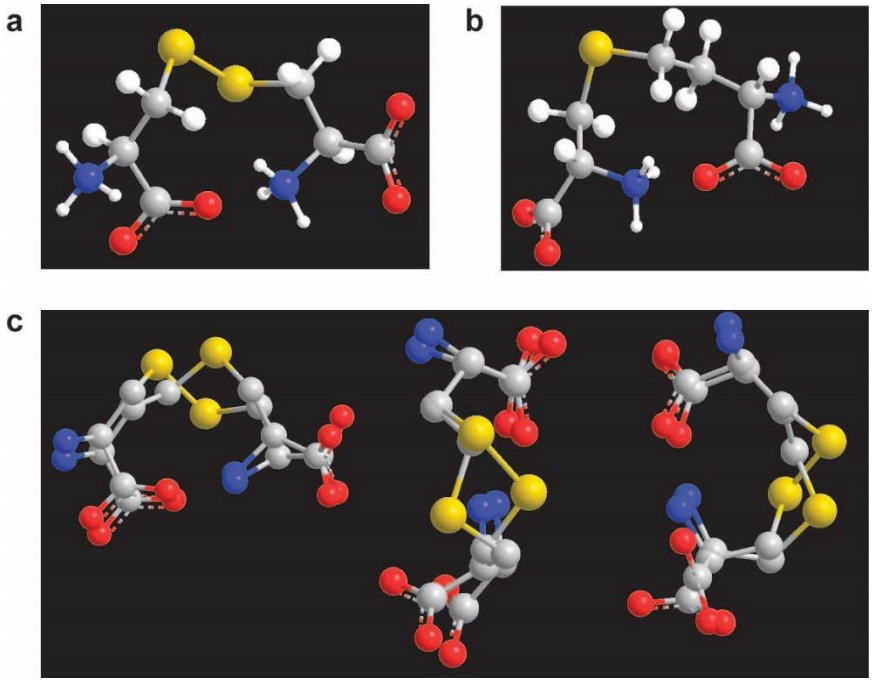
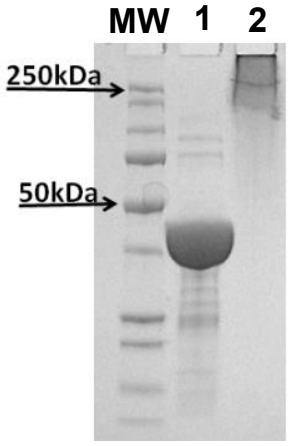


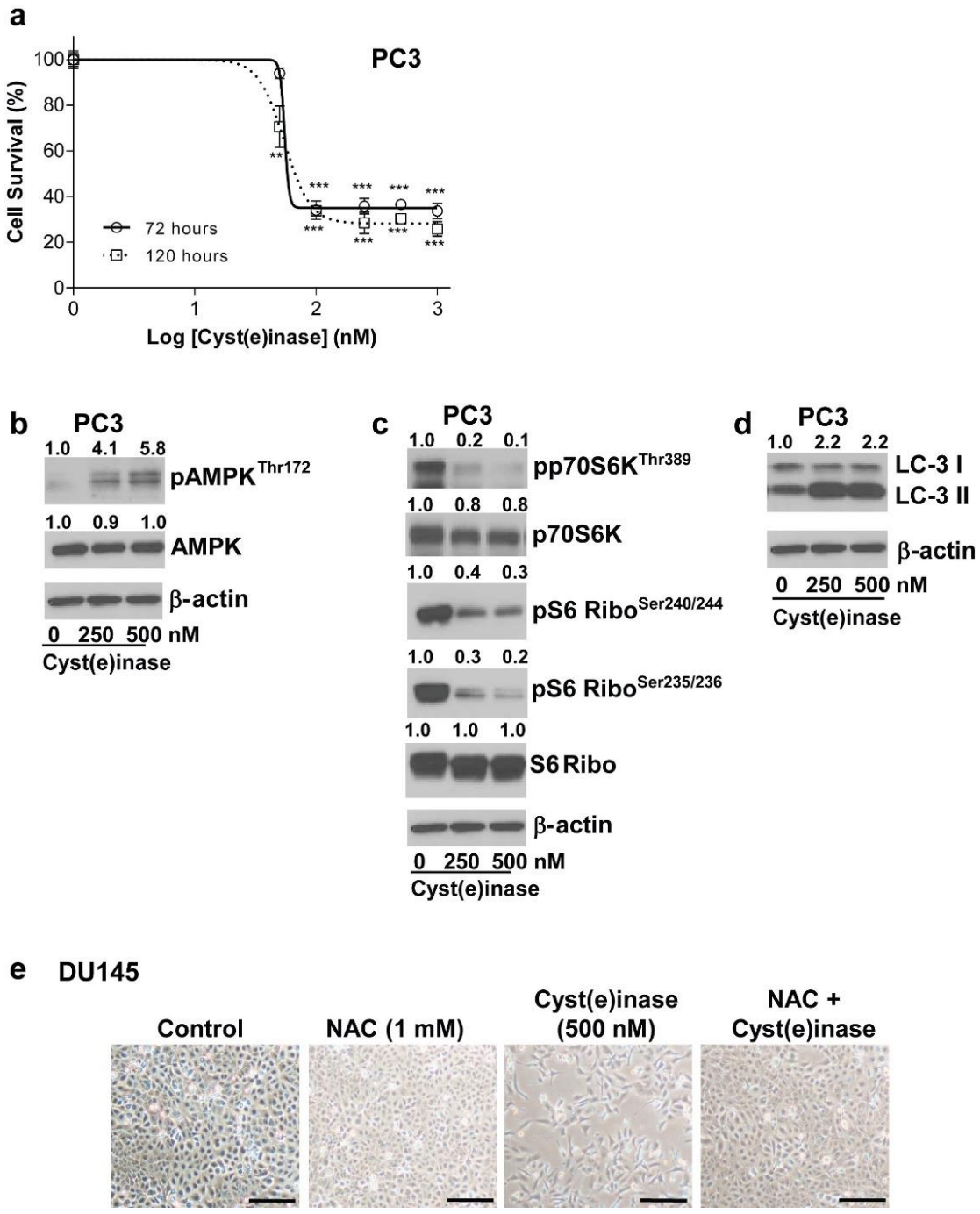
Supplementary Figure 1: Optimal conformations for L-Cystine and L-Cystathionine.

Molecular dynamic modeling using the MM2 force field with a target temperature of 310 K supplied in the Chem3D Pro software for the optimal conformations of **(a)** L-cystine (CSSC) and **(b)** L-cystathionine (L-Cysta). **(c)** Overlay between L-Cysta and CSSC shows that the C→S atomic substitution introduces an additional "kink" which is better accommodated by the engineered CGL thanks to the E59T and E339V amino acid substitutions (hydrogen atoms not shown). Overlay is shown from various angles.



Supplementary Figure 2: PEGylation analysis of Cyst(e)inase.

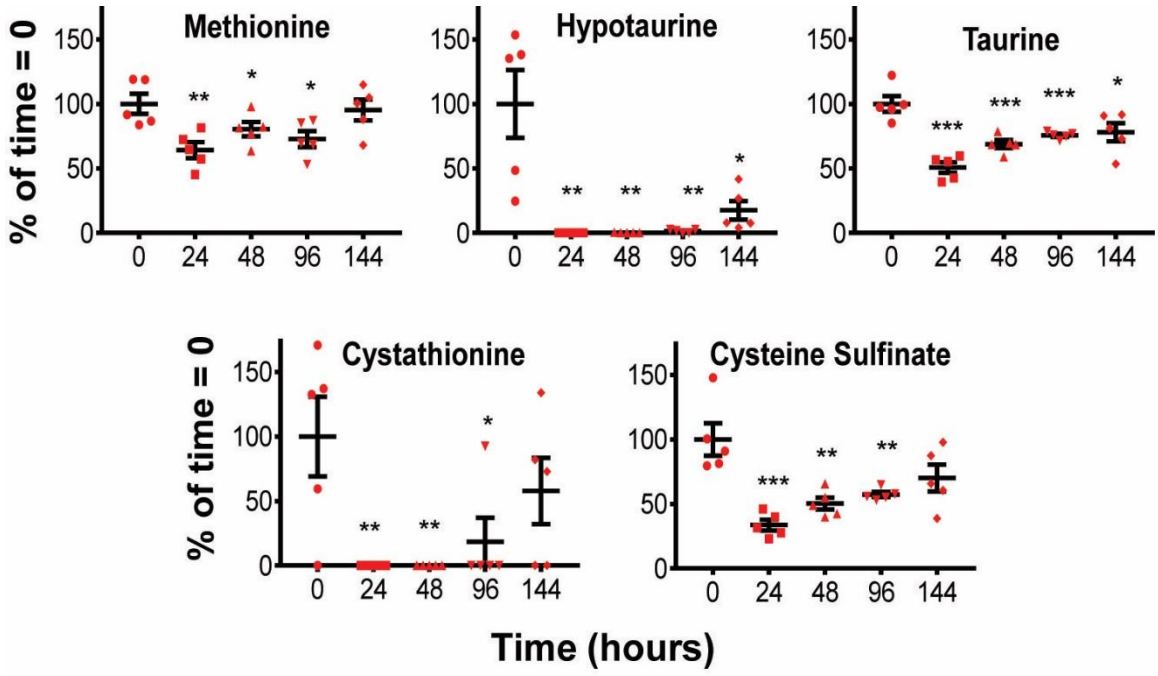
SDS-PAGE gel of purified CGL-E59T-E339V (Lane 1) and Cyst(e)inase after PEGylation with 100 fold molar excess of methoxy PEG succinimidyl carboxymethyl ester, MW 5000 Da (Lane 2).



Supplementary Figure 3: Effect of Cyst(e)inase in human prostate cancer cells.

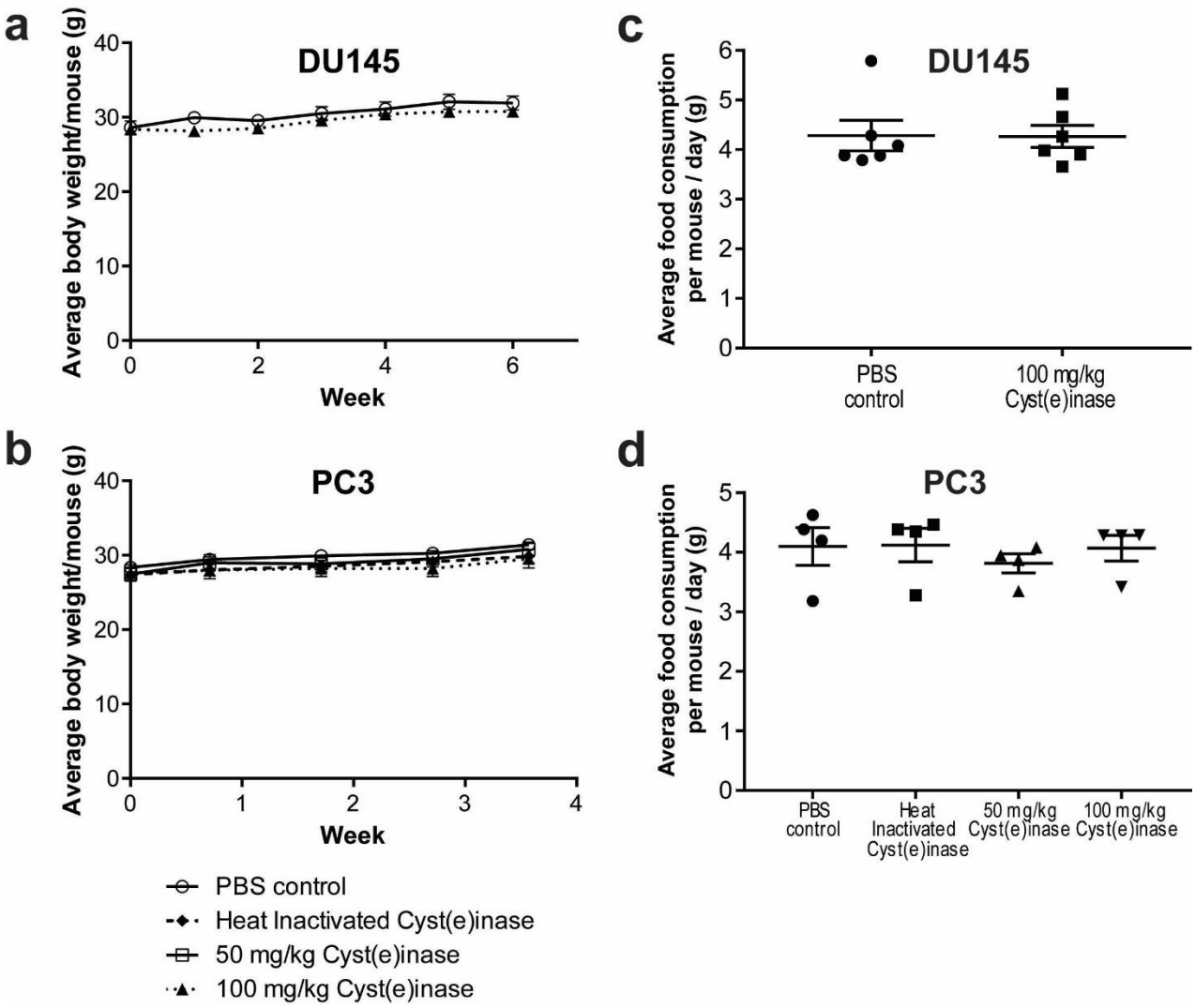
(a) Cell survival in PC3 prostate cancer treated with indicated concentrations of Cyst(e)inase as measured by cell counts at indicated time points. Data is expressed as mean ± s.e.m. **P<0.01; ***P<0.001; one-way ANOVA followed by Tukey's multiple comparison test. **(b-d)** Western blot analysis of protein lysates from PC3 cells after treatment with indicated concentrations of Cyst(e)inase for 48 hours: **(b)** pAMPK levels, **(c)** pp70S6K and pS6 ribosomal protein levels, and **(d)** autophagosome marker LC-3 II. Western blots are shown with representative β-actin

controls and numbers above blots indicate band intensity relative to no treatment. Images have been cropped for presentation, uncropped imaged are shown in Supplementary Fig. 14. **(e)** Treatment of DU145 prostate cancer cells with N-Acetyl-L-cysteine (NAC) reverses the effect of Cyst(e)inase on cell survival as assessed visually. Cells were treated with indicated concentrations of Cyst(e)inase and/or NAC for 48 hours, scale bar represents 200 μM.



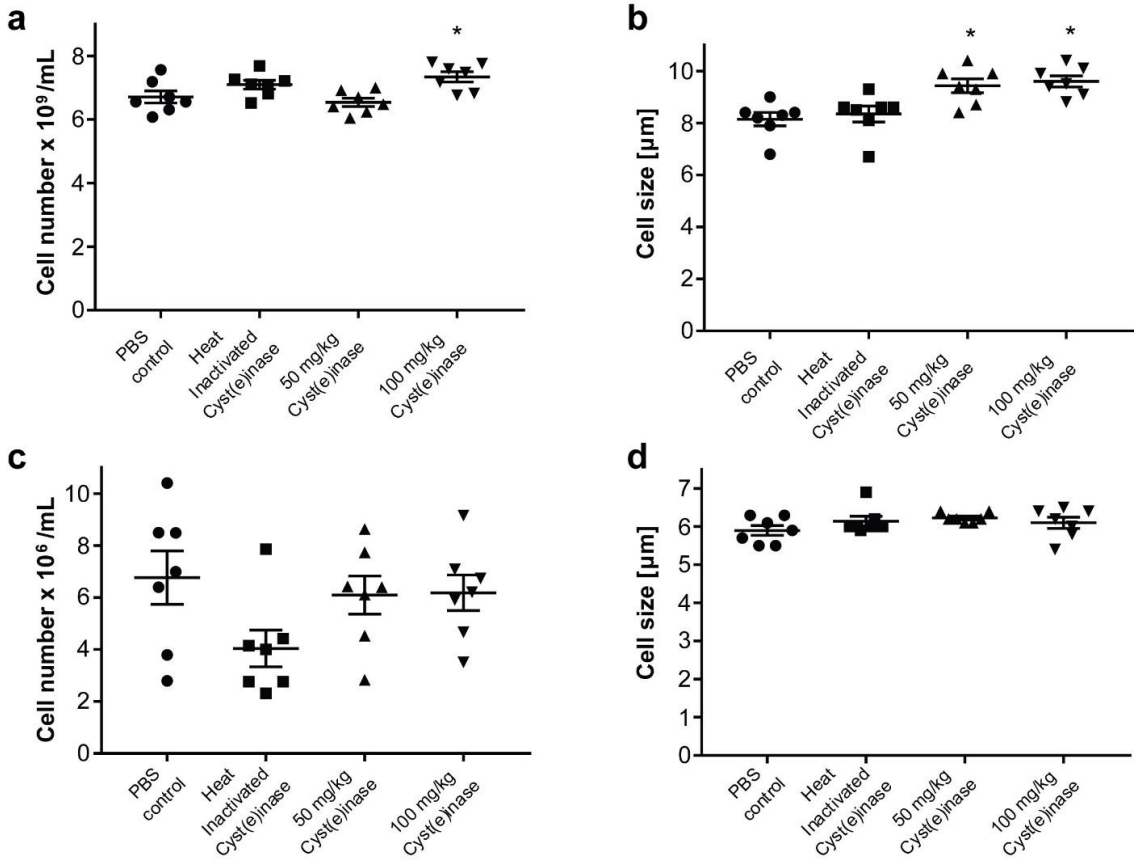
Supplementary Figure 4: Metabolic effects of single dose Cyst(e)inase administration in FVB mice.

Relative concentrations of non L-Cyst(e)ine sulfur-containing metabolites as a function of time as determined by MS (n = 5 per group). * P<0.05; ** P<0.01; *** P<0.001; two-sided Student's t-test. All data are expressed as mean ± s.e.m.



Supplementary Figure 5: Effect of Cyst(e)inase treatment on body weight and food consumption in male nude mice bearing prostate cancer xenograft tumors.

For each treatment group, average body weight in **(a)** DU145 and **(b)** PC3 PCa xenograft experiments; and average food consumption per mouse per day in **(c)** DU145 and **(d)** PC3 PCa xenograft experiments. Throughout, data are shown as mean ± s.e.m and were found to be not significant; one-way ANOVA followed by Tukey's multiple comparison test.

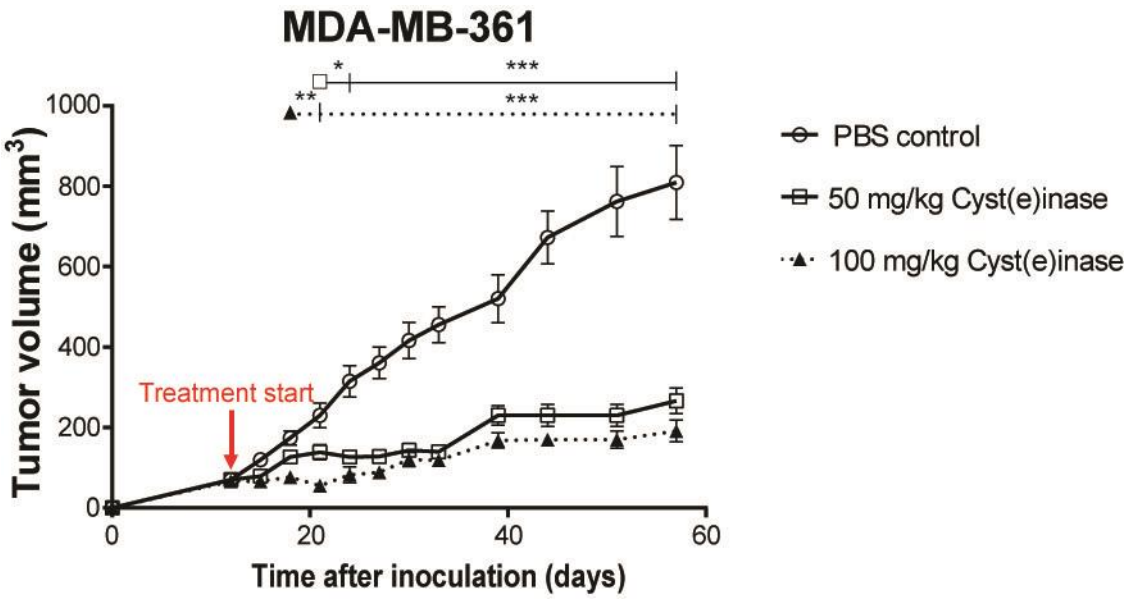


e

Treatment group	Serum urea concentration (µM/mL)	Alanine transaminase activity (milliunits/mL)	Aspartate transaminase activity (milliunits/mL)
PBS control	7.4 ± 0.5	8.0 ± 0.8	123 ± 2
Heat Inactivated Cyst(e)inase	7.0 ± 0.3	7.8 ± 0.2	125 ± 5
50 mg/kg Cyst(e)inase	6.6 ± 0.4	6.4 ± 0.7	119 ± 3
100 mg/kg Cyst(e)inase	6.3 ± 0.4	6.0 ± 0.5	115 ± 2

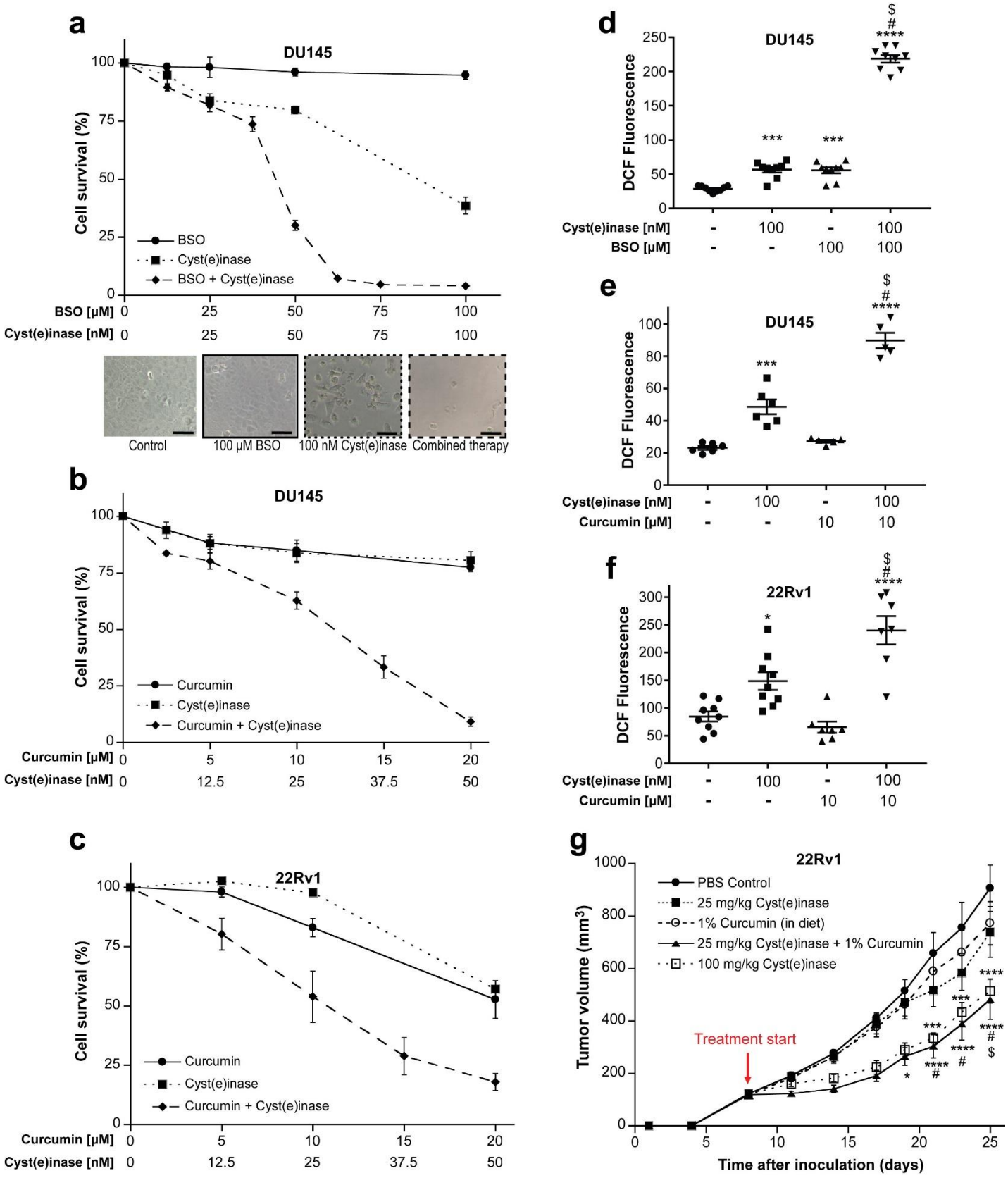
Supplementary Figure 6: Effect of Cyst(e)inase on blood cell counts, size and chemistry following PC3 prostate cancer xenograft studies.

Following termination of the PC3 xenograft studies, effect of Cyst(e)inase treatment in athymic nude mice on red blood cell **(a)** number and **(b)** size; and white blood cell **(c)** number and **(d)** size. **(e)** Renal function was assessed by monitoring serum urea concentrations, and markers of liver toxicity were assessed by alanine transaminase and aspartate transaminase activity. Throughout, data are expressed as mean ± s.e.m.; *P<0.05; one-way ANOVA followed by Tukey's multiple comparison test (n = 7 per group).



Supplementary Figure 7: Efficacy of Cyst(e)inase administration in a breast cancer xenograft mouse model.

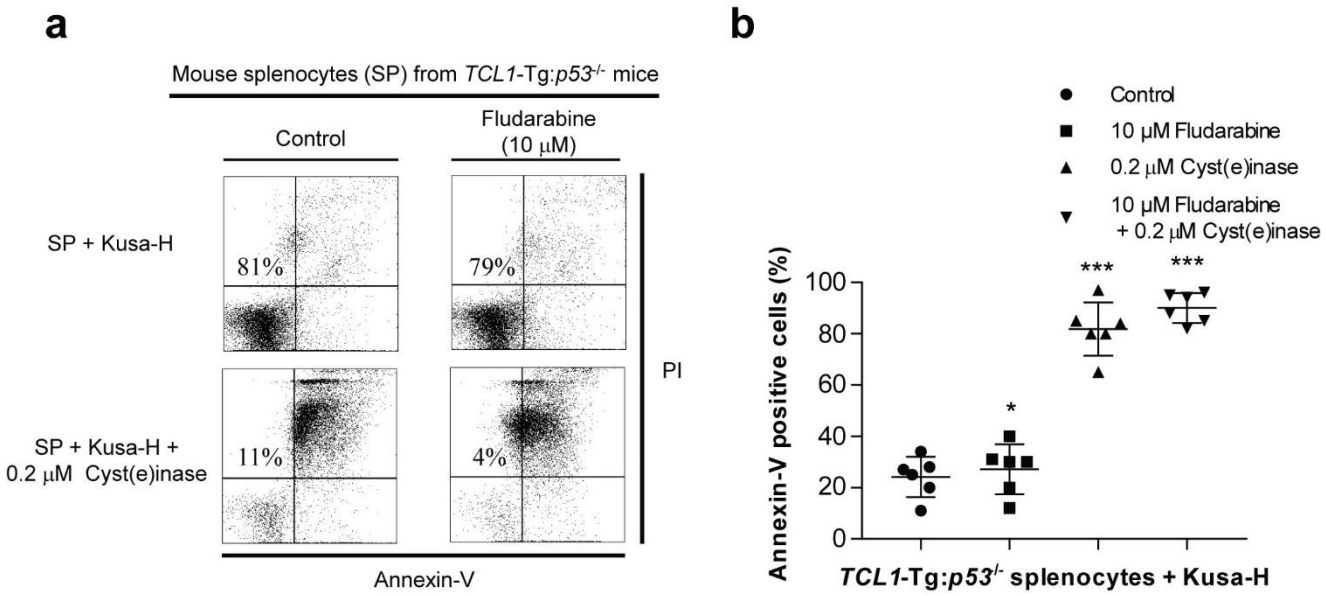
Quantification of tumor volume following treatment with active Cyst(e)inase or controls in female NOD SCID mice bearing xenograft tumors of MDA-MB-361 breast cancer cells orthotopically implanted (PBS, n = 9; 50 mg/kg Cyst(e)inase, n = 10; 100 mg/kg Cyst(e)inase, n = 9). Dosing was terminated when control tumors reached an IACUC predetermined maximum volume. *P<0.05; **P<0.001; *** P<0.0001; repeated measures two-way ANOVA followed by Bonferroni's multiple comparison test; data is expressed as mean ± s.e.m.



Supplementary Figure 8: Synergistic effects of Cyst(e)inase with other inhibitors of anti-oxidant pathways. **(a,b)** DU145 PCa cell survival as assessed by MTT assay following

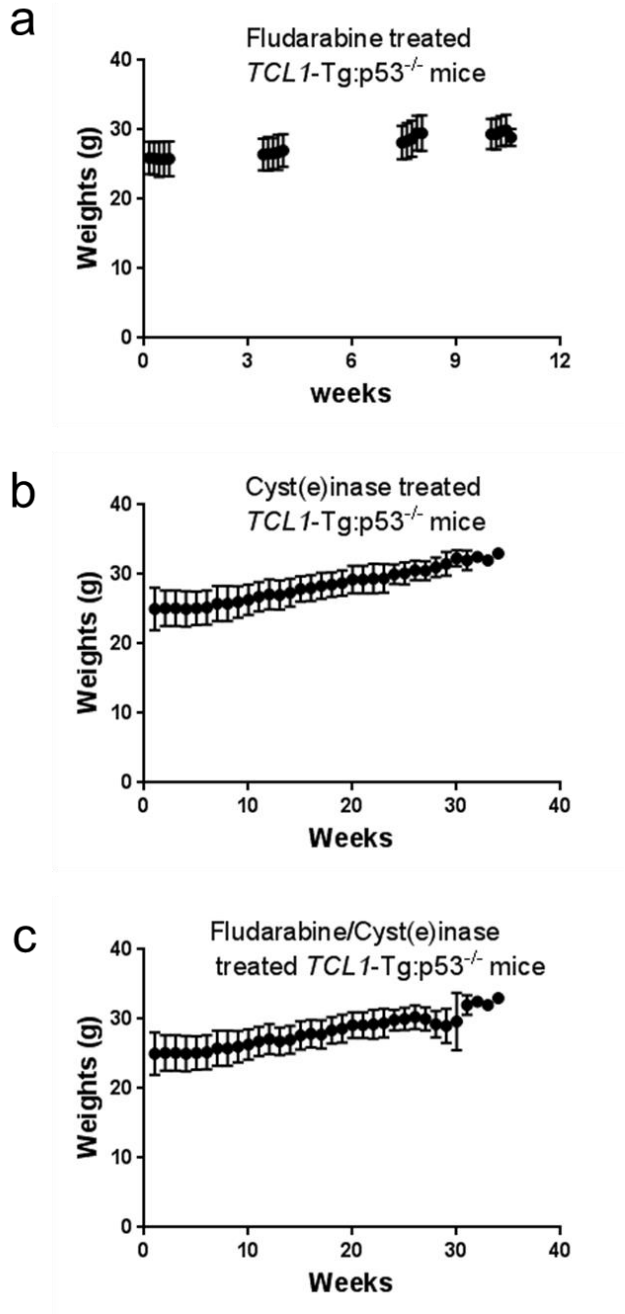
treatment for 72 hours with indicated concentrations of **(a)** Cyst(e)inase, BSO, or their combination (and representative images of remaining cells, scale bar represents 50 μM); and **(b)** Cyst(e)inase, curcumin, or their combination. **(c)** 22Rv1 PCa cell survival as assessed by MTT assay following treatment with indicated concentrations Cyst(e)inase, curcumin, or their combination for 72 hours. **(d-f)** Cellular ROS levels as assessed by DCFDA fluorescence 24 hours post-treatment with indicated concentrations of (**d**) Cyst(e)inase, BSO, or their combination in DU145 PCa cells; (**e**) Cyst(e)inase, curcumin, or their combination in DU145 PCa cells; and (**f**) Cyst(e)inase, curcumin, or their combination in 22Rv1 PCa cells. **(g)** Quantification of tumor volume in male nude mice bearing xenograft tumors 22Rv1 PCa cells following treatment with PBS, Cyst(e)inase, curcumin, or Cyst(e)inase and curcumin in combination (n = 7 per group).

For (**a-c**) combinatorial indices (CI) ranges: (**a**) 0.1-0.95; (**b**) 0.29-0.97; (**c**) 0.5-0.8. For (**a-f**), statistical analyses were performed using one-way ANOVA followed by Bonferroni's multiple comparison test. For (**a**, **b**): $P<0.05$; as compared to control: ≥ 25 nM Cyst(e)inase, ≥ 20 μM curcumin; ≥ 25 nM Cyst(e)inase/25 μM BSO; ≥ 12.5 nM Cyst(e)inase/5 μM curcumin; as compared to individual treatment: ≥ 37.5 nM Cyst(e)inase/37.5 μM BSO; ≥ 25 nM Cyst(e)inase/10 μM curcumin. For (**c**) $P<0.05$; as compared to control: ≥ 50 nM Cyst(e)inase, ≥ 20 μM curcumin; ≥ 25 nM Cyst(e)inase/10 μM curcumin; as compared to individual treatment: ≥ 25 nM Cyst(e)inase/10 μM curcumin. For (**d-f**): * $P<0.05$; *** $P<0.001$ **** $P<0.0001$ as compared to control; #$P<0.01$ as compared to Cyst(e)inase; $ $P<0.01$ as compared to curcumin or BSO. For (**g**) *$P<0.05$; *** $P<0.001$; ****$P<0.0001$; as compared to control; #$P<0.01$ as compared to curcumin alone, $$P<0.01$ as compared to 25 mg/kg Cyst(e)inase alone; two-way repeated measures ANOVA followed by Bonferroni's multiple comparison test. Throughout, data are expressed as mean ± s.e.m.



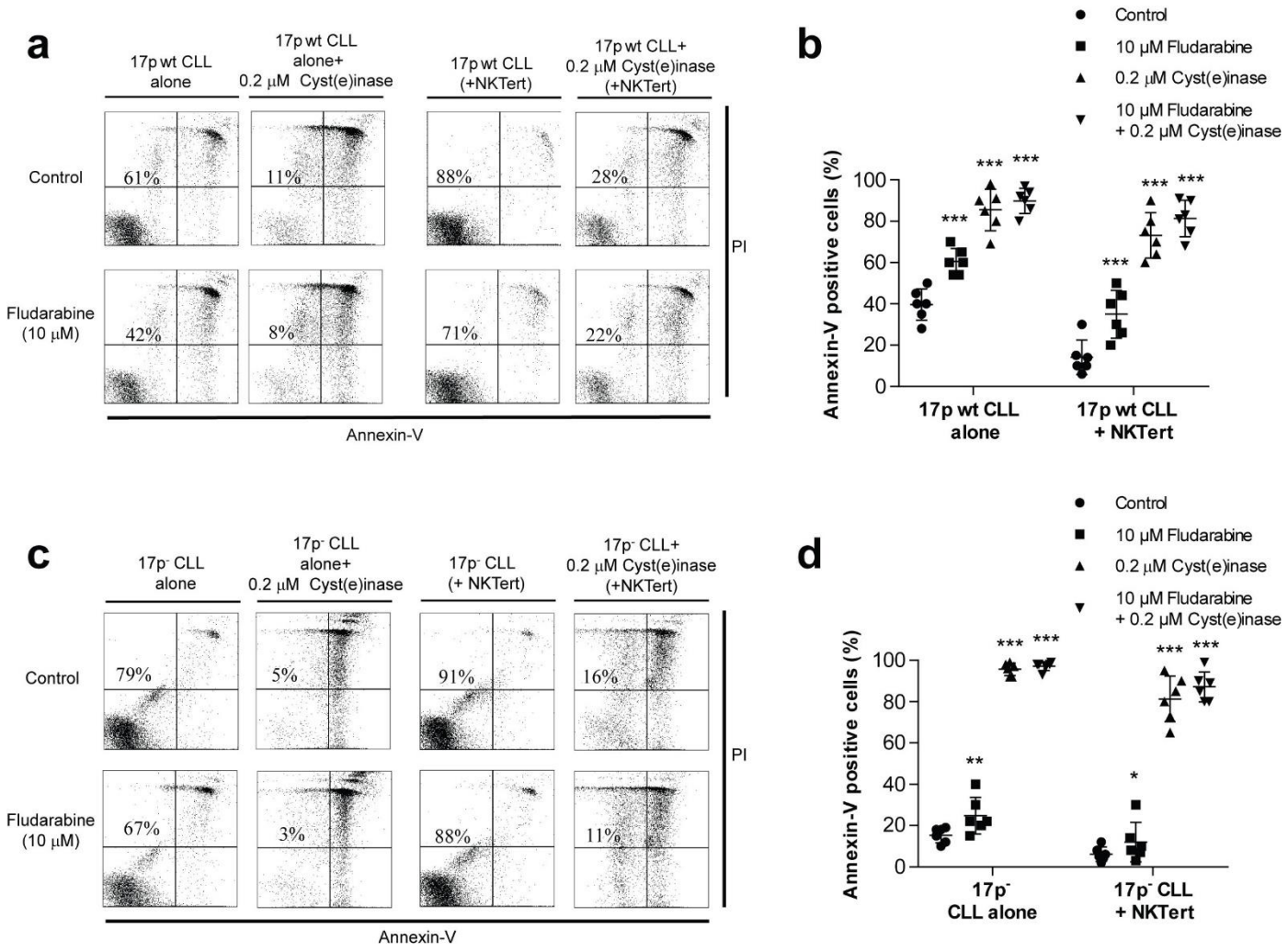
Supplementary Figure 9: *In vitro* efficacy of Cyst(e)inase in *TCL1*-Tg:*p53*$^{-/-}$ mouse splenocytes.

Cell viability 48 h after treatment with fludarabine, Cyst(e)inase, or their combination of mouse splenocytes (co-cultured with murine stromal Kusa-H) isolated from 4-month old *TCL1*-Tg:*p53*$^{-/-}$ mice (n = 6 per treatment group). Analysis was performed by flow cytometry after double staining with Annexin V-PI. Shown are **(a)** representative dot plots of 6 independent experiments using 6 different *TCL1*-Tg:*p53*$^{-/-}$ mice, and **(b)** percent Annexin-V positive cells. For (**b**) *P<0.05; ***P<0.001; two-sided Student's t-test; data are expressed as mean ± s.d.



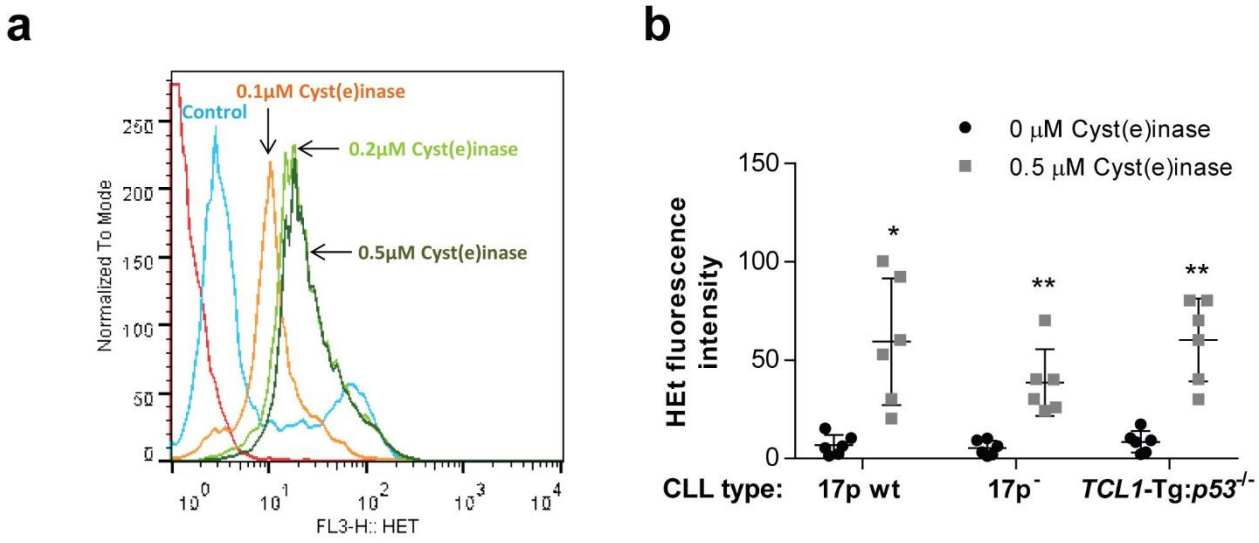
Supplementary Figure 10: *TCL1*-Tg:*p53*$^{-/-}$ mouse weights during treatment.

The weights of *TCL1*-Tg:*p53*$^{-/-}$ mice were monitored during a treatment course of **(a)** repeated cycles of 34 mg/kg daily i.p. injection of fludarabine for 5 days followed by three weeks respite until death (n=10), **(b)** 100 mg/kg i.p injection of Cyst(e)inase twice/week until death (n=10), or **(c)** their combination (n=10).



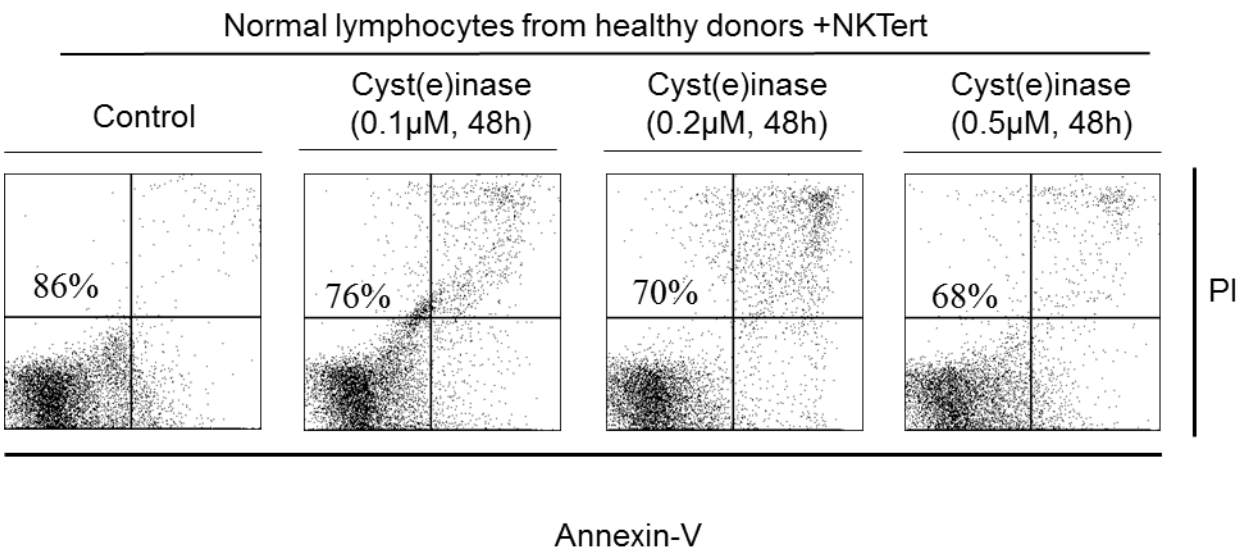
Supplementary Figure 11: ***In vitro*** **efficacy of Cyst(e)inase on primary patient CLL samples.**

Cell viability 48 h after treatment with fludarabine, Cyst(e)inase, or their combination of **(a,b)** primary 17p wt CLL cells cultured alone or co-cultured with stromal NKTert cells (n=6 different CLL patient samples); and **(c,d)** primary 17p- CLL cells cultured alone or co-cultured with stromal NKTert cells (n=6 different CLL patient samples). Analysis was performed by flow cytometry after double staining with Annexin-V-PI. Shown are **(a,c)** representative dot plots from 6 independent experiments using 6 different CLL patient samples for each CLL phenotype, and **(b,d)** percent Annexin-V positive cells for each CLL phenotype. For (**b, d**) *P<0.05; **P<0.01; ***P<0.001; two-sided Student's t-test; data are expressed as mean ± s.d.



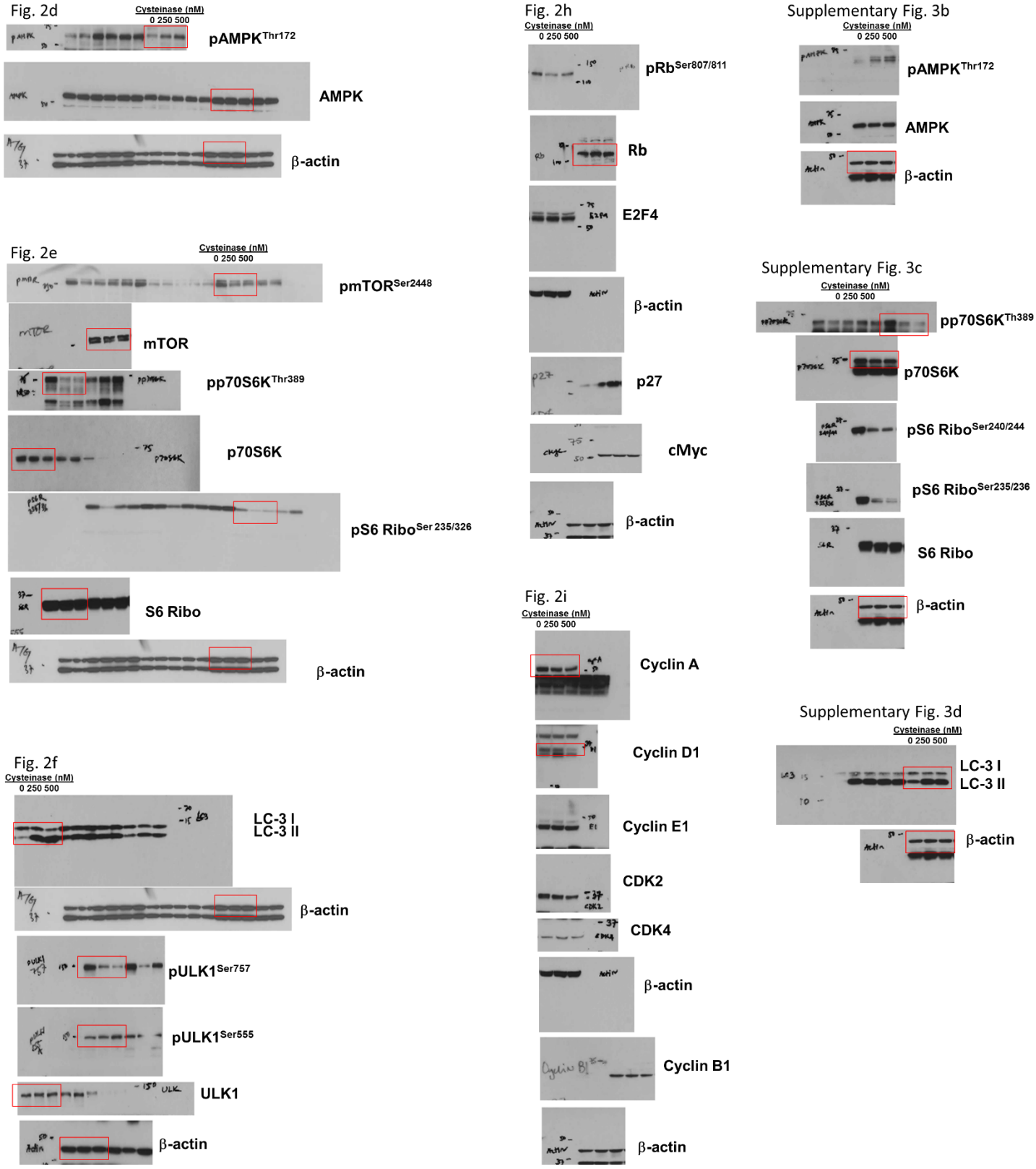
Supplementary Figure 12: Effect of Cyst(e)inase treatment on ROS levels in CLL samples.

Cellular O_2^- levels as assessed by flow cytometry using hydroethidine in CLL cells following treatment with Cyst(e)inase at indicated concentrations for 24 hours. **(a)** A representative FACS plot and **(b)** bar graph showing quantitative comparison of relative fluorescence intensity from 6 samples (n = 6 per group). For (**b**) *P<0.05, **P<0.01, two-sided Student's t-test; data are expressed as mean ± s.d.



Supplementary Figure 13: Effect of Cyst(e)inase treatment in normal lymphocytes from healthy donors.

Cell viability 48 h after Cyst(e)inase treatment at indicated concentrations in normal lymphocytes (co-cultured with NKTert stromal cells) isolated from healthy donors. Analysis was performed by flow cytometry after double staining with Annexin-V-PI. Shown are representative dot plots of 4 independent experiments using 4 different healthy donors (n = 4).



Supplementary Figure 14: Original uncropped images of western blots.

Supplementary Table 1: Data collection and refinement statistics for the X-ray crystal structure of CGL-E59T-E339V in complex with L-Cys.

PDB:5EIG	hCGL-E59T-E339V
Data collection	
Wavelength (Å)	0.97733
Resolution range (Å)	40 - 2.70 (2.75 - 2.70)[1]
Space group	P 2_1 2_1 2
Unit cell dimensions (Å)	163.445 181.624 113.375
Unique reflections	92940 (4621)
Redundancy	4.4 (4.3)
Completeness (%)	99.1 (99.8)
I/σ	10.385 (2.182)
R_{sym}	0.140 (0.545)
Refinement	
R_{work}	0.1898 (0.2058)
R_{free}[2]	0.2325 (0.2485)
Number of non-hydrogen atoms	24624
macromolecules	23981
ligands	83
water	560
Protein residues	3097
RMS Deviation	
Bond length (Å)	0.004
Bond angles (°)	0.96
Ramachandran plot	
Favored (%)	95.89
Outliers[3] (%)	0.3
B-factor (Å^2)	33.5
macromolecules	33.6
ligands	43
solvent	27.7

[1]Statistics for the highest-resolution shell are shown in parentheses.

[2] R_{free} is calculated with 5% of the data randomly omitted from refinement.

[3] Two residues in each monomer are outside the allowed range on the Ramachandran plot, Lys212 and Ser340. These unique Phi/Psi angles appear to be caused by the two mutations from WT CGL. Lys212 is the catalytic residue that forms an internal aldimine with the PLP cofactor, which may account for its unique Phi/Psi angle.

Supplementary Methods

Isolation of CGL-E59T-E339V.

Construction of a synthetic CGL gene and saturation mutagenesis libraries. A synthetic human CGL gene containing an N-terminal 6x histidine tag along with saturation mutagenesis libraries randomizing codons corresponding to amino acid positions E59, R119, and E339 were utilized as previously described[1]. The CGL saturation mutagenesis library containing randomized positions corresponding to R119 and E59 along with the E339V mutation was screened for L-Cys and CSSC activity as described below, and clones displaying improved activity were identified by sequencing, re-transformed into *E. coli*-BL21(DE3), purified, and kinetically characterized.

96-well plate screen. Libraries of CGL variants were screened for increased L-Cys and CSSC turnover using the aforementioned colorimetric assay (MBTH) adapted for detecting pyruvate production in a 96-well plate. Single colonies of *E. coli*-BL21(DE3), containing plasmids encoding either wild-type (CGL control) or variant CGL enzymes were picked into 96-well culture plates containing 75 μL of TB media/well and 50 μg/mL kanamycin. Cells were grown at 37°C on a plate shaker to an OD_{600} of ~0.8–1, cooled to 25°C, whereupon an additional 75 μL of TB media/well containing 50 μg/ml kanamycin, and 2 mM IPTG were added and incubation with shaking was continued for a minimum of 4 hours at 25°C. Subsequently, 30 μL of culture/well were transferred to a fresh 96 well plate and stored at 4°C for further characterization as needed. The original plates were centrifuged (10 min at 2500 x g) to pellet the cells, the media was removed, and the cells were chemically lysed by addition of 50 μL/well of bacterial protein extraction reagent (B-PER; Pierce, Rockford IL) and mixing for 15 minutes on a plate shaker. The plates were once again centrifuged (10 min at 2500 x g) to pellet cellular

debris, and 5 μL/well of the resulting supernatant was then incubated separately with 95 μL of CSSC (0.5 mM) and L-Cys (2.5 mM) (dissolved in 100 mM sodium phosphate buffer, 1 mM EDTA, pH 7.3) at 37ºC for approximately 12 hours. The pyruvate reaction product was then derivatized by addition of 146 μL of MBTH solution to 54 μL of reaction and heated for 40 min at 50°C. The absorbance at 320 nm was determined spectrophotometrically using a microtiter plate reader. Variants exhibiting high absorbance values indicative of pyruvate production were identified and selected for further characterization.

Crystallization.

E. coli-BL21(DE3) cells harboring plasmids with the CGL-E59T-E339V gene were grown to an OD_{600} of 0.6-0.8 upon which protein expression was induced with 0.5 mM IPTG at 16°C overnight. Cells were then collected by centrifugation, re-suspended in buffer A (50 mM Tris-HCl/ 500 mM NaCl/ 10 mM β-mercaptoethanol (BME)/ 10% glycerol/ 0.1% TritonX-100, pH 8.0), and lysed by sonication. The lysates were centrifuged at 27,000 x g for 30 minutes, and the resulting supernatant was applied to a nickel IMAC column, washed with buffer A containing 50 mM imidazole, and eluted with buffer A containing 300 mM imidazole. The eluted protein was dialyzed overnight into buffer B (50 mM HEPES/ 150 mM ammonium sulfate/ 5% glycerol/ 10 mM BME/ 200 μM PLP, pH 6.5). The protein was then polished by size exclusion chromatography with a Superdex 200 column (GE Healthcare) in buffer B. The associated PLP was monitored by spectroscopy at 412 nm. Following purification, CGL-E59T-E339V protein was concentrated to ~20 mg/mL using 10,000 MWCO Vivaspin 20 mL centrifugal concentrator tubes (Sartorius Stedim) before being flash frozen in liquid nitrogen and stored at -80°C for future crystallization experiments.

Diffracting crystals were obtained through vapor diffusion in sitting drops by mixing 2 parts CGL-E59T-E339V (20 mg/mL, buffer B) with 1 part crystallization solution (30% isopropanol/ 150 mM sodium citrate/ 100 mM sodium cacodylate, pH 6.5), followed by incubation at 4°C. Crystals were then transferred into crystallization solution containing an additional 1 mM L-Cys and soaked for 1 hour. After soaking, the crystals were cryo-protected with 2-methyl-2,4-pentanediol (MPD; 20%; Sigma) and flash frozen in liquid nitrogen prior to data collection.

Pharmacological optimization of CGL-E59T-E339V.

CGL-E59T-E339V was conjugated to lysyl residues using methoxy PEG succinimidyl carboxymethyl ester of MW 5000 (PEG-5K; JenKem Technology or NOF America Corporation) (conjugated protein henceforth referred to as Cyst(e)inase) to prevent renal clearance and impart long circulation persistence. Purification was performed as described above, with the inclusion of an additional on column wash step using 100 column volumes of Dulbecco's PBS (dPBS) additionally containing 0.1 mM PLP and 0.1% Triton X114 (Sigma, MO) in order to remove endotoxin. In addition, to minimize possible exposure to endotoxin, protein was eluted with dPBS containing 250 mM imidazole. Purified protein was then buffer exchanged into 100 mM potassium phosphate buffer, pH 8.4 using a 10,000 MWCO filtration device (Amicon or Pall Corporation). PEG-5K was added at a 100:1 molar ratio to enzyme and allowed to react for two hours at 25°C in a glass vial with gentle stirring. Cyst(e)inase was then extensively buffer exchanged using a 100,000 MWCO centrifugal filter device (Amicon or Pall Corporation) into dPBS, 10% glycerol (pH 7.4). Aliquots of Cyst(e)inase were flash frozen in liquid nitrogen and stored at -80°C. All PEGylated enzymes were analyzed for

lipopolysaccharide (LPS) content using the commercially available Limulus Amebocyte Lysate (LAL) kit (Cape Cod Incorporated or Thermo Scientific).

Cell viability assays.

Cell viability was measured by 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT; Sigma) assay[2]. Briefly, cells (1-10 $x10^3$/mL) in 96-well plates were titrated with varying concentrations of Cyst(e)inase, BSO (Sigma Aldrich), curcumin (Stanford chemicals, Irvine, CA), or combinations of the aforementioned agents. At indicated time points, the cells were treated with MTT solution (5 mg/mL), incubated for an additional 3 hours and dissolved in 20% sodium dodecyl sulfate (SDS; Fisher Scientific) solution. The absorbance was measured at 570 nm using a microplate reader (Tecan Group Ltd.). Cell viability was further confirmed by Alamar Blue assay, trypan blue dye exclusion test, or crystal violet assay as described elsewhere[3,4]. In combination studies, fixed ratio drug combinations were used. Combinatorial indices (CI) for drug combinations were calculated using CompuSyn software (ComboSyn, Inc). A CI value <1 indicates synergism whereas a value >1 indicates antagonism.

Measurement of reactive oxygen species.

The intracellular reactive oxygen species (ROS) concentration in HMVP2 cells was measured using 2′,7′-Dichlorofluorescin diacetate (DCFDA) fluorescence. HMVP2 cells in a 96 well plate were stained with DCFDA (20 μmol/L; Sigma) at 37°C for 30 minutes and then treated with indicated concentrations of Cyst(e)inase for 4 hours and fluorescence intensity was measured at the respective excitation and emission wavelengths of 485 nm and 535 nm using a fluorescent plate reader (Tecan Group Ltd.). For combination studies, cells (2-5$x10^5$) in 6 well plates were treated with indicated concentrations of Cyst(e)inase and their combinations for 24

hours following which, cells were stained with DCFDA (20 μmol/L; Sigma) at 37°C for 30 minutes, washed with PBS, trypsinized and the ROS level was measured by cytometry (Guava easyCyte 8HT, EMD Millipore). Determination of cellular O_2^- in CLL cells after treatment with Cyst(e)inase at indicated concentrations for 24 hours was detected by flow cytometry analysis using hydroethidine as described previously[5].

Measurement of intracellular glutathione levels.

HMVP2 cells were treated with indicated concentrations of Cyst(e)inase for 24 hours, the cells were then washed with PBS, pelleted and mixed with 3 volumes of 5% sulfosalicylic acid (Sigma). Following cell lysis (via 2 cycles of freeze thaw), the resulting mixture was centrifuged and glutathione level in the supernatant was measured using the commercially available glutathione detection kit (Sigma). For CLL cells, a glutathione assay kit (Cayman Chemical, Ann Arbor, MI) was used to measure cellular glutathione. After preparing cell extracts by sonication and deproteination, GSH was determined as previously described[6].

Cell cycle analysis.

HMVP2 cells were treated with various concentrations of Cyst(e)inase for 24 h, after which cells were harvested and washed twice with phosphate buffered saline (PBS) and fixed in ice cold 70% ethanol overnight at 20°C as previously described [7]. Following fixation, cells were washed, re-suspended in PBS containing RNase (250 μg/mL; Sigma), and incubated for 30 min at 37°C. Cells were then treated with PI (50 μg/mL; Sigma) solution, incubated for 30 min in the dark, and distribution of different phase of cell-cycle was analyzed by Guava-based flow cytometry (Millipore).

Deactivation of Cyst(e)inase as a control for mouse studies.

Cyst(e)inase samples were deactivated by incubation at 100°C for 10 minutes followed by centrifugation (16000 x g for 5 minutes) to ensure no protein precipitation. The samples (both before and after boiling) were subsequently tested using the aforementioned DTNB assay to ensure there was no remaining activity in the heat inactivated enzyme control.

Supplementary Methods References

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Supplementary Data Set 1: Preliminary toxicology study of Cyst(e)inase in male cynomolgus monkeys following a single bolus intravenous dose.

CLINICAL PATHOLOGY REPORT

TEST ARTICLE: Cyst(e)inase

TESTING FACILITY: MPI Research, Inc.
54943 North Main Street Mattawan,
Michigan 49071-8353

MPI RESEARCH STUDY NUMBER: 2327-002

1. RESULTS AND DISCUSSION

The clinical pathology data are presented in Appendix A.

1.1. Hematology

There were no test article-related effects among hematology parameters at either dose level. Animal number 202 who received 8 mg/kg had a mild increase in neutrophils at 168 hours postdose that was not considered likely to be test article-related based on the lack of a similar finding at 48 hours postdose.

All other individual hematology values were considered within expected ranges for biological variation.

1.2. Clinical Chemistry

There were no test article-related effects among clinical chemistry analytes. All individual clinical chemistry values were considered within expected ranges for biological variation.

Appendix A
Clinical Pathology Tables

Table 1
Individual Hematology Values

Abbreviations for Hematology Parameters

MCV	-	Mean Corpuscular Volume
MCH	-	Mean Corpuscular Hemoglobin
MCHC	-	Mean Corpuscular Hemoglobin Concentration

MPI Research Study Number 2327-002

Preliminary Toxicology Study of Cyst(e)inase in Male Cynomolgus Monkeys Following a Single Bolus Intravenous Dose

Individual Hematology Values - MALE

Predose

Group, Animal Number	Leukocytes $10^3/\mu L$	Erythrocytes $10^6/\mu L$	Hemoglobin g/dL	Hematocrit %	MCV fL	MCH pg	MCHC g/dL	Platelets $10^3/\mu L$
8 mg/kg								
202	8.8	6.10	14.0	47.6	78.0	22.9	29.4	287

MPI Research Study Number 2327-002

Preliminary Toxicology Study of Cyst(e)inase in Male Cynomolgus Monkeys Following a Single Bolus Intravenous Dose

Individual Hematology Values - MALE

Predose

Group, Animal Number	Absolute Reticulocytes $10^3/\mu L$	Neutrophils $10^3/\mu L$	Lymphocytes $10^3/\mu L$	Monocytes $10^3/\mu L$	Eosinophils $10^3/\mu L$	Basophils $10^3/\mu L$	Other Cells $10^3/\mu L$
8 mg/kg							
202	47.6	3.32	5.07	0.16	0.11	0.03	0.07

MPI Research Study Number 2327-002

Preliminary Toxicology Study of Cyst(e)inase in Male Cynomolgus Monkeys Following a Single Bolus Intravenous Dose

Individual Hematology Values - MALE

48 hours postdose

Group, Animal Number	Leukocytes $10^3/\mu L$	Erythrocytes $10^6/\mu L$	Hemoglobin g/dL	Hematocrit %	MCV fL	MCH pg	MCHC g/dL	Platelets $10^3/\mu L$
8 mg/kg								
202	7.1	6.03	13.9	46.8	77.7	23.1	29.8	286

MPI Research Study Number 2327-002

Preliminary Toxicology Study of Cyst(e)inase in Male Cynomolgus Monkeys Following a Single Bolus Intravenous Dose

Individual Hematology Values - MALE

48 hours postdose

Group, Animal Number	Absolute Reticulocytes $10^3/\mu L$	Neutrophils $10^3/\mu L$	Lymphocytes $10^3/\mu L$	Monocytes $10^3/\mu L$	Eosinophils $10^3/\mu L$	Basophils $10^3/\mu L$	Other Cells $10^3/\mu L$
8 mg/kg							
202	49.5	2.73	3.95	0.21	0.09	0.02	0.07

MPI Research Study Number 2327-002

Preliminary Toxicology Study of Cyst(e)inase in Male Cynomolgus Monkeys Following a Single Bolus Intravenous Dose

Individual Hematology Values - MALE

168 hours postdose

Group, Animal Number	Leukocytes $10^3/\mu L$	Erythrocytes $10^6/\mu L$	Hemoglobin g/dL	Hematocrit %	MCV fL	MCH pg	MCHC g/dL	Platelets $10^3/\mu L$
8 mg/kg								
202	15.9	5.81	13.2	45.3	77.9	22.8	29.2	310

MPI Research Study Number 2327-002

Preliminary Toxicology Study of Cyst(e)inase in Male Cynomolgus Monkeys Following a Single Bolus Intravenous Dose

Individual Hematology Values - MALE

168 hours postdose

Group, Animal Number	Absolute Reticulocytes $10^3/\mu L$	Neutrophils $10^3/\mu L$	Lymphocytes $10^3/\mu L$	Monocytes $10^3/\mu L$	Eosinophils $10^3/\mu L$	Basophils $10^3/\mu L$	Other Cells $10^3/\mu L$
8 mg/kg							
202	96.5	11.43	3.92	0.25	0.08	0.05	0.14

Table 2
Individual Clinical Chemistry Values

Abbreviations for Clinical Chemistry Parameters

GGT - Gamma Glutamyltransferase
AST - Aspartate Aminotransferase
ALT - Alanine Aminotransferase

MPI Research Study Number 2327-002

Preliminary Toxicology Study of Cyst(e)inase in Male Cynomolgus Monkeys Following a Single Bolus Intravenous Dose

Individual Clinical Chemistry Values - MALE

Predose

Group, Animal Number	Sodium mEq/L	Potassium mEq/L	Chloride mEq/L	Calcium mg/dL	Phosphorus mg/dL	Alkaline Phosphatase U/L	Total Bilirubin mg/dL
8 mg/kg							
202	150	5.5	104	10.3	4.7	711	0.1

MPI Research Study Number 2327-002

Preliminary Toxicology Study of Cyst(e)inase in Male Cynomolgus Monkeys Following a Single Bolus Intravenous Dose

Individual Clinical Chemistry Values - MALE

Predose

Group, Animal Number	GGT U/L	AST U/L	ALT U/L	Urea Nitrogen mg/dL	Creatinine mg/dL	Total Protein g/dL
8 mg/kg						
202	75	58	49	30	0.8	6.2

MPI Research Study Number 2327-002

Preliminary Toxicology Study of Cyst(e)inase in Male Cynomolgus Monkeys Following a Single Bolus Intravenous Dose

Individual Clinical Chemistry Values - MALE

Predose

Group, Animal Number	Albumin g/dL	Globulin g/dL	Albumin/ Globulin Ratio	Triglyceride mg/dL	Cholesterol mg/dL	Glucose mg/dL
8 mg/kg						
202	4.0	2.2	1.8	81	133	132

MPI Research Study Number 2327-002

Preliminary Toxicology Study of Cyst(e)inase in Male Cynomolgus Monkeys Following a Single Bolus Intravenous Dose

Individual Clinical Chemistry Values - MALE

48 hours postdose

Group, Animal Number	Sodium mEq/L	Potassium mEq/L	Chloride mEq/L	Calcium mg/dL	Phosphorus mg/dL	Alkaline Phosphatase U/L	Total Bilirubin mg/dL
8 mg/kg							
202	151	5.5	105	10.6	7.4	719	0.2

MPI Research Study Number 2327-002

Preliminary Toxicology Study of Cyst(e)inase in Male Cynomolgus Monkeys Following a Single Bolus Intravenous Dose

Individual Clinical Chemistry Values - MALE

48 hours postdose

Group, Animal Number	GGT U/L	AST U/L	ALT U/L	Urea Nitrogen mg/dL	Creatinine mg/dL	Total Protein g/dL
8 mg/kg						
202	75	51	36	28	0.7	6.8

MPI Research Study Number 2327-002

Preliminary Toxicology Study of Cyst(e)inase in Male Cynomolgus Monkeys Following a Single Bolus Intravenous Dose

Individual Clinical Chemistry Values - MALE

48 hours postdose

Group, Animal Number	Albumin g/dL	Globulin g/dL	Albumin/ Globulin Ratio	Triglyceride mg/dL	Cholesterol mg/dL	Glucose mg/dL
8 mg/kg						
202	4.2	2.6	1.6	53	158	91

MPI Research Study Number 2327-002

Preliminary Toxicology Study of Cyst(e)inase in Male Cynomolgus Monkeys Following a Single Bolus Intravenous Dose

Individual Clinical Chemistry Values - MALE

168 hours postdose

Group, Animal Number	Sodium mEq/L	Potassium mEq/L	Chloride mEq/L	Calcium mg/dL	Phosphorus mg/dL	Alkaline Phosphatase U/L	Total Bilirubin mg/dL
8 mg/kg							
202	149	4.8	103	10.3	8.1	643	0.2

MPI Research Study Number 2327-002

Preliminary Toxicology Study of Cyst(e)inase in Male Cynomolgus Monkeys Following a Single Bolus Intravenous Dose

Individual Clinical Chemistry Values - MALE

168 hours postdose

Group, Animal Number	GGT U/L	AST U/L	ALT U/L	Urea Nitrogen mg/dL	Creatinine mg/dL	Total Protein g/dL
8 mg/kg						
202	76	48	39	28	0.8	6.7

MPI Research Study Number 2327-002

Preliminary Toxicology Study of Cyst(e)inase in Male Cynomolgus Monkeys Following a Single Bolus Intravenous Dose

Individual Clinical Chemistry Values - MALE

168 hours postdose

Group, Animal Number	Albumin g/dL	Globulin g/dL	Albumin/ Globulin Ratio	Triglyceride mg/dL	Cholesterol mg/dL	Glucose mg/dL
8 mg/kg						
202	4.2	2.5	1.7	44	170	61