

Supplemental Information

Thymidine catabolism promotes NADPH oxidase-derived reactive oxygen species (ROS) signalling in KB and yumoto cells

Sho Tabata¹, Masatatsu Yamamoto², Hisatsugu Goto³, Akiyoshi Hirayama¹, Maki Ohishi¹, Takuya Kuramoto³, Atsushi Mitsuhashi³, Ryuji Ikeda⁴, Misako Haraguchi⁵, Kohichi Kawahara², Yoshinari Shinsato², Kentaro Minami², Atsuro Saijo³, Yuko Toyoda³, Masaki Hanibuchi³, Yasuhiko Nishioka³, Saburo Sone³, Hiroyasu Esumi⁶, Masaru Tomita¹, Tomoyoshi Soga¹, Tatsuhiko Furukawa², Shin-ichi Akiyama⁷

¹Institute for Advanced Biosciences, Keio University, 246-2 Mizukami, Kakuganji, Tsuruoka, Yamagata 997-0052, Japan.

²Department of Molecular Oncology, Graduate School Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan.

³Department of Respiratory Medicine and Rheumatology, Institute of Biomedical Sciences, Tokushima University Graduate School, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan.

⁴Department of Pharmacy, University of Miyazaki Hospital, 5200 Kihara, Kiyotake-cho, Miyazaki, 889-1692, Japan.

⁵Department of Biochemistry and Molecular Biology, Graduate School Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan.

⁶Clinical Research, Research Institute for Biomedical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-0022, Japan.

⁷Clinical Research Center, National Kyushu Cancer Center, 3-1-1 Notame Minami-ku, Fukuoka 811-1395, Japan.

SUPPLEMENTAL FIGURES AND TABLE

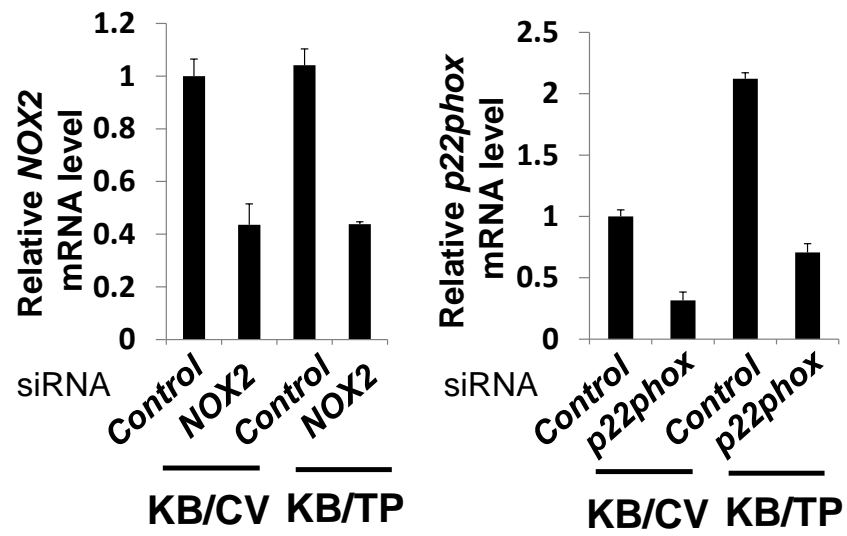


Figure S1. Knock down of *NOX2* and *p22phox* in KB/CV and KB/TP cells. KB/CV and KB/TP cells were transfected with *NOX2* siRNA or *p22phox* siRNA. mRNA levels of *NOX2* and *p22phox* were determined by real time PCR.

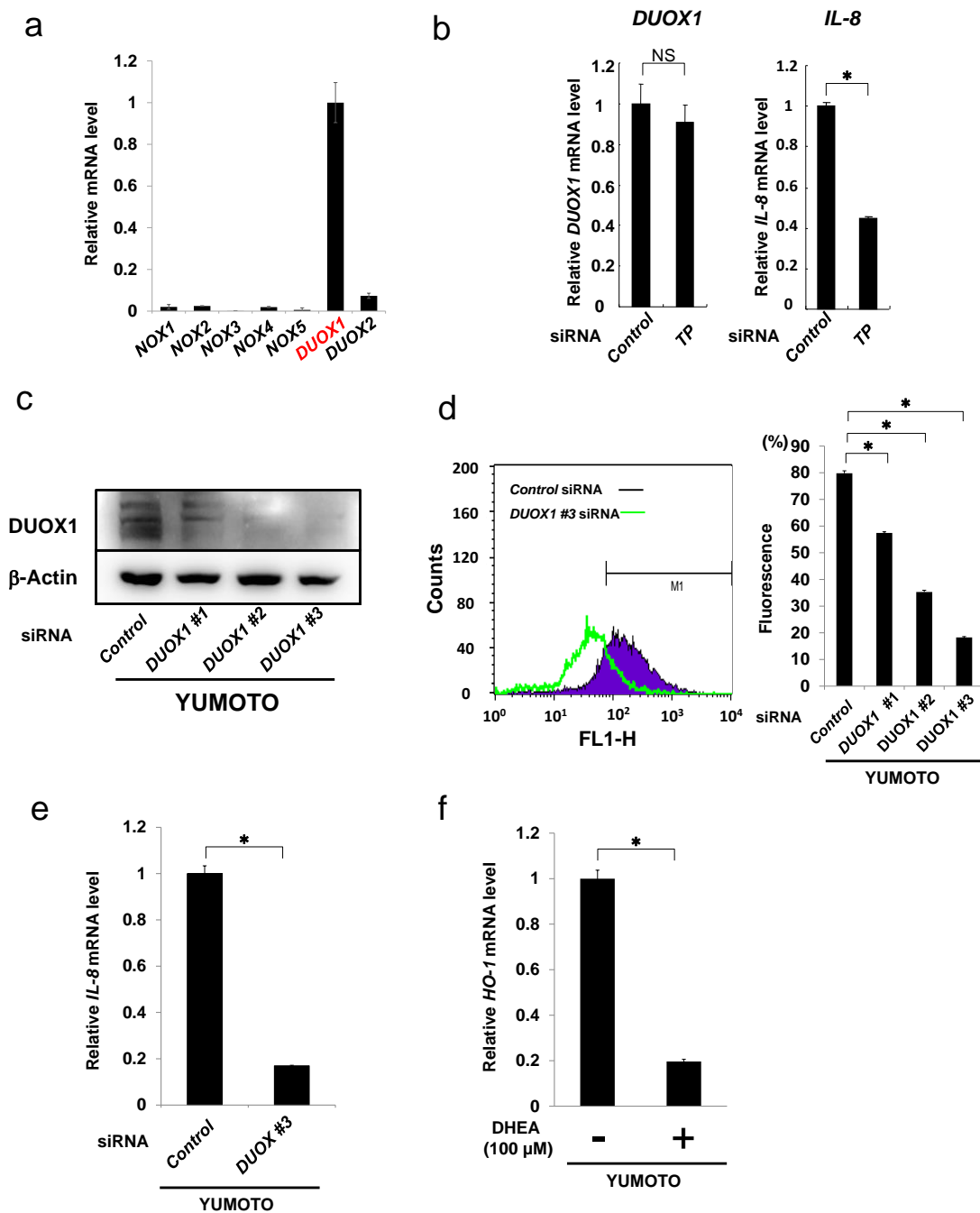


Figure S2. Knockdown of DUOX1 in Yumoto cells. (a) Expression of *NOX* isoforms in Yumoto. Expression levels of *NOX* isoforms in Yumoto cells were determined by real-time PCR. (b) Effect of TP silencing on *DUOX1* and *IL-8* expression in Yumoto cells. (c) Knockdown of *DUOX1* in Yumoto cells. (d) Effect of *DUOX1* downregulation on the generation of ROS in Yumoto cells. Yumoto cells transfected with *DUOX1* siRNA were treated with 10 μM H₂DCF-DA for 1h and the ROS levels were determined by using FACSscan. (d) Effect of *DUOX1* knockdown on *IL-8* expression in Yumoto cells. (f) Effect of DHEA on *HO-1* expression in Yumoto cells. Data are presented as mean ± SD. **P* < 0.01.

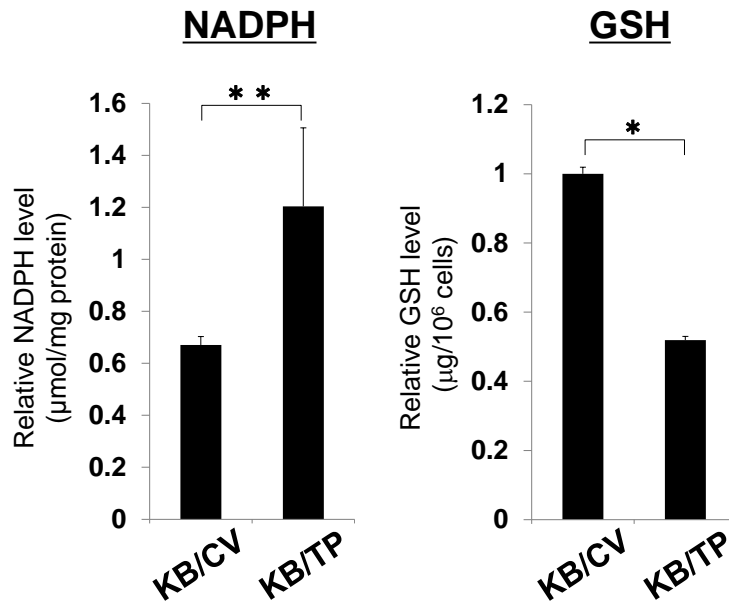
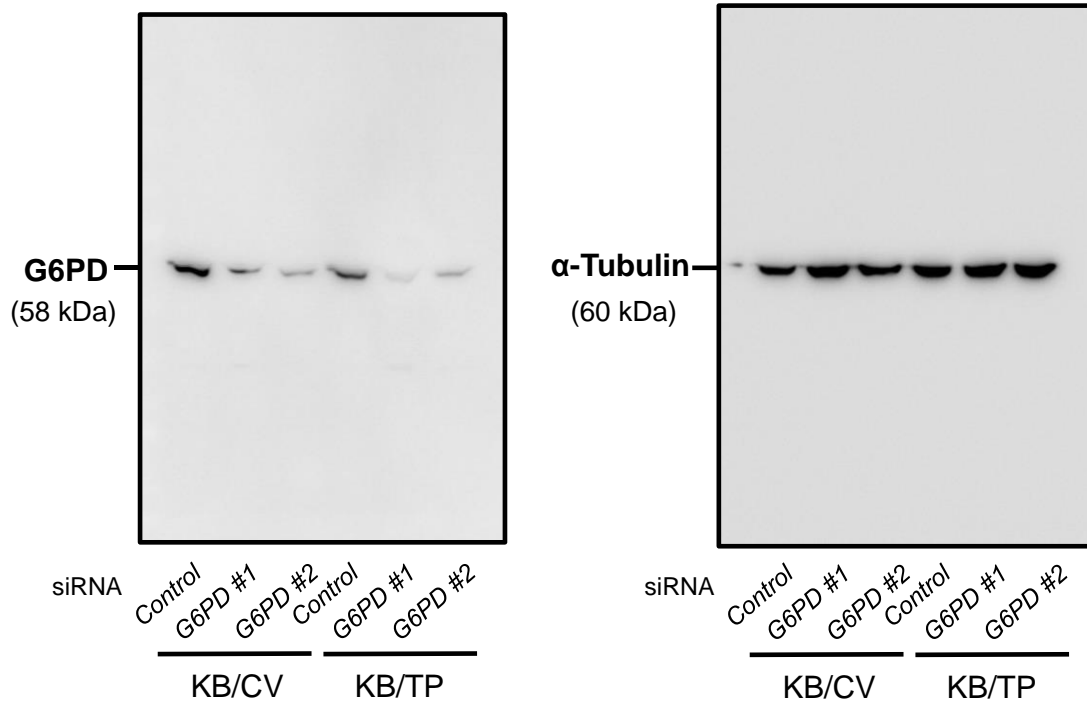


Figure S3. NADPH and GSH levels in KB/CV and KB/TP cells. Effect of TP on levels of NADPH (left) and GSH (right) in KB cells. NADPH levels in KB/CV and KB/TP cells were measured using a NADPH assay kit. GSH levels in KB/CV and KB/TP cells were determined using a GSH assay kit. Data are presented as mean \pm SD. * $P < 0.01$, ** $P < 0.05$.

Table S1. Primer sequences for real-time PCR assays.

Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')
<i>TP</i>	GCTGGAGTCTATTCCTGGATTC	ACTGAGAATGGAGGCTGTGATG
<i>IL-8</i>	CCTGATTTCTGCAGCTCTGTGT	GGTGGAAAGGTTTGGAGTATGTCT
<i>HO-1</i>	CGGGCCAGCAACAAAGTGCAAG	GTGTAAGGACCCATCGGAGAAG
<i>NOX1</i>	ACAAATTCCAGTGTGCAGACCA	AGACTGGAATATCGGTGACAGCA
<i>NOX2</i>	CTGCGATTCACACCATTGCAC	CGTGATGACAACCTCCAGTGATG
<i>NOX3</i>	ATGCAACCATCCACATCGTG	CGCCTGCTATTGTCCTTAGC
<i>NOX4</i>	CAGAAGGTTCCAAGCAGGAG	AAGTTGAGGGCATTACCAG
<i>NOX5</i>	CAGCTCTGCATGTGAAAGAG	CATCGATGTCATACACCTGG
<i>DUOX1</i>	CGACATTGAGACTGAGTTGA	CTGGAATGACGTTACCTTCT
<i>DUOX2</i>	AACCTAAGCAGCTCACAACCT	CAGAGAGCAATGATGGTGAT
<i>p22phox</i>	TCCTGCATCTCCTGCTCTC	CACAGCCGCCAGTAGGTAG
<i>GAPDH</i>	GTCAACGGATTTGGTCGTAT	TGGTGATGGGATTTCCATTG

• Figure 1E



• Figure S2

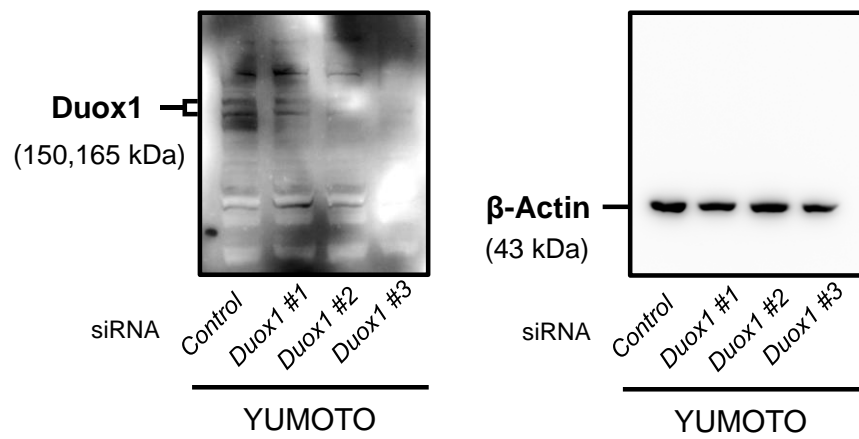


Figure S4. Uncropped images used in Figures 1E and S2.