SUPPLEMENTARY MATERIALS

Molecular characterisation of titin N2A and its binding of CARP reveals a titin/actin cross-linking mechanism

Zhou et al, 2021

Figure S1: Domains UN2A and Ig81 do not interact with each other

UN2A and Ig81 samples were produced independently and mixed post-production at a 1:1 molar ratio in 25mM HEPES pH 7.5, 100mM NaCl. The incubated mixture was subjected to size exclusion chromatography on a Superdex 75 16/60 column (GE Healthcare) and co-segregation monitored. The chromatogram showed two resolved peaks, indicating that UN2A and Ig81 species did not form a complex in solution. The chromatogram and associated SDS-PAGE are shown.

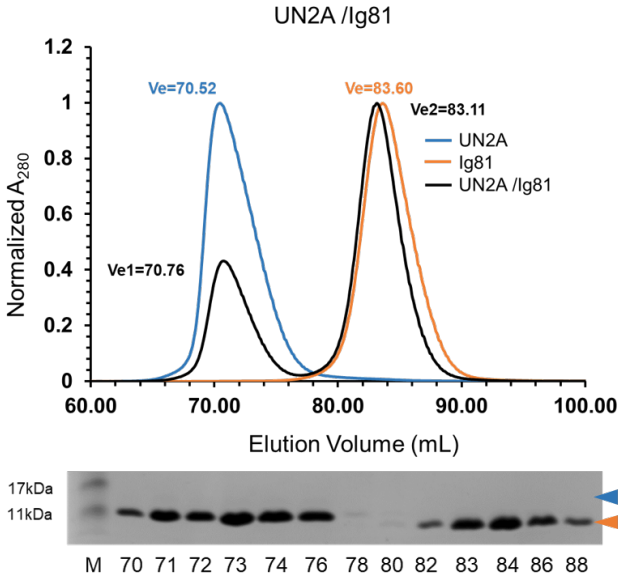


Figure S2: NMR-guided modelling of UN2A$^{34-73}$

A total of 32500 model decoys of UN2A$^{34-73}$ were calculated using CS-ROSETTA and subjected to cluster analysis in CALIBUR. **A.** Top ten models calculated in CS-ROSETTA with experimental NMR restraints ($RMSD_{C\alpha}$ = 2.3 Å); **B.** Top ten models from the largest CALIBUR cluster of 10216 models (i.e. most common conformation) with a corresponding $RMSD_{C\alpha}$ = 0.63 Å. **C.** and **D.** Plots of ROSETTA energy score versus $RMSD_{C\alpha}$ against the lowest-energy model for the calculated UN2A decoys. In C. the values for 32500 decoys calculated in CS-ROSETTA are shown. In D., the values correspond to 10216 decoys selected as primary conformational subpopulation with CALIBUR.

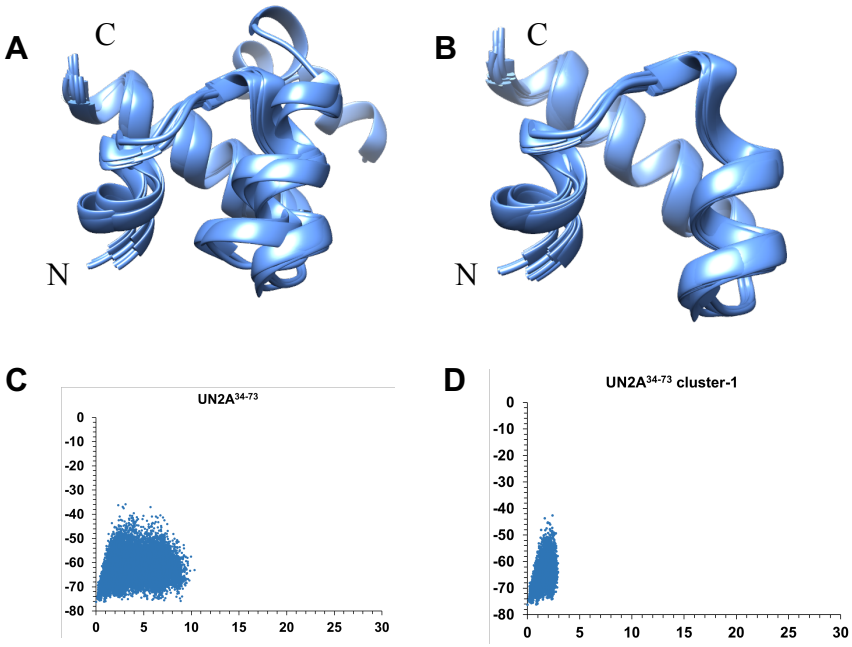


Figure S3: Relative fractional deuterium uptake (RFDU) of peptides forming the CARP interface in UN2A-Ig81 measured by HDX-MS

Shown are UN2A-Ig81 peptides presenting RFDU differences between their single state (blue) and in the presence of CARP$^{106-319}$ (red).

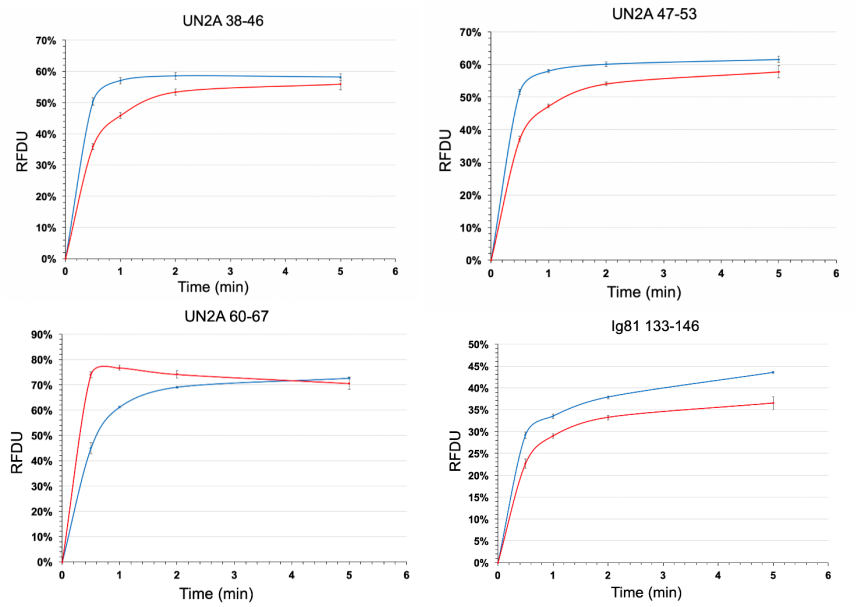


Figure S4: ^{1}H-^{15}N HSQC NMR spectrum of UN2A free and bound to CARP

A. ^{1}H-^{15}N HSQC spectrum of the UN2A/CARP$^{106-319}$ complex in a 1:2 molar ratio (red) compared to that of free UN2A (black). CARP$^{106-319}$ was not isotopically labelled; **B.** Titration process of three representative cross-signals (corresponding to the blue selection boxes in A). The CARP$^{106-319}$/UN2A molar ratio (C/U) is stated; **C.** The affected residues (total signal loss) upon CARP-interaction as monitored by NMR titration are mapped on the UN2A$^{34-73}$ model (red). The full-length UN2A sequence is shown below with the UN2A$^{34-73}$ sequence boxed and the amino acids with a changed NMR signal upon CARP presence indicated in red. NMR data show that the C-terminal helix is affected by CARP binding. HDX-MS data revealed that this helix is not direct part of the interface but, instead, that the helix is displaced upon binding of CARP to the frontal helical α-hairpin.

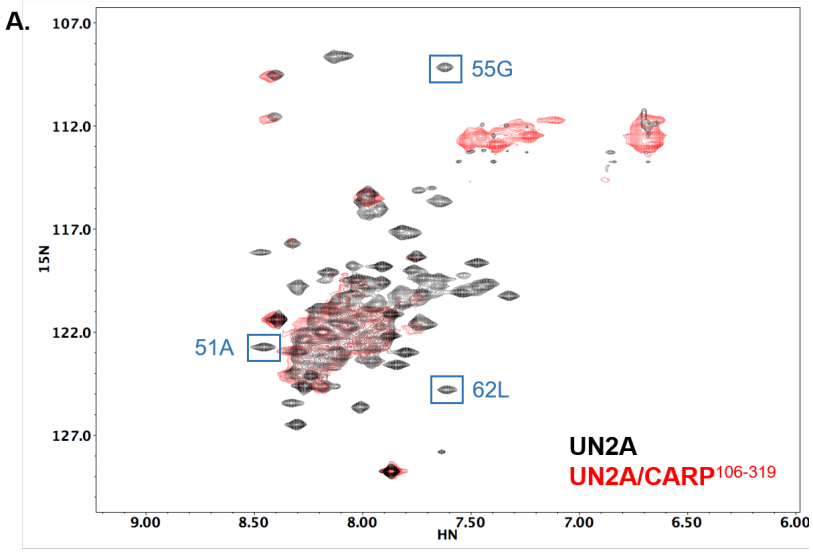


Figure S5: Identification of the titin-interface on CARP

Deuterium RFU values for free (blue) and UN2A-Ig81 bound (red) CARP$^{106-319}$. Values indicate the H/D exchange after 0.5 min incubation time;

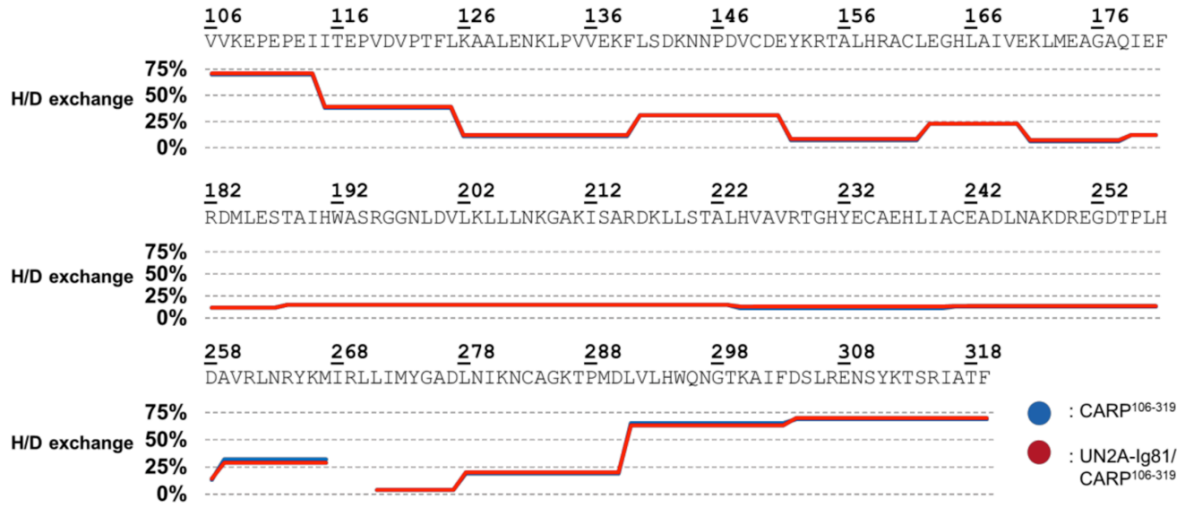
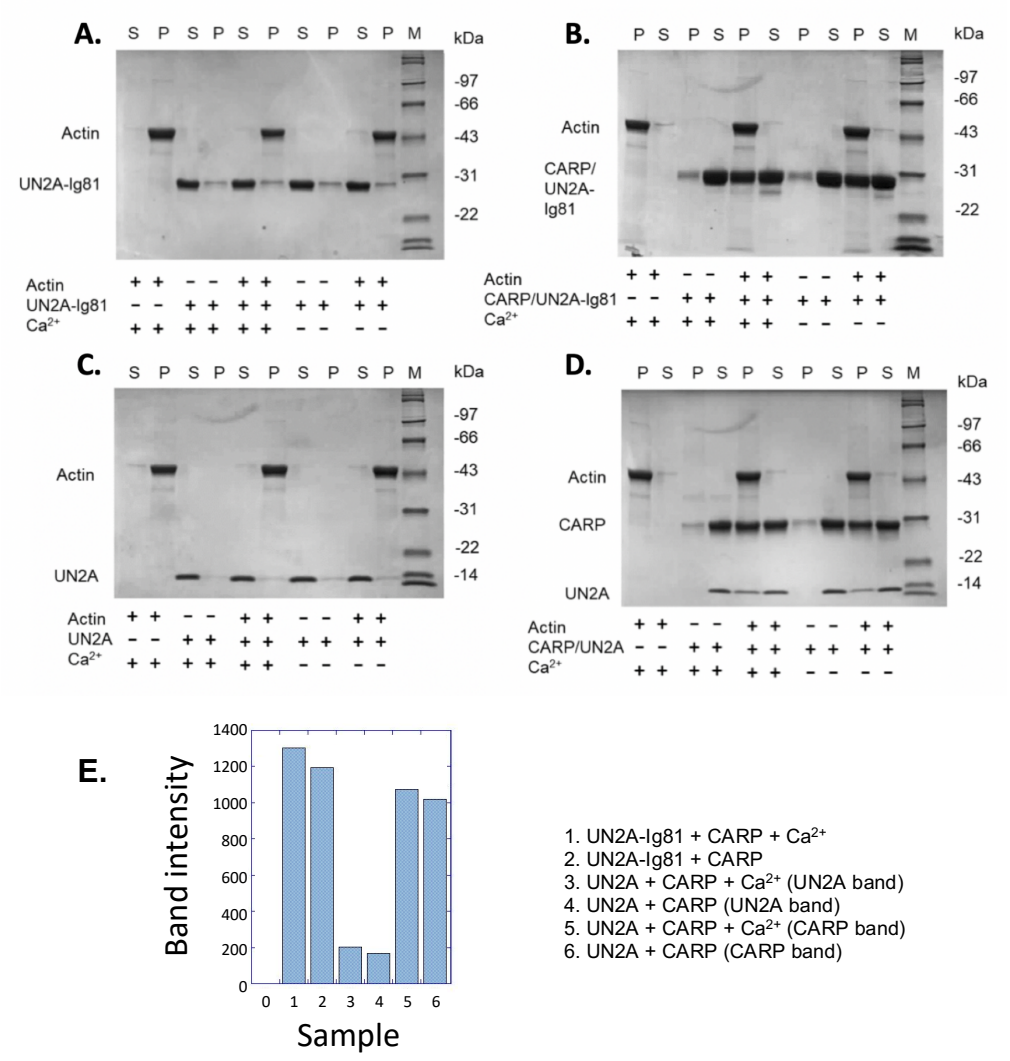


Figure S6: Ca^{2+}-dependence of actin co-sedimentation results

The actin co-sedimentation of (**A**) UN2A-Ig81, (**B**) UN2A, (**C**) $CARP^{106-319}$/UN2A-Ig81 and (**D**) $CARP^{106-319}$/UN2A was tested in the presence and absence of calcium. UN2A-Ig81 and UN2A do not co-sediment with actin significantly. In the presence of $CARP^{106-319}$, both titin segments co-sediment with actin noticeably. $CARP^{106-319}$ also co-sediments with actin as single species; **E.** SDS-PAGE band intensity as estimated by densitometric quantitation reporting on Ca^{2+}-sensitivity of F-actin binding. Samples where tested in the presence of calcium (2 mM $CaCl_2$) or in its absence (2 mM EGTA). Binding was not found to be dependent on calcium.



1. UN2A-Ig81 + CARP + Ca^{2+}
2. UN2A-Ig81 + CARP
3. UN2A + CARP + Ca^{2+} (UN2A band)
4. UN2A + CARP (UN2A band)
5. UN2A + CARP + Ca^{2+} (CARP band)
6. UN2A + CARP (CARP band)

Figure S7: CARP immunoprecipitation from heart lysates

Silver-stained SDS-PAGE of whole heart lysates (input) and immunoprecipitated proteins (IP pellet). As guidance, the locations where bands from selected proteins are expected are indicated. In this study, proteins were identified by mass spectrometry performed on the whole immuno-precipitate, not from bands excised from the gel.

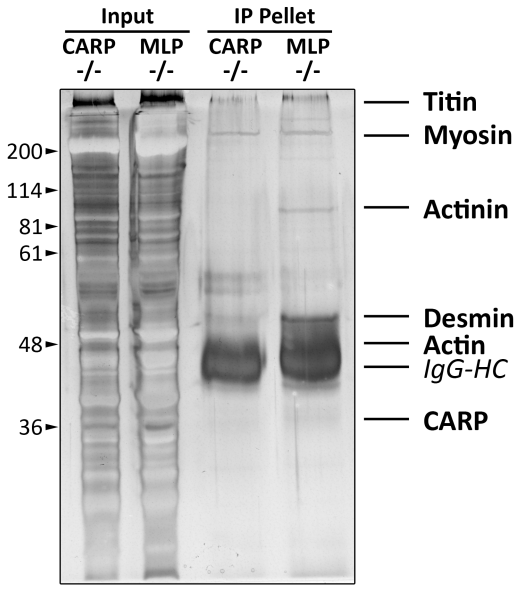


Table S1: Analysis of single nucleotide polymorphisms in human cohorts

Records as obtained from gnomAD v2.1.1 and v3. Regions for which structural models exist are indicated with a blue background. Residues mapping to the CARP/titin-N2A interface are shown in red. ΔΔG values over 2.5 kcal/mol calculated on the crystal structure of Ig81 using FoldX are indicative of potential onset of structural damage. This information, together with inspection of structural 3D-models using Missense3D as well as manual *in silico* residue replacement, lead us to identify two single nucleotide variants (W9617 and G9659, in bold) as potential destabilizers of this domain of titin. Analysis of N2A models did not reveal structural deleterious mutations in that domain. In Ig81, residue W9617 (W146 in this study) corresponds to a highly conserved tryptophan in the hydrophobic core of this domain that is signature of the Ig fold. Residue G9659 (G188 in this study) is the defining feature of the β-turn type II' of the FG β-hairpin in Ig81 and required for hairpin formation. The substitution of either of these residues, might destabilize Ig81 and weaken (but not abolish) CARP binding. Potentially, the destabilization might only manifest itself significantly in mechanically stretched states of titin.

rare sequence code	original bp	changed bp	original AA	AA number Q8WZ42	New AA	Occurrence	Meta-SNP	RI	FoldX ΔΔG (kcal/mol)	3D-Missense
rs760656457	C	T	Asp	9472 (1)	Asn	8,03E-06	Neutral	8		
rs1256487014	T	A	Asp	9472 (1)	Val	4,01E-06	Neutral	9		
rs775325497	T	A	Glu	9473 (2)	Asp	6,98E-06	Neutral	7		
rs771829036	A	G	Ile	9480 (9)	Thr	8,05E-06	Neutral	7		
rs1254367559	C	T	Gly	9482 (11)	Asp	1,15E-05	Neutral	5		
	C	T	Asp	9483 (12)	Asn	6,98E-06	Neutral	5		
rs1210593236	C	T	Ala	9486 (15)	Thr	6,98E-06	Neutral	7		
rs781737736	A	G	Ile	9493 (22)	Thr	2,98E-05	Neutral	9		
rs1356650510	T	C	Ile	9493 (22)	Val	6,98E-06	Neutral	8		
	T	G	Leu	9494 (23)	Phe	6,98E-06	Neutral	6		
rs755553000	T	G	Lys	9495 (24)	Gln	6,98E-06	Neutral	9		
rs1440897182	C	G	Gly	9499 (28)	Ala	4,40E-06	Neutral	7		
	C	T	Gly	9499 (28)	Glu	6,98E-06	Neutral	6		
rs780561066	C	A	Glu	9502 (31)	Asp	2,09E-05	Neutral	7		
rs758873870	C	A	Asp	9505 (34)	Tyr	1,40E-05				
rs962438568	A	G	Ile	9506 (35)	Thr	6,98E-06				
rs777309739	G	T	Leu	9509 (38)	Ile	2,79E-05	Neutral	7		
rs752150259	T	C	Asn	9512 (41)	Ser	8,09E-06	Neutral	6		
rs767075303	T	A	Asp	9514 (43)	Val	4,04E-06				
rs1223522531	T	C	Tyr	9518 (47)	Cys	9,45E-06	Disease	4		
rs578069922	G	A	Ala	9522 (51)	Val	4,02E-06				
	C	A	Ala	9522 (51)	Ser	6,98E-06				
rs558220856	C	T	Arg	9523 (52)	His	2,55E-05				
rs369990929	G	A	Arg	9523 (52)	Cys	1,40E-05				
rs1234119302	C	T	Met	9524 (53)	Ile	1,40E-05				
	C	T	Gly	9526 (55)	Glu	6,98E-06	Disease	4		
rs766144409	T	G	Thr	9528 (57)	Pro	6,98E-06	Disease	4		
rs773444238	C	T	Arg	9531 (60)	Gln	1,21E-05				
	G	A	Ala	9536 (65)	Val	6,98E-06	Neutral	5		
rs794727011	T	C	Glu	9538 (67)	Gly	6,98E-06	Neutral	6		
rs769913782	T	C	Lys	9541 (70)	Arg	4,02E-06	Neutral	6		
rs776836480	C	T	Glu	9547 (76)	Lys	1,40E-05	Neutral	6		
rs878919397	T	C	His	9549 (78)	Arg	4,03E-06	Neutral	7		
	G	C	His	9549 (78)	Asp	6,98E-06	Neutral	7		
rs777030251	A	G	Ile	9553 (82)	Thr	8,05E-06	Neutral	8		
rs768829835	C	G	Glu	9557 (86)	Gln	6,98E-06	Neutral	7		
rs760859295	C	T	Arg	9561 (90)	Lys	1,21E-05	Neutral	9		
	G	C	Asp	9562 (91)	Glu	1,40E-05	Neutral	9		
rs532933900	C	T	Glu	9563 (92)	Lys	2,79E-05	Neutral	7		
	T	G	Gln	9574 (103)	Pro	6,98E-06				
rs933913351	G	A	Thr	9580 (109)	Ile	1,15E-05	Neutral	5		
rs767705843	G	A	Pro	9582 (111)	Leu	7,73E-06			1,22	
	C	T	Val	9583 (112)	Ile	6,98E-06	Neutral	5	0,12	
rs1430193759	A	C	Leu	9585 (114)	Arg	6,98E-06			0,99	
rs367712022	A	G	Ile	9589 (118)	Thr	1,03E-05			2,69	
	C	G	Val	9594 (123)	Leu	1,40E-05	Neutral	4	5,22	

rs768314420	T	C	Asp	9599 (128)	Gly	1,03E-05	Neutral	5	2,14	
rs768314420	T	A	Asp	9599 (128)	Val	4,06E-06	Neutral	5	0,75	
rs373651676	A	G	Ile	9604 (133)	Thr	3,09E-05			2,81	
rs377147236	T	G	Asn	9609 (138)	Thr	7,73E-06			1,77	
rs377147236	T	A	Asn	9609 (138)	Ile	1,03E-05			-0,21	
rs1204774037	C	T	Glu	9612 (141)	Lys	2,79E-05			-0,08	
rs757077819	A	C	Ile	9613 (142)	Ser	2,06E-05	Disease	4	2,92	
rs373215746	T	C	Ile	9613 (142)	Val	6,98E-06	Neutral	6	1,04	
rs1300557715	A	G	Leu	9615 (144)	Pro	1,22E-05			3,02	
rs753689443	G	T	Leu	9615 (144)	Ile	6,98E-06	Neutral	5	1,25	
rs764352461	G	A	Ser	9616 (145)	Leu	2,15E-05			-0,59	
rs1046404085	**C**	**A**	**Trp**	**9617 (146)**	**Cys**	**6,98E-06**	**Disease**	**5**	**5,59**	Cavity altered
rs1428709009	T	C	Tyr	9618 (147)	Cys	4,08E-06			2,26	
rs766917273	C	G	Ser	9627 (156)	Thr	4,07E-06	Neutral	5	2,06	
rs766917273	C	T	Ser	9627 (156)	Asn	4,07E-06	Neutral	6	1,44	
rs763249086	T	C	Asp	9628 (157)	Gly	8,14E-06	Neutral	5	0,07	
rs1268571982	T	G	Lys	9629 (158)	Thr	4,07E-06			3,09	
	T	C	Ser	9633 (162)	Gly	6,98E-06	Neutral	5	0,12	
rs1482211815	A	G	Ile	9634 (163)	Thr	4,06E-06			0,9	
rs773555150	C	G	Asp	9635 (164)	His	6,98E-06	Neutral	5	0,55	
rs1201726118	C	T	Gly	9636 (165)	Asp	1,15E-05	Disease	4	4,75	
	C	A	Gly	9636 (165)	Cys	1,40E-05	Disease	4	4,2	
rs182332374	C	T	Arg	9638 (167)	Gln	1,03E-05	Neutral	4	-0,71	
rs1255328557	T	C	His	9639 (168)	Arg	4,05E-06			0,37	
rs778649000	T	C	Lys	9644 (173)	Arg	1,21E-05	Neutral	8	0,37	
rs1400936440	T	C	Lys	9649 (178)	Arg	4,03E-06	Neutral	9	-0,25	
	T	A	Lys	9649 (178)	Ile	1,40E-05	Neutral	7	0,42	
rs756024325	C	T	Gly	9652 (181)	Asp	8,05E-06	Disease	6	1,35	
rs961816213	C	T	Gly	9652 (181)	Ser	6,98E-06			3	
	T	C	Asn	9653 (182)	Ser	6,98E-06			0,68	
rs1183241604	**C**	**T**	**Gly**	**9659 (188)**	**Asp**	**4,02E-06**	**Disease**	**4**	**9,17**	**Disallowed phi/psi**
rs1254687485	G	A	Pro	9660 (189)	Ser	6,98E-06			1,71	
	C	A	Ala	9663 (192	Ser	1,40E-05	Neutral	8	-0,08	
	C	T	Ala	9663 (192)	Thr	2,65E-04			1,49	
rs200745162	C	T	Ala	9665 (194)	Thr	6,28E-05	Neutral	4	1,63	
	G	T	Leu	9667 (196)	Ile	6,98E-06	Neutral	4	2,02	
rs376644553	A	G	Ile	9669 (198)	Thr	3,36E-05	Neutral	8	1,05	
rs376644553	A	T	Ile	9670 (199)	Asn	4,55E-05	Neutral	7	1,06	
rs762584154	T	C	Glu	9671 (200)	Gly	1,21E-05	Neutral	8	0,49	