

SUPPLEMENTARY FIGURES

Supplementary Figure 1

(A) DNA-sequence of mini-gp130-ELP: normal print: cDNA coding for the LeB4-signal peptide, bold: cDNA coding for mini-gp130-ELP; Primer and restriction sites are indicated. The ATG start codon is underlined.

(B) Protein sequence of mini-gp130-ELP. Grey: LeB4-signal peptide; red: sgp130 domains 1-3; red, cursive: amino acid sequence determined by Edman-degradation of the mature purified mini-gp130-ELP; blue: c-myc-tag; green: 100 repeats of ELP; brown: ER-retention-signal.

Supplementary Figure 2

Protein stability of purified mini-gp130-ELP at 37°C and 4°C.

(A) 1 µg purified mini-gp130-ELP was diluted in 1 ml PBS and incubated for 48 h at 37°C. Aliquots of originally 10 ng mini-gp130-ELP were separated by SDS/PAGE and blotted onto a PVDF membrane. Proteins were detected with the c-myc specific antibody 9E10 and visualized by ECL detection. Lane 1+2: 0 h; lane 3+4: 12 h; lane 5+6: 24 h; Lane 7+8: 48 h.

(B) 1 µg purified mini-gp130-ELP was diluted in 1 ml PBS and incubated for 26 d at 4°C. Aliquots of originally 10 ng mini-gp130-ELP were separated by SDS/PAGE and blotted onto a PVDF membrane. Proteins were detected with a c-myc specific antibody and visualized by ECL detection. Lane 1: 0 d; lane 2: 7 d; lane 3: 19 d; Lane 4: 26 d.

Supplementary Figure 3

Expression of sgp130-variants in transgenic tobacco.

Leaves of transgenic lines were extracted in a mortar under liquid nitrogen in 50 mM Tris-HCl, 200 mM NaCl, 5 mM EDTA, 0.1% Tween 20, pH 8.0. The homogenate was centrifuged for 5 min at 4°C and 16,000 g. Proteins were separated on a 10% SDS polyacrylamide gel, blotted and c-myc-tag containing proteins were detected by Western blotting and ECL.

(A) Lane 1: 40 µg extract of sgp130Fc-ELP transgenic tobacco plants; lane 2: 40 µg extract of sgp130Fc transgenic tobacco plants; lane 3: 40 ng c-myc-tagged scFv control protein. M: molecular mass marker (kDa).

(B) Lane 1: 5 µg extract of mini-gp130-ELP transgenic tobacco plants; lane 2: 5 ng control protein; lane 3: 10 ng c-myc-tagged single chain Fv control protein; lane 4: 5 µg extract of mini-gp130-ELP transgenic tobacco plants. M: molecular mass marker (kDa).

Supplementary Figure 4 Calibration of size exclusion chromatography.

(A) For calibration the high molecular mass standard from Amersham Pharmacia Biotech was used. Peak 1: Thyroglobin 669 kD; Peak 2: Ferritin 490 kDa; Peak 3: Katalase 232 kDa; Peak 4: Aldolase 158 kDa.

(B) The known molecular masses of the purchased standard proteins and their respective elution volumes were subject to linear regression.

Supplementary Figure 5 Absorption spectra of pure mini-gp130-ELP

(A) The absorption spectrum of purified mini-gp130-ELP was recorded in the range of 240-320 nm.

(B) The protein concentration of purified mini-gp130-ELP (after inverse transition cycling and size exclusion chromatography) was calculated as indicated from the absorption at 280 nm.

Supplementary Figure 1

A

1 ATGGCTTCCA AACCTTTTCT ATCTTTGCTT TCAC~~TT~~TCCT TGCTTCTCTT

Primer: 5'gp130-Δsignal →

AflIII

51 TACAAGCACA TGT~~TT~~AGCAG AGCTGCTGGA TCCTTGCGGC TATATCTCCC
101 CTGAGTCTCC TGTGGTGCAG CTGCATTCTA ACTTCACCGC CGTGTGTGTG
151 CTGAAGGAAA AGTGCATGGA C~~T~~ACTTCCAC GTGAACGCCA ACTACATCGT
201 GTGGAAAACC AACCACTTCA CCATCCCCAA GGAGCAGTAC ACCATCATCA
251 ACCGGACCGC TTCTTCTGTG ACCTTCACCG ATATCGCCTC CCTGAATATC
301 CAGCTGACCT GCAACATCCT GACCTTTGGA CAGCTGGAGC AGAATGTGTA
351 CGGCATCACC ATCATCTCTG GCCTGCCTCC AGAGAAGCCT AAGAACCTGT
401 CCTGCATCGT GAATGAGGGC AAGAAGATGA GGTGTGAGTG GGATGGCGGC
451 AGAGAGACAC ATCTGGAGAC CAACTTCACC CTGAAGTCTG AGTGGGCCAC
501 CCACAAGTTT GCCGACTGCA AGGCCAAGAG AGATACCCTT ACCTCTTGCA
551 CCGTGGACTA CTCCACCGTG TACTTCGTGA ACATCGAGGT GTGGGTGGAG
601 GCTGAGAATG CTCTGGGCAA GGTGACCTCT GACCACATCA ACTTCGACCC
651 CGTGTACAAG GTGAAGCCTA ACCCTCCTCA CAACCTGTCC GTGATCAACT
701 CTGAGGAGCT GTCCTCTATC CTGAAGCTGA CCTGGACCAA CCTTCCATC
751 AAGTCCGTGA TCATCTGAA GTACAACATC CAGTACAGGA CCAAGGATGC
801 TTCTACCTGG TCTCAGATCC CTCTGAGGA TACCGCTTCC ACCAGATCCA
851 GCTTCACAGT GCAGGACCTG AAGCCTTTTA CCGAGTACGT GTTCAGGATC
901 CGGTGCATGA AGGAGGATGG CAAGGGCTAT TGGTCTGACT GGTCTGAGGA

Primer: 3'gp130-NaeI ←

NaeI

951 GGCTTCTGGC ATCACCTACG AGGACAGAGC CCGCGGACAA GCGGCCGAG
1001 AACAAAAACT CATCTCAGAA GAGGATCTGA ATGGGGCCGT CGAGATGGGC
1051 CACGGCGTGG GTGTTCCGGG CGTGGGTGTT CCGGGTGGCG GTGTGCCGGG
1101 CGCAGGTGTT CCTGGTGTAG GTGTGCCGGG TGTGGGTGTG CCGGGTGTG
1151 GTGTACCAGG TGGCGGTGTT CCGGGTGCAG GCGTTCGGG TGGCGGTGTG
1201 CCGGGCGTGG GTGTTCCGGG CGTGGGTGTT CCGGGTGGCG GTGTGCCGGG
1251 CGCAGGTGTT CCTGGTGTAG GTGTGCCGGG TGTGGGTGTG CCGGGTGTG
1301 GTGTACCAGG TGGCGGTGTT CCGGGTGCAG GCGTTCGGG TGGCGGTGTG
1351 CCGGGCGTGG GTGTTCCGGG CGTGGGTGTT CCGGGTGGCG GTGTGCCGGG
1401 CGCAGGTGTT CCTGGTGTAG GTGTGCCGGG TGTGGGTGTG CCGGGTGTG
1451 GTGTACCAGG TGGCGGTGTT CCGGGTGCAG GCGTTCGGG TGGCGGTGTG
1501 CCGGGCGTGG GTGTTCCGGG CGTGGGTGTT CCGGGTGGCG GTGTGCCGGG
1551 CGCAGGTGTT CCTGGTGTAG GTGTGCCGGG TGTGGGTGTG CCGGGTGTG
1601 GTGTACCAGG TGGCGGTGTT CCGGGTGCAG GCGTTCGGG TGGCGGTGTG
1651 CCGGGCGTGG GTGTTCCGGG CGTGGGTGTT CCGGGTGGCG GTGTGCCGGG
1701 CGCAGGTGTT CCTGGTGTAG GTGTGCCGGG TGTGGGTGTG CCGGGTGTG
1751 GTGTACCAGG TGGCGGTGTT CCGGGTGCAG GCGTTCGGG TGGCGGTGTG
1801 CCGGGCGTGG GTGTTCCGGG CGTGGGTGTT CCGGGTGGCG GTGTGCCGGG
1851 CGCAGGTGTT CCTGGTGTAG GTGTGCCGGG TGTGGGTGTG CCGGGTGTG
1901 GTGTACCAGG TGGCGGTGTT CCGGGTGCAG GCGTTCGGG TGGCGGTGTG
1951 CCGGGCGTGG GTGTTCCGGG CGTGGGTGTT CCGGGTGGCG GTGTGCCGGG
2001 CGCAGGTGTT CCTGGTGTAG GTGTGCCGGG TGTGGGTGTG CCGGGTGTG
2051 GTGTACCAGG TGGCGGTGTT CCGGGTGCAG GCGTTCGGG TGGCGGTGTG
2101 CCGGGCGTGG GTGTTCCGGG CGTGGGTGTT CCGGGTGGCG GTGTGCCGGG
2151 CGCAGGTGTT CCTGGTGTAG GTGTGCCGGG TGTGGGTGTG CCGGGTGTG
2201 GTGTACCAGG TGGCGGTGTT CCGGGTGCAG GCGTTCGGG TGGCGGTGTG
2251 CCGGGCGTGG GTGTTCCGGG CGTGGGTGTT CCGGGTGGCG GTGTGCCGGG
2301 CGCAGGTGTT CCTGGTGTAG GTGTGCCGGG TGTGGGTGTG CCGGGTGTG
2351 GTGTACCAGG TGGCGGTGTT CCGGGTGCAG GCGTTCGGG TGGCGGTGTG
2401 CCGGGCGTGG GTGTTCCGGG CGTGGGTGTT CCGGGTGGCG GTGTGCCGGG
2451 CGCAGGTGTT CCTGGTGTAG GTGTGCCGGG TGTGGGTGTG CCGGGTGTG
2501 GTGTACCAGG TGGCGGTGTT CCGGGTGCAG GCGTTCGGG TGGCGGTGTG
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Supplementary Figure 1

B

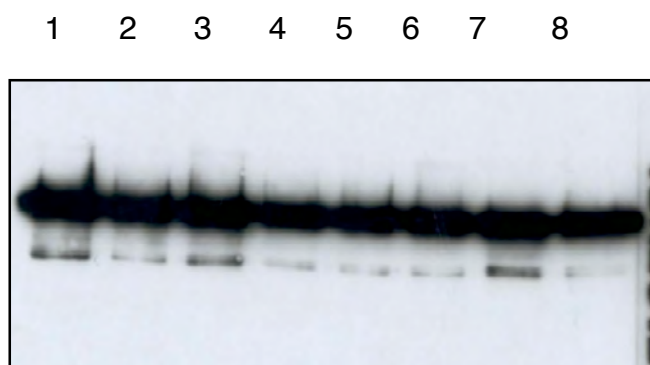
cleavage site of the signal peptide

LeB4-signal peptide ↓ **sgp130-ELP**

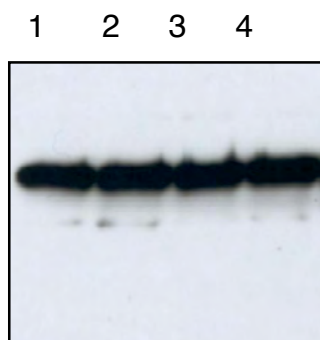
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LKEKCMDYFHVNANYIVWKTNHFTI**PKEQYTI**INRTASSVTF**TDIASLNI** 100
QLTCNILTFGQLEQNVYGITII**SGLPPEKPKNLS**CI**VNEGKMRCEWDGG** 150
RETHLETNFTL**KSEWATHKFADCKAKRDTPT**SCTVDY**STVYFVNIEVWVE** 200
AENALGKVTS**SDHINFDPVYKVKPNPPHNSV**INSEELSSIL**KLWTNPSI** 250
KSVIILK**YNIQYRTKDASTWSQIPPEDTAS**TRSSFT**VQDLKPFTEYVFRI** 300
RCMKEDGK**YWSDWSEEASGITYEDR**AGGQAA**AEQKLI**SEED**LNGAVEMG** 350
HG**VGPVGPVGP**GGG**VPAGVPGVGPVGPVGPVGPVGP**GGG**VPAGVPGGGV** 400
PGVGPVGPVGPGGG**VPAGVPGVGPVGPVGPVGPVGP**GGG**VPAGVPGGGV** 450
PGVGPVGPVGPGGG**VPAGVPGVGPVGPVGPVGPVGP**GGG**VPAGVPGGGV** 500
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PGGLAA**AEPKDEL***

Supplementary Figure 2

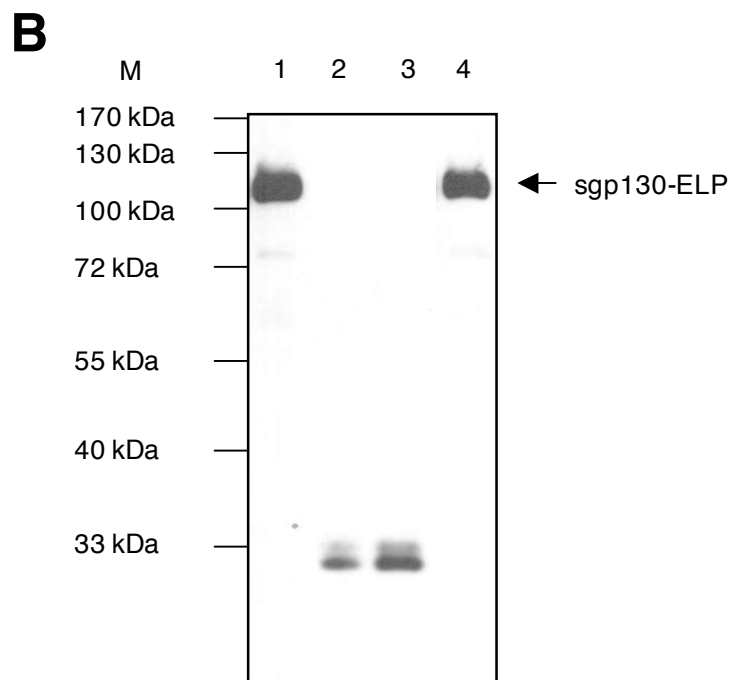
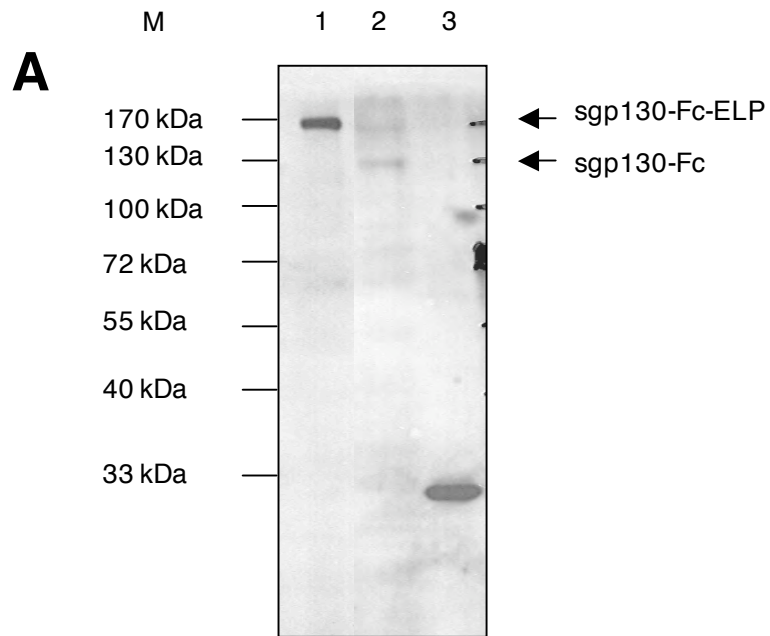
A



B

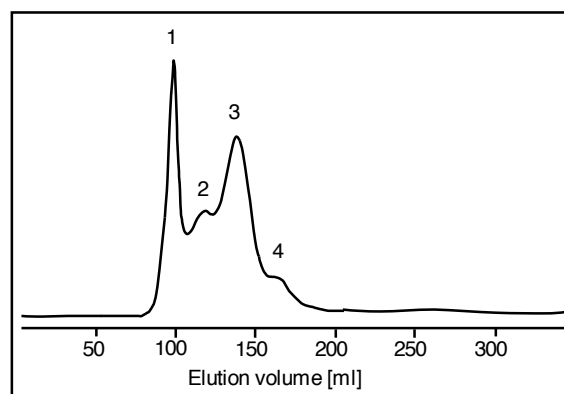


Supplementary Figure 3

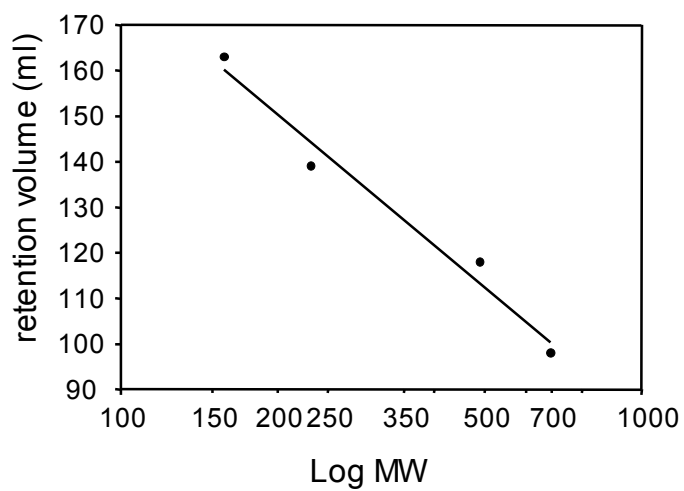


Supplementary Figure 4

A



B

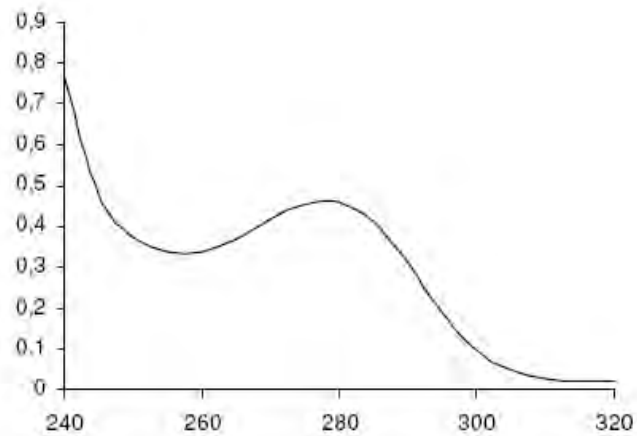


$$y = -95.5x + 370.1$$

$$r^2 = 0.973$$

Supplementary Figure 5

A



nm	Abs
240	0,766
245	0,478
250	0,374
255	0,336
260	0,339
265	0,37
270	0,417
275	0,455
280	0,458
285	0,413
290	0,316
295	0,199
300	0,098
305	0,048
310	0,029
315	0,022
320	0,021

B

$$c = \frac{0.458 \times 100,000 \text{ g/mol}}{64,890 \text{ l/cm} \times \text{mol} \times 1 \text{ cm}} = 0.705 \text{ mg/ml}$$