Supplementary Figures and Tables

G-quadruplexes are specifically recognized and distinguished by selected designed ankyrin repeat proteins

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	Salt and target oligonucleotide	Library
1C6	NaCl, tellong	N3C
1C7	NaCl, tellong	N3C
1C11	NaCl, tellong	N3C
1G2	KCl, tellong	N3C
1G11	KCl, tellong	N3C
1H4	KCl, teltt	N3C
2C10	NaCl, tellong	N3C
2D10	NaCl, teltt	N3C
2E4	KCl, tellong	N2C
2G7	KCl, tellong	N3C
2G10	KCl, tellong	N3C

Table ST1. Selection conditions of those DARPins that were characterized in detail

tel				
	k _{on}		k _{off}	K _D
	$M^{-1} \times s^{-1} \times 10^{-1}$	0 ⁶	s ⁻¹ × 10 ⁻³	$M \times 10^{-9}$
1C6	2.6 ±	0.9	40 ± 10	16 ± 2
1C7	1.2 ±	0.4	40 ± 1	37 ± 15
1C11	1.6 ±	1.1	74 ± 20	53 ± 22
1G2	$1.0 \pm$	0.4	45 ± 21	53 ± 44
1G11	0.055 ± 0.00	056	4.1 ± 2.9	72 ± 46
1H4	1.6 ±	1.0	82 ± 24	62 ± 34
2C10	1.5 ±	0.3	68 ± 10	48 ± 12
2D10	2.0 ±	0.4	64 ± 14	34 ± 16
2E4				
2G7	1.5 ±	0.1	30 ± 0	20 ± 2
2G10				

Table ST2. Kinetic data obtained with SPR in TBS

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	k _{on}		k _{off}	K _D
	M ⁻¹ × s ⁻¹ ×	< 10 ⁶	s ⁻¹ × 10 ⁻³	$M \times 10^{-9}$
1C6				
1C7	1.9 ±	0.6	62 ± 16	33 ± 8
1C11	1.3 ±	0.9	42 ± 2	43 ± 29
1G2	3.5 ±	0.7	90 ± 14	27 ± 9
1G11	0.14 ±	0.14	4.1 ± 1.8	44 ± 30
1H4				
2C10				
2D10				
2E4				
2G7				
2G10				

c-MYC

	k _{on}		k _{off}	K _D
	M ⁻¹ × s ⁻¹ ×	10 ⁻⁶	s ⁻¹ × 10 ⁻³	$M \times 10^{-9}$
1C6				
1C7	1.2 ±	0.6	79 ± 20	81 ± 38
1C11	2.1 ±	1.7	79 ± 27	49 ± 27
1G2	2.4 ±	0.9	95 ± 7	43 ± 19
1G11	0.12 ±	0.06	2.1 ± 0.9	19 ± 2
1H4				
2C10				
2D10				
2E4	2.7 ±	2.1	130 ± 30	64 ± 40
2G7				
2G10	0.95 ±	0.59	68 ± 2	90 ± 59

Table ST3. Kinetic data ol	btained with	SPR in 1	BS-KCI
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tel

	к _{оп, 1}	к _{оff, 1}	К _{D, 1}	к _{оп, 2}	к _{оff, 2}	К _{D, 2}
	$M^{-1} \times s^{-1} \times 10^{6}$	s ⁻¹ × 10 ⁻³	$M \times 10^{-9}$	$M^{-1} \times s^{-1} \times 10^{6}$	s ⁻¹ × 10 ⁻³	$M \times 10^{-9}$
1C6						
1C7	1.5 ± 0.2	23 ± 1.2	15 ± 1	0.062 ± 0.035	0.28 ± 0.36	3.3 ± 3.0
1C11	2.6 ± 2.7	33 ± 38	11 ± 2	0.83 ± 1.27	14 ± 24	8.9 ± 8.3
1G2	3.6 ± 1.0	28 ± 7	7.9 ± 1.9	1.0 ± 0.2	3.3 ± 0.5	3.3 ± 1.1
1G11						
1H4	2.5 ± 0.8	36 ± 4	15 ± 4	0.11 ± 0.05	1.3 ± 0.1	14 ± 7
2C10	4.2 ± 1.0	83 ± 17	20 ± 1	0.24 ± 0.04	7.8 ± 5.1	35 ± 28
2D10	4.5 ± 1.0	78 ± 13	18 ± 3	0.29 ± 0.10	6.2 ± 2.4	22 ± 2
2E4	4.3 ± 5.7	6.4 ± 6.3	4.6 ± 4.7	3.7 ± 2.3	100 ± 83	42 ± 48
2G7	2.4 ± 1.1	22 ± 10	10 ± 5	0.55 ± 0.08	2.9 ± 0.6	5.5 ± 1.9
2G10	3.5 ± 2.7	141 ± 87	65 ± 69	0.21 ± 0.12	4.0 ± 4.7	15 ± 11
ILPR						
	k _{on, 1}	к _{оff, 1}	К _{D, 1}	к _{оп, 2}	k _{off, 2}	К _{D, 2}
	$M^{-1} \times s^{-1} \times 10^{6}$	s ⁻¹ × 10 ⁻³	M × 10 ⁻⁹	$M^{-1} \times s^{-1} \times 10^{6}$	s ⁻¹ × 10 ⁻³	$M \times 10^{-9}$
1C6						
1C7	2.4 ± 0.2	50 ± 3	21 ± 2	0.047 ± 0.011	0.61 ± 0.16	14 ± 8

100						
1C7	2.4 ± 0.2	50 ± 3	21 ± 2	0.047 ± 0.011	0.61 ± 0.16	14 ± 8
1C11	9.3 ± 3.5	110 ± 50	12 ± 2	a)	a)	4.0 ± 4.6
1G2	16 ± 10	128 ± 25	9.4 ± 3.2	0.25 ± 0.21	0.68 ± 0.27	6.4 ± 7.4
1G11						
1H4						
2C10						
2D10						
2E4						
2G7						
2G10	8.5 ± 3.0	179 ± 36	22 ± 5	0.10 ± 0.08	1.1 ± 1.1	19 ± 24
	a) low values in	ndicate a differe	nt kinetic mode	of this DARPin		

	k _{on, 1}	k _{off, 1}	К _{D, 1}	к _{оп, 2}	к _{оff, 2}	К _{D, 2}
	$M^{-1} \times s^{-1} \times 10^{6}$	s ⁻¹ × 10 ⁻³	M × 10 ⁻⁹	M ⁻¹ × s ⁻¹ × 10 ⁶	s ⁻¹ × 10 ⁻³	M × 10 ⁻⁹
1C6						
1C7						
1C11	3.7 ± 0.7	64 ± 21	17 ± 3	0.015 ± 0.026	0.10 ± 0.17	6.8 ± 4.3
1G2	6.6 ± 2.4	97 ± 17	16 ± 5	0.095 ± 0.039	1.2 ± 0.3	14 ± 4
1G11						
1H4						
2C10						
2D10						
2E4	2.7 ± 0.9	67 ± 10	28 ± 13	1.9 ± 3.2	4.2 ± 4.7	19 ± 27
2G7						
2G10	2.8 ± 2.1	97 ± 61	104 ± 150	0.66 ± 0.96	7.0 ± 9.3	15 ± 5

Figure Legends:

Figure S1-S6. k_{on} , k_{off} and K_D values calculated from SPR data. S1: TBS, *tel*; S2: TBS-KCl, *tel*; S3: TBS, *ILPR*; S4: TBS-KCl, *ILPR*; S5: TBS, *c-Myc*; S6: TBS-KCl, *c-Myc*. For measurements in TBS-KCl, a heterogeneous ligand model was used; values for both binding events are given. Order of a group of bars is always: k_{on} , k_{off} , K_D

Figure S7. Sequences of selected DARPins. The differences to the consensus at randomized positions (indicated by X in consensus) and framework mutations are shown. Residues that had been randomized in the original design are boxed. DARPins 2E4 contains two internal repeats (N2C), all others contain 3 internal repeats (N3C).





















	2	DVNA											 	
	10	KXGA.	- H -	N -	- H -	- H -	- H -	N-	- <u>H</u> -	- <u>H</u> -	- <u>H</u> -	- <u>H</u> -	H H H	
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2. re	90	AXXG	- SW -	-GW-	- GY -	-SW-	- GY -	- GY-	-KF-	- GY-	-SR-	- д <u>7</u> -	- KW-	
	85	PLHLA												
	30	XdXTT	- <u>R</u> -	<u>и-М-</u>	- <u>-</u>	H	- <u>K</u> -	<u>7</u> - C	I-H-B	<u>ч-</u> п	W-R	- <u>-</u> 7-0	전-원-0	
	س-	AXDX		-R-RI	-H-I(-H-KJ	- H -	-K-IO	-F-N	H H	-M-HV	- K - I (-MDRI	
	7	ADVN												
	70	LKXG	- <u>7</u> -	- <u>N</u> -	YS	- <u>H</u>	- 7	- <u>N</u>	-HN-	- 7	- 7	- <u>N</u> -	- H	
	65	VEVL												
epeat	60	HLEI												
1. r	55	AAKX	LA-	RT-	RW-	Υр	KS-	AK	SH-	RT	WM	AK-	AW	
	0	PLHL.				1 1 1		1 1 1		R -				
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	40	ADVNAXD	- <u>0</u>	R-		N	<u> </u>		<u></u>	<u>S</u> -	K	<u>-</u>	- - - - -	
	35 40	MANGADVNAXD	-000	R		N	<u> </u>	<u>H</u>		<u>S</u> -	K		-I	
	30 35 40	EVRILMANGADVNAXD	000	<u>R</u> -	SS- K -			<u></u>	<u>T</u> -	<u>S</u> -	K			
	5 30 35 40	GQDDEVRILMANGADVNA <mark>X</mark> D	0	<u>R</u> -			<u> </u>	<u>_</u>				<u>_</u>	<u> </u>	
-cap	0 25 30 35 40	5. A A R A G Q D D E V R I L M A N G A D V N A X D	-0	<mark>R</mark> -	S- <u>K</u> -		<u></u>	<u></u>		<mark>S</mark> -		<u> </u>	<u>-</u>	
N-cap	20 25 30 35 40	KKLLEAARAGQDDEVRILMANGADVNA <mark>X</mark> D	-0		S- <mark>K</mark> -		<u></u>	<u></u>				<u></u>	<u> </u>	
N-cap	15 20 25 30 35 40	SDLGKKLLEAARAGQDDEVRILMANGADVNAXD	-0	<u></u>	S- <mark>K</mark> -		<u> </u>		<u></u>			<u></u>	<u>-</u>	
N-cap	10 15 20 25 30 35 40	IHHHGSDLGKKLLEAARAGQDDEVRILMANGADVNA <mark>XD</mark>	0	<u></u>		<u>-</u> N	<u>.</u>	<u></u>	<u> </u>	<u>S</u>	<u></u>	<u></u>	- <u>-</u>	
N-cap	5 10 15 20 25 30 35 40	GSHHHHHHGSDLGKKLLEAARAGQDDEVRILMANGADVNA <mark>XD</mark>	0	<u></u>			<u></u>	<u>L</u>		<u>S</u>		T	<u>-</u> <u>-</u>	
N-cap	5 10 15 20 25 30 35 40	s MRGSHHHHHHGSDLGKKLLEAARAGQDDEVRILMANGADVNAXD	0	<u></u>	S- <u>W</u> -	<u>N</u>	<u> </u>	<u>L</u>	T	<u>S</u>	<u>M</u>	<u> </u>	<u>-</u> <u>I</u> - <u>-</u> <u>-</u> - <u>-</u>	
N-cap	5 10 15 20 25 30 35 40	sensus Mrgshhhhhhgsdlgkklleaaragoddevrilmangadvna <mark>xp</mark>		<u></u>	1S- <mark>K</mark>	<u>N</u>	1		TC	SS	<u></u>		-II	

	3. repeat	C-cap
	110 115 120 125 130 135 140	145 150 155 160 165
	кркхфкгргнгаахснгетvevrrkgadvna	QDKFGKTAFDISIDNGNEDLAEILQKLN
1C6	I-AS-HTWYY	
1C7	M-KM-KXNX	
1C11	K-TT-RLWNVNV	
1G2	D-DS-HGIFYY	
1G11	S-VR-TFYKYY	
1H4	N-SS-THFNN	
2C10	R-YH-SHNMHH	
2D10	N-QL-DHWYY	
2E4		
2G7	N-IN-STYCC	
2G10	R-kK-Fuwuw	

Figure S7