## SIGNIFICANCE STATEMENT

Ephrin-B1 is known to be expressed at the slit diaphragm and to interact with nephrin, but its role and their precise functional association are not clearly established. In this study, we show that podocyte-specific ephrin-B1 conditional knockout mice displayed proteinuria, alteration of podocyte morphology, and disarrangement of the slit diaphragm. Ephrin-B1 bound to nephrin *via* extracellular domains, with nephrin-bound ephrin-B1 being phosphorylated through nephrin stimulation. The phosphorylated ephrin-B1 promoted c-Jun N-terminal kinase (JNK) phosphorylation independently of nephrin phosphorylation. The phosphorylation of JNK enhanced the cell motility. Nephrin-binding ephrin-B1 thus plays an essential role in maintaining the structure and barrier function of the slit diaphragm. Ephrin-B1 phosphorylation and the consequent promotion of JNK phosphorylation are involved in the development of podocyte injury.