Evidence for novel reticular corpuscle-connected filiform structures entangled on the fascia of the internal organs of rats: the implication for cell death

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Supplementary Figures, Table and Video

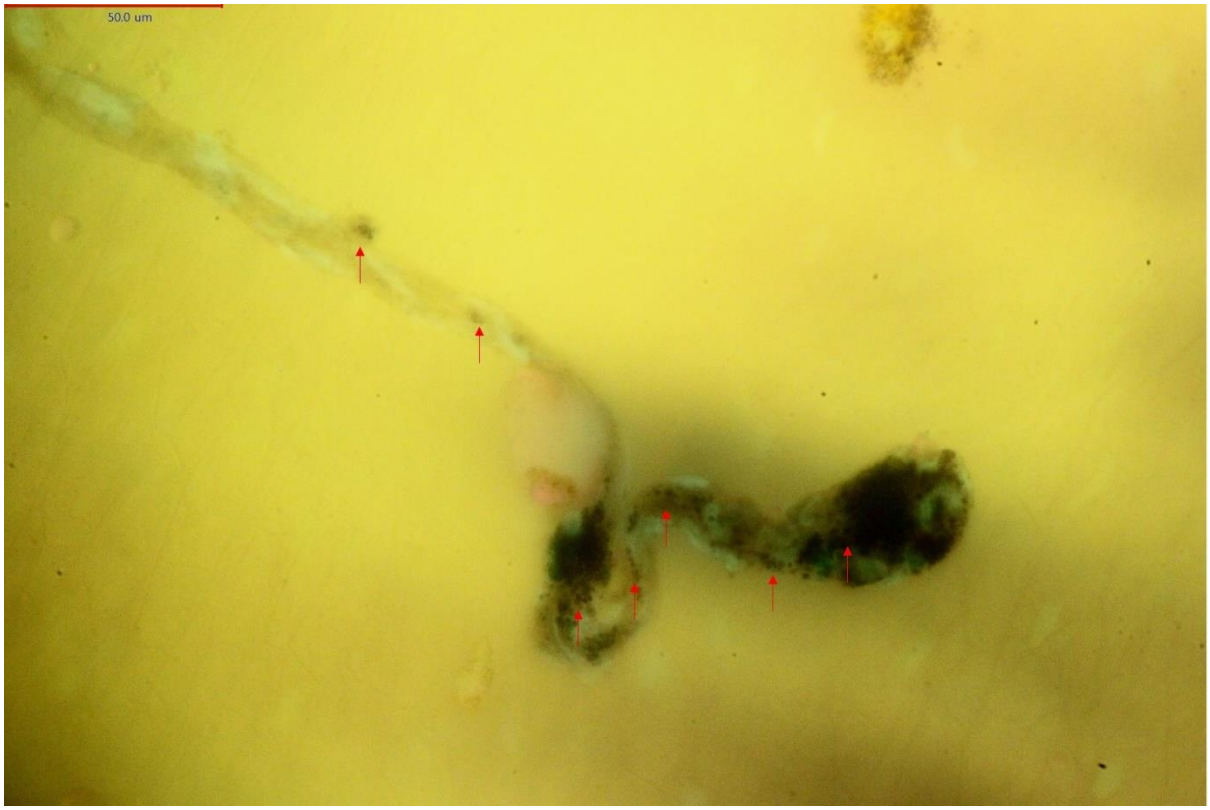


Figure S1: The DAPI-stained image of the CCFS shows that it has JGB-stained granules (blackish dots, red arrows) with distinctive nuclei. Noticeable thing is that DAPI-stained nuclei (bright bluish fluorescence) and JGB-stained granules are not merged. Scale bar: 50 μm

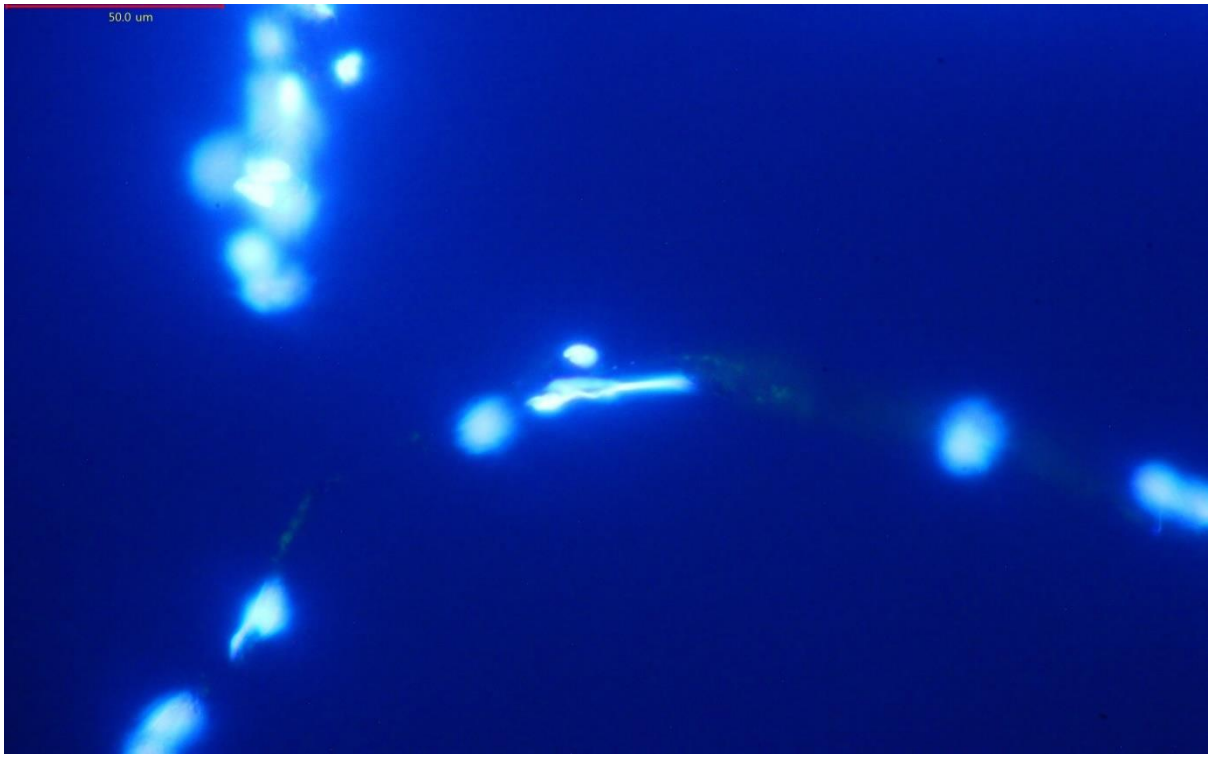


Figure S2: Original raw data for a6 of Figure 5 shows the DAPI-stained image of the CCFS with fragmented DNA molecules and extracellular DNA. Scale bar: 50 μm

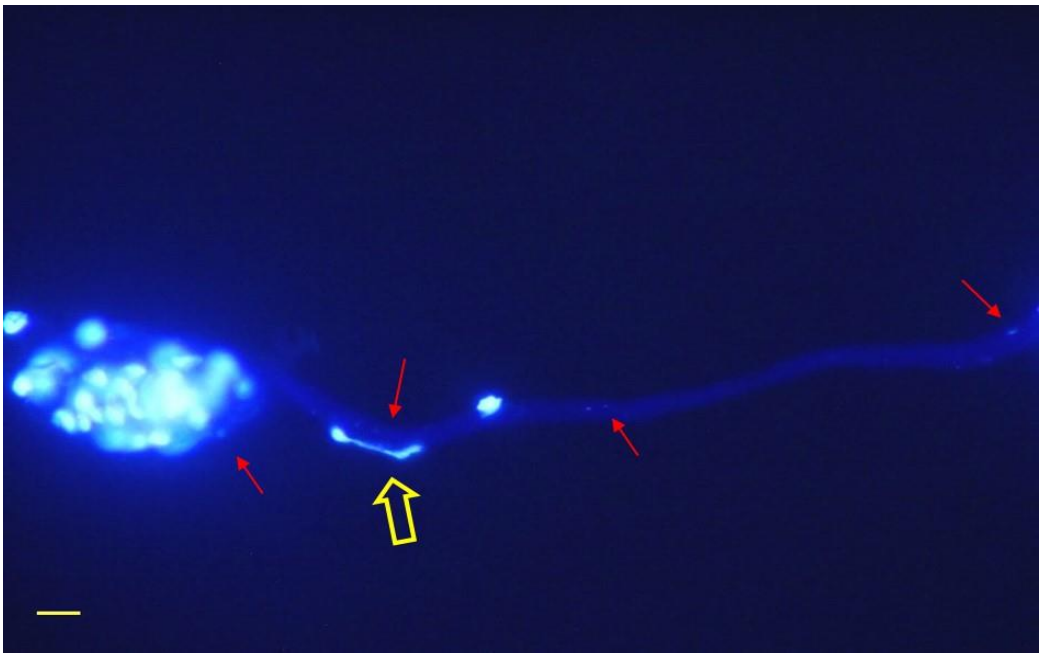


Figure S3: The DAPI-stained image of the CCFS shows that it has fragmented DNA molecules (red arrows) and extracellular DNA (open yellow arrow). Scale bar: 10 μm

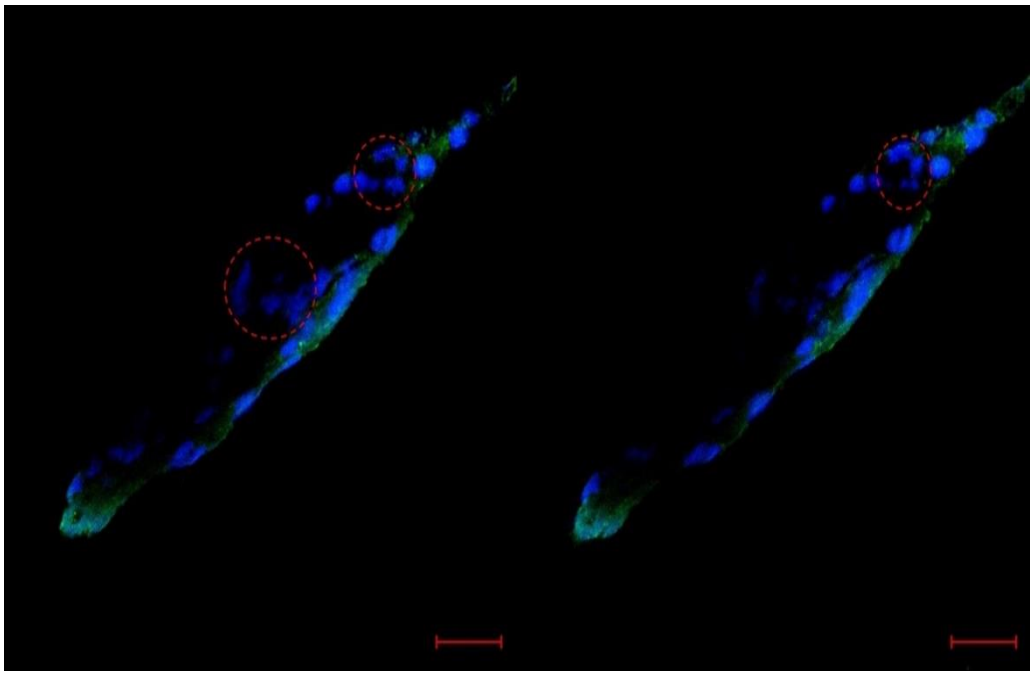


Figure S4: The DAPI-stained image of a corpuscle in the CCFS shown in Figure 5 (main manuscript) shows amorphous extracellular DNA (dotted red circles). These two images are serially optically sectioned by 1 μm. Scale bar: 20 μm

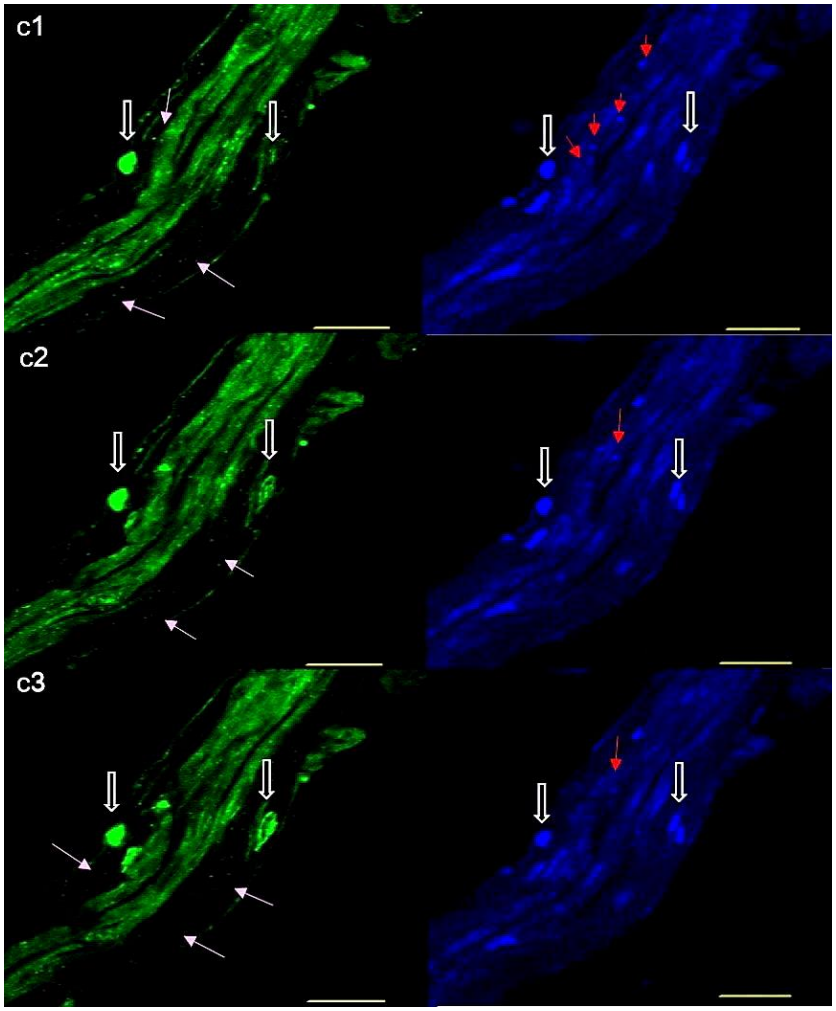


Figure S5: A rarely found CCFS, with a relatively thick filiform structure. Open arrows indicate distinctive DAPI-stained and f-actin images for nuclei; however, the red and the pink arrows are dotted DNA signals and images of fragmented actin, respectively. Even though this image is very clear, we did not inset it into the main manuscript as this type of CCFS is rarely found. All scale bars are 20 μm.

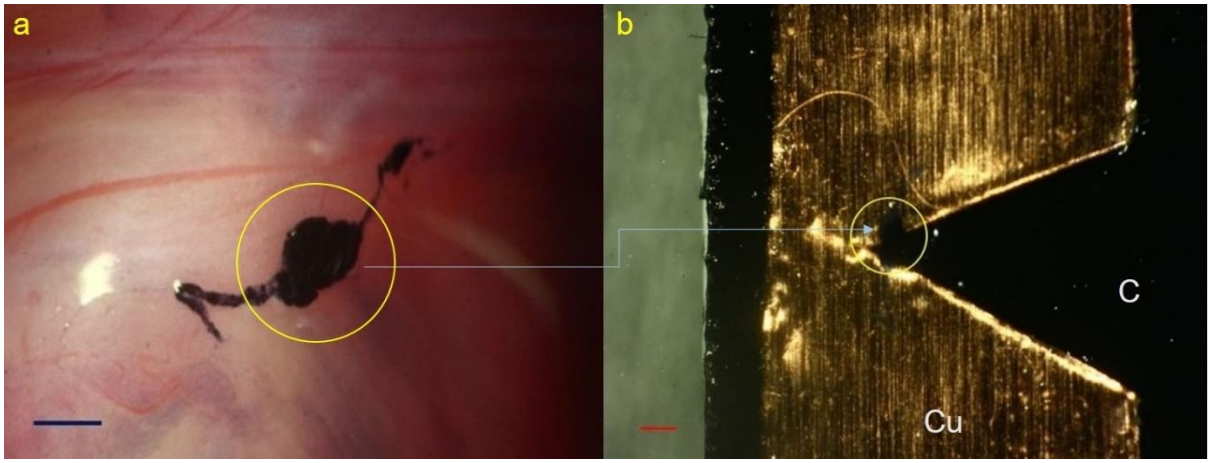
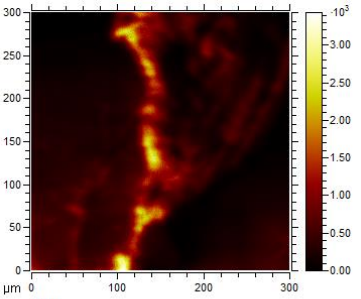
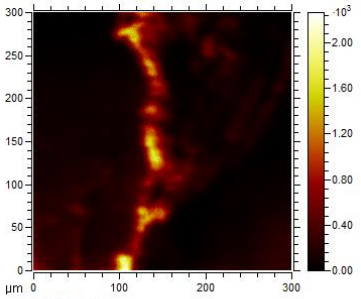
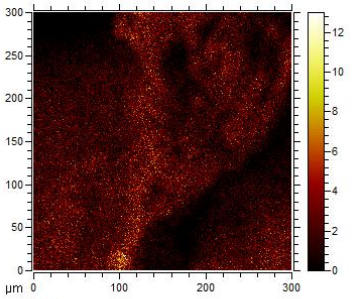
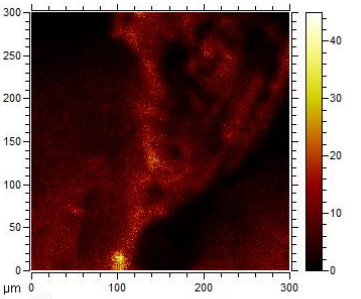


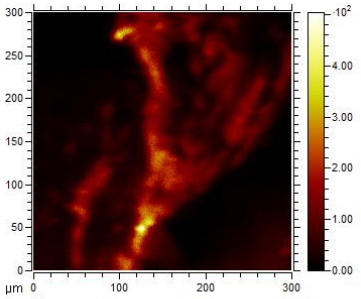
Figure S6: Sample preparation for TOF-SIMS (a): Stereoscopic image for CCFS which was loaded on the Cupper plate (Cu) on carbon tape (C) in (b). Yellow-circled corpuscular structure was target point for TOF-SIMS. Both scale bars are 500 μm.

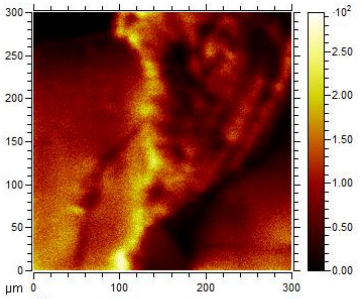

µm
total
MC: 3434; TC: 2.423e+007

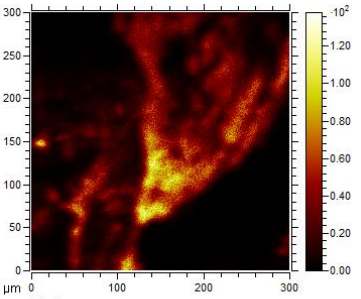

µm
sum of rest
MC: 2266; TC: 1.132e+007

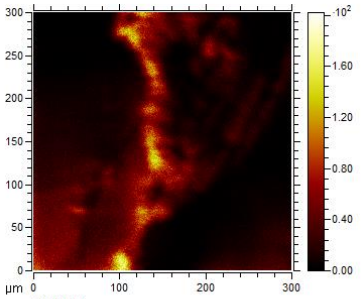

µm
C+
MC: 13; TC: 1.055e+005

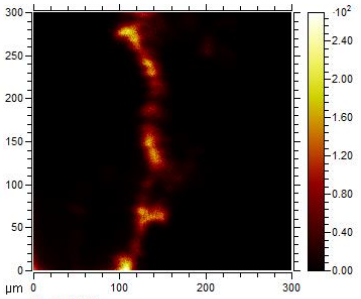

µm
CH2+
MC: 45; TC: 3.506e+005

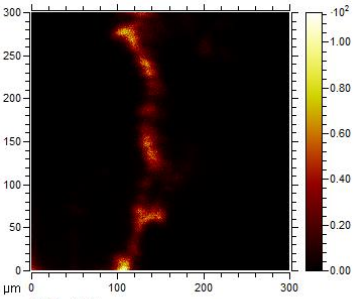

µm
Na+
MC: 504; TC: 3.581e+006

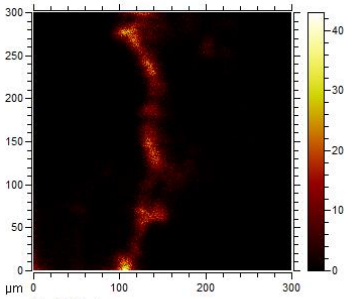

µm
Si+
MC: 295; TC: 5.526e+006

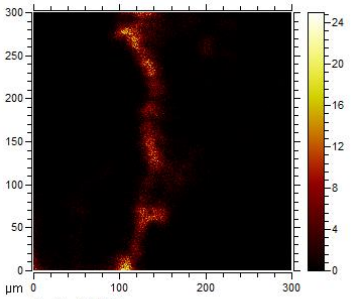

µm
Ca+
MC: 138; TC: 1.168e+006

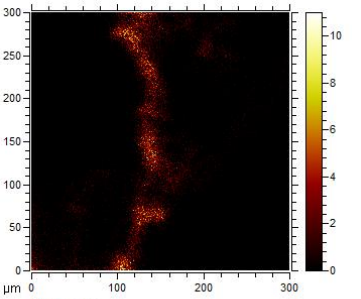

µm
CHNO+
MC: 202; TC: 1.380e+006


µm
C3H7NO+
MC: 270; TC: 5.096e+005


µm
C7H15SO+
MC: 113; TC: 1.706e+005


µm
C14H7O2+
MC: 43; TC: 6.400e+004


µm
C12H18O6Na+
MC: 25; TC: 3.444e+004


µm
C7O2F11+
MC: 11; TC: 1.473e+004

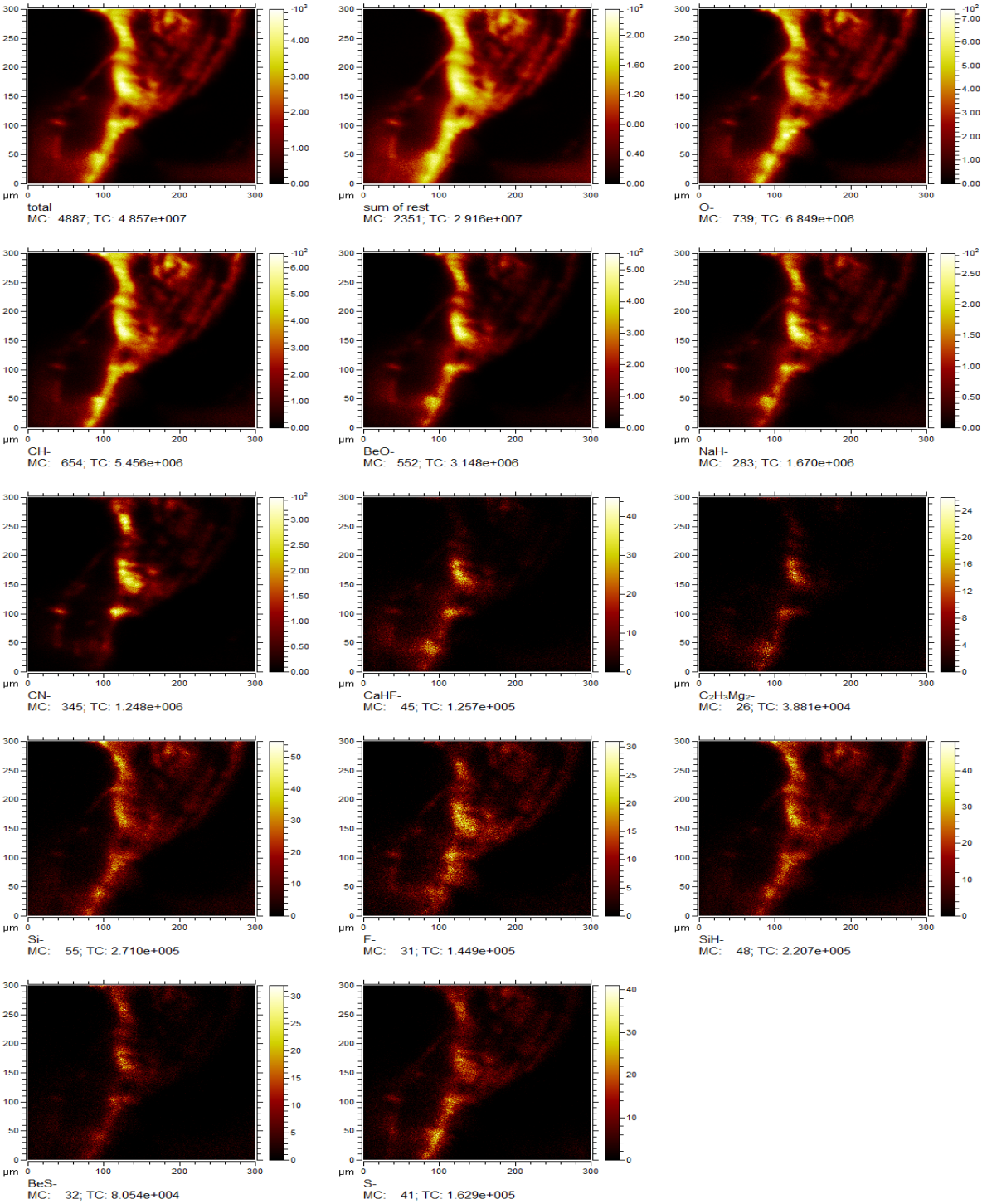


Figure S7: The raw data for 2D-imaged distributions of elements in the CCFS, as obtained using TOF-SIMS. The most abundant elements in the cations and the anions of the CCFS were silicone and oxygen molecules, respectively. The second most abundant cations in a CCFS were calcium ions. Various other elements were detected, as shown in this figure.

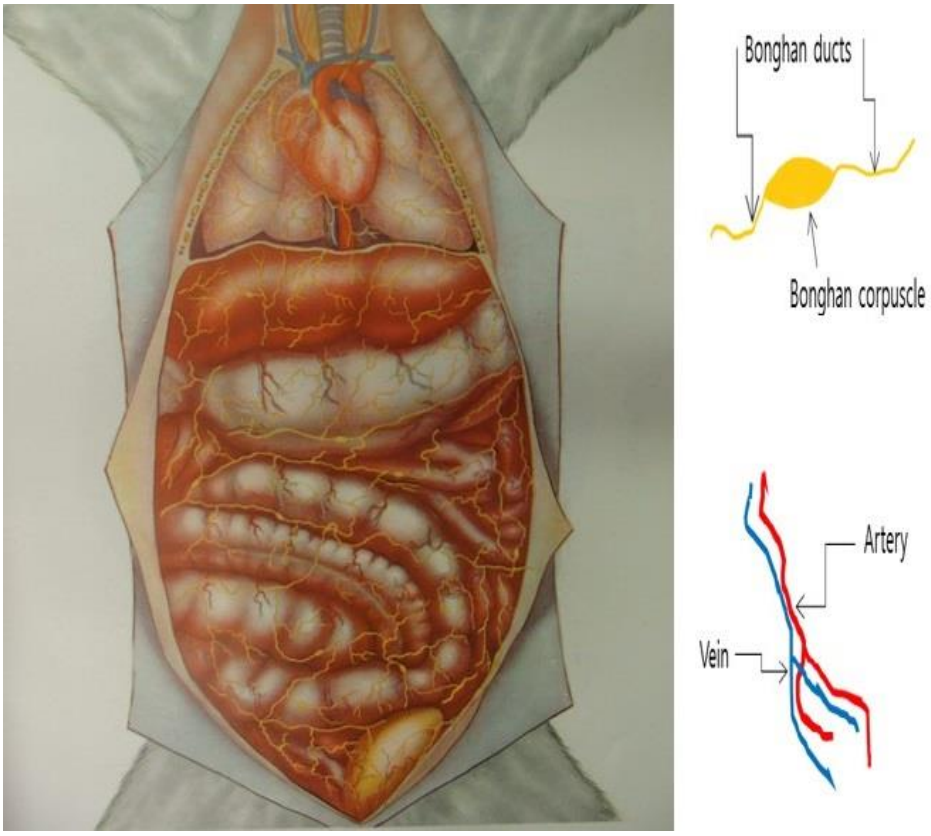


Figure S8: The Bonghan duct system: illustration of mysterious reticular networks (yellow filiform structures with corpuscles) on the internal organs of a rabbit (from the original image presented by Kim Bonghan). The right panel shows enlarged illustrations for the Bonghan duct, Bonghan corpuscle, vein, and artery.

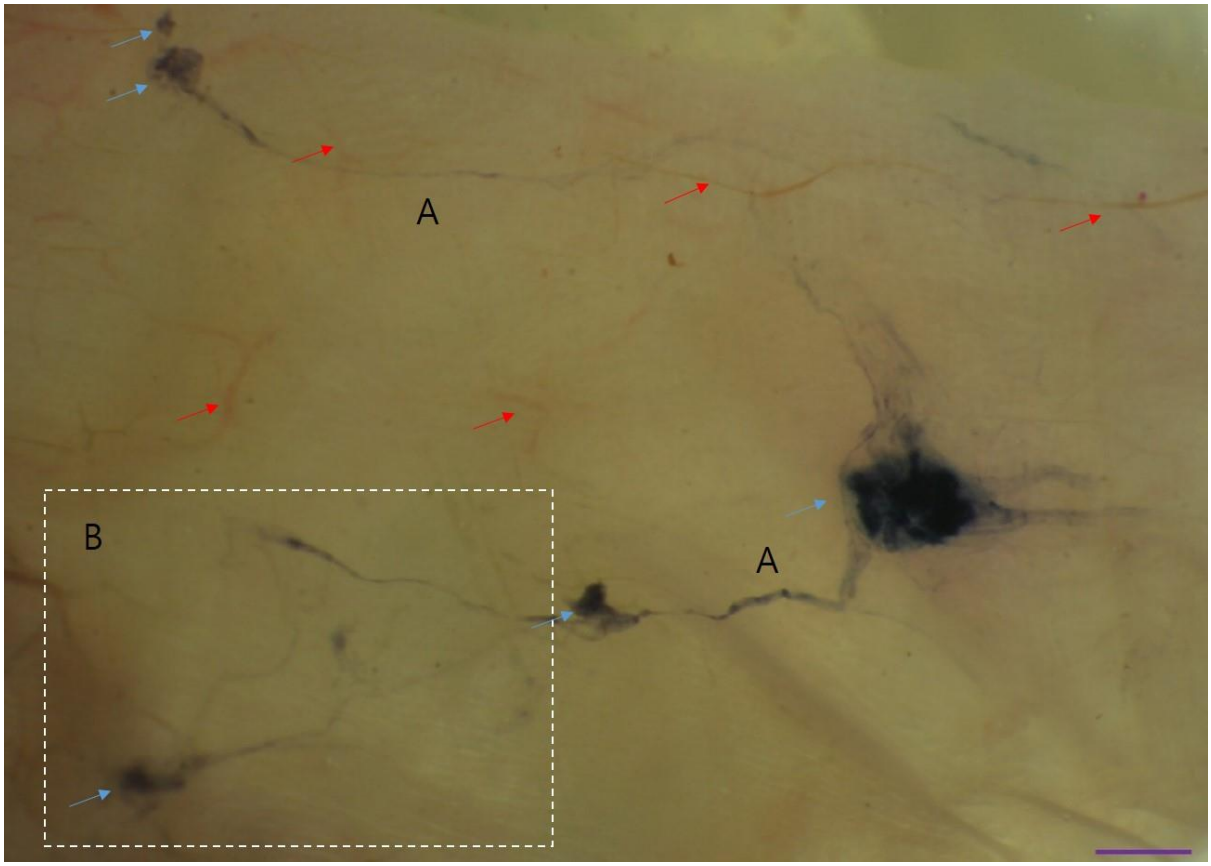


Figure S9: In situ horizontal slices of corpuscle-connected filiform structure (CCFS) visualized on the surface of the large intestine of rats. Note that blood capillaries (red arrows) near to corpuscles (blue arrows) and filiform structures are intact unstained. **A** parts are floating on the surface; however, **B** part of reticular CCFS is embedded (**Video S1**). Scale bar: 500 μm

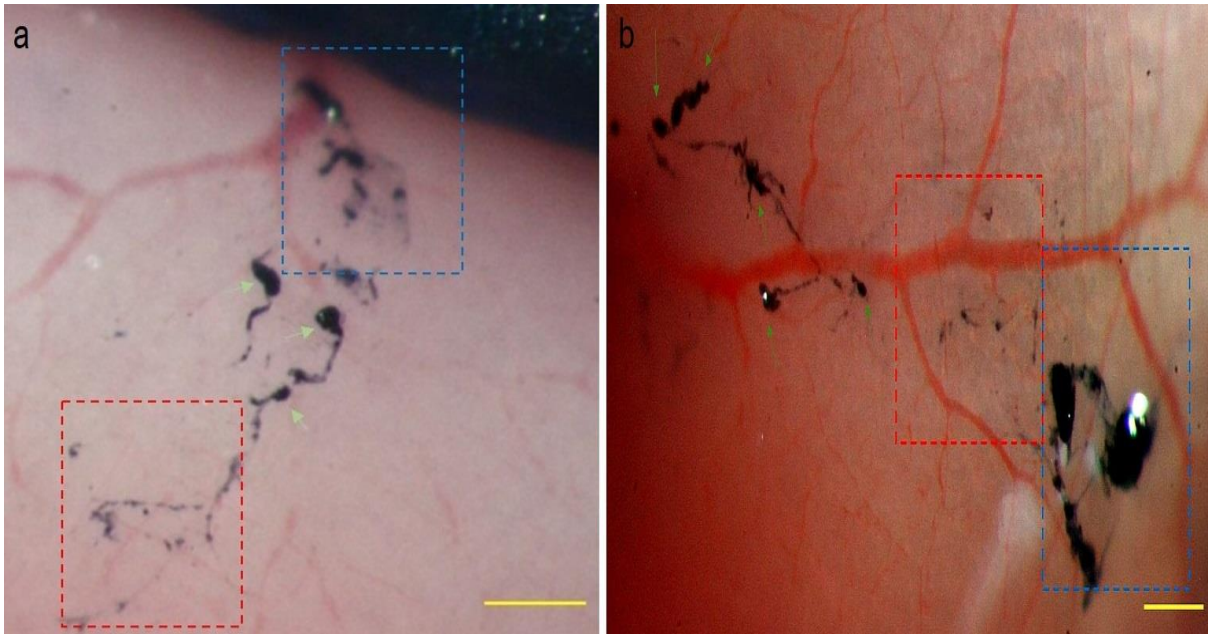


Figure S10: Raw data for stereoscopic microcopy of corpuscle-connected filiform structures (CCFSs) on the small intestine of a rat (a, b): distinctive corpuscular structures (green arrows) are present with filiform structures. Noticeably, the blood capillaries were not stained by JGB. In practice, field workers encounter the images in the blue-dotted and the red-dotted rectangles. The images in the blue-dotted rectangle (a, b) look somewhat bulky with large corpuscles. And those in the red-dotted rectangle (a, b) seem very thin filiform structures with small corpuscles. Scale bar: a: 500 μm, b: 250 μm.

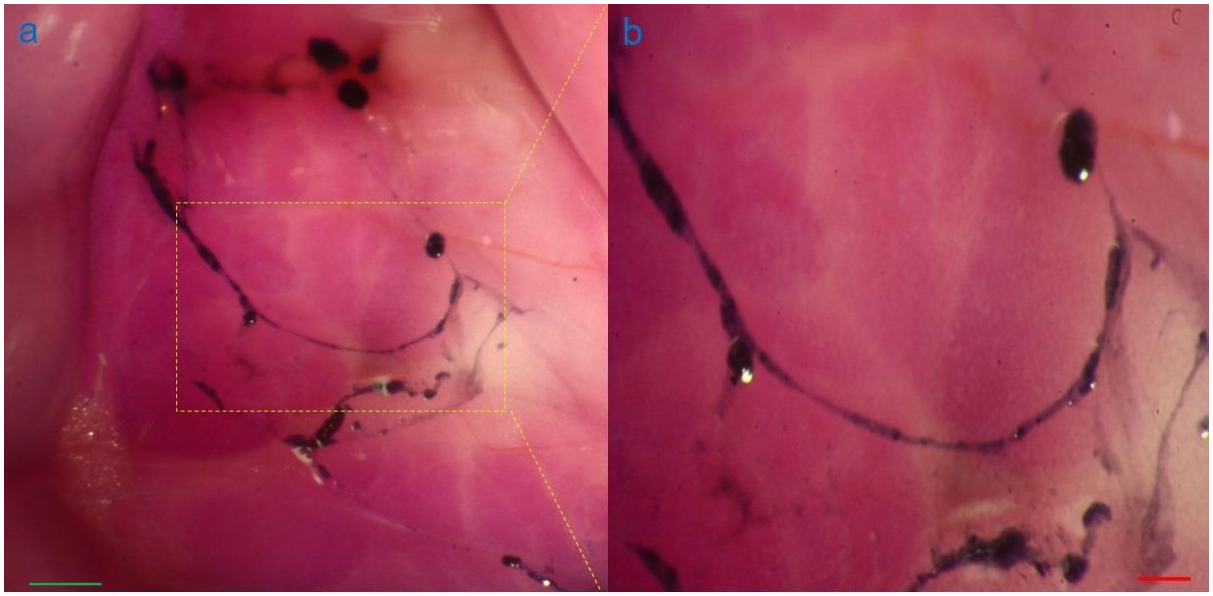


Figure S11: Raw data for stereoscopic microcopy of corpuscle-connected filiform structures (CCFSs) on the mesentery of internal organs of rats. The yellow-dotted rectangle of ‘a’ is magnified into ‘b’. Green scale bar: 500 μm; red scale bar: 200 μm.

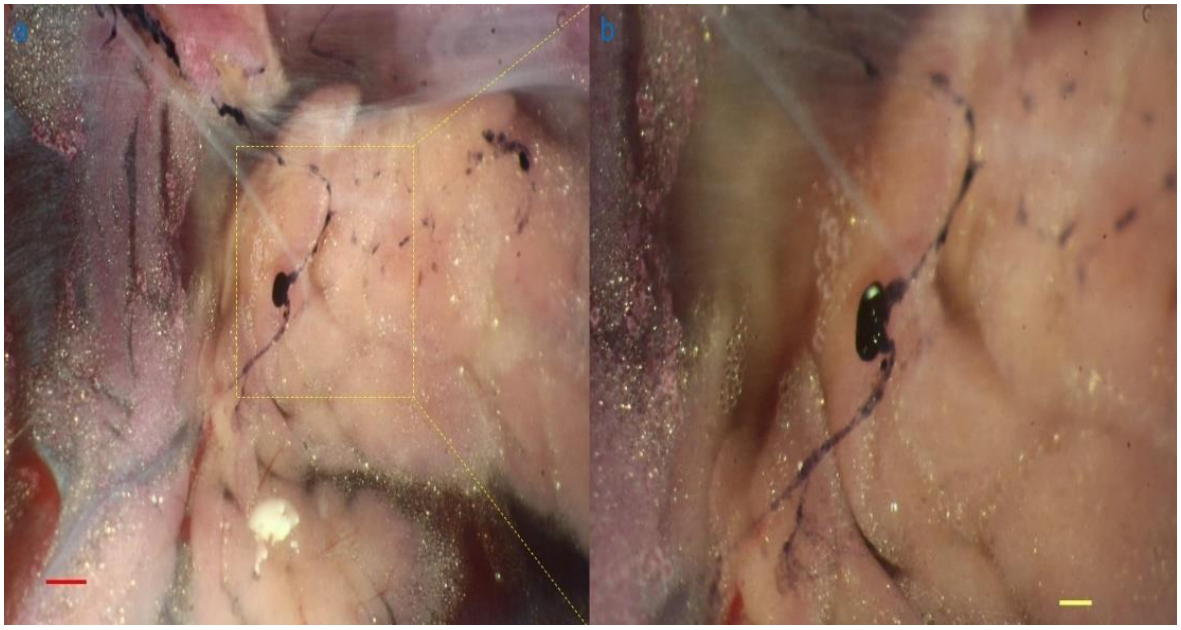


Figure S12: Raw data for stereoscopic microscopy of corpuscle-connected filiform structures (CCFSs) on fat tissues of rats. The yellow-dotted rectangle of ‘a’ is magnified into ‘b’. Red scale bar: 500 μm; yellow scale bar: 200 μm.

Table S1: Size distribution and locations of corpuscular structures in CCFSs in rats based on stereoscopic images

NF: names for the internal organs where the CCFSs were visualized; N: the numbers of CCFSs counted under SM; BC and SC: biggest and smallest corpuscles, respectively, among the CCFS; L and l: longest and shortest diameters of oval and round-shaped corpuscles, respectively; D: distance between two distinctive corpuscles.

Notes: (1) The units for each number are all μm. (2) The initial for each organ is the covering loose connective tissues (fascia) where the visualized CCFSs were entangled.

Abbreviations for the names of the internal organs: Aw (abdominal wall), Dp (diaphragm), Ed (epididymis), Ft (Fat tissue around the caudal vena cava), Li (large intestine), Lu (lung), Lv (liver), Ms (mesentery), Si (small intestine), Ts (testis), Pp (parietal pleura), and Ov (Ovary).

No	Sex	NF(N)	BC: Lxl	SC: Lxl	D	No	Sex	NF(N)	BC: Lxl	SC: Lxl	D
1	M	Aw(8)	821x107	107x71	357	8	F	Dp(10)	267x67	50x33	467
		Li(5)	252x86	60x40	320			Ft(8)	200x167	67x34	1,067
		Lv(5)	250x50	75x50	750			Lv(45)	667x111	56x56	1,111
		Ms(5)	320x180	200x60	1,180			Ms(14)	179x143	71x36	893

		Si(10)	800x80	133x80	133			Ov(10)	233x200	33x33	333
		Ts(5)	459x108	81x41	757			Si(8)	471x59	30x30	647
2	M	Aw(5)	556x333	122x94	1,222	9	F	Aw(15)	500x97	156x97	306
		Lv(4)	442x108	38x38	480			Lv(8)	571x286	71x71	189
		Ms(3)	750x333	192x83	1,550			Ms(5)	167x125	63x63	688
		Si(4)	94x94	73x70	288			Si(6)	250x229	188x63	725
3	M	Ft(2)	816x263	245x105	895	10	F	Si(9)	143x86	71x14	357
4	M	Aw(14)	108x99	54x43	326	11	F	Aw(20)	394x63	31x31	581
		Lv(8)	207x100	67x67	293			Lv(12)	190x133	33x33	600
		Ft(10)	275x91	31x31	544			Si(17)	94x56	42x28	181
5	M	Lv(17)	216x182	53x26	868	12	F	Aw(5)	333x146	42x21	479
		Ft(22)	263x187	33x33	290			Li(12)	313x227	100x67	333

		Si(10)	100x100	33x33	420			Lu(12)	217x139	111x56	556
		Ed(12)	1,656x186	250x125	750			Lv(12)	133x133	48x24	333
6	M	Aw(14)	110x51	26x26	252	13	F	Aw(17)	125x83	42x42	542
		Si(9)	466x308	38x35	380			Si(14)	45x32	32x21	223
7	F	Aw(16)	455x455	100x91	832	14	F	Ft(15)	100x50	33x17	150
		Lv(5)	163x110	30x25	375			Lv(22)	111x56	56x42	833
		Ms(18)	150x108	113x38	300			Si(14)	147x59	44x29	588
		Si(11)	167x167	105x57	145			Pp(6)	88x59	59x29	294

Video S1: Video of corpuscle-connected filiform structure (CCFS) visualized on the surface of the large intestine of rats. This video is associated with **Figure S9**.