

Supplemental materials

Wlader et al., <http://www.jcb.org/cgi/content/full/jcb.200703064/DC1>

Supplemental materials and methods

Cilium length quantitation

MTECs at day ALI + 3 were labeled with γ -tubulin and acetylated α -tubulin antibodies to identify cilia. Cilium length was measured by tracing the acetylated α -tubulin signal in images using AutoDeblur 9.3 and AutoVisualize 9.3 on $n \geq 20$ cells from each category. Length is reported as mean \pm standard error.

Quantitation of ciliated cells

Ciliated cells were quantitated as "ciliated cells per field" by counting the number of cells with γ -tubulin localization characteristic to ciliated cells at any of the defined stages of ciliogenesis present in the field of view from the eyepiece of an Axiovert 200M microscope using a 100 \times objective. This simplified metric was used in lieu of "ciliated cells per total cells," as the total cell number was found to be constant throughout ALI culture. Total cells per field was determined by counting the number of cells revealed by antibody labeling for the cell boundary protein ZO-1. Total cells per field was 326 ± 12 (mean \pm standard error) based on quantitation of multiple samples from various time points during ALI culture.