

SUPPLEMENTARY ONLINE MATERIALS

Prognostic Relevance of CCDC88C (Daple) Transcripts in the Peripheral Blood of Patients with Cutaneous Melanoma

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Supplementary Table 1 [Table S1]

Target gene	Primer and Probe sequence (TAQMAN ASSAY)
Daple-V1	Fwd: 5'-CGGGACCTCACCAAGCAA-3' Rev: 5'-CTGCTGAGCTGCTGGCTCTT-3' Probe: 5'-CAACTCTGAGGGAGGACCTGGTGCTC-3'
Daple-V2	Fwd: 5'-GGAGCCTCAGGATATACGTGCA-3' Rev: 5'-TCAAGGCTGCCTCTGTGTGG -3' Probe: 5'-CAGGATGTCCGTAATAAGCCCTGGGGATC-3'
S100A4	Fwd: 5'-CAAGCTCAACAAGTCAGAACTAAAGGA-3' Rev: 5'-TGTCCAAGTTGCTCATCAGCTTC-3' Probe: 5'- CCAGCTTCTTGGGGAAAAGGACAGATGAA-3'
GAPDH	Fwd: 5'- CCAATGTCACCGTTGTCCAGTT-3' Rev: 5'- CTCTACTTCAGCCTTAGGCTCAGGT-3' Probe: 5'-CCGTGCCCCGGTGACCAGAGC-3'
Target gene	Primer sequence (SYBR-GREEN ASSAY)
MLANA	Fwd: 5'- GAGAAAAACTGTGAACCTGTGGT -3' Rev: 5'- GACTGTTCTGCAGAGAGTTTCTCAT-3'
ABCB5	Fwd: 5'- CCCAAAATTTTATTGTTGGATGA-3' Rev: 5'-AAGGGCATGCTGAACCAC-3'
MCAM	Fwd: 5'-GGGTACCCCATTCCTCAAGT-3' Rev: 5'-CTGGGACGACTGAATGTGG-3'
GAPDH	Fwd: 5'-GGGTGTGAACCATGAGAAGT-3' Rev: 5'-GACTGTGGTCATGAGTCCT-3'

Supplementary Table 2 [Table S2]

		DAPLE-V1	DAPLE-V2	S100A4
DAPLE-V1	Spearman r	1.0000	0.5466	0.8014
	<i>p</i> -value	n/a	<0.0001	<0.0001
DAPLE-V2	Spearman r	0.5466	1.0000	0.3440
	<i>p</i> -value	<0.0001	n/a	<0.0001
S100A4	Spearman r	0.8014	0.3440	1.0000
	<i>p</i> -value	<0.0001	<0.0001	n/a

Supplementary Table 3 [Table S3]

	GENES	DAPLE-V1	DAPLE-V2	S100A4
AGE	Spearman r	0.04293	0.03299	0.1831
	<i>p</i> -value	0.5411	0.6387	0.0086
STAGE	Spearman r	-0.05526	-0.1624	-0.1327
	<i>p</i> -value	0.4313	0.02	0.0578
CLARK'S LEVEL	Spearman r	-0.0414	-0.1039	-0.09071
	<i>p</i> -value	0.579	0.1628	0.2233
BRESLOW THICKNESS	Pearson r	0.121	0.01823	-0.00617
	<i>p</i> -value	0.1515	0.8295	0.9419
ULCERATION	Spearman r	-0.02815	0.0732	-0.08334
	<i>p</i> -value	0.7006	0.3168	0.2542

Supplementary Table 4 [Table S4]

A

MARKERS STATUS (MLNA, ABCB5 OR MCAM)	EARLY STAGE PATIENTS WITH LOW V2(N)	EARLY STAGE PATIENTS WITH HIGH V2 (N)	TOTAL (N)
MARKERS (+) (%)	48 (64%)	29 (41%)	77
MARKERS (-) (%)	27 (36%)	41 (59%)	68
TOTAL (N)	75	70	145

B

ABCB5 STATUS	PATIENTS WITH LOW V2 (N) (ALL STAGES)	PATIENTS WITH HIGH V2 (N) (ALL STAGES)	TOTAL (N)
ABCB5 (+) (%)	37 (27%)	9 (13%)	46
ABCB5 (-) (%)	99 (73%)	60 (87%)	159
TOTAL (N)	136	69	205

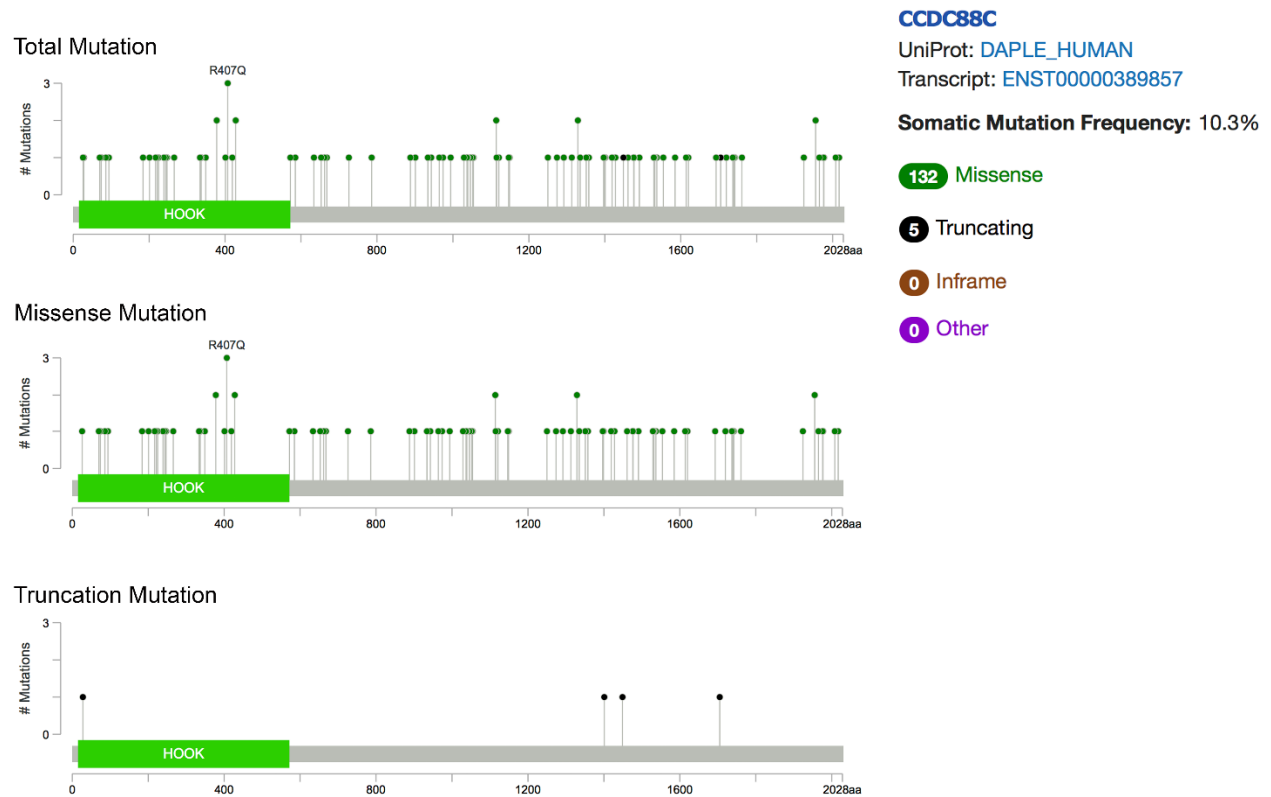
Altered in 112 (11%) of 983 sequenced cases/patients (1049 total)



Supplemental Figure 1

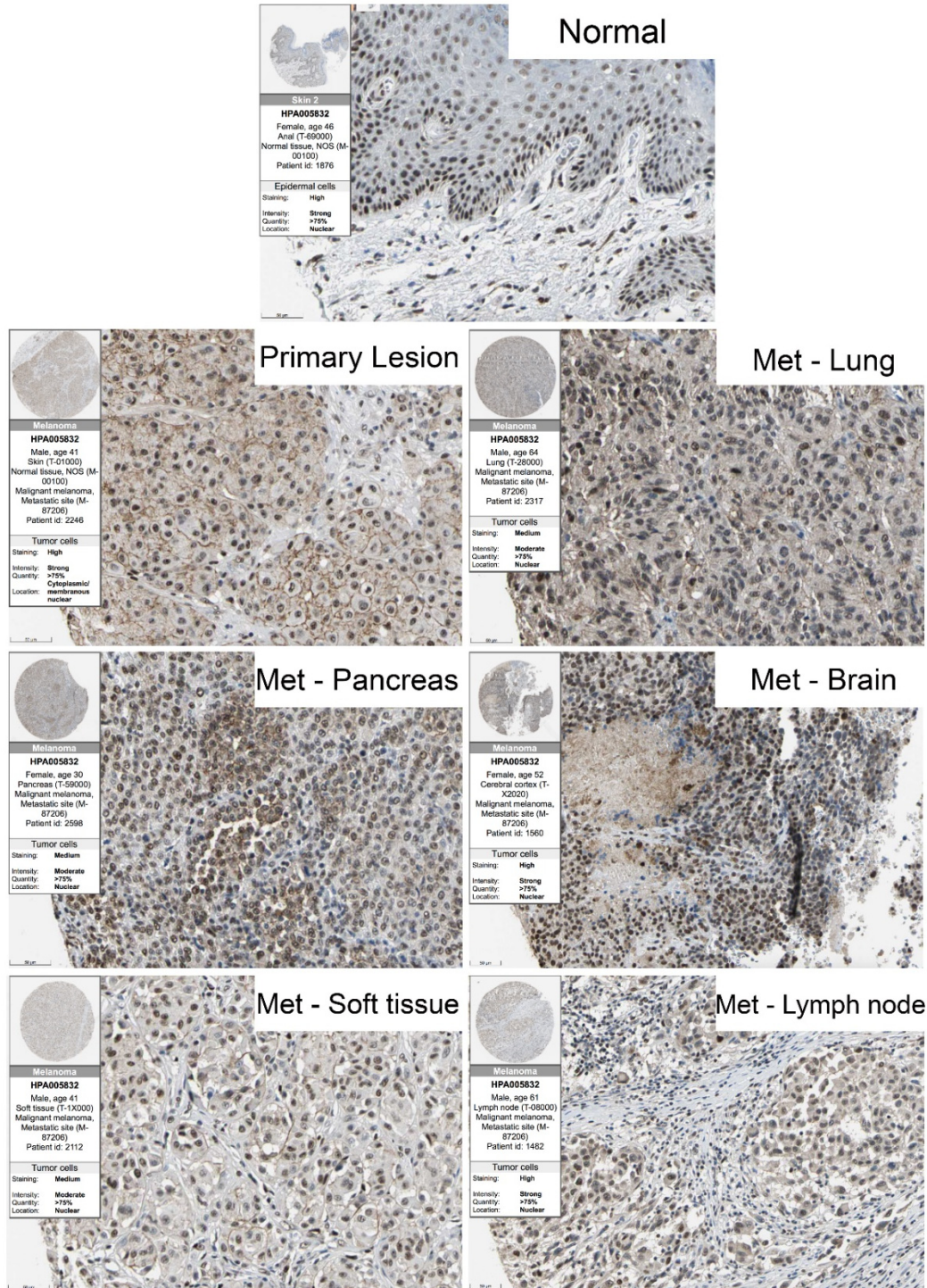
Summary of CCDC88C/ Daple gene alteration across multiple melanoma datasets.

Approximately 11% of patients carry a mutation in the gene. Missense mutations, truncating mutations, and homozygous deletions (deep deletions) have been identified in patients, with missense mutations accounting for most of the mutations.



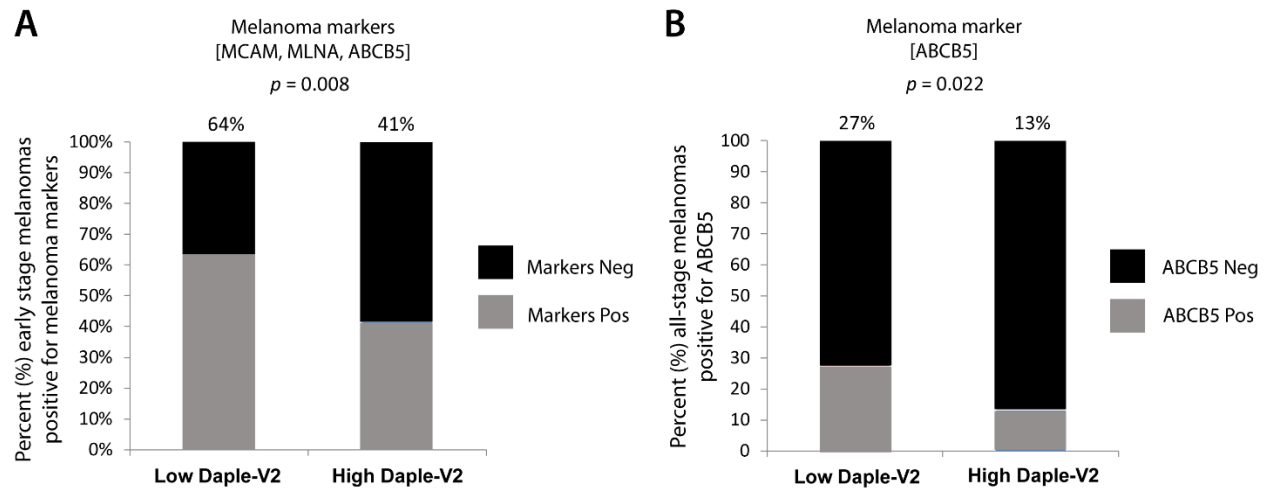
Supplemental Figure 2

Daple is frequently mutated in melanomas. Mutation diagram summarizing the number of mutations, mutation rate, and position of the mutation along the Daple protein in melanomas. The total landscape of mutation is illustrated in the top plot. Middle plot illustrates exclusively the missense mutations, whereas the bottom plot illustrates exclusively the truncating mutations.



Supplemental Figure 3

Daple is highly expressed in cutaneous melanomas. Expression of Daple, as determined by immunohistochemistry (IHC) on formaldehyde fixed paraffin embedded (FFPE) normal skin and primary and metastatic melanoma tissues as reported in The Human Protein Atlas (using antibody HPA005832). Of a total of 11 tumors, a 100% of the analyzed samples showed high (6) or very high (5) levels of expression. *Met*, Metastatic Site.



Supplemental Figure 4

Correlation between Daple-V2 expression and melanoma-associated markers. (A) Patients with early stage melanomas ($n = 145$) with known expression status (positive or negative) of melanoma associated markers (MCAM, MLNA and ABCB5) were analyzed in subgroups of patients with high ($n = 55$) or low ($n = 90$) expression levels for Daple-V2. Tumors were classified as “Markers Pos” if any one of the three melanoma-associated markers were detectable, whereas tumors were classified as “Markers Neg” if none of the three markers were detectable [see **Table S4-source data**]. Bar graphs display the incidence of marker positivity as percent (%; Y axis) of patients with low vs. high Daple-V2. (B) Patients with all stages of melanoma ($n = 205$) with known expression status (positive or negative) of the melanoma stem cell marker, ABCB5 alone was analyzed in subgroup of patients with low vs. high Daple-V2 as in A.