Supplementary Online Material for "Mutations in ribosomal proteins cause p53-mediated dark skin and pleiotropic effects"

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(Supplementary Tables 1 – 3, Supplementary Figures 1 – 5)

Supplementary Table 1. Differentially expressed genes in *Rps6*$^{lox/+}$*;Tg.K5Cre*/+ epidermis[a].

gene name	description	fold change	p-value
Ednrb	**endothelin receptor type B**	**11.9**	**0.05**
Si	**silver**	**11.4**	**0.05**
Ptgds	prostaglandin D2 synthase	9.99	0.05
Ddit4l	DNA-damage-inducible transcript 4-like	6.1	0.03
Mgmt	O-6-methylguanine-DNA methyltransferase	5.8	0.05
Syt4	synaptotagmin 4	4.7	0.03
Ptp4a3	protein tyrosine phosphatase 4a3	4.3	0.02
Ephx1	epoxide hydrolase 1, microsomal	3.9	0.05
Sox10	**SRY-box containing gene 10**	**3.6**	**0.05**
Trpm1	transient receptor potential cation channel M1	3.1	0.05
Aaas	achalasia, adrenocortical insufficiency, alacrimia	2.5	0.05
D630023F18Rik	RIKEN cDNA D630023F18	2.4	0.04
Gpnmb	glycoprotein, transmembrane nmb	2.3	0.05
Txnl2	thioredoxin-like 2	1.9	0.05
Pcolce2	procollagen C-endopeptidase enhancer 2	1.8	0.05
Matp	**membrane associated transporter protein**	**1.6**	**0.05**
Mdm2	transformed mouse 3T3 cell double minute 2	1.5	0.05
Nnt	nicotinamide nucleotide transhydrogenase	1.3	0.05
Cyp4f16	cytochrome P450, family 4f16	1.2	0.05
Zdhhc3	zinc finger, DHHC domain containing 3	1.2	0.03
5330417C22Rik	RIKEN cDNA 5330417C22	0.90	0.05
Fbxo38		0.86	0.05
Supt4h	suppressor of Ty 4 homolog	0.85	0.05
1500031N24Rik	RIKEN cDNA 1500031N24	0.76	0.05
Dido1		0.69	0.05

[a]Based on comparison of *Rps6*$^{lox/+}$*;Tg.K5Cre* to +/+ animals as described in text and in Figure 4e. The mean fold-change (n=3 for each of 2 hybridizations) is listed for genes whose false discovery-corrected p values are ≤0.05. Melanocyte-specific genes

upregulated due to melanocytosis are indicated in bold; genes known to be induced by p53 are indicated in red.

Supplementary Table 2. Effect of *Rps19*^{Dsk3} on body weight[a]

Time	P0	P3	P21
+/+	1.50 +/- 0.03 (21)	2.47 +/- 0.05 (42)	11.26 +/- 0.23 (10, 2:8)
Rps19^{Dsk3}/+	1.24 +/- 0.04 (11)	2.05 +/- 0.05 (43)	9.54 +/- 0.18 (13, 7:6)

[a]Weight in grams, +/- sem (No. of animals, with male:female at P21) of C3HeB/HeJ - *Rps19*^{Dsk3}/+ backcross progeny at different developmental time points. Two-tailed p values for mutant vs. nonmutant are 3.0×10^{-6}, 3.97×10^{-8} and 1.16×10^{-6} at P0, P3, and P21, respectively.

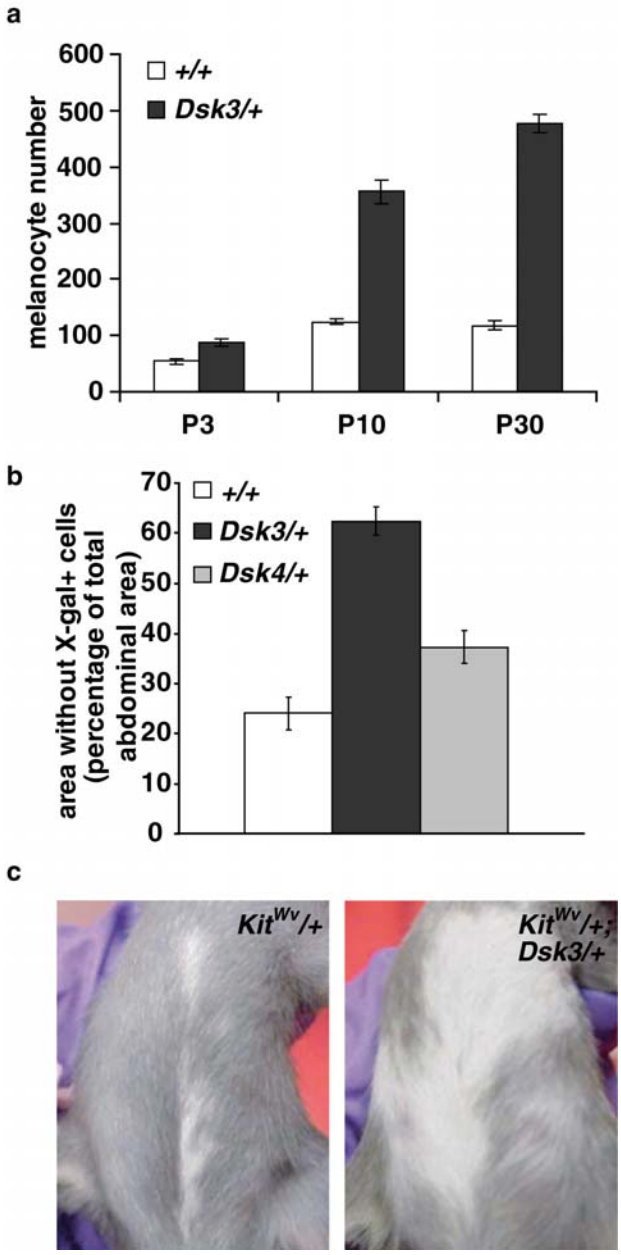
Supplementary Table 3. Effect of *Rps19* and *Trp53* mutations on erythrocytes

	inbred (C3HeB/FeJ)[a]		mixed[b]	
Trp53 genotype	+/+	+/+	+/+	*ko*/+
Rps19 genotype	+/+	*Dsk3*/+	+/+	+/+
No. of animals (male:female)	5 (2:3)	8 (3:5)	14 (7:7)	9 (6:3)
RBC	9.18 +/- 0.09	8.3 +/- 0.07 (p=0.00002)[a]	9.81 +/- 0.12	9.50 +/- 0.17
MCV	47.8 +/- 0.38	49.4 +/- 0.27 (p=0.009)[a]	48.9 +/- 0.26	49.6 +/- 0.34

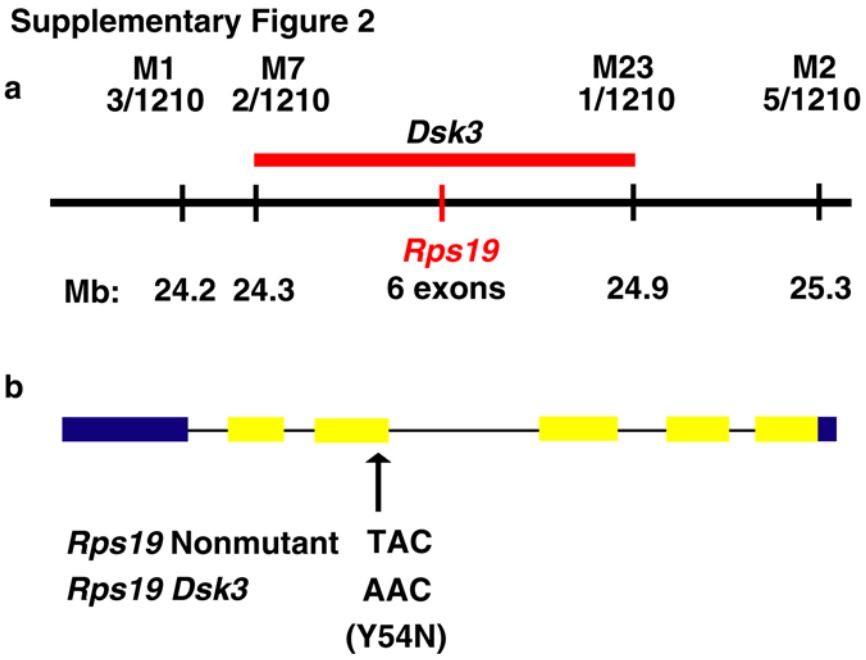
[a]Blood counts were obtained at 20 weeks of age on +/+ and $Rps19^{Dsk3/+}$ animals on an inbred background; all values are given as mean +/- sem (with p values based on multiple regression in which litter and sex are factors).

[b]Blood counts were obtained at 8 weeks of age on +/+ and $Trp53^{ko/+}$ animals on a mixed genetic background; all values are given as mean +/- sem.

Supplementary Figure 1

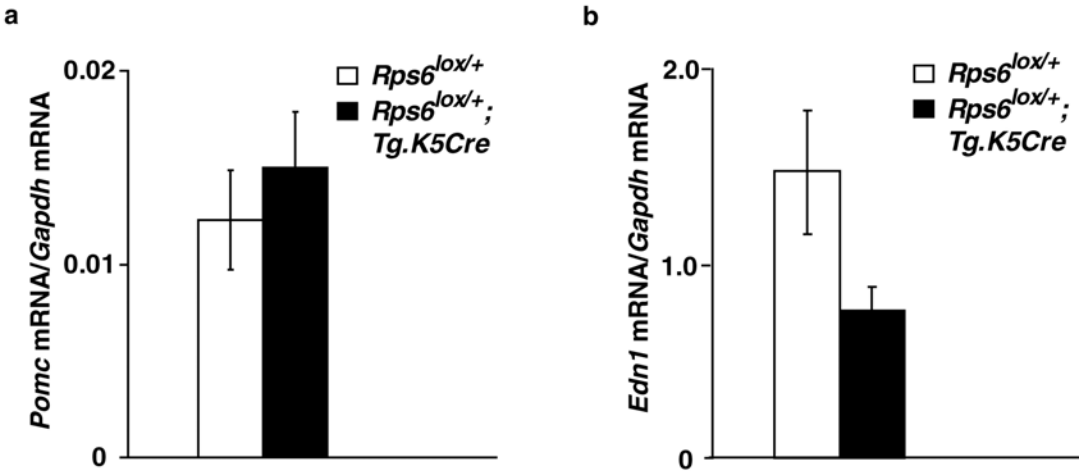


Supplementary Figure 1 Impaired pigment cell development in *Dsk3* and *Dsk4* mutant mice. **a,** The number of Xgal-positive cells (+/-sem) in the footpad epidermis (same anatomic location as shown in the inset to Figure 1e) of +/+ and *Dsk3*/+ animals at P3, P10, and P30; n=4 – 7. *Dsk3*/+ differed significantly at each time (p = 0.004, 0.0002, and 0.002, respectively) from +/+. **b**, Ventral surface area without Xgal-positive cells, expressed as a percentage of the total ventral surface area (measured from inguinal region to axillae) for E15.5 embryos of the indicated genotypes (+/- sem); n= 4 - 15. *Dsk3*/+ and *Dsk4*/+ differed significantly (p = 0.0018 and 0.04, respectively) from +/+. **c,** Example of how *Dsk3* enhances the size of the white belly spot in animals heterozygous for Kit^{Wv}.



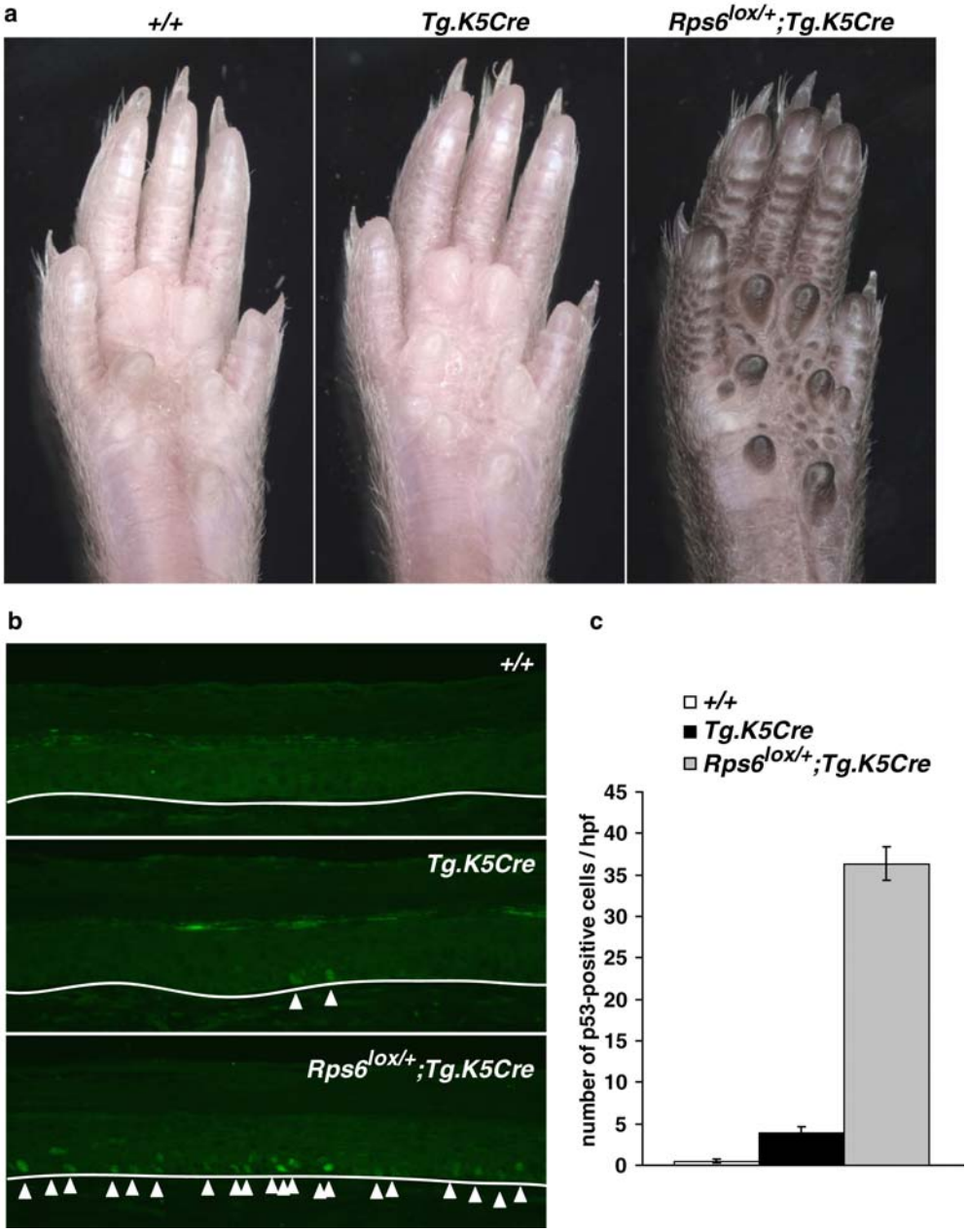
Supplementary Figure 2 Positional cloning of *Dsk3*. **a**, Genetic and physical maps of the *Dsk3* critical interval on mouse chromosome 7. Markers M1, M7, M2, and M23 represent single strand conformation polymorphisms as described in Supplementary Methods. Recombination frequencies (stated as the number of recombinant chromosomes between the marker and *Dsk3*, over the number of informative chromosomes evaluated) are given immediately below each marker. Approximate physical coordinates in megabases (Mb) are given below. **b**, The position and sequence of the *Dsk3* point mutation is shown relative to the exon-intron structure of *Rps19* where untranslated and protein-coding regions are represented by blue and yellow, respectively.

Supplementary Figure 3



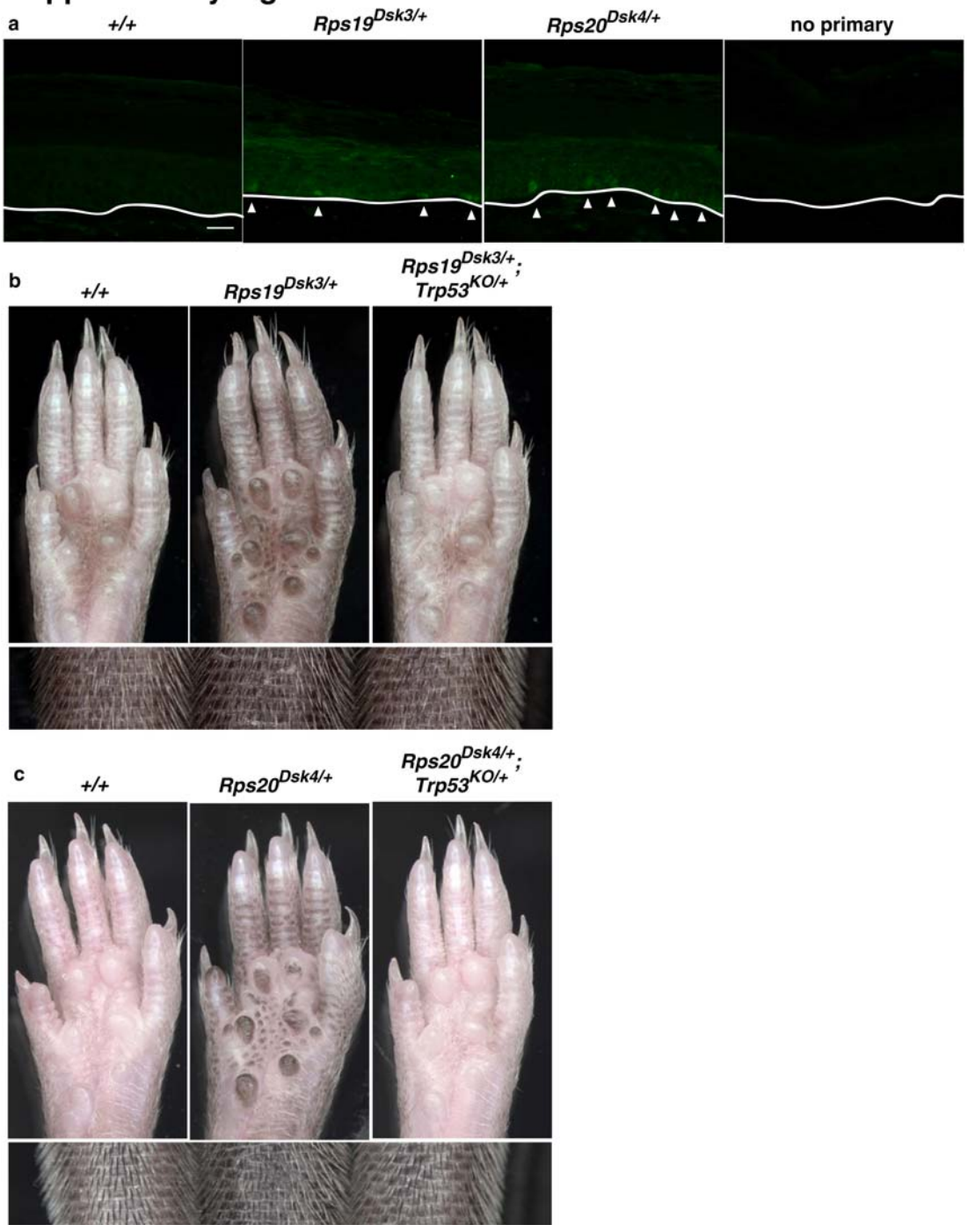
Supplementary Figure 3 Expression of *Pomc* (**a**) and *Edn1* (**b**) mRNA in *Rps6* mutant animals. Methods and samples are identical to those used for *Kitl* mRNA as depicted in Figure 4.

Supplementary Figure 4



Supplementary Figure 4 Expression of Trp53 in nonmutant, *TgK5Cre* and *Rps6*$^{lox/+}$;*Tg.K5Cre* animals. **a,** Footpad and tails from adult animals of the indicated genotypes. **b**, Immunofluorescence (green) for p53 on adult footpad skin from the indicated genotypes. White lines mark the dermal-epidermal junction and white arrowheads mark p53-positive cells. Scale bar: 40 M. **c**, The number of p53-postive cells per high powered field (hpf) (+/- sem) from animals of the indicated genotype at P30; n=4 for each genotype. *Tg.K5Cre* and *Rps6*$^{lox/+}$;*Tg.K5Cre* differed significantly from +/+ animals (p = 0.01 and 0.00004, respectively, based on a two tailed t test).

Supplementary Figure 5



Supplementary Figure 5 Expression and action of *Trp53* in *Dsk3* and *Dsk4*. **a**, Immunofluorescence (green) for p53 on adult footpad skin from the indicated genotypes. White lines mark the dermal-epidermal junction and white arrowheads mark p53-positive cells. The panel without primary antibody (no primary) controls for background fluorescence. Scale bar: 40 M. **b, c,** Footpad and tails from adult animals of the indicated genotypes.