

Supplementary Materials

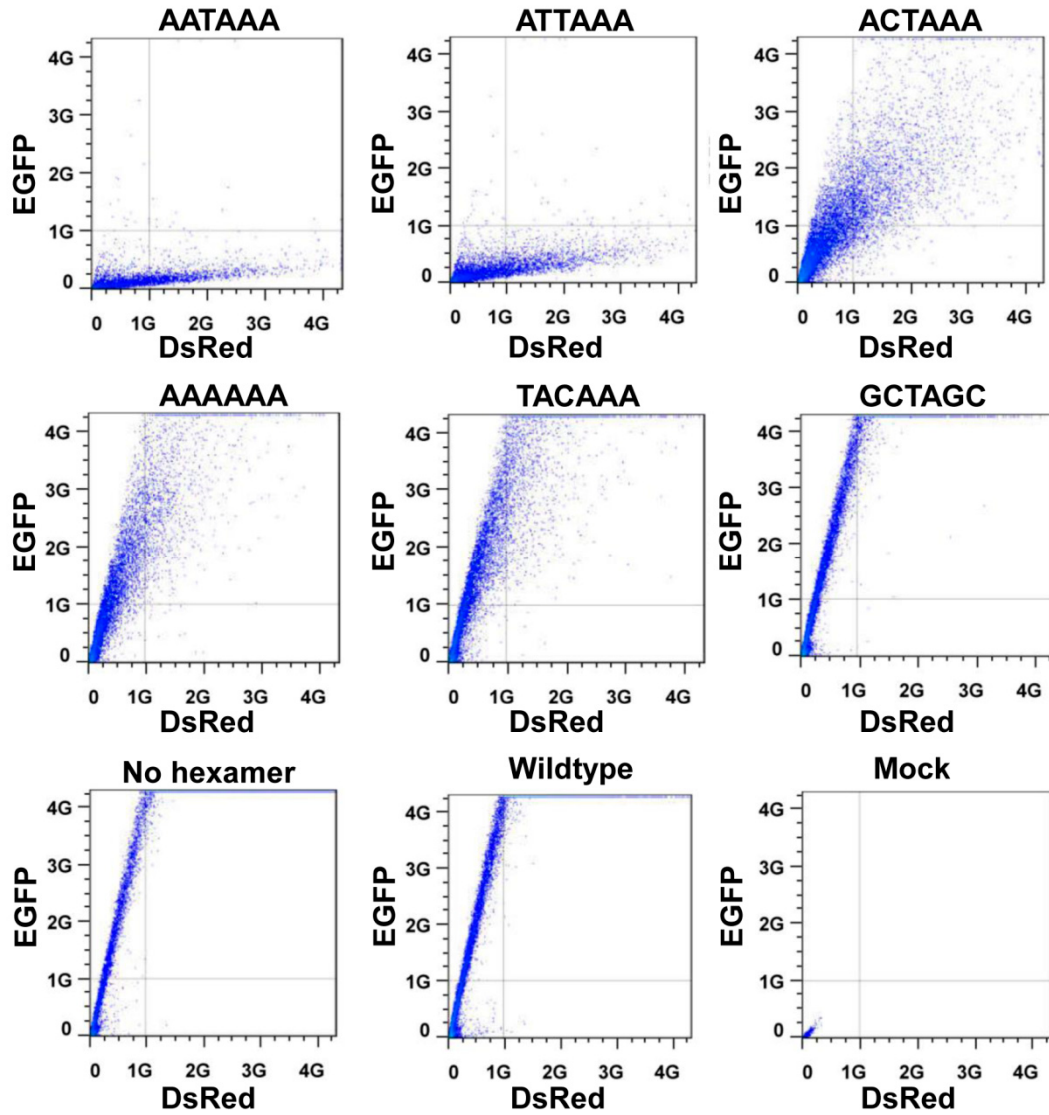


Figure S1. Fluorescent activated cell sorting (FACS) of HEK293 cells transfected with wildtype or mutant SPA constructs with different hexamer variants. HEK293 cells determined to be expressing both red and green were gated in the untransfected sample. The Y axis represents intensity of green fluorescence (EGFP) and the X axis represents the intensity of red fluorescence (DsRed). The slope represents the EGFP/DsRed ratio.

CD47-pA1 (200bp up-and downstream of the cleavage site)

AGTGAAGTGATGGACTCCGATTTGGAGAGTAGTAAGACGTGAAAGGAAT
ACACTTGTGTTTAAGCACCATTGGCCTTGATGATTCAGTGTGGGGAGAAG
AAACAAGAAAAGTAAGTGGTTGTCACCTATGAGACCCTTACGTGATTGTT
AGTTAAGTTTTATTCAAAGCAGCTGTAATTAGTTAATAAAATAATTATG
ATCTATGTTGTTTGCCCAATTGAGATCCA GTTTTTGTTGTTATTTTAAATC
AATTAGGGGCAATAGTAGAATGGACAATTTCCAAGAATGATGCCTTTCAG
GTCCTAGGGCCTCTGGCCTCTAGGTAACCAGTTTAAATTGGTTCAGGGTGA
TAACTACTTAGCACTGCCCTGGTGATTACCCAGAGATATCTATGAAAACC
AGTGG

CD47-pA2 (200bp up-and downstream of the cleavage site)

CATTTTAAGCTATTTTGTGTTGGGCTATTTCTATTGCTGCTACAGCAGACCAC
AAGCACATTTCTGAAAAATTTAATTTATTAATGTAATTTTAAGTTGCTTAT
ATTCTAGGTAACCAATGTAAGAATGATTTAAATATTAATTATGAA TTTT
TGAGTATAATACCCAATAAGCTTTTAATTAGAGCAGAGTTTTAATTAAAA
GTTTTAAATCAGTCCAATGTGTTTCATAATTATTTTTTAATTTCTCCATGG
TGCAGTGAAAACATCACATGAGAAAGCAGGAGACCTGGGTGCTAGTCCTG
GCTACACTACCAGCTAGCAGAGGACCTGGGAAAGCTACTTCCCTGGATCT
CAGTGTCCGTCTCTGCCTGTAGAATGAGATAAGTCTCTTCAAGACTGAAAT
GTATGGTTCTCCATTTTAATTATCAG

Figure S2. The sequences and *cis*-acting elements of the two pA sites of human CD47 gene. Yellow = AAUAAA or AAUAAA like hexamer, Arrowhead = cleavage site, Blue = downstream U/GU-rich element. Green = upstream auxiliary elements UGUA and U-rich element.

Table S1. Oligos used for construction of the wildtype reporter vectors and various constructs.

Oligo name	Sequence (5' to 3') ^a
Oligos for construction of the 4 wildtype dicistronic vectors	
DsRed-homology F	TCTCGAGCTCAAGCTTCATGGACAACACCGAGGACGTCATCA
DsRed-homology R	TGAGTCCGGACTGGGAGCCGAGTGGCGGGCCTCGGCGTGCTC
IRES-homology F	GTTTTTGTGTGAGGATCTGAATTCGCCCTCTCCCTCCCCCCCCCTAA
IRES-homology R	CTTAGCATCGGCCATGGTTGTGGCCATATTATCATCGTGTTTTC
pTK-homology F1	CGGAAGATCTAAATGAGTCTTCGGACCTCGCG
pTK-homology R1	GGCAGCTAGCGTGGCTTTACCAACAGTACCGGAAT
pTK-homology F2	AGATCTAAATGAGTCTTCGGACCTCGCG
pTK-homology R2	GCTAGCGTGGCTTTACCAACAGTACCGGAAT
pIRES2-EGFP-reverse F	TCCAGTCCGGACTCAGATCTCGAGCTCAA
pIRES2-EGFP-reverse R	GAAGCTTGAGCTCGAGATCTGAGTCCGGTAG
psiCHECK2-reverse F	ACCATGGCCGATGCTAAGAACATTA
psiCHECK2-reverse R	AGATCCTCACACAAAAAACCAACACAC
pIRES2-EGFPreverse F2	TGTTGGTAAAGCCACGCTAGCGCTACCGGACTCAGATCTCGAGCT
pIRES2-EGFPreverse R2	GTCCGAAGACTCATTTAGATCTGACCCCGTAATTGATTACTATTAATAACTA
psiCHECK2-reverse F2	TGTTGGTAAAGCCACGCTAGCCACCATGGCTTCCAAGGTGTACGACCCCGAGC
psiCHECK2-reverse R2	GTCCGAAGACTCATTTAGATCTGTCGAGCCATGTGAGCAAAAGGCCAGCAAAAG
Oligos for construction of SPA or SPA mutant constructs	

Red-GFP-PAS-wild-F	CTGCGGAATTCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGG
Red-GFP-PAS-R	CTGCGGGATCCTCACACAAAAACCAACACACAGATGTAATGAAAAT
PSI-wild-F	CTCCGCTCGAGAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGG
PSI-R	CTGCGGAATTCTCACACAAAAACCAACACACAGATGTAATGAAAAT
Red-GFP-PAS-mut1-F	CTGCGGAATTCANTAAAAATATCTTTATTTTCATTACATCTGTGTGTTGG
Red-GFP-PAS-mut3-F	CTGCGGAATTCNANAANATATCTTTATTTTCATTACATCTGTGTGTTGG
Red-GFP-PAS-rad-F	CTGCGGAATTCNNNNNNATATCTTTATTTTCATTACATCTGTGTGTTGG
Red-GFP-PAS-del-F	CTGCGGAATTCATATCTTTATTTTCATTACATCTGTGTGTTGG
PSI-mut1-F	CTCCGCTCGAGANTAAAAATATCTTTATTTTCATTACATCTGTGTGTTGG
PSI-mut3-F	CTCCGCTCGAGNANAANATATCTTTATTTTCATTACATCTGTGTGTTGG
PSI-rad-F	CTCCGCTCGAGNNNNNNATATCTTTATTTTCATTACATCTGTGTGTTGG
PSI-del-F	CTCCGCTCGAGATATCTTTATTTTCATTACATCTGTGTGTTGG
Oligos for construction of CD47 pA site constructs	
CD47PA1-F- <i>EcoRI</i>	GCGAGGAATTCAGTGAAGTGATGGACTCCGATT
CD47PA1-R- <i>BamHI</i>	GCGAGGGATCCCCACTGGTTTTCATAGATATCTC
CD47PA1-F- <i>XhoI</i>	GCGAGCTCGAGAGTGAAGTGATGGACTCCGATT
CD47PA1-R- <i>EcoRI</i>	GCGAGGAATTCCTGATAATTTAAATGGAGAACC
CD47PA1-F- <i>NotI</i>	AAGGAAAAAAGCGGCCCGCAGTGAAGTGATGGACTCCGATT
CD47PA1-R- <i>AflII</i>	GCGAGCTTAAGCCACTGGTTTTCATAGATATCTC
CD47PA1-F- <i>XbaI</i>	GCGAGTCTAGAAGTGAAGTGATGGACTCCGATT
CD47PA2-F- <i>EcoRI</i>	GCGAGGAATTCATTTTAAGCTATTTTGTGTTGGC
CD47PA2-R- <i>BamHI</i>	GCGAGGGATCCCTGATAATTTAAATGGAGAACC
CD47PA2-F- <i>XhoI</i>	GCGAGCTCGAGCATTTTAAGCTATTTTGTGTTGGC
CD47PA2-R- <i>EcoRI</i>	GCGAGGAATTCCTGATAATTTAAATGGAGAACC
CD47PA2-F- <i>NotI</i>	AAGGAAAAAAGCGGCCCGCATTTTAAGCTATTTTGTGTTGGC
CD47PA2-R- <i>AflII</i>	GCGAGCTTAAGCTGATAATTTAAATGGAGAACC
CD47PA2-F- <i>XbaI</i>	GCGAGTCTAGACATTTTAAGCTATTTTGTGTTGGC

^a The italic letters represent the shared homology sequences between the corresponding insert and the corresponding linearized plasmids or vectors.

Table S2. Primer pairs used for qRT-PCR analysis of the reporter or reference genes.

Gene name (abbreviation)	Sequence (5' to 3') ^a	Product length (bp)	Primer efficiency (%)	R ² ^b
DsRed	F:GCCCCGTAATGCAGAAGAAG	101	99.37	0.9999
DsRed	R:CTTCAGGGCGTGGGAGATCT			
EGFP	F:GGGCACAAGCTGGAGTACAAC	101	100.11	0.9991
EGFP	R:ATGTTGTGGCGGATCTTGAAG			
Kana	F:GCCGAATATCATGGTGGAAA	106	95.55	0.9998
Kana	R:AATATCACGGGTAGCCAACG			
Hluc	F:CGTGCCAGAGTCTTTCGACA	106	101.05	0.9997
Hluc	R:ACAGGCGGTGCGATGAG			
HRLuc	F:AAGAGCGAAGAGGGCGAGAA	217	95.96	0.9995
HRLuc	R:TGCGGACAATCTGGACGAC			
Amp	F:ACTCGGTCGCCGCATACACTA	178	103.30	0.9999
Amp	R:GGTTAGCTCCTTCGGTCCTCC			

^a F and R respectively indicate forward primer and reverse primer. ^b R² refers to the coefficient of determination.