SYNCELL APPLICATION NOTE

Insights from Stress Granule Proteomics

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Introduction

The composition of stress granule (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 SG proteome and shedding light on their functional implications.

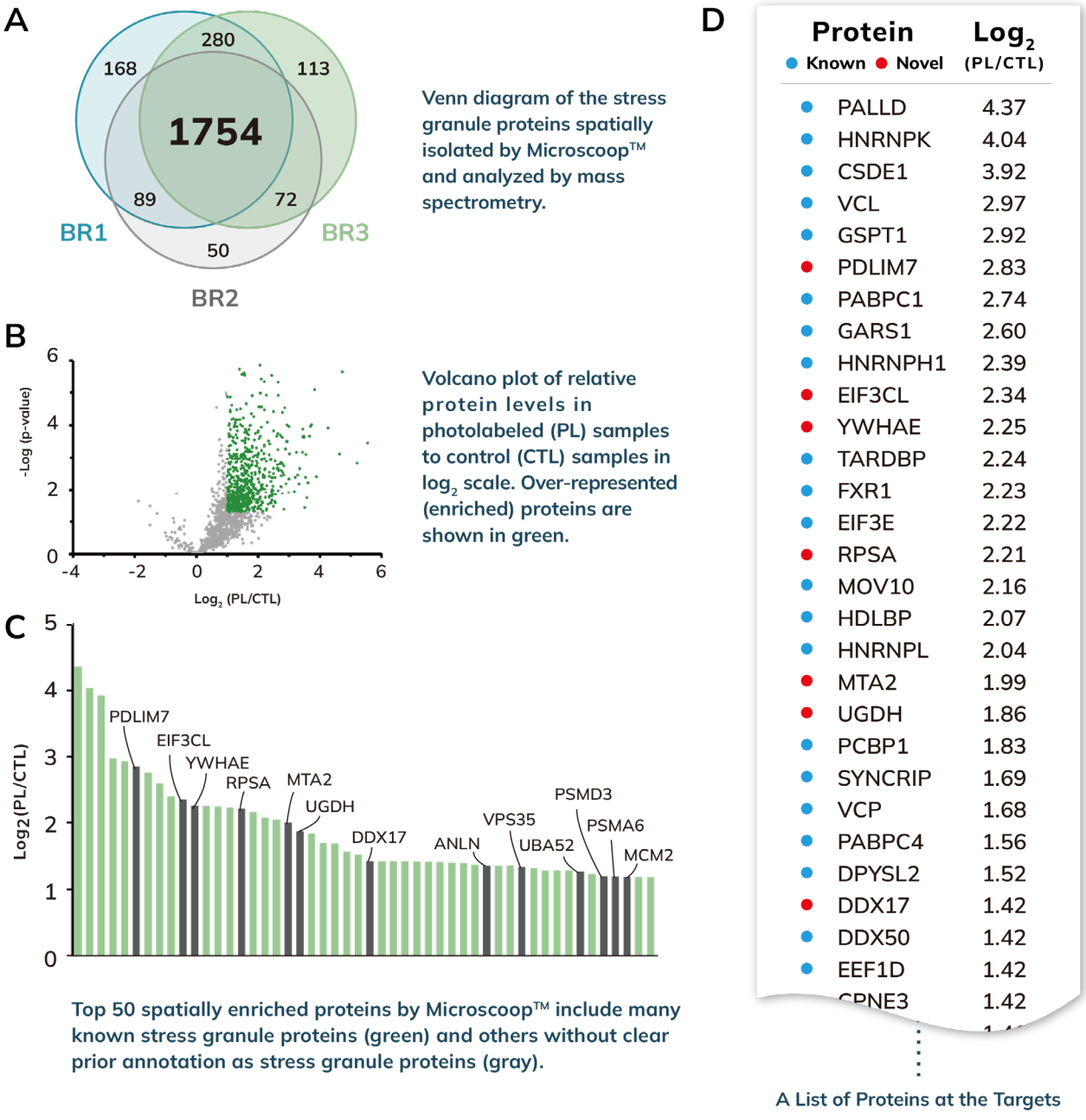

Fig. 1 | An example of a stress granule(SG) study showing the capability of protein biomarker discovery.

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-Independent Acquisition (DIA) method. Our comprehensive proteomic analysis identified 2,785 proteins with remarkable consistency across three biological replicates (**Figure 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 (**Figure 1B**).

Precision and Specificity

Figure 1C showcased 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 (**Figure 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 (**Figure 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 (**Figure 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 **Figure 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.

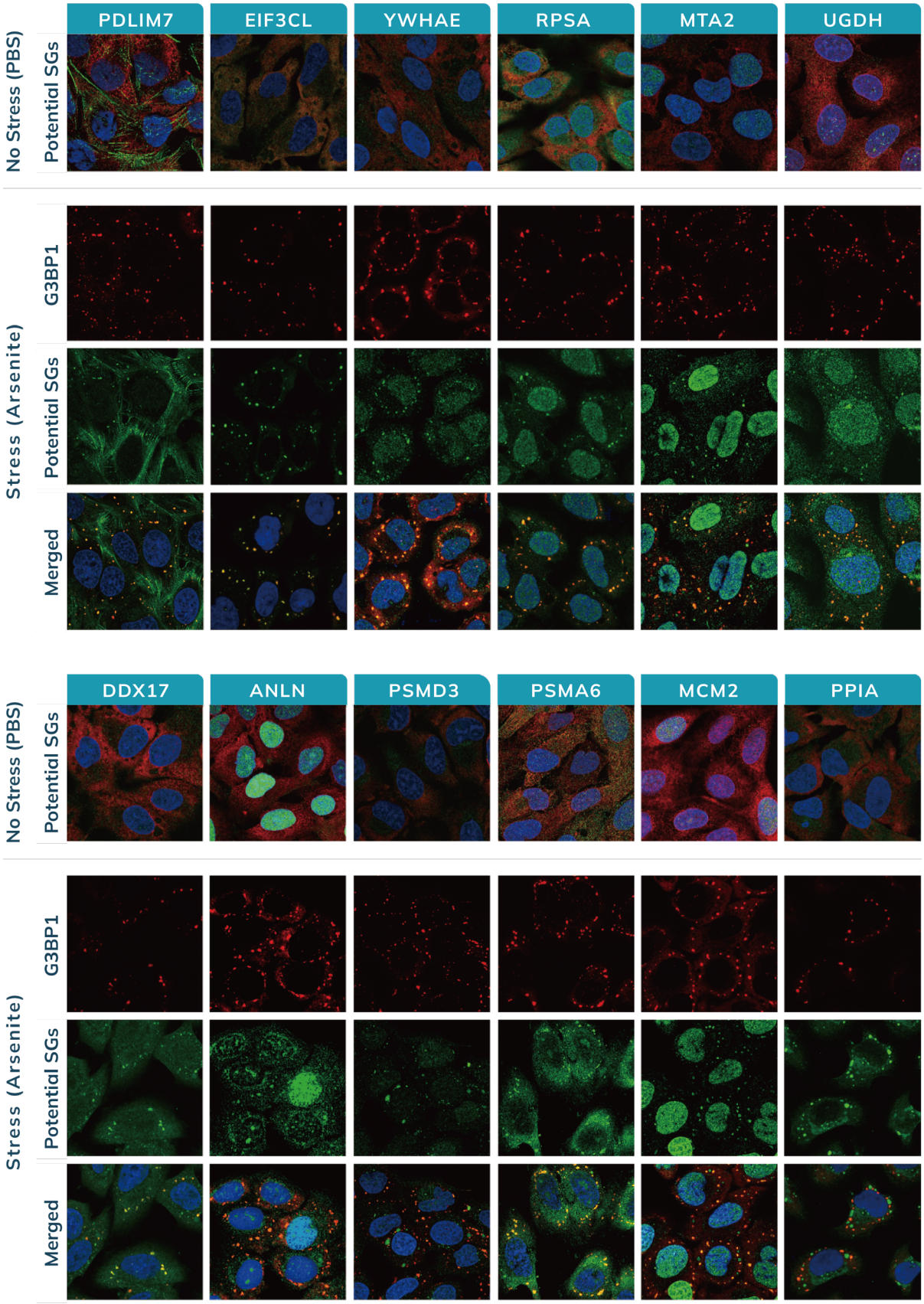

Fig. 2 | Confocal micrographs depict stress granule formation in U-2OS cells with or without an arsenite stress. Twelve proteins without clear prior annotation as stress granule proteins are highly colocalized with stress granule marker G3BP1. Green: proteins identified by Microscoop™; Red:G3BP1; Blue: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

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