SUPPLEMENTARY APPENDIX

Pbx3 and Meis1 cooperate through multiple mechanisms to support Hox-induced murine leukemia

Maria-Paz Garcia-Cuellar, Julia Steger, Elisa Füller, Katrin Hetzner, and Robert K. Slany

Department of Genetics, Friedrich-Alexander-University, Erlangen, Germany

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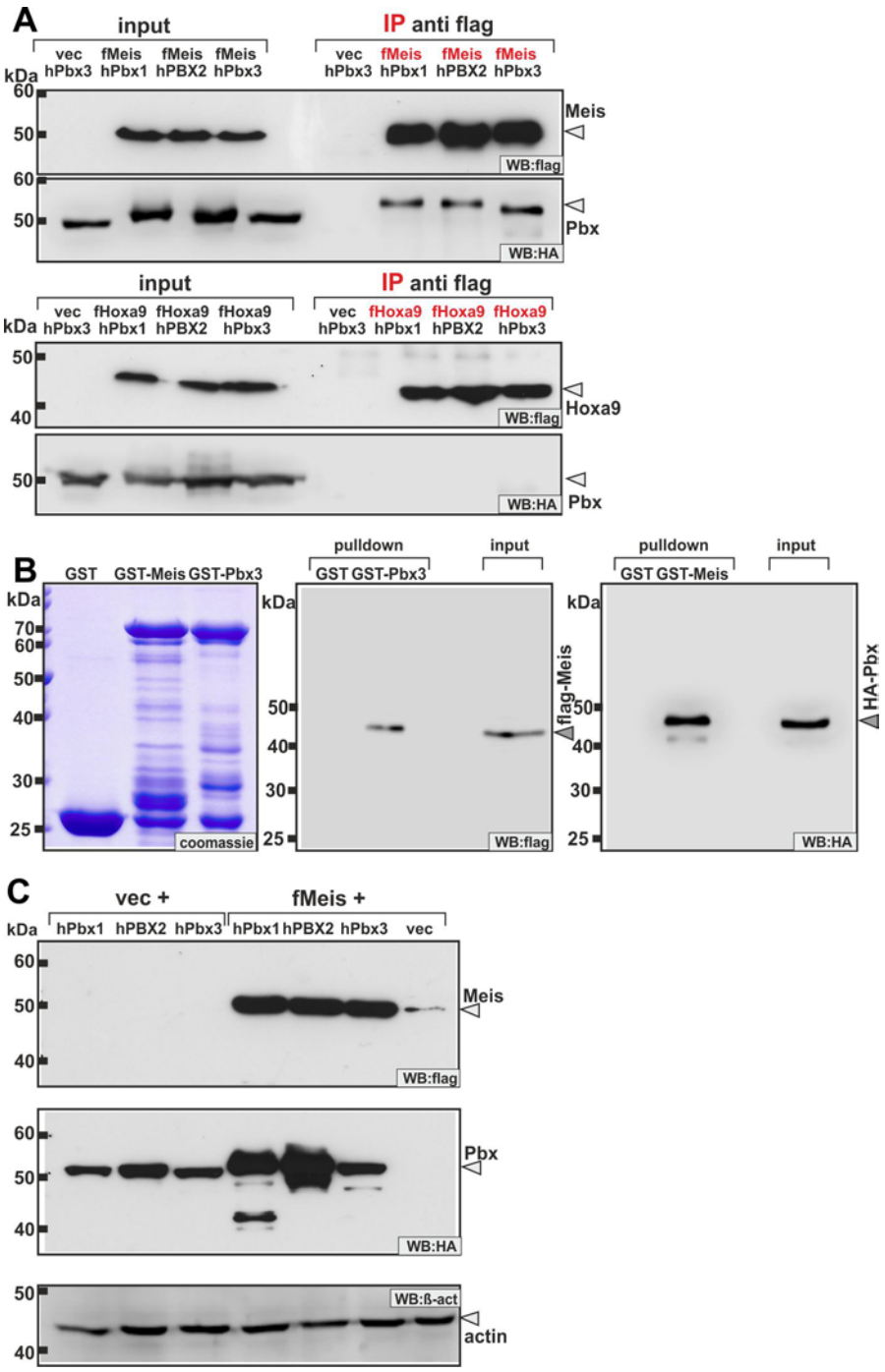
Correspondence: robert.slany@fau.de



Supplemental figure 1:

A: Co-immunoprecipitation of Meis1 with Pbx proteins. Flag-tagged Meis1 was coexpressed with HA-labeled Pbx1, PBX2 (human) and Pbx3 as indicated. Anti-flag immunoprecipitation done in DNase digested cell extracts brought down Meis1 and all three Pbx proteins (upper panels). f = flag tag, h = HA tag.

Control co-immunoprecipitation of Hoxa9 with Pbx proteins. Flag-tagged Hoxa9 was expressed together with Pbx proteins as above. Immunoprecipitation in the absence of DNA successfully precipitated flag-Hoxa9 but not Pbx (lower panels).

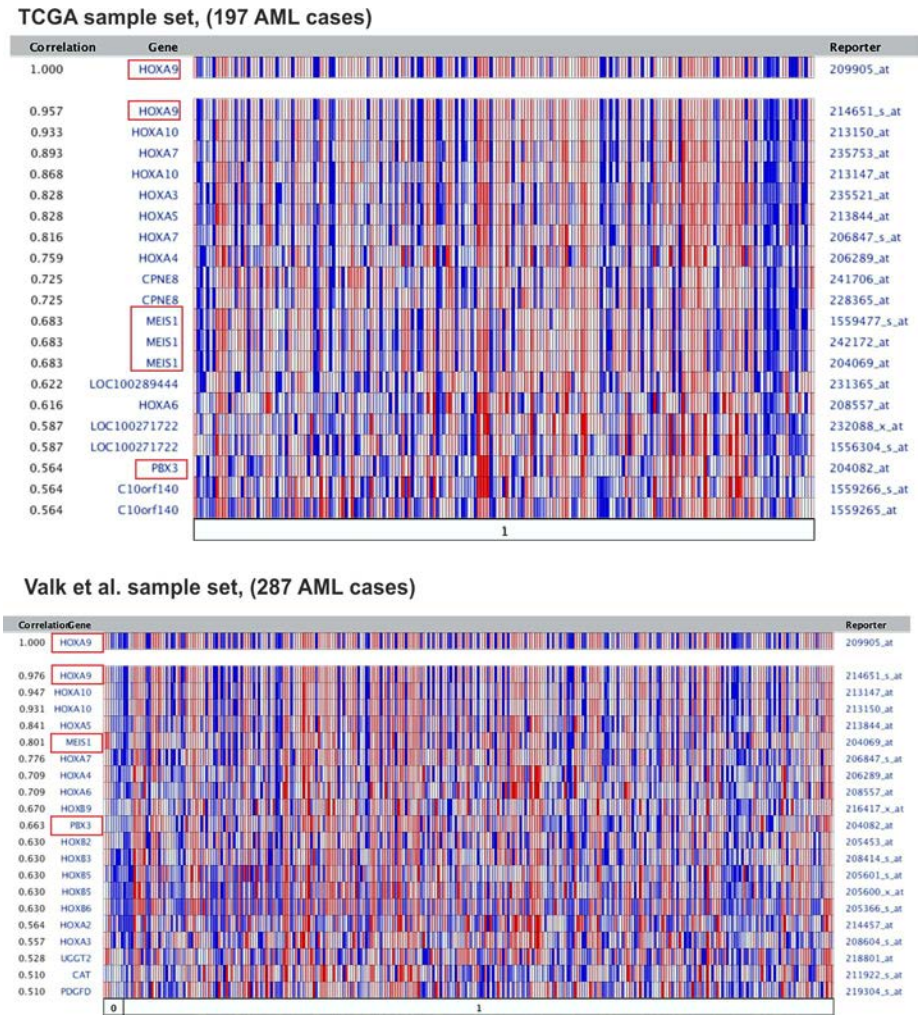
B: GST-pulldown experiments. GST-Meis1 and GST-Pbx3 fusion proteins were expressed in E.coli and affinity purified on gluthatione beads.

Left panel: Coomassie stained gel of proteins bound to glutathione beads used in the pulldown experiments. GST without fusion partner was used as control.

Middle panel: Loaded GST only or GST-Pbx3 beads were incubated with cellular extracts of HEK293T cells expressing flag-tagged Meis1. After intensive washing proteins bound to the gluthathione beads were released by boiling in SDS sample buffer and the eluate was probed for the presence of flag-Meis by immunoblotting. A sample of the input cell-extract was run alongside the pulldown products as indicated.

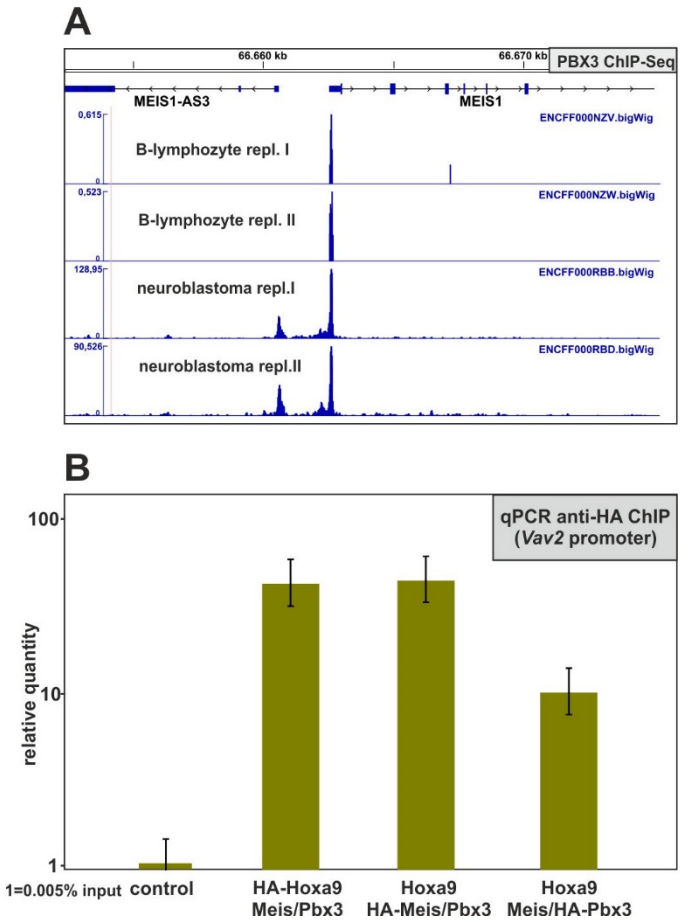
Right panel: Reciprocal experiment using GST-Meis fusion proteins loaded onto gluthatione beads to pull down HA-tagged Pbx3 from cellular extracts.

C: Mutual stabilization of Meis1 and Pbx proteins. HA-tagged Pbx1, PBX2, and Pbx3 were expressed together with flag-Meis1 or empty vector as control. Cell extracts were analyzed for the presence of Meis1 by anti-flag western blot (upper panel) and by anti-HA reagents to detect HA-Pbx proteins (middle panel). An anti ß-actin blot (lower panel) served as loading control.



Supplemental figure 2:

Oncomine analysis (www.oncomine.org) of correlated gene expression in the TCGA (197 cases unpublished) and the Valk et al. AML sample collection (287cases, Valk PJ, Verhaak RG, Beijen MA, et al. Prognostically useful gene-expression profiles in acute myeloid leukemia. N Engl J Med. 2004;350(16):1617-1628).



Supplemental figure 3:

A: Results of PBX3 specific ChIP-seq experiments as deposited with the ENCODE data collection. The data were visualized with the IGV browser (www.broad.edu). The region around the transcription initiation site of the human MEIS1 gene is shown. Samples show two independent replicates of precipitations done with a B-lymphoblastic and a neuroblastoma cell line. The original data accession numbers are noted to the right of each panel.

B: Hoxa9, Meis1 and Pbx3 can be detected on the same region of the *vav2* promoter by ChIP. Chromatin immunoprecipitation was performed using HA-tag specific antibodies with chromatin isolated from three cell lines transduced by a combination of Hoxa9/Meis1/Pbx3 and with a HA-tag fused to a different protein in each line as indicated. Analogous cells transformed by untagged proteins served as control. Binding to the Hoxa9 target gene *vav2* was tested by qPCR as described previously (Breitinger C, Maethner E, Garcia-Cuellar MP, et al. HOX genes regulate Rac1 activity in hematopoietic cells through control of Vav2 expression. Leukemia. 2013 Jan;27(1):236-238). Primers used for PCR were Vav2-2fw: 5'-atggttagttggtggcccacttc-3'; Vav2-2rev 5'-tctttctcctcacccattctctc-3'.