The 19S deubiquitinase inhibitor b-AP15 is enriched in cells and elicits rapid commitment to cell death

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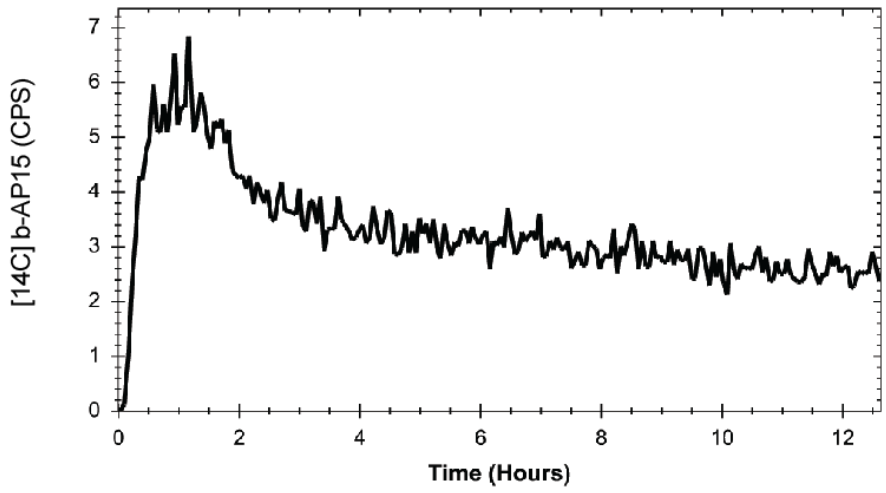
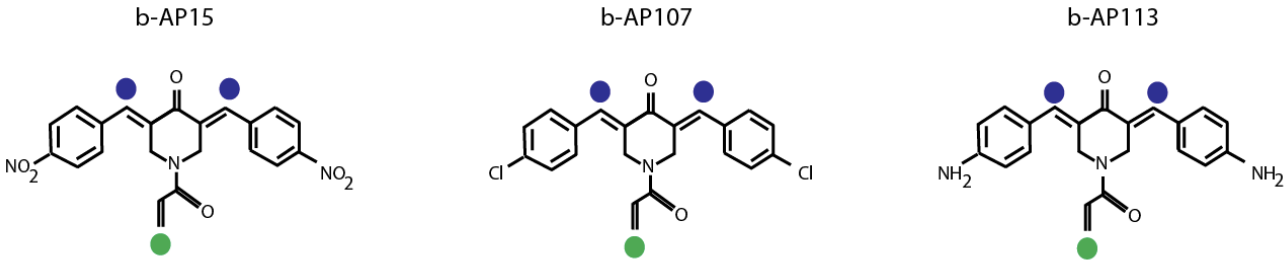
Supplementary Figure 1. Uptake of b-AP15 into cells.



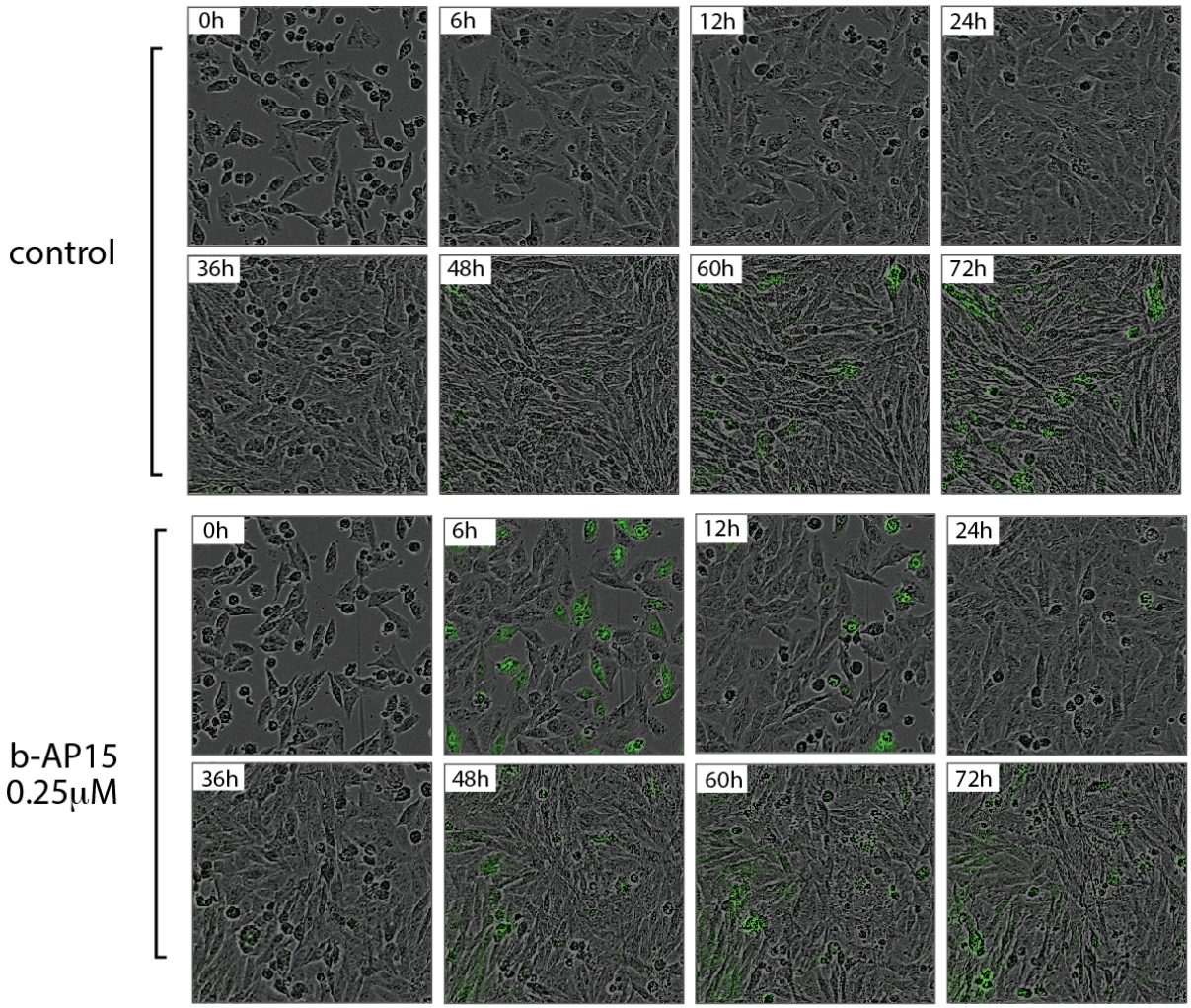
Uptake of [14C] b-AP15 over 12 hours was determined using LigandTracer® White. Association of radioactive drug with cells was determined in real-time.

Supplementary Figure 2. Structures of b-AP15 (NSC687852) and the analogues b-AP107 (NSC687449) and b-AP113 (NSC687853).

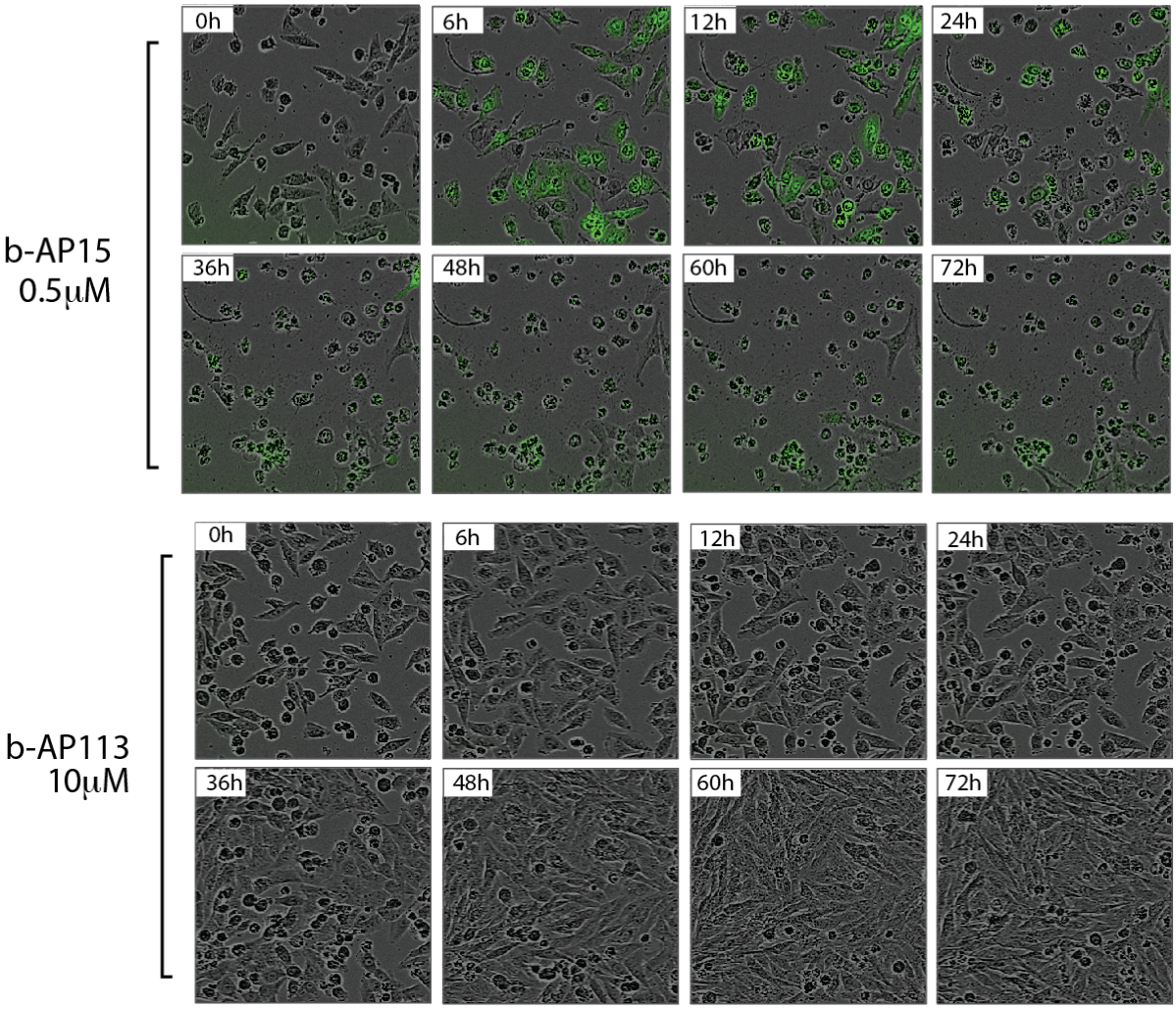


All compounds contain an α, β-unsaturated carbonyl units (blue dots) and an acrylamide moeity with a potential Michael acceptor (green dot). Note that the cytotoxic activity of the compounds correlates with the reactivity of the α, β-unsaturated carbonyl units (whereas compound b-AP113, which shows weak cytotoxic activity, contains the acrylamide).

Supplementary Figure 3. Response of MelJuSo Ub^{G76V}-YFP cells to b-AP15 and b-AP113.

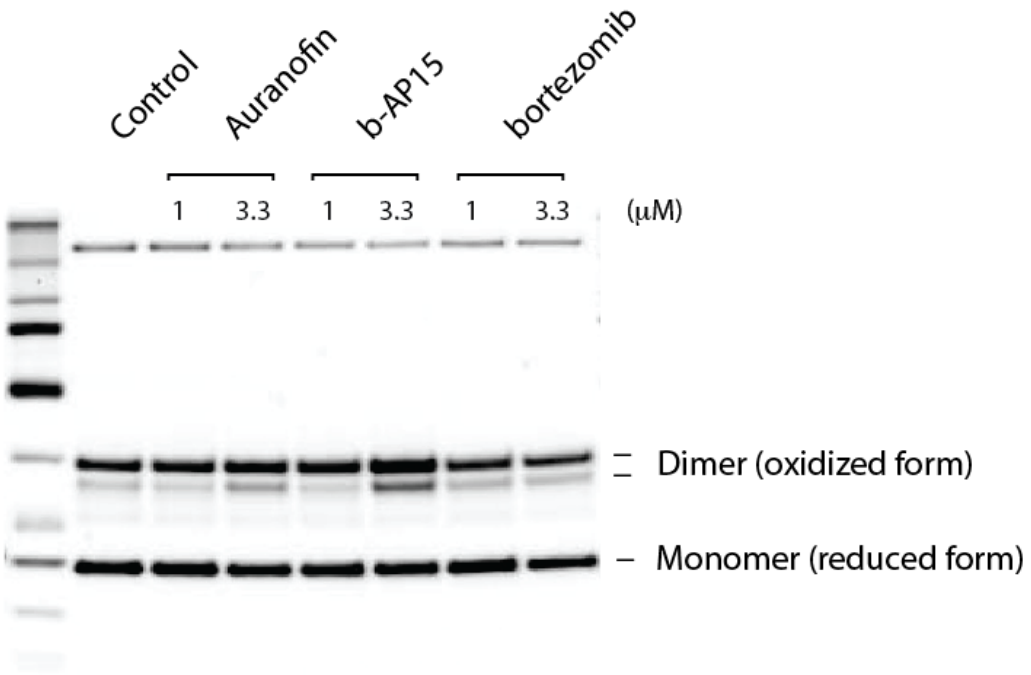


MelJuSo Ub^{G76V}-YFP cells were monitored after exposure to the indicated compounds. Photographs were taken at the indicated time-points using an IncuCyte instrument.



MelJuSo Ub^{G76V}-YFP cells were monitored after exposure to the indicated compounds. Photographs were taken at the indicated time-points using an IncuCyte instrument.

Supplementary Figure 4. Analysis of oxidative stress induction by b-AP15 and the thioredoxin reductase inhibitor auranofin.



Analysis of monomeric and dimeric forms of periredoxin 3 (Prx-3) as an indicator of oxidative stress. HCT116 cells were exposed to the indicated compounds for 45 minutes. Lysates were prepared as decribed in Methods and electrophoresed under non-reducing conditions. Note the increase in the oxidized form of Prx-3 (localized to the mitochondrial matrix) after exposure to auranofin and b-AP15. Both bands labelled as oxidized forms disappear with treatment with DTT. For details on the methodology, see Poynton and Hampton, Periredoxins as biomarkers of oxidative stress, *Biochim Biophys Acta* 1840 (2014) 906.