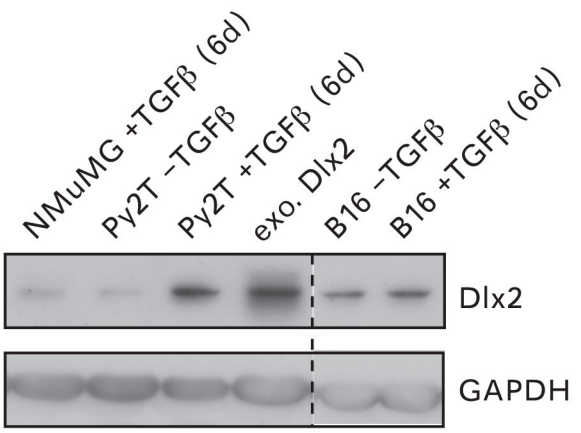
Yilmaz et al.,
Supplemental Figure S1

A



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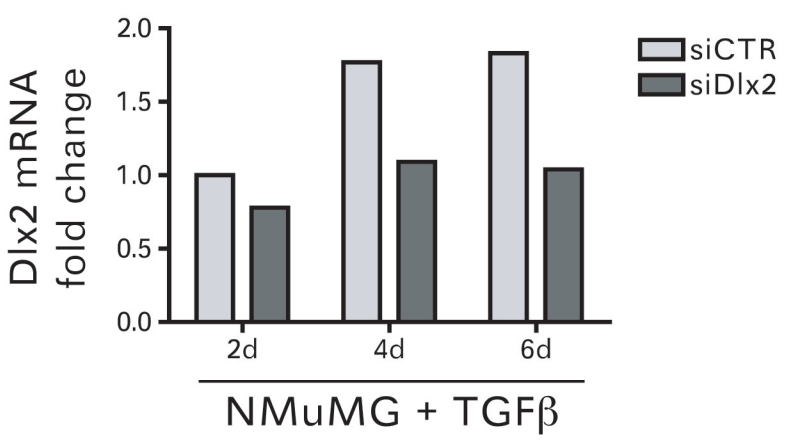

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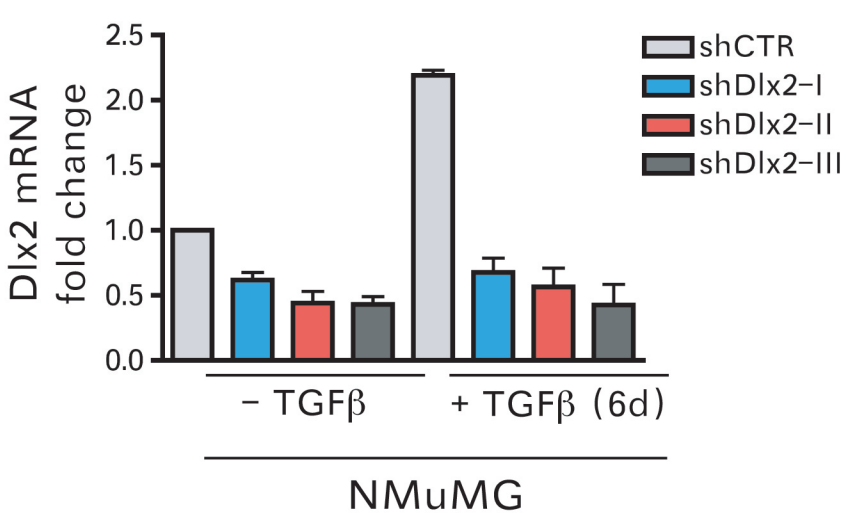
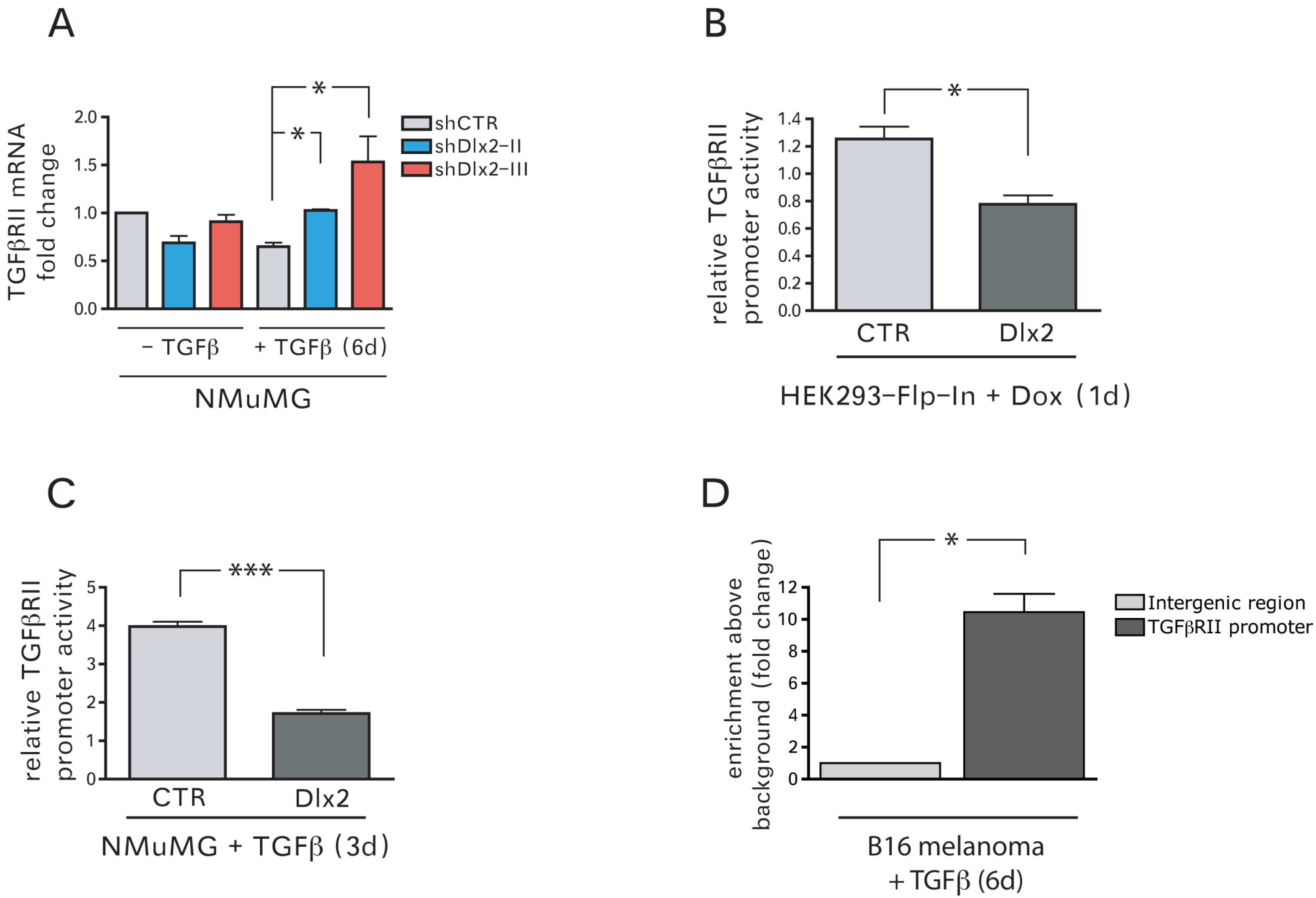
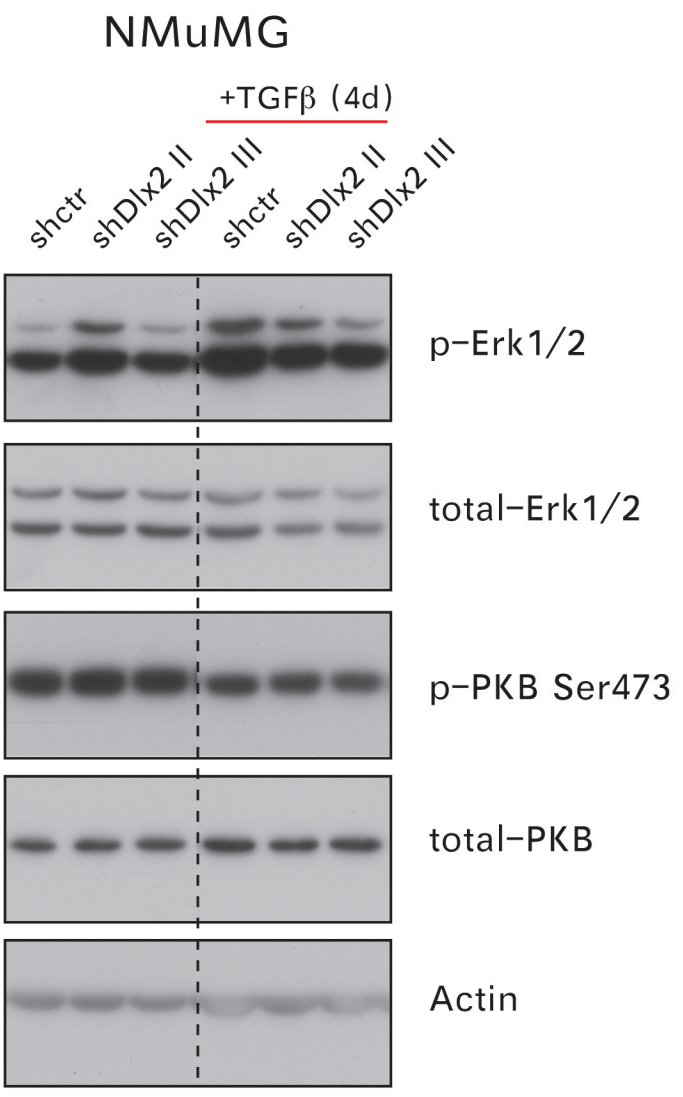


Yilmaz et al.,
Supplemental Figure S2



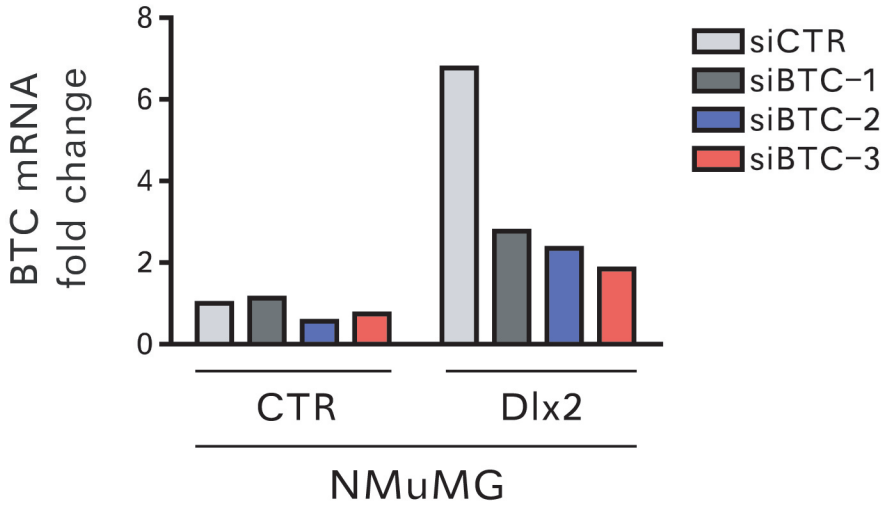
Yilmaz et al.,
Supplemental Figure S3

A



Yilmaz et al.,
Supplemental Figure S4

A



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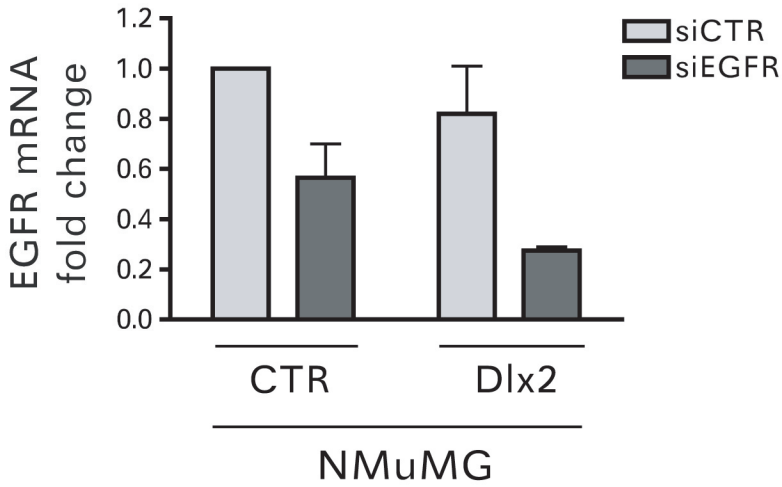

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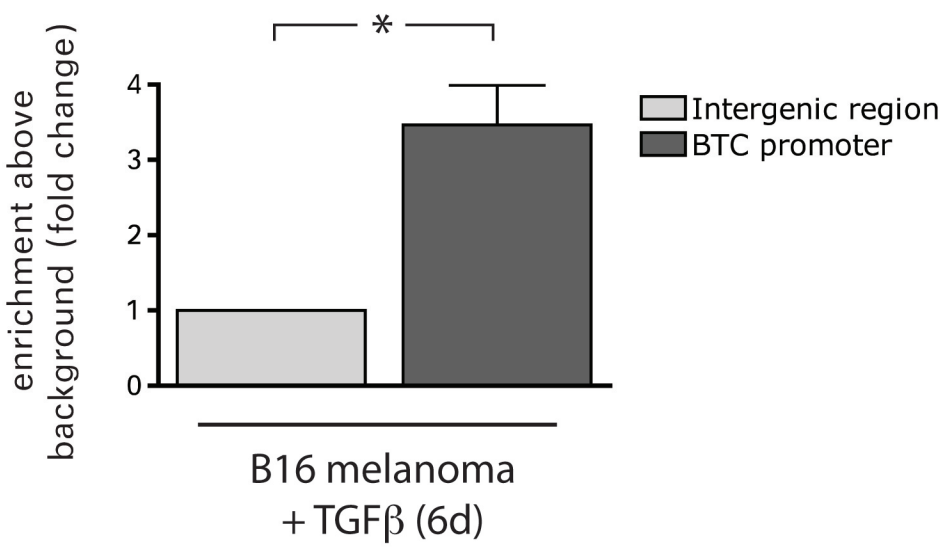
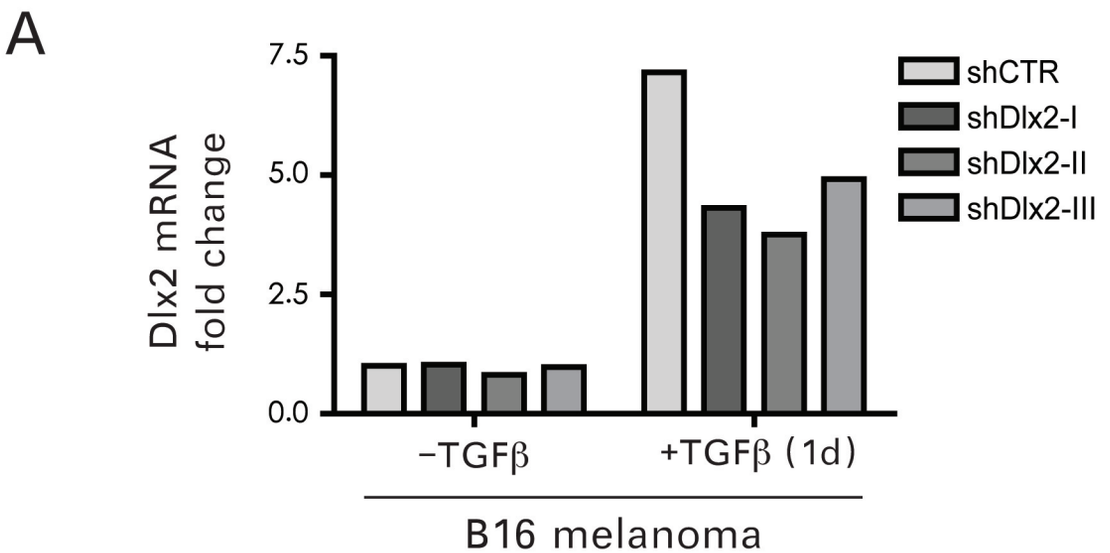
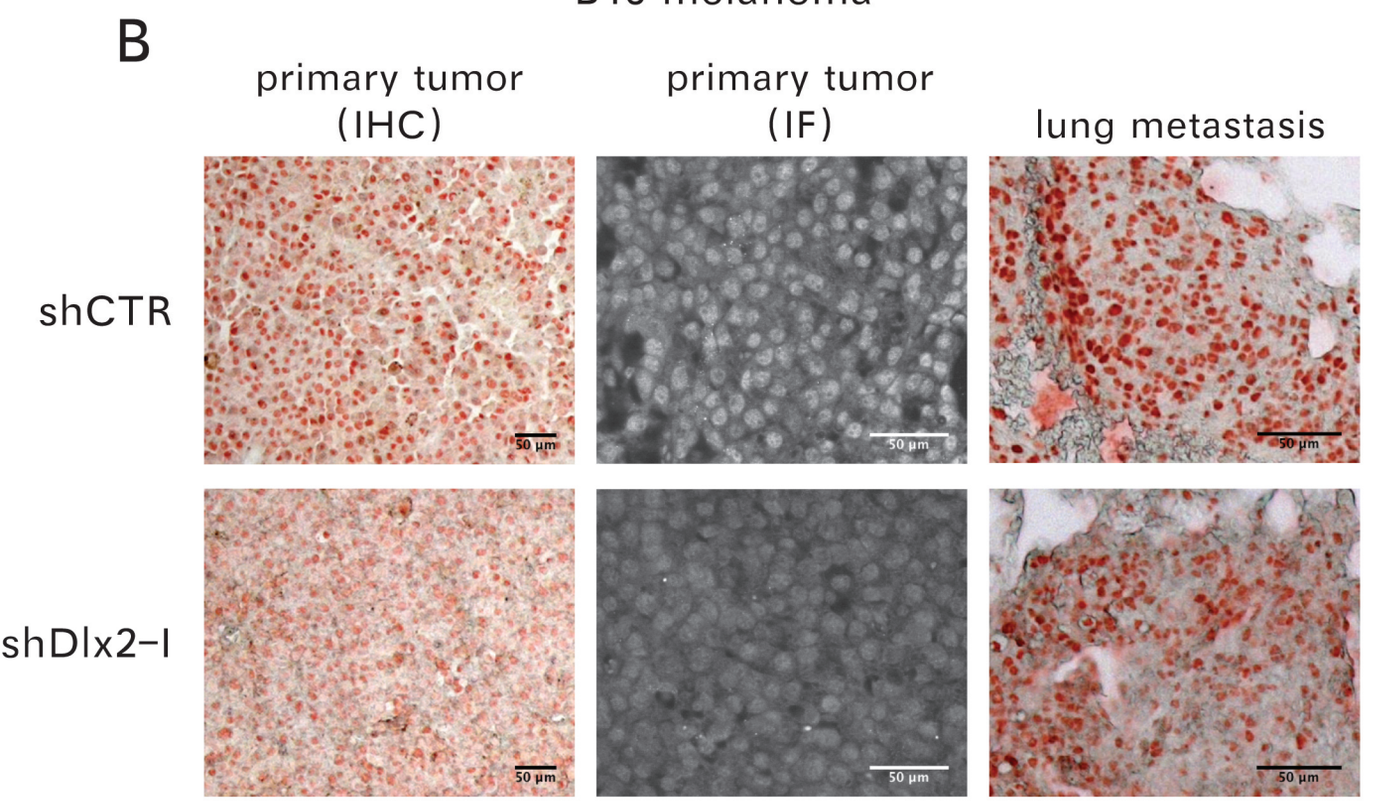
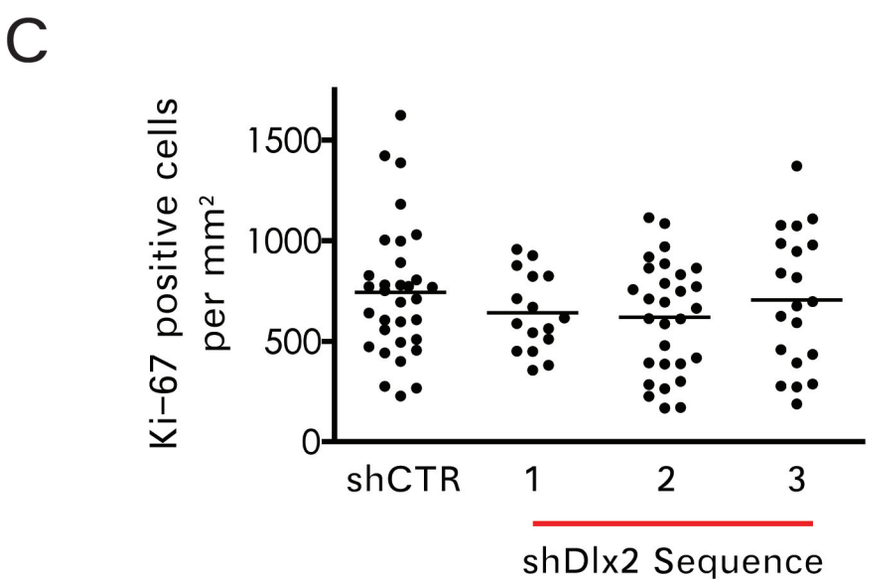


Yilmaz et al.,
Supplemental Figure S5







SUPPLEMENTAL INFORMATION

Supplemental Figure Legends

Supplemental Figure S1. TGFβ induces Dlx2 expression in several cellular systems.
(A) NMuMG, Py2T and B16 cells were treated for 6 days with TGFβ. Dlx2 protein levels compared to untreated and Dlx2-overexpressing cells were determined by immunoblotting analysis.
(B) Transient transfection of NMuMG cells with siRNA against Dlx2 (siDlx2) or control siRNA (siCTR) during TGFβ treatment for the days indicated. Expression of Dlx2 mRNA was determined by quantitative RT-PCR and presented as fold changes compared to control (siCTR).
(C) Stable lentiviral expression of three independent shRNAs against Dlx2 (shDLx2-I, II and III) and one control shRNA (shCTR) in NMUMG cells. Expression of Dlx2 mRNA was determined by quantitative RT-PCR and presented as fold changes compared to control (shCTR).

Supplemental Figure S2. Dlx2 regulates *TGFβRII* gene expression.
(A) Quantitative RT-PCR analysis of TGFβRII mRNA expression in shDlx2 cells and shCTR cells treated with TGFβ for 0 or 6 days. TGFβ treatment provokes an increase in TGFβRII mRNA expression in Dlx2-depleted cells as compared to shCTR cells.
(B) Doxycyclin-inducible expression of Dlx2 in HEK293 cells represses TGFβRII promoter activity. Doxycycline-inducible Flp-In T-Rex HEK293 cells expressing either GFP or N-terminal HA-tagged murine Dlx2 were transfected with a *firefly* luciferase-reporter plasmid containing 255 bp of the TGFβRII promoter sequence and treated for 1 day with doxycycline. Luciferase activity values were normalized to co-transfected *Renilla* luciferase activities.
(C) Dlx2-expressing NMuMG cells show a reduced TGFβRII promoter-reporter activity compared to control cells when treated for 3 days with TGFβ. Luciferase activities were quantified as described in panel B.
(D) Dlx2 binds directly to the *TGFβRII* gene promoter in B16 melanoma cells. Chromatin immunoprecipitation of Dlx2 was performed on B16 melanoma cells treated with TGFβ for 6 days. Immunoprecipitated DNA fragments were quantified

by quantitative PCR using primers covering basepairs -386 to -204 of the *TGFβRII* promoter region and primers covering an intergenic region as negative control.
Data are shown as mean ± SD and are representative of at least two independent experiments. Statistical values are calculated by using an unpaired, two-tailed t-test. *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

Supplemental Figure S3. Knockdown of Dlx2 expression does not inhibit Erk1/2 and PKB phosphorylation. Immunoblotting analysis of phosphorylated Erk1/2 and PKB comparing shControl and shDlx2 cells. Immunoblotting for actin was used as a loading control.

Supplemental Figure S4. Quantification of siRNA-mediated ablation of **(A)** betacellulin (BTC) and **(B)** EGFR expression in NMuMG cells transfected to express Dlx2 or transfected with a control vector (CTR). NMuMG cells were transfected with siRNA against betacellulin (siBTC-1 to 3), EGFR (siEGFR) or control siRNA (siCTR), and the mRNA levels of betacellulin and EGFR were determined by quantitative RT-PCR. Results are presented as fold changes compared to control (siCTR).
(C) Dlx2 binds directly to the betacellulin promoter in B16 melanoma cells. Chromatin immunoprecipitation of Dlx2 was performed on B16 melanoma cells treated for 6 days with TGFβ. Immunoprecipitated DNA fragments were quantified by quantitative RT-PCR using primers amplifying the promoter region of the *betacellulin* gene and primers covering an intergenic region as negative control.
Data are shown as mean ± SD and are representative of at least two independent experiments. Statistical values are calculated by using an unpaired, two-tailed t-test. *, $p \leq 0.05$.

Supplemental Figure S5. Dlx2 expression in B16 melanoma cells, tumors and metastases.
(A) Dlx2 expression is induced by TGFβ in B16 melanoma cells and reduced in expression by stable lentiviral expression of three independent shRNAs against Dlx2 (shDlx2-I, II, III) but not in cells expressing a control shRNA (shRNA). Dlx2 mRNA levels were determined by quantitative RT-PCR and are presented as fold changes

compared to control (shCTR, no TGFβ).

(B) Histological sections of primary tumors and lung metastasis, formed after subcutaneous injection of shCTR or shDlx2 (shDlx2-I) B16 melanoma cells, were stained for Dlx2, and Dlx2 was visualized by immunofluorescence or immunohistochemical stainings as indicated. Representative microphotographs of shControl and shDlx2-I samples are displayed. Scale bar = 50 μm

(C) Primary tumors formed by B16 melanoma cells either expressing shCTR or shDlx2 were analyzed for proliferation by counting Ki67-positive cells per mm^2.

Supplemental Material and Methods

Cell lines

The murine breast cancer cell line Py2T was established from a primary breast tumor of a MMTV-PyMT transgenic mouse. Py2T cells exhibit epithelial morphology under normal growth conditions. Upon treatment with TGFβ, these cells undergo full EMT within 10 days (Waldmeier et al., unpublished results).