Supplementary Appendix

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

Supplement to: Johnson DB, Balko JM, Compton ML, et al. Fulminant myocarditis with combination immune checkpoint blockade. N Engl J Med 2016;375:1749-55. DOI: 10.1056/NEJMoa1609214

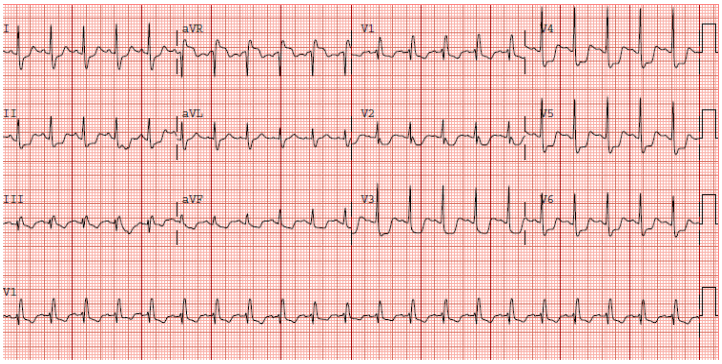
Supplementary Appendix

Supplement to: Johnson DB, Balko JM, Compton ML, et al. Fulminant Myocarditis with Combination Immune Checkpoint Blockade

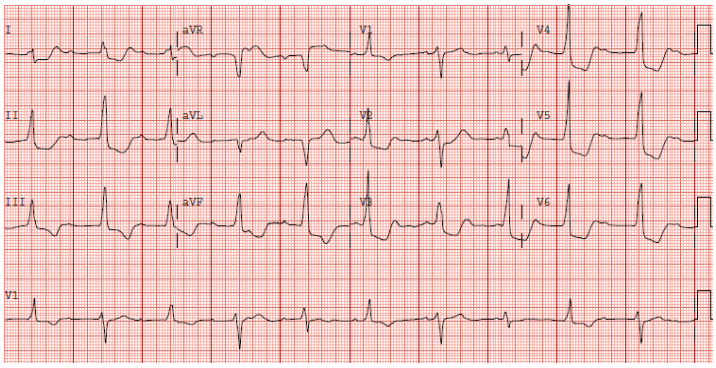
Contents

Figure S1

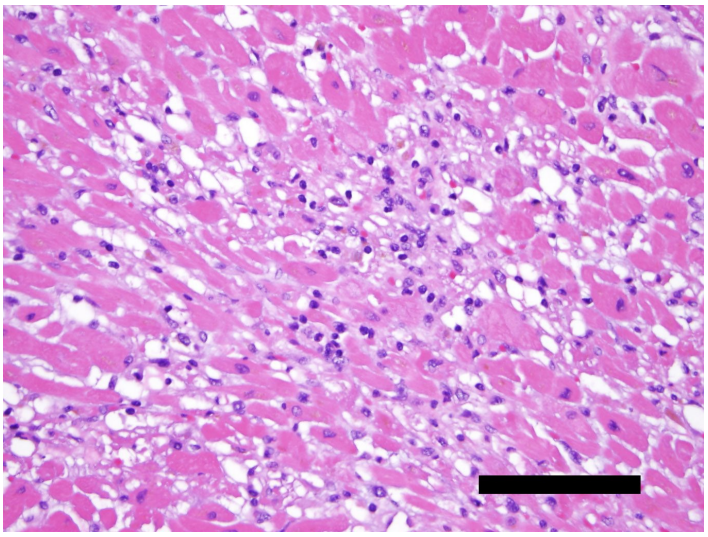
A. ECG with RBBB and ST depression



B. ECG with complete heart block

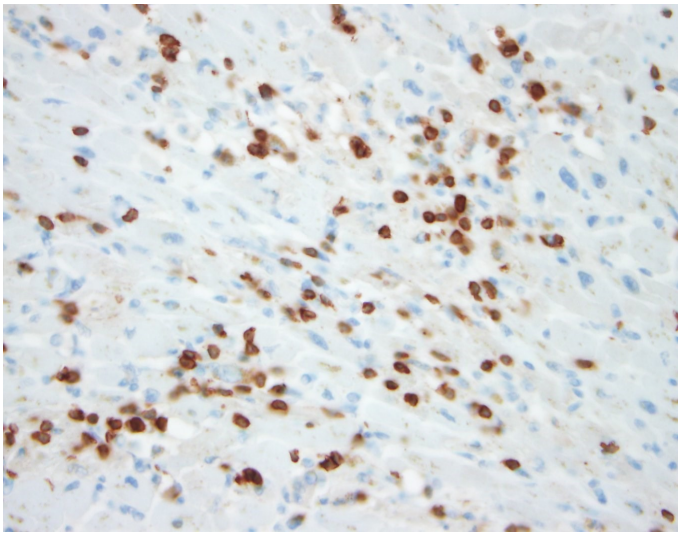


C. H&E, Myocardium

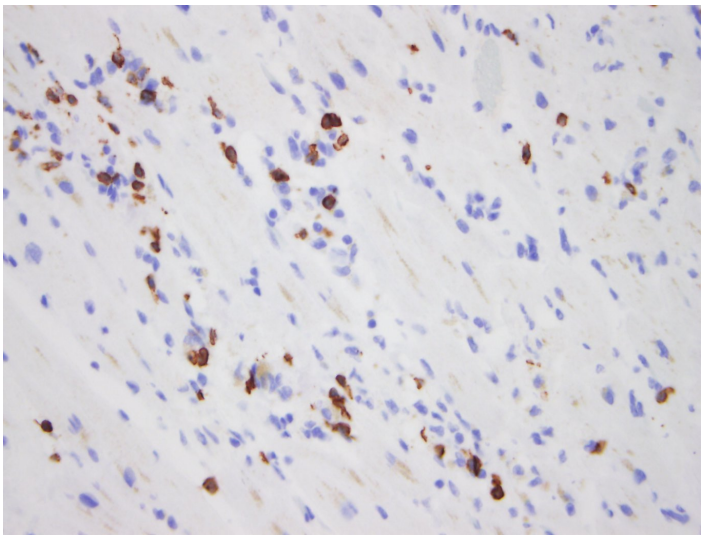


0.1 mm

D. CD3, Myocardium



E. CD8, Myocardium



F. CD68, Myocardium

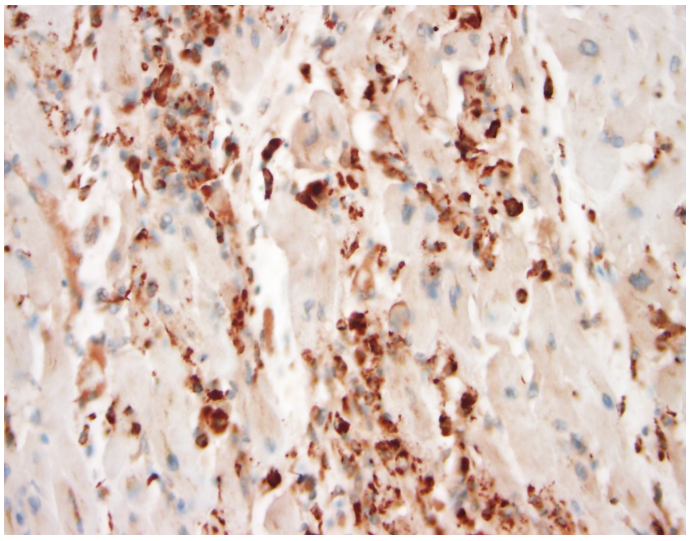
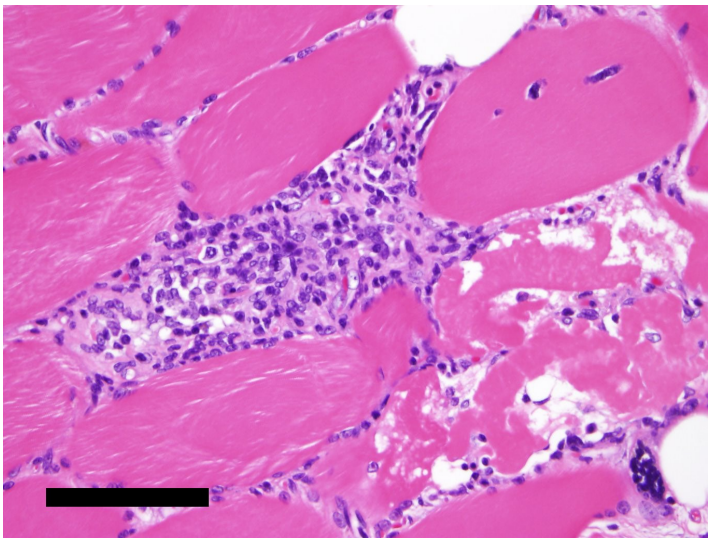
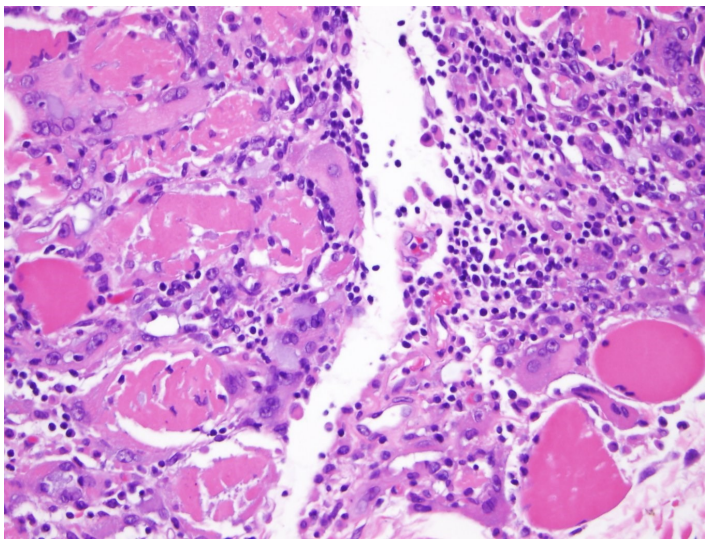


Figure S2

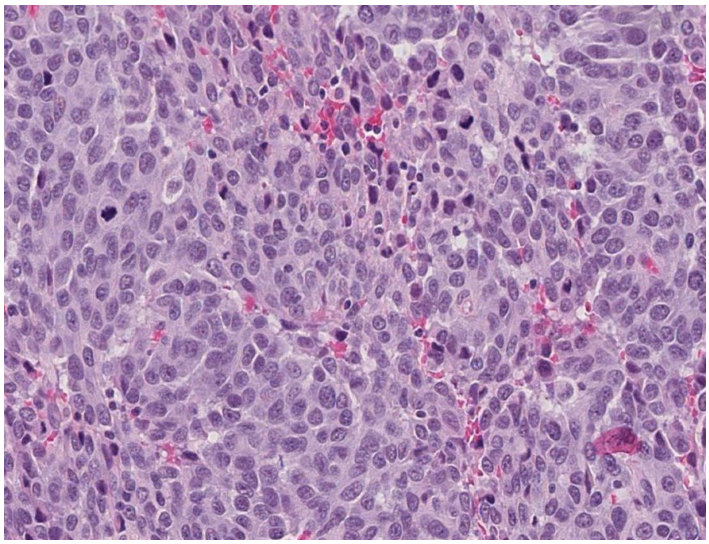
A. H&E, Skeletal muscle, Patient 1



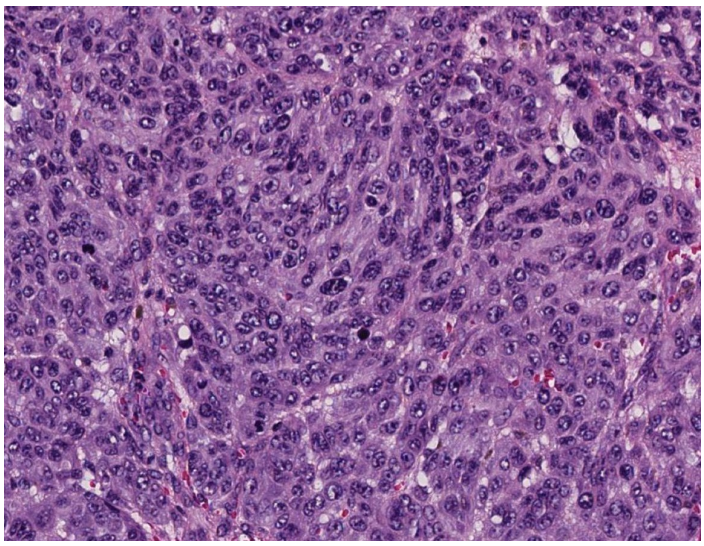
B. H&E, Skeletal muscle, Patient 2



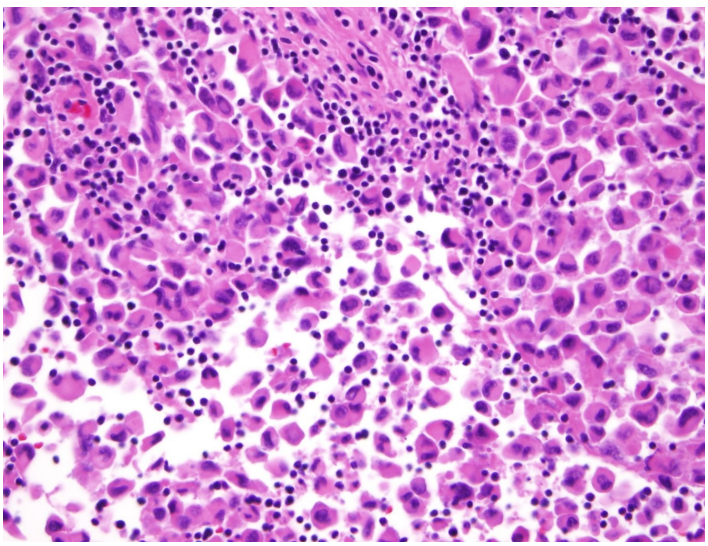
C. H&E, Pre-treatment Tumor, Patient 1



D. H&E, Pre-treatment Tumor, Patient 2



E. H&E, Post-treatment Tumor, Patient 1



F. H&E, Post-treatment Tumor, Patient 2

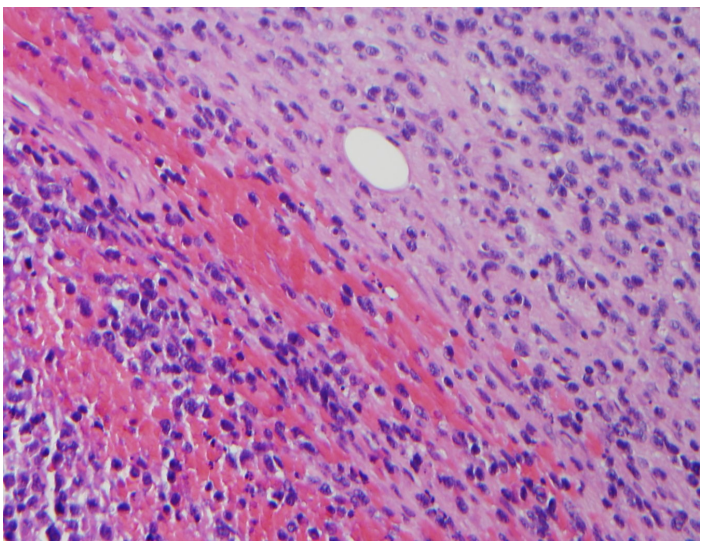
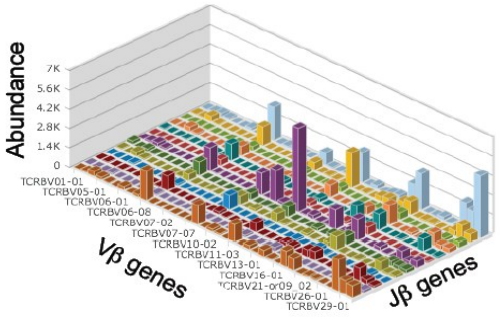


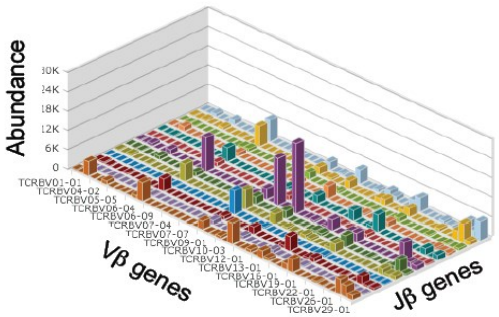
Figure S3

A

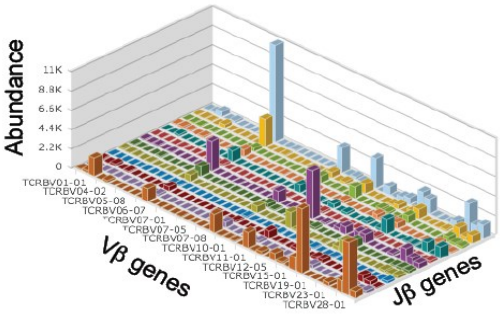
Patient 1 TCR repertoire by V-J gene pairing



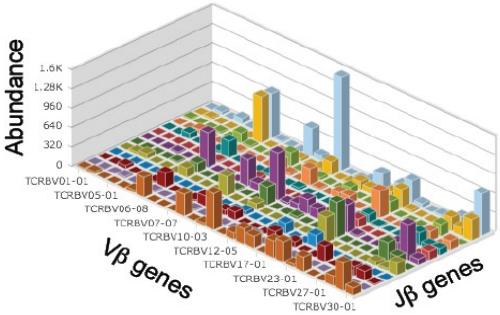
Pre-Treatment Tumor



Post-Treatment Tumor



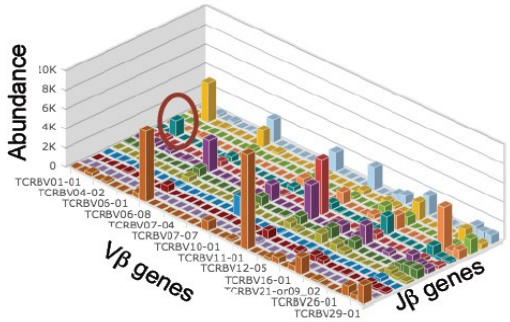
Cardiac



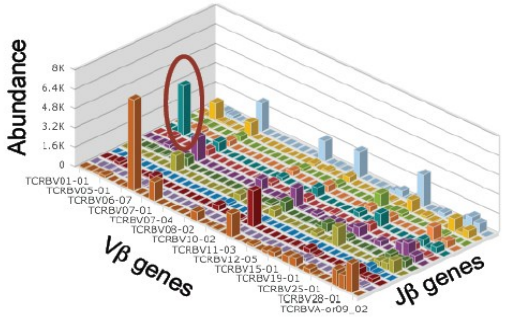
Skeletal muscle

B

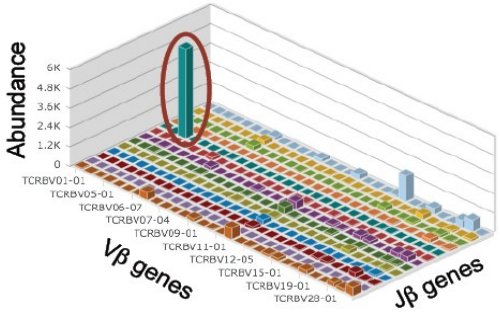
Patient 2 TCR repertoire by V-J gene pairing



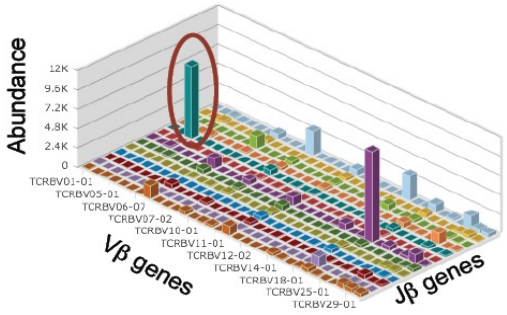
Pre-Treatment Tumor



Post-Treatment Tumor



Cardiac



Skeletal muscle

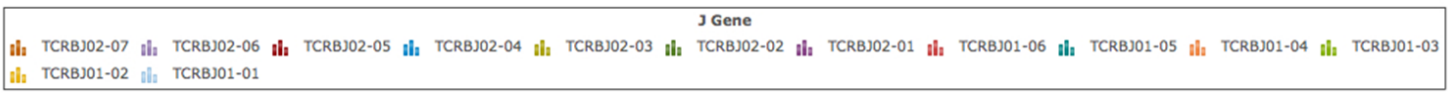
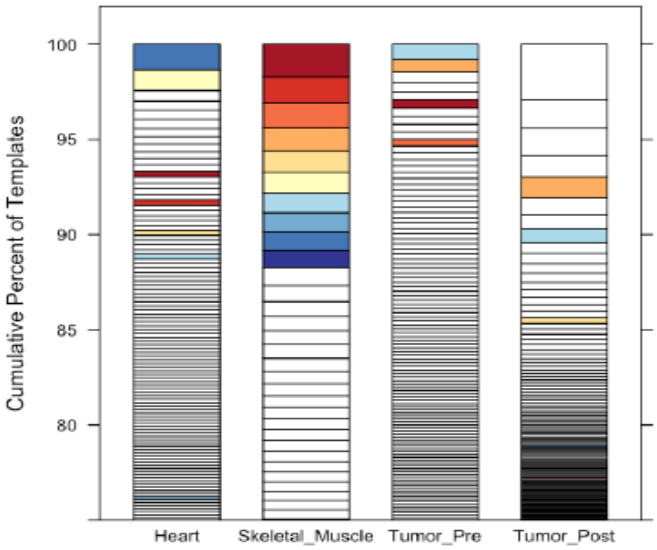
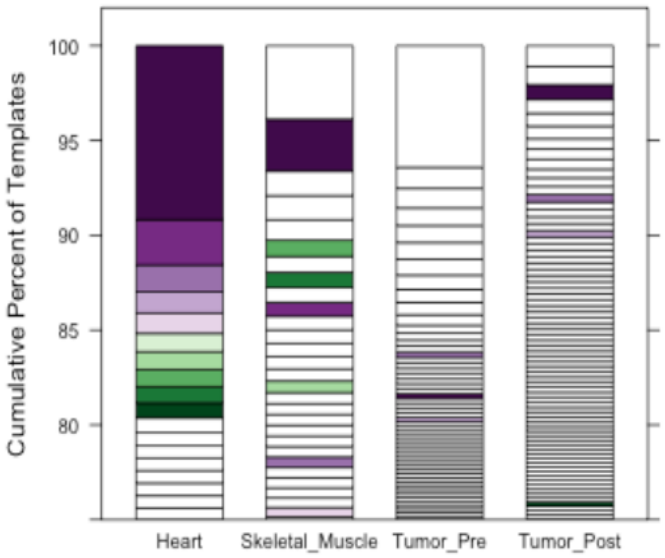


Figure S4

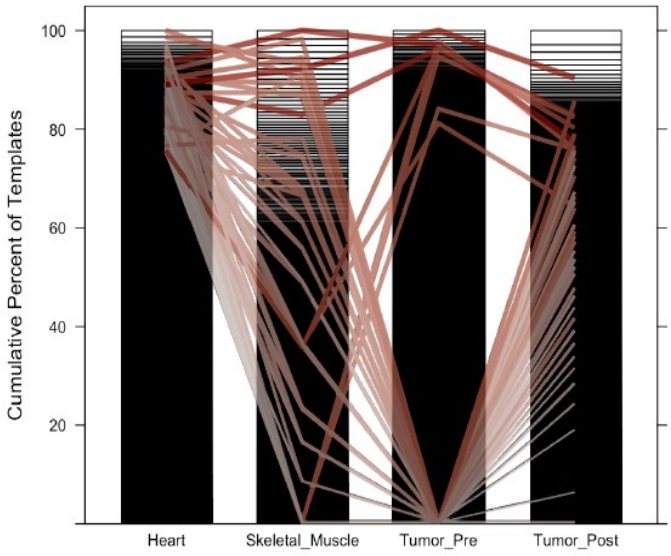
A. Abundant shared T cell clones, patient 1



B. Abundant shared T cell clones, patient 2



C. Tracked T cell clones, patient 1



D. Tracked T cell clones, patient 2

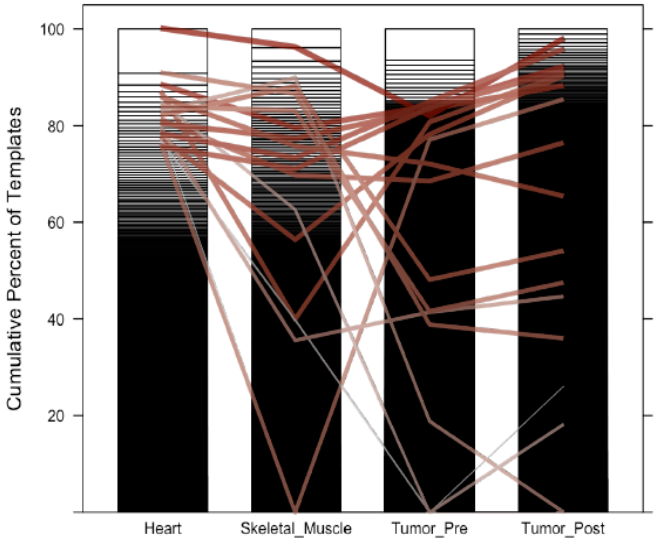


Figure S5

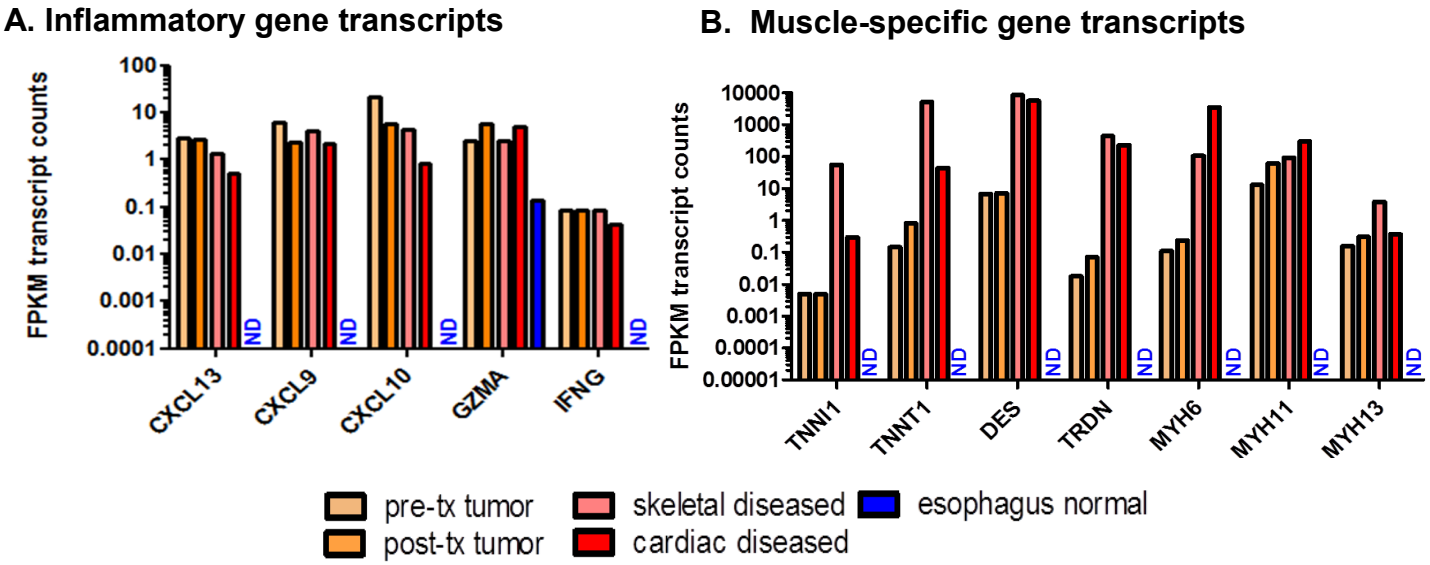
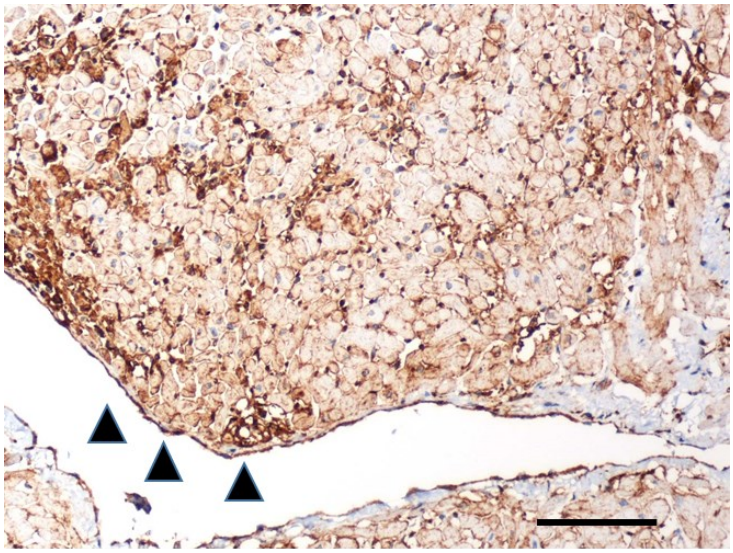

A. Inflammatory gene transcripts
B. Muscle-specific gene transcripts
FPKM transcript counts
100
10
1
0.1
0.01
0.001
0.0001
ND
CXCL13
CXCL9
CXCL10
GZMA
IFNG
FPKM transcript counts
10000
1000
100
10
1
0.1
0.01
0.001
0.0001
0.00001
ND
TNNI1
TNNT1
DES
TRDN
MYH6
MYH11
MYH13
pre-tx tumor
post-tx tumor
skeletal diseased
cardiac diseased
esophagus normal

Figure S6

A. PD-L1 expression, myocardium (200x)



B. PD-L1 expression, myocardium (400x)

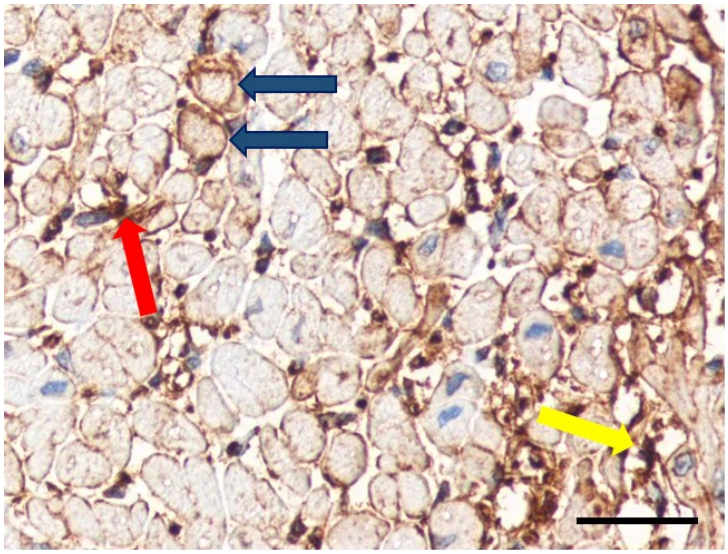


Figure S7

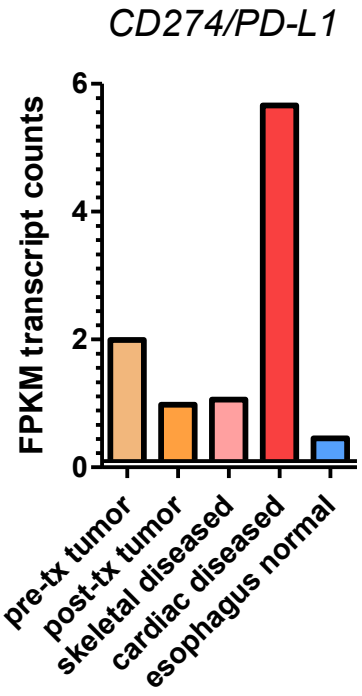

CD274/PD-L1
FPKM transcript counts
6
4
2
0
pre-tx tumor
post-tx tumor
skeletal diseased
cardiac diseased
esophagus normal

Table 1: Clinical characteristics of patients who experienced myocarditis or	
Myocarditis - no. (%)*	(N = 18)
Age – yr	
Median (range)	69 (32 – 86)
Sex - no. (%)[†]	
Female	5 (28)
Male	12 (67)
Cancer type - no. (%)	
Melanoma	9 (50)
Non-small cell lung cancer	3 (17)
Other	6 (33)
Time to Onset - days	
Median (range)	21 (13 – 64)
Pre-existing cardiac disease- no. (%)[‡]	6 (33)
Myositis - no. (%)	(N = 34)
Age – yr	
Median (range)	70 (32 – 90)
Sex - no. (%)[†]	
Female	10 (29)
Male	23 (68)
Cancer type - no. (%)	
Melanoma	15 (44)
Non-small cell lung cancer	12 (35)
Other	7 (21)
Time to Onset - days	
Median (range)	37 (17 – 437)
Receiving anti-cholesterol “statin” drug - no. (%)	5 (15)

Supplementary Figure Legends

Figure S1: Electrocardiographic and immune effects on myocardium following ipilimumab and nivolumab. Patient 2's ECG initially was significant for a right bundle branch block (RBBB) and profound ST depression (Panel A) progressing rapidly to complete heart block (Panel B) followed by ventricular tachycardia and cardiac arrest. Autopsy demonstrated lymphocytic infiltration in myocardium (intraventricular septum pictured, Panel C). Inflammatory infiltrate was comprised of CD3 positive T cells (Panel D), many of which were positive for CD8 (Panel E), as well as CD68 positive macrophages (Panel F).

Figure S2: Immune infiltration in skeletal muscle and tumor. Skeletal muscle at autopsy demonstrated infiltrating lymphocytes in both patients (Panel A, B). Pre-treatment tumor samples had minimal tumor infiltrating lymphocytes (Panels C, D), which increased following treatment (Panels E, F).

Figure S3: T cell receptor V-J pairing and shared clonotypes. The recombined V-J gene pairing for the TCR repertoire infiltrating tumor, cardiac or skeletal muscle shows a diverse TCR repertoire infiltrating the tissue of patient 1 (A) but a highly clonal expansion of TCR using TRBV 4-1 and TRBJ 1-5 (red circle) for patient 2 (B). TRBV = T-Cell Receptor Beta Variable. TRBJ = T-Cell Receptor Beta Joining.

Figure S4: T cell clones shared among the most abundant 25% of TCR sequences between samples are denoted in Patient 1 (Panel A) and Patient 2 (Panel B). Clones are ordered vertically based on decreasing relative abundance; colors denote identical T cell clones shared among tissues. T cell clones most frequent in the myocardium were shared across all affected tissues; select clones expanded between pre- and post-treatment tumor (Panels C and D).

Figure S5: Inflammatory and muscle-specific gene expression across affected tissue. RNA sequencing highlights inflammatory gene expression across affected tissues (Panel A) and muscle transcript expression in affected tissues but not in smooth muscle (Panel B) in patient 2.
Sig = Significant. ND= no transcript reads detected. CXCL13 = C-X-C motif ligand 13. CXCL9 =

C-X-C motif ligand 9. CXCL10 = C-X-C motif ligand 10. GZMA = Granzyme A. IFNG = Interferon gamma. TNNI1 = Troponin I Type 1. TNNT1 = Troponin T Type 1. DES = Desmin. TRDN = Tri-adin. MYH6 = Myosin Heavy Chain 6. MYH11 = Myosin Heavy Chain 11. MYH13 = Myosin Heavy Chain 13. FPKM = Fragments Per Kilobase of transcript per Million mapped reads

Figure S6: Immune characteristics of ipilimumab/nivolumab myocarditis and myositis. PD-L1 expression was identified on the myocardium. PD-L1 IHC was performed as previously described[1] with modifications (clone SP142 as the primary antibody and pH 6.0 for antigen retrieval), 200x (Panel A) and 400x (Panel B) original magnifications; black triangles show endomyocardium labeling for PD-L1; blue arrows show injured myocytes with cell surface / membranous PD-L1 staining, red arrow shows PD-L1+ inflammatory cell, yellow arrow shows a microvessel with PD-L1 expression.

Reference 1: Taube JM, Anders RA, Young GD, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Science translational medicine 2012;4:127ra37.

Figure S7: PD-L1 transcript expression in pre/post-treatment tumor, cardiac, skeletal, and smooth muscle.

Supplementary Video 1: Echocardiogram (apical four chamber view) of patient 1 demonstrating normal biventricular cardiac function 13 days after first dose of ipilimumab and nivolumab (1 day after hospital admission).

Supplementary Video 2: Echocardiogram (short axis view) of patient 2 demonstrating normal biventricular cardiac function 15 days after first dose of ipilimumab and nivolumab (the day of hospital admission).