Supplementary Information for

O-glycosylation modulates integrin and FGF signaling by influencing the secretion of basement membrane components

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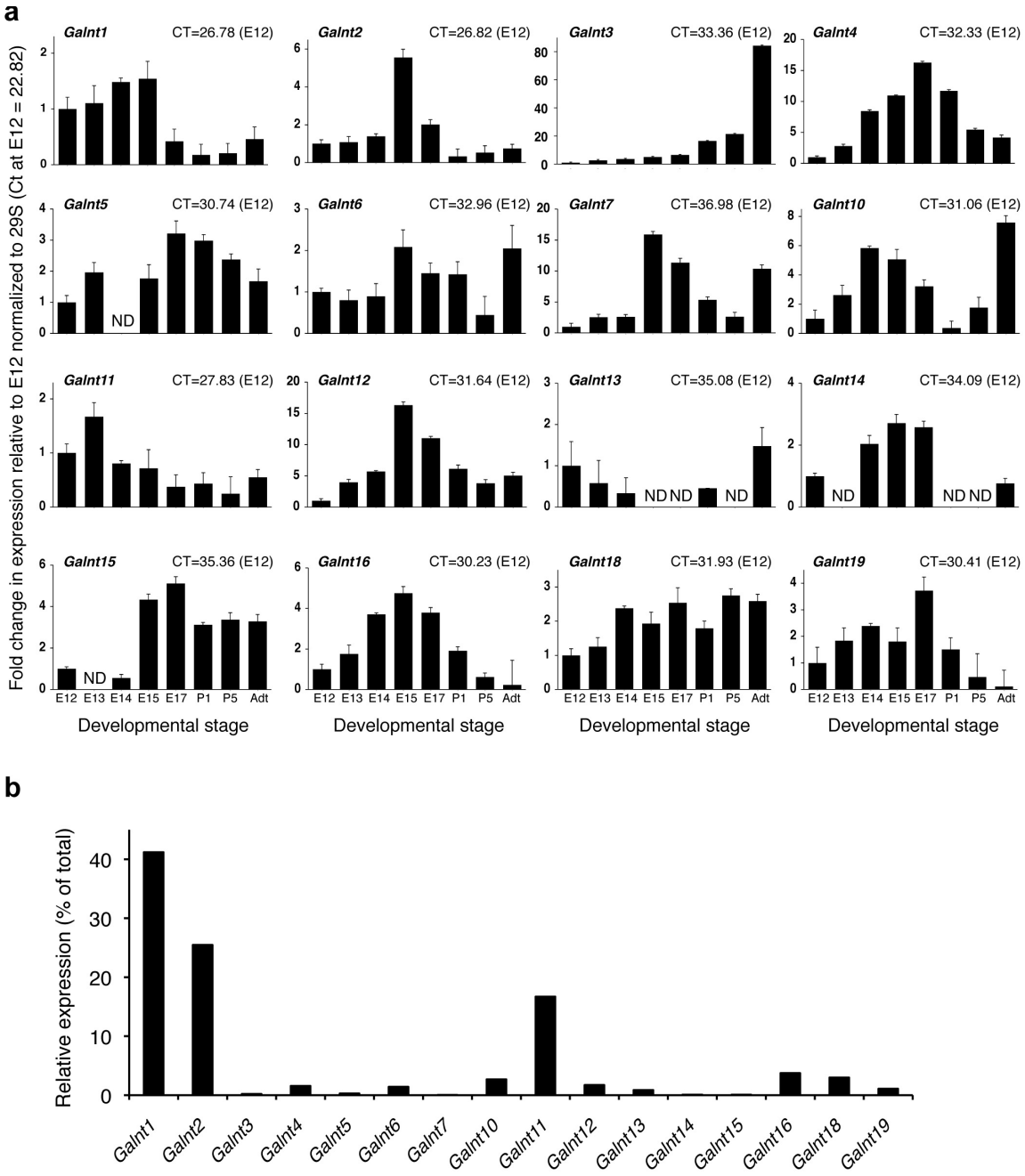
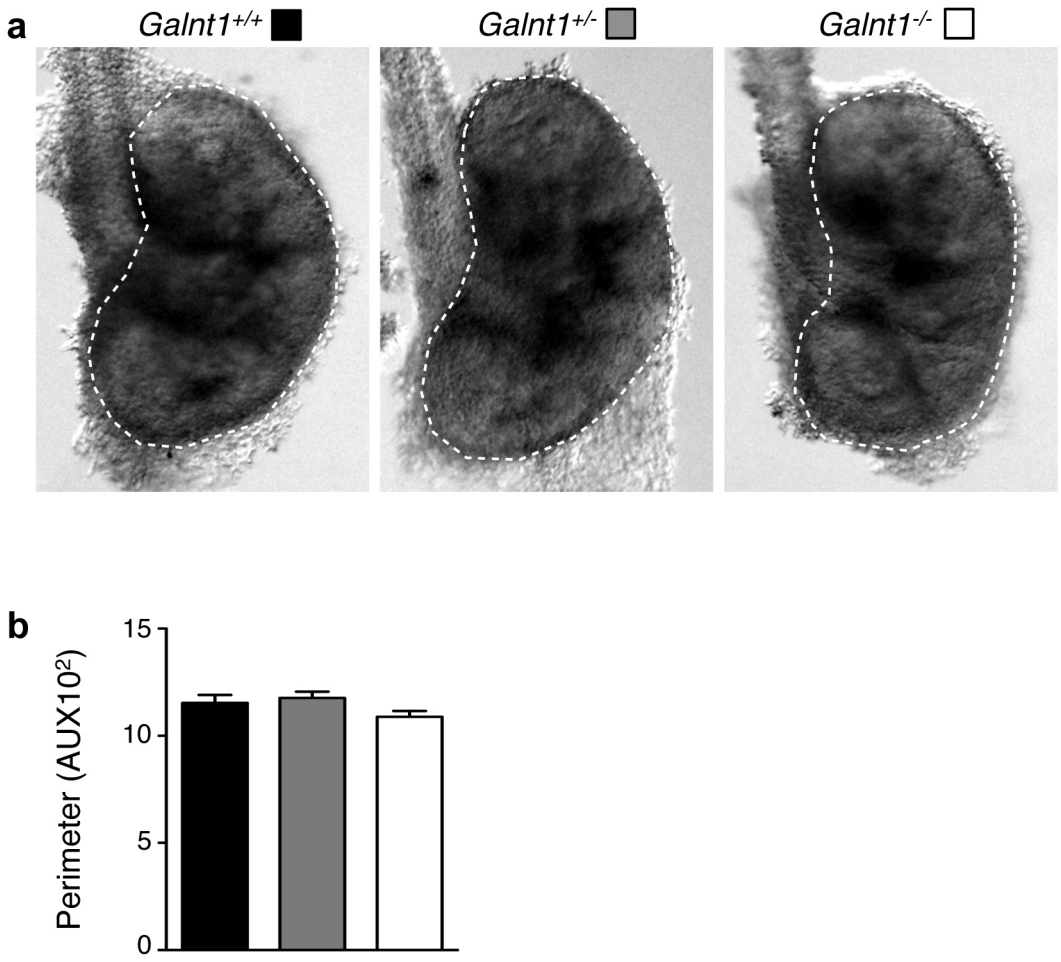
Supplementary Figures S1 to S6

Supplementary Tables S1 and S2

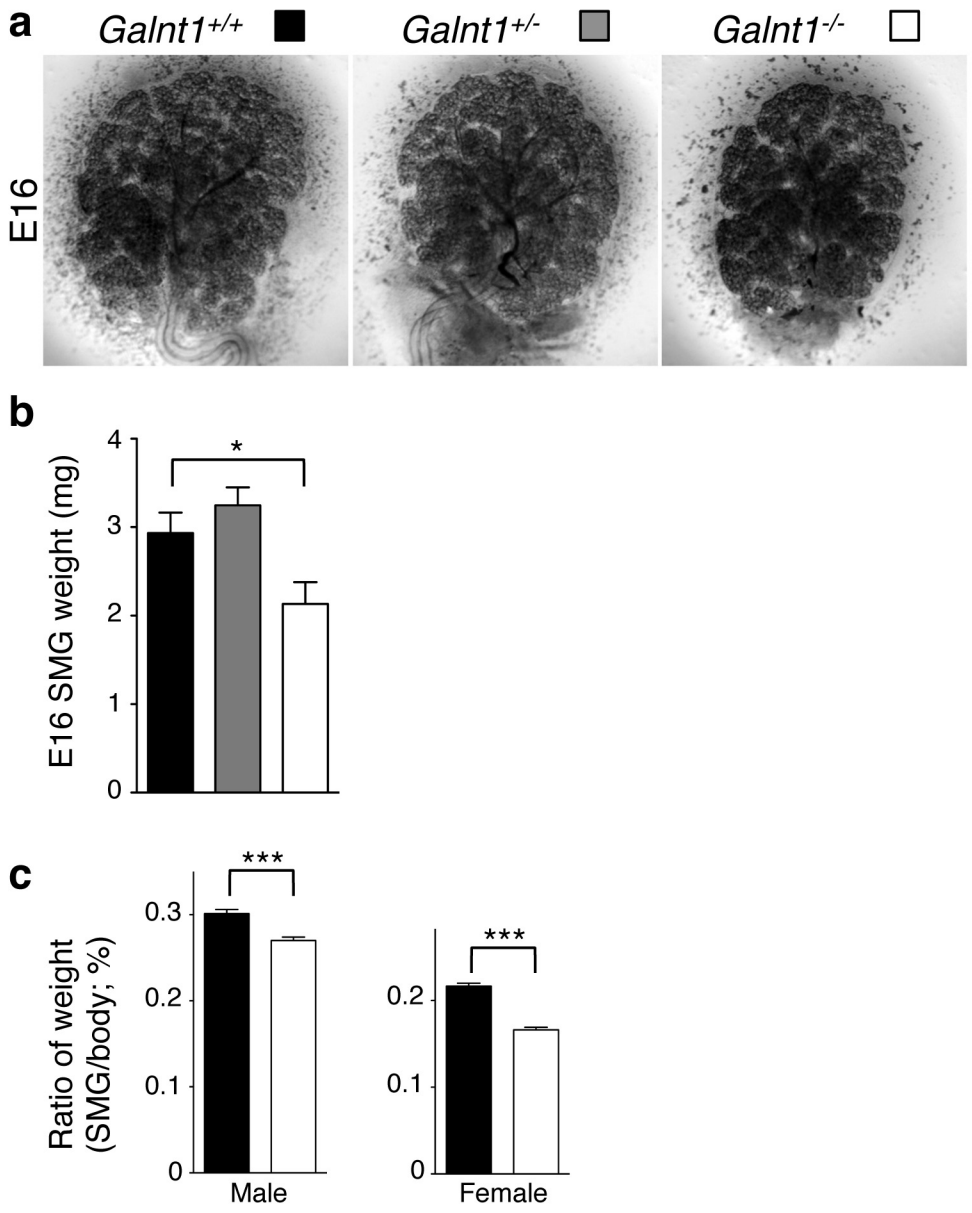
Supplementary Methods



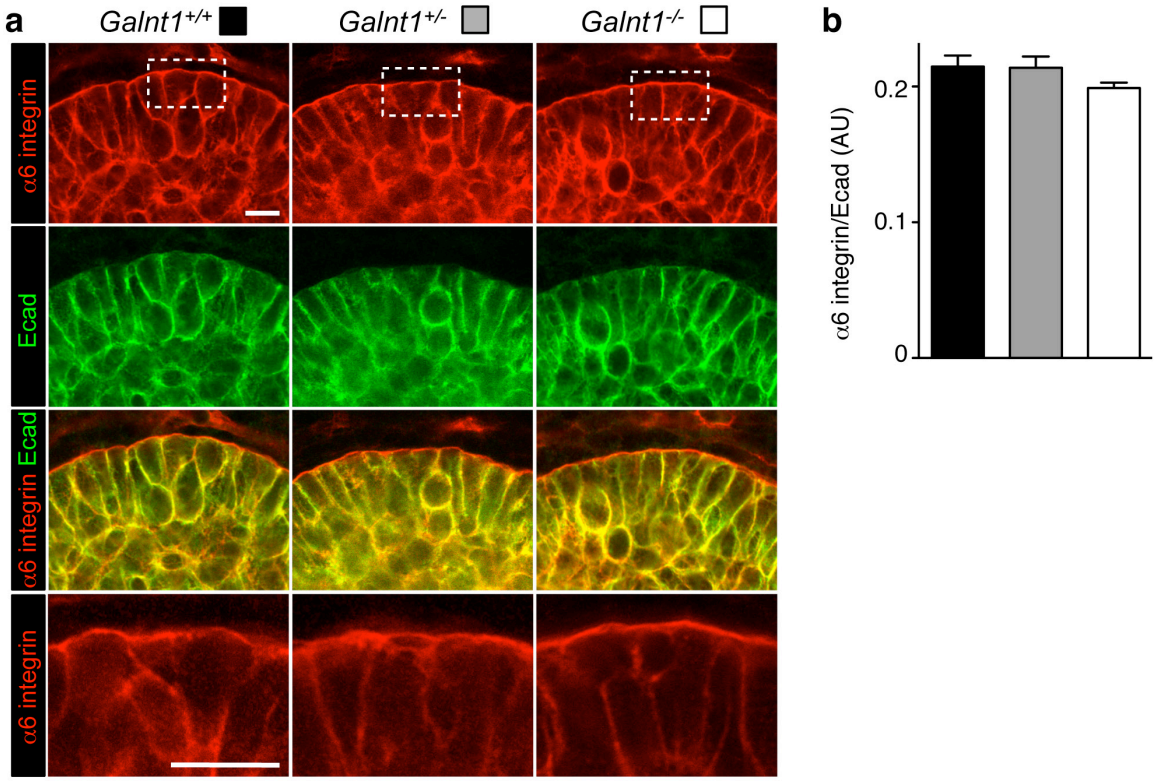
Supplementary Figure S1. The expression of *Galnt*s during SMG development. (**a**) Relative expression levels of *Galnt* family members at E12, E13, E14, E15, E17, P1, P5 and adult (Adt) stages, as determined by qPCR are shown. The relative expression levels of each *Galnt* family member are expressed as fold changes in expression compared to E12 SMGs. CT values for each gene at E12 are shown in the top right corner of each graph. (**b**) Relative expression levels of each *Galnt* family member in E12 SMGs, as determined by qPCR. *Galnt1* is the most abundant isoform expressed at E12.



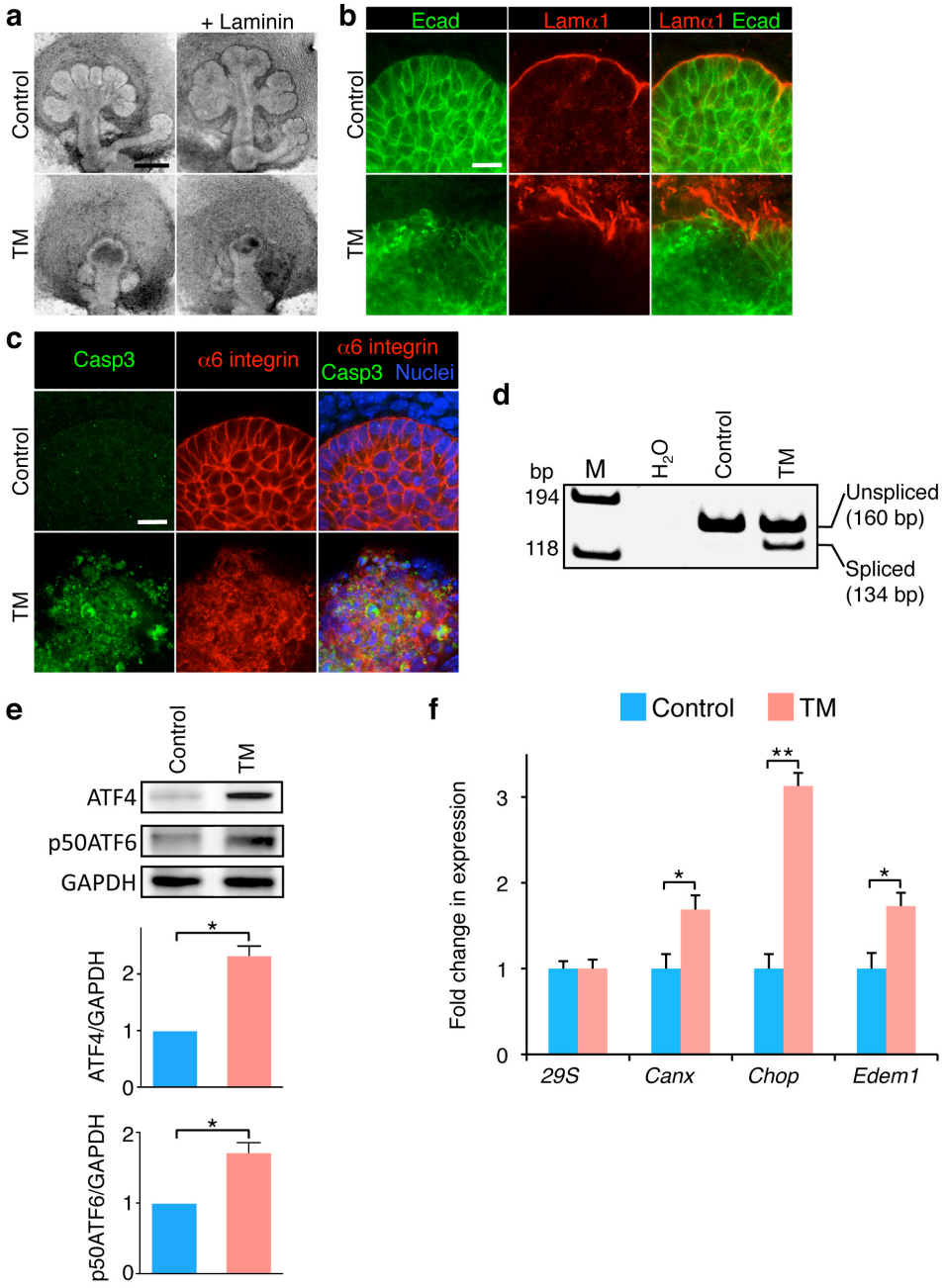
Supplementary Figure S2. Loss of *Galnt1* has no effect on kidney size at E12. (**a**) E12 kidneys from *Galnt1*$^{+/+}$ (n=10), *Galnt1*$^{+/-}$ (n=12) and *Galnt1*$^{-/-}$ (n=10) embryos were isolated and measured (white dashed line). (**b**) The average perimeter of E12 kidneys from each genotype was graphed. No significant difference in kidney perimeter was seen.



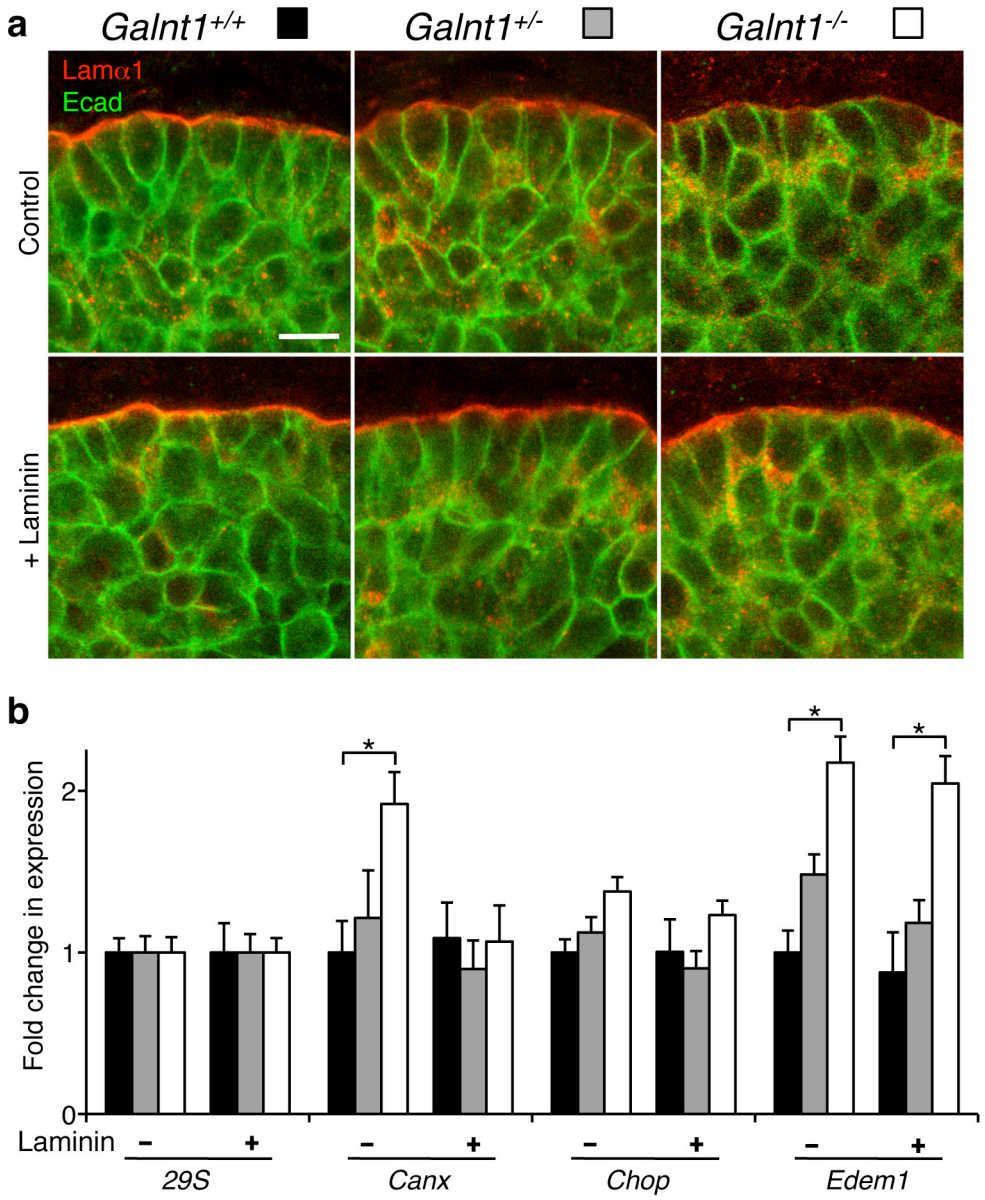
Supplementary Figure S3. *Galnt1*$^{-/-}$ SMGs remain smaller than *Galnt1*$^{+/+}$ SMGs throughout development and into adulthood. (**a, b**) E16 SMGs from *Galnt1*$^{+/+}$ (n=28), *Galnt* $^{+/-}$ (n=25) and *Galnt1*$^{-/-}$ (n=10) were collected and weighed. (**c**) The ratio of the weights of adult SMGs from male *Galnt1*$^{+/+}$ (n=14), male *Galnt1*$^{-/-}$ (n=6), female *Galnt1*$^{+/+}$ (n=12) and female *Galnt1*$^{-/-}$ (n=4) mice were calculated relative to total body weight. Student's *t*-test was used to calculate *P*-values. *, P<0.05; ***, P<0.001.



Supplementary Figure S4. Loss of *Galnt1* does not affect α6 integrin receptors present along the basal region of epithelial cells of E12 SMGs. (**a**) *Galnt1*$^{+/+}$, *Galnt1*$^{+/-}$ and *Galnt1*$^{-/-}$ E12 SMGs were stained with α6 integrin (red) to detect integrin receptors and E-cadherin (Ecad, green) as an internal control. The white dashed boxes represent areas of higher magnification that are shown in the last row of images. (**b**) The ratio of α6 integrin staining over E-cadherin staining was calculated across 3-4 regions for each SMG. Average ratios were calculated for each genotype and graphed. 4 SMGs of each genotype from 3 independent crosses were examined. Scale bar=10 μm.



Supplementary Figure S5. The effects of disruption of N-linked glycosylation are distinct from those seen upon loss of *Galnt1*. (**a**) Loss of N-glycosylation after 5 ng ml^{-1} tunicamycin (TM) treatment results in loss of SMG growth, which cannot be rescued by the addition of BM component Laminin. (**b**) Loss of N-glycosylation results in dispersed laminin α1 staining (Lamα1, red) and loss of E-cadherin staining (Ecad, green). (**c**) Loss of N-glycosylation causes disruption of α6 integrin staining (red) in epithelial cells and induction of apoptosis, as examined by caspase-3 staining (Casp3, green). Disruption of N-glycosylation causes ER stress-dependent splicing of Xbp1 (**d**), increases in ATF4 and the 50kDa cleaved form of ATF6 (p50ATF6) (**e**), and increases in the expression of ER stress genes (**f**). Expression was normalized to *29S*. M=size markers. H_2O=control reaction without cDNA added. Scale bar=100 μm for a; and 10 μm for b and c. Student's *t*-test was used to calculate *P*-values. *, P<0.05; **, P<0.01.



Supplementary Figure S6. Laminin-111 addition to E12 SMGs. (**a**) *Galnt1*$^{+/+}$ (n=11), *Galnt1*$^{+/-}$ (n=20) and *Galnt1*$^{-/-}$ (n=9) E12 SMGs were untreated (Control) or treated with 80 μg ml^{-1} laminin-111 (+ Laminin) for 48 hrs and glands were stained for laminin α1 (red) to demonstrate laminin accumulation along the BM. Intracellular accumulation is still observed in *Galnt1*$^{-/-}$ and *Galnt1*$^{+/-}$ SMG epithelial cells. (**b**) Expression of specific ER stress genes in *Galnt1*$^{-/-}$, *Galnt1*$^{+/-}$ and *Galnt1*$^{+/+}$ SMGs that were untreated (-) or treated with laminin-111 (+), as determined by qPCR. Expression was normalized to *29S*. Scale bar = 10μm. Student's *t*-test was used to calculate *P*-values. *, P<0.05.

Supplementary Table S1. Mouse *Galnt* gene primer sequence for qPCR

Gene	Primers
Galnt1	Sense: 5'- TCATCAAGAGCAGCGGCAAAGC -3' Anti-sense: 5'- ACAAGGCACATTCAGCAGAAACGG -3'
Galnt2	Sense: 5'- CGCCCTCTGCCTCCCTCTTTC -3' Anti-sense: 5'- TGATTGCTGCTTGCCCACTTGTTC -3'
Galnt3	Sense: 5'- TGCTACTCAGGGTGTCGTCCAG -3' Anti-sense: 5'- GCGTCACATGGCACTAAGTTTGG -3'
Galnt4	Sense: 5'- CCGCAATCGTATGTCCTGTCATCG -3' Anti-sense: 5'- AACGCCAGTCAAACCCACCAATC -3'
Galnt5	Sense: 5'- GCCGAGCAGAGATGGAAAGAAGG -3' Anti-sense: 5'- CTGGTGGTTGGGAGGTCATTGTG -3'
Galnt6	Sense: 5'- GTGTTGACCAGAAGTTCCG -3' Anti-sense: 5'- GATTTCATTCAGCAAGATGGC -3'
Galnt7	Sense: 5'- GGCTCGTGGTCCTCTGGTCTTC -3' Anti-sense: 5'- TCTCTGTCTTCCCTCATCCTGCTC -3'
Galnt10	Sense: 5'- CCGAGGCGAGGCTGCTTGG -3' Anti-sense: 5'- GGGTGACTGGGCTGGTGTGG -3'
Galnt11	Sense: 5'- CAGCAGTGGACCTTTGGGAAGAAC -3' Anti-sense: 5'- TGTTGAGAGGAGGAGCCATCGC -3'
Galnt12	Sense: 5'- CCGAGAGACCGTCCCAGAGAAC -3' Anti-sense: 5'- ACATTTCCTGCTGTGCTTGTGAAC -3'
Galnt13	Sense: 5'- CACCCGTCTTCAGTCTCCGTATTG -3' Anti-sense: 5'- GACATCAACAAGCACCCACATCAG -3'
Galnt14	Sense: 5'- GATGAGCGGCGGTATCTGAATGC -3' Anti-sense: 5'- GGTGATGATGATGCTGGTGTGAGG -3'
Galnt15	Sense: 5'- GGACTGGAGGACCGAAGAGGATG -3' Anti-sense: 5'- AGAGGATGACGCTGGCTGTAGG -3'
Galnt16	Sense: 5'- CGCCAATGCCATCGCCATCC -3' Anti-sense: 5'- GCTCGGTTGTCCTGCCATAAGTAG -3'
Galnt18	Sense: 5'- CCTGCCCTGCTCTCGGATTGC -3' Anti-sense: 5'- GCCTTGCGTGCGGTGATGTC -3'
Galnt19	Sense: 5'- TGGGTGTATGTTTGCGTGCTTGAG -3' Anti-sense: 5'- GCGTCCTTGTCCCTATCCACTGAG -3'

Supplementary Table S2. Mouse ER stress gene primer sequence for qPCR

Gene	Primers
Xbp1	Sense: 5'- GACGAGGTTCCAGAGGTG -3' Anti-sense: 5'- GAGGCAACAGTGTCAGAGT -3'
Canx	Sense: 5'- ACTGGTGGTGGTCATTGCCTTT -3' Anti-sense: 5'- GCACACGCTTAGCAGTTCTACCT -3'
Chop	Sense: 5'- CCTCGCTCTCCAGATTCCA -3' Anti-sense: 5'- GCCGCTCGTTCTCTTCAG -3'
Edem1	Sense: 5'- GCTGGTCAGAGGGTAGGG -3' Anti-sense: 5'- GCAGGGAAGAGGCACTAGA -3'

Supplementary Methods

Kidney size measurement. Kidneys were isolated from E12 *Galnt1*$^{+/+}$, *Galnt1*$^{+/-}$, *Galnt1*$^{-/-}$ embryos and perimeters were measured using NIH ImageJ software.

E16 and adult SMG size measurements. E16 SMGs were dissected from *Galnt1*$^{+/+}$, *Galnt1*$^{+/-}$, *Galnt1*$^{-/-}$ embryos and weighted. Adult SMGs were isolated from 2-3 month old male and female *Galnt1*$^{+/+}$ and *Galnt1*$^{-/-}$ mice. Both SMG weight and total body weight were determined for each individual. Ratios of SMG weight/body weight were calculated and averaged to obtain the values graphed in Supplementary Fig. S3c.

Ex vivo SMG organ culture with Tunicamycin (TM). E12 SMGs were dissected from ICR time-pregnant mice and cultured as described in the main text. 5 ng ml^{-1} TM (T7765, Sigma-Aldrich; 5 μg ml^{-1} stock solution in DMSO) was added to the culture medium and explants were cultured for 2 days[53]. SMGs were photographed and harvested for qPCR and immunostaining as described in the main text. Cleaved caspase-3 (Asp175) antibody (#9661, Cell Signaling Technology; 1:100) was used to detect apoptosis by immunostaining.