Supporting Information for:

Triggering of RNA Interference with RNA-RNA, RNA-DNA and DNA-RNA Nanoparticles

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Sequences used in this project

All DNAs and fluorescently labeled RNA and DNA molecules were purchased from IDT (http://www.idtdna.com/site)

RNA cube[1] 3'-end functionalized with antisense of Dicer substrate RNA (DS RNA)selected against eGFP[2]

Letter sequences below the RNA strands indicate the desired interactions between different strands. Dots indicate the parts of the strands that are per design single-stranded. Sequence characters in upper case represent the cube scaffold; Dicer substrate (DS) RNAs are denoted in lower case.

DS_A

GGCAACUUUGAUCCCUCGGUUUAGCGCCGGCCUUUUCUCCCACACUUUCACGuucgguggugca
gaugaacuucaggguca
MMMMMM...KKKKKKLLLL...FFFFFFGGGG...BBBBBBCCCC...NNNN..ZZZZZZZZZZ
ZZZZZZZZZZZZZZZ..

DS_B

GGGAAAUUUCGUGGUAGGUUUUGUUGCCCGUGUUUCUACGAUUACUUUGGUCuucgguggugca
gaugaacuucaggguca
QQQQQQ...PPPPPPPPPP...MMMMMMNNNN...EEEEEEEEEE...RRRR..ZZZZZZZZZZ
ZZZZZZZZZZZZZZZ..

DS_C

GGACAUUUUCGAGACAGCAUUUUUUCCCGACCUUUGCGGAUUGUAUUUUAGGuucgguggugca
gaugaacuucaggguca
IIIIII...OOOOOOOOOO...QQQQQQRRRR...DDDDDDDDDD...JJJJ..ZZZZZZZZZZ
ZZZZZZZZZZZZZZZ..

DS_D

GGCGCUUUUGACCUUCUGCUUUAUGUCCCCUAUUUCUUAAUGACUUUUGGCCuucgguggugca
gaugaacuucaggguca
FFFFFF...HHHHHHHHHH...IIIIIIJJJJ...AAAAAAAAAA...GGGG..ZZZZZZZZZZ
ZZZZZZZZZZZZZZZ..

DS_E

GGGAGAUUUAGUCAUUAAGUUUUACAAUCCGCUUUGUAAUCGUAGUUUGUGUuucgguggugca
gaugaacuucaggguca
BBBBBB...AAAAAAAAAA...DDDDDDDDDD...EEEEEEEEEE...CCCC..ZZZZZZZZZZ
ZZZZZZZZZZZZZZZ..

DS_F

GGGAUCUUUACCUACCACGUUUUGCUGUCUCGUUUGCAGAAGGUCUUUCCGAuucgguggugca
gaugaacuucaggguca
KKKKKK...PPPPPPPPPP...OOOOOOOOOO...HHHHHHHHHH...LLLL..ZZZZZZZZZZ
ZZZZZZZZZZZZZZZ..

DS RNA sense

pACCCUGAAGUUCAUCUGCACCACCG
ZZZZZZZZZZZZZZZZZZZZZZZZZ

DS RNA antisense
CGGUGGUGCAGAUGAACUUCAGGGUCA

RNA cube 3'-end functionalized with antisense of Dicer substrate RNA (DS RNA) selected against multiple sites (indicated in parenthesis) of HIV-1[3]

DS_A_(Primer Binding Site – Matrix)
GGCAACUUUGAUCCCUCGGUUUAGCGCCGGCCUUUUCUCCCACACUUUCACGuu
gacggacucgcacccaucucucuccuu
MMMMMM...KKKKKKLLLL...FFFFFFGGGG...BBBBBBCCCC...NNNN..SSSSSSSSSS
SSSSSSSSSSSSSS..
DS_B_(NEF)
GGGAAAUUUCGUGGUAGGUUUUGUUGCCCGUGUUUCUACGAUUACUUUGGUCuuggaggaaauu
agcccuuccagucccuu
QQQQQQ...PPPPPPPPPP...MMMMMMNNNN...EEEEEEEEEE...RRRR..TTTTTTTTTT
TTTTTTTTTTTTTT..
DS_C_(Protease)
GGACAUUUUCGAGACAGCAUUUUUUCCCGACCUUUGCGGAUUGUAUUUUAGGuuucuucuaaua
cuguaucaucugcuccu
IIIIII...OOOOOOOOOO...QQQQQQRRRR...DDDDDDDDDD...JJJJ..XXXXXXXXXX
XXXXXXXXXXXXXX..
DS_D_(ENV)
GGCGCUUUUGACCUUCUGCUUUAUGUCCCCUAUUUCUUAAUGACUUUUGGCCuuggacaauuugg
agaagugaauuauauu
FFFFFF...HHHHHHHHHH...IIIIIIJJJJ...AAAAAAAAAA...GGGG..ZZZZZZZZZZ
ZZZZZZZZZZZZZZ..
DS_E_(Capsid)
GGGAGAUUUAGUCAUUAAGUUUUACAAUCCGCUUUGUAAUCGUAGUUUGUGUuuccuggaaugc
ugucaucauuucuucuu
BBBBBB...AAAAAAAAAA...DDDDDDDDDD...EEEEEEEEEE...CCCC..WWWWWWWWWW
WWWWWWWWWWWWWW..
DS_F_(RT)
GGGAUCUUUACCUACCACGUUUUGCUGUCUCGUUUGCAGAAGGUCUUUCCGAuuauuuaucuac
uuguucauuuccucca
KKKKKK...PPPPPPPPPP...OOOOOOOOOO...HHHHHHHHHH...LLLL..CCCCCCCCCC
CCCCCCCCCCCCCC..

Corresponding sense strands

DS (Primer Binding Site – Matrix)
5'-pGGAGAGAGAUGGGUGCGAGUUCGUC
    SSSSSSSSSSSSSSSSSSSSSSSSS
DS (NEF)
5'-pGGGACUGGAAGGGCUAAUUUUCUCC
    TTTTTTTTTTTTTTTTTTTTTTTTT

DS (PRO)
5′-pGAGCAGAUGAUACAGUAUUAGAAGA
XXXXXXXXXXXXXXXXXXXXXXXXX
DS (ENV)
5′-pUAUAAUUCACUUCUCCAAUUGUCC
ZZZZZZZZZZZZZZZZZZZZZZZZZ
DS (Capsid)
5′-pGAAGAAAUGAUGACAGCAUUUCAGG
WWWWWWWWWWWWWWWWWWWWWWWWW
DS (RT)
5′-pGAGGAAAUGAACAAGUAGAUAAAU
CCCCCCCCCCCCCCCCCCCCCCCCC

DNA sequences designed for auto-recognizing RNA-DNA hybrids against eGFP[4]

Auto-recognizing toeholds are underlined.
DNA for antisense of eGFP DS RNA (hybrids for assembly with 3`-end functionalized RNA cubes)
Alexa546-GTCACGGTCTCCTGACCCTGAAGTTCATCTGCACCACCG
DNA for sense of eGFP DS RNA (cognate hybrids for 3`-end functionalized RNA cubes)
CGGTGGTGCAGATGAACTTCAGGGTCAGGAGACCGTGAC-Alexa488

DNA cube with three Ts at each corner

A
GGCAACTTTGATCCCTCGGTTTAGCGCCGGCCTTTTCTCCCACACTTTCACG
MMMMMM...KKKKKKLLLL...FFFFFFGGGG...BBBBBBCCCC...NNNN
B
GGGAAATTTCGTGGTAGGTTTTGTTGCCCGTGTTTCTACGATTACTTTGGTC
QQQQQQ...PPPPPPPPPP...MMMMMMNNNN...EEEEEEEEEE...RRRR
C
GGACATTTTCGAGACAGCATTTTTTCCCGACCTTTGCGGATTGTATTTTAGG
IIIIII...OOOOOOOOOO...QQQQQQRRRR...DDDDDDDDDD...JJJJ
D
GGCGCTTTTGACCTTCTGCTTTATGTCCCCTATTTCTTAATGACTTTTGGCC
FFFFFF...HHHHHHHHHH...IIIIIIJJJJ...AAAAAAAAAA...GGGG
E
GGGAGATTTAGTCATTAAGTTTTACAATCCGCTTTGTAATCGTAGTTTGTGT
BBBBBB...AAAAAAAAAA...DDDDDDDDDD...EEEEEEEEEE...CCCC
F
GGGATCTTTACCTACCACGTTTTGCTGTCTCGTTTGCAGAAGGTCTTTCCGA
KKKKKK...PPPPPPPPPP...OOOOOOOOOO...HHHHHHHHHH...LLLL

DNA cube 5'-end functionalized with auto-recognizing RNA-DNA hybrids carrying sense strand of DS RNA selected against eGFP

Auto-recognizing toeholds are underlined.

A

GGAGACCGTGACCGGTGGTGCAGATGAACTTCAGGGTCAttGGCAACTTTGATCCCTCGGTTTA
GCGCCGGCCTTTTCTCCCACACTTTCACG

B

GGAGACCGTGACCGGTGGTGCAGATGAACTTCAGGGTCAttGGGAAATTTCGTGGTAGGTTTTG
TTGCCCGTGTTTCTACGATTACTTTGGTC

C

GGAGACCGTGACCGGTGGTGCAGATGAACTTCAGGGTCAttGGACATTTTCGAGACAGCATTTT
TTCCCGACCTTTGCGGATTGTATTTTAGG

D

GGAGACCGTGACCGGTGGTGCAGATGAACTTCAGGGTCAttGGCGCTTTTGACCTTCTGCTTTA
TGTCCCCTATTTCTTAATGACTTTTGGCC

E

GGAGACCGTGACCGGTGGTGCAGATGAACTTCAGGGTCAttGGGAGATTTAGTCATTAAGTTTT
ACAATCCGCTTTGTAATCGTAGTTTGTGT

F

GGAGACCGTGACCGGTGGTGCAGATGAACTTCAGGGTCAttGGGATCTTTACCTACCACGTTTT
GCTGTCTCGTTTGCAGAAGGTCTTTCCGA

DNA for antisense of eGFP DS RNA (cognate hybrids for 5`-end functionalized DNA cubes)

TGACCCTGAAGTTCATCTGCACCACCGGTCACGGTCTCC

DNA cube 3'-end functionalized with auto-recognizing RNA-DNA hybrids carrying antisense strand of DS RNA selected against eGFP

A

GGCAACTTTGATCCCTCGGTTTAGCGCCGGCCTTTTCTCCCACACTTTCACGttTGACCCTGAA
GTTCATCTGCACCACCGGTCACGGTCTCC

B

GGGAAATTTCGTGGTAGGTTTTGTTGCCCGTGTTTCTACGATTACTTTGGTCttTGACCCTGAA
GTTCATCTGCACCACCGGTCACGGTCTCC

C

GGACATTTTCGAGACAGCATTTTTTCCCGACCTTTGCGGATTGTATTTTAGGttTGACCCTGAA
GTTCATCTGCACCACCGGTCACGGTCTCC

D

GGCGCTTTTGACCTTCTGCTTTATGTCCCCTATTTCTTAATGACTTTTGGCCttTGACCCTGAA
GTTCATCTGCACCACCGGTCACGGTCTCC

E

GGGAGATTTAGTCATTAAGTTTTACAATCCGCTTTGTAATCGTAGTTTGTGTttTGACCCTGAA
GTTCATCTGCACCACCGGTCACGGTCTCC

F

GGGATCTTTACCTACCACGTTTTGCTGTCTCGTTTGCAGAAGGTCTTTCCGAttTGACCCTGAA
GTTCATCTGCACCACCGGTCACGGTCTCC

DNA for sense of eGFP DS RNA (cognate hybrids for 3`-end functionalized DNA cubes)
GGAGACCGTGACCGGTGGTGCAGATGAACTTCAGGGTCA

Fluorescently labeled RNA and DNA molecules

DS RNA sense 3`-end labeled with Alexa488
pACCCUGAAGUUCAUCUGCACCACCG-Alexa488
ZZZZZZZZZZZZZZZZZZZZZZZZZ
DS RNA antisense 5`-end labeled with Alexa546
Alexa546-CGGUGGUGCAGAUGAACUUCAGGGUCA

DNA for antisense of eGFP DS RNA (hybrids for assembly with 3`-end functionalized RNA cubes) **5`-end labeled with Alexa546**
Alexa546-GTCACGGTCTCCTGACCCTGAAGTTCATCTGCACCACCG
DNA for sense of eGFP DS RNA (cognate hybrids for 3`-end functionalized RNA cubes) **3`-end labeled with Alexa488**
CGGTGGTGCAGATGAACTTCAGGGTCAGGAGACCGTGAC-Alexa488

Sgc8c aptamers[5, 6] for functionalization of RNA and DNA

Sgc8c aptamer part is in italic letters

Sgc8c_sense_DNA_cube
ACCCTGAAGTTCATCTGCACCACCG*GATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTA GATGA*

Supporting Figures

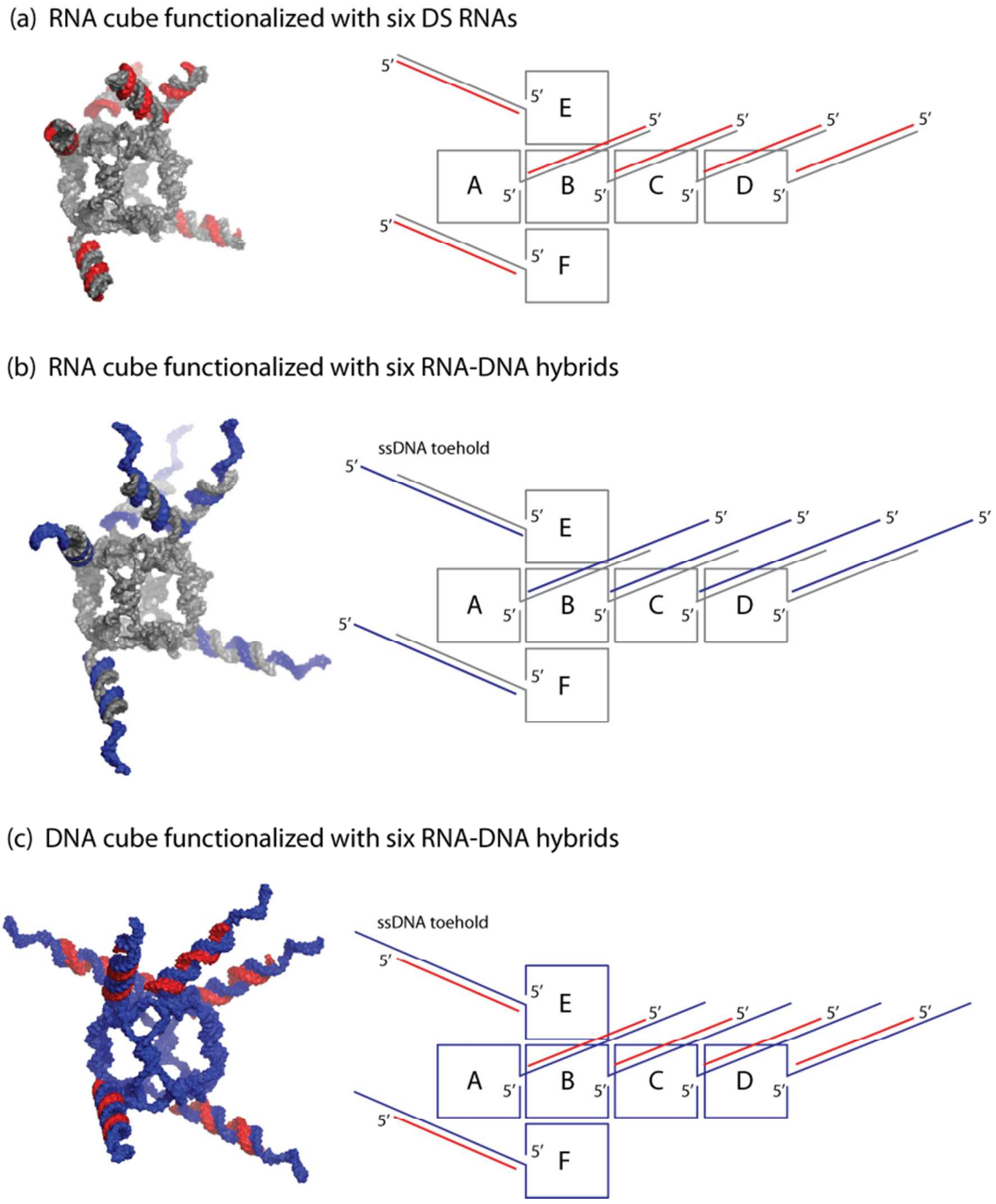

Figure S1. 3D models and corresponding 2D diagrams of RNA and DNA cubes functionalized through the 3'-side extensions of scaffold strands with six Dicer substrate siRNAs (DS RNAs) or RNA-DNA hybrids for conditional split function (RNAi) activation. RNA strands are colored in grey and red; DNA strands are in blue.

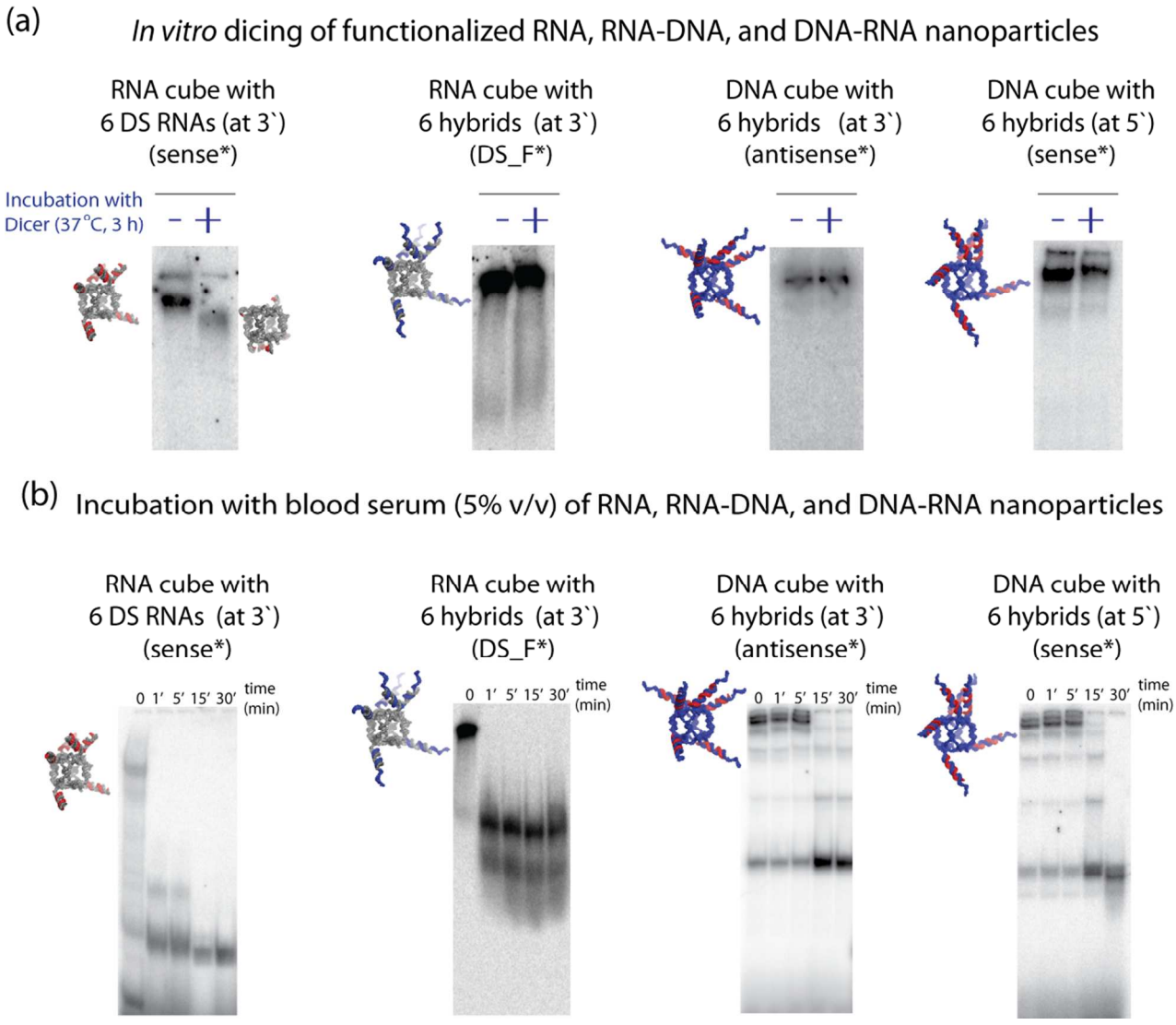

Figure S2. Dicing experiments shown in (**a**) and blood serum degradation assays shown in (**b**). Sense or antisense strands were radiolabeled with [32P]α –GTP at 3'-sides and "*" indicate radiolabeled strands. Native-PAGE gels were used to identify the dicing products after incubation with Dicer (indicated with "+") for 3 hours at 37°C and degradation products after incubation with 5% human blood serum at different time points. Radiolabled sense and antisense RNA strands (mixed with non-labeled RNAs) were added to the assembly mixture in 10% excess to guarantee a complete incorporation of radiolabels into the resulting cubes. The dicing of RNA cubes with six DS RNAs was also demonstrated in Afonin *et al*[7].

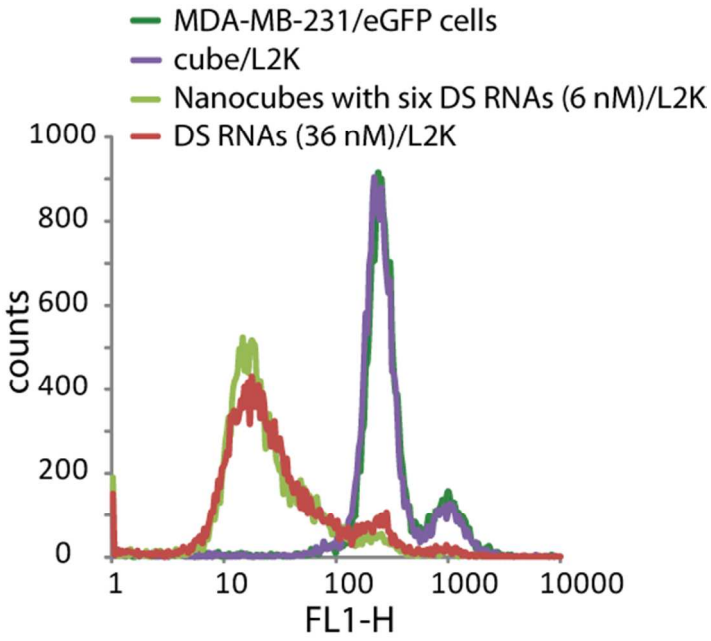

Figure S3. GFP knockdown assays with 3'-side functionalized RNA cubes for human breast cancer cells (MDA-MB-231/GFP) which stably express enhanced GFP (eGFP). Three after the transfection of cells with nanocubes, eGFP expression was statistically analyzed with flow cytometry experiments. As controls, non-functionialized RNA cube and individual DS RNA duplexes against eGFP were used.

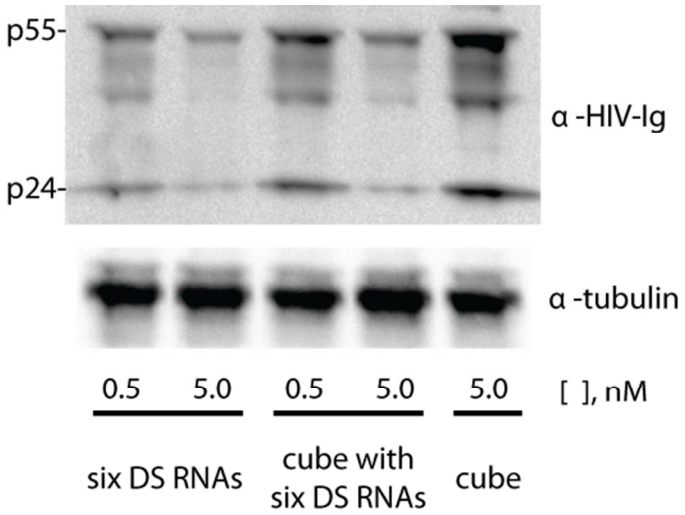

Figure S4. Western blot analysis using α-HIV-Ig confirms that both 3`-functionalized RNA nanocubes and the mixture of DS RNAs reduce the level of p55 (Gag polyprotein) and p24 in the virus producing cells compared to non-functionalized nanocubes.

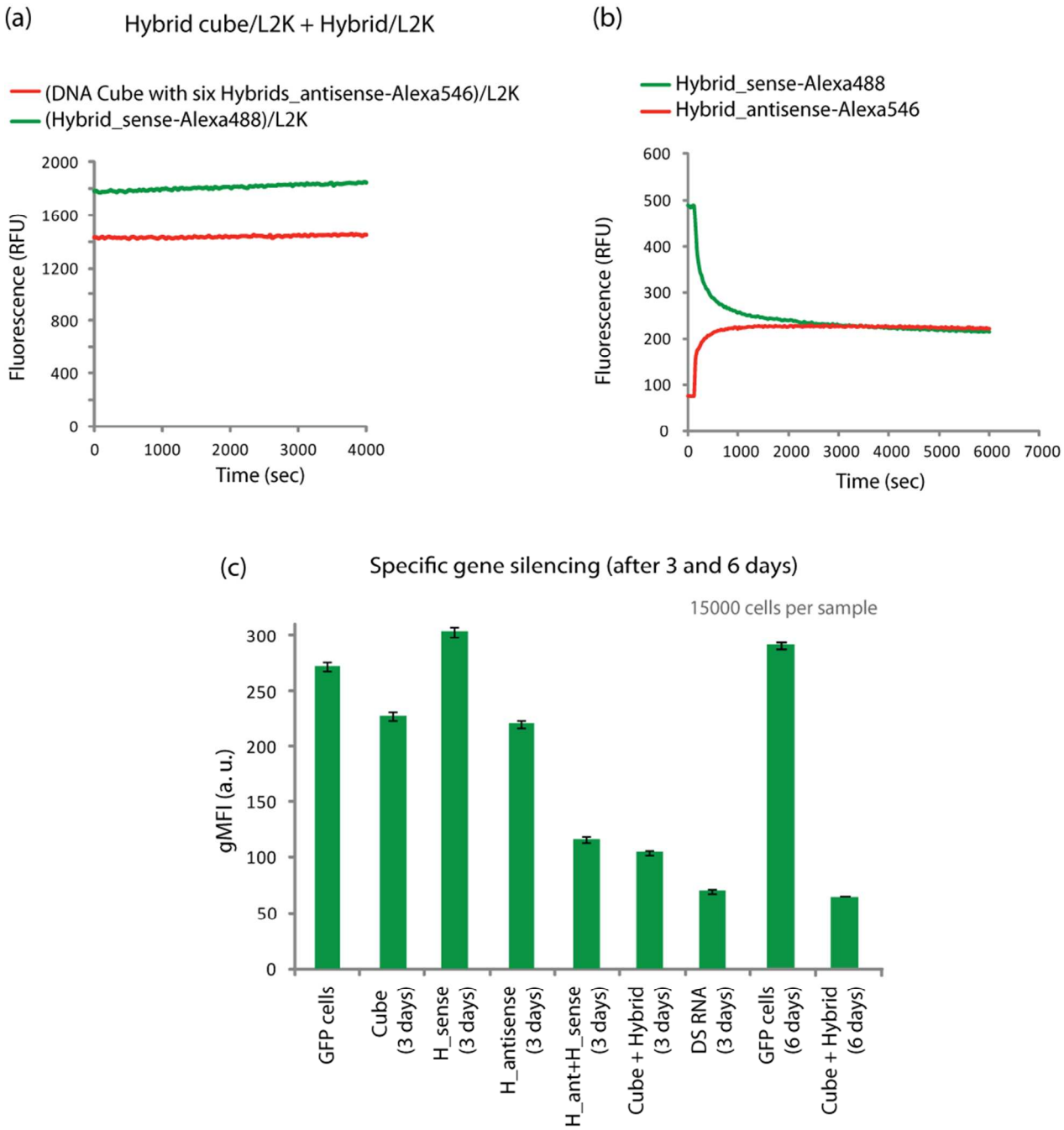

Figure S5. Activation of split functionalities with 3'-side functionalized DNA cubes carrying hybrids with antisenses of DS RNA and six cognate hybrids carrying sense strands of DS RNA. **(a)** Fluorescently labeled cubes and hybrids individually associated with L2K prior to mixing were followed by fluorescent time tracing. Please note that L2K forms complexes with cubes and hybrids thus, preventing their re-association. **(b)** FRET time traces during re-association of hybrids fluorescently labeled with Alexa488 and Alexa546. **(c)** GFP knockdown assays for human breast cancer cells expressing GFP (MDA-MB-231/GFP). Three and six days after the transfection of cells, eGFP expression was analyzed with flow cytometry experiments. As a control, siRNA duplexes against eGFP were used. Please note that the individual hybrids and DNA cubes decorated with hybrids cause no decrease in eGFP production. gMFI corresponds to the geometric mean fluorescence intensity. Error bars denote SEM

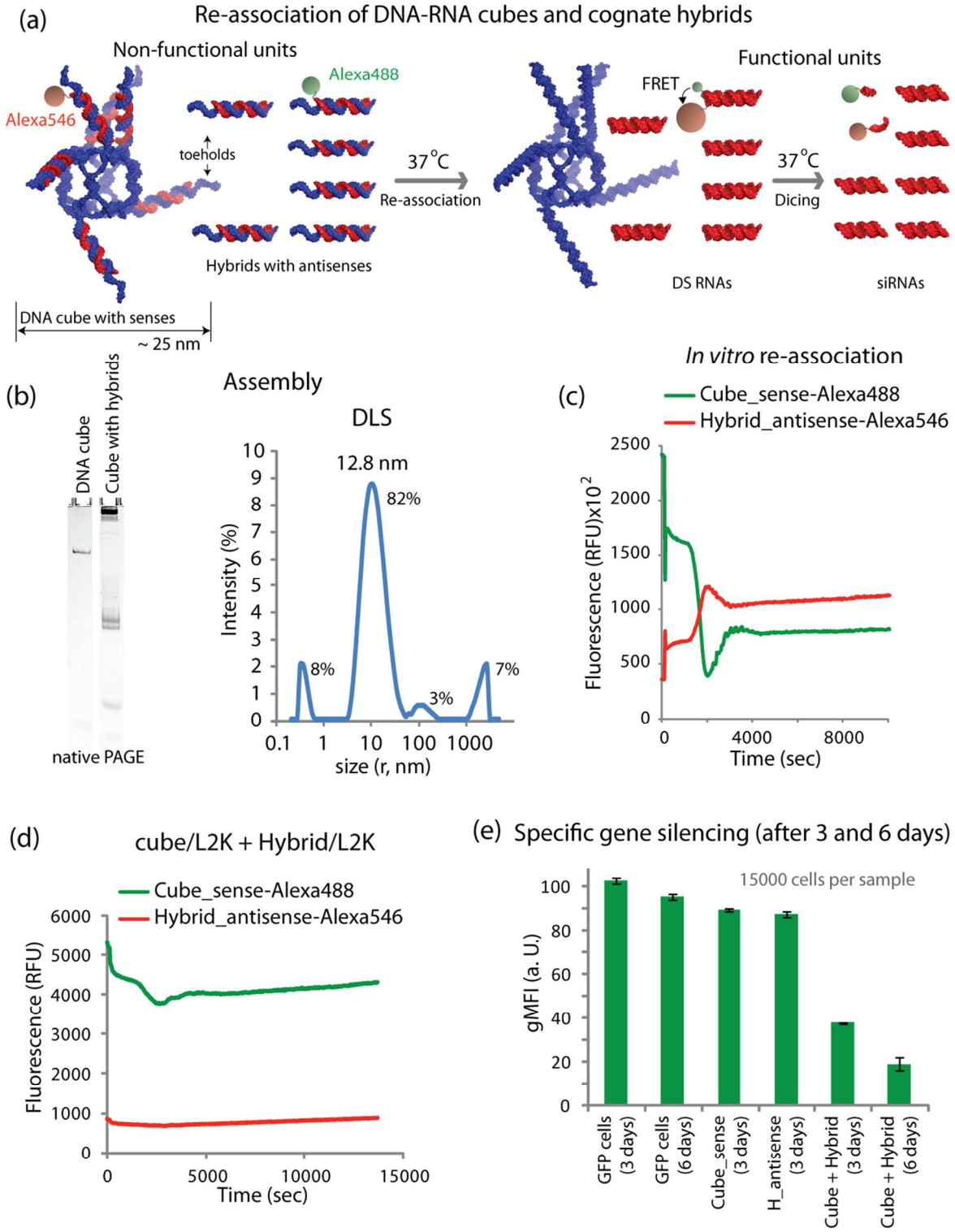

Figure S6. Activation of split functionalities with DNA cubes 5'-side decorated with RNA-DNA hybrids (carrying senses of DS RNA) and six cognate hybrids (carrying antisenses of DS RNA). **a)** Schematics of re-association and activation of FRET and RNAi. Preliminary modeling of cubic scaffolds with functionalized 5'-ends of the building strands indicates that RNA cubes may experience

structural collisions and entanglements in the self-assembly process, while the DNA-based cubes provide less sterically constraining scaffolds. **(b)** The formation of cubes was confirmed by total SYBR Gold staining native PAGE and DLS experiments. **(c)** FRET time traces during re-association of fluorescently labeled cubes and hybrids labeled with Alexa488 and Alexa546. **(d)** Fluorescently labeled cubes and hybrids individually associated with L2K prior to mixing were followed by fluorescent time tracing. Please note that L2K forms complexes with cubes and hybrids thus, preventing their re-association. **(e)** GFP knockdown assays for human breast cancer cells expressing enhanced GFP (MDA-MB-231/GFP). Three and six days after the transfection of cells, eGFP expression was analyzed with flow cytometry experiments. As the control, siRNA duplexes against eGFP were used. Please note that the individual hybrids and DNA cubes decorated with hybrids cause no decrease in eGFP production. gMFI corresponds to the geometric mean fluorescence intensity. Error bars denote SEM.

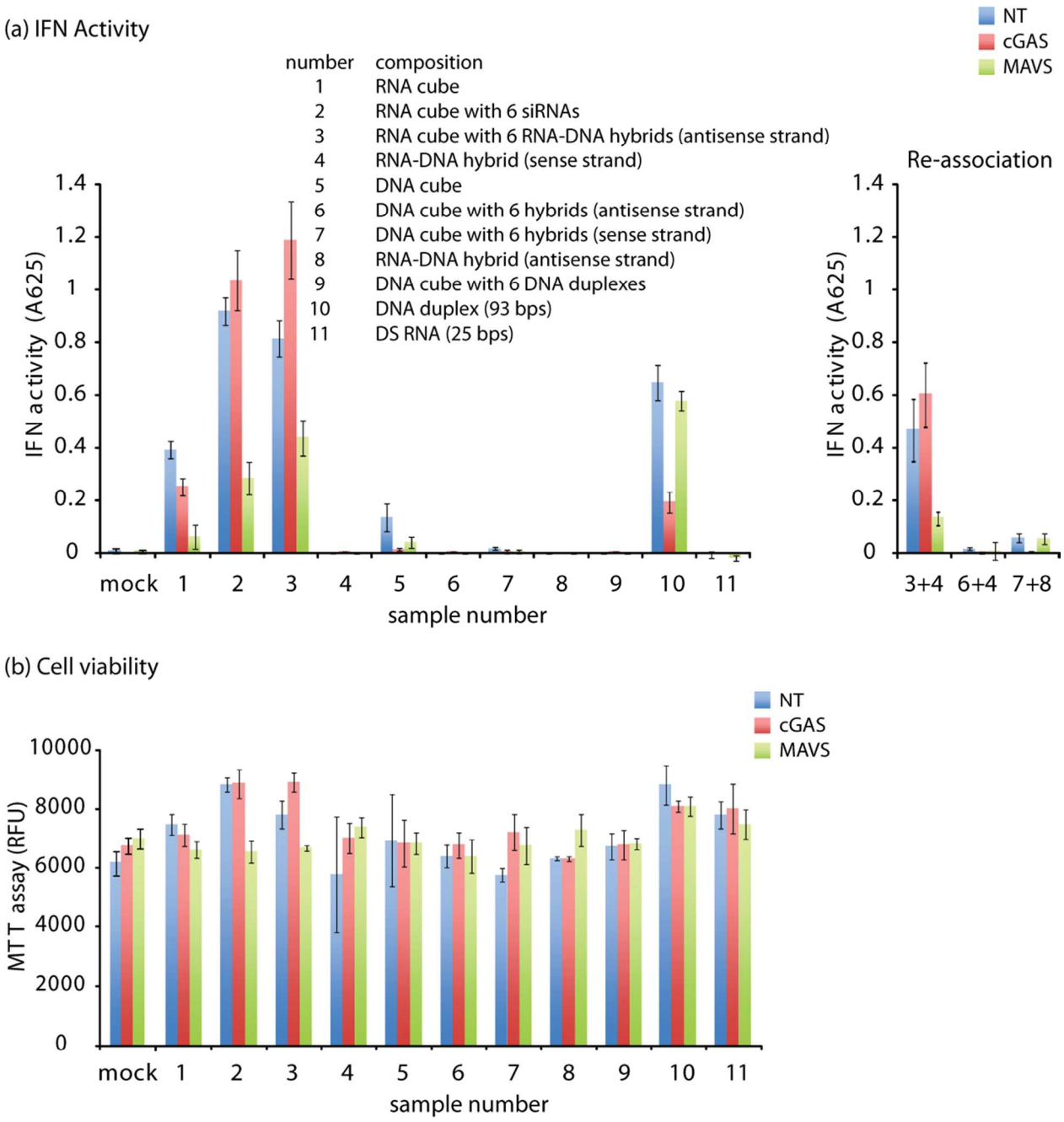

Figure S7. For IFN-activity experiments (**a**), THP-1 IFN reporter cells were depleted of cGAS or MAVS by siRNA and differentiated with PMA prior to experiments. Cells were transfected with indicated nucleic acids individually or in combination and secreted alkaline phosphatase activity was measured in culture supernatants 24 hours post-transfection. Cell viability (**b**) was assessed by MTT assay. Error bars denote SD; N=3.

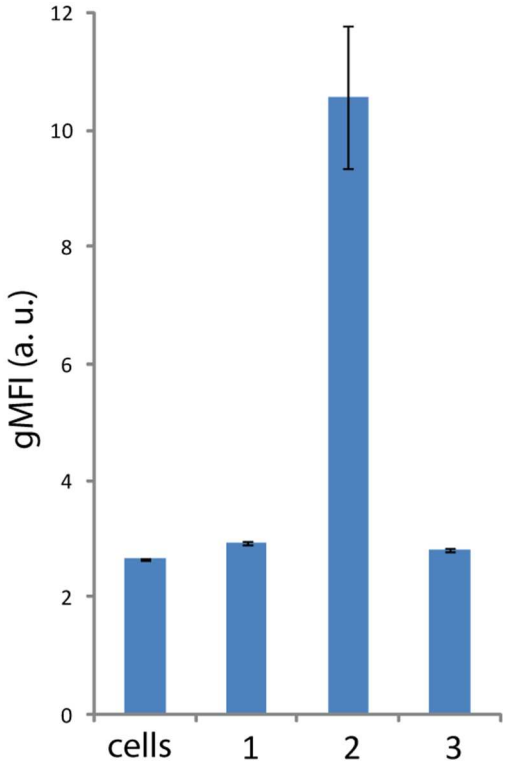

Figure S8. Cellular binding of fluorescently labeled RNA cubes functionalized with multiple DS RNAs and with DNA aptamer Sgc8c[5, 6] selected to specifically bind human protein tyrosine kinese-7 (PTK-7) presented on the cell surface of human acute lymphoblastic leukemia. CCRF-CEM cells were incubated at room temperature with (1) RNA cubes carrying 3 fluorescently labeled DS RNAs at 30 nM, (2) RNA cubes carrying 3 fluorescently labeled DS RNAs at 30 nM and one Sgc8c aptamers, and (3) Fluorescently labeled DS RNAs at three times higher concentration (100 nM). Samples were statistically analyzed 0.5 hour later using flow cytometry. The results demonstrate significant increase in cellular binding upon introduction of aptamer. More detailed studies on relative uptake and silencing efficiency with targeted RNA and/or DNA multifunctioinal nanoparticles are currently being conducted (Afonin et al. in preparation).

References

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