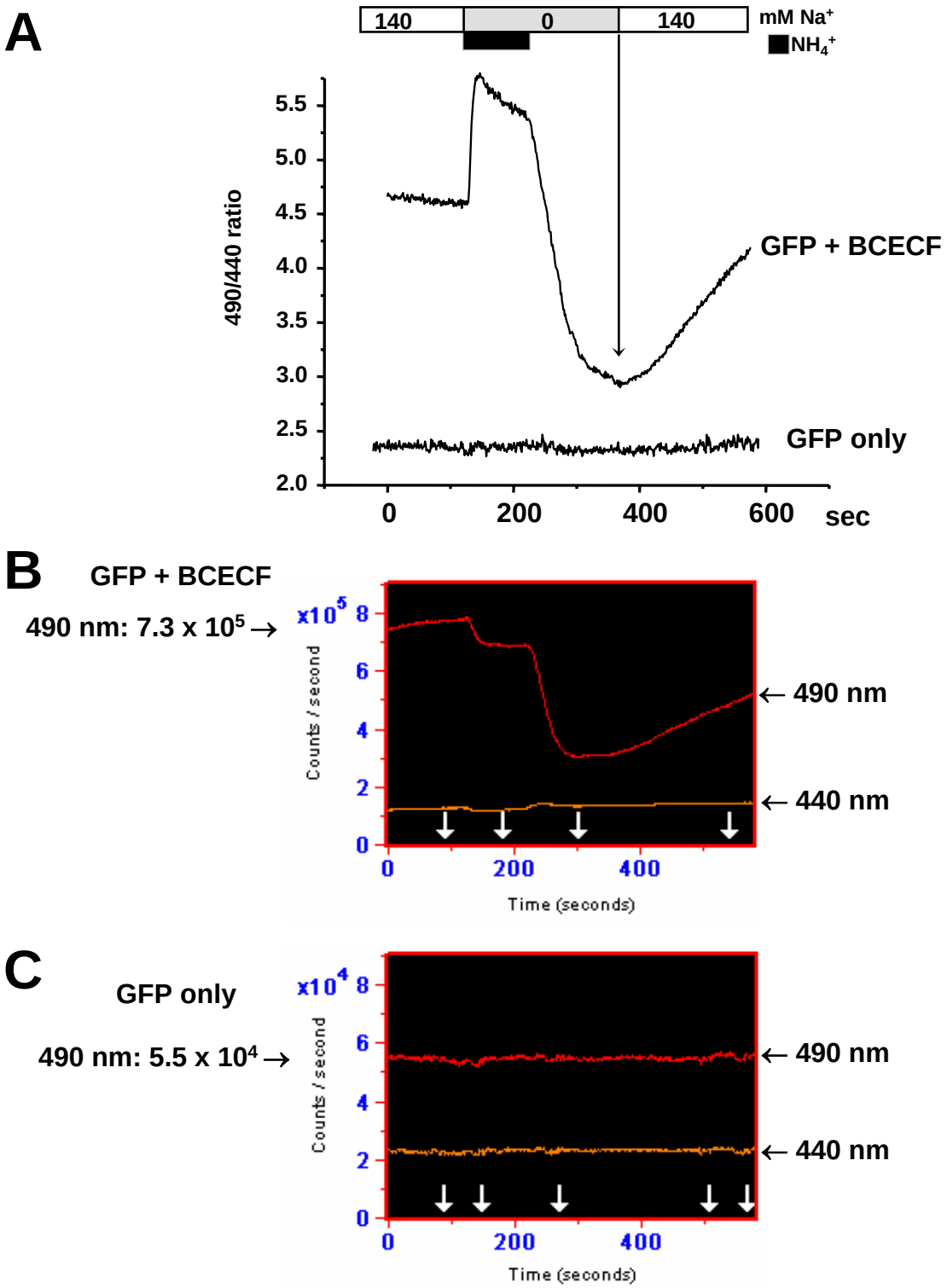
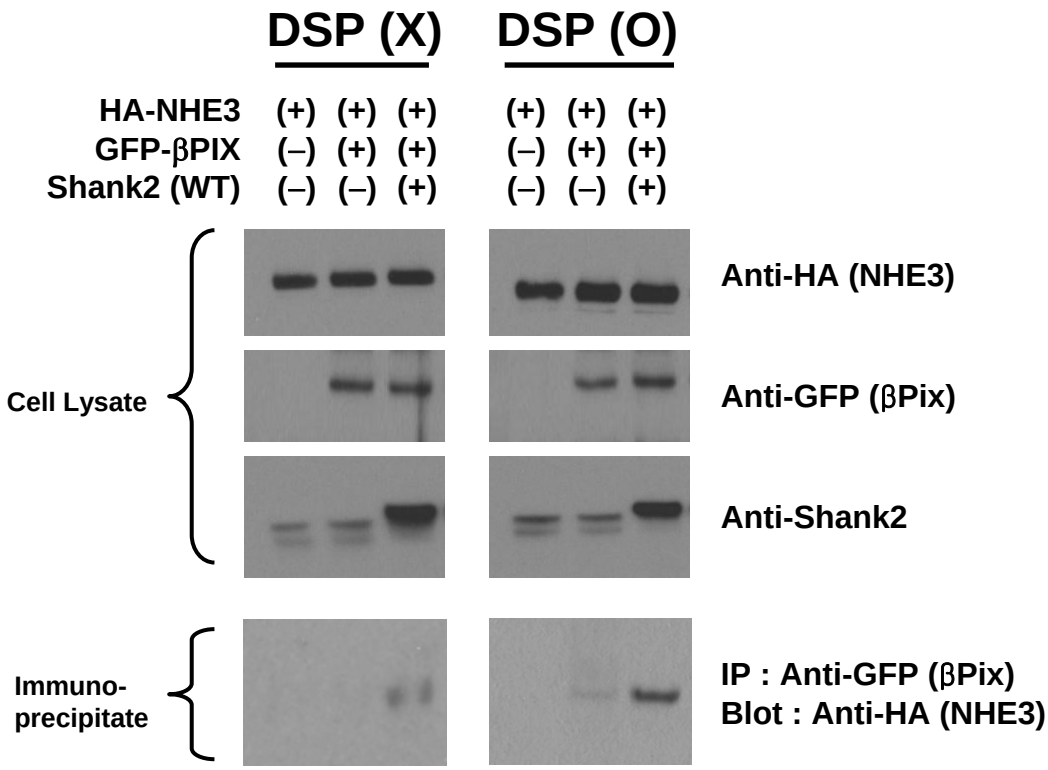
Supplementary Fig. 1



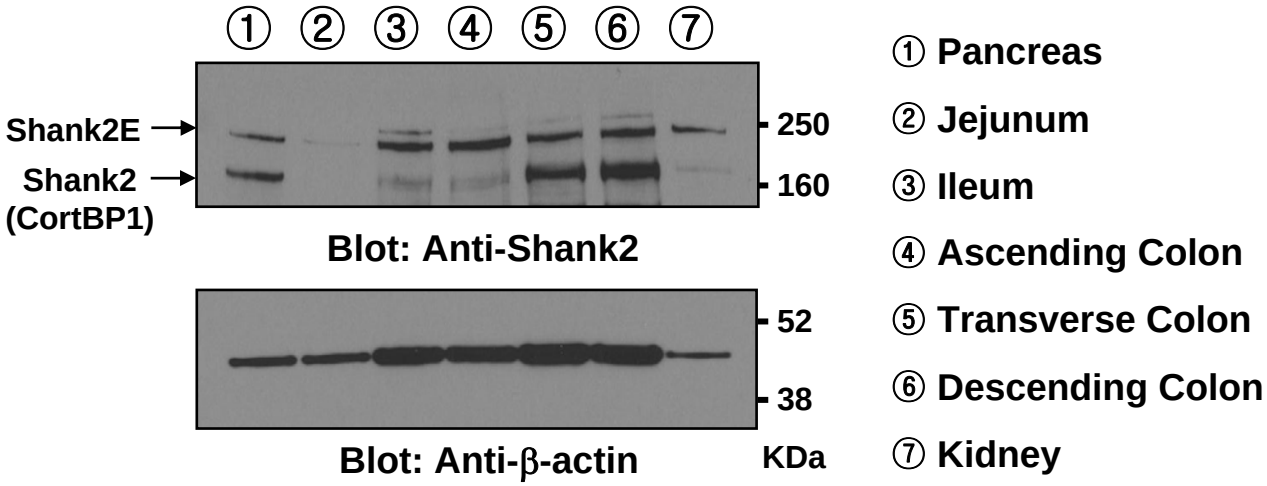
Supplementary Fig. 1. Measurements of Na^+/H^+ exchange activity after targeting the green fluorscent protein (GFP) expressing cells. When Shank2 and βPix constructs were transiently expressed, a GFP-expressing plasmid was co-transfected and pH_i measurements were performed with cells expressing high levels of GFP. PS120/NHE3 cells grown on glass coverslips were loaded with (panel ***B***) or without (panel ***C***) a pH-sensitive fluorescent dye BCECF and pH_i changes were measured using the protocols described in the Experimental Procedures. Panel **A** represents the 490/440 nm ratios calculated from traces shown in panels ***B*** and ***C***. Note that BCECF loaded cells showed at least 10-fold higher fluorescence intensity than the GFP only expressing cells and background GFP fluorescence did not affect pH_i measurements.

Supplementary Fig. 2



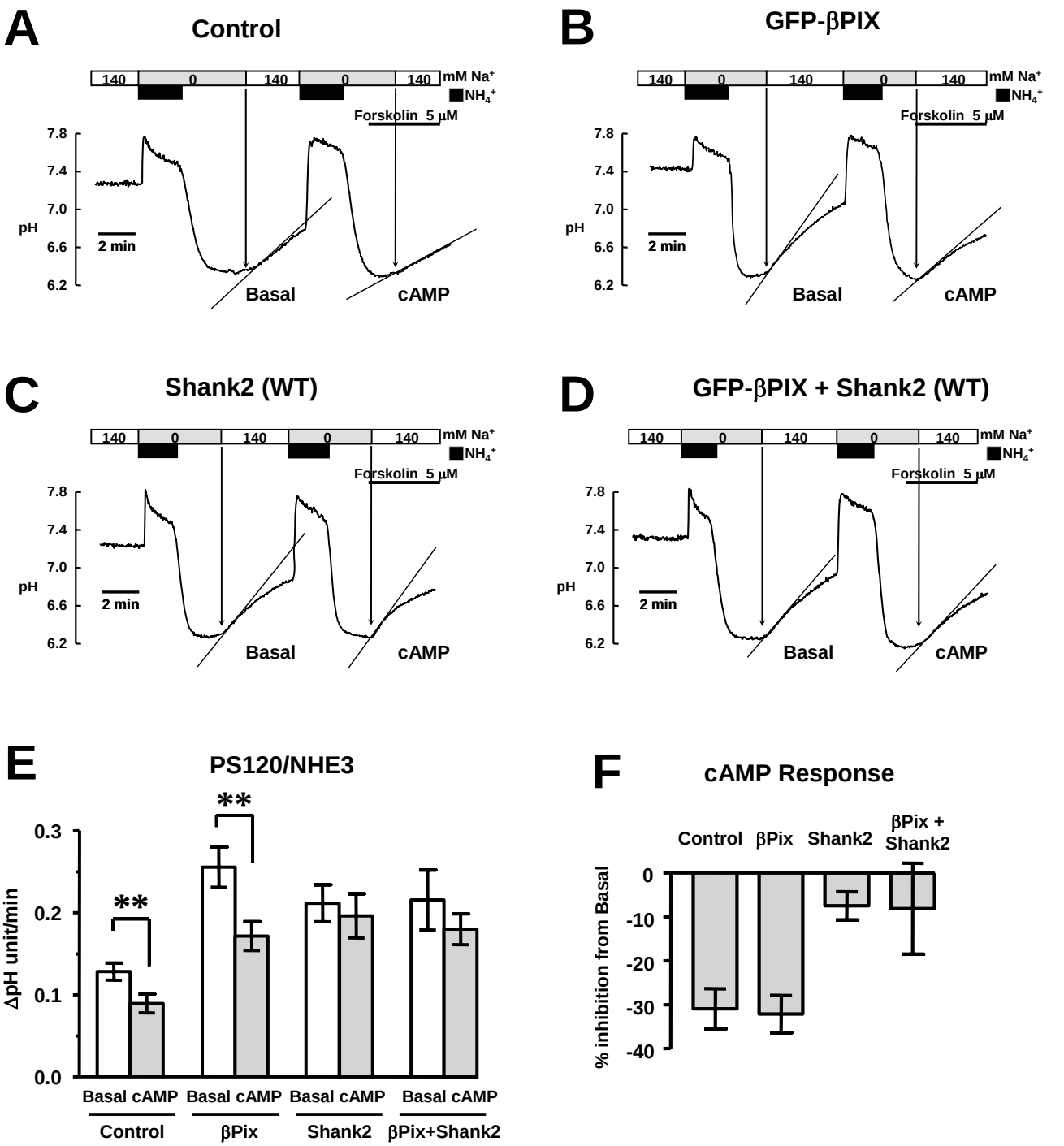
Supplementary Fig. 2. Effects of the crosslinking agent dithiobis-(succinimidyl propionate) (DSP) on the interaction between βPix and NHE3. Immunoprecipitation (IP) was performed in PS120/NHE3'$_{38HA3}$ cells that stably express HA epitope-tagged NHE3 (pCMV-rNHE3'$_{38HA3}$) using similar protocols described in the legend for Fig. 1. The PS120 cells were treated with or without DSP (2 mM) for 30 min at room temperature before harvesting. PS120/NHE3'$_{38HA3}$ cells were cotransfected with the plasmids expressing GFP-βPix (pEGFP-βPix) and Shank2 (pcDNA3.1-rShank2). Protein samples were precipitated with anti-GFP (βPix) antibodies and immunoblotting was carried out using monoclonal anti-HA, polyclonal anti-GFP, and polyclonal anti-Shank2 (#1136) antibodies. In immunoblotting of cell lysates, 50 μg of protein was loaded into each lane. IP was performed using a total of 500 μg cell lysate. Note that treatment with the crosslinking agent DSP greatly increased the association between βPix and NHE3 in Shank2-expressed cells.

Supplementary Fig. 3



Supplementary Fig. 3. Expression of Shank2 proteins in the rat gastrointestinal organs and kidney. In small intestines and colons, protein samples were prepared from the mucosal layer. Immunoblotting was carried out using polyclonal anti-Shank2 (#1136) and anti-β-actin (sc-1616) antibodies, and 50 μg of protein was loaded into each lane. In ileum, ascending colon, and kidney, the long isoform of Shank2 (Shank2E) is principally expressed, while transverse colon, descending colon, and pancreas express both the short (CortBP1) and the long (Shank2E) forms of Shank2 .

Supplementary Fig. 4



Supplementary Fig. 4. Effect of βPix expression on the cAMP-induced inhibition of Na^+/H^+ exchanger 3 (NHE3) activity. Basal and cAMP-stimulated (forskolin 5 μM) NHE activities were measured in PS120/NHE3 cells. Cells were transfected with each plasmid and NHE activities were measured using protocols detailed in the legend for Fig. 2. Panels ***A***-***D*** show representative traces of each experimental set, and panels ***E*** and ***F*** illustrate summaries of multiple (n=6) NHE activity measurements and cAMP response, respectively. ** P<0.01; difference from basal. βPix did not affect the cAMP-induced inhibition of NHE3 activity while Shank2 greatly reduced the cAMP effects, suggesting that βPix is not involved in the Shank2-mediated regulation of cAMP response.