A seventeen residue sequence from the MMP-9 hemopexin domain binds α4β1 integrin and inhibits MMP-9-induced functions in chronic lymphocytic leukemia B cells*

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Running title: *α4β1 integrin binding site in PEX9 as target in B-CLL*

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Supplemental Figure S1

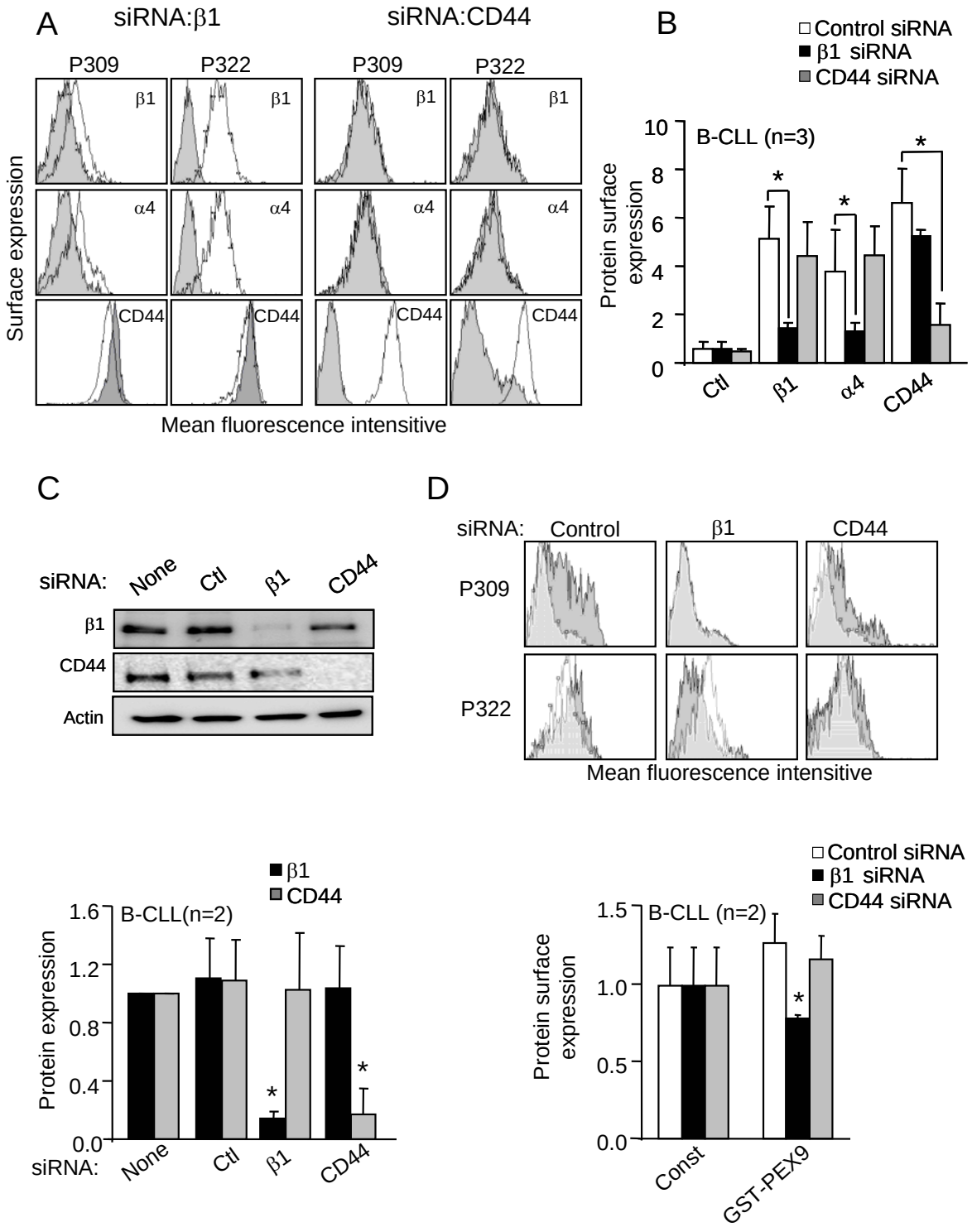

FIGURE S1. **Efficiency of B-CLL cell transfection with β1 integrin subunit or CD44 siRNA and effect on GST-PEX9 binding.** (A) B-CLL cells were transfected with control (Ctl), β1 or CD44 siRNA and analyzed by flow cytometry. Dark grey areas represent β1 or CD44 siRNA-transfected cells and white areas, control siRNA-trasfected cells. Two representative samples out of the three studied are shown. Quantitative values represent the average of the three patients analyzed (11, 17, 18), after normalizing constitutive values to 1. (B) Two of the samples used in (A) (patients 11 and 18) were lysed after 16 h (β1) or 24 h (control, CD44) upon transfection and lysates analyzed by western blotting. Representative results from patient 18 are shown. Quantitative values are the average of the two patients studied after normalizing untransfected cell values (None) to 1. (C) B-CLL cells (samples 11 and 18) transfected with the indicated siRNA were incubated for 30 min with or without 0.6 μM GST-PEX9, and analyzed by flow cytometry using anti–MMP-9 pAbs. Dark grey areas represent bound GST-PEX9; white areas, constitutive (pro)MMP-9 expression; light grey areas, overlapped regions of the two above. Average values of the two samples studied, after normalizing constitutive (Const.) values to 1 are also shown. *P ≤0.05.

Supplemental Figure S2

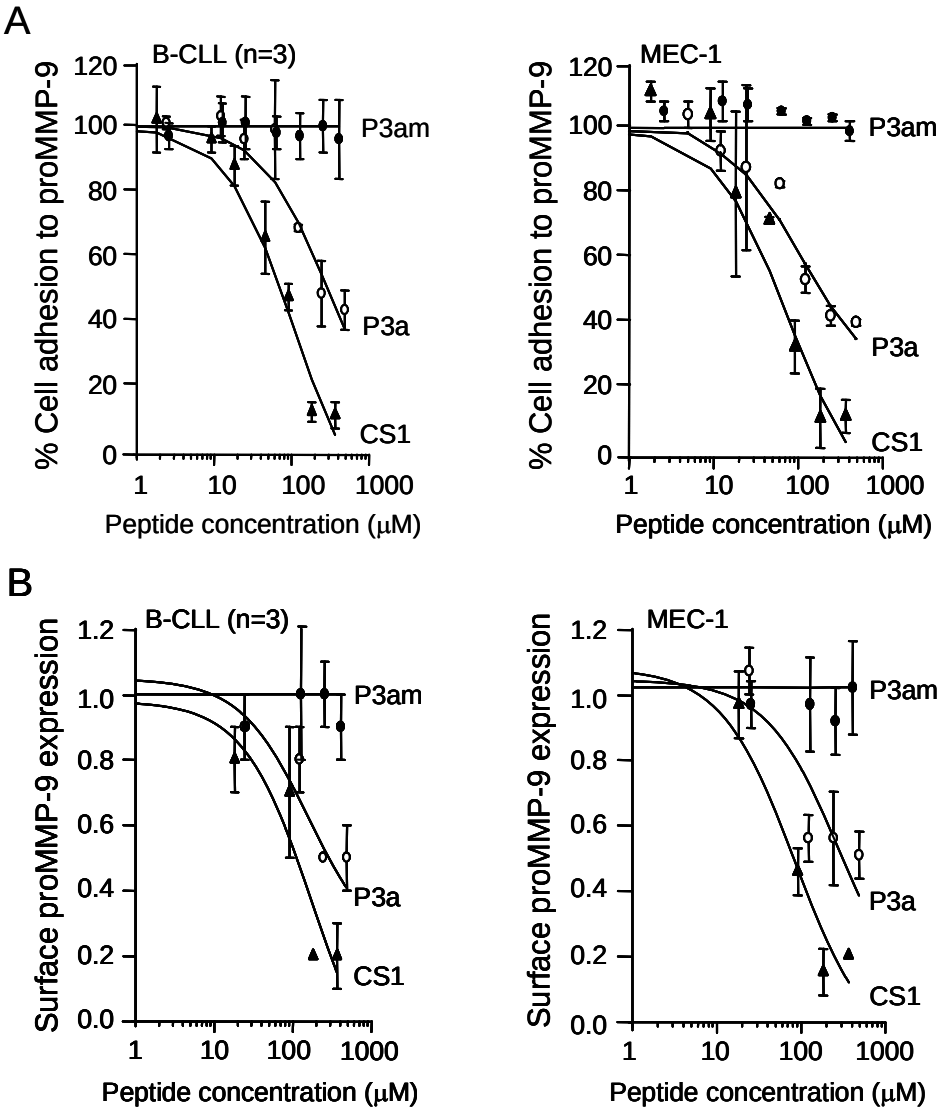

FIGURE S2. **Peptide dose-response inhibition of cell adhesion and soluble binding to proMMP-9.** (A) BCECF-AM-labeled primary B-CLL cells (patients 10, 11, 20) or MEC-1 cells (3 different experiments) were incubated (30 min, 37°C) with the indicated concentrations of P3a, P3am or CS1 peptides, and added to wells coated with 0.12 μM proMMP-9. After 60 minutes at 37°C, attached cells were quantitated using a fluorescence analyzer. Adhesion values in the absence of peptides were normalized to 100. (B) The same B-CLL samples listed in (A) or MEC-1 cells were treated for 30 min with the indicated peptide concentrations and incubated with 0.12 μM soluble proMMP-9. After 30 min, binding was analyzed by flow cytometry using an anti-MMP-9 antibody. Binding in the absence of peptides was normalized to 1. Average values ± std are shown. IC50 values for (A) and (B) were calculated using the SigmaPlot program.