Supplementary Information for:

Tick extracellular vesicles impair epidermal homeostasis through immune-epithelial networks during hematophagy

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Supplementary Figure Legends

Supplementary Figure 1: DETC flow cytometry gating strategy. 5 mm skin punch biopsies were obtained from the bite of ticks and compared to the naïve skin followed by flow cytometry analysis. Representative flow cytometry plots were gated for **(A)** DETCs ($Vγ5^+$) and **(B)** DETC co-receptors ($JAML^+$, $NKG2D^+$, $CD25^+$, $CD69^+$, $CD100^+$ or $CD44^+$).

Supplementary Figure 2: ScRNAseq data filtration. Composite datasets of FVB-Jax and FVB-Tac samples included 20,640 cells **(A)** before filtration by scran (R package). **(B)** tSNE plot of fixed threshold filtration, set to 2500-60,000 UMIs. **(C)** Doublet finder (R package) of dataset. tSNE was colored by the doublet score. **(D)** tSNE plot after fixed threshold filtration and doublet finder analysis.

Supplementary Figure 3: Expression of keratinocyte-specific markers. (A) Graphical illustration of keratinocyte stratified layers with select marker genes. tSNE of keratinocyte clusters depicting gene expression of **(B)** *Krt14*, **(C)** *Krt5*, **(D)** *Krt1*, **(E)** *Krt10* and **(F)** *Ivl*.

Supplementary Figure 4: Expression of hair follicle-specific markers. (A) Graphical illustration of hair follicle microanatomy with select marker genes. tSNE of keratinocyte clusters depicting gene expression of **(B)** *Shh*, **(C)** *Krt75*, **(D)** *Lgr5*, **(E)** *Mgst1* and **(F)** *Krt79*.

Supplementary Figure 5: Epidermally-enriched tSNE plots. (A-F) Individual tSNE plots of keratinocyte, T cell and antigen presenting cell clusters in **(A)** FVB-Jax=1174 cells, **(B)** FVB-Jax sc*V33*=651 cells, **(C)** FVB-Jax si*V33*=545 cells, **(D)** FVB-Tac=836 cells, **(E)** FVB-Tac sc*V33*=1139 cells and **(F)** FVB-Tac si*V33*=828 cells.

Supplementary Figure 6: Keratinocyte-specific markers along pseudotime trajectory. (A) *Krt14*, **(B)** *Krt1*, and **(C)** *Ivl* gene expression along pseudotime values (*x* axis) for naïve, sc*V33*-, or si*V33*-tick bites on FVB-Jax or FVB-Tac mice. Cells colored by clusters from keratinocyte tSNE plot (as shown in Figure **4H**) ordered across the pseudotime (x-axis).

Supplementary Figure 7: Flow cytometry gating strategy in keratinocyte. 5 mm punch biopsies were obtained from the bite site of ticks or naïve skin and processed for flow cytometry. Representative flow cytometry plots were gated for **(A)** $EpCAM^+$ keratinocytes, **(B)** $Ki67^+$, **(C)** Anti-rabbit IgG^+ for PI3K and **(D)** p-$PI3K^+$ keratinocytes.

Supplementary Table 1: Primers and siRNA sequences

Target	Type	Location Within mRNA	SiRNA/Primer Name	Strand	Primer Sequence
I. scapularis Vamp33	siRNA	Start at 300	Vamp33SiF	Forward	AAGGACACAGTGTGGAGAGATCCTGTCTC
			Vamp33SiR	Reverse	AAATCTCTCCACACTGTGTCCCCTGTCTC
	Scrambled		Vamp33ScF	Forward	AAGTGAGGGACGCATAGTAGACCTGTCTC
			Vamp33ScR	Reverse	AATCTACTATGCGTCCCTCACCCTGTCTC
	qRT-PCR	Start at 76	Vamp33qR-F	Forward	TCATACCTGAAGTTGTCCAA
		Start at 180	Vamp33qR-R	Reverse	CTCAAGAATGCCACTGTTG
D. variabilis Vamp33	siRNA	Start at 280	Vamp33Derm-SiF	Forward	AACCGGATGGTGATGTTAATCCCTGTCTC
			Vamp33Derm-SiR	Reverse	AAGATTAACATCACCATCCGGCCTGTCTC
	Scrambled		Vamp33Derm-ScF	Forward	AAGCCTCAGGTGGAATTAGTTCCTGTCTC
			Vamp33Derm-ScR	Reverse	AAAACTAATTCCACCTGAGGCCCTGTCTC
	qRT-PCR	Start at 78	Vamp33DqR-2F	Forward	TCACCTAAAGTTGTCCAACCC
		Start at 160	Vamp33DqR-2R	Reverse	TCAAGGATTCCACTGTTGGG
	qRT-PCR	Start at 64	Vamp33DqR-F	Forward	GACGTGGTTACTTCTCACCTAAA
		Start at 148	Vamp33DqR-R	Reverse	CTGTTGGGCCGTACACAATA
A. americanum Vamp33	siRNA	Start at 280	Vamp33AA-SiF	Forward	AACCAGATGGTGATGTGAACCCCTGTCTC
			Vamp33AA-SiR	Reverse	AAGGTTCACATCACCATCTGGCCTGTCTC
	Scrambled		Vamp33AA-ScF	Forward	AAGGGTGCTAACACGGACTTACCTGTCTC
			Vamp33AA-ScR	Reverse	AATAAGTCCGTGTTAGCACCCCCTGTCTC
	qRT-PCR	Start at 76	Vamp33AA qR-F	Forward	TCTCATCTAAAGCTTTCCAACCC
		Start at 160	Vamp33AA qR-R	Reverse	AAGGATGCCACTGTTGGG
I. scapularis β-actin	qRT-PCR	Start at 618	ActinIS 2F	Forward	GGTCATCACAATCGGCAAC
		Start at 742	ActinIS *2*R	Reverse	ATGGAGTTGTACGTGGTCTC
A. americanum β-actin	qRT-PCR	Start at 285	ActinAA F	Forward	GTCATGGTCGGCATGGG
		Start at 365	ActinAA R	Reverse	ATGCCGTGCTCAATGGG
D. variabilis β-actin	qRT-PCR	Start at 798	ActinIS 2F	Forward	GGTCATCACAATCGGCAAC
		Start at 905	ActinIS *2*R	Reverse	ATGGAGTTGTACGTGGTCTC

Supplementary Table 2: Cell markers

Cell type	Cell population	Gene	Ensembl
Antigen presenting cells	All	*H2-Aa*	ENSMUSG00000036594
		H2-Ab1	ENSMUSG00000073421
	Langerhans	*Cd207*	ENSMUSG00000034783
	Dendritic cells	*Itgax*	ENSMUSG00000030789
		Cd80	ENSMUSG00000075122
		Cd86	ENSMUSG00000022901
		Ly75	ENSMUSG00000026980
		Cd209a	ENSMUSG00000031494
	Macrophage	*Adgre1*	ENSMUSG00000004730
		Itgam	ENSMUSG00000030786
Keratinocytes	Undifferentiated (Stratum basale)	*Krt5*	ENSMUSG00000061527
		Krt14	ENSMUSG00000045545
		Krt15	ENSMUSG00000054146
	Differentiating (Stratum spinosum)	*Krt 1*	ENSMUSG00000046834
		Krt2	ENSMUSG00000064201
		Krt10	ENSMUSG00000019761
	Terminally differentiated (Stratum granulosum)	*Ivl*	ENSMUSG00000049128
		Flg	ENSMUSG00000102439
	Corneocytes (Stratum corneum)	*Klk5*	ENSMUSG00000074155
T cells	All	*Cd3d*	ENSMUSG00000032094
		Cd3e	ENSMUSG00000032093
		Cd3g	ENSMUSG00000002033
	αβ T cell	*Trac*	ENSMUSG00000076928
		Trbc1	ENSMUSG00000076490

		Trbc2	ENSMUSG00000076498
		CD4	ENSMUSG00000023274
		CD8a	ENSMUSG00000053977
	γδ T cell	*Trdc*	ENSMUSG00000104876
		Tcrg-C1	ENSMUSG00000076749
		Tcrg-C2	ENSMUSG00000076752
		Tcrg-C4	ENSMUSG00000076757
	DETCs	*Trdv4*	ENSMUSG00000076867
		Sema4d	ENSMUSG00000021451
		Jaml	ENSMUSG00000048534
		Klrk1	ENSMUSG00000030149
Endothelial		*Cldn5*	ENSMUSG00000041378
		Cdh5	ENSMUSG00000031871
		Egfl7	ENSMUSG00000026921
Fibroblasts		*Dcn*	ENSMUSG00000019929
		Fgf2	ENSMUSG00000037225
		Fgf7	ENSMUSG00000027208
Hair follicle	Upper hair follicle	*Krt79*	ENSMUSG00000061397
		Krt17	ENSMUSG00000035557
	Sebaceous gland	*Mgst1*	ENSMUSG00000008540
		Scd1	ENSMUSG00000037071
	Outer bulge	*Postn*	ENSMUSG00000027750
		Lgr5	ENSMUSG00000020140
	Inner bulge	*Krt6a*	ENSMUSG00000058354
		Krt75	ENSMUSG00000022986
	Base of follicle	*Shh*	ENSMUSG00000002633
	Hair germ	*Gli1*	ENSMUSG00000025407
Melanocytes		*Dct*	ENSMUSG00000022129

		Mlana	ENSMUSG00000024806
		Tyr	ENSMUSG00000004651
Red blood cells		*Hbb-bs*	ENSMUSG00000052305
		Hbb-bt	ENSMUSG00000073940
Schwann cells		*Mal*	ENSMUSG00000027375
		Plp1	ENSMUSG00000031425
		Sox10	ENSMUSG00000033006
Smooth muscle cells		*Acta2*	ENSMUSG00000035783
		Myh11	ENSMUSG00000018830
		Myl9	ENSMUSG00000067818
		Myocd	ENSMUSG00000067818
		Rgs5	ENSMUSG00000026678

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989 **Supplementary Table 3**: Resources and reagents available

Antibody	Source	Identifier	Dilution/ Concentration
7-AAD Viability Staining Solution	Biolegend	420404	1:500
Zombie Violet Fixable Live Dead stain	Biolegend	423113	1:500
Anti-mouse CD16/32 Antibody	Biolegend	156603	1:500
APC anti-mouse CD45 Antibody clone: 30-F11	Biolegend	103111	1:100
FITC anti-mouse CD3 Antibody clone:17A2	Biolegend	100203	1:100
BV650 anti-mouse Vy3 Antibody clone: 536	BD	743241	1:50
PE Mouse Anti-Rat IgM clone:G53-238	BD	553888	1:100
Monoclonal antibody 17D1	Adrian Hayday, Kings College London and Robert Tigelaar, Yale University	N/A	1:50
PE/Cyanine7 anti-mouse CD45 Antibody clone: 30-F11	Biolegend	103114	1:100
APC anti-mouse Thy1.2 Antibody clone: 30-H12	Biolegend	105312	1:100
PE anti-mouse JAML Antibody clone: 4E10	Biolegend	128503	1:100
BV711 anti-mouse CD100 Antibody clone: BMA-12	BD	745492	1:100
PE/Cyanine5 anti-mouse CD44 Antibody clone: IM7	Biolegend	103010	1:100
APC/Cyanine7 anti-mouse CD25 Antibody clone: PC61	Biolegend	102026	1:100
PerCP/Cyanine5.5 anti-mouse CD69 Antibody clone: H1.2F3	Biolegend	104522	1:100
APC anti-mouse CD314 (NKG2D) Antibody clone: CX5	Biolegend	130212	1:100
Alexa Fluor 700 anti-mouse Ki-67 Antibody clone: 16A8	Biolegend	652420	1:50
BV711 anti-mouse CD326 (Ep-CAM) Antibody clone: G8.8	Biolegend	118233	1:100
PE anti-mouse CD200 Antibody clone: OX-90	Biolegend	123807	1:100

PE/Cyanine5 anti-mouse CD34 Antibody clone: MEC14.7	Biolegend	119312	1:100
BV605 anti-mouse Sca1 Antibody clone: D7	Biolegend	108133	1:100
PE anti-mouse CD49f Antibody clone: GoH3	Biolegend	313612	1:100
FITC Phospho-PI3K p85/p55 (Tyr458, Tyr199) Monoclonal Antibody clone: PI3KY458-1A11	ThermoFisher Scientific	MA5-36955	1:50
PI3K p85 alpha Monoclonal Antibody clone: SU04-07	ThermoFisher Scientific	MA5-41128	1:50
Alexa Fluor 405 Goat anti-Rabbit IgG (H+L) Secondary Antibody	ThermoFisher Scientific	A-31556	1:100

Cell media			
Leibovitz's L-15 Medium, powder	Gibco	41300039	N/A
L-aspartic acid	Millipore-Sigma	11189	0.449 g/L
L-glutamine	Millipore-Sigma	G8540	0.500 g/L
L-proline	Millipore-Sigma	81709	0.450 g/L
L-glutamic acid	Millipore-Sigma	49449	0.250 g/L
alpha-ketoglutaric acid	Millipore-Sigma	K1128	0.449 g/L
Sodium hydroxide	Millipore-Sigma	S8045	10 N
D-glucose	Millipore-Sigma	G7021	18.018 g/L
FBS (USDA approved; for tick media)	Millipore-Sigma	F0926-500ML	0.1
Bacto™ Tryptose Phophate Broth	BD	260300	5%
Lipoprotein Concentrate	MP Biomedicals	191476	0.1%
Corning® 100 mL Penicillin-Streptomycin Solution, 100x	Corning	30-002-CI	1:100
Amphotericin B	Gibco	15290-026	1:100
Distilled water	Gibco	15-230-147	N/A
Materials			
Cell culture plate with lid (6 well, flat bottom)	Sigma	SIAL0516	N/A

Cellstart®, 96 well cell culture plate, F-bottom	Greiner bio-one	655 160	N/A
Costar® cell culture plate with lid (24 well, flat bottom)	Corning	CLS3526-1EA	N/A
Nunc™ 96-Well Polystyrene Round Bottom Microwell Plates	Thermo Scientific	262162	N/A
Vannas spring scissors, 4 mm cutting edge straight	Fine Science	15018-10	N/A
Integra Miltex 2 mm biopsy punches	Integra	95039-098	N/A
Integra Miltex 5 mm biopsy punches	Integra	33-35	N/A
AcuPunch biopsy punches 10 mm	Acuderm	P1050	N/A
FALCON® 14 ml Polypropylene round-bottom tube	Corning	352059	N/A
Corning® cell strainer size 40 µm, blue, sterile	Corning	431750	N/A
Falcon 5 mL Round Bottom Polystyrene Test Tube, with Cell Strainer Snap Cap	Corning	352235	N/A
12x75mm Plastic Tubes	Globe Scientific	110441	N/A
1.5 mL Eppendorf Safe-Lock Tubes	USA Scientific	1615-5500	N/A
1.5ml Microcentrifuge Tubes with Socket Screw Caps	VWR	525-1238	N/A
Lo-bind tubes	Eppendorf	22431048	N/A
Vannas Spring Scissors - 4mm	Fine Science Tools	15018-10	N/A
Reagents			
Trypsin/Lys-C Mix, Mass Spec Grade	Promega	V5071	N/A
Bovine Serum Albumin	Millipore-Sigma	A2058-5G	N/A
BenchMark™ FBS	Gemini Bio-products	100-106	1:10
Methanol anhydrous, 99.8%	Millipore-Sigma	322415-1L	0-20%
Ethyl alconol, Pure; 200 proof for molecular biology	Millipore-Sigma	E7023-1L	1:1
TRIzol reagent	Ambion	15596018	N/A
Liberase™ TL Research Grade	Roche	5401020001	10%
RPMI-1640 with L-Glutamine	Quality Biological	722461	N/A

DNase I	Roche	11284932001	N/A
10X Phosphate-buffered saline (PBS)	Quality Biological	119-069-131	1X
Phosphate-buffered saline (PBS)	Gibco	10010-023	N/A
0.5M Ethylenediaminetetraacetic acid (EDTA), pH 8.0	Boston BioProducts	BM-150	N/A
Sodium azide (NaN3)	Sigma-Aldrich	S2002-25G	N/A
Paraformaldehyde	Thermo Fisher Scientific	J61899-AP	N/A
RIPA lysis buffer	Millipore	20-188	1X
Halt Protease Inhibitor Cocktail	Thermo Fisher Scientific	87786	1X
Halt™ Phosphatase Inhibitor Cocktail	Thermo Fisher Scientific	78420	1X
Dispase II	Sigma-Aldrich	D4693-1G	NA
Dispase	Gibco	17105-041	4 U/mL
Magnesium chloride (MgCl2)	Sigma-Aldrich	M2393-100G	5mM
Calcium chloride (CaCl2)	Sigma-Aldrich	C1016-100G	0.4mM
Collagenase D	Sigma-Aldrich	11088866001	2.5mg/mL
Gum rosin	Millipore-Sigma	60895	75% (3/4 parts)
Beeswax	Thermo Fisher Scientific	S25192A	25% (1/4 parts)
Commercial Assays			
Pure Link RNA mini kit	Ambion	12183025	N/A
Silencer™ siRNA Construction Kit	Thermo Scientific	AM1620	N/A
Verso cDNA Synthesis Kit	Thermo Scientific	AB-1453B	N/A
Pierce BCA Protein Assay Kit	Thermo Scientific	23227	N/A
DNeasy® Blood and Tissue kit	Qiagen	69506	N/A
FOXP3 Fix/Perm Buffer Set	BioLegend	421403	N/A

Human KGF/FGF-7 Quantikine ELISA Kit	R&D Systems	DKG00	NA
Organisms			
Ixodes scapularis adult partially fed female ticks	Albert Mulenga, Texas A&M University	N/A	N/A
Ixodes scapularis adult partially fed female ticks	Adela Oliva Chavez, Texas A&M University	N/A	N/A
Ixodes scapularis nymph ticks	Jon Oliver and Ulrike Munderloh, University of Minnesota	N/A	N/A
Ixodes scapularis nymph ticks	Tick Lab, Oklahoma State University	N/A	N/A
Amblyomma americanum nymph ticks	Tick Lab, Oklahoma State University	N/A	N/A
Dermacentor variabilis nymph ticks	Tick Lab, Oklahoma State University	N/A	N/A
C57BL6J (WT) mice	University of Maryland	N/A	N/A
C57BL6J (WT) mice	Jackson Laboratories	#000664	N/A
FVB/NJ mice	The Jackson Laboratory	001800	N/A
FVB/NTac mice	Taconic Biosciences	N/A	N/A
Equipment			
CFX96 Touch Real-Time PCR Detection System	Biorad	N/A	N/A
Nanoject III	Drummond Scientific Company	3-000-207	N/A
NanoSight NS300	Malvern Panalytical	N/A	N/A
Percival I30BLL incubator	Percival	I30BLL	N/A
Software			
GraphPad Prism v9.1.2	https://www.graphpad.com/	N/A	N/A
Graphpad Quick Cals Outlier Calculator	https://www.graphpad.com/quickcalcs/Grubbs1.cfm	N/A	N/A
FlowJo (v10.6.1 – 10.8.1)	https://www.flowjo.com/	N/A	N/A
QIAGEN Ingenuity Pathway Analysis	https://digitalinsights.qiagen.com/products-overview/discovery-insights-	N/A	N/A

	portfolio/analysis-and-visualization/qiagen-ipa/		
Database for Annotation, Visualization and Integrated Discovery (DAVID) (version 2021)	https://david.ncifcrf.gov/	N/A	N/A

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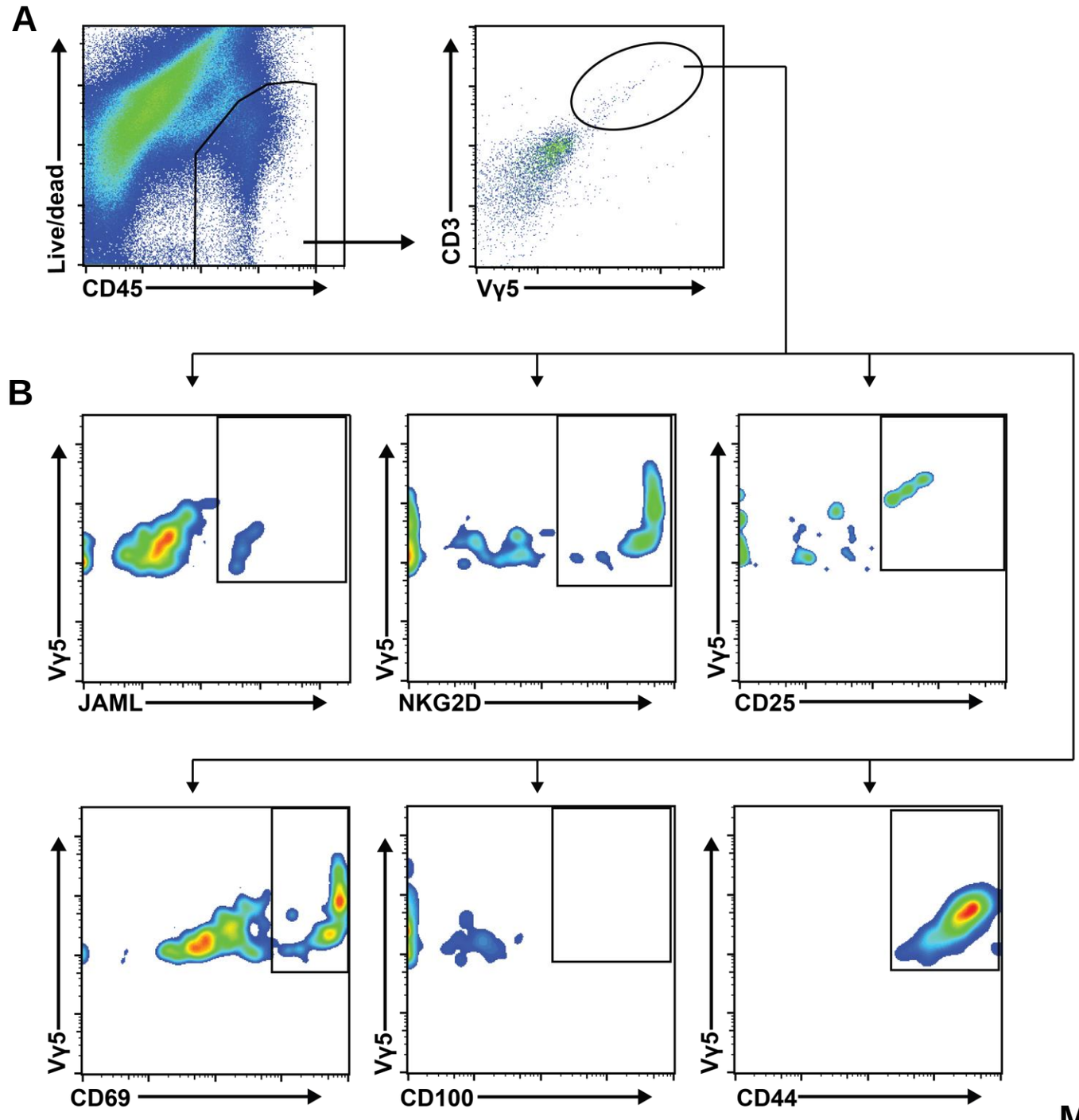
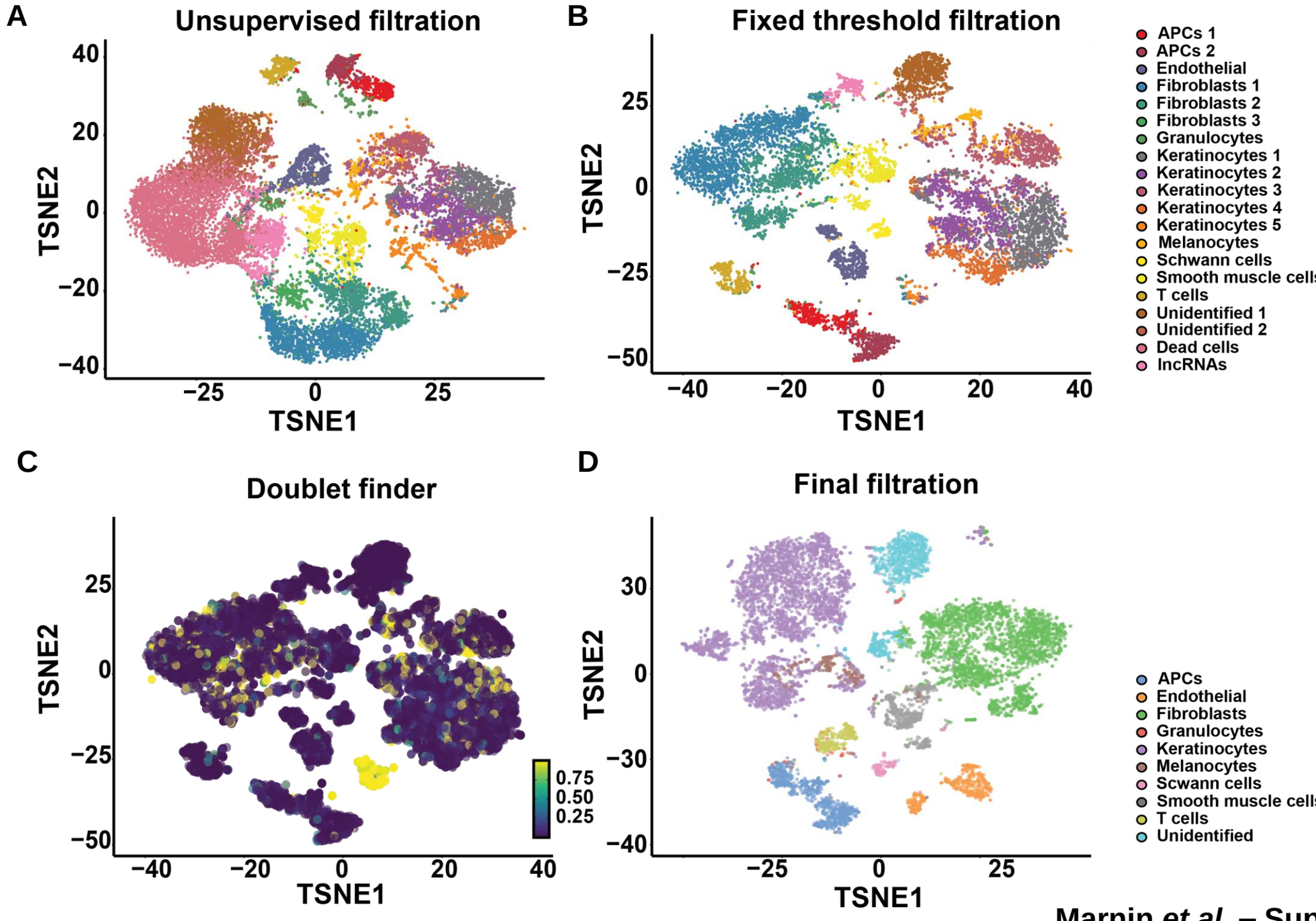


Marnin *et al.* – Supp. Figure 1



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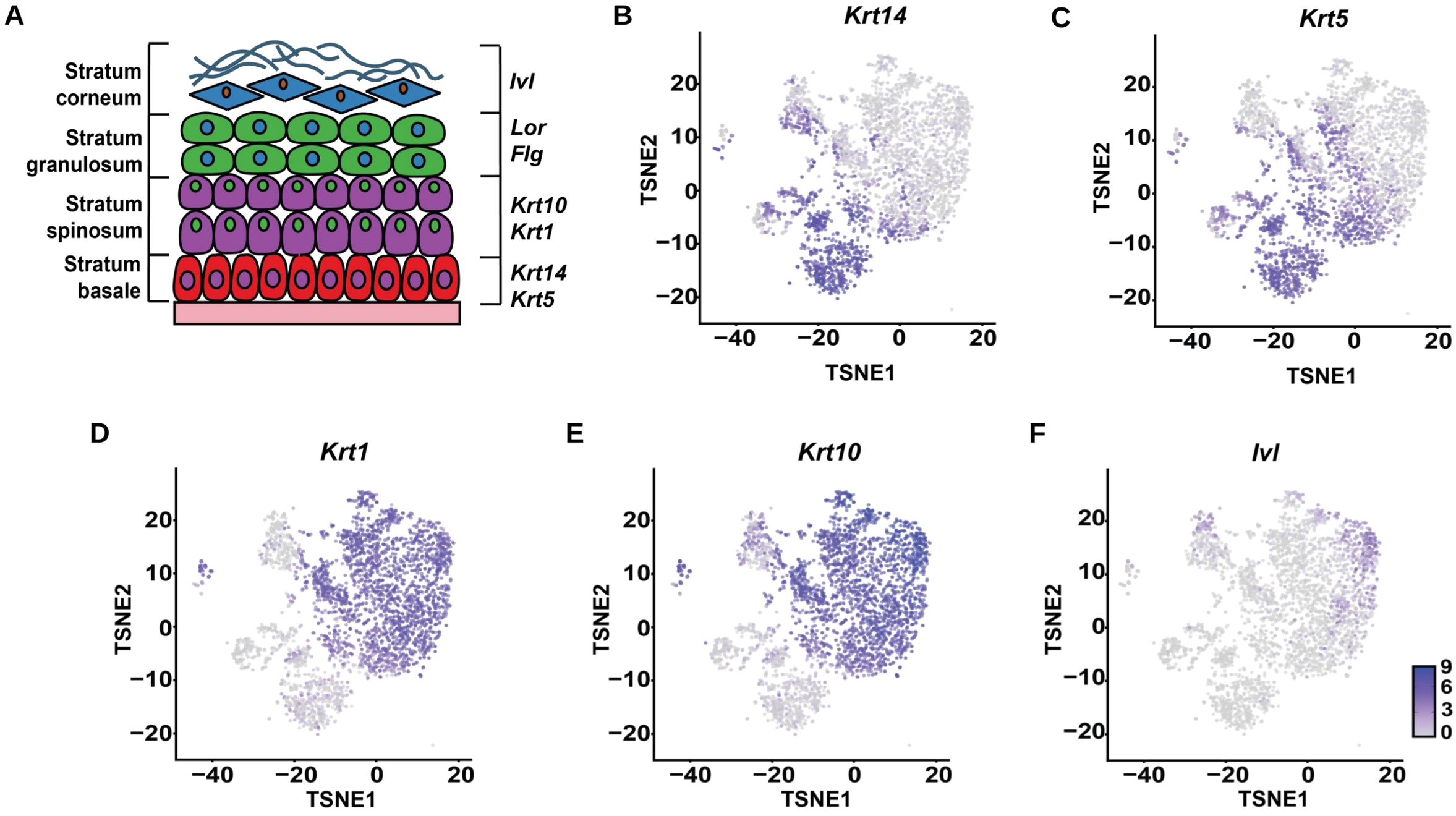

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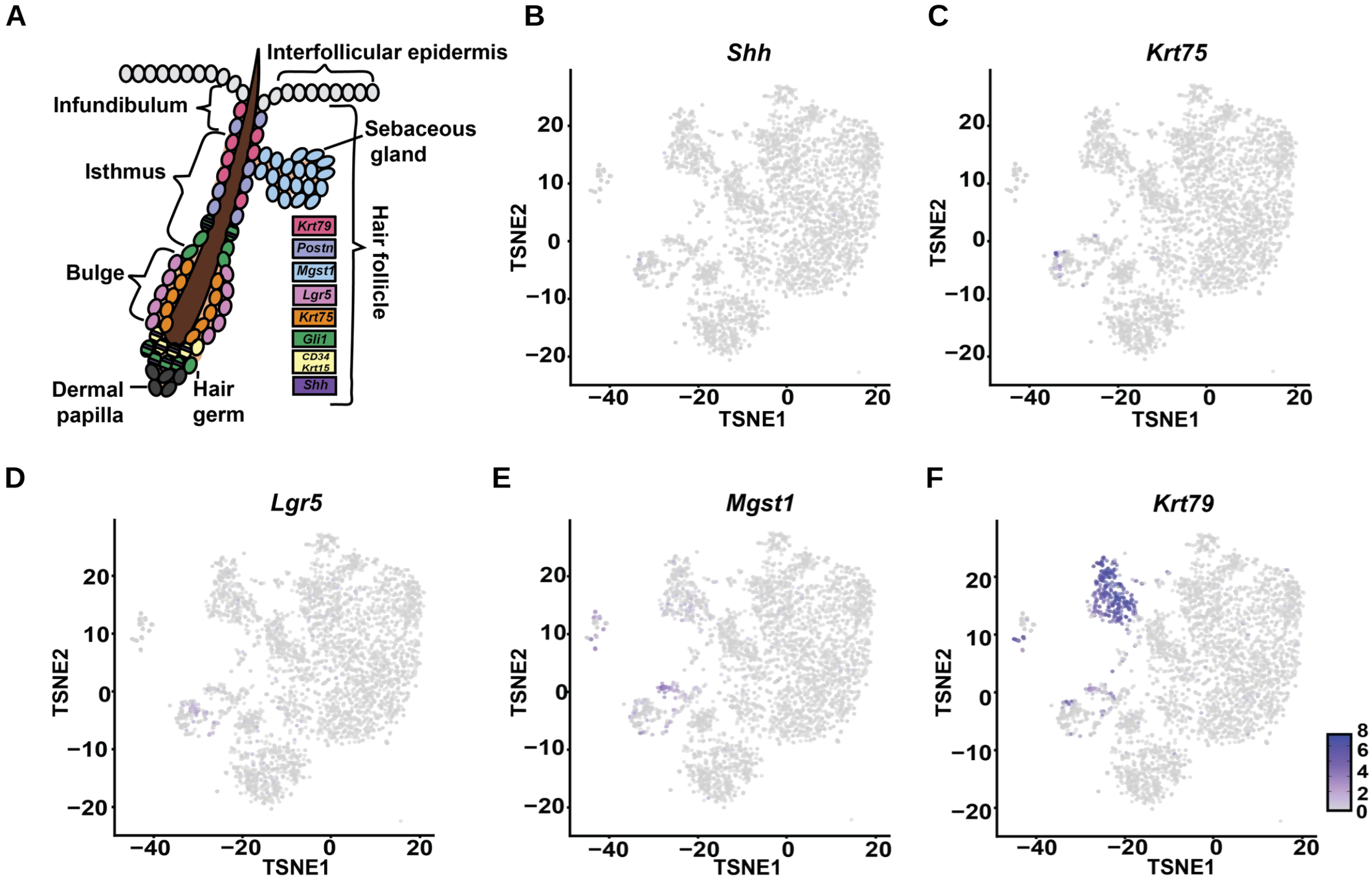

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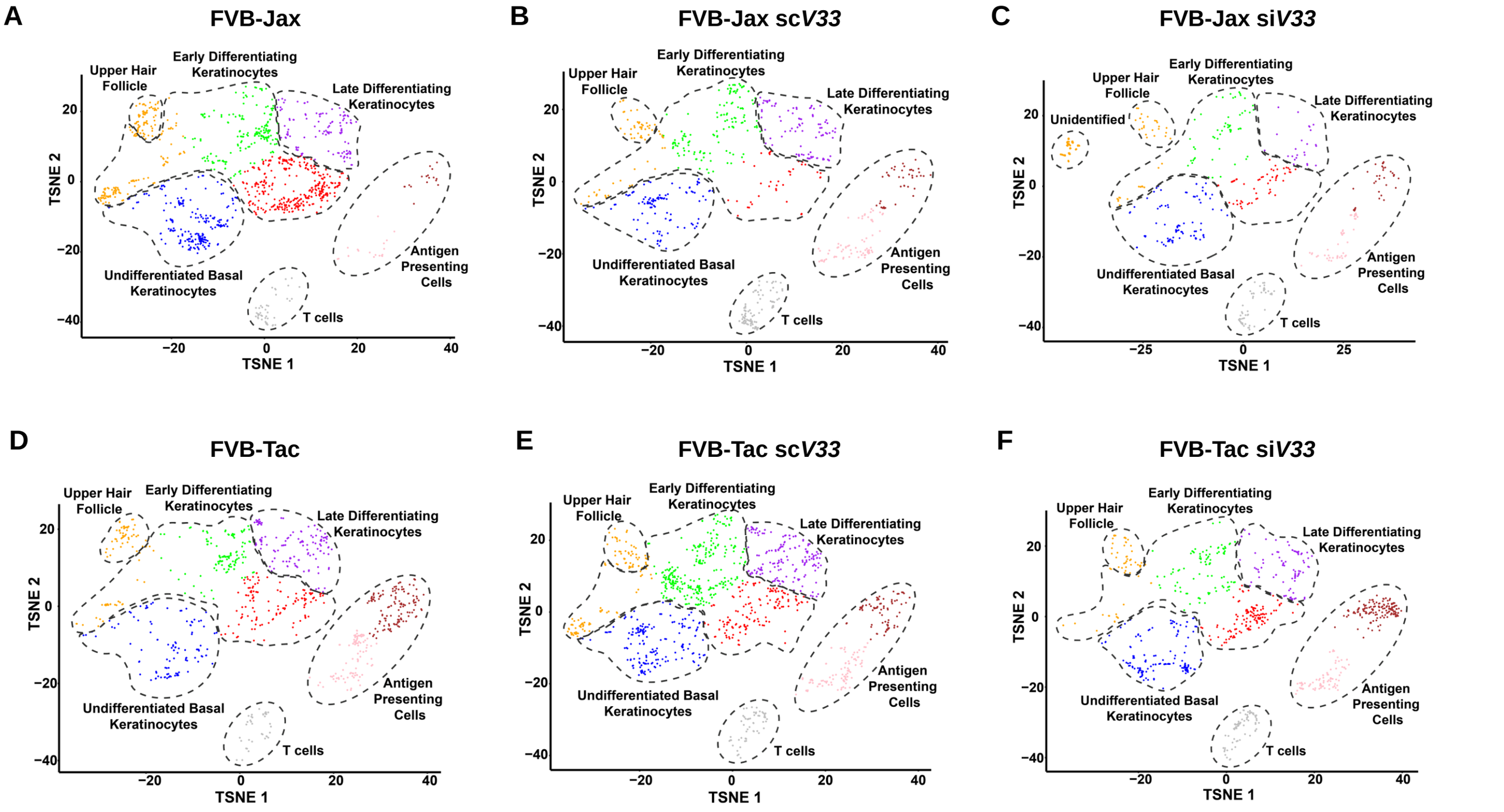

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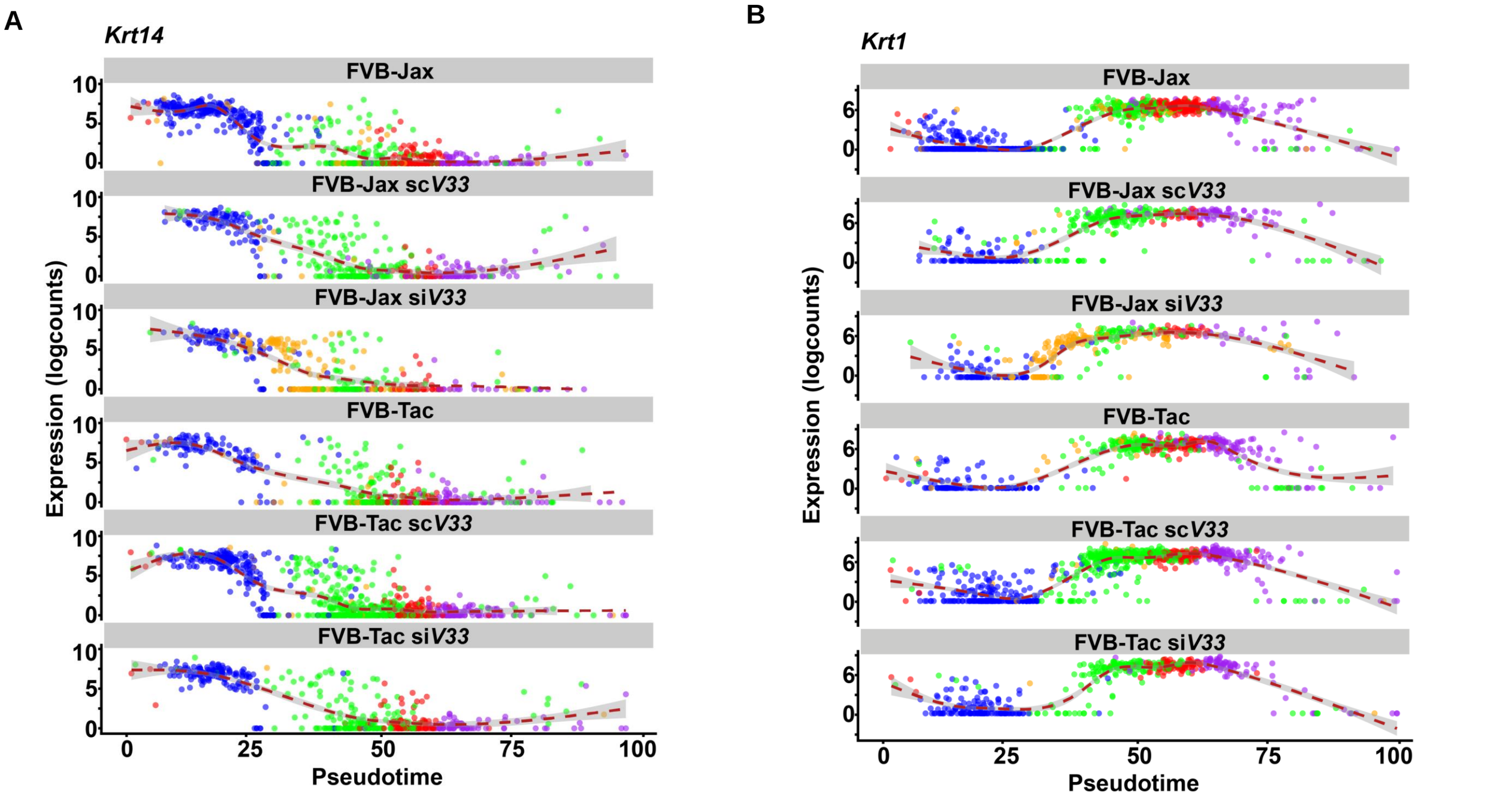

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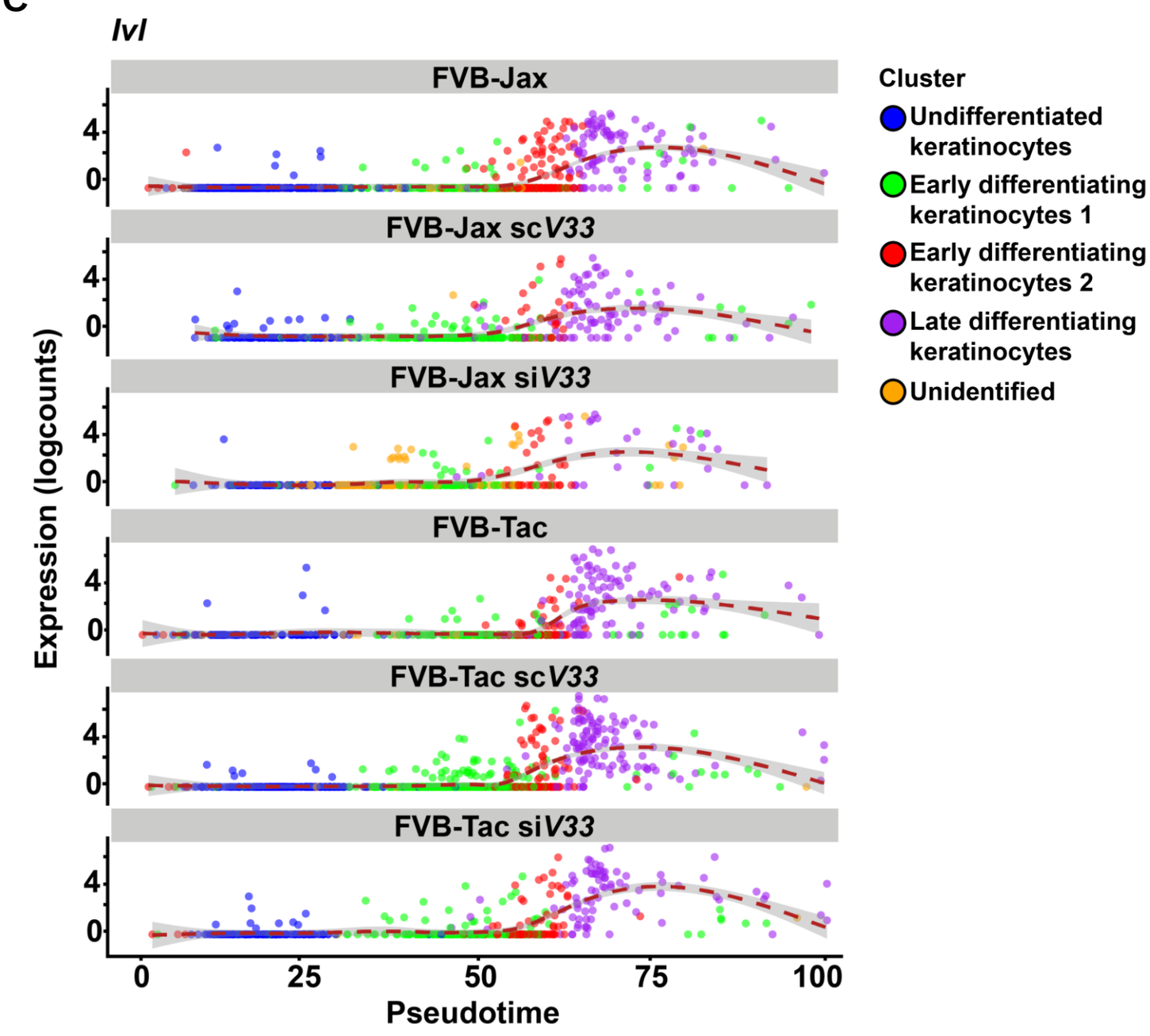

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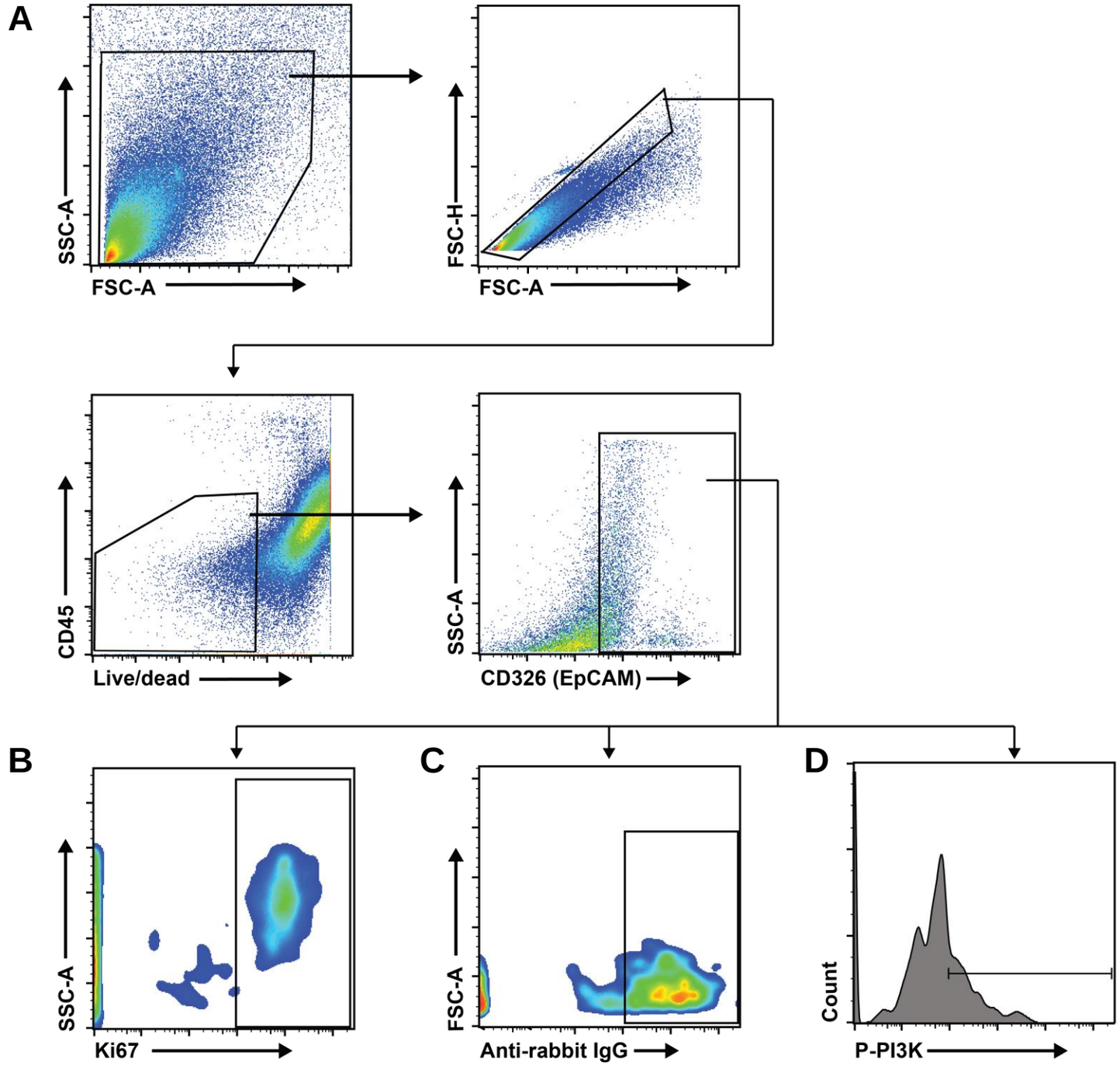

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