Supplemental Figure 1. *Spdef* mRNA in trachea and conducting airways.

In situ hybridization for *Spdef* mRNA was performed on sections of trachea and lungs from fetal (A) and postnatal (B-D) mice. *Spdef* mRNA was detected in the tracheal epithelium E17.5 (A and inset) and bronchi at postnatal days 5 (B), 10 (C), and 20 (D), not in peripheral lungs (**lu**) and blood vessels (**V**). **C**, Cartilage. Scale bars: A-D, 200 μm; A inset, 50 μm.

Supplemental Figure 2. *Spdef* mRNA was detected in various tissues in the mouse.

In situ hybridization for *Spdef* mRNA was performed on sections of adult mouse tissues. *Spdef* mRNA was detected in the epithelium of the dorsal (A) and ventral (B) prostate coagulating gland, seminal vesicle (C), stomach (not shown), small intestine (not shown), colon (D), and oviduct (E), but not in ovary (E) or uterus (F). Arrow indicates the infundibulum of the oviduct that was weakly labeled (E). No SPDEF expression was detected by *in situ* hybridization in heart, thymus, thyroid, esophagus, spinal cord, brain, bladder, testes or epididymus (not shown). u, uterus. Scale bars: 200 μm.

Supplemental Figure 3. Specificity of anti-sense probe for detection of *Spdef* mRNA.

In situ hybridization was performed on sections of mouse lungs using anti-sense (A, C) and sense (B, D) probes for *Spdef* mRNA. While signal for *Spdef* mRNA was detected with anti-sense probe in lung from PN10 (A) and trachea from E17.5 (C), no hybridization was observed with sense probe in comparable tissue sections (B, D). Scale bars: 200 μm.

Supplemental Figure 4. Specificity of SPDEF polyclonal antibody.

(A) Immunoblot analysis was performed on lysates of HeLa cells transfected with SPDEF plasmid and adult mouse lung (mLu) and trachea (mTra) using guinea pig polyclonal antibody (described in Methods). A single band of approximately 37 kDa (arrowheads) was detected in HeLa cells transfected with *Spdef* cDNA, in tracheal lysates, but not in lysates of normal HeLa

cells or lung parenchyma. (B) Immunocytochemistry was performed on HeLa cells transfected with SPDEF expression plasmid using SPDEF antibody. Nuclear staining was detected in transfected HeLa cells, but not in untransfected HeLa cells (arrow). DAPI was used to counterstain nuclei.

Supplemental Figure 5. SPDEF was expressed in the epithelium of prostate, oviduct, and intestine.

Immunohistochemistry for SPDEF was performed on sections of adult mouse tissues. Nuclear staining was detected in epithelium of the seminal vesicles and coagulating glands (A), epithelial cells lining oviduct (B), and subsets of epithelial cells in the colon (C). Scale bars: A-D, 50 μm.

Supplemental Figure 6. SPDEF activates the *Foxj1* promoter in vitro.

(A) A schematic diagram of the promoter region of the *Foxj1* gene. The locations of the putative ETS binding sites (GGAA/T) are indicated. HeLa cells were co-transfected with the increasing doses of plasmids expressing SPDEF and FOXJ1 reporter plasmids (B). All of the reporter plasmids were activated by SPDEF in a dose dependent manner (0.01, 0.02, and 0.05 pM). Mutations of the two putative ETS binding sites in 0.25 *Foxj1-luc* plasmid (m1 and m2) did not affect the activation of the reporter by SPDEF (C). Control (con) was the co-transfection of the reporter plasmid and the empty expression plasmid. Transfections were performed in triplicate and repeated three times with similar results. Values are mean ± SD.

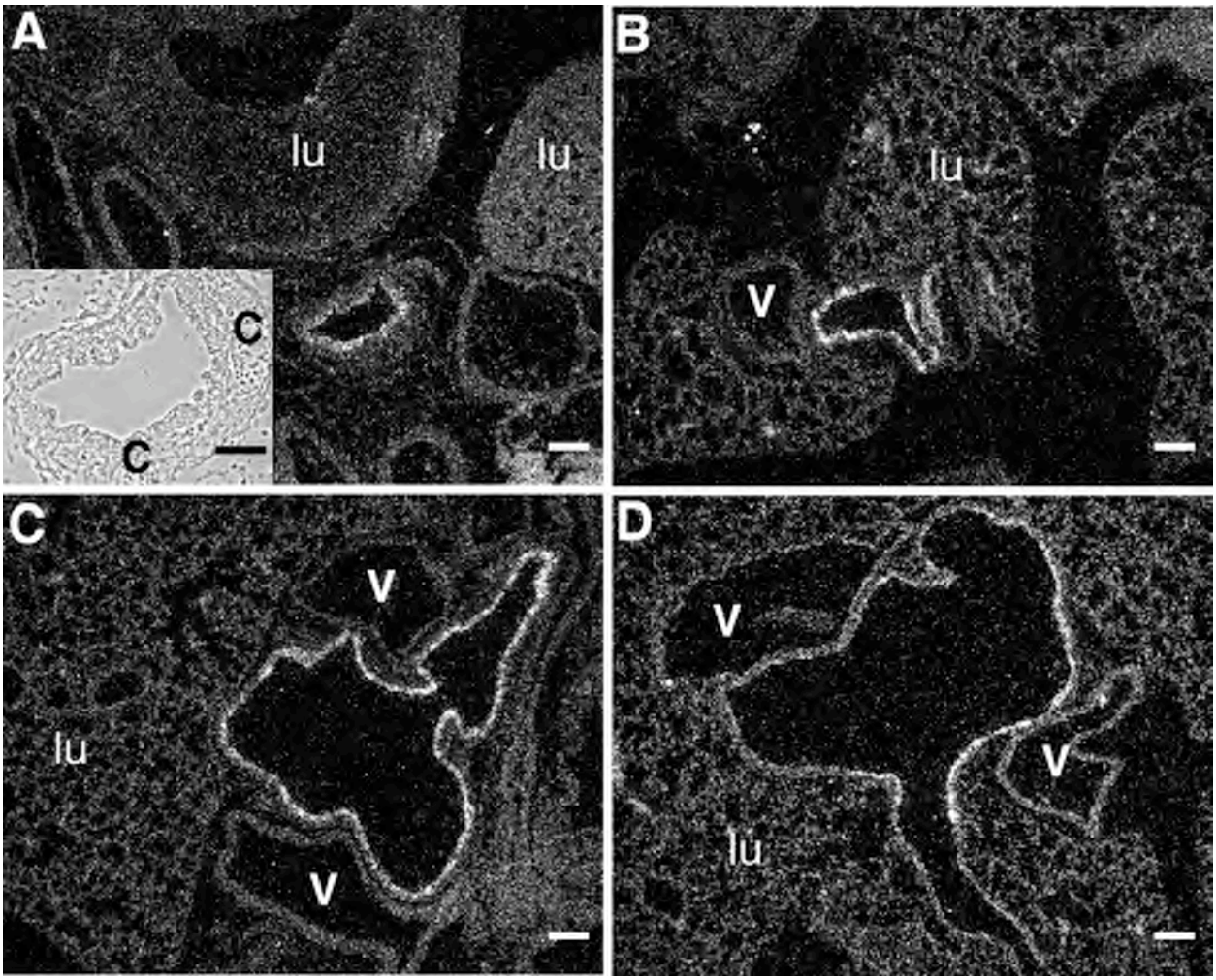
Supplemental Figure 7. Expression of SPDEF did not induce expression of proinflammatory mediators.

RT-PCR for *Spdef*, *IL-13*, *IL-4*, *IL-6*, *TGF-α*, *Heparin Binding (HB)-EGF* mRNAs was performed in lungs from *rCCSP-rtTA/TRE2-Spdef* mice treated with or without doxycycline. *Spdef* mRNA was increased more than three fold when the mice were treated with doxycycline.

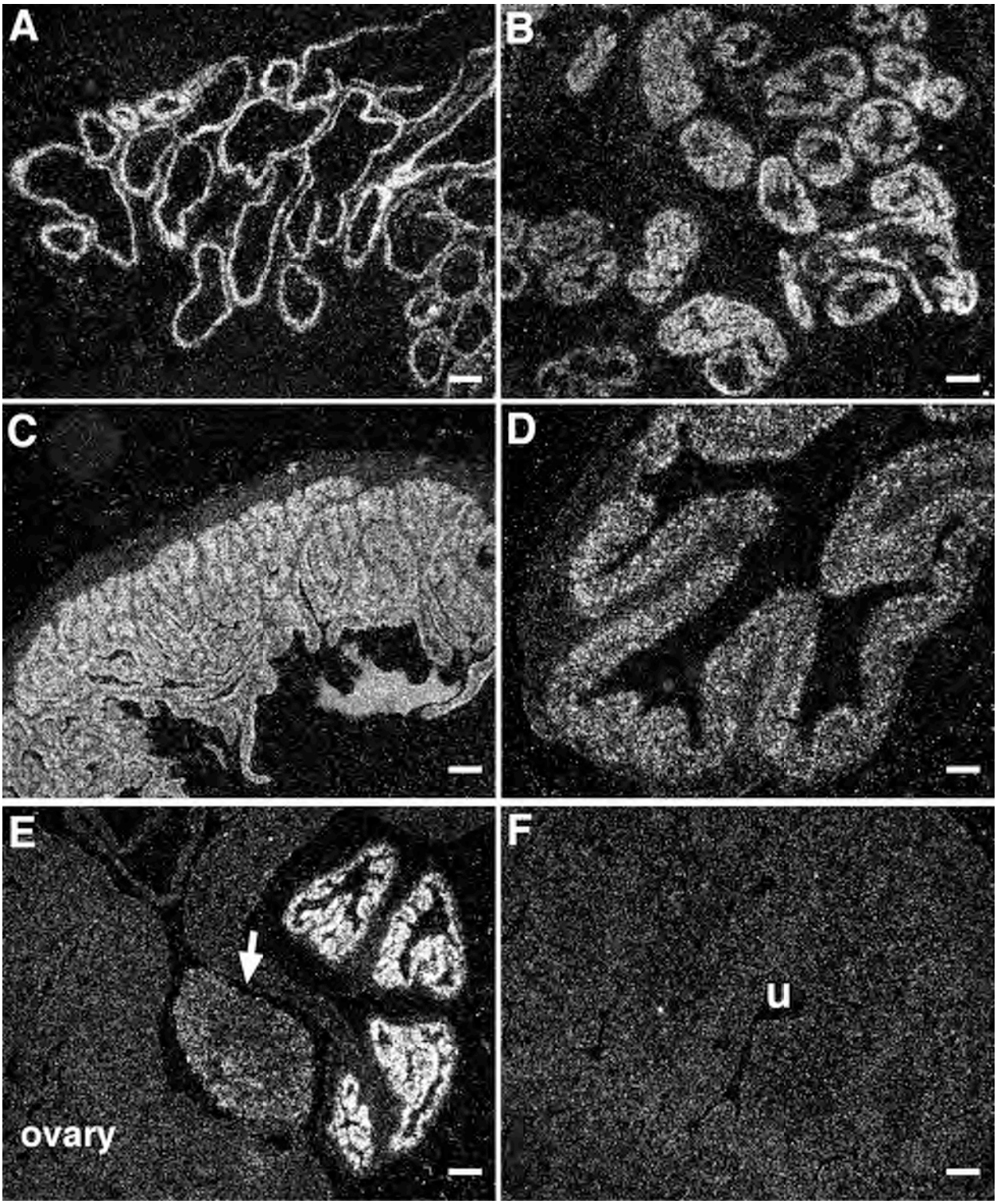
Levels of the cytokines and growth factors associated with goblet cell hyperplasia were not altered in the transgenic mice treated with doxycycline, *TGF-α* mRNA was not detected (ND). Sequence of PCR primers is in Supplemental Table 2. Values are mean ± SD, n=6 each group.

Supplemental Figure 8. Phospho-Histone 3 staining in lungs of *CCSP-rtTA/TRE2-Spdef* transgenic mice.

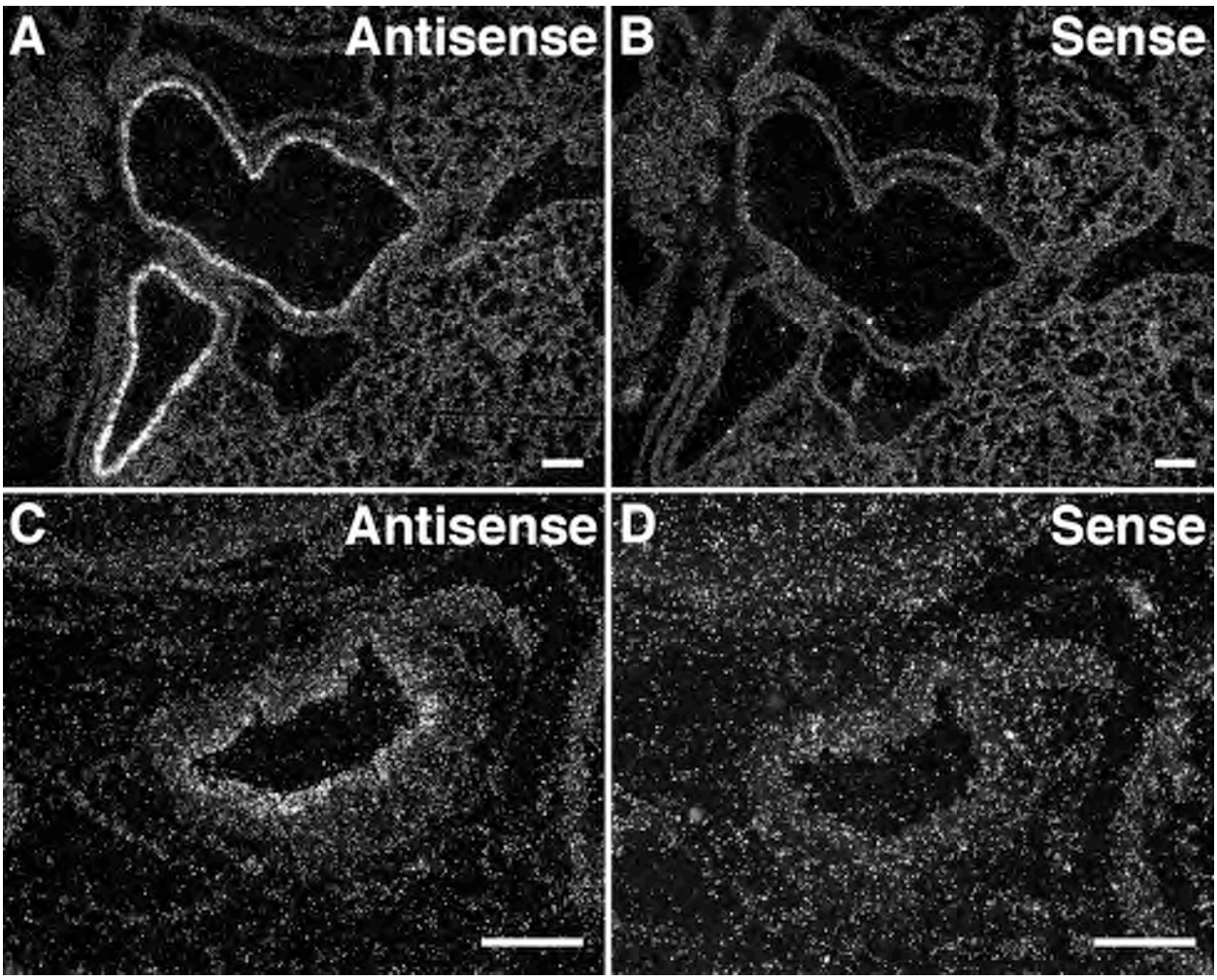
Immunohistochemistry for phospho-Histone 3 (pH3) was performed on the lung sections of the SPDEF expressing transgenic mice. pH3 staining was not detected in lung epithelial cells including the area of goblet cell hyperplasia (arrow) while detected in a few stromal cells and macrophages (arrowheads). The respiratory epithelium of non-transgenic littermates was also negative for pH3 staining (not shown).



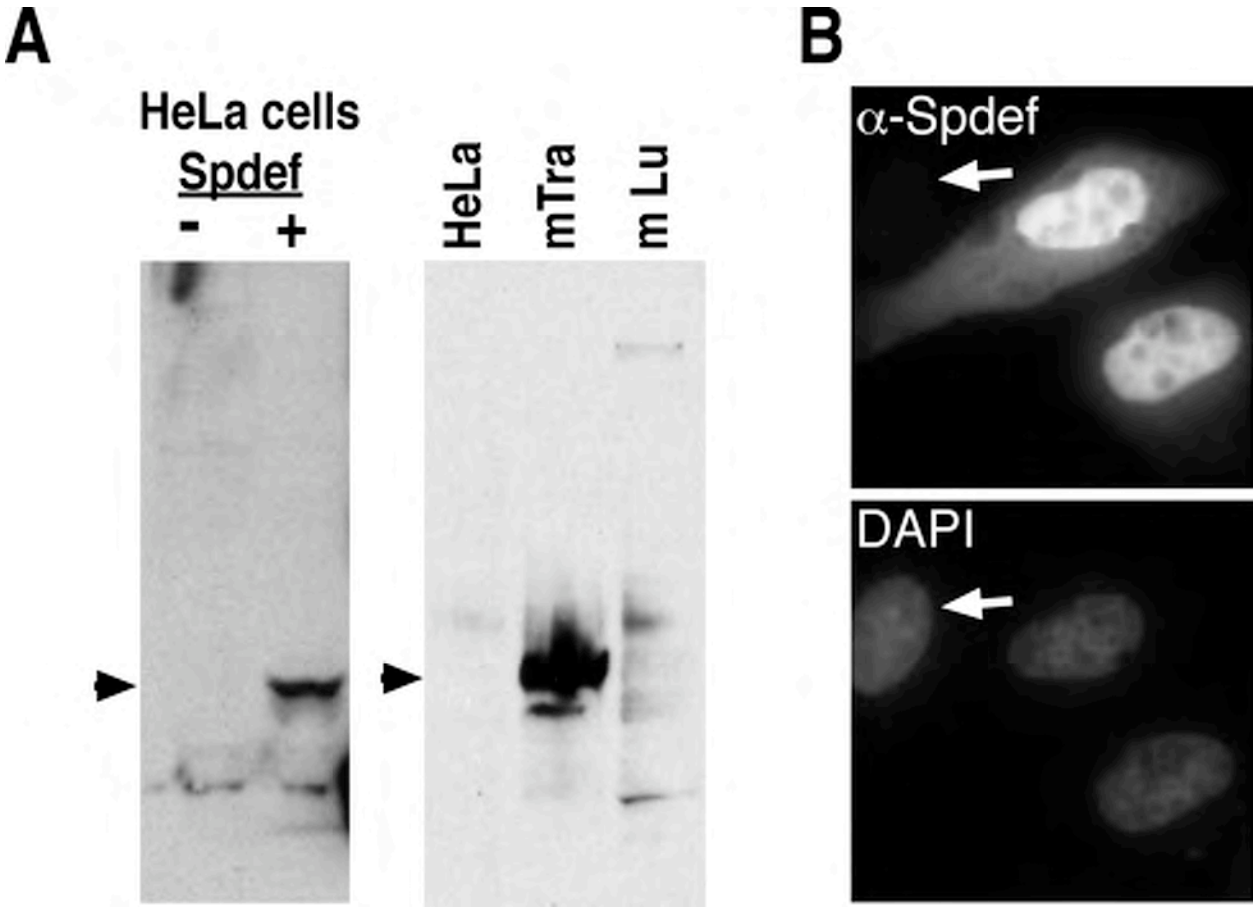
Supplemental Figure 1



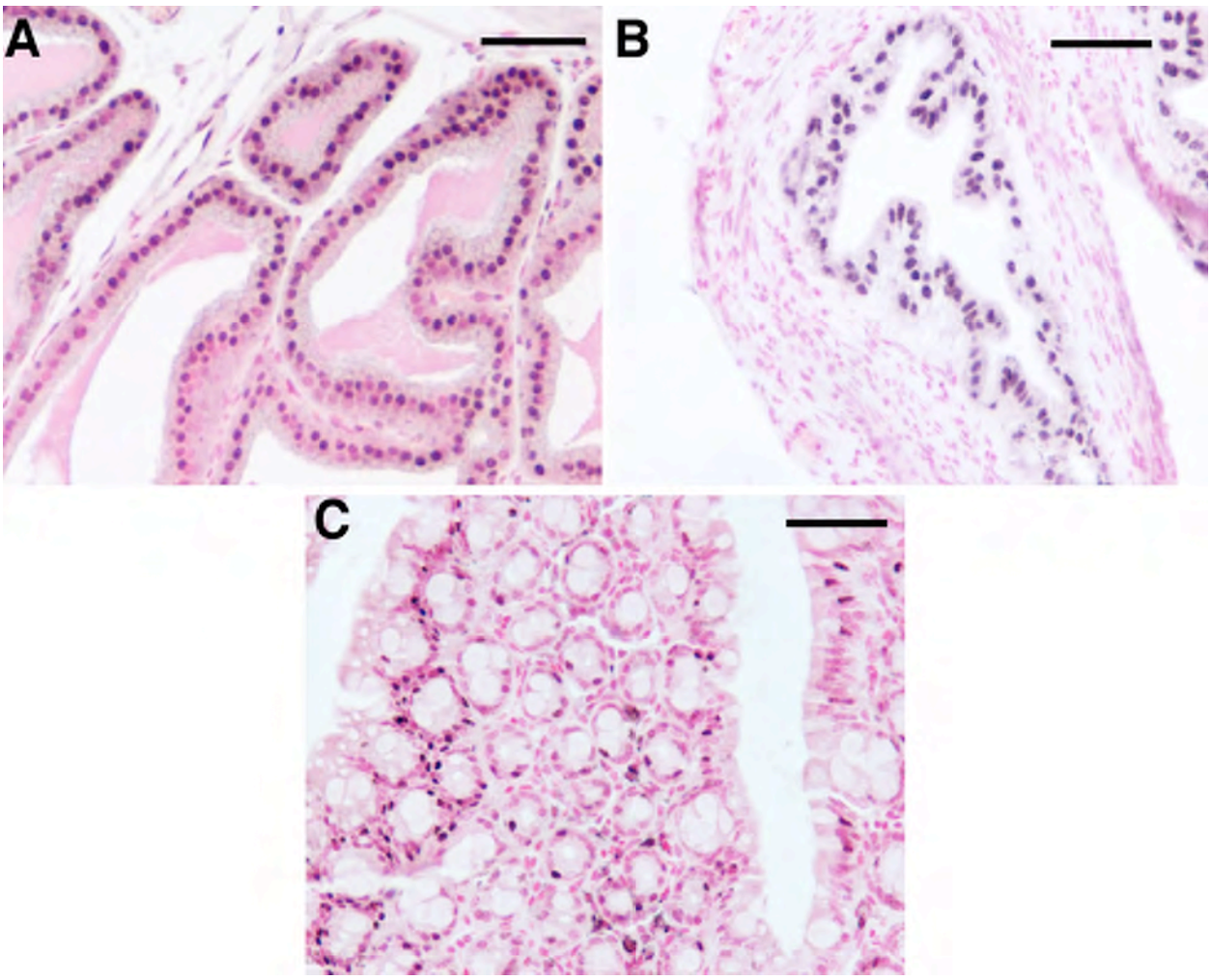
Supplemental Figure 2



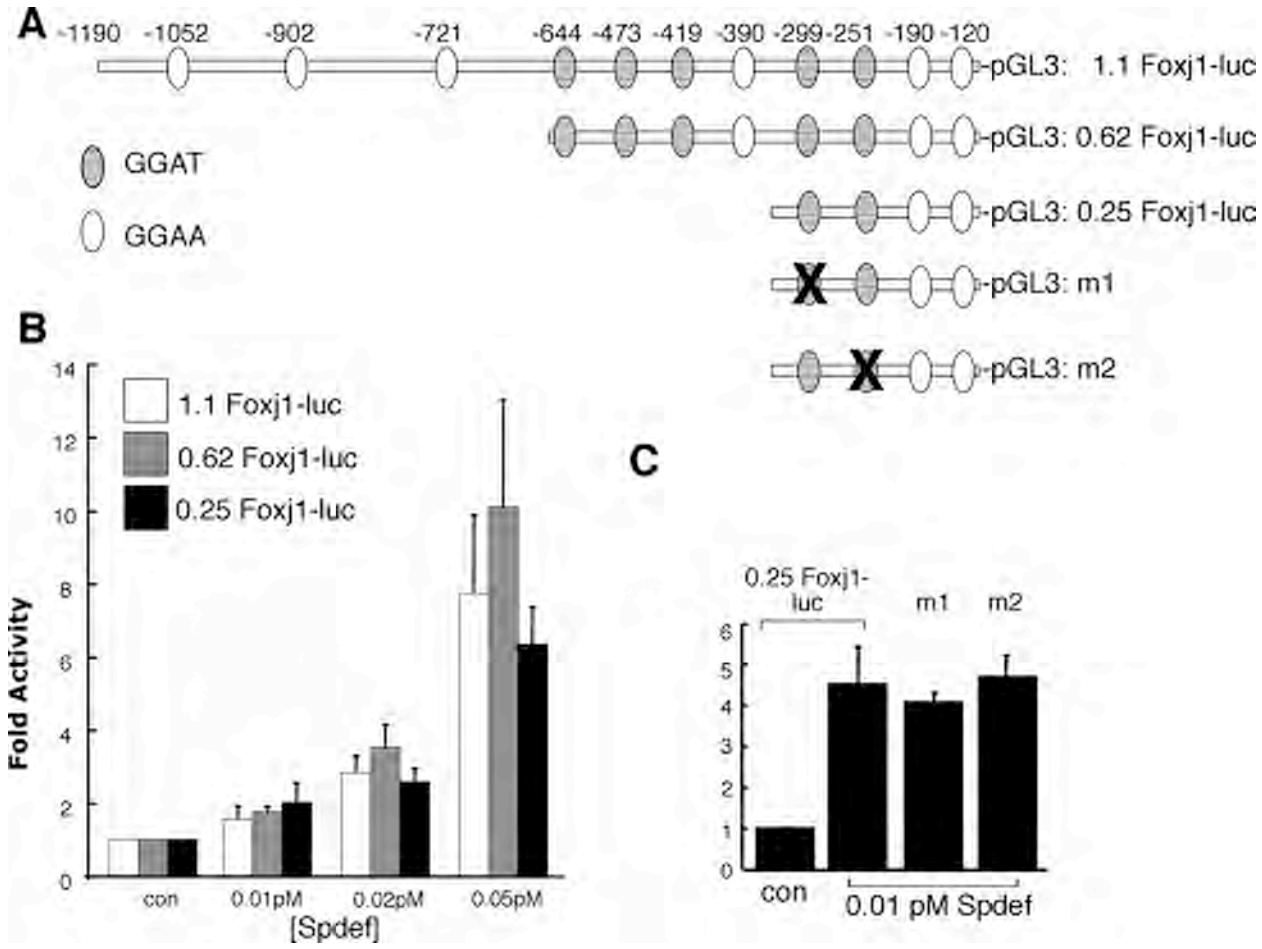
Supplemental Figure 3



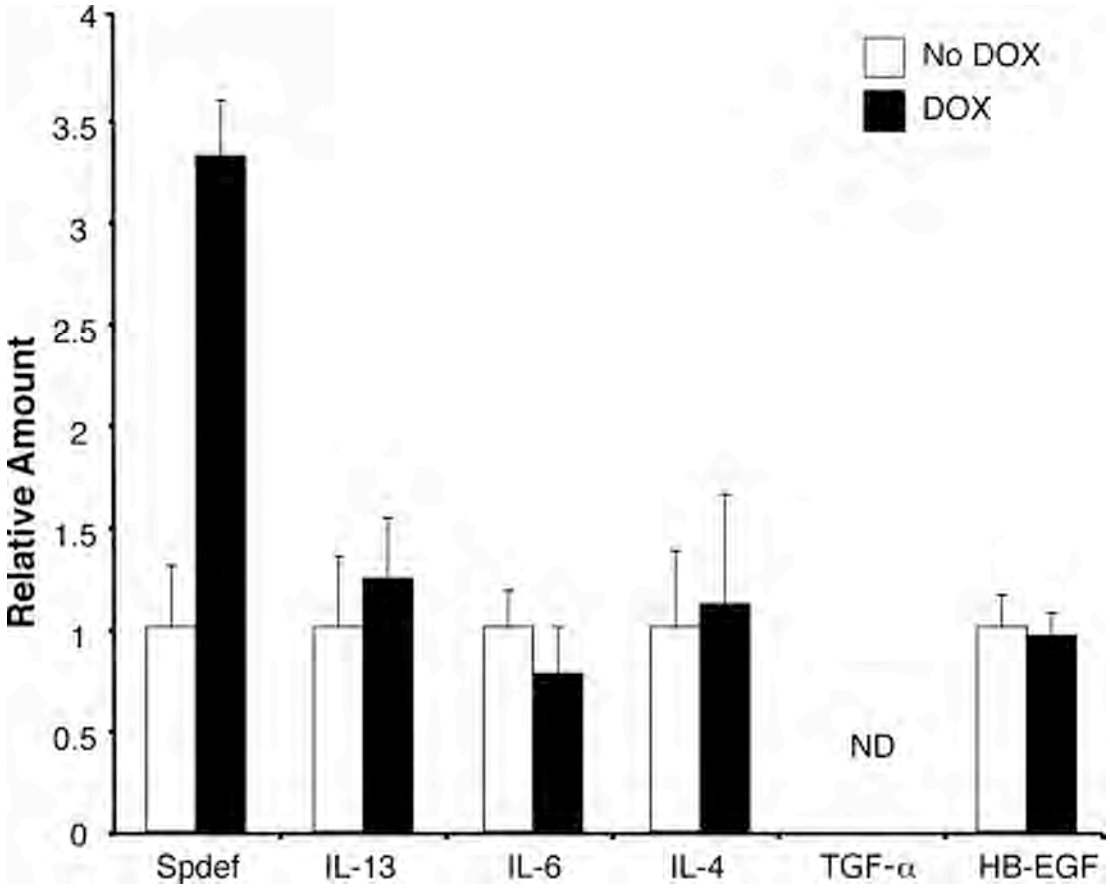
Supplemental Figure 4



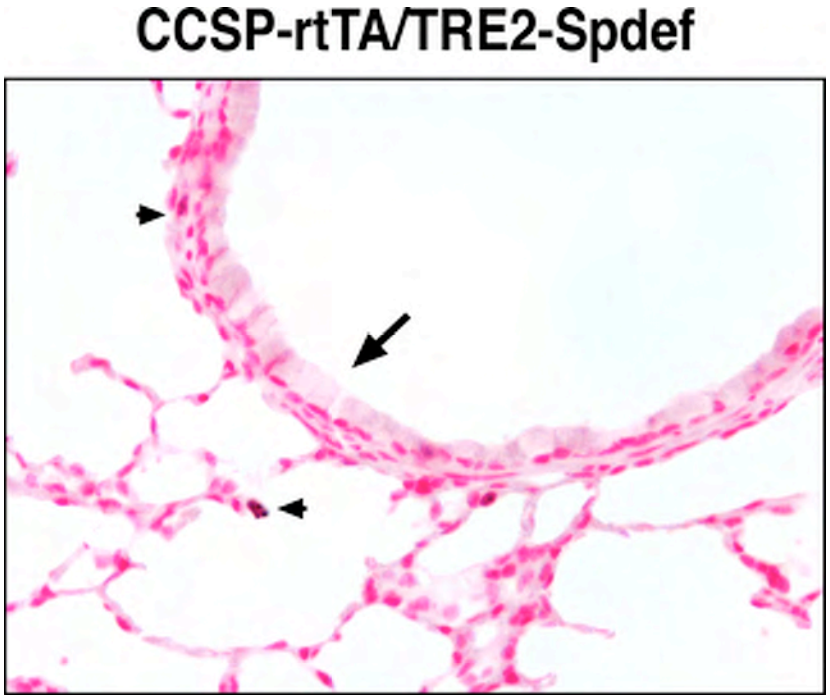
Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7



Supplemental Figure 8

Supplemental Table 1: **List of Constructs**

Name	**Insert**
pACT-Spdef	Spdef-(1-326)
pBIND-TTF-1	TTF-1-(1-372)
pBIND-T1-HD	TTF-1-(161-223)
pBIND-T1-C	TTF-1-(224-372)
pBIND-T1ΔN	TTF-1-(161-372)
pBIND-T1ΔC	TTF-1-(1-223)

Supplemental Table 2: **Sequence of the primers for PCR**

Target Gene	*Primer Sequences*
Spdef	forward 5′-TTC CAG GAG CTG GGC GGT AA-3′ reverse 5′-GGT CCA TGG TGA TAC AAG GGA CAT-3′
IL-13	forward 5′-TGA GCA ACA TCA CAC AAG ACC AG-3′ reverse 5′-GAG AAA GGA AAA TGA GTC CAC AGC-3′
IL-4	forward 5′-AAC CCC CAG CTA GTT GTC AT-3′ reverse 5′-GCT CTT TAG GCT TTC CAG GA-3′
IL-6	forward 5′-CCT CTG GTC TTC TGG AGT ACC AT-3′ reverse 5′-GGC ATA ACG CAC TAG GTT TGC CG-3′
TGF-α	forward 5′-CCT GTT CGC TCT GGG TAT TGT GTT-3′ reverse 5′-CGT GGT CCG CTG ATT TCT TCT CTA-3′
HB-EGF	forward 5′-GAC CAT GAA GCT GCT GCC GT-3′ reverse 5′-CGC CCA ACT TCA CTT TCT CTT C-3′