Supplementary Materials

GlcNAc6ST2/Chst4 is essential for the synthesis of R-10G-reactive keratan sulfate/sulfated *N*-acetyllactosamine oligosaccharides in mouse pleural mesothelium

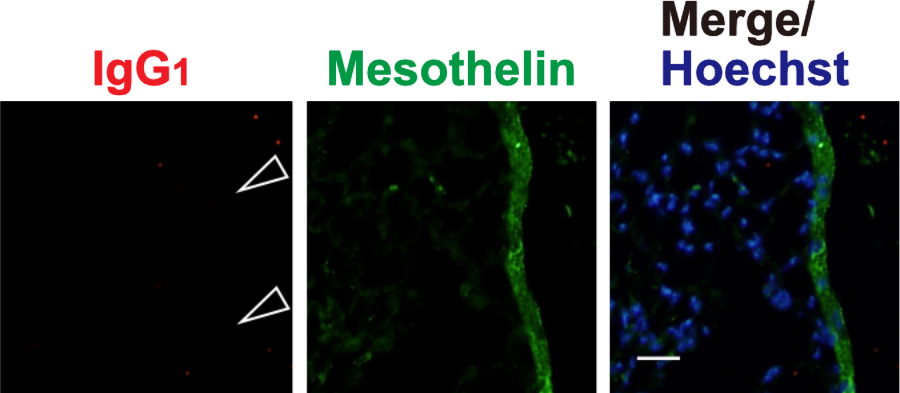
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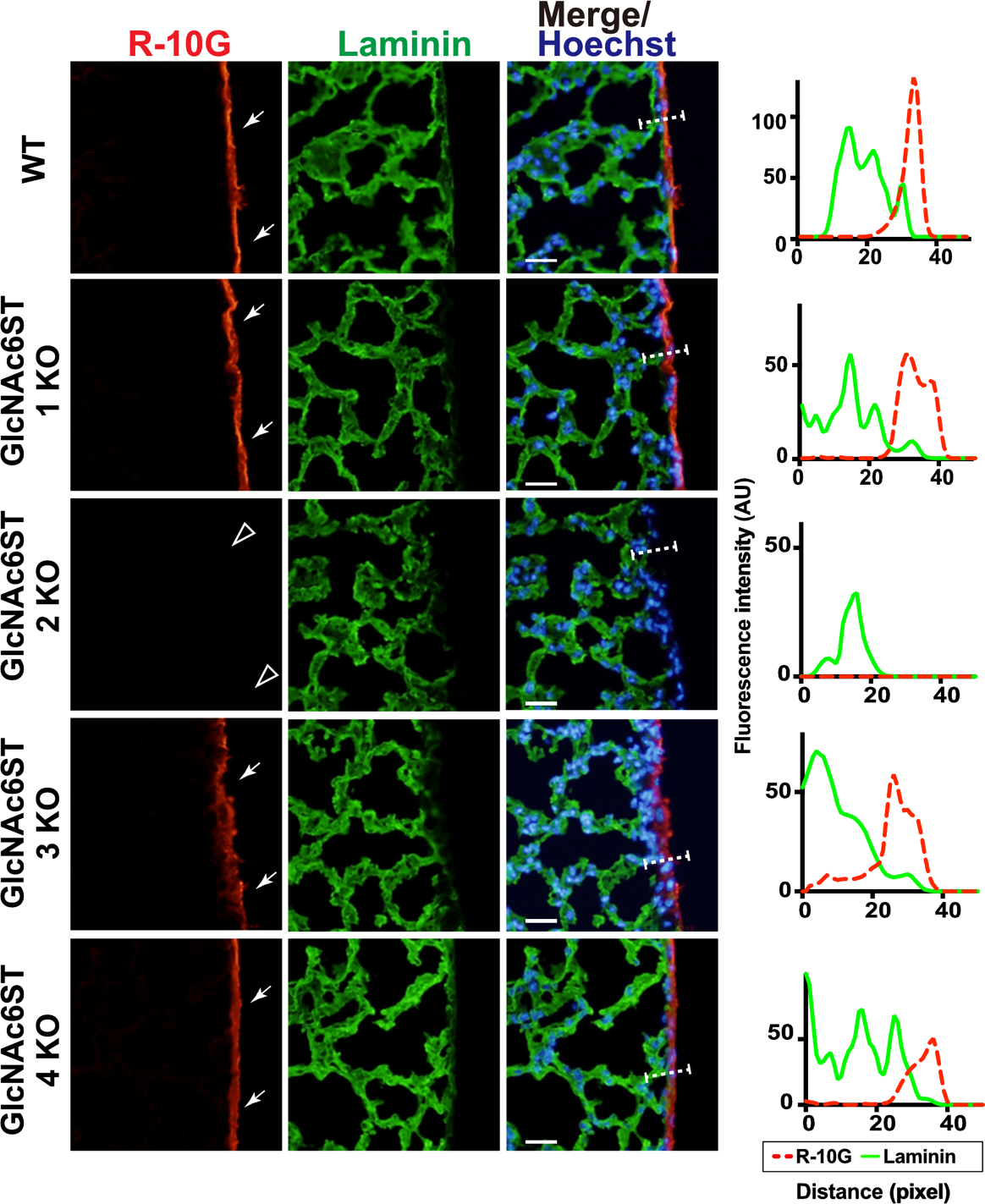
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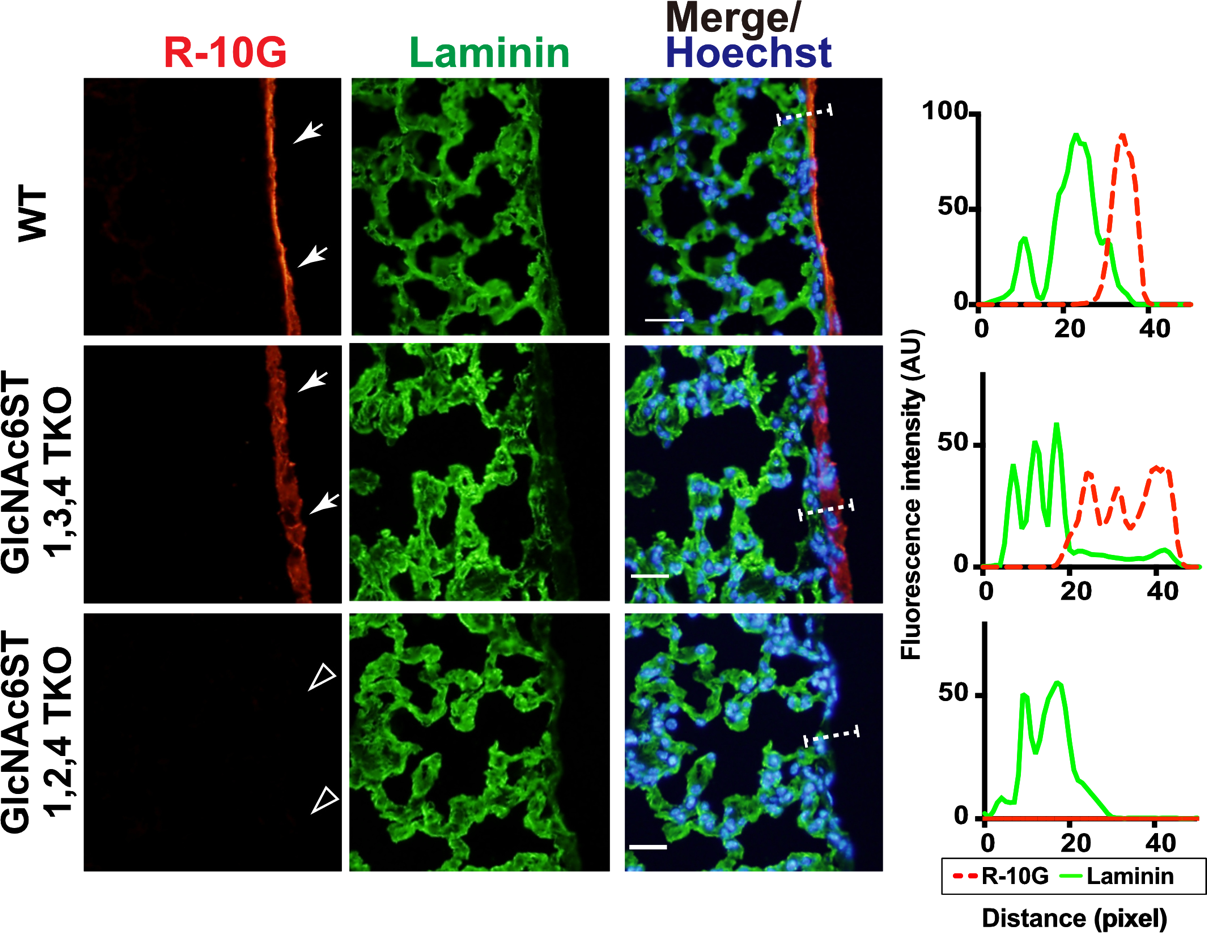
**Figure S1. Immunohistochemical analysis of the mouse lung with control IgG1.**

Lung sections prepared from normal wild-type mice were co-stained with control mouse IgG1 (*red*) and an anti-mesothelin (*green*) followed by Hoechst 33342 nuclear staining (*blue*). Signals with control IgG1 in the pleural mesothelium are negligible (*open arrowheads*) revealed by co-stained signals with a mesothelium marker, mesothelin. Representative fluorescence microscope images of the lower/middle region of left lung lobe are shown (n = 2). Scale bar: 20 µm.



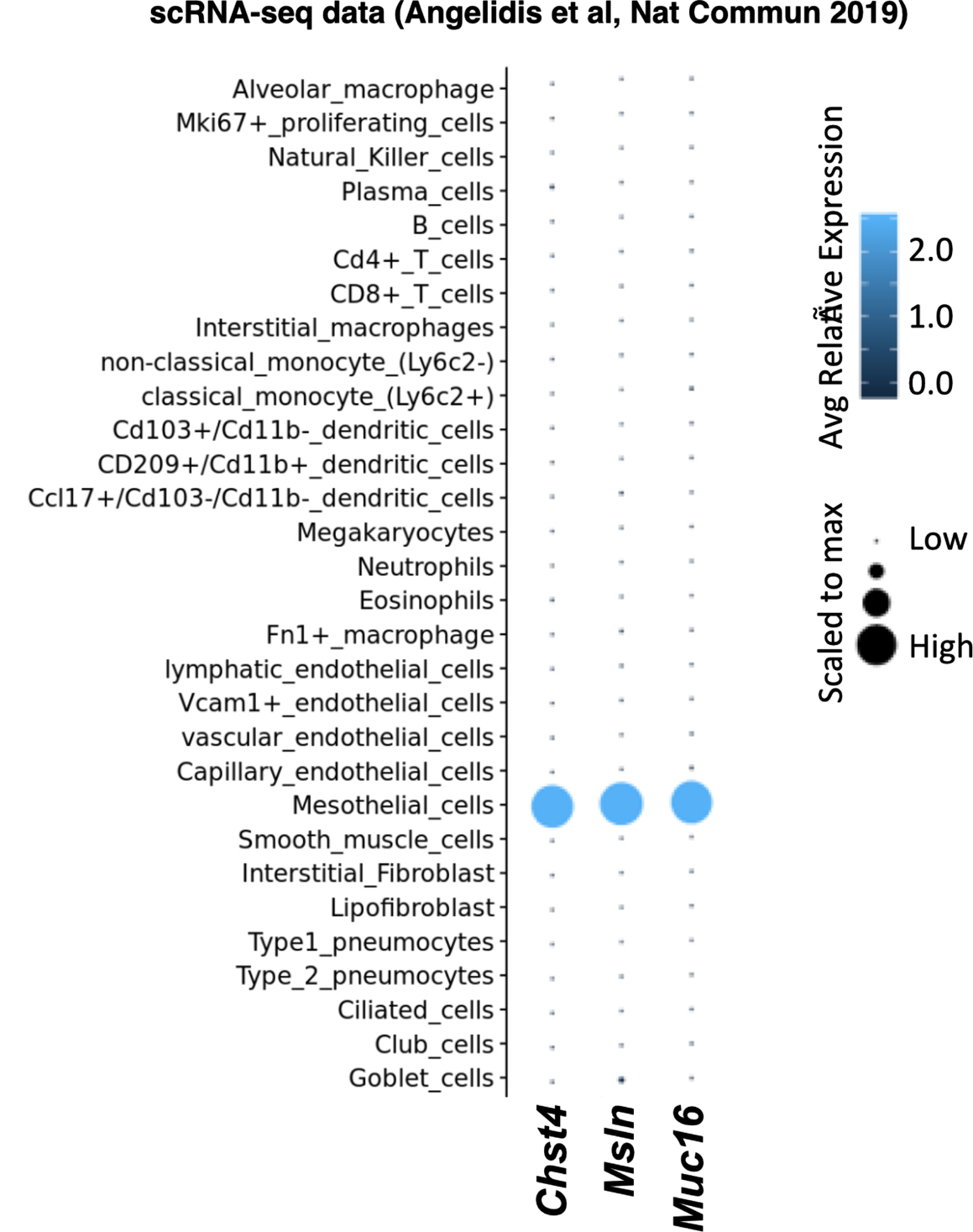
**Figure S2. Immunohistochemical analysis of the mouse lung with R-10G and anti-laminin.**

Lung sections prepared from normal wild-type (WT), *Chst2*-deficient (GlcNAc6ST1 KO), *Chst4*-deficient (GlcNAc6ST2 KO), *Chst5*-deficient (GlcNAc6ST3 KO), or *Chst7*-deficeint (GlcNAc6ST4 KO) mice were co-stained with R-10G (*red*) and anti-laminin (*green*) followed by Hoechst 33342 nuclear staining (*blue*). Dense R-10G staining in the pleural mesothelium is shown (*arrows*). Sections of GlcNAc6ST2 KO showed negligible levels of R-10G signals in the mesothelium (*open arrowheads*). Digital images were captured using the same settings for each staining. The plot profiles of R-10G and laminin staining are shown. The signal intensities along the path of the line marker (*dashed white line*) in the merged images were measured as described in Materials and Methods (n = 3 for each genotype). Scale bar: 20 µm.

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**Figure S3. Immunohistochemical analysis of the mouse lung with R-10G and anti-laminin.**

Lung sections prepared from normal wild-type (WT), *Chst2/Chst5/Chst7* triple-deficient (GlcNAc6ST1,3,4 TKO) and *Chst2/Chst4/Chst7* triple-deficient (GlcNAc6ST1,2,4 TKO) mice were co-stained with R-10G (*red*) and anti-laminin (*green*) followed by Hoechst 33342 nuclear staining (*blue*). Dense R-10G staining in the pleural mesothelium is shown (*arrows*). Sections of GlcNAc6ST1,2,4 TKO showed negligible levels of R-10G signals in the mesothelium (*open arrowheads*). Digital images were captured using the same settings for each staining. The plot profiles of R-10G and laminin staining are shown. The signal intensities along the path of the line marker (*dashed white line*) in the merged images were measured as described in Materials and Methods (n = 3 for each genotype). Scale bar: 20 µm.

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**Figure S4. Single cell RNA-seq data showing high, selective expression of *Chst4*, *Msln* and *Muc16* in mesothelial cells in mouse lung.**

Data pertinent to the genes of *Chst4, Msln and Muc16* were mined from a published single cellRNA sequencing (scRNA-Seq) analysis of adult mouse lung (Angelidis, L. et al., *Nat Commun* 2019, DOI: 10.1038/s41467-019-08831-9). Comparison of their transcription profiles in various cell types of the lung was performed using an scRNA-Seq transcriptome platform (https://theislab.github.io/LungAgingAtlas/).