Many studies have demonstrated nanomolar concentration of ouabain activates Src through Na/K-ATPase/Src receptor complex, and then consequently induce activation of several signaling pathways, including activation of MAPK pathway, mitochondrial release of reactive oxygen species (ROS), and transient release of intracellular Ca\(^{2+}\). The formation of Na/K-ATPase/Src complex involves the interaction between the third cytosolic domain (CD3) of α1 and kinase domain of Src. This interaction functionally keeps Src in inactive state, while ouabain binding will release the kinase domain and lead to Src activation. However, it is still unknown how the CD3 inhibits Src. Here, we further identified the binding motifs within the CD3. First, GST pulldown assay showed a direct interaction between the N-terminus (ND1) of N domain (nucleotide-binding domain) of α1 and Src. Functionally, ND1 significantly inhibited Src activity. Second, a 20-residue peptide, denoted as P3 peptide, encompassing amino acid residues from ND1, was capable to inhibit Src in a dose-dependent manner and delivery of P3 into LLC-PK1 cells by saponin-based protocol apparently inhibited Src. Accordingly, cell-permeable TAT-P3 consisting of P3 coupled to TAT significantly inhibited Src with \(IC_{50}\) around 1.5 nM, while no inhibitory effect on PKC activity. Consistently, TAT-P3 inhibited basal Src as well as FAK and ERK activity in PY-17 cells where these kinases activities were elevated specifically due to knockdown of Na/K-ATPase. Finally, preincubation with TAT-P3 abrogated ouabain-induced activation of Src and then blocked MAPK signaling pathway in neonatal cardiac myocytes. These new findings not only made it possible for us to further address the function role of digitalis-induced signaling through Na/K-ATPase, but also develop TAT-P3 as a Src inhibitor that may be a potential therapeutic intervention for certain types of cancers. Supported by the NIH grants HL-36573 and HL-67963 and GM-78565.