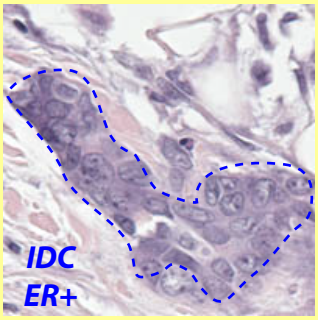
Pathological evaluation as part of clinical care

Formalin fixation of whole sample

Tissue block preparation, sectioning, histological stain

Pathological evaluation and diagnosis of carcinoma



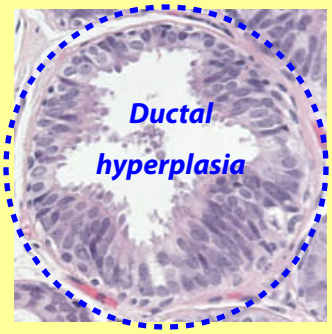
Deidentification of all samples of a case and classification as research specimens

Clinically informed evaluation of research specimens

Identification of neoplasias and DCIS associated with IDC

Histological characterization of tissue cores

Preparation of suitable samples for maximum tumor/neoplasia content



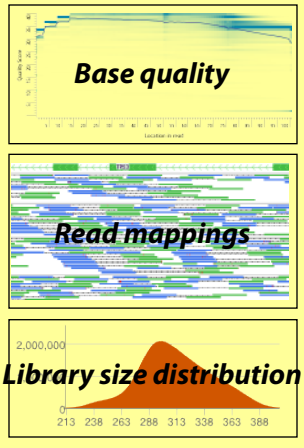
Transfer of core blocks from histology to molecular biology lab

Molecular biology and sequencing

Library construction

Test sequencing and read mapping to assess library quality and complexity

Full-scale sequencing of suitable libraries



Transfer of data to high performance computing environment

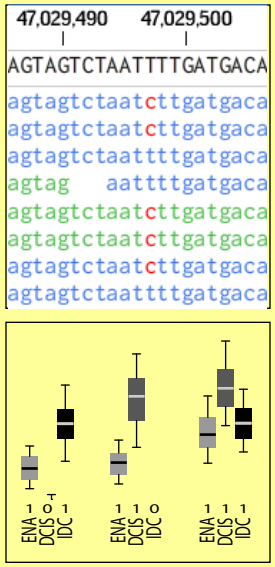
Computational sequence analysis

GATK multisample variant calling on realigned, recalibrated BAM files

Alternate allele frequency determination

Classification of SNVs into germline and candidate somatic variants

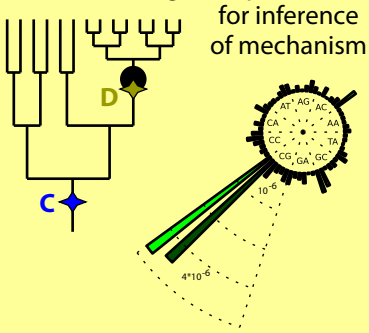
- Subclassification and refinement of candidate somatic variant calls based on read counts, presence-absence patterns in the samples, and alternate allele frequencies
- Identification of heterozygous germline variants for aneuploidy



Somatic SNVs

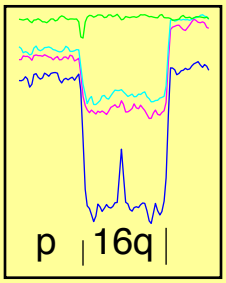
Lineage markers for tree building

Mutation spectrum for inference of mechanism



Germline SNVs

Aneuploidy detection using heterozygous SNPs

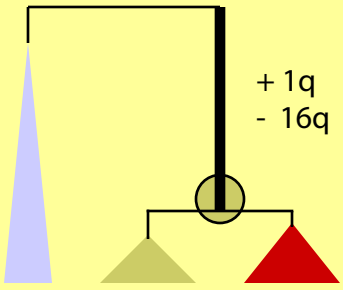


Determination of each case's evolutionary history

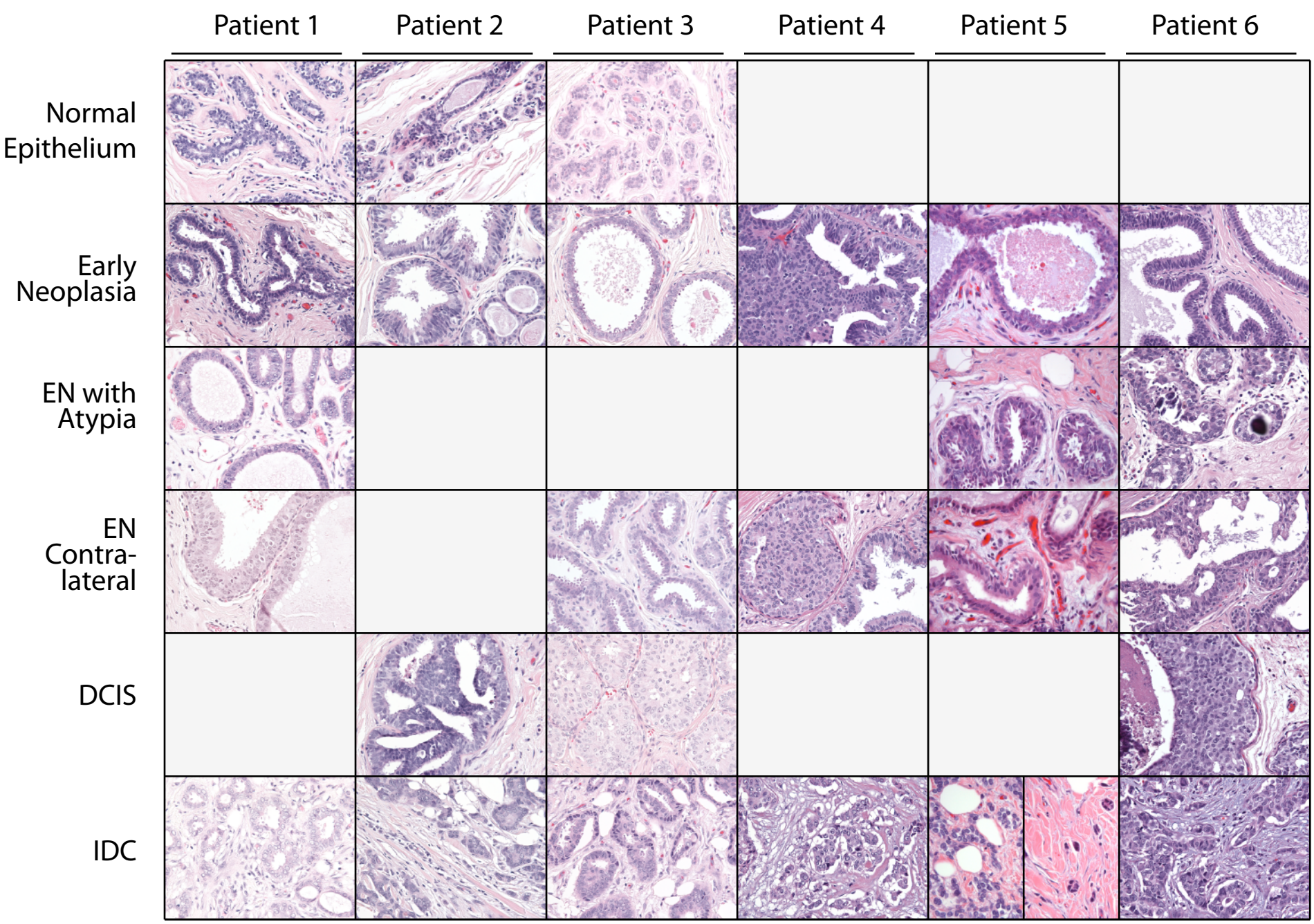
Quantification of branch lengths in terms of mutations that occurred during that evolutionary time

Mapping of aneuploidies onto the trees

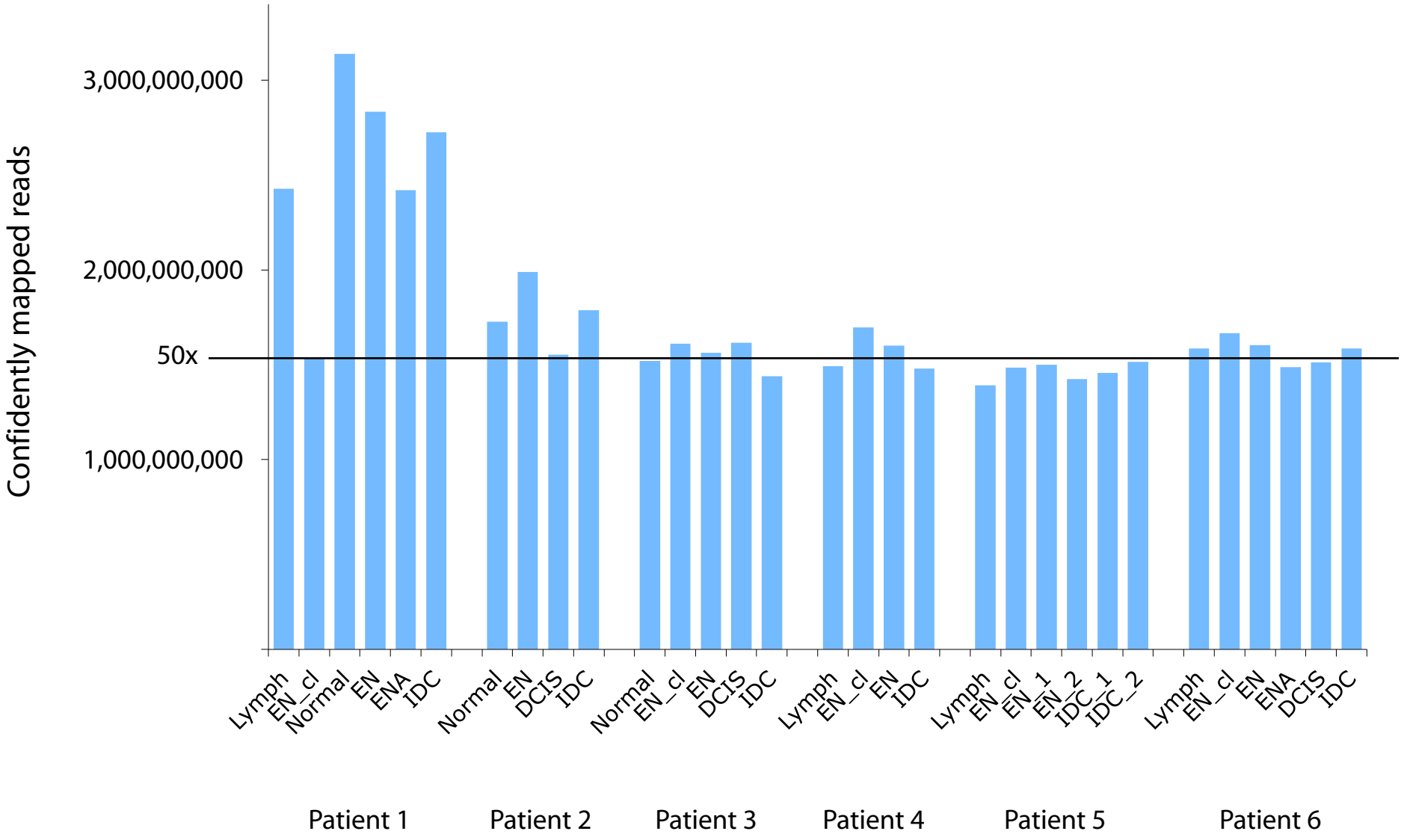
Inference of timing of genomic changes, and of genomic state of the last common ancestor well before the carcinoma



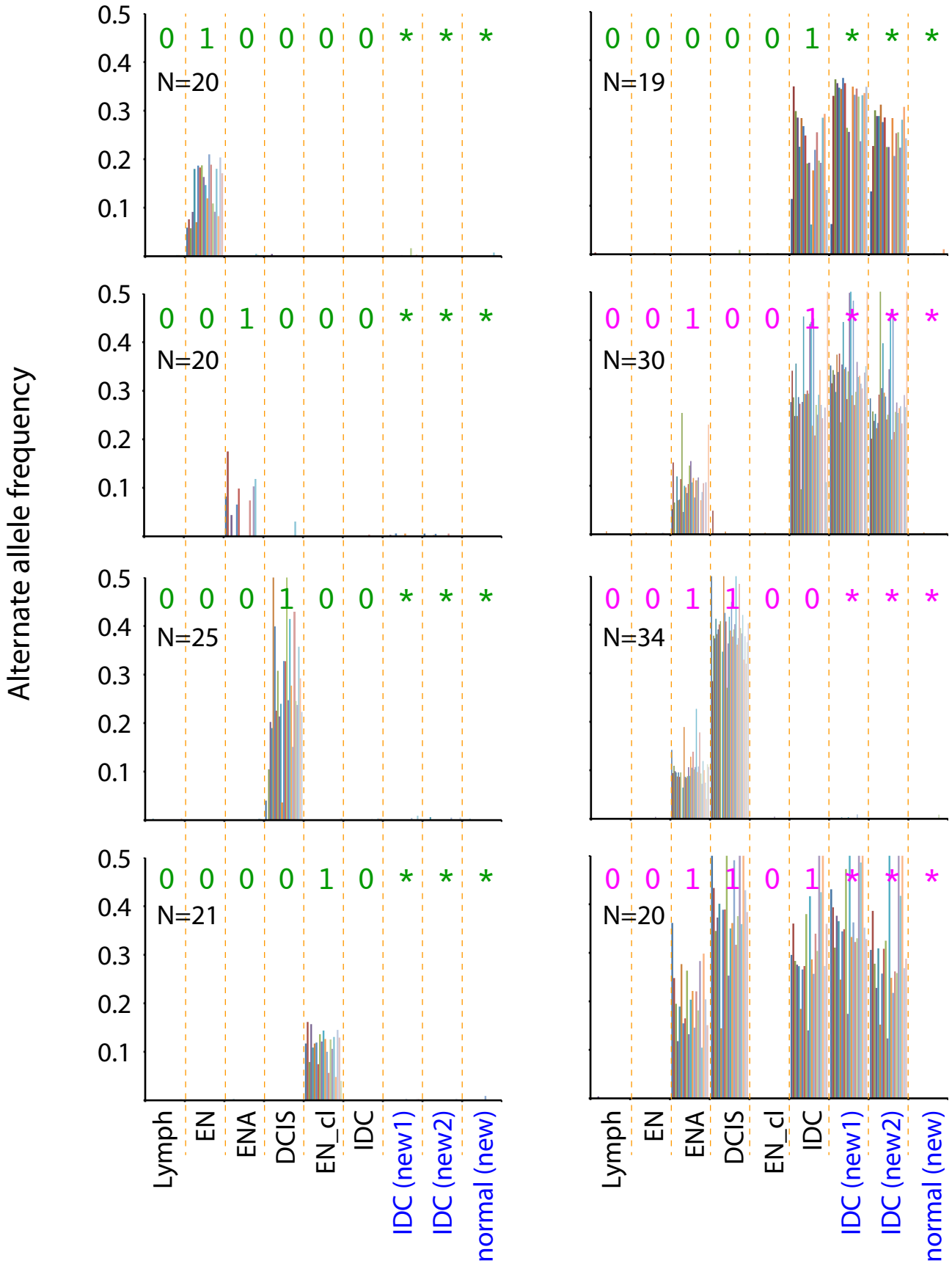
Supplemental Fig. S1



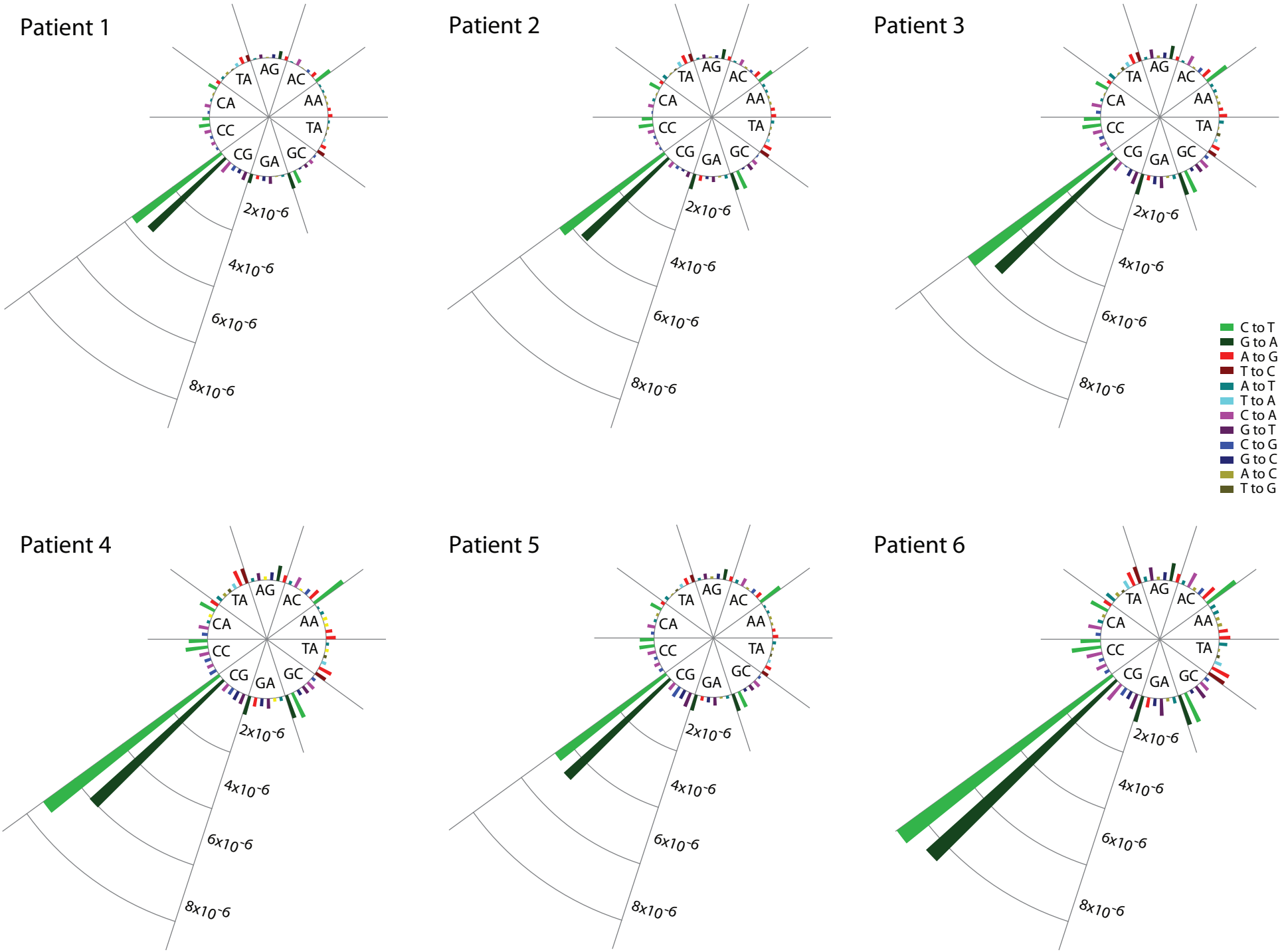
Supplemental Fig. S2



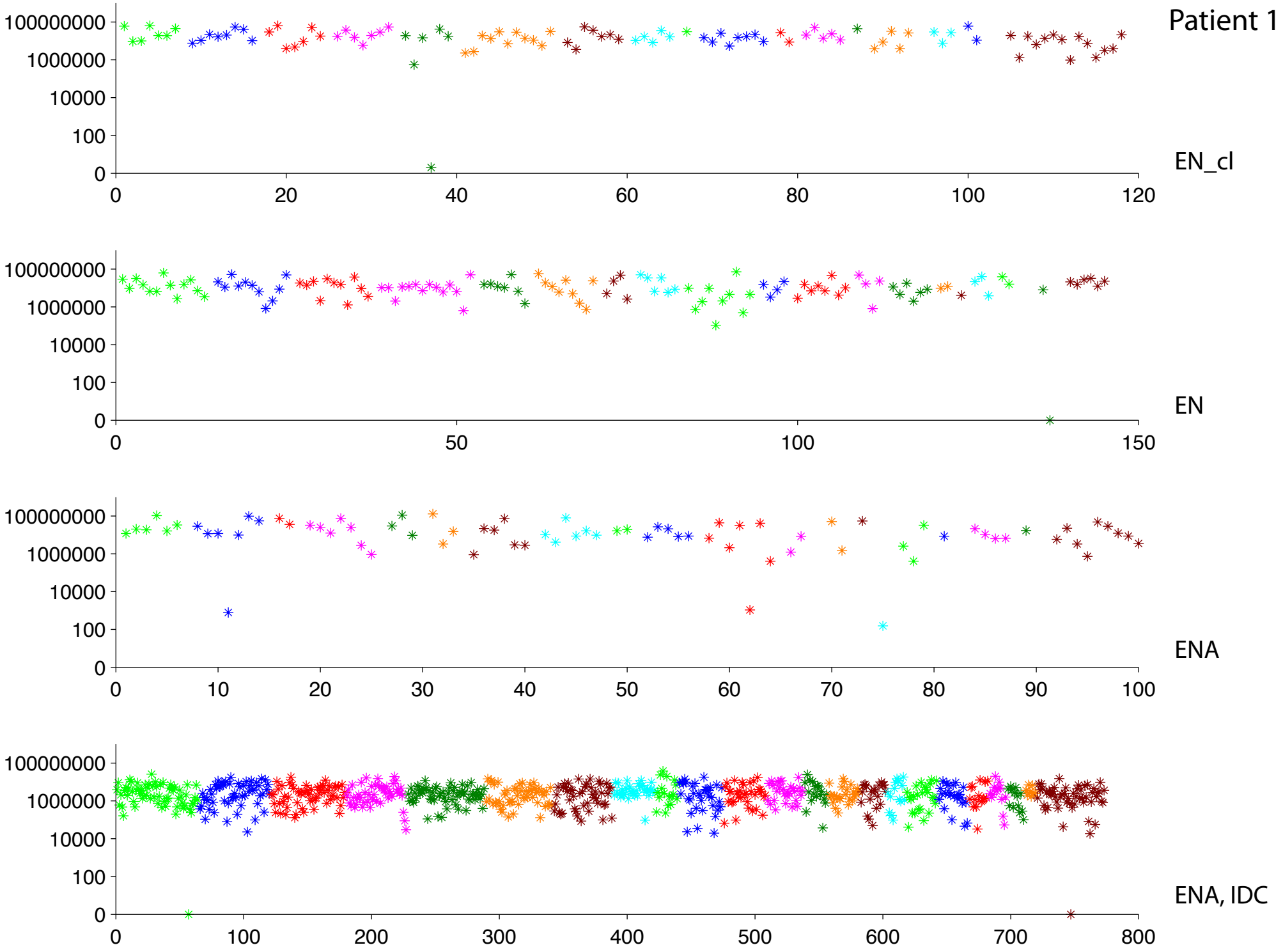
Supplemental Fig. S3



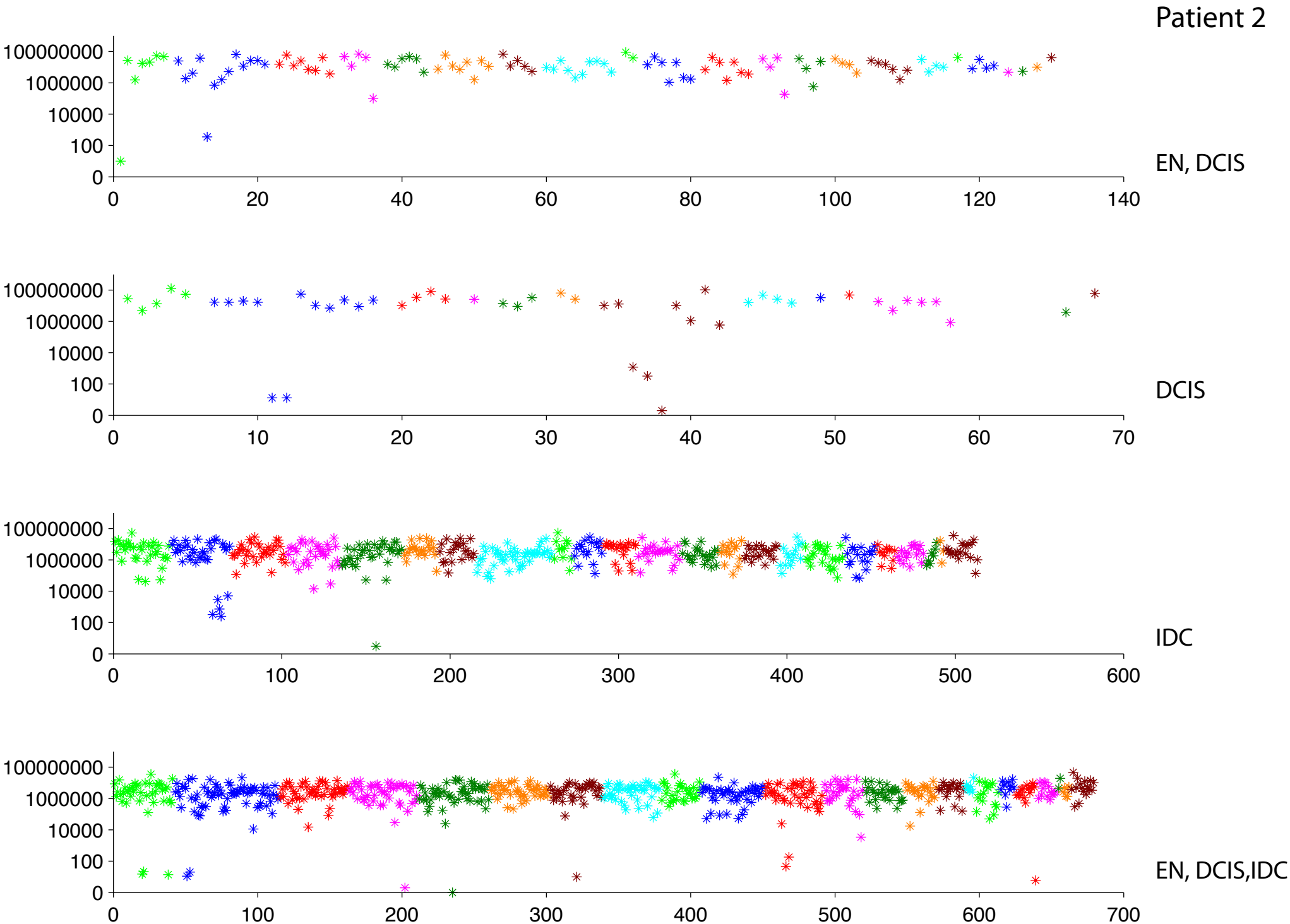
Supplemental Fig. S4



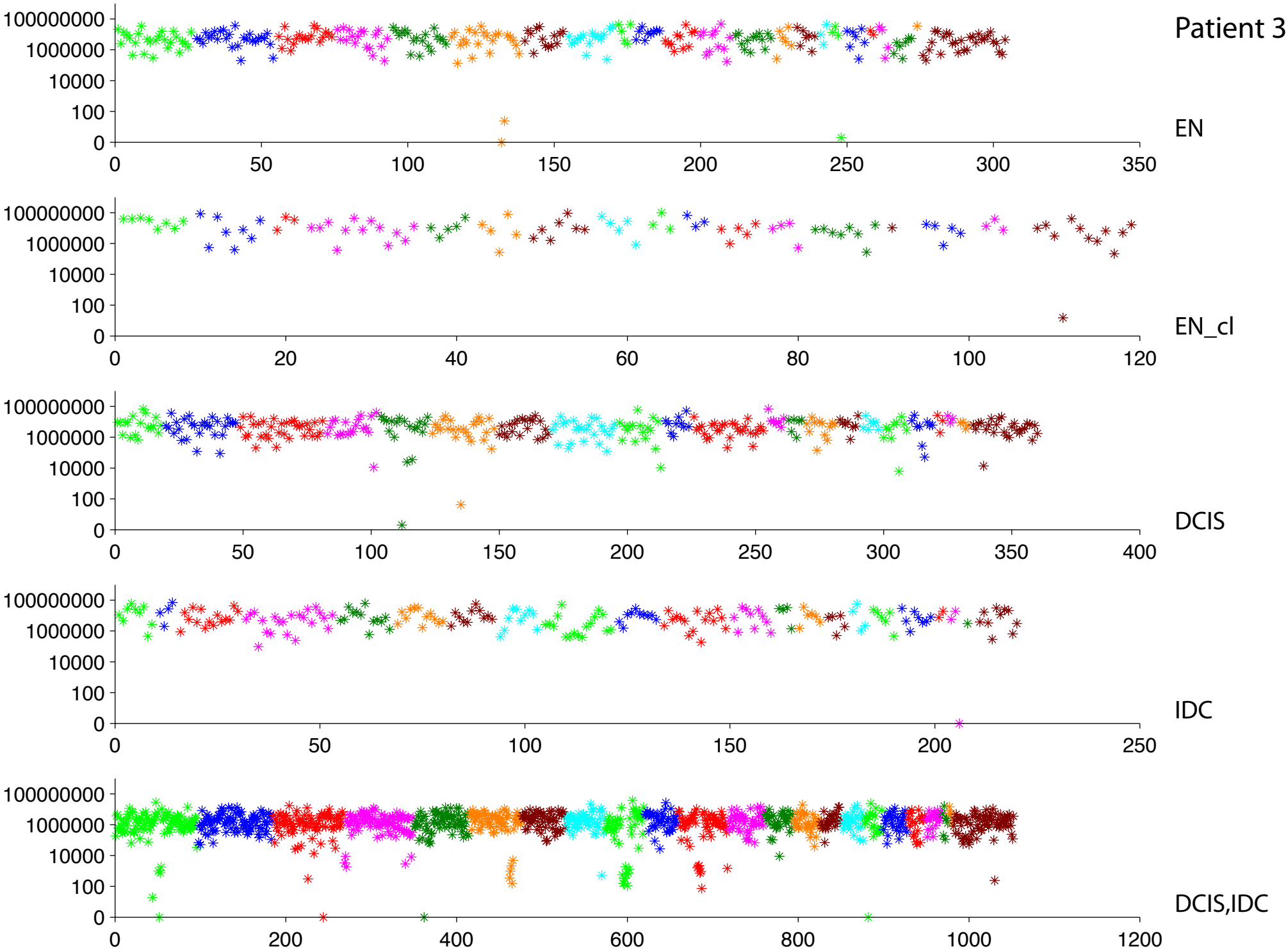
Supplemental Fig. S5



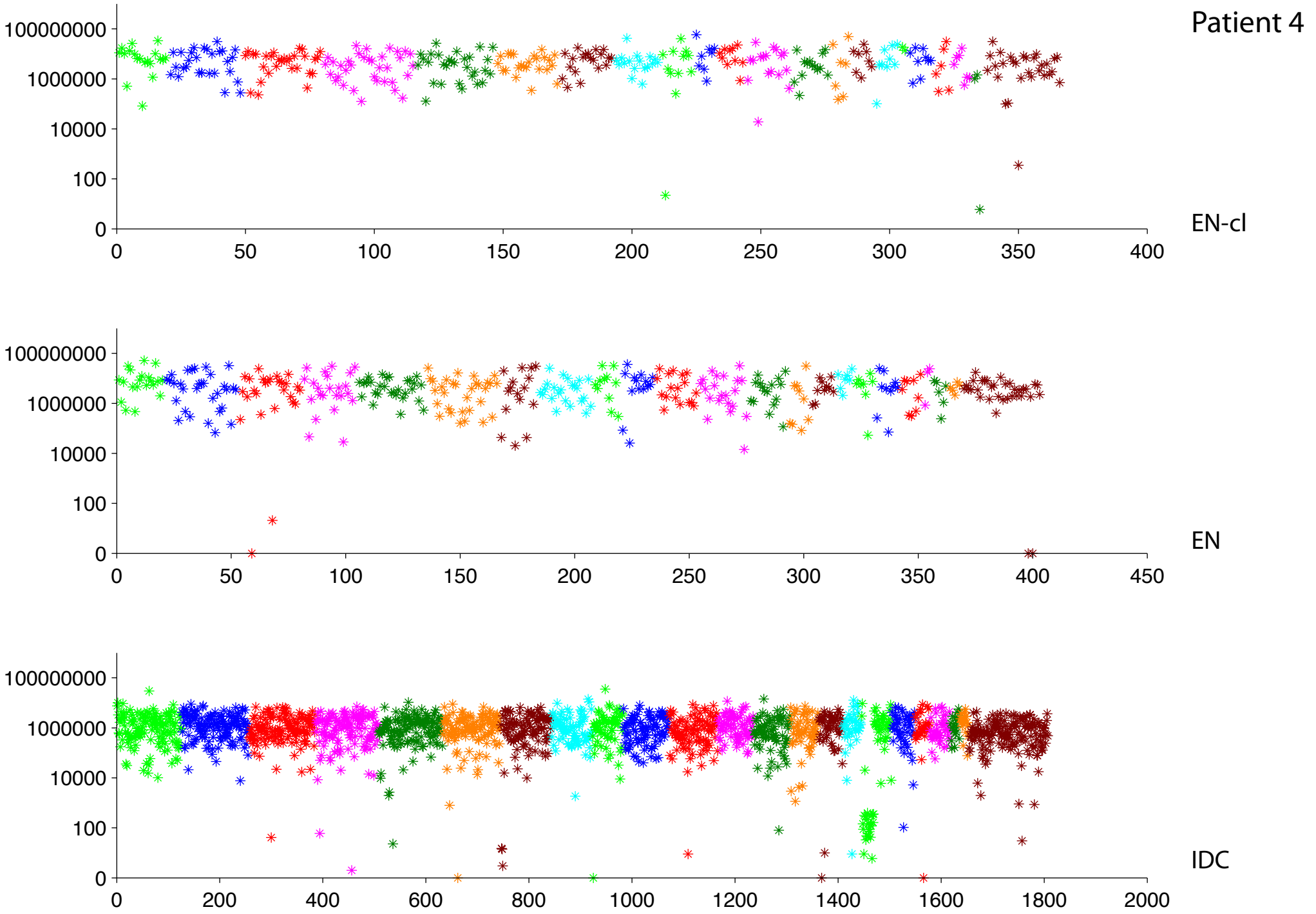
Supplemental Fig. S6



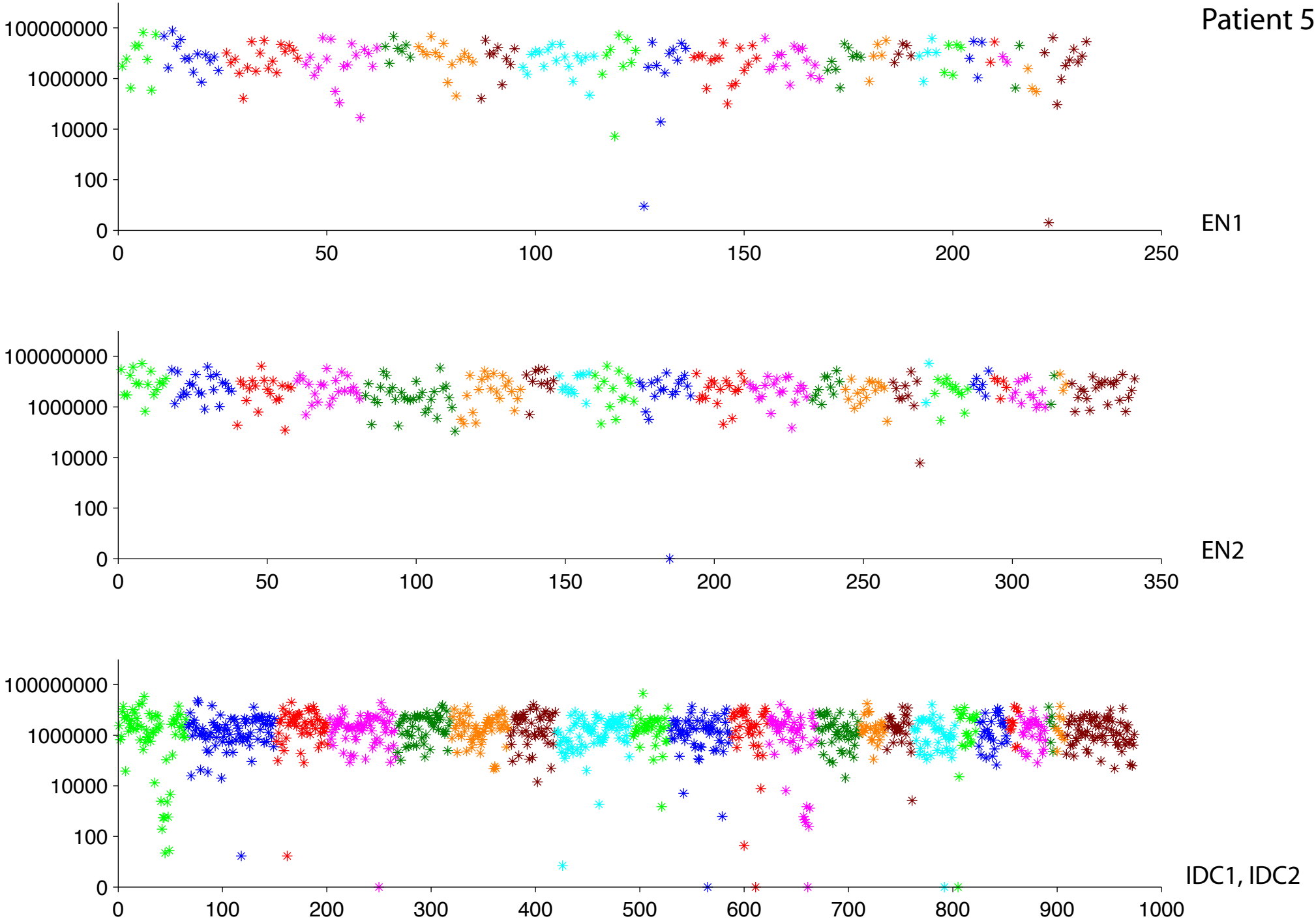
Supplemental Fig. S7



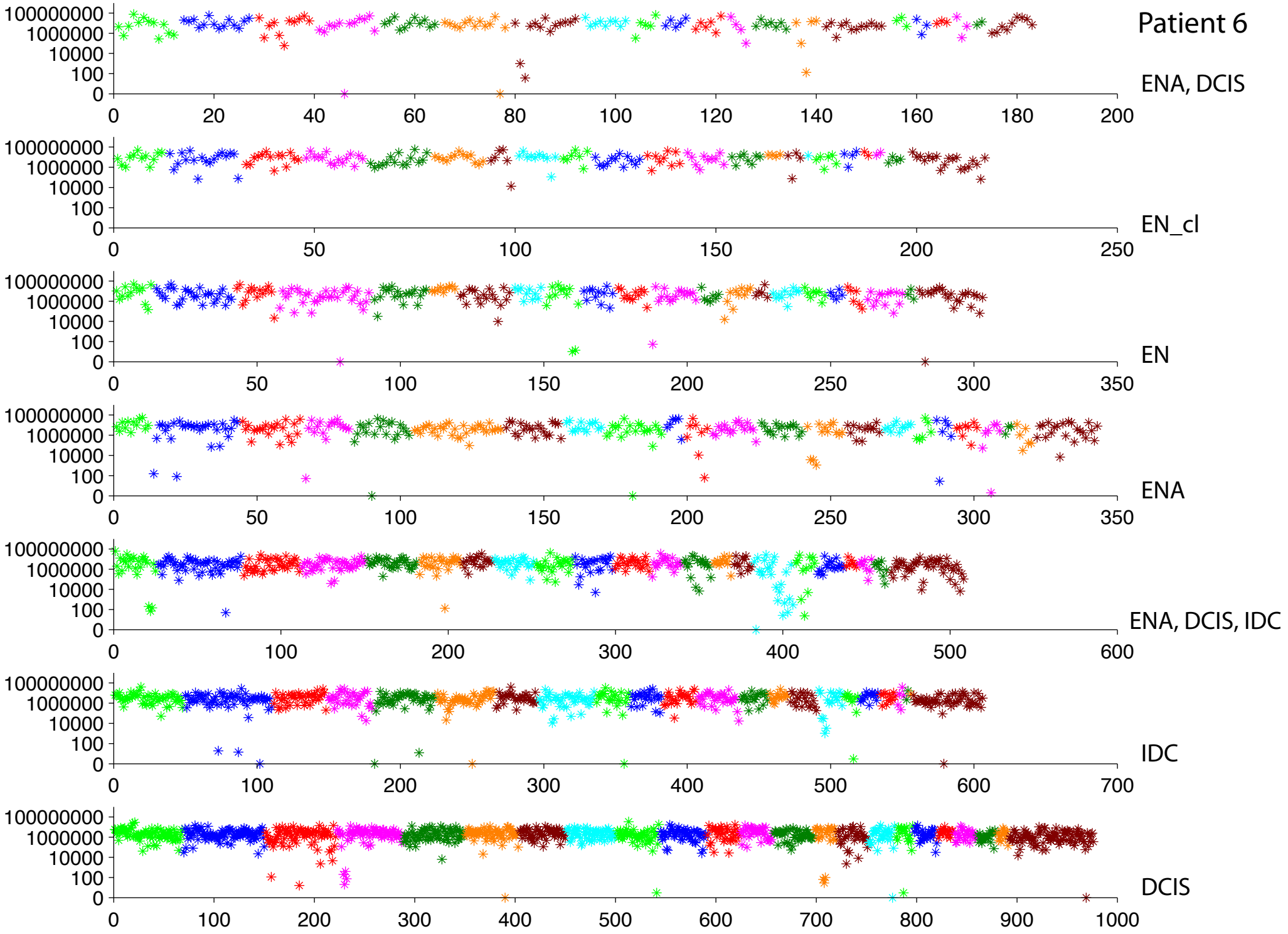
Supplemental Fig. S8



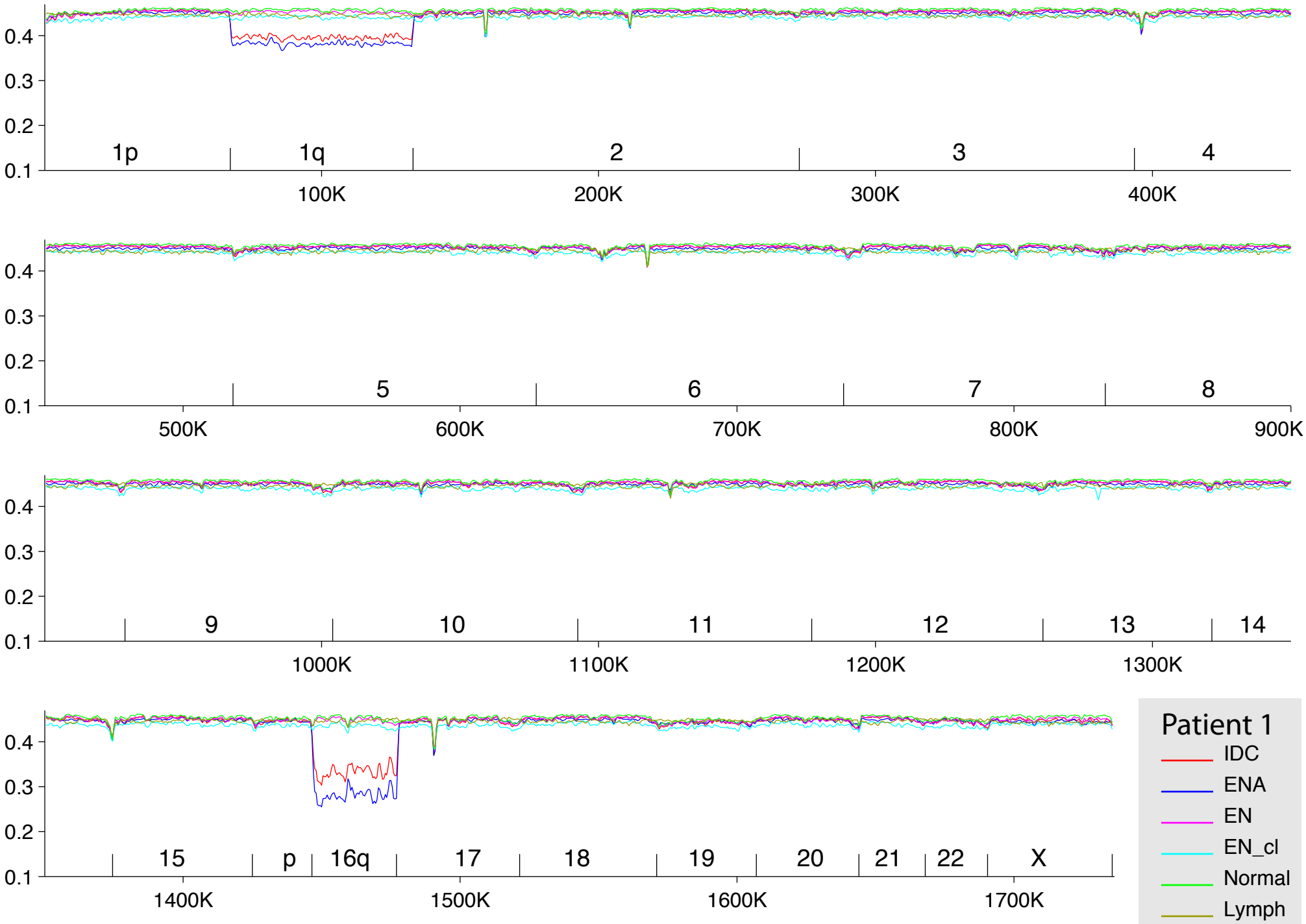
Supplemental Fig. S9



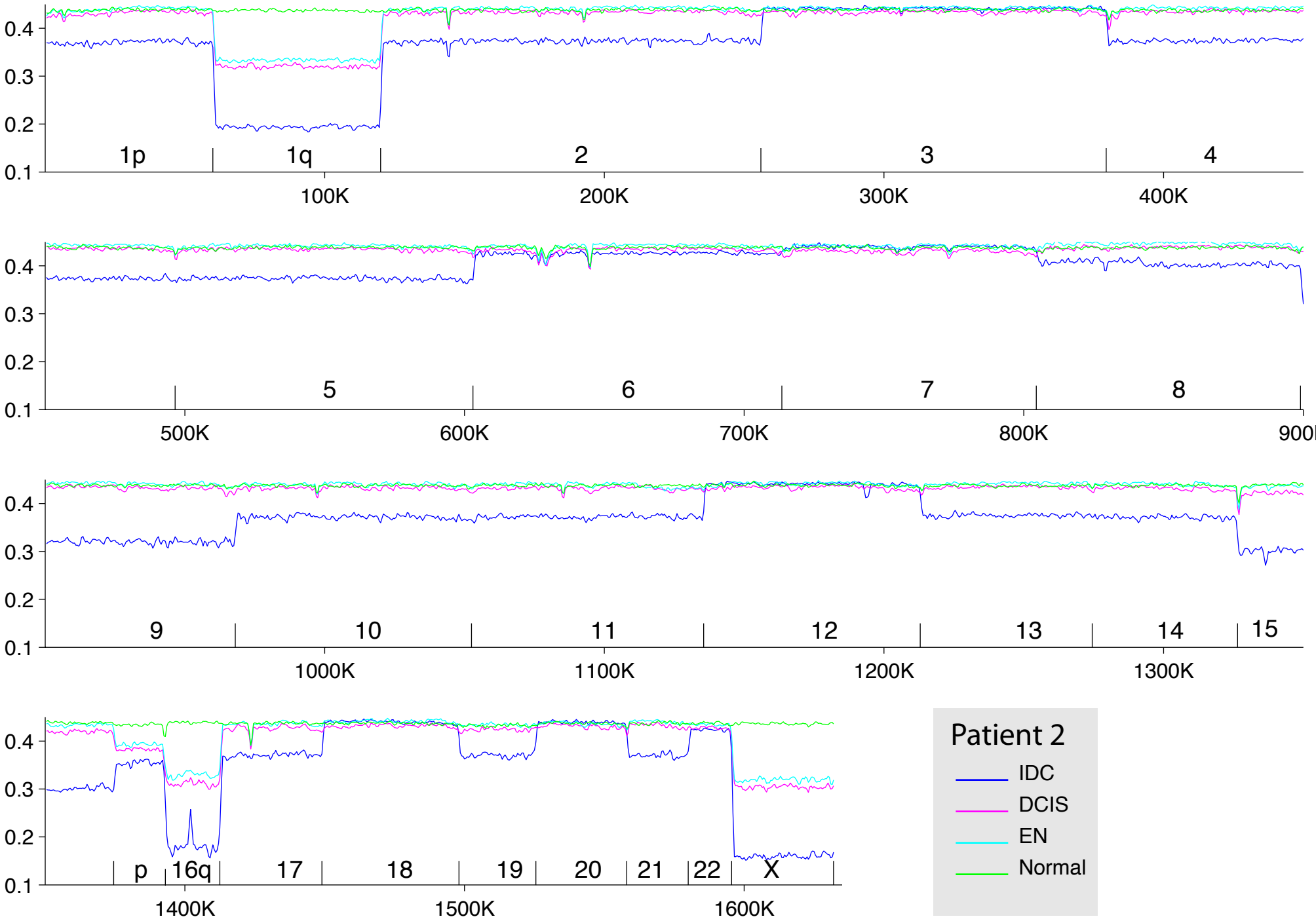
Supplemental Fig. S10



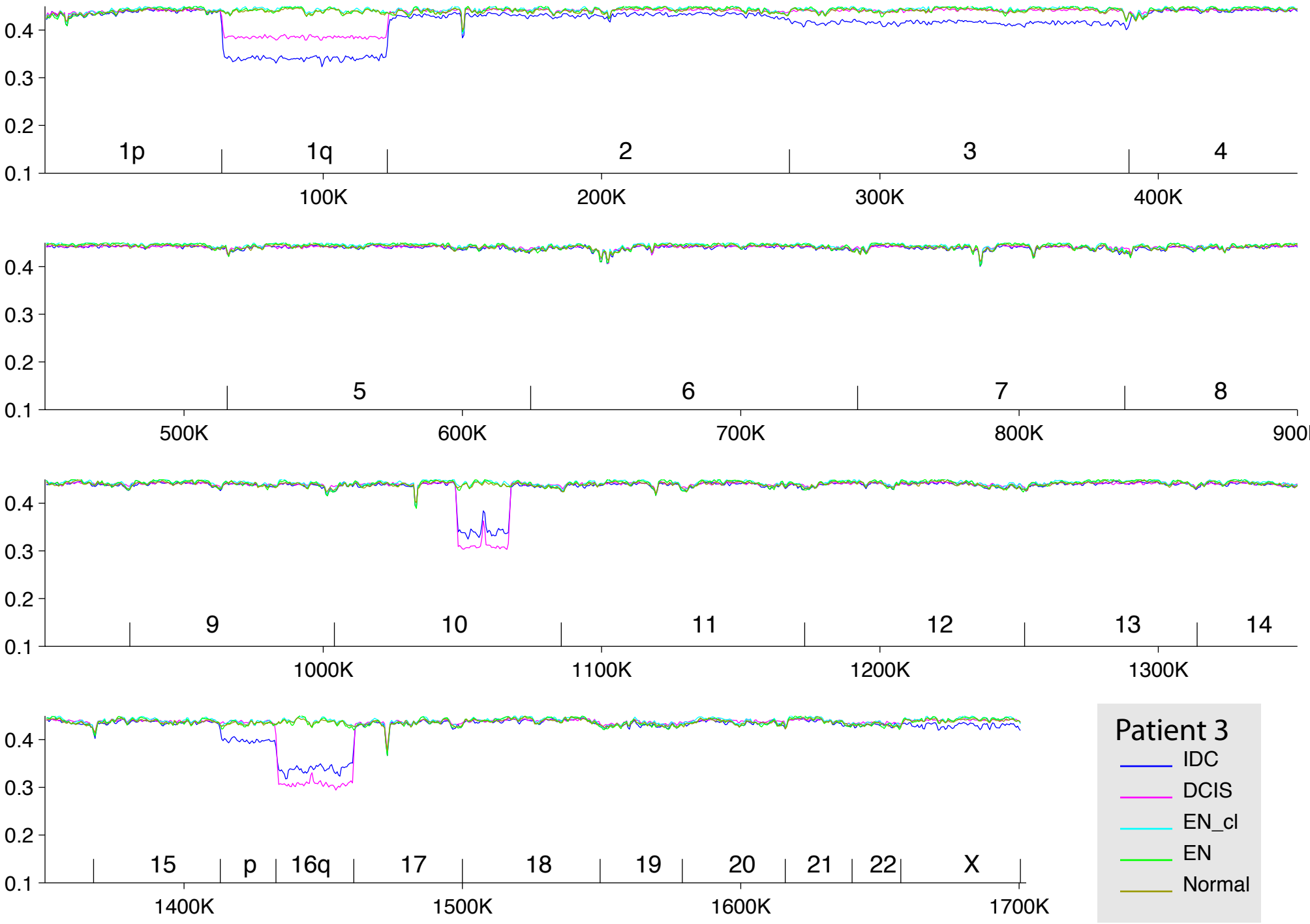
Supplemental Fig. S11



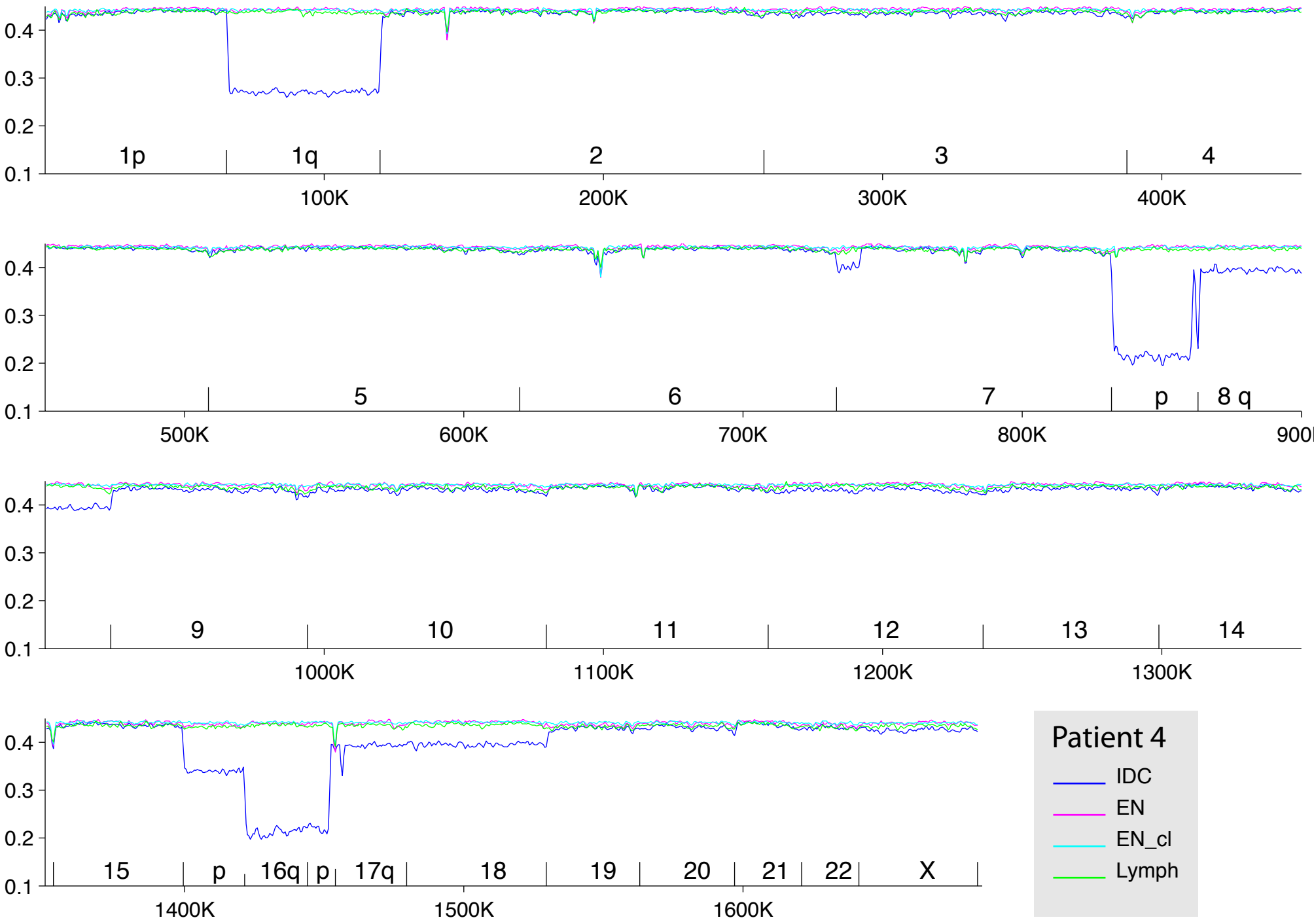
Supplemental Fig. S12



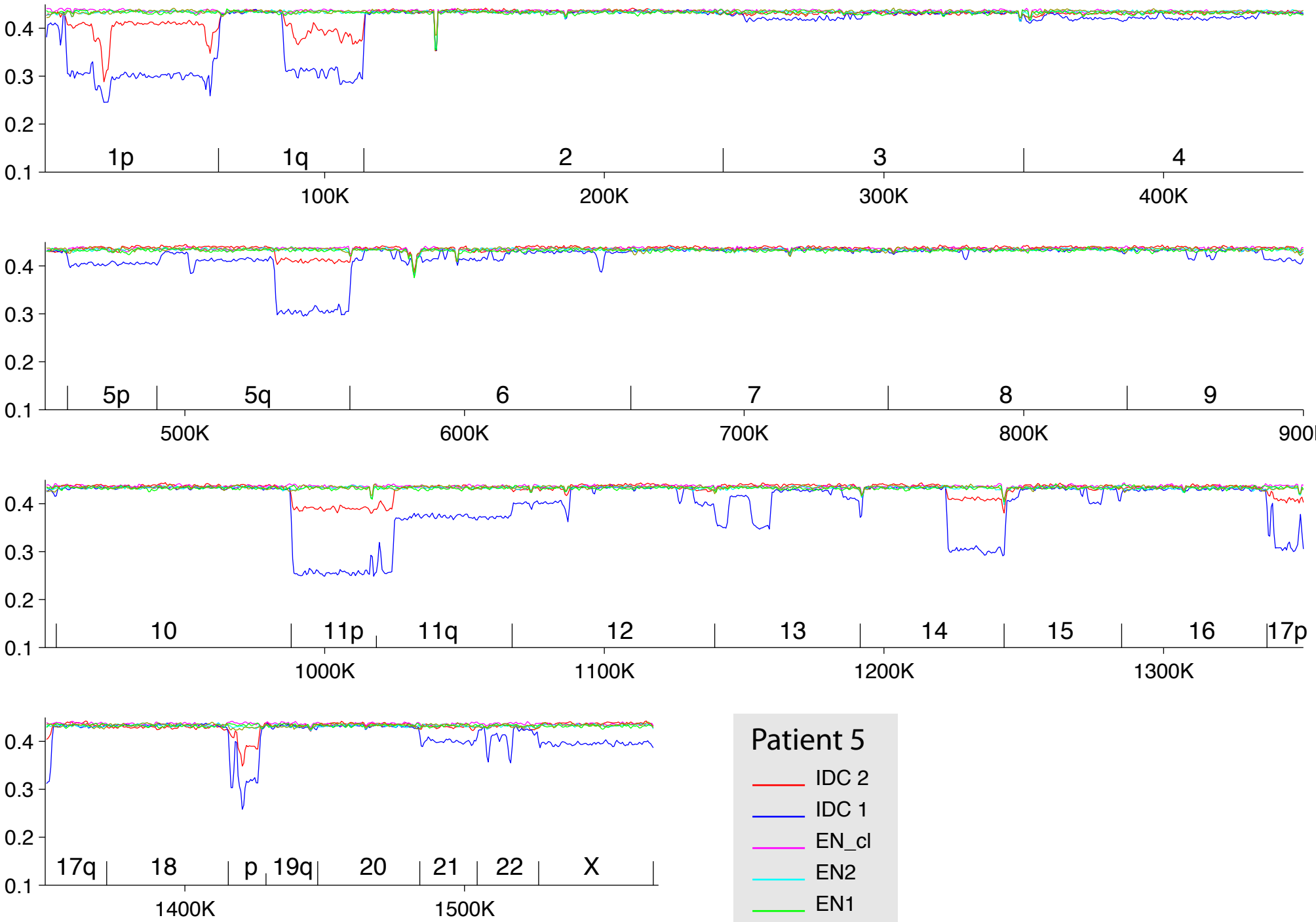
Supplemental Fig. S13



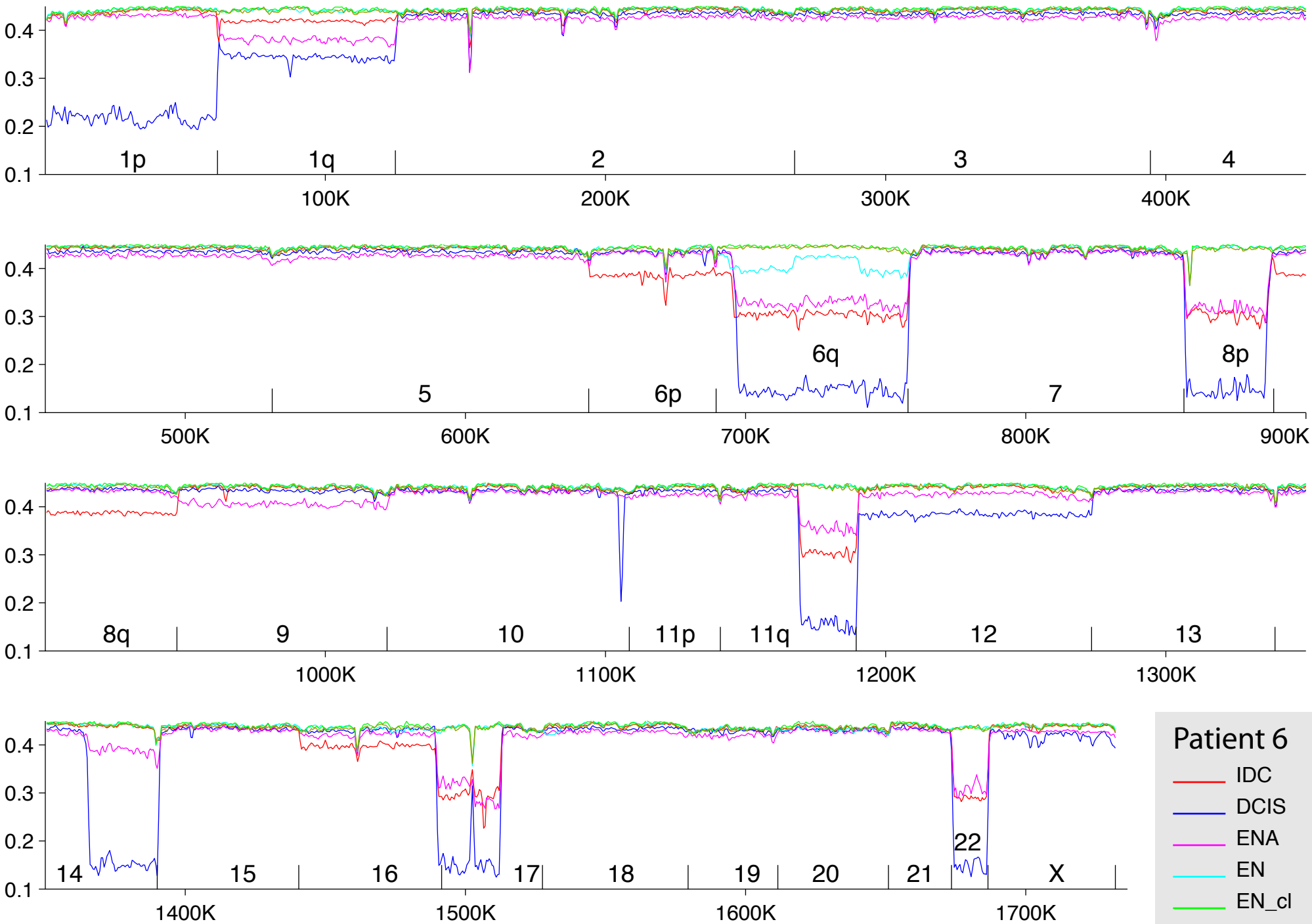
Supplemental Fig. S14



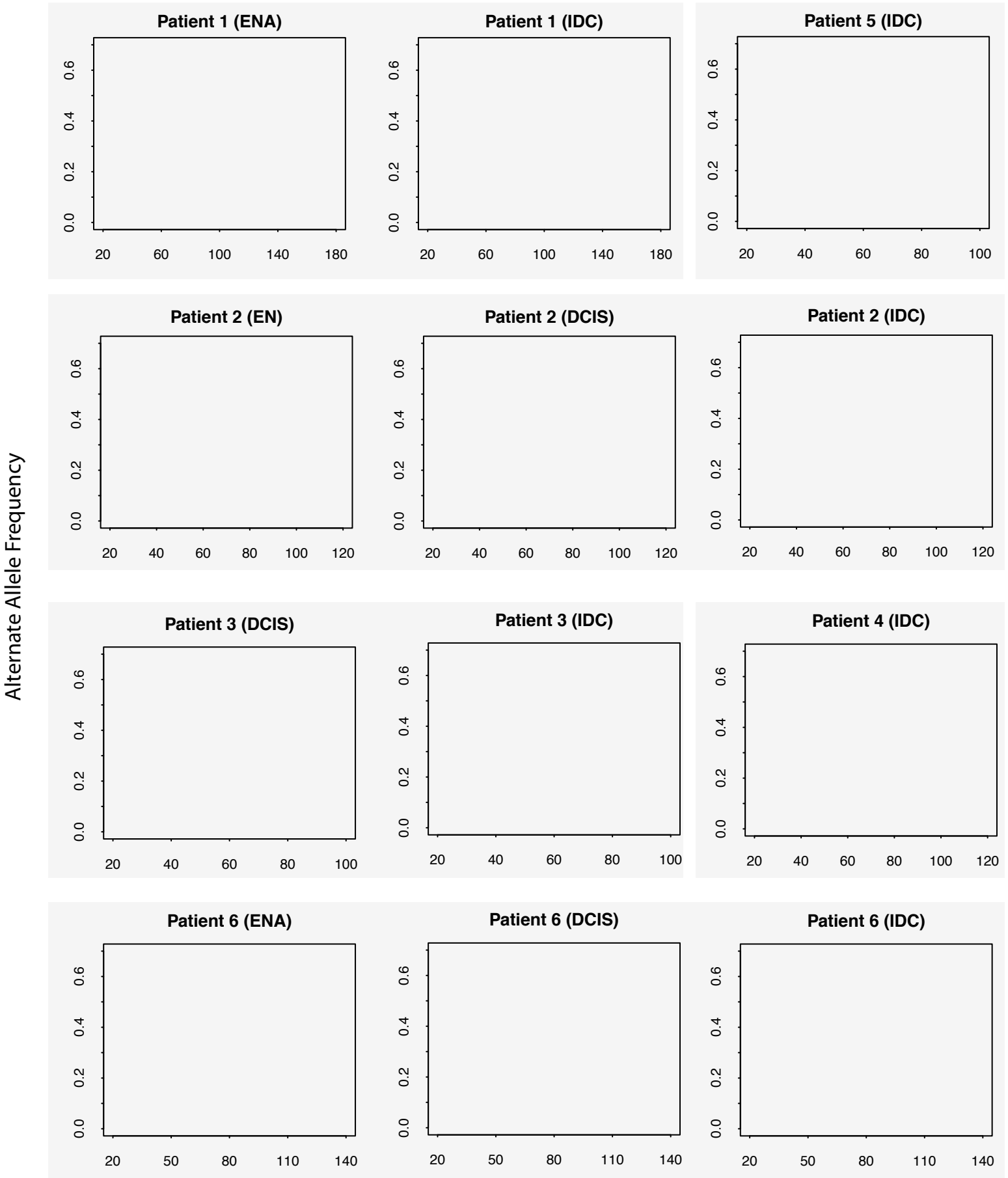
Supplemental Fig. S15



Supplemental Fig. S16



Supplemental Fig. S17



Chr1q SNVs that arose on the same ancestral branch as the first 1q ploidy gain
SNVs on all diploid chromosomes on the same branch

Supplemental Fig. S18

Supplemental Table 1. Robust SNV classes.

SNV class	Counts in each sample of the SNVs in that class							Phylogenetically informative?
Patient 1								
	Lymph	**Normal**	**EN_cl**	**EN**	**ENA**	**IDC**	**Sum**	
010000		89					89	
001000			120				120	
000100				147			147	
000010					102		102	
000001						46	46	
000011					775	775	775	Yes
							1279	
Patient 2								
	Normal	**EN**	**DCIS**	**IDC**	**Sum**			
0111		681	681	681	681			Yes
0001				515	515			
0110		133	133		133			Yes
0101		80		80	80			Yes
0010			70		70			
					1479			
Patient 3								
	Normal	**EN**	**EN_cl**	**DCIS**	**IDC**	**Sum**		
10000	39					39		
01000		306				306		
00100			121			121		
00010				362		362		
00001					222	222		
00011				1054	1054	1054		Yes
						2104		
Patient 4								
	Lymph	**EN_cl**	**EN**	**IDC**	**Sum**			
0001				1809	1809			
0010			405		405			
0100		368			368			
					2582			

Supplemental Table 1 continued

Patient 5								
	Lymph	**EN_1**	**EN_2**	**EN_cl**	**IDC_1**	**IDC_2**	**Sum**	
010000		343					343	
001000			234				234	
000100				58			58	
000010					183		183	
000001						23	23	
000011					793	793	793	Yes
011000		13	13				13	Yes
001010			26		26		26	Yes
001011			55		55	55	55	Yes
							1728	

Patient 6								
	Lymph	**EN**	**ENA**	**DCIS**	**EN_cl**	**IDC**	**Sum**	
010000		305					305	
001000			345				345	
000100				978			978	
000010					219		219	
000001						608	608	
001001			61			61	61	Yes
001100			185	185			185	Yes
001101			510	510		510	510	Yes
							3211	

Supplemental Table 2. All aneuploidies.

Patient	Chr	Arm	Partial?	Stage	Early	DCIS	IDC	IDC SC
1	1	q		1	Gain 1			
1	16	q		1	Loss 1			
2	1	q		1	Gain 2		Gain 2	
2	16	p		1	Gain 1		Gain 1	
2	16	q		1	Loss 1			
2	X			1	Loss 1			
2	1	p		4				Loss 1
2	2			4				Loss 1
2	4			4				Loss 1
2	5			4				Loss 1
2	8			4				Gain 1
2	9			4				Loss 1
2	10			4				Loss 1
2	11			4				Loss 1
2	13			4				Loss 1
2	14			4				Loss 1
2	15			4				Unknown
2	17			4				Loss 1
2	19			4				Loss 1
2	21			4				Loss 1
3	1	q		2		Gain 1	Gain 1	
3	10	q	1	2		Loss 1		
3	16	q		2		Loss 1		
3	16	p		3			Gain 1	
3	2			4				Loss 1
3	3			4				Loss 1
3	X			4				Loss 1
4	1	q		3			Gain 2	
4	8	p		3			Loss 1	
4	16	p		3			Gain 1	
4	16	q		3			Loss 1	
4	17	p		3			Loss 1	
4	8	q		4				Gain 1
4	17	q		4				Gain 1
4	18			4				Gain 1
5	1	p	1	3			Gain 2	
5	1	p	1	3			Loss 1	
5	1	q	1	3			Gain 2	
5	5	q	1	3			Loss 1	

Supplemental Table 2 continued.

5	11	p		3			Loss 1	
5	11	q	1	3			Loss 1	
5	14	q	1	3			Loss 1	
5	17	p		3			Loss 1	
5	19	p	1	3			Gain 2	
5	19	p	1	3			Loss 1	
5	3	p		4				Unknown
5	4			4				Unknown
5	5	p		4				Loss
5	9		1	4				Chromo
5	12		1	4				Chromo
5	13		1	4				Chromo
5	15		1	4				Chromo
5	21			4				Loss
5	22		1	4				Chromo
5	X			4				Loss
6	1	q		1	Gain 1		Gain 1	
6	6	q		1	Loss 1			
6	8	p		1	Loss 1			
6	9			1	Loss 1			
6	11	q	1	1	Loss 1			
6	14	q	1	1	Loss 1			
6	17			1	Loss 1			
6	22			1	Loss 1			
6	1	p		2		Loss 1		
6	6	p		3			Gain 1	
6	8	q		3			Gain 1	

Supplemental Table 3. Fisher's exact test results for high-AAF SNVs on Chr 1q

Patient	Branch* (see Figure 1)	Ploidy of 1q in that branch*	Number of SNVs in diploid chromosomes	Number of SNVs in Chr 1q	Number of SNVs with high AAF	P-value
1	B	3	723	37	3	1.07e-04
2	B	4	212	16	2	4.64e-03
3	D	3	671	45	3	2.33e-04
4	D	4	1579	63	24	7.80e-37
5	D	4	376	40	17	1.31e-19
6	B	3	294	18	4	7.90e-06

* Test was conducted only on the SNVs that occurred on that specific ancestral branch which had the first 1q ploidy gain.