

Figure 1. FSEC and mass spectrometry analyses of ENaC complexes isolated from homozygous ENaCy-VF. **a.** General workflow schematic for isolating ENaC complexes from mouse tissue. **b-d.** Representative traces of samples isolated from ENaCy-VF kidneys (b), colon (c), and lungs (d). For all FSEC traces, samples were analyzed using a Superose 6 Increase 10/300 column and mVenus fluorescence was monitored. Numbers in the x-axis are elution volumes in mL. **e.** Comparison of detected specific counts by mass spectrometry of four samples: WT kidneys, ENaCy-VF lungs, ENaCy-VF colon, and ENaCy-VF kidneys. WT kidneys were used as control to identify non-specific binding to the anti-FLAG resin.

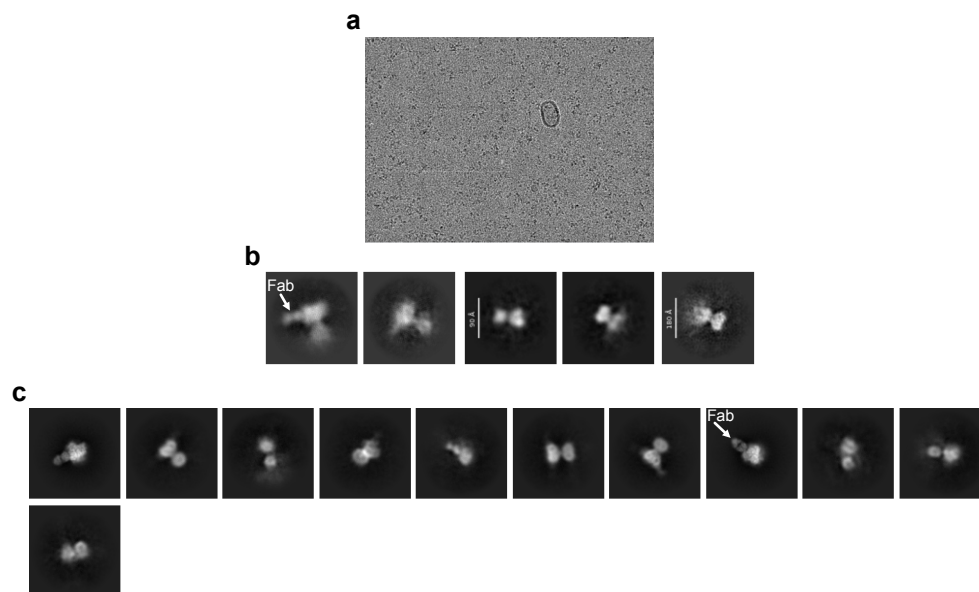


Figure 2. Cryo-EM analysis of purified ENaC complexes from ENaCy-VF mice.

a. Representative micrograph of lung ENaC complexes on 2/2 Quantifoil 2 nm carbon layer grid. **b.** Examples of 2D classes of mouse lung ENaC. Classes were generated using cryoSPARC. **c.** Examples of 2D classes of recombinant ENaC as reference.