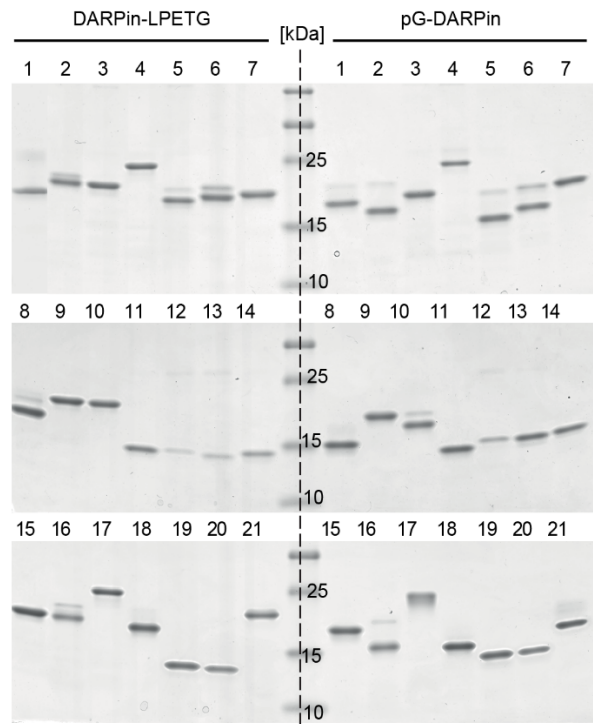


A

SrtA-ID	DARPin	Target	Format	Plückthun Reference
1	Ac2	EpCAM	N3C	Stefan et al., 2011
2	EC4	EpCAM	N3C	Stefan et al., 2011
3	E01	EGFR_ECD3	N3C	Steiner et al., 2008
4	E69	EGFR_ECD1	N4C	Steiner et al., 2008
5	E68	EGFR_ECD3	N3C	Steiner et al., 2008
6	E72	EGFR	N3C	Steiner et al., 2008
7	901	HER2_ECD1	N3C	Steiner et al., 2008
8	926E	HER2_ECD1	N3C	Tamaskovic et al., 2016
9	929	HER2_ECD1	N3C	Steiner et al., 2008
10	H14R	HER2_ECD4	N3C	Steiner et al., 2008
11	G3	HER2_ECD4	N2C	Zahnd et al., 2007
12	A1	HER3	N2C	this manuscript
13	F3	HER3, Ligand Competitor	N2C	this manuscript
14	C7x	HER3	N2C	this manuscript
15	B4_02	HER4	N3C	Steiner et al., 2008
16	4.05	HER4	N3C	Steiner et al., 2008
17	B4_50	HER4	N4C	Steiner et al., 2008
18	D2A	MET_ECD_A1	N2C	Andres et al., 2019
19	C9A	MET_ECD_B	N3C	Andres et al., 2019
20	A2C	MET_ECD_C3	N3C	Andres et al., 2019
21	Off7	MBP	N3C	Birz et al., 2004

B

Supplementary Figure S3. Summary of monovalent DARPins binding to different extracellular subdomains on the indicated receptors. **A**, Panel of monovalent DARPins and their corresponding ID number used for the construction of the bivalent DARPin constructs. ECD with a number refers to the extracellular domains containing the epitope, whenever it is known. The specific epitope and a relevant reference are indicated in each case. **B**, SDS-PAGE of monovalent DARPins educts expressed either as an MRGSmyc_DARPin_LPETG_His₆ (left) or as MRGSHis₆_TEV_Gly₅_DARPin (right). Proteins were purified by IMAC. Note that different sizes are due to the size of the DARPin (cf. panel **A**) as well as the well-known fact that some DARPins do not completely denature on SDS gels, giving rise to faster running bands or two bands, even though the DARPin is completely pure. Numbers correspond to IDs above. **C**, Sequences of monovalent DARPins binding to different extracellular subdomains, as summarized in Supplementary Figure S3. The DARPin consensus sequence is shown on top, randomized residues are indicated as "X", point mutations are indicated by shading, and deletions by darker shading. The C-cap regions come from different designs and the details can be found in the original references (see **A**).

		N-cap															1st repeat																																																				
SrtA-ID	DARPin	D	L	G	K	K	L	L	E	A	A	R	A	G	Q	D	D	E	V	R	I	L	M	A	N	G	A	D	V	N	A	X	D	X	X	G	X	T	P	L	H	L	A	A	X	X	G	H	L	E	I	V	E	V	L	L	K	X	G	A	D	V	N	A					
1	Ac2	D	L	G	K	K	L	L	E	A	A	R	A	G	Q	D	D	E	V	R	I	L	M	A	N	G	A	D	V	N	A	X	D	E	Y	G	S	T	P	L	H	L	A	A	T	L	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A					
2	Ec4	D	L	G	K	K	L	L	E	A	A	R	A	G	Q	D	D	E	V	R	I	L	M	A	N	G	A	D	V	N	A	X	D	F	T	G	W	T	P	L	H	L	A	A	T	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A					
3	E01	D	L	G	K	K	L	L	E	A	A	R	A	G	Q	D	D	E	V	R	I	L	M	A	N	G	A	D	V	N	A	X	D	D	T	W	G	T	P	L	H	L	A	A	Y	Q	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A				
4	E69	D	L	G	K	K	L	L	E	A	A	R	A	G	Q	D	D	E	V	R	I	L	M	A	N	G	A	D	V	N	A	X	D	D	N	A	G	R	T	P	L	H	L	A	A	F	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A				
5	E68	D	L	G	K	K	L	L	E	A	A	R	A	G	Q	D	D	E	V	R	I	L	M	A	N	G	A	D	V	N	A	X	D	F	D	Y	W	G	M	T	P	L	H	L	A	A	D	N	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A			
6	E72	D	L	G	K	K	L	L	E	A	A	R	A	G	Q	D	D	E	V	R	I	L	M	A	N	G	A	D	V	N	A	X	D	I	D	S	T	G	T	P	L	H	L	A	A	S	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A				
7	901	D	L	G	K	K	L	L	E	A	A	R	A	G	Q	D	D	E	V	R	I	L	M	A	N	G	A	D	V	N	A	X	D	A	E	D	E	G	R	T	P	L	H	L	A	A	S	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A			
8	926E	D	L	G	K	K	L	L	E	A	A	R	A	G	Q	D	D	E	V	R	I	L	M	A	N	G	A	D	V	N	A	X	D	F	Y	G	I	T	P	L	H	L	A	A	Y	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A					
9	929	D	L	G	K	K	L	L	E	A	A	R	A	G	Q	D	D	E	V	R	I	L	M	A	N	G	A	D	V	N	A	X	D	H	F	Y	G	I	T	P	L	H	L	A	A	F	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A				
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11	G3	D	L	G	K	K	L	L	E	A	A	R	A	G	Q	D	D	E	V	R	I	L	M	A	N	G	A	D	V	N	A	X	D	K	E	D	E	G	L	T	P	L	H	L	A	A	T	A	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A		
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16	4.05	D	L	G	K	K	L	L	E	A	A	R	A	G	Q	D	D	E	V	R	I	L	M	A	N	G	A	D	V	N	A	X	D	N	A	R	V	F	G	W	T	P	L	H	L	A	A	V	D	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A	
17	B4_50	D	L	G	K	K	L	L	E	A	A	R	A	G	Q	D	D	E	V	R	I	L	M	A	N	G	A	D	V	N	A	X	D	N	A	R	H	D	R	Y	G	V	T	P	L	H	L	A	A	F	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A
18	D2A	D	L	G	K	K	L	L	E	A	A	R	A	G	Q	D	D	E	V	R	I	L	M	A	N	G	A	D	V	N	A	X	D	F	D	Y	G	Q	T	P	L	H	L	A	A	F	L	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A			
19	C9A	D	L	G	K	K	L	L	E	A	A	N	N	G	H	D	E	V	R	I	L	M	A	N	G	A	D	V	N	A	X	D	A	D	W	G	R	T	P	L	H	L	A	A	S	M	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A				
20	A2C	D	L	G	K	K	L	L	E	A	A	R	A	G	Q	D	D	E	V	R	I	L	M	A	N	G	A	D	V	N	A	X	D	K	L	F	G	T	P	L	H	L	A	A	W	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A					
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		2nd repeat															3rd repeat																																																								
SrtA-ID	DARPin	X	D	X	X	G	X	T	P	L	H	L	A	A	X	X	G	H	L	E	I	V	E	V	L	L	K	X	G	A	D	V	N	A	X	D	X	X	G	X	T	P	L	H	L	A	A	X	X	G	H	L	E	I	V	E	V	L	L	K	X	G	A	D	V	N	A						
1	Ac2	D	D	A	T	G	L	T	P	L	H	L	A	A	W	N	G	H	L	E	I	V	E	V	L	L	K	Y	G	A	D	V	N	A	X	D	F	E	G	W	T	P	L	H	L	A	A	H	F	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A					
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9	929	F	D	Y	D	N	T	P	L	H	L	A	A	D	A	K	Y	N	G	H	L	E	I	V	E	V	L	L	K	Y	G	A	D	V	N	A	X	D	S	D	R	D	G	T	P	L	H	L	A	A	R	E	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A		
10	H14R	N	D	W	R	G	F	T	P	L	H	L	A	A	L	N	K	Y	N	G	H	L	E	I	V	E	V	L	L	K	Y	G	A	D	V	N	A	X	D	T	D	A	G	N	T	P	L	H	L	A	A	W	F	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A	
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12	A1	S	D	N	F	G	Y	T	P	L	H	L	A	A	Y	N	K	Y	N	G	H	L	E	I	V	E	V	L	L	K	Y	G	A	D	V	N	A	X	D	S	D	N	F	G	Y	T	P	L	H	L	A	A	I	N	H	G	H	L	E	I	V	E	V	L	L	K	D	G	A	D	V	N	A
13	F3	S	D	N	F	G	Y	T																																																																	