Conformational flexibility of the oncogenic protein LMO2 primes the formation of the multi-protein transcription complex

H. Sewell, T. Tanaka, K. El Omari, E.J. Mancini, A. Cruz,

N. Fuentes-Fernandez, J. Chambers, T.H. Rabbitts

Supplementary Table one: Data Collection

Data collection statistics	
Space group	P6
Unit cell Dimensions *a, b, c* (Å) a, β, γ (°)	a=124.3, b=124.3, c=81.4 and α =90.0° β = 90.0° γ = 120.0°
Resolution (Å)	50-(2.95-2.80)
Completeness (%)	99.8 (100)
Redundancy	2.9(3.0)
I/ σ (I)	10.8 (2.3)
Rmerge (%)	10.9 (72.9)
Refinement Statistics	
Resolution	50.00-2.80
Number of reflections	16834
Rwork/Rfree (%)	23.99/25.83
R.m.s.d. bond (Å)	0.005
R.m.s.d. angle (°)	0.904
Mean B-factor ($Å^2$)	75.405
Molecules in the a.u.	2
Ramachandran plot (%) Favored/allowed/outliers	93.7/5.56/0.74

Supplementary Table 2: Contacts of Structural Restraint

Antibody region	VH residue	LMO2 residue	Interaction type
CDR1	His 31	Arg 63	Charged groups
Framework	Gln 39	Phe 129 Cys 130	Hydrogen bond Charged groups
Framework Former VH-VL interface	Leu 45	Leu 117 Phe 120 Phe 120 Val 131	Hydrophobic Hydrophobic Hydrophobic Hydrophobic
Framework Former VH-VL interface	Trp 47	Met 106 Leu 117	Hydrophobic Charged groups
CDR2	Tyr 50	Cys 60 Met 106	Hydrogen bond Hydrophobic
CDR2	Tyr 53	Asp 53	Hydrogen bond
CDR2	Asn 54	Asp 53	Hydrogen bond
CDR2	Ser 57	Leu 59 Cys 60 Gly 61	Charged groups Charged groups Hydrophobic
Framework	Tyr 59	Leu 59 Tyr 104	Hydrogen bond Hydrophobic
Framework	Tyr 95	Val 131	Hydrophobic
CDR3	Leu 100	Arg 109 (also contacts LID)	Hydrophobic
CDR3	Thr 101	Glu 66	Charged groups
CDR3	Glu 102	Cys 80 Arg 81 Arg 82	Charged groups Charged groups Hydrogen bond
CDR3	Ser 103	Cys 60 Asp 83 Asp 86 (hinge region)	Hydrogen bond Hydrogen bond Charged groups
CDR3	Leu 104	Leu 59 Tyr 104 Glu 105 Met 106 Thr 107	Hydrophobic Hydrophobic Charged groups Hydrophobic Hydrogen bond
CDR3	Glu 105	Arg 82 Arg 86 (hinge) Thr 107 Arg 109 Val 114	Hydrophobic Hydrogen bond Charged groups Hydrogen bond Charged groups
CDR3	Leu 106	Met 106 Thr 107 Met 108 Arg 109 Leu 117	Hydrophobic Charged groups Hydrophobic Hydrogen bond Hydrophobic
CDR3	Thr 107	Arg 109	Hydrogen bond
CDR3	Ala 108	Val 110 Phe 120 Tyr 135	Charged groups Hydrophobic Hydrophobic
CDR3	Trp 110	Phe 120 Cys 130 Val 131	Hydrophobic Hydrophobic Hydrophobic

		Gly 132 Asp 133 Tyr 135	Charged groups Hydrogen bond Hydrophobic
Framework	Trp 114	Met 108 Val 131	Hydrophobic Hydrophobic

Hydrogen bonds XML

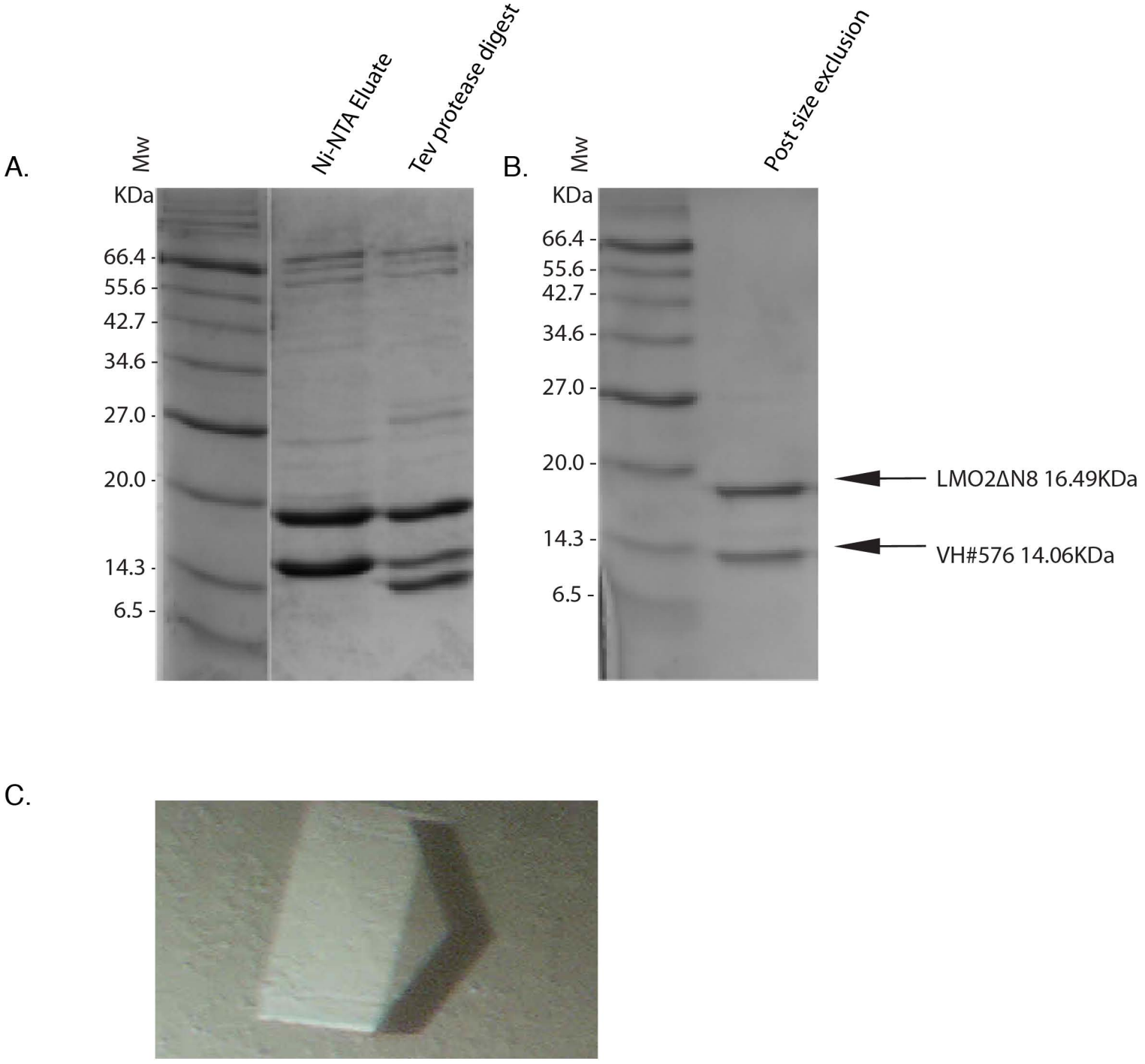
##	Structure 1	Dist. [Å]	Structure 2
1	C:GLN 39[NE2]	3.35	A:PHE 129[O]
2	C:TYR 50[OH]	2.66	A:CYS 60[O]
3	C:TYR 53[OH]	3.44	A:ASP 53[OD1]
4	C:TYR 53[OH]	3.09	A:ASP 53[O]
5	C:ASN 54[ND2]	3.65	A:ASP 53[O]
6	C:ASN 54[ND2]	3.86	A:SER 56[OG]
7	C:TYR 59[OH]	2.61	A:LEU 59[O]
8	C:SER 103[N]	3.36	A:CYS 60[SG]
9	C:SER 103[OG]	2.57	A:ASP 83[OD1]
10	C:LEU 106[N]	2.73	A:THR 107[O]
11	C:THR 107[OG1]	3.04	A:ARG 109[O]
12	C:ALA 108[N]	3.43	A:ARG 109[O]
13	C:ALA 108[N]	3.58	A:MET 108[SD]
14	C:TRP 110[NE1]	2.97	A:ASP 133[O]
15	C:GLU 102[OE1]	3.10	A:ARG 82[N]
16	C:LEU 104[O]	3.53	A:THR 107[N]
17	C:LEU 104[O]	3.32	A:THR 107[OG1]
18	C:GLU 105[OE1]	3.81	A:ARG 109[NE]
19	C:GLU 105[OE2]	3.13	A:ARG 86[NH1]
20	C:LEU 106[O]	2.75	A:ARG 109[N]
21	C:ALA 108[O]	3.32	A:TYR 135[OH]

Salt bridges XML

##	Structure 1	Dist. [Å]	Structure 2
1	C:GLU 105[OE1]	3.81	A:ARG 109[NE]
2	C:GLU 105[OE2]	3.13	A:ARG 86[NH1]

No disulfide bonds found
No covalent bonds found

Supplementary Figure S1.



Purification and crystallisation of anti-LMO2 VH576 and LMO2

(A) LMO2 and VH#576 were co-purified using Nickel agarose chromatography. The protein purity was analysed by 15% SDS-PAGE, stained with Coomassie Brilliant Blue, pre and post digestion with his-tagged Tobacco Etch virus protease.

(B) The protein purity was again assessed after gel filtration chromatography with a Hi-Load Superdex 75 column.

(C) The microscope image shows a crystal grown by sitting drop vapour diffusion, Greiner 96 well plate and reagent 100mM MES monohydrate pH 6.0, 0.8 M ammonium sulphate with additive: 1,3 Butanediol. The drop consisted of 200nl VH576/LMO2 at 8mg/ml, 100nl reagent and 100nl additive. The reservoir (100µl) reagent was at a dilution of 90%. The hexagonal, plate shaped crystal, measured 100µm by 40µm

Supplementary Figure 2

A. Amino Acid sequence of anti-LMO2 VH protein

```
      1         10         20         30 CDR1    40
GGSMAEVQLLESGGGLVQPGGSLRLSCAASGFSFSHSPMNWVRQAPGKG
     50   CDR2   60         70         80         90
LEWVSYISYNSSSIYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV
     100   CDR3 110        120
YYCARGLTESLELTADWFDYWGQGTLVTVSS
```

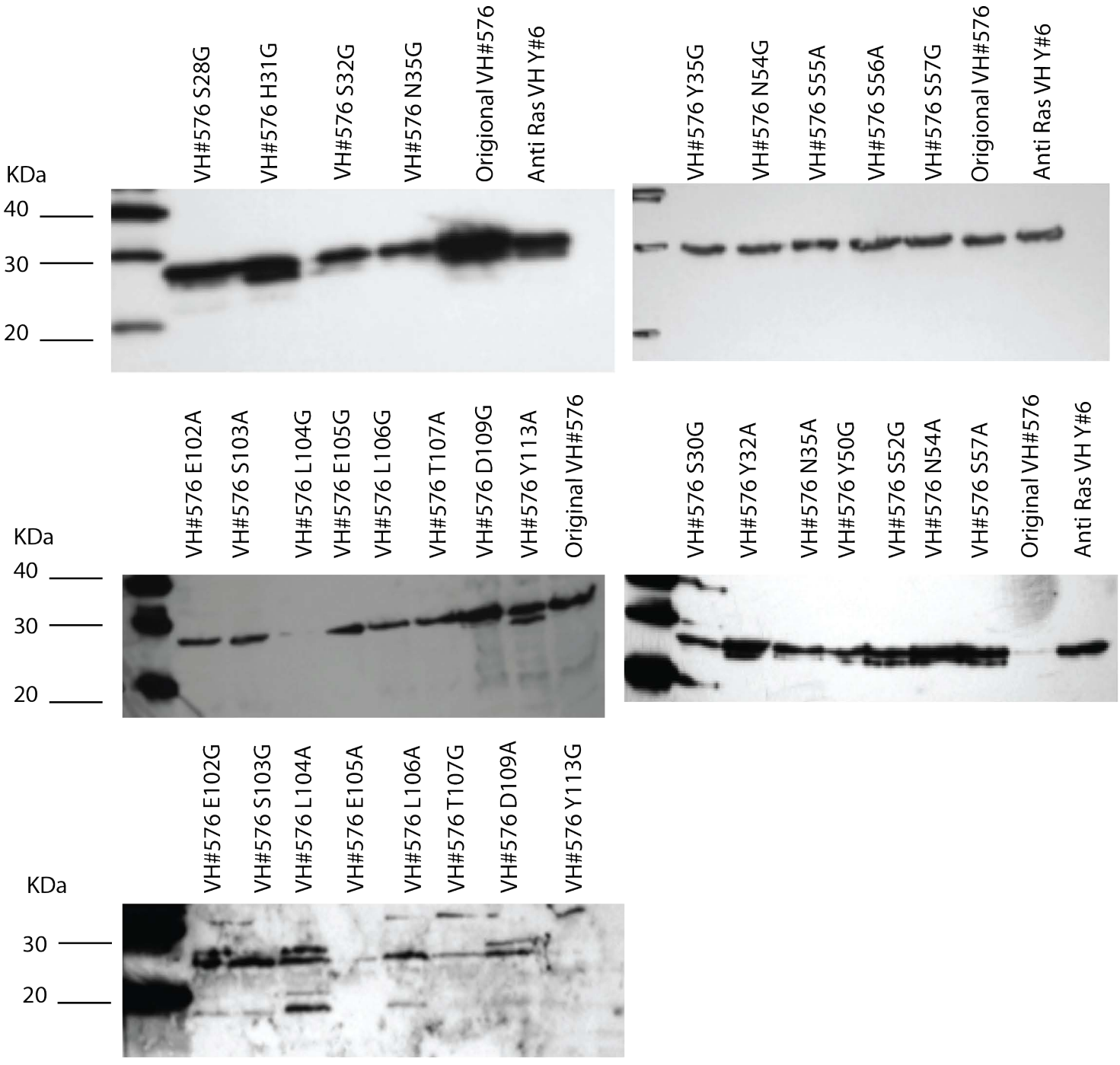
Footnote: The CDR residues are highlighted in yellow and the key CDR amino acids determined by mutagenesis (see main text figure 2) are shown in red.

B. Amino Acid sequence of LMO2ΔN8 protein

```
10         20         30         40         50
SLDPSEEPVDEVLQIPPSLLTCGGCQQNIGDRYFLKAIDQYWHEDCLSC
 60         70         80         90        100
DLCGCRLGEVGRRLYYKLGRKLCRRDYLRLFGQDGLCASCDKRIRAYEM
 110        120        130        140        150
TMRVKDKVYHLECFKCAACQKHFCVGDRYLLINSDIVCEQDIYEWTKIN
158
GMI
```

Footnote: The LMO2ΔN8 lacks the first eight amino acids of the mature protein (MSSAIERK).
The zinc binding residues are highlighted in yellow (LIM1 domain) or grey (LIM2 domain).
The anti-parallel beta sheets of LMO2 (when in complex with LDB1-LID) are highlighted in green.

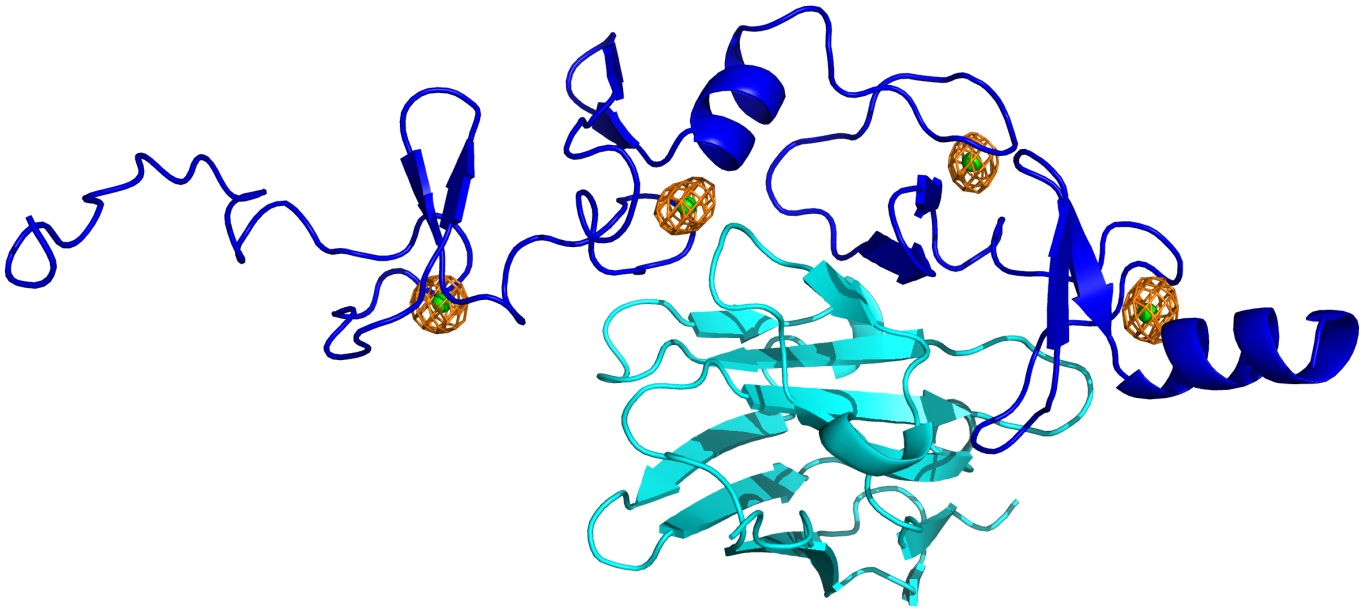
Supplementary Figure S3



Western blot analysis of mutated versions of VH#576

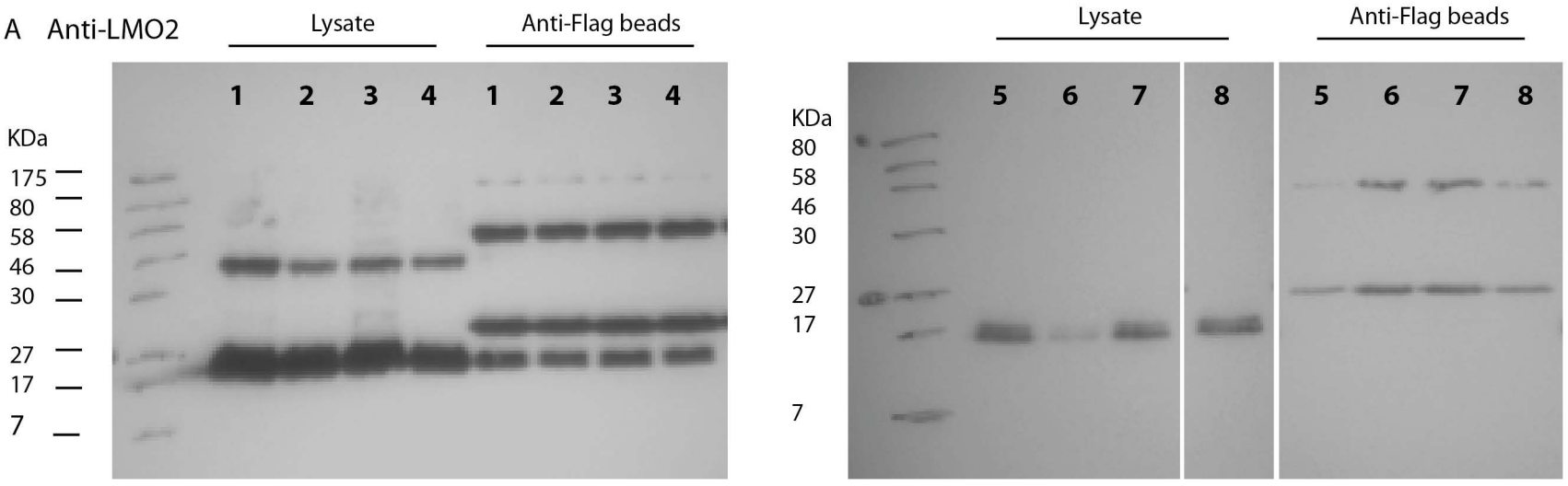
Anti-VP16 Western blot analysis was employed to analyse the prey (VH#576-VP16) expression levels. All mutated versions of VH#576 were expressed, except for the L104G, E105A, Y113G mutated forms. All blots, except the final panel, show a positive control of the original VH#576 and/or VHY#6.

Supplementary Figure 4

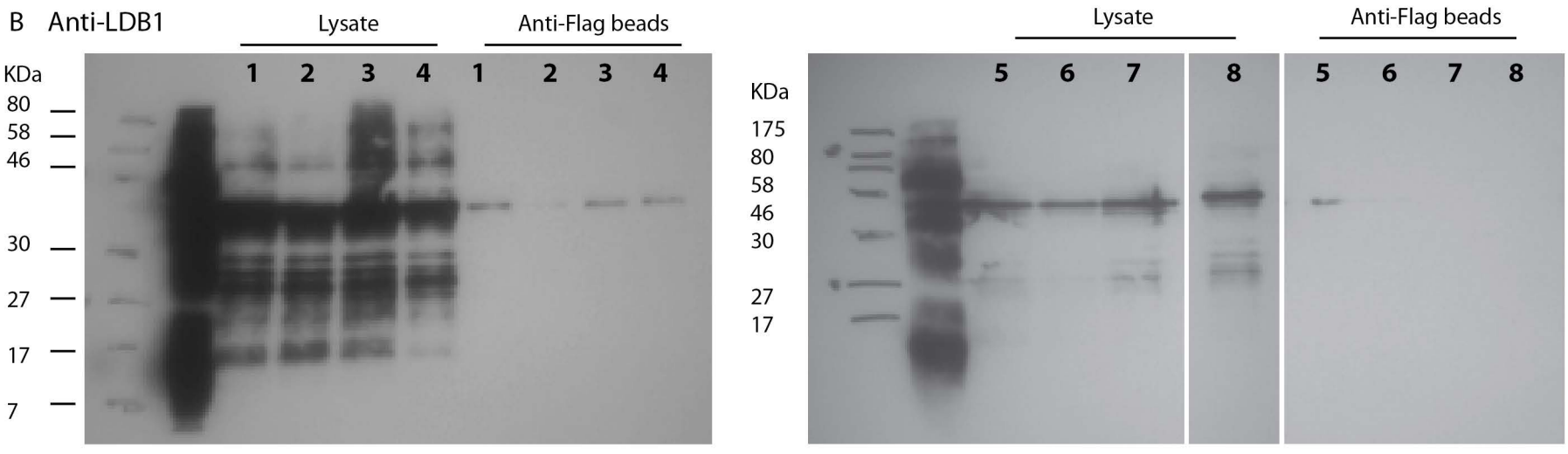


An anomalous difference map positioning zinc atoms in the LMO2-VH complex
An anomalous difference Fourier map contoured at 4.0σ (orange mesh) is shown to highlight the positions of the zinc ions (green spheres). The map was calculated using the peak wavelength anomalous differences and phases after refinement with all zinc ions omitted. LMO2 and VH#576 are coloured in blue and cyan respectively.

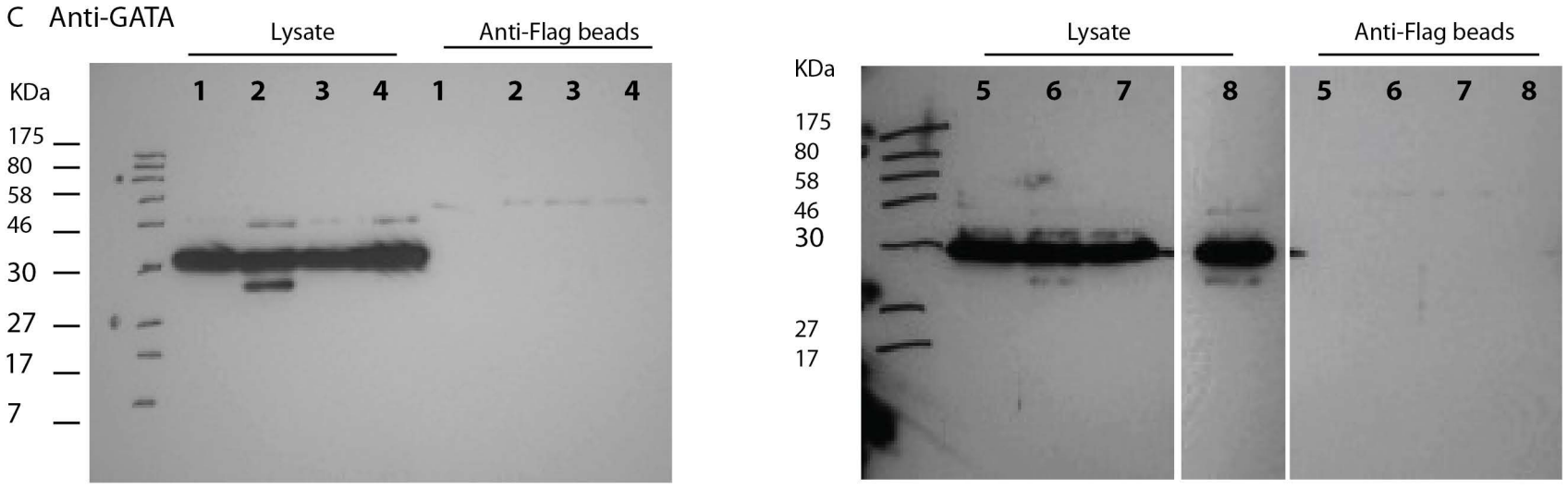
A Anti-LMO2



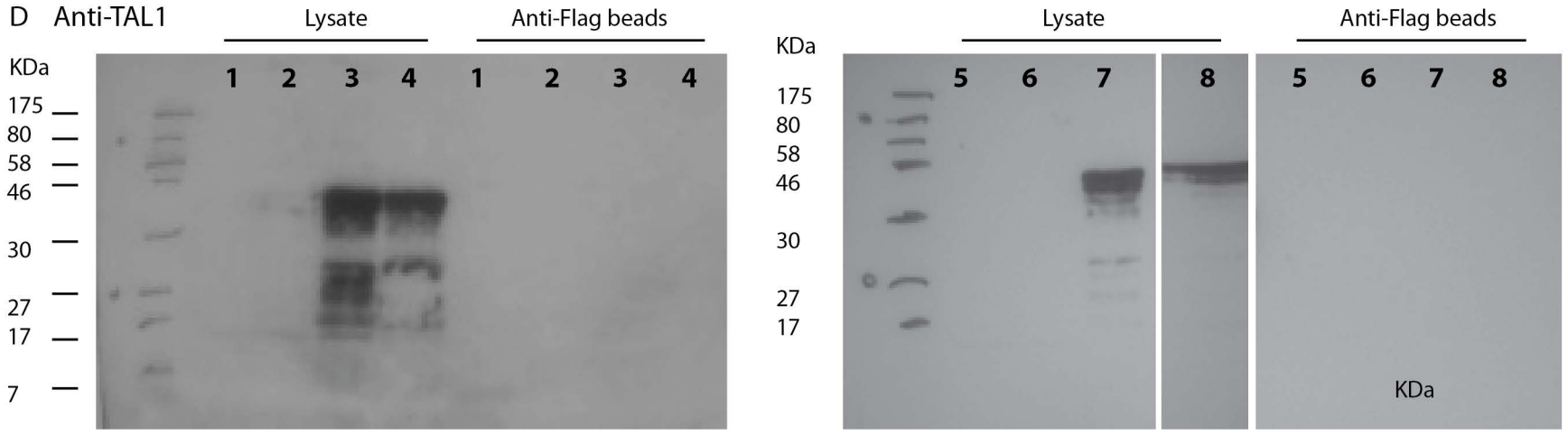
B Anti-LDB1



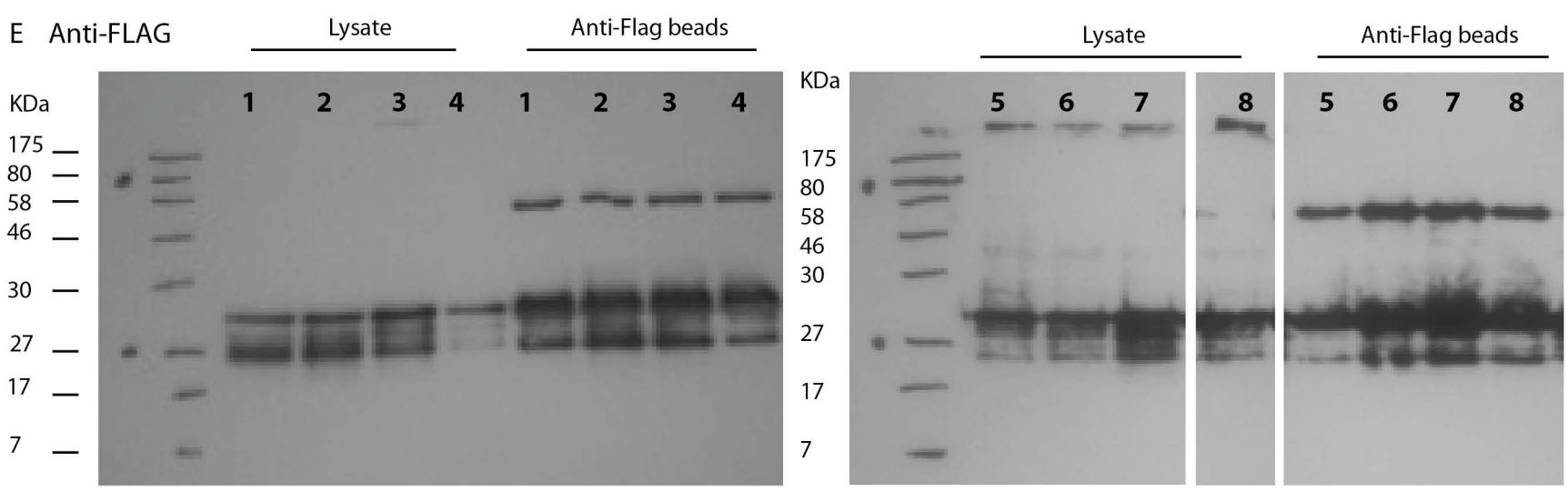
C Anti-GATA



D Anti-TAL1



E Anti-FLAG



Lane key:

Transfection	1	2	3	4	5	6	7	8
pGL3-ElbLUC-(Ebox-GATA)$_2$	+	+	+	+	+	+	+	+
Ldb1/Lmo2	+	+	+	+	+	+	+	+
GATA-1	-	+	-	+	-	+	-	+
TAL1/E2A	-	-	+	+	-	-	+	+
3XFlag-VH576-NLS	+	+	+	+	-	-	-	-
3XFlag-VHY6-NLS	-	-	-	-	+	+	+	+

Figure 5: Expanded data for figure 4C, Western blotting of pull-down proteins. COS-7 cells were co-transfected various combinations of plasmids coding for LMO2, LDB1, TAL1, E47 and GATA-1 and either Flag-tagged VH#576 or Flag-tagged VH#6 (a non-relevant VH) as shown in the inset table. Protein complexes were isolated with anti-Flag antibody beads and purified proteins separated by SDS-PAGE. The presence of LMO2 (A), LDB1 (B), GATA-1 (C), TAL1 (D) Flag tagged VH (E) was detected in both the lysate and pull down material by Western blotting. Bands from lanes 4 and 8 are shown in figure 4C.

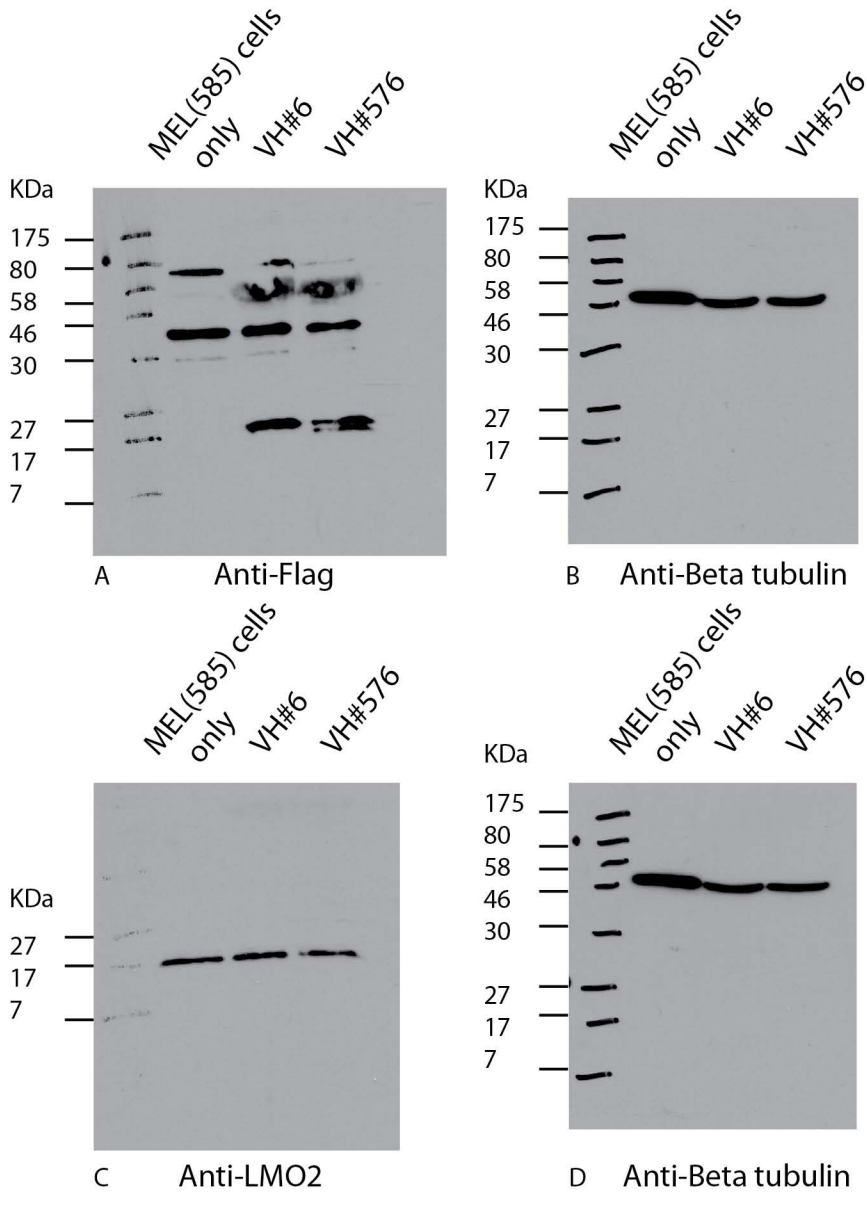

MEL(585) cells only
VH#6
VH#576
KDa
175
80
58
46
30
27
17
7
A
Anti-Flag
MEL(585) cells only
VH#6
VH#576
KDa
175
80
58
46
30
27
17
7
B
Anti-Beta tubulin
MEL(585) cells only
VH#6
VH#576
KDa
27
17
7
C
Anti-LMO2
MEL(585) cells only
VH#6
VH#576
KDa
175
80
58
46
30
27
17
7
D
Anti-Beta tubulin

Figure 6: Expanded data for figure 4D, the effect on protein stability of binding VH#576 to LMO2.

Extracts from MEL585 cells expressing VH#576 (anti-LMO2) or VH#6 (anti-RAS) or untransfected were separated by SDS-PAGE and transferred to membrane for Western analysis with anti-flag mouse monoclonal antibody (A) and anti-tubulin (as a protein loading control) (B); or anti-LMO2 monoclonal antibody (AbD Serotec) (C) and anti-tubulin (D).