

RB155, RB156 and RB189 antibodies do not recognize the *D. discoideum* Tsg101 protein by western blot

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Abstract

The recombinant antibodies RB155, RB156 and RB189 do not detect by western blot the full-length Tsg101 protein from *Dictyostelium discoideum*.

Introduction

Tsg101 (DDB_G0286797, UniProt #Q54LJ3) is a member of the ESCRT-I complex in the amoeba *D. discoideum*. Here we describe that three recombinant antibodies (RB155, RB156 and RB189) directed against the N-terminus of Tsg101 were not able to detect the full-length Tsg101 protein by western blot.

Materials & Methods

Antibodies: ABCD_RB155, ABCD_RB156, and ABCD_RB189 antibodies (ABCD nomenclature, <https://web.expasy.org/abcd/>) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies; Blanc *et al.*, 2014) as mini-antibodies with the antigen-binding scFv fused to a mouse IgG2A Fc (MRB155, MRB156 and MRB189). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~50 mg/L) were collected after 5 days. The rabbit polyclonal J81 antibody recognizing Tsg101 was a kind gift of Dr. L. Aubry (CEA, Grenoble, France).

Antigen: The antibodies were originally raised against a GST protein fused to the 20 first residues of Tsg101 (MYGHHGYPMHAHQQQMVNPT). This chimeric GST-Tsg101 was used as antigen for antibody selection. GST was used as negative control.

Protocol: *D. discoideum* cells were collected, washed in Sorensen-120 mM Sorbitol, counted and resuspended as to have 5×10^7 cells/ml in Laemmli Buffer (125 mM Tris pH 6.8, 4% (w/v) SDS, 20% glycerol, 0.01% (w/v) bromophenol blue, 10 mM DTT). 10 μ L of each sample was migrated (50 V stacking and 150 V running, 1h30) in a 10% homemade acrylamide gel and transferred to a nitrocellulose membrane (Amersham, Protran GE10600002) in 25 mM Tris, 192 mM glycine, 20% MeOH, 0.01% SDS at 4 °C, 30 V, 16 h. After checking transfer by Ponceau Red staining, the membranes were blocked during 1 hour in PBS containing 5% (w/v) BSA (bovine serum albumin fraction V, pH 7.0 (SERVA Electrophoresis GmbH 11930)). The membranes were

then incubated with each of the three MRB antibodies (dilution 1:2 in PBS with or without Tween and 3% (w/v) milk) or the J81 antibody (dilution 1:500 in PBS with Tween and 3% (w/v) milk), overnight at 4 °C, then washed three times for 10 minutes in PBS. The membranes probed with the MRB antibodies were then incubated during 1 h with goat anti-mouse IgG coupled to horseradish peroxidase (Brunschwig, dilution 1:10'000 in PBS and 3% (w/v) BSA) and washed three times for 10 minutes in PBS. The membrane probed with the J81 antibody was incubated during 1 h with goat anti-rabbit coupled to horseradish peroxidase (dilution 1:10'000 in PBS-Tween and 3% (w/v) milk). The signal was revealed by enhanced chemiluminescence (ECL) (Amersham Biosciences RPN2232) using a Fusion Fx device (Vilbert Lourmat).

Results

Antibodies were tested on the GST-antigen fusion as a positive control, lysates from wild-type AX2 (Ka) *D. discoideum* cells, and as negative control a clone of *tsg101* knock-out cells (López-Jiménez *et al.*, 2018). Antibodies MRB155, MRB156 and MRB189 recognize the GST-Tsg101 antigen, but not the endogenous Tsg101 in *D. discoideum* cells (Fig. 1). Antibodies were then tested on lysates from wild-type cells overexpressing GFP-Tsg101. As a control, the J81 antibody that specifically recognizes Tsg101 (Fig. 2) was used. The J81 antibody recognized both the endogenous and exogenous Tsg101, while MRB155, MRB156 and MRB189 did not.

References

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López-Jiménez AT, Cardenal-Muñoz E, Leuba F, Gerstenmaier L, Barisch C, Hagedorn M, King JS, Soldati T. The ESCRT and autophagy machineries cooperate to repair ESX-1-dependent damage at the *Mycobacterium*-containing vacuole but have opposite impact on containing the infection. *PLOS Pathog*. 2018; 14:e1007501. PMID: 30596802

Conflict of interest

The authors declare no conflict of interest.

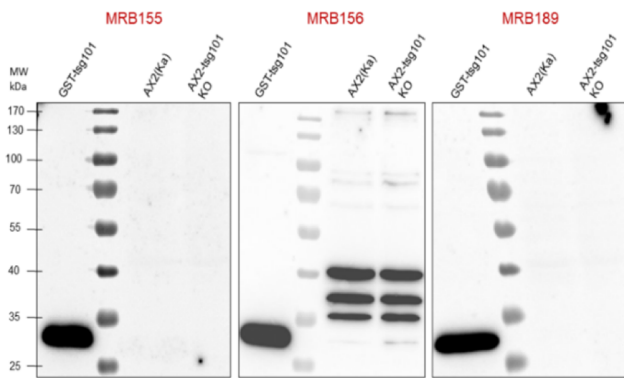


Fig. 1. Western blot with MRB155, MRB156, MRB189 on GST-Tsg101, lysates from wild-type AX2(Ka) and *tsg101* KO cells.

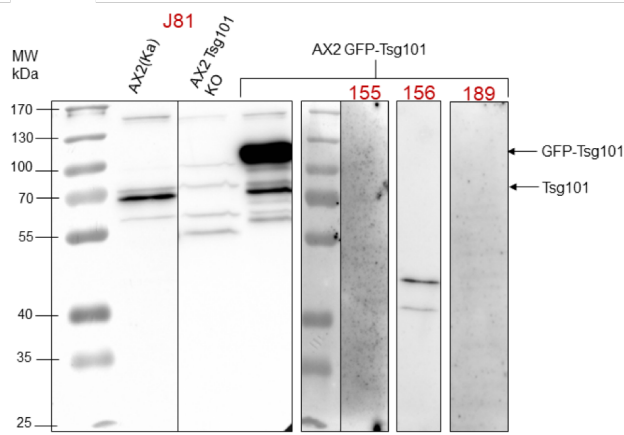


Fig. 2. Western blot with MRB155, MRB156, MRB189 and J81 on lysates from wild-type AX2(Ka), *tsg101* KO and AX2(Ka) expressing GFP-Tsg101 cells.