Ultrastructural support in paediatric pathology

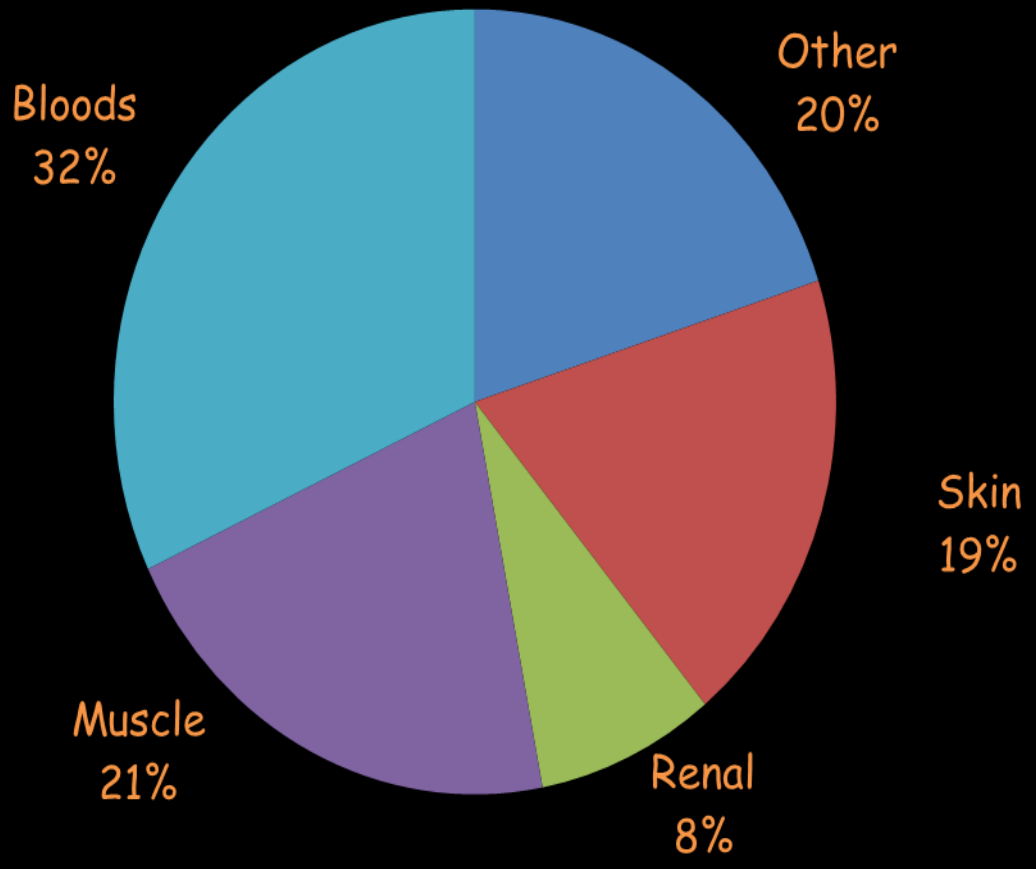
Glenn Anderson
Clinical Electron Microscopist
Great Ormond Street Hospital for Children
London, UK

Paediatric EM training day, Southampton University, 4th October 2013

Sample types received at GOSH

- Blood
- Muscle
- Skin
- Renal
- Tumour
- Other – lung, heart, GI, liver, cornea, CVS

Sample types 2012/13 - GOSH

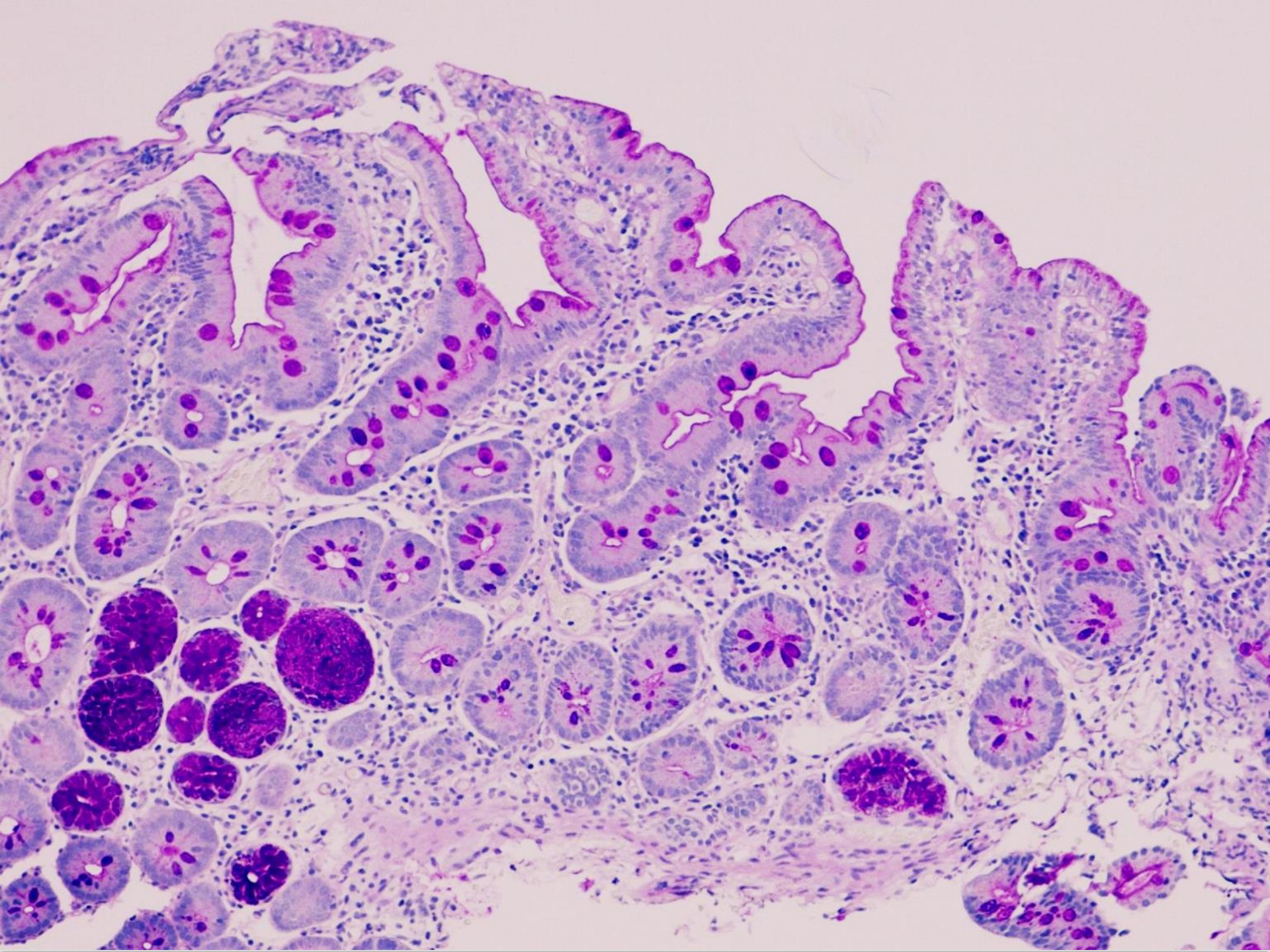


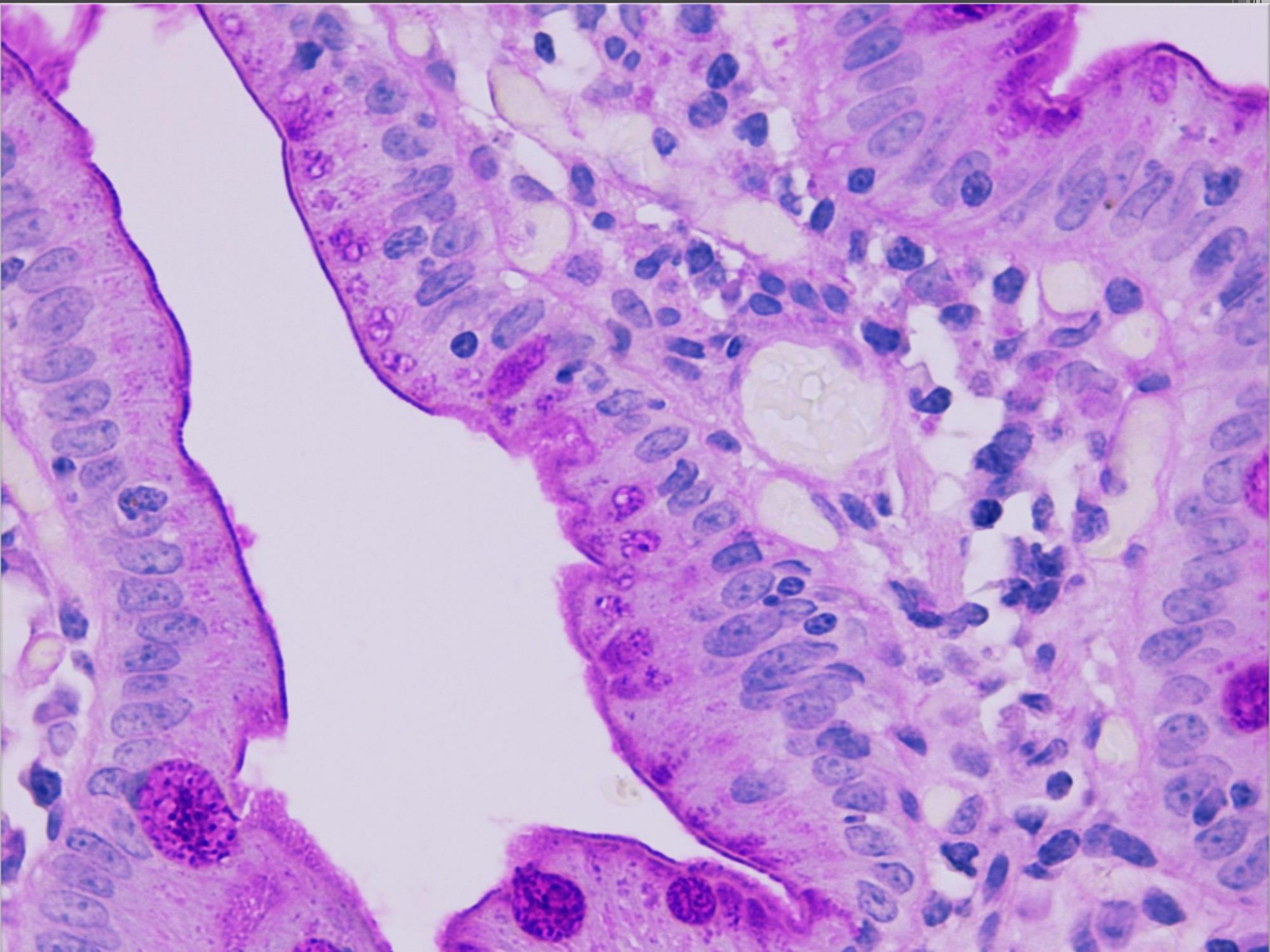
Gastrointestinal conditions

- Infections – enteropathic E coli, giardia, viruses
- Microvillous inclusion disease
- Tufting enteropathy
- Chronic Intestinal Pseudo-Obstruction – ‘smooth muscle myopathies, neuropathies’
- Other miscellaneous

Microvillous Inclusion Disease

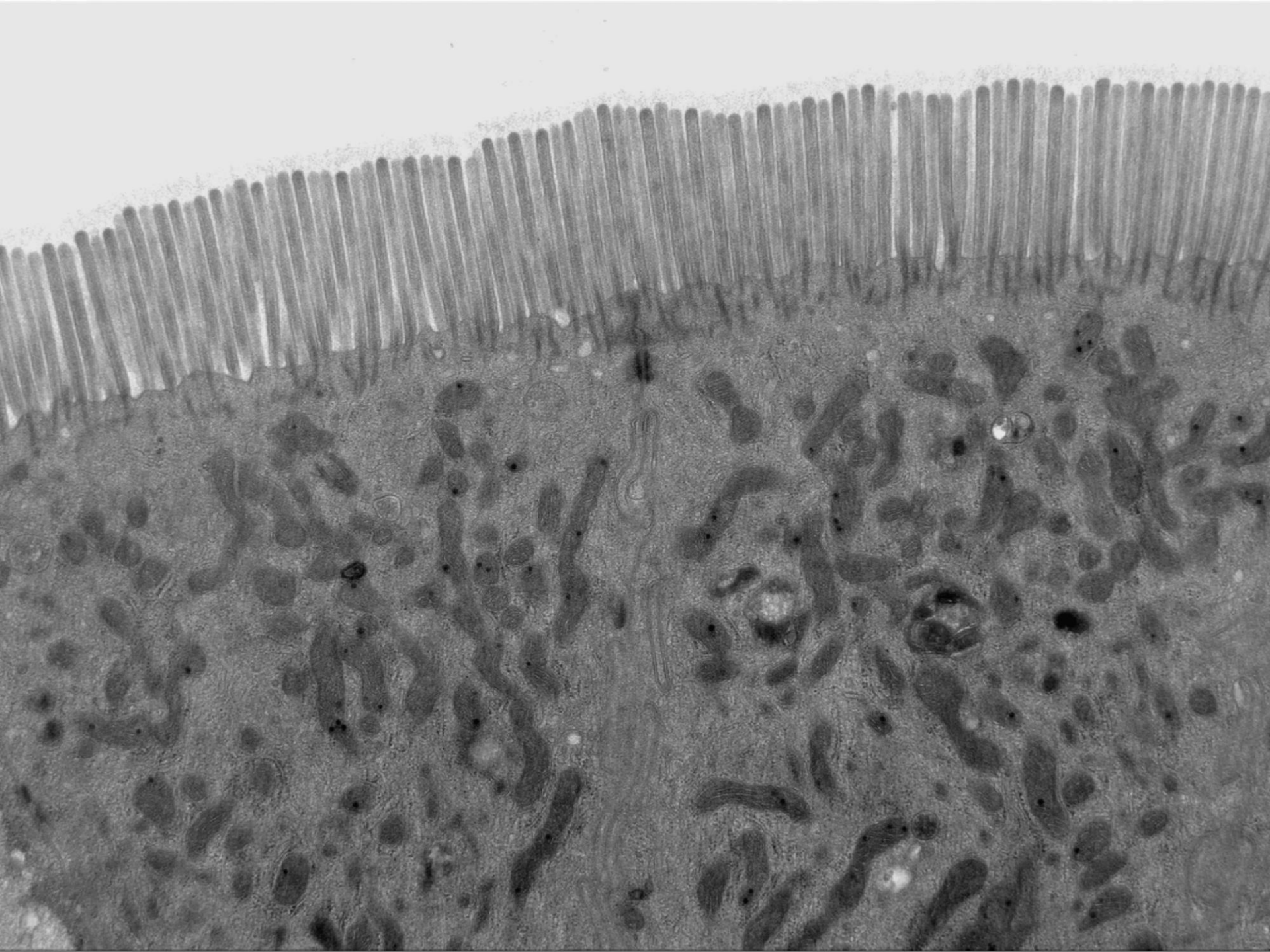
- Neonatal enteropathy
- Intestinal failure due to defective microvillous border
- Small and large bowel
- Thought incompatible with life – MVA
- Less severe forms – MVD
- Autosomal recessive – MYO5B mutation

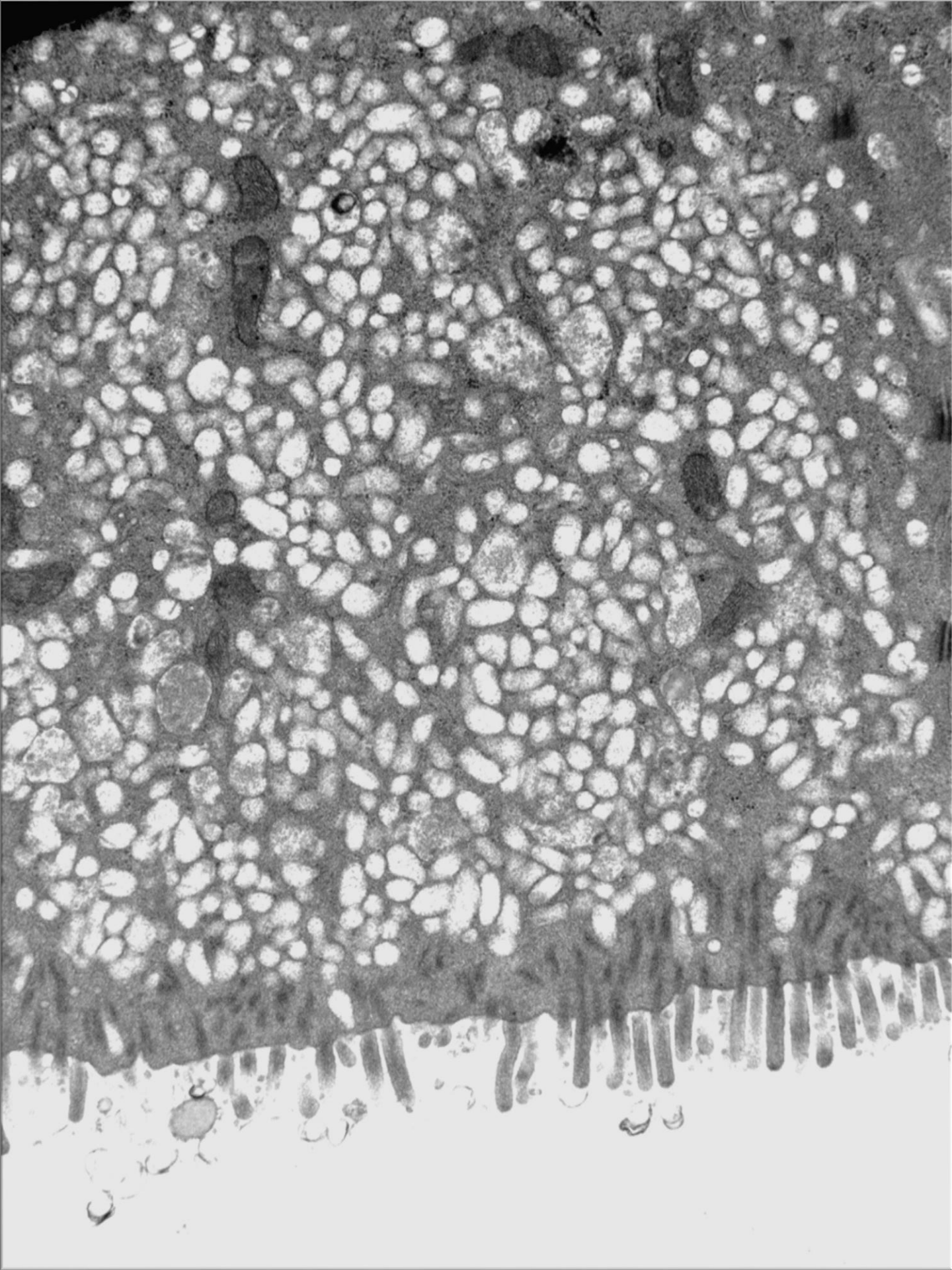


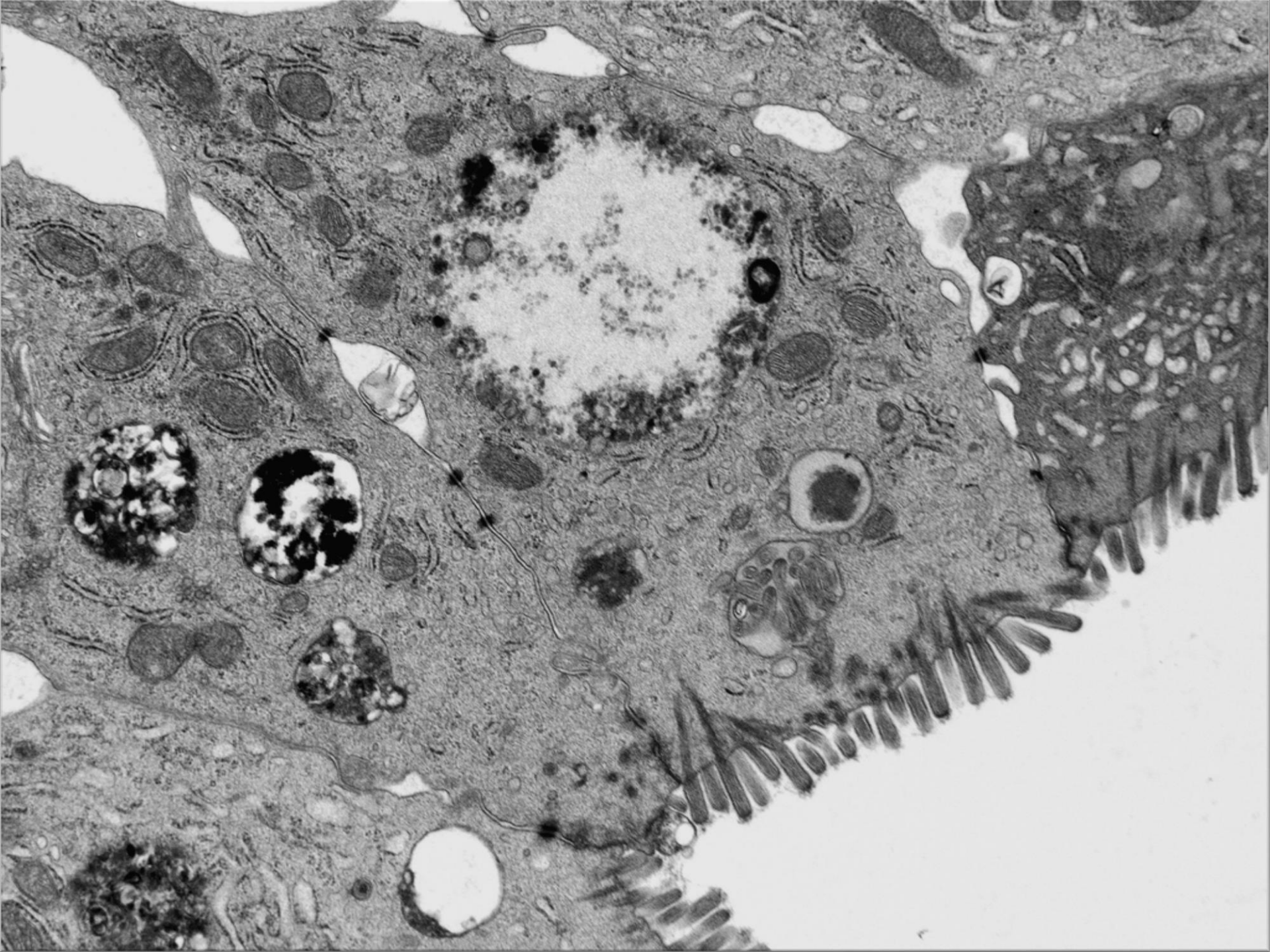


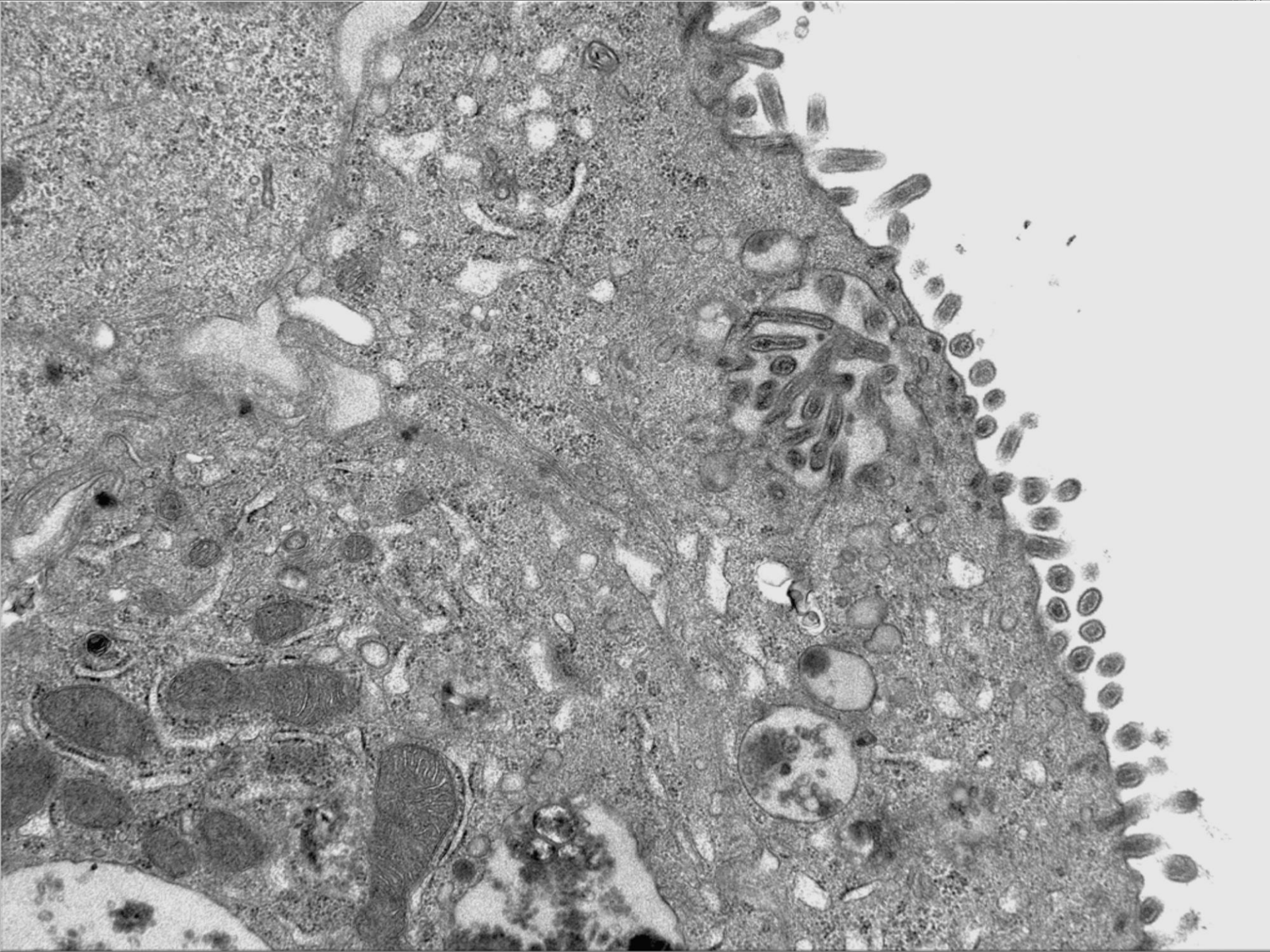
MVID ultrastructure

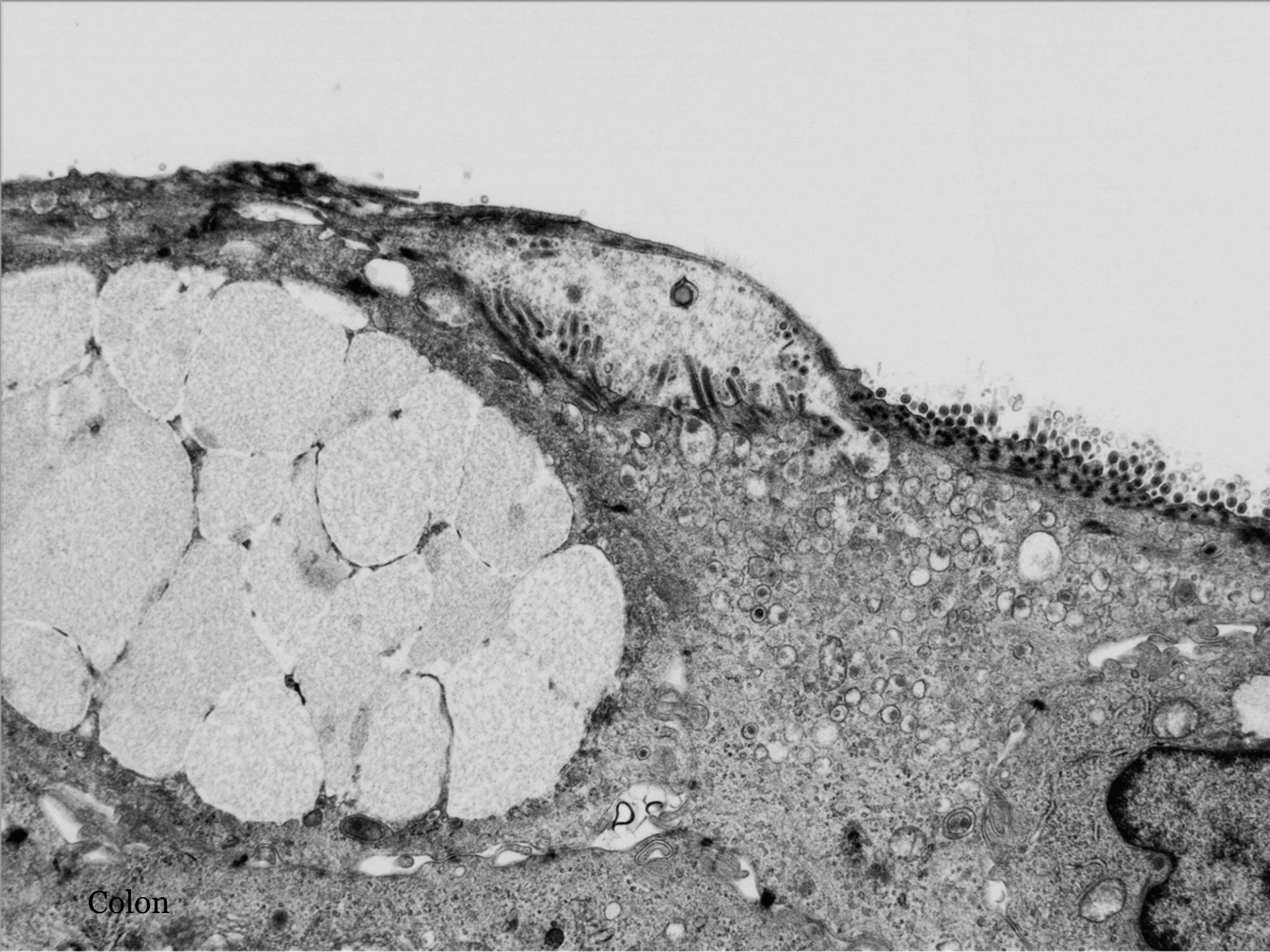
- Patchy loss of microvilli
- Numerous secretory vesicles
- Large or frequent cytolysosomes
- Apical internal microvilli
- Crypt region shows increased electron dense vesicles





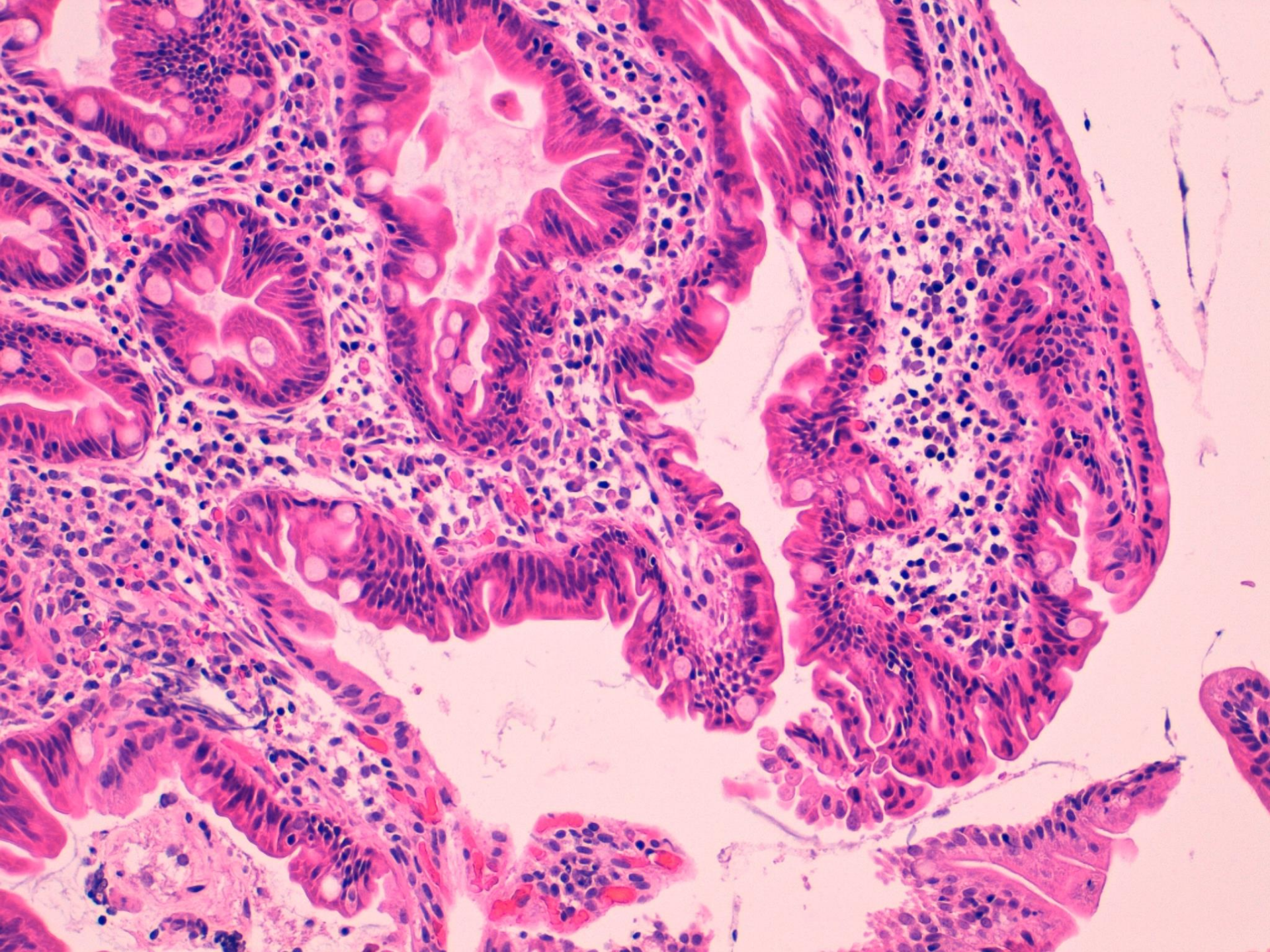


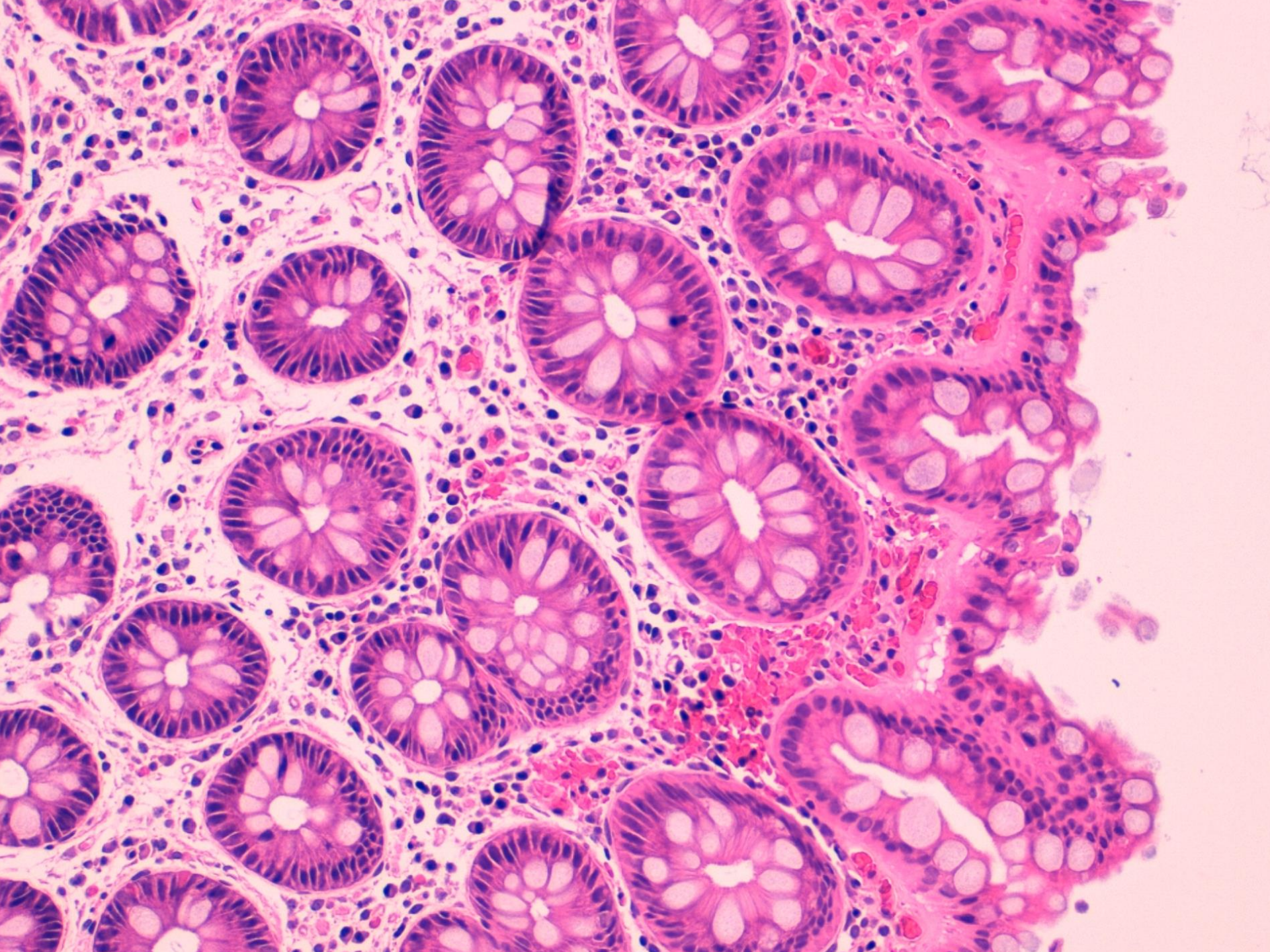


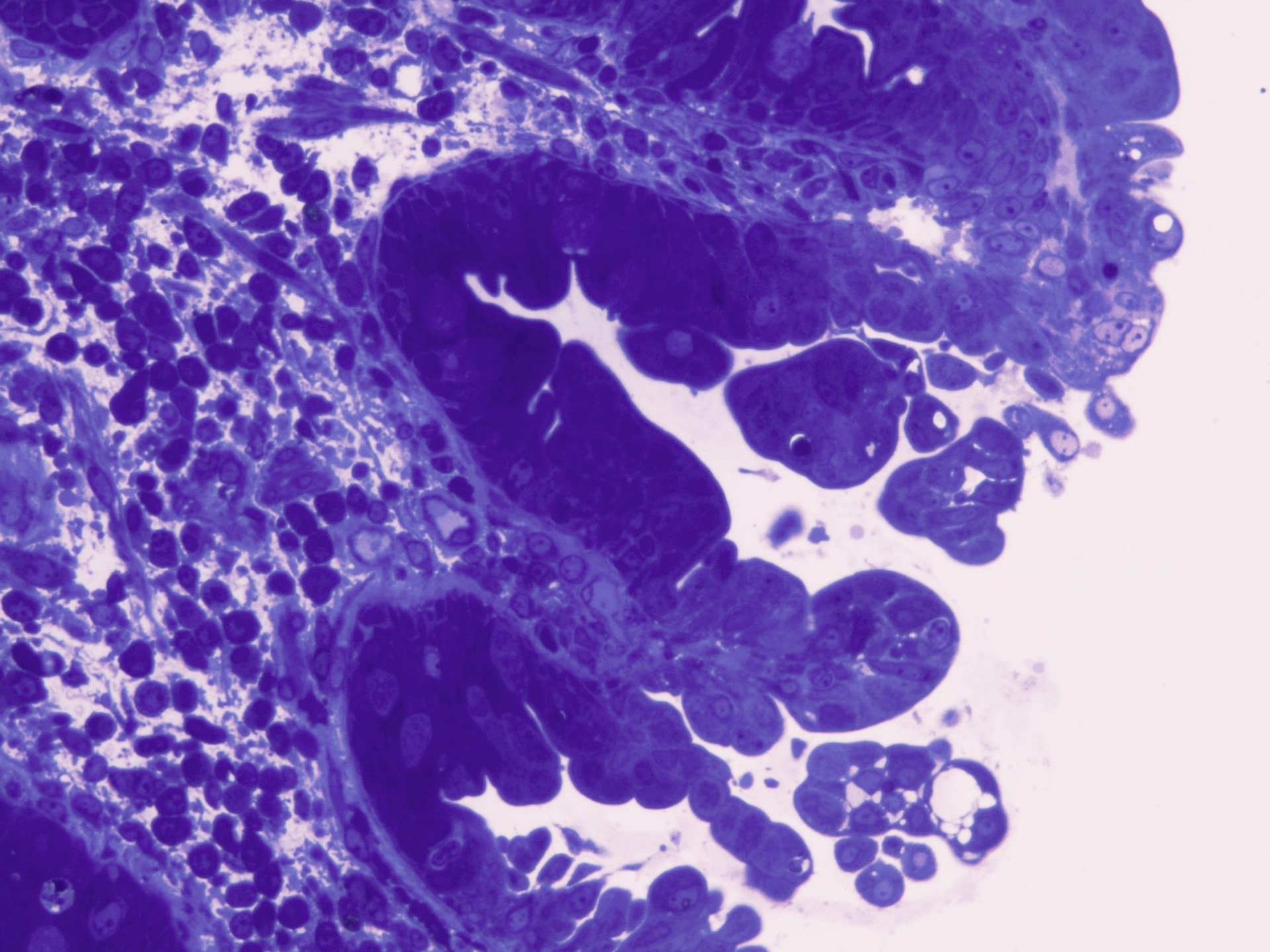

Colon

Tufting enteropathy - Intestinal epithelial dysplasia

- Early-onset severe intractable diarrhoea
- Villous atrophy and inflammation – variable
- Surface epithelial irregularities
- 'Tufts' of rounded enterocytes extruding into lumen
- Mutation in gene encoding EpCAM (epithelial cell adhesion molecule)

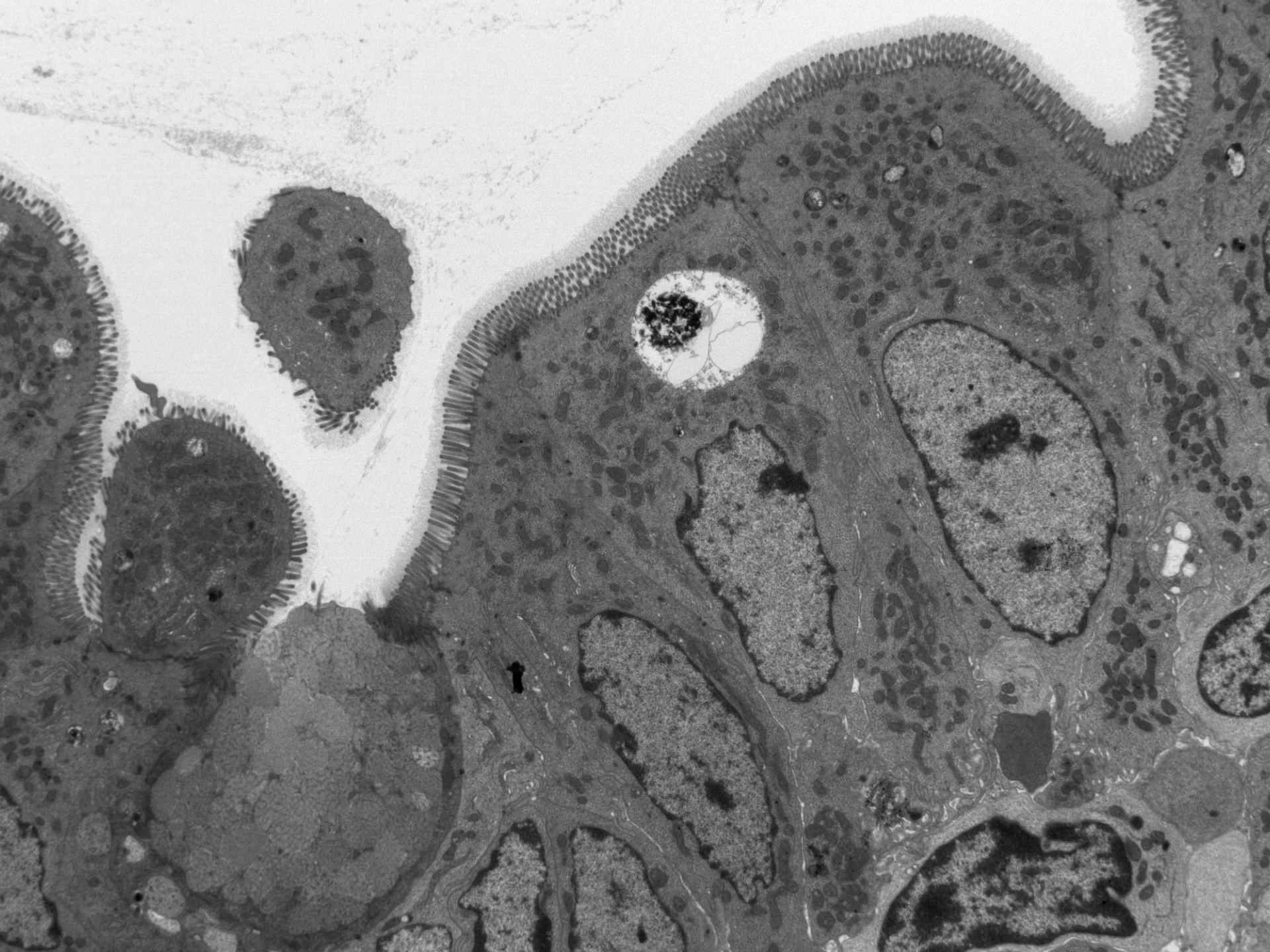


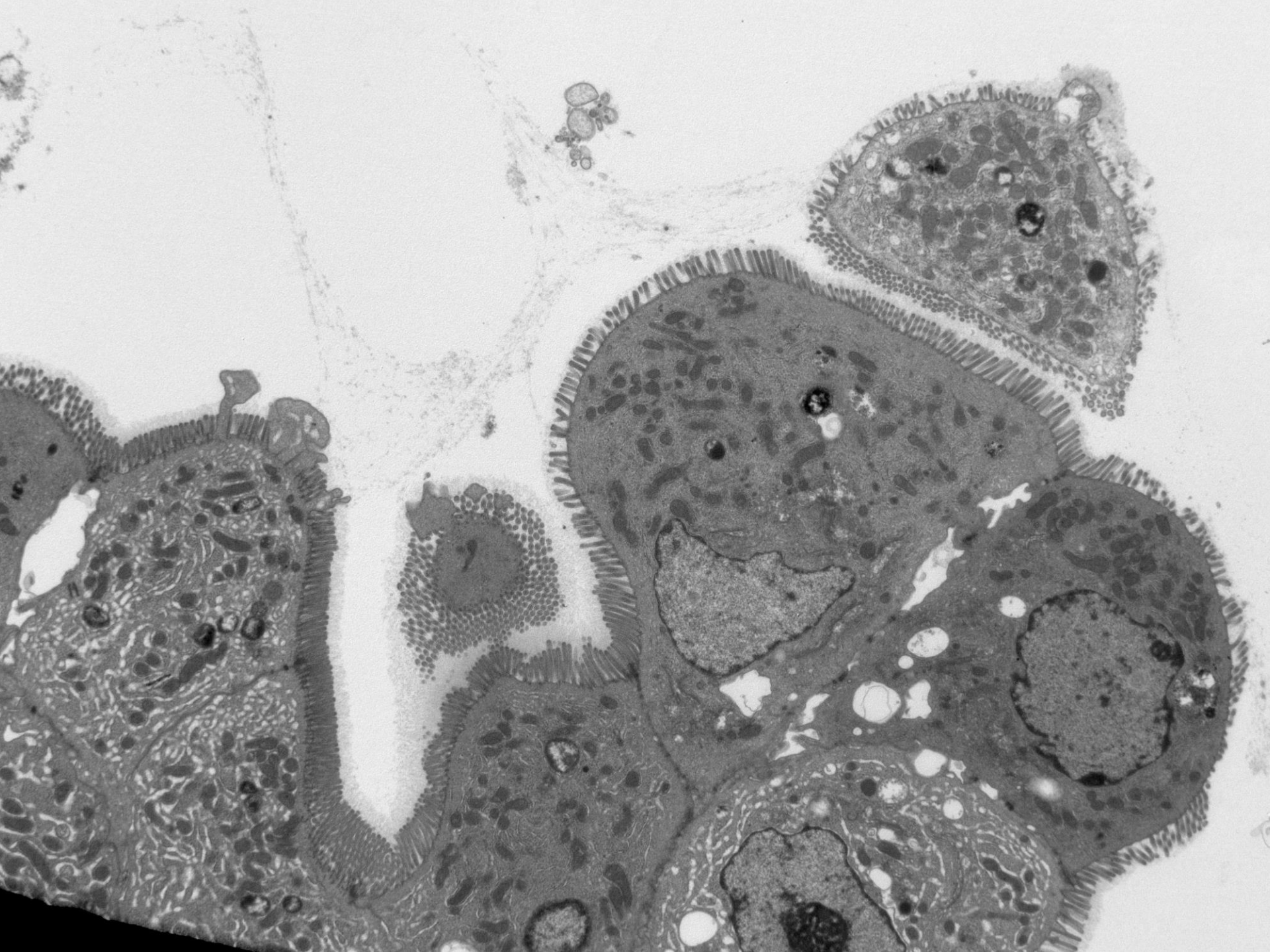


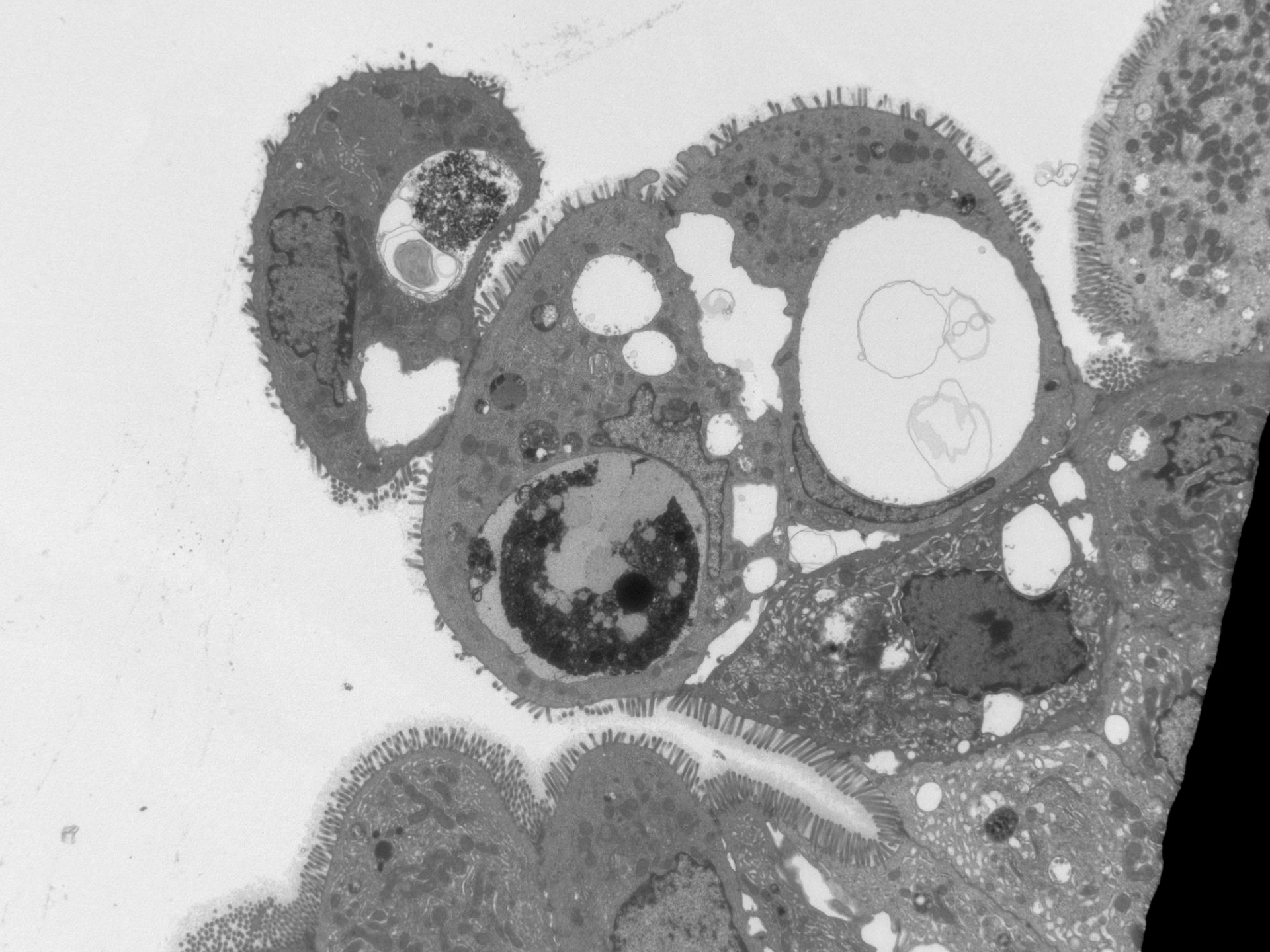


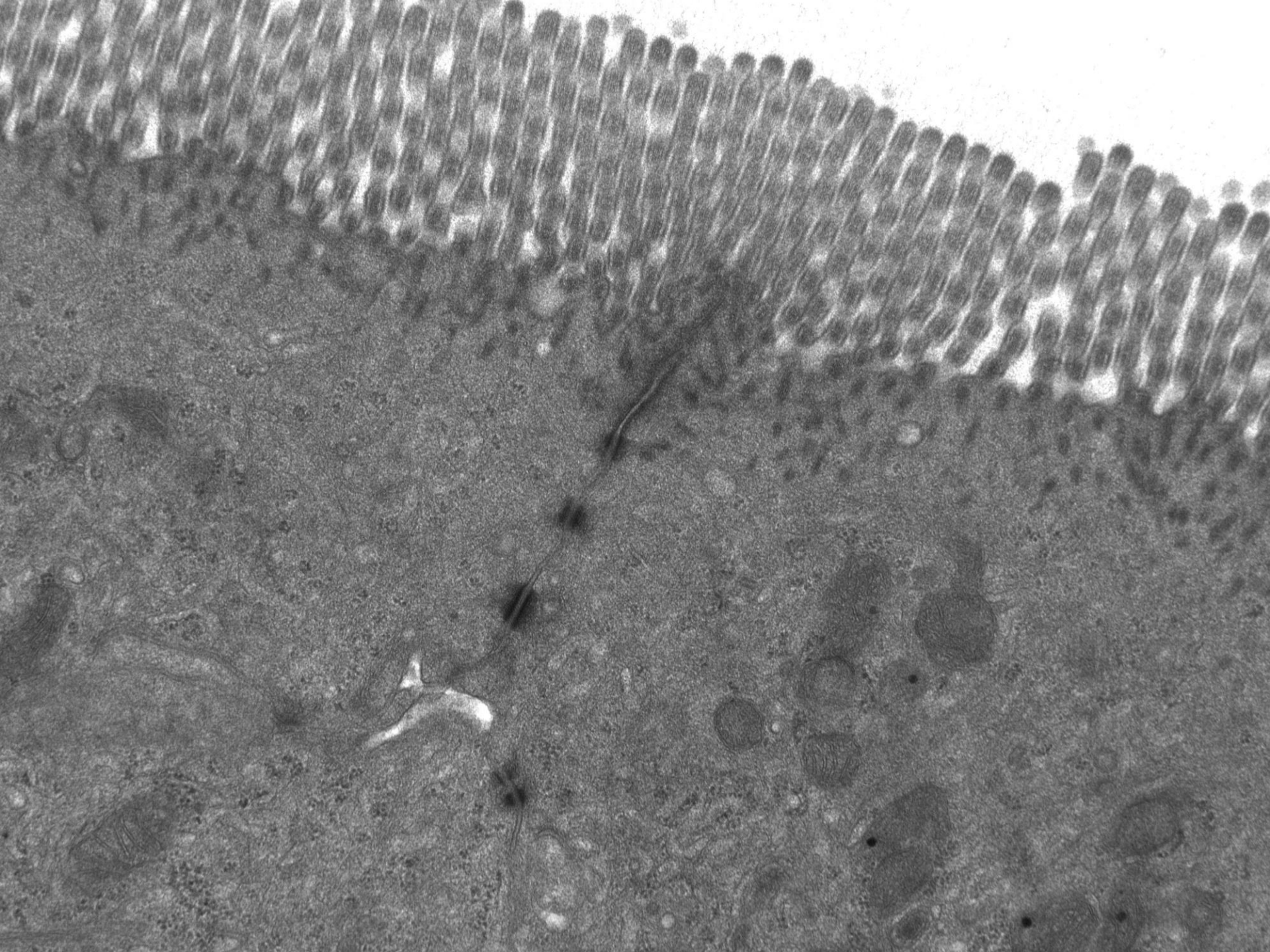
Tufting ultrastructure

- Disrupted microvilli
- Disorganisation of enterocyte architecture
- Focal crowding – tufts in lumen
- Desmosomes increased in length and numbers
- Changes also described in colon









Lung disease

- Congenital surfactant deficiency
- Pulmonary interstitial glycogenosis
- Neuroendocrine cell hyperplasia of infancy
- Respiratory Niemann-Pick disease

Congenital surfactant deficiency

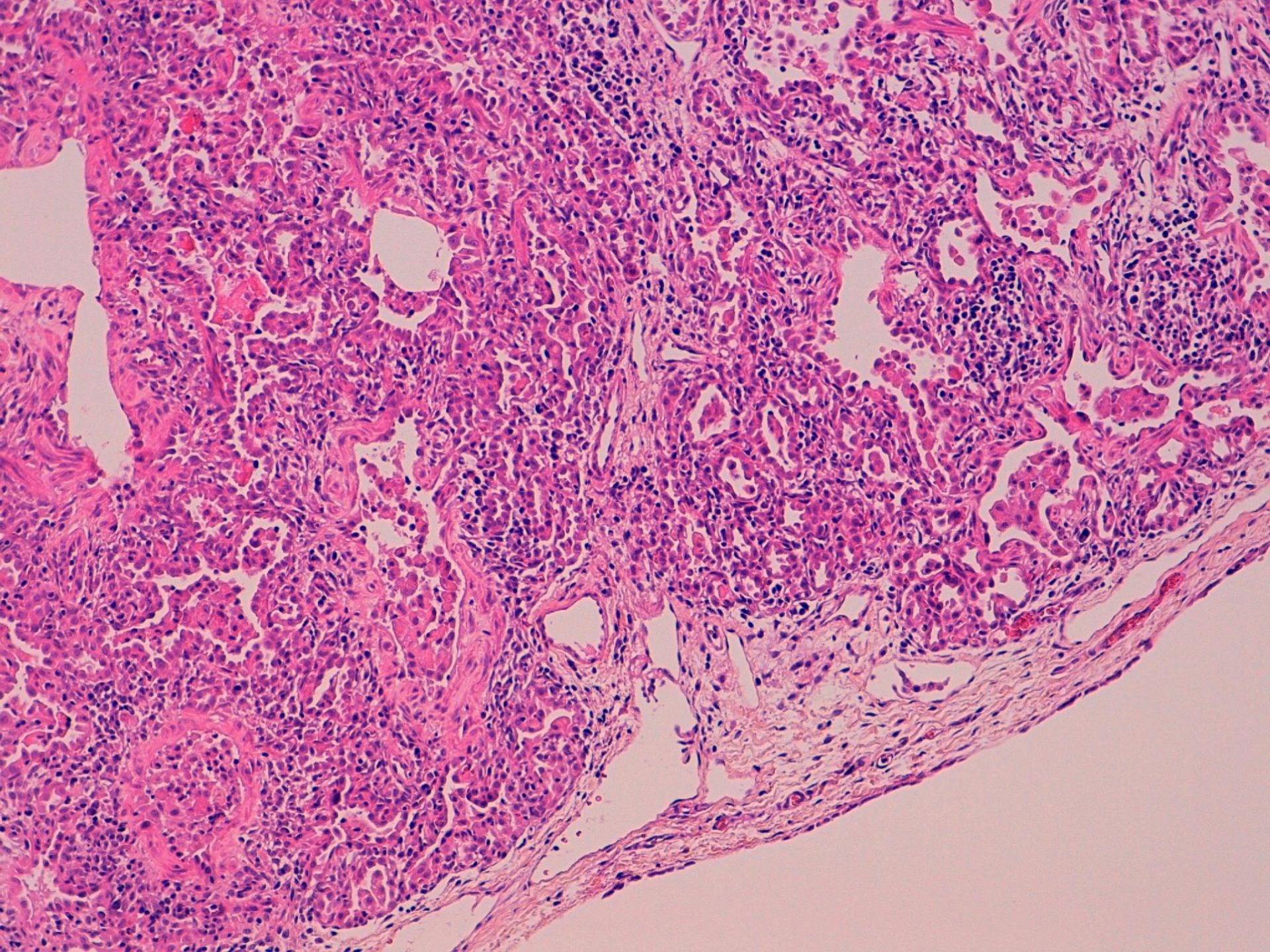
- Can present as:
  - acute respiratory failure in neonates
  - interstitial lung disease in older children

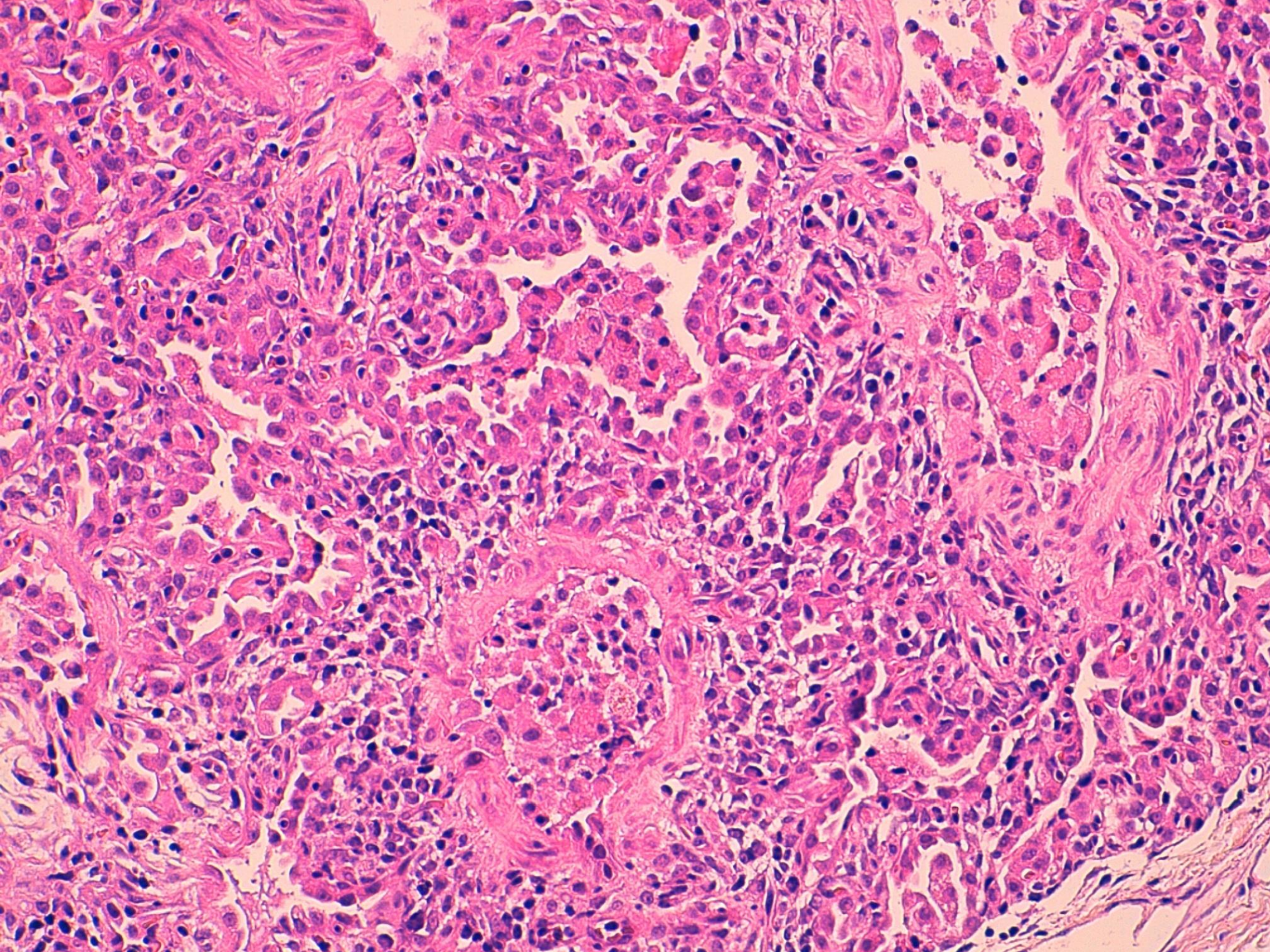
- Pathologic features include:
  - alveolar proteinosis
  - type 2 pneumocyte hyperplasia
  - foamy macrophages
  - diffuse interstitial widening

Surfactant genetics

3 genes identified

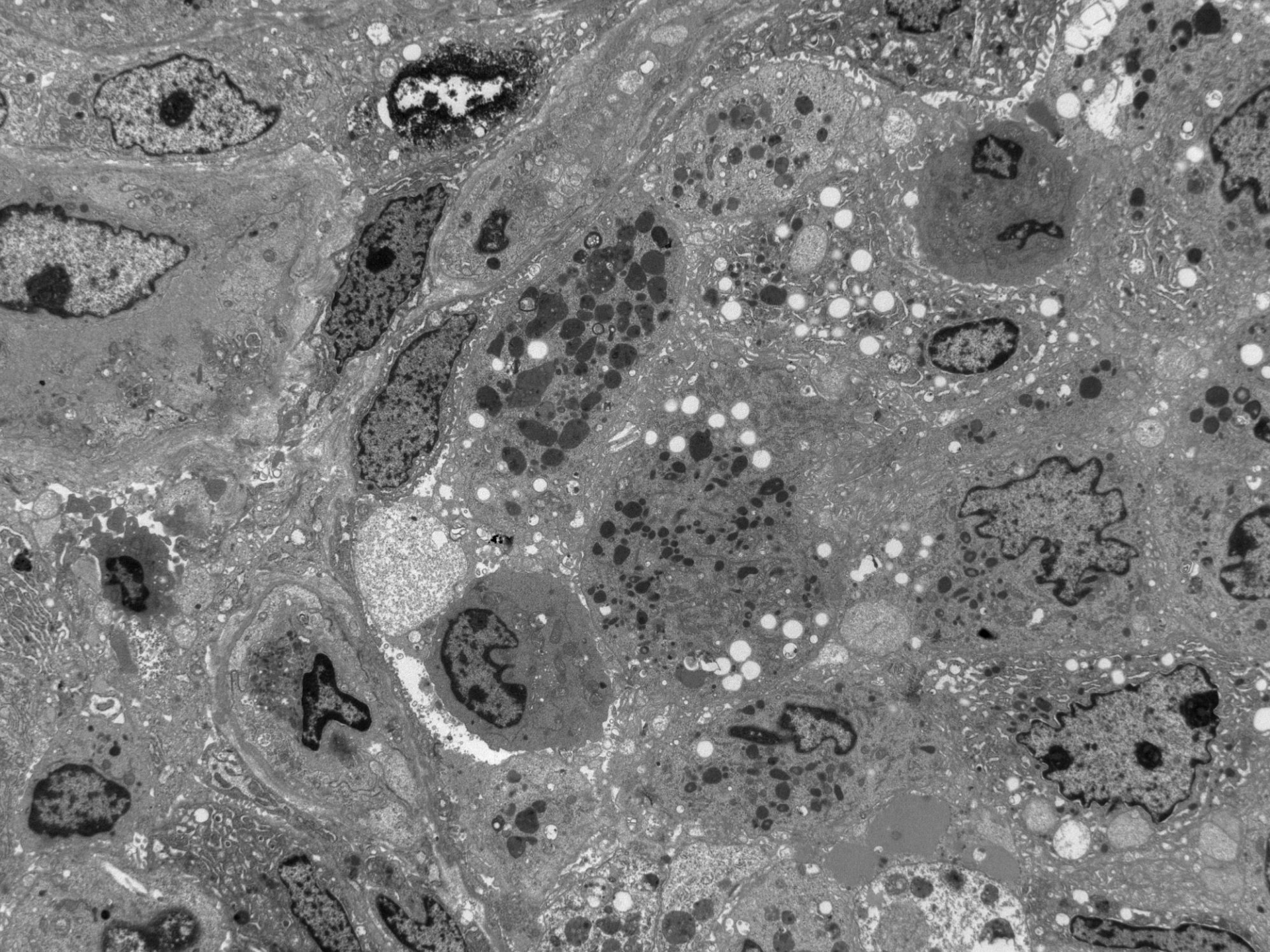
- Surfactant protein B – *SFTPB*
  - severe neonatal onset, AR inheritance
- Surfactant protein C – *SFTPC*
  - AD inheritance, onset often later
- *ABCA3 mutation*
  - most common type of defect

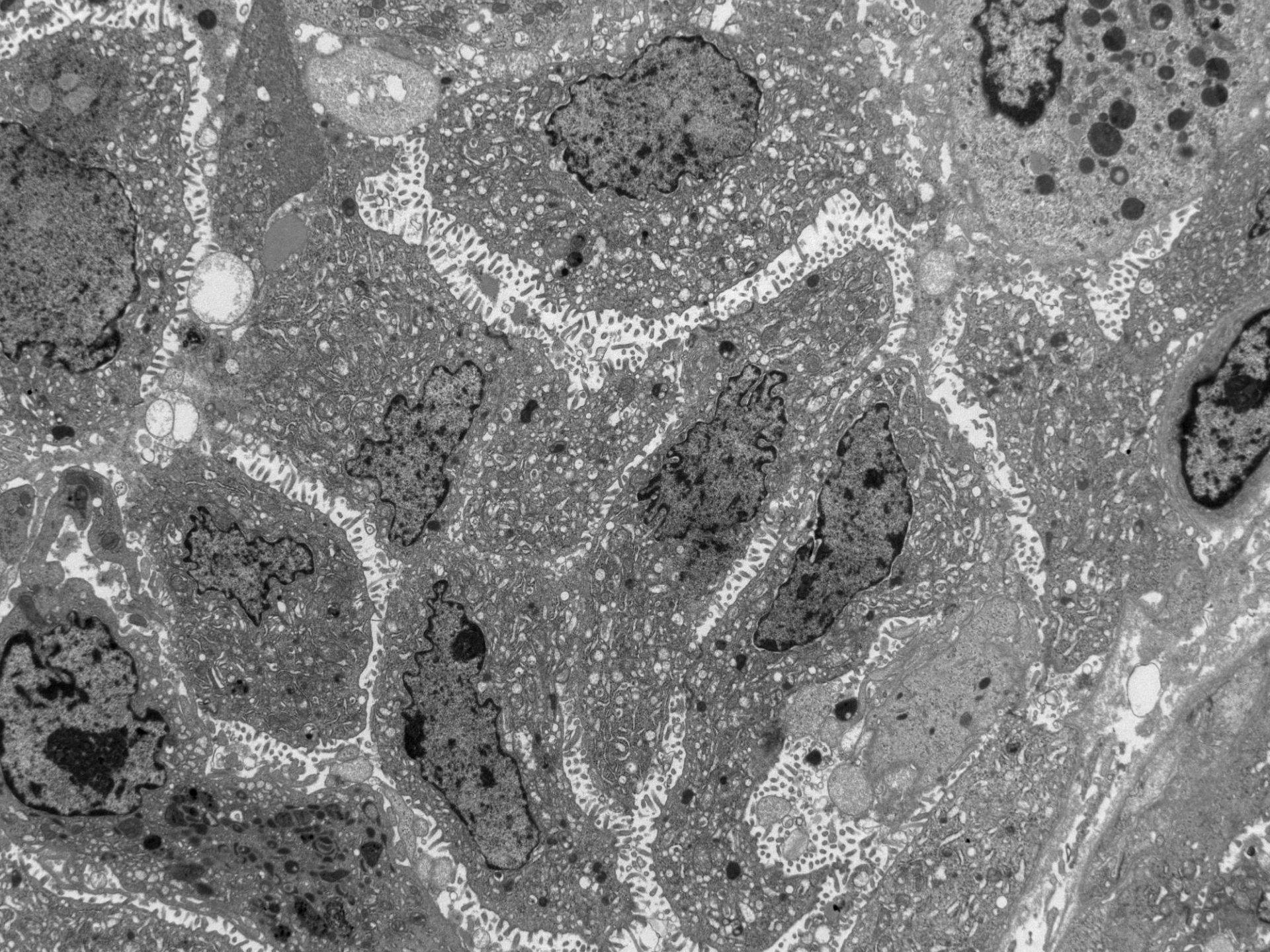


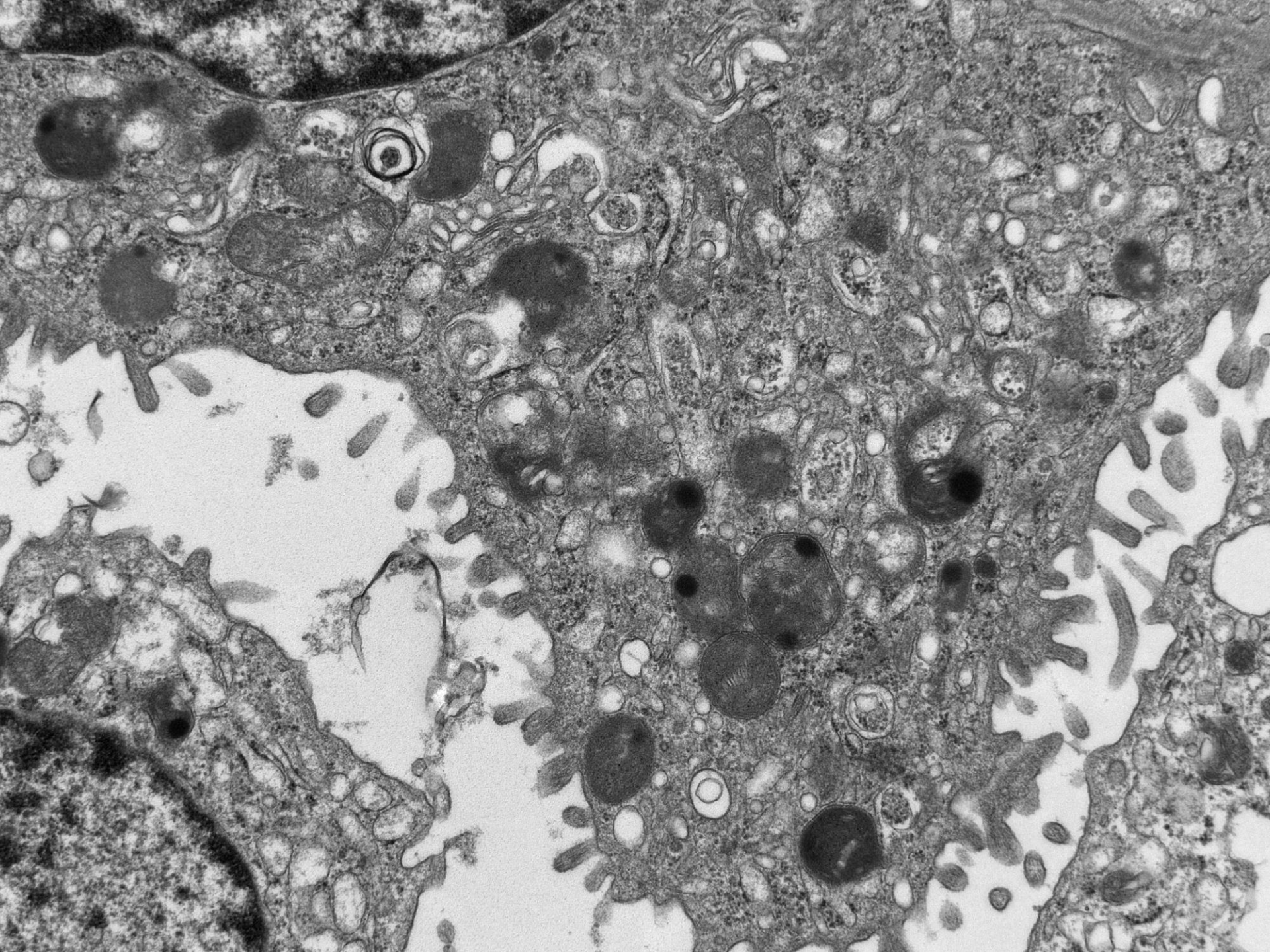


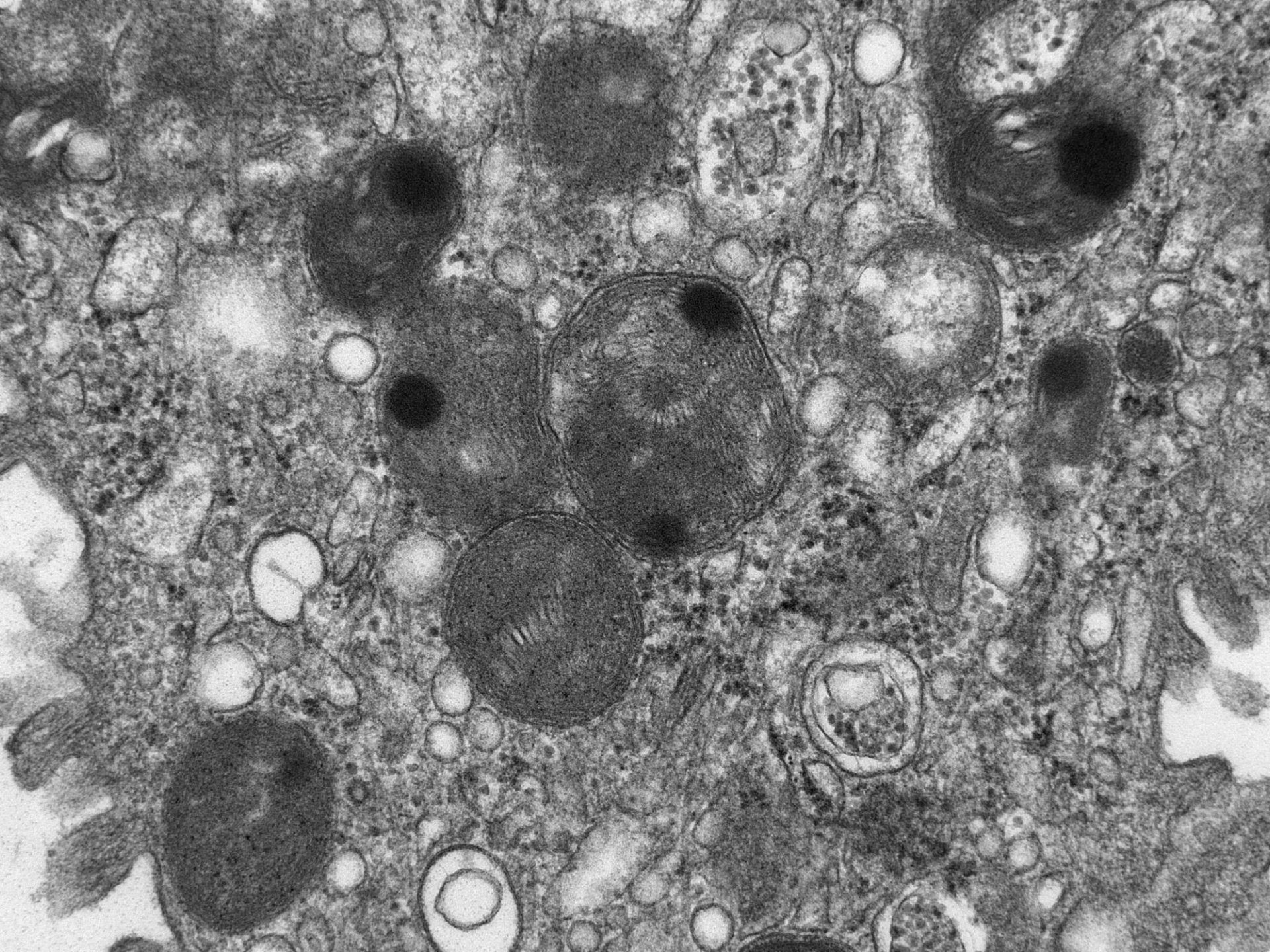
Surfactant body ultrastructure

- Surfactant protein B - lack of tubular myelin, disorganized lamellar bodies and irregular multivesicular bodies
- Surfactant protein C - normal lamellar bodies and infrequent disorganized lamellar bodies
- ABCA3 mutation - small lamellar-like bodies with concentric phospholipid membranes and eccentric dense cores.



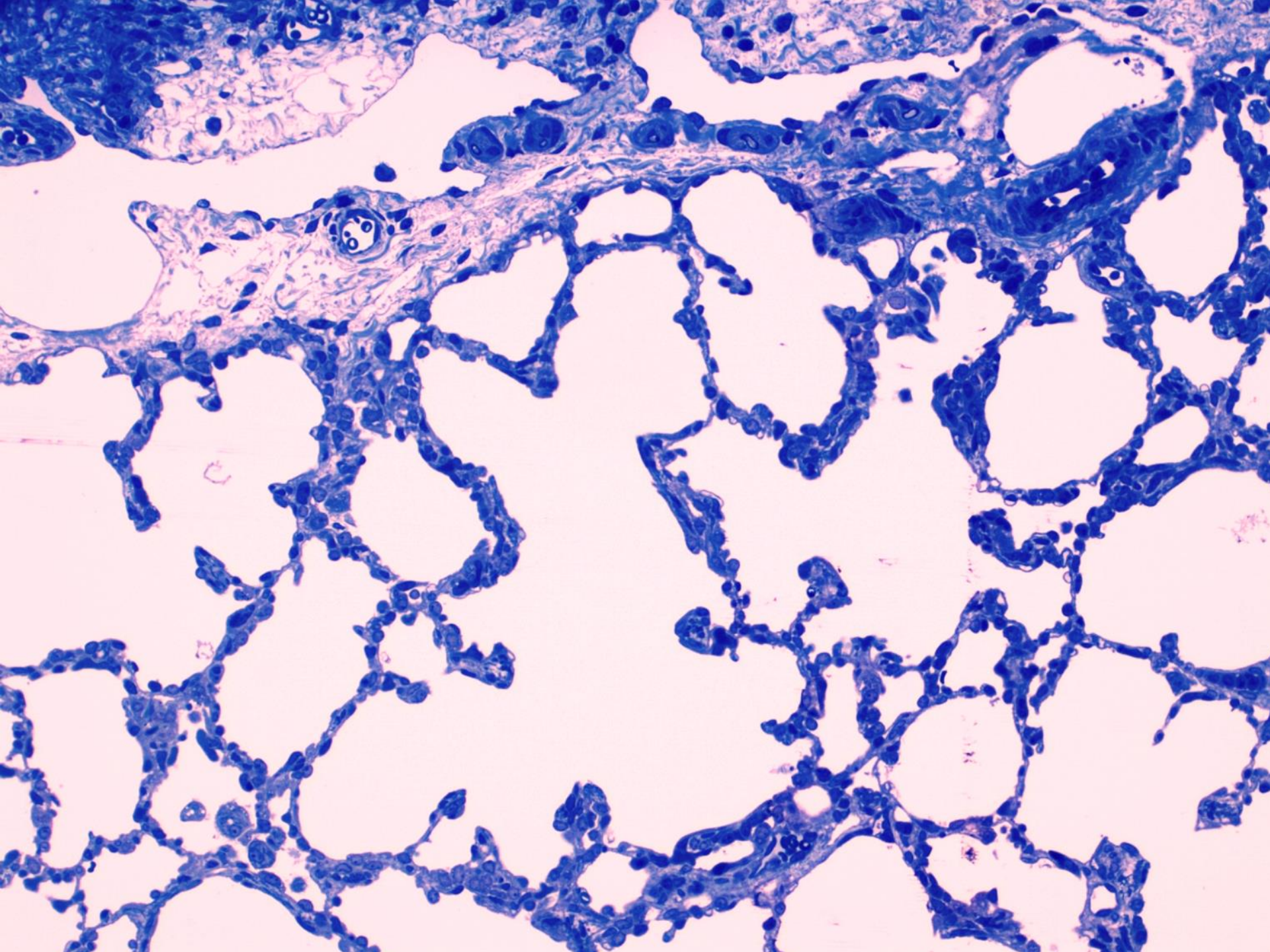


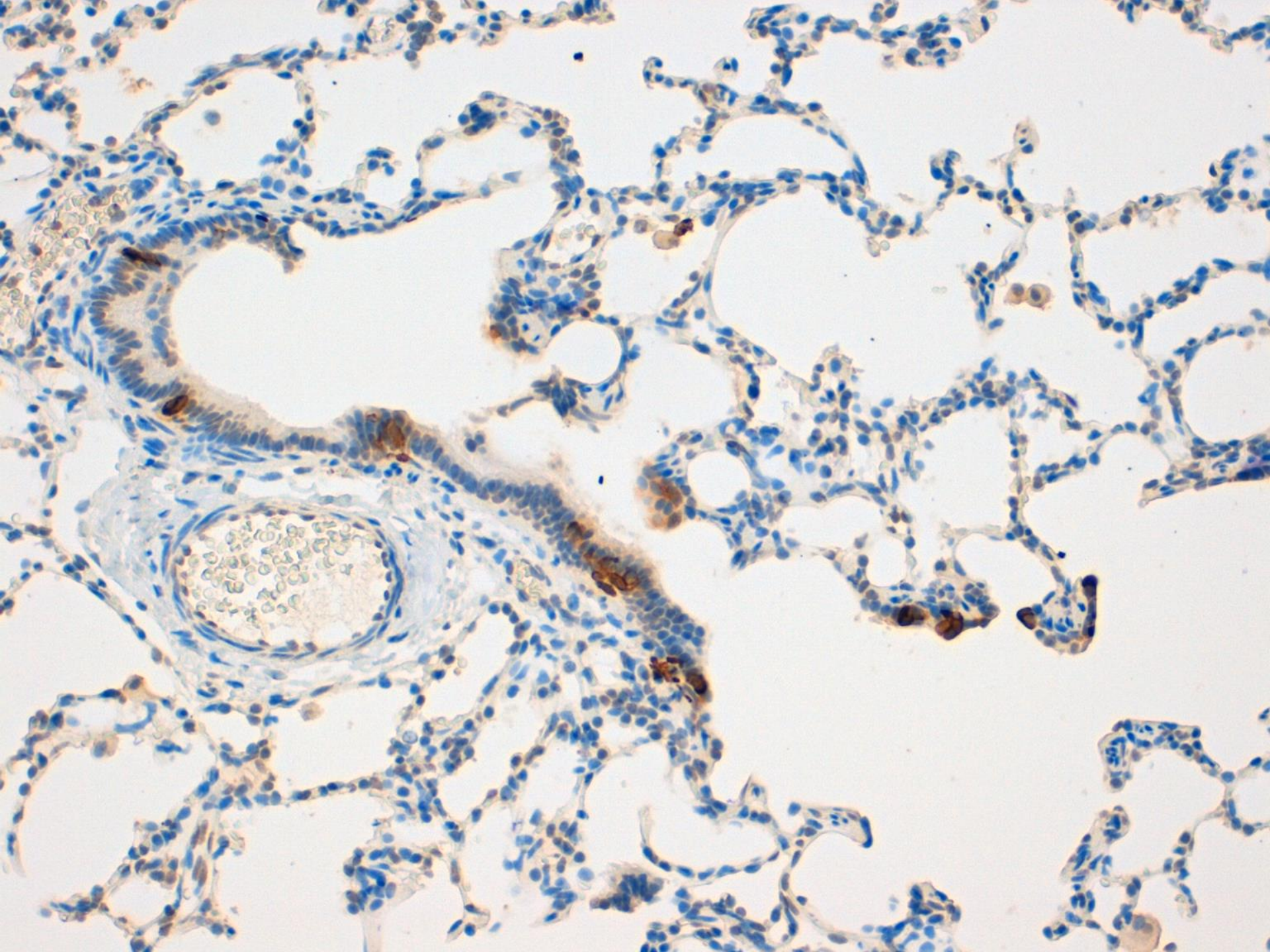


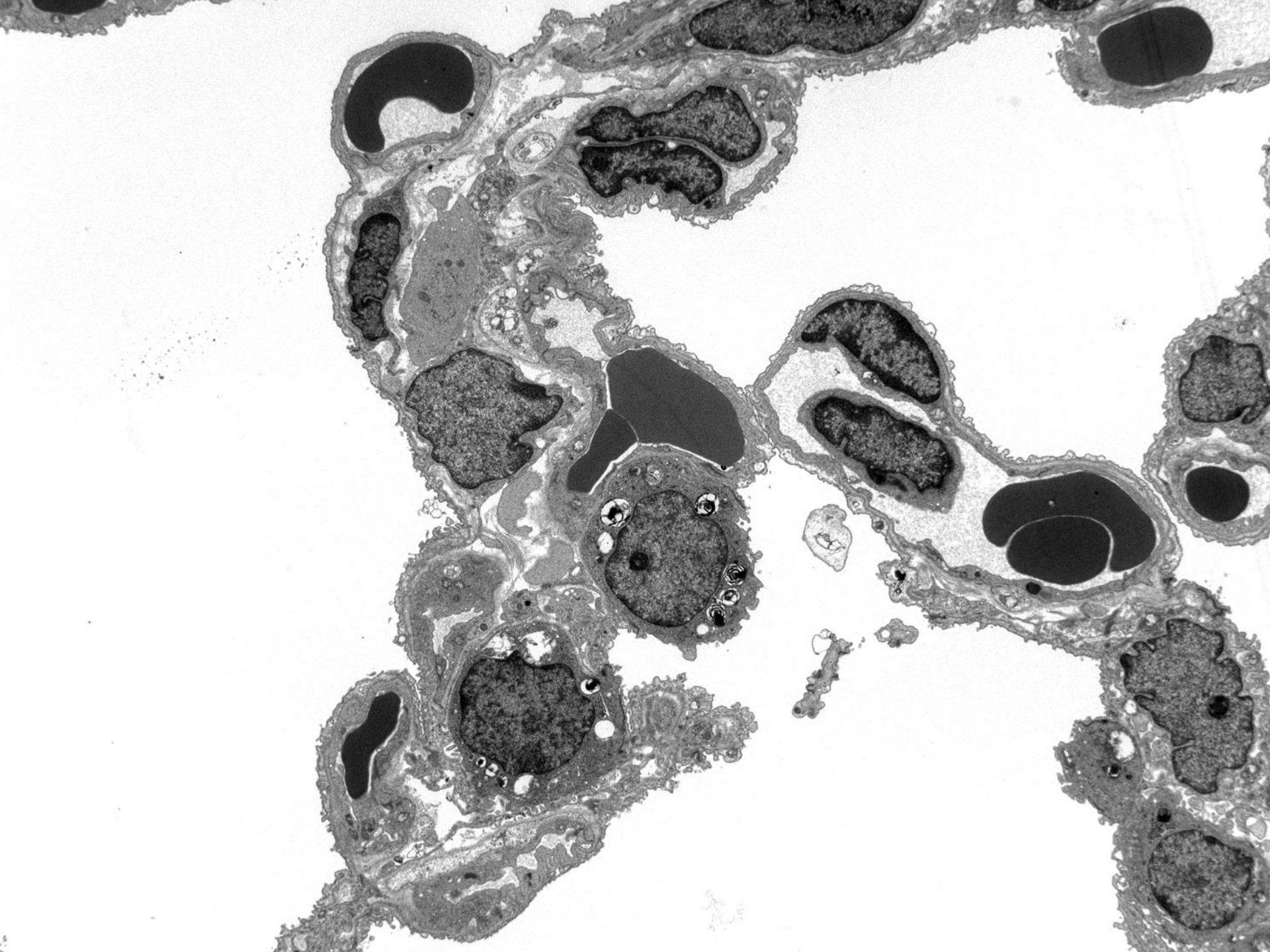


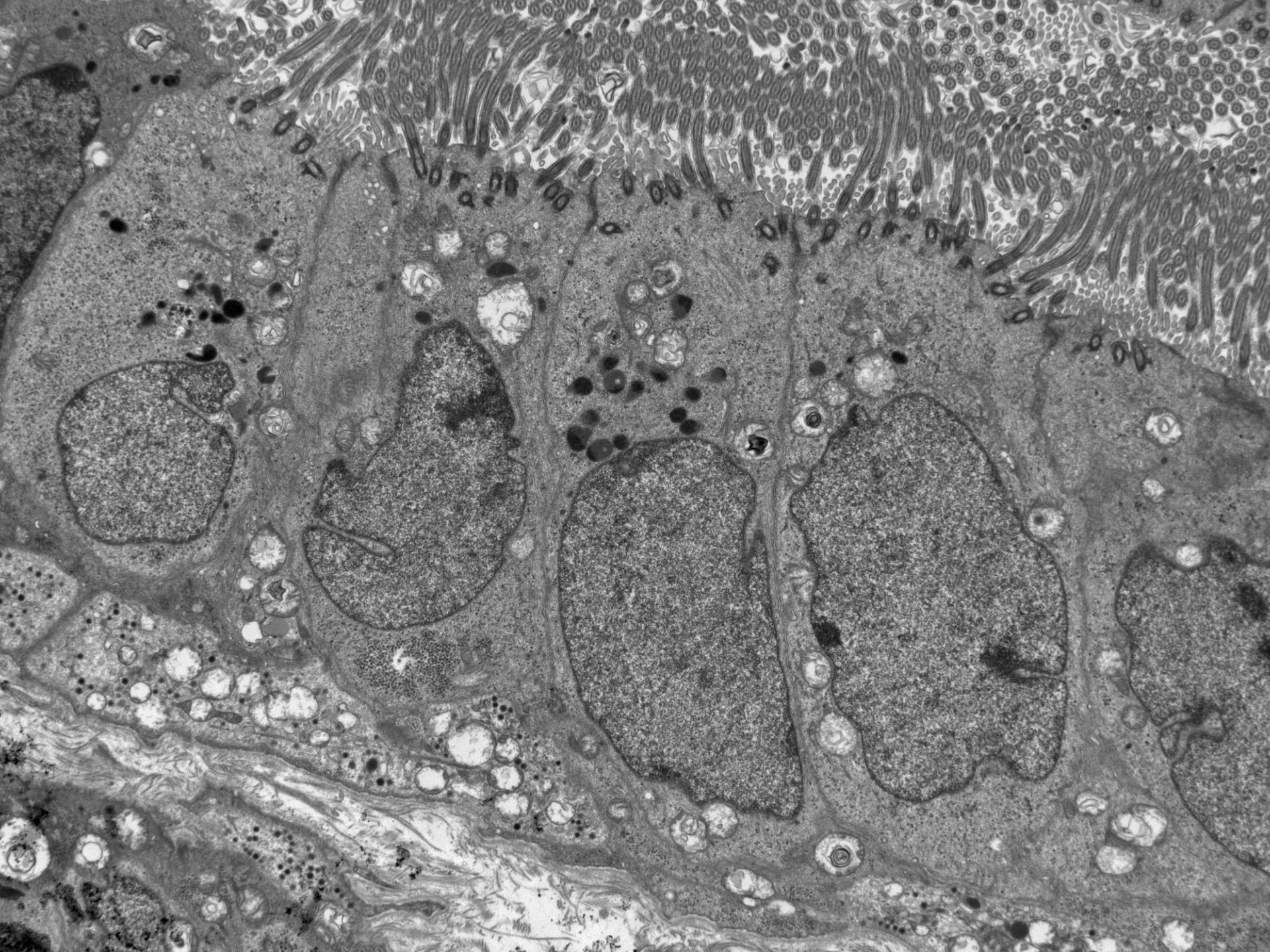
Neuroendocrine cell hyperplasia of infancy

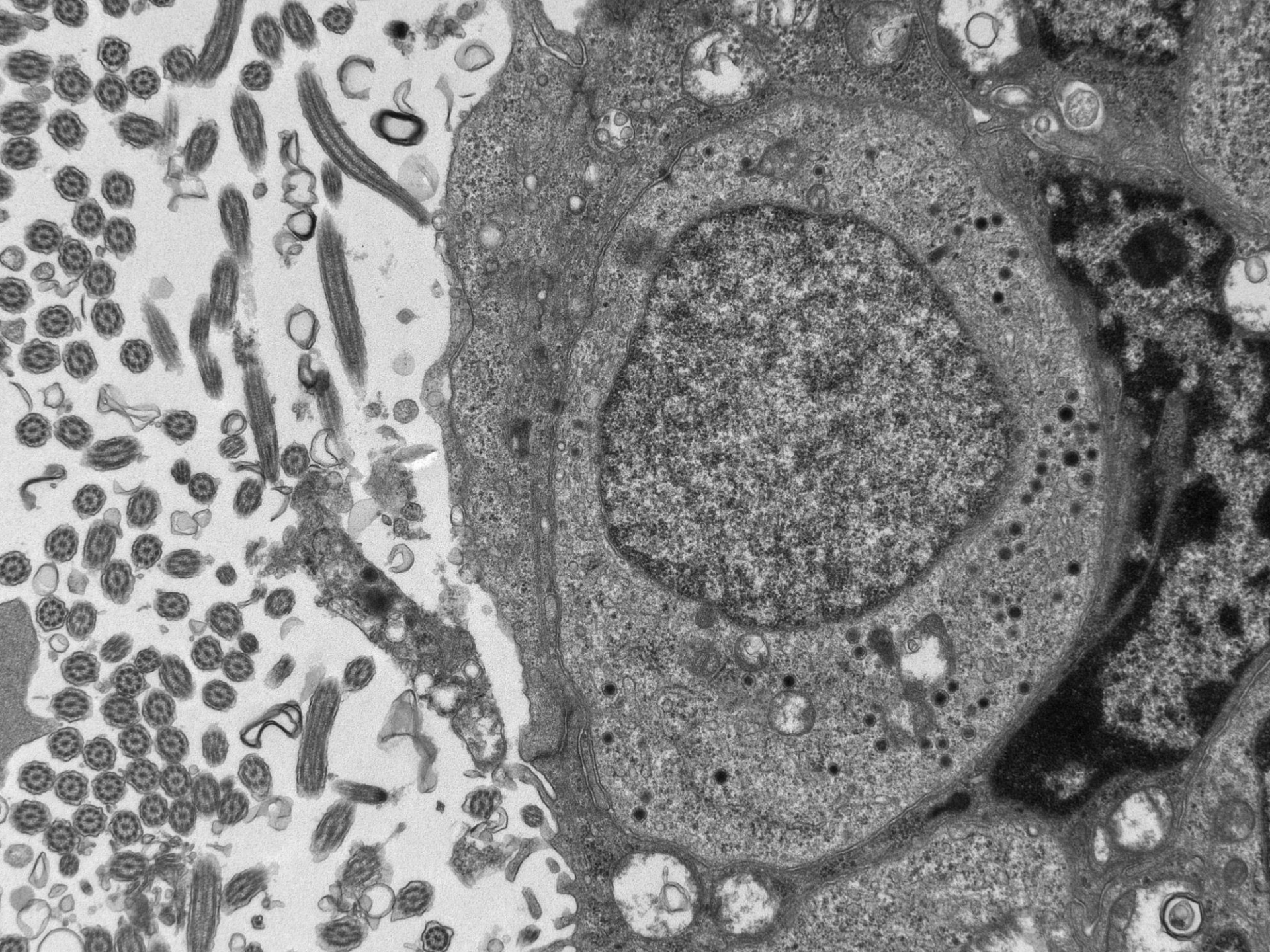
- Small airway disorder
- Increased numbers of NE-C
- Histology can look normal
- Bombesin immuno , +10%
- Patient requires O2 supplement, no aggressive therapy, prognosis good











Neuromuscular Disease

- Major cause of disability
- Numerous genes identified – but
- Muscle biopsy still important for diagnosis
  - rapid answer
  - aids direction of genetic testing
  - can identify broader differential diagnoses

Neuromuscular Disease

- Primary genetic muscle disease
  - muscular dystrophy - Duchenne
  - Congenital myopathies - central core, nemaline
- Neurogenic disorders - spinal muscular atrophy
- Metabolic myopathies - glycogenoses, mitochondrial
- Inflammatory myopathies – dermatomyositis
- Ion channel disorders - periodic paralysis
- Neuromuscular junction defects - myasthenia gravis

Standard histological staining panels

- Tinctorial stains
  - H&E, Gomori trichrome, HVG, ORO, PAS

- Histochemistry
  - Oxidative enzymes COX, SDH (mitochondria)
    reduced NADH (mitochondria, myofibre architecture)
  - ATPase (fibre typing)
  - Acid phosphatase (macrophages, storage)

Standard immunocytochemistry panels

- Dystrophy panel

  - Dystrophin, merosin, sarcoglycans, utrophin, desmin, dysferlin, dystroglycan, collagen VI, spectrin

- Inflammatory panel

  - CD3, CD4, CD8, CD20, CD68
  - MHC 1, MAC, CD31, dysferlin

- Fibre typing

  - Fast and slow myosin, developmental or neonatal myosin

Muscle – Juvenile Dermatomyositis

- Commonest inflammatory myopathy of childhood – 5 to 15 years
- Genetic predisposition
- Can affect various system – muscle/skin
- Muscle weakness and skin rash - calcium deposition
- Inflammation of small blood vessels, circulating immune complexes

JDM - histopathology

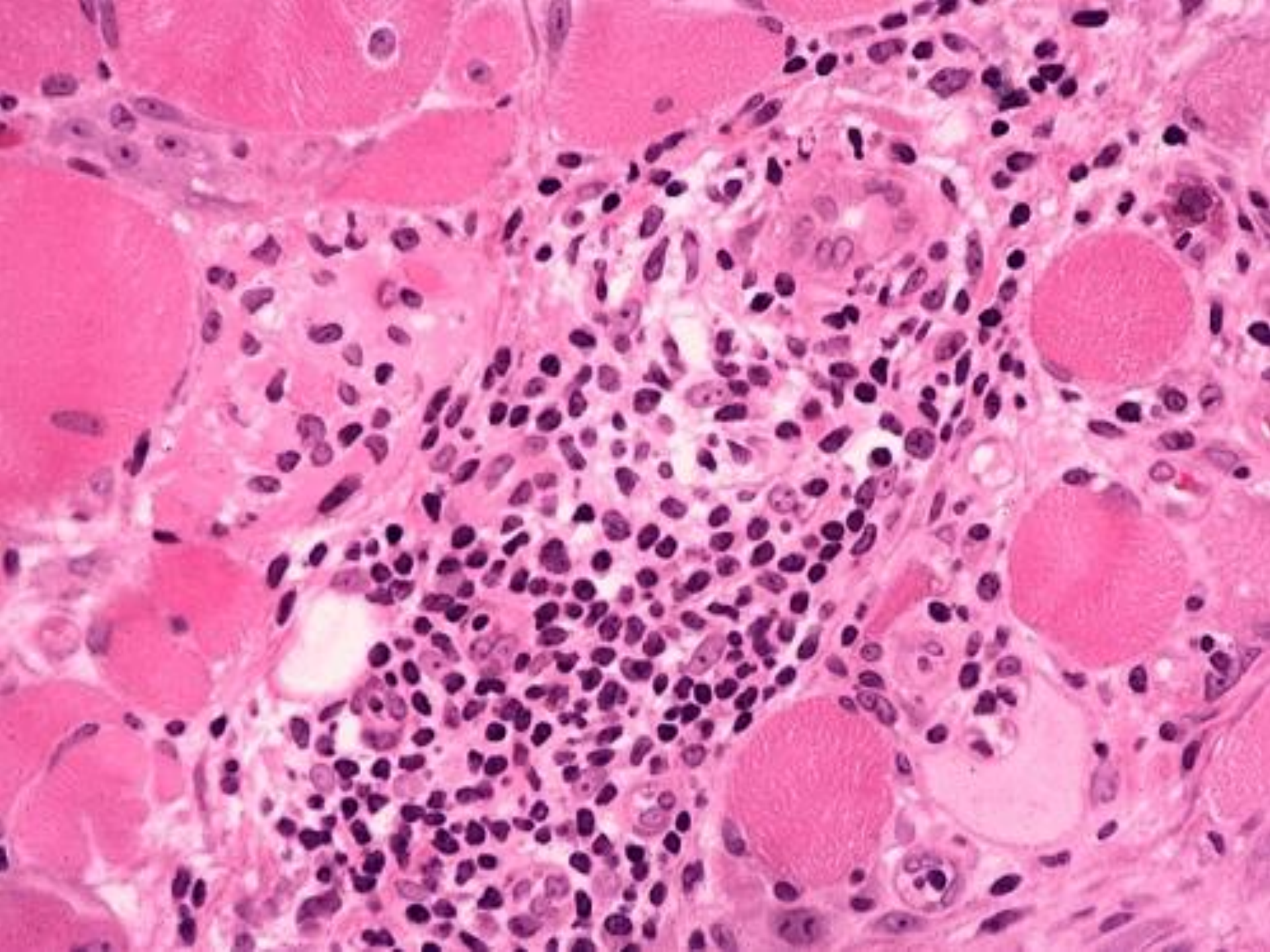
- Destructive myopathy – necrosis, regeneration, atrophy, internal nuclei and fibrosis
- Inflammation – lymphocytes – perifascicular or perivascular distribution

JDM – muscle biopsy

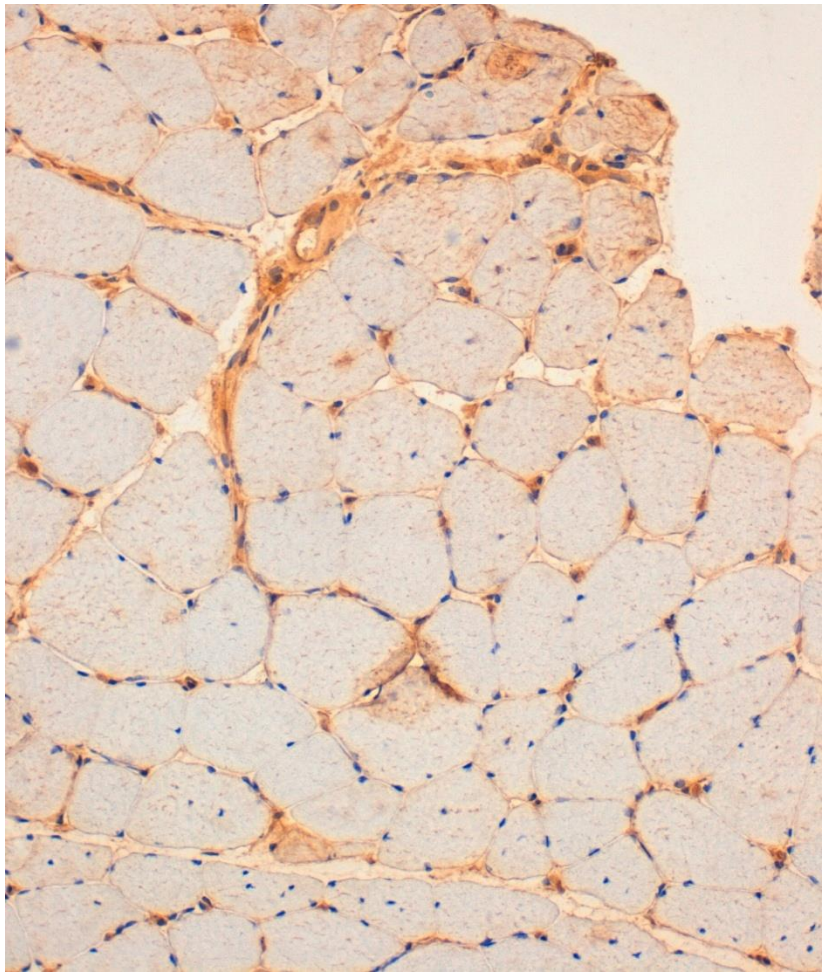
- Histochemical & tinctorial stains

- Immunocytochemistry
  - inflammatory panel CD3, CD4, CD8, CD20, CD68
  - sarcolemmal staining MHC
  - membrane attack complex
  - endothelial markers CD31

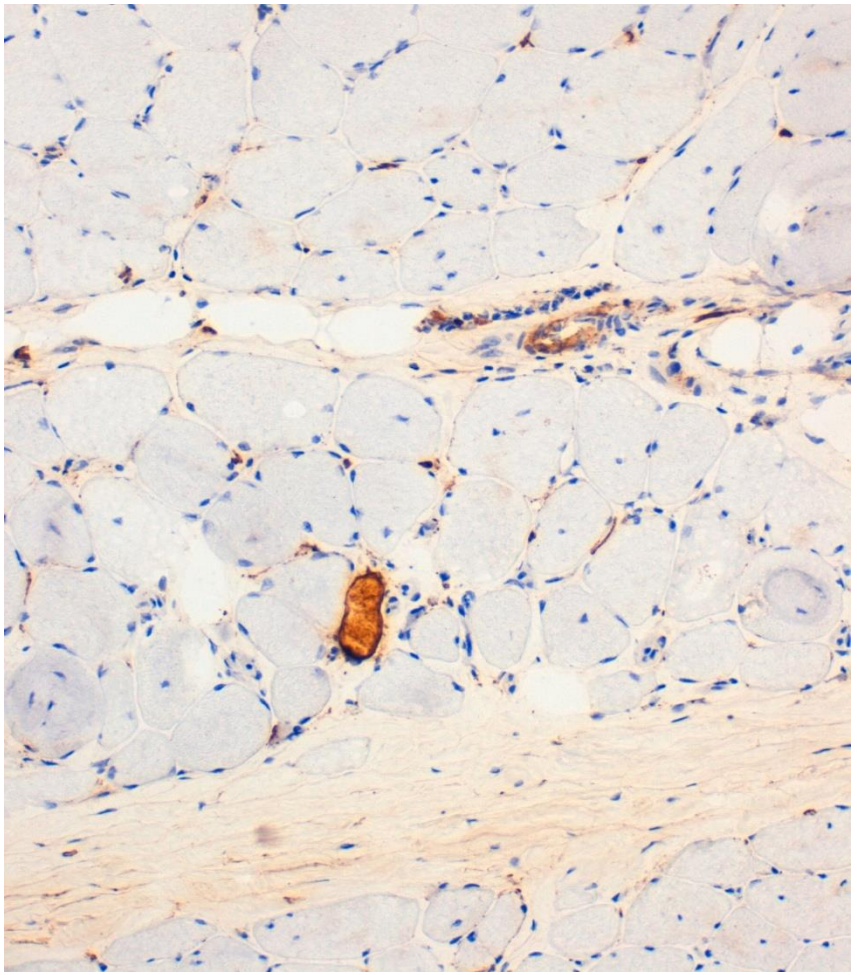
- Electron microscopy



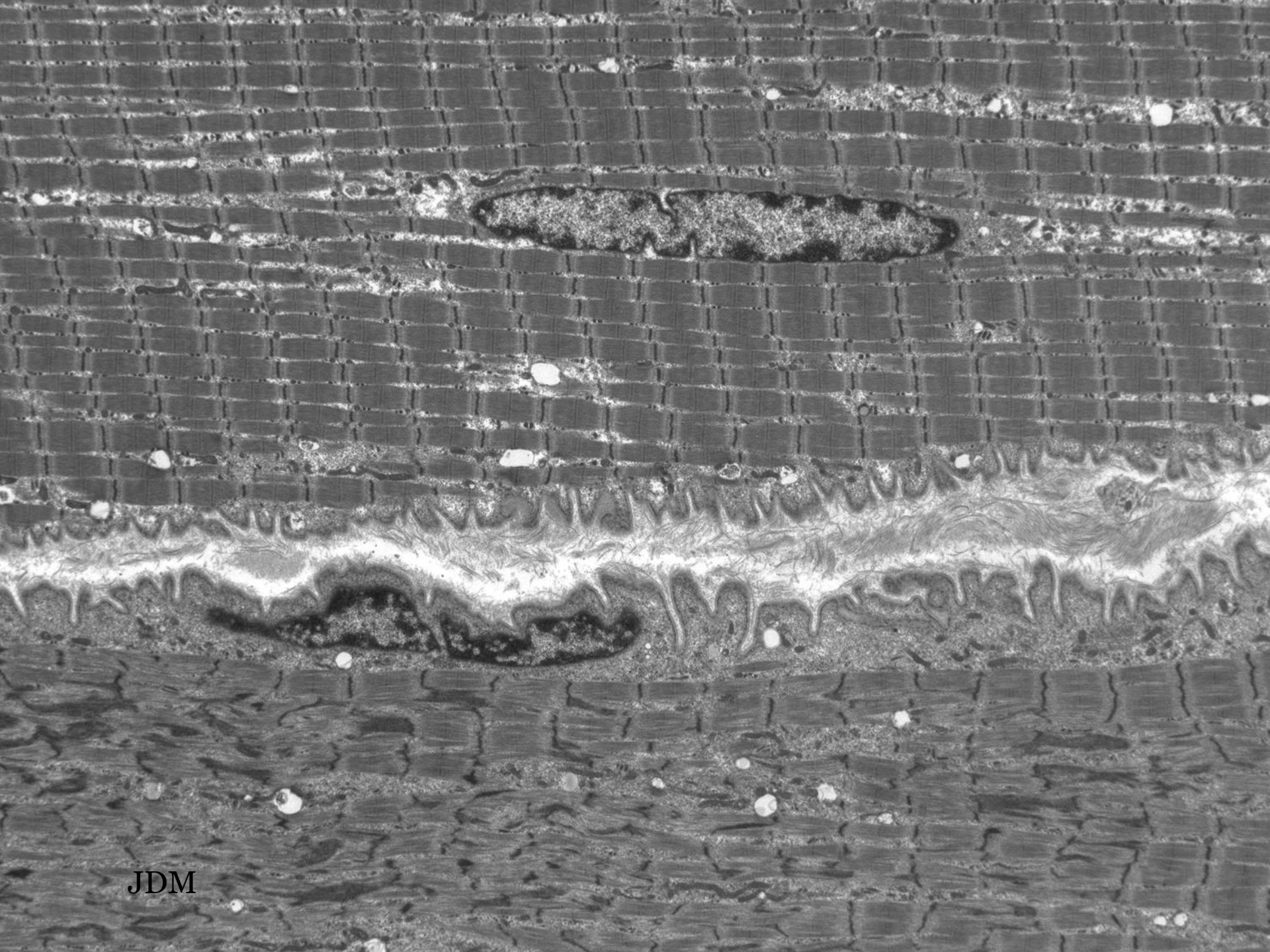
JDM inflammatory panel

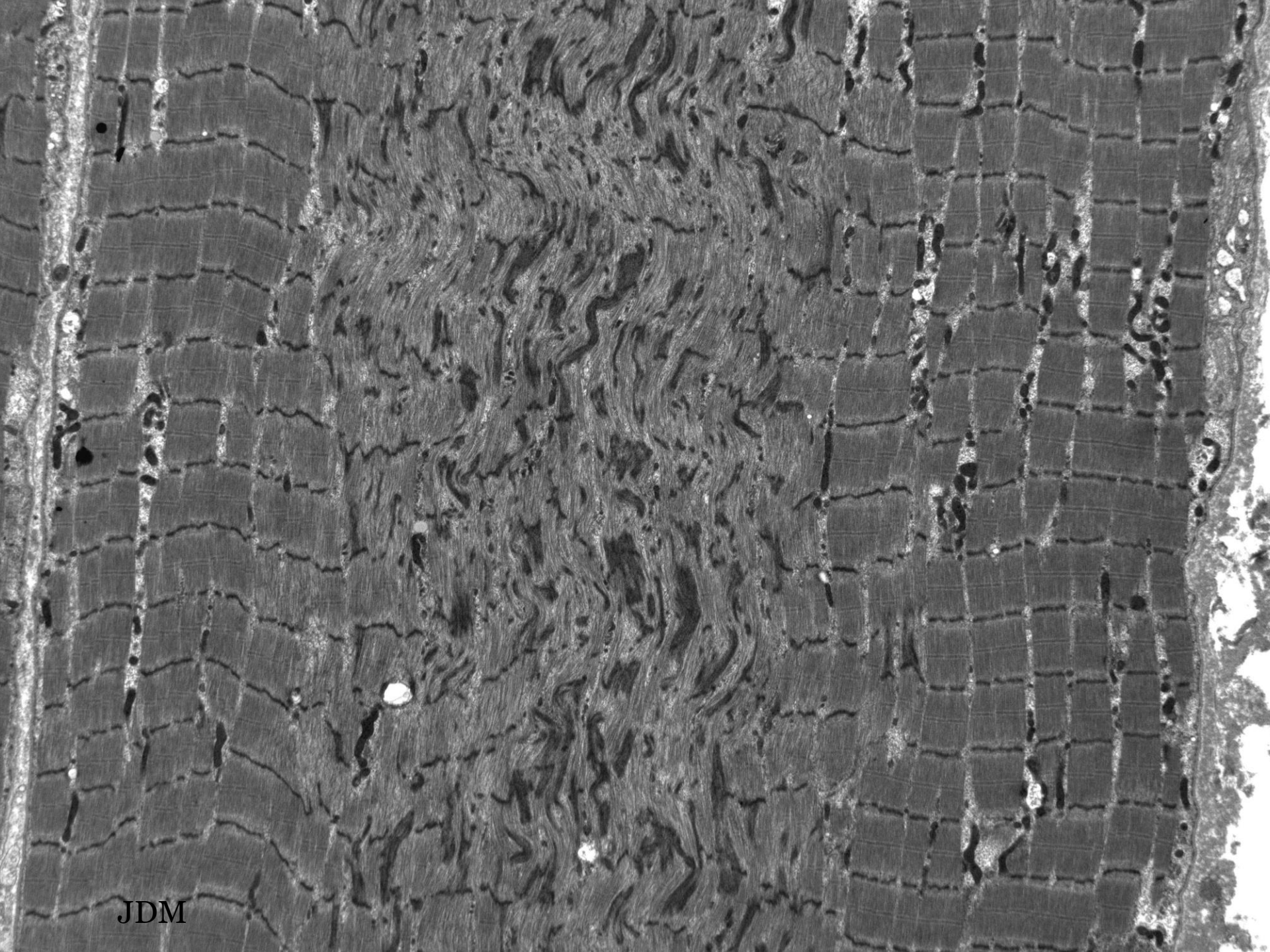


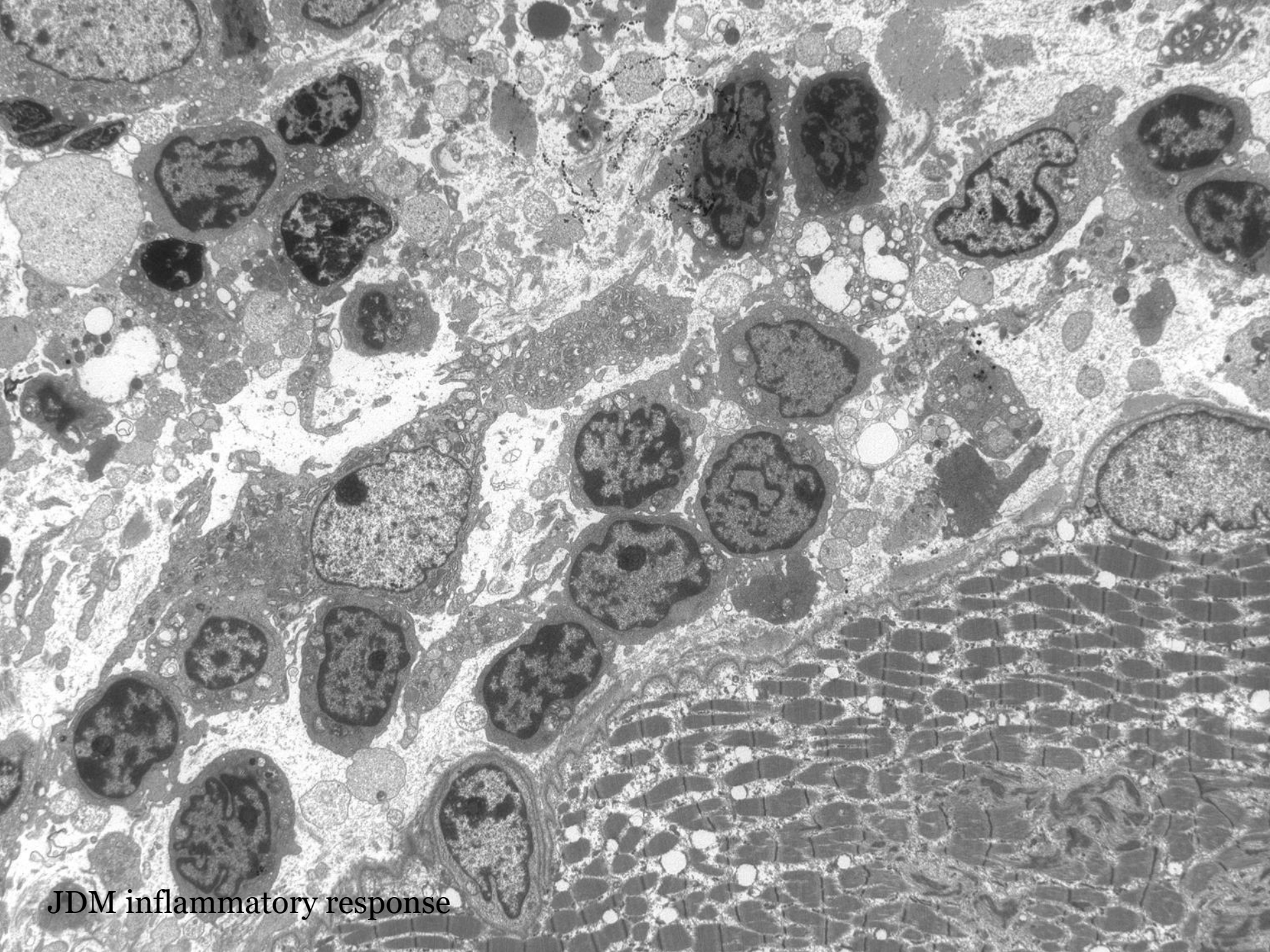
MHC-1

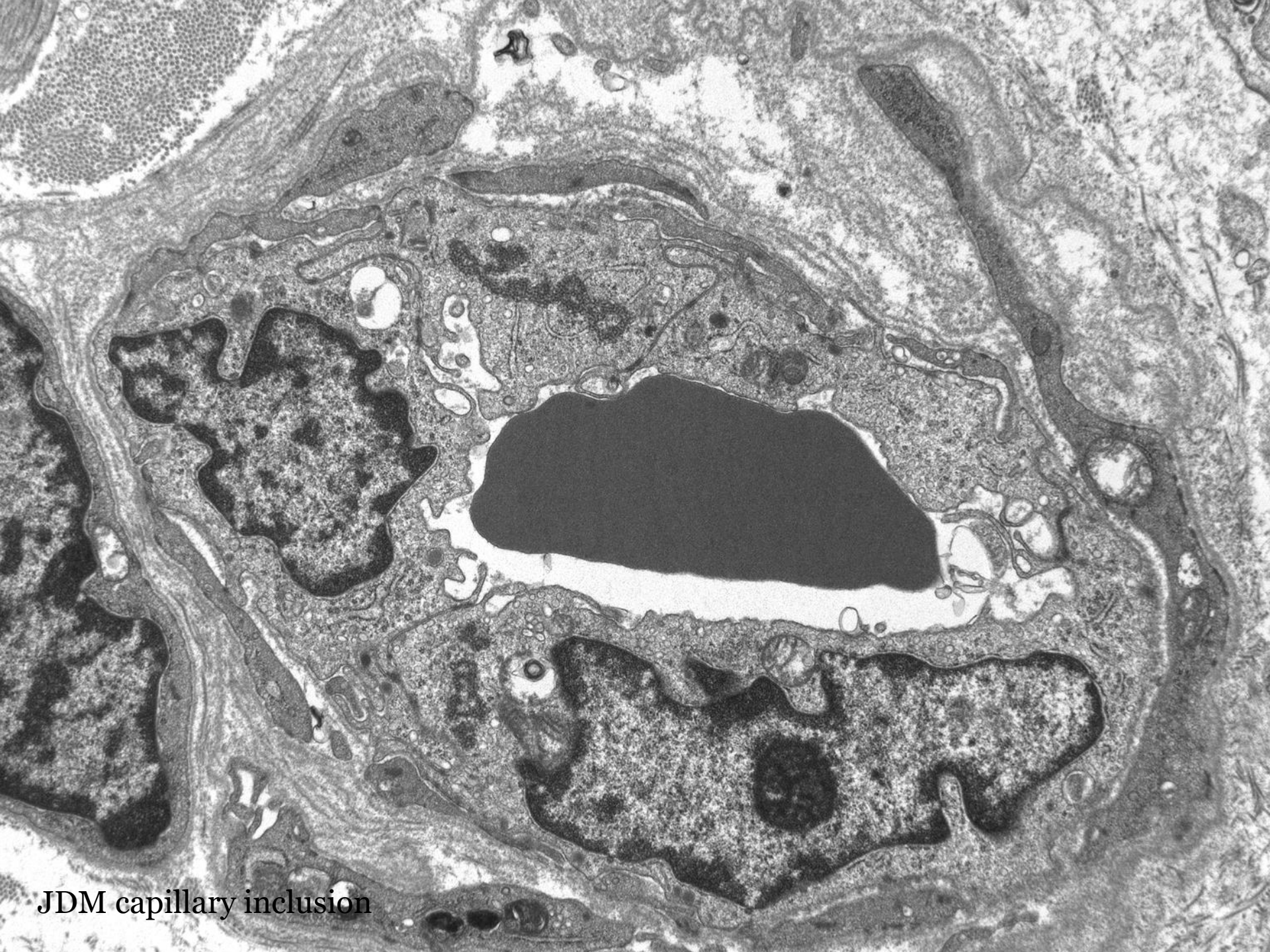


MAC

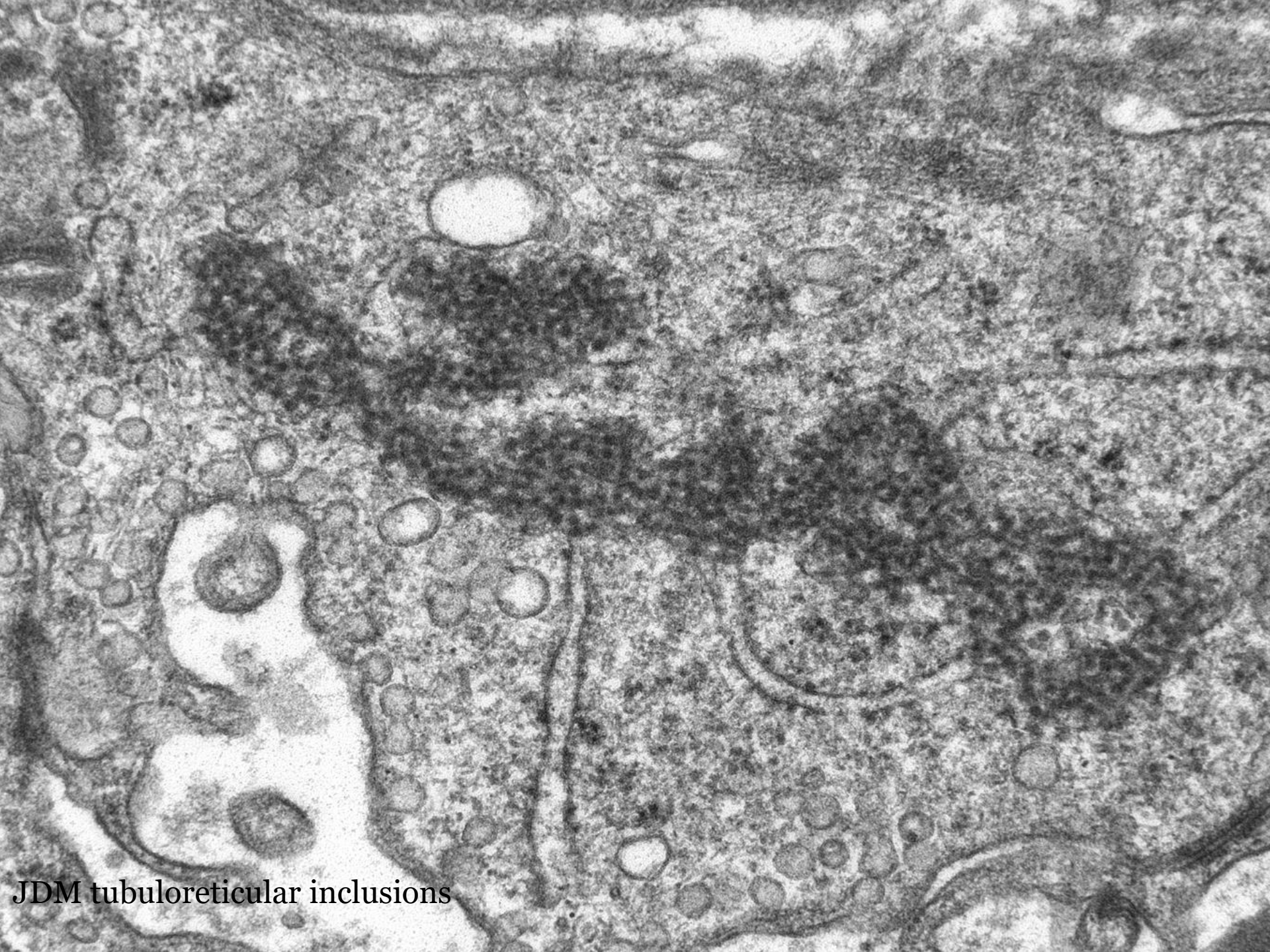

JDM


JDM


JDM inflammatory response



JDM capillary inclusion



JDM tubuloreticular inclusions

Platelet Function Disorders

- Platelets with absent or decreased receptor sites

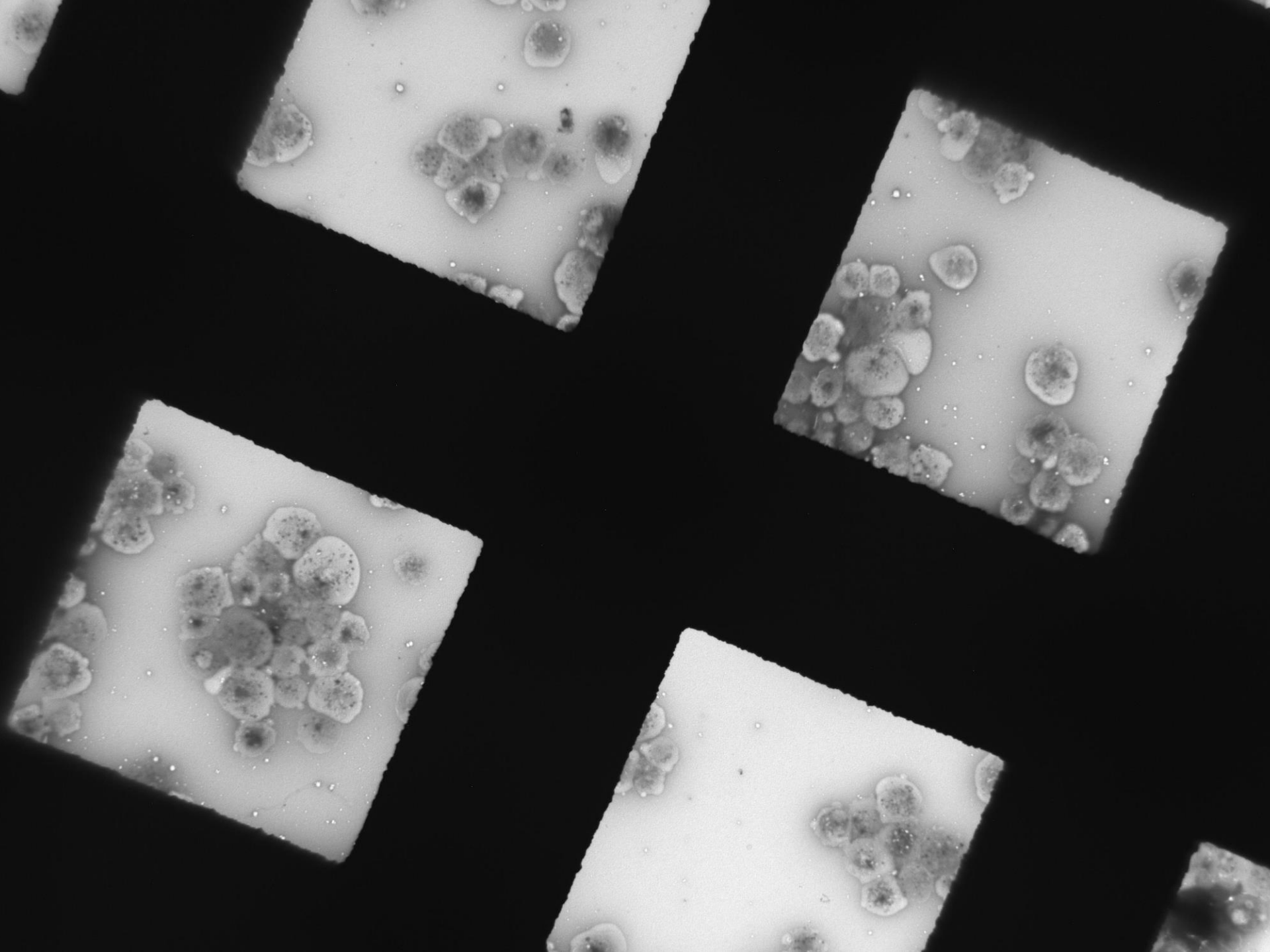
- Defects in granule content “Storage Pool Disorders”
  - not associated with a systemic disorder
  - associated with other systemic inherited disorders

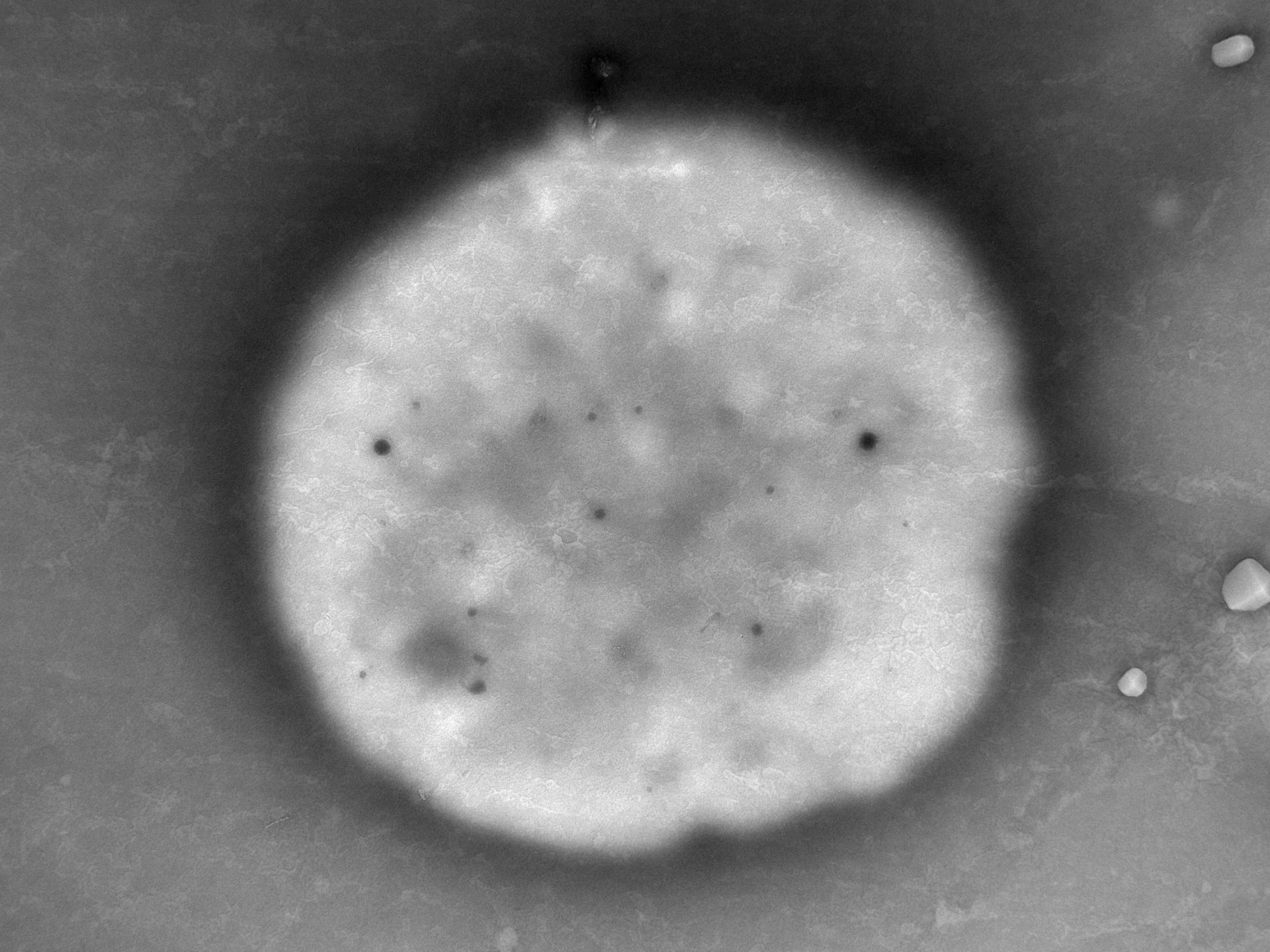
Platelet Ultrastructure

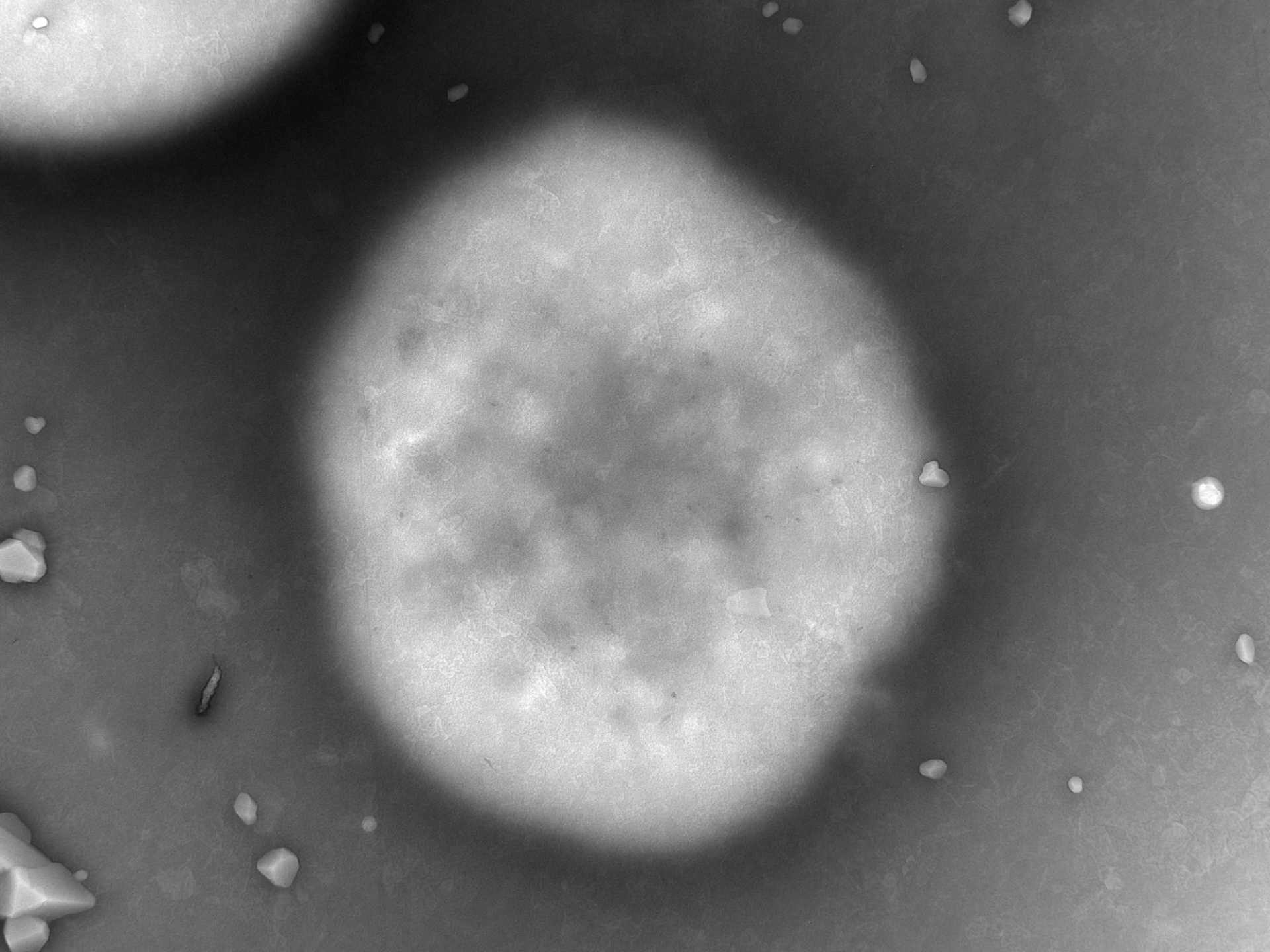
- Whole mount preparation – dense granule estimation, Hermansky Pudlak syndrome
- Ultrathin sections – fine detail, Grey platelets
- Bone marrow aspirate –megakaryocyte/platelet formation

Whole mount platelet preparation

- EDTA blood 3 - 5 ml (preferably delivered within 24hrs)
- Centrifuge - 1000 rpm, 5 min
- Platelet rich plasma
- Drop on carbon coated grid
- Brief wash, air dry
- Examine

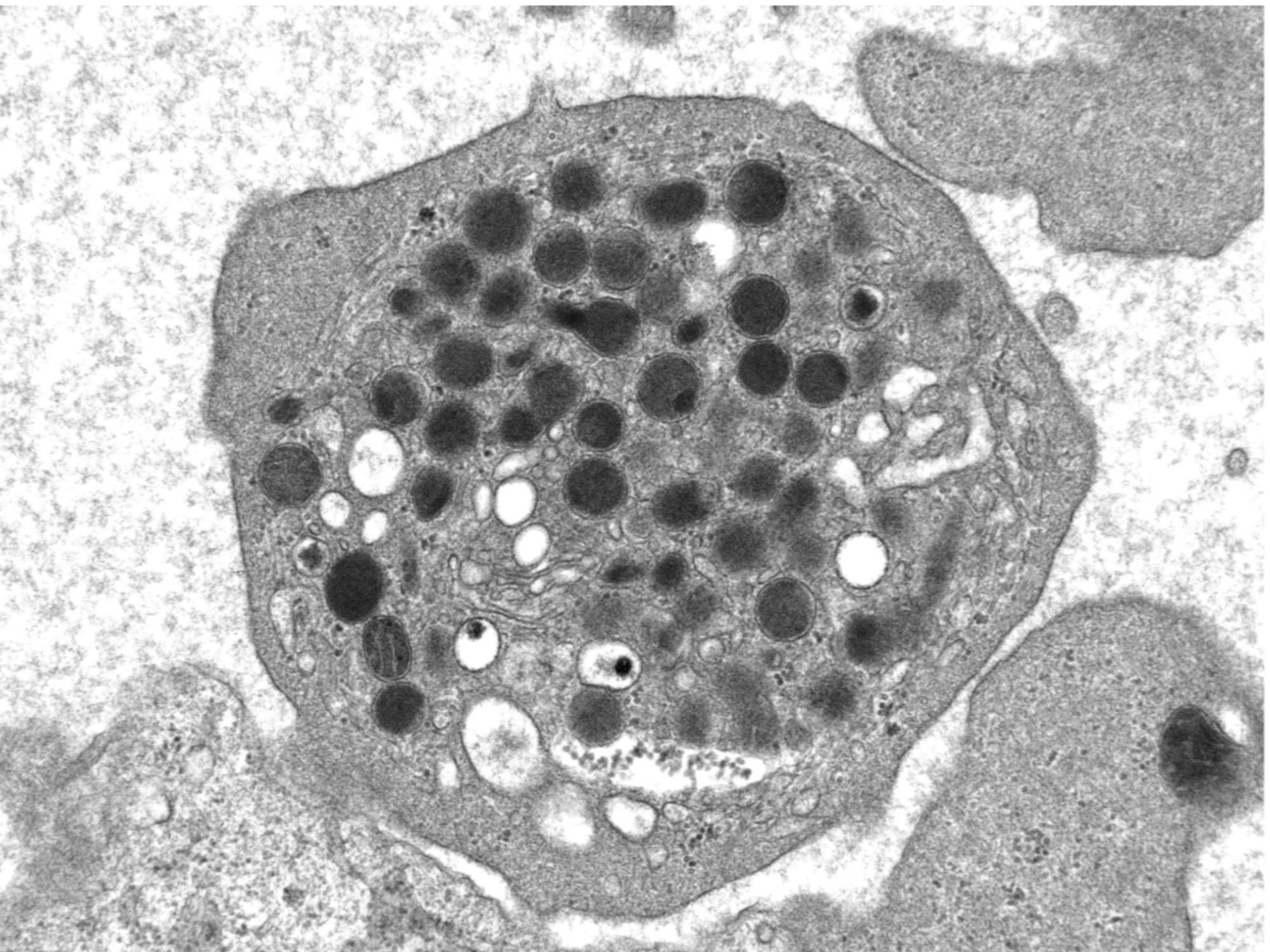


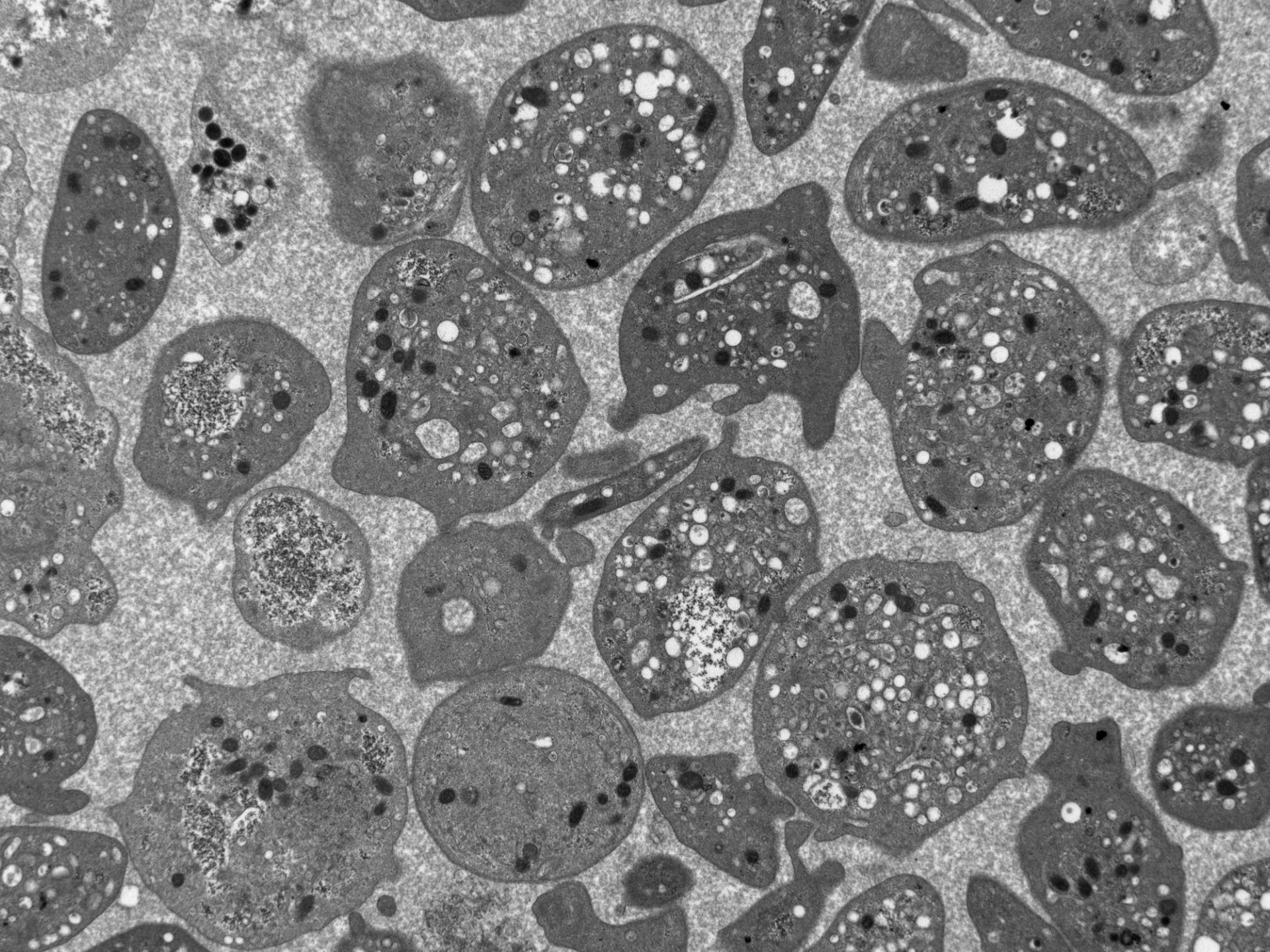


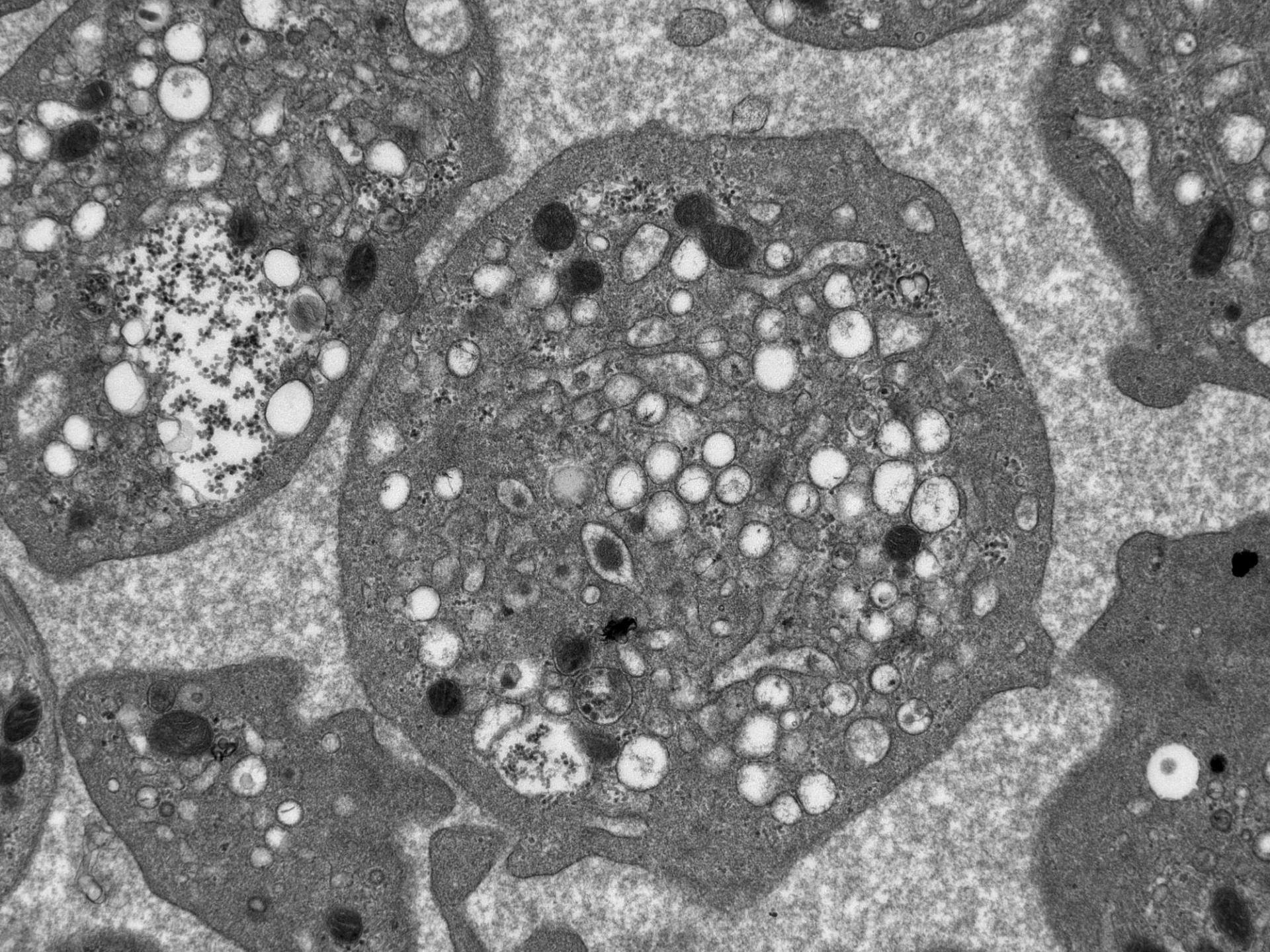


Platelet EM

- Dense granule disorders – Hermansky Pudlak syndrome, Chediak Higashi sydrome
- Alpha granule disorders – Paris-Trousseau or Jacobsen syndrome (giant granules)
- Alpha and dense granule disorders – rare
- Grey platelet syndrome – large, alpha granule deficiency
- Small platelets – Wiskott-Aldrich syndrome







Thank you