SUPPLEMENTARY FIGURES

Fig. S1. Affinity purification of FLAG-tagged collagen VII with M2-agarose. To demonstrate the specificity of the affinity purification by M2-agarose, conditioned medium of cells transfected with a cDNA for either FLAG- or His-tagged collagen VII was applied into the M2-agarose column, and the flow-through, washing and elution fractions were analyzed by immunoblotting with antibodies to the FLAG-tag (A: upper panel) or His-tag (A: lower panel). The lanes in the immunoblots (from left to right) show: the total amount of tagged collagen in the medium; unbound fraction not retained in the M2-agarose column; the first two washing steps preceding the elution; and the elution fraction. The blots demonstrate that FLAG-tagged collagen is bound to the column quite efficiently. In contrast, most of Histagged collagen passed the column directly, and residual amounts were completely rinsed off within the first two washing steps. No His-tagged collagen was found in the elution fraction. B: As a control, a 1:1 mixture of FLAG- and His-tagged collagen VII was applied to the M2-agarose column. This resulted in His-tagged molecules appearing in the flow-through (unbound) and washing fraction one. Importantly, under these conditions, where WT: mutant hybrid formation was impossible, no His-tagged collagen VII was visible in the elution fraction (bound).

Figure S1

