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eQTL colocalization analyses identify *NTN4* as a candidate breast cancer risk gene

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19 **ABSTRACT**

20 Breast cancer genome-wide association studies (GWAS) have identified 150 genomic risk regions
21 containing more than 13,000 credible causal variants (CCVs). The CCVs are predominantly
22 noncoding and enriched in regulatory elements. However, the genes underlying breast cancer risk
23 associations are largely unknown. Here, we used genetic colocalization analysis to identify loci at
24 which gene expression could potentially explain breast cancer risk phenotypes. Using data from the
25 Breast Cancer Association Consortium (BCAC) and quantitative trait loci (QTL) from the Genotype-
26 Tissue Expression (GTEx) project and The Cancer Genome Project (TCGA), we identify shared
27 genetic relationships and reveal novel associations between cancer phenotypes and effector genes.
28 Seventeen genes, including *NTN4,* were identified as potential mediators of breast cancer risk. For
29 *NTN4,* we showed the rs61938093 CCV at this region was located within an enhancer element that
30 physically interacts with the *NTN4* promoter, and the risk allele reduced *NTN4* promoter activity.
31 Furthermore, knockdown of *NTN4* in breast cells increased cell proliferation *in vitro* and tumor growth
32 *in vivo*. These data provide evidence linking risk-associated variation to genes that may contribute
33 to breast cancer predisposition.

34

35

36 The influence of common genetic variation on gene expression underlies a considerable proportion
37 of the heritability associated with complex traits. Mapping of expression QTL (eQTL), where genetic
38 variants are tested for association with gene expression levels, is widely used to identify genes that
39 are regulated by trait-associated variants. Several studies have shown that eQTLs are enriched in
40 cell types relevant to the trait of interest[1; 2]. For example, T cell-specific eQTLs are over-represented
41 for autoimmune risk alleles and monocyte-specific eQTLs for Alzheimer's [MIM: 104300] and
42 Parkinson's [MIM: 168600] disease alleles[2]. For breast cancer [MIM: 114480], several studies have
43 used eQTL data from tumor and normal tissues datasets to identify candidate target genes[3-6]. Recent
44 studies have also showed that breast cancer risk variants could regulate genes in cells of the tumor
45 microenvironment, such as immune cells and fibroblasts[7; 8]. Because eQTLs are widespread, overlap
46 between GWAS and eQTL signals is likely to occur by chance when using nominal significance

levels. To mitigate false positive findings it is therefore important to show that the same genetic signal underlies gene expression and disease susceptibility.

Several statistical colocalization approaches have been developed to determine whether molecular traits (e.g. gene expression) and a disease trait share common causal variants. The simplest Bayesian model used in tools such as QTLMatch[9] and COLOC[10] tests for colocalization for two traits and determines whether they are driven by distinct variants or share a single causal signal. For example, Parker et al used COLOC to identify 32 emphysema-associated [MIM: 130700] regions where it is likely that colocalized GWAS and eQTL signals arise from the same causal variant[11]. Additional functional studies then showed that the emphysema-associated variant rs1690789 regulates *TGFB2* (encoding transforming growth factor beta 2 [MIM: 190220]) expression in human lung fibroblasts. A recent implementation of COLOC, called HyPrColoc (Hypothesis Prioritization in multi-trait Colocalization), identifies colocalized association signals using summary statistics on large number of traits[12]. This method has been used to identify regulatory loci underlying quantitative haematopoietic traits[13].

In this study, we extracted eQTL association effect estimates and standard errors for all variants at the 150 breast cancer risk loci previously analysed by BCAC[14] (mean region size = 1.09 Mb). GWAS summary data were available for overall breast cancer risk from 122,977 cases and 105,974 controls[3]; and for estrogen receptor negative (ER-) breast cancer risk from 21,468 cases and 100,594 controls, combined with 18,908 *BRCA1* mutation carriers (9414 with breast cancer)[15] all of European ancestries. Variant IDs were converted to GRCH38 build co-ordinates and harmonized with GTEx data (0.86% failed conversion and were dropped from the analysis). The GTEx version 8 release includes data from normal breast tissue from 396 individuals. GTEx eQTL association data for variants within ±1 Mb windows of transcription start sites were extracted based on the variants present in the breast cancer risk data. Colocalization of the GWAS and eQTL signals were calculated using the HyPrColoc R package[12]. Breast cancer risk phenotypes and each proximal gene were analyzed separately with default parameters. Signals were considered to be plausibly colocalizing if posterior probability for colocalization (PPFC) > 0.7, resulting in a false discovery rate of 5%[12].

We identified 17 genes at 14 loci where the GTEx eQTL association *P* values are < 10^{-6} (**Table 1**). For every locus, all candidate SNPs met the GWAS significance *P* value threshold ($5x10^{-8}$) for overall or ER- breast cancer risk (**Table 1**). For 11 loci (*NTN4, PIDD1* [MIM: 605247]*, CBX8* [MIM: 617354]*, L3MBTL3* [MIM: 618844], *RCCD1* [MIM: 617997], *PRC1-AS1*, *SSBP4* [MIM: 607391], *MARCH11* [MIM: 613338], *ZNF596*, *RP5-855D21.3 and RP11-53O19.1*), the candidate colocalized SNPs have been previously nominated as strong candidate causal signals using multivariate logistic regression[14] (**Table 1** and **Figure 1**). However, at six loci (*ATG10* [MIM: 610800]*, CCDC88C* [MIM: 611204]*, PPM1K* [MIM: 611065]*, RP11-250B2.3, RP1-265C24.5* and *RP11-250B2.5*), the colocalized signals are independent secondary signals based on stepwise multinomial logistic regression analysis ($10^{-6} < P < 10^{-4}$)[14]. While this does not rule out causality, larger GWAS would be required to confirm genome-wide significance (**Figure S1**)[14].

Published computational predictions of target genes at breast cancer risk loci using the INQUISIT pipeline (which interrogates data including ChIA-PET, Hi-C, ChIP-seq and eQTL data independent of GTEx) provide further support for ten colocalized genes (**Table S1**)[3; 14]. Of these, *NTN4, PIDD1, L3MBTL3* and *RCCD1* have the strongest evidence from functional genomics data. Transcriptome-wide association studies also suggest that 13 of the 17 genes are regulated by breast cancer risk variants[5-7; 16; 17] (**Table S1**). Moreover, previous eQTL analysis based on TCGA breast tumor data have identified three of these candidate genes[3; 14] (**Figure S2**). For three genes (*PIDD1, L3MBTL3* and *SSBP4*), CCVs are located in the promoter regions, and for *PIDD1* previous reporter assays indicate the risk haplotype increases promoter activity[3]. Our recent capture Hi-C data showed also chromatin looping occurs between putative regulatory regions containing CCVs and the promoters of four genes (*NTN4, PRC1-AS1, ATG10* and *RP1-265C24.5*) in breast cell lines[18]. For the remaining loci, multiple CCVs were located in the introns of target genes and/or intergenic regions, but lacked demonstrable CCV-gene interactions. It is possible that some *cis*-regulatory interactions are only detected in specific breast cell subpopulations, or that CCVs are acting through other mechanisms such as perturbation of pre-messenger RNA splicing or altered noncoding RNA stability, structure and/or function. Of note, three genes (*PIDD1*, *CBX8*, and *L3MBTL3*) contain breast cancer CCVs in

105 their exons which are predicted to change the amino acid sequence, thus we cannot rule out that
106 these are functional variants that affect the protein product.
107

108 One high probability colocalization signal, targeting *NTN4,* was detected at a locus at 12q22 (**Table**
109 **1**, **Figures 1** and **2**). Genetic fine-mapping studies have identified one risk signal at 12q22 that
110 contains two CCVs (rs61938093 and rs17356907; odds ratio = 1.094, r^2 = 1)[14]. Both CCVs fall within
111 putative regulatory elements (PREs) marked by open chromatin in B80T5 and MCF10A non-
112 tumorigenic breast cell lines (**Figure 3A**). The PREs map to a large intergenic region between
113 *USP44* [MIM: 610993] (encoding ubiquitin-specific protease 44) and *NTN4* (encoding Netrin 4;
114 **Figure 3A**). Using promoter capture HiC data[18], we observed that the PREs frequently participate in
115 long-range chromatin interactions with the *NTN4* promoter in non-tumorigenic and tumorigenic
116 breast cell lines (**Figures 3A** and **S2A**). Notably, no other eQTLs or chromatin interactions from the
117 PRE to promoter regions were detected in the breast cell lines we examined (**Figures 3A** and
118 **S2A)**[18], suggesting *NTN4* is the likely target gene at this signal.
119

120 To determine how the PRE alters *NTN4* transcriptional activity, we targeted a nuclease-defective
121 dCas9 fused to the Kruppel-associated box (lentiviral vector pHR-SFFV-dCas9-BFP-KRAB; a gift
122 from Stanley Qi & Jonathan Weissman, Addgene plasmid #46911) to the PRE. Two independent
123 single-guide RNAs (sgRNAs) targeting the PRE were designed (**Table S2**) and cloned into the
124 lentiviral vector pgRNA-humanized (a gift from Stanley Qi, Addgene plasmid #44248). Lentiviral
125 particles were produced from HEK293 cells transfected with accessory plasmids pCMV-dR8.91 and
126 pCMV-VSV-G (gifts from David Harrich, QIMR Berghofer), and with dCas9-KRAB or pgRNA
127 constructs using Lipofectamine 2000 (Life Technologies). Supernatants from dCas9-KRAB and
128 pgRNA cultures were mixed and transduced into Bre80-TERT1 breast cells. Cells expressing both
129 dCas9-KRAB (co-expressing blue fluorescent protein) and pgRNA (co-expressing mCherry) were
130 enriched by FACS on the Aria IIIu platform (Becton-Dickinson). Notably, silencing of the PRE
131 significantly reduced *NTN4* expression in Bre80-TERT1 cells, suggesting that the PRE acts as an
132 transcriptional enhancer (**Figure 3B**).
133

134 The regulatory capability of the PRE, combined with the effects of the CCVs, was further examined
135 in reporter assays. An *NTN4* promoter-driven luciferase reporter construct was generated by the
136 insertion of a PCR amplified genomic fragment into the KpnI/HindIII sites of pGL3-basic (Promega).
137 A 1010 base pairs (bp) fragment containing a PRE1, with either the risk or protective alleles of
138 rs61938093, or a 983 bp fragment containing a PRE2, with either the risk or protective alleles of
139 rs17356907, were synthesized as gBlocks (Integrated DNA Technologies) and then cloned into the
140 BamHI/SalI sites of the *NTN4*-promoter construct (genomic coordinates and primers are listed in
141 **Table S2**). MCF10A and Bre80-TERT1 breast cells were transfected with the reporter constructs
142 and luciferase activity was measured 24 h post-transfection using the Dual-Glo Luciferase System
143 (Promega). To correct for any differences in transfection efficiency, *Firefly* luciferase activity was
144 normalized to *Renilla*. Reporter assays confirmed strong enhancer activity of the PRE1 on the *NTN4*
145 promoter in MCF10A and Bre80-TERT1 cells and inclusion of the rs6198093 risk allele significantly
146 reduced *NTN4* promoter activity (**Figure 3C**). In contrast, inclusion of PRE2 with the protective or
147 risk alleles of rs17356907 had no significant effects on the NTN4 promoter activity. pGL3-promoter
148 luciferase results ???? These results suggest that rs61938093 alters transactivation of NTN4, but it
149 is possible that the CCVs also influence recruitment of key proteins required for chromatin looping
150 between the enhancer and *NTN4* promoter, which would not be observed in a reporter assay.
151

152 To assess the potential impact of the CCVs on chromatin looping, quantitative allele-specific 3C was
153 performed in heterozygous MCF10A and T47D breast cell lines. 3C libraries were generated using
154 HindIII as previously described[19]. Three independent 3C libraries or genomic input DNA were
155 amplified for 15 cycles with two separate xxxxx 3C specific or genomic DNA PCR primers (primers
156 are listed in **Table S2**) and purified by QIAGEN columns. Allele-specific PCR products were then
157 quantified using a custom TaqMan SNP genotyping assay for rs61938093 (Life Technologies) on
158 the Rotor-Gene 6000 platform. Purified PCR products were Sanger sequenced by the Australian
159 Genome Research Facility (AGRF). The results showed a preference for the protective *t*-allele
160 (**Figures 3D, 3E** and **S2B-S2E**), indicating that risk alleles may abrogate looping between the
161 enhancer and *NTN4* which in turn may reduce *NTN4* expression.
162

Electrophoretic mobility shift assays (EMSAs) then assessed transcription factor (TF) binding for the protective and risk alleles of the CCVs. Nuclear lysates were prepared from Bre80-TERT1 and MCF10A breast cells using the NE-PER nuclear and cytoplasmic protein extraction kit (ThermoFisher). Biotinylated oligonucleotides representing either the risk or protective allele were synthesized (Integrated DNA Technologies; **Table S2**) and annealed to form double-stranded duplexes. Duplex-bound complexes were resolved by electrophoresis in 10% (w/v) Tris-borate-EDTA polyacrylamide (Lonza) and transferred to positively-charged nylon membranes by semi-dry transfer (Bio-Rad). Membranes were processed using the LightShift Chemiluminescent EMSA kit (ThermoFisher) and visualized with the C-DiGit blot scanner. The EMSAs showed that rs61938093 and rs17356907 altered protein binding *in vitro* in Bre80-TERT1 and MCF10A cell lysates (**Figures 3F, S3A** and **S3B**). *In silico* prediction tools including HaploReg[20] and Alibaba2[21] predicted both CCVs to alter TF binding. However, EMSAs using competitor DNA against predicted and other breast-relevant TFs were unable to identify the specific protein(s) binding to the alleles (**Figures S3C** and **S3D**).

We examined expression of *NTN4* in matched normal and cancerous breast tissues using TCGA RNA-seq data. *NTN4* was more highly expressed in normal tissue, a mixture of cell types, compared to adjacent tumor samples (**Figure 4A**) and is expressed across the histological subtypes, albeit with lower expression in the basal subtype (**Figure 4B**). To explore the effect of reduced *NTN4* on breast cancer cell proliferation, MCF7 cells were transfected with ON-TARGETplus negative control or *NTN4* siRNA smartpools (Dharmacon) using RNAiMAX (Life Technologies). *NTN4* silencing was confirmed by TaqMan qPCR gene expression assay 72 h post-transfection (**Figures S3E** and **S3F**). Notably, *NTN4* depletion promoted anchorage-dependent and -independent cell growth in MCF7 cells (**Figures 4C** and **4D**). To assess the effect of reduced *NTN4* on tumor growth, we stably depleted *NTN4* in MCF7 cells by targeting dCAS9-KRAB to the promoter of *NTN4,* and injected the cells in the mammary fat pad of nude mice*.* Female BALB/c-Foxn1^{nu}/Arc mice were first subcutaneously implanted with 17β-estradiol (0.72 mg/pellet, 90 day release; Innovative Research of America) at 8 weeks of age. MCF7 control-CRISPRi or *NTN4*-CRISPRi cells were orthotopically injected into mammary fatpads 3 days later at 10^7 cells per mouse (6-7 mice per cell line). Tumor

192 volumes were measured every 2 days until experimental end, at which point mice were euthanized
193 and their tumors excised and weighed. All animal procedures were conducted in accordance with
194 Australian National Health and Medical Research regulations on the use and care of experimental
195 animals, and approved by the QIMR Berghofer Medical Research Institute Animal Ethics Committee
196 (P1499). Compared to control MCF7 cells containing non-targeting sgRNA, *NTN4* depletion led to a
197 marked increase in tumor growth (**Figures 4E, 4F** and **S3G**), which was reflected in increased tumor
198 weight (**Figure 4G**).
199
200 *NTN4* encodes the Netrin-4 secreted protein which has been implicated in various developmental
201 processes including axon guidance, angiogenesis, mammary and lung morphogenesis[22]. Several
202 studies show that NTN4 is involved in cancer, but the exact role of NTN4 appears to be dependent
203 on the cancer type. For example, NTN4 knockdown reduces cell proliferation and motility in gastric
204 cancer [MIM: 613659] and melanoma [MIM: 155600][23; 24], but promotes cell migration and invasion
205 in colorectal cancer [MIM: 114500] and breast cancer[25; 26]. *NTN4* has also been implicated in breast
206 cancer progression. For example, reduced NTN4 is reported to promote migration and invasion of
207 breast cancer cells through epithelial to mesenchymal transition[26]. In addition, NTN4 has been
208 shown to be an independent biomarker for prognosis of survival in breast cancer[27; 28]. We and others
209 have demonstrated that SNPs can alter chromatin loop formation between promoters and
210 enhancers[29; 30]. Here, we provide evidence that the same mechanism may explain how breast cancer
211 CCVs alter *NTN4* expression and that suppressed *NTN4* increases cancer-related processes
212 including cell proliferation and tumor growth. However, we acknowledge that further functional
213 studies will be required to clarify how NTN4 contributes to breast tumor initiation.
214
215 Seven additional colocalized target genes have prior evidence for a functional role in cancer. For
216 example, PIDD1 (p53-induced death domain protein 1) is implicated in DNA-damage-induced
217 apoptosis and tumorigenesis[31]. CBX8 is overexpressed in breast cancer and correlates with poor
218 survival[32]. CBX8 functions by interacting with the H3K4 methyltransferase complex component
219 WDR5 to activate genes involved in Notch signaling and promote breast tumorigenesis[32].
220 Furthermore, Guo et al showed ectopic ATG10 (autophagy related 10) overexpression decreases

221 colony formation of MDA-MB-231 breast cancer cells, suggesting it may play a role in breast
222 tumorigenesis[5]. Notably, ten genes have no reported involvement in breast tumorigenesis and may
223 represent new genes that influence the susceptibility to breast cancer. This list includes five lncRNAs
224 which are arguably more challenging to investigate as they can have multiple functions. However,
225 there is increasing evidence that dysregulated lncRNAs contribute to breast cancer etiology[29; 33; 34].
226
227 In summary, we used eQTL colocalization to link breast cancer risk variants to seventeen target
228 genes, including some potential cancer driver genes, but many with no reported role in breast cancer
229 etiology. However, even with demonstration of shared genetic signals, it is as yet unknown how
230 genes implicated by statistical colocalization analyses reflect true molecular mechanisms. It is
231 therefore important to perform functional assays, as we have done for *NTN4,* to provide evidence
232 that the variant affects gene expression and the gene plays a role in the disease etiology. Future
233 work confirming the role of these genes or associated pathways in breast cancer development could
234 ultimately lead to new avenues for breast cancer prevention or therapy.
235
236 **SUPPLEMENTAL DATA**
237 Supplemental Data include three figures and two tables.
238
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252

253 **DECLARATION OF INTERESTS**

254 The authors declare no conflict of interests.
255

256 **WEB RESOURCES**

257 Breast Cancer Association Consortium,
258 http://bcac.ccge.medschl.cam.ac.uk/bcacdata/oncoarray/gwas-icogs-and-oncoarray-summary-
259 results/
260 GTEx Portal, https://gtexportal.org/home/
261 LDlink, https://ldlink.nci.nih.gov/
262 Bioconductor, https://www.bioconductor.org/
263

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373

374 **FIGURE LEGENDS**

375 **Figure 1. Comparison of association signals from BCAC and GTEx v8 for breast tissue.**
376 LocusCompare plots[35] for eleven high-probability colocalized signals**.** Gene names and the relevant
377 breast cancer phenotypes are shown in the plot headings. Points are coloured based on linkage
378 disequilibrium (LD) bins relative to the candidate SNP prioritized by HyPrColoc (purple diamond
379 labelled with rsID; red: ≥ 0.8, orange: 0.6 – 0.8, green: 0.4 – 0.6, light blue: 0.2 – 0.4, and dark blue:
380 < 0.2). LD data from 1000 Genomes phase 3, version 5 were retrieved from the LDlink portal[36].
381 Strong candidate causal variants for breast cancer risk are annotated as small diamonds and weaker
382 secondary risk variants as squares.

383

384 **Figure 2. Regional association plots at the 12q22 breast cancer risk locus.** Single variant
385 associations with overall breast cancer risk (top panel) and with *NTN4* expression in normal breast
386 tissue from GTEx v8 (lower panel). Variants are represented by points colored relative to linkage
387 disequilibrium (LD) with the candidate variant detected by HyPrColoc (rs17356907; red: ≥ 0.8,
388 orange: 0.6 – 0.8, green: 0.4 – 0.6, light blue: 0.2 – 0.4, and dark blue: < 0.2).

389

390 **Figure 3. Breast cancer CCVs distally regulate *NTN4*.** (**A**) WashU genome browser showing
391 topologically associating domains (TADs) as horizontal gray bars above GENCODE annotated

coding genes (blue). The promoter capture Hi-C (PCHi-C) baits are depicted as black boxes. The putative regulatory element (PRE) containing the CCVs is shown as red colored vertical lines. The ATAC-seq tracks for B80T5 and MCF10A breast cells are shown as blue histograms. PCHi-C chromatin interactions are shown as black arcs. Red arcs depict chromatin looping between CCVs and the *NTN4* promoter region. (**B**) dCAS9-KRAB was targeted to the PRE using two different sgRNAs (sgPRE1 and sgPRE2) in Bre80-TERT1 breast cells. SgCON contains a non-targeting control guide RNA. Gene expression was measured by qPCR and normalized to *beta-glucuronidase* (*GUSB*) expression. Error bars, SEM (n=3). *P*-values were determined by one-way ANOVA followed by Dunnett's multiple comparisons test (**p < 0.01). (**C**) Luciferase reporter assays following transient transfection of MCF10A and Bre80-TERT1 breast cells. A PRE1 containing the protective (Prot.) or risk alleles of rs61938093 and a PRE2 containing the protective (Prot.) or risk alleles of rs1735907 were cloned into *NTN4*-promoter driven luciferase constructs. Error bars, SEM (n=3). *P*-values were determined by two-way ANOVA followed by Dunnett's multiple comparisons test (**p < 0.01, ****p < 0.0001). (**D,E;** *left panels*) 3C interaction profiles between the *NTN4* promoter and the genomic region containing the PRE in MCF10A (**D**) and T47D (**E**) 3C libraries generated with HindIII. A physical map of the region interrogated by 3C is shown above; the blue shading represents the position of the PRE and the anchor point set at the *NTN4* promoter. Representative 3C profiles are shown. Error bars, SD (n=3). (**D,E;** *right panels*) Allele-specific qPCR to quantify the allelic ratio at CCV rs61938093. Error bars, SEM (n=3). *P*-values were determined using a Student's t-test (***p < 0.001). (**F**) EMSA for oligonucleotide duplexes containing CCVs rs61938093 or rs17356907 with either the risk allele (R) or protective allele (P) as indicated, assayed using Bre80-TERT1 nuclear extracts. Competitor oligonucleotides are listed above each panel and were used at 100-fold molar excess: (-) no competitor; (Neg) a non-specific competitor; (Self) an identical oligonucleotide with no biotin label. Red arrowheads indicate band mobility differences between alleles.

Figure 4. *NTN4* depletion promotes breast cell proliferation and tumor formation. (**A**) Boxplot showing *NTN4* expression in normal breast and paired tumor tissue samples from TCGA. Boxplots indicate median (centre line), interquartile range (box limits) and range (whiskers). P-value was determined using a two-tailed t-test. (**B**) Boxplot showing *NTN4* expression in breast tumors from

421 TCGA stratified by PAM50 molecular subtypes (n=841). Boxplots indicate median (centre line),
422 interquartile range (box limits) and range (whiskers). (**C**) Proliferation of MCF7 cells transfected with
423 a non-targeting control (siCON) or *NTN4* (siNTN4) ON-TARGETplus siRNAs. Cells were grown in
424 24-well plates and confluency of the wells was measured by the IncuCyte live-cell imaging system.
425 Results represent relative cell growth rates. Error bars, SD (n=2). P-value was determined by
426 Student's t-test comparing confluency at the last time point measured (***p < 0.001). (**D**) MCF7 cells
427 were transfected with the siCON or siNTN4 and grown over 7 days in ultra low-attachment
428 conditions. Cell growth was assessed using the CellTiter-Glo luminescent cell viability assay. Graph
429 shows fold change in luminescence of siNTN4 treated cells relative to siCON treated cells. Error bar,
430 SEM (n=3). P-value was determined by Student's t-test test (**p < 0.01). (**E**) MCF7-control (PgCON)
431 or MCF7-dCas9-KRAB NTN4 repressed cells (SgNTN4-P1/P2) were orthotopically injected into the
432 mammary fat pads of nude mice. Tumor growth curves for each group are shown. Values are shown
433 as average tumor volumes at each time point. Error bars, SEM (n=6-7 mice per group). (**F**) Tumors
434 of individual mice were dissected at day 38 post-injection. The scale bars represent 1 cm. (**G**) Plot
435 of the individual weights of tumors with mean and SEM shown by cross-bar and error bars. (**E, G**)
436 Mann-Whitney *U* test was used to compare differences between groups (*p < 0.05, **p < 0.01, ***p
437 < 0.001).

438 **Table 1**. Candidate breast cancer risk genes identified by eQTL colocalization analyses.

439

Ensembl ID	Gene symbol	Breast cancer risk association	Genomic coordinates (hg19)	Posterior probability	Candidate SNP	Posterior explained by SNP	GTEx eQTL *P* value	Breast cancer risk *P* value
ENSG00000074527.11	*NTN4*	Overall risk	chr12:95527759-96527759	0.9466	rs17356907	0.97	8.01E-09	1.02E-39
ENSG00000141570.10	*CBX8*	Overall risk	chr17:77281387-78281725	0.9178	rs9905914	0.49	7.92E-23	4.00E-09
ENSG00000198945.7	*L3MBTL3*	Overall risk	chr6:129849119-130849119	0.7998	rs7740107	1.00	5.88E-40	2.90E-11
ENSG00000183654.8	*MARCH11*	Overall risk	chr5:15687358-16687528	0.8369	rs1013018	0.16	3.05E-09	1.65E-11
ENSG00000177595.17	*PIDD1*	Overall risk	chr11:303017-1303017	0.9695	rs6597981	0.22	6.53E-27	1.35E-12
ENSG00000166965.12	*RCCD1*	Overall risk	chr15:91009215-92009215	0.9633	rs113343095	0.60	2.44E-24	3.37E-15
ENSG00000130511.15	*SSBP4*	Overall risk	chr19:18050434-19071141	0.7800	rs7258465	0.09	7.87E-08	2.79E-28
ENSG00000172748.13	*ZNF596*	ER- risk	chr8:0-670692	0.9059	rs35346588	0.79	2.17E-08	1.39E-08
ENSG00000258725.1	*PRC1-AS1**	Overall risk	chr15:91009215-92009215	0.9302	rs2290202	0.22	5.89E-10	1.87E-15
ENSG00000251141.5	*RP11-53O19.1**	Overall risk	chr5:44013304-45206498	0.9347	rs10941679	1.00	4.41E-07	5.61E-73
ENSG00000272812.1	*RP5-855D21.3**	ER- risk	chr8:0-670692	0.9769	rs3008281	0.81	6.11E-08	6.23E-09
ENSG00000152348.15	*ATG10*#	Overall risk	chr5:80928261-82038046	0.7904	rs144580806	0.36	2.56E-40	8.07E-12
ENSG00000015133.18	*CCDC88C*#	Overall risk	chr14:91341069-92368623	0.9465	rs8018155	0.50	9.15E-11	4.03E-12
ENSG00000163644.14	*PPM1K*#	Overall risk	chr4:88743818-89743818	0.9935	rs10022462	0.58	1.60E-08	1.55E-09
ENSG00000233967.6	*RP11-250B2.3**#	Overall risk	chr6:80594287-81594287	0.8473	rs9448940	0.22	4.65E-11	9.85E-09
ENSG00000260645.1	*RP11-250B2.5**#	Overall risk	chr6:80594287-81594287	0.8227	rs1436864	0.08	1.97E-08	3.89E-09
ENSG00000219392.1	*RP1-265C24.5**#	Overall risk	chr6:26180698-27180698	0.9901	rs35768595	0.38	5.95E-10	3.16E-09

440

441 * Noncoding RNAs

442 # Weaker secondary signals[14]

443

444

445