

Okamoto *et al* Supplemental Material

Legends to the Supplementary Figures

Figure S1: Sequence alignment of all 6 identified M11L orthologs

Residues marked in cyan are located in the binding groove of M11L are conserved between M11L and SPPV14. Green indicates residues that are located in the binding groove of M11L are conserved between M11L and SPPV14 and appear as a group to determine anti-apoptotic activity within the 6 M11L orthologs. Magenta indicates residues that are different between DPV83gp022 and DPV84gp022. & indicate additional residues that are conserved in all 6 M11L orthologs and are located in the M11L binding groove. * indicates residues fully conserved across all sequences, : highly conserved and . relatively conserved residues.

Figure S2: The putative SPPV14 binding groove

Ribbon diagram of M11L (green) bound to the Bak BH3 peptide (yellow, N-terminus at bottom); PDB:2JBY (1). Highlighted in cyan are four M11L residues (I37, Y41, A82 and F122) that are strictly conserved across a family of related poxvirus sequences. Highlighted in magenta are four M11L residues (M52, T67, L68, A71) that display sequence variation between functionally active and inactive proteins described here (see Discussion). Particular constellations of these four residues can be correlated with pro-survival activity.

Figure S3: SPPV14 does not inhibit Fas-induced cell death

Bax/Bak (*bax*^{-/-}/*bak*^{-/-}) deficient or wild-type MEFs infected with retrovirus carrying M11L, SPPV14 or B14 were treated with (A) FasL (100 ng/mL) alone or additionally, with (B) cycloheximide (1 μg/mL) and anti-FLAG antibody (2 μg/mL). Cell viability was determined by propidium iodide (PI) exclusion at each time point. Data represent means ± SD from 2 independent experiments.

SM References

1. Kvensakul M, van Delft MF, Lee EF, Gulbis JM, Fairlie WD, Huang DC, *et al.* A structural viral mimic of prosurvival Bcl-2: a pivotal role for sequestering proapoptotic Bax and Bak. *Mol Cell* 2007 Mar 23; **25**(6): 933-942.

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M11L -----MMSRLKTAVYDYLDNDVDITE-----CTEMDLLCQLSNCCDFINETYAKNYDTLYDIMERDILS 58
SPPV14 ---MDNCNYNIEKVLNVYLRDLRIES----LNNNELEILIMIRECCEVIKKDYKTEFNEICNFILQNNVK 63
GP011L -----MSRLKEVVYTYLNGGDITE-----CTEIDLCCQLVNCNFINNTYAKNYDVLCDIMERDILS 57
LD17 ---MDNCNYNIEKVLNVYLRDLRIES----LNNNELAILIMIRECCEVIKKDYKTEFNEICNFILRNNVK 63
SPV12L MYKKYNSNVCIRNVLYVYLYKYNTINK----LSRYERMIYTKIKNQCEAIKYRYCNDFNSVTCILEYDENK 66
DPV83gp022 MEAAIEFDEIVKLLNIYINDICTMGEKRLLNNYEKSILDRIYKSCHEYIKKNEYELDFNSMYNQININDIT 70
DPV84gp022 MEAAIEFDEIVKLLNIYINDICTMGEKRLLNNYEKSILDRIYKSCHEYIKKNEYELDFNSMYNQININDIT 70
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# &
M11L YN--IVNIKNITTFALR-DASPSVKLATLTLASVIKKLN---KIQHTDAAMFSEVIDGIVAEEQQVIGF 122
SPPV14 SCYDINDVKNIIETINSDFRPSVILASISLLSIIIKKKKDENNEVVDDDLALNELINKFSSYQKDIISF 133
GP011L YN--IENIKKALGFALL-DASPSVKLATLALLSIIILKKNL---KIRHTEACVFSVDVIDGITAEENKVIGF 121
LD17 SCYDINDVKNIIETINSDFRPSVILASISLLSIIIKKKKNENNEVVNDLALNELINTFSSYQKDIISF 133
SPV14 YID---NVHKEVISILLSDSRPSIKLAAISLLSIIIDKLCIRNIR--IAKYIIDDINIISEDGIYIILF 131
DPV83gp022 TS---DIKSKIIEALLIDSRPSVKLATLSFISLIAEKWG-EKNRAKIMEILSNEIVEEKISNNGKDFIDF 135
DPV84gp022 TS---DIKSKIIESLLIDSRPSVKLATLSFISLIAEKWG-EKNRTKIMEILSNEIVEEKISNNGKDFIDF 135
      : : : * ** : ** : : : : : . *

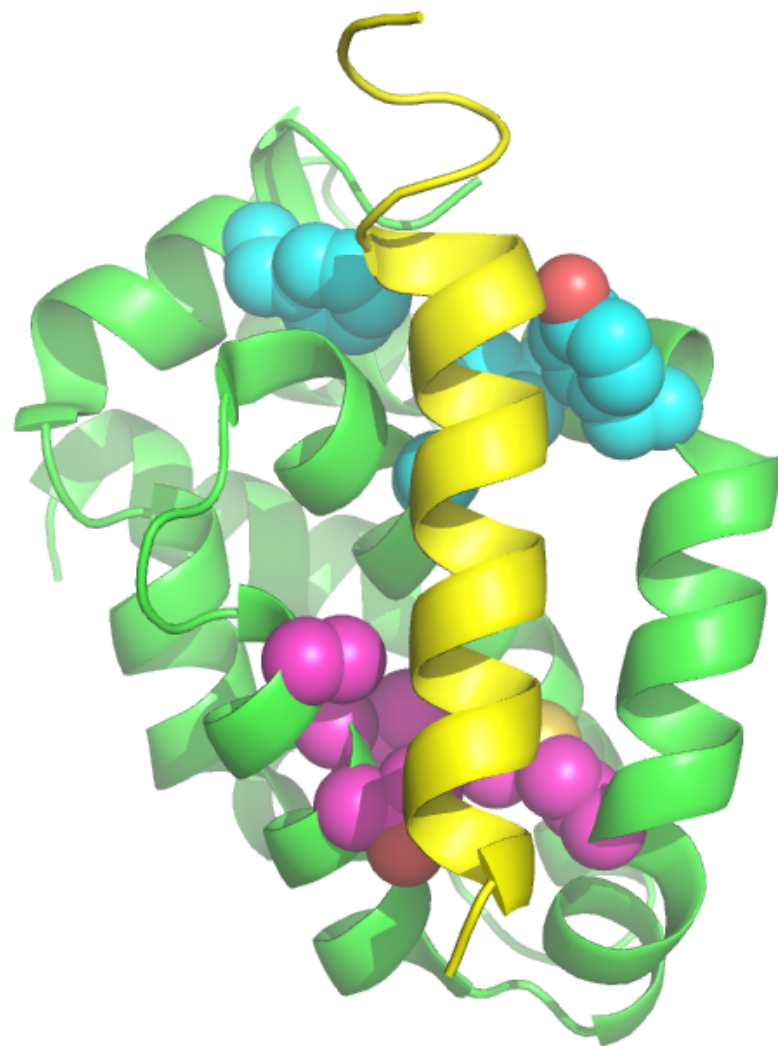
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&
M11L IQKKCKYNTTYYNVRSGGCKISVYLTAAVVG-FVAYGILKWYRGT--- 166
SPPV14 VEKNKKKNKQNDFFIFS---IINFFVIVGSII--ITYYLLKIIIGRIRWK 176
GP011L IQEKYKYNTTYYNKRS---KLPVYLTAMVATLIVYGVIKWRRGT--- 163
LD17 VEKNKKNNEHNDFFIFS---IINFFVMVGSII--IAYYLLKIIIGRIRWK 176
SPV14 LDEFDKYTDTR---CR---RRGLSMMIASIV---TYYCLRYVLKI--- 167
DPV83gp022 IDRDDDDIVDDYVLIT---NYLKITIFGAILGITAYYICKYLLKSIF- 179
MPV84gp22 IDRDDDDIVDDYVLIT---NYLKITIFGAILGITAYYICKYLLKSIF- 179
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Okamoto *et al* Fig. S3

