**Supplementary File 5. Description of the CBF analysis algorithm**

To quantify cilia beating frequency (CBF), image series were analysed using a custom-built script in MATLAB (MathWorks, Natick, MA) as previously described1.

BeatingCiliaBatchOMEfiles\_JOVE.m algorithm uses Open Microscopy Environment (OME) package of scripts (bfmatlab) to load the microscopy files, in this case an .nd2 file. The user has choice to run a single or multiple (batch) processing. User is prompted to input a frame time in seconds and then algorithm proceeds to calculate CBF for each pixel in the field of view (FOV) as well as an average value per FOV.

One of the first and essential steps in the analysis is to use Fourier space filtering to remove the immobile (static) component from each pixel in the time series. This is usually seen as stationary elements of cell bodies and mucus that do not move during the 1000 frames of the acquisition. Next the algorithm proceeds to compute CBF pixel by pixel. At each pixel, a temporal spectrum is computed using Fast Fourier Transforms (FFT). Then peaks in the spectrum are detected using MATLAB function ‘findpeaks’ and setting the allowed peak prominence to 0.05 and peak signal-to-noise cut-off (see MATLAB ‘findpeaks’ for details) at 5, so that noisy peaks in spectrum are filtered out. For each pixel, the main 3-5 peaks with highest amplitudes are saved with their corresponding frequencies. From these 3-5 peaks, the highest amplitude peak within the physiological range of 3-30 Hz is assigned the CBF for this pixel. The average spectrum of all pixels in the FOV is computed.

**Reference**

1 Awatade, N. T. *et al.* Significant functional differences in differentiated Conditionally Reprogrammed (CRC)- and Feeder-free Dual SMAD inhibited-expanded human nasal epithelial cells. *Journal of Cystic Fibrosis.* **20** (2), 364-371, (2021).