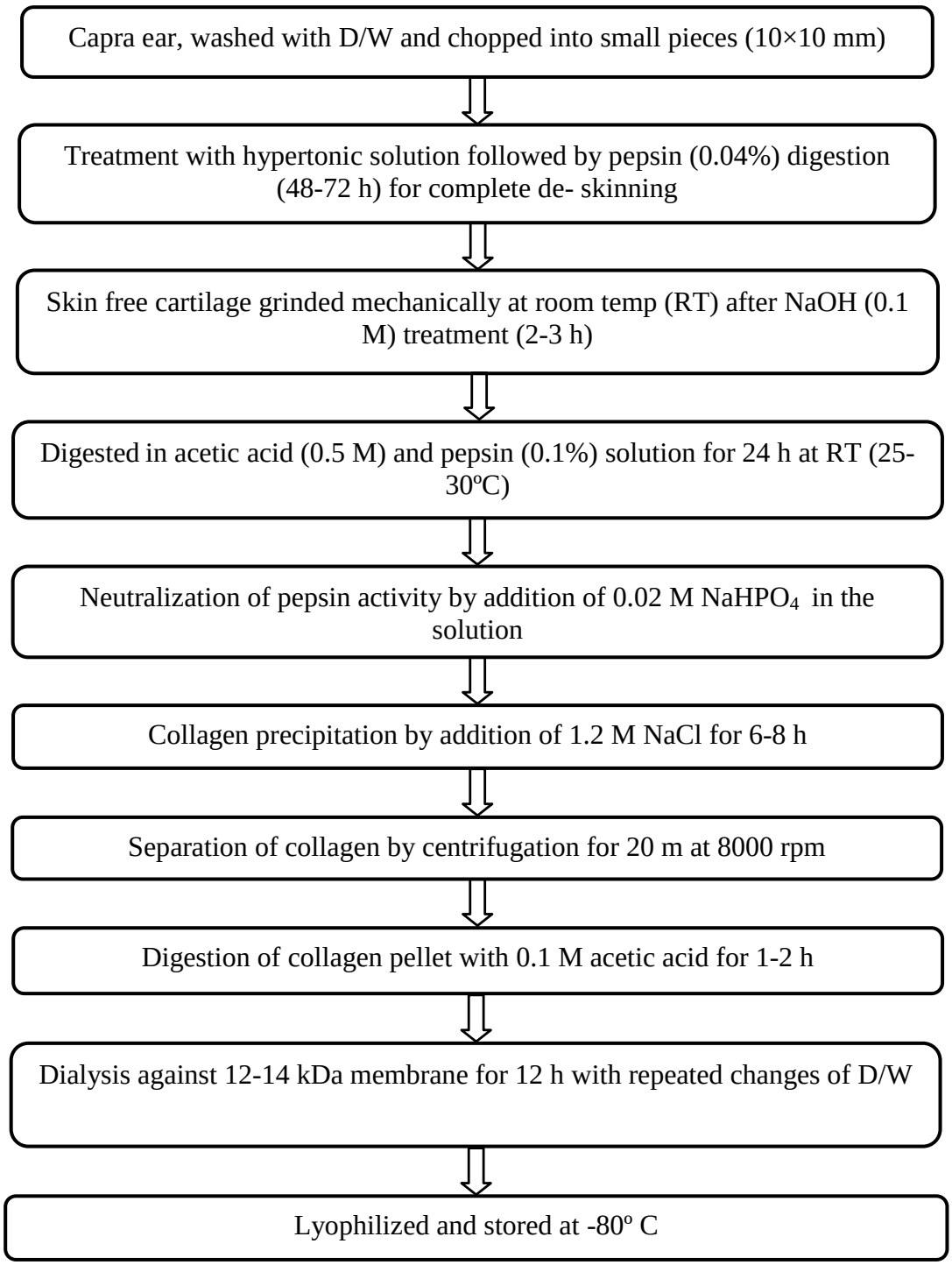
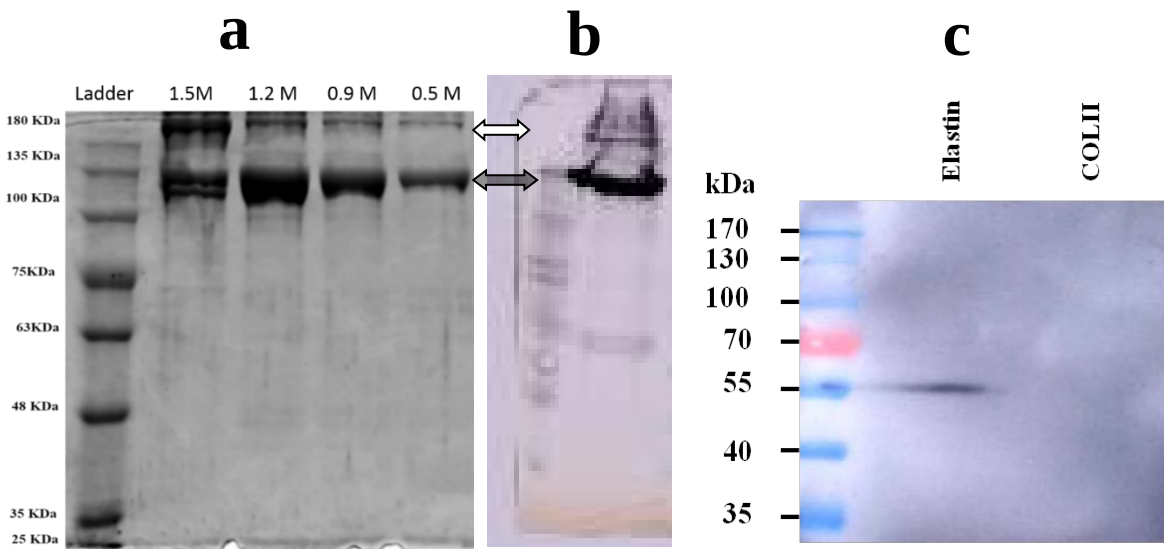
Supplementary

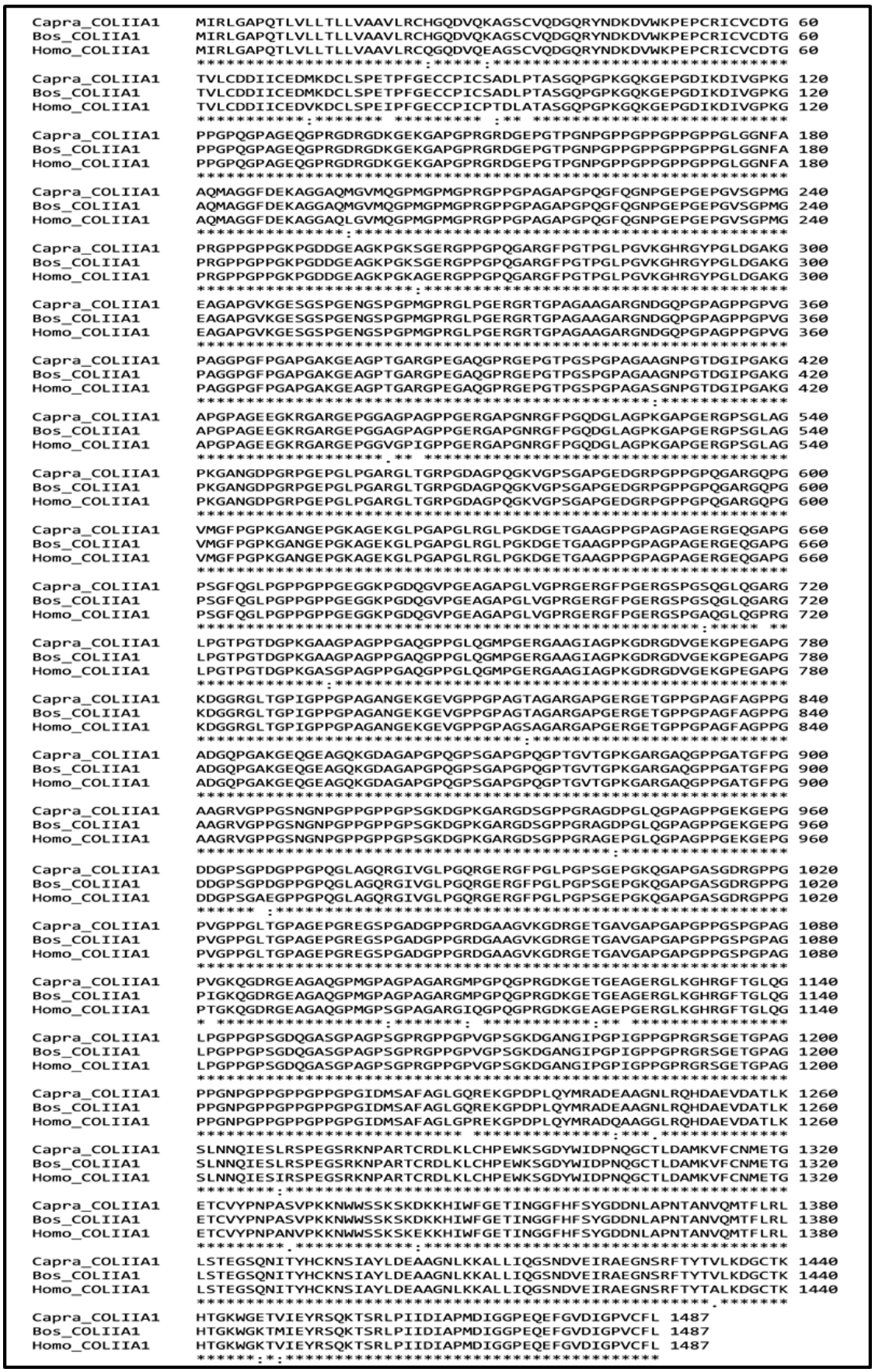
Isolation and mass spectrometry based hydroxyproline mapping of type II collagen derived from *Capra hircus* ear cartilage



Supplementary Figure 1. Schematic representation of collagen II isolation from Capra ear.

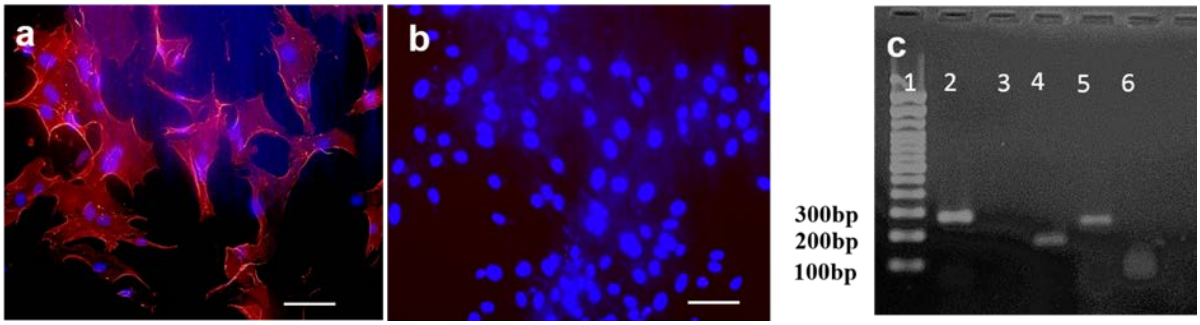


Supplementary Figure 2. Identification of purified COLII by SDS-PAGE and western blot analysis. (a) SDS-PAGE showing the isolated COLII with two prominent bands where filled arrow indicating α chain and open arrow indicating β chain, (b) α chain was identified using anti-COLII antibody using western blot analysis and (c) showing prominent band of bovine elastin (Sigma, USA) as a control and absence of band in *Capra* ear derived COLII indicating isolated protein was devoid of elastin.

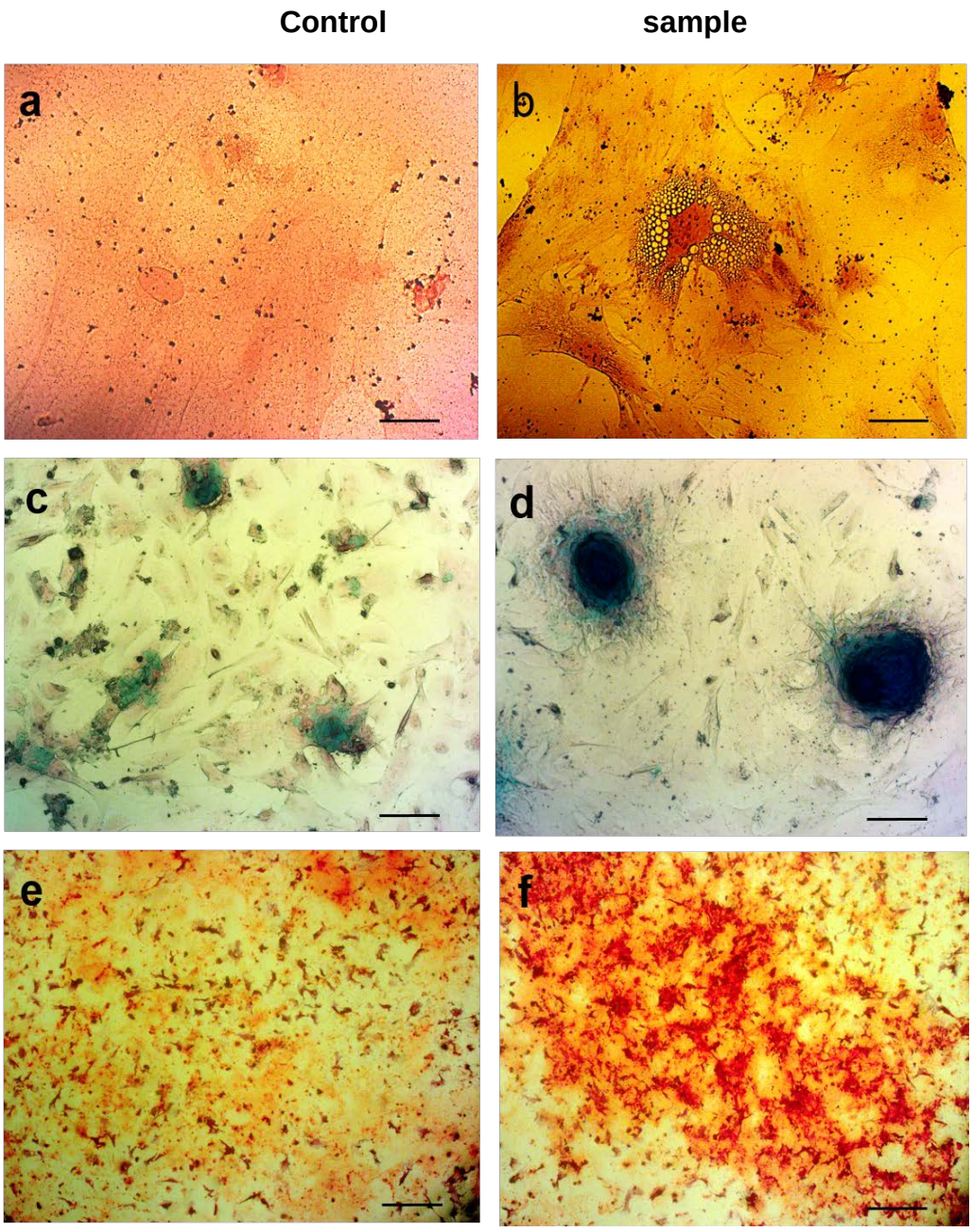


Supplementary Figure 3. Amino acid sequence alignment of COLIIA1 from *Capra hircus, Bos taurus* and *Homo sapiens* showing 98% identity.

Supplementary Figure 4. Highlighted amino acid sequences identified from Capra COLIIA1 by peptide mass finger printing using *Bos taurus* COLIIA1 as template sequence.



Supplementary Figure 5. Identification of isolated capra ADMSCs. Protein expression analysis showed (A) positive expression of CD 44 marker and (B) negative expression of CD 31 marker (pseudo colors, Scale bars: 100 μm); (C) RT-PCR study revealed expression of GAPDH (lane 2), CD 73 (lane 4), CD 90 (lane 5) and CD 105 (lane 6) and negative expression of CD 45 marker associated genes (lane 3) in reference to 100 bp ladder (lane 1).



Supplementary Figure 6. Assessment of trilineage differentiation potential of ADMSCs to adipogenic, chondrogenic and osteogenic lineage. (A-B) showing significant accumulation of lipid droplets by Oil Red O staining in cytoplasm after cells cultured under adipogenic differentiation media compared to control (Scale bars: 50 µm); (C-D) depicting cartilage micro-tissue formation as stained by Alcian Blue after cells cultured under chondrogenic differentiation media compared to control (Scale bars: 100 µm); (E-F) identified significant calcium deposition by Alizarin Red S staining after cells cultured under osteogenic differentiation media as compared to control (Scale bars: 500 µm).

Supplementary Table 1: Predictive values of percentage denaturation of COL II secondary structure

Temp. (^{o}C)	α-helix	β-strand	% of denature	% of intactness
25	53.23	8.04	0	100
30	53.26	7.68	0	100
35	52.49	7.93	1.4	98.60
40	50.26	8.25	5.58	94.42
41	44.02	8.74	17.31	82.69
42	37.61	9.71	29.35	70.65
43	**25.98**	**11.68**	**51.2**	**48.80**
44	19.02	12.20	64.27	35.73
45	17.38	12.07	67.35	32.65
46	17.73	11.99	66.7	33.30
47	16.16	12.07	69.55	30.35
48	16.12	12.35	69.72	30.28
49	15.69	12.11	70.53	29.47
50	15.33	12.47	77.21	28.79

Supplementary Table 2: Comparative analysis of amino acid residues in Capra ear cartilage derived COLII with Chicken samples as reported by Cao *et al*., 2008[16]

Amino Acid	COL II (Cao *et al.*, 2008)(residues/1000 residues)	Capra ear cartilage derived COL II in present study (residues/1000 residues)
Glycine	313	302
Alanine	102	105
Glutamic acid	94	77
Proline	94	99
Hydroxyproline	118	118
Arginine	53	55
Leucine	31	29
Histidine	4	5
Lysine	15	19
Phenylalanine	15	21
Isoleucine	13	14
Methionine	2	10
Valine	22	25
Serine	25	27
Threanine	30	28
Aspartic Acid	47	41
Tyrosine	5	5
Cysteine	17	20

Supplementary Table 3: Comparisons of COLIIA1 MASCOT score with other proteins

Protein	Mass	Score
COLIIA1_Bos taurus	134858	82
COLIIA1_Bos mutus	134842	82
COLIIA1_Bos indicus	142919	73
COLIIA1_Pantholops hodgsonii	134852	76
COLIIA1_Bubalus bubalis	134844	76
COLIIA1_Homo sapiens	142782	56

Supplementary Table 4: HPLC solvent system for amino acid analysis

A-Buffer (0.01M Na_2HPO_4); B- Acetonitrile, Excitation:230, Emission: 460						
Time	**A%**	**B%**	**C%**	**D%**	**Flow rate ml/min**	**Max. Pressure limit (Bar)**
0.00	91.0	9.0	0.0	0.0	1.000	400.00
2.00	80.0	20.0	0.0	0.0	1.000	400.00
35.00	68.0	32.0	0.0	0.0	1.000	400.00
55.00	20.0	80.0	0.0	0.0	1.000	400.00
57.00	91.0	9.0	0.0	0.0	1.000	400.00

Supplementary Table 5: Designed Primers for RT-PCR

Genes	Sequence	Annealing Temp.(ºC)	Product Size (bp)	References
GAPDH-FP	*GGCGCTGCCAAGGCCGTGGGCAAG*	73.0	273	KEGG_100860743
GAPDH-RP	*GGCAATGCCAGCCCCAGCATCGAAGG*			
CD90 -FP	*GCACCATGAACCCTACCATC*	55	241	Mohamad-Fauzi et al., 2015 [22]
CD90 -RP	*TTGGTTCGGGAGCTGTATTC*			
CD73-FP	*CTGAGACACCCGGATGAGAT*	52	160	
CD73-RP	*ACTGGACCAGGTCAAAGGTG*			
CD105-FP	*AGATGCCAACATCACACAGC*	49	129	
CD105-RP	*TCCAGACGAAGGAAGATGCT*			
CD45-FP	*GGGAGGAGGGAAAGCAAACC*	55	146	
CD45-RP	*GCAGCTCTTCCCCATTCCAG*			
COLIIA1-FP	*GGCGCTGCCAAGGCCGTGGGCAAG*	64	269	KEGG_100860743
OLIIA1-RP	*GGCAATGCCAGCCCCAGCATCGAAGG*			