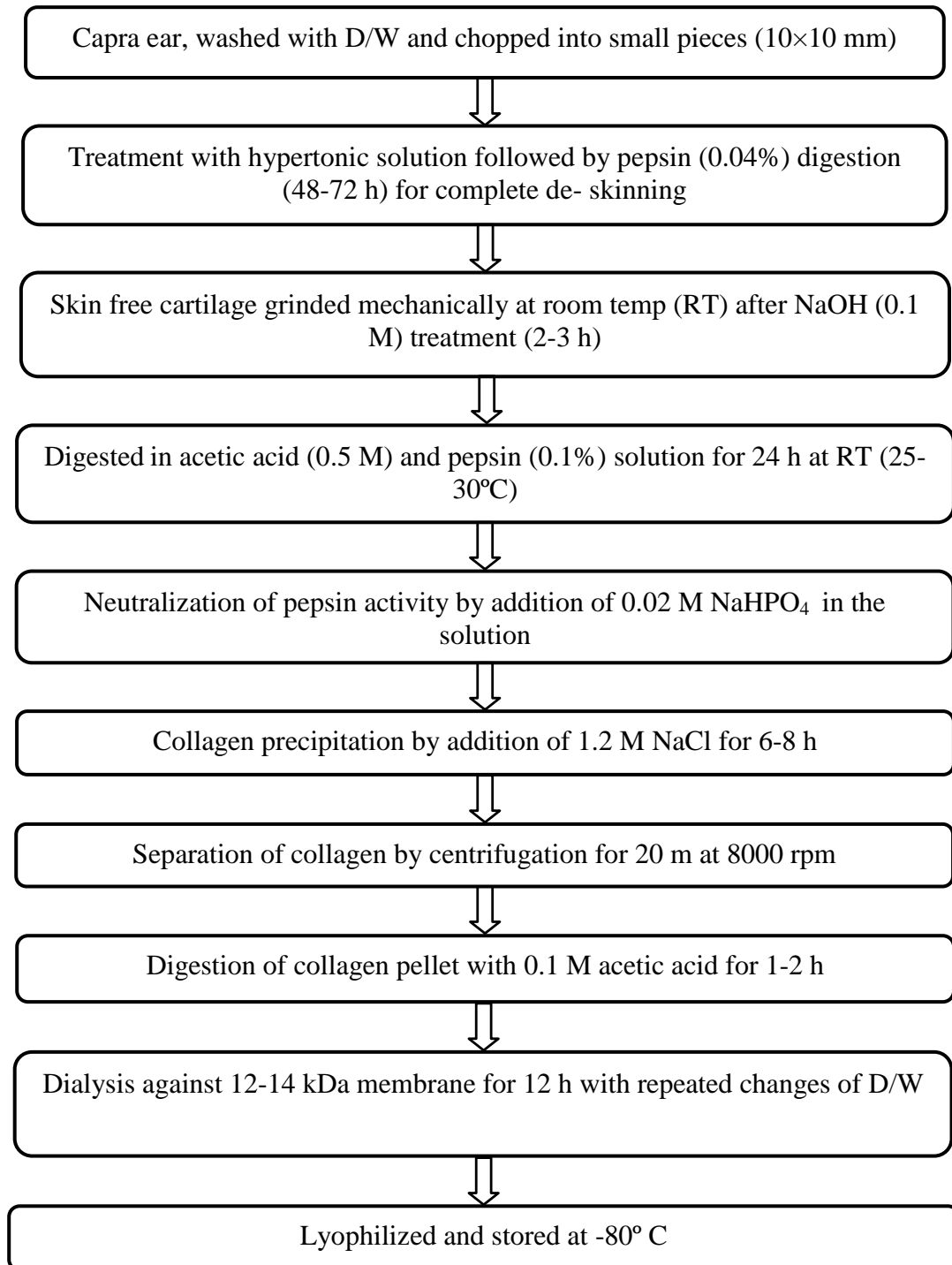
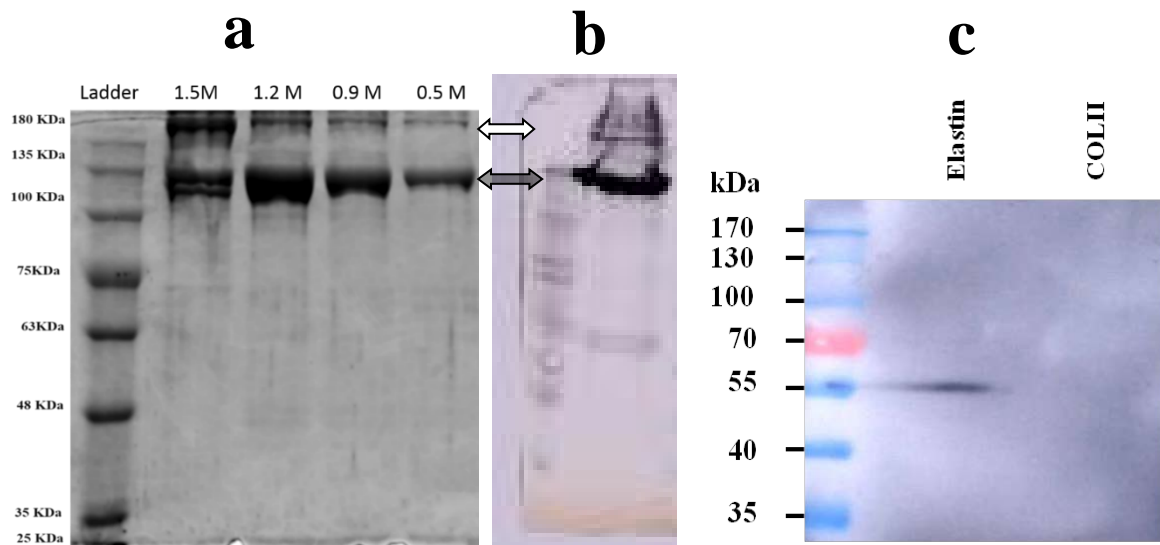


## Supplementary

### 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  $\alpha$  chain and open arrow indicating  $\beta$  chain, (b)  $\alpha$  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.

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Capra_COLIIA1 MIRLGAPQTLVLLTLLVAAVLRCHGQDVQKAGSCVQDQQRYNKDKVWKPEPCRCVCDTG 60
Bos_COLIIA1 MIRLGAPQTLVLLTLLVAAVLRCHGQDVQKAGSCVQDQQRYNKDKVWKPEPCRCVCDTG 60
Homo_COLIIA1 MIRLGAPQTLVLLTLLVAAVLRCHGQDVQKAGSCVQDQQRYNKDKVWKPEPCRCVCDTG 60
*****

Capra_COLIIA1 TVLCDDIICEDMKDCLSPETPFGECCPICADLPTASGQPGPKGQKGEKGEKIDIVGPKG 120
Bos_COLIIA1 TVLCDDIICEDMKDCLSPETPFGECCPICADLPTASGQPGPKGQKGEKGEKIDIVGPKG 120
Homo_COLIIA1 TVLCDDIICEDVKDCLSPETPFGECCPICADLPTASGQPGPKGQKGEKGEKIDIVGPKG 120
*****

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Bos_COLIIA1 PPGPQGPAGEQGPGRDRGDKGEKAGPGRGRDGEPTGPNPGRPDPGPPGPPGLGGNFA 180
Homo_COLIIA1 PPGPQGPAGEQGPGRDRGDKGEKAGPGRGRDGEPTGPNPGRPDPGPPGPPGLGGNFA 180
*****

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Homo_COLIIA1 APGPAGEEGKRGARGEPGGVGPJGPPGERGAPGNRFPQDGLAGPKGAPGERGSPGLAG 540
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Homo_COLIIA1 LPGTPTDGPKGAAGPAGPPGAGQPPGLQGMPPERGAAGIAGPKGDRGDVGEKGEAGAP 780
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Homo_COLIIA1 KDGGRGLTGPVIGPPGAGANGEKGEVPPGAGTAGARGAPGERGETGPPGAPGAPGPPG 840
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Homo_COLIIA1 ADGQPGAKGEQGEAGQKGDAGAPGQPPGAPGPPGPTGVTGPKGARGAQQPPGATGFP 900
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Homo_COLIIA1 DDGSPGAEPPGPPGQGLAGQRGIVGLPGQRGERGFPGLPGSPGEPGKQAGPAGSADRGGPPG 1020
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Homo_COLIIA1 PVGPPGLTGPAGEPREGSPGADGPPGRDGAAGVKGDRGETGAVGAPGAPGPPGSPGAP 1080
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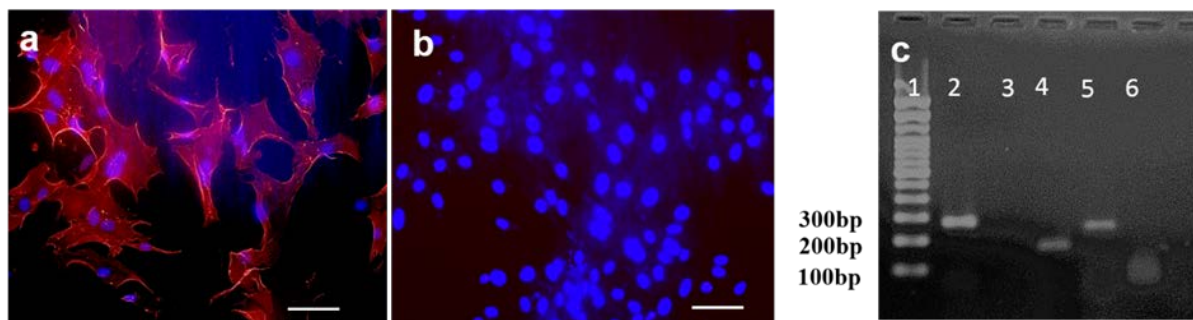
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Supplementary Figure 3. Amino acid sequence alignment of COLIIA1 from *Capra hircus*, *Bos taurus* and *Homo sapiens* showing 98% identity.

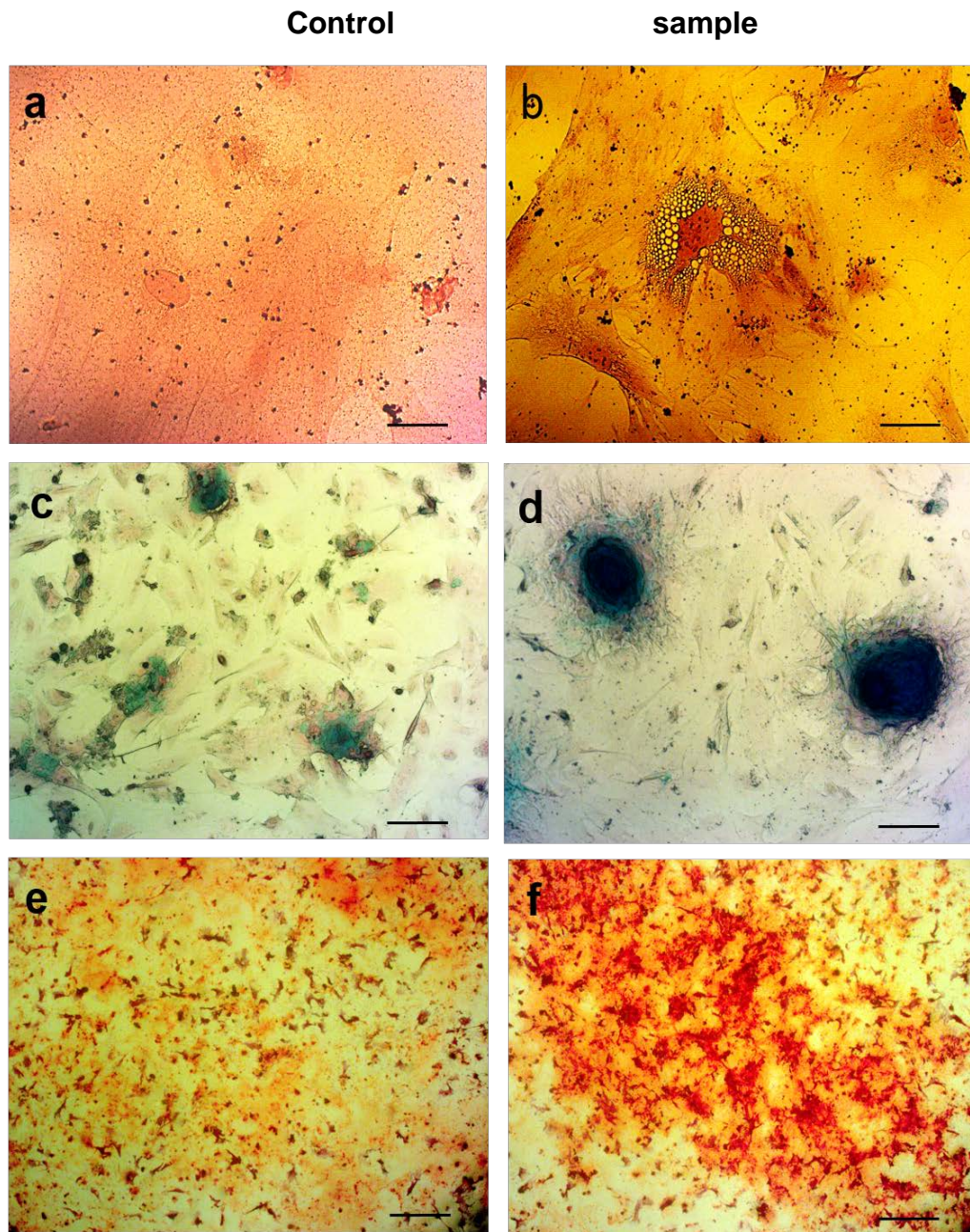
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101	GPKGQKGEFG	DIKDIVGPKG	PPGPQGPAGE	QGPRGDRGDK	<b>GEKGAPGPRG</b>
151	RDGEPGTPGN	PGPPGPPGPP	GPPGLGGNFA	AQMAGGFDEK	<b>AGGAQMGMVMQ</b>
201	<b>GPMGPMGPRG</b>	PPGPAGAPGP	QGFQGNPGEP	GEPGVSGPMG	PRGPPGPPGK
251	PGDDGEAGKP	GKSGER <b>GPPG</b>	<b>PQGARGFPPT</b>	<b>PGLPGVKGHR</b>	GYPGLDGAKG
301	EAGAPGVKGE	SGSPGENGSP	GPMGPRGLPG	ERGR <b>TGPAGA</b>	<b>AGARGNDGQP</b>
351	GPAGPPGPVG	PAGGPGFPGA	PGAKEAGPT	GARGPEGAQG	PRGEPGTPGS
401	PGPAGAAGNP	GTDGIPGAKG	<b>SAGAPGIAGA</b>	<b>PGFPGPRGPP</b>	GPQGATGPLG
451	PKGQTGEPGI	AGFKGEQGP	GEPGPAGPQG	APGPAGEEGK	RGARG <b>EPGGA</b>
501	<b>GPAGPPGERG</b>	<b>APGNRGFPQG</b>	<b>DLAGPKGAP</b>	<b>GERGPSGLAG</b>	<b>PKGANGDPGR</b>
551	<b>PGEPLPGAR</b>	<b>GLTGRPGDAG</b>	<b>PQKVGPSGA</b>	<b>PGEDGRGPP</b>	<b>GPQGARGQPG</b>
601	VMGFPGPKGA	NGEPGKAGEK	<b>GLPGAPLRG</b>	<b>LPKDGGETGA</b>	<b>AGPPGPAGPA</b>
651	<b>GERGEQGAPG</b>	PSGFQGLPGP	PGPPGEGGKP	GDQGVFGEAG	APGLVGRGE
701	<b>RGFPGERGS</b>	GSQGLQGARG	<b>LPGTPGTDGP</b>	<b>KGAAGPAGPP</b>	<b>GAQPPGLQG</b>
751	<b>MPGERGAAGI</b>	<b>AGPKGDRGDV</b>	GEKGPEGAPG	KDGGRGLTGP	IGPPGPAGAN
801	GEKGEVGGPP	PAGTAGARGA	PERGET <b>GPP</b>	<b>GPAGFAGPPG</b>	<b>ADGQPGAKGE</b>
851	QGEAGQKQDA	GAPGPQGPSG	APGPQGPQTV	TGPKGARG <b>AG</b>	<b>GPPGATGFPG</b>
901	<b>AAGRVGPPGS</b>	NGNPGPPGPP	GPSGKDGP	ARGDSGPPGR	AGDPGLQGPA
951	GPPGEKGEFG	DDGPSGPDGP	PGPQGLAGQR	<b>GIVGLPGQRG</b>	ERGF <b>PGLPGP</b>
1001	<b>SGEPGKQGAP</b>	<b>GASGDRGPPG</b>	<b>PVGPPLTGP</b>	<b>AGEPREGSP</b>	GADGPPGRDG
1051	<b>AAGVKGDRGE</b>	<b>TGAVGAPGAP</b>	<b>GPPGSPGAP</b>	<b>PIGKQGDRGE</b>	<b>AGAQQPMGPA</b>
1101	<b>GPAGARGMPG</b>	<b>PQGPRGDKGE</b>	TGEAGERGLK	GHR <b>GFTGLQG</b>	<b>LPGPPGPSGD</b>
1151	<b>QGASGPAGPS</b>	<b>GPRGPPGPVG</b>	PSGKD <b>GANGI</b>	<b>PGPIGPPGPR</b>	GRSGETGPAG
1201	PPGNPGPPGP	PGPPGPGIDM	SAFAGLGQRE	KGPDPLQYMR	ADEAAGNLRQ
1251	HDAEVDATLK	SLNNQIESLR	SPEGSRKNPA	RTCRDLKLCH	PEWKSGDYWI
1301	DPNQGCTLDA	MKVFCNMETG	ETCVYPNPAS	VPKKNWSSK	SKDKKHIWFG
1351	ETINGGFHFS	YGDDNLAPNT	ANVQMTFLRL	LSTEGSQNIT	YHCKNSIAYL
1401	DEAAGNLKKA	LLIQGSNDVE	IRAEGNSRFT	YTVLKDGGCTK	HTGKWKGTMI
1451	EYRSQKTSRL	PIIDIAPMDI	GGPEQEFGVD	IGPVCFL	

**Supplementary Figure 4.** Highlighted amino acid sequences identified from *Capra COLIA1* by peptide mass finger printing using *Bos taurus COLIA1* 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  $\mu$ m); (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  $\mu\text{m}$ ); (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  $\mu\text{m}$ ); (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  $\mu\text{m}$ ).

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

<b>Temp. (°C)</b>	<b><math>\alpha</math>-helix</b>	<b><math>\beta</math>-strand</b>	<b>% of denature</b>	<b>% of intactness</b>
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
<b>43</b>	<b>25.98</b>	<b>11.68</b>	<b>51.2</b>	<b>48.80</b>
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<sup>16</sup>**

Amino Acid	COL II (Cao <i>et al.</i> , 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
Threonine	30	28
Aspartic Acid	47	41
Tyrosine	5	5
Cysteine	17	20



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

<b>Protein</b>	<b>Mass</b>	<b>Score</b>
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**

<b>A-Buffer (0.01M Na<sub>2</sub>HPO<sub>4</sub>); B- Acetonitrile, Excitation:230, Emission: 460</b>						
<b>Time</b>	<b>A%</b>	<b>B%</b>	<b>C%</b>	<b>D%</b>	<b>Flow rate ml/min</b>	<b>Max. Pressure limit (Bar)</b>
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.(°C)	Product Size (bp)	References	
<i>GAPDH-FP</i>	<i>GGCGCTGCCAAGGCCGTGGGCAAG</i>	73.0	273	KEGG_10086074 3	
<i>GAPDH-RP</i>	<i>GGCAATGCCAGCCCCAGCATCGAAGG</i>				
<i>CD90 -FP</i>	<i>GCACCATGAACCCTACCATC</i>	55	241	Mohamad-Fauzi et al., 2015 <sup>22</sup>	
<i>CD90 -RP</i>	<i>TTGGTTCGGGAGCTGTATTC</i>				
<i>CD73-FP</i>	<i>CTGAGACACCCGGATGAGAT</i>	52	160		
<i>CD73-RP</i>	<i>ACTGGACCAGGTCAAAGGTG</i>				
<i>CD105-FP</i>	<i>AGATGCCAACATCACACAGC</i>	49	129		
<i>CD105-RP</i>	<i>TCCAGACGAAGGAAGATGCT</i>				
<i>CD45-FP</i>	<i>GGGAGGAGGGAAAGCAAACC</i>	55	146		
<i>CD45-RP</i>	<i>GCAGCTCTTCCCCATTCCAG</i>				
<i>COL1A1-FP</i>	<i>GGCGCTGCCAAGGCCGTGGGCAAG</i>	64	269		KEGG_10086074 3
<i>COL1A1-RP</i>	<i>GGCAATGCCAGCCCCAGCATCGAAGG</i>				