Supporting Information

Khan et al. 10.1073/pnas.1217991110

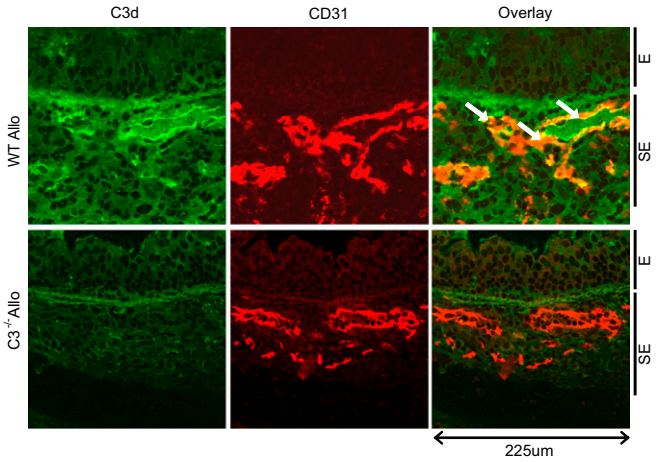

Fig. S1. Deposition of vascular complement component 3d (C3d) in orthotopic tracheal transplants in WT allograft (Allo) and $C3^{-/-}$ Allo recipients. Representative images showing absence of colocalization of C3d on $CD31^{+}$ [platelet endothelial cell adhesion molecule-1 (PECAM-1)] vascular endothelial cells (white arrows) on day 6 in allograft rejection in BALB/c→C57BL/6 $C3^{-/-}$ grafts compared with BALB/c→C57BL/6 grafts. E, epithelial layer; SE, subepithelial area in tracheal sections. Original magnification, 40× (n = 4–6 per group).

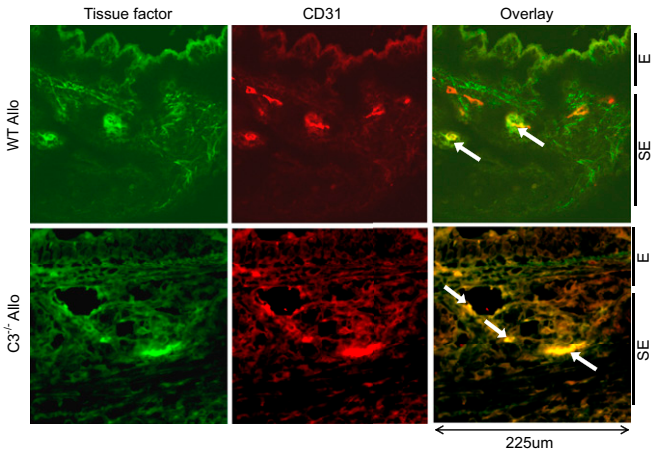

Fig. S2. Deposition of vascular tissue factor in orthotopic tracheal transplants in WT Allo and $C3^{-/-}$ Allo recipients. Representative images showing colocalization of tissue factor on $CD31^{+}$ vascular endothelial cells (white arrows) on day 6 in allograft rejection in BALB/c→C57BL/6 $C3^{-/-}$ grafts compared with BALB/c→C57BL/6 grafts. E, epithelial layer; SE, subepithelial area in tracheal sections. Original magnification, 40× (n = 4–6 per group).

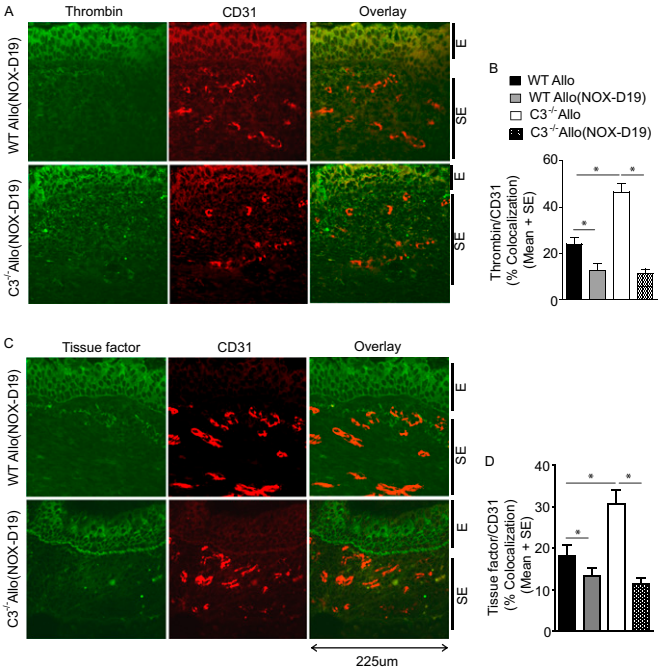

Fig. S3. Decreased vascular thrombin and tissue factor in WT Allo and C3$^{-/-}$ Allo NOX-D19–treated allografts. (*A*) Representative images showing decreased colocalization of thrombin on CD31^{+} vascular endothelial cells on day 6 of allograft rejection. (*B*) Morphometric assessments of thrombin/CD31^{+} colocalization compared with WT Allo, C3$^{-/-}$ Allo groups which were previously presented in Fig. 1*B*. (*C*) Representative images showing decreased colocalization of tissue factor on CD31^{+} vascular endothelial cells on day 6 of allograft rejection compared with WT Allo, C3$^{-/-}$ Allo groups which were previously presented in Fig S2. (*D*) Morphometric assessments of tissue factor/CD31^{+} colocalization. Data are shown as means with SEM. *$P < 0.05$. ($n = 4$–6 per group). E, epithelial layer; SE, subepithelial area in tracheal sections. Original magnification, 40× ($n = 4$–6 per group).

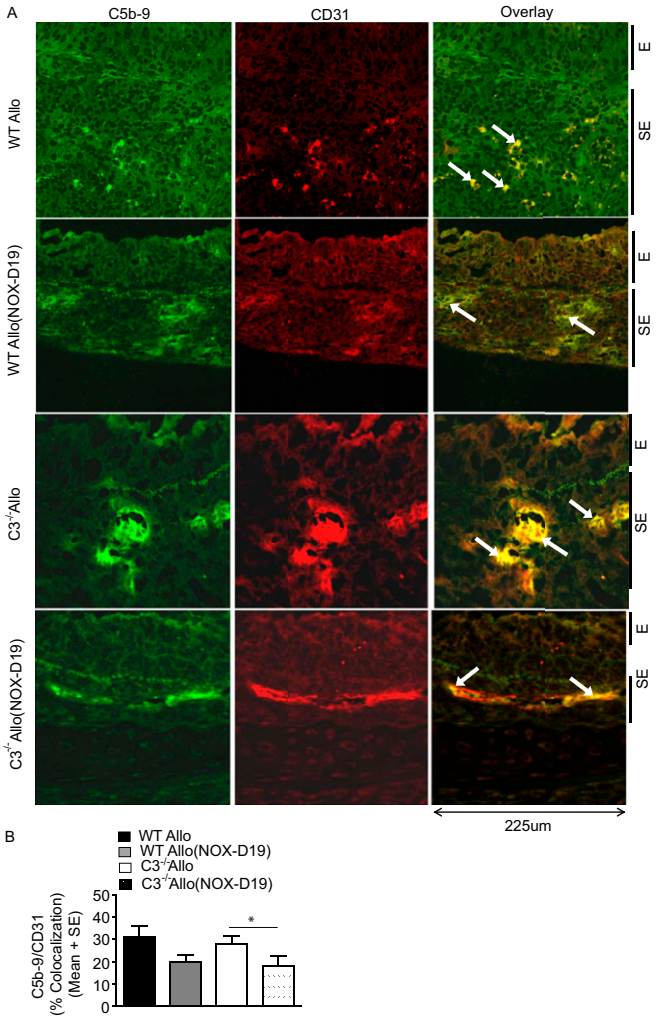

Fig. S4. Deposition of vascular C5b-9 in orthotopic tracheal transplants in $C3^{-/-}$ and NOX-D19–treated allografts. (*A*) Representative images showing co-localization of C5b-9 on $CD31^{+}$ vascular endothelial cells (white arrows) on day 6 of allograft rejection in BALB/c→C57BL/6 $C3^{-/-}$ grafts compared with BALB/c→C57BL/6 grafts. (*B*) Morphometric assessments of C5b-9/$CD31^{+}$ colocalization. E, epithelial layer; SE, subepithelial area in tracheal sections. Original magnification, 40× (n = 4–6 per group).

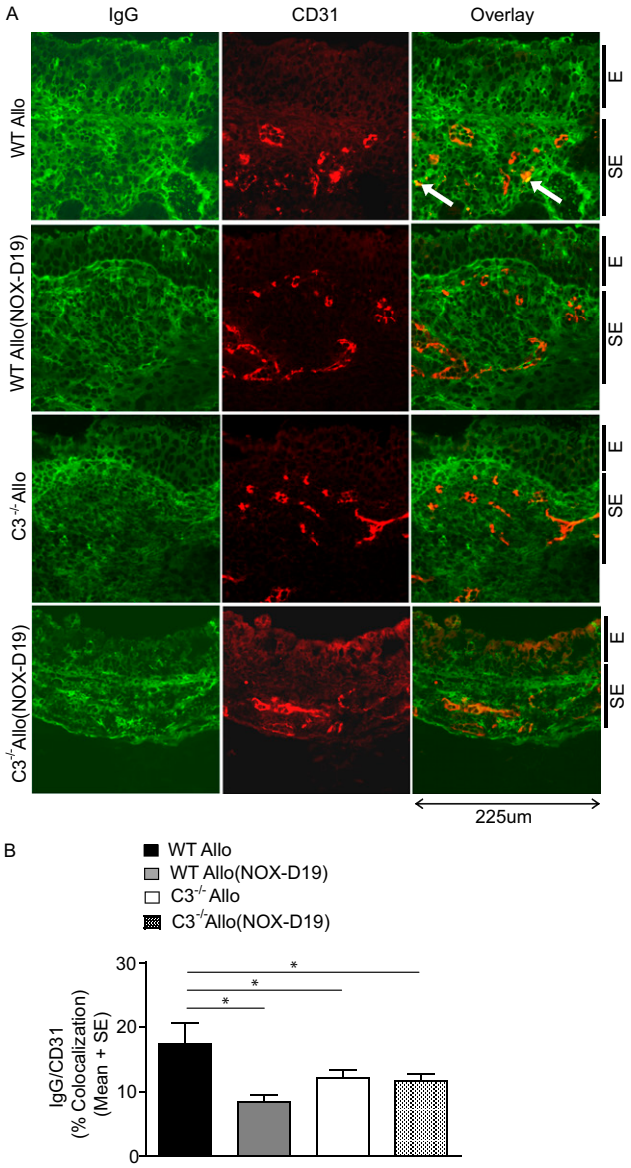

Fig. S5. Decreased vascular IgG in orthotopic tracheal transplants in $C3^{-/-}$ and NOX-D19– treated allografts. (*A*) Representative images showing colocalization of IgG on $CD31^{+}$ vascular endothelial cells (white arrows) on day 6 of allograft rejection in BALB/c→C57BL/6 $C3^{-/-}$ grafts compared with BALB/c→C57BL/6 grafts. E, epithelial layer; SE, subepithelial area in tracheal sections. Original magnification, 40×. (*B*) Morphometric assessments of IgG/$CD31^{+}$ colocalization. Data are shown as means with SEM. $*P < 0.05$ (n = 4–6 per group).

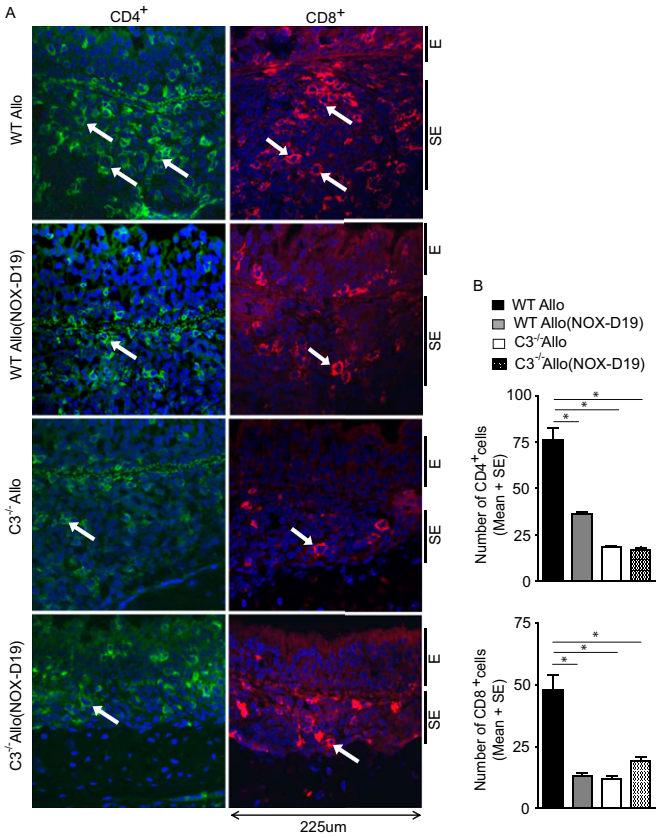

Fig. S6. (*A*) $CD4^+$ and $CD8^+$ T cells in orthotopic tracheal transplants in WT Allo, WT Allo (NOX-D19), $C3^{-/-}$ Allo, and $C3^{-/-}$ Allo (NOX-D19)–treated allografts. (White arrows shows the deposition of $CD4^+$ or $CD8^+$ cells on day 6). E, epithelial layer; SE, subepithelial area in tracheal sections. Original magnification, 40×. (*B*) Quantitative analysis of $CD4^+$ and $CD8^+$ cells in a given high power field. Data are shown as means with SEM. $^*P < 0.05$ (n = 4–6 per group).

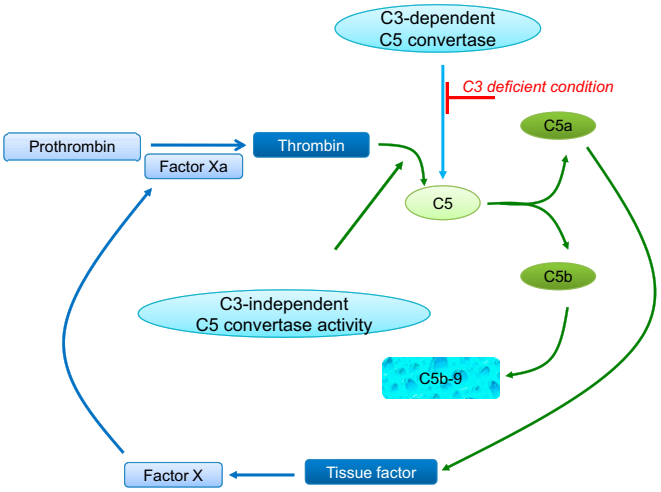

Fig. S7. Model illustrates how, during allograft rejection, increased C3-independent C5 convertase activity in C3-deficiency states may lead to increased production of C5a through production of thrombin and how generation of C5a could further contribute to a feed-forward amplification of this process.