Supplementary Material and Methods

Immunofluorescence

Cultured cells on glass coverslips were stained by an indirect immunofluorescence method. Cells were rinsed in PBS and fixed with 3.7% formaldehyde in PBS for 10 minutes at room temperature. They were permeabilized with PBS plus 0.5% Triton X-100 for 5 minutes. Cells were treated overnight at 4°C, with PBS containing 1% BSA to block nonspecific binding sites. They were then incubated with primary antibodies: rabbit anti–ZEB-1 (1:50; Santa Cruz Biotechnology) or mouse anti–E-Cadherin (1:100; BD Biosciences) in PBS for 2 hours at room temperature. The same anti–ZEB-1 antibodies were used for Western blot analysis and ChIP assay. After three washes with PBS, 5 minutes at room temperature, cells were incubated for 30 minutes with goat antirabbit IgG conjugated to the fluorescent Alexa 488 dye (1:400; Invitrogen Molecular Probe, Leiden, Netherlands) or with goat antimouse IgG conjugated to the fluorescent Alexa 555 dye (1:400; Invitrogen Molecular Probe) in PBS containing the fluorescent nuclear probes TOPRO3 (1:250; Invitrogen Molecular Probe). After three washes, samples were mounted in Vectashield Medium (Vector Laboratories Inc, Abcys, Paris, France) for viewing with a confocal microscope (FV 1000, Olympus IX-81, Tokyo, Japan).

Table W1. Primer Sequences for Real-time Quantitative RT-PCR (A), ChIP assays (B), ZEB-1 and SEMA3F siRNA (C), E-box Site–Directed Mutagenesis (D), and Luciferase Reporter Construct with Restriction Sites Underlined (E).

Primer Name	Primer Sense	Primer Sequence (5′–>3′)	Amplicon Size (bp)
(A) Real-time quantitative RT-PCR primers			
GAPDH	Forward	TGCACCACCAACTGCTTAGC	87
	Reverse	GGCATGGACTGTGGTCATGAG	
SEMA3F	Forward	AGCAGACCCAGGACGTGAG	114
	Reverse	AAGACCATGCGAATATCAGCC	
E-Cadherin	Forward	CGGGAATGCAGTTGAGGATC	201
	Reverse	AGGATGGTGTAAGCGATGGC	
ZEB-1	Forward	AGCAGTGAAAGAGAAGGGAATGC	226
	Reverse	GGTCCTCTTCAGGTGCCTCAG	
ZEB-2	Forward	AGGCATATGGTGACGCACAA	78
	Reverse	CTTGAACTTGCGGTTACCTGC	
(B) ChIP primers			
ChIP E-Cadherin	Forward	GGCCGGCAGGTGAACCCTCA	108
	Reverse	GGGCTGGAGTCTGAACTGA	
A	Forward	GGCGTATGGATGTGTGGATGA	90
	Reverse	TATGAGAGCACCCACCCAGAAC	
B	Forward	GATTCTGAAGGTGGCAATGCC	100
	Reverse	AAACTTCCAGCTCCGCCTCTA	
C	Forward	GGAGCCCATGAGGATAACCAA	86
	Reverse	CCGTAAGCCTCCTTCCTCACA	
D	Forward	TGTGCCTCGGTTTCTCCATC	89
	Reverse	CCCCTTCACAACACCCACC	
E	Forward	TTGATGCTGTTGCGTACCCTG	99
	Reverse	CACCCAAAGCTAAACCCCTGA	
F	Forward	CCCTACAGTTCCAGCAGCCC	90
	Reverse	CCACCAACCCAGACCCTGAT	
(C) Primer sequences for ZEB-1 silencing by RNA interference			
ZEB-1	Sense	AATGCAGATTGAGGCTGATCACCTGTCTC	
	Antisense	AATGATCAGCCTCAATCTGCACCT GTCTC	
SEMA3F genome smart pool M-0176644-02 (Dharmacon)		GAACCGAACACACCUGUAU	
		GCAAGGAUGUCAACGGCGA	
		CAACACAACCGACUACCGA	
		GCUGGUGUGUACAUCGAUU	
(D) Site-directed mutagenesis primers			
E-Box mut1	Forward	GAAGGGGGTGCTTATATTAGCGCAGGCTCGTTGCTC	
	Reverse	GAGCAACGAGCCTGCGCTAATATAAGCACCCCCTTC	
E-Box2 mut2	Forward	GGGGCTGCACCGCCATTACCTGAGCAGCCCGGG	
	Reverse	CCCGGGCTGCTCAGGTAATGGCGGTGCAGCCCC	
(E) Primers for luciferase reporter construct			
*Xho*I-5836	Forward	GCGCTCGAGCTCAGCAGGATCCCTAGTGCC	
4013-*Hind*III	Reverse	GTTAAGCTTGCACTCACCTCTTCCGCAG	

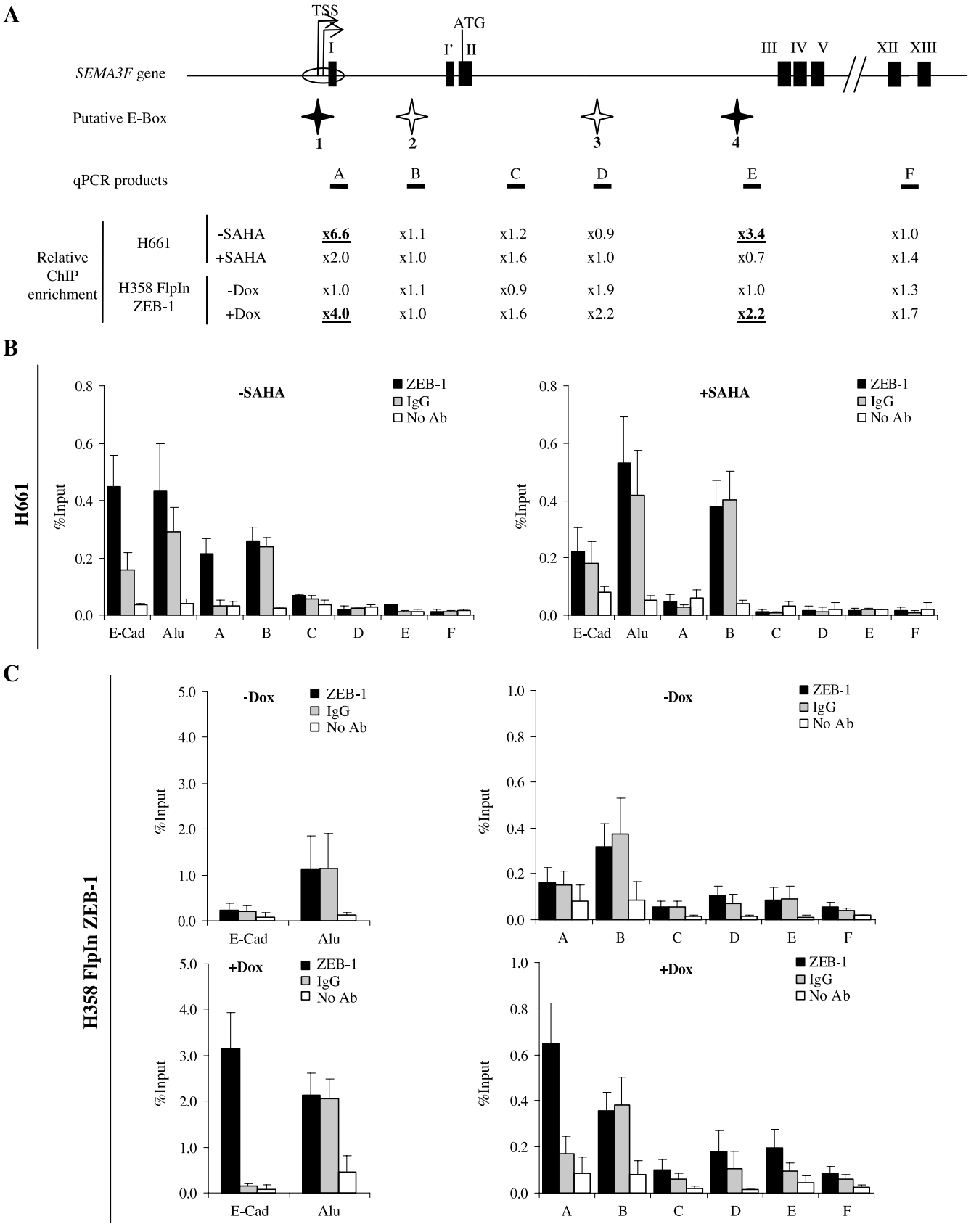

Figure W1. Summary of ZEB-1 binding sites in *SEMA3F* in H661 and H358 FlpIn ZEB-1 cells. (A) E-Box sites that bound ZEB-1 are indicated by black stars (sites 1 and 4), whereas those invalidated are in white (sites 2 and 3). The enrichment factors are given for H661 cells without or with HDAC inhibition by SAHA and for H358 FlpIn ZEB-1 cells without or with ZEB-1 induction by Dox. Percentage of input for the ChIP experiments in H661 cells without or with SAHA (B; left and right, respectively) and in H358 cells (C) without (−Dox) or with (+Dox).

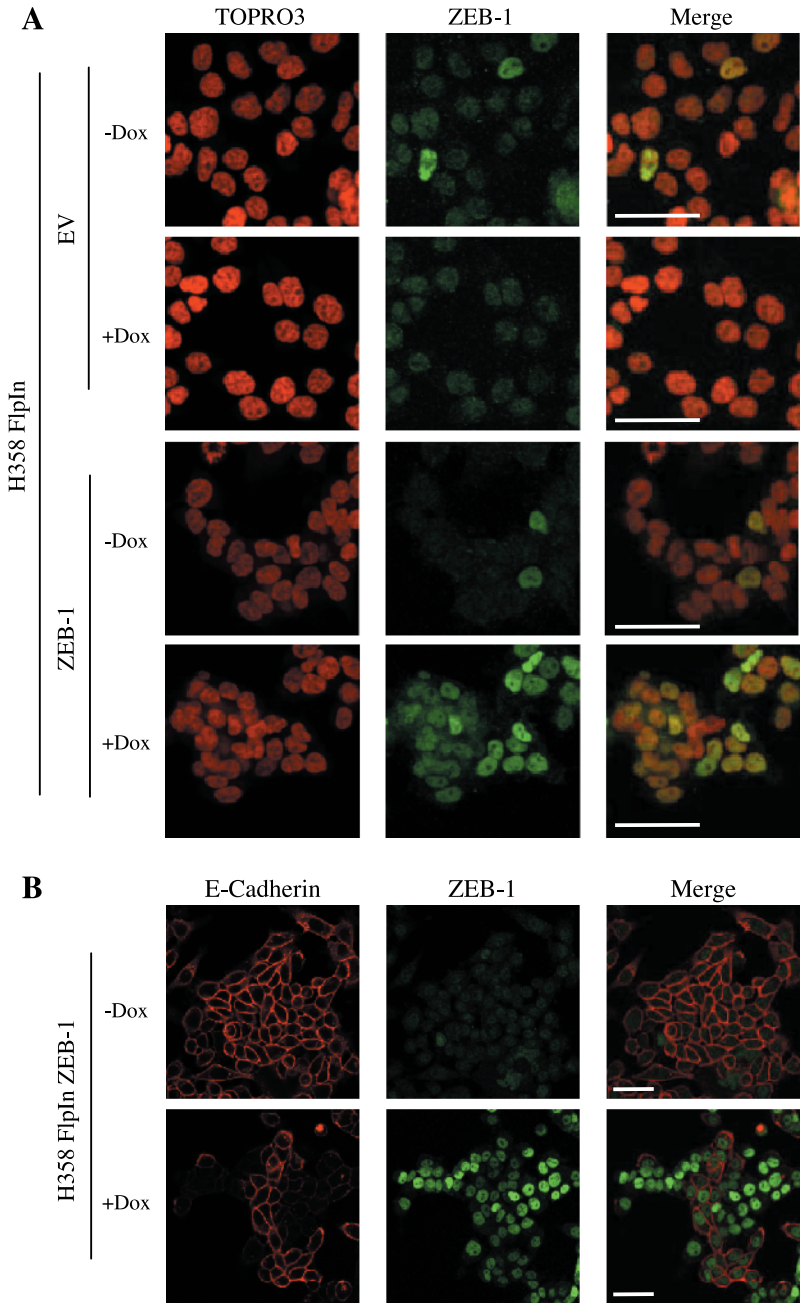

Figure W2. Induced ZEB-1 is localized in the nuclei of H358 FlpIn ZEB-1 cells and is present in cells where E-Cadherin is reduced. (A) Immunostaining with ZEB-1 antibody in H358 FlpIn EV or FlpIn ZEB-1 cells induced or not by Dox was performed to check the localization of ZEB-1. Nuclei were stained with the TOPRO3 probe. Three independent experiments were done. (B) Immunostaining with anti–E-Cadherin and anti–ZEB-1 antibodies was done in H358 FlpIn ZEB-1 cells induced or not with Dox. This experiment was done twice.

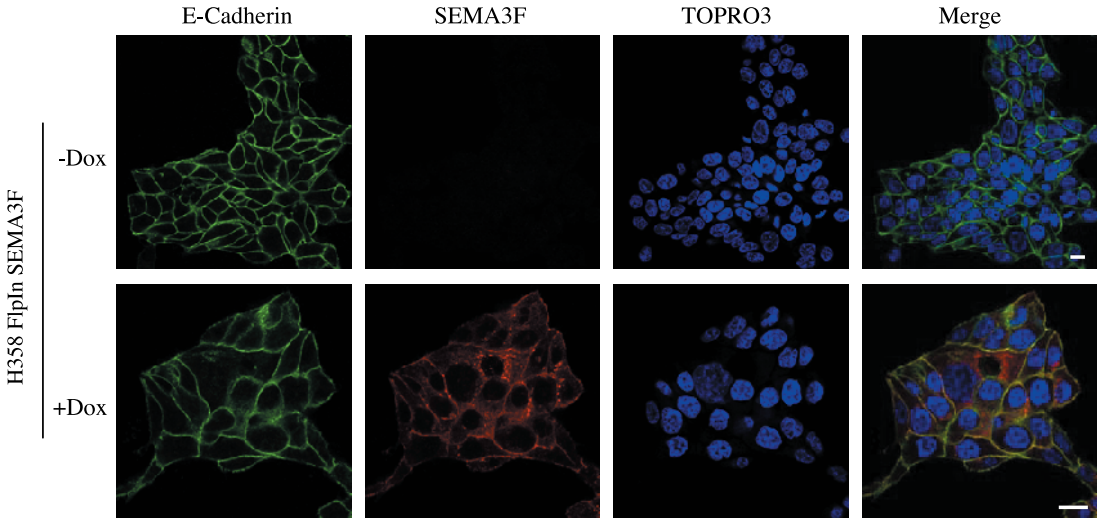

Figure W3. E-Cadherin localization is not modified by SEMA3F in H358 cells. Myc-tagged *SEMA3F* cDNA was cloned into pcDNA5/FRT/TO (Invitrogen) at the *Eco*RV site and introduced into H358 FlpIn cells. SEMA3F was induced for 3 days with Dox at 200 ng/ml. Cells were fixed with 3.7% formaldehyde for 20 minutes. Immunostaining with anti–E-Cadherin and anti-Myc antibodies was done with or without induction. Nuclei were stained with the TOPRO3 probe.