

Supplementary Information
Table S1. Circular dichroism melting temperatures and percentage of folded fraction at 37°C for DNA oligonucleotides under study

No.	Name	Circular Dichroism T _m , °C	Percentage of folded ODN at 37°C, %
1	HD1	39.2 [1], 49.1 [2]	>50%
2	biHD1	32 [1]	<50%
3	biHD1-C3	37	50
4	biHD1+Ba	-	-
5	biHD1-T4A,T20A	39	61
6	biHD1-5'-Δ2G	35	47

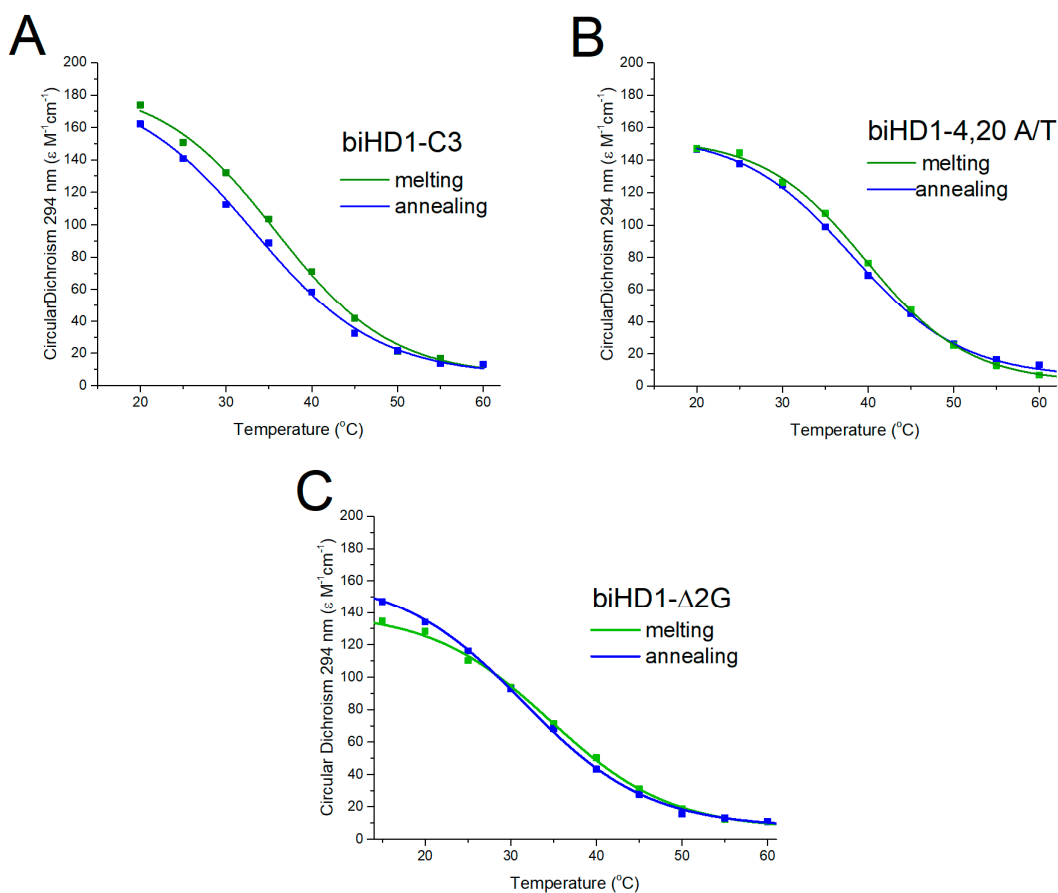


Figure S1. CD-melting (green) and annealing (blue) curves are plotted as functions of biHD1-T4A,T20A (A), biHD1-C3 (B) and biHD1-Δ2G (C) amplitude at 294 nm of temperature. CD data were recorded in a cuvette with a path length of 1 cm in the temperature range of 20–70°C with a scan rate of 30°C/h at 2.5 μM ODN strand concentration in working buffer solution (10 mM NaH₂PO₄/Na₂HPO₄, 10 mM KCl, 140 mM NaCl, pH 7.5).

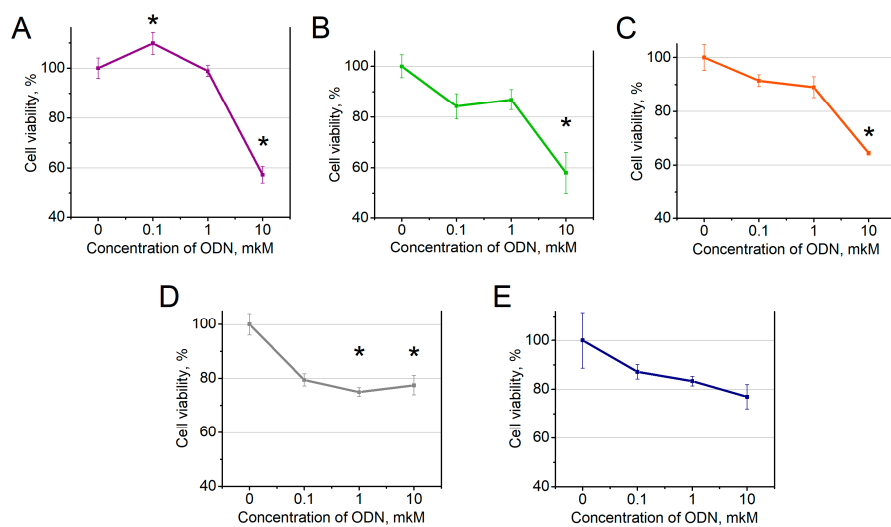


Figure S2. MTT test results of biHD1 treatment for different cell lines: **A** - HeLa, **B** - HCT116, **C** - MCF7, **D** - mS, **E** - PC3. Cells were incubated for 72 hr without oligonucleotide, with 0.10 μ M, 1.0 μ M, and 10 μ M biHD1 oligonucleotide. Data are presented as mean \pm S.D. Asterisk - $p < 0.05$ compared with control (w/o oligo).

Table S2. Investigation of biHD1 IC50 for different cell lines

Cell Line	biHD1 IC50, μ M
RL-67	1
U87	7
HeLa	>10
HCT116	>10
MCF7	>10
mS	>10
PC3	>10
hEF	>10

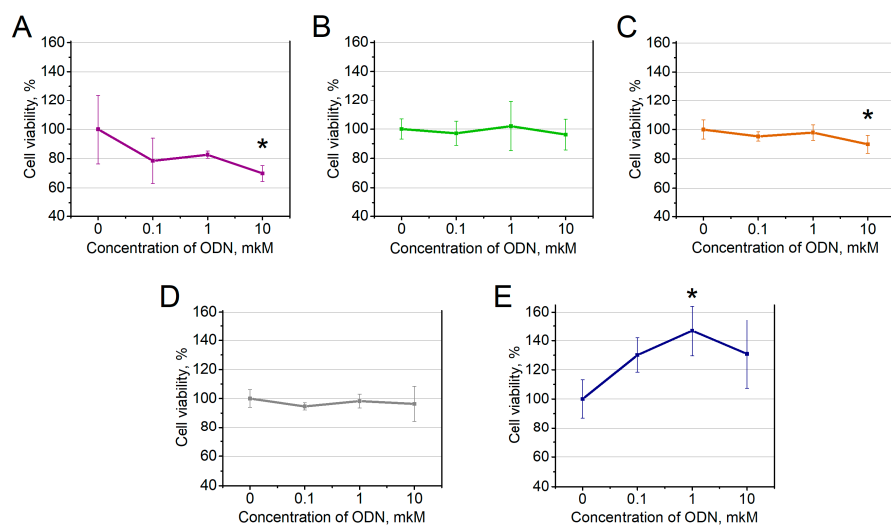


Figure S3. MTT test results of biHD1-C3 treatment for different cell lines: **A** - HeLa, **B** - HCT116, **C** - MCF7, **D** - mS, **E** - PC3. Cells were incubated for 72 hr without oligonucleotide, with 0.10 μM , 1.0 μM , and 10 μM biHD1-C3. Data are presented as mean \pm S.D. Asterisk - $p < 0.05$ compared with control (w/o oligo).

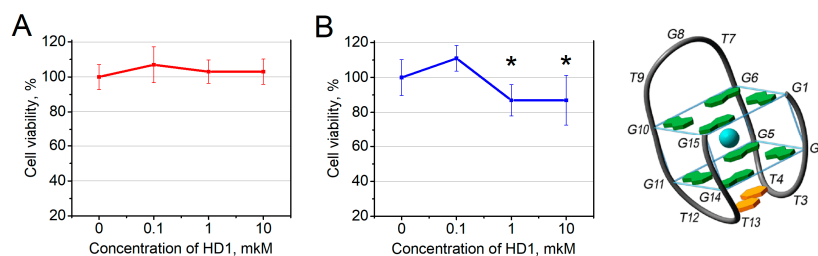


Figure S4. MTT test results for U87 (A) and embryo fibroblast (B) cell lines after 72 hr incubation of cells with different concentrations of single-modular HD1: control experiment without oligonucleotide, 0.10 μM HD1, 10 μM oligonucleotide, and 10 μM oligonucleotide. Data are presented as mean \pm S.D. Asterisk - $p < 0.05$ compared with control (w/o oligo).

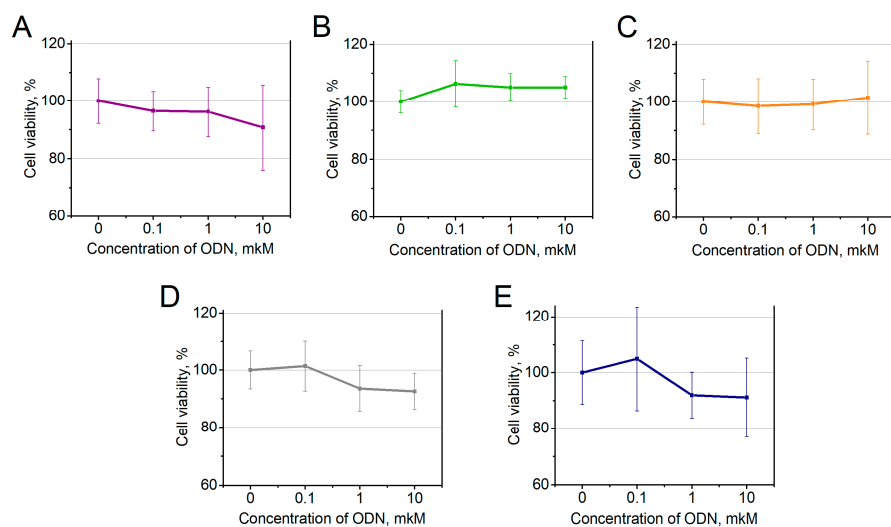


Figure S5. MTT test results of biHD1-A4TA20T treatment for different cell lines: **A** - HeLa, **B** - HCT116, **C** - MCF7, **D** - mS, **E** - PC3. Cells were incubated for 72 hr without oligonucleotide, with 0.10 μ M, 1.0 μ M, and 10 μ M biHD1-A4T, A20T. Data are presented as mean \pm S.D. Asterisk - $p < 0.05$ compared with control (w/o oligo).

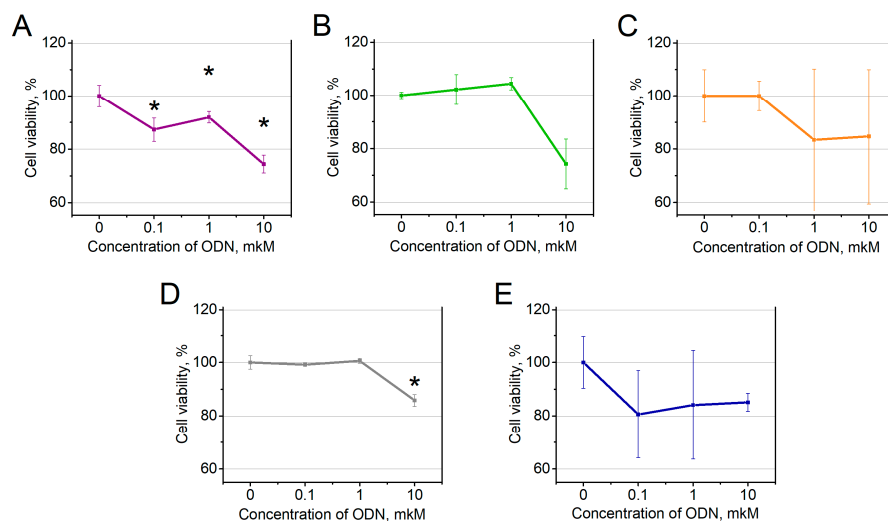


Figure S6. MTT test results of biHD1-5'- Δ 2G treatment for different cell lines: **A** - HeLa, **B** - HCT116, **C** - MCF7, **D** - mS, **E** - PC3. Cells were incubated for 72 hr without oligonucleotide, with 0.10 μ M, 1.0 μ M, and 10 μ M biHD1-5'- Δ 2G. Data are presented as mean \pm S.D. Asterisk - $p < 0.05$ compared with control (w/o oligo).

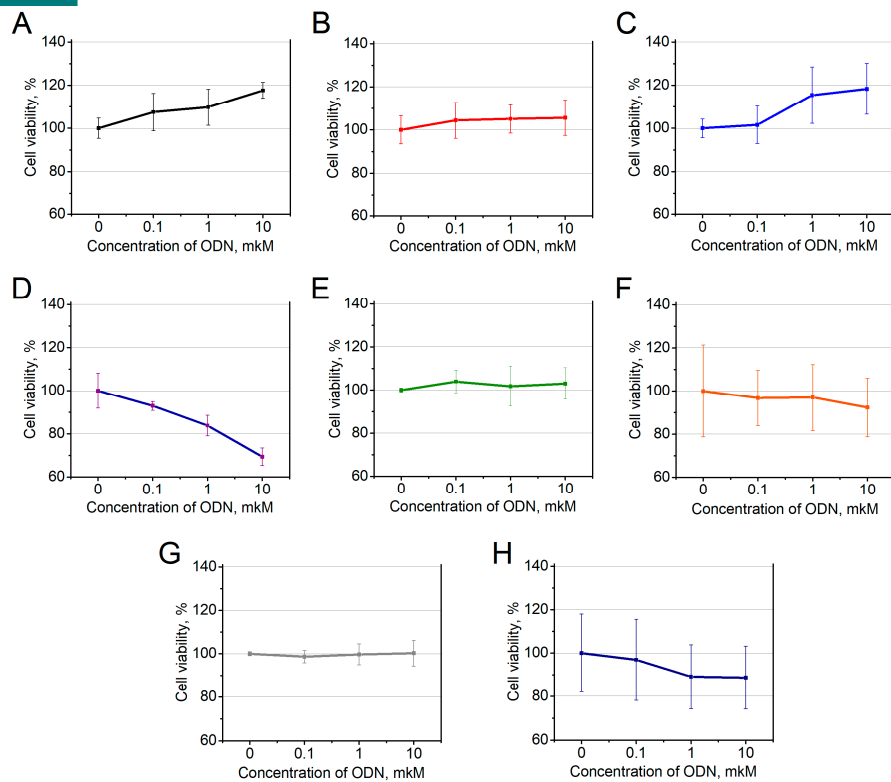


Figure S7. MTT test results of biHD1+Ba treatment for different cell lines: **A** - RL-67, **B** - U87, **C** - embryonic fibroblasts, **D** - HeLa, **E** - HCT116, **F** - MCF7, **G** - mS, **H** - PC3. Cells were incubated for 72 hr without oligonucleotide, with 0.10 μ M, 1.0 μ M, and 10 μ M biHD1+Ba. Data are presented as mean \pm S.D.

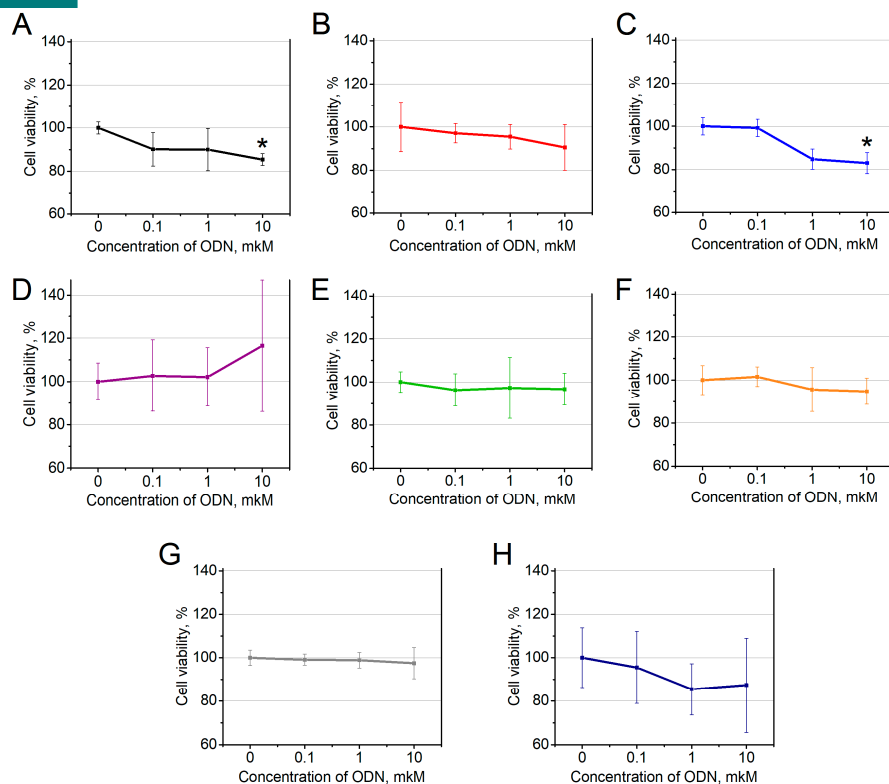


Figure S8. MTT test results of BaCl₂ treatment for different cell lines: **A** - RL-67, **B** - U87, **C** - embryonic fibroblasts, **D** - HeLa, **E** - HCT116, **F** - MCF7, **G** - mS, **H** - PC3. Cells were incubated for 72 hr without salt, with 0.20 μM, 2.0 μM, and 20 μM BaCl₂. Data are presented as mean ± S.D. Asterisk - p < 0.05 compared with control (w/o oligo).

References

- Amato, T.; Virgilio, A.; Pirone, L.; Vellecco, V.; Bucci, M.; Pedone, E.; Esposito, V.; Galeone, A. Investigating the properties of TBA variants with twin thrombin binding domains. *Sci Rep.* **2019**, *9*(1):9184. doi: 10.1038/s41598-019-45526-z.
- Zavyalova, E.G.; Legatova, V.A.; Alieva, R.S.; Zalevsky, A.O.; Tashlitsky, V.N.; Arutyunyan, A.M.; Kopylov, A.M. Putative mechanisms underlying high inhibitory activities of bimodular DNA aptamers to Thrombin. *Biomolecules.* **2019**, *9*(2), E41. doi: 10.3390/biom9020041