

# Supporting Information

## **Novel dynamic residue network analysis approaches to study allosteric modulation: SARS-CoV-2 M<sup>pro</sup> and its evolutionary mutations as a case study**

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# Equally contributed first authorship

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**Table S1.** List of mutations and sample IDs extracted from GISAID database.

<b>Mutation Position</b>	<b>Strain ID</b>	<b>Mutation Position</b>	<b>Strain ID</b>	<b>Mutation Position</b>	<b>Strain ID</b>
A7V	EPI_ISL_425319	A116V	EPI_ISL_425284	T198I	EPI_ISL_421515
G15D	EPI_ISL_420422	A129V	EPI_ISL_422860	T201A	EPI_ISL_423642
G15S	EPI_ISL_420181	R105H	EPI_ISL_419984	L220F	EPI_ISL_419256
M17I	EPI_ISL_423772	A129V	EPI_ISL_422860	Y237H	EPI_ISL_416720
V20L	EPI_ISL_425342	P132L	EPI_ISL_420579	D248E	EPI_ISL_425886
T45I	EPI_ISL_421312	T135I	EPI_ISL_425655	A255V	EPI_ISL_418075
M49I	EPI_ISL_425839	I136V	EPI_ISL_420182	I259T	EPI_ISL_422919
R60C	EPI_ISL_418269	N151D	EPI_ISL_420510	A260V	EPI_ISL_423725
K61R	EPI_ISL_420306	V157I	EPI_ISL_415503	V261A	EPI_ISL_425498
A70T	EPI_ISL_421763	C160S	EPI_ISL_417413	A266V	EPI_ISL_421380
G71S	EPI_ISL_413021	A173V	EPI_ISL_418082	N274D	EPI_ISL_420610
L89F	EPI_ISL_415643	P184L	EPI_ISL_420241	R279C	EPI_ISL_425643
K90R	EPI_ISL_420059	P184S	EPI_ISL_423288	S301L	EPI_ISL_422184
P99L	EPI_ISL_419756	T190I	EPI_ISL_423007	G15S, D48E	EPI_ISL_425242
Y101C	EPI_ISL_425132	A193V	EPI_ISL_415610		
P108S	EPI_ISL_421005	T196M	EPI_ISL_424470		

**Table S2. Molecular interactions established between hits and their respective allosteric site residues in SARS-CoV-2 M<sup>pro</sup>. In black and red labels are residues from protomer A and B respectively. Functional group of hits are indicated in brackets closer to the residue. H-bond lengths for all H-bonds are shown.**

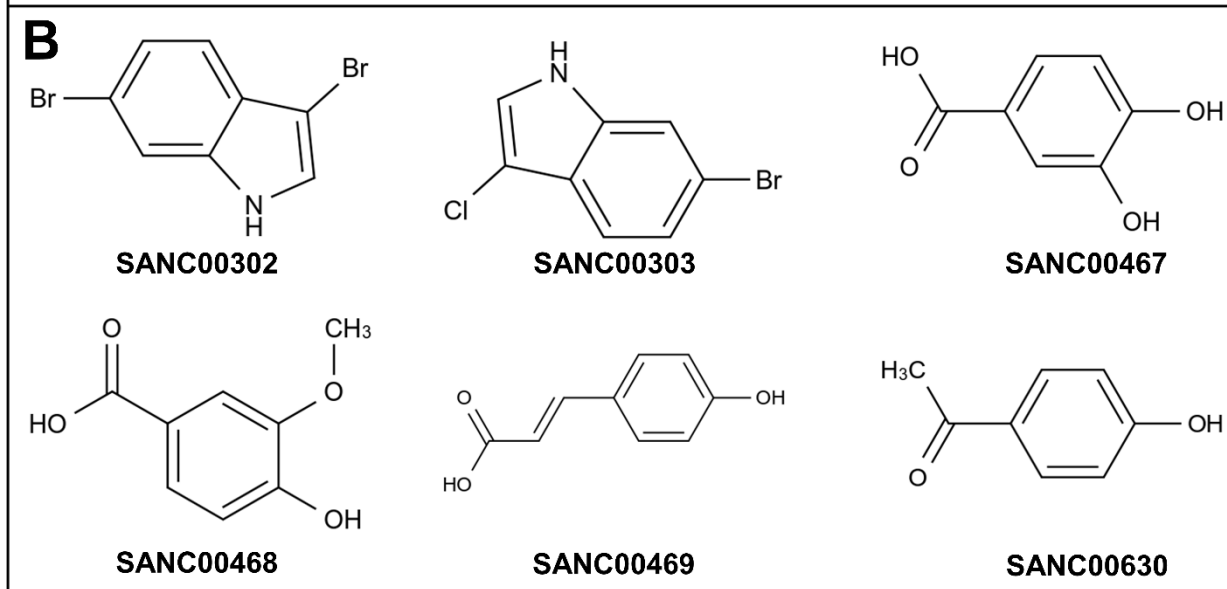
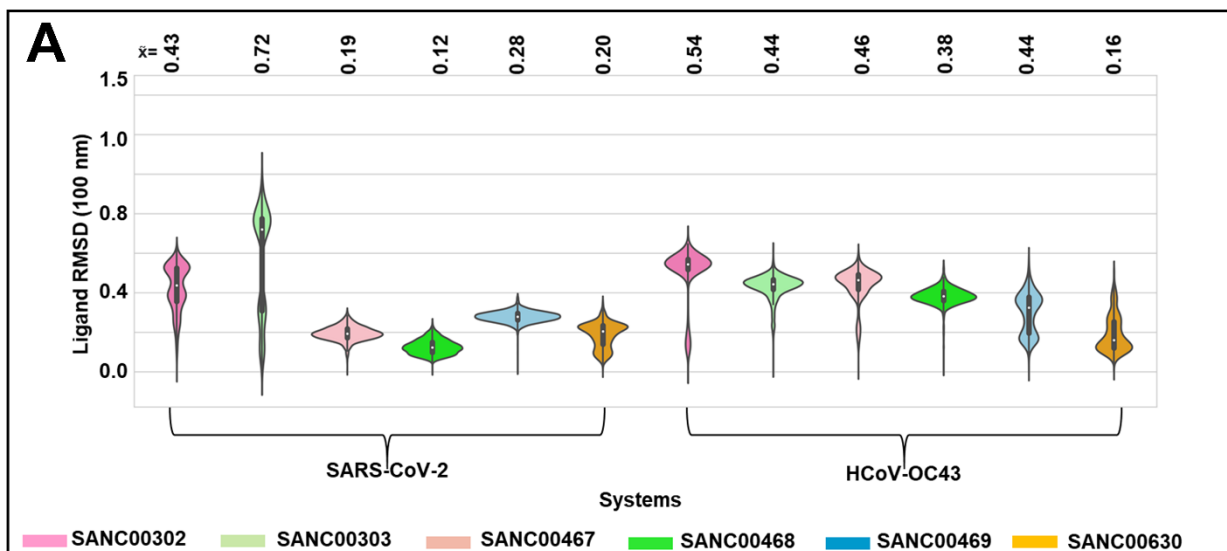
Compound ID	H-bonds	H-bond length (Å)	hydrophobic	Pi-	Compound ID	H- bonds	H-bond length (Å)	Hydrophobic	Pi-
SANC00302			PHE3, ALA7, <b>PHE8</b> , PRO9, <b>SER123</b> , <b>GLY124</b> , GLU290, GLN299	MET6, PHE291, ASP295, ARG298	SANC00468	MET6 (O9) <b>SER123</b> (O18) GLN299 (O8)	3.10 2.67 2.94	PHE3, ALA7, <b>PHE8</b> , PRO9, SER113, <b>GLY124</b> , GLN127, PHE291, ASP295, GLY302, VAL303	MET6, ARG298
SANC00303			PHE3, ALA7, <b>PHE8</b> , PRO9, SER113, <b>SER123</b> , <b>GLY124</b> , GLN127, GLU290, GLN299	MET6, PHE291, ASP295, ARG298	SANC00469	MET6 (O13) <b>Val303</b> (O11)	2.45 2.52	ALA7, PHE8, GLY124, SER113, PHE291, TYR118, GLN299,	ASP229, ARG298
SANC00467	<b>MET6 (O3)</b> <b>MET6 (O4)</b> <b>VAL303 (O2)</b>	3.12 2.82 3.10		<b>ARG298, PHE8,</b> <b>MET6</b>	SANC00630	<b>SER123 (O2)</b> GLN299 (O1)	2.71 2.88	PHE3, ALA7, PHE8, THR111, SER113, <b>GLY124</b> , GLN127, GLU90, F291	MET6, ARG298

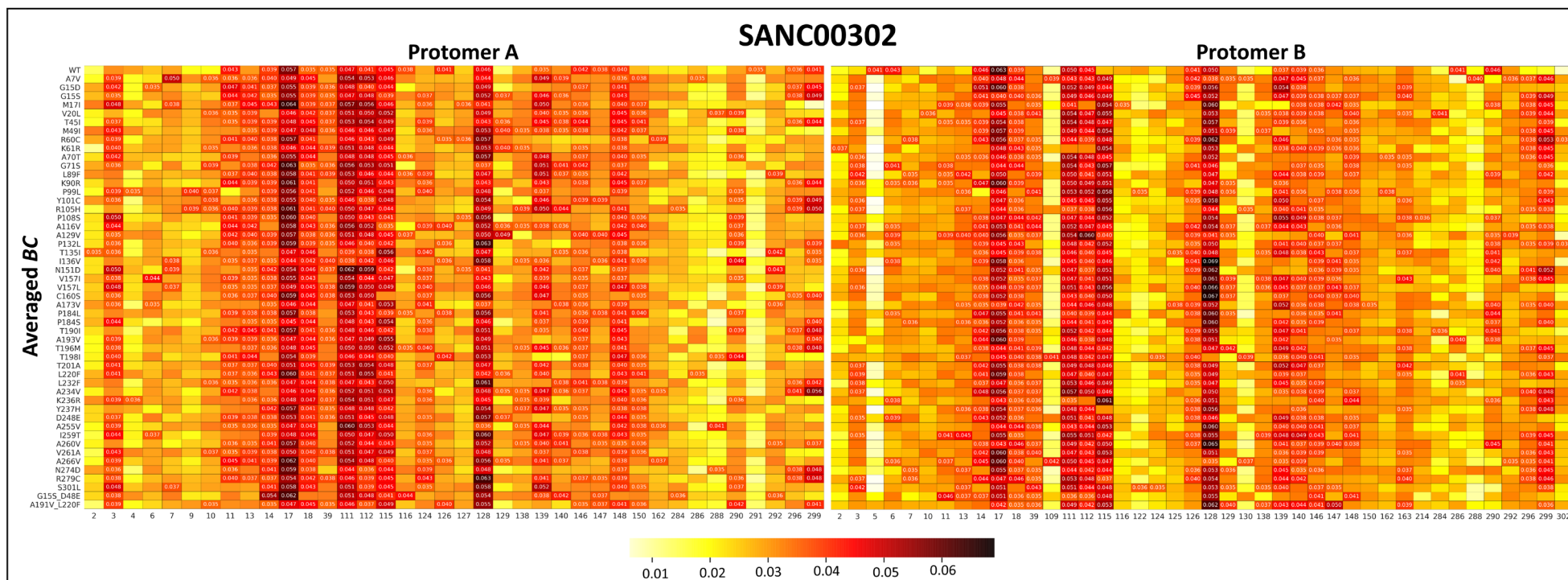
**Table S3. The distribution of ligand stability across mutant samples.** The symbol ‘✓’ indicates highly stable motion. The consensus score across each mutant system is the number of ‘✓’ entries in that row.

Mutation Position	Strain ID	302	303	467	468	469	630	Consensus
A7V	EPI_ISL_425319	✓	✓			✓		3
G15D	EPI_ISL_420422	✓		✓	✓	✓		4
G15S	EPI_ISL_420181	✓	✓	✓		✓		4
M17I	EPI_ISL_423772			✓	✓	✓	✓	4
V20L	EPI_ISL_425342		✓	✓	✓	✓		4
T45I	EPI_ISL_421312	✓	✓		✓		✓	4
M49I	EPI_ISL_425839		✓	✓	✓	✓	✓	5
R60C	EPI_ISL_418269	✓			✓	✓		2
K61R	EPI_ISL_420306		✓		✓	✓		3
A70T	EPI_ISL_421763			✓	✓	✓	✓	4
G71S	EPI_ISL_413021	✓		✓	✓	✓		4
L89F	EPI_ISL_415643		✓	✓	✓			3
K90R	EPI_ISL_420059	✓	✓			✓		3
P99L	EPI_ISL_419756				✓	✓	✓	3
Y101C	EPI_ISL_425132	✓		✓	✓	✓		4
R105H	EPI_ISL_419984	✓			✓	✓	✓	4
P108S	EPI_ISL_421005		✓		✓		✓	3
A116V	EPI_ISL_425284		✓	✓	✓		✓	4
A129V	EPI_ISL_422860		✓			✓	✓	3
P132L	EPI_ISL_420579	✓		✓			✓	3
T135I	EPI_ISL_425655			✓	✓	✓	✓	4
I136V	EPI_ISL_420182			✓	✓	✓		3
N151D	EPI_ISL_420510				✓	✓		2
V157I	EPI_ISL_415503		✓	✓				2
V157L	EPI_ISL_426028		✓		✓	✓	✓	4

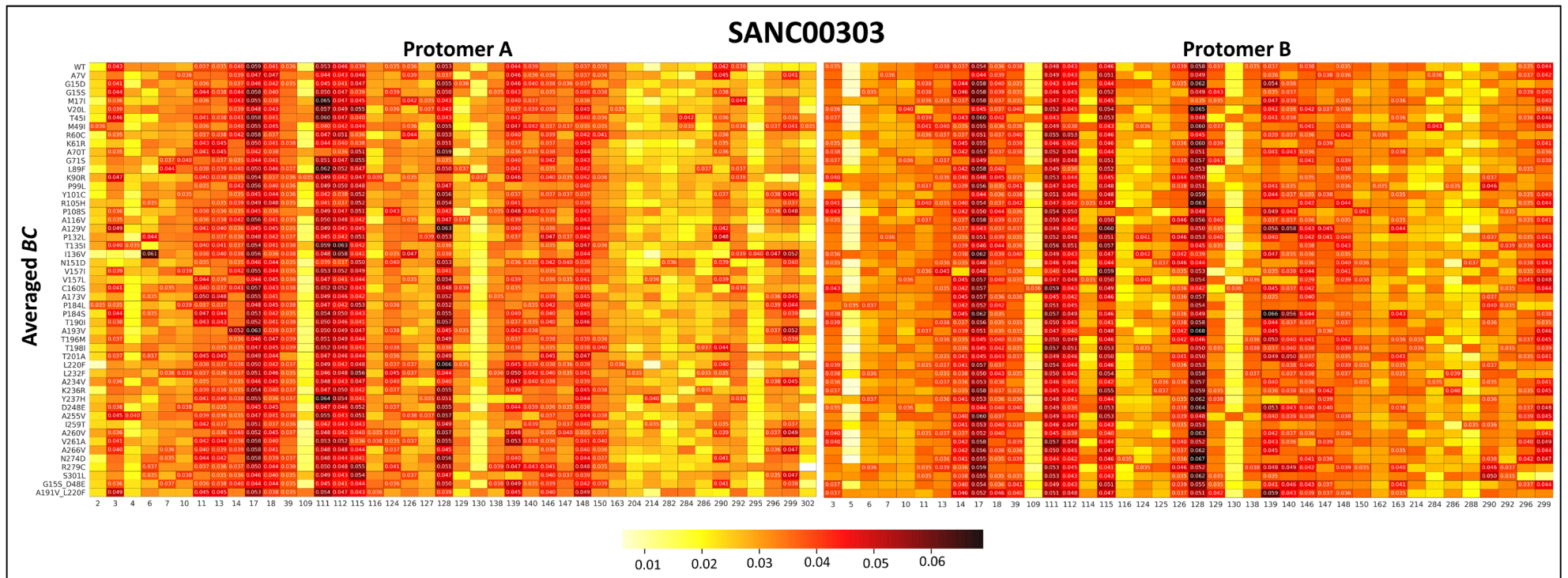
Mutation position	Strain ID	302	303	467	468	469	630	Consensus
C160S	EPI_ISL_417413				✓	✓		2
A173V	EPI_ISL_418082	✓	✓	✓	✓	✓	✓	6
P184L	EPI_ISL_420241				✓		✓	2
P184S	EPI_ISL_423288	✓			✓	✓	✓	4
T190I	EPI_ISL_423007	✓			✓	✓	✓	4
A193V	EPI_ISL_415610	✓	✓	✓	✓	✓		5
T196M	EPI_ISL_424470	✓			✓	✓	✓	4
T198I	EPI_ISL_421515			✓	✓	✓		3
T201A	EPI_ISL_423642			✓	✓	✓	✓	4
L220F	EPI_ISL_419256				✓	✓	✓	3
L232F	EPI_ISL_421506	✓	✓		✓	✓		4
A234V	EPI_ISL_425235	✓				✓	✓	3
K236R	EPI_ISL_426097	✓	✓	✓	✓	✓		5
Y237H	EPI_ISL_416720			✓	✓	✓		3
D248E	EPI_ISL_425886			✓	✓	✓	✓	4
A255V	EPI_ISL_418075				✓		✓	2
I259T	EPI_ISL_422919			✓	✓		✓	3
A260V	EPI_ISL_423725		✓	✓	✓	✓	✓	5
V261A	EPI_ISL_425498			✓	✓	✓		3
A266V	EPI_ISL_421380		✓	✓	✓	✓	✓	5
N274D	EPI_ISL_420610	✓	✓	✓	✓	✓	✓	6
R279C	EPI_ISL_425643	✓	✓	✓	✓	✓	✓	6
S301L	EPI_ISL_422184				✓	✓	✓	3
G15S, D48E	EPI_ISL_425242		✓	✓	✓	✓	✓	5
A191V, L220F	EPI_ISL_419710			✓	✓	✓	✓	4

20 22 29 43 41 30

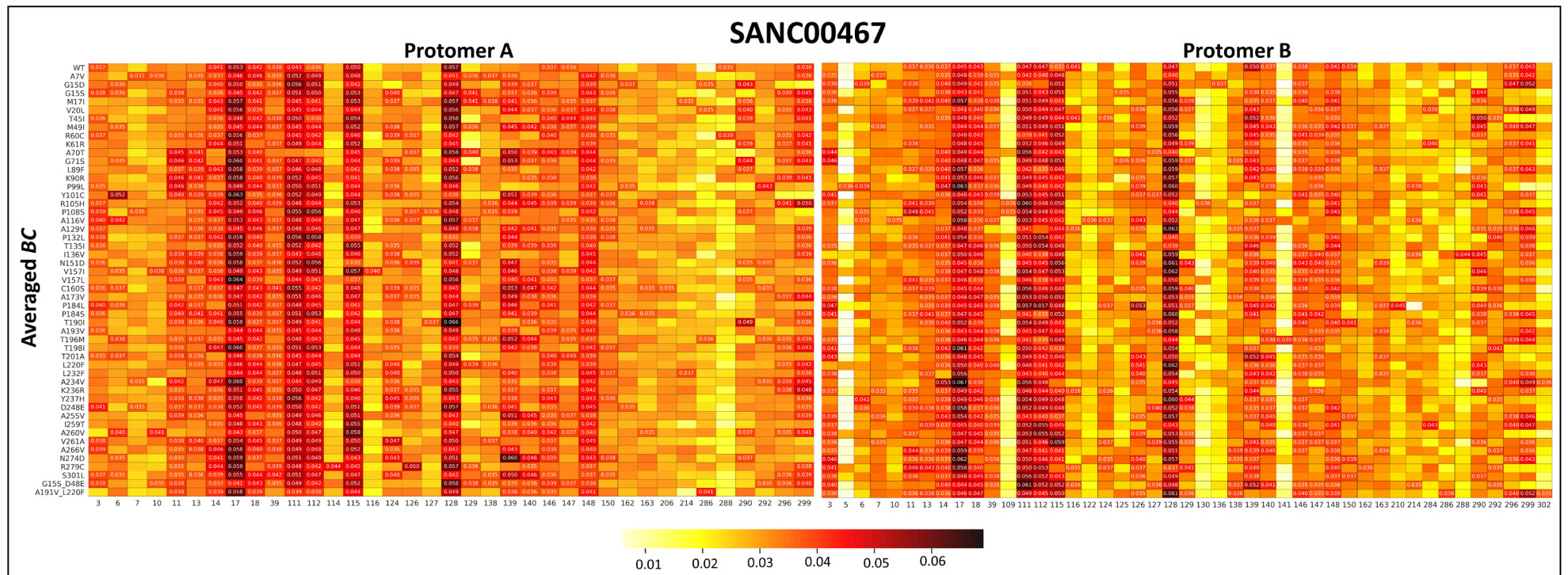




**Figure S2A.** Heatmaps of the potential hubs as per to the global top 5% of the *BC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00302. *BC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.

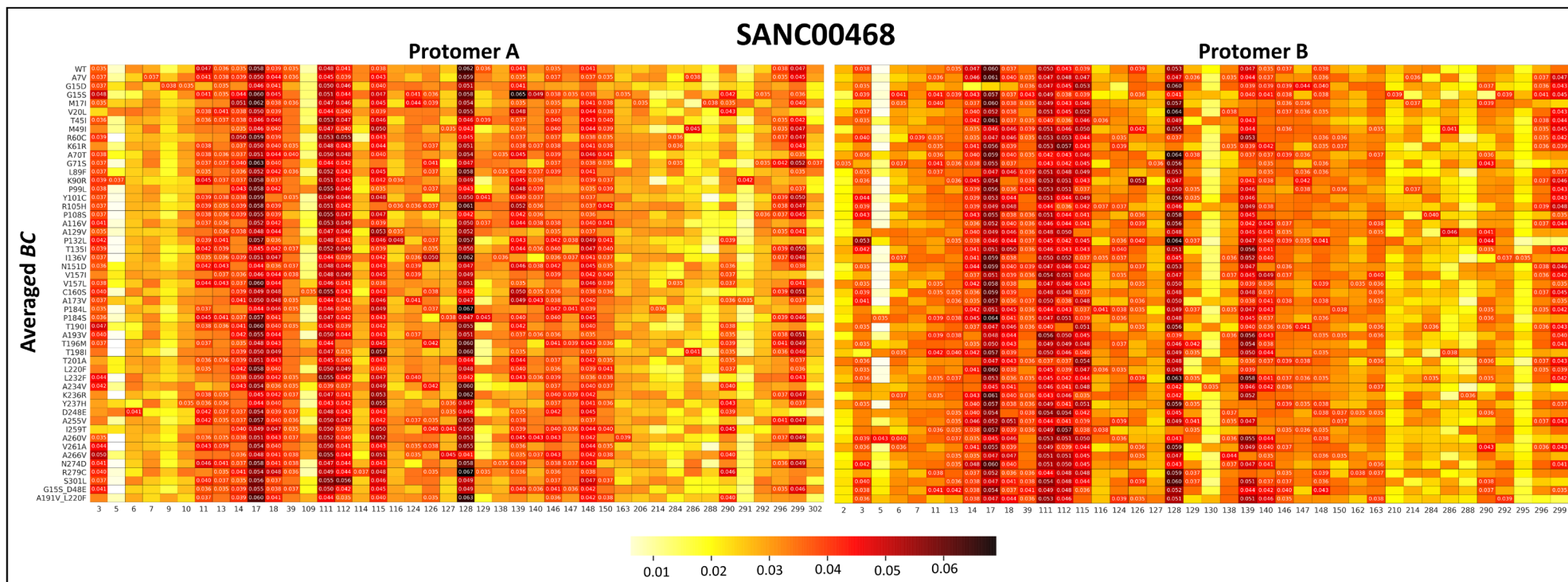


**Figure S2B.** Heatmaps of the potential hubs as per to the global top 5% of the *BC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00303. *BC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.

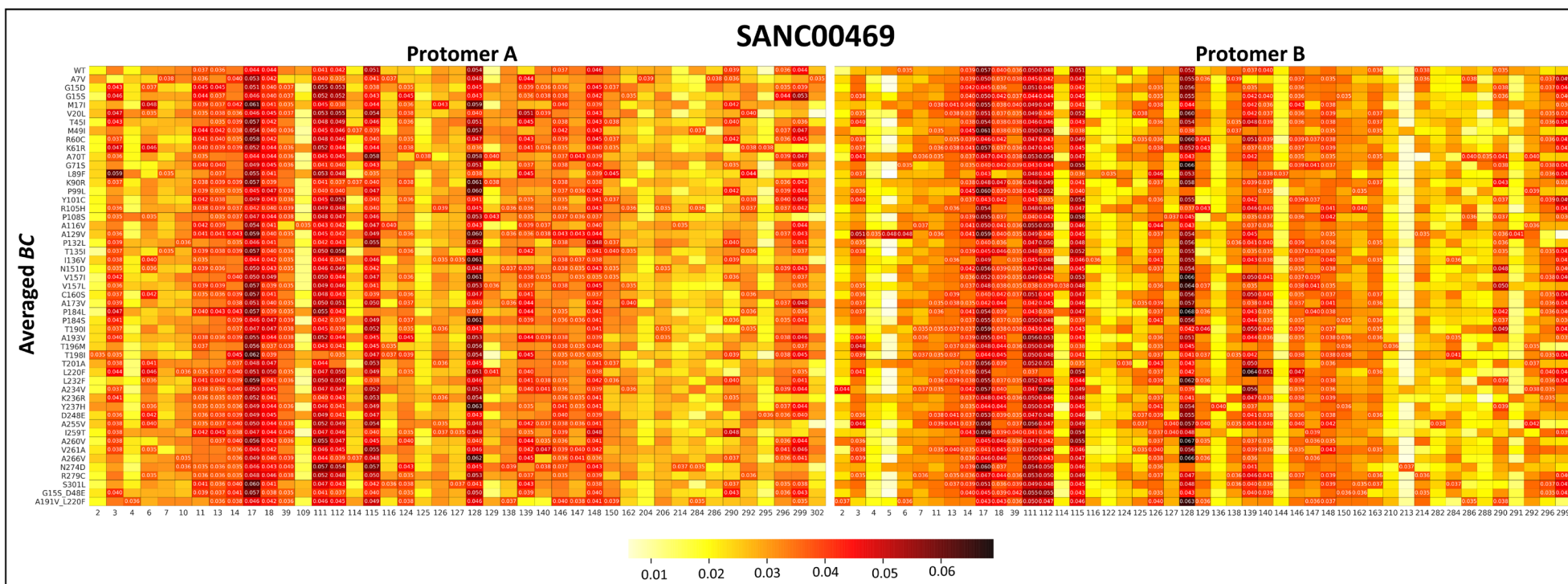


**Figure S2C.** Heatmaps of the potential hubs as per to the global top 5% of the *BC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00467. *BC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The color scale from white, through yellow, orange and red to black indicates the degree of residue centrality.

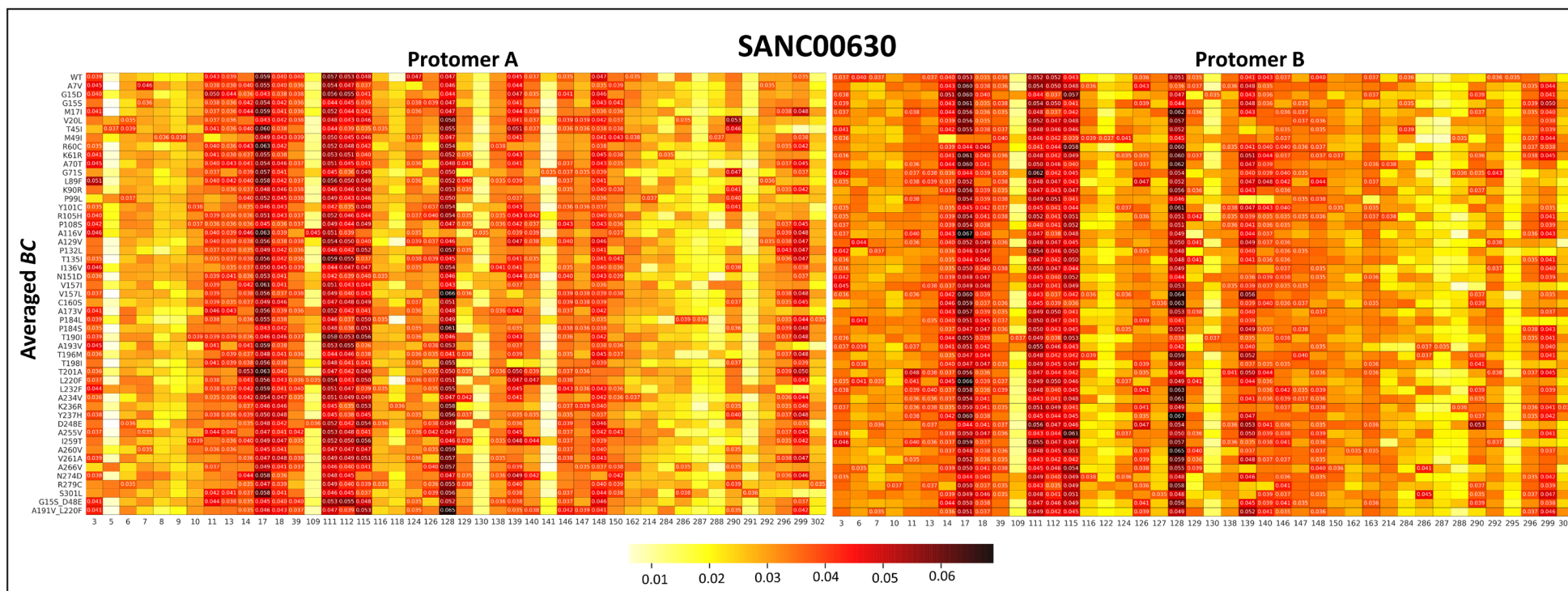




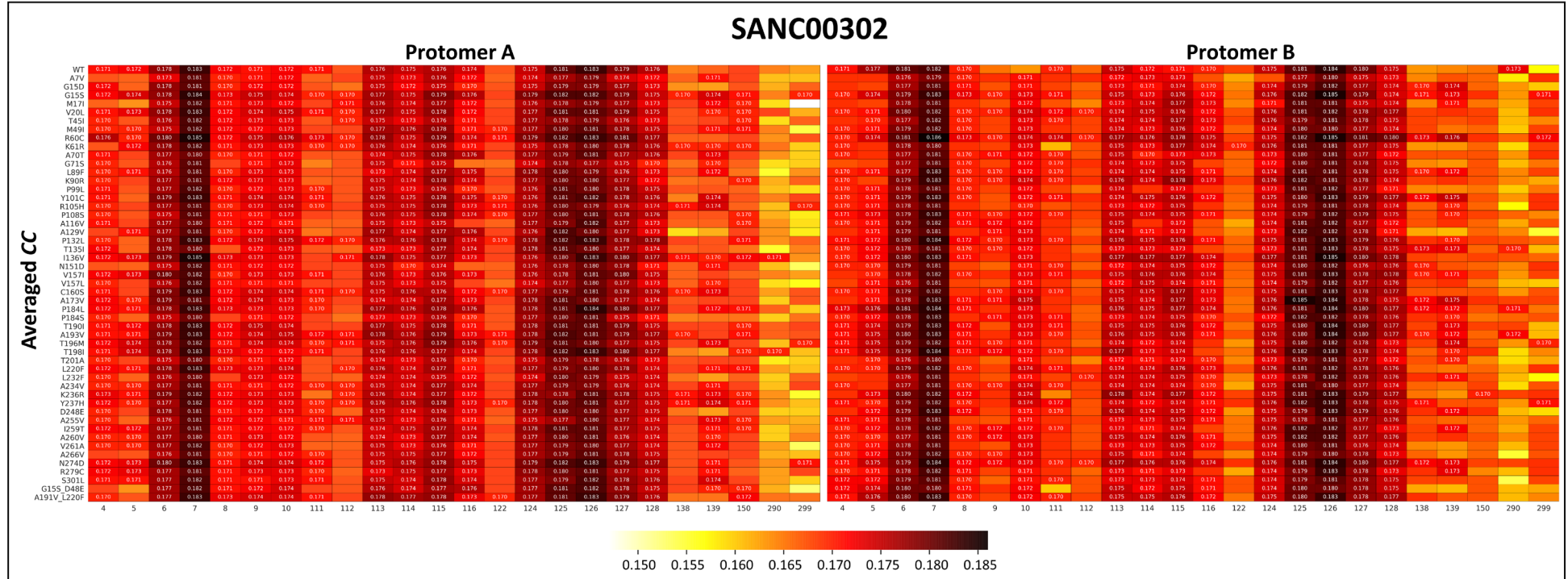
**Figure S2D.** Heatmaps of the potential hubs as per to the global top 5% of the *BC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00468. *BC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



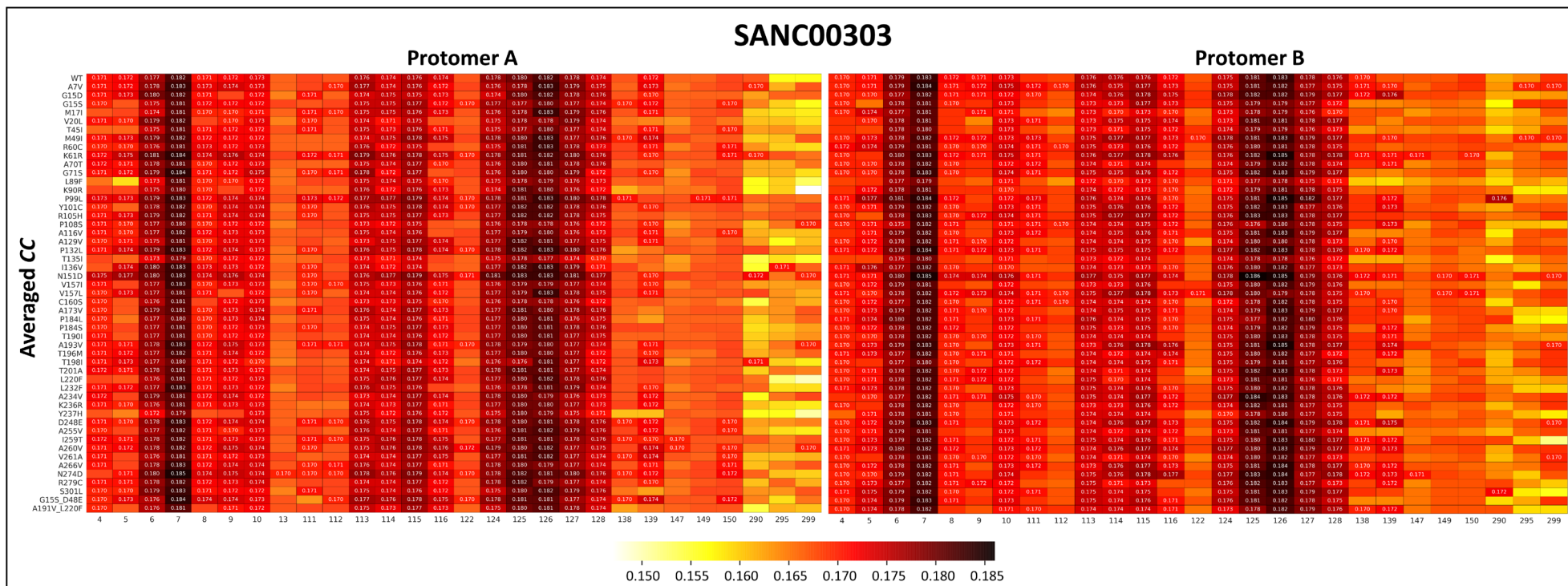
**Figure S2E.** Heatmaps of the potential hubs as per to the global top 5% of the *BC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00469. *BC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



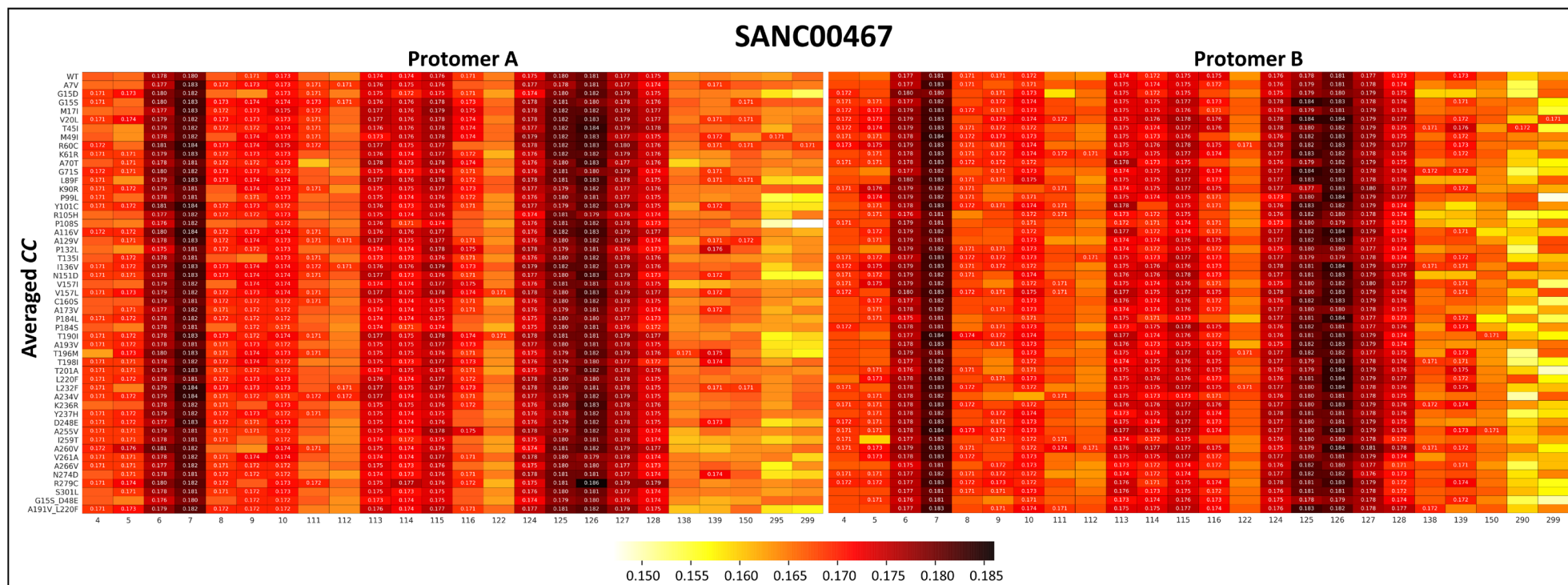
**Figure S2F.** Heatmaps of the potential hubs as per to the global top 5% of the *BC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANCO0630. *BC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



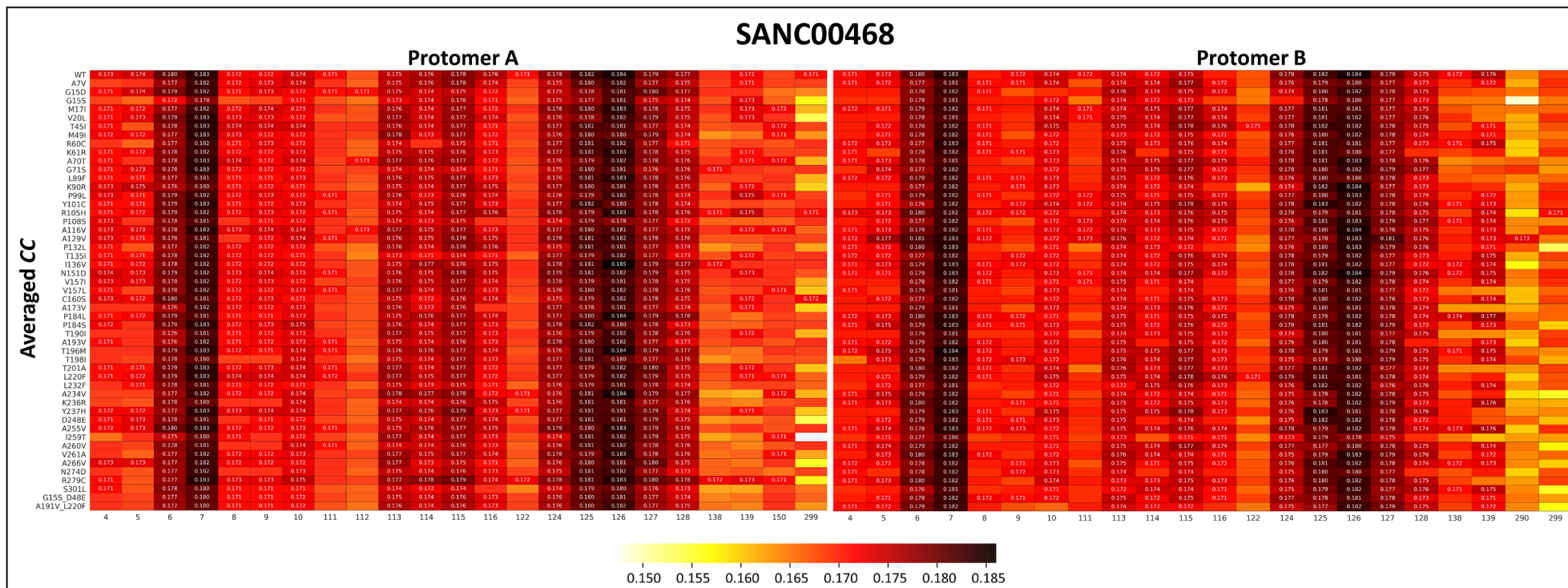
**Figure S3A.** Heatmaps of the potential hubs as per to the global top 5% of the *CC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00302. *CC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The color scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



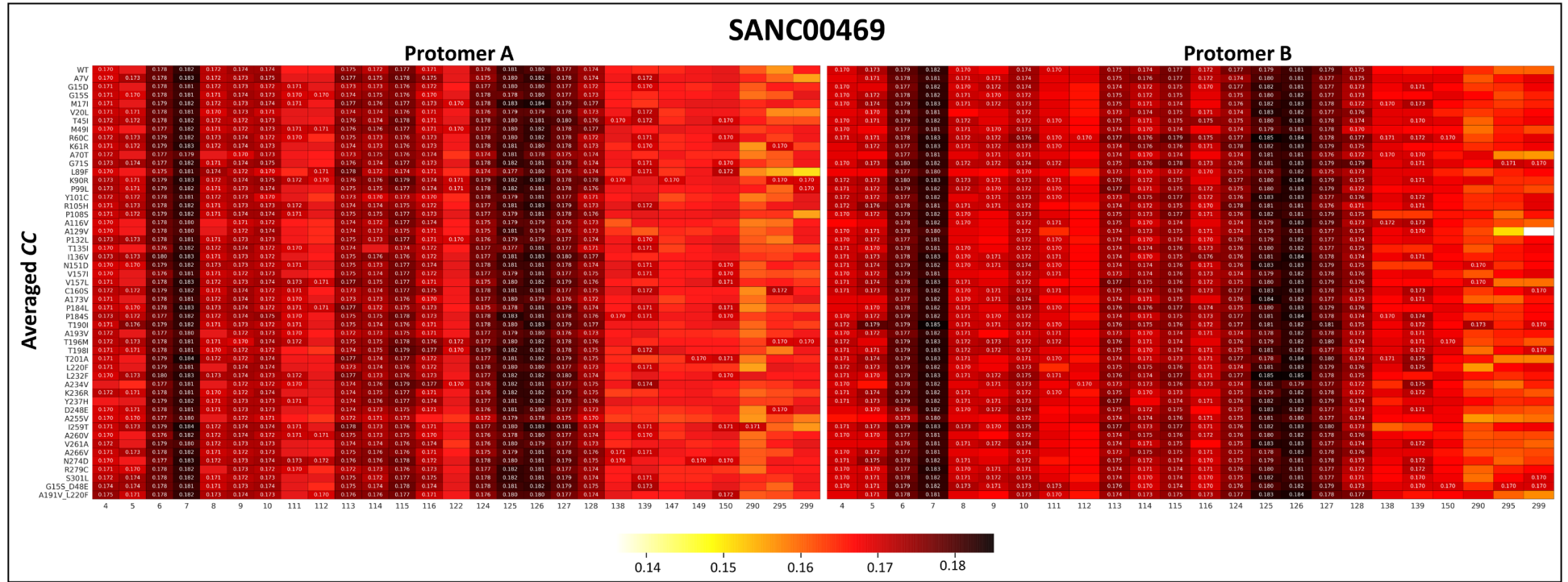
**Figure S3B.** Heatmaps of the potential hubs as per to the global top 5% of the CC DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00303. CC measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



**Figure S3C.** Heatmaps of the potential hubs as per to the global top 5% of the CC DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00467. CC measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.

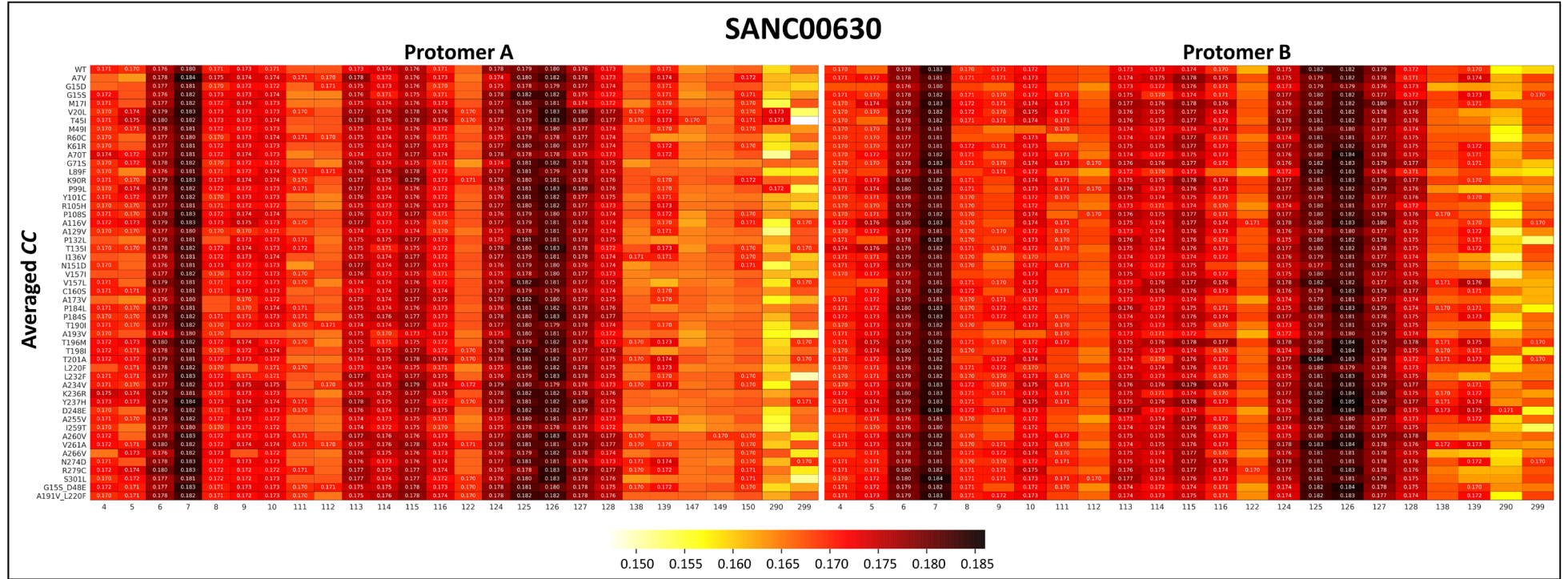


**Figure S3D.** Heatmaps of the potential hubs as per to the global top 5% of the CC DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00468. CC measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.

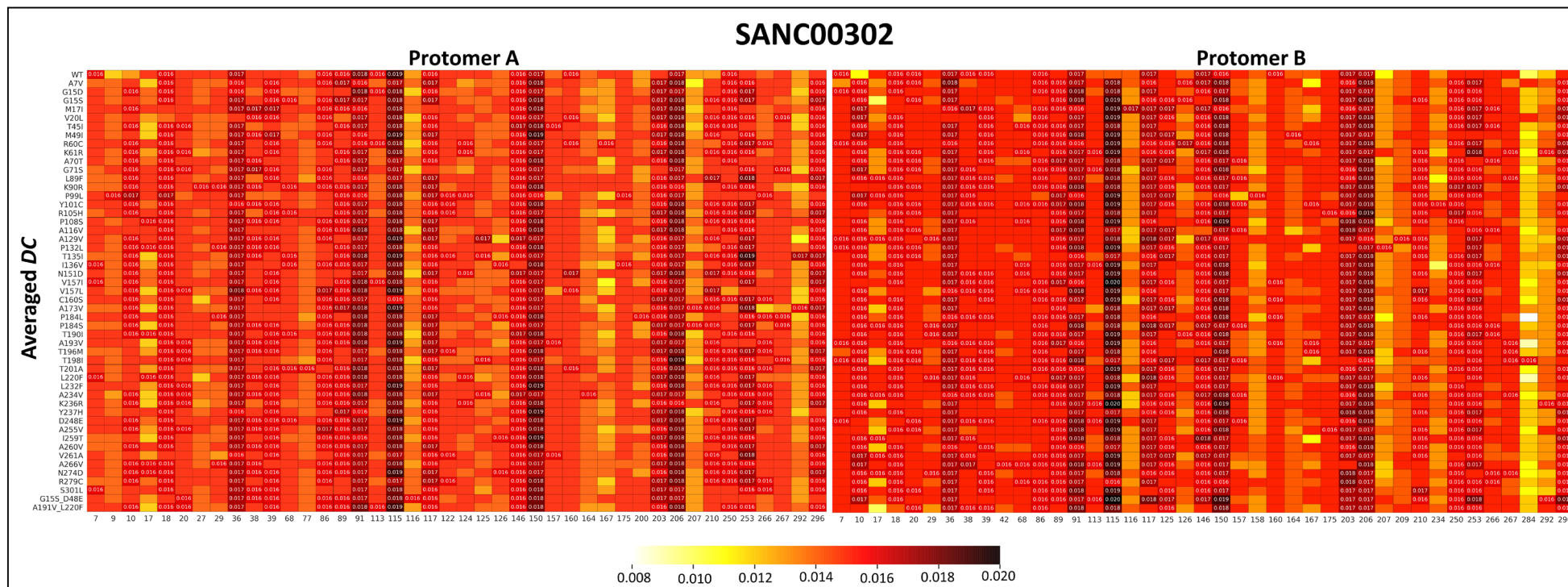


**Figure S3E.** Heatmaps of the potential hubs as per to the global top 5% of the *CC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00469. *CC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.

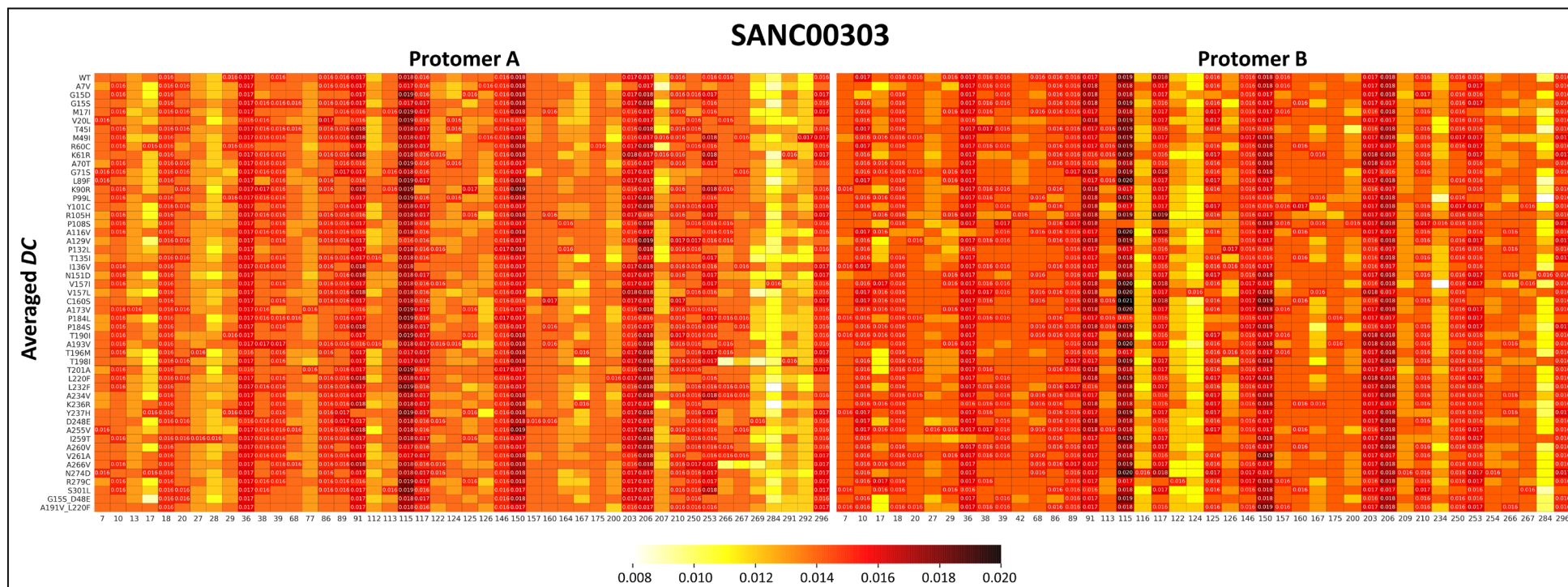




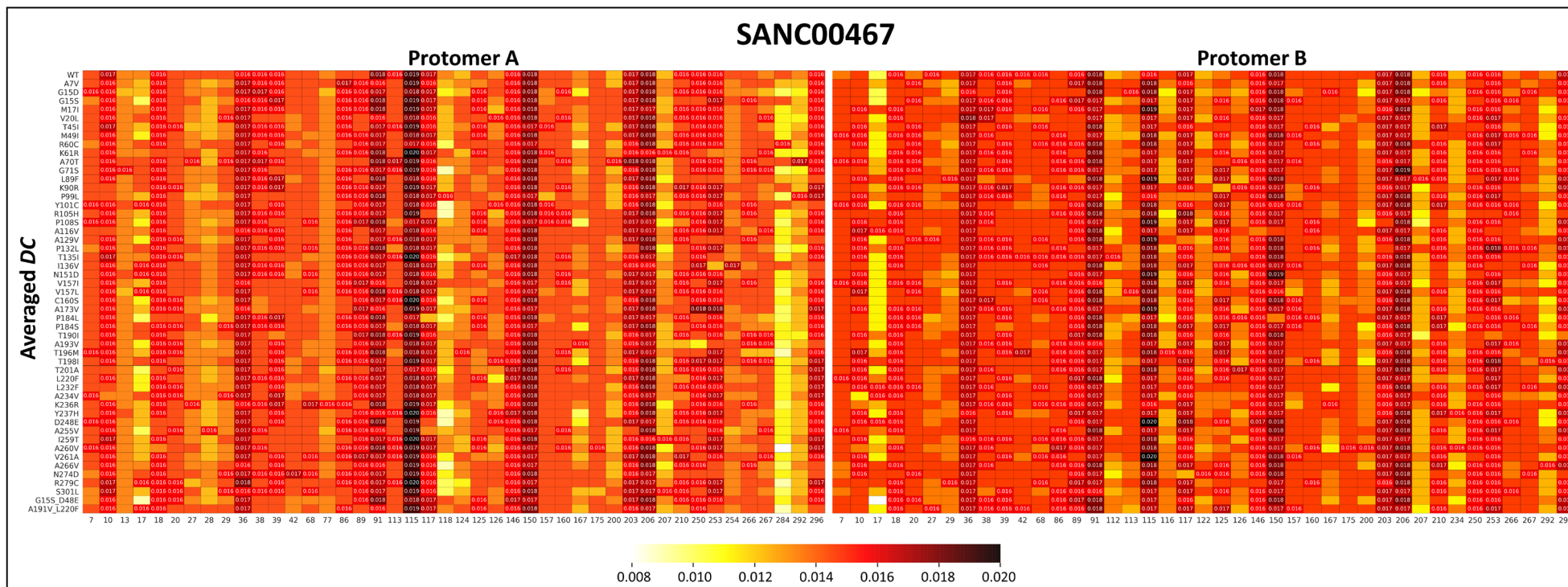
**Figure S3F.** Heatmaps of the potential hubs as per to the global top 5% of the *CC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00630. *CC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



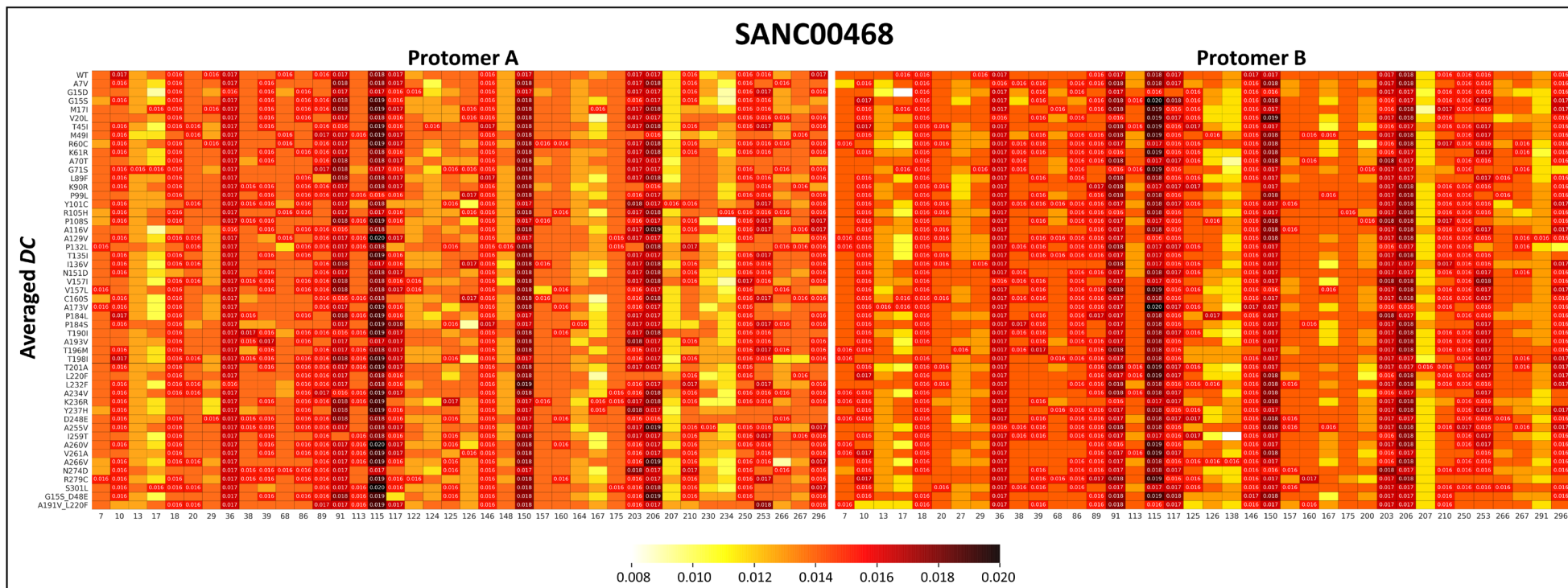
**Figure S4A.** Heatmaps of the potential hubs as per to the global top 5% of the *DC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00302. *DC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



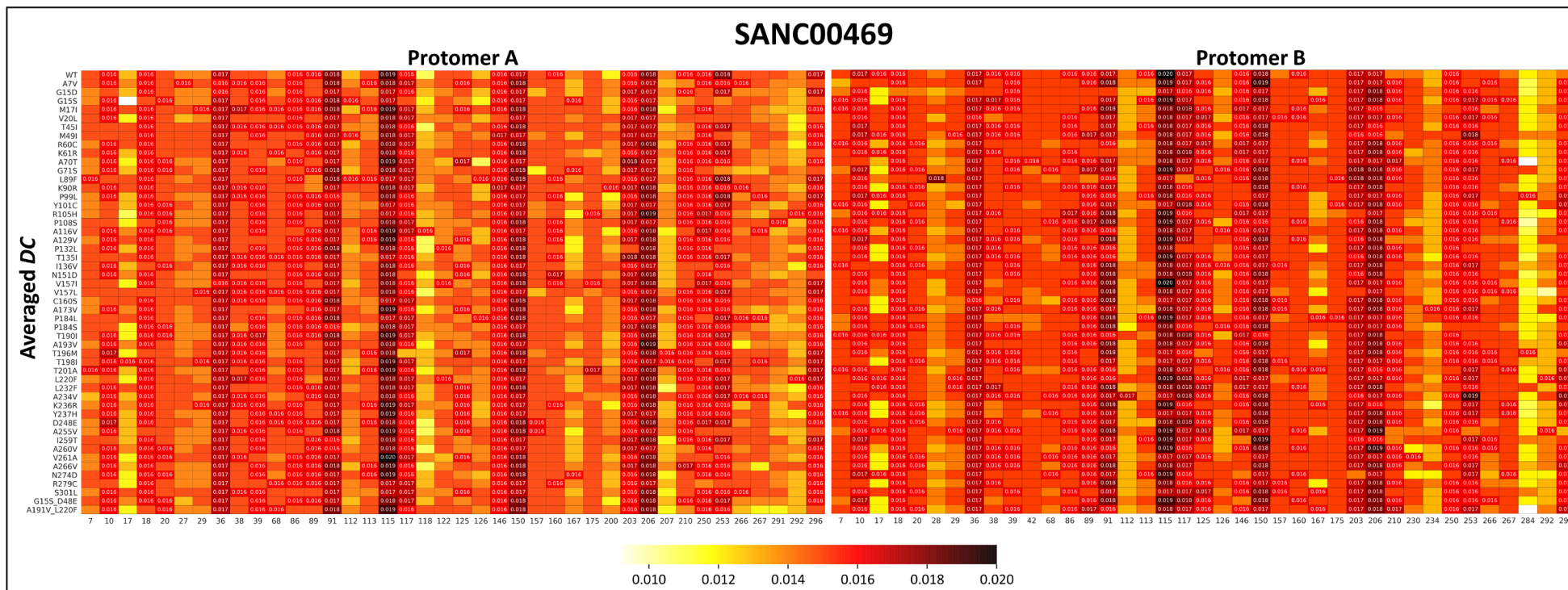
**Figure S4B.** Heatmaps of the potential hubs as per to the global top 5% of the *DC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00303. *DC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



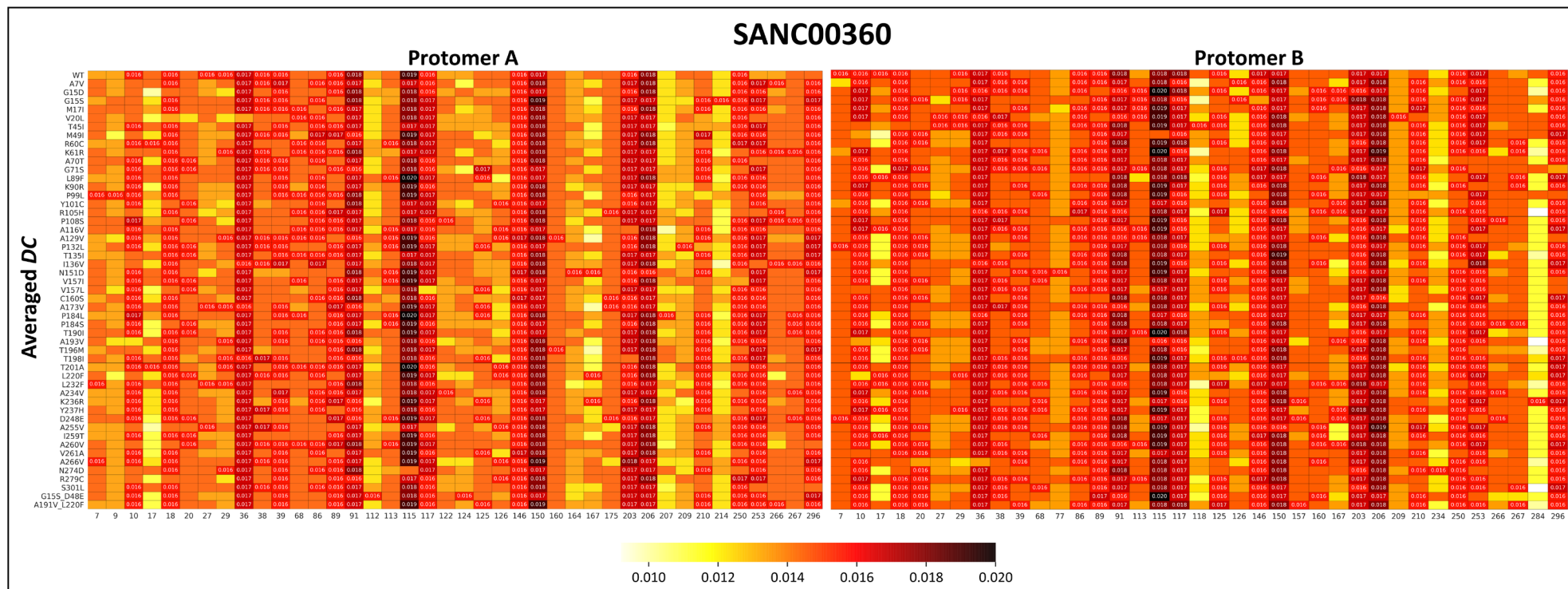
**Figure S4C.** Heatmaps of the potential hubs as per to the global top 5% of the *DC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00467. *DC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



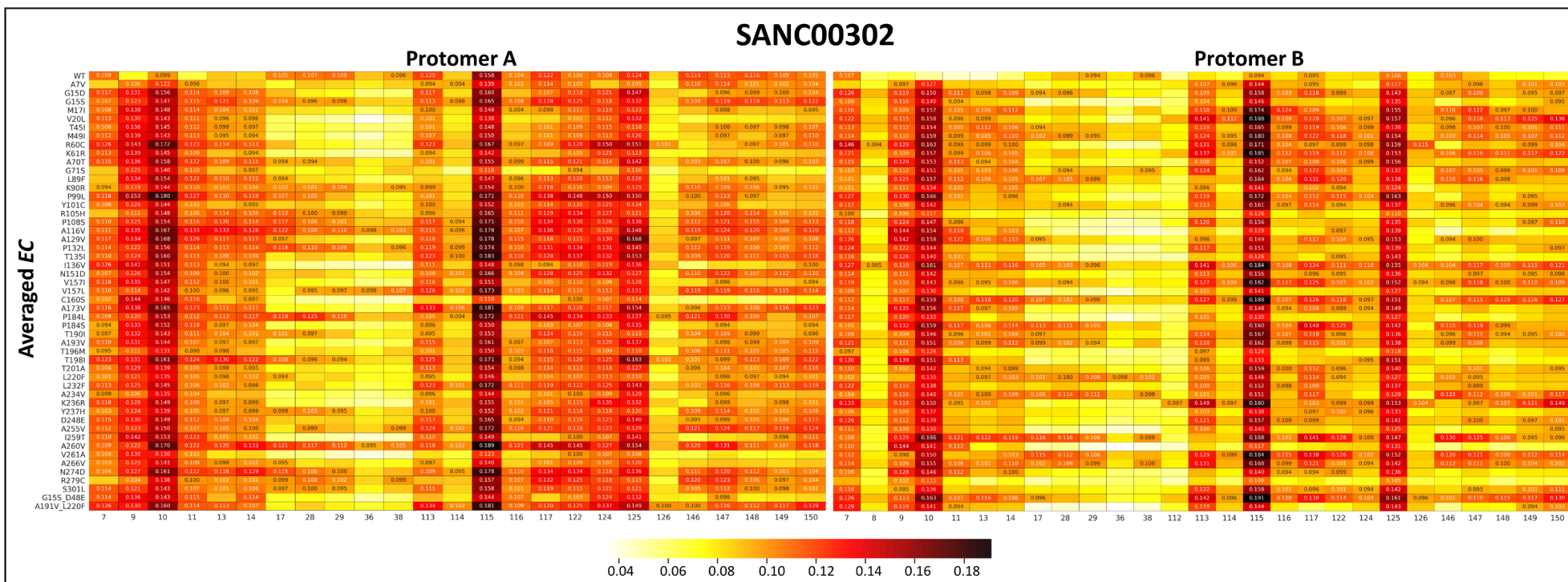
**Figure S4D.** Heatmaps of the potential hubs as per to the global top 5% of the *DC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00468. *DC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



**Figure S4E.** Heatmaps of the potential hubs as per to the global top 5% of the *DC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00469. *DC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.

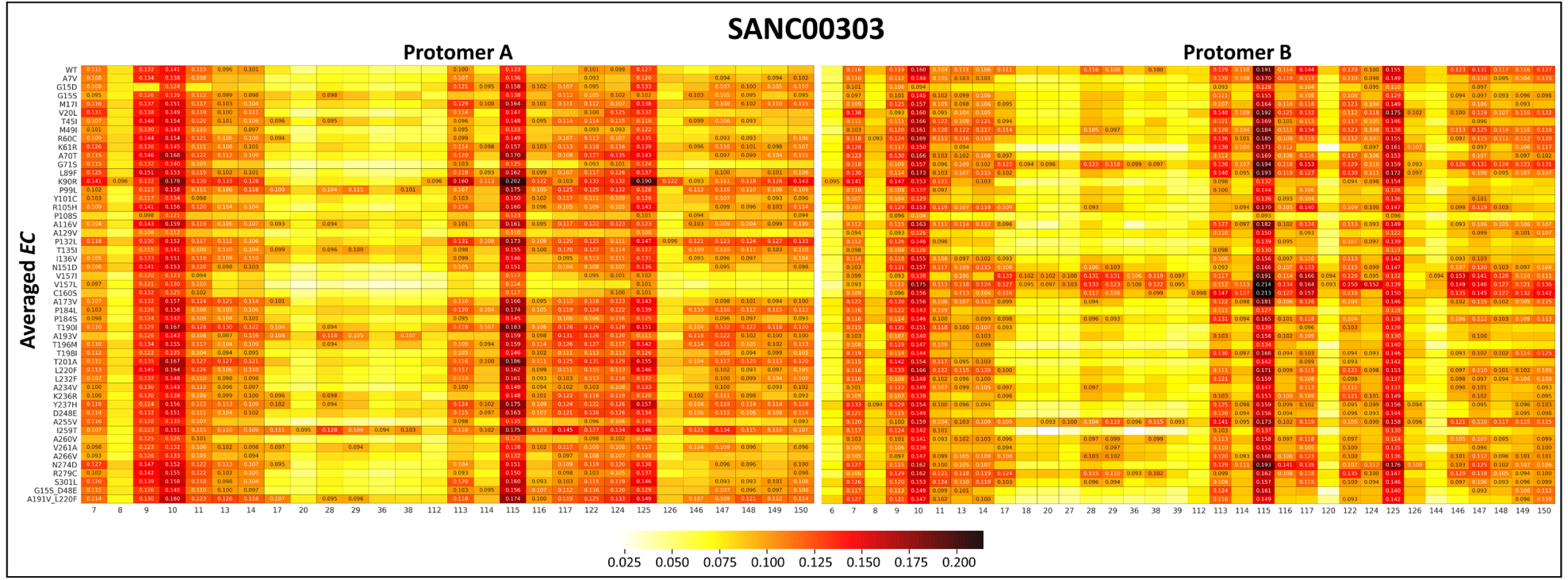


**Figure S4F.** Heatmaps of the potential hubs as per to the global top 5% of the *DC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00630. *DC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.

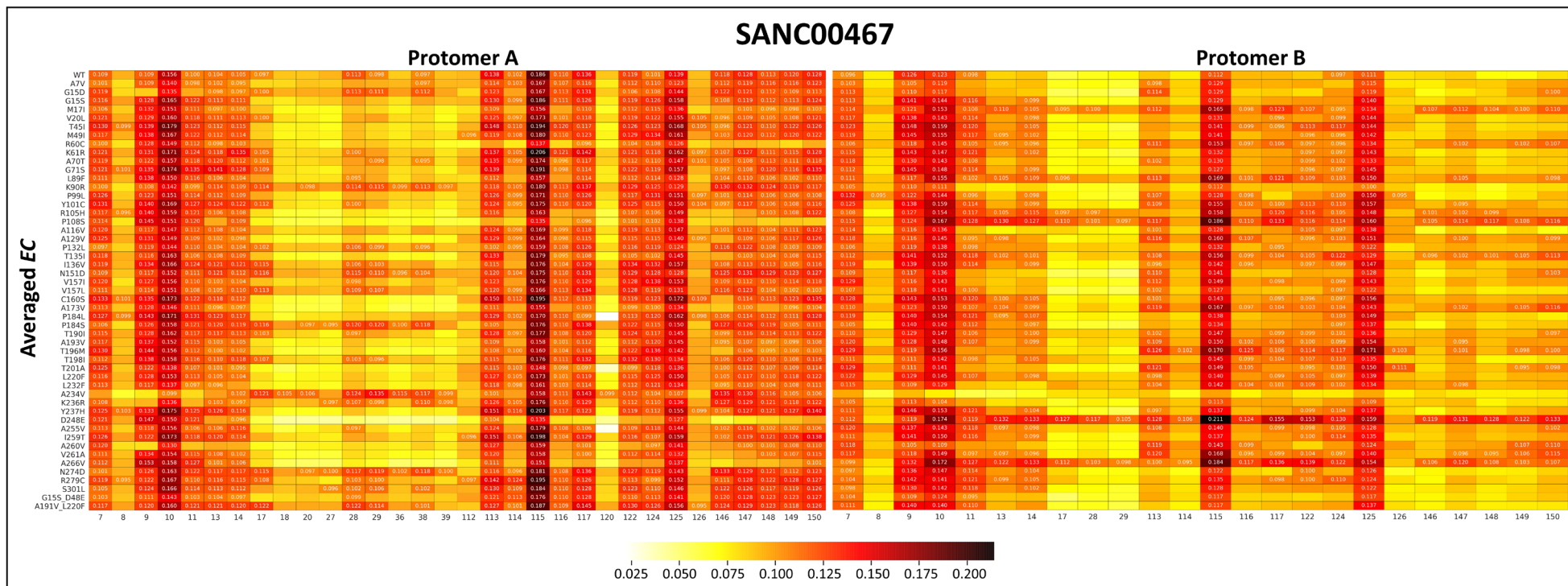


**Figure S5A.** Heatmaps of the potential hubs as per to the global top 5% of the *EC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00302. *EC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.

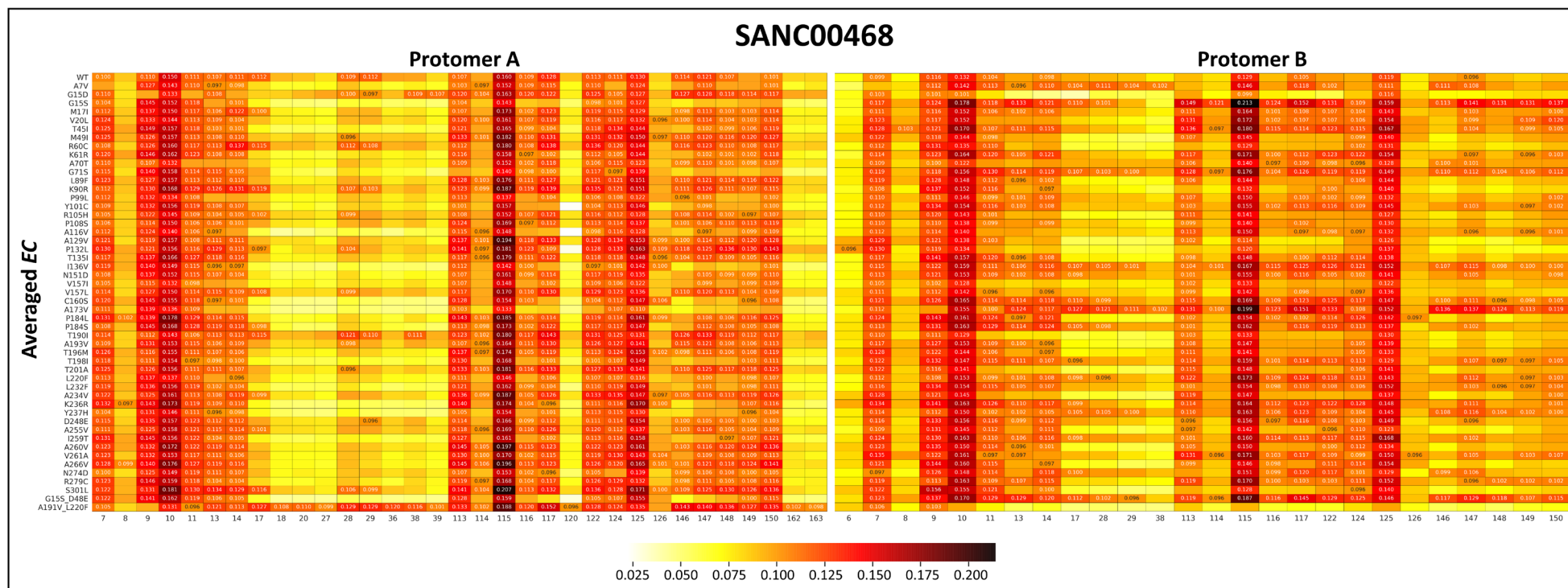




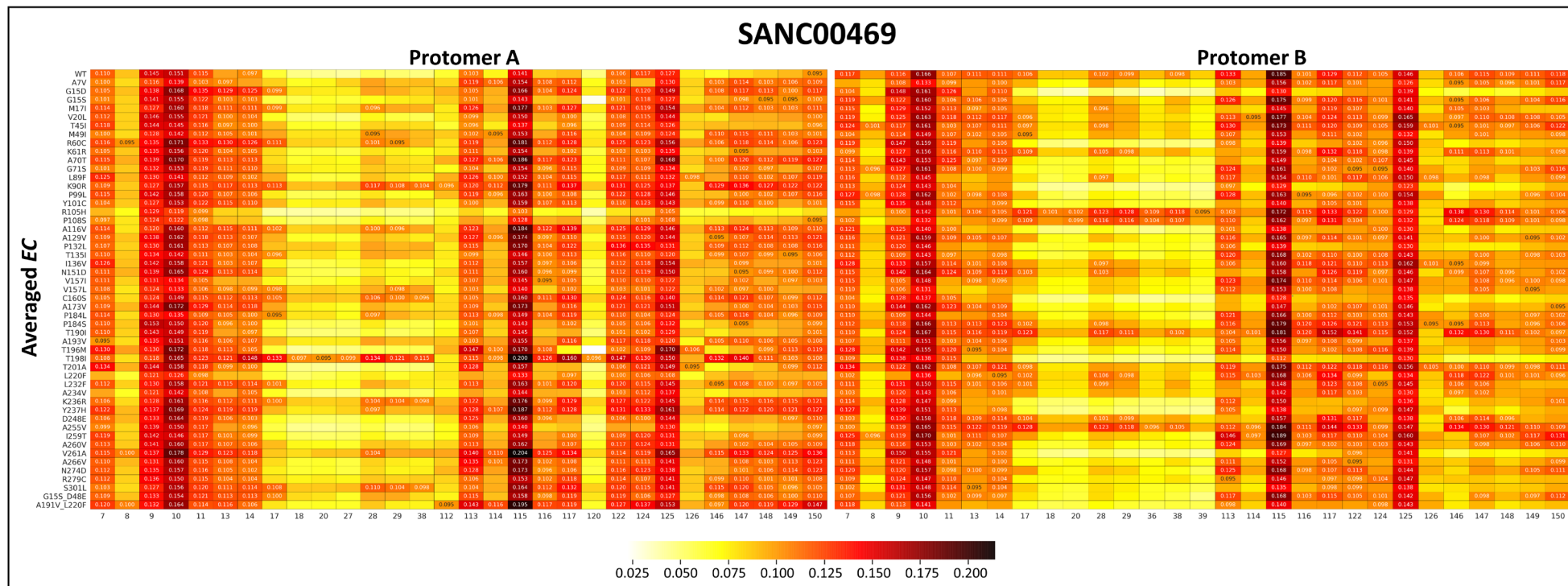
**Figure S5B.** Heatmaps of the potential hubs as per to the global top 5% of the *EC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00303. *EC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



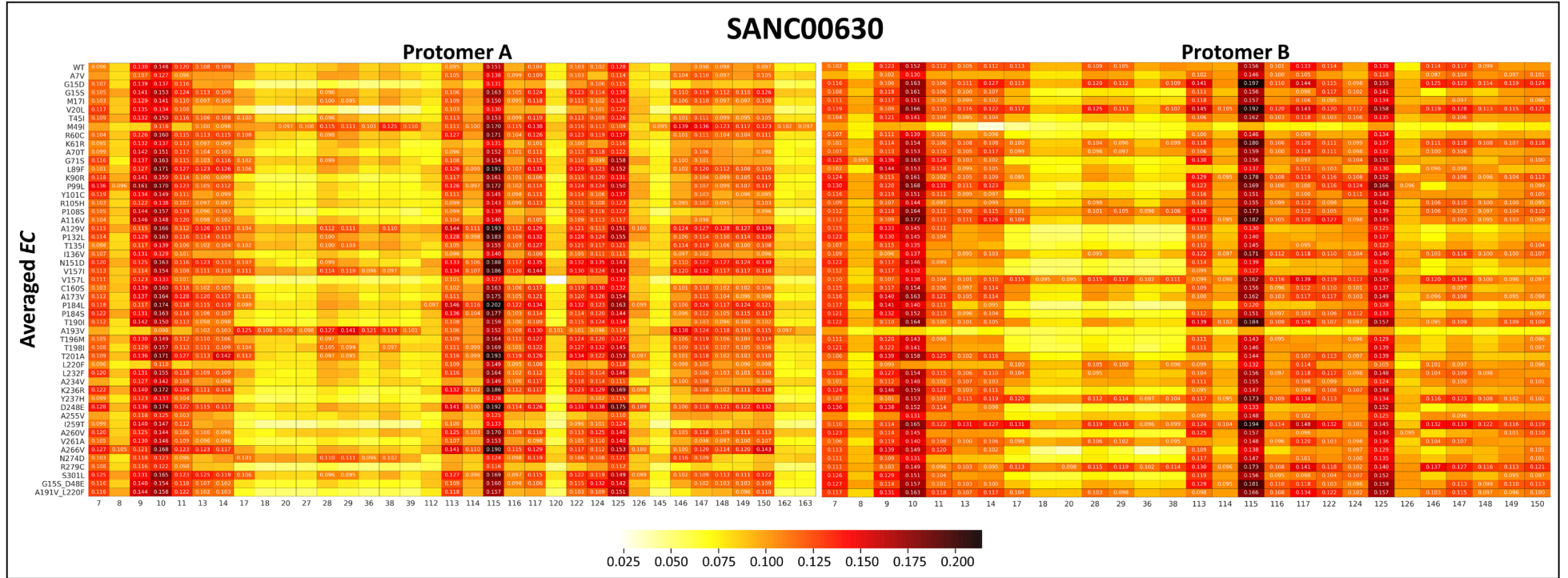
**Figure S5C.** Heatmaps of the potential hubs as per to the global top 5% of the *EC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00467. *EC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



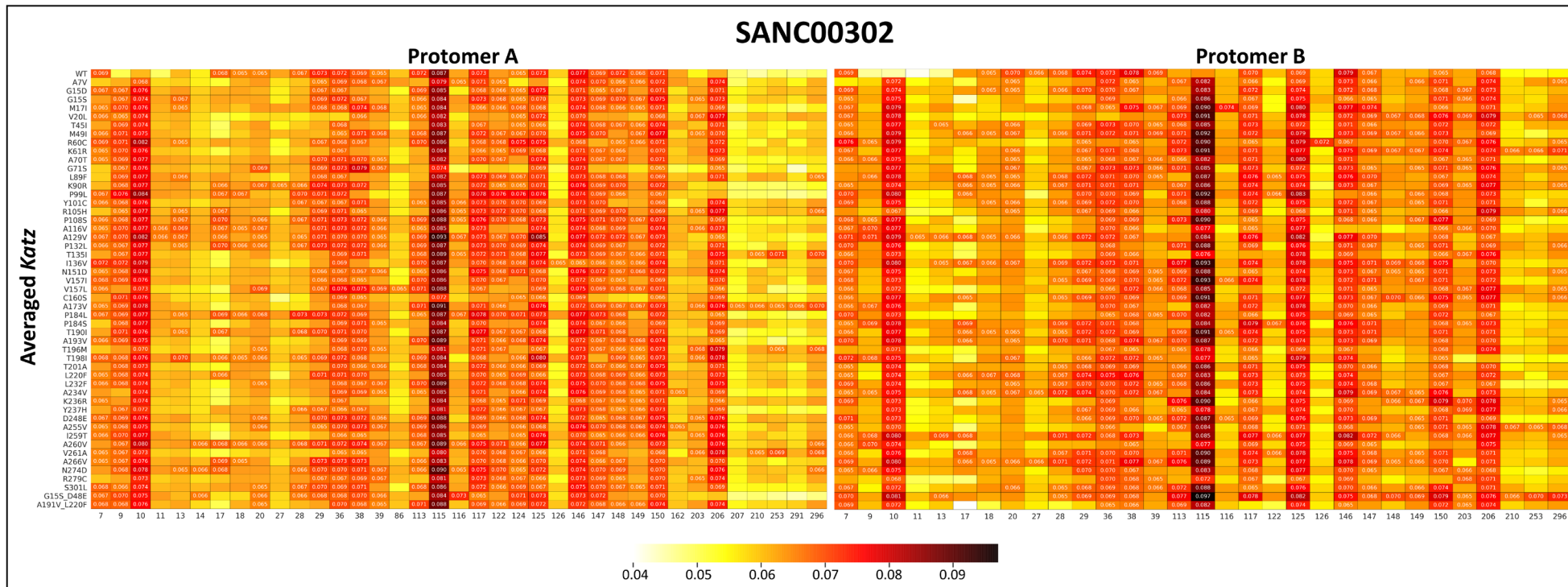
**Figure S5D.** Heatmaps of the potential hubs as per to the global top 5% of the *EC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANCO0468. *EC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



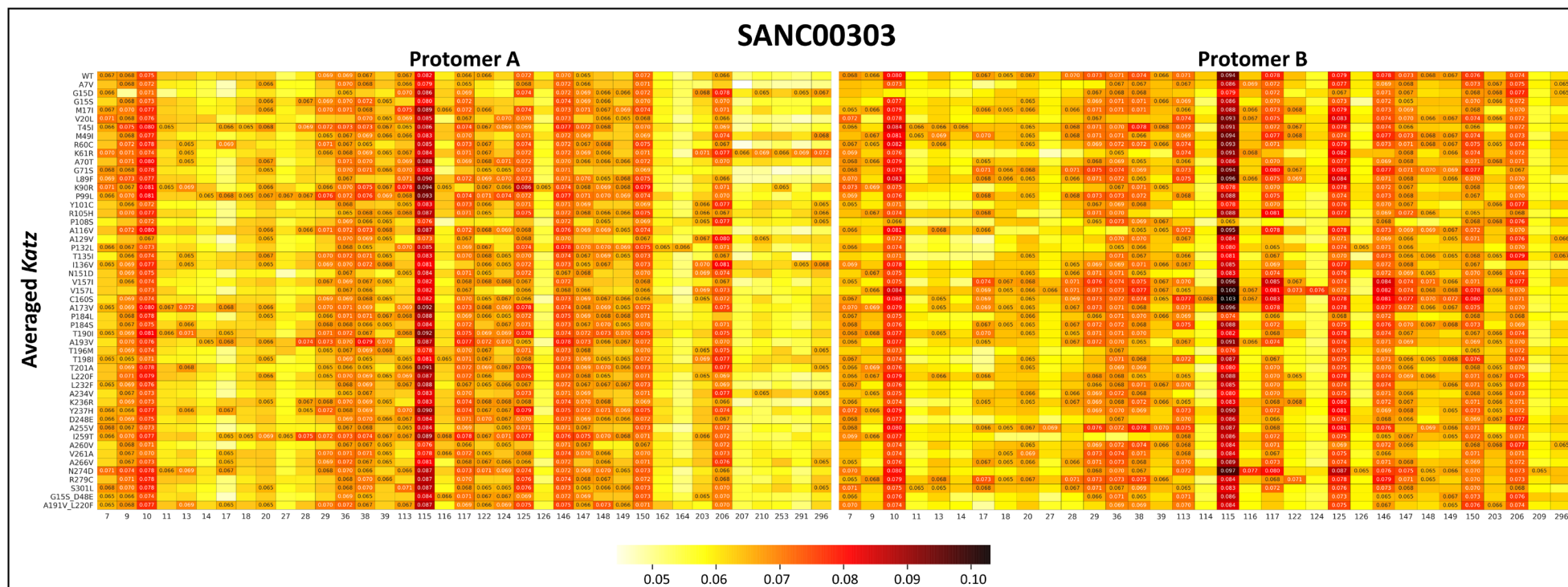
**Figure S5E.** Heatmaps of the potential hubs as per to the global top 5% of the *EC* DRN metric for the reference (WT) and mutant samples in complex with allosteric modulator: SANC00469. *EC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



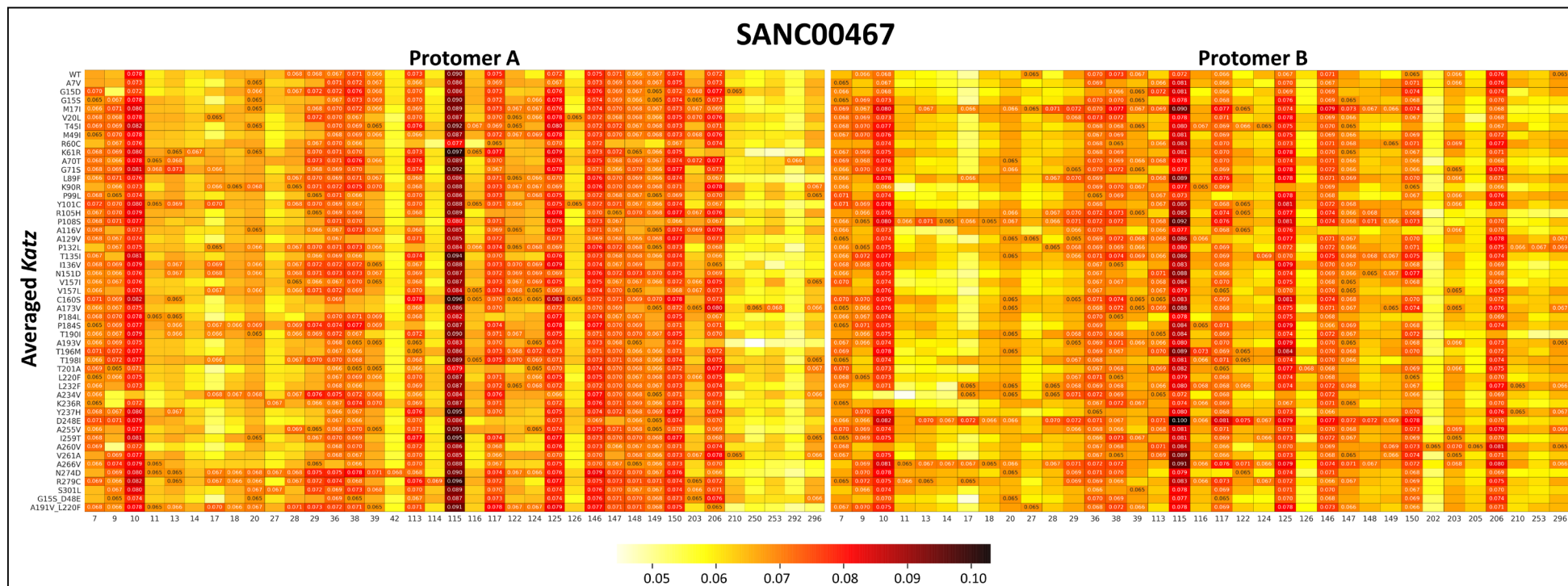
**Figure S5F.** Heatmaps of the potential hubs as per to the global top 5% of the *EC* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00630. *EC* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



**Figure S6A.** Heatmaps of the potential hubs as per to the global top 5% of the *Katz* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANCO0302. *Katz* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.

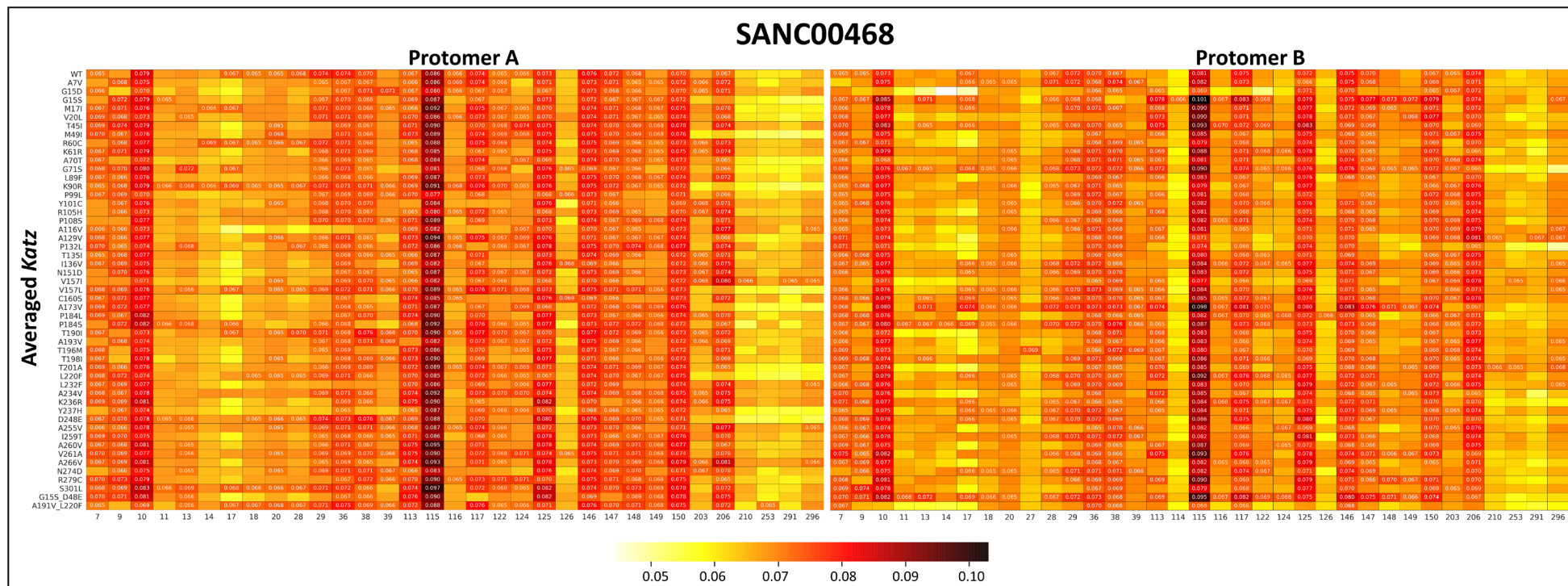


**Figure S6B.** Heatmaps of the potential hubs as per to the global top 5% of the *Katz* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00303. *Katz* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.

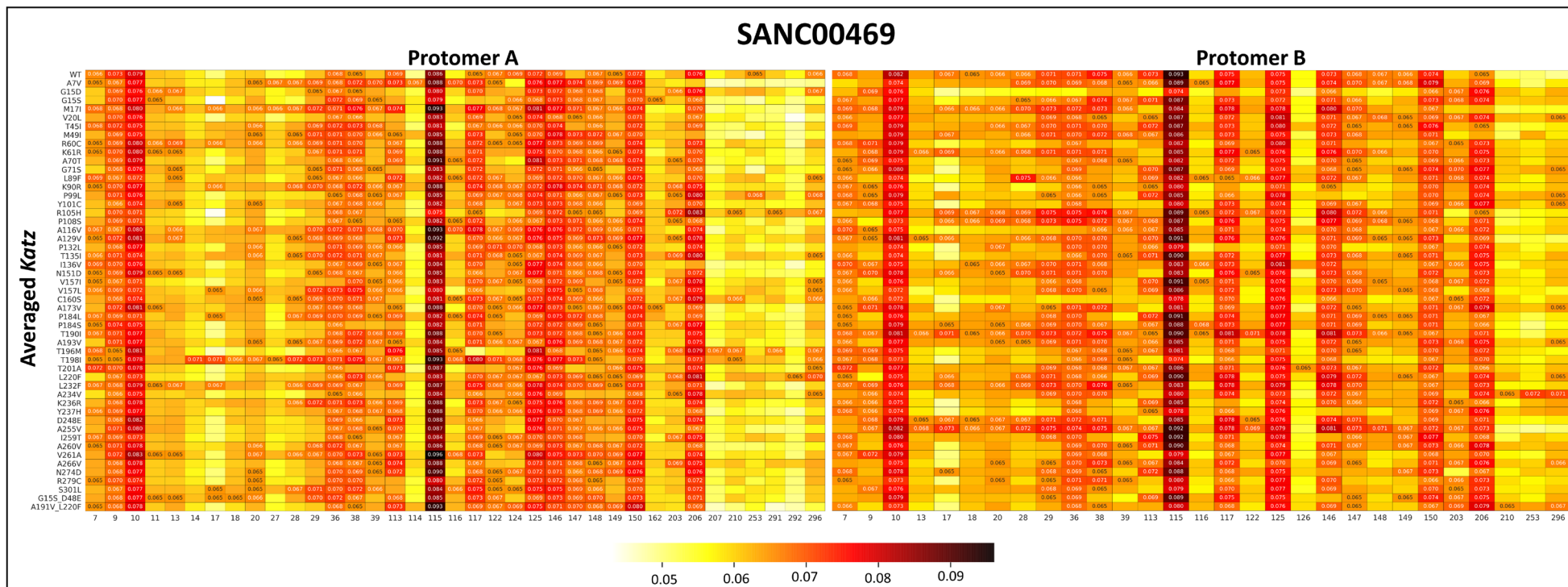


**Figure S6C.** Heatmaps of the potential hubs as per to the global top 5% of the *Katz* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00467. *Katz* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.

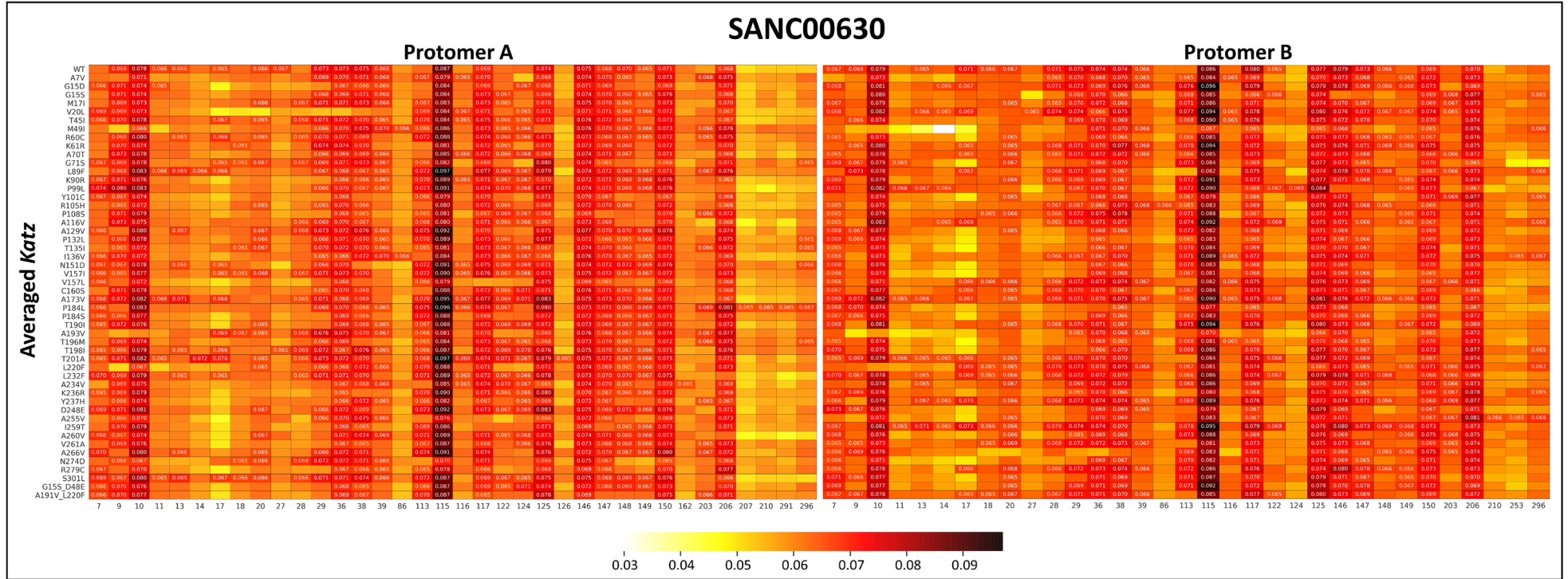




**Figure S6D.** Heatmaps of the potential hubs as per to the global top 5% of the *Katz* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00468. *Katz* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



**Figure S6E.** Heatmaps of the potential hubs as per to the global top 5% of the *Katz* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00469. *Katz* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.



**Figure S6F.** Heatmaps of the potential hubs as per to the global top 5% of the *Katz* DRN metric for the reference (WT) and mutant systems in complex with allosteric modulator: SANC00630. *Katz* measurements of ligand-bound protomer (chain A) are shown on the left while those of the unbound protomer B are on the right. Residue hubs are annotated with their centrality values, while their homologous residues in alternate samples are not. The colour scale from white, through yellow, orange and red to black indicates the degree of residue centrality.