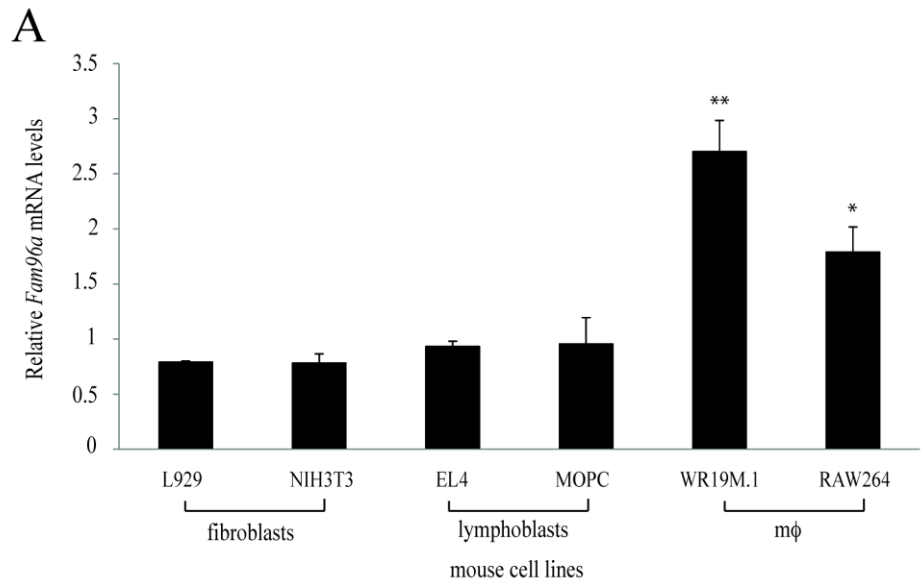
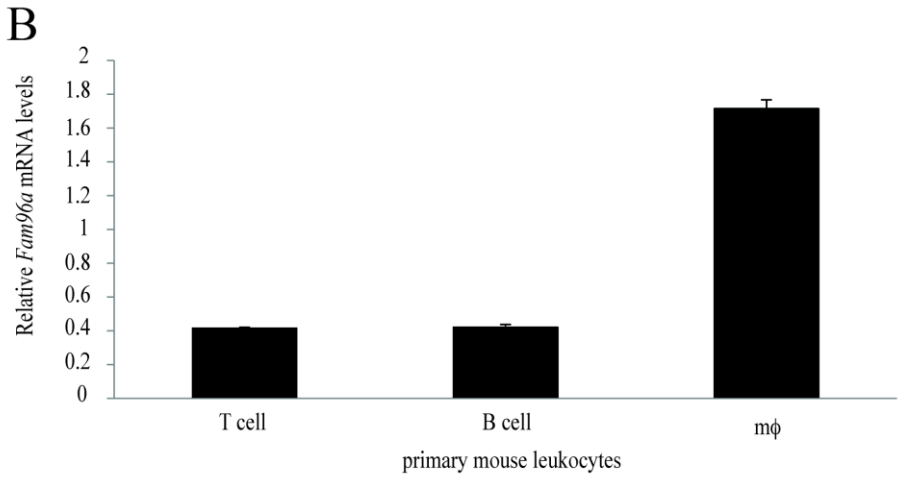
SUPPLEMENTARY MATERIAL

Table S1. Structural comparison of hFam96a major and minor dimers with three bacterial DUF59-containing proteins

	PDB ID	Z score[§]	RMSD[§] (Å)	No. aligned residues	Sequence identity (%)
Major dimer					
TM0487	1UWD	0.8	3.4	47	13
***B. anthracis* DUF59**	3LNO	1.5	3.1	49	8
TTHB138	3CQ1	0.4	3.6	40	5
Minor dimer					
TM0487	1UWD	7.9	3.1	83	17
***B. anthracis* DUF59**	3LNO	11.7	1.9	85	21
TTHB138	3CQ1	8.6	2.0	77	22

[§] Calculated using DALI (Holm and Rosenstrom, 2010)


A
Relative Fam96a mRNA levels
3.5
3
2.5
2
1.5
1
0.5
0
**
*
L929
NIH3T3
EL4
MOPC
WR19M.1
RAW264
fibroblasts
lymphoblasts
mϕ
mouse cell lines


B
Relative Fam96a mRNA levels
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
T cell
B cell
mϕ
primary mouse leukocytes

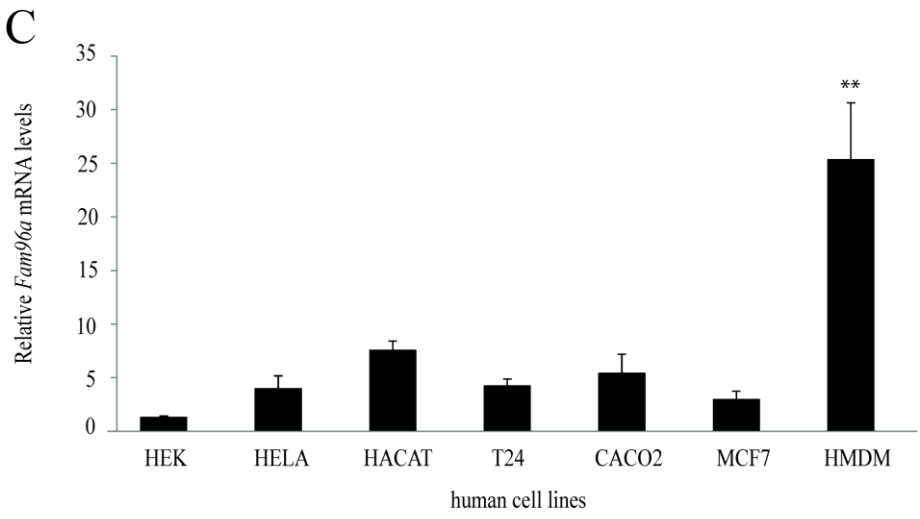

C
Relative Fam96a mRNA levels
35
30
25
20
15
10
5
0
**
HEK
HELA
HACAT
T24
CACO2
MCF7
HMDM
human cell lines

Figure S1. Quantitative real-time PCR analysis of *Fam96a*. Quantitative real-time PCR profiles of *Fam96a* mRNA expression in (**A**) different mouse cell lines; (**B**) three primary mouse leukocyte populations; and (**C**) human cell lines versus primary human monocyte-derived macrophages (HMDM). Data represent mean ± SEM for three independent RNA preparations (**A**, **C**) or mean ± range for two independent RNA preparations (**B**). * $P<0.05$, ** $P<0.01$ (compared to non-macrophage cell types, one-way ANOVA).

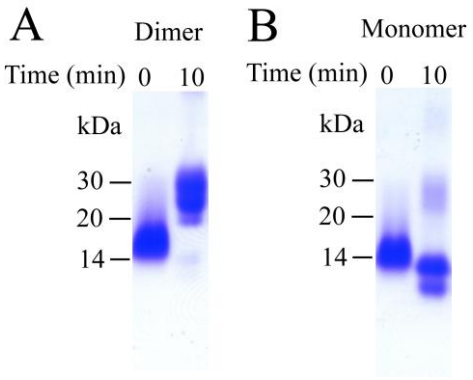


Figure S2. Fam96a forms monomers and dimers. (A) Fam96a peak 2 crosslinked with BS3 for 0 min and 10 min, revealing the presence of Fam96a dimer. (B) Fam96a peak 3 crosslinked with BS3 for 0 min and 10 min, revealing the sample is monomeric. The monomer runs as two types of BS3 cross-linked protein. Both run faster than the uncrosslinked protein on SDS-PAGE, as crosslinked proteins can form SDS complexes with a smaller Stokes radius than uncrosslinked protein:SDS complexes (Staros and Anjaneyulu, 1989). This experiment was performed twice giving the same result each time.

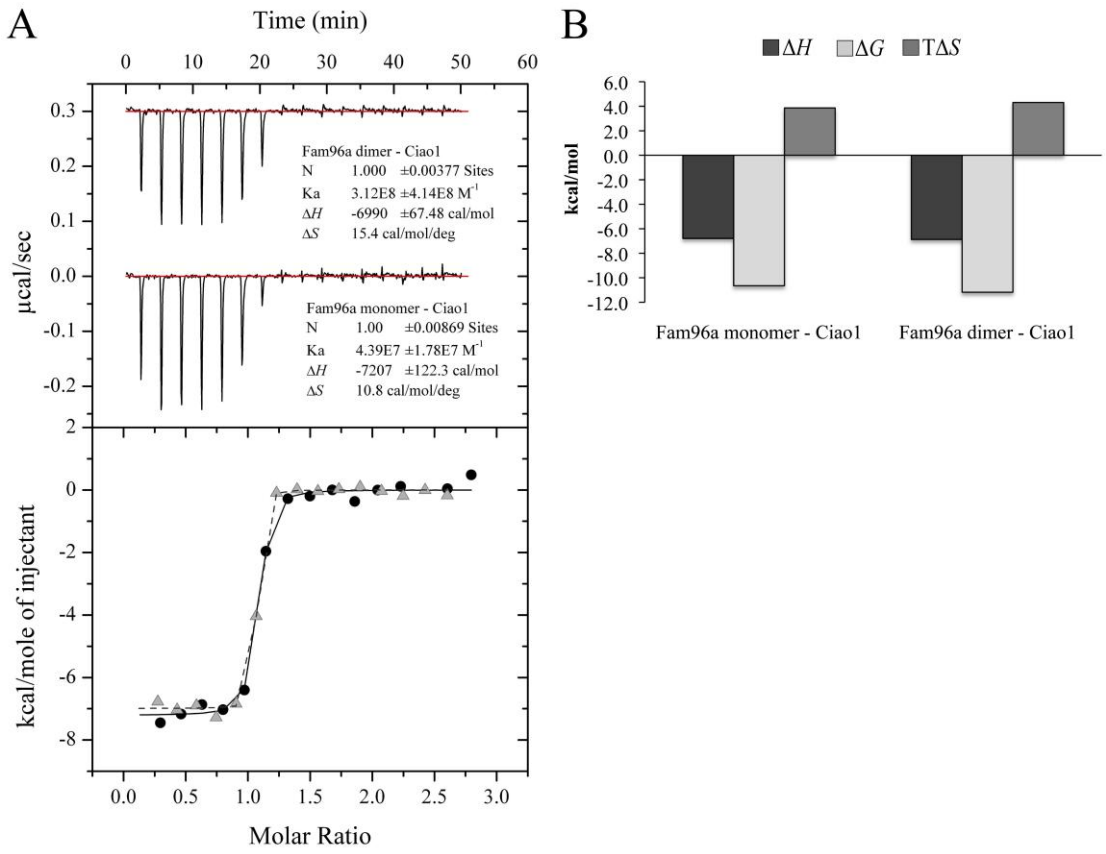


Figure S3. Isothermal titration calorimetry data for the Fam96a-Ciao1 interaction. (A) Raw data (top) and integrated normalized data (bottom) of dimer-Ciao1 (grey line and triangle) and monomer-Ciao1 (black line and circle) complex. The image shown is representative of three independent experiments. (B) Histogram of enthalpy (Δ*H*), Gibbs free energy (Δ*G*) and entropy (TΔ*S*) of dimer-Ciao1 and monomer-Ciao1 complex.

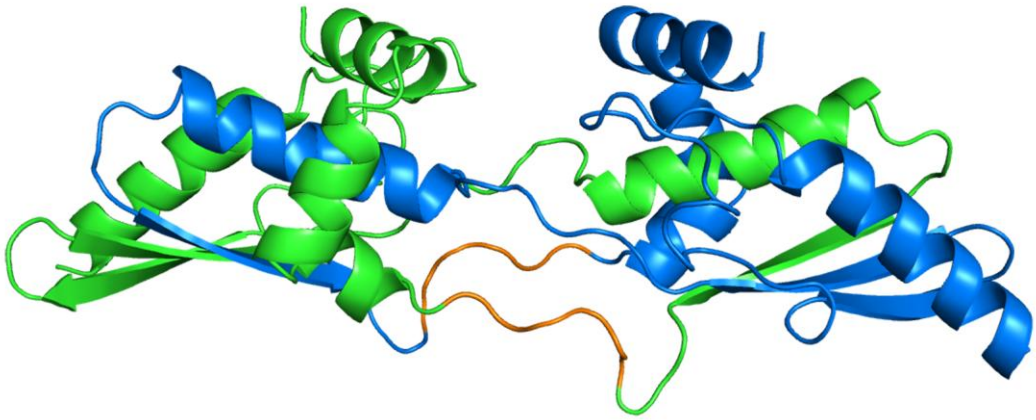


Figure S4. Schematic representation of Fam96a major dimer structure with modelled flexible loop. The flexible region (highlighted in orange) comprising residues 122 to 127 has no electron density, and was modelled here using ARP/wARP (Langer et al., 2008).

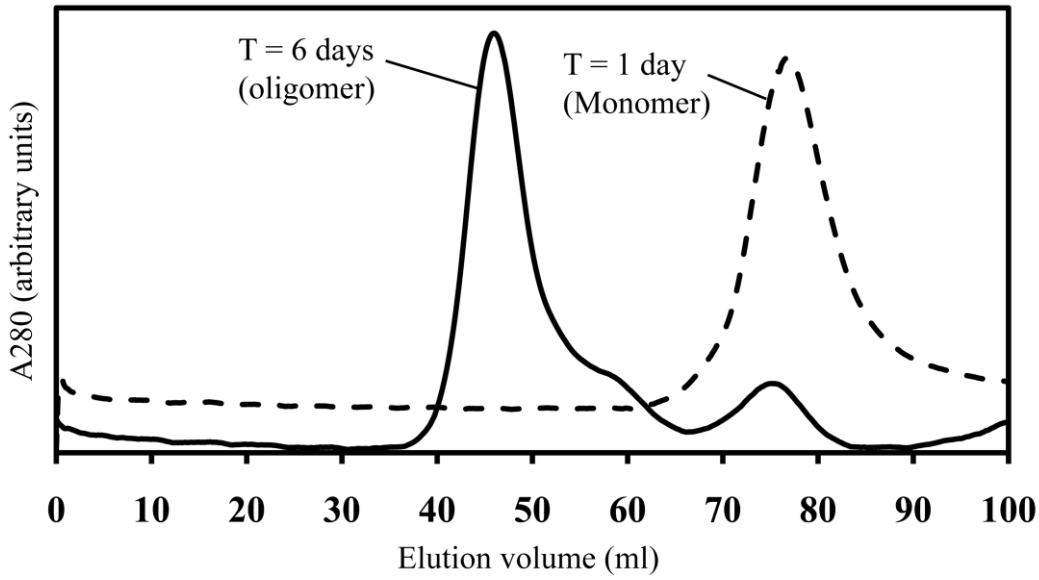


Figure S5. SEC analysis of Fam96a monomer after zinc incubation. The dashed line represents SEC analysis of purified Fam96a monomer that had been incubated in 1 mM zinc after 1 day at 4°C. The solid line represents SEC analysis of purified Fam96a monomer incubated with 1 mM zinc after 6 days at 4°C. The protein had precipitated over the 6 days and the precipitated protein was re-solubilised in the same buffer without zinc. The monomeric Fam96a had converted almost entirely to oligomer with a shoulder peak suggesting the presence of a small amount of dimer.

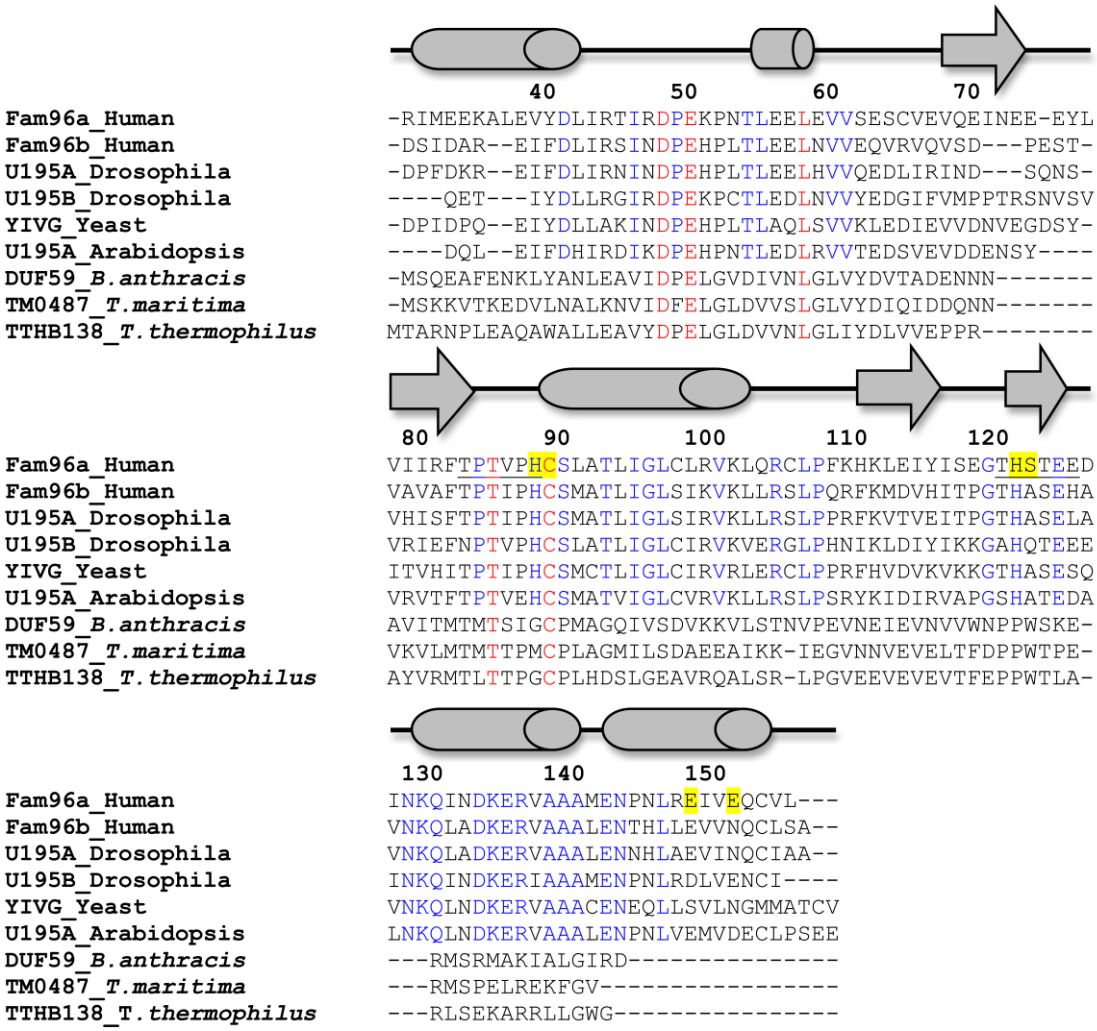


Figure S6. Sequence alignment of eukaryotic and prokaryotic DUF59-containing proteins. Conserved residues in all the proteins are highlighted in red and conserved residues in MIP18/DUF59-containing proteins are highlighted in blue. The two hinge loops of Fam96a are underlined and the zinc-binding residues are highlighted in yellow. Sequence alignment was generated using CLUSTALW from Network Protein Sequence Analysis using default parameters (Combet et al., 2000). These proteins only contain a DUF59 domain.

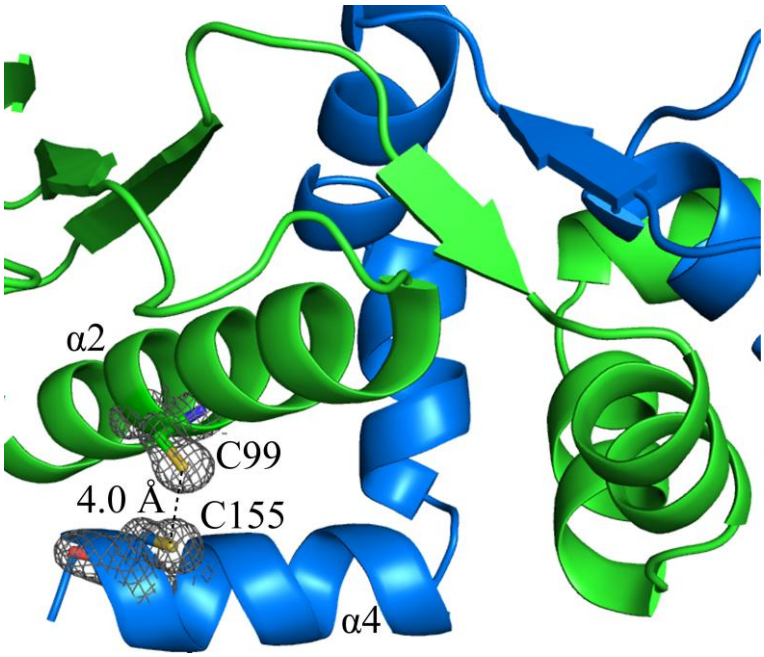


Figure S7. Electron density for C99 and C155. The sulfur atom of C155 from the C-terminus of one protomer (blue) is located within 4 Å of the sulfur atom of C99 from the other protomer (green). Shown is the structure of the minor dimer, though the same placement of these cysteines occurs in the major dimer structure. The electron density map ($2F_o$-F_c map shown at 1σ) indicates a disulfide is not present in the structure, but the proximity of the two cysteines suggests this could occur under appropriate conditions.

REFERENCES

Combet, C., Blanchet, C., Geourjon, C., and Deleage, G. (2000). NPS@: network protein sequence analysis. Trends Biochem Sci *25*, 147-150.

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