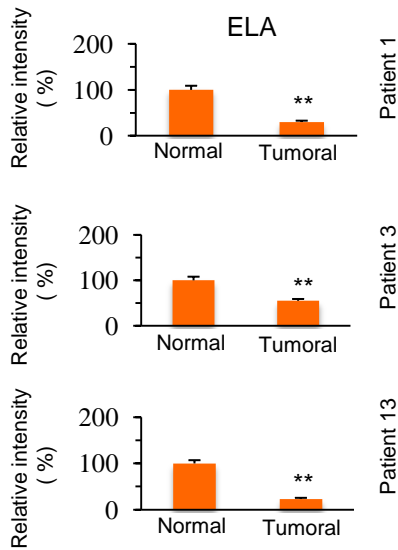


Reagents and antibodies

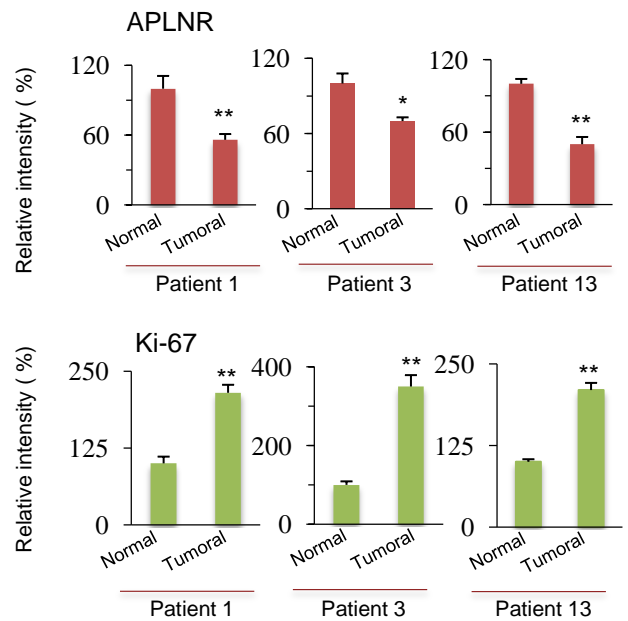
Antibodies used in this study were as follows: Anti-ELA (Custom made, dilution 1:1,000) from Eurogentec, anti-APLNR (#ab84296, dilution 1:1,500) from abcam, anti-Ki67 (#sc-23900, dilution 1:1,500) from Santa Cruz Biotechnology, anti-CD31 (#553370, dilution 1:1,500) from BD Pharmingen™, and anti-phosphoAKT(Ser473) (#4060, dilution 1:1,000), anti-AKT (#4691, dilution 1:1,000), anti-phospho ERK (#9106S, dilution 1:1,000), anti-ERK (#4695, dilution 1:1,000), anti-phosphoS6 (Ser235/236) (#4856, dilution 1:1,000), anti-S6 (#2217, dilution 1:1,000), anti-phospho-S6K (T389) (#9205, dilution 1:1,000), anti-S6K (#2708, dilution 1:1,000), anti-phospho-4EBP1(T37/46) (#2855, dilution 1:1,000), anti-4EBP1 (#9452, dilution 1:1,000), anti-LC3 AB (#12741, dilution 1:1,000), anti-P62 (#5114, dilution 1:1,000), anti-cleaved caspase 3 (#9664, dilution 1:1,000), anti-cleaved caspase 8 (#9746, dilution 1:1,000), anti-cleaved PARP (#5625, dilution 1:1,000), anti-P62 (#5114, dilution 1:1,000), anti-LC3 (#12741, dilution 1:1,000) and anti- β -actin (#4967, dilution 1:1,000) were derived from Cell Signaling Technology. Anti-CD31 (#M0823, dilution 1:200), anti-Cytokeratin (#M3515 Dako, dilution 1:200) from Dako and anti-CD34, (dilution 1:500) from Abcam. Secondary antibodies anti-rabbit (#7074, dilution 1:1,000) and anti-mouse (#7076, dilution 1:1,000) were obtained from Cell Signaling Technology.

All powders and reagents were from Sigma.

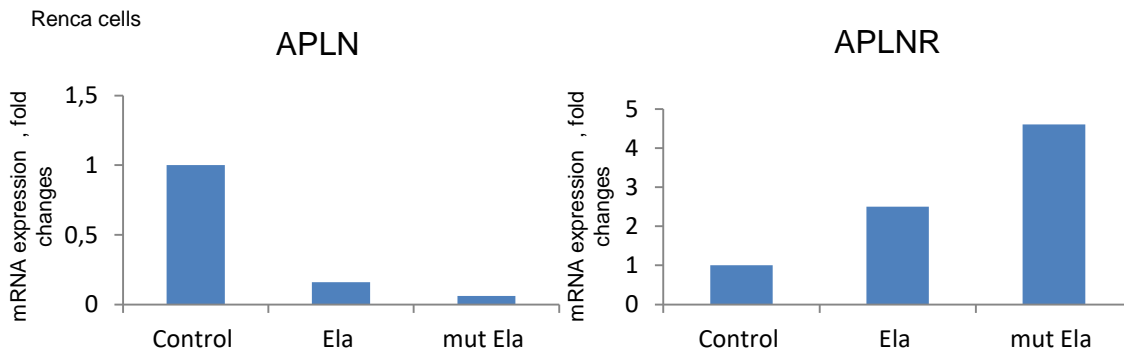
Clinisciences synthesized ELA11, ELA32 and mutant ELA32 (mutELA) peptides.



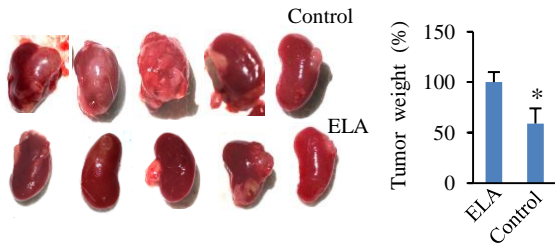
Supplementary Figure 1. Bars indicate the percentage of relative staining intensity of ELA for tumor samples and adjacent healthy tissue for each patient corresponding to Figure 1F. Unpaired *t* tests were used to analyze the data. (n= 11), **p < 0.01



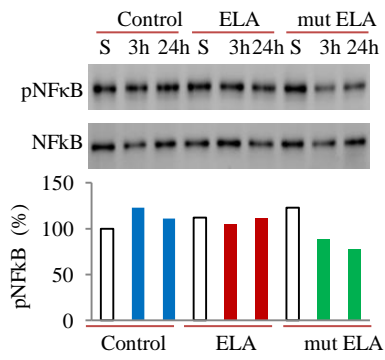
Supplementary Figure 2. Bars indicate the percentage of relative staining intensity of APLNR and Ki67 for tumor samples and adjacent healthy tissue for each patient corresponding to Figure 2A. Unpaired *t* tests were used to analyze the data. (n=11), *p < 0.05, **p < 0.01



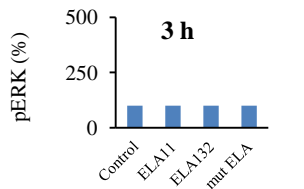
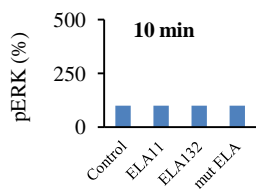
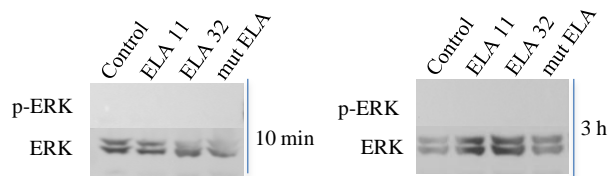
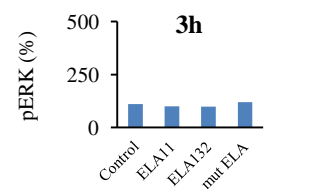
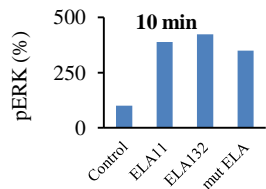
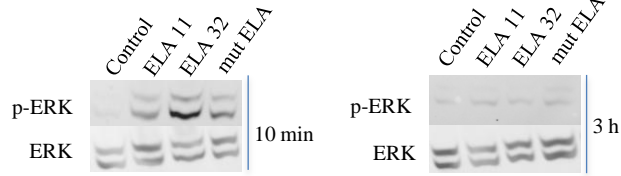
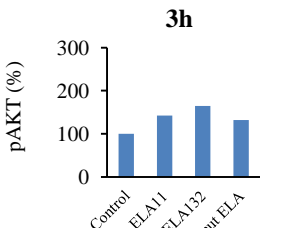
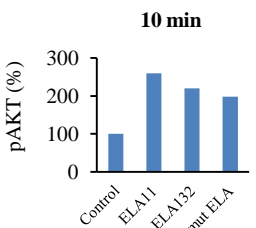
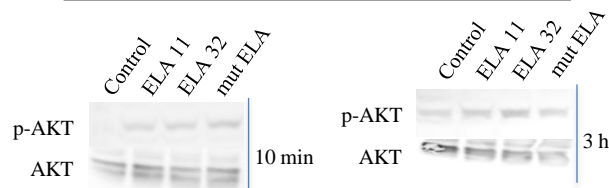
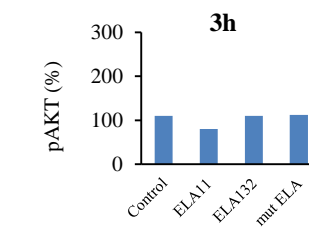
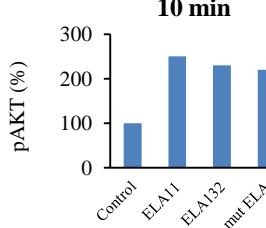
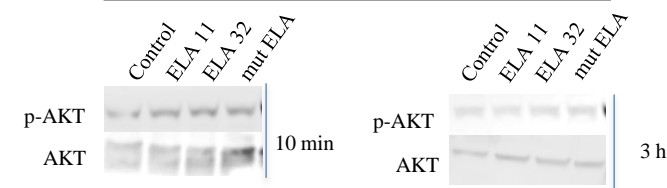
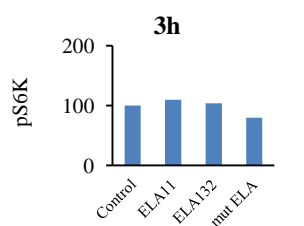
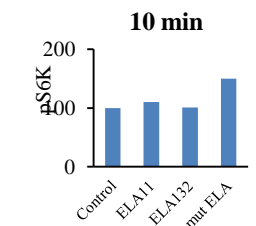
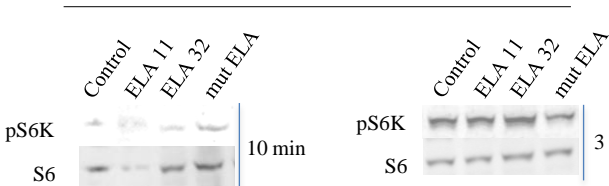
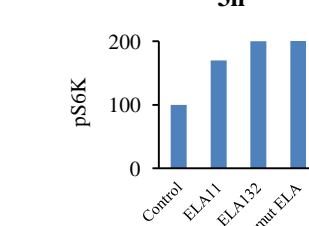
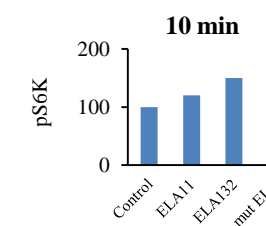
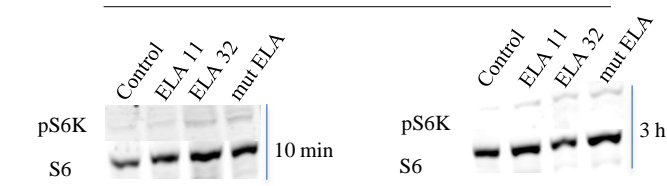
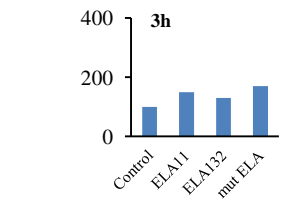
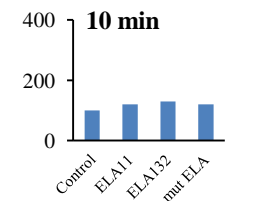
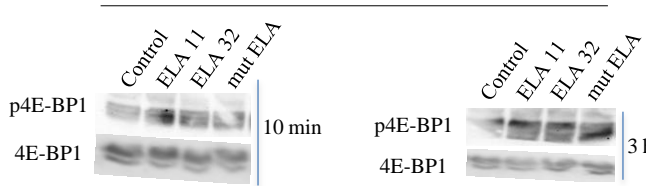
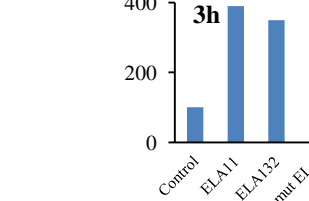
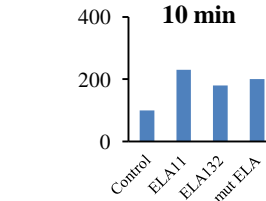
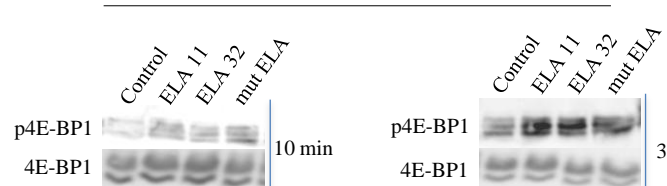
Supplementary Figure 3. ELA, APLN and APLNR mRNA transcript expression in Renca cells. Relative expression of APLN and APLNR mRNA level in Renca cells control and following the expression of ELA or mut ELA, as assessed by real-time PCR analysis using specific primers for APLN, APLNR or GAPDH. Expression of GAPDH that was evaluated in each sample was used as endogenous control. The relative amounts of mRNA were normalized against GAPDH mRNA and expressed relative to the mRNA abundance in control cells assigned 1. Results shown are representative of 2 experiments.



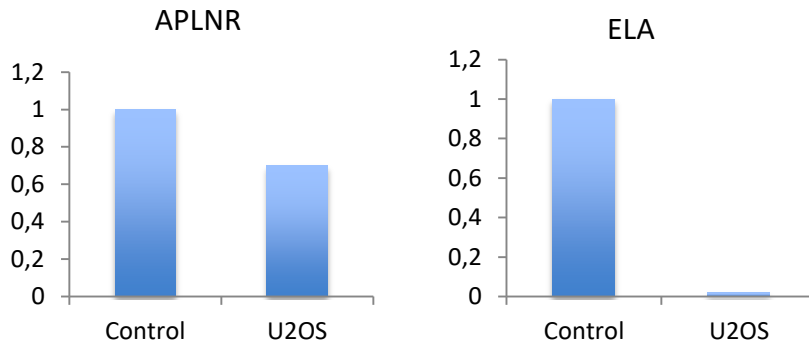
Supplementary Figure 4. 1×10^6 Control Renca cells or the same cells stably expressing ELA using lentiviral vectors were injected into the sub-capsular space of BALB/c mice ($n=7$ tumors per group) and tumor weight was analyzed two weeks later. Unpaired t tests were used to analyze the data. * $p < 0.05$.



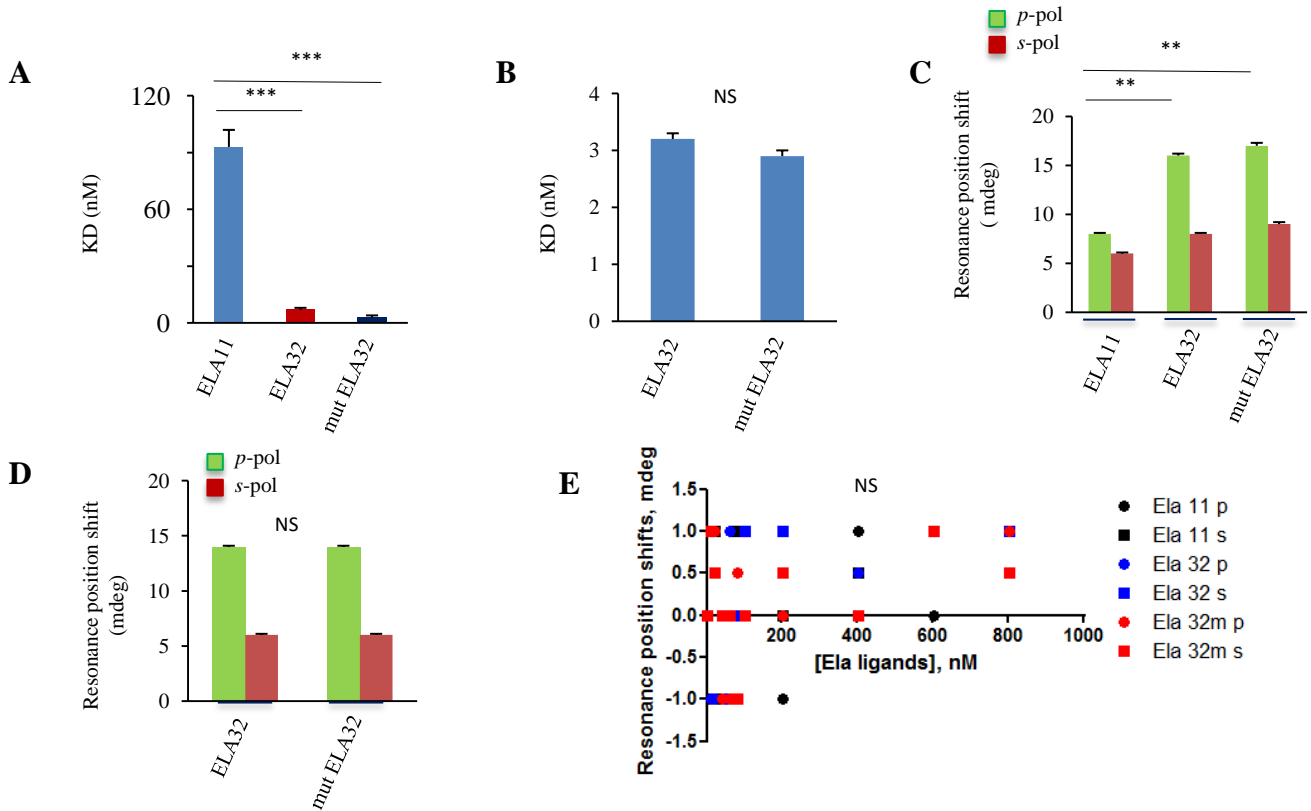
Supplementary Figure 5. Western blot analysis of the activation of NFkB-pS536 in control, ELA and mutELA expressing cells starved for the indicated time period or in the presence of serum (S).

HEK 293/Control**HEK 293/APLNr****HEK 293/Control****HEK 293/APLNr****HEK 293/Control****HEK 293/APLNr****HEK 293/Control****HEK 293/APLNr**

Supplementary Figure 6. Control HEK cells and HEK-APLNR cells were serum starved and incubated with ELA11, ELA32 or mutELA32 peptides for indicated time periods and the activation of AKT, ERK, S6K and 4EBP-1 were analyzed by Western blot analysis. Bars denote the corresponding percentages of phosphorylated proteins.



Supplementary Figure 7. ELA and APLNR mRNA transcript expression in U2OS cells. Relative expression of ELA and APLNR mRNA level in U2OSa cells as assessed by real-time PCR analysis using specific primers for ELA, APLNR or GAPDH. Expression of GAPDH that was evaluated in each sample was used as endogenous control. The relative amounts of mRNA were normalized against GAPDH mRNA and expressed relative to the mRNA abundance in the healthy human kidney tissue derived from kidney cancer sample assigned 1. Results shown are representative of 2 experiments.



Supplementary Figure 8. Plasmon Waveguide Resonance. (A) Bar graph showing the results of KD values ($n = 3$) corresponding to figures 6F-6H. (B) KD values ($n=3$) obtained with ELA and mutELA interaction with APLNR in the presence of 200 nM of ELA11. (C, D) Conformational changes of APLNR in response to ELA peptides. (C) Spectral changes induced by ELA11, ELA32 and mutELA32 binding to APLNR with two polarizations (p -pol and s -pol) ($n=3$). (D) The pre-incubation of the cell fragments with ELA11 did not alter the receptor conformational changes during APLNR interaction with ELA32 or mutELA32 ($n=3$). (E) No significant ELA11, ELA32 and mutELA32 binding to cellular membrane lacking APLNR was observed. P: p - (perpendicular to the sensor surface) polarized light S: s - (parallel to the sensor surface). Data shown as the mean \pm s.e.m. ** $P < 0.01$, *** $P < 0.001$ determined by 1-way ANOVA with Tukey's multiple comparison tests. NS: not significant.

Species	Amino acids sequence	compared to human sequence	PC site 1	PC site 2	Accession number
Homo sapiens	QRPVNLTMR ^{RR} KL ^{RK} HNCLQ ^{RR} C ^{MP} L ^{HS} R ^{VP} FP	100%	³¹ RR ³²	⁴² RR ⁴³	NP_001284479
Bos taurus	QRQANLAMR ^{RR} KL ^{HR} HNCLQ ^{RR} C ^{MP} L ^{HS} R ^{VP} FP	76%	³¹ RR ³²	⁴² RR ⁴³	NP_001295178
Rattus norvegicus	EKSVNFP ^{RR} R ^{RR} KLY ^{RH} NC ^{FR} ^{RR} C ^{IS} L ^{HS} R ^{VP} FP	61%	³¹ RR ³²	⁴² RR ⁴³	XP_008770257
Mus musculus	QKPVNFP ^{RR} R ^{RR} KLY ^{RH} NC ^{FR} ^{RR} C ^I L ^{HS} R ^{VP} FP	69%	³¹ RR ³²	⁴² RR ⁴³	NP_001284483
Gallus gallus	QRPANLALR ^{RR} KL ^{HR} HNCSH ^{RR} C ^{MP} L ^{HS} R ^{VP} FP	71%	³¹ RR ³²	⁴² RR ⁴³	NP_001295179
Xenopus laevis	QKPANLAQR ^{RR} I ^{HR} HNCF ^L ^{KR} C ^I L ^{HS} R ^{VP} FP	61%	³¹ RR ³²	⁴² KR ⁴³	XP_018097195
Danio rerio	DKHG ^T KHDFLNL ^{RR} KY ^{RR} HNCP ^K ^{KR} C ^L L ^{HS} R ^{VP} FP	70%	³⁵ RR ³⁶	⁴⁶ KR ⁴⁷	NP_001284476



Supplementary Table-1. Comparison of Ela peptide sequences from various species.

Represented are the putative peptide sequences of indicated species. Note that the two proprotein convertases (PC) general motifs (K/R)-(X)n-(K/R)Q are conserved.

The NCBI accession number for each sequence and the homology percentage of each sequence to human sequence is given. The mature peptide ELA11 is shown in green.

The sequence logos of the proprotein convertase cleavage motifs at the bottom was deduced following the alignment for ELA peptides of different species (see supplemental Table-1 suite for other species).

The proprotein convertase motif associated with the proprotein convertase-mediated cleavage is located in the positions 31–32 and 42-43. The size of each logo represent the proportion of conserved indicated amino acid.

	10	20	30	40	50
Xenopus tropicalis	M D F Q K L F F A L F F I L M	S L L L I N G Q K P A N	L A Q R R R I H R H N C	F L K R C I P L H S R V P F F	
Mouse	M R F Q P L F W V F F I F A M	S L L F I S E Q K P V N	F P R R R K L Y R H N C	F R R R C I P L H S R V P F F	
Rabbit	M K L Q R F F F M F L I L M M	S L L F I N G Q R P V N	L A M R R K F H R H N C	L Q R R C V P L H S R V P F F	
Pig	M R E R Q F F L V F F I F M M	N L L L I C G Q R P A N	L A V R R K L H R H N C	L Q R R C M P L H S R V P F F	
Cat	M R L Q Q F F C V F F I F V M	S L L L I D G Q R P A N	L A L R R K L H R H N C	L Q R R C M P L H S R V P F F	
Squirrel	M R F Q Q F L C V F L I F V M	S L L L I N G Q R P A N	L A L R R K L H R H N C	L Q R R C M P L H S R V P F F	
Chinchilla	M R E R Q L F C V L L I F L T	S L L L S N G Q R P A N	L V L R R K L H R H N C	L Q R R C M P L H S R V P F F	
Cow	M R E H Q F F L L F V I F M L	S L L L I H G Q R Q A N	L A M R R K L H R H N C	L Q R R C M P L H S R V P F F	
Dolphin	M R L Q Q F F F L F L V F M M	S L L L T P G Q R P A N	L A M R R K L H R Y N C	L Q R R C M P L H S R V P F F	
Alpaca	M R L Q Q F F F L F L I F M M	S P L L I H G Q R S A N	L A M R R K L P R Y N C	L Q R R C I P L H S R V P F F	
Panda	M R L Q Q F L L A F L L F A M	S L L L I N G Q R P A N	L A M R R K S H R H S C	L Q R R C M P L H S R V P F F	
Squirrel monkey	M R F Q K F L F A F F I F L M	S L L L I S G Q R P V N	L A M R R K L R K H N C	L Q R R C V P L H S R V P F F	
Chimp	M R F Q Q F L F A F F I F I M	S L L L I S G Q R P V N	L T M R R K L R K H N C	L Q R R C M P L H S R V P F F	
Human	M R F Q Q F L F A F F I F I M	S L L L I S G Q R P V N	L T M R R K L R K H N C	L Q R R C M P L H S R V P F F	
Rhesus	M K F Q Q F L F A F C I F I M	S L L L I S G H R P V N	L T M R R K L R K H N C	L Q R R C I P L H S R V P F F	
Horse	M R F Q Q F F Y V F L I F M M	S L - L I N G Q R P A N	L A M R R K L H R H N C	L Q R R C M P L H S R V P F F	
Elephant	M R C R Q F F F I F F I L I M	S L - L I N G Q R P A N	F V M R R K L H R Q N C	L Q R R C M P L H S R V P F F	
Microbat	M R F Q P L F F V F L I V M M	S L L L I N G Q R P A N	L A M R R K L H R L S C	H Q R R C M P L H S R V P F F	
Megabat	M R F Q Q F F F V F F I F I T	S L L L I N G Q R P A N	L A T R R K L H R L S C	H Q R R C M P L H S R V P F F	
Shrew	M R I Q Q V F F V - V I E L L	S L V L T D G Q R T A T	R A T R R K S Y R K N C	H K R R C R P L H S R V P F F	
Green seaturtle	M R F Q Q L L Y I L F F L M M	S L L L I N G Q R P A N	L A L R R K L H R H N C	S H R R C M P L H S R V P F F	
Tibetan ground jay	M R L Q L L L W V V A V L L A	S L L P A Y G Q R P A N	L A L R R K L H R H G C	S H R R C V P L H S R V P F F	
Zebra finch	M R L Q L L L W I V A V L L A	S L L P A S G Q R P A N	L A L R R K L H R H G C	S H R R C M P L H S R V P F F	
Parrot	M R L R L V L C V V F F L L V	S L L P A D G Q R T A N	L A L R R K L H R H G C	S H R R C V P L H S R V P F F	
Lizard	M R F Q Q L L L T W F L L L A	G A L L I N G Q R P A N	L A S R R K L H R H C	S H R R C M P L H S R V P F F	
Dog	M R L R R L F L A A C A L M L	S L L V I N G Q R P A N	L A M R R K L H R H N C	L Q R R C M P L H S R V P F F	

Supplementary Table-1 suite . Comparison of Ela peptide sequences from various species.