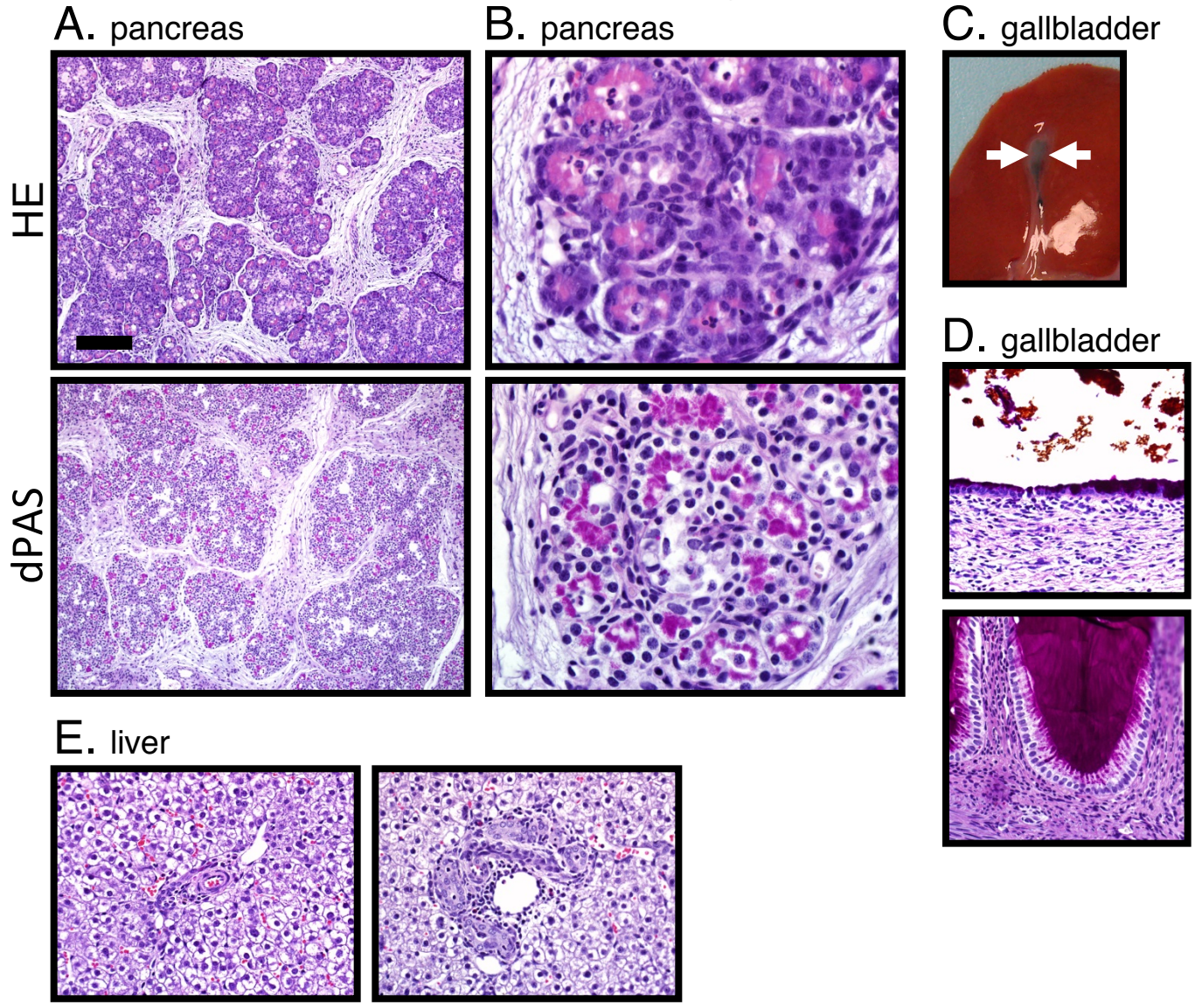
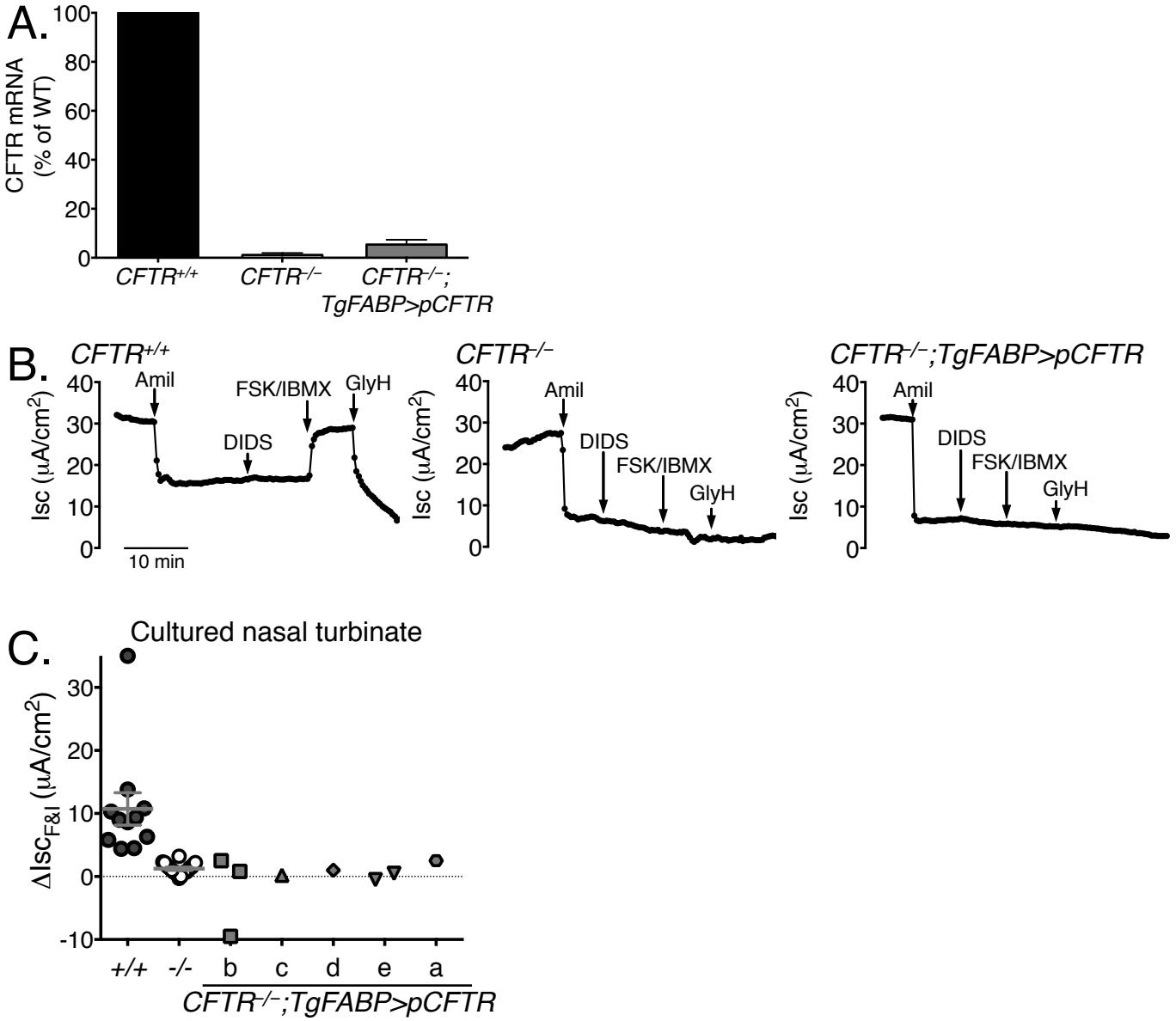
Supplemental Figure 1



Supplemental Figure 1. The extra-intestinal abdominal phenotype is preserved in *CFTR$^{-/-}$;TgFABP>pCFTR* newborn pigs. (**A-B**) Newborn *CFTR$^{-/-}$;TgFABP>pCFTR* pig pancreas, top panels – H & E staining; bottom panels – dPAS staining. (**A**) Low-magnification image showing loss of lobularity. (**B**) High-magnification image showing variable extent of zymogen staining in acinar cells. (**C**) Gross image of *CFTR$^{-/-}$; TgFABP>pCFTR* micro-gallbladder (denoted by arrows). (**D**) Microscopic images of *CFTR$^{-/-}$;TgFABP>pCFTR* gallbladders. Gallbladders varied from moderate sized and patent with focal mucous change in the epithelium (top panel) to severe micro-gallbladder that was obstructed by mucus with diffuse mucinous change in the epithelium (bottom panel). (**E**) Microscopic images of livers from *CFTR$^{-/-}$;TgFABP>pCFTR* newborn pigs. There was minimal evidence (left panel) of portal change to prominent focal biliary cirrhosis-like changes (right panel). Scale bar = 200 μm for (A), 33 μm for (B), and 68 μm for (D) and (E).

Supplemental Figure 2



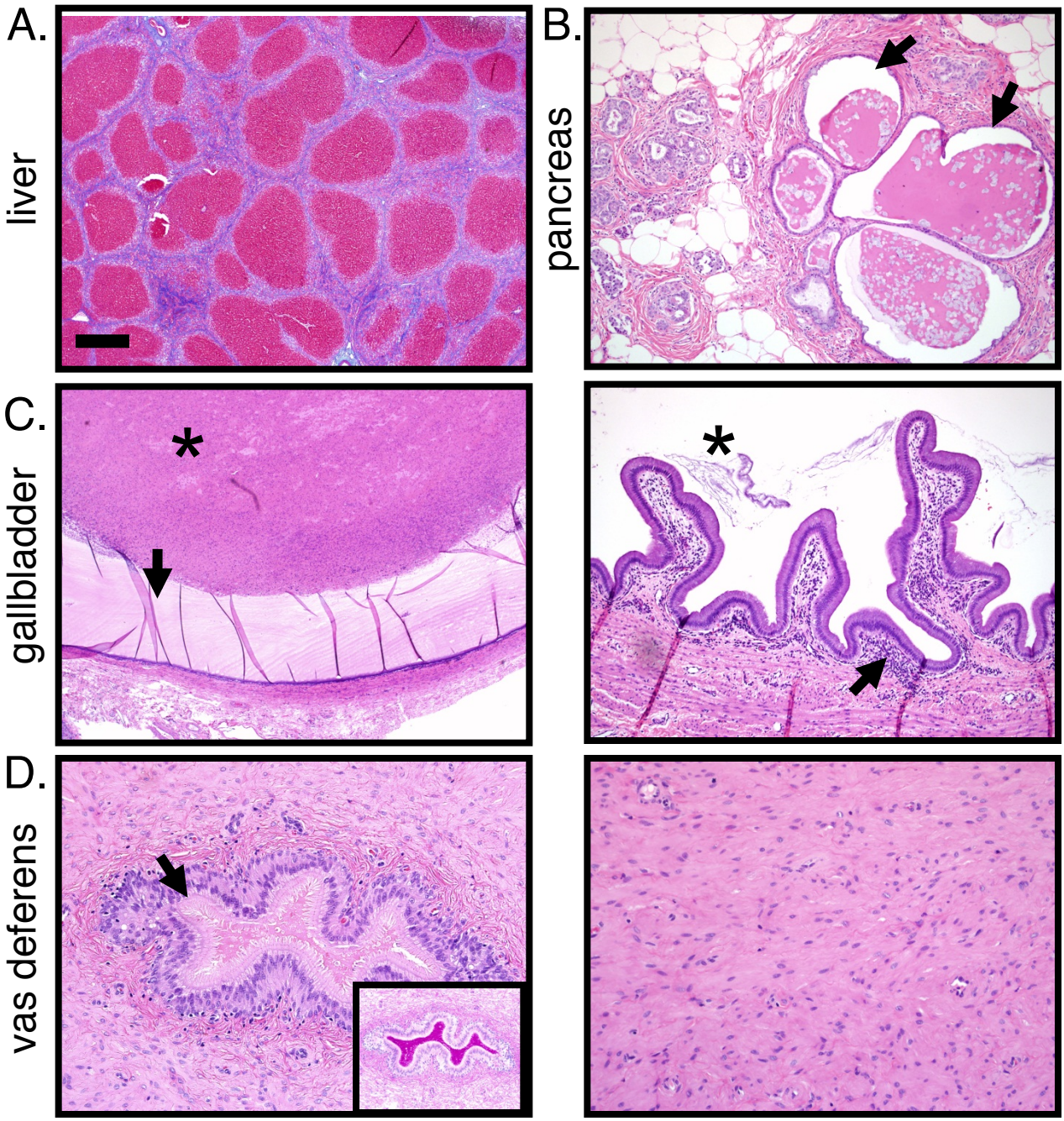
Supplemental Figure 2. CFTR expression and function in *CFTR$^{-/-}$;TgFABP>pCFTR* pig airway. (**A**) *CFTR* mRNA expression in trachea from *CFTR$^{+/+}$*, *CFTR$^{-/-}$*, and *CFTR$^{-/-}$;TgFABP>pCFTR* piglets (line e). *CFTR* mRNA levels were determined with quantitative RT-PCR (relative to *β-actin*) and values are expressed as a percentage relative to *CFTR* mRNA levels in *CFTR$^{+/+}$* tracheas . n = 3 - 4 per genotype. Bars represent mean ± SEM. (**B**) Representative short-circuit current (Isc) tracings from excised tracheas mounted in Ussing chambers for electrophysiological studies. Tracheal samples were obtained from newborn piglets. The following agents were added sequentially: apical 100 μM amiloride, apical 100 μM DIDS, apical 100 μM forskolin(FSK)/10 μM IBMX, and apical 100 μM GlyH. (**C**) $\Delta Isc_{F\&I}$ in cultured nasal turbinate epithelia from newborn *CFTR$^{+/+}$*, *CFTR$^{-/-}$*, and *CFTR$^{-/-}$;TgFABP>pCFTR* piglets. Prior to FSK/IBMX treatment, tissues were sequentially exposed to amiloride and DIDS. Symbols represent individual animals. Line represents mean ± SEM.

Supplemental Table 1

CASE	AGE	REASON FOR EUTHANASIA
1	4 wk	gastric ulcer
2	11 mo	elective
3	14 mo	elective
4	3 wk	respiratory symptoms
5	3 wk	respiratory symptoms
6	3 wk	elective

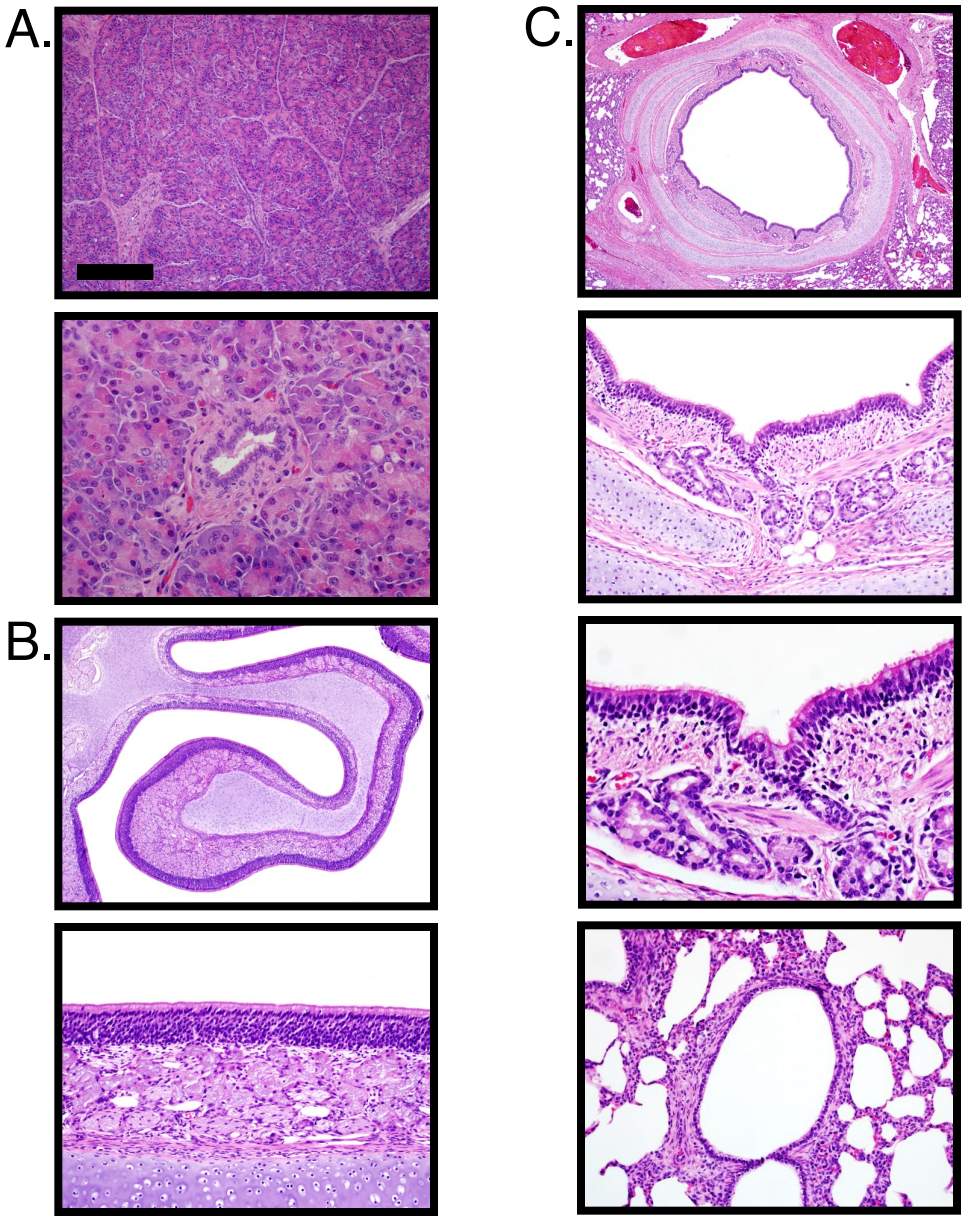
Supplemental Table 1. ***CFTR$^{-/-}$;TgFABP>pCFTR*** **transgenic pigs three or more weeks old.** All pigs were from line e. Gastric ulcer has previously been reported to occur in both non-CF and CF pigs (Stoltz *et al*. Science Translational Medicine, 2010 and Ostedgaard *et al*. Science Translational Medicine, 2011).

Supplemental Figure 3



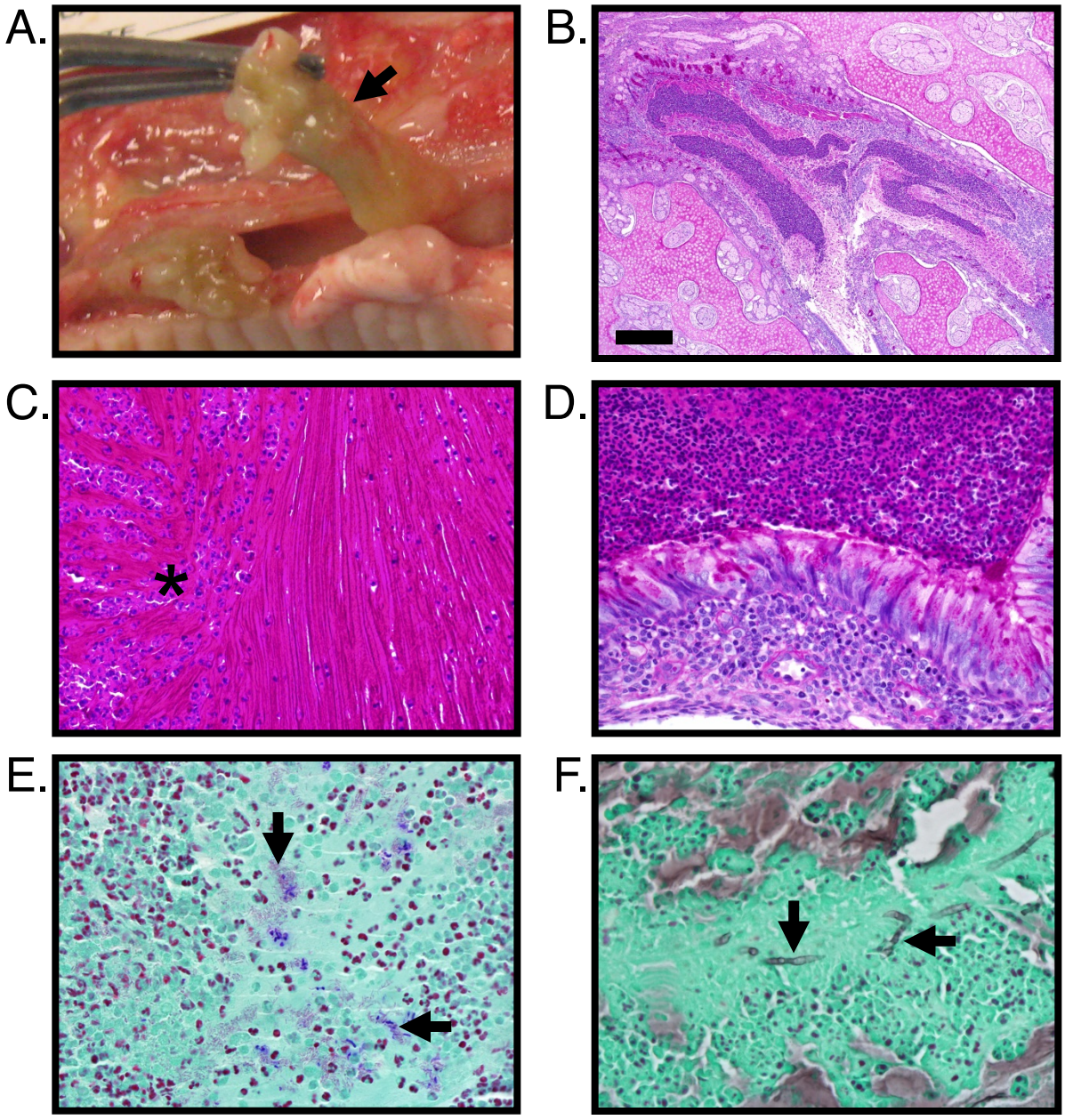
Supplemental Figure 3. Liver, pancreas, gallbladder, and vas deferens disease progression in older *CFTR*$^{-/-}$*;TgFABP>pCFTR* pigs. (**A**) Microscopic image of the liver from Case #1. At necropsy, the changes in the liver were heterogeneous. Some regions were normal-appearing and others had evidence of severe bridging cirrhosis. Masson's Trichrome stain. (**B**) Microscopic image of the pancreas from Case #2. Large obstructed cystic ducts (arrows) and fibrosis were also present. HE stain. (**C**) Microscopic images of the gallbladder from Cases #2 and #3. Left panel - There was mucus (arrow) and purulent/necrotic debris (asterisk) in the gallbladder lumen. Right panel - inflammatory cells (arrow) and luminal mucus (asterisk) were present. HE stain. (**D**) Microscopic images of the vas deferens from Case #3. The vas deferens epithelium (arrow, left panel), when detected, was often collapsed with eosinophilic material in the lumen that was dPAS positive (inset) or more often completely absent within the fibromuscular matrix (right panel). HE stain. Scale bar = 840 μm for (A), 167 μm for (B), 418 μm (left panel) and 167 μm (right panel) for (C), and 84 μm for (D).

Supplemental Figure 4



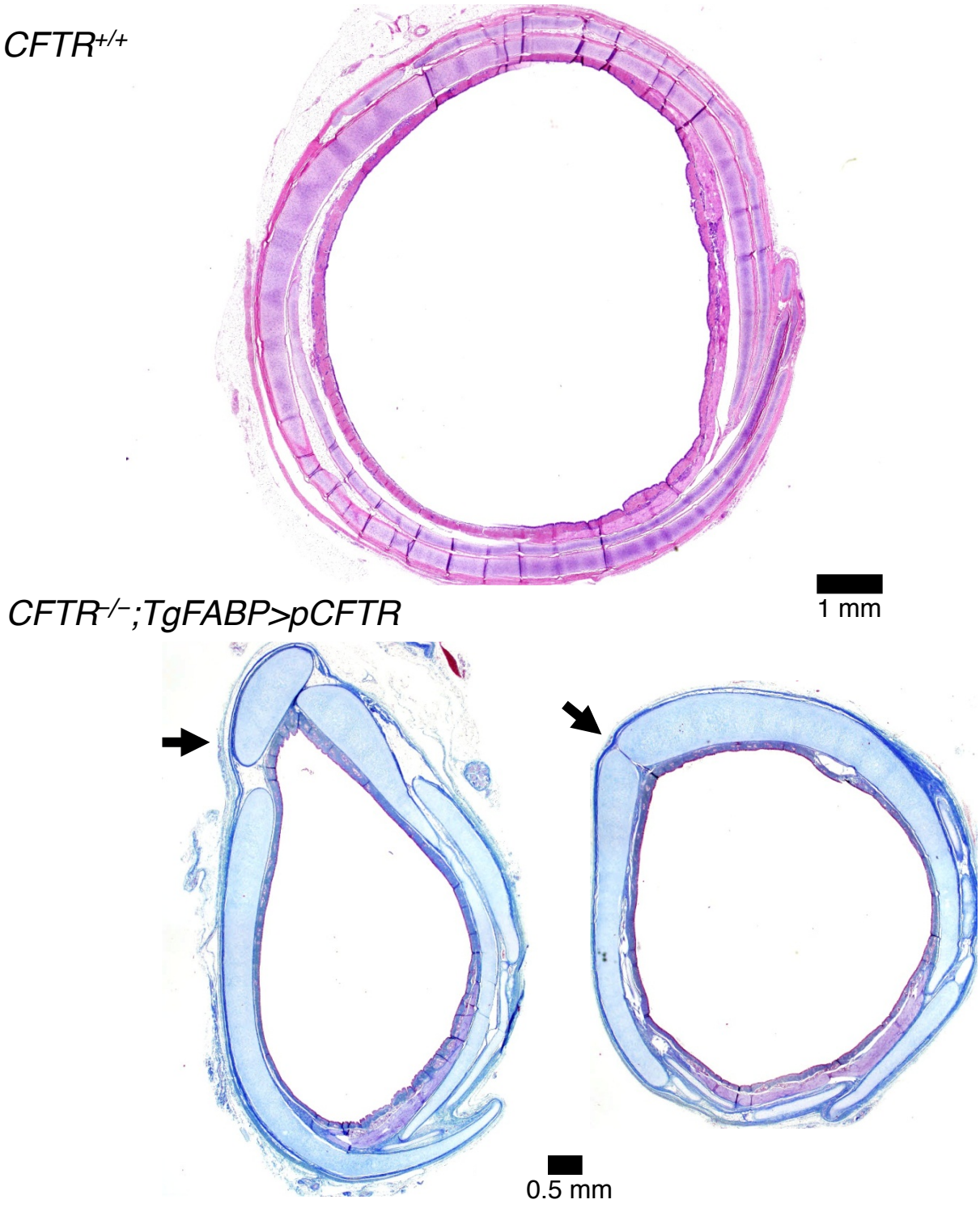
Supplemental Figure 4. Sections of normal pancreas, ethmoid sinus, and lung from older non-CF pigs. (**A**) Pancreas from a non-CF pig, bar = 348 (top panel) and 86 (bottom panel) μm. (**B**) Ethmoid sinus from a non-CF pig, bar = 870 (bottom panel) and 174 (top panel) μm. **(C**) Lung - Bronchus (top 3 panels) and bronchiole (bottom panel) from a non-CF pig, bar = 870, 174, 86 and 174 μm, respectively.

Supplemental Figure 5



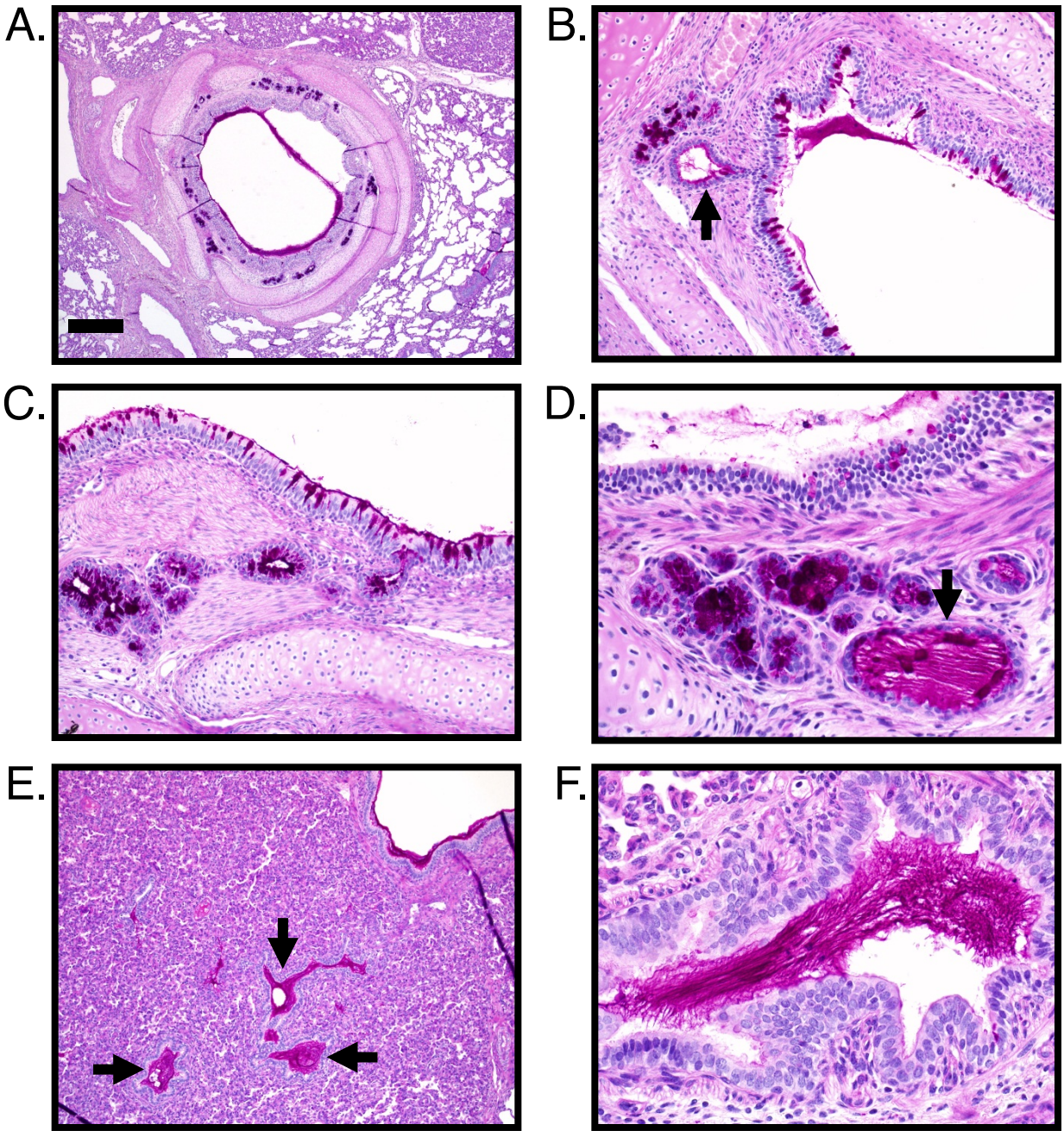
Supplemental Figure 5. ***CFTR*$^{-/-}$*;TgFABP>pCFTR*** **pigs develop sinus disease postnatally.** (**A**) Case # 4, on necropsy, thick mucus (arrow) occluded the ethmoid sinuses. (**B**) Case #4, histopathologic analysis showed purulent, necrotizing mucinous sinusitis. dPAS stain. (**C**) Case #3, oftentimes luminal mucus had a lamellar appearance that demarcated areas of neutrophil accumulation between layers (asterisk). dPAS stain. (**D**) Case #6, moderate sinus disease with epithelial cell hyperplasia and mucopurulent obstruction of the lumen. dPAS stain. (**E**) Case #6, Gram staining of luminal sinus material showed large colonies (arrows) of both gram-negative (pink stained) and gram-positive (purple stained) bacteria of varying morphologies. Microbiologic cultures revealed a number of different bacterial species including *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus spp*., and *Klebsiella pneumonia*e. (**F**) Case #3, Gomori's methenamine silver stain showed irregular hyphae (arrows) consistent with secondary zygomycosis. Scale bar = 450 μm for (B), 45 μm for (C), 45 μm for (D), 30 μm for (E), and 30 μm for (F).

Supplemental Figure 6



Supplemental Figure 6. Airway structural abnormalities persist in older *CFTR$^{-/-}$;TgFABP>pCFTR* pigs. Cross section of trachea from CFTR$^{+/+}$ (HE stain) and *CFTR$^{-/-}$;TgFABP>pCFTR* pigs (Masson's Trichrome stain). Bottom panel – note that trachea was irregularly shaped and had cartilage ring defects in the anterior trachea (arrows).

Supplemental Figure 7



Supplemental Figure 7. ***CFTR*$^{-/-}$*;TgFABP>pCFTR*** **pigs spontaneously develop airway disease.** (**A**) Case #4, large bronchus with mucus often lining the airway wall. dPAS stain. (**B**) Case #5, bronchus with mucus lining the epithelial wall with a dilated submucosal gland duct (arrow). dPAS stain. (**C**) Case #4, bronchus with a thin rim of mucus lining the epithelial wall and goblet cell hyperplasia. dPAS stain. (**D**) Case #6, small bronchus with luminal dPAS positive mucus and a dilated mucus filled submucosal gland duct (arrow). dPAS stain. (**E**) Case #4, diffuse atelectasis with magenta colored mucus lining large bronchioles and filling/ obstructing the small bronchioles. dPAS stain. (**F**) Case #4, mucus filled bronchioles had an interesting lamellar to stringy appearance. Note the paucity of dPAS staining in the adjacent epithelium. dPAS stain. Scale bar = 435 μm for (A), 87 μm for (B), 87 μm for (C), 43 μm for (D), 174 μm for (E), and 43 μm for (F).

Supplemental Figure 8

A.

Case	Age	Total Cells/ml	% Alveolar Macrophages	% Neutrophils
#4	3 wk	188,235	100	0
#5	3 wk	106,666	100	0
#6	3 wk	800,000	99	1

B.

CASE #4				
Lung tissue	Left caudal lobe	7.5E+04	cfu/ml	*Enterococcus spp.*
	Left cranial lobe	5.5E+04	cfu/ml	*Enterococcus spp.*
	Right tracheal lobe	8.5E+04	cfu/ml	*Enterococcus spp.*
BAL Liquid	Left lung	2.0E+01	cfu/ml	*Streptococcus spp.*
		2.0E+01	cfu/ml	*Erysipelothrix rhusiopathiae*
	Right lung	2.0E+01	cfu/ml	*Enterococcus spp.*
		5.0E+01	cfu/ml	*Enterococcus spp.*
		8.0E+01	cfu/ml	*Escherichia coli*
		5.5E+02	cfu/ml	*Erysipelothrix rhusiopathiae*
		1.3E+02	cfu/ml	*Streptococcus spp.*
Sinus	Left ethmoid	1.0E+04	cfu/ml	*Klebsiella pneumoniae*
		2.5E+06	cfu/ml	*Escherichia coli*
		2.5E+06	cfu/ml	*Streptococcus spp.*
		4.0E+06	cfu/ml	*Staphylococcus aureus*
	Right ethmoid	5.0E+06	cfu/ml	*Staphylococcus aureus*
		1.6E+06	cfu/ml	*Streptococcus spp.*
		1.0E+03	cfu/ml	*Escherichia coli*
CASE #5				
Lung tissue		no growth		
BAL liquid	Left lung	5.0E+01	cfu/ml	*Klebsiella pneumoniae*
		3.0E+01	cfu/ml	*Enterococcus spp.*
	Right lung	6.0E+01	cfu/ml	*Enterococcus spp.*
		1.0E+01	cfu/ml	*Staphylococcus aureus*
		3.4E+02	cfu/ml	*Erysipelothrix rhusiopathiae*
		1.0E+01	cfu/ml	*Escherichia coli*
CASE #6				
Lung tissue		no growth		
BAL liquid		no growth		
Sinus	Left ethmoid	8.0E+05	cfu/ml	*Staphylococcus aureus*
		5.0E+05	cfu/ml	*Klebsiella pneumoniae*
		1.0E+06	cfu/ml	*Escherichia coli*
		7.0E+05	cfu/ml	*Enterococcus spp.*
	Right ethmoid	6.0E+04	cfu/ml	*Escherichia coli*
		6.5E+05	cfu/ml	*Klebsiella pneumoniae*
		1.9E+02	cfu/ml	*Staphylococcus aureus*

Supplemental Figure 8. Summary of lung and sinus microbiology from cases #4-6. (**A**) Bronchoalveolar liquid cell counts and differentials from Cases #4, #5, and #6 at time of necropsy. (**B**) Quantitative bacteriology from lung tissue, BAL liquid, and sinus samples at the time of necropsy. Samples were not available from Case #1 because of complications from a gastric ulcer.