**Supplemental Digital Content 1**

**DETAILED METHODS**

**Animal Preparation**

Five Yorkshire pigs (32.1±2.9 kg) were anesthetized with intramuscular injections of ketamine (20–25 mg/kg) and xylazine (2 mg/kg). Anesthesia was maintained by continuous intravenous infusion of ketamine (7–11 mg/kg/h) and midazolam (0.2–0.7 mg/kg/h) through a peripheral catheter. The femoral artery was catheterized for blood pressure monitoring and arterial blood gas measurement. Pigs were intubated with a 6.5 mm cuffed endotracheal tube. SpO2, heart rate and body temperature were monitored continuously. All animals received intravenous hydration with normal saline (10 ml/kg bolus and 1 ml/kg/hinfusion). Data from these pigs were included in a previously published article1; there is no overlap in analysis between those results and the current imaging dataset.

**Mechanical Ventilation and Lung Injury**

Animals were ventilated using a custom-made mechanical ventilator with tidal volumes (TV) 8 ml/kg, respiratory rate 15 breaths per minute, and inspired fraction of oxygen 0.6. Healthy baseline imaging was performed while pigs were ventilated with PEEP 5 and 10 cmH2O in the supine and in the prone positions. Each combination of position and PEEP level was applied in random order and maintained for 10 minutes prior to imaging. Five pigs then received 2 ml/kg hydrochloric acid (HCl, pH 1.25), injected through the endotracheal tube in two equally divided doses with the animals in the right and in the left lateral position. Pigs were then stabilized supine for 60 minutes with PEEP 10 cmH2O. After stabilization, imaging was repeated with identical protocol as in the healthy baseline. Blood gases were obtained after each image acquisition.

**CT Imaging**

CT scans were acquired with a Siemens SOMATOM Force scanner. End-inspiratory (EI) and end-expiratory (EE) images were obtained during 5-second-long inspiratory and expiratory pauses. The settings were: 120 kVp, 200 mAs, pitch 0.95, slice thickness 0.75 mm, collimation 57.6x0.6 mm, estimated dosage 3–5 mSv. All images were reconstructed to a resolution of 1x1x1 mm with QR44 kernel. Only images obtained at PEEP 5 cmH2O were analyzed for the purposes of this study.

**Image Registration and Segmentation**

The methodology of CT analysis (summarized in **Figure 1**, main manuscript) was designed to match lung densities in paired supine and prone images. To measure density changes in individual voxels, images were registered to correct for the effects of body position on chest wall geometry and on regional tissue inflation. The outlines of the lungs were segmented (separated) from non-pulmonary tissue.

All the registration steps were implemented using the open-source ANTs package2. The entire registration code in ANTs is shown in the Supplemental Online Material. During *Step 1* (**Figure 1** of the main manuscript) of the image processing pipeline, all the images were segmented using a semiautomatic registration-based segmentation technique3. For this purpose, the whole lung masks of the baseline supine and prone images at PEEP 5 cmH2O were manually outlined by trained operators and automatically applied to all the remaining images in the corresponding position. This was possible because all images obtained in each position were registered to the same coordinates. With this methodology, the segmented binary masks were >95% accurate3; in addition, the segmentation was further refined by trained operators, using ITK-SNAP to manually improve accuracy of the lung boundaries. This was more often necessary in posterior and diaphragmatic lung regions after injury, where pulmonary opacifications were more prominent and borders more blurred.

In Step 2 (**Figure 1**, main manuscript), registration steps were performed to align the two segmented binary masks of each set of paired prone-supine images. The prone (moving) images were aligned to the supine (target) images, resulting in a warped prone binary mask. For registration, we applied (in sequence) rigid, affine, and symmetric diffeomorphic transformation models with third-order B-spline regularization (BSplineSyn)4. Mutual information served as the similarity function of rigid and affine registration5. Mean squared difference was used as the similarity metric for diffeomorphic transformation. A transformation matrix (ΦM) between the moving and target image mask was acquired.

During Step 3, whole-lung CT scans were aligned using symmetric diffeomorphic registration6 (**Figure 1**, main manuscript). The region of interest of the final registration step was confined to the lung parenchyma only (ignoring the ribcage and the remaining intrathoracic tissue) to reduce the computation cost. Third-order B-spline regularization was used on the real moving and target images, with ΦM acquired at step 2 as the initial moving transform. Normalized cross correlation was used as the similarity metric.

**ANTs Registration Code**

***# Binary mask registration***

${ANTsPATH}/antsRegistration -d 3 \

-o ${outputPrefix} \

-r [${maskfix},${maskmov},1] \

-t Rigid[0.2] \

-m MI[${maskfix},${maskmov},1,8,Regular,0.15] \

-c 100x100x50 \

-s 4x2x1 \

-f 8x6x4 \

-t Affine[0.2] \

-m MI[${maskfix},${maskmov},1,8,Regular,0.15] \

-c 100x100x50 \

-s 4x2x1 \

-f 8x6x4 \

-t BSplineSyN[0.2,40,0] \

-m MSQ[${maskfix},${maskmov},1,1] \

-c 100x100x50x0 \

-s 4x2x1x0 \

-f 8x4x2x1 \

***# CT mask dilation***

maskfixDilated=${inputPath}/${fixname}\_m\_Dilated.nii.gz

maskmovDilated=${inputPath}/${movname}\_m\_Dilated.nii.gz

${ANTsPATH}/ImageMath 3 ${maskfixDilated} MD ${maskfix} 10

${ANTsPATH}/ImageMath 3 ${maskmovDilated} MD ${maskmov} 10

***# CT image registration***

${ANTsPATH}/antsRegistration -d 3 \

-o ${outputPrefix} \

-r ${outputPrefix}1Warp.nii.gz \

-r ${outputPrefix}0GenericAffine.mat \

-t BSplineSyN[0.2,14,0] \

-m CC[${ctImagefix},${ctImagemov},1,4] \

-c 50x20x5 \

-s 2x1x0 \

-f 4x2x1 \

-x [${maskfixDilated},${maskmovDilated}]

***# Apply the transform matrix on moving image***

${ANTsPATH}/antsApplyTransforms -d 3 \

-o $fix2movWarped \

-n Linear \

-r ${ctImagemov} \

-i ${ctImagefix} \

-t [${outputPath}/${subject}/${fixname}\_To\_${movname}\_Results0GenericAffine.mat,1] \

-t ${outputPath}/${subject}/${fixname}\_To\_${movname}\_Results1InverseWarp.nii.gz \

-t ${outputPath}/${subject}/${fixname}\_To\_${movname}\_Results2InverseWarp.nii.gz

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