Supplementary Methods:

DNA Methylation Analysis by HELP assay

The HELP assay was carried out as described previously[1,2] to determine methylome of 50,000 CpGs corresponding to 14,000 genes. Detailed description provided in supplementary methods.

. Methylation data if obtained from a human hg17 custom-designed oligonucleotide array (50-mers) covering 50,000 CpGs corresponding to 14,000 gene promoters with extensive quality control [2] . The $\log_2$(HpaII/MspI) was used as a representative for methylation and analyzed as a continuous variable (GEO (GSE60233). Unsupervised clustering of HELP data by hierarchical clustering was performed using the statistical software R version 2.6.2. A two-sample *t* test was used for each gene to summarize methylation differences between groups. Genes were ranked on the basis of this test statistic and a set of top differentially methylated genes with an observed log fold change of >0.5 between group means was identified.

Pathway Analysis was performed using the IPA software (Redwood City, CA) [3]. The list of hypermethylated genes was examined for enrichment of conserved gene-associated regions using the Molecular Signatures Database (MSigDB) [4]. Transcription factor binding sites in the differentially methylated regions was determined by the HOMER algorithm [5]

Mice

The doxycycline-inducible constitutively active β-catenin mice (*KH2-Col1A1-tetO-CTNN1S33Y/Rosa-rtTA; S33Y*) were generated by the Hochedlinger laboratory [6]. This

model consists of a β-catenin S33Y point mutation under the control of a tetOP minimal promoter into the *Col1a1* locus crossed to a *Rosa26* promoter- driven M2 reverse tetracycline transactivator allele. These mice were crossed to transgenic mice expressing a *NUP98-HOXD13* fusion gene in hematopoietic tissues in the C57BL6 background, resulting in a transgenic *NUP98-HOXD13* mouse with doxycycline-inducible constitutively active β-catenin. All experimental mice were heterozygous in both *Col1a1* (S33Y under tetOP) and *Rosa26* (rtTA) loci and for the *NUP98-HOXD13* transgene. Transgenic primary *NUP98-HOXD13* mice aged 14-18 months were used for analysis of MDS hematopoietic and stromal cells. Analyzed mice were verified to display clinical hallmarks of MDS and the transgenic *NUP98-HOXD13* model (including peripheral blood pancytopenia) compared to age-matched mice.

Hematopoietic cell isolation and bone marrow transplantation

Hematopoietic cells were isolated from tibias, femurs, pelvis and humeri. Transplantation experiments were performed using bone marrow from 2 month old litter mates was transplanted into CD45.1 mice (3x10^6 BM with 0.3x10^6 of CD45.1 helper bone marrow). To induce the expression of constitutively active β-catenin, mice were fed doxycycline feed (Harlan Teklad) at 625mg doxycycline hyclate per kg diet, with individual mice receiving approximately 2.2 mg of doxycycline per day according to the manufacturer.

Murine Flow Cytometry

Bone marrow, spleen peripheral blood cells were analyzed on a BD LSRFortessa instrument. The following antibodies were used for various analyses: Lineage antibody cocktail (CD3, CD4, CD8, Gr1, B220, CD19, TER119 conjugated with PeCy5), Sca-

PacBlue, CD34-FITC, CD150-APC, CD48-PE, cKIT-APC-Cy7, CD45.1-PE-TexasRed, CD45.2-PacBlue, Gr1-FITC, Mac1/CD11b-APC, and B220-PECy7. For intracellular staining, cells were fixed with 1.5% paraformaldehyde for 15 minutes at room temperature and permeabilized with ice-cold methanol. Cells were washed 3 times with PBS and incubated with a PE conjugated β-catenin antibody (eBioscience, 12-2567-41) in 2% FBS in PBS for 1 hour at room temperature. Additional antibodies for cell surface proteins were added following the intracellular stain, and incubated in 2% FBS in PBS for 30 minutes at room temperature.

Analysis of WNT signature in MDS cohort

WNT target genes obtained from a comprehensive database (http://web.stanford.edu/group/nusselab/cgi-bin/wnt/target_genes) that were expressed in MDS derived and healthy CD34+ cells were analyzed in a large cohort of gene expression profiles [7]. Average gene expression values of all healthy controls was calculated for all downstream target genes. A composite score for each subject was obtained by tabulating the sum of the differences between the gene expression of each gene and the average value in healthy controls. A higher score was reflective of greater magnitude of target gene response and this score was then correlated with disease subtypes and overall patient survival by Kaplan Meir analysis.

Primary MDS mesenchymal cell isolation and RNA-seq analysis

Patient characteristics are shown in Supplemental Table 2. Control marrow was obtained from donors for allogeneic transplantation (median age: 45 (35-61), after written informed consent. The use of human samples was approved by the Institutional Review Board of Erasmus Medical Center, the Netherlands, in accordance with the

declaration of Helsinki. Mesenchymal cells from human MDS patients were FACS sorted using the FACSAria III systems (BDBioscience) with the following antibodies using optimized dilutions: CD45-PE-Cy7 (1:200), CD235a-BV421-A (1:100), CD271-PE (1:100), CD105-APC (1:50), CD31-APC-CY7 (1:50). Sorted cells were kept in TRIzol (Ambion). Smarter Ultra Low RNA kit for Illumina Sequencing (Clonetech) was used for cDNA synthesis according to the manufacturer's protocol. Sample preparation, sequencing, demultiplexing and alignment were performed as previously described [8] with modifications specific to the application of Smarter kit.

The resulting sequences were aligned to the human Ensemble transcriptome using TopHat [9]. Normalization and quantification was performed using cufflinks [10]. The resulting gene expression values are measured as FPKM (Fragments Per Kilo of transcript per million mapped reads). Differential expression analysis was performed on the fragment counts using the DESeq26 package in the R environment and the False Discovery Rate (FDR) multiple testing correction was applied. The required per-gene fragment counts were measured using the htseq-count program using the strict intersection option. Gene set enrichment analysis (GSEA) was performed on the gene expression values using the curated C2 collection of MSigDB.[4]

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