Supplement: Qualitative comparison of histopathology and ex-vivo MRI of Case 2 and 3

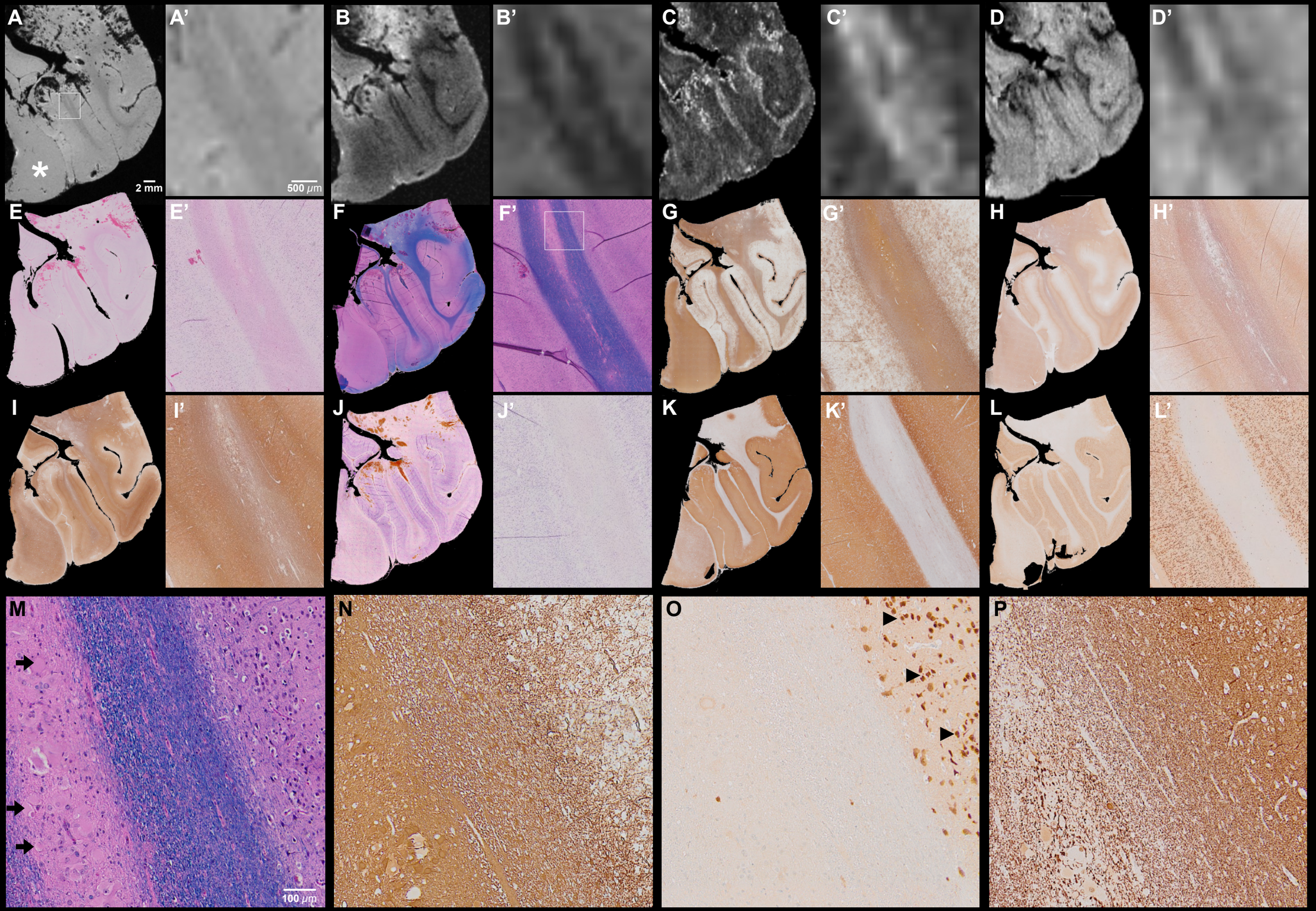
Supplemental Figure 1

Case 2 - Left occipital tuber with white matter microtuber. The top row shows high resolution ex-vivo images (A-D), and next to each image a higher magnification (A'-D'), indicated by the rectangle in (A). The second and third row show registered histopathology stains (E-H) and (I-J), again with an adjacent higher magnification image (E'-H') and (I'-J'). (A) T1w image (B) T2w image (C) FA image (D) MD image (E) H&E stain (F) H&E stain counterstained with LFB (G) GFAP stain (H) Neurofilament stain (I) SMI 31 stain (J) cresyl violet stain (K) and (L) NeuN stain.

In the T1w image (A), a tuber is indicated by an asterisk. It is GFAP positive in (G), and has a paucity of neurons (L).

The most salient feature is a fusiform inclusion of tuber pathology in the white matter, referred to as a microtuber. The rectangle in (A) indicates the area of higher magnification, and indeed this area is also evident on T1w (A'), T2w (B'), FA image (C') and MD images (D'). The H&E stains (E) and (F) show its fairly sharp borders. Strong GFAP positivity of the microtuber and of adjacent perituber can be seen in (G'), better seen in (N), and quantified in Figure 3. There is no neurofilament (H') or phosphorylated neurofilament (I') in the core of this satellite tuber lesion, and cresyl violet (J'), synaptophysin (K') and NeuN (L') all show only rare neurons in the white matter and microtuber.

(M-P) depict a higher magnification of the cortex, white matter and part of the microtuber, indicated by the rectangle in (F'). In the tuber, balloon cells are noted (arrows in M), and cells with variable positivity for glial markers (N). The cortex is full of neurons indicated in (O) by an arrowhead, with preserved cortical lamination (L'). There is a paucity of neurons in the white matter. In (P), the axons are stained by phosphorylated neurofilament, and faintly a 90 degree turn into the cortex can be seen.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Supplemental Figure 2

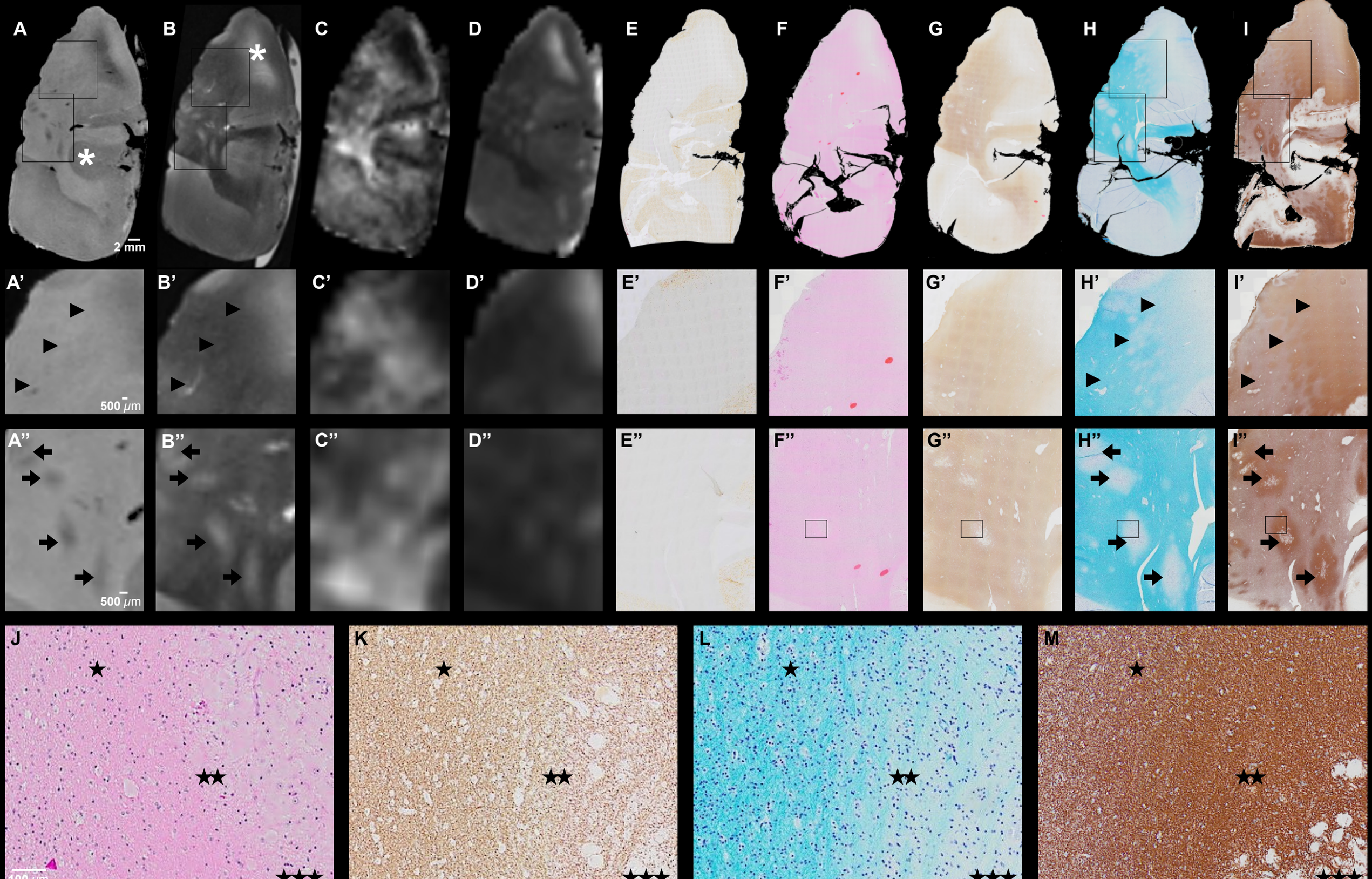
Case 3 - Left frontal tuber and white matter microtubers. The top row shows high resolution ex-vivo images (A-D) and registered histopathology (E-I). In (A), (H) and (I), two rectangles indicate areas of higher magnification shown in corresponding second row (A'-I') and third row (A''-I''). (A) T1w image (B) T2w image (C) FA image (D) MD image (E) NeuN stain (F) H&E stain (G) SMI31 stain (H) LFB stain and (I) GFAP stain.

In the center, a U-shaped band of white matter is seen (asterisk in A). This u-fiber served as an easy to recognize anatomical marker for identification of the resection specimen in the pre-operative images (Figure 2), and is also visible in the (C) FA and (D) MD images. There is a paucity of neurons (E) in the white matter and in the tuber.

The top rectangle demonstrates a gradual transition from healthy white matter, via perituber white matter, to the tuber (asterisk in B). This transition is indicated in the second row by the three black arrowheads in the structural images (A') and (B'), and is evident also in the changing histopathology from white matter towards tuber: (G') SMI31 (reduction in phosphorylated neurofilament), (H') LFB (reduction in myelination), and (I') GFAP (increased gliosis).

The bottom rectangle shows 4 islets of tuber pathology, known as microtubers. In the (A'') T1w and (B'') T2w images, they are easier to appreciate than in the diffusion images (C'' and D''). These are associated with a lack of (G'') SMI31, and (H'') LFB, and increased gliosis (H'').

(J-M) depict a magnified part of a microtuber, indicated by the rectangle in F''-I''. In the (J) H&E stain, an increase of balloon cells is noted from white matter (*) to perilesional white matter (**) and tuber rim (***). There is decreasing (K) neurofilament and (L) myelin, and increasing (M) gliosis – except in the tuber center, where balloon cells are predominant.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