Supplementary

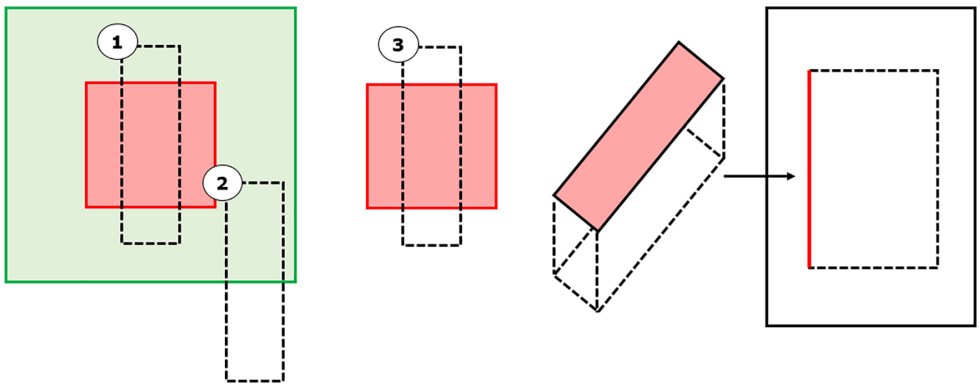

Figure S1 Standardized methodology for cutting histological samples. Left image: from the samples with a lung sealant applied, two standardized samples are taken: ① through the middle of the lesion; and ② with half of the sample comprising healthy, uncovered pleura. Middle image: from the negative control samples, a single sample is taken: ③ straight through the lesion. Right image: all samples are embedded with the pleural side to one direction. Green: patch; red: lesion.

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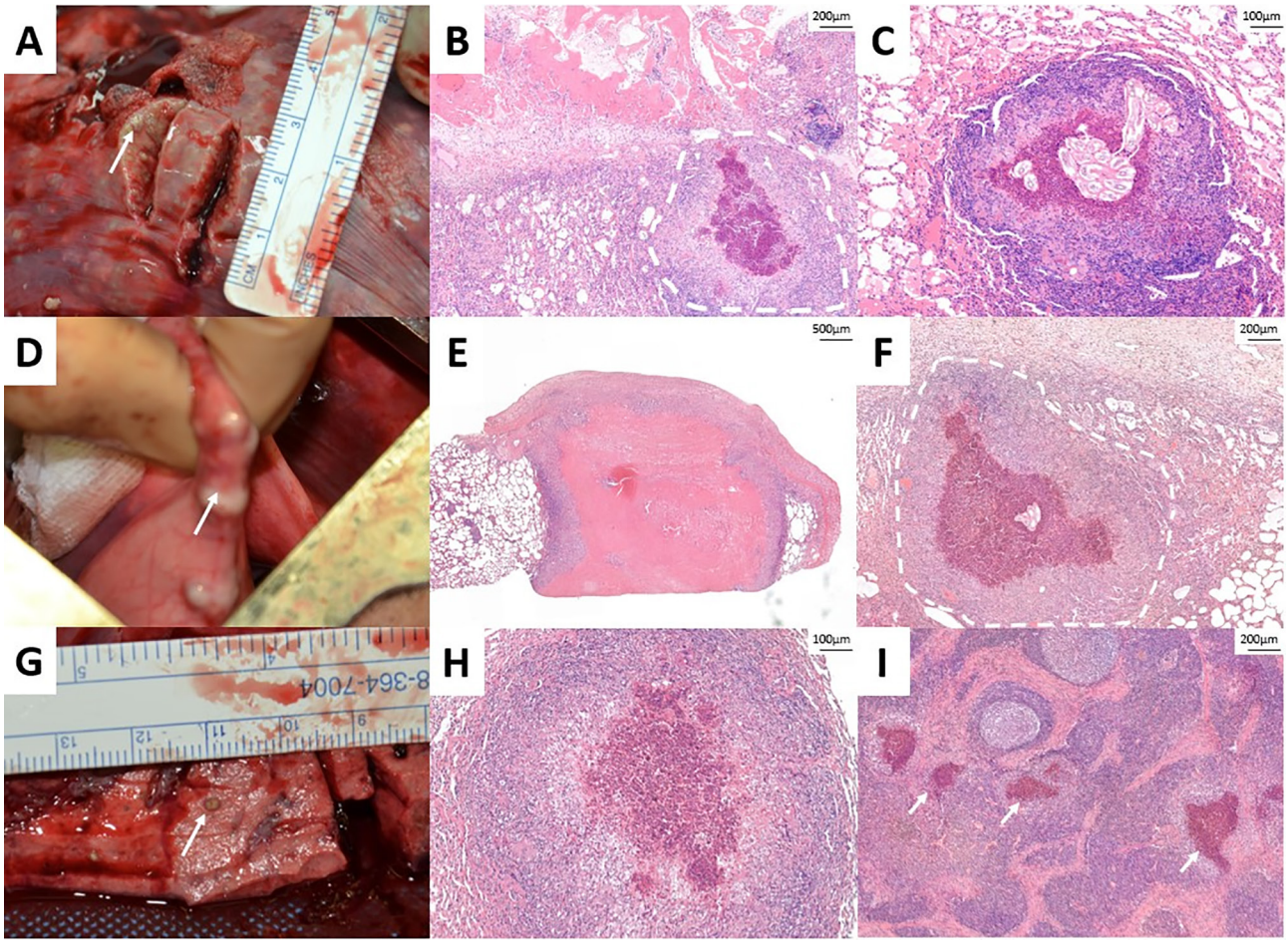

Figure S2 Macro- and microscopic aspects of infiltrates, granulomas and parasitic infestations. (A) Macroscopic infiltrates below fibrin patch sample at 5 days. (B) Aspect of infiltrate of A on histology, showing an eosinophilic granuloma with close relation to the pleura. The immune response of this granuloma, encircled by the white dotted line, is not counted towards the inflammatory response of the sample. (C) Eosinophilic granuloma with associated parasites. (D) Aspect of macroscopic infiltrates during surgery. (E) Aspect of necrotizing granuloma [biopsy of (D)]. (F) Aspect of eosinophilic granuloma close to pleural surface. Immune response in white dotted area is not counted towards the inflammatory response of the sample. (G) Aspect of macroscopic infiltrate found during lung sectioning, confirmed to be an eosinophilic granuloma in (H). (I) Aspect of eosinophilic granulomas (arrows) in lymph node. All samples are stained with H&E. H&E, hematoxylin-eosin.

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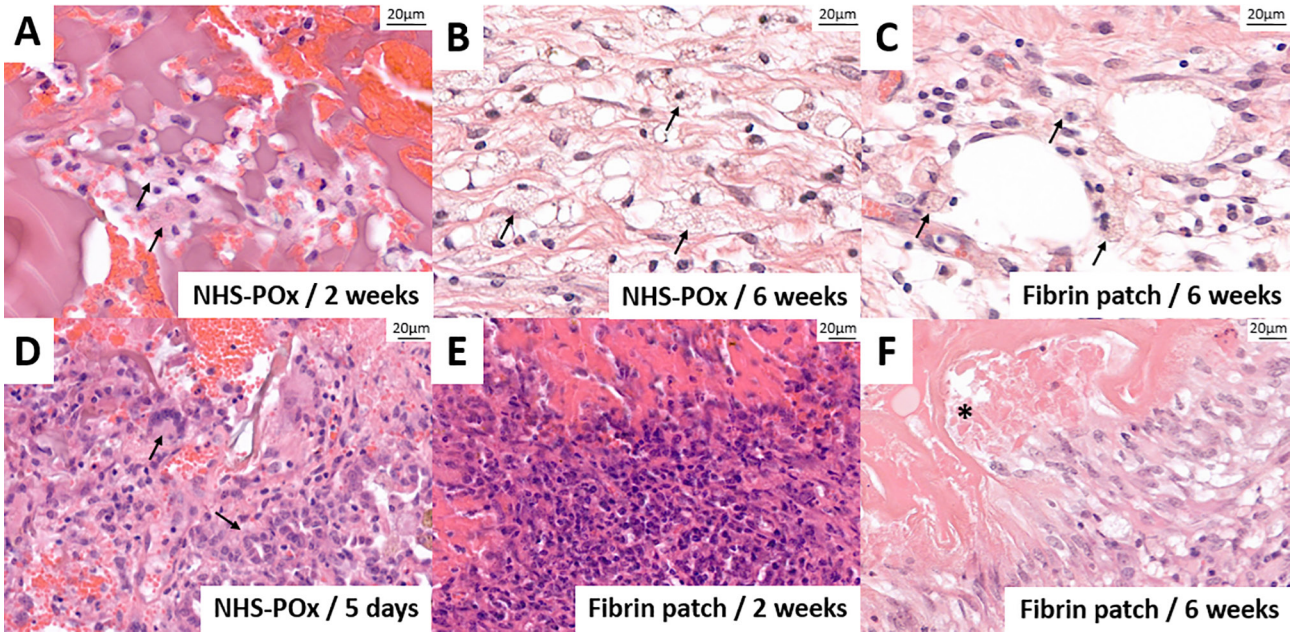

Figure S3 Detailed aspects of histological findings. (A) Aspect of several foamy macrophages associated with NHS-POx remnant material at 2 weeks (arrows). (B) Aspect of foamy macrophages (arrows) and fatty tissue in the pleural scar at 6 weeks in the NHS-POx patch group, no associated material remnants. (C) Aspect foamy macrophages in a fibrin patch sample at 6 weeks. (D) Aspect of horseshoe shaped giant cell (top arrow) and type II pneumocyte proliferation (lower arrow), as seen just below an NHS-POx sample at 5 days. (E) Aspect of adaptive immune response with lymphocytes and plasma cells to a fibrin patch sample at 2 weeks. (F) Aspect of granulomatous reaction with necrosis to fibrin patch remnant (asterisk) at 6 weeks. All samples are stained with H&E. H&E, hematoxylin-eosin.

https://dx.doi.org/10.21037/jtd-24-1733

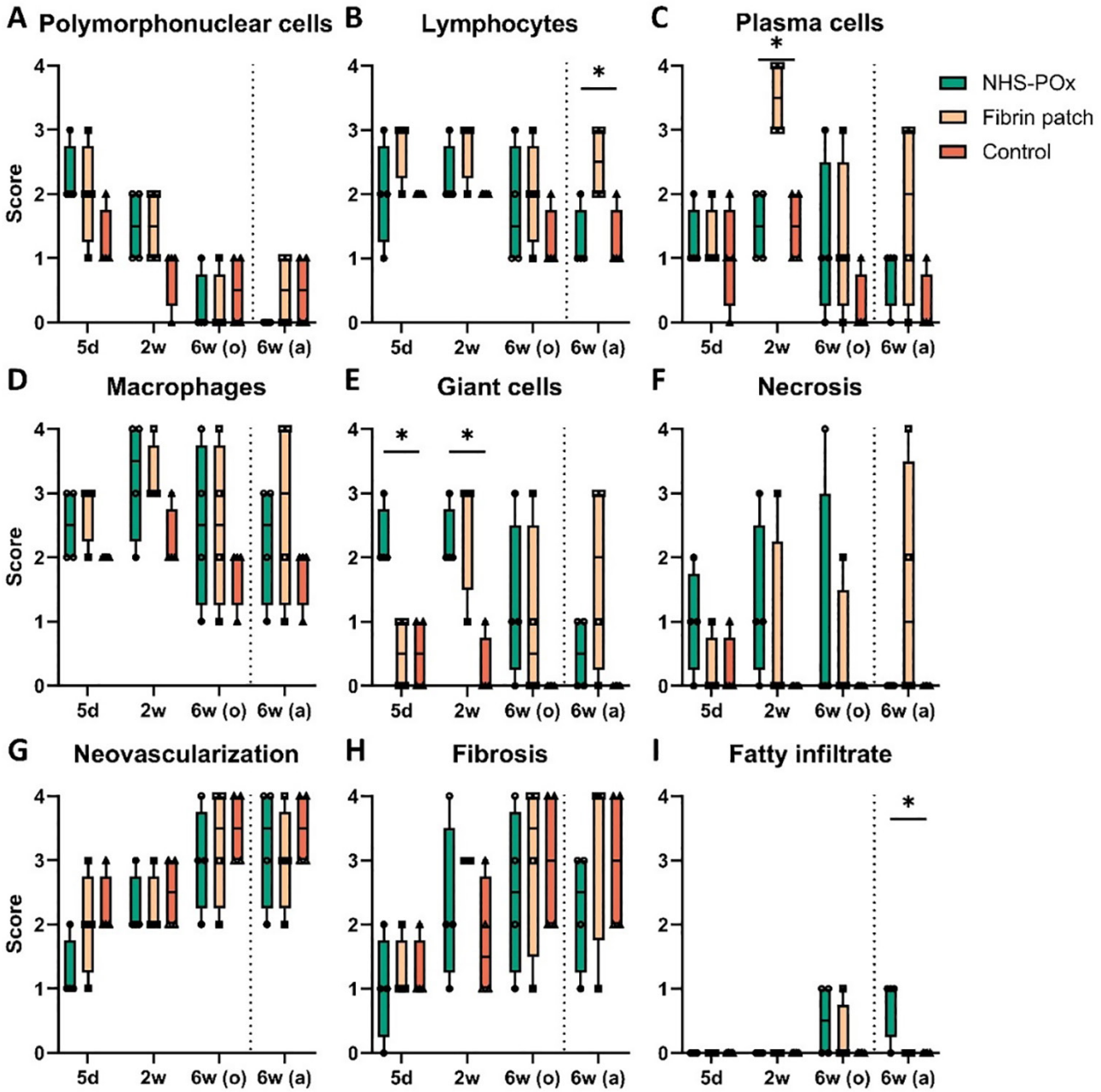

Figure S4 Between-group sub-analysis of histological parameters at different timepoints based on central histological sections. All items are scored on a semi-quantitative scale 0–4. In or directly adjacent to the pleura, cellular response is scored, based on polymorphonuclear cells (A), lymphocytes (B), plasma cells (C), macrophages (D), giant cells (E) and necrosis (F), and biomaterial response is scored based on neovascularization (G), fibrosis (H), and fatty infiltrate (I). Statistical testing performed with Friedman's test. *, P<0.05. a, adapted data; d, day; o, original data; w, week.

https://dx.doi.org/10.21037/jtd-24-1733

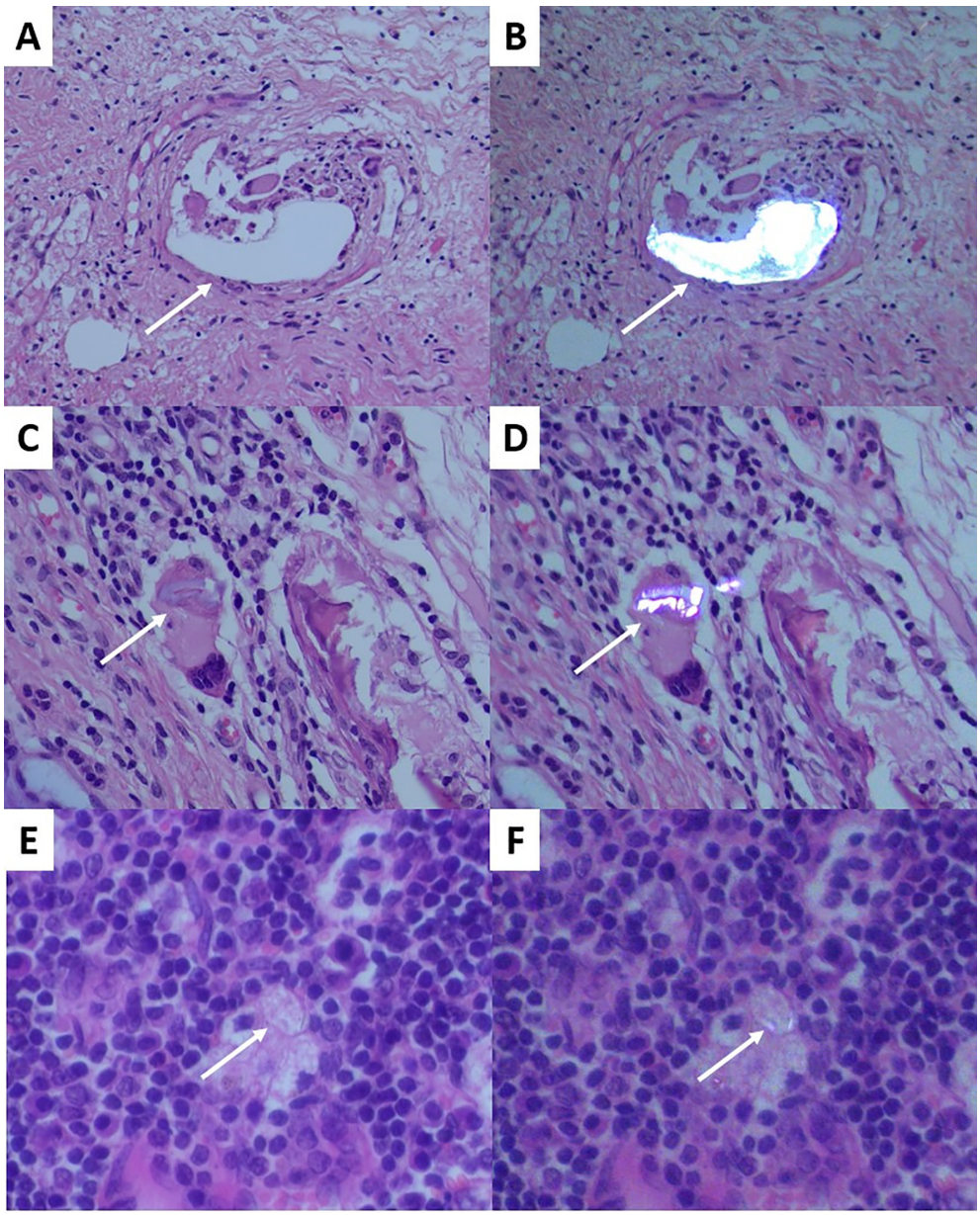

Figure S5 Additional findings on polarized light microscopy. (A) NHS-POx sample at 6 weeks, demonstrating a foreign-body structure with associated giant cells, not clearly visible in the H&E staining, but showing evident birefringence in (B) (after mix-up correction). (C) Fibrin patch sample at 6 weeks, demonstrating a giant cell containing birefringent foreign body material (D). (E) Lymph node at 6 weeks that demonstrates a needle-like birefringent structure in a foamy macrophage (F). These arrows point towards the birefringent structures. H&E, hematoxylin-eosin.

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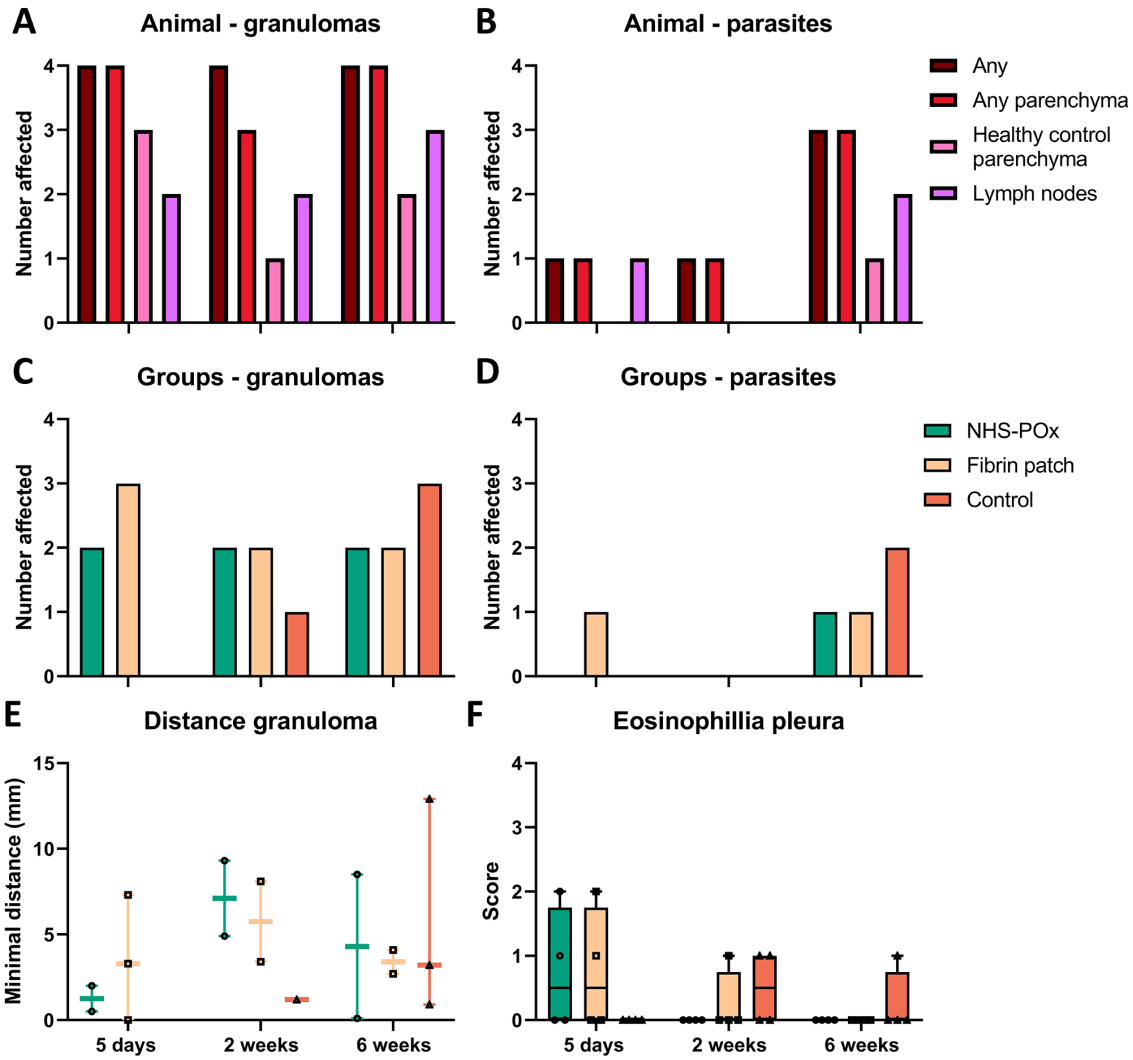

Figure S6 Distribution of chronic granulomatous inflammation and parasitic infestation. (A) Number of animals with presence of (eosinophilic) granulomas per survival term based on different histological sections (any section, any lung parenchyma section, any healthy control parenchyma section, any lymph node section). (B) Number of animals also affected by parasites associated with this granulomatous inflammation in (A). (C) Distribution of (eosinophilic) granulomatous inflammation in central slides of the lesion samples. (D) Number of central slides that also show parasites associated with granulomatous inflammation. (E) Minimal distance from granuloma to pleural interface in central slides. In case of close proximity to pleura, the immune response of the granuloma is not counted towards the histological scoring of the slide (see *Figure S3*). (E) Eosinophilia within the pleural interface. Original and adapted data at 42 days are identical.

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Giant cells at 5 days	P-value
NHS-POx - Fibrin patch	**0.034**
NHS-Pox - Control	**0.034**
Control - Fibrin patch	>0.99
Cell response at 5 days	
NHS-POx - Fibrin patch	0.157
NHS-Pox - Control	**0.005**
Control - Fibrin patch	0.157
Giant cells at 14 days	
NHS-POx - Fibrin patch	0.724
NHS-Pox - Control	0.052
Control - Fibrin patch	**0.022**
Cell response at 14 days	
NHS-POx - Fibrin patch	0.724
NHS-Pox - Control	0.052
Control - Fibrin patch	**0.022**

Total response at 14 days	
NHS-POx - Fibrin patch	0.289
NHS-Pox - Control	0.112
Control - Fibrin patch	**0.008**
Lymphocytes at 42 days	
NHS-POx - Fibrin patch	0.112
NHS-Pox - Control	>0.99
Control - Fibrin patch	0.112
Fatty infiltrate at 42 days	
NHS-POx - Fibrin patch	0.112
NHS-Pox - Control	0.112
Control - Fibrin patch	>0.99
Plasma cells at 14 days	
NHS-POx - Fibrin patch	**0.034**
NHS-Pox - Control	>0.99
Control - Fibrin patch	**0.034**

Chronic at 14 days	
NHS-POx - Fibrin patch	**0.034**
NHS-Pox - Control	>0.99
Control - Fibrin patch	**0.034**
Phago at 5 days	
NHS-POx - Fibrin patch	0.077
NHS-Pox - Control	**0.013**
Control - Fibrin patch	0.480
Phago at 14 days	
NHS-POx - Fibrin patch	>0.99
NHS-Pox - Control	**0.034**
Control - Fibrin patch	**0.034**

Figure S7 Pairwise comparisons with Wilcoxon signed-rank test on all variables that are significant in the between group analysis, P values not adjusted. Cell response = polymorphonuclear cells + lymphocytes + plasma cells + macrophages + giant cells + necrosis. Biomaterial response = neovascularization + fibrosis + fatty infiltrate. Total response = cell response + biomaterial response.

Table S1 Anesthesia protocol in experiment two until experiment twelve.

Moment	Medication	Route	Dose	Frequency
Pre-medication	Midazolam	Intra-muscular	0.7 mg/kg	Once
	Ketamine	Intra-muscular	10 mg/kg	Once
Induction	Propofol	Intra-venous	2 mg/kg	Once
	Ketamine	Intra-venous	1 mg/kg	Once
	Methadone	Intra-venous	0.2 mg/kg	Once
Multimodal anesthesia during surgery	Meloxicam	Intra-muscular	0.5 mg/kg	Once during induction
	Magnesium sulphate	Intra-venous	40 mg/kg	Once (in 20 min)
	Fentanyl	Trans-dermal	100 µg/h	Continuous for 72 h
	Ropivacaine	Intercostal block at three levels	1.5 mg/kg	Once right after thoracotomy
Maintenance	Remifentanil	Intra-venous	0.06 mg/kg/h[1]	Continuous during surgery
	Propofol	Intra-venous	Titrated[1]	Continuous during surgery
	Ketamine	Intra-venous	0.2 mg/kg/h	Continuous during surgery
	Isoflurane	Inhalation	Titrated[1]	Continuous during surgery[2]
Post-operative	Fentanyl	Trans-dermal	100 µg/h	Continuous for 72 h, repeat if required
	Ketamine	Intra-muscular	0.5 mg/kg	In case of discomfort: max 2×/day
	Meloxicam	Oral	0.4 mg/kg	1×/day for five days, phase out if possible
Antibiotic prophylaxis	Amoxicillin	Intra-venous	10 mg/kg	Once before incision
	Ampicillin	Intra-muscular	15 mg/kg	Right after surgery and again after 48 h

[1], doses were titrated to maintain the mean arterial pressure between 50 and 100 mmHg. Noradrenaline is additionally titrated in case of hypotension. [2], inhalation anesthesia is switched of when a lesion is made on the lung.

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Table S2 Anesthesia protocol in the first experiment

Moment	Medication	Route	Dose	Frequency
Pre-medication	Midazolam	Intra-muscular	0.7 mg/kg	Once
	Ketamine	Intra-muscular	10 mg/kg	Once
Induction	Propofol	Intra-venous	2 mg/kg	Once
	Remifentanil	Intra-venous	0.01 mg/kg	Once
Multimodal anesthesia during surgery	Meloxicam	Intra-muscular	0.5 mg/kg	Once during induction
	Lidocaine/bupivacaine 20/5 mg/mL	Intercostal block at three levels	0.4 mL/kg	Once right after thoracotomy
Maintenance	Remifentanil	Intra-venous	0.06 mg/kg/h[1]	Continuous during surgery
	Propofol	Intra-venous	Titrated[1]	Continuous during surgery
	Isoflurane	Inhalation	Titrated[1]	Continuous during surgery[2]
Post-operative	Buprenorphine	Intra-muscular	0.05 mg/kg	Every 12 h for five doses total
	Meloxicam	Oral	0.4 mg/kg	1×/day for five days, phase out if possible
Antibiotic prophylaxis	Amoxicillin	Intra-venous	10 mg/kg	Once before incision
	Ampicillin	Intra-muscular	15 mg/kg	Right after surgery and again after 48 h

[1], doses were titrated to maintain the mean arterial pressure between 50 and 100 mmHg. Noradrenaline is additionally titrated in case of hypotension. [2], inhalation anesthesia is switched of when a lesion is made on the lung.

Table S3 Scoring system to asses cellular and biomaterial response, based on ISO 10993-6:2016 with adjustments made for a semi-quantitative analysis

	Score				
	0	1	2	3	4
Cell type/response					
Polymorphonuclear cells	Absent	Rare	Minimal	Moderate infiltrate	Packed infiltrate
Lymphocytes	Absent	Rare	Minimal	Moderate infiltrate	Packed infiltrate
Plasma cells	Absent	Rare	Minimal	Moderate infiltrate	Packed infiltrate
Macrophages	Absent	Rare	Minimal	Moderate infiltrate	Packed infiltrate
Giant cells	Absent	Rare	Minimal	Moderate infiltrate	Packed infiltrate
Necrosis	None	Minimal	Mild	Moderate	Severe
Biomaterial response					
Neovascularization	None	Minimal capillary proliferation	Groups of capillaries with supporting fibroblastic structures	Broad band of capillaries with supporting fibroblastic structures	Extensive band of capillaries with supporting fibroblastic structures
Fibrosis	None	Narrow band	Moderately thick band	Thick band	Extensive band
Fatty infiltrate	None	Minimal fat associated with fibrosis	Several layers of fat and fibrosis	Elongated and broad accumulation of fat cells	Extensive fat completely surrounding implant

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Table S4 Description of minor deviations from protocol

	Pre-operative symptom	Deviation		
		Index procedure	Lesion/group	Obduction
Term				
5 days (n=4)	Mastitis (n=1)	No ivermectin prophylaxis (n=2)		Mechanical sample manipulation due to extensive bleeding during obduction (n=1)
	Paw infection (n=1)	Increased blood loss from pulmonary ligament laceration (n=1)		Difficult obduction due to severe adhesions, also present at index surgery (n=1)
		Pre-existing adhesions (grade 2-3), torn upper lobe which was sutured, bone wax on rib fracture (E4, n=1)		
14 days (n=4)	Swelling of paw (n=1) and parasternal (n=1)	N/A		N/A
42 days (n=4)	Swelling mammary glands (n=1) and parasternal (n=1)	Pre-existing adhesions, torn middle lobe which was sutured, re-thoracotomy before drain removal for suspected tension pneumothorax (E9, n=1)		Difficult obduction due to severe adhesions, also present at index surgery (n=1)
		Biopsy of infiltrate during index surgery (n=2)		Serosanguinous effusion possibly caused by bleeding during obduction (n=1)
		Delayed anesthesia recovery (n=1)		
Group				
NHS-POx (n=12)			Ventilator on during lesion creation (n=1)	
			Imprecise lesions due to difficult exposure (n=1)	
			Ventilation on during first seconds of application (n=1)	
Fibrin patch (n=12)			Ventilator resumed early during application (n=5)	
			Bubble under patch after application (n=1)	
			Extra pressure applied (n=1)	
			New patch applied after inadequate placement (n=2)	
Negative control (n=12)			Aberrant MLP measurement (n=1)	
			RML not usable due to laceration, placed on right upper lobe (n=1)	
			Accidental aberrant lesion location (RML instead of RLLv) due to complicated procedure (n=1)	

RLLv, right lower lobe ventral aspect; RML, right middle lobe.

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Table S5 Lesion characteristics at baseline

	NHS-POx (n=12)	Fibrin patch (n=12)	Control (n=12)	P value
Location				N/A
RML	3 [25]	4 [33]	4 [33]	
RLLv	5 [42]	3 [25]	3 [25]	
RLLd	4 [33]	5 [42]	3 [25]	
Other	0 [0]	0 [0]	2 [17]	
SBSS grade				0.17
Minimal	1 [8]	0 [0]	2 [17]	
Mild	9 [75]	12 [100]	10 [83]	
Moderate	2 [17]	0 [0]	0 [0]	
Hemostasis time (min)	2.5 [2–8]	2 [2–5]	2 [2–4]	0.17
Macchiarini scale				0.97
0	7 [58]	7 [58]	6 [50]	
I	0 [0]	1 [8]	2 [17]	
II	2 [17]	1 [8]	2 [17]	
III	3 [25]	3 [25]	2 [17]	
MLP				
Measured	5 [42]	4 [33]	5 [42]	0.91
Value (cmH_2O)	8 [6–22]	11 [5–22]	14 [7–17]	N/A

Data presented as number [%] or median [range]. Statistical testing performed with Friedman's test or Cochran's Q test. Not performed on lesion location (equal assignment in randomized allocation) and MLP value (Friedman's test not possible because MLP could not be measured on all lesions per animal). MLP, minimal leakage pressure; RLLd, right lower lobe dorsal aspect; RLLv, right lower lobe ventral aspect; RML, right middle lobe; SBSS, bleeding scale.

Table S6 Leakage capacity based on lesion location

	MLP measured[1]	P value
RML	10/11 [91]	<0.001
RLLv	4/11 [36]	
RLLd	0/12 [0]	
Other	0/2 [0]	

Data are presented as number/total [%]. Statistical testing with Fischer's exact test. [1], MLP can only be measured in case the lesion shows leakage at normal ventilation pressures. MLP, minimal leakage pressure; RLLd, right lower lobe dorsal aspect; RLLv, right lower lobe ventral aspect; RML, right middle lobe.

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Table S7 Description of adverse events and causality to implanted patches

	Adverse event	Causality	Explanation
5 days (n=4)	Groin hematoma (n=1)	Unrelated	Caused by arterial line placement
	Elevated respiratory rate (n=1)	Possible	Parasitic pneumonia, might be exacerbated by patch(es)
	Low appetite (n=1)	Possible	Unknown cause, differential diagnosis: opioids, surgical trauma, anesthetics, systemic inflammation effects of patches, parasitic infection
14 days (n=4)	Incisional seroma (n=2)	Unrelated	Surgical wound complication
	Thickened udder (n=1)	Unrelated	Possibly mastitis
42 days (n=4)	Encapsulated intrathoracic gauze, asymptomatic (n=1)	Unrelated	Gauze left behind during index surgery

Table S8 Between-group comparisons based on central slides of main histological outcomes

	5 d	14 d	42 d (o)	42 d (a)
Polymorphonuclear cells	0.223	0.232	0.717	0.264
Lymphocytes	0.105	0.097	0.368	0.050*
Plasma cells	0.779	0.032*	0.368	0.368
Macrophages	0.097	0.178	0.717	0.717
Giant cells	0.032*	0.038*	0.174	0.174
Necrosis	0.232	0.202	0.607	0.135
Neovascularization	0.150	0.368	0.807	0.807
Fibrosis	0.905	0.223	0.936	0.627
Fatty infiltrate	>0.99	>0.99	0.368	0.050*
Cell response	0.018*	0.038*	0.257	0.223
Biomaterial response	0.424	0.420	0.936	0.936
Total response	0.368	0.022*	0.607	0.526
Chronic inflammation (plasma cells + lymphocytes)	0.116	0.032*	0.150	0.082
Phagocytosis (macrophages + giant cells)	0.039*	0.050*	0.319	0.424
Neovascularization + fibrosis	0.424	0.420	0.936	0.936
Healing score	0.223	0.905	0.368	0.368
Microscopic biodegradation	0.317	0.141	0.655	0.180

Data are show in P values. Cell response = polymorphonuclear cells + lymphocytes + plasma cells + macrophages + giant cells + necrosis. Biomaterial response = neovascularization + fibrosis + fatty infiltrate. Total response = cell response + biomaterial response. The details are shown in Figure S7. *, statistically significant values (P<0.05). a, adapted data; d, day; o, original data; w, week.

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Table S9 Within-group comparisons based on central *vs.* pleural overlap slides on main histological outcome measures

	NHS-POx				Fibrin patch			
	5 d	14 d	42 d (o)	42 d (a)	5 d	14 d	42 d (o)	42 d (a)
Polymorphonuclear cells	0.157	0.083	0.317	0.317	0.655	>0.99	0.102	0.102
Lymphocytes	0.564	>0.99	0.157	0.157	0.102	0.317	0.317	0.317
Plasma cells	>0.99	>0.99	0.317	0.317	0.317	0.157	0.180	0.180
Macrophages	0.564	0.157	0.317	>0.99	0.414	0.317	>0.99	0.655
Giant cells	0.414	0.414	0.564	>0.99	0.655	0.317	>0.99	0.317
Necrosis	0.102	0.180	0.317	>0.99	0.317	0.564	0.317	0.157
Neovascularization	>0.99	0.157	0.157	0.564	0.102	0.157	>0.99	0.317
Fibrosis	>0.99	0.180	0.276	0.157	0.317	0.157	0.655	>0.99
Fatty infiltrate	>0.99	>0.99	0.157	0.083	>0.99	>0.99	>0.99	0.317
Cell response	0.197	0.063	0.414	0.414	0.461	>0.99	0.581	0.581
Biomaterial response	>0.99	0.180	0.461	0.581	0.102	>0.99	0.655	0.655
Total response	0.461	0.276	0.414	0.593	0.285	0.655	0.854	0.705
Chronic inflammation (plasma cells + lymphocytes	0.564	>0.99	0.102	0.180	0.102	0.564	0.180	0.180
Phagocytosis (macrophages + giant cells)	0.414	0.102	>0.99	>0.99	0.785	0.180	>0.99	0.414
Neovascularization + fibrosis	>0.99	0.180	0.276	0.276	0.102	>0.99	>0.99	>0.99
Healing score	0.564	0.317	>0.99	>0.99	0.317	>0.99	>0.99	0.317

Data are show in P values. Cell response = polymorphonuclear cells + lymphocytes + plasma cells + macrophages + giant cells + necrosis. Biomaterial response = neovascularization + fibrosis + fatty infiltrate. Total response = cell response + biomaterial response. The details are shown in *Figure S7*. a, adapted data; d, day; o, original data; w, week.

Table S10 Between group comparisons based on macroscopic findings

	5 d	14 d	42 d (o)	42 d (a)
Adhesion presence	0.264	0.368	0.264	0.264
Adhesion severity	0.264	0.867	0.264	0.264
Macroscopic biodegradation	>0.99	0.180	0.564	0.083

Data are show in P values. a, adapted data; d, day; o, original data; w, week.

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Table S11 Quantitative synthesis of qualitative histological findings on additional histological samples

Term	Sample location	N	Qualitative finding(s)
5 days	Lung biopsy without lesion	4	Thickening/fibrosis of pleura (n=1, 25%)
			Irritated pleura[2] (n=2, 50%)
			Granuloma (n=3, 75%)
	Lymph node[1]	8	Granuloma (n=3, 37.5%)
			Parasites associated with granuloma (n=1, 12.5%)
	Parietal pleura biopsy[3]	9	Irritated pleura[2] (n=3, 33.3%)
	Infiltrate (obduction)	3	Thickening/fibrosis of pleura (n=1, 33%)
			Irritated pleura[2] (n=1, 33%)
			Granuloma (n=2, 67%)
	Extra patch biopsy	1	NHS-POx patch with underlying granulation tissue and fibrinous exudate (n=1, 100%)
14 days	Lung biopsy without lesion	4	Thickening/fibrosis of pleura (n=4, 100%)
			Irritated pleura[2] (n=3, 75%)
			Granuloma (n=1, 25%)
			Bronchopneumonia (n=1, 25%)
	Lymph node[1]	3	Granuloma (n=2, 67%)
	Parietal pleura biopsy[3]	24	Thickening/fibrosis of pleura (n=11, 45.8%)
			Irritated pleura[2] (n=17, 70.8%)[4]
	Infiltrate (obduction)	5	Thickening/fibrosis of pleura (n=5, 100%)
			Irritated pleura[2] (n=5, 100%)
			Granuloma (n=2, 40%)
			Parasites associated with granuloma (n=1, 20%)
	Extra patch biopsy	1	NHS-POx patch with lymphohistiocytic reaction. Presence of necrotic fatty and striated muscle tissue, likely originating from parietal pleura (n=1)
42 days	Lung biopsy without lesion	4	Thickening/fibrosis of pleura (n=4, 100%)
			Granuloma (n=2, 50%)
			Parasites associated with granuloma (n=1, 25%)
	Lymph node[1]	8	Granuloma (n=4, 50%)
			Parasites associated with granuloma (n=2, 25%)
	Parietal pleura biopsy[3]	23	Thickening/fibrosis of pleura (n=8, 34.8%)[4]
			Irritated pleura[2] (n=3, 13%)
	Infiltrate (obduction)	9	Thickening/fibrosis of pleura (n=3, 33%)
			Granuloma (n=8, 89%). One sample was not a granuloma, but necrotic/bloody tissue with macrophages
			Parasites associated with granuloma (n=2, 22%)
	Infiltrate (index surgery)	2	Granuloma (n=2, 100%)

[1], relevant nodes of 2/4/10/11R. [2], based on immune cell infiltration, fibrinoid pleuritis, reactive mesothelial cells, neovascularization. [3], apical, lateral, costodiaphragmatic recess, across NHS-POx, fibrin patch and control lesion samples. [4], in two parietal pleura samples (n=1 at 14 and n=1 at 42 days), the inflammatory response was also noted in the intercostal muscles.

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Table S12 Between animal comparisons of chronic granulomatous infections and parasitic infestations

	Granuloma	Parasites
Any section	N/A	0.455
Any parenchyma	>0.99	0.455
Healthy parenchyma	0.766	>0.99
Lymph node	>0.99	0.709

Data are show in P values.

Table S13 Between group comparisons of chronic granulomatous infections and parasitic infestations

	5 d	14 d	42 d (o)	42 d (a)
Granuloma	0.097	0.607	0.607	0.607
Parasites	0.368	N/A	0.717	0.717
Distance (granuloma – pleura)	0.655	0.368	0.368	0.368
Eosinophilia	0.135	0.223	0.368	0.368

Data are show in P values. a, adapted data; d, day; o, original data; w, week.

https://dx.doi.org/10.21037/jtd-24-1733