Electronic Supplementary Information (ESI)

Glucose Oxidase Mediated Targeted Cancer-Starving Therapy by Biotinylated Self-Assembled Vesicle

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Materials: Silica gel of 60-120 mesh and 100-200 mesh, triethylamine (Et_3N), tert-butoxycarbonyl (Boc) anhydride, dicyclohexylcarbodiimide (DCC), 4-(N,N-dimethylamino) pyridine (DMAP), 1-hydroxybenzotriazole (HOBT), N-hydroxy succinimide (NHS), 6-amino caproic acid, polyethylene glycol methyl ether (PEG550), trifluoroacetic acid, dextrose, pyrogallol, celite545 and all other reagents were procured from SRL, India. Milli-Q water was used throughout the study. Trimesoyl chloride, D-biotin, Nα-Boc-Nε-Cbz-L-lysine, sephadex-G50, 1,6-diphenyl-1,3,5-hexatriene (DPH), 8-anilino-1-naphthalenesulfonic acid (ANS), coumarin 153 (C153), horseradish peroxidase (HRP), Glucose oxidase (GOx), MTT, apoptosis kit and other deuteriated solvents for NMR were purchased from Sigma Aldrich. Live-dead kit for eukaryotic cells was procured Genetix, India. All materials used in the cell culture study such as Dulbecco's Modified Eagles' Medium (DMEM), heat inactivated fetal bovine serum (FBS), trypsin from porcine pancreas were obtained from Himedia. ^{1}H NMR spectra were recorded in AVANCE 300 and 500 MHz (Bruker) spectrometer. MALDI-TOF spectrum was recorded on a Bruker spectrometer with 2,5-dihydroxy benzoic acid (DHB) as the matrix.

Synthesis of Biotinylated Triple Tailed Amphiphile (TMB). In a typical experiment, Nα-Boc-Nε-Cbz-L-lysine (1.0 equivalent) and methyl ester of 6-amino caproic acid (1.1 equivalent) were coupled together using N,N'-dicyclohexylcarbodiimide (DCC, 1.1 equivalent) in presence of DMAP (1.1 equivalent) and catalytic amount of HOBt in dry DCM under nitrogen atmosphere (Scheme S1). After overnight stirring, the reaction mixture was filtered and the filtrate was concentrated in a rotary evaporator. The whole residue was extracted in DCM and the product was consecutively washed with HCl (1N), sodium carbonate (1M), and brine to neutrality. The organic part was dried over anhydrous sodium sulphate and purified through column chromatography using 100-200 mesh silica gel where chloroform/methanol was used as the eluent to get the compound I in pure form (Scheme S1). The obtained intermediate, I was dissolved in methanol and subjected to alkaline hydrolysis by stirring 14-16 h with NaOH (1N). The reaction mixture was acidified with HCl (1N) and extracted with ethyl acetate followed by washing with brine to neutrality. Ethyl acetate part was dried over anhydrous sodium sulfate and concentrated to get the solid material. This solid residue purified through column chromatography using 60-120 mesh silica gel and chloroform/methanol used as the eluent to get the pure acid (II). The acid terminal of intermediate II was coupled with PEG_{550} (Polyethylene glycol methyl ether) using DCC (1.1 equivalent) and a catalytic amount of DMAP in the presence of 1.1 equivalent of HOBT in dry DCM under nitrogen atmosphere. The produced ester was purified through column chromatography using 60-120 mesh silica gel and chloroform/methanol as the eluent to obtain PEG linked compound III. The intermediate III was then subjected to deprotection by trifluoroacetic acid (1.5 equivalent) in dry DCM. After stirring for 4h, the solvent was removed on a rotary evaporator and the material was dried in a vacuum pump. It was then dissolved in MeOH and treated with solid sodium carbonate. The solution was

filtered and concentrated in a rotary evaporator followed by drying in a vacuum pump to get the free amine (IV).

In a separate reaction, NHS linked biotin was prepared by taking D-biotin (1 equivalent), DCC (1.1 equivalent) and NHS (1.1 equivalent) in dry DMF (8 mL) and stirred overnight under N_2 atmosphere. To this activated biotin (NHS-biotin) mixture, free amine, IV and triethylamine were added. The solution was stirred overnight and the DMF was distilled out under vacuum. The residue mixture was then purifid using 100-200 mesh silica gel and chloroform/methanol as the eluent to obtain pure compound V. The compound V was dissolved in MeOH. Then Pd on activated charcoal was added to the mixture under hydrogen (H_2) atmosphere and the reaction mixture was stirred overnight at room temperature to deprotect the benzyl protection. The whole mixture was filtered in a celite$_{545}$ column where hot methanol was used as eluent. Solvent was evaporated on a rotary evaporator and dried under high vacuum. Finally crystallization from methanol/diethyl ether afforded the pure amine (VI). The trimesoyl chloride (1 equivalent) was then mixed with prepared amine (VI, 3.5 equivalent) in freshly distilled anhydrous tetrahydrofuran (THF) in presence of triethylamine (3.5 equivalent) under nitrogen atmosphere for 6 h. The THF was then removed completely with vacuum pump to get yellow gelatinous material. The obtained yellow gummy material was thoroughly washed with hot ether to get purified final product **TMB** (Scheme S1).

Characterization of TMB. (*400 MHz, DMSO-d6, 25 °C*). δ = 1.13-1.16 (m, 18H, methelyne protons of D-biotin, L-lysine and 6-aminocaproic acid residue) 1.59-2.11 (m, 36H, methelyne protons of D-biotin, L-lysine and 6-aminocaproic acid residue), 2.08-2.25 (m, 12H, -C$\underline{H}_2$-CO-), 2.39-2.51 (m, 6H, -C$\underline{H}_2$-S- of D-biotin), 2.49 and 2.71 (s, 9H, -O-C$\underline{H}_3$), 2.98-3.68, 3.69-3.74 and 4.23-4.26 (m, 147H, three different position of PEG moiety and -C$\underline{H}$-S- of D-biotin), 4.98-5.11

(m, 3H, chiral proton of L-lysine moiety), 5.26-5.37 and 5.51-5.62 (m, 6H, -NH-CH-CH-NH- of D-biotin), 5.88 (s, 6H, -NH-CH-CH-NH- of D-biotin), 8.60 (s, 3H, aromatic proton of trimesic acid). MALDI-TOF MS: m/z; calculated for $C_{150}H_{267}N_{15}O_{54}S_3$: 3246.0791 [M + K^+]; found: 3246.0770 [M + K^+].

Preparation of Vesicles. Triskelion amphiphile, **TMB** (5 mg) was taken in a glass vial and simply dissolved in Milli-Q water (1 mL) that spontaneously produced a translucent vesicular solution. All the microscopic and spectroscopic characterizations were performed with these vesicular solutions.

Determination of Critical Association Concentration (CAC) for TMB. To determine the critical association concentration (CAC) for **TMB** amphiphile, the Du Noüy ring method was applied by measuring surface tension using a tensiometer at 25±0.1 °C. Stock solutions of **TMB** was prepared (10 mM) in Milli-Q water and measured the surface tension of specific concentrations of each diluted solution prepared from the stock solution. The CAC value was calculated by plotting surface tension versus log molar concentration of the amphiphile (Fig. S1) with an accuracy of ±2% in triplicate experiments.

Microscopic studies.

Transmission Electron Microscopy (TEM). For TEM studies, 3μL of the translucent solution of **TMB** in pure water (5 mg/mL) was deposited on a 300-mesh carbon coated copper grid. It was kept for 1 min to adsorb. The excess solution was blotted out with a filter paper. The copper grid was negatively stained with freshly prepared aqueous solution of uranyl acetate (1 μL, 1% w/v) and the excess solution was immediately blotted out with a filter paper. The sample was then dried for 4 h in vacuum before taking the image. The TEM images were obtained in JEOL JEM 2010 microscope.

Field-Emission Scanning Electron Microscopy (FESEM). FESEM images were obtained on a JEOL-6700F microscope. 6 μL of the translucent solution **TMB** in pure water (5 mg/mL) were placed on a piece of cover slip and dried for overnight. Then it was kept few hours under vacuum before taking the images.

Atomic Force Microscopy (AFM). The AFM images of **TMB**-vesicles were taken on a Veeco, model AP0100 in noncontact mode. 10 μL aqueous translucent solution of **TMB** (5 mg/mL) was deposited on freshly cleaved (1 cm × 1 cm) mica and dried overnight before imaging.

Spectroscopic Studies.

Dynamic Light Scattering (DLS). Mean hydrodynamic diameter of the vesicle formed by **TMB** in aqueous medium was determined by DLS using a fixed-angle apparatus (Zen 3690 Zetasizer Nano ZS instrument (Malvern Instruments Ltd)). The scattering intensity was measured at an angle of 175°. **TMB** solutions with varying concentrations were prepared in Milli-Q water. The scattering intensity data collected in different solution concentrations were computed by a data processor with the necessary software.

Solvent Dependent ^{1}H NMR Measurements. Solvent dependent ^{1}H NMR spectra of **TMB** were recorded in AVANCE 300 MHz (Bruker) spectrometer where the amphiphile concentration was maintained at 0.5 mg/mL for each ^{1}H NMR spectrum. Solvents were varied from [D6]DMSO (non-self assembled) to D_2O (self-assembled state). ^{1}H NMR spectra were also recorded in an intermediate solvent system i.e. [D6]DMSO-D_2O (1:1 v/v).

UV-vis Study. Solvent dependent UV-vis spectroscopic study was carried out using 8-anilino-1-naphthalenesulfonic acid (ANS) as the probe. The minute amount of ANS (1×10^{-5} M) was doped within **TMB** solution and the corresponding UV-vis spectra were recorded in a PerkinElmer Lambda 25 spectrophotometer. The solvents were varied from non-self assembled (DMSO) to

self-assembled (water). DMSO-water (1:1 v/v) was chosen as an intermediate solvent. The amphiphile concentration was maintained at 0.5 mg/mL.

X-ray Diffraction. X-ray diffraction (XRD) spectrum (Fig. S2) of dried film of **TMB** vesicular solution was obtained on a Bruker D8 Advance diffractometer and the source was CuKα radiation (α = 0.15406 nm) with a voltage 40 kV and current 30 mA. The vesicular solution of **TMB** in water (5 mg/mL) were placed over a glass slide and dried to form a thin film. The samples were scanned from 0° to 70°.

Fluorescence Anisotropy. Fluorescence spectra were recorded in a Varian Cary Eclipse fluorescence spectrophotometer using fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH). DPH is a well known hydrophobic dye to attach in the hydrophobic region of the self-aggregates. The steady-state anisotropy (*r*) of DPH was evaluated in aqueous solution of **TMB** with varying concentrations (Table S1). A stock solution of DPH (0.2 mM) was prepared in tetrahydrofuran (THF) and the final concentration of DPH was maintained at 1 μM in each solution. The DPH doped in **TMB**-vesicles was excited at 370 nm. The emission intensity was measured at 450 nm using an emission cut off filter at 430 nm to avoid any scattering due to turbidity of the solution. The excitation and emission slit widths were kept at 5 nm. The fluorescence anisotropy value (*r*) was calculated (Table S1) by the instrumental software following equation 1

$$r=(I_{VV}-GI_{VH})/(I_{VV}+2GI_{VH}) \quad (1)$$

where I_{VV} and I_{VH} are the intensities of the emission spectra obtained with vertical and horizontal polarization (for vertically polarized light), respectively, and $G = I_{HV}/I_{HH}$ is the instrumental correction factor, where I_{HV} and I_{HH} are the emission intensities obtained with vertical and horizontal polarization (for horizontally polarized light), respectively. The fluorescence measurements were performed at least five times for each sample at 25 °C.

Time Resolved Study. Time correlation single photon count (TCSPC) measurement was performed in a picosecond diode laser IBH-405. A stock solution of coumarin 153 (C153) dye (1 mM) was prepared in dry THF and was added to aqueous solution of **TMB**-vesicle. In the prepared solution, the final concentration amphiphile was 0.5 mg/mL while the dye concentration was maintained at 2 μM. In addition, free C153 solution in pure water (2 μM) was prepared for control experiment. All samples were excited at 405 nm. The fluorescence decays were analyzed with IBH DAS6 software. Equation (2) was used to analyze the experimental time resolved fluorescence decay *p(t)*.

$$p(t) = b + \sum_{i}^{n} \alpha_i \exp\left(-\frac{t}{\tau_i}\right) \qquad (2)$$

where n is the number of discrete emissive species, b is a baseline correction ("dc" offset), and i and i are pre-exponential factors and excited state fluorescence lifetimes associated with the ith component, respectively. For multi exponential decays the average lifetime $<\tau>$ was calculated from equation (3).

$$<\tau> = \sum_{i=1}^{n} \alpha_i \tau_i \qquad (3)$$

Where $\alpha_i = \dfrac{\alpha_i}{\sum \alpha_i}$ which indicates the contribution of a decay component.

Enzyme Activity Assay. The enzyme activity of GOx was estimated spectrophotometrically in a UV-vis spectrophotometer. In a typical experiment, the stock solutions in PBS buffer (pH = 7.4) of horseradish peroxidase (HRP) (1 mg/mL), glucose (500 mg/mL), GOx (10 mg/mL) and pyrogallol (1 g/mL in acetone) were prepared separately. All the substances except GOx were mixed together in such a way that the overall concentration of glucose, pyrogallol and HRP

inside the cuvette was maintained at 25 mg/mL, 30 mg/mL and 6 μg/mL, respectively. Finally, upon addition of 1 μL of GOx solution in the cuvette ([GOx] = 20 μg/mL), the progress of the reaction was monitored by the formation of purpurogallin, the oxidized product of pyrogallol at 420 nm (λ_{max} of purpurogallin) for the initial 5 min at 25 °C. The initial rate (V) of this enzymatic oxidation was determined from the slope of the absorption versus time curve, using the molar absorption coefficient of purpurogallin at 420 nm (4400 M^{-1} cm^{-1}). The working solution expect the GOx was used as background. The activity of GOx was proportional to the slope of the absorbance versus time curve. Activity calibration curve was also prepared by plotting of the activity of GOx against different known concentration of GOx.

The activity of vesicle entrapped GOx was also measured in a similar way after rupturing the vesicular membrane by using Triton X-100 and percentage loading was calculated from activity calibration curve (Fig. S3a).

GOx Loading and Release. In a glass vial, 10 mg of solid **TMB** was mixed with 1 mL of glucose oxidase (GOx) solution (10 mg/mL) in water and kept overnight under stirring condition. The solution was then loaded into a sephadex G-50 column (10 cm height and 1.2 cm diameter) pre-equilibrated with Milli-Q water. GOx loaded **TMB**-vesicular solutions were eluted right after the void volume. The filtration was carried out until un-entrapped GOx was gel-filtrated and removed completely. The eluent was collected in 2 mL fraction each. To confirm the presence of GOx in each fraction the absorbance for all the fractions was taken at 274 nm. Eluent was collected till no detectable absorbance of GOx obtained. Finally 0.5% (v/v) Triton X-100 was used to disintegrate the vesicles and estimate the amount of loaded GOx. Percentage loading of the GOx was determined using the standard absorbance calibration curve of free GOx as well as by comparing the activity of released GOx with the enzyme activity calibration plot (Fig. S3). To

further ensure the successful loading of GOx within the vesicle, fluorescence spectra of free GOx and entrapped GOx within vesicles were recorded at λ_{em}= 335 nm (λ_{ex}= 295 nm). For release experiments, 0.5% (v/v) Triton X-100 was mixed with the GOx loaded vesicular solution and the fluorescence spectra was taken after the addition of Triton X-100 (Fig. S4). Horseradish peroxidase (HRP) was also loaded in the **TMB**-vesicle in a similar manner as stated above for the control experiments. Loading was confirmed by measuring the absorbance of HRP at 404 nm and comparing it with standard calibration plot (Fig. S9).

Stability. The long time stability of free GOx and vesicle entrapped GOx was investigated by measuring the enzyme activity for 10 days in different experimental systems i.e. water, PBS (20 mM, pH = 7.4), 10% FBS-DMEM media. Stock solutions of GOx and vesicle entrapped GOx were prepared separately in water, PBS, 10% FBS-DMEM media ([GOx] = 2 mg/mL in each case). An aliquot of each solution was taken separately at different time intervals (maintaining the [GOx] = 20 μg/mL in cuvette), and the enzyme activity was estimated by following the procedures as mentioned above. This process was continued for 10 days (Fig. S5a-c). Moreover, the stability of **TMB**-vesicle encapsulated GOx compared to native GOx ([GOx] = 2 mg/mL in each case) was also investigated using FBS containing DMEM media having varying concentration of FBS (0-75%) and all the solutions were kept for 48 h. An aliquot of each solution was taken separately (maintaining the [GOx] = 20 μg/mL in cuvette) and the enzyme activity was estimated by following the procedures as mentioned above (Fig. S5d).

Cell Culture. Cancerous cells HeLa, B16F10 melanoma and normal cells CHO, NIH3T3 were collected from National Centre for Cell Science, Pune and cultured in 10% FBS supplemented DMEM media in presence of streptomycin (100 mg/L) and penicillin (100 IU/mL). The cells were adhered in 25 mL culture flask and maintained at 37 °C under humidified atmosphere of

5% CO_2 to ~70-80% confluence. Sub-culture of cells was carried out in every 2-3 days. FBS supplemented media was replaced after 24 h. Trypsinization was done to detach the seeded cells from the culture flask surface. These cells were utilized for all the cytotoxicity and GOx delivery experiments.

MTT Assay. Cytotoxicity of **TMB**-vesicle was tested by the microculture MTT reduction method. This method involves the conversion of tetrazolium salt to a colored formazan (water insoluble) product by mitochondrial dehydrogenase of live cells. The quantity of formazan was estimated by measuring the absorbance value of the product upon dissolving in DMSO. The formation of formazan is directly related to the number of live cells. HeLa, B16F10, CHO and NIH3T3 cells were separately seeded at a density of 15,000 cells per well in four 96-well microtiter plate 18-24 h before the assay. MTT assay with **TMB** was investigated over a concentration range of 100 to 500 μg/mL in all the microtiter plates. All types of cells were separately incubated for 12 h at 37 °C under 5% CO_2 atmosphere. MTT stock solution (10 μL from 5 mg/mL) in phosphate buffer saline was added to the microtiter plates and the cells were further incubated for another 4 h. The precipitated water insoluble formazan was dissolved thoroughly in DMSO and absorbance at 570 nm was measured using BioTek Elisa Reader. The number of surviving cells were expressed as percent viability = [A_{570} (treated cells)-background/A_{570} (untreated cells)-background] ×100 (Fig. S6).

MTT Assay of free GOx and GOx loaded TMB-vesicle. Free GOx and **TMB**-vesicle entrapped GOx were separately added into four separate 96 well chambered plates containing confluent HeLa, B16F10, CHO and NIH3T3 cells in 10% FBS-DMEM culture media. In each set free GOx and **TMB**-vesicle loaded GOx were separately added in such a way that the GOx concentration was varied from 100-500 μg/mL. MTT assay was carried out by the usual

procedure as described above. Percentage killing of the treated cells by free GOx (Fig. S7) and vesicle loaded GOx was investigated after 12 h of incubation.

The half inhibitory concentration (IC_{50}) for different cell line upon incubation with **TMB**-vesicle encapsulated GOx was determined from the percent killing plot (Fig. S8). For control experiment HRP entrapped **TMB**-vesicle was utilized in a similar fashion as stated above and the corresponding killing effect has been checked by MTT assay for all the four cell lines (HeLa, B16F10, CHO and NIH3T3 cells) (Fig. S10).

Cell Viability Determined by Live/Dead Viability Kit. Cell viability of GOx entrapped vesicle was determined using a live/dead assay kit by reported protocol (Fig. S11). The kit is composed of two nucleic acid binding stains, Calcein AM (acetomethoxy) and ethidium homodimer-1 (EthD-1). Approximately 4 μM EthD-1 solution was prepared by adding 4 μL of the supplied 2 mM EthD-1 solution within 2 mL of PBS buffer (autoclaved). To that, 1 μL of the 4 mM calcein AM stock solution was added, and the resulting solution was vortexed. A mixture of 2 μM calcein AM and 4 μM EthD-1 was added to HeLa, B16F10, CHO and NIH3T3 cells pre-treated with GOx (300 μg/mL) entrapped vesicle (12 h at 37 °C) followed by incubation for 30 min. After the incubation period, cells were observed under an Olympus IX51 inverted microscope using a BP460−495 nm excitation filter and a band absorbance filter covering wavelengths below 505 nm calcein intercalation. A BP530-550 excitation filter and band absorbance filter covering wavelengths below 570 nm were used for imaging of EthD-1 intercalation.

Flow Cytometry. HeLa, B16F10, CHO and NIH3T3 cells pre-treated with GOx (300 μg/mL) entrapped vesicle were further incubated with live/dead assay kit for 30 min. These cells were repeatedly washed with PBS buffer to ensure complete removal of the extracellular hybrids. The treated cells were then trypsinized, centrifuged and suspended in 500 μL of PBS. The cells were

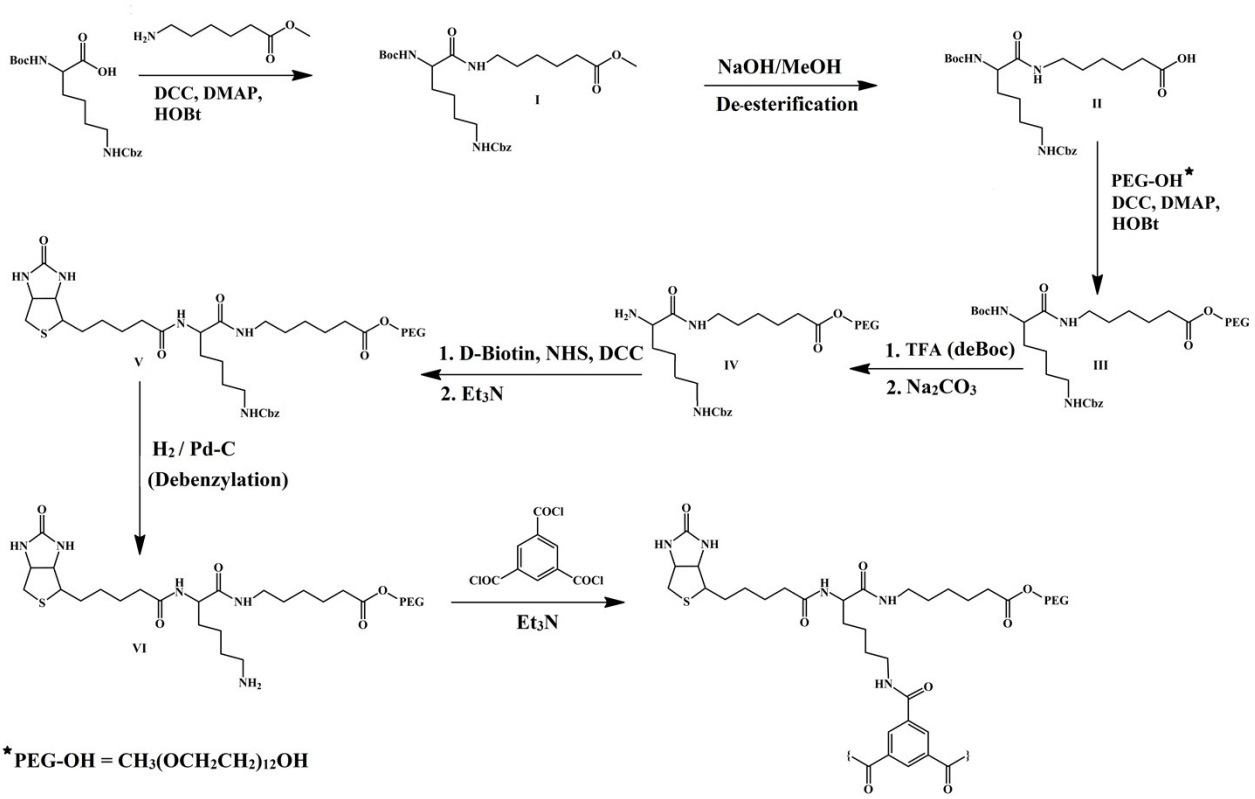
analyzed using a flow cytometer, BD Accuri C6 operating at 488 nm excitation wavelength and emission wavelength using a 533±30 nm (FL-1), 675±25 nm (FL-4) bandpass filters for Calcein AM (acetomethoxy) and ethidium homodimer-1 (EthD-1) intercalation, respectively. Initially, 50,000 cells were taken for seeding and the data was collected from 10,000 cells (Fig. S11).

MTT Assay of GOx Loaded TMB-vesicle of Biotin Pre-treated Cancer Cells. Confluent Hela and B16F10 cells were seeded into 96 well plates 24 h before incubation. The adhered cells were then treated with 2 mM biotin for 1 h to ensure blocking of the receptors with biotin. This was followed by incubation of the cells with GOx loaded **TMB**-vesicle in 10% FBS-DMEM culture media where GOx concentration was varied from 100-500 μg/mL.

Apoptosis. GOx entrapped **TMB**-vesicle was taken in two separate 24-well plate having confluent HeLa and B16F10 cells in 10% FBS-DMEM media. The final concentration of GOx was maintained at 300 μg/mL in 500 μL of media. After 6 h incubation at 37 °C, cells were washed with PBS and trypsinized, followed by centrifugation to obtain the cell pellet. Pellets were suspended in 500 μL of Annexin V-FITC (0.25 μg) and propidium iodide (PI, 1.0 μg)-containing binding buffer. The cellular mixture was incubated for 15 min at room temperature in the dark followed by trypsinization, centrifugation, and suspension in PBS buffer (500 μL). Cells were immediately analyzed in a BD Accuri C6 flow cytometer by exciting the sample at 488 nm and recording the emission at a 533 ± 30 (FL-1) bandpass filter for Annexin V-FITC and 585 ± 40 nm (FL-2) bandpass filter for PI (Fig. S12).

Table S1. Steady-state fluorescence anisotropy (*r*) of DPH with varying concentrations of **TMB** in water

Concentration of **TMB**/ mg/mL	*r*-value
1	0.09
2	0.09
4	0.11
8	0.16
16	0.16



Scheme S1 Synthetic scheme for **TMB**.

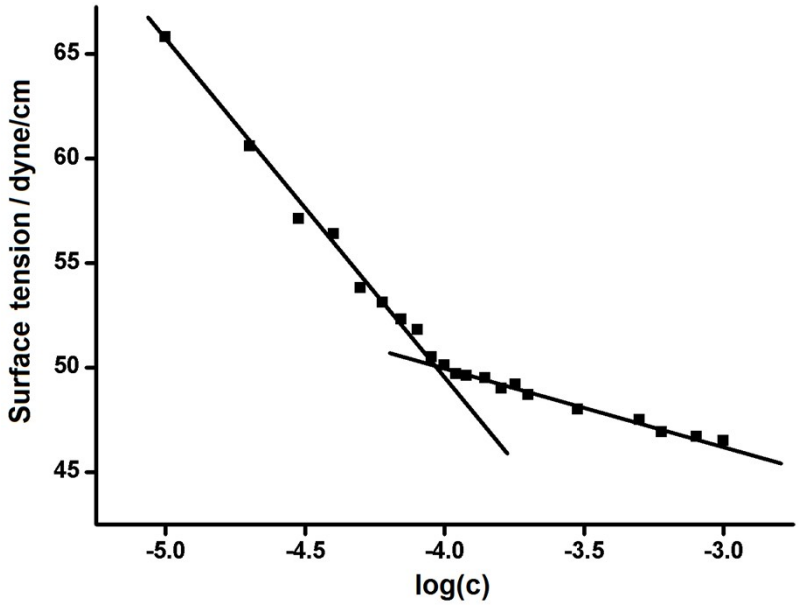


Fig. S1. Plot of surface tension versus log (molar concentration of **TMB** (c)) at 25 °C.

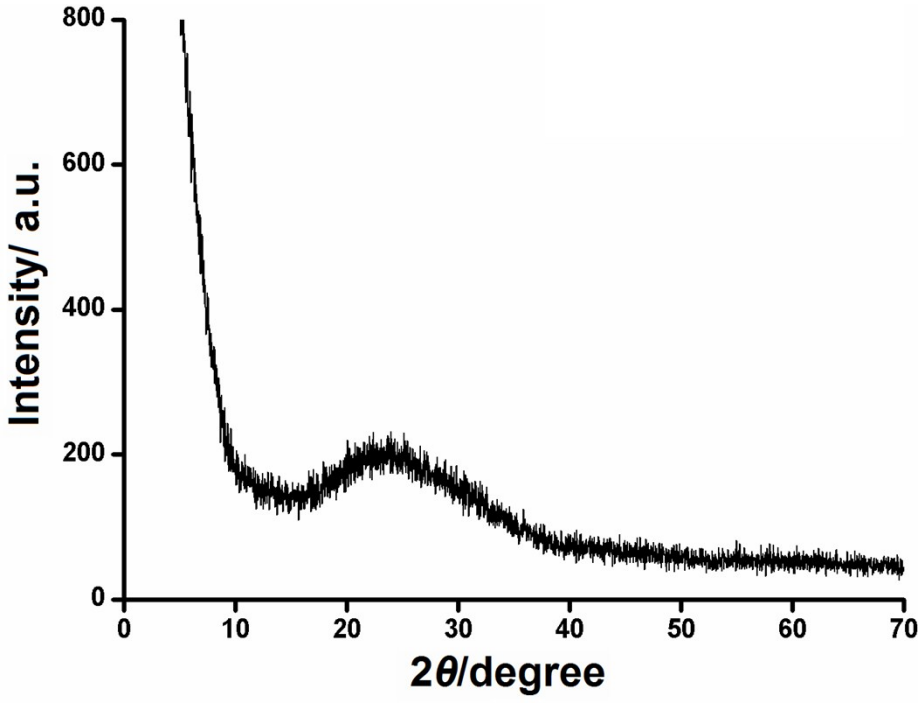


Fig. S2 XRD of **TMB** (5 mg/mL) film prepared in water.

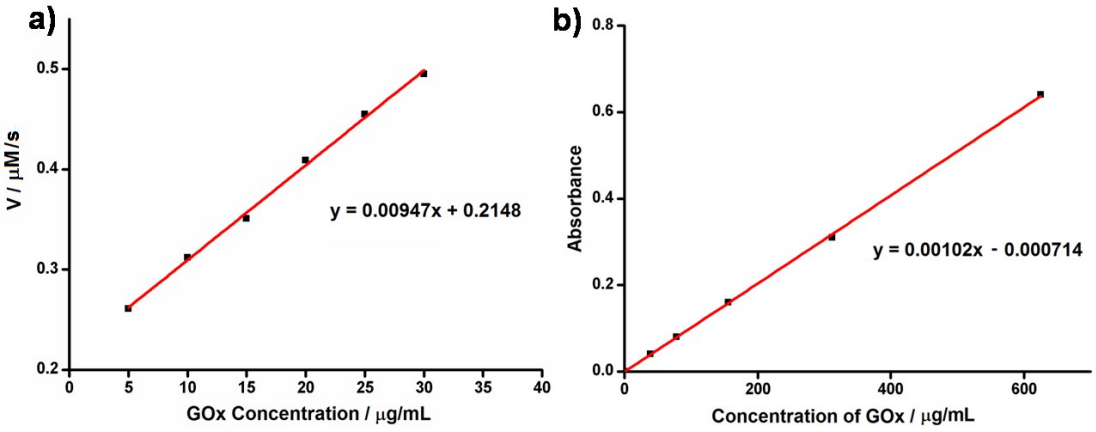


Fig. S3 a) Calibration plot of GOx activity with its concentration and b) calibration plot of GOx in water (λ_{max}= 274 nm, absorbance vs concentration).

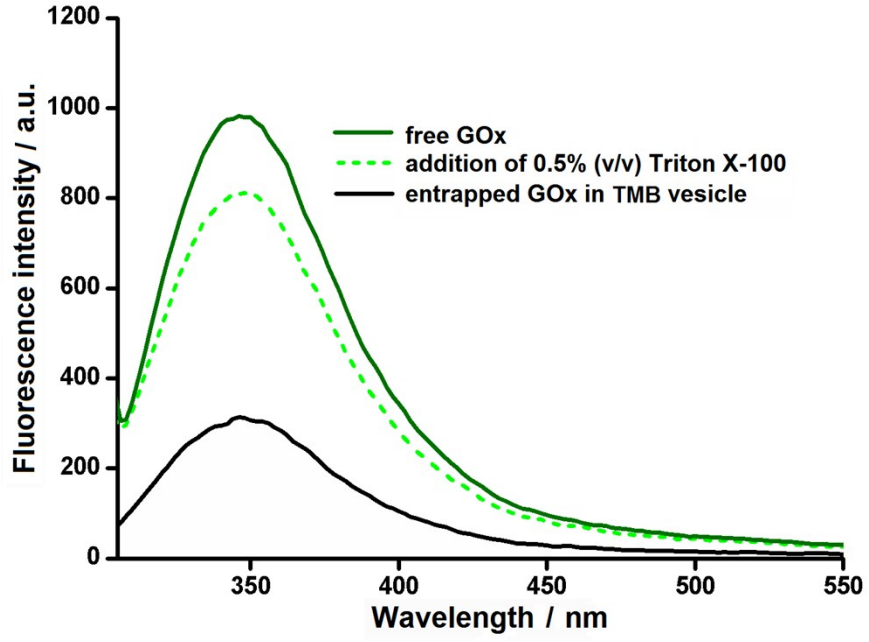


Fig. S4 Emission spectra of free GOx, GOx entrapped within **TMB**-vesicle, after treating with Triton X-100 GOx.

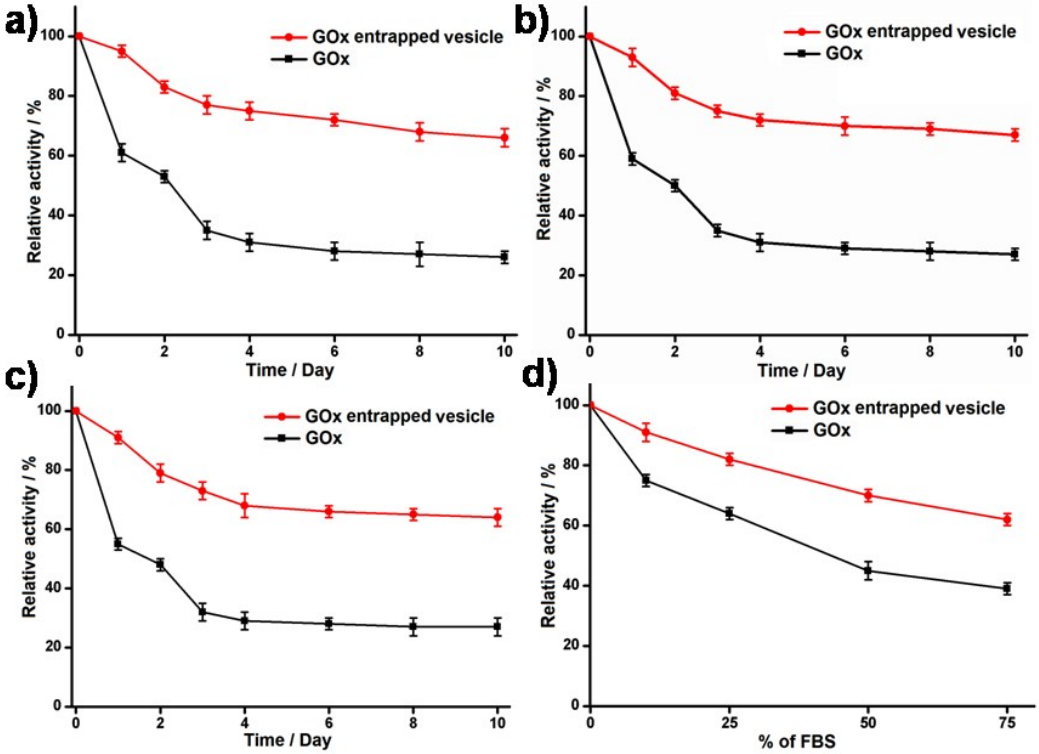


Fig. S5 a) Change of enzymatic activity of free GOx and **TMB**-vesicle encapsulated GOx with respect to its initial activity (expressed as % relative activity) for ten (10) days at 25 °C in a) water, b) PBS (20 mM) c) 10% FBS-DMEM media and d) in varying FBS concentration in DMEM media (Concentration of GOx in each experimental system = 2 mg/mL; and the concentration of GOx in cuvette = 20 μg/mL as described above in the enzyme assay).

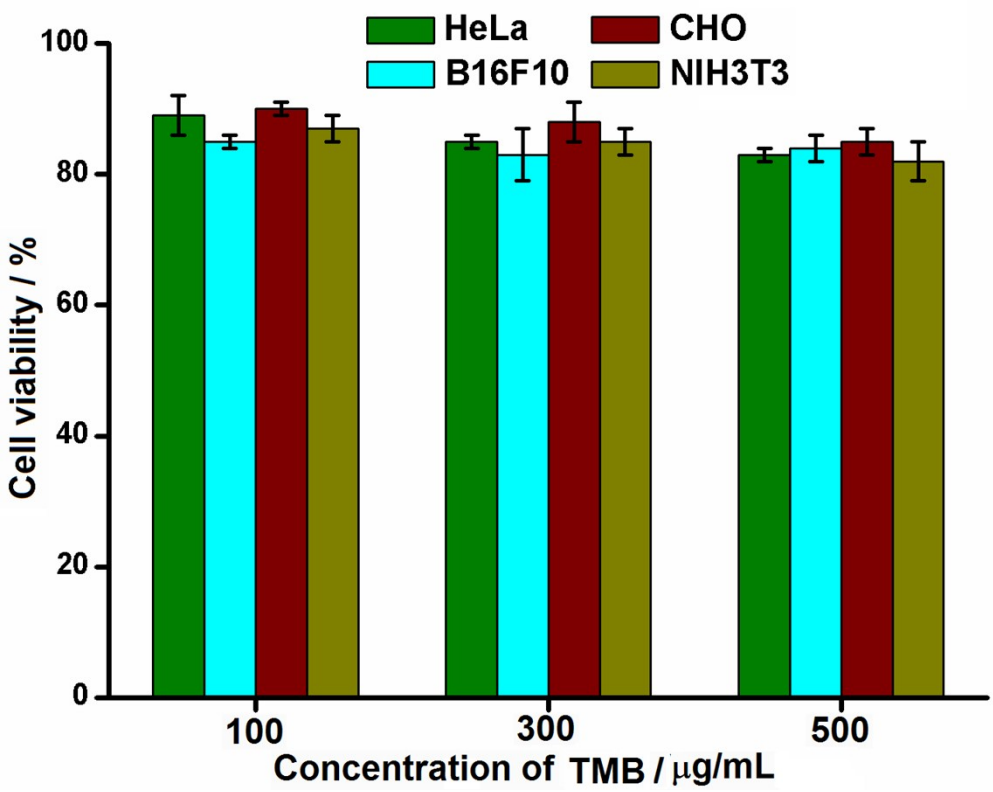


Fig. S6 Cell viability of **TMB** against HeLa, B16F10, CHO and NIH3T3 cells upon 12 h of incubation. Percent errors are within ±5% in triplicate experiments.

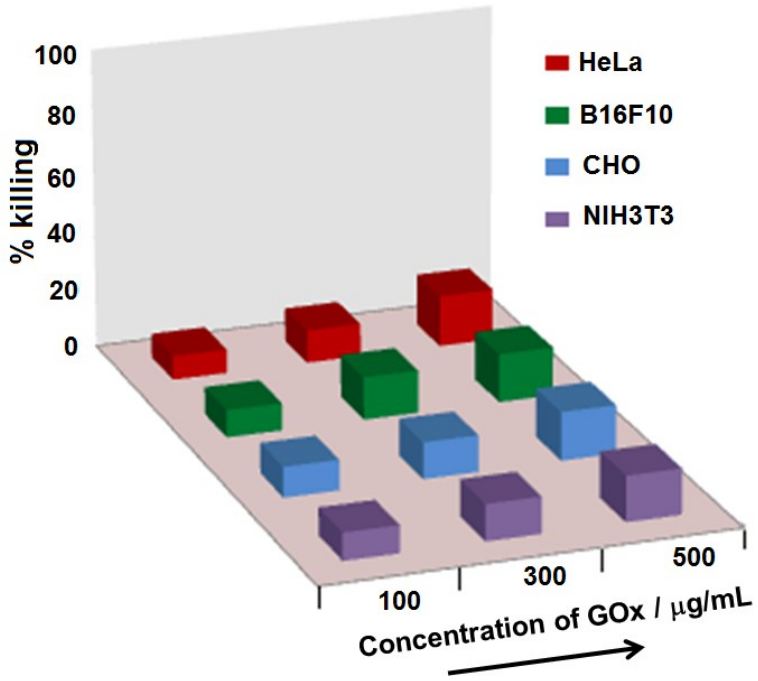


Fig. S7 % Killing of cancerous (HeLa, B16F10) and non-cancerous (CHO, NIH3T3) cells incubated with free GOx for 12 h with varying concentration. Percent errors are within ±5% in triplicate experiments.

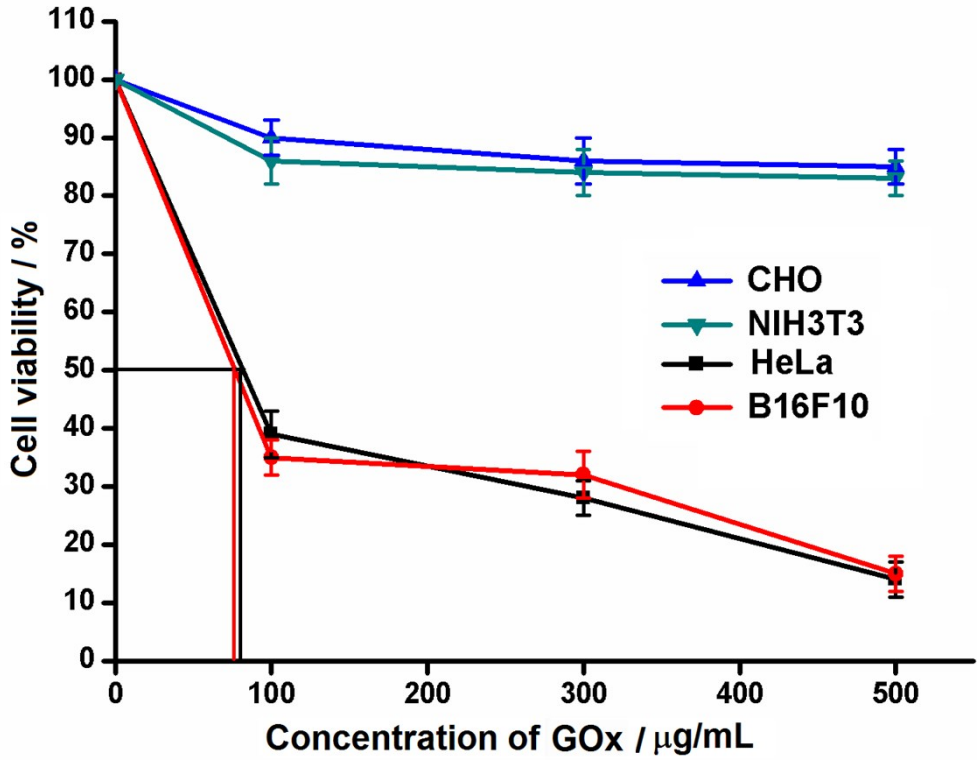


Fig. S8 IC_{50} determination of **TMB**-vesicle entrapped GOx after 12 h incubation for HeLa, B16F10, CHO and NIH3T3 cells. The experimental errors were in the range of ±5% in triplicate experiments.

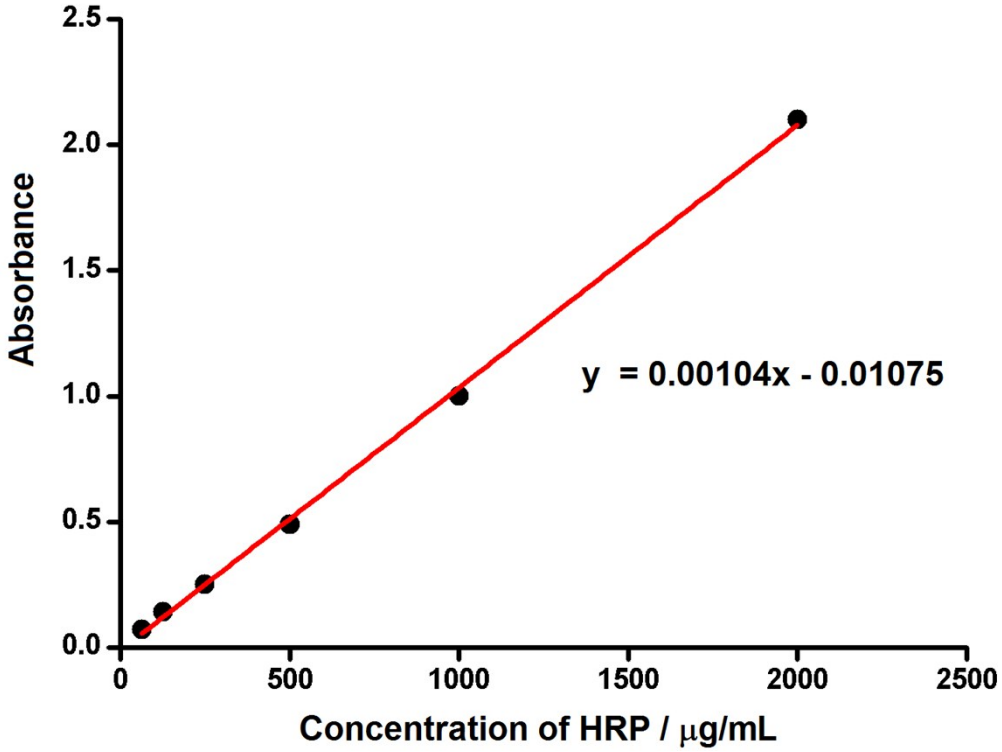


Fig. S9 Calibration plot of absorbance vs concentration for horseradish peroxidase (HRP) in water (λ_{max}= 404 nm).

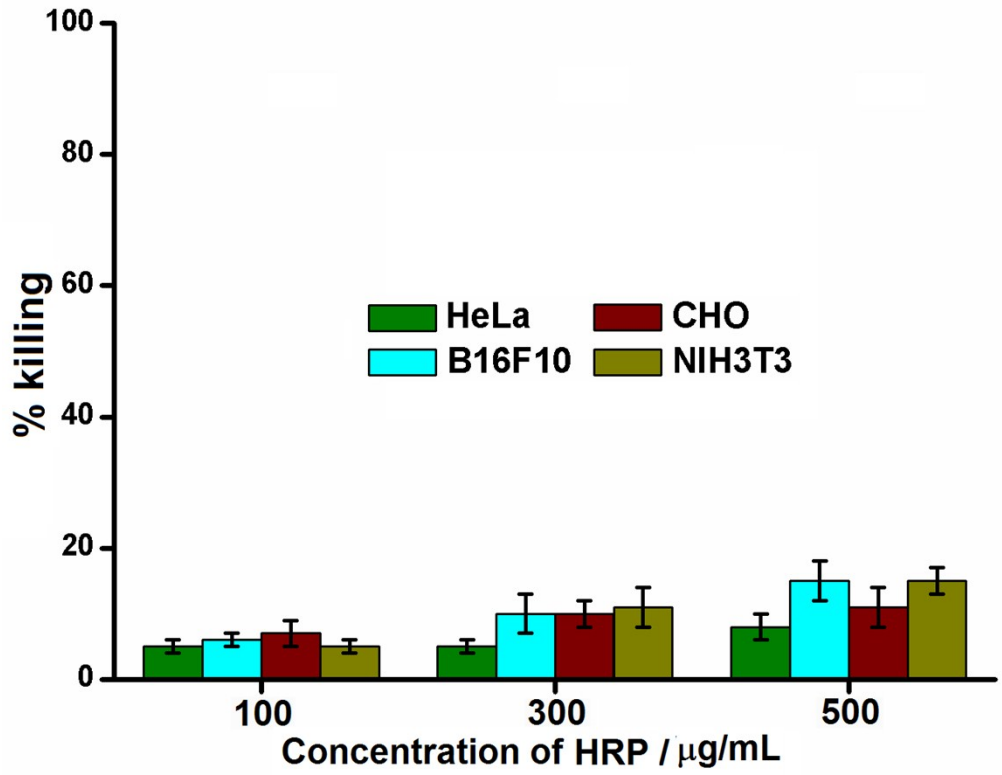


Fig. S10 % Killing of cancerous (HeLa, B16F10) and non-cancerous (CHO, NIH3T3) cells incubated with **TMB**-vesicle loaded HRP for 12 h with varying HRP concentration. Percent errors are within ±5% in triplicate experiments.

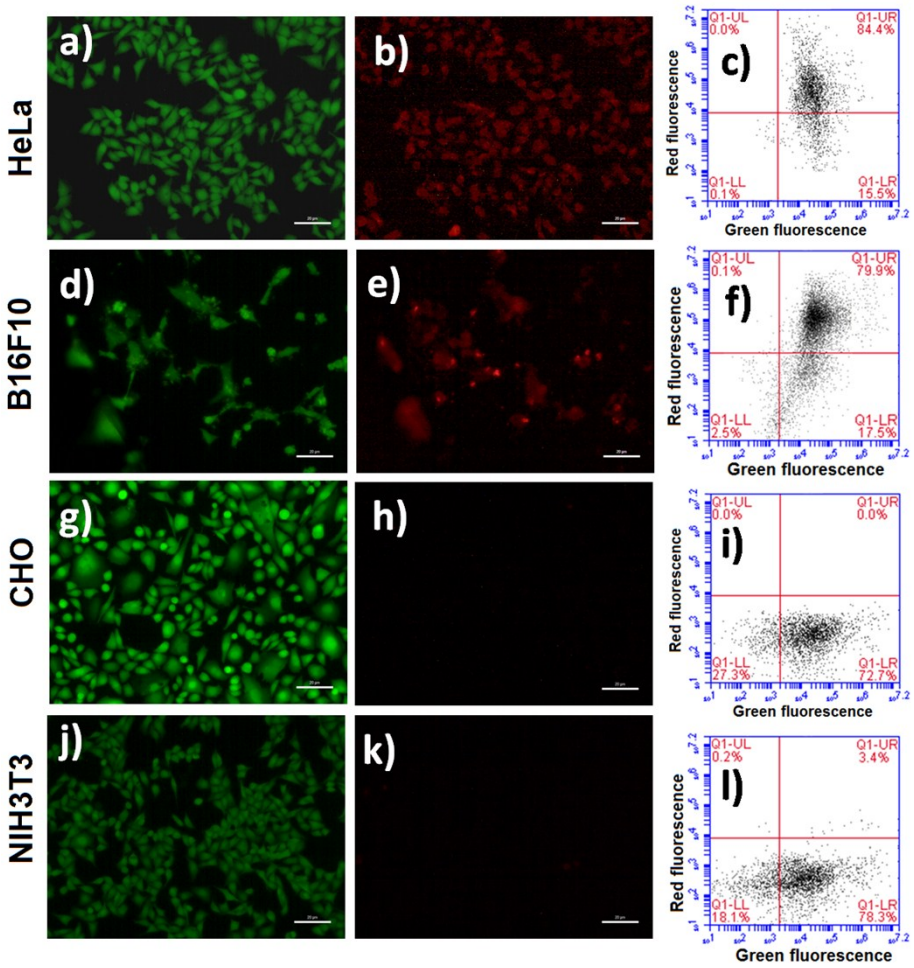


Fig. S11 LIVE/DEAD fluorescence microscopic images (a, b, d, e, g, h, j, k) of the cells incubated for 12 h with GOx loaded **TMB**-vesicle ([GOx] = 300 μg/mL). (a and b) HeLa cells, c) corresponding flow cytometric plot for HeLa cells, (d and e) B16F10 cells, f) corresponding flow cytometric plot for B16F10 cells, (g and h) CHO cells, i) corresponding flow cytometric plot for CHO cells and (j and k) NIH3T3 cells, l) corresponding flow cytometric plot for NIH3T3 cells. Scale bars correspond to 20 μm.

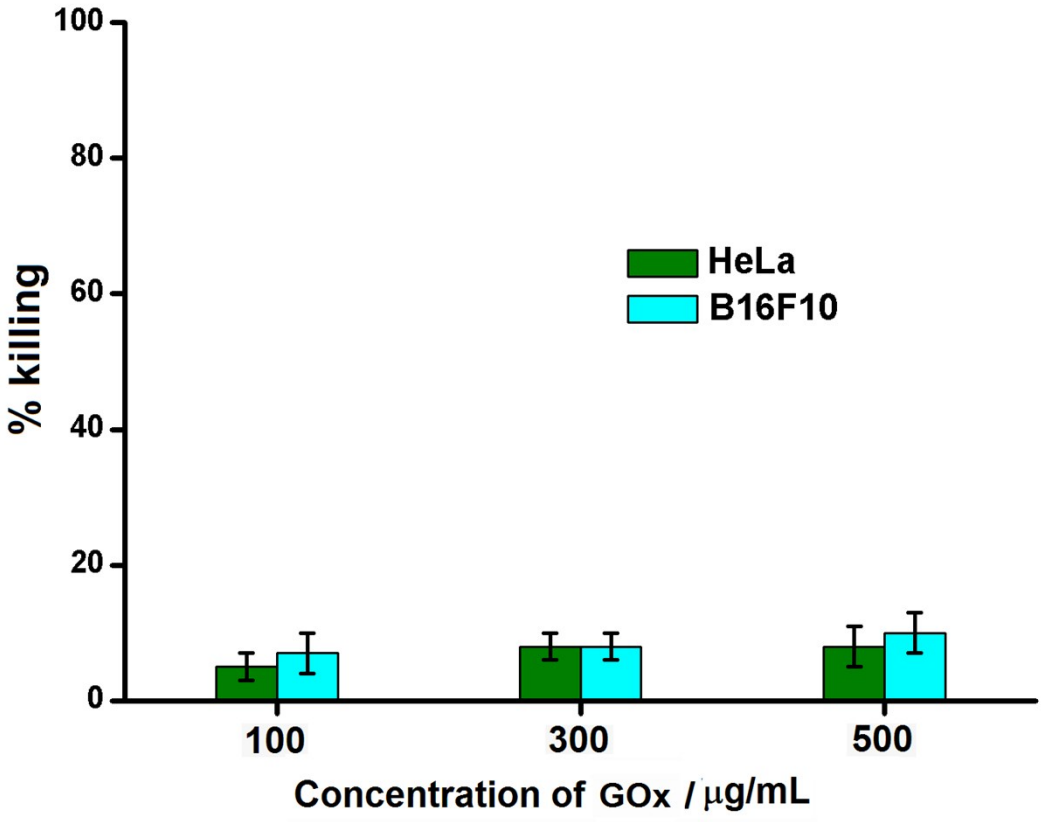


Fig. S12 % Killing of biotin pre-treated cancer cells (HeLa, B16F10) incubated with GOx loaded **TMB**-vesicle for 12 h with varying GOx concentration. Percent errors are within ±5% in triplicate experiments.

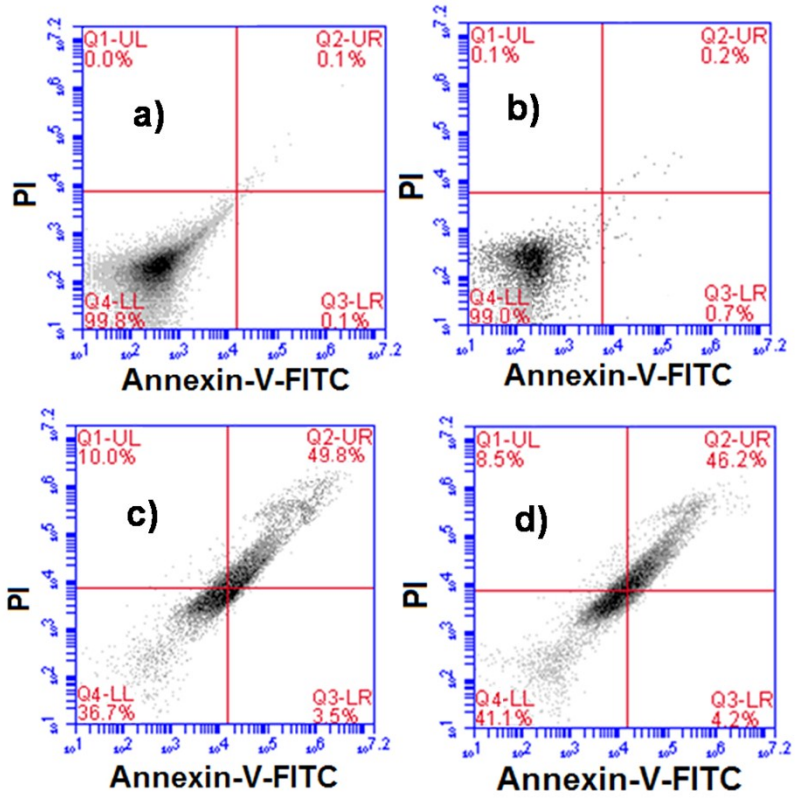


Fig. S13 Flow cytometric analysis of apoptosis using Annexin V-FITC/PI in a) untreated HeLa cells, b) untreated B16F10 cells and c) treated HeLa, d) treated B16F10 cells with GOx entrapped vesicle for 6 h, where [GOx] = 300 μg/mL.