

Fig. S1. Differences in donkey milk peptides from M1 (TCA precipitation) and M2 (Ultrafiltration) samples identified by database search. Peptides density charts show the physicochemical properties of each sample: molecular weight distribution of peptides (A), distribution of peptides average isoelectric point (B), peptides net charge at pH 7.4 (C), peptides gravity score (D), and distribution of amino acid composition (E). In the molecular weight distribution graph, the inner ring represents the molecular weight distribution of the endogenous peptide identified by TCA precipitation method, and the outer ring represents the molecular weight distribution of the endogenous peptide identified by ultrafiltration method.

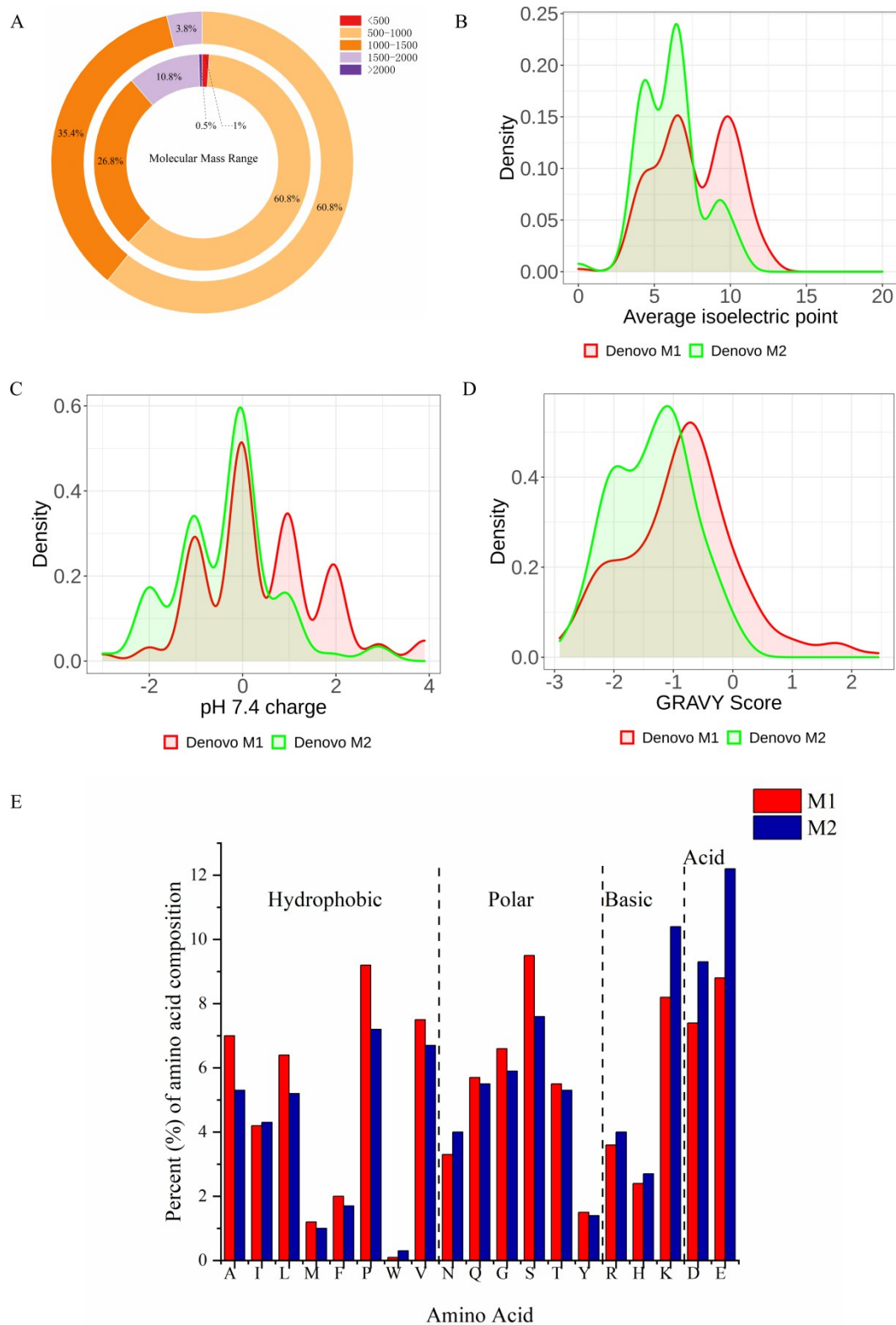


Fig. S2. Differences in donkey milk peptides from M1 (TCA precipitation) and M2 (Ultrafiltration) samples identified by *de novo*. Peptides density charts show the physicochemical properties of each sample: molecular weight distribution of peptides (A), distribution of peptides average isoelectric point (B), peptides net charge at pH 7.4 (C), peptides gravity score (D), and distribution of amino acid composition (E). In the molecular weight distribution graph, the inner ring represents the molecular weight distribution of the endogenous peptide identified by TCA precipitation method, and the outer ring represents the molecular weight distribution of the endogenous peptide identified by ultrafiltration method.

Table S1

Enzyme patterns predicted by Enzyme Predictor ²⁵.

Enzyme name	P4	P3	P2	P1	P1'	P2'
Arg-C proteinase	-	-	-	R	-	-
Asp-N endopeptidase	-	-	-	-	D	-
Caspase 1	F, W, Y, or L	-	H, A or T	D	not P, E, D, Q, K or R	-
Caspase 2	D	V	A	D	not P, E, D, Q, K or R	-
Caspase 3	D	M	Q	D	not P, E, D, Q, K or R	-
Caspase 4	L	E	V	D	not P, E, D, Q, K or R	-
Caspase 5	L or W	E	H	D	-	-
Caspase 6	V	E	H or I	D	not P, E, D, Q, K or R	-
Caspase 7	D	E	V	D	not P, E, D, Q, K or R	-
Caspase 8	I or L	E	T	D	not P, E, D, Q, K or R	-
Caspase 9	L	E	H	D	-	-
Caspase 10	I	E	A	D	-	-
Chymotrypsin-high specificity (C- term to [FYW], not before P)	-	-	-	F or Y	not P	-
	-	-	-	W	not M or P	-
	-	-	-	F,L or Y	not P	-
Chymotrypsin-low specificity (C- term to [FYWML], not before P)	-	-	-	W	not M or P	-
	-	-	-	M	not P or Y	-
	-	-	-	H	not D,M,P or W	-
Enterokinase	D or N	D or N	D or N	K	-	-
Factor Xa	A,F,G,I,L,T,V or M	D or E	G	R	-	-
Formic acid	-	-	-	D	-	-
Glutamyl endopeptidase	-	-	-	E	-	-

GranzymeB	I	E	P	D	-	-
Hydroxylamine	-	-	-	N	G	-
Iodosobenzoic acid	-	-	-	W	-	-
LysC	-	-	-	K	-	-
NTCB (2-nitro-5-thiocyanobenzoic acid)	-	-	-	-	C	-
Pepsin (pH1.3)	-	not H,K, or R	not P	not R	F,L,W or Y	not P
Pepsin (pH>2)	-	not H,K or R	not P	F,L,W or Y	-	not P
Proline-endopeptidase	-	-	H,K or R	P	not P	-
Staphylococcal peptidase I	-	-	not E	E	-	-
Thrombin	A,F,G,I,L,T,V or M	A,F,G,I,L,T,V,W or A	G	R	G	-
Trypsin (please note the exceptions!)	-	-	P	R	not D or E	not DE
Modified-chymotrypsin	-	-	-	K or R	not P	-
elastase	-	-	W	K	P	-
Cyanogen-bromide	-	-	M	R	P	-
Proline-endopeptidase_diff	-	-	-	F,W,Y or L	not P	-
Plasmin	-	-	-	A,V,I,L,G or R	G,P,A,L or F	-
CathepsinD	-	-	-	M	-	-
	-	-	-	P	-	-
	-	-	-	K or R	-	-
	-	-	-	A,V,L,I,P,M ,F or W	A,V,L,I,P,M or F	-

Table S2.

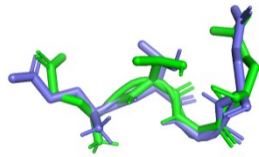
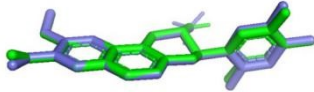
Predicted Enzymes, cleavage sites, and Counts for donkey milk main proteins.

Enzymes	N-terminus cleavage count	C-terminus cleavage count	Total cleavage	Unique cleavage	Number of expected cleavages within the peptide	Number of proteins cleaved	Odds Ratio	Std. Error
Plasmin	806	541	1347	0	3598	11	1.65	1.03
Trypsin1	803	539	1342	0	3522	11	1.68	1.03
Clostripain	465	214	679	0	1084	11	2.74	1.04
Argcproteinase	465	214	679	0	1084	11	2.74	1.04
CathepsinD	426	598	1024	0	6386	11	0.65	1.03
Elastase	414	444	858	0	5623	11	0.62	1.04
Modifiedchymotrypsin	404	419	823	0	2532	11	1.40	1.04
Chymotrypsinlow1	382	411	793	0	2480	11	1.38	1.04
AspNendopeptidase	349	96	445	0	3475	11	0.53	1.05
LysC	341	327	668	0	2514	11	1.13	1.04
Pepsin(pH1.3)2	239	179	418	0	1509	11	1.18	1.05
Formicacid	239	233	472	0	3645	11	0.53	1.05
Pepsin(pH>2)2	201	141	342	0	1196	11	1.22	1.06
staphprotease	189	140	329	0	2633	11	0.52	1.06
Pepsin(pH1.3)1	189	424	613	0	2073	11	1.26	1.04
Glutamylendopeptidase	189	140	329	0	2633	11	0.52	1.06
StaphylococcalpeptidaseI	173	132	305	0	2398	11	0.53	1.06
Pepsin(pH>2)1	170	382	552	0	1791	11	1.32	1.04
Chymotrypsinhigh1	163	137	300	0	840	11	1.52	1.06
Prolineendopeptidasediff	141	174	315	1	3106	11	0.42	1.06
Chymotrypsinlow4	63	107	170	0	540	8	1.34	1.08
Cyanogenbromide	39	23	62	3	220	10	1.19	1.14
CNBr	39	23	62	3	220	10	1.19	1.14
Chymotrypsin	39	23	62	3	217	10	1.21	1.14

nlow3									
Prolineendop eptidase	30	4	34	1	162	4	0.89	1.19	
Iodosobenzoi cacid	22	8	30	0	49	3	2.60	1.23	
iodosobenzoa te	22	8	30	0	49	3	2.60	1.23	
Chymotrypsi nlow2	22	8	30	0	49	3	2.60	1.23	
Chymotrypsi nhigh2	22	8	30	0	49	3	2.60	1.23	
Hydroxylami ne	8	5	13	0	60	3	0.92	1.34	
Thrombin1	4	0	4	0	8	1	2.12	1.87	

Table S3.

RMSD value for ligand ETGE, HL1, and NXA docked with Keap1, DPP-IV, and ACE.

Ligand ID	Proteins	RMSD	3D diagram of ligands before and after docking
ETGE	Keap1 (PDB ID: 2FLU)	0.813	
HL1	DPP-IV (PDB ID: 5J3J)	0.002	
NXA	ACE (PDB ID: 1O8A)	0.504	