

Functional and molecular characterisation of EO771.LMB tumours, a new C57BL/6-mouse-derived model of spontaneously metastatic mammary cancer.

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Supplementary Figure Legends

Figure S1: Histological classification of murine mammary tumours. (A) EO771 and EO771.LMB primary tumours were sectioned (3 μ m), stained with haematoxylin and eosin (H&E), and compared to 67NR and 4T1.2 primary tumours. H&E stained sections from at least two primary tumours of each tumour type were assessed by a qualified breast pathologist in accordance with the Bloom Richardson Ellis classification system (Elston and Ellis, 2002). Circles indicate highly pleomorphic nuclei. (B) An overall grade was calculated dependent on tubular differentiation/formation (score 1: >75% of tumor area forming glandular/tubular structures, score 2: 10% to 75% and score 3: <10%), nuclear pleomorphism (score 1: nuclei with little increase in size in comparison with normal breast epithelium with minimal variation in size, score 2: larger nuclei with moderate variability and visible nucleoli and score 3: marked nuclear variation with prominent nucleoli) and tumour mitotic count determined by numbers of mitosis seen in 10 consecutive high power fields (score 1: <9 mitosis per 10 HPF, score 2: 9-17 mitosis per 10 HPF, score 3: 18 or more mitosis per 10 HPF). Adding the scores for each parameter determined tumour grade as follows: grade 1 - scores 3,4 and 5, grade 2 - scores 6 and 7, grade 3 - scores 8 and 9.

Black scale bars represent 100 μ m. White circles denote highly pleomorphic nuclei.

Figure S2: Whole lungs were dissected from mice inoculated with the indicated tumour lines and stained with India ink. The number of metastatic nodules on the surface of each lung was counted. $P = 0.20$ (Mann-Whitney test).

Figure S3: Experimental lung metastasis assay. EO771 or EO771.LMB cells were inoculated into C57Bl/6 mice via the tail vein. 19 days later, the metastatic burden in the lung was analyzed by TaqMan qPCR. $P = 0.51$ (Student's t test).

Figure S4: The cell lines indicated were cultured on plastic or monomeric type I bovine collagen (PureCol, Advanced Biomatrix, Carlsbad, CA) for at least 2 days. Images (x100 magnification) were generated using a Leica DM IRB inverted microscope (Leica Microsystems, North Ryde, NSW, Australia).

Figure S5: Anchorage independent growth in soft agar. Colonies in soft agar were stained with calcein^{AM} and fluorescent images generated. The number of colonies > 50 μm in size were counted in three fields per well and averaged. The graph depicts the average number of colonies per field across three wells \pm SD. Differences in colony forming ability between EO771 and EO771.LMB were not significant (Student's t test).

Figure S6: Mammosphere forming capacity of EO771 and EO771.LMB cells in vitro. Representative images from primary (A) and secondary (B) mammosphere cultures from EO771 and EO771.LMB (LMB) are shown. Scale bar = 200 μm . (C) Mammospheres from primary or secondary cultures were enumerated after 10 or 7 days of growth, respectively, and expressed as the mean number of mammospheres per 10 fields \pm SEM. No significant differences in mammosphere number was found between EO771 and EO771.LMB in either primary or secondary cultures. (D) The graph depicts mean mammosphere area in $\mu\text{m}^2 \pm$ SEM for primary and secondary cultures after 10 or 7 days of growth, respectively. The number of individual

mammospheres measured in each condition is indicated above the bars. n/s, not significant.

Figure S7: Adhesion of 67NR and 4T1.2 tumour cells to different substrates after 30min. Adhesion is presented as the percentage of total cell input (mean of triplicate wells \pm SD of one of three representative experiments). *, $P < 0.05$.

Figure S8: ER α -positive human breast cancer cells (MCF7, top panels) or ER α -negative human breast cancer cells (MDA-MB-231, bottom panels) were grown as xenografts in the mammary glands of immunodeficient mice. Tumour sections were stained with anti-human ER α antibody (left panels) or an isotype control antibody (right panels). Scale bars represent 100 μ m.

Figure S9: Distribution of eight gene expression signatures across 67NR, 4T1.2, EO771, and EO771.LMB primary tumours displayed as heat maps. Triplicate tumour specimens are indicated along the X axes and individual genes are shown on the Y axes. Gene expression signatures were obtained from the following references or from the Molecular Signatures Database (<http://www.broadinstitute.org/gsea/msigdb/index.jsp>). (A) Basal epithelial (54 genes) (Hupér and Marks, 2007), (B) luminal epithelial (59 genes) (Hupér and Marks, 2007), (C) proliferation (97 genes) (Ghazoui et al., 2011), (D) hypoxia-regulated (75 genes) (Ghazoui et al., 2011), (E) interferon-regulated (27 genes) (Einav et al., 2005), (F) cancer invasion (64 genes) (Kim et al., 2010), (G) EMT (91 genes) (Taube et al., 2010), (H) breast cancer stem cells (94 genes) (Creighton et al., 2009).

Figure S10: Venn diagrams were generated from Affymetrix array data showing genes significantly (unadjusted $P < 0.05$) upregulated (**A**), or downregulated (**B**) in 4T1.2 v 67NR (red) and EO771.LMB v EO771 (green). P values were determined by one-way ANOVA using Partek Genomics Suite v6.6. 205 genes were commonly upregulated and 220 genes commonly downregulated in both isogenic tumour comparisons.

Figure S11: qRT-PCR data showing mRNA expression levels of MMP-3 (A), Pthrp (B), S100a8 (C), S100a9 (D), Cd36 (E) and GlyCAM1 (F) in whole 67NR, 4T1.2, EO771 and EO771.LMB (LMB) primary tumours. Three different primary tumours were analysed in duplicate for each tumour model. Thus, each data point represents the mean \pm SD of 6 qRT-PCR reactions across 3 tumours. Expression levels in normal adult mouse mammary gland (MG) were included for each gene for comparison and set to 1. Values on the Y axis represent mRNA expression levels of the gene of interest normalised to Rps27a. *, $P = 0.05$; **, $P < 0.01$; ***, $P < 0.005$; n/s, not significant.

Figure S12: qRT-PCR data showing mRNA expression levels of MMP-3 (A), Pthrp (B), S100a8 (C), S100a9 (D), Cd36 (E) and GlyCAM1 (F) in 67NR, 4T1.2, EO771 and EO771.LMB (LMB) cell cultures. Each data point represents the mean \pm SD of triplicate qRT-PCR reactions. Expression levels in the immortalised mouse mammary epithelial cell line NMuMG were included for comparison and set to 1. Values on the Y axis represent mRNA expression levels of the gene of interest normalised to Rps27a. *, $P < 0.05$; **, $P < 0.01$; n/s, not significant; n/e, not expressed.

Figure S13: Kaplan-Meier survival curves (disease-free survival) for selected genes in primary human breast cancer samples. The BreastMark database (<http://glados.ucd.ie/BreastMark/index.html>) was analyzed for the individual genes indicated above the curves for all patients (all, upper row) or in basal-like tumours only (basal, lower row). Gene expression was measured in primary tumours using Affymetrix microarrays (Madden et al., 2013). A median split was used to allocate 50% of tumours to the "high expression" category (blue curve) and 50% to the "low expression" category (red curve). Patient clinical follow-up was for at least 200 months (X axes). A logrank P value ≤ 0.05 was considered statistically significant. s, significant, n/s, not significant.

Supplementary Table 1: Eight different expression signatures were analysed across four murine mammary cancer models (67NR, 4T1.2, EO771, EO771.LMB) using the R program. The Table depicts the number of genes and percentage of genes (in parentheses) within each signature that were significantly ($P < 0.05$) upregulated (UP), or significantly ($P < 0.05$) downregulated (DOWN) in 67NR, 4T1.2, EO771, or EO771.LMB tumours relative to the mean expression level of each gene across all 15 tumours analyzed (see Materials and Methods). The number and percentage of genes within each signature that were not significantly deregulated is also indicated. Yellow highlighting indicates tumour types where $\geq 25\%$ of the genes within the signature were significantly upregulated, and were thereby considered enriched for the signature. The enriched gene expression signatures are summarised at the bottom of the Table.

Supplementary Table 2: Genes significantly **downregulated** (unadjusted $P < 0.05$, $n=220$) in both 4T1.2 v 67NR and EO771.LMB v EO771 comparisons. P values were determined by one-way ANOVA using Partek Genomics Suite v6.6.

Supplementary Table 3: Genes significantly **upregulated** (unadjusted $P < 0.05$, $n=205$) in both 4T1.2 v 67NR and EO771.LMB v EO771 comparisons. P values were determined by one-way ANOVA using Partek Genomics Suite v6.6.

Supplementary Table 4: Sequences of oligonucleotide primers used for SYBR-green qRT-PCR.

Supplementary References

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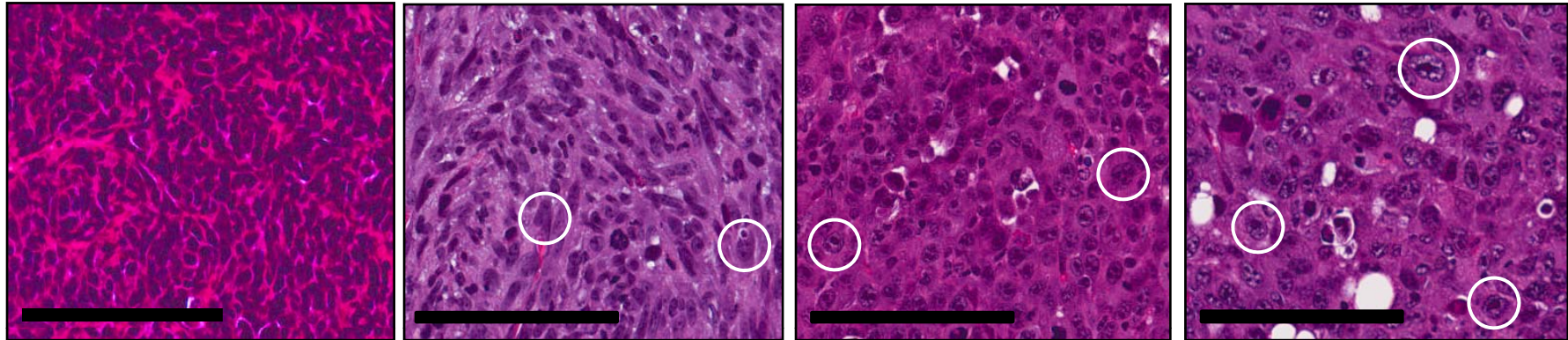
A

67NR

4T1.2

EO771

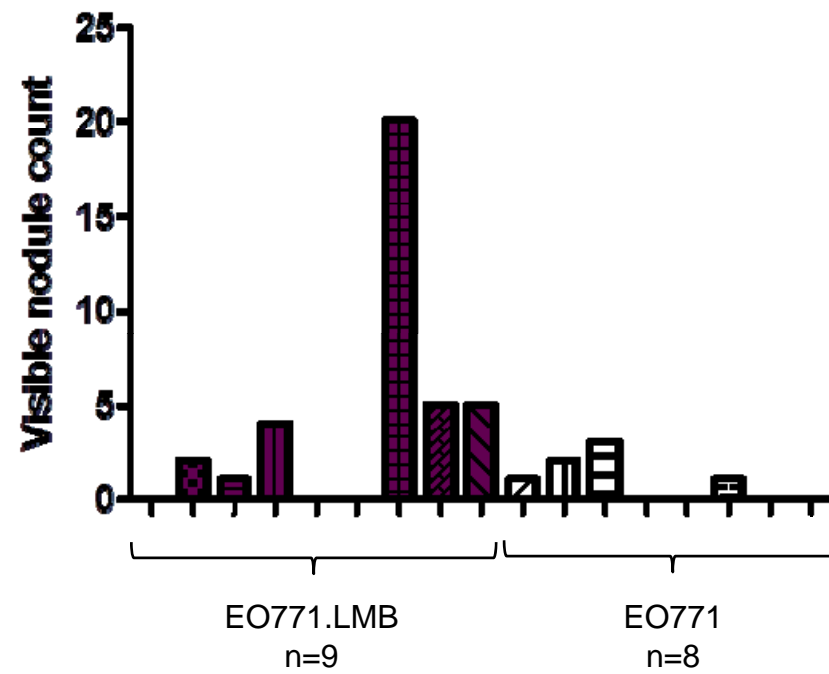
EO771.LMB



B

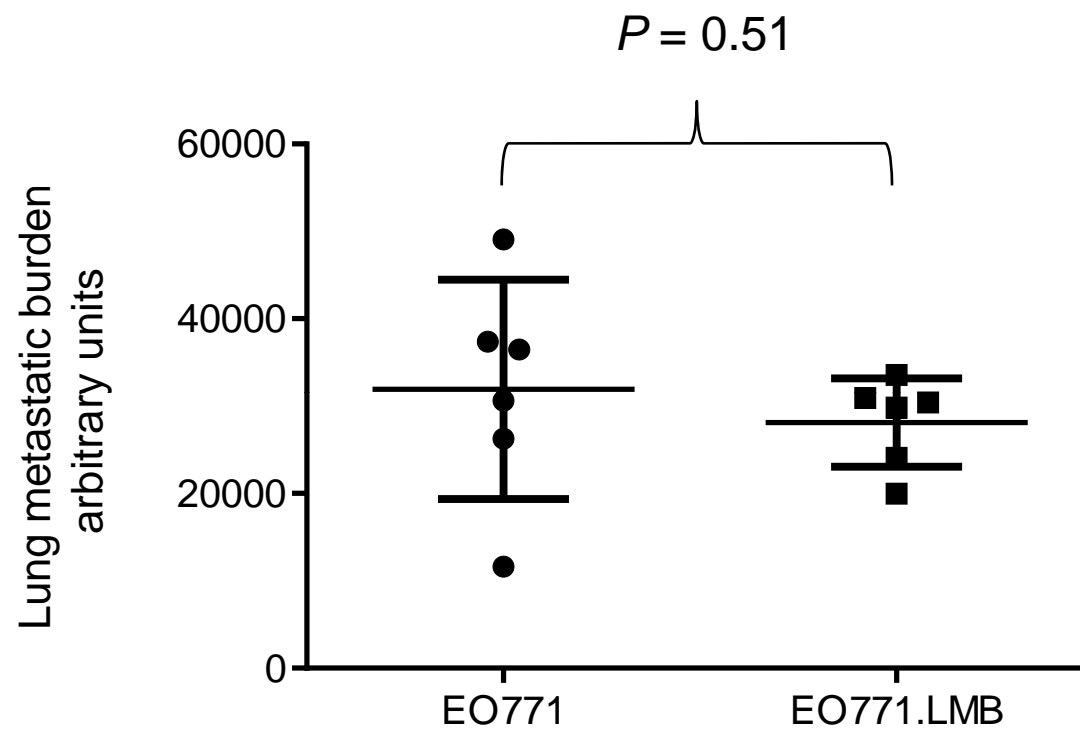
Tumour	Overall Grade	Tubule Formation Score	Nuclear Pleomorphism Score	Mitosis Score	Mitoses per 10 HPF
67NR-A	3	3	2	3	29
67NR-B	3	3	2	3	32
4T1.2-A	3	3	3	2	15
4T1.2-B	3	3	3	3	42
EO771-A	3	3	3	3	27
EO771-B	3	3	3	3	90
EO771.LMB-A	3	3	3	3	57
EO771.LMB-B	3	3	3	3	66

Supp. Figure 1

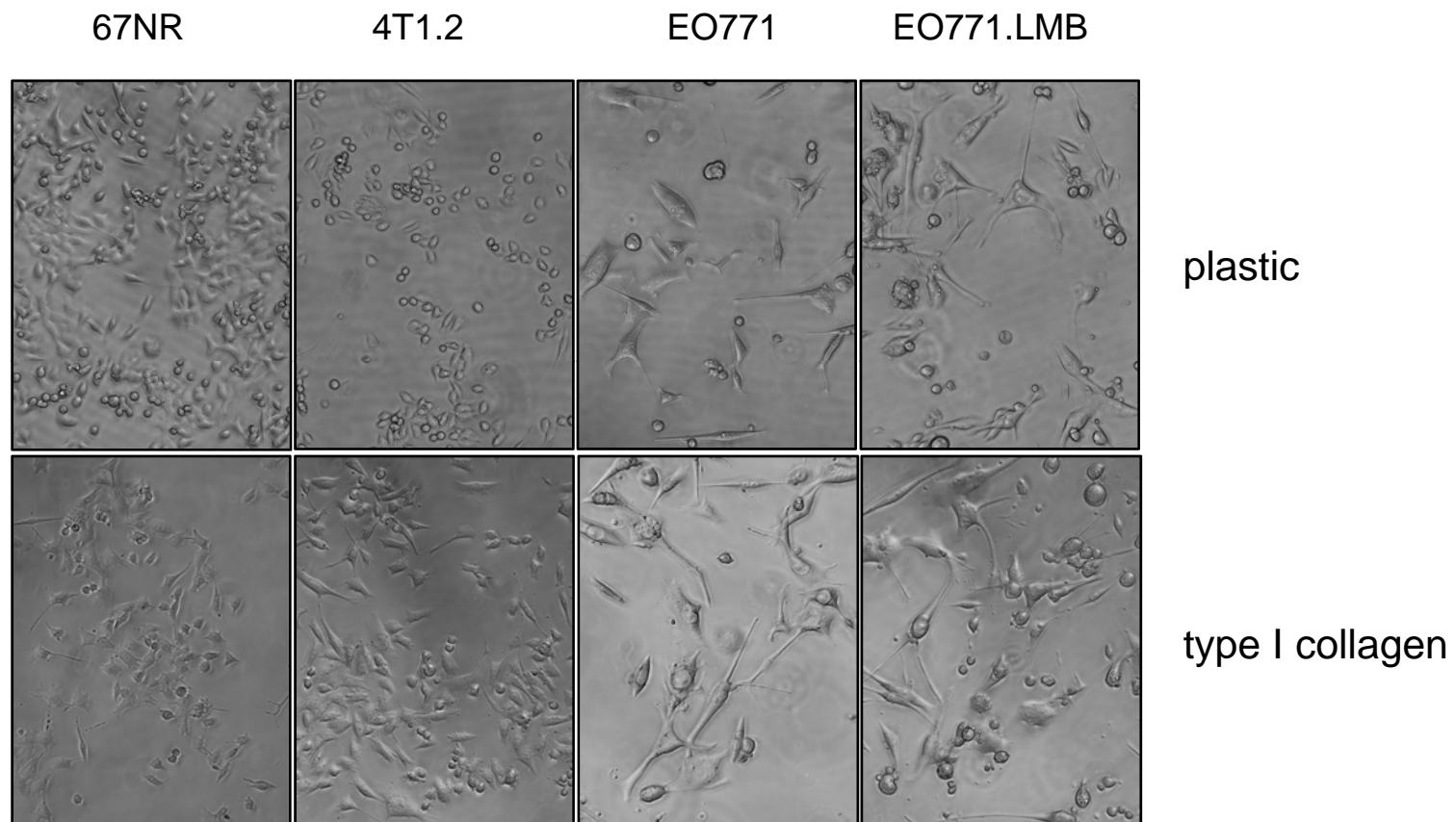


$P = 0.20$

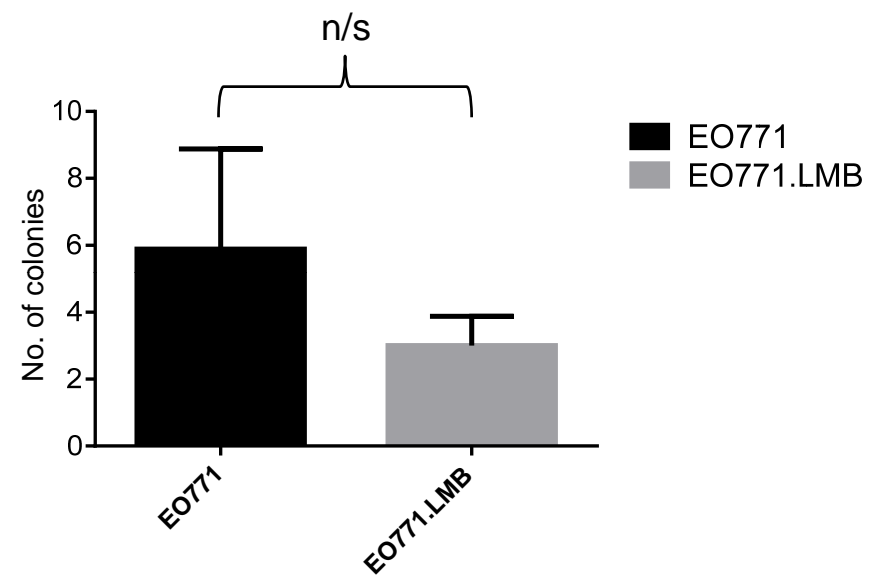
Supp. Fig. 2



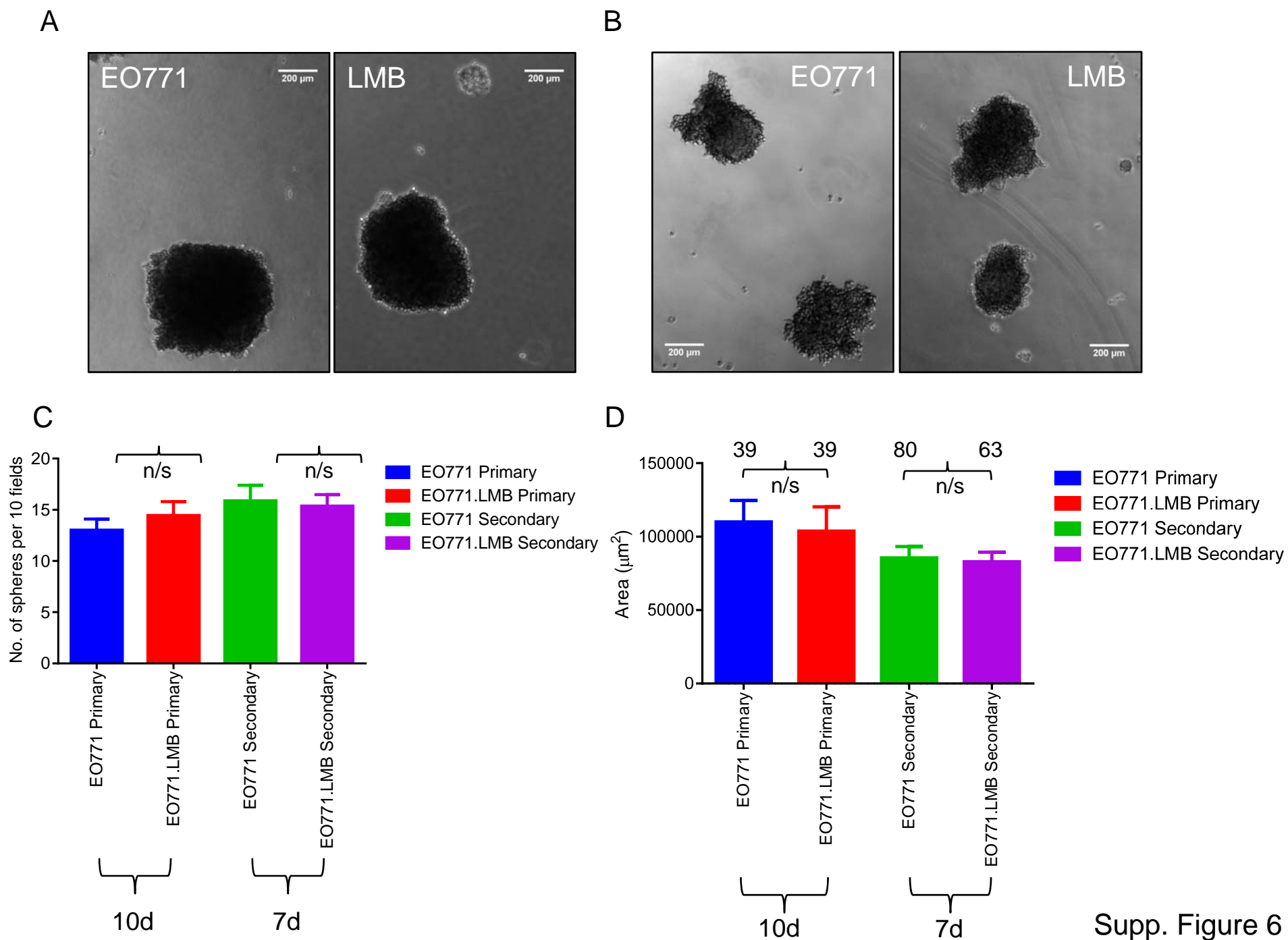
Supp. Figure 3



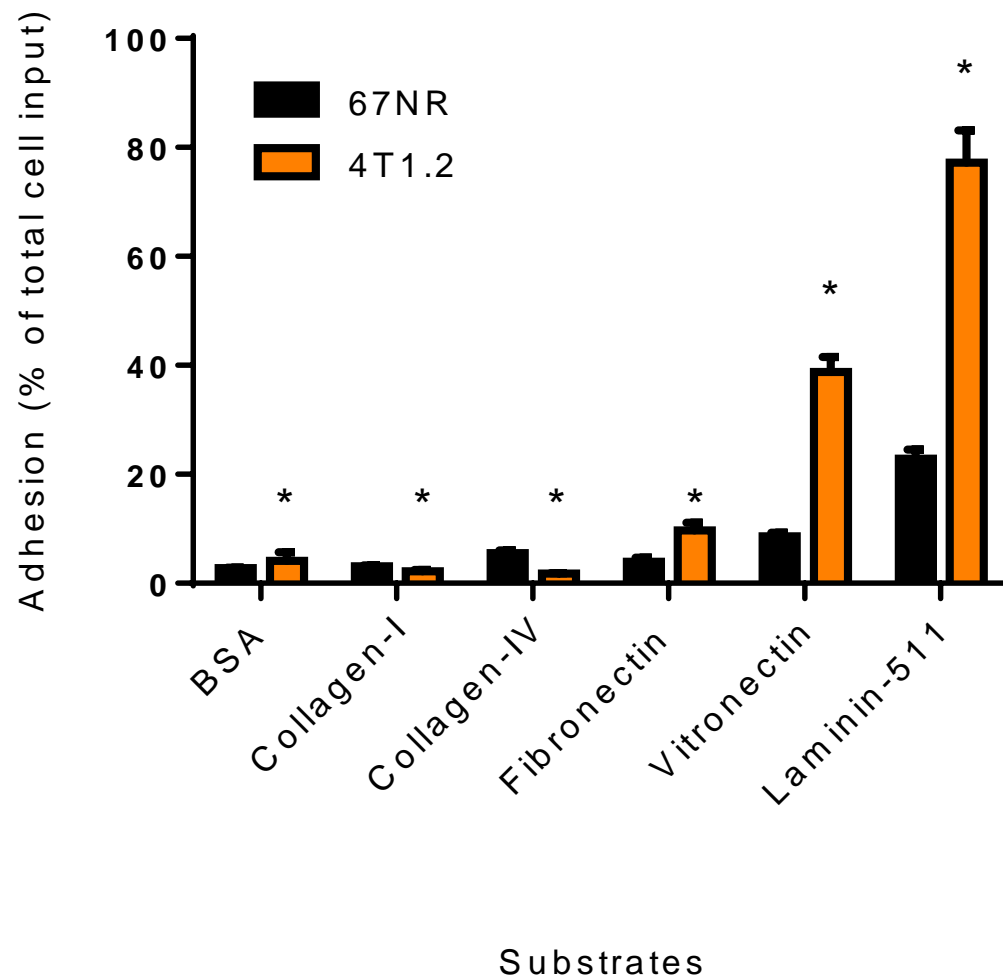
Supp. Figure 4



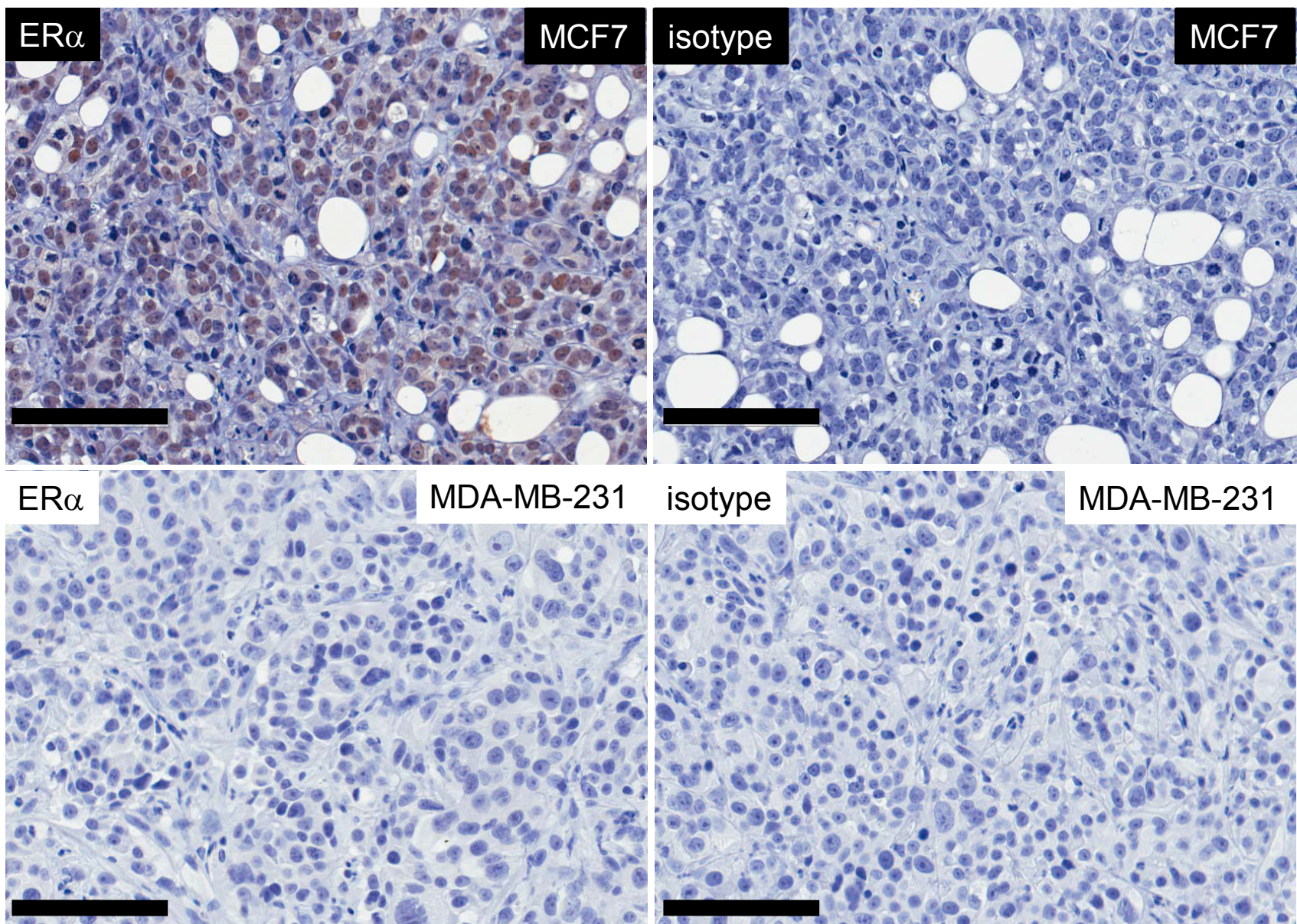
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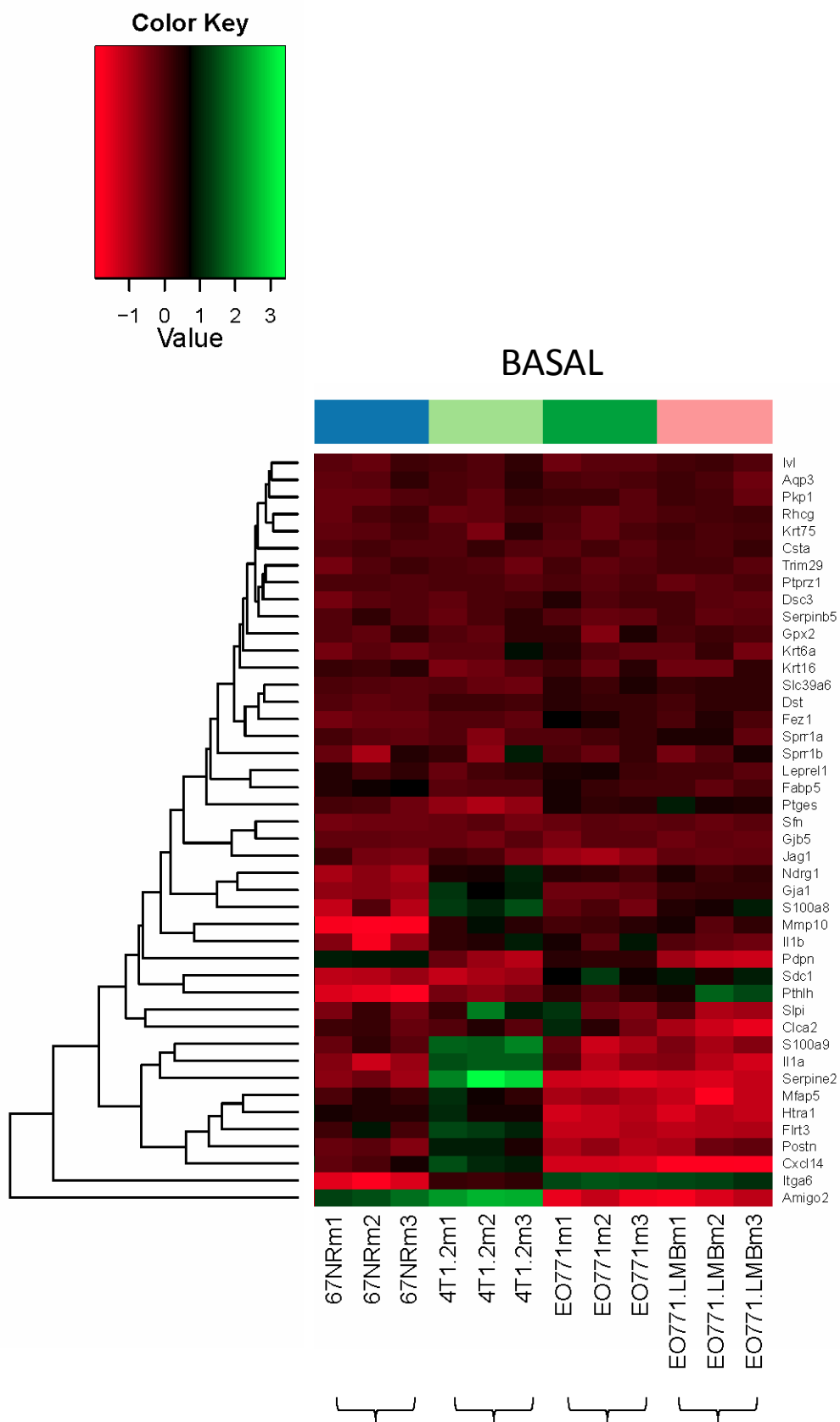
Supp. Figure 6



Supp. Figure. 7

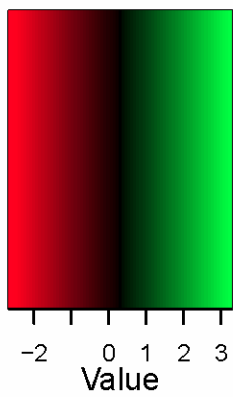


Supplementary Figure 8

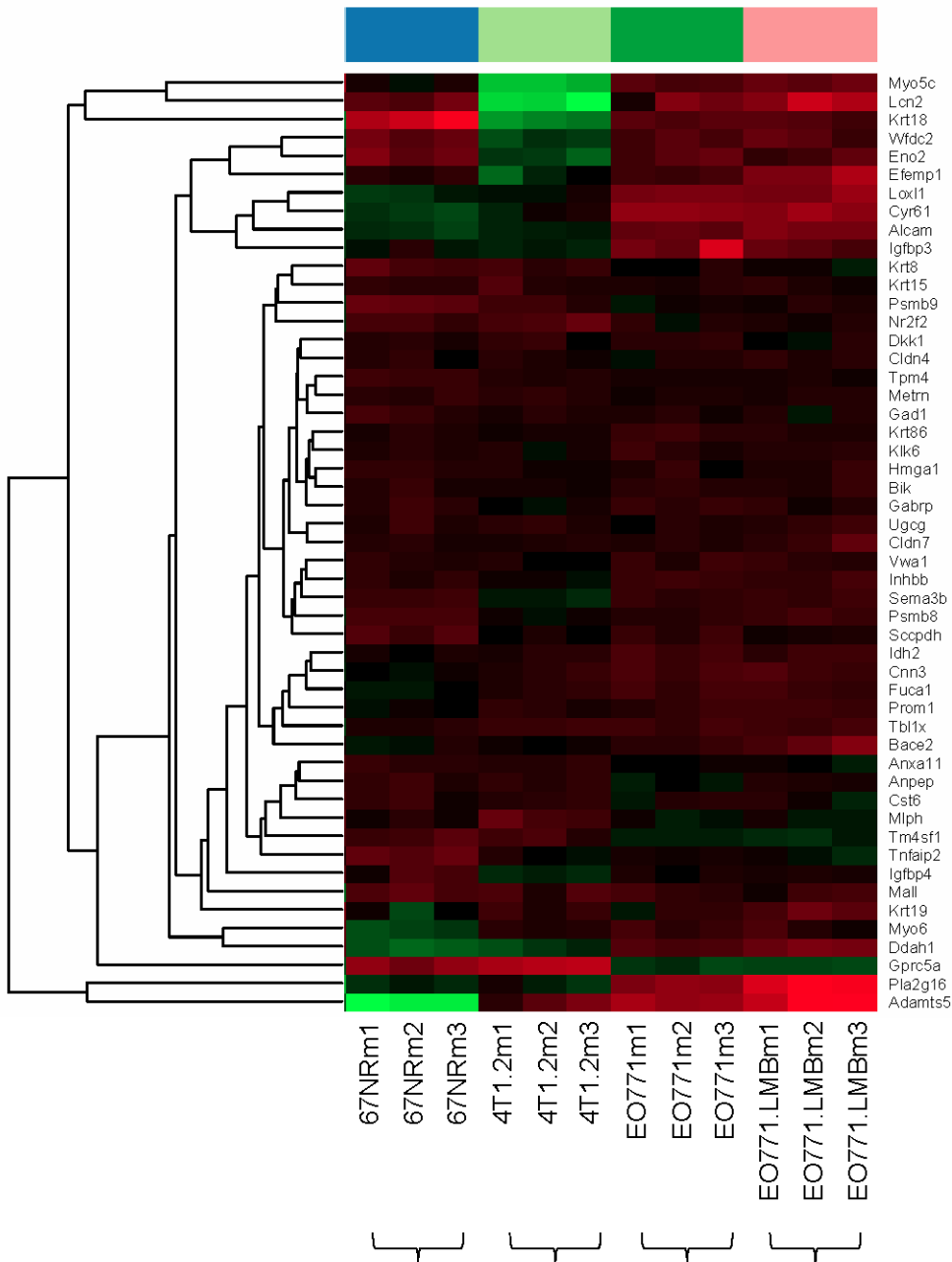


Supp. Figure 9A

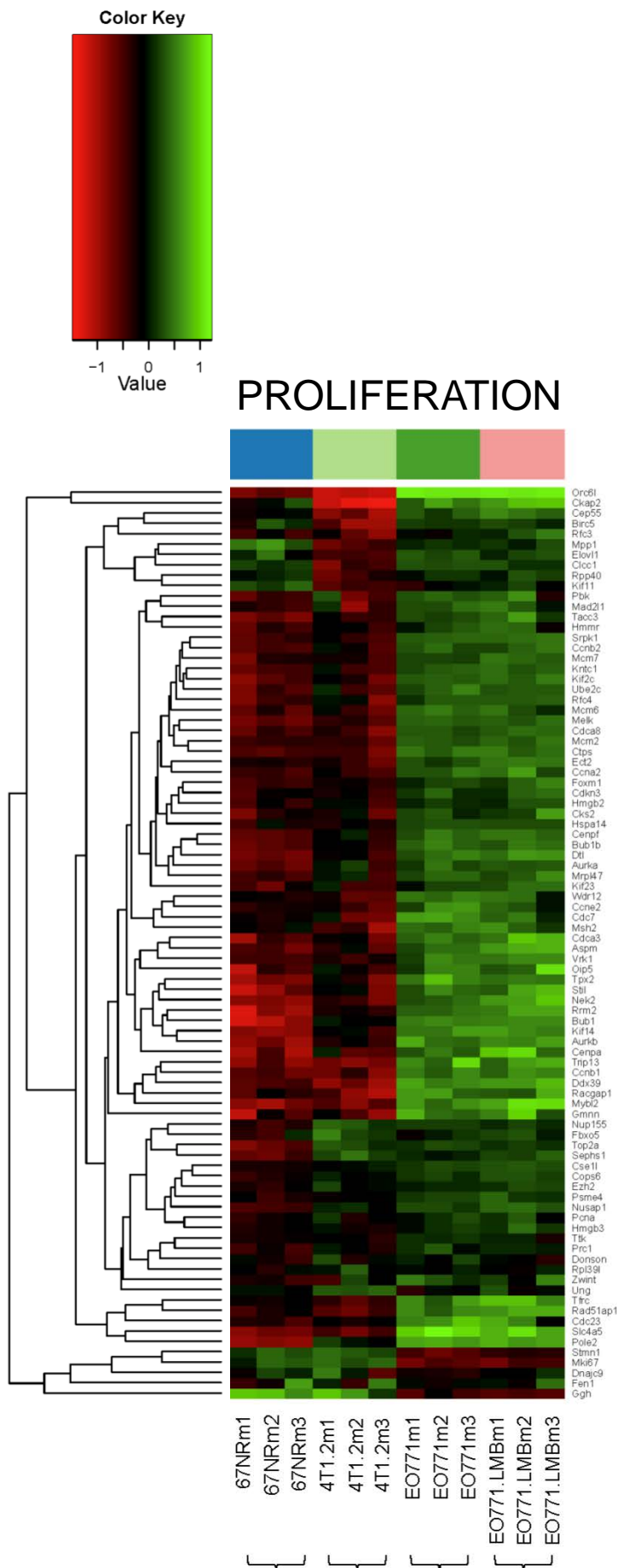
Color Key



LUMINAL

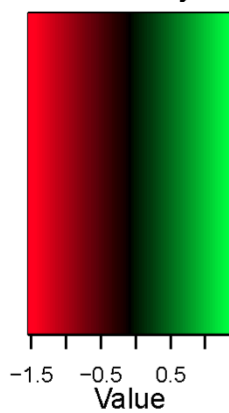


Supp. Figure 9B

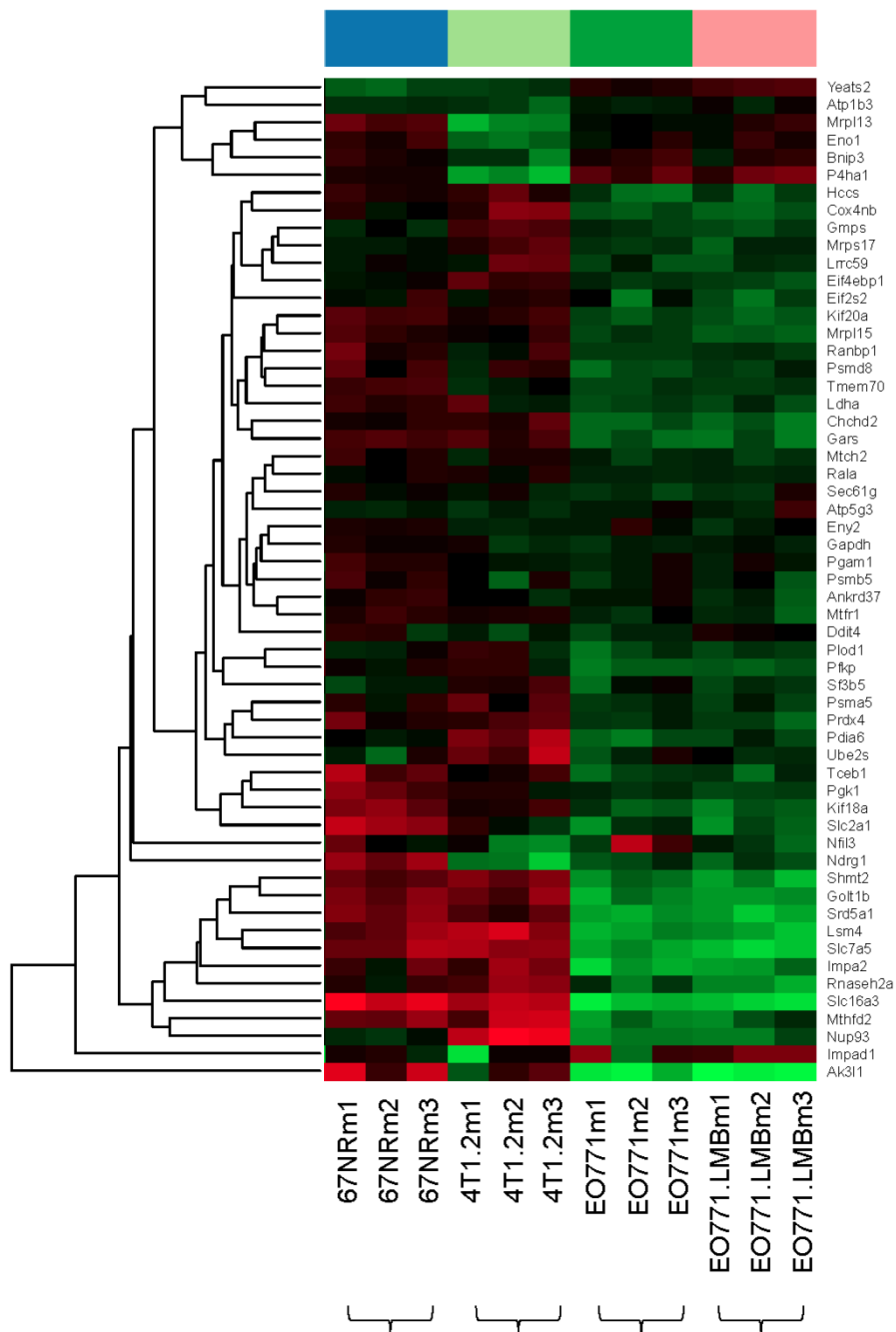


Supp. Figure 9C

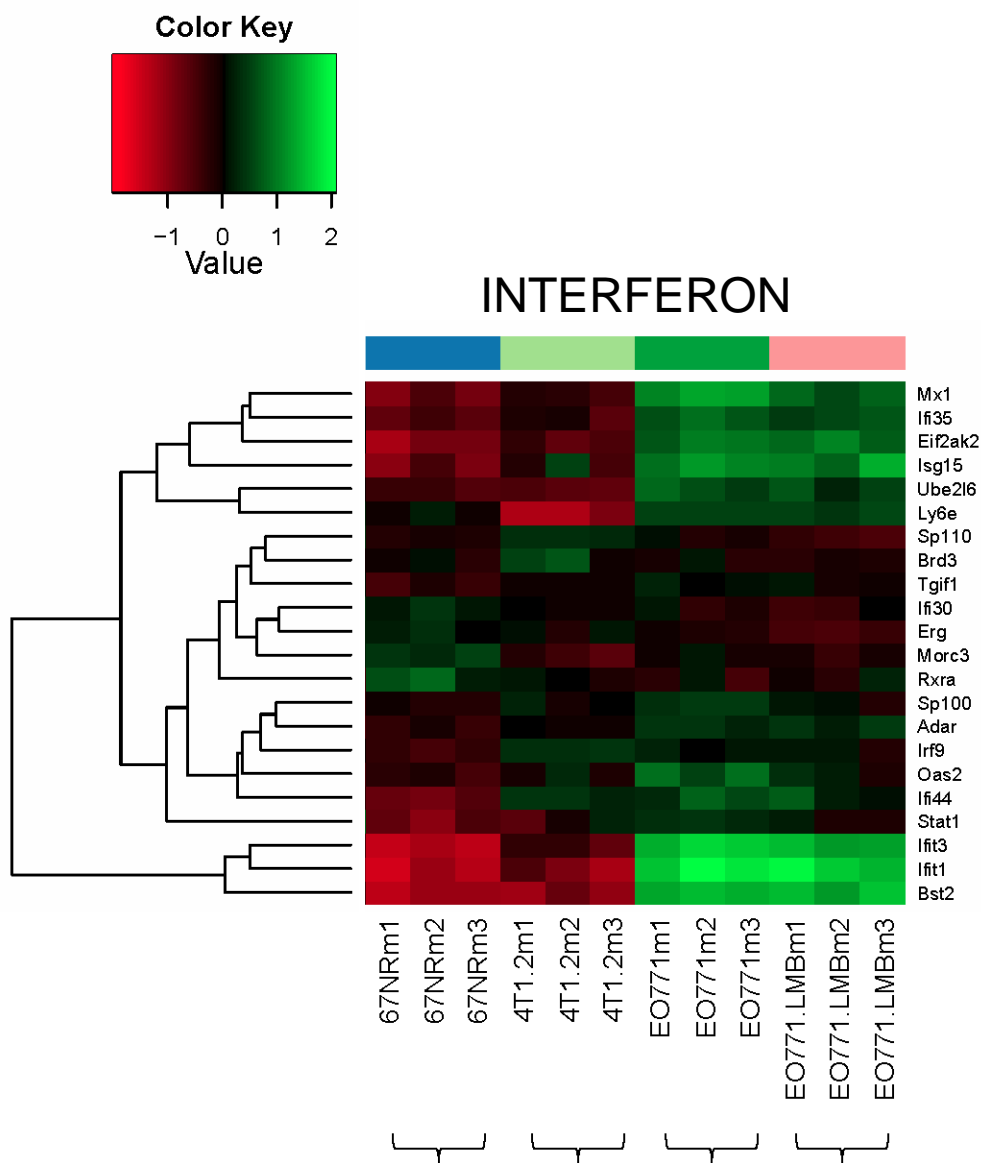
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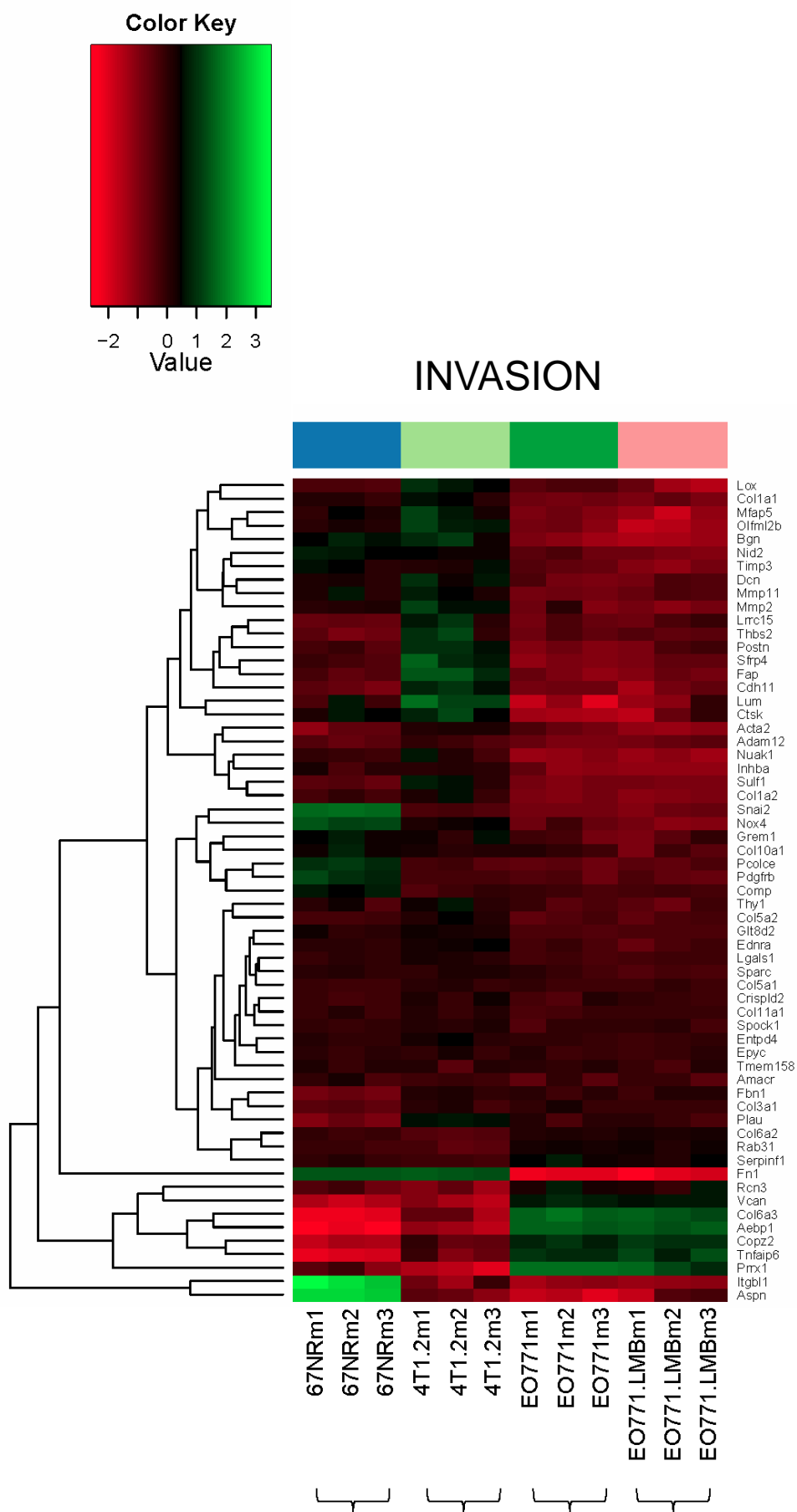
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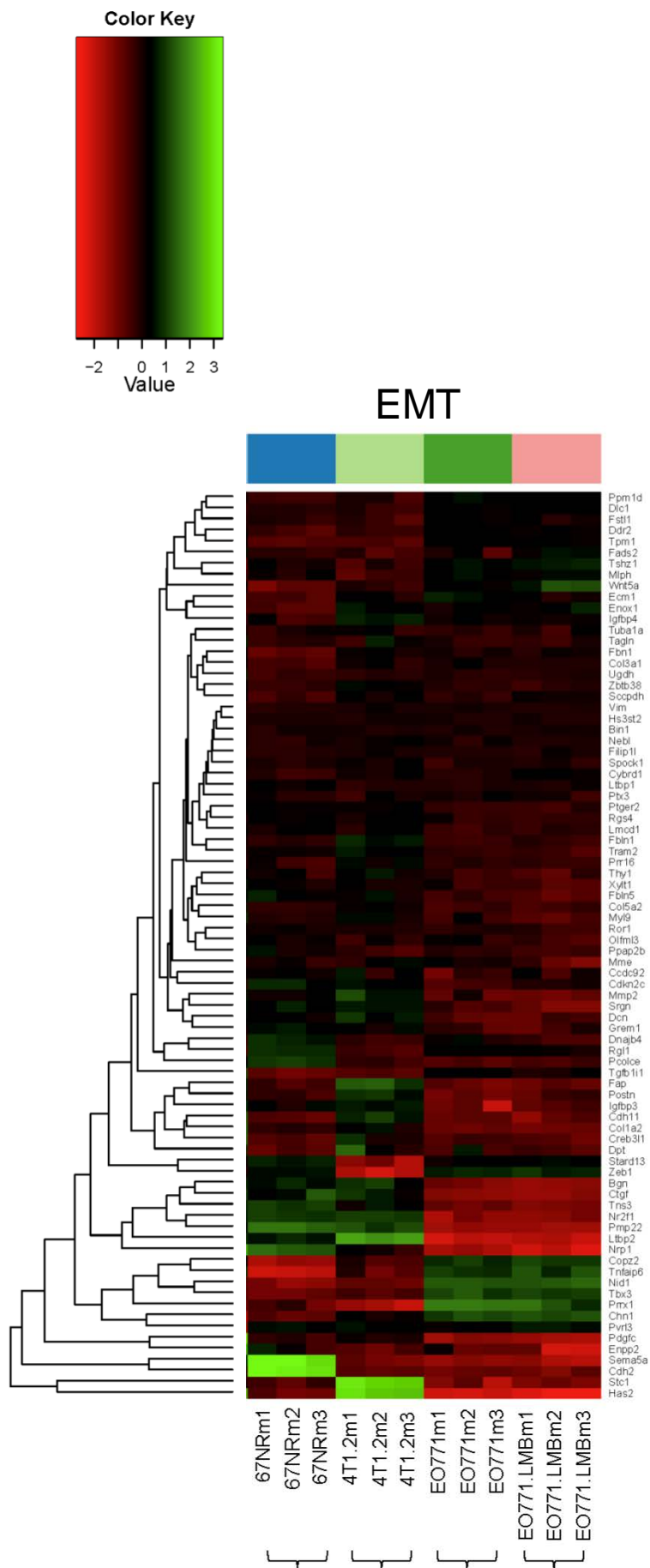
Supp. Figure 9D



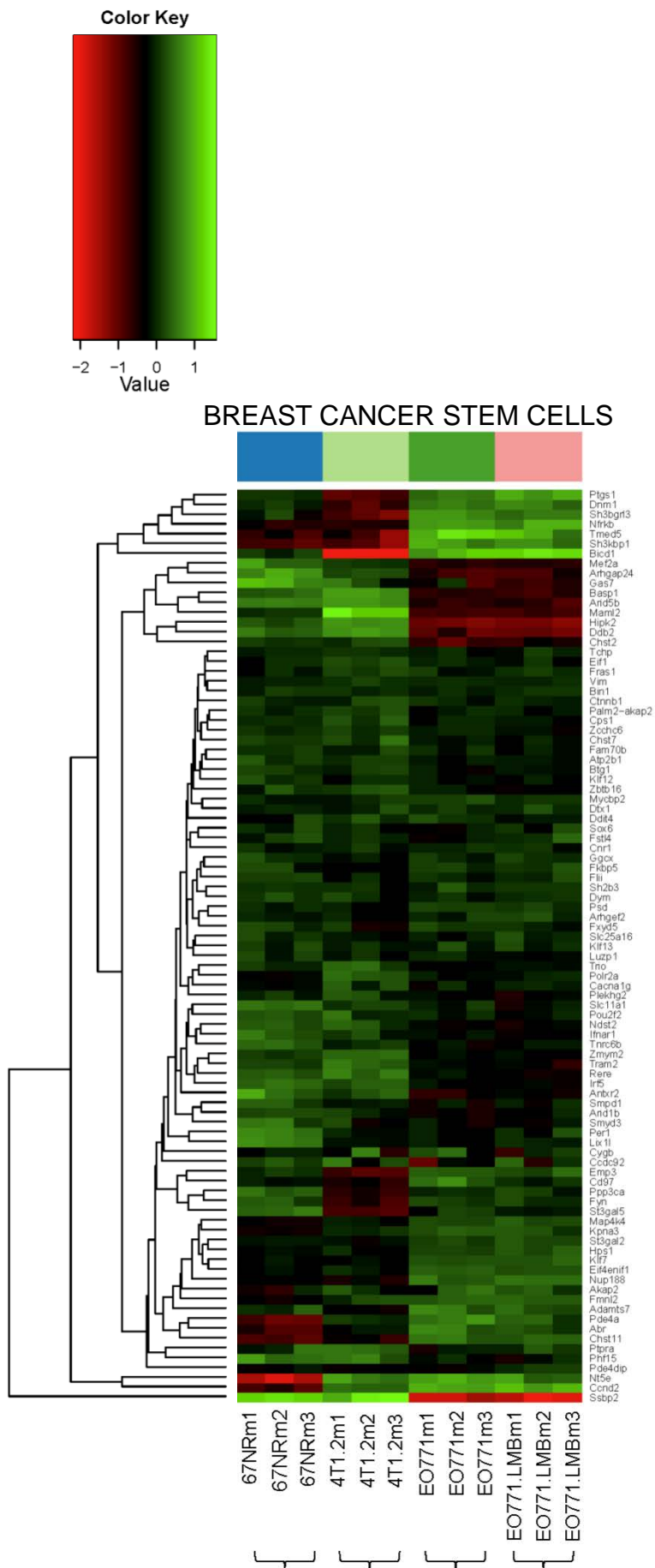
Supp. Figure 9E



Supp. Figure 9F

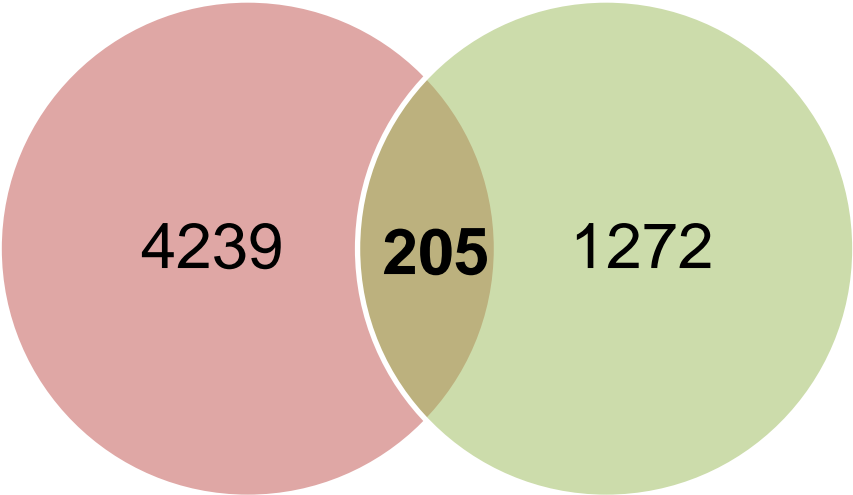


Supp. Figure 9G



Supp. Figure 9H

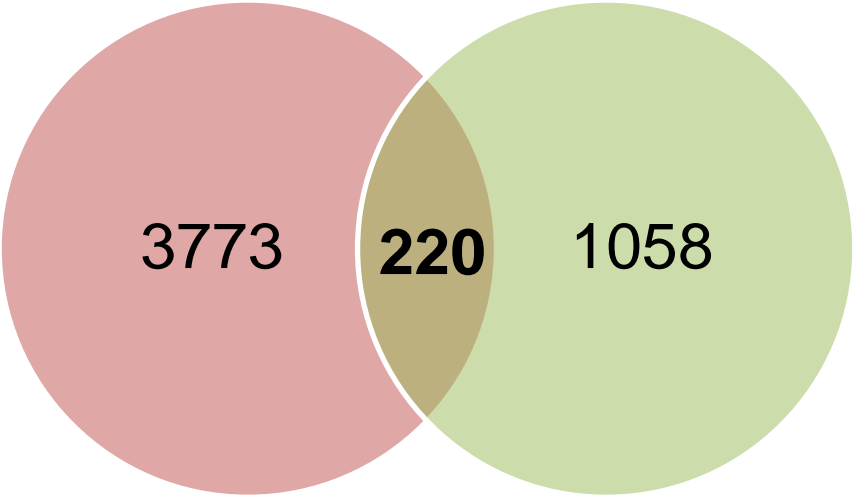
A. Upregulated



4T1.2 v 67NR
n=4444

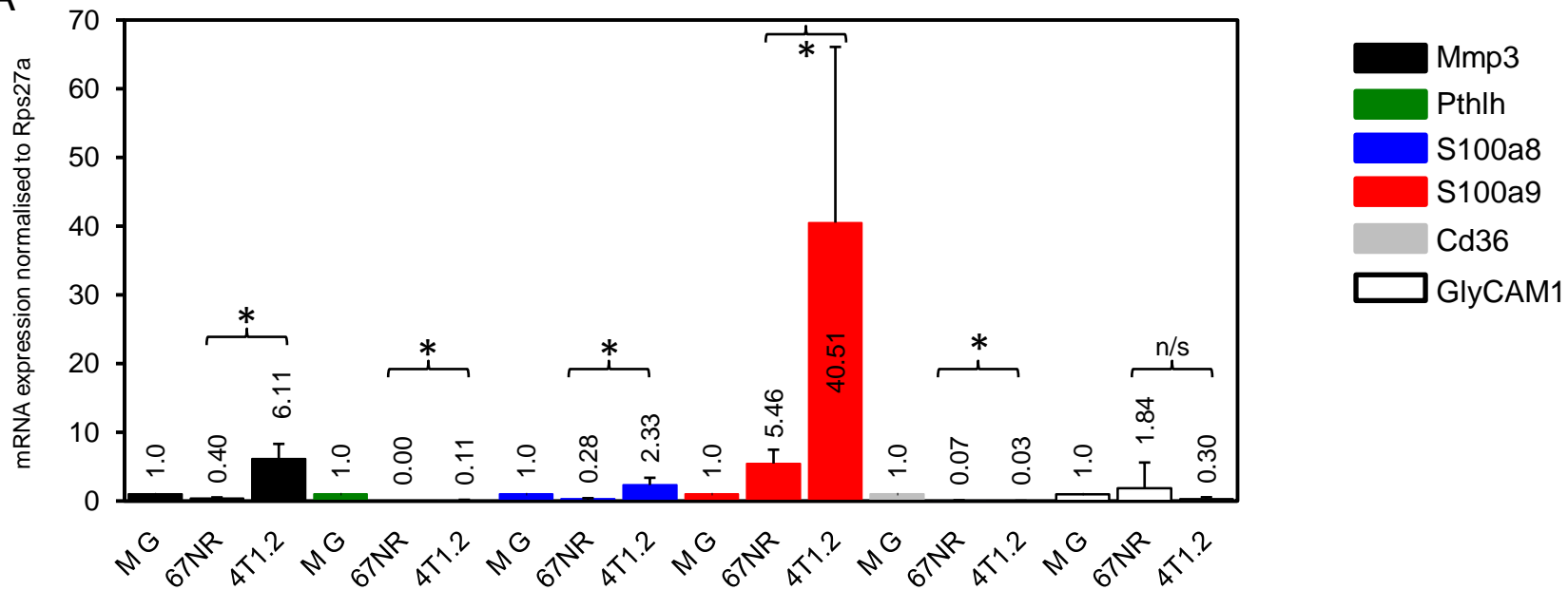
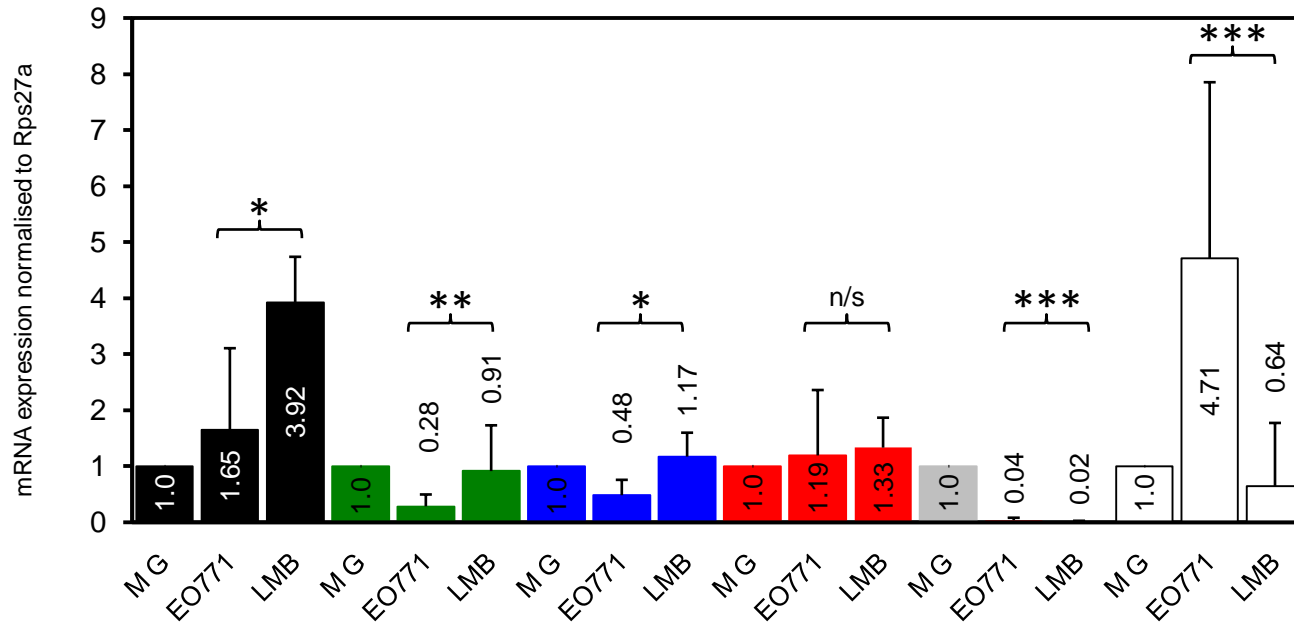
EO771.LMB v EO771
n=1477

B. Downregulated

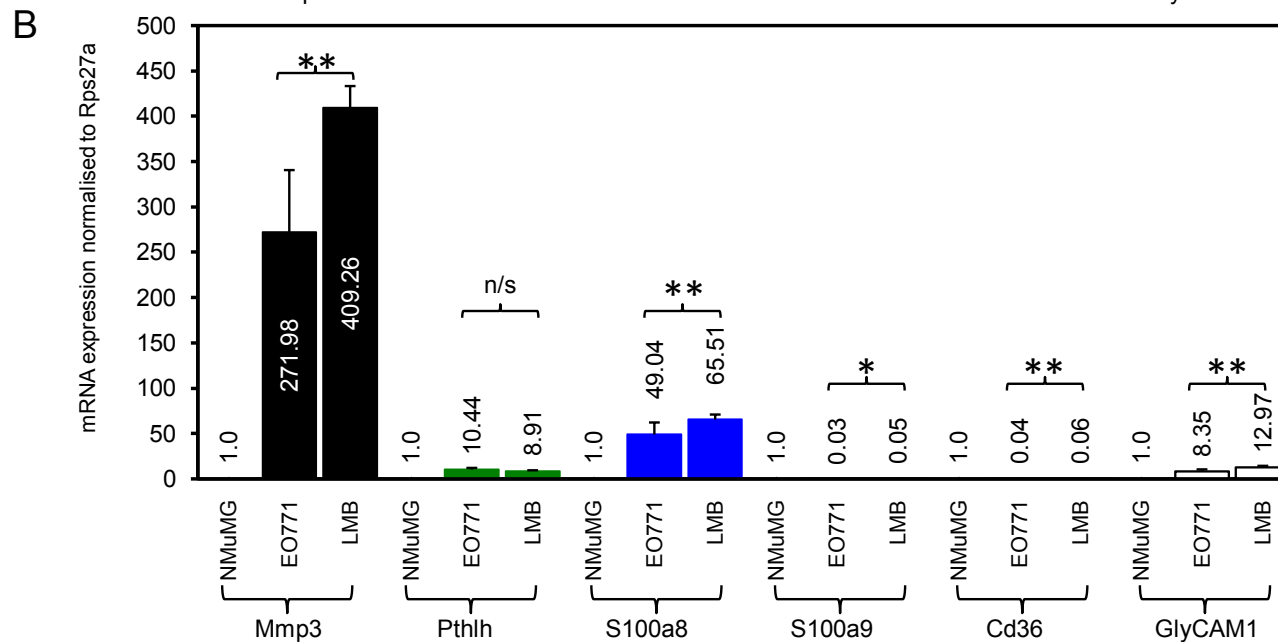
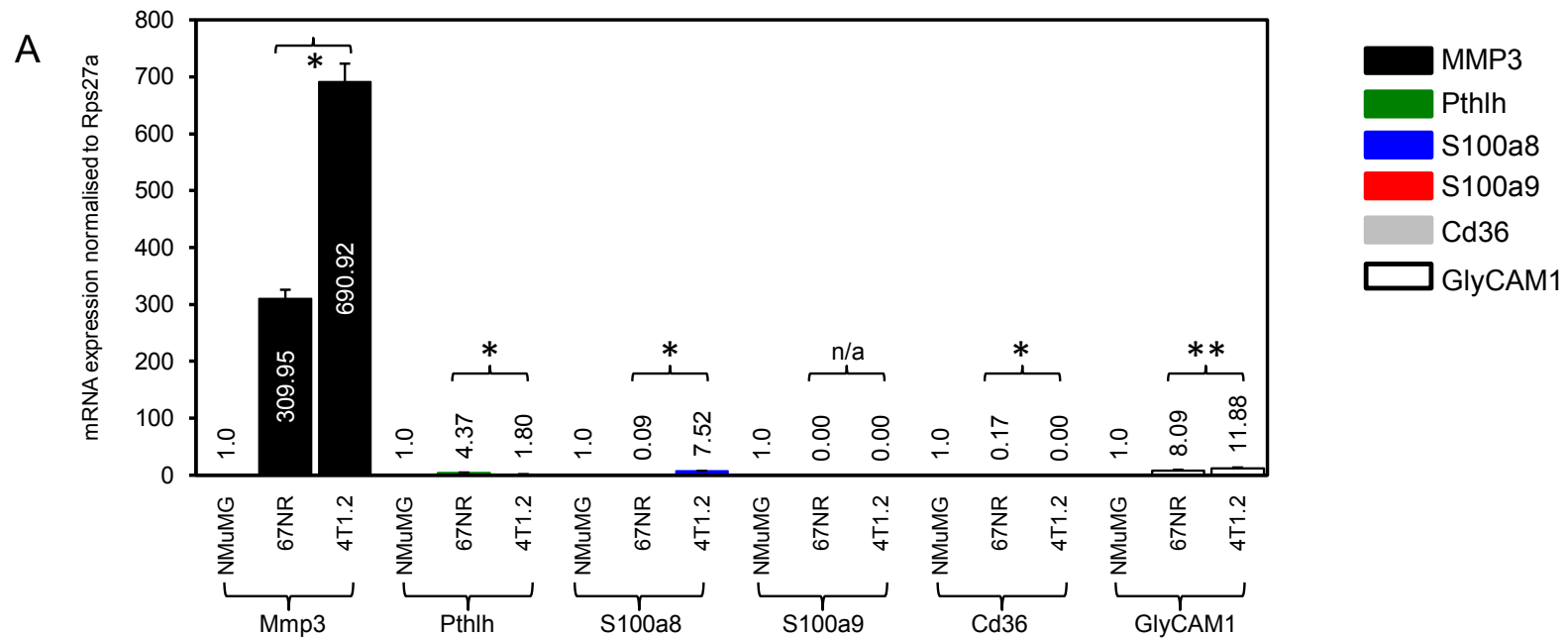


4T1.2 v 67NR
n=3993

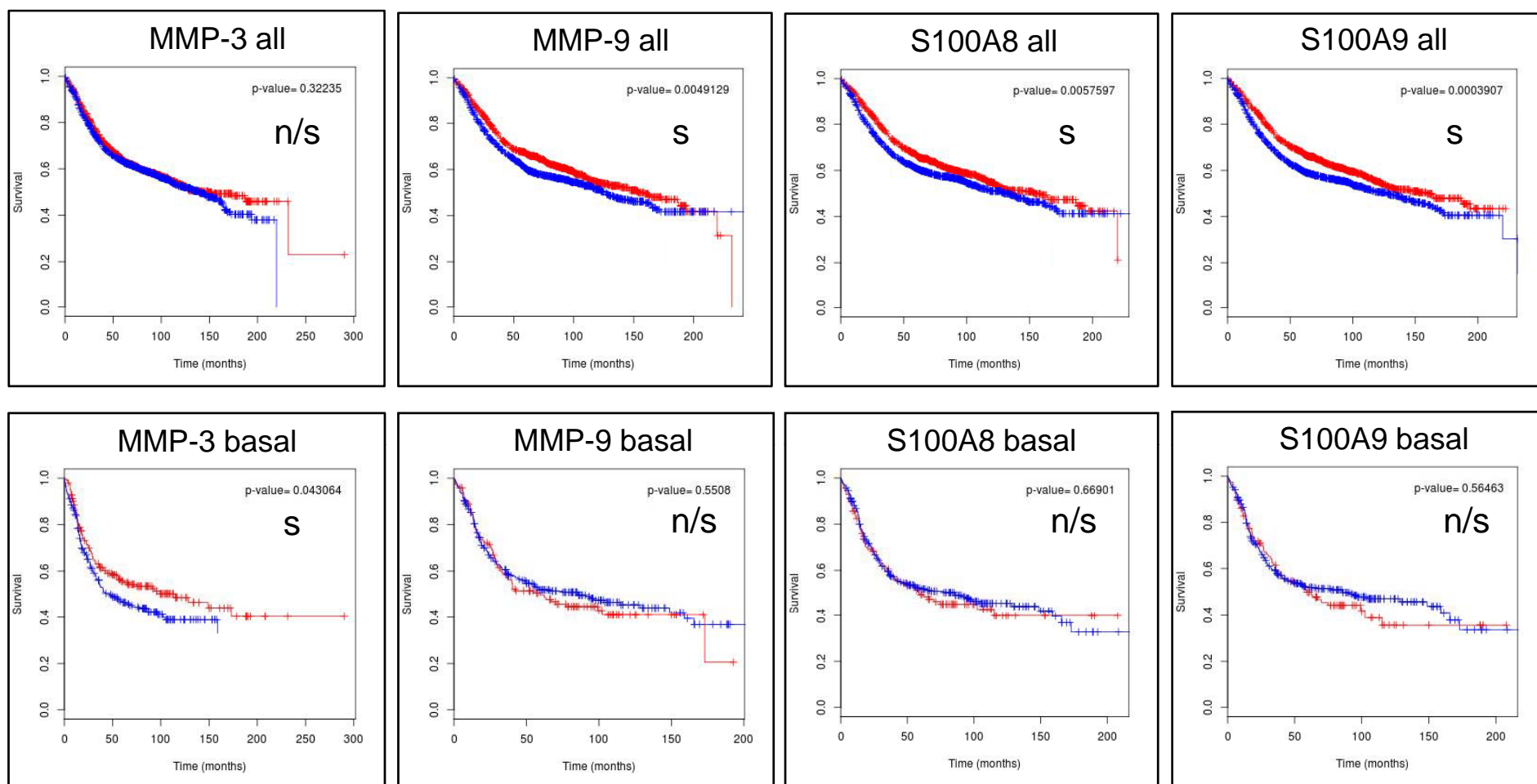
EO771.LMB v EO771
n=1278

A**B**

Supp. Figure 11



Supp. Figure 12



Supplementary Figure 13

Supplementary Table 1

Signature	67NR	4T1.2	EO771	EO771.LMB
BASAL sig-UP	4 (9%)	11 (23%)	5 (11%)	5 (11%)
BASAL sig-DOWN	11 (23%)	3 (6%)	11 (23%)	10 (21%)
BASAL insignificant	32 (68%)	33 (70%)	31 (66%)	32 (68%)
LUMINAL sig-UP	10 (18%)	15 (27%)	4 (7%)	8 (15%)
LUMINAL sig-DOWN	14 (25%)	3 (5%)	17 (31%)	15 (27%)
LUMINAL insignificant	31 (56%)	37 (67%)	34 (62%)	32 (58%)
PROLIFERATION sig-UP	6 (6%)	4 (4%)	50 (48%)	57 (54%)
PROLIFERATION sig-DOWN	61 (58%)	24 (23%)	5 (5%)	4 (4%)
PROLIFERATION insignificant	38 (36%)	77 (73%)	50 (48%)	44 (42%)
HYPOXIA sig-UP	3 (3%)	6 (5%)	28 (25%)	29 (25%)
HYPOXIA sig-DOWN	43 (38%)	17 (15%)	5 (4%)	2 (2%)
HYPOXIA insignificant	68 (60%)	91 (80%)	81 (71%)	83 (73%)
EMT sig-UP	22 (24%)	16 (17%)	16 (17%)	15 (17%)
EMT sig-DOWN	27 (29%)	13 (14%)	29 (31%)	28 (30%)
EMT insignificant	44 (47%)	64 (69%)	48 (52%)	50 (54%)
INTERFERON sig-UP	1 (4%)	3 (12%)	9 (35%)	13 (50%)
INTERFERON sig-DOWN	13 (50%)	3 (12%)	3 (12%)	1 (4%)
INTERFERON insignificant	12 (46%)	20 (77%)	14 (54%)	12 (46%)
CANCER STEM CELLS sig-UP	23 (23%)	20 (20%)	17 (17%)	20 (20%)
CANCER STEM CELLS sig-DOWN	15 (15%)	17 (17%)	18 (18%)	18 (18%)
CANCER STEM CELLS insignificant	62 (62%)	63 (63%)	65 (65%)	62 (62%)
INVASION sig-UP	13 (20%)	14 (22%)	8 (13%)	9 (14%)
INVASION sig-DOWN	17 (27%)	10 (16%)	30 (47%)	31 (48%)
INVASION insignificant	34 (53%)	40 (63%)	26 (41%)	24 (38%)
SIGNIFICANT GENE EXPRESSION SIGNATURES		LUMINAL	PROLIFERATION HYPOXIA INTERFERON	PROLIFERATION HYPOXIA INTERFERON

Supplementary Table 2: Commonly down-regulated genes [with unadjusted P value of 0.05 n= 220]

[illegible]

Supplementary Table 3: Commonly up-regulated genes [with unadjusted P value of 0.05 n= 205]

Cluster ID	Gene name	Gene Symbol	Ref Seq ID	Unadjusted P value (41.1 ± 67NR)	Adjusted P value (41.1 ± 67NR)	Ratio (41.1 ± 67NR)	Fold Change (41.1 ± 67NR)	Unadjusted P value (LMB vs E0771)	Adjusted P value (LMB vs E0771)	Ratio (LMB vs E0771)	Fold Change (LMB vs E0771)
1058071	NM_010697 // Mmp1 // matrix metalloproteinase 1 // 9 A 119 15 10 M // 17932 // ENS	Mmp1	NM_010697	0.000005	0.000705	1.2174600	1.2174600	0.005274	0.013300	2.130000	2.130000
1045455	NM_201362 // Cnab1 // colloid coat domain containing protein 1 // 8 1 12 // 181175 // ENS	Cnab1	NM_201362	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045254	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353170	0.0617700	0.1683200	1.6483200	1.6483200
1045213	NM_020493 // Cnab2 // colloid coat domain containing protein 2 // 8 1 11 11 15 14 M // 1	Cnab2	NM_020493	0.000008	0.000806	0.9353170	0.9353				

1006640	NM_172203 // <i>Nov1</i> // NADPH oxidase 1 // X 15 // 23708 // ENSMUST0000033630 //	Nov1	NM_172203	0.0441651	0.1914710	1.2752400	1.2752400	0.0456763	0.6350840	1.3056600	1.3105600
10481626	NM_010504 // <i>Dna2</i> // DNA topoisomerase II (2) C2 4 A3.1 cm // 13192 // ENDM4	Dna2	NM_010504	0.0432535	0.1413830	1.2740300	1.2740300	0.0208966	0.6209360	1.2621800	1.3021500
10523068	NM_01081105 // 4933407H180k // <i>Riken</i> cDNA 4933407H18 gene // 5 kb // 71101 //	4933407H180k	NM_01081101	0.0377139	0.1804450	1.2712400	1.2712400	0.0163674	0.5851120	1.2541300	1.2541300
10460782	NM_130453 // <i>Gph2a</i> // glycoprotein hormone alpha 2 // 19 A // 170468 // ENDM0JST	Gph2a	NM_130453	0.0389703	0.1853650	1.2695900	1.2695900	0.0001728	0.3887110	1.1764400	1.1764400
10334448	NM_17052 // <i>Ki6a</i> // <i>kinase family member 6</i> // 17 C // 131991 // ENDMJST00000	Ki6a	NM_17052	0.0416402	0.2000120	1.2695400	1.2695400	0.0001979	0.5966630	1.2900000	1.2900000
10445839	NM_145489 // <i>A061453</i> // expressed sequence A061453 // 17 C // 124813 // BC006	A061453	NM_145489	0.0317381	0.1138910	1.2674400	1.2674400	0.0181295	0.5851120	1.1041100	1.1041100
10345678	NM_178207 // <i>Thb27</i> // <i>hemoglobin beta 2</i> // 18 C C2 // 43207 // ENDMJST0	Thb27	NM_178207	0.0390517	0.1814030	1.2674200	1.2674200	0.0191761	0.5890040	1.2149100	1.2149100
10361846	NM_009048 // <i>Reps1</i> // <i>Reps1</i> associated Eps domain containing protein // 10 A3 //	Reps1	NM_009048	0.0096347	0.1267080	1.2670800	1.2670800	0.0228651	0.5966630	1.1247800	1.1247800
10356065	NM_001024720 // <i>Hmcs1</i> // <i>hemocytin 1</i> // 1 G1 // 545370 // ENDMJST0000074783 //	Hmcs1	NM_001024720	0.0472177	0.2045160	1.2652400	1.2652400	0.0480029	0.6420950	1.1460800	1.1460800
10359138	NM_001031480 // <i>R4301700130k</i> // <i>Riken</i> cDNA R430170013 gene // 1 G1 // 71932 //	R4301700130k	NM_001031480	0.0319640	0.1196430	1.2642200	1.2642200	0.0180074	0.5851120	1.2851200	1.2851200
10488323	NM_001081262 // 4932431H170k // <i>Riken</i> cDNA 4932431H17 gene // 3 D // 54552 //	4932431H170k	NM_001081262	0.0070742	0.0215685	1.2637500	1.2637500	0.0045377	0.5213400	1.1621200	1.1621200
10472598	NM_177784 // <i>Wht2</i> // <i>leucine like 23 (Drosophila)</i> // 2 C2 // 277396 // ENDMJST0	KR23	NM_177784	0.0413307	0.1064800	1.2589700	1.2589700	0.0009181	0.4730920	1.0846300	1.0846300
10397975	NM_172544 // <i>Nov3</i> // <i>nov3</i> // 20024 (2.45.0A.01) // ENDMJST0000000	Nov3	NM_172544	0.0707198	0.2852030	1.2587600	1.2587600	0.0030033	0.5207630	1.2475800	1.2475800
10594825	NM_020208 // <i>Aqap3</i> // <i>septatrin 9</i> // 9 D // 64008 // ENDMJST0000074465 // <i>Aqap3</i>	Aqap3	NM_020208	0.0219558	0.1343070	1.2494800	1.2494800	0.0325958	0.6144630	1.1198400	1.1198400
10502780	NM_133892 // <i>Lao1</i> // <i>L-aminine acid oxidase 1</i> // 1 D2.1 // 100470 // ENDMJST0000000	Lao1	NM_133892	0.0338348	0.1709150	1.2469100	1.2469100	0.0370077	0.6149790	1.1848700	1.1848700
10490590	ENSMJST0000020548 // <i>Nov2</i> // <i>nov2</i> // 20024 (2.45.0A.01) // ENDMJST0000000	Nov2	ENSMJST0000020548	0.0707198	0.2852030	1.2468000	1.2468000	0.0030033	0.5207630	1.2475800	1.2475800
10521136	NM_001081102 // <i>Whsc1</i> // <i>Wahl-Reichenow syndrome candidate 1 (human)</i> // 5 B1 //	Whsc1	NM_001081102	0.0056497	0.0245070	1.2450700	1.2450700	0.0045377	0.5213400	1.2025000	1.2025000
10396278	NM_026102 // <i>Daam1</i> // <i>disseminated activator of morphogenesis 1</i> // 12	Daam1	NM_026102	0.0046071	0.0547162	1.2405200	1.2405200	0.0494036	0.6430890	1.1631300	1.1631300
10519589	NM_008642 // <i>Mtp</i> // <i>microtubule triglyceride transfer protein</i> // 9 G31 68.1 cm	Mtp	NM_008642	0.0605600	0.0648978	1.2402000	1.2402000	0.0040517	0.5001000	1.1851300	1.1851300
10502755	NM_133948 // <i>Pip1</i> // <i>PC4 and SFPS1 interacting protein 3</i> // 4 C3 // 101739 //	Pip1	NM_133948	0.0005001	0.0064307	1.2387600	1.2387600	0.0368218	0.6187790	1.1112800	1.1112800
10550022	NM_133948 // <i>Pip1</i> // <i>PC4 and SFPS1 interacting protein 3</i> // 4 C3 // 101739 //	Pip1	NM_133948	0.0005001	0.0205357	1.2231400	1.2231400	0.0031854	0.5153990	1.1521900	1.1521900
10564149	NM_0242154 // <i>Scor116</i> // <i>small nuclear RNA, C/D box 116 cluster</i> // 7 C17 29.0	Scor116	AF241256	0.0274040	0.1374940	1.2230500	1.2230500	0.0285367	0.6031410	1.2999600	1.2999600
10444086	NM_013860 // <i>Crm1</i> // <i>cysteine rich transmembrane BMP regulator 1 (chordin like)</i>	Crm1	NM_013860	0.0403016	0.2088000	1.2212600	1.2212600	0.0313908	0.5184900	1.1484400	1.1484400
10496077	NM_027907 // <i>Agp21</i> // <i>aspartate glyoxylate aminotransferase 2-like 1</i> // 3 G3 //	Agp21	NM_027907	0.0163949	0.1767900	1.2211800	1.2211800	0.0016189	0.4825080	1.0607900	1.0607900
10361507	NM_030796 // <i>Smyd2</i> // <i>S1 and MPR9 domain containing 2</i> // 1 H6 // 236810 // 195	Smyd2	NM_030796	0.0489162	0.1884440	1.2211900	1.2211900	0.0142171	0.6172700	1.2211900	1.2211900
10451782	NM_133066 // <i>Gid4</i> // <i>gap junction protein, delta 4</i> // 18 A1 // 275152 // ENDM425	Gid4	NM_133066	0.0316691	0.1694830	1.2200100	1.2200100	0.0044127	0.6443000	1.0987900	1.0987900
10461856	NM_008137 // <i>Gna14</i> // <i>guanine nucleotide binding protein, alpha 14</i> // 19 A 8.19	Gna14	NM_008137	0.0168781	0.1172790	1.2168800	1.2168800	0.0151827	0.5813830	1.2639400	1.2639400
10474207	NM_212413 // <i>Fluo3</i> // <i>F-box protein 1</i> // 2 C2 // 57443 // NM_020593 // <i>Fluo3</i> //	Fluo3	NM_212413	0.0460184	0.1448010	1.2109700	1.2109700	0.0045120	0.5213400	1.1569100	1.1569100
10330446	NC138179 // <i>ABR1100</i> // expressed sequence ABR1100 // 1 H2.1 // 22651 // ENDM4	ABR1100	NC138179	0.0451127	0.1997820	1.2091900	1.2091900	0.0051660	0.5213400	1.1639000	1.1639000
10427661	---	---	---	0.0013233	0.2053400	1.2053400	1.2053400	0.0080597	0.5362340	1.2110300	1.2110300
10420148	NM_001081129 // <i>Cttnp3</i> // <i>connectin associated protein like 3</i> // 13 B1 // 23868	Cttnp3	NM_001081129	0.0884408	0.2801170	1.2031700	1.2031700	0.0347380	0.6172700	1.1258800	1.1258800
10521183	NM_007418 // <i>Adra2c</i> // <i>adrenergic receptor, alpha 2c</i> // 5 B3.1 20.0 cm // 11551	Adra2c	NM_007418	0.0295531	0.1852160	1.2012700	1.2012700	0.0217295	0.5966630	1.0901800	1.0901800
10359422	NM_007453 // <i>Prd6</i> // <i>perlecan domain 6</i> // 1 H2.1.1 83.6 cm // 13758 // ENDMJST00	Prd6	NM_007453	0.0439768	0.1964760	1.1996900	1.1996900	0.0291687	0.6047360	1.1236600	1.1236600
10545057	NM_008210 // <i>H3f5a</i> // <i>H3 histone, family 3A</i> // 1 D2.3 // 15078 // BC008353 // H	H3f5a	NM_008210	0.0470108	0.2039880	1.1993500	1.1993500	0.0175517	0.5851120	1.0691600	1.0691600
10380741	ENSMJST0000074598 // 3110043A150k // <i>Riken</i> cDNA 3110043A15 gene // 13 B2 // 71	3110043A150k	ENSMJST0000074598	0.0603655	0.1872150	1.1955700	1.1955700	0.0061170	0.5123400	1.1407800	1.1407800
10481818	NM_144852 // <i>Sct4a</i> // <i>solute carrier family 7 (cationic amino acid transporter,</i>	Sct4a	NM_144852	0.0348129	0.1728970	1.1892900	1.1892900	0.0204823	0.5966630	1.2164200	1.2164200
10517058	NM_001083916 // 18100101160k // <i>Riken</i> cDNA 1810010116 gene // 4 D2.3 // 69073	18100101160k	NM_001083916	0.0001141	0.1595320	1.1885500	1.1885500	0.0402122	0.6251770	1.1297100	1.1297100
10501210	NM_146558 // <i>Ctrf6a</i> // <i>cellular receptor like 6</i> // 17 B85.1 // ENDMJST00000	Ctrf6a	NM_146558	0.0029165	0.0465227	1.1882500	1.1882500	0.0035498	0.5103990	1.1046100	1.1046100
10554140	BC116191 // <i>Zck</i> // <i>zinc finger protein of the cerebellum 4</i> // 9 E3.319 61.0 cm	Zck	BC116191	0.0114328	0.0958844	1.1859600	1.1859600	0.0082483	0.5362340	1.1053000	1.1053000
10373660	NM_146777 // <i>Qtrf18</i> // <i>cellular receptor 18</i> // --- // 258773 // ENDMJST00000	Qtrf18	NM_146777	0.0261747	0.1472200	1.1842600	1.1842600	0.0158094	0.5813830	1.1566000	1.1566000
10538571	NM_015113 // <i>Vsr1</i> // <i>vesicular nuclear 1 receptor</i> // 18 B1 // 131838 // NM_134185	Vsr1	NM_015113	0.0013865	0.0612626	1.1810600	1.1810600	0.0151341	0.6112700	1.1251100	1.1251100
10538631	NM_001024720 // <i>Hmcs1</i> // <i>hemocytin 1</i> // 1 G1 // 545370 // ENDMJST0000074783 //	Hmcs1	NM_001024720	0.0511771	0.1105820	1.1810200	1.1810200	0.0448210	0.6308800	1.0158800	1.0158800
10437737	NM_025546 // <i>Rut1a1</i> // <i>ribosomal L1 domain containing 1</i> // 16 B1 // 66409 // 176	Rut1a1	NM_025546	0.0327645	0.1872130	1.1810200	1.1810200	0.0448754	0.6308800	1.0679500	1.0679500
10534253	NM_001081342 // <i>Gtf1a1</i> // <i>general transcription factor 1 repeat domain cont-</i>	Gtf1a1	NM_001081342	0.0441150	0.1978850	1.1801600	1.1801600	0.0122651	0.5717140	1.1059800	1.1059800
10408367	NM_008232 // <i>Hdgf1</i> // <i>hepatoma derived growth factor-like 1</i> // 13 A3.1 // 15192	Hdgf1	NM_008232	0.0201178	0.1282400	1.1775200	1.1775200	0.0018780	0.4852320	1.1927500	1.1927500
10398136	---	---	---	0.0575408	0.1134790	1.1753200	1.1753200	0.0411185	0.6296050	1.1580200	1.1580200
10477495	NM_001012392 // <i>U4608</i> // <i>cDNA sequence U4608</i> // 2 H1 // 22880 // NM_153418 //	U4608	NM_001012392	0.0441138	0.1944130	1.1746600	1.1746600	0.0000120	0.5061480	1.1877900	1.1877900
10564159	AF241256 // <i>Scord116</i> // <i>small nuclear RNA, C/D box 116 cluster</i> // 7 C17 29.0 c-	Scord116	AF241256	0.0275760	0.1530940	1.1733300	1.1733300	0.0378411	0.6190170	1.2204400	1.2204400
10503374	---	---	---	0.0465304	0.2027780	1.1709600	1.1709600	0.0482236	0.6417260	1.1164400	1.1164400
10601545	---	---	---	0.0494560	0.1843950	1.1681900	1.1681900	0.0048514	0.5213400	1.1807100	1.1807100
10502070	NM_008718 // <i>Npas1</i> // <i>neuronal PAS domain protein 1</i> // 7 A217 4.0 cm // 18142 //	Npas1	NM_008718	0.0413704	0.1851050	1.1672100	1.1672100	0.0379938	0.6166390	1.1065800	1.1065800
10480631	NM_009045 // <i>Rela</i> // <i>v-rel reticuloendotheliosis viral oncogene homolog A (avian</i>	Rela	NM_009045	0.0317343	0.1643500	1.1651500	1.1651500	0.0377845	0.6190170	1.1435700	1.1435700
10473276	---	---	---	0.0207779	0.1672580	1.1609900	1.1609900	0.0450586	0.6351460	1.2043900	1.2043900
10584986	NM_146806 // <i>Orf143</i> // <i>orphan receptor 143</i> // --- // 258802 // ENDMJST00000	Orf143	NM_146806	0.0122221	0.0947448	1.1594200	1.1594200	0.0245680	0.5966630	1.0959700	1.0959700
10471298	NM_001199634 // <i>Batzf</i> // <i>HLA-B associated transcript 2 like 7 B</i> // 22772 //	Batzf	NM_001199634	0.0064942	0.0705273	1.1589000	1.1589000	0.0252456	0.5966630	1.1493200	1.1493200
10510957	NM_172990 // <i>Par1a</i> // <i>parathormone kinase 1</i> // 1 E2 // 206814 // ENDMJST0000000	Par1a	NM_172990	0.0210579	0.0977279	1.1478900	1.1478900	0.0040296	0.6077970	1.1789900	1.1789900
10461713	NM_146684 // <i>Orf1440</i> // <i>orphan receptor 1440</i> // --- // 258679 // ENDMJST00000	Orf1440	NM_146684	0.0489931	0.2084230	1.1452900	1.1452900	0.0259882	0.5966630	1.1837000	1.1837000
10461818	NM_146636 // <i>Orf1487</i> // <i>orphan receptor 1487</i> // --- // 258629 // ENDMJST00000	Orf1487	NM_146636	0.0389115	0.1585130	1.1450900	1.1450900	0.0308615	0.6077970	1.1653900	1.1653900
10389997	AK048022 // <i>CL30030630k</i> // <i>Riken</i> cDNA CL30030630 gene // --- // 320644	CL30030630k	AK048022	0.0494058	0.1717790	1.1406200	1.1406200	0.0497865	0.6169010	1.1763800	1.1763800
10521088	ENSMJST0000011153 // <i>ENSMJST0000007304</i> // predicted gene, ENSMUS0000007304 //	ENSMJST0000011153	ENSMJST0000007304	0.0546017	0.1851180	1.1352100	1.1352100	0.0484772	0.6141660	1.1581100	1.1581100
10461101	NM_001034881 // <i>EG432870</i> // predicted gene, EG432870 // 14 D114 // 432870 // E	EG43									

Supplementary Table 4: Sequences of oligonucleotide primers used for qRT-PCR

Gene	Forward Sequence	Reverse Sequence
Pthrp	5' TTC GGT GGA GGG GCT TGG CC	5' CGG CGG CGC AAG TCT TGG AT
S100a8	5' CAC CAT GCC CTC TAC AAG AAT G	5' TCA CCA TCG CAA GGA ACT CC
S100a9	5' GGA AGC ACA GTT GGC AAC	5' TCC AGG TCC TCC ATG ATG TCA
Egfr	5' CTG TCG CAA AGT TTG TAA TG	5' GAA TTT CTA GTT CTC GTG GG
MMP-9	5' CCA GAC ACT AAA GGC CAT TCG A	5' TCC CAC TTG AGG CCT TTG AAG
Rps27a	5' GAC CCT TAC GGG GAA AAC CAT	5' AGA CAA AGT CCG GCC ATC TTC