Supporting Information

Synthesis of Tetrahedron DNA Nanostructures for Detecting the Activation of Cell Signal Transduction via Specifically Binding to Transcriptional Factors

Ying Zhang[a], Yue Chen[a], Bing Wu[a], Danqing Liu[a], Lengxi Fu[a], Fei Huang[a] *

[a]Central Laboratory, Fujian Key Laboratory of Precision Medicine for Cancer, Key Laboratory of Radiation Biology of Fujian Higher Education Institutions, The First Affiliated Hospital, Fujian Medical University, Fuzhou, Fujian, 350005, China.

*Corresponding author:
Fei Huang, PhD
Central Lab,
First Affiliated Hospital of Fujian Medical University,
No.20, Chazhong Road, Taijiang District, Fuzhou, 350005
China
Tel.: 86-591-87981768
Fax: 86-591-87981028
Email: feifeigood2148@126.com

Table S1 Oligonucleotide sequences and modifications.

Name	Sequences (5'-3')
TA	**FAM-** ACACGCTCACGACCACGACCACGAACACGCACTACCA CTTTTTTACATTCCTAAGTCTGAAACATTACAGCTTGC TACACGAGAAGAGCCGCCATAGTA
TB	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATA GATGCGAGGGTCCAATAC
TC	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAAT CTACTATGGCGGCTCTTC
TD	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTT GTATTGGACCCTCGCAT
cDNA	GCGTGTTCGTGGTCGTGGTCGTGAGCGTGT
Displacement strands (BHQ1)	AGTGGTAGTGCGTGTTCGTGGTCGTGGTCGTGAGCGTG T-**BHQ1**
TA-Smads	**FAM-** TCTGTCTGTCTGTCTGTCTGTCTGTCTGTCTGACTACCA CTTTTTTACATTCCTAAGTCTGAAACATTACAGCTTGC TACACGAGAAGAGCCGCCATAGTA
cD-Smads	CAGACAGACAGACAGACAGACAGACAGACAGA
Displacement strands (BHQ1-Smads)	AGTGGTAGTCAGACAGACAGACAGACAGACAGACAG ACAGA-**BHQ1**

1. FAM-labeled TDNs for hypoxia transcriptional factor α (HIF-1α) consisted of TA, TB, TC, TD, cDNA. The breaklined sequence in TA could hybridize to cDNA, which was the bind motif to HIF-1α.
2. FAM-labeled TDNs for TGF-β signaling consisted of TA-Smads, TB, TC, TD, cD-Smads. The underlined sequence in TA-Smads could hybridize to cD-Smads, which was the bind motif to Smads.

Figure S1

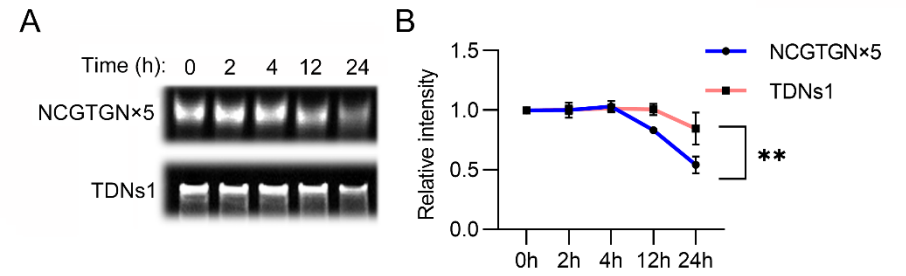

Figure S1 The stability of TDNs. (A) TDNs1 and NCGTGN x5 double strands were treated with 10% FBS-containing medium for the indicated time. Then DNAs (2 μM) were harvested and subjected to PAGE (12%). (B) The band intensity of DNAs in (A) was normalized to the time point 0h and the curves were drawn. $**P<0.01$

Figure S2

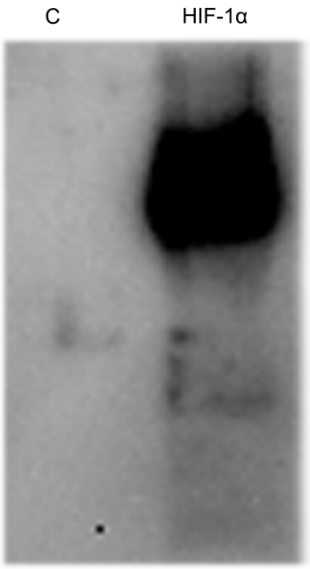

Figure S2 The purified HIF-1α protein was harvested and subjected to immunoblotting.

Figure S3

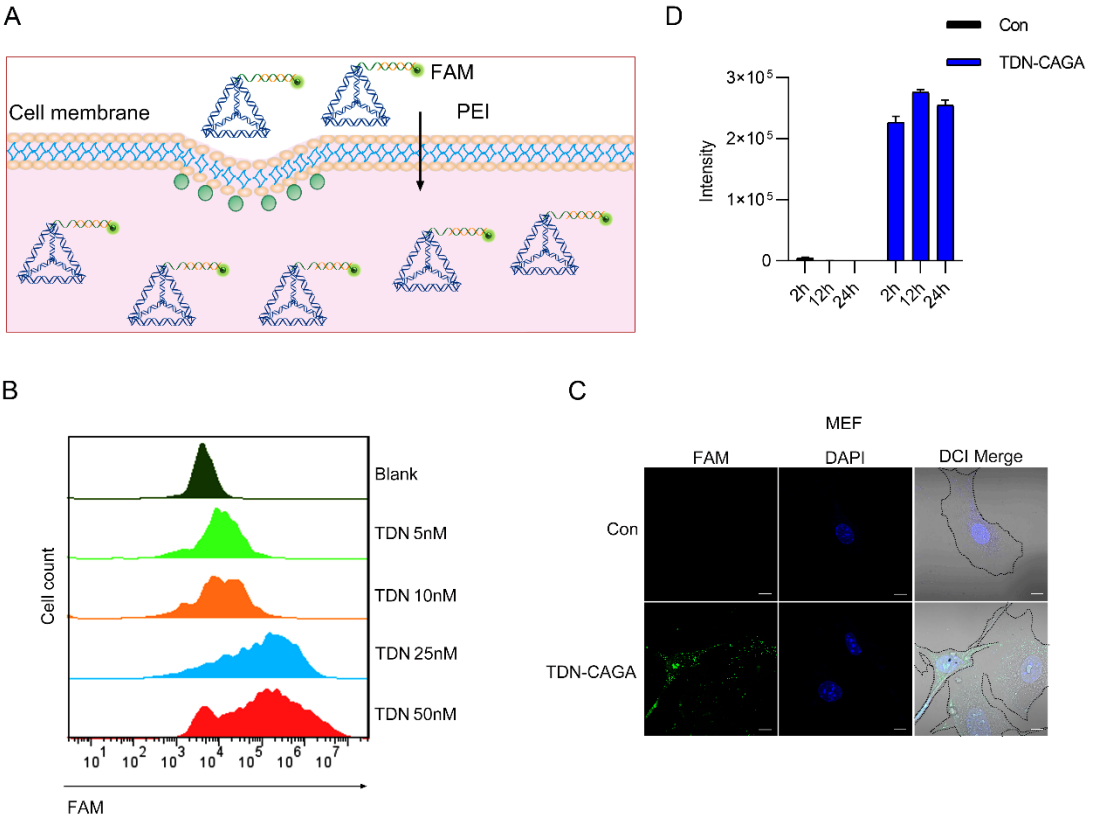

Figure S3. TDNs could be transfected into cells. (A) Graph shows that TDNs could be transfected in to cells via PEI. (B) The flow cytometry analysis of MEF cells incubated with 0 nM, 5 nM, 10 nM, 25 nM and 50 nM TDNs. (C) Confocal microscopic images of MEF cells 24 h after TDNs were transfected. The nucleus was stained with DAPI (blue). TDN positive particles (green). Scale bar: 10 μm. (D) The fluorescence intensity of FAM-labeled TDNs in MEF cells detected by the fluorescence spectrometer 2 h, 12 h and 24 h after the transfection reagents were depleted.

Figure S4

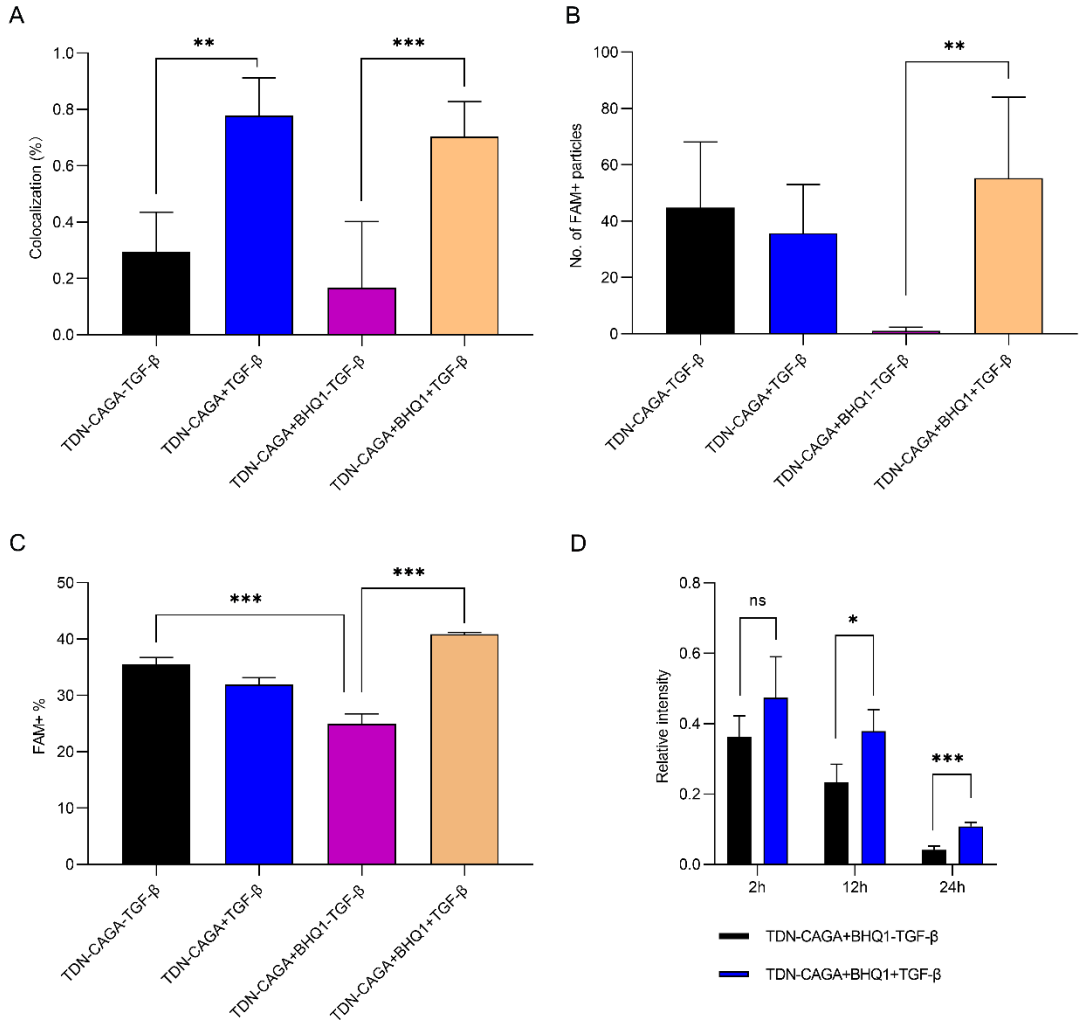

Figure S4. TGF-β signaling pathway activity examined by TDNs. (A) Statistical FAM positive particles comparison of individual cells in Figure 5(B) (error bars represent SD). ** indicates $P < 0.01$. (B) Statistical colocalization percentages of FAM positive particels with Smad2/3 of individual cells in Figure 5(B) (error bars represent SD). ** indicates $P < 0.01$, *** indicates $P < 0.001$. (C) Statistical FAM positive cells percentage in each group of Figure 5(C) (error bars represent SD). *** indicates $P < 0.001$. (D) The fluorescence intensity of FAM-labeled TDNs2 in MEF cells incubated with TGF-β ligands or control vesicles for 2 h was detected. BHQ1-labeled

displacement strands were hybridized with TDNs within cells for 2 h, 12 h and 24 h. The fluorescence intensity of each group was normalized with the fluorescence intensity before hybridization. (error bars represent SD). * indicates $P < 0.05$, *** indicates $P < 0.001$.

Figure S5

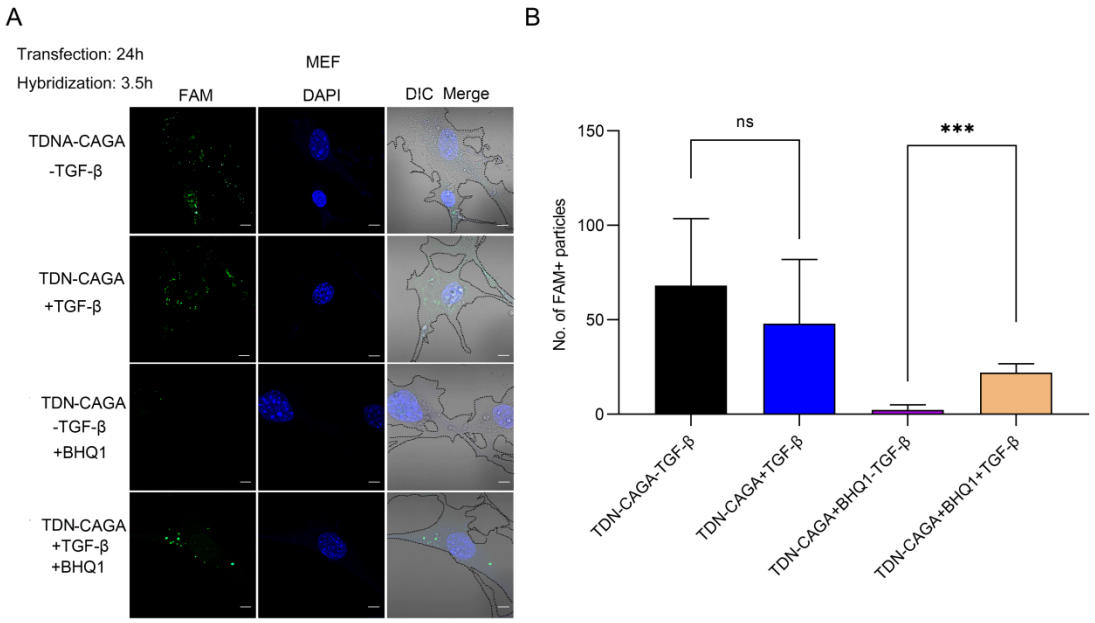

Figure S5 TGF-β signaling activity examined by TDNs2. (A) Confocal microscopic images of TDNs2 transfected MEF cells incubated with TGF-β ligands or control vesicles for 2 h. BHQ1-labeled displacement strands were hybridized with TDNs2 within cells for 3.5 h. The nucleus was stained with DAPI (blue). Scale bar: 10 μm. (B) Statistical FAM positive particles comparison of individual cells in (A) (error bars represent SD). *** indicates $P < 0.001$.