SYNCELL APPLICATION NOTE

Insights from Stress Granule Proteomics

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Introduction

The composition of stress granules (SGs) has been a complex puzzle in cellular stress biology due to their transient and membrane-less nature. This application note reports the groundbreaking contribution of Microscoop® in unraveling the SG proteome and shedding light on its functional implications.

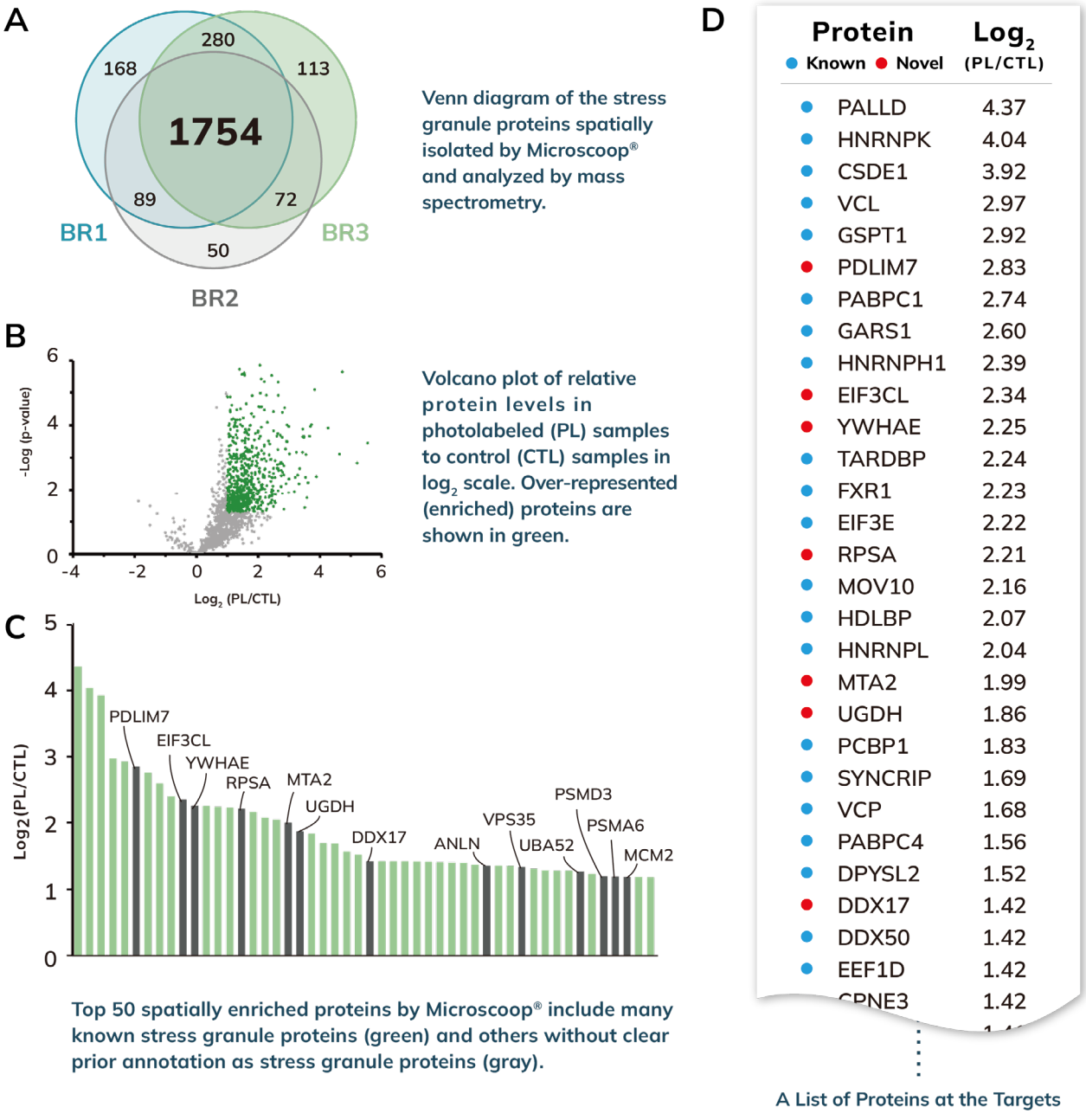

Fig. 1 | An example of a stress granule(SG) study showing the capability of protein biomarker discovery.

Robust Proteomic Analysis

To induce the formation of Stress Granules (SGs), U2-OS cells were exposed to arsenite. Following the photolabeling process by the Microscoop® system, the biotinylated proteins were extracted using the SynPull™ kit. Subsequently, LC-MS/MS analysis was performed in the Orbitrap Fusion Lumos MS (Thermo Scientific™) using a Data-dependent Acquisition (DDA) method. Our comprehensive proteomic analysis identified 2,785 proteins with remarkable consistency across three biological replicates (Fig. 1A). Notably, 1,754 proteins were consistently identified. Stringent filtering criteria, including a $\log_2$ fold-change cutoff of 0.585, a minimum of 3 unique peptides, and a Sequest HT score of 100, were applied, leading to 124 significantly enriched proteins for further analysis (Fig. 1B).

Precision and Specificity

Fig. 1C showcases the known stress granule proteins in green and unknown proteins in gray within the top 50 ranking. Microscoop® system exhibited exceptional precision, identifying 74% of true positive SGs among the top 50 proteins (37/50). Among the enriched proteins, well-known SG proteins such as hnRNPs, eRF3a, PABP1, TADBP, FXR1, and eIF3s exhibited notably high PL/CTL ratios (Fig. 1D), emphasizing their importance in SG dynamic and confirming the efficacy of the Microscoop® proteomic strategy.

Validation and Discovery

To validate the specificity of our proteomic results and confirm the presence of novel SG-associated proteins, immunostaining experiments were conducted to examine the co-localization of G3BP1 with proteins lacking prior SG annotation (Fig. 2). Among the 13 proteins tested, 11 exhibited co-localization with G3BP1, including PDLIM7, EIF3CL, YWHAE, RPSA, MTA2, UGDH, DDX17, ANLN, PSMD3, PSMA6, and MCM2 (Fig. 2). Considering all known SG proteins and these validated SG-localized proteins, the SG specificity of the obtained proteome reached an impressive 96% among the top 50 identified proteins (48/50), highlighting Microscoop®'s unparalleled discovery potential. The catalog of newly discovered Stress Granule proteins in Fig. 1D, highlighted in red, represents a blend of precision and novel discoveries with the potential to unlock previously hidden insights into cellular processes.

Conclusion

Microscoop® emerges as a transformative tool in proteomics, redefining our understanding of SG biology and paving the way for innovative therapeutic interventions.

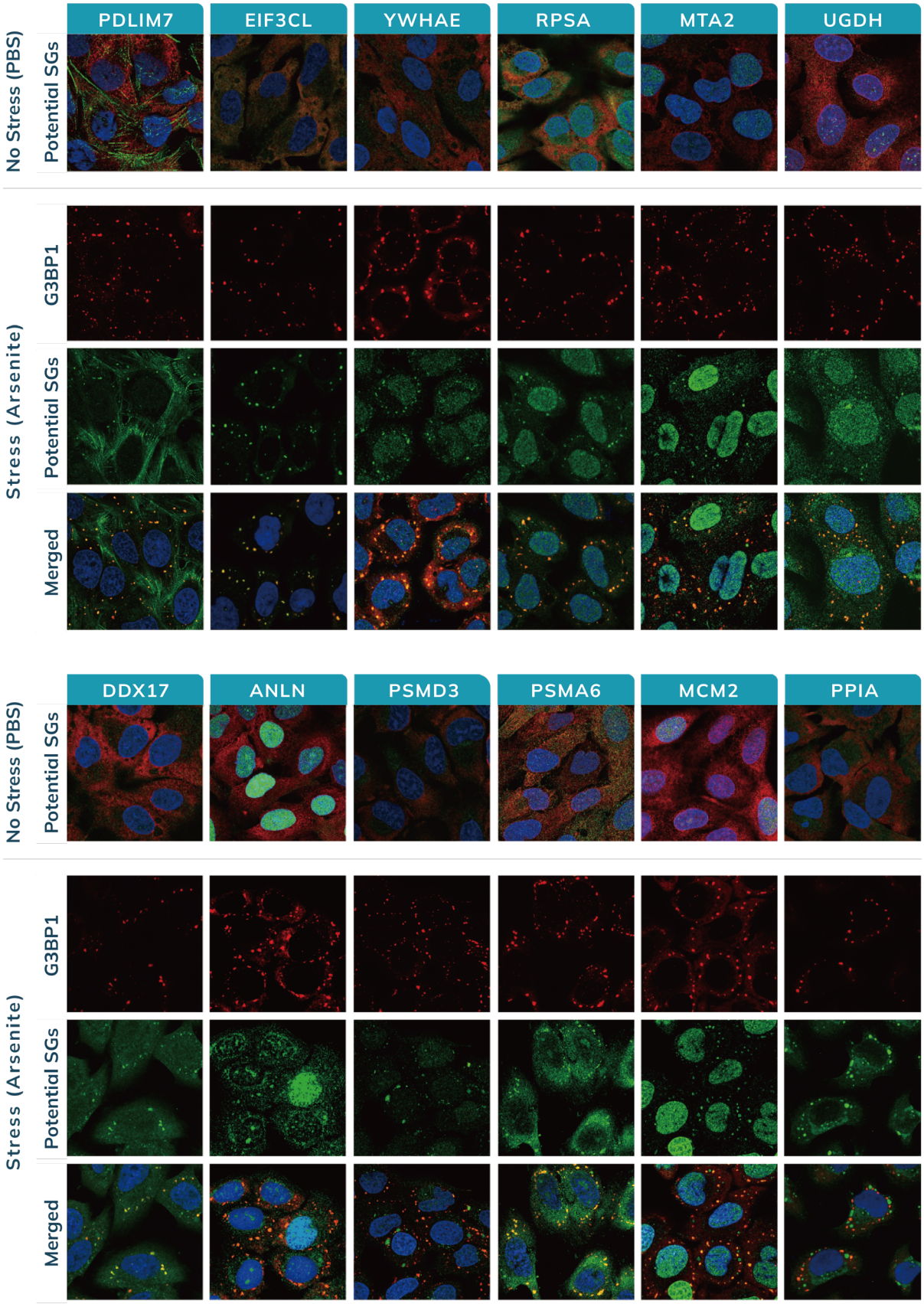

Fig. 2 | Confocal micrographs depict stress granule formation in U-2OS cells with or without an arsenite stress. Twelve proteins without clear prior annotation as stress granule proteins are highly colocalized with stress granule marker G3BP1.Green: proteins identified by Microscoop®; Red:G3BP1; Blue: DAPI.

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