SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Reintroduction of HDAC1 in HDAC1-/- ES cells reverts the phenotype of HDAC1-/- teratomas. $3x10^6$ HDAC1 reintroduced (HDAC1-/-re) and empty vector infected (HDAC1-/-ev*)* knockout ES cells were subcutaneously injected in SCID/Balb/c mice and teratoma formation as well as tumor size was monitored every 4 days. (A) Statistical comparison of the tumor volume of HDAC1-/-re (grey bars) and HDAC1-/-ev (black bars) teratomas. (B) Western Blot analysis of protein extracts from ES cells used for the injection and three individual HDAC1-/-re (lanes 1-3) and HDAC1-/-ev teratomas (lanes 4-6). The membrane was probed with antibodies against HDAC1, HDAC2 and Actin was used as loading control. (C) Immunohistochemistry of representative HDAC1-/-re and HDAC1-/-ev teratoma paraffin sections with antibodies against HDAC1 and HDAC2 (red AEC staining). The percentage in each picture window represents positively stained cells evaluated by the HistoQuest Software. All pictures were taken in a 20x magnification.

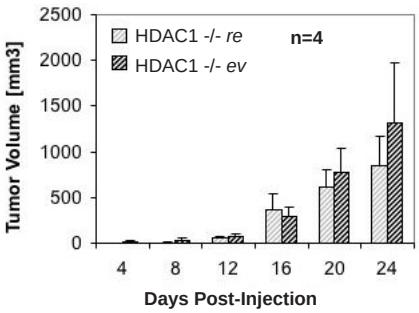
Supplementary Figure 2. Immunohistochemistry staining of HDAC1+/+ and HDAC1-/- teratomas with an antibody against cytokeratin (red AEC staining). Cells were counterstained with Hemalaun (blue staining). All pictures were taken in a 20x magnification.

Supplementary Table I

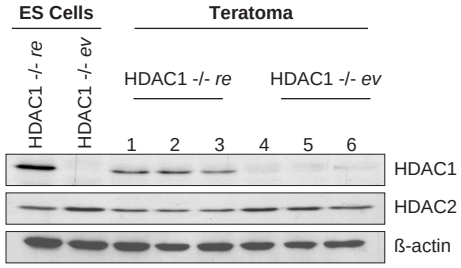
List of injection experiments with all ES cell lines used for teratoma generation. The number of individual experiments, total number of mice injected and duration of each individual experiment are listed.

Supplementary Figure 1

A



B



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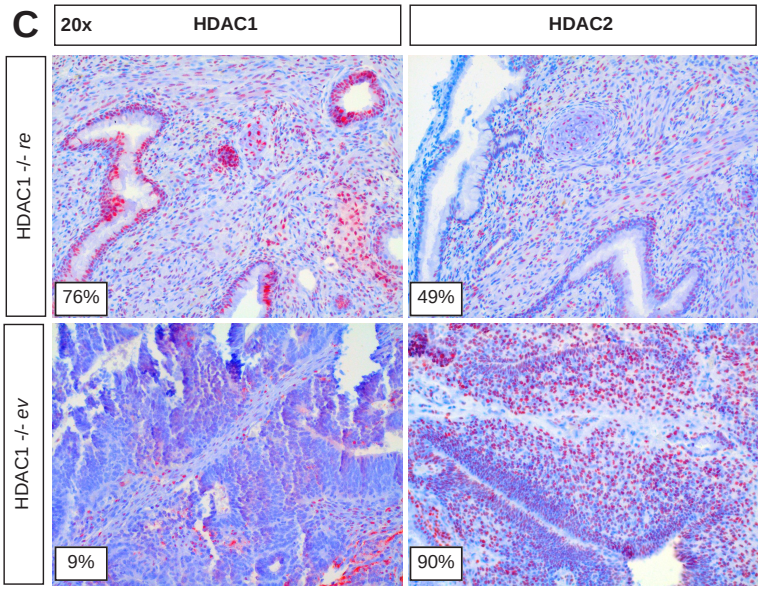
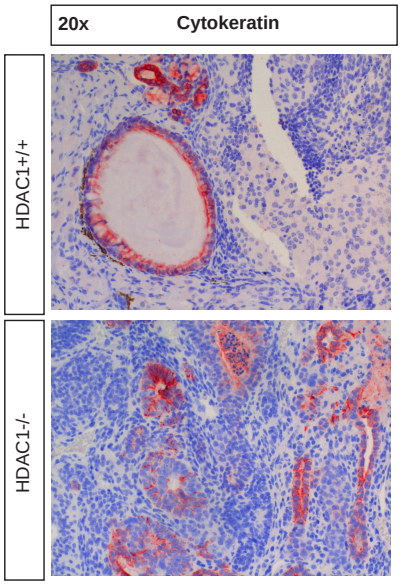


Supplementary Figure 2



Supplementary Table 1

ES Cells injected	HDAC1 +/+ and HDAC1 -/-	HDAC1 -/- *re* and HDAC1 -/- *ev*	HDAC1 +/+ and HDAC1 -/- (Alternate Cell Lines)
Number of Individual Experiments	3	1	1
Total Number of Mice injected	11	4	3
Duration of Experiments (days)	28	23	28

ES Cells injected	HDAC1 +/+ and HDAC1 -/- Snail1 NT and Snail1-1	HDAC1 +/+ and HDAC1 -/- Snail1 NT and Snail1-2
Number of Individual Experiments	1	1
Total Number of Mice injected	8	4
Duration of Experiments (days)	20	24

SUPPLEMENTARY INFORMATION

qRT-PCR primers

The following primer pairs were used to analyze mRNA expression by qRT-PCR: Hdac1 (*5' AAGCAGCAGACGGACATCG 3'* and *5' GCCTCTTCCACGCCATCG 3'*), Hdac2 (*5' CGGTGTTTGATGGACTCTTTG 3'* and *5' CCTGATGCTTCTGACTTCTTG 3'*), E-cadherin (*5' GAAGGAGGTGGAGAAGAAG 3'* and *5' CTCGTTCAGATAATCGTAGTC 3'*), Snail1 (*5' ACTCGGATGTGAAGAGATACC 3'* and *5' GCTGTGTCCAGAGGCTAC 3'*), Col2a1 (*5' GCGGAGACTACTGGATTG 3'* and *5' AGTGGTAGGTGATGTTCTG 3'*), Mmp9 (*5' ACGACATAGACGGCATCCA 3'* and *5' GCTGTGGTTCAGTTGTGGTG 3'*) (Olmeda et al 2007, Oncogene), Pcna (*5' CTGAAGAAGGTGCTGGAG 3'* and *5' GACATGCTGGTGAGGTTC 3'*), Hprt (*5' GCTGGTGAAAAGGACCTCT 3'* and *5' CACAGGACTAGAACACCTGC 3'*) and Gapdh (*5' GTCGTGTGAACGGATTTG 3'* and *5' GACTCCACGACATACTCA 3'*).

ChIP primers

Primer pairs used for ChIP analyses were designed to cover the E-box of the mouse Snail1 gene (*5' GCCCAAGCGGAATCTCAG 3*' and *5' GTTGGTCACGCCCCTTTG 3'*) and the E-pal sequence of the mouse E-cadherin gene (*5' GACCGTGGAATAGGAAG 3'* and *5' CAGGAGTCTAGCAGAAG 3'*).

Construction and Prevalidation of shRNA Vectors

To generate shRNA expression cassettes targeting mouse Snail1, Hdac1, and Hdac2 the pLKO.1 lentiviral vector system was used (Moffat J. et al. Cell. 2006 Mar 24. 124(6):1283-98) as described by Addgene (http://www.addgene.org/plko). Briefly, specific oligonucleotides (Sigma) corresponding to the following Broad TRC RNAi shRNA library (The RNAi Consortium) sequences were introduced into the Age I – EcoR I sites of pLKO.1 (Addgene plasmid # 10878):

mouse Snail1 (Snail shRNA#1: TRCN0000218784, Snail shRNA#2: TRCN0000234035, Snail shRNA#3: TRCN0000234034, Snail shRNA#4: TRCN0000234036, Snail shRNA#5: TRCN0000234033 and the 19nt long shRNA specific for Snail1 obtained from Olmeda et al. 2007 Oncogene); mouse Hdac1 (Hdac1 shRNA#1: TRCN0000218222, Hdac1 shRNA#2: TRCN0000229438, Hdac1 shRNA#3: TRCN0000229440); mouse Hdac2 (Hdac2 shRNA#1: TRCN0000039395, Hdac2 shRNA#2:TRCN0000039397, Hdac2 shRNA#3:TRCN0000039398).

To test for efficiency and specificity shRNA constructs were analyzed for target mRNA degradation using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's directions. The inhibitory effects generated by shRNA constructs were expressed as normalized ratios between the activities of the reporter luciferase gene (firefly) and the luciferase reporter target gene fusion (renilla) relative to the negative control vector containing scrambled shRNA (Addgene plasmid # 1864).

For mSnail1 *in vivo* knock-down experiments mismatch (MM) controls were constructed corresponding to the two most potent target sequences which are mutated at crucial positions 4, 10, 11, and 18. All shRNA expression cassettes were sequence verified.

Viral Particle Production and Target Cell Infection

Described shRNA-pLKO.1 constructs were co-transfected with the packaging plasmid pPax2 (Addgene plasmid # 12260) and the envelop plasmid pMD2.G (Addgene plasmid #12259) into human embryonic kidney 293FT cells using Lipofectamine 2000 (Invitrogen). Virus was harvested 24, 48, and 72 h posttransfection and combined viral supernatants (3 x 8 ml) were concentrated by sucrose gradient ultracentrifugation. Infections of mouse embryonic stem cells and F9 cells were carried out in the presence of 10 μg/ml hexadimethrine bromide (Sigma). Following transduction, cells were selected with 2 μg/ml puromycin.