



FIGURE S6: Global point-MLE solutions, segmentation, and statistical analyses for 4-plex and 24-plex gene targeting, related to Figures 5-6. (A-B) Point-MLE solutions for samples 1 and 2, respectively. Grid-lines are used to denote spacings of L_{diff} . (C-D) Position-agnostic cells segmentation for samples 1 and 2, respectively: grey = ACTB/beacon, white = GAPDH, green = GFP, and red = RFP. (E-F) Point-MLE solutions for samples 4 and 5 (see gene sets in Tables S5 and S6). All targeted genes were found at non-zero frequencies except for GRIN2D, MEA1, FAM170B, and C11ORF44. Additional gene colorings include hypothetically MDA-MB-231 enriched genes (yellow) and hypothetically BT-549 enriched genes (magenta). (G-H) Position-agnostic cells segmentation for samples 4 and 5, respectively. (I-J) Rarefaction plots for samples 4 and 5, respectively (with top and bottom curves indicating the same data subsets described in Figure 2). (K-L) Zoomed-in portions of the image windows outlined in panels E-F, with *de novo* sequenced transcript variants of the CDC25B gene shown, and their divergent sub-sequences highlighted. (M-N) Correlograms of log-transformed read-abundances from Klijn et al 2015 compared to the mean fraction of total UMIs observed in each putative cell (assigned as MDA-MB-231 if it had more GFP than RFP, or as BT-549 otherwise). (O-R) Mean RMS distance traversed for each UEI associating the specified target gene versus the size of the corresponding gene insert for samples 1, 2, 4, and 5, respectively.