Histone deacetylase 2 is required for chromatin condensation and subsequent enucleation of cultured mouse fetal erythroblasts

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Supplementary Appendix

Sequences of short hairpin RNA against individual histone deacetylases. Five different short hairpin RNA were used for each histone deacetylase.

HDAC1: GCTTGGGTAATAGCAGCCATT; CCGGTATTTGATGGCTTGTTT; CCCTACAATGACTACTTTGAA; GCCAGTCATGTCCAAAGTAAT; GCGTTCTATTCGCCCAGATAA.

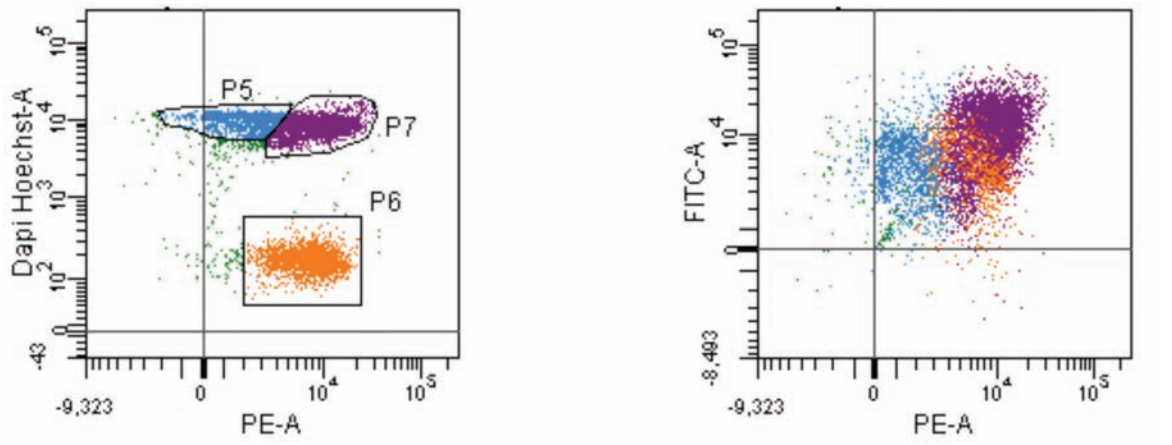
HDAC2: CCCAATGAGTTGCCATATAAT; GCTGTGAAATTAAACCGGCAA; CGAGCATCAGACAAACGGATA; CGATCAATAAGACCAGATAAT.

HDAC3: CCTGCATTATGGTCTCTATAA; GTGTTGAATATGTCAAGAGTT; GAGGCCATTAGTGAGGAACTT; GAGTTCTATGATGGCGACCAT; CGTGGCTCTCTGAAACCTTAA.

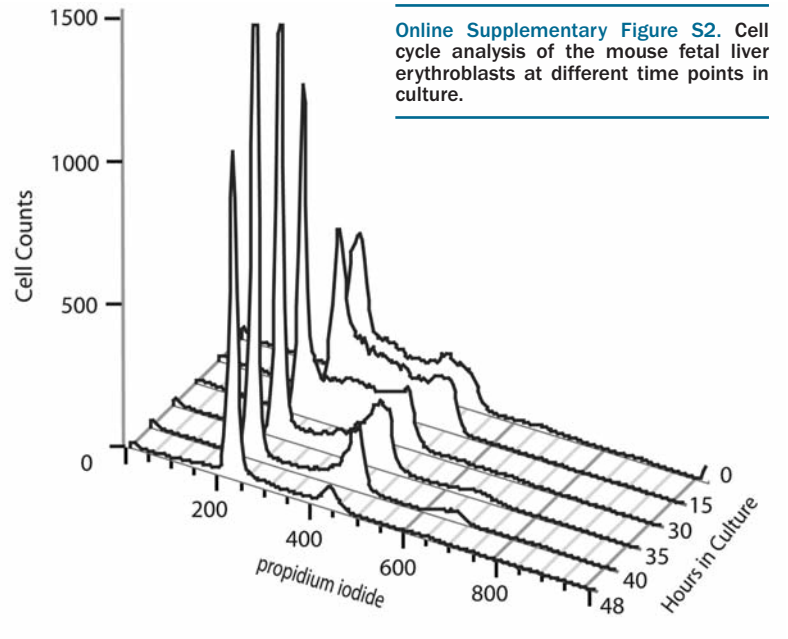
HDAC5: CTGGGCAAGATCCTTACCAAA; CCAAACCAGTTCAGCCTCTAT; GCTCAAGAATGGATTTGCTAT; GCACCAGTGTATGTGCGGAAA; CCAGGAATTCCTGTTGTCCAA.

Quantitative polymerase chain reaction

For Figure 3A, total mRNA from indicated cells was extracted using an RNeasy mini kit (Qiagen). RNA was reverse transcribed and quantified using SYBR green real-time polymerase chain reaction (PCR) and an ABI Prism 7000 sequence detection system (Applied Biosciences). The primers used in quantitative PCR are as follows: HDAC1 forward, TAATGAGCTGCCCTACAACG; HDAC1 reverse, CTGGTTGGTCATGTTGGAAG; HDAC2 forward, GGAGAAGATTGTCCGGTGTT; HDAC2 reverse, TTGTTGCCGGTTTAATTTCA; HDAC3 forward, TGGCATTGATGACCAGAGTT; HDAC3 reverse, CCTCGAATGCTGAGATTGAA; HDAC4 forward, AGGCACACAGAGACCCAAG; HDAC4 reverse, GCTGTCTCGCTTCTTCCTTC; HDAC5 forward, ACAGAGAAAGCACGAGACCA; HDAC5 reverse, TCCTCTTTCTCCCTGTCTGG; HDAC6 forward, AGACAAACCCAAAGGACCAG; HDAC6 reverse, CCCGTTGTCTCCTTCTGTCT; HDAC7 forward, GACAGCAGGAGCAAGAACC; HDAC7 reverse, GCTGGGTGGCTGTTCTCT; HDAC8 forward, GACCACCAGACCCAAGAAAG; HDAC8 reverse, GCTGTGTTGTCCTCCTCCTT; HDAC9 forward, ACCACAAGACACAACCAGGA; HDAC9 reverse, TCCTCTCTGCCCTTTCTGTT; HDAC10 forward, GAGGAGGAGACGGCAAGA; HDAC10 reverse, CTCCCGTGGTCCTTTGTC; HDAC11 forward, CAGCAGACCAGAGAAACGC; HDAC11 reverse, GCTTCTCTTCTTTCGGGTTG;



Online Supplementary Figure S1. The CD71^{med}TER119^{low} population represents extruded nuclei. TER119-negative mouse fetal liver erythroblasts were cultured as in Figure 1. After 48 h, the enucleation and differentiation status of cultured cells were analyzed by flow cytometric analysis using staining with Hoechst 33342 and TER119–PE (left) and FITC–CD71 and TER119–PE (right), respectively. P5, P6, and P7 represent extruded nuclei, incipient reticulocytes, and nucleated erythroblasts, respectively, establishing that extruded nuclei (blue in both panels) do indeed exhibit lower TER119 staining than nucleated erythroblasts.



Online Supplementary Figure S2. Cell cycle analysis of the mouse fetal liver erythroblasts at different time points in culture.