Supplementary Information

Self-assembly of amphiphilic peptide nanoparticles for the efficient entrapment and delivery of 100 nucleotide DNA fragments

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Table S1. Sequence of DNA fragments

	oligonucleotide sequence
22nt ssDNA	5′-TAA CAG GAT TAG CAG AGC GAG G-3′ Atto550 conjugation to the 5′ end
22nt ssDNA complementary strand	5′-CCT CGC TCT GCT AAT CCT GTT A-3′
100nt ssDNA	5′-AAA CTC CAA CCA AAA CCT CCT CCC CCA CCC TAA CTC ATT ATC CCC CCT TAC CCA TTT ACC CCA ACA CAC CAA CCT CCC AAC AAC CTC CCT CCA CCA ACC A-3′ Atto550 conjugation to the 5′ end
100nt ssDNA complementary strand	5′-TGG TTG GTG GAG GGA GGT TGT TGG GAG GTT GGT GTG TTG GGG TAA ATG GGT AAG GGG GGA TAA TGA GTT AGG GTG GGG GAG GAG GTT TTG GTT GGA GTT T-3′

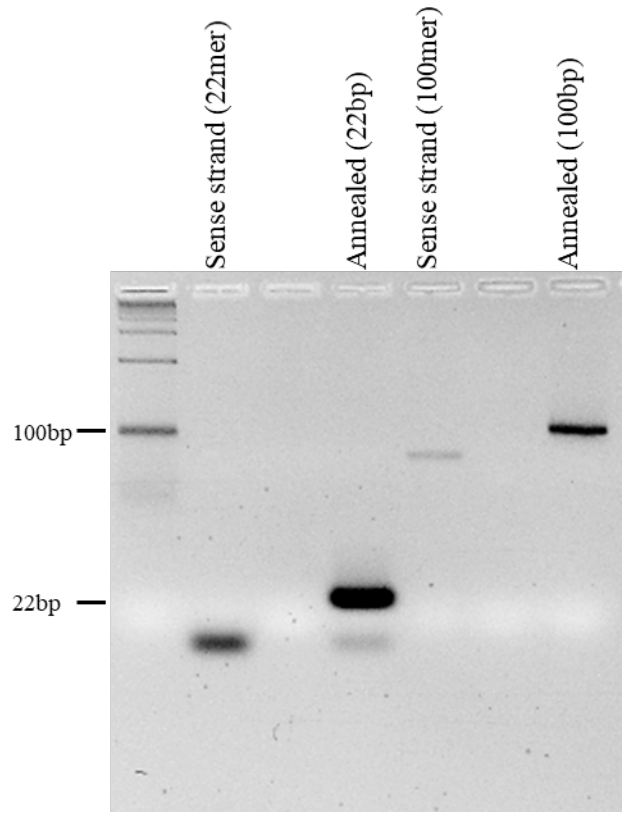

Fig. S1: 5% MetaPhor agarose gel showing single stranded DNA fragments and annealing of complementary 22nt ssDNA and 100nt ssDNA. For annealing equimolar sense and antisense oligonucleotides in 10mM Tris-HCl/1mM EDTA, pH 8 supplemented with 50 mM NaCl were heated to 95°C and gradually cooled down to 10°C over 50min.

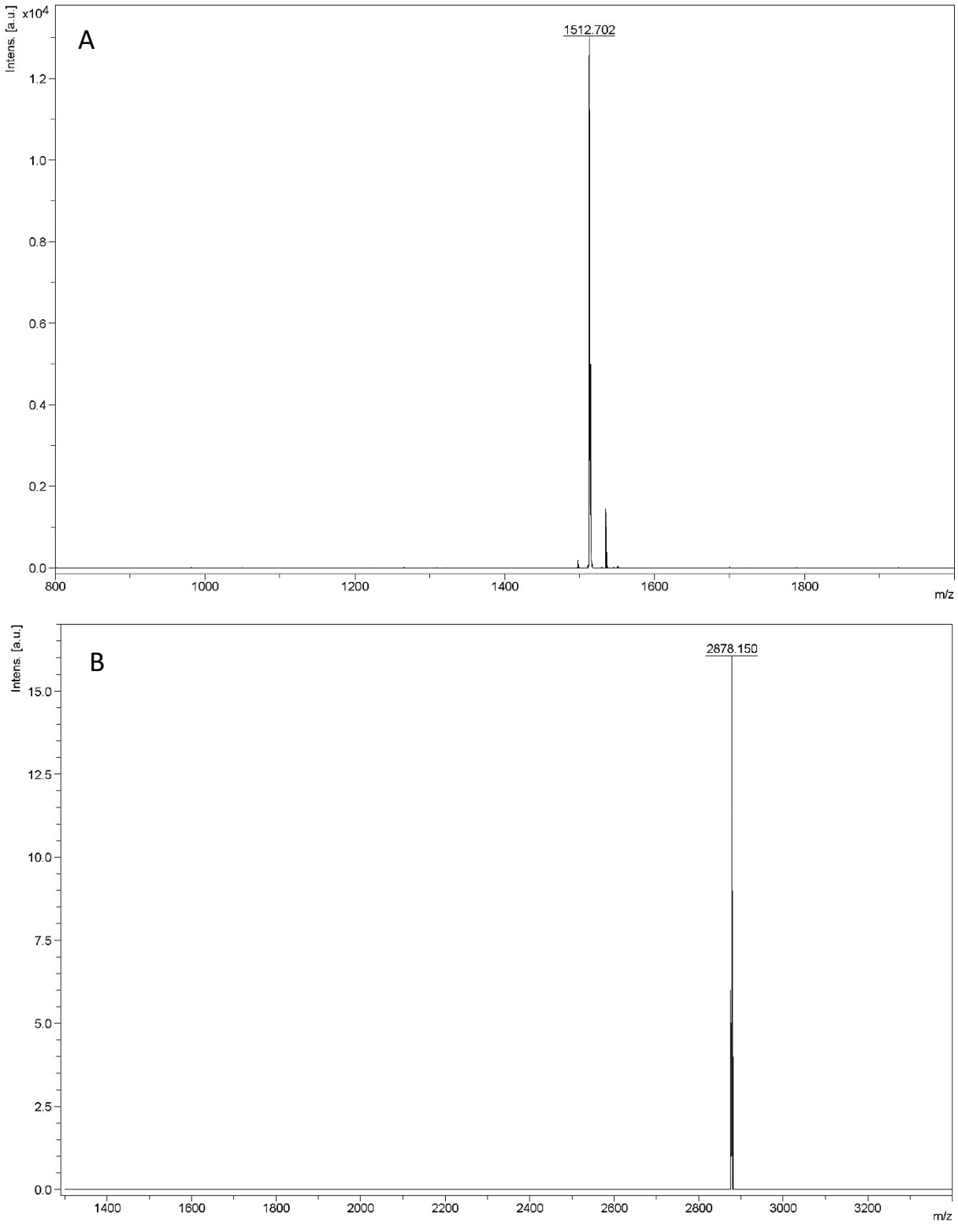

Fig. S2: MALDI-TOF mass spectra of H3gT (A) and (HR)3gT (B) peptides.

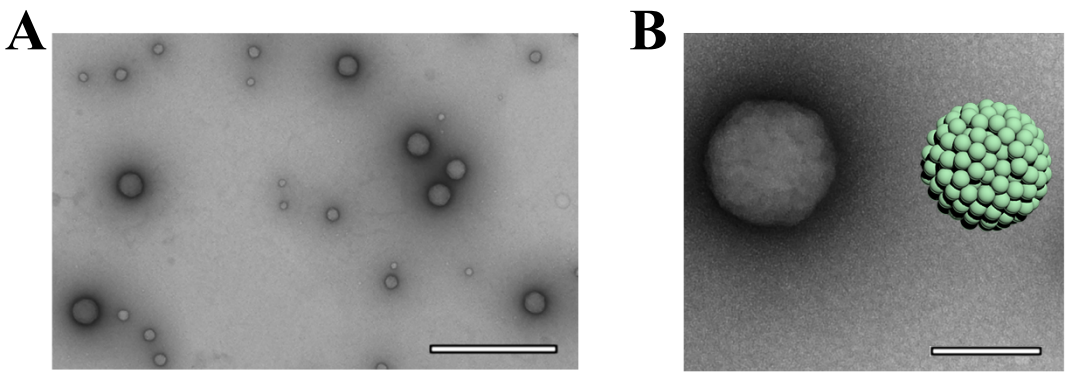

Fig. S3: TEM images of (HR)3gT MCM-NPs. *(A)* Scale bar=500nm, *(B)* Scale bar=100nm.

Solvent effect on self-assembly of (HR)3gT peptide and 22nt ssDNA

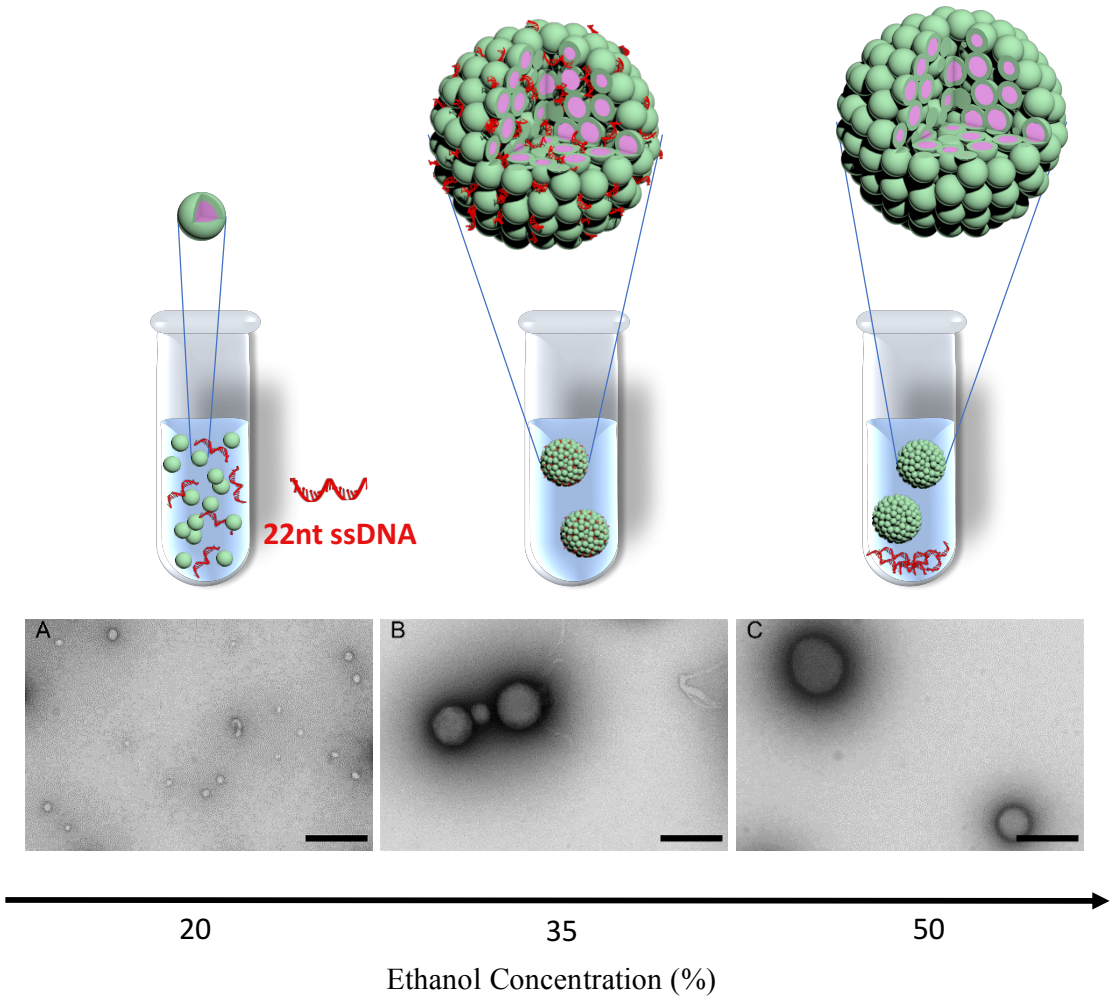

Fig. S4: (HR)3gT self-assembly process with 22nt ssDNA under different final ethanol concentrations, 20% *(A)*, 35% *(B)*, 50% *(C)*, Scale bars=200nm.

Table S2: Characterization of DNA-free and DNA-loaded multicompartment micellar (MCM) H3gT peptide NPs in water, pH 7.

(HR)3gT MCM-NPs	PDI	D_H (nm) DLS	D_H (nm) NTA	D_H(nm) FCS	Zeta potential (mV)
H3gT NPs	0.32±0.024	211±46	236±57	NA*	3.42±3.62
22nt ssDNA loaded H3gT NPs	0.34±0.027	241±73	242±12	233±29	0.6±2.3

*not applicable

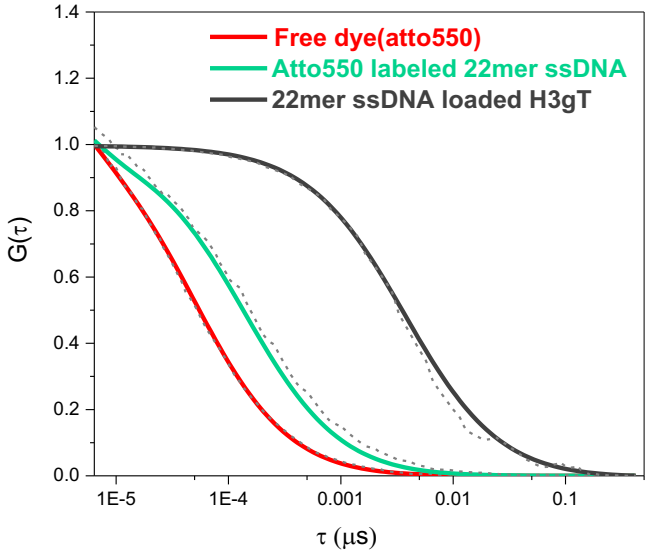

Fig. S5: Normalised FCS autocorrelation curves of free dye-Atto550, Atto550-labelled 22nt ssDNA, and 22nt ssDNA-loaded H3gT NPs.

Table S3: FCS characterization of singly and loaded DNA in to MCM H3gT NPs.

Fluorescent species	Diffusion time (µs)	DNA/NP
Atto550 fluorescent dye	48±3	N/A
Atto550-labelled 22nt ssDNA	139±5	N/A
22nt ssDNA-loaded H3gT NPs	5322±382	3.95±2.39

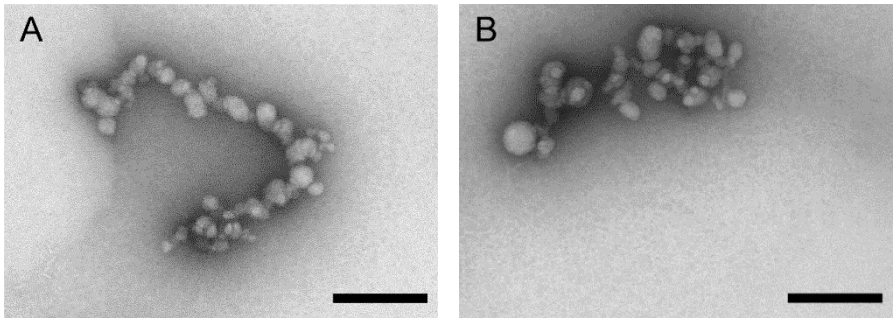

Fig. S6: TEM micrograph of H3gT NPs *(A)* and 22nt ssDNA-loaded H3gT NPs *(B)* after 5h incubation at 37°C. Scale bars=200nm.

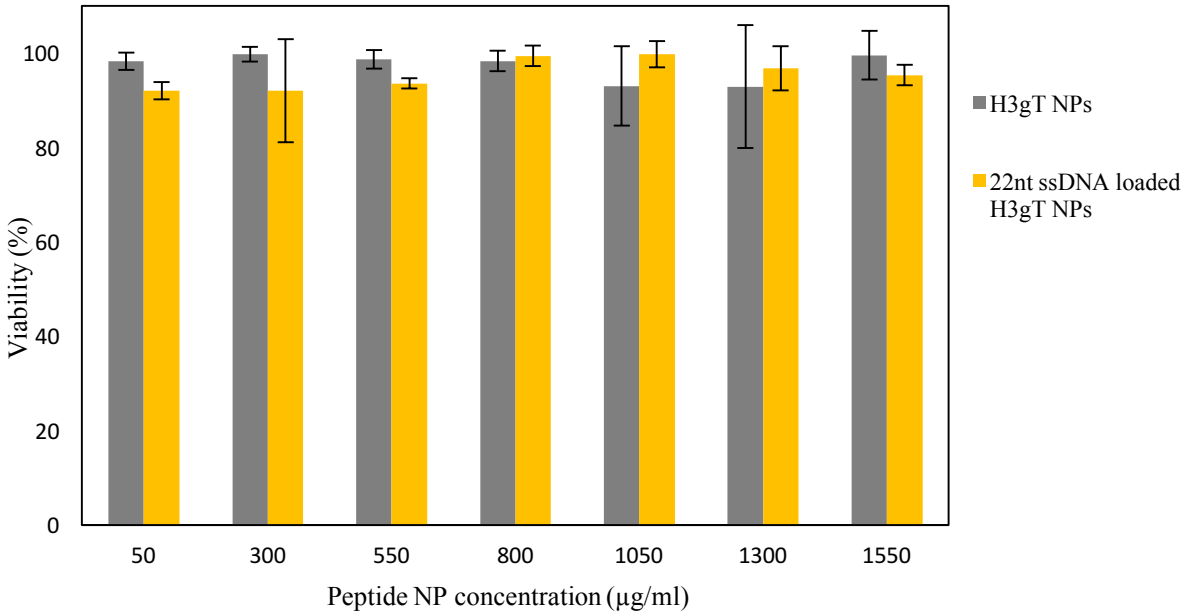

Fig. S7: HeLa cell viability (MTS assay). Cells treated for 24h with H3gT NPs (gray) and 22nt ssDNA-loaded H3gT NPs (yellow). Cell viability was normalized to untreated HeLa cells (negative control, 100% viability). All data presented as the mean ± SD (n=3).

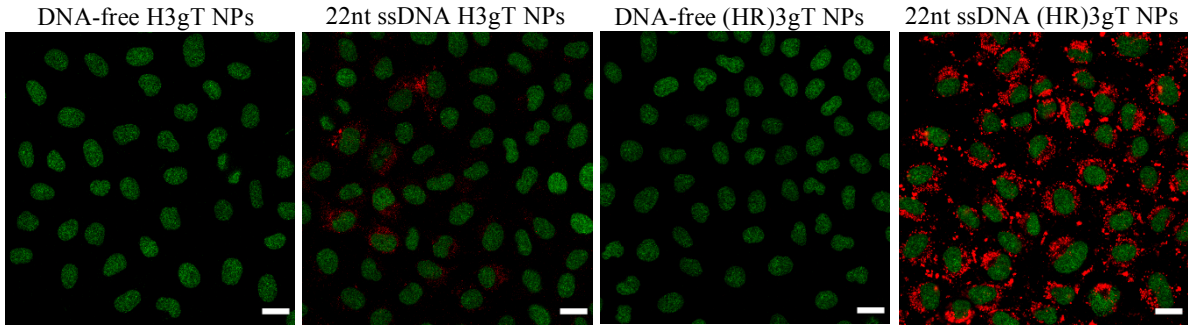

Fig. S8: CLSM merged images (GFP and atto550) of H2B-GFP expressing HeLa cells treated with 22nt ssDNA-loaded H3gT and (HR)3gT MCM NPs after 48h. Scale bars=20µm.

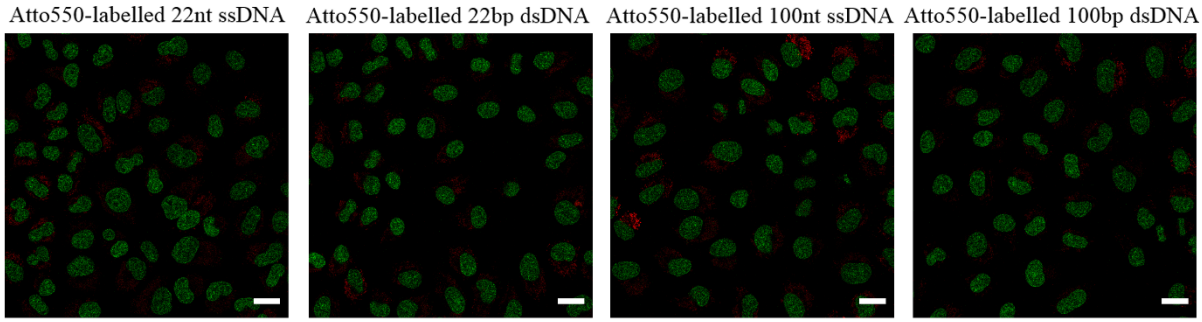

Fig. S9: CLSM merged images (GFP and Atto550) of H2B-GFP expressing HeLa cells treated with different free Atto550-labelled DNAs. Images are recorded under the same conditions as in Fig. 7. Scale bars=20µm.

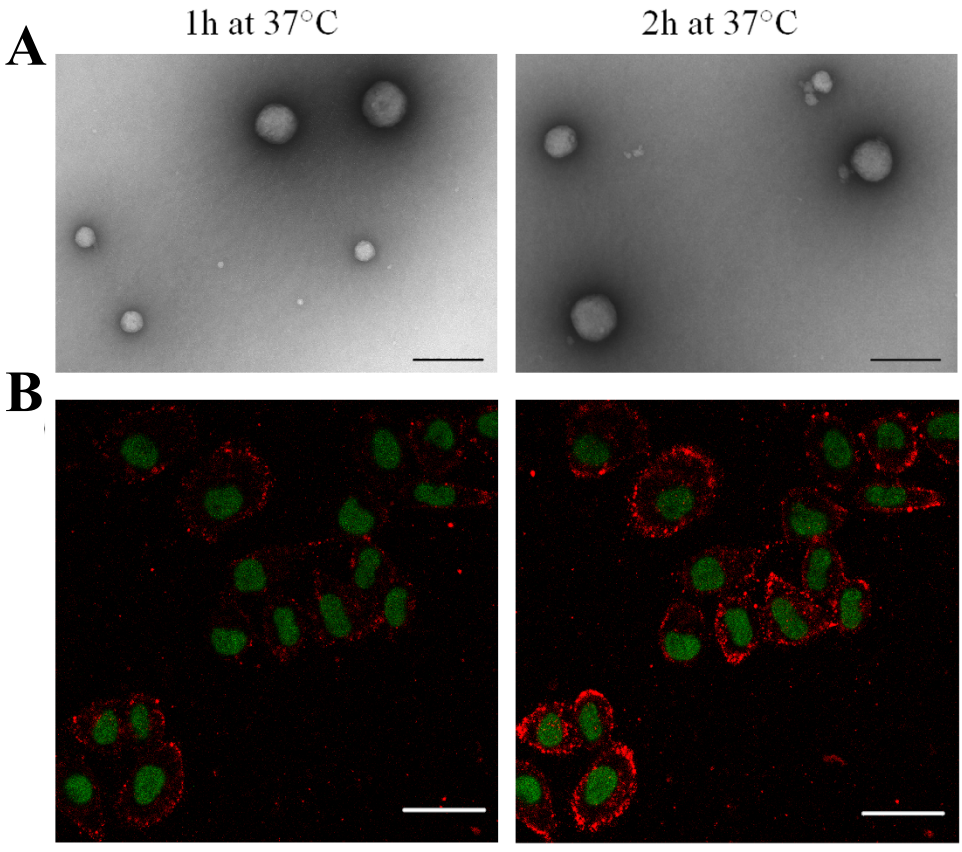

Fig. S10: *(A)* TEM images of 100bp dsDNA-loaded (HR)3gT MCM-NPs after 1h (*left*) and 2h (*right*) incubation at 37°C. Scale bars: 200nm. *(B)* CLSM merged images (GFP and Atto550) of H2B-GFP expressing HeLa cells treated with 100bp dsDNA-loaded (HR)3gT MCM NPs for 1h (*left*) and 2h (*right*). Scale bars=40µm.