

Supplementary Figures

Chen and Richards *et al*

“The crystal structure of the caspase recruitment domain of BinCARD reveals that all three cysteines can be oxidized”

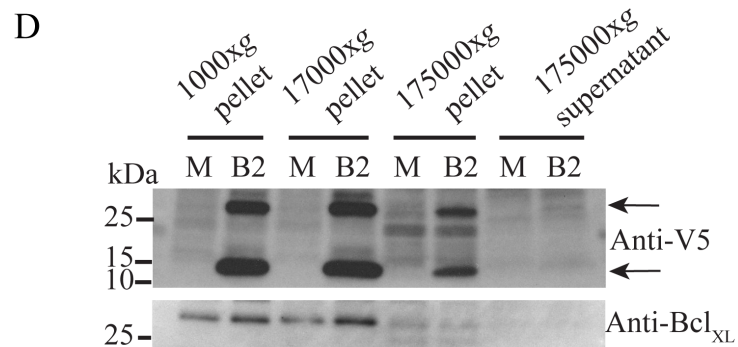
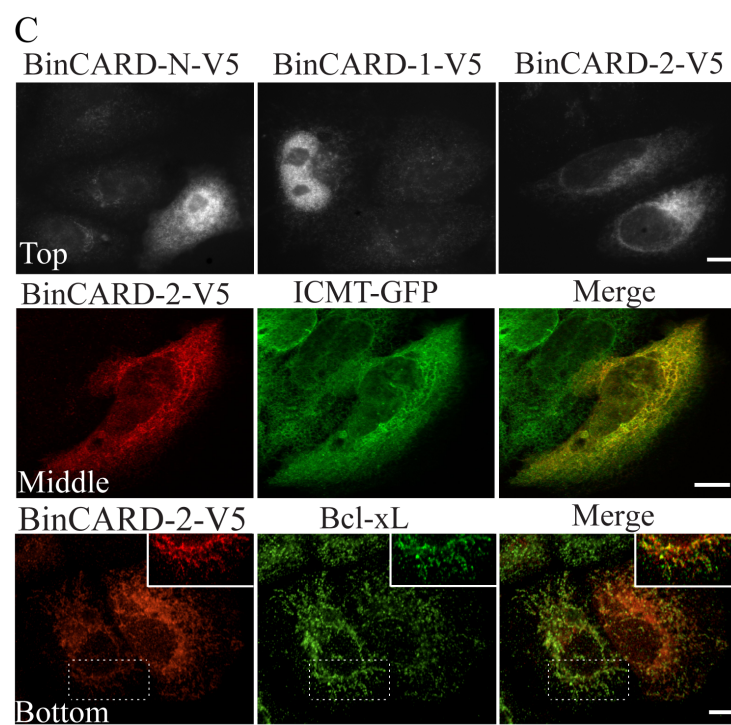
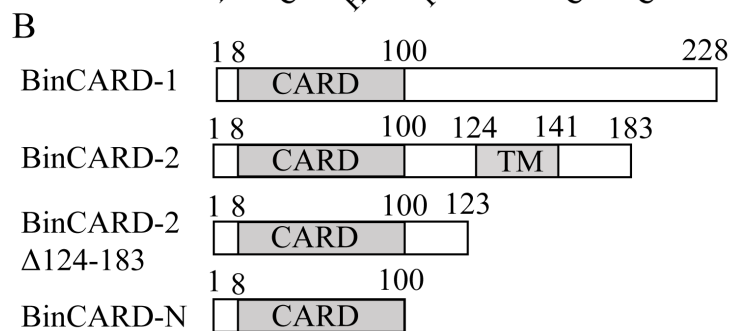
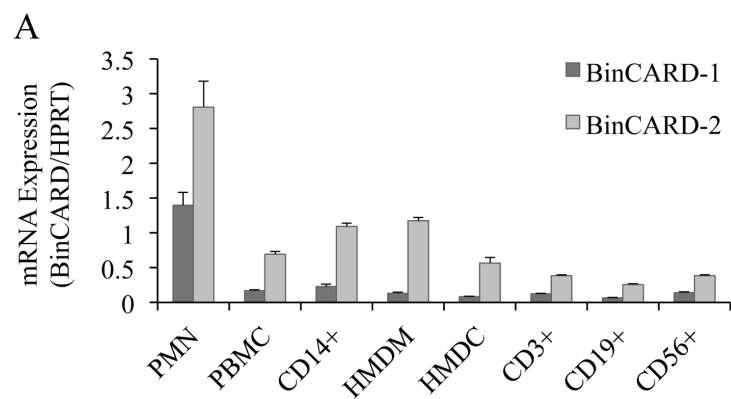


Figure S1. Expression and localization of BinCARD isoforms (A) Real time qPCR of Bincards 1 and 2 in human peripheral blood mononuclear cell fractions. CD14⁺ monocytes, CD4⁺ T cells, CD19⁺ B cells and CD56⁺ natural killer (NK) cells were purified directly, whereas human monocyte-derived dendritic cells (HMDC) and human monocyte-derived macrophages (HMDM) were differentiated as described in Materials and Methods. (B) Schematic representation of BinCARD constructs, BinCARD-1 (BinCARD isoform 1), BinCARD-2 (BinCARD isoform 2), BinCARD-2-Δ124-183 (BinCARD-2 lacking the C-terminal residues) and BinCARD-N (CARD alone) used in this study. (C) HeLa cells were seeded on glass coverslips and transfected with V5/His-tagged BinCARD-N, BinCARD-1 or BinCARD-2 (top panels). Cells were fixed 20 h post-transfection and immunolabelled for V5 before imaging by epifluorescence microscopy. Confocal imaging of cells co-expressing BinCARD-2 with the ER marker, ICMT-GFP (middle panels), or cells transfected with BinCARD-2 and colabelled for the outer mitochondrial membrane protein, Bcl-x_L (bottom panels), revealed clear colocalization of BinCARD-2 with both proteins, indicating an ER and mitochondrial distribution for this isoform. Images shown are representative of three or more independent experiments. (D) Cells transfected with BinCARD-2-V5 (B2) or mock-transfected (M) were fractionated by a series of centrifugation steps of increasing g-force. Full-length BinCARD-2-V5 and an N-terminally truncated form were found to be enriched in the 1000 x g pellet and 17,000 x g pellet along with the mitochondrial marker, Bcl-x_L. Images shown are representative of three or more independent experiments. Scale bars, 5 μm.

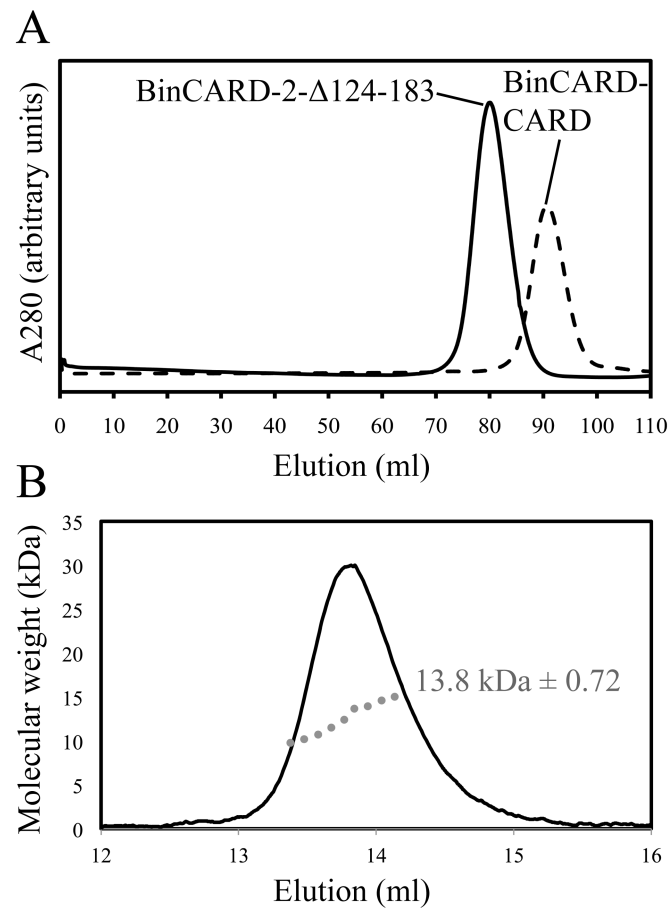


Figure S2. Characterization of the oligomeric status of BinCARD-CARD and BinCARD-2-Δ124-183 (A) Purified human BinCARD-2-Δ124-183 and BinCARD-CARD both elute as a single peak on size exclusion chromatography (SEC). (B) SEC-MALLS of BinCARD-2-Δ124-183 indicates that this soluble form of BinCARD-2 is monomeric in solution.

Human	3	DQTYCDRLVQDTPFLTGHRGLSEQQVDRIILQLNRYYPQILTNKEAEKF	51
Mouse	3	DQTYCDRLVQDTPFLTGQGRGLSEQQVDRIILQLNRYYPQILTNKEAEKF	51
Rat	23	DQTYCDRLVQDTPFLTGQGRGLSEQQVDRIILQLNRYYPQILTNKEAEKF	71
Bovine	3	EQTYCDRLVQDTPFLTSLGRGLSEQQVDRIILQLNRYYPQILSNKDAEKF	51
Chicken	6	HQSYCHRLQHDMYFLTSNSRLNEQVVDKIILQLNRYYPQILTNTEAEKF	54
Dog	506	EQTYCDRLVQDTPFLLGRLSEQQVDRIILQLNRYYPQILSNKDAQKF	554
Cat	3	EQTYCDRLVQDTPFLMGHRGLSEQQVDRIILQLNRYYPQILSNNDAEKF	51
Pig	3	EQTYCDRLVQDTPFLTGLGRGLSEQQVDRIILQLNRYYPQILSNKDAEKF	51
Rhesus macaque	3	DQTYCDRLVQDTPFLTGHRGLSEQQVDRIILQLNRYYPQILTNKEAEKF	51
Atlantic salmon	2	GDSFHDQLLEDRLSDRFLRTDRRLDTLVDKLILQLNRYYPQILTDKEATKF	50
Tasmanian devil	135	DQTYCDRLVQDTPFLTSNGRLSEQQVDKIILQLNRYYPQILTNKEAEKF	183
Pygmy chimpanzee	3	DQTYCDRLVQDTPFLTGHRGLSEQQVDRIILQLNRYYPQILTNKEAEKF	51
Green anole	4	DQTYCDRLQDTPFLTSNNRLSEQLVDKIILQLNRYYPQILTNKEAEKF	52
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Human	52	RNPKASLRVRLCDLLSHLQRSGERDCQEFYRALYIHAQPLHSRLPSRH	99
Mouse	52	RNPKASLRVRLCDLLSHLQQRGERHCQEFYRALYIHAQPLHSHLPSRY	99
Rat	72	RNPKASLHRLCDLLSHLQQRGERHCQEFYRALYIHAQPLHSHLPSRY	119
Bovine	52	RNPKLSLRVRLCDLLGHLQRSGERDCQEFYRALYIHAQPLHSCLP SRH	99
Chicken	55	RNPKASLHTRLSDLIKHLQKKGERHCQEFYRALQINAEQLYDDLPSRK	102
Dog	555	RNPKASVRVRLCDLLGHLQRSGERDCQEFYRALYIHAQPLHGLLPSRL	602
Cat	52	RNPKASLRVRLCDLLGHLQRSGERDCQEFYRALYIHAQPLHSRLPSRL	99
Pig	52	RNPKASLRVRLCDLLGHLQRSGERDCQEFYRALYIHAQPLHSGLP SRH	99
Rhesus macaque	52	RNPKASLRVRLCDLLSHLQRSGERDCQEFYRALYIHAQPLHSCLP SRH	99
Atlantic salmon	51	RDLDVPTCVRLAEALLAHLQKGEEACREFYRALHLHVEEVYFSLPTRL	98
Tasmanian devil	184	RNPKTSLRVRLCDLLTHLQRKGERDCQEFYRALYINAQHLYVSLPSRK	231
Pygmy chimpanzee	52	RNPKASLRVRLCDLLSHLQRSGERDCQEFYRALYIHAQPLHSRLPSRH	99
Green anole	53	RSPKASLHSRLSNLIAHLQKKGDKPCQEFYRALQINAEQLYNNLPSRK	100
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Figure S3. Multiple sequence alignment of BinCARD-CARD in 13 different species. Residues of each BinCARD-CARD sequence are numbered at the left end and right ends of each row. BinCARD-CARD residues identical across all species are shown in red and labeled with symbol *. Conserved and weakly conserved residues across all species are labeled with the symbols : and . respectively. The three cysteine residues are highlighted in yellow and residues Y39 and P40 involved in the *cis*-peptide bond are highlighted in blue. The sequence alignment was generated using CLUSTALW (Combet *et al.*, 2000) from the Network Protein Sequence Analysis using default parameters. Sequence conservation was based on a TCooffee multiple sequence alignment (Notredame *et al.*, 2000).

References:

Combet, C., Blanchet, C., Geourjon, C. & Deleage, G. (2000). *Trends Biochem. Sci.* **25**, 147-150.

Notredame, C., Higgins, D. G. & Heringa, J. (2000). *J. Mol. Biol.* **302**, 205-217.