## SUPPLEMENTARY FIGURE LEGENDS

**Table 1. RT-PCR and q-PCR primers.**

**Table 2. CHIP assay primers.**

**Figure S1. Interaction assays of ERRα/β, SIRT6, SIRT7 and SMILE through *in vivo* GST pull-down.** (**A**) HepG2 cells were cotransfected with expression vectors for Flag-ERRα, Flag-ERRβ and pEBG-SMILE (GST-SMILE) or pEBG alone (GST). (**B**) HepG2 cells were cotransfected with expression vectors encoding Flag-SIRT6, Flag-SIRT7 and pEBG-SMILE (GST-SMILE) or pEBG alone (GST). The complex formation (top panel, GST puri.) and the amount of Flag-ERRα, Flag-ERRβ, Flag-SIRT6, Flag-SIRT7 used for the *in vivo* binding assay (bottom panel, Lysate), were determined via Western blot analysis using an anti-Flag antibody. The same blot was stripped and reprobed with anti-GST antibody (middle panel) to confirm the expression levels of the GST fusion protein (GST-SMILE) and the GST control (GST). The data shown are representative of at least three independent experiments.

**Figure S2. Acetylation assays of FOXO1, ERRγ and SMILE.** (**A**) Deacetylation of FOXO1 by wild type SIRT1. (**B** and **C**) No acetylation/deacetylation occurs with ERRγ and SMILE. HepG2 cells were cotransfected with expression plasmids for Flag-FOXO1, Flag-ERRγ or Flag-SMILE, together with expression vectors for wild type Myc-SIRT1 or the dominant negative mutant Myc-SIRT1H363Y as indicated. 36 h after transfection, the cells were treated with or without 10 μM of EX527. Cell extracts prepared from the cells were subjected to immunoprecipitation assays using anti-Flag M2 antibody. The acetylation levels of FOXO1, ERRγ and SMILE were detected by anti-acetylated lysine antibodies (upper panels). Precipitated Flag-FOXO1, Flag-ERRγ, Flag-SMILE and the expression of myc-SIRT/SIRT1H363Y fusions in cell lysates were determined via Western blot analysis using indicated antibodies (middle and lower panels). The data shown are representative of at least three independent experiments.

**Figure S3. Homodimerization of SMILE is not required for the repression of SMILE on ERRγ.** (**A**) Structure of human SMILE. The basic region and leucine zipper domains are shown. The leucine zipper mutant SMILE-L(239-267)V indicates the leucine residues between position 239 and 267 were mutated to valine, as indicated by the arrows. The numbers in the figure indicate the amino acid (aa) residues. (**B**) *In vivo* homodimerization possibility analysis of wt SMILE and SMILE-L(239-267)V. HepG2 cells were cotransfected with expression vectors for Flag-SMILE, Flag-SMILE-L(239-267)V with pEBG-SMILE (GST-SMILE wt) or pEBG-SMILE-L(239-267)V or pEBG alone (GST) as indicated. Protein interactions were examined via *in vivo* GST pull-down. The top and middle panels (GST puri) show GST beads-precipitated Flag-SMILE and GST fusions, respectively. The bottom panel shows the protein expression levels of Flag-SMILE and

Flag-SMILE-L(239-267)V in cell lysates. (**C**) The effect of SMILE-L(239-267)V on ERRγ transactivation. HepG2 cells were cotransfected with sft4-Luc luciferase reporter vectors, together with expression vectors for ERRγ, wt SMILE or SMILE-L(239-267)V as indicated. 48h after tranfection, luciferase activity was measured. Wt, wild-type. The data shown are representative of at least three independent experiments.

**Figure S4. Transcription initiation site on the SMILE promoter.** Human SMILE sequences from -338 to +231 to the putative transcription start site. The guanine at the transcription start site (arrow) is designated +1. The translation start codon ATG is underlined with the encoded amino acids shown below the codon.

# Supplementary data

**Table 1. RT-PCR and q-PCR Primers**

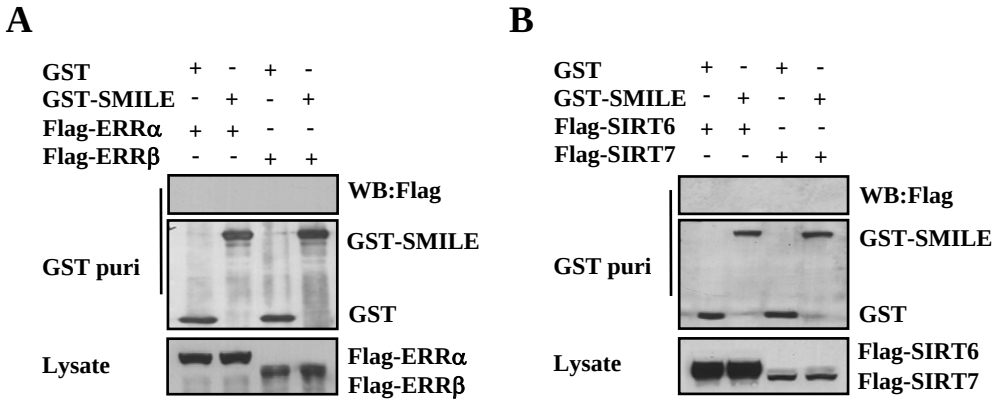
| Name | Forward Primers (5'-3') | Reverse Primers (5'-3') |
|---|---|---|
| ERRγ | GACTTGACTCGCCACCTCTC | GTGGTACCCAGAAGCGATGT |
| PDK4 | CCC GCTGTCCATGAAGCAGC | CCAATGTGGCTTGGGTTTCC |
| PDK2 | GAAGAATGCGTCCCTGGCAG | GGTTCCGGATGGTGACCAGG |
| SIRT1 | GCAGATTAGTAGGCGGCTTG | TCTCCATCAGTCCCAAATCC |
| SHP | CTTCCTCAGGAACCT | CCCAGTGAGCCTCCT |
| SMILE | AAAAGAGGCGGA GAAAGTCC | CTCTGAAGAGCGAGGTGGTC |
| β -actin | GTCATCACCATTGGCAATGAG | CGTCATACTCCTGCTTGCTG |

**Table 2. CHIP Assay Primers**

| Name | Positions Targeting[1] | Forward Primers (5'-3') | Reverse Primers (5'-3') |
|---|---|---|---|
| SMILE | -996 → -735 | CAGGAAGACGAAGGAAGACAAAGAG | GCCAGGAAAGGCTATGAAGAGAG |
| | -220 → -19 | TGTTGAGTCTGGGTATTGGGTGG | TAGCCGGAAGTCAGTGGTTTTCG |
| PDK4 | -1699 → -1493 | TAGACGAAGAGGGCAAAGGA | TGGTGAAGATGGGGTTTCTC |
| | -1056 → -886 | GGAATCACAAAGCTGCATCA | GCTGCAAAAGGTCTTCCTTG |

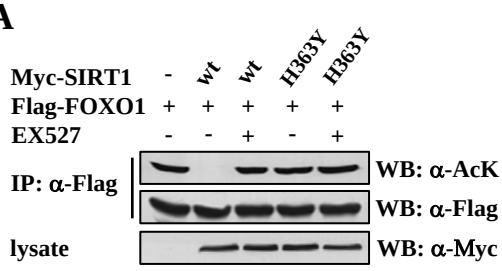
1 The transcription start site (TSS) is numbered +1.

**Figure. S1**

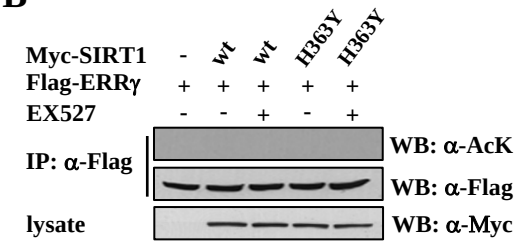

A
GST + - + -
GST-SMILE - + - +
Flag-ERRα + + - -
Flag-ERRβ - - + +
WB:Flag
GST puri
GST-SMILE
GST
Lysate
Flag-ERRα
Flag-ERRβ
B
GST + - + -
GST-SMILE - + - +
Flag-SIRT6 + + - -
Flag-SIRT7 - - + +
WB:Flag
GST puri
GST-SMILE
GST
Lysate
Flag-SIRT6
Flag-SIRT7

## Figure. S2

**A**




**B**




**C**

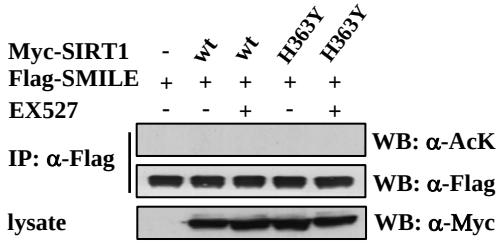


**Figure. S3**

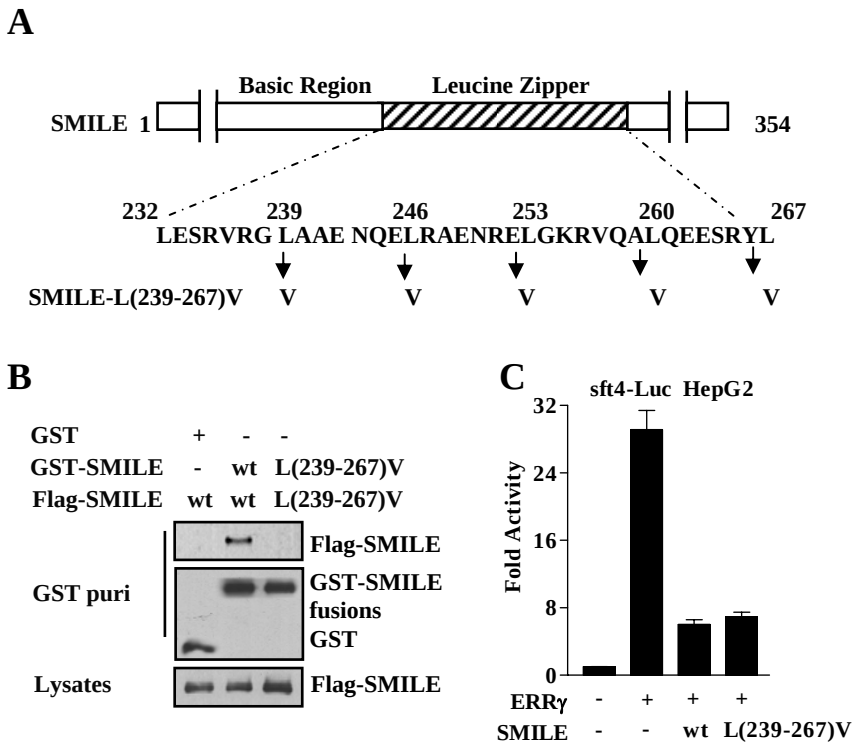
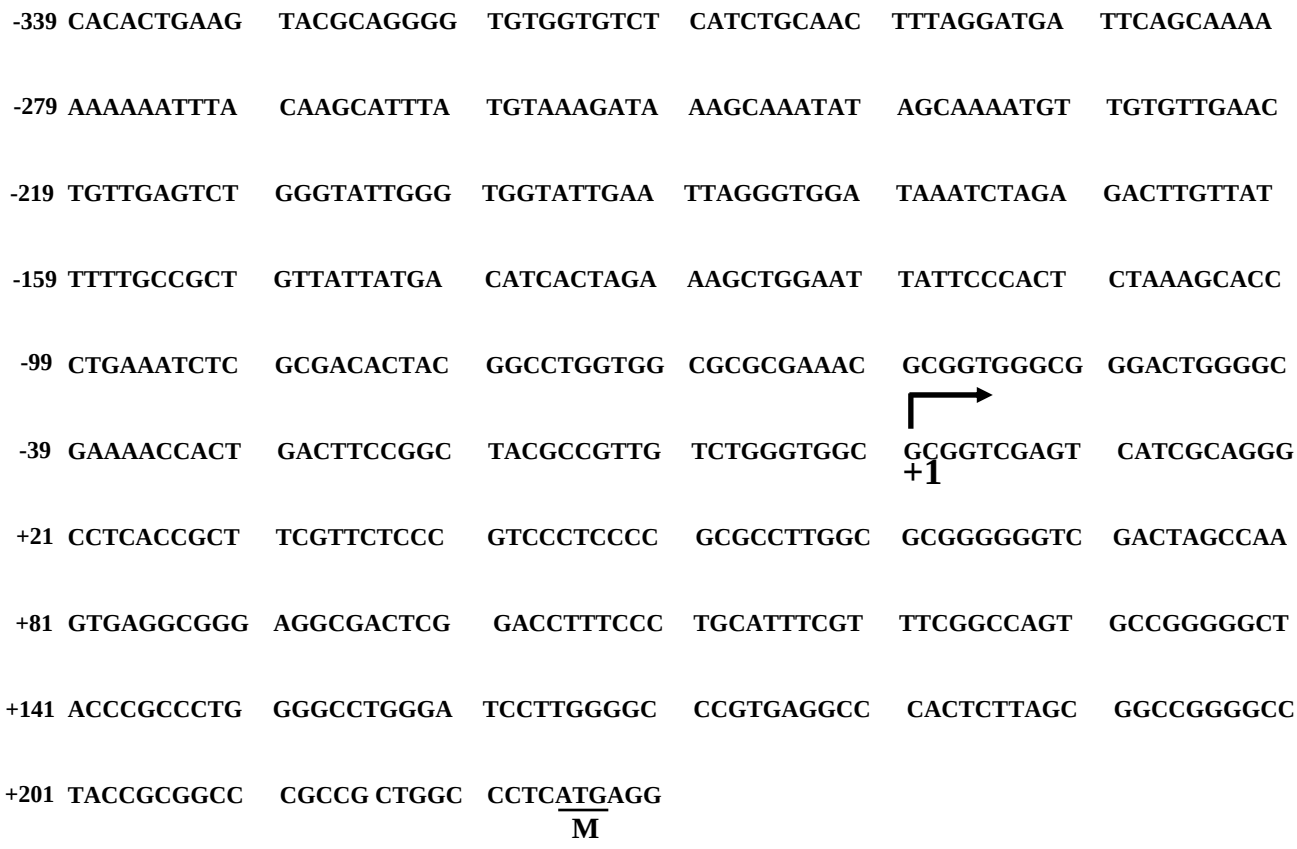

A
Basic Region
Leucine Zipper
SMILE 1
354
232
239
246
253
260
267
LESRVRG LAAE NQELRAENRELGKRVQALQEESRYL
SMILE-L(239-267)V
V
V
V
V
V
B
GST + - -
GST-SMILE - wt L(239-267)V
Flag-SMILE wt wt L(239-267)V
GST puri
Flag-SMILE
GST-SMILE fusions
GST
Lysates
Flag-SMILE
C
sft4-Luc HepG2
Fold Activity
32
24
16
8
0
ERRγ - + + +
SMILE - - wt L(239-267)V

**Figure. S4**


-339 CACACTGAAG TACGCAGGGG TGTGGTGTCT CATCTGCAAC TTTAGGATGA TTCAGCAAAA
-279 AAAAAATTTA CAAGCATTTA TGTAAAGATA AAGCAAATAT AGCAAAATGT TGTGTTGAAC
-219 TGTTGAGTCT GGGTATTGGG TGGTATTGAA TTAGGGTGGA TAAATCTAGA GACTTGTTAT
-159 TTTTGCCGCT GTTATTATGA CATCACTAGA AAGCTGGAAT TATTCCCACT CTAAAGCACC
-99 CTGAAATCTC GCGACACTAC GGCCTGGTGG CGCGCGAAAC GCGGTGGGCG GGACTGGGGC
-39 GAAAACCACT GACTTCCGGC TACGCCGTTG TCTGGGTGGC GCGGTCGAGT CATCGCAGGG
+1
+21 CCTCACCGCT TCGTTCTCCC GTCCCTCCCC GCGCCTTGGC GCGGGGGGTC GACTAGCCAA
+81 GTGAGGCGGG AGGCGACTCG GACCTTTCCC TGCATTTCGT TTCGGCCAGT GCCGGGGGCT
+141 ACCCGCCCTG GGGCCTGGGA TCCTTGGGGC CCGTGAGGCC CACTCTTAGC GGCCGGGGCC
+201 TACCGCGGCC CGCCG CTGGC CCTCATGAGG
M