

**Disulfiram/copper suppresses cancer stem cell activity in differentiated thyroid cancer cells by inhibiting BMI1 expression**

Yung-Lun Ni<sup>1</sup>, Peng-Ju Chien<sup>2</sup>, Yang-Chih Hsieh<sup>2</sup>, Huan-Ting Shen<sup>1</sup>, Hsueh-Te Lee<sup>3</sup>, Shih-Ming Chen<sup>4\*</sup>, Wen-Wei Chang<sup>2, 5\*</sup>

**Supporting information**

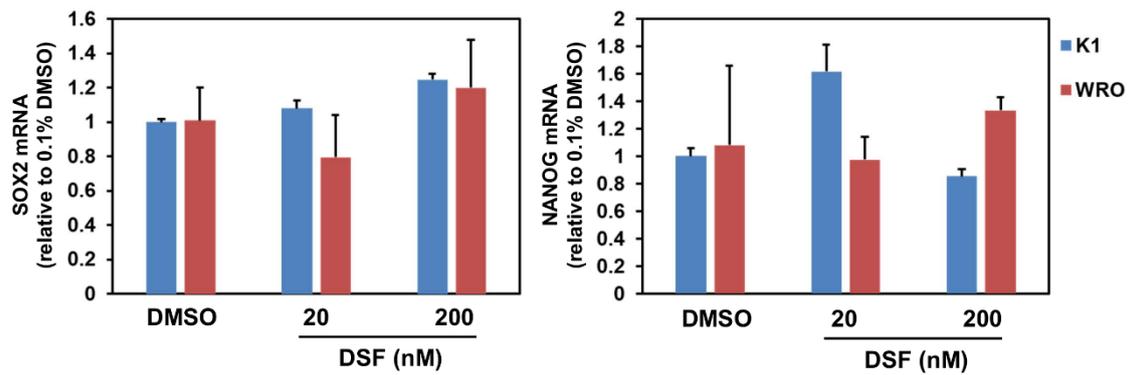
**Table S1. Primer sequences used in this study**

<b>Target</b>	<b>Forward (5' to 3')</b>	<b>Reverse (5' to 3')</b>
BMI1	AATCCCCACCTGATGTGTGT	GCTGGTCTCCAGGTAACGAA
OCT4	GGTCCGAGTGTGGTTCTGTA	CGAGGAGTACAGTGCAGTGA
SOX2	AACCCCAAGATGCACAACCTC	CGGGGCCGGTATTTATAATC
NANOG	CATGAGTGTGGATCCAGCTTG	CCTGAATAAGCAGATCCATGG
MRPL19	GGGATTTGCATTCAGAGATCAG	GGAAGGGCATCTCGTAAG
BMI1- promoter (ChIP use)	CCGGGGAGAAAGAAAGAACG	CGGCCTGGGAATTAGTGTC

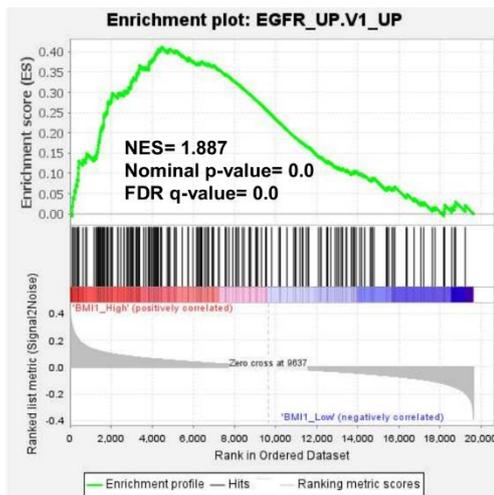
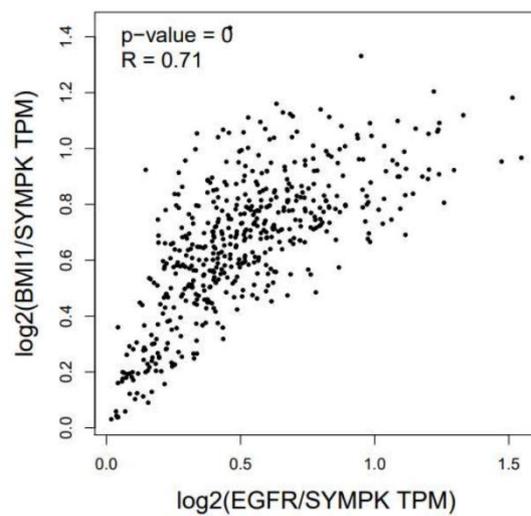
**Table S2. Antibodies used in this study**

<b>Target</b>	<b>Source</b>	<b>Cat. No.</b>
BMI1	Cell Signaling Technology, Inc.	6964s
c-Myc	GeneTex International Corporation	GTX109636
E2F1 (western blot)	Proteintech group Inc	12171-1-AP
E2F1 (ChIP)	BD Pharmingen	554213
Lamin B1	GeneTex International Corporation	GTX103292
GAPDH	GeneTex International Corporation	GTX100118
Tubulin	Proteintech group Inc	66031-1-Ig
HA-Tag	Santa Cruz Biotechnology, Inc	sc-7392
Mouse IgG (HRP conjugated)	GeneTex International Corporation	GTX221667-01
Rabbit IgG (HRP conjugated)	GeneTex International Corporation	GTX221666-01

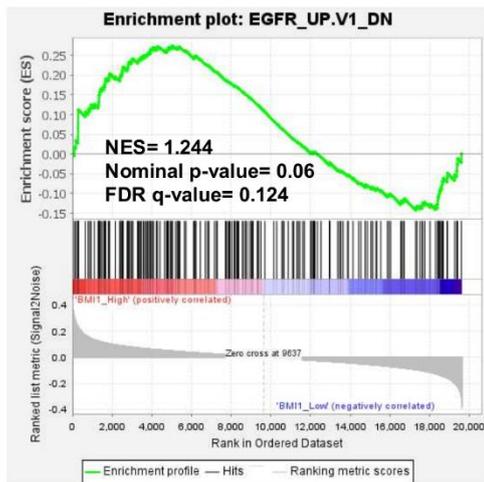
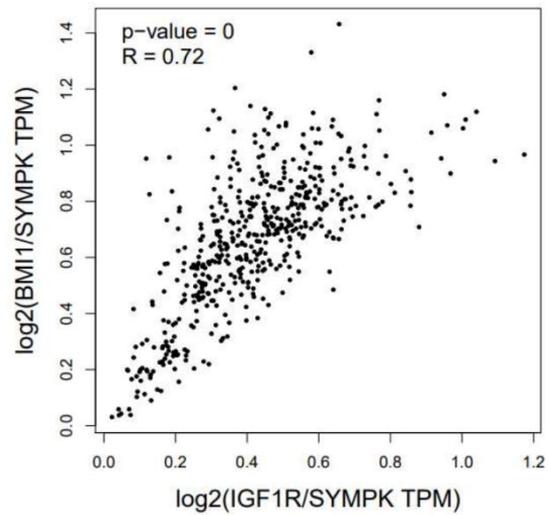
## Figures



**Figure S1. SOX2 or NANOG is not suppressed by DSF/copper treatment.** K1 or WRO cells were treated with 20 nM or 200 nM of DSF in presence of 1  $\mu$ M CuCl<sub>2</sub> for 48 hours. The mRNA expressions of SOX2 or NANOG were determined by real-time RT-PCR method.

**A****B**

**Figure S2. The positive correlation between BMI1 and EGFR among THCA samples.** (A) THCA RNA-Seq dataset was obtained from TCGA database and used median expression level of BMI1 mRNA as a cutoff value followed by gene set enrichment analysis using GSEA software. BMI1 high expression samples was enriched in the gene set of EGFR\_UP.V1\_UP. (B) The correlation between BMI1 and EGFR in THCA dataset of TCGA data was obtained from GEPIA\_2 website.

**A****B**

**Figure S3. The positive correlation between BMI1 and IGF1R among THCA samples.** (A) THCA RNA-Seq dataset was obtained from TCGA database and used median expression level of BMI1 mRNA as a cutoff value followed by gene set enrichment analysis using GSEA software. BMI1 high expression samples was enriched in the gene set of EGFR\_UP.V1\_DN. (B) The correlation between BMI1 and IGF1R in THCA dataset of TCGA data was obtained from GEPIA\_2 website.