**Supplementary Methods**

***Human Patient Derived Xenografts (PDX) and Tumor Monitoring***

Human breast cancer tissues were obtained from consenting patients through the Royal Melbourne Hospital Tissue Bank and the Victorian Cancer Biobank with relevant institutional review board approval. Human Ethics approval was obtained from the Walter and Eliza Hall Institute (WEHI) Human Research Ethics Committee. NOD-SCID-IL2Rγ–/– mice were bred and maintained according to institutional guidelines. Animal experiments were approved by the WEHI Animal Ethics Committee.

A cohort of 40 female NOD-SCID-IL2Rγ–/– mice was seeded with a single cell suspension of early passage of the human breast PDX 315, as previously described (1). Briefly, cells were resuspended in transplantation buffer (50% fetal calf serum, 0.04% trypan blue in PBS and Matrigel [BD] at a ratio of 3:1), and injected (150,000 cells in 10 μl) into the cleared mammary fat pads of 3-4 week-old female mice. Mice were monitored for tumor development using electronic vernier calipers. Tumor volume was estimated by measuring the minimum and maximum tumor diameters using the formula: (minimum diameter)2(maximum diameter)/2. Once tumor volume reached 80–120 mm3, mice were randomized into treatment arms and treatment was initiated. Mice receiving tamoxifen were injected daily (7 days per week) with tamoxifen solution (5 mg/ml in ethanol:sunflower oil, 1:9). Mice receiving venetoclax were oral gavaged daily (5 days per week) with venetoclax solution (2.5 or 10 mg/ml in ethanol:PEG 400:Phosal-50G, 1:3:6). Mice were sacrificed when the tumor volume exceeded 600 mm3 or animal health deteriorated. Tumor fragments were archived in neutral buffered formalin and also snap frozen for further analysis.

***Immunohistochemistry***

Patient samples were fixed in neutral buffered formalin before embedding in paraffin. Sections were subjected to antigen retrieval and then incubated with antibodies against ER (Novocastra), PR (Novocastra), HER2 (Dako), BCL-2 (Dako), Ki67 (BD Pharmingen) and cleaved caspase-3 (Cell Signaling) for 30 min at room temperature, followed by biotinylated anti-IgG secondary antibodies (Vector Labs). Signal detection was performed using ABC Elite (Vector Labs) for 20 min and 3,3'-diaminobenzidine (Dako) 5 min at room temperature. Ki67 was quantified using a custom-made ImageJ (FIJI) plugin.

***Sample processing and plasma circulating tumour DNA extraction***

Blood was collected in EDTA tubes. Whole blood was first centrifuged at 1,600 g for 10 min to separate the plasma from the peripheral blood cells, followed by a further centrifugation step at 20,000 g for 10 min to pellet any remaining cells and/or debris. The plasma was then stored at -80°C until DNA extraction. DNA was extracted from up to 2 ml aliquots of plasma using the QIAmp Circulating Nucleic Acid Kit (Qiagen) according to manufacturer’s instructions. The DNA was eluted into 50 μl buffer AVE (Qiagen) and stored at -20◦ C. Matched normal germline DNA (from buffy coat) was obtained from study participants to enable confirmation of somatic status of identified genetic events.

***Targeted amplicon sequencing***

Targeted amplicon sequencing was performed on plasma-derived DNA and matched germline DNA using the 48.48 Access Array™ system (Fluidigm) as described previously (2). A panel of 394 amplicons with average amplicon length of 164 bases was designed across 39 genes recurrently mutated in breast cancer (see Supplementary Tables 5-6 for full list of genes/primers). All samples were analyzed in duplicate to control for PCR artefacts. The libraries were then sequenced with the Next Seq or MiSeq system (Illumina) using paired end sequencing with v2 150-bp kits. Bcl2fastq V.2.17.1 was used to perform sample demultiplexing and to convert BCL files generated from the Next Seq/MiSeq instrument into FastQ files containing short-read data. Using the primer sequences that are present in the data, short reads were first assigned to their respective amplicon. Samples were annotated through the ‘PathOS’ pipeline: a web-based variant management system (3). To restrict our analysis to high confidence variants, only variants which were called in both technical replicates or validated by droplet digital PCR were included.

***Droplet digital PCR***

Droplet digital PCR (ddPCR) analysis was performed using the Bio-Rad Droplet Digital PCR system following manufacturer’s protocols. Allele-specific PCR assays to specifically detect and quantify the absolute copies as well as the fractional abundance of point mutations and corresponding wild-types alleles were either custom designed or commercially obtained (Primer PCRTM PCR Primer and Assays, BioRad Laboratories) (Supplementary Table 7). Each sample was analysed by at least two technical replicates with 1-5 µl input per well. A Poisson correction was applied to determine the number of amplifiable molecules, which was used to further derive the number of copies of DNA carrying a particular mutation per millilitre of plasma. Data analysis was carried out using the QuantaSoft Software, version 1.7 (Bio-Rad). The limit of blank for the *PIK3CA* and *ESR1* assays was determined to be zero copies by running plasma DNA from healthy controls (data not shown).

***Flow Cytometry Profiling of Leukocyte Subsets***

Blood was collected from each patient by venepuncture into lithium heparin tubes. All samples were collected in the morning and stored at room temperature. For flow cytometric analysis, red blood cells were lysed and cells recovered by centrifugation at room temperature prior to resuspension in Dulbecco’s Phosphate-Buffered Saline. Cell numbers were determined by counting with the Invitrogen Countess Automated Cell Counter. Five million cells were added to a 10 ml polystyrene round bottomed tube (BD Falcon), washed with 3 ml DPBS and centrifuged at 1,500 rpm for 5 min at room temperature. The cell pellet was resuspended in 6 ml DPBS and 6 μl live/dead dye (Invitrogen) was added and cells were incubated for 30 min at 37° C. Unbound label was washed from the cells twice with 5 ml DPBS and cells recovered by centrifugation at 1,500 rpm for 5 min at room temperature. The cells were split into 5 wells (1 million for compensation control, T cell, T regulatory and NK/Monocyte/Dendritic stain; 2 million for B cell stain) of a 96 well round-bottomed plate and stained with one of the four antibody mixtures then for 30 min on ice in the dark. Unbound antibody was washed from the cells twice with 150 μl DPBS. Cells were fixed, permeabilized and stained with anti-FOXP3 according to manufacturer’s instruction (eBioscience, Foxp3/Transcription Factor Staining Buffer Set) Flow cytometry was performed on a BD LSRFortessa running FACSDiva software, and manually analysed using FlowJo v9.9.6 (Treestar). Cell populations were analyzed with statistical tests using Graphpad Prism v7.

***RNA-Seq Analysis***

RNA was extracted from breast cancer tissue taken from patients before and after treatment with tamoxifen. Patient samples were either snap frozen and stored at -80o C, or placed in RNA-later. Samples were homogenized and total RNA extracted using RNAeasy mini Kit (Qiagen). The RNA (50 ng) samples were prepared and indexed for Illumina sequencing using the TruSeq RNA V2 sample Prep Kit (Illumina) as per manufacturer’s instruction. The library was quantified using the Agilent Tapestation and the Qubit™ RNA assay kit for Qubit 2.0® Fluorometer (Life technologies). The indexed libraries were then prepared for paired end 80 bp sequencing on a NextSeq500 instrument using the 150 cycle kit v2 chemistry (Illumina) as per manufacturer’s instructions.Reads were mapped to the human genome (hg19) with Rsubread (4), and then featureCounts (5)was used to count the number of reads mapping to Entrez genes specified by NCBI refSeq annotation. edgeR (6) was used to normalize the data with the TMM method (7).

**REFERENCES**

1. Vaillant F, Merino D, Lee L, Breslin K, Pal B, Ritchie ME, et al. Targeting BCL-2 with the BH3 mimetic ABT-199 in estrogen receptor-positive breast cancer. Cancer Cell 2013;24:120-9.

2. Wong SQ, Raleigh JM, Callahan J, Vergara IA, Ftouni S, Hatzimihalis A, et al. Circulating Tumor DNA Analysis and Functional Imaging Provide Complementary Approaches for Comprehensive Disease Monitoring in Metastatic Melanoma. JCO Prec Oncol 2017;DOI: 10.1200/PO.16.00009.

3. Doig KD, Fellowes A, Bell AH, Seleznev A, Ma D, Ellul J, et al. PathOS: a decision support system for reporting high throughput sequencing of cancers in clinical diagnostic laboratories. Genome Med 2017;9:38.

4. Liao Y, Smyth GK, Shi W. The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. Nucleic Acids Res 2013;41:e108.

5. Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 2014;30:923-30.

6. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 2010;26:139-40.

7. Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol 2010;11:R25.