Supporting Information

Hydrophobic CPP/HDO Conjugates: A New Frontier in Oligonucleotide-Warheaded PROTAC Delivery

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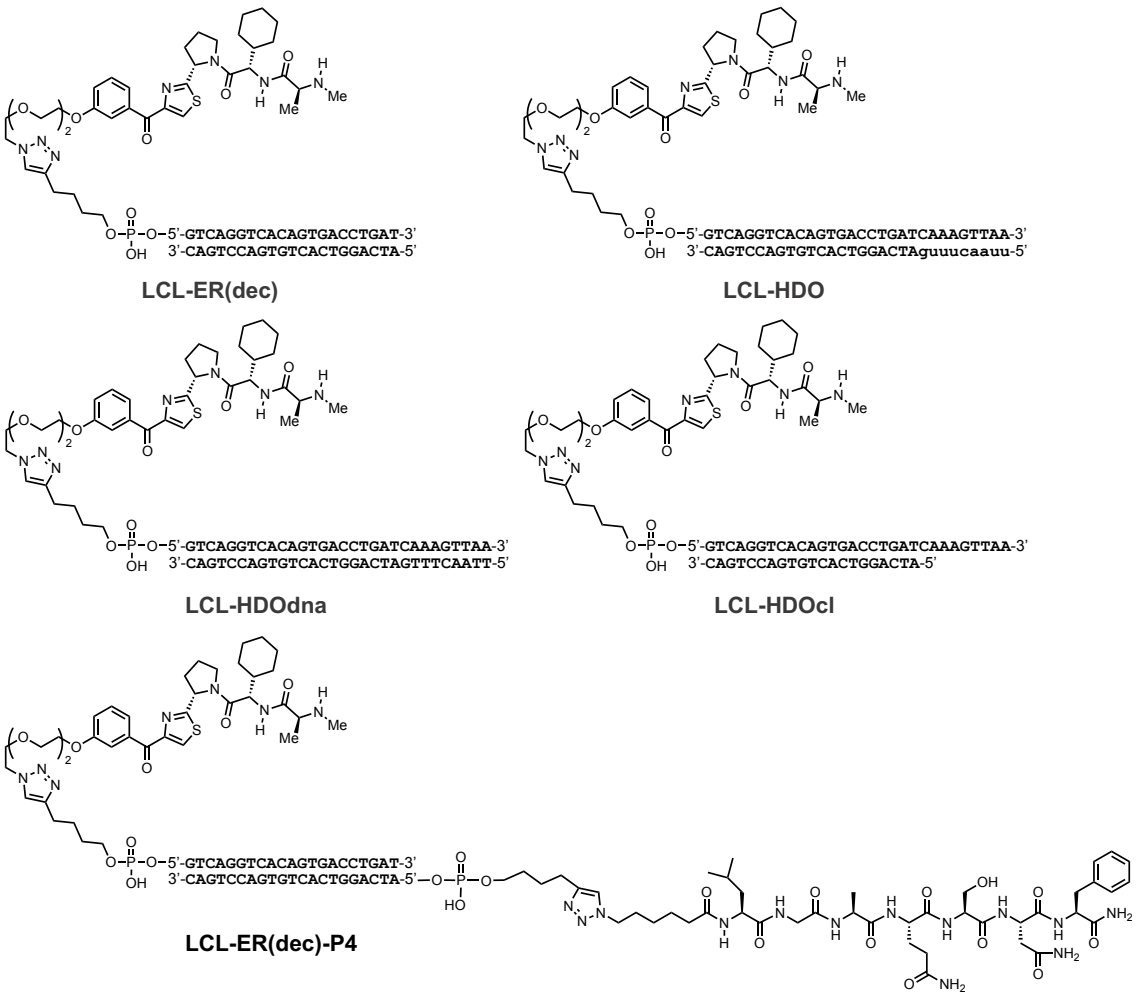


Figure S1. Molecules synthesized in this study.

Table S1. Sequence of decoys and peptide used in this study.

	Compounds	Sequence
1	**LCL-ER(dec)**	Forward (**LCL-ER(dec)-F**): 5'-**LCL**-GTCAGGTCACAGTGACCTGAT-3' Reverse (**ER(dec)-R**): 3'-CAGTCCAGTGTCACTGGACTA-5'
2	**LCL-HDO**	Forward (**LCL-HDO-F**): 5'-**LCL**-GTCAGGTCACAGTGACCTGATCAAAGTTAA-3' Reverse (**HDO-R**): 3'-CAGTCCAGTGTCACTGGACTAguuucaauu-5'
3	**LCL-HDOdna**	Forward (**LCL-HDO-F**): 5'-**LCL**-GTCAGGTCACAGTGACCTGATCAAAGTTAA-3' Reverse (**HDOdna-R**): 3'-CAGTCCAGTGTCACTGGACTAGTTTCAATT-5'
4	**LCL-HDOcl**	Forward (**LCL-HDO-F**): 5'-**LCL**-GTCAGGTCACAGTGACCTGATCAAAGTTAA-3' Reverse (**ER(dec)-R**): 3'-CAGTCCAGTGTCACTGGACTA-5'
5	**CPP/HDO-PROTAC**	Forward (**LCL-HDO-F**): 5'-**LCL**-GTCAGGTCACAGTGACCTGATCAAAGTTAA-3' Reverse (**P4-HDO-R**): 3'-CAGTCCAGTGTCACTGGACTAguuucaauu-5'-**P4**
6	**FP-probe**	Forward (**5'-FAM-ER(dec)-F**) 5'-**FAM**-GTCAGGTCACAGTGACCTGAT-3' Reverse (**5'-FAM-ER(dec)-R**): 3'-CAGTCCAGTGTCACTGGACTA-**FAM**-5'
7	**FAM-ER(dec)**	Forward (**5'-FAM-ER(dec)-F**) 5'-**FAM**-GTCAGGTCACAGTGACCTGAT-3' Reverse (**ER(dec)-R**): 3'-CAGTCCAGTGTCACTGGACTA-5'
8	**FAM-HDOdna**	Forward (**5'-FAM-HDO-F**) 5'-**FAM**-GTCAGGTCACAGTGACCTGATCAAAGTTAA-3' Reverse (**HDOdna-R**): 3'- CAGTCCAGTGTCACTGGACTAGTTTCAATT-5'
9	**FAM-HDO-P4**	Forward (**5'-FAM-HDO-F**) 5'-**FAM**-GTCAGGTCACAGTGACCTGATCAAAGTTAA-3' Reverse (**P4-HDO-R**): 3'-CAGTCCAGTGTCACTGGACTAguuucaauu-5'-**P4**
10	**LCL-ER(dec)-P4**	Forward (**LCL-ER(dec)-F**): 5'-**LCL**-GTCAGGTCACAGTGACCTGAT-3' Reverse (**P4-ER(dec)-R**): 3'-CAGTCCAGTGTCACTGGACTA-5'-**P4**

LCL: LCL161, lowercase : RNA, **FAM**: fluorescein, P4: **N_3-C6-LGAQSNF-NH_2**

HPLC analysis of decoys

HPLC conditions

Column : CAPCELL PAK MG-II (C18, 4.6 x 250 mm, 5 μm; OSAKA soda).

Mobile phase : A = 0.1 M triethylammonium acetate (TEAA) buffer (pH7.0), B = CH_3CN.

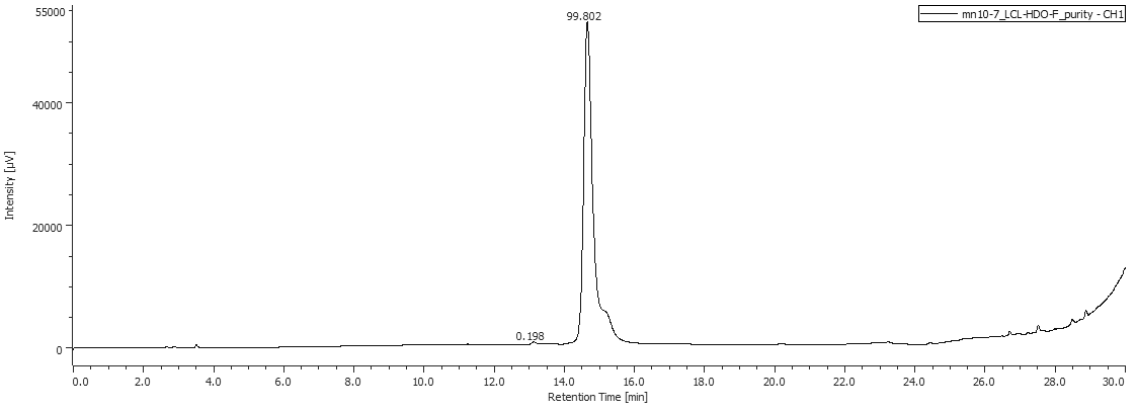
Gradient : B% = 10-40 over 20 min, 40-100 over 5 min.

Flow rate : 1.0 mL/min.

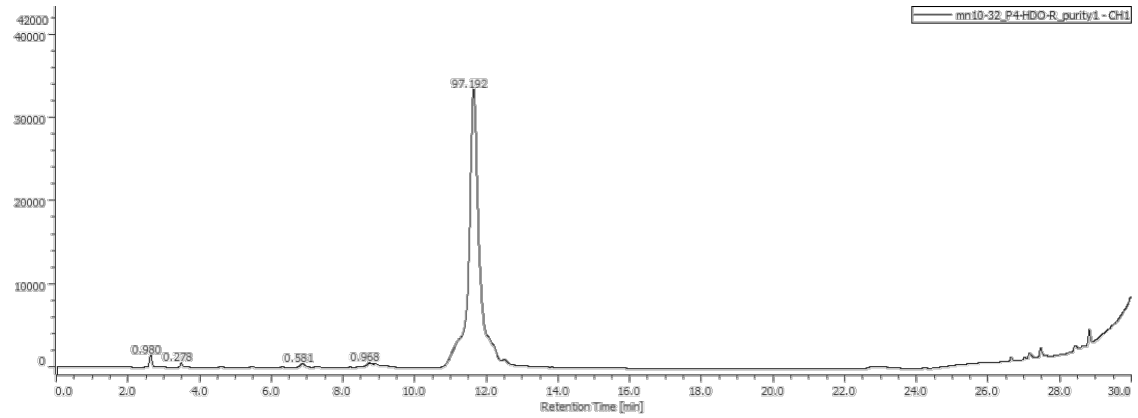
Column temperature : 35°C.

Detection : 260 nm.

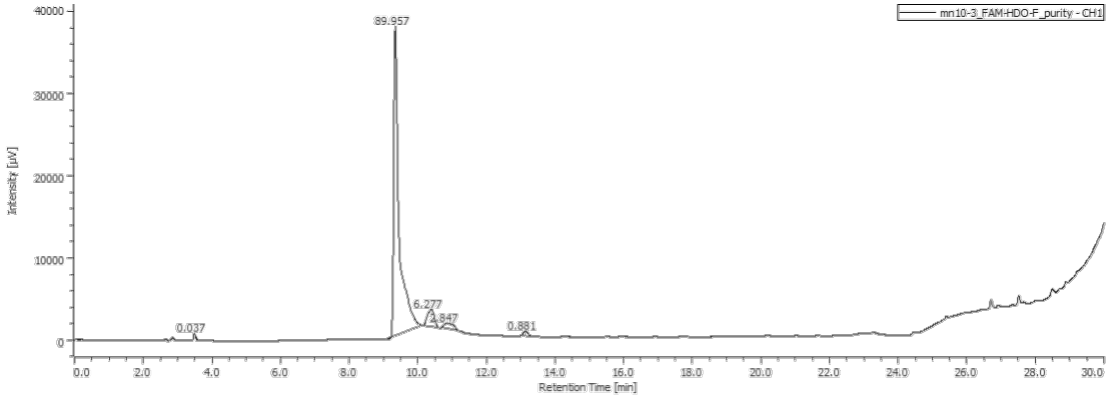
LCL-HDO-F purity 99%



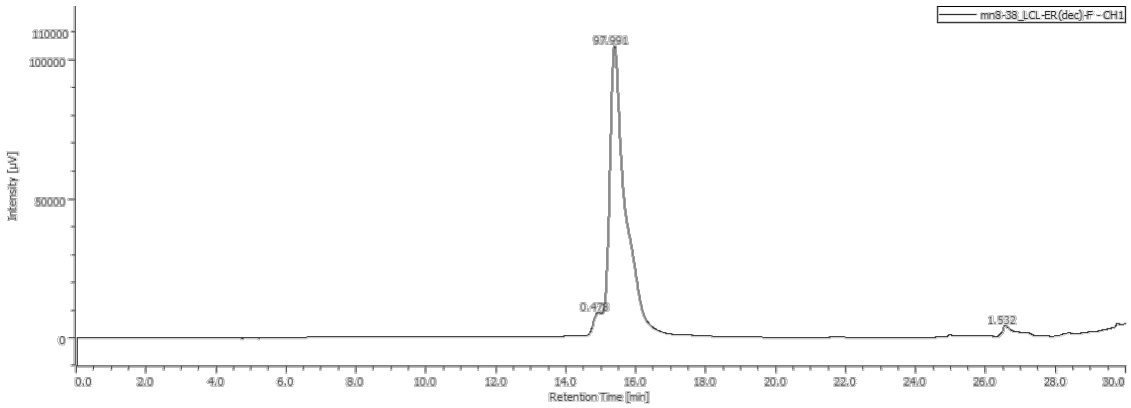
P4-HDO-R purity 97%



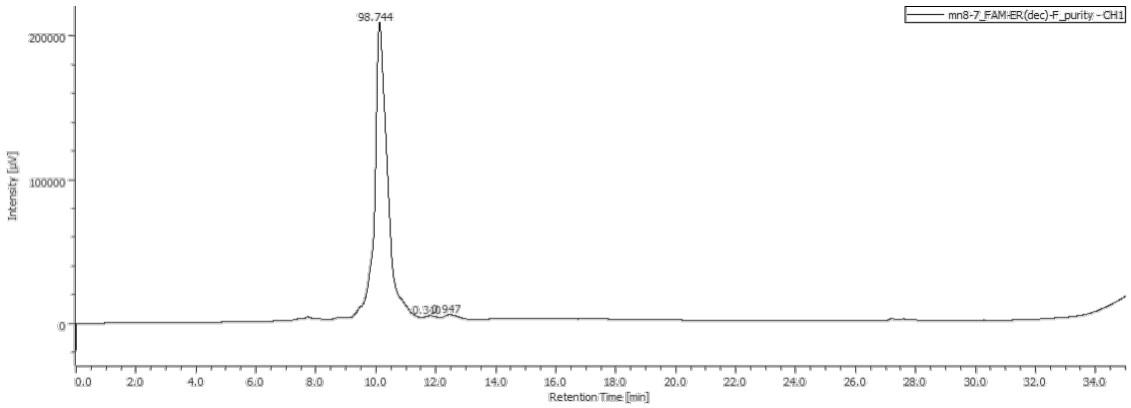
FAM-HDO-F purity 90%



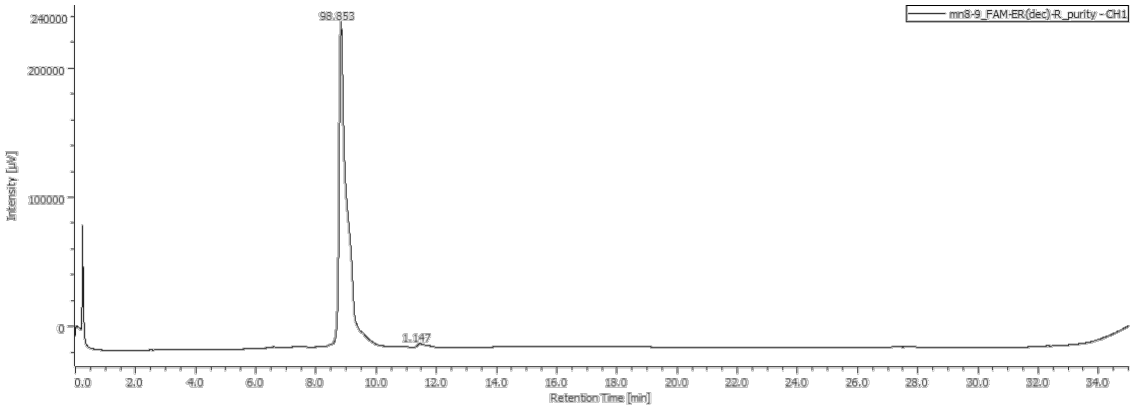
LCL-ER(dec)-F purity 98%



FAM-ER(dec)-F purity 99%



FAM-ER(dec)-R purity 99%



UPLC analysis of peptide

UPLC conditions

Column : ACQUITY UPLC® BEH C18 1.7 µm column (2.1 × 50 mm)

Mobile phase : A = 0.1% TFA/water, solvent, B: 0.1% TFA/CH_3CN gradient: 10-90% gradient of solvent B over 4 min).

flow rate: 0.5 mL/min

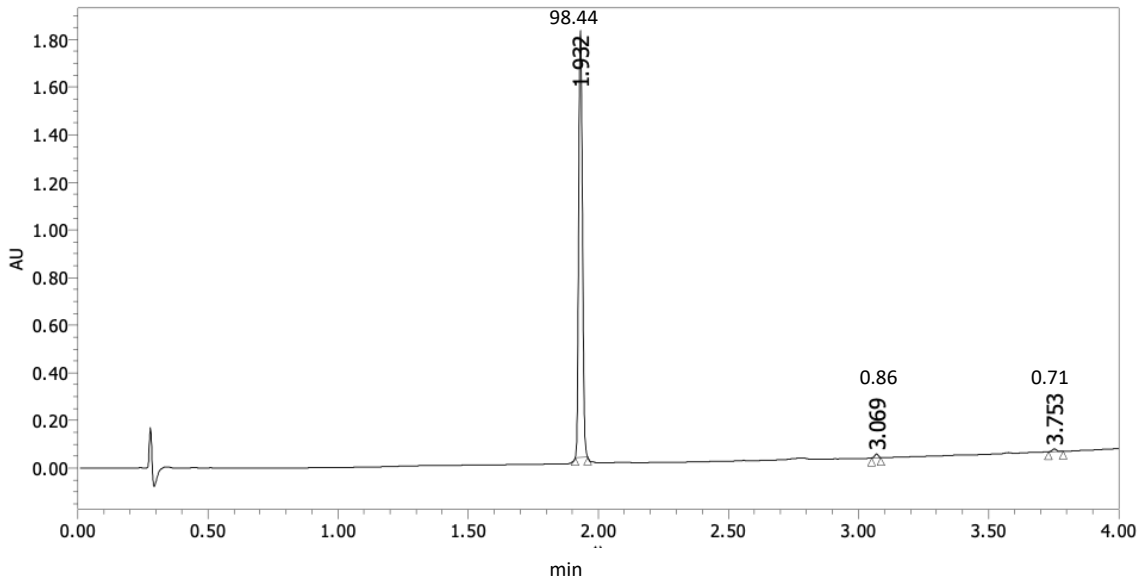
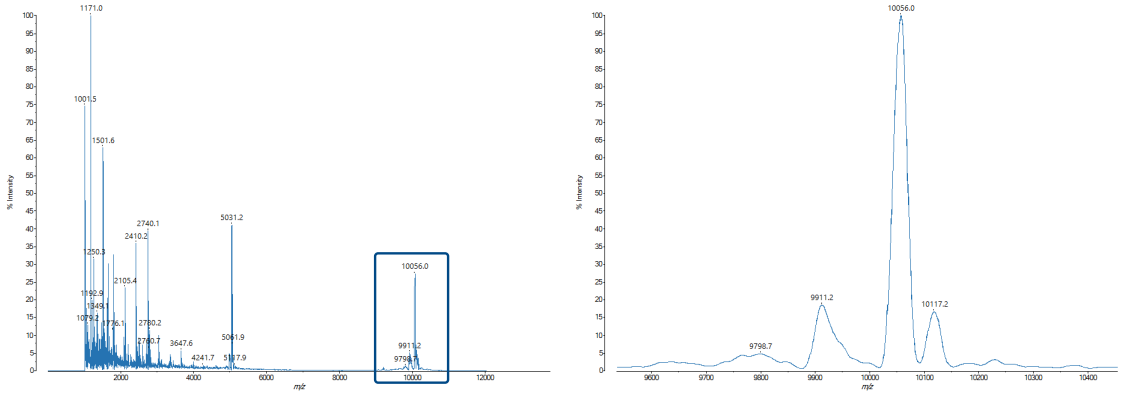


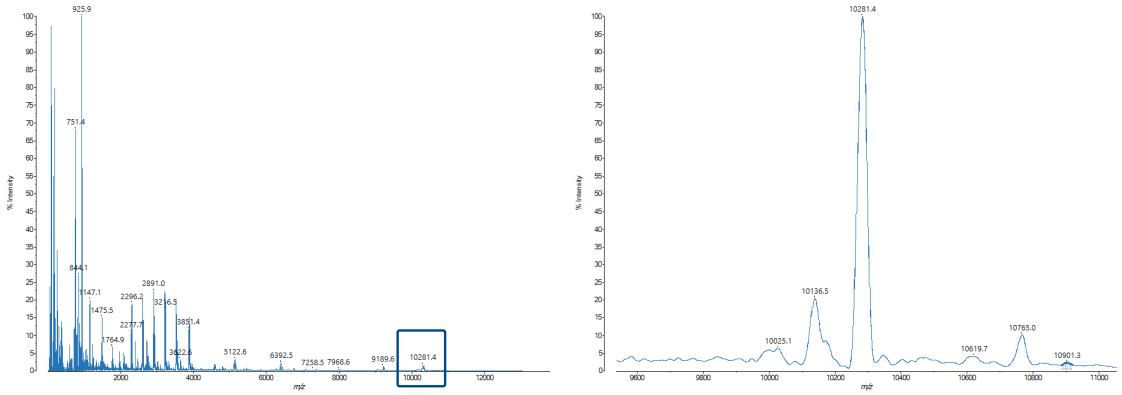
Figure S2. HPLC trace of the synthesized decoys, peptide and chimeric molecules.

MALDI TOF MS

LCL-HDO-F



P4-HDO-R



FAM-HDO-F

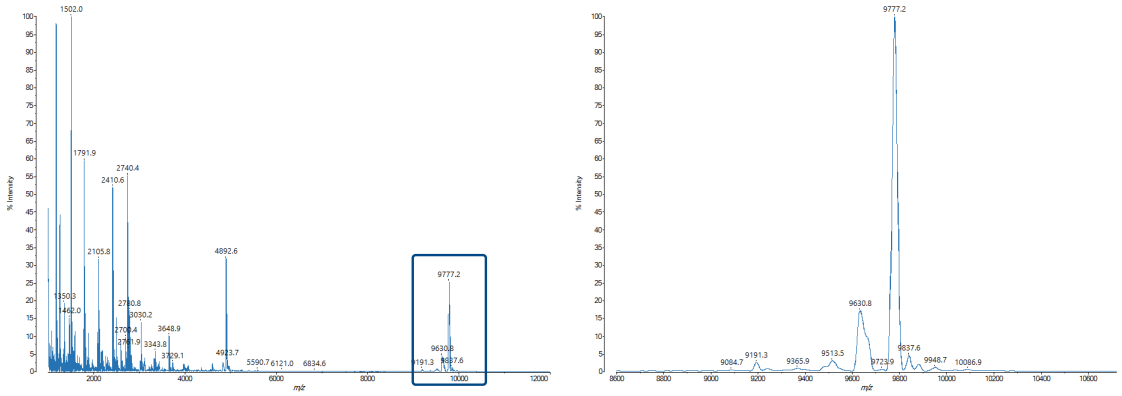
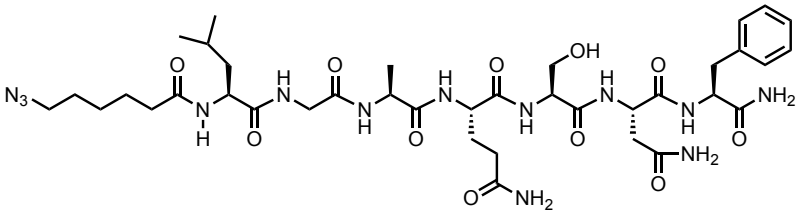


Figure S3. MALDI TOF MS spectrum of decoys and chimeric molecules.

HR-MS (ESI)

P4-N_3 N_3-C6-LGAQSNF-NH_2



m/*z*: calcd for $C_{38}H_{58}N_{12}O_{12}$ $[M+H]^+$: 874.4535, found : 874.4218

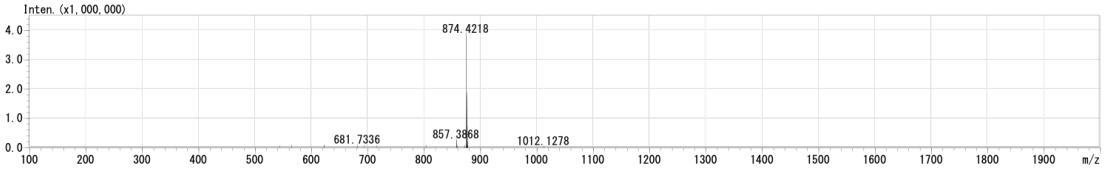


Figure S4. ESI TOF MS spectrum of peptide.

Fluorescence polarization assay

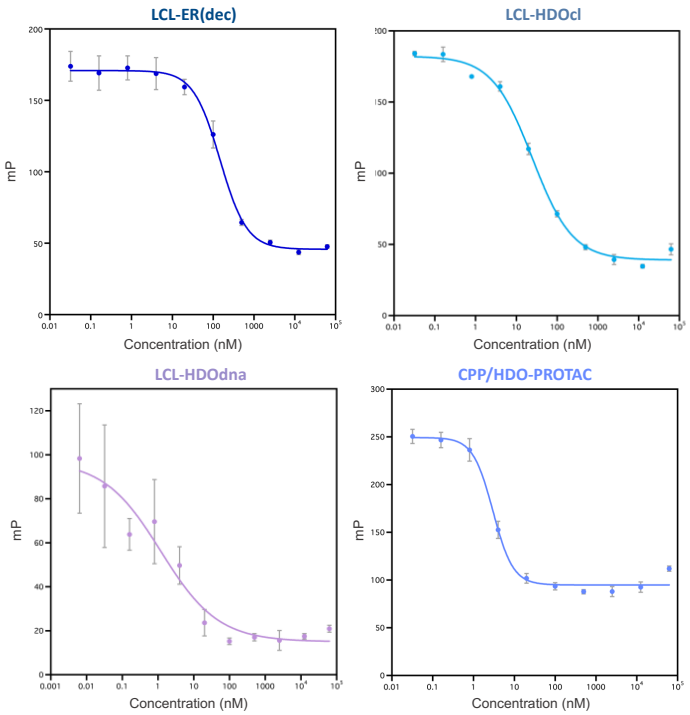


Figure S5. The competitive binding assay of **LCL-ER(dec)**, **LCL-HDOcl**, **LCL-HDOdna** and **CPP-HDO-PROTAC** to the ERα. Condition: 62.5 μM-0.032 nM decoy in TE buffer (pH 7.5) containing 25 mM NaCl, measured at 25°C. The data represent means S.D. (n = 3).

Biology

Western blotting

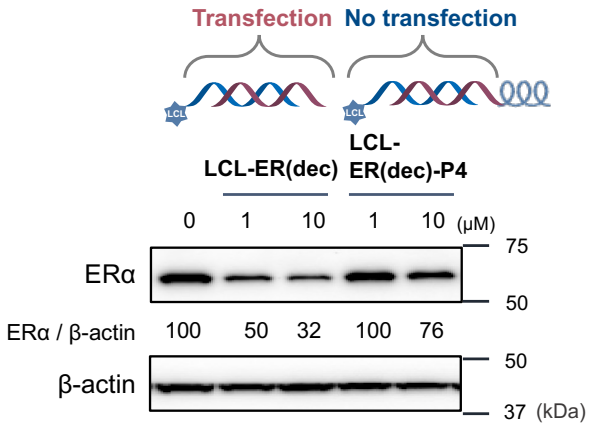


Figure S6. Degradation of ERα by **LCL-ER(dec)** and **LCL-ER(dec)-P4**. MCF-7 cells were treated (for 24 h) with the indicated concentrations of **LCL-ER(dec)** and **LCL-ER(dec)-P4**.

Cell proliferation assay

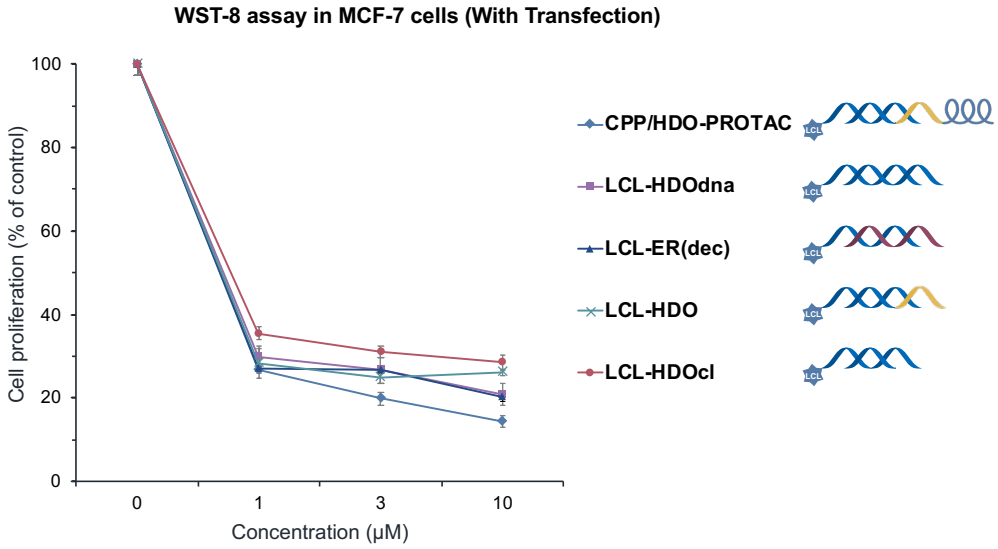


Figure S7. Effects of PROTACs on the proliferation of ERα-positive breast cancer cells. Growth inhibition of ERα-positive breast cancer cells by PROTACs. MCF-7 cells were transfected with 1–10 μM of the indicated PROTACs for 72 h, and cell proliferation was then evaluated using a cell viability assay. Data represent the mean ± standard deviation (n = 5).

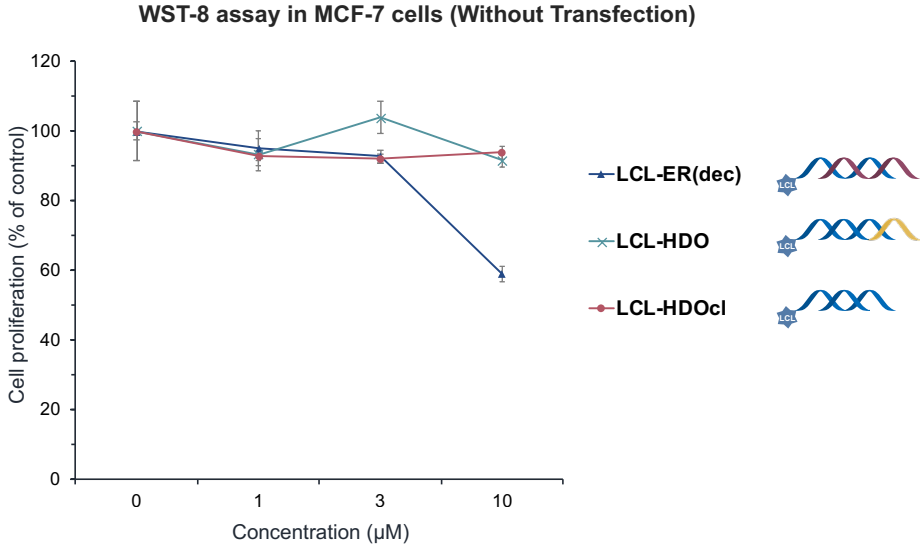


Figure S8. Effects of PROTACs on the proliferation of ERα-positive breast cancer cells. Growth inhibition of ERα-positive breast cancer cells by PROTACs. MCF-7 cells were treated with 1–10 μM of the indicated PROTACs for 72 h, and cell proliferation was then evaluated using a cell viability assay. All PRORACs were added without transfection reagents. Data represent the mean ± standard deviation (n = 5).