Supplemental Information

Materials in this Supplemental Information:

Figure S1. Four modes of interaction between dimers of IF proteins (Related to Figure 4).

Figure S2. Crystal contacts of K5/K14 wildtype 2B complex and of K5/K14-C367A mutant 2B complex (Related to Figures 1 and 2).

Figure S3. Sequence alignment of type I and type II keratins in the 2B region of the rod domain (Related to Figures 1 and 2).

Figure S4. Transmission electron microscopy images of the negative-stained filaments of K5/K14 wildtype, ID1_AAA mutant or ID7_AAA mutant (Related to Figure 3).

Table S1. List of ID contacts found in K5/K14-C367A 2B complex (Related to Figures 1 and 2)

Table S2. Summary of the results from PISA and the mutated residues for interaction deficiencies (Related to Figures 1 and 2)

Table S3. Sequences of the oligonucleotide primers used for mutagenesis to produce ID-deficient mutants (Related to Key Resources Table of the STAR METHODS).

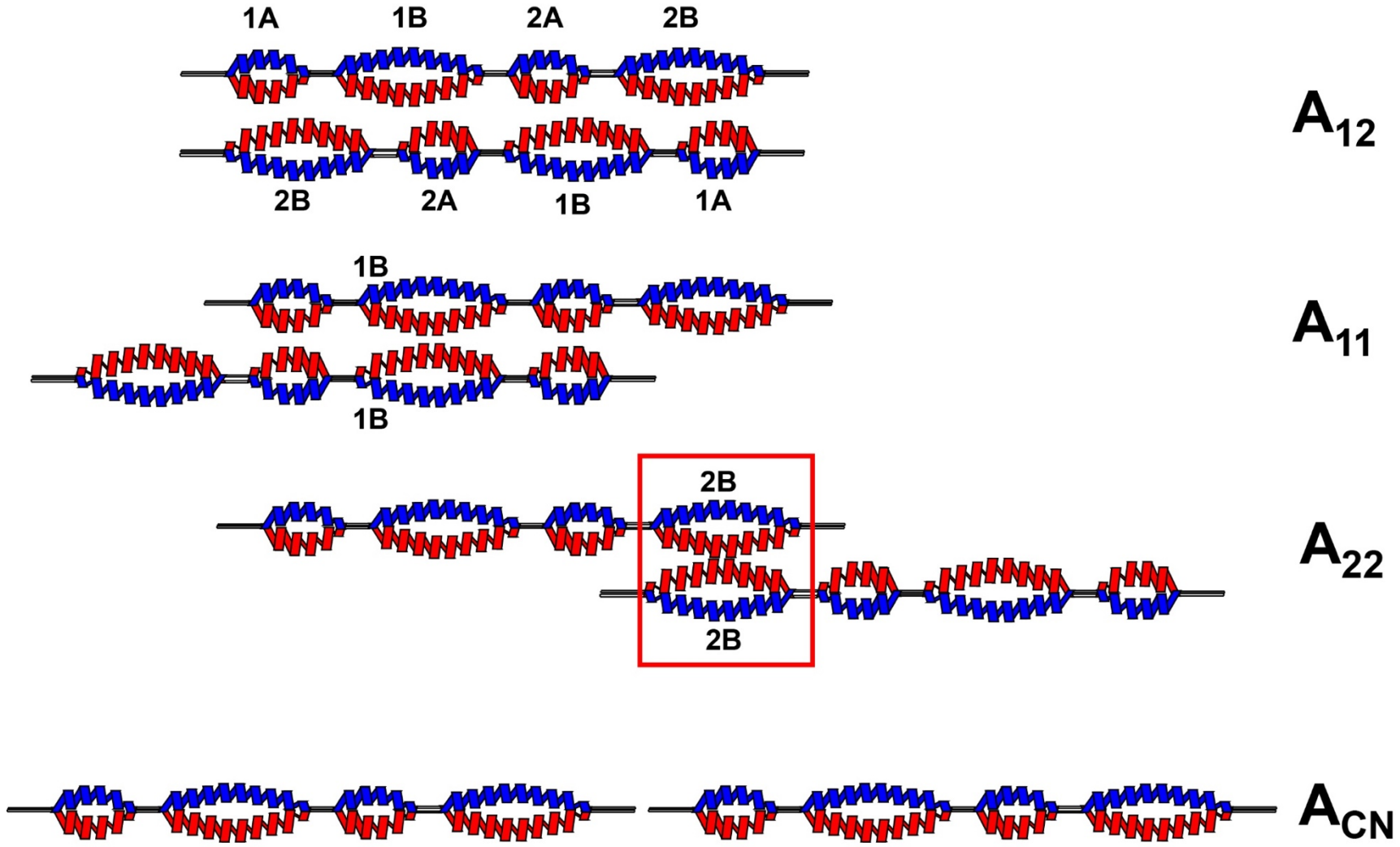
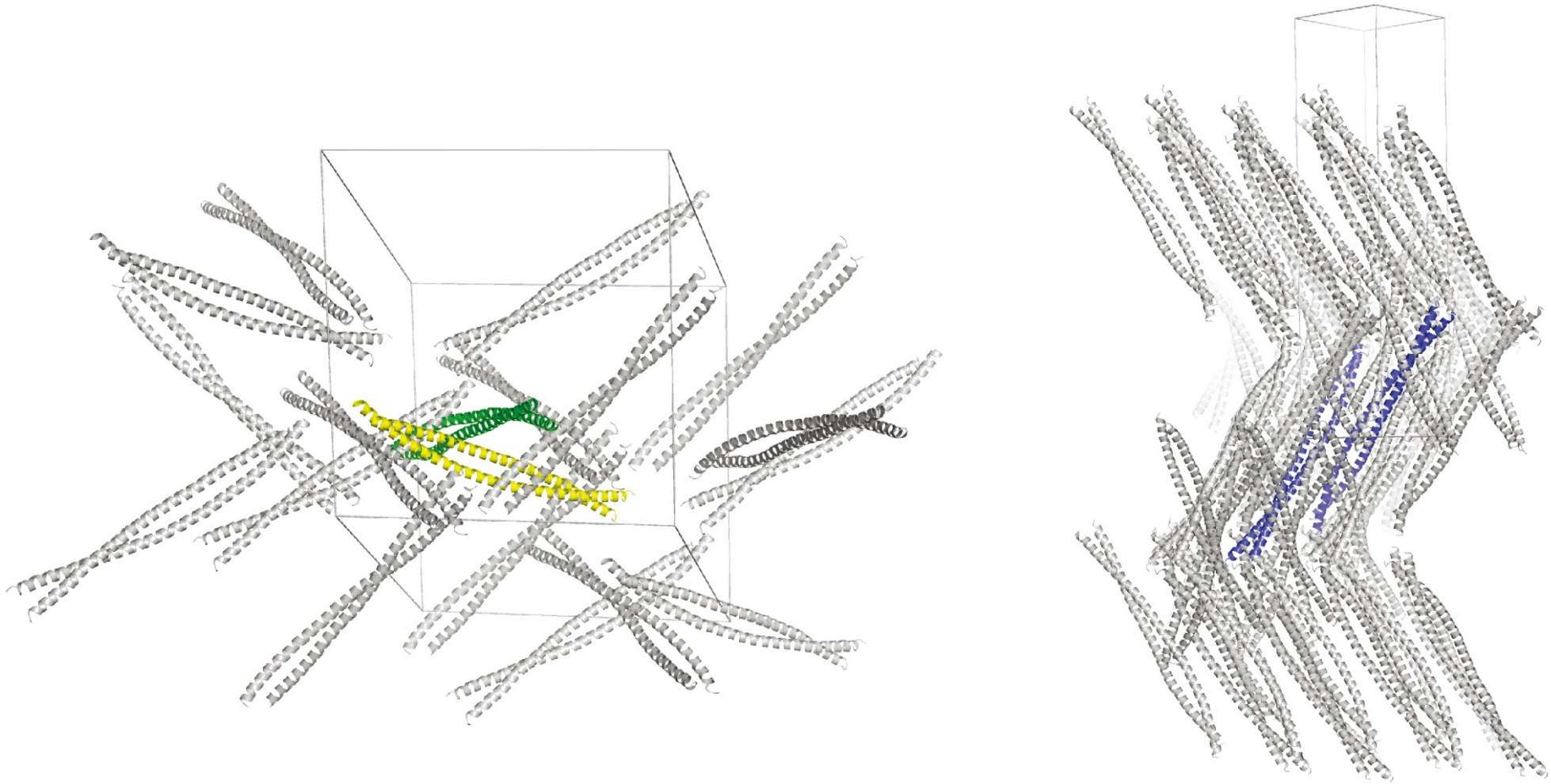
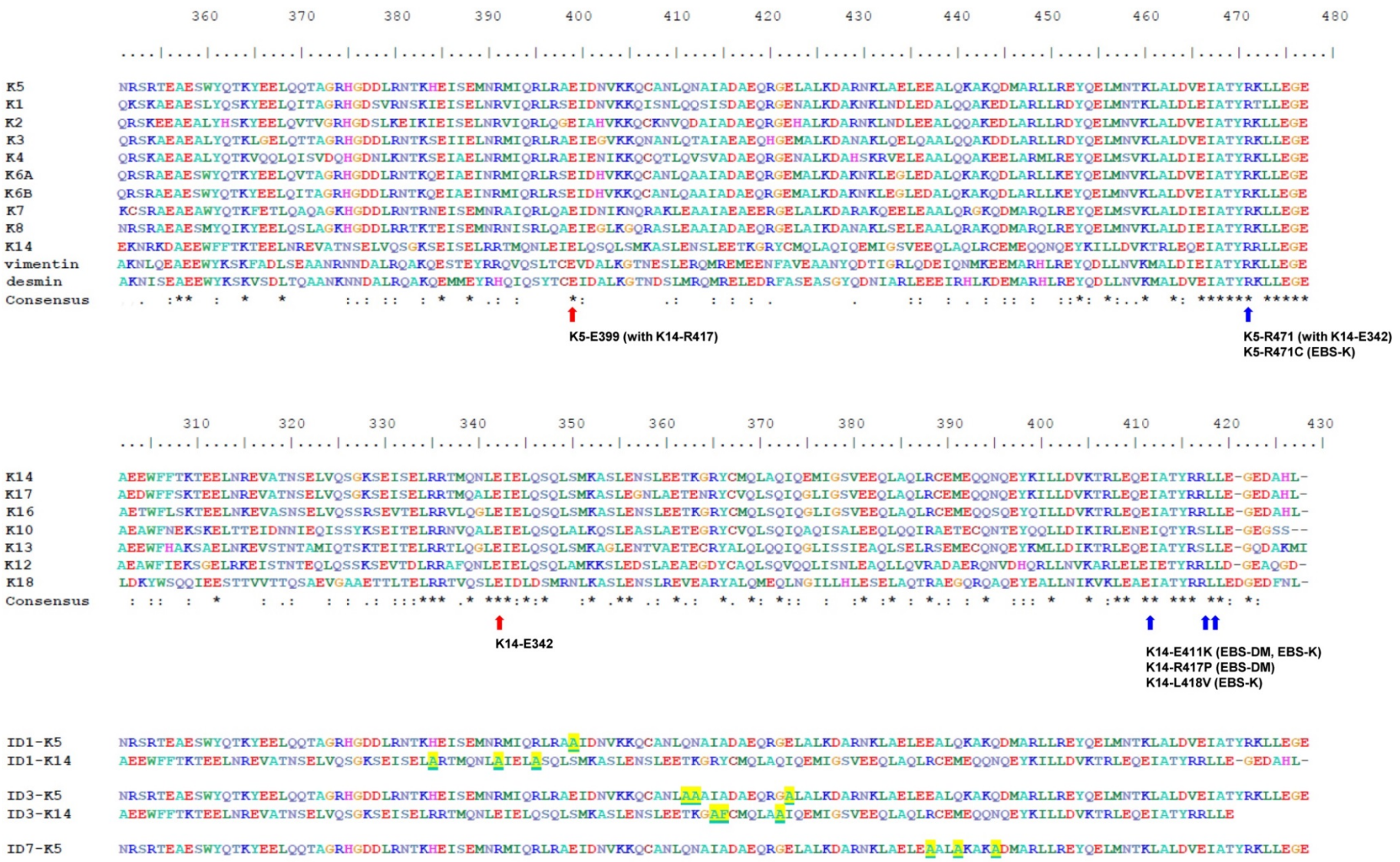


Figure S1. Four modes of interaction between dimers of IF proteins (Related to Figure 4). These alignments are named A_{11}, A_{12}, A_{22}, and A_{CN} reflecting the name of the interacting domains involved (Herrmann and Aebi, Curr Opin Struct Biol. 8:177-185. 1998). The interaction modes were suggested from nearest neighbor analysis using chemical cross-linking coupled to mass analysis, complemented by site-directed mutagenesis (Strelkov et al., BioEssays 25:243-251, 2003). The red chains and the blue chains represent type I keratins and type II keratins, respectively.



<u>Figure S2.</u> Crystal contacts of K5/K14 wildtype 2B complex and of K5/K14-C367A mutant 2B complex (Related to Figures 1 and 2). (Left) The crystal contact of wild-type K5/14 2B (PDB ID : 3TNU). the single K5/14 2B heterodimeric coiled-coil helix in asymmetric unit shown in green color and symmetry related molecule making disulfide bond shown in yellow color. (Right) The crystal contact of C367A mutant of K5/14 2B complex (PDB ID: 6JFV). Two heterodimeric molecules in asymmetric unit are shown in blue color. The unit cell is also shown in line.



<u>Figure S3</u>. Sequence alignment of type I and type II keratins in the 2B region of the rod domain (Related to Figures 1 and 2). Note that key amino acids found in both the ID1 and ID3 contacts (Fig. 1) are highly conserved, and some of them are mutated in individuals with EBS. Highlighted and underlined residues are amino acids that were mutated to disrupt dimer interfaces. Red arrows identify key residues not implicated in EBS. Blue arrows identify residues mutated in EBS.

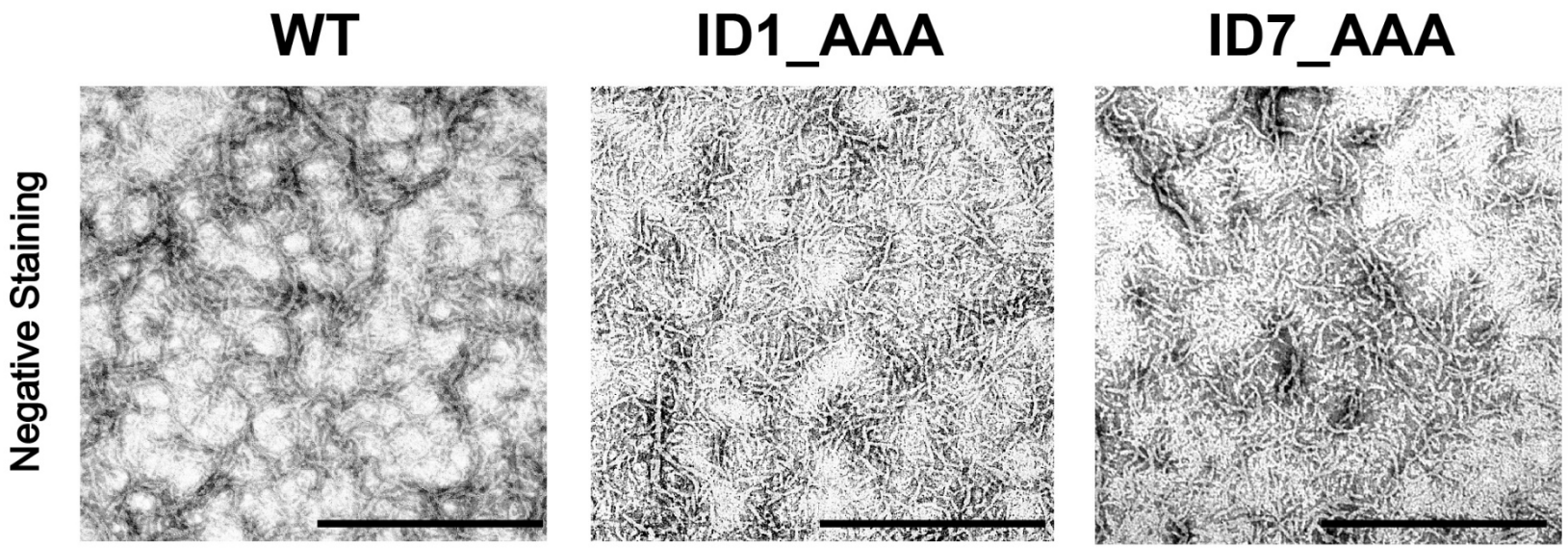


Figure S4. Transmission electron microscopy images of the negative-stained filaments of K5/K14 wildtype, ID1_AAA mutant or ID7_AAA mutant (Related to Figure 3). Bar, 200 nm.

Contact ID	Interface area (Å^2)	Coiled-coil Alignment	Symmetry Operation
ID1	805.7	Anti-parallel	x, y-1, z
ID3	760.9	parallel	-y+1, x, z+1/4
ID5	362.3	T-shape	-y+1, x, z+1/4
ID7	215.3	Anti-parallel	x, y, z
ID8	190.8	N/A	-x, -y+1, z-1/2
ID9	40.3	N/A	-y+1, x+2, z+1/4
ID10	16.3	N/A	-x, -y+2, z-1/2
ID11	6.5	N/A	-y+1, x+1, z+1/4

Table S1. List of ID contacts found in K5/K14-C367A 2B complex (Related to Figures 1 and 2)

	ID1			ID3			ID7		
average values	interface area (A^2)		Δ^iG (kcal/mol)	interface area (A^2)		Δ^iG (kcal/mol)	interface area (A^2)		Δ^iG (kcal/mol)
	805.7		-4.5	362.3		-2.1	215.3		1.7
	dimer 1	interacting residue (inter-dimer)	Comment	dimer 1	interacting residue (inter-dimer)	comment	dimer 1	interacting residue (inter-dimer)	comment
	K14-I331	-		K14-M351	-		K5-K426	-	
	K14-R335	K5-E475	mutated to Alanine for deficiency	K14-S354	K5-Q454		K5-N430	-	
	K14-M338	K5-L474		K14-L355	-		K5-A433	-	
	K14-Q339	K5-E475		K14-S358	K5-Q454		K5-E434	-	
	K14-E342	K5-R471 K14-Y415	mutated to Alanine for deficiency	K14-E361	K5-L461		K5-E437	K5-R451	mutated to Alanine for deficiency
	K14-I343	K5-R471		K14-T362	-		K5-Q440	K5-Q444	mutated to Alanine for deficiency
	K14-L345	K14-T414		K14-R365	K5-D464	mutated to Alanine for deficiency	K5-Q444	K5-Q440	mutated to Alanine for deficiency
	K14-Q346	K14-E411	mutated to Alanine for deficiency	K14-Y366	K5-K460	mutated to Phenylalanine for deficiency	K5-R448	-	
	K14-L349	K14-Q410 K14-E411		K14-Q369	-		K5-N458	-	
	K14-S350	-		K14-Q372	K5-R471	mutated to Alanine for deficiency	dimer 2	interaction	note
	K14-K352	K14-Q410		K5-K404	-	K404E (WC*)	K5-K426	-	
	K14-A353	K14-R407		K5-C407	-		K5-N430	-	
	K14-E356	K14-Q410		K5-A408	-		K5-A433	-	
	K14-N357	K14-D403, K14-K399		K5-Q411	K14-Q394 K5-L450	mutated to Alanine for deficiency	K5-E434	-	
	K14-E360	K14-K399		K5-N412	K14-E397	mutated to Alanine for deficiency	K5-E437	K5-R451	
	K14-E361	K14-K399		K5-I414	K5-Y453 K5-M457		K5-Q440	K5-Q444	mutated to Alanine for deficiency
	K5-E385	-		K5-A415	K14-E397		K5-K441	-	
	K5-E388	-		K5-D416	-		K5-Q444	K5-Q440	mutated to Alanine for deficiency
	K5-M389	K5-E475		K5-E418	K5-K460 K14-L401		K5-D445	-	
	K5-M392	K5-L474 K14-L419		K5-Q419	-		K5-R448	-	
	K5-R395	K14-E420		K5-E422	K14-R407	mutated to Alanine for deficiency	K5-R451	K5-E437	mutated to Alanine for deficiency
	K5-L396	-		K5-R429	-		K5-N458	-	
	K5-E399	K14-R417	mutated to Alanine for deficiency	dimer 2	interaction	note			
	K5-V403	-		K14-Q394	K5-Q411				
	dimer 2	interaction	note	K14-E397	K5-N412 K5-A415				
	K14-K399	K14-E360 K14-E361		K14-I400	-				
	K14-D403	K14-N357		K14-L401	K5-E418	L401P (WC*)			
	K14-T406	-		K14-V404	-				
	K14-R407	K14-A353		K14-R407	K5-E422				
	K14-Q410	K14-E356		K14-E411	-	E411K (DM*, K*)			
	K14-E411	K14-Q346 K14-L349	E411K (DM*, K*)	K5-M446	-				
	K14-T414	K14-L345		K5-A447	-				
	K14-Y415	K14-E342	Y415H (K*, DM*) Y145C (WC*)	K5-L450	K5-Q411				
	K14-R417	K5-E399	R417P (DM*)	K5-R451	-				
	K14-L418	-	L418V (K*)	K5-Y453	K5-I414				
	K14-L419	K5-M392	L419Q (DM*)	K5-Q454	K14-S354 K14-S358				
	K14-G421	-		K5-M457	K5-I414				
	K5-I467	-	I467L (WC*) I467M (K*) I467T (DM*)	K5-N458	-				
	K5-R471	K14-E342 K14-I343	R471C (K*)	K5-K460	K5-E418 K14-Y366				
	K5-L474	K14-M338		K5-L461	K14-E361				
	K5-E475	K14-R335 K14-Q339	E475K (DM*) E475G (DM*)	K5-D464	K14-R365				
	K5-G476	-	G476D (WC, EPPK*)	K5-V465	-				
				K5-I467	-	I467L (WC*) I467M (K*) I467T (DM*)			
				K5-A468	-				
				K5-R471	K14-Q372	R471C (K*)			
				K5-E475	-	E475K (DM*) E475G (DM*)			

Table S2. Summary of the results from PISA and the mutated residues for interaction deficiencies (Related to Figures 1 and 2)

*Note: Relevance to genetic skin disease is highlighted. Abbreviations are as follow: **DM**, Epidermolysis bullosa simplex, Dowling-Meara variant; **EPPK**, Epidermolytic palmoplantar keratoderma; **K**, Epidermolysis bullosa simplex, Koebner (generalized) variant; **WC**, Epidermolysis bullosa simplex, Weber-Cockayne (localized) variant.

Mutant	Primer Name	Sequence (5'->3')
ID1	ID1_K5E_PvuI_F	CCAGAGGCTGAGAGCCGCGATCGACAATGTCAAGAAACAGTGC
	ID1_K5E_PvuI_R	GCACTGTTTCTTGACATTGTCGATCGCGGCTCTCAGCCTCTGGATCA TCCGG
	ID1_K14REQ_F	CGAGATCTCGGAGCTCGCGCGCACCATGCAGAACCTGGCGATTGAGCTGGCGTCCC AGCTCAGCATGAAAGC
	ID1_K14_K14REQ_R	CCAGGTTCTGCATGGTGCGCGCGAGCTCCGAGATCTCGCTCTTGCCGC TCTGC
ID3	ID3_K5QNE_NotI_F	GCCGAGATTGACAATGTCAAGAAACAGTGCGCCAATCTGGCGGCCGCCATTGCGG ATGCCGAGCAGCGTGGGGCGCTGGCCCTCAAGGATGCC
	ID3_K5QNE_NotI_R	GCGGCCGCCAGATTGGCGCACTGTTTCTTGACATTGTCAATCTCGGCTCTCAGCC TCTGGATCATCCGG
	ID3_K14RYQ_F	GGAGGAGACCAAAGGTGCCTTCTGCATGCAGCTGGCCGCGATCCAGGAGATGATTGG
	ID3_K14RYQ_R	GGCCAGCTGCATGCAGAAGGCACCTTTGGTCTCCTCCAGGCTGTTCTCCAGGG
ID7	ID7_K5AAA_F	GGATGCCAGGAACAAGCTGGCCGAGCTGGAGGCAGCGCTGGCGAAGGCCAAGG CGGACATGGCCCGGCTGCTGCGTGAGTACCAGG
	ID7_K5AAA_R	CCTGGTACTCACGCAGCAGCCGGGCCATGTCCGCCTTGGCCTTCGCCAGCGCTGC CTCCAGCTCGGCCAGCTTGTTCCTGGCATCC

Table S3. Sequences of the oligonucleotide primers used for mutagenesis to produce ID-deficient mutants (Related to Key Resources Table of the STAR METHODS).