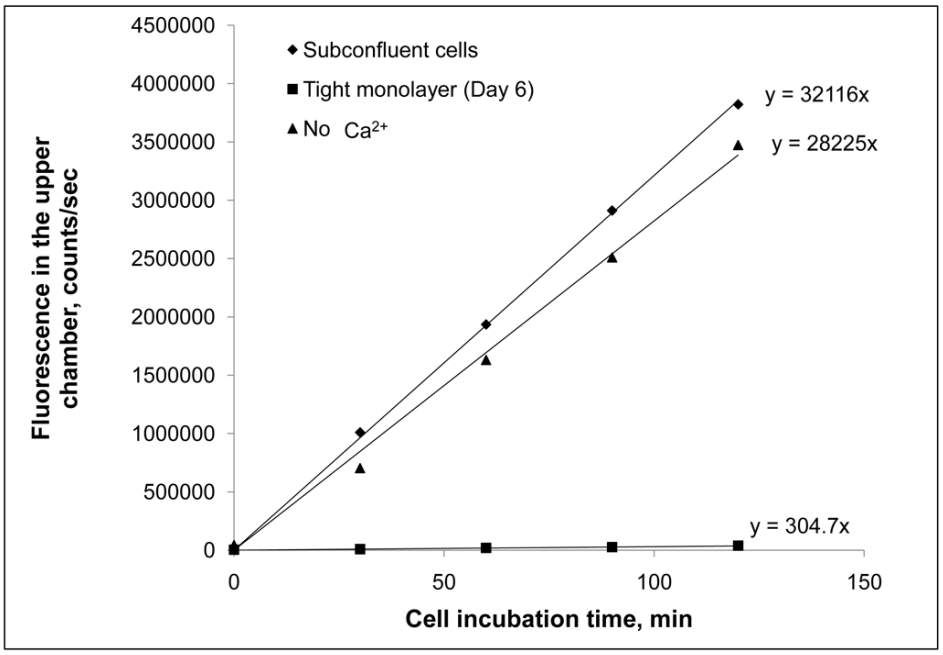
Supplemental Table 1. Primers used for RT real-time PCR of the Na,K-ATPase β_1 subunit and glycosyltransferases.

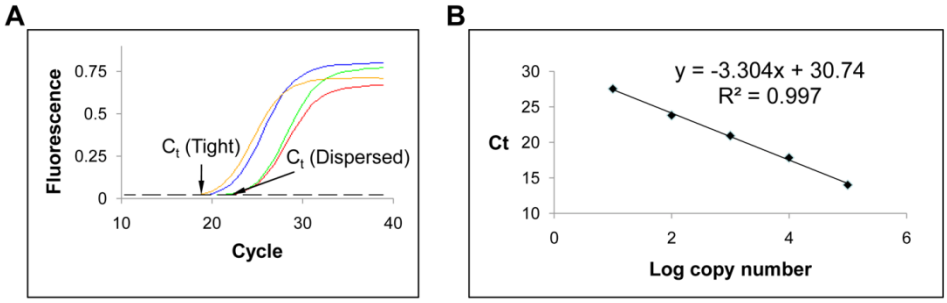
Enzymes	Accession #	Primers
Na,K-ATPase β_1 subunit	NM001003283	Sense: TCAGCGAATTCAAGCCCACATACC Antisense: CGCCCAGGCCAAAATACTCCA
GT*	XM538701	Sense: GCCTCAGCTTCTCCATCCTCATAA Antisense: ATAGCATTCGGGCGAGATACAGAC
ST	XM843848	Sense: GCCGCTGCGTGGTGGTG Antisense: ATCTTGCGTGGCTGTTTCATTGG
GnT-III	XM538365	Sense: GTCATCAACGCCATCAACATCAA Antisense: GGTCAGGAAGGTGCGCAGGTAGTC
GnT-IVA	XM531790	Sense: TGGAAGAACGGGAGTGTCAATAG Antisense: GGTTTTGCTTTGTTCTCCATCTTA
GnT-IVB	XM538579	Sense: GACTTCATCCGCTTCCGCTTCTT Antisense: GGTCCACCTCGCCCTCTGC
GnT-IVC	XM532641	Sense: AAATGAGATGCCTGCGAAAAC Antisense: TAAAAGACCCCGGAAATGAGTT
GnT-V	XM541015	Sense: GCAGCGCATTGGCAAGTTAGAGTC Antisense: GGCCACCACTGAAAGCTGTCTCTG

*Abbreviations: GT - UDP-Gal:betaGlcNAc beta-1,4-galactosyltransferase 1, membrane-bound form; ST – beta-galactoside-alpha-2,3-sialyltransferase 4, GnT –N-acetylglucosamine-glycosyltransferase

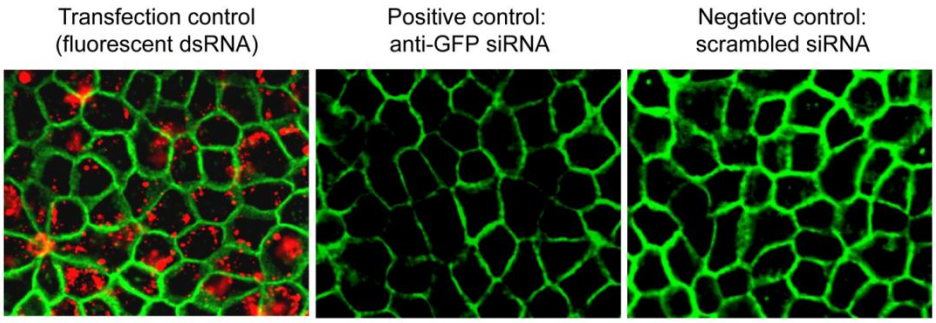
Supplemental Figure 1



Supplemental Figure 2

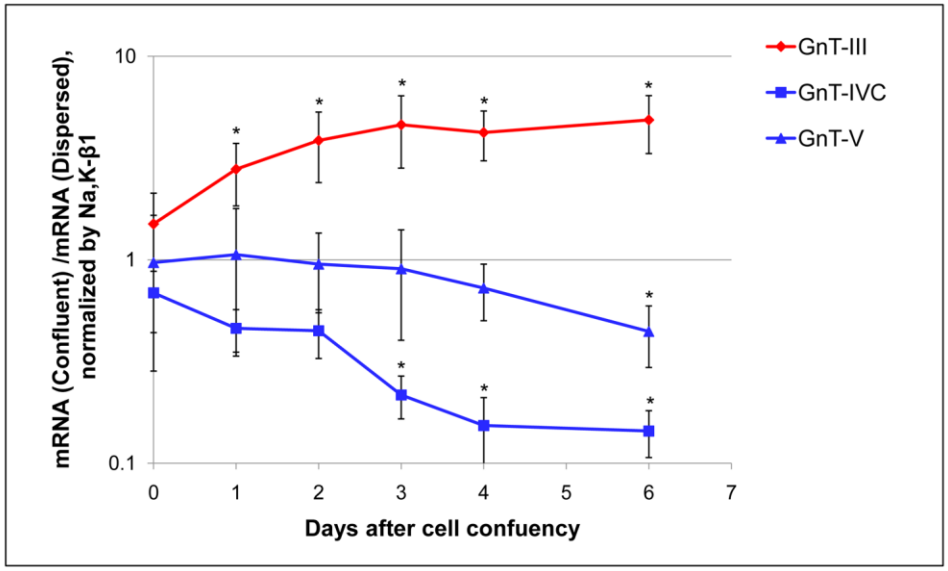


Supplemental Figure 3



Green – YFP-β_1, red – fluorescent double-stranded RNA

Supplemental Figure 4



Supplemental Fig. 1. The paracellular flux of the membrane impermeable dye, BCECF free acid, decreases about 100-fold as a result of formation of the confluent MDCK cell monolayer and its maturation for 6 days. Conversely, disruption of intercellular junctions by pre-incubation of the tight cell monolayers in Ca^{2+}-free PBS buffer for 30 min followed by cell incubation with BCECF free acid in PBS for 2 hours increases the paracellular dye flux to the level observed in sub-confluent MDCK cells.

Supplemental Fig. 2. Representative graphs show results of the real-time PCR and a standard curve used for quantification of mRNA. Accumulation of the PCR product for GnT-III using the same amounts of total RNA isolated from the dispersed MDCK cells and the tight MDCK cell monolayers as templates is shown (*A*). The PCR product obtained using primers for GnT-III was purified as described in Experimental Procedures and used as a template for the real-time PCR. The range of PCR product concentrations was chosen so that the standard curve included the values of C_t observed for the RNA samples from the tight monolayers and dispersed cells (19.2 and 21.1 for GnT-III). The resulting values of C_t were plotted against the logarithms of the PCR copy number to generate a standard curve (*B*) that was used to determine the efficiency and sensitivity of amplification. Similarly, a standard curve for mRNA quantification was obtained for each primer set. The values of slopes for the standard curves varied in the range between 3.25 and 3.41.

Supplemental Fig. 3. The efficiency of transfection with siRNA was close to 100% as detected by using a fluorescently labeled transfection control (IDT). MDCK cells stably expressing YFP-β_1 were transfected with transfection control (fluorescently labeled double-stranded RNA), positive control (anti-GFP siRNA) and negative control (scrambled double-stranded RNA) (IDT). Confocal microscopy images were taken 72 hrs after transfection. Almost each cell was transfected (*left panel*). About 50% of YFP-β_1 silencing was achieved by transfection of anti-GFP siRNA as seen from comparing the *middle* and *right* panels.

Supplemental Fig. 4. Expression of the genes encoding GnT-III, GnT-IVC and GnT-V gradually changes during development of the tight cell monolayer from dispersed MDCK cells as determined by RT real-time PCR. Abbreviations are as in the legend to Fig. 4. Error bars, ±s.d. (n=3). *Significant difference with dispersed cells. $P<0.01$, Student's *t*-test.