Benign prostatic hyperplasia (BPH) is characterized by an enlargement of the prostate, causing lower urinary tract symptoms in elderly men worldwide. However, the molecular mechanism underlying the pathogenesis of BPH is unclear. Anoctamin1 (ANO1) encodes a Ca2+-activated chloride channel (CaCC) that mediates various physiological functions. Here we demonstrate that it is essential for testosterone-induced BPH. ANO1 was highly amplified in dihydrotestosterone (DHT)-treated prostate epithelial cells, whereas the selective knockdown of ANO1 inhibited DHT-induced cell proliferation. Three androgen-response elements were found in the ANO1 promoter region, which is relevant for the DHT-dependent induction of ANO1. Administration of the ANO1 blocker or Ano1 small interfering RNA, inhibited prostate enlargement and reduced histological abnormalities in vivo. We therefore concluded that ANO1 is essential for the development of prostate hyperplasia and is a potential target for the treatment of BPH.