Supplementary Information

A sequential methodology for the rapid identification and characterization of breast cancer-associated functional SNPs

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Supplementary Figure 1 and 2

Supplementary Tables 1-4
Supplementary Figure 1. Dot plot of 3436 SNPs in the function of Slope (x axis) versus P value (y axis).

P value for HTP Reel-seq screen was calculated with 5 technical replicates using Student’s t-test with 2 tails. Source data are provided as a Source Data file.
Supplementary Figure 2. Regulation of FGFR2 expression by OCT-1 (A), RUNX2 (B) and FOXA1 (C) via rs2981578.

A. AIDP-Western blot showing the no binding of OCT-1 (Upper), allele-imbalanced binding of RUNX2 (Middle), and allele-balanced binding of FOXA1 (Lower) to the two allele of rs2981578 (n=3 biological independent experiments). NE input: nuclear extract control for antibody staining; TEAD1: positive control for allele-imbalanced binding. PARP-1: used for loading control. B. Western blot (n=3 biological independent experiments) and C. qPCR (n=3 biological independent experiments with each in duplicate) showing the downregulation of FGFR2 expression by OCT-1 (Upper) and RUNX2 (Middle) knockdown and no effect on FGFR2 expression in FOXA1 knockdown cells (Lower). P value was calculated using Student’s t-test with 2 tails. Error bars in all the plots represent the median with S.E. Source data are provided as a Source Data file.
### Supplementary Table 1. Epigenetic and functional annotations of SNPs on the BC-associated FGFR2 locus.

| SNPs     | Promoter histone Marks | Enhancer Histone Marks | DNAse | Proteins bound | Motifs changed | Score | Average |
|----------|-------------------------|------------------------|-------|----------------|----------------|-------|---------|
| rs7895676 | SKIN                    | 8 tissues              |       |                |                |       | 3       |
| rs2981578 | SKIN                    | 5 tissues              | 13 tissues | FOXA1, HIC1 | 4 altered motifs | 4     | 3       |
| rs2981584 | SKIN                    | 11 tissues             | 13 tissues |                |                |       | 2       |
| rs4752570 | SKIN                    | 13 tissues             | 5 tissues |                |                |       | 2       |
| rs1219642 | SKIN                    | 6 tissues              |         |                |                |       | 2       |
| rs2912774 | SKIN                    | 7 tissues              |        |                |                |       | 3       |
| rs2912779 | SKIN                    | 7 tissues              |        |                |                |       | 3       |
| rs2981575 | SKIN                    | 7 tissues              |        |                |                |       | 3       |
| rs2912780 | SKIN                    | 13 tissues             | 8 tissues |                |                |       | 3       |
| rs3155718 | SKIN                    | 10 tissues             | 6 tissues | 13 tissues | 4 altered motifs | 4     | 3       |
| rs10736303 | SKIN                    | 9 tissues              |        |                |                |       | 2       |
| rs1078806 | SKIN                    | 10 tissues             |        |                |                |       | 2       |
| rs150005405 | SKIN                    | 6 tissues              |        |                |                |       | 2       |
| rs2162540 | SKIN, BRN, GI           | 7 tissues              |        |                |                |       | 2       |
| rs2912778 | SKIN, GI                | 12 tissues             | 7 tissues |                |                |       | 2       |
| rs2912781 | SKIN                    | 7 tissues              |        |                |                |       | 2       |
| rs2981582 | SKIN, BRN, GI           | 6 tissues              |        |                |                |       | 2       |
| rs34032268 | SKIN                    | 6 tissues              |        |                |                |       | 2       |
| rs35054928 | SKIN                    | 6 tissues              |        |                |                |       | 2       |
| rs48631539 | IPSC                    | 6 tissues              |        |                |                |       | 2       |
| rs1219648 | IPSC                    | 7 tissues              |        |                |                |       | 2       |
| rs2936870 | IPSC, SKIN              | 7 tissues              |        |                |                |       | 2       |
| rs2860197 | SKIN                    | 6 tissues              |        |                |                |       | 2       |
| rs2420946 | SKIN                    | 6 tissues              |        |                |                |       | 2       |
| rs3155718 | SKIN                    | 10 tissues             | 6 tissues |                |                |       | 2       |
| rs11200314 | SKIN, GI                | 9 tissues              |        |                |                |       | 2       |
| rs2981579 | SKIN, BRN, SKIN         | 13 tissues             |        |                |                |       | 2       |
| rs11599804 | SKIN                    | 13 tissues             | 4 tissues |                |                |       | 2       |
| rs4752571 | SKIN                    | 8 tissues              |        |                |                |       | 2       |
| rs1219651 | SKIN                    | 8 tissues              |        |                |                |       | 2       |
### Supplementary Table 2. Primers used in this work

| EMSA Primer (5'-3') |
|---------------------|
| rs7895676-C-F       |
| rs7895676-C-R       |
| rs7895676-T-F       |
| rs7895676-T-R       |
| rs2981578-G-F       |
| rs2981578-G-R       |
| rs2981578-A-F       |
| rs2981578-A-R       |
| rs1219642-A-F       |
| rs1219642-A-R       |
| rs1219642-G-F       |
| rs1219642-G-R       |
| rs4752570-T-F       |
| rs4752570-T-R       |
| rs4752570-A-F       |
| rs4752570-A-R       |
| rs4752570-C-F       |
| rs4752570-C-R       |
| rs1686034-T-F       |
| rs1686034-T-R       |
| rs1686034-C-F       |
| rs1686034-C-R       |
| rs60054381-T-F      |
| rs60054381-T-R      |
| rs60054381-G-F      |
| rs60054381-G-R      |
| rs4762363-T-F       |
| rs4762363-T-R       |
| rs4762363-A-F       |
| rs4762363-A-R       |
| rs77371588-T-F      |
| rs77371588-T-R      |
| rs77371588-G-F      |
| rs77371588-G-R      |
| rs111968853-C-F     |
| rs111968853-C-R     |
| rs111968853-T-F     |
| rs111968853-T-R     |
| rs79321361-G-F      |
| rs79321361-G-R      |
| rs79321361-A-F      |
| rs79321361-A-R      |
| rs8101691-C-F       |
| rs8101691-C-R       |
| rs8101691-T-F       |
| rs8101691-T-R       |

Continue table 2 on next page
**Q-PCR Primer (5'-3')**

- **Q-TEAD1-F**: AAGCTCAAACACTTACCAGAGA
- **Q-TEAD1-R**: GCCATGCAGAGTAGAGTTT
- **Q-TEAD3-F**: GAAACGGTGCTGGGAGA
- **Q-TEAD3-R**: GTTGGAGACTTCGAAGGAAATG
- **Q-TFAM-F**: GTCTGTTCTTATCACCACCCCG
- **Q-TFAM-R**: GGCAAAATCAGGAGAGGTTTA
- **Q-NFIB-F**: GCCAGAATCAGGAGAGGTGAATG
- **Q-NFIB-R**: TGGAGTCTGCGCGAAGAAGA
- **Q-PARP2-F**: GGCACAAATCAGGAGAGGTGAATG
- **Q-PARP2-R**: TGGAGTCTGCGCGAAGAAGA
- **Q-FGFR2-F**: ACTTGGATCGAATTCTCACTCTC
- **Q-FGFR2-R**: GTGTCAAAGTTCTAGTTGAAATAC
- **Q-GAPDH-F**: CGACCACTTTGTCAAGCTCA
- **Q-GAPDH-R**: AGGGGTCTACATGGCAACTG
- **Q-FOXA1-F**: GCAATACTCGCCTTACGGCT
- **Q-FOXA1-R**: TACACACTCCCTGAGGAGTG
- **Q-RUNX2-F**: TCAACGATCTGAGATTTGTGGG
- **Q-RUNX2-R**: GGGAGGATTTGTGAAAGG
- **Q-POU2F1-F**: ATGAACAATCCGTCAGAAACCAG
- **Q-POU2F1-R**: GATGGAGATGTCCAAGGAAAGC

**Allele-imbalanced DNA Pull Down Western Blot Primer (5'-3')**

(Reserve same as EMSA)

| Allele  | Primer Sequence  |
|---------|------------------|
| C       | GTCTGTGTTCCGGTTGCGTCCGAATAATCGGGATCGCATCGCACTGCCAC |
| T       | GTCTGTGTTCCGGTTGCGTCCGAATAATCGGGATCGCATCGCACTGCCAC |
| G       | GTCTGTGTTCCGGTTGCGTCCGAATAATCGGGATCGCATCGCACTGCCAC |
| A       | GTCTGTGTTCCGGTTGCGTCCGAATAATCGGGATCGCATCGCACTGCCAC |

**SDCP-MS Primer (5'-3')**

| Allele  | Primer Sequence  |
|---------|------------------|
| C       | GTCTGTGTTCCGGTTGCGTCCGAATAATCGGGATCGCATCGCACTGCCAC |
| T       | GTCTGTGTTCCGGTTGCGTCCGAATAATCGGGATCGCATCGCACTGCCAC |
| G       | GTCTGTGTTCCGGTTGCGTCCGAATAATCGGGATCGCATCGCACTGCCAC |
| A       | GTCTGTGTTCCGGTTGCGTCCGAATAATCGGGATCGCATCGCACTGCCAC |

**SDCP-MS Primer (5'-3')**

| Allele  | Primer Sequence  |
|---------|------------------|
| C       | GTCTGTGTTCCGGTTGCGTCCGAATAATCGGGATCGCATCGCACTGCCAC |
| T       | GTCTGTGTTCCGGTTGCGTCCGAATAATCGGGATCGCATCGCACTGCCAC |
| G       | GTCTGTGTTCCGGTTGCGTCCGAATAATCGGGATCGCATCGCACTGCCAC |
| A       | GTCTGTGTTCCGGTTGCGTCCGAATAATCGGGATCGCATCGCACTGCCAC |
### Supplementary Table 3. Antibodies, siRNAs and shRNAs used in this work

| Antibody       | Manufacturer       | Cat#   | Dilution | Source |
|----------------|--------------------|--------|----------|--------|
| TEAD1          | ABCLONAL           | A6768  | 1:1000   | Rabbit |
| TEAD3          | ABCLONAL           | A7454  | 1:1000   | Rabbit |
| TFAM           | ABCLONAL           | A13552 | 1:1000   | Rabbit |
| NFIB           | ABCLONAL           | A15294 | 1:1000   | Rabbit |
| PARP2          | ABCLONAL           | A16475 | 1:1000   | Rabbit |
| FGFR2          | LifeSpan Biosciences | LS-C172060 | 1:1000   | Rabbit |
| Ku86(C-20)     | Santa Cruz         | sc-1484| 1:500    | Goat   |
| PARP-1(H-250)  | Santa Cruz         | sc-7150| 1:500    | Rabbit |
| α-Tubulin      | Sigma              | T6074  | 1:1000   | Mouse  |
| m-IgGk BP-HRP  | Santa Cruz         | sc-516102| 1:5000 | Mouse  |
| mouse anti-rabbit IgG-HRP | Santa Cruz             | SC-2357 | 1:5000 | Mouse  |
| donkey anti-goat IgG-HRP | Santa Cruz             | SC-2020 | 1:5000 | Donkey |
| FOXA1          | ABCLONAL           | A15278 | 1:1000   | Rabbit |
| RUNX2          | ABCLONAL           | A2851  | 1:1000   | Rabbit |
| POU2F1         | ABCLONAL           | A1682  | 1:1000   | Rabbit |

| shRNA lentivirus | Accession number   | TRC number   | Target Region | Manufacturer |
|-----------------|--------------------|--------------|---------------|--------------|
| TEAD1           | NM_021961          | TRCN0000297070 | 3UTR | sigma |
| TEAD3           | NM_003214          | TRCN0000015948 | 3UTR | sigma |
| TFAM            | NM_003201          | TRCN0000016093 | 3UTR | sigma |
| NFIB            | NM_005596          | TRCN0000274124 | 3UTR | sigma |
| PARP2           | NM_005484          | TRCN0000235999 | CDS | sigma |

| siRNA           | Cat                | Manufacturer |
|-----------------|--------------------|--------------|
| HUMAN PARP2 siRNA | J-010127-05-0002 | Horizon discovery |
| HUMAN TEAD1 siRNA | J-012603-07-0002 | Horizon discovery |
| HUMAN TEAD3 siRNA | J-012604-05-0002 | Horizon discovery |
| HUMAN TFAM siRNA  | J-019734-05-0002 | Horizon discovery |
| HUMAN TFAM siRNA  | J-019734-05-0002 | Horizon discovery |
| HUMAN NFIB siRNA   | J-008456-05-0002 | Horizon discovery |
| HUMAN FOXA1 siRNA  | J-010319-05-0002 | Horizon discovery |
| HUMAN RUNX2 siRNA  | J-012665-07-0002 | Horizon discovery |
| HUMAN POU2F1 siRNA | J-019689-06-0002 | Horizon discovery |
Supplementary Table 4. Primer used in this work for luciferase reporter assay

| Primer | 5' (3') Primer |
|--------|---------------|
| Luci-rs1219642-A-F | CCGACCCTGAGTACAAATGTCGAATGTTGCT | |
| Luci-rs1219642-A-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs1219642-G-F | CCGACCCTGAGTACAAATGTCGAATGTTGCT | |
| Luci-rs1219642-G-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs4752570-A-F | CCTCTGGGACTGGGAAAAT | |
| Luci-rs4752570-A-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs4752570-G-F | CCTCTGGGACTGGGAAAAT | |
| Luci-rs4752570-G-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs4752570-C-F | CCTCTGGGACTGGGAAAAT | |
| Luci-rs4752570-C-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs16886034-A-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs16886034-A-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs16886034-C-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs16886034-C-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs6054381-T-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs6054381-T-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs6054381-A-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs6054381-A-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs6054381-C-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs6054381-C-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs74762363-T-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs74762363-T-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs74762363-A-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs74762363-A-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs74762363-C-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs74762363-C-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs7731588-T-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs7731588-T-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs7731588-G-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs7731588-G-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs7731588-A-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs7731588-A-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs7731588-C-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs7731588-C-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs79321361-G-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs79321361-G-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs79321361-A-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs79321361-A-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs79321361-C-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs79321361-C-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs8131691-G-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs8131691-G-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs8131691-A-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs8131691-A-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs8131691-C-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs8131691-C-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs7895676-C-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs7895676-C-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs7895676-T-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs7895676-T-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs2981578-G-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs2981578-G-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs2981578-A-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs2981578-A-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs2981578-C-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs2981578-C-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs2981578-T-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs2981578-T-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |
| Luci-rs2981578-A-F | CAGTGGCAGGTACCCCTGACATGTCACCTGACATCC | |
| Luci-rs2981578-A-R | TCGAGGCTGACTTGACATGTCACCTGACATCC | |