

Supplementary data

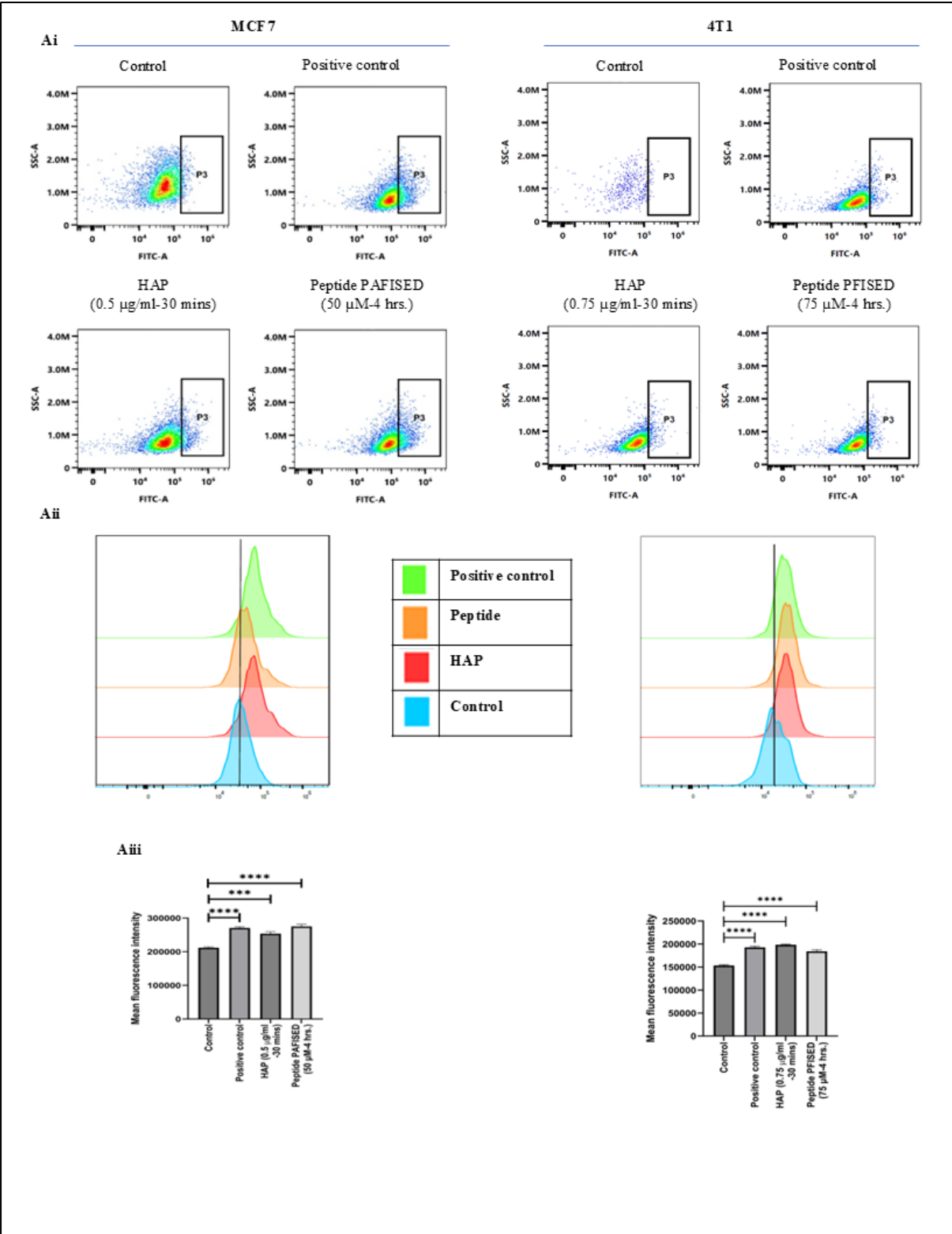
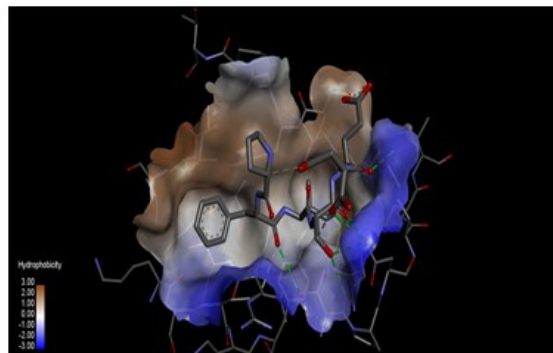
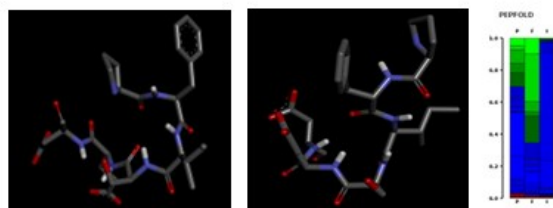


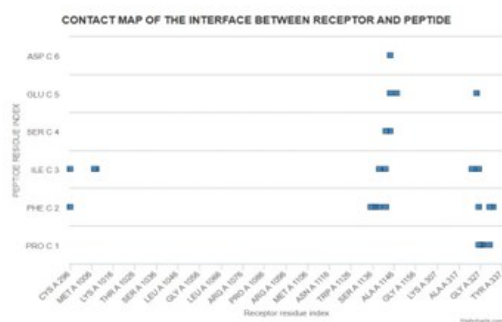
Figure S1: Autophagy assay analysis. (Ai) Representative flowcytometric images of MCF7 and 4T1 cells treated with HAP (0.5 and 0.75 $\mu\text{g/ml}$ for MCF-7 and 4T1 respectively) for 30 minutes and with the peptides (50 μM of PAFISED for MCF-7 and 75 μM of PFISED for 4T1) for 4 hours and stained using the Autophagy assay kit (ab139484) of Abcam. Cells treated with rapamycin and chloroquine simultaneously for 24 hours was kept as positive control. Fluorescence was measured under FITC channel of a flowcytometer. All the representative histograms in each cell type are arranged vertically for a better understanding of comparative analysis. **(Aii)** Graphical analysis of Mean fluorescence intensity of FITC.

PFISED



CONTACT MAP OF THE INTERFACE BETWEEN RECEPTOR AND PEPTIDE

Peptide Residue Index	Receptor Residue Index	Contact
ASP C 7	LEU A 11	Yes
GLU C 6	LEU A 11	Yes
SER C 5	LEU A 11	Yes
ILE C 4	LEU A 11	Yes
PHE C 3	LEU A 11	Yes
ALA C 2	LEU A 11	Yes
PRO C 1	LEU A 11	Yes



CLUSTERS COMPOSITION

Model index	Trajectory Index
100	5
600	9
900 - 1000	9

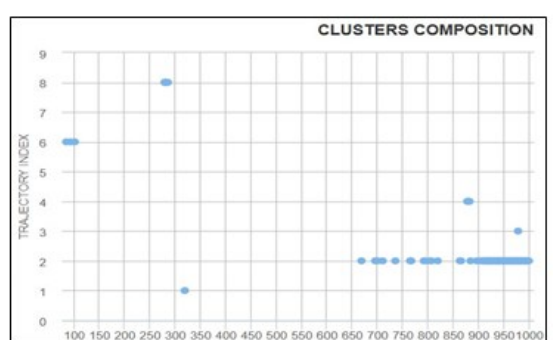
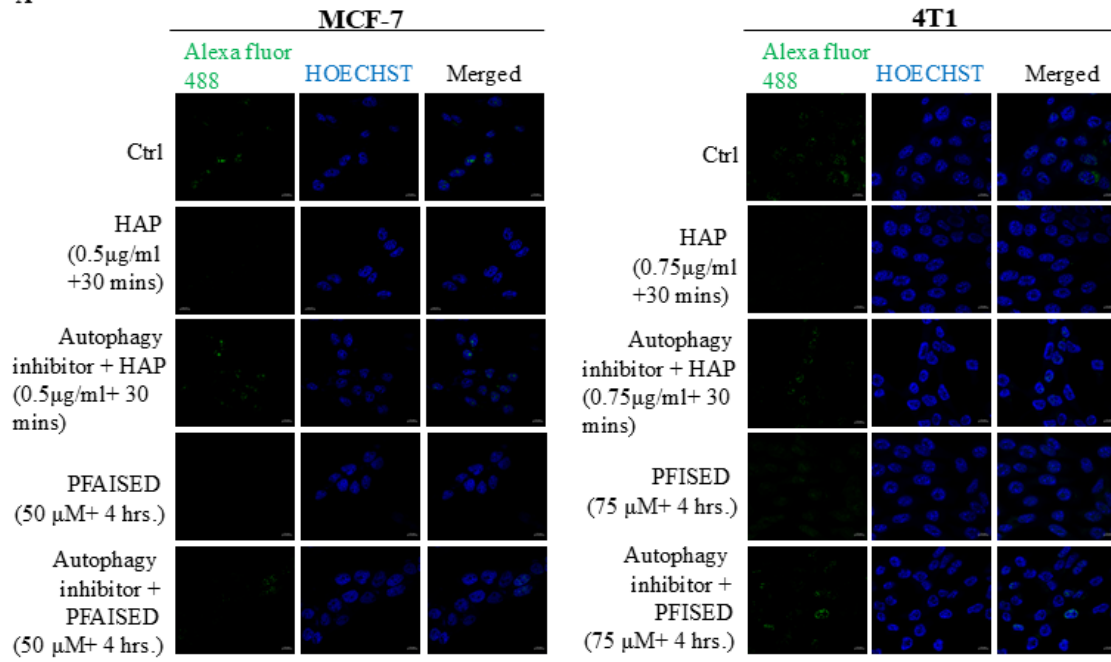
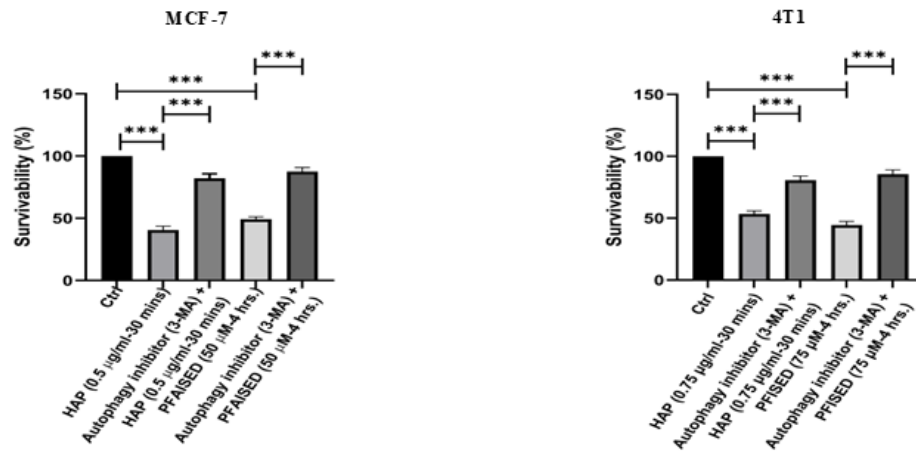


Figure S2: Peptide Structure prediction and Blind Docking Analysis of the peptides PAFISED (Left Side) & PFISED (Right side). (A) Top 2 predicted peptide structure by PEP-FOLD4 with local structure prediction profile which shows the probabilities of structural alphabet. (B) hydrophobicity representation of peptide with PAR1 by CABS dock. (C) Contact Map of receptor residues and Peptide of top docked pose. (D) Cluster composition vs trajectory affiliations of top docked pose.

A



B



C

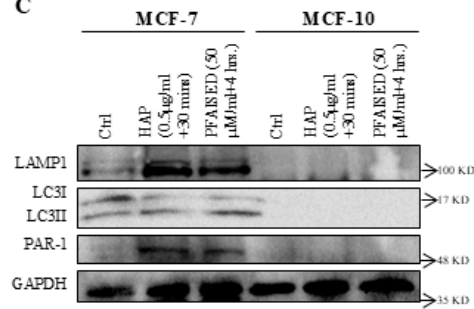


Figure S3: Effect of HAP and peptide induced autophagy on the proliferation and viability of cancer cells: (A) Representative immunofluorescence images of MCF-7 and 4T1 cells treated with HAP (0.5 and 0.75 $\mu\text{g/ml}$ for MCF-7 and 4T1 respectively) for 30 minutes and with the peptides (50 μM of PAFISED for MCF-7 and 75 μM of PFISED for 4T1) for 4 hours with or without pre-treatment of autophagy inhibitor 3-MA and analyzed for proliferation using anti-Ki-67 antibody and AlexaFluor 488 tagged corresponding secondary antibody. Nuclei were stained with Hoechst 33342. Images were captured using a confocal microscope. (B) Survivability analysis of MCF-7 and 4T1 cells treated with HAP (0.5 and 0.75 $\mu\text{g/ml}$ for MCF-7 and 4T1 respectively) for 30 minutes and with the peptides (50 μM of PAFISED for MCF-7 and 75 μM of PFISED for 4T1) for 4 hours with or without pre-treatment of autophagy inhibitor 3-MA by MTT assay. Absorbance was measured using a spectrophotometric plate reader and survivability was calculated considering the control cells have a 100% survivability. The survivability percentages are presented graphically. (C) Immunoblots of LAMP1, LC3 and PAR-1 in the whole cell lysates of MCF7 and MCF10A, treated with HAP (0.5 $\mu\text{g/ml}$ for 30 minutes) and the peptide PAFISED (50 μM for 4 hours).

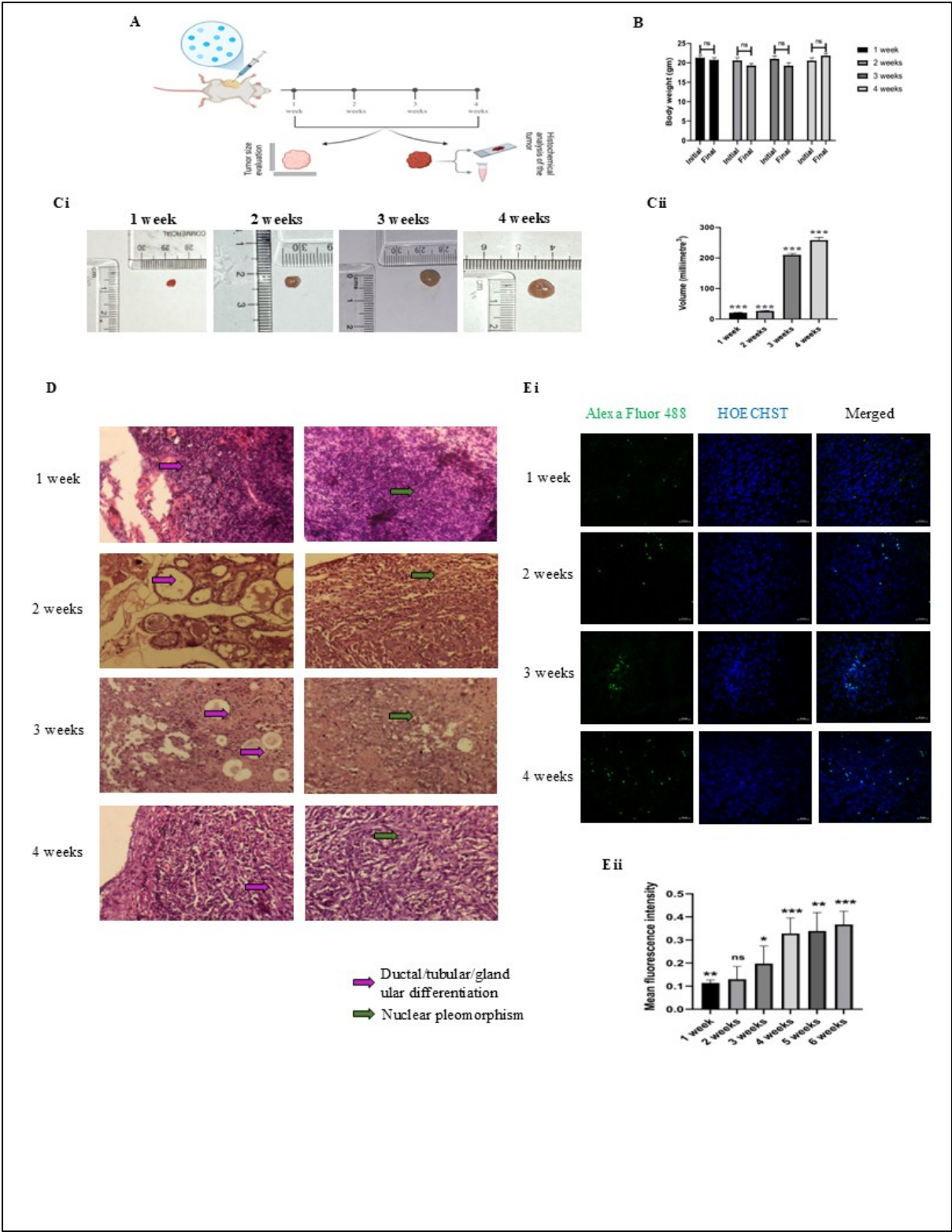
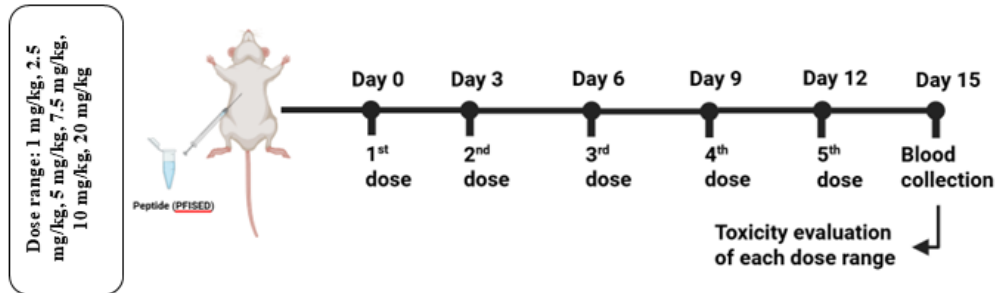


Figure S4: Gradation of murine breast cancer. **(A)** Schematic diagram of the outline of the experiments done to determine the grades of breast cancer developed by 4T1 cells in BALB/c mice upto six weeks. **(B)** Graphical presentation of body weights of mice with breast cancer at each week's interval upto 6 weeks starting from the day of injecting 4T1 cells. **(Ci)** Images of breast tumors developed by 4T1 cells in BALB/c mice. **(Cii)** Graphical comparison of the volumes of tumors of different time points. **(D)** Representative histological images of tumors of different time points with H&E staining indicating ductal/ tubular/ glandular differentiation (purple arrow) and nuclear pleomorphism (green arrow). **(Ei)** Representative immunohistochemical images of tumor tissues of different time points stained against Ki-67. **(Eii)** Quantification of mean fluorescence intensity of Ki-67, normalized against nuclear staining with Hoechst 33342.

A



B

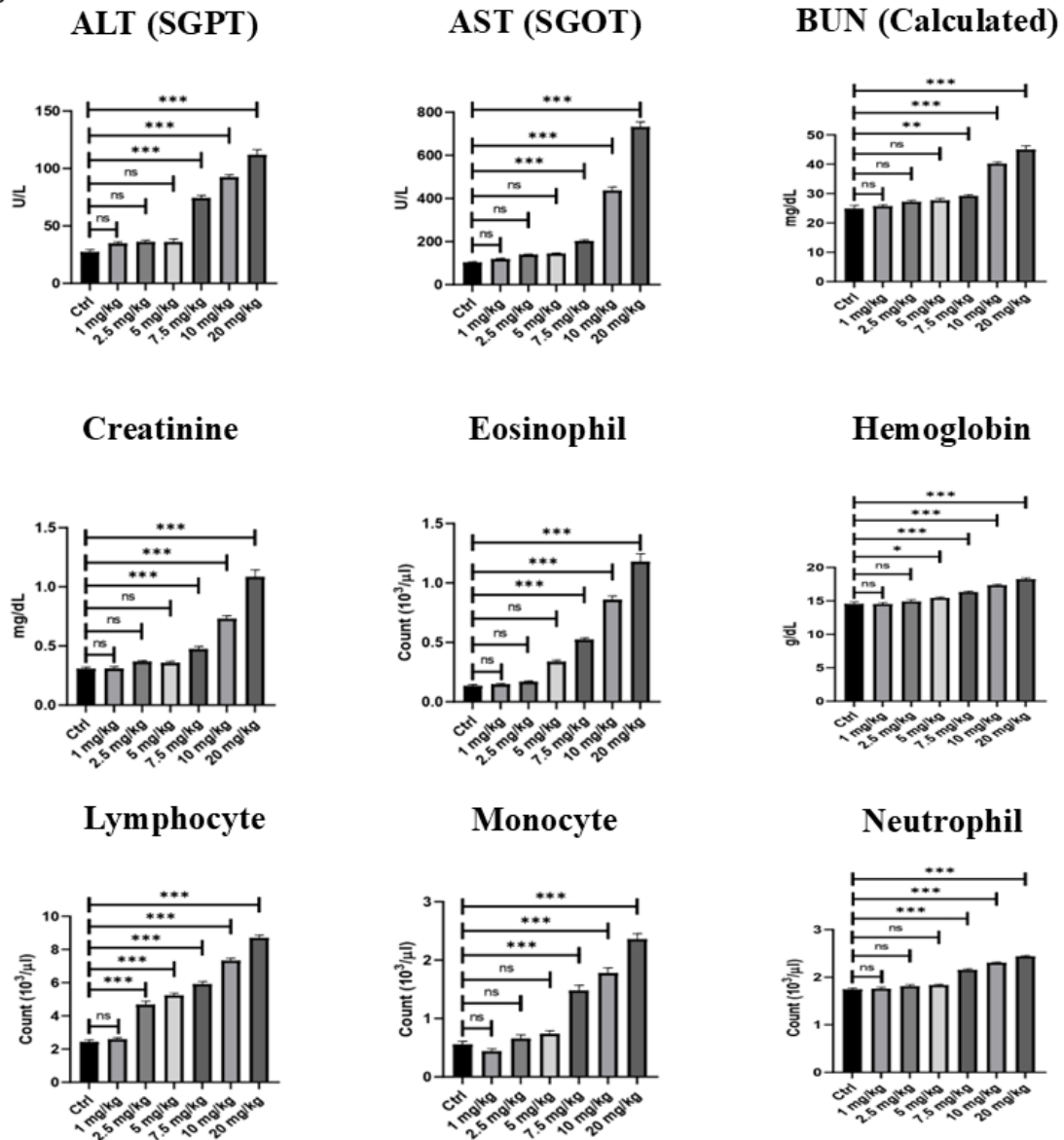


Figure S5: Optimal peptide dose determination. (A) Schematic diagram of the design of the experiment for toxicity evaluation of the peptide PFISED in BALB/c mice. (B) Assessment of hepatotoxicity parameters (ALT and AST), nephrotoxicity parameters (creatinine and calculated BUN) and hematological parameters (eosinophil, hemoglobin, lymphocyte, monocyte and neutrophil) in BALB/c mice given peptide of different dosages ranging from 1 mg/kg body weight to 20 mg/kg body weight at an interval of 3 days for 12 days.

Table S1: The physico-chemical properties of Peptide checked by SwissADME

ADMET	PAFISED	PFISED
Formula	C35H51N7O13	C32H46N6O12
Molecular Weight	777.82 g/mol	706.74 g/mol
H-bond Acceptors	14	13
H-bond Donors	11	10
Lipinski	No	No
Veber	No	No
Bioavailability	0.11	0.11
GI Absorption	Low	Low
Water Solubility	Soluble	Soluble
Synthetic Accessibility	6.46	5.95
Blood-Brain Barrier Permeant	No	No
Toxicity	No	No

An analysis of the drug-like properties, pharmacokinetics, toxicity, and physicochemical properties of the peptides

Table S2: The hydrogen bond interactions of top docked pose by CABS-dock

Hydrogen Bond	PAFISED	PFISED
	A:LYS135:NZ - C:ALA2:O	A:THR1142:OG1 - C:PHE2:O
	A:TYR1025:OH - C:SER5:OG	A:ASN1144:ND2 - C:SER4:OG
	C:SER5:OG - A:LEU216:O	A:ASN1144:ND2 - C:SER4:O
	C:GLU6:N - A:ASP1127:OD2	A:ASN1144:ND2 - C:ASP6:O
	A:LYS1135:CE - C:PRO1:O	A:LYS1147:NZ - C:GLU5:O
		C:ILE3:N - A:CYS296:SG
		C:SER4:OG - A:THR1142:O
		A:ARG1137:CD - C:PHE2:O
		C:PRO1:CA - C:ASP6:OD1
		C:SER4:CB - C:ASP6:O

The hydrogen bond interactions between receptor residue and peptides of top docked pose by CABS-dock. The interactions were analysed by Biovia Discovery Studio visualizer.

Table S3: Identification of low-grade breast carcinoma by gradation of tumor

Tumor sample	Ductal/tubular/glandular differentiation	Nuclear pleomorphism	Mitotic figure	Cumulative score	Grade of tumor
1 week	2	1	1	4	I
2 weeks	1	2	1	4	I
3 weeks	2	2	1	5	I
4 weeks	3	2	1	6	II

Scoring pattern of the tumors harvested at four different time points. Scores were attributed upon histopathological analysis of the three parameters, *viz.* Ductal/tubular/glandular differentiation, Nuclear pleomorphism and Mitotic figure and gradation of tumors based on their cumulative scores according to Nottingham Grading System.