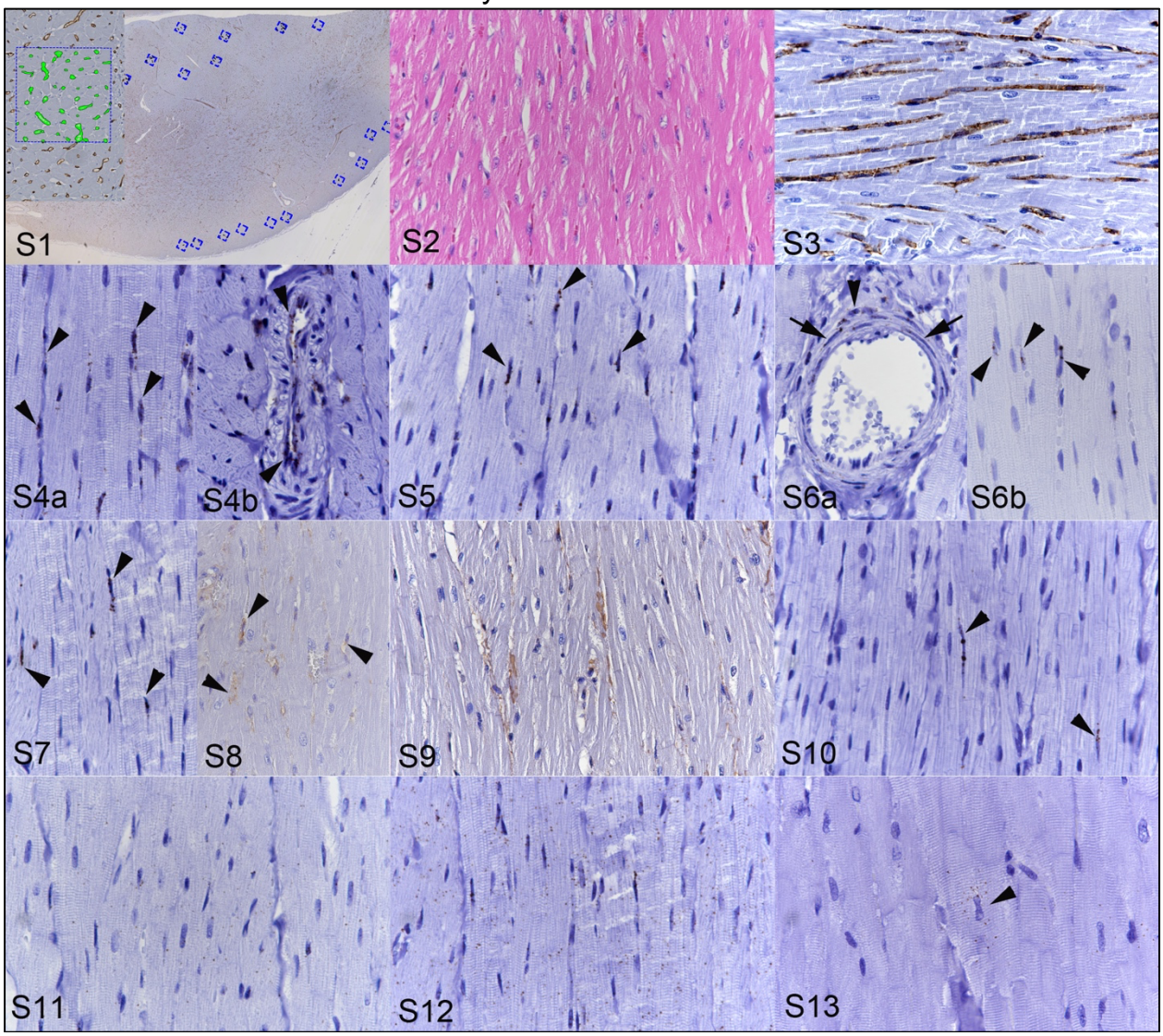


**Supplemental Figures S1-13.** Control heart, left ventricular free wall, cat. **S1.** Case 13. Example of a cross section stained for CD31 subjected to the morphometric evaluation of interstitial capillaries. Inset. Closer view of a region of interest. CD31+ vascular structures are labelled in green. Immunohistochemistry (IHC). **Figure S2.** Case C10, male. Normal myocardium. Hematoxylin eosin (HE). **Figure S3.** Case C10, male. Interstitial capillaries (CD31+) are arranged in parallel to and in tight association with the cardiomyocytes. IHC. **Figure S4.** Case 4, female. CD34 mRNA expression is seen in elongate cells in the interstitium (arrowheads) that are morphologically consistent with endothelial cells of interstitial capillaries (**a**) and of a small myocardial artery (**b**). RNA-in situ hybridization (RNA-ISH). **Figure S5.** Case C4, female. VEGFR2 mRNA expression is seen in spindle shaped cells in the interstitium (arrowheads) that are morphologically consistent with endothelial cells of interstitial capillaries. RNA-ISH. **Figure S6.** Case C4, female. **a.** PDGFRB mRNA expression is seen in medial smooth muscle cells (arrows) and periadventitial cells (pericytes; arrowhead) of a small myocardial artery. **b.** There are also scattered interstitial spindle shaped cells with a PDGFRB signal (arrowheads). RNA-ISH. **Figure S7.** Case C4, female. A few interstitial cells with the morphology of fibroblasts express Col1A1 mRNA (arrowheads). RNA-ISH. (*continued on next page*)

**Supplemental Figures S1-S13** (continued). **Figure S8.** Case C8, male. There are a few interstitial cells with the morphology of fibroblasts that show procollagen I expression (arrowheads). IHC. **Figure S9.** Case C8, male. The interstitium contains variable, but generally small amounts of collagen I. IHC. **Figure S10.** Case C3, female. Rare individual elongate to spindle shaped cells in the interstitium show Kit mRNA expression (arrowheads). RNA-ISH. **Figure S11.** Case C3, female. Cardiomyocytes exhibit a weak MEF2C signal. RNA-ISH. **Figure S12.** Case C4, female. Cardiomyocytes exhibit a weak to moderate CD29 signal. RNA-ISH. **Figure S13.** Case C4, female. Occasional individual cardiomyocytes exhibit a perinuclear Kit signal (arrowhead). RNA-ISH.

**Supplemental Table S1.** Animals with hypertrophic cardiomyopathy included in the study, with information on the cases subjected to RNA-in situ hybridization and transmission electron microscopy.

| Animal No | Breed | Sex | Age (y) |
|---|---|---|---|
| 1[a] | DSH | M | 11 |
| 2[a] | Siberian Forest | M | 1 |
| 3[a] | BSH | MN | 7.5 |
| 4 [a,b] | Main Coon | MN | 8 |
| 5 [a] | ESH | MN | 9 |
| 6 [a] | ESH | MN | 11 |
| 7 [a,b] | Devon Rex | MN | 5 |
| 8 [a] | ESH | F | 10 |
| 9 [a] | Main Coon | MN | 1 |
| 10 [a,b] | BSH | FN | 10 |
| 11 [a] | Main Coon | M | 7 |
| 12 [a,b] | ESH | FN | 11 |
| 13 [a,b] | Chartreux | MN | 15 |
| 14 [a,b] | ESH | MN | 8 |
| 15[b] | ESH | MN | 15 |
| 16 [a] | DSH | MN | 7 |

Abbreviations: BSH, British Shorthair; DSH, Domestic Shorthair; ESH, European Shorthair; F, female; M, male; N, neutered; y, years.
Immunohistochemistry for CD31, Iba1, calprotectin, Ki67, Kit, α-SMA and cleaved caspase 3 was performed in all cases. Staining for collagens was done on selected cases: procollagen I (n=6), collagen I (n=7), collagen IV (n=3).
[a] RNA in situ hybridisation; [b] transmission electron microscopy

**Supplemental Table S2.** Histological features described in the myocardium of cats with hypertrophic cardiomyopathy (HCM) and their presence and extent in the cases included into the present study. Interstitial fibrosis is not listed here, as this was morphometrically assessed (see Table 2).

| **Histological feature** | **Animal number** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
| CM disarray | 0 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 1 | 2 | 2 | 2 | 0 |
| CM degeneration | 2 | 1 | 1 | 2 | 2 | 1 | 3 | 2 | 1 | 2 | 3 | 2 | 3 | 3 | 1 | 1 |
| Cell-rich RF | 2 | 0 | 0 | 2 | 2 | 0 | 3 | 2 | 1 | 0 | 2 | 2 | 2 | 3 | 1 | 0 |
| Arteriosclerosis | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |

CM – cardiomyocyte; CT – connective tissue; Cell-rich RF – cell-rich replacement fibrosis.

CM dissarray: Cardiomyocytes are irregularly arranged, interweaving and branching. 0 – not observed; 1 – disarray of small groups of CM (up to 5 CM); 2 – disarray of large groups of CM (>5 CM)
CM degeneration: Sarcoplasmic swelling and loss of cross striation. 0 – not observed; 1 – degeneration of small groups of CM; 2 – multifocal areas of CM degeneration; 3 – large extensive areas of CM degeneration.
Cell-rich RF: CM loss and replacement by cell rich fibrous connective tissue. 0 – not observed; 1 – replacement of <10% of the CM compartment; 2 – replacement of 10-50% of CM compartment; 3 – replacement of >50% of CM compartment
Arteriosclerosis: thickening of arterial wall due to smooth muscle cell hypertrophy or deposition of collagen with narrowing of the arterial lumen. 0 – not observed; 1 –observed.

**Supplemental Table S3.** Antibodies and immunohistochemical methods

| Antigen | Antibody (clone) | Source | Dilution | Pretreatment | Detection system |
|---|---|---|---|---|---|
| Calprotectin | Mouse mAB (MAC387) | Neomarkers | 1:700 | EDTA | Envision-HRP Mouse[a] |
| CD31 | Rabbit pAb | Santa Cruz | 1:1000 | EDTA | Envision-HRP Rabbit[a] |
| Cleaved caspase 3 | Rabbit pAb | Cell Signaling | 1:400 | CC1 | Discovery OmniMap anti-Rb HRP[b] |
| Collagen I | Rabbit pAb | Abcam | 1:500 | EDTA | Discovery OmniMap anti-Rb HRP[b] |
| Collagen IV | Mouse mAb (CIV 22) | Agilent | 1:30 | Citrate | Envision-HRP Rabbit[a] |
| Iba1 | Rabbit pAb | Wako | 1:750 | Citrate | Envision-HRP Rabbit[a] |
| Ki67 | Mouse mAb (MIB-1) | Agilent | 1:50 | EDTA | MACH 4 Universal HRP-Polymer[c] |
| Kit | Rabbit pAb | Agilent | 1:400 | CC1 | Discovery UltraMap anti-Rb HRP[b] |
| Procollagen I | Rat mAb (M-58) | Abcam | 1:400 | EDTA | Envision-HRP Rabbit[a] |
| α-SMA | Mouse mAb (1A4) | Agilent | 1:400 | n/a | MACH 4 Universal HRP-Polymer[c] |

Abbreviations: anti-Rb, anti-rabbit; CC1, Cell conditioning 1; CD, cluster of differentiation; citrate, citrate buffer pretreatment; EDTA, EDTA pretreatment; HRP, horseradish peroxidase; Iba1, ionized calcium binding adaptor molecule 1; mAb, monoclonal antibody; n/a, not applicable; pAb, polyclonal antibody; α-SMA, α-smooth muscle actin.
[a]Agilent Technologies, Inc.; [b]F. Hoffmann-La Roche Ltd; [c]Biocare Medical, LLC.