



Watanabe et al., Supplementary Fig. 1

**Supplementary Figure 1: Characterization of the antibodies used in the present study.**

The immunocytochemical findings of the present study were confirmed by using two different sources of antibodies against each antigen, as far as possible. Thick cryosections of the rat

pituitary gland were immunostained simultaneously with two different sources of anti-GM130 (A2, A3), anti-TGN38 (B2, B3), anti- $\alpha$ -tubulin (C2, C3), or anti- $\gamma$ -tubulin (E2, E3) antibodies. The immunostaining patterns of anti-acetylated  $\alpha$ -tubulin (mouse monoclonal; D2) and anti- $\alpha$ -tubulin (rabbit polyclonal; D3) antibodies were also compared. In the left panels, merged images of immunostaining patterns with two different sources of the antibodies (labeled with Alexa Fluor 594 (red) and 488 (green)) and an anti-LH antibody (labeled with Alexa Fluor 405 (blue)) were demonstrated (A1 -E1). Ms: mouse monoclonal antibody, Rb: rabbit polyclonal antibody, Sh: sheep polyclonal antibody. Bar = 10  $\mu$ m.

The specificity of these antibodies was also confirmed by immunoblot analyses in extracts of rat representative endocrine tissues including pituitary (Pit), thyroid (Thy) and adrenal (Adr) glands. Ten  $\mu$ g proteins of each tissue extract were separated onto 12% SDS-PAGE under reducing conditions, transferred to PVDF membranes, and immunostained with the antibodies, as described previously (Sakai et al., 2003). At the right of each immunocytochemical figure panels, blots immunostained with two different sources of anti-GM130 (A4), anti-TGN38 (B4), anti- $\alpha$ -tubulin (C4), and anti- $\gamma$ -tubulin (E4) antibodies are demonstrated side by side, indicating that these two different sources of antibodies properly recognized identical antigens of appropriate molecular weight (GM130 (130 kDa), TGN38

(85-95 kDa),  $\alpha$ -tubulin (55 kDa), and  $\gamma$ -tubulin (48-50 kDa)).