SIGNIFICANCE STATEMENT

With No Lysine kinase (WNK) signaling regulates mammalian renal epithelial ion transport to maintain electrolyte and BP homeostasis. Patients with WNK gain-of-function mutations have hypertension and hyperkalemia, whereas loss of WNK pathway signaling in animal models results in lower BP and serum potassium concentrations. WNK pathway inhibition could, therefore, be a useful strategy for treating hypertension and hyperkalemia. In this study, we examine regulators of WNK signaling in the *Drosophila* renal tubule. We show cooperative roles for epithelial intracellular chloride concentration and the scaffold protein mouse protein 25 (Mo25)/calcium binding protein 39 (Cab39) in regulating transepithelial ion transport *via* the WNK signaling pathway. This has implications for mechanisms of WNK pathway regulation in the mammalian nephron.