Kumarapeli and co-workers presented in the FASEB journal an in vivo reporter model for the ubiquitin/proteasome system (UPS) (1). Similarly to the strategy that we used previously for the generation of our transgenic reporter model (2), they generated mice ubiquitously expressing a modified green fluorescent protein (GFP) that is constitutively targeted for proteasomal degradation.

While we welcome new mouse models for the UPS, we regret that the authors, in an attempt to make a direct comparison of the two models based on the literature, misrepresented some data from our earlier study. The authors argue that the proteasome inhibitor provoked in their UPS reporter mice accumulation of the reporter in several tissues that did not respond in earlier experiments with our reporter mice (1). In addition, it is stated that this ‘unresponsiveness’ of tissues is a serious shortcoming of our reporter system.

The authors correctly state that they used the same proteasome inhibitor (MG262), the same inhibitor concentration (5 µmol/kg) and analyzed the mice at the same time after inhibitor administration (20 hrs), but fail to point out that they injected the inhibitor intravenously while we used intraperitoneal administration (1, 2). We feel it is important to point out to the readers that variations in responsiveness can be alternatively explained as a direct consequence of differences in the bioavailability of the inhibitor due to the route of administration. Moreover, we showed that several primary cultures obtained from our reporter mice (cardiomyocytes, neurons and fibroblasts) properly responded with GFP accumulation to treatment with proteasome inhibitors in vitro (2). Unfortunately, Kumarapeli and co-workers ignored this important observation even though they used the same approach to further validate the responsiveness in their own mouse model (1). We anticipate that, since these mouse models are based on different type of UPS substrates (3), it is indeed likely that there will be variations in the responsiveness of these model substrates (which may reflect how different classes of substrates are handled by the UPS) but would like to emphasize that the data presented by Kumarapeli and co-workers do not allow drawing conclusions on this important issue.

We are pleased to see that the usage of GFP reporter substrates of the UPS is becoming more widespread. In vivo UPS models have been instrumental in gaining new insights in pathologic processes as well as actions of proteasome inhibitors (3–5). We are convinced that, also in the future, the different UPS reporter mice will be important tools to decipher the role of the UPS in various pathologies and are looking forward to detailed studies with UPS reporter mice (6).

**REFERENCES**


**Response from the Authors:**

We sincerely welcome the comments by Lindsten et al concerning our recent article (1). As previously indicated, we created our reporter (GFPdgn) mouse model independently in the search for an effective tool to dissect in vivo UPS proteolytic function. It was not designed to overcome any pitfalls of UbG76V-GFP mice. GFPdgn was engineered by the fusion of the degron CL1 to the carboxyl terminus of a GFP. The creation of GFPdgn mouse was started immediately after Bence et al...
reported the GFP\textsuperscript{u} reporter for cell culture (2), more than 2 years before the Ub\textsuperscript{G76V}-GFP mice were reported (3). A careful analysis on the testing results from our GFPdgn mice and the data reported for Ub\textsuperscript{G76V}-GFP mice made us feel obligated to discuss the important differences in the suitability of these reporter mice for monitoring in vivo UPS proteolytic function. Indeed, for some of the data reported by Lindsten et al, we provided a likely alternative interpretation that differs from the ones offered by them; but we have never misrepresented any data.

Lindsten et al (3) reported accumulation of Ub\textsuperscript{G76V}-GFP in the liver, small intestine, pancreas, kidney, and a small fraction of the cells in the lung and spleen but no fluorescent cells detected in the brain, heart, and skeletal muscles 20 hours after intraperitoneal (i.p.) injection of MG-262 (5 \mu mol/kg). It is possible but highly unlikely that the variation in responses is caused by a potential difference in the bioavailability of the inhibitor. This is because MG-262 is cell membrane permeable and easily enters the blood flow when used via i.p. injection. Evidently, Lindsten et al observed a dose-dependent decrease in the chymotrypsin-like activities (CTLA) of the proteasome in the lysates of not only the liver but also the kidney and spleen (3). To produce effects in the kidney, spleen, and lungs, an i.p. injected pharmacological agent needs to get into the systemic circulation first. It is very unlikely that MG-262 absorbed into the systemic circulation was insufficient to inhibit the proteasome in the heart and skeletal muscles while it was evidently effective in the kidney and lungs. Unfortunately, no data were presented by Lindsten et al concerning the CTLA in the heart, skeletal muscles, or the brain in their systemic proteasome inhibition experiments.

To clarify this issue, we have repeated the same proteasomal inhibition experiments with our GFPdgn mice using i.p. injections. As expected, the same degree of CTLA inhibition and GFPdgn accumulation were detected in the heart, brain, and skeletal muscles as we previously observed with intravenous injections. This confirms our proposition that the GFPdgn mice are much better suited to report in vivo UPS proteolytic function in the heart, skeletal muscles, and brain.

Experimental results from in vitro cell culture do not necessarily reflect in vivo situations and vice versa. We did carry out cell culture tests to validate the platform of in vitro use of GFPdgn in adult cardiomyocytes (1); but they were neither intended to nor utilized to validate GFPdgn as a reliable reporter for in vivo UPS proteolytic function.

We agree that substrates carrying different degradation signals are likely handled differentially by the UPS. The objective of a study ultimately dictates its choice of reporter systems to monitor in vivo UPS proteolytic function.

The research into the (patho)physiological significance of the UPS in the heart, skeletal muscles, and brain is inarguably important and the reliable readouts for in vivo UPS proteolytic function in these organs will undoubtedly facilitate the research endeavor. It is gratifying that GFPdgn mice have been successfully used to delineate the deregulation of UPS proteolytic function in the heart of several disease models (1, 4, 5). We anticipate that all sensitive and reliable fluorescence-based UPS reporters will be more extensively employed in further unveiling the regulation of UPS proteolytic function and its roles in physiology and pathology.

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