

SOX9 indirectly regulates CEACAM1 expression and immune resistance in melanoma cells

Supplementary Material

Supplementary Table 1: Primers

Real-time primers

Gene	Forward primer	Reverse Primer
SOX9	5'- CCCTATCGACTTCCGCGAC	5'-TCGTTGACATCGAAGGTCTCG
CEACAM1	5'- GAGTAGTGGCCCTGGTTGCTC	5'- CGCTGGTCGCTTGCCCT
ETS1	5'- CTGACCCAGATGAGGTG	5'- CGCTGTCTTGTGGATGAT
GAPDH	5'- TGCACCACCAACTGCTTAGC	5'- CGCATGGACTGTGGTCATGAG

Cloning primers

Insert	Forward primer	Reverse Primer
SOX9 cDNA	5'- CATGAATCTCCTGGACCCC	5'- TCAAGGTCGAGTGAGCTGT
pCEACAM1 full	5'- GTTCCAGTGATTCTCCTGC	5'- GGTGTCTCCTGCTGGC
pCEACAM1 600bp	5'- CTGAGACCCCTGGACTTGGGTCT	5'- GGTGTCTCCTGCTGGCCCTGT
pCEACAM1 500bp	5'- CCACCTCTGTCACCTCC	5'- GGTGTCTCCTGCTGGC
pCEACAM1 400bp	5'- GGATATGCCAGGGTTCTCT	5'- GGTGTCTCCTGCTGGC
pCEACAM1 300bp	5'- CTGATGGGGACAGAGGTC	5'- GGTGTCTCCTGCTGGC
pCEACAM1 200bp	5'- AGGACAAAGGTAGGAATGAGAG	5'- GGTGTCTCCTGCTGGC

Mutations / deletions primers

	Position	Location upstream to start codon	Primers
Deletion of SOX9 site 1 in pCEACAM1	Chr19, 42530146 - 42530151	(-1777) - (-1772) bp	Fw: 5'- GACCACACCTGGCTAATTTTTTT TAGTAGAGACGGGG
			Rev: 5'- CCCCCTCTCTACTAAAAAAAATT AGCCAGGTGTGGTC
Deletion of SOX9 sites 2 & 3 in pCEACAM1	Chr19, 42529777 - 42529793	(-1419) - (-1403) bp	Fw: 5'- ATTCTCAAGCCCCCAAAGGCACT TTTTGTTTTGTTTTGTTTTGC
			Rev: 5'- GCAAAAACAAAACAAAACAAAA AGTGCCTTTGGGGGCTTGAGAAT
Deletion of SOX9 sites 4-7 in pCEACAM1	Chr19, 42529740 - 42529776	(-1402) - (-1366) bp	Fw: 5'- GCCCCCAAAGGCACCCTCTGGG ACAGAT
			Rev: 5'- ATCTGTCCAGAGGGTGCCTTTG GGGGC
Deletion of SOX9 site 8 in pCEACAM1	Chr19, 42528704 - 42528710	(-336) - (-330) bp	Fw: 5'- CCCATAGCCAGGACAGGTCAGT GCTGGA
			Rev: 5'- TCCAGCACTGACCTGTCCTGGCT ATGGG
Mutation of Sp1 site in	Chr19,	(-165) - (-152) bp	Fw: 5'- AATGAGAGGAGAGGAAACAGA GCTTCCTGGACAGTAACCGAACAGCACACA

pCEACAM1	42528526 - 42528539		TGATCAG
			Rev: 5'- CTGATCATGTGTGCTGGTTCGGTTAC TGTCAGGAAGCTCTGTTTCCTCTCCTCTCATT
Mutation of ETS1 site 1 in pCEACAM1	Chr19, 42528569 - 42528565	(-199) - (-195) bp	Fw: 5'- AGAGAAAGTAAGGACAAAGGT AAAAATGAGAGGAGAGGAAACAGAG
			Rev: 5'- CTCTGTTTCCTCTCCTCTCATTTT TACCTTTGTCCTTACTTTCTCT
Mutation of ETS1 site 2 in pCEACAM1	Chr19, 42528555 - 42528559	(-185) - (-181) bp	Fw: 5'- CAAAGGTAAAAATGAGAGGA GAAAAACAGAGCTTCTGGACAAAC
			Rev: 5'- GTTTGTCCAGGAAGCTCTGTTTT TTCTCTCTCATTTTTACCTTTG
Mutation of ETS1 site 3 in pCEACAM1	Chr19, 42528543 - 42528547	(-173) - (-169) bp	Fw: 5'- AAAATGAGAGGAGAAAAAAC AGAGCTTTTTGGACAAACCCCGC
			Rev: 5'- GCGGGGTTTGTCCAAAAGCTC TGTTTTTCTCCTCTCATTTT
Mutation of ETS1 site 4 in pCEACAM1	Chr19, 42528425 - 42528429	(-55) - (-51) bp	Fw: 5'- CAGCCGTGCTCGAAGCGTTTTTG GAGCCAAG
			Rev: 5'- CTTGGGCTCCAAAACGCTTCGA GCACGGCTG
Deletion of AP-2 binding site in pCEACAM1	Chr19, 42528485 - 42528500	(-126) - (-111) bp	Fw: 5'- GATCAGACAAAGCTCTGCTCAG CACAGAGAGTGG
			Rev: 5'- CCACTCTCTGTGCTGAGCAGAGC TTTGTCTGATC

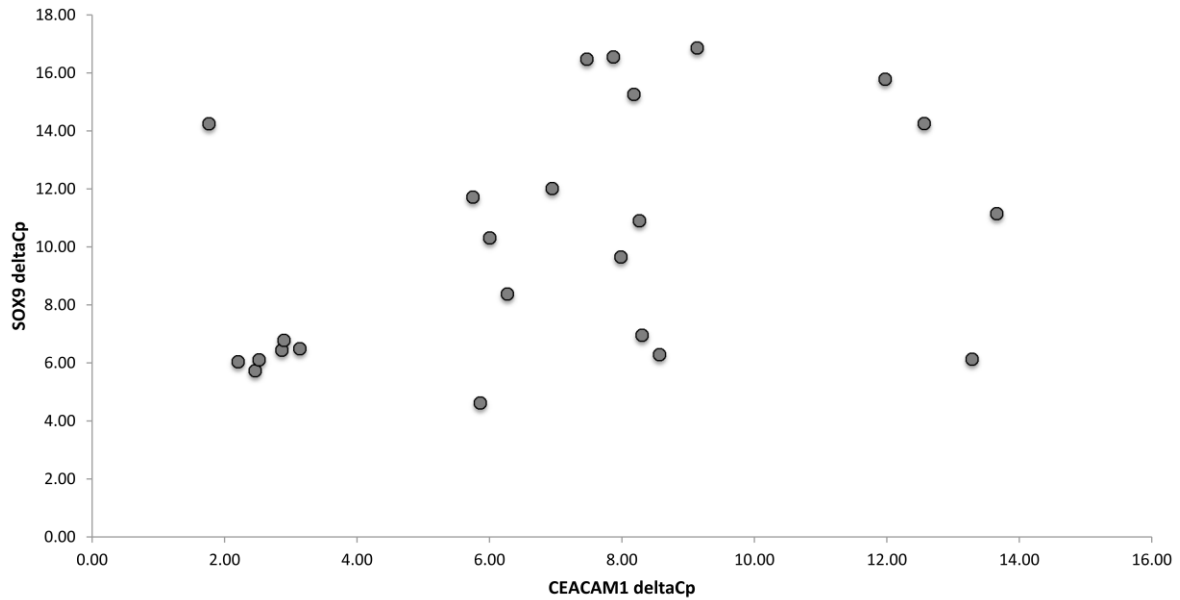


Figure 1: SOX9 and CEACAM1 mRNA expression in melanoma cell cultures. SOX9 and CEACAM1 mRNA expression in melanoma cell lines and low passage metastatic melanoma cultures, as determined by qPCR. Data represent one experiment performed in triplicates.