Supplementary Material for:

Portable NMR for the investigation of models of mammographic density ex vivo: Androgens antagonise the promotional effect of oestrogen

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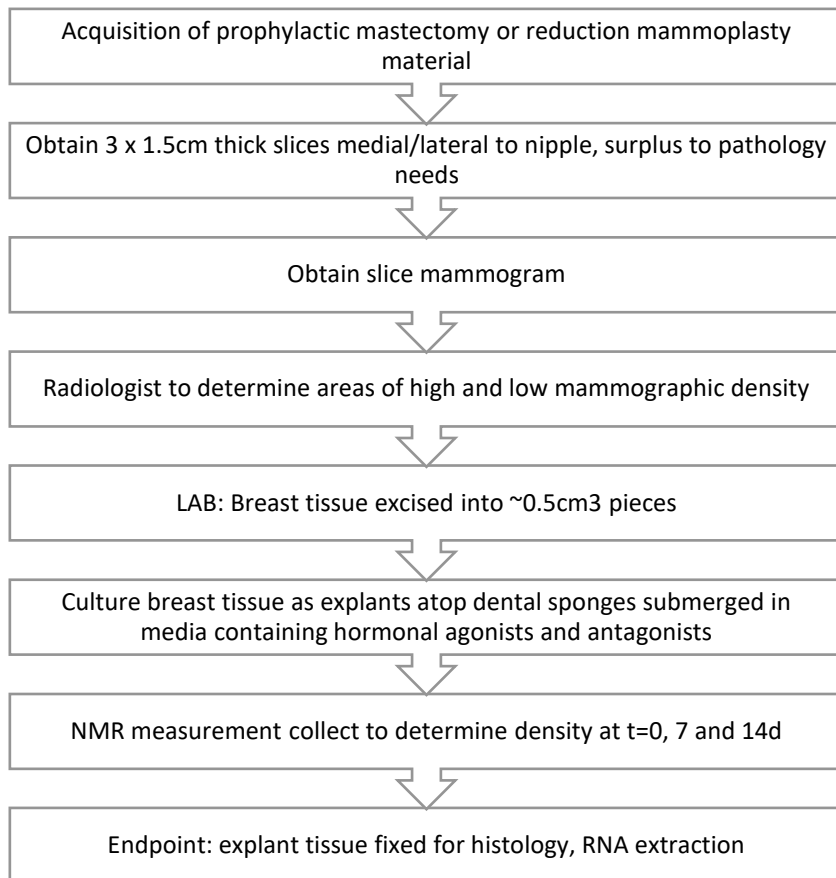
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Supplementary Figure 1. Outline of study design.

Acquisition of prophylactic mastectomy or reduction mammoplasty material

Obtain 3 x 1.5cm thick slices medial/lateral to nipple, surplus to pathology needs

Obtain slice mammogram

Radiologist to determine areas of high and low mammographic density

LAB: Breast tissue excised into ~0.5cm3 pieces

Culture breast tissue as explants atop dental sponges submerged in media containing hormonal agonists and antagonists

NMR measurement collect to determine density at t=0, 7 and 14d

Endpoint: explant tissue fixed for histology, RNA extraction

Supplementary Table 1. Effect of ethanol control (Eth) compared with estradiol (E2) on expression across ER, PR or AR gene signatures. P value derived from Student's paired t test. Gene expression values within each receptor grouping were normalised using respective hormone receptor expression values. The respective receptor grouping *p* values and their associated average expression values considered significant (p<0.05) have been bolded.

Receptor grouping	ID	Average expression		Eth vs E2 *p* value
		Eth	E2	
aveERGs	PAH040M	-0.819	0.831	0.087
	PAH032M	-1.022	1.186	
	PAH043M	0.424	0.932	
	PAH044M	0.030	0.339	
	PAH045M	0.576	0.622	
avePRGs	PAH040M	-0.653	0.251	0.185
	PAH032M	-0.071	1.340	
	PAH043M	0.660	0.896	
	PAH044M	0.246	0.008	
	PAH045M	-0.365	-0.287	
aveARGs	PAH040M	1.129	0.377	0.188
	PAH032M	1.395	1.503	
	PAH043M	2.026	1.732	
	PAH044M	1.692	0.493	
	PAH045M	0.454	0.572	

Supplementary Table 2. Effect of E2 compared with E2 + Enobosarm (SARM), E2 + DHR or E2 + AR agonists combined on expression across ER, PR or AR gene signatures. P value derived from Student's paired t test. Gene expression values within each receptor grouping were normalised using respective hormone receptor expression values. The respective receptor grouping *p* values and their associated average expression values considered significant (p<0.05) have been bolded.

Receptor grouping	ID	Average expression				E2 vs SARM *p* value	E2 vs DHT *p* value	E2 vs AR agonists *p* value
		E2	E2+SARM	E2+DHT	E2+AR agonists			
aveERGs	PAH032M	1.186	0.200	-2.337	-1.068	**0.007**	0.194	0.090
	PAH043M	0.932	0.479	-2.631	-1.076			
	PAH044M	0.339	-0.406	0.390	-0.008			
	PAH045M	0.622	-0.290	0.707	0.208			
avePRGs	PAH032M	1.340	0.210	-2.335	-1.062	**0.011**	0.206	0.098
	PAH043M	0.896	0.380	-2.528	-1.074			
	PAH044M	0.008	-0.644	0.235	-0.205			
	PAH045M	-0.287	-1.353	-0.262	-0.807			
aveARGs	PAH032M	1.503	1.180	-0.665	0.258	0.081	0.218	0.141
	PAH043M	1.732	0.776	-1.430	-0.327			
	PAH044M	0.493	0.384	1.148	0.766			
	PAH045M	0.572	-0.519	0.000	-0.260			

Supplementary Table 3. Effect of E2 compared with ethanol control (ETH) on expression of ER, PR or AR associated genes. P value derived from Student's paired t test. Gene expression values within each receptor grouping were normalised using respective hormone receptor expression values. Specific gene *p* values and their associated average expression values considered significant (p<0.05) have been bolded.

Receptor grouping	Gene	Average expression		Eth vs E2 *p* value
		ETH	E2	
ERGs	PDZK1	-3.377	-1.783	0.128
	SERPINA3	6.354	6.935	0.373
	CELSR2	**-3.484**	**-2.092**	**0.045**
	AR	**-0.918**	**0.448**	**0.014**
	ATP6V1B1	-0.209	0.579	0.261
	GREB1	**0.890**	**2.230**	**0.021**
	IGSF1	-2.748	-1.788	0.168
	CLIC6	**-0.508**	**1.317**	**0.009**
	MSMB	-2.559	-1.785	0.290
	TFF1	0.011	0.605	0.219
	MYB	-1.639	-1.253	0.150
	PGR	0.301	0.739	0.103
	ALCAMN	2.588	3.026	0.349
	STMN1	2.999	3.736	0.074
PRGs	RASSF5	-0.990	-0.788	0.636
	SHROOM3	0.947	0.867	0.786
	FKBP5	3.374	3.868	0.133
	RANK	-1.653	-1.318	0.452
	RANKL	-2.749	-3.289	0.285
ARGs	SPC2	2.088	1.187	0.096
	EAF2	0.483	-0.577	0.109
	SEC14L2	**2.799**	**1.711**	**0.014**
	ZBTB16	4.079	3.300	0.088
	GPRC5A	**3.272**	**2.185**	**0.042**
	ELOVL5	4.918	4.439	0.219

Supplementary Table 4. Effect of E2 compared with Enobosarm (SARM) on expression of ER, PR or AR associated genes. P value derived from Student's paired t test. Gene expression values within each receptor grouping were normalised using respective hormone receptor expression values. Specific gene *p* values and their associated average expression values considered significant (p<0.05) have been bolded.

Receptor grouping	Gene	Average expression		E2 vs SARM *p* value
		E2	E2 + SARM	
ERGs	PDZK1	-2.186	-2.245	0.912
	SERPINA3	**7.114**	**6.043**	**0.043**
	CELSR2	-2.097	-2.535	0.676
	AR	0.667	0.030	0.146
	ATP6V1B1	**0.596**	**-0.793**	**0.024**
	GREB1	**2.250**	**0.899**	**0.011**
	IGSF1	-1.975	-1.242	0.455
	CLIC6	**1.499**	**-0.748**	**0.018**
	MSMB	-1.473	-1.113	0.105
	TFF1	**0.261**	**-2.415**	**0.013**
	MYB	-1.510	-1.611	0.711
	PGR	0.747	0.523	0.448
	ALCAMN	3.071	2.442	0.263
	STMN1	3.919	2.745	0.130
PRGs	RASSF5	-0.857	-0.693	0.780
	SHROOM3	0.644	-0.165	0.122
	FKBP5	4.000	2.696	0.074
	RANK	**-0.986**	**-1.995**	**0.019**
	RANKL	-2.819	-3.037	0.682
ARGs	SPC2	1.452	0.732	0.327
	EAF2	-0.645	-1.168	0.407
	SEC14L2	1.870	1.434	0.447
	ZBTB16	3.572	2.954	0.407
	GPRC5A	2.492	2.237	0.747
	ELOVL5	4.490	3.973	0.378

Supplementary Table 5. Effect of E2 compared with E2+DHT on expression of ER, PR or AR associated genes. P value derived from Student's paired t test. Gene expression values within each receptor grouping were normalised using respective hormone receptor expression values.

Receptor grouping	Gene	Average expression		E2 vs E2+DHT *p* value
		E2	E2+ DHT	
ERGs	PDZK1	-2.186	-3.966	0.531
	SERPINA3	7.114	3.975	0.292
	CELSR2	-2.097	-1.712	0.973
	AR	0.667	0.188	0.600
	ATP6V1B1	0.596	-1.771	0.176
	GREB1	2.250	-0.404	0.218
	IGSF1	-1.975	-2.526	0.522
	CLIC6	1.499	-1.207	0.234
	MSMB	-1.473	-2.178	0.355
	TFF1	0.261	-4.347	0.084
	MYB	-1.510	-2.060	0.274
	PGR	0.747	0.753	0.991
	ALCAMN	3.071	1.524	0.145
	STMN1	3.919	1.320	0.242
PRGs	RASSF5	-0.857	-0.260	0.526
	SHROOM3	0.644	-1.733	0.285
	FKBP5	4.000	1.075	0.241
	RANK	-0.986	-2.933	0.089
	RANKL	-2.819	-3.168	0.864
ARGs	SPC2	1.452	-0.149	0.221
	EAF2	-0.645	-0.808	0.806
	SEC14L2	1.870	-0.408	0.151
	ZBTB16	3.572	2.879	0.527
	GPRC5A	2.492	2.447	0.975
	ELOVL5	4.490	1.945	0.281

Supplementary Table 6. Effect of E2 compared with E2+AR agonists on expression of ER, PR or AR associated genes. P value derived from Student's paired t test. Gene expression values within each receptor grouping were normalised using respective hormone receptor expression values. Specific gene *p* values and their associated average expression values considered significant (p<0.05) have been bolded.

Receptor grouping	Gene	Average expression		E2 vs E2+AR agonists *p* value
		E2	E2+ AR agonists	
ERGs	PDZK1	-2.186	-2.983	0.520
	SERPINA3	7.114	5.009	0.163
	CELSR2	-2.097	-2.260	0.960
	AR	0.667	0.109	0.298
	ATP6V1B1	0.596	-1.282	0.067
	GREB1	2.250	0.247	0.088
	IGSF1	-1.975	-1.884	0.917
	CLIC6	1.499	-0.977	0.081
	MSMB	-1.473	-1.646	0.681
	TFF1	**0.261**	**-3.381**	**0.048**
	MYB	-1.510	-1.836	0.304
	PGR	0.747	0.638	0.777
	ALCAMN	**3.071**	**1.983**	**0.020**
	STMN1	3.919	2.032	0.079
PRGs	RASSF5	-0.857	-0.476	0.603
	SHROOM3	0.644	-0.949	0.204
	FKBP5	4.000	1.886	0.106
	RANK	**-0.986**	**-2.464**	**0.049**
	RANKL	-2.819	-3.095	0.573
ARGs	SPC2	1.452	0.292	0.243
	EAF2	-0.645	-1.014	0.432
	SEC14L2	1.870	0.513	0.171
	ZBTB16	3.572	2.916	0.472
	GPRC5A	2.492	2.342	0.893
	ELOVL5	4.490	2.959	0.282

Supplementary Table 7. Parameter estimate outputs of generalised estimated equation analysis of genes significantly associated with MD, shown in table 1, where direction of association (positive or negative) is indicated in red. Model: (Intercept); MD.

Dependent variable	Parameter	B	Std. Error	95% Wald Confidence Interval	
				Lower	Upper
GREB1	(Intercept)	-0.563	0.8262	-2.183	1.056
	MD	0.02	0.0099	0	0.039
	(Scale)	2.993			
IGSF1	(Intercept)	-0.17	0.8556	-1.847	1.507
	MD	-0.024	0.0116	-0.047	-0.002
	(Scale)	0.913			
MSMB	(Intercept)	4.228	1.7227	0.852	7.604
	MD	-0.078	0.0215	-0.12	-0.036
	(Scale)	1.211			
PGR	(Intercept)	3.724	1.2359	1.301	6.146
	MD	-0.038	0.0149	-0.067	-0.009
	(Scale)	0.484			
ALCAM	(Intercept)	5.179	0.672	3.862	6.496
	MD	-0.035	0.0084	-0.051	-0.018
	(Scale)	1.055			
SHROOM3	(Intercept)	-5.871	1.3383	-8.494	-3.248
	MD	0.075	0.018	0.04	0.111
	(Scale)	3.271			
RANK	(Intercept)	-6.753	1.2194	-9.143	-4.363
	MD	0.059	0.0144	0.03	0.087
	(Scale)	1.752			
RANKL	(Intercept)	-14.938	4.436	-23.632	-6.243
	MD	0.145	0.0478	0.051	0.238
	(Scale)	4.376			
GPRC5A	(Intercept)	6.833	1.8436	3.22	10.447
	MD	-0.054	0.022	-0.097	-0.011
	(Scale)	1.461			

Supplementary Table 8. Generalised estimated equations of SHROOM3 expression versus MD separated by BRCA 1 and BRCA 2 mutation status. The correlation was analysed among all treated explants.

	BRCA mutated		BRCA WT	
	Wald Chi-Square	P value	Wald Chi-Square	P value
SHROOM3				
L32	16591.591	<0.001	2.784	.095
HR	14956.425	<0.001	1.032	.310