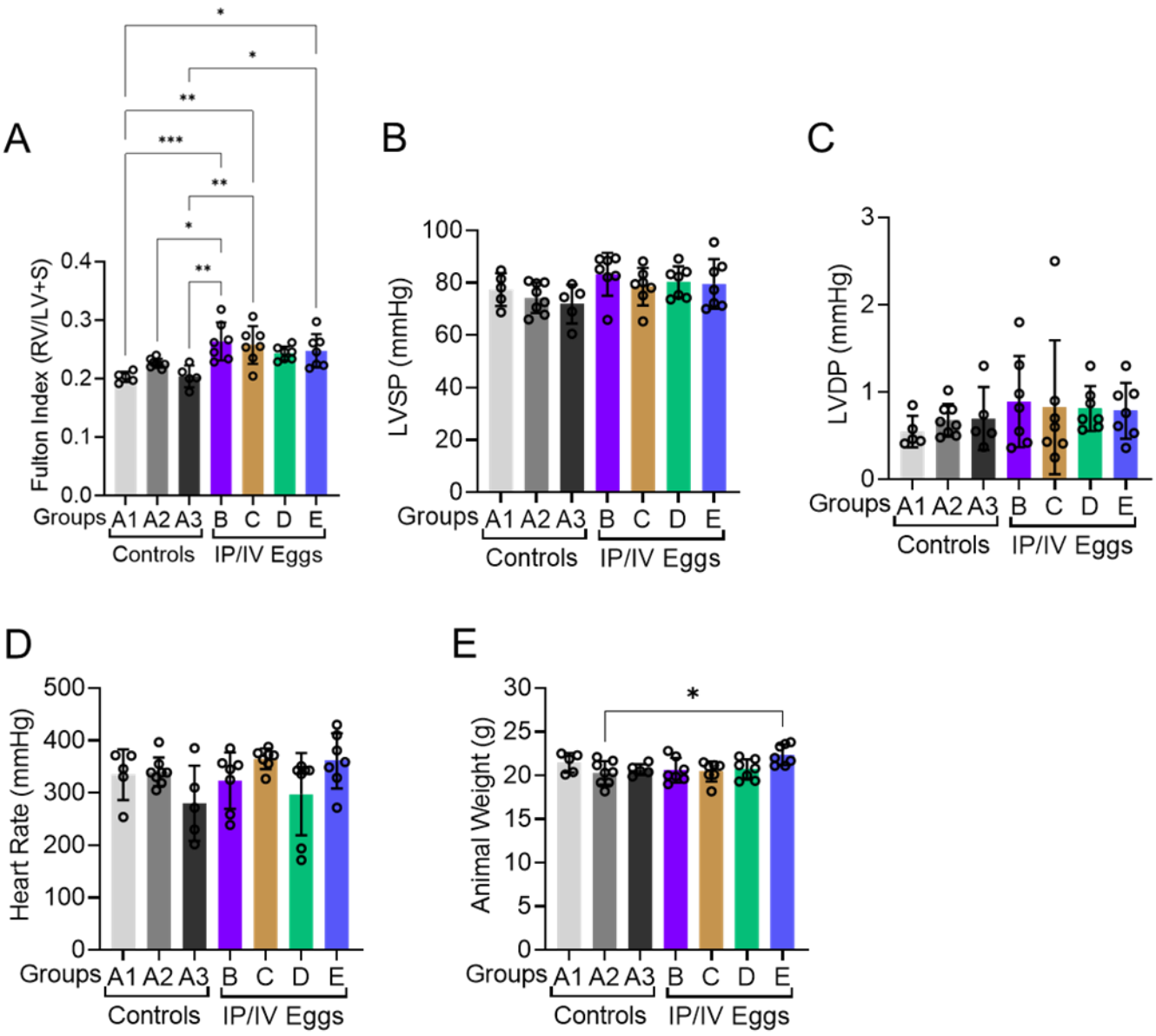
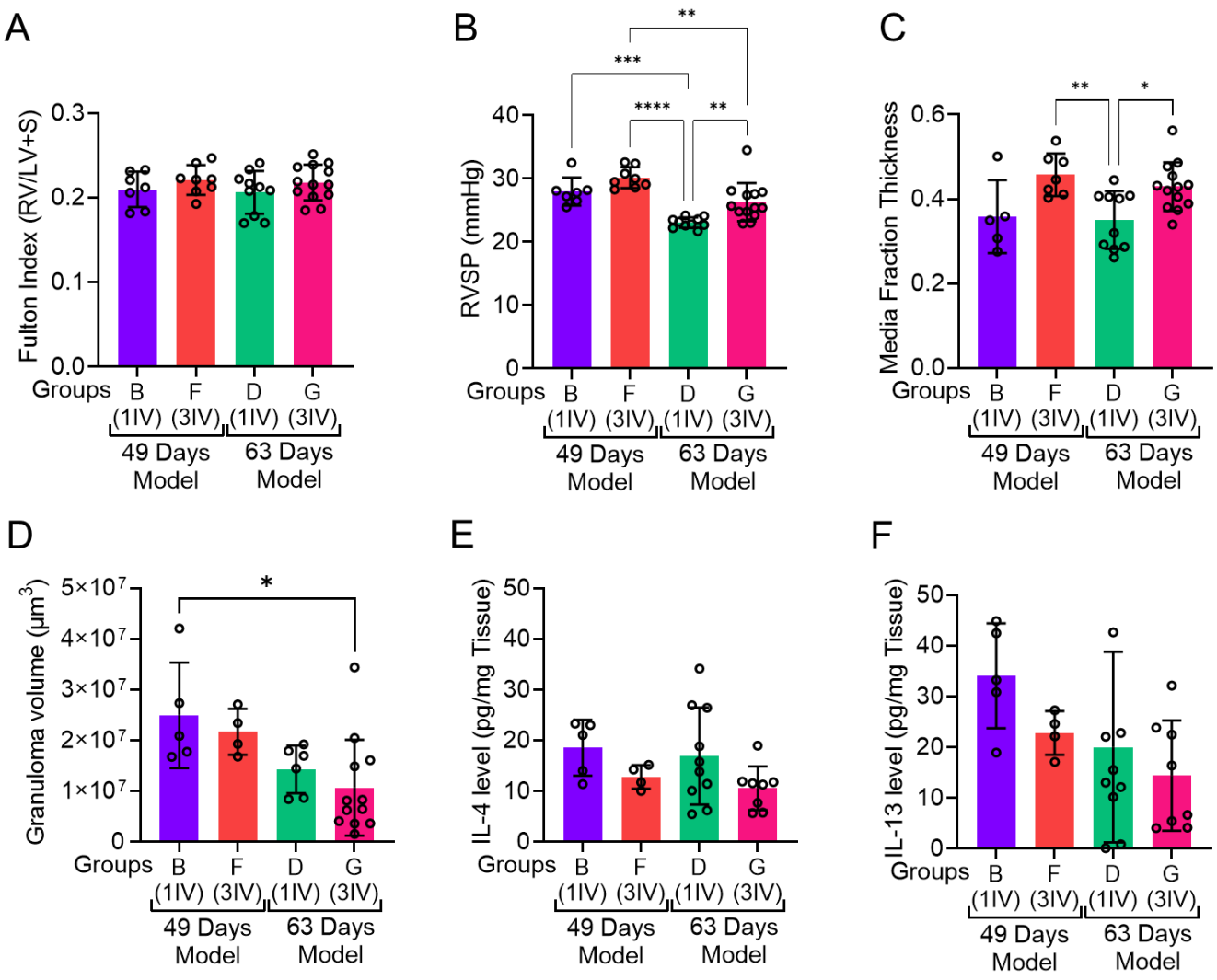
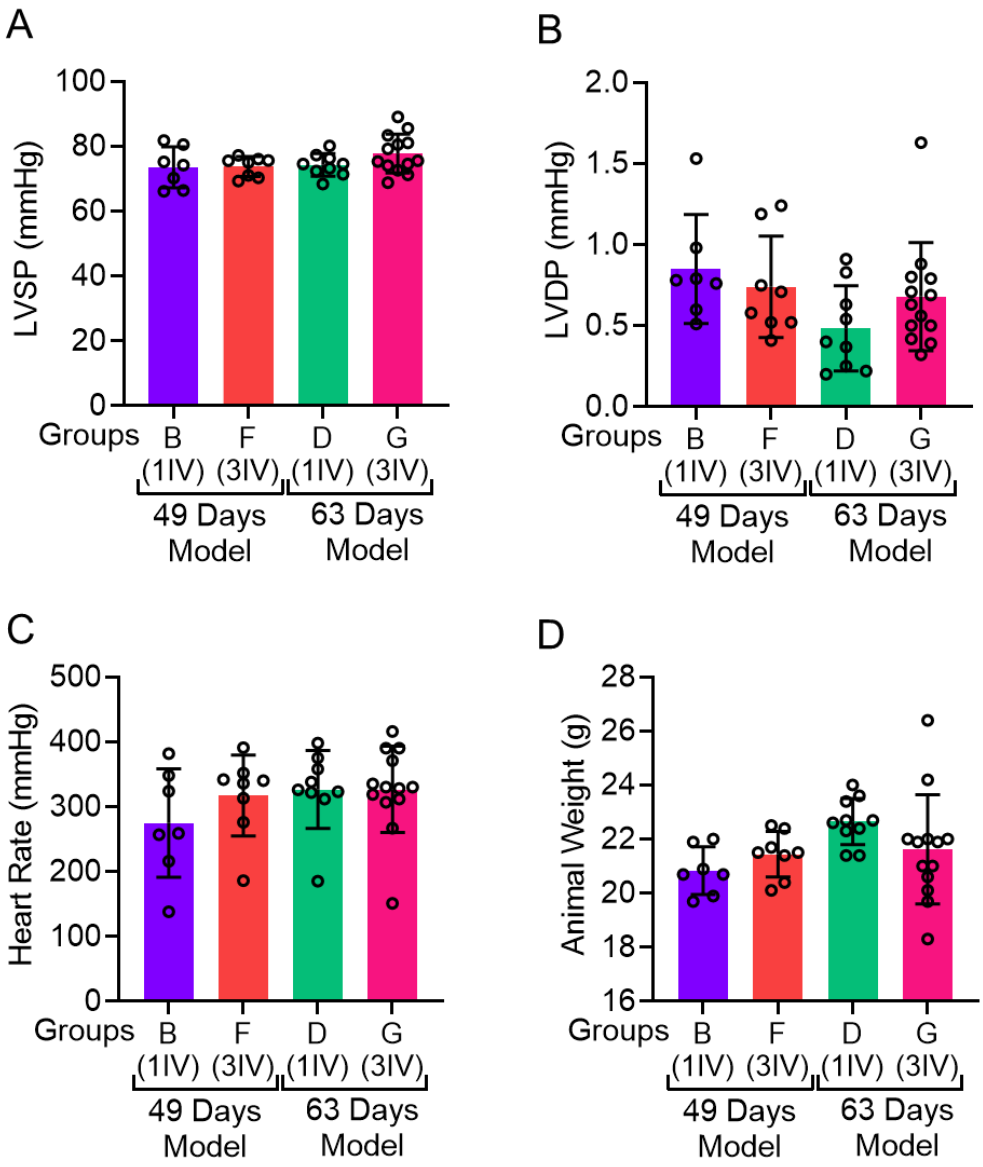


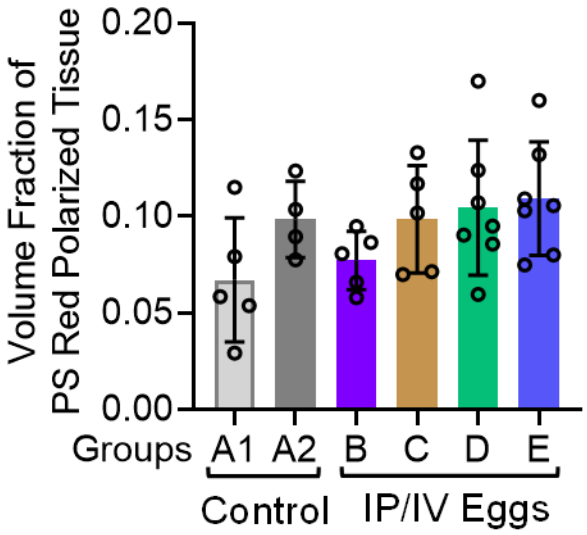
Supplementary Figure 1. The timecourse following a single intravenous dose of *Schistosoma* eggs reveals an increase in right ventricle hypertrophy, but no change in left heart hemodynamics. (A) Right ventricular hypertrophy as measured by Fulton index: the ratio of the mass of the right ventricle (RV) divided by the combined mass of the left ventricle (LV) and septum (S). (B) Left ventricle systolic pressure (LVSP). (C) Left ventricle diastolic pressure (LVDP). (D) Heart rate (HR). (E) Animal body weight. See **Figure 1A** for the group nomenclature. Mean ± SD plotted; analysis of variance (ANOVA) $P<0.001$ for Fulton index with post hoc Tukey tests shown; *$P<0.05$, **$P<0.01$. RV: right ventricle; LV: left ventricle; S: septum; IP: intraperitoneal *Schistosoma* eggs; IV: intravenous *Schistosoma* eggs.



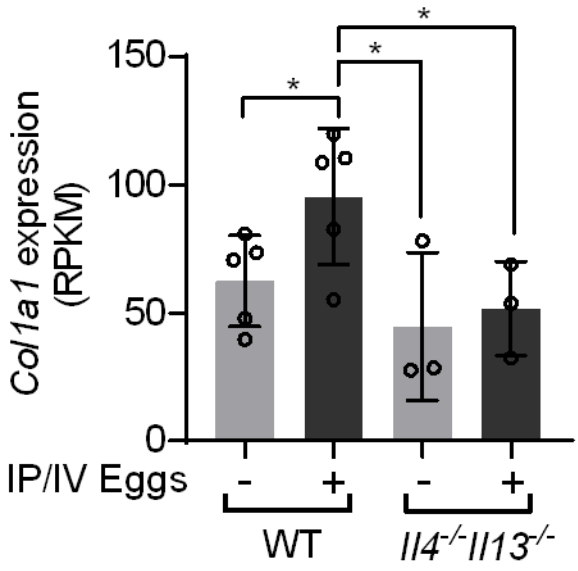
Supplementary Figure 2. Pathology of multiple *Schistosoma* challenged mice. (A) Right ventricular hypertrophy as measured by Fulton index. (B) Right ventricular systolic pressure (RVSP). (C) Quantitative fractional thickness of the pulmonary vasculature. (D) Peri-egg granuloma volumes. (E) IL-4 and (F) IL-13 protein concentrations by ELISA. See **Figures 1A and 2A** for the group nomenclature. Mean ± SD plotted; t tests shown; **P*<0.05, ***P*<0.01, ****P*<0.005, *****P*<0.001. IP: intraperitoneal *Schistosoma* eggs; IV: intravenous *Schistosoma* eggs.)



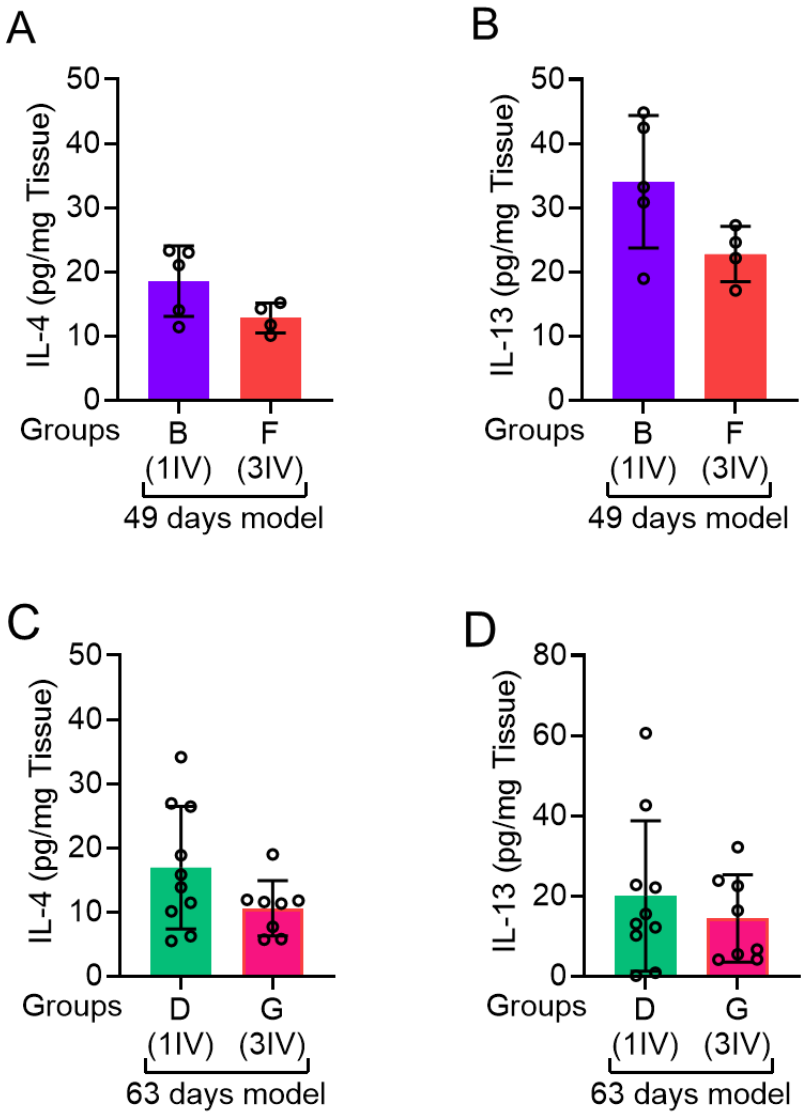
Supplementary Figure 3: The persistent pathology after repeated *Schistosoma* doses is not confounded by changes in left heart hemodynamics or body weight. (A) Left ventricular systolic pressure (LVSP). (B) Left ventricular diastolic pressures (LVDP). (C) Heart rate (HR). (D) Animal body weight. See **Figure 2A** for group nomenclature. Mean ± SD plotted; IV: intravenous *Schistosoma* eggs.



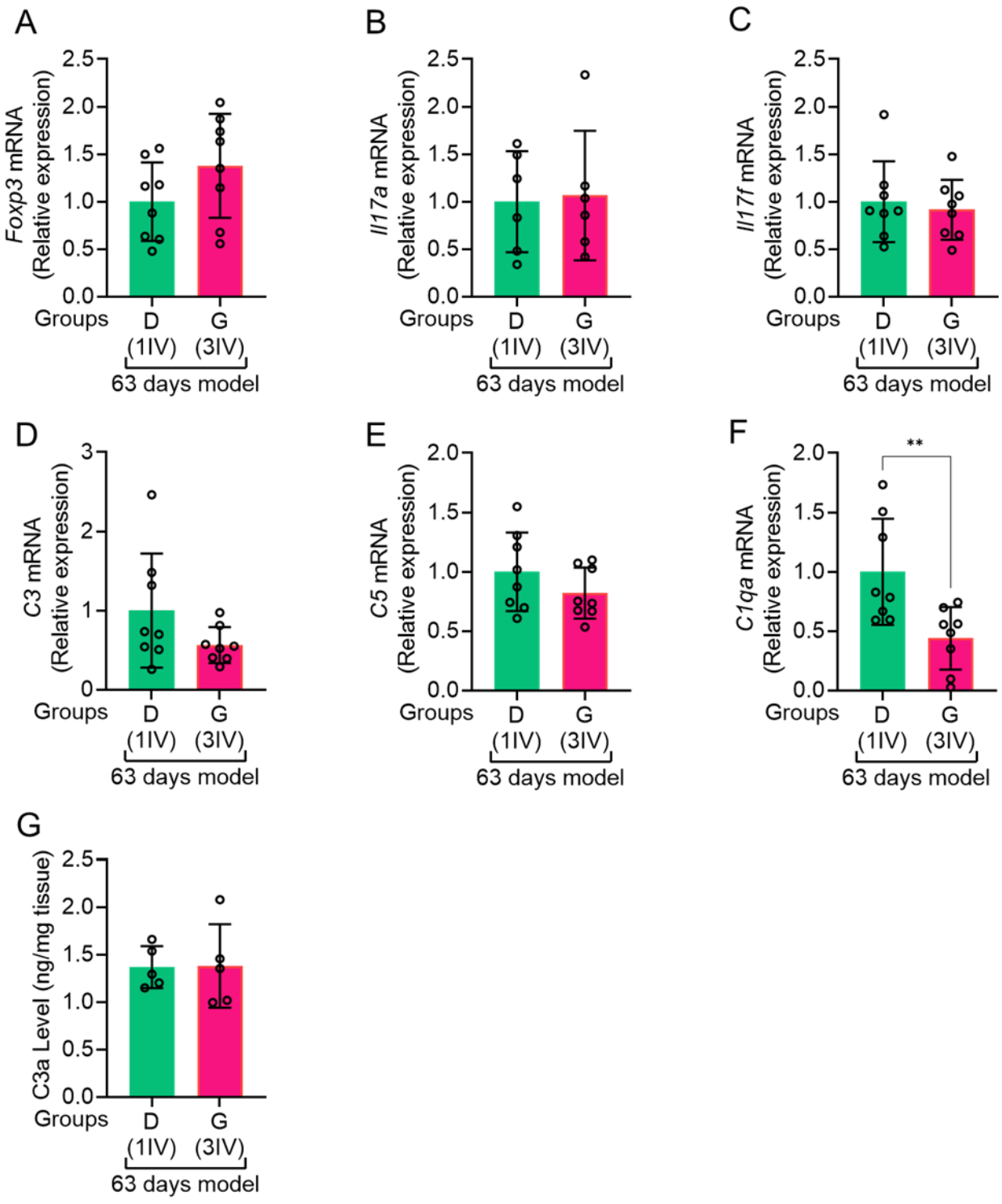
Supplementary Figure 4. No difference in the perivascular collagen as quantified by picrosirus red following a single *Schistosoma* challenge at different timepoints. See **Figure 1A** for group nomenclature; mean ± SD plotted; IP/IV: intraperitoneal/intravenous *Schistosoma* eggs.



Supplemental Figure 5. Col1a1 mRNA is increased in wildtype mice following *Schistosoma* exposure, which is suppressed in Il4-/-Il13-/- mice. Analysis of previously published (GEO Series accession number GSE49116) mRNA quantification of whole lung lysates for *Col1a1* expression by RNAseq in wildtype (N=5/group) and *Il4*$^{-/-}$*Il13*$^{-/-}$ (N=3/group) mice. Mean ± SD plotted; *: t-test with pairwise comparisons $P<0.05$; IP/IV: intraperitoneal/intravenous *Schistosoma* eggs.

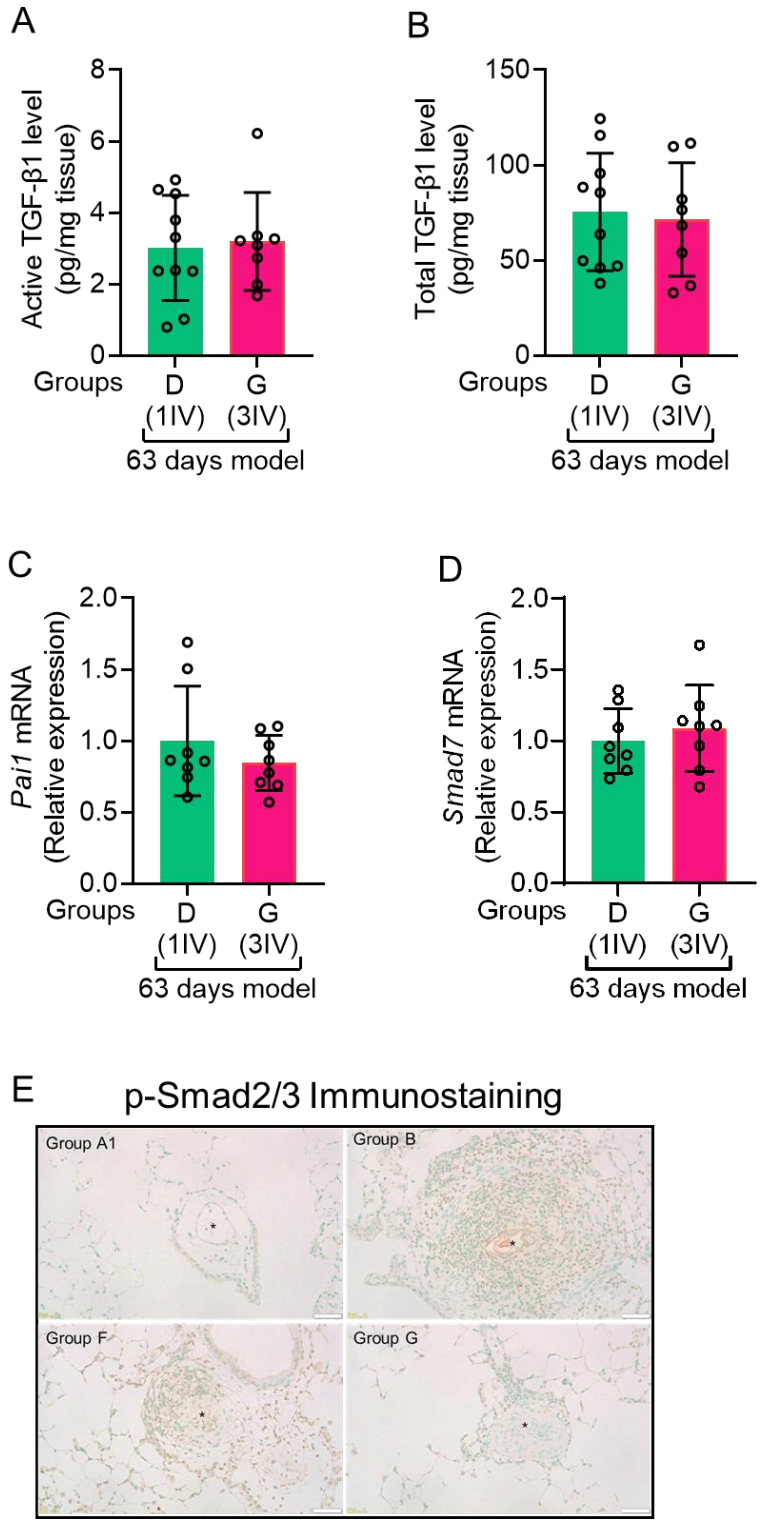


Supplemental Figure 6. IL-4 and IL-13 levels in whole lung lysates are not different between mice singly or multiple challenged with intravenous eggs. (A) IL-4 and (B) IL-13 protein concentrations in whole lung lysates 7d after the final IV eggs, following either 1 or 3 repeated IV egg challenges (*Groups B and F*; N=5-5/group). (C) IL-4 and (D) IL-13 protein concentrations in in whole lung lysates 21d after the final IV eggs, following either 1 or 3 repeated IV egg challenges (*Groups D and G*; N=8-10/group).



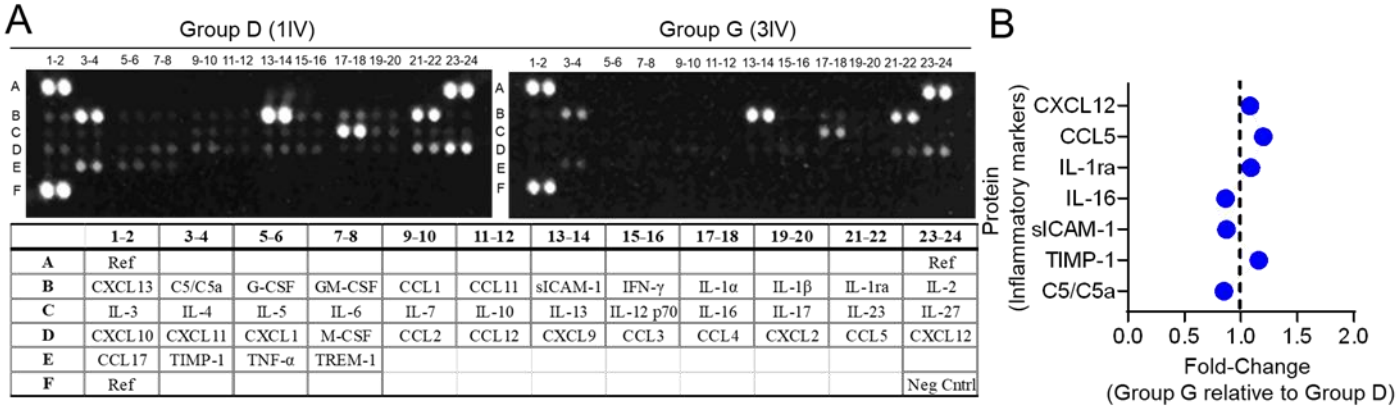
Supplementary Figure 7: The persistent pathology after repeated *Schistosoma* doses is independent of mRNA and protein markers of T cell phenotype and the complement pathway. (A-F) RT-PCR of whole lung lysates; 2-ΔCt method; relative to β-actin housekeeping gene. (A) Foxp3, a marker of Tregs. (B) Il17a and (C) IL17f, markers of Th17 cells. Complement

pathway members (D) C3, (E) C5 and (F) C1qa. (G) ELISA for C3a of whole lung lysates. Mean ± SD plotted; IV: intravenous *Schistosoma* eggs.



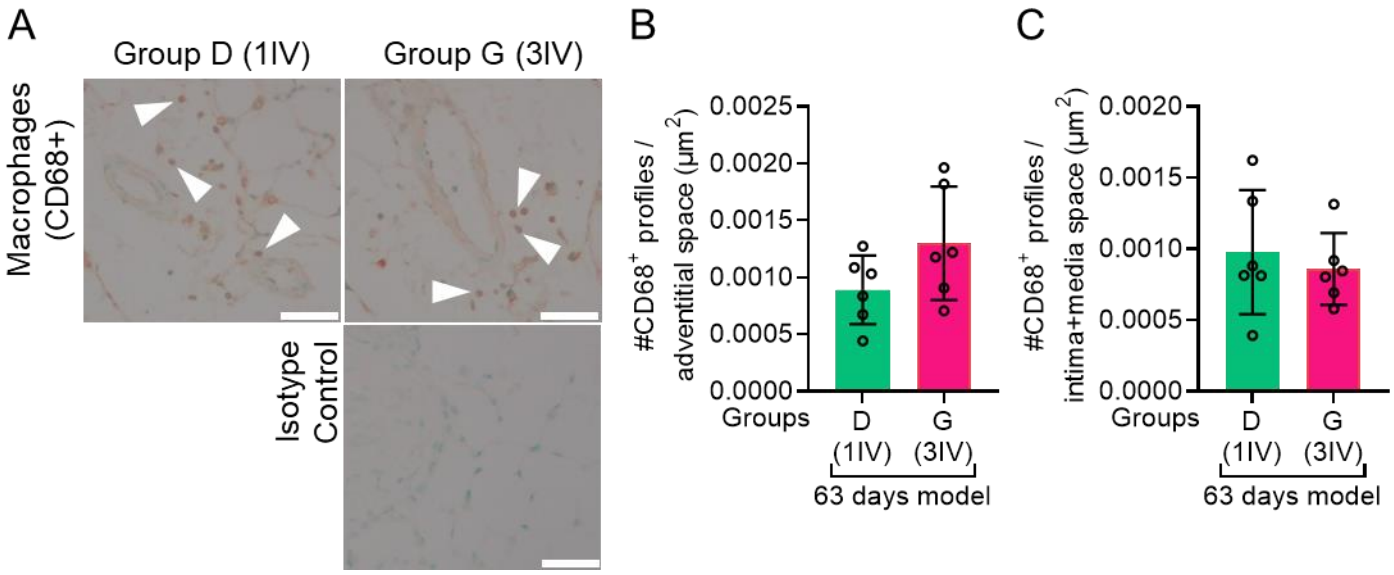
Supplementary Figure 8. The persistent pathology after repeated *Schistosoma* doses is independent of TGF-β signaling. (A) Active and (B) total TGF-β1 in whole lung lysates by ELISA. (C) Pai1 and (D) Smad7 mRNA in whole lung lysates (RT-PCR; $2^{-\Delta Ct}$ method; relative to β-actin housekeeping gene). (E) Immunostain for phospho-Smad2/3, intracellular signaling

mediators of canonical TGF-β signaling. *: vessel lumen. Mean ± SD plotted; IV: intravenous *Schistosoma* eggs.

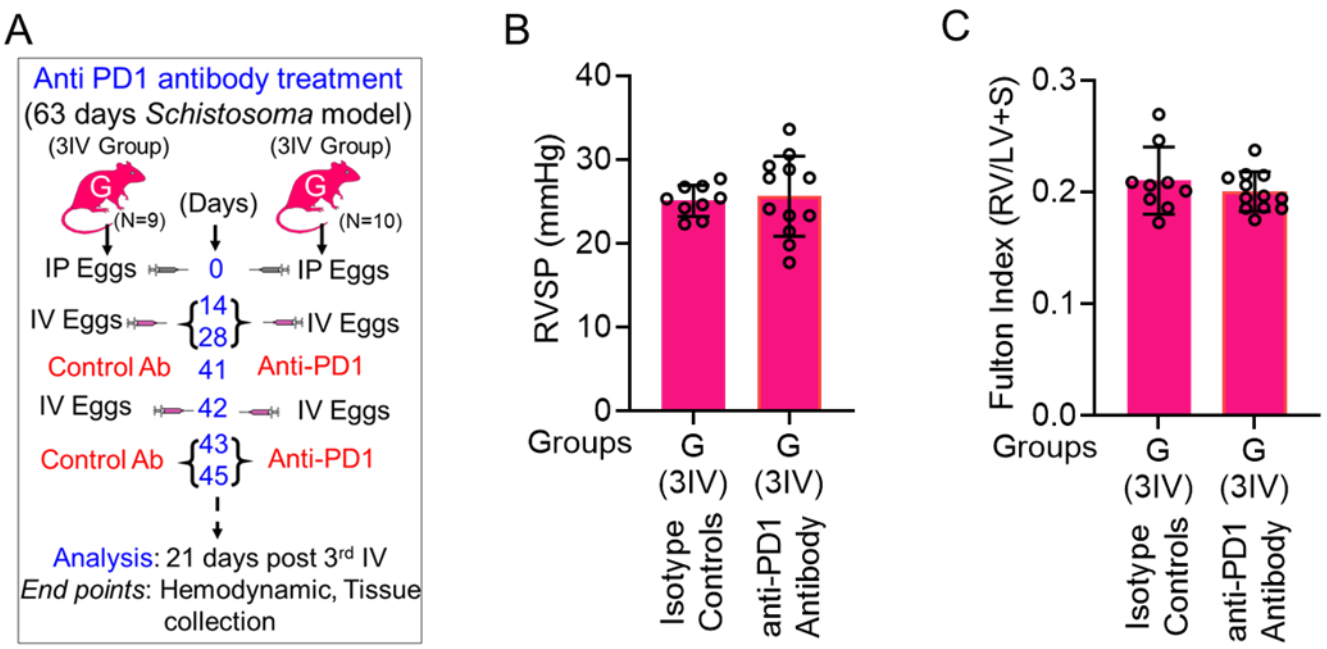


	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22	23-24
A	Ref											Ref
B	CXCL13	C5/C5a	G-CSF	GM-CSF	CCL1	CCL11	sICAM-1	IFN-γ	IL-1α	IL-1β	IL-1ra	IL-2
C	IL-3	IL-4	IL-5	IL-6	IL-7	IL-10	IL-13	IL-12 p70	IL-16	IL-17	IL-23	IL-27
D	CXCL10	CXCL11	CXCL1	M-CSF	CCL2	CCL12	CXCL9	CCL3	CCL4	CXCL2	CCL5	CXCL12
E	CCL17	TIMP-1	TNF-α	TREM-1								
F	Ref											Neg Cntrl

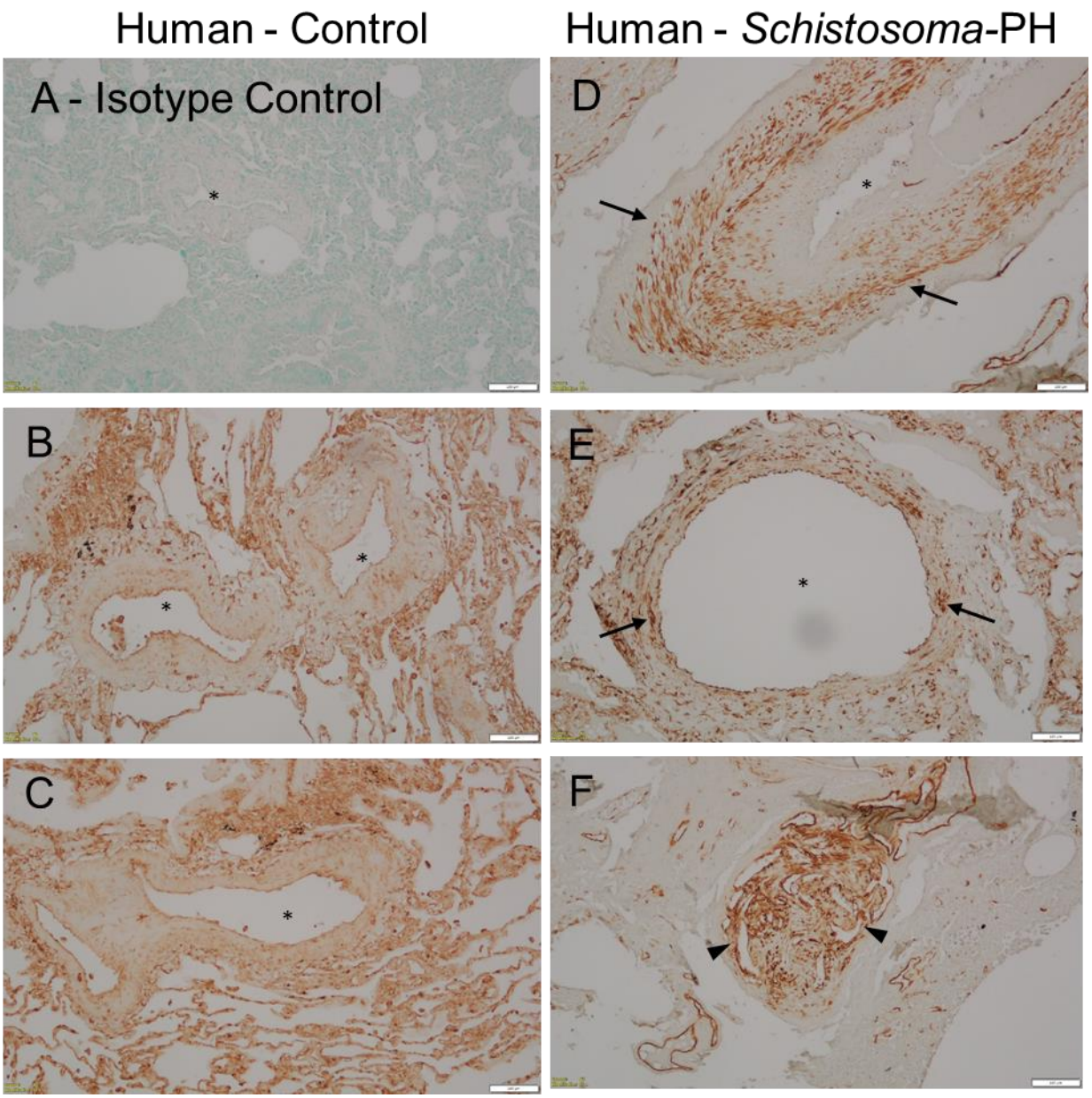
Supplementary Figure 9. No significant differences in lung cytokine protein levels between 3 IV challenged versus 1 IV challenged mice. (A) Representative protein arrays for mouse cytokines/chemokines of lung lysates. The table shows the location of analytes on the membrane. (B) Relative expression of cytokines, fold-change of Group G relative to Group D (ratio of means of N=2/group). IV: intravenous *Schistosoma* eggs.



Supplementary Figure 10. No significant differences in the density of CD68-positive cells (macrophages) in the mice that received multiple *Schistosoma* challenges. (A) Representative images of anti-CD68 stain of Groups D and G, and an isotype control. Arrowheads mark CD68-positive cells; scale bars: 50µm. Density of CD68-positive cells in the (B) perivascular/adventitial and (C) intima and media combined (within the adventitia) spaces (N=6/group), *P*=NS for both. Mean ± SD plotted; IV: intravenous *Schistosoma* eggs.



Supplementary Figure 11. Blockade of PD-1 does not attenuate PH pathology in multiple *Schistosoma* challenged mice. (A) Schematic of anti-PD-1 neutralization in *Schistosoma*-exposed mice. (B) Right ventricle systolic pressure (RVSP). (C) RV hypertrophy. Mean ± SD plotted; **P*<0.05, ***P*<0.01, *****P*<0.0001. IP: intraperitoneal; IV: intravenous *Schistosoma* eggs.



Supplementary Figure 12. Evidence of increased fibroblast density assessed by vimentin staining in the medial vascular compartment in lung tissue from autopsy cases of patients who died of schistosomiasis-associated PH, as compared to control lung tissue. (A) Isotype control of control lung tissue. (B and C) Representative images of vimentin immunostained lung tissue from unsuccessful lung donors. (D and E) Two of three cases of *Schistosoma*-PH analyzed demonstrated substantial vimentin staining in the media. (One of the 4 cases available did not have any significant immunostaining in the entire specimen.) (F) Representative image of a plexiform lesion in *Schistosoma*-PH with significant vimentin immunostaining. *: vessel lumen. Arrows: representative positive staining in the medial compartment. Arrowheads: positive staining in a plexiform lesion. Scale bars: 100 μm.

Supplemental Table 1. Primers used for mouse mRNA quantification by RT-PCR.

All primers are TAQMAN Gene Expression Assays (Life Technologies Corporation, Carlsbad, CA, USA).

Target	Catalog #	Interrogated Sequence	Amplicon Length
Pai1	Mm00435858_m1	NM_008871.2	87
Smad7	Mm00484742_m1	NM_001042660.1	119
Foxp3	Mm00475157_g1	NM_054039.2	87
Thbs1	Mm01335413_g1	NM_011580.3	84
Il17a	Mm00439618_m1	NM_010552.3	80
Il17f	Mm00521423_m1	NM_145856.2	85
C3	Mm01232779_m1	BC029976.1	88
C5	Mm00439275_m1	NM_010406.2	104
C1qa	Mm00432142_m1	NM_007572.2	80
Actb	Mm02619580_g1	AK075973.1	143
Gapdh	Mm99999915_g1	NM_008084.3	107
Pdcd1 (PD1)	Mm00435532_m1	NM_008798.2	65
Cd274 (PDL1)	Mm00452054_m1	NM_021893.3	77
Pdcd1lg2 (PDL2)	Mm00451734_m1	NM_021396.2	95

Supplemental Table 2. Reagents for immunostaining mouse tissue. All rinses between steps in TBST unless noted otherwise.

Immunostain	Antigen Retrieval	Block	Primary Antibody	Secondary Antibody	Tertiary Reagent
αSM-actin	Citrate Buffer 20 min in steamer (Vector H-3300)	Avidin 10 min, Biotin 10 min, Mouse on Mouse (MOM) kit blocking solution (Vector BMK-2202) 1hr at RT	1:100 30min at RT (Dako #M0851)	MOM Biotinylated anti-Mouse Reagent (Vector BMK-2202) 10min at RT	Texas Red-Strepavidin 1:2000 (Invitrogen #S872), Vectashield with DAPI (Vector H-1500)
p-Smad2/3	Borg Decloaker RTU (BioCARE MEDICAL BD1000G1)	10% Goat Serum in TBS 1hr at RT	1:200 1hr at RT (Thermo-Fisher #PA5-99378)	1:200 Goat anti-Rabbit in TBS 1hr at RT (Vector BA-1000)	SA-HRP 30min (Vector SA-5704), DAB 5min at RT (Vector, SK-4100), Methyl Green Counterstain (Vector H3402)
HSP47	Borg Buffer 20 min in steamer (Biocare Borg Decloaker)	Avidin 10 min, Biotin 10 min, 3% H_2O_2 in PBS (5min), Endogenous Enzyme Block (5min; Dako S2003), 10% goat serum in PBS 1hr at RT	1:500 1hr at RT (Abcam ab254015)	1:200 Biotinylated Goat anti-Rabbit (Vector BA-1000)	
CD68	Citrate Buffer 20 min in steamer (Vector H-3300)		1:100 1hr at RT (Invitrogen #PA5-89134)		

Supplemental Table 3. Reagents for immunostaining human tissue. All rinses between steps in TBST unless noted otherwise.

Immunostain	Antigen Retrieval	Block	Primary Antibody	Secondary Antibody	Tertiary Reagent
Vimentin	Borg Buffer 20 min in steamer (Biocare Borg Decloaker)	3% H_2O_2 in PBS (5min), Endogenous Enzyme Block (5min; Dako S2003)	1:500 in PBS 1hr at RT (abcam ab92547)	Anti-Rabbit poly-HRP (Dako EnVision+ K4003)	DAB 5min at RT (Vector, SK-4100), Methyl Green Counterstain (Vector H3402)

Supplemental Table 4. Demographic data on the control human specimens (N=3). (Data on the schistosomiasis-associated pulmonary arterial hypertension specimens was not available.)

Age	Median = 45, Range 27 – 55
Sex	2 Female, 1 Male
Race	3 White