Supplement

Loss of tumorigenic potential upon transdifferentiation from keratinocytic into melanocytic lineage

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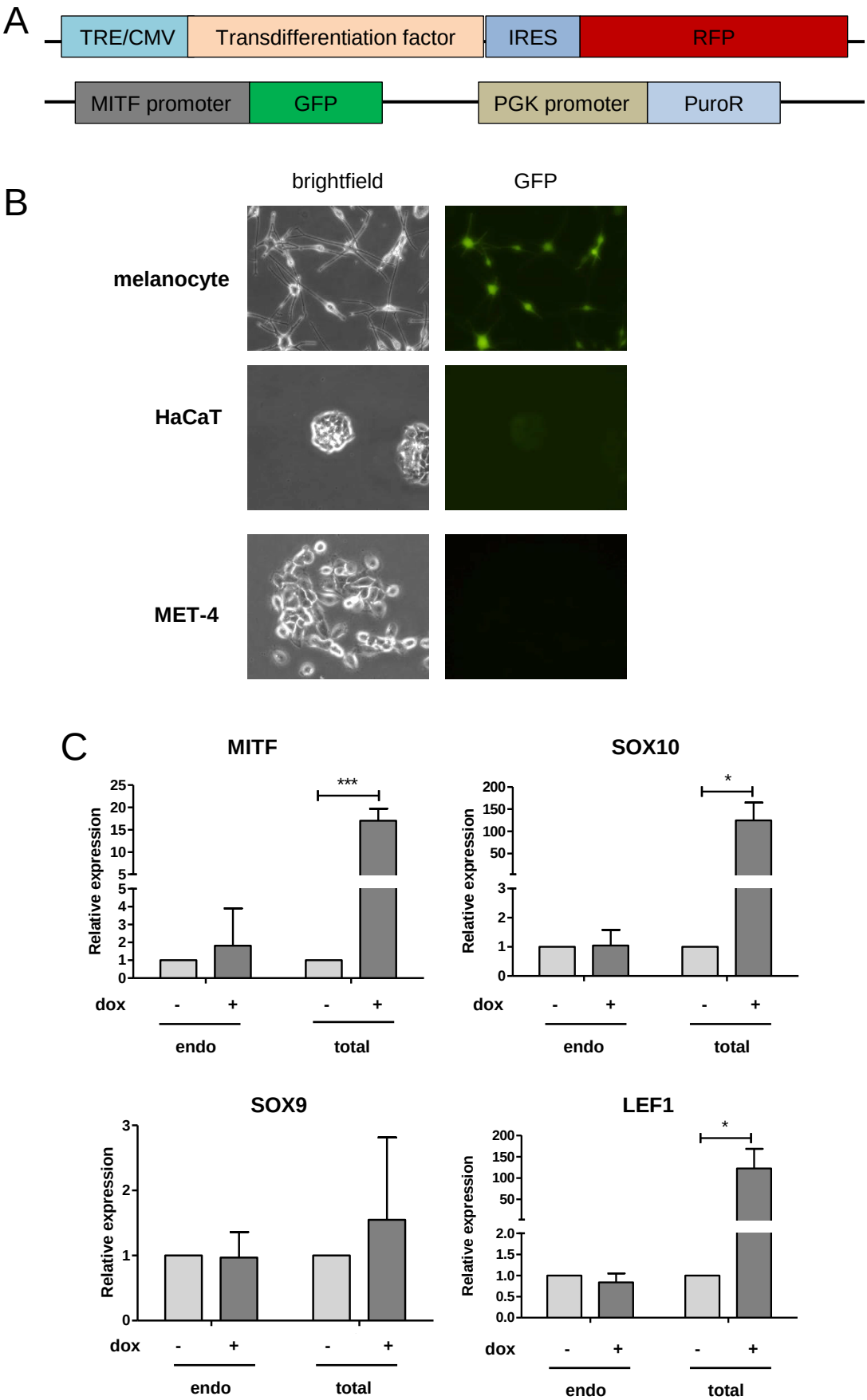
Supplementary Figure 1: A) Scheme of lentiviral vector constructs. Vectors carrying the transdifferentiation factors also harbor RFP to visualize transgene expression. The MITF promoter was linked to GFP to make endogenous MITF expression visible with this reporter construct. B) Validation of the functionality of the melanocyte-specific reporter construct. 12 days after infection with the reporter construct reporter activity was observed in melanocytes as indicated by strong GFP signal. No activity of the melanocyte-specific reporter construct was detectable in HaCaT cells and MET-4 cells 12 days after infection. C) Endogenous (endo) and total expression of the four transcription factors MITF, SOX10, SOX9 and LEF1 with and without induction with doxycycline in MET-4 cells. Error bars depict the SD of three independent experiments. P values were calculated by two-tailed, paired sample t-test. Asterisks indicate t-test p-value <0.05 (** p-value <0.01, *** p-value <0.005).

Supplementary Figure 2: A) MITF alone is not sufficient to induce neither MITF reporter activity nor morphological changes in MET-4 cells. MET-4 cells could not be cultured in the melanocyte medium MCDB. These cells died within a week in the MCDB medium. B) Strong RPF signal was observed in MET-4 cells after transfection with the transdifferentiation factors MITF, LEF-1, SOX9 and SOX10 and induction with doxycycline. After doxycycline withdrawal RFP signal was absent indicating a complete shutdown of transgene expression. However, the cells retained their changed morphology.

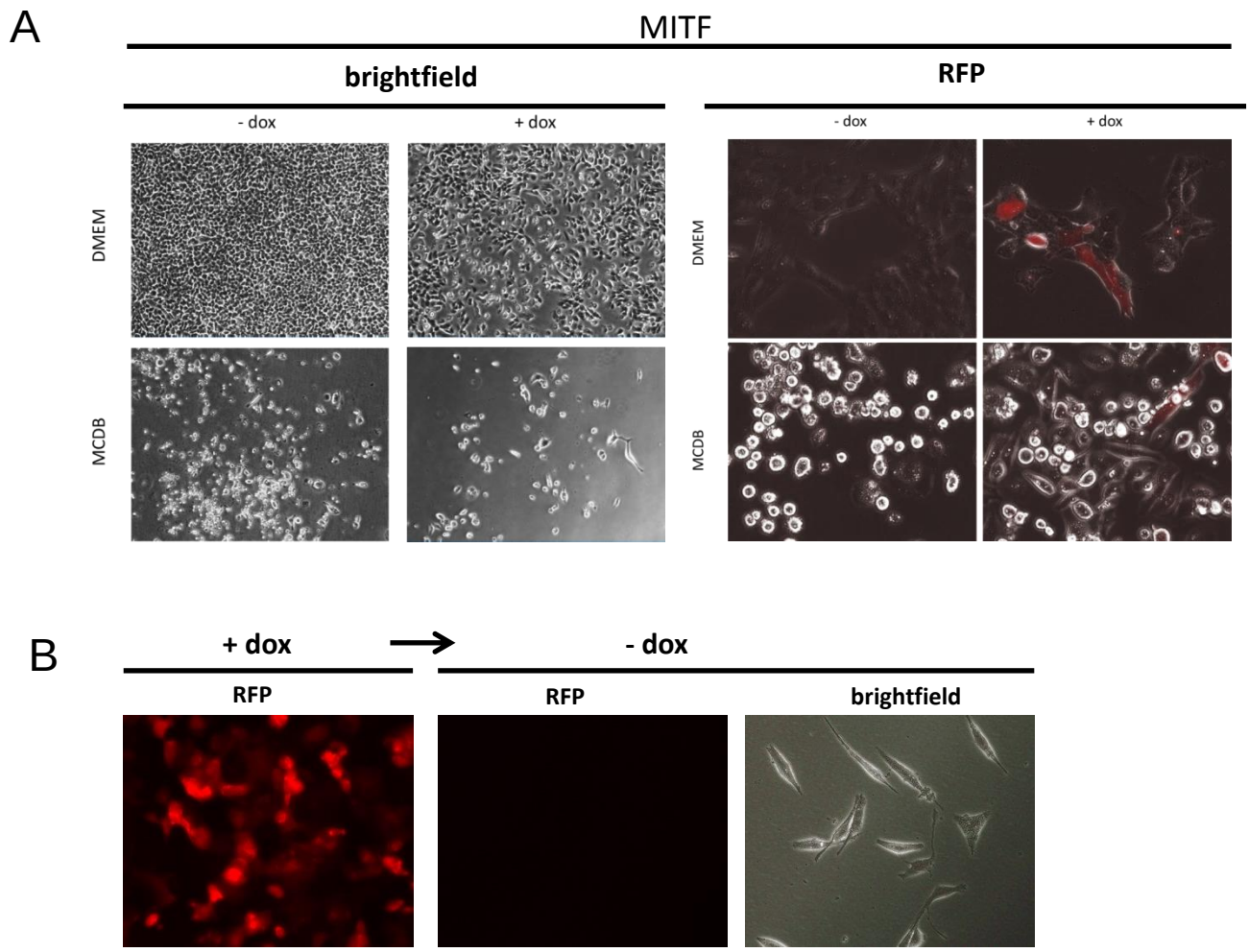
Supplementary Figure 3: Overview of senescence marker expression in MET-4 and MT-MET-4 (TD) cells

Supplementary Figure 4: IL-24 mRNA expression in MET-4 cells and MT-MET-4 cells cultured with and without TPA.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3

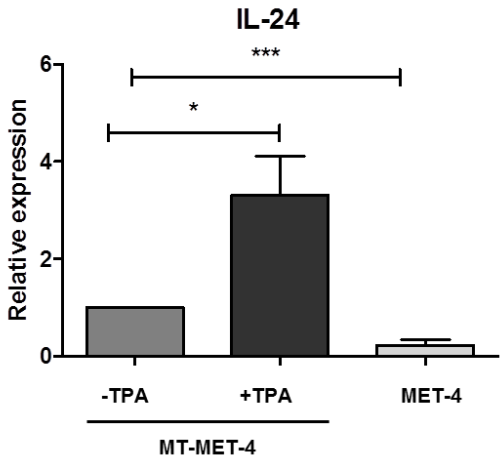
A

	-1<FC<+1	MET4	TD
cellular senescence-associated genes (GO:0090398)	ABL1	9,31	9,46
	ARNTL	6,75	6,96
	BCL2L12	7,16	7,15
	BCL6	6,54	6,54
	BMPR1A	6,71	6,54
	C2orf40	6,47	6,48
	CALR	11,24	11,09
	CAV1	13,00	12,94
	CDK6	10,91	10,63
	CDKN1A	6,53	6,58
	CDKN2A	6,53	6,69
	H2AFX	9,92	10,19
	HMGA1	6,74	6,87
	HMGA2	6,76	7,15
	HRAS	9,79	9,18
	ING2	9,08	9,35
	KAT6A	8,15	8,29
	MAGEA2	6,44	6,41
	MAP2K1	10,44	9,63
	MAPK14	6,89	7,44
	MAPKAPK5	9,07	9,82
	NEK4	6,69	6,82
	NEK6	9,15	8,63
	NSMCE2	9,49	9,09
	NUAK1	10,24	9,78
	OPA1	8,28	8,74
	PLA2R1	6,91	6,51
	PML	7,03	6,77
	PNPT1	7,37	7,90
	PRKCD	9,20	8,40
	PRKDC	9,61	10,64
	PRMT6	8,46	8,96
	RSL1D1	10,34	11,12
	SIRT1	7,87	7,47
	SMC5	6,57	6,48
	SMC6	7,03	7,27
	SRF	10,37	10,16
	TBX2	6,45	6,69
	TBX3	6,59	6,66
	TERF2	7,91	8,08
	TP53	7,79	7,96
	TWIST1	9,40	10,32
	ULK3	6,82	6,96
	VASH1	6,82	6,51
	WNT16	6,68	6,60
	ZKSCAN3	7,24	7,41
	ZNF277	7,62	7,77

B

	-1<FC<+1	MET4	TD
SASP-associated genes	CCL13	6,61	6,84
	CCL2	6,49	6,54
	CCL27	6,63	6,59
	CCL3	6,62	6,60
	CCL8	6,67	6,61
	CSF3	6,61	6,96
	CXCL12	6,46	6,62
	CXCL13	6,52	6,53
	CXCL2	9,31	10,23
	IFNG	6,41	6,44
	IGFBP7	12,06	11,78
	IL13	6,42	6,45
	IL1B	10,31	10,57
	IL7	6,55	6,52
	IL8	8,37	8,51

Supplementary Figure 4



Suppl. Table 1: Candidate transcription factors used for transdifferentiation from keratinocytic into melanocytic lineage

Candidate transcription factors for transdifferentiation			
MITF-M	NM_000248.3	cMET	XM_011516223.1
SOX2	NM_003106.3	TFAP2A	NM_003220.2
SOX5	XM_011520846.1	NFIX	NM_002501.3
SOX9	NM_000346.3	IRF4	NM_002460.3
SOX10	NM_006941.3	ETS1	NM_005238.3
LEF1	NM_016269.4	FOSB	NM_006732.2
β-catenin	XM_005264886.2	HAND1	NM_004821.2
PAX3A	NM_000438.5	HES1	NM_005524.3
PAX3B	NM_013942.4	HOXB7	NM_004502.3
PAX3C	NM_181457.3	IFI16	NM_005531.2
PAX3D	NM_181458.3	KLF9	NM_001206.2
PAX3I	NM_001127366.2	PITX1	NM_002653.4
SNAI2	NM_003068.4		

Suppl. Table 2: Q-PCR primers

Q-PCR Primer	Forward primer	Reverse primer
MITF	GCTCACAGCGTGTATTTTTCC	GCTCACAGCGTGTATTTTTCC
DCT	GGTTCCTTTCTTCCCTCCAG	GGTTCCTTTCTTCCCTCCAG
TRP1	AGCAGTAGTTGGCGCTTTGT	AGCAGTAGTTGGCGCTTTGT
TYR	TTGTACTGCCTGCTGTGGAG	TTGTACTGCCTGCTGTGGAG
K10	GCTGACCTGGAGATGCAAAT	GCTGACCTGGAGATGCAAAT
K14	AGGAGGTCACATCTCTGGATGACTG	AGGAGGTCACATCTCTGGATGACTG
Integrin beta 4	CTGTACCCGTATTGCGACT	CTGTACCCGTATTGCGACT
Integrin alpha 6	GCTGGTTATAATCCTTCAATATCAATTGT	GCTGGTTATAATCCTTCAATATCAATTGT
Loricrin	GGAGTTGGAGGTGTTTTCCA	GGAGTTGGAGGTGTTTTCCA
Involucrin	CTCCATGTGTCATGGGATATG	CTCCATGTGTCATGGGATATG
IL-24	GACTTTAGCCAGCAGACCCTT	GGTTGCAGTTGTGACACGAT
18S	GAGGATGAGGTGGAACGTGT	GAGGATGAGGTGGAACGTGT
MITF endo	ACCGTCTCTCACTGGATTGG	CGTTGGGCTTGCTGTATGTG
MITF total	TGCCTGTCTCGGGAAACTTG	CCAGTGCTCTTGCTTCAGAC
SOX9 endo	AGACCTTTGGGCTGCCTTAT	TAGCCTCCCTCACTCCAAGA
SOX9 total	CTGAGCAGCGACGTCATCTC	GTTGGGCGGCAGGTACTG
SOX10 endo	GGCTTTCTGTCTGGCTCACT	TAGAGGGTCATTCCTGGGGG
SOX10 total	AGCCCAGGTGAAGACAGAGA	ATAGGGTCCTGAGGGCTGAT
LEF1 endo	GCATCAGGTACAGGTCCAAGA	TCTCCAGAAGAGGTCCTGGG
LEF1 total	AGAGCGAATGTCGTTGCTGA	TCTTGGACCTGTACCTGATGC