Supporting Information

Cell-Membrane-Anchored DNA Nanoplatform for Programming Cellular Interactions

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Table of Contents

1. Reagents and materials ……..…….…... S3

2. Cell lines and cell culture ……..…… S3

3. Experimental section ……..…...… S3

3.1 Fabrication of DNA tetrahedral structures ……... S3

3.2 Native polyacrylamide gel electrophoresis (PAGE) ……..…... S3

3.3 Atomic force microscopy (AFM) imaging ……..…….. S3

3.4 Confocal Laser Scanning Microscopy (CLSM) Imaging ……..…….…..... S3

3.5 Flow cytometry assay ……..…….... S3

3.6 DNA hybridization-based cell-cell interactions ……..……...…….... S4

3.7 Aptamer recognition-based cell-cell interactions ……...…… S4

3.8 Intracellular signaling events triggered by intercellular contacts ……...…… S4

3.9 Construction of multicellular assembly ……..…….……...……..... S4

4. Supplementary Figures ……..……..……. S5

Figure S1-S16 ……..……..……..……. S5-S12

5. Supplementary

Tables ……..……..…..…… S13

Table S1-S3 …….. S13-S14

1. Reagents and materials

All DNA oligonucleotides were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). CellTracker™ Dyes (green, blue and red) and DNase I were purchased from Thermo Fisher Scientific (New York, USA). Bovine serum albumin (BSA), Triton X-100 and 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) were bought from Beyotime biotechnology. (Jiangsu, China). 4% Paraformaldehyde were purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. Rabbit polyclonal IgG were bought from Santa Cruz Biotechnology. Goat anti-rabbit IgG were purchased from Immunoreagents Inc., Raleigh, NC. Tumor necrosis factor (TNF) kit were bought from Mercker Biotechnology Co., Ltd. (Wuhan, China). Lipopolysaccharide (LPS) were purchased from sigma.

2. Cell lines and cell culture.

All cells used in this experiment, including CCRF-CEM, Ramos, K562, A549, Hela, MCF-7 and Raji B cells were purchased from ATCC. All the cells were cultured in fresh 1640 medium supplemented with 10% FBS (Gibco) and 0.5 mg/mL penicillin–streptomycin (Cellgro) at 37 ℃ with 5% CO_2. FBS used in all experiments was heat-inactivated. Unless other stating, CEM was used as the testing cell line.

3. Experimental section

3.1 Fabrication of DNA tetrahedral structures. Four DNA oligonucleotides (S1, S2, S3 and S4) were mixed together in 1x PBS (pH 7.4) plus 5 mM $MgCl_2$, and the final concentration of each oligonucleotide was 2 μM. The mixture was slowly annealed from 95 ℃ to room temperature (RT). The resultant DNA products were stored at 4 ℃ for further use.

3.2 Native polyacrylamide (PAGE) gel electrophoresis. To characterize the construction of DNA tetrahedron structures, each DNA sample (10 μL, 1 μM) was mixed with 6× loading buffer (2 μL). Electrophoresis was performed in 1×Tris-acetic acid-EDTA/Mg^{2+} buffer (40 mM Tris-acetic acid, 12.5 mM magnesium acetate and 1 mM EDTA, pH 8.0) at 110 V for 45 min. After that, the polyacrylamide gel was stained with Stains-All (Sigma-Aldrich) for 15 min and washed 2 times before imaging on a Bio-Rad molecular imager under UV light. To assess the structural stability, Cy5-labeled DNA tetrahedrons (150 nM) were incubated in fresh 1640 medium plus 10% FBS at 37 ℃ for different time spans. Then, 10 μL of the sample was mixed with glycerin (2 μL). Electrophoresis was performed in 1×Tris-acetic acid-EDTA/Mg^{2+} buffer (40 mM Tris-acetic acid, 12.5 mM magnesium acetate and 1 mM EDTA, pH 8.0) at 110 V for 30 min. Finally, gels were imaged on a SageCapture™ molecular imager under 635 nm red light excitation. The data were analyzed with ImageJ software.

3.3 Atomic force microscopy (AFM) imaging. The DNA sample (15 μL) was mixed with $NiCl_2$ (300 μM, 5 μL) for 5 min. The mixture was deposited on mica and dried for 10 min. After being rinsed with ultrapure water three times and dried in nitrogen atmosphere, the sample was imaged on a Multimode 8 (Bruker/USA) using the ScanAsyst mode and analyzed with the Nanoscope analysis software.

3.4 Confocal Laser Scanning Microscopy (CLSM) Imaging. 1) For the colocalization experiment, CEM cells (10^5) were first incubated with a membrane indicator dye (DiI) in fresh 1640 medium for 15 min. After washing 3 times with 1×PBS, the cells were resuspended in 1×PBS and further incubated with a FAM-labeled T-cho3 (250 nM, 100 μL) at RT for 10 min. Finally, cells were washed and resuspended in 1×PBS supplemented with 5 mM $MgCl_2$ for imaging. 2) To evaluate the orientation of DNA probes on the membrane, CEM cells (10^5) were first dispersed in 1×PBS and incubated with a FAM-labeled T-cho3 (250 nM, 100 μL) at RT for 10 min. After being washed with 1×PBS for 3 times, the cells were imaged with CLSM. Subsequently, DNase I （1U/μL） was added to the confocal dish and allowed to incubate for 1 min. After that, the cells were imaged with CLSM again. 3) To evaluate membrane-anchoring capability of different amphiphilic DNA probes, CEM cells (10^5) were first incubated with FAM-labeled S-cho1, S-cho3, T-cho0, T-cho1, T-cho2 or T-cho3 (250 nM, 100 μL) at RT for 10min. After that, cells were washed 3 times with 1×PBS and further incubated in fresh 1640 medium plus 10% FBS at 37 ℃ with 5% CO_2 for different time spans. After being washed with 1×PBS again, the cells were imaged with the FV1000-X81 confocal microscope (Olympus), and data were analyzed with ImageJ software. 4) To evaluate the internalization of different amphiphilic DNA probes, CEM cells (10^5) were incubated with FAM-labeled S-cho1, T-cho1, or T-cho3 (250 nM, 100 μL) at RT for 10 min. Then, the cells were washed with 1×PBS, resuspended in 1×PBS and allowed to further incubate at 37 ℃ for 15 min. After being washed, the cells were imaged with CLSM, and date were analyzed with an ImageJ software.

3.5 Flow cytometry assay. 1) To optimize the initial concentration of T-cho3, CEM cells (10^5) were first washed 3 times with 1xPBS and resuspended in 100 μL 1×PBS. Then, the cells were incubated with FAM-labeled T-cho3 of desired concentrations at RT for 10 min. After being washing 3 times with 1×PBS, the cells were resuspended in 1×PBS and detected with a BD FACSVerse™ flow cytometer. 2) To optimize the incubation time of T-cho3, CEM cells (10^5) were first washed 3 times with 1xPBS and incubated with 250 nM FAM-labeled T-cho3 for a certain length of time. Then, the cells were washed 3 times with 1×PBS and analyzed with flow cytometry. 3) To characterize DNA hybridization-based cell-cell interactions, CEM cells (10^5) were first stained with an APC dye or an FITC dye by following the protocol mentioned bellow. Then, the APC-stained cells were modified with T-cho3-probe1 (250 nM) and the FITC-

stained cells were modified with T-cho3-probe2 (250 nM). After that, these two cell batches were mixed together at a 1:10 ratio in 1×PBS supplemented with 5 mM $MgCl_2$. Subsequently, a DNA linker $L_{1\text{-}2}$ that could link the protruded probe1 and the protruded probe2 together through DNA hybridization was added to the cell mixture (the final concentration of $L_{1\text{-}2}$ = 50 nM). After shaking at 240 rpm for 30 min, the cells were detected with Cytek DxP Athena flow cytometer.

3.6 DNA hybridization-based cell-cell interactions. 1) To stain cells with color, cells (10^5) were first washed 3 times with 1xPBS and incubated with prewarmed CellTracker™ working solution (1 μM) at 37 °C for 15 min. After removal of the staining solution by centrifuging, the cells were resuspended in fresh 1640 medium plus 10% FBS at 37 °C with 5% CO_2 for further use. 2) To regulate cell assembly and disassembly, red-stained CEM cells (10^5) were incubated with T-cho3-probe1 (250 nM, 100 μL) at RT for 10 min and unstained CEM cells (10^5) were incubated with T-cho3-probe2 (250 nM, 100 μL) at RT for 10 min. Then the two cell batches were washed with 1xPBS and mixed together at a 1:10 ratio in 1×PBS supplemented with 5 mM $MgCl_2$. A DNA linker (termed $L_{1\text{-}2}$, final concentration = 50 nM) that could link the protruded probe1 and the protruded probe2 together through DNA hybridization was added. The mixture was shaken at 240 rpm for 30 min. After that, the cells were analyzed with CLSM. To induce cell disassembly, a cDNA of $L_{1\text{-}2}$ ($cL_{1\text{-}2}$, 250 nM) was added. After shaking at 240 rpm for another 20 min, the resultant samples were imaged with CLSM.

3.7 Aptamer recognition-based cell-cell interactions. 1) For cell type-specific assembly, K562 cells were labeled with a red dye and modified with T-cho3-sgc8 (250 nM). CEM cells and Ramos cells were labeled with a green and a blue dye, respectively. Then these three cell batches were mixed together at a 1:10:10 ratio under shaking for 30 min in 1×PBS with 5 mM Mg^{2+}. The resultant samples were imaged with CLSM. 2) For tunable binding strength, red-stained Ramos cells were modified with S-cho1-sgc8, T-cho1-sgc8, T-cho2-sgc8 or T-cho3-sgc8, and then mixed with untreated CEM cells at a ratio of 1:10 in 1×PBS supplemented with 5 mM $MgCl_2$ and 2 mg/mL BSA. After shaking for certain time spans, the samples were analyzed with CLSM.

3.8 Intracellular signaling events triggered by intercellular contacts. A549 cells were seeded in a 35-mm confocal dish and incubated for 24 h. Raji B cells ($3x10^5$) were first washed three times with 1xPBS and then cultured in fresh 1640 medium (10% FBS) with 10 μg/mL LPS for 3 h. Subsequently, A549 cells and Raji B cells were modified with T-cho3-probe1 (250 nM) and T-cho3-probe2 (250 nM), respectively. After removal of free probes by washing with 1x PBS, $L_{1\text{-}2}$ (50 nM) was added to the mixture of T-cho3-probe1-modified A549 cells and T-cho3-probe2-modified Raji B cells. The resultant cells mixture was gently shaken at RT for 10 min, supplemented with fresh 1640 medium plus 1% FBS, and further incubated at 37 °C for 3 h. After washing three times with 1xPBS, the cells were fixed with 4% paraformaldehyde (PFA) for 20 min, permeabilized with 0.2% triton for 30 min, and then blocked with 5% BSA in PBS for 1 h. The cells were stained with a primary antibody against NF-κB at 4 °C overnight, and then stained with a PE-labeled secondary antibody for 1 h. After being stained with Hoechst 33258 at RT for 5 min, the cells were imaged with CLSM. The imaging data were analyzed with ImageJ software.

3.9 Construction of multicellular assembly. A549 cells were seeded in a confocal dish and cultured for 24 h before experiments. To distinguish three batches of cells in the mixed population, A549 cells, CEM cells and Ramos cells were labeled with a blue cytosolic fluorescence dye, a green cytosolic fluorescence dye and a red cytosolic fluorescence dye, respectively. Then both A549 cells and Ramos cells were modified with T-cho3-probe1, and CEM cells were modified with T-cho3-probe2 by following the above-mentioned protocol. Excess T-cho3-probe2-modified CEM cells and $L_{1\text{-}2}$ (50 nM) were added to the dish of T-cho3-probe1-modified A549 and allowed to incubate at RT under gentle shaking for 10 min. After washing to remove unbound cells, excess T-cho3-probe1-modified Ramos cells and $L_{1\text{-}2}$ (50 nM) were added and allowed to incubate at RT under gentle shaking for 10 min. After removal of unbound Ramos cells and free $L_{1\text{-}2}$, the resultant cell samples were imaged samples were imaged.

4. Supplementary Figures

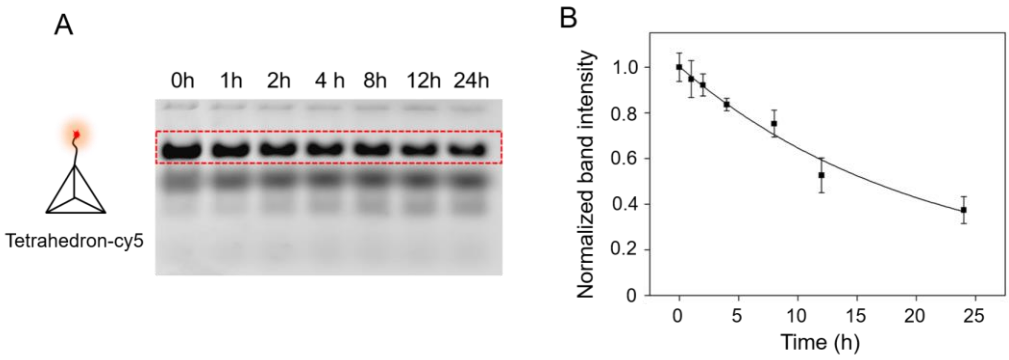

Figure S1. (A) PAGE gel assay. The DNA tetrahedron was incubated in fresh culture medium plus 10% FBS at 37 °C for different time spans before gel testing. (B) Normalized band intensity of corresponding samples of A. The band intensity at the time point of 0 h was set as 100%. Three independent experiments were performed.

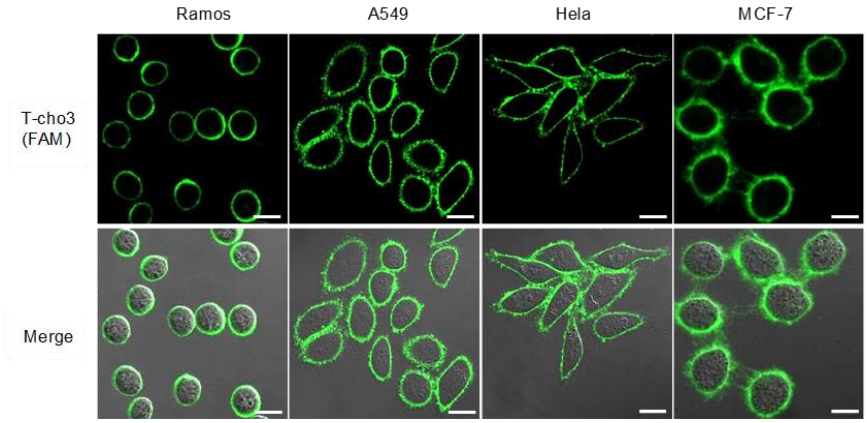

Figure S2. CLSM images of different cell lines after treatment with FAM-labeled T-cho3 (250 nM)) at RT for 10 min. Scale bars represent 10 μm.

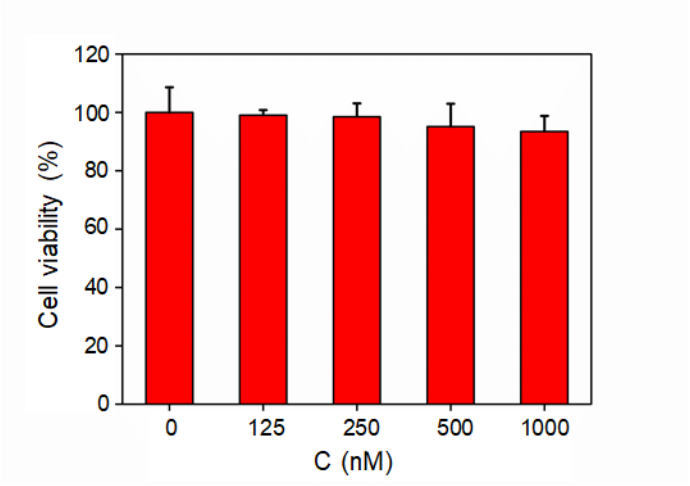

Figure S3. Cytotoxicity assay of T-cho3. CEM cells (3×10^4) were incubated with T-cho3 of different concentrations in culture medium at 37 ºC with 5% CO_2 for 48 h, followed by analysis with 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetra-zolium (MTS) assay. Error bars represent the standard deviation of three independent experiments.

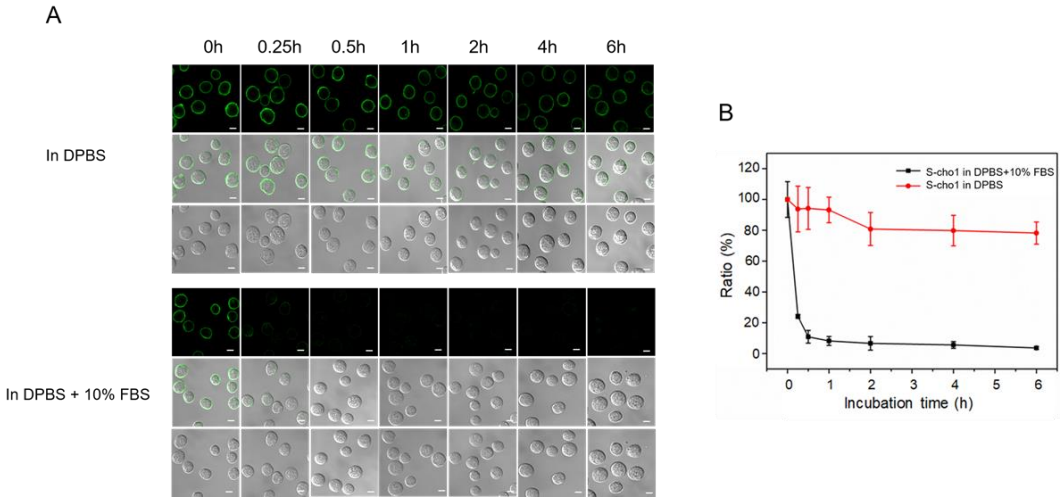

Figure S4. Evaluating the membrane-anchoring ability of S-cho1 in different conditions. (A) CLSM images. CEM cells were first incubated with 250 nM FAM-labeled S-cho1 at RT for 10 min. After being washed with DPBS for three times, the cells were resuspended in DPBS or 10% FBS-containing DPBS, and allowed to incubate for different time spans at 4 ºC before CLSM imaging. Scale bars represent 10 μm. (B) Relative fluorescence reduction of corresponding samples of A. The relative fluorescence intensity at the time point of 0 h was set as 100%. Over 100 cells were measured with an ImageJ software for each sample group, and three independent experiments were performed. Error bars represent the standard deviation of three independent experiments.

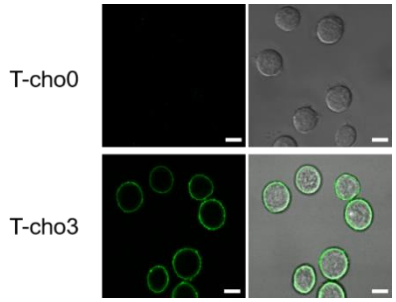

Figure S5. CLSM images of CEM cells after incubation with FAM-labeled T-cho0 (top) or FAM-labeled T-cho3 (down) at RT for 10 min. The concentration of both T-cho0 and T-cho3 was 250 nM. Scale bars represent 10 μm.

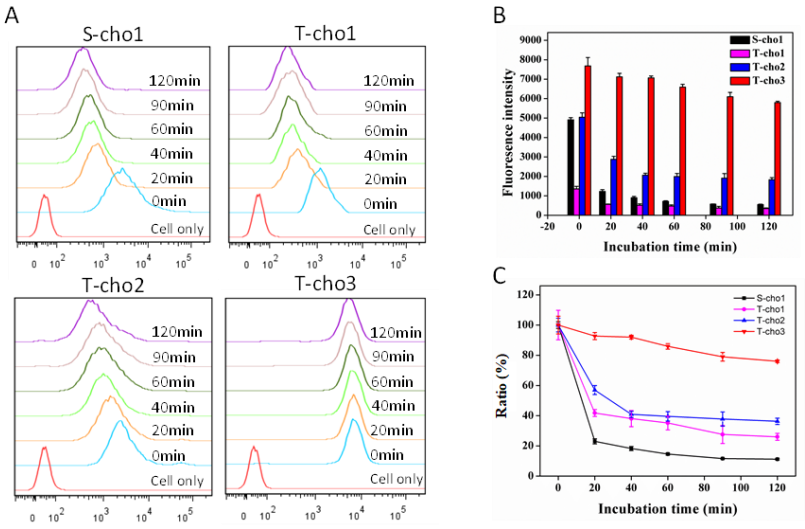

Figure S6. Evaluating the membrane-anchoring capability of different amphiphilic DNA probes. (A) Flow cytometry assay of cells modified with fluorescent S-cho1, T-cho1, T-cho2 or T-cho3, resuspended in culture medium plus 10% FBS, and further incubated at 37 ºC for different time spans. (B) Statistical analysis of the absolute fluorescence intensity of corresponding samples. (C) Relative fluorescence change of corresponding samples. The fluorescence intensity of each sample at the time point of 0 min was set as 100%. Error bars represent the standard deviation of three independent experiments.

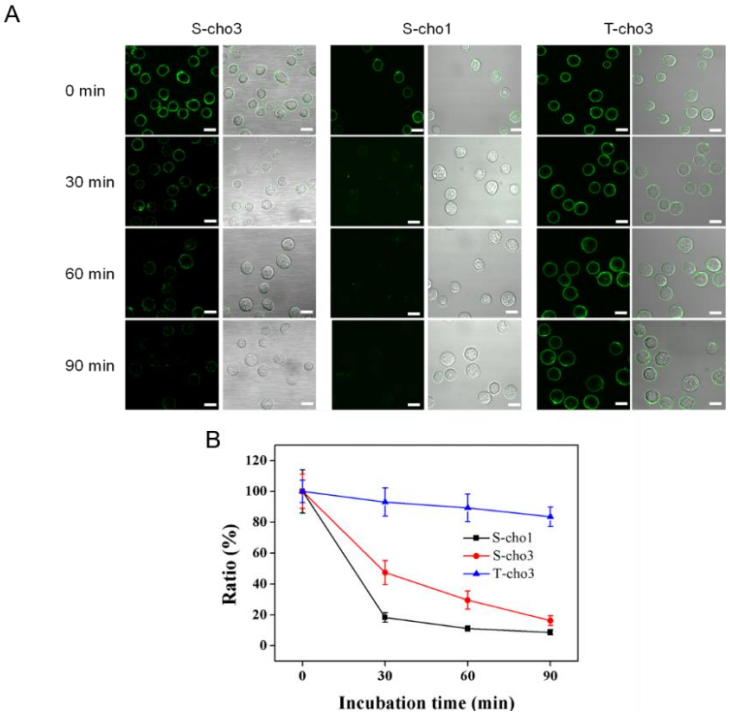

Figure S7. (A) CLSM images. CEM cells (10^5) were first modified with 250 nM FAM-labeled S-cho3, S-cho1 or T-cho3. After being washed 3 times with 1×PBS, the cells were further incubated in fresh 1640 medium plus 10% FBS at 37 °C with 5% CO_2 for different time spans. Scale bars represent 10 μm. (B) Relative fluorescence change of corresponding samples of A. The fluorescence intensity of each sample at the time point of 0 min was set as 100%. Over 100 cells were measured with an ImageJ software for each sample group, and three independent experiments were performed. Error bars represent the standard deviation of three independent experiments.

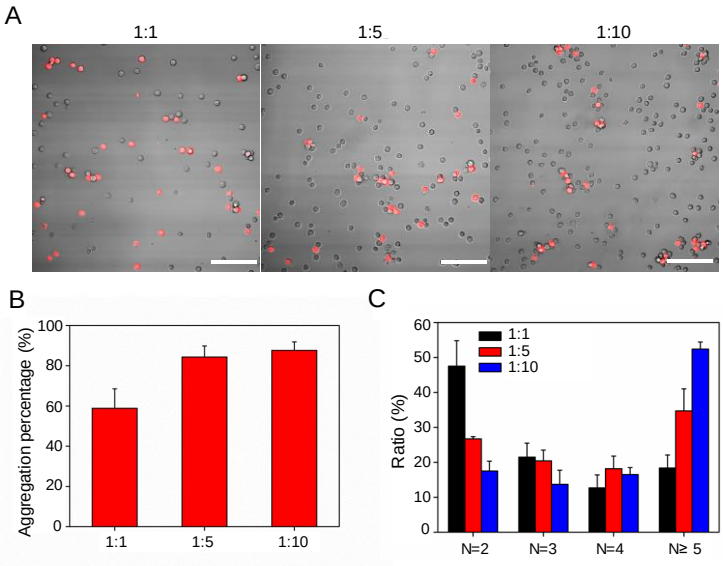

Figure S8. Impact of the cell ratio on the efficiency of cell aggregation. (A) CLSM imaging. T-cho3-probe1-modified cells (red) and T-cho3-probe2-modified cells (colorless) were mixed together at a ratio of 1:1, 1:5 or 1:10 in the presence of DNA linker. (B) Efficiency of cell aggregation at different cell ratios. (C) Percentages of the cell constructs at different cell ratios. The scale bar represents 100 μm.

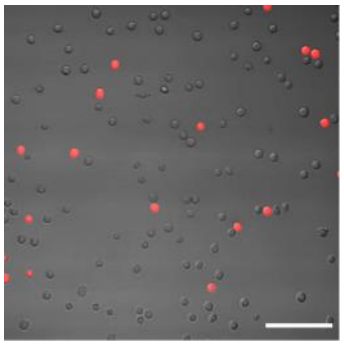

Figure S9. CLSM imaging. Two batches of unmodified cells were mixed together at a ratio of 1:10 (red and colorless). The scale bar represents 100 μm.

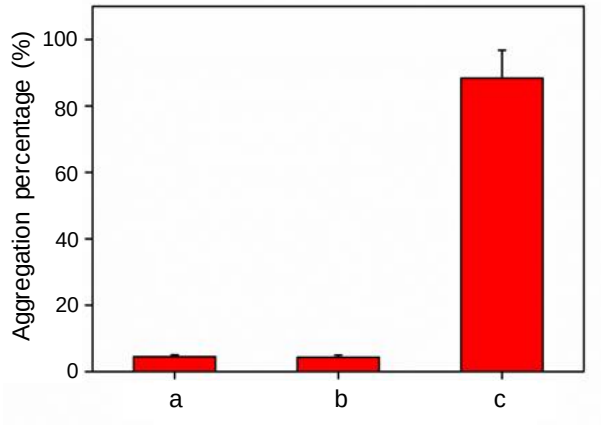

Figure S10. Percentage of cell aggregation in different conditions: the mixture of two unmodified cells (sample a); the mixture of T-cho3-probe1-modified cells (red staining) and T-cho3-probe2-modified cells (colorless) in the absence (sample b) and presence of the DNA linker (sample c). In all conditions, the red cells and the colorless cells were mixed at a ratio of 1:10. The aggregation percentage was calculated according to the percentage of red cells that were bound with at least one colorless cell.

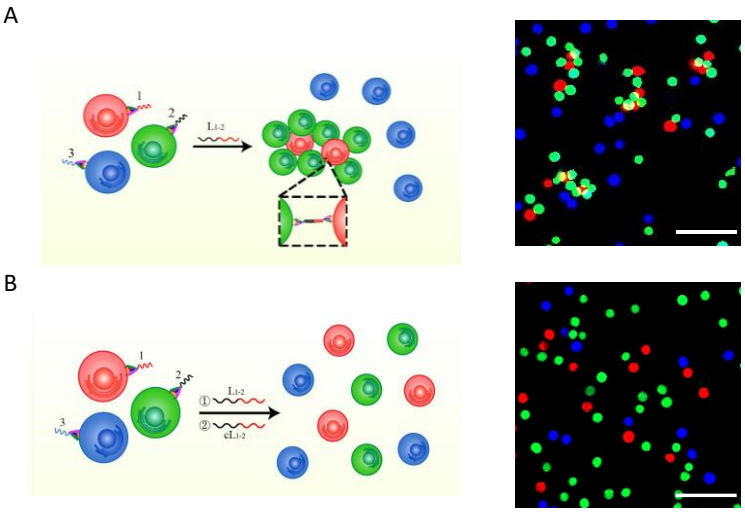

Figure S11. Specific control over cell-cell interactions via DNA hybridization. (A) Schematic illustration (left); CLSM imaging, T-cho3-probe1-modified cells (red), T-cho3-probe2-modified cells (green) and T-cho3-probe3-modified cells (blue) were mixed together at a ratio of 1:10:10 in the presence of DNA linker $L_{1\text{-}2}$ able to join together protruded probe1 and protruded probe2 (right). (B) Schematic illustration (left); CLSM images of the mixture described above with addition of a cDNA of $L_{1\text{-}2}$ (right). The scale bar represents 100 μm.

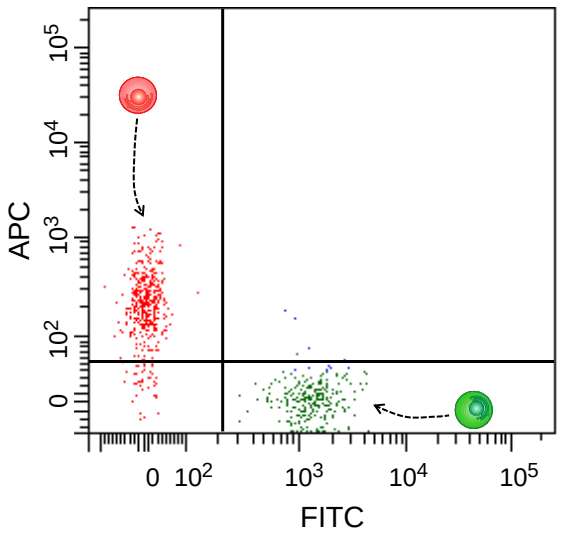

Figure S12. Flow cytometry assay. T-cho3-probe1-modified cells (stained with an APC dye) and T-cho3-probe2-modified cells (stained with an FITC dye) were mixed and shaken at 240 rpm for 30 min before flow cytometry testing.

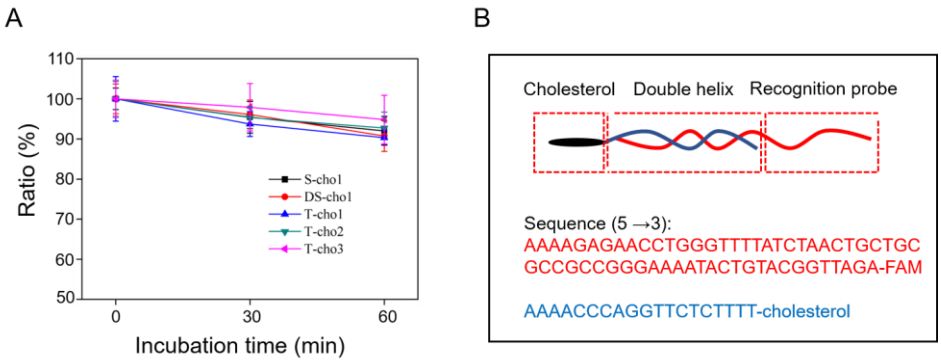

Figure S13. (A) Evaluating the membrane-anchoring stability of S-cho1, DS-cho1, T-cho1, T-cho2 and T-cho3 in FBS-free culture medium. After modification with FAM-labelled S-cho1, DS-cho1, T-cho1, T-cho2 or T-cho3, CEM cells were washed and resuspended in FBS-free culture medium and allowed to incubate for different time spans before CLSM imaging. The data were analyzed with an ImageJ software. The relative fluorescence intensity at the time point of 0 h was set as 100%. Error bars represent the standard deviation of three independent experiments. (B) Schematic diagram of DS-cho1.

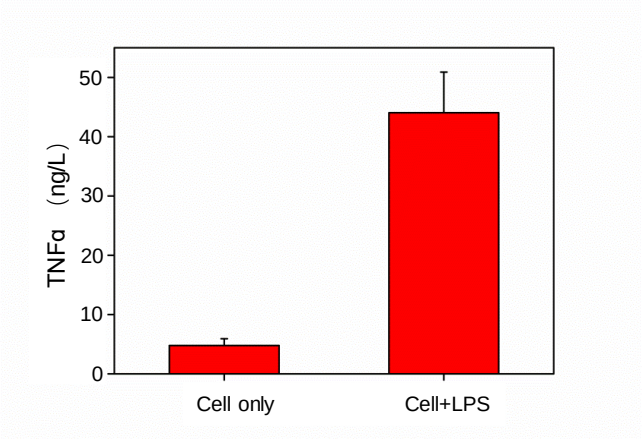

Figure S14. Quantification of TNFα secretion with ELISA assay. Raji B cells ($3x10^5$) were stimulated by Lipopolysaccharide (LPS, 10 μg/mL) in fresh 1640 medium (200μl) supplemented with 10% FBS for 3 h. Subsequently, the secreted TNFα in the supernatant was tested with an ELISA kit by following the manufacturer's manual.

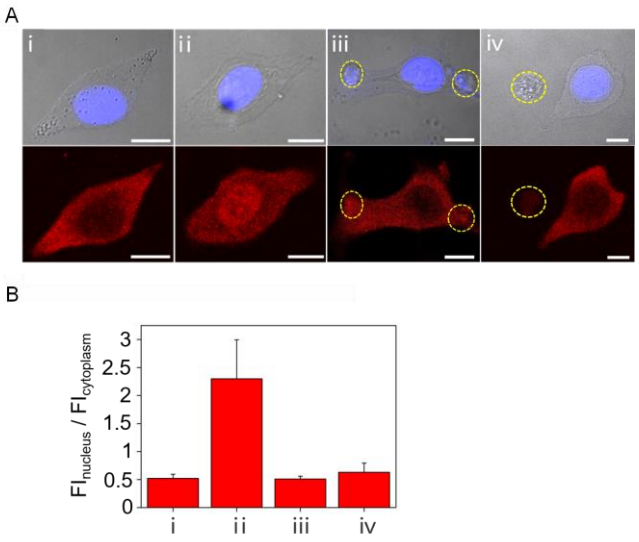

Figure S15. (A) CLSM images of T-cho3-probe1-modified A549 cells (i), T-cho3- probe1-modified A549 cells stimulated with 2.5 ng/mL TNFα (ii), T-cho3-probe1-modified A549 cells bound with unstimulated Raji B cells (iii) and A549 cells mixed with LPS-stimulated Raji B cells (iv). Scale bars represent 10 μm. From top to bottom: overlay of the DAPI channel and the bright-field, the fluorescence channel of PE-anti NF-κB. (B) Corresponding normalized ratio between the PE fluorescence intensity in nucleus and that in cytoplasm. At least 30 A549 cells were calculated for each sample group, and three independent experiments were performed.

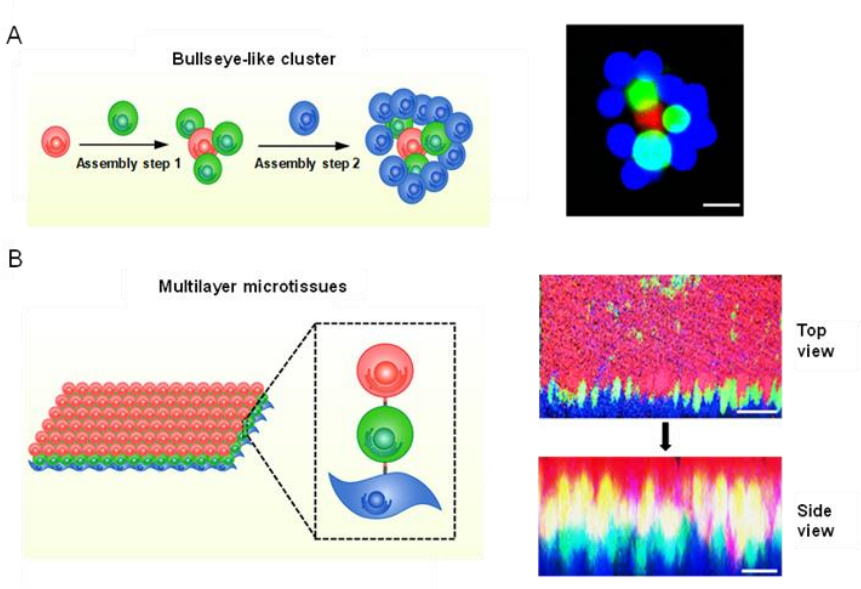

Figure 16. Fabrication of multicellular networks with defined connectivity. (A) Schematic illustration (left) and fluorescence images (right) of assembly of bullseye-like cell cluster. Scale bar represents 10 μm. (B) Schematic illustration (left) and fluorescence images (right) of assembly of multilayer cell assembly. Scale bars represent 50 μm (up) and 10 μm (down), respectively.

5. Supplementary Tables

Table S1. DNA sequences used in this study.

Name	Strand components(5'→3')
S1	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATACTT
S2	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTCTT
S3	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCATTT
S1-cho	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATACTT-cholesterol
S2-cho	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTCTT-cholesterol
S3-cho	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCATTT-cholesterol
S4	CCCAGGTTCTCTTTTTTTACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
S4-FAM	FAM-CCCAGGTTCTCTTTTTTTTACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
cDNA	AAGAGAACCTGGGGGAGTATTGCGGAGGAAGGT
S-cho1	CCCAGGTTCTCTT-/i6FAMdT/-TTTTTTTTTTTTTT-cholesterol
S-cho3	CCCAGGTTCTCTT-/i6FAMdT/-TTTTTTTTTTTTTT-cholesterol-cholesterol-cholesterol
Probe1	TTGTATCCAGTGGCTCATTTTTTTTACATTCCTAAGTCTGAAACATTACAGCTTGCTACCGAGAAGAGCCGCCATAGTA
Probe2	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTATTTTTTCCAGAGACATCCAGT
Probe3	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTATTTCGTAGGCAGTCGGCGACAAT
L-11	ACTGGATACAATAGCAGGTTCTCTACTGGATSTCT
L-13	CCACTGGATACAATAGCAGGTTCTCTACTGGATGTCTCT
L-15(L1-2)	AGCCACTGGATACAATAGCAGGTTCTCTACTGGATGTCTCTGG
cL1-2	CAGAGACATCCAGTGAGAGAACCTGCTAATGTATCCAGTGGCT
Probe sgc8	AAAAGAGAACCTGGGTTTTATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA
Lib	AAAAGAGAACCTGGGTTTTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN

Table S2. Quantification of the cell surface density of different DNA probes.

Name	T-cho3	T-cho2	T-cho1	S-cho1
Number of probes / μm^2	587.3±20.7	424.2±23.1	113.1±3.9	411.4±11.2

Note: The cell surface density was estimated based on a cell diameter of 10 μm.

Table S3. The percentage of the cell clusters formed with 2, 3, 4 and ≥5 cells in the samples of L-11, L-13 or L-15.

Linker	Incubation time (min)	Percentage of 2-cell clusters (mean±s.d.%)	Percentage of 3-cell clusters (mean±s.d.%)	Percentage of 4-cell clusters (mean±s.d.%)	Percentage of ≥5-cell clusters (mean±s.d.%)
L-11	10	89.0 ±6.7	5.5 ±2.6	4.1 ±1.6	0.0 ±0.0
	20	71.8 ±11.2	16.5 ±6.0	8.7 ±3.0	2.9 ±1.9
	30	55.4 ±11.8	19.0 ± 6.9	14.3 ±5.0	11.3 ±3.6
	40	44.6 ±8.3	21.7 ±1.85	5.4 ±5.1	18.3 ±6.0
	60	35.6 ±4.0	25 ±3.9	16.9 ±6.6	22.5 ±8.5
	90	32.7 ±3.7	23 ±6.4	16.3 ±3.6	28.1 ±6.4
	120	30.8 ±2.4	23.7 ±2.2	17 ±2.4	28.5 ±5.3
L-13	10	40.8 ±4.5	25.1 ±0.7	20.9 ±5.4	13.2 ±3.4
	20	36.8 ±4.3	20.3 ±1.2	22.6 ±4.8	20.3 ±9.3
	30	30.2 ±4.0	19 .3 ±2.4	24.9 ±6.5	25.6 ±3.9
	40	22.8 ±3.7	21.3 ±7.1	24.2 ±4.5	31.7 ±7.7
	60	20.2 ±4.0	22.6 ±2.1	17.7 ±5.3	39.5 ±.32
	90	19.7 ±2.5	15.3 ±4.2	19.7 ±3.8	45.3 ±4.7
	120	18.3 ±0.7	20.1 ±4.3	18.3 ±6.8	43.2 ±3.1
L-15	10	35.5 ±12.9	24.2 ±2.8	16.1 ±3.8	23.9 ±5.2
	20	29.2 ±1.7	20.8 ±4.2	17.2 ±3.4	32.8 ±6.8
	30	24.9 ±2.9	19.6 ±2.6	14.3 ±4.3	41.3 ±8.8
	40	20.3 ±3.5	17.1 ±1.0	15.2 ±0.86	47.2 ±2.5
	60	13.5 ±2.1	12.1 ±2.1	20.5 ±6.0	53.9 ±8.9
	90	15.8 ±3.0	14.3 ±2.0	15.4 ±3.0	54.4 ±0.68
	120	19.4 ±4.9	15.6 ±2.0	14.5 ±3.2	50.5 ±10.1