

## Supplemental Online Content

Patel H, Sintou A, Chowdhury RA, et al; DIAMONDS consortium. Evaluation of autoantibody binding to cardiac tissue in multisystem inflammatory syndrome in children and COVID-19 vaccination–induced myocarditis. *JAMA Netw Open*. 2023;6(5):e2314291. doi:10.1001/jamanetworkopen.2023.14291

**eTable 1.** Cardiac Donors and Details of Cardiac Tissue Collection and Storage

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**eFigure 2.** IgM Immunohistochemistry Images—Donor A

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**eFigure 6.** IgG Immunohistochemistry Images for Adult Myocarditis/Inflammatory Cardiomyopathy Patients—Donor A

This supplemental material has been provided by the authors to give readers additional information about their work.

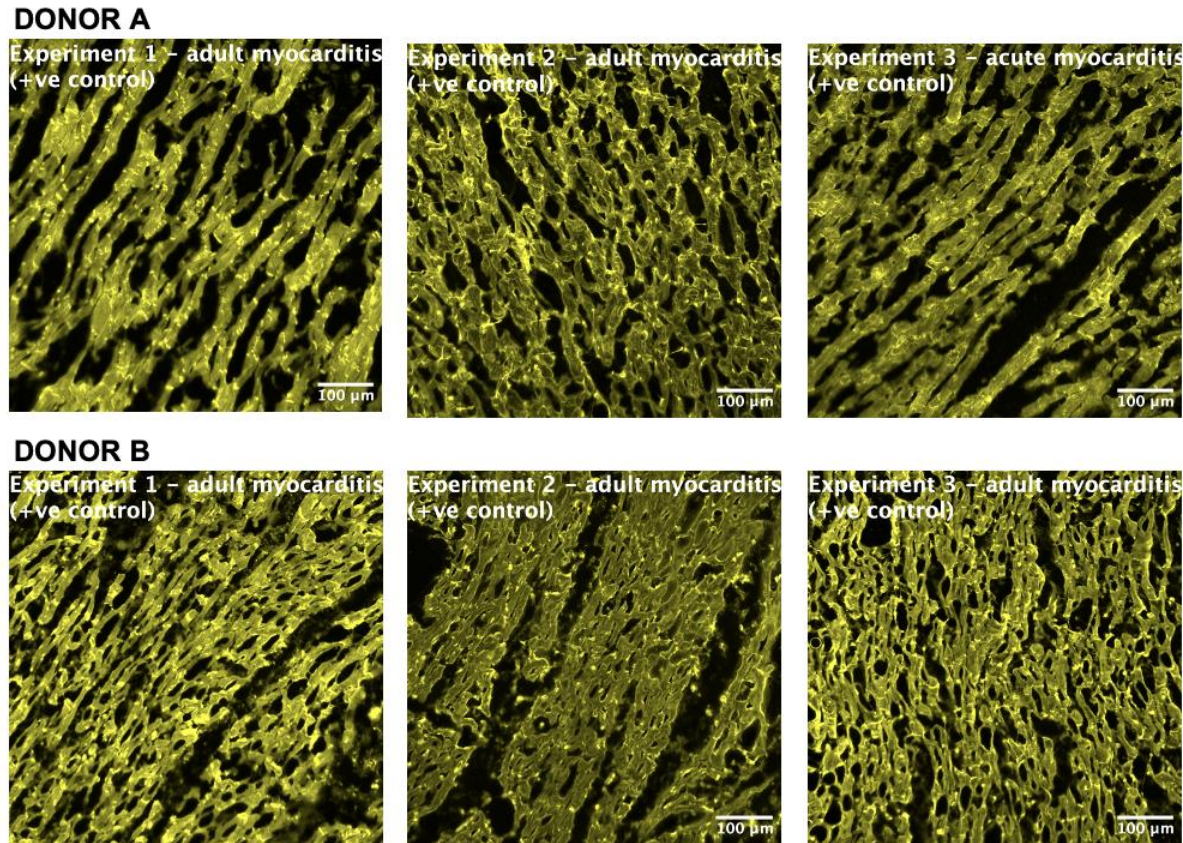
**eTable 1.** Cardiac donors and details of cardiac tissue collection and storage

|   | <b>Donor A</b>  | <b>Donor B</b>   |
|---|---|--|
| <b>Age (years)</b>                                  | 48  | 17   |
| <b>Sex</b>  | Male  | Female   |
| <b>Past medical history</b>                         | Nil recorded  | No history of: HTN/cardi thoracic disease/liver disease/diabetes/smoking/alcohol drinking. |
| <b>Cause of death</b>                               | Hemorrhage  | Status Epilepticus   |
| <b>Reason for unsuitability as transplant organ</b> | Poor function   | Deterioration on organ care system   |
| <b>Sample collection and storage</b>                | The hearts were flash-frozen in liquid nitrogen, then stored at $-80\text{ }^{\circ}\text{C}$ for cryosectioning. Heart tissue was cut into $7\mu\text{m}$ thick sections using a cryostat (CM1850, Leica, Germany) and air-dried for 15 minutes before storing long-term at $-80\text{ }^{\circ}\text{C}$ freezer. |  |

**eTable 2.** Clinical and demographic details of seven adult myocarditis/inflammatory cardiomyopathy cases

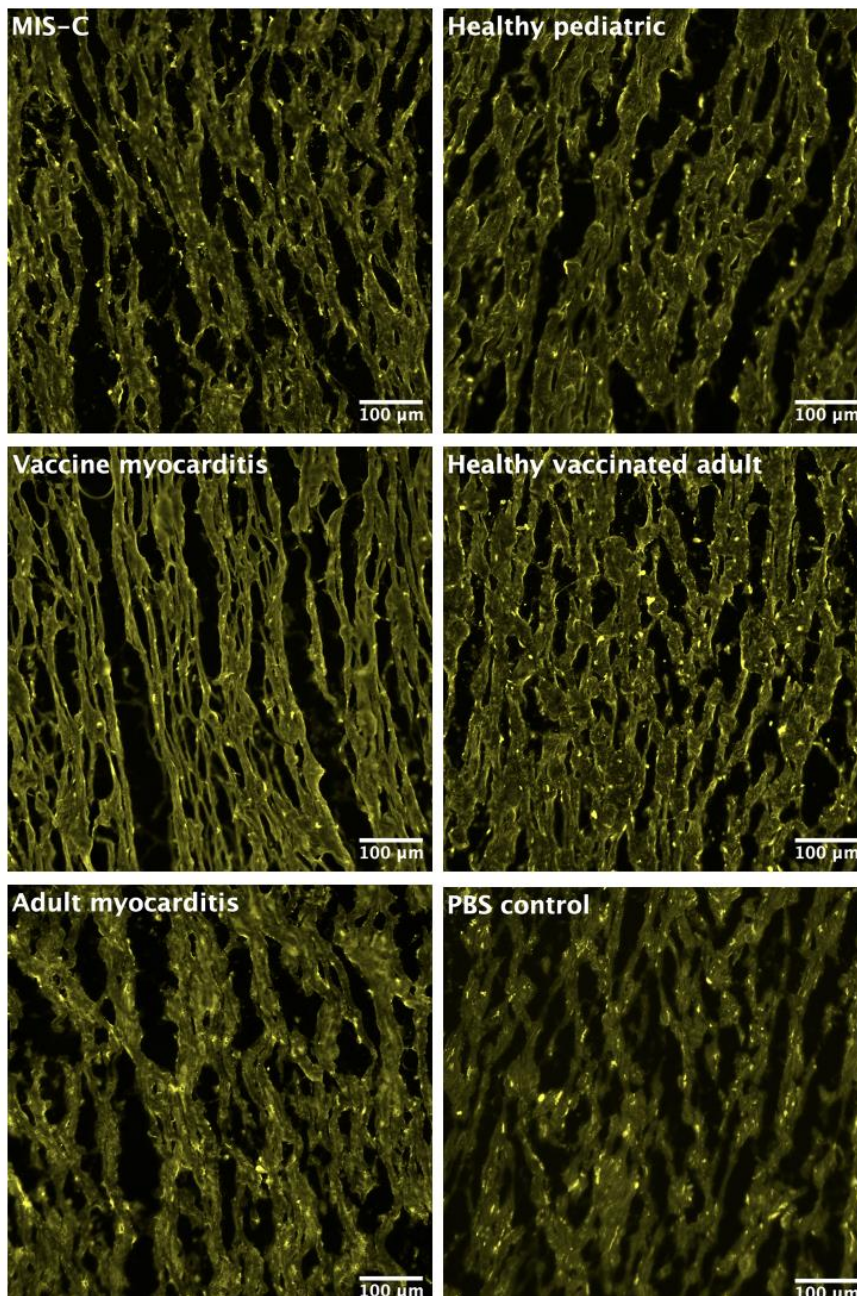
|  | Patient - 1                          | Patient - 2                 | Patient - 3                                      | Patient - 4                                  | Patient - 5                 | Patient - 6                 | Patient - 7                                     |
|--|--------------------------------------|-----------------------------|--|--|-----------------------------|-----------------------------|---|
| <b>Age (years)</b>   | 61                                   | 48                          | 68   | 76   | 48                          | 61                          | 27  |
| <b>Sex</b>   | Female                               | Male                        | Female   | Male   | Male                        | Male                        | Male  |
| <b>Self-reported ethnicity</b>                                       | Caucasian                            | Caucasian                   | Caucasian  | Caucasian                                    | Caucasian                   | Caucasian                   | Caucasian                                       |
| <b>Maximum CRP (mg/L)<br/>[normal range &lt; 3 mg/L]</b>             | 136                                  | 4                           | 14.3   | 270.5  | 3                           | 31.4                        | 56.6  |
| <b>Maximum cardiac Troponin (ng/L)<br/>[normal range:&lt;14ng/L]</b> | 353                                  | 48                          | 610  | 34   | 20                          | 318                         | 2179  |
| <b>Maximum NT-proBNP (ng/L)<br/>[normal range &lt; 50 ng/L]</b>      | 38744                                | 256                         | 824  | 9004   | 8131                        | 208886                      | 266   |
| <b>Ejection fraction on echocardiogram<br/>[normal range 50-70%]</b> | 19%                                  | 40%                         | 59%  | 20%  | 15%                         | 33%                         | 56%   |
| <b>Significant Cardiac Magnetic Resonance Imaging findings</b>       | Not done                             | late gadolinium enhancement | No significant abnormality                       | late gadolinium enhancement, perimyocarditis | late gadolinium enhancement | No significant abnormality  | late gadolinium enhancement, edema, myocarditis |
| <b>Cardiac biopsy diagnosis</b>                                      | inflammatory cardiomyopathy          | inflammatory cardiomyopathy | acute myocarditis                                | inflammatory cardiomyopathy                  | inflammatory cardiomyopathy | inflammatory cardiomyopathy | inflammatory cardiomyopathy                     |
| <b>Final diagnosis</b>   | terminal inflammatory cardiomyopathy | inflammatory cardiomyopathy | Immune Checkpoint Inhibitors-induced myocarditis | inflammatory cardiomyopathy                  | inflammatory cardiomyopathy | inflammatory cardiomyopathy | recurrent myocarditis                           |

**eFigure 1.** IgG Immunohistochemistry images of adult myocarditis (positive control) repeated on 3 separate experiments



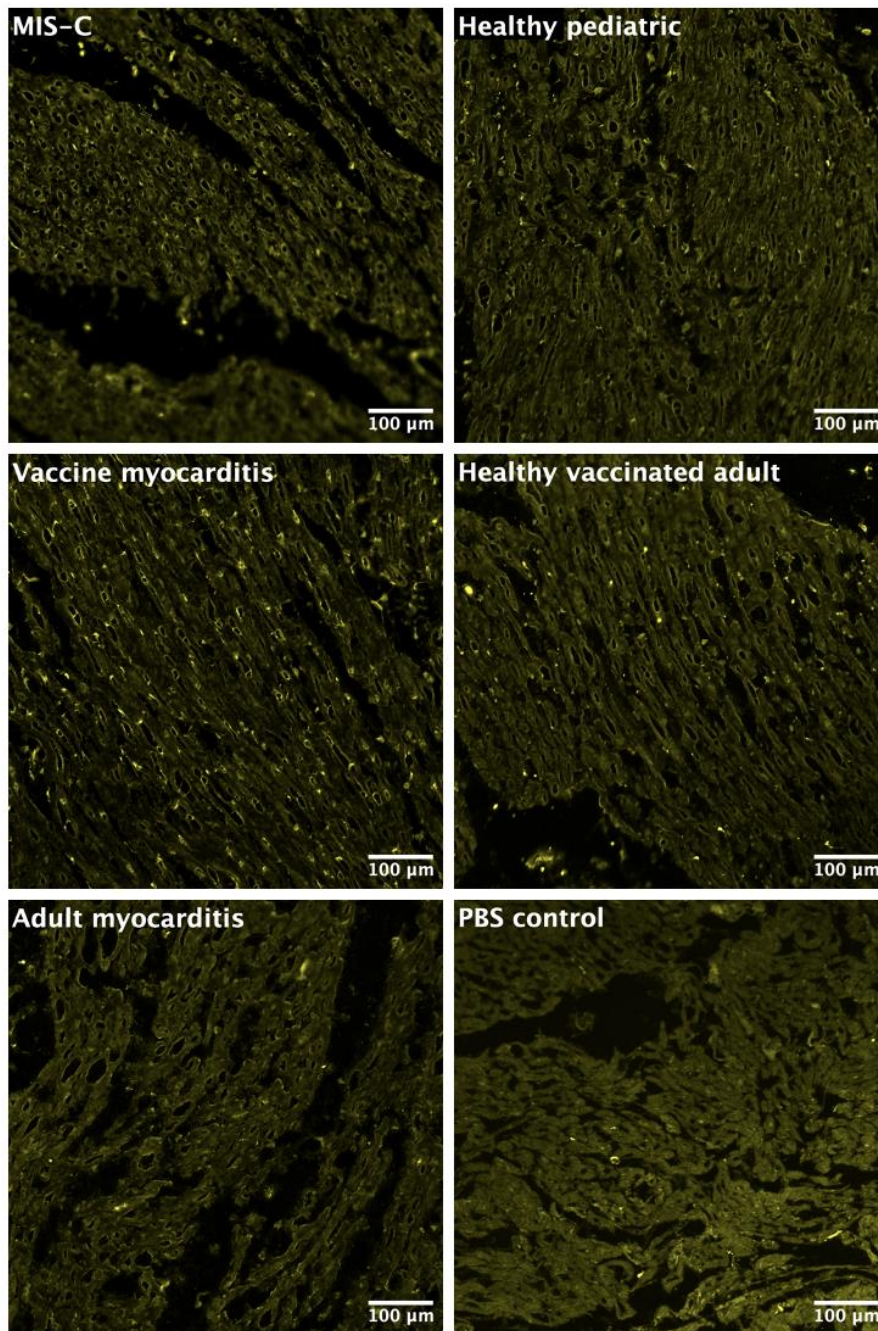
**Supplementary eFigure 1:** Immunohistochemistry images of cardiac tissue (Donor A and Donor B) treated with serum (1:50) from an adult with non-SARS-CoV-2 myocarditis and stained with Fluorescein isothiocyanate (FIT-C) conjugated anti-human IgG, repeated on three separate experiments. Images were taken on widefield microscope at using a 20x 0.8na objective.

**eFigure 2.** IgM immunohistochemistry images – Donor A



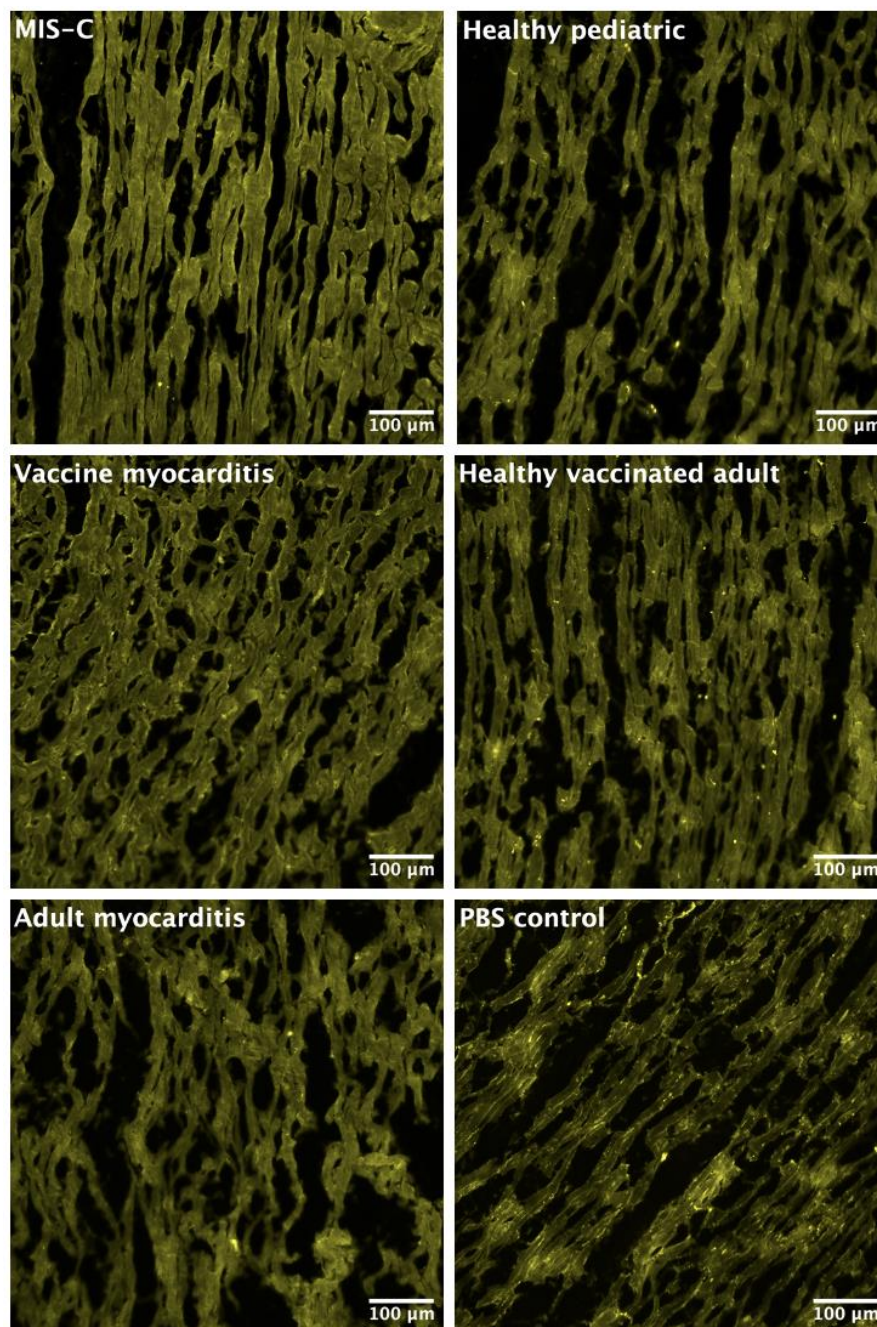
**Supplementary eFigure 2:** Immunohistochemistry images of cardiac tissue (Donor A) treated with serum (1:50) from patients and controls and stained with Fluorescein isothiocyanate (FIT-C) conjugated anti-human IgM. Images were taken on widefield microscope at using a 20x 0.8na objective. An example image from each of the following groups has been shown: Phosphate buffered saline (PBS) – negative control, healthy pre-pandemic paediatric control, healthy adults  $\geq 14$  days following COVID-19 vaccination, COVID-19 vaccine associated myocarditis and Multisystem Inflammatory Syndrome in Children (MIS-C).

**eFigure 3.** IgM immunohistochemistry images – Donor B



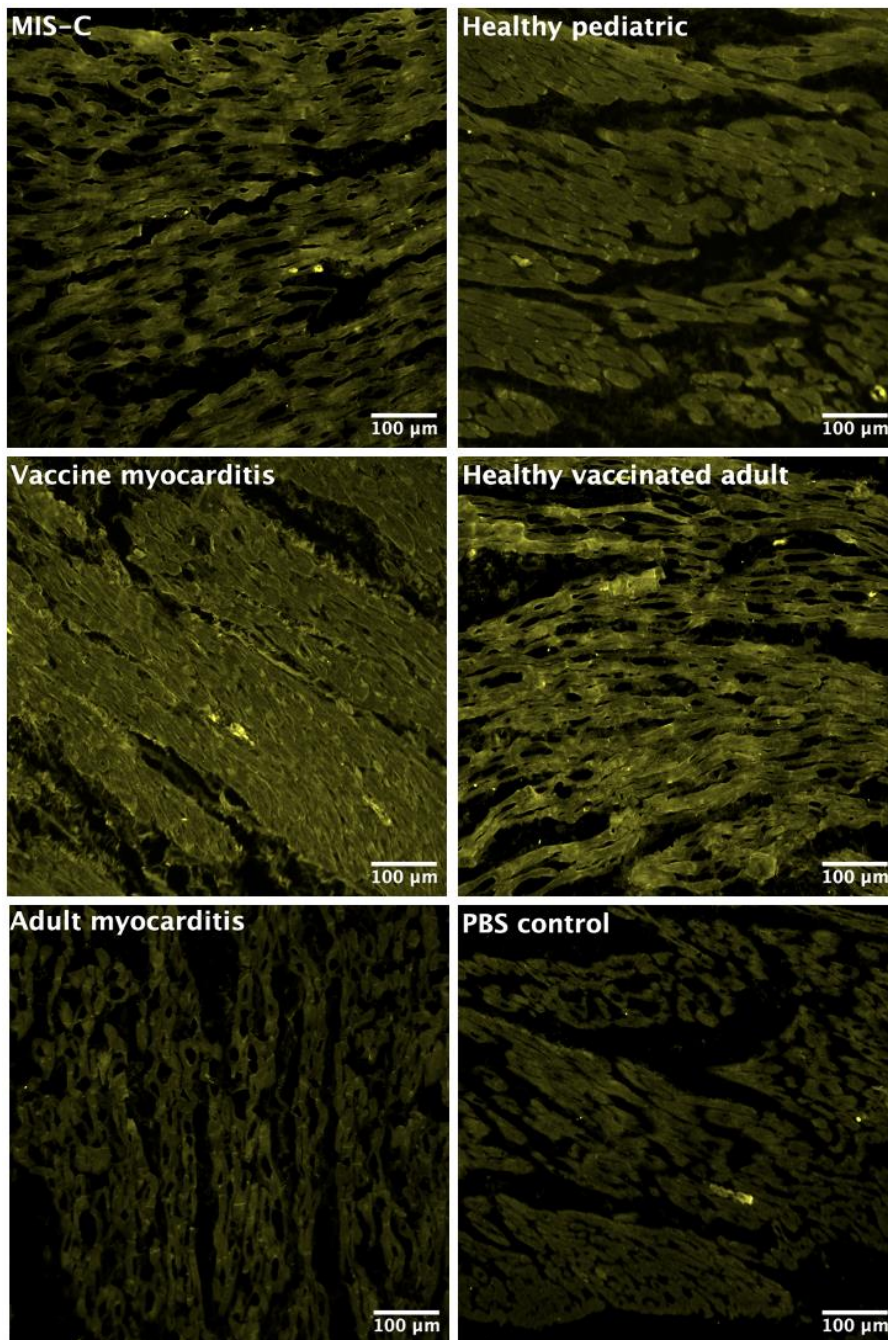
**Supplementary eFigure 3:** Immunohistochemistry images of cardiac tissue (Donor B) treated with serum (1:50) from patients and controls and stained with Fluorescein isothiocyanate (FIT-C) conjugated anti-human IgM. Images were taken on widefield microscope at using a 20x 0.8na objective. An example image from each of the following groups has been shown: Phosphate buffered saline (PBS) – negative control, healthy pre-pandemic paediatric control, healthy adults  $\geq 14$  days following COVID-19 vaccination, COVID-19 vaccine associated myocarditis and Multisystem Inflammatory Syndrome in Children (MIS-C).

**eFigure 4.** IgA immunohistochemistry images – Donor A



**Supplementary eFigure 4:** Immunohistochemistry images of cardiac tissue (Donor A) treated with serum (1:50) from patients and controls and stained with Fluorescein isothiocyanate (FIT-C) conjugated anti-human IgA. Images were taken on widefield microscope at using a 20x 0.8na objective. An example image from each of the following groups has been shown: Phosphate buffered saline (PBS) – negative control, healthy pre-pandemic paediatric control, healthy adults  $\geq 14$  days following COVID-19 vaccination, COVID-19 vaccine associated myocarditis and Multisystem Inflammatory Syndrome in Children (MIS-C).

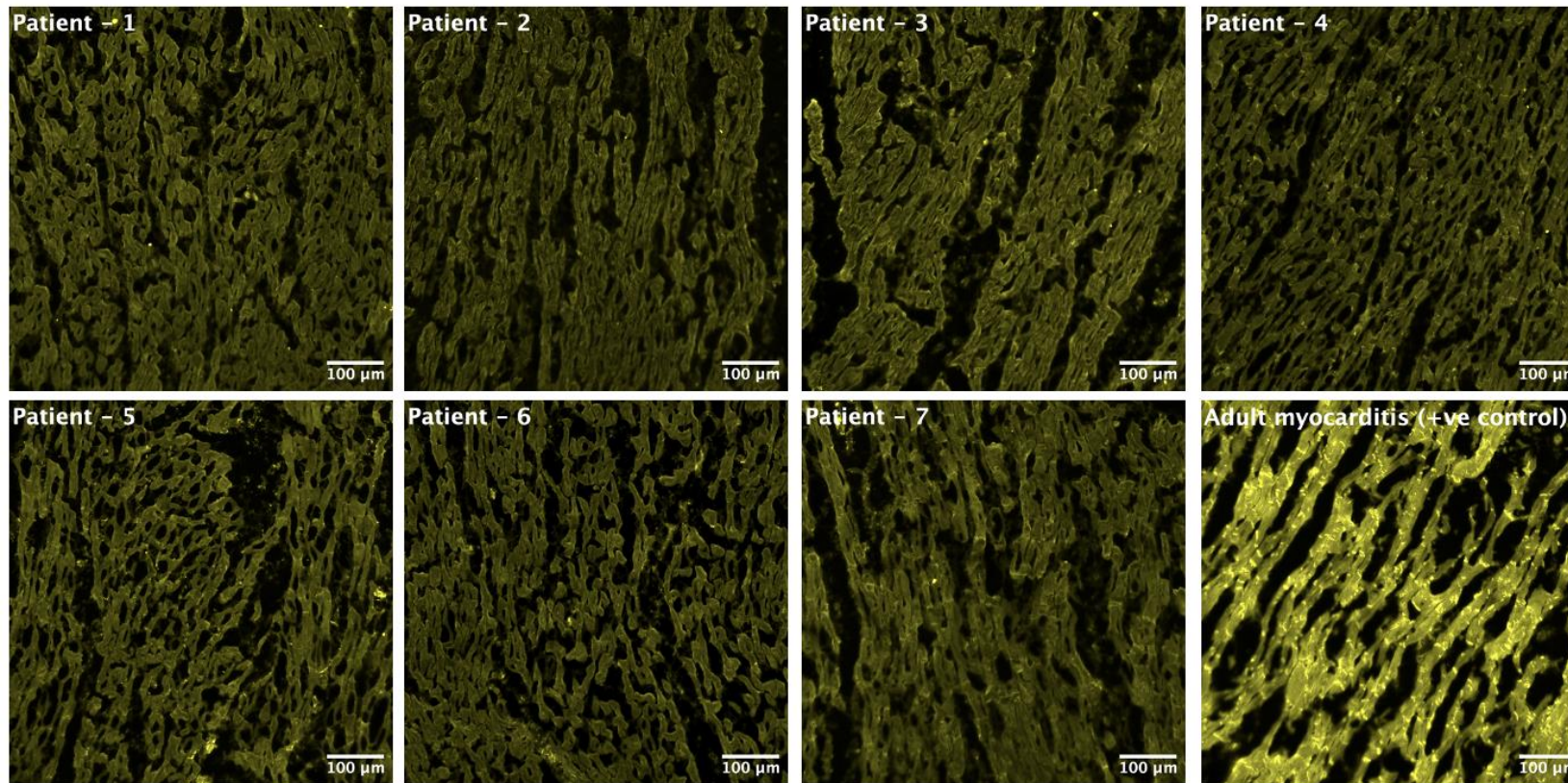
**eFigure 5.** IgA immunohistochemistry images – Donor B



**Supplementary eFigure 5:** Immunohistochemistry images of cardiac tissue (Donor B) treated with serum (1:50) from patients and controls and stained with Fluorescein isothiocyanate (FIT-C) conjugated anti-human IgA. Images were taken on widefield microscope at using a 20x 0.8na objective. An example image from each of the following groups has been shown: Phosphate buffered saline (PBS) – negative control, healthy pre-pandemic paediatric control, healthy adults  $\geq 14$  days following COVID-19 vaccination, COVID-19 vaccine associated myocarditis and Multisystem Inflammatory Syndrome in Children (MIS-C).



**eFigure 6.** IgG immunohistochemistry images for adult myocarditis/inflammatory cardiomyopathy patients – Donor A



**Supplementary eFigure 6:** Immunohistochemistry images of cardiac tissue (Donor B) treated with serum (1:50) from adults with myocarditis/inflammatory cardiomyopathy stained with Fluorescein isothiocyanate (FIT-C) conjugated anti-human IgA. Images were taken on widefield microscope at using a 20x 0.8na objective. An example image from each patient has been shown.