

Supporting Information

Plasma treatment causes structural modifications in lysozyme, with possible applications for cancer treatment

Table S1: Amino acids affected in control lysozyme at pH 2 and 7. Eight amino acids (Met, Tyr, Asp, Phe, His, Trp, Asn, and Pro) were chosen as variable oxidation sites for the analysis with Mascot and Sequest.

Identifying Node	Annotated Sequence	Modifications	Master Protein Accessions	Protein Accessions	m/z [Da]	MH+ [Da]	DeltaM [ppm]
Sequest HT	[R].cELAAAMK.[R]	C1(Carbamidomethyl)	B8YJT7	B8YJT7; Q6LEL2; P00698; B8YJN9; B8YK79; B8YJP1	893.33335	893.33335	-99.15
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.75001	1428.49275	-110.23
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.83335	1428.65941	6.43
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.91668	1428.82608	123.09
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.83335	1428.65941	6.43

Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.91668	1428.82608	123.09
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	715.00001	1428.99275	239.75
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.83335	1428.65941	6.43
Sequest HT	[R].NTDGSTDYGILQINSR.[W]		B8YJT7	P00698; B8YK79	585.50001	1754.48547	370.81
Sequest HT	[R].NTDGSTDYGILQINSR.[W]		B8YJT7	P00698; B8YK79	877.75002	1754.49276	374.96
Sequest HT	[R].NTDGSTDYGILQINSR.[W]		B8YJT7	P00698; B8YK79			

Table S2: Number of CAP-induced oxidations and type of amino acids affected by plasma-treated lysozyme at pH 7. Eight amino acids (Met, Tyr, Asp, Phe, His, Trp, Asn, and Pro) were chosen as variable oxidation sites for the analysis with Mascot and Sequest.

Identifying Node	Annotated Sequence	Modifications	Master Protein Accessions	Protein Accessions	m/z [Da]	MH+ [Da]	DeltaM [ppm]
Sequest HT	[R].NTDGSTDYGILQINSR.[W]		B8YJT7	P00698; B8YK79; B8YJT7	877.83335	1754.65942	469.99
Sequest HT	[R].NTDGSTDYGILQINSR.[W]		B8YJT7	P00698; B8YK79; B8YJT7	877.50002	1753.99276	89.88
Sequest HT	[R].NTDGSTDYGILQINSR.[W]		B8YJT7	P00698; B8YK79; B8YJT7	877.75002	1754.49276	374.96

Sequest HT	[R].GYSLGNWVcAAK.[F]	C9(Carbamidomethyl)	B8YJT7	P00698; B8YJT7; B8YK79	663.50001	1325.99275	273.12
Sequest HT	[R].GYSLGNWVcAAK.[F]	W7(Oxidation); C9(Carbamidomethyl)	B8YJT7	P00698; B8YJT7; B8YK79	671.33334	1341.65941	25.2
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.91668	1428.82608	123.09
Sequest HT	[R].NTDGSTDyGILQINSR.[W]	Y8(Oxidation)	B8YJT7	P00698; B8YK79; B8YJT7	885.50002	1769.99276	91.94
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.91668	1428.82608	123.09
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	715.00001	1428.99275	239.75
Sequest HT	[R].NTDGSTDYGILQINSR.[W]		B8YJT7	P00698; B8YK79; B8YJT7	877.25002	1753.49276	-195.21
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.91668	1428.82608	123.09
Sequest HT	[R].cELAAAMK.[R]	C1(Carbamidomethyl)	B8YJT7	B8YJT7; Q6LEL2; P00698; B8YJN9; B8YK79; B8YJP1	893.58335	893.58335	180.67
Sequest HT	[K].GTDVQAWIR.[G]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	1045.50002	1045.50002	-40.65

Table S3: Number of CAP-induced oxidations and type of amino acids affected by plasma-treated lysozyme at pH 2. Eight amino acids (Met, Tyr, Asp, Phe, His, Trp, Asn, and Pro) were chosen as variable oxidation sites for the analysis with Mascot and Sequest.

Identifying Node	Annotated Sequence	Modifications	Master Protein Accessions	Protein Accessions	m/z [Da]	MH+ [Da]	DeltaM [ppm]
Sequest HT	[R].NTDGSTDYGILQINSR.[W]		B8YJT7	P00698; B8YK79; B8YJT7	877.58335	1754.15942	184.91
Sequest HT	[R].NTDGSTDyGILQINSR.[W]	Y8(Oxidation)	B8YJT7	P00698; B8YK79; B8YJT7	885.41668	1769.82609	-2.23
Sequest HT	[K].fESNFNTQATNR.[N]	F1(Oxidation)	B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	722.91668	1444.82608	125.25
Sequest HT	[R].NTDGSTDYGILQInSR.[W]	N14(Oxidation)	B8YJT7	P00698; B8YK79; B8YJT7	885.41668	1769.82609	-2.23
Sequest HT	[K].FESNfNTQATNR.[N]	F5(Oxidation)	B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	722.83335	1444.65942	9.88
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.91668	1428.82608	123.09
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.83335	1428.65941	6.43
Sequest HT	[R].GYSLGNWVcAAK.[F]	C9(Carbamidomethyl)	B8YJT7	P00698; B8YJN9; B8YK79; B8YJT7	663.25001	1325.49275	-104.05

Table S4: Oxidized amino acids in control lysozyme peptide at pH 2. Eight amino acids (Met, Tyr, Asp, Phe, His, Trp, Asn and Pro) were chosen for analysis. Samples were analyzed with Mascot and Sequest algorithms.

Identifying Node	Annotated Sequence	Modifications	Master Protein Accessions	Protein Accessions	m/z [Da]	MH+ [Da]	DeltaM [ppm]
Sequest HT (A7)	[R].NTDGSTDYGILQINSR.[W]		B8YJT7	B8YJT7; P00698	877.50002	1753.99276	89.88
Sequest HT (A7)	[R].GYSLGNWVcAAK.[F]	C9(Carbamidomethyl)	B8YJT7	B8YJT7; P00698	663.50001	1325.99275	273.12
Sequest HT (A7)	[R].GYSLGNWVcAAK.[F]	C9(Carbamidomethyl)	B8YJT7	B8YJT7; P00698	663.58334	1326.15941	398.85
Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698	714.83335	1428.65941	6.43
Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698	715.16668	1429.32608	473.07
Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698	714.91668	1428.82608	123.09
Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698	715.00001	1428.99275	239.75

Table S5: Oxidized amino acids in control lysozyme peptide at pH 7. Eight amino acids (Met, Tyr, Asp, Phe, His, Trp, Asn and Pro) were chosen for analysis. Samples were analyzed with Mascot and Sequest algorithms.

Identifying Node	Annotated Sequence	Modifications	Master Protein Accessions	Protein Accessions	m/z [Da]	MH+ [Da]	DeltaM [ppm]
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Sequest HT (A7)	[R].NTDGSTDYGILQINSR.[W]		B8YJT7	B8YJT7; P00698	877.50002	1753.99276	89.88
Sequest HT (A7)	[R].GYSLGNWVcAAK.[F]	C9(Carbamidomethyl)	B8YJT7	B8YJT7; P00698	663.50001	1325.99275	273.12
Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698	715.16668	1429.32608	473.07
Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698	715.00001	1428.99275	239.75

Table S6: Oxidized amino acids in plasma-treated lysozyme peptide at pH 7. Eight amino acids (Met, Tyr, Asp, Phe, His, Trp, Asn and Pro) were chosen for analysis. Samples were analyzed with Mascot and Sequest algorithms.

Identifying Node	Annotated Sequence	Modifications	Master Protein Accessions	Protein Accessions	m/z [Da]	MH+ [Da]	DeltaM [ppm]
Sequest HT	[R].NTDGSTDYGILQINSR.[W]		B8YJT7	P00698; B8YK79	877.75002	1754.49276	374.96
Sequest HT	[R].NTDGSTDYGILQINSR.[W]		B8YJT7	P00698; B8YK79	585.33334	1753.98547	85.72
Sequest HT	[R].NLcNIPcSALLSSDITASVncAK.[K]	C3(Carbamidomethyl); C7(Carbamidomethyl); C21(Carbamidomethyl)	B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	836.75002	2508.23549	18.5
Sequest HT	[R].NTDGSTDYGILQINSR.[W]		B8YJT7	P00698; B8YK79	877.83335	1754.65942	469.99
Sequest HT	[R].GYSLGNWVcAAK.[F]	C9(Carbamidomethyl)	B8YJT7	P00698; B8YJN9; B8YK79	663.41668	1325.82608	147.4
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.91668	1428.82608	123.09

Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	715.00001	1428.99275	239.75
Sequest HT	[R].GYSLGnwVcAAK.[F]	N6(Oxidation); W7(Oxidation); C9(Carbamidomethyl)	B8YJT7	P00698; B8YJN9; B8YK79	679.33334	1357.65941	28.65
Sequest HT	[R].GYSLGNWVcAAK.[F]	C9(Carbamidomethyl)	B8YJT7	P00698; B8YJN9; B8YK79	663.50001	1325.99275	273.12
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.83335	1428.65941	6.43
Sequest HT	[R].GYSLGNWVcAAK.[F]	C9(Carbamidomethyl)	B8YJT7	P00698; B8YJN9; B8YK79	663.41668	1325.82608	147.4
Sequest HT	[R].GYSLGNWVcAAK.[F]	C9(Carbamidomethyl)	B8YJT7	P00698; B8YJN9; B8YK79	663.41668	1325.82608	147.4
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	715.08335	1429.15941	356.41
Sequest HT	[R].GYSLGNWVcAAK.[F]	C9(Carbamidomethyl)	B8YJT7	P00698; B8YJN9; B8YK79	663.50001	1325.99275	273.12
Sequest HT	[R].GYSLGnwVcAAK.[F]	N6(Oxidation); W7(Oxidation); C9(Carbamidomethyl)	B8YJT7	P00698; B8YJN9; B8YK79	679.33334	1357.65941	28.65
Sequest HT	[K].IVSDGNGMNAWVAVR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	838.50002	1675.99276	114.46
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.91668	1428.82608	123.09

Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.83335	1428.65941	6.43
Sequest HT	[R].GYSLGNwVcAAK.[F]	W7(Oxidation); C9(Carbamidomethyl)	B8YJT7	P00698; B8YJN9; B8YK79	671.41668	1341.82608	149.43
Sequest HT	[R].GYSLGNwVcAAK.[F]	W7(Oxidation); C9(Carbamidomethyl)	B8YJT7	P00698; B8YJN9; B8YK79	671.41668	1341.82608	149.43
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.91668	1428.82608	123.09
Sequest HT	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	1428.4167	1428.4167	-163.46
Sequest HT	[R].cELAAAmK.[R]	C1(Carbamidomethyl); M7(Oxidation)	B8YJT7	B8YJT7; Q6LEL2; P00698; B8YJN9; B8YK79; B8YJP1	909.50002	909.50002	91.45

Table S7: Oxidized amino acids in plasma-treated lysozyme peptide at pH 2. Eight amino acids (Met, Tyr, Asp, Phe, His, Trp, Asn and Pro) were chosen for analysis. Samples were analyzed with Mascot and Sequest algorithms.

Identifying Node	Annotated Sequence	Modifications	Master Protein Accessions	Protein Accessions	m/z [Da]	MH+ [Da]	Delta M [ppm]
Sequest HT (A7)	[R].NTDGSTDyGILQINSR.[W]	Y8(Oxidation)	B8YJT7	B8YJT7; P00698	885.41668	1769.82609	-2.23
Sequest HT (A7)	[R].NTDGSTDYgILQINSR.[W]		B8YJT7	B8YJT7; P00698	585.50001	1754.48547	370.81
Sequest HT (A7)	[R].NTDGSTDYgILQINSR.[W]		B8YJT7	B8YJT7; P00698	877.33335	1753.65942	-100.18

Sequest HT (A7)	[R].NTDGSTDYGILQINSR.[W]		B8YJT7	B8YJT7; P00698	877.83335	1754.6 5942	469.99
Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.91668	1428.8 2608	123.09
Sequest HT (A7)	[R].GYSLGnwVcAAK.[F]	N6(Oxidation); W7(Oxidation); C9(Carbamidomethyl)	B8YJT7	B8YJT7; P00698; B8YK79	679.41668	1357.8 2608	151.42
Sequest HT (A7)	[R].GYSLGnwVcAAK.[F]	N6(Oxidation); W7(Oxidation); C9(Carbamidomethyl)	B8YJT7	B8YJT7; P00698; B8YK79	679.33334	1357.6 5941	28.65
Sequest HT (A7)	[R].NTDGSTDyGILQINSR.[W]	Y8(Oxidation)	B8YJT7	B8YJT7; P00698	885.83335	1770.6 5942	468.62
Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	715.00001	1428.9 9275	239.75
Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	715.16668	1429.3 2608	473.07
Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	715.16668	1429.3 2608	473.07
Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.83335	1428.6 5941	6.43
Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	715.14166	1429.2 7605	438.05
Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	715.00001	1428.9 9275	239.75
Sequest HT (A7)	[R].GYSLGNwVcAAK.[F]	C9(Carbamidomethyl)	B8YJT7	B8YJT7, P00698; B8YJN9	663.41668	1325.8 2608	147.4
Sequest HT (A7)	[R].GYSLGnwVcAAK.[F]	N6(Oxidation); W7(Oxidation); C9(Carbamidomethyl)	B8YJT7	B8YJT7; P00698; B8YJN9	679.58334	1358.1 5941	396.94
Sequest HT (A7)	[R].GYSLGNwVcAAK.[F]	W7(Oxidation); C9(Carbamidomethyl)	B8YJT7	B8YJT7; P00698; B8YJN9	671.33334	1341.6 5941	25.2

Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.91668	1428.8 2608	123.09
Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.75001	1428.4 9275	- 110.23
Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.75001	1428.4 9275	- 110.23
Sequest HT (A7)	[K].FESNFNTQATNR.[N]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	714.91668	1428.8 2608	123.09
Sequest HT (A7)	[R].GYSLGnwVcAAK.[F]	N6(Oxidation); W7(Oxidation); C9(Carbamidomethyl)	B8YJT7	B8YJT7; P00698; B8YJN9	679.58334	1358.1 5941	396.94
Sequest HT (A7)	[K].GTDVQAWIR.[G]	W7(Oxidation)	B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	531.50001	1061.9 9274	428.91
Sequest HT (A7)	[K].GTDVQAWIR.[G]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	523.33334	1045.6 594	111.79
Sequest HT (A7)	[K].GTDVQAWIR.[G]	W7(Oxidation)	B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	531.41667	1061.8 2607	271.9
Sequest HT (A7)	[K].GTDVQAWIR.[G]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	523.50001	1045.9 9274	430.61
Sequest HT (A7)	[K].GTDVQAWIR.[G]		B8YJT7	B8YJT7; P00698; B8YJN9; B8YK79; B8YJP1	523.41667	1045.8 2607	271.2

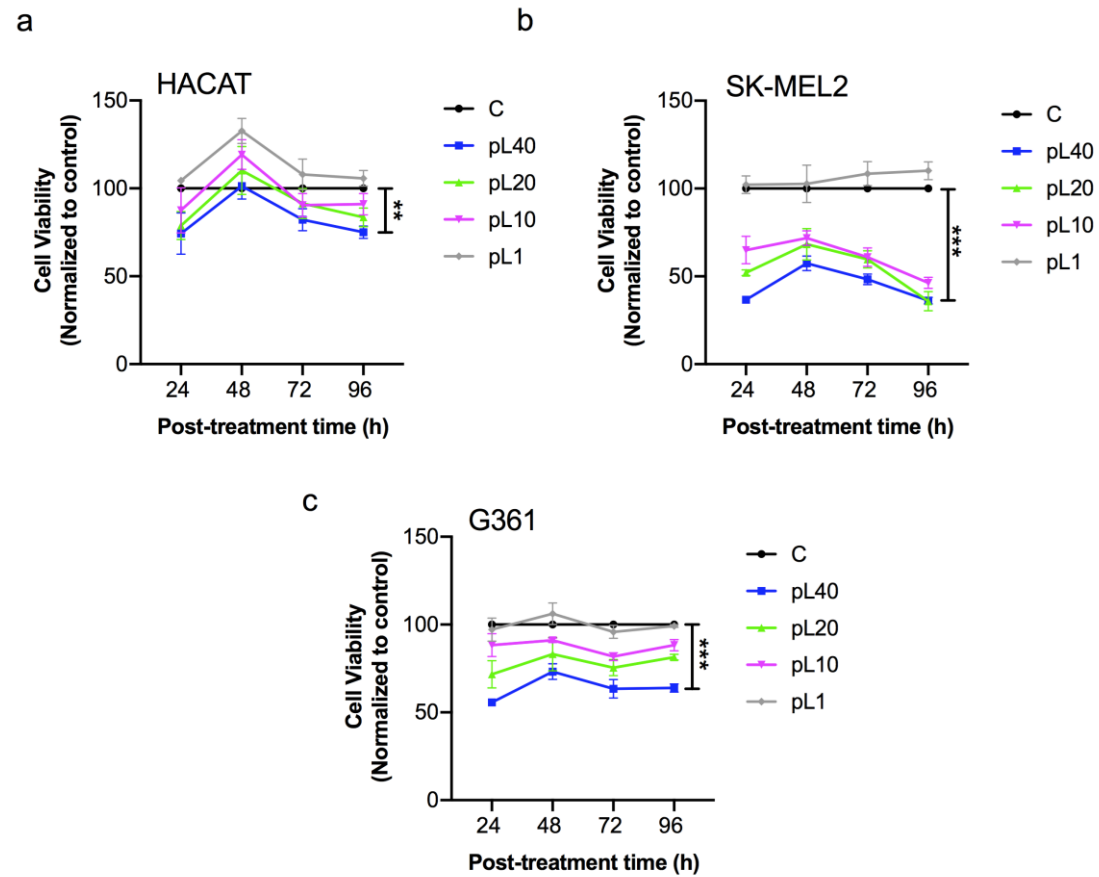


Figure S1. Cell viability analysis as a function of time, of HACAT (a), SK-MEL-2 (b) and G361 (c) cells, treated with plasma-treated lysozyme solutions. Results are presented as means \pm SEM ($n = 3$). Statistically significant differences are indicated as ** $p < 0.01$, and *** $p < 0.001$ between untreated lysozyme and corresponding treated groups. Significance was determined using student's t-test.

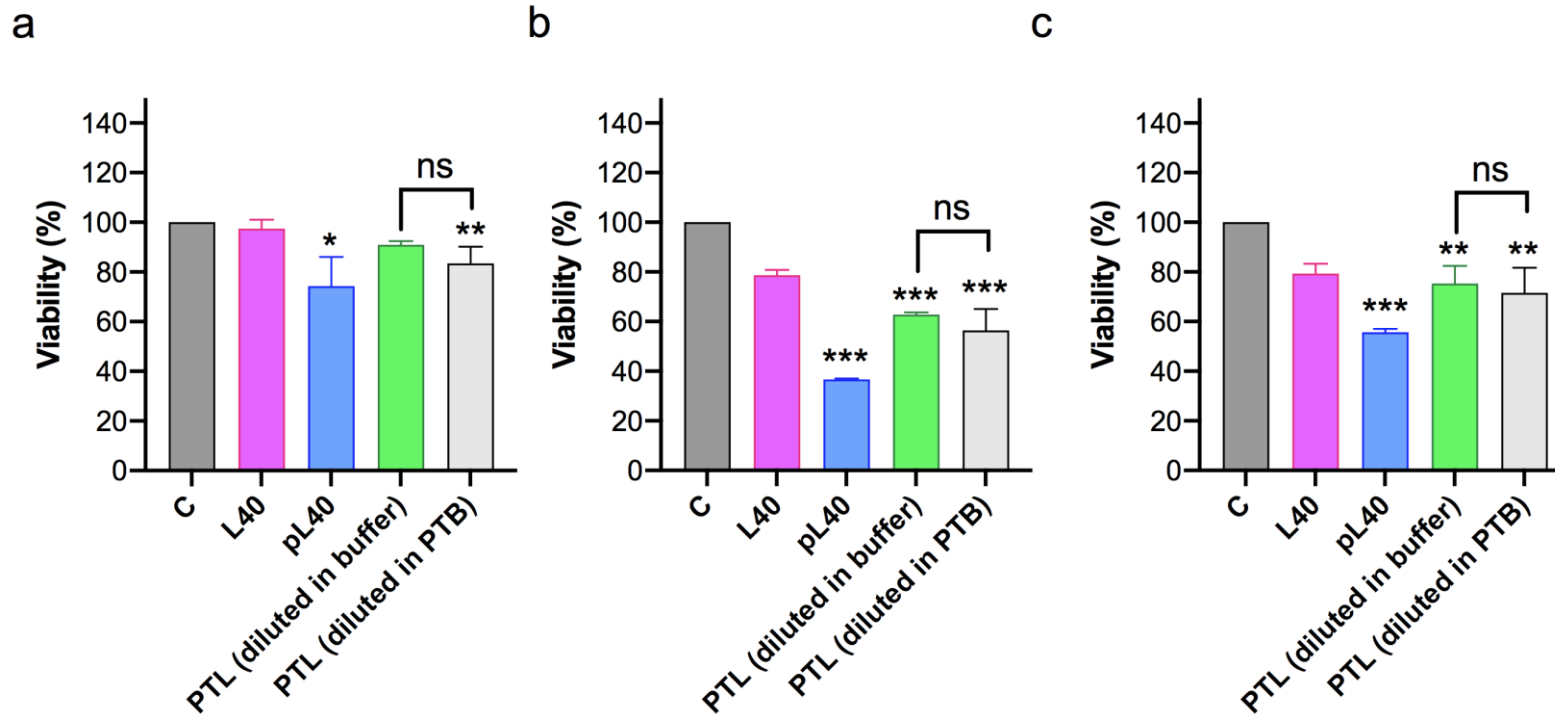


Figure S2. Comparison analysis of plasma-treated and untreated lysozyme solution under different conditions. (a-c) Viability assays of HACAT (a), SK-MEL-2 (b) and G361 (c) cells treated as indicated in the x-axis. ns = not significant. Results are presented as means \pm SEM (n = 3). Statistically significant difference are indicated as * p < 0.05, ** p < 0.01, and *** p < 0.001 between untreated lysozyme and corresponding treated groups. Significance was determined using student's t-test.