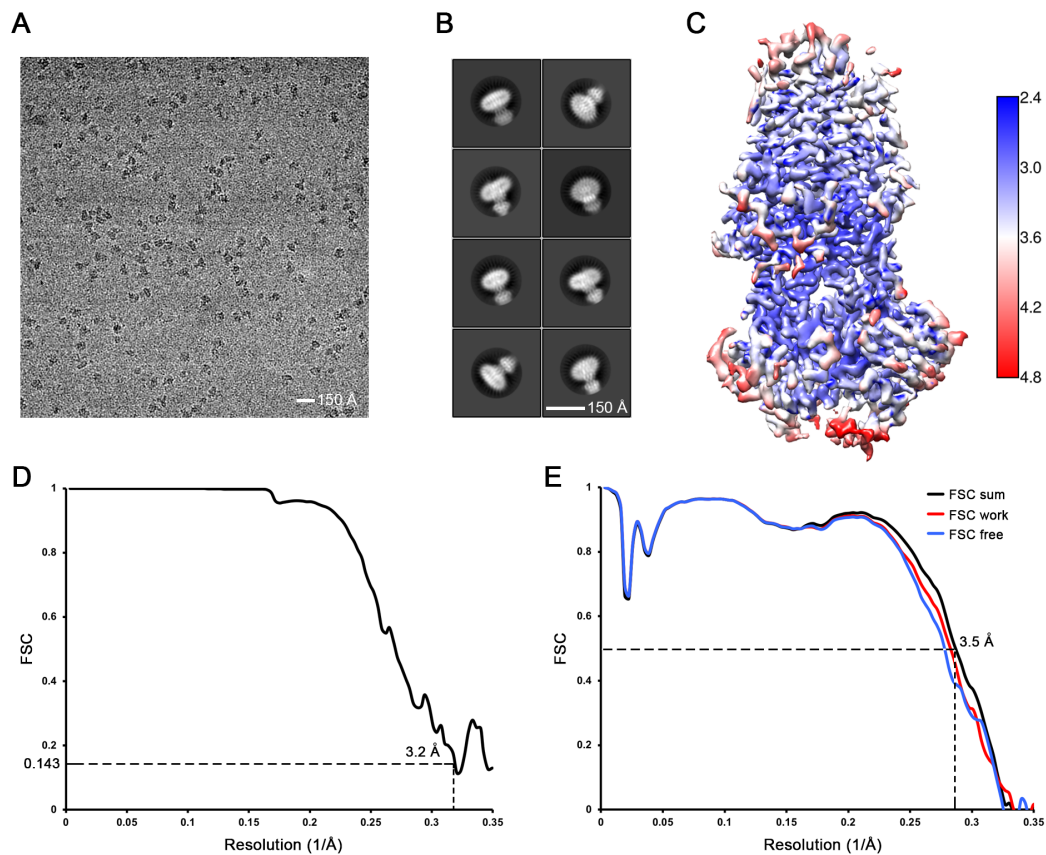**Supplemental Figures**

A

B

C

D

E

Fig. S1. Cryo-EM analysis. (A) A representative summed image at the 2.4 μm defocus level. **(B)** 2-D class average of the top 8 classes. **(C)** Local resolution distribution estimated by Blocres (48). **(D)** Fourier Shell Correlation (FSC) curves between two half-datasets calculated by Frealign (42). **(E)** FSC curves between the refined structure and the map calculated from the full dataset (sum, black), the half-map used in refinement (work, red), and the other half-map (free, blue).
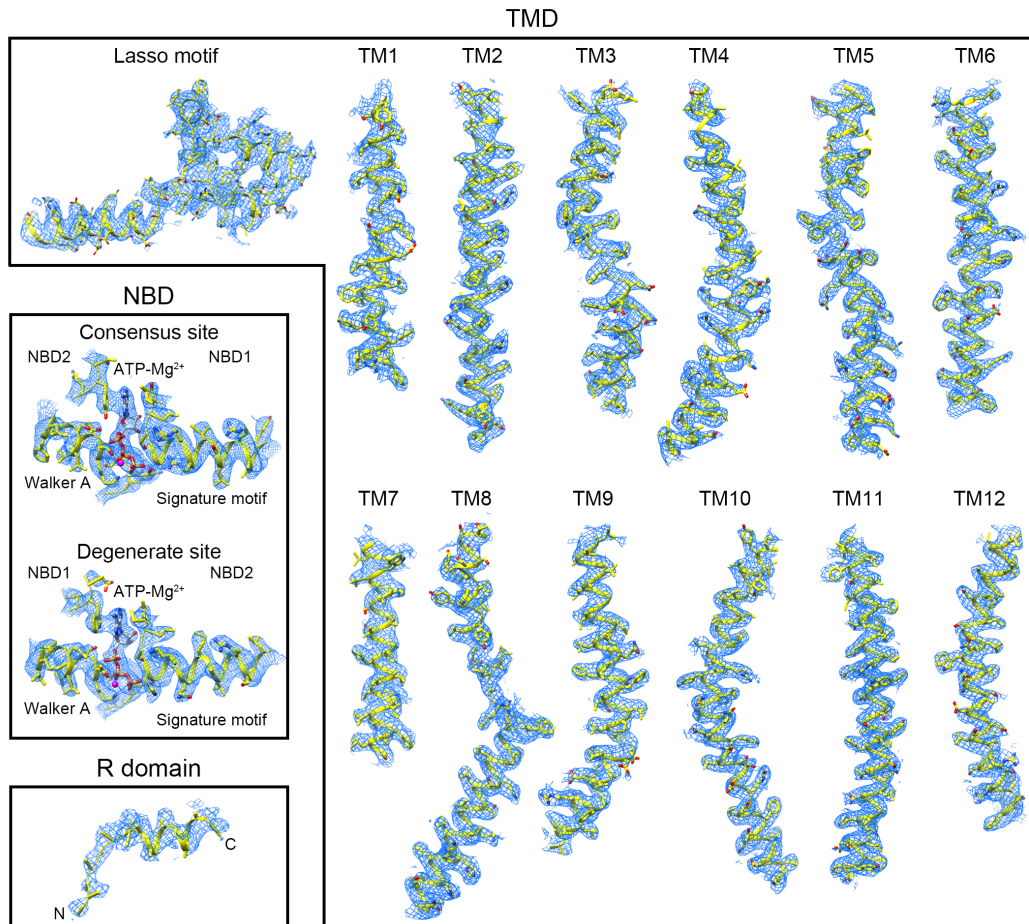
Fig. S2. B-factor sharpened densities of different regions of CFTR.

Table S1. Summary of EM data and structure refinement statistics.

Data collection

Microscope	Titan Krios (FEI)
Voltage (kV)	300
Detector	K2 Summit (Gatan)
Pixel size (Å)	1.03
Defocus range (μm)	0.8 to 2.5
Movies	2,093
Frames/movie	50
Dose rate (electrons/pixel/s)	8
Total dose (electrons/Å^2)	75
Number of particles	677,308

Model composition

Non-hydrogen atoms	9,694
Protein residues	1,197
Lipids	6
ATP	2
Mg^{2+}	2

Refinement

Resolution (Å)	3.2
Sharpening B-factor (Å^2)	-50
RMS deviations	
Bond lengths (Å)	0.013
Bond angles (°)	1.509

Validation

Molprobity score	1.70
Clashscore, all atoms	7.46
Favored rotamers (%)	94.7
Ramachandran plot (%)	
Favored	95.9
Allowed	4.1
Outliers	0.0