SUPPLEMENTARY INFORMATION

Figure S1
Figure S1. GDNF signaling up-regulates RET expression and localization in the VMP of the developing prostate. UGSs from E15.5 embryos were cultured with 10 nM DHT and 100 ng/mL GDNF or vehicle for 2 days and 7 days. (A) QPCR analysis shows that Ret mRNA expression increased 6-fold and 18-fold in UGSs co-treated with DHT and GDNF compared to those treated with DHT alone on day 2 and day 7, respectively. Data represent the mean ± SEM (n = 6) in log2 scale, *P < 0.05 and **P < 0.01. (B) In addition, Spry1 mRNA increased 5-fold in UGSs co-treated with DHT and GDNF compared to those treated with DHT alone on day 7 (n = 6). (C) Immunoblot analysis of lysates from UGSs and prostate rudiments reveals that RET expression increased 4-fold and 7-fold in UGSs co-treated with DHT and GDNF compared to those treated with DHT alone on day 2 and day 7, respectively. Relative RET to GAPDH protein ratios are shown using the mean ± SEM (n = 5). (D) Immunohistochemistry revealed the localization of RET protein expression (red) during prostate development ex vivo. DAPI-stained nuclei (blue) identify all cells in the sections, and immunodetection of keratins (green) identifies the UrE and the ventral, lateral, and dorsal prostatic epithelium (VPE, LPE, DPE). Boundaries between the PUM and UrE/PrE have been traced (dashed white line), and the VMP has been labeled. (E) Quantification of the relative RET protein per cell detected by immunohistochemistry revealed that RET expression increased 2-fold per cell in DHT-induced UGSs co-treated with GDNF for 2 days compared to UGSs treated with DHT alone (n = 100). Significantly increased RET expression per cell was also present in DHT-induced UGSs co-treated with GDNF for 7 days compared to UGSs treated with DHT alone (n = 160). (F) Immunodetection of FGF10 (green), RET (red), and DAPI-stained nuclei (blue) reveals robust co-localization of FGF10 and RET in the VMP of postnatal day 0 (P0) prostate cultured with 10 nM DHT and 100 ng/mL GDNF for 2 days. RET expression is localized primarily in the VMP and to a lesser extent in the PUM of the developing prostate. The ductus deferens (DD) and seminal vesicles (SV) have been labeled. Scale bars represent 100 µm (D) or 200 µm (F).
**Figure S2.** Smooth muscle actin expression and localization are unaffected by GDNF signaling in the developing prostate. (A) E15.5 UGSs were cultured for 7 days with 10 nM DHT and 100 ng/mL GDNF or vehicle. Immunodetection of SMA (green), RET (red), and DAPI-stained nuclei (blue) are shown. SMA staining identifies layers of smooth muscle cells, which separate the PUM from the VMP layers of the UrM and encase the PrE buds. Since RET expression is localized in the VMP of the developing prostate, there is a lack of co-localization of SMA and RET in the smooth muscle and VMP layers. (B) Immunoblot analysis of prostate rudiment lysates shows similar levels of SMA expression in UGSs co-treated with DHT and GDNF compared to those treated with DHT alone. Relative SMA to GAPDH protein ratios are shown using the mean ± SEM (n = 5). (C) E15.5 UGSs were cultured with 10 nM DHT and 100 ng/mL GDNF or vehicle for 2 days and 7 days. Immunohistochemistry revealed similar localization of SMA protein (green) at emerging and elongating prostatic epithelial buds during prostate development *ex vivo* at 2 days and 7 days. (D) Quantification of the relative SMA protein per cell detected by immunohistochemistry also revealed similar SMA expression per cell in DHT-induced UGSs co-treated with GDNF compared to UGSs treated with DHT alone (n = 160). The ventral, lateral, dorsal, and anterior prostatic epithelium (VPE, LPE, DPE, APE), ductus deferens (DD), and seminal vesicles (SV) have been labeled. Scale bars represent 100 µm (A, C).