Jinglin Zhou[1], Abigail Gaffar[1], Audrey M. Michel[2], Nicholas T. Crump[1], Holger W. Auner[1,3,4,5] and Mónica Román-Trufero[1,3,4]
[1]Department of Immunology and Inflammation, Imperial College London, London. [2]EIRNA Bio Ltd, Cork.[3]Division of Hematology and Central Hematology Laboratory, Lausanne University Hospital (CHUV), Lausanne. [4]The Francis Crick Institute, London. [5]Faculty of Biology and Medicine, University of Lausanne (UNIL), Lausanne.

# Ribosome profiling defines the translational sequelae of proteasome inhibition in multiple myeloma

## Experimental Hematology/Oncology

## Background & objective

Multiple myeloma (MM) is a plasma cell cancer that primarily develops in the bone marrow and remains incurable despite therapeutic advancements. MM cells are characterised by a heavy reliance on the ubiquitin-proteasome system (UPS), which has made proteasome inhibitors (PIs) the backbone of MM treatments. PIs induce cytotoxicity mainly through proteotoxic mechanisms such as misfolded protein accumulation and disruption of amino acid (AA) recycling, yet the mechanisms of PI resistance are poorly understood.

The Integrated Stress Response (ISR) is a critical signalling pathway in eukaryotic cells, activated by different stress conditions through four kinases (PERK, PKR, HRI and GCN2) with the aim of restoring cellular homeostasis. GCN2 specifically senses amino acid deficiency or translation block due to ribosome collisions (RCs). Its activation leads to the phosphorylation of eIF2α, which in turn reduces global protein synthesis and activates a transcriptional program driven by ATF4 to reestablish proteostasis. GCN2 is critical for cancer cell growth and plays complex roles in MM cells, particularly in recovery from PI treatment and amino acid depletion.

Here, we use ribosome profiling to investigate the proteostatic responses that allow MM cells to overcome PI-induced stress.

## Methods

RPMI-8226 MM cells were exposed to a 1h pulse of carfilzomib (Cfz) to reduce cell viability by approximately 50%, closely matching clinical pharmacokinetics and anti-tumor effects observed in MM patients. Cells were also treated with a specific GCN2 inhibitor (GCN2iB, 1 μM) following Cfz treatment. Cell viability was determined by Trypan Blue exclusion. Translation was measured by puromycin incorporation. Samples for ribosome profiling (Ribo-seq), Western blot, and metabolic (LC-MS) analyses were collected at 4 hours, 24-48 hours, and 6 days post-Cfz pulse, corresponding to the acute stress, early recovery, and late recovery phases, respectively (**Fig. 1**).

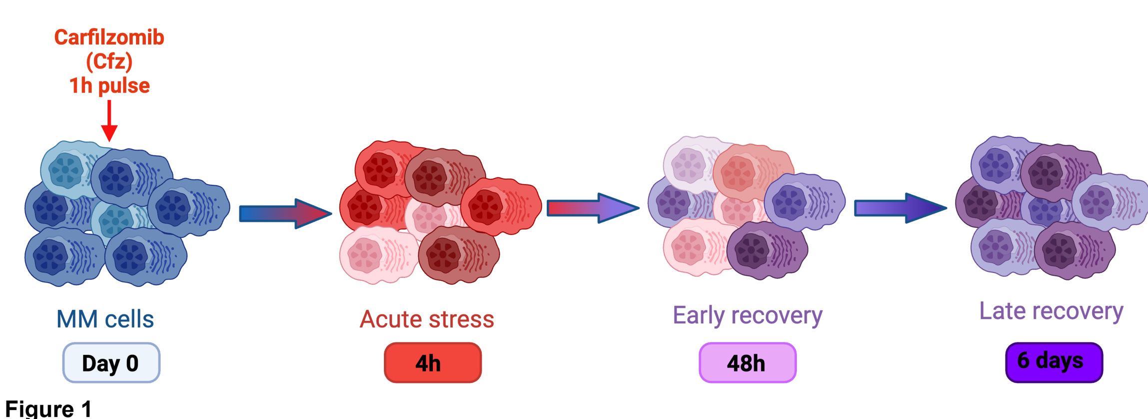


Figure 1

Presented at SOHC 2024, from 20 - 22 November 2024

## Results

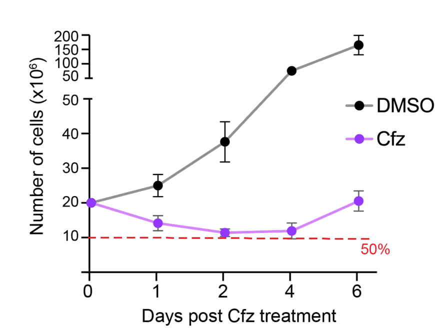


Figure 2

The 1h Cfz pulse reduced the number of viable RPMI-8226 to a nadir of 50% on day +2, followed by a return to pre-treatment levels of viable cells by day +6 (**Fig. 2**)

Cfz treatment caused an immediate, significant decrease in intracellular AA levels (**Fig. 3A**), alongside increased RCs, as indicated by elevated RPS10 ubiquitination (**Fig. 3B**). This response was accompanied by an increase in ATF4 levels and a marked decrease in translation rates (**Fig. 3B,C**). Ribo-seq revealed 125 genes with differential translation upon Cfz treatment (**Fig. 3D**), with Gene Ontology (GO) analysis showing an acute anti-stress response (**Fig. 3E**).

ACUTE STRESS

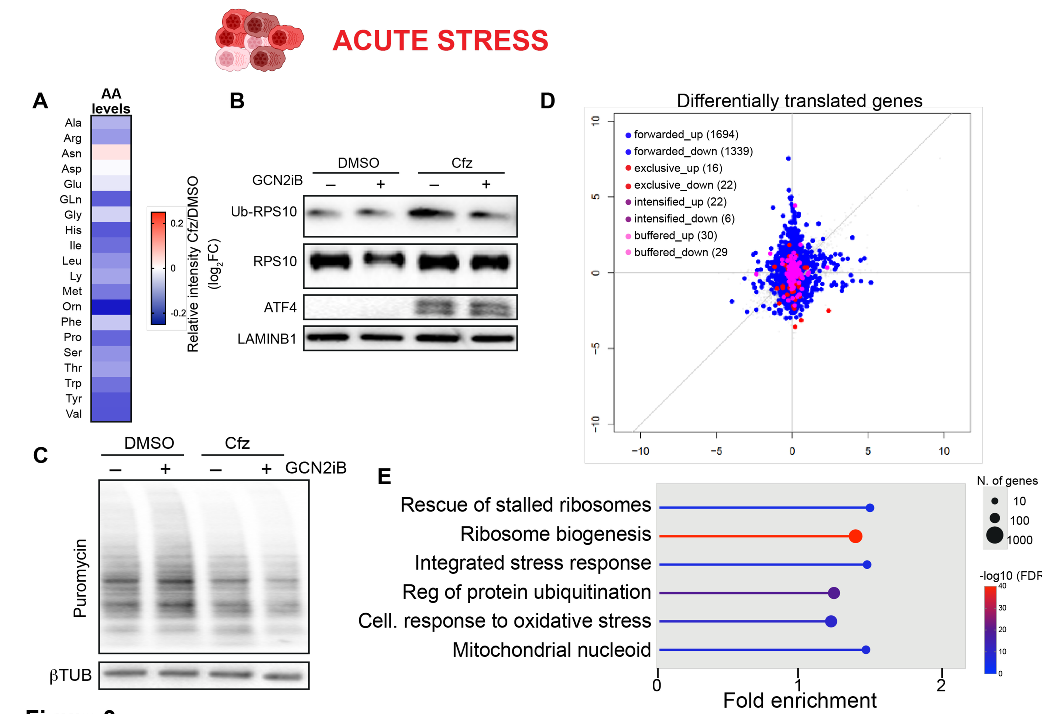


Figure 3

EARLY RECOVERY

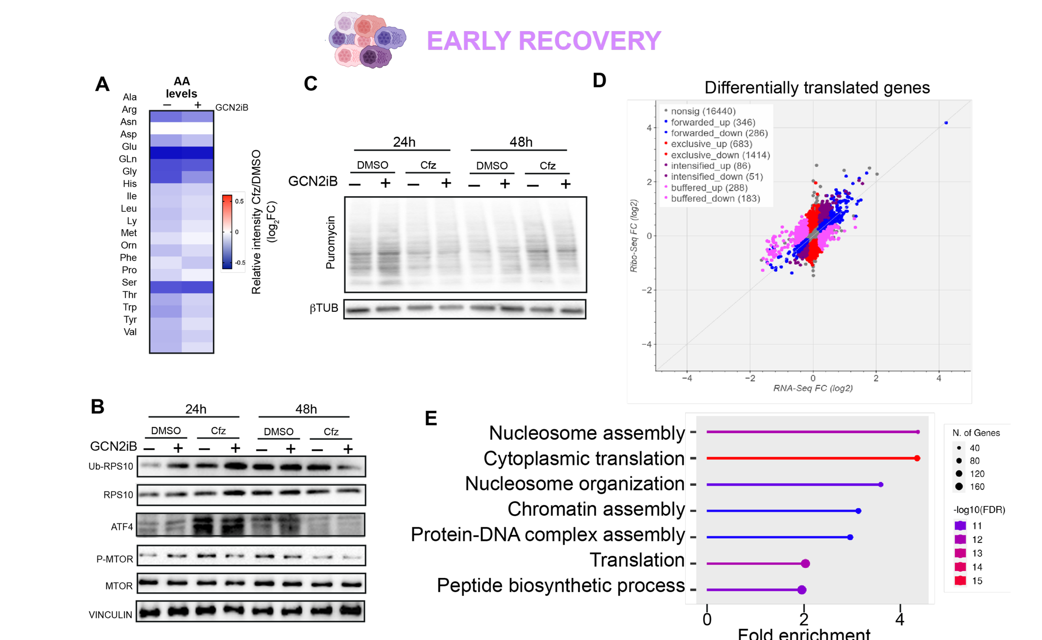


Figure 4

By 48h post-treatment, intracellular AA levels remained low, but RCs and ISR signalling had diminished (**Fig. 4A,B**). Notably, protein synthesis rates increased significantly at 48 h (**Fig. 4C**), prompting an assessment of mTOR activity, which was elevated at 24h (**Fig. 4B**). Additionally, GCN2 inhibition impacted translation, with higher puromycin incorporation observed in control cells treated with GCN2iB but not in Cfz-treated cells (**Fig. 4C**) Ribo-seq identified over 2700 genes with differential translation in Cfz-treated cells (**Fig. 4D**), with enriched GO categories relating to nuclear organization and translation processes (**Fig. 4E**).

During late recovery, no evidence of RCs or ISR activation was observed, though mTOR activity remained slightly elevated compared to DMSO controls (**Fig. 5A**). Protein synthesis rates were comparable to controls, though GCN2 inhibition resulted in a slight increase in control cells, as observed during early recovery (**Fig. 5B**). Ribo-seq data showed 326 genes with differential translation in Cfz-treated cells (**Fig. 5C**), with translation-related genes prominently represented among those with increased translation efficiency (TE) (**Fig. 5D**).

LATE RECOVERY

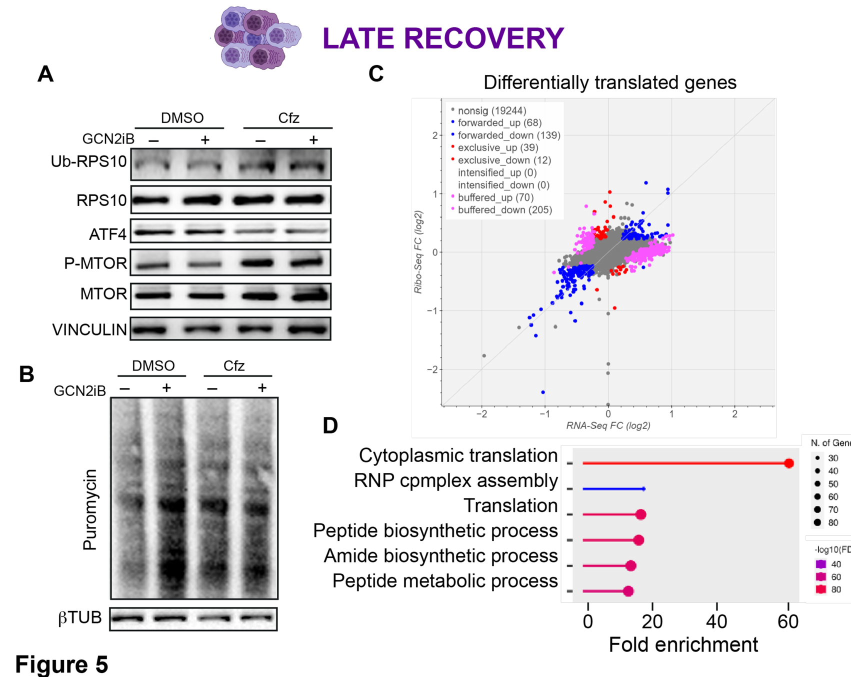


Figure 5

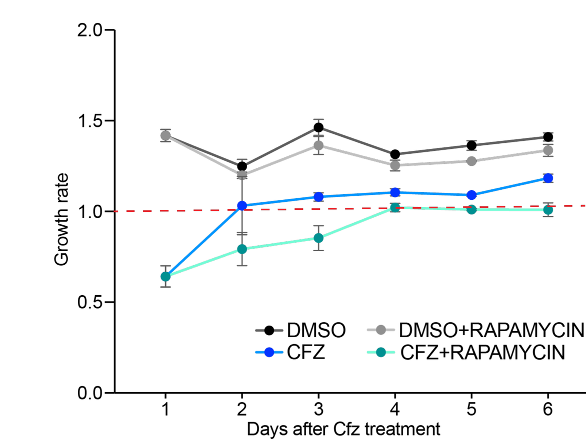
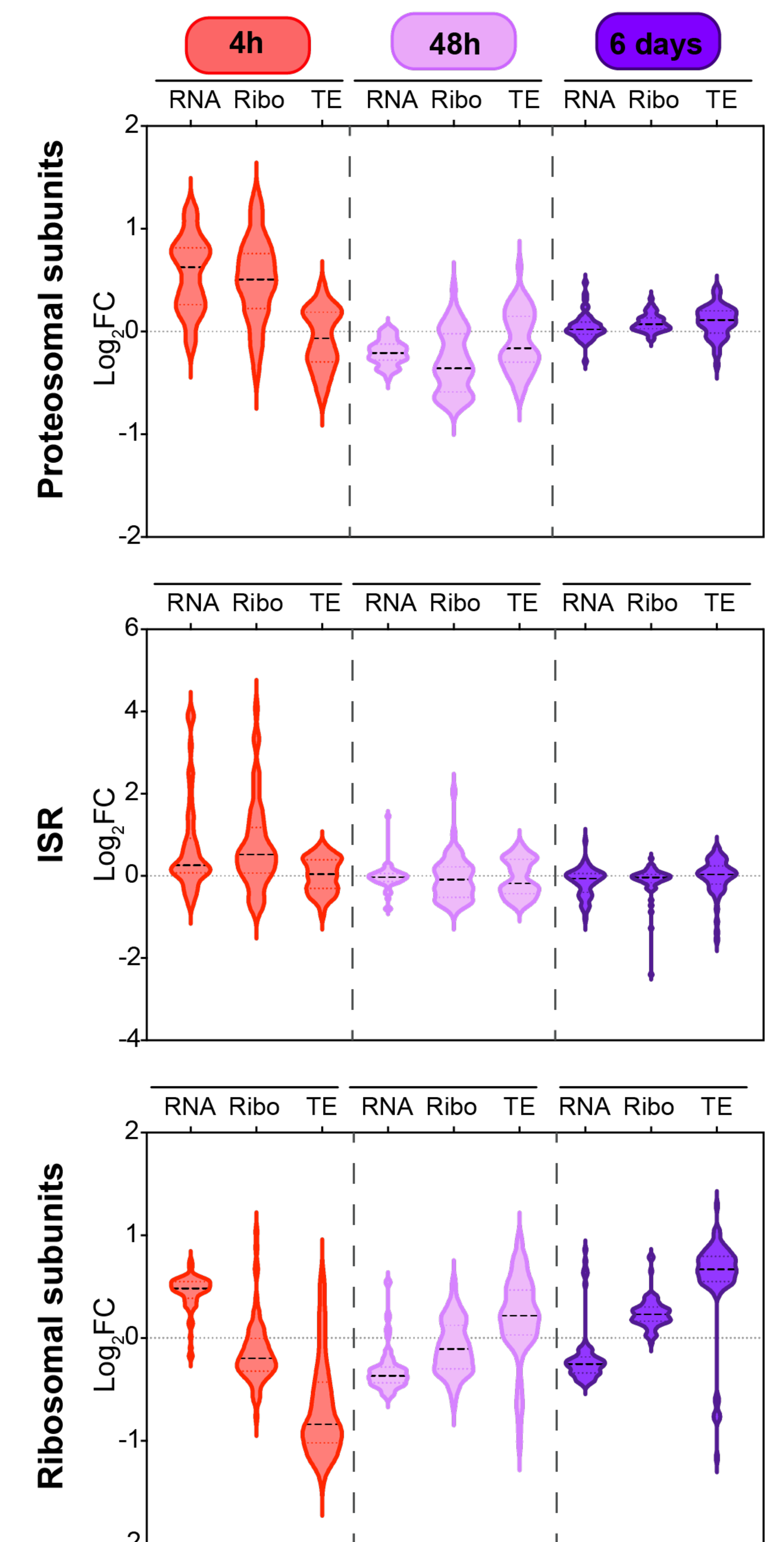
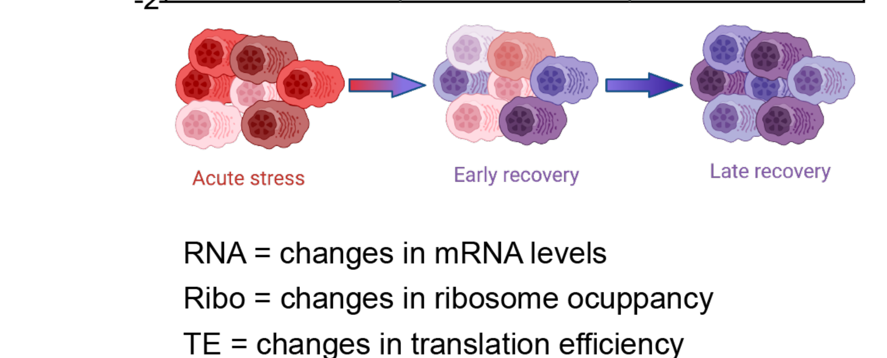


Figure 6

Inhibition of MTOR by rapamycin hinders MM cell recovery from Cfz (**Fig. 6**)

PI-resistant MM cells exhibit dynamic changes in their proteostatic mechanisms. Ribo-seq revealed a rapid response to Cfz treatment, with increased transcription and translation of proteasome subunits as early as 4h post-treatment. However, by 48 hours, this trend reversed in a proteasome bounce-back effect. A similar pattern was observed in the mRNA levels and ribosome occupancy of ISR components. In contrast, analysis of ribosomal subunits and translation factors showed an initial decrease in TE shortly after treatment, followed by a marked increase during the early recovery phase. Notably, during late recovery, PI-resistant cells maintained elevated ribosome biogenesis levels (**Fig. 7**).




RNA = changes in mRNA levels
Ribo = changes in ribosome ocuppancy
TE = changes in translation efficiency

Figure 7

## Conclusion

Our study reveals that proteasome inhibition rapidly triggers RCs and a suppression of protein translation, which is followed by upregulated translational activity linked to MTOR activation in surviving MM cells.

## Acknowledgements

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