

Table S1. Vectors, Northern probes, and qPCR primers used in this study

Vector	Template/source	Primer For and Rev (5'→3') // restriction enzyme site
pcDNA3-Flag	Imai et al., 2001 ^a	
pcDNA3-Flag-Msi1	Imai et al., 2001 ^a	
pcDNA3-Flag-Msi2	Imai et al., 2001 ^a	
pcDNA3-Flag-Msi1	pcDNA3-Flag-Msi1	GATGGTCACTgcGACGgcGgcGATCTTCGTG /
NLSmutB ^b		CACGAAGATCgcCgcCGTCgcAGTGACCATC //
pcDNA3-Flag-Msi1	pcDNA3-Flag-Msi1NLSmutB	GACCCCTGACCgcAgcATCCgcGGGTCTCGGC /
NLSmutAB ^b		GCCGAgACCCgcGGATgcTgcGGTCAGGGGGTC //
p3xFlag-GST	Kawahara et al., 2008 ^c	
pcDNA3-Flag-Lin28		tctagaATGGGCTCGGTGTCCAACCAGCAGTTTGCAGG /
		gaattcTCAATTCTGGGCTTCTGGGAGCAGGGCAGG // <i>XbaI-EcoRI</i>
pcDNA3-Flag-mPasha		tctagaATGGAGACATATGAGAGTCCCTCTCCCTCTCC /
		gaattcTCATACATCGACTGTGCACAAGGGCTCAC // <i>XbaI-EcoRI</i>
pcDNA3-mDrosha-Flag ^d		ATGCCCCACGGCCAGACTTTGTCCCTTACCCTCCC /
		GGATCCCAAAGTGAACGATAATCGGGAAAGTGATCAC //
		GGATCCGCCCCGCACAGTTGAGTTATGCAGGAGAC /
		CTTCTTGATGTCTTCAGCCTCCTCCGGCTC //
pEBG-Msi1	Kawahara et al., 2008 ^c	
pGEX6-P	GE Healthcare	
pGEX6P-hnRNP K	Provided by Dr. Yano ^e	
pGEX-6P-Flag-Msi1	pcDNA3-Flag-Msi1	
pGEX-6P-myc-Msi2	Provided by Dr. Shibata ^f	
pGEX6P-Lin28	pcDNA3-Flag-Lin28	
pGEX6P-mKpna1 (α -5)	Fig. 5A	ATGTCCACACCAGGAAAAGAGAAGTTCGCCTG /
		TCAAAGCTGGAAACCTTCCATAGGAGCTTAC//
pGEX6P-mKpna4 (α -3)	Fig. 5A	ATGGCGGACAACGAGAAATTGGACAACCAACGG/
		CTAAACTGGAACCCCTCTGTTGGTACATTGG //
pGEX6P-mKpna2 (α -1)	Fig. 5A	ATGTCCACGAACGAGAATGCTAAGTACCAGC /
		TTAGAAGTTAAAGTCCCAGGAGCTCCATCCT //
	Kaneko	
3xFLAG hZCCHC11 (hTUT ²)	Provided by Prof. Yoshimura ^f	
pT7-pri-miR98		CCAAAGCCTACATAATATTTTAAAAGCACTGAAGAG /
		GCAAGTCATTTAACATTATTTGCCTCACTCTATC //
pT7-pri-let-7b		CACCTCCCTCCCCACCCCGCCTGTGCCCTATG /
		CATCTTTATTTATACCCAGGTCCCACGTCATAGC//
U6 snRNA	Fig. 2C	CACGAATTTGCGTGTATCCTT
mmu-miR-98 (39209-00)	miRCURY LNA TM Detection probe	AACAATACAACCTTACTACCTCA
mmu-let-7b (39001-00)	miRCURY LNA TM Detection probe	AACCACACAACCTTACTACCTCA

^a Imai, T., A. Tokunaga, T. Yoshida, M. Hashimoto, K. Mikoshiba, G. Weinmaster, M. Nakafuku, and H. Okano. 2001. *Mol Cell Biol.* 21:3888-3900.

^b Site-Directed Mutagenesis was performed as the previous study (Sunabori et al., *J. Cell Sci.* 121: 1204-12); it was based on "QuikChange Site-Directed Mutagenesis Kit" (Stratagene) and used *KOD+* (TOYOBO) and *DpnI* (NEB).

^c Kawahara, H., T. Imai, M. Tuzimoto, K. Matsumoto, and H. Okano. 2008 *J. Cell Biology* 22:139-153.

^d Cloning of mDrosha was amplified two-fragments and legated.

^e Yano, M., H.J. Okano, and H. Okano. 2005. *J Biol Chem.* 280:12690-12699.

^f Dr. Shibata kindly gift

Table S2. Buffers used in this study

Buffer	Composition	Use
TEV cleavage (TC) ^a	10 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.1% NP-40, 0.5 mM EDTA, and 1 mM DTT	Fig. 1B, 1C, 2A, 2B, 2D, 4B, 5A 5B, and S1B
Buffer A ^b	10mM HEPES at pH 7.9, 10mM KCl, 1mM DTT and 0.1mM EDTA, and Complete Protease Inhibitor Cocktail-EDTA	Fig. 1A, 4E, and S1A
Buffer D ^b	20mM Tris-HCl at pH 8.0, 100mM KCl, 0.2mM EDTA and Protease Inhibitor cocktail EDTA-complete	Fig. 1A, 4E, and S1A
Hybridization solution ^a	0.2 M Phosphate Buffer pH7.2, 7% SDS, and 1mM EDTA	Fig. 2A, 2D
Lysis buffer ^c	20 mM Tris-HCl (pH 8.0), 100 mM KCl, and 0.2 mM EDTA	Fig. 3A to 3C
RNA elution buffer ^c	0.3 M sodium acetate, pH 5.5 and 2% SDS	Fig. 3A to 3C

^aKawahara, H., T. Imai, M. Tuzimoto, K. Matsumoto, and H. Okano. 2008 *J. Cell Biology* 22, 139-153.

^bHeo, I., Joo, C., Cho, J., Ha, M., Han, J., and Kim, V.N. 2008. *Mol. Cell* 32, 276-284.

^cLee, Y., and Kim, V.N.. 2007. *Methods Enzymol* 427, 89-106.

Table S3. Antibodies used in this study

Antibody	Animal species	Identity/source
Msi1 (14H1)	Rat monoclonal antibody 14H1	Kaneko et al., 2000 ^a
Lin28	Goat polyclonal antibody	R&D Systems, Inc.
Hu-R	Mouse monoclonal antibody 3A2	Santa Cruz Biotechnology, Inc.
Msi2	Rabbit polyclonal antibody	Sakakibara et al., 2001 ^b
Nanog	Rabbit polyclonal antibody	ReproCELL Inc.
β -tubulin	Mouse monoclonal antibody SDL3D10	Sigma-Aldrich
α -tubulin	Mouse monoclonal antibody DM-1A	Sigma-Aldrich
β -Actin	Mouse monoclonal antibody AC-15	Sigma-Aldrich
Flag	Mouse monoclonal antibody M2	Sigma-Aldrich
GST	Rabbit polyclonal antibody (G7781)	Sigma-Aldrich

^aKaneko, Y., S. Sakakibara, T. Imai, A. Suzuki, Y. Nakamura, K. Sawamoto, Y. Ogawa, Y. Toyama, T. Miyata, and H. Okano. 2000. *Dev Neurosci*. 22:139-153.

^bSakakibara, S., Nakamura, Y., Satoh, H., and Okano, H. 2001 *J Neurosci* 21, 8091-8107.