Methods (online-only)

miniCoopR Assay. miniCoopR was constructed by inserting a zebrafish *mitfa* minigene (promoter + open reading frame + 3'UTR) into the BglII site of pDestTol2pA2[19]. Individual MiniCoopR clones were created by Gateway multisite recombination using human, full-length open reading frames (Invitrogen). Recombination junctions were sequence verified. 25pg of each MiniCoopR-Candidate clone and 25pg of tol2 transposase mRNA were microinjected into one-cell embryos generated from an incross of *Tg(mitfa:BRAF^{V600E}); p53(lf); mitfa(lf)* zebrafish. Transgenic animals were selected based on the presence of rescued melanocytes at 48 hours post-fertilization. Rescued animals were scored weekly for the presence of visible tumor.

Tumor Invasion Assay. Zebrafish with dorsal melanomas between the head and dorsal fin were isolated, and tumors were allowed to progress for two weeks, at which time animals were sacrificed. Tumors were formalin fixed, embedded, and sectioned transversely to assess invasion.

Senescence Assay. SA-βGal staining was performed as described[10], except that scales plucked from the dorsum of melanocyte-rescued zebrafish were stained. This assay was performed in an *albino(b4)* mutant background so melanin pigment would not obscure βGal staining. Experimental animals were injected with 20pg miniCoopR-*SETDB1* + 10pg miniCoopR-*EGFP* and controls with 30pg miniCoopR-*EGFP*. Rescued melanocytes were recognized as EGFP-positive cells.

Gene Expression and GSEA. From zebrafish, total RNA was extracted from four miniCoopR-*SETDB1* melanomas and four miniCoopR-*EGFP* melanomas. Total RNA

from each was used for cDNA synthesis, which was hybridized to a Nimblegen 385K array (catalog 071105_Zv7_EXPR). Zebrafish genes downregulated by *SETDB1* were selected by fold change (*EGFP/SETDB1*) > 5 and filtered by a '*SETDB1* specificity score', which was defined as a fold change of >3 when comparing *Tg(mitfa:BRAF^{V600E}); p53(lf)* melanomas to miniCoopR-*SETDB1* melanomas. Human orthologs of *SETDB1*-downregulated genes were identified for GSEA analysis (http://www.broadinstitute.org/gsea/). For GSEA of *SETDB1*-downregulated and SETDB1 "bound-bound" genes, a rank-ordered gene list was derived from expression profiles of 93 melanoma cell lines and short-term cultures[6] using *SETDB1* expression level as a continuous variable. In WM451Lu cells, the dose of *SETDB1* lentiviral infection was titrated to achieve *SETDB1* expression levels comparable to those of *SETDB1*-high melanoma lines. Total RNA was extracted then amplified and hybridized to an Affymetrix human gene 1.0 ST array. Control gene expression values were obtained from WM451Lu cells infected with *EGFP* lentivirus.

Chromatin Immunoprecipitation. Chromatin immunoprecipitation was performed from short-term cultures of WM262 and WM451Lu and ChIP-Seq data analyzed as previously described[14].

Methyltransferase complex reconstitution. In vitro translated Flag-GLP, HA-G9a and untagged SETDB1 (WT or the indicated mutants) were incubated for 4 h at 4°C with 5 mg of either GST, GST-Suv39h1 WT or GST-Suv39h1(H324K) mutant immobilized on agarose-glutathione beads. Beads were then extensively washed, as described in Fritsch et al. [16], and protein complexes eluted with free glutathione. The eluate was then subjected to an overnight Flag immunoprecipitation at 4°C using Flag-agarose. After

extensive washing, protein complexes were eluted with 0.1 M glycine pH 3.0. Glycine was then neutralized with NaOH, the eluate renatured for 1 h at room temperature then incubated overnight at 4°C with HA-resin. The HA-resin was then washed and protein complexes eluted with SDS. 10% of the input and 100% of the HA eluate were resolved by SDS-PAGE and analyzed by western blot with the indicated antibodies. The top of the membrane was revealed with 3 different antibodies (anti-SETDB1, anti-HA and anti-Flag) with 2 stripping steps.

Histone methylation assay. Purified complex was incubated with 5 mg of core histones (Upstate, 13-107) and 1.5 mCi of Adenosyl-L-Methionine, S-[methyl-3H] (PerkinElmer, NET155050UC) in a buffer containing 50 mM Tris pH 8.0, 100 mM NaCl, 1% NP40, 1 mM DTT and protease inhibitors (reaction volume 30 µl). The mixture was incubated 1 hour at 30°C followed by SDS-PAGE analysis. The gel was stained by SimplyBlue kit (Invitrogen) and analyzed by fluorography using an FLA-7000 phosphoimager (Fuji).

Immunohistochemistry. Human melanoma tissue microarrays (TMAs) were analyzed by immunohistochemistry for SETDB1 using rabbit polyclonal Ab (Sigma HPA018142, 1:200) and mouse monoclonal Ab 4A3 (Sigma WH0009869M7, 1:400) with a purple substrate for the secondary antibody (Vector Labs, VIP Substrate). A methyl green counterstain was used. Melanoma TMAs were obtained from U.S. Biomax (ME1003, ME482). A modified visual semiquantification method was used to score staining as previously described[21], using a two-score system for immunointensity (II) and immunopositivity (IP). II and IP were multiplied. SETDB1 immunostaining was also performed on formalin-fixed, paraffin-embedded zebrafish melanomas.

References (online-only)

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