

Supplemental Information

Targeting SOX2 Protein with Peptide

Aptamers for Therapeutic Gains

against Esophageal Squamous Cell Carcinoma

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Supplemental Information.

Figure S1. Establishment of a peptide aptamer library for screening based on BiFc.

(A) Schematics of aptamer library construction and screening process.

(B) TrxA-MCS-TrxA fragment and pBiFc-VC155-TrxA-MCS-TrxA vector were generated after high-fidelity PCR, ligation and enzyme identification.

(C) A peptide aptamer expression library containing 33 random nucleotide sequences was obtained with high-fidelity PCR.

(D) The peptide aptamer library was validated with bacterial liquid PCR using specific primers.

(E) Constructs expressing SOX2 protein and peptide aptamers used for immunoprecipitation validation.

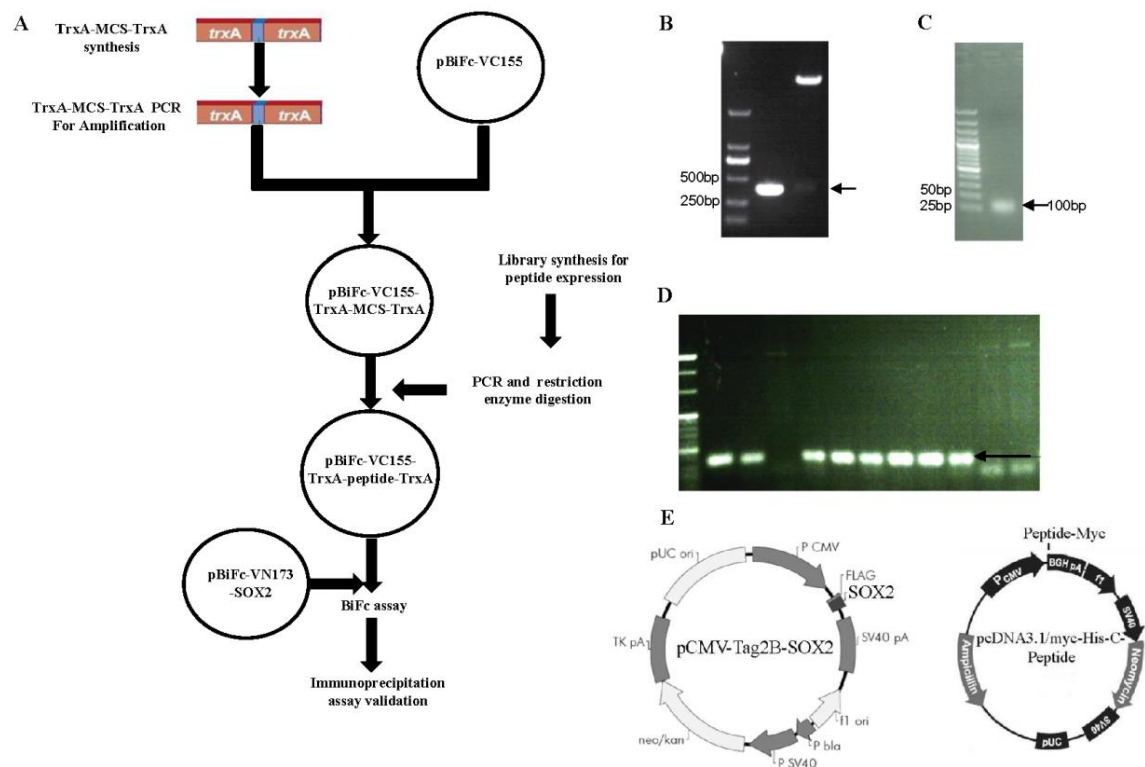


Figure S2. Production of lentivirus that is used to express peptide aptamers P15, P18, P42 and GFP. Note that control contains GFP and the package cell line is HEK293T. Scale bar: 100 μm .

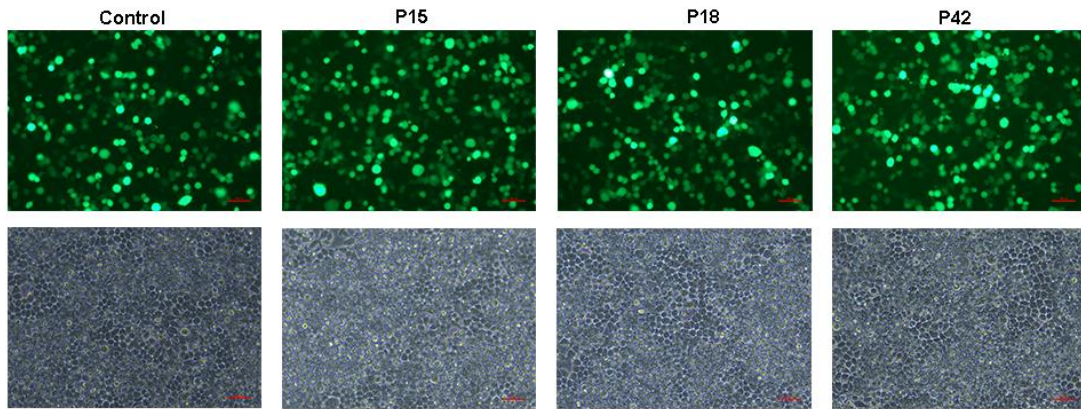
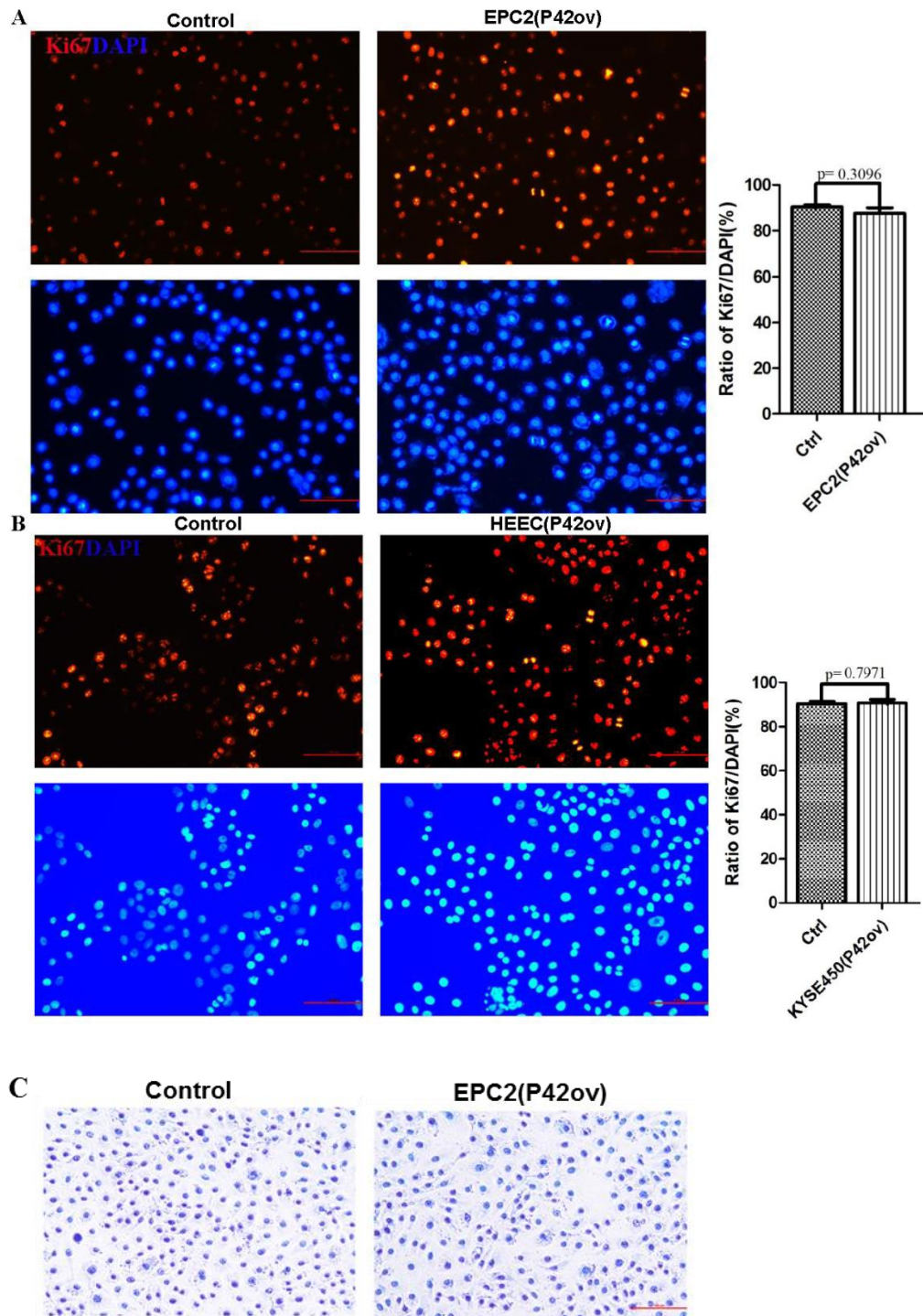


Figure S4. P42 aptamer overexpression does not impact the proliferation and apoptosis of normal esophageal epithelial cell lines (EPC2 and HEEC). Note apoptotic cells are rarely detected in EPC2 control and p42 overexpression groups (C).

Scale bar: 100 μ m.



Supplementary Table S1. The clinicopathological characteristics of 75 ESCCs are closely correlated with the levels of SOX2 protein.

		Cases in each group	Percent
Histopathological grade	I	11	14.7%
	I-II	14	18.7%
	II	34	45.3%
	II-III	5	6.7%
	III	11	14.7%
TNM Stage	T Stage	24	32%
	N Stage	46	61.3%
	M Stage	5	6.7%

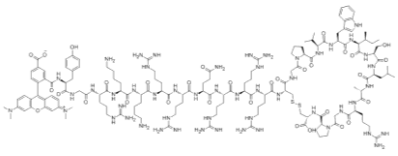
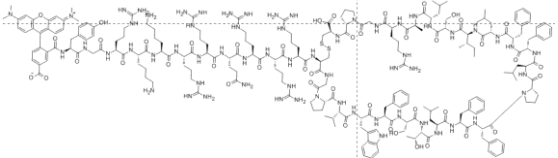
Supplementary Table S2. Primers used for lentiviral plasmid construction

Lentiviral plasmid	Primer sequence(5'-3')
For pCDH-CMV-Oligo- IRES-GFP-Puro construction	Forward:5'-CTAGAGCTAGCGAATTCTTCGAAACCGGTGATATCC TCGAGG-3'
	Reverse:5'-GATCCCTCGAGGATATCACCGGTTTCGAAGAATTCCG CTAGCT-3'
For pCDH-CMV-Peptide- IRES-GFP-Puro construction	Forward:5'-CGGAATTCGCCACCATGAGCGATAAAATTATTCAC-3'
	Reverse: 5'-CGGGATCCTCACAGGTTAGCGTCGAGGAA-3'

Supplementary Table S3. The most prominent biological process, cellular component and molecular function of changed protein upon ectopic expression of P42 aptamer.

	Biological Process	Cellular Component	Molecular Function
Up-regulated Protein	cellular process	cell	binding
	single-organism process	organelle	catalytic activity
	biological regulation	extracellular region	molecular function regulator
	metabolic process	membrane	signal transducer activity
	response to stimulus	membrane-enclosed lumen	structural molecule activity
Down-regulated Protein	cellular process	cell	binding
	single-organism process	organelle	catalytic activity
	biological regulation	membrane	molecular function regulator
	metabolic process	extracellular region	molecular transducer activity
	response to stimulus	membrane-enclosed lumen	transporter activity

Supplementary Table S4. The detailed information of chemically synthetic control peptide and peptide 42.

	Control peptide	Peptide 42
Sequence	TAMRA-YGRKKRRQRRRCGPV WISLARGPC(C12-C24)	TAMRA-YGRKKRRQRRRCGPVWFSTLFFPLF FLISLARGPC(C12-C35)
Structure		

Supplementary Table S5. Primers used for peptide library construction and BiFc

	sequence(5'-3')
Synthesized Sequence for TrxA-MCS-TrxA	5'-ATGAGCGATAAAAATTATTCACCTGACTGACGACAGTTTTGACACGGATGTA CTCAAAGCGGACGGGGCGATCCTCGTCGATTTCTGGGCAGAGTGGTGCGGTCCAGTGTGCTGGGCCCAGCCGGCCAGATCTGAGCTCGCGGCCGCGATATCGCTAGCTCGAGGTCCGTGCAAAATGATCGCCCCGATTCTGGATGAAATCGCTGACGAATATCAGGGCAAACCTGACCGTTGCAAAACTGAACATCGATCAAAACCCTGGCACTGCGCCGAAATATGGCATCCGTGGTATCCCGACTCTGCTGCTGTTCAAAAACGGTGAAGTGGCGGCAACCAAAGTGGGTGCACTGTCTAAAGGTCAGTTGAAAGAGTTCCTCGACGCTAACCTG-3'
For pBiFc-VC155-TrxA-MCS-TrxA construction	Forward: 5'- <u>ACGCGTCGACCATGAGCGATAAAAATTATTCAC</u> -3'
	Reverse: 5'- <u>GGGTACCCAGGTTAGCGTCGAGGAA</u> -3'
Synthesized random sequence for peptide expression	5'-CTGCAGA <u>ACCAGTGTGCTGGN32GATATCGCTAGCTCGAGC</u> -3'
For random sequence cloning	Forward: 5'-CTGCAGA <u>ACCAGTGTGCT</u> -3'
	Reverse: 5'- <u>GCTCGAGCTAGCGATATC</u> -3'
For pBiFc-VC155-TrxA-peptide-TrxA identification	Forward: 5'-TGCGGTCCAGTGTGCTGG-3'
	Reverse: 5'-ACCTCGA GCTAGCGATATC-3'
For pBiFc-VN173-SOX2 construction	Forward: 5'- <u>CGAATTCAATGTACAACATGATGGAGAC</u> -3'
	Reverse: 5'- <u>TGCTCTAGACATGTGTGAGAGGGGCAG</u> -3'

Supplementary Table S6. Primers used for immunoprecipitation

	sequence(5'-3')
For pcDNA3.1-myc-hisC- TrxA-Peptide-TrxA construction	Forward:5'-cg <u>GGATCCA</u> ACCATGAGCGATAAAAATTATTCAC-3'
	Reverse:5'- <u>CGGAATTC</u> CCAGGTTAGCGTCGAGGAA-3'
For pCMV-Tag2B-SOX2 construction	Forward: 5'- <u>CGGAATTC</u> ATGTACAACATGATGGAGACG-3'
	Reverse: 5'- <u>CCCAAGCTT</u> TCACATGTGTGAGAGG GGCA-3'