

Supplementary material for

**Specific targeting of human caspases
using designed ankyrin repeat proteins**

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Supplementary Figure 1

| Casp-Selection | Round n°1 surface panning | | | | Round n°2 solution panning | | | Round n°3 surface panning | | | | Round n°4 solution panning | | |
|----------------|------------------------------|---|---|-------------|-------------------------------|--------------|-------------------------|------------------------------|---|---|--------------|-------------------------------|--------------|--------------------------|
| Caspase-1 | ■ | 4 | 5 | 1 | ■ | 1 | off-rate | ■ | 4 | 5 | 1 | ■ | 1 | off-rate |
| Caspase-4 | ■ | 1 | 5 | 4 | ■ | 4 | off-rate | ■ | 1 | 5 | 4 | ■ | 4 | off-rate |
| Caspase-5 | ■ | 1 | 4 | 5 | ■ | 5 | off-rate | ■ | 1 | 4 | 5 | ■ | 5 | off-rate |
| Caspase-8 | ■ | 2 | 9 | 8 | ■ | 8 | off-rate | ■ | 2 | 9 | 8 | ■ | 8 | off-rate |
| Caspase-9 | ■ | 2 | 8 | 9 | ■ | 9 | off-rate | ■ | 2 | 8 | 9 | ■ | 9 | off-rate |
| Caspase-3 | ■ | 6 | 7 | 3 | ■ | 3 | off-rate | ■ | 6 | 7 | 3 | ■ | 3 | off-rate |
| Caspase-6 | ■ | 3 | 7 | 6 | ■ | 6 | off-rate | ■ | 3 | 7 | 6 | ■ | 6 | off-rate |
| Caspase-7 | ■ | 3 | 6 | 7 | ■ | 7 | off-rate | ■ | 3 | 6 | 7 | ■ | 7 | off-rate |
| | MBP pre-panning | | | Wash 2 x 5' | MBP | Wash 2 x 10' | 5 minutes 100 excess | MBP pre-panning | | | Wash 3 x 30' | MBP | Wash 4 x 30' | 10 minutes 500 excess |

Supplementary Figure 1: Ribosome display selection strategy

To increase the binders specificity for a single caspase family member, additional pre-panning steps in round no°1 and no°3 with the closest homologues of the targeted caspase was introduced. In addition, round n°2 and n°4 was extended with k_{off} maturation steps with a final washing step containing an excess of targeted caspase.

Supplementary Figure 2

| | N-cap | | | 1st repeat | | |
|-----------|-----------------|----------------|--------------|----------------|-------------------------------|---------------|
| | 10 | 20 | 30 | 40 | 50 | 60 |
| Consensus | MRGSHHHHHGSDLGK | KLLEAARAGQDDEV | RILMANGADVNA | XDXXGXT | PLHLAA | XXGH L |
| D1.73 | | | | | F.QH.H..... | YD... |
| AR_F8 | | | | | T. WL .H..... | KT ... |
| D3.4S76R | | | | | | |
| D3.8 | | | | | N.FV.K.-..... | SV... |
| 5.15 | | R..... | | | D.WL.S..... | VA... |
| 6.11 | | | | | H.ID.T..... | VH... |
| 7.18 | | | | | D. AW . Q | QN ... |
| 7.43 | | | | | N.KH.W..... | FF... |
| D8.1 | | | | | E.AS.W..... | FN... |
| D8.4 | | | | | E. AS . W | FN ... |
| D9.2 | | | I..V..... | | TSWN.S..... | SL.R. |

| | 1st repeat | 2nd repeat | | 3rd repeat | | | |
|-----------|-----------------|-----------------|---------------------------------------|-----------------|-----------------|-------------------------------|-------------|
| | 70 | 80 | 90 | 100 | 110 | 120 | |
| Consensus | EIVEVLLKZGADVNA | XDXXGXT | PLHLAA | XXGH | EIVEVLLKZGADVNA | XDXXGXT | PLHLA |
| D1.73 | | Y..... | F.SE.W...V.. | FS..... | H..... | F.YS.F..... | |
| AR_F8 | | Y..... | W. NY . A | DN | H..... | K. YE . F | |
| D3.4S76R | | | M . DA . V | KR | H..... | R. IW . R | |
| D3.8 | | H..... | A.DA.V.S..... | SY..... | N..... | R.IW.R..... | |
| D5.15 | | MN | D.HY.K..... | IE..... | H..... | D.LW.D..... | |
| D6.11 | | H..... | Y.SR.Y..Q.. | MY..... | V..... | N..... | R.DN.M..... |
| D7.18 | | HD..... | T. WV . M | DD | A..Y..... | Y. QL . N | N.. |
| D7.43 | | N..... | V.NM.T..... | | | | |
| D8.1 | | N..... | V.HA.M...R.. | LF..... | N..... | N.ME.H..... | |
| D8.4 | | N..... | V. HA . M ...R.. | LF | N..... | N. ME . H | |
| D9.2 | | N..... | D.R.LR.D..... | TW...GA..... | EY..... | S.T.DL.D..... | R.. |

| | 3rd repeat | C-cap | | |
|-----------|---------------------------------------|-------------------|-------|-------|
| | 130 | 140 | 150 | 160 |
| Consensus | AXXGH LEIVEVLLKZGADVNAQDKFGKTA | FDISIDNGNEDLAEILQ | | |
| D1.73 | .HS..... | N..... | | |
| AR_F8 | . YD | Y..... | | |
| D3.4S76R | .TV..... | Y..... | | |
| D3.8 | .VS..... | Y..... | | |
| D5.15 | .KW.Y..... | H..... | | |
| D6.11 | .WS..... | N..... | | |
| D7.18 | . TD | Y..... | | |
| D7.43 | .HM.R..... | Y..... | | |
| D8.1 | .MF..... | H...A..... | | |
| D8.4 | . MF | N..... | | |
| D9.2 | .ME..... | H..... | | |

Supplementary Figure 2: Caspase-targeted DARPIn sequences

The DARPIn consensus sequence is shown on top in black with randomized residues in red (X = All amino acids except Cys, Gly and Pro; Z = N, H or Y). Unintended framework mutations are depicted in green. Interacting residues were determined by the EPPIC server (1) (<http://www.eppicweb.org>) and are marked bold and underlined.

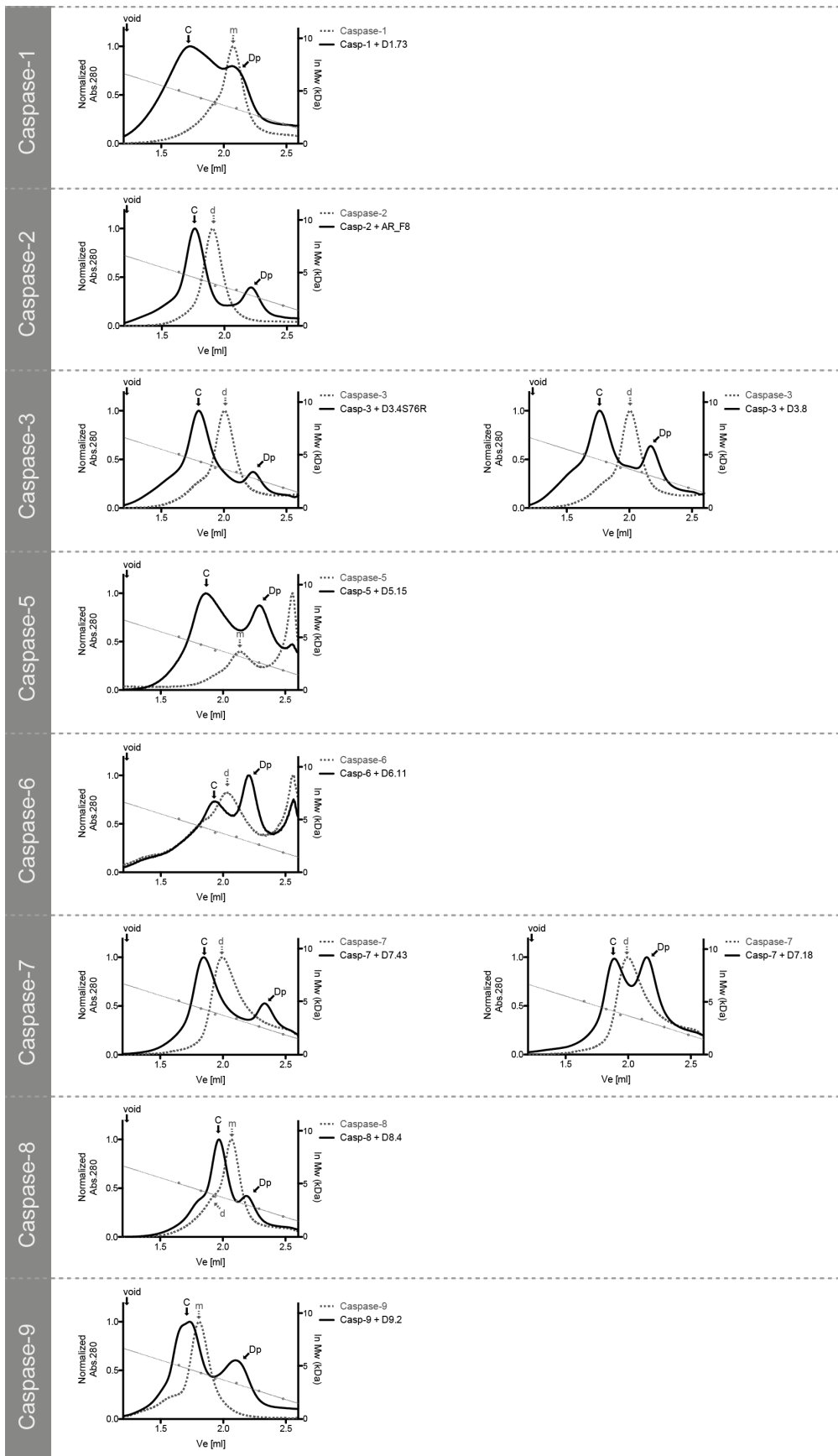
Supplementary Table 1

| DARPin | Target | Type | No# of AA | Molecular weight | Extinction coefficient | Theoretical isoelectric point | Comments | Ref. |
|----------|--------|------|-----------|------------------|------------------------|-------------------------------|-----------------------------------|------|
| D1.73 | Casp-1 | N3C | 169 | 18358 | 9970 | 5.34 | No inhibition of small substrates | |
| AR_F8 | Casp-2 | N3C | 169 | 18417 | 18450 | 5.28 | Allosteric inhibitor | (2) |
| D3.4S76R | Casp-3 | N2C | 136 | 14786 | 6690 | 5.56 | Competitive Inhibitor | (3) |
| D3.8 | Casp-3 | N3C | 168 | 17964 | 8480 | 5.51 | Competitive Inhibitor | (3) |
| D5.15 | Casp-5 | N3C | 169 | 18324 | 19480 | 4.99 | No inhibition of small substrates | |
| D6.11 | Casp-6 | N3C | 169 | 18300 | 9970 | 5.46 | No inhibition of small substrates | |
| D7.18 | Casp-7 | N3C | 169 | 18273 | 15470 | 4.74 | Zymogen activation inhibitor | (4) |
| D7.43 | Casp-7 | N2C | 136 | 14812 | 6990 | 5.68 | Zymogen activation inhibitor | (4) |
| D8.1 | Casp-8 | N3C | 169 | 18157 | 5500 | 5.28 | No inhibition of small substrates | |
| D8.4 | Casp-8 | N3C | 169 | 18190 | 5500 | 5.30 | No inhibition of small substrates | |
| D9.2 | Casp-9 | N3C | 169 | 18112 | 12490 | 5.01 | No inhibition of small substrates | |

Supplementary Table 1: Biophysical parameters of selected DARPins

Molecular weight, extinction coefficient and theoretical isoelectric point were calculated using the ProtParam Tool provided on expasy.ch (<http://web.expasy.org/protparam/>). Eight of eleven binders are N3C DARPins and five DARPins have reported inhibitory effects on the targeted caspase (2-4). All other selected DARPins did not show an inhibitory effect using peptide substrates. However, procaspase binding and interference with caspase activation as reported for caspase-7 DARPins (4) was not tested and thus can not be excluded.

Supplementary Figure 3



Supplementary Figure 3: Caspase-DARPin complex formation in solution

The reported DARPins form stable protein complexes with the targeted caspase in solution and can be separated on size exclusion chromatography. Without a present DARPin, Caspases-1, -5 and -9 run as monomers (dotted line, peak marked with dotted arrow and (m) for monomer), whereas caspases-2, -3, -6 and -7 run as dimers (peak marked with dotted arrow and (d) for dimer). Caspase-8 shows a monomer-dimer equilibrium (marked with (m) and (d) respectively). In presence of a selected DARPin, all caspases elute at earlier retention volumes (solid lines, complex peak marked with (C), for complex and (Dp) for DARPin excess). Caspase-5 and -6 were used directly after NiNTA purification (5) displaying an elution peak at 2.6 ml originating from imidazole. Calibration curve (right ordinate) was made using aldolase (158 kDa), conalbumin (75 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), ribonuclease A (13.7 kDa) and aprotinin (6.5 kDa).

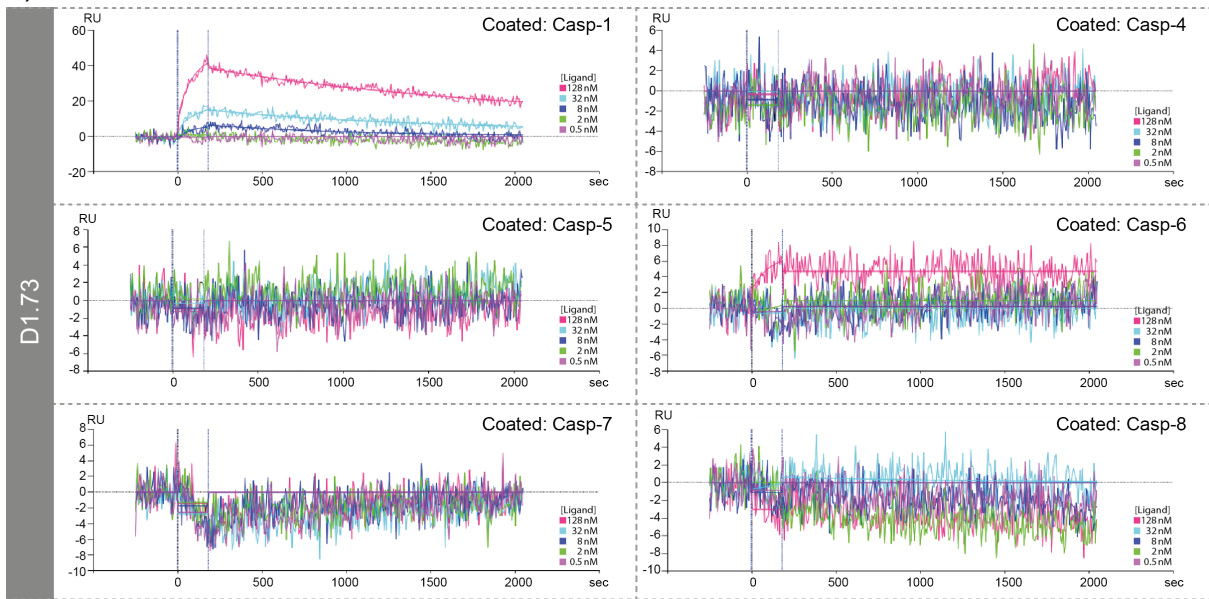
Supplementary Table 2

| Data collection | |
|--------------------------------------|--|
| Space group | P2 ₁ 2 ₁ 2 ₁ |
| Cell dimensions | |
| <i>a</i> , <i>b</i> , <i>c</i> (Å) | 61.0, 81.60, 163.20 |
| α, β, γ (°) | 90.0, 90.0, 90.0 |
| Resolution (Å) | 48.86 – 1.8 |
| Wavelength (Å) | 1.0 |
| R _{sym} (%) | 9.8 (51.5) |
| Completeness (%) | 99.4 (98.9) |
| I/σ(I) | 13.29 (3.76) |
| Refinement | |
| Resolution [Å] | 1.8 |
| R _{work} /R _{free} | 18.0 / 21.8 |
| No. atoms | |
| Protein | 6246 |
| Water | 685 |
| Ligands | 3 · SO ₄ ²⁻ , 5 · C ₂ H ₆ O ₂ |
| <i>B</i> -factors | |
| Protein | 22.9 |
| Water | 33.9 |
| rmsd | |
| Bond length [Å] | 0.009 |
| Bond angle [°] | 1.2 |
| Ramachandran | |
| Preferred [%] | 97.0 |
| Allowed [%] | 3.0 |
| Outliers [%] | 0 |

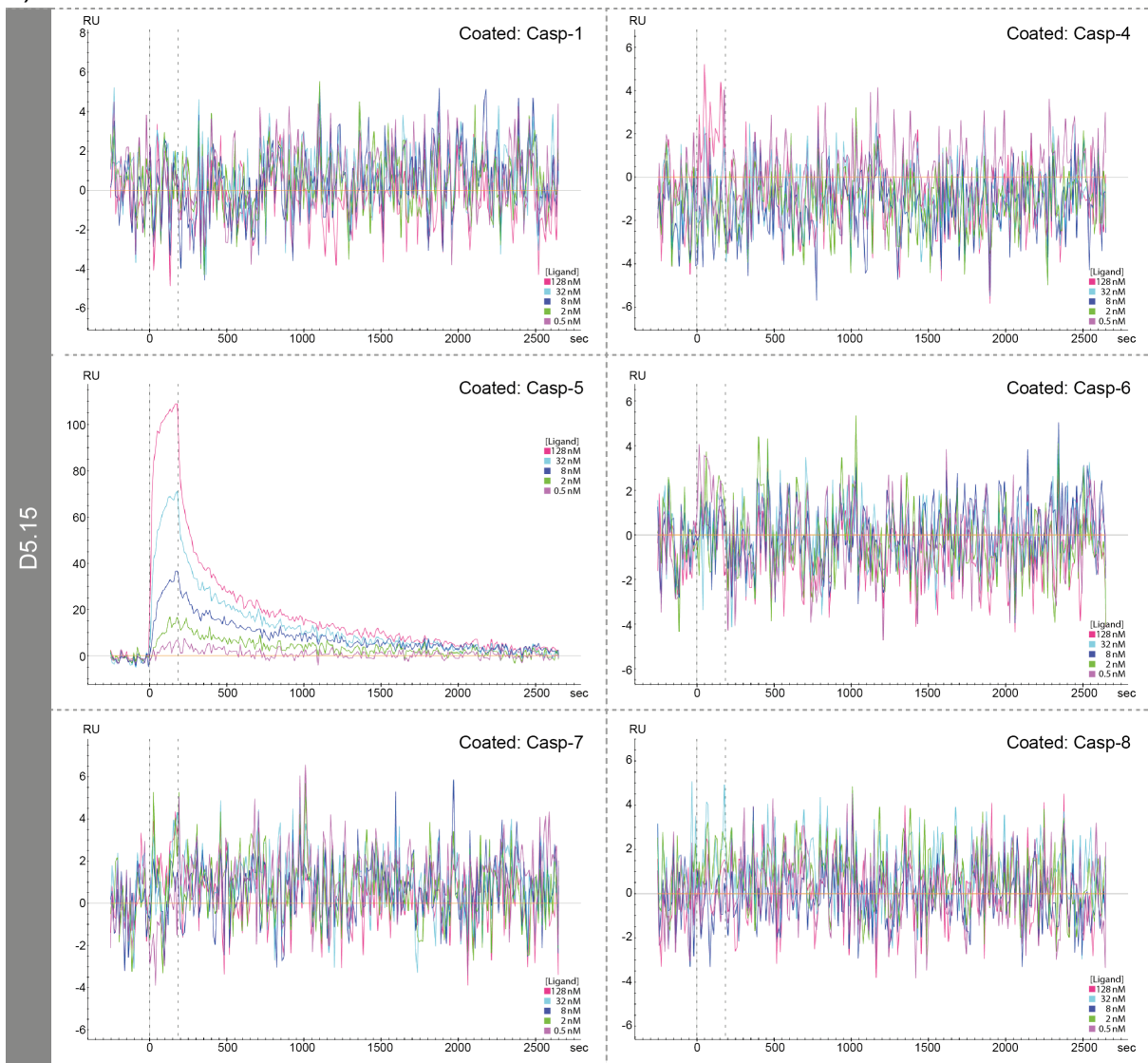
Supplementary Table 2: Data collection and refinement statistics

Supplementary Figure 4

a)

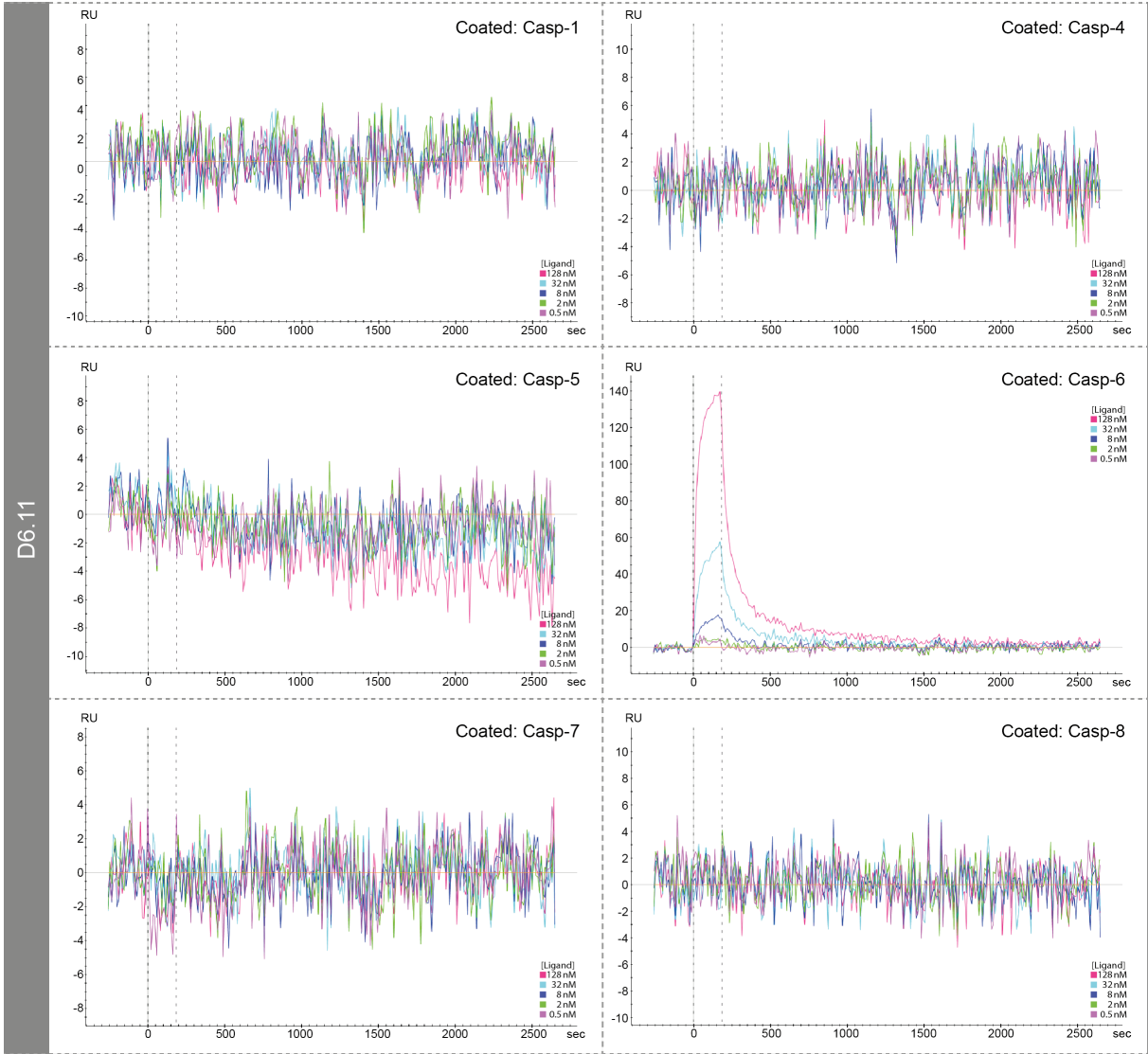


b)



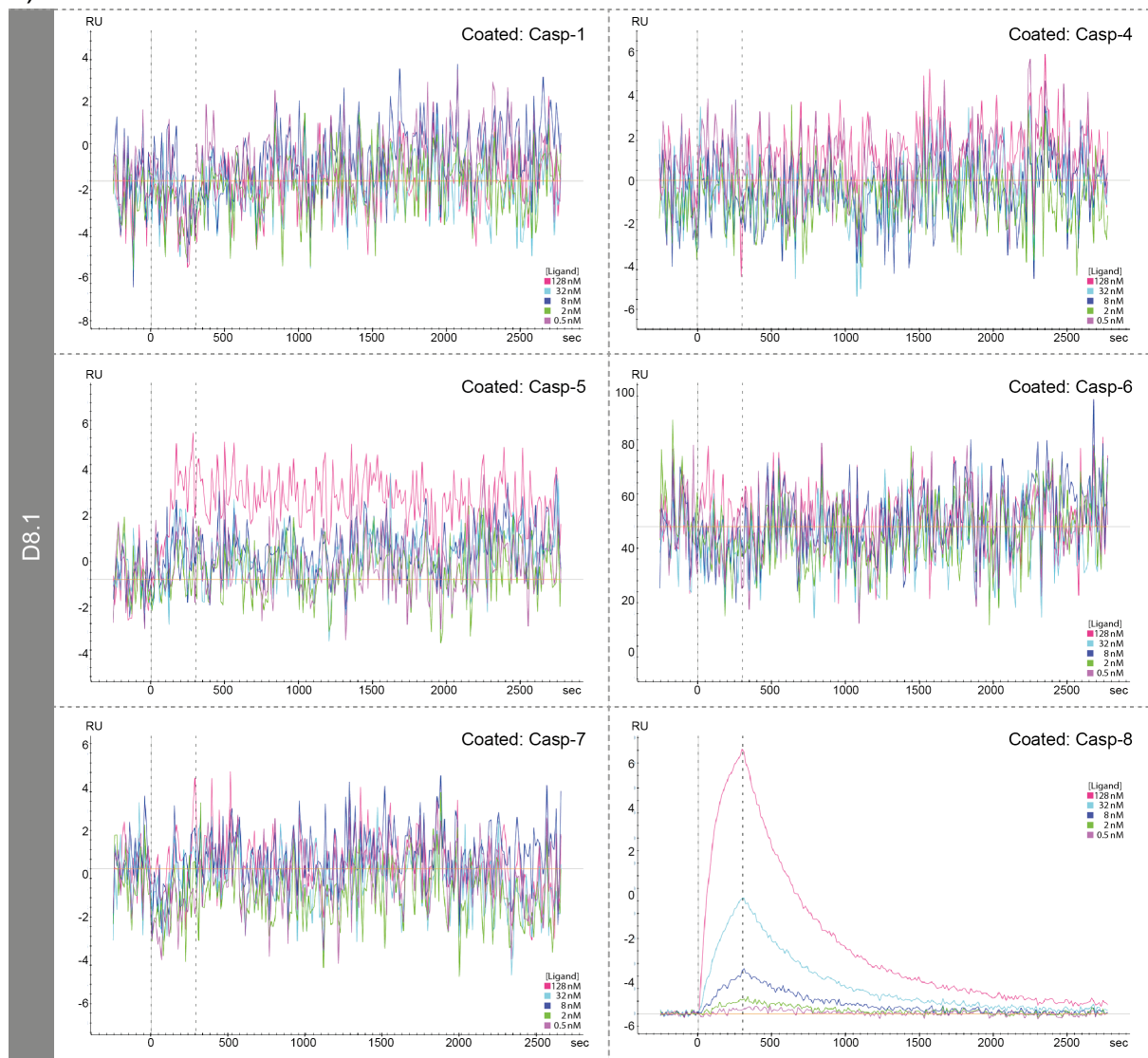
Supplementary Figure 4

c)



Supplementary Figure 4

d)



Supplementary Figure 4: Caspase specificity of selected DARPins

Caspase specificity of (a) D1.73, (b) D5.15, (c) D6.11 and (d) D8.1 was measured by surface plasmon resonance on a Proteon XPR36 (Bio-Rad Laboratories, Inc) machine using a NLC sensor chip. Six ligand channels were coated with biotinylated caspases-1, -4, -5, -6, -7 and -8 and binding of the DARPins was simultaneously observed at six different concentrations (0 nM to 128 nM). Caspase specificity of AR_F8 for caspase-2, D3.4S76R for caspase-3 and D7.18 as well as D7.43 for caspase-7 is reported elsewhere (2-4). D9.2 was not tested against other caspase family members.

Supplementary Figure 5

a)

| | | | |
|-----------|---|------------|------------|
| | N-cap | 1st repeat | |
| | 10 20 30 40 50 60 | | |
| Consensus | MRGSHHHHHGSDLGKKLLEAARAGQDDEVRIILMANGADVNA DXXGX TPLHLA AXX GH | | |
| D8.1 |E.AS.W.....FN... | | |
| D8.4 | <u>R</u>E. <u>AS.W</u> <u>FN</u> ... | | |
| | 1st repeat | 2nd repeat | 3rd repeat |
| | 70 80 90 100 110 120 | | |
| Consensus | EIVEVLLKZGADVNA DXXGX TPLHLA AXX GHLEIVEVLLKZGADVNA DXXGX TPLHLA | | |
| D8.1 |N.....V.HA.M...R...LF.....N.....N.ME.H..... | | |
| D8.4 |N.....V.HA. <u>M</u> ... <u>R</u> ... <u>LF</u>N.....N. <u>ME</u> . <u>H</u> | | |
| | 3rd repeat | C-cap | |
| | 130 140 150 160 | | |
| Consensus | A XX GHLEIVEVLLKZGADVNAQDKFGKTA FDISID NGNEDLAEILQ | | |
| D8.1 | .MF.....H...A..... | | |
| D8.4 | . <u>MF</u>N..... | | |

b)

| | 231 | 232 | 235 | 246 | 249 | 250 | 280 | 282 | 284 | 288 | 290 | 291 | 294 | 295 | 297 | 298 | 299 | 301 | 302 | 303 | 305 | 306 | 307 | 327 | 347 |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Caspase-8 | K | P | Y | K | E | K | E | K | H | T | E | Q | E | I | K | I | Y | L | M | D | S | N | M | D | S |
| Caspase-10 | N | H | L | - | - | - | T | H | H | T | V | E | M | V | Q | K | Q | C | P | A | A | D | G | D | R |
| Caspase-9 | E | P | H | R | - | - | M | E | K | T | K | K | L | A | L | E | L | Q | Q | D | G | A | L | D | S |
| Caspase-3 | P | E | L | K | - | - | E | R | K | T | E | E | E | L | R | D | V | K | E | D | S | K | R | N | S |
| Caspase-7 | E | K | K | K | - | - | D | I | Y | S | A | K | D | L | K | K | A | E | E | D | T | N | A | D | T |
| Caspase-6 | R | R | I | W | - | - | E | K | F | K | E | E | L | K | H | E | V | T | V | S | A | D | A | D | S |
| Caspase-4 | R | R | L | H | - | - | S | D | E | T | R | D | S | A | R | A | F | T | P | E | K | S | S | K | S |
| Caspase-5 | R | R | L | H | - | - | T | V | E | T | R | D | S | V | R | A | F | A | P | E | K | S | S | K | S |
| Caspase-1 | K | R | L | S | - | - | S | D | K | T | S | D | T | E | E | A | F | H | P | E | K | T | S | V | S |
| Caspase-14 | A | A | L | - | - | - | E | T | K | T | E | Q | E | E | E | K | F | Q | I | D | R | E | D | D | A |
| Caspase-2 | R | P | L | G | - | - | D | H | L | T | Q | E | E | K | Q | N | F | Q | P | A | R | V | T | D | S |

| | | | | | | | | |
|-------------------|---|---|---|---|---|---|---|---|
| Bulky Positive | R | K | | | | | | |
| Bulky Negative | D | E | | | | | | |
| Bulky uncharged | M | F | Y | W | H | N | Q | C |
| Small Hydrophobic | G | A | V | I | L | P | | |
| Small Hydrophilic | S | T | | | | | | |
| No Homologue | - | | | | | | | |

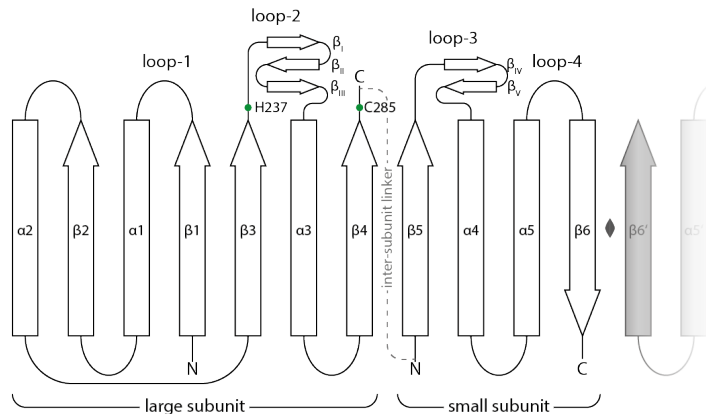
Supplementary Figure 5: DARPin D8.4/Caspase-8 interacting residues

(a) DARPin sequence D8.1 and D8.4 are aligned with the DARPin consensus sequence displaying two unintended framework mutations: H85R and V139A in D8.1; Q26R and H85R in D8.4 (marked in green). Interacting residues determined by the EPPIC server (1) (<http://www.eppicweb.org>) are marked in bold/underlined. In total, 18 framework and 13 randomized residues are involved in a binding interface of overall 938 Å². Notably, the D8.1 sequence is almost identical and besides Arg-26, all interacting residues of D8.4 are present in D8.1 suggesting the same interaction.

(b) Comparison of caspase-8 residues involved in D8.4 binding by sequence alignment (ClustalW2, <http://www.ebi.ac.uk/Tools/msa/clustalw2/>) with other human caspases. Amino acids are classified and colored in five groups. Caspase sequence accession numbers are indicated in supplementary figure 6.

Supplementary Figure 6

a)



b)

| | | |
|---------------------|--|-----|
| Caspase-3 (P42574) | -----SYKMDYP--E | 43 |
| Caspase-7 (P55210) | -----TYQYNMNF--K | 66 |
| Caspase-6 (P55212) | -----KYKMDHR--R | 43 |
| Caspase-8 (Q14790) | -----SQTLDKVYQMKS--E | 232 |
| Caspase-10 (Q92851) | KHAGSNGNRATNGAPSLVSRGMQGASANTLNSETSTKRAAVYRMNRR--H | 282 |
| Caspase-9 (P55211) | -----NADLAYILSME--P | 159 |
| Caspase-4 (P49662) | -----RAEEIYPIKERNNR | 134 |
| Caspase-5 (P51878) | -----NHDEIYPIKKREDR | 191 |
| Caspase-1 (P29466) | -----KSAEIYPIMDKSSR | 161 |
| Caspase-14 (P31944) | -----EKYDMSGARLA | 20 |
| Caspase-2 (P42575) | -----HFQLAYRLQSR--P | 198 |

* :

| | | |
|---------------------|--|-----|
| Caspase-3 (P42574) | MGLCIIINNKNFHKST-----GMTSRSGTDVDAANLRETFRNLKYEVR | 86 |
| Caspase-7 (P55210) | LGKCIINNKNFDKVT-----GMGVRNGTDKDAEALFKCFRSLGFDVI | 109 |
| Caspase-6 (P55212) | RGIALIFNHERFFWHL-----TLPERRGTCADRDNLTTRRFSDLGFEVK | 86 |
| Caspase-8 (Q14790) | RGYCLIIINNHNFAAREKVPKLHSIRDNRGTHLDAGALTTTFEELHFEIK | 282 |
| Caspase-10 (Q92851) | RGLCVIVNHNHST-----SLKDRQGTHKDAEILSHVFQWLGFVTH | 322 |
| Caspase-9 (P55211) | CGHCLIIINNHNFCRES-----GLRTRTGSNIDCEKLRRRFSSLHFMVE | 202 |
| Caspase-4 (P49662) | TRLALIICNTEFDHLP-----PRNGADFDTGMKELLEGLDYSVD | 174 |
| Caspase-5 (P51878) | RRLALIICNTEFDHLP-----ARNGAHYDIVGMKRLQLGLGYTVV | 231 |
| Caspase-1 (P29466) | TRLALIICNEEFDSIP-----RRTGAEVDITGMTMLLQNLGYSVD | 201 |
| Caspase-14 (P31944) | LILCVTK-----AREGSEEDLDALEHMFRQLRFEST | 51 |
| Caspase-2 (P42575) | RGLALVLSNVHFTGEK-----ELEFRSGGDVDHSTLVTLFKLLGYDVH | 241 |



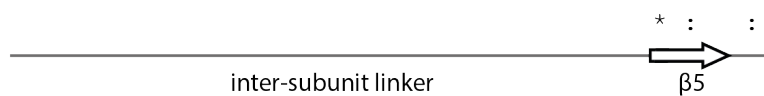
| | | |
|---------------------|--|-----|
| Caspase-3 (P42574) | NKNDLTREEIVELMRDVSK-EDHSKR--SSFVCVLLSHGEEG-----I | 126 |
| Caspase-7 (P55210) | VYNDCSCAKMQDILKKAASE-EDHTNA--ACFACILLSHGEEEN-----V | 149 |
| Caspase-6 (P55212) | CFNDLKAEEELLLKIHEVST-VSHADA--DCFVCFVLSHGEGN-----H | 126 |
| Caspase-8 (Q14790) | PHDDCTVECIYEILKIYQL-MDHSNM--DCFICILSHGDKG-----I | 322 |
| Caspase-10 (Q92851) | IHNNTKVMEMVQLQKQKCNPAHADG--DCFVFCILTHGRFG-----A | 363 |
| Caspase-9 (P55211) | VKGDLTAKKMVLALELAQ-QDHGAL--DCCVVVILSHGCQASHLQFPGA | 249 |
| Caspase-4 (P49662) | VEENLTARDMESALRAFATRPEHKSS--DSTFLVLSHGILEG--ICGTV | 220 |
| Caspase-5 (P51878) | DEKNLTARDMESVLRFAAARPEHKSS--DSTFLVLSHGILEG--ICGTA | 277 |
| Caspase-1 (P29466) | VKKNLTASDMTTELEAFARPEHKTS--DSTFLVLSHGIREG--ICGKK | 247 |
| Caspase-14 (P31944) | MKRDPTAEQFQEELEKQQAIDSREDPVSCAFVVLMAHGREG-----F | 94 |
| Caspase-2 (P42575) | VLCDQTAQEMQEKLQNFAPLPAHRVT--DSCIVALLSHGVGEG-----A | 282 |



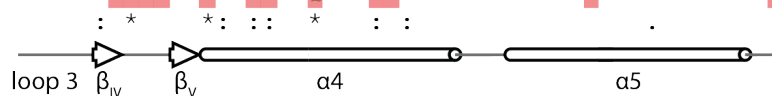
| | | | |
|------------|----------|---|-----|
| Caspase-3 | (P42574) | IFGTNG-PVDLKKITNFFRGDRCSRSLTGKPKLFI IQACRG----- | 165 |
| Caspase-7 | (P55210) | IYGRDG-VTPIKDLTAHFRGDRCKTLLLEKPKLFFI IQACRG----- | 188 |
| Caspase-6 | (P55212) | IYAYDA-KIEIQTLTGLFKGDKCHSLVGGPKIFIIQACRGNQHDVPIPL | 175 |
| Caspase-8 | (Q14790) | IYGTGQGEAPIYELTSQFTGLKCP LAGKPKVFFI IQACQGDNYQKG---- | 368 |
| Caspase-10 | (Q92851) | VYSSDEALIPIREIMSHFTALQCPRLAEKPKLFFI IQACQGEEIQPS---- | 409 |
| Caspase-9 | (P55211) | VYGTGDCPVSVVEKIVNIFNGTSCPSLGGKPKLFFI IQACGGEQKDHGFEVA | 299 |
| Caspase-4 | (P49662) | HDEKKPDVLLYDTIFQIFNNRNCLESLKDKPKVIVQACRGANRGEWVRD | 270 |
| Caspase-5 | (P51878) | HKKKKPDVLLYDTIFQIFNNRNCLESLKDKPKVIVQACRGEKHGELWVRD | 327 |
| Caspase-1 | (P29466) | HSEQVPDILQLNAIFNMLNTKNCPSLKDKPKVII IQACRGDSPGVVWFKD | 297 |
| Caspase-14 | (P31944) | LKGEDGEMVKLENLFEALNNKNCQALRAKPKVYIIQACRGE----- | 135 |
| Caspase-2 | (P42575) | IYGVDGKLLQLQEVFQLFDNANCP SLQNKPKMFFI IQACRGDETRDGVDDQ | 332 |



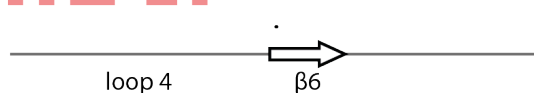
| | | | |
|------------|----------|--|-----|
| Caspase-3 | (P42574) | TELDGCIETDSG-VDDDMA--C-----HKIPVEADFLYAYSTAP | 201 |
| Caspase-7 | (P55210) | TELDGDIQADSGPINDTDANPR-----YKIPVEADFLFAYSTVP | 227 |
| Caspase-6 | (P55212) | DVVDNQTEKLDNTITEVDAASV-----YTLPAGADFLMCSVAE | 214 |
| Caspase-8 | (Q14790) | IPVETDSEEQPYLEMDLSSPQT-----RYIPDEADFLMGMATVN | 407 |
| Caspase-10 | (Q92851) | VSIEADALNPEQAPTSLOQS-----IPAEADFLGLLATVP | 444 |
| Caspase-9 | (P55211) | STSPEDESPGSNPEPDATPFQEGRLTFDQLDAISSLPTPSDIFVSYSTFP | 349 |
| Caspase-4 | (P49662) | SPASLEVASSQSSENLEEDAVY-----KTHVEKDFIAFCSSTP | 308 |
| Caspase-5 | (P51878) | SPASLALISSQSSENLEEDAVY-----KIHEEKDFIAFCSSTP | 365 |
| Caspase-1 | (P29466) | SVGVSGNLSLPTTEEFEDDAIK-----KAHIEKDFIAFCSSTP | 335 |
| Caspase-14 | (P31944) | QRDPGETVGGDEIVMVIKDSPO-----TIPTYTDALHVVYSTVE | 173 |
| Caspase-2 | (P42575) | DGKNHAGSPGCEESDAGKEKLP-----KMLRPLTRSDMICGYACLK | 372 |



| | | | |
|------------|----------|--|-----|
| Caspase-3 | (P42574) | GYYSWRNSKDGSWFIQSLCAMLKQYA-DKLEFMHILTRVN---RKVATEF | 247 |
| Caspase-7 | (P55210) | GYYSWRSPGRGSWFVQALCSILEEHG-KDLEIMQILTRVN---DRVARHF | 273 |
| Caspase-6 | (P55212) | GYYSHRETVNGSWYIQDLCEMLGKYG-SSLEFTELLTLVN---RKVSQRR | 260 |
| Caspase-8 | (Q14790) | NCVSYRNPAGETWYIQSLCQSLRERCPRGDDILTILTEVN-----Y | 448 |
| Caspase-10 | (Q92851) | GYVSFRHVEEGSWYIQSLCNHLKLVPRMLKFLKTEMEIRGRKRTVWGAK | 494 |
| Caspase-9 | (P55211) | GFVSWRDPKSGSWYVETLDDIFEQWAHSEDLQSLLLRVAN----- | 389 |
| Caspase-4 | (P49662) | HNVSWRDSTMGSIFITQLITCFQKYS-WCCHLEEVFRKVQ----- | 347 |
| Caspase-5 | (P51878) | HNVSWRDRTRGSIFITELITCFQKYS-CCCHLMEIFRKVQ----- | 404 |
| Caspase-1 | (P29466) | DNVSWRHPTMGSVFIGRLIEHMQEYA-CSCDVEEIFRQV----- | 374 |
| Caspase-14 | (P31944) | GYIAYRHDQKGSFCFIQTLVDVFTKRKGHILELLTEV--TR-----RM | 213 |
| Caspase-2 | (P42575) | GTAAMRNTRKGSWYIEALQVFSSEA-CDMHVADMLVKVN----ALIKDR | 417 |



| | | | |
|------------|----------|--------------------------------------|-----|
| Caspase-3 | (P42574) | ESFSFDATFHAKKQIPCIVSMLTKELYFYH----- | 277 |
| Caspase-7 | (P55210) | ESQSDDPHFHEKKQIPCVSMLTKELYFSQ----- | 303 |
| Caspase-6 | (P55212) | VDFCKDPSAIGKKQVPCFASMLTKKLHFFPKSN-- | 293 |
| Caspase-8 | (Q14790) | EVSNDKDDKKNMGKQMPQPTFTLRKKLVFPSD---- | 479 |
| Caspase-10 | (Q92851) | QISATSLPTAISAQTTPRPPMRRWSSVS----- | 521 |
| Caspase-9 | (P55211) | ----AVSVKGIYKQMPGCFNLRKKLFFKTS---- | 416 |
| Caspase-4 | (P49662) | ---QSFETPRAKAQMPTIERLSMTRYFYLFPGN-- | 377 |
| Caspase-5 | (P51878) | ---KSFEVPQAKAQMPTIERATLTRDFYLFPGN-- | 434 |
| Caspase-1 | (P29466) | ---FSFEQPDGRAQMPTTERTVTLTRCFYLFPGH-- | 404 |
| Caspase-14 | (P31944) | AEAELVQEGKARKTNPEIQSTLRKRLYLQ----- | 242 |
| Caspase-2 | (P42575) | EGVAPGTEFHRCKEMSEYCSLTCRHLYLFPGHPPT | 452 |



Supplementary Figure 5: Caspase sequence alignment and interacting residues

(a) Caspase topology map with active site residues (His-237 and Cys-285) indicated with green dots. Active-site forming loops are marked with loop-1 to loop-4. They are also known as 179-loop (=loop-1), 240-loop (=loop-2), 341-loop (=loop-3) and 381-loop (=loop-4) (6).

(b) Sequence alignment (ClustalW2, <http://www.ebi.ac.uk/Tools/msa/clustalw2>) of all human caspase family members with sequence accession numbers as indicated in the figure. DARPIn interacting residues were determined using the EPPIC server (1) (<http://www.eppicweb.org>) and are marked in red. Primary structure elements are indicated below the sequence and labeled according the caspase topology map depicted in panel (a). Consensus symbols are depicted below the sequence with (*) indicating fully conserved residues and conservation of strongly (:) and weakly (.) similar properties.

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