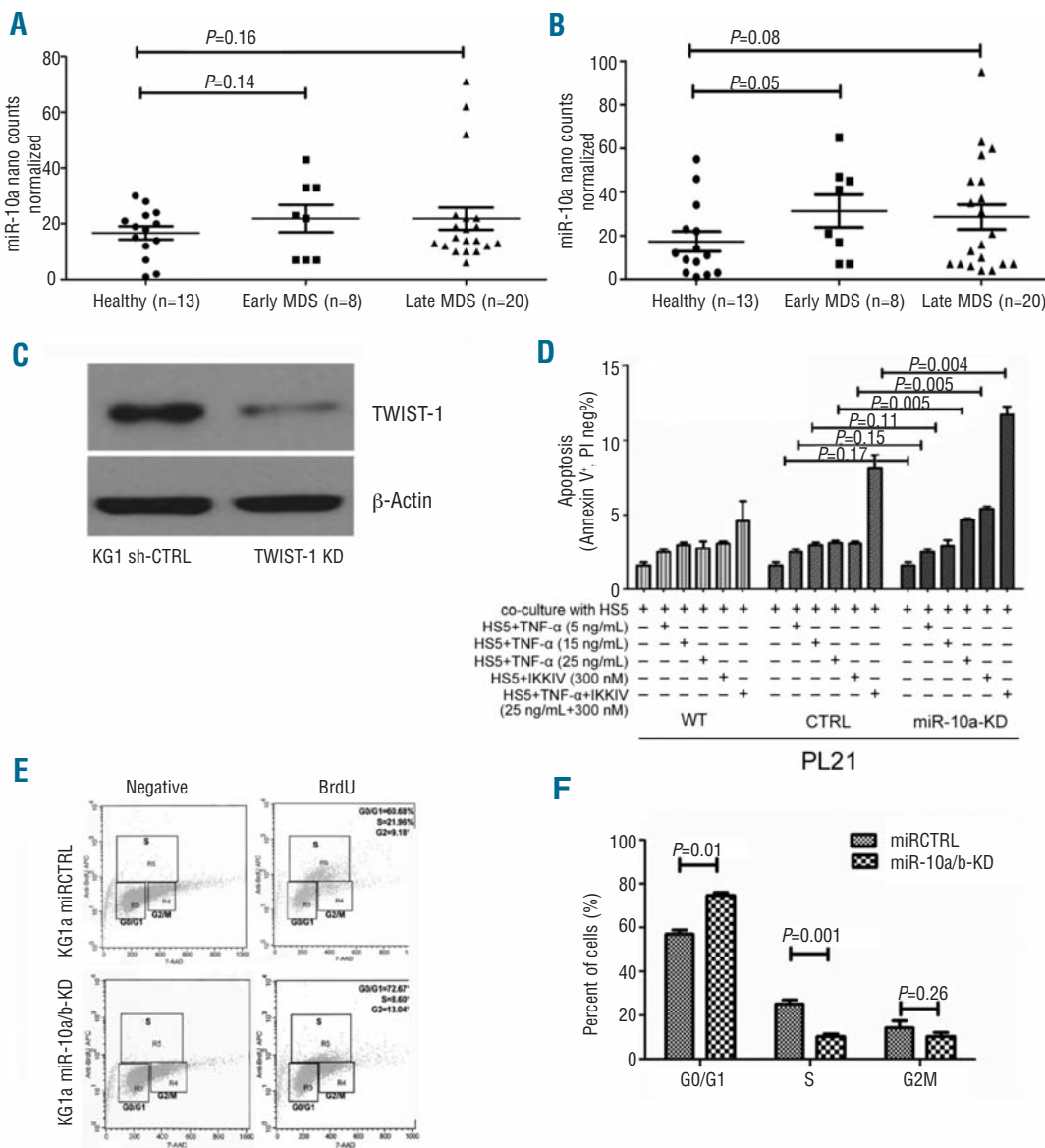


Transcriptional regulation of miR-10a/b by TWIST-1 in myelodysplastic syndromes

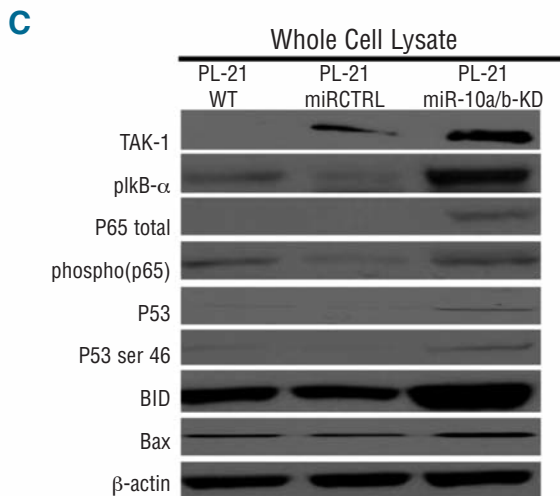
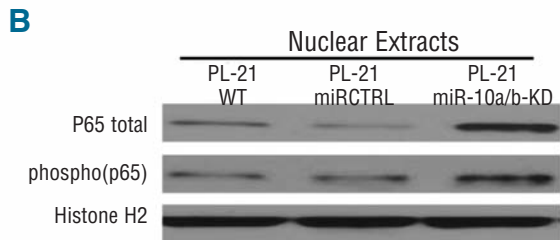
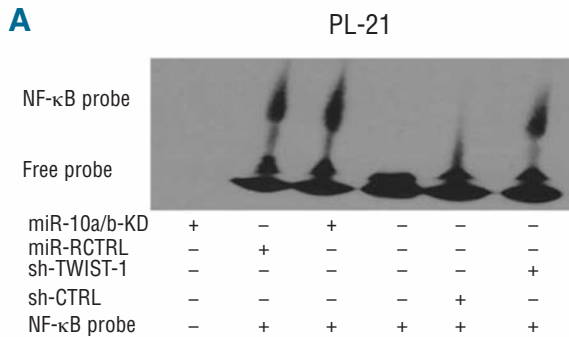
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Online Supplementary Figure S1. TWIST-1 and miR10a/b levels and their effects on apoptosis and proliferation. (A) miR10a and (B) miR10b levels in CD34⁺ marrow cells from healthy donors and patients with MDS, as determined by NanoString array, normalized to 7 internal controls (see *Design and Methods*). Relative expression of miR10a was higher in patients with low grade (P=0.14) and advanced MDS (P=0.16) than in healthy donors, although this was without statistical significance. Relative expression of miR10b was statistically significantly higher in patients with low grade (P=0.05) and showed a strong trend for advanced MDS (P=0.08) in comparison to healthy donors (mean \pm SEM, Student's t-test for comparison of continuous variables) (C) Western blot shows levels of TWIST-1 in KG1a cells with or without knockdown, using a TWIST-1-specific construct (shRNA TWIST-1). ShCTRL indicates a scrambled control construct. (D) Apoptosis rate in PL-21 cells with miR10a/b knockdown (PL-21 miR10a/b KD) in comparison to unmodified cells (wt) and cells transfected with a control vector (PL-21 miRCTRL). Cells were co-cultured with HS5 stroma and treated with TNF α (5-25 ng/mL), IKK inhibitor (300 nM) or both. The rate of early stage apoptosis (Annexin V⁺ PI⁻) is shown. (mean \pm SEM of 3 experiments; Student's t-test for comparison of continuous variables). (E) Effect of miR10a/b knockdown on proliferation (as determined by BrdU uptake) and (F) cell-cycle progression of KG1a cells as determined by BrdU uptake and cell cycle phase (G1/G0, S, G2/M). (mean \pm SEM of 3 experiments; Student's t-test for comparison of continuous variables).



Online Supplementary Figure S2. TWIST-1-dependent miR10a/b expression in PL-21 cells impacts downstream targets of NF-κB. (A) Activation of NF-κB in PL-21 miR10a/b KD and PL-21 TWIST-1-KD cells (shRNA TWIST-1) as shown by Electrophoretic Mobility Shift Assay (EMSA); (B) Nuclear protein lysates from PL-21 wt, miRCTRL and miR10a/b KD cells, separated on 4%-12% Bis-Tris gels and immunoblotted with antibodies against p65 total and phospho-p65. Histone H2 served as loading control. (1 of 2 similar experiments). (C) Protein lysates from PL-21wt, miRCTRL and miR10a/b KD cells, were separated on 4-12% Bis-Tris gels and immunoblotted with antibodies against TAK-1, plkB-α, p65 total, phospho-p65, p53, p53-ser46, BID and Bax, respectively; β-actin served as loading control.