

## Supplementary Materials

### Characterization of long-term *ex vivo* expansion of tree shrew spermatogonial stem cells

Cong Li<sup>1,2,3</sup>, Rui Bi<sup>2,3</sup>, Lin Wang<sup>1,2</sup>, Yu-Hua Ma<sup>4</sup>, Yong-Gang Yao<sup>2,3,4,5,\*</sup>, Ping Zheng<sup>1,2,3,4,5,\*</sup>

<sup>1</sup> State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650201, China

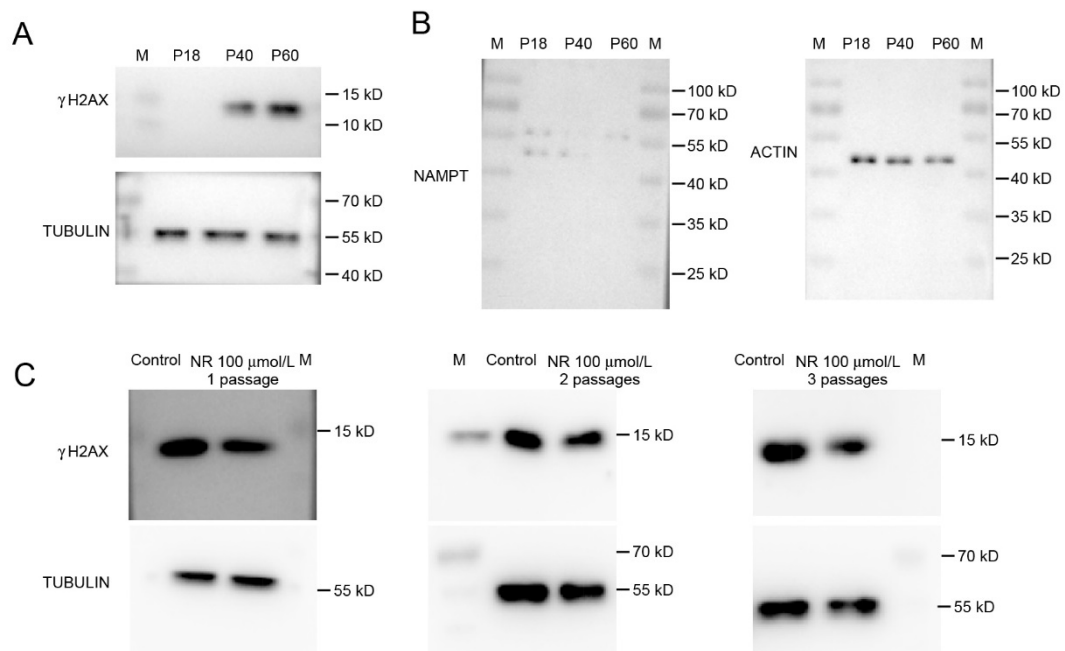
<sup>2</sup> Key Laboratory of Animal Models and Human Disease Mechanisms of Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650201, China

<sup>3</sup> Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan 650204, China

<sup>4</sup> National Resource Center for Non-Human Primates, National Research Facility for Phenotypic & Genetic Analysis of Model Animals (Primate Facility), Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650107, China

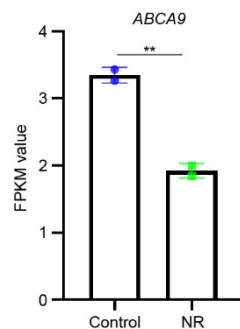
<sup>5</sup> KIZ/CUHK Joint Laboratory of Bioresources and Molecular Research in Common Diseases, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650204, China

\*Corresponding authors, E-mail: yaoyg@mail.kiz.ac.cn; zhengp@mail.kiz.ac.cn



### Supplementary Figure S1 Uncropped versions of blots are shown

A: Long-term culture of undifferentiated tree shrew SPGs increased  $\gamma$ H2AX levels, indicative of greater DNA damage. As shown in Figure 5A. B: Immunoblotting analysis of NAMPT protein level in SPGs at different passages. As shown in Figure 7B. C: Immunoblotting showed that NR treatment in undifferentiated SPGs (at P39) over three passages decreased  $\gamma$ H2AX levels. As shown in Figure 7F.



### Supplementary Figure S2 FPKM value of *ABCA9* gene

Results were based on RNA-seq data between undifferentiated SPGs treated with or without NR for four passages at P39 ([Supplementary Dataset S2](#)).

**Supplementary Table S1 Information on tree shrew undifferentiated SPG cell lines used in each experiment**

Assay	Tree shrew undifferentiated SPG cell line	Related to figure
Cell morphology	Line 1	Figure 1A
Cell cycle analysis		Figure 1B
Sub-G1 analysis		Figure 1F
Cell proliferation assay		Figure 1C
Karyotype analysis		Figure 5D
Alkaline phosphatase staining		Figure 2A
Telomere length		Figure 1G
Cell growth rate analysis	Line 2	Figure 1D
TUNEL		Figure 1E
ROS		Figure 6D, 7J
NAD <sup>+</sup>		Figure 7C, 7D, 7E
ATP & mtDNA	Line 1	Figure 6A, 6C
	Line 2	Figure 7H, 7I
Alkaline comet assay	Line 1	Figure 5C
	Line 2	Figure 7G
Immunofluorescence staining	Line 1	Figure 2B (PLZF)
	Line 2	Figure 2B (GFRA1)
		Figure 5B
		Figure 6B
RNA-seq	Line 1	Figure 4
	Line 2	Figure 7K
qRT-PCR	Line 1	Figure 4D, 7A
	Line 2	Figure 2C, 7L
Immunoblotting	Line 1	Figure 5A, 7B
	Line 2	Figure 7F
Undifferentiated SPG transplantation	Line 1 & Line 2 & Line 3	Figure 3

**Supplementary Table S2 Antibody information**

Antibody	Source	Dilution
Rat monoclonal anti-BrdU (clone BU1/75)	Novus; Cat# NB500-169	1:1 000 (Flow cytometry)
Goat anti-rat IgG secondary antibody, Alexa Fluor Cy3	Thermo Fisher Scientific; Cat# A-10522	1:500 (Flow cytometry)
Rabbit polyclonal anti- $\gamma$ -H2AX (clone 20E3)	Cell Signaling Technology; Cat# 9718	1:1 000 (WB/IF)
Beta-tubulin (10B1) mouse mAb	Abmart; Cat# M20005S	1:1 000 (WB)
Beta-actin (8H10D10) mouse mAb	Cell Signaling Technology; Cat# 3700S	1:5 000 (WB)
GFP-Tag (7G9) mouse mAb	Abmart; Cat# M20004S	1:1 000 (WB)
NAMPT antibody	Abmart; Cat# TD6059S	1:500 (WB)
Goat anti-mouse IgG (H+L) Secondary antibody, HRP	Thermo Fisher Scientific; Cat# 31430	1:5 000 (WB)
Goat anti-rabbit IgG (H+L) Secondary antibody, HRP	Thermo Fisher Scientific; Cat# 31460	1:5 000 (WB)
PLZF	Santa Cruz; Cat# SC-22839	1:100 (IF)
GFRA1	R&D Cat# AF560	1:100 (IF)
Tom20 (D8T4N) rabbit mAb	Cell Signaling Technology; Cat# 42406S	1:100 (IF)
Anti-GFP antibody	Abcam; Cat# ab13970	1:2 000 (IF)
Goat anti-rabbit IgG (H+L) Secondary antibody, Alexa Fluor 488	Thermo Fisher Scientific; Cat# A-32731	1:500 (IF)
Donkey anti-goat IgG (H+L) Secondary antibody, Alexa Fluor 555	Thermo Fisher Scientific; Cat# A-21432	1:500 (IF)
Goat anti-chicken IgG (H+L) Secondary antibody, Alexa Fluor 488	Thermo Fisher Scientific; Cat# A-11039	1:500 (IF)

**Supplementary Table S3 Primer pairs for PCR and quantitative real-time PCR (qPCR)**

Gene / Fragment	Primer pairs for qRT-PCR (5'-3')	
<i>Telomeres</i>	Forward	5'- GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTG AGGGT-3'
	Reverse	5'- TCCCGACTATCCCTATCCCTATCCCTATCCCTAT CCCTA -3'
<i>HBB</i>	Forward	5'-ACAGCTCCTGGGCAACGT-3'
	Reverse	5'-CAGGGCATTAGCCACACC-3'
<i>mtDNA</i>	Forward	5'-ACTTTCCGTCCCATTAGCCA-3'
	Reverse	5'-AATCAGCCGGCTAGAGGTAT-3'
<i>FMR1</i>	Forward	5'-CGACAAATGTGTGCCAAGGA-3'
	Reverse	5'-GTGCTCGCTTTGAGGTGACT-3'
<i>CDK17</i>	Forward	5'-TTGTGGCATGTTTGGGGGTA-3'
	Reverse	5'-CAGTTTGTTAGGCTACGGGGT-3'
<i>SMC5</i>	Forward	5'-CAGGGGATGGACCCAATCAAT -3'
	Reverse	5'-GGTTCAGCATATGAGGACCAT -3'
<i>FANCM</i>	Forward	5'-GCTCTAAGACCGGTTTGCCT -3'
	Reverse	5'-GCAGTTGGTAGTCTCGCACT -3'
<i>ATP5MG</i>	Forward	5'-AGCCTCGATTGGCCACATTT -3'
	Reverse	5'-AAACCACATCCACACCTCGG -3'
<i>NDUFA8</i>	Forward	5'-AAGTGAAAACGGATCGGCCT -3'
	Reverse	5'-TTCTTCGGTCGTTGTCTGGG -3'
<i>NAMPT</i>	Forward	5'-AAAACGTGCTAAGGGGCGTA -3'
	Reverse	5'-TACAGCTTTTCGCGTCCACT -3'
<i>LIN28A</i>	Forward	5'-CCAGGATGCCAACTGCACTA -3'
	Reverse	5'-GCTGCTGCTGACTAATCCCT -3'
<i>STRA8</i>	Forward	5'-TCTGACGGGGAATGGGATCA -3'
	Reverse	5'-ACCACTGTCCTTCATGCTGC -3'
<i>SOHLH1</i>	Forward	5'-CAGGTTGTTCTCGTCCCCTC -3'
	Reverse	5'-CTTGTTCTCGTCCACACCCA -3'
<i>FGFR3</i>	Forward	5'-GTGGAGACACCCTGGAAGT -3'
	Reverse	5'-GGGAGGCATTCAACACCTGA -3'
<i>ETV5</i>	Forward	5'-ACCTGGACCACAACAGCAAA -3'
	Reverse	5'-GTAGCTGGGGCGGTTATCTC -3'
<i>DDX4</i>	Forward	5'- AACAGAGATGCTGGCGAGTC -3'
	Reverse	5'- TCTCTTGGAAAACCCTCGGC -3'
<i>DAZL</i>	Forward	5'- ACGGATCGAACTGGTGTGTC -3'
	Reverse	5'- TTAAAGACCAGAGGACGCGG -3'
<i>MSL3</i>	Forward	5'-CGTTTGATTACACGCTCCCG -3'
	Reverse	5'-ACTGTGGTGTGGATGGCTTC -3'

<i>ID4</i>	Forward	5'- GCACGTTTCATAAGCATTCTGCAT -3'
	Reverse	5'- TCACGAAGGCTTCCAAGGTT -3'
<i>MORC1</i>	Forward	5'-AACGTCTACCGTCCATCCCT -3'
	Reverse	5'-AGTGCTTTGTGGGACTGTGG -3'
<i>ZBTB43</i>	Forward	5'-GCCTTATGGCTGTGGTGTCT -3'
	Reverse	5'-TCAGCCTTTGCAGCTTCGTA -3'
<i>ID3</i>	Forward	5'-ATCCTGCCATCTCCAGAACG-3'
	Reverse	5'-TTTCAGGCCACCCAAGTTCA -3'
<i>NANOS3</i>	Forward	5'-TCCCTGCCTTCCAGAGACTT -3'
	Reverse	5'-ACGGACTGGGAACCTCTACCT -3'
<i>DMRTB1</i>	Forward	5'-CTCGGATTGTGTGATGGGGT -3'
	Reverse	5'-TGGTAATGGCTGCAGGAGAC -3'
<i>GAPDH</i>	Forward	5'- CGCCAACATCAAATGGGGTG -3'
	Reverse	5'- ATGGTTCACGCCCATCACAA -3'
Primer pairs for regular PCR (5'-3')		
<i>EGFP</i>	Forward	5'- ACCGGTATGGTGAGCAAGGGCGAGGA-3'
	Reverse	5'- CAAAGGCATTAAAGCAGCGTATC -3'
<i>GAPDH</i>	Forward	5'- CGTGAACCATGAGAAGTACG -3'
	Reverse	5'- ACGACCTGGTCCTCAGTGTA -3'