Supplementary Online Content

Chao J, Bedell V, Lee J, et al. Association between spatial heterogeneity within nonmetastatic gastroesophageal adenocarcinomas and survival. *JAMA Netw Open*. 2020;3(4):e203652. doi:10.1001/jamanetworkopen.2020.3652

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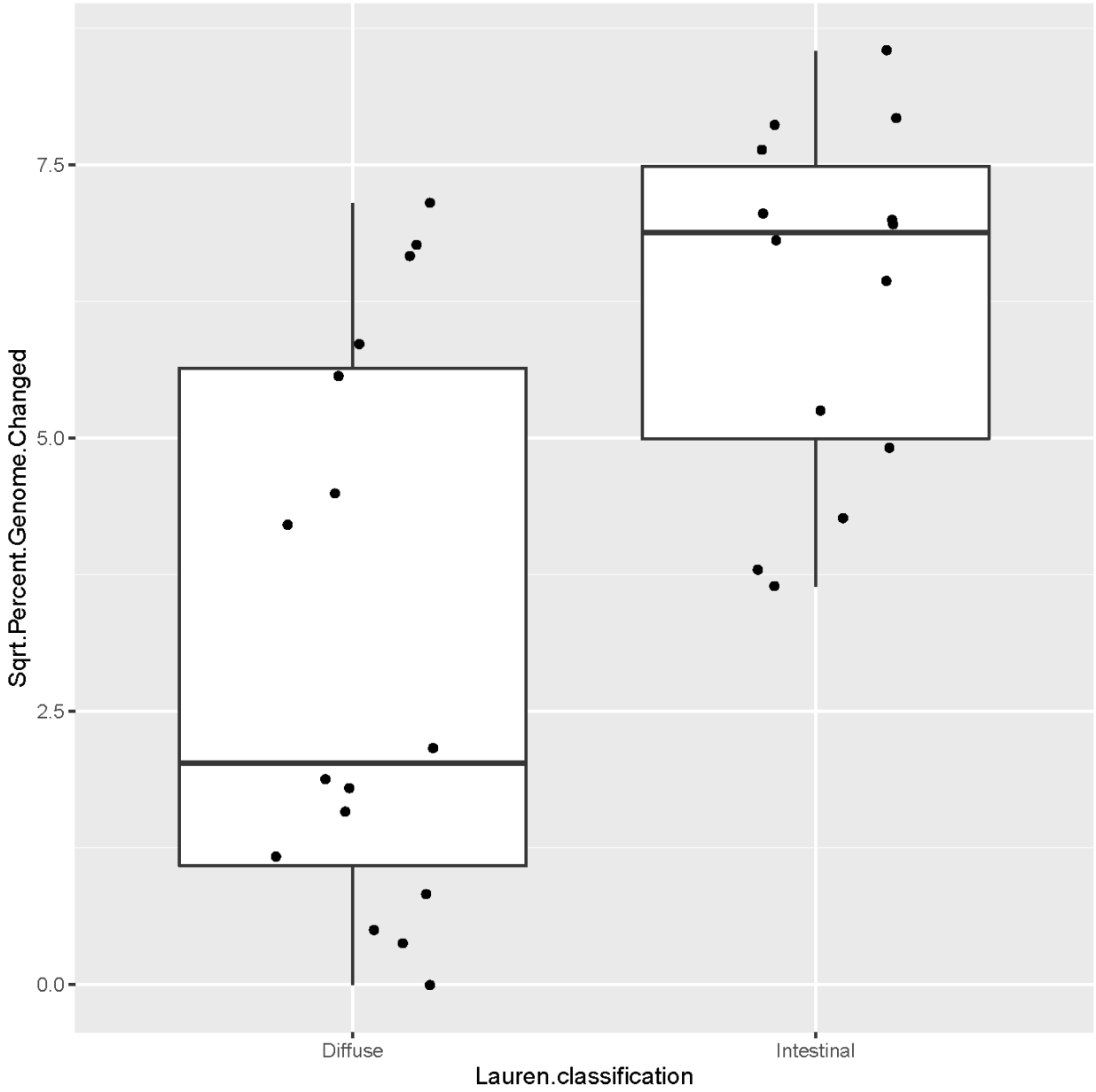
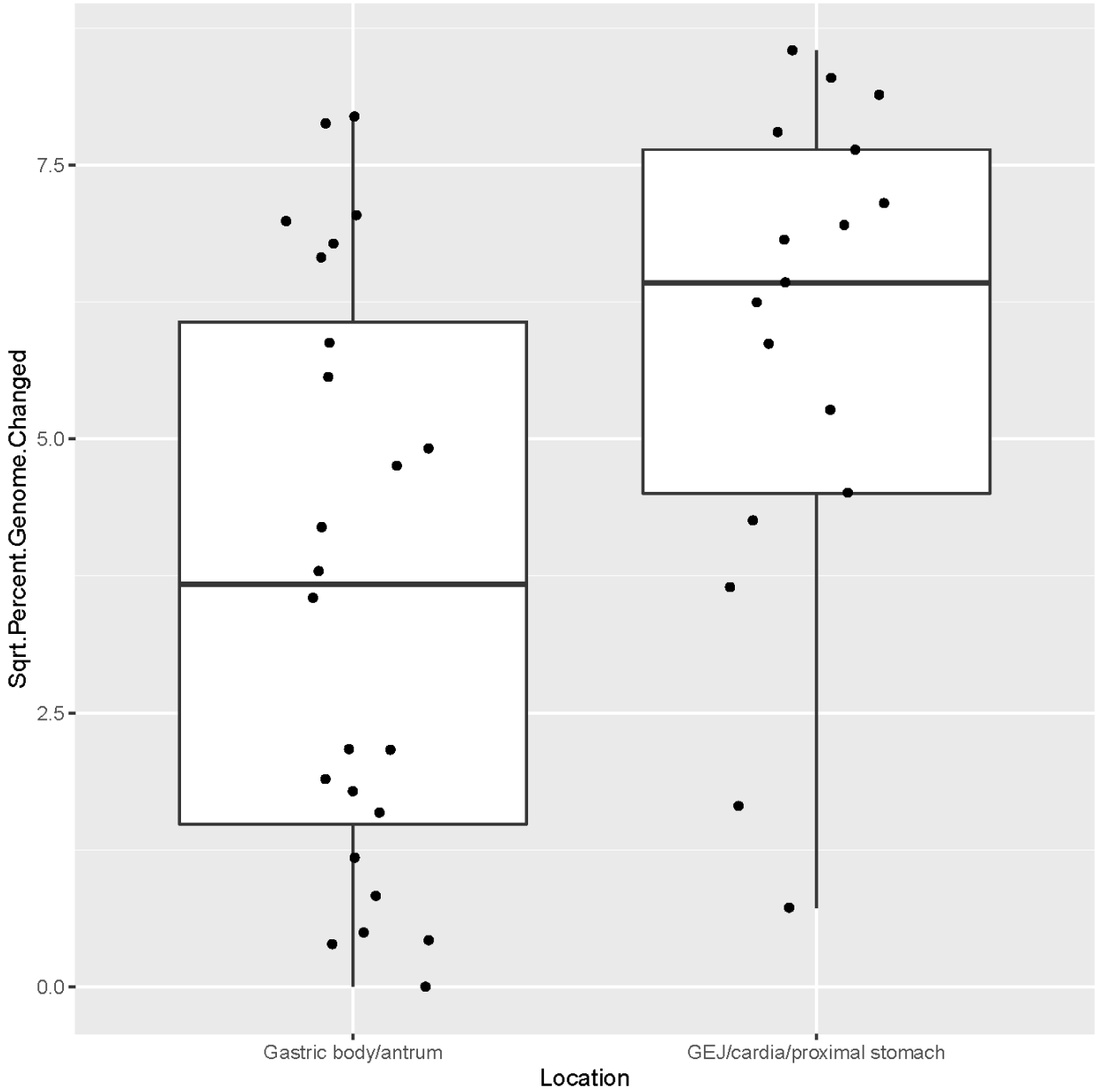
This supplementary material has been provided by the authors to give readers additional information about their work.

eFigure 1. Distribution of Percentage of Genomic Changes

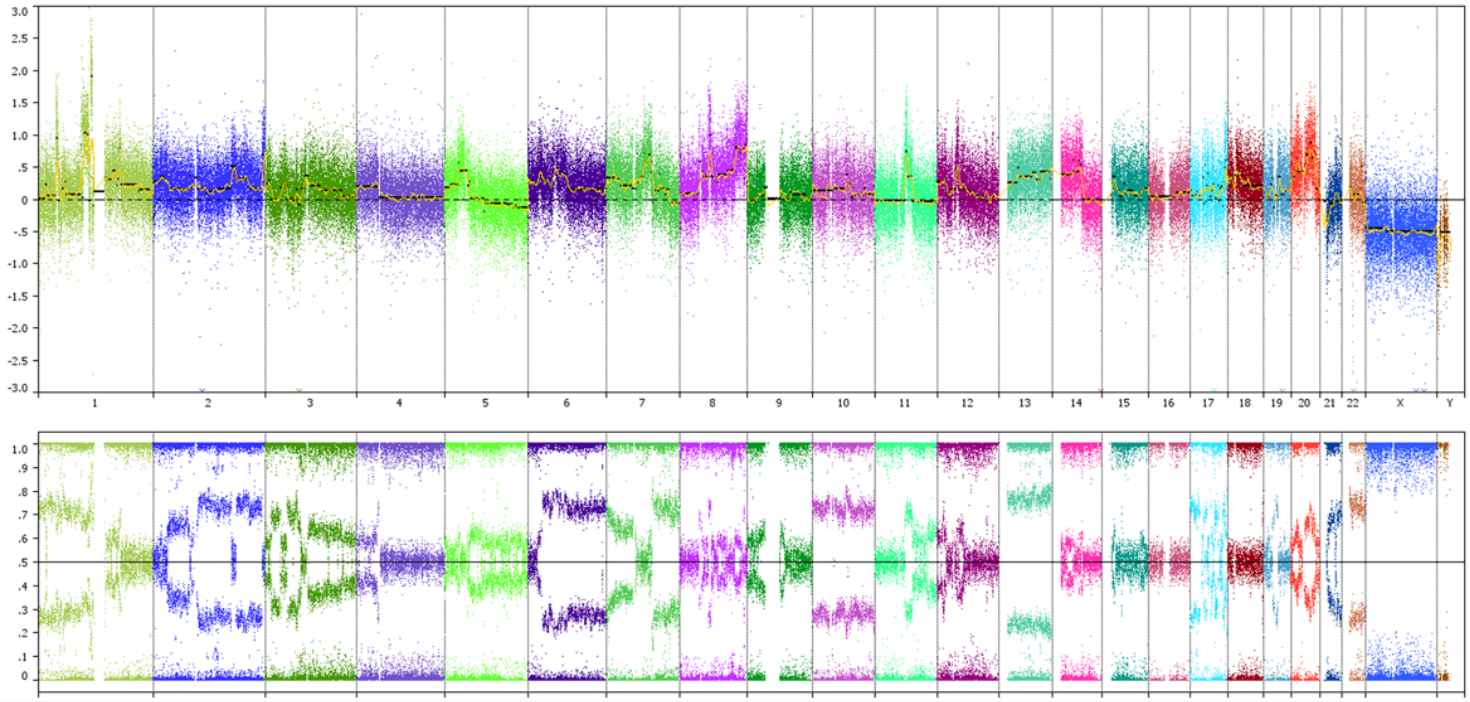
A. Box plots representing distribution of percentage genomic changes between Lauren intestinal vs. diffuse subtype histology. Lines represent the median and 25th and 75th percentiles.



B. Box plots representing distribution of percentage genomic changes between tumors arising from the GEJ/cardia/proximal stomach vs. Gastric body/antrum. Lines represent the median and 25th and 75th percentiles.

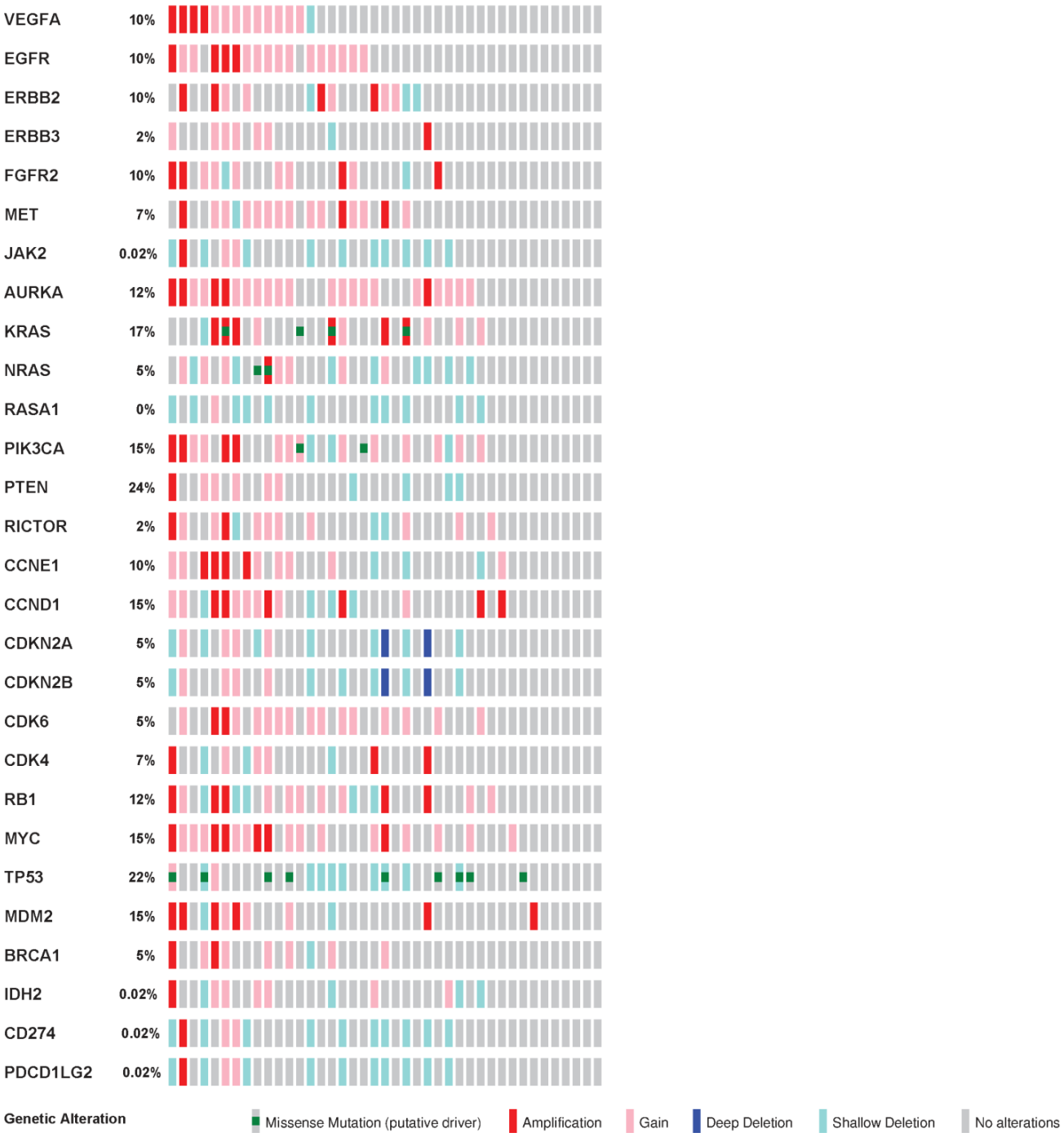


eFigure 2. A Case With Significant Alterations in Whole-Genome Copy Number and B-Allele Frequency Plot



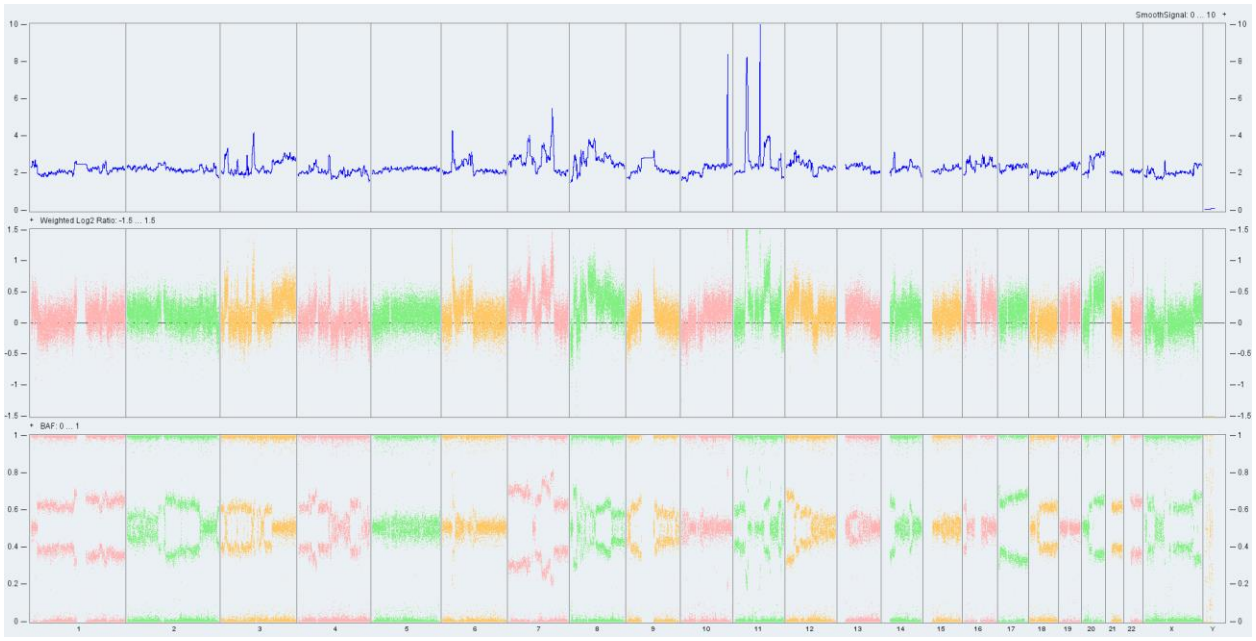
Top panel representing a case with significant alterations in whole genome copy number (y-axis, log2 scale) across multiple chromosomes (x-axis). The bottom panel represents the BAF (B allele frequency) plot (y-axis) in which multiple areas of the genome exhibit alternating retention and disruption of BAF representing both chromosomes (x-axis) with retention and loss of heterozygosity. This is indicative of chromothripsis being a major molecular event during this tumor's evolution.

eFigure 3. Oncoprint of CNAs and Mutations Detected With the OncoScan Platform Demonstrating Interpatient Tumoral Heterogeneity of Genomic Alterations of Selected Genes of Interest
A variety of oncogenic CNAs were observed across patients including high copy gains in *EGFR, JAK2, FGFR2, MET, VEGFA, KRAS, NRAS, PIK3CA, CCNE1, CCND1, CDK4, CDK6,* and *AURKA*. One sample exhibited co-amplification of *CD274* and *PDCD1LG2* (encoding PD-L1 and PD-L2), in addition to concurrent amplification of *ERBB2, JAK2, FGFR2, MET, KRAS, PIK3CA,* and *MDM2*.

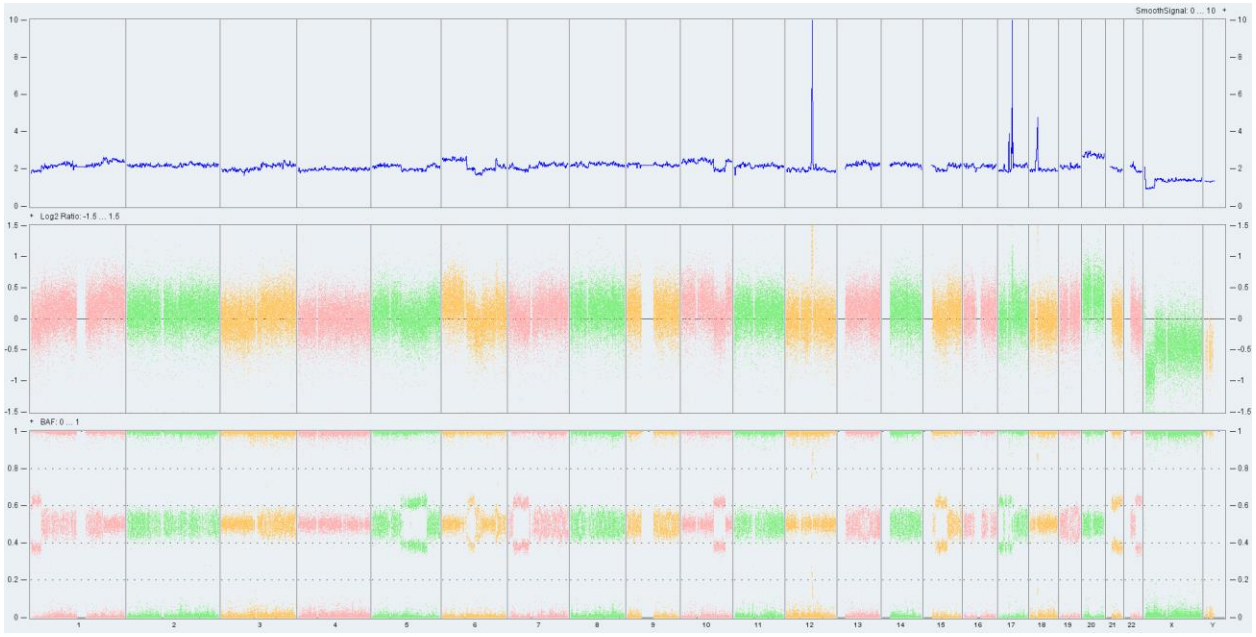


eFigure 4. OncoScan Genomic Data

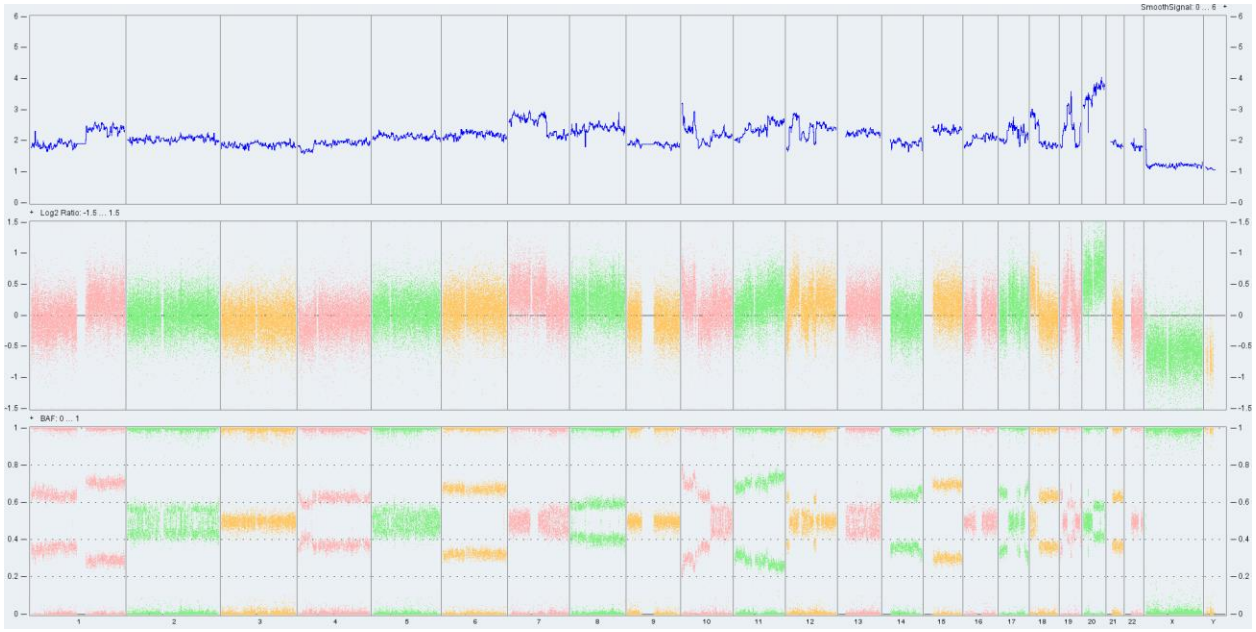
A. Oncoscan genomic data for Case 29.



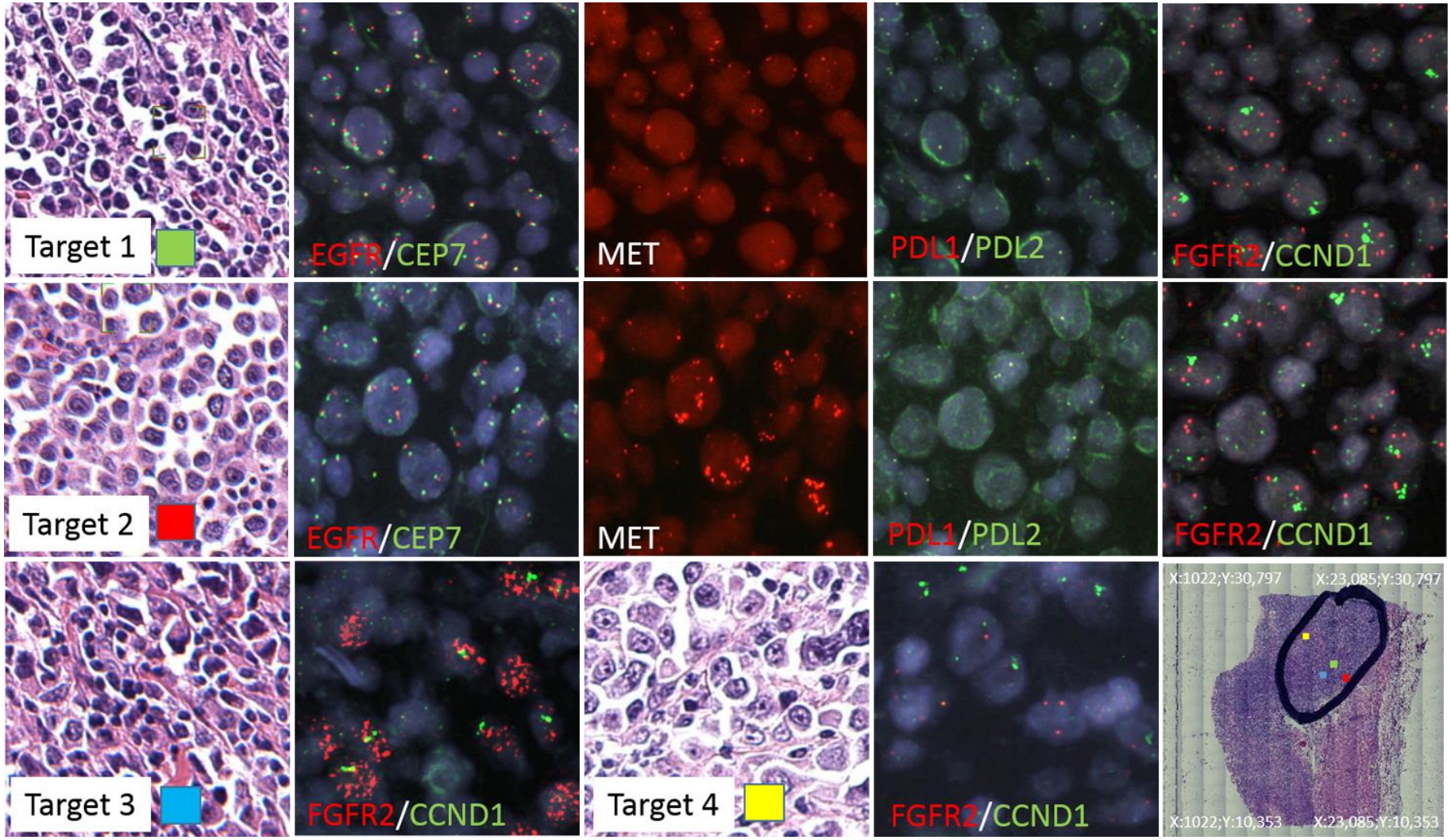
B. Oncoscan genomic data for Case 37.



C. Oncoscan genomic data for Case 21.



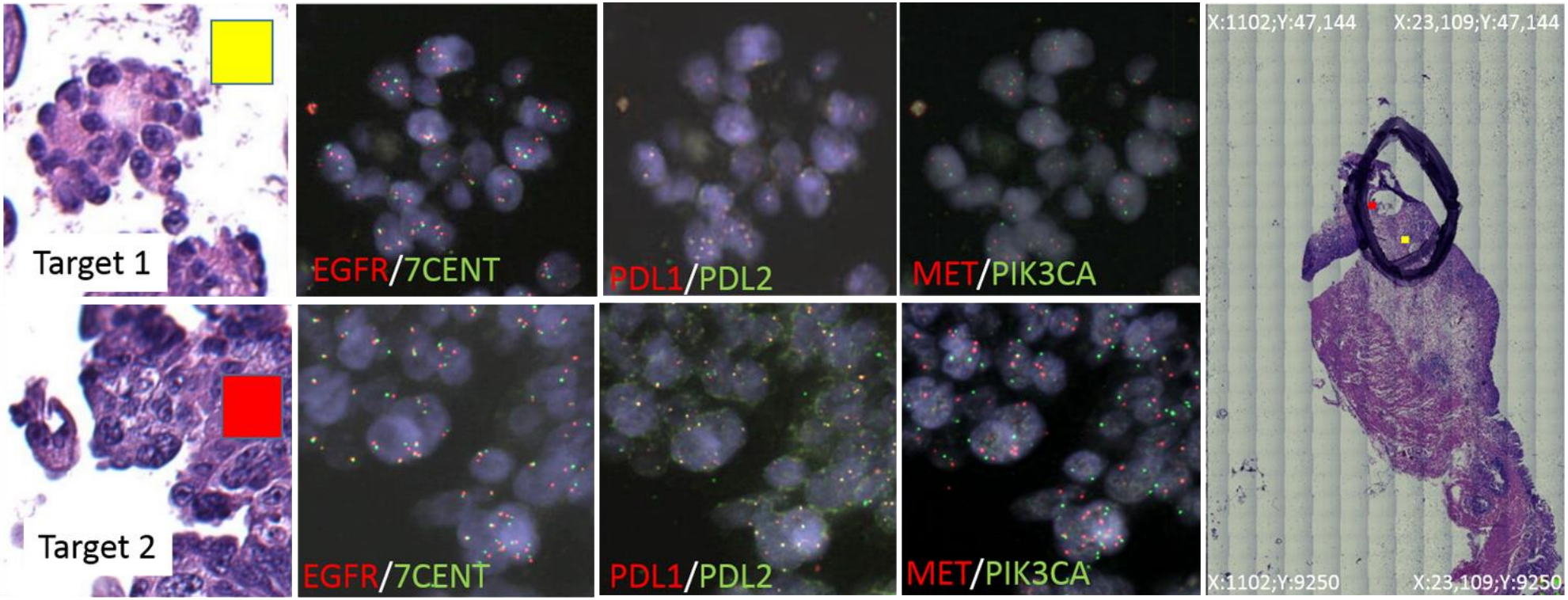
eFigure 5. Patient 29 Representing a pT3N0 Lauren Diffuse Subtype Adenocarcinoma Arising From the GEJ With CNAs in *MET, FGFR2, CCND1, EGFR, CD274*, and *PDCD1LG2*



All fluorescent images are taken at 60X magnification, whole H&E tumor slide section image is at 5X, and zoomed in H&E is at 20X. The lower right panel is the tumor area of interest circled on the H&E slide section with the x,y reference coordinates at the four corners of the image displayed in micrometer distances. The top row encompasses images from Target 1 (green square) residing at coordinates x = 14,124 µm; y = 23,706 µm and exhibited 4-7 copies of *EGFR*, 3 copies of *MET*, 1 copy of the genes encoding PD-L1/PD-L2, amplified *CCND1*, and 3-4 copies of *FGFR2*. The middle row represents Target 2 (red square) residing at coordinates x = 15,145 µm; y = 23,424 µm and exhibited 3-4 copies *EGFR*, amplified *MET*, biallelic loss of the genes encoding PD-L1/PD-L2, amplified *CCND1* and

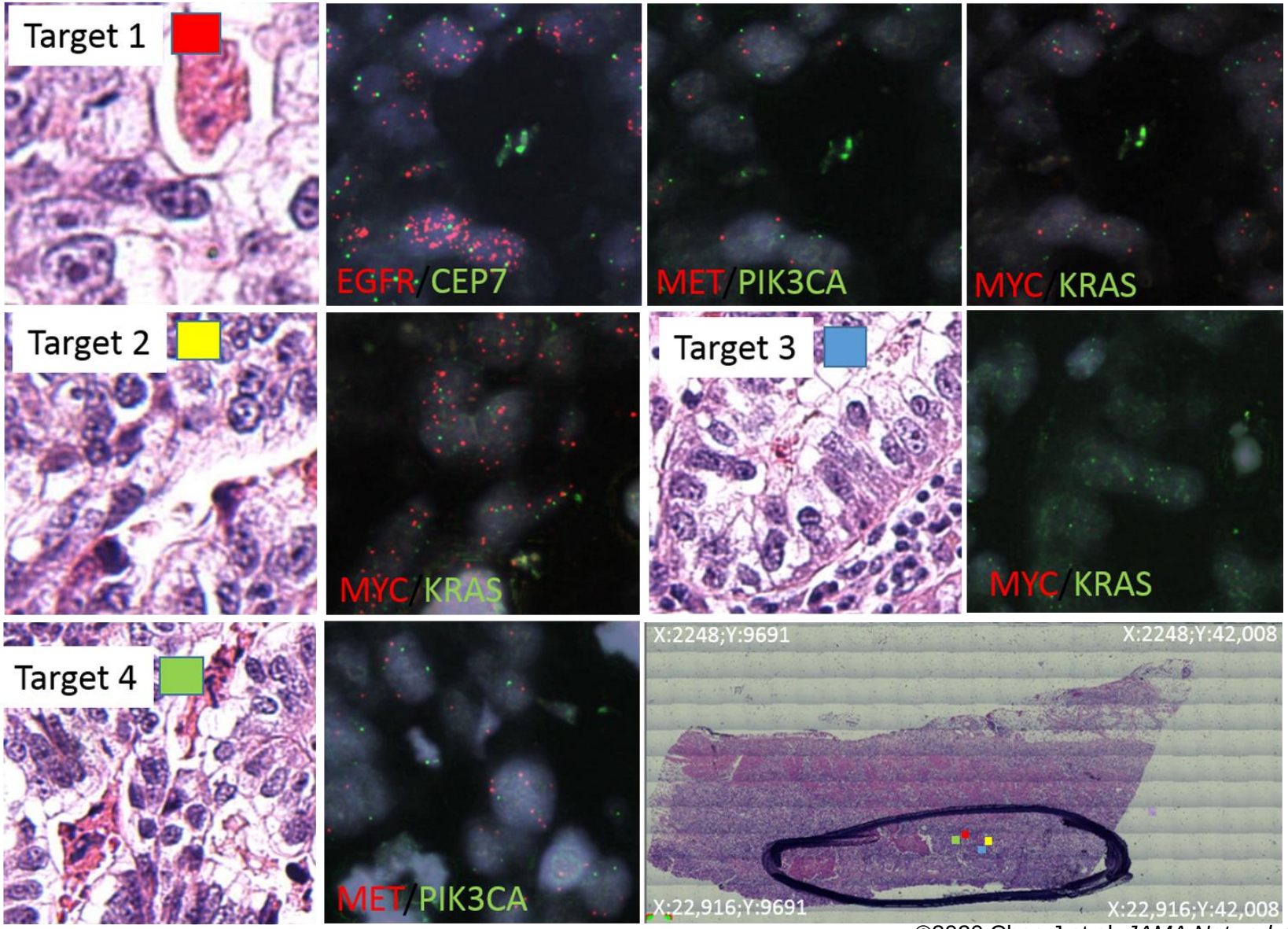
3-4 copies of *FGFR2*. Based on Oncoscan data reporting FGFR2 amplification, additional targets were captured after initial analysis. An area of highly amplified *FGFR2* was subsequently identified in Target 3 (blue square, x = 13,505 µm; y = 23,449 µm). Target 4 (yellow square) resided the greatest distance from Target 1 at coordinates x = 11,002 µm; y = 27,428 µm and exhibited normal copy number for *CCND1* and *FGFR2*.

eFigure 6. Patient 37 Representing a pT2N3a Lauren Intestinal Subtype Adenocarcinoma Arising From the Gastric Cardia



Oncoscan analysis revealed major CNAs of *CD274, PDCD1LG2, EGFR, MET*, and *PIK3CA.* Far right panel exhibits the tumor area of interest circled on the H&E slide section with the x,y reference coordinates at the four corners of the image displayed in micrometer distances. Top row encompasses Target 1 (yellow square) at coordinates x = 13,654 µm; y = 33,594 µm exhibiting amplification of the genes encoding PD-L1 and PD-L2, but modest copy number gains of *EGFR, MET,* and *PIK3CA*. Bottom row represents Target 2 (red square) at coordinates x = 11,292 µm; y = 34,466 µm exhibiting amplification in all 5 oncogenes. All fluorescent images are taken at 60X magnification, whole H&E tumor slide section image is at 5X, and zoomed in H&E is at 20X.

eFigure 7. Patient 21 Representing a pT3N0 Lauren Intestinal Subtype Adenocarcinoma Arising From the Gastric Antrum



Oncoscan analysis reported amplification of *EGFR, MYC, KRAS*, *MET* and *PIK3CA.* Bottom right panel exhibits the tumor area of interest circled on the H&E slide section with the x,y reference coordinates at the four corners of the image displayed in micrometer distances. Top row encompasses Target 1 (red square) at coordinates x = 16,854 µm; y = 24,633 µm and exhibited amplified *EGFR* with normal copy numbers of *MET, MYC*, and *PIK3CA* and modest copy number gain in *KRAS*. Target 2 (yellow square) resided at coordinates x = 17,387 µm; y = 25,310 µm and demonstrated amplified *MYC* with normal *KRAS* copy number. Target 3 (blue square) at coordinates x = 17,682 µm; y = 25,188 µm exhibited the converse of Target 2 with amplified *KRAS* but normal *MYC* copy number. Target 4 (green square) at coordinates x = 17,407 µm; y = 24,485 µm demonstrated amplified *MET* with normal copy number for *PIK3CA*. All fluorescent images are taken at 60X magnification, whole H&E tumor slide section image is at 5X, and zoomed in H&E is at 20X.