Supplemental Information

for

ELABELA and its fragment protect against acute kidney injury

Running title: ELA protects against AKI

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Table S1 Effects of ELA peptides on physiological parameters of I/R injured mice.

	CREA (μmol/L)	uCREA (mmol/L)	uBUN (mmol/L)	Urea volume (mL/day)	Urea protein (mg/day)
CT	3.0±0.8	2427.5±90.8	282.5±0.5	0.28±0.05	0.91±0.1
I/R	6.6±1.0*	3767±264.7*	290.1±2.1*	0.66±0.07*	2.2±0.2*
I/R+E32	4.6±0.3$^{\#}$	2954.7±246.7$^{\#}$	278.3±2.6$^{\#}$	0.46±0.05$^{\#}$	1.5±0.1$^{\#}$
I/R+E11	4.3±0.2$^{\#}$	2979.3±237.6$^{\#}$	283.2±5.5$^{\#}$	0.43±0.02$^{\#}$	1.3±0.06$^{\#}$
I/R+AE11C	6.2±1.5	3847.4±133.4	290.7±1.7	0.55±0.02	1.8±0.06

CT, non-injured mice; I/R, I/R injured mice; E32/E11/AE11C, ELA32/ELA11/AE11C treated I/R-injured mice, n = 5-7 per group. CREA, serum creatinine; uBUN, urea nitrogen; uCREA, urea creatinine, $^{*}p < 0.05$ compared to CT mice; $^{\#}p < 0.05$ compared to I/R-injured mice.

Table S2 Primers used in the present study.

Gene	Forward	Reverse
M *Apela*	TTTGCAGAGACTTCCCGCTT	GCTCACCCCACATCCTATGG
M *Apln*	GCTGCTGCTGCTCTGGCTCT	GGGGGCGCTGTCTGCGAAAT
M *Aplnr*	GCCTGTCATGGTGTTCCG	CTCAATGCGCTCCTTTCGG
M *Il6*	CACTTCACAAGTCGGAGGCT	CTGCAAGTGCATCATCGTTGT
M *Il8*	TTGGAGCCAAGGCAAGAACA	AATGGAGAGGCATCCGGTTC
M *Collagen1a*	GCACGTCTGGTTTGGAGAGA	ACATTAGGCGCAGGAAGGTC
M *Tgfb1*	GACTCTCCACCTGCAAGACC	GGACTGGCGAGCCTTAGTTT
M *Fibronectin*	TCAGAAGAGTGAGCCCCTGA	CAGGGTTGGTGATGAAGGGG
M *Mcp1*	ACAAGAGGATCACCAGCAG	GGACCCATTCCTTCTTGGGG
M *Icam1*	CCATCACCGTGTATTCGTTT	GAGGTCCTTGCCTACTTGCT
R *Il6*	AGCCAGAGTCATTCAGAGCAA	AGAGCATTGGAAGTTGGGGT
R *Tnfa*	TCTCTTCAAGGGACAAGGCT	TCCTGGTATGAAATGGCAAA
R *Kim1*	CTCCAGGAAGCCGAGCAAAC	AAGCACTGGGTACAGATCCAAA
R *Mcp1*	TTGCTGCCTGTAGCATCC	GAGTAGCAGCAGGTGAGTGG
R *Icam1*	TACAAGTGCCGTGCCTTTAG	CATGGTACAGCACTGTCAGGT
R *Vcam1*	GCTACATCCACACTGACGCT	CAGGGAATGAGTAGACCTCCA
H/R/M *Rn18s*	CTCAACACGGGAAACCTCAC	CGCTCCACCAACTAAGAACG

APJ primer	CCGGAATTCATGGAGGAAGGTGGTGATTT	CGCGGATCCATGTCAACCACAAGGGTCTCCT
E32-GFP primer	CCGGAATTCATGCAGAGACCAGTTAATTTG	TGCTCTAGAGGGAAAGGGTACTCGTGA
E11-GFP primer	CCGGAATTCATGTGTATGCCTCTCCATTCA	TGCTCTAGAGGGAAAGGGTACTCGTGA

M: *Mus musculus*; H: *Homo sapiens*; R: *Rattus norvegicus*.

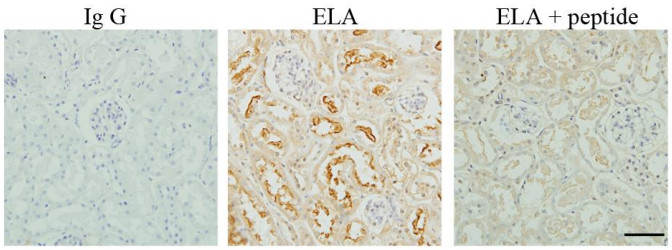

Figure S1. ELA staining on the mouse renal section. Representative images of ELA (brown) staining. IgG (left): section stained with IgG instead of primary antibody; ELA (middle): section stained with ELA antibody; ELA + peptide (right): section stained with ELA antibody pre-incubated with blocking peptide at ratio 1:10 for 30 min, scale bar = 50 μm.

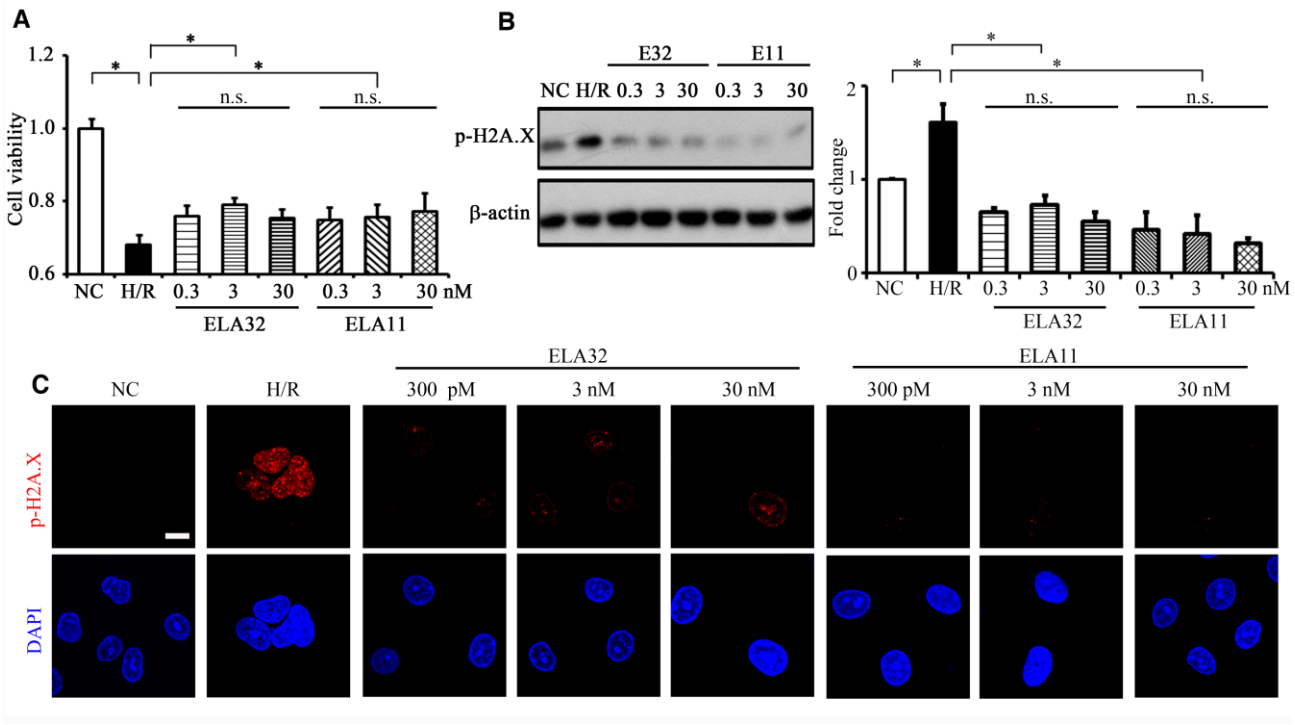

Figure S2. ELA32 and ELA11 dose-independently suppress H/R-induced cell death and DNA damage in cultured renal tubular cells. (A) Relative cell viability. (B) Representative western blots (left) with densitometric quantitative results (right) of p-H2A.X in different experimental groups. (C) Representative images of p-H2A.X staining in different groups, scale bar = 10 μm. NC, non-injured cells; H/R, H/R-injured cells; E32 or E11, 300 pM/3 nM/30 nM, 300 pM/3 nM/30 nM ELA32/ELA11 treated H/R-injured cells.

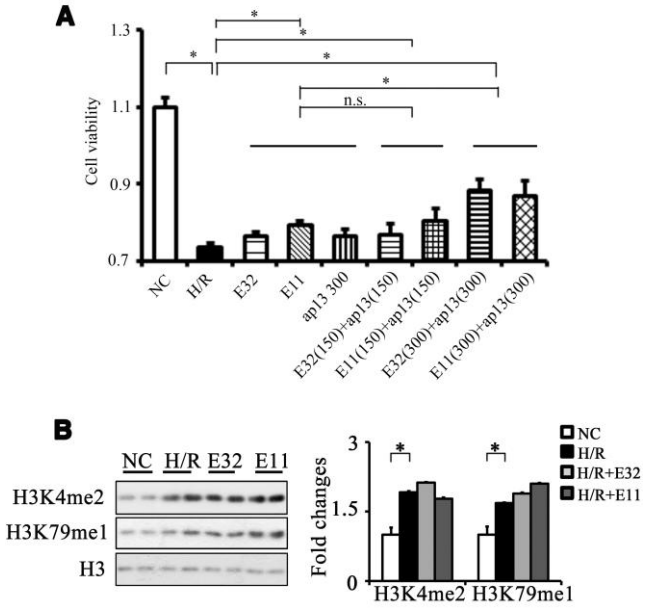

Figure S3. ELA32, ELA11 and apelin-13 suppress H/R injury induced cell death in cultured renal tubular cells. (A) Relative cell viability. (B) Representative western blots (left) with densitometric quantitative results (right) of H3K4me2, H3K79me1 and H3 in different experimental groups. NC, non-injured cells; H/R, H/R-injured cells; E32/E11/ap13 300, 300 pM ELA32/ELA11/apelin-13 treated H/R injured cells; E32(150)+ap13(150), 150 pM ELA32 and 150 pM apelin-13 treated H/R injured cells; E11(150)+ap13(150), 150 pM ELA11 and 150 pM apelin-13 treated H/R injured cells; E32(300)+ap13(300), 300 pM ELA32 and 300 pM apelin-13 treated H/R injured cells; E11(300)+ap13(300), 300 pM ELA11 and 300 pM apelin-13 treated H/R injured cells.

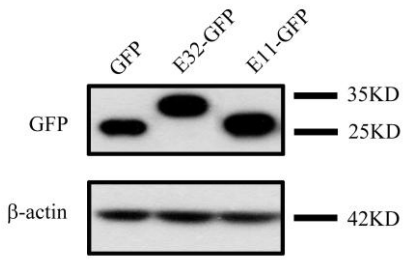

Figure S4. Successful overexpression of E32-GFP and E11-GFP in NRK-52E cells. Representative western blots of GFP and β-actin in different experimental groups.

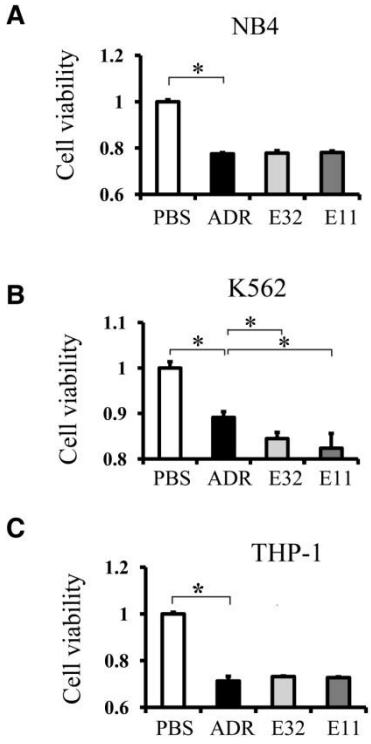

Figure S5. ELA32 and ELA11 do not influence the efficacy of ADR-induced cell death in K562 leukemia cell line. Relative cell viability measured by MTT assay of NB4 cells (A), K562 cells (B), and THP-1 cells (C), treated with or without ADR, or with or without ELA32 or ELA11. PBS, PBS treated NRK-52E cells; ADR, ADR treated NRK-52E cells; E32/E11, ELA32/ELA11 treated ADR injured cells, n = 10 per group.

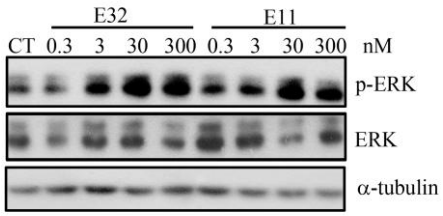

Figure S6. High dosages of ELA32 and ELA11 activate p-ERK in HEK293 cells under normal cultured conditions. Representative western blots of p-ERK, ERK and α-tubulin in different experimental groups.

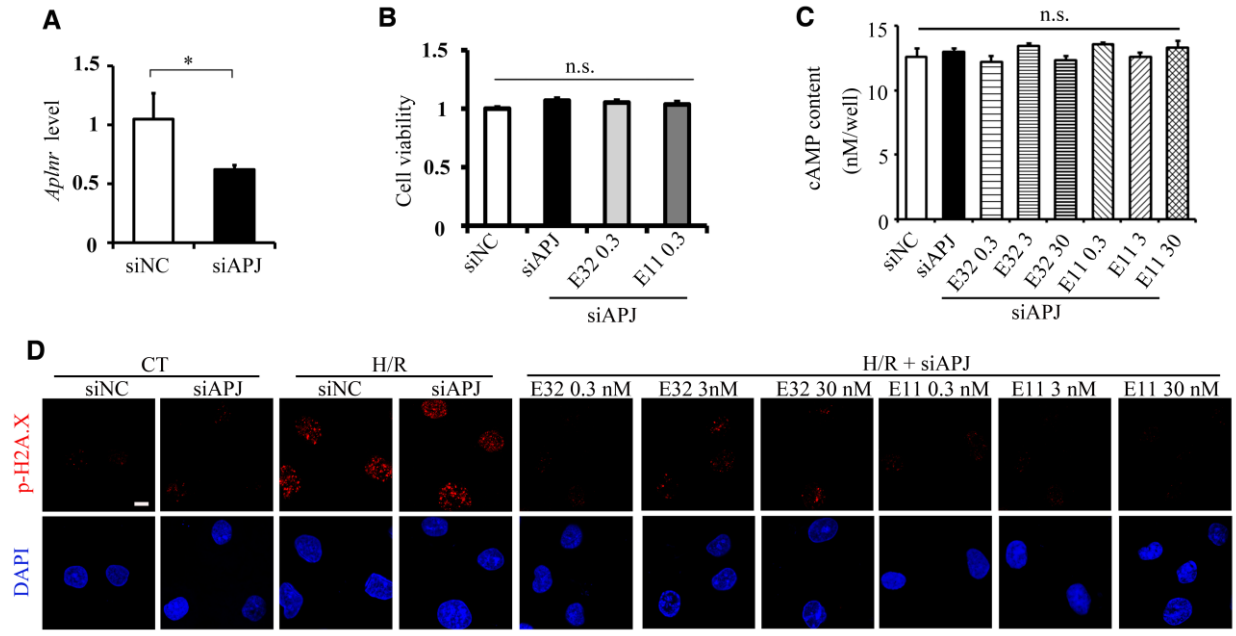

Figure S7. ELA and its short fragment dose-independently inhibit H/R-induced cell death and DNA damage in APJ knockdown NRK-52E cells. (A) qPCR result of *Aplnr* in NRK-52E cells. (B) Relative cell viability measured by MTT assay in APJ knockdown NRK-52E cells. (C) cAMP assay of ELA32 and ELA11 in APJ knockdown NRK-52E cells. (D) Representative images of p-H2A.X in different groups, scale bar = 10 μm. NC, non-injured cells; H/R, H/R injured cells; siNC or siAPJ, siNC or siAPJ transfected NRK-52E cells; E32 or E11 0.3/3/30 nM, 0.3/3/30 nM ELA32/ELA11 treated APJ knockdown NRK-52E cells, n.s., not significant.

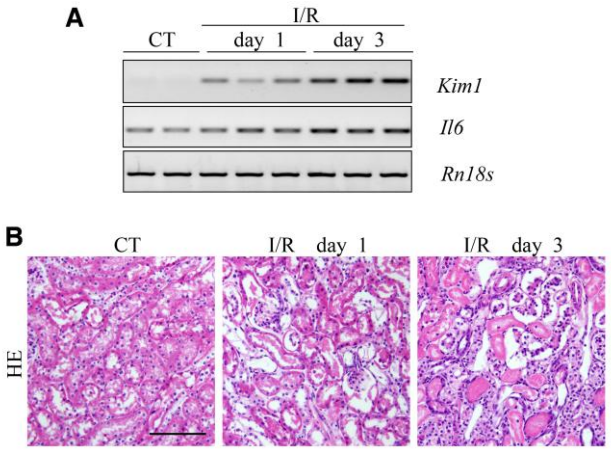

Figure S8. Assessment of renal I/R injury. (A) RT-PCR results of *Kim1*, *Il6* and *Rn18s* in different experimental groups. (B) Representative images of H&E staining in different experimental groups, scale bar = 100 μm.