SUPPLEMENTARY DATA

The EMT transcription factor ZEB2 promotes proliferation of primary and metastatic melanoma while suppressing an invasive, mesenchymal-like phenotype

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# Share equal contributions.

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Supplementary Figure Legends

Supplementary Figure S1. Zeb2-wildtype cells outcompete Zeb2-negative cells in terms of expansion and differentiation. (A) Morphological appearance of the mice and skins of Tyr::NRAS^{Q61K} p53$^{fl/fl}$ Zeb2$^{fl/fl}$ mice and their wildtype controls. (B) Hematoxylin-eosin (H&E) staining (left) and immunostaining against Zeb2 (right) on sections isolated from indicated skin regions. For Zeb2 immunostaining, sections were depigmented via peroxide-bleaching.

Supplementary Figure S2. Zeb2 does not accelerate melanoma development on a p53-wildtype background. (A) Induced transgenic ZEB2 expression upon Cre activation. (B) Kaplan-Meier tumour-free survival curves for Tyr::NRAS^{Q61K} Rosa26-Zeb2 mice and their wildtype controls. Tumor-free percentages were compared using the Log-Rank (Mantel-Cox) test.

Supplementary Figure S3. ZEB2 affects the phenotypical switch between proliferation and invasion in mouse and human melanoma cells. (A) Relative proliferation after Zeb2 knockdown in primary melanoma cell lines established from from five different NRAS^{Q61K} Rosa-Zeb2 mice (Mel1 to Mel5) and B16 melanoma cells. (B) Differential expression of melanoma phenotype-specific genes after siZeb2 depletion of B16 cells, determined via q-RT-PCR and normalised to the median of the expression levels and represented in a simplified bar chart. (C-D) Relative invasion and/or migration of B16 melanoma cells after knockdown of Zeb2 or Mitf, or after Zeb1 induction. (E) Top: Schematic diagram of an in vitro extravasation assay. Bottom: Human HUVEC cells are used as endothelial monolayer and challenged with B16 iZeb1 cells. (F) Immunoblot analysis confirming Zeb1 induction or knockdown in Lu1205 cells, below relative invasion measurements in a transwell invasion assay. Data (panel A,C,D,F) are compared by using unpaired Student's t-test and are presented as means± 95%SD. P-values are indicated with ***P<0.005, **P<0.05 and *P<0.01. (G) *Left*: Log2(fold change) expression values of differentially expressed phenotype-specific genes in 501 melanoma cells after ZEB modulation. Differential expression determined via q-RT-PCR and analysed in qBase+, normalized to the median of the expression levels and represented in a heat-map. Pro: Proliferative gene signature, Inv: Invasive gene signature. *Right*: Relative proliferation of ZEB1-induced 501MEL melanoma cells measured via SRB assay, data are represented as mean+SEM and compared using Two-way ANOVA, post-hoc Tukey' HSD. (H) Boyden-Chamber measurements of migration in presence of the proliferation blocker

aphidicolin (1.6µg/ml) of ZEB1-inducible human melanoma cell lines 501MEL and SK-MEL28. Error bars represent SD and means of repeats were compared with a Student's t test. (I) Immunoblot analysis of the respective cell lines 72h after doxycycline administration.

Supplementary Figure S4. ZEB2 affects the phenotypical switch between proliferation and invasion in human melanoma. (A) Immunoblot analysis of ZEB2, MITF and ZEB1 in SK-MEL28 cells transduced with lentiviral vector expressing doxycyclin-inducible shRNA (sh1) directed against ZEB2. Cells in withdrawal condition where treated with doxycyclin for 7 days and subsequently doxycyclin was washed away and ommited for 11 consecutive days. (B) Immunoblot analysis of ZEB2, MITF and ZEB1 in SK-MEL28 cells transduced with a second independent lentiviral vectors expressing a doxycyclin-inducible shRNA directed against ZEB2 (sh2-ZEB2). (C) Left: images of colony formation in doxycyclin-induced SK-MEL28 cells (sh2-ZEB2) and Mel501 cells (sh1-ZEB2 and sh2-ZEB2). Cells were untreated (-DOX) or pre-treated (+DOX) with doxycylin and grown in soft agar in the absence (-DOX) or presence (+DOX) of doxycylin. After 15 days, cell colonies were imaged using an inverted microscope. Right: Relative proliferation after ZEB2 knockdown in the respective melanoma cells measured via SRB assay. Data are represented as mean+SEM and compared using Two-way ANOVA, post-hoc Tukey' HSD. (D) Relative proliferation measured via SRB assay of 501MEL melanoma cells with rescue and complementation settings with RNAi-mediated knockdown and cDNA expression vectors. Equimolar siRNA concentrations were used for all settings. Data were compared by using Student's t-test and presented as averages+SD. P-values are indicated with ***P<0.001, **P<0.01 and *P<0.05. (E) Log2(fold change) mRNA expression for cultures used in panel D.

Supplementary Figure S5. TGF-ß treatment mediates the switch from ZEB2 to ZEB1 and induces an invasive gene signature. Differential gene expression of phenotype-specific genes is determined via q-RT-PCR, normalized to the timepoint zero. Data were compared by using Student's t-test and presented as averages+SD. P-values are indicated with ***P<0.001, **P<0.01 and *P<0.05.