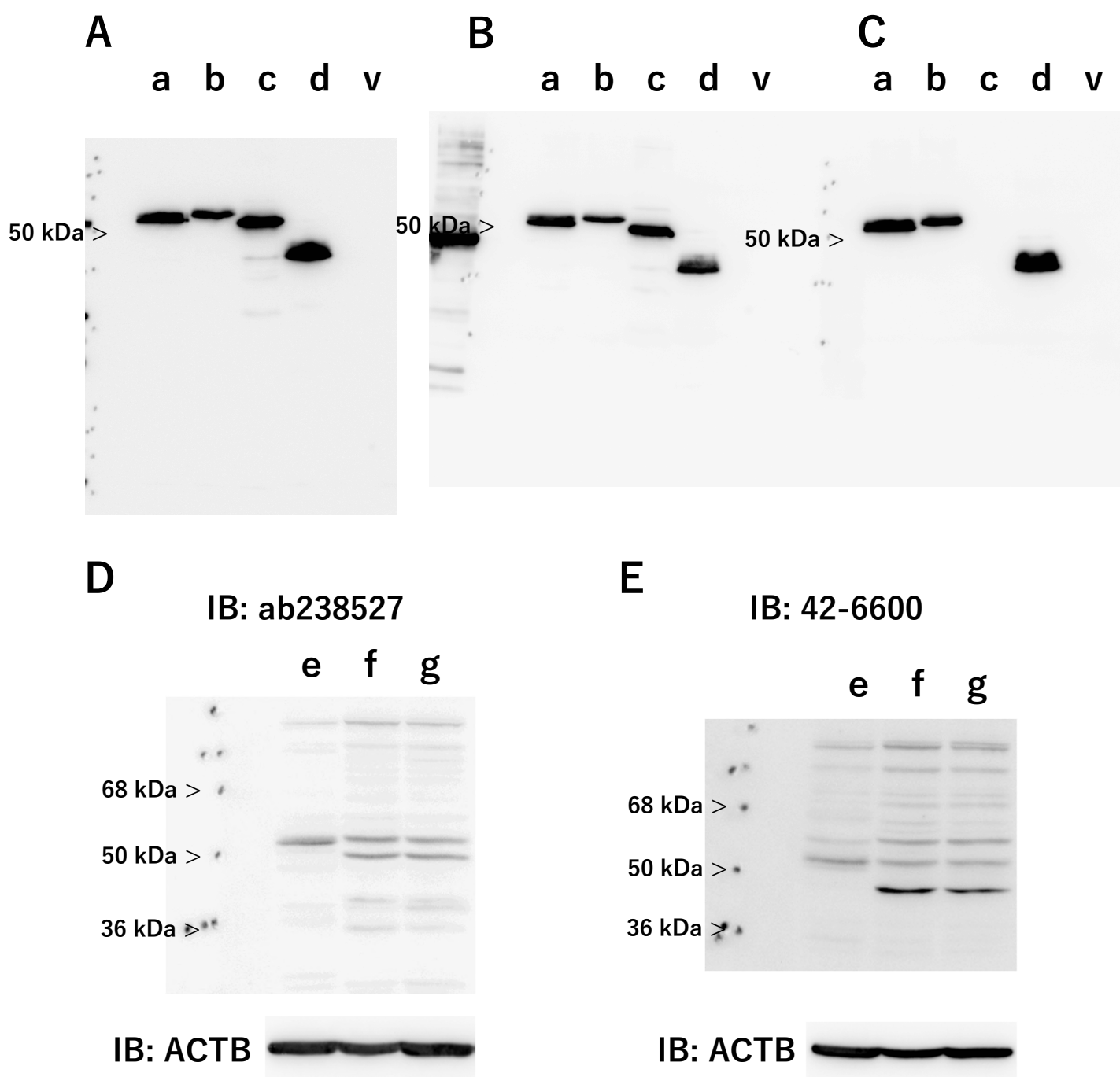


Fig. S1



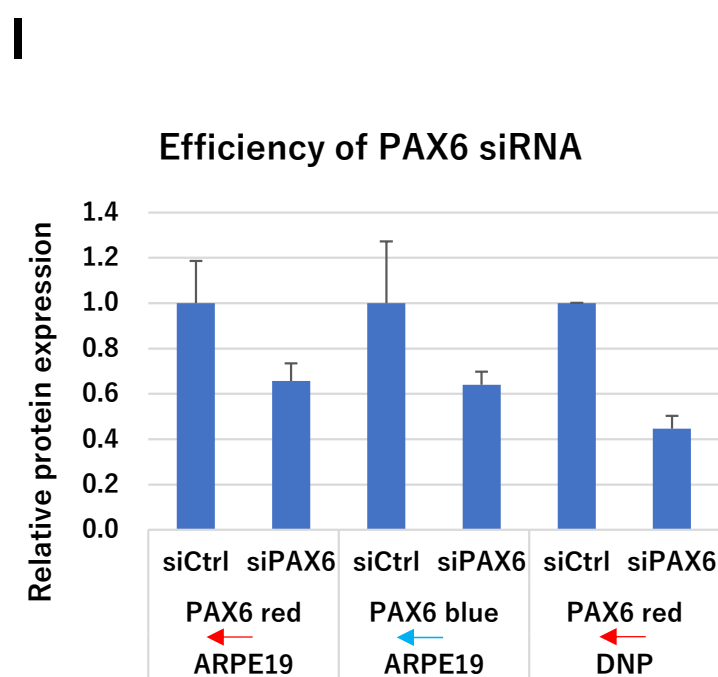
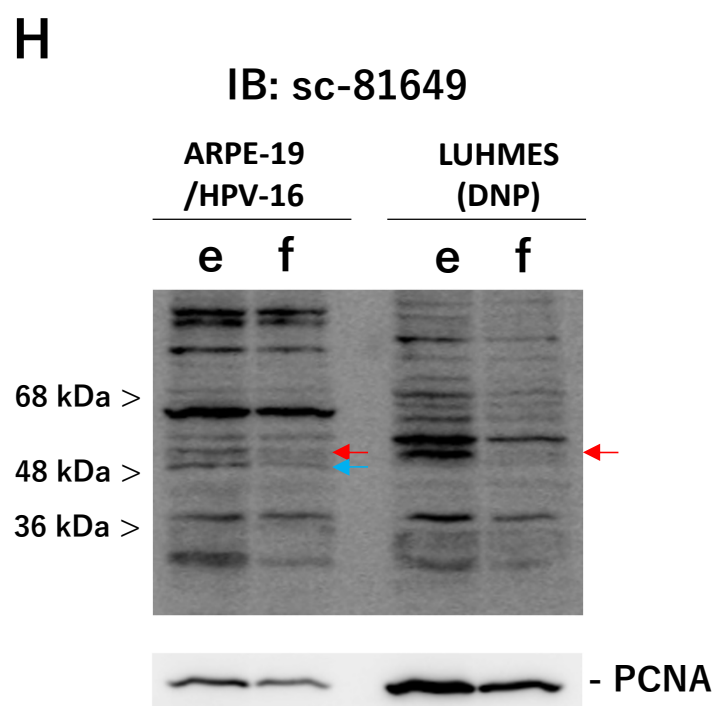
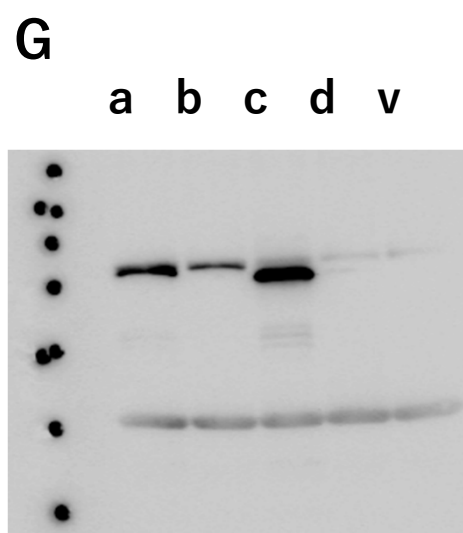
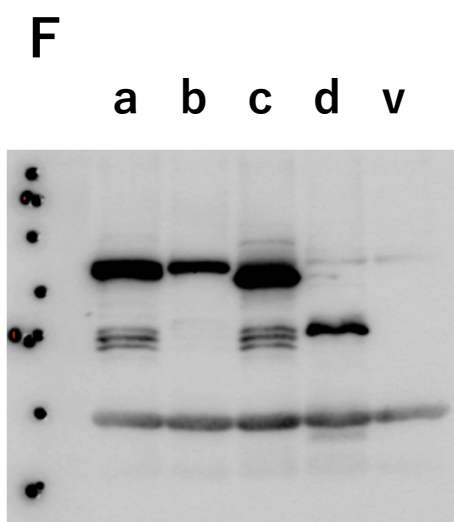


Fig. S1 Evaluation of sensitivity and specificity of commercially available antibodies.

Method: Flag-PAX6 isoforms (isoform a; NM_000280, isoform b/5a; NM_001604, isoform c; NM_001310159, isoform d; NM_001310161) and vector alone (v) were transiently expressed in PC3 cells and immunoprecipitated with an anti-Flag antibody. The transferred membranes were treated with anti-Flag antibody (Sigma; M2-HRP) (A), anti-PAX6 antibody (Abcam; ab238527) and anti-rabbit IgG antibody conjugated HRP (B), anti-PAX6 antibody (Invitrogen; 42-6600) and anti-rabbit IgG antibody conjugated HRP (C). (D)(E) Whole cell lysate (50 µg) of ARPE19/HPV16 (e), LUHMES (f) and PC3 (g) cells were subjected with SDS-PAGE. Each membrane was blotted with anti-PAX6 antibodies (ab238527 and 42-6600) and anti-rabbit IgG antibody conjugated HRP. These transfer membranes were also blotted with anti-ACTB antibody conjugated HRP (Sigma; A3854) for internal control. (F) Similar to (A), Flag-PAX6 isoforms a, b/5a, c, d and vector alone (v) were transiently expressed in PC3 cells and immunoprecipitated with an anti-Flag antibody. The transferred membrane was treated with anti-Flag antibody (Sigma; M2-HRP), and also treated with anti-PAX6 antibody (Santa Cruz; sc-81649) and anti-mouse IgG antibody conjugated HRP (G). (H) ARPE19/HPV16 and LUHMES cells were transfected with siRNA for control (e) and PAX6 (f; Silencer® Select s529239, Thermo Fisher Scientific Inc.). After 48 hours, cells were collected and separated into nucleoplasm and cytoplasm. Each nuclear protein (50 µg) was subjected with SDS-PAGE and blotted with anti-PAX6 antibody (Santa Cruz; sc-81649) and anti-mouse IgG antibody conjugated HRP. This transfer membrane was also blotted with anti-PCNA antibody (Santa Cruz; sc-56) and anti-mouse IgG antibody conjugated HRP for internal control. (I) In (H), the decreased signal around the predicted molecular weight of PAX6(5a) in (f) compared to (e) was quantified (n=2). siCtrl and siPAX6 indicate siRNA for control and that for PAX6, respectively.

Result: Three anti-PAX6 antibodies, ab238527, 42-6600 and sc-81649, detected the Flag-PAX6 isoforms b/5a. However, no signal of PAX6 (5a) was observed for antibodies (ab238527 and 42-6600) in ARPE19/HPV16 and LUHMES cells compared to PC3 cells (D and E). On the other hand, using the anti-PAX6 antibody (sc-81649), several signals whose expression was reduced by siPAX6 treatment were observed, suggesting that PAX6(5a) may be among these signals (H and I). However, the PAX6(5a) signal with anti-PAX6 antibody (sc-81649) has low specificity, so it was judged difficult to identify it.