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Sandia-Biophagy NMSBA Project, 2015

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Test of 7 Compounds for Autophagy-Modulating Activity

- HeLa cells, 12-well plates, 3e5/well plated 18hrs prior to compound addition.
- Compounds added at either 11 or 33uM at hour 0.
- Bafilomycin (100nM) added at hour 22 of 24.
- MG132 (250nM) added at hour 0.
- At hour 24, supernatant collected (ice) and 50ul lysis buffer (NuPAGE sample buffer) added to each well, scraped with 1ml syringe plunger.
- Well supernatant centrifuged at 500xg to collect non-adherent cells, aspirate supe, combine with adherent cell lysate. This was done because many conditions caused many or most cells to detach.
- Gels: 4-12% NuPAGE, MES buffer.
- Blot quality was negatively affected by numerous stripping operations due to initial use of defective secondary (G-anti-R-HRP) followed by replacement and re-probing.
- Experiment should be repeated with fewer samples per run and lysis in sample buffer compatible with total protein assay to provide better normalization across samples.

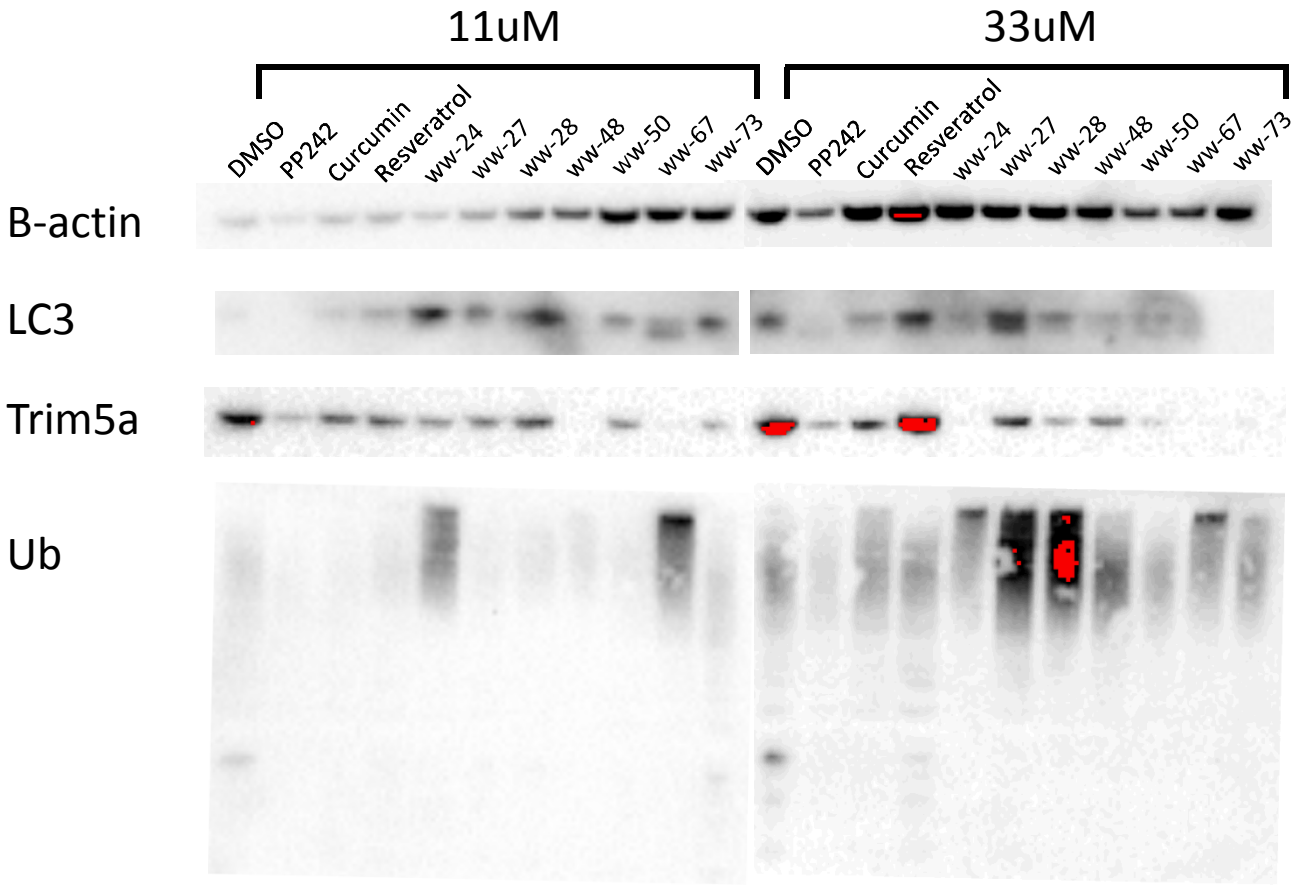
Qualitative Cell Health at T=22hrs

(Compounds alone, no Baf or MG-132, assessed by bright field at 10x)

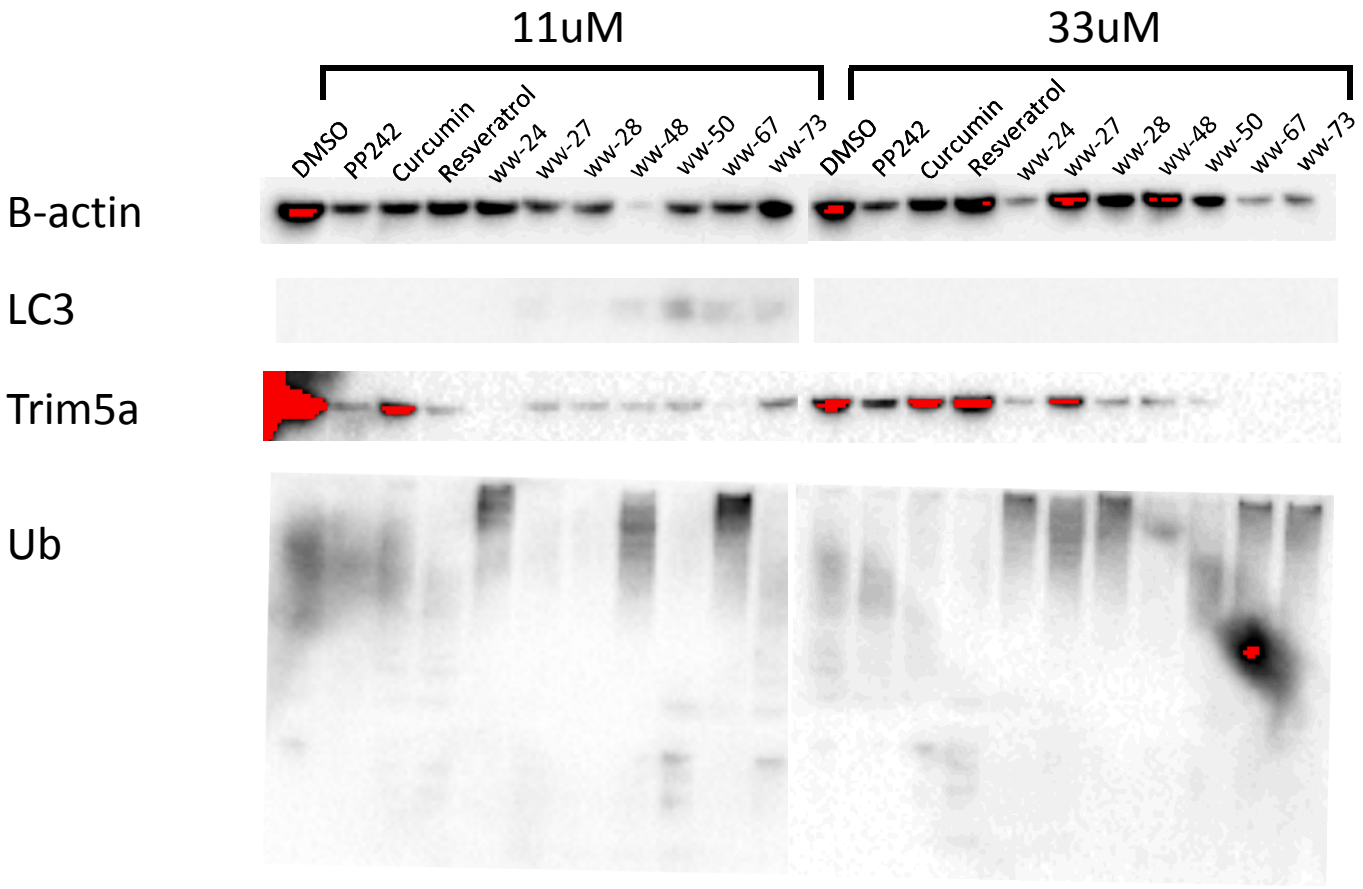
Key: +++=healthy, ++=sick/rounded/partly detached, +=very sick/dying/detached

Compound	11uM	33uM
ww-24	+++	+
ww-27	+++	+
ww-28	+++	+++/++
ww-48	++/+	+
ww-50	+++	++
ww-67	+	+
ww-73	+/++	+
DMSO	+++	+++
PP242	+++/++	++
Curcumin	+++	+++
Resveratrol	+++	+++

Compounds Alone 11,33uM (24hrs)



Compounds 11,33uM (24hrs) + Bafilomycin 100nM (hrs 22-24)



Compounds 11,33uM (24hrs) + MG132 250nM (24hrs)