SUPPLEMENTARY MATERIAL

Polymeric Nanoparticles as Cancer-specific DNA Delivery Vectors to Human Hepatocellular Carcinoma

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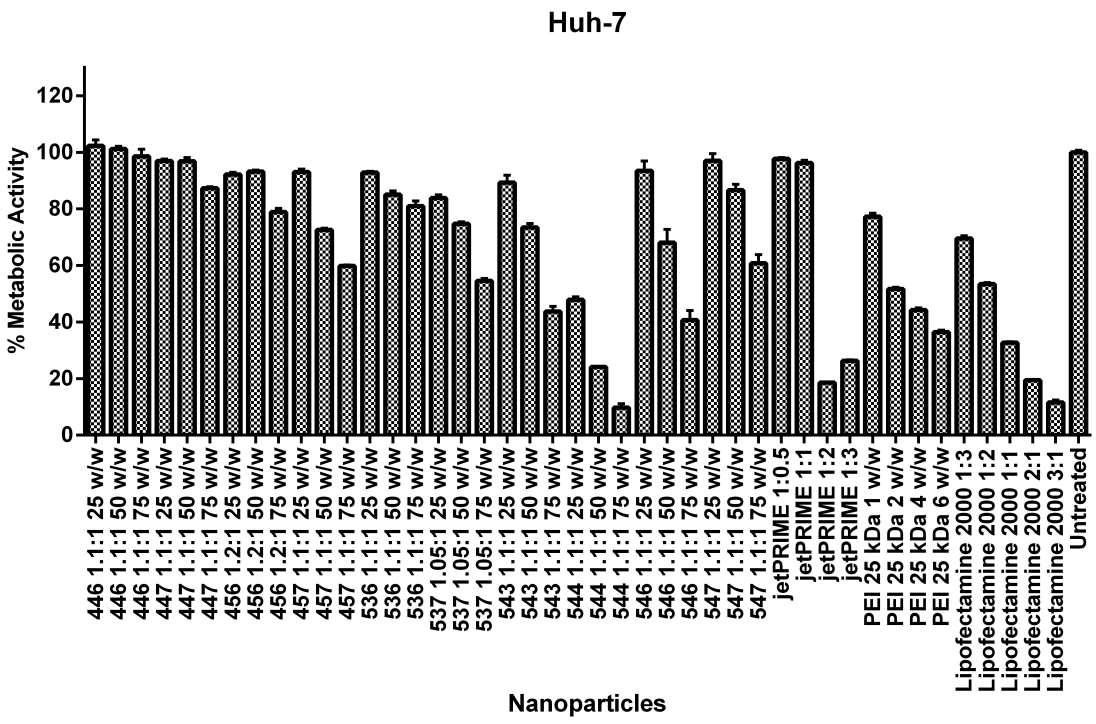
Polymer	B:S Ratio	M_n (Da)	M_w (Da)	PDI
446	1.1:1	3382	4936	1.46
447	1.1:1	4938	11868	2.40
456	1.2:1	3622	5124	1.41
457	1.1:1	7812	16679	2.13
536	1.1:1	4637	8229	1.77
537	1.05:1	4361	9211	2.11
543	1.1:1	5564	11216	2.02
544	1.1:1	6529	14051	2.15
546	1.1:1	4114	7278	1.77
547	1.1:1	6700	14796	2.21

Supplemental Fig. 1 GPC characterization of all PBAE polymers screened in this study. The results presented are relative to monodisperse polystyrene standards. The samples were diluted at 20 mg/mL in a solution of 95% THF, 5% DMSO and 0.1 M piperidine and run at 0.5 mL/min in a Waters 2414 Refractive Index Detector System with 3 Styragel Columns in series (7.8 × 300 mm; HR 1, HR 3, and HR 4), kept at 40ºC. M_n: number average, M_w: weight average, PDI: polydispersity.


Huh-7
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40
60
80
100
120
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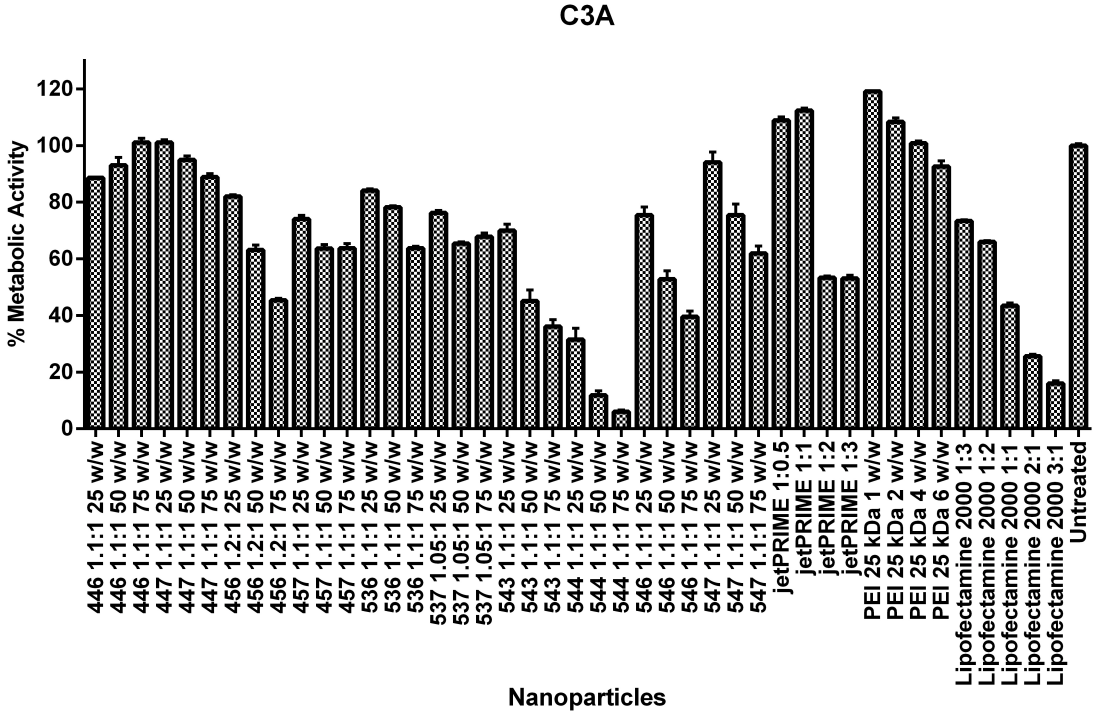
446 1.1:1 50 w/w
446 1.1:1 75 w/w
447 1.1:1 25 w/w
447 1.1:1 50 w/w
447 1.1:1 75 w/w
456 1.2:1 25 w/w
456 1.2:1 50 w/w
456 1.2:1 75 w/w
457 1.1:1 25 w/w
457 1.1:1 50 w/w
457 1.1:1 75 w/w
536 1.1:1 25 w/w
536 1.1:1 50 w/w
536 1.1:1 75 w/w
537 1.05:1 25 w/w
537 1.05:1 50 w/w
537 1.05:1 75 w/w
543 1.1:1 25 w/w
543 1.1:1 50 w/w
543 1.1:1 75 w/w
544 1.1:1 25 w/w
544 1.1:1 50 w/w
544 1.1:1 75 w/w
546 1.1:1 25 w/w
546 1.1:1 50 w/w
546 1.1:1 75 w/w
547 1.1:1 25 w/w
547 1.1:1 50 w/w
547 1.1:1 75 w/w
jetPRIME 1:0.5
jetPRIME 1:1
jetPRIME 1:2
jetPRIME 1:3
PEI 25 kDa 1 w/w
PEI 25 kDa 2 w/w
PEI 25 kDa 4 w/w
PEI 25 kDa 6 w/w
Lipofectamine 2000 1:3
Lipofectamine 2000 1:2
Lipofectamine 2000 1:1
Lipofectamine 2000 2:1
Lipofectamine 2000 3:1
Untreated
Nanoparticles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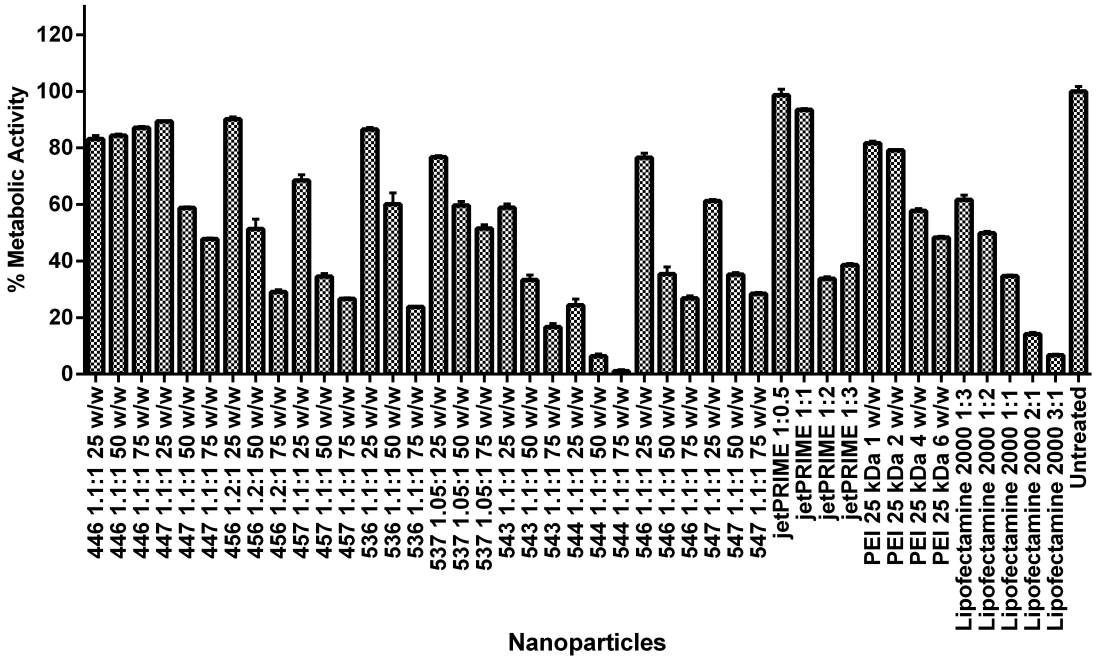
447 1.1:1 75 w/w
456 1.2:1 25 w/w
456 1.2:1 50 w/w
456 1.2:1 75 w/w
457 1.1:1 25 w/w
457 1.1:1 50 w/w
457 1.1:1 75 w/w
536 1.1:1 25 w/w
536 1.1:1 50 w/w
536 1.1:1 75 w/w
537 1.05:1 25 w/w
537 1.05:1 50 w/w
537 1.05:1 75 w/w
543 1.1:1 25 w/w
543 1.1:1 50 w/w
543 1.1:1 75 w/w
544 1.1:1 25 w/w
544 1.1:1 50 w/w
544 1.1:1 75 w/w
546 1.1:1 25 w/w
546 1.1:1 50 w/w
546 1.1:1 75 w/w
547 1.1:1 25 w/w
547 1.1:1 50 w/w
547 1.1:1 75 w/w
jetPRIME 1:0.5
jetPRIME 1:1
jetPRIME 1:2
jetPRIME 1:3
PEI 25 kDa 1 w/w
PEI 25 kDa 2 w/w
PEI 25 kDa 4 w/w
PEI 25 kDa 6 w/w
Lipofectamine 2000 1:3
Lipofectamine 2000 1:2
Lipofectamine 2000 1:1
Lipofectamine 2000 2:1
Lipofectamine 2000 3:1
Untreated
Nanoparticles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100
120
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447 1.1:1 50 w/w
447 1.1:1 75 w/w
456 1.2:1 25 w/w
456 1.2:1 50 w/w
456 1.2:1 75 w/w
457 1.1:1 25 w/w
457 1.1:1 50 w/w
457 1.1:1 75 w/w
536 1.1:1 25 w/w
536 1.1:1 50 w/w
536 1.1:1 75 w/w
537 1.05:1 25 w/w
537 1.05:1 50 w/w
537 1.05:1 75 w/w
543 1.1:1 25 w/w
543 1.1:1 50 w/w
543 1.1:1 75 w/w
544 1.1:1 25 w/w
544 1.1:1 50 w/w
544 1.1:1 75 w/w
546 1.1:1 25 w/w
546 1.1:1 50 w/w
546 1.1:1 75 w/w
547 1.1:1 25 w/w
547 1.1:1 50 w/w
547 1.1:1 75 w/w
jetPRIME 1:0.5
jetPRIME 1:1
jetPRIME 1:2
jetPRIME 1:3
PEI 25 kDa 1 w/w
PEI 25 kDa 2 w/w
PEI 25 kDa 4 w/w
PEI 25 kDa 6 w/w
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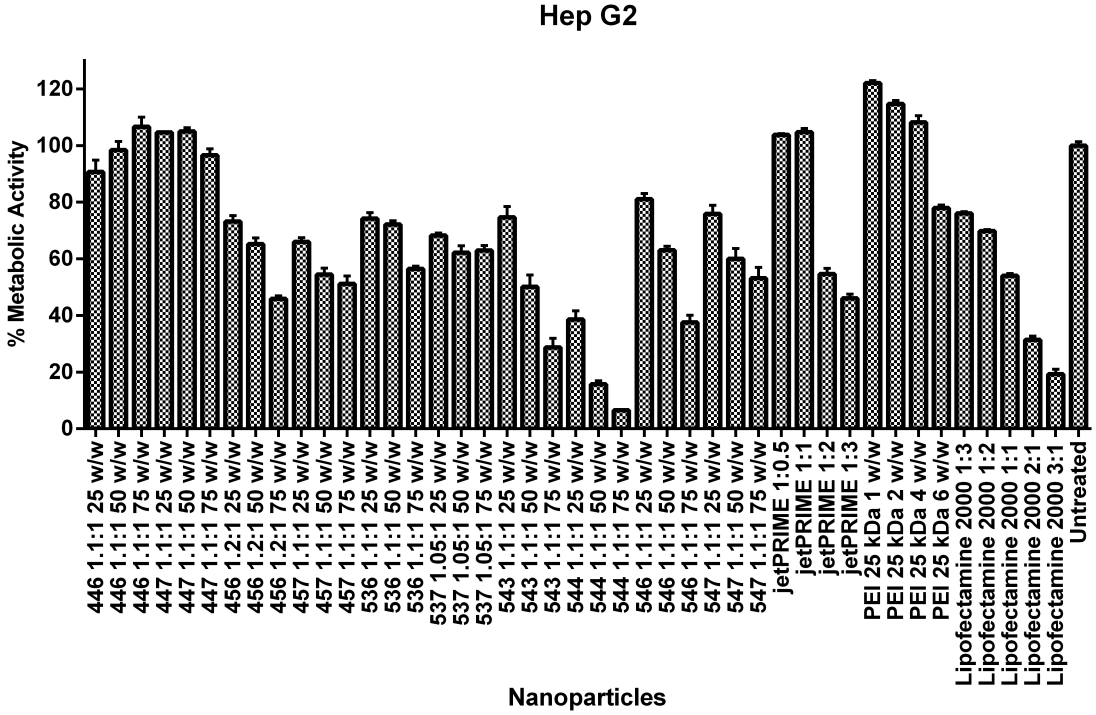
Lipofectamine 2000 1:2
Lipofectamine 2000 1:1
Lipofectamine 2000 2:1
Lipofectamine 2000 3:1
Untreated
Nanoparticles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60
80
100
120
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446 1.1:1 50 w/w
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447 1.1:1 25 w/w
447 1.1:1 50 w/w
447 1.1:1 75 w/w
456 1.2:1 25 w/w
456 1.2:1 50 w/w
456 1.2:1 75 w/w
457 1.1:1 25 w/w
457 1.1:1 50 w/w
457 1.1:1 75 w/w
536 1.1:1 25 w/w
536 1.1:1 50 w/w
536 1.1:1 75 w/w
537 1.05:1 25 w/w
537 1.05:1 50 w/w
537 1.05:1 75 w/w
543 1.1:1 25 w/w
543 1.1:1 50 w/w
543 1.1:1 75 w/w
544 1.1:1 25 w/w
544 1.1:1 50 w/w
544 1.1:1 75 w/w
546 1.1:1 25 w/w
546 1.1:1 50 w/w
546 1.1:1 75 w/w
547 1.1:1 25 w/w
547 1.1:1 50 w/w
547 1.1:1 75 w/w
jetPRIME 1:0.5
jetPRIME 1:1
jetPRIME 1:2
jetPRIME 1:3
PEI 25 kDa 1 w/w
PEI 25 kDa 2 w/w
PEI 25 kDa 4 w/w
PEI 25 kDa 6 w/w
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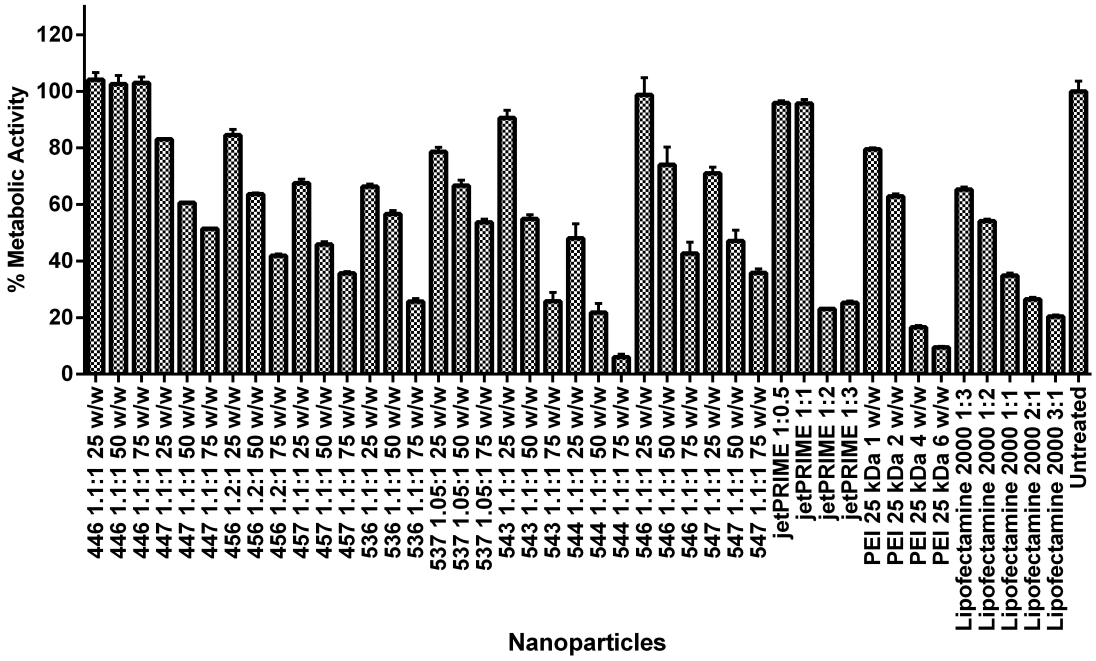
Lipofectamine 2000 1:2
Lipofectamine 2000 1:1
Lipofectamine 2000 2:1
Lipofectamine 2000 3:1
Untreated
Nanoparticles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60
80
100
120
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446 1.1:1 50 w/w
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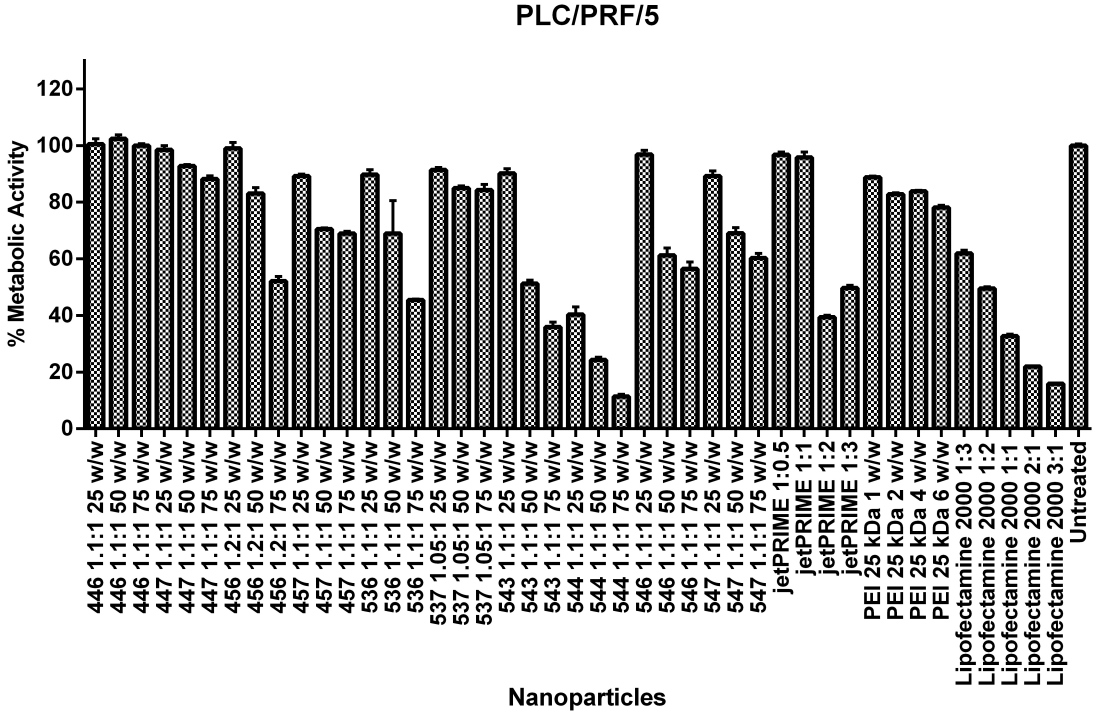
447 1.1:1 25 w/w
447 1.1:1 50 w/w
447 1.1:1 75 w/w
456 1.2:1 25 w/w
456 1.2:1 50 w/w
456 1.2:1 75 w/w
457 1.1:1 25 w/w
457 1.1:1 50 w/w
457 1.1:1 75 w/w
536 1.1:1 25 w/w
536 1.1:1 50 w/w
536 1.1:1 75 w/w
537 1.05:1 25 w/w
537 1.05:1 50 w/w
537 1.05:1 75 w/w
543 1.1:1 25 w/w
543 1.1:1 50 w/w
543 1.1:1 75 w/w
544 1.1:1 25 w/w
544 1.1:1 50 w/w
544 1.1:1 75 w/w
546 1.1:1 25 w/w
546 1.1:1 50 w/w
546 1.1:1 75 w/w
547 1.1:1 25 w/w
547 1.1:1 50 w/w
547 1.1:1 75 w/w
jetPRIME 1:0.5
jetPRIME 1:1
jetPRIME 1:2
jetPRIME 1:3
PEI 25 kDa 1 w/w
PEI 25 kDa 2 w/w
PEI 25 kDa 4 w/w
PEI 25 kDa 6 w/w
Lipofectamine 2000 1:3
Lipofectamine 2000 1:2
Lipofectamine 2000 1:1
Lipofectamine 2000 2:1
Lipofectamine 2000 3:1
Untreated
Nanoparticles


PLC/PRF/5
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40
60
80
100
120
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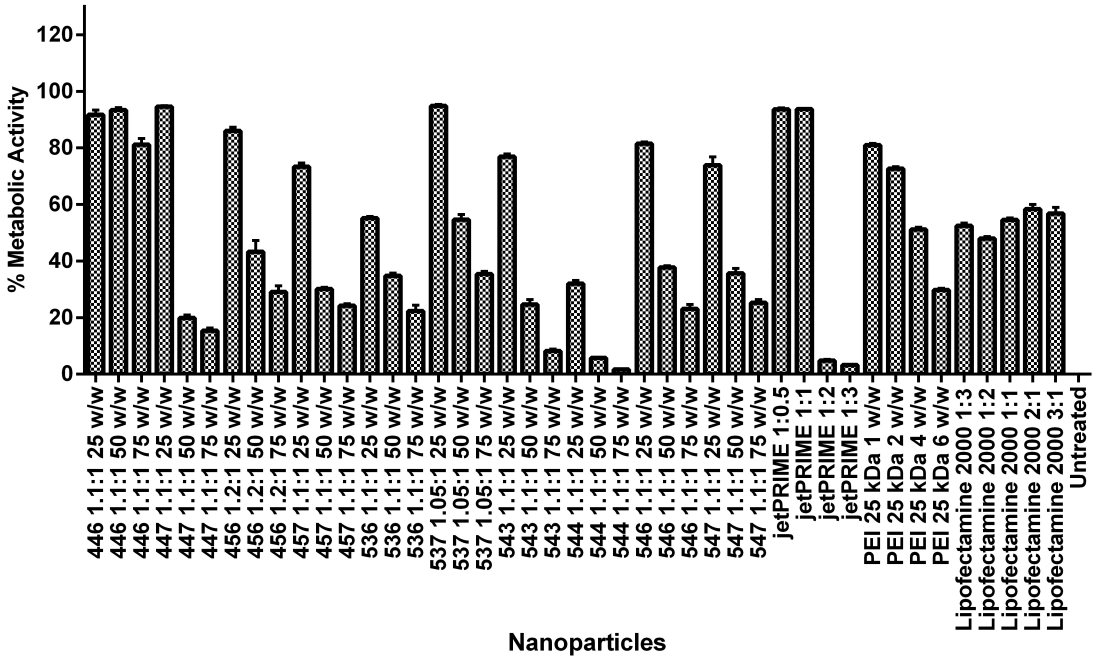
446 1.1:1 75 w/w
447 1.1:1 25 w/w
447 1.1:1 50 w/w
447 1.1:1 75 w/w
456 1.2:1 25 w/w
456 1.2:1 50 w/w
456 1.2:1 75 w/w
457 1.1:1 25 w/w
457 1.1:1 50 w/w
457 1.1:1 75 w/w
536 1.1:1 25 w/w
536 1.1:1 50 w/w
536 1.1:1 75 w/w
537 1.05:1 25 w/w
537 1.05:1 50 w/w
537 1.05:1 75 w/w
543 1.1:1 25 w/w
543 1.1:1 50 w/w
543 1.1:1 75 w/w
544 1.1:1 25 w/w
544 1.1:1 50 w/w
544 1.1:1 75 w/w
546 1.1:1 25 w/w
546 1.1:1 50 w/w
546 1.1:1 75 w/w
547 1.1:1 25 w/w
547 1.1:1 50 w/w
547 1.1:1 75 w/w
jetPRIME 1:0.5
jetPRIME 1:1
jetPRIME 1:2
jetPRIME 1:3
PEI 25 kDa 1 w/w
PEI 25 kDa 2 w/w
PEI 25 kDa 4 w/w
PEI 25 kDa 6 w/w
Lipofectamine 2000 1:3
Lipofectamine 2000 1:2
Lipofectamine 2000 1:1
Lipofectamine 2000 2:1
Lipofectamine 2000 3:1
Untreated
Nanoparticles


SNU-387
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80
100
120
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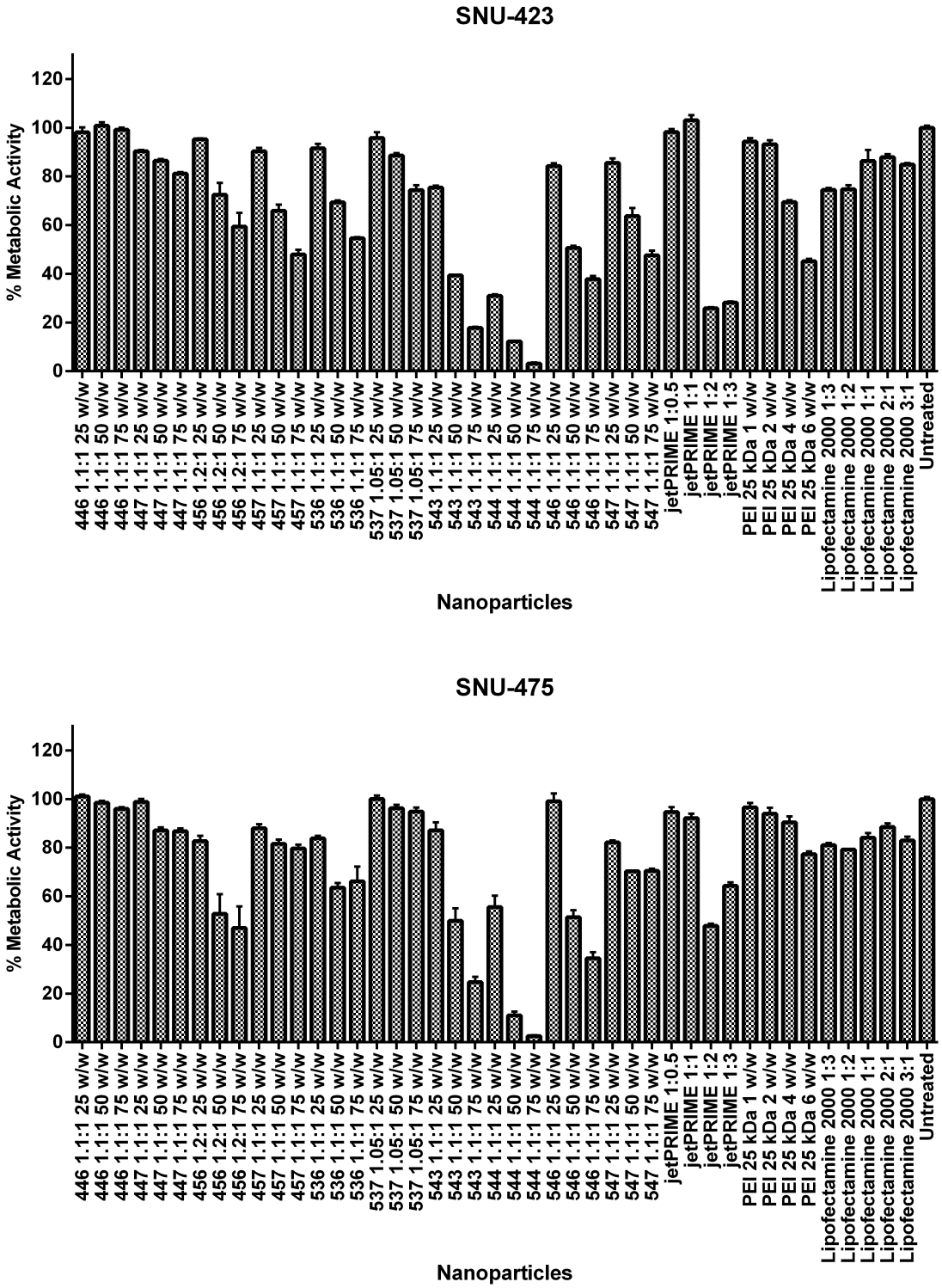
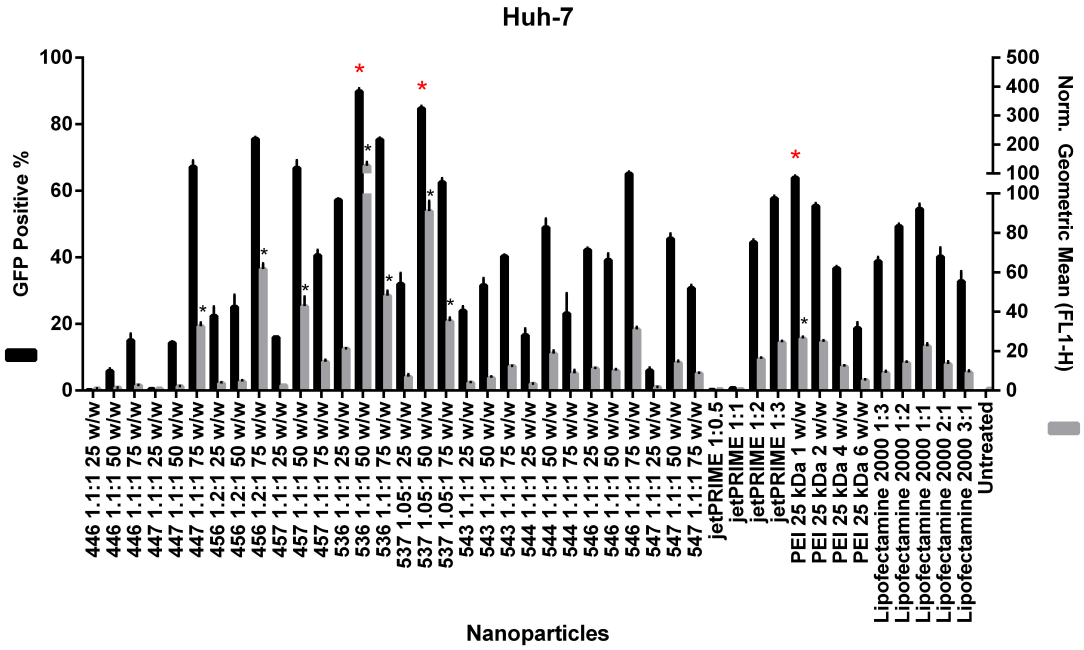
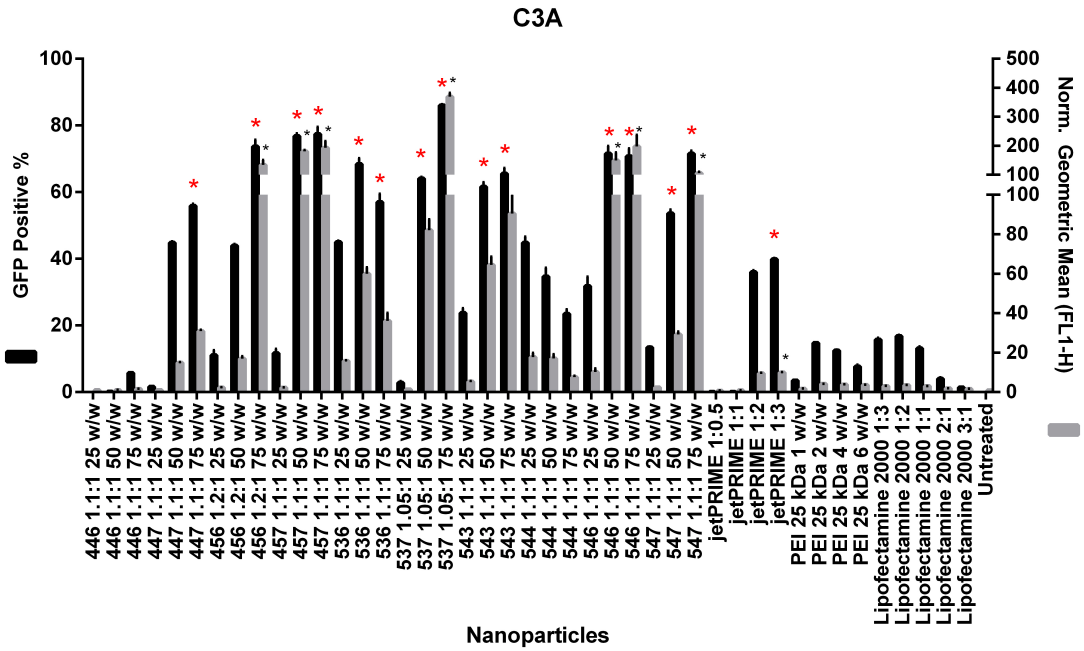
447 1.1:1 25 w/w
447 1.1:1 50 w/w
447 1.1:1 75 w/w
456 1.2:1 25 w/w
456 1.2:1 50 w/w
456 1.2:1 75 w/w
457 1.1:1 25 w/w
457 1.1:1 50 w/w
457 1.1:1 75 w/w
536 1.1:1 25 w/w
536 1.1:1 50 w/w
536 1.1:1 75 w/w
537 1.05:1 25 w/w
537 1.05:1 50 w/w
537 1.05:1 75 w/w
543 1.1:1 25 w/w
543 1.1:1 50 w/w
543 1.1:1 75 w/w
544 1.1:1 25 w/w
544 1.1:1 50 w/w
544 1.1:1 75 w/w
546 1.1:1 25 w/w
546 1.1:1 50 w/w
546 1.1:1 75 w/w
547 1.1:1 25 w/w
547 1.1:1 50 w/w
547 1.1:1 75 w/w
jetPRIME 1:0.5
jetPRIME 1:1
jetPRIME 1:2
jetPRIME 1:3
PEI 25 kDa 1 w/w
PEI 25 kDa 2 w/w
PEI 25 kDa 4 w/w
PEI 25 kDa 6 w/w
Lipofectamine 2000 1:3
Lipofectamine 2000 1:2
Lipofectamine 2000 1:1
Lipofectamine 2000 2:1
Lipofectamine 2000 3:1
Untreated
Nanoparticles



Supplemental Fig. 2 Viability of all HCC cell lines 24 hours after transfection with PBAE or positive control NPs.


Huh-7
GFP Positive %
Norm. Geometric Mean (FL1-H)
Nanoparticles
446 1.1:1 25 w/w
446 1.1:1 50 w/w
446 1.1:1 75 w/w
447 1.1:1 25 w/w
447 1.1:1 50 w/w
447 1.1:1 75 w/w
456 1.2:1 25 w/w
456 1.2:1 50 w/w
456 1.2:1 75 w/w
457 1.1:1 25 w/w
457 1.1:1 50 w/w
457 1.1:1 75 w/w
536 1.1:1 25 w/w
536 1.1:1 50 w/w
536 1.1:1 75 w/w
537 1.05:1 25 w/w
537 1.05:1 50 w/w
537 1.05:1 75 w/w
543 1.1:1 25 w/w
543 1.1:1 50 w/w
543 1.1:1 75 w/w
544 1.1:1 25 w/w
544 1.1:1 50 w/w
544 1.1:1 75 w/w
546 1.1:1 25 w/w
546 1.1:1 50 w/w
546 1.1:1 75 w/w
547 1.1:1 25 w/w
547 1.1:1 50 w/w
547 1.1:1 75 w/w
jetPRIME 1:0.5
jetPRIME 1:1
jetPRIME 1:2
jetPRIME 1:3
PEI 25 kDa 1 w/w
PEI 25 kDa 2 w/w
PEI 25 kDa 4 w/w
PEI 25 kDa 6 w/w
Lipofectamine 2000 1:3
Lipofectamine 2000 1:2
Lipofectamine 2000 1:1
Lipofectamine 2000 2:1
Lipofectamine 2000 3:1
Untreated


C3A
GFP Positive %
Norm. Geometric Mean (FL1-H)
Nanoparticles
446 1.1:1 25 w/w
446 1.1:1 50 w/w
446 1.1:1 75 w/w
447 1.1:1 25 w/w
447 1.1:1 50 w/w
447 1.1:1 75 w/w
456 1.2:1 25 w/w
456 1.2:1 50 w/w
456 1.2:1 75 w/w
457 1.1:1 25 w/w
457 1.1:1 50 w/w
457 1.1:1 75 w/w
536 1.1:1 25 w/w
536 1.1:1 50 w/w
536 1.1:1 75 w/w
537 1.05:1 25 w/w
537 1.05:1 50 w/w
537 1.05:1 75 w/w
543 1.1:1 25 w/w
543 1.1:1 50 w/w
543 1.1:1 75 w/w
544 1.1:1 25 w/w
544 1.1:1 50 w/w
544 1.1:1 75 w/w
546 1.1:1 25 w/w
546 1.1:1 50 w/w
546 1.1:1 75 w/w
547 1.1:1 25 w/w
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547 1.1:1 75 w/w
jetPRIME 1:0.5
jetPRIME 1:1
jetPRIME 1:2
jetPRIME 1:3
PEI 25 kDa 1 w/w
PEI 25 kDa 2 w/w
PEI 25 kDa 4 w/w
PEI 25 kDa 6 w/w
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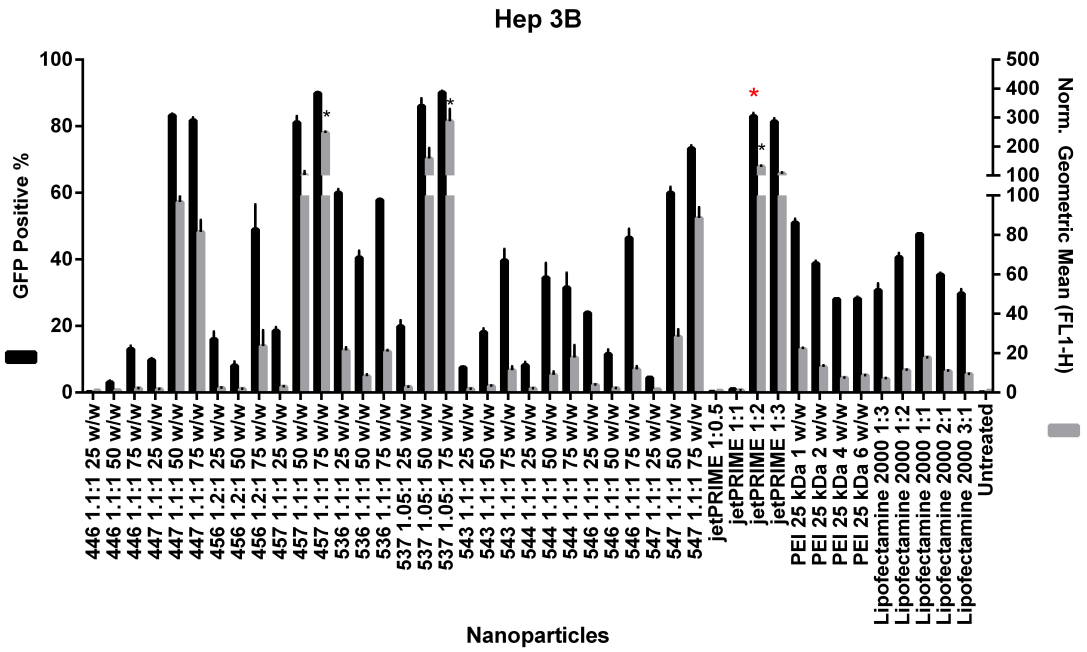
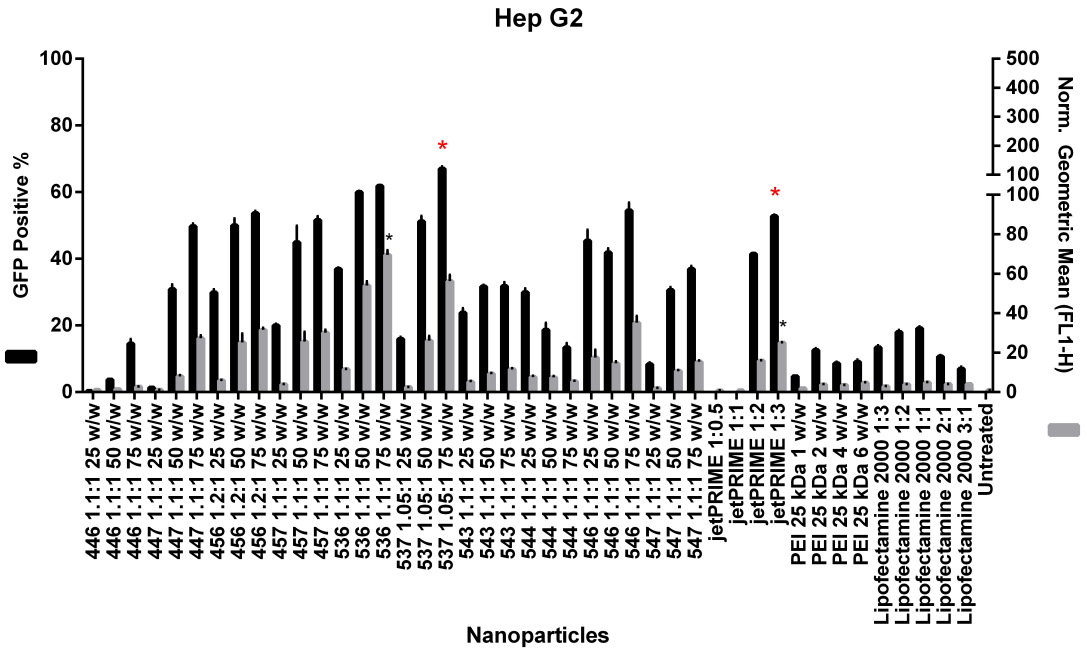
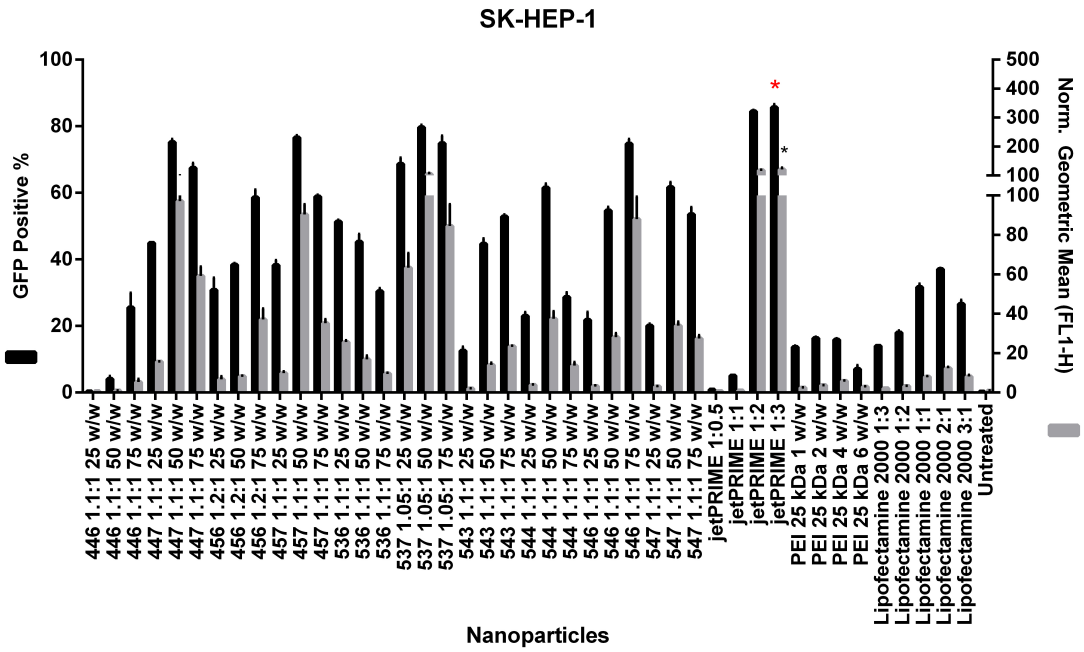
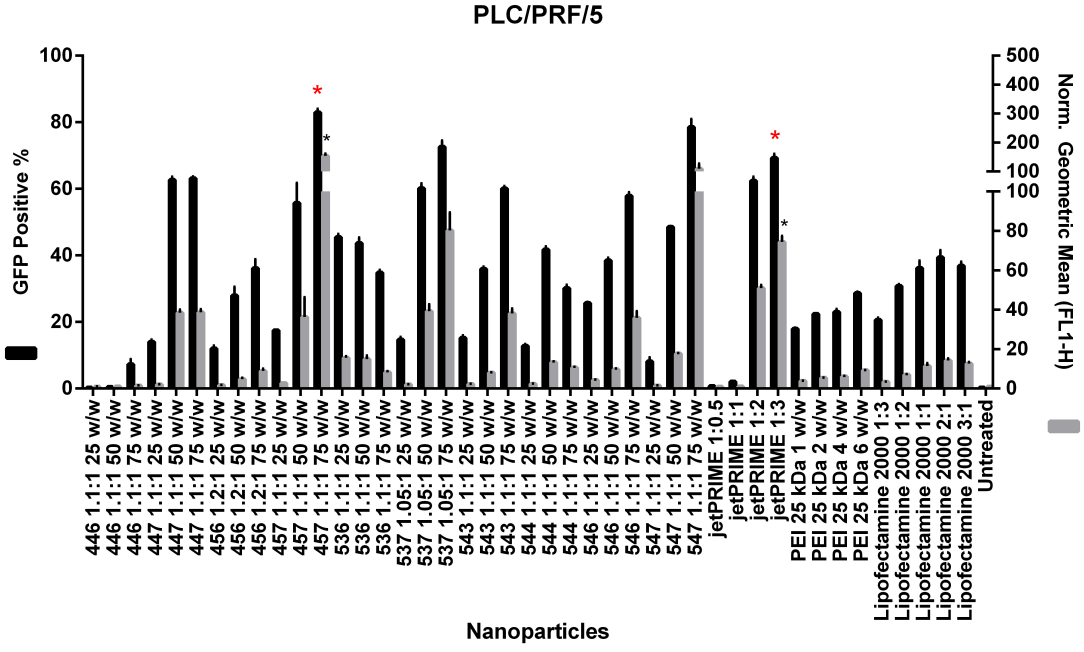
Lipofectamine 2000 1:2
Lipofectamine 2000 1:1
Lipofectamine 2000 2:1
Lipofectamine 2000 3:1
Untreated


Hep 3B
GFP Positive %
Norm. Geometric Mean (FL1-H)
Nanoparticles
446 1.1:1 25 w/w
446 1.1:1 50 w/w
446 1.1:1 75 w/w
447 1.1:1 25 w/w
447 1.1:1 50 w/w
447 1.1:1 75 w/w
456 1.2:1 25 w/w
456 1.2:1 50 w/w
456 1.2:1 75 w/w
457 1.1:1 25 w/w
457 1.1:1 50 w/w
457 1.1:1 75 w/w
536 1.1:1 25 w/w
536 1.1:1 50 w/w
536 1.1:1 75 w/w
537 1.05:1 25 w/w
537 1.05:1 50 w/w
537 1.05:1 75 w/w
543 1.1:1 25 w/w
543 1.1:1 50 w/w
543 1.1:1 75 w/w
544 1.1:1 25 w/w
544 1.1:1 50 w/w
544 1.1:1 75 w/w
546 1.1:1 25 w/w
546 1.1:1 50 w/w
546 1.1:1 75 w/w
547 1.1:1 25 w/w
547 1.1:1 50 w/w
547 1.1:1 75 w/w
jetPRIME 1:0.5
jetPRIME 1:1
jetPRIME 1:2
jetPRIME 1:3
PEI 25 kDa 1 w/w
PEI 25 kDa 2 w/w
PEI 25 kDa 4 w/w
PEI 25 kDa 6 w/w
Lipofectamine 2000 1:3
Lipofectamine 2000 1:2
Lipofectamine 2000 1:1
Lipofectamine 2000 2:1
Lipofectamine 2000 3:1
Untreated


Hep G2
GFP Positive %
Norm. Geometric Mean (FL1-H)
Nanoparticles
446 1.1:1 25 w/w
446 1.1:1 50 w/w
446 1.1:1 75 w/w
447 1.1:1 25 w/w
447 1.1:1 50 w/w
447 1.1:1 75 w/w
456 1.2:1 25 w/w
456 1.2:1 50 w/w
456 1.2:1 75 w/w
457 1.1:1 25 w/w
457 1.1:1 50 w/w
457 1.1:1 75 w/w
536 1.1:1 25 w/w
536 1.1:1 50 w/w
536 1.1:1 75 w/w
537 1.05:1 25 w/w
537 1.05:1 50 w/w
537 1.05:1 75 w/w
543 1.1:1 25 w/w
543 1.1:1 50 w/w
543 1.1:1 75 w/w
544 1.1:1 25 w/w
544 1.1:1 50 w/w
544 1.1:1 75 w/w
546 1.1:1 25 w/w
546 1.1:1 50 w/w
546 1.1:1 75 w/w
547 1.1:1 25 w/w
547 1.1:1 50 w/w
547 1.1:1 75 w/w
jetPRIME 1:0.5
jetPRIME 1:1
jetPRIME 1:2
jetPRIME 1:3
PEI 25 kDa 1 w/w
PEI 25 kDa 2 w/w
PEI 25 kDa 4 w/w
PEI 25 kDa 6 w/w
Lipofectamine 2000 1:3
Lipofectamine 2000 1:2
Lipofectamine 2000 1:1
Lipofectamine 2000 2:1
Lipofectamine 2000 3:1
Untreated


SK-HEP-1
GFP Positive %
Norm. Geometric Mean (FL1-H)
Nanoparticles
446 1.1:1 25 w/w
446 1.1:1 50 w/w
446 1.1:1 75 w/w
447 1.1:1 25 w/w
447 1.1:1 50 w/w
447 1.1:1 75 w/w
456 1.2:1 25 w/w
456 1.2:1 50 w/w
456 1.2:1 75 w/w
457 1.1:1 25 w/w
457 1.1:1 50 w/w
457 1.1:1 75 w/w
536 1.1:1 25 w/w
536 1.1:1 50 w/w
536 1.1:1 75 w/w
537 1.05:1 25 w/w
537 1.05:1 50 w/w
537 1.05:1 75 w/w
543 1.1:1 25 w/w
543 1.1:1 50 w/w
543 1.1:1 75 w/w
544 1.1:1 25 w/w
544 1.1:1 50 w/w
544 1.1:1 75 w/w
546 1.1:1 25 w/w
546 1.1:1 50 w/w
546 1.1:1 75 w/w
547 1.1:1 25 w/w
547 1.1:1 50 w/w
547 1.1:1 75 w/w
jetPRIME 1:0.5
jetPRIME 1:1
jetPRIME 1:2
jetPRIME 1:3
PEI 25 kDa 1 w/w
PEI 25 kDa 2 w/w
PEI 25 kDa 4 w/w
PEI 25 kDa 6 w/w
Lipofectamine 2000 1:3
Lipofectamine 2000 1:2
Lipofectamine 2000 1:1
Lipofectamine 2000 2:1
Lipofectamine 2000 3:1
Untreated


PLC/PRF/5
GFP Positive %
Norm. Geometric Mean (FL1-H)
Nanoparticles
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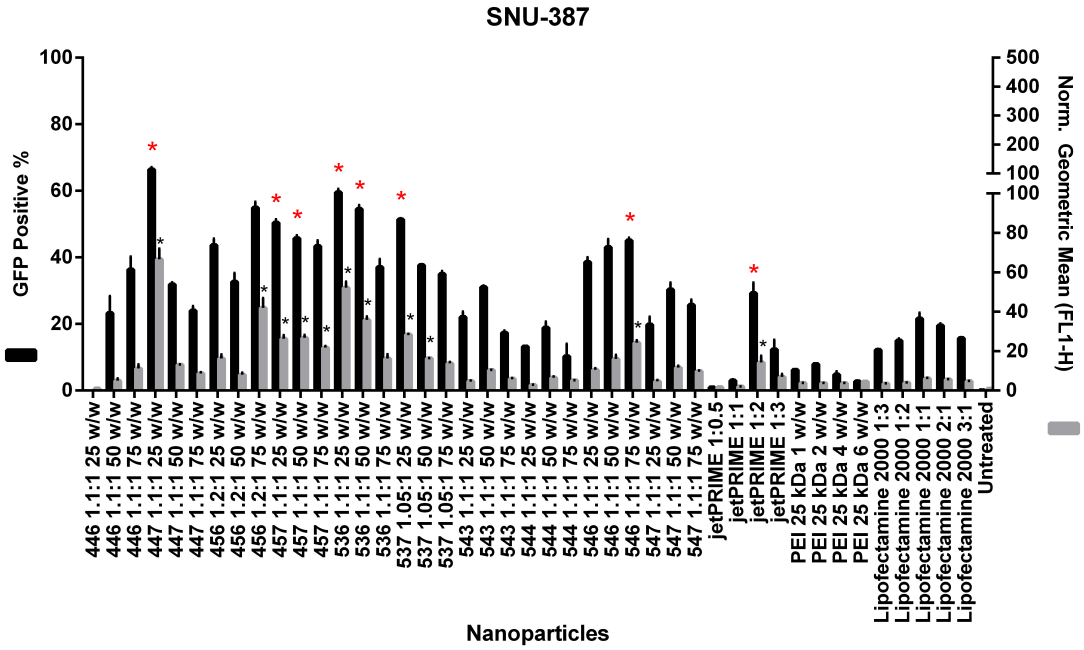
446 1.1:1 50 w/w
446 1.1:1 75 w/w
447 1.1:1 25 w/w
447 1.1:1 50 w/w
447 1.1:1 75 w/w
456 1.2:1 25 w/w
456 1.2:1 50 w/w
456 1.2:1 75 w/w
457 1.1:1 25 w/w
457 1.1:1 50 w/w
457 1.1:1 75 w/w
536 1.1:1 25 w/w
536 1.1:1 50 w/w
536 1.1:1 75 w/w
537 1.05:1 25 w/w
537 1.05:1 50 w/w
537 1.05:1 75 w/w
543 1.1:1 25 w/w
543 1.1:1 50 w/w
543 1.1:1 75 w/w
544 1.1:1 25 w/w
544 1.1:1 50 w/w
544 1.1:1 75 w/w
546 1.1:1 25 w/w
546 1.1:1 50 w/w
546 1.1:1 75 w/w
547 1.1:1 25 w/w
547 1.1:1 50 w/w
547 1.1:1 75 w/w
jetPRIME 1:0.5
jetPRIME 1:1
jetPRIME 1:2
jetPRIME 1:3
PEI 25 kDa 1 w/w
PEI 25 kDa 2 w/w
PEI 25 kDa 4 w/w
PEI 25 kDa 6 w/w
Lipofectamine 2000 1:3
Lipofectamine 2000 1:2
Lipofectamine 2000 1:1
Lipofectamine 2000 2:1
Lipofectamine 2000 3:1
Untreated


SNU-387
GFP Positive %
Norm. Geometric Mean (FL1-H)
Nanoparticles
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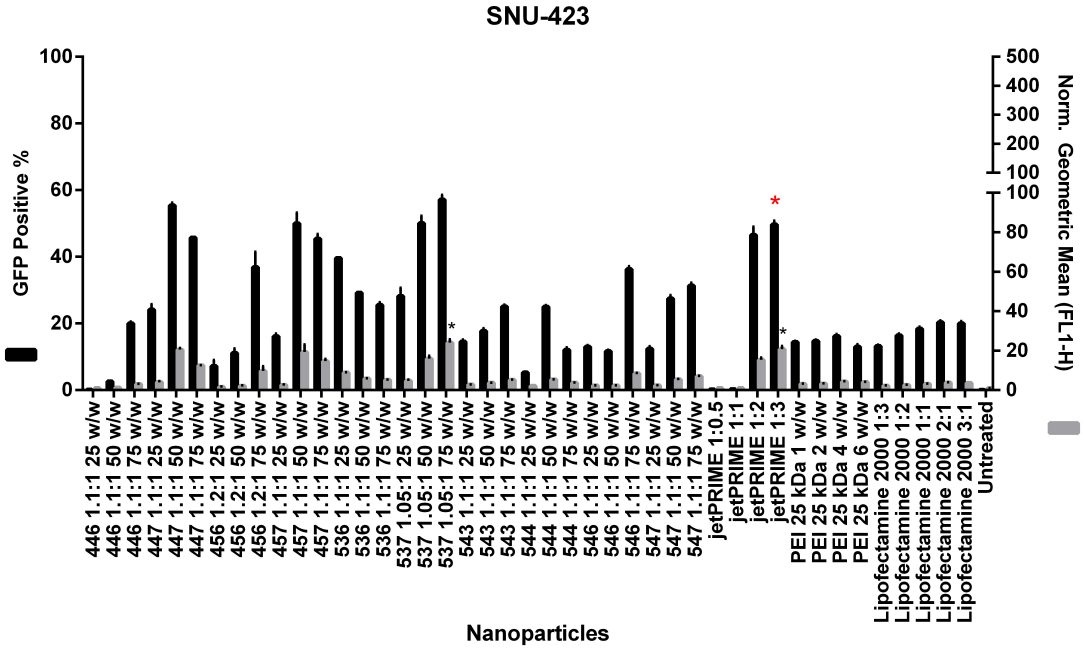
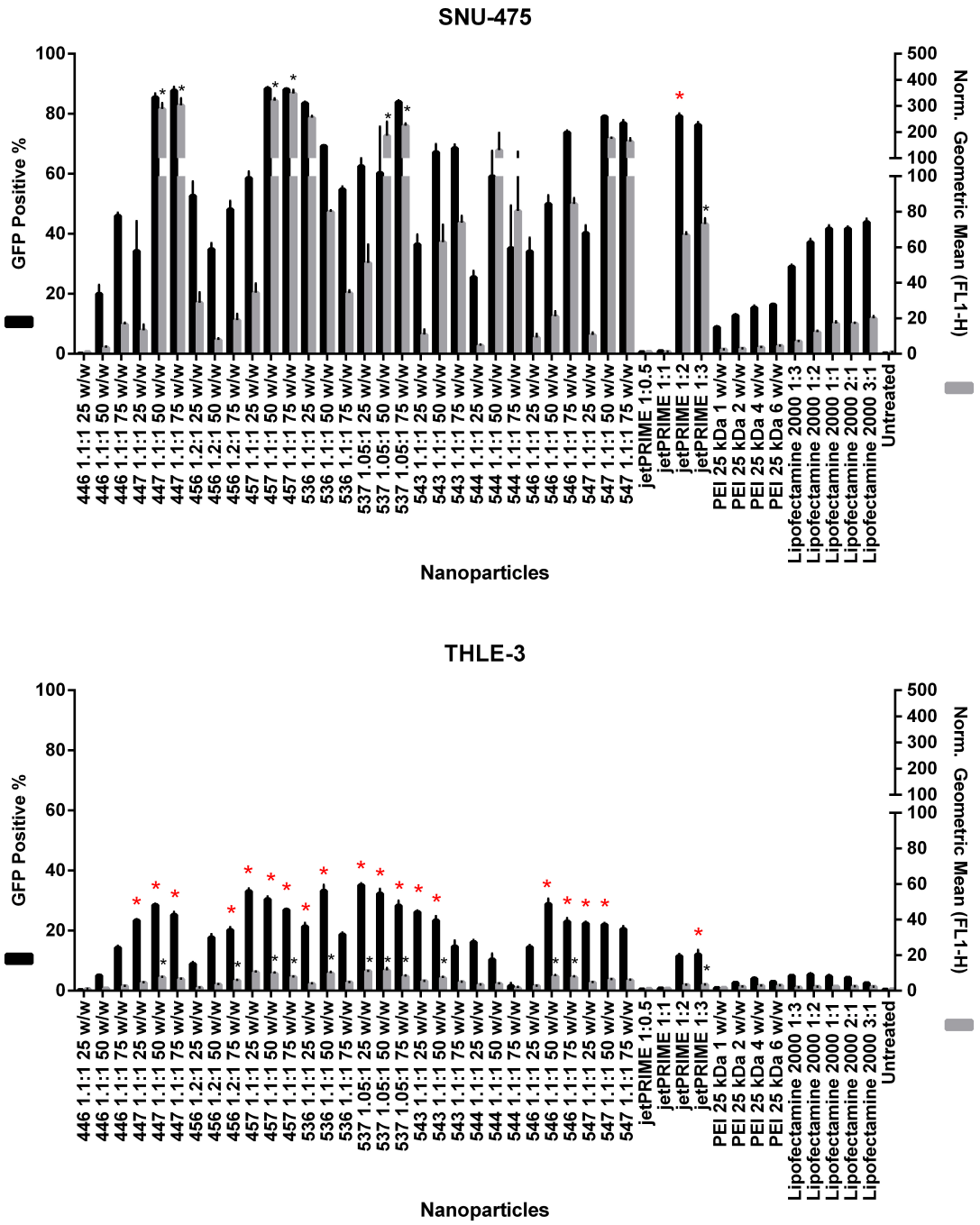
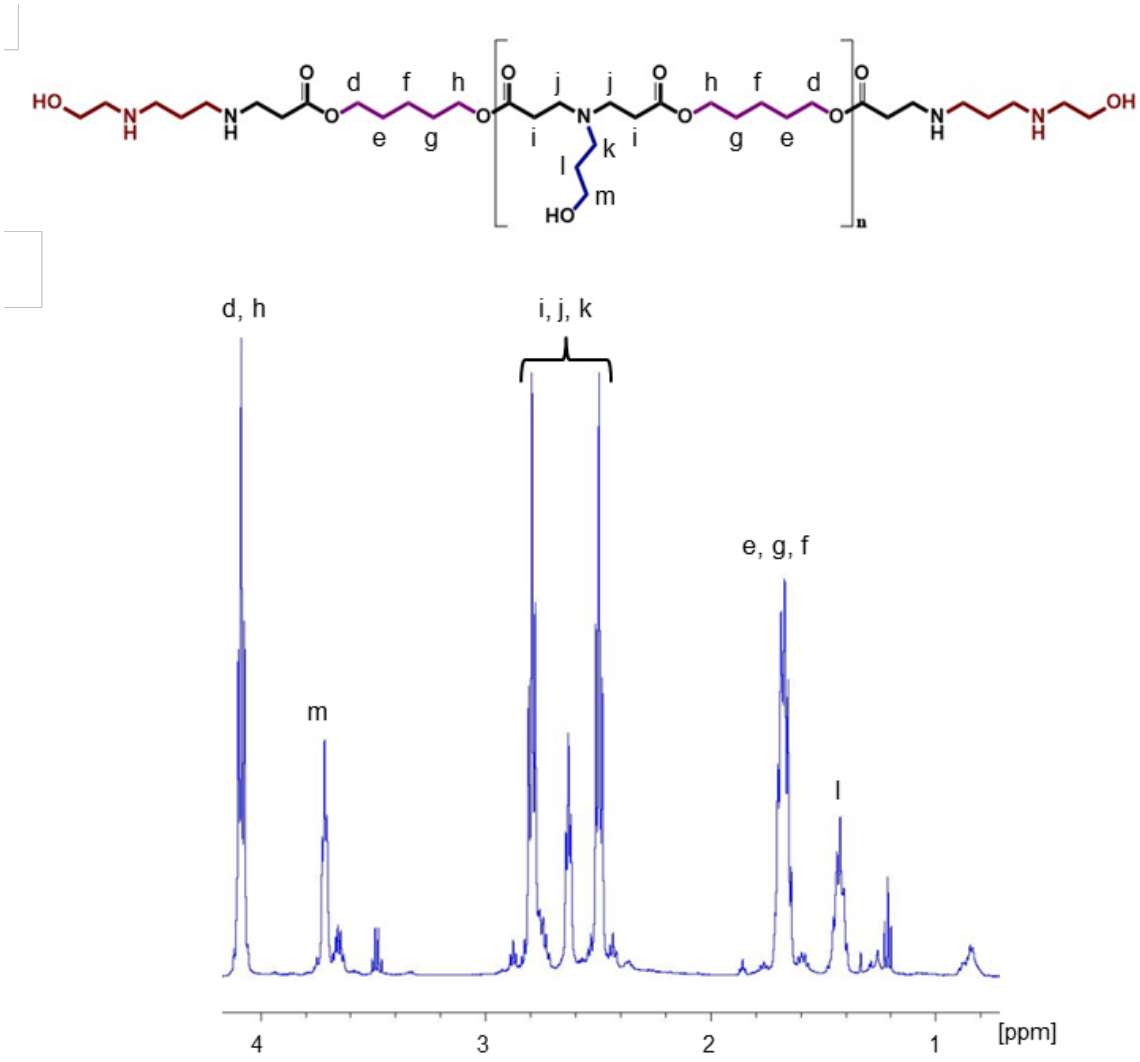
446 1.1:1 50 w/w
446 1.1:1 75 w/w
447 1.1:1 25 w/w
447 1.1:1 50 w/w
447 1.1:1 75 w/w
456 1.2:1 25 w/w
456 1.2:1 50 w/w
456 1.2:1 75 w/w
457 1.1:1 25 w/w
457 1.1:1 50 w/w
457 1.1:1 75 w/w
536 1.1:1 25 w/w
536 1.1:1 50 w/w
536 1.1:1 75 w/w
537 1.05:1 25 w/w
537 1.05:1 50 w/w
537 1.05:1 75 w/w
543 1.1:1 25 w/w
543 1.1:1 50 w/w
543 1.1:1 75 w/w
544 1.1:1 25 w/w
544 1.1:1 50 w/w
544 1.1:1 75 w/w
546 1.1:1 25 w/w
546 1.1:1 50 w/w
546 1.1:1 75 w/w
547 1.1:1 25 w/w
547 1.1:1 50 w/w
547 1.1:1 75 w/w
jetPRIME 1:0.5
jetPRIME 1:1
jetPRIME 1:2
jetPRIME 1:3
PEI 25 kDa 1 w/w
PEI 25 kDa 2 w/w
PEI 25 kDa 4 w/w
PEI 25 kDa 6 w/w
Lipofectamine 2000 1:3
Lipofectamine 2000 1:2
Lipofectamine 2000 1:1
Lipofectamine 2000 2:1
Lipofectamine 2000 3:1
Untreated

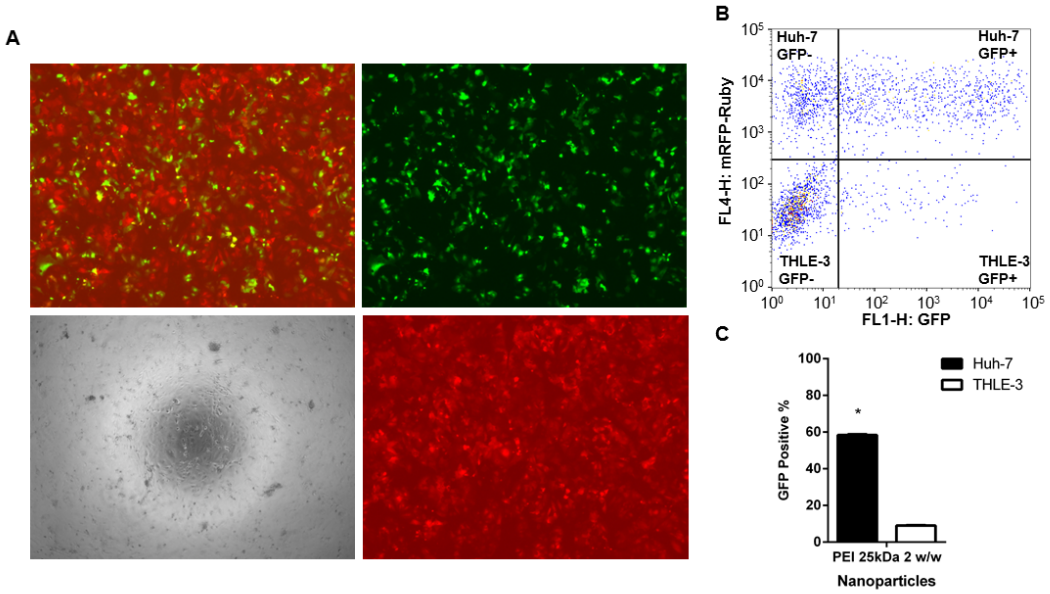

SNU-423
GFP Positive %
Norm. Geometric Mean (FL1-H)
Nanoparticles
446 1.1:1 25 w/w
446 1.1:1 50 w/w
446 1.1:1 75 w/w
447 1.1:1 25 w/w
447 1.1:1 50 w/w
447 1.1:1 75 w/w
456 1.2:1 25 w/w
456 1.2:1 50 w/w
456 1.2:1 75 w/w
457 1.1:1 25 w/w
457 1.1:1 50 w/w
457 1.1:1 75 w/w
536 1.1:1 25 w/w
536 1.1:1 50 w/w
536 1.1:1 75 w/w
537 1.05:1 25 w/w
537 1.05:1 50 w/w
537 1.05:1 75 w/w
543 1.1:1 25 w/w
543 1.1:1 50 w/w
543 1.1:1 75 w/w
544 1.1:1 25 w/w
544 1.1:1 50 w/w
544 1.1:1 75 w/w
546 1.1:1 25 w/w
546 1.1:1 50 w/w
546 1.1:1 75 w/w
547 1.1:1 25 w/w
547 1.1:1 50 w/w
547 1.1:1 75 w/w
jetPRIME 1:0.5
jetPRIME 1:1
jetPRIME 1:2
jetPRIME 1:3
PEI 25 kDa 1 w/w
PEI 25 kDa 2 w/w
PEI 25 kDa 4 w/w
PEI 25 kDa 6 w/w
Lipofectamine 2000 1:3
Lipofectamine 2000 1:2
Lipofectamine 2000 1:1
Lipofectamine 2000 2:1
Lipofectamine 2000 3:1
Untreated



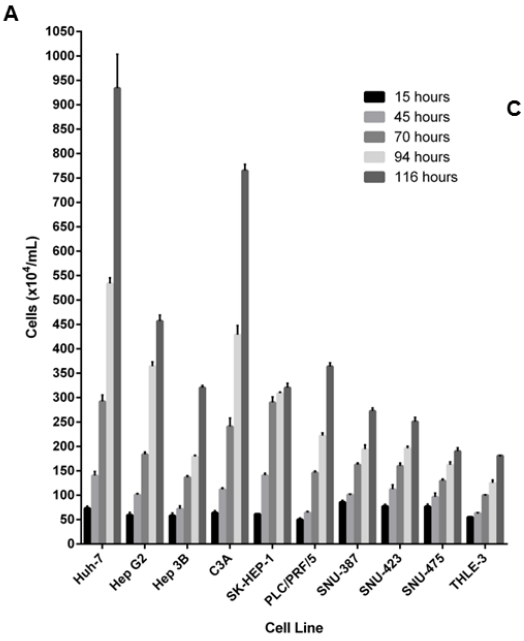
Supplemental Fig. 3 Flow cytometry results indicating eGFP expression 48 hours following transfection with varied PBAE and positive controls NPs to all HCC cell lines.



Supplemental Fig. 4 H^1-NMR spectrum of the polymer 536. Proton peaks were identified and matched with their corresponding positions in the 536 structure.

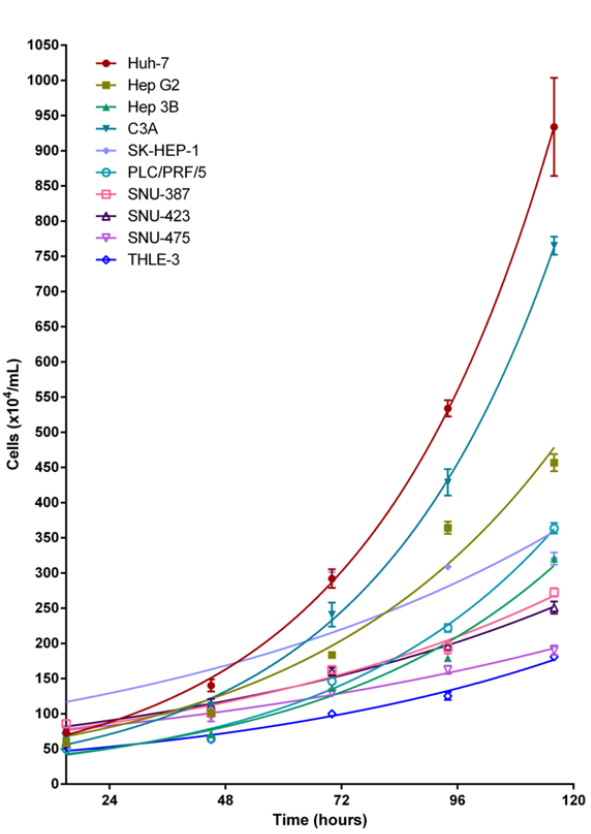


Supplemental Fig. 5 Transfection efficacy and cancer-specificity of PEI 25kDa 2 w/w NPs in a Huh-7/ THLE-3 co-culture model. **A.** Microscopy images and **B.** flow cytometry gating of a PEI 25kDa 2 w/w-treated co-culture representative 48 hours after eGFP transfection. Microscopy images show: merged eGFP and RFP channels (top left), eGFP channel only (top right), bright-field only (bottom left), and RFP channel only (bottom right). **C.** Percentage of GFP positive cells among Huh-7 (RFP positive) and THLE-3 (RFP negative) by flow cytometry analysis.



B

Cell Line	Doubling time (hours ± SE)	R^2
Huh-7	27 ± 0.4	0.9997
Hep G2	36 ± 5	0.9677
Hep 3B	35 ± 4	0.9779
C3A	27 ± 0.6	0.9992
SK-HEP-1	24 ± 0.3	0.9999*
PLC/PRF/5	32 ± 2	0.9935
SNU-387	56 ± 5	0.9817
SNU-423	62 ± 3	0.9943
SNU-475	76 ± 4	0.9939
THLE-3	53 ± 5	0.9826



Supplemental Fig. 6 A. Number of cells counted per line at five different time points (15, 45, 70, 94 and 116 hours) **B.** Doubling times for all cell lines based on fitting exponential growth curves (R^2 values are shown). **C.** Fitting exponential curves showing expected (solid lines) and actual (symbols) cell number for each line at the recorded time points. For calculation of the growth rate, cells were seeded in 12 well plates at 71,250 cells/ well. Fifteen wells were prepared per line, allowing the analysis to be performed in triplicate for each time point (5 total). At the established time points, three wells per line were trypsinized and individually counted using a hemocytometer. * Calculated during the exponential growth phase (from 15 to 70 hours).