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## Supplementary information

# UFM1 E3 ligase promotes recycling of 60S ribosomal subunits from the ER

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# SI Guide

## UFM1 E3 ligase promotes recycling of 60S ribosomal subunits from the ER

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## Legends for Supplementary Information Figures:

**Supplementary Figure 1: Uncropped immunoblots from Figure 1 and SDS-PAGE gel from Figure 2a. a,** Immunoblots from Fig 1d of polysome profiles (sucrose gradient sedimentation of cell lysate) in different genetic backgrounds. *Left*, WT; *center*, UFM1$^{KO}$; *right*, UFSP2$^{KO}$. For each cell line, two gels were run and the associated membranes (membrane 1 and membrane 2) were cut into upper and lower sections. Membrane 1 was probed for NEMF on the upper section; the lower section was probed for uL24 followed by anti-CDK5RAP3. Membrane 2 was probed for UFL1 on the upper section; the lower section was probed for eIF6, followed by an immunoblot for DDRGK1. **b,** Immunoblots from Fig 1e of sucrose gradient sedimentation after *in vitro* UFMylation of 60S ribosomes without (*left*) or with (*right)* ATP present. Samples were run on a single gel and the resulting membrane was cut into an upper section and a lower section. The upper section was probed for CDK5RAP3 followed by UFL1. The lower section was probed for uL24, followed by an immunoblot for DDRGK1. **c,** Immunoblots from Fig 1f of *in vitro* UFMylation of 60S and 80S ribosomes over time. **d,** Coomassie-stained SDS-PAGE from **Fig. 2a** of 3x-FLAG-UFM1 pulldown from cell lysate. Red rectangles indicate the approximate cropping used in the main figures.

**Supplementary Figure 2: Uncropped immunoblots and gels from Figures 4a, 4c, and 4e. a,** Coomassie-stained SDS-PAGE from **Fig. 4a** of 3x-FLAG-UFL1 pulldown from cell lysate. **b,** Immunoblots from **Fig. 4c** of inputs (right *panels*) and ribosome pellets (left *panels*) from UFL1$^{KO}$ cells rescued with 3xFLAG tagged UFL1 truncations. The ribosome pellet immunoblot (*left column*) was probed for UFM1 followed by a probe for the control uL22 in a separate detection channel. Two gels were run for the input resulting in two membranes. One membrane was probed for the FLAG tag of UFL1 followed by GAPDH (*center column*) as a loading control in a separate detection channel. The second membrane was probed for CDK5RAP3, followed by DDRGK1, and finally blotted for GAPDH as a

loading control in a separate detection channel (*right column*). **c,** Immunoblots from **Fig. 4d** of whole cell (WC) lysate (*left*) and cytosolic and membrane fractions (*right).* Ribosome pellet membranes (as indicated) were probed for UFM1 followed by uL22 as a ribosome loading control in a separate detection channel. Input membranes were cut into upper and lower sections. The upper section was probed for DDRGK1 followed by GAPDH in a separate detection channel. The lower section was probed for SEC61β. **d,** Immunoblots from **Fig. 4e** showing representative immunoblots of inputs (*left)* and all replicate ribosome pellets (*right*) for SEC61β (n = 4) (first biological replicate for SEC61β consists of 4 technical replicates) and SEC61α (n=2) translocon association with ribosomes during time courses of puromycin treated WT and UFC1$^{KO}$ cells. Two gels were run for input samples. One membrane was probed for UFC1, second membrane was cut and probed for GAPDH on the upper section, the lower section was probed for SEC61β. Two gels were run for each set of ribosome pellets resulting in two membranes. The first membrane was cut and probed for UFM1 and uL22 (in separate detection channels) on the upper section, the lower section was probed for SEC61β. The second membrane was cut and probed for SEC61α on the upper section, and probed for SEC61β, followed by uL22 (in a separate detection channel) on the lower section. Immunoblot images in **Fig. 4e** are of replicate 2 (Rep 2) as indicated. The additional biological replicates are associated with the quantifications in **Fig. 4e** and **Extended Data Fig. 9c**. Densitometry was taken from the SEC61α, SEC61β, and uL22 blots for quantification in **Fig. 4e** and **Extended Data Fig. 9g**. Lanes in replicate immunoblots were run in the same order as those present in **Fig. 4e**. Red rectangles indicate the approximate cropping used in the main figures or the analogous bands in replicates.

**Supplementary Figure 3: Uncropped immunoblots and gels from Figures 4f, 4h, and 4i**. **a,** Immunoblots from **Fig. 4f** showing representative immunoblots of inputs (*left)* and ribosome pellets (*right*) and replicate ribosome pellets (n = 3) for SEC61 translocon association with ribosomes during time courses of harringtonine treated WT and UFC1$^{KO}$ cells. One gel was run for each set of input samples. The whole membrane was first probed with SEC61α, then SEC61β, followed by UFC1, and

finally GAPDH (in a separate detection channel). Two gels were run for each set of ribosome pellet samples resulting in two membranes. The first membrane was cut and probed for SEC61α on the upper section, and probed for SEC61β, followed by uL22 (in a separate detection channel) on the lower section. The second membrane was probed for UFM1. Immunoblot images in **Fig. 4f** are of replicate 1 (Rep 1) as indicated. The additional biological replicates are associated with the quantifications in **Fig. 4f** and **Extended Data Fig. 9h**. Densitometry was taken from the SEC61α, SEC61β, and uL22 blots for quantification in Fig 4f and **Extended Data Fig 9h**. Lanes in replicate immunoblots were run in the same order as those present in **Fig. 4f**. **b,** Immunoblots from **Fig. 4h** (*far left*) of arrest peptide accumulation in DDRGK1$^{KO}$ cells expressing WT DDRGK1 or DDRGK1 mutants (UFIM$^{mt}$ or ΔEBM) and for all replicates experiments (n = 5) used in the quantification present in **Fig. 4i** (replicates 1, 2, 3, 4, and 5). In replicate 2 (**Fig. 4h**), a single membrane was probed for the FLAG-tagged arrest peptide followed by GAPDH (in the same detection channel) and finally DDRGK1 (in a separate detection channel). In replicate 3, a single membrane was first probed for FLAG and then for GAPDH in the same detection channel. For replicates 1, 4, and 5, the whole membrane was first probed for FLAG and subsequently for GAPDH in two separate channels. For quantification in **Fig. 4i**, densitometry was taken from the relevant bands indicated by the red rectangles corresponding to the following samples: DDRGK1-KO + empty vector, DDRGK1-KO + WT DDRGK1, DDRGK1-KO + UFIM$^{mt}$ DDRGK1, and DDRGK1-KO + ΔEBM DDRGK1. On each replicate immunoblot, lanes were ordered as in **Fig. 4h**. Red rectangles indicate the approximate cropping used in the main figures or the analogous bands in replicates.

**Supplementary Figure 4: Uncropped immunoblots from Figure 5. a,** Immunoblots from **Fig. 5a** of polysome profiles (sucrose gradient sedimentation) of UFSP2$^{KO}$ cell lysate treated with mUFSP1 (*right*) or NEM-inactivated mUFSP1 (*left)*. Two gels were run for each set of samples resulting in two membranes. The first membrane was cut and probed for UFL1 on the upper section, and probed for UFM1, followed by uL22 on the lower section in separate detection channels. The second membrane was probed for CDK5RAP3 followed by a probe for DDRGK1 in the same detection channel. **b,** Immunoblots

from F**ig. 5b** of polysome profiles (sucrose gradient sedimentation) of *in vitro* UFMylated 60S treated with inactive (*left*) or active (*right*) deUFMylase, mUFSP1. One gel was run for each polysome profile and the resulting membranes were cut into upper and lower sections. The lower sections were probed for uL24, followed by DDRGK1 (in the same detection channel), and the upper sections were probed for UFL1. Red rectangles indicate the approximate cropping used in the main figures.

**Supplementary Figure 5: Uncropped immunoblots from Extended Data Figure 1. a,** Immunoblot from **Extended Data Fig. 1b** of miniTurbo knock-in cells. **b,** Immunoblot from **Extended Data Fig. 1c** showing ribosome pelleting experiment gels. Nitrocellulose was stained with Licor Revert Total Protein Stain (Total Protein) prior to immunoblotting for UFM1. **c,** Immunoblots from **Extended Data Fig. 1d** of streptavidin isolated material after increasing time of miniTurbo (mT) knock-in cells with biotin. The membrane was probed for UFC1 followed by DDRGK1(in one detection channel), and finally biotin (via fluorescent streptavidin) in a separate detection channel. **d,** Immunoblots from **Extended Data Fig. 1e** showing SBP-UFM1 and eL36-SBP pulldowns. Total protein stained membranes for eL36-SBP transfected cells (*top*) was a separate experiment from the two lower images. For the SBP-UFM1 and UFM1 pulldown (*middle and bottom*), nitrocellulose was first stained for total protein (as in **b**) followed by immunoblotting for uL24 (RPL26). **e,** Immunoblots from **Extended Data Fig. 1g** of K562 cell lysate sucrose density sedimentation (polysome profile). Three gels (Gels 1, 2, and 3) were run to accomplish blotting for different proteins with rabbit antibodies: the membrane from Gel 1 was immunoblotted for UFM1, the Gel 2 membrane was cut into lower and upper sections and probed for eIF6 and NEMF, respectively, and the membrane for Gel 3 was probed for uL24. Densitometry of uL24-UFM1 (monoUFMylated), eIF6, and NEMF were performed to show the correlation between the co-sedimentation of UFMylated uL24 with 60S subunits and is plotted in **Extended Data Fig. 1h**. **f,** Immunoblots from **Extended Data Fig. 1i** showing fractionation of materials used in **Fig. 1d**. Westerns were probed for UFM1 followed by UFSP2 in separate detection channels. **g,** Immunoblots from **Extended Data Fig. 1j** showing the additional fractionation controls relevant to **Extended Data Fig. 1i**

and **Fig. 1d**. A single gel was run and the nitrocellulose membrane was cut into two sections. The top section was probed for DDRGK1 in one detection channel, followed by GAPDH and finally uL22 in a second detection channel. The bottom section of the gel was probed for SEC61β. **h,** Immunoblots from **Extended Data Fig. 1k** and related biological replicate data quantified via densitometry and shown in **Extended Data Fig. 1l**. The data shown in **Extended Data Fig. 1k** of ribosome pellets with or without anisomycin (ANS) came from Replicate 2 (Rep 2) as indicated. Biological replicates for **Extended Data Fig. 1k** were run on the same gel in the same order (as shown). Two gels were run to probe for all proteins. The membrane from one gel was cut into two sections and probed for UFL1 (top section) and DDRGK1 (bottom section). The membrane from the second gel was cut into two sections also and probed for UFM1 (lower section) followed by uL22 (in a separate detection channel). The upper section was probed for CDK5RAP3. **i,** Immunoblots from **Extended Data Fig. 1m** of a sucrose gradient sedimentation of an *in vitro* UFMylated mixture of 60S and 80S ribosomes. A single gel was run and the corresponding membrane was cut into upper and lower sections. The upper section was probed for UFL1 and the lower section was probed for uL24. **j,** Immunoblots from **Extended Data Fig. 1n** of a sucrose gradient sedimentation of *in vitro* UFMylated 80S ribosomes. A single gel was run and the corresponding membrane was cut into an upper and lower section. The upper section was probed for UFL1 and the lower section was probed for uL24. Red rectangles indicate the approximate cropping used in the Extended Data figures or the analogous bands in replicates.

**Supplementary Figure 6: Uncropped immunoblots from Extended Data Figure 9. a,** Immunoblots from **Extended Data Fig. 9c** and related triplicate ribosome pellet blots for quantifications in **Extended Data Fig. 9d**. Replicate 3 (Rep 3) was used for the blot shown in **Extended Data Fig. 9c**. For biological replicates 2 and 3 (*left*) a single gel was run for each of the ribosome pellets and the inputs. The ribosome pellet membrane was cut into upper and lower sections. The upper section was probed for UFL1. The lower section was probed for CDK5RAP3 followed by UFM1 (in one detection channel) followed by uL22 (in a separate detection channel). The input membrane was also cut into two sections; the upper

section was probed for UFL1 while the lower section was probed for DDRGK1 followed by GAPDH (in a separate detection channel). Biological replicate 1 data was collected from two gels, one membrane was probed for UFL1, the second membrane was probed for CDK5RAP3 followed by UFM1, followed by uL22 as above. Densitometry was taken from the CDK5RAP3 and UFL1 blots for quantification in **Extended Data Fig. 9d**. **b,**. Immunoblots from **Extended Data Fig 9i** showing representative immunoblots of inputs (*left)* and ribosome pellets (*right*) and replicate ribosome pellets (n = 3) for SEC61 translocon association with ribosomes during harringtonine time courses with WT and UFM1$^{KO}$ cells. One gel was run for each set of input samples. The whole membrane was first probed with SEC61α, then SEC61β in one detection channel, followed by uL22, and finally GAPDH (in a separate detection channel). Two gels were run for each set of ribosome pellet samples. The membrane of the first gel was cut and probed for SEC61α on the upper section, and probed for SEC61β, followed by uL22 (in a separate detection channel) on the lower section. The membrane of the second gel was probed for UFM1. Immunoblot images in **Extended Data Fig. 9i** are of replicate 1 (Rep 1) as indicated. The additional biological replicates are associated with the quantifications in **Extended Data Fig. 9i**. Densitometry was taken from the SEC61α, SEC61β, and uL22 blots for quantification in **Extended Data Fig 9i**. Red rectangles indicate the approximate cropping used in the Extended Data figures or the analogous bands in replicates.

**Supplementary Information Tables:** Tables named below are present in a separate supplementary tables spreadsheet file with each table in the indicated tab:

**Supplementary Table 1 |** TMT-MS3 analysis of miniTurbo-UFM1 affinity purification in WT and UBA5 KO cells. Analyzed with Comet (2019.01 rev. 5) (Related to Fig. 1a, and Extended Data Fig. 1a)

**Supplementary Table 2 |** Identified SBP-UFM1 affinity captured proteins from crude ribosomes (Related to Fig. 1b, c)

**Supplementary Table 3 |** Quantification/densitometry of bands of fractions in Extended Data Fig. 1g for correlations between UFMylated uL24 and NEMF and eIF6. (Related to Extended Data Fig. 1g, h )

**Supplementary Table 4 |** Quantification/densitometry of mono-UFMylated uL24, UFL1, DDRGK1, and CDK5RAP3 bands from biological replicates used to generate Extended Data Fig. 1l (Related to Extended Data Fig. 1k, l)

**Supplementary Table 5 |** Quantification/densitometry of bands of co-sedimenting UFL1 and CDK5RAP3 with ribosomes used to generate Extended Data Fig. 9d for DDRGK1 rescues (Related to Extended Data Fig. 9c, d)

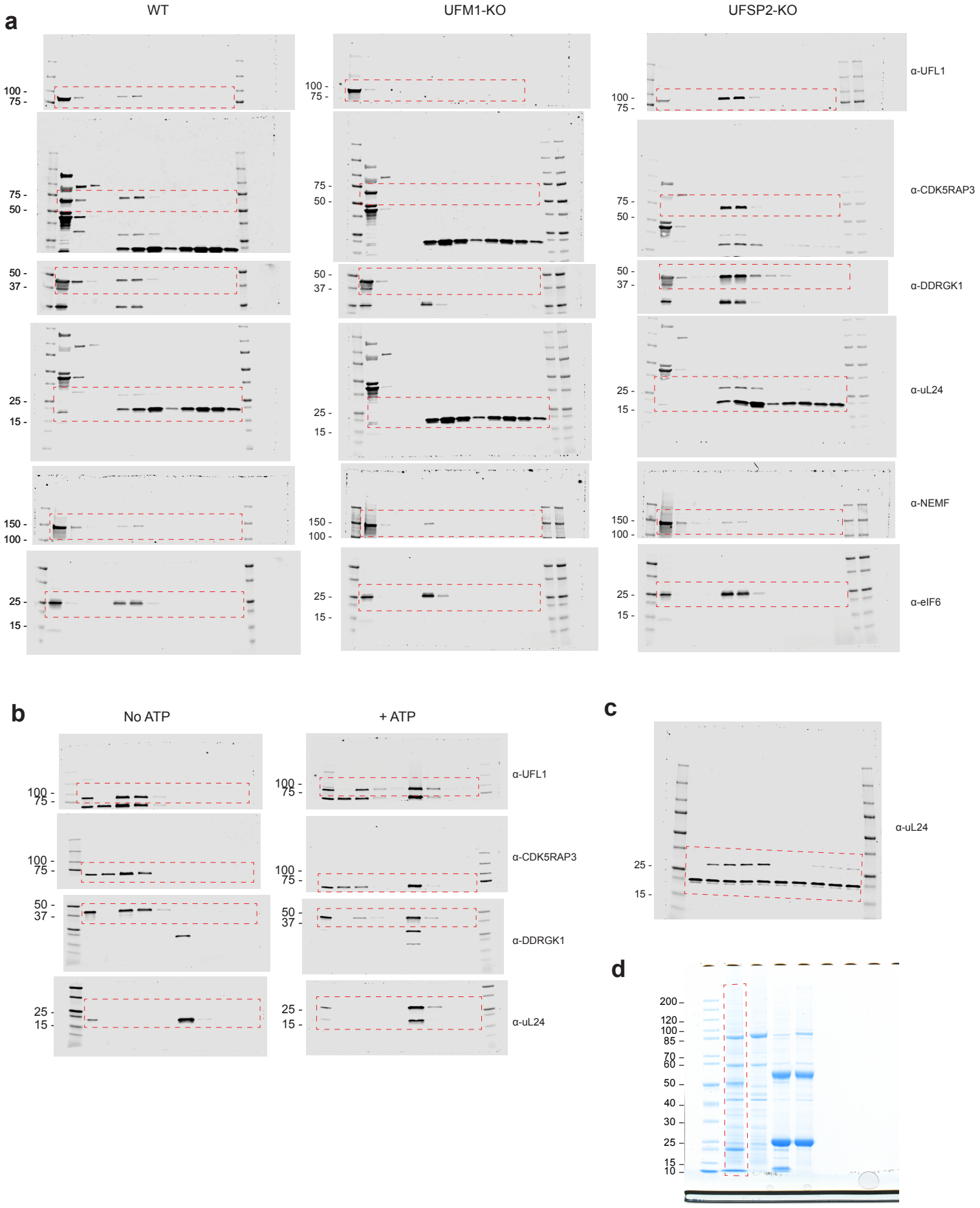
**Supplementary Table 6 |** Quantification/densitometry of SEC61α or SEC61β band intensities in ribosome pellets from biological replicates of puromycin treated WT and UFC1-KO cells (Related to Fig. 4e, Extended Data Fig. 9g )

**Supplementary Table 7 |** Quantification/densitometry of SEC61α or SEC61β band intensities in ribosome pellets from biological replicates of HT treated WT and UFC1-KO cells (Related to Fig. 4f, Ext. Data Fig. 9h)

**Supplementary Table 8 |** Quantification/densitometry of SEC61α or SEC61β band intensities in ribosome pellets from biological replicats of HT treated WT and UFM1-KO cells (Related to Ext. Data Fig 9i)

**Supplementary Table 9 |** Quantification/densitometry of ER-AP intensities from data represented in Fig. 4h with n = 5 biological replicates (Related to Fig. 4h, i)

DaRosa et al., Sup. Figure 1



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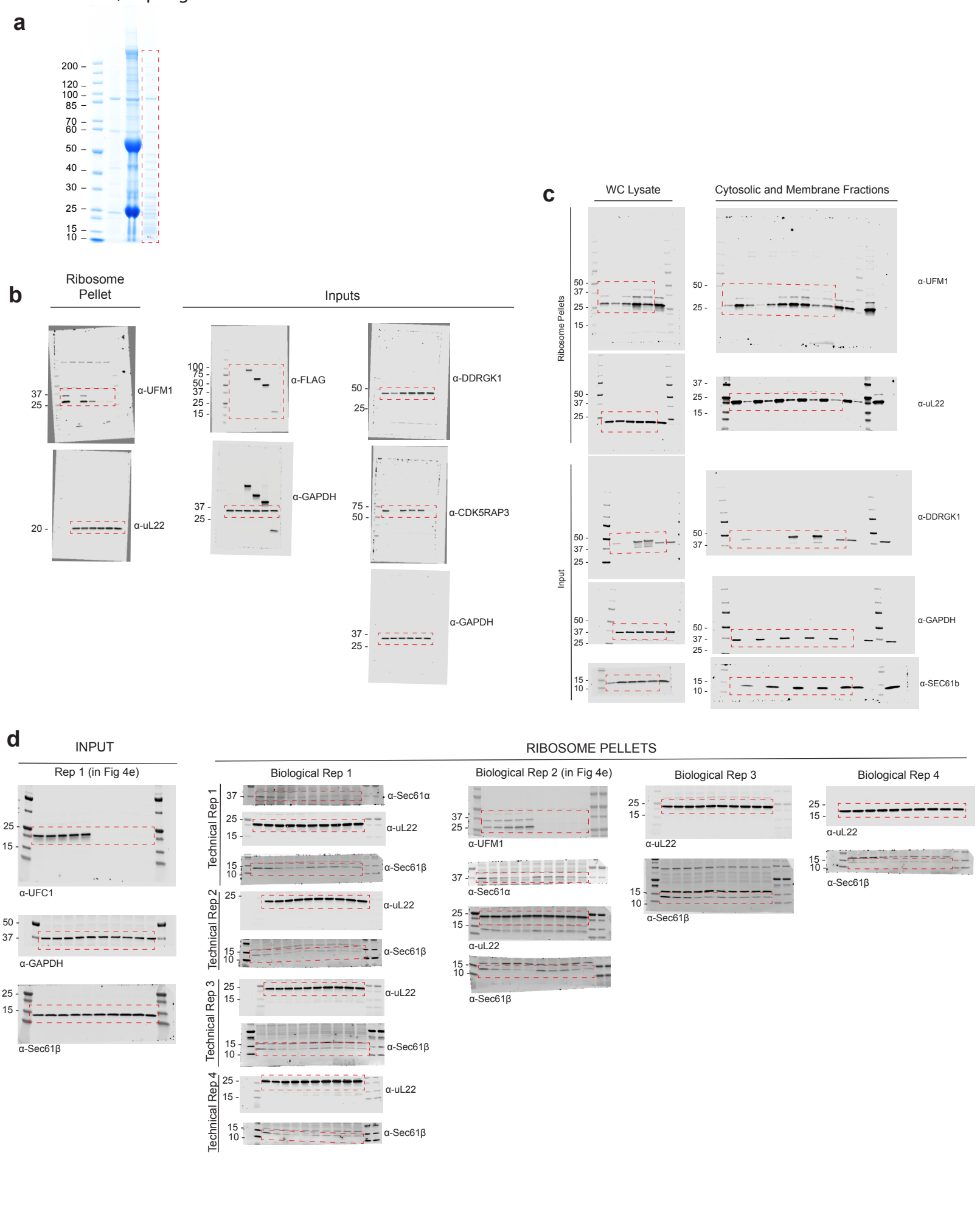

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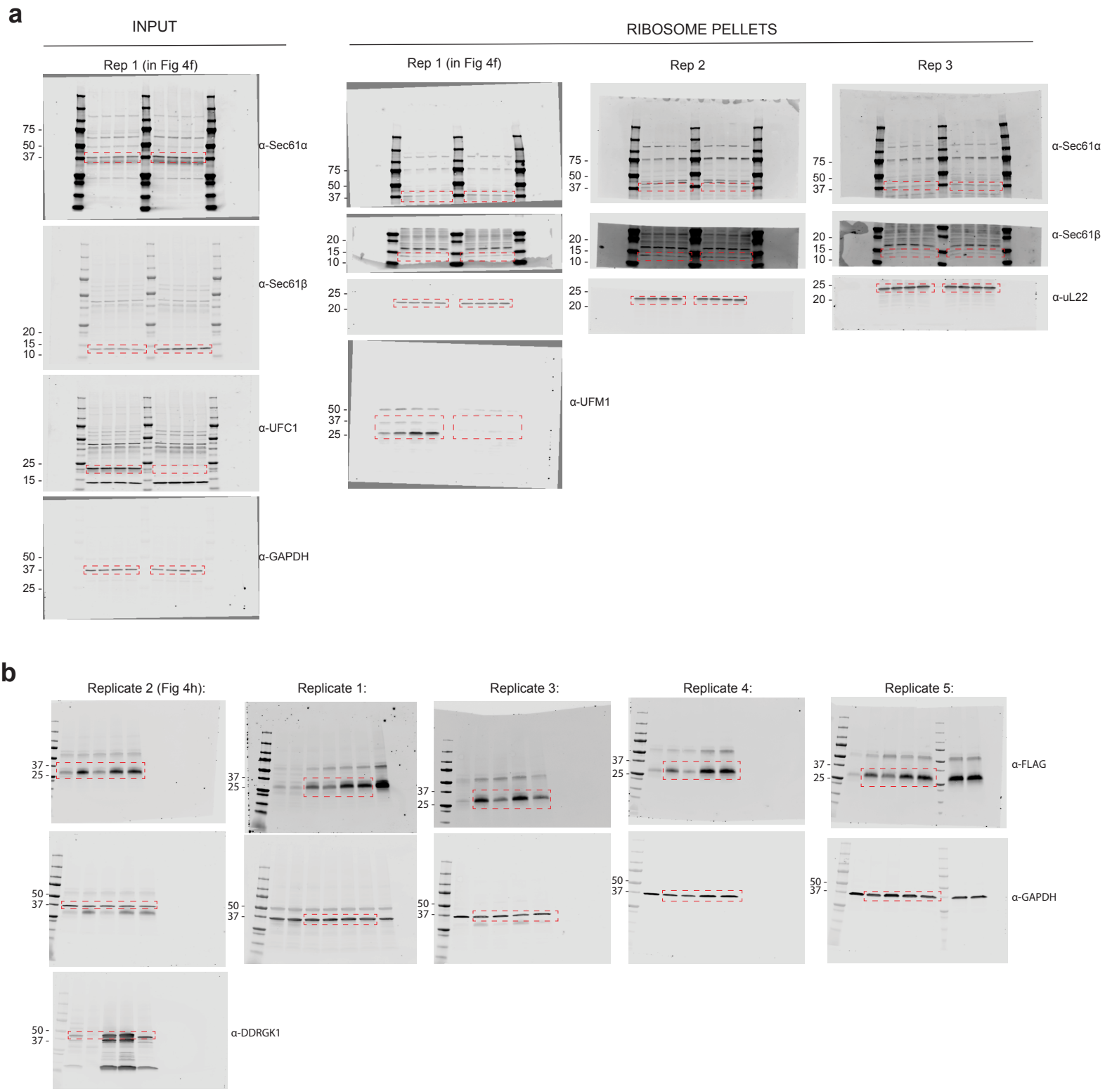

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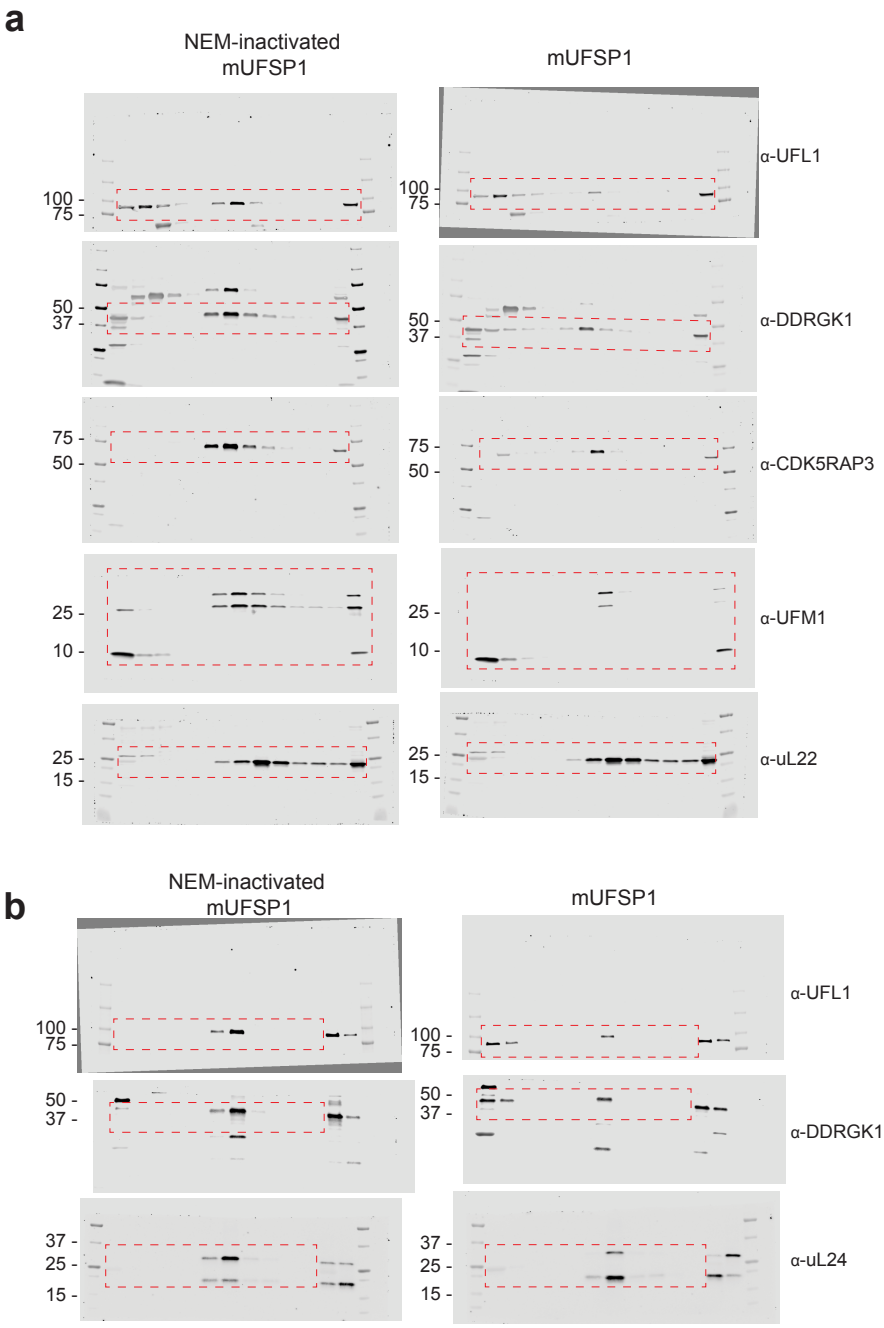

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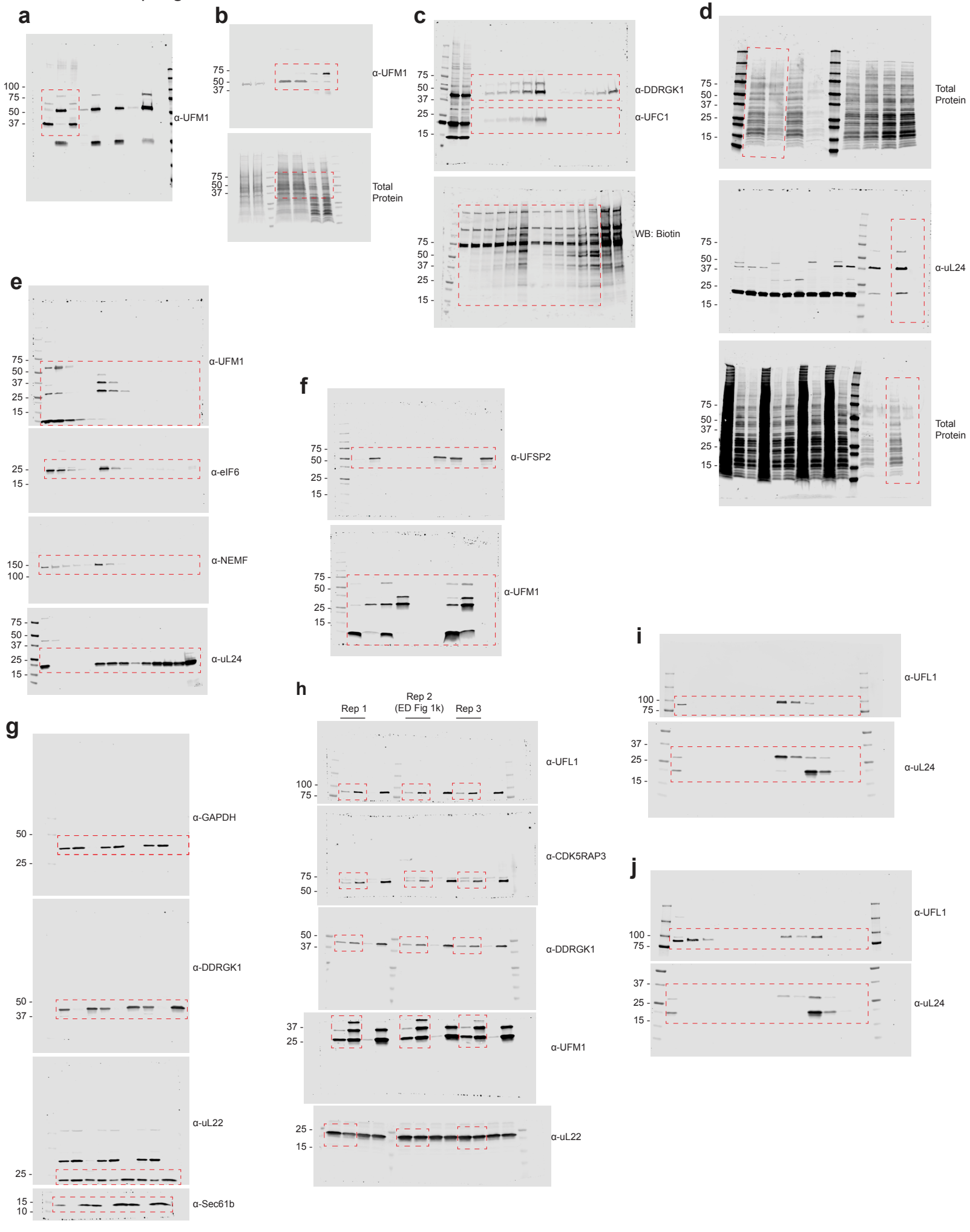

DaRosa et al., Sup. Figure 6