

SUPPLEMENTARY FIGURE/TABLE LEGENDS:

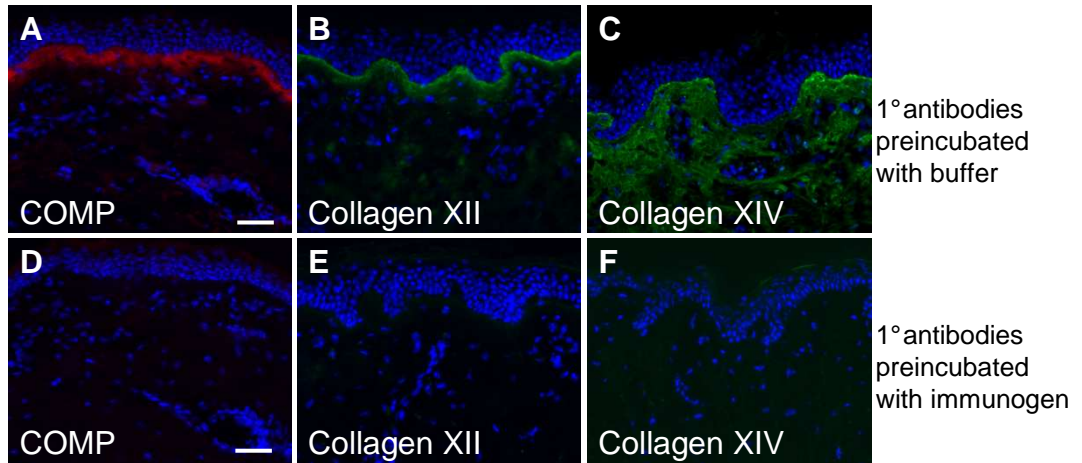
Figure S1: Specificity of primary antibodies directed to recombinant COMP, collagen XII and collagen XIV. A. Cryosections of human skin were stained with primary antibodies raised in rabbit against full-length murine COMP (A, D) or in guinea pig against fibronectin type III repeats 14-18 of human collagen XII (B, E) or in guinea pig against fibronectin type III repeats 5-8 of human collagen XIV (C, F). Before application to tissues, antibodies were either pre-incubated with buffer (A-C) or with the corresponding recombinant immunogen (20-40-fold excess, wt/wt) overnight at 4°C. Bar indicates 50 µm. B. Proteins extracted from human cartilage (10 µg) were separated by 7.5% SDS-PAGE under reducing conditions and immunoblotted with antibodies directed to murine COMP that had been preincubated with buffer (-) or with recombinant COMP (+) as described above. Proteins extracted from human skin (30 µg) were separated by 4-12% gradient SDS-PAGE under reducing conditions and immunoblotted with antibodies recognizing human collagen XII or XIV, preincubated with buffer (-) or with recombinant immunogens (+). The recombinant proteins used for pre-absorption were added in 40-fold excess (wt/wt) of antibodies. Ponceau staining of membranes served as loading control.

Figure S2: Specificity of secondary gold-conjugated antibodies. Specimens of human skin were incubated with either one of the three primary antibodies (rabbit antibodies recognizing murine COMP, guinea pig antibodies recognizing human collagen XII or human collagen XIV), followed by incubation in a combination of goat-anti rabbit antibodies (coupled to 10 nm gold particles) or goat-anti guinea pig antibodies (coupled to 6 nm gold particles). Gold particles were measured in random fields and the graph shows the frequency distribution of gold-conjugated antibodies. The size of the gold particles within the secondary conjugates '6 nm' and '10 nm' varies. To determine the overlap, they were spread onto formvar-coated grids (the same substrate used to support sections) and measured in photographs taken of randomly selected fields. All micrographs were collected at 150,000x and imported into Adobe Photoshop CS5. The microscope was calibrated using a carbon grating replica (Ted Pella Inc., order no. 603) and the calculated measurement scale was 250 pixels = 100 nm following import and enlargement in Adobe Photoshop.

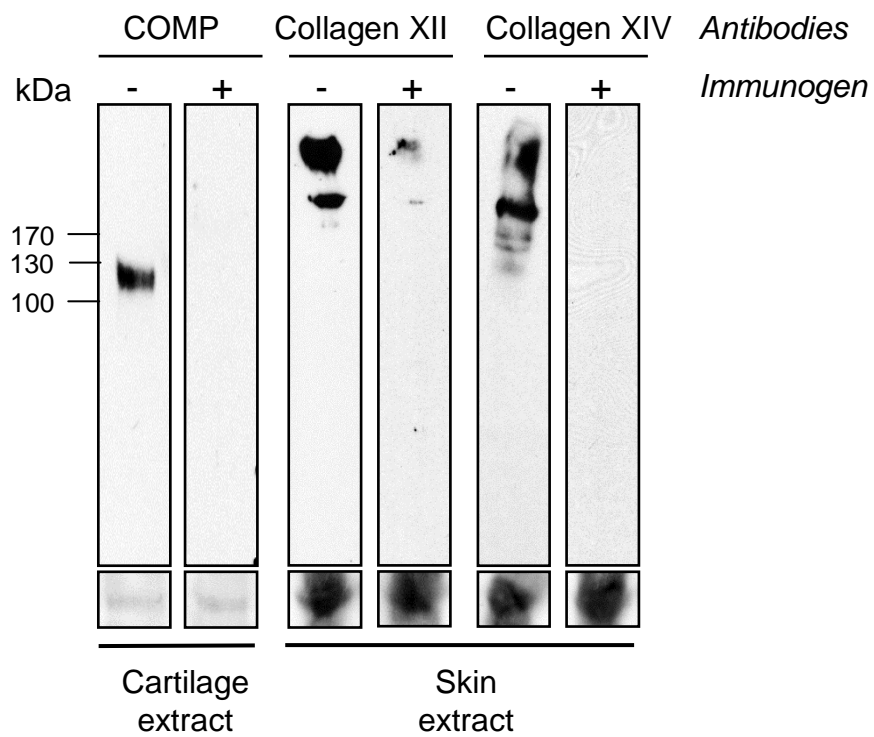
Table S1: List of PCR primers used for vector construction. The table provides the nucleotide sequences of primers used to amplify the recombinant full-length proteins and fragments. The nucleotide sequences displayed in capital letters represent the corresponding restriction sites. F stands for forward and R stands for reverse primers, respectively.

Supplementary Figure S1

A



B



Supplementary Figure S2

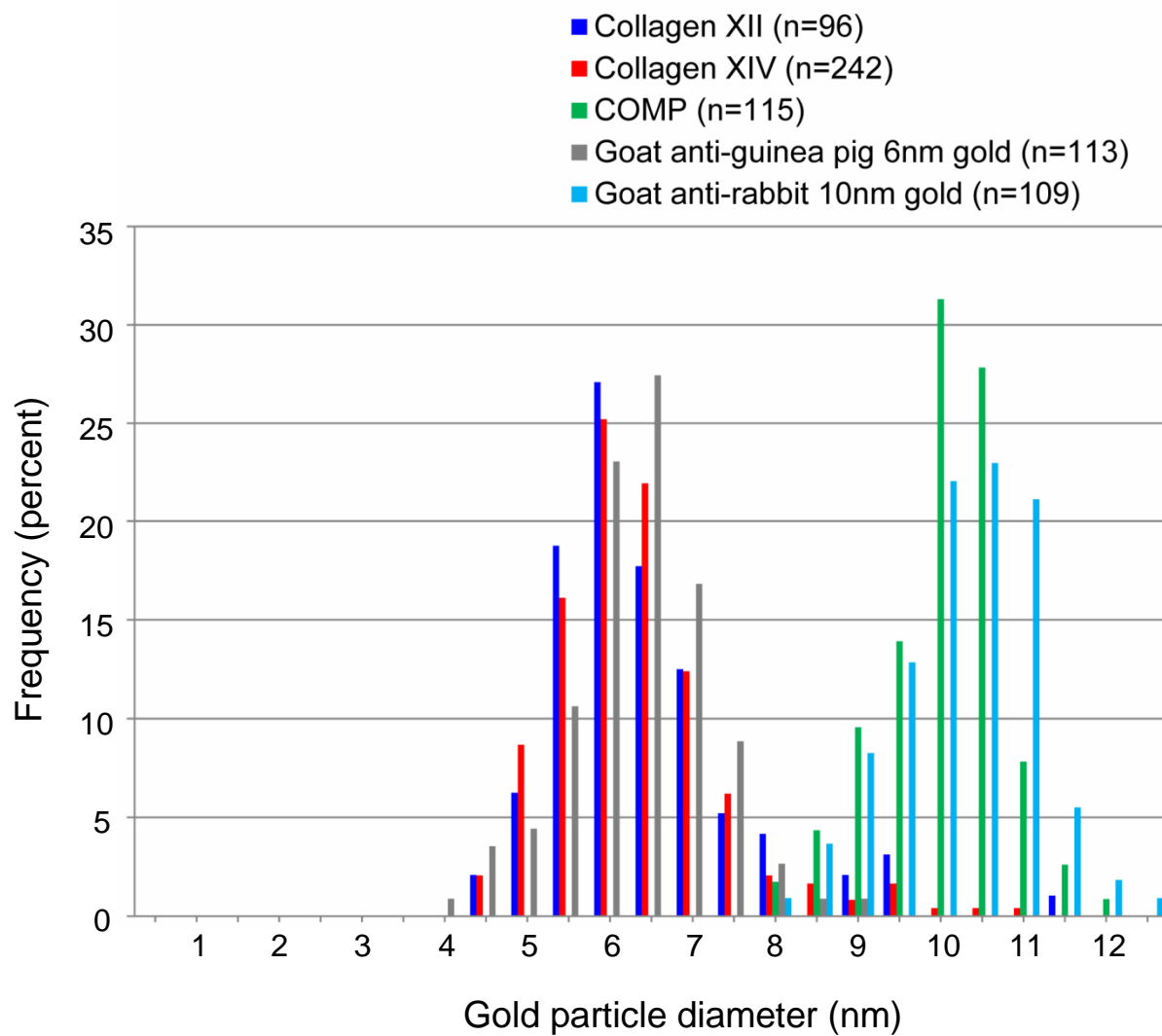


Table S1: List of PCR primers used for vector construction

Protein	Primers	Forward (f) /Reverse (r)	Sequence (5' → 3')	Restriction sites
XII-lsv	P145	f	caaGCTAGCgaagaccacctccgacttg	Nhe I
	P146	r	ttgCTCGAGttacacATGCATagtggggggcc	Xho I, Nsi I
	P143	f	aaaGCTAGCgcatatgacacaacccggc	Nhe I
	P8	r	ttgGGATCCttagccggaacctggatagccttgc	Bam HI
XII-ssv	M854	f	caaGCTAGCgaaggatggattgtctcaccagag	Nhe I
	M893	r	caaGCGGCCGcttaggtcttcccctccggtcatc	Not I
	P143	f	aaaGCTAGCgcatatgacacaacccggc	Nhe I
	P144	r	caaGCGGCCGcttaagaTCCGGAttccaaagagac	Not I, Bsp
	P3	f	aaaGCTAGCcaggatggctacacatcaccagg	Nhe I
	P8	r	ttgGGATCCttagccggaacctggatagccttgc	Bam HI
Nt-XII	P145	f	caaGCTAGCgaagaccacctccgacttg	Nhe I
	T374	r	catCTCGAGctaagaagatagaaatgggtgc	Xho I
Mid-XII	T375	f	cacGCTAGCgggatggattgtctcaccag	Nhe I
	T376	r	tttACTCGAGTttatggatgtgtagccatcctga	Psp XI
Ct-XII	P3	f	aaaGCTAGCcaggatggctacacatcaccagg	Nhe I
	P8	r	ttgGGATCCttagccggaacctggatagccttgc	Bam HI
XIV	M850	f	cacGCTAGCcaagtggctccaccacaagg	Nhe I
	P148	r	cacGCGGCCGcttaggtCCTGCAGGacctgtttc	Not I, Sbf I
	P18	f	caaGCTAGCgtgcacaaggatggagttgatc	Nhe I
	P19	r	tttGGATCCttagatgcctgcgctccacagatcc	Bam HI
Nt-XIV	M850	f	cacGCTAGCcaagtggctccaccacaagg	Nhe I
	T377	r	cattcGTCGACTtacaattcatctcaattttctt	Sal I
Ct-XIV	P18	f	caaGCTAGCgtgcacaaggatggagttgatc	Nhe I
	P20	r	tttGGATCCttagtgtgtaatcattgtacgg	Bam HI
COMP	P987	f	acaGCTAGCcagatcccgtgggtggagacc	Nhe I
	P988	r	aggAGATCTctagactctctgcagccggtgac	Bgl II