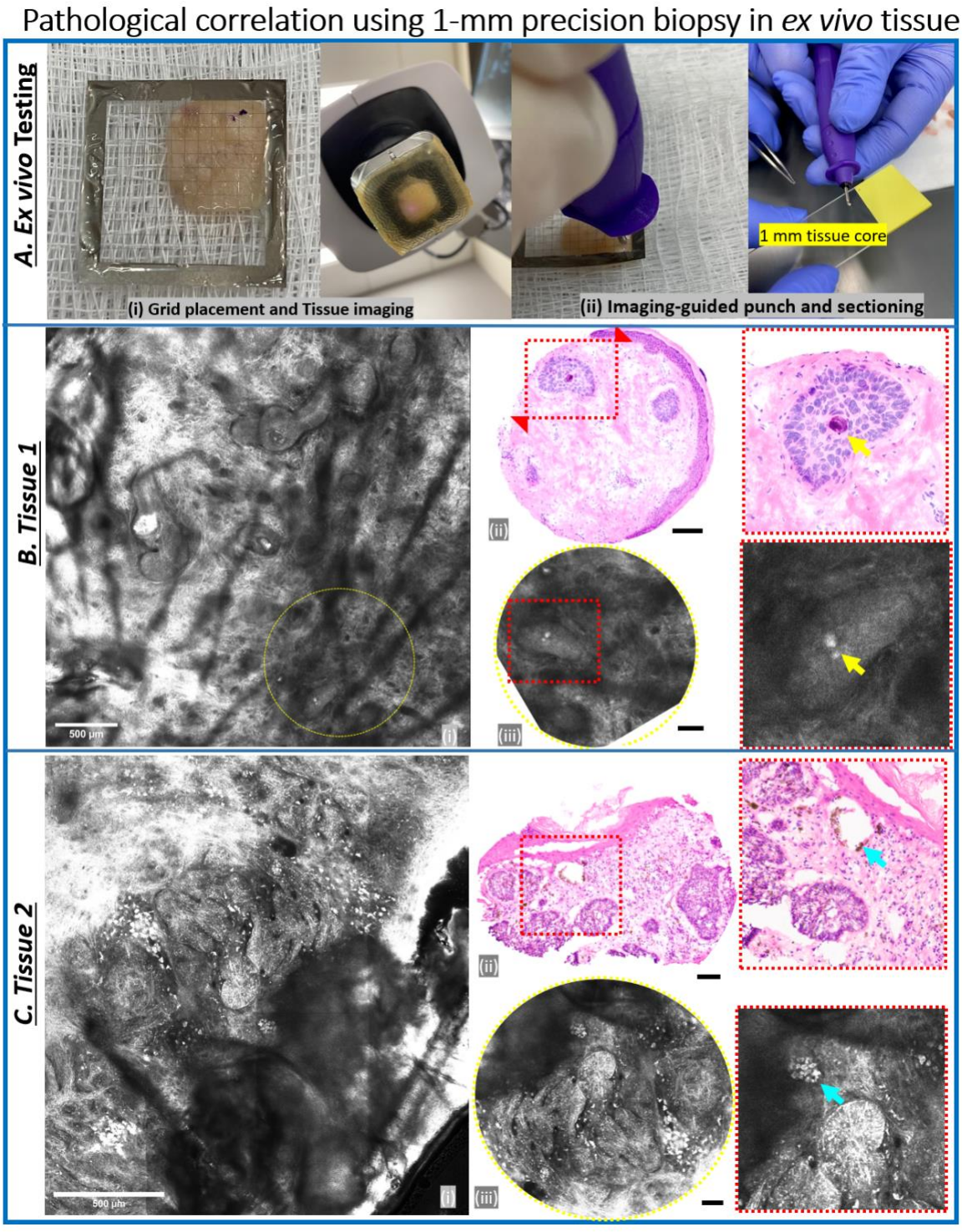
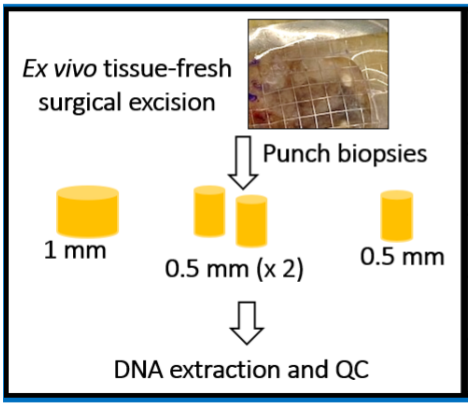
Supplementary Figures



Supplementary Figure 1. Prior to *in vivo* testing, we verified the feasibility of 1-mm imaging-

guided precision biopsy for feature correlation in debulked BCC tumor tissue procured during Mohs surgery (n=2). ***A. Ex vivo testing.*** Grid placement, imaging and biopsy was optimized in *ex vivo* tissue. Successful imaging, biopsy and histopathology correlation was demonstrated as shown in two exemplars. ***B, C. Tissue 1, Tissue 2.*** RCM image of the *ex vivo* tissue with the area selected for precision biopsy (yellow circle) (i) Histopathology (20 x, scale bar=100 micron) of the yellow circular area sectioned *en face* to match RCM plane of imaging (ii), RCM correlate of the 1-mm samples area (scale bar = 100 micron) that corresponds to the 1-mm targeted biopsy (iii). The red boxes in histopathology (H&E sections) and RCM correspond to the specifically located unique feature, in example 1, we successfully correlate a BCC tumor nest containing a minute calcification on RCM with histopathology (yellow arrow), while in example 2, we successfully correlate a round subepidermal collection of melanophages (cyan arrow) above the BCC tumor nests.

DNA yield and quality



	1 mm	0.5 mm (x2)	0.5 mm
Tumor volume, h=1 mm	0.78 mm^3	0.39 mm^3	0.19 mm^3
Tumor volume, h=2 mm	1.57 mm^3	0.78 mm^3	0.39 mm^3
DNA conc (ng/µl)	5.7-9.5	2.2-4.1	1.8-3.1
DNA mass (ng)	260.4-438.2	101.4-187.8	83.4-140.5
DIN range	7.9-9.1	7.4-8.2	6.5-8.6

Supplementary Figure 2. We also performed an initial *ex vivo* experiment on freshly excised debulked BCC tumor tissue procured during Mohs surgery (n=2) to verify the DNA quality and yield from 0.5 mm and 1-mm biopsies for downstream next generation sequencing. The DNA yield was proportional to the tumor volume obtained through punch biopsies (volume of tumor= $\pi r^2 h$). The DNA quantity (260-438 ng/sample) and quality (7.9-9.1) from the 1-mm micro-biopsy was adequate for next-generation sequencing approaches such as whole exome sequencing or gene panels such as MSK-IMPACT to help guide optimal targeted therapies through precision medicine. The DNA yield (83-187 ng/sample) and quality (6.5-8.6) from the smaller single or double 0.5-mm precision biopsies was found to be enough for targeted sequencing of a few hundred genes. We selected 1-mm punches for optimal DNA quantity/quality for *in vivo* experiments to facilitate any potential downstream next-gen applications.