

## **Supplementary methods**

### **Molecular Dynamics**

Molecular dynamics (MD) simulations were performed using the GROMACS version 4.0.5 with OPLS force field (50). The heterodimer model was placed in a cubic box with the box-edges at least 10 nm apart from the protein surface. The system was solvated with Simple Point Charge (SPC216) water molecules and appropriate number of counterions were added in the box to neutralize the system. In order to remove the possible clashes between atoms, the energy minimization was set to run for 5000 steps or until convergence to machine precision. After energy minimization, simulations were performed for 100 ps at constant temperature and pressure with periodic boundary conditions, particle-mesh Ewald summation, and a 2-fs time step to heat and equilibrate the system. Then the system was subjected to production MD simulations for 5 ns. Structures were saved every 10 ps for analysis. The output files from the GROMACS 4.0.5 was analysed using XMGRACE software (51).

The overall stability of the dimer was measured by estimating the root mean square deviation (RMSD) of the molecule, its radius of gyration (RGYR) and solvent accessible surface area (ASA). We observed from our MD analysis that the system is stable after 1ns. This confirms that the heterodimer is stable.

We analysed the hydrogen bonds and hydrophobic interactions at the interface in each simulated structure. Interaction between hydrophobic side chains are identified using a distance cutoff of 5 Angstrom between apolar groups in the apolar side chains (52). The hydrogen bonds formed between subunits are identified using HBOND program which is a part of JOY suite (51). The interactions that exist in at least 50% of the simulated structures were considered as dynamically stable and used for the interpretation of stability.

### **Co-expression and purification of Zfp206SCAN-Zfp110SCAN heterodimer**

Co-expressed proteins were produced as follows. A DNA fragment of Zfp206 SCAN (amino acids 36-128) was amplified from the full-length Zfp206 cDNA (IMAGE: 30006755) by PCR using the primers 5'-CACTCAGCATATGAGGCCTAGGCCTGAGGTGGCC-3' which includes a *Nde*I

restriction enzyme site and 5'- GCGAAGCTCGAGCTATCAGTGGTGAT GGTGATGATGCATGTGGCTGATGTCTCTGGG-3' which includes a *XhoI* restriction enzyme site and 6×His tag. DNA fragment of Zfp110 SCAN (amino acids 154-247) was amplified from the full-length Zfp110 cDNA (IMAGE: 3500984) using the primers 5'- CACTCAGGGATCCGCGTTTACTGACACTGAAGCT-3' which includes a *BamHI* restriction site and 5'- AAGTATGCGGCCGCCTATCATTTTTTCG AACTGCGGGTGGCTCCAAGCGCTGGCAAGCGAAGCATCGTCCTT-3' which includes a *NotI* restriction site and strep tag. The PCR product of 206SCAN was cloned into pCOLADuet1 plasmid (53) at *NdeI/XhoI* restriction sites and the PCR product of 110SCAN was cloned into pCOLADuet1 plasmid at *BamHI/NotI* restriction sites. The DNA sequence of Zfp206SCAN-Zfp110SCAN in the produced vector pCOLA-Zfp206SCAN-Zfp110SCAN was confirmed by sequencing.

The pCOLA-Zfp206SCAN-Zfp110SCAN expression plasmid was transformed into *E. coli* BL21 (DE3) cells (Invitrogen). 200 ml overnight culture of the cells transformed with pCOLA-Zfp206SCAN-Zfp110SCAN expression plasmid was used to inoculate 6 L Terrific Broth (TB) medium with 50 µg/ml kanamycin. The cells were then grown at 37 °C until OD<sub>600</sub> reached 0.8. To induce protein expression, isopropyl β-D-1-thiogalactopyranoside (IPTG) was added into the culture with a final concentration of 0.5 mM. The cells were further grown overnight at 20 °C. The harvested cells were lysed in buffer containing 50 mM Tris-Cl, pH 8.0, 150 mM NaCl, 30 mM imidazole, 10 mM 2-mercaptoethanol. The initial purification was conducted by using affinity technique with Ni-NTA<sup>+</sup> (Invitrogen). Subsequent purification of Zfp206SCAN-Zfp110SCAN protein was conducted by strep-tactin (Qiagen). The purified Zfp206SCAN-Zfp110SCAN protein were pooled and stored in appropriate buffer for further experiments.

### **Circular dichroism (CD) spectroscopy**

The CD spectrums of the Zfp206-Zfp110 heterodimer and other mutants (100 µM) in 50 mM Hepes, pH 7.3, 150 mM sodium chloride buffer was collected at 25° C in the range of 190–250 nm using a Chirascan spectropolarimeter (Applied Photophysics, UK) in continuous scanning mode. The acquisition parameters for the CD spectrum were 20 nm/min with a 4 s response and a 2-nm bandwidth. For the consistency of the experiment, the protein concentration was also determined at the end of the run.

## Supplementary Table 1

Primers for 20 SCAN domains (attB sites (underline) and a tobacco etch virus (TEV) protease recognition and cleavage site (**bold**))

Gene name	IMAGE ID	Forward primer	Reverse primer
ZSCAN4	8069244	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTCGAAGTCTCAAGAATGGTGG-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
Zfp110	3500884	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTCGAAGTCTCAAGAATGGTGG-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
Zfp167	4006804	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTCGAAGTCTCAAGAATGGTGG-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
ZNF75	40034784	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTAGAGAAATCTGGTCTGAG-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
MZF1	4301723	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTATGGGACCCAGGCCCTGAA-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
ZNF24	4072598	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTCAGAGATTTCGACAGGGA-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
H213	9387888	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTCCGAACTGCGCCAGCGG-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
ZNF165	4837021	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTGAACTCCTTAAGCAGGAG-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
ZNF174	3461658	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTGAACTCCTGAGCTGAG-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
Zfp192	4006900	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTGAAAGTAACCTCTTGGG-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
ZNF193	4132636	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTATATCCATCGGCAAGGAA-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
ZNF197	4828392	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTGCTCTCTGTTGGGAGCC-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
ZNF232	4635999	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTAAGATCTCCGCCAACCC-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
ZNF390	9052071	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTGAGCCGCGCCAGAGG-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
ZNF394	4641268	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTCGAAGTCTGACTGAC-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
ZNF435	3809511	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTGCACTCAGAGGAA-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
ZNF452	6499884	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTGAGGCACTCTGCTGCGC-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
Zfp445	8068992	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTCAACCGCCGCGCCAGG-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
Zfp449	30346713	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTGAAAGTTCGCTCAGG-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
Zfp496	3051320	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTGAGCTCCAGCCGCCAGTCC-3'	5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'

## Supplementary Table 2

Primers for deletion mutations of Zfp206SCAN-Zfp110SCAN

Zfp206SCAN-ZNF174SCAN	primers for Zfp206SCAN 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCAGGCCTAGGCCGTGAGGTGGCC-3' 5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3' linker 5'-CCCAGAGACATCAGCCACATGGGTGGTCCGGTAAAACCTGCCAGATCCTGAG-3' 5'-CTCAGGATCGGGCAGTGTTTACCAGAACCCATGTGGCTGATGTCTCTGGG-3'
	primers for ZNF174SCAN 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCAAAAACCTGCCAGATCCTGAG-3'
Zfp206SCAN-ZNF174SCAN(H1)-Zfp110SCAN (H2-H5)	primers for Zfp206SCAN (as shown above) reverse primer for ZNF174SCAN (H1) connecting Zfp206SCAN 5'-TAACAAAAGCGTCTGAAAGCTCTGGCGGAGAGCTCAGGATCTGGACCGGAACCCCATGTGGCTGATGTCTCTCTG primers for Zfp110SCAN (H2-H5) connecting ZNF174SCAN (H1) 5'-AGCTTCAGACGCTTTTGTATGAAGAGTCAAGTCTGAGTGGTCCAG-3' 5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
Zfp206SCAN-ΔH1Zfp110SCAN	primers for Zfp206SCAN (as shown above) reverse primer for ΔH1Zfp110SCAN connecting Zfp206SCAN 5'-CTGGGACCAGCTGACTTTCACCGGAACCCATGTGGCTGATGTCTCTGGG-3' primers for ΔH1Zfp110SCAN connecting Zfp206SCAN 5'-CCCAGAGACATCAGCCACATGGGTGGTCCGGTGAAGATCAGTGGTCCAG-3' 5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
Zfp206SCAN-Zfp110SCAN (H1)-ZNF174SCAN (H2-H5)	primers for Zfp206SCAN (as shown above) reverse primer for Zfp110SCAN (H1) connecting Zfp206SCAN 5'-ATACTGGAATGTCGGAATCTGGCGAGAAGCTTCAAGTCAAGTCAAGCAGCCGGAACCCCATGTGCTGATG primers for ZNF174SCAN (H2-H5) connecting Zfp110SCAN (H1) 5'-AAGTTCAGACATTTCCAGTATTTTGTATCAAGAGGTGCTCT-3' 5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3'
ΔH1Zfp206SCAN-Zfp110SCAN	primers for ΔH1Zfp206SCAN 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAACCTGTATTTTCAGGGCTATCAGGAAGATATGGGGCCA-3' 5'-GGGGACCACCTTTGTACAAAAGAGCTGGGTTTAAAGTGGATTTATGCTGTCTATC-3' linker and primers for Zfp110SCAN are the same as those of Zfp206SCAN-Zfp110SCAN

## Supplementary Table 3

Primers for site mutagenesis in Zfp206SCAN-Zfp110SCAN

Zfp206SCAN48AL49A-Zfp110SCAN	I48AL49A	5'-CTGCACACCAAGAAGCAGGCCGCGGAGCTGCTGGTATGGA-3'
Zfp206SCANR13AR31A-Zfp110SCAN	I48AL49A_antisense	5'-TCCAGTACCAGCAGCTCAGCCGCTGCTCTTGGTGTGAGC-3'
	R13A	5'-TGCCCCACACAGCTTTTCGCATGCTTCCAGTATCAGG-3'
	R13A_antisense	5'-CCTGATACTGGAAGCATGCGAAAAGCTGGTGGGCCA-3'
	R30A	5'-CCTGGGCCGGCTCAGGGAACCTGCAAC-3'
	R30A_antisense	5'-GTTGCGAGTTCGCGAGCCGGCCAGG-3'
Zfp206SCAN-Zfp110SCANC36AL40AL51A	C36AL40A	5'-CAGCTTCGTAAGCTGGCTCACCAGTGGGCGCAGCCCAATACAG-3'
	C36AL40A_antisense	5'-CGTGTATTGGGCTGCGCCCACTGGTGAAGCAGCTTACGAAGCTG-3'
	L50A	5'-CGTTCAAAGAAGCAGATGCGAGAGTTCGCTGGTCTCGA-3'
	L50A_antisense	5'-TCGAGCACCAAGCACTTGCATCTGCTTCTTTGAACG-3'
Zfp206SCAN-Zfp110SCANE52AQ58A	E52A	5'-AAGAAGCAGATCCTAGCGTTCGCTGGTGTCTGAG-3'
	E52A_antisense	5'-CTCAGCACCAGCAACGCTAGGATCTGCTCTT-3'
	Q58A	5'-GTTGCTGGTGTCTGAGCGTTCCTGAATGCATG-3'
	Q58A_antisense	5'-CAGTGCATTGAGAACGCTCGAGCACCAGCAAC-3'

#### Supplementary Table 4

Functions of zinc finger transcription factors of SCAN family. ZSCAN4, Zfp110, Zfp167, ZNF24, ZNF75, Zfp213 and MZF1 are partners of Zfp206.

<b>SCAN family member</b>	<b>Function</b>
Zfp206	pluripotency maintenance in embryonic stem cells(54,55)
ZSCAN4	elongation of telomere in ES cells (56)
Zfp110	programmed cell death (interact with p75 <sup>NTR</sup> ) in mouse embryonic neural retina (57)
Zfp167	gene expression repression (58)
ZNF24	early embryonic development and cell proliferation (59)
ZNF75	not clear
Zfp213	not clear
MZF1	delay apoptosis and differentiation in leukemia cells (60)
ZNF165	a novel cancer-testis antigen (61)
ZNF174	repression on DNA binding (62)
Zfp192	predominant isoenzymes, tumor related (63)
ZNF193	interact with the hITF protein, which may contribute to the mucosal repair (64)
ZNF197	overexpression in thyroid carcinomas (65)
ZNF232	not clear
ZNF390	not clear
ZNF394	inhibition transcription activity of AP-1 and c-jun (66)
ZNF435	inhibition transcription activity of AP-1 (67)
Zfp445	activate transcription activity of AP-1 and SRE (68)

Zfp449

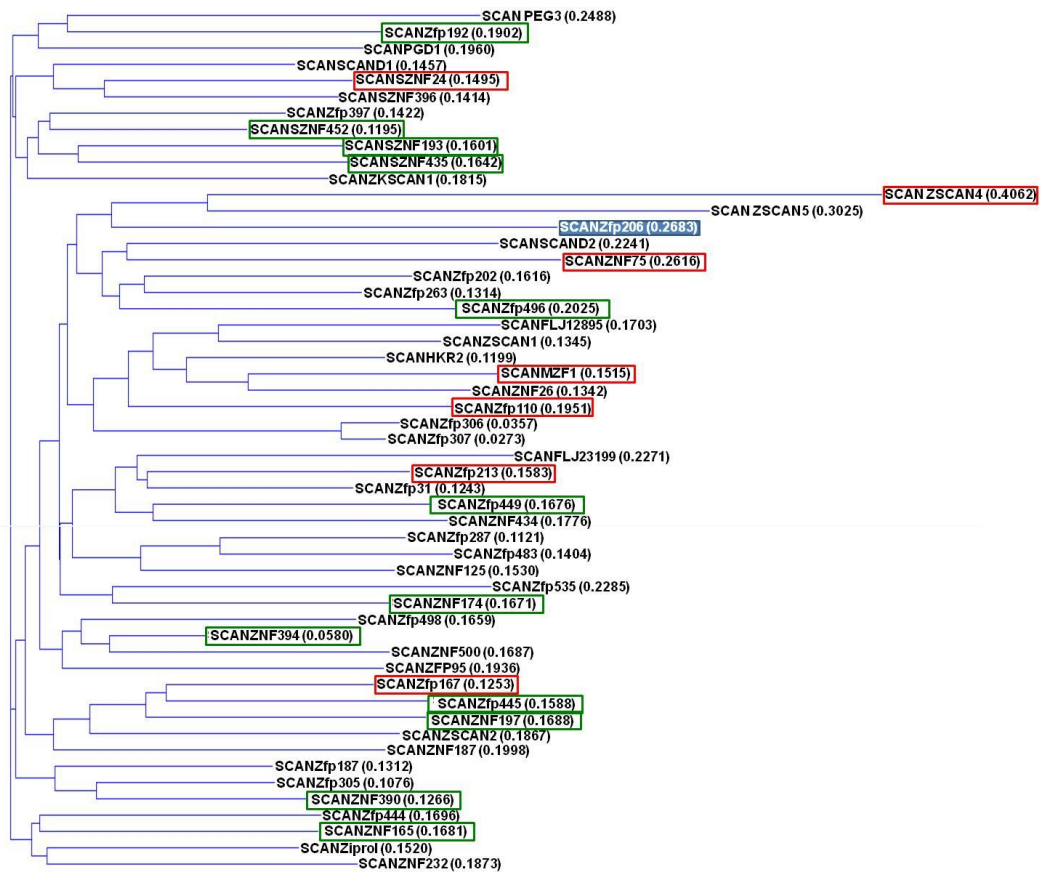
not clear

Zfp452

a moderate transcriptional factor for Rpe65 gene upregulation (69)

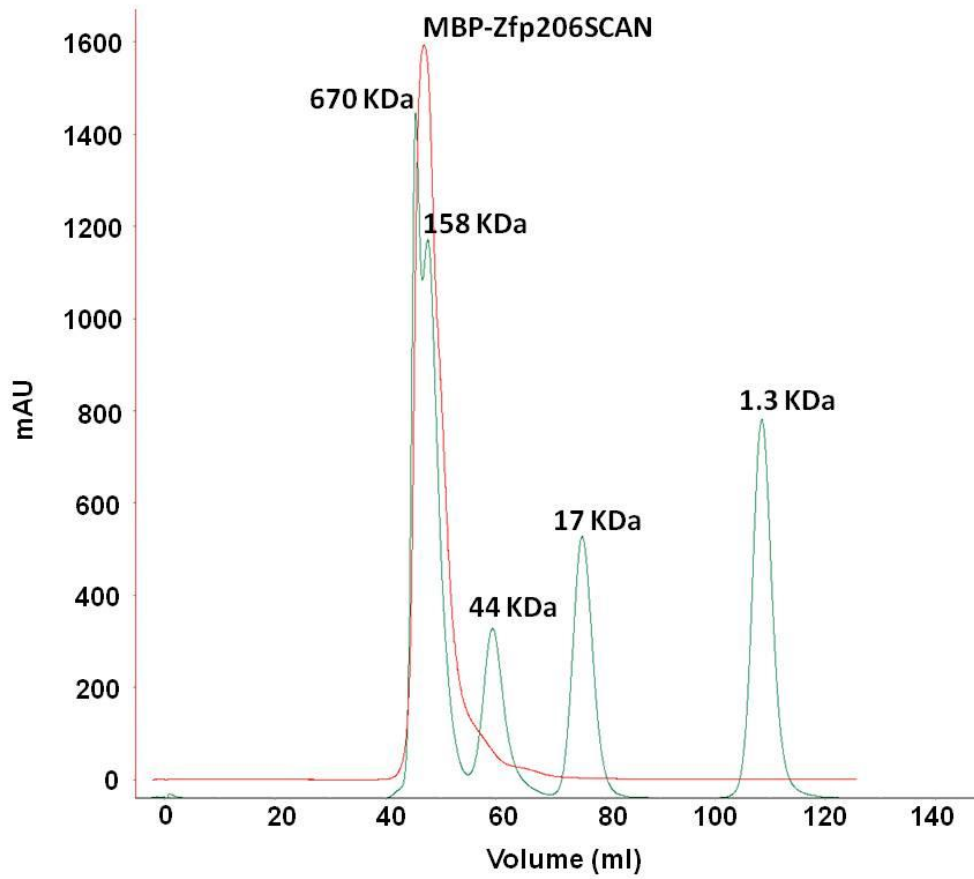
### Supplementary Figure 1

Phylogenetic tree relating 52 SCAN domain sequences. Zfp206SCAN is shown in blue box. The SCAN domains in red box are those interacting with Zfp206SCAN shown in MBP pull down assay. The SCAN domains in green box are those found to not interact with Zfp206SCAN in the MBP pull down assay.



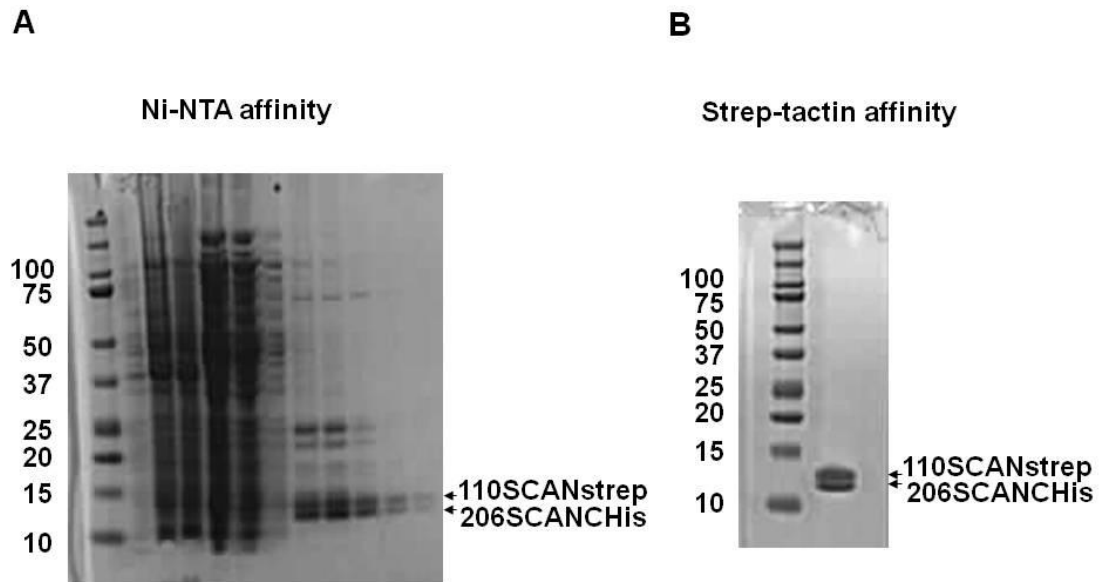
### Supplementary Figure 2

Analysis of MBP fused Zfp206SCAN by Size-exclusion chromatography. MBP-Zfp206SCAN elutes at the volume corresponding to the theoretical molecular weight of a dimeric fusion protein 104 KDa.



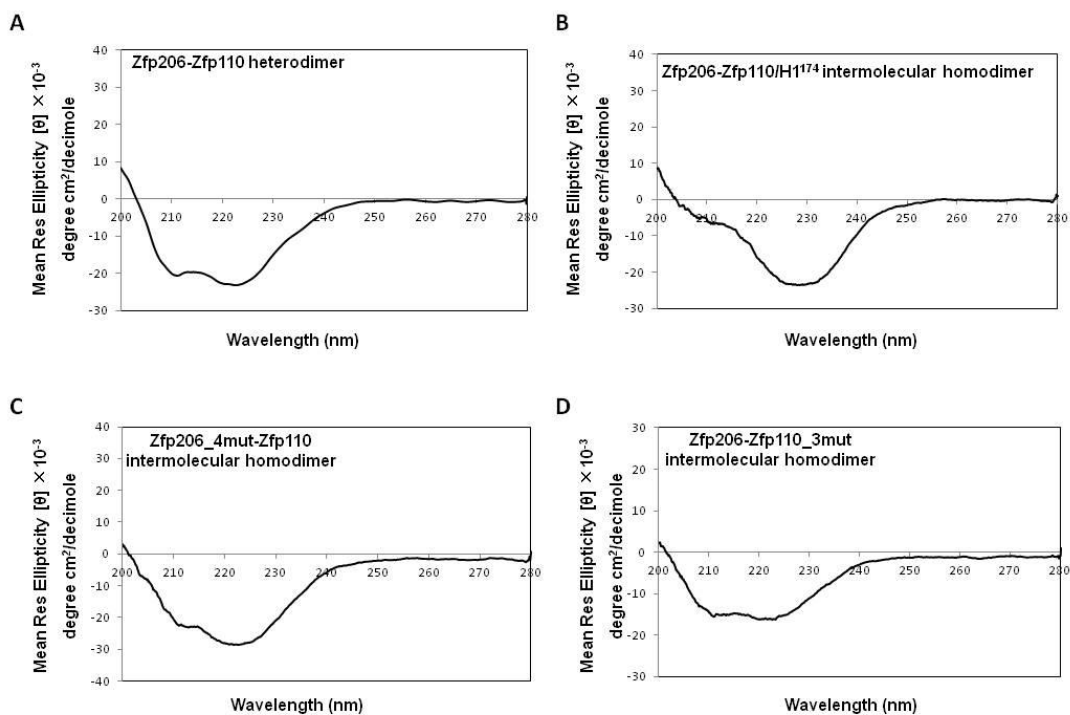
### Supplementary Figure 3

Co-expression and purification of Zfp206SCAN-Zfp110SCAN heterodimer. (A) Zfp206SCAN-Zfp110SCAN heterodimer was first purified by Ni-NTA affinity column. (B) Zfp206SCAN-Zfp110SCAN heterodimer was then purified by streptactin beads.



### Supplementary Figure 4

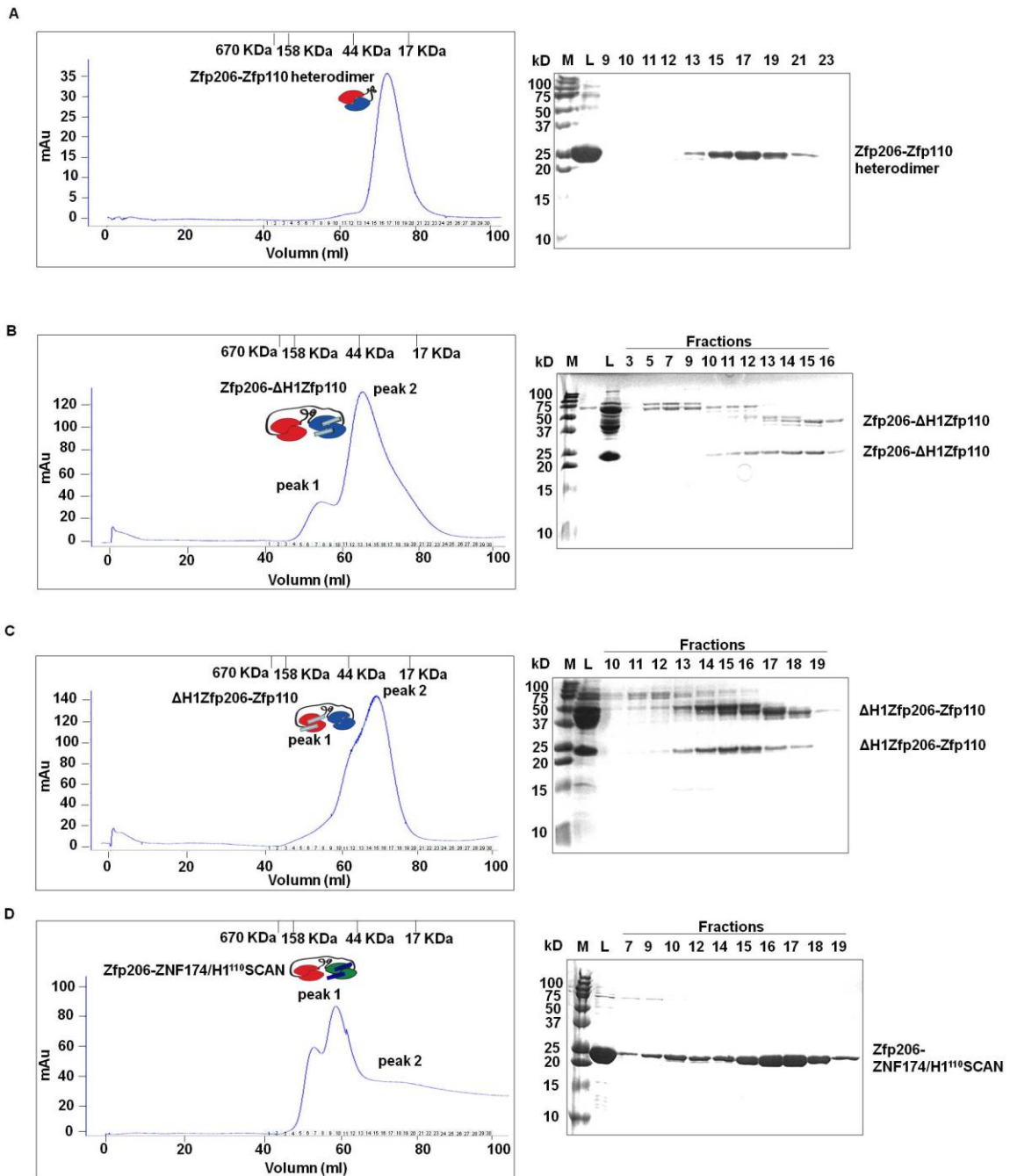
Secondary structure character of Zfp206-Zfp110 heterodimer and other mutants analyzed by CD spectrometry. (A) Zfp106-Zfp110, (B) the Zfp206-/Zfp110\_H1<sup>174</sup>, (B) Zfp206-4mut-Zfp110 intermolecular homodimer (Zfp206SCANI48AL49AR13AR31A-Zfp110SCAN), (F) Zfp206-Zfp110-3mut intermolecular homodimer (Zfp206SCAN-Zfp110SCANC36AL40AL51A).





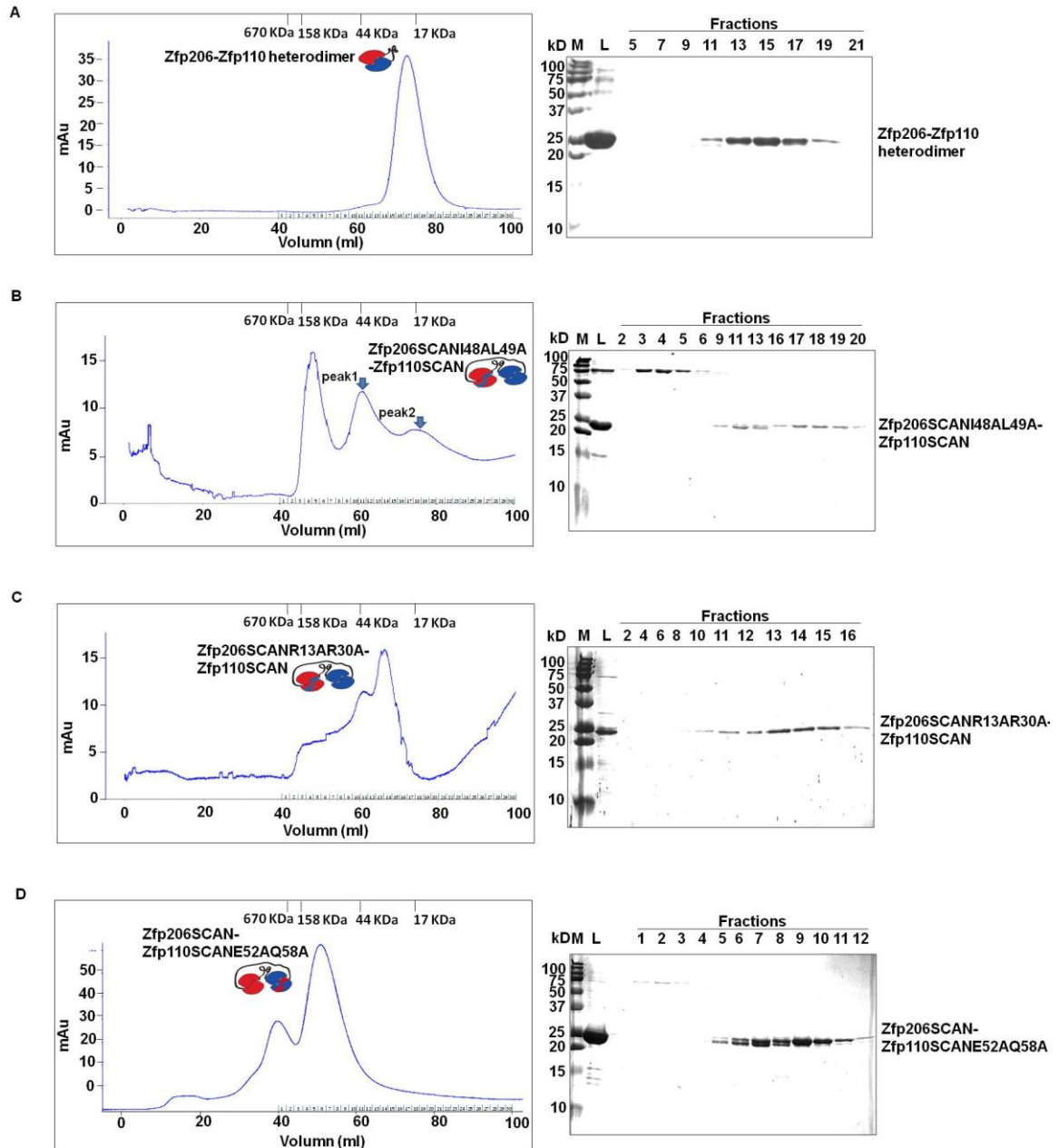
### Supplementary Figure 5

Role of the amino-terminal helix 1 for Zfp206SCAN and Zfp110SCAN heterodimerization. (A-D) Left, elution profiles of size-exclusion chromatography. Right, 15% SDS-PAGE gel analysis of the elutions. (A) Zfp206-Zfp110 heterodimer, (B) Zfp206- $\Delta$ H1Zfp110, (C)  $\Delta$ H1Zfp206-Zfp110, (D) Zfp206-H1Zfp110-ZNF174(H2-H5).



### Supplementary Figure 6

Analysis of potential Zfp206SCAN-Zfp110SCAN interface residues by size-exclusion chromatography. (A-D) Left, elution profiles of size-exclusion chromatography. Right, 15% SDS-PAGE gel analysis of the elutions. (A) Zfp206-Zfp110 heterodimer, (B) Zfp206SCANI48AL49A-Zfp110SCAN, arrows indicate the elution peaks, (C) Zfp206SCANR13AR31A-Zfp110SCAN, (D) Zfp206SCAN-Zfp110SCANE52AQ58A, arrows indicate the elution peaks.



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