

Fig. S1. ER α does not affect TFIIIC₆₃ expression. MCF-10A and MCF-7 cells were treated with 25mM ethanol, 5nM E2 or both for 60min. RT-qPCR was performed using RNAs derived from these cells to determine cellular levels of TFIIIC₆₃ and GAPDH mRNAs. The fold change was calculated by normalizing to the amount of GAPDH mRNA. The values represent mean \pm SE from three independent experiments.

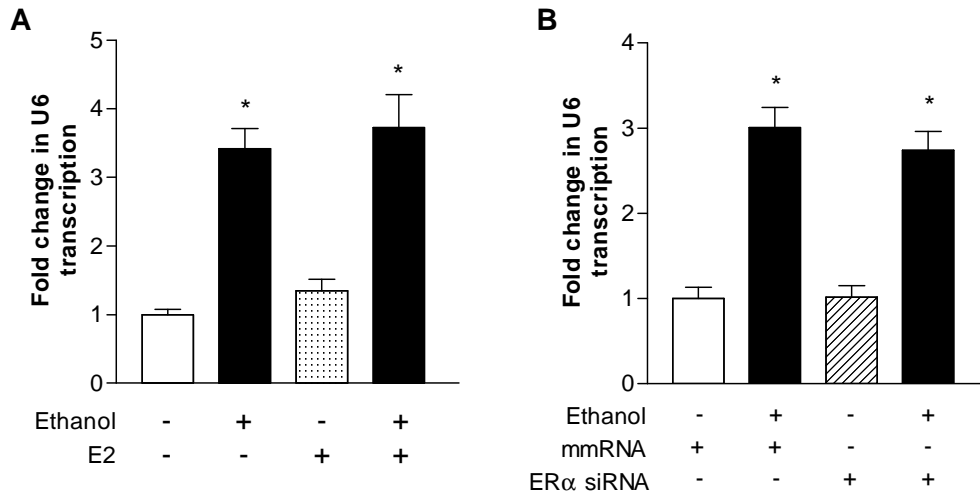


Fig. S2. E2 or ER α does not affect U6 gene transcription. (A) MCF-7 cells were treated with 25mM ethanol, 5nM E2 or both for 60min. (B) MCF-7 cells were transfected with mismatch (mm) RNA or ER α siRNA for 48h and treated with 25 mM ethanol for another 60min. RT-qPCR was performed using RNAs derived from these cells to determine cellular levels of U6 gene and GAPDH (6). The fold change was calculated by normalizing to the amount of GAPDH mRNA. The values represent mean \pm SE from three independent experiments. *: $p < 0.01$ control compared with ethanol or ethanol + E2 (A) and mmRNA compared with mmRNA + ethanol or ER α siRNA + ethanol (B).

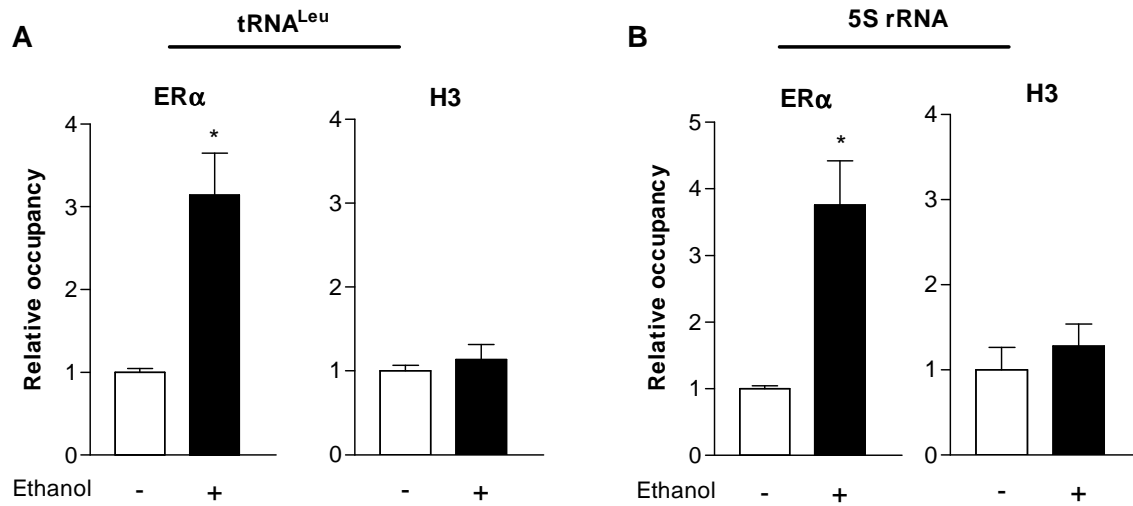


Fig. S3. Ethanol induces occupancy of ER α to Pol III gene. (A) *Ethanol-mediated binding of ER α to the tRNA^{Leu}.* (B) *Ethanol increases ER α occupancy to 5S rRNA.* MCF-7 cells were treated with or without ethanol and ChIP assays were performed using ER α or histone H3 antibodies and qPCR to quantify the amplified DNA. The relative occupancy of the proteins was calculated based on the control (no ethanol treatment). All values shown are the means \pm SE of at least three independent chromatin preparations. *: $p < 0.01$ control compared with ethanol.

SUPPLEMENTS

Table S1. siRNA targets	
Targets	Sequences
Mismatch	sense 5'UUC UCC GAA CGU GUC ACG U 3' antisense 5'ACG UGA CAC GUU CGG AGA A 3'
Human Brf1 siRNA	A: sense 5' GGA AGA UCU GUU GUU ACU U 3' (1) antisense 5' AAG UAA CAA CAG AUC UUC C 3' B: sense 5' CCC GUG CCU GUA UAU UCC A 3' (1) antisense 5' UGG AAU AUA CAG GCA CGG G 3' C: sense 5' GAG CAU AGC GCC AGU GCC A 3' antisense 5' UGG CAC UGG CGC UAU GCU C 3'
Human ER α siRNA:	A: sense 5' GGC CAA AUU CAG AUA AUC G 3' (2) antisense 5' CGA UUA UCU GAA UUU GGC C 3' B: sense 5' AAU GAU GAA AGG UGG GAU A 3' antisense 5' UUA CUA CUU UCC ACC CUA U 3' C: sense 5' UGA UGA AUC UGC AGG GAG A 3' antisense 5' ACU ACU UAG ACG UCC CUC U 3'

Table S2. Primer Sets for Quantitative RT-PCR		
Target	Primers	Annealing Temperature
Pre-tRNA ^{Leu} (3)	(F) 5'-GTC AGG ATG GCC GAG TGG TCT AAG-3' (R) 5'-CCA CGC CTC CAT ACG GAG AAC CAG AAG ACC C-3'	61°
5S rRNA (4)	(F) 5' GGC CAT ACC ACC CTG AAC GC 3' (R) 5' CAG CAC CCG GTA TTC CCA GG 3'	61°
Human TBP	(F) 5' GCT AGG TTT CTG CGG TCG CGT C -3' (R) 5' CTG TAC TGA GGC TGC TGC AGT TGC TAC -3'	60°
Human Brf1	(F) 5' CCT CGG GCC TCT GCG GAG CAG -3' (R) 5' TCA TCA ATG GTC AAC TGA CTG GTG G -3'	6°
GAPDH (4)	(F) 5'-TCC ACC ACC CTG TTG CTG TA-3' (R) 5'-ACC ACA GTC CAT GCC ATC AC-3'	61°

Abbreviations: (F) = forward, (R) = reverse.

Table S3 Primer Sets for ChIP		
Target	Primers	Annealing Temperature
hBrf1 promoter -233/+42	(F) 5' CGT CCA GCT TTA GTC CCC GAC 3' (R) 5' CGA GCC CAA GGC GGC TTC G 3'	63°
hBrf1 promoter -1153/-941	(F) 5' GAG ACA GAG TTT CAC TAT TGT C 3' (R) 5' GGT ACA ACC GGT CCG ACC AGA GC 3'	62°
tRNA ^{Leu} gene (3)	(F) 5'-GTC AGG ATG GCC GAG TGG TCT AAG-3' (R) 5'-CCA CGC CTC CAT ACG GAG AAC CAG AAG ACC C-3'	61°

Chromatin immunoprecipitation (ChIP) assays. MCF-7 cells (3×10^6 cells) were cultured in 15 cm dishes and treated with ethanol. The cells were fixed with formaldehyde (1% final concentration) at 24°C for 10 min. Soluble chromatin were prepared as described previously (5). The chromatin were then diluted 1:10 with buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris–HCl and 167 mM NaCl) and were subjected to immunoprecipitation (IP) in lysis buffer (50 mM Tris–HCl, 10 mM EDTA, 1% SDS) and a protease inhibitor cocktail set (CalBiochem). Pre-immune serum was used as a control and antibodies of Brf1, ER α or histone H3 were used for IP. The chromatin and antibodies were incubated at 4°C overnight. Complex of chromatin/antibody were recovered by adding 45 μ l of protein A/G PLUS–agarose beads and incubated at 4°C for 2 h. The beads were sequentially washed for 10 min each in 1 ml of low salt, high salt and LiCl immune complex wash buffer. Immunocomplexes were eluted off the beads by incubation with 200 μ l of 1% SDS and 50 mM NaHCO₃. The eluents were incubated at 65°C for 6 h to reverse the formaldehyde-induced protein–DNA crosslinks. Extracted DNAs were resuspended in 100 μ l of TE and qPCR were performed for amplification (5). The primer sequences that were used are shown in **Table S3** in Supplementary Data. The fold change in promoter occupancy was calculated by setting the level of promoter occupancy in the cells without ethanol treatment at 1.

Supplemental References

1. Marshall L, *et al.* (2008) Elevated tRNA(iMet) synthesis can drive cell proliferation and oncogenic transformation. *Cell* **133**, 78-89.
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5. Zhang Q, *et al.* (2011) Phosphorylation of histone H3 serine 28 modulates RNA polymerase III-dependent transcription. *Oncogene* **30**:3943-3952.
6. Goodfellow SJ, *et al.* (2006) Regulation of RNA polymerase III transcription during hypertrophic growth. *EMBO J.* **25**, 1522-1533.