

## Supplementary Information

### Development of a modularized aptamer targeting the nuclear T-cell suppressor PAC1

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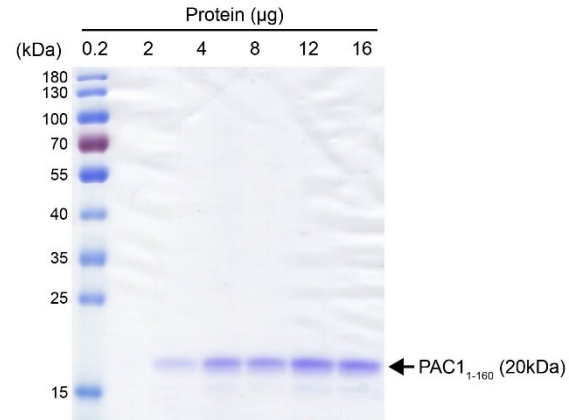
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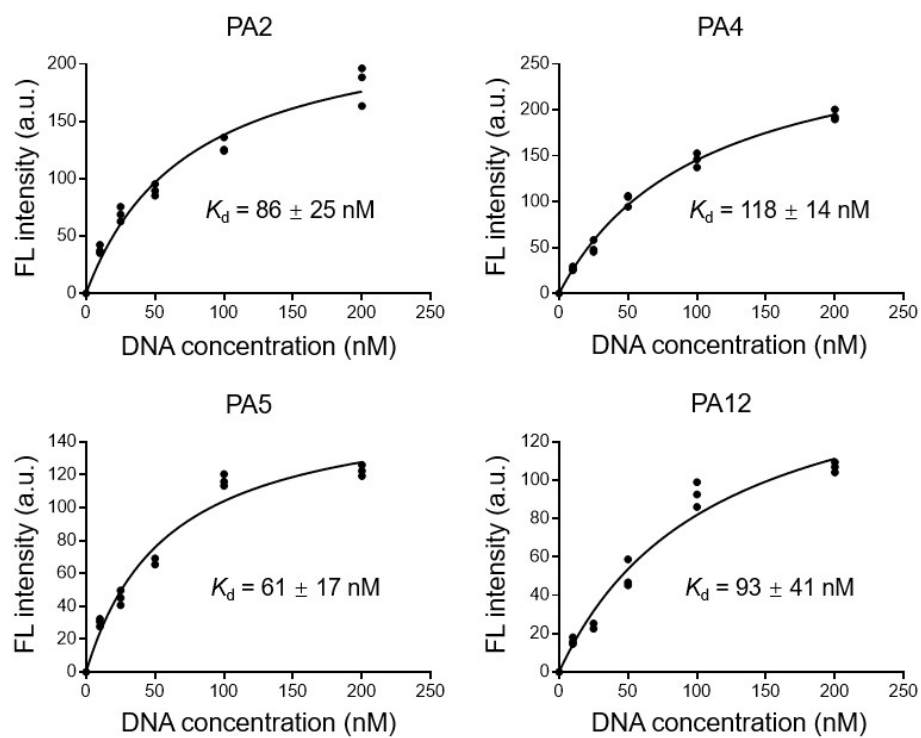
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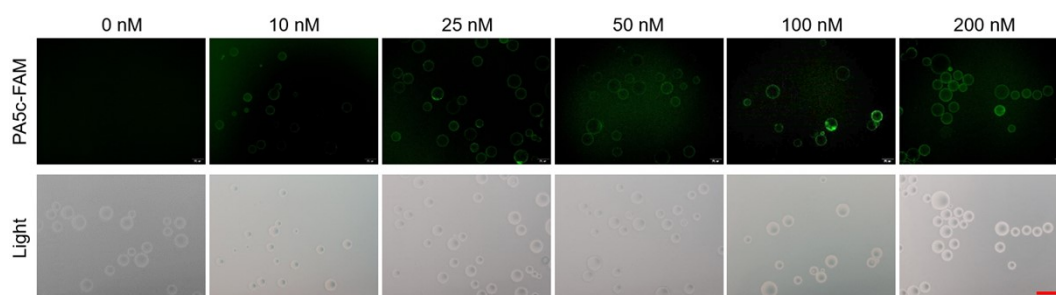
† First authors: These authors contributed equally to the work. Zixi Hu and Zhongyu Jiang



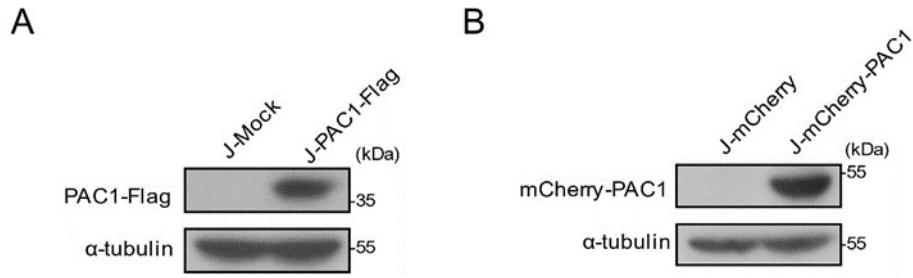
**Fig. S1** The incubation ratio of His-tagged PAC1<sub>1-160</sub> protein and Ni-beads was optimized.



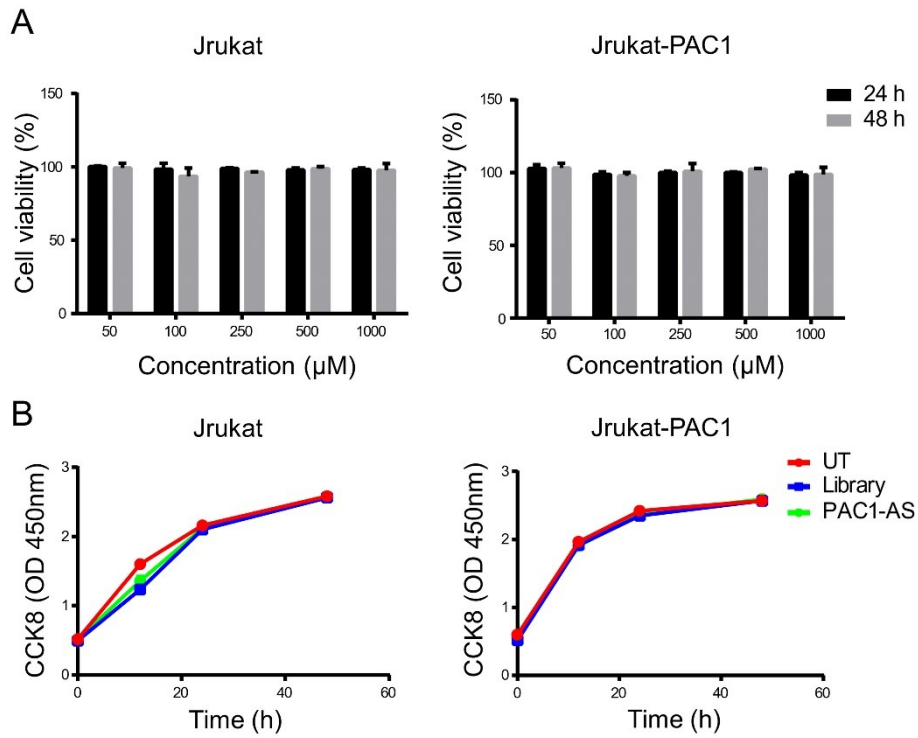
**Fig. S2** The  $K_d$  value of candidate aptamers PA2, PA4, PA5 and PA12.



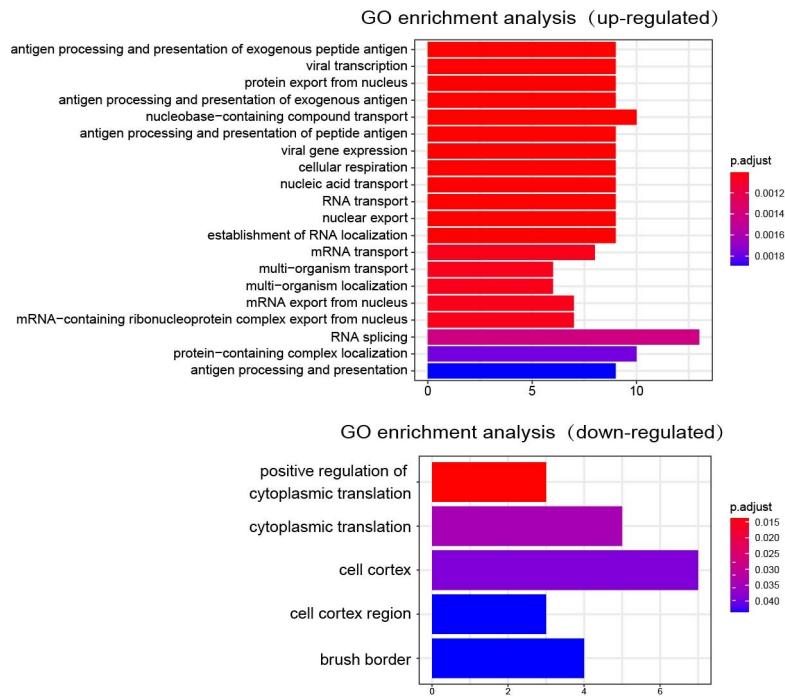
**Fig. S3** Inspected the binding ability of PA5c in different concentrations with P-beads by fluorescence microscopy. Scale bars: 100  $\mu$ m



**Fig. S4** The expression of PAC1 in J-PAC1-Flag cells (A) and J-mCherry-PAC1 cells (B) compared to J-Mock.



**Fig. S5** The toxicity of aptamer PAC1-AS-p to Jurkat and Jurkat-PAC1 cells was detected by CCK8 kit.



**Fig. S6** Jurkat cells untreated or stimulated with PMA plus ionomycin for 3 h. Mass spectrometry was used to identify the PAC1 interactome after treated with or without PAC1-AS. Then, the GO enrichment analysis up/down-regulated proteins from the data of mass spectrometry. All the target proteins in the GO enrichment analysis were significant ( $P < 0.05$ ). The abscissa represents the number of enriched proteins.

**Table. S1** Conditions used in SELEX.

Round	Dosage of library (pmol)	Negative selection		Positive selection		Temperature (°C)	Washing buffer volume (mL)	PCR cycles
		Ni-beads (μL)	duration (min)	Protein (μg)	duration (min)			
1	5000	-	-	60	60	4	1	5
2	1000	-	-	60	60	4	1	7
3	500	-	-	60	60	4	1	7
4	200	5	30	30	60	4	1 × 2	7
5	200	5	40	30	50	4	1 × 2	11
6	200	10	50	30	40	4	1 × 3	7
7	200	10	60	30	30	4	1 × 3	7
8	200	10	60	30	30	4	1 × 3	7

**Table. S2** Sequences of aptamer candidates.

Name	Sequence 5'-3'	$K_d$
	ATCCAGAGTGACGCAGCACCCACCCGCACG	
PA2	TCATTTCCACCCTTCTCTACTTCTCTCTGGAC ACGGTGGCTTAGT	$86 \pm 25$ nM
	ATCCAGAGTGACGCAGCACCTTACATCCCGC	
PA4	ATACAGCCCGCTCACACCCTCCCTTAGTGGA CACGGTGGCTTAGT	$118 \pm 14$ nM
	ATCCAGAGTGACGCAGCACCTACATTCACCG	
PA5	TCTCACTTCTCCCCTCTCGTTCCCCTCTGGAC ACGGTGGCTTAGT	$61 \pm 17$ nM
	ATCCAGAGTGACGCAGCAGTGCTCGTGCCCC	
PA12	CGTACCCTTGCTCTAGACCTCTCCCTCTGGAC ACGGTGGCTTAGT	$93 \pm 41$ nM

**Table. S3** The truncated sequences of PA5.

<b>Name</b>	<b>Sequence 5'-3'</b>
PA5a (1-58bp)	ATCCAGAGTGACGCAGCACCTACATTCACCGTCTCAC TTCTCCCCTCTCGTTCCCCTC
PA5b (19-76bp)	CCTACATTCACCGTCTCACTTCTCCCCTCTCGTTCCCCT CTGGACACGGTGGCTTAGT
PA5c (19-58bp)	CCTACATTCACCGTCTCACTTCTCCCCTCTCGTTCCCCT C
PA5d (25-52bp)	TTCACCGTCTCACTTCTCCCCTCTCGTT



**Table. S4** Aptamer with artificial bases. (Phosphorodithiate, \*)

<b>Name</b>	<b>Sequence 5'-3'</b>
AS1411-L	<i>GCGTTTTCGCGGTGGTGGTGGTTGTGGTGGTGGTGG</i>
PA5c-L	<i>GCGAAAACGCCCTACATTCACCGTCTCACTTCTCCCC TCTCGTTCCCCTC</i>
AS1411-p	<i>GCGTTTTCGCGGTGGTGGTGGTTGTGGTGGTGG*T*G* G</i>
PA5c-p	<i>GCGAAAACGCCCTACATTCACCGTCTCACTTCTCCCC TCTCGTTCCC*C*T*C</i>

**Table. S5** The sequences of the qRT-PCR primers.

<b>Name</b>	<b>Sequence 5'-3'</b>
<i>Tnf</i> -F	AACTTGTGTTTCACAGTCCGTTT
<i>Tnf</i> -R	GCCTCCCTCTCATCAGTTCTATG
<i>Gzmb</i> -F	GAGAGGACTTTGTGCTGACTGC
<i>Gzmb</i> -R	GCTGGGTCTTCTCCTGTTCTTTG
<i>Il2</i> -F	CGACCAGAACATCCAGAAGAAT
<i>Il2</i> -R	AGAGACATAAACAGCAGGTCCA
<i>Ifng</i> -F	GGGTTCTCTTGGCTGTTACT
<i>Ifng</i> -R	GAGTTCCATTATCCGCTACATCT
<i>Pac1</i> -F	CAAGAGTATCCCTGTGGAGGAC
<i>Pac1</i> -R	GAAACTGAAGTTGGGGGAGATG
<i>Pd-1</i> -F	CAGGATGGTTCTTAGACTCCC
<i>Pd-1</i> -R	GCTCATGCGGTACCAGTTTA
<i>Ctla4</i> -F	CAGTGGAAATCAAGTGAACCTC
<i>Ctla4</i> -R	GAGGAGGAAGTCAGAATCTGG
<i>Tim3</i> -F	GGAGCCTCCCTGATATAAATCTAAC
<i>Tim3</i> -R	GCTCCGATGTAGATGCCTATTC
<i>Tigit</i> -F	GCCAGGTTCCAGATTCCATT
<i>Tigit</i> -R	GGATTCTGAGGGCTTTCTTCTT
<i>Gapdh</i> -F	ACCCACTCCTCCACCTTTGA
<i>Gapdh</i> -R	CTGTTGCTGTAGCCAAATTCGT