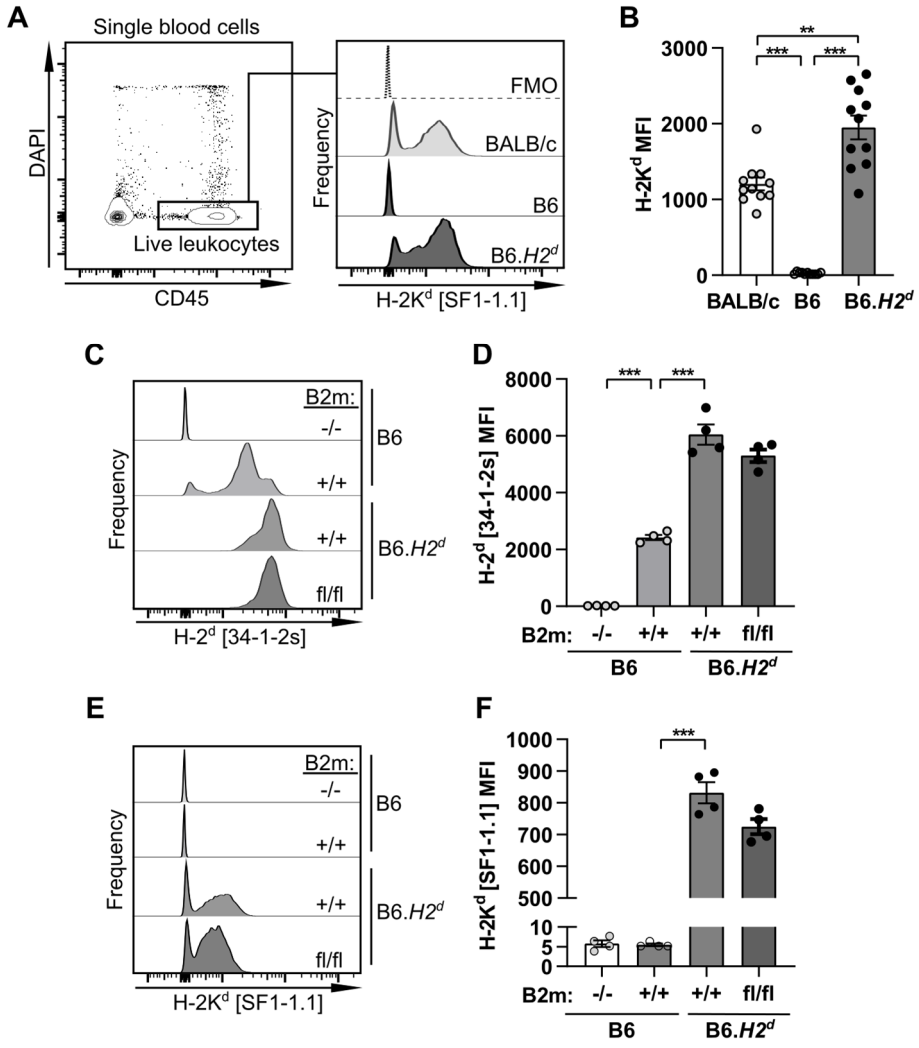
Supplemental Movie 1. Lung retention of anti-H-2^{d} after intravenous injection. Two-photon intravital lung imaging of LPS-primed C57BL/6 (B6) or B6.*H2^{d}* mice given 70 kDa FITC-dextran i.v. to label blood plasma (green) and then 1 mg/kg anti-H-2^{d}-PE i.v. (magenta) during acquisition to image the location, extent and kinetics of anti-H-2^{d} deposition in lungs of TRALI-resistant B6 mice and TRALI-susceptible B6.*H2^{d}* mice.

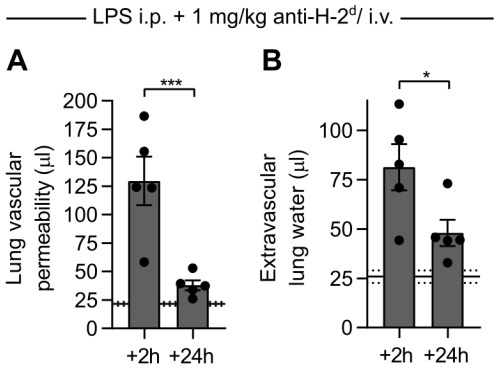
Supplemental Movie 2. Responses of platelets and LysM+ cells in lungs to anti-H-2^{d} intravenous injection. Two-photon intravital lung imaging using an LPS-primed BALB/c-congenic LysM-GFP × PF4-Cre × Ai14 mouse (LysM+ cells: green, PF4+ platelets: red). Evans blue was given i.v. to label blood plasma (blue), and 1 mg/kg anti-H-2^{d} was given during acquisition to study responses to antibody injection. Maximum intensity projections over 10 z planes 1 μm apart, with zoom to show a small blood vessel, and a surface rendering of the same vessel.

Supplemental Movie 3. Platelet thrombosis, microvascular ischemia, plasma extravasation, and neutrophil swarming in lungs following anti-H-2^{d} injection. Two-photon intravital lung imaging using LPS-primed BALB/c-congenic LysM-GFP × PF4-Cre × Ai14 mice (LysM+ cells: green, PF4+ platelets: red). Evans blue was given i.v. to label blood plasma (blue), and 1 mg/kg anti-H-2^{d} was given during acquisition to study responses to antibody injection. The final movie shows a response to 4.5 mg/kg anti-H-2^{d} (high dose used for survival experiments). Maximum intensity projections over 10 z planes 1 μm apart.

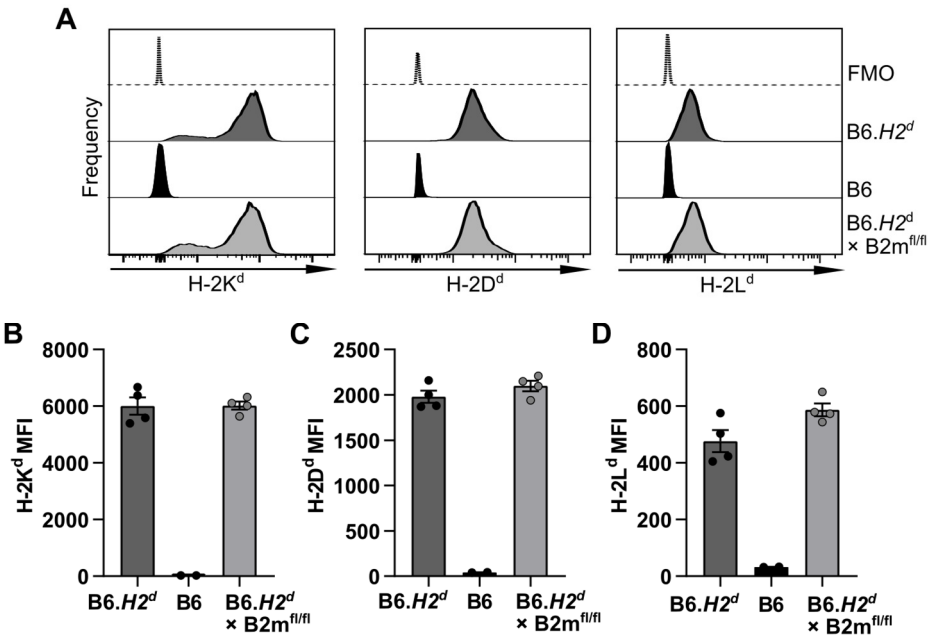
Supplemental Movie 4. Release of DNA in lung microvasculature in response to anti-H-2^{d} injection. Two-photon intravital lung imaging using an LPS-primed B6.*H2^{d}* × PF4-Cre × mTmG mouse (GFP-labelled platelets: blue, other cells in lung: red) given SYTOX Green i.v. to label cell-free DNA or DNA in cells with compromised membranes (green), and 4.5 mg/kg anti-H-2^{d} during acquisition. Maximum intensity projections over 5 z planes 1 μm apart.



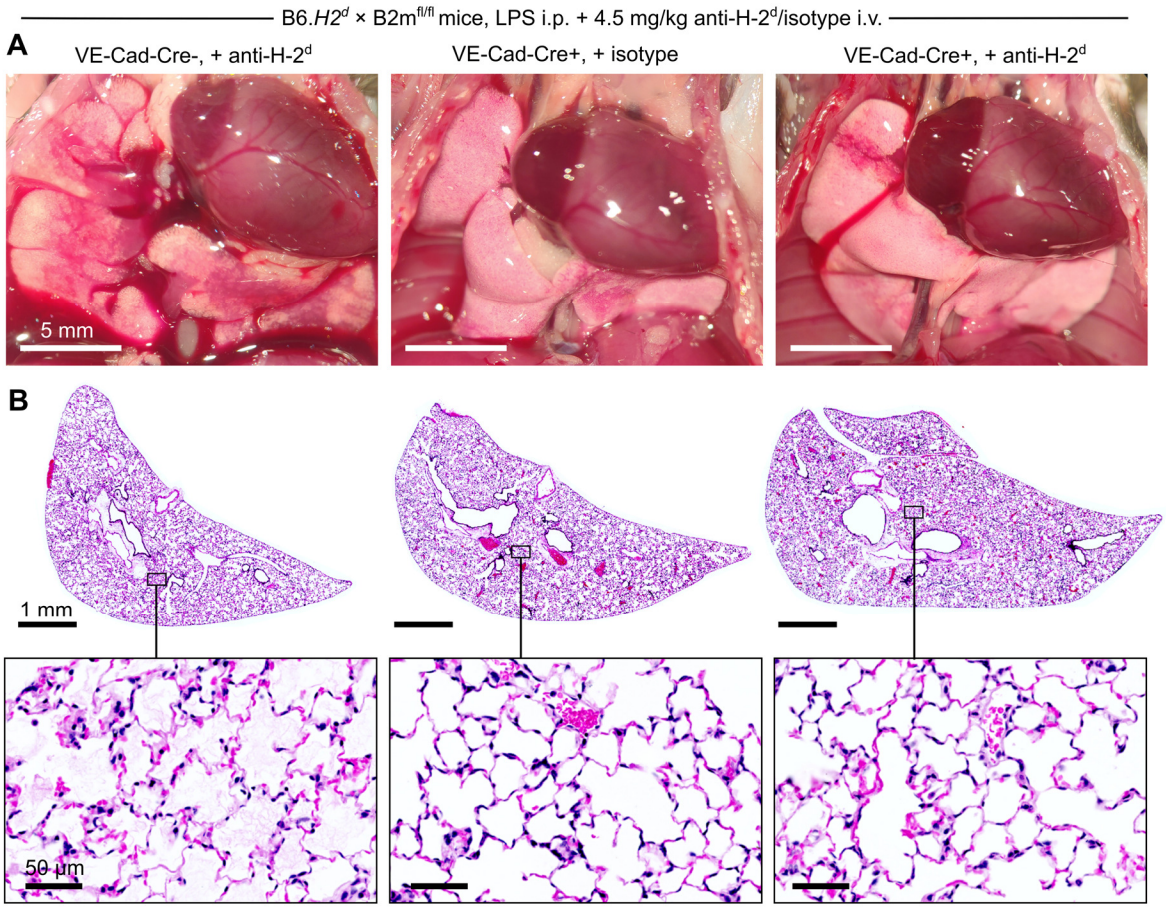
Supplemental Figure 1. MHC I expression on leukocytes from inbred strains and B6.*H2*^{d} × B2m$^{fl/fl}$ mice. Fc-blocked, live blood leukocytes were analyzed using flow cytometry for surface MHC I expression. (**A**) Gating strategy and representative histograms showing H-2K^{d} MHC I expression on BALB/c, C57BL/6 (B6) and B6.*H2*^{d} leukocytes, and (**B**) quantification of expression as MFI. Histograms and MFI quantifications showing (**C, D**) MHC I-dependent binding of anti-H-2^{d} clone 34-1-2S to B6.*H2*^{d} and B6 leukocytes, (**E, F**) and H-2^{d}-dependent binding of anti-H-2K^{d} clone SF1-1.1 to B6.*H2*^{d} leukocytes. Mean ± SEM, (**B**) n=10, (**D, F**) n=4, one-way (**B**,) or 2-way (**D, F**) ANOVA with Holm's test, ***P* <0.01, ****P* <0.001.



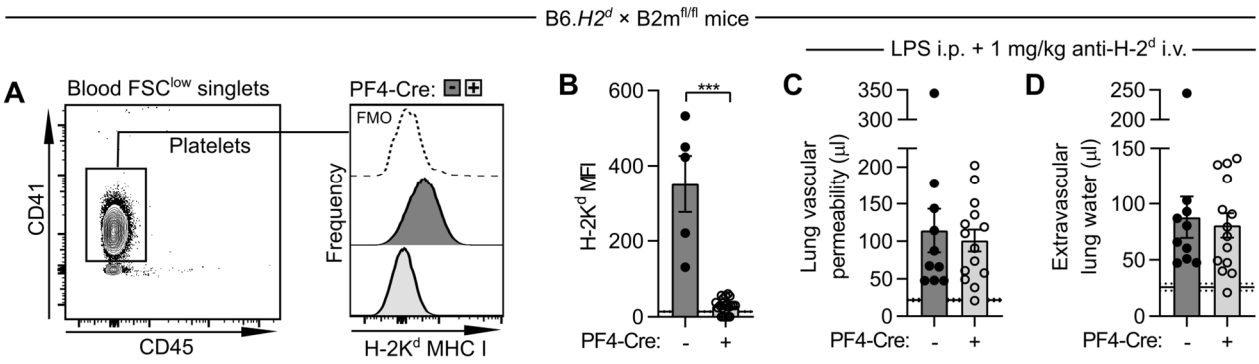
Supplemental Figure 2. Acute lung injury resolution at 24 hours post-antibody injection in B6.*H2^d* mice. LPS-primed B6.*H2^d* mice were injected with 1 mg/kg anti-H-2^d. At 2 hours or 24 hours post antibody injection, lungs and blood were collected for assessment of (**A**) lung vascular permeability and (**B**) extravascular lung water, with I^{125}-albumin tracer given i.v. 2 hours before sample collection in each group. Horizontal lines are reference mean ± SEM from isotype controls in Figure 1. Mean ± SEM, n=5, unpaired t-test, **P* <0.05, ****P* <0.001.



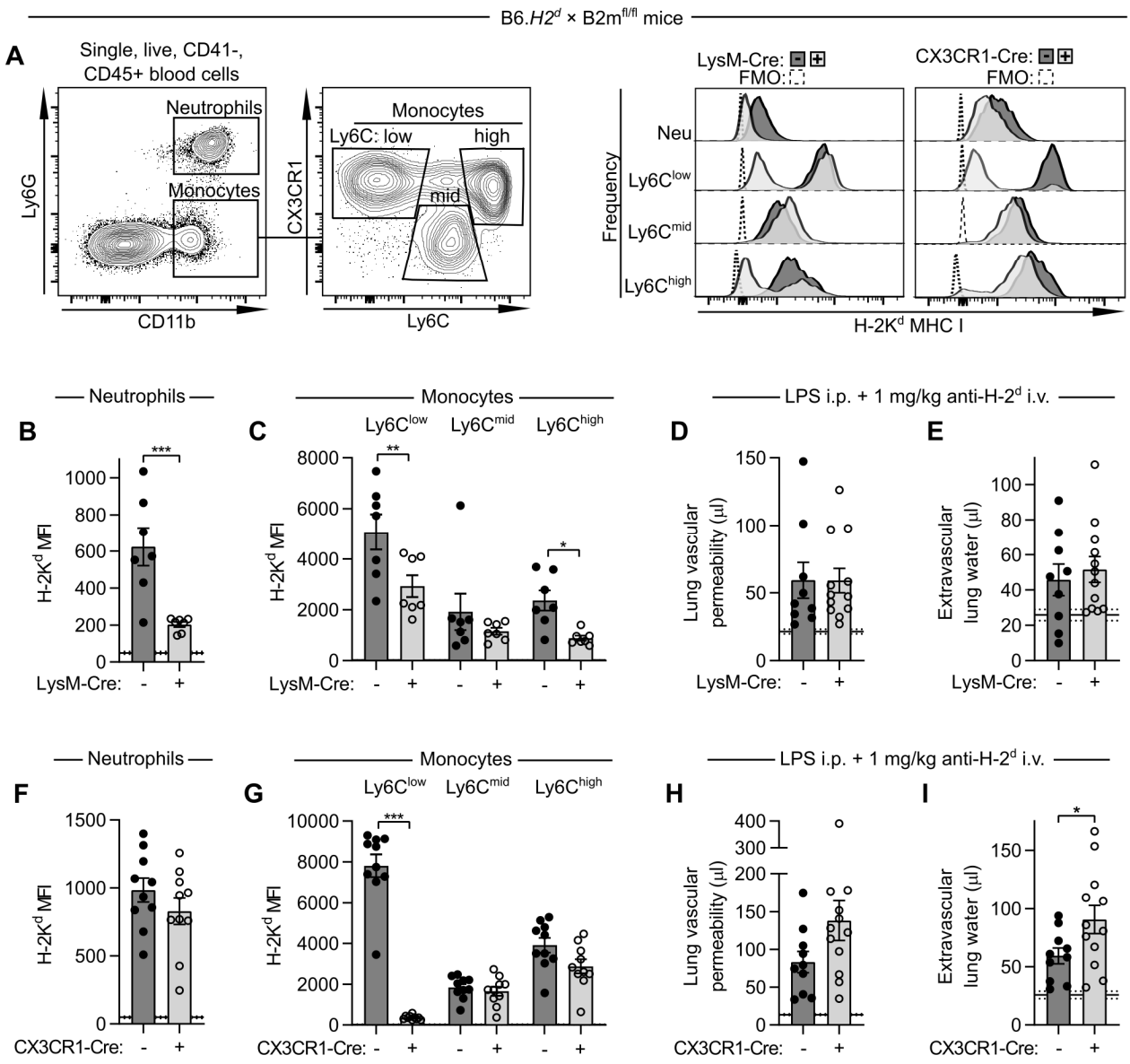
Supplemental Figure 3. Full expression of MHC I on leukocytes from B6.*H2*^{d} × B2m$^{fl/fl}$ mice. Fc-blocked, live blood leukocytes were analyzed using flow cytometry to assess surface expression of the three classical MHC I proteins of the H-2^{d} type. **A**) Histograms, and (**B-D**) MFI quantifications showing successful transmission of all H-2^{d}-type MHC I proteins expressed at normal levels to B6.*H2*^{d} × B2m$^{fl/fl}$ mice. Mean ± SEM, n=2-4, two-way ANOVA with Holm's test.



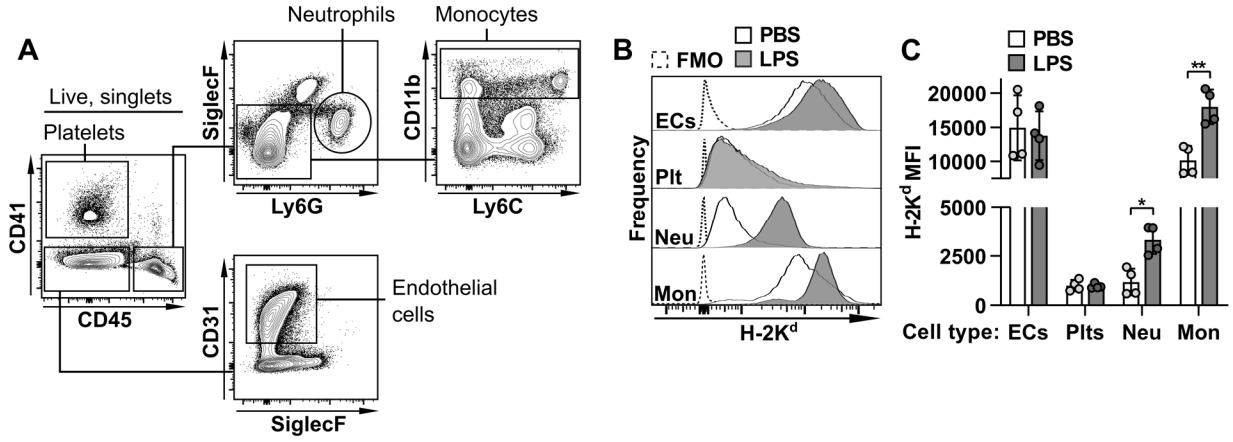
Supplemental Figure 4. Effect of removal of endothelial MHC I on gross pathology and histological features in anti-MHC I-mediated lung injury. LPS-primed B6.*H2*^{d} × B2m$^{fl/fl}$ mice expressing VE-Cad-Cre, or their Cre-negative littermates were challenged with 4.5 mg/kg anti-H-2^{d}/isotype control. At 2 hours post antibody injections or cessation of respiratory movements, photographs were taken of lungs, which were then fixed for histological analysis. (**A**) Photographs show gross lung pathology in situ. (**B**) Micrographs of right lung lobe sections (4 µm thick), which were stained with hematoxylin & eosin and imaged with a 20× objective. Images are representative of 2 mice sampled per group.



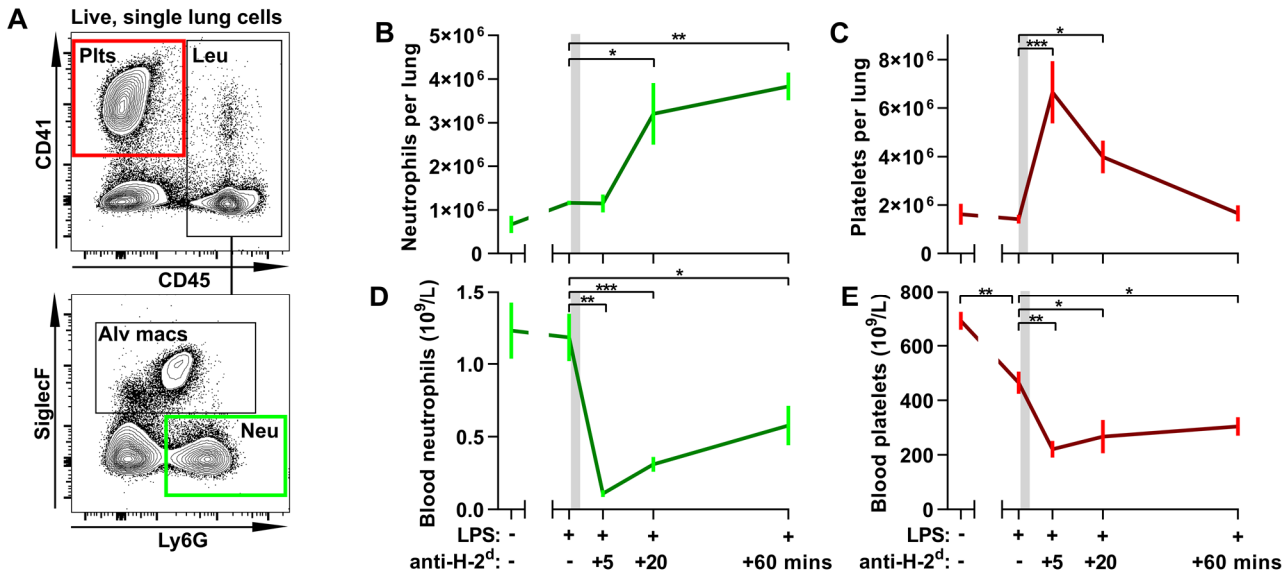
Supplemental Figure 5. Removal of platelet MHC I does not alter anti-MHC I-mediated lung injury. Blood was collected from B6.*H2*^{d} × B2m$^{fl/fl}$ mice either positive or negative for PF4-Cre for measurement of H-2K^{d} MHC class I (MHC I) expression on platelets using flow cytometry. (**A**) Gating strategy and representative histograms and (**B**) platelet surface H-2K^{d} MHC I median fluorescence intensity (MFI) quantification (Cre-: n=5, Cre+: n=20). LPS-primed mice were given anti-H-2^{d} i.v. and used for assessment of (**C**) lung vascular permeability and (**D**) extravascular lung water (Cre- n=10, Cre+ n=14). Mean ± SEM with unpaired t-tests. Horizontal lines are reference data from (**B**) Cre- fluorescence minus one (FMO) controls, or (**C**, **D**), reference data from Figure 1 from mice given isotype control i.v., ***P <0.001.



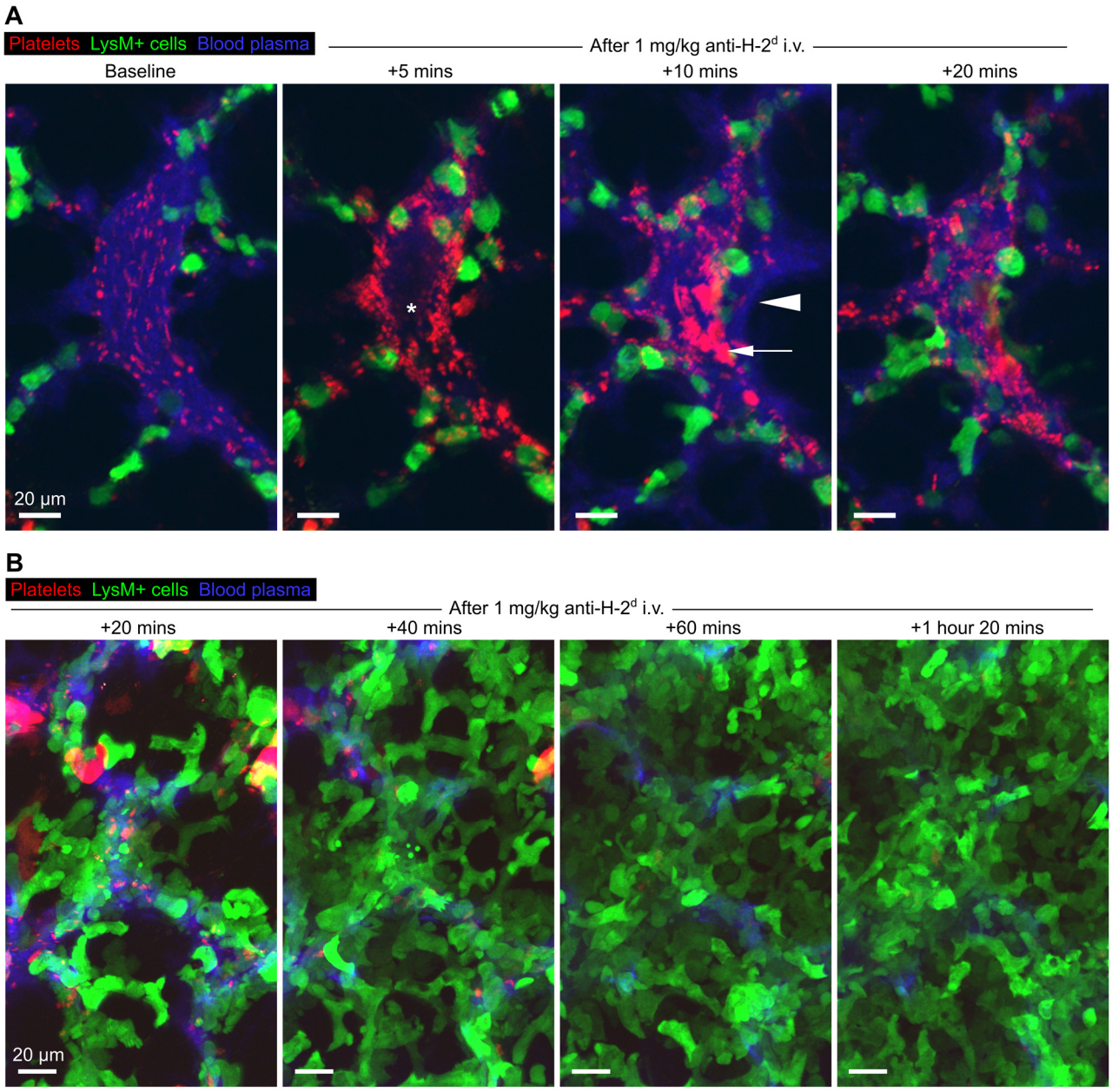
Supplemental Figure 6. Anti-MHC I-mediated lung injury occurs with removal of neutrophil or Ly6C^{low} monocyte MHC I. Blood was collected from B6.*H2*^{d} × B2m$^{fl/fl}$ mice either positive or negative for LysM-Cre or CX3CR1-Cre for measurement of H-2K^{d} MHC I surface expression on CD41- live leukocytes using flow cytometry. (**A**) Gating strategy and representative histograms showing H-2K^{d} expression on leukocyte populations with fluorescence minus one (FMO) controls. (**B**, **C**, **F**, **G**) Quantification of H-2K^{d} median fluorescence intensity (MFI) (LysM-Cre: n=7, CX3CR1-Cre: Cre- n=10, Cre+ n=11). LPS-primed mice were given anti-H-2^{d} i.v. and used for assessment of (**D**, **H**) lung vascular permeability and (**E**, **I**) extravascular lung water (LysM-Cre: Cre- n=9, Cre+ n=12; CX3CR1-Cre: Cre- n=10, Cre+ n=12). Mean ± SEM with unpaired t-tests. Horizontal lines are reference data from (**B**, **F**) Cre- fluorescence minus one (FMO) controls, or (**D**, **E**, **H**, **I**), reference data from Figure 1 from mice given isotype control i.v., **P* <0.05, ***P* <0.01, ****P* <0.001.



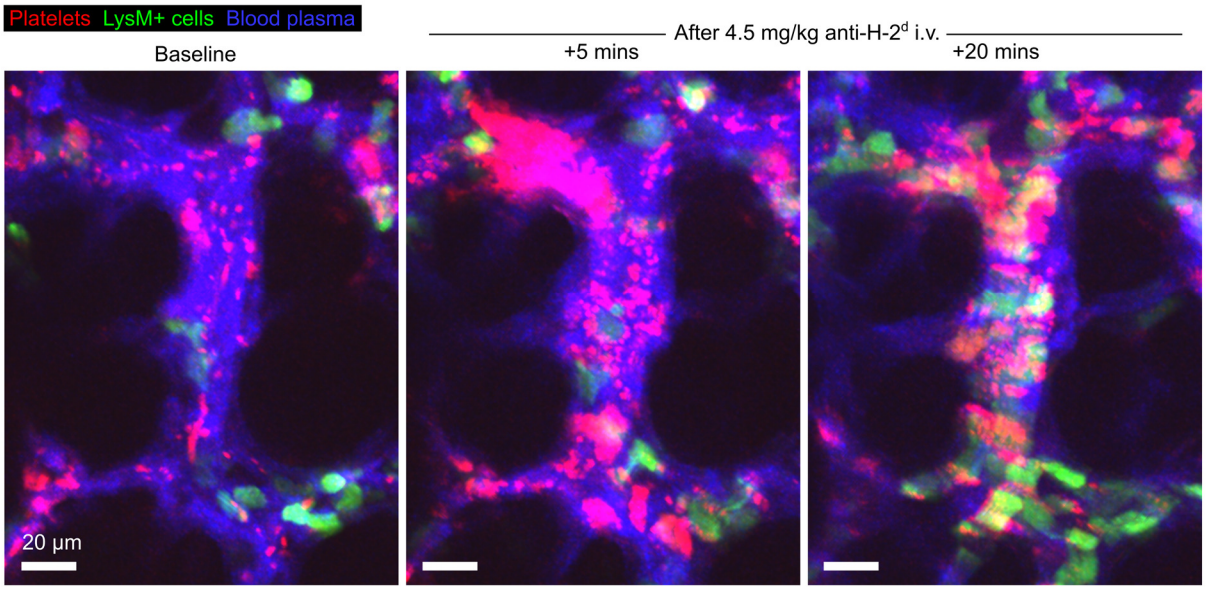
Supplemental Figure 7. Surface MHC I expression across mouse lung cell types. BALB/c mice were either primed with LPS, or given PBS as vehicle control and 24 hours later lungs were collected for dissociation and flow cytometry analysis of H-2K^d MHC I expression across lung cell subsets. (**A**) Gating strategy for identification of endothelial cells, platelets, neutrophils and monocytes. (**B**) MFI quantification of surface H-2K^d expression. Mean ± SEM, n=4, 2-way ANOVA with Holm's test for effect of LPS, **P*< 0.05, ***P* <0.01.



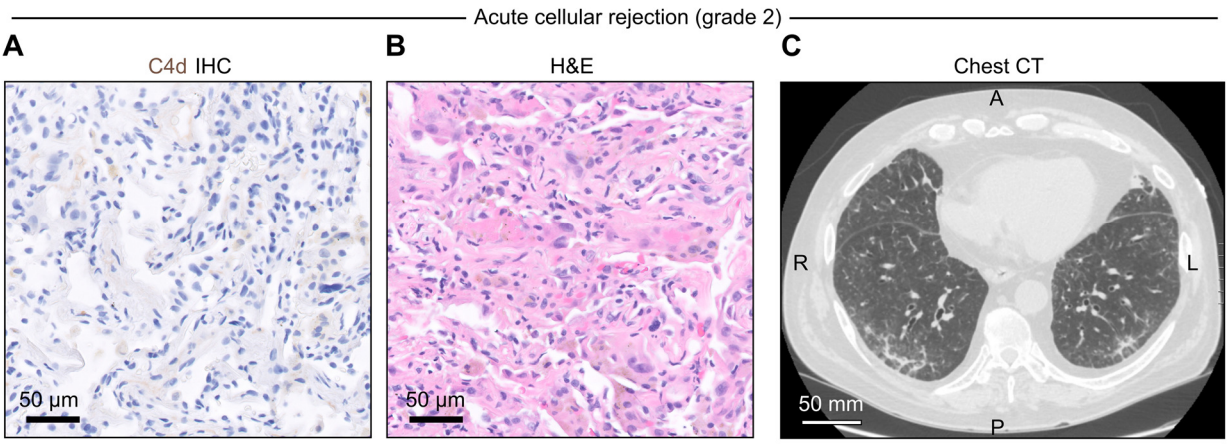
Supplemental Figure 8. Time course of anti-MHC I-mediated platelet and neutrophil retention in lungs and removal from blood. BALB/c mice were administered LPS or PBS vehicle and 24 hours later were given either i.v. isotype control or anti-H-2^{d} i.v. with blood samples and lungs collected at the given time points. (**A**) Flow cytometry gating strategy to identify platelets and neutrophils from dissociated lungs. (**B**) Neutrophil, and (**C**) platelet counts from dissociated lungs. (**D**) Neutrophil, and (**E**) platelet counts from blood samples. Mean ± SEM, all n=4. One-way ANOVA with Dunnett's post hoc test vs. LPS + isotype control group, **P* <0.05, ***P* <0.01, ****P* <0.001.



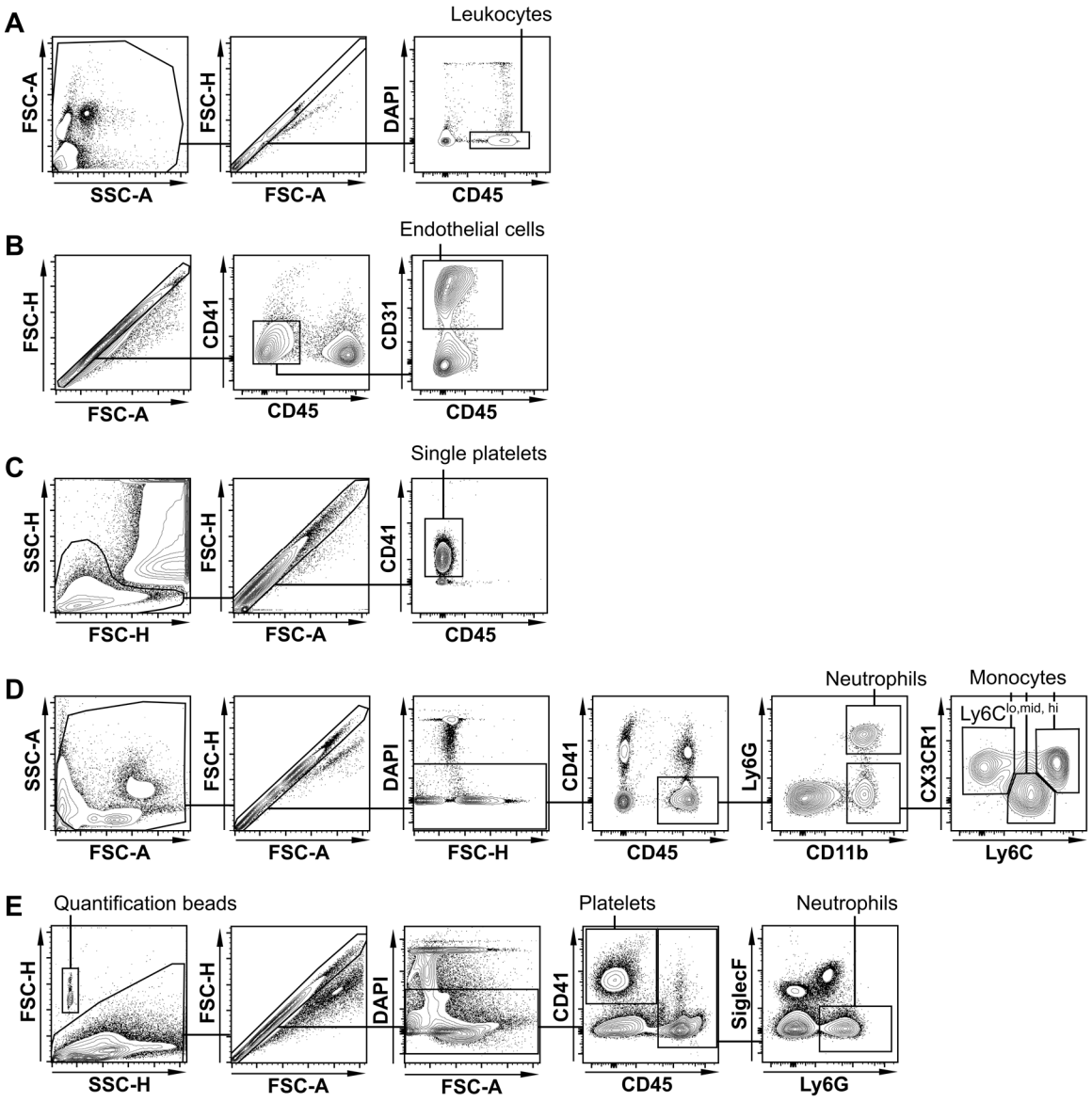
Supplemental Figure 9. Anti-MHC I-induced transient ischemia, platelet aggregation, plasma extravasation and neutrophil swarming in lungs. Two-photon intravital lung imaging using LPS-primed BALB/c-congenic LysM-GFP × PF4-Cre × Ai14 mice (LysM+ cells: green, PF4+ platelets: red). Evans blue was given i.v. to label blood plasma (blue), and 1 mg/kg anti-H-2^d was given during acquisition to study responses to antibody injection. (**A**) Transient ischemic event (*loss of intravascular plasma fluorescence due to stopped flow) with lung microvascular platelet aggregation (arrow) and plasma extravasation (arrowhead – plasma signal outside of blood vessel). (**B**) Formation of neutrophil swarm over 20 min – 1 hour 20 min after anti-H-2^d i.v. injection.



Supplemental Figure 10. Platelet aggregation in lungs with high-dose anti-MHC I injection. Two-photon intravital lung imaging using LPS-primed BALB/c-congenic LysM-GFP × PF4-Cre × Ai14 mice (LysM+ cells: green, PF4+ platelets: red). Evans blue was given i.v. to label blood plasma (blue), and 4.5 mg/kg anti-H-2^d (high dose) was given during acquisition to study responses to antibody injection. In addition to margination to endothelium, platelets could also be observed forming intravascular aggregates.



Supplemental Figure 11. Absence of lung allograft endothelial C4d positivity in acute cellular rejection. Transbronchial biopsies with (**A**) C4d immunohistochemical (IHC) staining (C4d positivity = brown, hematoxylin counterstain = blue) or (**B**) hematoxylin and eosin staining, and (**C**) chest computed tomography (CT) scan from a lung transplant recipient with grade 2 acute cellular rejection, which does not require donor specific antibodies.



Supplemental Figure 12. Gating strategies used in flow cytometry experiments. Full gating strategies showing methods used in flow cytometry experiments to isolate populations of (**A**) live leukocytes, (**B**) pulmonary endothelial cells, (**C**) single blood platelets, (**D**) blood myeloid cells, (**E**) lung cells and platelets together with quantification beads.

Supplemental Table 1. Demographic characteristics of TRALI and control subjects. Values are number (percentage) unless otherwise indicated.

	Control subjects (n=18)	TRALI cases (n=9)
Age in years: median (Q1-Q3)	57 (45-57)	61 (45-70)
Sex		
Female	13 (72)	7 (78)
Male	5 (28)	2 (22)
Race		
White	14 (78)	6 (67)
Other	4 (22)	3 (33)
Ethnicity		
Hispanic	0 (0)	1 (11)
Not Hispanic	18 (100)	7 (78)
Not reporting ethnicity	0 (0)	1 (11)
Transfusion strata		
High (≥10 units)	5 (28)	1 (11)
Medium (3-9 units)	6 (33)	5 (56)
Low (1-2 units)	7 (39)	3 (33)
Patient location at time of transfusion		
Floor	9 (50)	1 (11)
Hematology/oncology floor	3 (17)	1 (11)
ICU	2 (11)	3 (33)
OR/PACU	4 (22)	4 (44)
Surgery before edema		
No	5 (28)	6 (67)
Yes	13 (72)	3 (33)
Mechanical ventilation before transfusion		
No	5 (28)	6 (67)
Yes	13 (72)	3 (33)
Shock before transfusion		
Hemorrhagic shock	0 (0)	1 (11)
No	12 (67)	4 (44)
Other Shock	6 (33)	4 (44)

Supplemental Table 2. Baseline demographic characteristics of lung transplant recipients providing data for effect of anti-HLA DSAs on CLAD-free survival. Values are number (percentage) unless otherwise indicated.

Subjects (n)	215
Age at transplantation, median years	59 (IQR 50 – 65)
Male gender (%)	61
Transplant type: n (%)	
Double	189 (88)
Other	26 (12)
Race/Ethnicity: n (%)	
White	149 (69)
Other	66 (31)
Transplant indication: n (%)	
Pulmonary Fibrosis	154 (59)
Other	61 (41)
Donor Specific Antibodies: n (%)	44 (20)
HLA class I only	15
HLA class II only	22
Both HLA class I & class II	7

Supplemental Table 3. Origins of mouse strains. Origins and stock numbers of inbred and transgenic mouse strains used in this study. Where mice are not commercially available, references are given to first descriptions of strains and previous studies from laboratories who provided the mice.

Strain	Source	Reference/stock number
BALB/c*	Charles River	028
B6.*H2*^{d}	Jackson Laboratory	000359
C57BL/6*	Jackson Laboratory	000664
B2m^{flox}	Craig Morrell, Univ. Rochester	(1)
B2m$^{-/-}$	Jackson Laboratory	002087
PF4-Cre	Jackson Laboratory	008535
VE-Cad-Cre	Jackson Laboratory	006137
LysM-Cre	Jackson Laboratory	004781
CX3CR1-Cre	Jackson Laboratory	025524
B2m^{inv}	James Zimring, Univ. Virginia	First reported in this manuscript
LysM-GFP	Ellen Robey, UC Berkeley	(2-4)
Ai14	Jackson Laboratory	007914
mTmG	Jackson Laboratory	007676

*Bred at supplier site. Other lines were maintained at UCSF.

References:

1. Hilt ZT et al. Platelet-derived β2M regulates monocyte inflammatory responses. JCI Insight 2019;4(5). doi:10.1172/JCI.INSIGHT.122943
2. Ortiz-Muñoz G et al. Aspirin-triggered 15-epi-lipoxin A4 regulates neutrophil-platelet aggregation and attenuates acute lung injury in mice. Blood 2014;124(17):2625–34.
3. Coombes JL et al. Motile invaded neutrophils in the small intestine of Toxoplasma gondii-infected mice reveal a potential mechanism for parasite spread. Proc. Natl. Acad. Sci. U. S. A. 2013;110(21):E1913–E1922.
4. Faust N, Varas F, Kelly LM, Heck S, Graf T. Insertion of enhanced green fluorescent protein into the lysozyme gene creates mice with green fluorescent granulocytes and macrophages. Blood 2000;96(2):719–726.

Supplemental Table 4. Antibodies used for flow cytometry and immunohistochemistry.

Antibody (target-fluorophore)	Clone	Dilution	Supplier	Catalog number
Mouse flow cytometry				
CD41-PE-Cy7	MWReg30	1:400	Biolegend	133916
CD45-PerCP	30-F11	1:400	Biolegend	103130
SiglecF-AF647	E50-2440	1:400	BD	562680
Ly6G-FITC	1A8	1:400	BD	551460
Ly6C-APC-Cy7	HK1.4	1:400	Biolegend	128026
CD11b-BV711	M1/70	1:400	BD	563168
H-2K^{d}-PE	SF1-1.1	1:400	BD	553566
H-2^{d}-PE	34-1-2S	1:400	ThermoFisher	12-5998-83
H-2L^{d}-FITC	30-5-7S	1:400	ThermoFisher	MA5-18006
H-2D^{d}-AF647	34-2-12	1:400	Biolegend	110612
CD31-PE	MEC 13.3	1:400	BD	561073
CX3CR1-PerCP-Cy5.5	SA011F11	1:400	Biolegend	149009
C3-APC-Cy7	11H9	1:1600	Novus Biologicals	NB200-540APCCY7
CD62L-PE	MEL-14	1:400	BD	553151
Fc block	2.4G2	1:7000	UCSF mAb core	AM004
Human flow cytometry				
HLA-ABC-APC	G46-2.6	1:400	BD	562006
CD41a-PE-Cy7	HIP8	1:400	BD	561424
CD45-APC-Cy7	2D1	1:400	BD	368515
CD11b-PE	ICRF44	1:400	BD	555388
CD31-Brilliant Violet 605	WM59	1:400	BD	562855
CD14-PerCP	MφP9	1:400	BD	340585
CD66b-FITC	G10F5	1:400	BD	555724
Mouse immunohistochemistry				
CD41	MWReg30	1:500	BD	553847
Collagen IV	Polyclonal	1:1000	Cosmo Bio	LSL-LB-1403
C3-APC-Cy7	11H9	1:1000	Novus Biologicals	NB200-540APCCY7
CD31	2H8	1:500	ThermoFisher	MA3105
Ly6G-FITC	1A8	1:400	BD	551460
Immunohistochemistry secondaries				
Donkey anti-rat IgG-Cy3	Polyclonal	1:500	Jackson ImmunoResearch	712-165-153
Donkey anti-goat IgG-AF488	Polyclonal	1:500	Jackson ImmunoResearch	705-546-147
Donkey anti-rabbit IgG-AF647	Polyclonal	1:500	Jackson ImmunoResearch	711-606-152
Goat anti-Arm. hamster IgG-AF488	Polyclonal	1:500	Jackson ImmunoResearch	127-545-160