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# A yeast selection system for the detection of proteasomal activation

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## Abstract

The ubiquitin proteasome system (UPS) is a complex cellular machinery that catalyzes degradation of misfolded or damaged proteins and regulates turnover of native proteins in eukaryotic cells, thus playing a crucial role in maintaining protein homeostasis. The UPS has emerged as a drug target for a diverse range of diseases characterized by accumulation of misfolded or aggregated proteins. While enhancement of UPS activity is widely recognized as a promising strategy to prevent accumulation of aberrant, off-pathway protein conformations and ameliorate the phenotypes of a wide range of protein misfolding diseases, the molecular mechanisms underlying activation of proteasomal degradation are poorly characterized. We report the development of a yeast selection platform for genome-wide selection of UPS activators. We engineered the Saccharomyces cerevisiae selection marker orotidine-5′-phosphate decarboxylase (URA3) to function as a substrate of proteasomal degradation through fusion to UPS-sensitive tags. The resulting UPS-sensitive URA3 variant links UPS activity to cell growth. The yeast selection platform reported in this study will open the way to high-throughput, genome-wide studies aimed at identifying modulators of UPS function that might provide novel target for therapeutic applications.

Key words: degron, proteasomal degradation, UPS activators, URA3, yeast

## Introduction

Protein degradation in eukaryotic cells relies on the ubiquitin proteasome system (UPS). The UPS regulates turnover of native proteins and mediates disposal of misfolded or damaged conformations, thus playing a fundamental role in the maintenance of protein homeostasis (Amm et al., 2014) and in the regulation of a wide variety of fundamental cellular processes, including cell cycle, DNA repair, immune response and apoptosis (Konstantinova et al., 2008). Dysfunction or deregulation of the UPS is associated with development of multiple disease conditions, such as neurodegenerative disorders (Gadhave et al., 2016) and cancer (Mata-Cantero et al., 2015), pointing to the role of the UPS as a potential target for therapeutic intervention (Huang and Dixit, 2016).

Modulation of UPS activity has been extensively investigated through pharmacological agents that inhibit the proteasome (Ausseil et al., 2007; Blackburn et al., 2010) and genome-wide screens of genetic repressors (Paddison et al., 2004; Webster et al., 2017), providing a detailed understanding of the molecular mechanisms underlying inhibition of proteasomal degradation. Strategies to enhance UPS activity, on the other hand, remain largely unexplored. Increase in proteasome activity was reported upon overexpression of proteasomal subunits, such as polypeptides of the 20 S catalytic core particle (CP; Chondrogianni et al., 2003), the 11 S proteasome activator 28 (Li et al., 2011) and the 19 S subunit regulatory particles Rpn11 (Tonoki et al., 2009) and PSMD11 (Vilchez et al., 2012). Overexpression of E3 ligases, such as carboxy-terminus of