Biomolecules and Biomedicine
ISSN: 2831-0896 (Print) | ISSN: 2831-090X (Online)

Journal Impact Factor® (2022): 3.4
CiteScore® (2022): 5.3
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SUPPLEMENTAL DATA

Reconsideration of the laminin receptor 67LR in colorectal cancer cells

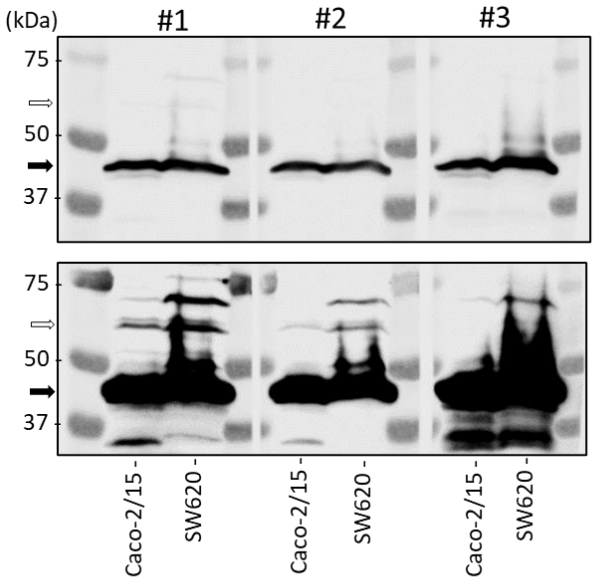

Figure S1. **Protein immunodetection with different anti-RPSA antibodies.** Representative western blot analysis of Caco-2/15 and SW620 cell lysates using three different anti-RPSA polyclonal antibodies: Antibody #1 (Ab90073) generated against a peptide derived from an unknown sequence of RPSA, antibody #2 (Ab246651) generated against the synthetic peptide corresponding to RPSA amino acid sequence 1-50 both detected a 42 kDa immunoreactive protein

https://doi.org/10.17305/bb.2024.10323

corresponding to RPSA (black arrows) as well as extra-immunoreactive bands including one at 67 kDa (white arrows) after longer exposure (lower panel) and antibody #3 (Ab99484) generated against a peptide derived from an unknown sequence of RPSA only detected the RPSA component (back arrows).

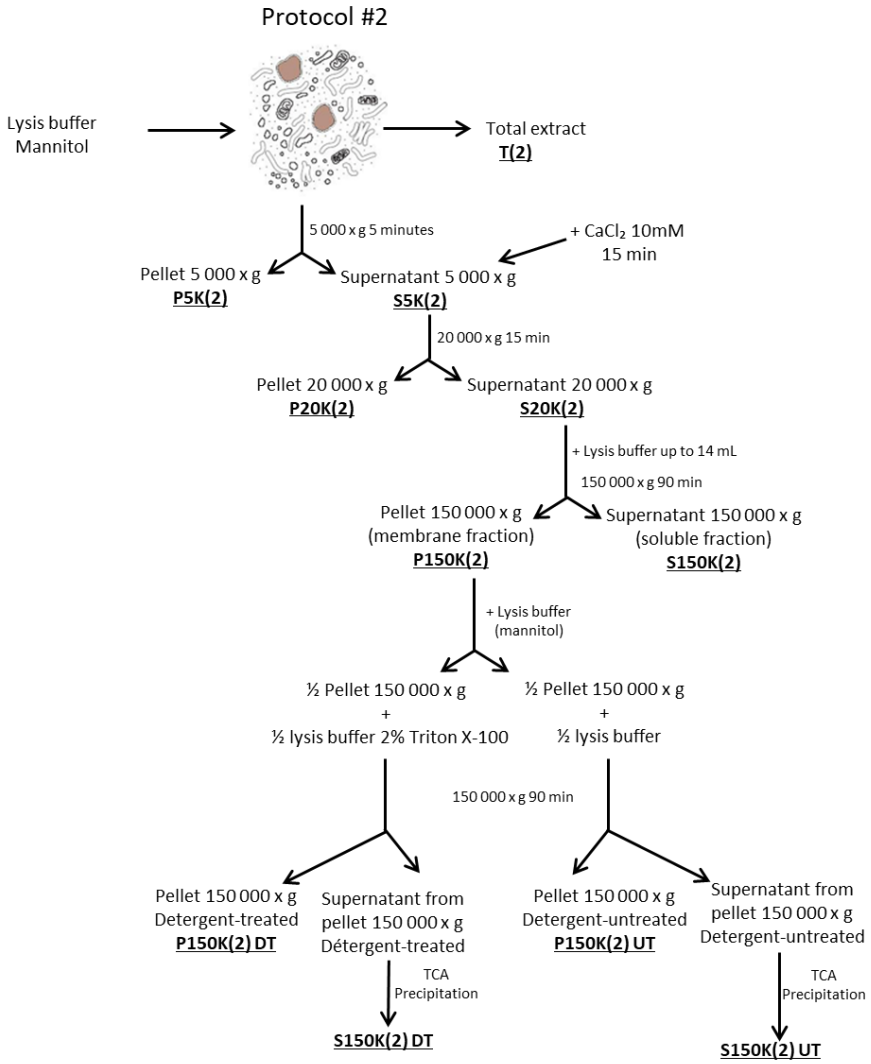

Figure S2. Schematic representation of the differential centrifugation cell fractionation protocol #2. Hypotonic cell lysis and ribosome precipitation were performed in mannitol buffer. The first centrifugation at 5,000 x g allowed the precipitation of lysed cells and cellular debris. Addition of $CaCl_2$ to the 5,000 x g supernatant allowed ribosome aggregation and precipitation in the pellet from the 20,000 x g centrifugation. Hypotonic cell lysis was followed by differential

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centrifugation and detergent treatment of plasma membrane-containing pellets from the cells using buffer containing 2% Triton X-100. Proteins solubilized by Triton X-100 were recovered after TCA precipitation. Protocol #2 was conducted in Caco-2/15 cells. DT: Detergent treated, UT: Untreated.

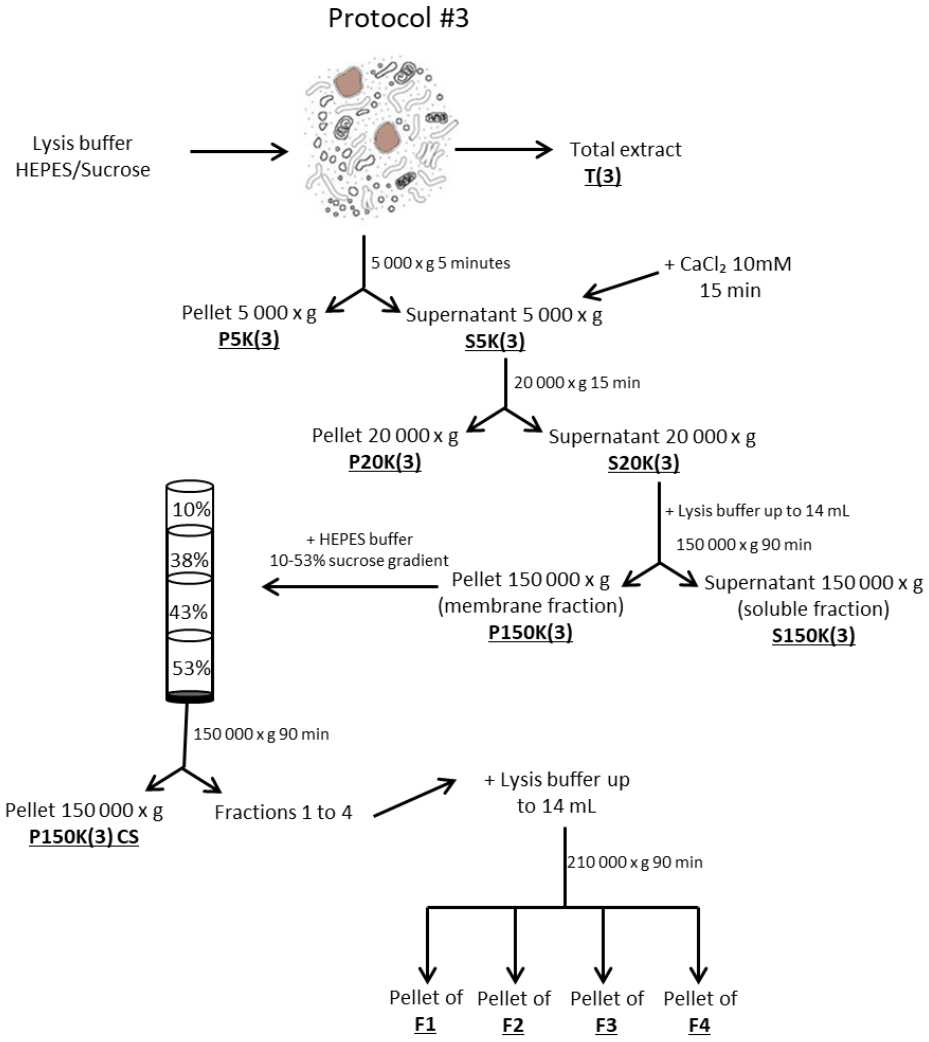

Figure S3. Schematic representation of the differential centrifugation cell fractionation protocol #3. Hypotonic cell lysis and ribosome precipitation were performed in HEPES/sucrose buffer. The first centrifugation at 5,000 x g allowed the precipitation of lysed cells and cellular debris. Addition of $CaCl_2$ to the 5,000 x g supernatant allowed ribosome aggregation and precipitation in the pellet from the 20,000 x g centrifugation. Hypotonic cell lysis was followed by differential centrifugation and sucrose cushion separation. The differential centrifugation and

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separation of plasma membrane containing pellets on sucrose cushions was conducted in Caco-2/15, SW480 and SW620 cells.

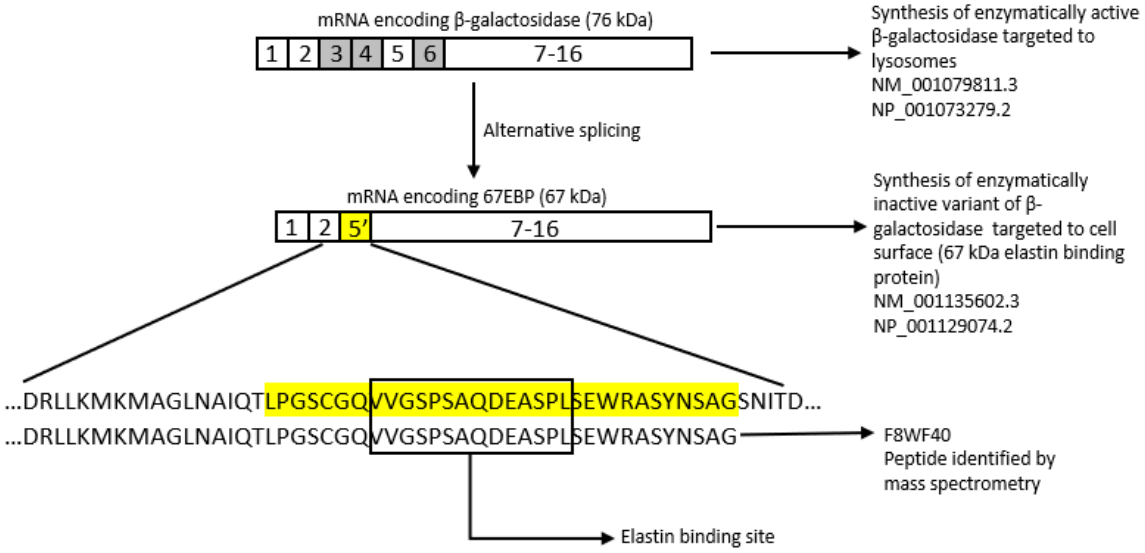

Figure S4. β-galactosidase mRNA splicing to generate 67EBP. Alternative splicing of mRNA encoding the enzymatic active form of β-galactosidase can result in an isoform of 67EBP mRNA encoding an enzymatically inactive 67 kDa protein with elastin and laminin binding affinity. A first change in reading frame results in a deletion of exons 3 and 4 resulting in a different exon 5 (yellow) and leads to the emergence of an elastin binding site (framed sequence) for the isoform corresponding to 67EBP. A second change in the reading frame causes a deletion of exon 6 leading to synthesis of an isoform of 67EBP that shares exons 7 to 16 with β-galactosidase exons 7 to 16. Sequence alignment of the F8WF40 peptide, identified in the 67 kDa gel bands by mass spectrometry analyses is specific to 67EBP. The 67EBP mRNA including exon 1, 2, 5 and 7-16 was confirmed by RTqPCR.

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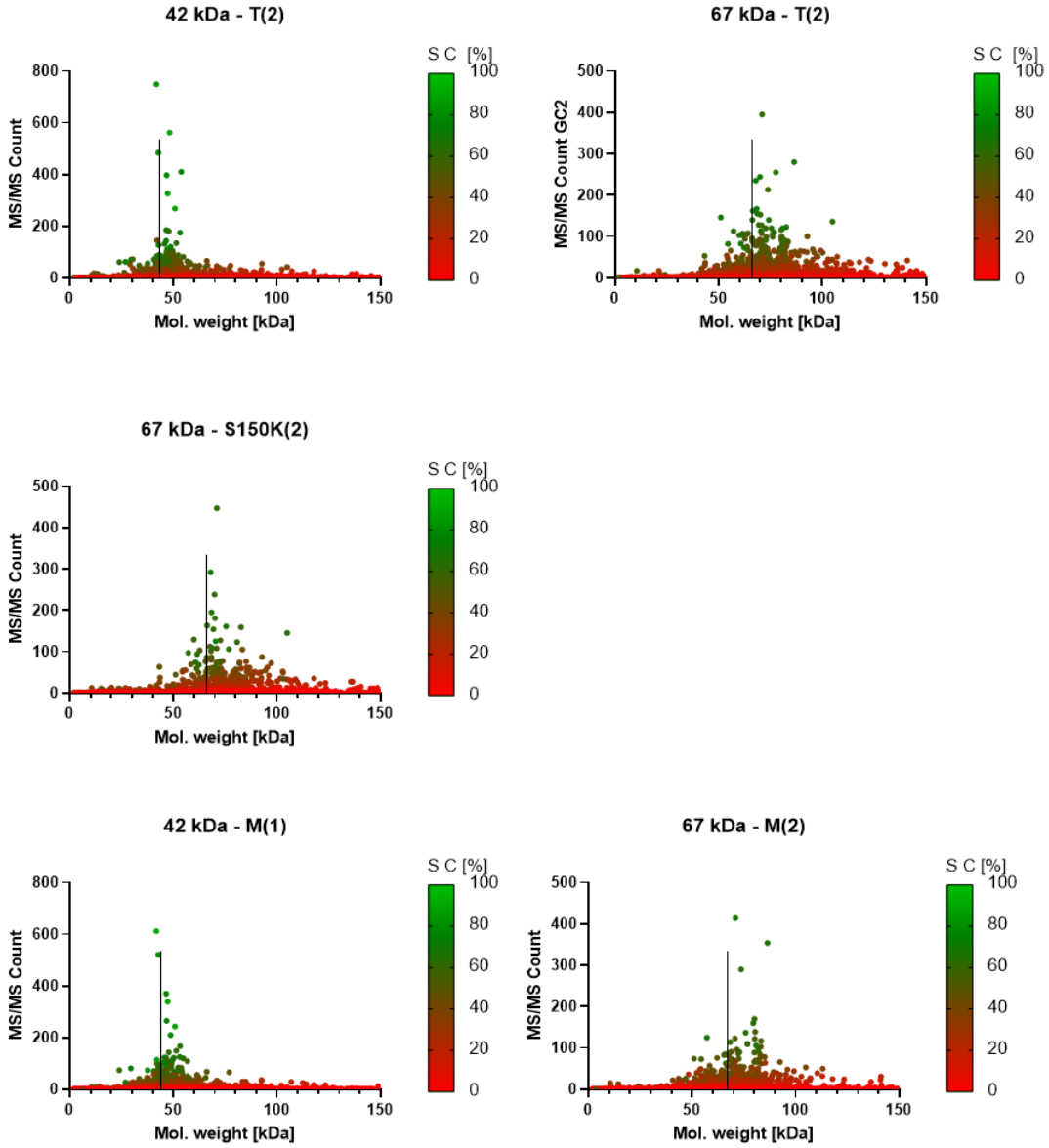

Figure S5. **Validation of proteins identified in isolated gel bands analyzed via mass spectrometry (first set of analyses).** MS/MS count of protein-associated peptides found in isolated gel bands. The associated molecular weight represents the theoretical molecular weight of each protein. The SC of each protein by the protein-associated peptides identified is represented on a scale of % (from green to red). The vertical line represents the targeted molecular weight of the cut gel band. The distribution of proteins with high sequence coverage is primarily clustered around the

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targeted molecular weight, increasing the probability of these proteins being present in the gel band and confirming the accurate isolation of the intended molecular weight gel band. MS/MS: Tandem mass spectrometry; SC: Sequence coverage.

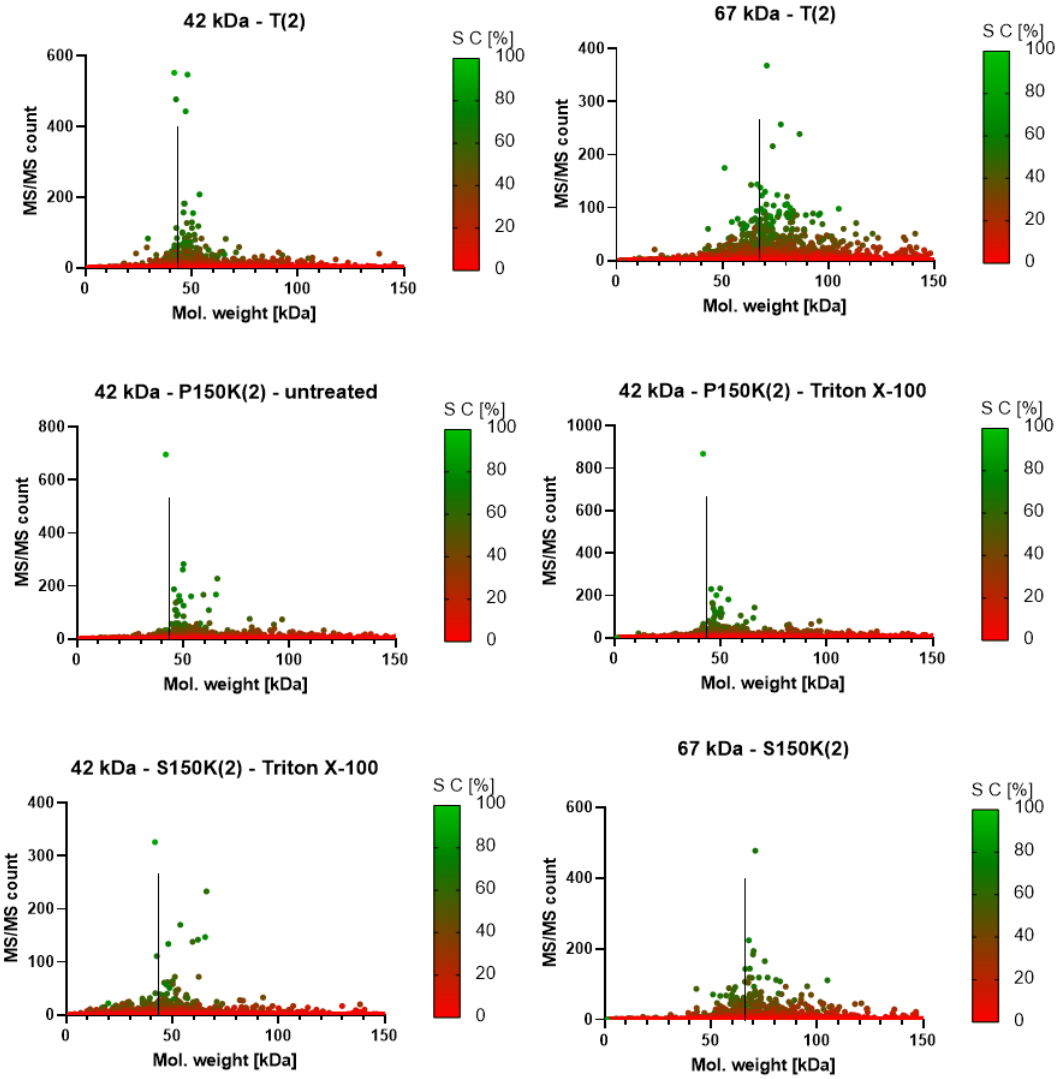

Figure S6. **Validation of proteins identified in isolated gel bands analyzed via mass spectrometry (second set of analyses).** MS/MS count of protein-associated peptides found in isolated gel bands. The SC of each protein by the protein-associated peptides identified is represented on a scale of % (from green to red). The vertical line represents the targeted molecular weight of the cut gel band. The distribution of proteins with high sequence coverage is primarily

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clustered around the targeted molecular weight, increasing the probability of these proteins being present in the gel band and confirming the accurate isolation of the intended molecular weight gel band. MS/MS: Tandem mass spectrometry; SC: Sequence coverage.

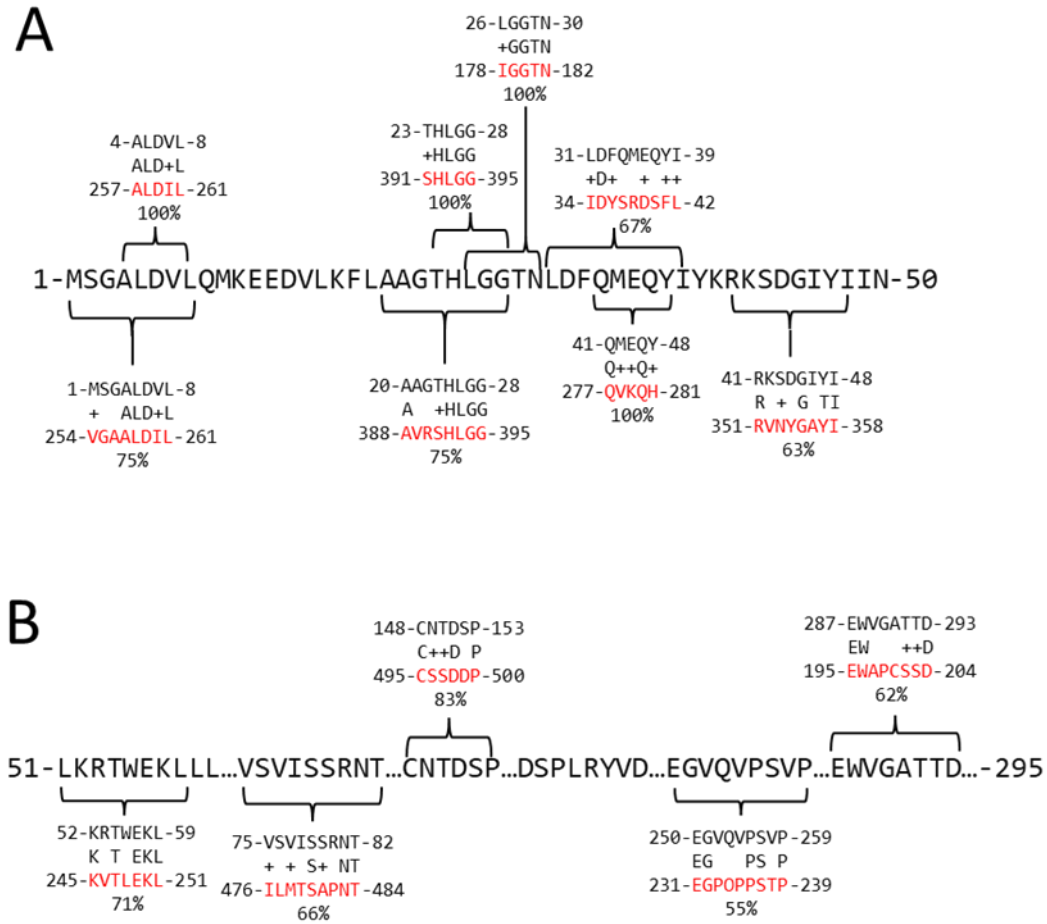

Figure S7. Schematic representation of putative sequences responsible for the cross-reactivity of anti-RPSA antibodies with 67EBP. +Similar amino acid; Amino acid sequence comparison between RPSA (black sequence) and the β-galactosidase (red sequence). (A) Amino acid sequence 1 to 50 of RPSA used as immunogen to generate the antibody AB246651 (referred to as antibody #2); (B) Amino acid sequence 51 to 295 of RPSA. The similarity between RPSA and GLB1 sequence was indicated in %.