Online Supplement

Chymotrypsin-like Elastase-1 Mediates Progressive Emphysema in Alpha-1 Antitrypsin Deficiency

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Supplemental Table 1: Genes 2-fold increased or decreased protein abundance in AAT-deficient vs wildtype lungs

Two-fold reduced in AAT-deficient compared to wildtype	adjusted p-value	log(AAT/WT)
Alpha-1-antitrypsin 1-4	0.005402969	-7.658939793
Alpha-1-antitrypsin 1-1	0.104629553	-4.127926977
Alpha-1-antitrypsin 1-2	0.24021247	-3.840431444
CapZ-interacting protein	0.76976043	-2.962319502
Phosphatidylinositol 5-phosphate 4-kinase type-2 alpha	0.710098202	-2.818698064
Ubiquinone biosynthesis monooxygenase COQ6, mitochondrial	0.548562953	-2.526899803
USP6 N-terminal-like protein	0.76976043	-2.320869555
Geranylgeranyl pyrophosphate synthase	0.635451325	-2.241314546
Histone H2A type 1-F	0.710098202	-1.869754004
Nuclear autoantigen Sp-100	0.716466787	-1.680303091
CD99 antigen	0.706555434	-1.256946612
[Pyruvate dehydrogenase (acetyl-transferring)] kinase isozyme 2,	0.76976043	-1.215477898
Non-histone chromosomal protein HMG-14	0.76976043	-1.201008833
Ras-specific guanine nucleotide-releasing factor 2	0.76976043	-1.167508425
cAMP-dependent protein kinase type II-beta regulatory subunit	0.670739978	-1.100712009
Ig heavy chain V region AC38 205.12	0.772417645	-1.055441928
Enhancer of rudimentary homolog	0.635451325	-1.036661021

Two-fold increased in AAT-deficient compared to wildtype	adjusted p-value	log(AAT/WT)
Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 2	0.635451325	4.356620473
Septin-5	0.814392232	2.569137722
Mannose-6-phosphate isomerase	0.814392232	2.409962705
Endoplasmic reticulum metallopeptidase 1	0.706555434	2.202271146
SLIT-ROBO Rho GTPase-activating protein 2	0.814392232	2.155599029
Carboxypeptidase Q	0.814392232	2.118892851
Golgin subfamily A member 2	0.814392232	2.110520803
Collagen alpha-2(V) chain	0.814392232	2.050161614
Plasma kallikrein	0.814392232	2.037018714
Transmembrane protein 256	0.787335326	1.999844798
Ubiquitin-like domain-containing CTD phosphatase 1	0.814392232	1.983197913
Active breakpoint cluster region-related protein	0.710098202	1.964948073
Zinc finger and BTB domain-containing protein 20	0.836830423	1.922906569
Matrix metalloproteinase-9	0.814392232	1.899151387
Gamma-interferon-inducible lysosomal thiol reductase	0.814392232	1.84450418
Serum amyloid A-4 protein	0.814392232	1.79859424
Complement C2	0.710098202	1.791135782
Prefoldin subunit 3	0.799651701	1.76560095
Alpha-1-acid glycoprotein 1	0.786781732	1.746757615
Dynein light chain Tctex-type 3	0.799651701	1.653570064
Secretory carrier-associated membrane protein 1	0.836830423	1.631691006
SH3 domain-containing kinase-binding protein 1	0.799651701	1.626019142
Mitochondrial import receptor subunit TOM40 homolog	0.814392232	1.601646268
Cytoplasmic dynein 2 heavy chain 1	0.793717161	1.597597047
IST1 homolog	0.814392232	1.587979889
Plakophilin-2	0.706555434	1.575039769
Aldehyde oxidase 1	0.814392232	1.517322151
Double-strand-break repair protein rad21 homolog	0.836830423	1.506272632
Disks large-associated protein 4	0.814392232	1.476735135
RNA helicase aquarius	0.799651701	1.460264526
AP-1 complex subunit mu-2	0.836830423	1.363440153
Centrin-2	0.799651701	1.359339453
Epidermal growth factor receptor	0.832446546	1.358962695
Conserved oligomeric Golgi complex subunit 1	0.814392232	1.348375208
Volume-regulated anion channel subunit LRRC8C	0.814392232	1.330912411
BolA-like protein 2	0.814392232	1.316034624
Bifunctional epoxide hydrolase 2	0.814392232	1.31568963
C-type lectin domain family 11 member A	0.706555434	1.284167018
DnaJ homolog subfamily B member 11	0.836830423	1.279985812
Actin-related protein 2/3 complex subunit 5-like protein	0.814392232	1.272532949
Transmembrane protein 214	0.799651701	1.25521081
Retinoid-inducible serine carboxypeptidase	0.814979184	1.253613023
H-2 class II histocompatibility antigen gamma chain	0.814392232	1.250222229
Protein AMBP	0.848218401	1.24815933
Collagen alpha-1(VIII) chain	0.814392232	1.243450557
Ragulator complex protein LAMTOR5	0.841242473	1.234339674
Interferon-induced helicase C domain-containing protein 1	0.814392232	1.228872136
WD repeat-containing protein 82	0.814392232	1.208879749
E3 ubiquitin-protein ligase HECTD1	0.814392232	1.205309685
Serine protease 56	0.821064821	1.197847018
AH receptor-interacting protein	0.832446546	1.197386863
Haptoglobin	0.76976043	1.188611838
U6 snRNA-associated Sm-like protein LSm2	0.814392232	1.168714164
Neutrophil gelatinase-associated lipocalin	0.814392232	1.152929464
Charged multivesicular body protein 2a	0.799651701	1.146736093
GTP-binding protein Rit1	0.849719788	1.140625331
Polyprenol reductase	0.814392232	1.139162093
Integrin alpha-M	0.814392232	1.111257911
Bromodomain-containing protein 4;Bromodomain-containing protein 3	0.814392232	1.094684836

Supplemental Table 2: Genes 2-fold increased or decreased protein abundance in Cela1&AAT-deficient vs AAT-deficient lungs

Two-fold deceased in Cela1&AAT-deficient compared to AAT-deficient	adjusted p-value	log(DKO/AAT)
Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 2	0.635451325	-4.356620473
Septin-5	0.814392232	-2.569137722
Mannose-6-phosphate isomerase	0.814392232	-2.409962705
Zinc finger and BTB domain-containing protein 20	0.836830423	-1.922906569
Matrix metalloproteinase-9	0.814392232	-1.899151387
Serum amyloid A-4 protein	0.814392232	-1.79859424
Prefoldin subunit 3	0.799651701	-1.76560095
Alpha-1-acid glycoprotein 1	0.786781732	-1.746757615
Plakophilin-2	0.706555434	-1.575039769
Double-strand-break repair protein rad21 homolog	0.836830423	-1.506272632
Disks large-associated protein 4	0.814392232	-1.476735135
BolA-like protein 2	0.814392232	-1.316034624
C-type lectin domain family 11 member A	0.706555434	-1.284167018
Actin-related protein 2/3 complex subunit 5-like protein	0.814392232	-1.272532949
Serine protease 56	0.821064821	-1.197847018
U6 snRNA-associated Sm-like protein LSm2	0.814392232	-1.168714164
Bromodomain-containing protein 4;Bromodomain-containing protein 3	0.814392232	-1.094684836
Vacuolar protein sorting-associated protein VTA1 homolog	0.835350159	-0.966865802
Tumor protein p63-regulated gene 1-like protein	0.814392232	-0.963464717

Two-fold increased in Cela1&AAT-deficient compared to AAT-deficient	adjusted p-value	log(DKO/AAT)
USP6 N-terminal-like protein	0.76976043	2.320869555
RNA-binding motif protein, X chromosome	0.814392232	2.28054377
Geranylgeranyl pyrophosphate synthase	0.635451325	2.241314546
Septin-10	0.814392232	2.171613508
Polyadenylate-binding protein 2	0.814392232	1.76735785
Protein phosphatase 1 regulatory subunit 12B	0.814392232	1.757948104
Homer protein homolog 3	0.814392232	1.751469843
PRKC apoptosis WT1 regulator protein	0.814392232	1.624204285
E3 ubiquitin-protein ligase Itchy	0.814392232	1.606612821
Alpha-1,3/1,6-mannosyltransferase ALG2	0.814392232	1.592098663
Nostrin	0.814392232	1.580235527
NHP2-like protein 1	0.814392232	1.535833642
ER membrane protein complex subunit 7	0.814392232	1.534010735
Dynactin subunit 3	0.836830423	1.481142994
Equilibrative nucleobase transporter 1	0.814392232	1.443035311
60S ribosomal protein L36	0.816225412	1.425669401
LIM and cysteine-rich domains protein 1	0.814392232	1.398942749
Sphingosine-1-phosphate lyase 1	0.814392232	1.377943636
Palmdelphin	0.814392232	1.365293779
Cytoglobin	0.814392232	1.280099477
Serine protease HTRA2, mitochondrial	0.821183794	1.219441125
Vacuolar protein sorting-associated protein 33A	0.847893608	1.215416973
Oxidation resistance protein 1	0.814392232	1.194977614
PTB domain-containing engulfment adapter protein 1	0.836830423	1.068995294
Ig heavy chain V region AC38 205.12;Ig heavy chain V region J558;Ig heavy chain V region MO	0.772417645	1.055441928
Calcineurin B homologous protein 1	0.836830423	1.053178882
TIP41-like protein	0.836830423	1.04728927
Malectin	0.833615817	0.981350537

Supplemental Figure Titles and Legends

Figure S1: Titration in Lipopolysacharide Model of AAT-deficient Emphysema. (A) Middle lobe sections of AAT-deficient mice treated with PBS (B) 1 unit of LPS at day 1 and 0.5 units at day 10 (C) 2 and 1 units, (D) 5 & 2.5 units, and (E) 10 & 5 units. (F) Middle lobes of wildtype (WT) mice treated with 10 & 5 units of LPS. (G) Airspace diameter percentile values of tile scanned lung lobe sections of the mice above showing that emphysema was no worse in *AAT*-deficient mice than WT.

Figure S2: Titration of tracheal PPE in *AAT*-deficient Mice. Tile scanned images of *AAT*-deficient (AAT) and wildtype (WT) mice 21 days after different doses of porcine pancreatic elastase (PPE). AAT mice had evidence of substantial emphysema at the lowest tested dose (0.25 units).

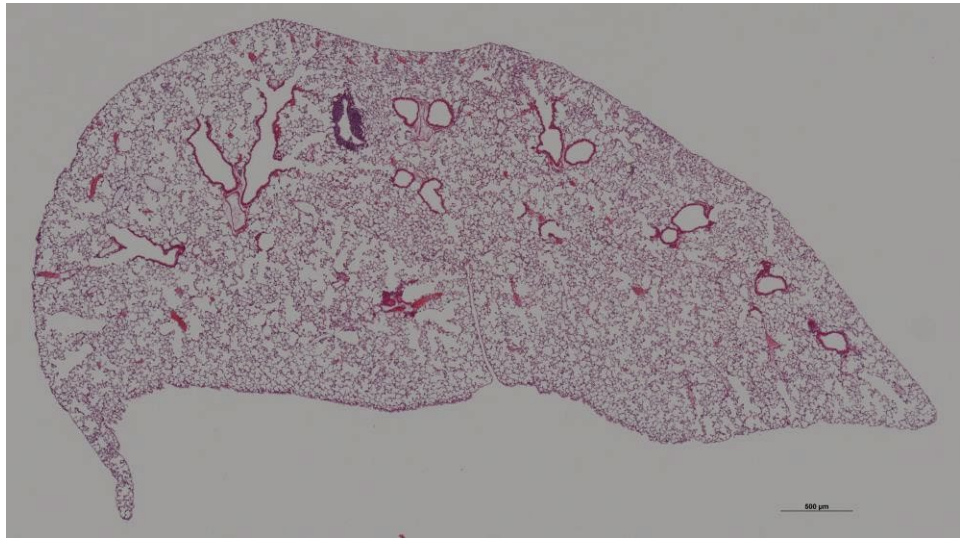
Figure S3: Complete Protein-protein Interaction Network for $AAT^{-/-}$ vs wildtype mice. The complete protein-protein interaction network of proteins with significantly different abundance between *AAT*-deficient and wildtype mouse lungs 42 days after low-dose tracheal PPE.

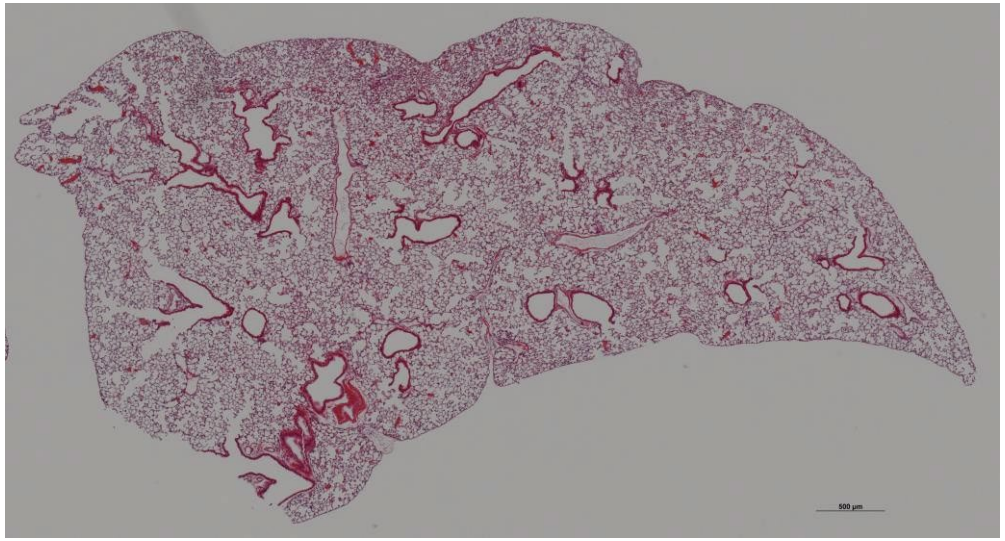
Figure S4: Complete Protein-protein Interaction Network for $AAT^{-/-}$&$Cela1^{-/-}$ mice vs $AAT^{-/-}$ mice. The complete protein-protein interaction network of proteins with significantly different abundance between *AAT-/-&Cela1*$^{-/-}$ vs $AAT^{-/-}$mouse lungs 42 days after low-dose tracheal P

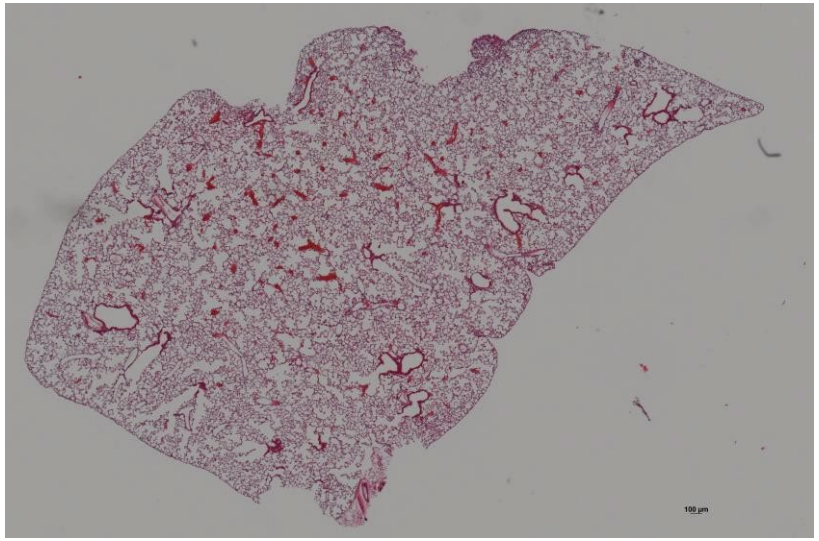
Supplemental Figure 1

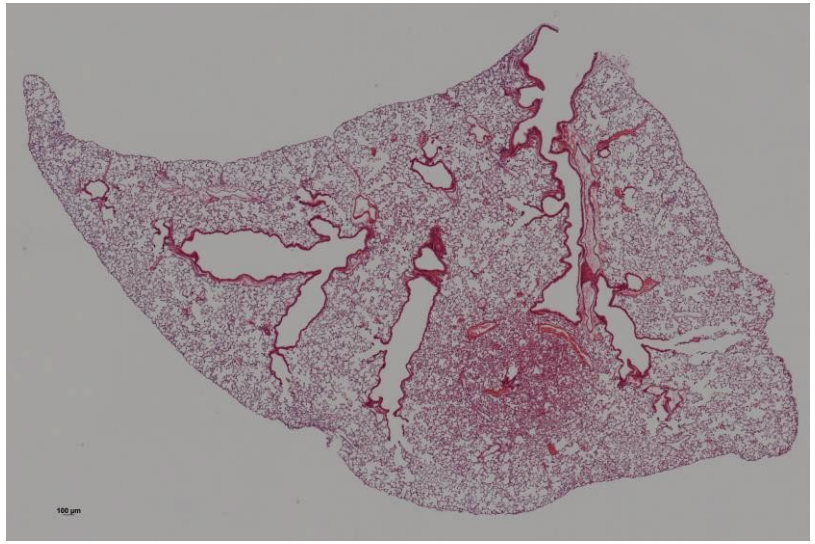
AAT KO PBS

A



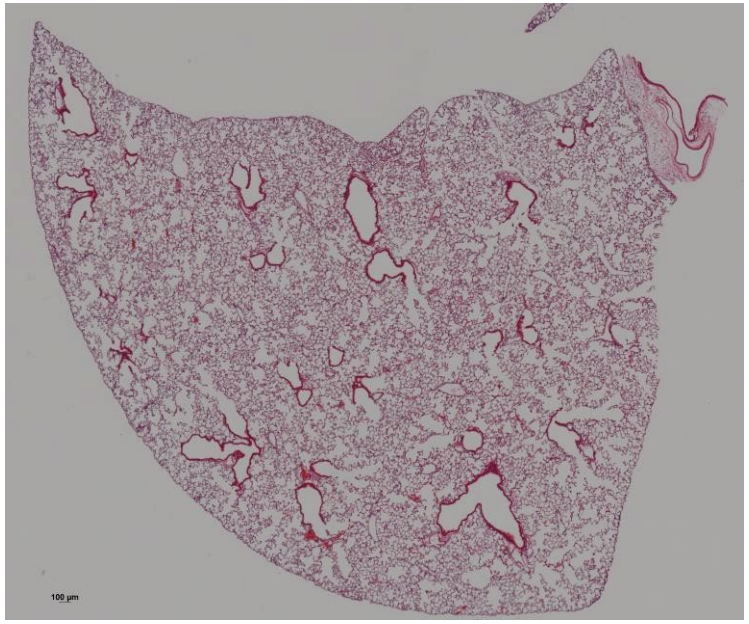


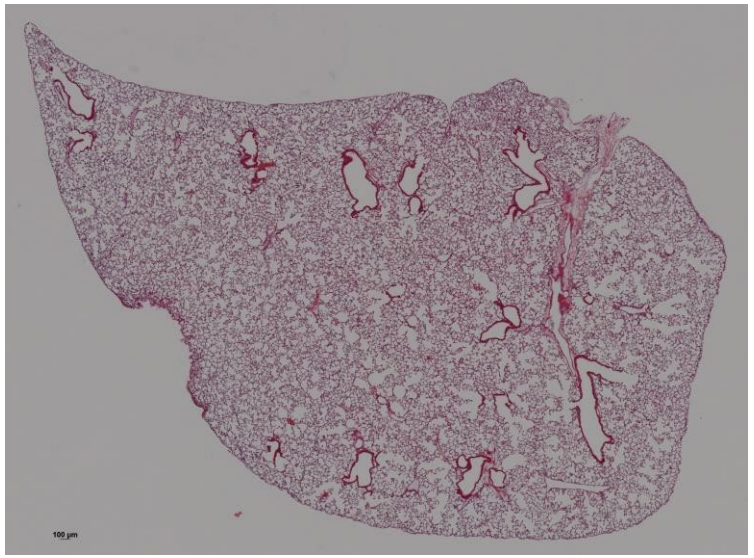


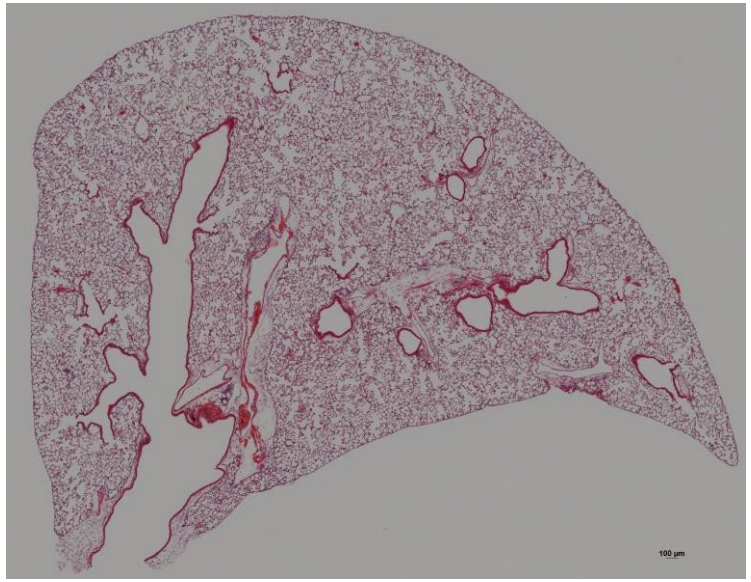


WT 21 days 1&0.5 Units LPS

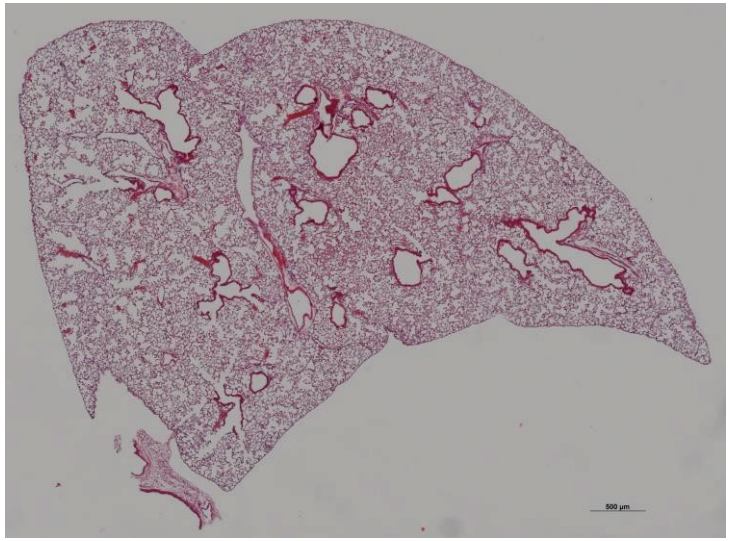
B

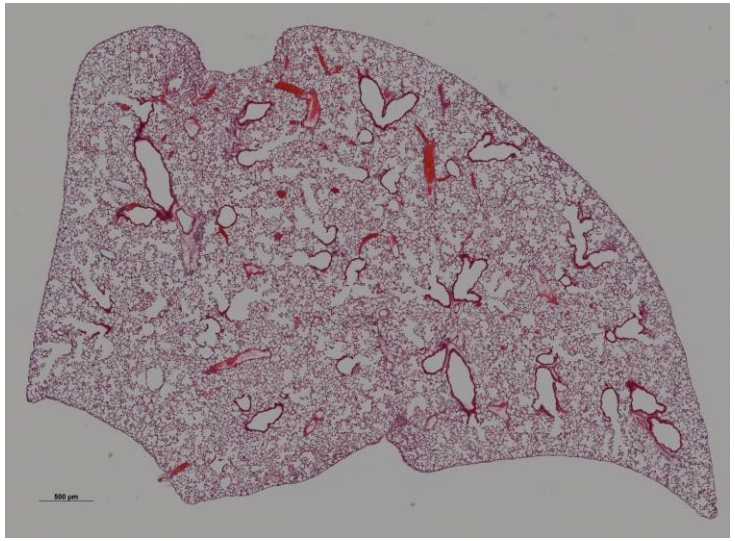


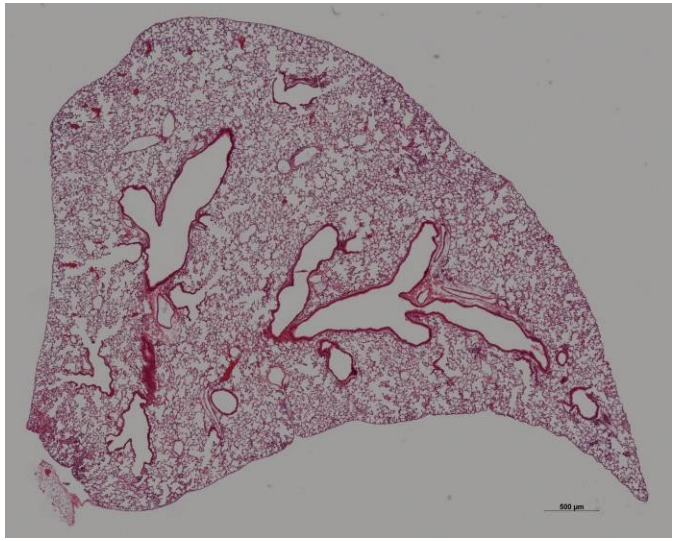


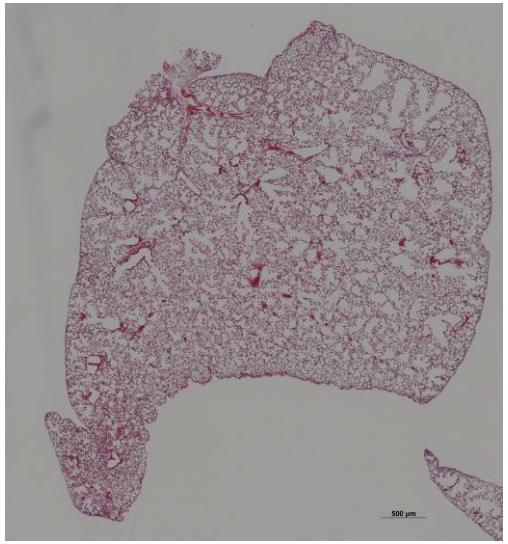


AAT KO 21 days 1&0.5 Units LPS



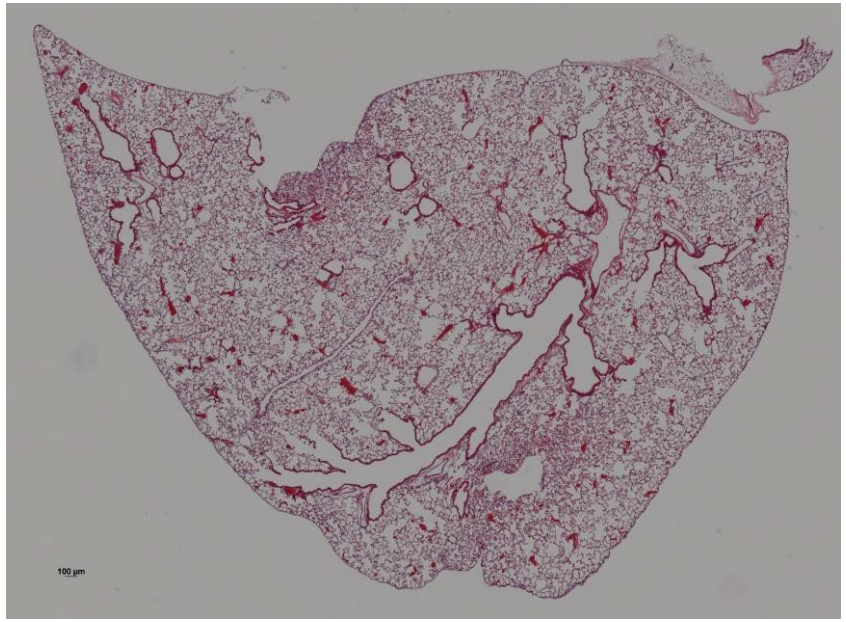


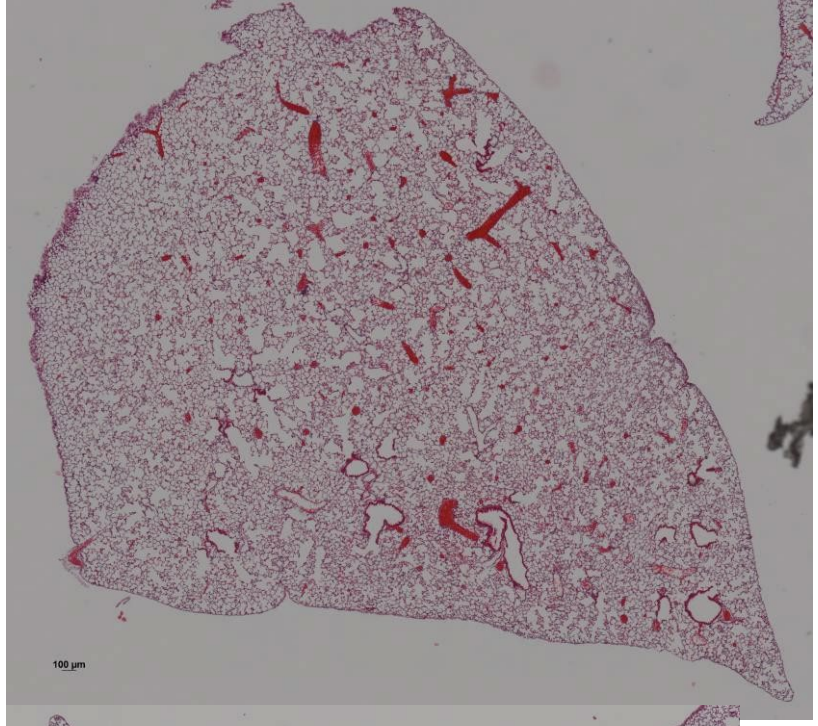


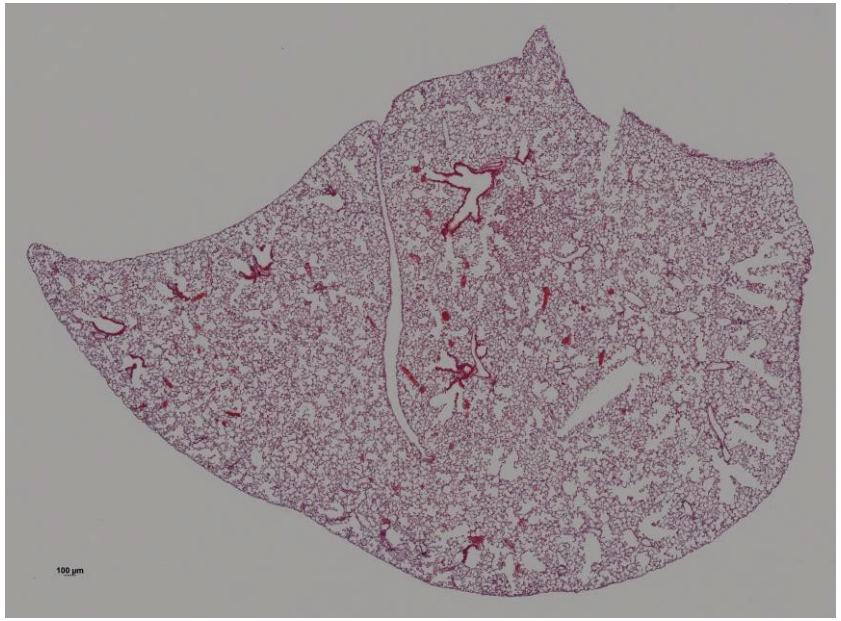


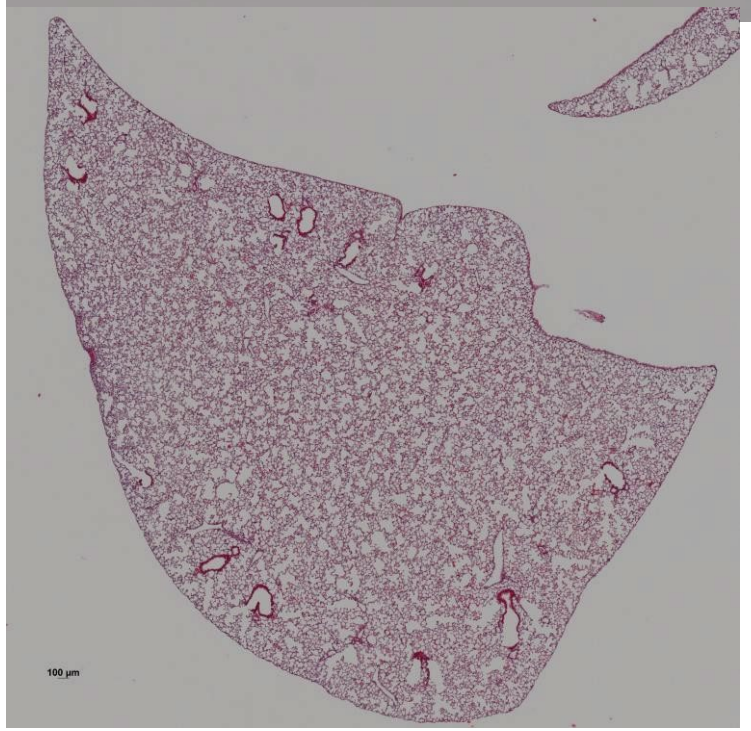
C

AAT KO 2&1 Units LPS



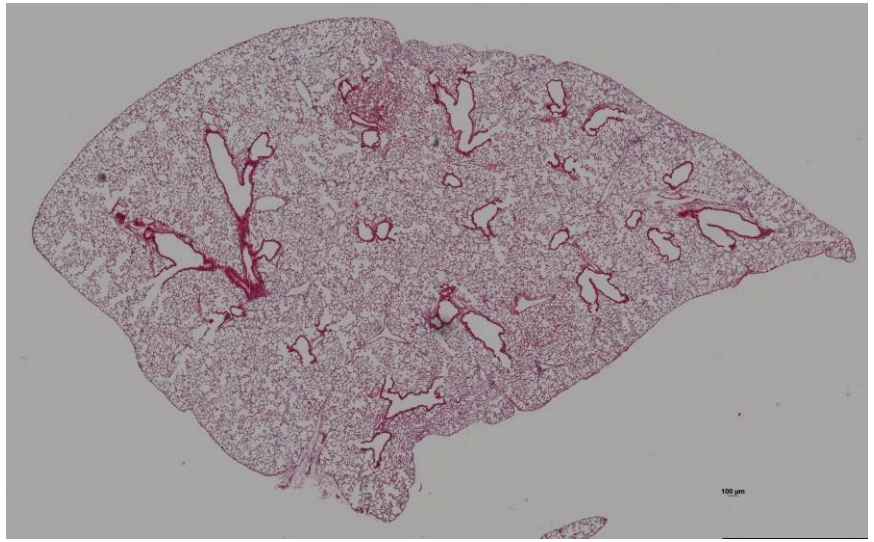


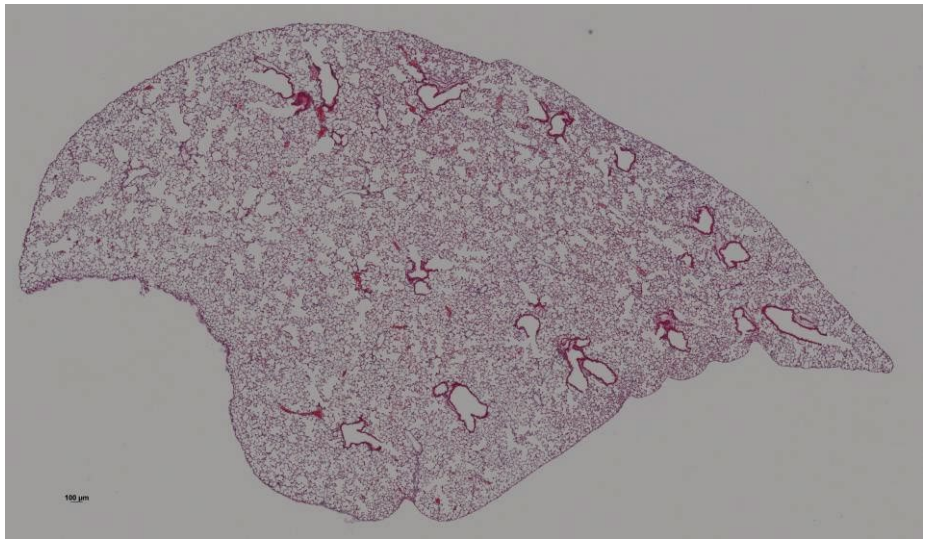


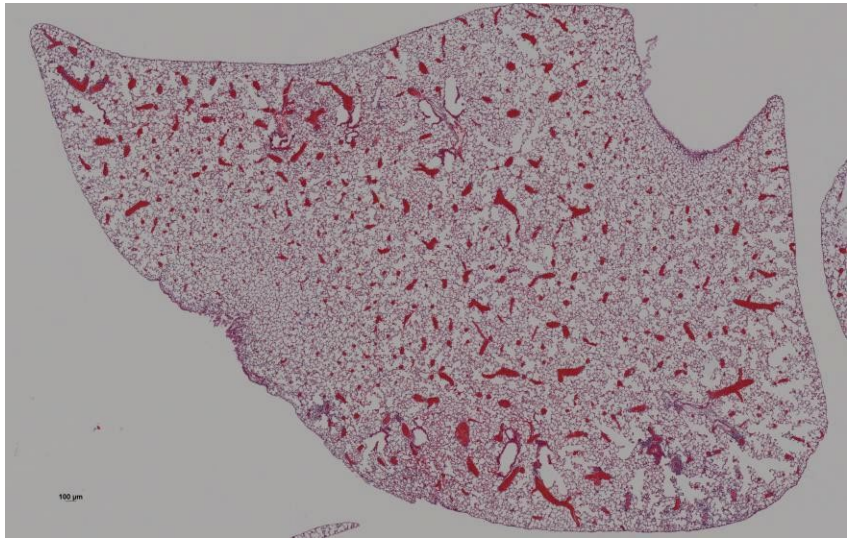


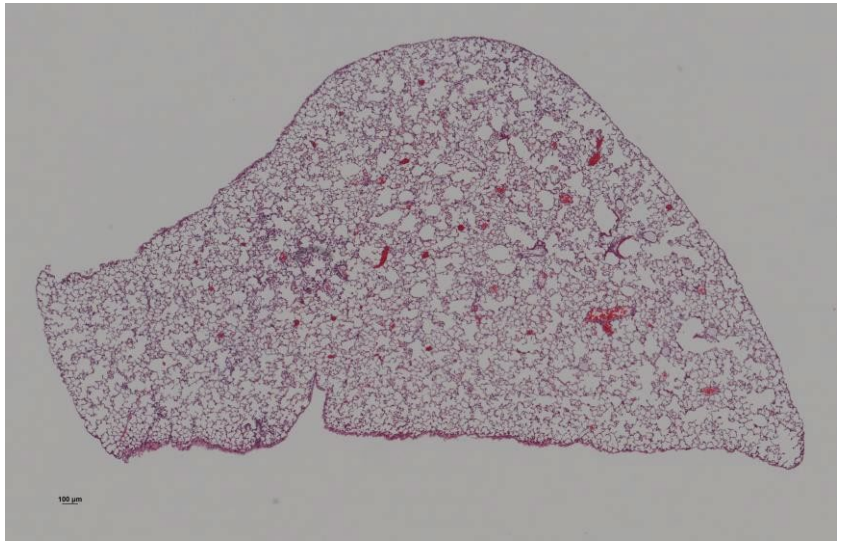
AAT KO 5&2.5 Units LPS

D



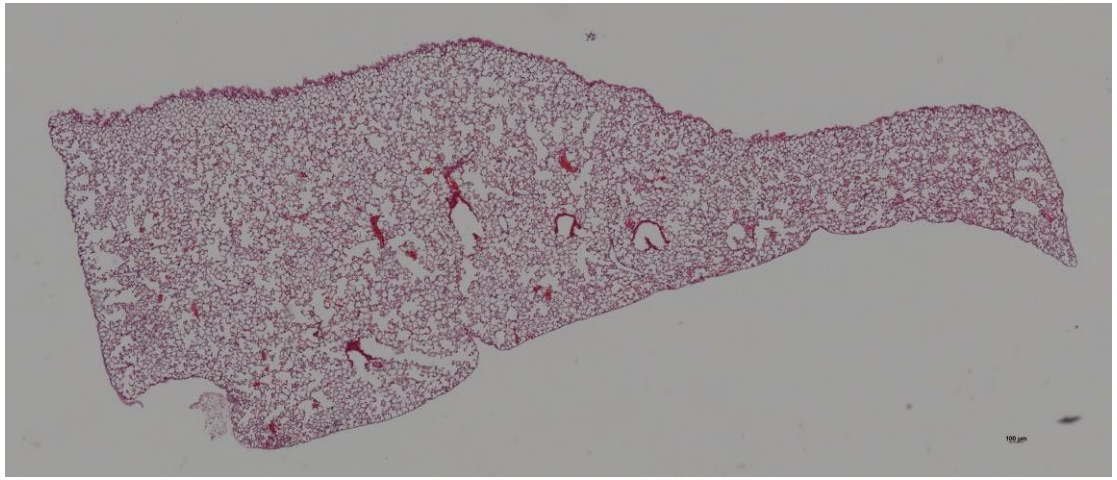


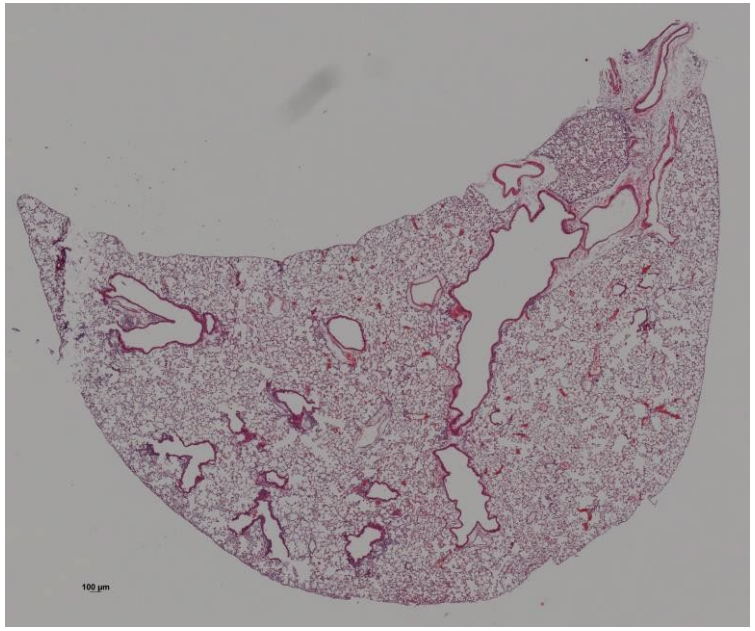


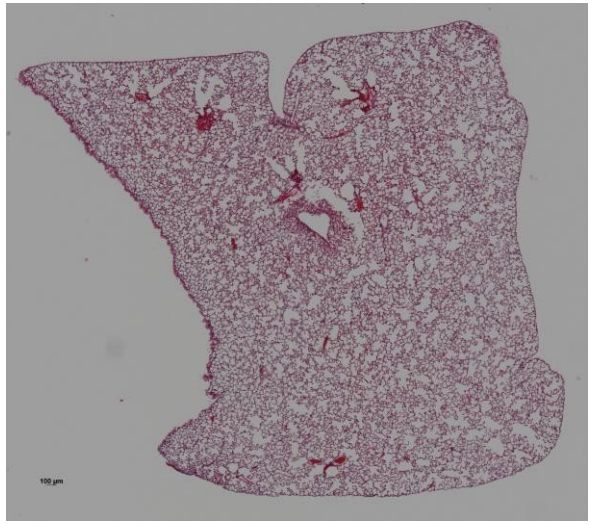


E

AAT KO 10&5 Units LPS

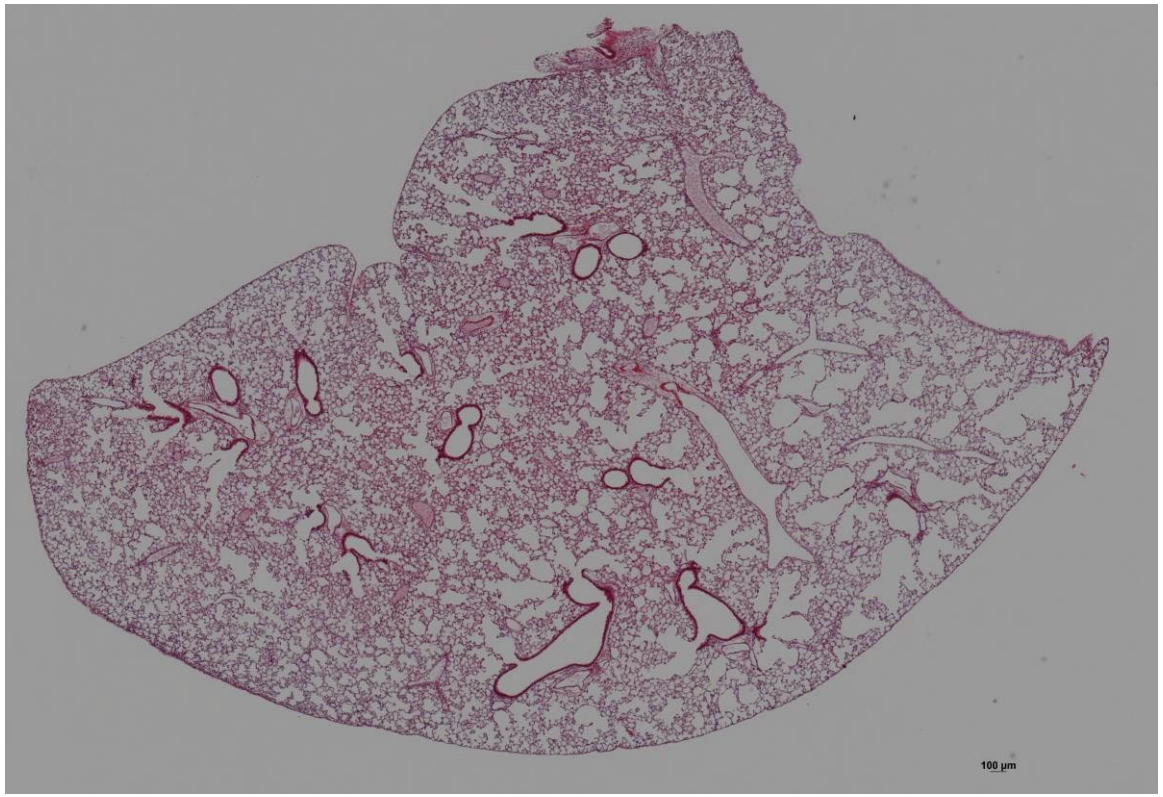


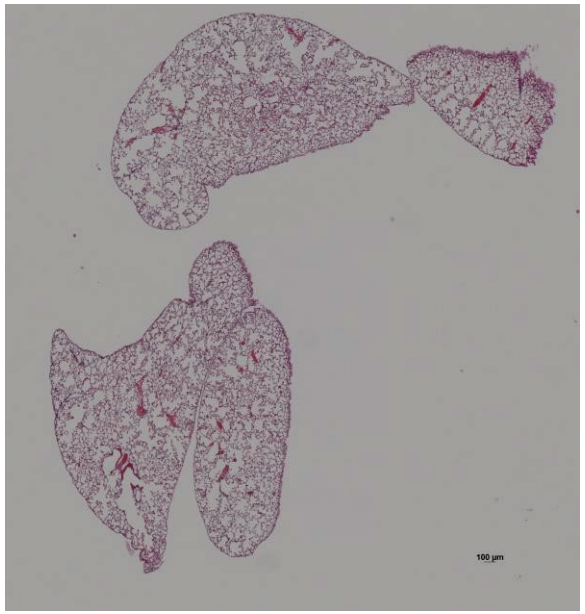




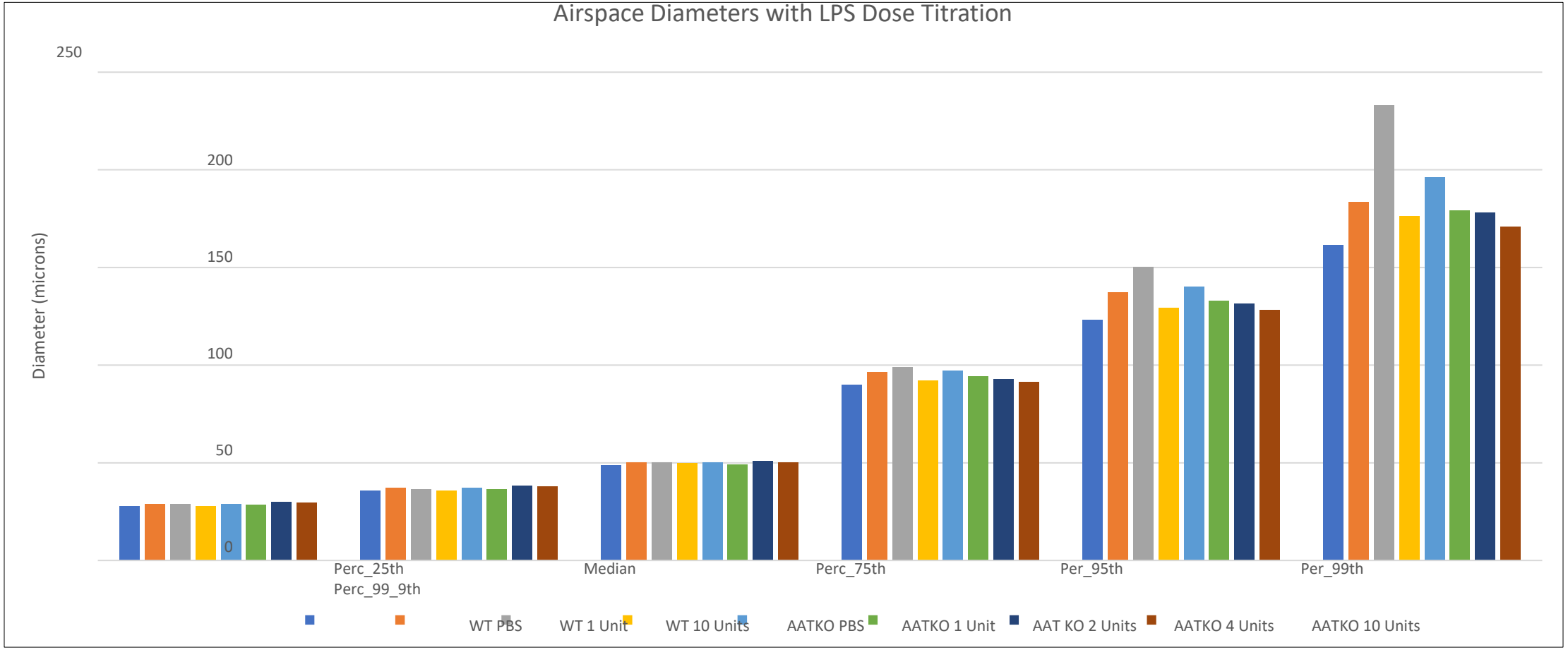
F

WT 10&5 Units LPS



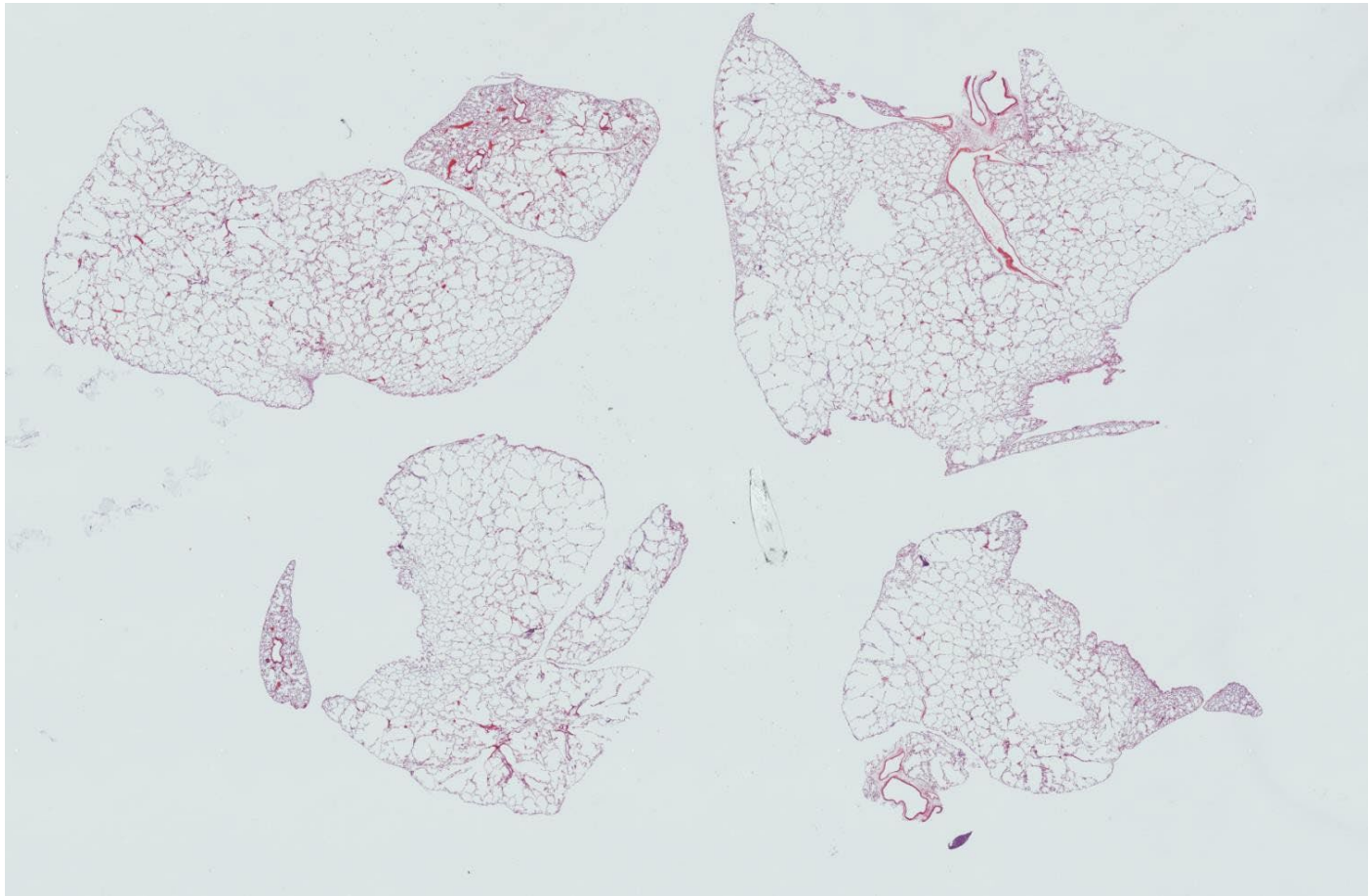


G


Airspace Diameters with LPS Dose Titration
Diameter (microns)
250
200
150
100
50
0
Perc_25th
Perc_99_9th
Median
Perc_75th
Per_95th
Per_99th
WT PBS
WT 1 Unit
WT 10 Units
AATKO PBS
AATKO 1 Unit
AAT KO 2 Units
AATKO 4 Units
AATKO 10 Units

Titration of Tracheal PPE Dose in AAT-deficient Mice

2659 AAT-KO 0.25 U



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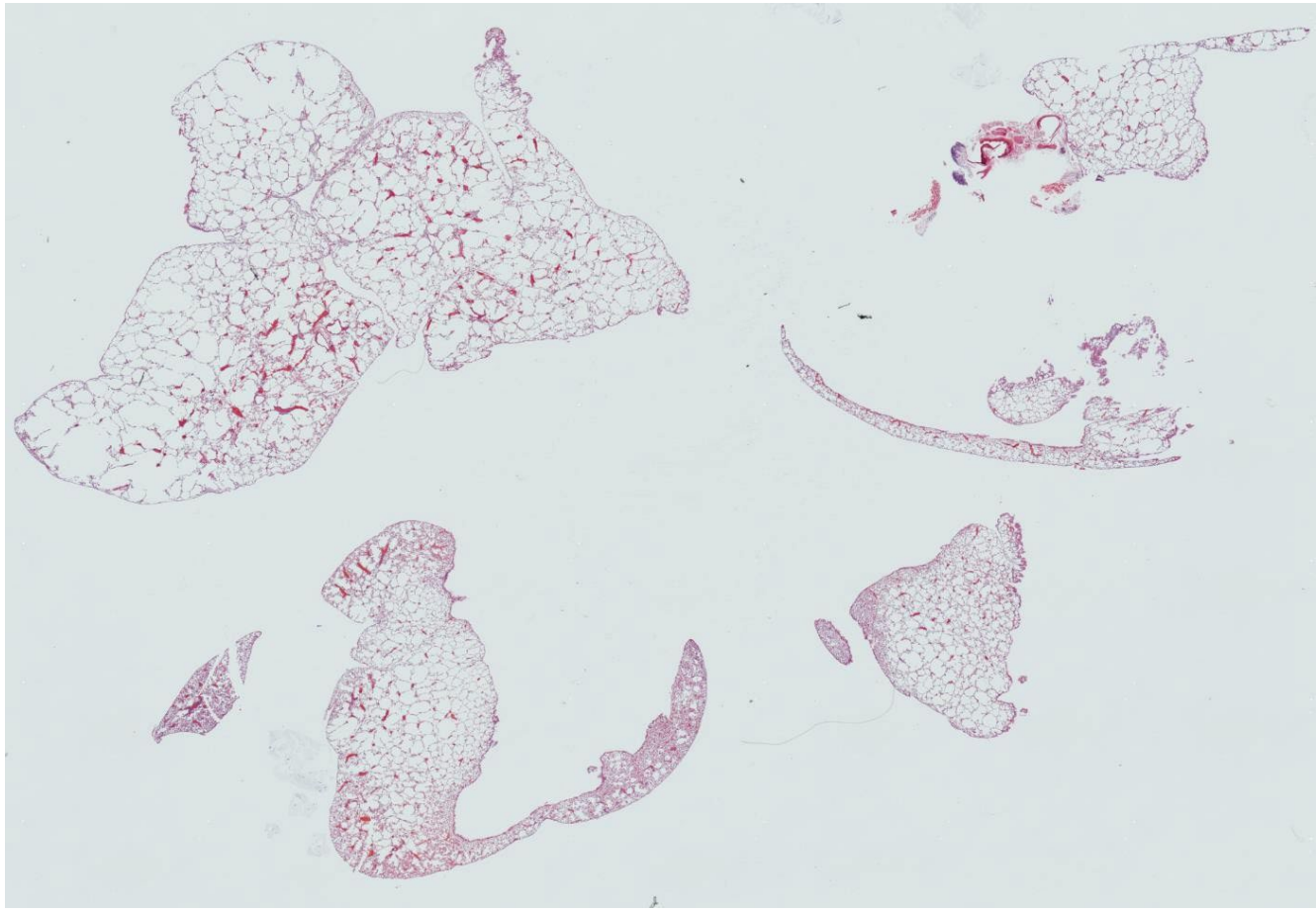

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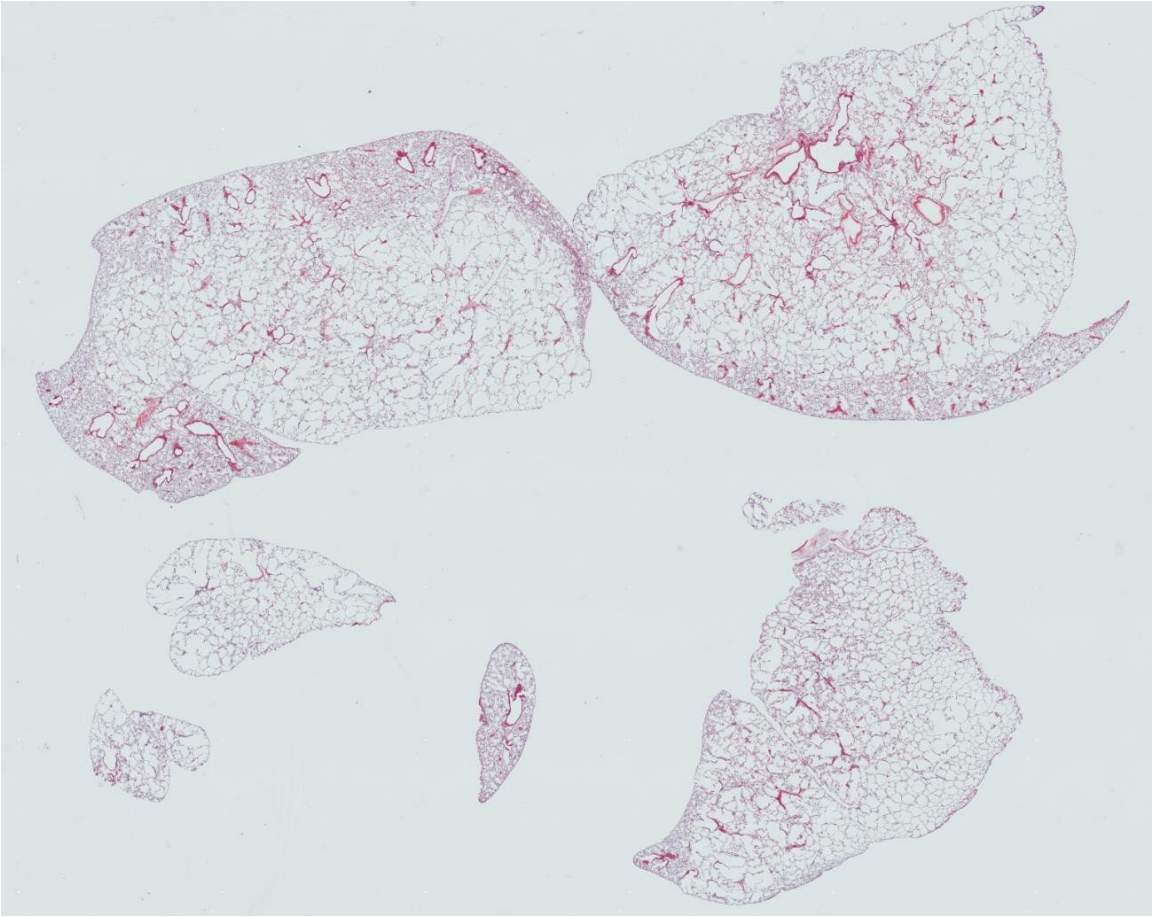

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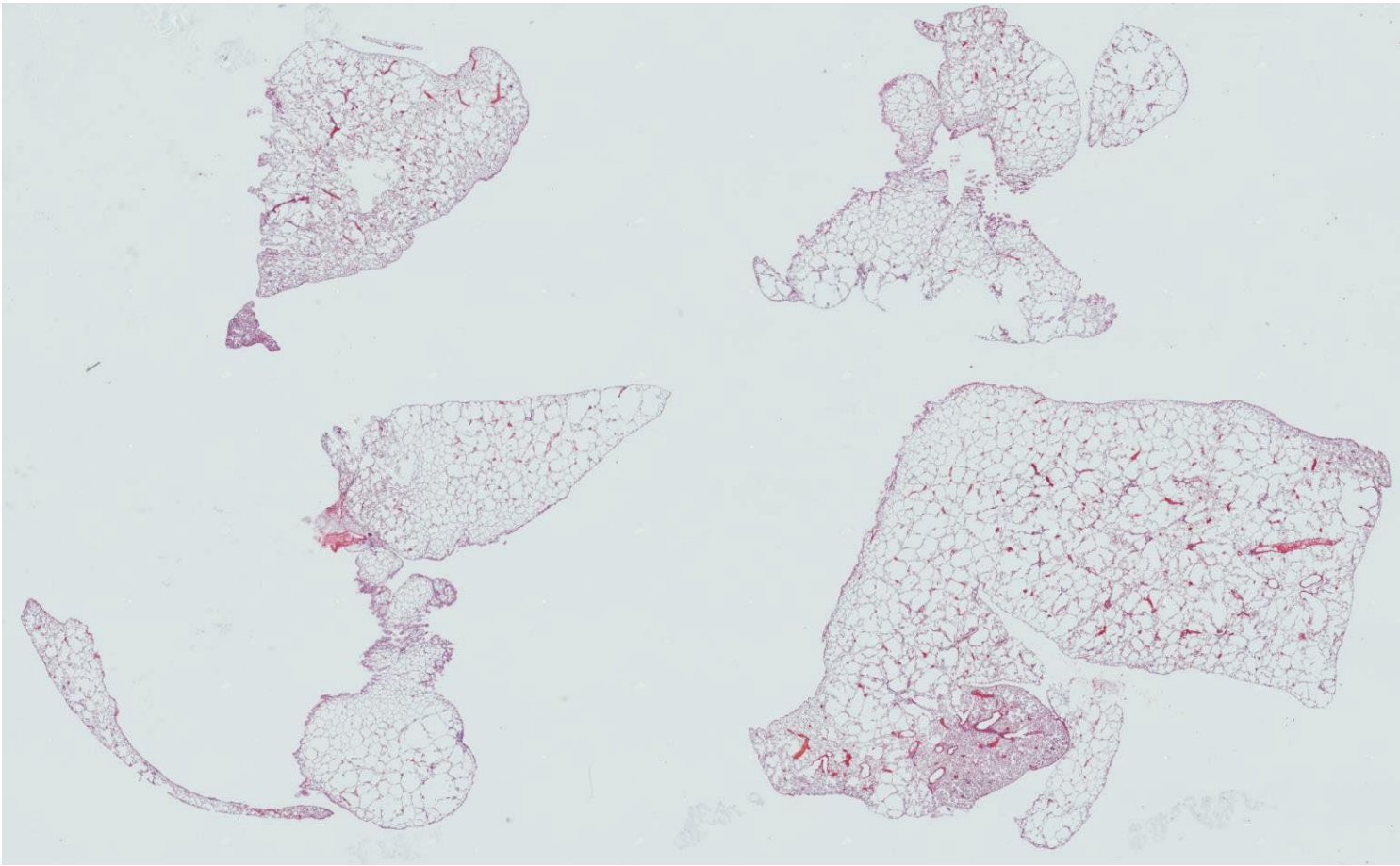

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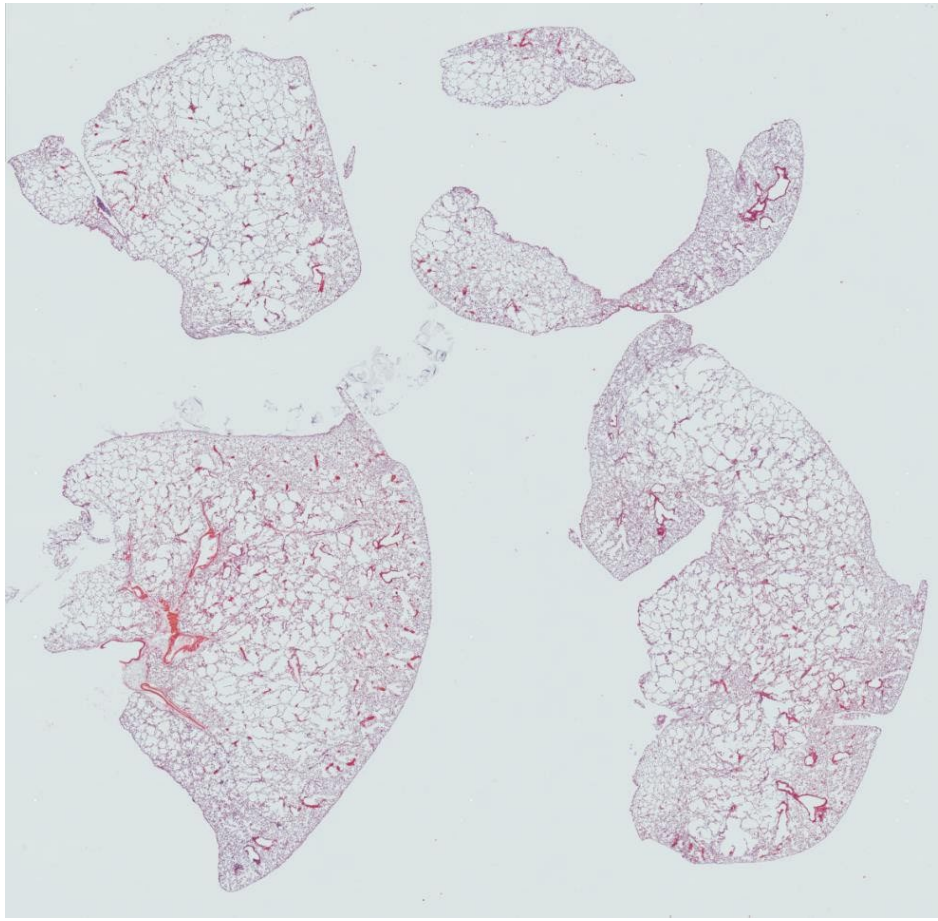

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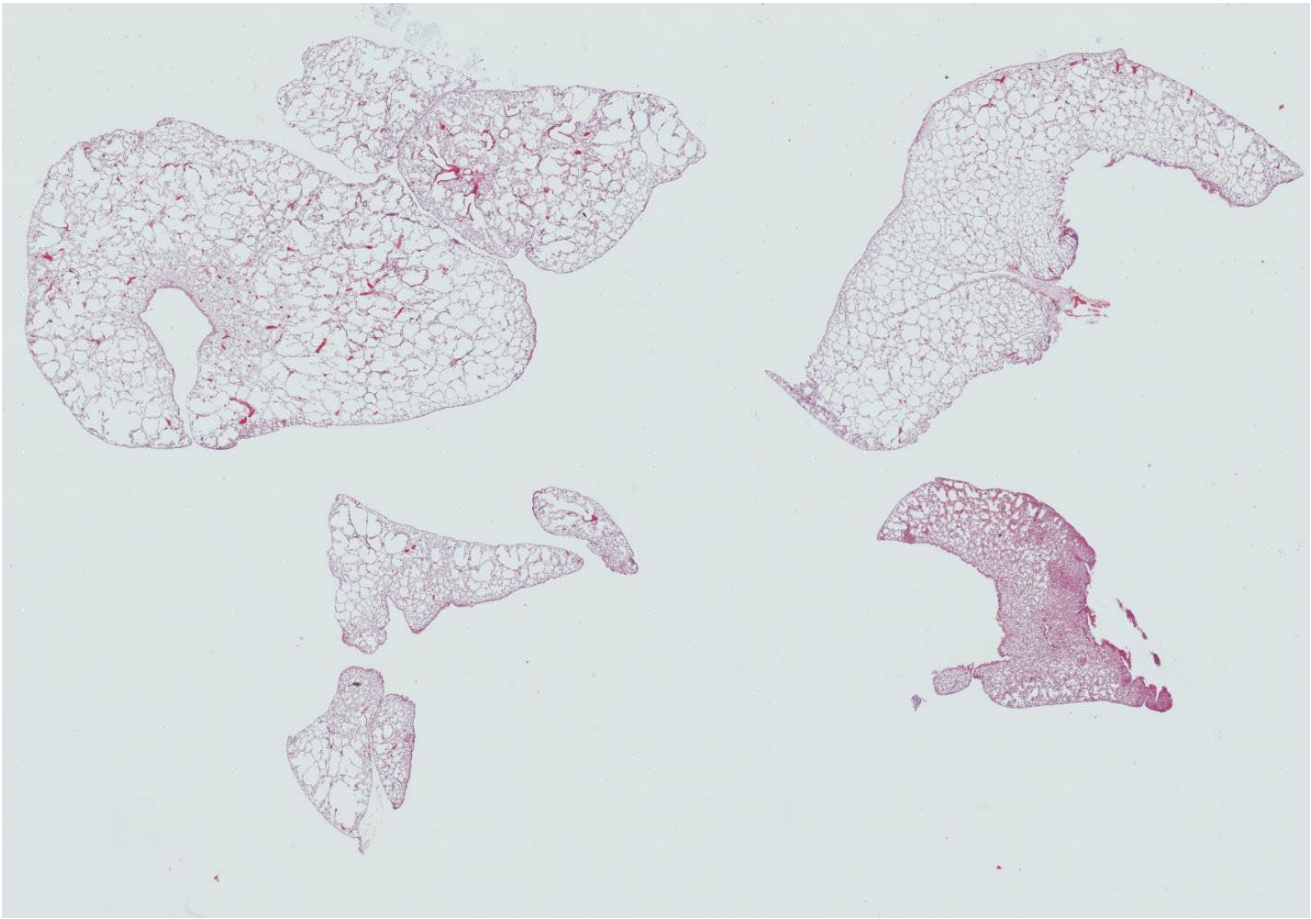
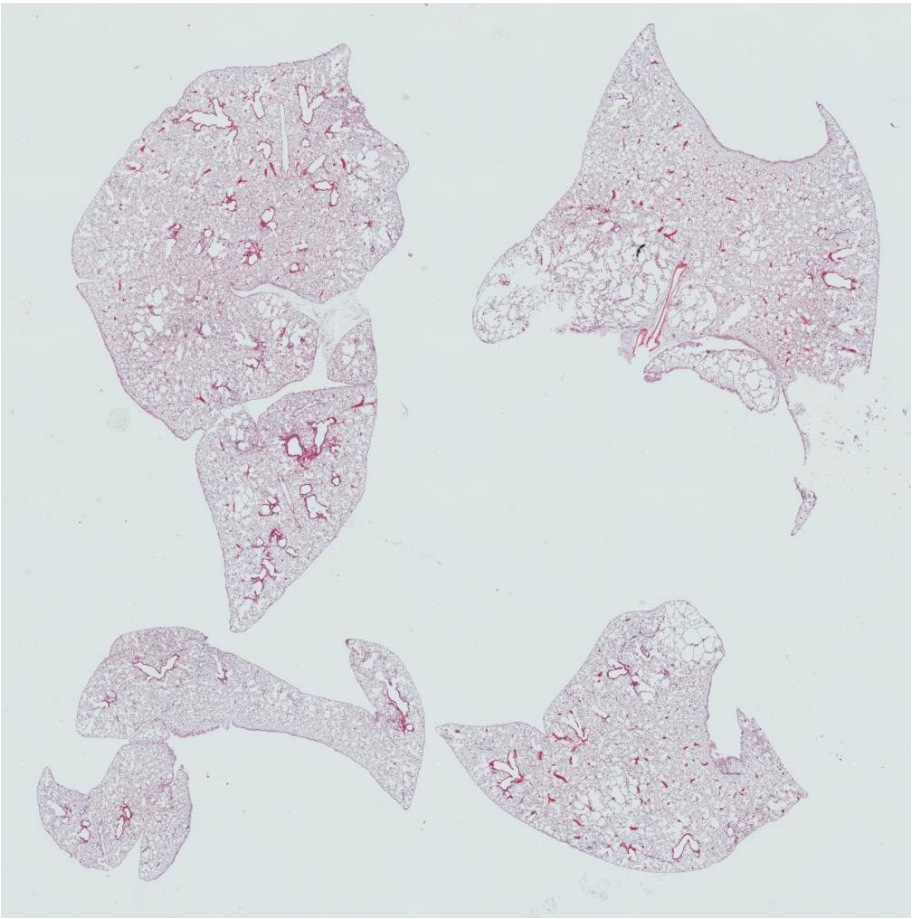


2677 AAT-KO 0.5 U



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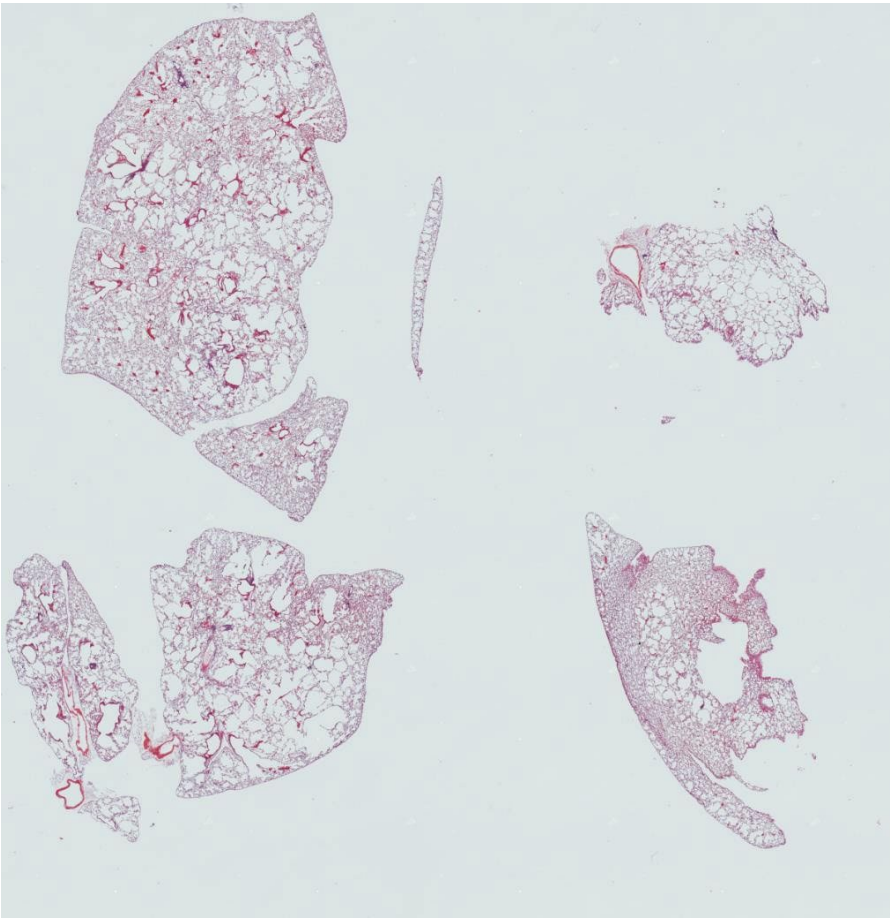

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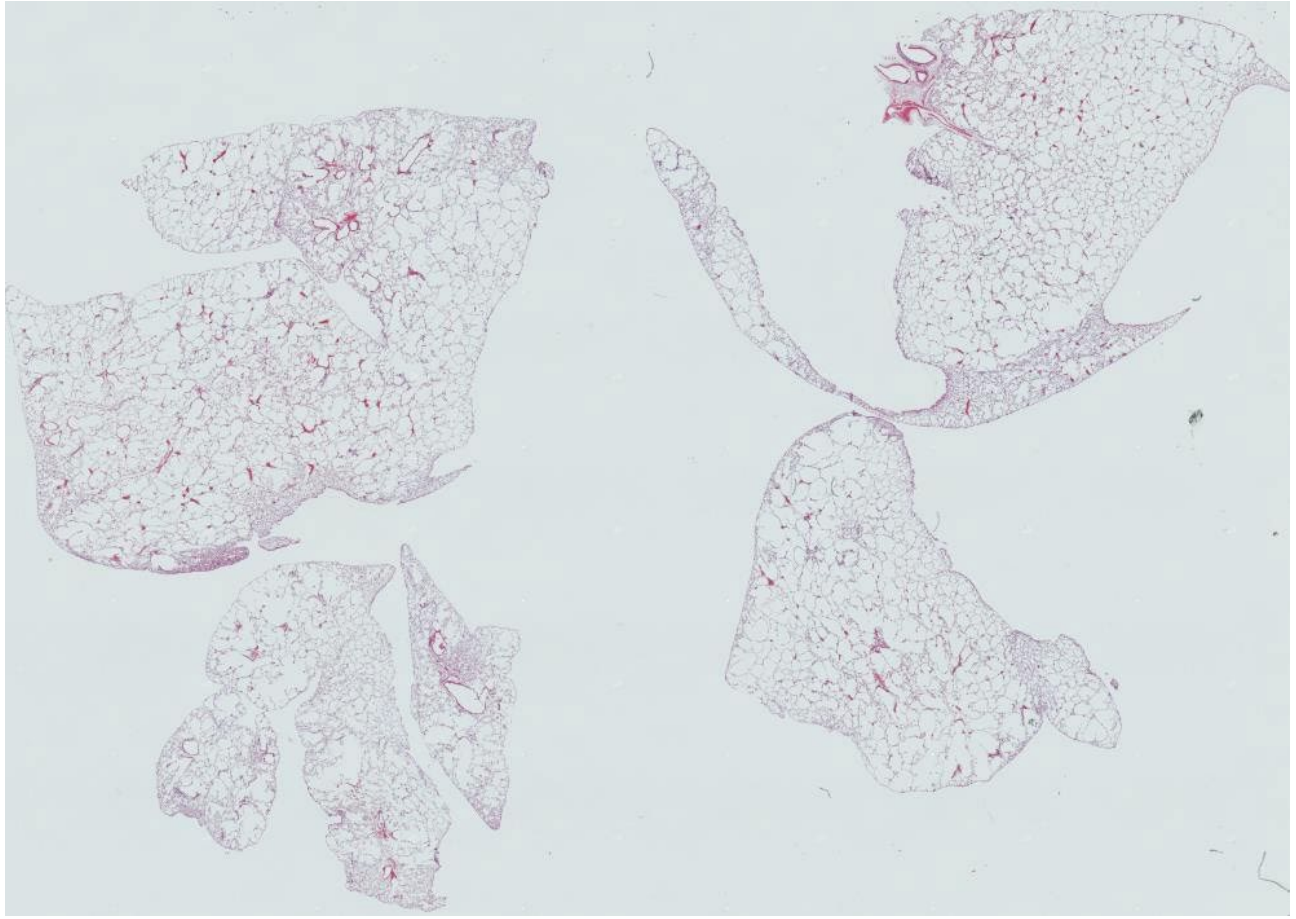

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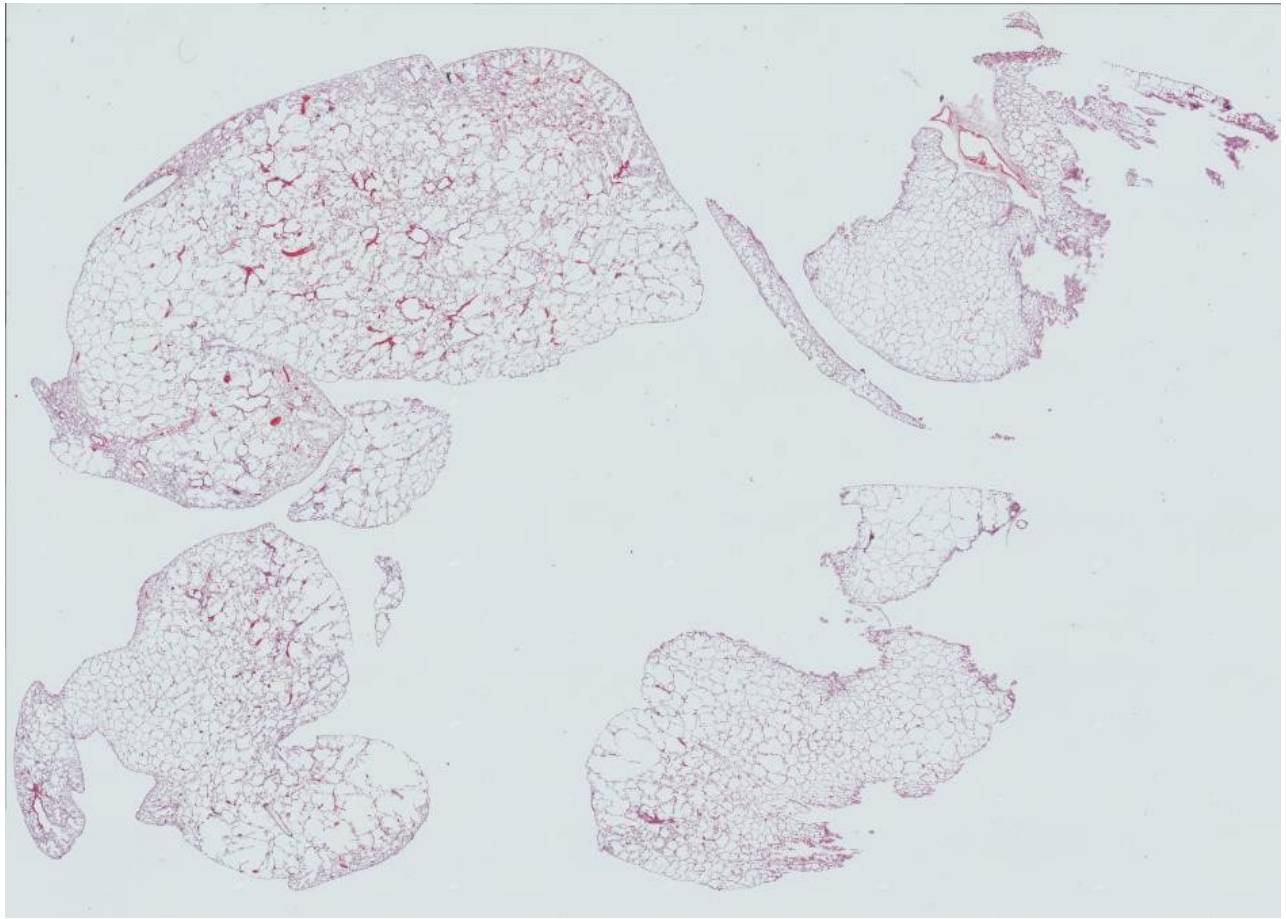

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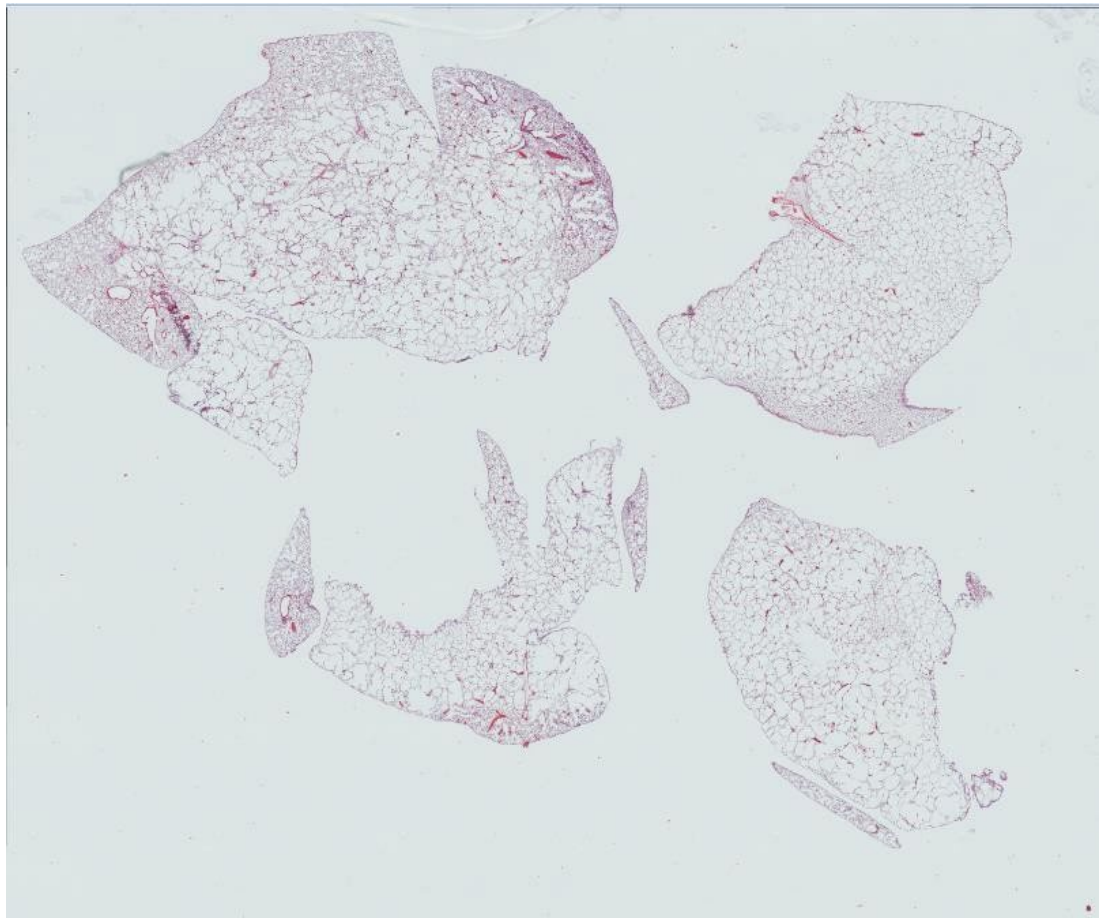

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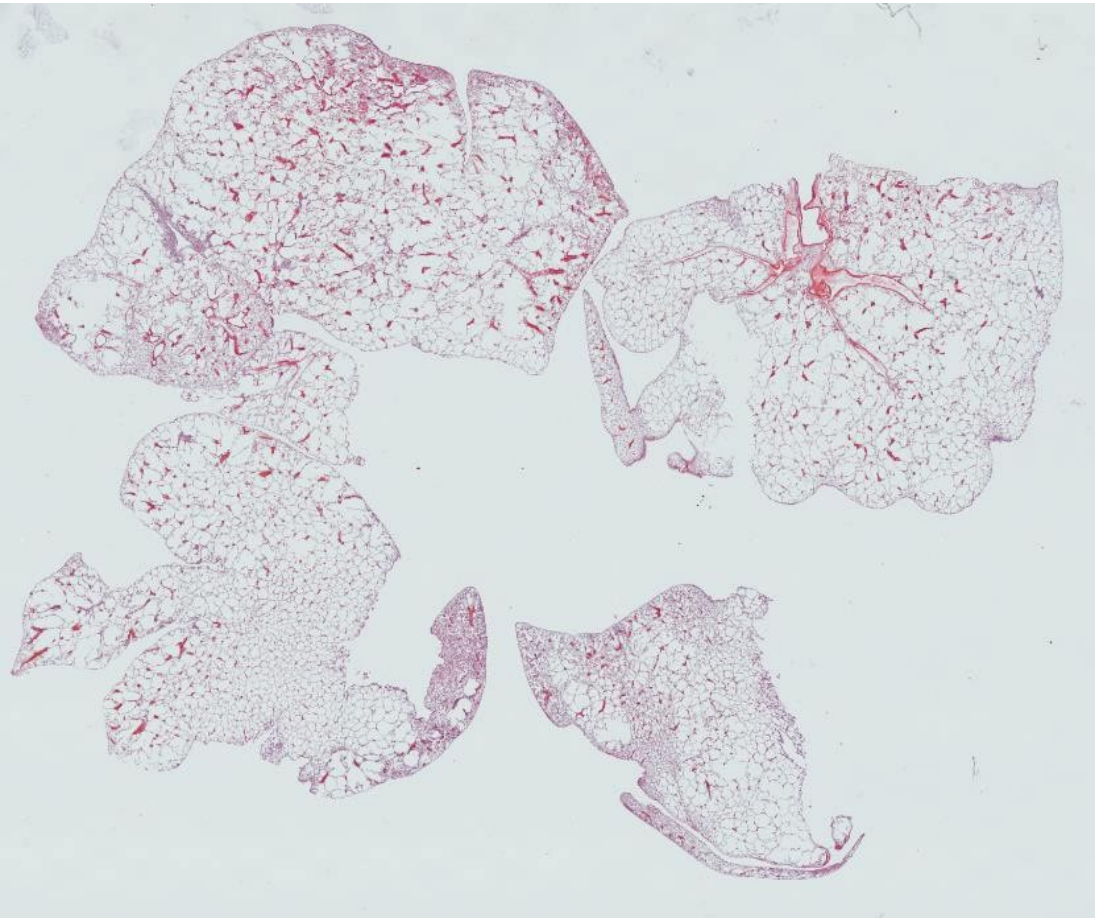

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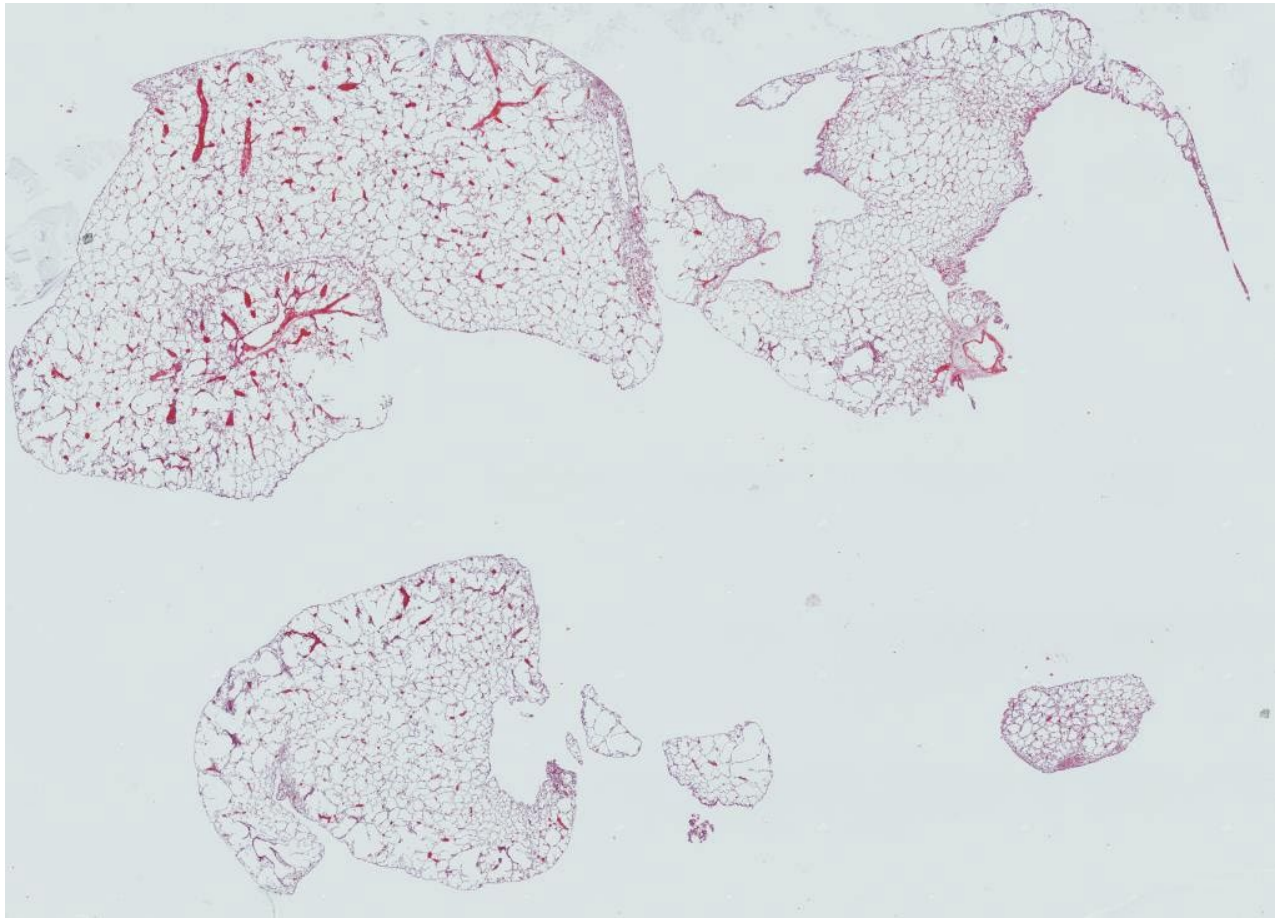

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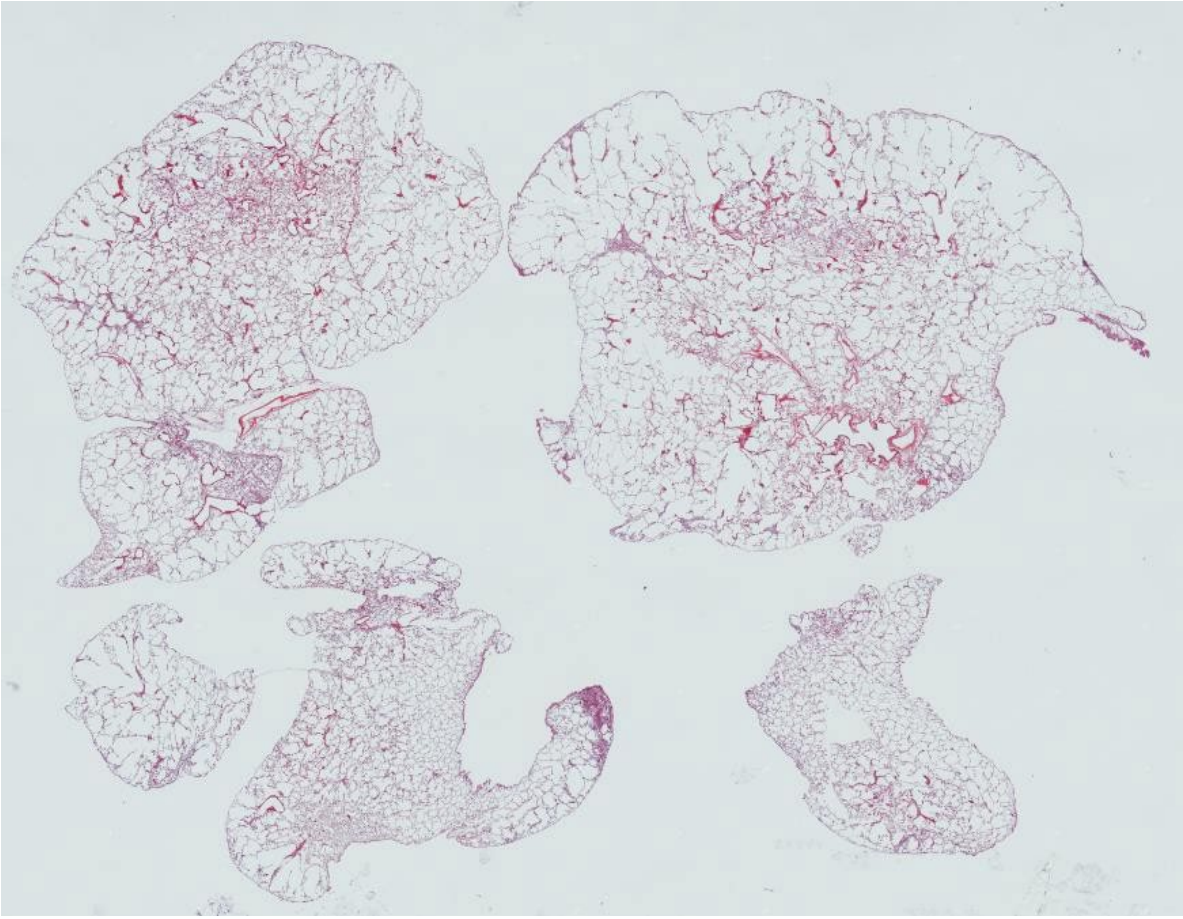

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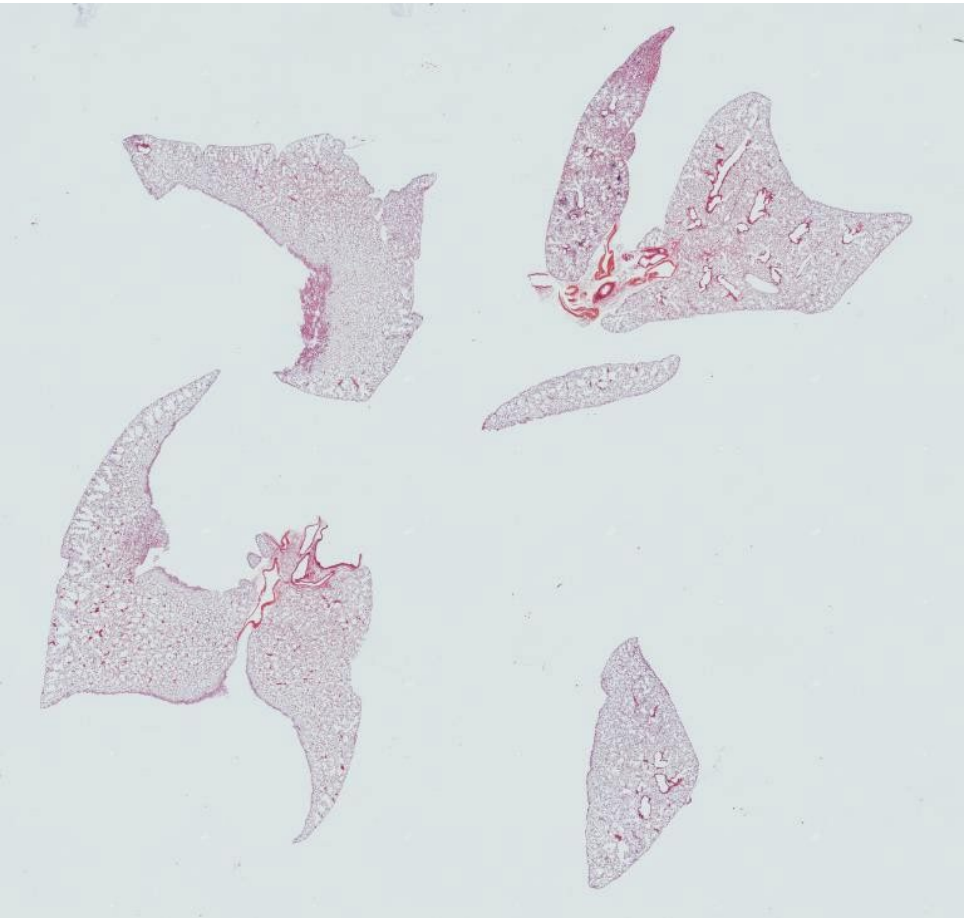

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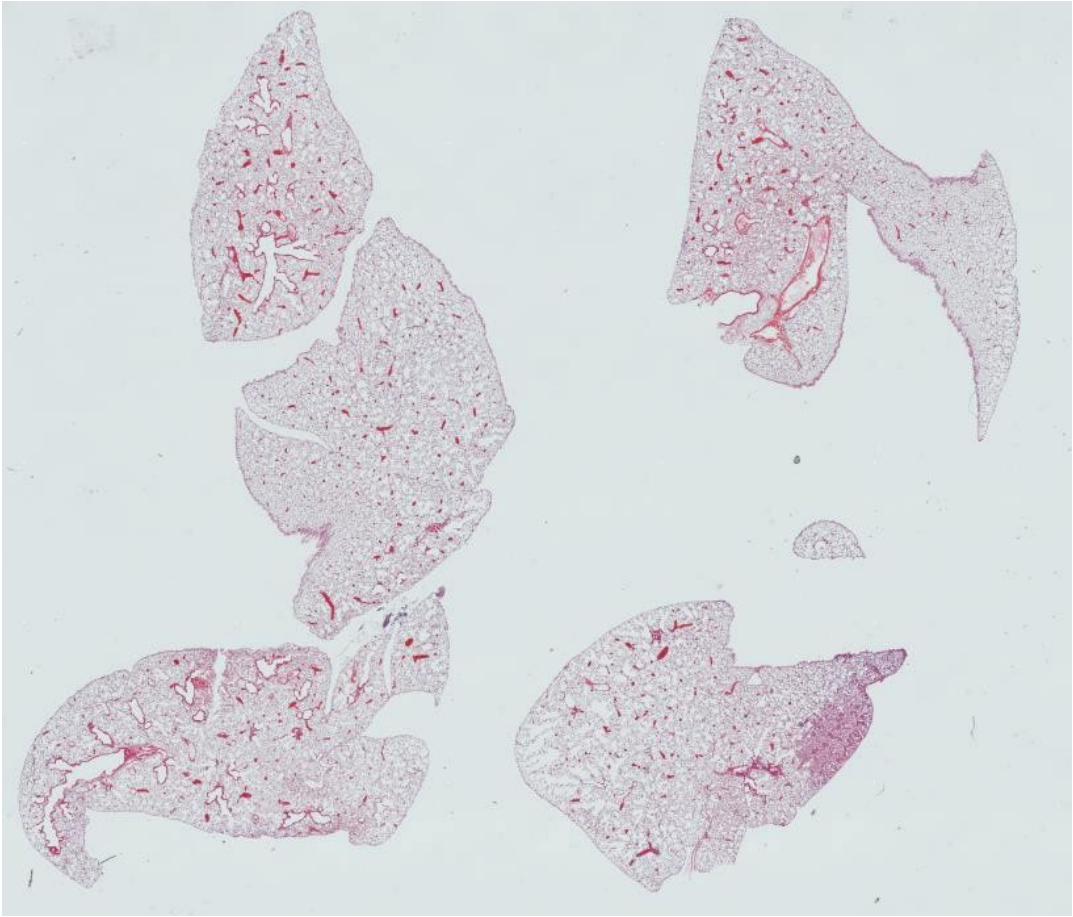

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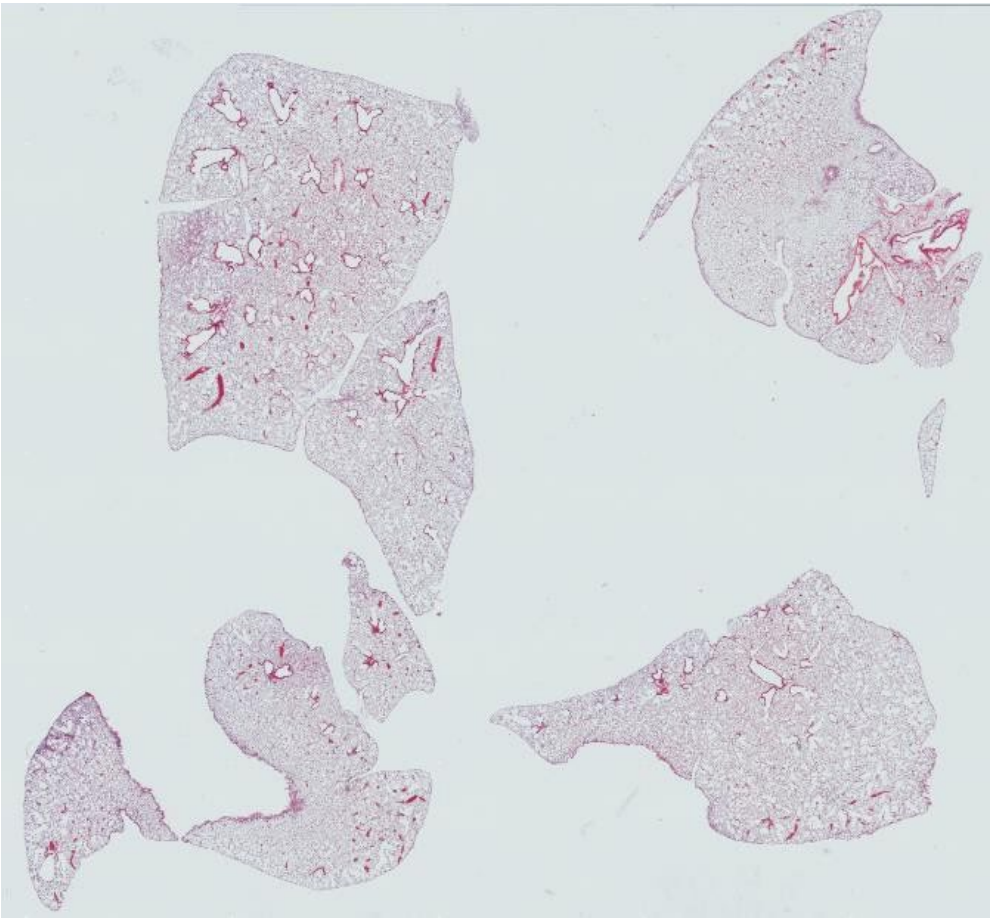
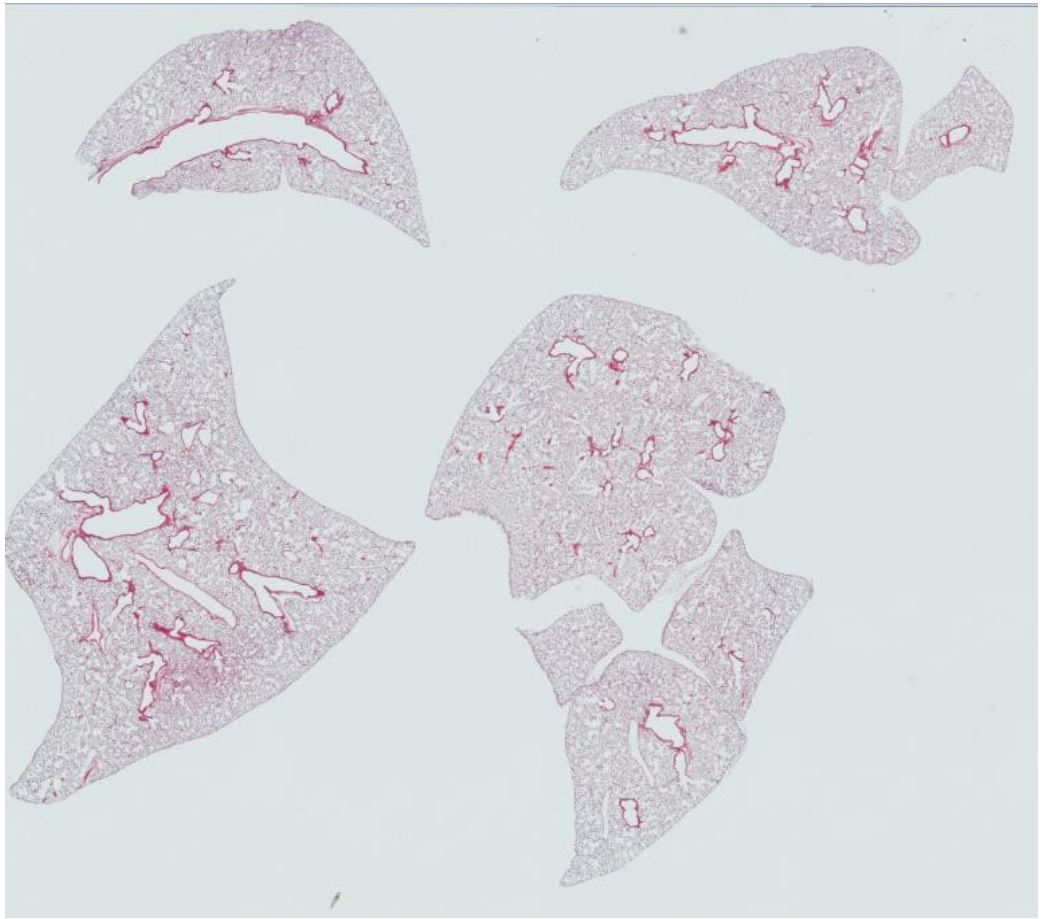


2453 WT 0.25 U



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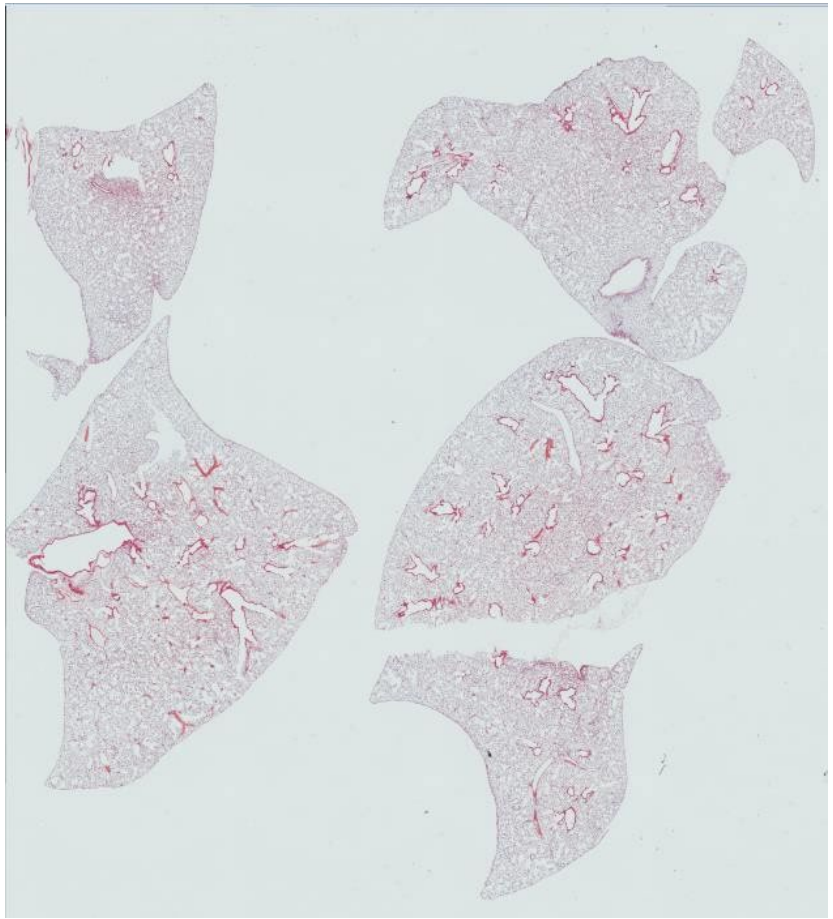

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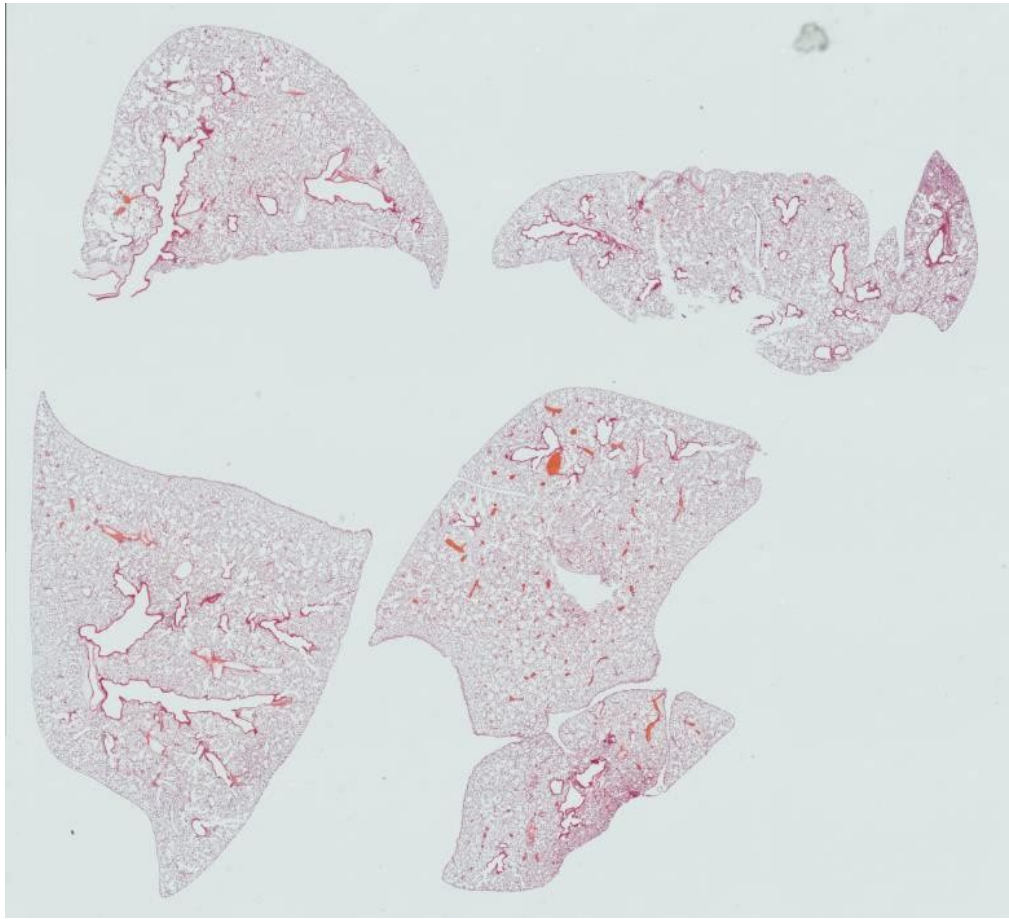

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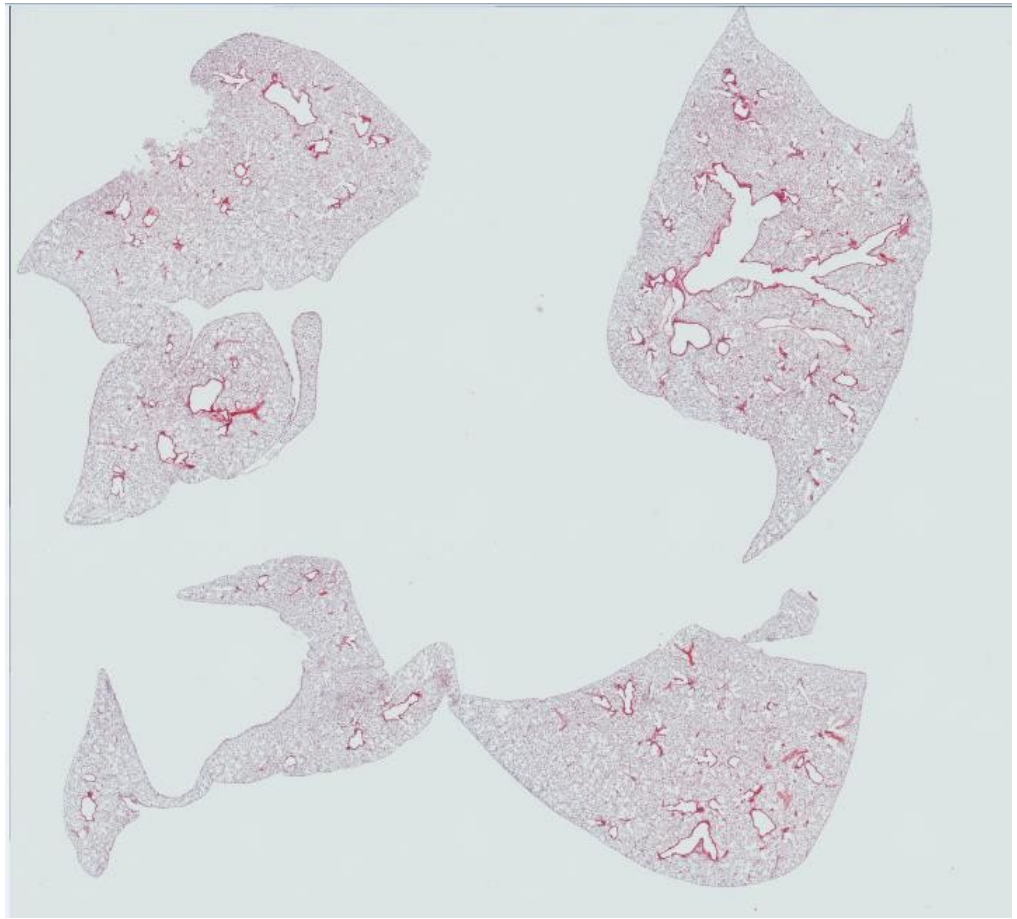

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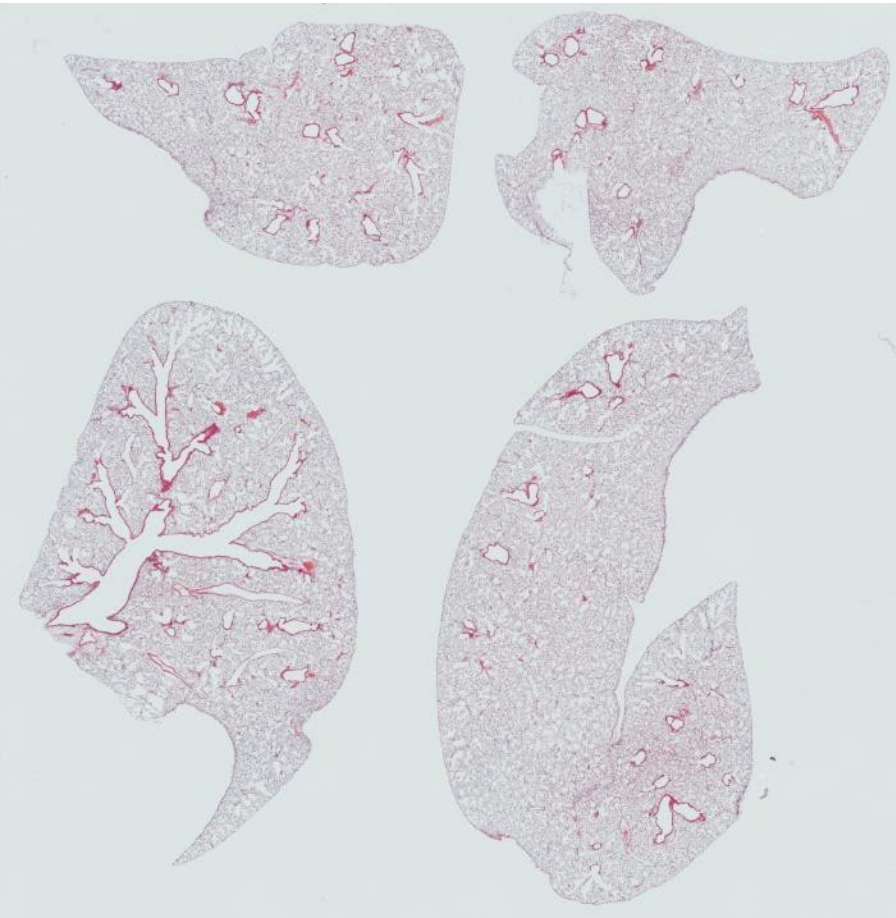

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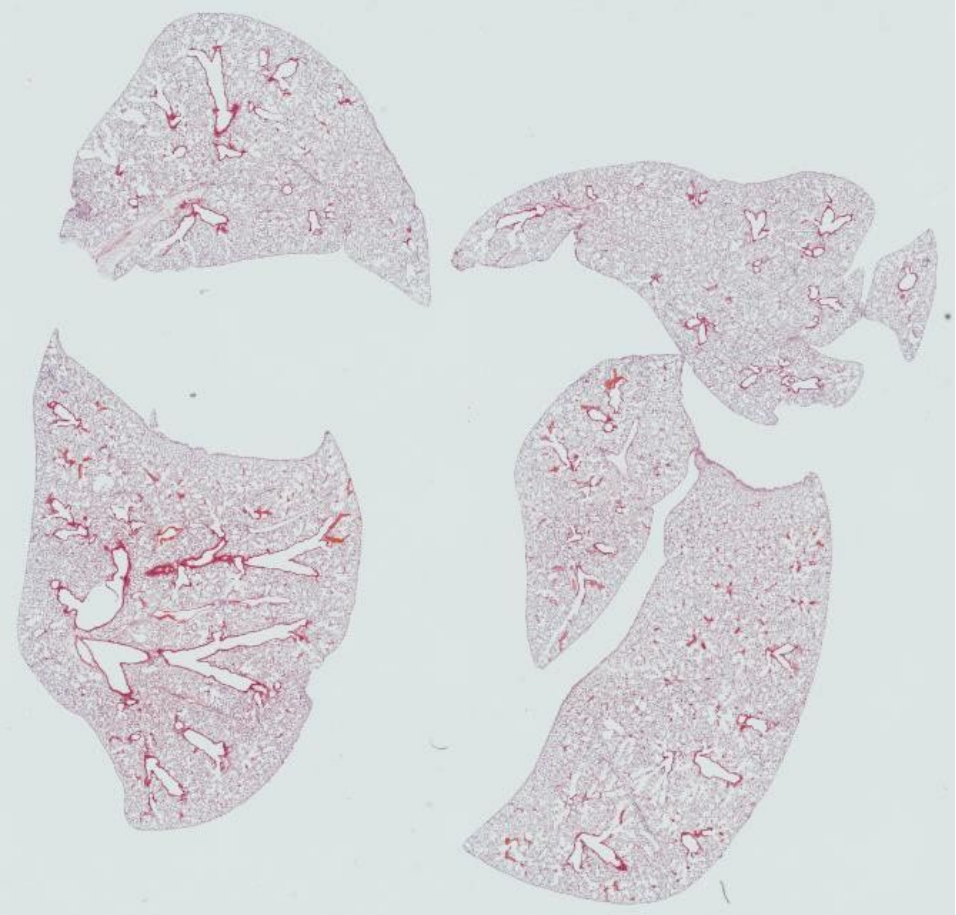

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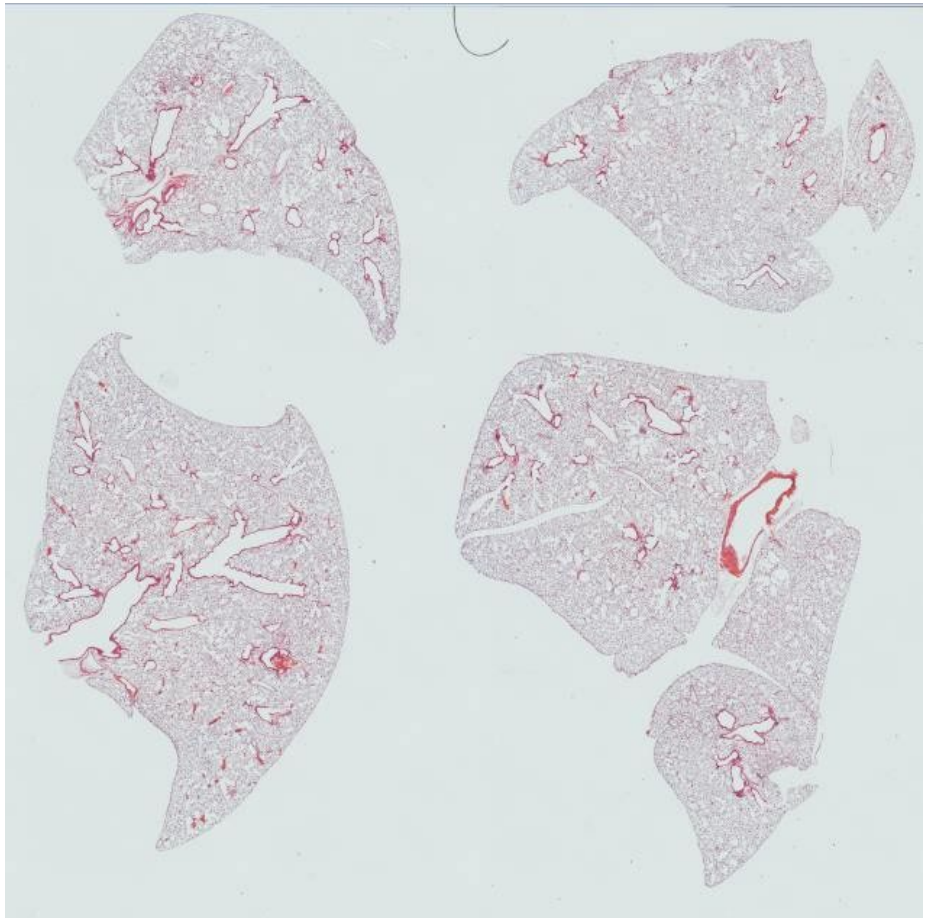

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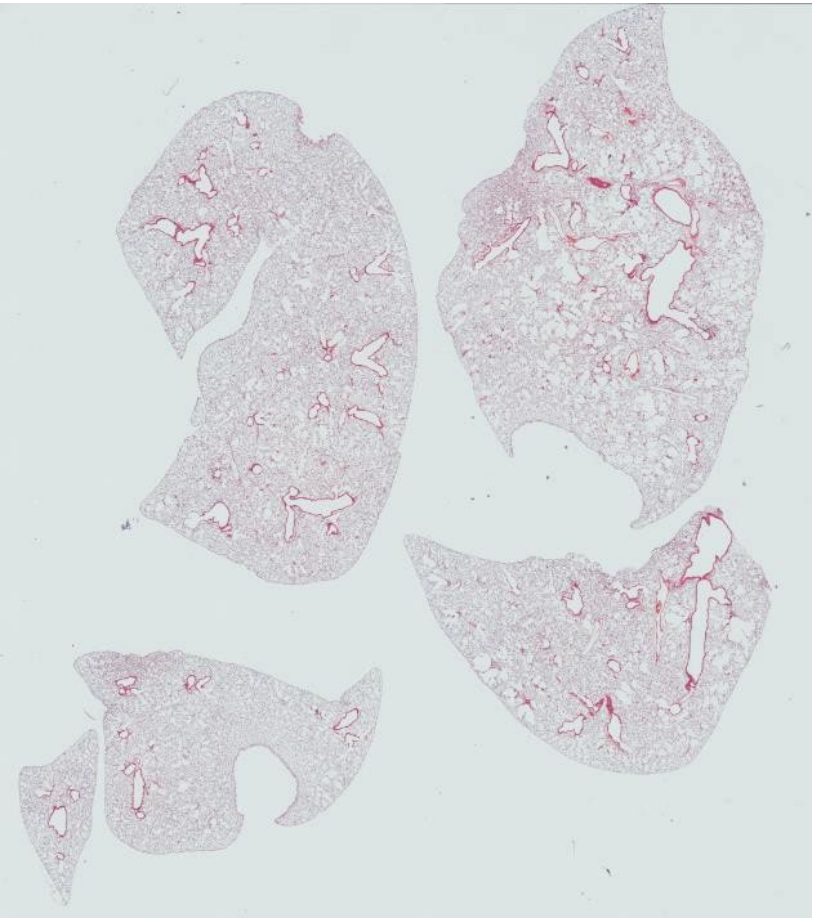

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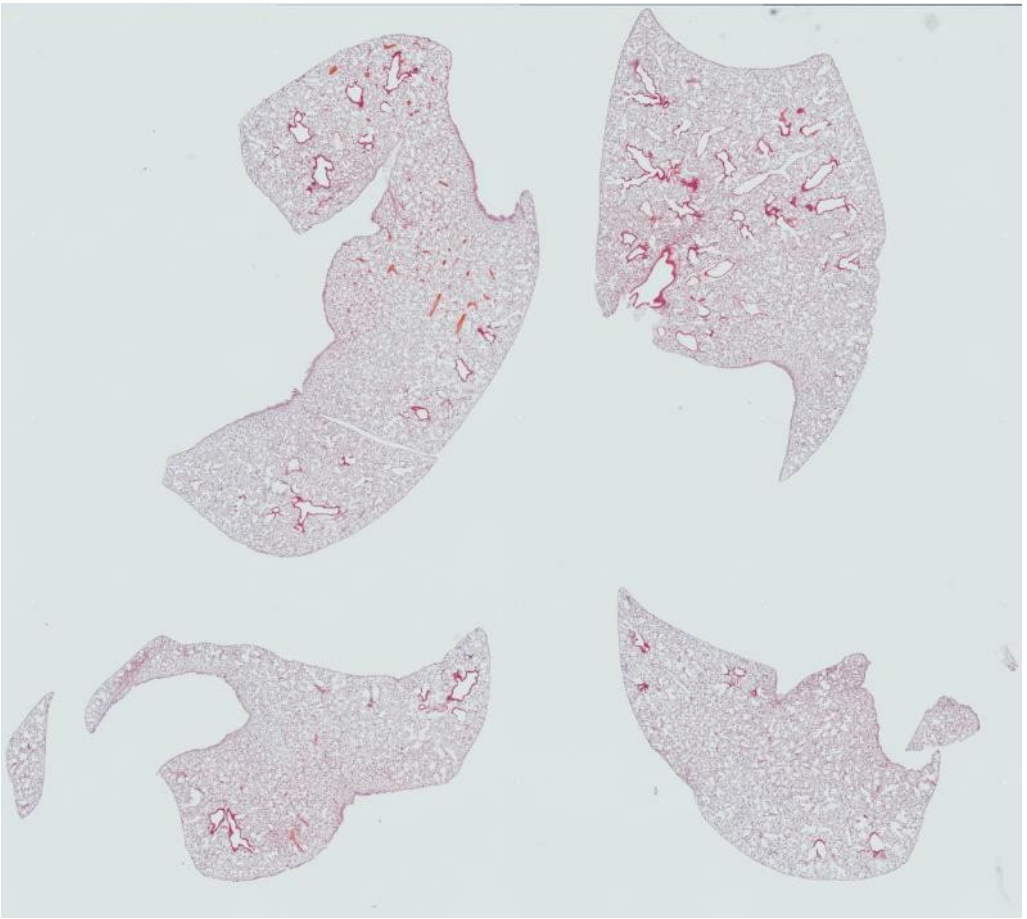

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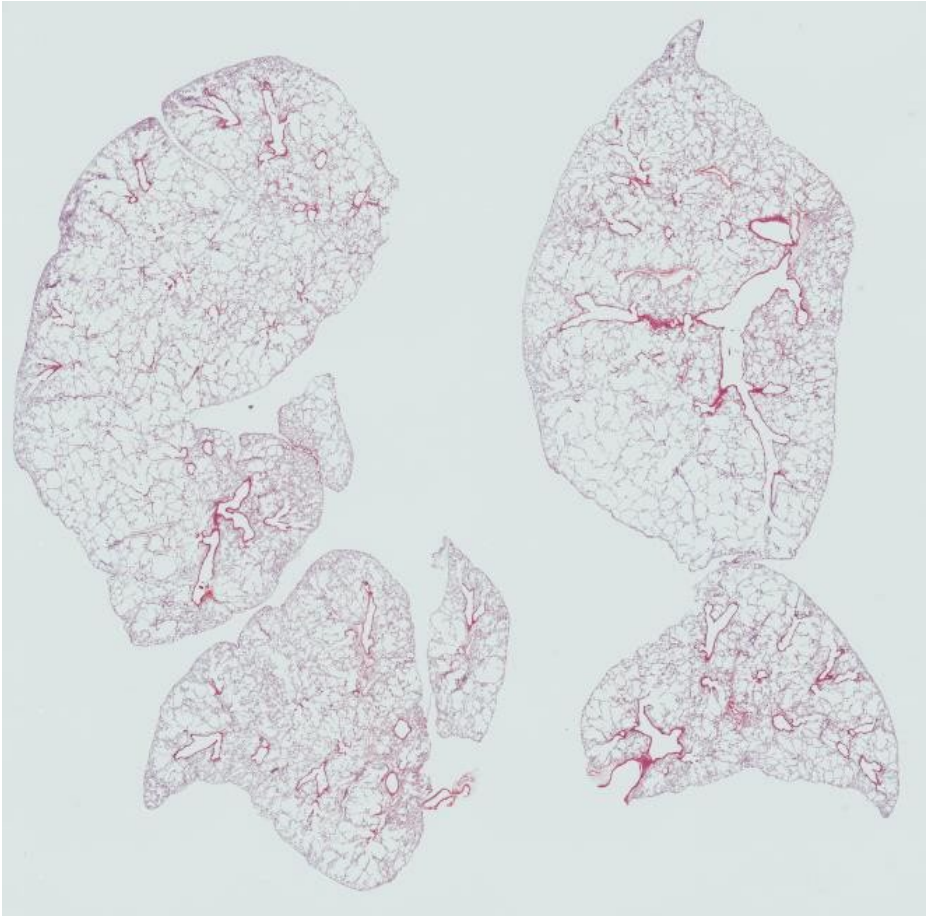

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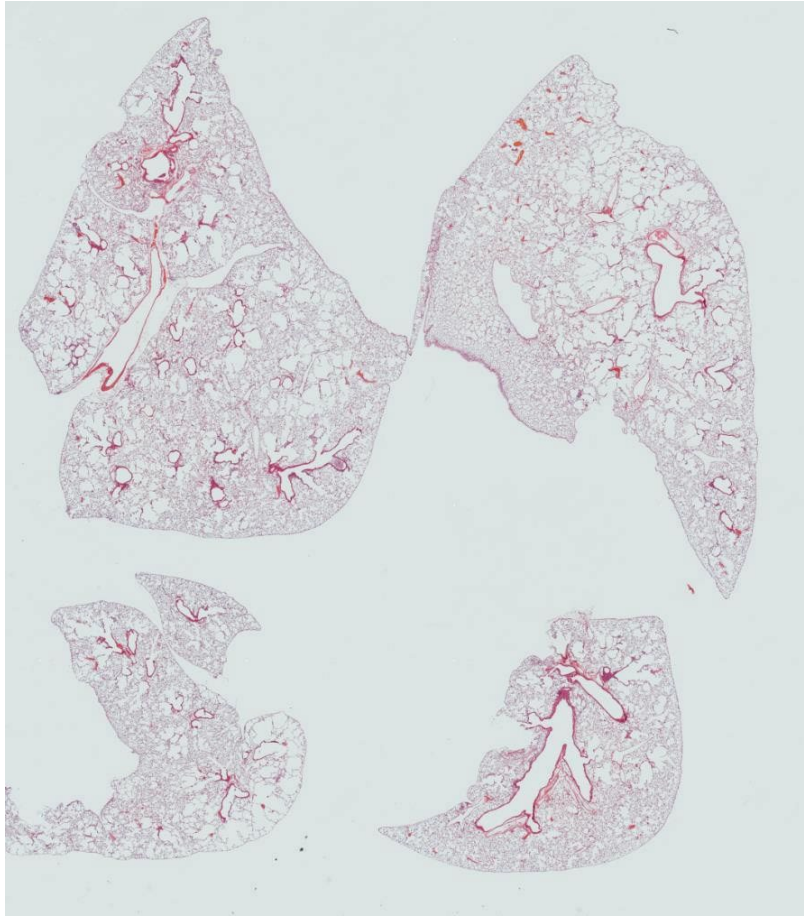

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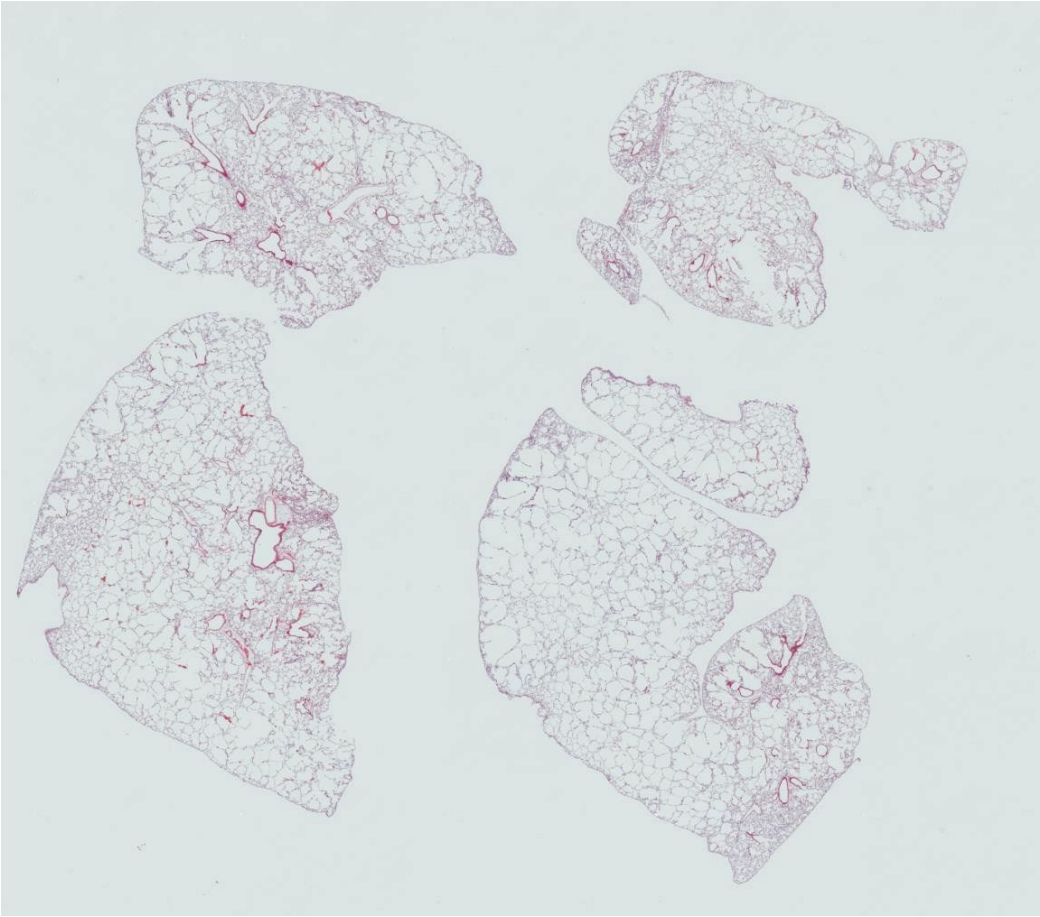

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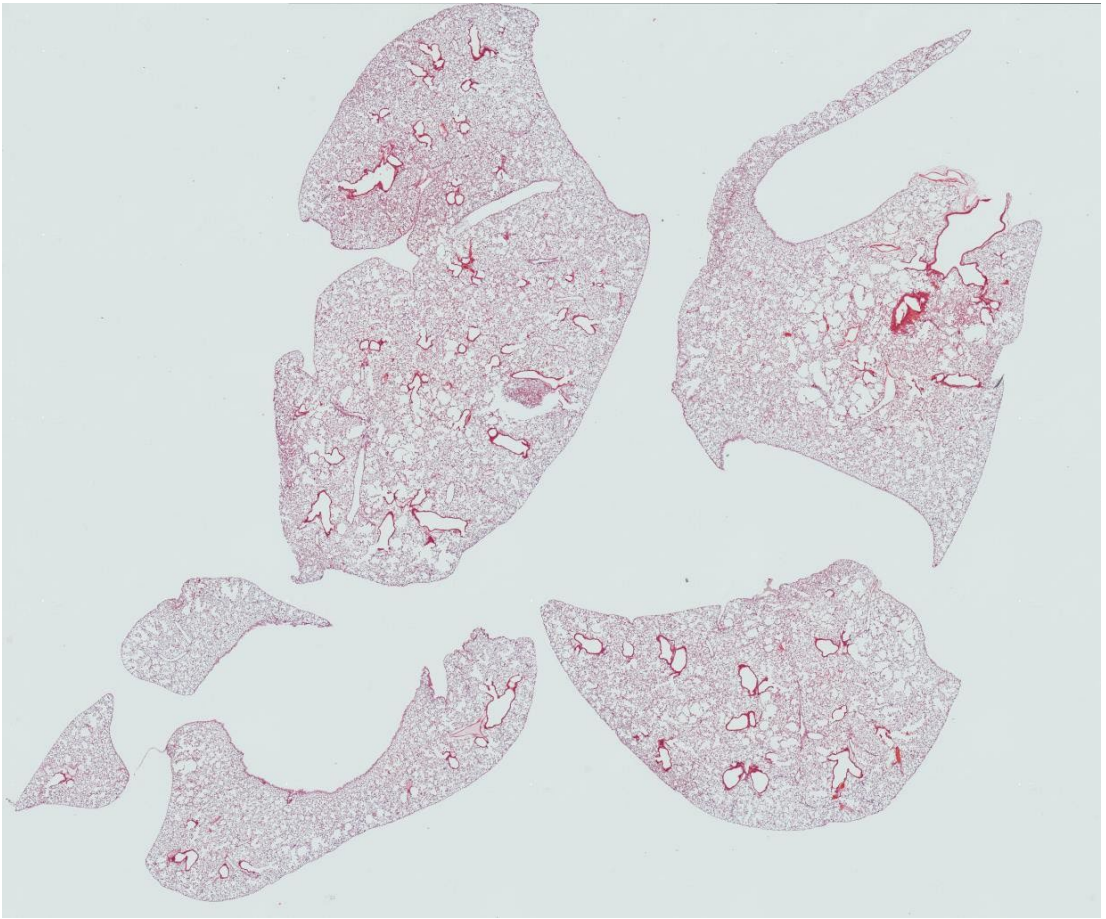
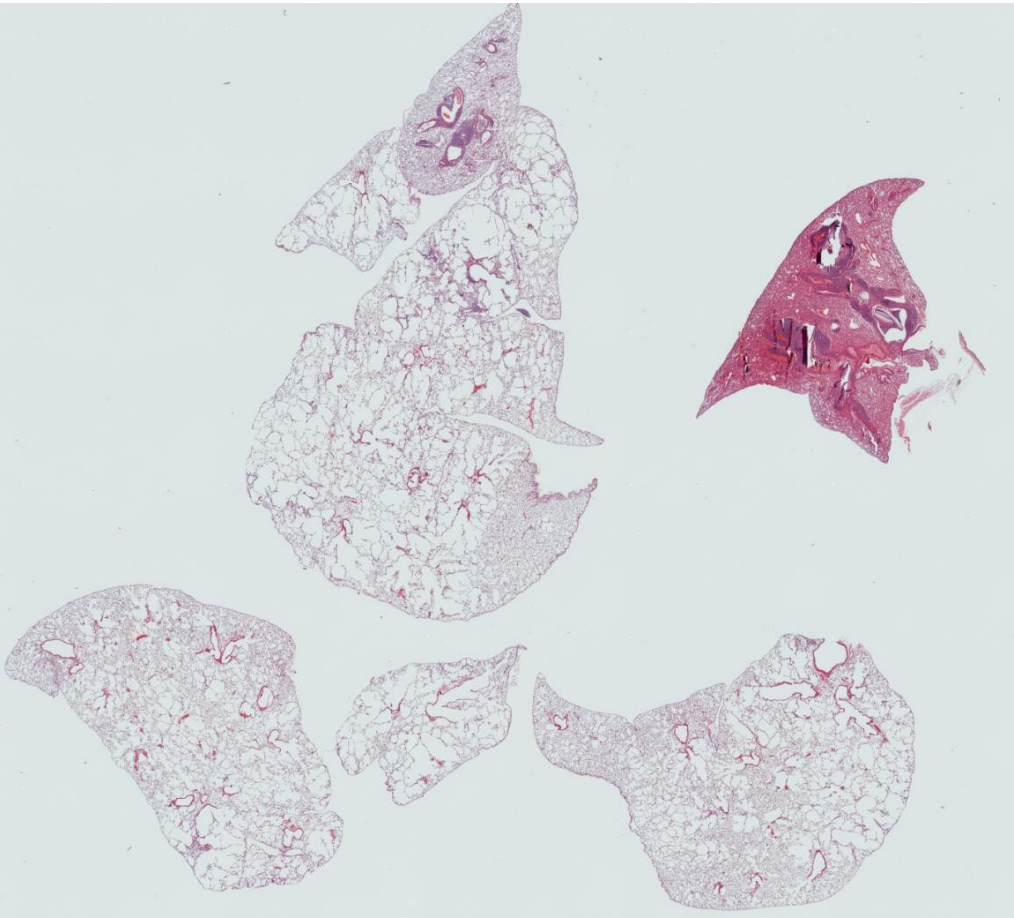
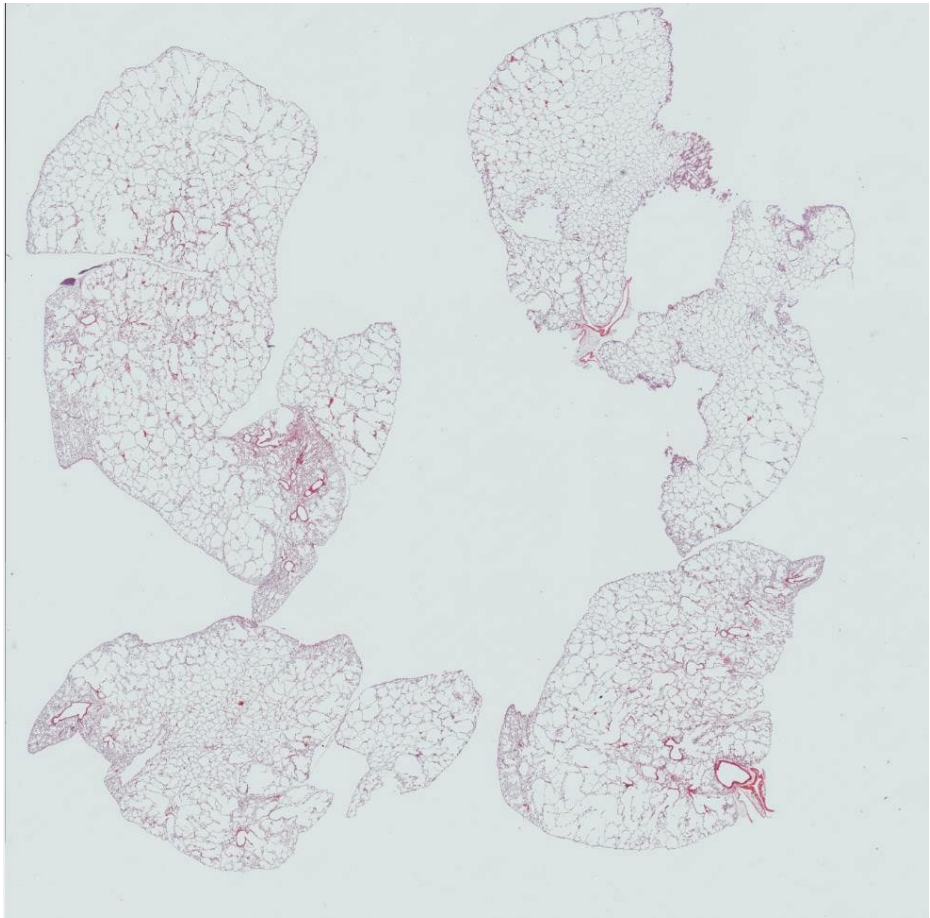
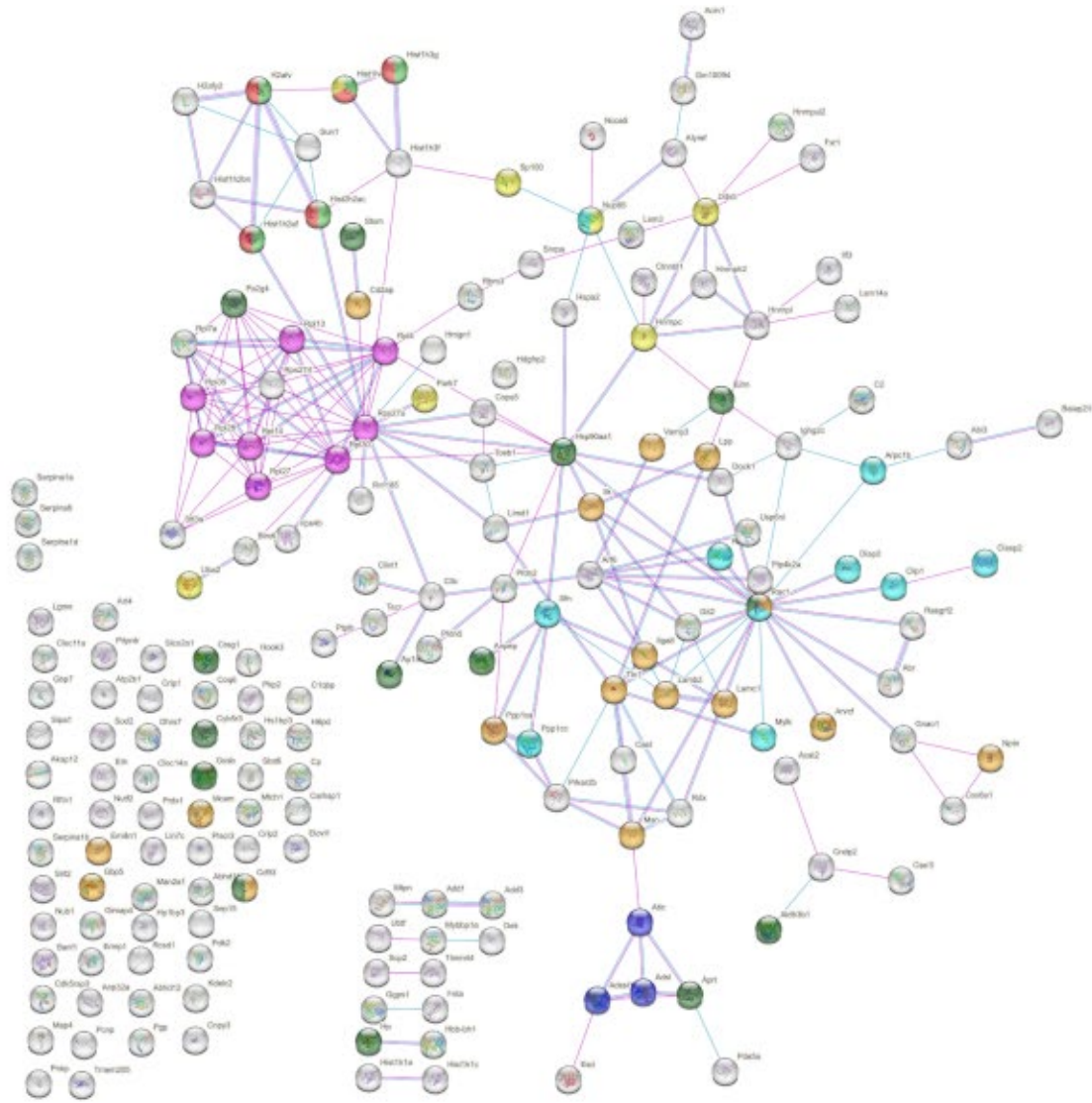




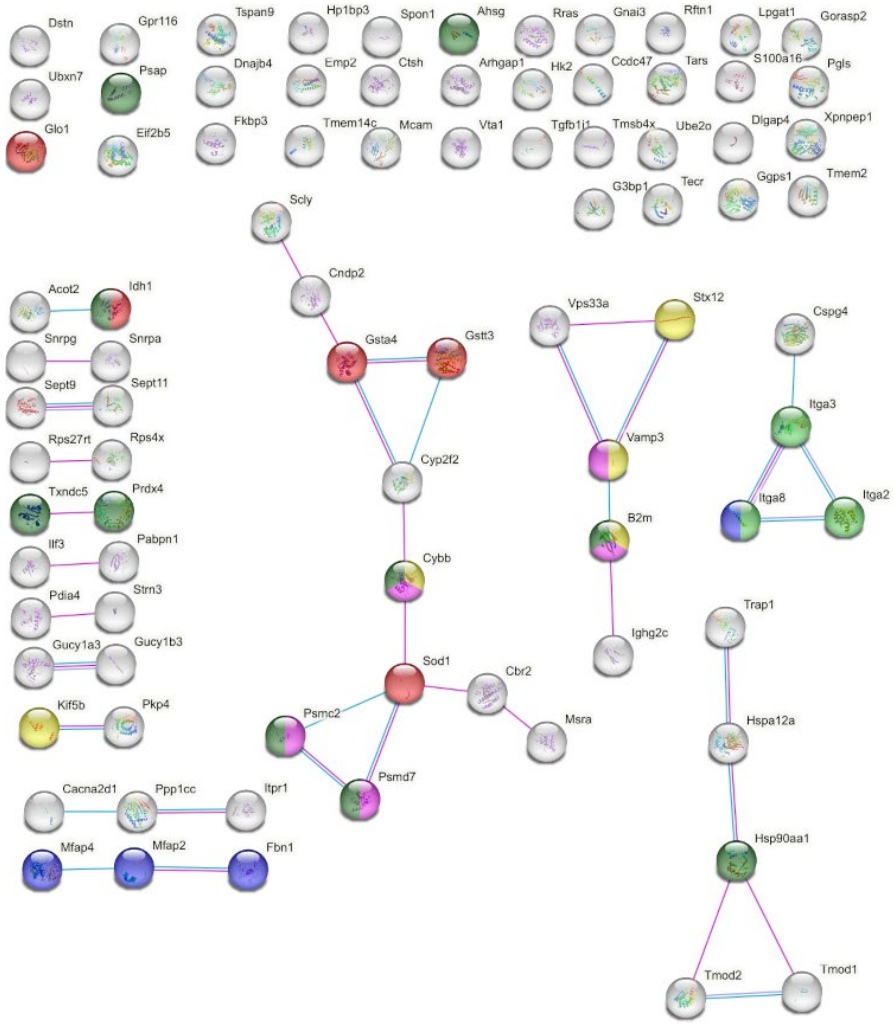


Supplemental Figure 3



pathway	description	count in network	strength	false discovery rate
MMU-73817	Purine ribonucleoside monophosphate biosynthesis	3 of 12	1.47	0.0095
MMU-110330	Recognition and association of DNA glycosylase with site c...	5 of 33	1.26	0.0016
MMU-9670095	Inhibition of DNA recombination at telomere	5 of 34	1.24	0.0017
MMU-110331	Cleavage of the damaged purine	5 of 35	1.23	0.0018
MMU-2299718	Condensation of Prophase Chromosomes	5 of 38	1.2	0.0019
MMU-212300	PRC2 methylates histones and DNA	5 of 40	1.17	0.0022
MMU-5689901	Metalloprotease DUBs	3 of 26	1.14	0.0493
MMU-1799339	SRP-dependent cotranslational protein targeting to membra...	9 of 88	1.09	0.00019
MMU-8936459	RUNX1 regulates genes involved in megakaryocyte different...	5 of 48	1.09	0.0041
MMU-975956	Nonsense Mediated Decay (NMD) independent of the Exon ...	9 of 90	1.08	0.00019
MMU-2559586	DNA Damage/Telomere Stress Induced Senescence	6 of 59	1.08	0.0017
MMU-212165	Epigenetic regulation of gene expression	7 of 71	1.07	0.00054
MMU-3214858	RMTs methylate histone arginines	4 of 41	1.07	0.0191
MMU-72689	Formation of a pool of free 40S subunits	9 of 97	1.04	0.00019
MMU-73884	Base Excision Repair	6 of 70	1.01	0.0025
MMU-72706	GTP hydrolysis and joining of the 60S ribosomal subunit	9 of 108	1.0	0.00019
MMU-156827	L13a-mediated translational silencing of Ceruloplasmin exp...	9 of 107	1.0	0.00019
MMU-975957	Nonsense Mediated Decay (NMD) enhanced by the Exon Ju...	9 of 110	0.99	0.00019
MMU-75153	Apoptotic execution phase	4 of 49	0.99	0.0314
MMU-606279	Deposition of new CENPA-containing nucleosomes at the c...	4 of 52	0.96	0.0365
MMU-68875	Mitotic Prophase	7 of 96	0.94	0.0018
MMU-3214815	HDACs deacetylate histones	4 of 55	0.94	0.0428
MMU-6806834	Signaling by MET	5 of 78	0.88	0.0219
MMU-5357801	Programmed Cell Death	7 of 116	0.86	0.0041
MMU-2559583	Cellular Senescence	7 of 124	0.83	0.0055
MMU-6791226	Major pathway of rRNA processing in the nucleolus and cyt...	9 of 171	0.8	0.0018
MMU-2990846	SUMOylation	7 of 150	0.75	0.0136
MMU-72163	mRNA Splicing - Major Pathway	8 of 178	0.73	0.0073
MMU-5663220	RHO GTPases Activate Formins	6 of 133	0.73	0.0337
MMU-195258	RHO GTPase Effectors	10 of 246	0.69	0.0033
MMU-3108232	SUMO E3 ligases SUMOylate target proteins	6 of 145	0.69	0.0478
MMU-68886	M Phase	14 of 364	0.66	0.00054
MMU-72203	Processing of Capped Intron-Containing Pre-mRNA	9 of 234	0.66	0.0086
MMU-2262752	Cellular responses to stress	14 of 395	0.63	0.0011
MMU-8953854	Metabolism of RNA	19 of 556	0.61	0.00019
MMU-194315	Signaling by Rho GTPases	13 of 381	0.61	0.0019
MMU-422475	Axon guidance	9 of 278	0.59	0.0231
MMU-73894	DNA Repair	9 of 293	0.56	0.0314
MMU-6798695	Neutrophil degranulation	14 of 519	0.51	0.0069
MMU-1640170	Cell Cycle	15 of 574	0.49	0.0060
MMU-168249	Innate Immune System	21 of 949	0.42	0.0033
MMU-392499	Metabolism of proteins	27 of 1609	0.3	0.0181
MMU-168256	Immune System	27 of 1621	0.3	0.0194
GO:0022610	Biological adhesion	17 of 751	0.43	0.0450
GO:0003735	Structural constituent of ribosome	8 of 155	0.79	0.0171

Supplemental Figure 4



pathway	description	count in network	strength	false discovery rate
MMU-2129379	Molecules associated with elastic fibres	4 of 35	1.47	0.0255
MMU-1236975	Antigen processing-Cross presentation	5 of 92	1.14	0.0255
MMU-6798695	Neutrophil degranulation	10 of 519	0.69	0.0255
GO:0006749	Glutathione metabolic process	5 of 53	1.38	0.0155
GO:0008305	Integrin complex	3 of 30	1.41	0.0301
GO:0045335	Phagocytic vesicle	5 of 112	1.06	0.0134