

SUPPLEMENTARY MATERIALS

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Table S1. Prevalence of somatic single gene CNVs in the study cohort of 93 HR+/HER2 MBC patients, ET, and no-ET subgroup

Abbreviations: CNVs: Copy Number Variations; ET: endocrine therapy; N: number

| Gene (CNVs) | No-ET N. (%) | ET N. (%) | Total N. (%) | P value (Fisher exact test) |
|---------------|-----------------|--------------|-----------------|-----------------------------|
| <i>KIT</i> | 1 (5·0) | 0 (0·0) | 1 (1·1) | 0·21 |
| <i>PDGFRA</i> | 1 (5·0) | 0 (0·0) | 1 (1·1) | 0·21 |
| <i>BRAF</i> | 1 (5·0) | 1 (1·4) | 2 (2·2) | 0·39 |
| <i>ERBB2</i> | 0(0·0) | 0(0·0) | 0 (0·0) | -- |
| <i>FGFR1</i> | 3 (15·0) | 9 (12·3) | 12 (12·9) | 0·72 |
| <i>CDK4</i> | 2 (10·0) | 0 (0·0) | 2 (2·2) | 0·04 |
| <i>CCND1</i> | 4 (20·0) | 6 (8·2) | 10 (10·8) | 0·21 |
| <i>KRAS</i> | 2 (10·0) | 2 (2·7) | 4 (4·3) | 0·20 |
| <i>AR</i> | 1 (5·0) | 1 (1·4) | 2 (2·2) | 0·39 |
| <i>RAF1</i> | 3 (15·0) | 0 (0·0) | 3 (3·2) | 0·009 |
| <i>CDK6</i> | 1 (5·0) | 2 (2·7) | 3 (3·2) | 0·52 |
| <i>PIK3CA</i> | 3 (15·0) | 3 (4·1) | 6 (6·5) | 0·11 |
| <i>EGFR</i> | 3 (15·0) | 2 (2·7) | 5 (5·4) | 0·06 |
| <i>MYC</i> | 4 (20·0) | 2 (2·7) | 6 (6·5) | 0·02 |
| <i>MET</i> | 0 (0·0) | 0 (0·0) | 0 (0·0) | -- |
| <i>CCNE1</i> | 2 (10·0) | 0 (0·0) | 2 (2·2) | 0·04 |
| <i>CCND2</i> | 1 (5·0) | 0 (0·0) | 1 (1·1) | 0·21 |
| <i>FGFR2</i> | 1 (5·0) | 0 (0·0) | 1 (1·1) | 0·21 |
| <i>ERBBS</i> | 0 (0·0) | 0 (0·0) | 0 (0·0) | -- |
| <i>ESR1</i> | 0 (0·0) | 0 (0·0) | 0 (0·0) | -- |

Table S2. Prevalence of somatic single gene SNVs in the study cohort of 93 HR+/HER2 MBC patients, ET and no-ET subgroups

Abbreviations: ET: endocrine therapy; N: number; SNVs: Single Nucleotide Variations

| Gene (SNVs) | No-ET N. (%) | ET N. (%) | Total N. (%) | P value (Fisher exact test) |
|---------------|-----------------|--------------|-----------------|-----------------------------|
| <i>KIT</i> | 0 (0·0) | 0 (0·0) | 0 (0·0) | -- |
| <i>PDGFRA</i> | 0 (0·0) | 0 (0·0) | 0 (0·0) | -- |
| <i>BRAF</i> | 1 (5·0) | 2 (2·7) | 3 (3·2) | 0·52 |
| <i>ERBB2</i> | 1 (5·0) | 2 (2·7) | 3 (3·2) | 0·52 |
| <i>FGFR1</i> | 0 (0·0) | 0 (0·0) | 0 (0·0) | -- |
| <i>CCND1</i> | 0 (0·0) | 0 (0·0) | 0 (0·0) | -- |
| <i>KRAS</i> | 0 (0·0) | 5 (6·8) | 5 (5·4) | 0·58 |
| <i>RAF1</i> | 0 (0·0) | 0 (0·0) | 0 (0·0) | -- |
| <i>PIK3CA</i> | 9 (45·0) | 26 (35·6) | 35 (37·6) | 0·45 |
| <i>EGFR</i> | 0 (0·0) | 2 (2·7) | 2 (2·2) | 0·99 |
| <i>FGFR2</i> | 0 (0·0) | 1 (1·4) | 1 (1·1) | 0·99 |
| <i>ESR1</i> | 12 (60·0) | 31 (42·5) | 43 (46·2) | 0·21 |
| <i>TP53</i> | 6 (30·0) | 23 (31·5) | 29 (31·2) | 0·99 |
| <i>ARID1A</i> | 1 (5·0) | 4 (5·5) | 5 (5·4) | 0·99 |
| <i>CDKN2A</i> | 0 (0·0) | 1 (1·4) | 1 (1·1) | 0·99 |
| <i>NF1</i> | 0 (0·0) | 3 (4·1) | 3 (3·2) | 0·99 |
| <i>PTEN</i> | 0 (0·0) | 5 (6·8) | 5 (5·4) | 0·58 |
| <i>CDH1</i> | 0 (0·0) | 4 (5·5) | 4 (4·3) | 0·57 |
| <i>AKT1</i> | 0 (0·0) | 2 (2·7) | 2 (2·2) | 0·99 |
| <i>GATA3</i> | 1 (5·0) | 8 (11·0) | 9 (9·7) | 0·68 |
| <i>SMAD4</i> | 0 (0·0) | 2 (2·7) | 2 (2·2) | 0·99 |
| <i>RB1</i> | 1 (5·0) | 2 (2·7) | 3 (3·2) | 0·52 |
| <i>FBXW7</i> | 0 (0·0) | 0 (0·0) | 0 (0·0) | -- |
| <i>HRAS</i> | 1 (5·0) | 0 (0·0) | 1 (1·1) | 0·21 |
| <i>GNAS</i> | 0 (0·0) | 1 (1·4) | 1 (1·1) | 0·99 |
| <i>BRCA2</i> | 1 (5·0) | 1 (1·4) | 2 (2·2) | 0·39 |
| <i>IDH1</i> | 0 (0·0) | 0 (0·0) | 0 (0·0) | -- |
| <i>NRAS</i> | 0 (0·0) | 0 (0·0) | 0 (0·0) | -- |
| <i>BRCA1</i> | 0 (0·0) | 2 (2·7) | 2 (2·2) | 0·99 |
| <i>VHL</i> | 0 (0·0) | 0 (0·0) | 0 (0·0) | -- |
| <i>RHOA</i> | 0 (0·0) | 0 (0·0) | 0 (0·0) | -- |
| <i>APC</i> | 0 (0·0) | 1 (1·4) | 1 (1·1) | 0·99 |
| <i>JAK2</i> | 0 (0·0) | 0 (0·0) | 0 (0·0) | -- |
| <i>STK11</i> | 0 (0·0) | 1 (1·4) | 1 (1·1) | 0·99 |

| | | | | |
|---------------|---------|---------|---------|------|
| <i>PTPN11</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>ATM</i> | 0 (0-0) | 1 (1-4) | 1 (1-1) | 0-99 |
| <i>RET</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>TERT</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>IDH2</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>FGFR3</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>MAPK1</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>RIT1</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>CTNNB1</i> | 0 (0-0) | 1 (1-4) | 1 (1-1) | 0-99 |
| <i>CDK12</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>TSC1</i> | 0 (0-0) | 1 (1-4) | 1 (1-1) | 0-99 |
| <i>ARAF</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>NFE2L2</i> | 0 (0-0) | 1 (1-4) | 1 (1-1) | 0-99 |
| <i>MPL</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>HNFI1A</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>NOTCH1</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>CHEK2</i> | 0 (0-0) | 2 (2-7) | 2 (2-2) | 0-99 |
| <i>JAK3</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>MAP2K2</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |

Table S3. Prevalence of somatic single gene Syn alterations in the study cohort of 93 HR+/HER2 MBC patients, ET and no-ET subgroups

Abbreviations: ET: endocrine therapy N: number; Syn: Synonymous

| Gene (syn) | No-ET No. (%) | ET No. (%) | Total No. (%) | P value (Fisher exact test) |
|---------------|------------------|---------------|------------------|-----------------------------|
| <i>KIT</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>PDGFRA</i> | 0 (0-0) | 2 (2-7) | 2 (2-2) | 0-99 |
| <i>BRAF</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>ERBB2</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>FGFR1</i> | 0 (0-0) | 1 (1-4) | 1 (1-1) | 0-99 |
| <i>RAF1</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>PIK3CA</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>EGFR</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>FGFR2</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>ESR1</i> | 1 (5-0) | 0 (0-0) | 1 (1-1) | 0-21 |
| <i>TP53</i> | 1 (5-0) | 1 (1-4) | 2 (2-2) | 0-39 |
| <i>ARID1A</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>CDKN2A</i> | 0 (0-0) | 2 (2-7) | 2 (2-2) | 0-99 |
| <i>NF1</i> | 0 (0-0) | 2 (2-7) | 2 (2-2) | 0-99 |
| <i>PTEN</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>CDH1</i> | 0 (0-0) | 1 (1-4) | 1 (1-1) | 0-99 |
| <i>AKT1</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>GATA3</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>SMAD4</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>RB1</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>HRAS</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>BRCA2</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>IDH1</i> | 1 (5-0) | 0 (0-0) | 1 (1-1) | 0-21 |
| <i>BRCA1</i> | 0 (0-0) | 3 (4-1) | 3(3-2) | 0-99 |
| <i>VHL</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>RHOA</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>APC</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>STK11</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>PTPN11</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>ATM</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>RET</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>TERT</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>FGFR3</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>MAP2K1</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |

| | | | | |
|---------------|---------|---------|---------|------|
| <i>RIT1</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>CTNNB1</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>CDK12</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>TSC1</i> | 1 (5-0) | 0 (0-0) | 1 (1-1) | -- |
| <i>ARAF</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0-21 |
| <i>NFE2L2</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>HNFI1A</i> | 0 (0-0) | 1 (1-4) | 1 (1-1) | -- |
| <i>NOTCH1</i> | 0 (0-0) | 1 (1-4) | 1 (1-1) | 0-99 |
| <i>MAP2K2</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0-99 |
| <i>AR</i> | 0 (0-0) | 3 (4-1) | 3(3-2) | -- |
| <i>MET</i> | 0 (0-0) | 2 (2-7) | 2 (2-2) | 0-99 |
| <i>MTOR</i> | 0 (0-0) | 1 (1-4) | 1 (1-1) | 0-99 |
| <i>CCNE1</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0-99 |
| <i>NTRK3</i> | 0 (0-0) | 1 (1-4) | 1 (1-1) | -- |
| <i>NTRK1</i> | 0 (0-0) | 1 (1-4) | 1 (1-1) | 0-99 |
| <i>ALK</i> | 0 (0-0) | 1 (1-4) | 1 (1-1) | 0-99 |
| <i>FBXW7</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0-99 |
| <i>MAPK3</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>MYC</i> | 0 (0-0) | 1 (1-4) | 1 (1-1) | -- |
| <i>MLH1</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0-99 |
| <i>ROS1</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>CCND2</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>SMO</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>GNAI1</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>CDKN2B</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>CCND1</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>CDK4</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| <i>CDK6</i> | 0 (0-0) | 1 (1-4) | 1 (1-1) | -- |
| <i>DDR2</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0-99 |
| <i>MSH6</i> | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |

Table S4. Prevalence of somatic pathway mutations in the study cohort of 93 HR+/HER2 MBC patients, ET and no-ET subgroup

Abbreviations: CNVs: Copy Number Variations; N: number; Syn: Synonymous; SNVs, Single Nucleotide Variations, VUS: variant of uncertain significance

| Pathway | No-ET N. (%) | ET N. (%) | Total N. (%) | P value (Fisher exact test) |
|-----------------|-----------------|--------------|-----------------|-----------------------------|
| PI3K SNVs | 9 (45.0) | 30 (41.1) | 39 (41.9) | 0.80 |
| ER SNVs | 12 (60.0) | 34 (46.6%) | 46 (49.5) | 0.32 |
| p53 SNVs | 6 (30.0) | 26 (35.6) | 32 (34.4%) | 0.79 |
| RTK SNVs | 1 (5.0) | 4 (5.5) | 5 (5.4) | 0.99 |
| Cell-cycle SNVs | 1 (5.0) | 3 (4.1) | 4 (4.3) | 0.99 |
| RAS SNVs | 1 (5.0) | 7 (9.6) | 8 (8.6) | 0.99 |
| NOTCH1 SNVs | 0 (0.0) | 0 (0.0) | 0 (0.0) | -- |
| RAF SNVs | 1 (5.0) | 2 (2.7) | 3 (3.2) | 0.52 |
| WNT SNVs | 0 (0.0) | 2 (2.7) | 2 (2.2) | 0.99 |
| MEK SNVs | 0 (0.0) | 0 (0.0) | 0 (0.0) | -- |
| NRF2 SNVs | 0 (0.0) | 1 (1.4) | 1 (1.1) | 0.99 |
| RTK CNVs | 5 (25.0) | 10 (13.7) | 15 (16.1) | 0.30 |
| RAF CNVs | 3 (15.0) | 1 (1.4%) | 4 (4.3) | 0.03 |
| Cell-cycle CNVs | 5 (25.0) | 6 (8.2) | 11 (11.8) | 0.054 |
| RAS CNVs | 2 (10.0) | 2 (2.7) | 4 (4.3) | 0.20 |
| ER CNVs | 1 (5.0) | 1 (1.4) | 2 (2.2) | 0.39 |
| PI3K CNVs | 3 (15.0) | 3 (4.1) | 6 (6.5) | 0.11 |
| MYC CNVs | 4 (20.0) | 2 (2.7) | 6 (6.5) | 0.02 |
| ER vus | 1 (5.0) | 6 (8.2) | 7 (7.5) | 0.99 |
| RAF vus | 0 (0.0) | 1 (1.4) | 1(1.1) | 0.99 |
| RTK vus | 2 (10.0) | 19 (26) | 21 (22.6) | 0.22 |
| NOTCH1 vus | 0 | 2 (2.7) | 2 (2.2) | 0.99 |
| PI3K vus | 3 (15.0) | 5 (6.8) | 8 (8.6) | 0.36 |
| Cell-cycle vus | 2 (10) | 5 (6.8) | 7 (7.5) | 0.64 |
| RAS vus | 1 (5.0) | 7 (9.6) | 8 (8.6) | 0.99 |
| p53 vus | 1 (5.0) | 8 (11.0) | 9 (9.7) | 0.68 |
| MYC vus | 0 (0.0) | 2 (2.7) | 2 (2.2) | 0.99 |
| WNT vus | 1 (5.0) | 6 (8.2) | 7 (7.5) | 0.99 |
| MEK vus | 0 (0.0) | 1 (1.4) | 1 (1.1) | 0.99 |
| NRF2 vus | 0(0.0) | 0(0.0) | 0 (0.0) | -- |
| RTK syn | 0 (0.0) | 7 (9.6) | 7 (7.5) | 0.34 |
| RAF syn | 0 (0.0) | 0 (0.0) | 0 (0.0) | -- |
| WNT syn | 0(0.0) | 0(0.0) | 0(0.0) | -- |

| | | | | |
|-----------------------|---------|---------|---------|------|
| NOTCH1 syn | 0 (0-0) | 1 (1-4) | 1 (1-1) | 0-99 |
| ER syn | 1 (5-0) | 3 (4-1) | 4 (4-3) | 0-99 |
| PI3K syn | 1 (5-0) | 1 (1-4) | 2 (2-2) | 0-39 |
| RAS syn | 0 (0-0) | 2 (2-7) | 2 (2-2) | 0-99 |
| Cell-cycle syn | 0 (0-0) | 3 (4-1) | 3 (3-2) | 0-99 |
| MEK syn | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |
| P53 syn | 1 (5-0) | 3 (4-1) | 4 (4-3) | 0-99 |
| MYC syn | 0 (0-0) | 1 (1-4) | 1 (1-1) | 0-99 |
| NRF2 syn | 0 (0-0) | 0 (0-0) | 0 (0-0) | -- |

Table S5. Clinical-pathological characteristics of the CD4/6i naïve control cohort of 181 HR+/HER2- patients
Abbreviations: CNS: central nervous system; ET: endocrine treatment ; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; MXD: mixed histology N: number; NA: not applicable

| Variable | CDK4/6i naïve cohort N=181 (%) |
|----------------------------|-----------------------------------|
| Histotype (N=164) | |
| IDC | 121 (73·8) |
| ILC | 33 (20·1) |
| mixed | 10 (6·1) |
| Lung (N=181) | |
| No | 135 (74·6) |
| Yes | 46 (25·4) |
| Liver (N=181) | |
| No | 136 (75·1) |
| Yes | 45 (24·9) |
| Bone (N=181) | |
| No | 48 (26·5) |
| Yes | 133 (73·5) |
| Lymph node (N=181) | |
| No | 114 (70·0) |
| Yes | 67 (30·0) |
| Soft tissue (N=181) | |
| No | 160 (88·4) |
| Yes | 21 (11·6) |
| CNS (N=181) | |
| No | 169 (93·4) |
| Yes | 12 (6·6) |
| De novo (N=181) | |
| No | 153 (84·6) |
| Yes | 28 (15·5) |

Table S6. Prevalence of somatic SNVs e CNVs pathway mutations in the post-CDK4/6i and CDK4/6i naïve cohorts

Abbreviations: CNVs: Copy Number Variations; N: number; SNVs: Single Nucleotide

| | Post CDK4/6i cohort (N=93) N (%) | CDK4/6i naïve cohort (N=181) N (%) | P value (Chi-square test) |
|-------------------|-------------------------------------|---------------------------------------|---------------------------|
| SNVs | | | |
| PI3K | 39 (41.9) | 67 (37.0) | 0.43 |
| ER | 46 (49.5) | 28 (15.5) | <0.001 |
| p53 | 32 (34.4) | 59 (32.6) | 0.76 |
| RTK | 5 (5.4) | 13 (7.2) | 0.57 |
| Cell-cycle | 4 (4.3) | 4 (2.2) | 0.33 |
| RAS | 8 (8.6) | 13(7.2) | 0.68 |
| NOTCH1 | 0 (0.0) | 2 (1.1) | 0.31 |
| RAF | 3 (3.2) | 2 (1.1) | 0.21 |
| WNT | 2 (2.2) | 5 (2.8) | 0.76 |
| MEK | 0 (0.0) | 1 (0.6) | 0.47 |
| NRF2 | 1 (1.1) | 0 (0.0) | 0.16 |
| CNVs | | | |
| RTK | 15 (16.1) | 35 (19.3) | 0.51 |
| RAF | 4 (4.3) | 4 (2.2) | 0.33 |
| Cell-cycle | 11 (11.8) | 21 (11.6) | 0.96 |
| RAS | 4 (4.3) | 3 (1.7) | 0.19 |
| ER | 2 (2.2) | 2 (1.1) | 0.45 |
| PI3K | 6 (6.5) | 8(4.4) | 0.47 |
| MYC | 6 (6.5) | 7 (3.9) | 0.34 |

Table S7. Prevalence of somatic single gene CNVs in the post-CDK4/6i and CDK4/6i naïve cohorts
Abbreviations: CNVs: Copy Number Variations; N: number

| Gene (CNVs) | Post CDK4/6i cohort (N=93) N (%) | CDK4/6i naïve cohort (N=181) N (%) | P value (Chi - square test) |
|---------------|-------------------------------------|---------------------------------------|--------------------------------|
| <i>KIT</i> | 1 (1·1) | 1 (0·6) | 0·63 |
| <i>PDGFRA</i> | 1 (1·1) | 4 (2·2) | 0·51 |
| <i>BRAF</i> | 2 (2·2) | 3 (1·7) | 0·77 |
| <i>ERBB2</i> | 0 (0·0) | 1 (0·6) | 0·47 |
| <i>FGFR1</i> | 12 (12·9) | 22 (12·2) | 0·86 |
| <i>CDK4</i> | 2 (2·2) | 5 (2·8) | 0·76 |
| <i>CCND1</i> | 10 (10·8) | 11 (6·1) | 0·17 |
| <i>KRAS</i> | 4 (4·3) | 3 (1·7) | 0·19 |
| <i>AR</i> | 2 (2·2) | 1 (0·6) | 0·23 |
| <i>RAF1</i> | 3 (3·2) | 3 (1·7) | 0·40 |
| <i>CDK6</i> | 3 (3·2) | 4 (3·2) | 0·61 |
| <i>PIK3CA</i> | 6 (6·5) | 8 (4·4) | 0·47 |
| <i>EGFR</i> | 5 (5·4) | 14 (7·7) | 0·47 |
| <i>MYC</i> | 6 (6·5) | 7 (3·9) | 0·34 |
| <i>MET</i> | 0 (0·0) | 3 (1·7) | 0·21 |
| <i>CCNE1</i> | 2 (2·2) | 6 (3·3) | 0·59 |
| <i>CCND2</i> | 1 (1·1) | 0 (0·0) | 0·16 |
| <i>FGFR2</i> | 1 (1·1) | 1 (0·6) | 0·63 |
| <i>ERBB3</i> | 0 (0·0) | 0 (0·0) | -- |
| <i>ESR1</i> | 0 (0·0) | 1 (0·6) | 0·47 |

Table S8. Prevalence of somatic single gene SNVs in the post-CDK4/6i and CDK4/6i naïve cohorts
Abbreviations: N: number; SNVs: Single Nucleotide

| Gene (SNVs) | Post CDK4/6i cohort (N=93) N (%) | CDK4/6i naïve cohort (N=181) N (%) | P value (Chi - square test) |
|---------------|----------------------------------------|------------------------------------------|--------------------------------|
| <i>KIT</i> | 0 (0.0) | 0 (0.0) | -- |
| <i>PDGFRA</i> | 0 (0.0) | 1 (0.6) | 0.47 |
| <i>BRAF</i> | 3 (3.2) | 1 (0.6) | 0.08 |
| <i>ERBB2</i> | 3 (3.2) | 6 (3.3) | 0.97 |
| <i>FGFR1</i> | 0 (0.0) | 0 (0.0) | -- |
| <i>CCND1</i> | 0 (0.0) | 0 (0.0) | -- |
| <i>KRAS</i> | 5 (5.4) | 8 (4.4) | 0.72 |
| <i>RAF1</i> | 0 (0.0) | 0 (0.0) | -- |
| <i>PIK3CA</i> | 35 (37.6) | 54 (29.8) | 0.19 |
| <i>EGFR</i> | 2 (2.2) | 1 (0.6) | 0.22 |
| <i>FGFR2</i> | 1 (1.1) | 2 (1.1) | 0.98 |
| <i>ESR1</i> | 43 (46.2) | 12 (6.6) | <0.001 |
| <i>TP53</i> | 29 (31.2) | 52 (28.7) | 0.67 |
| <i>ARID1A</i> | 5 (5.4) | 14 (7.7) | 0.47 |
| <i>CDKN2A</i> | 1 (1.1) | 2 (1.1) | 0.98 |
| <i>NF1</i> | 3 (3.2) | 2 (1.1) | 0.21 |
| <i>PTEN</i> | 5 (5.4) | 3 (1.7) | 0.083 |
| <i>CDH1</i> | 4 (4.3) | 7 (3.9) | 0.86 |
| <i>AKT1</i> | 2 (2.2) | 14 (7.7) | 0.062 |
| <i>GATA3</i> | 9 (9.7) | 16 (8.8) | 0.82 |
| <i>SMAD4</i> | 2 (2.2) | 2 (1.1) | 0.49 |
| <i>RB1</i> | 3 (3.2) | 2 (1.1) | 0.21 |
| <i>FBXW7</i> | 0 (0.0) | 1 (0.6) | 0.47 |
| <i>HRAS</i> | 1 (1.1) | 2 (1.1) | 0.98 |
| <i>GNAS</i> | 1 (1.1) | 3 (1.7) | 0.70 |
| <i>BRCA2</i> | 2 (2.2) | 4 (2.2) | 0.97 |
| <i>IDH1</i> | 0 (0.0) | 1 (0.4) | -- |
| <i>NRAS</i> | 0 (0.0) | 2 (1.1) | 0.31 |
| <i>BRCA1</i> | 2 (2.2) | 0 (0.0) | 0.048 |
| <i>VHL</i> | 0 (0.0) | 1 (0.6) | 0.47 |
| <i>RHOA</i> | 0 (0.0) | 1 (0.6) | 0.47 |
| <i>APC</i> | 1 (1.1) | 5 (2.8) | 0.37 |

| | | | |
|---------------|---------|----------|--------------|
| <i>JAK2</i> | 0 (0-0) | 1 (0-6) | 0·47 |
| <i>STK11</i> | 1 (1·1) | 1 (0-6) | 0·63 |
| <i>PTPN11</i> | 0 (0-0) | 0 (0-0) | -- |
| <i>ATM</i> | 1 (1·1) | 7 (3-9) | 0·19 |
| <i>RET</i> | 0 (0-0) | 2 (1·1) | 0·31 |
| <i>TERT</i> | 0 (0-0) | 0 (0-0) | -- |
| <i>IDH2</i> | 0 (0-0) | 4 (2-2) | 0·165 |
| <i>FGFR3</i> | 0 (0-0) | 0 (0-0) | -- |
| <i>MAPK1</i> | 0 (0-0) | 1 (0·46) | 0·47 |
| <i>RIT1</i> | 0 (0-0) | 1 (0-6) | 0·47 |
| <i>CTNNB1</i> | 1 (1·1) | 0 (0-0) | 0·16 |
| <i>CDK12</i> | 0 (0-0) | 1 (0-6) | 0·47 |
| <i>TSC1</i> | 1 (1·1) | 0 (0-0) | 0·16 |
| <i>ARAF</i> | 0 (0-0) | 1 (0-6) | 0·47 |
| <i>NFE2L2</i> | 1 (1·1) | 0 (0-0) | 0·16 |
| <i>MPL</i> | 0 (0-0) | 0 (0-0) | -- |
| <i>HNF1A</i> | 0 (0-0) | 0 (0-0) | -- |
| <i>NOTCH1</i> | 0 (0-0) | 1 (06) | 0·47 |
| <i>CHEK2</i> | 2 (2·2) | 0 (00) | 0·048 |
| <i>JAK3</i> | 0 (0-0) | 0 (0-0) | -- |
| <i>MAP2K2</i> | 0 (0-0) | (0-0) | -- |

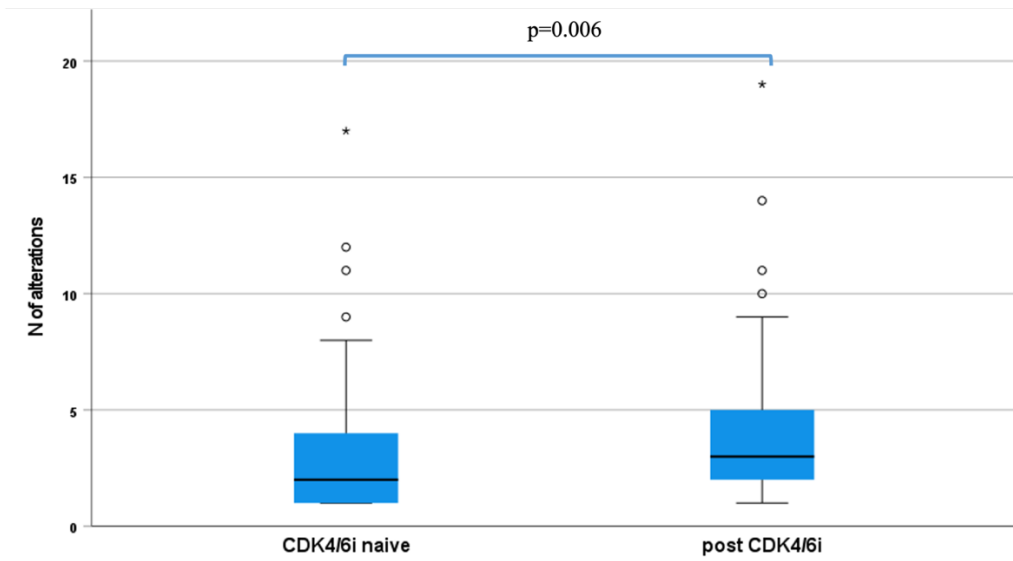
Table S9. Multivariable logistic regression analysis of ESR1 and ER pathway between post-CDK4/6i and CDK4/6i naïve cohort

Abbreviations: CDK4/6i: CDK4/6 inhibitors; mets: metastasis.

| | OR | 95% C.I. | | P value |
|------------------------------|--------|----------|--------|---------|
| <i>ESR1</i> SNVs | | | | |
| Lung mets | 0.958 | 0.368 | 2.497 | 0.930 |
| Liver mets | 1.699 | 0.790 | 3.657 | 0.175 |
| Bone mets | 4.938 | 1.513 | 16.117 | 0.008 |
| Lymphnode mets | 1.199 | 0.512 | 2.810 | 0.676 |
| Soft tissue | 0.493 | 0.143 | 1.705 | 0.264 |
| De novo | 1.334 | 0.587 | 3.031 | 0.491 |
| CDK4/6i exposure (yes vs no) | 14.456 | 6.349 | 32.914 | <0.001 |
| Path ER SNVs | | | | |
| Lung mets | 1.070 | 0.472 | 2.429 | 0.871 |
| Liver mets | 1.415 | 0.726 | 2.769 | 0.308 |
| Bone mets | 3.655 | 1.443 | 9.254 | 0.006 |
| Lymphnode mets | 1.052 | 0.511 | 2.165 | 0.890 |
| Soft tissue | 0.351 | 0.113 | 1.087 | 0.801 |
| De novo | 1.099 | 0.5256 | 2.296 | 0.801 |
| CDK4/6i exposure (yes vs no) | 5.857 | 3.098 | 11.073 | <0.001 |

*18 patients in the CDK4/6i-naïve cohort with prior endocrine therapy in the (neo)adjuvant setting were excluded to avoid treatment-related bias.

a



b

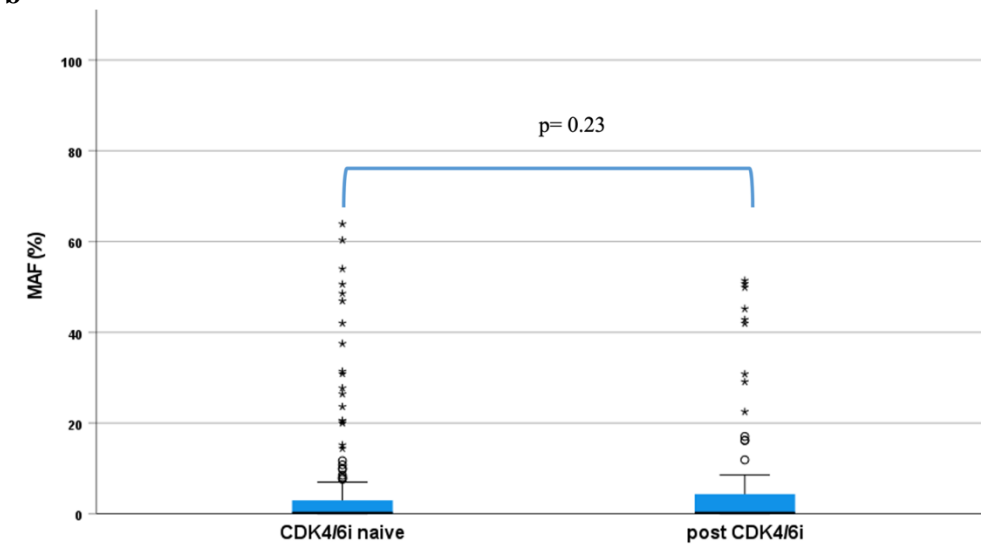


Figure S1· Differences in MAF (a) and NOA (b) between post-CDK4/6i and CDK4/6i naïve cohorts represented by box-plot. A statistically significantly higher incidence of NOA after the selective pressure driven by the drug exposure ($p=0.006$) was showed, while no difference was observed in terms of MAF ($p=0.23$)

Abbreviations: CDK4/6i: CDK4/6 inhibitors; MAF: mutant allele frequency; NOA: Number of alterations