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"

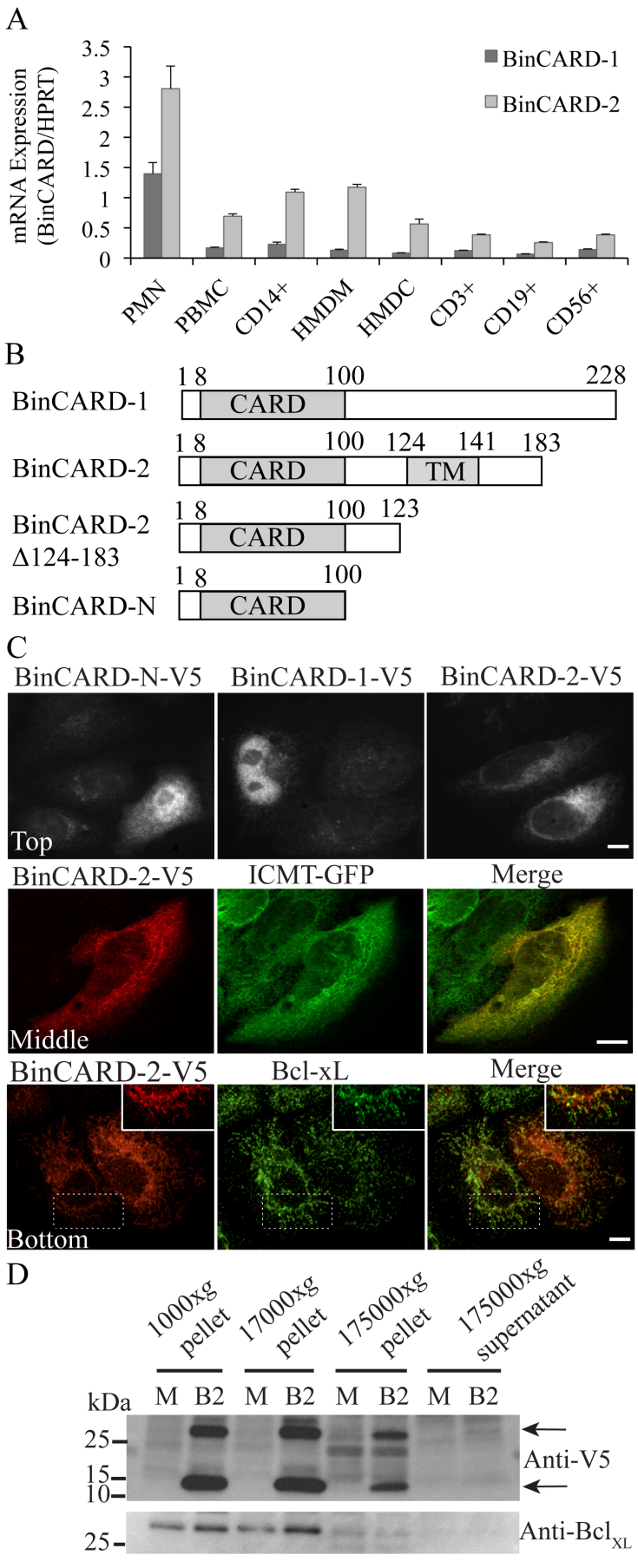
A
mRNA Expression (BinCARD/HPRT)
3.5
3
2.5
2
1.5
1
0.5
0
BinCARD-1
BinCARD-2
PMN
PBMC
CD14+
HMDM
HMDC
CD3+
CD19+
CD56+
B
BinCARD-1
1 8
100
228
CARD
BinCARD-2
1 8
100
124
141
183
CARD
TM
BinCARD-2 Δ124-183
1 8
100
123
CARD
BinCARD-N
1 8
100
CARD
C
BinCARD-N-V5
BinCARD-1-V5
BinCARD-2-V5
Top
BinCARD-2-V5
ICMT-GFP
Merge
Middle
BinCARD-2-V5
Bcl-xL
Merge
Bottom
D
1000xg pellet
17000xg pellet
175000xg pellet
175000xg supernatant
kDa
M B2 M B2 M B2 M B2
25
15
10
Anti-V5
25
Anti-Bcl$_{XL}$

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.

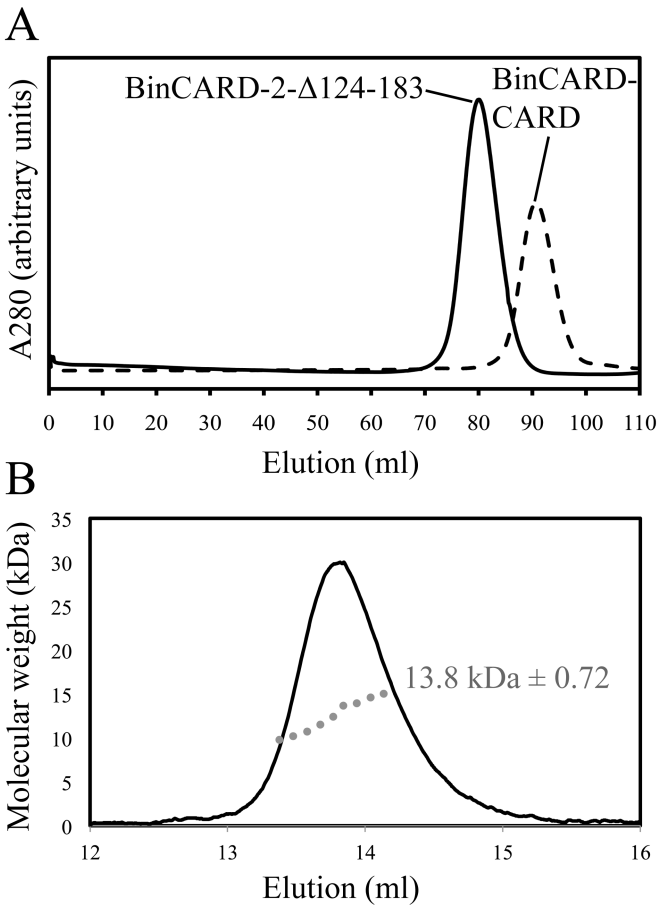

Figure S2. Characterization of the oligomeric status of BinCARD-CARD and BinCARD-2-Δ124-183 (A) Purified human BinCARD-2-Δ124-183 and BinCARD-CARD both elute as a single peak on size exclusion chromatography (SEC). (B) SEC-MALLS of BinCARD-2-Δ124-183 indicates that this soluble form of BinCARD-2 is monomeric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

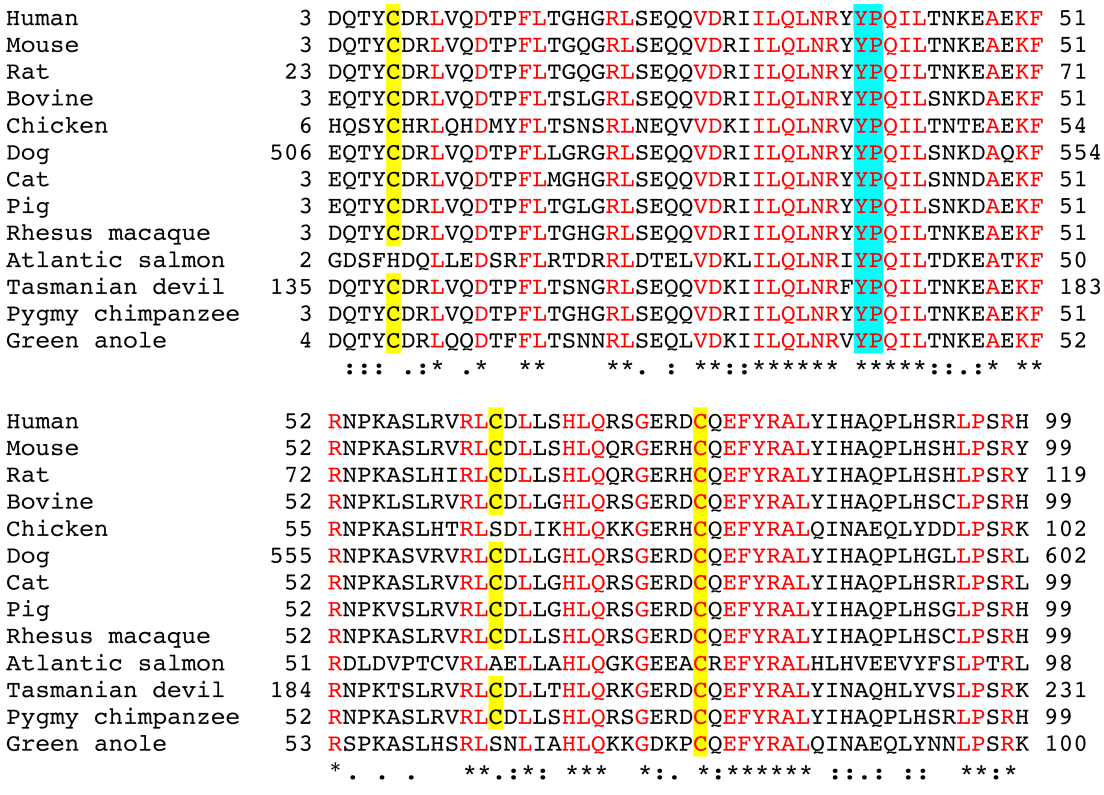

Figure S3. Multiple sequence alignment of BinCARD-CARD in 13 different species. Residues of each BinCARD-CARD sequence are numbered at the left end and right ends of each row. BinCARD-CARD residues identical across all species are shown in red and labeled with symbol *. Conserved and weakly conserved 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 TCoffee 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.