Supporting Information

Blouin et al. 10.1073/pnas.1314177110

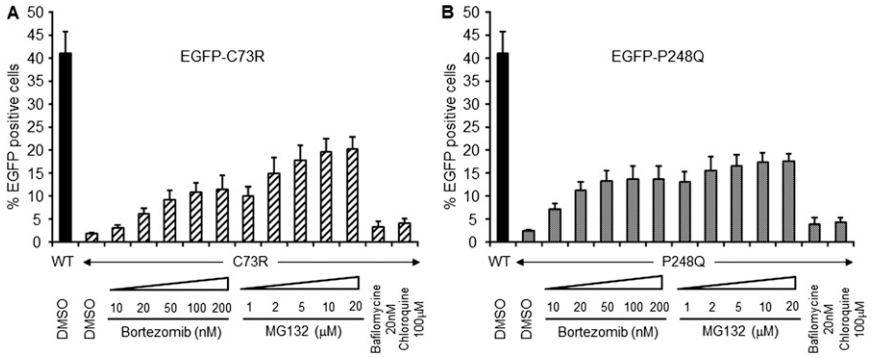

Fig. S1. UROS^{P248Q} and UROS^{C73R} mutants trigger premature degradation by the proteasome pathway in human erythroid K562 cells. (*A* and *B*) Human erythroleukemic K562 cells were stably transfected with plasmids expressing EGFP fused to the C terminus of WT, C73R, or P248Q UROS cDNA. Stably transfected cells were treated with DMSO or the indicated concentration of lysosome inhibitor (bafilomycine or chloroquine) or proteasome inhibitor (MG132 or bortezomib) for 16 h. EGFP expression was monitored by flow cytometry analysis. Results are expressed as the mean of three independent experiments; error bars represent SD. *Significant difference ($P < 0.001$) vs. mutant UROS-EGFP treated with DMSO.

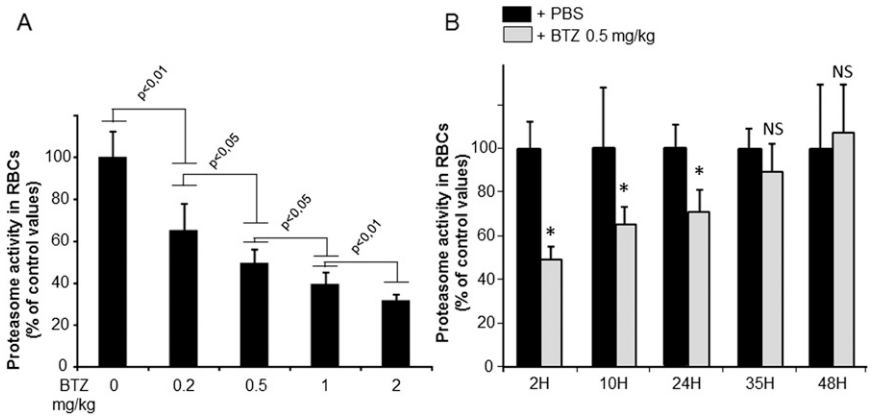

Fig. S2. Dose-dependent inhibition of proteasome activity was evaluated in normal mice after a single bortezomib injection. (*A*) Proteasome activity was quantified in blood lysates from mice ($n = 6$ per group) at 2 h after a single dose of bortezomib (0.2–2 mg/kg). A dose-dependent inhibition of proteasome activity was observed in vivo. (*B*) Proteasome activity was quantified over time (from 2 h to 48 h) in peripheral RBCs from mice injected with a single dose of bortezomib (0.5 mg/kg). Proteasome activity recovered completely within 2 d after bortezomib injection. *Significant difference ($P < 0.01$) vs. PBS-injected mice.

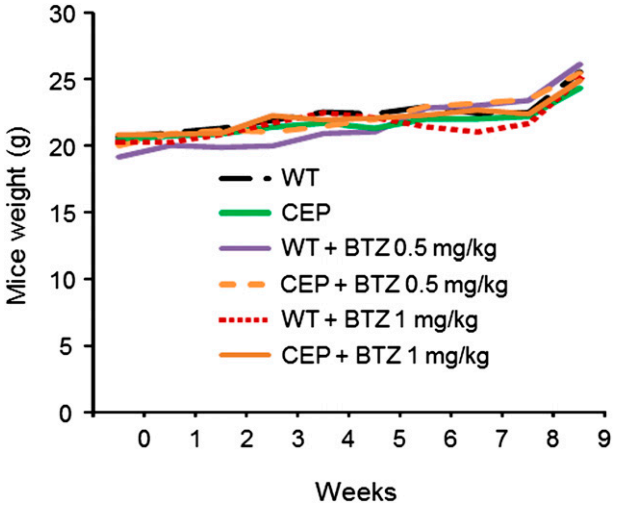

Fig. S3. Bortezomib injection does not induce toxicity in WT and CEP mice. WT and CEP mice were treated with bortezomib (0.5 or 1 mg/kg) every 48 h and weighted over a 9-wk period. Toxicity (e.g., cachexia) was not observed in mice treated with proteasome inhibitors.

Table S1. Hematologic parameters were analyzed in bortezomib-treated WT and CEP mice after a 9-wk period

Mice (no.)	Treatment	RBC, 10^6/mL	WBC, 10^3/mL	Platelets, 10^3/mL	Hb, g/dL	Hematocrit, %	Spleen/body weight, %
WT (10)	Saline	10 ± 0,6	12.3 ± 4,6	976 ± 657	16.2 ± 0,6	54 ± 2.1	0.4 ± 0.1
CEP (6)	Saline	6.5 ± 0,2	21.7 ± 4.8	1,180 ± 302	8,9 ± 0,2	31 ± 1.3	5.2 ± 0.6
		$P < 0.05$ vs. WT	$P < 0.05$ vs. WT	$P < 0.05$ vs. WT	$P < 0.05$ vs. WT	$P < 0.05$ vs. WT	$P < 0.05$ vs. WT
WT (5)	Bortezomib 0.5 mg/kg	9.3 ± 0.4	12.1 ± 2.3	1,419 ± 353	16.8 ± 0.4	52.3 ± 2.4	0.5 ± 0.1
		P = NS vs. WT	P = NS vs. WT	P = NS vs. WT	P = NS vs. WT	P = NS vs. WT	P = NS vs. WT
WT (5)	Bortezomib 1 mg/kg	9 ± 0.2	13.3 ± 3.8	837 ± 285	14.5 ± 0.4	50.4 ± 1	0.6 ± 0.1
		P = NS vs. WT	P = NS vs. WT	P = NS vs. WT	P = NS vs. WT	P = NS vs. WT	P = NS vs. WT
CEP (5)	Bortezomib 0.5 mg/kg	6.3 ± 1	26.7 ± 16.6	2,049 ± 158	8.9 ± 1.2	31.4 ± 5	4 ± 0.4
		P = NS vs. CEP	P = NS vs. CEP	$P < 0.05$ vs. CEP	P = NS vs. CEP	P = NS vs. CEP	$P < 0.01$ vs. CEP
CEP (7)	Bortezomib 1 mg/kg	6.6 ± 0.3	18.2 ± 7.5	1,793 ± 308	8.9 ± 0.3	31.5 ± 1.1	4.1 ± 0.5
		P = NS vs. CEP	P = NS vs. CEP	P = NS vs. CEP	P = NS vs. CEP	P = NS vs. CEP	$P < 0.01$ vs. CEP

Statistical significance is indicated in groups compared with untreated WT or CEP mice used as controls. NS indicates not statistically significant.