

***L-Arginine dependence of breast cancer –
molecular subtypes matter.***

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Supplementary Material

Supplementary Table

Supplementary Table 1. Specific TaqMan gene expression assays for real-time quantitative PCR for each of the genes analyzed in this study.

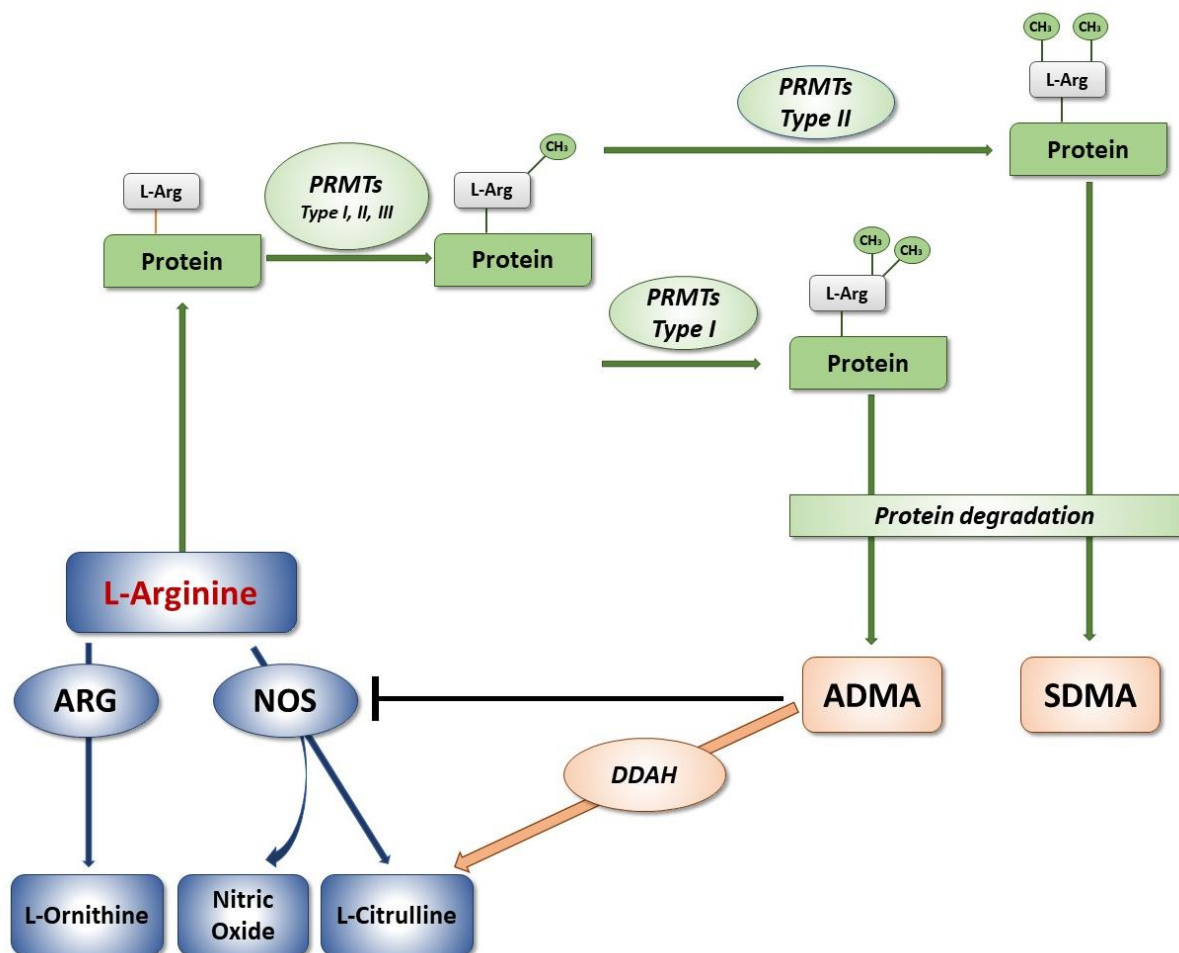
Target Gene	Taqman gene expression assay identifier
ARG1	Hs00968979_m1
ARG2	Hs00982833_m1
DDAH1	Hs00201707_m1
DDAH2	Hs00967863_g1
ASS1	Hs01597989_g1
ASL	Hs00902699_m1
PRMT1	Hs01587651_g1
PRMT2	Hs00895397_m1
PRMT3	Hs00969596_m1
PRMT4	Hs00406354_m1
PRMT5	Hs01047356_m1
PRMT6	Hs05054640_s1
PRMT7	Hs00219300_m1
PRMT8	Hs00998598_m1
PRMT9	Hs00378858_m1

Gene names: ARG, arginase; DDAH, dimethylarginine dimethylaminohydrolase; ASS, L-arginine succinate synthase; ASL, L-arginine succinate lyase; PRMT, protein-arginine methyltransferase.

Supplementary Figures

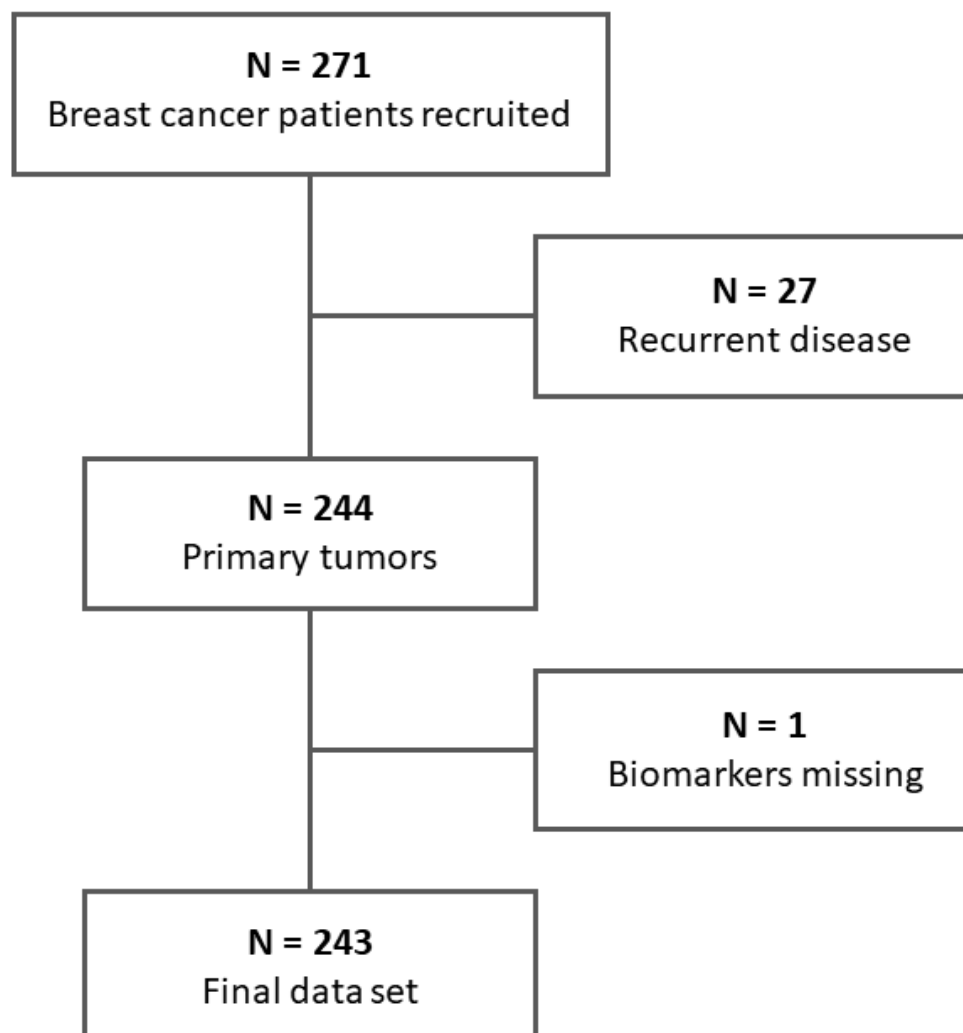
Supplementary Figure 1.

Schematic representation of major L-arginine-metabolizing pathways. L-arginine is converted to L-citrulline and nitric oxide by nitric oxide synthases or to L-ornithine and urea by arginases. Further, it can be methylated by a family of protein-arginine N-methyltransferases (PRMTs), of which three distinct subtypes can be discriminated. The dimethylarginines, ADMA and SDMA, are released upon hydrolytic degradation of methylated proteins. ADMA is metabolically cleaved to L-citrulline and dimethylamine by dimethylarginine dimethylaminohydrolases (DDAHs).



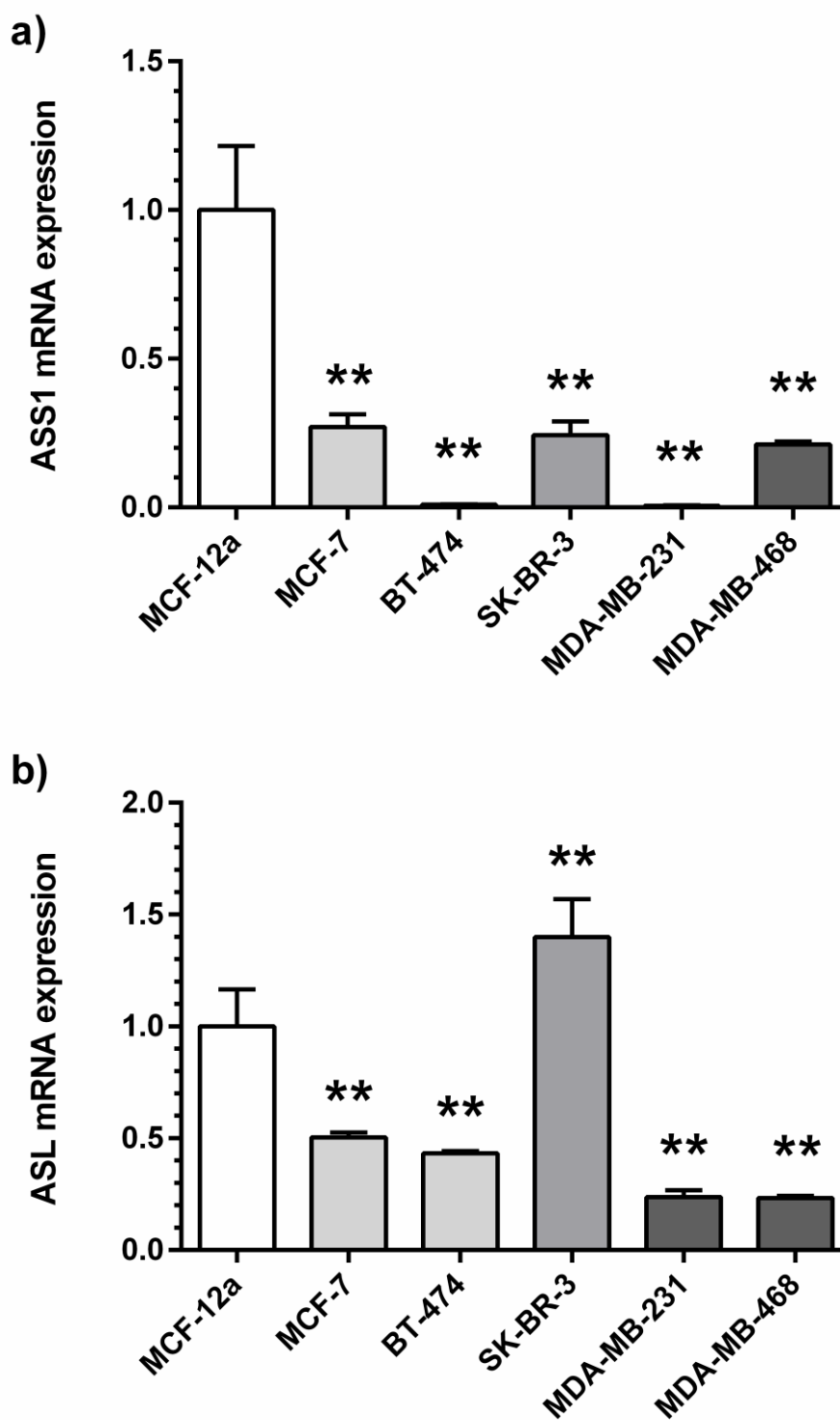
Supplementary Figure 2.

CONSORT flow diagram of the breast cancer study population.



Supplementary Figure 3.

Gene expression measured by quantitative real-time RT-qPCR for genes involved in the L-arginine salvage pathway: a) argininosuccinate synthase-1 (ASS1), b) argininosuccinate lyase (ASL). ** $p < 0.001$ as compared to MCF-12A normal breast epithelial cells in two-way ANOVA followed by Dunnett's multiple comparisons test.



Participant No: _____

Data documentation for study participant No. _____

Date of documentation: _____

General Patient Characteristics

Date of birth	_____	Date of diagnosis	_____
Body weight at diagnosis	_____ (kg)	Height	_____ (cm)
Date of surgery	_____	Serum creatinine (pre-surgery)	_____ (mg/dL)

Diagnosis / Medical History

- ☐ Primary breast cancer ☐ Recurrent carcinoma ☒ left ☐ right breast
- ☐ IDC (Invasive, ductal carcinoma)
- ☐ ILC (Invasive, lobular carcinoma)
- ☐ DCIS (Ductal carcinoma *in situ*)
- ☐ LCIS (Lobular carcinoma *in situ*)

Remarks: _____
(e.g., IDC with DCIS components;
bifocal;
microcalcifications) _____

Histological Type

- | | | | |
|---------------------------------------|---------------------------------------|---|--|
| <input type="checkbox"/> ductal | <input type="checkbox"/> lobular | <input type="checkbox"/> papillary | <input type="checkbox"/> serous |
| <input type="checkbox"/> endometrioid | <input type="checkbox"/> mucinous | <input type="checkbox"/> serous-papillary | <input type="checkbox"/> clear-cell |
| <input type="checkbox"/> tubular | <input type="checkbox"/> inflammatory | <input type="checkbox"/> other | <input type="checkbox"/> not specified |

Participant No: _____

Previous Malignant Disease(s)

☐ No

☐ Yes

If yes, which
(type, date,
etc.)

Family History of Breast Cancer or other Malignant Diseases

TNM-State & Grading

Grading: ☐ 1 ☐ 2 ☐ 3

Tumor size **pT** _____

Sentinel node **N** _____ positive LN/dissected LN ____ / ____

Metastasis **M** _____ If positive, where? _____

Lymphatic invasion ☐ L0 ☐ L1

Vascular invasion ☐ V0 ☐ V1 ☐ V2

Resection ☐ R0 ☐ R1 ☐ R2

Date of secondary resection _____ Thereafter R0? ☐ yes ☐ no

Participant No: _____

Receptor State / Molecular Markers

ER	_____ /12	<input type="checkbox"/> N/A	PR	_____ /12	<input type="checkbox"/> N/A
HER2	<input type="checkbox"/> 0 (neg)	<input type="checkbox"/> 1 (pos)	<input type="checkbox"/> N/A	Ki-67	_____ % <input type="checkbox"/> N/A
E-cadherin	<input type="checkbox"/> neg	<input type="checkbox"/> pos	<input type="checkbox"/> N/A		

Therapy

Surgery

☐ Mastectomy ☐ Lumpectomy ☐ _____ ☐ None

Chemotherapy

☐ None ☐ Yes, regimen: _____

Radiotherapy

☐ Yes ☐ No

Endocrine Therapy

☐ None ☐ Yes, which: _____

*Patient Questionnaire
for Breast Cancer Follow-up Study, UKE*

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