Podocytes are highly specialized epithelial cells in glomeruli, with a complex morphology composed of a cell body, primary processes, and foot processes, which maintain barrier function in glomerular filtration. The microtubule-based cytoskeleton is necessary for podocyte morphology. Microtubule structure and function can be affected by post-translational modification of tubulin, including detyrosination. Recent studies have shown that vasohibin-1 (VASH1), an antiangiogenic factor, has tubulin carboxypeptidase activity that causes detyrosination of α-tubulin. We aimed to examine the role of VASH1 in regulating α-tubulin detyrosination in podocytes and the potential involvement of VASH1 deficiency in renal morphology. In normal mouse kidneys, detyrosinated α-tubulin was mainly identified in glomeruli, especially in podocytes; meanwhile, in cultured immortalized podocytes, α-tubulin detyrosination was promoted with cell differentiation. Notably, α-tubulin detyrosination in glomeruli was diminished in *Vash1* homozygous knockout (*Vash1*$^{-/-}$) mice, and knockdown of *VASH1* in cultured podocytes prevented α-tubulin detyrosination. Although VASH1 deficiency-induced downregulation of detyrosination caused no remarkable glomerular lesions, urinary albuminuria excretion and glomerular volume were significantly higher in *Vash1*$^{-/-}$ mice than in wild-type mice. Furthermore, decreased glomerular nephrin expression and narrower slit diaphragms width were observed in *Vash1*$^{-/-}$ mice. Taken together, we demonstrated that α-tubulin detyrosination in podocytes was mainly regulated by VASH1 and that VASH1 deficiency-mediated decreases in α-tubulin detyrosination led to minor alterations in podocyte morphology and predisposition to albuminuria. VASH1 expression and α-tubulin detyrosination may be novel targets for maintaining glomerular filtration barrier integrity.