Downregulation of E-cadherin in pluripotent stem cells triggers partial EMT

Aban C.E.[1]

Lombardi A.[1]

Neiman G.[1]

Biani M.C.[1]

La Greca A.[1]

Waisman A.[1]

Moro L.N.[1]
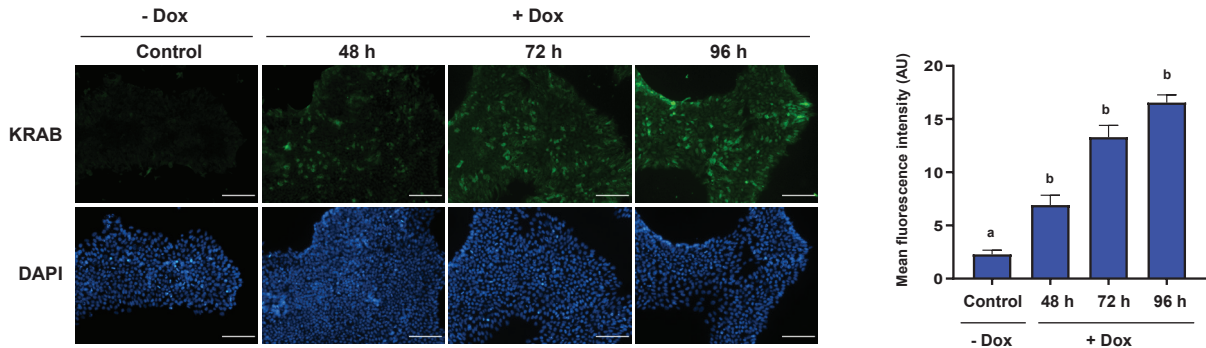
Sevlever G.[1]

Miriuka S.[1]

Luzzani C.[1,*]

[1] LIAN-CONICET, FLENI, Buenos Aires, Argentina

[*] Corresponding author: cluzzani@fleni.org.ar

S1

Figure S1. Expression of KRAB increased gradually after Dox incubation. Left panel: Immunofluorescence images of KRAB protein expression in cells incubated with Dox during 48, 72 and 96 h. Nuclei were stained with DAPI. Representative images of at least three experiments are shown. Scale bar 100 μm. Right panel: Quantification of the mean fluorescence intensity of three images from three independent experiments. Results are represented as mean ± SD (n=3). Different letters indicate significant differences of groups compared to control condition (p<0.0001) for ANOVA with post hoc Tukey.

S2
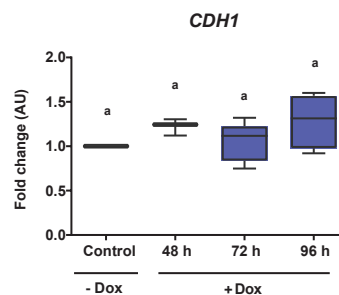
Figure S2. E-cadherin levels were not affected in parental HES3 cells after Dox incubation. Relative mRNA levels of CDH1 in parental HES3 wild type cells after incubation with Dox at different times assessed by qPCR. AU is the abbreviation for arbitrary units. Results are represented as mean ± SD (n=5). Different letters indicate significant differences of groups compared to control condition for ANOVA with post hoc Tukey.

S3
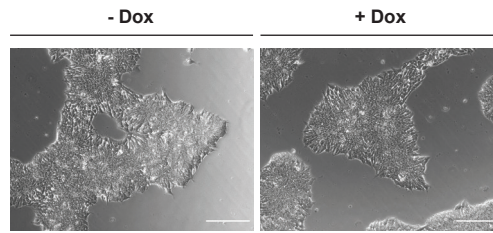
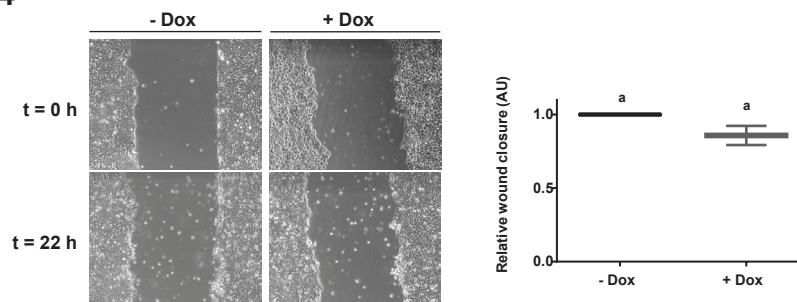**Figure S3. Cell morphology was not affected in parental HES3 cells incubated with Dox.** Representative images of parental HES3 wild type cells incubated with (+Dox) or without Dox (-Dox) for 96h. Scale bar 200 µm.

S4

Figure S4. Cell migration was not enhanced in parental HES3 cells after Dox incubation. Wound healing assay was made after parental HES3 wild type cells were treated with or without Dox (+Dox and -Dox, respectively) (t=0h). Representative images of at least three experiments are shown. Images were taken at t=0 h and after 22 h of recovery (t=22h).

Table S1 - sgRNA sequences

Name	Sequence
sgRNA1 Coding	ACACCGCAGTTCCGACGCCACTGAGG
sgRNA1 Template	AAAACCTCAGTGGCGTCGGAACTGCG
sgRNA2 Coding	ACACCGCGCCGGGTGCGGTCGGGTCG
sgRNA2 Template	AAAACGACCCGACCGCACCCGGCGCG

Table S2 - Primers sequences

Gene	Sequence	
CDH1	Forward	AAAGGCCCATTTCCTAAAAACCT
	Reverse	TGCGTTCTCTATCCAGAGGCT
TJP1	Forward	GGCAGCAAGAGATGGCAATA
	Reverse	ACGGTAGCCCGTTCAATCTCT
OCLN	Forward	GTTGCGGCGAGCGGATTG
	Reverse	TGGACTTTCAAGAGGCCTGG
CLDN3	Forward	ACGCGAGAAGAAGTACACGG
	Reverse	GTAGTCCTTGCGGTCGTAGC
GJA1	Forward	GTGCCTGAACTTGCCTTTTC
	Reverse	CCCTCCAGCAGTTGAGTAGG
DSC2	Forward	AACGGAGGTCAGGAGACCAT
	Reverse	TTTTTCACCAAGACGGGGCT
DSG2	Forward	TTCGGGAGGGAGAGGATCTG
	Reverse	TCCTCTTGCATCCAAAGCGT
DSP	Forward	GTGTCCTGGCACTACTGCAT
	Reverse	CTTCTGGGCATCGGTGAACT
NANOG	Forward	AAAGGATCTTCACCTATGCC
	Reverse	GAAGGAAGAGGAGAGACAGT

OCT4	Forward	CTGGGTTGATCCTCGGACCT
	Reverse	CACAGAACTCATACGGCGGG
LIN28A	Forward	TCAGGCTTGGGTTCACACCATCAC
	Reverse	GGTTGCCCCAGAACCCTCAC
SNAI1	Forward	ATGCACATCCGAAGCCACA
	Reverse	GAGGGTCAGCGGGGACATC
SNAI2	Forward	TCAAGGACACATTAGAACTCAC
	Reverse	CTACACAGCAGCCAGATTC
ZEB1	Forward	TTACACCTTTGCATACAGAACCC
	Reverse	TTTACGATTACACCCAGACTGC
ZEB2	Forward	GGAGACGAGTCCAGCTAGTGT
	Reverse	CCACTCCACCCTCCCTTATTTC
TBX6	Forward	CAGCCTACCAGAACCCACAG
	Reverse	GTGTGTCTCCGCTCCCATAG
MIXL1	Forward	GGTACCCCGACATCCACTT
	Reverse	TGGAAGGATTTCCCACTCTG
LINC-ROR	Forward	CACTCCAGCTATGCAGACCA
	Reverse	CTGACCTGTTGACCCACCTT
MALAT1	Forward	TGTTCTGATCCCGCTGCTATT
	Reverse	ACGACTGCTTAAAACTGCAGAAA
CCND1 (cyclin D1)	Forward	GATCAAGTGTGACCCGGACT
	Reverse	TCCTCCTCCTCTTCCTCCTC
MYCBP (c-myc)	Forward	CCTGGCTCCCCTCCTGCCTCGA
	Reverse	GCTCCCTCTGCCTCTCGCTGGA
RPL7	Forward	AATGGCGAGGATGGCAAG
	Reverse	TGACGAAGGCGAAGAAGC
HPRT1	Forward	TGACACTGGCAAAACAATGCA
	Reverse	GGTCCTTTTCACCAGCAAGCT

NKX2.5	Forward	CCCACGCCCTTCTCAGTCAA
	Reverse	GTAGGCCTCTGGCTTGAAGG
GATA4	Forward	CATCAAGACGGAGCCTGGCC
	Reverse	TGACTGTCGGCCAAGACCAG
TUBB3	Forward	TGGATTCGGTCCTGGATGTG
	Reverse	ACCTTGCTGATGAGCAACGT