Supplemental Information

Supplementary Materials and Methods

Transwell assay

Exponentially growing cells were seeded in transwell chambers with 8 µm pore size (BD Biosciences, Franklin Lakes, NJ, USA) at a density of $4x10^4$ per well in medium with 5% FCS and treated for 72 h with FGF2 or FGF5 (20 ng/ml) or a combination of FGF2 and FGF5 (100 ng/ml each). Cells at the bottom of the lower chamber were fixed in methanol and stained with crystal violet. Staining was quantified with Lucia software (Nikon). Assays were performed in duplicates and repeated three times.

Growth curve

Melanoma cells were seeded in 6-well plates at a density of $1x10^5$ cells per well in medium with 10% FCS and 24 h later transduced with adenoviruses expressing dnFGFR1, dnFGFR3, dnFGFR4 or GFP (used as control). Cell number was determined 2 and 5 days after transduction with a Casy cell counter (Roche Innovatis AG, Bielefeld, Germany). Triplicate counts of three independent experiments were performed.

Anchorage independent growth

Melanoma cells were transduced with dnFGFR1 or GFP and seeded in soft agar (1% agar in growth medium with 10% FCS) at a density of $3x10^4$ cells per well of a 6-well plate. Number of clones was determined microscopically after 2 weeks. Experiments were performed in duplicates and repeated three times.

Western blot analysis

For analysis of the activity of the dn receptor construct, cells were seeded into 6-well plates at a density of $5x10^4$ cells/well. After 24 hours they were transduced with dnFGFR1 or GFP adenovirus and cultured for 5 days. For analysis of the effects of PD166866 and sorafenib, cells were grown to 80% confluence in six-well plates and treated with the inhibitors for 6 h in medium with 5% FCS. Cells were harvested in a buffer containing 50 mM Hepes pH 7.5, 150 mM NaCl, 10% glycerol, 1 mM EDTA, 10 mM NaF, 0.5 mM Na_3VO_4, 1% Triton X 100, 1.5 mM $MgCl_2$, and protease inhibitors (Complete, Roche). Proteins were separated by polyacrylamide gel electrophoresis and blotted onto PVDF membranes (GE Healthcare). Membranes were incubated with primary antibodies at 4°C overnight. HRP-conjugated secondary antibodies (Dako, Denmark) and Western C reagent (Biorad) were used for blot development. For a list of antibodies and dilutions see Table S4.

Flow cytometry

For analysis of cell cycle distribution, cells were grown to 80% confluence in T25 flasks and treated with PD166866 and sorafenib for 18 h in medium with 5% FCS. Cells were fixed in ethanol, treated with 50 µg/ml RNAse A, stained with 50 µg/ml propidium iodide (PI) and analysed on a FACSCalibur flow cytometer with Modfit LT software (Verity Software House).

Apoptosis assay

Cells were seeded into 24-well plates at a density of $5x10^4$ cells/well and treated with PD166866 and sorafenib for 48 h in medium with 5% FCS. Cells were double stained with Hoechst 333258 (5 µg/ml) and PI (2 µg/ml) and percentages of viable and apoptotic cells were determined microscopically.

Supplementary Table S1: FGF transcript expression in melanoma cell lines and normal melanocytes (NM)

	NM	Cell Lines (%)		NM	Cell Lines (%)
FGF1	**+**	**83**	**FGF12**	**-**	**75**
FGF2	**+**	**92**	**FGF13**	**+**	**100**
FGF3	**-**	**0**	**FGF14**	**+**	**58**
FGF4	**-**	**0**	**FGF16**	**+**	**58**
FGF5	**-**	**75**	**FGF17**	**-**	**0**
FGF6	**-**	**0**	**FGF18**	**+**	**100**
FGF7	**-**	**0**	**FGF19**	**-**	**0**
FGF8	**-**	**25**	**FGF20**	**-**	**25**
FGF9	**-**	**0**	**FGF21**	**+**	**42**
FGF10	**-**	**0**	**FGF22**	**+**	**67**
FGF11	**+**	**100**	**FGF23**	**-**	**50**

Supplementary Table S2: Nucleotide sequences of the primer pairs used for detection of FGFR variants

Target	Primer Sequence
FGFR1 IIIb	5-TCCAGTGGCTAAAGCACATC-3 5-CCGCATCCGAGCTATTAATC-3
FGFR1 IIIc	5-GACAAAGAGATGGAGGTGCT-3 5-GTTGTAGCAGTATTCCAGCC-3
FGFR2 IIIb	5-AACGGGAAGGAGTTTAAGCAG-3 5-CTCGGTCACATTGAACAGAG-3
FGFR2 IIIc	5-AACGGGAAGGAGTTTAAGCAG-3 5-TGGCAGAACTGTCAACCATGC-3
FGFR3 IIIb	5-AACGGCAGGGAGTTCCGCGGC-3 5-CCCGTCCCGCTCCGACACATTG-3
FGFR3 IIIc	5-AACGGCAGGGAGTTCCGCGGC-3 5-CCCGGCGTCCTCAAAGGTG-3
FGFR4	5-TGCCTGGGCCTCCAGTCTTG-3 5-CTGCAGCTGGACAGCGGAAC-3
GAPDH	5-CTGGCGTCTTCACCACCAT-3 5-CTGGCGTCTTCACCACCAT-3

Supplementary Table S3: Taqman assays used for qRT-PCR. All assays were obtained from Applied Biosystems.

Target	Assay
FGFR1	Hs00915135 m1
FGFR2	Hs01552926 m1
FGFR3	Hs00179829 m1 Hs00997397 m1
FGFR4	Hs00608751 g1 Hs00242558 m1 Hs00608744 g1
FGF2	Hs00960934 m1
FGF5	Hs00170454 m1 Hs00738132 m1
FGF18	Hs00826077 m1
Beta 2 microglobulin	Hs99999907 m1
18S RNA	Hs99999901 s1

Supplementary Table S4: Primary antibodies used for immunoblotting.

Target	Type	Supplier	Number	Dilution
Caspase 3	Rabbit polyclonal	Cell Signaling Technology	#9662	1:1000
Cleaved PARP	Rabbit polyclonal	Cell Signaling Technology	#9541	1:1000
Beta actin	Mouse monoclonal	Sigma	Clone AC-15	1:5000
pAkt (Ser 473)	Rabbit polyclonal	Cell Signaling Technology	#9271	1:1000
Akt	Rabbit polyclonal	Cell Signaling Technology	#9272	1:1000
pERK1/2	Rabbit polyclonal	Cell Signaling Technology	#9101	1:1000
ERK1/2	Rabbit polyclonal	Cell Signaling Technology	#9102	1:1000
pStat3 (Ser727)	Rabbit polyclonal	Cell Signaling Technology	#9134	1:1000
Stat3	Rabbit polyclonal	Cell Signaling Technology	#9132	1:1000

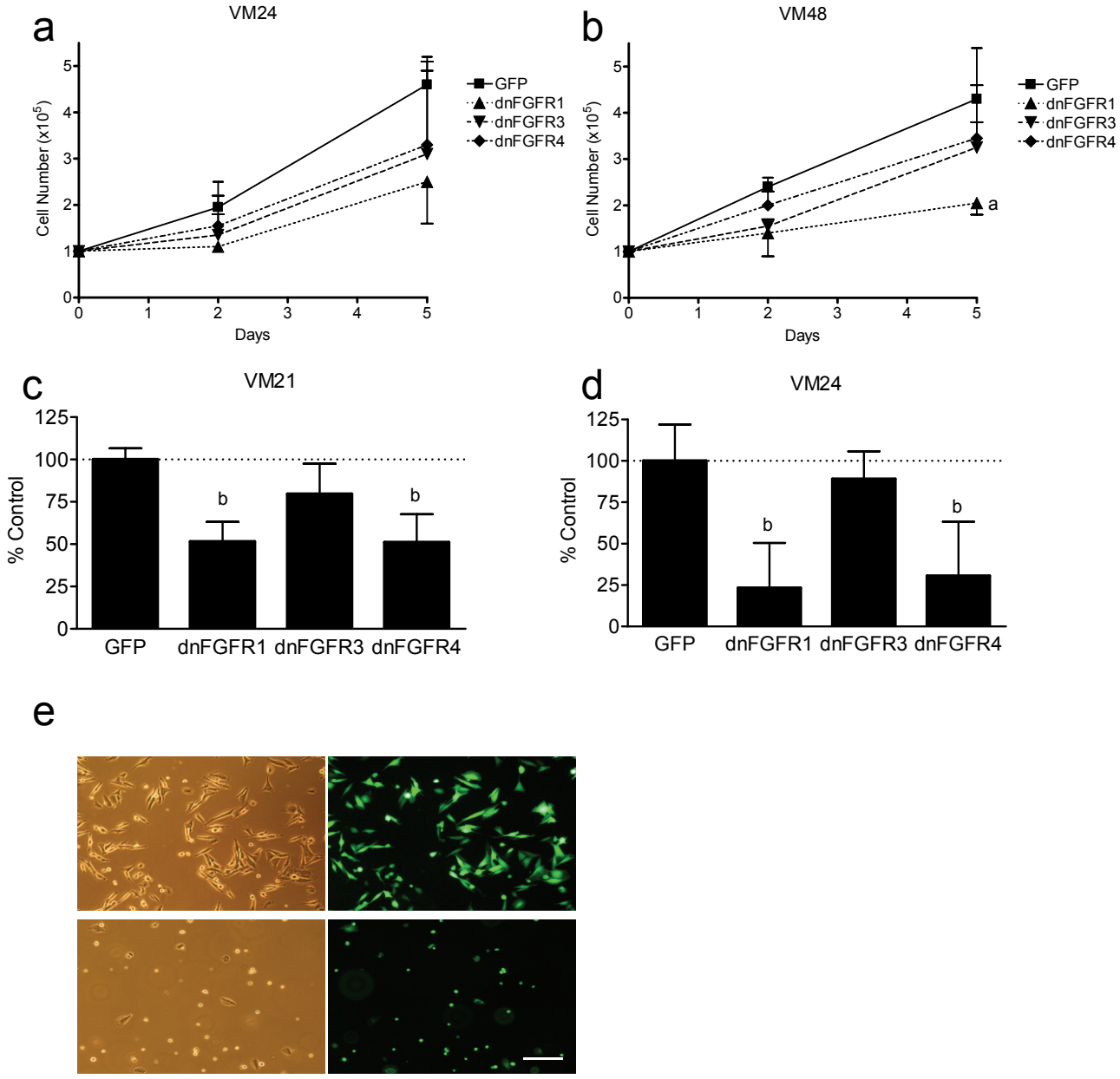

Figure S1: DnFGFR expression impairs melanoma cell growth and induces cell death. (**a**, **b**) VM24 and VM48 cells were transduced with dnFGFR1, dnFGFR3, dnFGFR4, or GFP adenovirus. Cell number was determined after 2 and 5 days. (**c, d**) For clonogenic assays VM21 and VM28 cells were transduced as above and the number of colonies was determined after 14 days. a, $p < 0.05$; b, $p < 0.01$ versus control (GFP). (**e**) VM1 cells were seeded in 6-well plates and transduced with GFP (upper panels) or dnFGFR1 (lower panels). Cells were reseeded and photographed after 5 days with phase contrast (left panels) and GFP (right panels) filter settings. Scale bar represents 50 µm.