Supplementary Methods

1. GeoMx library preparation and sequencing

ROIs were exposed to 385-nm UV light to release indexing oligonucleotides, which were collected into a 96-well plate. Sequencing libraries were prepared using the GeoMx Seq Code Pack (NanoString) through PCR amplification of photo-released oligonucleotides with ROI-specific Illumina adapter sequences and unique dual indices. PCR products were pooled and purified using AMPure XP beads (Beckman Coulter). Libraries were sequenced on an Illumina NovaSeq 6000 platform (2×101 bp paired-end).

2. Quality control metrics and filtering

AOI quality was pre-evaluated using QC thresholds recommended by the GeoMx DSP Control Center. The applied criteria were as follows: read depth >1,000 reads, alignment rate >80%, sequencing saturation >50%, and nuclei count ≥100 per AOI. All AOIs sufficiently met these criteria; the actual alignment rate exceeded 90% and sequencing saturation exceeded 95% across all AOIs, indicating overall sequencing quality was excellent. No AOIs were excluded on the basis of sequencing quality.

"Low Negative Probe Count" and "Low Surface Area" flags were identified in a subset of AOIs. Negative probes serve as a technical indicator for background signal estimation and are distinct from core sequencing quality metrics such as read depth, alignment rate, and sequencing saturation. Q3 normalization was applied in this study, which minimizes the influence of background signal variability on overall normalization outcomes. Surface area is an indirect proxy for RNA yield, and biological content adequacy is a more relevant determinant of analytical suitability. All AOIs met the nuclei count threshold of ≥100, and the number of targets above background was comparable across AOIs. Therefore, no AOIs were excluded based on these flags.

Q3 normalization factors were assessed for outliers using the Tukey rule based on the interquartile range (IQR × 1.5). No extreme outliers exceeding the statistical range were identified, and no AOIs were excluded on the basis of normalization factor.

The limit of quantification (LOQ) was calculated per AOI according to the GeoMx DSP platform specification, using the formula: LOQ = geometric mean (negative control probe counts) × geometric SD (negative control probe counts)^2, where the standard deviation multiplier was set to 2. LOQ-based filtering was applied to assess gene detectability; all 18,677 genes exceeded the LOQ in at least one ROI and were therefore retained. Additionally, a secondary filter requiring a raw count greater than 2 in

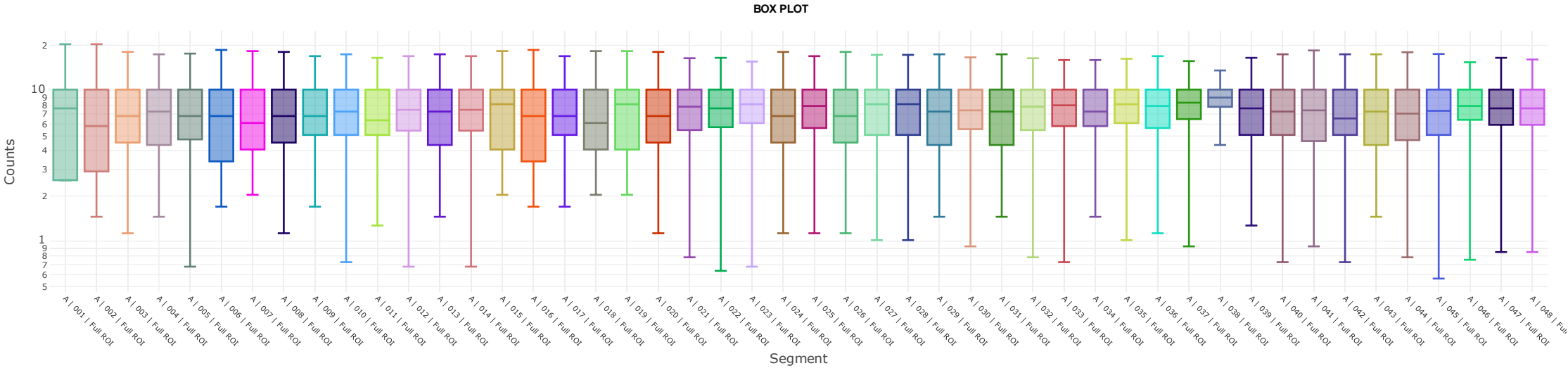
at least 20% of AOIs was applied; again, all 18,677 genes satisfied this criterion. Consequently, no genes were removed by either filtering step, and the full panel of 18,677 genes was carried forward for downstream analysis.

Gene expression distributions following Q3 normalization were assessed by boxplot across all ROIs and biopsy samples. The distributions were comparable across ROIs and biopsy specimens, with no evidence of systematic batch effects attributable to individual slides.

3. Spatial cell type deconvolution

Cell type deconvolution was conducted using SpatialDecon (v1.16.0) with the safeTME (Safe Tumor Microenvironment) reference profile. The matrix was adopted for cortical analysis by excluding medullary cell types (thick ascending loops of Henle) and merging cell subtypes into single categories (e.g., peritubular capillary endothelium, proximal tubule). Background correction using negative control probes and deconvolution with nuclei count normalization and automatic gene alignment were performed.

Gene expression distribution by ROI following Q3 normalization



Gene expression distribution by group following Q3 normalization