Supplementary Information

The R882H DNMT3A hotspot mutation stabilizes the formation of large DNMT3A oligomers with low DNA methyltransferase activity

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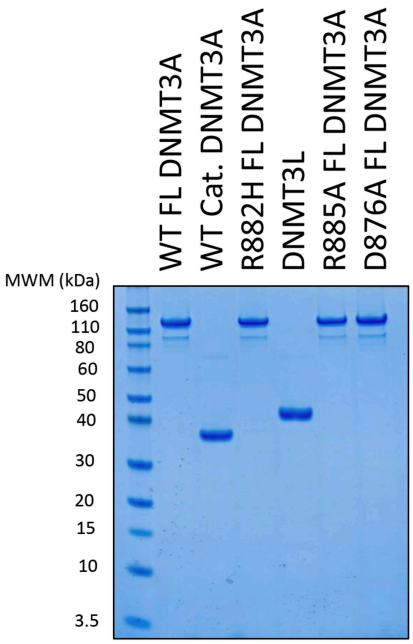
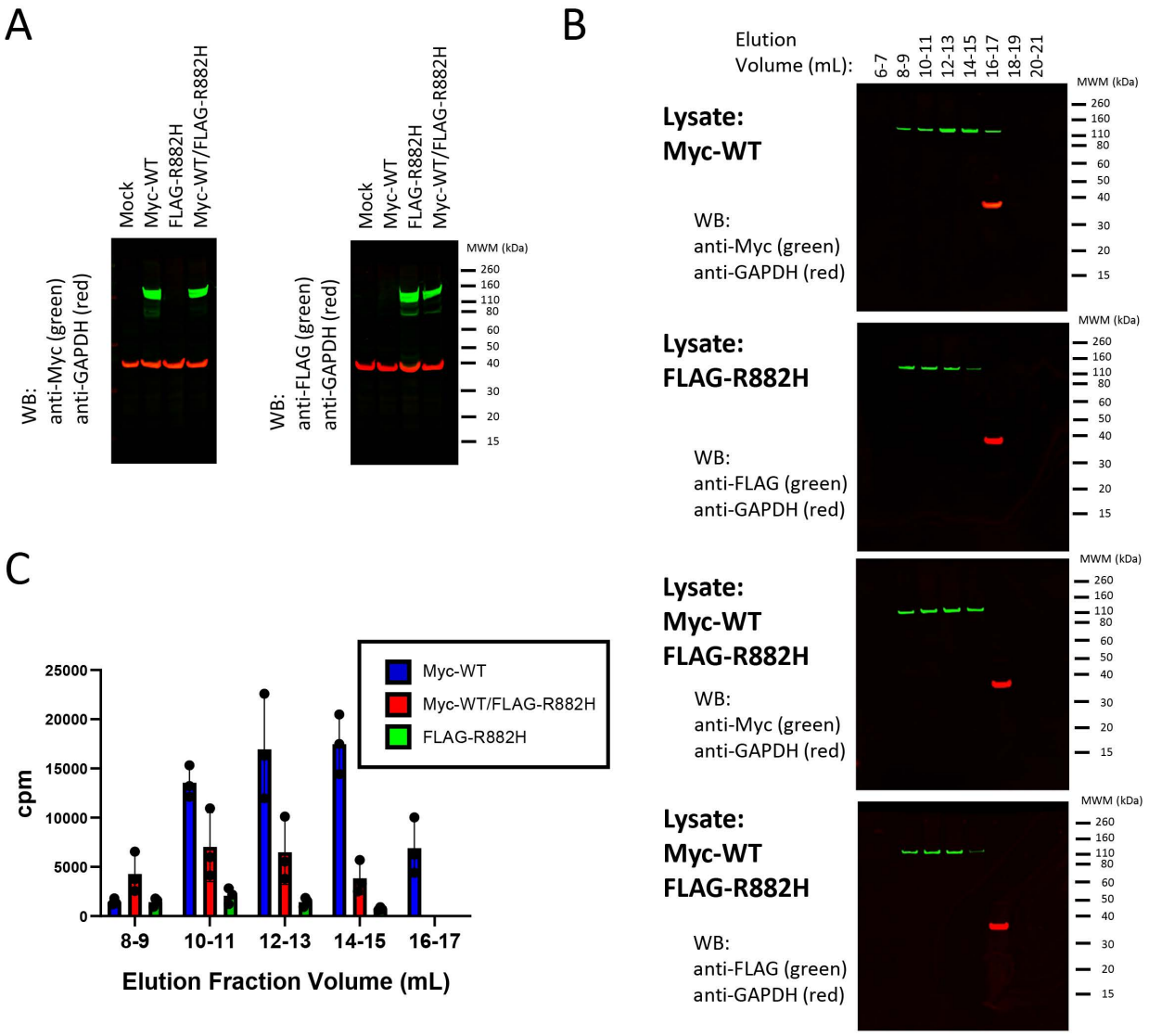
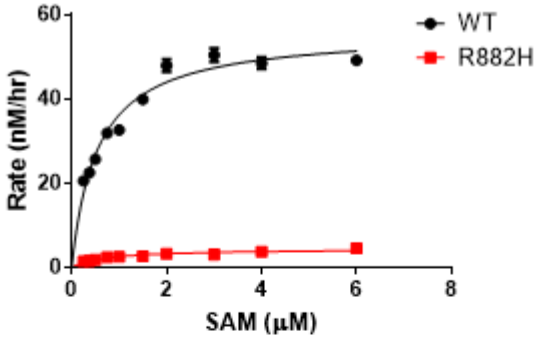


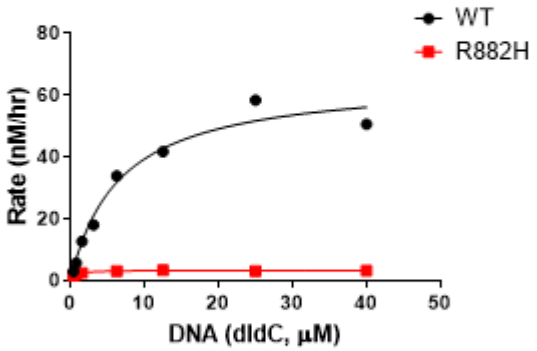
Supplementary Figure 1: SDS-PAGE gel of DNMT3A and DNMT3L proteins used in this study. All proteins were purified from *E. coli* as described in the Methods section. 2 µg of each protein was run on a NuPAGE 4-12% Bis-Tris gel with Novex Prestained molecular weight marker and visualized by coomassie staining.



Supplementary Figure 2: WT and R882H DNMT3A oligomer distribution and activity in HEK293 cell lysates. *A*, Western blots of whole cell lysate of Myc-WT and FLAG-R882H DNMT3A transiently expressed alone or together in HEK293 cells. *B*, Western blots of SEC elution fractions from HEK293 cell lysates expressing Myc-WT alone, FLAG-R882H alone or Myc-WT and FLAG-R882H together. After exogenous expression of tagged DNMT3A proteins, HEK293 cells were lysed by Dounce homogenization and lysates passed over a Superose 6 size exclusion column. *C*, DNA methyltransferase activity of each

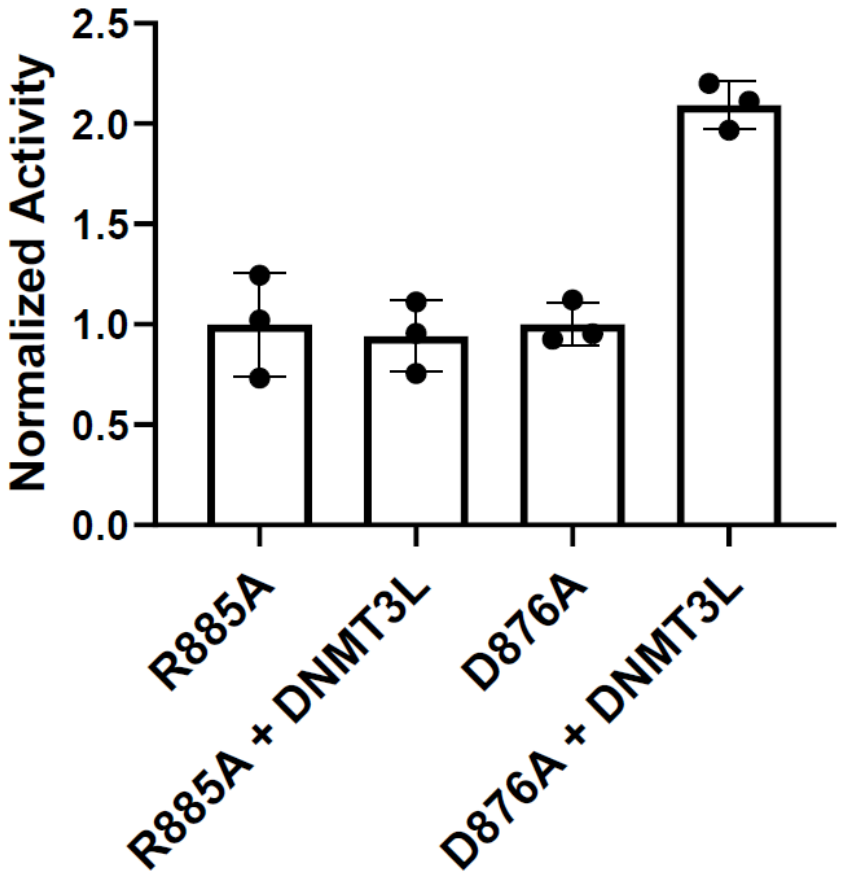
fraction containing DNMT3A from *B*. in the presence of 3 μM ^{3}H-SAM and 10 μM base pair DNA substrates. Error bars represent SD, n = 3.





	dIdC app K_m (µM)	SAM app K_m (µM)	app k_{cat} (hr^{-1})	SAH K_i (µM)
WT	6.6 +/- 0.8	0.55 +/- 0.04	2.61 +/- 0.11	0.18 +/- 0.05
R882H	0.49 +/- 0.04	0.73 +/- 0.08	0.13 +/- 0.01	0.15 +/- 0.03

Supplementary Figure 3. Apparent kinetic parameters of full length WT and R882H DNMT3A. WT and R882H apparent K_m curves for SAM at 30 µM dIdC. WT and R882H apparent K_m curves for dIdC DNA substrate at 5 µM SAM. IC50 curves for SAH inhibition of WT and R882H DNMT3A activity. K_i values for SAH were calculated using the Cheng-Prusoff equation. Table of apparent kinetic parameters measured for full length WT and R882H DNMT3A.



Supplementary Figure 4. Activation of R885A and D876A by DNMT3L. Methyltransferase activity of 200 nM R885A and 200 nM D876A DNMT3A alone and in the presence of an equal amount of DNMT3L after a 2 hr pre-incubation. Reactions were stopped after 4 hrs. Activity of R885A and D876A alone is normalized to 1. Error bars represent SD, n = 3.