**Figure S4. Ubiquitination of Rpn13 but not Rpt1 is reversible in vivo.** HEK293F cells were treated with DMSO or proteasome inhibitors (10 µM MG132, 1 µM BTZ, 400 nM Epoxomycin) for 4 hours. Then the inhibitor was removed and the cells were continued to grow for 1, 4, and 20 hours. Crude cell extracts were prepared and analyzed by Western blot for **A)** accumulation of ubiquitin conjugates, **B)** modification of proteasome subunits with ubiquitin, **C)** content of proteasome-associated ubiquitin ligases, and **D)** modification of proteasome-associated DUBs with ubiquitin.

<table>
<thead>
<tr>
<th>Hours after wash out</th>
<th>DMSO</th>
<th>MG132</th>
<th>BTZ</th>
<th>EPX</th>
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### A) Ubiquitin

### B) α1-7

- **Rpt1-Ub**
- **Rpt5-Ub**
- **Rpn13-Ub**
- **Rpn13-Ub**

### C) Proteasome-associated ubiquitin ligases

- **Ubr4**
- **Huwe1**
- **Ube3a**
- **Ube3c**
- **Rnf181**

### D) Proteasome-associated DUBs

- **Uch37**
- **Usp14**