SUPPLEMENTAL METHODS

Cell lines and culture conditions. OCI-Ly7 and OCI-Ly19 human DLBCL lines are of germinal center (GC) phenotype, and OCI-Ly3 and OCI-Ly10 are human DLBCL lines of activated B-cell phenotype (ABC), which were kind gifts from Dr. Louis Staudt at the National Cancer Institute, Bethesda MD. Cells were cultured in RPMI medium supplemented with 10% fetal calf serum. The Granta and Rec-1 MCL lines were obtained from ATTC, and were cultured similarly.

Normal donor peripheral blood mononuclear cells (PBMCs) were obtained under a University of Rochester Institutional Reviewed Board (IRB) approved protocol. The cells were counted and washed once in PBS. Dynabeads CD19 Pan B (Invitrogen) was used to isolate B-cells, and the DETACHaBEAD CD19 (Invitrogen) was used to detach the B-cells from the Dynabeads according to the manufacturer's suggested protocols. Isolated B-cells were rinsed in PBS and divided into three fractions. One fraction of cells was spun down, and the cell pellet frozen at -80ºC. The two remaining fractions of cells were re-suspended in RPMI containing 10% FBS. The cells ($2x10^6$ cells/ml) were either activated by adding anti-IgM (2 μg/ml) and CpG oligonucleotides (1 μg/ml) into the culture medium, or not activated by culturing in medium alone for 72 hrs before harvesting the cells for immunoblotting.

Patient derived lymphoma biopsy specimens were obtained under a University of Rochester IRB approved protocol. CLL/SLL01 was obtained from a lymph node from a patient with this disease; DLCL01 and 02 were obtained from the spleen and pleural fluid, respectively from two individuals with this disease; MCL01 was obtained from a lymph node from a patient with this disease. Single cell suspensions from these samples were made and subjected to ficoll-hypaque separation. In each of the samples, the proportion of mononuclear cells that were malignant was > 80%.

MTT assays. Cells were incubated with CDDO or DMSO (vehicle control) at a density of 6 x 10^4 per well on a 96-well flat-bottomed microtiter plate, and an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, (MTT) assay was done as previously described [1]. The results are presented as the percent of cells that reduce MTT, as compared with DMSO-treated control cells.

Reagents. CDDO and its derivatives were synthesized by Dr. T. Honda and kindly provided by Dr. Michael Sporn, both of Dartmouth College (Hanover, NH). Biotinylated CDDO was synthesized as previously described [2, 3].

Lon Purification. Rosetta *E. coli* expressing human Lon with an amino-terminal hexa-histidine tag was used to purify recombinant protein as previously described [4]. An overnight culture was used to inoculate 1 L of Terrific Broth prepared from Modified EZMix powder (Sigma) to an OD_{600} of 0.07-0.10, and the bacteria were cultured at 37°C to an OD_{600} of 0.8-.09 before induction with IPTG (1 mM) for 90 min. Bacteria were pelleted and suspended to 0.25 g wet weight/ml in Buffer A (50 mM Hepes pH 8.0, 0.3 M NaCl, 10 mM $MgCl_2$, 20% (w/v) glycerol), sonicated and the homogenate centrifuged at 100,000 x g at 4°C for 10 min. The extract was loaded directly onto a Ni-NTA column (1 ml) equilibrated in Buffer A. The column was washed with Buffer A (2X with 2 ml), Wash Buffer (50 mM Hepes pH 8.0, 0.15 M NaCl, 10 mM $MgCl_2$, 20% (w/v) glycerol) containing 40 mM imidazole (1X 2 ml, 4X 1 ml) and then eluted with E1 buffer (Wash Buffer containing 0.1M imidazole) (1X with 1.5 ml) and with E3 buffer (Wash Buffer containing 0.3 M imidazole) (2X with 1.5 ml). Protein concentration of the eluted protein was determined using the bicinchoninic acid (BCA) assay (Pierce, Thermo Scientific) and the purity of Lon was determined by Coomassie Brilliant Blue staining (Suppl. Fig. S1).

Transmission electron microscopy. Samples were processed as previously described [5]. Briefly, after fixation at 4°C overnight, in 2.5% glutaraldehyde/4.0% paraformaldehyde in 0.1 M phosphate buffer, the samples were rinsed in 2 changes of 0.1 M sodium phosphate buffer. Cells were post-fixed in 1.0% osmium tetroxide in the same buffer, dehydrated through a graded series of ethanol to 100% and placed into liquid Spurr epoxy resin overnight. The next day, size 3 BEEM capsules were filled with fresh Spurr resin and inverted and placed over the cells of interest on the slides. The slides/capsules were polymerized overnight at 70°C. The next day the slides were dipped several times in liquid nitrogen to break the surface tension between the glass and the polymerized capsules until they "popped off" the slide. The capsules with the entrapped cells were trimmed down to a trapezoid and thin sectioned with a diamond knife at 70 nm and placed onto 200 mesh rhodium/copper grids. The grids were stained with uranyl acetate and lead citrate and examined using a Hitachi 7650 transmission electron microscope with an attached Gatan Erlangshen 11 megapixel digital camera.

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1. Brookes P, Morse K, Ray D, et al. The triterpenoid CDDO and its derivatives elicit human lymphoid cell apoptosis through a novel pathway involving the unregulated mitochondrial permeability transition pore. *Cancer Res.* 2007;67(4):1793-1802.

2. Samudio I, Konopleva M, Hail N, Jr., et al. 2-Cyano-3,12-dioxooleana-1,9-dien-28-imidazolide (CDDO-Im) directly targets mitochondrial glutathione to induce apoptosis in pancreatic cancer. *J Biol Chem.* 2005;280(43):36273-36282.

3. Samudio I, Konopleva M, Pelicano H, et al. A novel mechanism of action of methyl-2-cyano-3,12 dioxoolean-1,9 diene-28-oate: direct permeabilization of the inner mitochondrial membrane to inhibit electron transport and induce apoptosis. *Mol. Pharmacol.* 2006;69(4):1182-1193.

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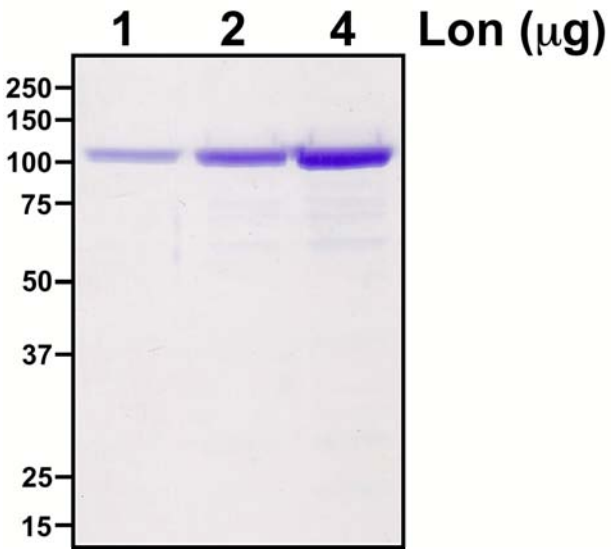
5. di Sant'Agnese PA, De Mesy-Jensen KL. Diagnostic electron microscopy on reembedded ("popped off") areas of large Spurr epoxy sections. *Ultrastruct Pathol.* 1984;6(2-3):247-253.

Supplementary Figure S1

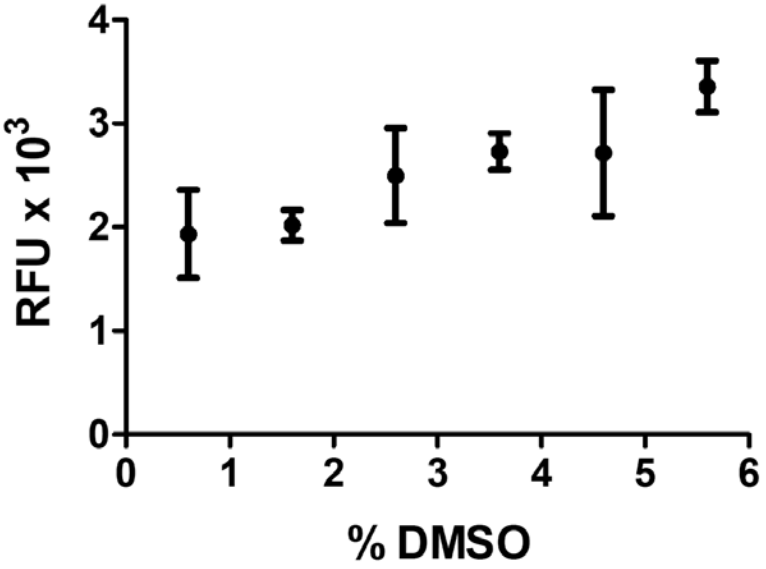
Purified recombinant human Lon



Supplementary Figure S1. The purity of recombinant Lon was estimated at >95% by Coomassie Brilliant Blue staining of protein separated on 10% SDS-PAGE.

Supplementary Figure S2

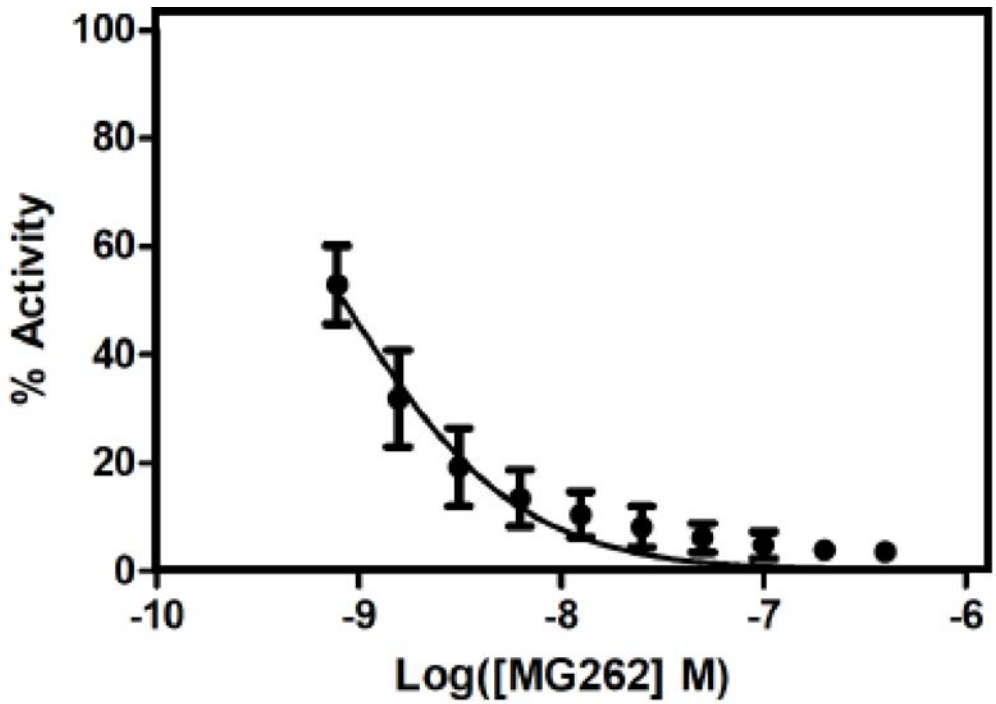
DMSO-tolerance of Lon peptidase activity



Supplementary Figure S2. Purified human Lon (800 nM monomer) was incubated in buffer containing ATP (2.0 mM), (Ala-Ala)$_2$-Rhodamine-110 (6 μM) and varying concentrations of DMSO as indicated for 3 hours at 37°C. The relative fluorescence units (RFU) were measured. The error bars represent the standard deviation of 4 independent experiments.

Supplementary Figure S3

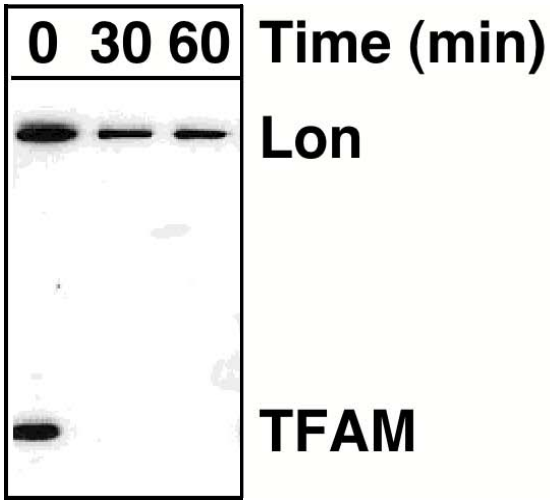
MG262 inhibition of the 20S proteasome



Supplementary Figure S3. The 20S proteasome-mediated cleavage of AA_2-Rh110 is inhibited by the proteasome inhibitor MG262 with an IC_{50} = 0.7-1.0 nM.

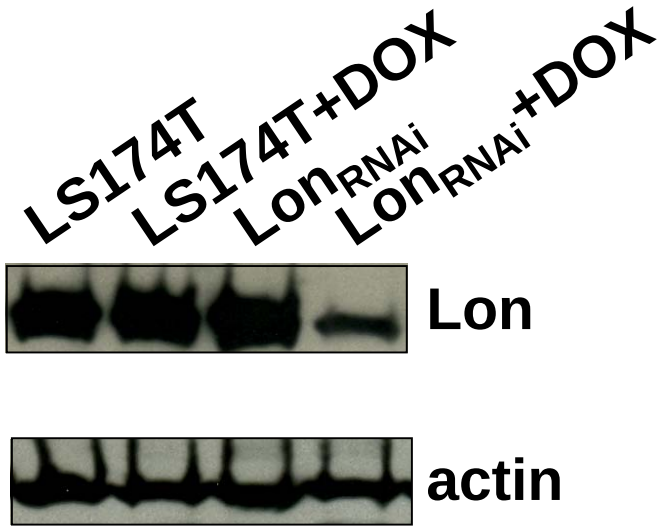
Supplementary Figure S4

Purified TFAM is degraded by Lon with a half-life less than 30 min



Supplementary Figure S4. Lon (200 nM) was incubated with TFAM (80 nM) for the time periods as shown. Samples were immunoblotted with antibodies recognizing Lon and TFAM.

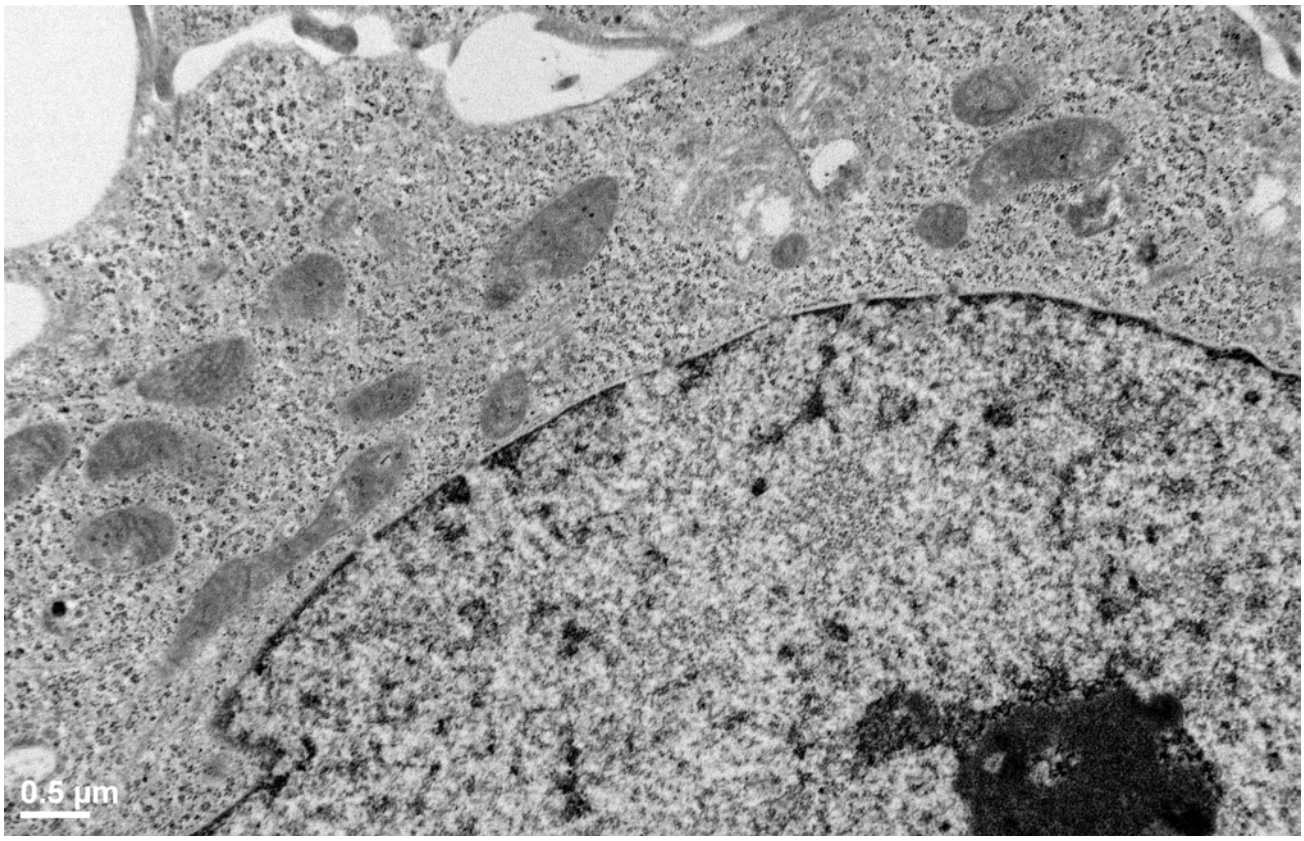
Supplementary Figure S5



Supplementary Figure S5. LS174T and Lon_{RNAi} were cultured with and without doxycycline (DOX, 50 ng/ml) for 2.5 weeks. Cell extracts (20 µg) were immunoblotted with antibodies recognizing Lon or actin.

Supplementary Figure S6A

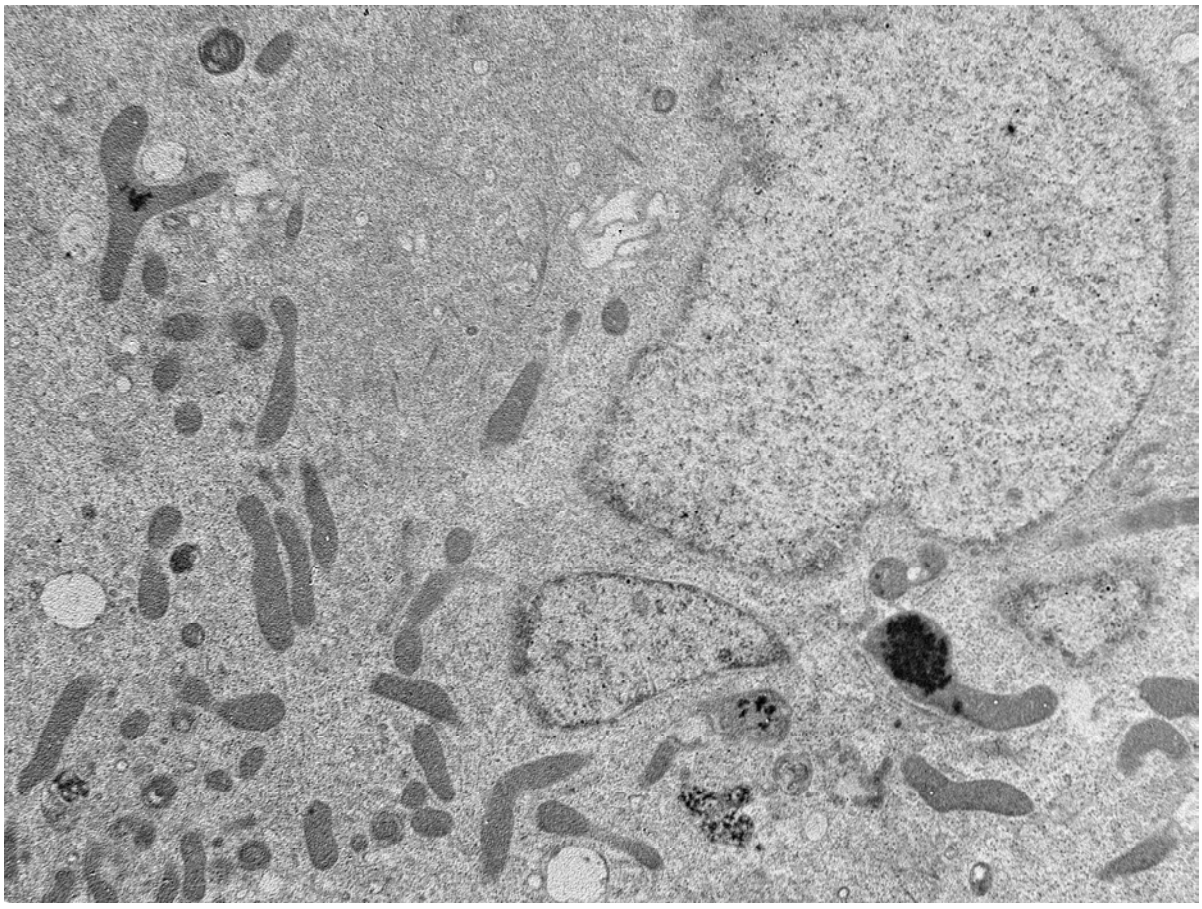
Lon_{RNAi}- control

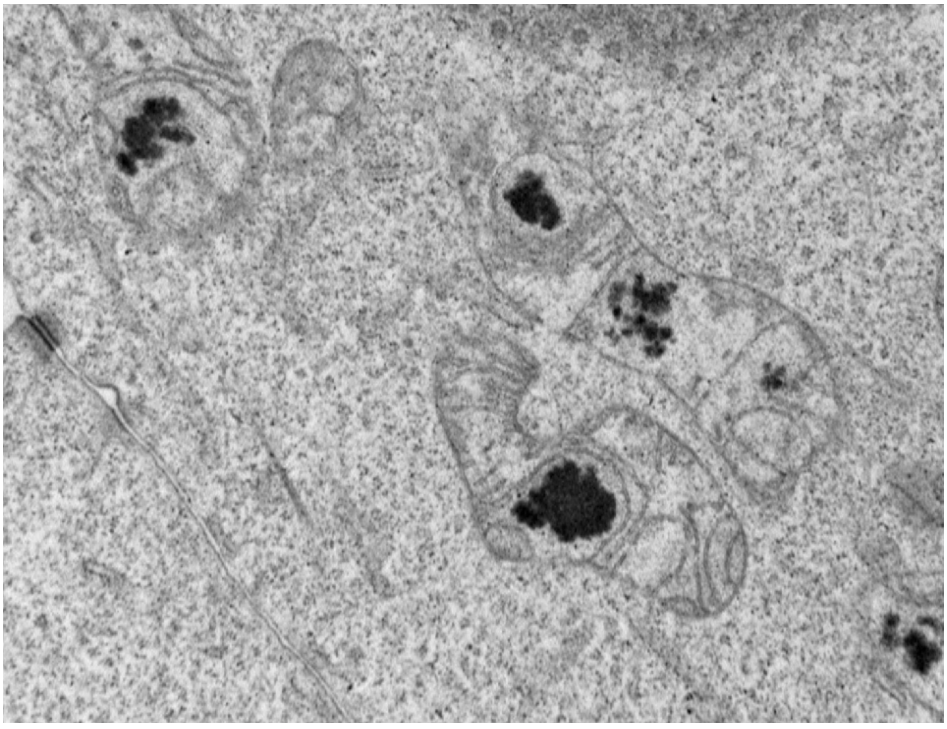


Supplementary Figure S6. Lower magnification images of Lon_{RNAi} control (**A**) and doxycycline (DOX)-treated (**B**, 50 ng/ml for 2.5 weeks) cells, demonstrating that the knockdown of Lon (as shown in Suppl. Fig. S4), results leads to the accumlulation of inclusion bodies in mitochondria, but not in other cellular compartments.

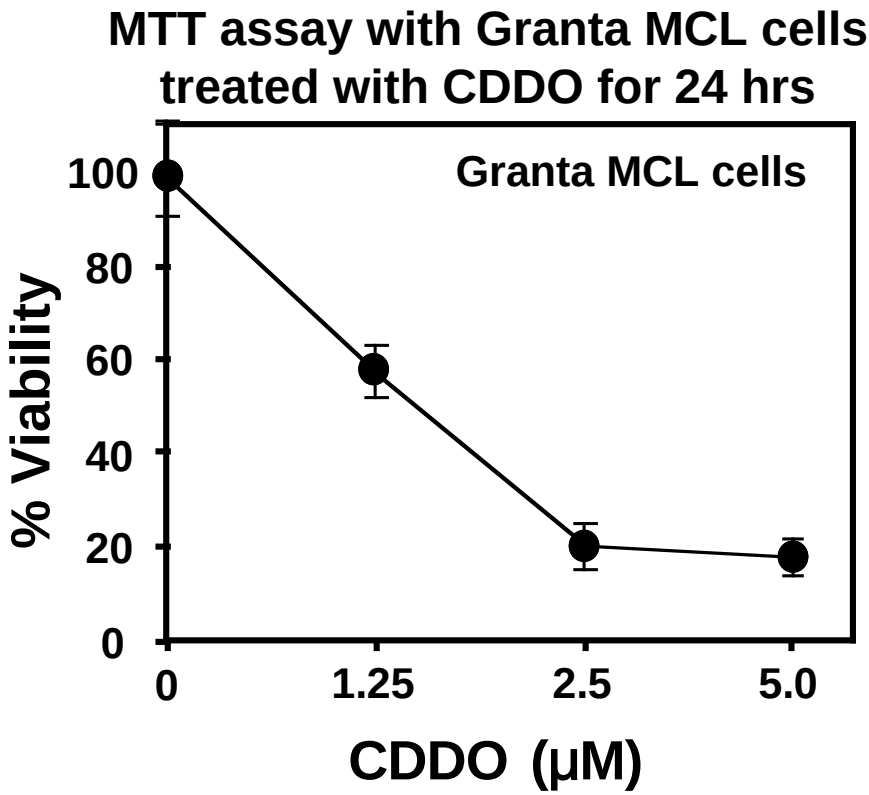
Supplementary Figure S6B

Lon_{RNAi} knockdown + DOX (2 µg/ml), 2.5 weeks





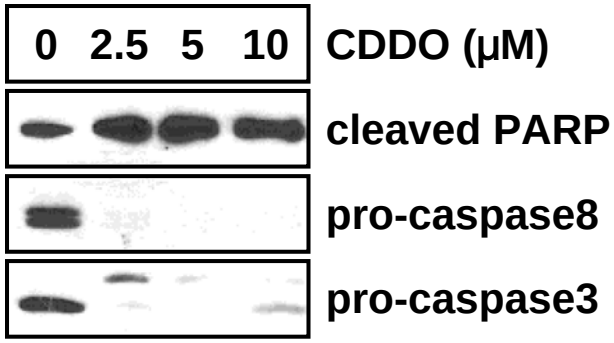
Supplementary Figure S7A



Supplementary Figure S7A. Granta MCL cells were exposed to increasing concentrations of CDDO for 24 hours at which time an MTT assay was performed; results are expressed as the percent MTT activity compared with that of the vehicle-treated control cells. Data are presented as the mean of triplicates ± the standard deviation and are representative of three separate experiments.

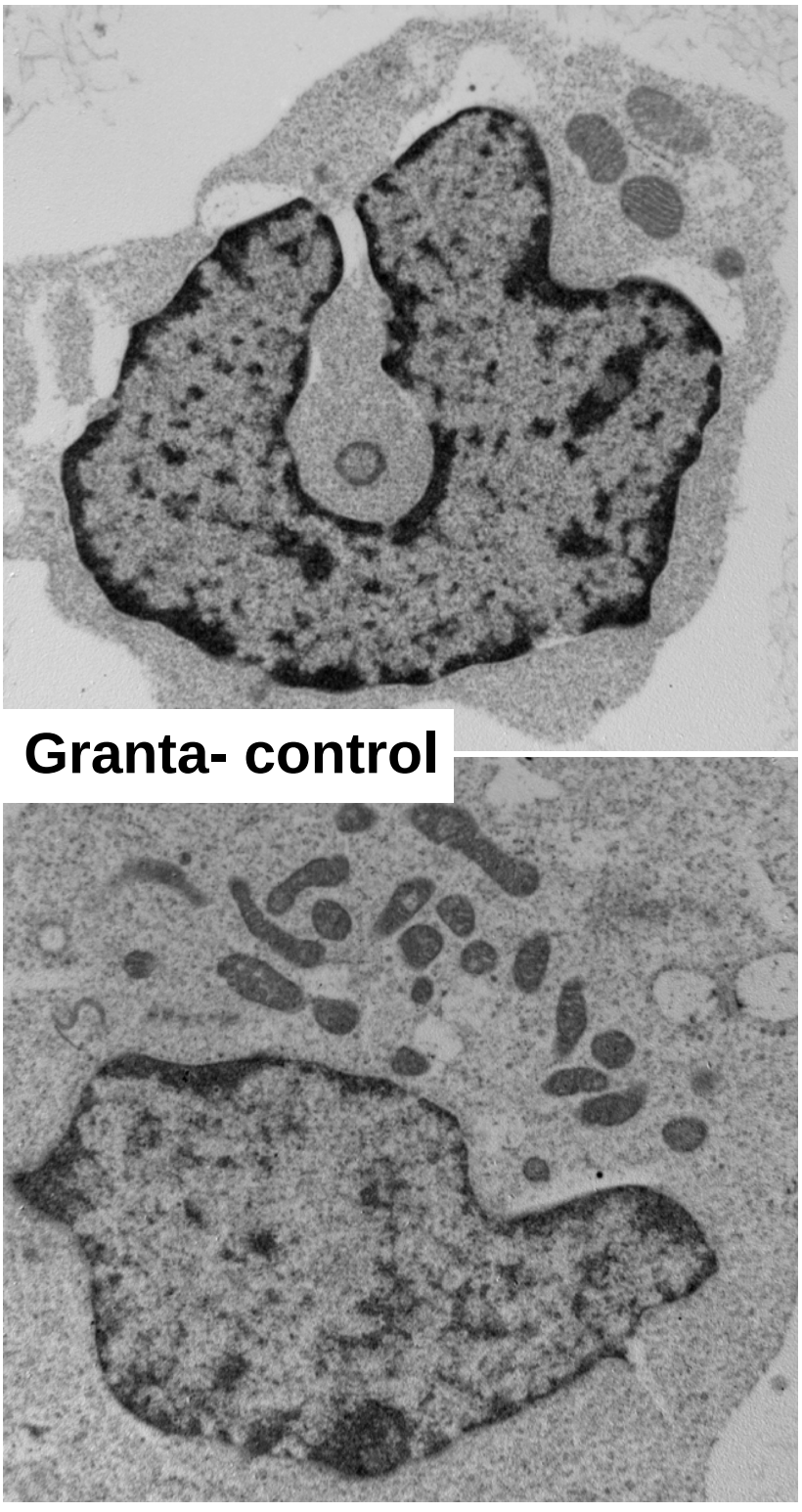
Supplementary Figure S7B

CDDO-induced apoptosis in Granta cells



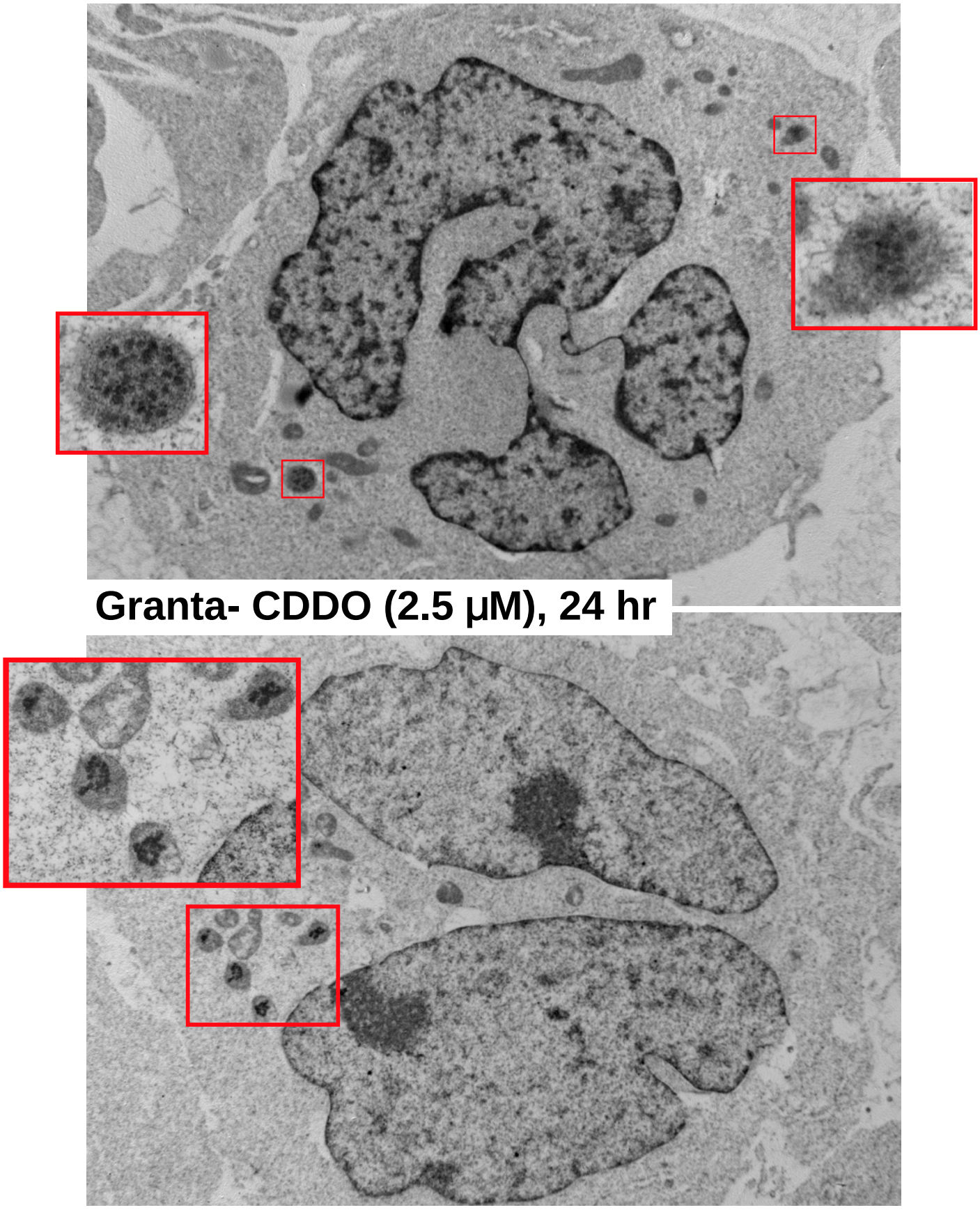
Supplementary Figure S7B. Cell extracts (20 ug) from Granta cells treated with CDDO for 25 hrs were immunoblotted with antibodies recognizing cleaved PARP, pro-caspases -3 or -8.

Supplementary Figure S8A

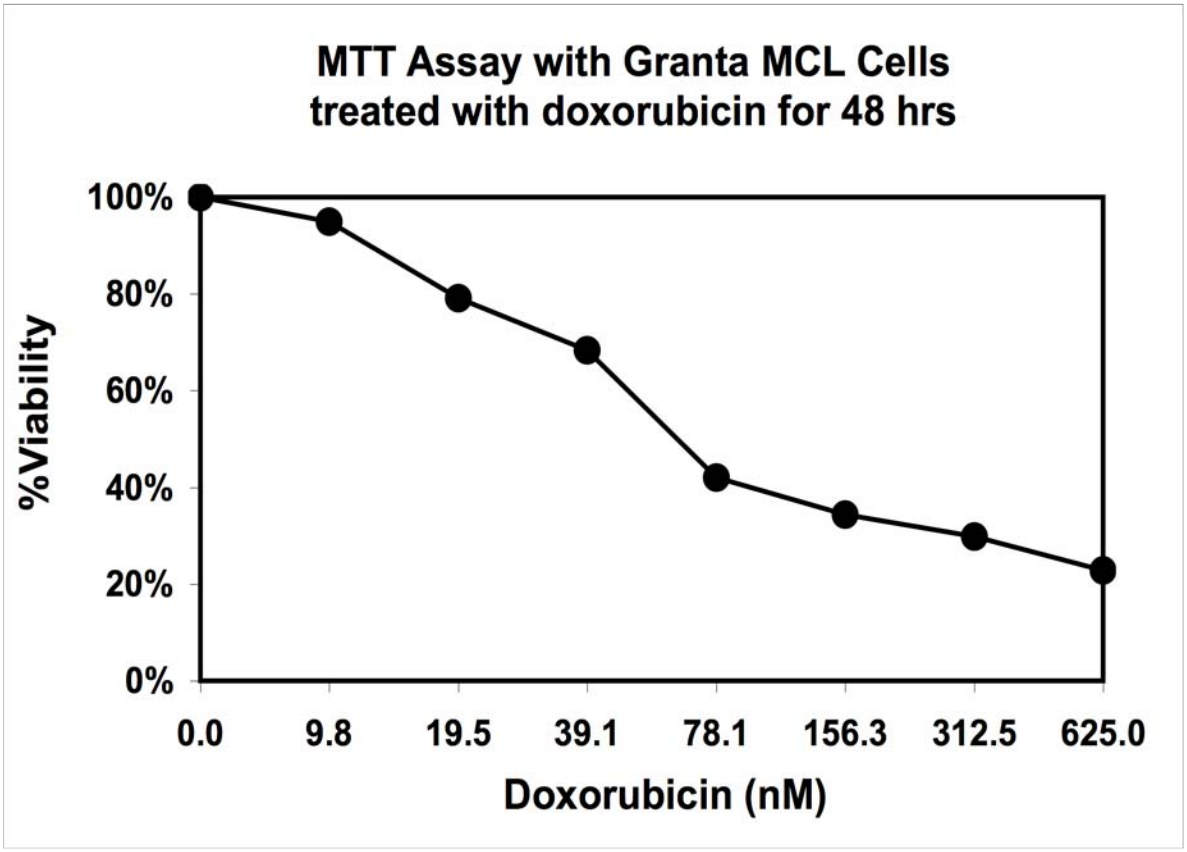


Supplementary Figure S8. Lower magnification images of Granta control (A) and CDDO-treated (B, 2.5 μM, 24 hrs) cells. CDDO-treatment leads to electron-dense aggregates in mitochondria but not in other cellular compartments.

Supplementary Figure S8B



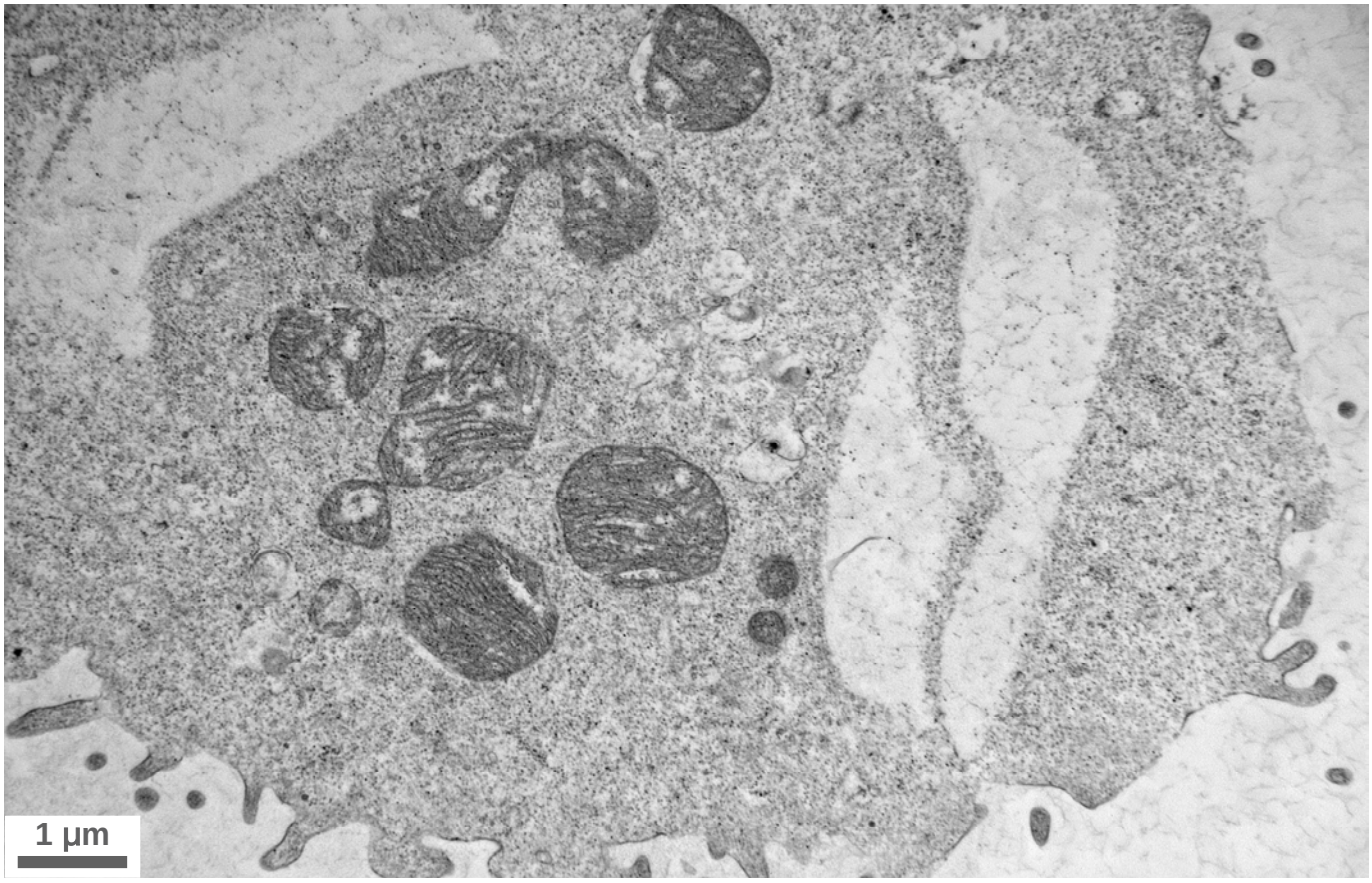
Supplementary Figure S9



Supplementary Figure S9. Granta MCL cells were exposed to increasing concentrations of doxorubicin for 48 hrs at which time an MTT assay was performed; results are expressed as the percent MTT activity compared with that of the vehicle-treated control cells. Data are presented as the mean of triplicates ± the standard deviation and are representative of three separate experiments.

Supplementary Figure S10A

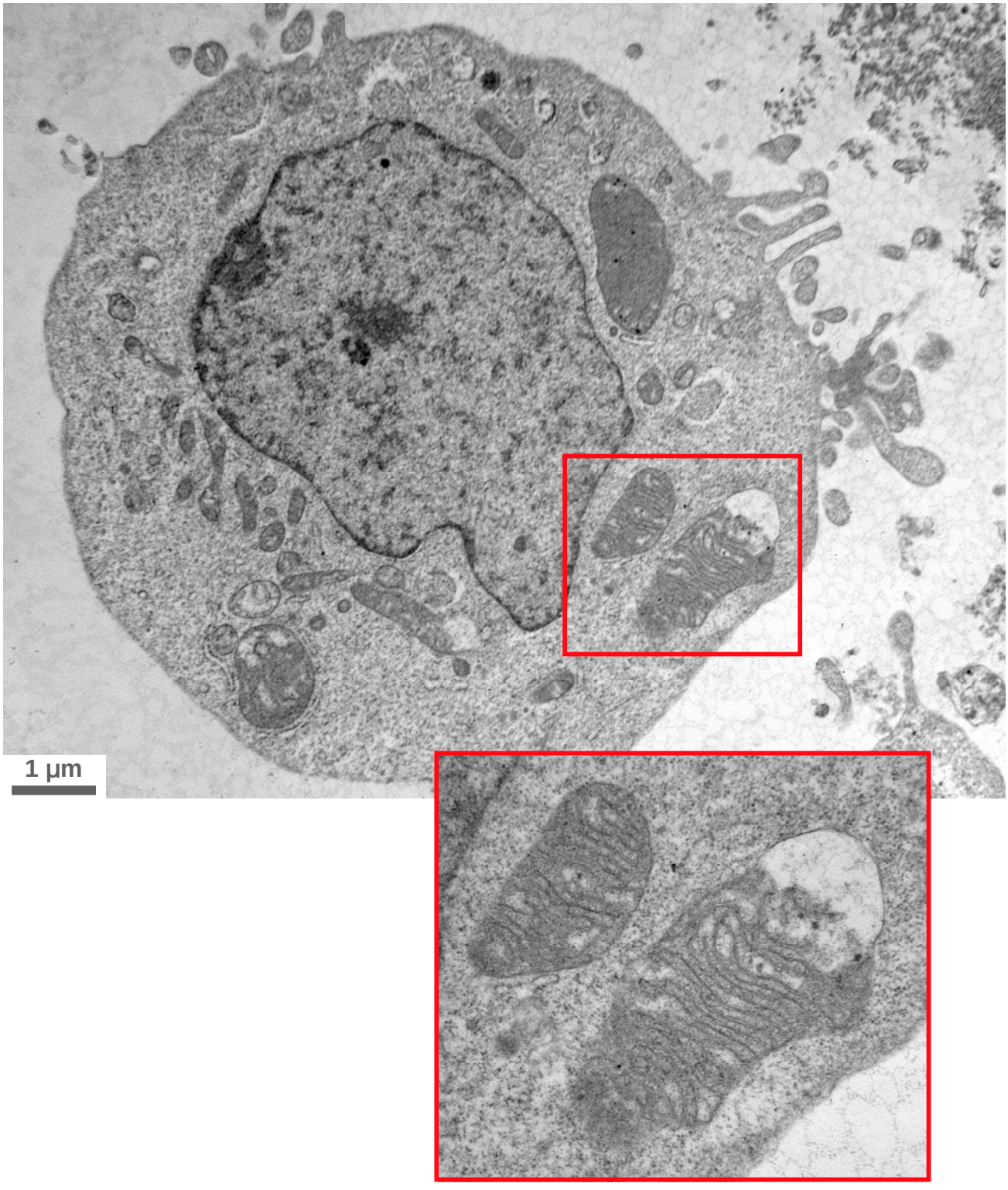
Granta- control



Supplementary Figure S10. Lower magnification images of Granta control (A) and doxorubicin-treated (B, 50 nM, 48 hrs) cells. Doxorubicin-treatment does not lead to the accumulation of inclusion bodies in any intracellular compartments.

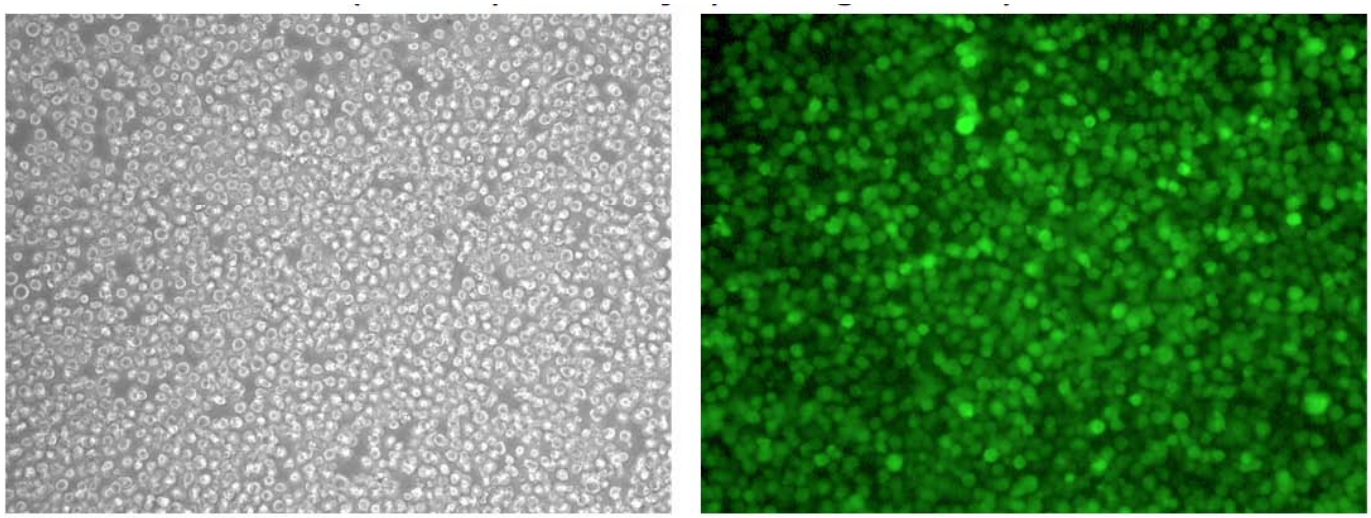
Supplementary Figure S10B

Granta- doxorubicin (50 nM), 48 hr



Supplementary Figure S11

Lentivirus transduction efficiency



Supplementary Figure S11. Granta cells (2×10^5) were transduced with GFP lentivirus particles (MOI 5). Images were captured 4 days post-transduction (20X magnification).