S1_raw_images for

Development and characterization of Protein Kinase B/AKT isoform-specific Nanobodies

Tijs Merckaert^{1,2}, Olivier Zwaenepoel¹, Kris Gevaert^{1,2}, Jan Gettemans*¹

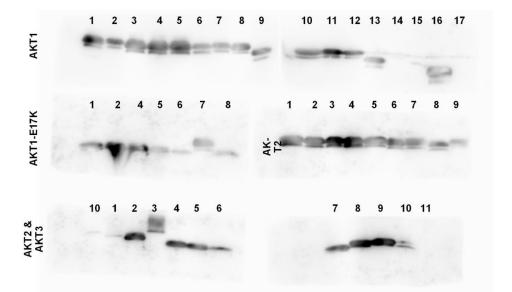
¹Department of Biomolecular Medicine, Faculty of Medicine and Health Sciences, Ghent University, Ghent,

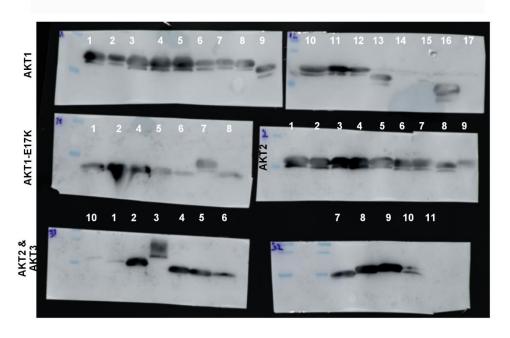
Belgium

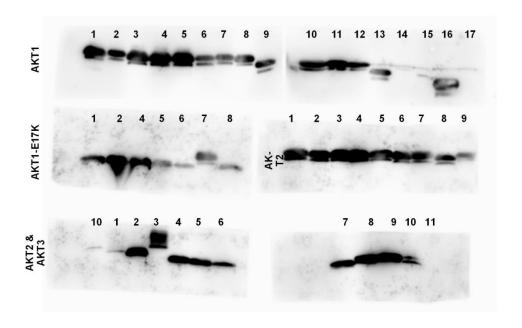
²VIB-UGent Center for Medical Biotechnology, Ghent, Belgium

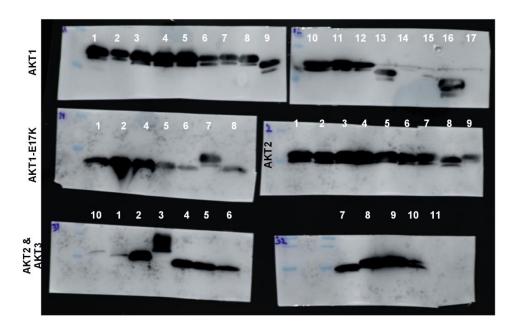
*Corresponding author

E-mail: jan.gettemans@ugent.be







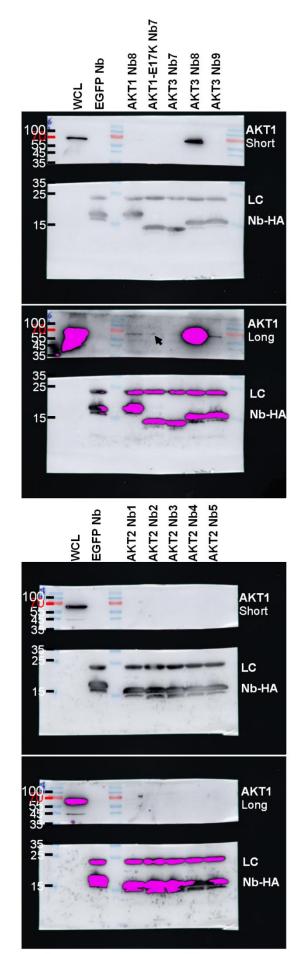


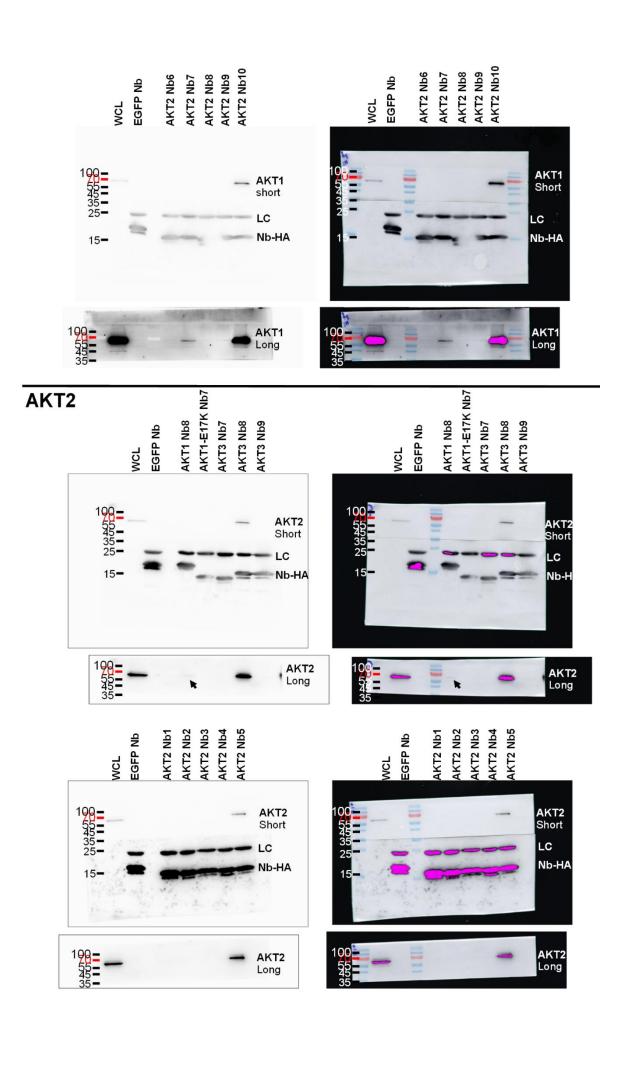
Raw images for Fig 1: Expression of AKT nanobodies in WK6 *E. coli*. Uncropped Western blot images for Fig1 recorded using the Amersham Imager 680 blot and gel imager. AKT nanobodies present in the crude periplasmatic extract were detected through enhanced-chemiluminescence using an anti-HA Ab and a secondary HRP-linked anti-mouse Ab. Nb signal is present at approximately 15kDa. Membranes are labelled with the library antigen and lanes with the Nb clone number.

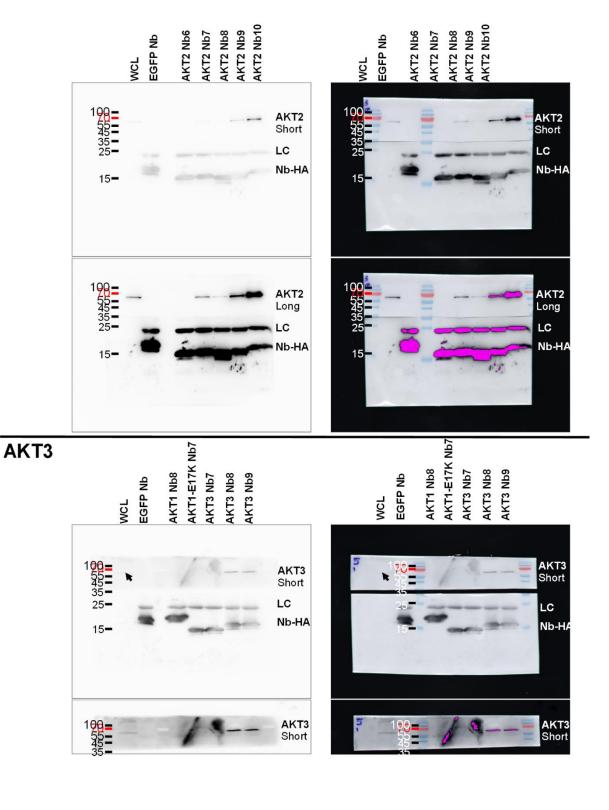
15-

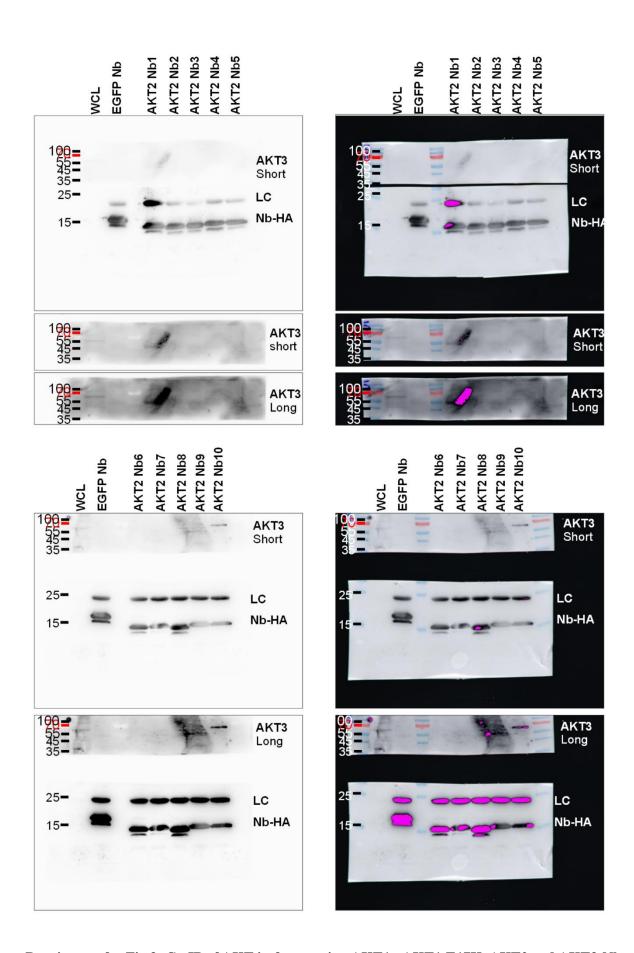
LC

Nb-HA

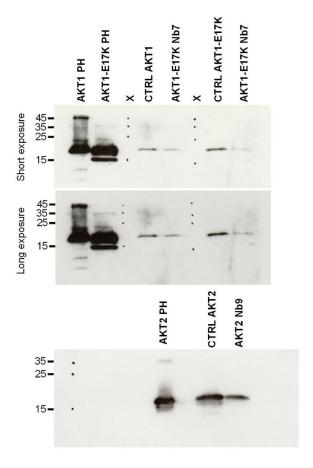




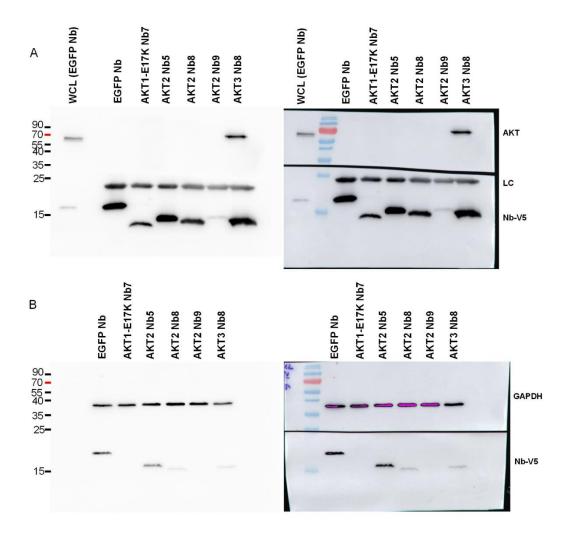




Raw images for Fig 3: Co-IP of AKT isoforms using AKT1-, AKT1-E17K, AKT2 and AKT3-Nbs.



Raw images for Fig 5: AKT nanobodies interfere with AKT-PH-PIP3 interaction. Uncropped western blots for Fig5, pull-down experiments using PIP3-coated beads.



Raw images for Fig6: Co-IP of endogenous AKT from MDA-MB-231 cells with Nbs transiently expressed as intrabodies. Raw images for Fig 6. (A) Co-IP of AKT and Nbs. (B) Nb expression. Images were recorded using the Amersham Imager 680 blot and gel imager, the ECL signal is shown on the left. The right half of the figure shows the ECL signal superimposed on a photo of the blot membranes.