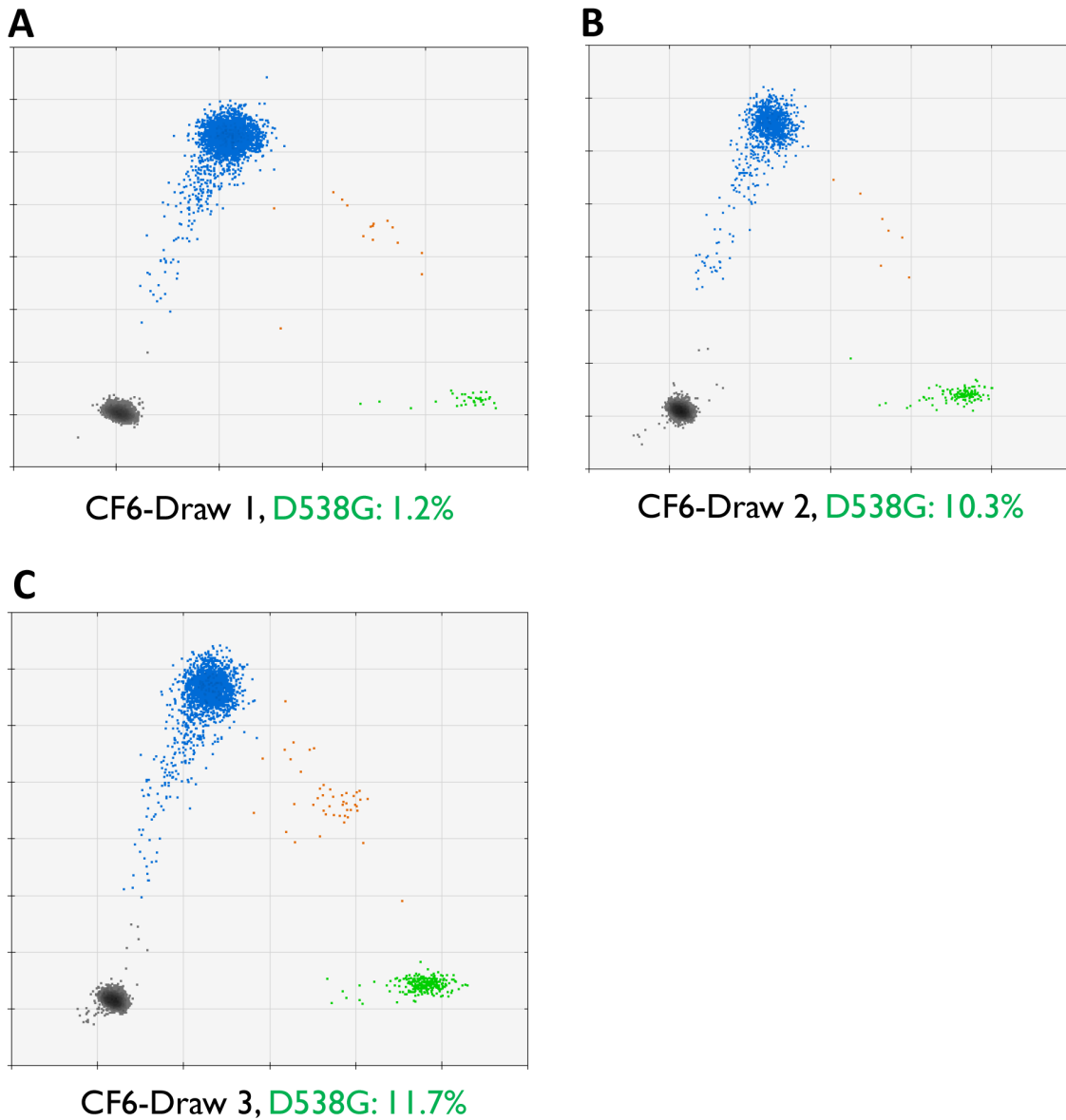
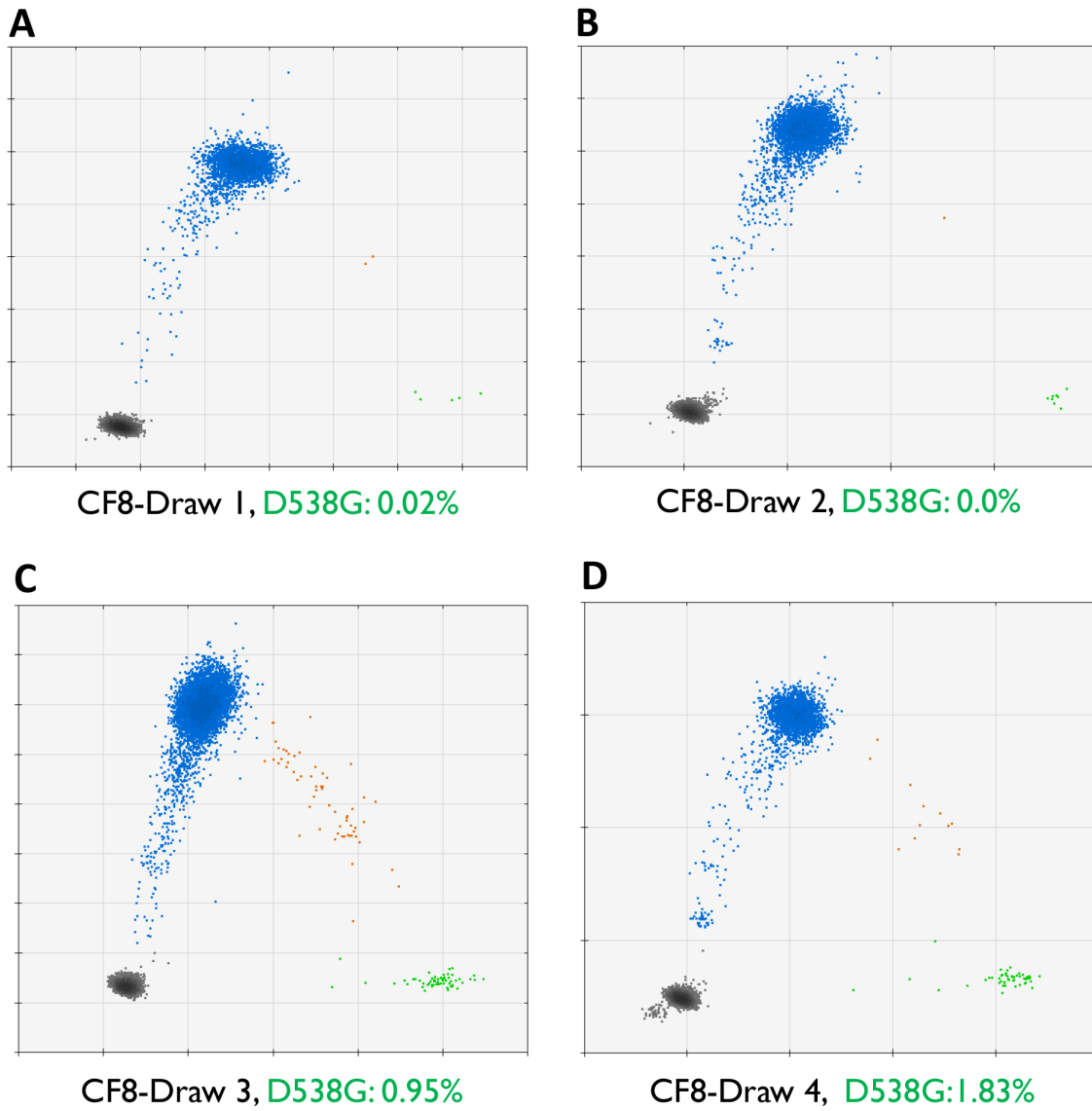


## Detection of ESR1 mutations in circulating cell-free DNA from patients with metastatic breast cancer treated with palbociclib and letrozole

### SUPPLEMENTARY FIGURES AND TABLES

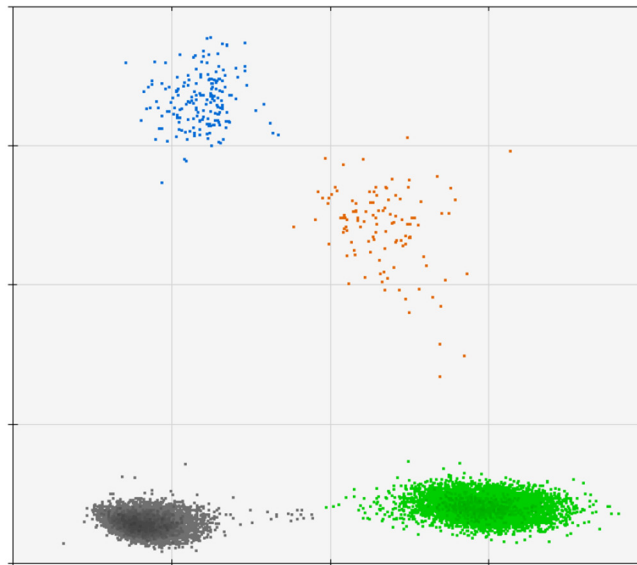


Supplementary Figure S1: ESR1-D538G mutant allele frequency in serial blood draws is indicated for patient CF6 using data from a representative experiment (after subtraction of background noise).



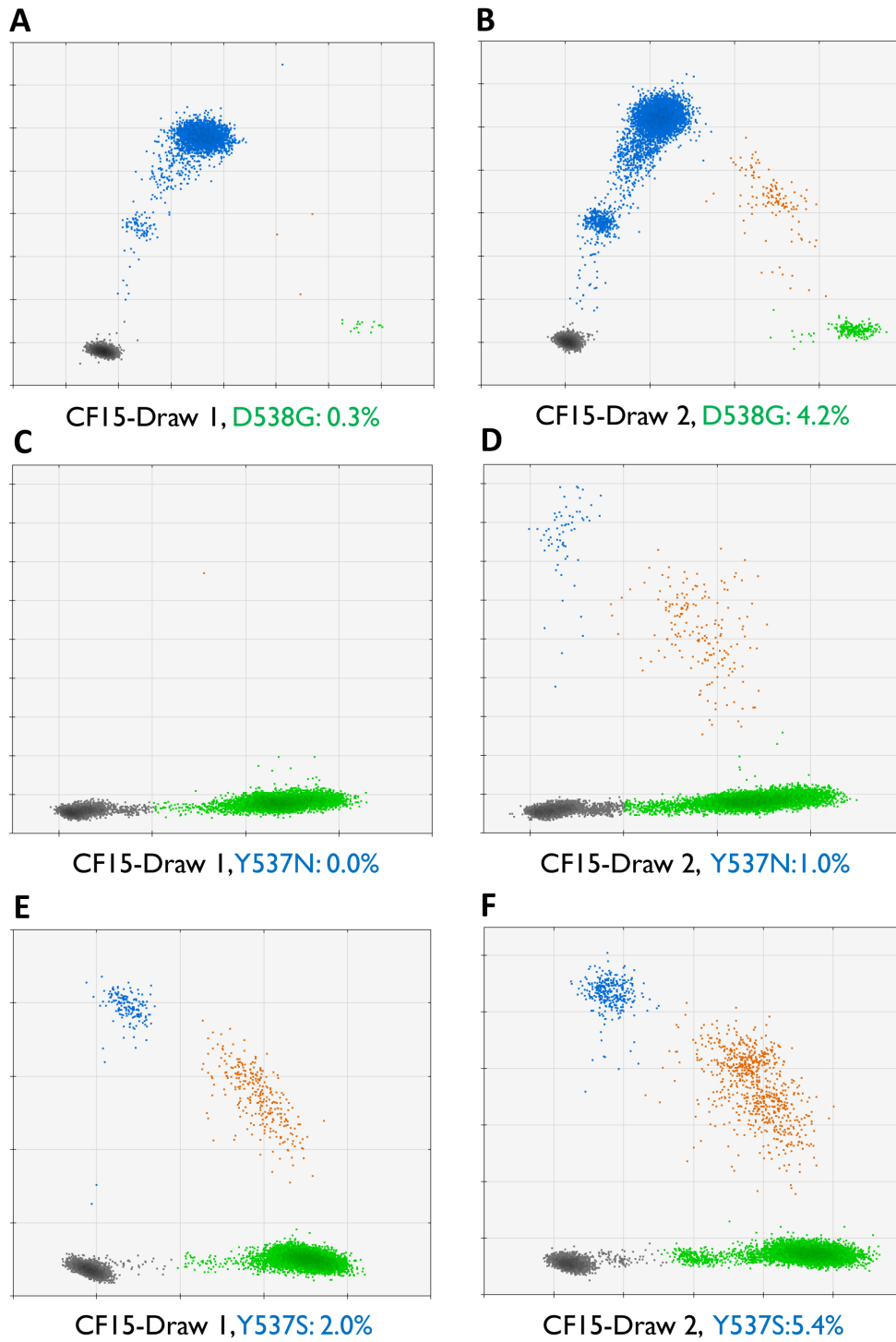
Supplementary Figure S2: ESR1-D538G mutant allele frequency in serial blood draws is indicated for patient CF8 using data from a representative experiment (after subtraction of background noise).

**A**

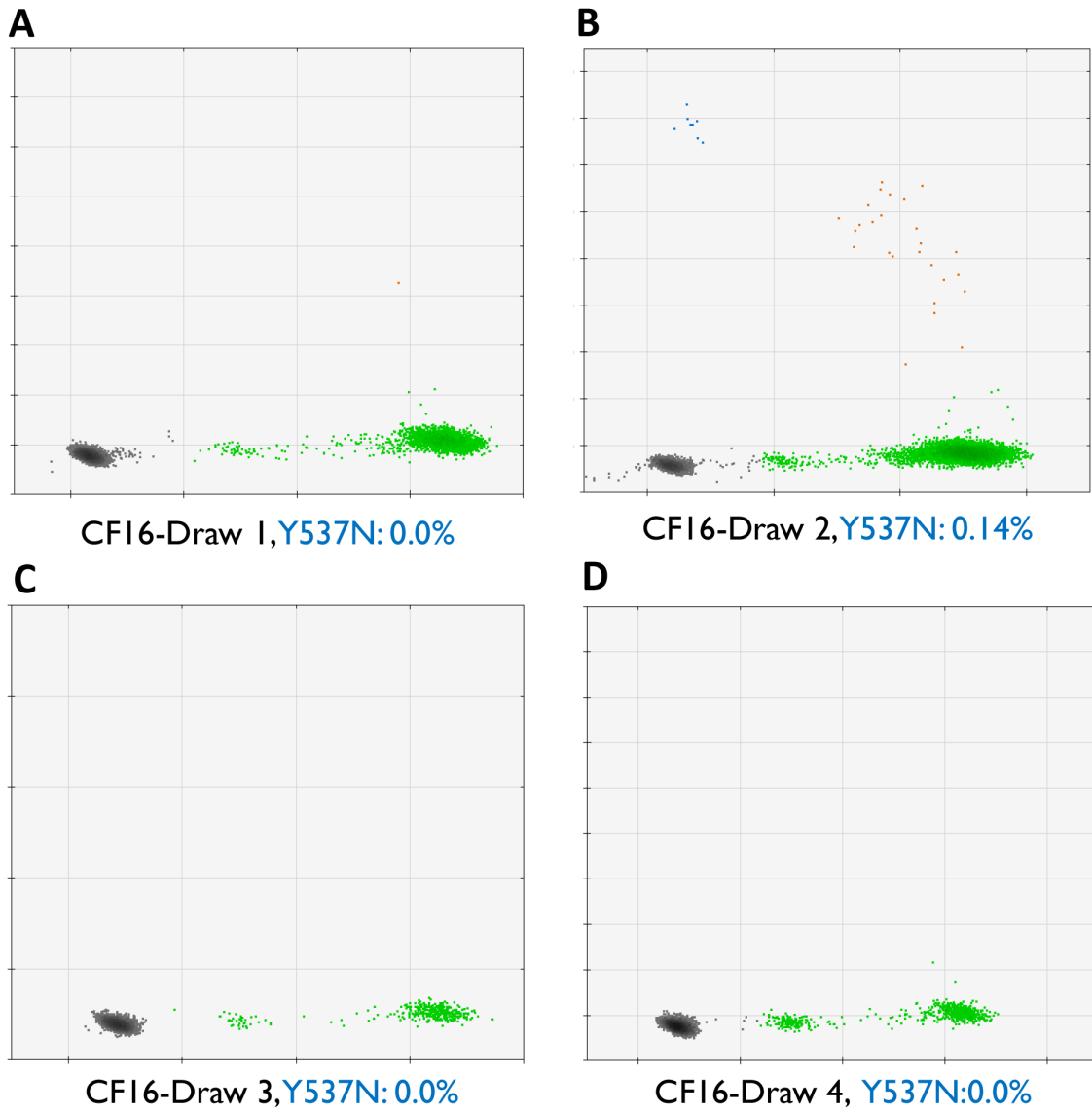


CF14-Draw I, Y537S: 3.3%

Supplementary Figure S3: ESR1-Y537S mutant allele frequency is indicated for patient CF14 using data from a representative experiment (after subtraction of background noise).



Supplementary Figure S4: ESR1-D538G, -Y537N, and -Y537S mutant allele frequencies in serial blood draws are indicated for patient CF15 using data from a representative experiment (after subtraction of background noise).



Supplementary Figure S5: ESR1-Y537N mutant allele frequency in serial blood draws is indicated for patient CF16 using data from a representative experiment (after subtraction of background noise).

**Supplementary Table S1: Clinical characteristics and treatment data for all patients**

See Supplementary File 1

Supplementary Table S2: Sequence of targeted amplification primers

| Mutations  | Forward primer        | Reverse primer     | Amplicon size |
|--|-----------------------|--------------------|---------------|
| <i>ESR1</i> Y537C <i>ESR1</i> Y537N<br><i>ESR1</i> Y537S <i>ESR1</i> D538G | CAAAGGCATGGAGCATCTGTA | TGAAGTAGAGCCCGCAGT | 169           |

Supplementary Table S3: Sequence of ddPCR primer and probes

| Mutation               | Forward primer               | Reverse primer          | Mutant Probe                  | WT probe                      | Fluorescence |
|------------------------|------------------------------|-------------------------|-------------------------------|-------------------------------|--------------|
| <i>ESR1</i> -<br>Y537C | CAGCATGAAGTGC<br>AAGAACGT    | TGGGCGTCCAGCA<br>TCTC   | CCCCTCTGTG<br>ACCTG           | TGCCCCTCTAT<br>GACCTG         | FAM/VIC      |
| <i>ESR1</i> -<br>Y537N | CTGTACAGCATGA<br>AGTGCAAGAAC | TGGGCGTCCAGCA<br>TCTC   | TGCCCCTC<br>AATGAC            | TGGTGCCCCT<br>CTATGAC         | FAM/VIC      |
| <i>ESR1</i> -<br>Y537S | CAGCATGAAGTGC<br>AAGAACGT    | TGGGCGTCCAGCA<br>TCTC   | CCCTCTCTG<br>ACCTGC           | CCCCTCTAT<br>GACCTGC          | FAM/VIC      |
| <i>ESR1</i> -<br>D538G | GCATGAAGTGCAA<br>GAACGTG     | AAGTGGCTTTGGTC<br>CGTCT | TCTATGGCCTGCTGCT<br>GGAGATGCT | TCTATGACCTGCT<br>GCTGGAGATGCT | HEX/FAM      |