Mixed-lineage leukemia protein modulates the loading of *let-7a* onto AGO1 by recruiting RAN

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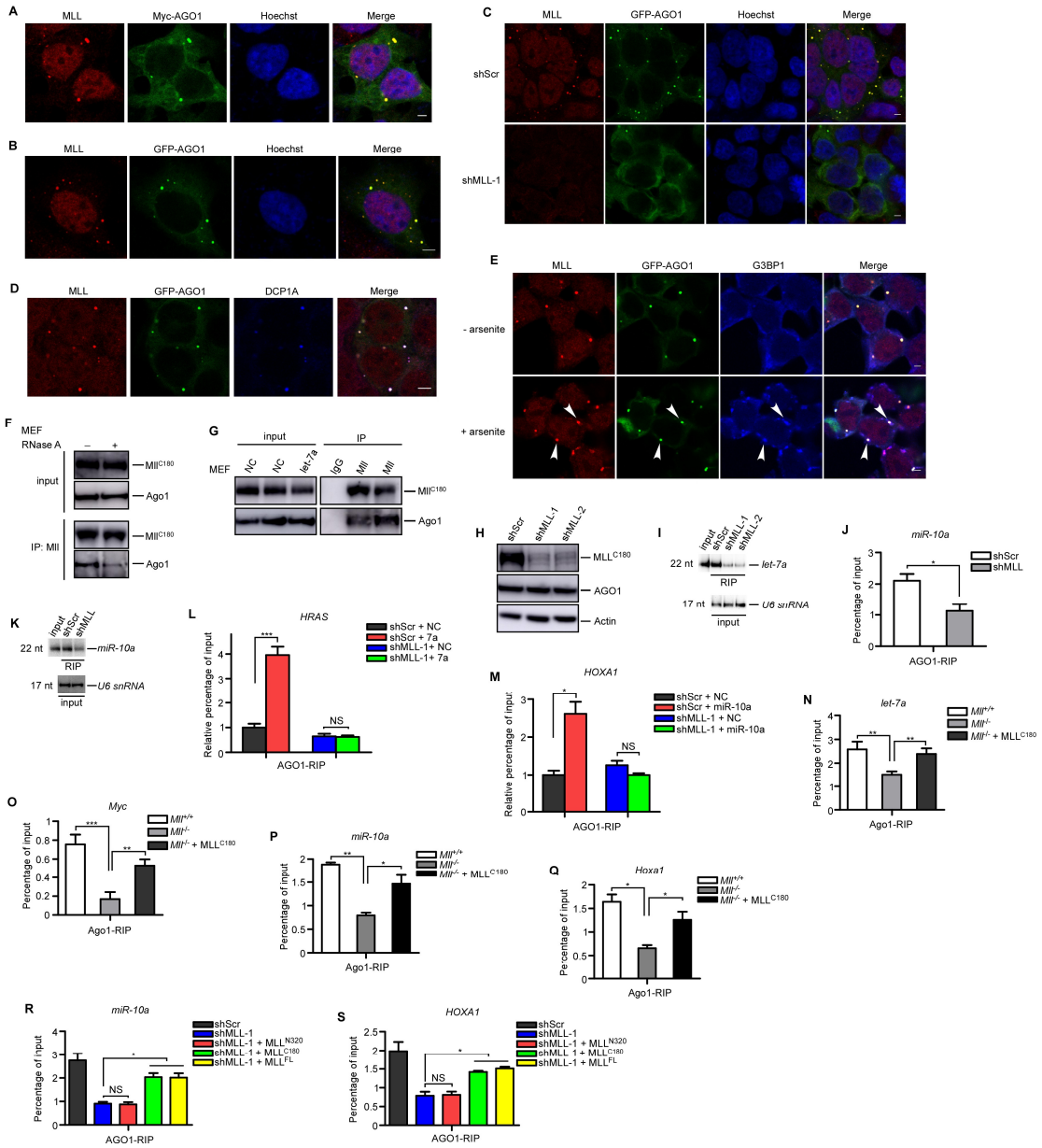
Supplementary Figure and Figure Legends

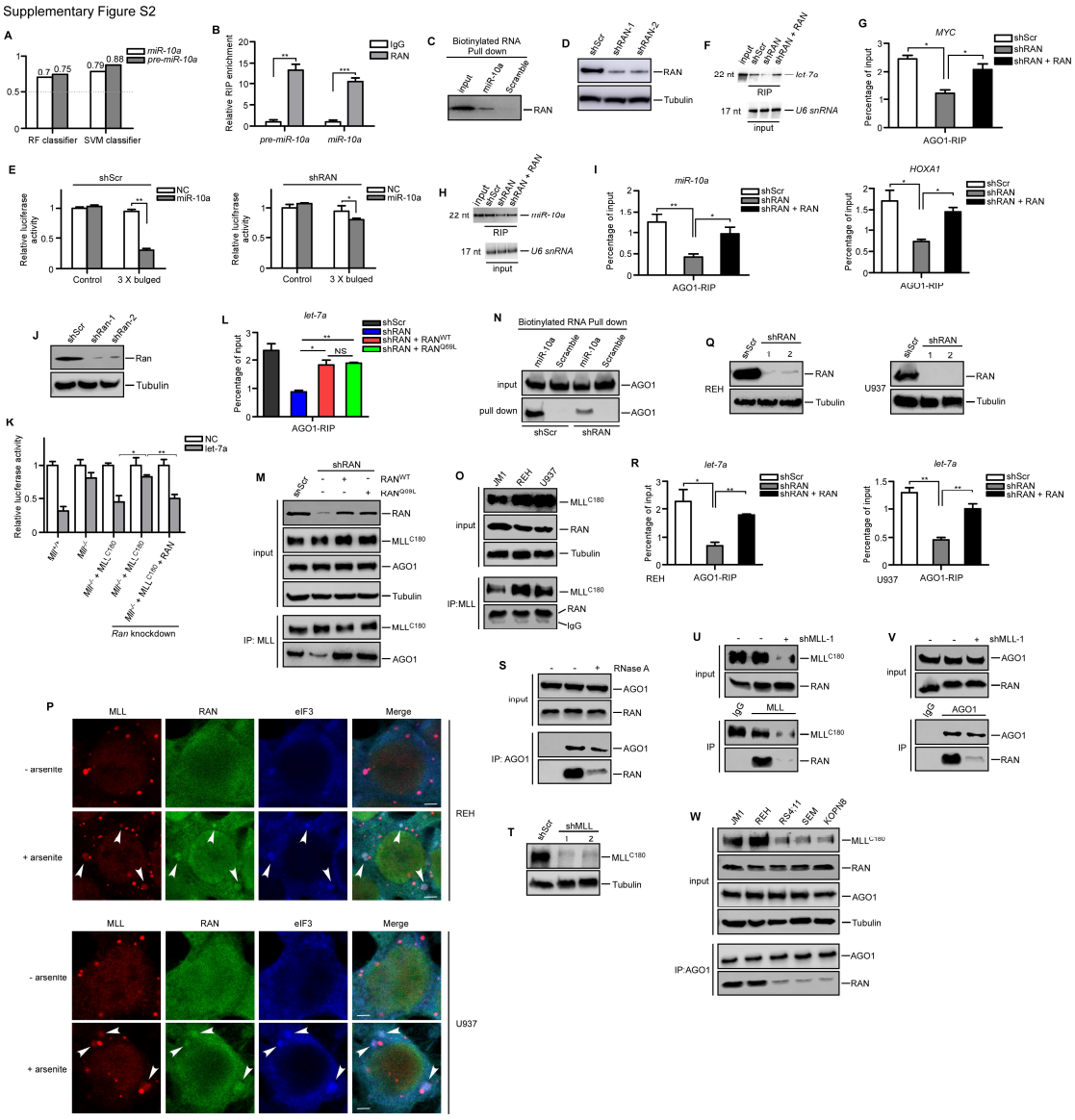
Supplementary Figure S1



Supplementary Figure S1. MLL plays a causal role in the recruitment of *let-7a* and *miR-10a* to miRISC.

(A) 293T cells were transfected with Myc-AGO1. Immunofluorescence experiments were performed to visualize the localization of Myc-AGO1 and MLL. anti-Myc antibody was used to detect Myc-AGO1. MLL-CT antibody that recognizes MLL^{C180} (aa2829-2883) was used to detect MLL. Scale bar, 5 um. (B) 293T cells were transfected with GFP-AGO1. Immunofluorescence experiments were performed to visualize the localization of GFP-AGO1 and MLL. A300-374A antibody recognizing aa2725-2775 of MLL was used to detect MLL. Scale bar, 5 um. (C) The localization of AGO1 after *MLL* depletion. 293T-shScr and 293T-shMLL cells were transfected with GFP-AGO1 and visualized

by confocal microscopy at 24 hours post-transfection. Scale bar, 5 um. (D) 293T cells were transfected with GFP-AGO1. Endogenous MLL and P-body proteins DCP1A were probed using indicated antibodies. The localization of GFP-AGO1, MLL and P-bodies were visualized by immunofluorescence assays. Scale bar, 5 um. (E) 293T cells were untreated (upper panels) or arsenite treated (0.5 mM, 45 min) (lower panels), then fixed and stained with indicated antibodies. Note that G3BP1 is specific for the stress granule. Scale bar, 5 um. (F) MEF cell lysates were untreated or treated with RNase A followed by anti-Mll immunoprecipitation. Immunopurified complexes were analyzed by Western blot with indicated antibodies. (G) The interaction between Mll and Ago1 in MEF cells was assessed after *let-7a* transfection. Anti-Mll immunoprecipitation assays were performed and analyzed by immunoblots using indicated antibodies. (H) AGO1 expression level upon *MLL* depletion were confirmed by Western blot assays using indicated antibodies. (I) 293T-shScr and 293T-shMLL cells were prepared for the RIP analysis using anti-AGO1 antibody, then the bound miRNAs were subjected to the Northern blot assays against *let-7a*. *U6* snRNA was used as loading control. (J) Extracts of 293T-shScr and 293T-shMLL cells were subjected to RIP analysis using anti-AGO1 antibody, and pull-downed RNAs were analyzed by qRT-PCR using specific primers for *miR-10a*. (K) 293T-shScr and 293T-shMLL cells were prepared for the RIP analysis using anti-AGO1 antibody, then the bound miRNAs were subjected to the Northern blot assays against *miR-10a*. *U6* snRNA was used as loading control. (L) 293T-shScr and 293T-shMLL cells were transfected with negative control (NC) and let-7a followed by anti-AGO1 RIP experiments at 24h post-transfection. Total RNAs were isolated to analyze the *HRAS* mRNA level by qRT-PCR. (M) 293T-shScr and 293T-shMLL cells were transfected with negative control (NC) and miR-10a followed by anti-AGO1 RIP experiments at 24h post-transfection. Total RNAs were isolated to analyze the *HOXA1* mRNA level by qRT-PCR. (N-O) Extracts of *Mll* wild-type ($Mll^{+/+}$) and *Mll* knockout ($Mll^{-/-}$) MEF cells with the latter being rescued by MLL^{C180} were subjected to anti-Ago1 RIP assays. Pull-downed RNAs were analyzed by qRT-PCR using specific primers for *let-7a* (N) and Myc (O). (P-Q) Extracts of *Mll* wild-type ($Mll^{+/+}$) and *Mll* knockout ($Mll^{-/-}$) MEF cells with the latter being rescued by MLL^{C180} were subjected to anti-Ago1 RIP assays. Pull-downed RNAs were analyzed by qRT-PCR using specific primers for *miR-10a* (P) and *Hoxa1* (Q). (R-S) 293T-shScr and 293T-shMLL cells with the latter being rescued by exogenous shRNA-resistant MLL^{N320}, MLL^{C180} or full-length *MLL* (MLL^{FL}) were performed with anti-AGO1 RIP experiments at 24 hours post-transfection. Total RNAs were isolated to analyze the *miR-10a* (R) and *HOXA1* (S) levels by qRT-PCR using specific primers. NS, no significant difference. * for $P<0.05$, ** for $P<0.01$, *** for $P<0.001$. Data represent mean and s.e.m of three independent experiments.

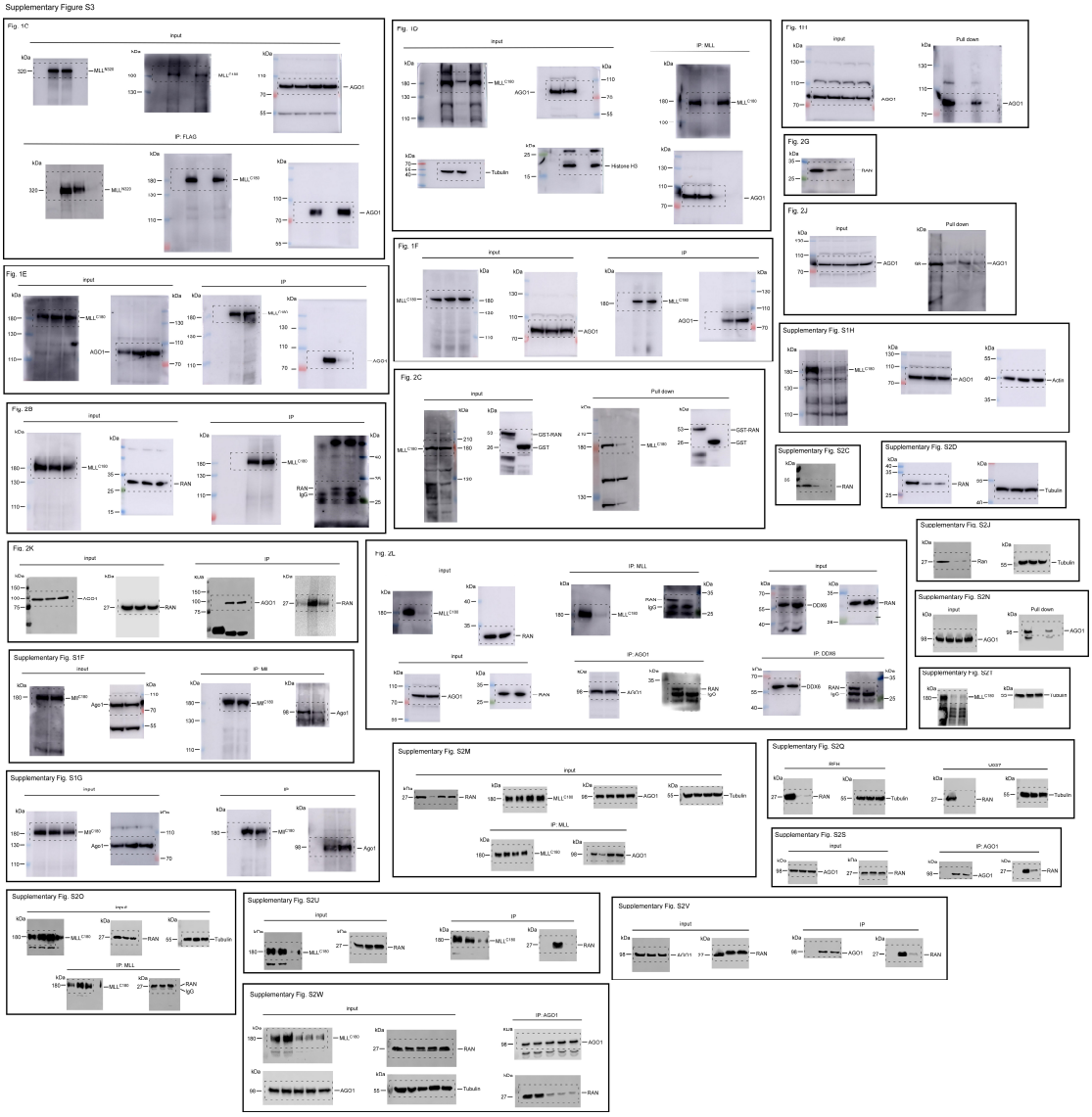


Supplementary Figure S2. MLL-RAN interaction contributes to the loading of *let-7a* and *miR-10a* onto AGO1.

(A) The RPISeq tool was used to predict the interactions between RAN and *miR-10a* or *pre-miR-10a*. The RF classifier and SVM classifier represent the confidence of the prediction. In performance evaluation experiments, predictions with probabilities > 0.5 were considered “positive”. (B) 293T cells lysate were prepared and anti-RAN RIP experiments were performed. Pull-downed RNAs were isolated, *pre-miR-10a* and mature *miR-10a* were analyzed by qRT-PCR using specific primers. (C) 293T cellular lysates were subjected to Biotinylated-miR-10a RNA pull down assay. Then *miR-10a*-immunoprecipitated RAN proteins were subjected to the Western blot analysis. Scrambled miRNAs were used as negative control. (D) 293T cells stably expressing control or RAN shRNAs were harvested for the Western blot assays using the indicated antibodies. (E) 293T-shScr and shRAN cells transfected with Agomir-negative

control (NC) or Agomir-miR-10a mimic (miR-10a) were subjected to dual luciferase reporter assays. The ratio of luciferase activity was measured and normalized to the value of the cells transfected with the control reporter and NC. (F) 293T-shScr and 293T-shRAN cells with the latter being rescued by exogenous shRNA-resistant *RAN* were prepared for the RIP analysis using anti-AGO1 antibody, then the bound miRNAs were subjected to the Northern blot assays against *let-7a*. *U6* snRNA was used as loading control. (G) Extracts of 293T-shScr and shRAN cells with the latter being rescued by shRNA-resistant *RAN* were subjected to anti-AGO1 RIP assays. Pull-downed RNAs were analyzed by qRT-PCR using specific primers for *MYC*. (H) 293T-shScr and 293T-shRAN cells with the latter being rescued by exogenous shRNA-resistant *RAN* were prepared for the RIP analysis using anti-AGO1 antibody, then the bound miRNAs were subjected to the Northern blot assays against *miR-10a*. *U6* snRNA was used as loading control. (I) Extracts of 293T-shScr and shRAN cells with the latter being rescued by shRNA-resistant *RAN* were subjected to anti-AGO1 RIP assays. Pull-downed RNAs were analyzed by qRT-PCR using specific primers for *miR-10a* and *HOXA1*. (J) *Mll* knockout ($Mll^{-/-}$) MEF cells were transduced with lentivirus specifically targeting control or the 3'UTR region of *Ran*. Cells were harvested for the Western blot assays using the indicated antibodies. (K) *Mll* knockout ($Mll^{-/-}$) MEF cells transfected with Agomir-negative control (NC) or Agomir-let-7a mimic (let-7a) were subjected to dual luciferase reporter assays. shRNA-resistant MLL^{C180} with or without *RAN* were reintroduced into the *Ran*-depleted $Mll^{-/-}$ MEF cells to determine the linking role of RAN in the MLL^{C180}-mediated miRNA function. The ratio of luciferase activity was measured and normalized to the value of the cells transfected with the control reporter and NC. (L) 293T-shScr and 293T-shRAN cells with the latter being rescued by shRNA-resistant RAN^{WT} *or* RAN^{Q69L} were subjected to anti-AGO1 RIP assays. Pull-downed RNAs were analyzed by qRT-PCR using specific primers for *let-7a*. (M) Transfections of *RAN* knockdown 293T cells with shRNA-resistant wild-type *RAN* (RAN^{WT}) or GTPase-deficient mutant *RANQ69L* (RAN^{Q69L}) were confirmed by Western blot assays. Co-IP assays were performed to check the interaction between MLL and AGO1. Antibodies were used as indicated. (N) 293T-shScr and 293T-shRAN cellular lysates were subjected to Biotinylated-miR-10a RNA pull down assay. Then miR-10a-immunoprecipitated AGO1 proteins were subjected to the Western blot analysis. Scrambled miRNAs were used as negative control. (O) JM1, REH and U937 cells were collected followed by anti-MLL immunoprecipitation. Western blots were performed using indicated antibodies. (P) REH (upper panels) and U937 (lower panels) cells were untreated or arsenite treated (0.1 mM, 30 min), then fixed and stained with indicated antibodies. Note that eIF3 is specific for the stress granules. Arrowheads show the localization of MLL with the RAN and eIF3. Scale bar, 2 um. (Q) REH (left panels) and U937 (right panels) cells stably expressing control or RAN shRNAs were harvested for the Western blot assays

using the indicated antibodies. (R) Extracts of scramble control and shRAN cells with the latter being rescued by shRNA-resistant *RAN* were subjected to anti-AGO1 RIP assays. Pull-downed RNAs were analyzed by qRT-PCR using specific primers for *let-7a*. Left: REH cells; Right: U937 cells. (S) REH cell lysates were treated with RNase A followed by anti-AGO1 immunoprecipitation. Western blots were performed using indicated antibodies. (T) REH cells stably expressing control or MLL shRNAs were harvested for the Western blot assays using the indicated antibodies. (U-V) Extracts of REH-shScr and REH-shMLL cells were collected and Co-immunoprecipitation assays were performed and analyzed using the indicated antibodies. (W) JM1, REH, RS4;11, SEM and KOPN8 cells were collected followed by anti-AGO1 immunoprecipitation. Western blots were performed using indicated antibodies. JM1 and REH were wild-type *MLL* harboring cell lines, RS4;11 and SEM were *MLL-AF4* harboring cell lines, KOPN8 was the *MLL-ENL* harboring cell line. * for $P<0.05$, ** for $P<0.01$. Data represent mean and s.e.m of three independent experiments.



Supplementary Figure S3. Unprocessed original Western blot images of this manuscript.