

**PDE3 inhibitor and EGCG combination treatment suppress cancer stem cell properties
in pancreatic ductal adenocarcinoma**

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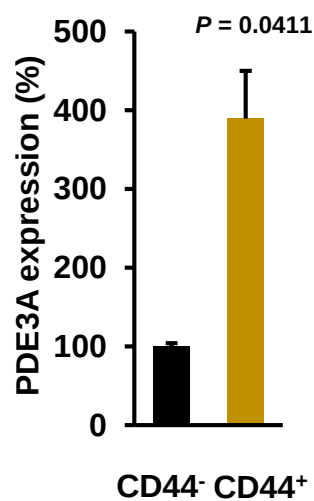
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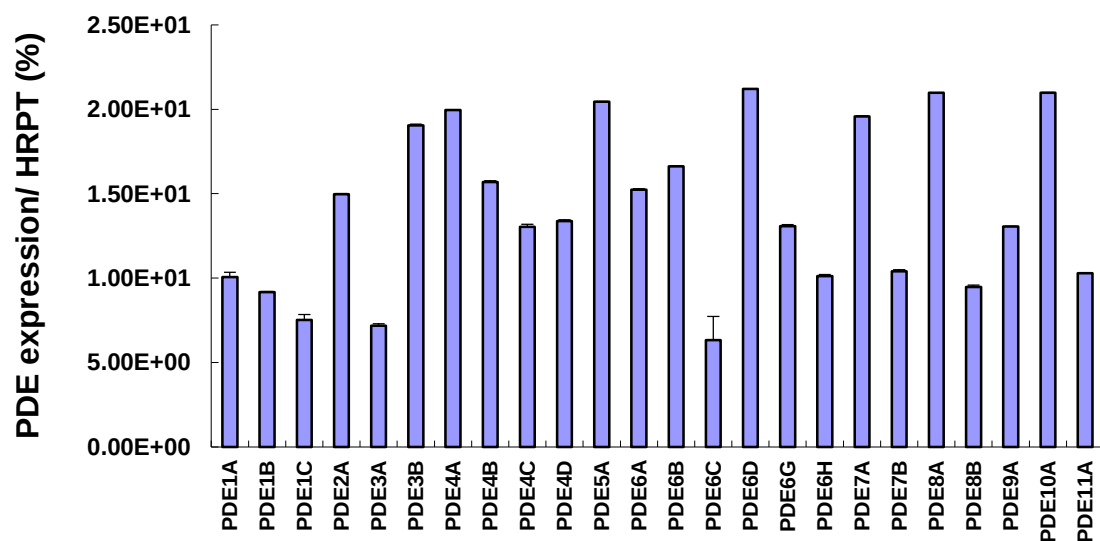
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Keywords: CSCs; PDAC; EGCG; CD44; FOXO3

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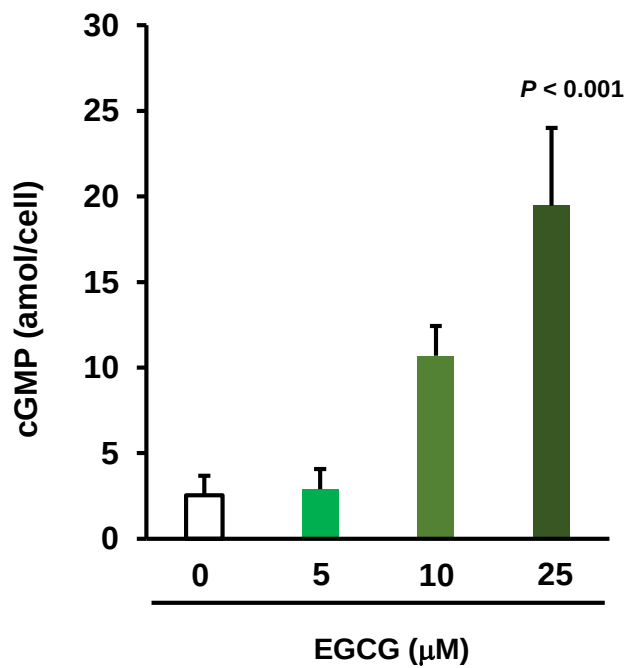


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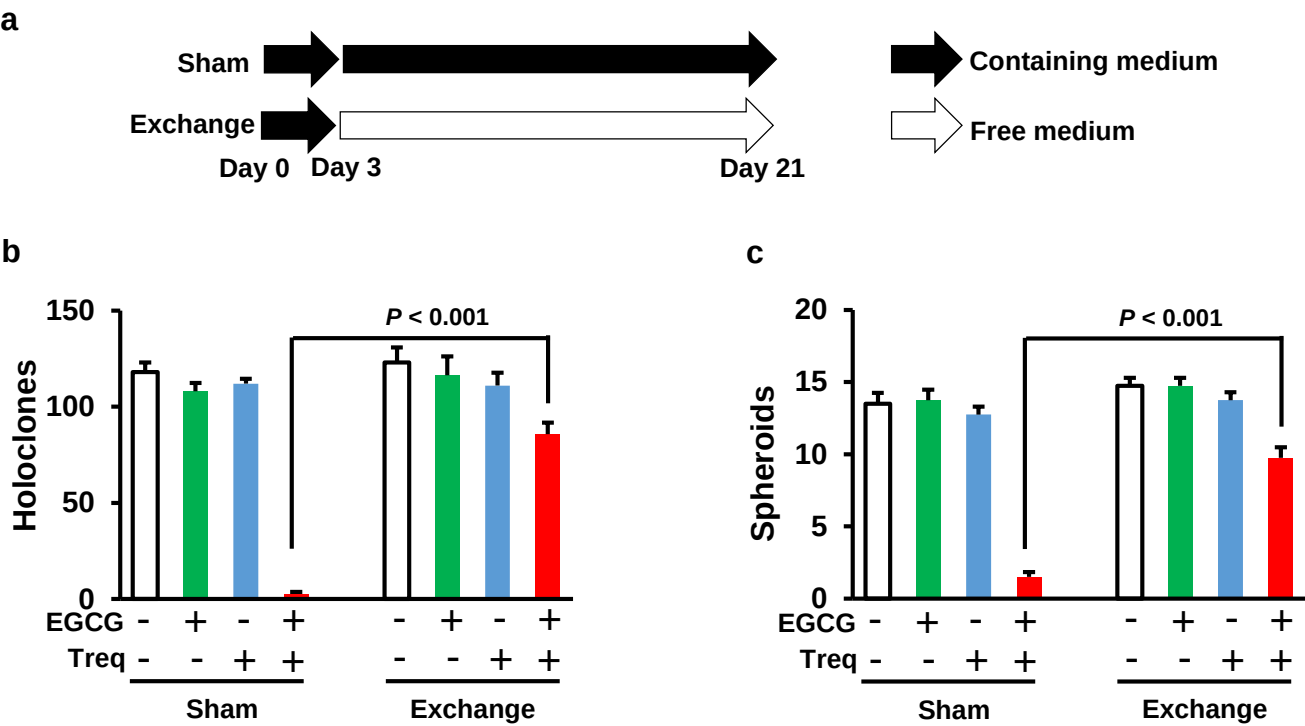
Supplementary Figure 1: Comparison of PDE3A expression between CD44⁺ and CD44⁻ cells.

(a) PDE3A expression was compared between CD44⁺ and CD44⁻ cells using FCM ($n = 3$). (b) PDE expression in Panc-1 cells was assessed using qRT-PCR. All data are presented as mean $\pm$ SEM.

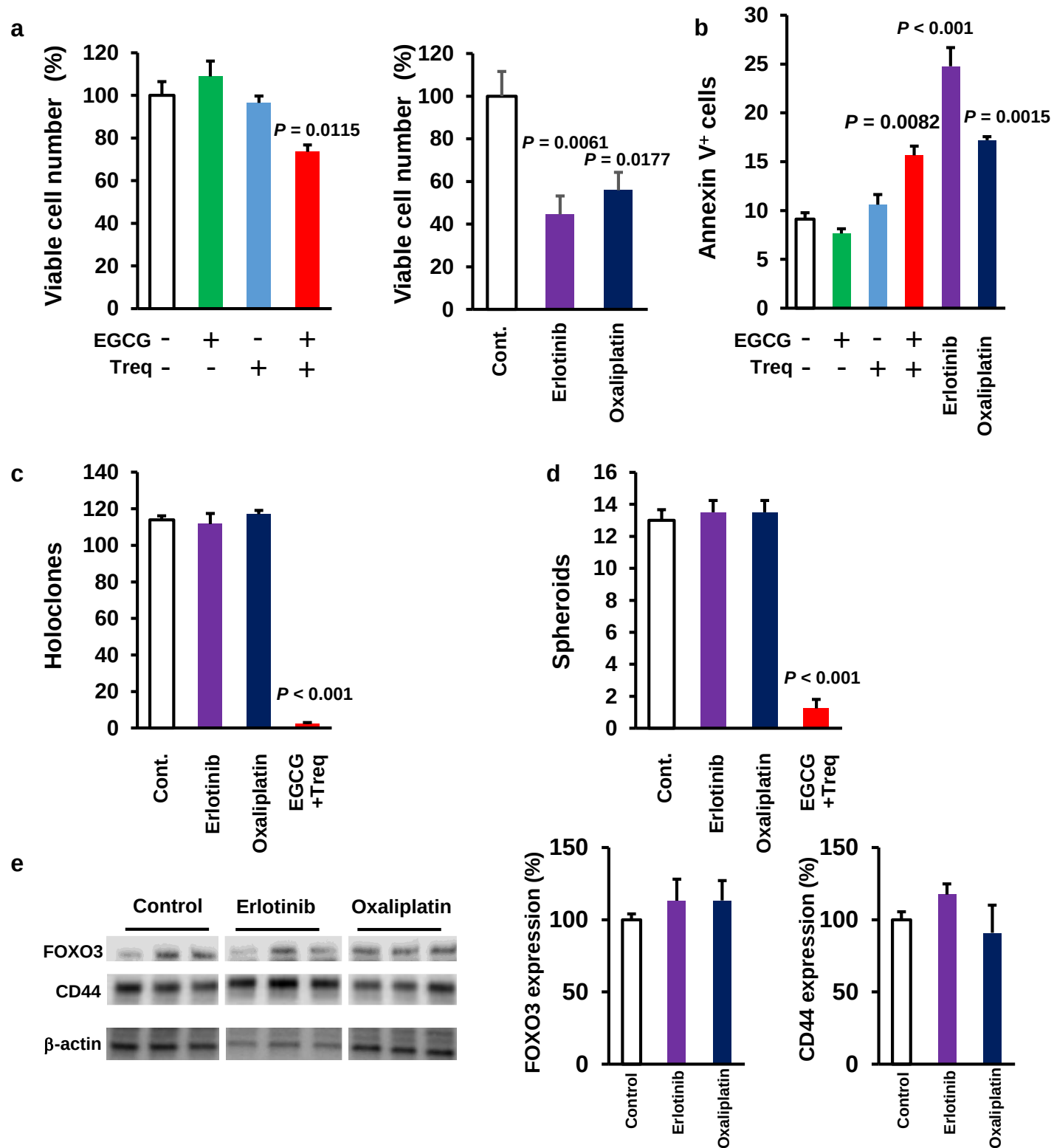


Supplementary Figure 2: Effect of high dose of EGCG on cGMP levels in Panc-1 cells.

The effect of a high dose of EGCG on cGMP levels in Panc-1 cells for 3 h ($n = 4$). All data are presented as mean $\pm$ SEM.

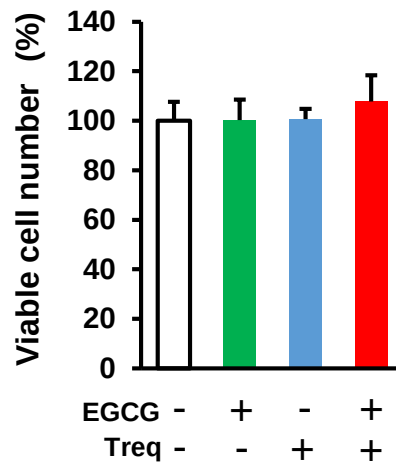


Supplementary Figure 3: Effect of short-term combined treatment of PDE3 inhibitor and EGCG. (a) Experiment schedule of the assessment of the effect of short-term combined treatment of PDE3 inhibitor and EGCG. (b) The effect of short-term combined treatment of PDE3 inhibitor (2.5 μ M) and EGCG (5 μ M) on colony formation ($n = 3$). (c) The effect of short-term combined treatment of PDE3 inhibitor (2.5 μ M) and EGCG (5 μ M) on spheroid formation ($n = 4$). All data are presented as mean $\pm$ SEM.



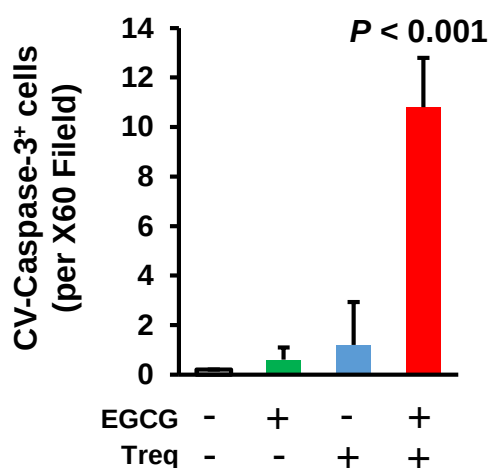
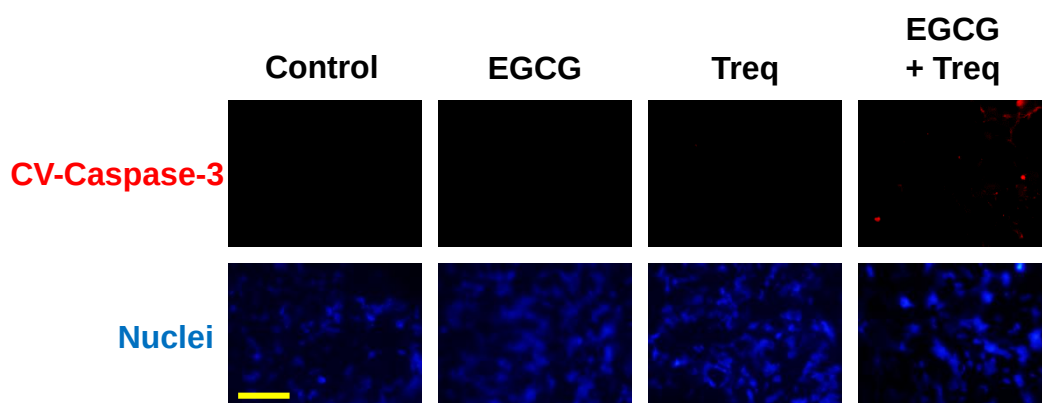
Supplementary Figure 4: Effect of anti-cancer agents on CSC properties.

(a) The effect of PDE3 inhibitor (2.5 μ M), EGCG (5 μ M), or erlotinib (25 μ M)/oxaliplatin (25 μ M) treatment for 72 h on viable cell number was determined by the ATPlite assay ($n = 3$). (b) The apoptosis-inducing effect of PDE3 inhibitor (2.5 μ M), EGCG (5 μ M), or erlotinib (25 μ M)/oxaliplatin (25 μ M) treatment for 72 h was determined by flow cytometry ($n = 3$). (c, d) The effect of erlotinib (25 μ M)/oxaliplatin (25 μ M) treatment for 21 days on (c) colony formation ($n = 3$) and (d) spheroid formation ($n = 4$). (e) The effect of erlotinib (25 μ M) and oxaliplatin (25 μ M) treatment on FOXO3 and CD44 expression in Panc-1 cells was assessed for 72 h ($n = 3$). Lanes were run on the same gel but were noncontiguous, white line. All data are presented as mean $\pm$ SEM.



Supplementary Figure 5: Combined effects of PDE3 inhibitor and EGCG on HUVECs.

The effect of PDE3 inhibitor (2.5 μ M) and EGCG (5 μ M) treatment for 72 h on viable cell number in HUVECs was determined by the ATPlite assay ($n = 3$). All data are presented as mean $\pm$ SEM.



Supplementary Figure 6: Combined effects of PDE3 inhibitor and EGCG on CV-Caspase-3⁺ cells.

CV-Caspase-3⁺ cells were evaluated by immunofluorescence analyses for 36 days. Nude mice implanted with Panc-1 cells were injected with EGCG (10 mg/kg/2 days i.p.) and trequinsin (5 mg/kg/2 days i.p.) ($n = 5$). Scale bar: 50 μ m. Original magnification, $\times 60$. All data are presented as mean $\pm$ SEM.

Supplementary Table1 :Primer list.

Gene	FORWARD (5'→3')	REVERSE (5'→3')
PDE1A	TGAAGGGATTGACAGAGC	ATGGTCCACCGATAATGC
PDE1B	CGCCGAGCAGAGGAGAAG	AGAACCGCAGTAGAGTAAGTGG
PDE1C	TGGAAGTGGGATACAGCAAGC	CTCCGTGAGCCAGTTCGC
PDE2A	ATCTTTGCCTTGTTTATTTCTG	CAGCCAGCACAGATTTTCG
PDE3A	GATGATAAATACGGATGTCTGTC	ACCGCCTGAGGAGCACTAG
PDE3B	TGCCTTCTTCTTCCTCACCTG	GACCACCACTGCCACACC
PDE4A	TTCACGGACCTGGAGATTC	TGAGGAACTGGTTGGAGAC
PDE4B	CAAGCCTAAACAATACAAGCATC	TGAGAATATCCAGCCACATTAAAG
PDE4C	CACCTGGCTGTGGGCTTC	ACTCAGTCGCTGCTTGGC
PDE4D	CTACTGGCTGATTTGAAGACTATG	GCTGGAGAGGCTTTGTTGG
PDE5A	ATCAGGAAACGGTGGGACATTTAC	CTTGTTCTCCAGCAGTGAAGTCTC
PDE6A	TGGCAAAGAGGACATCAAAGT	TAATCATCCATCCAGACTCATCC
PDE6B	GCAGAACAAATAGGAAAGAGTGGA	CAGGATACAGCAGGTTGAAGA CT
PDE6C	AAGAATGTTTTGTCCCTGCCTA	AAGAGTGGCTTTGGTTTGTT
PDE6D	AATGGTTCTTCGAGTTTGGC	AAAGTCTCACTCTGGATGTGCT
PDE6G	TTTAAGCAGCGACAGACCAG	ATATTGGGCCAGCTCGTG
PDE6H	TGAGTGACAACACTACTCTGC CT	ATGCAATTCCAGGTGGCT
PDE7A	AGATAGGTGCTCTGATACTAG	ATGTCTGTGTCTGGTGTC
PDE7B	GGCTTCTTGCTCATTTGC	CCTGTTGATGTCTGTTGC
PDE8A	ATGTTTGCTCGCTTTGGAATC	CAGAATGTGTAGAATTGTGGTAGG
PDE8B	CAAATCCCTCCGAGCACAC	CTCCATAAATCTCCTGTTGAAGC
PDE9A	CGTGGAATTGGAAGGACTAAAAG	GAGTCAACTTCTTGTTGTTATCC
PDE10A	AACTATCGGCGGGTTCCTTATC	GCGTGTGATTGTTCTGAAGTATGG
PDE11A	CTGCTGGGTTTCAAGACATTC	GCTTGGAAGGCATTGTTGG