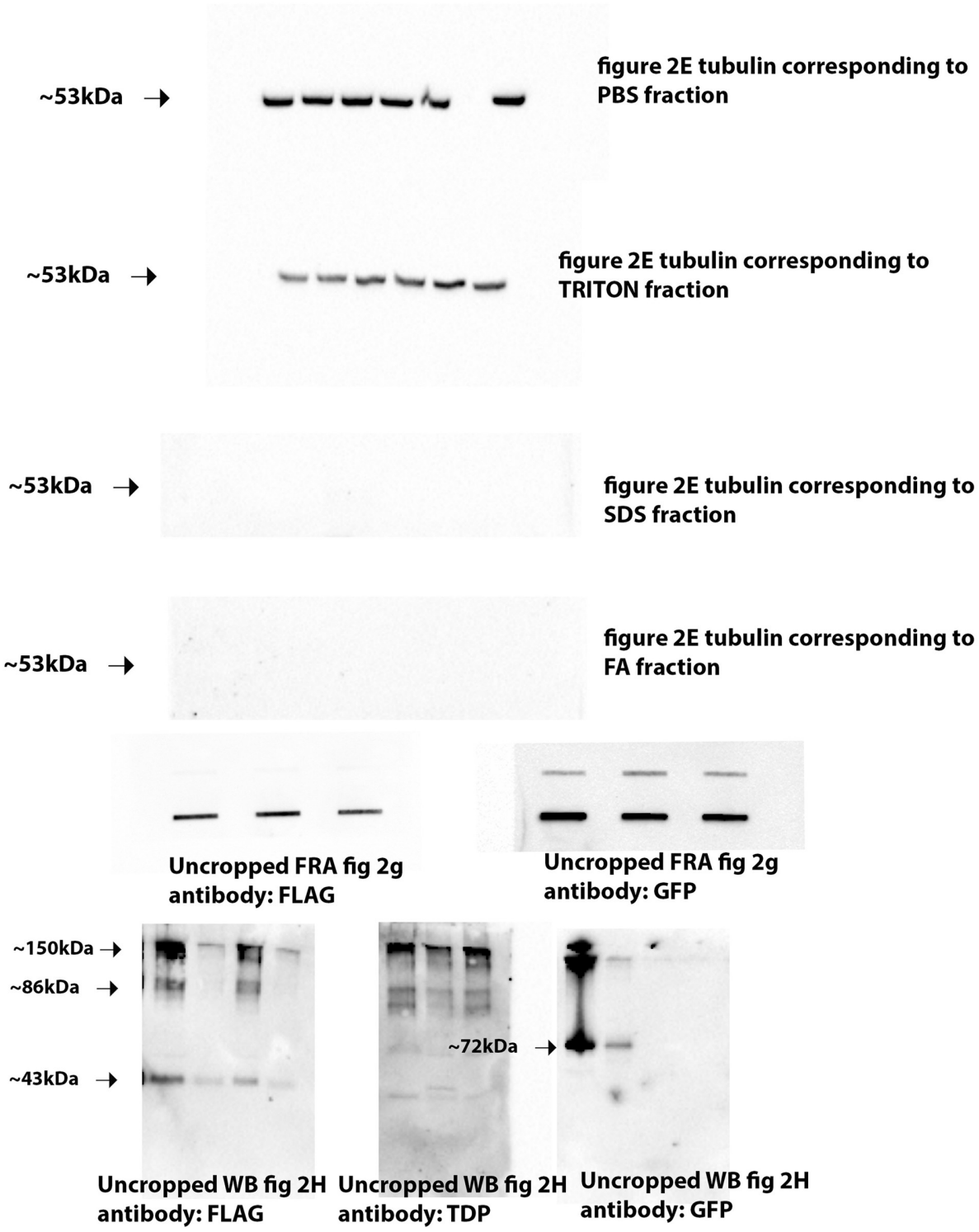

Figure S6: Uncropped Western Blot and Filter Retardation Assay from Figure 2 E, G, H.
Blots were halved in order to process the membrane against different antibodies, so the entire blots are not available.

FIGURE S7

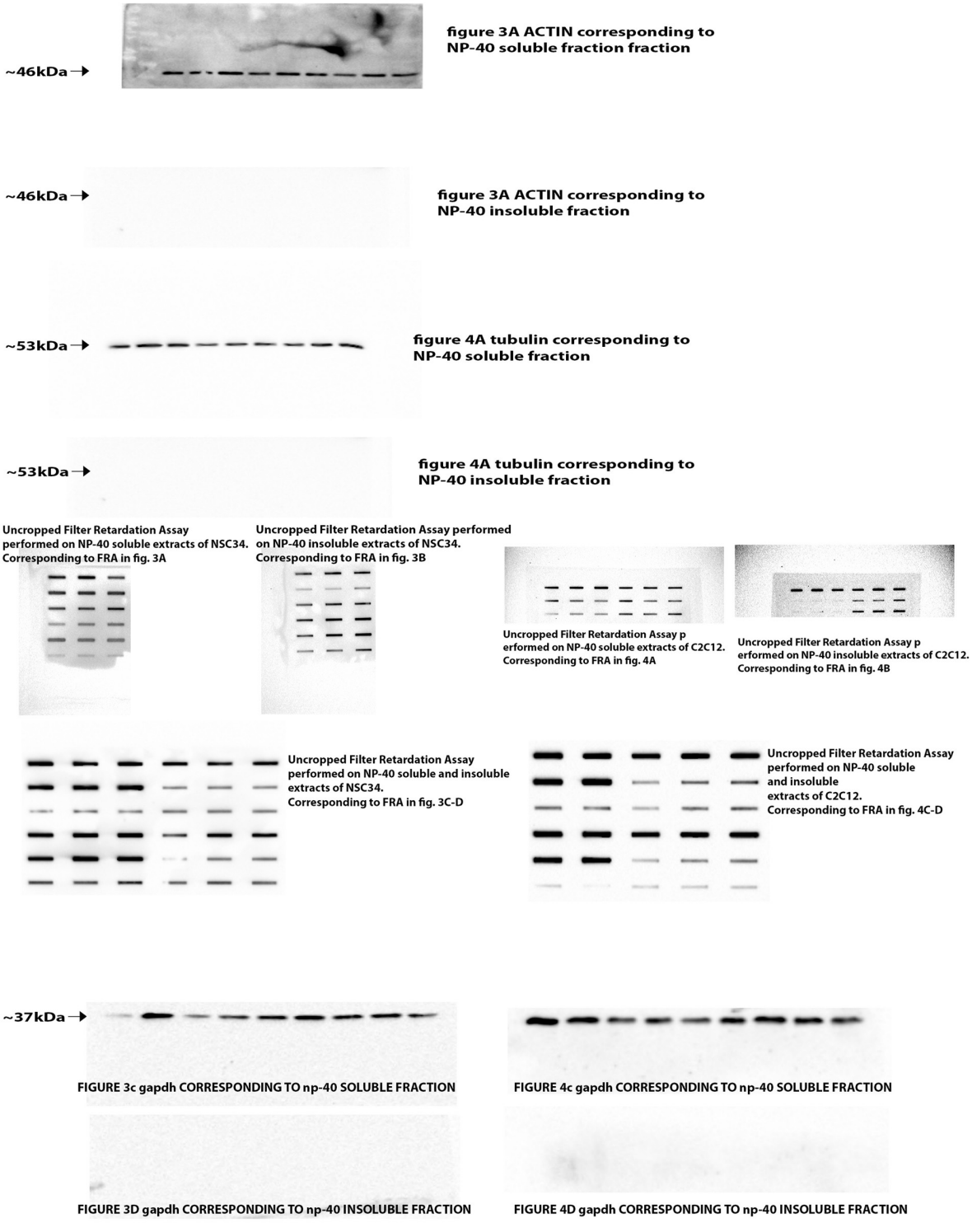

Figure S7: Uncropped Western Blot and Filter Retardation Assay from Figure 3 and Figure 4.
Blots were halved in order to process the membrane against different antibodies, so the entire blots are not available.

FIGURE S8

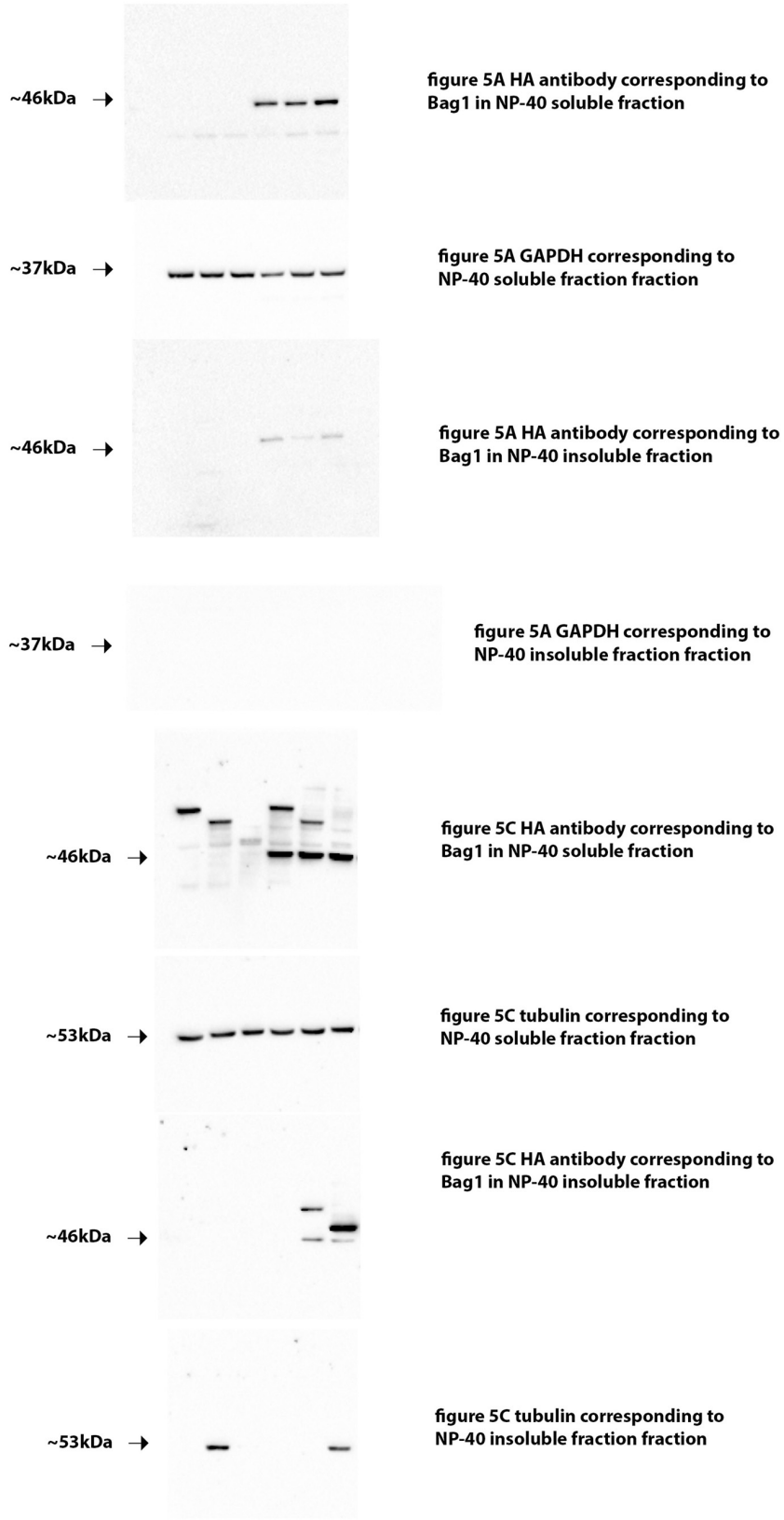

Figure S8: Uncropped Western Blot from Figure 5.
Blots were halved in order to process the membrane against different antibodies, so the entire blots are not available.

FIGURE S9

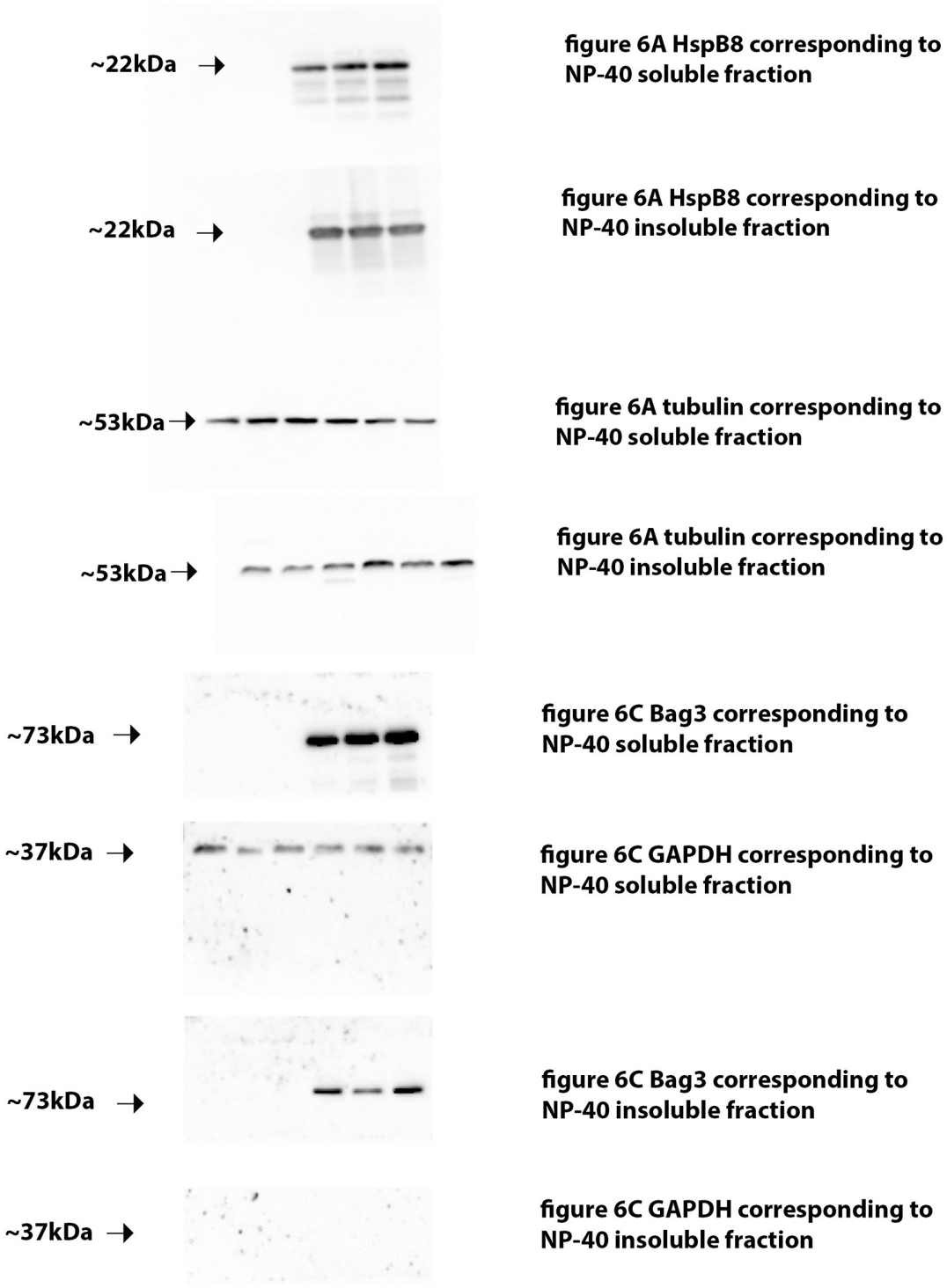

Figure S9: Uncropped Western Blot from Figure 6.
Blots were halved in order to process the membrane against different antibodies, so the entire blots are not available.

FIGURE S10

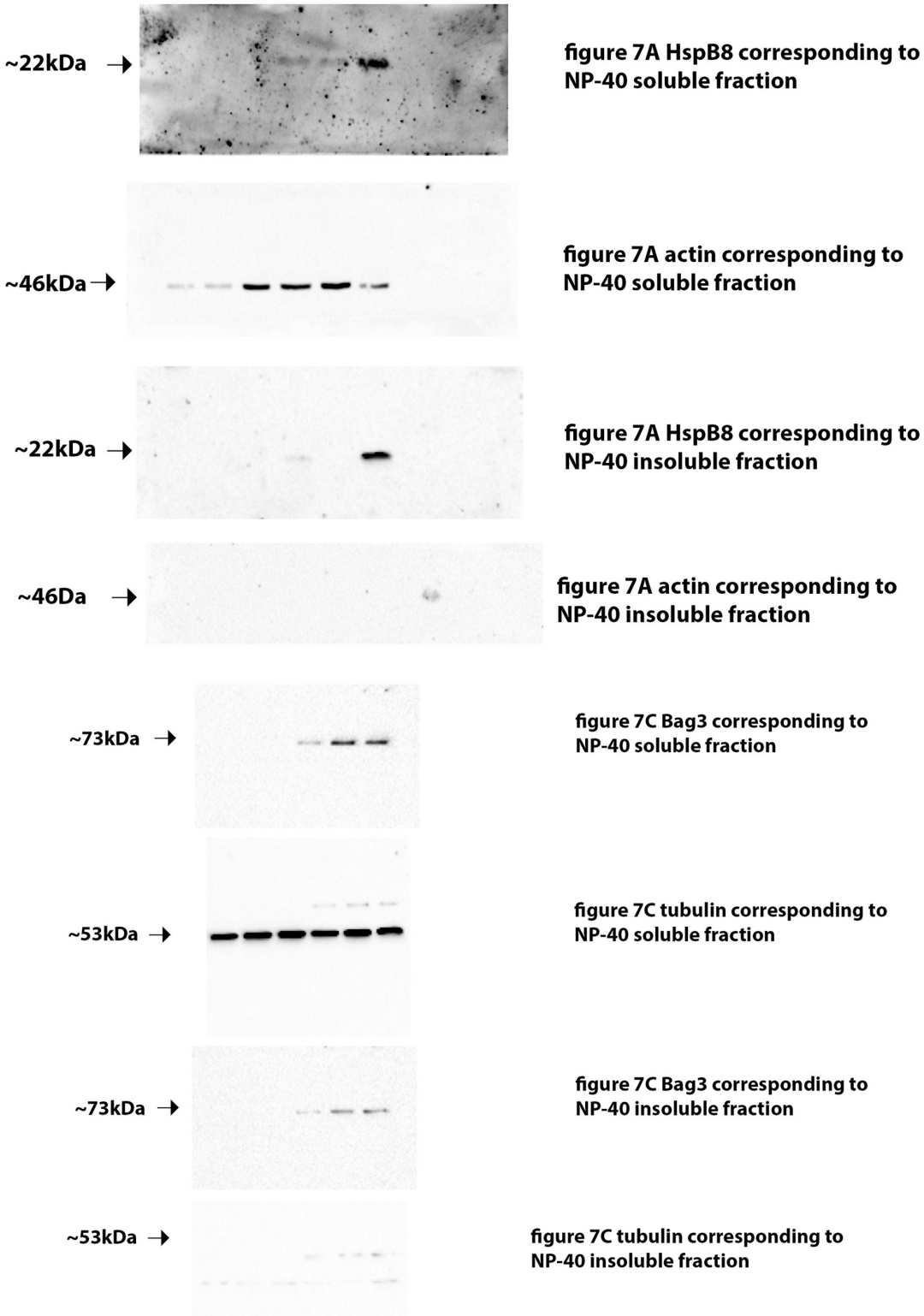

Figure S10: Uncropped Western Blot from Figure 7.
Blots were halved in order to process the membrane against different antibodies, so the entire blots are not available.