Absolute quantification of somatic DNA alterations in human cancer

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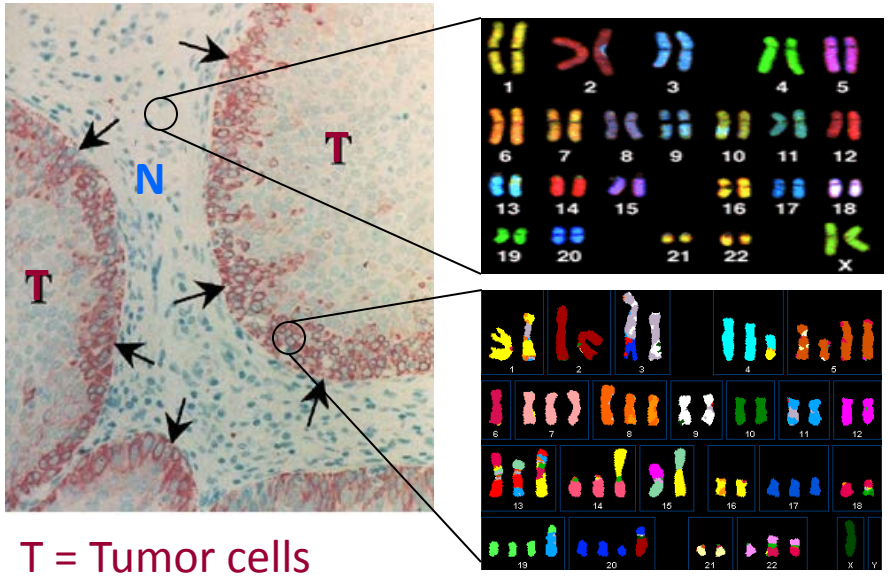
Overview

1) Inference of tumor purity / ploidy, copy-numbers per cell (ABSOLUTE)

1) Analysis of somatic point-mutations using ABSOLUTE

1) Analysis of genome doublings in human cancer development

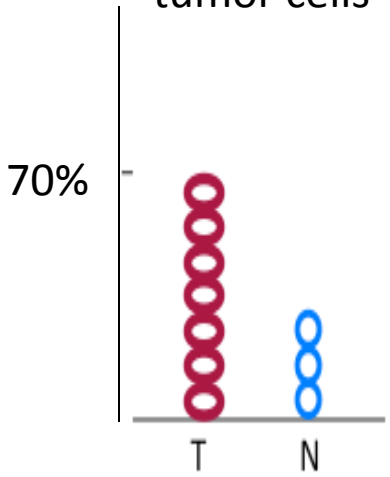
High throughput characterization of cancer genomes



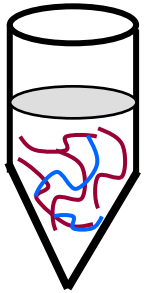
T = Tumor cells
N = Normal cells

Ploidy = mass of DNA in units of normal haploid genome mass. Here ~2.7.

Purity = fraction of tumor cells



Aliquot of mixed tumor and normal DNA

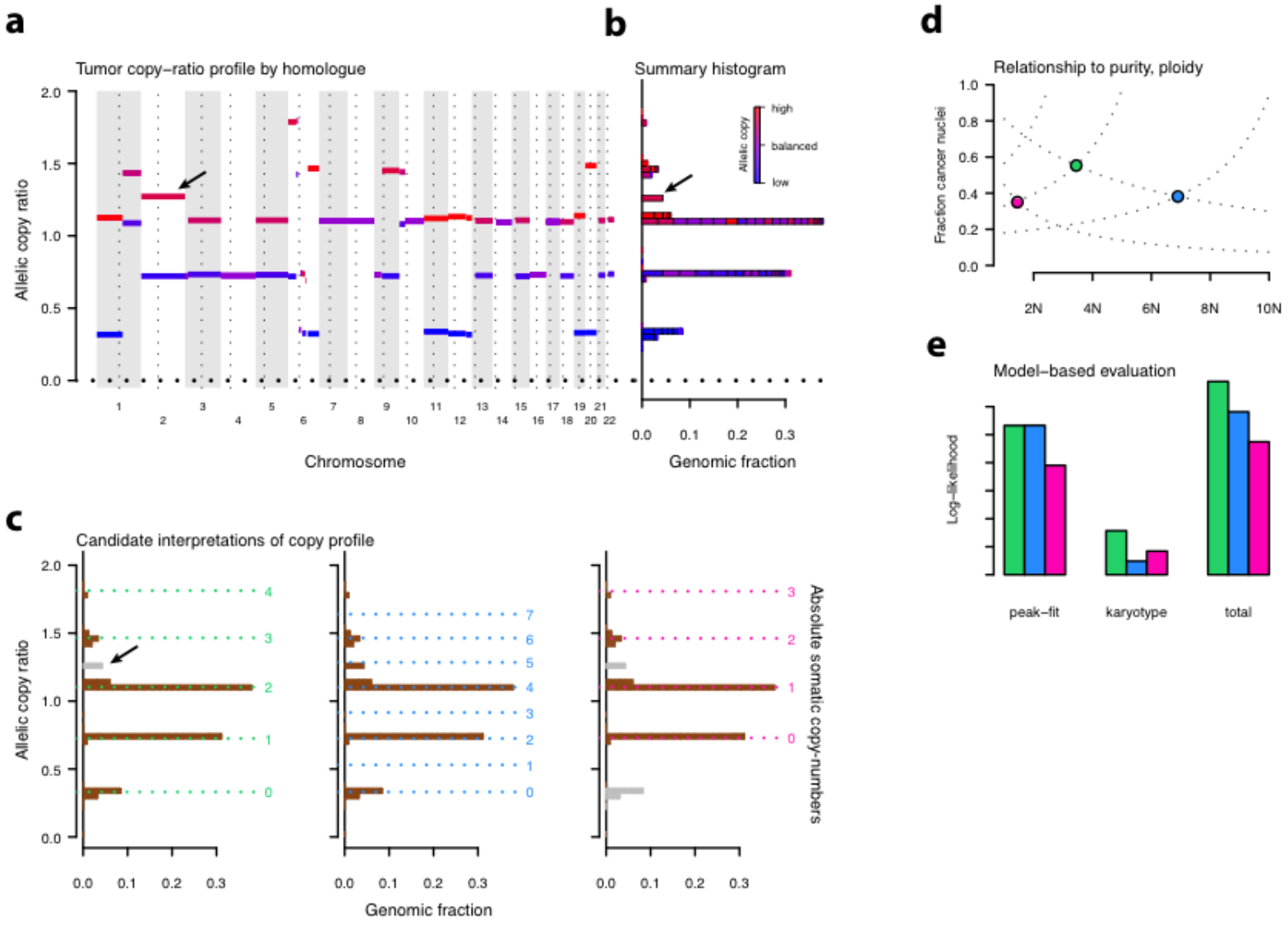


Illumina sequencing

SNP-array hybridization

Observed copy-number signal is proportional to *locus concentration*, both for sequencing and hybridization methods: dependant on sample purity and ploidy.

Inference of purity and ploidy (ABSOLUTE)



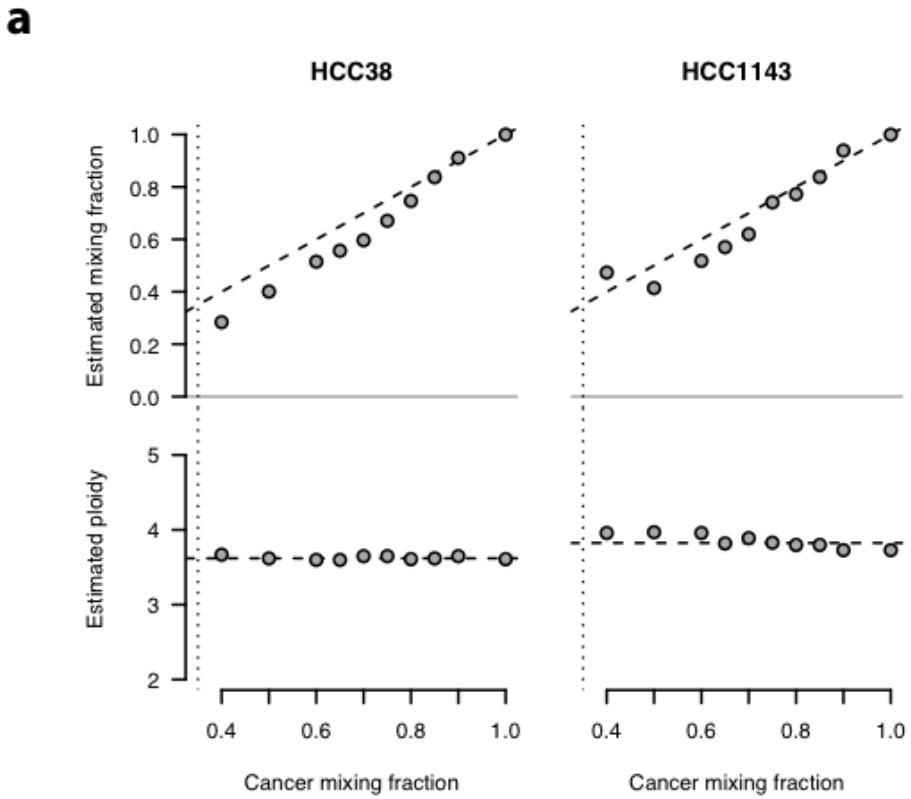
Validation

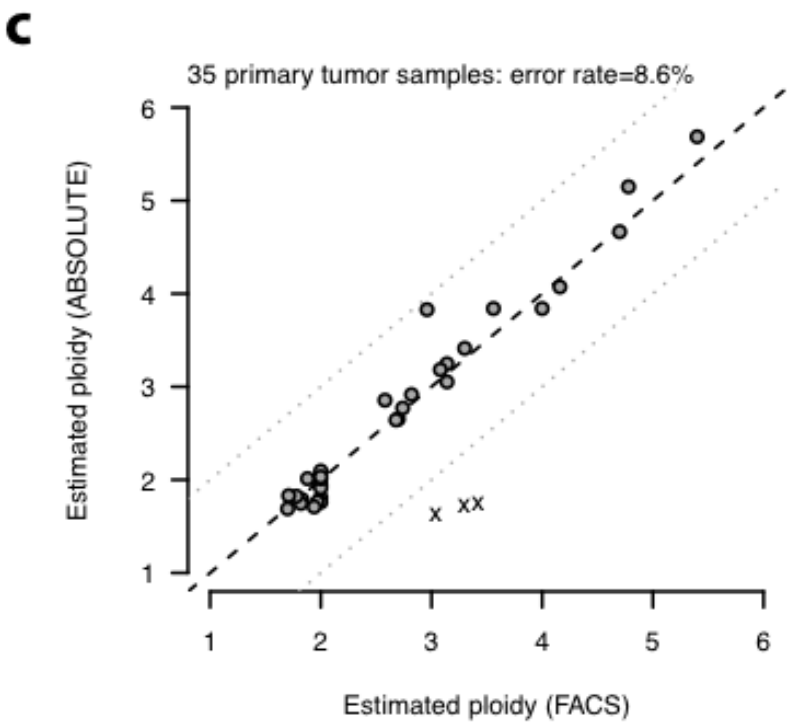
Purity

Cancer / normal mixing experiment

Ploidy

FACS analysis of primary OvCa samples





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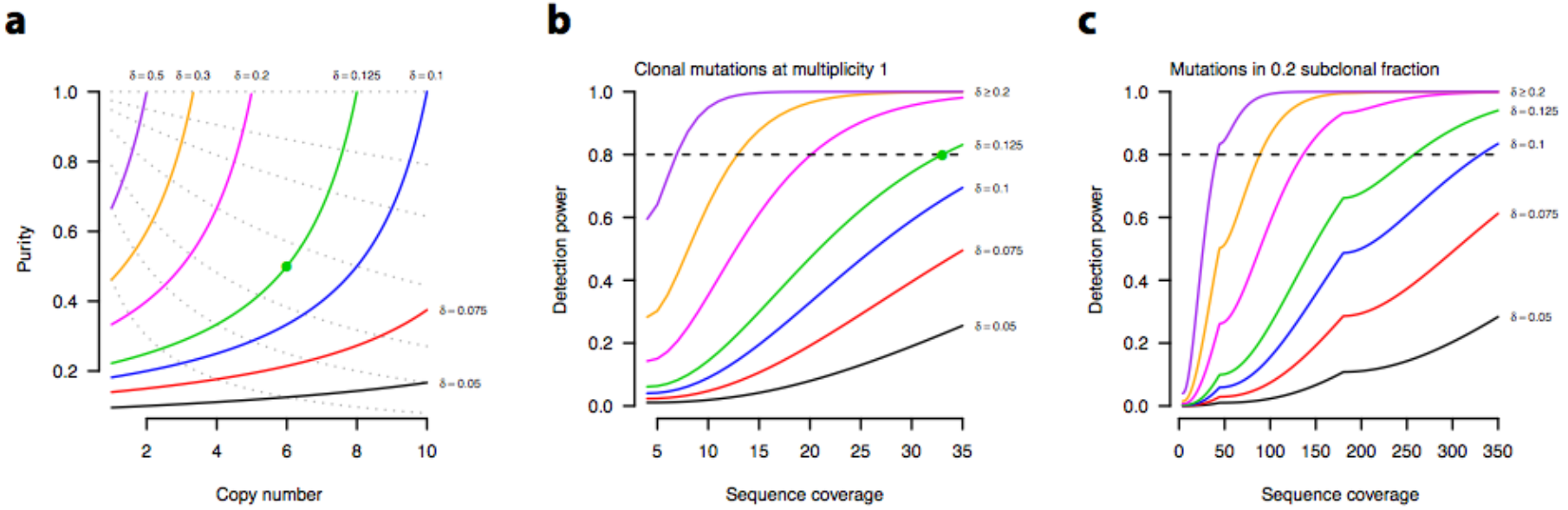
Purity and ploidy determine power to detect mutations



Clonal

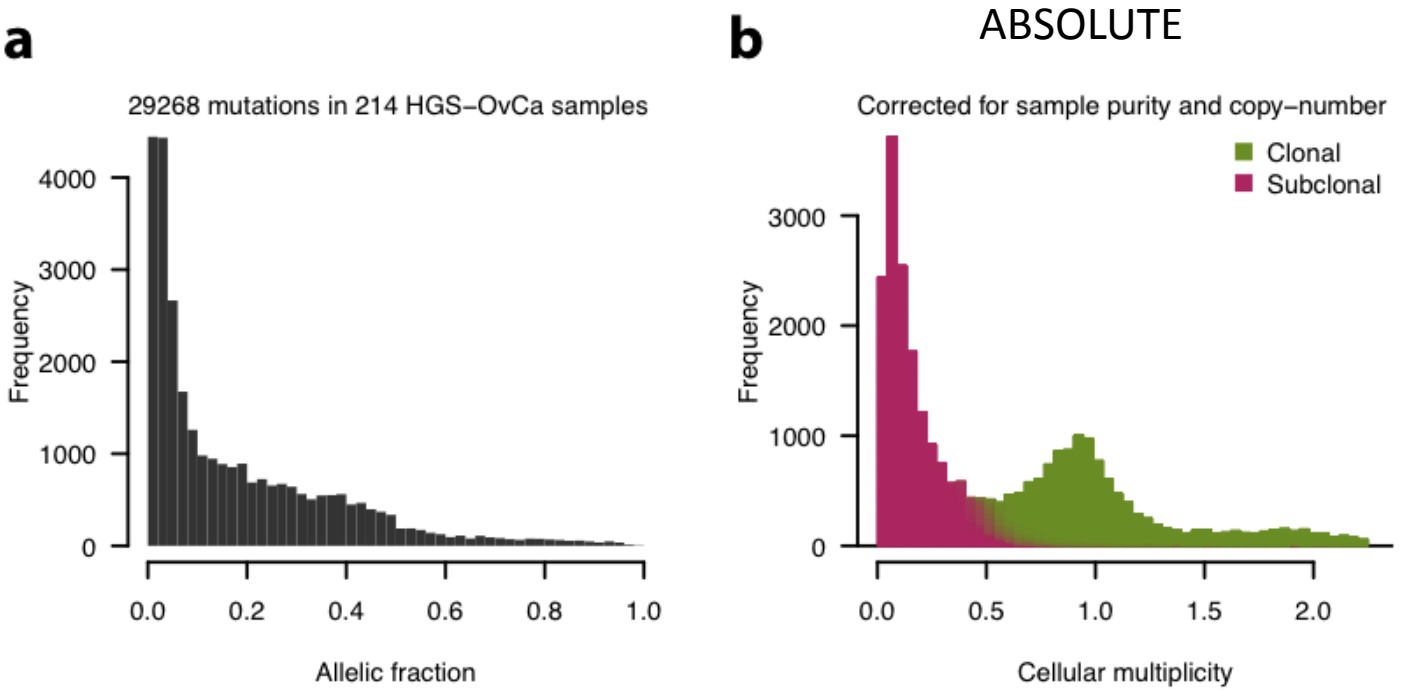
Subclonal

Identification of subclonal point-mutations by sequencing

E.g., sequencing results in *x* A's and *y* G's at a mutated locus: allelic- fraction is *x* / (*x*+*y*)

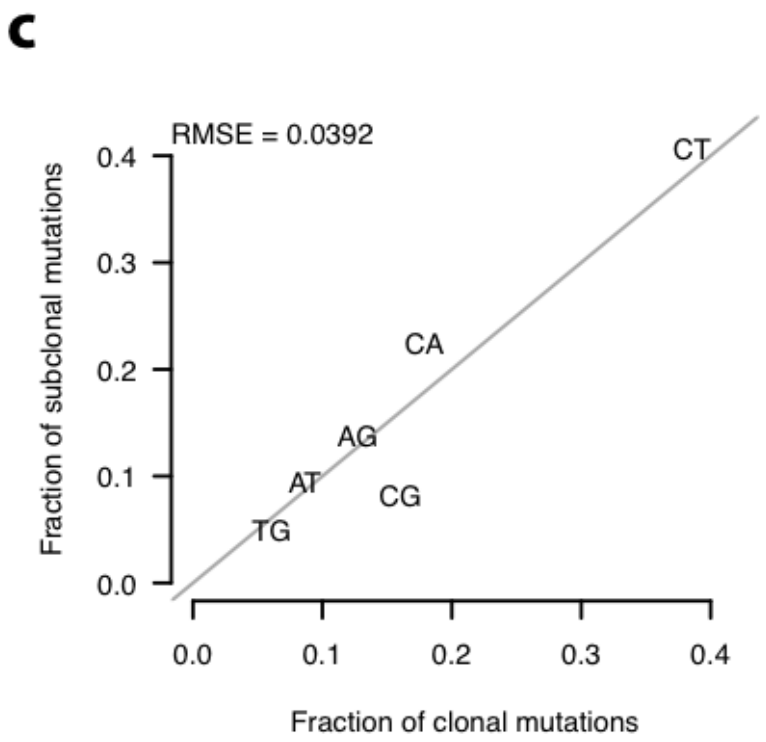
Discrete allelic-fractions are obscured by tumor purity and local copy-number.

Resolved with ABSOLUTE: change units to *cellular multiplicity* (integral allele-count)

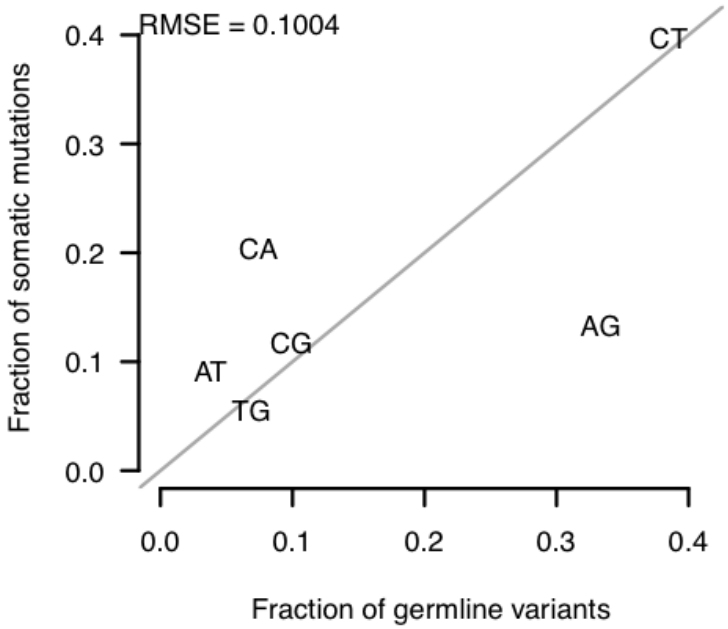


Common mechanism for clonal / subclonal mutations

Equivalent nucleotide substitution frequencies for clonal and subclonal point-mutations. Rules out contamination



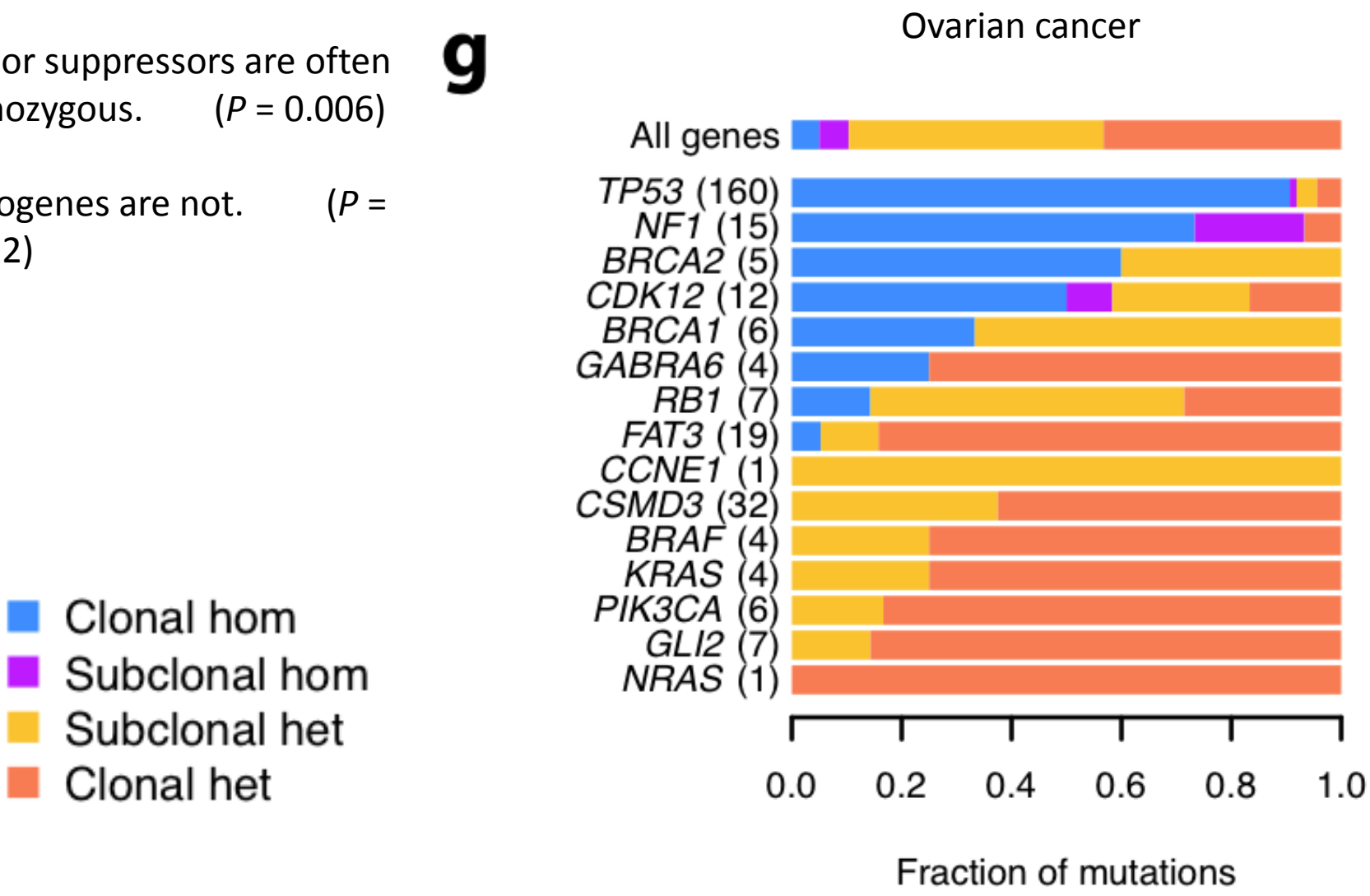
Compare to germline SNPs



Classification of point-mutations by multiplicity

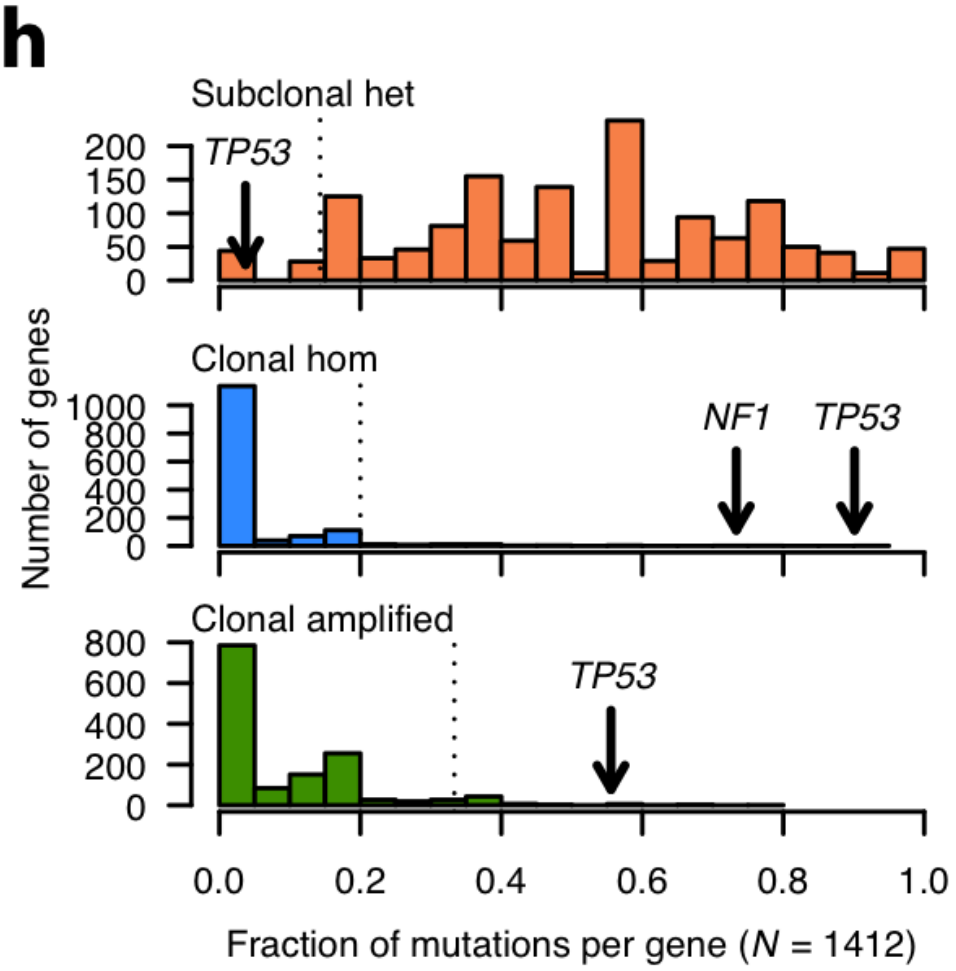
Tumor suppressors are often homozygous. (P = 0.006)

Oncogenes are not. (P = 0.012)



Identification of *TP53* as early event in ovarian cancer

TP53 mutations occur prior to gain of chr17



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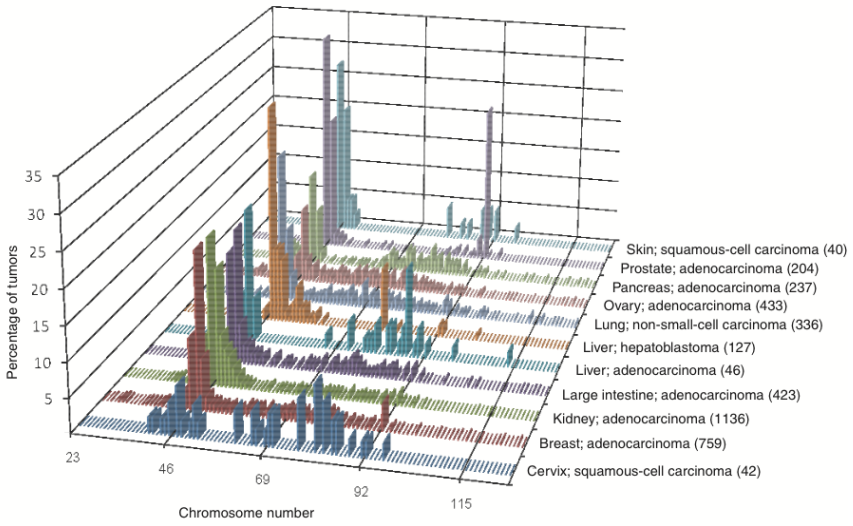
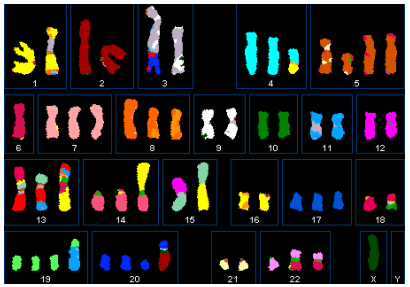
Bimodal distribution of ploidy in human cancer

Mitelman data (Storchova *et al*. 2008)



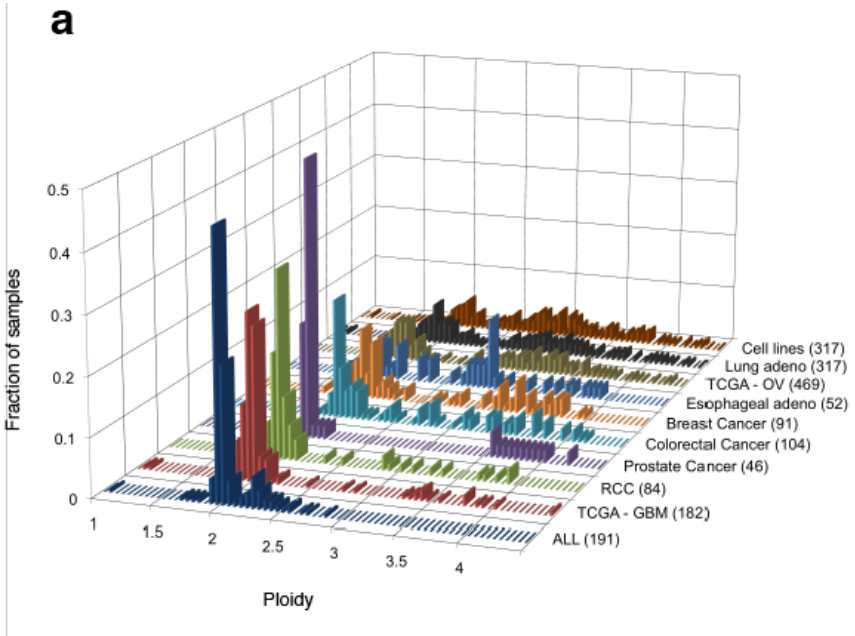
Cytogenetics (SKY)



e.g. 57 chromosomes

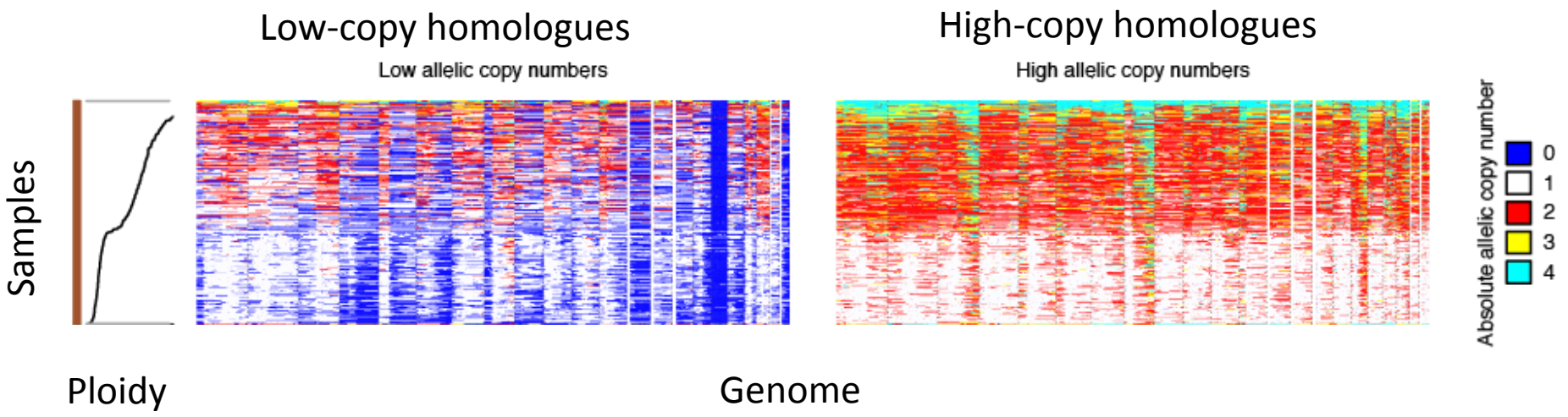
ABSOLUTE

Tumor-derived DNA (SNP arrays)



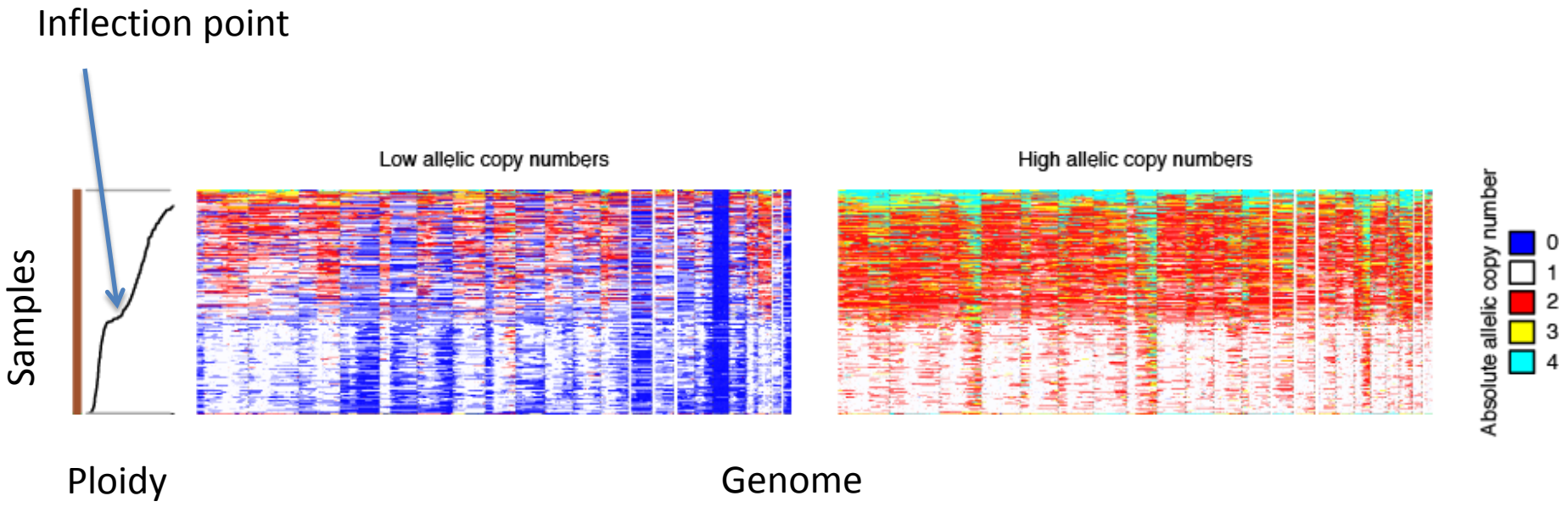
Visualizing absolute allelic copy-numbers

Example: High-grade serous ovarian carcinoma

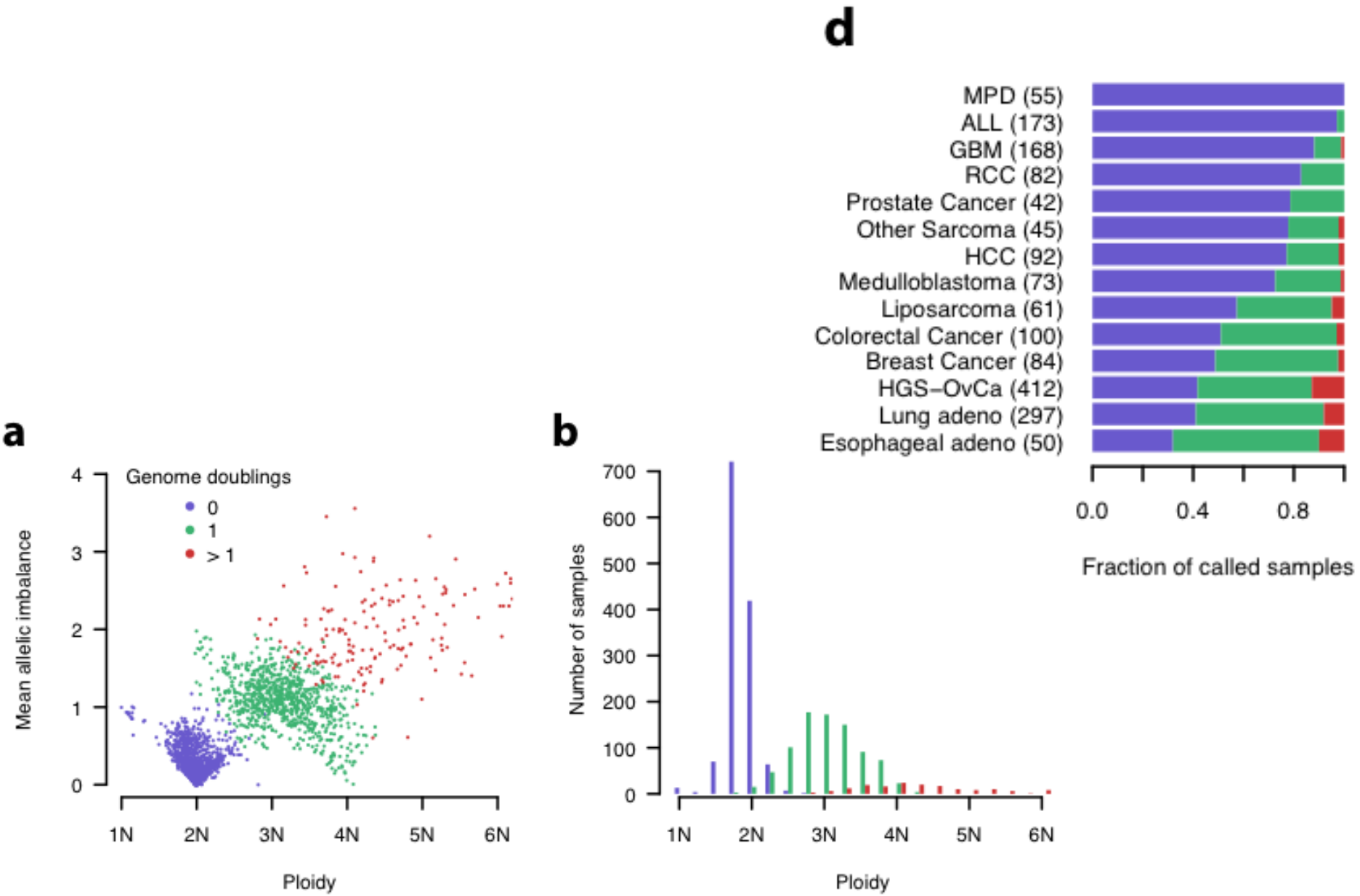


Inference of genome doubling

High ploidy samples evolved via a genome doubling event



Frequent whole genome doublings in human cancers

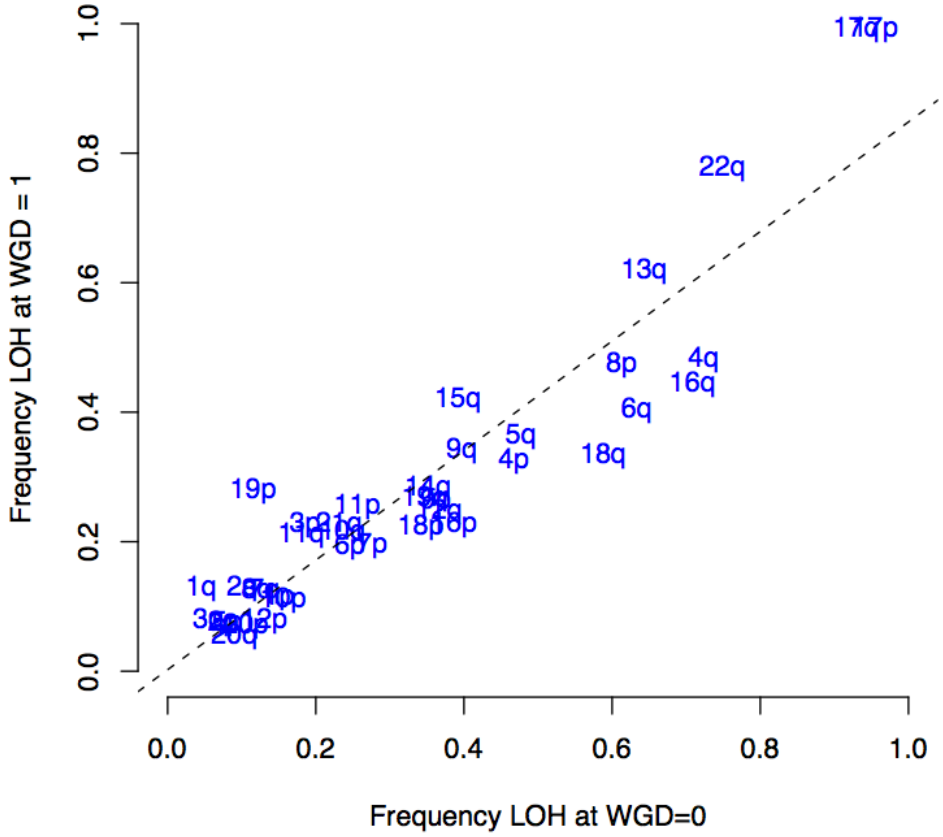


Genome doubling occurs *after* aneuploidy

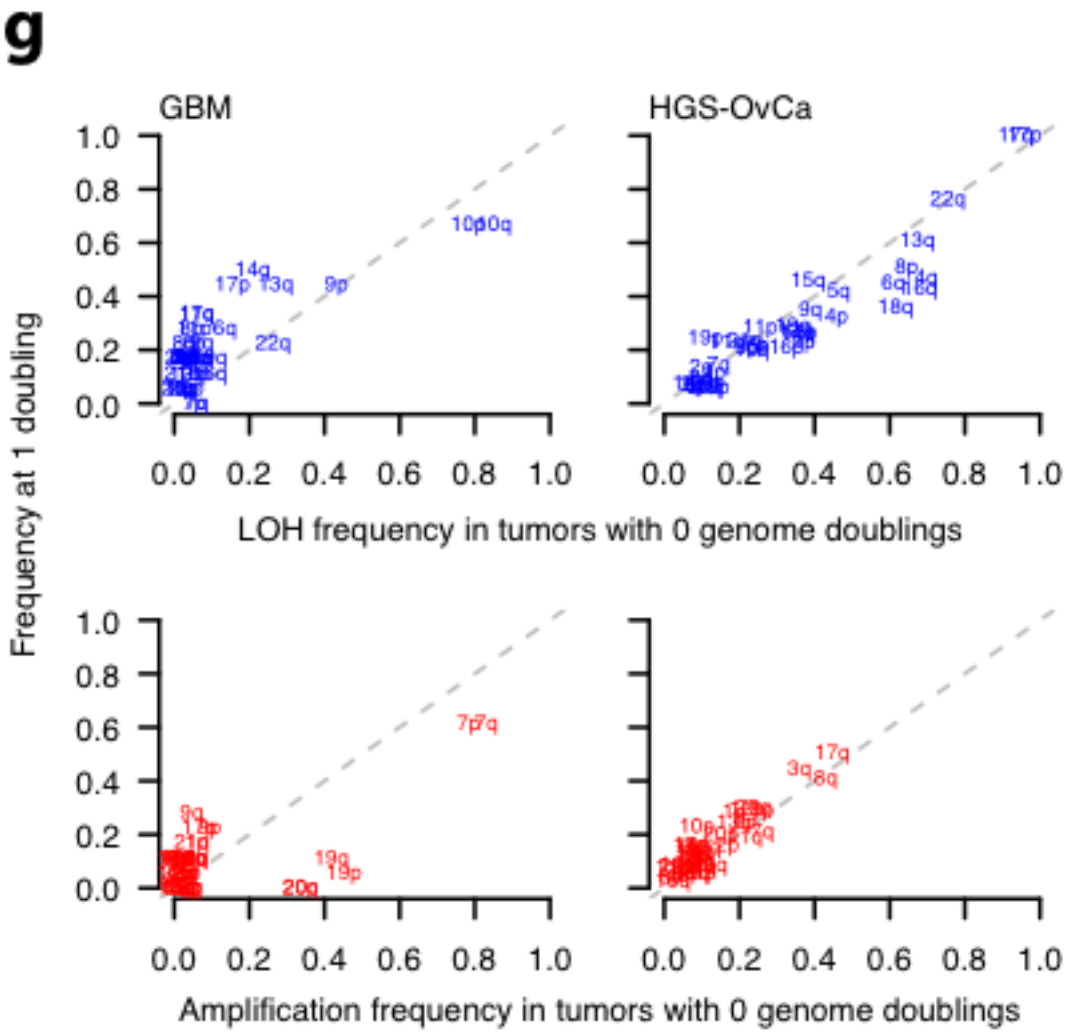
Similar frequencies of arm-level deletion (LOH) with and without genome doubling

Simplest explanation: LOH precedes doubling

Tetraploidization is not an initiating oncogenic event in ovarian cancer



Genome doubling occurs *after* aneuploidy



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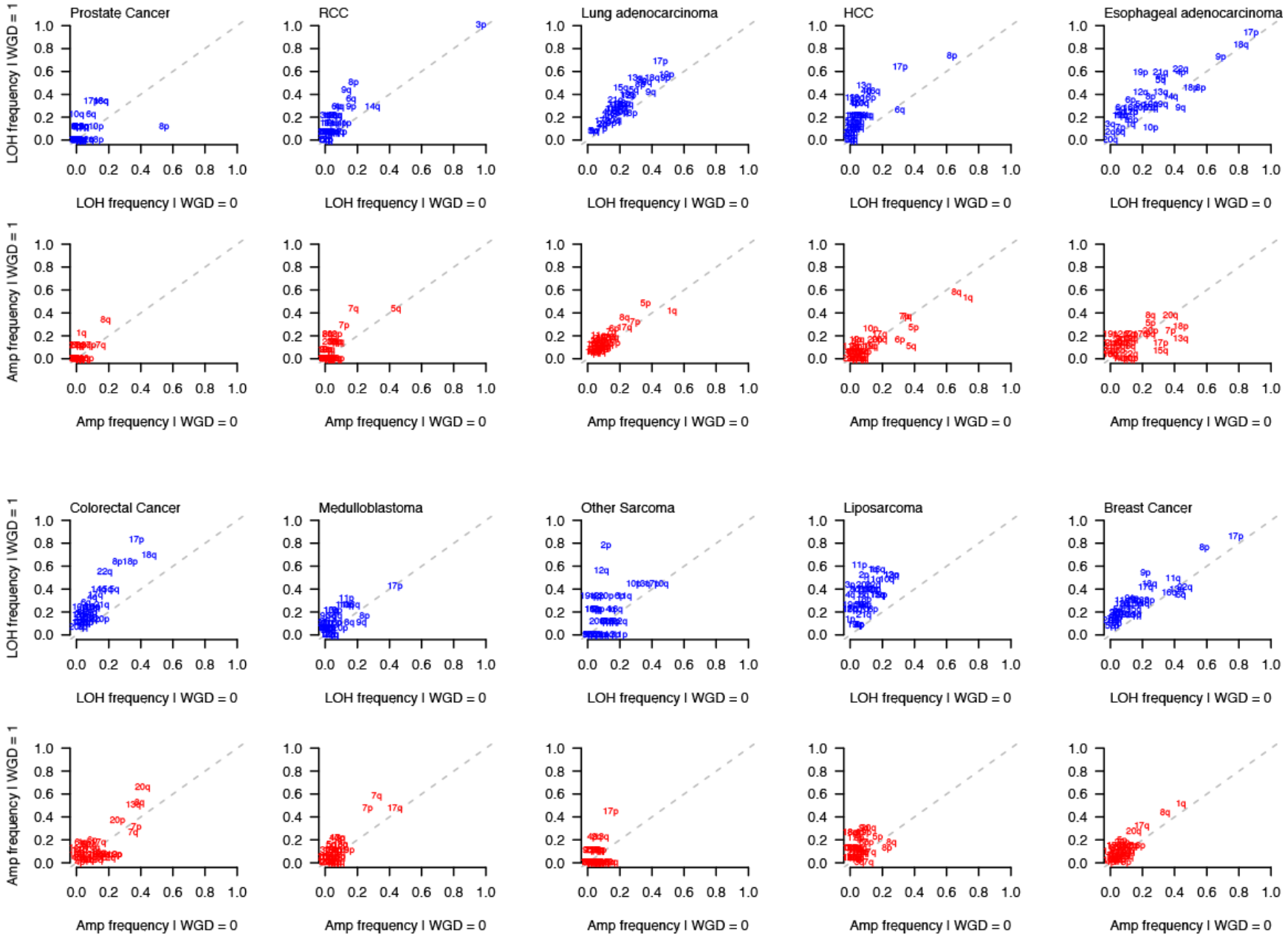
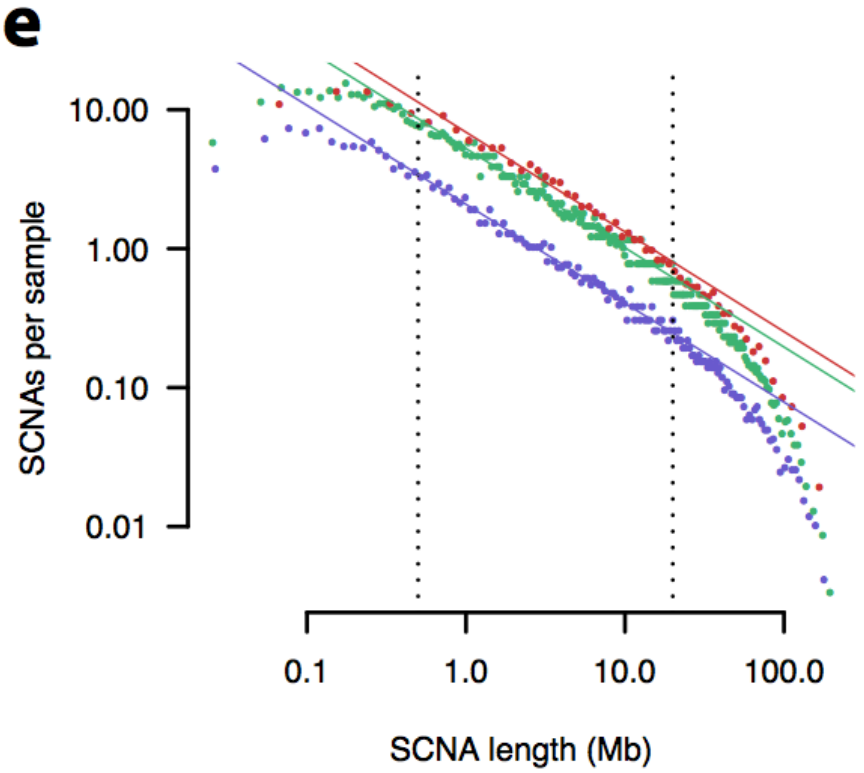


Genome doubled samples have more copy alterations

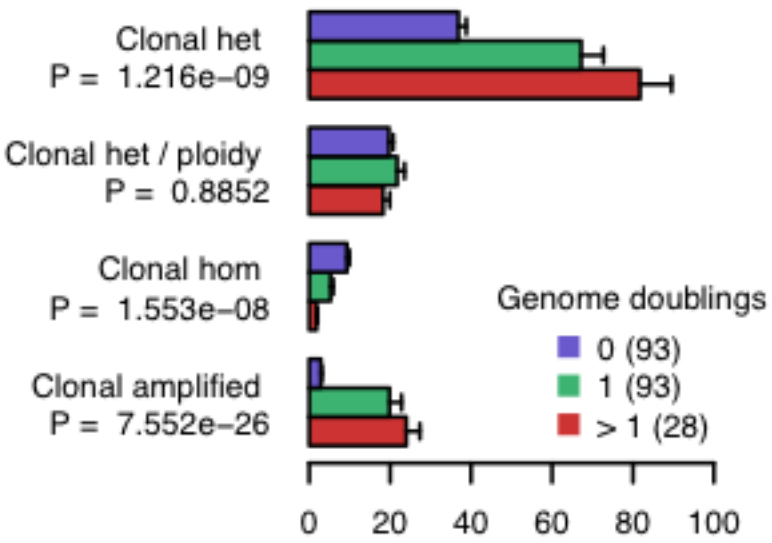
Linear fit to log length vs. log frequency: power law scaling with exponent ~0.71, *regardless of genome doubling*

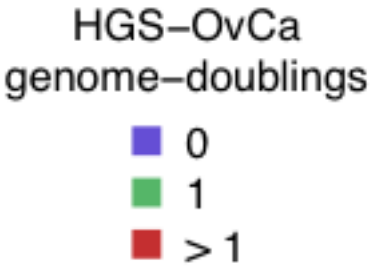


HGS–OvCa genome–doublings

- 0
- 1
- > 1

Genome doubled ovarian cancer evolves differently





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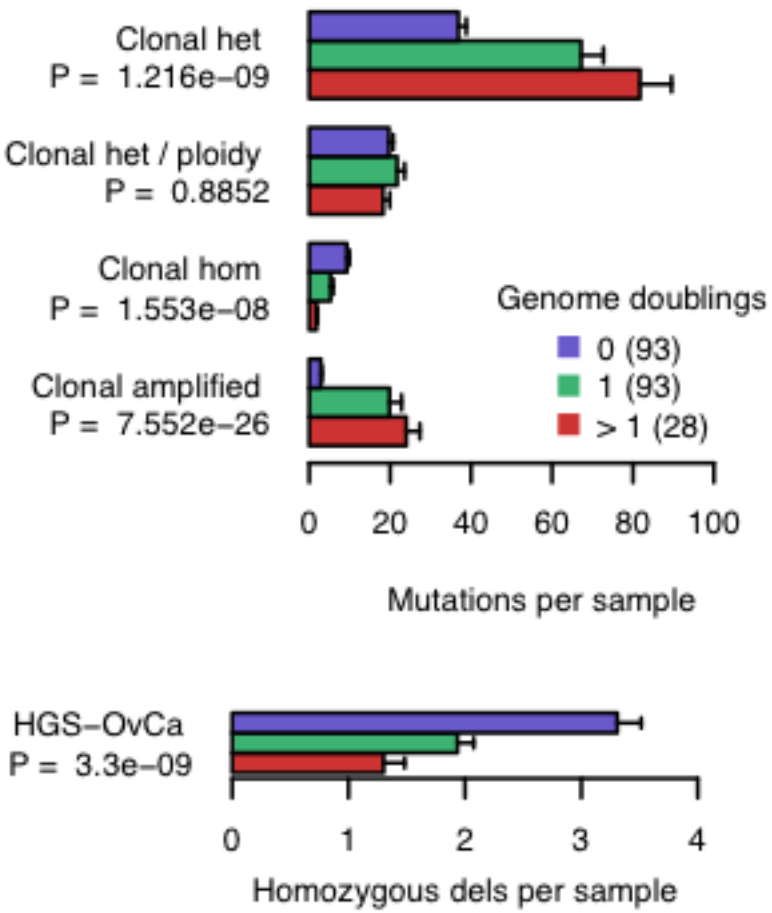
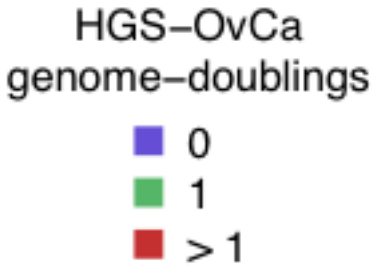




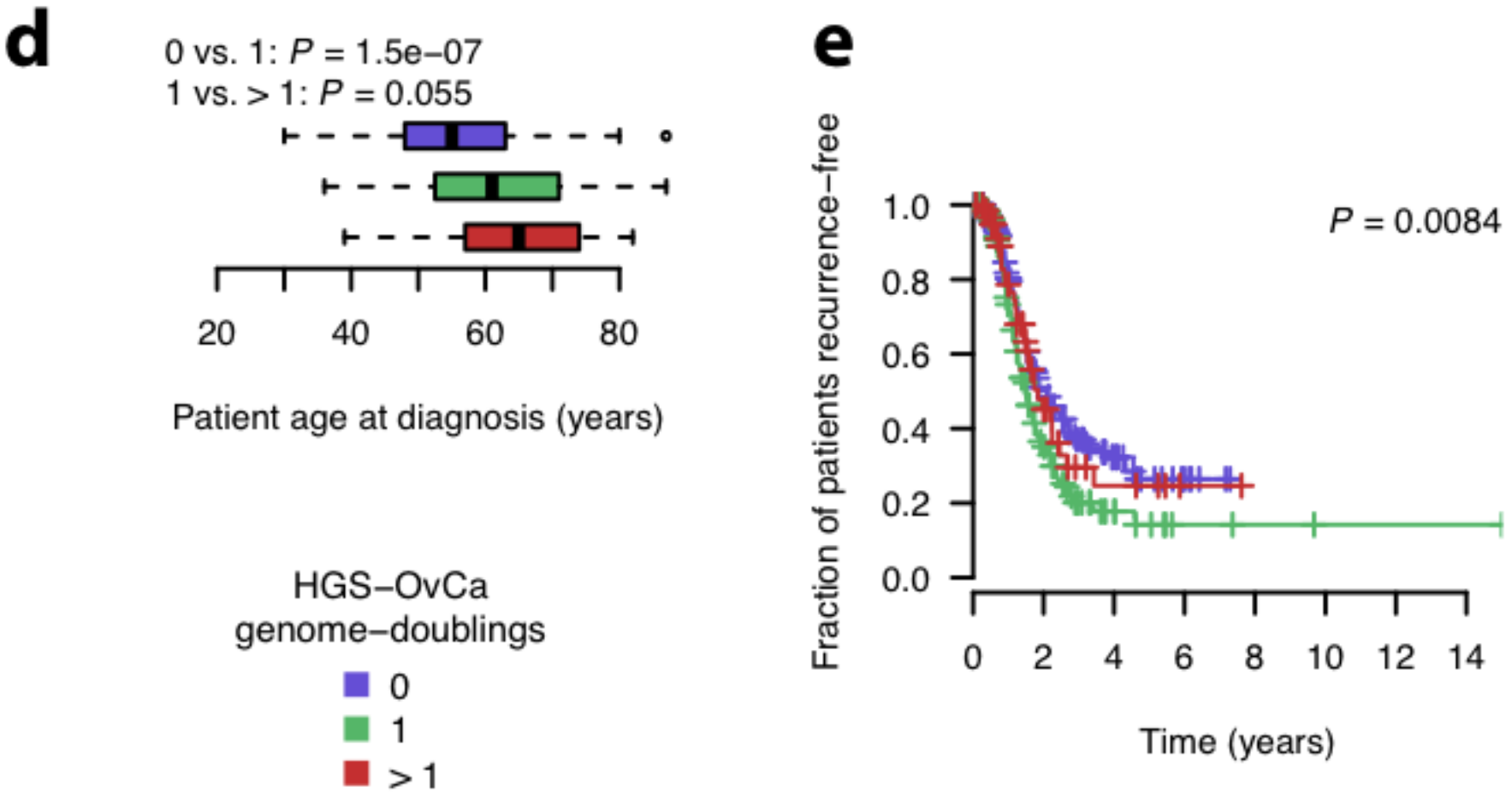
13/15 mutations in *NF1* occurred in non-doubled samples, in which case they were homozygous (*P* = 0.002)

Selection acts specifically on *recessive* inactivation of NF1.

No *amplified* mutations in *NF1* were observed in doubled samples; *NF1* mutators *do not progress via genome doubling.* In contrast to p53

Clinical correlations with genome doubling

Ovarian carcinoma



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