Melanoma Microsatellites Exhibit a Metastatic Signature by Spatial Transcriptomics and Overexpress Mediators of Immune Evasion

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Disclosures

I do not have any relevant financial relationships to disclose.

Microsatellites in Melanoma

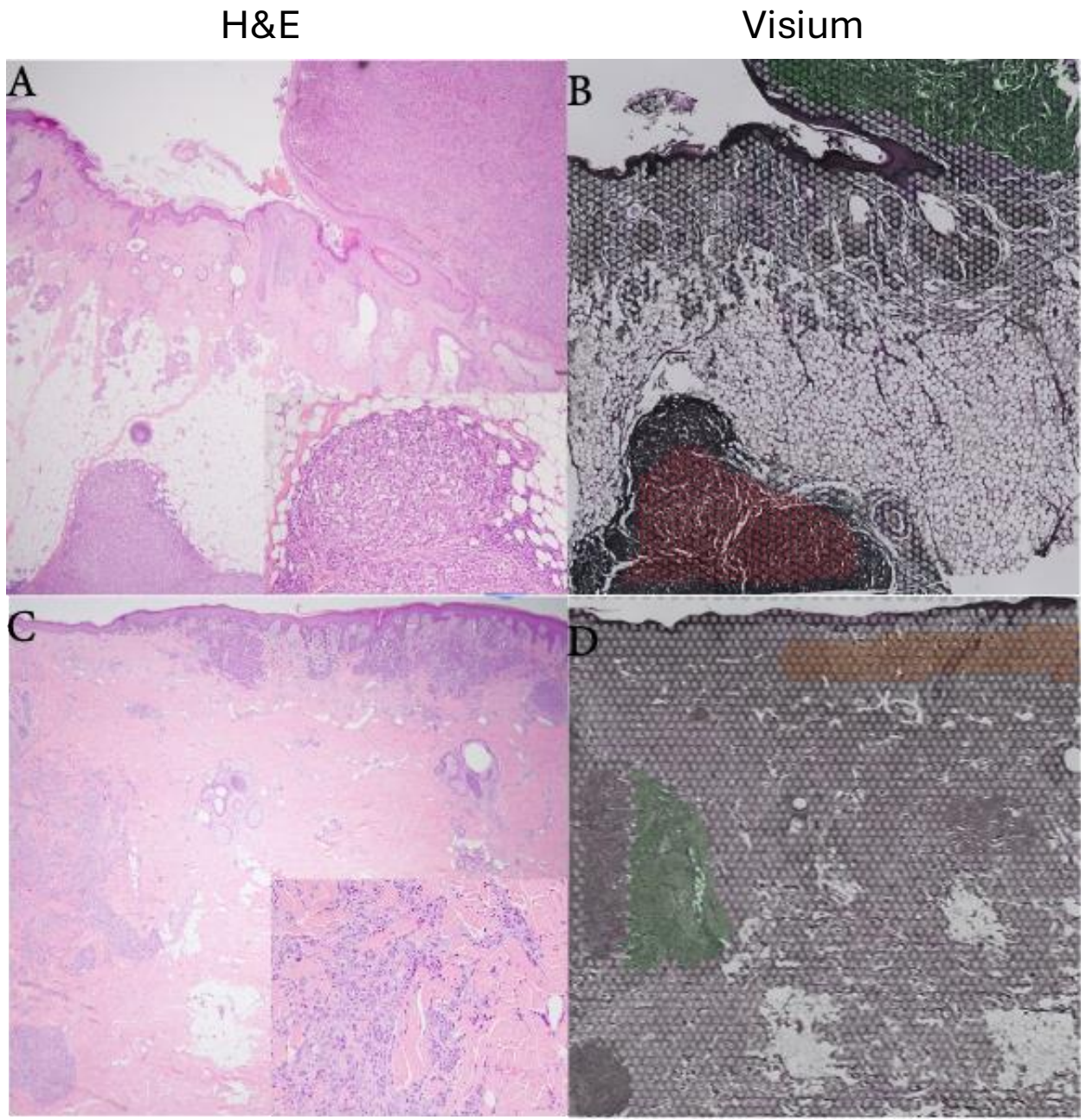
- Are hypothesized to arise by lymphovascular invasion then exit similar to other metastases
- Are associated with sharply increased risk of disease progression (5 year disease-free survival of 21% compared to 73% in a group of *matched* melanoma controls lacking microsatellites*)
- *Have not been previously studied at the molecular level.*

**Niebling MG The prognostic significance of microsatellites in cutaneous melanoma. Mod Pathol. 2020*

What we did:

- We compared gene expression in microsatellites with the matched primary melanoma in 2 patients using spatial transcriptomics on Visium platform.
- Immunostaining for select genes to confirm protein expression in a larger group
- Gene expression analysis compared to defined melanoma gene sets to understand overall trends

Selection of areas of primary melanomas and microsatellites for analysis



We identified *recurrently* overexpressed genes in microsatellites contributing to:

- vascular invasion (ITGA4, MCAM)
- survival in the circulation (CXCL8, PDGFRB, CDH1)
- vascular exit (ITGB2)
- survival at metastatic sites (C3, TGBFI)
- matrix remodeling (MMP9, VCAN, FN1,BGN)
- angiogenesis (EMILIN2)
- immune evasion (PAEP, GDF15, CD74, HLA-DRA)

Normalized expression for selected *recurrent* overexpressed genes, ranked by fold increase in microsatellite over primary melanoma

Gene Case 1	Melanoma	Microsatellite	Fold Change
PAEP	0.05	15.29	274.90
MMP9	0.12	2.57	20.53
EMILIN2	0.24	4.83	20.22
CXCL8	0.15	1.94	12.54
CD53	0.15	1.56	10.08
CD37	0.28	2.75	9.62
ITGB2	0.28	2.13	7.57
C3	0.61	4.51	7.43
MICAL1	0.52	3.65	7.05
ITGA4	0.15	1.02	6.65
GSDME	0.18	1.17	6.42
CXCL13	0.19	1.09	5.70
GDF15	1.75	7.26	4.15
CD74	6.24	25.02	4.01
VCAN	0.53	1.76	3.32
HLA-DRA	4.36	13.28	3.04
BGN	1.27	3.76	2.96
PDGFRB	0.72	1.96	2.71
TGFBI	1.10	2.25	2.05
MCAM	6.73	15.35	2.28
FN1	10.12	16.61	1.64

Gene Case 2	Melanoma	Microsatellite	Fold Change
PAEP	0.07	1.95	24.85
MMP9	1.04	12.00	11.49
BGN	2.76	20.53	7.42
GDF15	1.50	11.16	7.41
C3	1.25	7.87	6.26
CXCL8	0.91	4.53	4.95
TGFBI	4.31	16.81	3.89
VCAN	2.02	6.73	3.32
GSDME	0.42	1.15	2.72
ITGA4	0.68	1.72	2.51
MICAL1	3.30	8.14	2.46
ITGB2	1.04	2.51	2.40
HLA-DRA	14.59	33.84	2.31
CXCL13	1.38	3.11	2.25
CD53	0.72	1.58	2.18
PDGFRB	3.57	7.58	2.12
CD37	0.69	1.44	2.08
CD74	19.50	40.09	2.05
EMILIN2	1.04	1.92	1.85
MCAM	15.25	24.69	1.62
FN1	49.01	98.28	2.00

- immuno-evasion
- immune response
- tissue remodeling
- tissue remodeling, fibroblast derived
- melanoma chemokines
- angiogenesis
- lymphovascular invasion
- anoikis resistance
- diapedesis
- survival at metastatic site
- pyroptosis
- negative regulator of apoptosis

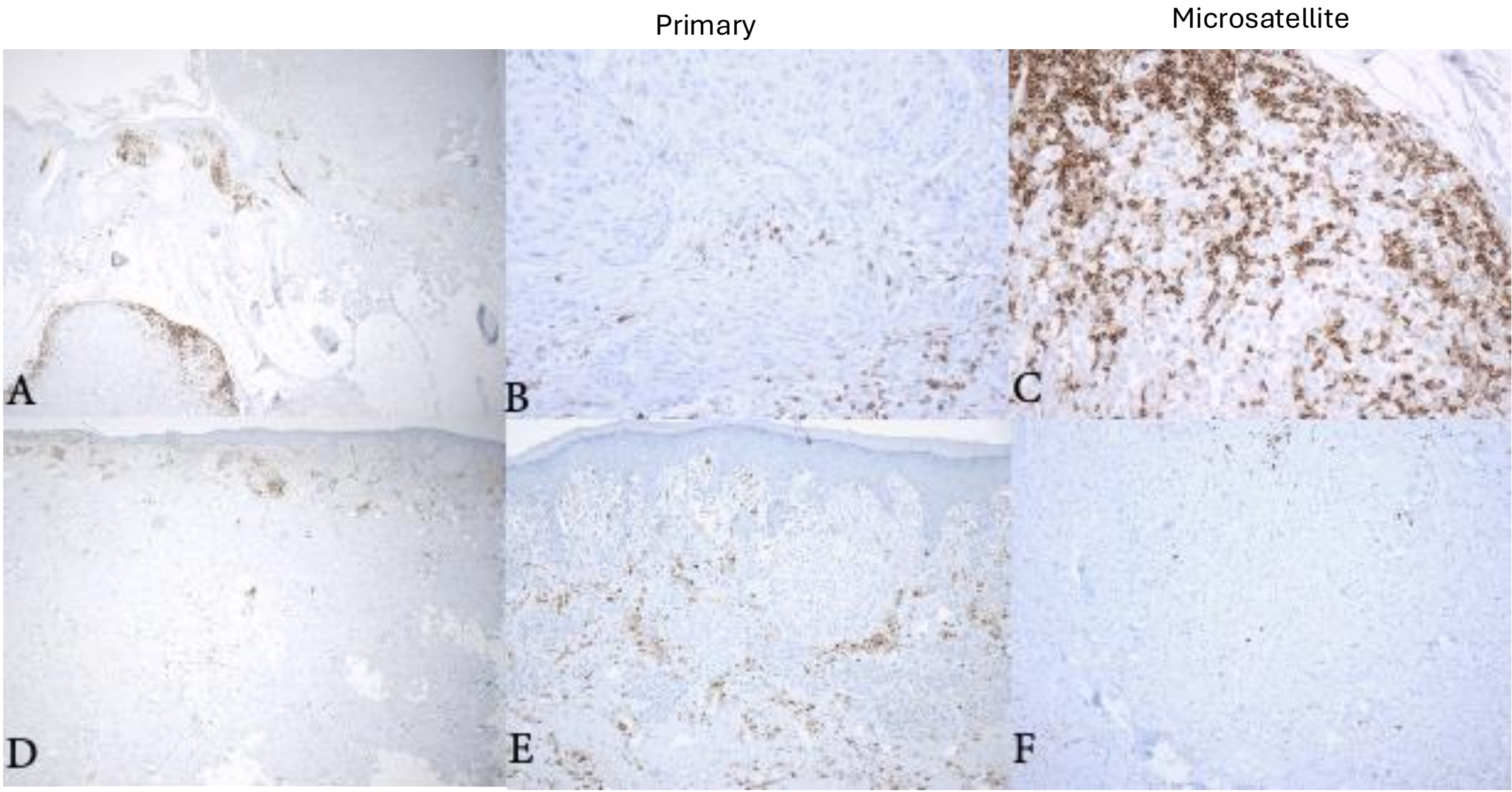
Normalized expression for selected *non-recurrent* overexpressed genes, ranked by fold increase in microsatellite over primary melanoma

Gene Case 1	Melanoma	Microsatellite	Fold change
QPCT	0.37	21.84	58.39
IGLV3-1	0.12	4.67	37.95
IGHM	0.06	1.75	26.75
TRBJ2-2	0.05	1.06	20.70
IL7R	0.07	1.36	18.95
LTB	0.16	2.93	18.04
FCMR	0.09	1.42	15.31
IGLC1	0.26	3.97	15.25
FRZB	0.16	2.23	13.47
IGHG3	0.32	4.31	13.36
CD3D	0.11	1.42	13.30
CD3E	0.09	1.13	12.86
IGHG2	0.16	2.02	12.85
TRBC1	0.20	2.59	12.69
SKAP1	0.12	1.49	12.68
IL32	0.83	10.25	12.28
TRAC	0.23	2.59	11.12
ACAP1	0.12	1.23	10.36
CD52	0.13	1.36	10.25
TRBC2	0.34	3.43	10.01
IL2RG	0.14	1.28	9.21
RAC2	0.17	1.58	9.18
CXCR4	0.36	3.23	9.06
CHST11	0.24	2.05	8.55
PTPRC	0.39	3.15	8.13
UNC13D	0.16	1.32	8.00
CXCL9	0.42	3.20	7.68
PLSCR1	1.24	9.34	7.51
ARHGAP9	0.16	1.20	7.49
ICAM1	1.22	8.79	7.19
IRF8	0.15	1.04	6.97
BST2	1.32	8.72	6.57
CCND1	0.22	1.42	6.34
IRF1	0.50	2.14	4.28
CD276	2.05	7.91	3.86
ITGB3	1.43	3.18	2.22

Gene Case 2	Melanoma	Microsatellite	Fold change
MMP13	0.18	13.37	73.97
TFPI2	0.09	6.35	64.73
MMP3	0.06	3.09	49.31
SFRP4	0.02	1.02	43.41
SULF1	0.20	7.58	37.11
SPP1	2.06	68.01	32.96
PRR4	0.06	1.95	31.16
OLR1	0.07	2.03	27.26
PMP2	0.06	1.58	25.17
GPC6	0.06	1.61	24.13
PI16	0.47	6.89	14.50
DSG2	0.11	1.59	14.49
TGM2	0.19	2.53	13.17
ABI3BP	0.33	3.03	9.09
COLEC12	0.45	3.75	8.32
TREM2	0.16	1.35	8.04
MGP	0.27	2.05	7.59
TIMP3	7.76	53.15	6.84
CD86	0.20	1.18	5.68
TGFB3	0.28	1.55	5.51
MMP1	2.82	15.16	5.37
MRC1	0.52	2.64	5.05
ADAM12	0.62	3.00	4.84
TIMP1	10.62	39.59	3.72
SERPINE1	1.62	5.00	3.08
NOTCH4	0.37	1.13	3.08
ITGA5	0.98	2.82	2.88
FBN1	5.52	14.39	2.60
HEG1	0.76	1.94	2.53
CDH5	1.07	2.61	2.41
NRP1	2.01	4.57	2.26
AXL	0.82	1.88	2.29
SPARC	41.43	85.41	2.06
SFRP2	1.52	3.01	1.97
FXYD3	10.70	20.74	1.93
COL1A1	83.55	156.24	1.87

- immuno-evasion
- immune response
- tissue remodeling
- tissue remodeling, fibroblast derived
- chemokines
- angiogenesis
- vasculogenic mimicry
- adhesion
- EMT
- MAP kinase activation
- diapedesis

LCA (Leukocyte common antigen) in primary melanomas and their microsatellites.



Immune evasion in microsatellites

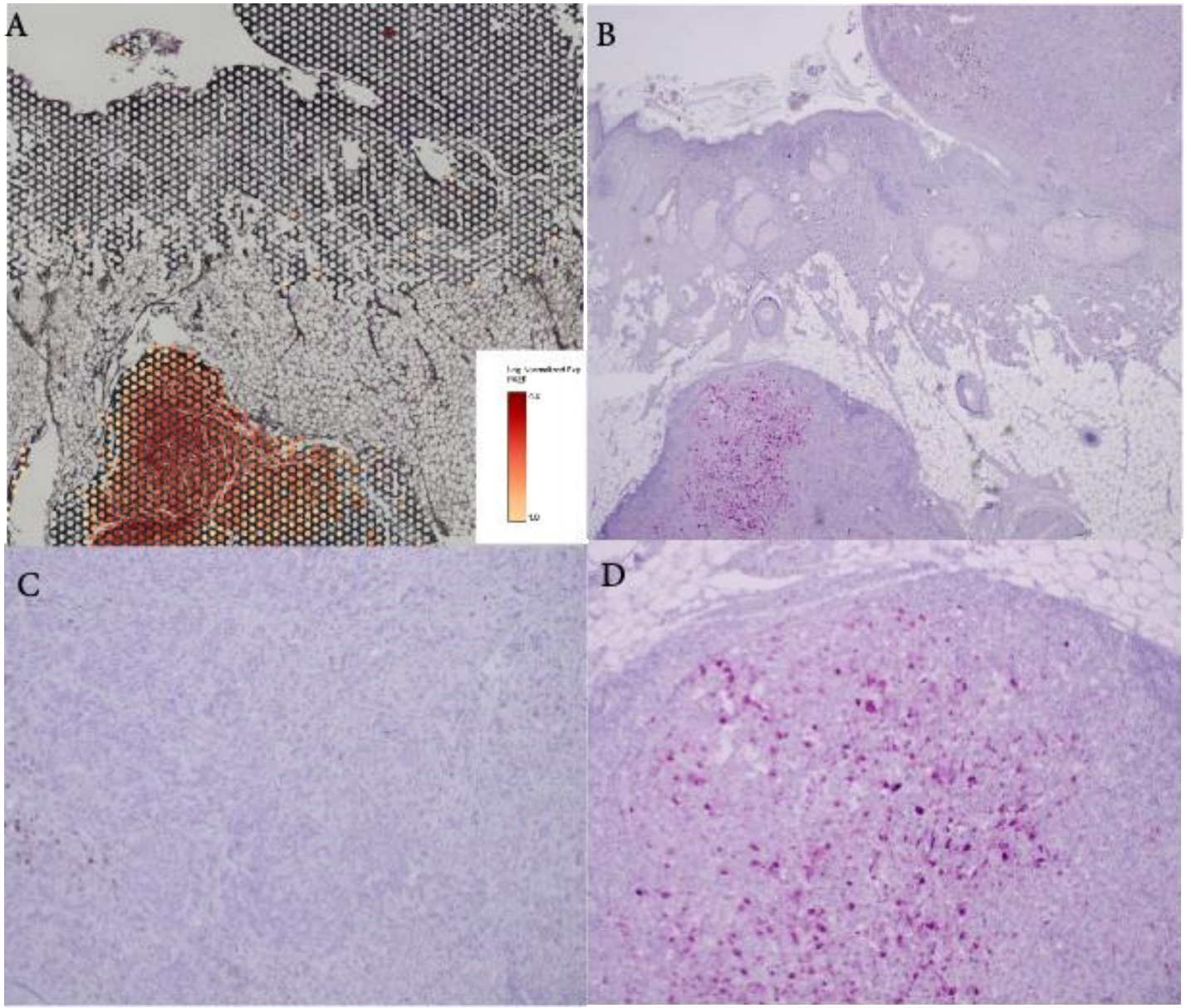
- Microsatellites exhibited increased host immune responses
- Four recurrently overexpressed mediators of immune-evasion were present (PAEP, GDF15, CD74, HLA-DRA)
- Implies selection pressure from the increased immune response.
- Seven additional non-recurrent mediators of immune-evasion were identified in microsatellites, overexpressed up to 58 fold in microsatellites

PAEP
(progestagen associated endometrial protein)

- PAEP was 275-fold and 25-fold overexpressed respectively in microsatellites in 2 cases.
- We studied PAEP protein expression by immunohistochemistry in a larger group of 12 and found overexpression in microsatellites in 5 of 12 patients.

PAEP RNA and protein expression in primary melanoma and microsatellite

PAEP RNA (Visium)

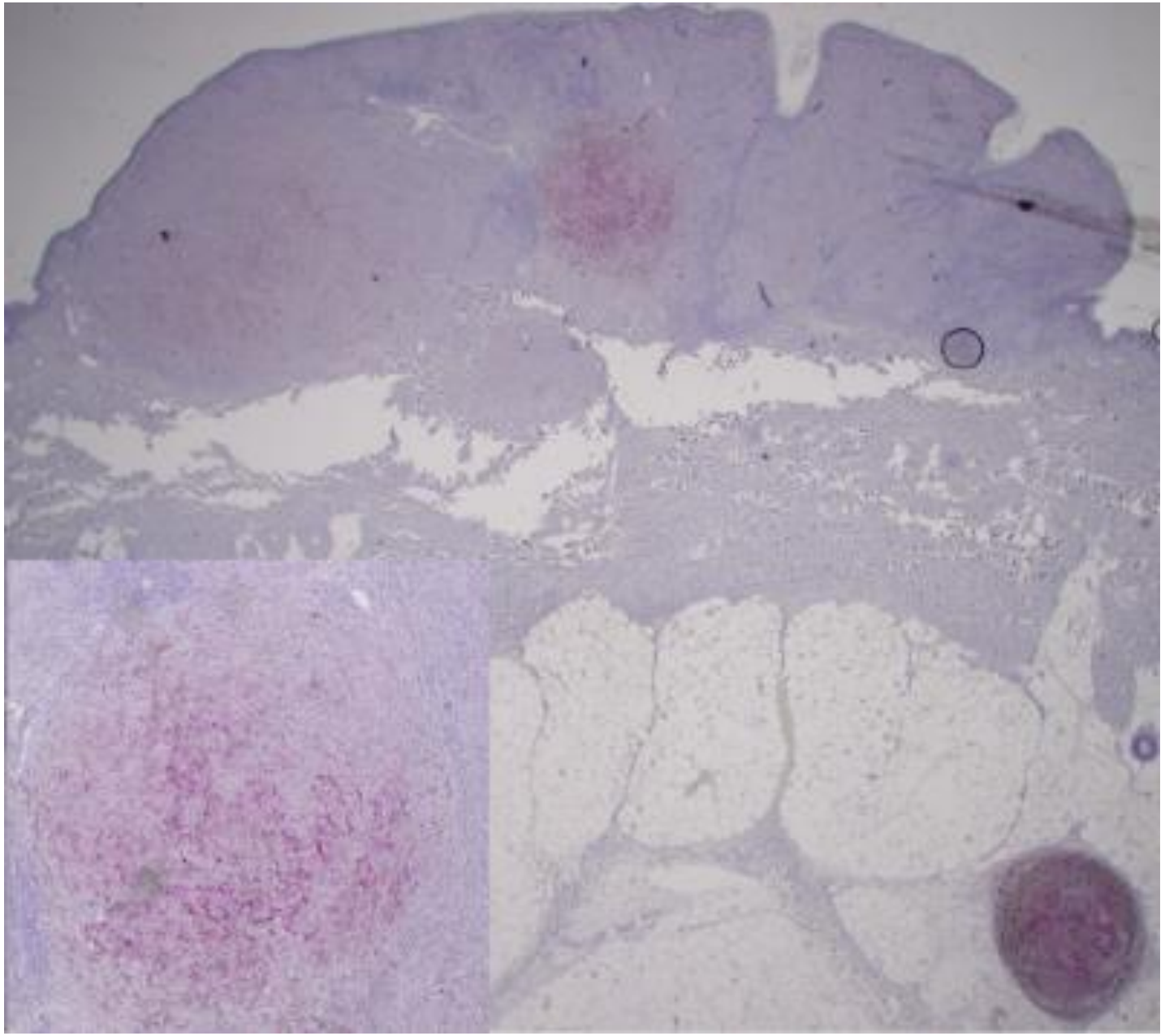


PAEP IHC

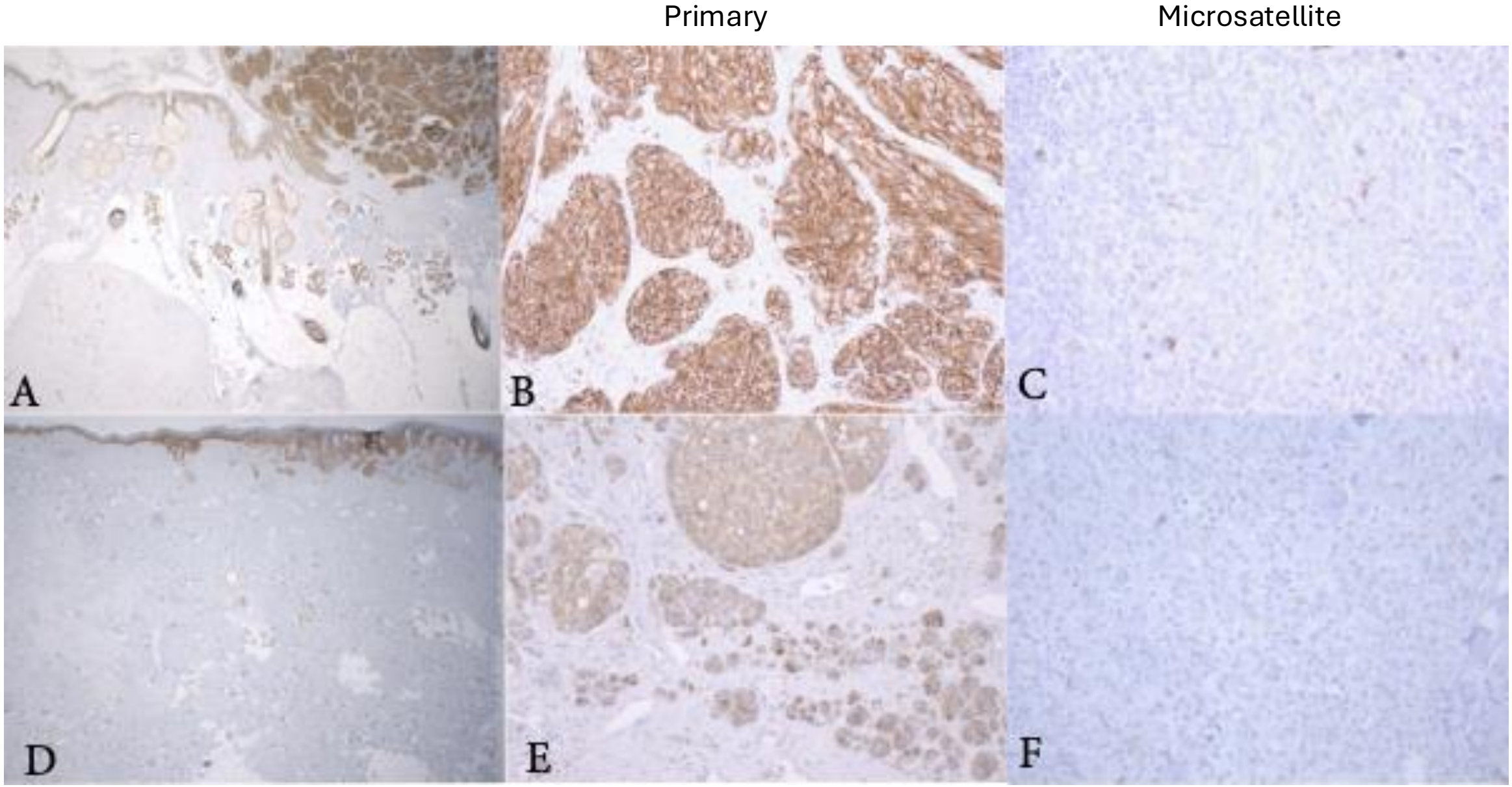
Primary

Microsatellite

PAEP immunostain is positive in the microsatellite and a subclone in the primary melanoma



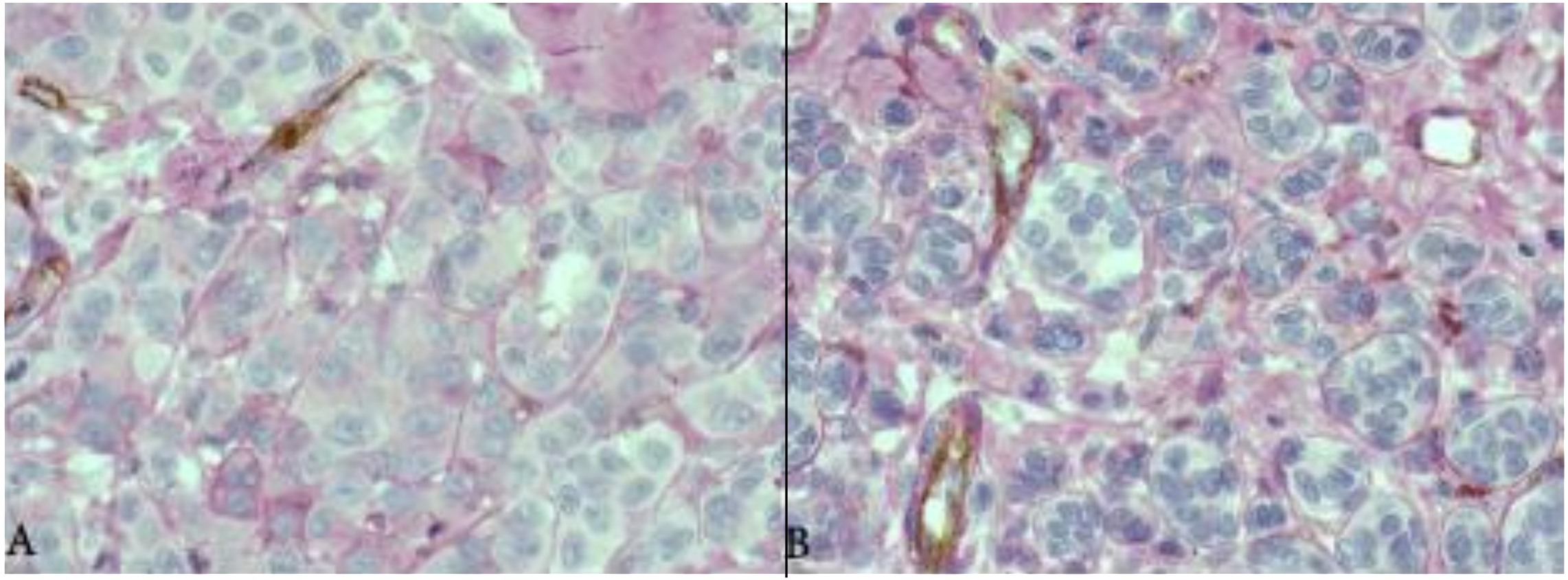
E Cadherin immunostain is positive in the primary melanomas and lost in the microsatellites



Combined CD31/ PAS stain supports that vasculogenic mimicry is present in the microsatellite in case 2 but not its primary

Microsatellite

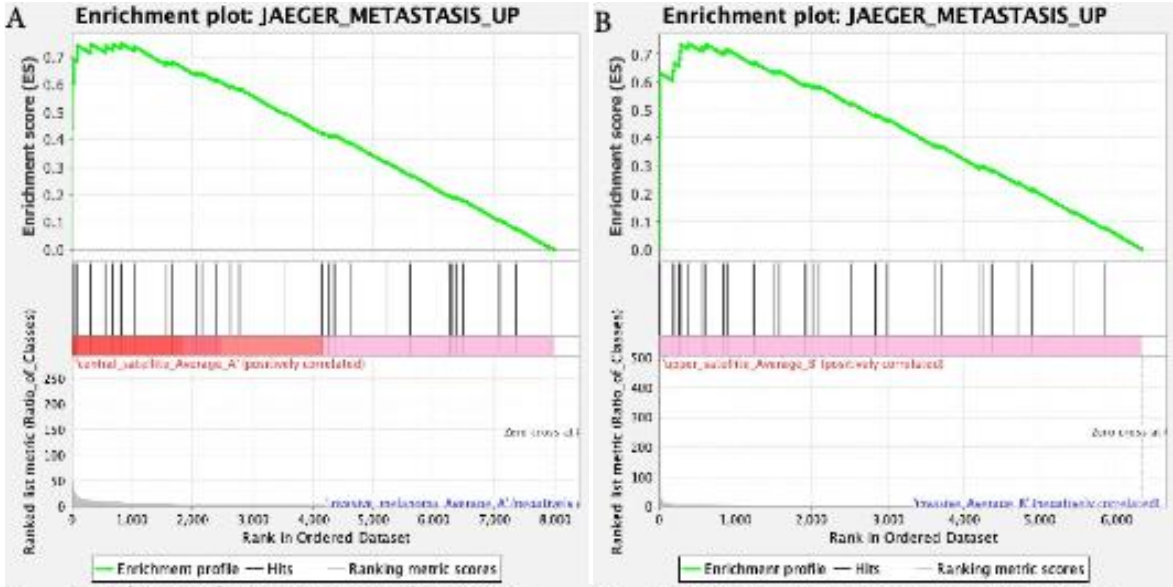
Primary



Comparison to defined set of genes from 19 primary melanoma and 22 *distant* metastases

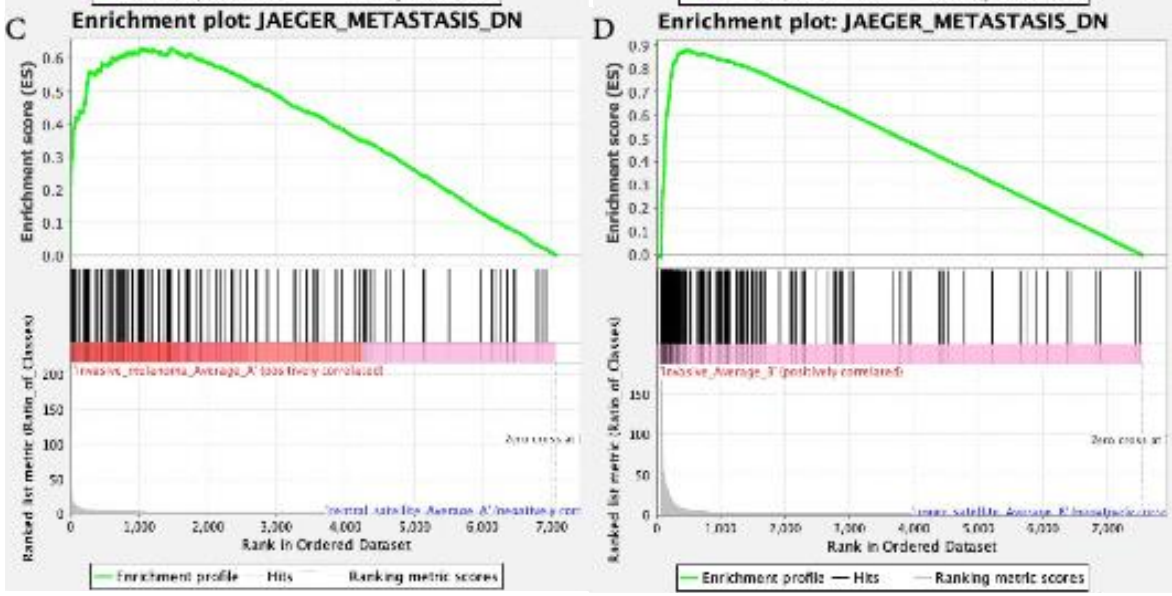
Case 1 | Case 2

p=0.003



p=0.003

p=0.0



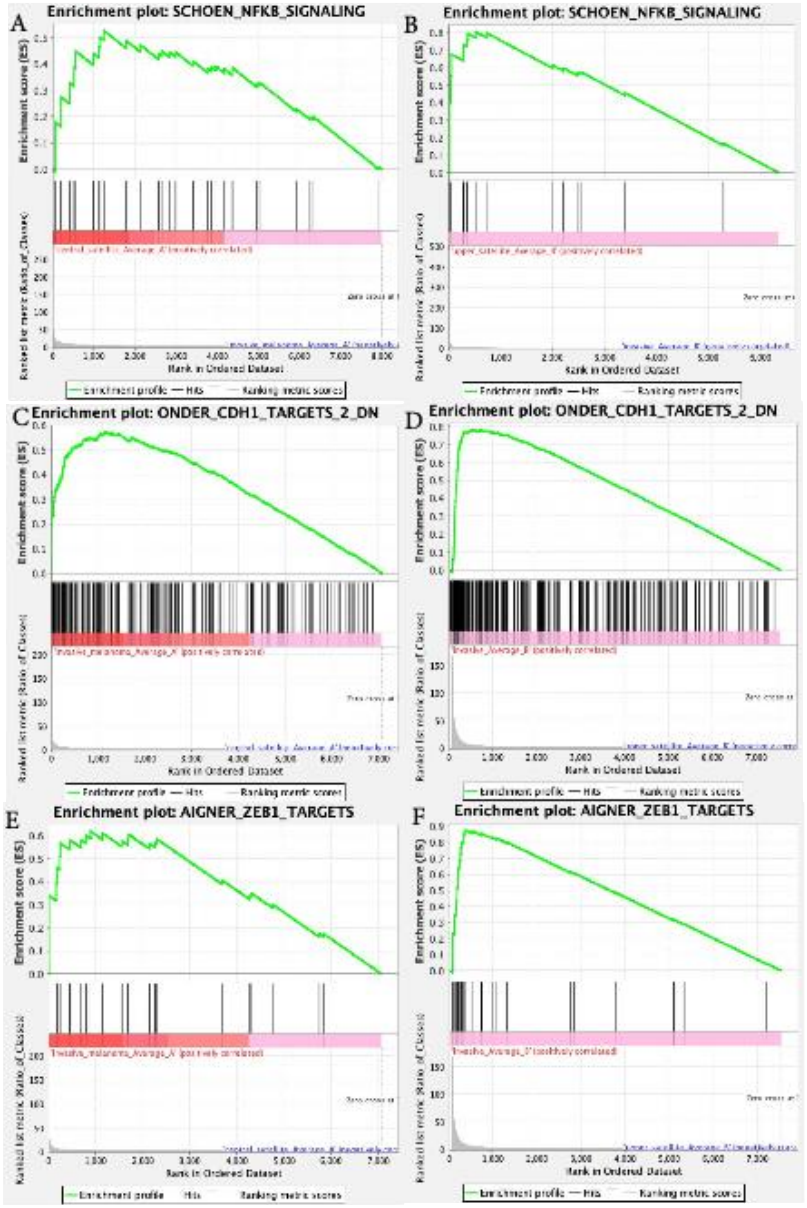
p=0.0

Comparison to defined sets of genes for NFKB, CDH1 and ZEB1 pathway signaling

Case 1 Case 2

p=0.11

p=0.011



p=0.0

p=0.0

p=0.03

p=0.0

Conclusions

- These preliminary findings suggest that melanoma microsatellites are true metastases at the level of gene expression
- Important processes in evolution of microsatellites likely include vascular invasion, survival in the circulation, vascular exit, survival at metastatic sites, matrix remodeling, angiogenesis, immuno-evasion
- These findings provide molecular context to the sharply increased risk of disease progression in patients with microsatellites.
- *Larger studies needed*