Cella Technologies

yeast two-hybrid system services

28 Peptide PRTN1–PRTN2 Interaction Inhibitor Candidates

Protein–protein interaction (PPI) function of a protein–protein interaction domain (PPID) that is involved in PPI between (fictional) Protein 1 (PRTN1) and a protein that interacts with PRTN1 may be prevented specifically by an appropriate short linear peptide. Such short linear peptides / peptide protein–protein interaction inhibitors are highly specific (Figure 1) and have many applications in research—using these tools can lead to new insights.

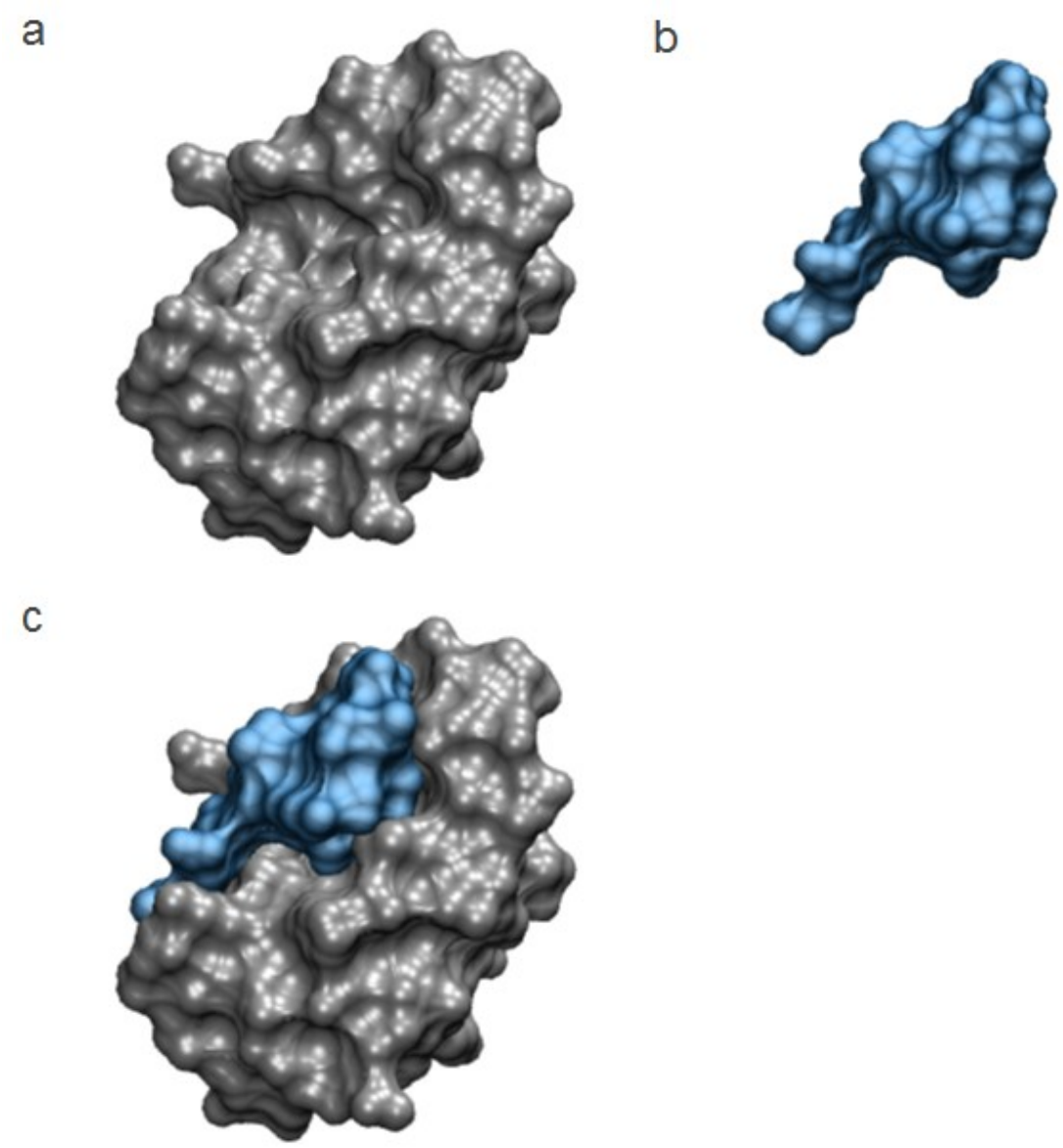

Figure 1. Protein–protein interaction (PPI) function of a protein–protein interaction domain (PPID) prevented specifically. **(a)** *Target*—a representation of the tertiary structure of a PPID of MDM2 protein that has strong protein–protein interaction activity towards a PPID of p53 protein (PDB ID: 1YCR). **(b)** *Tool*—a PPID-fragment (short linear peptide) of p53 protein that has strong PPI activity towards a PPID of MDM2 protein (PDB ID: 1YCR). **(c)** *Tool bound to target*—PPI function of a specific PPID can be prevented by an appropriate short linear peptide (PDB ID: 1YCR).

PRTN1-relevant peptide protein–protein interaction inhibitor development

One or more of the twenty-eight peptides that are indicated in Table 6 below may be able to prevent protein–protein interaction (PPI) function of a protein–protein interaction domain (PPID) of PRTN1 that has PPI activity towards (fictional) Protein 2 (PRTN2).

Using the yeast two-hybrid (Y2H) system (for references and explanation, see our Yeast two-hybrid (Y2H) system web page at https://cellatechnologies.com/tech/yeast-two-hybrid-system) it is possible to demonstrate binding between Rb protein and LTP (a linear peptide of 13 amino acid residues) experimentally (Figure 2a (related tertiary structures are shown in Figures 2b-d))—this showed that the Y2H system/assay can be used to map short protein–protein interaction domains (PPIDs) experimentally.

A hypothetical example—in Table 1 to Table 5 hypothetical PPI between PRTN1(1-500) and PRTN2(1-600) (or a part thereof) is used to demonstrate a straightforward strategy to identify the primary structure of a segment of PRTN2 that contains a protein–protein interaction domain that is involved in PRTN1–PRTN2 interaction; from that segment short linear peptides can be derived (Table 6) that potentially can prevent protein–protein interaction between PRTN1 and PRTN2.

If yeast/bacterial two-hybrid system assay A1 is positive and control assays A2 and A3 are negative (Project 1) (Table 1), then hypothetical PRTN1(1-500)–PRTN2(1-600) interaction is confirmed. In Project 2 (Table 2) three fragments of PRTN2(1-600) are used as prey and, in this example, positive assays A4 and A5 (Table 2) suggest that PRTN2(151-300) contains a PPID that is involved in binding between PRTN1 and PRTN2—it is less likely that PRTN2(1-150) and PRTN2(301-450) both contain such a PPID. Segments of this putative positive region can be tested in similar assays and by repeating this procedure several times, a short region of PRTN2 that seems to be able to significantly bind to PRTN1 can be systematically identified (Table 3 to Table 5). In Project 6, based on the primary structure of PRTN2(226-244) (Table 5), twenty-eight tool candidates are generated (Table 6).

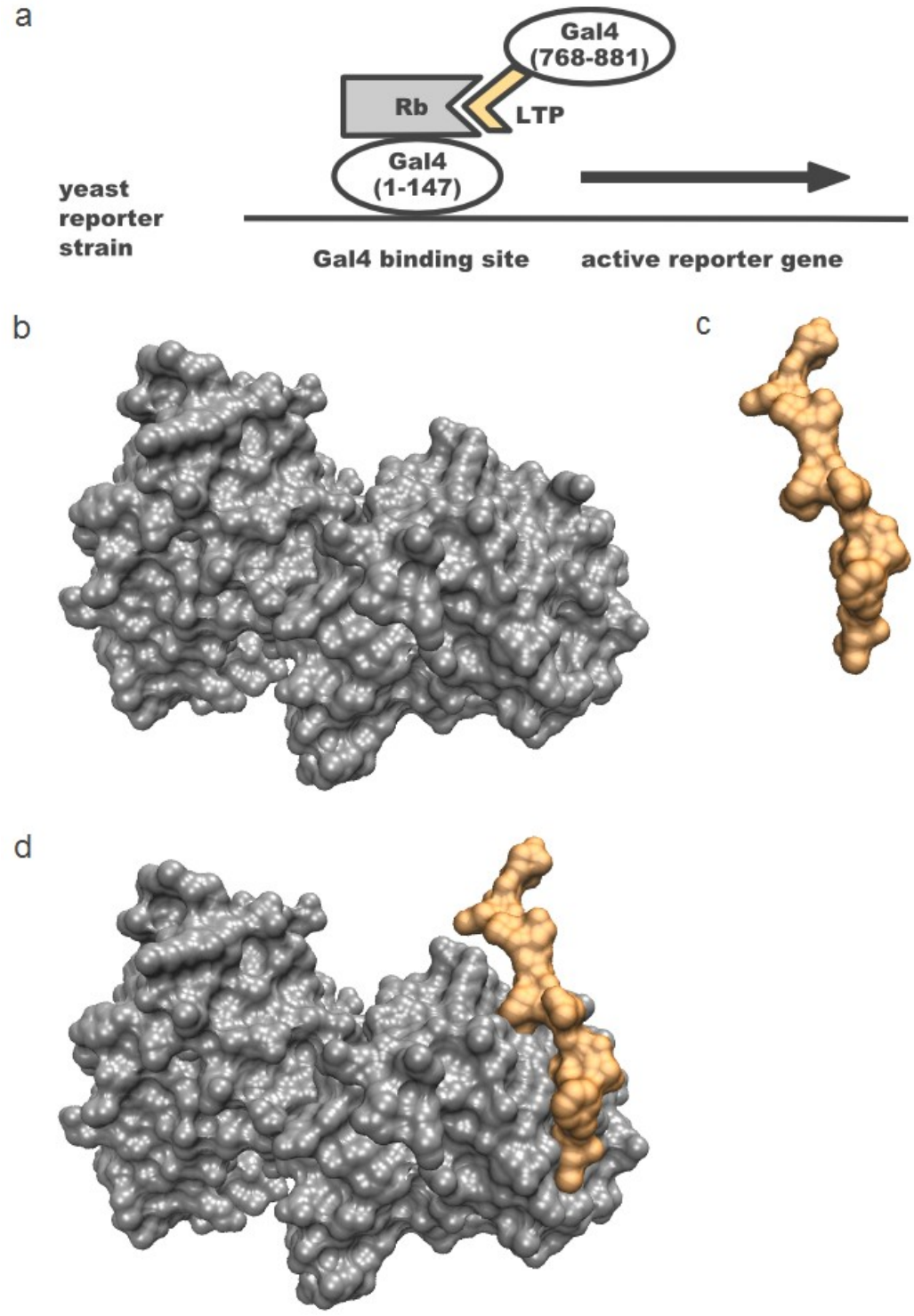

Figure 2. Protein–protein interaction (PPI) between retinoblastoma protein and large T antigen protein. **(a)** A cell of a reporter strain of a yeast two-hybrid (Y2H) system that has been cotransformed with a plasmid that expresses Gal4(1-147)-Rb and a plasmid that expresses Gal4(768-881)-LTP has an active reporter gene in its genome—data acquired and diagram adapted from NAR 23:1152, Oxford Journals; Rb: Retinoblastoma protein (301-918); LTP (for Large T antigen Peptide): SV40 Large T Antigen (residues 103-115). Coordinates from PDB ID: 1GH6 were used to generate representations of: **(b)** Retinoblastoma protein (residues 378-772 minus residues 578-644); **(c)** SV40 Large T Antigen (residues 103-115); and **(d)** Retinoblastoma protein (residues 378-772 minus residues 578-644) and SV40 Large T Antigen (residues 103-115).

Table 1. Project 1. Testing of protein–protein interaction between PRTN1(1-500) and PRTN2(1-600) in the yeast/bacterial two-hybrid system. **(a)** C3 expresses System Domain 1 (SD1) fused to PRTN1(1-500) (accession.version N/A); C4 expresses System Domain 2 (SD2) fused to PRTN2(1-600) (accession.version N/A). **(b)** A1 is a bait–prey interaction assay; A2 is a prey-dependency control assay; A3 is a bait-dependency control assay.

(a)	**Construct**	**Plasmid**	**Source**[1]	**Price**[2]	
	C1	pSD1	Empty vector	N/A	
	C2	pSD2	Empty vector	N/A	
	C3	pSD1-PRTN1(1-500)	This Project	290 EUR	
	C4	pSD2-PRTN2(1-600)	This project	320 EUR	
(b)	**Assay**[3]	**Cotransformant**[4]	**Expressed Constitutively**[5]	**Price**[2]	**Reporter**[6]
	A1	Cell + C3 & C4	SD1-PRTN1(1-500) & SD2-PRTN2(1-600)	65 EUR	Active
	A2	Cell + C3 & C2	SD1-PRTN1(1-500) & SD2	65 EUR	Inactive
	A3	Cell + C1 & C4	SD1 & SD2-PRTN2(1-600)	65 EUR	Inactive

(1) Compatible hybrid-encoding plasmids that were generated under a previous agreement between Client and Supplier can be used in the assays.

(2) For extensive information about our services see our website at https://cellatechnologies.com. The indicated prices are subject to change; taxes, surcharges and or additional costs may apply; USD and some other currencies are also supported.

(3) The assays are explained on our Yeast two-hybrid (Y2H) system web page at https://cellatechnologies.com/tech/yeast-two-hybrid-system.

(4) A cell of a yeast/bacterial two-hybrid system reporter strain cotransformed with the two indicated constructs.

(5) Note: in hybrids of most two-hybrid systems SD1 and SD2 are located N-terminally (with regard to the bait/prey), however, placing them C-terminally may be possible.

(6) For the purpose of this example, hypothetical results are shown; active/inactive means that the observed phenotype suggests that a specific reporter in the cotransformant is active/inactive.

Table 2. Project 2. Testing of protein–protein interaction between PRTN1(1-500) and fragments of PRTN2(1-600) in the yeast/bacterial two-hybrid system. **(a)** C3 expresses System Domain 1 (SD1) fused to PRTN1(1-500) (accession.version N/A); C5/C6/C7 expresses System Domain 2 (SD2) fused to the N-terminal 50% / middle 50% / C-terminal 50% of PRTN2(1-600) (accession.version N/A). **(b)** A4 to A6 are bait–prey interaction assays; A7 to A9 are bait-dependency control assays.

(a)	**Construct**	**Plasmid**	**Source**[1]	**Price**[2]	
	C1	pSD1	Empty vector	N/A	
	C3	pSD1-PRTN1(1-500)	Project 1 (Table 1A)	N/A	
	C5	pSD2-PRTN2(1-300)	This project	220 EUR	
	C6	pSD2-PRTN2(151-450)	This project	220 EUR	
	C7	pSD2-PRTN2(301-600)	This project	220 EUR	
(b)	**Assay**[3]	**Cotransformant**[4]	**Expressed Constitutively**[5]	**Price**[2]	**Reporter**[6]
	A4	Cell + C3 & C5	SD1-PRTN1(1-500) & SD2-PRTN2(1-300)	45 EUR	Active
	A5	Cell + C3 & C6	SD1-PRTN1(1-500) & SD2-PRTN2(151-450)	45 EUR	Active
	A6	Cell + C3 & C7	SD1-PRTN1(1-500) & SD2-PRTN2(301-600)	45 EUR	Inactive
	A7	Cell + C1 & C5	SD1 & SD2-PRTN2(1-300)	45 EUR	Inactive
	A8	Cell + C1 & C6	SD1 & SD2-PRTN2(151-450)	45 EUR	Inactive
	A9	Cell + C1 & C7	SD1 & SD2-PRTN2(301-600)	45 EUR	Inactive

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(4) A cell of a yeast/bacterial two-hybrid system reporter strain cotransformed with the two indicated constructs.

(5) Note: in hybrids of most two-hybrid systems SD1 and SD2 are located N-terminally (with regard to the bait/prey), however, placing them C-terminally may be possible.

(6) For the purpose of this example, hypothetical results are shown; active/inactive means that the observed phenotype suggests that a specific reporter in the cotransformant is active/inactive.

Table 3. Project 3. Testing of protein–protein interaction between PRTN1(1-500) and fragments of PRTN2(151-300) in the yeast/bacterial two-hybrid system. **(a)** C3 expresses System Domain 1 (SD1) fused to PRTN1(1-500) (accession.version N/A); C8/C9/C10 expresses System Domain 2 (SD2) fused to the N-terminal 50% / middle ≈50,67% / C-terminal 50% of PRTN2(151-300) (accession.version N/A). **(b)** A10 to A12 are bait–prey interaction assays; A13 to A15 are bait-dependency control assays.

(a)	**Construct**	**Plasmid**	**Source**[1]	**Price**[2]	
	C1	pSD1	Empty vector	N/A	
	C3	pSD1-PRTN1(1-500)	Project 1 (Table 1A)	N/A	
	C8	pSD2-PRTN2(151-225)	This project	160 EUR	
	C9	pSD2-PRTN2(188-263)	This project	160 EUR	
	C10	pSD2-PRTN2(226-300)	This project	160 EUR	
(b)	**Assay**[3]	**Cotransformant**[4]	**Expressed Constitutively**[5]	**Price**[2]	**Reporter**[6]
	A10	Cell + C3 & C8	SD1-PRTN1(1-500) & SD2-PRTN2(151-225)	45 EUR	Inactive
	A11	Cell + C3 & C9	SD1-PRTN1(1-500) & SD2-PRTN2(188-263)	45 EUR	Active
	A12	Cell + C3 & C10	SD1-PRTN1(1-500) & SD2-PRTN2(226-300)	45 EUR	Inactive
	A13	Cell + C1 & C8	SD1 & SD2-PRTN2(151-225)	45 EUR	Inactive
	A14	Cell + C1 & C9	SD1 & SD2-PRTN2(188-263)	45 EUR	Inactive
	A15	Cell + C1 & C10	SD1 & SD2-PRTN2(226-300)	45 EUR	Inactive

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(4) A cell of a yeast/bacterial two-hybrid system reporter strain cotransformed with the two indicated constructs.

(5) Note: in hybrids of most two-hybrid systems SD1 and SD2 are located N-terminally (with regard to the bait/prey), however, placing them C-terminally may be possible.

(6) For the purpose of this example, hypothetical results are shown; active/inactive means that the observed phenotype suggests that a specific reporter in the cotransformant is active/inactive.

Table 4. Project 4. Testing of protein–protein interaction between PRTN1(1-500) and fragments of PRTN2(188-263) in the yeast/bacterial two-hybrid system. **(a)** C3 expresses System Domain 1 (SD1) fused to PRTN1(1-500) (accession.version N/A); C11/C12/C13 expresses System Domain 2 (SD2) fused to the N-terminal 50% / middle 50% / C-terminal 50% of PRTN2(188-263) (accession.version N/A). **(b)** A16 to A18 are bait–prey interaction assays; A19 to A21 are bait-dependency control assays.

(a)	**Construct**	**Plasmid**	**Source**[1]	**Price**[2]	
	C1	pSD1	Empty vector	N/A	
	C3	pSD1-PRTN1(1-500)	Project 1 (Table 1A)	N/A	
	C11	pSD2-PRTN2(188-225)	This project	160 EUR	
	C12	pSD2-PRTN2(207-244)	This project	160 EUR	
	C13	pSD2-PRTN2(226-263)	This project	160 EUR	
(b)	**Assay**[3]	**Cotransformant**[4]	**Expressed Constitutively**[5]	**Price**[2]	**Reporter**[6]
	A16	Cell + C3 & C11	SD1-PRTN1(1-500) & SD2-PRTN2(188-225)	45 EUR	Inactive
	A17	Cell + C3 & C12	SD1-PRTN1(1-500) & SD2-PRTN2(207-244)	45 EUR	Active
	A18	Cell + C3 & C13	SD1-PRTN1(1-500) & SD2-PRTN2(226-263)	45 EUR	Inactive
	A19	Cell + C1 & C11	SD1 & SD2-PRTN2(188-225)	45 EUR	Inactive
	A20	Cell + C1 & C12	SD1 & SD2-PRTN2(207-244)	45 EUR	Inactive
	A21	Cell + C1 & C13	SD1 & SD2-PRTN2(226-263)	45 EUR	Inactive

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(4) A cell of a yeast/bacterial two-hybrid system reporter strain cotransformed with the two indicated constructs.

(5) Note: in hybrids of most two-hybrid systems SD1 and SD2 are located N-terminally (with regard to the bait/prey), however, placing them C-terminally may be possible.

(6) For the purpose of this example, hypothetical results are shown; active/inactive means that the observed phenotype suggests that a specific reporter in the cotransformant is active/inactive.

Table 5. Project 5. Testing of protein–protein interaction between PRTN1(1-500) and fragments of PRTN2(207-244) in the yeast/bacterial two-hybrid system. **(a)** C3 expresses System Domain 1 (SD1) fused to PRTN1(1-500) (accession.version N/A); C14/C15/C16 expresses System Domain 2 (SD2) fused to the N-terminal 50% / middle ≈52,63% / C-terminal 50% of PRTN2(207-244) (accession.version N/A). **(b)** A22 to A24 are bait–prey interaction assays; A25 to A27 are bait-dependency control assays.

(a)	**Construct**	**Plasmid**	**Source**[1]	**Price**[2]	
	C1	pSD1	Empty vector	N/A	
	C3	pSD1-PRTN1(1-500)	Project 1 (Table 1A)	N/A	
	C14	pSD2-PRTN2(207-225)	This project	160 EUR	
	C15	pSD2-PRTN2(216-235)	This project	160 EUR	
	C16	pSD2-PRTN2(226-244)	This project	160 EUR	
(b)	**Assay**[3]	**Cotransformant**[4]	**Expressed Constitutively**[5]	**Price**[2]	**Reporter**[6]
	A22	Cell + C3 & C14	SD1-PRTN1(1-500) & SD2-PRTN2(207-225)	45 EUR	Inactive
	A23	Cell + C3 & C15	SD1-PRTN1(1-500) & SD2-PRTN2(216-235)	45 EUR	Inactive
	A24	Cell + C3 & C16	SD1-PRTN1(1-500) & SD2-PRTN2(226-244)	45 EUR	Active
	A25	Cell + C1 & C14	SD1 & SD2-PRTN2(207-225)	45 EUR	Inactive
	A26	Cell + C1 & C15	SD1 & SD2-PRTN2(216-235)	45 EUR	Inactive
	A27	Cell + C1 & C16	SD1 & SD2-PRTN2(226-244)	45 EUR	Inactive

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Table 6. Project 6. Construction of plasmids that encode GFP-linker-peptide. All possible 13- to 19-residue peptides that can be derived from PRTN2(226-244) (accession.version N/A) are covered.

Plasmid	Fusion Protein	Potential Effect[1]	Price[2]
1	GFP-linker-PRTN2(226-244)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
2	GFP-linker-PRTN2(226-243)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
3	GFP-linker-PRTN2(226-242)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
4	GFP-linker-PRTN2(226-241)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
5	GFP-linker-PRTN2(226-240)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
6	GFP-linker-PRTN2(226-239)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
7	GFP-linker-PRTN2(226-238)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
8	GFP-linker-PRTN2(227-244)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
9	GFP-linker-PRTN2(227-243)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
10	GFP-linker-PRTN2(227-242)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
11	GFP-linker-PRTN2(227-241)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
12	GFP-linker-PRTN2(227-240)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
13	GFP-linker-PRTN2(227-239)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
14	GFP-linker-PRTN2(228-244)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
15	GFP-linker-PRTN2(228-243)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
16	GFP-linker-PRTN2(228-242)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
17	GFP-linker-PRTN2(228-241)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
18	GFP-linker-PRTN2(228-240)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
19	GFP-linker-PRTN2(229-244)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
20	GFP-linker-PRTN2(229-243)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
21	GFP-linker-PRTN2(229-242)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
22	GFP-linker-PRTN2(229-241)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
23	GFP-linker-PRTN2(230-244)	Loss of PPI function of targeted PPID of PRTN1	100 EUR

24	GFP-linker-PRTN2(230-243)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
25	GFP-linker-PRTN2(230-242)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
26	GFP-linker-PRTN2(231-244)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
27	GFP-linker-PRTN2(231-243)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
28	GFP-linker-PRTN2(232-244)	Loss of PPI function of targeted PPID of PRTN1	100 EUR

(1) If the fusion protein is expressed in transfected cells, then the PRTN2-part of the fusion protein may bind to the targeted PRTN2-binding protein–protein interaction domain (PPID) of endogenous PRTN1 and cause loss of protein–protein interaction (PPI) function of the targeted PPID. Note: with these plasmids it may also be possible to confirm this peptide–protein interaction by co-immunoprecipitation analysis using anti-GFP antibodies and antibodies against PRTN1. (2) For extensive information about our services see our website at https://cellatechnologies.com. The indicated prices are subject to change; taxes, surcharges and or additional costs may apply; USD and some other currencies are also supported.

Notes

- Proteins that (putatively/potentially) bind to (and work with) your protein of interest (to facilitate a specific cellular process) may be found through databases such as BioGRID (https://thebiogrid.org/), CCSB Interactome Database (http://interactome.dfci.harvard.edu/), HPRD (http://www.hprd.org/), IntAct (https://www.ebi.ac.uk/intact/) and STRING (http://string-db.org/).

- To the extent applicable, on request we will review project-related text and figures of your manuscript-in-progress; we will be glad to do this (free of additional charges).

- Acknowledgments to the developers of the molecular visualization program VMD (http://www.ks.uiuc.edu/Research/vmd/), which was used to generate the pictures in this document that show representations of protein structures.

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