Supplementary methods

qRT PCR primers and qRT PCR arrays

PCR Arrays used were: Stem Cell Transcription Factors (PAMM-501), Transcription Factors (PAMM-075) and Stem Cells (PAMM-405). Primers used for qRT PCR assays of individual genes were: Barx1 (PPM28978A), Barx2 (PPM35833A), Runx1 (PPM04445A), Runx2 (PPM04449C), Pax6 (PPM04498B), Rbm3 (PPM31141A), Krt5 (PPM59967F) (SABiosciences, Quagen). Primers for Runx3 were (F-CCGGCAGAAGATAGAAGACCAG and R-CTGAAGTGGCTCGTGGTGCT), PrS13 (F-TCCCTCCCAGATAGGTGTAATCC and R-TCCTTTCTGTTCCTCTCAAGGT), and PrL27 (F-AAAGCCGTCATCGTGAAGAAC and R-GCTGTCACTTTCCGGGGATAG).

Primers for RT PCR

The following primers were used for RT PCR: Runx1 (F-GGC AAC TAA CTG CTG GAA CT and R-CTC ATC TTG CCG GGG CTC AG); Runx2 (F-GCACAGACAGAAGCTTGATG and R- CACGTCAGTGATGGCAGGT). Runx3, Barx1, Barx2, GAPDH primers were described previously[1, 2].

siRNAs and optimization of siRNA treatment

Silencer® Select Pre-Designed and Validated siRNAs (Ambion, Invitrogen) were used in all experiments. The sequences of siRNAs are listed below: Mouse Runx1, NM_009821 (s63435)

GCAGAACUGAGAAAUGCUATT
UAGCAUUUCUCAGUUCUGCCG

Mouse Runx2, NM_009820 (s229351)

CCAUAUCUCUACUAUGGUATT
UACCAUAGUAGAGAUAUGGAG

Mouse Runx3, NM_019732 (s63432)
CACCAACCUUCAUACGAGATT
UCUCGUAUGAAGGUUGGUGTA

Negative control (4390846)

UCGUAAGUAAGCGCAACCCtt
GGGUUGCGCUUACUUACGAtt

Cy3 labeled Negative Control (AM4621)

AGUACUGCUUACGAUACGGTT
CCGUAUCGUAAGCAGUACUTT

Optimal conditions for siRNA-mediated inhibition of Runx1, 2, and 3 expression were determined using siRNA concentrations ranging from 10 to 200 nM. The efficient inhibition of Runx expression was observed at 20-200 nM siRNA concentrations. In all subsequent experiments a 30 nM concentration of siRNAs was used. Embryonic (E15.5) LGs (five-six glands/filter, 2-3 filters (10-15 glands) per each condition) were transfected with either 30 nM Runx1, Runx2, Runx3 or their siRNA combinations or with a non-silencing scrambled control siRNA, or non-specific-Cy3 labeled (penetration control) siRNA using RNAiMAX (Invitrogen). Each embryo served as a source of one LG for siRNA and one for a non-silencing control. Transfected LGs were grown for 24–48 hrs and processed for estimation of branching and cell proliferation, and/or qRT-PCR analysis. Total RNA was purified using a miRNeasy Mini Kit (Qiagen) and used for cDNA synthesis to assess Runx1, 2, and 3 and Barx2 (non-targeted control) gene expression by qRT PCR using the ABI-7300 real time PCR system.

X-Gal staining

Fixed tissue was rinsed in 0.1M PBS pH 7.4 for 20-30 minutes, rinsed 3X with PBS, and stained overnight at room temperature in the dark in a X-gal staining solution. Solution comprised 5 mM $K_3[Fe(CN)_6]$, 5 mM $K_4[Fe(CN)_6]$, 0.1 mM $MgCl_2$, 0.02% NP40, 0.02% Deoxycholate, and 1mg/ml of Xgal in 0.1M PBS pH 7.4. 50 mM stock solution of X-gal (Sigma #B4252) in dimethylformamide was stored at -80°C.

Tyrode solution.

Tyrode solution pH 7.2 (Ca, Mg free solution) was used for cell dissociation. Tyrode solution contained NaCl (8g/L), KCl (0.2 g/L), $NaH_2PO_4XH_2O$ (0.05 g/L), D(+) glucose (1g/L) (H_2O free), $NaHCO_3$ (1g/L). Solution was filter sterilized and stored at 4°C.

References

1. Yarmus M, Woolf E, Bernstein Y, et al. Groucho/transducin-like Enhancer-of-split (TLE)-dependent and -independent transcriptional regulation by Runx3. *Proc Natl Acad Sci U S A* 2006;103:7384-7389.
2. Tsau C, Ito M, Gromova A, Hoffman MP, Meech R, Makarenkova HP. Barx2 and Fgf10 regulate ocular glands branching morphogenesis by controlling extracellular matrix remodeling. *Development* 2011;138:3307-3317.