

Figure S1 Nuclei flow-sorting strategy to enrich for tumor cells. Representative example for three different sorting strategies depending on the tissue type. (A) Fresh-frozen tissue was stained with DAPI (DNA content/ploidy) represented on the x -axis. Autofluorescence is depicted on the y -axis. Where only DAPI sorting was possible, diploid populations were considered as germline for WES analysis. Aneuploid populations represent a mixture of tumor cells with proliferating G2M cells, tumor cells that underwent WGD and proliferating G2M non-tumor cells. Identification of diploid tumor cells is not possible by pure DAPI sorting. Diploid sorted samples with flat diploid genomes were considered as germline for sequencing (B) To distinguish tumor cells from proliferating G2M cells, pan-cytokeratin (pCK) was used (y -axis) in FFPE samples. Aneuploid tumor population were pCK-positive and distinguishable from pCK-negative cells. Diploid pCK-negative populations were used as germline. (C) Some samples displayed four populations. Tumor population (diploid pCK+) and non-tumor population (diploid pCK-) were diploid and ‘aneuploid’ in the G2M phase. Therefore, the ploidy is approximately the doubled amount (aneuploid pCK- and aneuploid pCK+). In general, all populations were sorted and sequenced, if amount of DNA was sufficient.

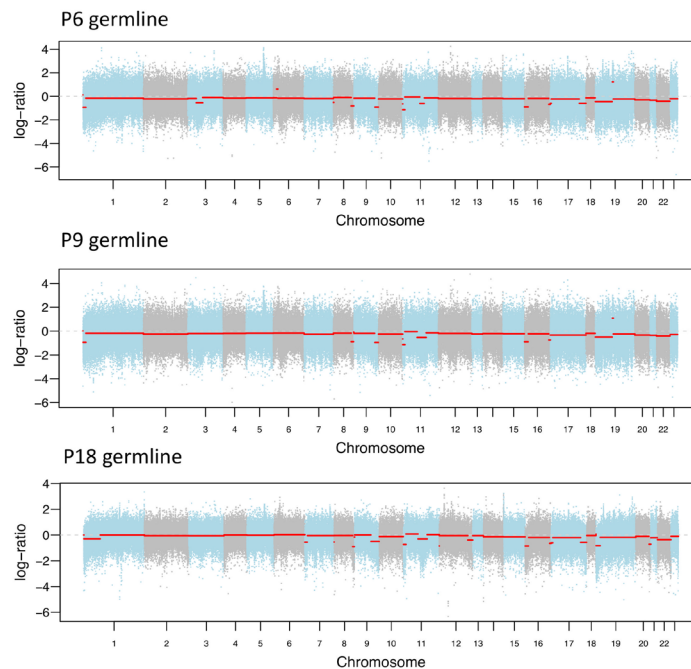


Figure S2 Copy number plots of diploid populations in samples sorted by DAPI alone. Illustrations show no copy number aberration in the diploid populations of P6, P9 and P18, using the P103 germline sample (sorted by DAPI and pCK) as reference. Data was generated using Whole Exome Sequencing. The slight irregularities of CNA plots are technical noise resulting from the comparison between FF and FFPE samples.

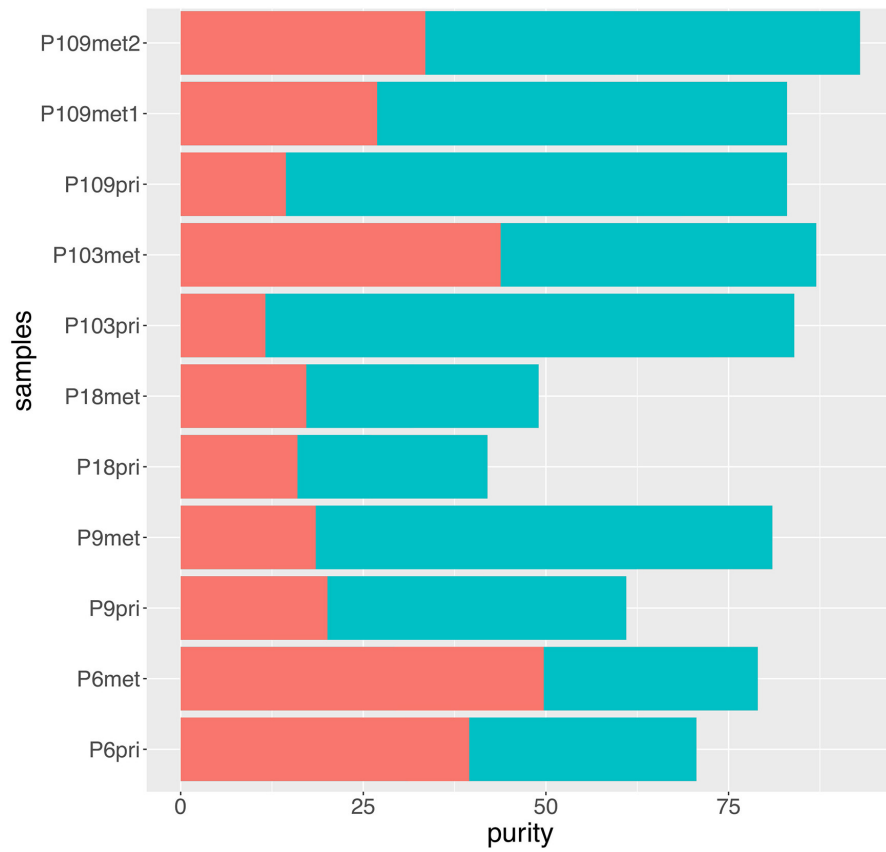


Figure S3 Purity before and after sorting. Bar plot displays the purity before sorting (red) and after sorting (blue). Purity after sorting was calculated by FACETS. pri = primary tumor; met = metastasis.

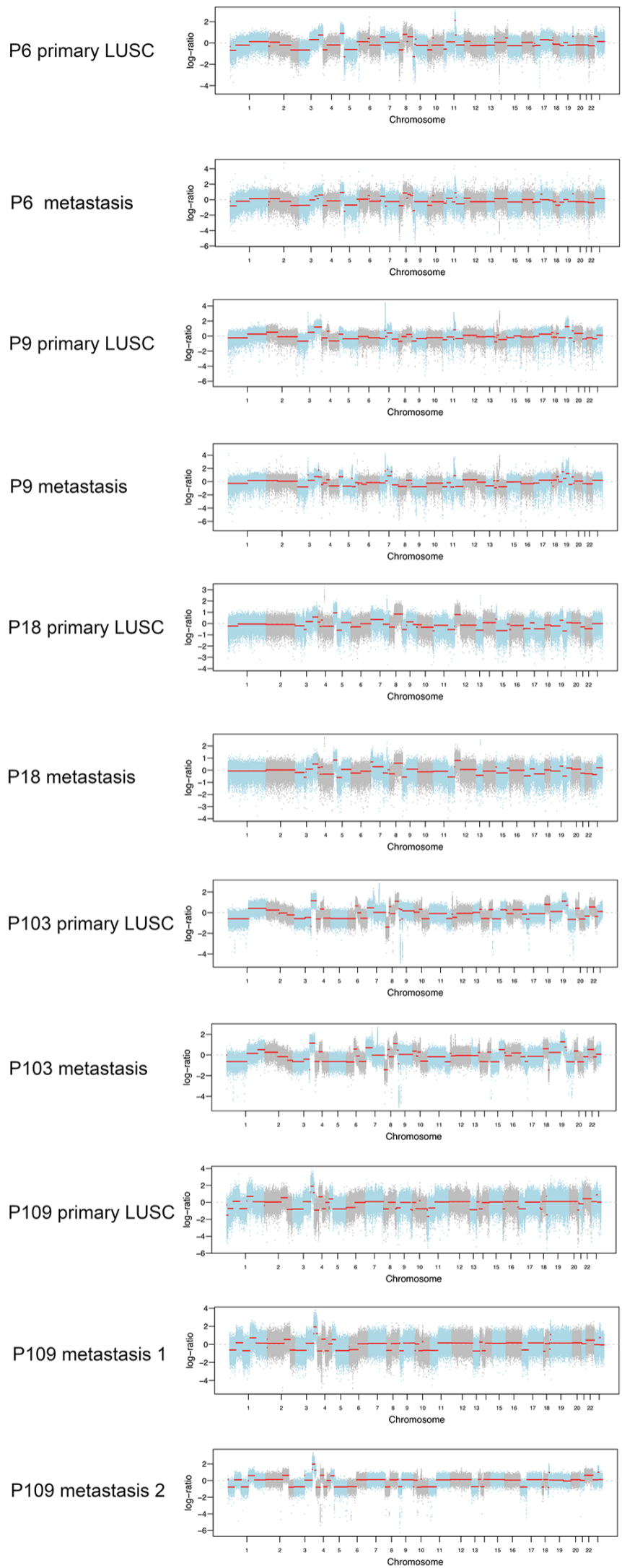


Figure S4 Copy number aberration plots of all tumors. Illustration shows copy number aberration plots of all 11 tumor samples from five patients. Plots were generated by FACETS.