

# Antistoffen Huid

Hendri H. Pas

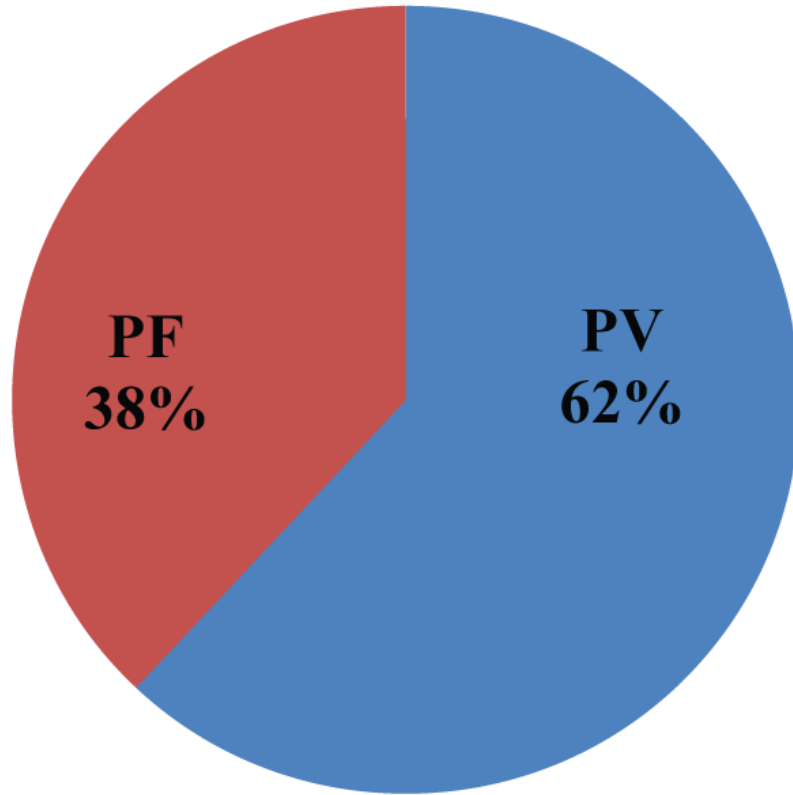


SKML-HIM 10 maart 2016

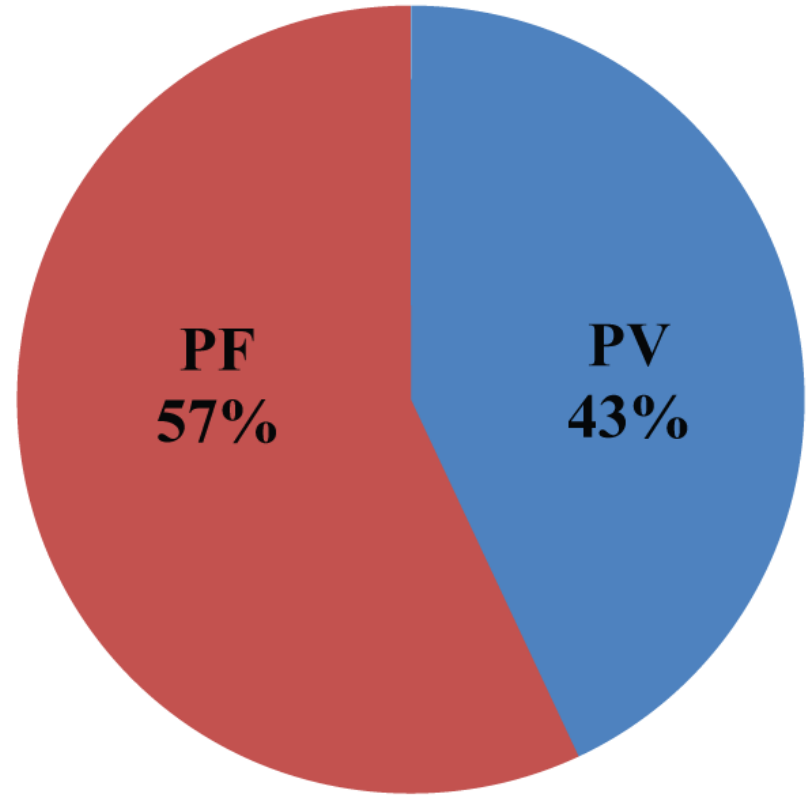
# Nabespreking rondzendingen 2013-2015

	A	B	C	D	E	F	G
1		var	expertwaarde	IIF score	afw	opm	overall
2	2013 A1	Geen	IIF negatief	7/7			7/7
3	2013 B1	Geen	IIF negatief	7/7			7/7
4	2013 C1	BP	IIF BMZ sterk	7/7			7/7
5	2013 D1	PV	IIF ICS sterk	7/7			7/7
6	2013 E1	BP	IIF BMZ zwak	7/7	1x ICS		6/7
7	2013 F1	PF?	IIF negatief	7/7			7/7
8							
9	2014 A1	BP	IIF negatief	7/7		2 labs alsnog BP via salt-split skin	2/7
10	2014 B1	BP	IIF BMZ zwak	7/7	1 x BMZ dub		6/7
11	2014 C1	BP	IIF BMZ sterk	6/7	1 x BMZ dub		6/7
12	2014 D1	Geen	IIF negatief	6/7	1 x BMZ		6/7
13	2014 E1	PF	IIF negatief	6/7	1 x BMZ/ICS (Euroimmun)	3 labs alsnog PF via ELISA of mozaiek	3/7
14	2014 F1	PV	IIF ICS sterk	7/7	1 x BMZ/ICS (Euroimmun)		6/7
15							
16	2015 A1	PV	IIF ICS sterk	7/7			7/7
17	2015 B1	PF	IIF ICS sterk	5/7	2 x dub ICS		5/7
18	2015 C1	PF	IIF ICS zwak	3/7		Bij neg IIF 2 labs alsnog PF via ELISA of mozaiek	5/7
19	2015 D1	BP	IIF BMZ sterk	6/7			6/7
20	2015 E1	BP	IIF negatief	7/7		IIF zwak vals positief, NC16A negatief	6/7
21	2015 F1	Geen	IIF negatief	7/7			7/7

# PV versus PF in IIF (DIF positief)

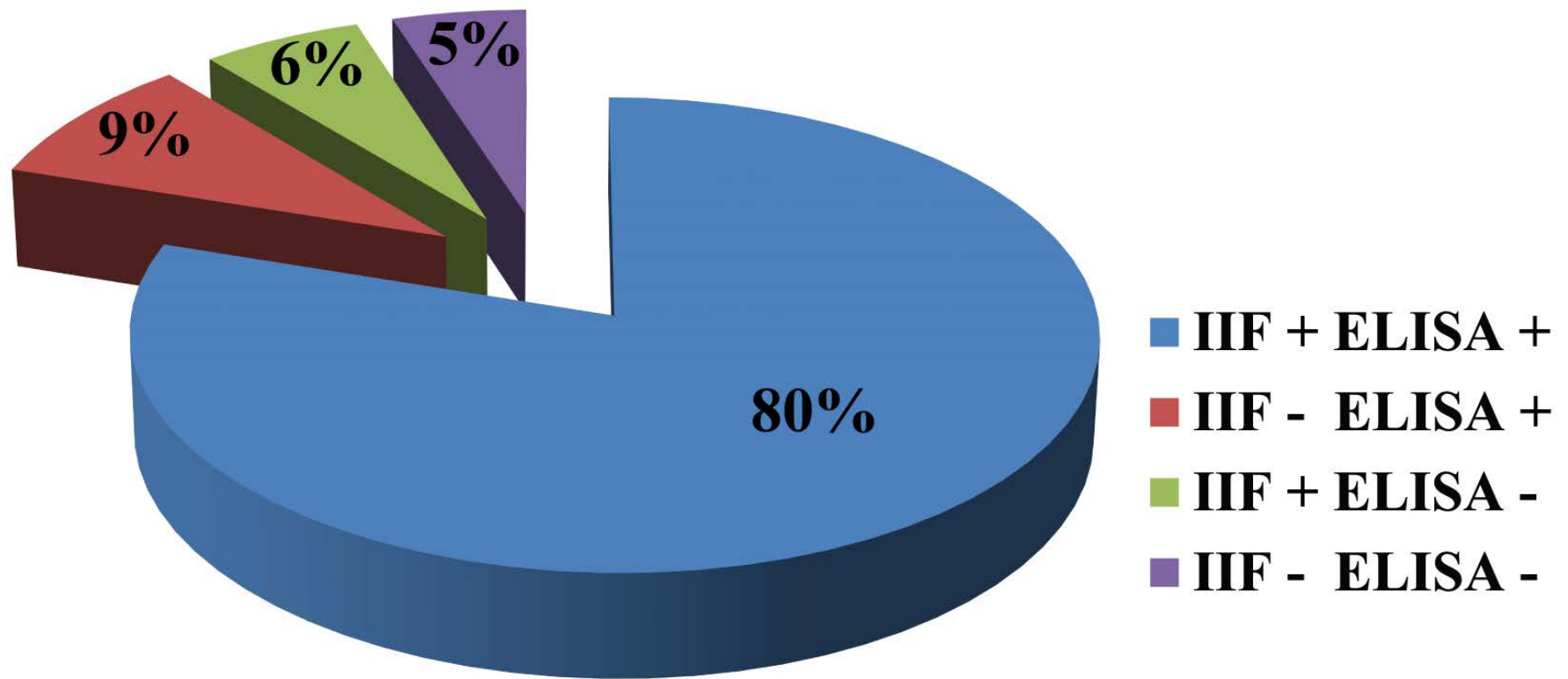


**IIF pos**



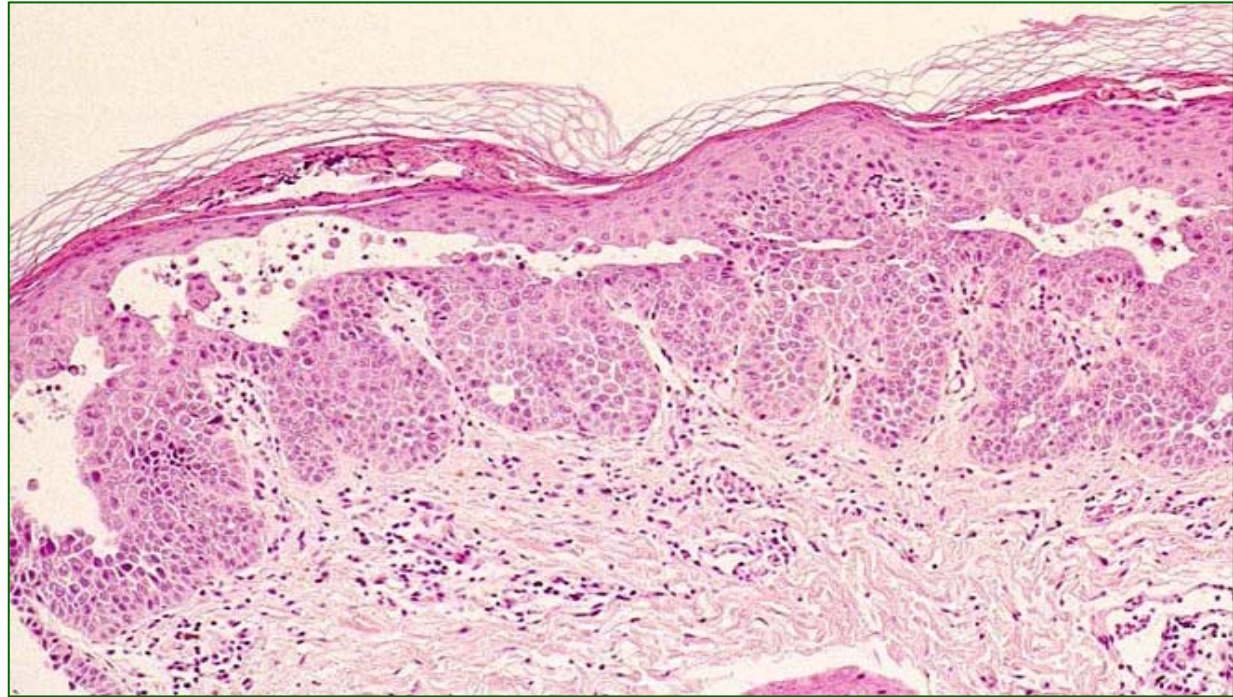
**IIF neg**

# IIF versus ELISA (DIF positief)

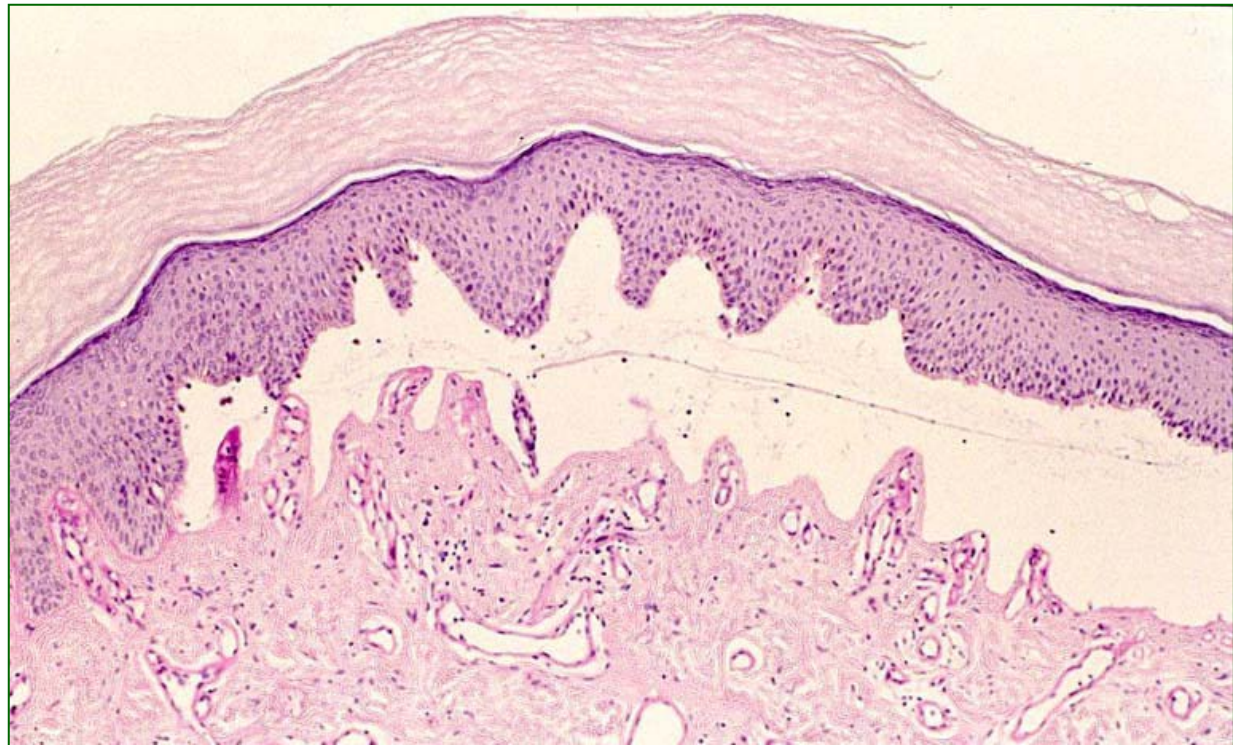


# Epitheliaal splijtingsnivo

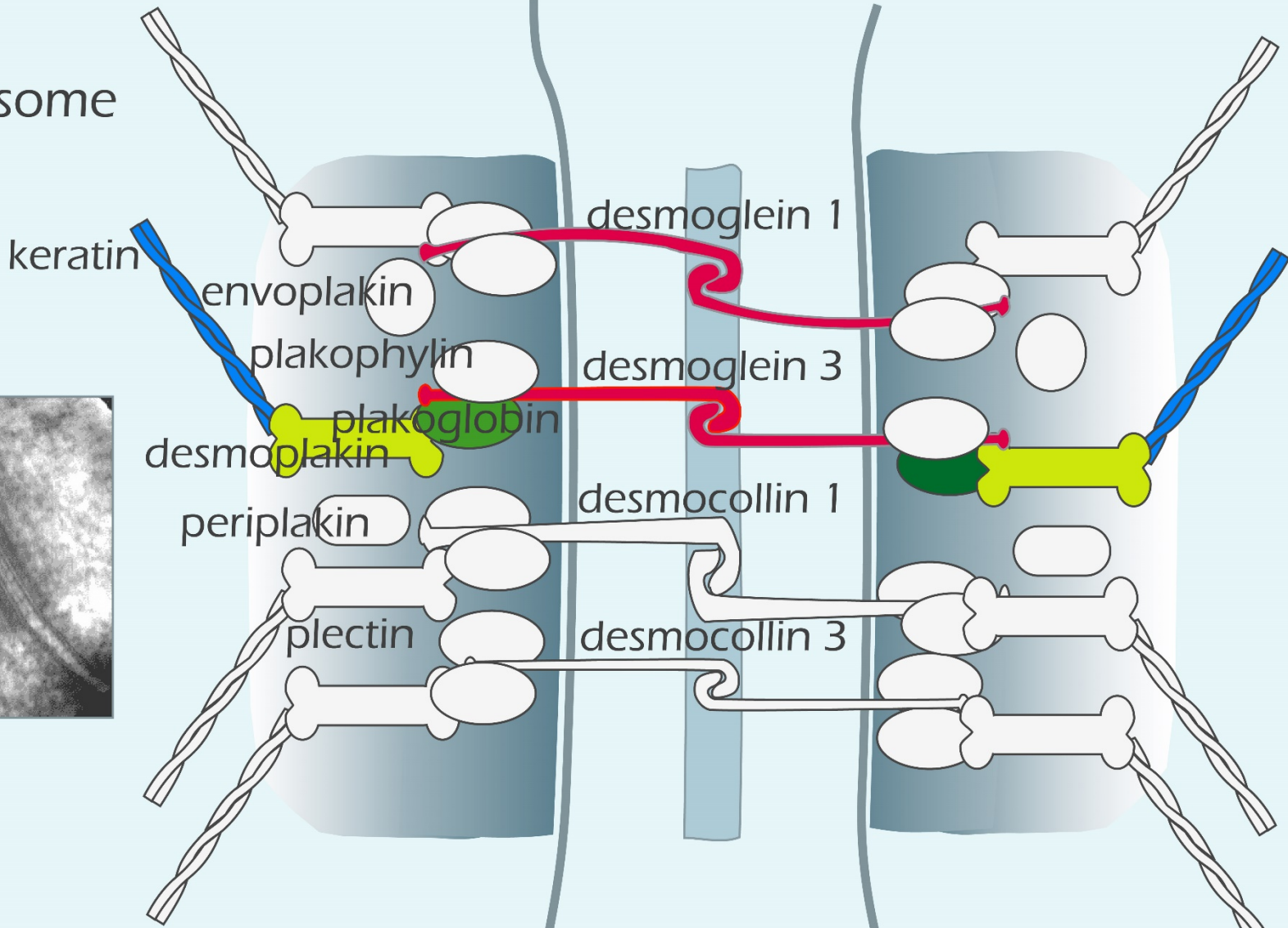
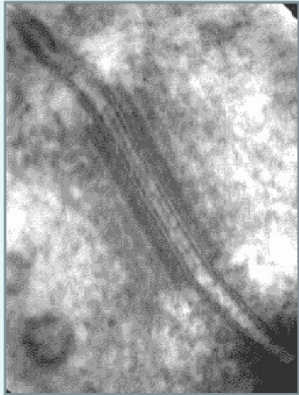
intra-epidermaal  
pemphigus



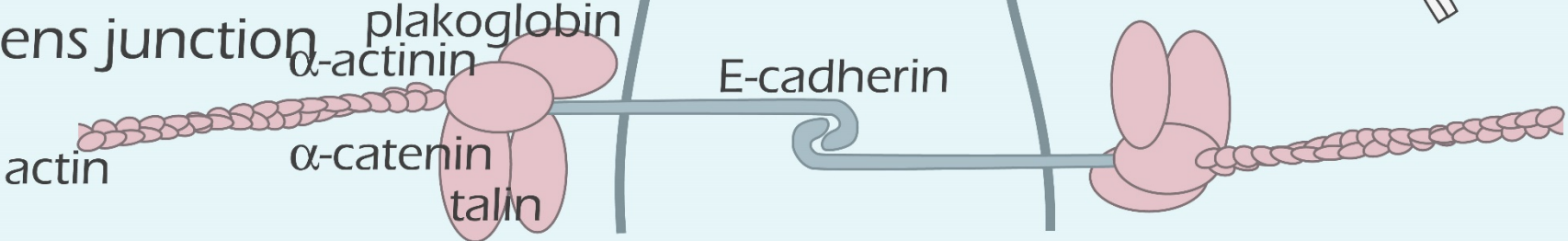
sub-epidermaal  
pemfigoïd

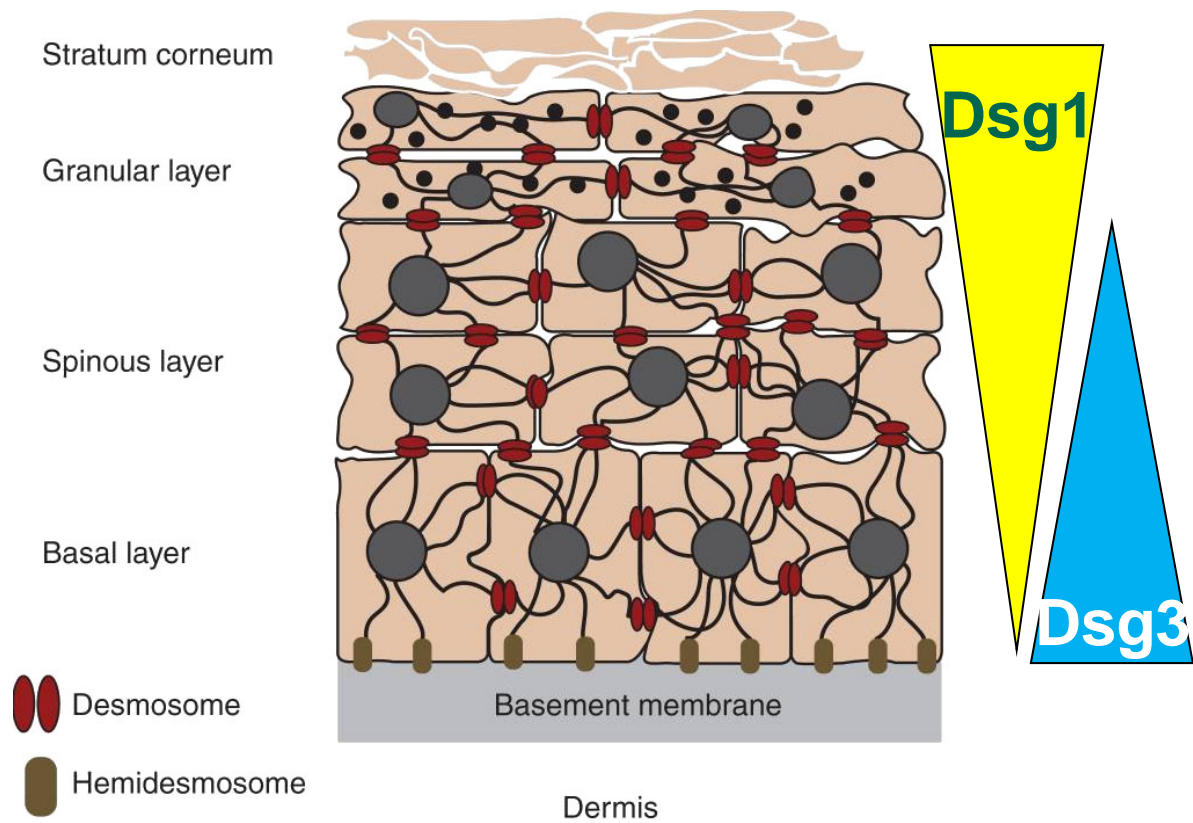
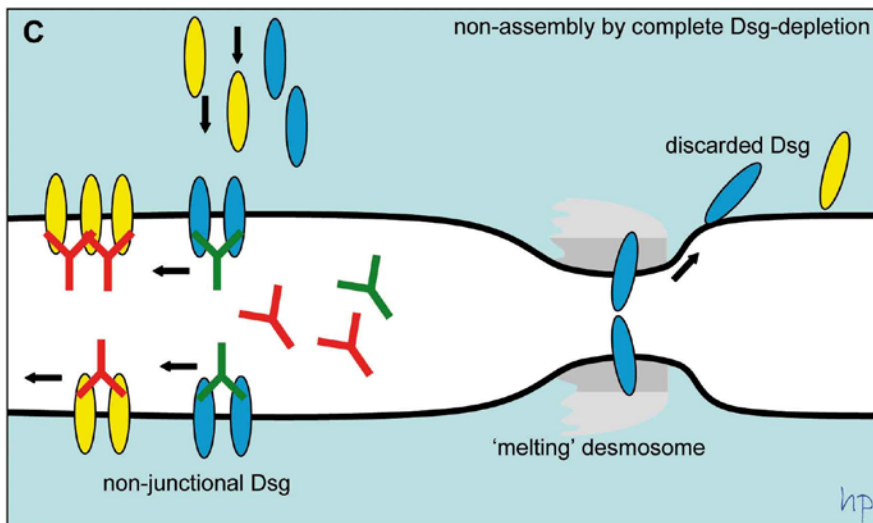
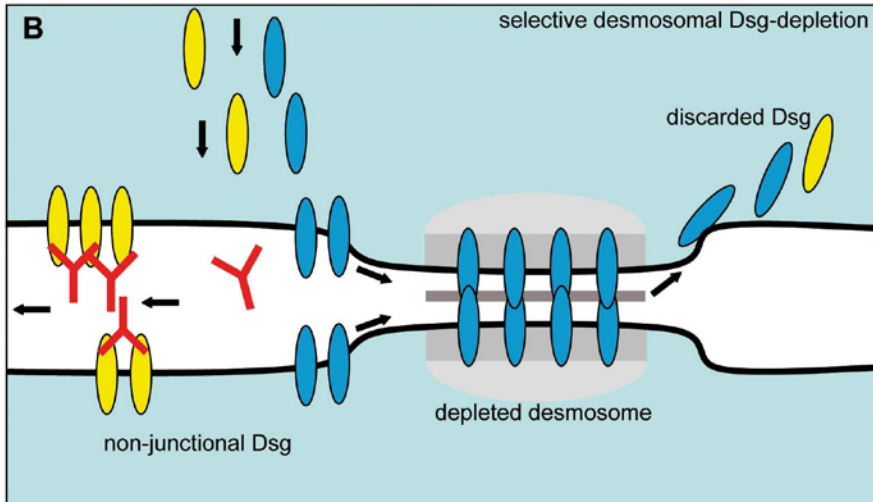
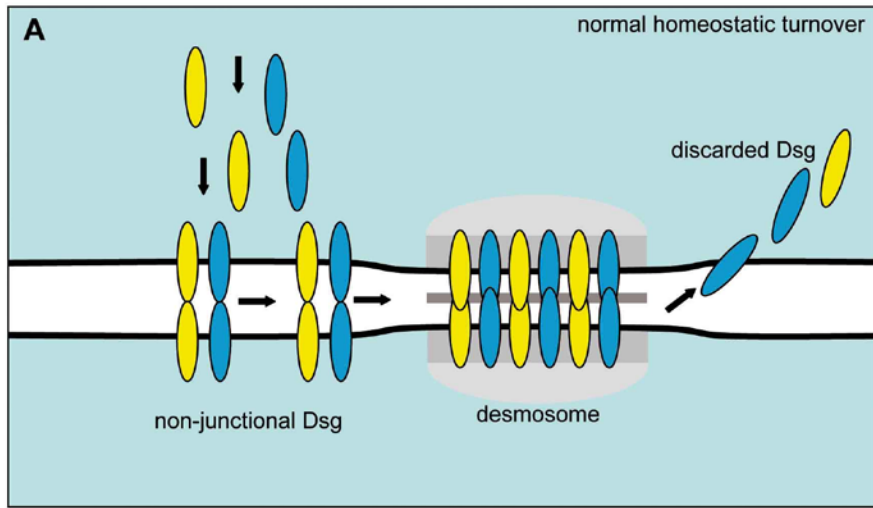


# Desmosome



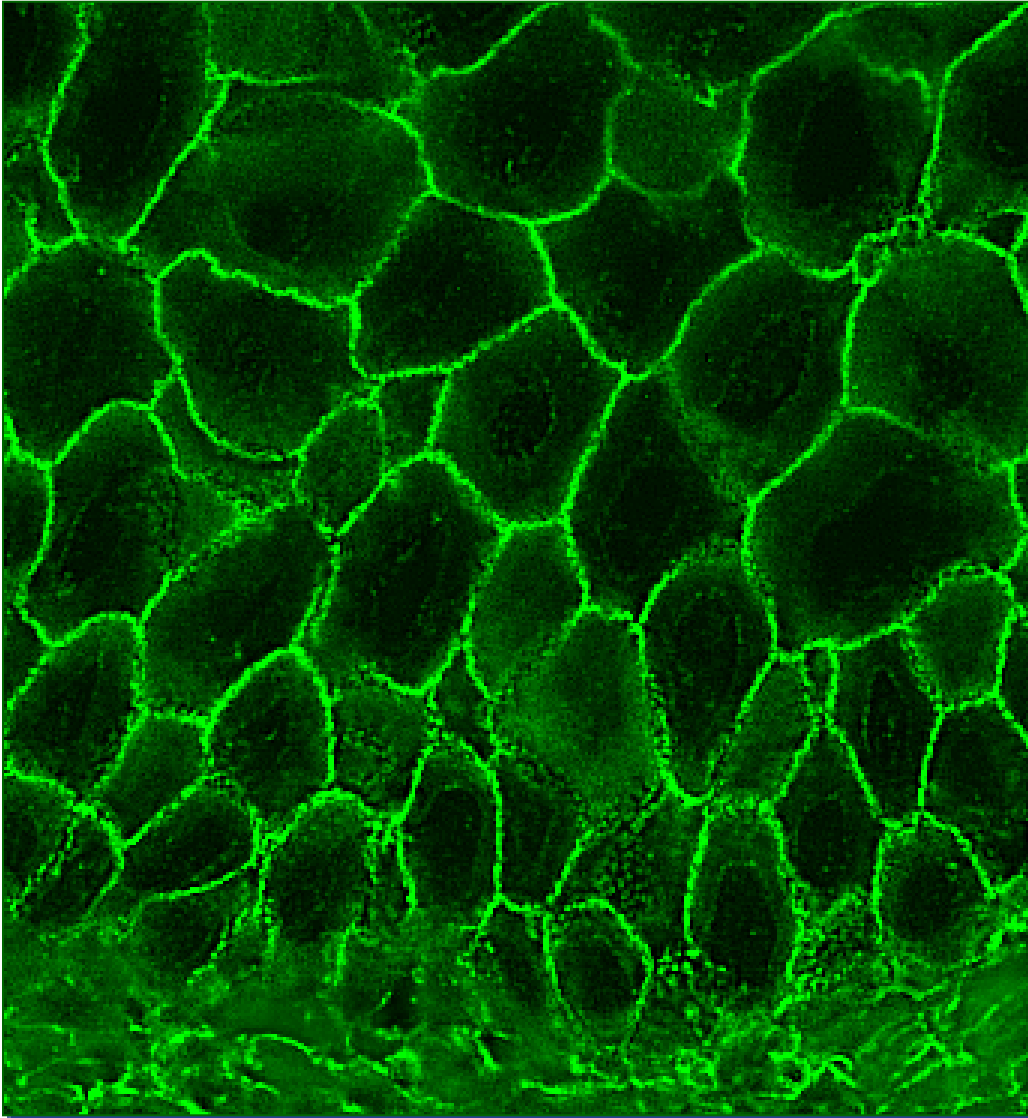
# Adherens junction



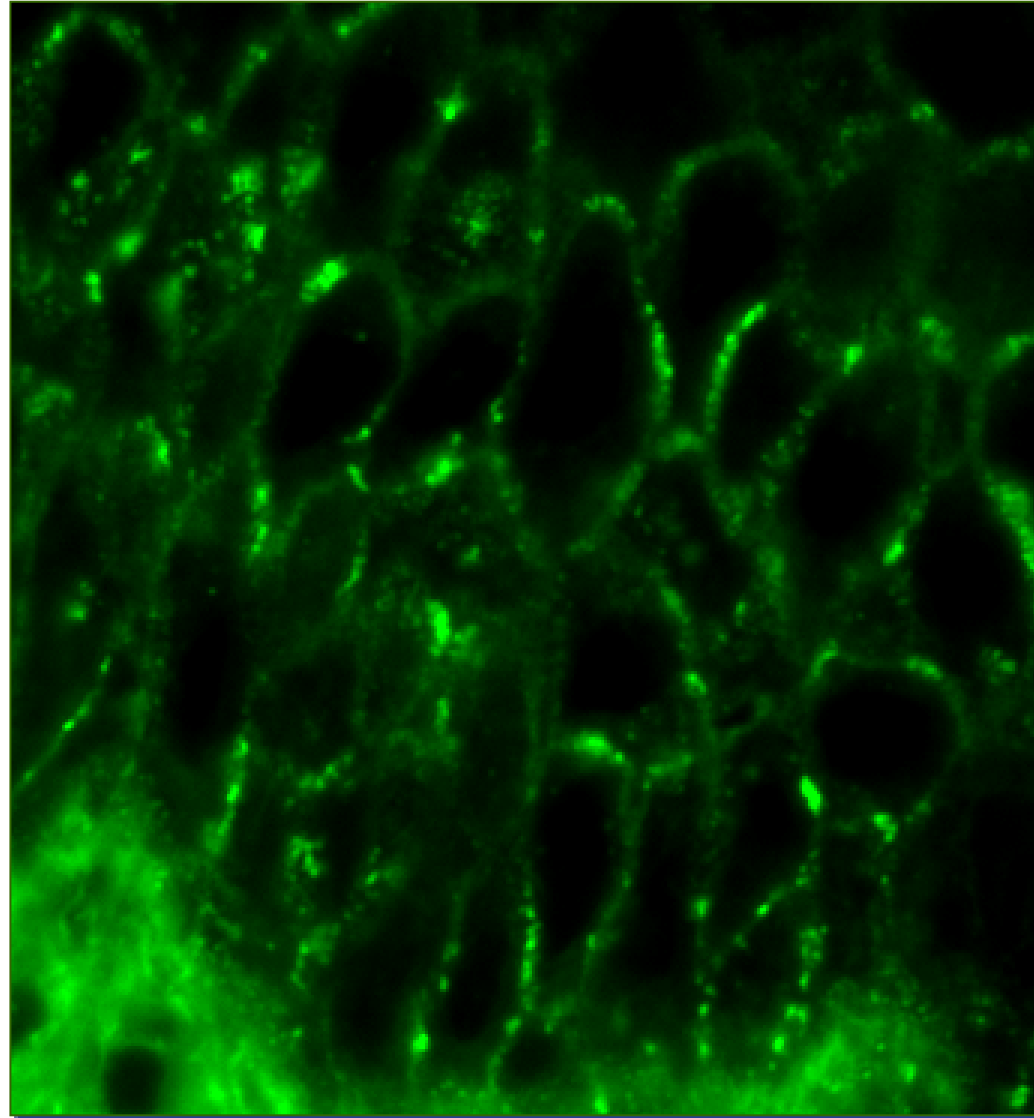


Oktarina DA, van der Wier G, Diercks GF, Jonkman MF, Pas HH. IgG-induced clustering of desmogleins 1 and 3 in skin of patients with pemphigus fits with the desmoglein nonassembly depletion hypothesis.

# Serum patterns versus skin patterns



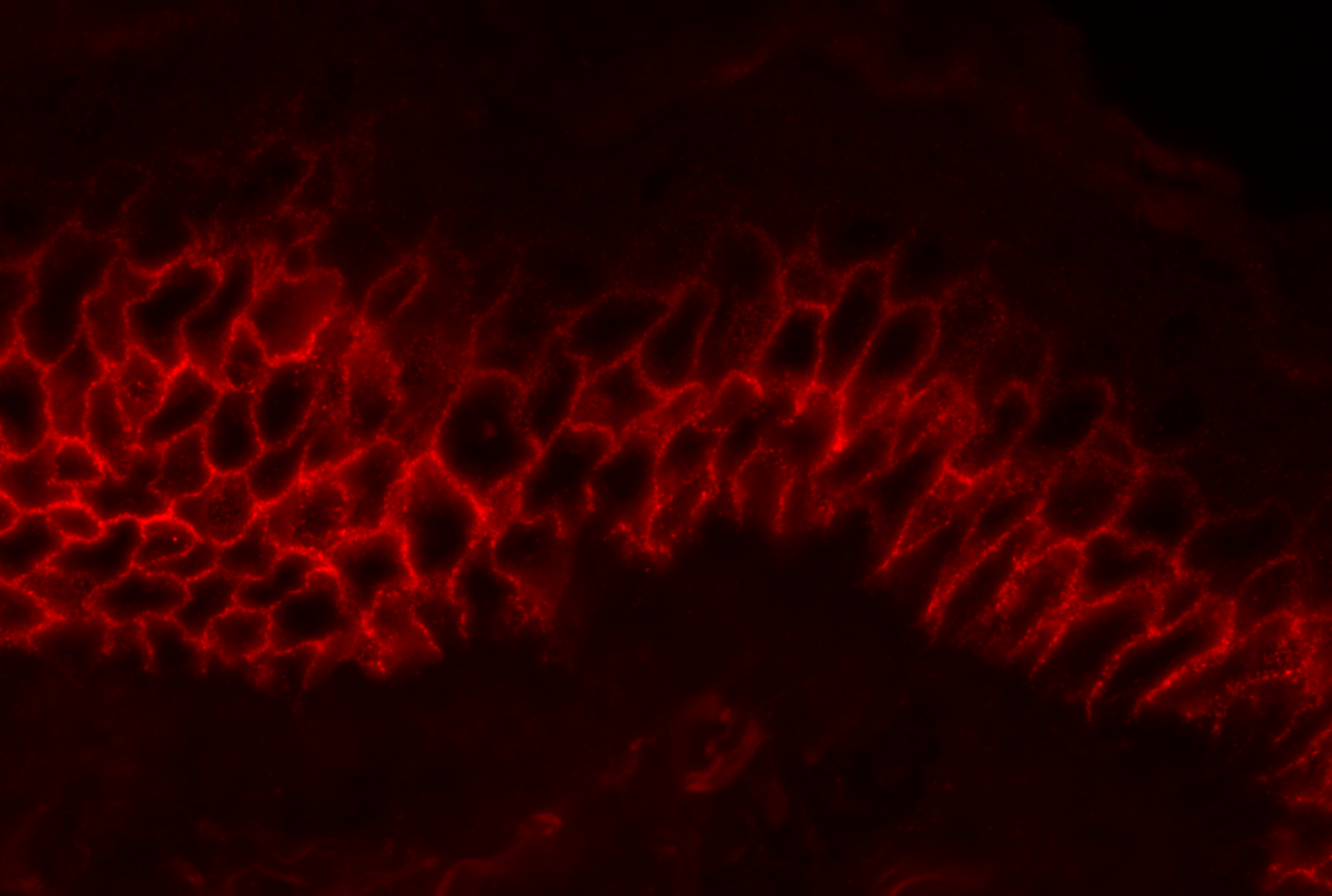
Patiënt IgG on monkey esophagus



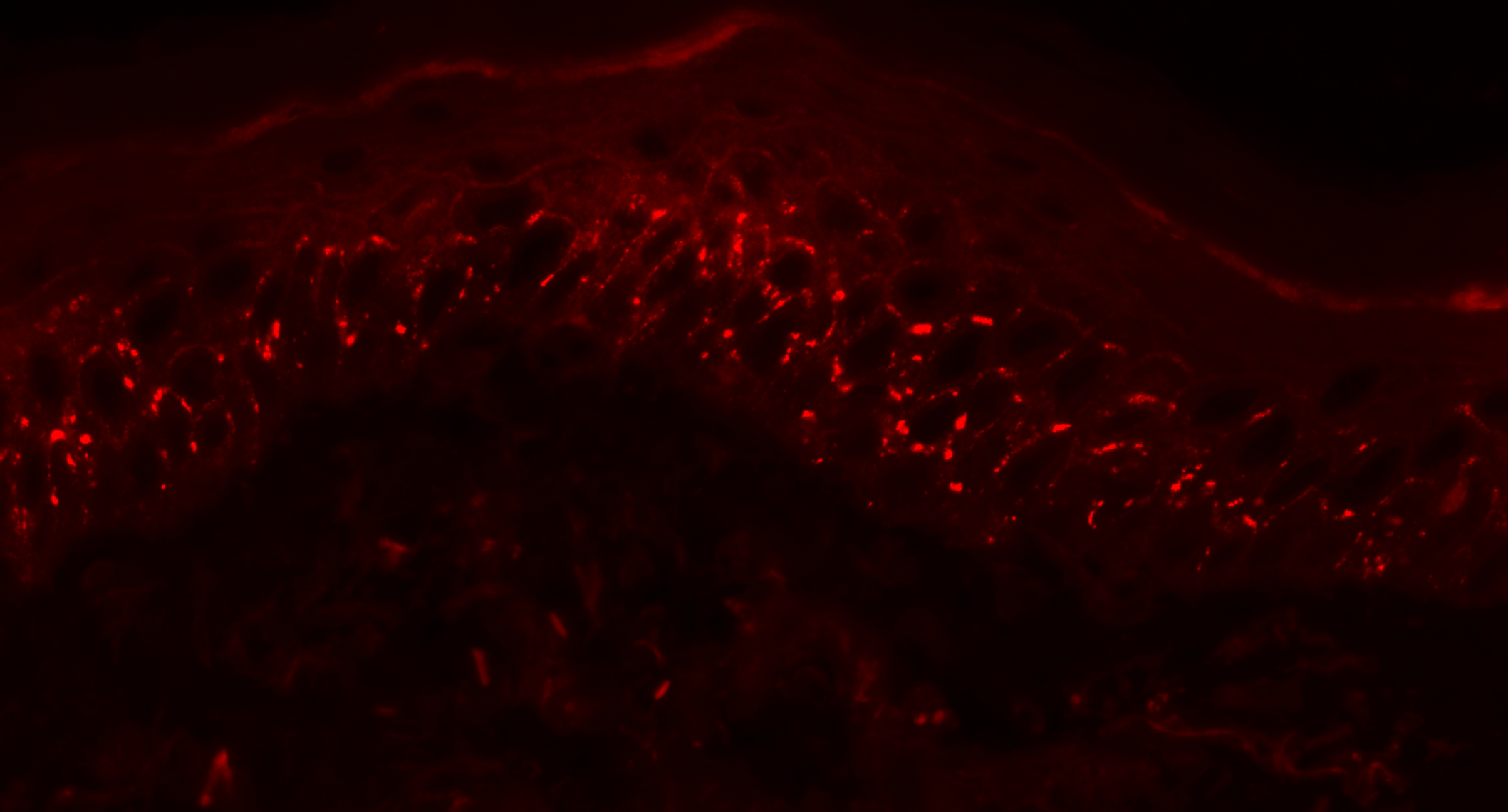
IgG deposition in patient skin



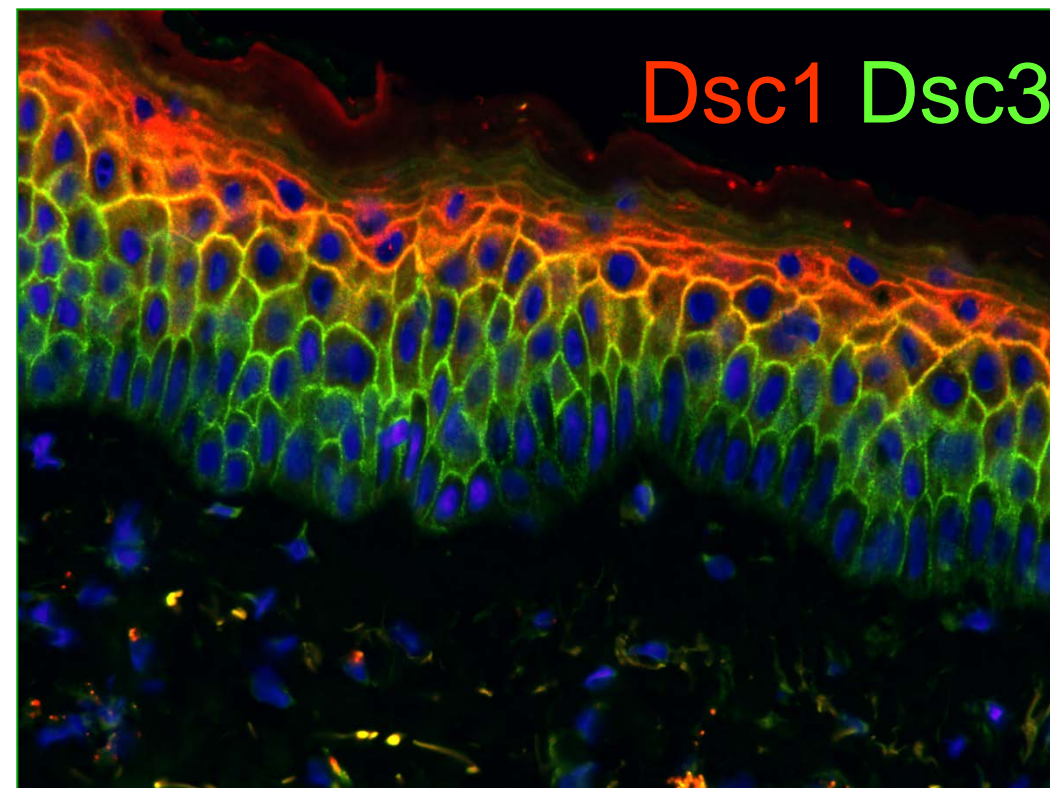
desmoglein 3: normal human skin



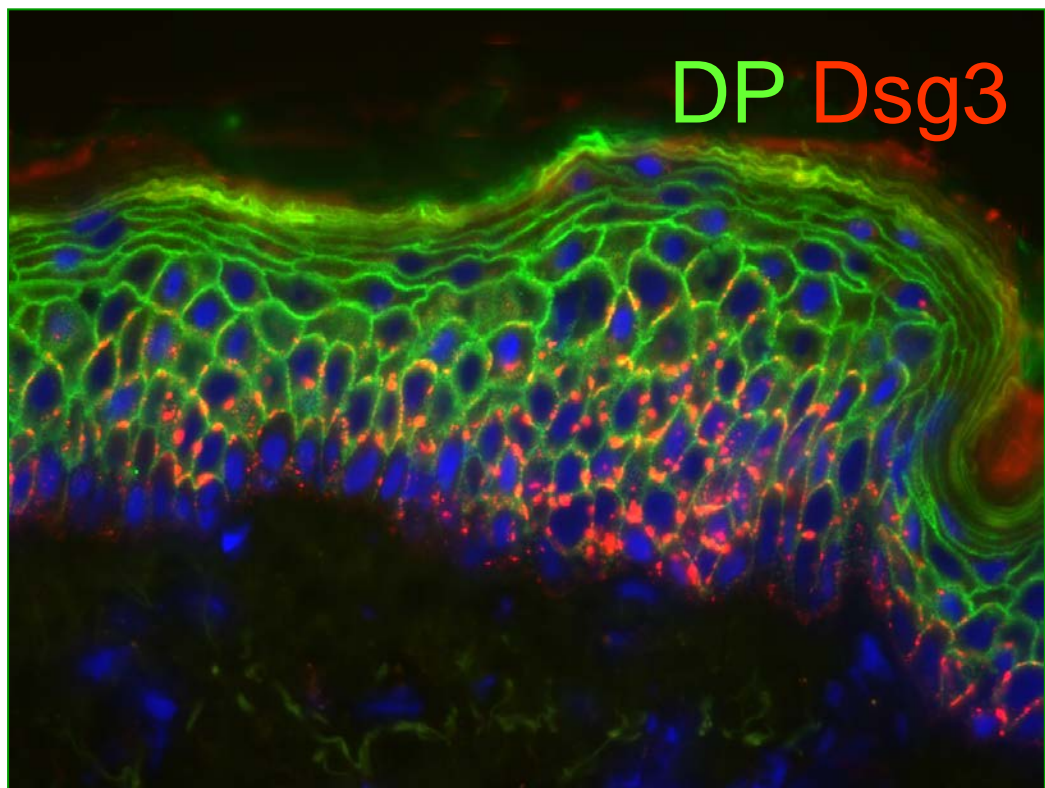
desmoglein 3: mdPV skin



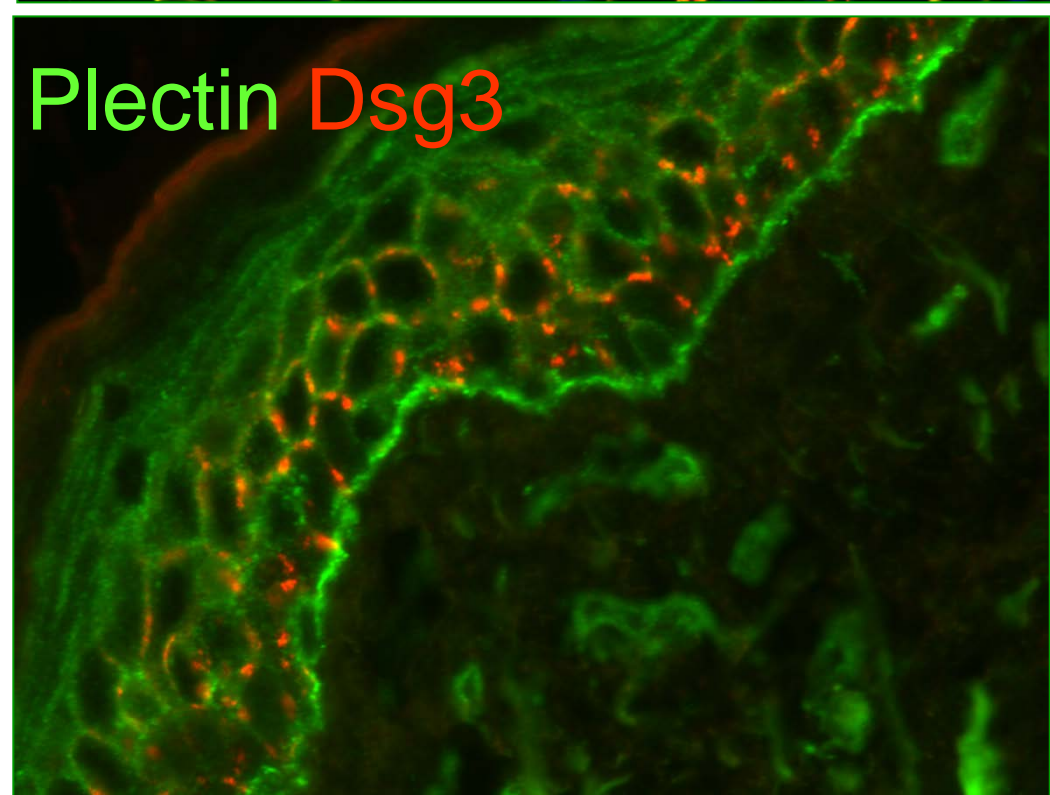
Dsc1 Dsc3



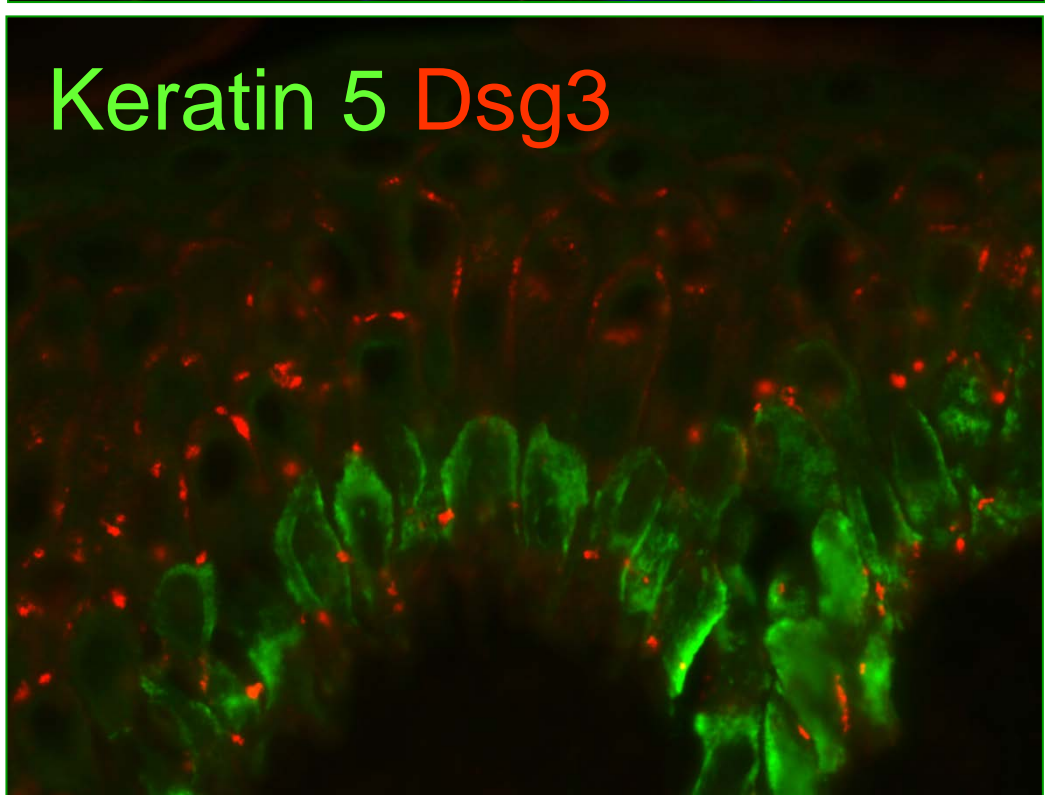
DP Dsg3



Plectin Dsg3

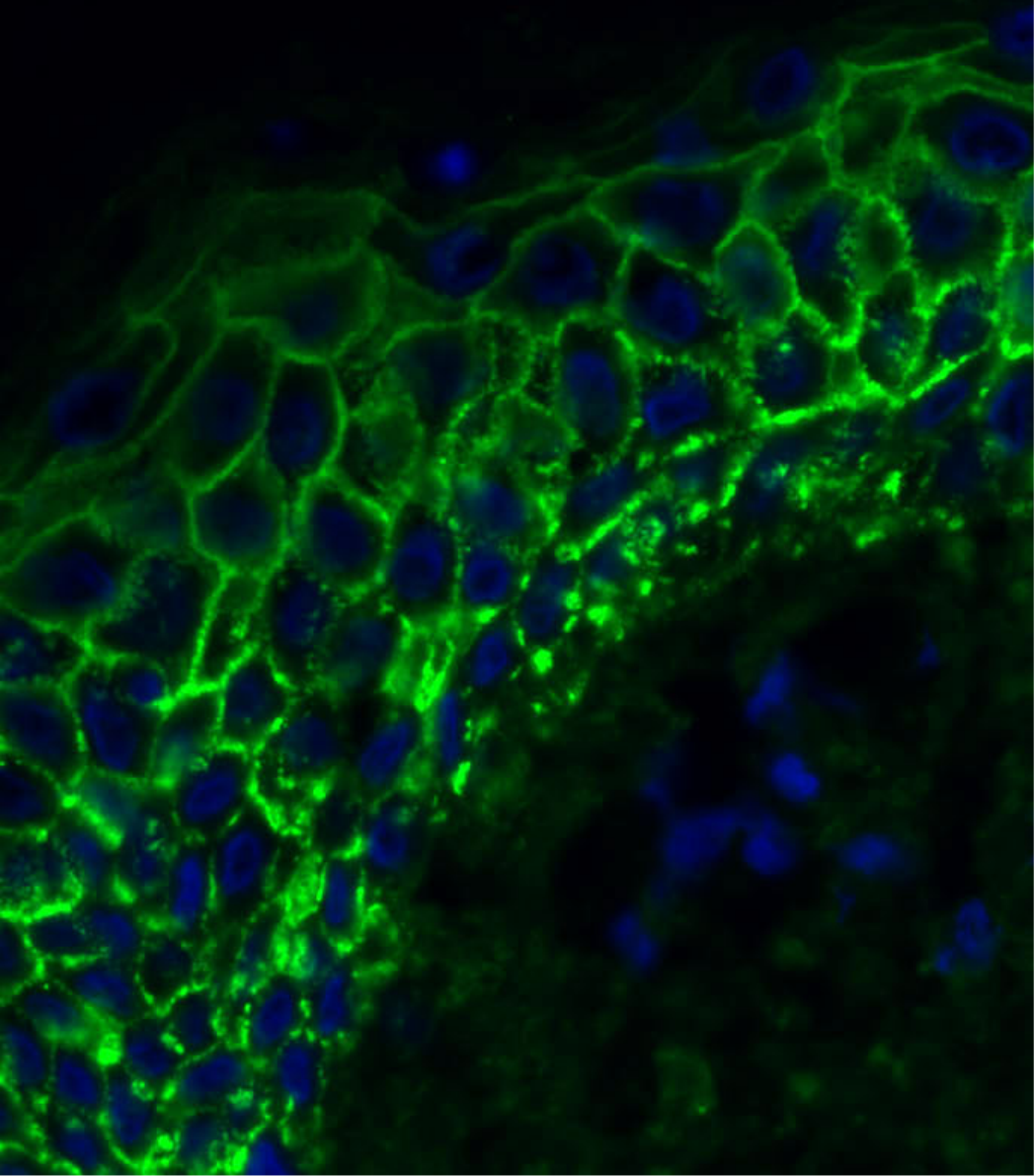


Keratin 5 Dsg3

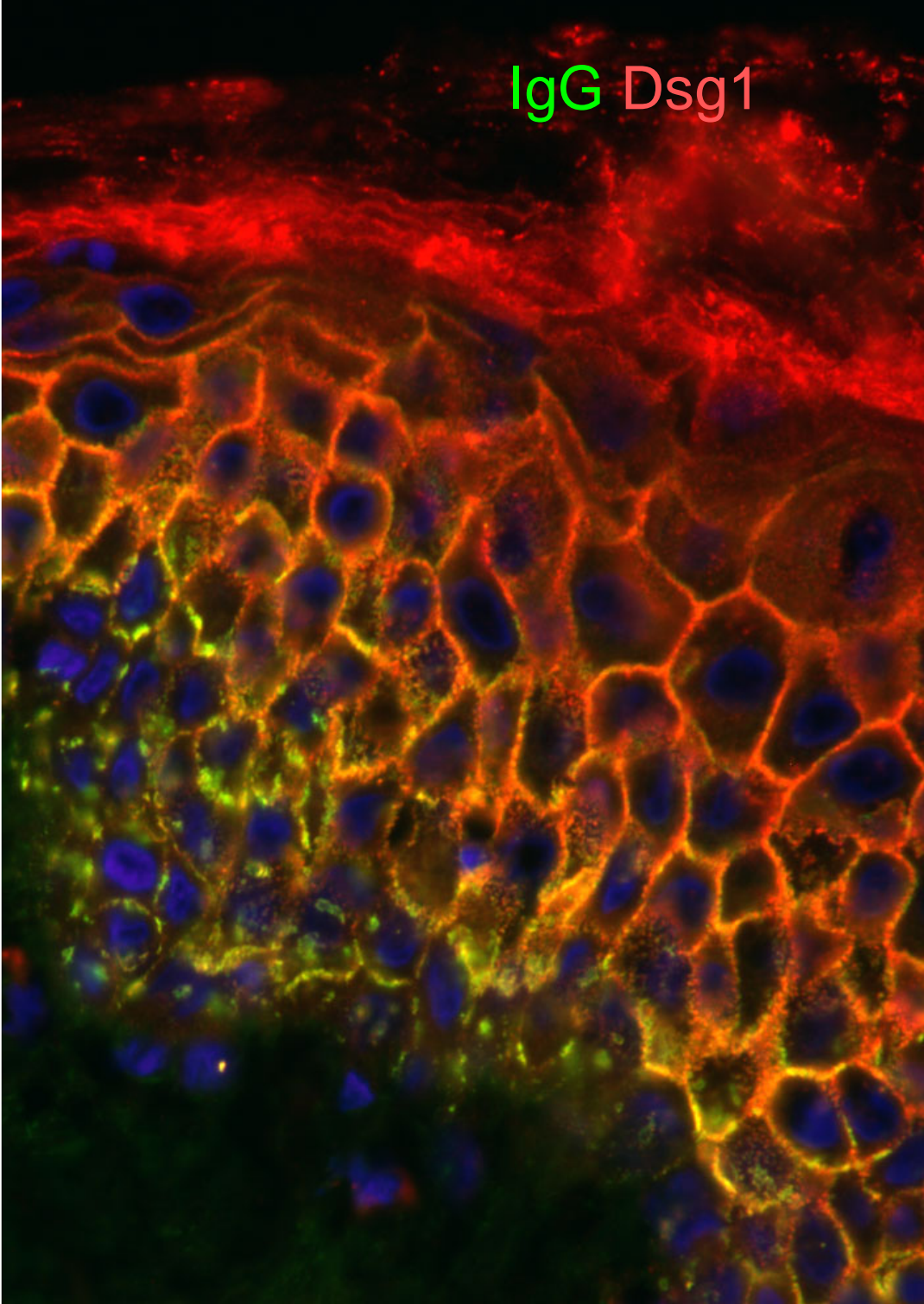


non-lesional PF skin

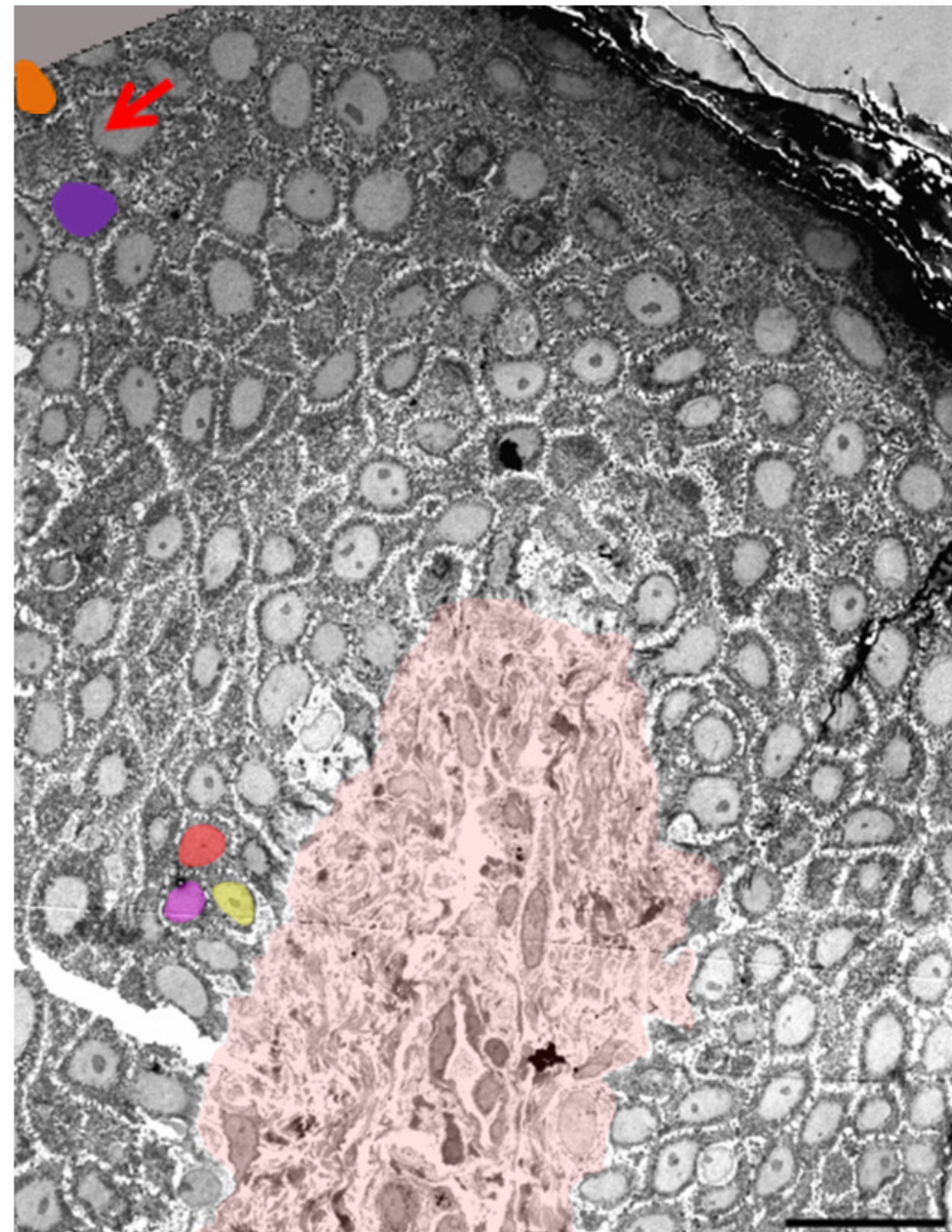
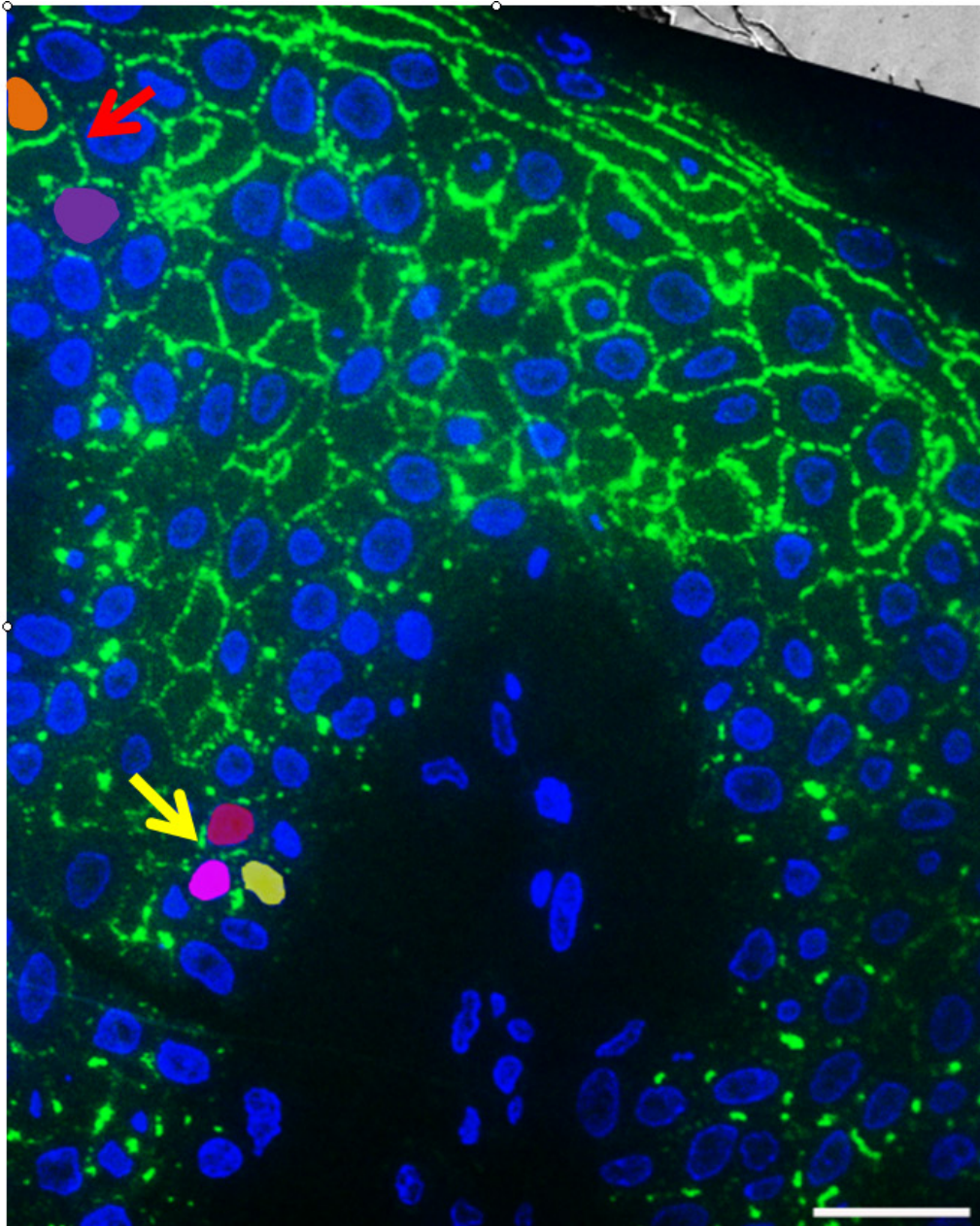
IgG



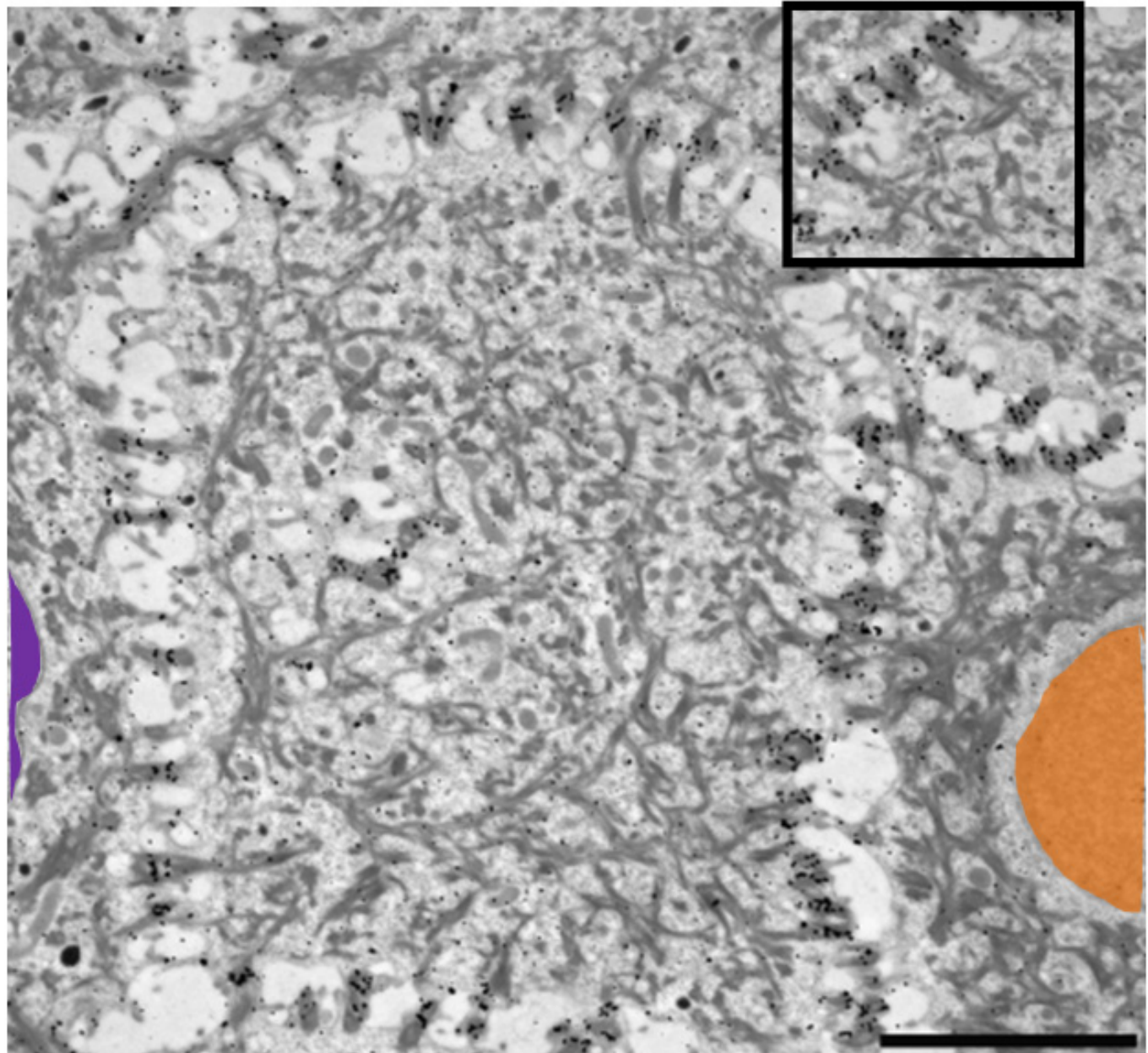
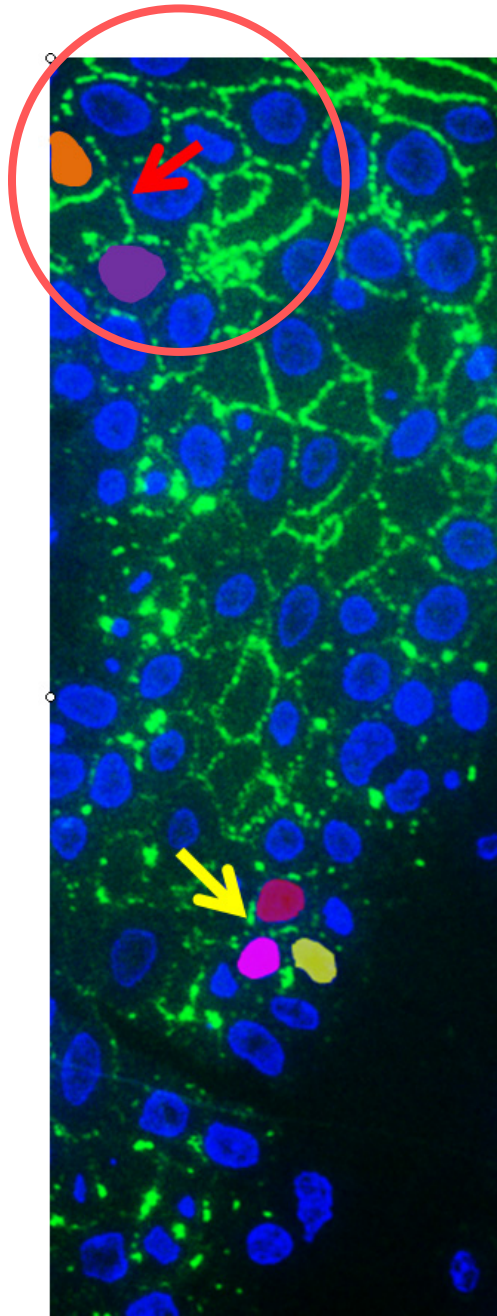
IgG Dsg1



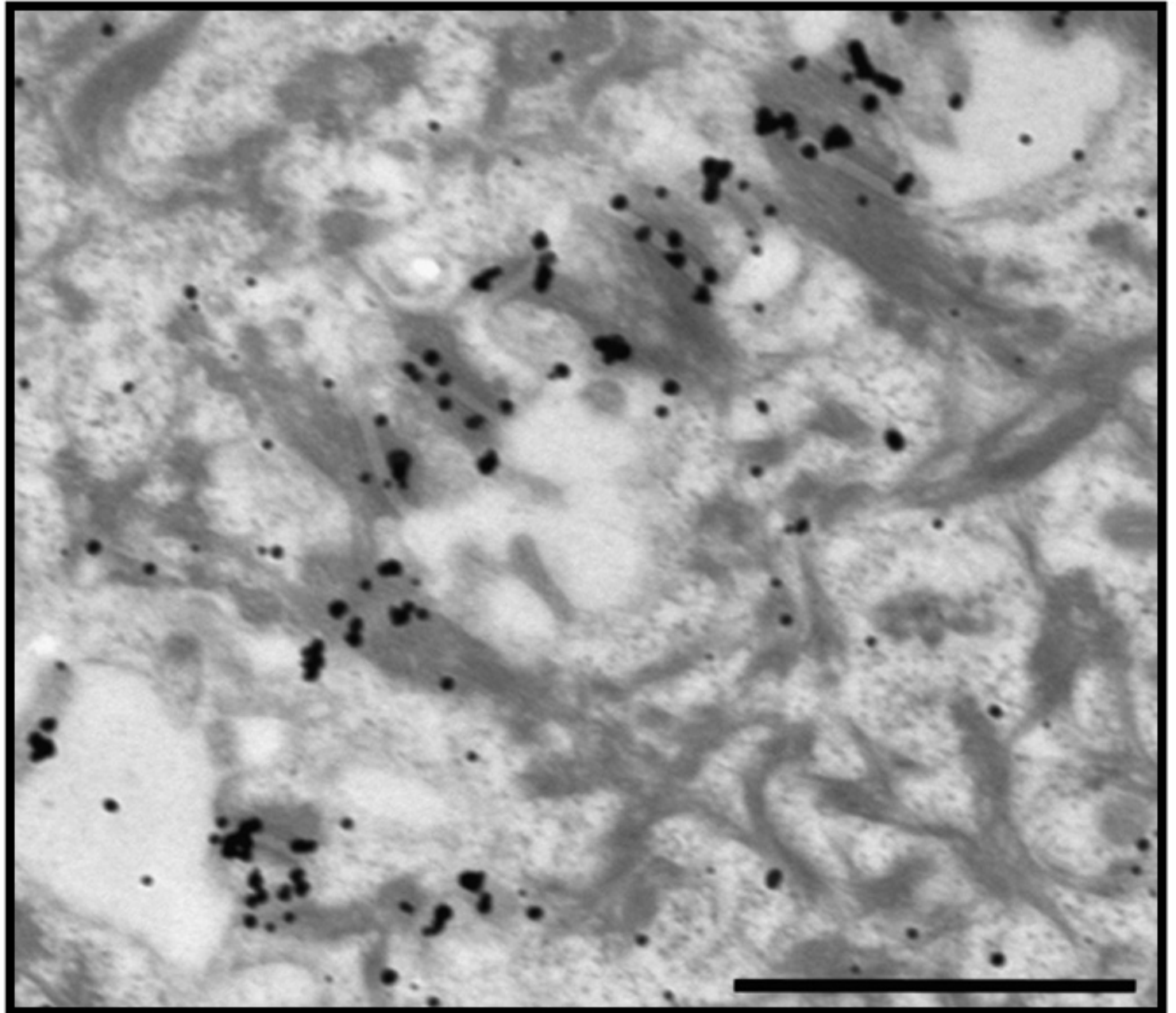
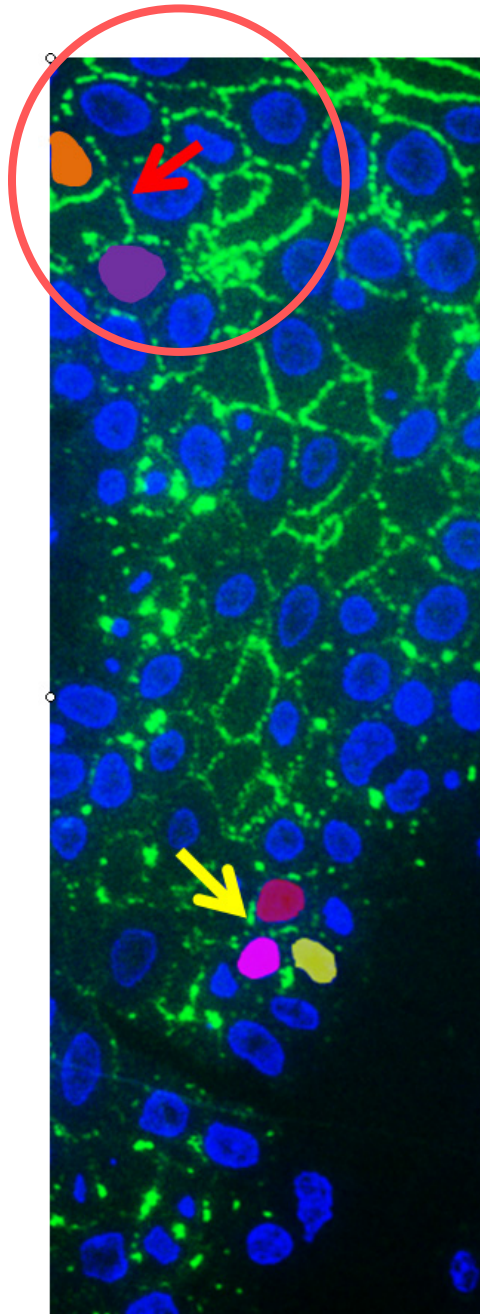
# Ultrastructural characterization of IgG clusters by CLEM



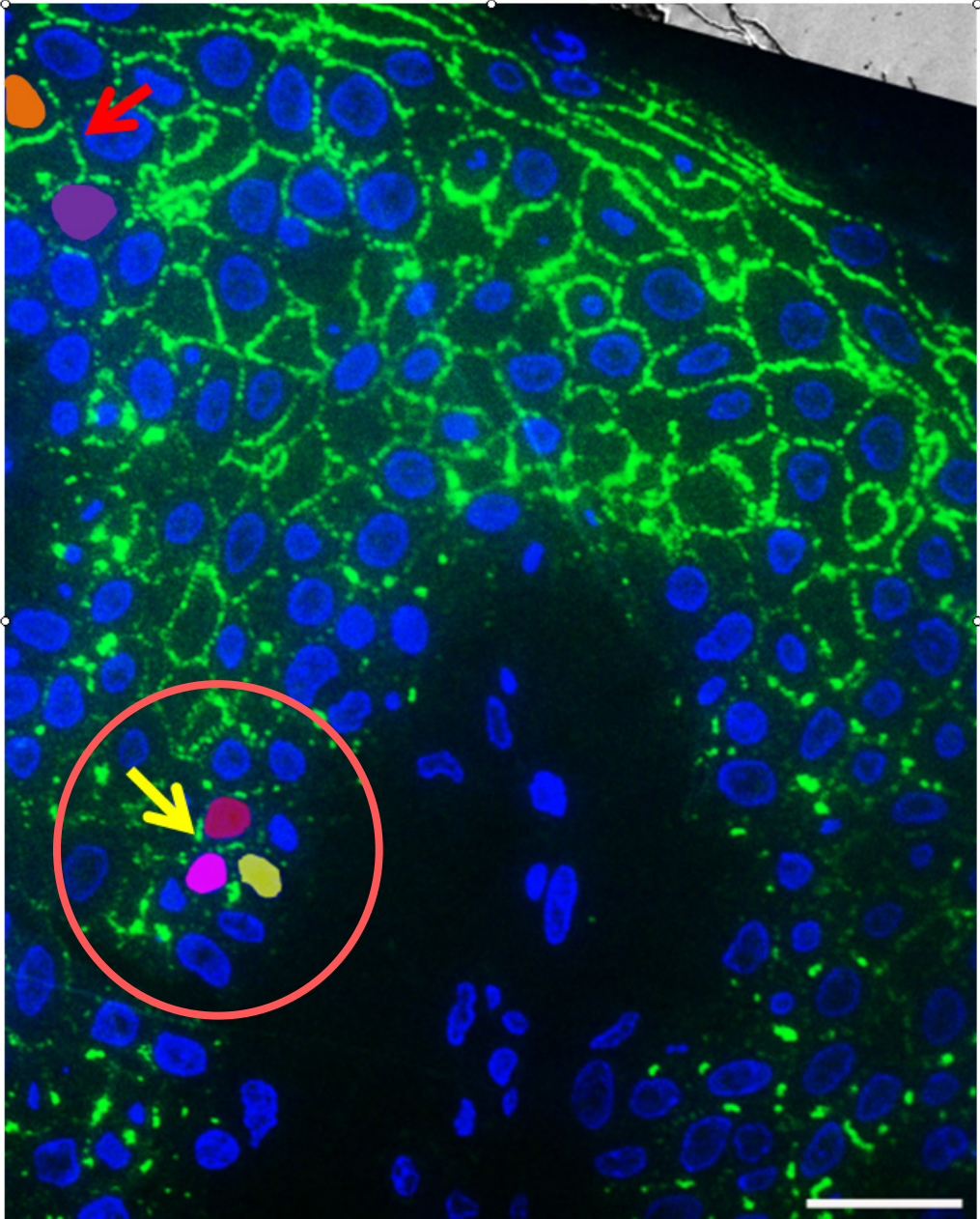
# Ultrastructural characterization of IgG clusters by CLEM



# Ultrastructural characterization of IgG clusters by CLEM

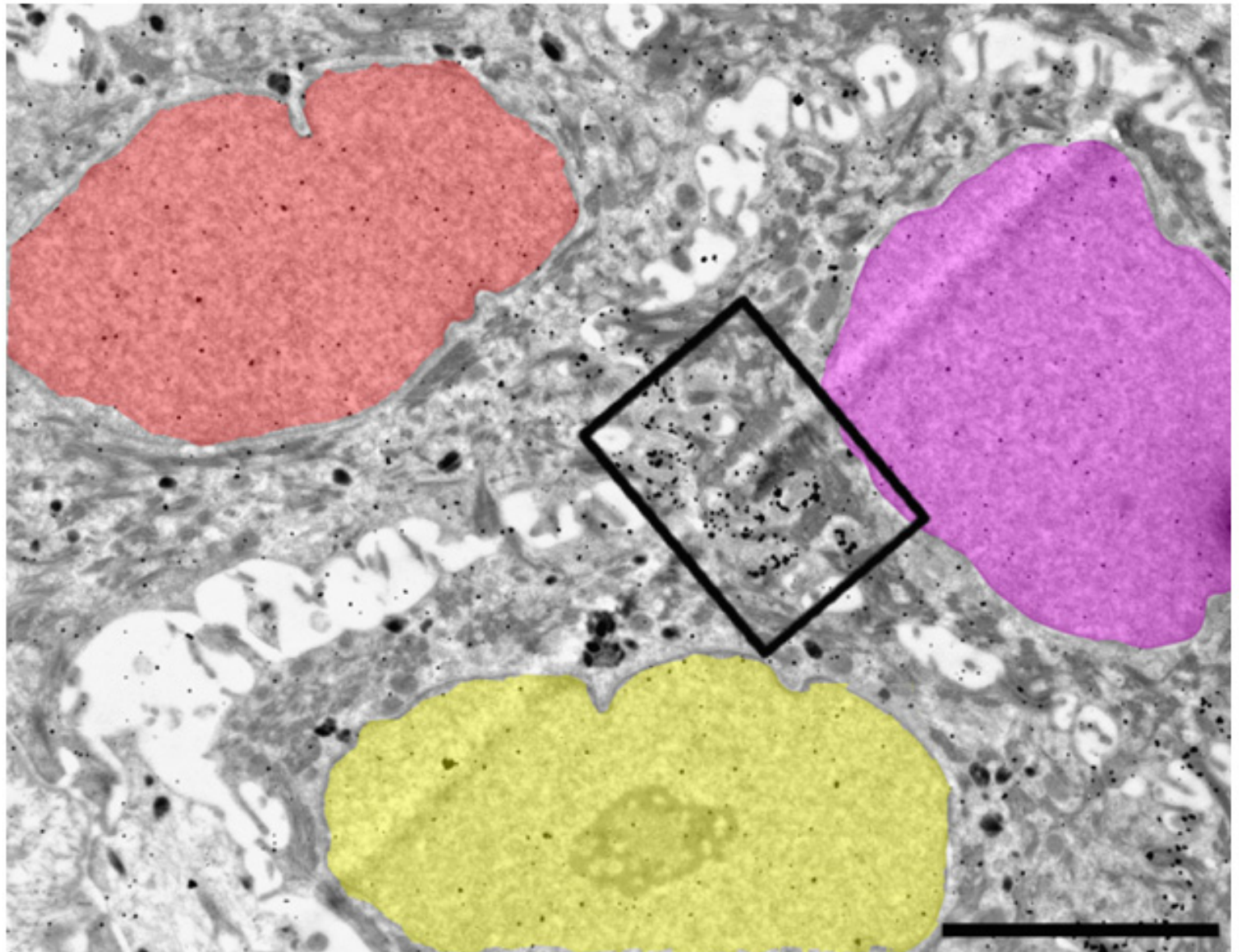
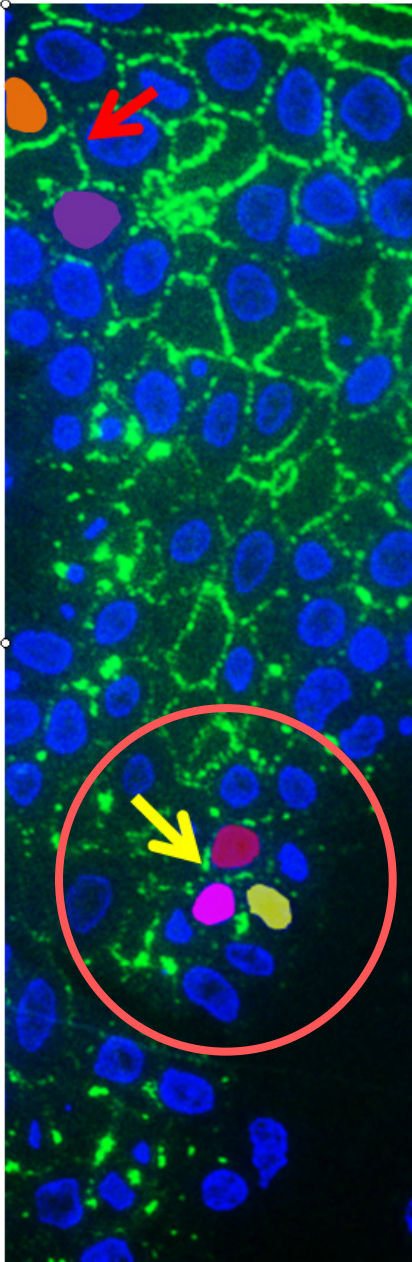


# Ultrastructural characterization of IgG clusters by CLEM

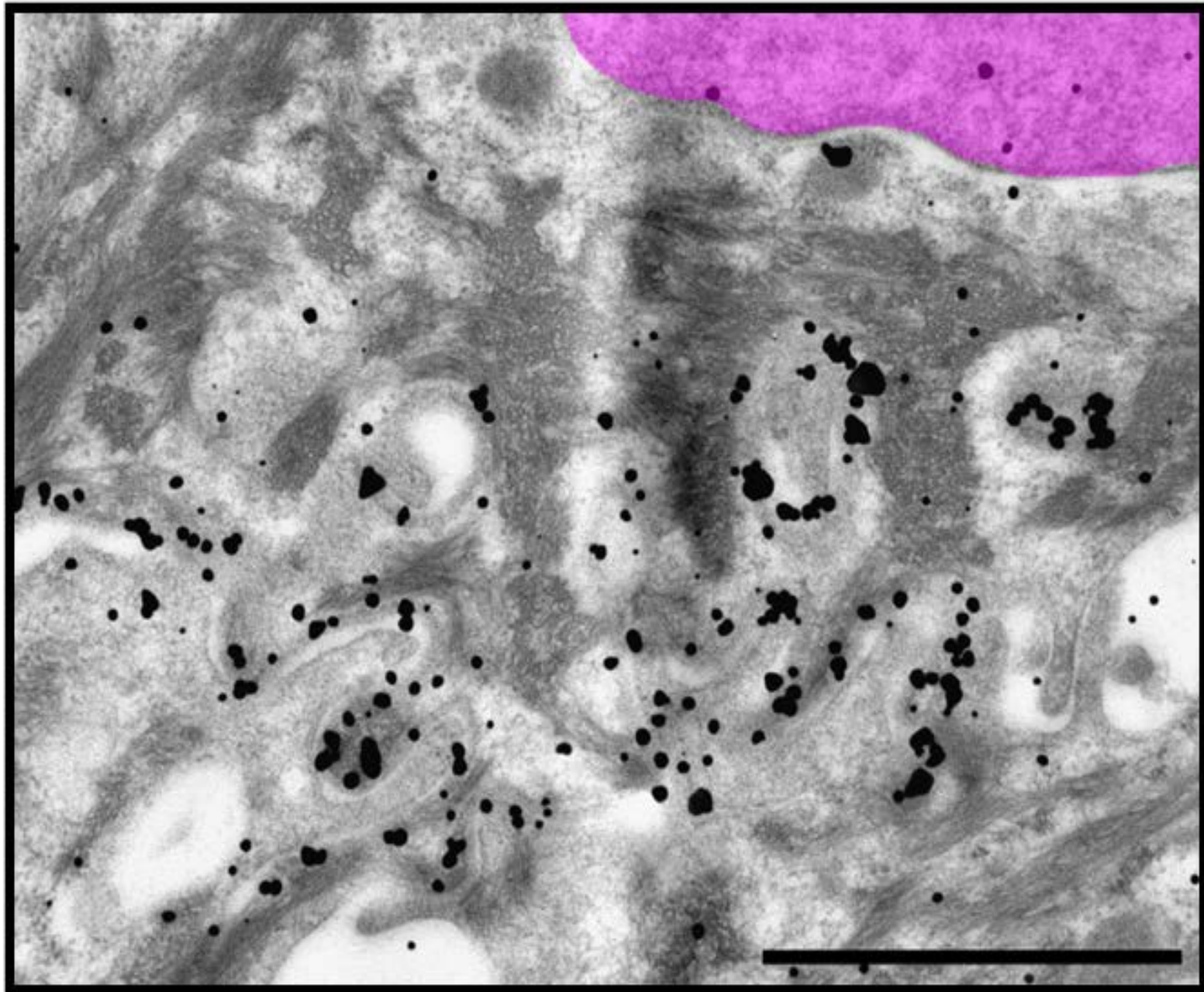
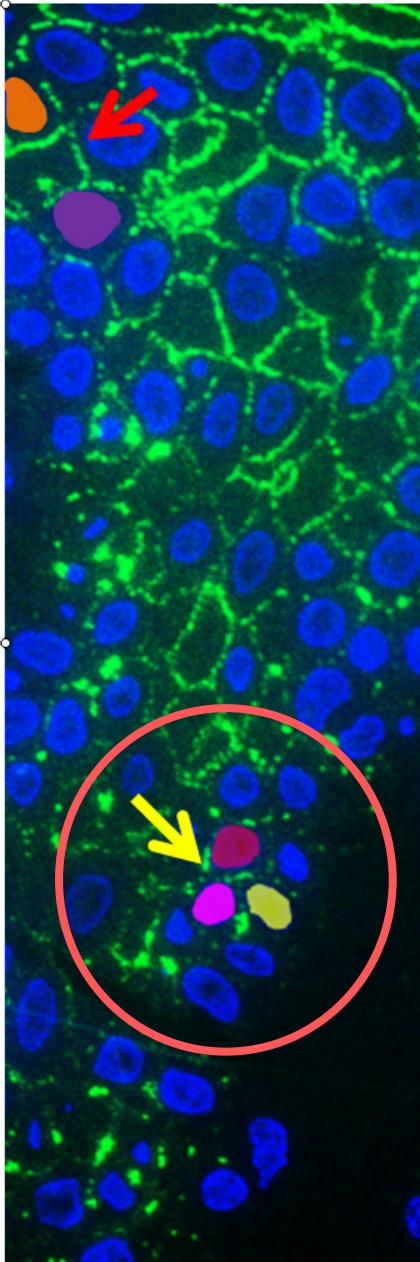




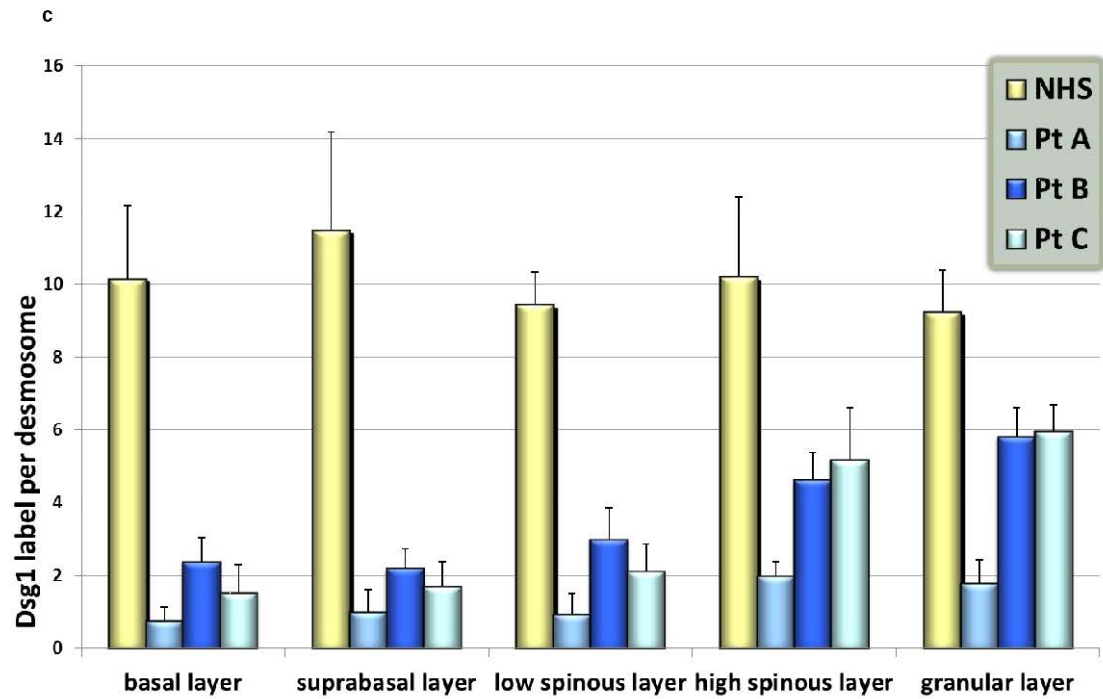
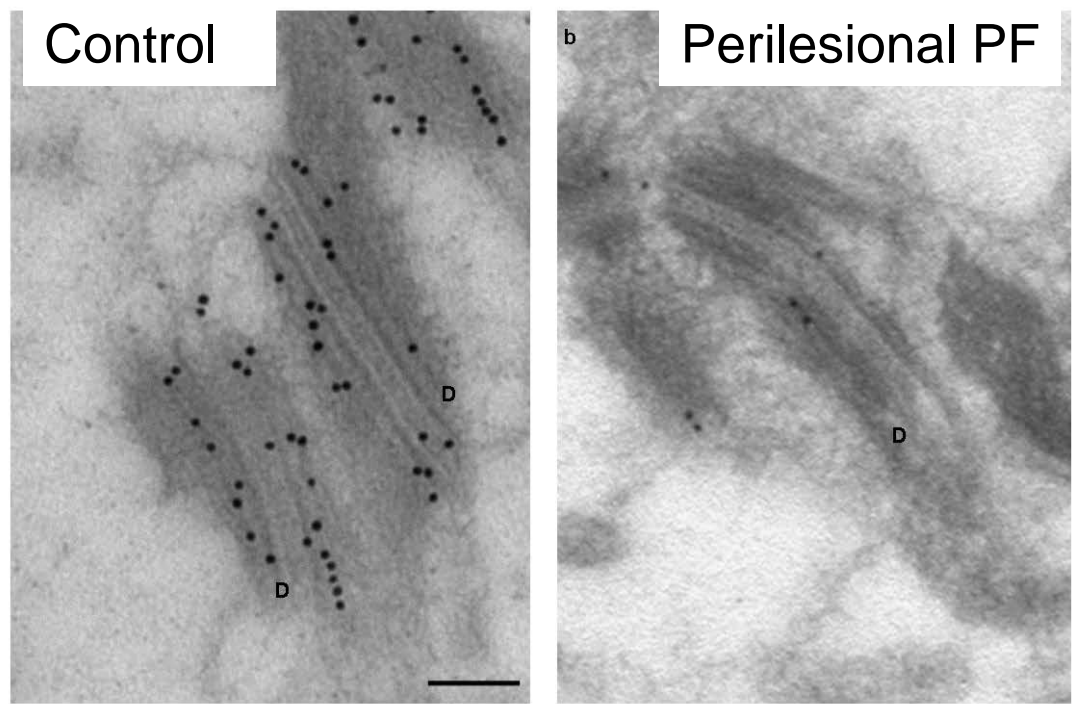
# Ultrastructural characterization of IgG clusters by CLEM



# Ultrastructural characterization of IgG clusters by CLEM

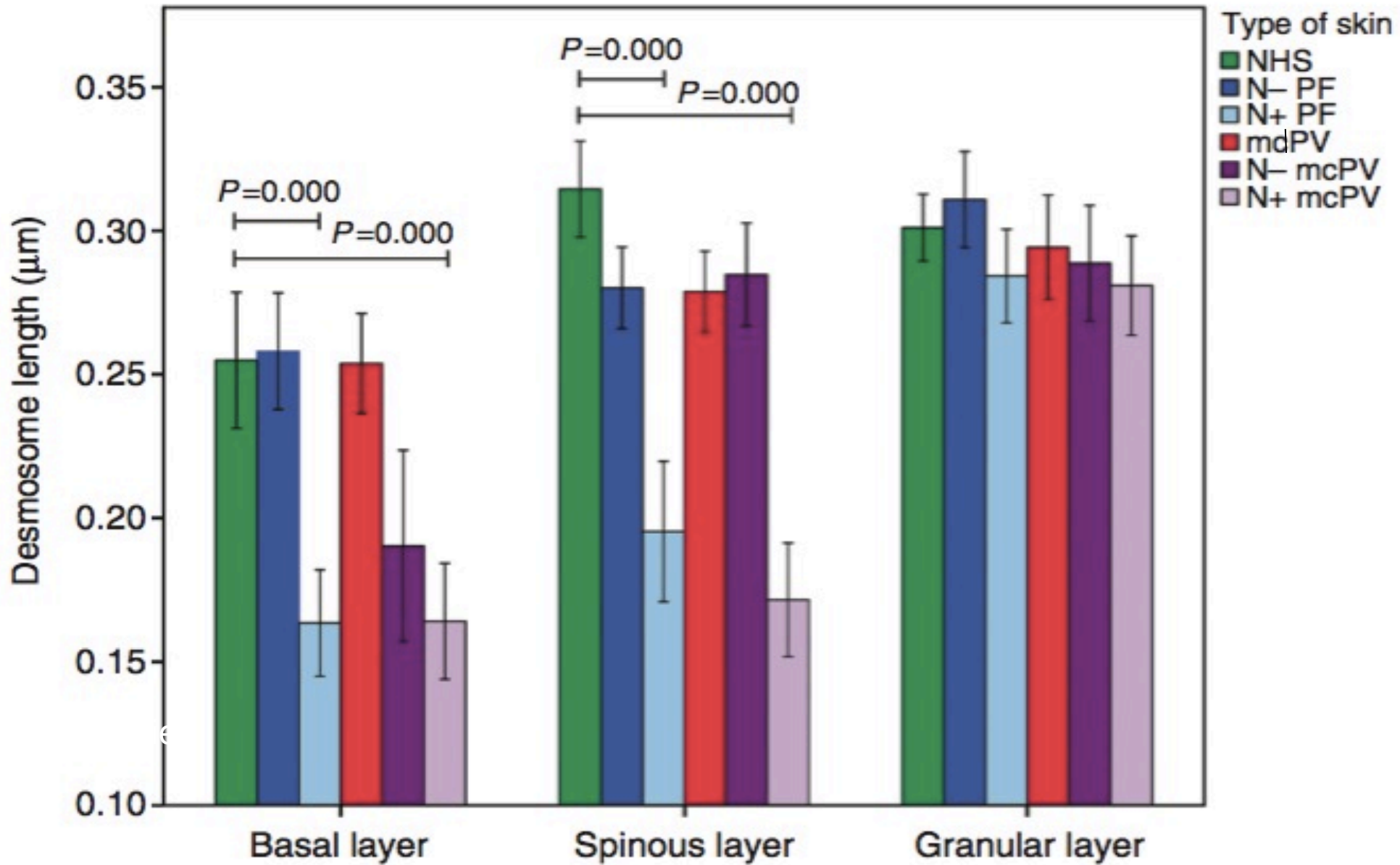


Immuno-EM morphometry  
Non-lesional PF skin



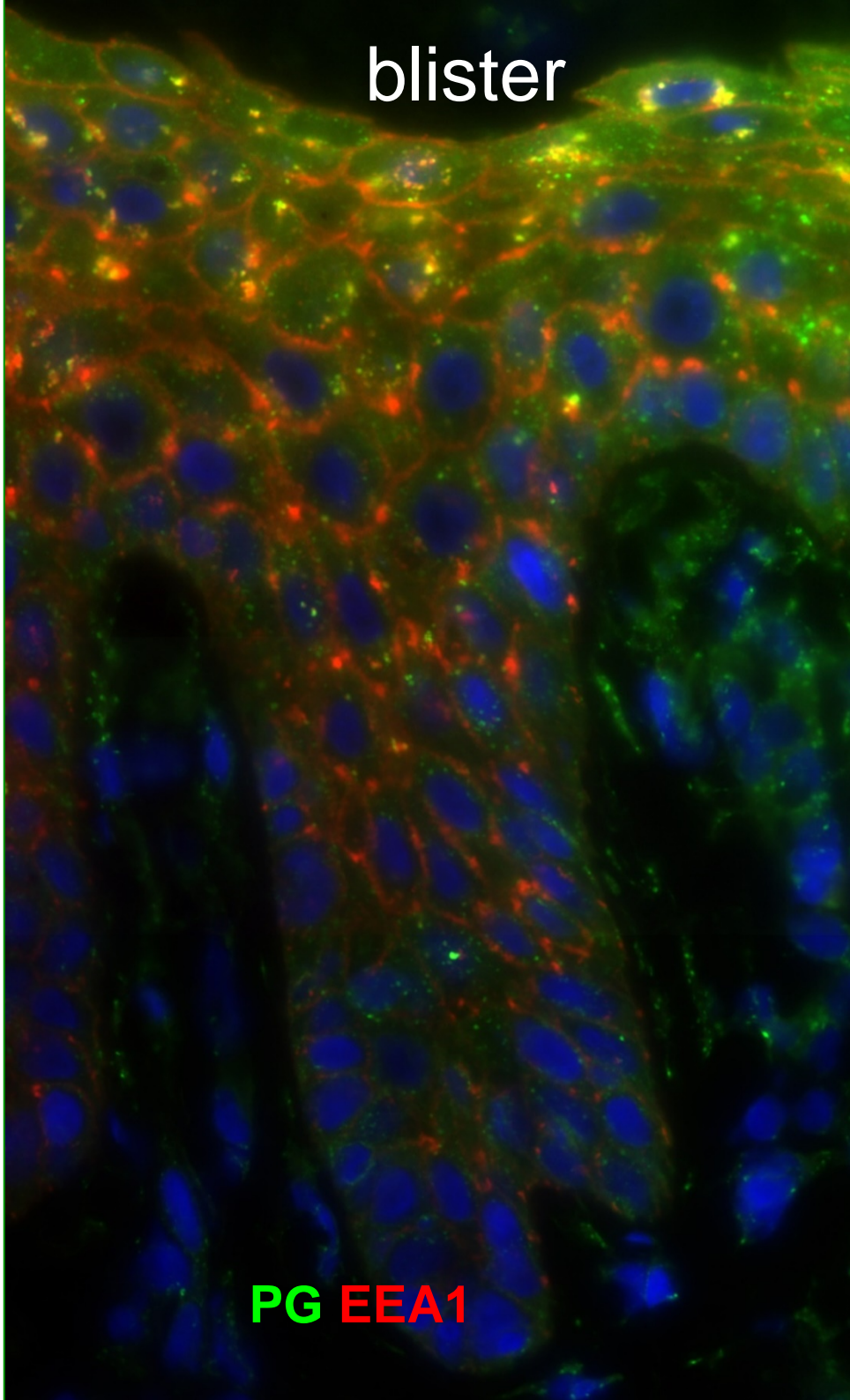
Dsg1 is depleted from desmosomes.

# EM morphometry of desmosomes in PF skin

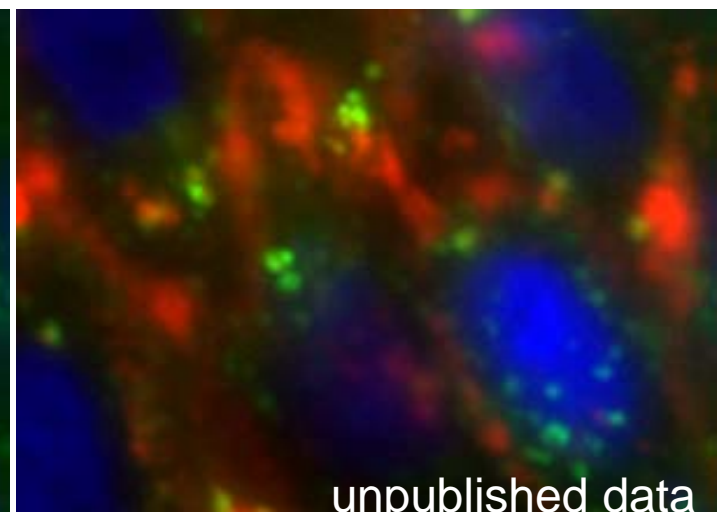
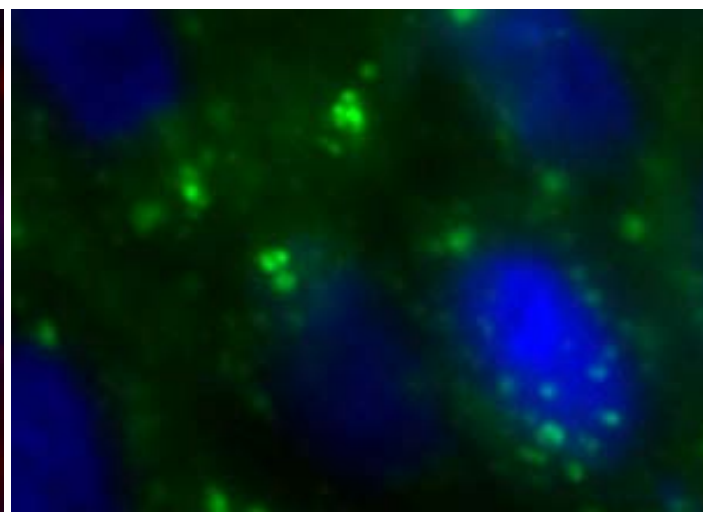
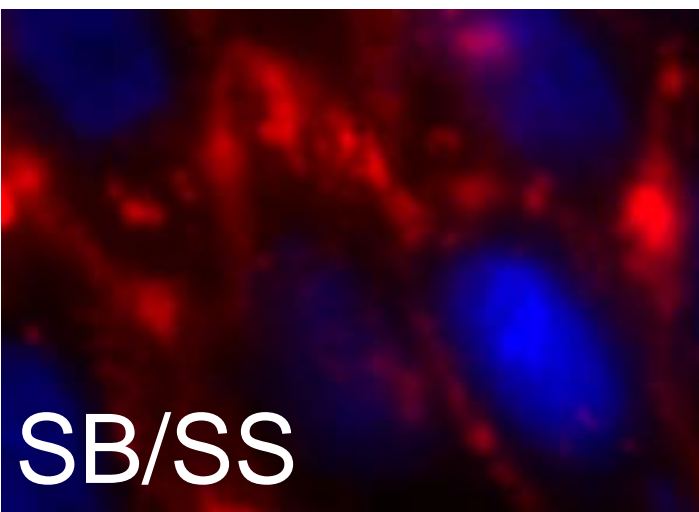
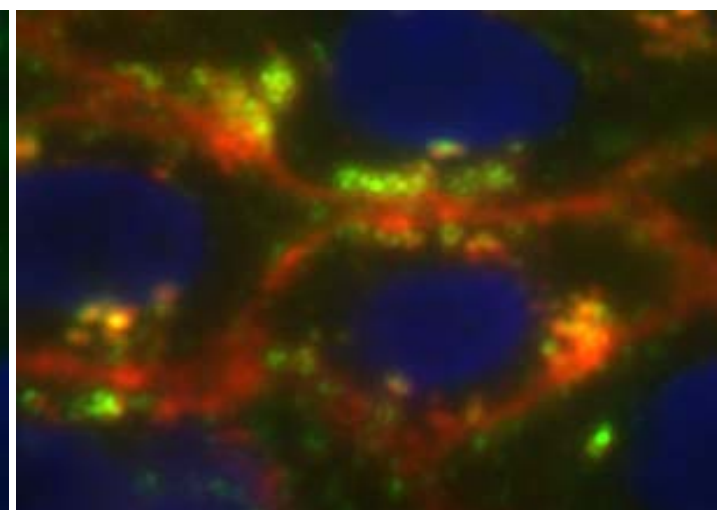
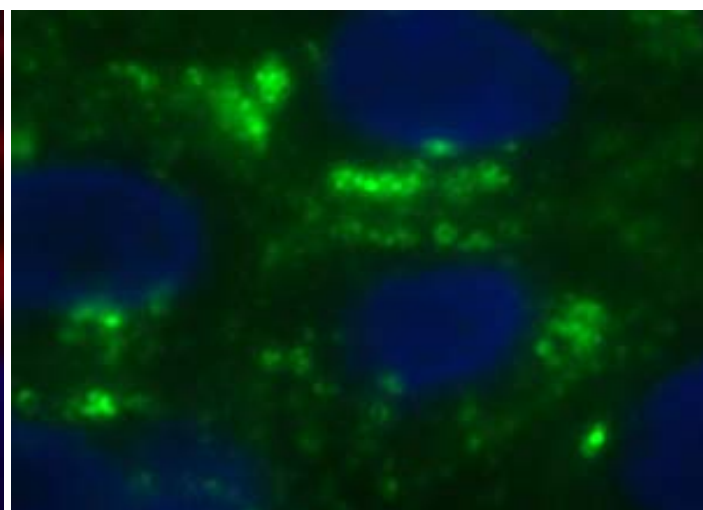
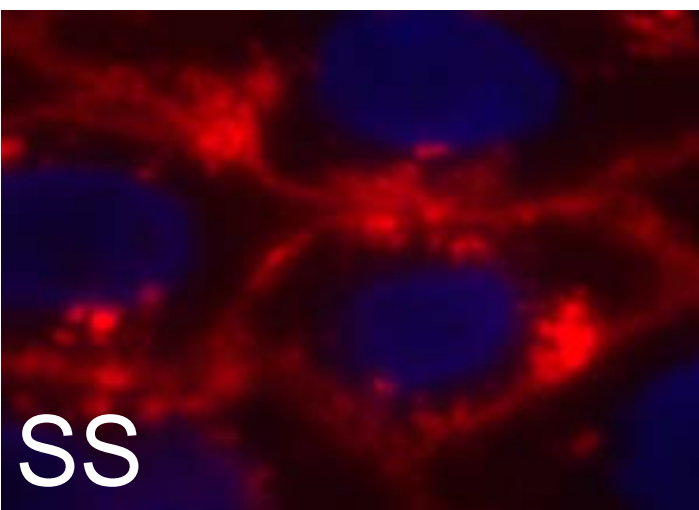
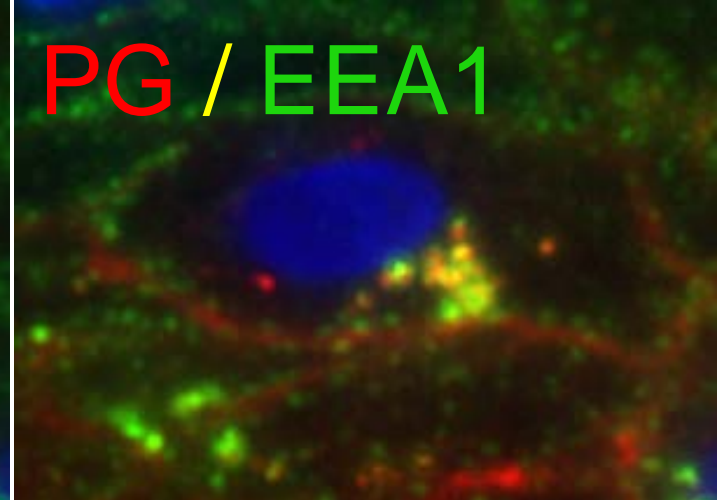
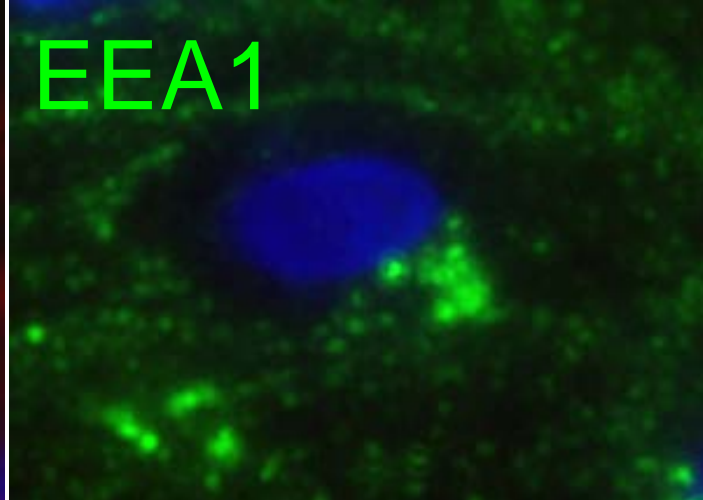
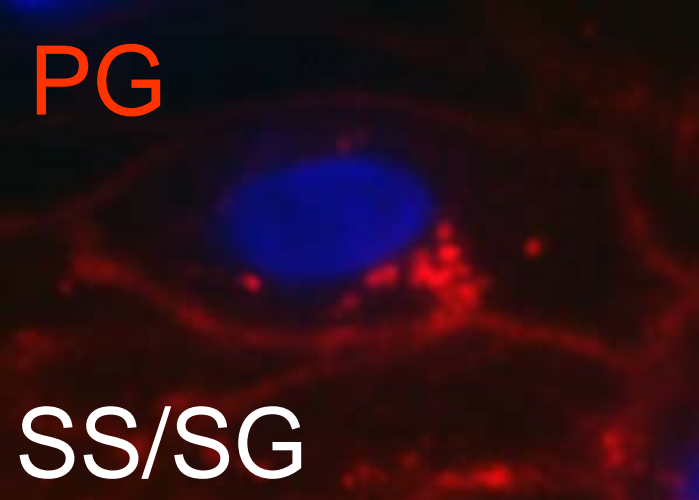


**Lesional PF skin**

**blister**



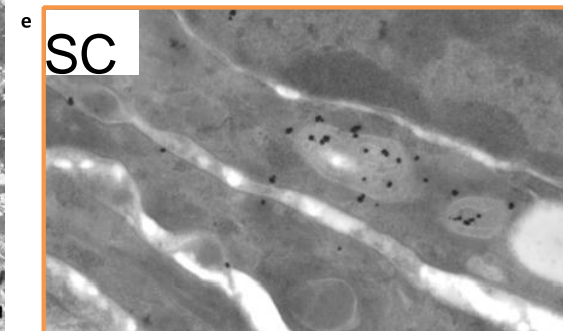
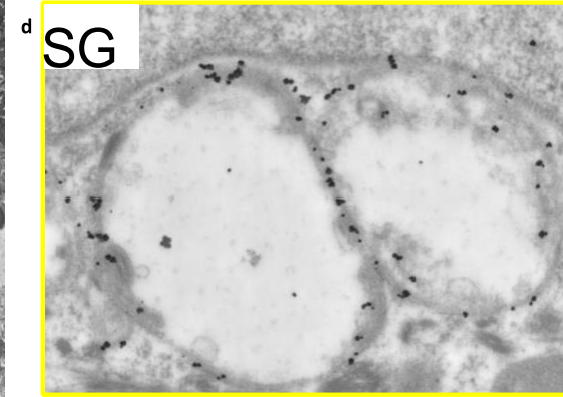
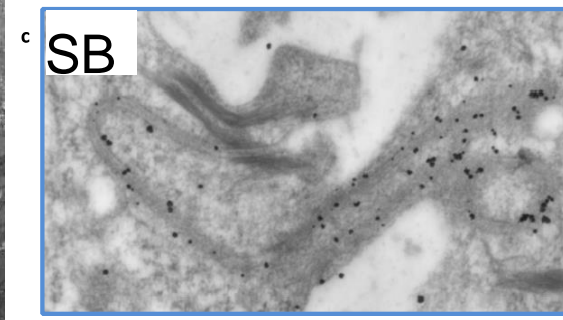
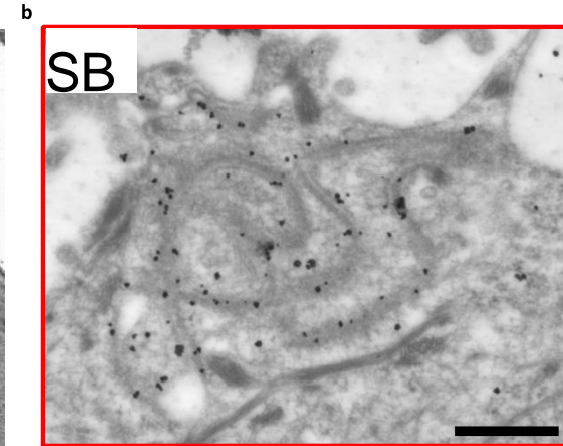
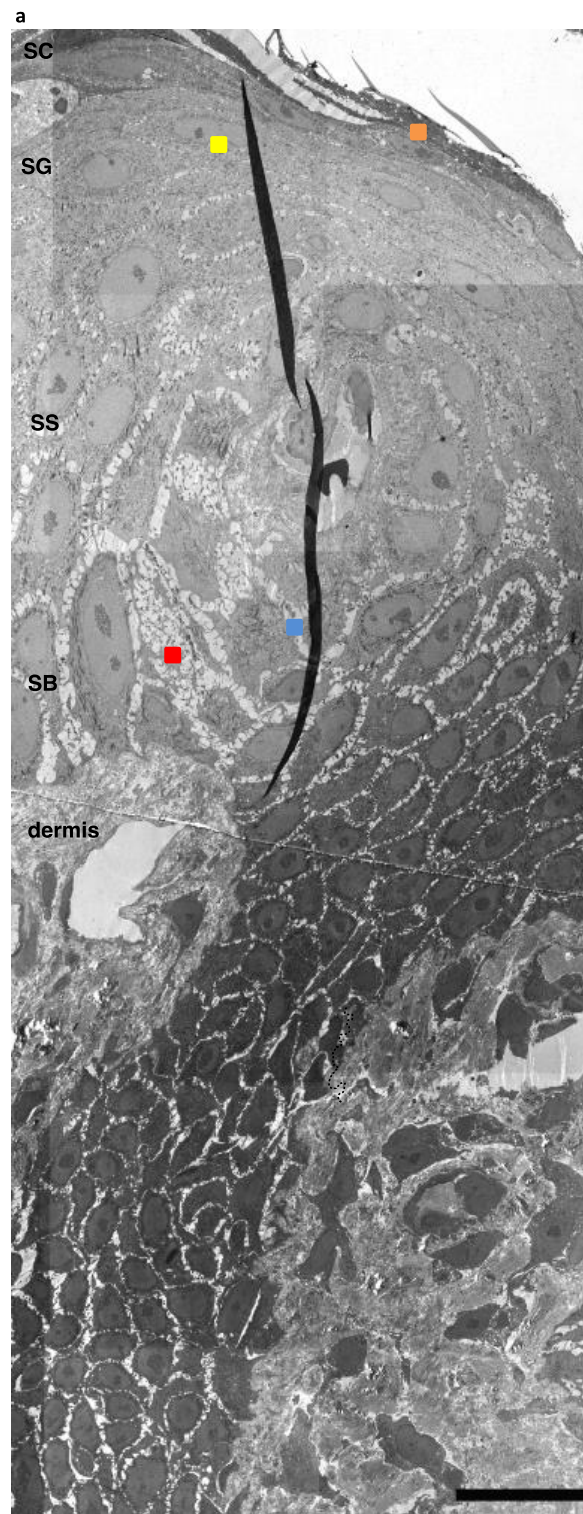
**PG EEA1**

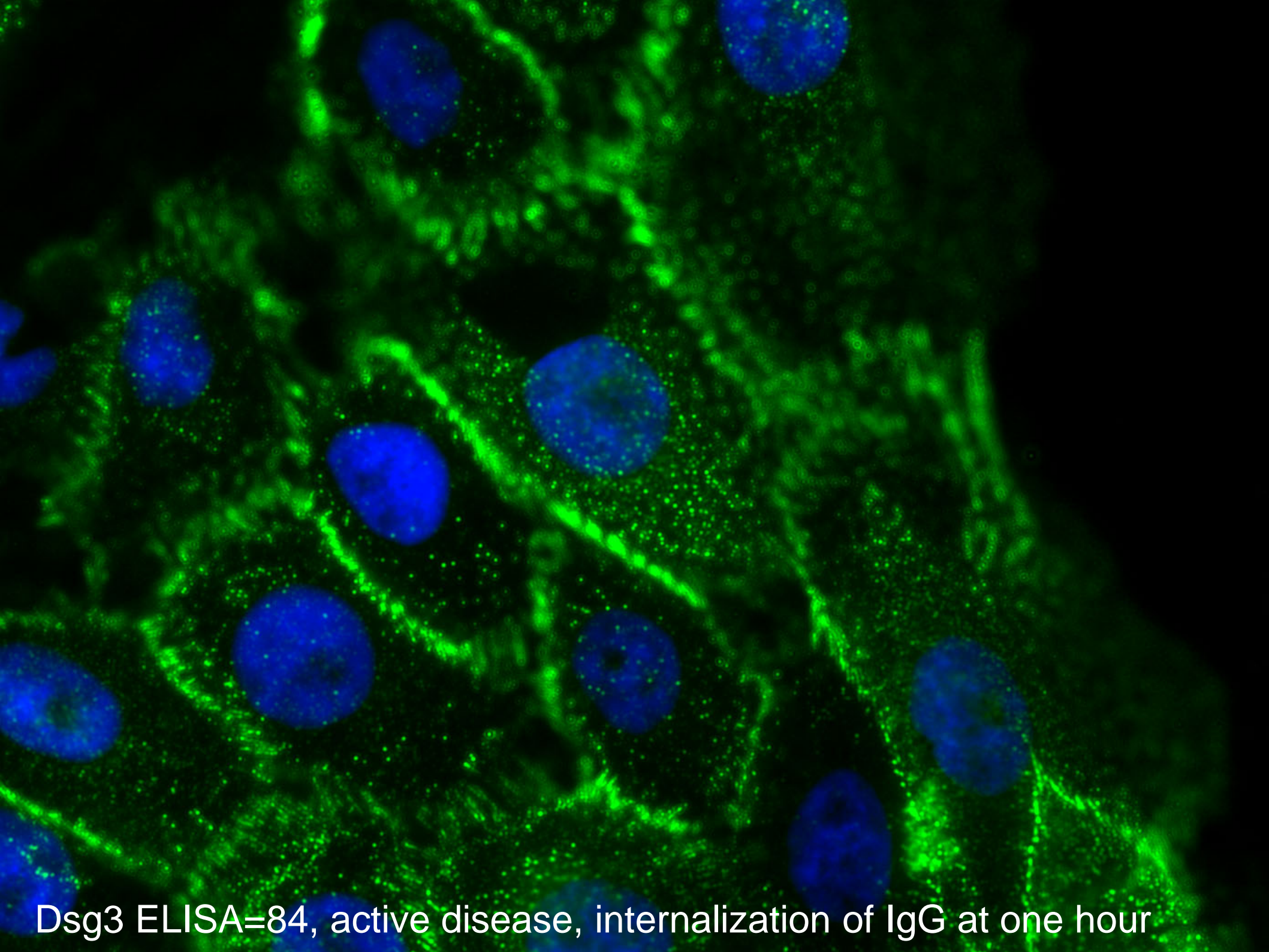


# Immuno-EM

## Non-lesional PF skin

Remaining Dsg1 in PF is sequestered in double membrane invaginations (SB+SS), then vacuoles (SG), and finally in corneocyte inclusions (SC).

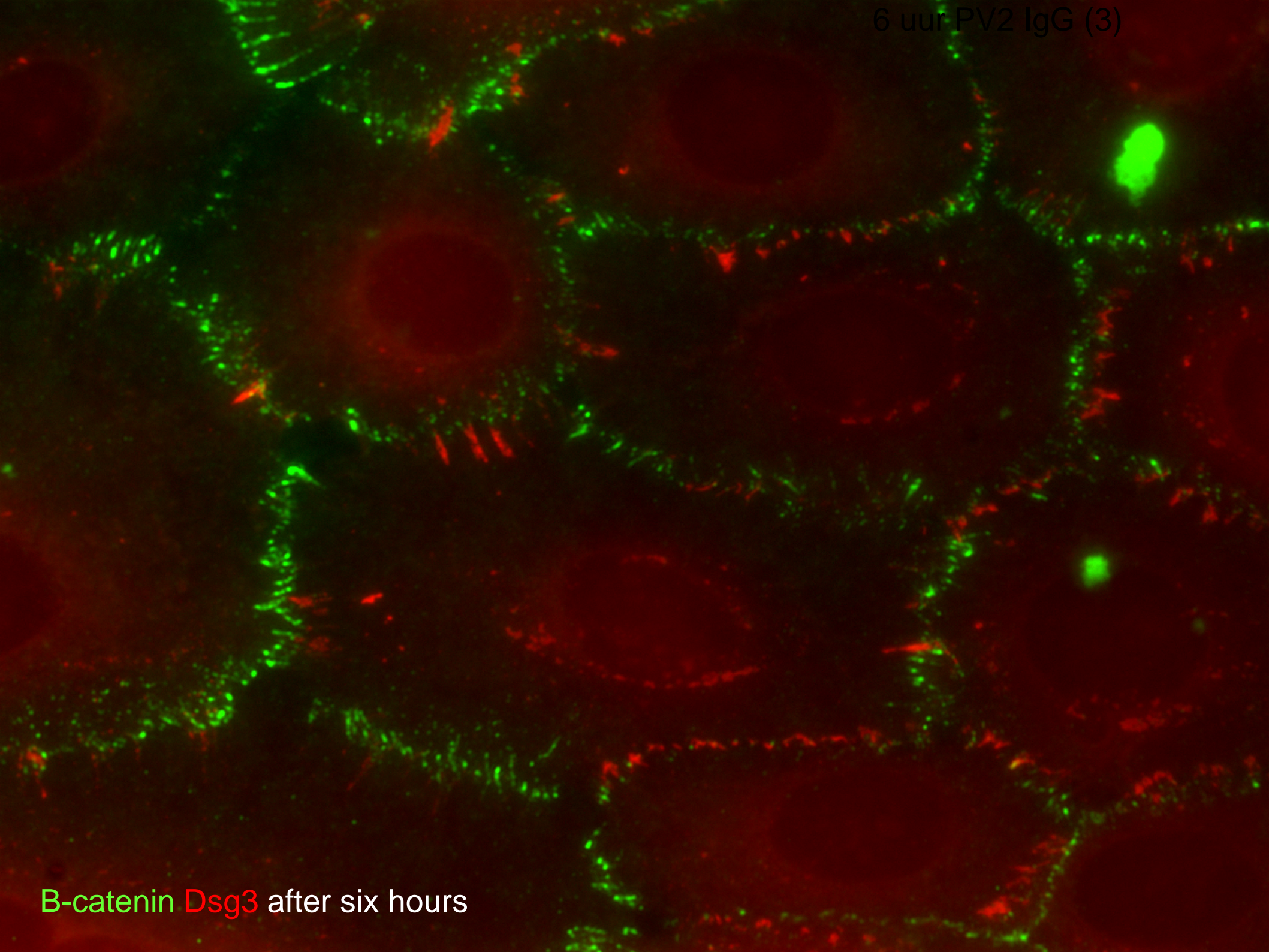




Dsg3 ELISA=84, active disease, internalization of IgG at one hour

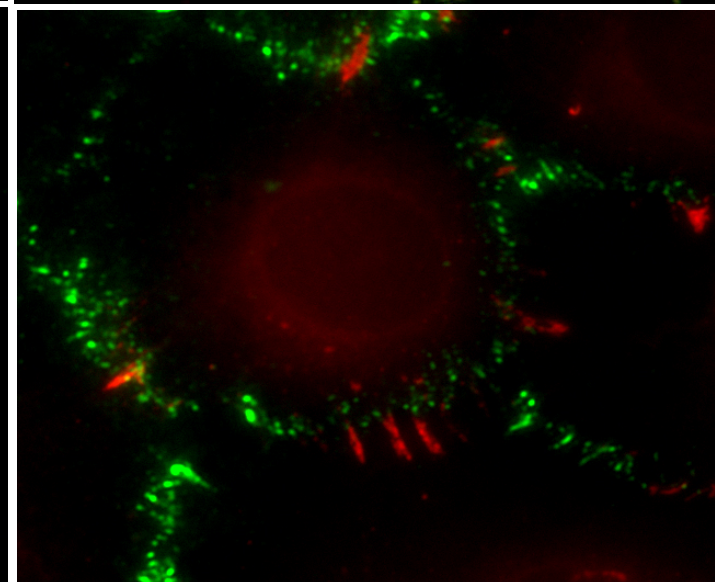
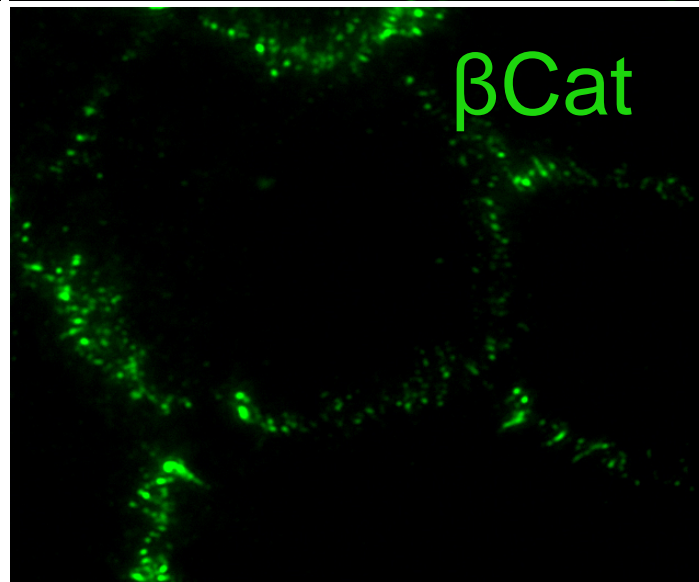
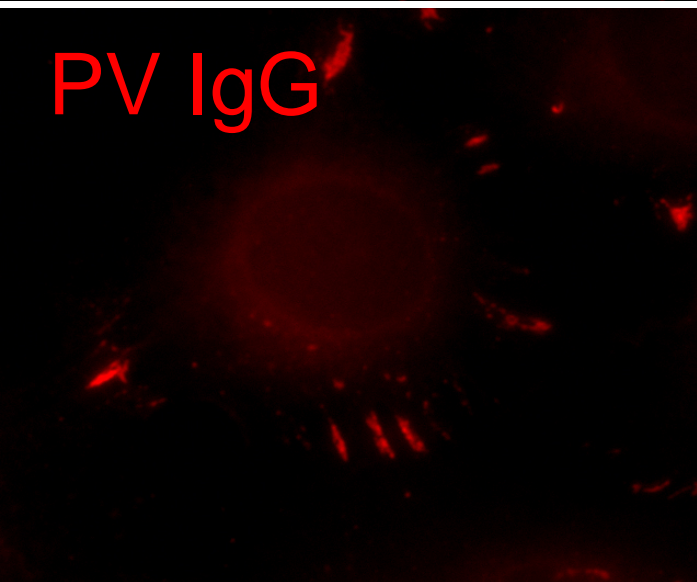
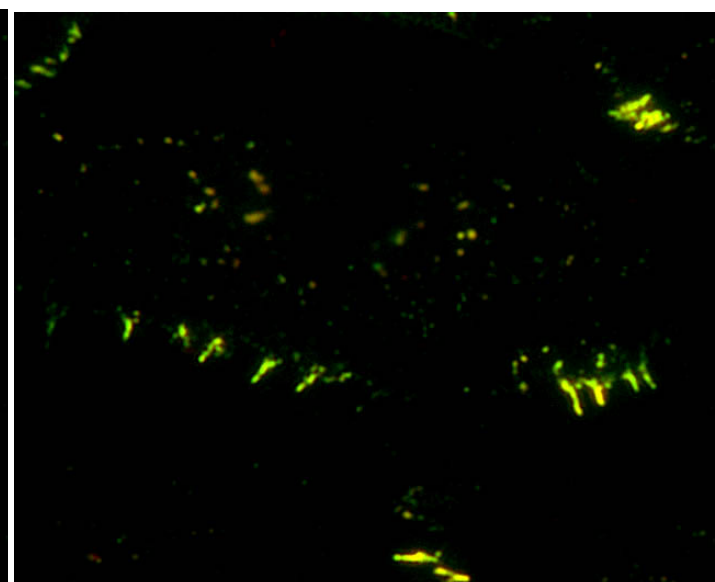
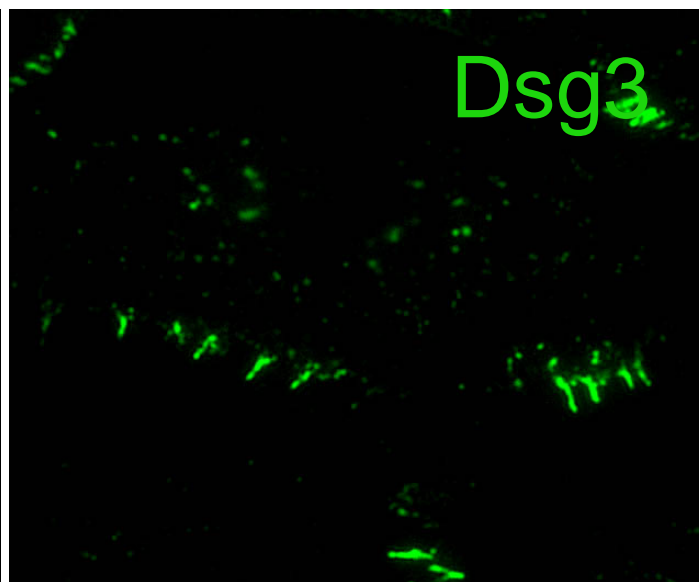
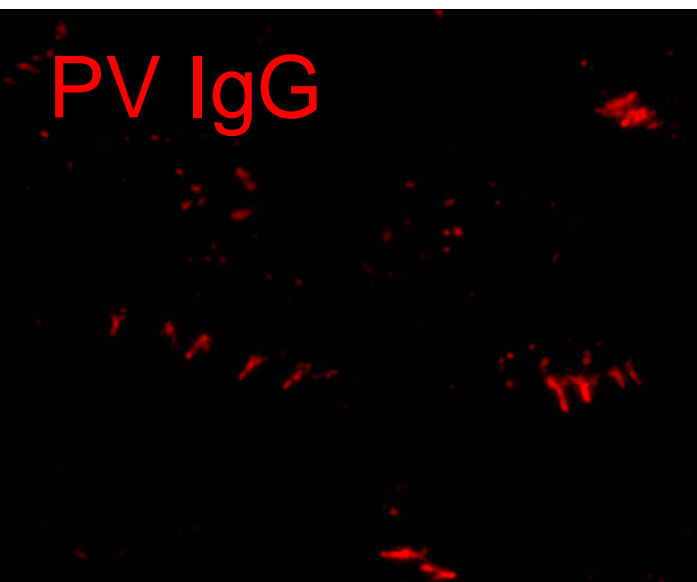


6 uur PV2 IgG (3)



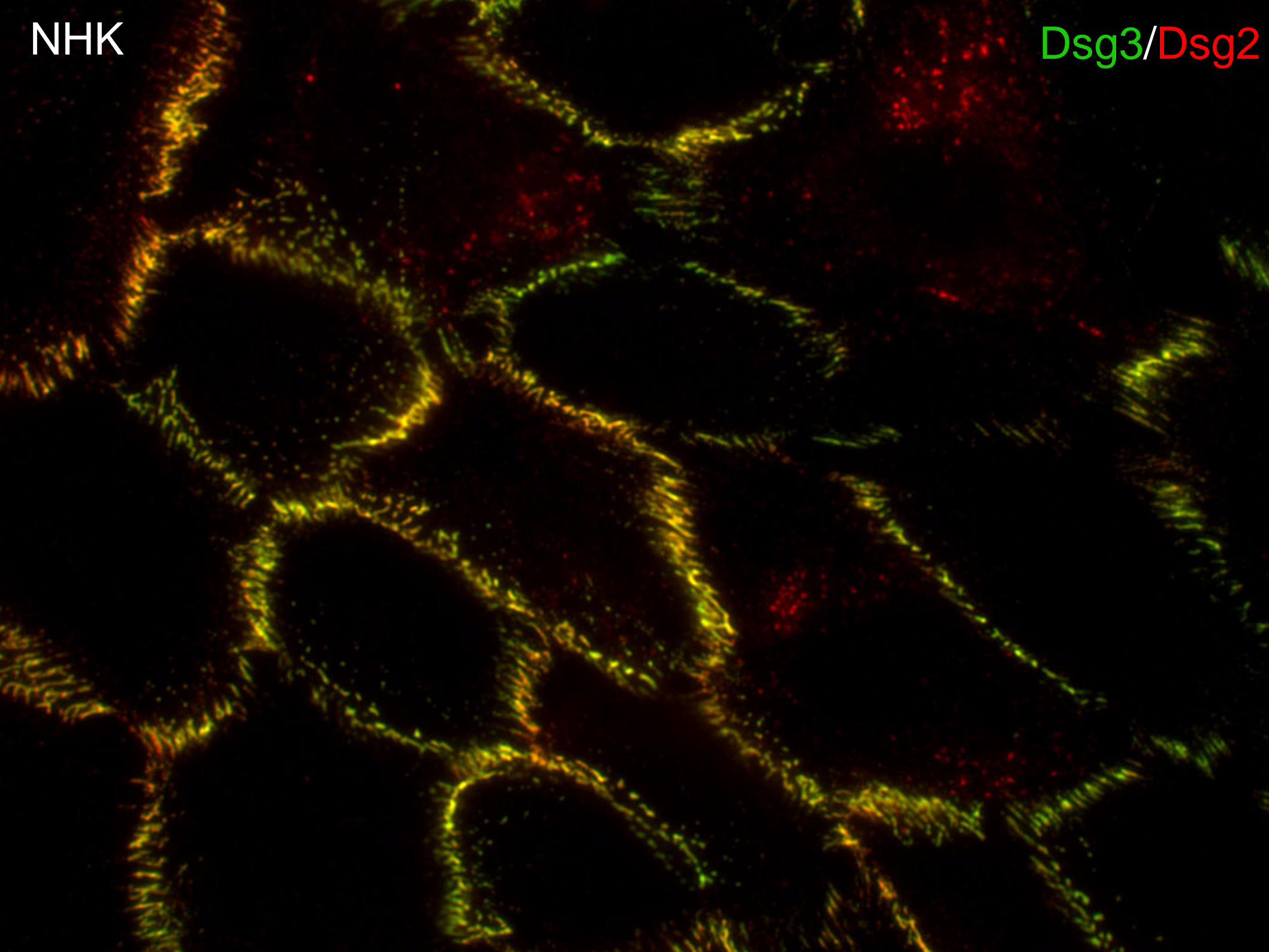
B-catenin Dsg3 after six hours

Between cells arrays form mostly unilateral ('donor/acceptor')



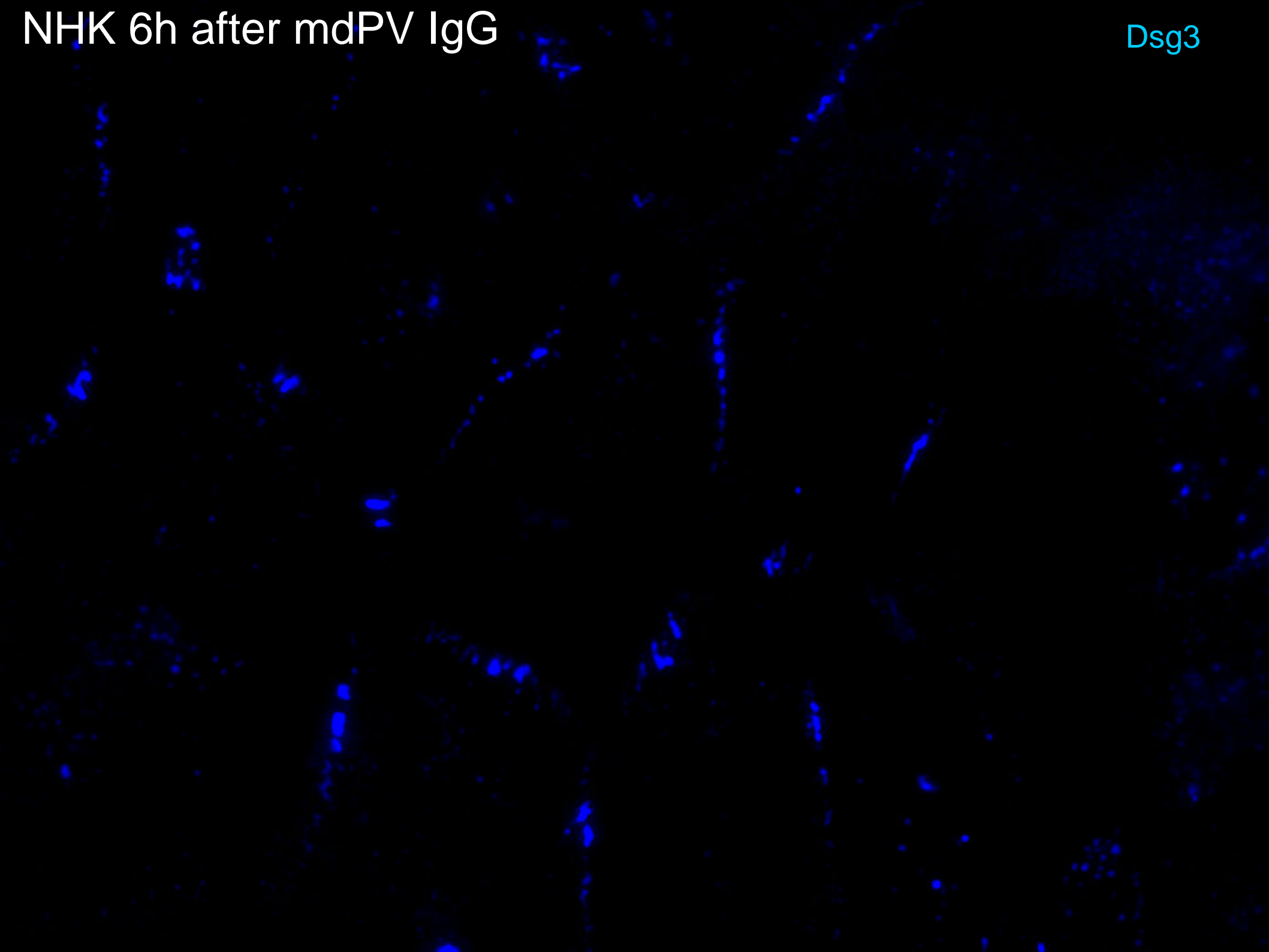
NHK

Dsg3/Dsg2



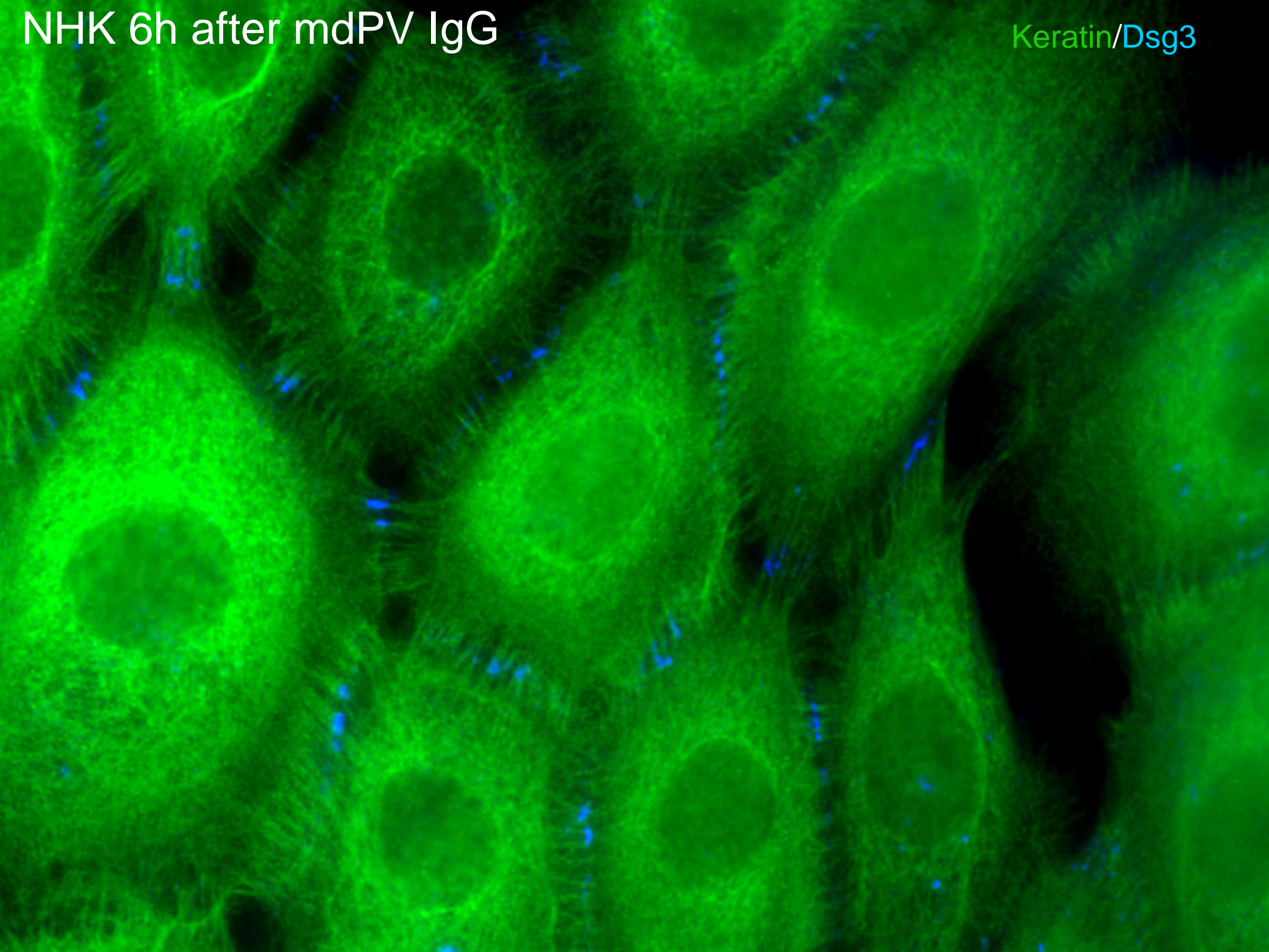
NHK 6h after mdPV IgG

Dsg3



NHK 6h after mdPV IgG

Keratin/Dsg3



NHK 6h after mdPV IgG

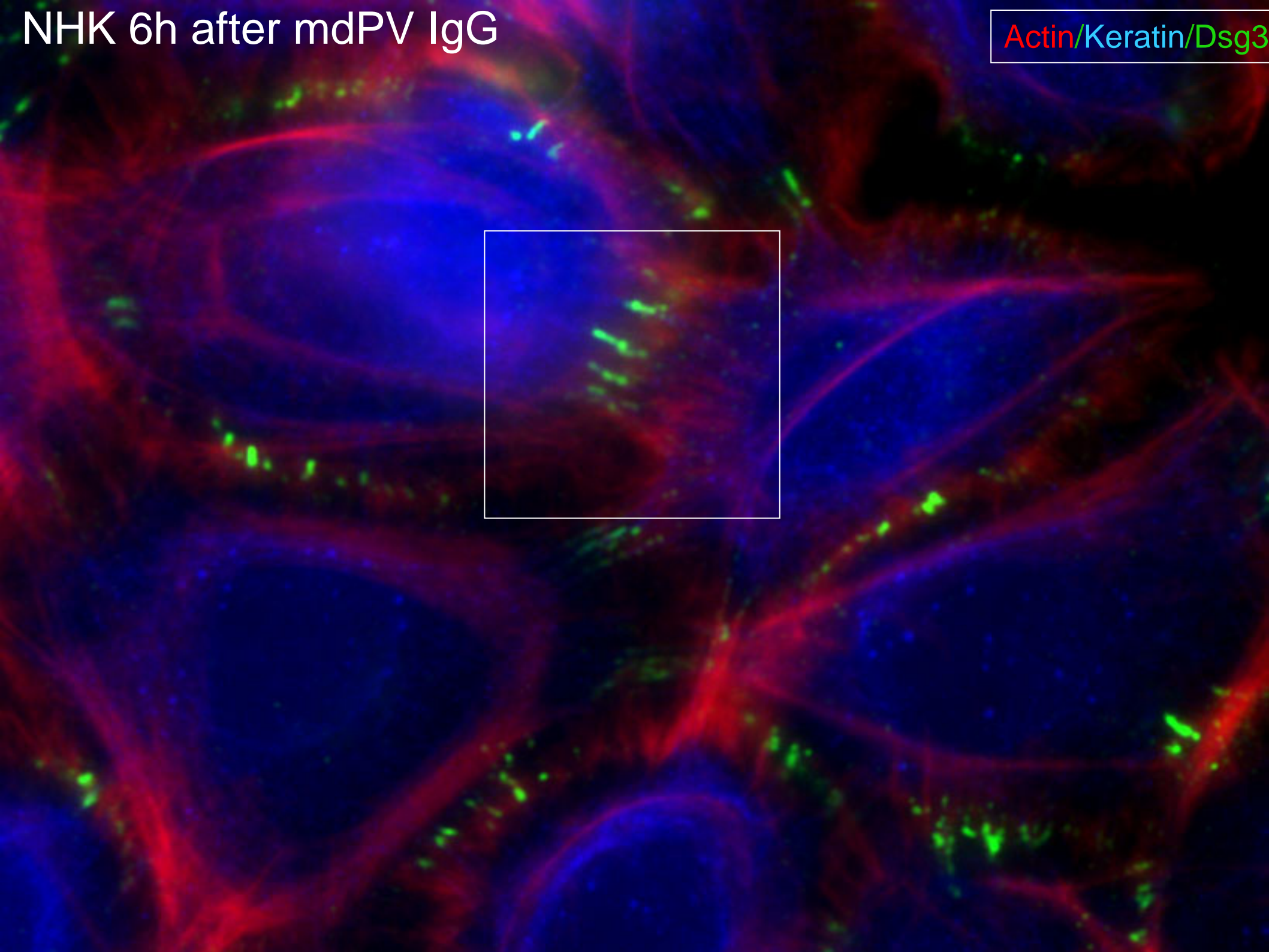
Keratin/Dsg3/Dsg2



IgG depletes desmosomes of Dsg3

NHK 6h after mdPV IgG

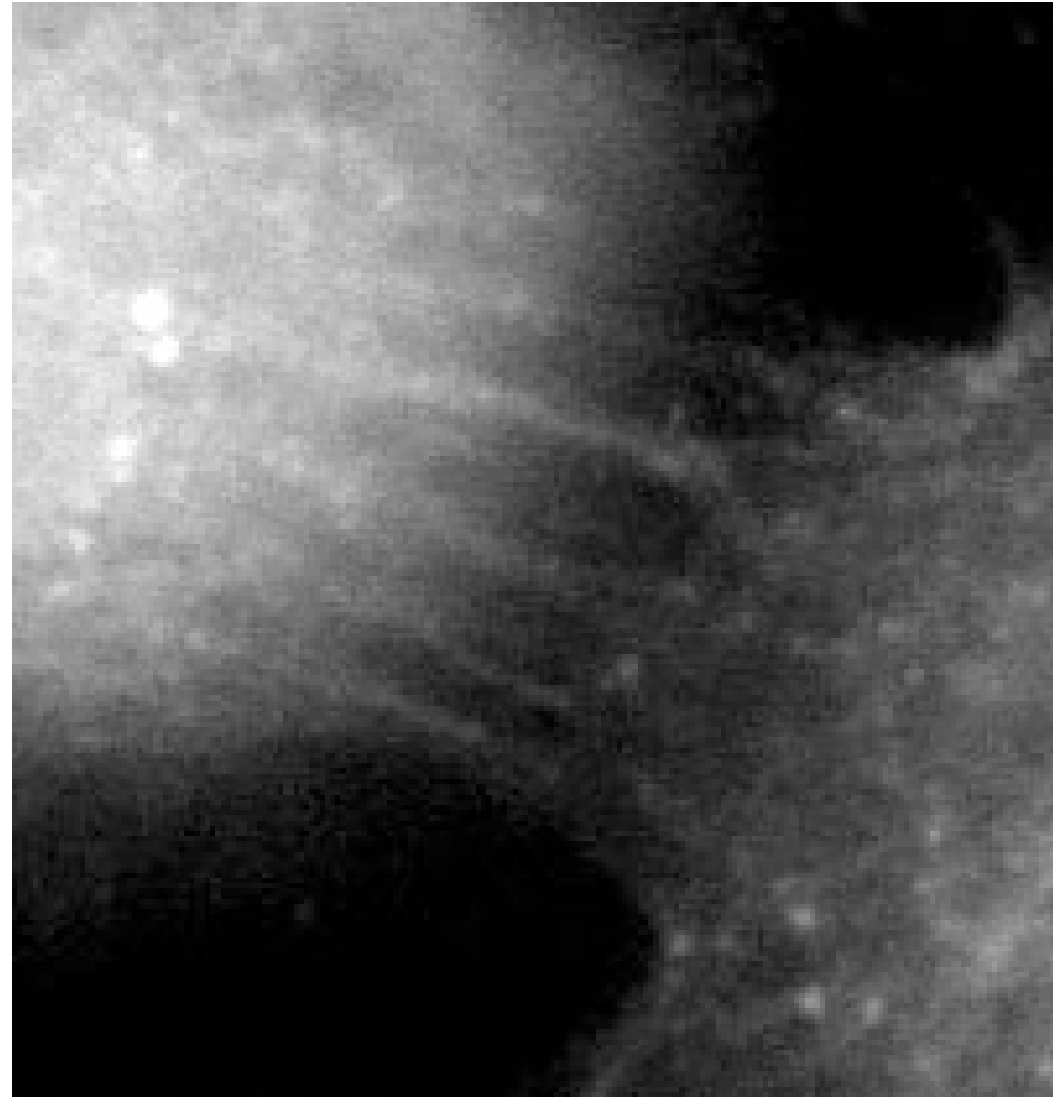
Actin/Keratin/Dsg3



# Location of Dsg3



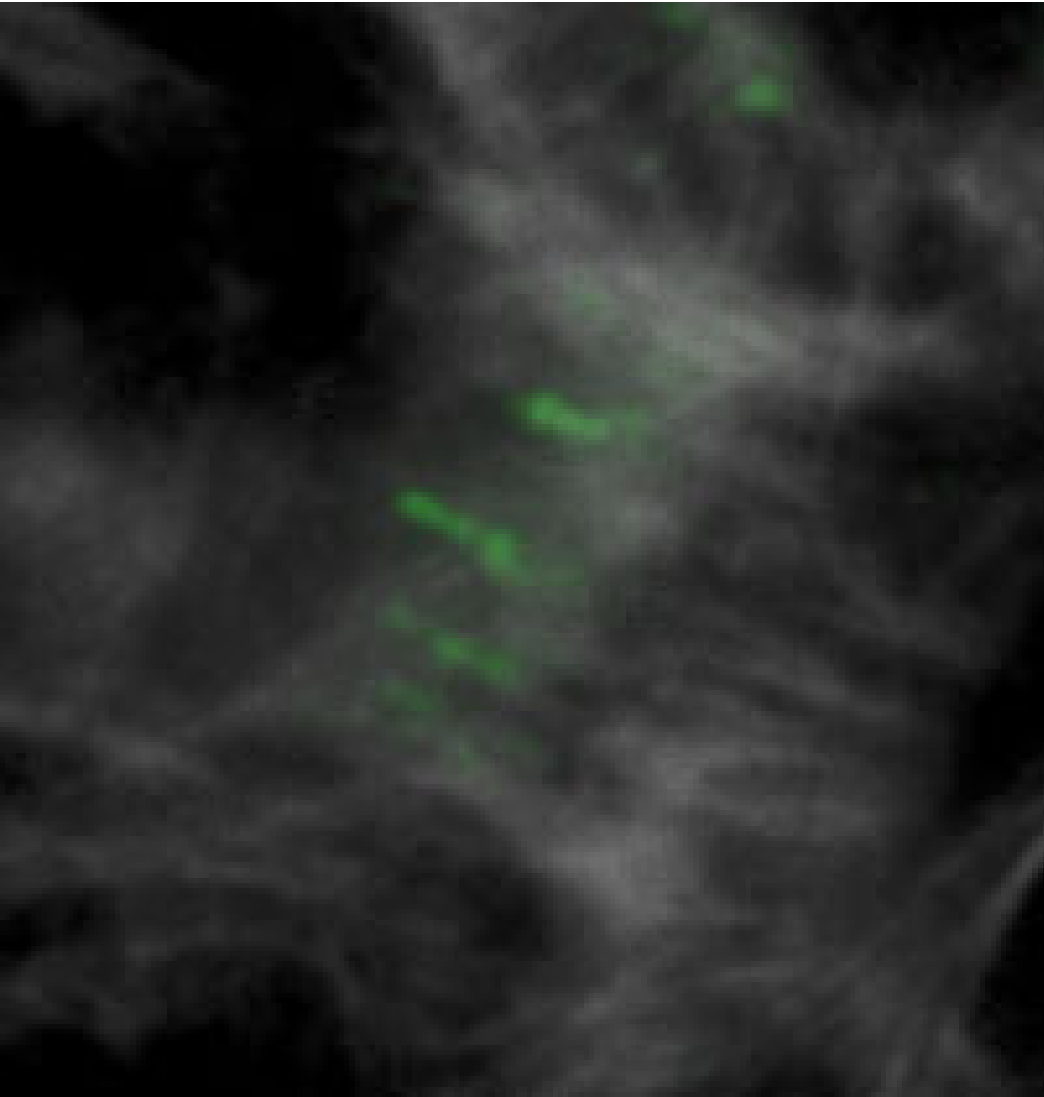
Actin



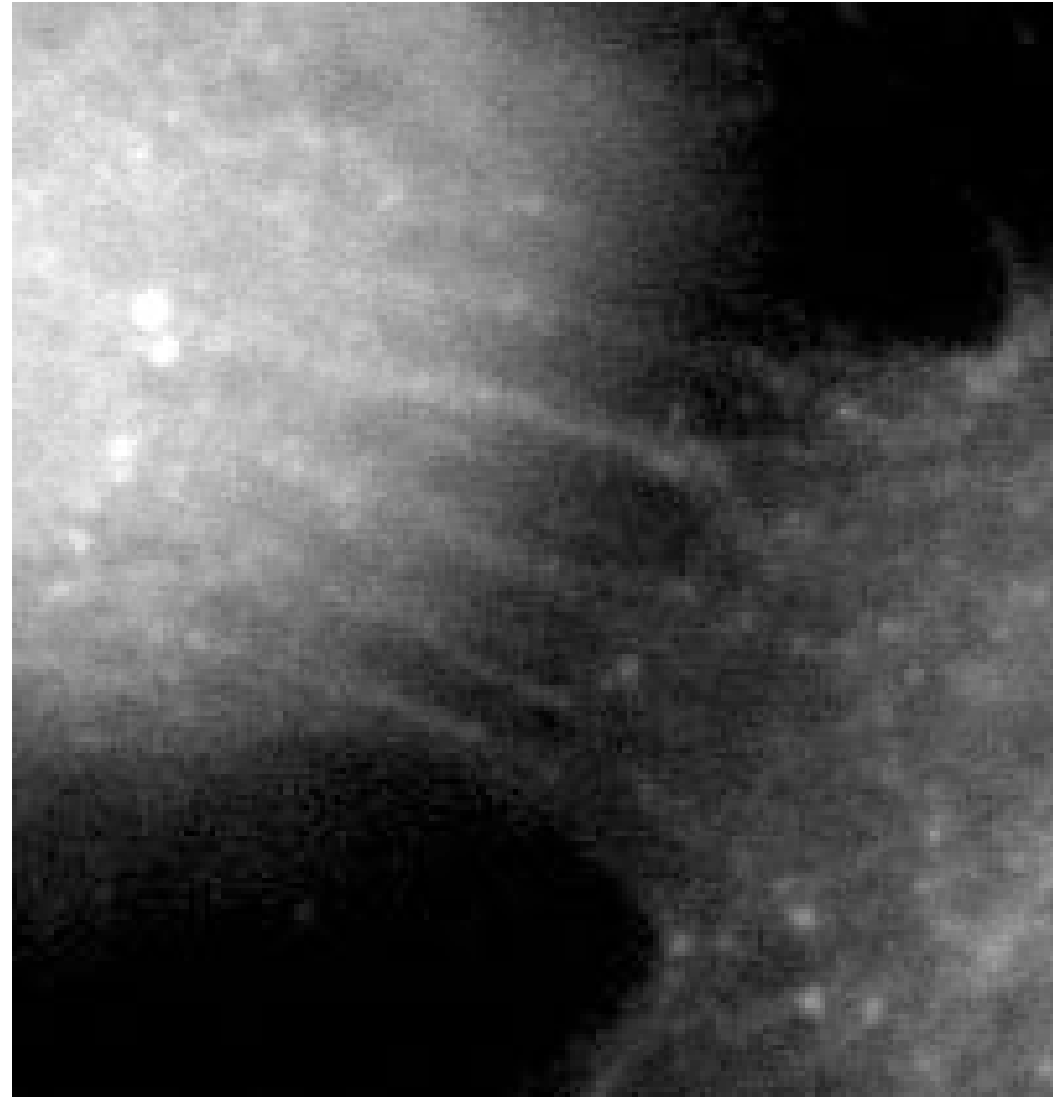
Keratin



# Location of Dsg3



Actin

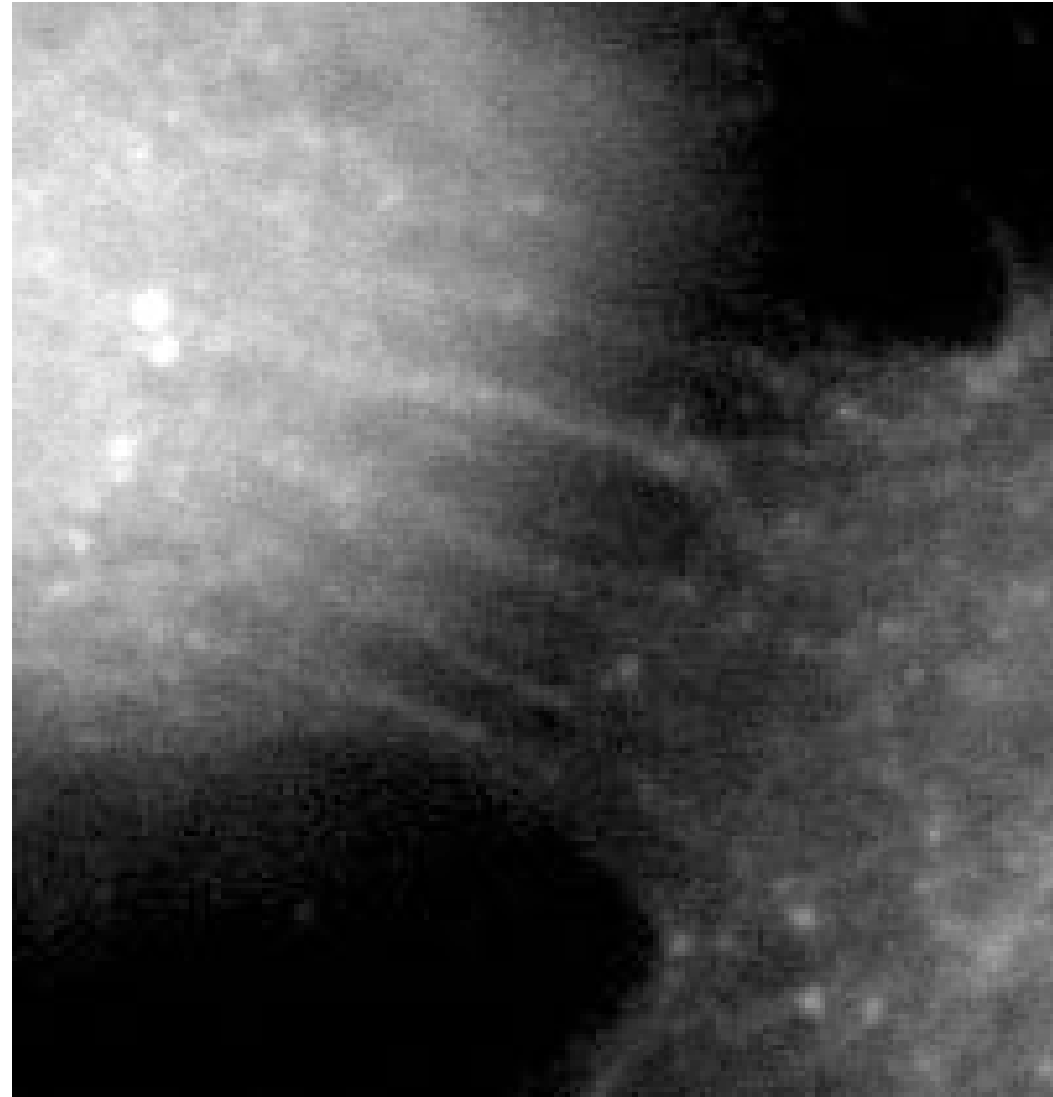


Keratin

# Location of Dsg3

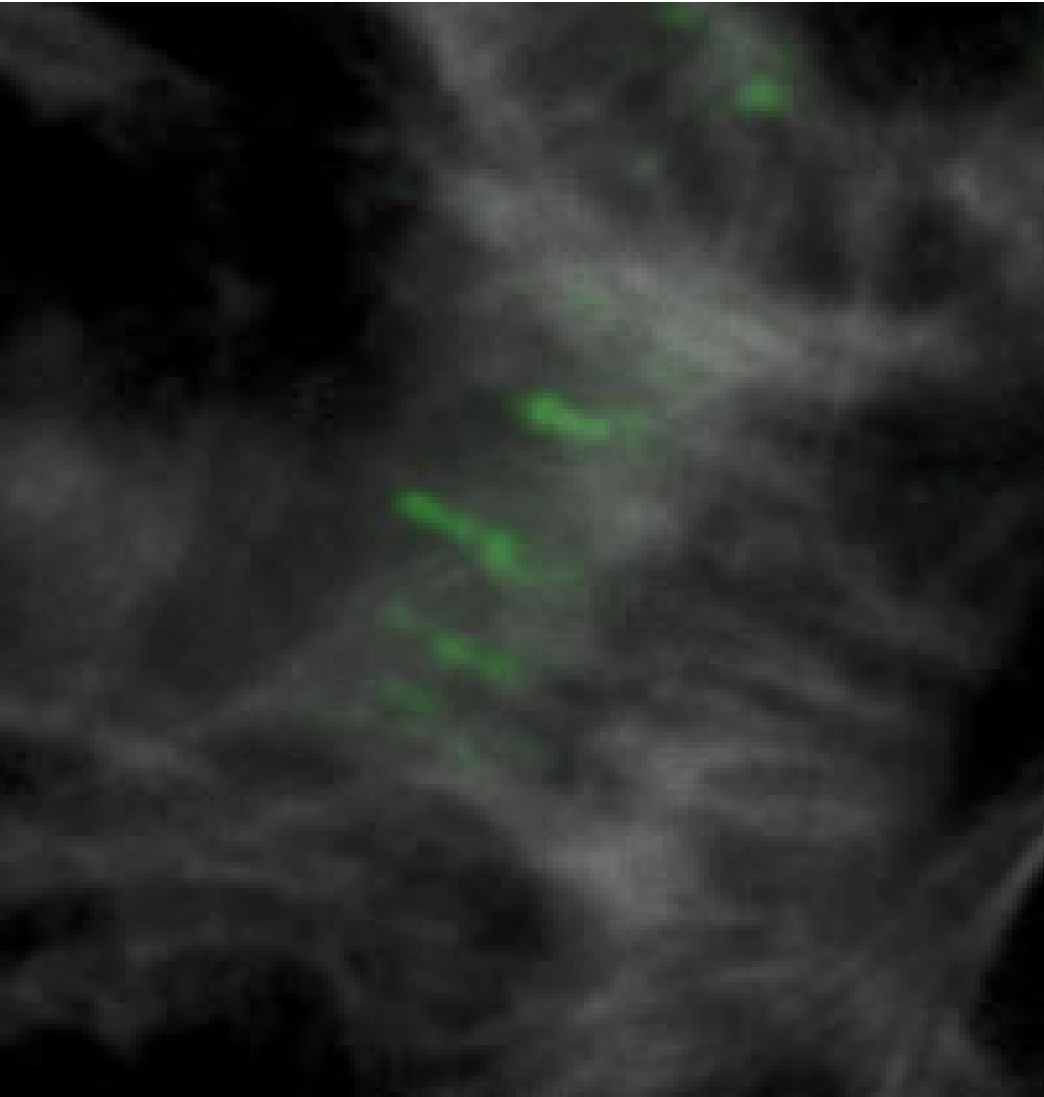


Actin

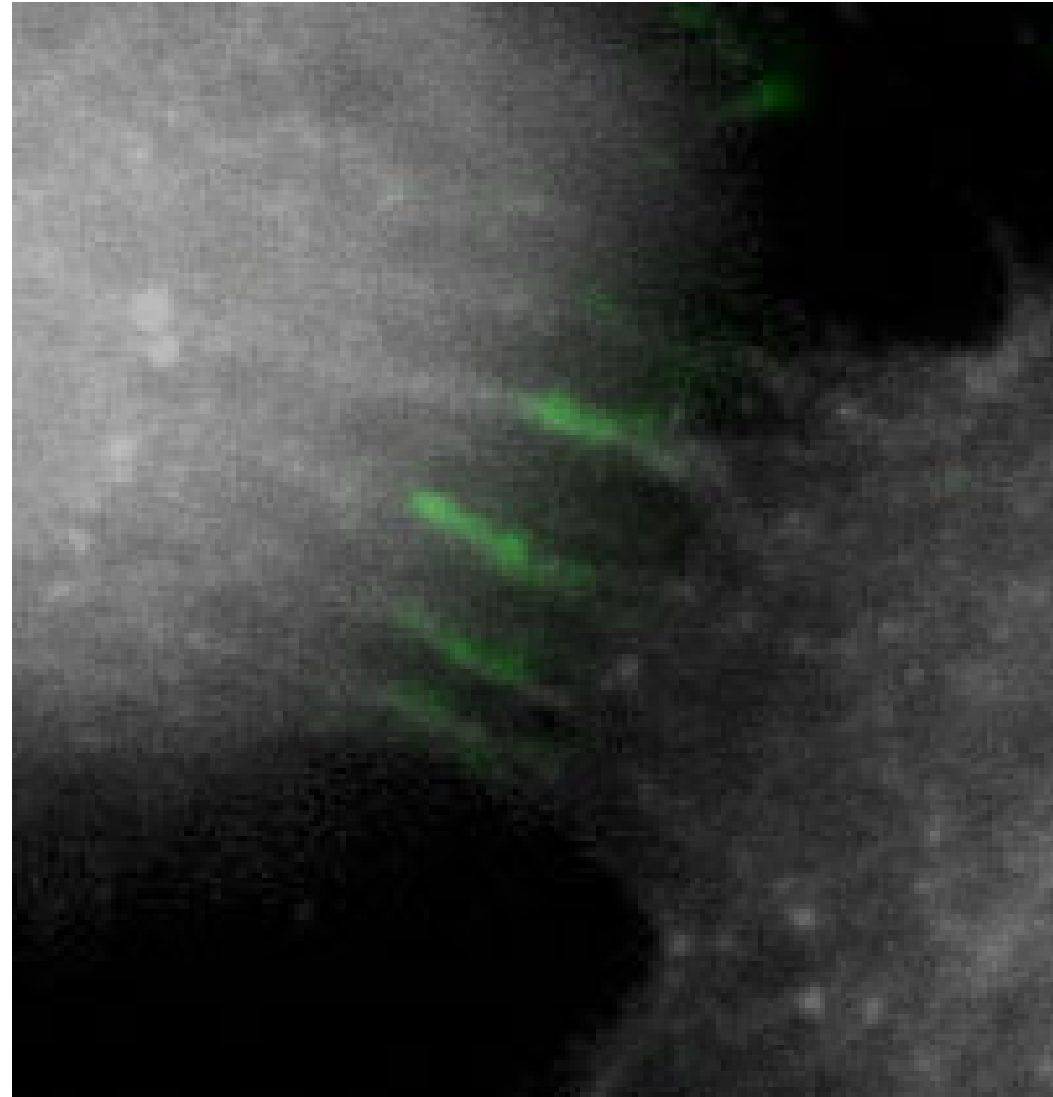


Keratin

# Location of Dsg3



Actin

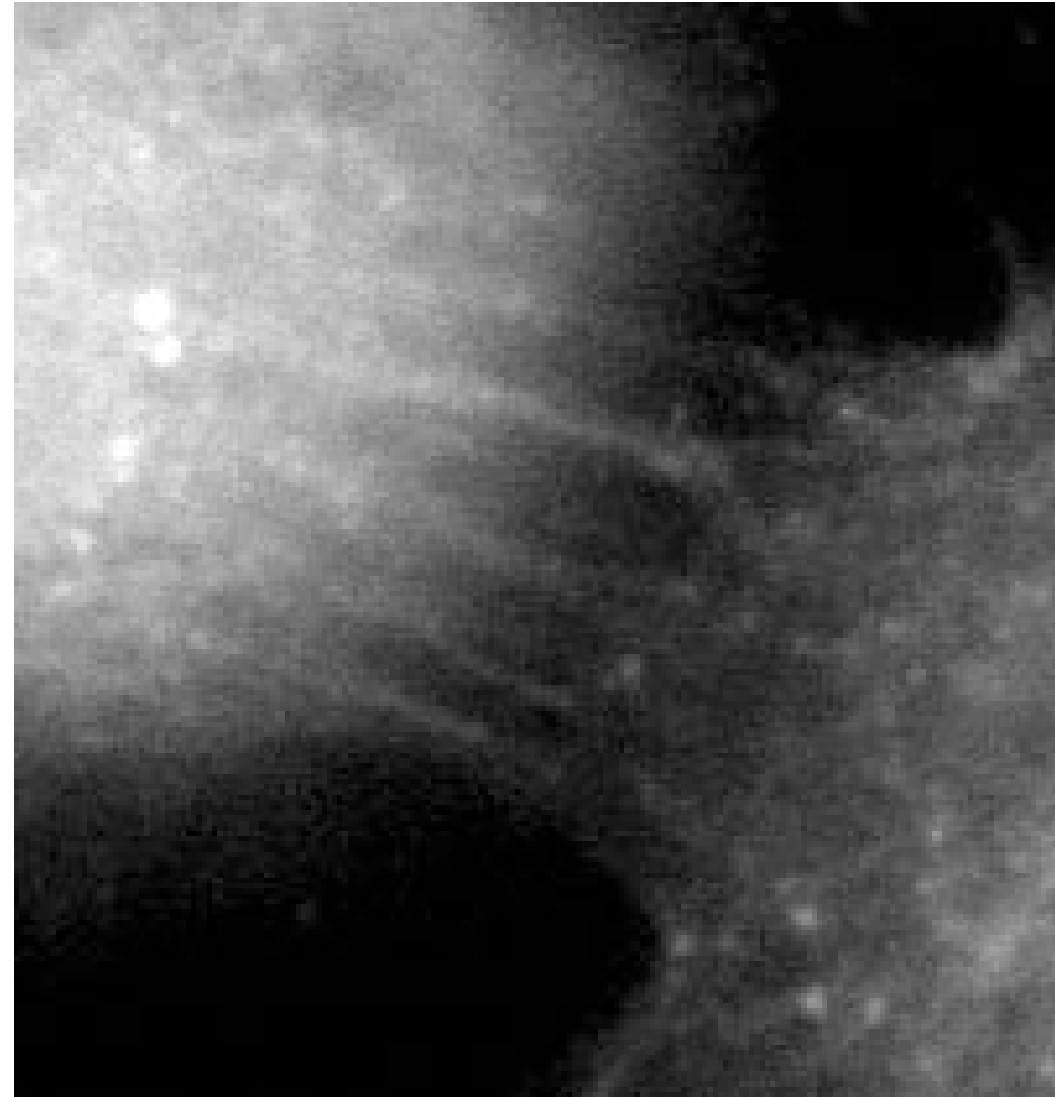


Keratin

# Location of Dsg3



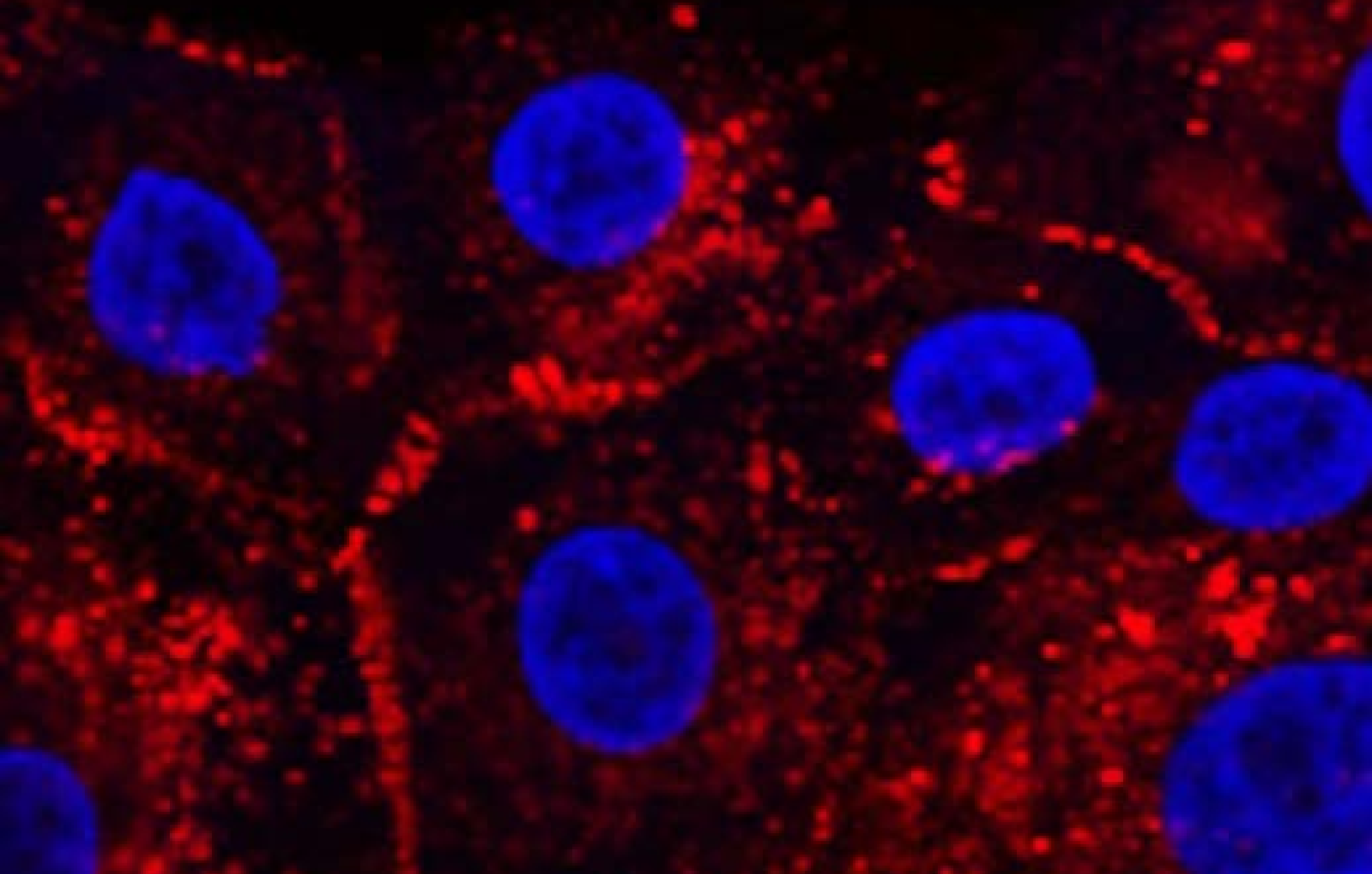
Actin



Keratin

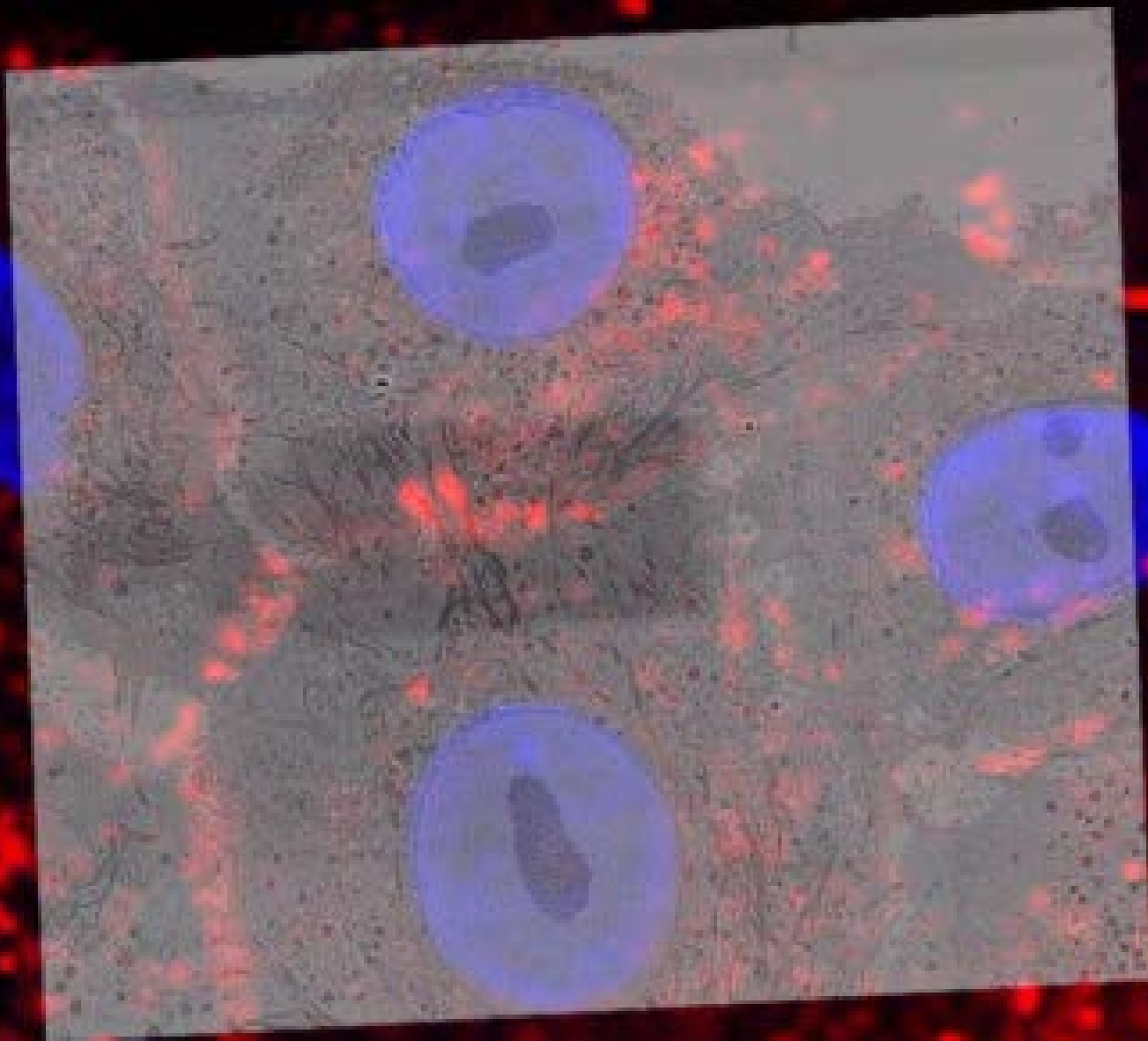
Arrays mainly localize with keratin

# Ultrastructural analysis of arrays by correlative light and electron microscopy

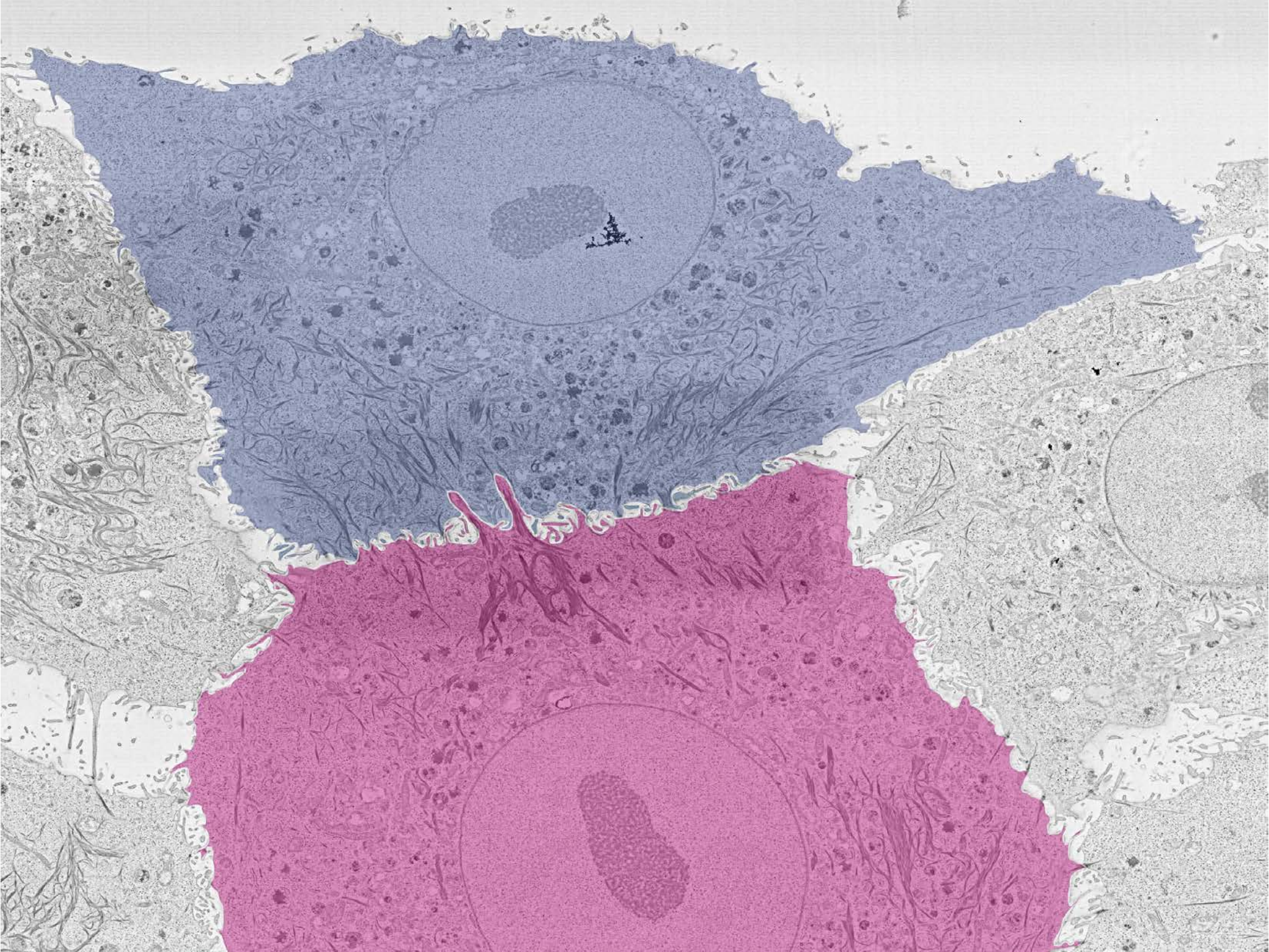


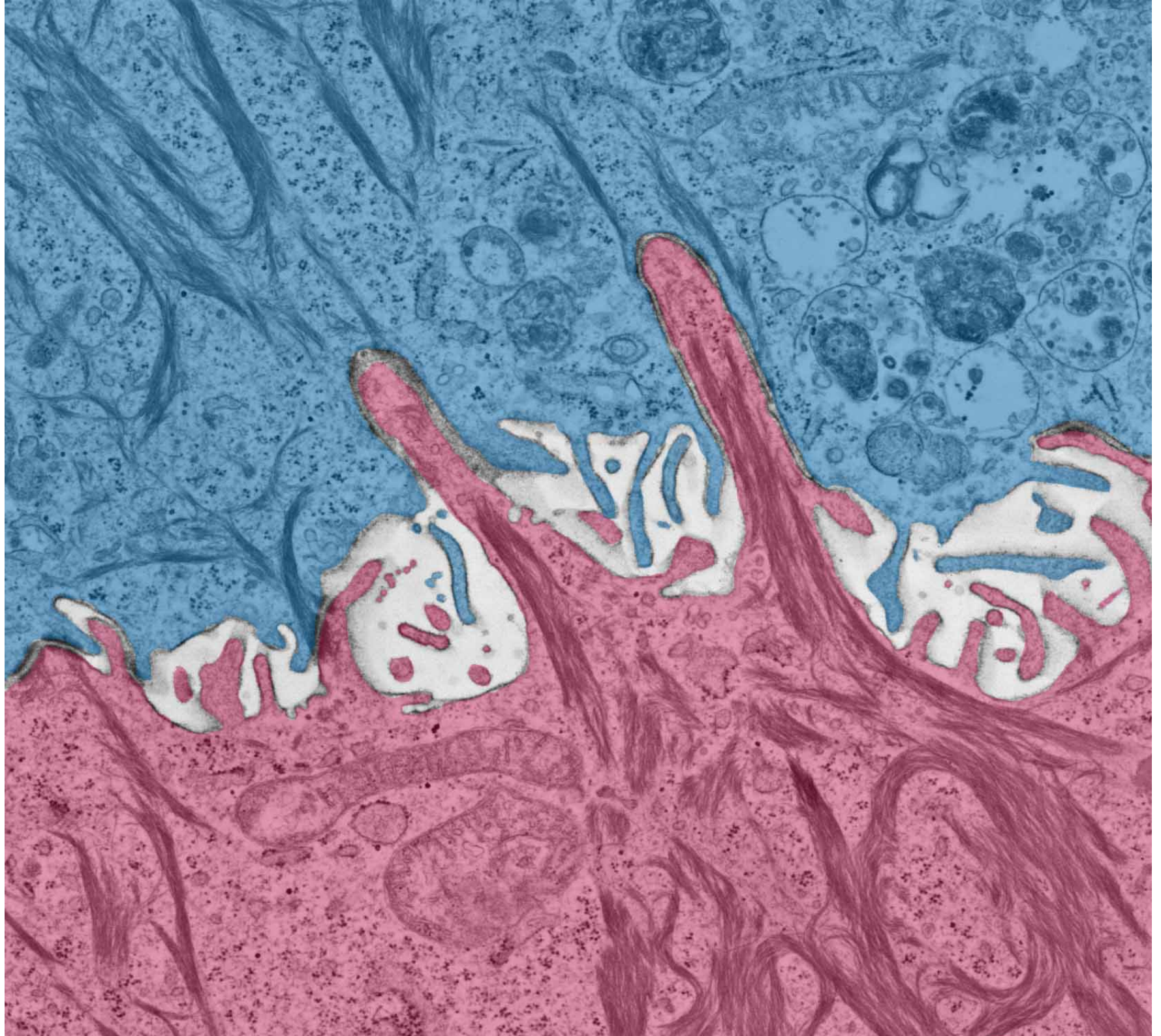
Alexa 568 labeled patient IgG



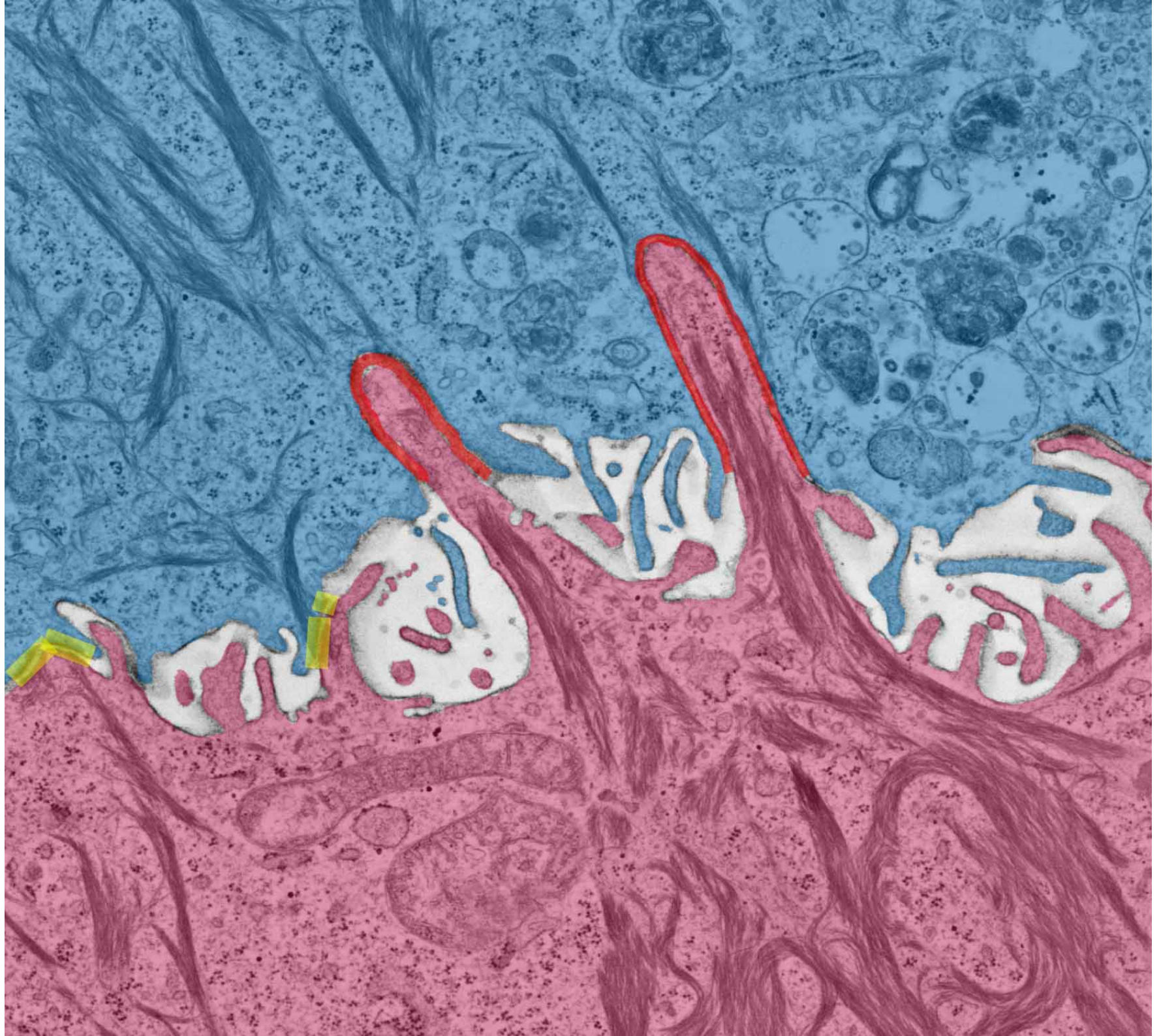


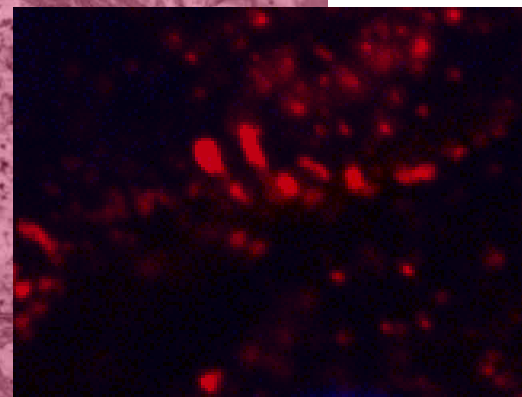
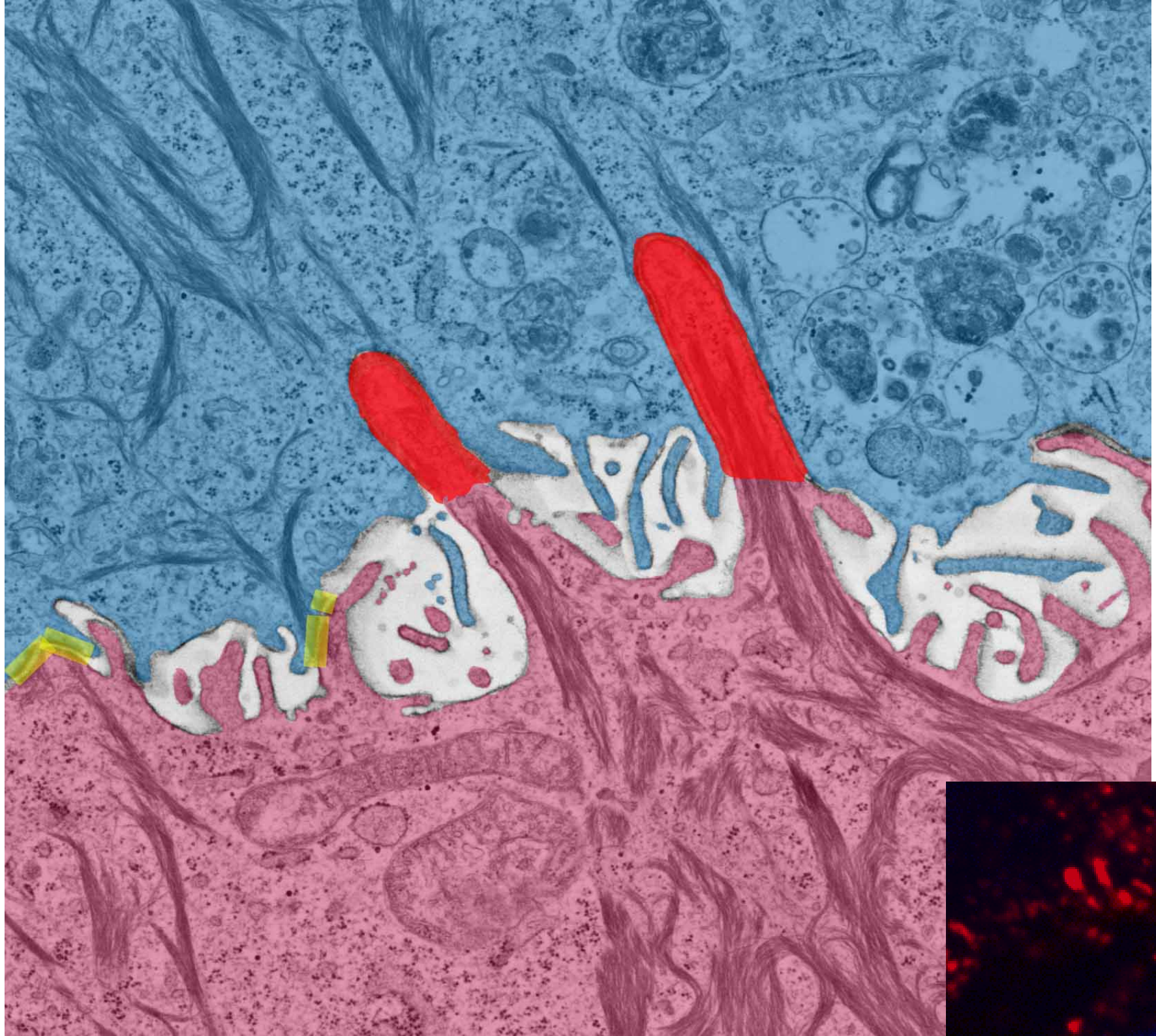
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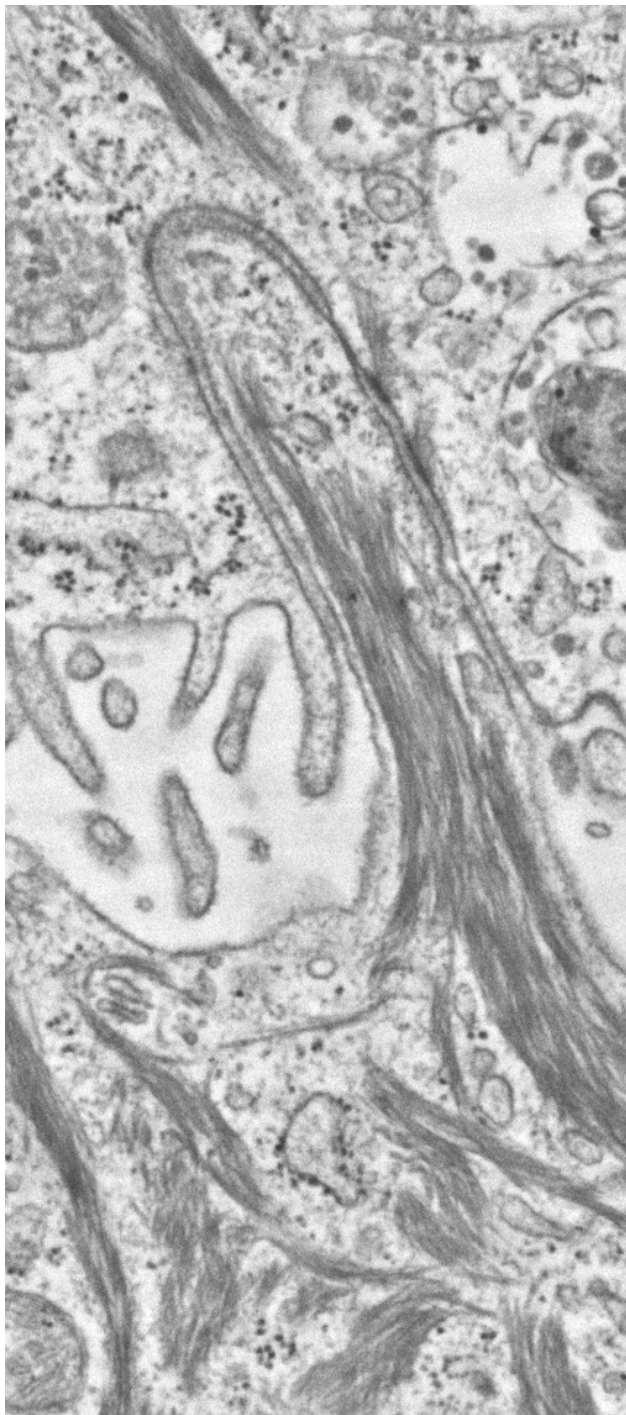




# Ultrastructural 3D-reconstruction using serial sections



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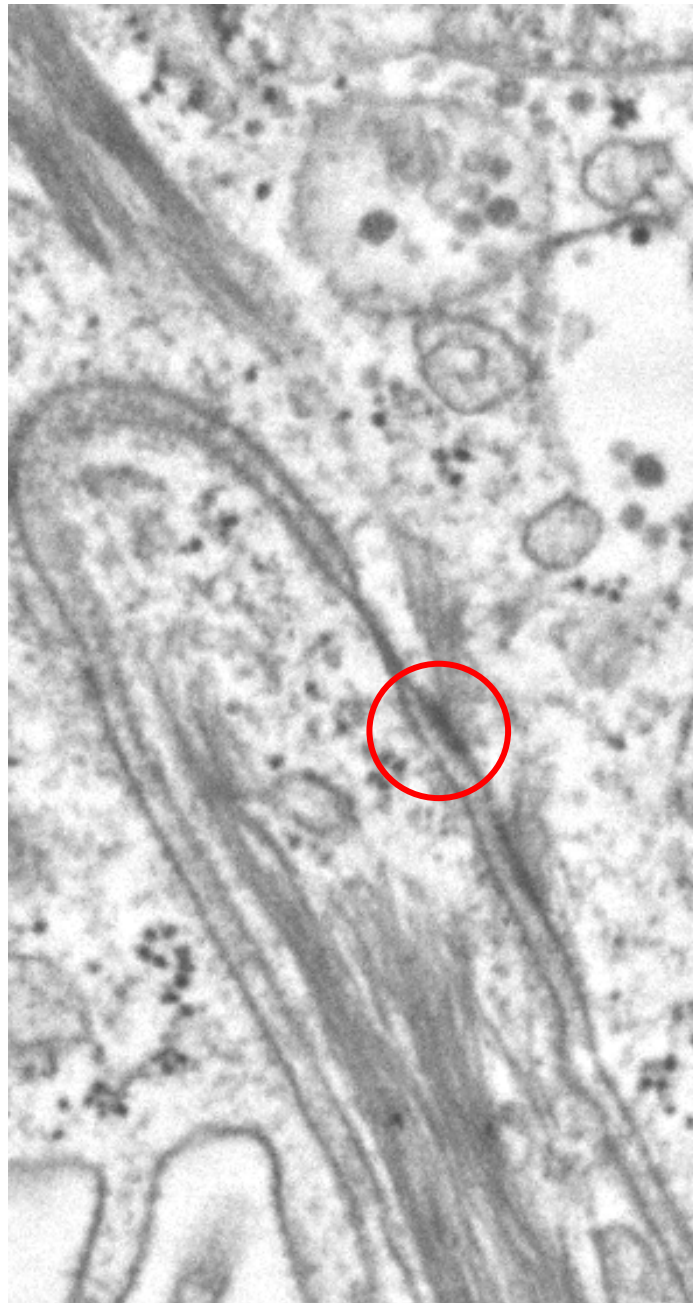
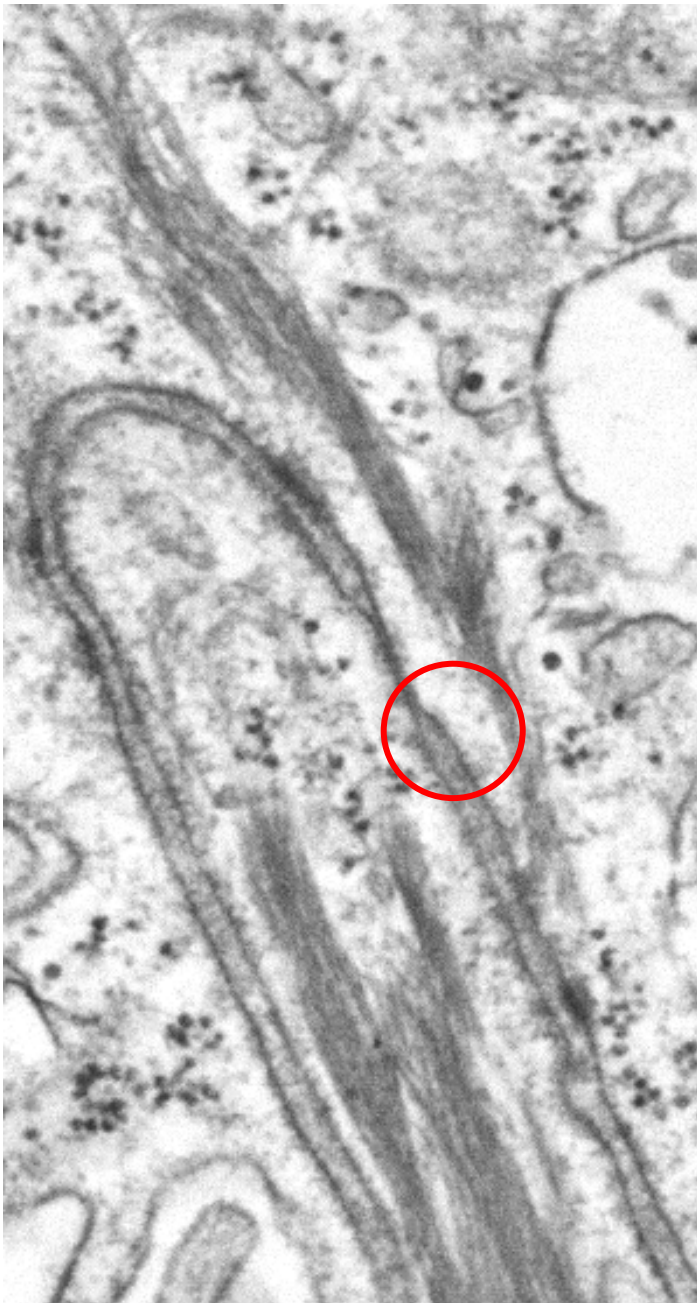


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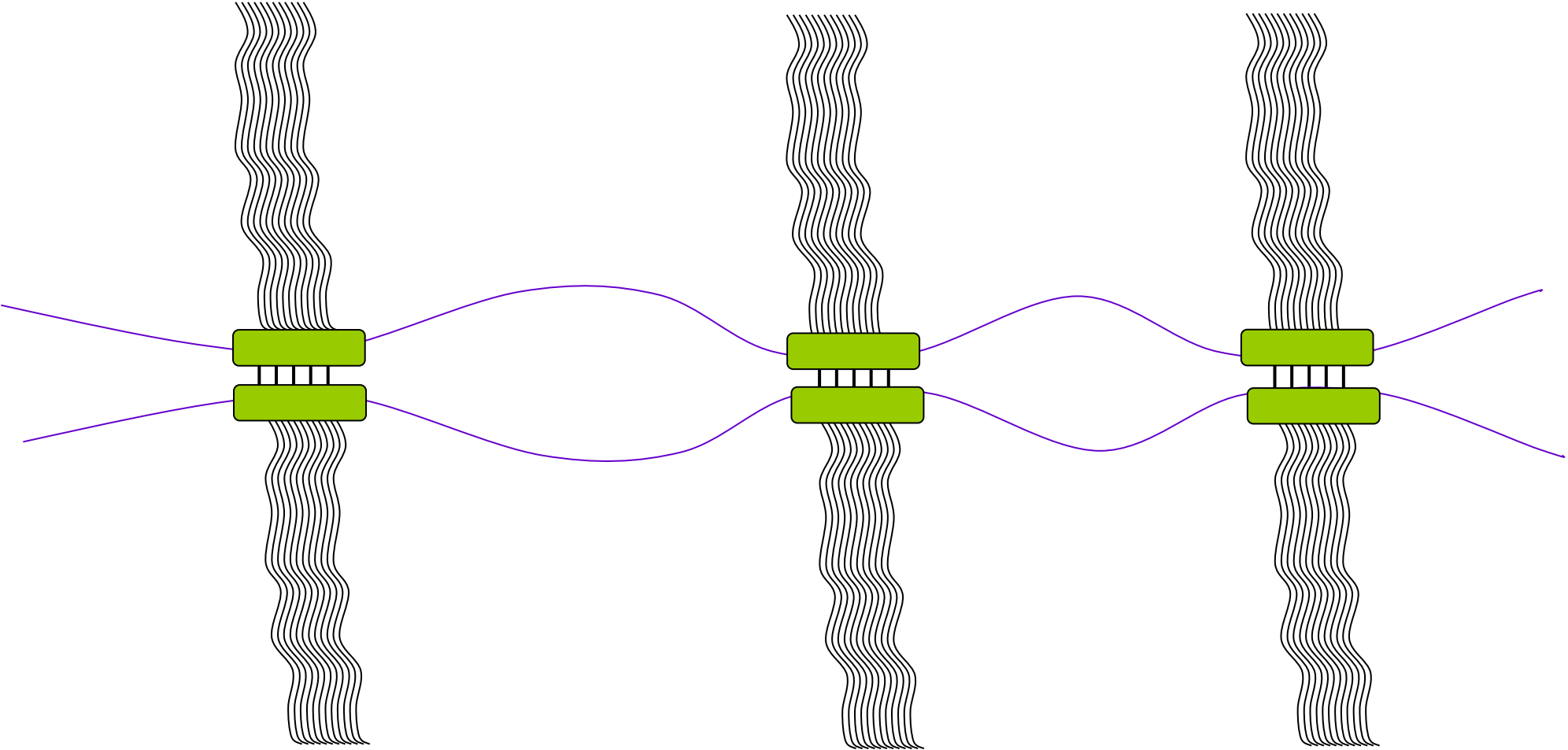


31

# Ultrastructural 3D-reconstruction using serial sections

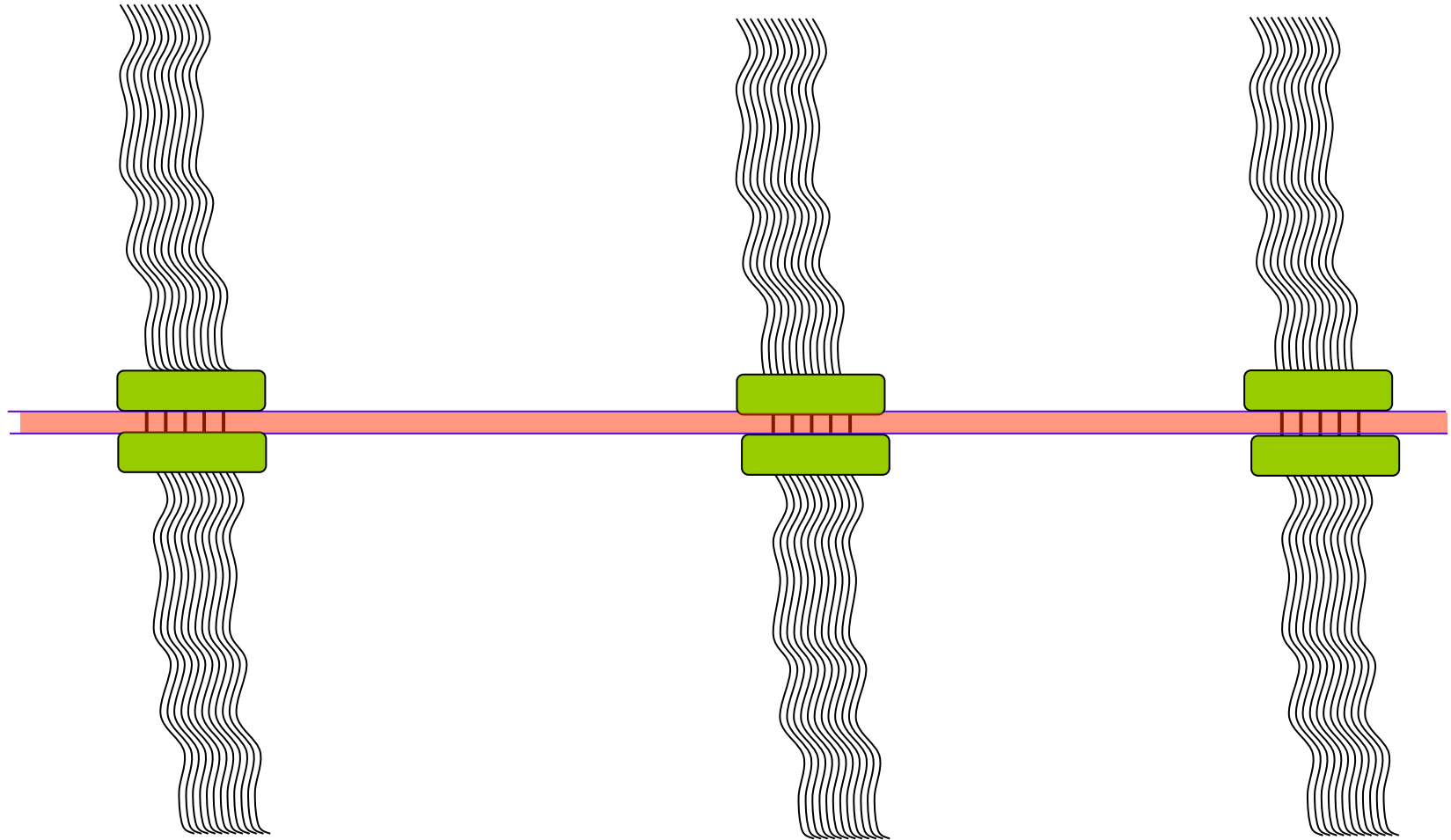


acceptor cell



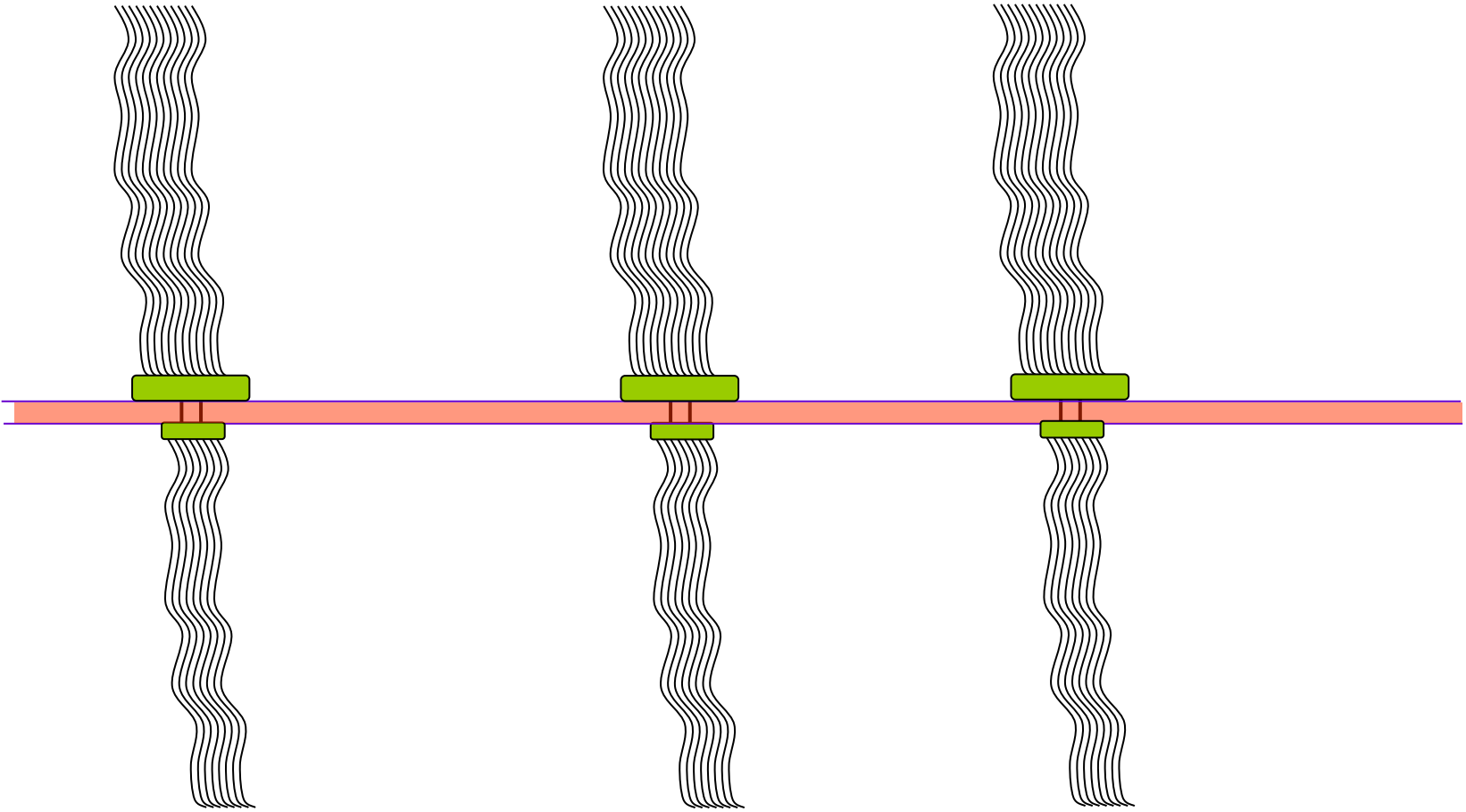
donor cell

acceptor cell



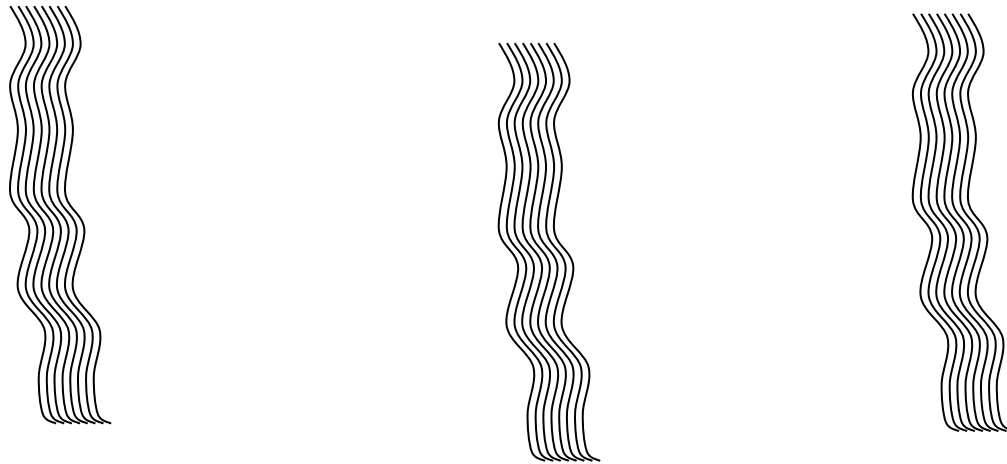
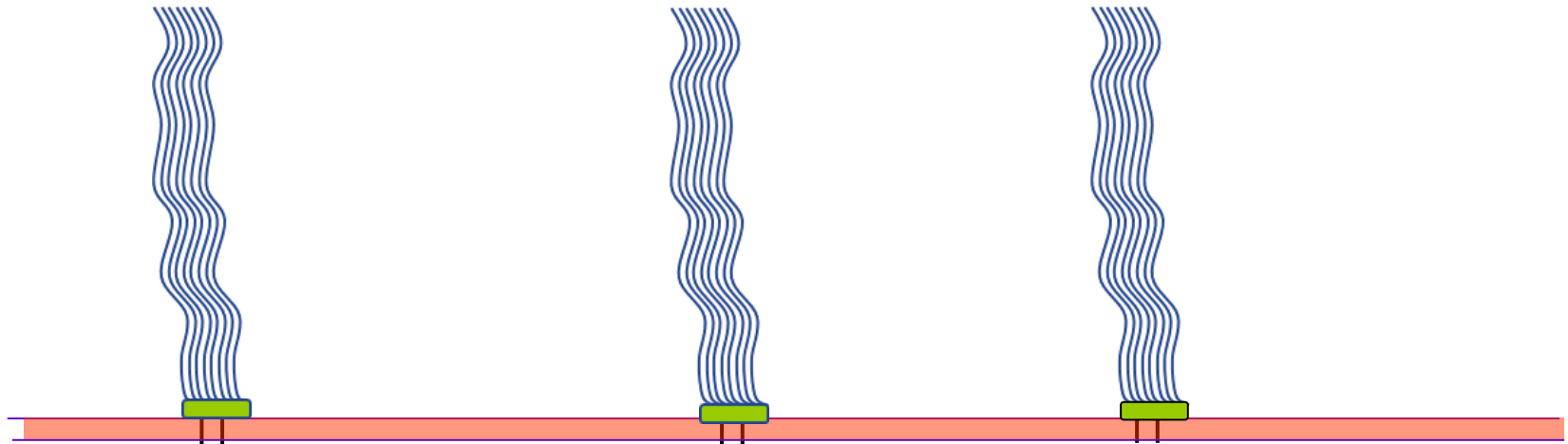
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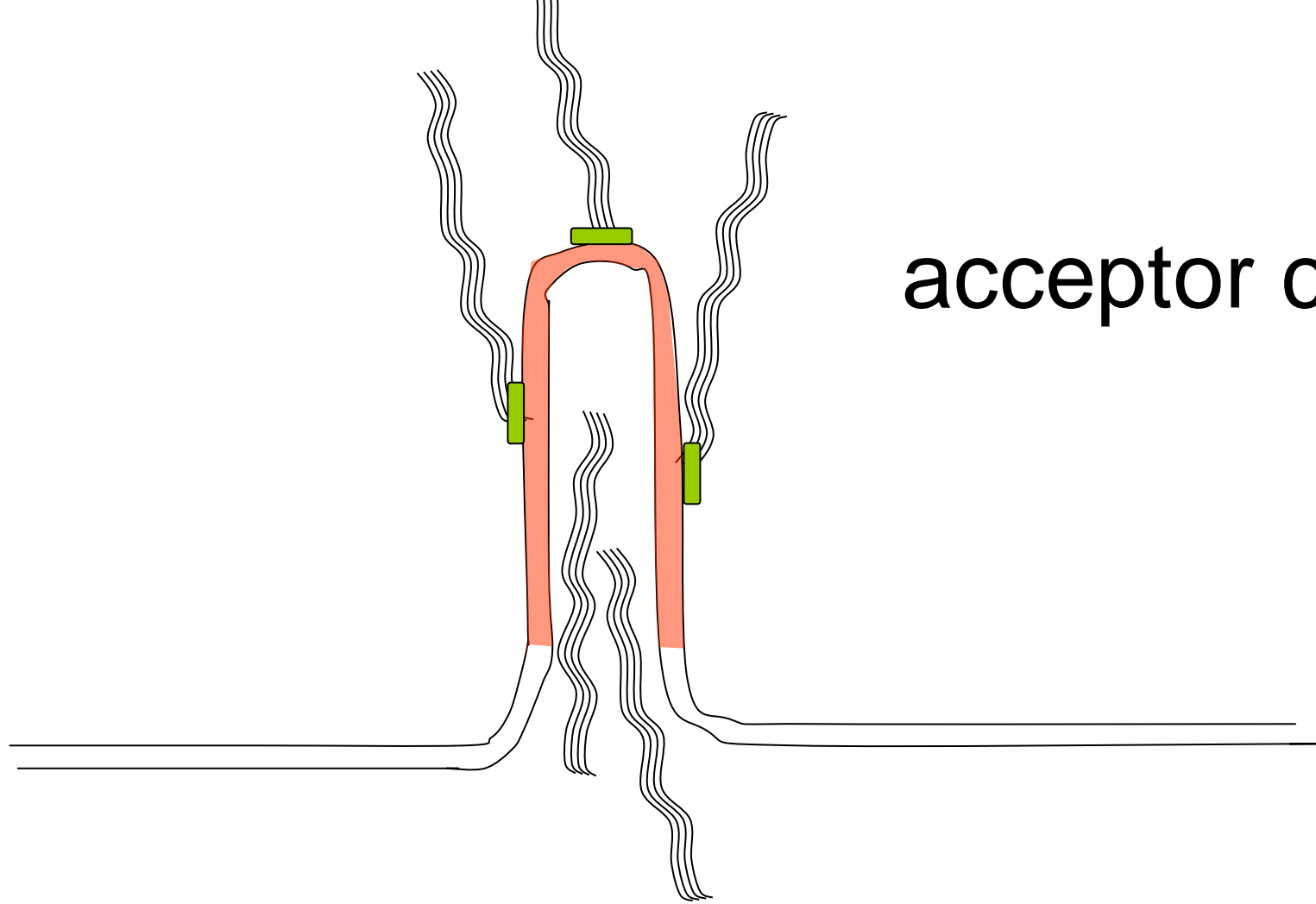
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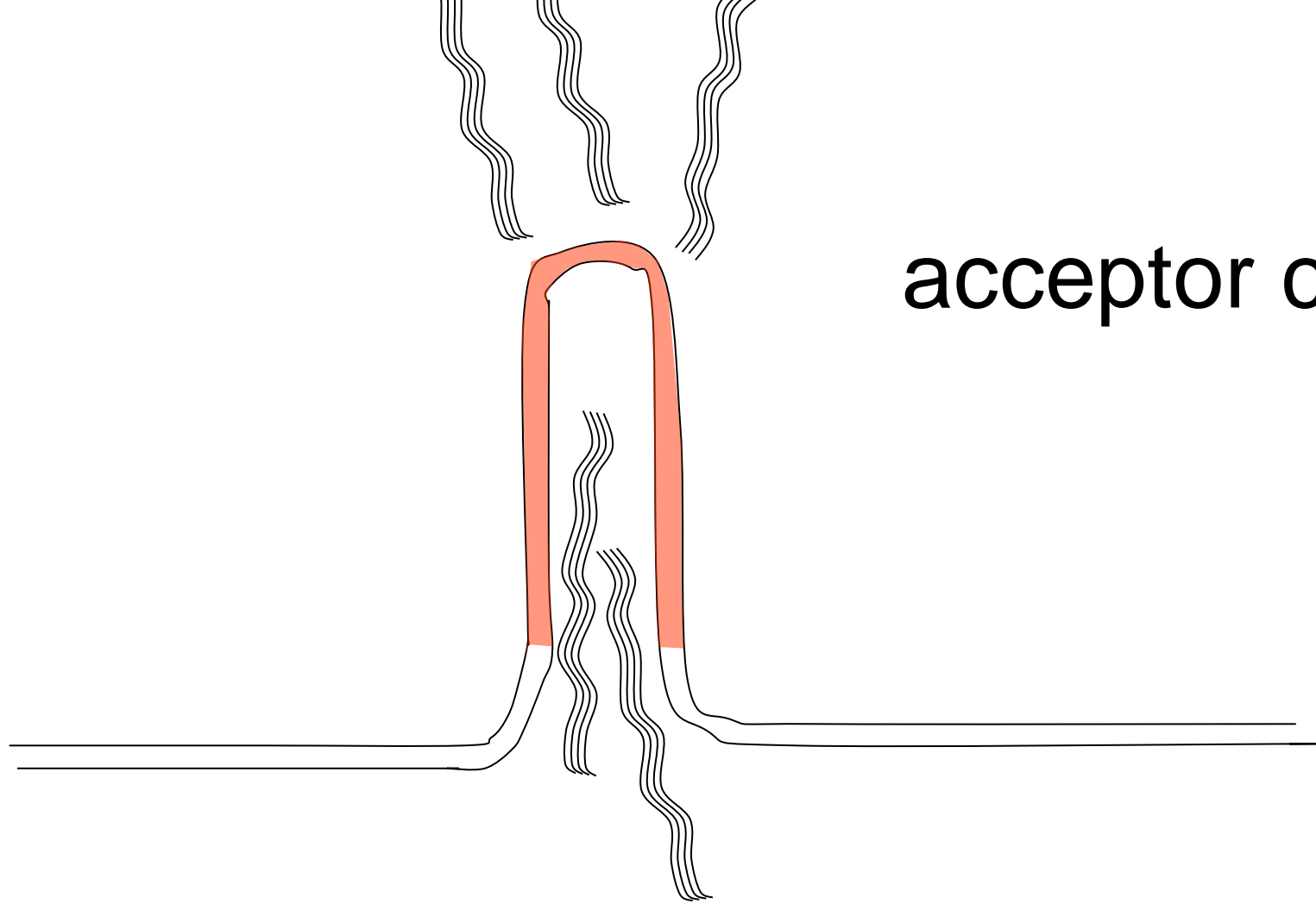
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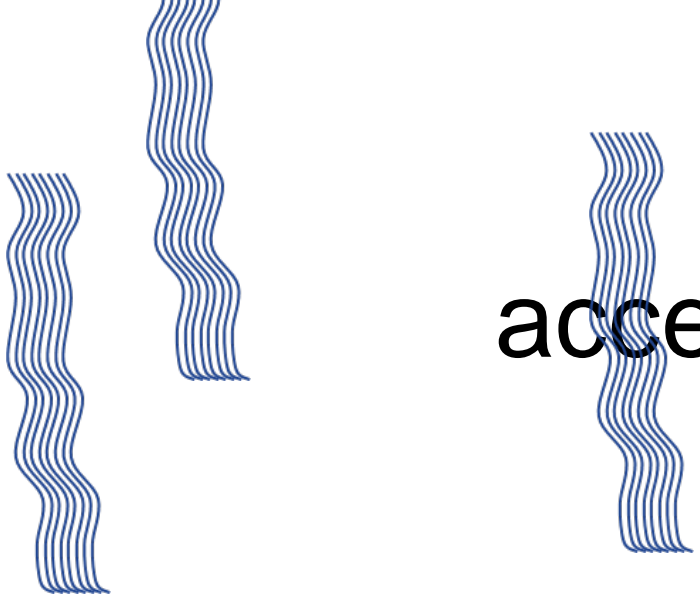
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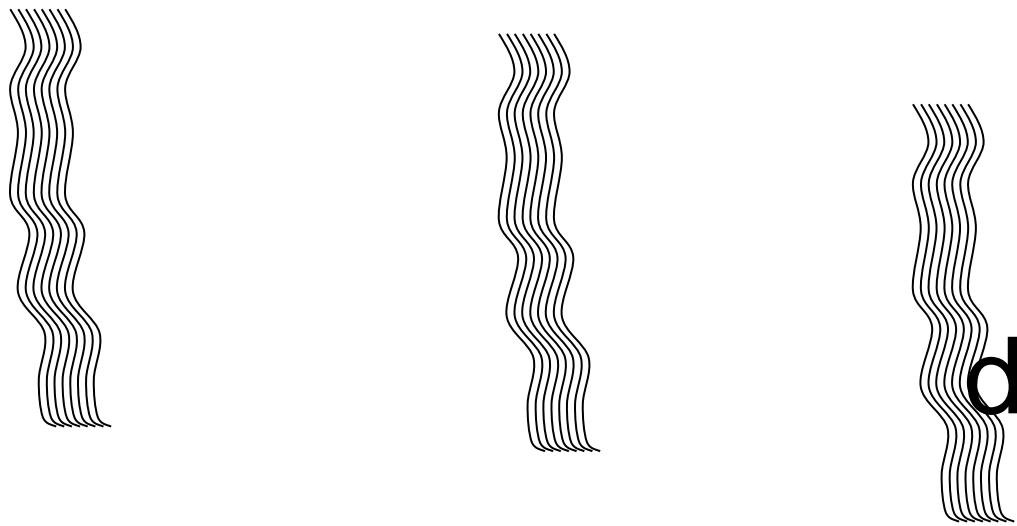


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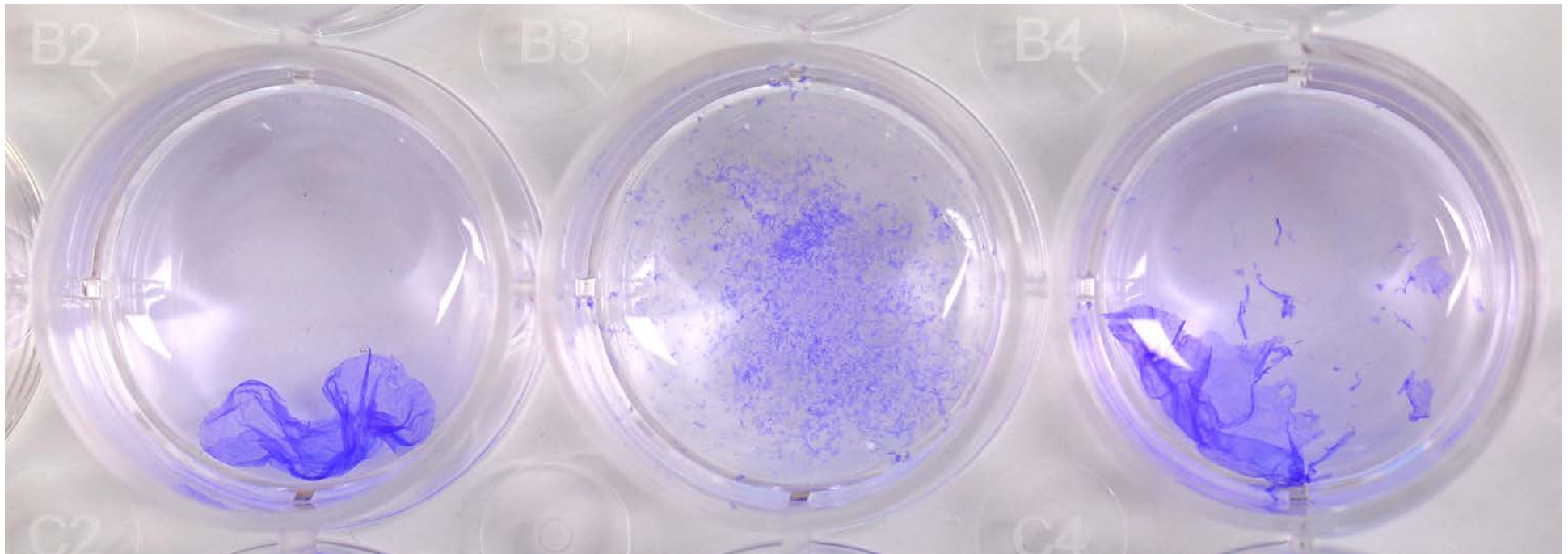
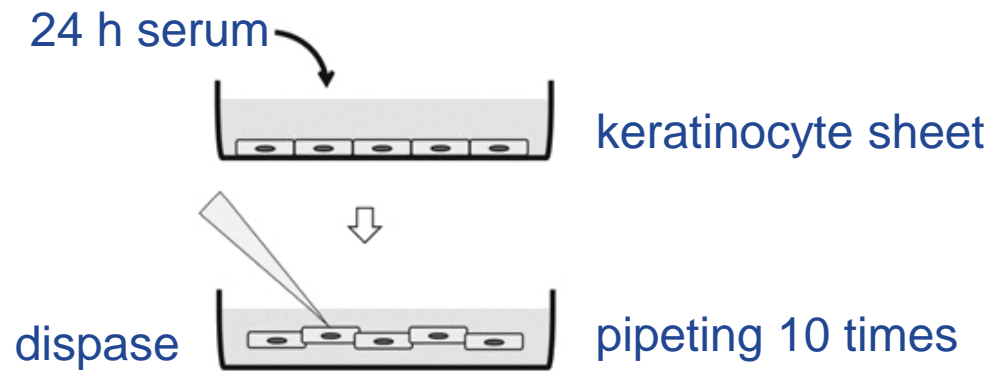


acceptor cell



donor cell

# In vitro keratinocyte dissociation assay: serum pathogenicity



Normal human serum

Active disease  
Dsg3 titer >150

Remission off medication  
Dsg3 titer 83

# In Vitro Keratinocyte Dissociation Assay

## Evaluation of the Pathogenicity of Anti-Desmoglein 3 IgG Autoantibodies in Pemphigus Vulgaris

						ELISA Dsg3	Dissociation assay	ELISA Dsg1	
Pt. 1	40	Male	PV-mc	Severe	3 y	1515.9	106.0	57.9	PSL 60 mg per d+plasmapheresis
				Remission		32.4	6.0	0.3	
Pt. 2	63	Male	PV-mc	Severe	5 mo	369.1	72.9	7.6	Methylprednisolone pulse+
				Remission		29.7	18.2	0	PSL 60 mg per d
Pt. 3	33	Male	PV-m	Severe	4 mo	514.9	180.0	1.2	PSL 60 mg+plasmapheresis
				Remission		73.2	30.0	0.1	
Pt. 4	47	Female	PV-m	Severe	10 mo	2553.7	111.1	4.8	PSL 40 mg per d
				Remission		5.7	28.7	0.3	
Pt. 5	35	Female	PV-m	Severe	2 y	443.3	143.4	1.9	Topical steroid application
				Remission		415.8	31.0	2.5	
Pt. 6	52	Male	PV-m	Severe	1 y	77.8	129.1	6.8	PSL 60 mg per d
				Remission		111	14.3	0.1	