RA11 and RA12 antibodies recognize a peptide of the D. discoideum Mucolipin protein by western blot
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Abstract
Recombinant antibodies RA11 and RA12 detect by western blot a peptide of the Dictyostelium discoideum Mucolipin protein fused to a GST protein.

Introduction
Mucolipin (Mcln, DDB_G0291275, UniProt #Q54EY0) is a calcium channel involved in lysosomal calcium homeostasis in D. discoideum (Lima et al., 2012). Here we describe the ability of the RA11 and RA12 antibodies to detect by western blot a fragment of the Mucolipin protein fused to a GST protein.

Materials & Methods
Antibodies: ABCD_RA011 and ABCD_RA012 antibodies (ABCD nomenclature, web.expasy.org/abcd/; Lima et al., 2019) 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 (MRA11 and MRA12). HeLa cells (growing in DMEM GlutaMAX® (Gibco, #31966) supplemented with 8% Fetal Bovine Serum (Gibco, #10270)) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~1 mg/L) were collected after 4 days.

Antigen: The antibodies were originally raised against a GST protein fused to the residues 529 to 669 of the Mucolipin protein. This chimeric GST-Mcln protein was used as antigen for detection. GST was used as a negative control.

Protocol: Expression of the GST-Mcln recombinant protein was induced in E. coli bacteria growing exponentially (OD600, 0.5) at 37°C (in 50 ml of Luria-Bertani (LB) medium containing 20% glucose and 100 μM ampicillin) by addition of 1.5 mM IPTG. After 3 h, bacteria were pelleted and resuspended in lysis buffer (4 ml of PBS + 1% Triton X100 + aprotinin 10 μg/ml + leupeptin 20 μg/ml + iodoacetamide 1.8 mg/ml + PMSF 18 μg/ml) and lysed by sonication. GST was purified on glutathione-coupled sepharose 4 Fast Flow beads (GE Healthcare Life Sciences #17-5132-01), then eluted in 500 μl of reducing sample buffer (20.6% (w/v) sucrose, 100 mM Tris pH 6.8, 10 mM EDTA, 0.1% (w/v) bromophenol blue, 4% (w/v) SDS, 6% (v/v) β-mercaptoethanol). 15 μL of each sample was migrated (200 V, 30 min) in a 12% acrylamide gel (Mini-PROTEAN® TGX™ Precast Gel, Biorad #456-1043), and transferred to a nitrocellulose membrane using a dry transfer system for 10 minutes (iBlot gel transfer device, Invitrogen #IB1001EU). The membranes were blocked overnight at 4°C in PBS containing 0.1% (v/v) Tween20 and 5% (w/v) milk, and washed three times (5 minutes) in PBS + 0.1% (v/v) Tween20. The membranes were then incubated with each of the tested antibodies (undiluted), for 1h at room temperature, and washed three times (5 minutes) in PBS-Tween. The membranes were then incubated with horseradish peroxidase-coupled goat anti-mouse (Biorad #170-6516, dilution 1:3000) for 1h at room temperature, and washed three times (5 minutes) in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) using a PXi-4 gel imaging systems (Syngene).

Results
MRA11 and MRA12 antibodies specifically recognize the GST-Mcln fusion protein (~43 kDa), as well as a probable partial degradation product at ~35kDa. The antibodies do not bind the GST negative control (Fig. 1).

References
Blanc C, Zufferey M, Cosson P. Use of in vivo biotinylated GST fusion proteins to select recombinant antibodies. ALTEX. 2014;31(1):37-42. PMID:24100547

Conflict of interest
The authors declare no conflict of interest.