RB252, RB253, RB254 and RB255 antibodies recognize human Miner1 protein by immunofluorescence

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Abstract

The recombinant antibodies RB252, RB253, RB254 and RB255 detect by immunofluorescence the human Miner1 protein in paraformaldehyde-fixed cells.

Introduction

Miner1/CISD2 (MitoNEET-related 1 protein, CDGSH iron-sulfur domain-containing protein 2; UniProt #Q8N5K1) is an integral protein of the endoplasmic reticulum (Wiley *et al.*, 2007). Here we describe the ability of four recombinant antibodies to successfully recognize Miner1 by immunofluorescence in MEF cells overexpressing Miner1.

Materials & Methods

Antibodies: RB252, RB253, RB254 and RB255 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (https://www.unige.ch/medecine/antibodies/; Blanc *et al*., 2014) as mini-antibodies with the antigen-binding scFv fused to a mouse Fc (MRB252, MRB253, MRB254 and MRB255). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (~50 mg/L) were collected after 5 days.

Antigen: The antibodies were raised against a GST protein fused to the 75 C-terminal residues of Miner1 protein (PKKKQQKDSLINLKIQKENPKVVNEINIEDL CLTKAAYCRCWRSKTFPACDGSHNKHNELTGDNV GPLILKKKEV). MEF cells (growing in DMEM GlutaMAX™ supplemented with 10% Fetal Bovine Serum) cultured on a glass coverslip (Menzel-Gläser, 22x22 mm) transfected 3 days before the experiment with Miner1 were used to detect the full-length protein.

Protocol: The whole procedure was carried out at room temperature. Transfected MEF cells were fixed with PBS + 4% paraformaldehyde (w/v) (Applichem, #A3013) for 30 min, and blocked with PBS + 40 mM ammonium chloride (NH_4Cl) (Applichem, #A3661) for 5 min. Cells were then permeabilized in PBS + 0.2% saponin (w/v) (Sigma, #S7900) for 5 min, washed once (5 min) with PBS + 0.2% (w/v) BSA (PBS-BSA), and incubated for 30 min with the antibody-containing supernatants (dilution 1:10). After 3 washes (5 min) with PBS-BSA, cells were incubated for 30 min in PBS-BSA with secondary goat anti-mouse IgG conjugated to AlexaFluor-488 (1:400, Molecular Probes, #A11029). After 3 washes (5 min) with PBS-BSA, cells were mounted on slides (Menzel-Gläser, 76x26 mm) with Möwiol (Hoechst) + 2.5% (w/v) DABCO (Fluka, #33480). Pictures were taken using a Zeiss LSM700 confocal microscope, with a 40x Neofluar oil immersion objective.

Results

The antibodies MRB252, MRB253, MRB254 and MRB255 specifically detect a signal in Miner1-transfected cells, resembling the endoplasmic reticulum network (Fig. 1). Endogenous Miner1 is detected with a very faint signal. No signal was detected when the primary antibody was omitted (Fig. 1A).

References

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Conflict of interest

The authors declare no conflict of interest.

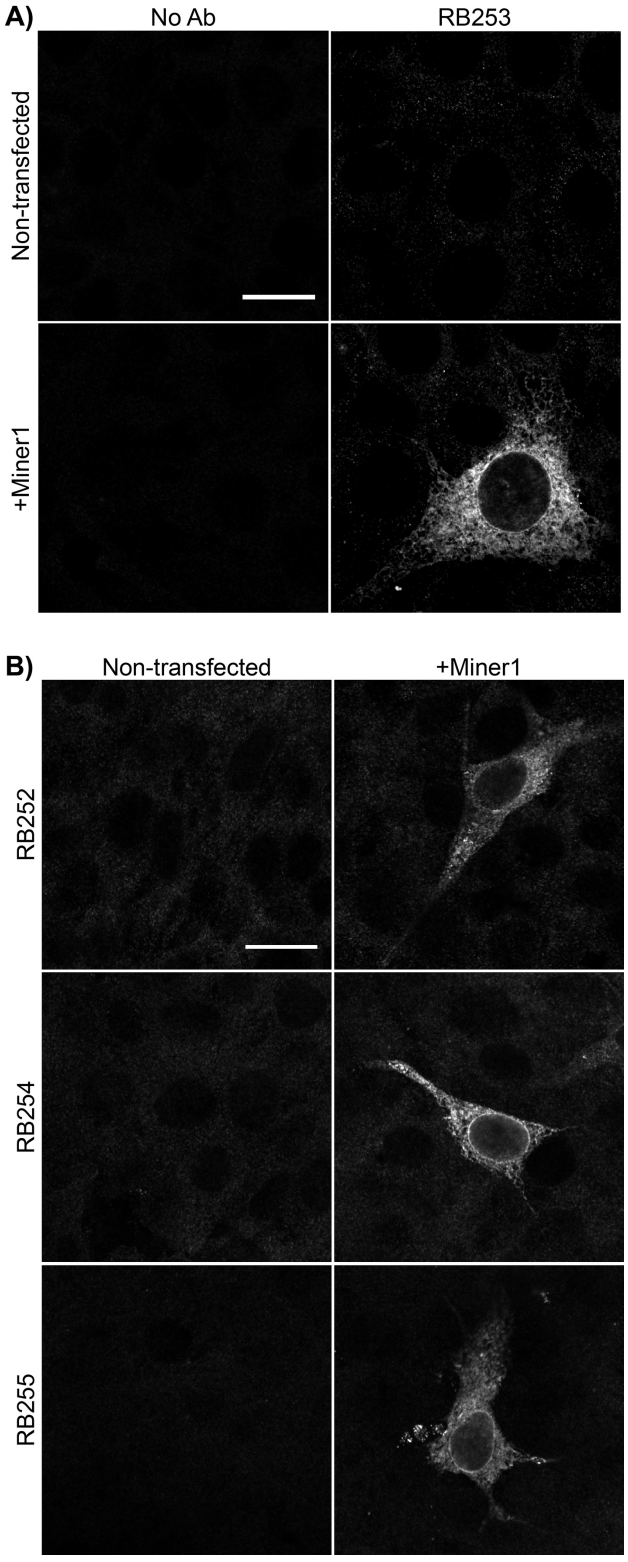

Fig. 1. MRB antibodies successfully label MEF cells over-expressing the Miner1 protein. No labelling was seen when the primary antibody was omitted (No Ab panel in *A*), and a very faint signal (corresponding to endogenous Miner1) can be detected in non-transfected cells. Scale bar: 10 μm.