

siRNA screening identifies METTL9 as a histidine N^π -methyltransferase that targets the pro-inflammatory protein S100A9

Hiroaki Daitoku*, Momoka Someya, Koichiro Kako, Takahiro Hayashi, Tatsuya Tajima, Hikari Haruki, Naoki Sekiguchi, Toru Uetake, Yuto Akimoto, Akiyoshi Fukamizu*

*Hiroaki Daitoku and Akiyoshi Fukamizu

Email: hiroakid@tara.tsukuba.ac.jp and akif@tara.tsukuba.ac.jp

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Table S1

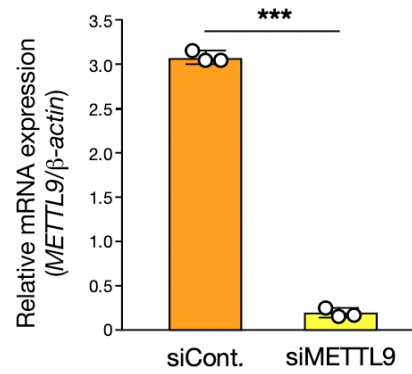


Figure S1. siRNA knockdown of *METTL9* markedly reduces its mRNA. HEK293T cells were transfected with either control or *Mettl9*-specific siRNAs. The mRNA levels of *Mettl9* were measured by qPCR analysis and normalized against the mRNA levels of β -actin. Mean \pm s.d. ($n=3$ independent experiments). *** $P<0.001$; two-tailed Student's t test.

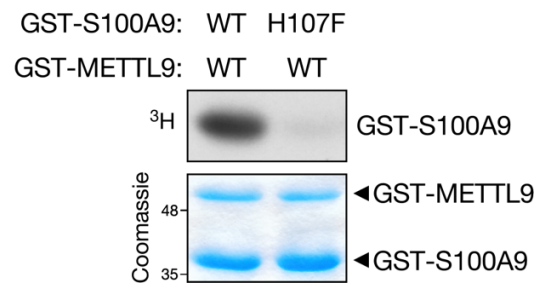


Figure S2. METTL9 specifically methylates S100A9 at His-107 *in vitro*. GST-S100A9 wild-type or H107F mutant was incubated with GST-METTL9 in the presence of ³H-SAM. The incorporation of [³H]-methyl into proteins was visualized by autoradiography (*Top*). Coomassie blue staining of proteins used in the reaction is shown as a loading control (*Bottom*).

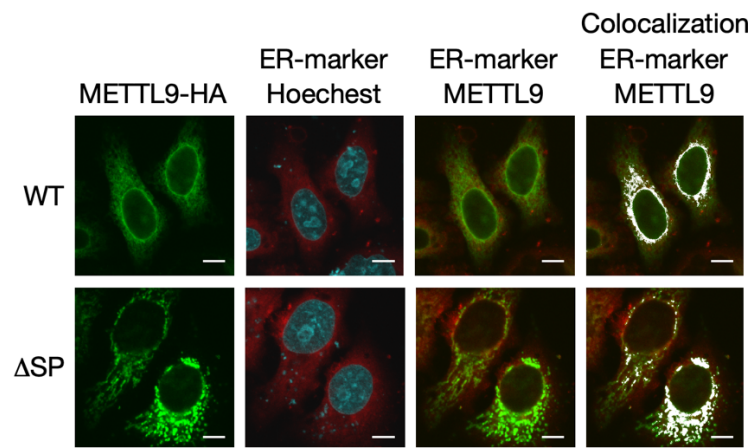


Figure S3. METTL9 localizes to the ER in HeLa cells. Representative immunofluorescence images of METTL9 wild-type (*Top*) or Δ SP mutant (*Bottom*) localization in HeLa cells. Green, METTL9-HA; red, ER-marker (Alexa594-conjugated Concanavalin A); blue, nucleus (Hoechst33258); scale bar, 10 μ m. Images of ER-marker were merged with Hoechst and METTL9-HA staining. Colocalization indicates overlay of the ER-marker and METTL9-HA, shown as white pixels.

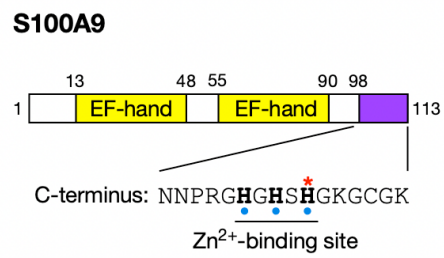


Figure 4. A schematic model of mouse S100A9.

Top, domain structure of S100A9 containing the two EF-hand (yellow) and a C-terminal Zn²⁺-binding site (purple). *Bottom*, the C-terminal sequence of mouse S100A9. Histidine residues involved in zinc coordination are marked with blue spheres, and methylated histidine at 107 is indicated with a red asterisk.

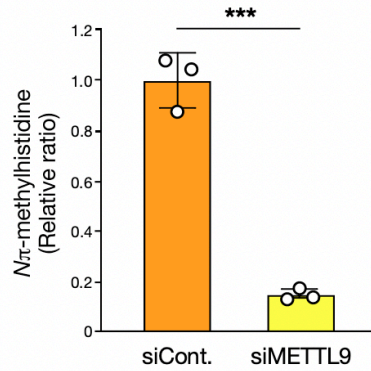


Figure S5. siRNA knockdown of *METTL9* reduces N π -methylhistidine levels of Flag-S100A9. HEK293T cells transfected with control or *METTL9* siRNAs were further transfected with Flag-S100A9 expression plasmid, followed by immunopurification and acid hydrolysis. Their methylhistidine contents were determined by LC-MS/MS and shown as a relative ratio compared to control siRNA knockdown. Mean \pm s.d. ($n=3$ independent experiments).

*** $P < 0.001$; two-tailed Student's t test.

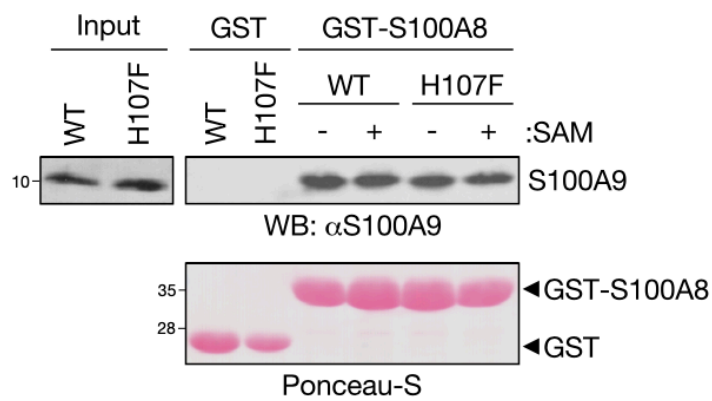


Figure S6. METTL9-mediated methylation of S100A9 has no effect on its heterodimer formation with S100A8.

Recombinant S100A9 wild-type and H107F proteins were methylated by GST-METTL9 in the absence or presence of SAM and then subjected to GST pull-down assay with GST alone or GST-fused S100A8, followed by Western blotting using an anti-S100A9 antibody (*Top*). The input indicates 5% of whole-cell lysates used for the GST pull-down assay. Ponceau-S staining of GST proteins used in the reaction is shown as a loading control (*Bottom*).

Supplementary Table S1: The sequences of siRNA pools used in the screening.

Gene Symbol	Gene Accession	Sequence
Non-targeting Control		UGGUUUACAUGUCGACUAA
		UGGUUUACAUGUUGUGUGA
		UGGUUUACAUGUUUCUGA
		UGGUUUACAUGUUUCCUA
METTL18	NM_033418	GAUUAAACAGUAUGGUGA
		GCAUGUUAAAGUUUACAGUA
		GAUCACUUGUGGGAACAUA
		GUUUUAGAGGGAGGCUUAA
METTL20	NM_001135864	CGCUGAAGGAAUUGAUUA
		CUAUJAGAACUCGAGUACUG
		GAGGACAACUGUGAUGUAA
		CCUUGUUGUUCUUGGCGAU
METTL21A	NM_145280	AGUGUGAGAUUUCGAUUUA
		UGACUAUCACGGAUCGAAA
		AUGAGGAGACCACGGAAUU
		CCUGAAAUCUGGCCGGAUG
METTL21B	NM_015433	GUGCAAUUUUUCGAGAGU
		GAGCCUUGAUCACGGAAACA
		GGCAAGAAGGUGAUCGAAC
		CUUCAGUCCAUUCCGAAA
METTL21C	NM_001010977	GGGCAAACAAUUCAGGUU
		GCUAUGGCCUUGUGUCAAU
		ACUUGAAAUUGGUGCCGGA
		GGAUUGAGAAGGUAGUGCA
METTL21D	NM_001040662	GCUCUAACAACCCUGGGUAA
		UGGUACAGUGCUACGACUA
		CUAAAAGAUUACAGCGGAU
		CAGCUUAGGUUACUUGAUU
METTL22	NM_024109	CAUGUGAAGCCUACGAUCA
		CAAGGCAGCCCGCACGAUA
		UAGCACAGGAAGAAGACGA
		CCGAAGUGUUUACGACGA
METTL23	NM_001080510	AAGGACAGUCUCUGAAUUA
		GGACUAACAUGGGGUCAUA
		UUGAGAAUGCAGUGGGUCU
		UCACCUAGACAACACUUA
METTL25	NM_032230	GGGAGGAGCUGGUCGACUU
		UGACUUGGGUUCGGUAAA
		AUAUUGAACUACUACGAGA
		GUUGUUUGAUCCCGUGAAA
CAMKMT	NM_024766	GCAUAAUAGUGGAUCCUUG
		CCGGACAUAUUGAAGAAA
		CGCCGAGGGAUACUUUAA
		GGUGCAUGGGUCCAAUUA

Gene Symbol	Gene Accession	Sequence
EEF2KMT	NM_201598	GAAAUCAAGGCCAACCAAA
		GCCUCUACGUGCUGCCCAU
		GCACGGCCAUCUCCCUA
		GUGCUGCUCGAAAGGGUUU
FAM86B1	NM_001083537	AAUCAAACUGCCUCUAA
		GAGCAGAGCUUACUCUAAU
		UGGAAUGUUUGGAGAUUUA
		CAUGAAGAACAAGAGACAG
FAM86B2	XM_928336	GGGCAUACAUCUUCAGCGA
		CAUUAGAGGCAGACAUAC
		CCGGCAGCCUUCAUUAACA
		GCCAUCAUCUCCACGGUA
FAM86C1	NM_152563	GAGACCAAUUGCAGGGUAA
		CUAAUAAGACAUCGGCUAU
		GAGCAGAGCUUACUUCUAAU
		ACUGGAAUGUUUGGAGAAU
CARNMT1	NM_152420	UCUUGUUUGUGGUCGGUAA
		CUAAGAAAACUAGCGUAA
		AGGAGAGCCUUGAGCGUGA
		UAGCAUCACAGGCCGGUAA
HNMT	NM_001024074	CGGAAAUAUGUUGAAUCU
		CCACGGAACACCAGUGCAU
		CAUCUUCUAGAGGAGCUU
		AGGAAUUAUGGACAAGAA
METTL6	NM_152396	UAGAAGAAGAUCCGAAUUA
		AGAAGUGGUAAACGAGUUA
		UCCAAGAGCCAUGAAUUA
		GGAUUGUUUAUGUUGAUA
METTL9	NM_016025	AUAAACACGUGGAGGUCGA
		UGGUUAUAAAGCCGAAUUA
		GUGAAGGCAGCAUGAUUAA
		AGAGUUGGGUUUUGUGAUU
SETD3	NM_199123	GGUAAGAGAGUCGAGUAA
		GCUAAUGACUGUUGAAUCU
		GCACUGGCCUUCUUAUUGC
		ACACUCCUCUCUACUUGA
SETD4	NM_001007259	GUUACGGCCUCACGAUAA
		GAAACUACUUCUACGAAA
		CCAGGUAAGCAGCGUUU
		GAGCCCAGGUCAGGAGUU
SMYD1	NM_198274	GUGAUUACUGCAACGGUU
		AAGAAUGAAUUGUCGGCCA
		GUUAUCAGAGGUUGUGAAA
		GCCCAACUGUACUGUCAUA