LRRC32 monoclonal antibody (Plato-1)

LRRC32 (leucine rich repeat containing 32; also known as GARP or Garpin; Glycoprotein A repetitions predominant) is a glycoprotein expressed on the cell surface of megakaryocytes, platelets and activated regulatory T (Treg) cells.

This antibody is covered by our Worry-Free Guarantee.

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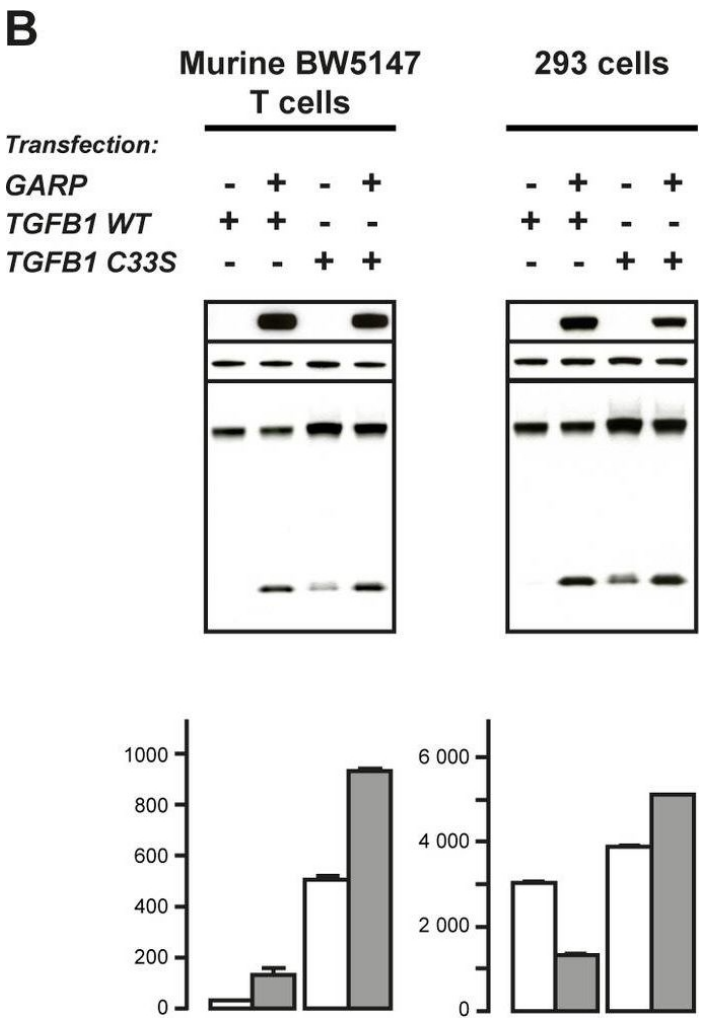
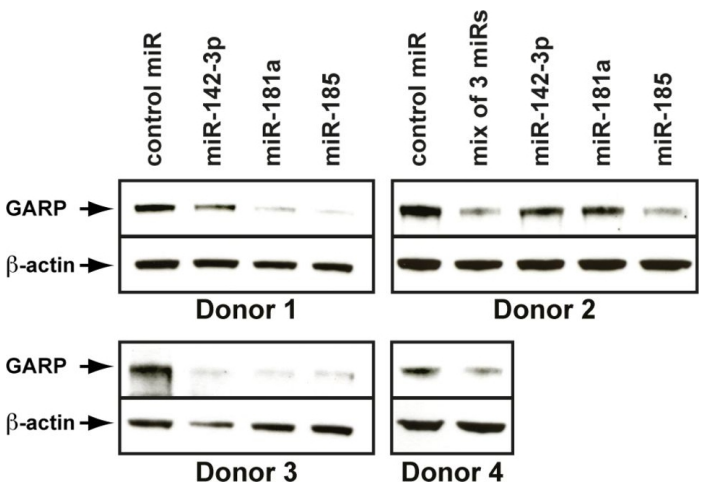
ALX-804-867-C100	100µg

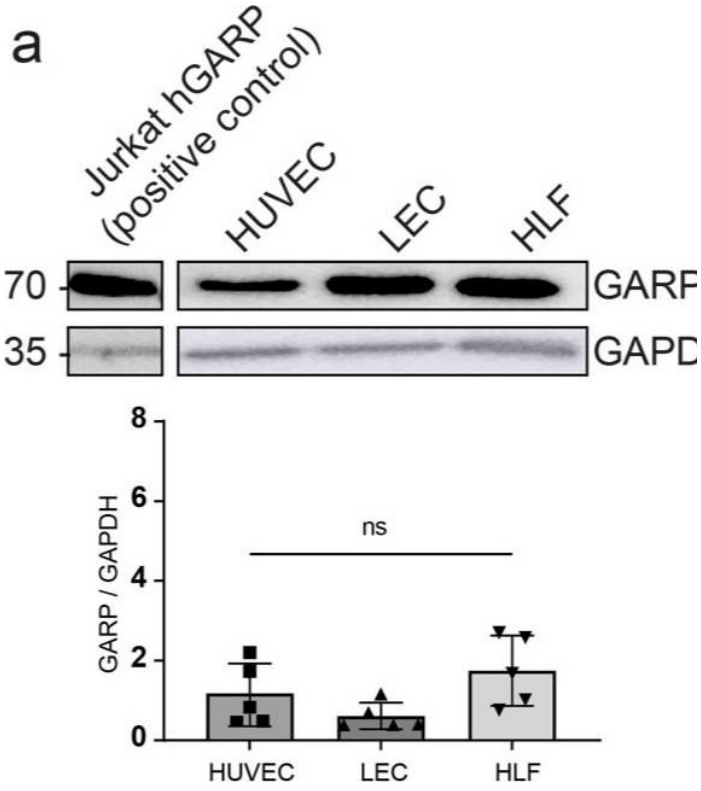
Manuals, SDS & CofA View Online »



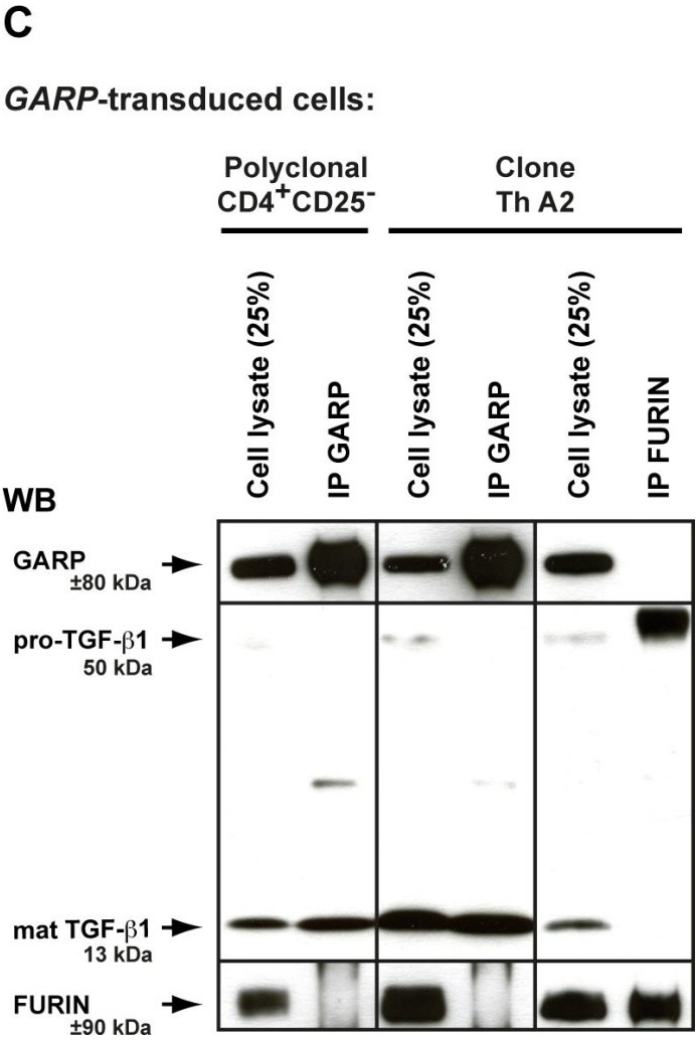
GARP increases cleavage of the pro-TGF-β1 precursor and secretion of latent TGF-β1 in T lymphocytes.Cell lysates were analyzed by WB after SDS-PAGE under reducing conditions with antibodies against GARP, β-actin and a C-terminal epitope of the TGF-β1 peptide (top panels). Supernatants were treated or not with acid and analyzed by ELISA to measure concentrations of total (latent + active) and active TGF-β1, respectively (bottom panels). Total TGF-β1 detected in the acid-treated samples corresponds to latent TGF-β1 because no active TGF-β1 was detected in the non-treated samples. Values represent means of duplicates + SD. A. Analysis of human T cell lines transduced or not with lentiviruses coding GARP or GFP. T cells were left resting (Rest) or stimulated for 24 hours with anti-CD3/CD28 antibodies (Stim) in serum-free medium. B. Analysis of stable clones of murine BW5147 T cells and 293 cells transiently transfected with GARP and WT or C33S mutant TGFB1. Untransfected BW5147 and 293 cells express low levels of endogenous TGF-β1 that are not detectable by WB in these conditions (not shown). By comparison to WT, transfection of mutant C33S results in increased production of total TGF-β1 (pro- + mature), as previously described [49].



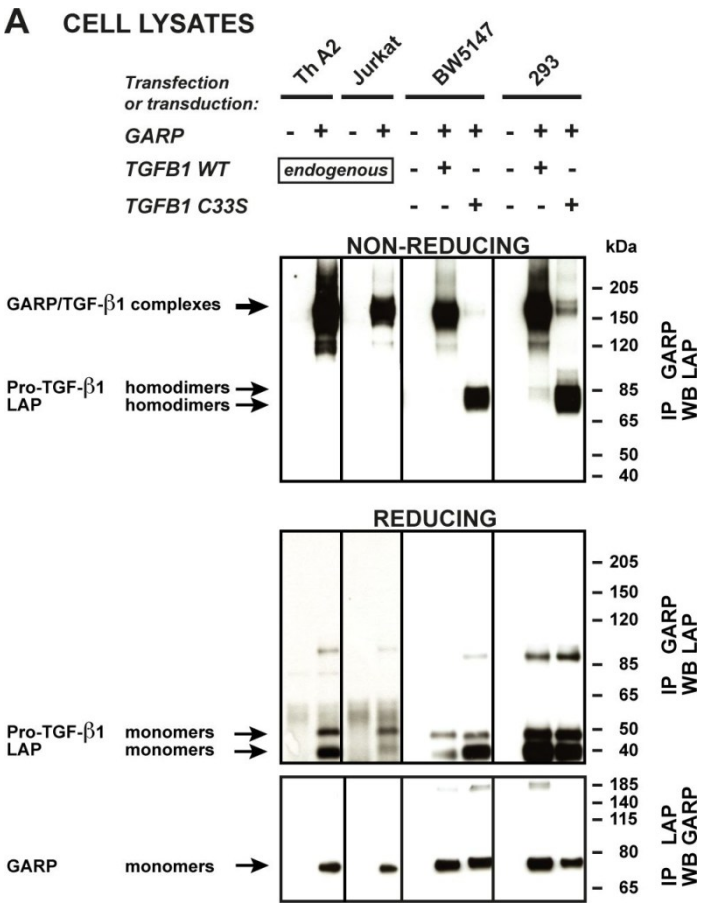
Endogenous GARP levels in Tregs are reduced after transfection of miR-181a, miR-142-3p and miR-185 mimics.Polyclonal CD4+CD25+CD127lo populations were purified from human PBMCs and amplified invitro. Amplified cells were electroporated with the indicated miRNA mimics and stimulated 6 hours later with anti-CD3/CD28 antibodies. Cell lysates were collected 24 hours later and analyzed by WB with anti-GARP and anti-β-ACTIN antibodies.



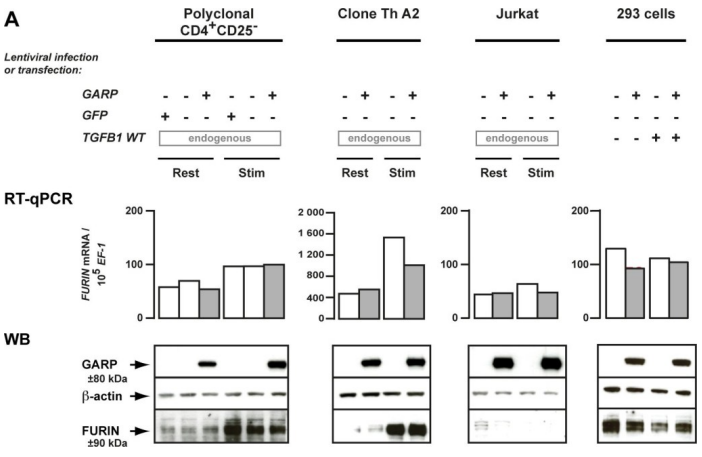
Evaluation of GARP in HUVEC, LEC, and HLF cells cultured under basal conditions. (a) Western blot analysis of GARP expression. The blot is a representative blot out of 4 independent experiments. The bar graph shows the quantification of GARP protein levels relative to the GAPDH protein signal (GARP/GAPDH signals) (n = 4, means ± SD, n.s. determined by one-way ANOVA). (b) Flow cytometry analysis of GARP at the surface of primary cells. Jurkat cells overexpressing GARP (Jurkat−hGARP) were used as a positive control. The isotype control is represented in grey, and the positive signal is depicted in red as a percentage of the maximum. The relative MFI of GARP in flow cytometry is represented with a bar graph (n ≥ 3, means ± SD, n.s., no significance, determined by one-way ANOVA).



GARP does not increase FURIN expression or activity, and does not co-immunoprecipitate with FURIN.A. Expression of FURIN mRNA and protein were analyzed by RT-qPCR and WB in the human cells described in Figure 2. B. FURIN activity was measured 24 hours after transfection of 293 cells. Lysates of transfected cells were incubated with a FURIN fluorogenic substrate directly (top panel), or after capture on plastic-coated anti-FURIN antibody (bottom panel), to measure FURIN-like or FURIN specific activity, respectively. Graphs show mean fluorescence intensity at the indicated time (min) after addition of the substrate. The FURIN inhibitor Dec-RVKR-CMK was added to one condition to verify the specificity of the assay. C. Lysates of cells described in Figure 2 were immunoprecipitated with anti-GARP (IP GARP) or anti-FURIN (IP FURIN) antibodies. Immunoprecipitation products or total cell lysates (25% of input used for IPs) were analyzed by WB with anti-GARP, anti-TGF-β or anti-FURIN antibodies, as indicated.



Disulfide-linked GARP/TGF-β1 complexes are released in the supernatant of T cells, but not 293 cells.A. Cells described in Figure 2 were lysed and immunoprecipitated (IP) with anti-GARP or anti-LAP antibodies. IP products were submitted to SDS-PAGE under non-reducing or reducing conditions, followed by WB with anti-LAP antibodies (top and middle panels), or anti-GARP antibodies (bottom panels). Pro-TGF-β1 and LAP homodimers in the top panels are not clearly resolved, but can be distinguished better with longer migrations or higher concentrations of polyacrylamide. The +/- 85-90 kDa bands that appear in the middle panel correspond to non-specific bands, or to incompletely reduced pro-TGF-β1. B. Cells (2×106/ml for murine and human T cells, 2.5×105/ml for transfected 293 cells) were incubated in serum free medium during 24 hours. Different cell concentrations were used to adjust for the different amounts of secreted TGF-β1 (see Figure 2). Human Th A2 and Jurkat cells were stimulated with anti-CD3/CD28 antibodies to increase secretion. Supernatants (0.5-10 µl) were analyzed by WB under non-reducing conditions with anti-GARP and anti-LAP antibodies. * Band that also appears when the secondary anti-IgG2b-HRP antibody is used alone (without anti-GARP antibody), due to cross reactivity against the anti-CD3/CD28 antibodies used for T cell stimulation.



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Handling & Storage

Use/Stability	Stable for at least 1 year after receipt when stored at -20°C.
Handling	For long term storage keep unconjugated antibody at -20°C. Avoid freeze/thaw cycles. Protect from light.
Short Term Storage	+4°C
Long Term Storage	-20°C
Shipping	Blue Ice

Regulatory Status

RUO - Research Use Only

Product Details

Alternative Name	Leucine-rich repeat-containing protein 32, GARP, Garpin, Glycoprotein A repetitions predominant
Application	ELISA, IP, WB
Application Notes	Cited applications include flow cytometry.
Clone	Plato-1
Formulation	Liquid. In PBS containing 50% glycerol and 0.02% sodium azide.
Host	Mouse
Immunogen	Recombinant human LRRC32 (aa 20-627). Detects a band of ~100kDa (Fc Fusion ALX-522-117) by Western blot. Native protein is ~72kDa.
Isotype	IgG2b
Purity Detail	Protein G-affinity purified.
Recommendation Dilutions/Conditions	Immunoprecipitation (1:200)Western Blot (1:1,000)Suggested dilutions/conditions may not be available for all applications.Optimal conditions must be determined individually for each application.

Source	Purified from concentrated hybridoma tissue culture supernatant.
Species Reactivity	Human, Mouse
Technical Info / Product Notes	1 test means: 1µl of MAb is used to stain 500’000 cells in a sample volume of 50µl.
UniProt ID	Q14392
Worry-free Guarantee	**This antibody is covered by our Worry-Free Guarantee .**

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