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Supplemental Information

Aptamer-Functionalized Drug Nanocarrier Improves Hepatocellular Carcinoma toward Normal by Targeting Neoplastic Hepatocytes

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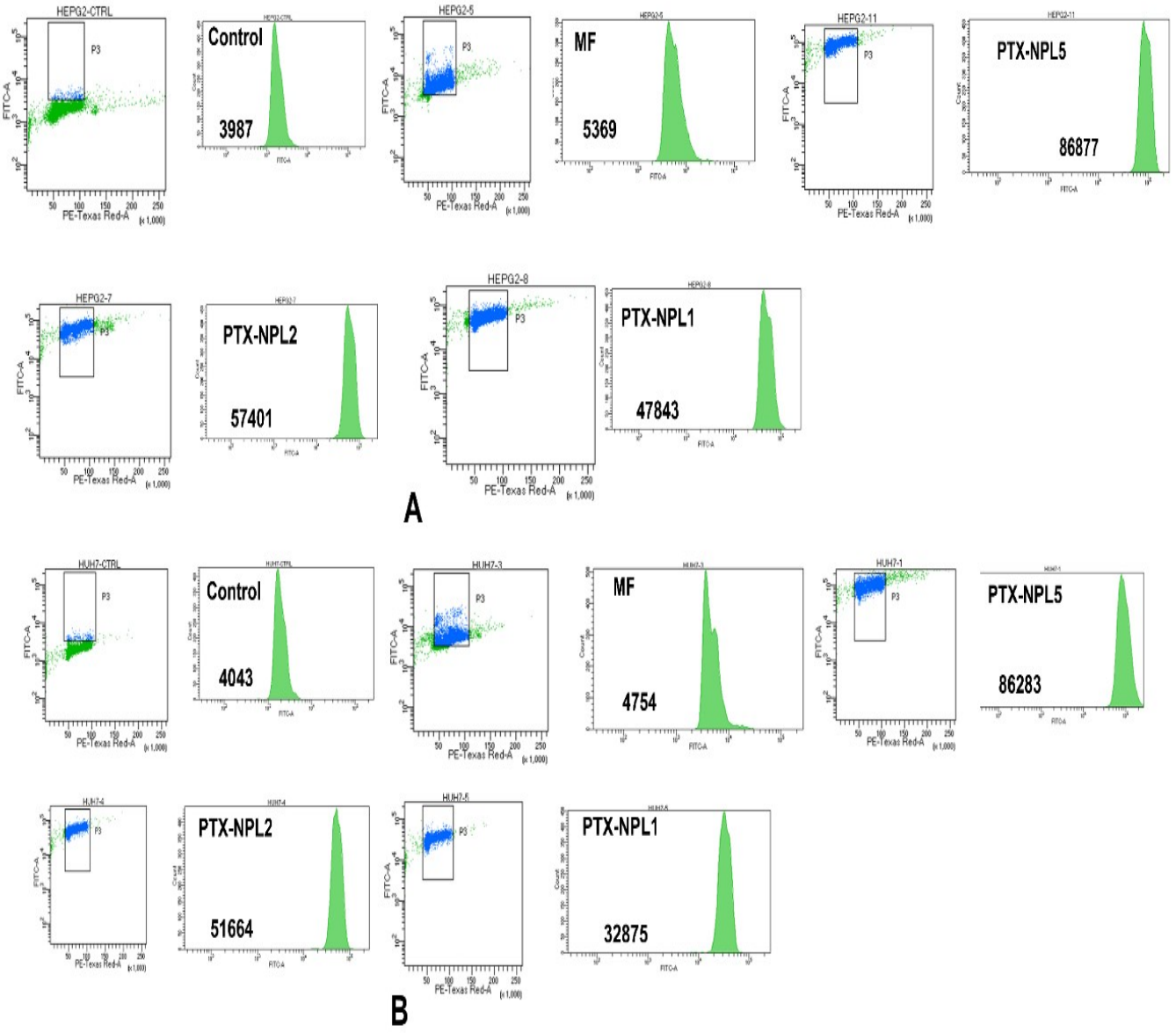

Figure S1 Quantitative determination of extent of DNA fragmentations measured by Apo-BrdU DNA fragmentation assay kit (A) in HepG2 and (B) in Huh-7 cells respectively upon treatment with MF/PTX-NPL5/PTX-NPL2/PTX-NPL1. Numbers in each block denotes the levels of fluorescent signals. Control cells depicts cells without treatment and various treatments are shown for cells treated with various experimental formulations as mentioned in each block. Rise in fluorescent signal directly proportional to DNA strand-breaks which in turn signify DNA fragmentation.

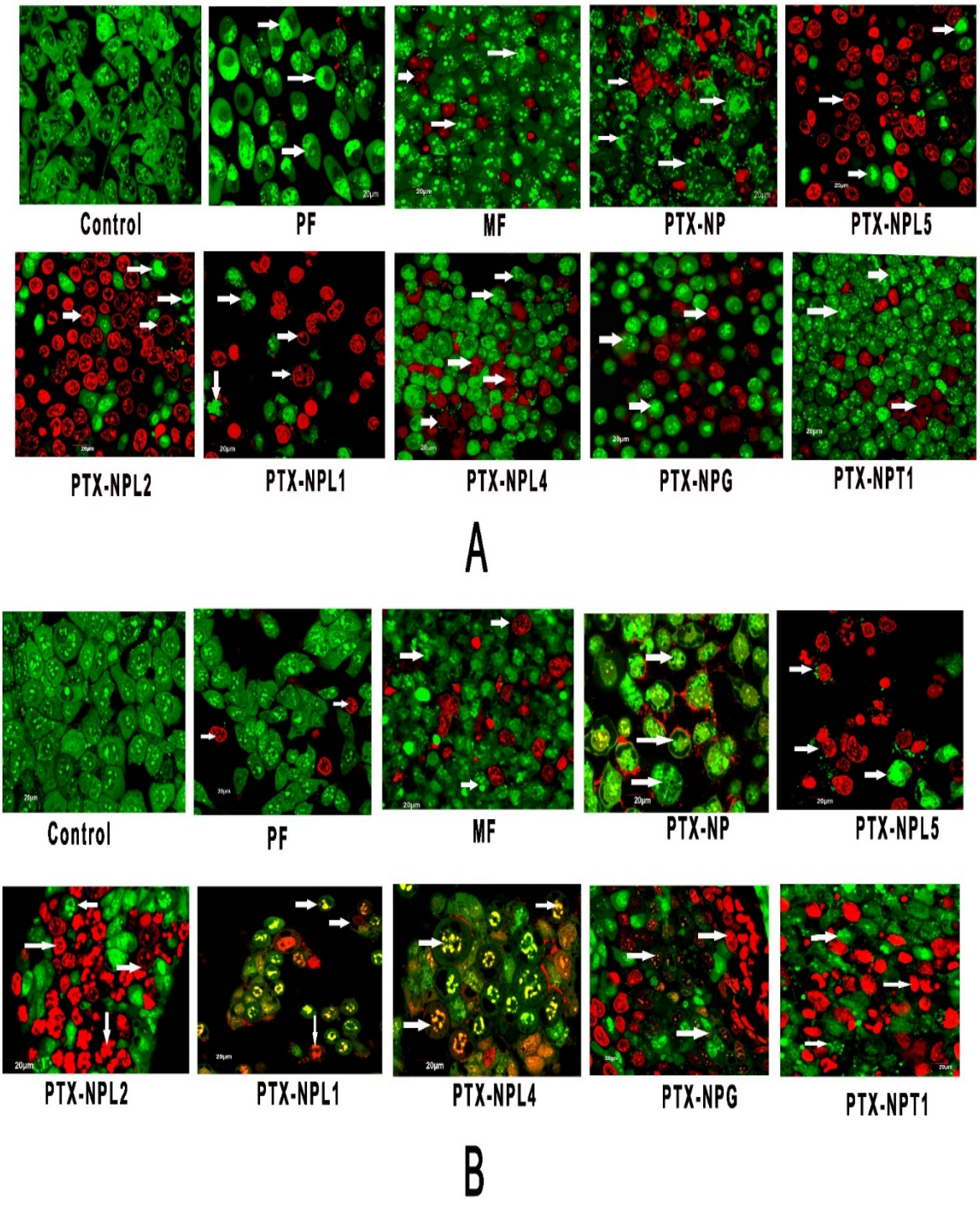

Figure S2 Morphological changes in (A) HepG2 cells and (B) Huh-7 cells respectively, treated with different treatments as mentioned under each image. Cells without treatment considered as control. Morphological changes are indicated by arrowheads. (Scale bar - 20µm)

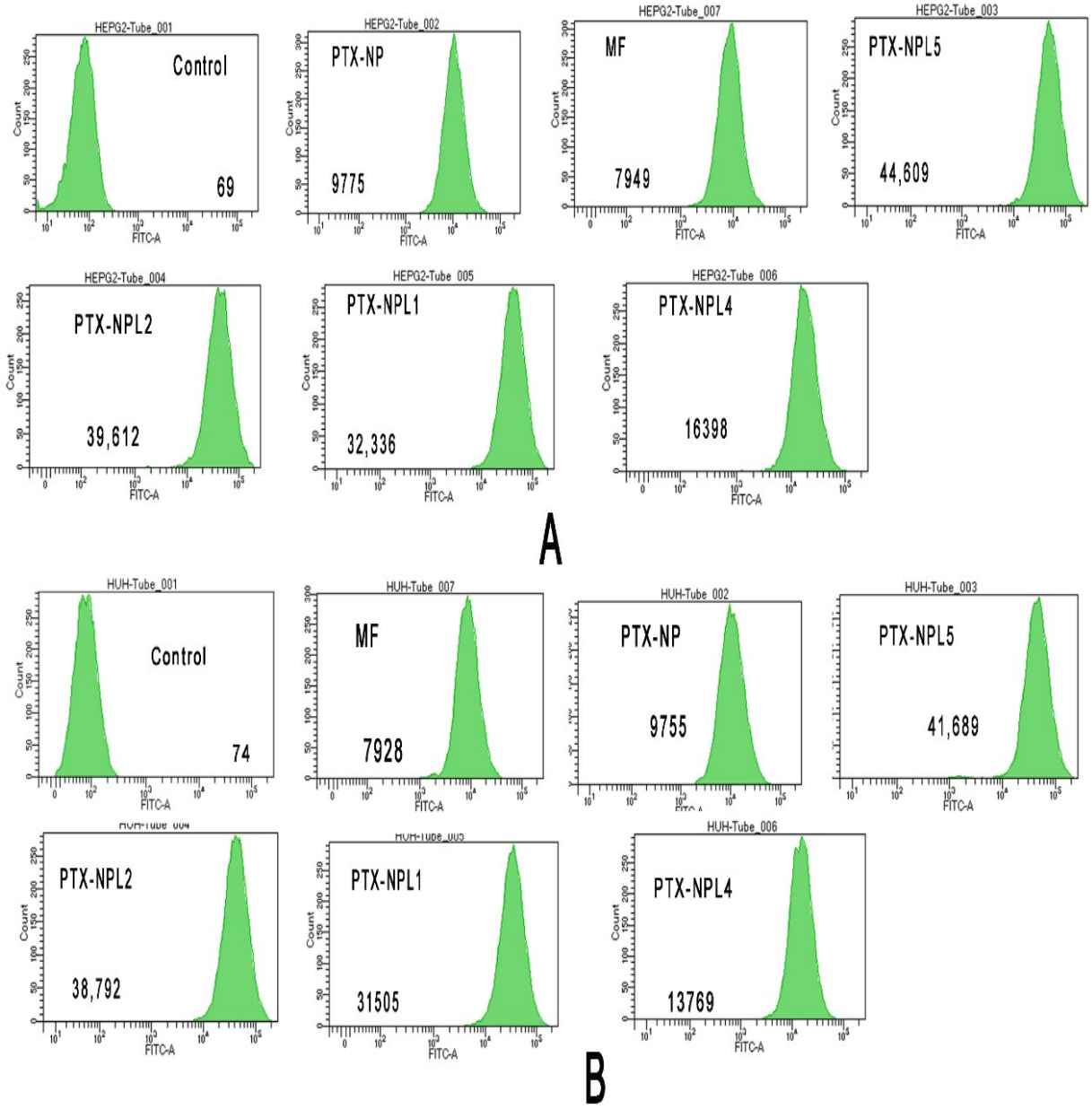

Figure S3 Levels of reactive oxygen species (ROS) (as denoted by numbers), (A) in HepG2 and (B) in Huh7 cells upon treatment with different experimental formulations as mentioned in each block. Cells without treatment considered as control.

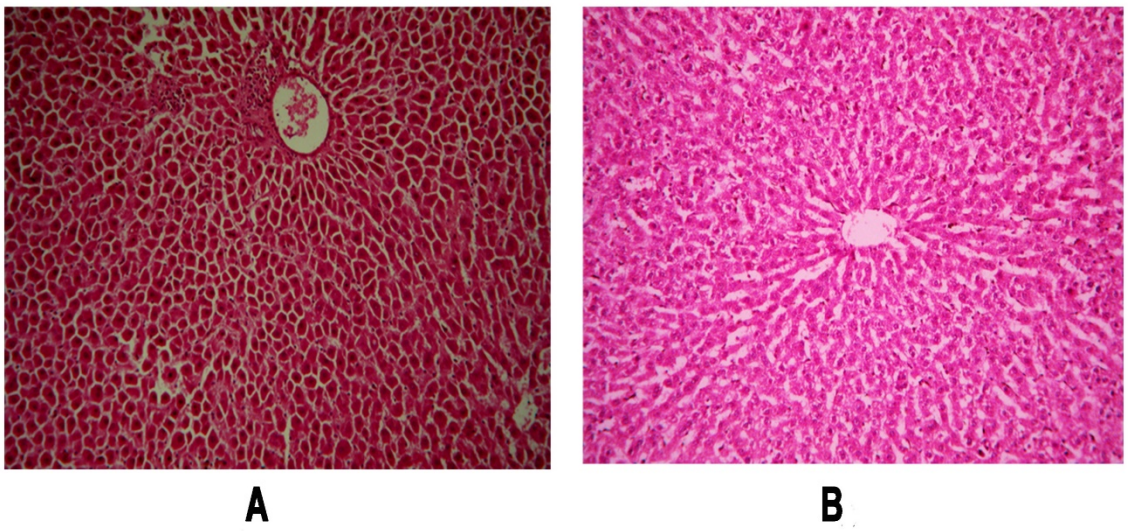

Figure S4 Histopatological examination of liver of normal rats upon treatment with PTX-NPL5. A. Image of liver of normal (control) rats, at 100 X magnification upon H&E staining B. image of liver of normal rats at 28^{th} day upon treatment with PTX-NPL5, at 100 X magnification upon H&E staining. No distinctive changes in liver architecture was observed, indicating its extremely low or no-toxicity against normal hepatocytes.

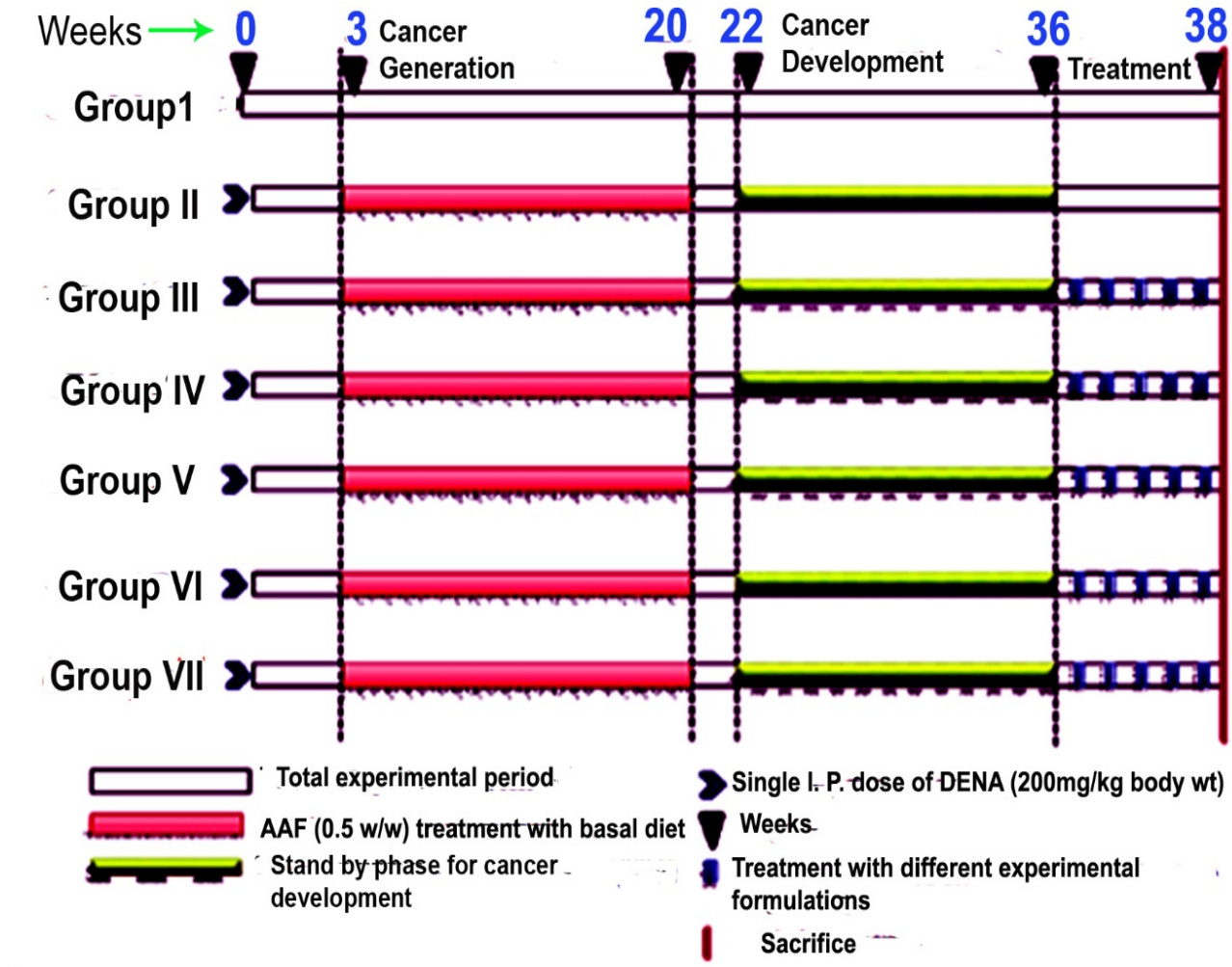

Figure S5 Scheme for induction of chemically-induced hepatocarcinogenesis in rats. Group-I (normal control rats), Group-II (carcinogen control rats), Group-III (carcinogen treated rats received MF), Group-IV (Carcinogen treated rats received PTX-NPL5), Group –V(carcinogen treated rats received PTX-NP), Group VI (carcinogen treated rats received PTX-NPL2), Group VII(carcinogen treated rats received PF)

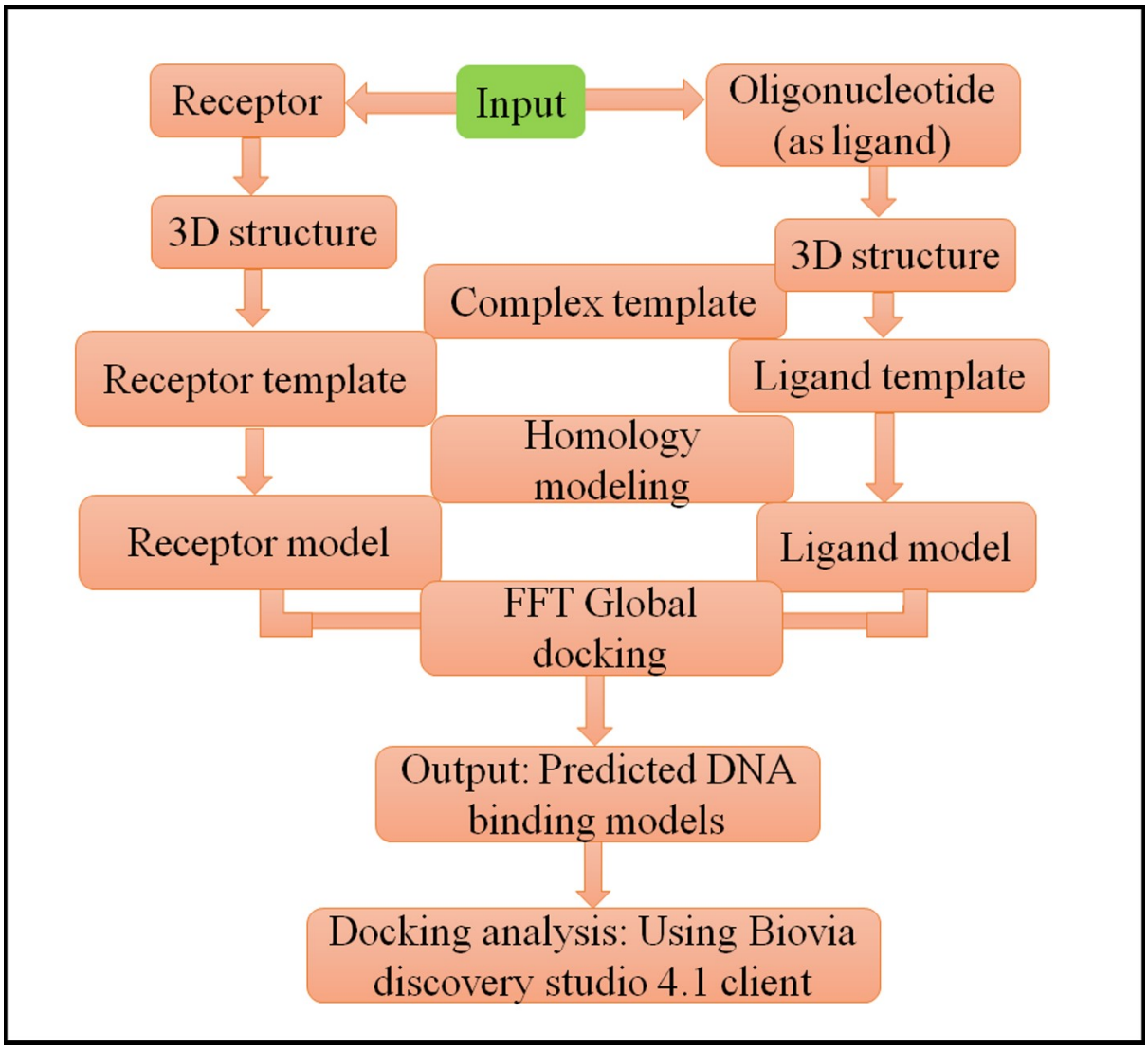

Figure S6 Schematic representation of blind docking protocol to analyse ligand-receptor interaction of L2 and L5

Table S1 Physical characterizations of experimental nanoparticles

Name of the formulation	Average particle diameter (Z-average) (nm)	Polydispersity index	Zeta potential (mV)	Drug-loading (%)	Entrapment efficiency (%)
PTX-NP	181.5± 12.25	0.334	-10.7± 4.27	5.98±0.55	65.78±4.04
PTX-NPL1	217.9± 8.04	0.246	-15.0± 5.27	6.31±0.46	69.41± 1.65
PTX-NPL2	236.1±17.34	0.532	-17.7± 5.19	6.38±0.63	70.18±2.14
PTX-NPL3	229.6±15.44	0.459	-17.2± 3.16	6.05±0.43	66.55 ±2.07
PTX-NPL4	220.2± 18.25	0.323	-16.3±3.71	6.14±0.37	67.54±4.18
PTX-NPL5	211.9±13.43	0.285	15.6± 4.06	6.45± 0.45	70.95±3.69
PTX-NPG	240.9±17.09	0.356	-14.6± 3.71	6.35±0.62	69.85± 2.76
PTX-NPT1	242.4±19.67	0.329	-13.0±5.46	6.23±0.53	68.53±3.19

[a]Data show mean ± SD (n=3)

Table S2 IC_{50} doses and % inhibition in various cancerous and normal cell types, upon the treatment of experimental formulations, free-drug suspension (PF) and commercial formulation (MF)

Treatment Groups	IC_{50} dose in HepG2 cells (nM)	IC_{50}dose in Huh-7 cells (nM)[a]	% inhibition in Chang liver cells[a]	% inhibition in WRL-68 cell
PF	995.76 ± 3.2 [a]	989.16 ± 6.43	63.41 ± 5.88 [a]	64.51± 4.08
PTX-NP	194.71 ± 6.23	198.01 ± 4.45	8.57 ± 2.50	8.89 ± 3.50
PTX-NPL1	73.61 ± 5.68	75.48 ± 3.84	9.25 ± 2.75	9.83 ± 2.28
PTX-NPL2	64.54 ± 5.21	67.82 ± 6.34	9.65 ± 3.68	9.7 ± 4.91
PTX-NPL3	192.61 ± 5.71	194.28 ± 5.45	8.62 ± 3.59	8.72 ± 2.75
PTX-NPL4	95.06 ± 5.48	98.27 ± 5.86	9.82 ± 3.94	9.58 ± 3.73
PTX-NPL5	42.87 ± 2.56	46.64 ± 6.48	8.04 ± 2.44	8.36 ± 2.91
PTX-NPT1	175.56 ± 5.68	178.19 ± 5.36	19.64 ± 4.61	19.2 ± 2.4
PTX-NPG	147.23 ± 6.45	152.99 ± 6.43	18.21 ± 3.1	19.81 ± 2.33
MF	380.06 ± 5.83	363.51± 4.51	71.85 ± 6.56	74.45 ± 3.98

[a]Data show mean ± standard deviation (n=3)

Table S3 Tumor incidences, numbers and size distribution of hepatic altered foci (HAF) and area of neoplastic lesions on rats-treated with hepatocarcinogen (carcinogen control rats) and carcinogen-treated rats treated with different experimental formulations

Groups	Numbers of tumor bearing rats/ total no. of rats	Size distribution of HAF (% of total no.)			Area of lesion (% of total hepatic area observed)
		<1mm	>1mm to <3mm	> 3mm	
Normal control rats	0/6	00	00	00	00
Carcinogen-control rats	6/6	16.07±1.16	40.65±.098	43.51±3.12	HAF = 83.23±3.02[a]
Carcinogen-treated rats treated with free-drug (PF)	6/6	16.38±1.32	41.01±1.15	42.61±2.67	HAF= 82.04±3.21
Carcinogen-treated rats treated with commercial (MF)	5/6	13.19±1.06*	48.53±2.27	38.28±2.19	HAF = 71.62±4.18
Carcinogen-treated rats treated with PTX-NP	4/6	32.45±3.62*	42.39±4.21*	25.14±4.01*	HAF= 52.43±2.33
Carcinogen-treated rats treated with PTX-NPL2	2/6	58.45±4.21*	26.87±3.86*	14.68±3.06*	HAF = 31.45±2.85
Carcinogen-control rats – treated with PTX-NPL5	0/6	82.47±3.48*	14.56±4.5*	3.08±0.056*	HAF = 19.23±3.53

Data show mean ± SD (n=6). "*" indicates $p<0.05$ when compared with data of carcinogen-control rats.

Table S4 Determination of body weight of rats belong to different to different groups used for in vivo study during the course of the experiment

Days/weeks	Normal-control Rats	Carcinogen-control rats	Carcinogen-treated rats treated with free-drug suspension (PF)	Carcinogen-treated rats treated with commercial formulation (MF)	Carcinogen-treated rats treated with PTX-NP	Carcinogen-treated rats treated with PTX-NPL2	Carcinogen-treated rats treated with PTX-NPL5
1st Day	141.2± 5.2[a, b]	144.4 ± 7.5	143.4 ± 6.8 [a, b]	148.7 ± 5.4	147.5 ± 4.6	146.6 ± 4.3	140.4 ± 5.8
7th day (1st week)	145.8 ± 6.3	143.7 ± 6.3	141.8 ± 9.2	146.5 ± 4.9	144.4 ± 6.6	144.4 ± 5.2	141.01 ± 4.7
2nd week	148.5 ± 6.5	142.4± 6.3	141.9 ± 3.9	145.2 ± 5.6	140.6 ± 3.7	142.2 ± 6.3	139.6 ± 5.2
3rd week	152.3 ± 4.6	140.8 ± 7.5*	139.7 ± 5.3*	144.4 ± 3.8*	139.1 ± 5.2*	140.7 ± 8.3*	140.2 ± 7.5*
4th week (First month)	156.4 ± 5.5	139.8 ± 4.8*	138.6 ± 6.4*	145.8 ± 5.3*	138.4 ± 3.5*	138.9 ± 6.2*	138.7 ± 8.1*
5th week	162.8 ± 6.3	138.5 ± 5.8*	138.1± 4.6*	143.9 ± 5.8*	137.6 ± 4.4*	136.6 ± 5.3*	137.2 ± 4.1*
6th week	170.4 ± 6.2	136.6 ± 5.9*	137.8 ± 3.6 *	142.3 ± 6.4*	136.8 ± 5.3*	136.5 ± 4.5*	136.8 ± 7.1*
7th week	178.6 ± 8.2	134.4 ± 10.2*	136.2 ± 5.8*	139.8 ± 4.4*	134.4 ± 5.2*	135.3 ± 7.8*	134.8 ± 3.4*
8th week (2nd Month)	186.4 ± 9.2	130.5 ± 5.5*	132.7 ± 8.6*	135.8 ± 6.8*	129.8 ± 4.8*	130.5 ± 5.6*	131.8 ± 6.5*
Third month	196 ± 5.8	127.3 ± 5.8*	129.8 ± 6.4*	128.8 ± 3.6*	126.6 ± 5.5*	127.8 ± 6.2*	128.6 ± *4.6
Fourth month	204.6 ± 5.7	125.8 ± 4.3*	126.4 ± 4.6	125.6 ± 4.8*	124.6 ± 6.4*	124.4 ± 5.1*	125.1 ± 3.7*
Fifth Month	212.3 ± 6.9	123.4 ± 5.8*	123.6 ± 5.8*	122.2 ± 5.8*	122.6 ± 7.4*	121.8 ± 4.8*	122.4 ± 6.5*
Sixth Month	220 ± 9.8	120.6 ± 5.8*	121.8 ± 5.8*	120.4 ± 8.2*	119.7 ± 5.1*	119.6 ± 7.4*	118.4 ± 4.8*
Seventh Month	225.7 ± 9.5	117.6± 5.8*	118.8 ± 4.7*	116.8 ± 7.6*	116.7 ± 5.8*	115.4 ± 5.2*	114.8 ± 3.6*
Eight month	229.6 ± 8.8	114.7 ± 4.5*	112.7± 4.7*	113.4 ± 5.5*	113.8 ± 4.8*	111.6 ± 5.6*	110.7 ± 4.4*
Nine month	235.6 ± 10.52	109.6 ± 3.5*	108.8 ± 5.3*	108.9 ± 6.2*	107.5 ± 4.2*	107.8 ± 3.3*	105.4 ± 5.1*
After treatment							
One day			110.4 ± 5.4	110.7 ± 5.8	112.3 ± 4.8	113.4 ± 3.7	111.3 ± 4.8
3rd day			111.4 ± 4.1	116.9 ± 4.7	117.1 ± 7.5	119.6 ± 8.4	120.8 ± 5.8
7th day			113.8 ± 3.2	118.6 ± 3.9#	122.4 ± 4.3#	125.6 ± 5.1#	126.8 ± 2.6#

10th Day	113.6 ± 6.8	119.8 ± 5.8#	130.6 ± 4.5#	133.6 ± 4.8#	134.8± 5.4#
14th Day	112.4 ± 7.2	118.8 ± 4.6#	132.8 ± 8.6#	136.6 ± 6.4#	139.8 ± 3.7#

[a]weight of the rats were expressed in gram, [b]data are expressed as mean ±SD (n=6). "*" indicates $p < 0.05$ when the weights of rats belong to carcinogen-control group and different treatments groups were compared withthe rats of normal control group before the treatment. "#" indicates $p < 0.05$ when the weights of rats of different treatments groups were compared with those of the rats carcinogen-control group.

Table S5 Levels of different biochemical parameters in liver of normal rats treated with different treatments

Group	SGPT (IU/l) ±SD(n=3)		SGOT (IU/l) ±SD(n=3)		ALK (IU/l) ±SD(n=3)	
	0 days	28 days	0 days	28 days	0 days	28 days
Control	43.73 ±1.51[a]	46.85 ±1.45	58.77±2.81	60.61± 2.09	191.32± 2.70	213.58± 1.57
PF	47.37±1.64*	64.03 ±1.59*	61.16±2.42	96.50± 1.15*	196.62± 3.90*	252.29± 3.70*
MF	49.22 ±1.00*	95.68 ±3.94*	62.31± 1.08*	122.71± 2.71*	195.56± 4.15*	294.03± 3.55*
PTX-NP	41.27 ±1.01	57.43 ±1.02*	54.96± 3.88	75.30± 3.09*	194.89± 2.17*	225.99± 2.13*
PTX-NP L2	41.57 ±1.53	51.91± 1.16	56.66± 3.08	72.69± 3.71*	196.01± 2.93	227.11± 4.05*
PTX-NP L5	40.28 ±1.07	49.62± 1.46	55.24± 1.18	70.57± 1.70	192.10± 1.15	222.43± 3.92

[a] Data mean ± SD (n=6) ; "*"indicates $p<0.05$ with respect to the value of control group of rats

Table S6 Changes in bodyweight of normal rats–treated with different treatments

Days	Normal control rats	Normal control rats-treated with free-drug suspension (PF)	Normal control rats-treated with commercial formulation (MF)	Normal control rats-treated with PTX-NP	Normal control rats-treated with PTX-NPL2	Normal control rats-treated with PTX-NPL5
1st Day	140.5 ± 5.4[a, b]	139.8 ± 4.5[a, b]	141.4 ± 3.6	142.3 ±6.2	145 ± 6.5	143± 5.8
2nd Day	140.8 ± 4.8	135.2 ± 5.2*	129.8 ± 4.4*	140± 5.6	143 ±4.8	142± 3.5
3rd Day	141.4 ± 4.4	126.5 ± 3.4*	117.6 ±5.9*	138.6 ± 4.8	142 ±5.1	140 ± 3.8
4TH day	143.6 ± 6.4	115 ± 7.6*	102.6 ± 4.8*	137.4 ± 5.5	140.8 ±2.8	139.4 ± 4.4
7th day	144.8 ± 4.5	108 ± 5.6*	90.5 ± 5.4*	138.5 ±3.2	141.5 ± 4.8	140.4 ± 6.6
14th day	146.2 ± 6.5	98.9 ± 4.4*	75.3 ± 3.8*	137.4 ±5.8	140.4 ± 6.4	140.8 ±3.6
21st day	147.8 ± 5.8	90.5 ± 6.3*	62.5 ± 4.5*	138.7 ±2.8	141.3 ±5.7	141.4 ±5.4
28th day	149.2 ±4.3	79.7 ± 2.6*	48.7 ± 6.4*	137.3 ± 5.5	139.8 ± 4.2	140.6 ±4.3

[a] weight of rats were expressed in gram, [b]data were expressed as mean ± SD (n=6). Statistical level of significance is indicated as "*"($p<0.05$), when the data of the treatment groups were compared with those of the normal (control) rats.

Table S7: Concentration of PTX (ng/g liver) in normal SD rats upon i.v. administration of experimental formulations

Time (h)	Upon administration of free-drug (PF)	Upon administration of commercial formulation (MF)	Upon administration of PTX-NP	Upon administration of PTX-NPL2	Upon administration of PTX-NPL5
1	97.17 ± 2.34[*]	230.16 ± 8.21[c]	116.12± 2.56[b,e]	120.45± 3.28[c,e]	118.24± 4.45[b,e]
2	219.45 ± 4.13	328.12 ± 2.19[c]	198.12± 6.28[b,e]	203.34± 3.48[a,e]	201.24± 6.67[b,e]
4	267.71± 9.82	431.25 ± 7.73[c]	231.19± 4.21[c,e]	235.72± 2.17[c,e]	233.57± 5.13[c,e]
6	92.74 ± 7.48	418. 25 ± 5.45[c]	320.16± 4.13[c,e]	322.68± 5.32[c,e]	325.48± 2.26[c,e]
8	18.83 ± 3.86	226.27 ± 4.32[c]	202.37± 2.62[c,e]	205.81± 4.18[c,e]	208.41± 3.19[c,d]
10	BLQ	130.48 ± 6.18	104.16± 5.13	106.38 ± 3.18	108.51± 4.82
24	BLQ	56.57 ± 3.28	44.82 ± 7.32	46.58 ± 8.23	42.34 ± 5.21
48	BLQ	26.12 ± 4.38	14.47 ± 5.29[d]	15.65 ± 4.15[d]	13.46 ± 7.14[d]

[*]Data show mean ± SD (n=3). [(a,b,c,d,e)]Significant difference when compared to the data obtained upon free-drug treatment ([a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$), commercial formulation treatment ([d]$p<0.01$, [e]$p<0.001$).

BLQ denotes below the quantification limit (<2ng/ml)

Table S8: Concentration of PTX (ng/g of liver) in carcinogen-treated SD rats upon i.v. administration of experimental formulations.

Time (h)	Upon administration of free-drug (PF)	Upon administration of commercial formulation (MF)	Upon administration of PTX-NP	Upon administration of PTX-NPL2	Upon administration of PTX-NPL5
1	94.26 ± 4.12*	141.82 ± 3.58[a]	169.41± 5.14[a,b]	234.13 ± 4.17[a,b,c]	249.42 ± 5.35[a,b,c]
2	215.61 ± 5.32	342.56 ± 5.16[a]	239.71± 4.22[a,b]	398.41± 4.23[a,b,c]	418.12 ± 5.28[a,b,c]
4	261.84± 3.59	451.78 ± 3.74[a]	272.56± 3.39[a,b]	528.81± 7.24[a,b,c]	563.19 ± 3.20[a,b,c]
6	96.88± 4.84	429.17 ± 4.77[a]	471.91± 7.87[a,b]	702.76± 3.36[a,b,c]	728.26 ± 4.69[a,b,c]
8	32.18± 6.71	232.61 ± 5.16[a]	335.63±3.38[a,b]	615.57± 7.20[a,b,c]	641.56 ± 7.89[a,b,c]
10	11.78 ± 5.43	145.27 ± 2.29[a]	153.69± 4.27[a,b]	414.53± 5.49[a,b,c]	436.18 ± 6.43[a,b,c]
24	4.12 ± 1.68	66.41 ± 4.52[a]	107.18± 6.17[a,b]	245.26± 5.23[a,b,c]	268.93 ± 4.87[a,b,c]
48	BLQ	12.62 ± 9.12	51.29± 2.28[a,b]	132.65 ± 4.15[a,b,c]	156.45 ± 2.97[a,b,c]

*Data show mean ± SD (n=3). [(a,b,c)]Significant difference when compared to the data obtained upon free-drug treatment ([a]$p<0.01$), commercial formulation treatment ([b]$p<0.01$) and PTX-NP treatment ([c]$p<0.01$).

BLQ denotes below the quantification limit (<2ng/ml)

Table S9 Docking results of 39 and 63 base pair oligonucleotides with surface biomarker proteins of neoplastic hepatocytes.

S. No	Name of Protein	PDB ID	(-) Docking Score	Interacting Residues	Interactions
			Oligonucleotide with 39 base pairs		
1	Tumor Associated Glycoprotein 72 (TAG-72)	6bsb	227.82	ALA: 1106, PHE: 1042, LEU: 1089, THR: 1104, PHE: 1146, ASN: 1091, ASP: 1143, SER: 1142, PHE: 1044, SER: 1137, ASP: 1138, HIS: 1138, GLU: 1118, ASN: 1122 and LYS: 1125	Hydrogen bonding (classical and non-classical) interactions, Salt bridge, Attractive charges, π-Anion, π-π-T-shaped, π-πstacked and π-sigma and π-alkyl
2	Heat shock protein-70 (HSP- 70)	6do2	235.68	LYS: 163, THR: 29, LYS: 46, ASN: 47, GLY: 407, LEU: 405, ASP: 26, VAL: 27, MET: 196, ARG: 49, GLY: 40, GLU: 51, TYR: 160, SER: 406, LYS: 185, ILE: 199, ILE: 198, ARG: 197, LYS: 213, GLY: 240	Hydrogen bonding (classical and non-classical) interactions, Salt bridge, attractive charges, π-π stacked, π-alkyl, π-sigma
			Oligonucleotide with 63 base pairs		
1	Tumor Associated Glycoprotein 72 (TAG-72)	6bsb	205.23	HIS: 1048, ASP: 1138, SER: 1140, SER: 1046, GLN: 1102, SER: 1074, ASN: 1091, GLY: 1088, LEU: 1087, ARG: 1108, PHE: 1044, SER: 1142, VAL: 1144 and PHE: 1146	Hydrogen bonding (classical and non-classical) interactions, Attractive charges, π-π-T shaped, π-πstacked, π-sigma

2	Heat shock protein-70 (HSP- 70)	6do2	230.46	MET: 196, VAL: 27, ASP: 26, GLY: 407, ASN: 47, LYS: 46, TYR: 160, GLU: 51, LYS: 46, ARG: 49, SER: 406, THR: 29, GLN: 401, ILE: 207, LYS: 163, ILE: 198, ILE: 199, ARG: 197, GLY: 204LYS: 213, LYS: 185	Hydrogen bonding (classical and non-classical) interactions, Salt bridge, attractive charges, π-cation, π-πstacked, π-alkyl, π-sigma, π-Sulphur