

Legends to supplementary Figures

Suppl. Figure 1: Unfolded maps of the myeloarchitecture in three monkeys (Mk6, Mk12 and Mk13) and series of photomicrographs of frontal sections from Mk13 at levels indicated in the corresponding unfolded map and on lateral view of the left hemisphere (middle panels). Zones 1 to 6 correspond to increasing density of myelin across cortical layers and emergence of an outer band of Baillarger in zones 5 and 6. The terms 3β and 4β designate areas with overall similar densities of myelinated fibers as in zones 3 and 4, but with some differences in laminar distributions. For other conventions see Figures 3 and 4 legends. Scale bar (left photomicrograph): 1mm

Suppl. Figure 2: Unfolded maps of AChE in three monkeys (Mk8, Mk10 and Mk12) and series of photomicrographs of frontal sections from Mk10 at levels indicated in the corresponding unfolded map and on lateral view of the left hemisphere (middle panels). Zones 1 to 6 correspond to increasing density of AChE-containing fibers in deeper layers in antero-ventral and in middle layers in posterior insula. Light or moderate AChE is expressed in antero-dorsal and middle parts of the insula (zones 1-3). The terms 3β , 4α and 5α designate zones with overall similar density of AChE fibers as in zones 3-5, but with different laminar distributions. For other conventions see Figures 3 and 4 legends. Scale bar (left photomicrograph): 1mm

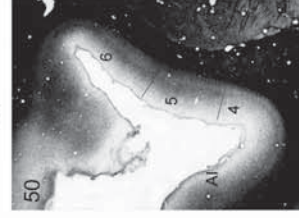
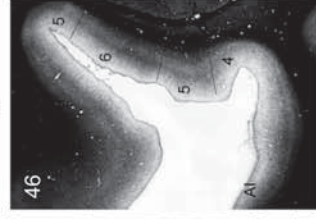
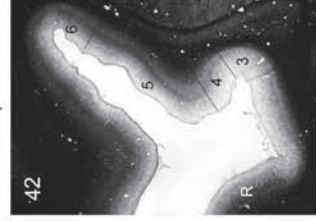
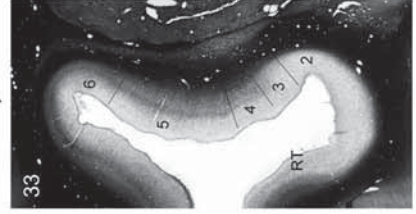
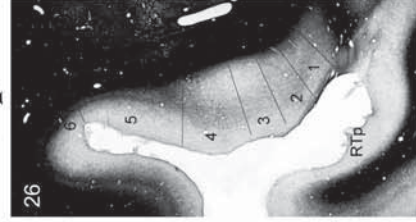
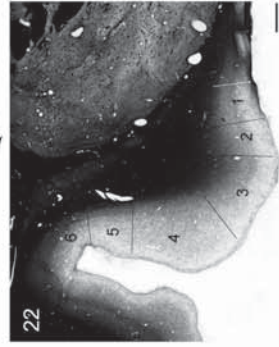
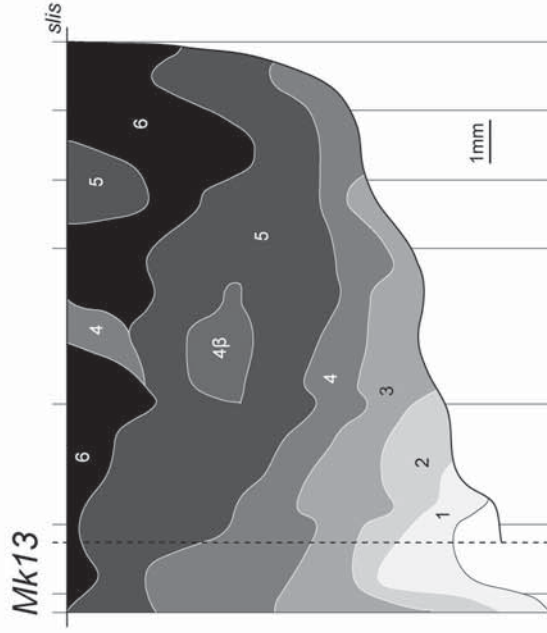
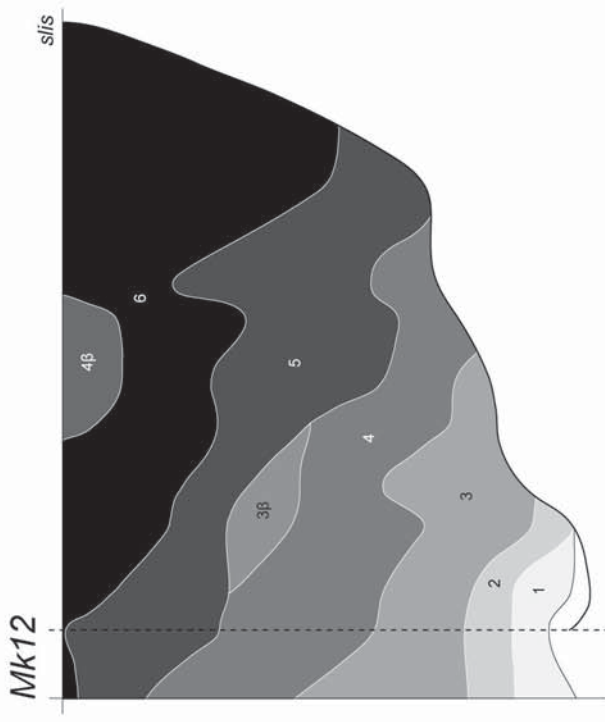
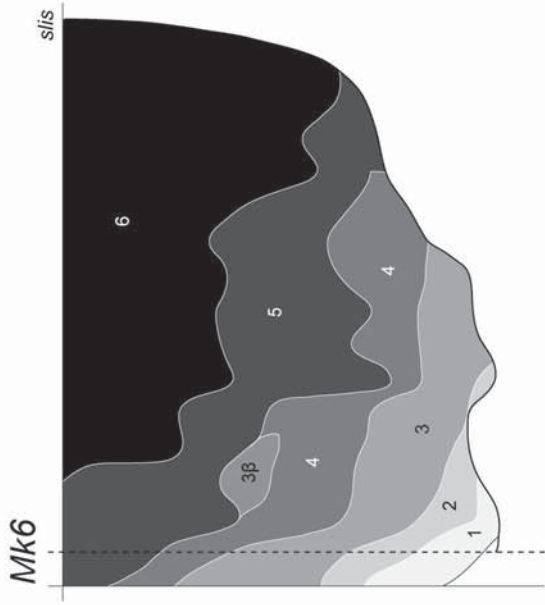
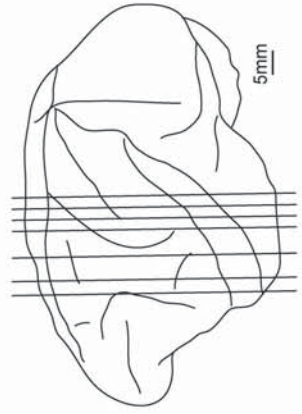
Suppl Figure 3: Comparison of unfolded maps of Nissl, myelin, SMI-32, PV and AChE from one monkey (MK12), and “interstaining” variability map (top left diagram). The individual maps were also illustrated in Figures 4-6 and suppl. Figures 1-2 for comparison with architectonic maps obtained with the same markers in other monkeys. The maps were superposed graphically using the sliis and anterior limit of the “morphological” insula (vertical dotted red line) as references for alignment. Boundaries of myelin, SMI-32, PV and AChE, which most correspond with those of Nissl were selected (Nissl unfolded map is in this case taken as reference) and the variability range for each subdivision estimated by the surface

between the most dorsal and most ventral limits of the area selected. The same procedure was followed for the other subdivisions. This graphical representation of variability of the different architectonic subdivisions is shown in upper left panel where **a** corresponds to Ia1-Ia2; **b**, to Ia-Id; **c**, to Id1-Id2; **d**, to Id2-Id3; **e**, to Id-Ig; and **f**, to Ig-G. The same overall low-to-high, antero-ventral to dorsal and posterior staining gradient is seen in all maps, except for AChE (see text and legend of suppl. Fig. 2 for details). However, in contrast to Nissl and myelin, patterns of PV, SMI-32 and AChE exhibit a ventral, temporal-opercular extension of high-density staining (zones 6 or 5 α) surrounding a strip of lighter staining. This is also represented by the yellow strip (zone f) surrounding the blue one (zone e) in the “variability” graph and explains the relative large variability in the posterior insula. In the antero-ventral insula, the differences observed reflect in large part variations due to shrinkage factors that differ between staining procedures.

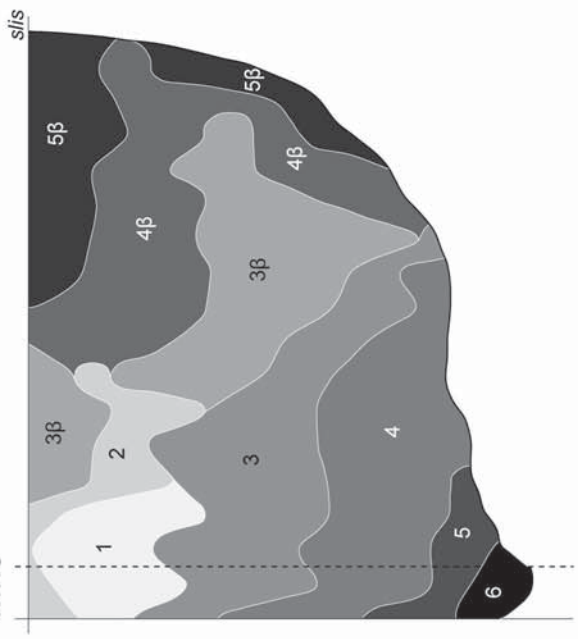
Supplementary list of Abbreviations

1/2	somatosensory areas 1 and 2
3a/b	somatosensory areas 3a and 3b
4	primary motor cortex
7b	somatosensory area 7b
Amg	Amygdala
AI	primary auditory area
acs	arcuate sulcus
BB	bands of Baillarger
Cd	Caudate nucleus
Cl	claustrum
cs	central sulcus
cis	circular sulcus
CM	caudomedial area (belt auditory)
G	hypergranular (insular) area
Gu	gustatory area
GPe	globus pallidus, external segment
Ia	agranular insula
Id	dysgranular insula
Ig	granular insula
lis (slis, ilis)	limiting sulcus (superior and inferior limbs)
ios	inferior occipital sulcus
ips	inferior parietal sulcus
ls	lateral sulcus
lus	lunate sulcus
OPf	frontal opercular area

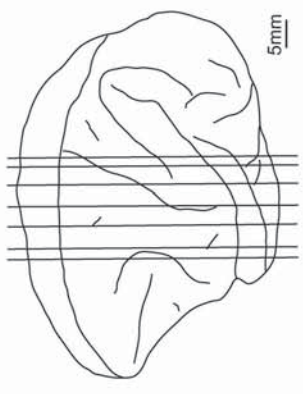
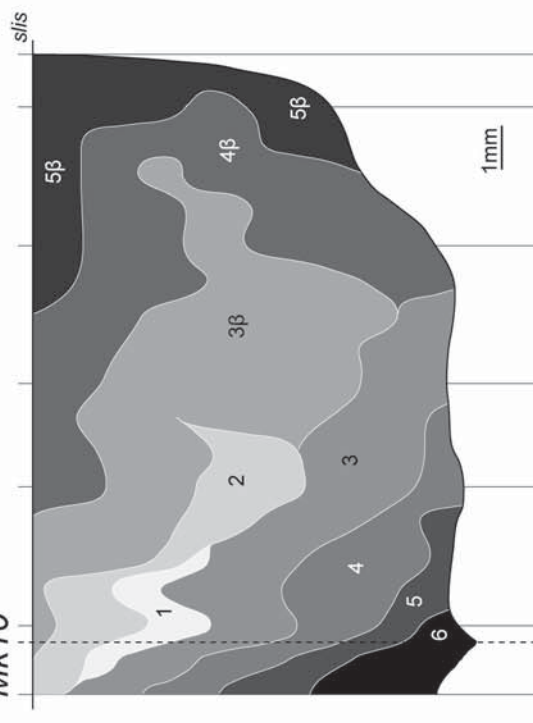
Pi	parainsular area
PMv	ventral premotor cortex
Poc	piriform olfactory cortex
PR	rostroventral parietal area
ps	principal sulcus
PuT	Putamen
PVs	parietal ventral somatosensory area
R	rostral area (core auditory)
Ri	retroinsular area
RM	rostromedial area (belt)
RT	rostrotemporal area (core auditory)
RTM	medial rostrotemporal area (belt auditory)
RTp	polar rostrotemporal area (belt auditory)
SII	secondary somatosensory area
sts	superior temporal sulcus
VS	ventral somatosensory area



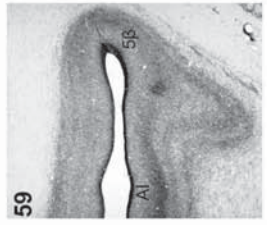
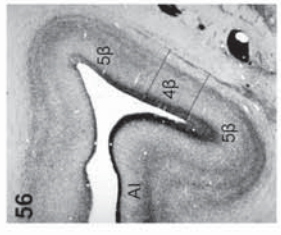
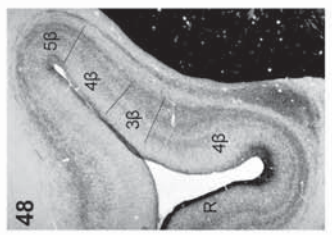
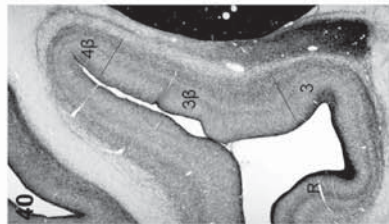
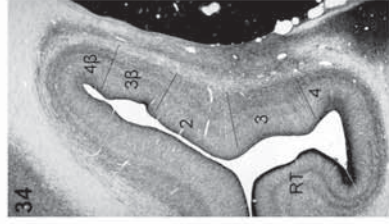
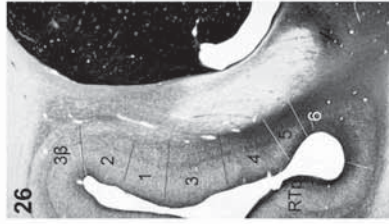
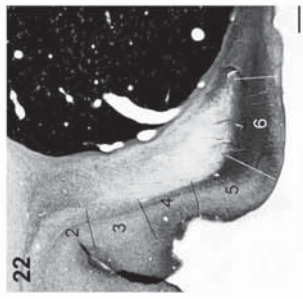
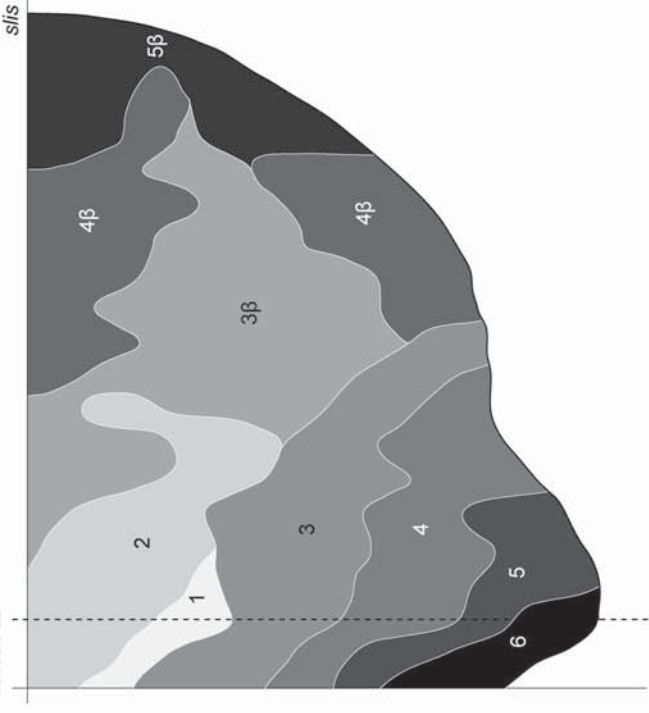
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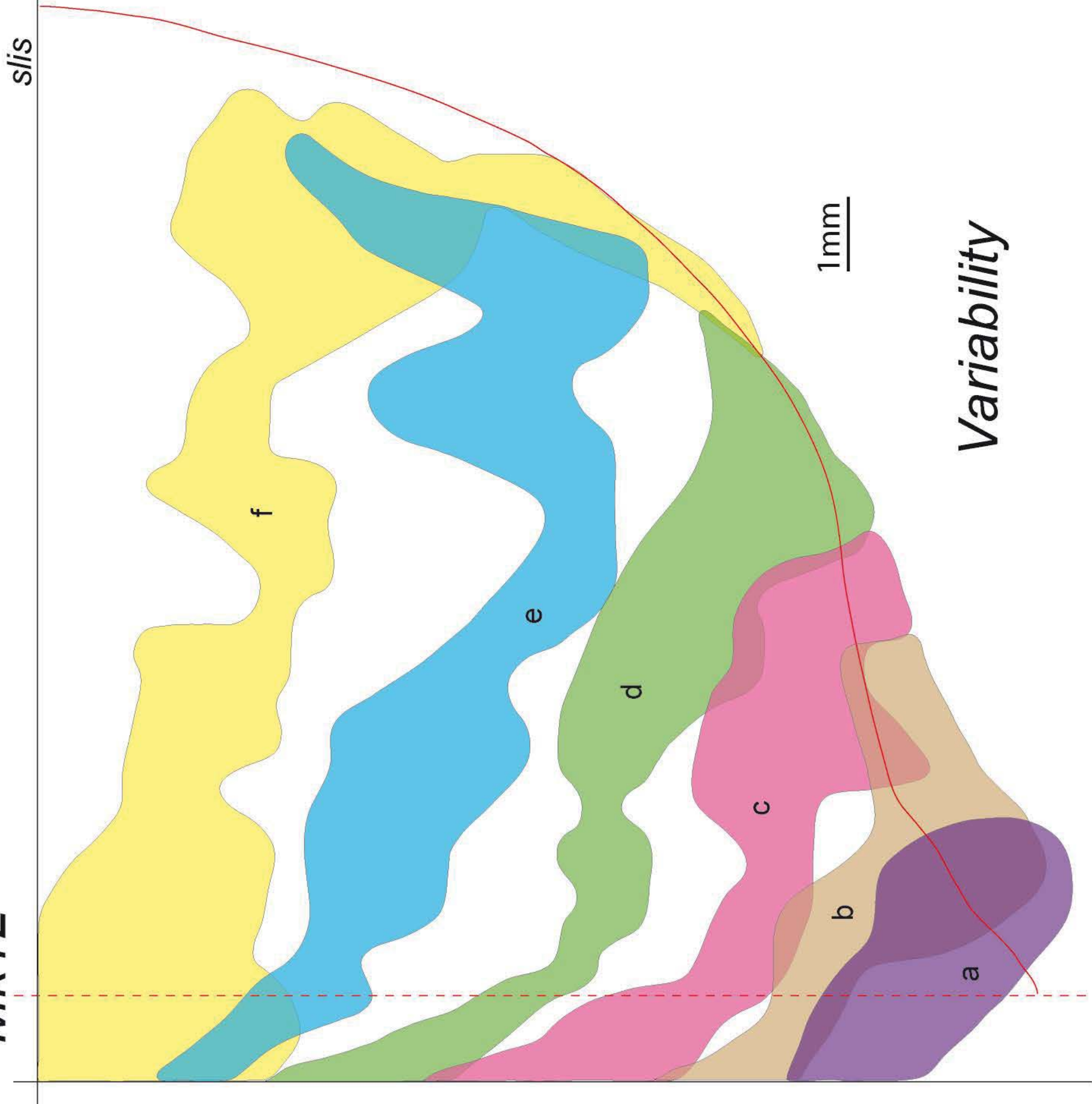
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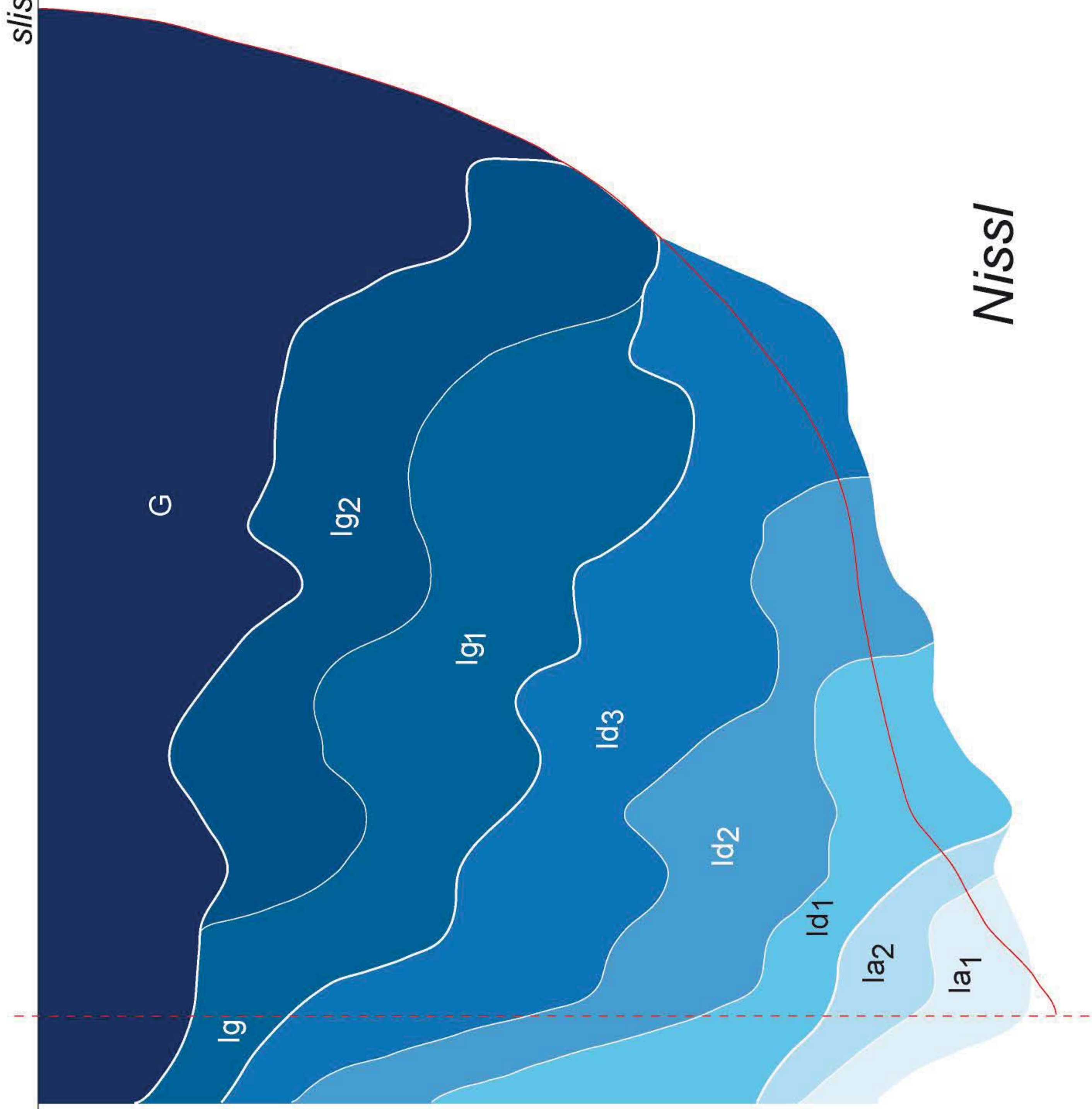
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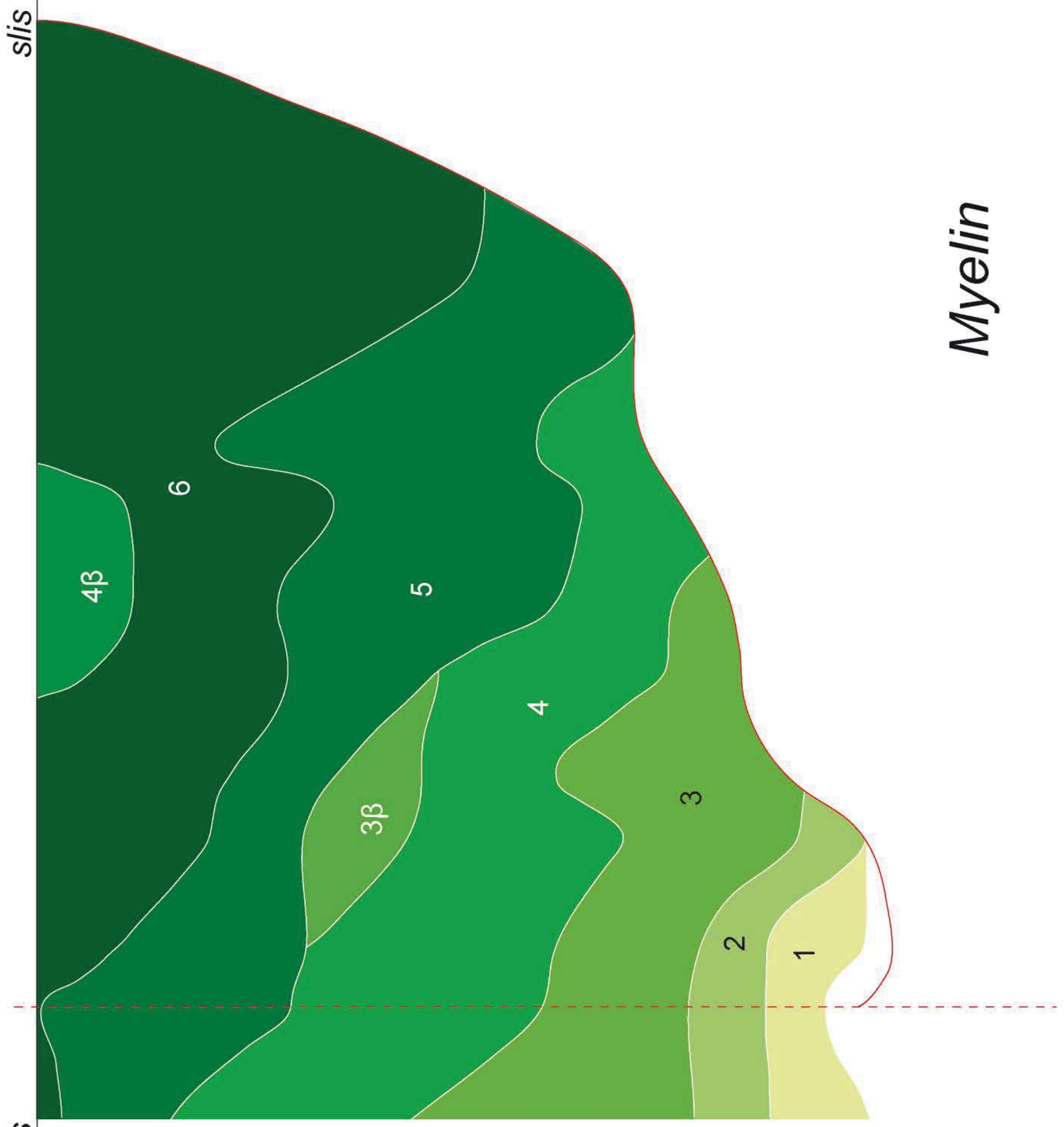
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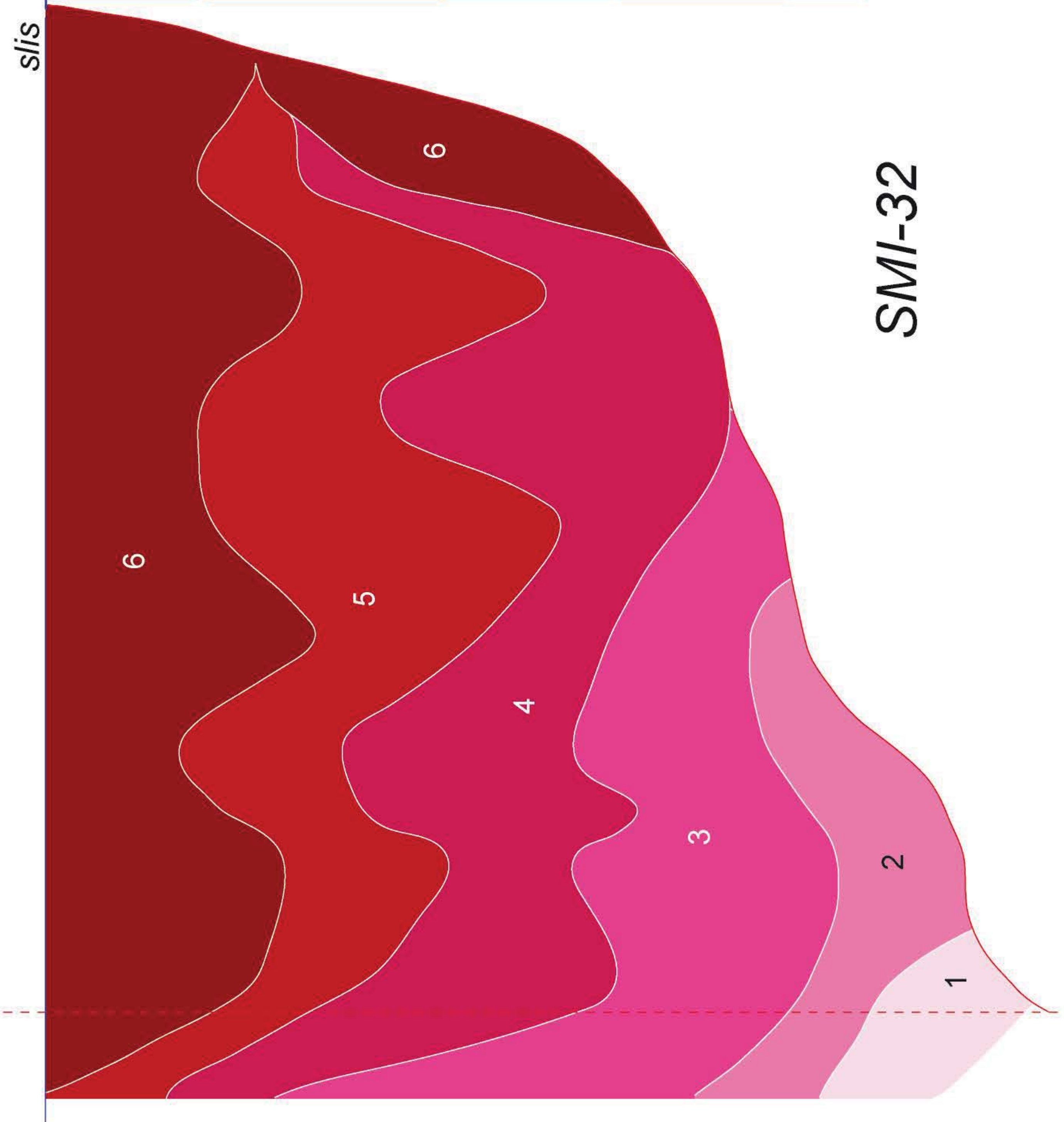
Variability



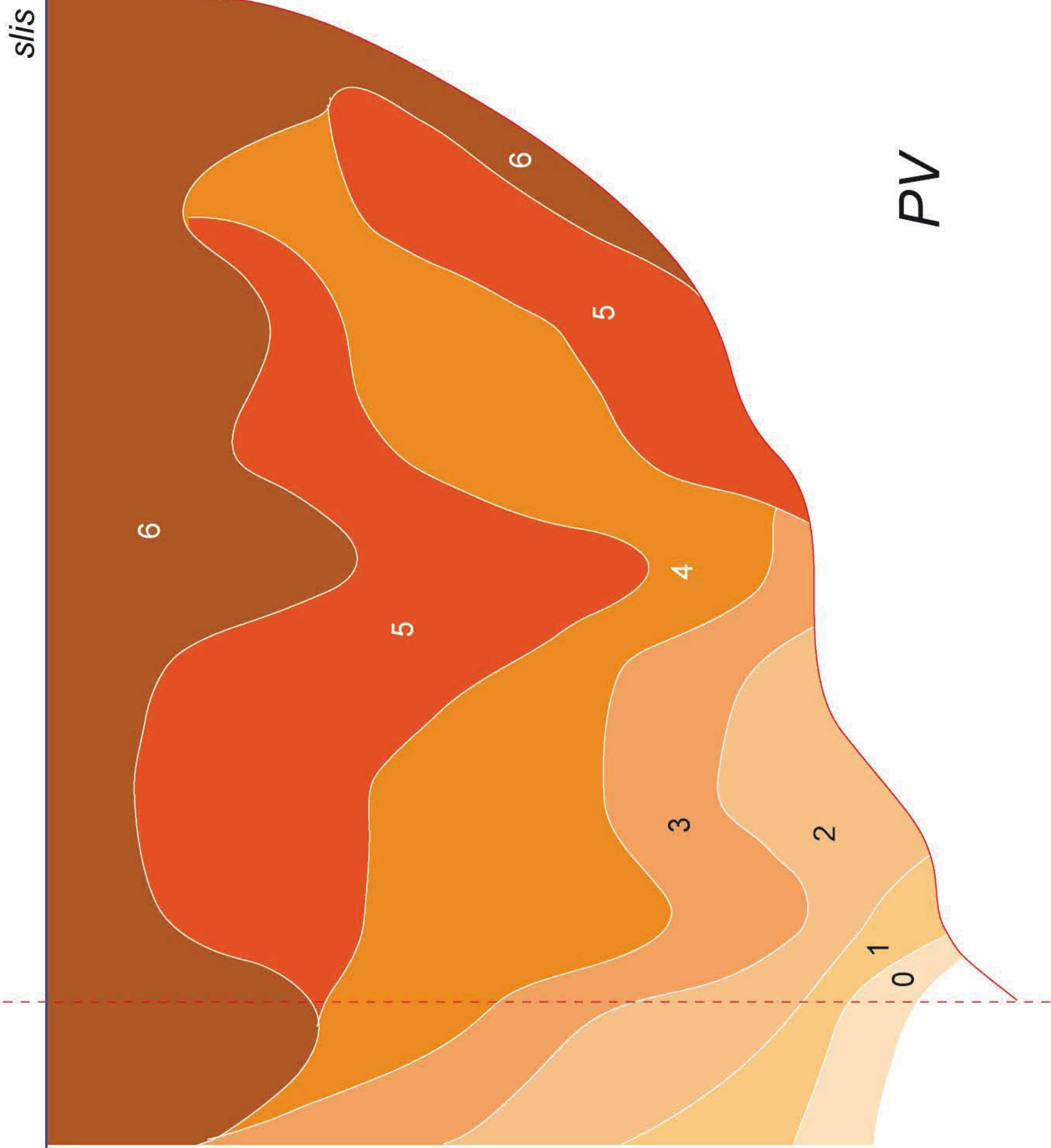
Nissl



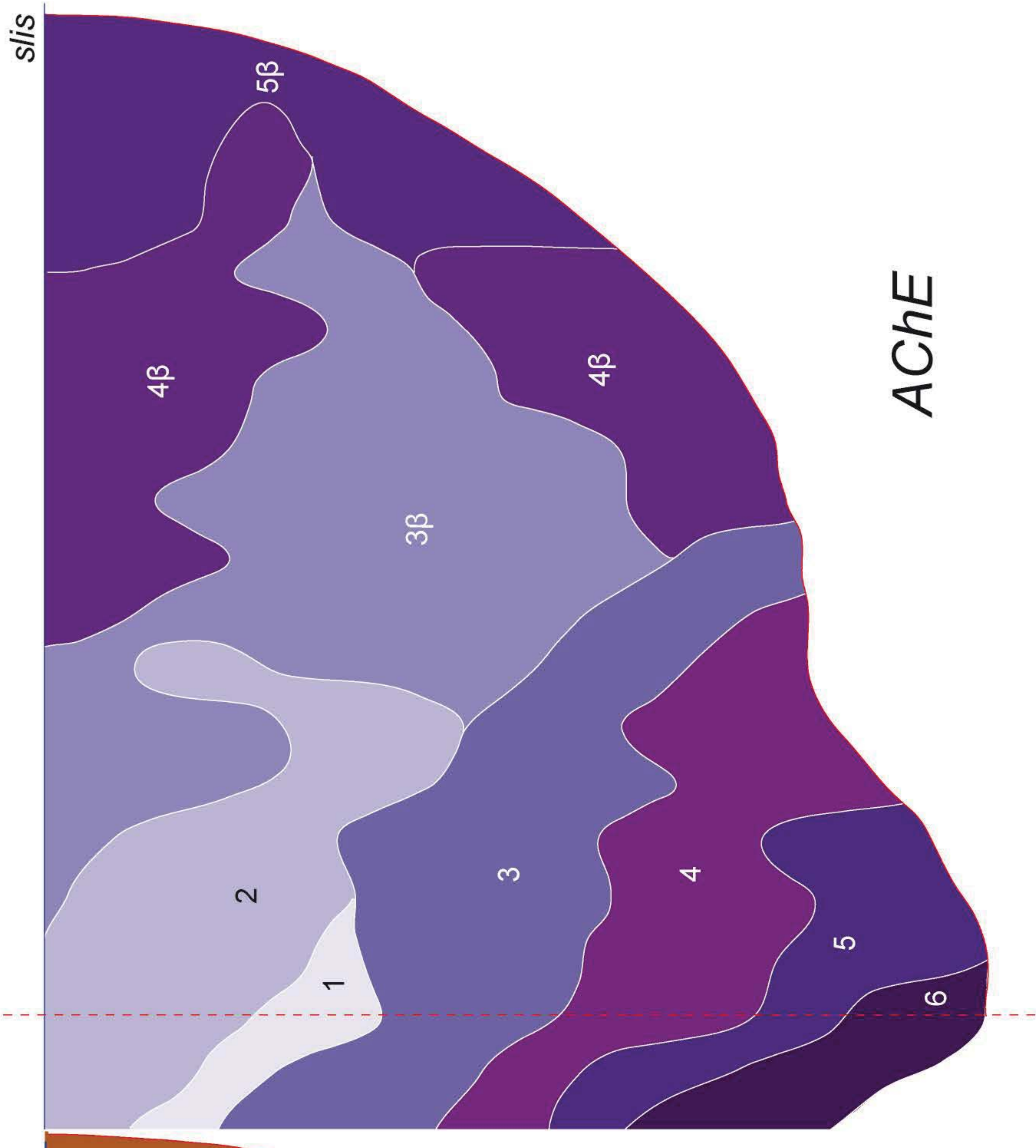
Myelin



SMI-32



PV



AChE

Supplementary Material and Methods

Immunocytochemistry

Free-floating sections were first preincubated for 10 min in 1.5% H₂O₂ in phosphate-buffered saline to remove endogenous peroxidase activity. After several rinses in phosphate-buffered saline, sections were incubated overnight in primary antibodies, 2% normal horse or goat serum, and 0.2% Triton-X-100. The antibodies used were mouse monoclonal directed against PV (Sigma, St Louis, MO, USA) and mouse monoclonal against a nonphosphorylated epitope on neurofilament proteins (SMI-32, Sternberger Monoclonals Inc., Lutherville, MD, USA). Dilutions between 1:3000 and 1:5000 for PV, and between 1:1000 and 1:2000 for SMI-32 yielded best results. After several rinses, sections were incubated for 30–60 min at room temperature in biotinylated secondary antibody (1 : 200, Vector Laboratories, Burlingame, CA, USA) and stained with the avidin–biotin complex immunoperoxidase method (Vectastain Elite kits, Vector Laboratories). The reaction was visualized with 3,3'-diaminobenzidine tetrahydrochloride as the chromogen, diluted 0.05% in Tris-saline with 0.001% H₂O₂. Sections were then washed thoroughly and immediately mounted on gelatin-coated slides, dehydrated, and coverslipped. As a control, the primary antibody was omitted for some sections while the rest of the procedure remained the same.

Supplementary Table 1: List of monkeys and histological processing

<i>Monkey #</i>	<i>Nissl</i>	<i>Myelin</i>	<i>AChE</i>	<i>PV</i>	<i>SMI-32</i>
Mk1(AD)	+	-	+	+	+
*Mk2(BA)	+	-	+	+	+
Mk3(EV)	+	-	+	-	+
Mk4(HE)	+	-	-	+	+
Mk5(JO)	+	-	+	+	+
*Mk6(M3)	+	+	-	+	+
Mk7(MA)	+	-	+	+	+
Mk8(OS)	+	-	+	+	+
Mk9(RH)	+	-	+	+	+
Mk10(RU)	+	-	+	-	+
Mk11(SC)	+	-	+	+	+
Mk12(UL)	+	+	+	+	+
*Mk13(Z1)	+	+	+	-	+

*Macaca fascicularis (all others are Macaca mulatta)