LEGENDS OF SUPPLEMENTAL FIGURES

Supplemental Figure 1. EtOH exposure does not affect the viability and morphology of 1C11 cells

a. Absorbance (450 nm) of 1C11 cells grown in 96-well plates, treated with 300 mM EtOH for 16h or 24h, and incubated with the XTT assay solution. Absorbance is represented in arbitrary units and is proportional to the amount of viable cells in the sample.

b. Phase contrast pictures of 1C11 cells in control conditions or treated with 300 mM EtOH for 8h (scale bar: 20 μm).

Supplemental Figure 2. Range of EtOH concentrations that activate the HSF pathway in 1C11 cells

a. HSF1 and HSF2 activity in unstressed and EtOH-treated 1C11 cells. Gel-shift analysis (EMSA) of HSF1 and HSF2 DNA-binding activity in 1C11 cells in control conditions and exposure to 150 mM for 2h, 4h, 6h, or 8h or to 300 mM EtOH for 4h or 6h, or untreated (CTR). The presence of HSF1 or HSF2 in the HSF-HSE complex was analyzed by supershifting with anti-HSF1 (α1; arrowhead) or anti-HSF2 antibodies (α2). 1C11 control cells exhibit a low constitutive HSF1 activity. In response to EtOH exposure, both HSF1 and HSF2 activities are increased by EtOH exposure as shown by supershifting of the HSE-HSF complex by anti-HSF1 or disruption of the HSE-HSF complex by anti-HSF2 antibodies. CHBA: constitutive HSE-binding activity, which is not carried by HSFs (Abravaya et al. 1991; Mosser et al. 1988); ns: non-specific DNA-protein complex; free: unbound double-stranded HSE oligonucleotide. Control human neuroblastoma cells SHSY-5Y heat-shocked SHSY-5Y and iMEFs were used a positive control for HSF2 and HSF1 DNA-binding activity, respectively.

b. *Hsp70* mRNA levels in 1C11 cells exposed to 150 mM or 300 mM EtOH for 2h, 4h, 6h, or 8h**.** RNA preparations were assayed for *Hsp70* mRNA. Results from RT-qPCR assays are shown as the average *Hsp70* mRNA level ± SEM over unstimulated cells in 3 independent experiments, normalized to *hmbs* and *Rpl13a* levels. One-way ANOVA followed by adjustment for multiple comparisons using Dunnett's method was performed: * p<0,05, ** p<0,01, *** p<0,001 vs 0h.

c*.* Immunoblot analysis of HSP70 protein levels in nuclear extracts of 1C11 cells treated with EtOH (300 mM) for 8h or 16h. GAPDH was used as an internal loading control. A representative immunoblot of 3 independent experiments is shown

Supplemental Figure 3. EtOH exposure increases *Dnmt* transcript levels in 1C11 neural precursors

a. *Dnmt* mRNA levels in 1C11 cells exposed to 150 mM of EtOH for 2h, 4h, 6h, and 8h. RNA preparations were assayed by RT-qPCR for *Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l* mRNAs. Results are shown as the average *Dnmt* mRNA levels over unstimulated cells ± SEM in 3 independent

experiments, normalized to *hprt1 and pgk1* levels. One-way ANOVA followed by adjustment for multiple comparisons using Dunnett's method was performed: * p<0.05, *** p<0.001 vs 0h.

b. Comparison of *Dnmt3a1*, *Dnmt3a2*, *Dnmt3b* and *Dnmt3l* mRNA levels in 1C11 cells exposed to 300 mM EtOH for 8h. Cycles obtained from RT-qPCR analysis of a representative experiment were used to estimate the relative abundance of the *Dnmt* transcripts.

Supplemental Figure 4. EtOH exposure augments *Tet1, Tet2 and Tet3* transcript, but not protein levels in 1C11 cells.

a. *Tet* mRNA levels in 1C11 cells exposed to 150 mM or 300 mM EtOH for 2h, 4h, 6h or 8h. RNA preparations were assayed by RT-qPCR for *Tet1, Tet2, Tet3* mRNA. Results are shown as the mean *Tet* mRNA levels over unstimulated cells (ctrl) ± SEM in 2 independent experiments, normalized against *hmbs* and *Rpl13a* levels.

b. Immunoblot analysis of TET protein levels in nuclear extracts of 1C11 cells treated with EtOH (300 mM) for 8h, 16h or 24h. Beta-ACTIN was used as an internal loading control. A representative immunoblot of 2 independent experiments is shown.

Supplemental Figure S5. mRNA levels of epigenetic factors in primary *Hsf1^{WT}* and *Hsf1$^{-/-}$* MEF exposed to ethanol.

mRNA levels of epigenetic modifiers in primary *Hsf1^{WT}* and *Hsf1$^{-/-}$* MEF exposed to 430 mM EtOH for 8h. RNA preparations were assayed by RT-qPCR for epigenetic enzymes, which were found among the HSF1 or HSF2 targets in ChIP-seq analyses (see Online Resource 2, Suppl. Table 2). Results are shown as the mean mRNA levels ± SD over unstimulated WT cells of 2 independent experiments, normalized to *B2m* and *cyclophilin* levels. The sole gene whose regulation is HSF-dependent is *Kdm6a* (*n=5* for this gene). One-way ANOVA followed by adjustment for multiple comparisons using Sidak's method was performed: * p<0,05.

Supplemental Figure S6. The increase in *Dnmt3a* mRNA levels upon EtOH treatment is not abolished by inhibition of NADPH oxidase.

a. *Dnmt3a* mRNA levels in 1C11 cells exposed to 300 mM EtOH for 6h, in the presence of DMSO or 4 µM DPI. RNA preparations were assayed by RT-qPCR for *Dnmt3a* mRNA. Results are shown as the average *Dnmt* mRNA levels ± SEM over unstimulated cells in 4 independent experiments, normalized to *hprt1* and *rpl13a* levels. One-way ANOVA followed by adjustment for multiple comparisons using Sidak's method was performed: *** p<0,001.

b. Immunoblot analysis of DNMT3A1, AKT, and phospho-Serine473-AKT levels in nuclear extracts of WT MEFs exposed or not to EtOH for 16h or 24h at 430 mM.

c. Immunoblot analysis of AKT, and phospho-Serine473-AKT levels in total extracts of 1C11 cells exposed or not to EtOH for 16 h at 300 mM.

SUPPLEMENTARY INFORMATION

Supplementary Materials and Methods

Electrophoretic mobility shift assay

Whole cell extracts were prepared as previously described (Mezger et al. 1989) and incubated with a (32P)-labeled HSE oligonucleotide (5'-CTAGAACGTTCTAGAAGCTTCGAGA-3'). Complexes were separated on a native 4% gel polyacrylamide gel as described (Rallu et al. 1997). The components of the retarded complexes were analyzed by supershift using antibodies against HSF1 (Ab4 Neomarker; 1:150 final dilution) or HSF2 (rabbit polyclonal H57; kind gift from Lea Sistonen Lab; 1:100 final dilution).

References related to supplemental information

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Supplementary Table 1. ***Primers used for RT-qPCR experiments***

	Primer - F	Primer - R
Epigenetic actors		
	DNA methylation	
Dnmt1	CGTTGTGGTGGATGACAAGA	GAACCAGGACAGTGGCTCT
Dnmt3a	GTGCAGAAACATCGAGGACA	ATGCCTCCAATGAAGAGTGG
Dnmt3b	ACAACCGTCCATTCTTCTGG	GTGAGCAGCAGACACCTTGA
Dnmt3l	CTGCTGACTGAGGATGACCA	ACCCGCATAGCATTCTGGTA
	DNA demethylation	
Tet1	AACTGGCTTCCTGTCAGGTG	TCGGCGTAGATCTCCTCACT
Tet2	TGCCTCCAGATCACCATACA	TGCCGTGTAGCTGTAGATCG
Tet3	CTTGCCAGGCTTTGTCTAGC	TTGACTGGTCCCAGCCTAAC
	Histone/Lysine acetylation	
Ncoa3	GGCAGGCACTTGAAATGAAA	GCCATTTGGGCATTAAAGAA
	Histone/Lysine deacetylation	
Hdac4	CACACCTCTTGGAGGGTACAA	GATGGCTGTCAGGTCATGG
Hdac5	GTGACACGGTGTGGAATGAG	CCGGATGATAGCAAATCCAT
Hdac7	GAGGCCTGTGTAGCTGCTCT	GGCACTGAGGTTGGGTTTCT
Hdac9	CCTGCCCAATATCACTCTGG	GCATCTGTGTCTCGCACTTC
Hdac11	TCATGGGTGACAAGCGAGTA	CCTGATGGCCTCTTTAGCAA
	Histone/Lysine methylation	
Dot1l	CAACTGTTGCTGGCCTCTTT	GTGATCTCCAGTGGCTGGTT
Ehmt1	ATTGACGCTCGGTTCTATGG	AATCAGGCGGGTACTGAAGA
Prmt2	CACCACACACTGGAAGCAGA	AACAGAACCCGTGACCACAT
Prmt3	GAAGAAGTGAGTCTTCCTGTGGA	GGACAGAATCCAACATCGACT
Setd5	GAGTCCCATCTGCTCCTCAG	CCCACCAGAATTCTCCTTAGC
	Histone demethylation	
Jarid2	CAAAGCAACCACCAACAATG	TTCTCCCGTGCTGACCTACT
Jmjd6	TGACCTCCAGGAGTCCACA	TCTGAGTCCGAGTCTGACGA
Kdm1a	GGCCATTCTCAAAGGGATTT	GGATCCTGCAGCCACATAAG
Kdm3a	TGAGAAAGCGCCTCTATCAAG	GCTCCTGCTGGGATAAACAC
Kdm4b	GATGACTGGCCCTATGTGGT	GCGATAGTAGAGCCCATTGC
Kdm6a	TTGTAGTATTTGTGAGGTGGAGGT	GTGCACAATCTTGGCAATGT
Kdm6b	AAGAGCTGGTGCTGAGCAAG	GGCTGCCATTCTCACTTGTA
Kdm8	GGGCTCAAGGTACACAGATGA	AAGAGCTGGTGCTGAGCAAG
Smyd3	GGAGGTTCAAGAGTCGCTGA	CCGGTTGGAATTGCTGTTTA
Heat Shock Pathway		
Hsp70	GGCCACATTGTTGATACATGC	CTACAGTGCAACCACCATGC
Reference genes		
B2m	AGAATGGGAAGCCGAACATA	CGTTCTTCAGCATTTGGATT
Cyclophilin	TGCCATCCAGCCAGGAGGTC	CCATCGTGTCATCAAGGACTT
Hmbs	GCTGAAAGGGCTTTTCTGAG	TGCCCATCTTTCATCACTGT
Hprt1 3'	TGTCAGTTGCTGCGTCCCCAGA	TCTACCAGAGGGTAGGCTGGCC
Hprt1 5'	GCCATTGCTGAGGCGGCGAG	CCGGCGGAGGAGGTGCTACC
Pgk1	ACCTGCTGGCTGGATGGGCT	CTCGACCCACAGCCTCGGCA
Rpl13a	CGGATGGTGGTCCCTGCTG	GAGTGGCTGTCACTGCCTGG

Supplementary Table 2. ***Enzymes involved in epigenetic mechanisms, whose genes are potential HSF targets, identified in HSF1 and HSF2 ChIP seq data*.**

Symbol	RefSeq	Description	HSF-ChIP-Seq		
			Mendillo	Charos	Vihervaara
DNA methylation					
Dnmt1	NM_010066	DNA methyltransferase 1	+	+	
Dnmt3a	NM_007872	DNA methyltransferase 3A		+	
Dnmt3b	NM_010068	DNA methyltransferase 3B	+	+	+
Dnmt3l	NM_001081695.2	DNA methyltransferase 3-like	+		+
DNA hydroxymethylation					
Tet1	NM_001253857.1	Ten-eleven-translocation-methylcytosine dioxygenase 1			
Tet2	NM_001040400.2	Ten-eleven-translocation-methylcytosine dioxygenase 2			
Tet3	NM_183138.2	Ten-eleven-translocation-methylcytosine dioxygenase 3	+		
Histone/Lysine acetylation					
Atf2	NM_009715	Activation transcription factor 2	+		
Cdyl	NM_009881	Chromodomain protein, Y chromosome-like	+		
Csrp2bp	NM_181417	Cysteine and glycine-rich protein 2 binding protein		+	
Esco1	NM_001081222	Establishment of cohesion homolog 1 (*S. cerevisiae*)			+
Esco2	NM_028039	Establishment of cohesion homolog 2 (*S. cerevisiae*)			
Kat2A (GCN5)	NM_020004	K(lysine) acetyltransferase 2A			+
Kat7 (Myst2)	NM_177619	MYST histone acetyltransferase 2	+		
Kat8 (Myst1)	NM_026370	MYST histone acetyltransferase 1			
Ncoa3	NM_008679	Nuclear receptor coactivator 3	+	+	
Histone/Lysine deacetylation					
Hdac2	NM_008229	Histone deacetylase 2	+		
Hdac4	NM_207225	Histone deacetylase 4		+	
Hdac5	NM_010412	Histone deacetylase 5	+	+	
Hdac7	NM_019572	Histone deacetylase 7	+		+
Hdac8	NM_027382	Histone deacetylase 8			
Hdac9	NM_024124	Histone deacetylase 9	+		+
Hdac11	NM_144919	Histone deacetylase 11		+	
Histone/Lysine methylation					
Carm1	NM_021531	Coactivator-associated arginine methyltransferase 1			
Kmt1b (Suv39h1)	NM_011514	Suppressor of variegation 3-9 homolog 1 (*Drosophila*)			
Kmt1c (Ehmt2, G9A)	NM_145830	Euchromatin histone N-methyltransferase 2			
Kmt1d (Ehmt1, GLP)	NM_172545	Euchromatin histone methyltransferase 1	+		+
Kmt1e (Setdb1)	NM_018877	SET domain, Bifurcated 1	+		
Kmt1f (Setdb2)	NM_001081024	SET domain, Bifurcated 2	+		
Kmt2a (MLL)	NM_005933	myeloid/lymphoid or mixed-lineage leukemia	+		+
(Kmt2c) Mll3	NM_001081383	myeloid/lymphoid or mixed-lineage leukemia 3	+		
(Kmt2e) Mll5	XM_893956	myeloid/lymphoid or mixed-lineage leukemia 5	+		
Kmt3a (Setd2)	NM_001081340	SET domain containing 2	+		
Kmt3b (Nsd1)	NM_008739	Nuclear Receptor Binding SET Domain			

		Protein 1			
Ktm3g (Whsc1, NSD2)	NM_001081102	Wolf-Hirshhom syndrome candidate 1 (human)	+		
Ktm4 (Dot1l)	NM_199322	DOT1-like Histone H3 methyltransferase (*S. cerevisiae*)	+		+
Mllt1	NM_022328.2	myeloid/lymphoid or mixed-lineage leukemia; translocated to, 1	+		
Mllt4	NM_010806.13	myeloid/lymphoid or mixed-lineage leukemia; translocated to, 4	+		
Mllt6	NM_139311.2	myeloid/lymphoid or mixed-lineage leukemia; translocated to, 6	+	+	
Prmt1	NM_019830	Protein arginine N-methyltransferase 1			
Prmt2	NM_133182	Protein arginine N-methyltransferase 2		+	+
Prmt3	NM_133740	Protein arginine N-methyltransferase 3		+	+
Prmt5	NM_013768	Protein arginine N-methyltransferase 5		+	
Setd4	NM_145482	SET domain containing 4	+		
Setd5 (KIAA1757)	NM_028385	SET domain containing 5	+	+	
Setd6	NM_001035123	SET domain containing 6			
Setd7	NM_080793	SET domain containing (lysine methyltransferase) 7	+		
Smyd1	NM_009762	SET and MYND domain containing 1			
Smyd3	NM_027188	SET and MYND domain containing 3	+		+
Ktm5b (Suv4-20h1)	NM_144871	Suppressor of variegation 4-20 homolog 1 (*Drosophila*)	+		
		Histone/Lysine demethylation			
Kdm1a (AOF2, KDM1, LSD1, BHC110)	NM_133872	Lysine (K)-specific demethylase 1	+		+
Kdm4c (JHDM3C, JMJD2C, TDRD14C)	NM_144787	Lysine (K)-specific demethylase 4C	+		
Kdm5b (JARID1B)	NM_152895	Lysine (K)-specific demethylase 5B	+		
Kdm8 (JMJD5)	NM_029842.5	Lysine (K)-specific demethylase 8	+	+	
Jarid2 (JMJ)	NM_001205043.1	Jumonji, AT rich interactive domain 2	+		+
Kdm6a (UTX)	NM_009483.1	Lysine (K)-specific demethylase 6A	+	+	+
Kdm4b (JMJD2B, TDRD14B)	NM_172132.2	Lysine (K)-specific demethylase 4B	+		+
Kdm3a (JHDM2A, JHMD2A, JMJD1,JMJD1A	NM_001038695.3	Lysine (K)-specific demethylase 3A	+	+	
Kdm3b (JMJD1B)	NM_001081256.1	Lysine (K)-specific demethylase 3B	+		
Jmjd1c	NM_001242396.1	Lysine (K)-specific demethylase 1C	+		
Jmjd4	NM_001205068.1	Lysine (K)-specific demethylase 4		+	
Kdm5a (JARID1A)	NM_145997.2	Lysine (K)-specific demethylase 5A	+		
Kdm6b (JMJD3)	NM_001017426	KDM1 Lysine (K)-specific demethylase 6B	+	+	+

Enzymes involved in chromatin remodeling that were found among the HSF1 or HSF2 targets identified in ChIP-seq analyses from Charos et al. (2012), Mendillo et al. (2012), and Vihervaara et al. (2013). A plus indicates that the gene is bound by HSFs. The genes chosen for qPCR analysis are present in at least two ChIP-seq analyses and are highlighted in yellow.