Supplementary information (SI)

Feedback loop between miR-155/Pdcd4/AP-1 is essential for tumorigenic growth of tongue cancer cells.

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CONFLICT OF INTEREST: No competing interests.

Running tiltle: miR-155/Pdcd4/AP-1 feedback loop in tongue cancer

Table S1

Plasmid I (20ng)	Plasmid II (200ng)	Plasmid III (750ng)
Renilla	pGL3 Control	pcDNA3.1
Renilla	pGL3 Control	pcDNA-Pdcd4
Renilla	pGL3-BIC (Full length)	pcDNA3.1
Renilla	pGL3-BIC (Full length)	pcDNA-Pdcd4
Renilla	pGL3-BIC (AP1 mutant)	pcDNA3.1
Renilla	pGL3-BIC (AP1 mutant)	pcDNA-Pdcd4
Renilla	pGL3-BIC (NFkB mutant)	pcDNA3.1
Renilla	pGL3-BIC (NFkB mutant)	pcDNA-Pdcd4

Table S2

Plasmid I (50ng)	Plasmid II (200ng)	Plasmid III (750ng)
Renilla	pGL3	pcDNA3.1
Renilla	pGL3	pcDNA-pmiR155
Renilla	pGL3-BIC (Full length)	pcDNA3.1
Renilla	pGL3-BIC (Full length)	pcDNA-pmiR-155
Renilla	pGL3-BIC (AP1 mutant)	pcDNA3.1
Renilla	pGL3-BIC (AP1 mutant)	pcDNA-pmiR-155
Renilla	pGL3-BIC (NFkB mutant)	pcDNA3.1
Renilla	pGL3-BIC (NFkB mutant)	pcDNA-pmiR-155

Table S3

Plasmid I (20ng)	Plasmid II (200ng)	Plasmid III (750ng)
Renilla	pGL3	pLCE
Renilla	pGL3	pLCE-miR-155 sponge
Renilla	pGL3-BIC (Full length)	pLCE
Renilla	pGL3-BIC (Full length)	pLCE-miR-155 sponge
Renilla	pGL3-BIC (AP1 mutant)	pLCE
Renilla	pGL3-BIC (AP1 mutant)	pLCE-miR-155 sponge
Renilla	pGL3-BIC (NFkB mutant)	pLCE
Renilla	pGL3-BIC (NFkB mutant)	pLCE-miR-155 sponge

Table S4

Plasmid I (20ng)	Plasmid II (200ng)	Plasmid III (750ng)
Renilla	pGL3 Control	pLCE
Renilla	pGL3 Control	pLCE-miR-155 sponge
Renilla	pGL3- 4X AP-1	pLCE
Renilla	pGL3- 4X AP-1	pLCE-miR-155 sponge
Renilla	pGL3 Control	pcDNA3.1
Renilla	pGL3 Control	pcDNA-Pdcd4
Renilla	pGL3- 4X AP-1	pcDNA3.1
Renilla	pGL3- 4X AP-1	pcDNA-Pdcd4

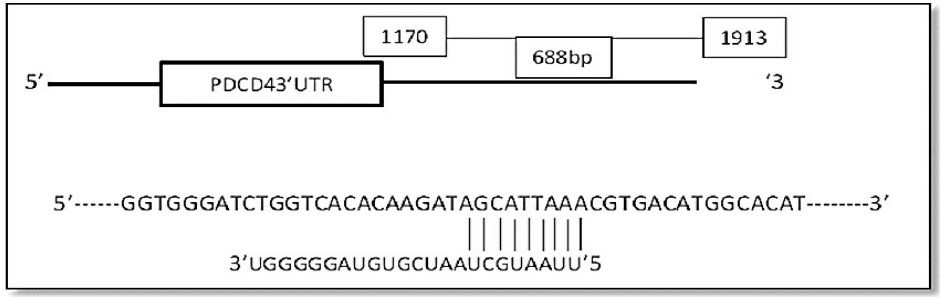
Supplementary Fig. 1

Human Bic promoter components and In silico analysis of miR-155 binding sites on Pdcd4 3'UTR.

A

miRNA	Gene	DIANAmT	miRanda	miRWalk	Targetscan
has-miR-155	Pdcd4	1	1	1	1

B



A. Structure of human BIC promoter
B. Target prediction algorithm software used
C. Schematic representation of complementarity between 3'UTR of Pdcd4 and miR-155.

Supplementary Fig. 2

A

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WT Pdcd4 UTR        5'    ...CUGGUCACACAAGAUAGCAUUAA...........'3
                                  | | | |           | | | | | | | |
        miR-155     3'UGGGGAUAGUGCUAA-----UCGUAAUU '5
                                  | | | |           |     |      |
Mut Pdcd4 UTR       5'........CUGGUCACACAAGAUAUUACCUA..........'3
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miR-155 directly targets Pdcd4 3'UTR in SCC745 cells.

A. Sequence alignment between 3'UTR of Pdcd4, miR-155 and 3'UTR Pdcd4 mutated at miR-155 binding site.