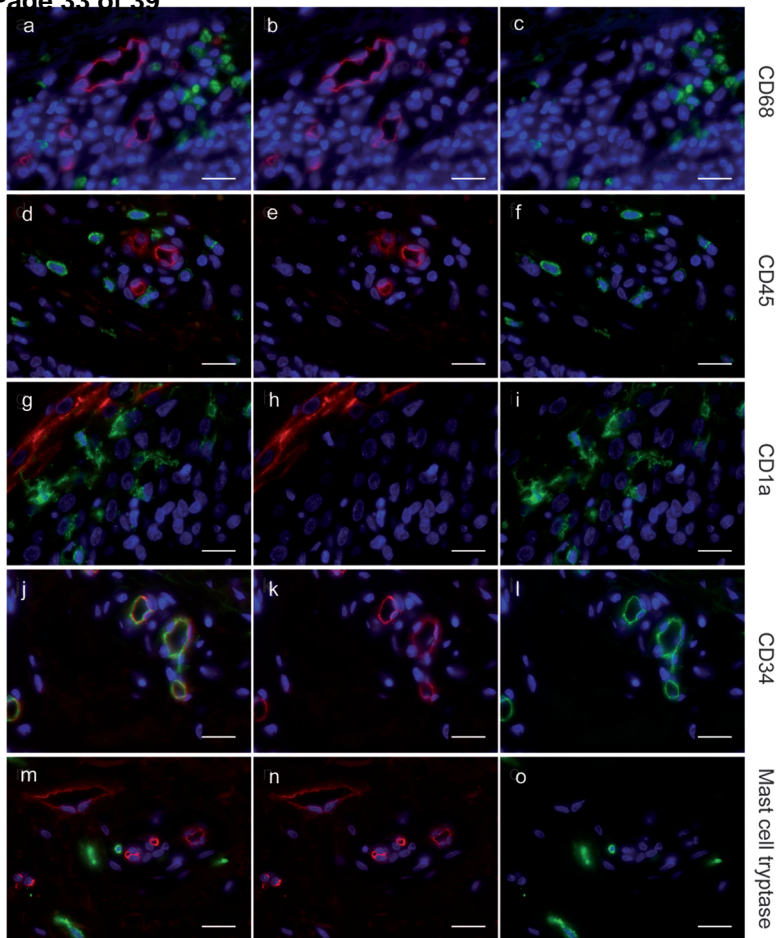
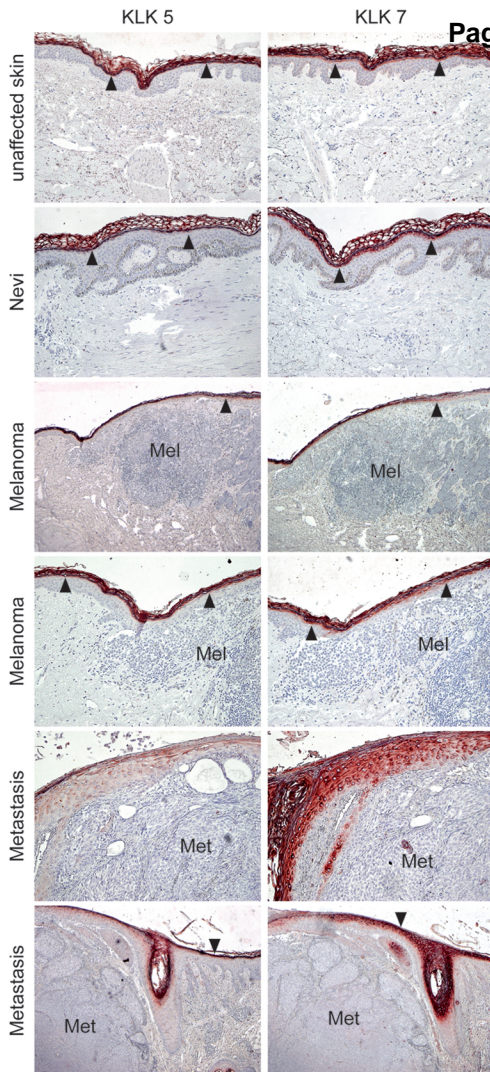


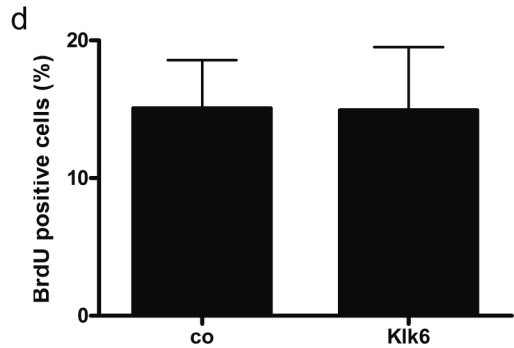
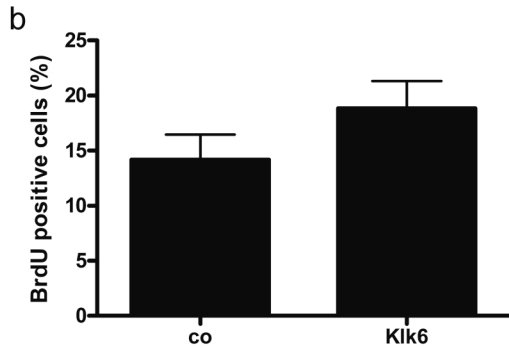
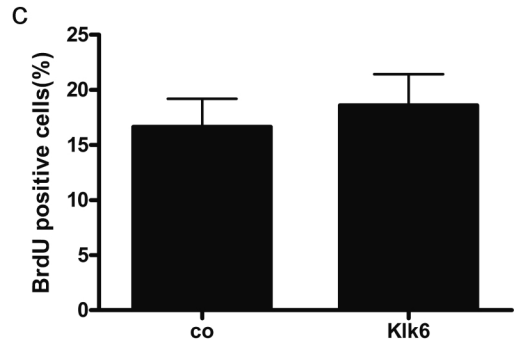
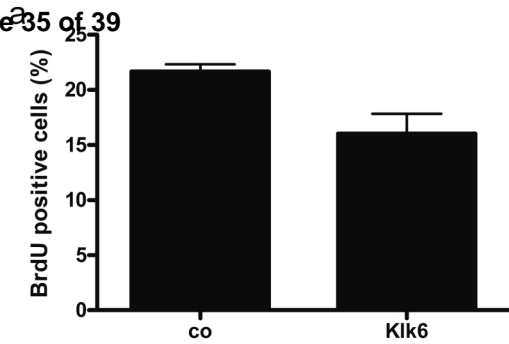
Supplemental Figure S1. KLK6 protein expression in human melanoma. IHC analysis with tissue sections from unaffected skin (a-b), dysplastic nevi (c-f) and primary melanoma (g-j) was performed with an anti-KLK6 antibody and revealed specific staining (brown signal) in epidermal keratinocytes (black arrowheads) and blood vessels (open arrowheads). Right panels show a higher magnification of areas marked by black boxes. Staining with an anti-S100 antibody was used as a control to detect transformed melanoma cells (c-d, g-h). Sections were counterstained with hematoxylin. Scale bars, 40 μ m. Mel, Melanoma.



Supplemental Figure S2. KLK6 protein expression in endothelial cells of human melanoma. Co-immunofluorescence analysis with tissue section from primary melanoma were performed using anti-KLK6 antibody (b, e, h, k, n, red staining), anti-CD68 antibody (c, green staining), anti-CD45 (f, green staining), anti-CD1a (i, green staining), anti-CD34 (l, green staining), and anti-Mast cell tryptase (o, green staining). Left panels show the merge of the co-staining. Scale bars, 40 μ m.



Supplemental Figure S3. KLK5 and KLK7 protein expression in epidermal keratinocytes of human melanoma tissue sections. IHC analysis with tissue sections of unaffected skin, dysplastic nevi, primary melanoma, and epidermotropic metastasis was performed with antibodies raised against KLK5 and KLK7 proteins and revealed specific staining (red signal) mainly in terminally differentiated epidermal keratinocytes (black arrowheads). Sections were counterstained with hematoxylin. Scale bars, 40 μ m. Mel, Melanoma and Met, Metastasis.



Supplemental Figure S4. Measurement of BrdU positive cells. Cells were seeded in a 12-well plate and stimulated for 4 (a, c) and 8 hours (b, d) with 40 nM recombinant rat Klk6 and BrdU incorporation was quantified by FACS analysis. BrdU incorporation of MeWo cells (a, b) and Skmel23 cells (c, d) were quantified in the absence (control) and presence (Klk6) of 40 nM recombinant rat Klk6.

Supplemental Table S1: Summary of the patient characteristics

Probe No.	Age	Sex	Type	Classification	Location	Progress
572/07B	49	m	SSM	AJCC IA	Chest	no
1079/06C	72	m	ulc. amelan. MM	AJCC IIC	Lower leg	yes
1973/06C	56	m	SSM	AJCC IA	Back	no
2703/06D	88	m	SSM	AJCC IIC	Back	-
2737/06C	55	f	SSM	AJCC IB	Lower leg	no
3672/06A	52	f	SSM	AJCC IA	Lower leg	no
388/07B	75	m	SSM	AJCC IB	Chest	no
339/07C	69	m	ulc. SSM	AJCC IIB	Back	yes
573/07B	49	m	SSM	-	-	no
666/07L	58	f	ulc. ALM	AJCC IIC	Ankl joint	yes
905/07	72	m	SSM	AJCC IA	Shoulder	yes

SSM: Superficial spreading melanoma, ALM: Acral lentiginous melanoma, ulc: ulceration
AJCC: American Joint Committee on Cancer Staging System for Cutaneous Melanoma
m: male, f: female

Supplemental Table S2: Summary of the antibodies used in the study

Name	Number	Assay	Antigen	Company	Dilution
anti-human KLK5	AF1108	IHC	KLK5	R&D systems, Minneapolis, USA	1:200
anit-human KLK6	AF2008	IHC	KLK6	R&D systems, Minneapolis, USA	1:100
anti-human KLK7	AF2624	IHC	KLK7	R&D systems, Minneapolis, USA	1:200
anti-human S100	SI691R06	IHC	S-100	DCS, Hamburg, Germany	ready to use
biotinylated horse-anti-mouse IgG	BA-2000	IHC		Vector, Burlingame, CA	1:200
biotinylated horse-anti-goat IgG	BA-9500	IHC		Vector, Burlingame, CA	1:200
anti-human PAR-1	sc-13503	Western Blot	PAR-1	Santa Cruz Biotechnology, Santa Cruz, CA	1:500
anti-human β -actin	sc-1615	Western Blot	β -actin	Santa Cruz Biotechnology, Santa Cruz, CA	1:2000
anti-goat IgG, HRP-linked antibody	sc-2020	Western Blot		Santa Cruz Biotechnology, Santa Cruz, CA	1:5000
anti-mouse IgG, HRP-linked antibody	#7076	Western Blot		Cell Signaling Technology, Boston, USA	1:2000
anti human PAR-1	AF3855	FACS analysis	PAR-1	R&D systems, Minneapolis, USA	2.5 μ g/ml
anti-goat Alexa 488	A11055	FACS analysis		Invitrogen, Karlsruhe, Germany	1:200
anti-human CD45	M0701	IF	CD45	Dako Cytomation	1:100
anti-human mast cell tryptase	M7052	IF	mast cell tryptase	Dako Cytomation	1:100
anti-human CD34	NCL-END	IF	CD34	Novocastra Laboratories	1:50
anti-human CD1a	M3571	IF	CD1a	Dako Cytomation	1:50
anti-human CD68	M0876	IF	CD68	Dako Cytomation	1:100

Supplemental Table S3: shRNA against human PAR-1 cloned into the lentivirus vector pLKO.1-puro were purchased from Sigma Aldrich

Name	Sequence 5'-3' and region targeted by the shRNA
shRNA 1349	CCGGGCATTACTCATTCTTTCTCACTCGAGTGAGAAAGGAATGAGTAATGCTTTTT Region: CDS
shRNA 2152	alternate species: NM_001992.2 CCGGGACGGCAAGGTTTAAGTTATTCTCGAGAATAACTTAAACCTTGCCGTCTTTTT Region: CDS
co shRNA	alternate species: NM_001992.2 catalogue number: SHC002V

Supplemental Table S4: Summary of primers used for qRT-PCR

Primer name	Sequence
h Lamin b1 for	5'- GCT GCT CCT CAA CTA TGC TAA GAA - 3'
h Lamin b1 rev	5'- TTT GAC GCC CAG AAT CCA C -3'
h PAR-1 for	5'- GGC CCG CAG GCC AGA ATC AA -3'
h PAR-1 rev	5'- AGT GTC AGC CAG GAG CTG GTC A -3'