

## **Supplemental Material to:**

**Martinet et al.**

**High endothelial venules (HEVs) in human melanoma lesions: Major gateways for tumor-infiltrating lymphocytes**

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**Supplementary Table S1.** Spearman correlation matrix between HEVs and immune parameters

	HEV number	HEV/mm <sup>2</sup>	CD3 level	CD8 level
HEV/mm <sup>2</sup>	<b>0.8840*</b>			
CD3 level	<b>0.7050*</b>	<b>0.7089*</b>		
CD8 level	<b>0.6631*</b>	<b>0.6672*</b>	<b>0.9540*</b>	
CD20 level	<b>0.6128*</b>	<b>0.5625*</b>	<b>0.7075*</b>	<b>0.7199*</b>

**Supplementary Table S2.** Characteristics of melanomas (n=225) according to CD3<sup>+</sup> tumor-infiltrating lymphocyte density

	CD3			
	Grade 1	Grade 2	Grade 3	
HEV /mm <sup>2</sup>				<b>P &lt; 0.001</b>
Median	0.0	2.4	5.4	
(Range)	(0.0: 17.5)	(0.0: 18.7)	(0.0: 27.3)	
Type				
SSM	42 (44.2)	23 (43.4)	33 (50.8)	<b>p &lt; 0.001</b>
Autre	17 (17.9)	12 (22.6)	14 (21.5)	
Acral-lentiginous	24 (25.3)	2 ( 3.8)	1 ( 1.5)	
Dubreuilh	12 (12.6)	16 (30.2)	17 (26.2)	
Missing	4	2	1	
Breslow thickness				<b>p &lt; 0.02</b>
<=1mm	43 (47.8)	36 (70.6)	37 (57.8)	
1.01 - 2 mm	16 (17.8)	3 ( 5.9)	15 (23.4)	
>2.0mm	31 (34.4)	12 (23.5)	12 (18.8)	
Missing	9	4	2	
Ulceration				<b>p = 0.58</b>
No	84 (88.4)	47 (92.2)	55 (85.9)	
Yes	11 (11.6)	4 ( 7.8)	9 (14.1)	
Missing	4	4	2	
Regression				<b>p &lt; 0.001</b>
No	86 (89.6)	38 (74.5)	42 (65.6)	
Yes	10 (10.4)	13 (25.5)	22 (34.4)	
Missing	3	4	2	
Clark Level				<b>p &lt; 0.03</b>
I-II	30 (31.6)	22 (43.1)	24 (36.4)	
III	20 (21.1)	17 (33.3)	23 (34.8)	
IV-V	45 (47.4)	12 (23.5)	19 (28.8)	
Missing	4	4	0	

**Supplementary Table S3.** Characteristics of melanomas (n=225) according to CD8<sup>+</sup> tumor-infiltrating lymphocyte density

	CD8			
	Grade 1	Grade 2	Grade 3	
HEV /mm <sup>2</sup>				<b>p &lt; 0.001</b>
Median	0.0	2.8	5.4	
(Range)	(0.0: 17.5)	(0.0: 13.4)	(0.0: 27.3)	
Type				<b>p &lt; 0.001</b>
SSM	40 (43.0)	24 (54.5)	27 (49.1)	
Others	18 (19.4)	6 (13.6)	14 (25.5)	
Acral-lentiginous	21 (22.6)	1 ( 2.3)	0 ( 0.0)	
Dubreuilh	14 (15.1)	13 (29.5)	14 (25.5)	
Missing	4	2	1	
Breslow thickness				<b>p = 0.17</b>
<=1mm	49 (55.1)	29 (69.0)	31 (57.4)	
1.01 - 2 mm	12 (13.5)	7 (16.7)	12 (22.2)	
>2. mm	28 (31.5)	6 (14.3)	11 (20.4)	
Missing	8	4	2	
Ulceration				<b>p = 0.20</b>
No	84 (90.3)	40 (95.2)	45 (83.3)	
Yes	9 ( 9.7)	2 ( 4.8)	9 (16.7)	
Missing	4	4	2	
Regression				<b>p &lt; 0.005</b>
No	82 (87.2)	29 (69.0)	35 (64.8)	
Yes	12 (12.8)	13 (31.0)	19 (35.2)	
Missing	3	4	2	
Clark Level				<b>p &lt; 0.03</b>
I-II	35 (37.2)	18 (41.9)	21 (37.5)	
III	19 (20.2)	18 (41.9)	16 (28.6)	
IV-V	40 (42.6)	7 (16.3)	19 (33.9)	
Missing	3	3	0	

**Supplementary Table S4.** Characteristics of melanomas (n=225) according to CD20<sup>+</sup> tumor-infiltrating lymphocyte density

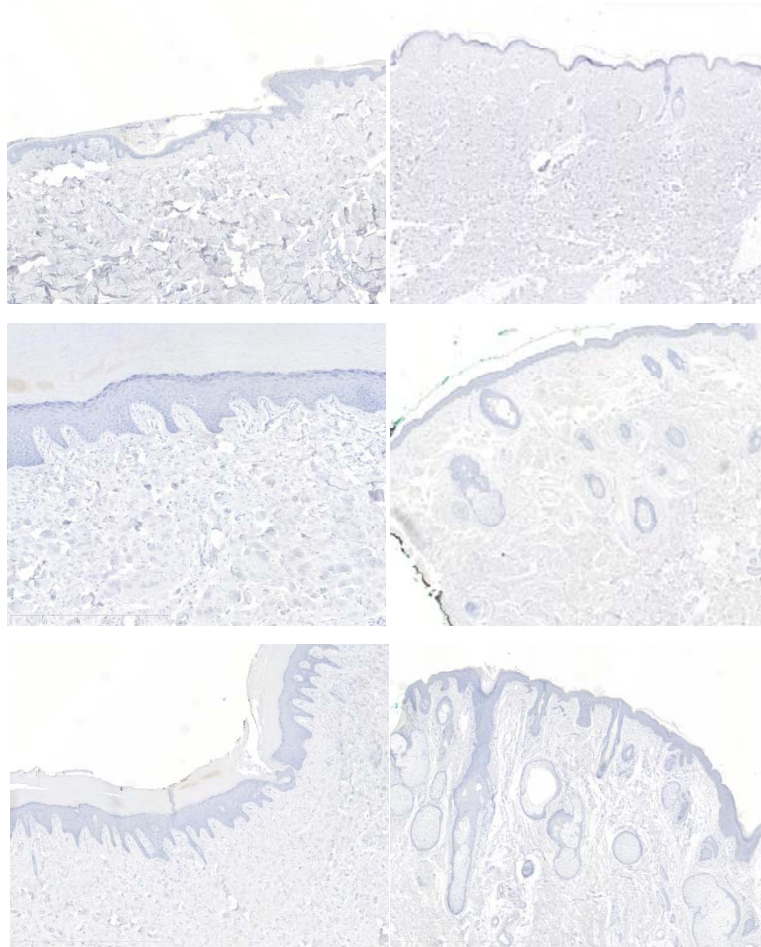
	CD20			
	Grade 1	Grade 2	Grade 3	
HEV /mm <sup>2</sup>				<b>p &lt; 0.001</b>
Median	0.1	5.2	7.9	
(Range)	(0.0: 17.5)	(0.0: 18.9)	(0.2: 27.3)	
Type				<b>p = 0.09</b>
SSM	70 (47.3)	18 (42.9)	11 (47.8)	
Others	26 (17.6)	11 (26.2)	5 (21.7)	
Acral-lentiginous	25 (16.9)	1 ( 2.4)	1 ( 4.3)	
Dubreuilh	27 (18.2)	12 (28.6)	6 (26.1)	
Missing	6	1	0	
Breslow Thickness				<b>p = 0.86</b>
<=1mm	84 (58.7)	20 (51.3)	12 (52.2)	
1.01 - 2 mm	22 (15.4)	8 (20.5)	4 (17.4)	
2.01 - 4 mm	37 (25.9)	11 (28.2)	7 (30.4)	
Missing	11	4	0	
Ulceration				<b>p = 0.58</b>
Non	132 (89.2)	35 (89.7)	19 (82.6)	
Oui	16 (10.8)	4 (10.3)	4 (17.4)	
Missing	6	4	0	
Regression				<b>p &lt; 0.05</b>
Non	123 (82.6)	29 (74.4)	14 (60.9)	
Oui	26 (17.4)	10 (25.6)	9 (39.1)	
Missing	5	4	0	
Clark level				<b>p = 0.72</b>
I-II	54 (36.7)	16 (38.1)	6 (26.1)	
III	42 (28.6)	9 (21.4)	8 (34.8)	
IV-V	51 (34.7)	17 (40.5)	9 (39.1)	
Missing	7	1	0	

**Supplementary Table S5** : List of antibodies used in immunofluorescence and immunohistochemistry

Antigen	Antibody	Dilution	Species	Retrieval method	Tissue fixative
MECA-79	MECA-79 (BD)	1/2	Rat IgM	95°C TRS PH low (Dako)	R, F, D
CD3	SP7 (Abcam)	1/100	Rabbit IgG	95°C TRS PH low (Dako)	R, F, D
CD3	F7.2.38 (Dako)	1/100	Mouse IgG	95°C TRS PH low (Dako)	R, F
CD8	C8/144B (Dako)	1/100	Mouse IgG	95°C TRS PH high (Dako)	F, D
CD20	L26 (Beckman coulter)	1/100	Mouse IgG	95°C TRS PH low (Dako)	R, F, D
CD31	Ab28364 (Abcam)	1/50	Rabbit IgG	95°C TRS PH low (Dako)	R, F
CD45RO	UCHL1 (Dako)	1/100	Mouse IgG	95°C TRS PH low (Dako)	R, F, D
vWB	vWB (Dako)	1/100	Rabbit IgG	95°C TRS PH low (Dako)	R, F
DARC	Fy6 (BD)	1/50	Mouse IgG	95°C TRS PH low (Dako)	R, F
ICAM1	1H4 (Abcam)	1/50	Mouse IgG	95°C TRS PH low (Dako)	R, F
G72	G72	1/10	Mouse IgM	95°C TRS PH low (Dako)	R
G152	G152	1/10	Mouse IgM	95°C TRS PH low (Dako)	R
HECA-452	HECA-452 (BD)	1/50	Rat IgM	95°C TRS PH low (Dako)	R, F, D
CD45RA	(Beckman coulter)	1/50	Mouse IgG	95°C TRS PH low (Dako)	R, F, D
Foxp3	236A/E7 (Abcam)	1/50	Mouse IgG	95°C TRS PH high (Dako)	R, F
DC-LAMP	<sup>E</sup> 1010 1.01(Dendritics)	1/50	Rat IgG	95°C TRS PH high (Dako)	F, D
Fascin	55K2 (Dako)	1/50	Mouse IgG	95°C TRS PH low (Dako)	R, F, D
Melan-A	A103 (Dako)	1/50	Mouse IgG	95°C TRS PH high (Dako)	R, F, D

R: RCL-2, F: Formalin, D: Dubosc. The HEV-specific antibodies G72 and G152 were kindly provided by Dr Reiji Kannagi (Aichi Cancer Center, Nagoya, Japan).

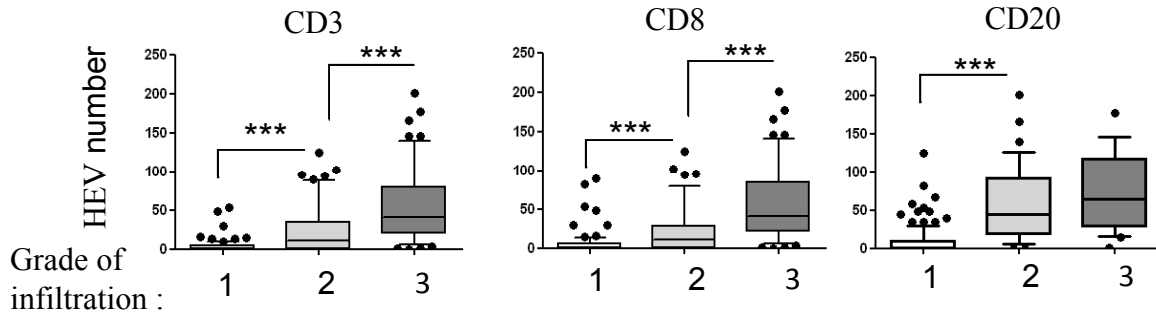
## Supplementary Figure S1



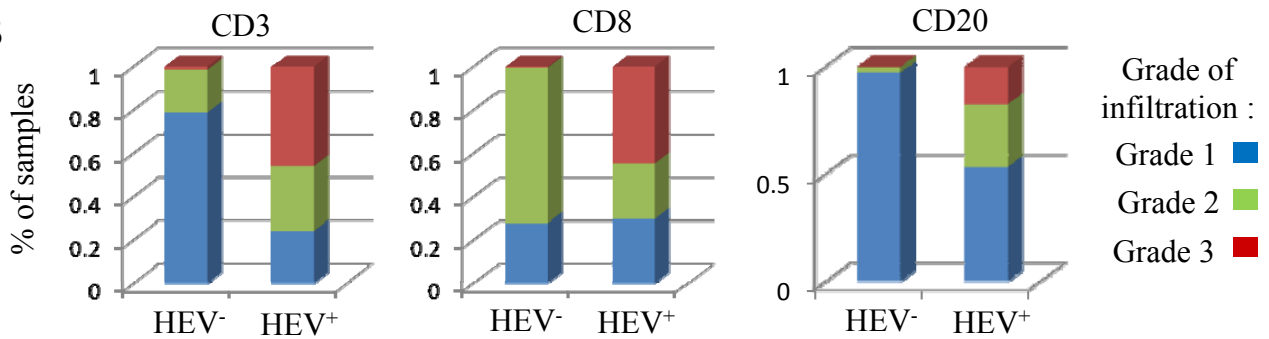
**Figure S1.** Absence of HEV blood vessels in normal skin. Representative pictures of normal skin tissue sections stained with MECA-79 antibody demonstrating the absence of HEV blood vessels in non-pathologic tissues distant from primary melanoma site.

## Supplementary Figure S2

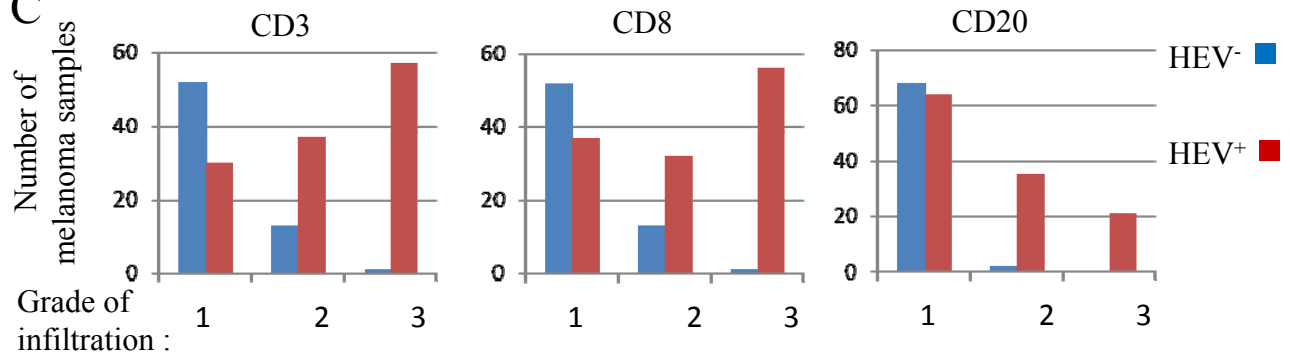
A



B



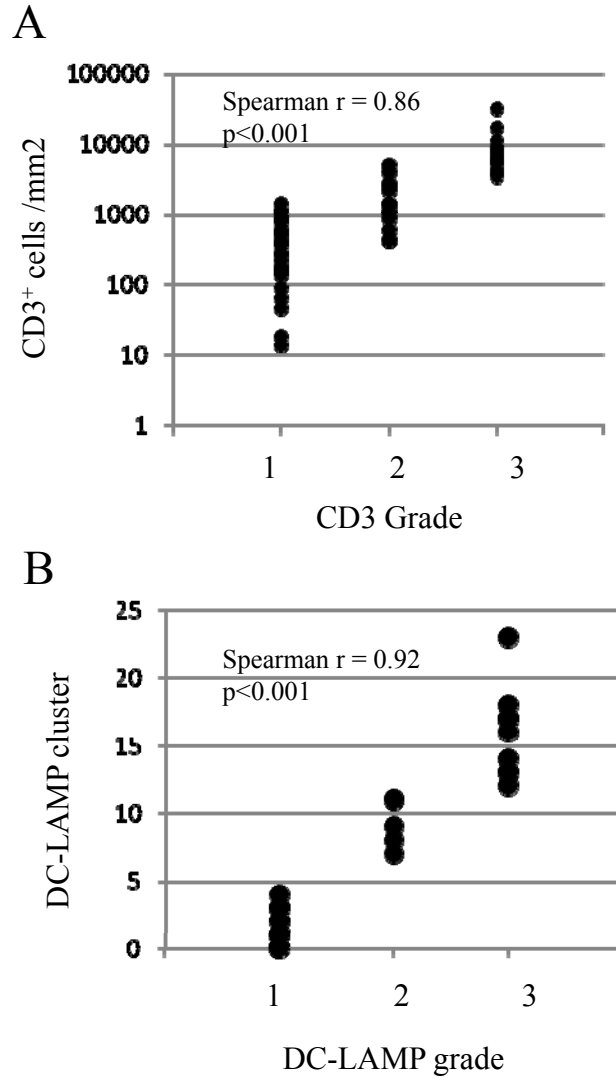
C



**Figure S2.** Tumor HEVs predict lymphocyte infiltration in melanoma. (A) Graphs showing the absolute numbers of HEVs in melanoma according to the density of CD3<sup>+</sup>, CD8<sup>+</sup> and CD20<sup>+</sup> TILs. (B) Graphs showing the percentage of melanoma with grade 1 (blue bars), grade 2 (green bars) and grade 3 (red bars) levels of CD3<sup>+</sup>, CD8<sup>+</sup> or CD20<sup>+</sup> TILs according to the presence or absence of tumor HEVs. (C) Graphs showing the number of melanoma tumors with grade 1, grade 2 and grade 3 density of CD3<sup>+</sup>, CD8<sup>+</sup> or CD20<sup>+</sup> TILs according to the presence (red bars) or absence of tumor HEVs (blue bars). \*\*\* p < 0.001; Mann Whitney test.



### Supplementary Figure S3



**Figure S3.** Graphs showing the correlation between optical grading and automatic cell count for CD3<sup>+</sup> (A) and DC-LAMP<sup>+</sup> tumor infiltrating cells (B).