Supplemental information

Humanized mice reveal a macrophage-enriched gene signature defining human lung tissue protection during SARS-CoV-2 infection

Devin J. Kenney, Aoife K. O'Connell, Jacquelyn Turcinovic, Paige Montanaro, Ryan M. Hekman, Tomokazu Tamura, Andrew R. Berneshawi, Thomas R. Cafiero, Salam Al Abdullatif, Benjamin Blum, Stanley I. Goldstein, Brigitte L. Heller, Hans P. Gertje, Esther Bullitt, Alexander J. Trachtenberg, Elizabeth Chavez, Evans Tuekam Nono, Catherine Morrison, Anna E. Tseng, Amira Sheikh, Susanna Kurnick, Kyle Grosz, Markus Bosmann, Maria Ericsson, Bertrand R. Huber, Mohsan Saeed, Alejandro B. Balazs, Kevin P. Francis, Alexander Klose, Neal Paragas, Joshua D. Campbell, John H. Connor, Andrew Emili, Nicholas A. Crossland, Alexander Ploss, and Florian Douam
Figure S1
Figure S1. Related to Figure 1. Engraftment of NRGL mice with human fetal lung xenografts (fLX).

A. Left and right fLX following extraction from an NRGL mouse 4 weeks post engraftment.

B. Bilateral fetal lung xenografts (fLX) subcutaneously implanted in NRG mice. Recruitment of subcutaneous murine blood vessels is highlighted in the inset (black arrows).

C-D. Representative integration of murine and human blood vessels in human NRGL-LX following engraftment. (C) Integration of murine and human blood vessels at the interface of murine subcutis (left of the black hash line) and fLX (right of the black hash line). (D) Both human and murine blood vessels are present within the fetal lung xenograft interstitium. Purple (murine CD31) and brown (human CD31) duplex IHC, 200x (top, bar=100um), 400x total magnification (bottom, bar=50um).

E-N. Representative NRGL-LX tissue sections displaying heterogenous stages of lung maturation and differentiation affiliated with differential expression of ACE2 (host receptor for SAS-CoV-2), Prosurfactant Protein C (SFTPC-Alveolar type II pneumocyte differentiation), and CD31 (endothelium-human specific). (E-F) Pseudoglandular phenotype, columnar to cuboidal pneumocytes with high expression of ACE2 and SFTPC, forming glandular like structures supported by prominent mesenchymal stroma with high vascular density. (I-J) Canalicular phenotype, low cuboidal pneumocytes with moderate sporadic expression of ACE2 and SFTPC, retraction of mesenchymal stroma, decreased vascular density, and formation of coalescing airspaces with loss of distinct glandular like structures. (K-L) Saccular phenotype, further enlargement of airspaces lined by squamous epithelium with low to moderate expression of ACE2 and SFTPC, formation of distinct septal like structures, and low vascular density. (M-N) Bronchiole differentiation, columnar to pseudostratified ciliated epithelium with apical ACE2 expression and absence of SFTPC. E,I,K,M: Hematoxylin and Eosin (H&E), magnification 100x, scale bar=200um. F,J,L,N: fluorescent multiplex immunohistochemistry (mIHC), magnification 200x, scale bar =100um. DAPI-grey, ACE2 (magenta), SFTPC (teal), & CD31-Human (green).
Figure S2. Related to Figure 1. Human hematopoietic engraftment in HNFL-LX.

A. Gating strategy for analyzing the human hematopoietic compartment in naïve HNFL-LX following HSC engraftment. The same gating strategy was used for NRGL-LX.

B. Frequencies of AT1 (green), AT2 (purple), ciliated cells (blue) and club cells (red) within the human epithelial compartment in naïve NRGL-LX (3 fLX, 2,650 cells) and HNFL-LX (2 fLX, 2,159 cells) as determined by scRNAseq analysis.

C. Relative human ACE2 expression in HNFL-LX (blue) and NRGL-LX (red). Quantification was performed by RTqPCR. ns, non-significant.

D. t-SNE plots displaying clustered expression (scaled expression) of several transcripts coding for several human myeloid, inflammatory, and regulatory markers (CD33, CD14, CD68, IL1β, IL10) in naïve HNFL-LX (n=2). Cluster defining genes (i.e. whose expression level is significantly associated with a given cluster) have their name followed with an asterisk, and Log2FC value are indicated near the corresponding cluster(s). MP, macrophages.
E. Volcano plots displaying differentially expressed proteins in naïve HNFL-LX (n=4) vs. NRGL-LX (n=4) following side-by-side analysis by mass spectrometry run. Proteins with $p \leq 0.05$ (horizontal dashed line) and with logFC$\geq$1 or $\leq$-1 (vertical dashed lines) are considered significantly up- or downregulated respectively.

F. Quantification of tissue area immunoreactive for CD68 (% of analyzed tissue) in naïve HNFL-LX and NRGL-LX. n=3-8. Mean±SD, Welch’s t test ****$p \leq 0.0001$. 
Figure S3
Figure S3. Related to Figure 2. Susceptibility of NRGL mice to SARS-CoV-2 infection.

A-C. Probability of survival (A), weight loss (B), and temperature change (C) of NRGL mice following fLX inoculation with 10^4 PFU (n=6, blue), 10^6 PFU (n=9, red) or with PBS (n=1, black).

D. Macroscopic representative evidence of pathology in inoculated NRGL-LX. (Left) Uninoculated control, soft doughy, homogenous pale tan to white fLX. (Right) 7 days post SARS-CoV-2 infection, areas of red (black arrow) and pale tan to yellow (asterisk) were histologically confirmed to represent hemorrhage and coagulative necrosis, respectively.

E-H. Representative SARS-CoV-2 N IHC on inoculated NRGL-LX tissue section (2DPI: E,G; 7DPI: F,H) using 10^4 PFU. E,F: 200x, scale bar=100 µm; G,H: 400x, scale bar=50 µm.

I-K. Representative SARS-CoV-2 N IHC on naive (I) and inoculated (2DPI: J; 7DPI: K; 10^6 PFU) NRGL-LX. 400x, scale bar=50 µm.

L. Representative SARS-CoV-2 N IHC on inoculated NRGL-LX tissue section (2DPI; 10^6 PFU), with a focus on the bronchiole epithelium. 200x, scale bar=100 µm.

M. Representative SARS-CoV-2 N IHC on contralateral NRGL-LX tissue section (7DPI) from infected NRGL mice (10^6 PFU). 200x, scale bar=100 µm.

N. SARS-CoV-2 viral RNA quantification in the serum of non-infected (naïve) and infected NRGL mice (10^6 PFU) at 2DPI and 7DPI (n=4-10 fLX). Limit of detection (LOD, dotted line) represents mean viremia (n=4) in non-infected mice. n=4-10. Mean±SEM, Kruskal-Wallis test, ns, non-significant.

O. Planar in vivo imaging of SARS-CoV-2 infection in an NRGL mouse, following inoculation with rSARS-CoV-2-NL (10^6 PFU) in the left fLX. These images were used to calculate bioluminescent signals (photons/second/mm^3) reported in Figure 2O and 2P, using defined regions of interest (ROI) that are shown in red (inoculated fLX) and yellow (contralateral fLX).

P. SARS-CoV-2 viral RNA quantification in inoculated (10^6 PFU) NRGL-LX or NRGFL-LX (n=3 fLX per group). Limit of detection (LOD, dotted line) represents mean viral load in naïve NRGL-LX and NRGFL-LX (n=7). Mean±SEM, Kruskal-Wallis test, ns non-significant.
Figure S4
Figure S4. Related to Figure 3. Histopathological characterization of SARS-CoV-2 infection in NRGL and HNFL mice.

A. Histopathologic score of ten specific histopathological manifestations observed in inoculated NRGL-LX (10⁴ or 10⁶ PFU) at 2 and 7DPI. Sum of the ten score for each mouse was used to calculate cumulative histopathologic score shown in Figure 2A. n=5-10 flX. mean±SEM, two-way ANOVA *p≤0.05, **p≤0.01.

B. Histopathologic score of ten specific histopathological manifestations observed in inoculated NRGL-LX (10⁶ PFU) at 2 and 7DPI in comparison to naïve/Contralateral (CL) NRGL-LX. Sum of the ten score for each mouse was used to calculate cumulative histopathologic score shown in Figure 2B. n=8-12 flX. Mean±SEM, Kruskal-Wallis test *p≤0.05, **p≤0.01, ***p≤0.001.

C. Cumulative histopathologic score of inoculated (10⁶ PFU) NRGL-LX (n=12) and NRGFL-LX (n=5) at 2DPI. Mean±SEM. Kolmogorov-Smirnov t-test *p≤0.05.

D. Suspected viral budding events (asterisks) in inoculated NRGL-LX (10⁶ PFU). Inset magnification is shown at the top right. Spike proteins can be observed (arrow).

E. Binucleate type II pneumocyte syncytial cell with chromatin condensation and nuclear pyknosis with extracellular virions admixed with necrotic cellular debris and lamellar bodies in inoculated NRGL-LX (10⁶ PFU).

F-G. (F) Dying pneumocyte in the airspace filled with viral particles being released in the extracellular milieu in inoculated NRGL-LX (10⁶ PFU). (G) Magnified inset (2X) from panel C.

H. Virus replication centers within the cytoplasm of type II pneumocyte in inoculated NRGL-LX (10⁶ PFU).

I. Blood vessel occluded by an aggregate of platelets at 7DPI. Scale bar dimensions are indicated for all pictures in inoculated NRGL-LX (10⁶ PFU).

J. Occlusion of vascular lumen by polymerized fibrin in inoculated NRGL-LX (10⁶ PFU). Adjacent endothelium is abruptly absent suggestive of necrosis.

K. Histopathologic score of four selected specific histopathological manifestations observed in inoculated (10⁶ PFU) NRGL-LX (n=8-12) and HNFL-LX (n=4) at 2 and 7DPI. Sum of all the histopathologic score recorded for each flX analyzed was used to calculate cumulative histopathologic score shown in Figure 5K. Mean±SEM, two-way ANOVA *p≤0.05, **p≤0.01, ***p≤0.001.
Figure S5. Related to Figure 4. Human hematopoietic infiltration in HNFL-LX upon SARS-CoV-2 inoculation.

A. Flow cytometry gating strategy used to delineate the human hematopoietic compartment in naïve and inoculated HNFL-LX.

B. t-SNE plots displaying clustered expression (scaled expression) of several transcripts coding for human myeloid, inflammatory, and regulatory markers expression in inoculated HNFL-LX at 7DPI. Cluster of interest (dotted circles) are labelled in a plot at the left of the panel (AIM, activated inflammatory macrophages; ARM, activated regulatory macrophages; Mon, monocytes). Cluster defining genes are labelled with an asterisk, and Log2FC value for such genes are indicated near the corresponding cluster(s). 3 fLX, 11,269 cells.
Figure S6
Figure S6. Related to Figure 5. Molecular signature of SARS-CoV-2 infection in NRGL-LX.

A. Cluster heatmap representing proteins significantly ($p \leq 0.05$) up- ($z$-score $>0$) and downregulated ($z$-Score $<0$) in NRGL-LX at 2DPI ($10^6$ PFU) in comparison to naïve NRGL-LX. Naïve, $n=4$; 2DPI, $n=4$.

B-C. Host transcript frequencies (B; human, mouse, and SARS-CoV-2) and SARS-CoV-2 gene counts (C) in naïve ($n=4$), as well as in 2DPI ($n=4$), 7DPI ($n=8$) and contralateral NRGL-LX ($n=3$) samples used for bulk transcriptomic.

D. PCA plots of 2DPI (left; $n=4$), 7DPI (middle; $n=6$) and contralateral (right; $n=3$) NRGL-LX samples vs. naïve samples following bulk transcriptomic analysis.

E. Number of downregulated (left) and up-regulated (right) transcripts overlapping or not between 2DPI ($n=4$), 7DPI ($n=6$) and contralateral ($n=3$) NRGL-LX samples.

F. Normalized count of IFNB1 and IFNL1 transcripts in naïve, 2DPI ($n=4$), 7DPI ($n=6$) and contralateral ($n=3$) NRGL-LX samples. Adjusted p-values in comparison to naïve fLX are indicated.

G. Enriched IPA canonical pathway analysis (Qiagen) in inoculated (2, 7DPI) and contralateral (CL) NRGL-LX. Color intensity is proportional to Z-Score. Naïve, $n=3$; 2DPI, $n=4$; 7DPI, $n=6$; CL/Contralateral, $n=3$. 
**Figure S7**

**Figure S7. Related to Figure 7. Comparison of PDG expression between monocyte/macrophage subsets derived from HNFL-LX and from the lung of patients with severe COVID19.** Violin plots displaying combined scaled mean expression of all PDG in monocyte and macrophage clusters identified in human lung tissues from patients with severe COVID19 disease (autopsy samples, cluster 1 to 6) and in activated macrophages identified in inoculated HNFL-LX (2DPI, AIM and ARM clusters). For each cluster, scaled mean expression of each PDG was normalized on the mean scaled expression of CD163 (a monocyte/macrophage marker) and median of the scaled mean expression of all PDG is shown as a red line. Statistically significant differences in combined PDG expression between HNFL mice and human patient clusters was calculated using a RM one-way ANOVA with Geisser Greenhouse correction, and p values (-Log10(p.value)) are reported as an heatmap at the right of the panel. *p* ≥ 0.05 (-Log10[0.05] = 1.30) are shown in grey and are considered non-significant. AIM, activated inflammatory macrophages; ARM, activated regulatory macrophages.