Antibody validation

In our current study, we used mouse monoclonal antibodies against KIBRA (clone 2A5), which is gifted from Dong Jixin's lab [1]. This antibody was non-commercial antibodies, so we performed experiments to validate the specificity and reactivity for western blotting according to the methods [2]. Cell culture supernatant (clone 2A5) was used for western blotting.

The HT22 cell line stably overexpressing the mouse KIBRA (referred as KIBRA-Flag cells) were generated by cloning KIBRA into the vector Ubi-MCS-3FLAG-SV40-puromycin. Stably overexpressing cells were selected by 3 μg/mL puromycin (P8032, Solarbio Science & Technology Co., Ltd., Beijing, China). The stable cell line was maintained in DMEM with 1 μg/mL puromycin. We examined the expression of KIBRA (clone 2A5) and Flag (Sigma, F1804) by western blotting and found that the result of probing a tagged fusion protein (Flag) with an antibody toward KIBRA produces a detection pattern that is similar to that of the Flag-specific antibody (Fig. 1), thus demonstrating the specificity and reactivity of the antibody under these conditions. In addition, the fragments (about 35 kDa-55 kDa) was also detected by Ab and anti-Flag, which may be the non-specific site.

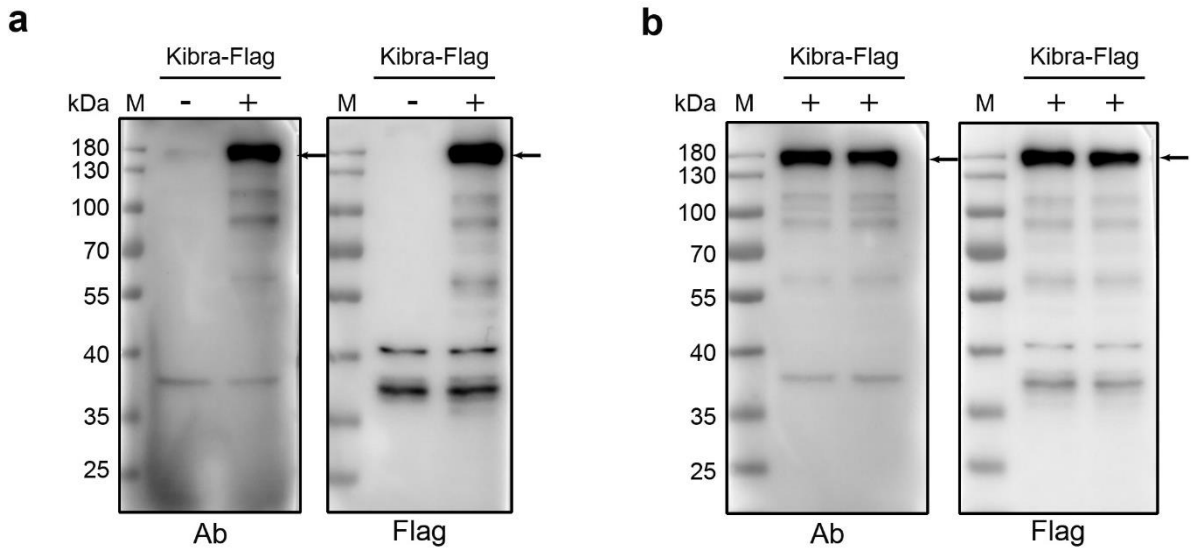

Fig. 1. Validation of antibodies in western blotting applications.

(**a**) A fusion of KIBRA with a peptide Flag was stained with either the antibody to be validated (Ab) or a Flag-specific antibody (Flag) in cell lysates from HT22 cells stably overexpressing either control or KIBRA-Flag. The black arrows indicate the theoretical sizes of the target protein. (**b**) A fusion of KIBRA with a peptide Flag was stained with either the antibody to be validated (Ab, anti-KIBRA (clone 2A5)) or a Flag-specific antibody (Flag) in cell lysates from KIBRA-Flag cells (Labeled 1-2). The black arrows indicate the theoretical sizes of the target protein.

Reference

1. Xiao L, Chen Y, Ji M, Volle DJ, Lewis RE, Tsai MY, et al. KIBRA protein phosphorylation is regulated by mitotic kinase aurora and protein phosphatase 1. J Biol Chem. 2011;286(42):36304-15.
2. Uhlen M, Bandrowski A, Carr S, Edwards A, Ellenberg J, Lundberg E, et al. A proposal for validation of antibodies. Nat Methods. 2016;13(10):823-7.