Supplementary Information Desmedt et al.

"Characterization and clinical evaluation of CD10+ stroma cells in the breast cancer microenvironment"

1. **Supplementary methods**
2. **Supplementary figures and tables**

1. **Supplementary methods**

Gene expression profiling experiments

Isolation of RNA was performed using the RNeasy Micro kit according to the manufacturer's instructions (Qiagen). For the RNA obtained from cell cultures, the quality was assessed based on the RNA profile generated by the bioanalyzer (Agilent Inc). RNA amplification, hybridization and image scanning were done according to standard Affymetrix protocols. For the RNA extracted from the CD10+ cells isolated from the tumor and normal breast tissues, RNA quantity was assessed by quantifying the GUS gene by RT-PCR (reverse transcription with the Superscript™ First-Strand Synthesis System for RT-PCR from Invitrogen, GUS forward primer: GAGTGGTGCTGAGGATTGGC,GUS reverse primer: TCTAGCGTGTCGACCCATT, SYBR Green PCR Master Mix). For those samples, a double amplification step was performed using the TargetAMP 2-Round Aminoallyl-aRNA Amplification kit 1.0 kit from Epicentre Biotechnologies. We then used the Human Genome U133-2.0 plus GeneChips.

For the co-culture experiments, RNA was extracted from the cells collected in the lysis buffer with the same kit from Agilent (Absolutely RNA microprep kit). Quality was assessed based on the RNA profile generated by the bioanalyzer (Agilent Inc) and the quantity was assessed using the Nanodrop. One hundred ng of total RNA was then used to generate the expression data using the Affymetrix GeneChip 3' IVT Expression Kit and theHuman Genome U133-2.0 plus GeneChips. Of note, for 3 co-culture experiments with the MSCs (with T47D, with MDAMB361 and with BT474), the RNA yield was not sufficient so that these conditions could not be analyzed in duplicate.

2. **Supplementary Figures and Tables**

Supplementary Figures (in this document):

Supplementary Figure 1: Schematic tissue fractioning

Supplementary Figure 2: Line plot of the expression values of the CD10+ stroma signature genes in the Neve et al. invasive breast cancer cell lines. The cell lines are ranked according to their molecular subgroup (ER-/HER2-: from BT20 to ZR751, HER2+: from AU565 to UACC812 and ER+/HER2-: from ZR7530 to ZR75B). The colours of the lines represent the following: orange= median expression of the array, light and dark blue= 25th and 75th percentile expression of the array, green= expression values of the individual probesets, brown=median expression of the gene, pink=standard deviation.

Supplementary Figure 3: Boxplots representing the CD10+ signature genes in the different CD10+ cell types (co-cultures with different subtypes of breast cancer cell lines and controls are represented separately).

Supplementary Figure 4: Proliferation results of the co-culture experiments. The Y-axis represents the number of cells after 4 days of co-culture. Nr of cells seeded at day 0: 200.000 for the breast cancer cell lines (except 400.000 for the SKBR3), 700.000 for the fibroblasts and 600.000 for the myoepithelial cells. Confluent dishes were used for the

MSCs. The X-axis represents the subtype of the breast cancer cell line used in the co-culture. The p-values between the different plots are illustrated on the horizontal bars.

Supplementary Tables (in separate Excel file):

Supplementary Table 1: Description of the different cell populations used for the cell co-culture experiments.

Supplementary Table 2: Description of the invasive breast cancer datasets used for prognosis and correlation analyses. DMFS: distant metastasis free survival; RFS: relapse free survival.

Supplementary Table 3: List of genes differentially expressed between tumor and normal CD10+ cells (with absolute fold change, FC, >1.5 and false discovery rate, FDR, <0.05).

Supplementary Table 4: Most significant (FDR<5%) gene ontology (GO) biological processes terms from the genes listed in Supplementary Table 3.

Supplementary Table 5: Gene overlap between the genes listed in Supplementary Table 4 and various published signatures.

Supplementary Table 6: The CD10+ stroma signature.

Supplementary Table 7: Comparison of the variances of the CD10+ stroma signatures in tumor versus normal breast CD10+ samples using the F-test.

Supplementary Table 8: Comparison of expression values of the CD10+ stroma signature genes across the 3 different CD10+ cell types. FC= fold change.

Supplementary Table 9: Comparison of the fibroblasts expression values of the CD10+ stroma signature genes across the 4 different subgroups (co-culture with ER+/HER2- cell lines, co-culture with HER2+ cell lines, co-culture with ER-/HER2- cell line, controls). FC= fold change.

Supplementary Table 10: Comparison of the MSCs expression values of the CD10+ stroma signature genes across the 4 different subgroups (co-culture with ER+/HER2- cell lines, co-culture with HER2+ cell lines, co-culture with ER-/HER2- cell line, controls). FC= fold change.

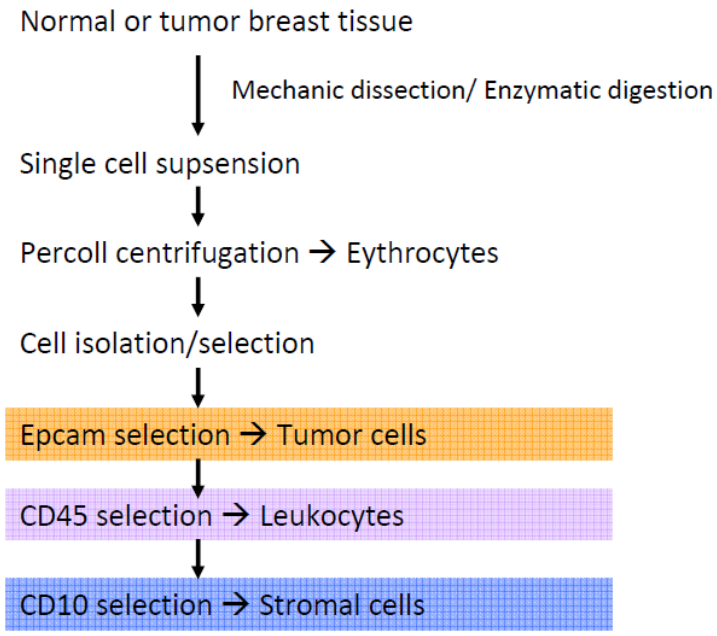
Supplementary Table 11: Comparison of the myoepithelial cells expression values of the CD10+ stroma signature genes across the 4 different subgroups (co-culture with ER+/HER2- cell lines, co-culture with HER2+ cell lines, co-culture with ER-/HER2- cell line, controls). FC= fold change.

Supplementary Table 12: Univariate analysis of the CD10+ stroma signature, the PLAU stroma metagene (Desmedt et al. 2008) and SDPP (Finak et al. 2008) per molecular subgroup of untreated breast cancer patients.

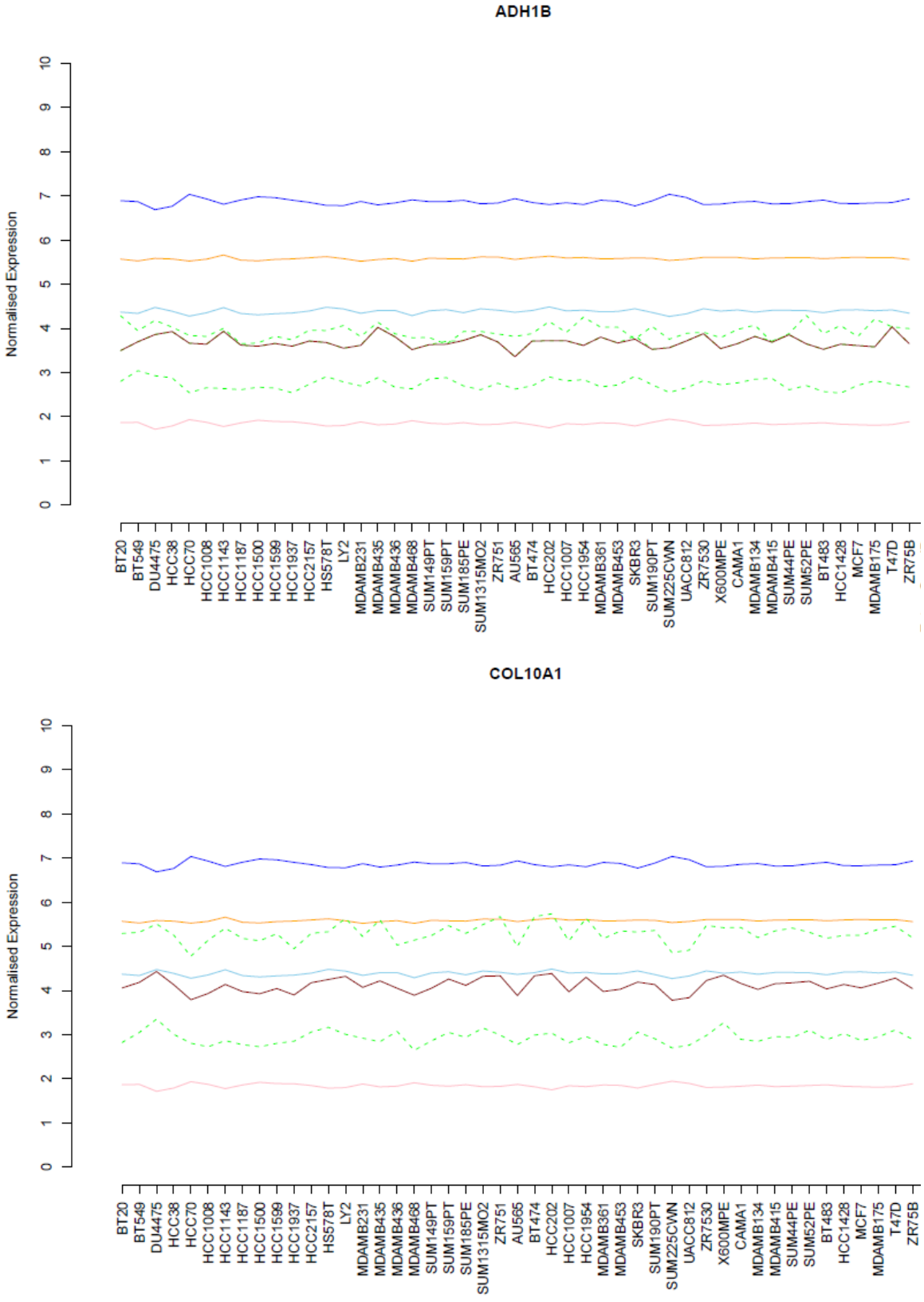
Supplementary Table 13: Correlation values between our CD10+ stroma signature (CD10+ stroma sign), the PLAU stroma metagene (Desmedt et al. 2008) and SDPP (Finak et al. 2008).

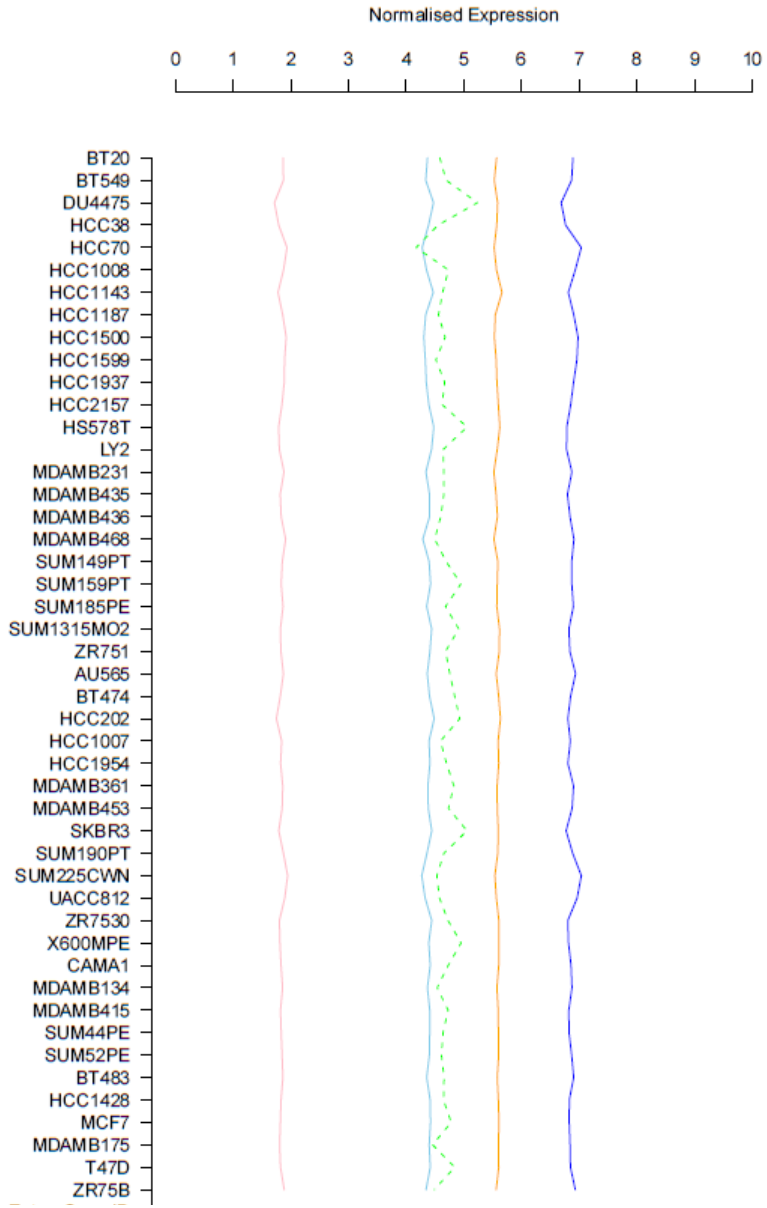
Supplementary Table 14: Results of the predictive ability (AUC, 95% CI and p-value) of the CD10+ stroma signature, the PLAU stroma metagene (Desmedt et al. 2008), the DCN stroma metagene (Farmer et al. 2009) and SDPP (Finak et al. 2008) in the TOP trial.

Supplementary Figure 1:



Supplementary Figure 2

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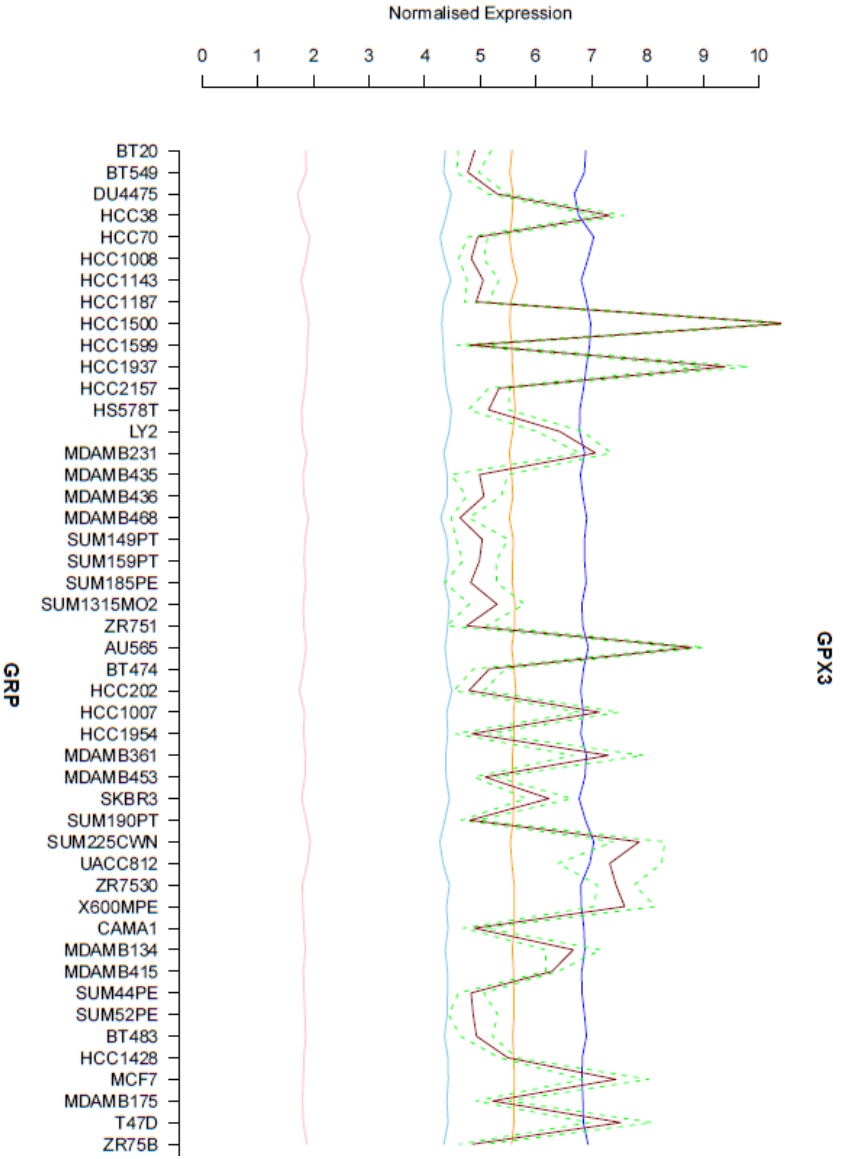
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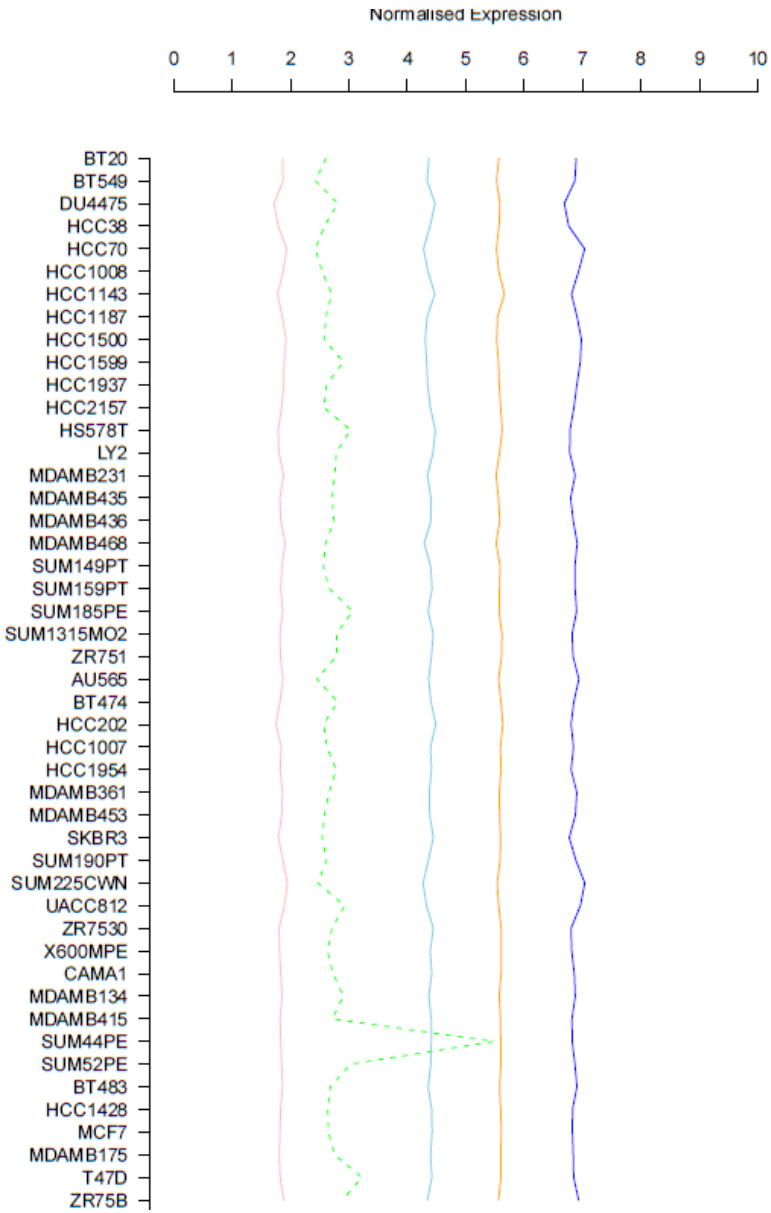
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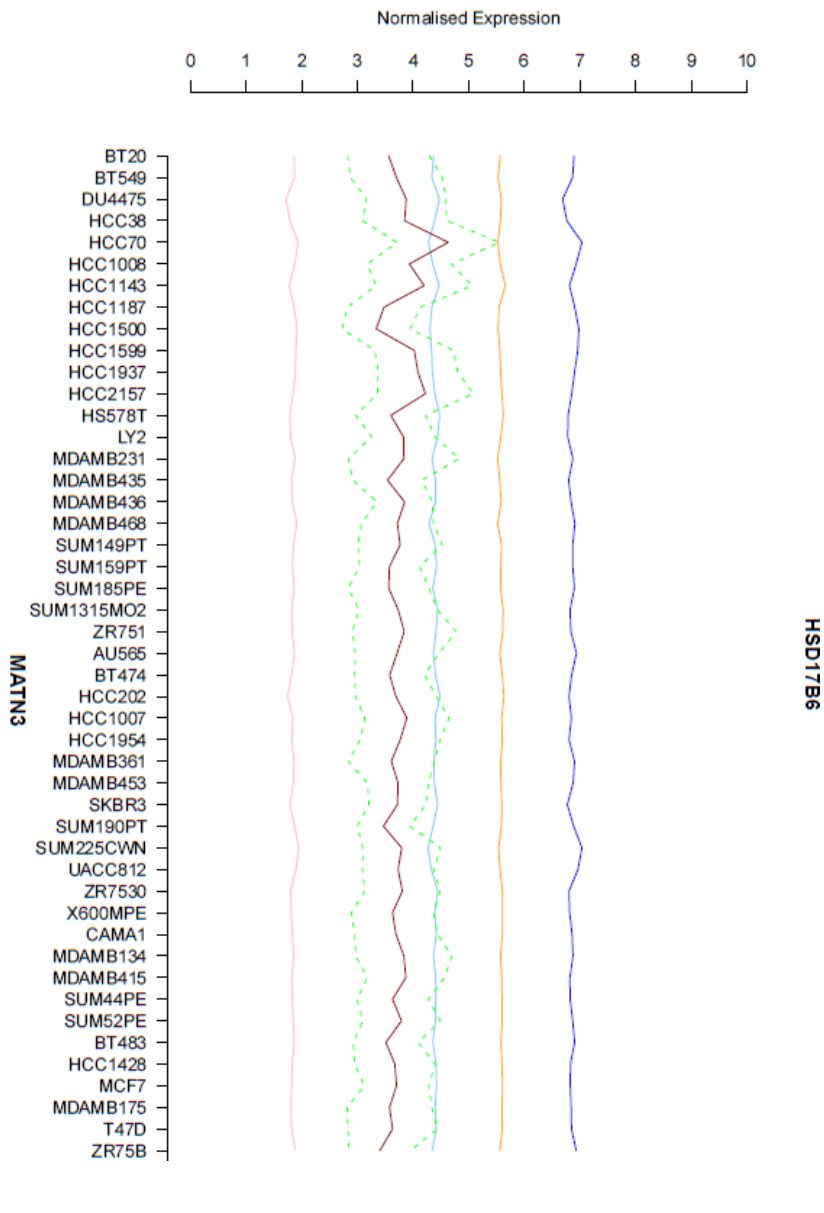
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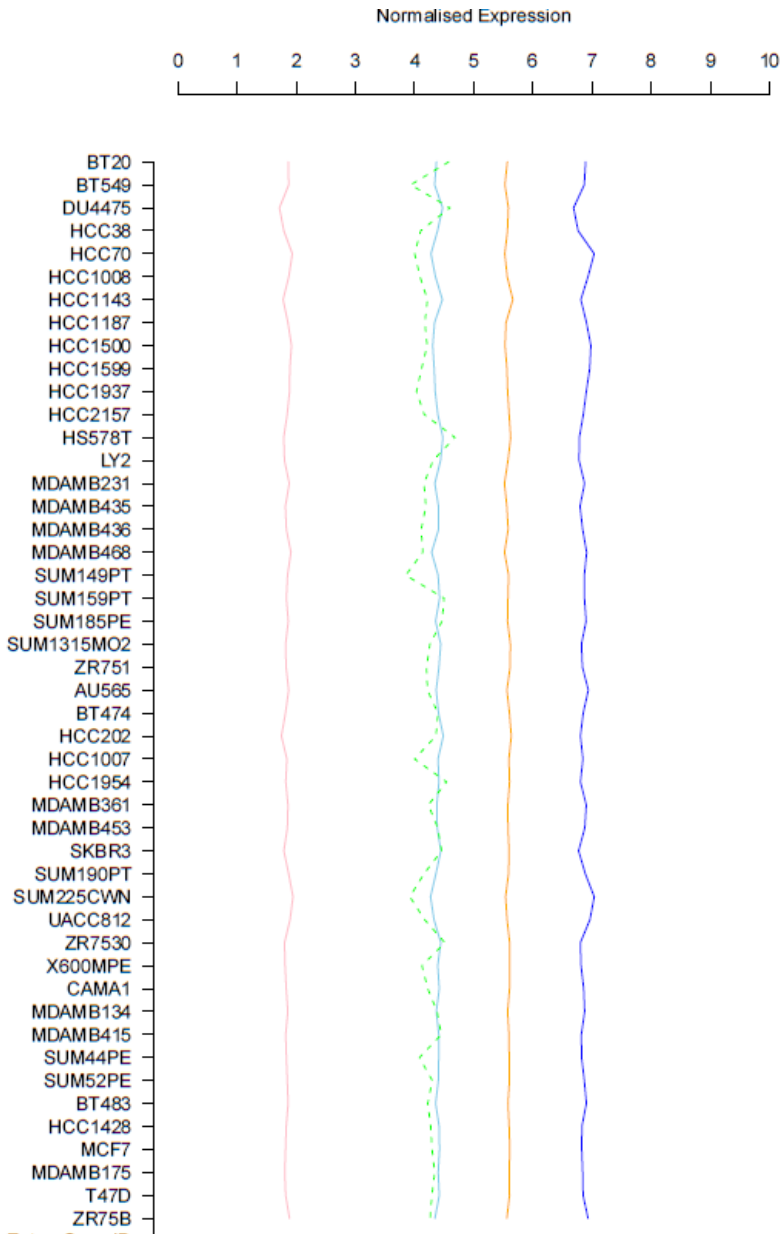
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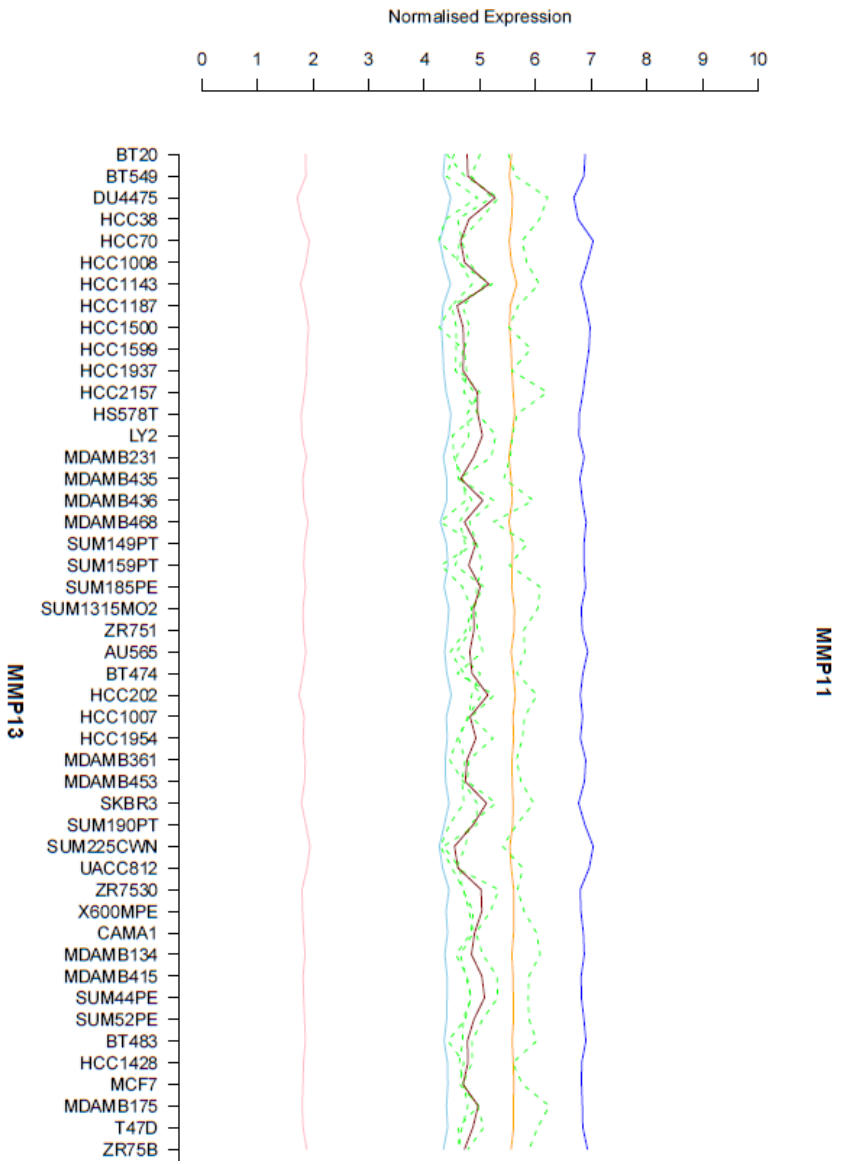
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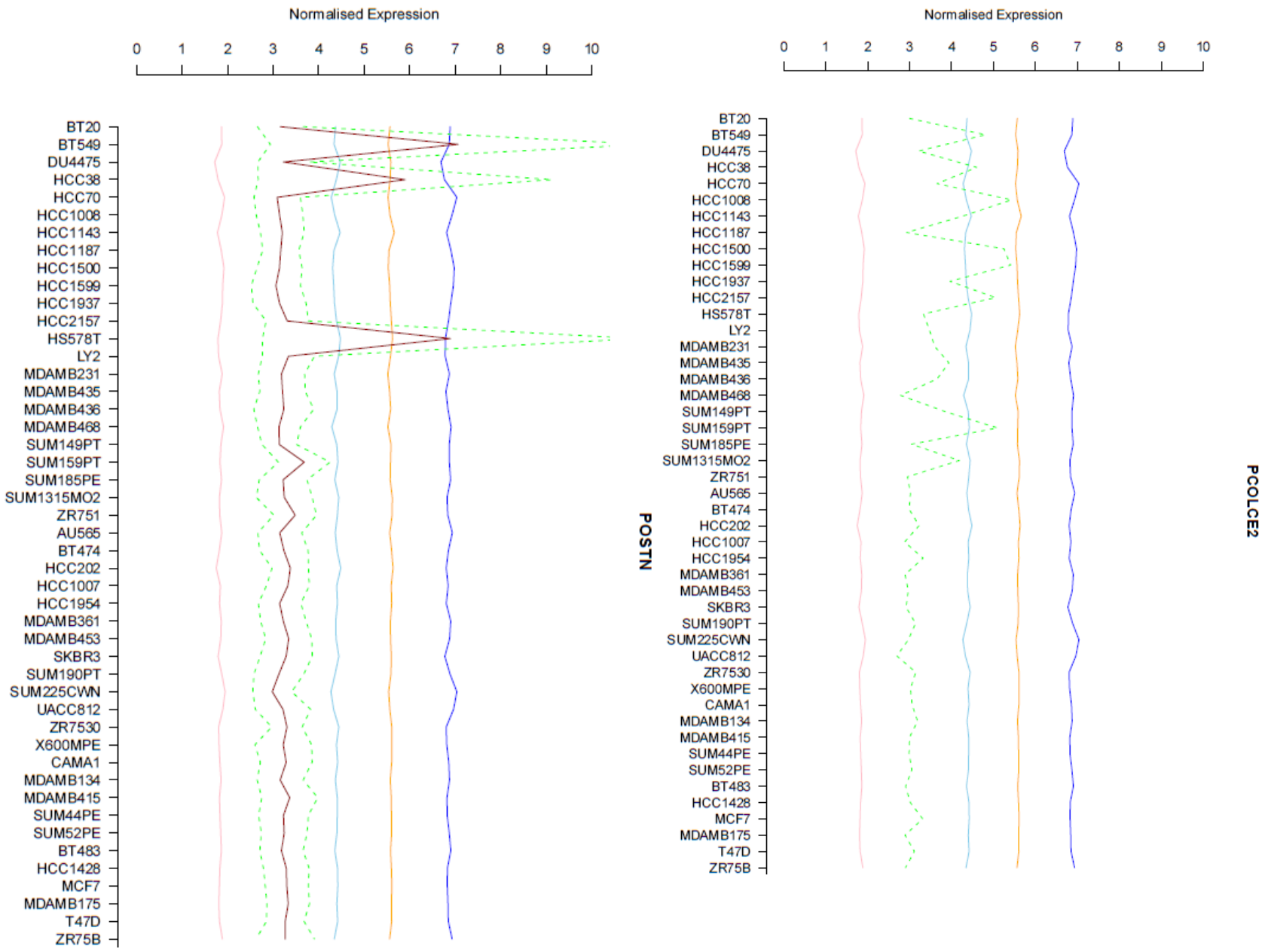
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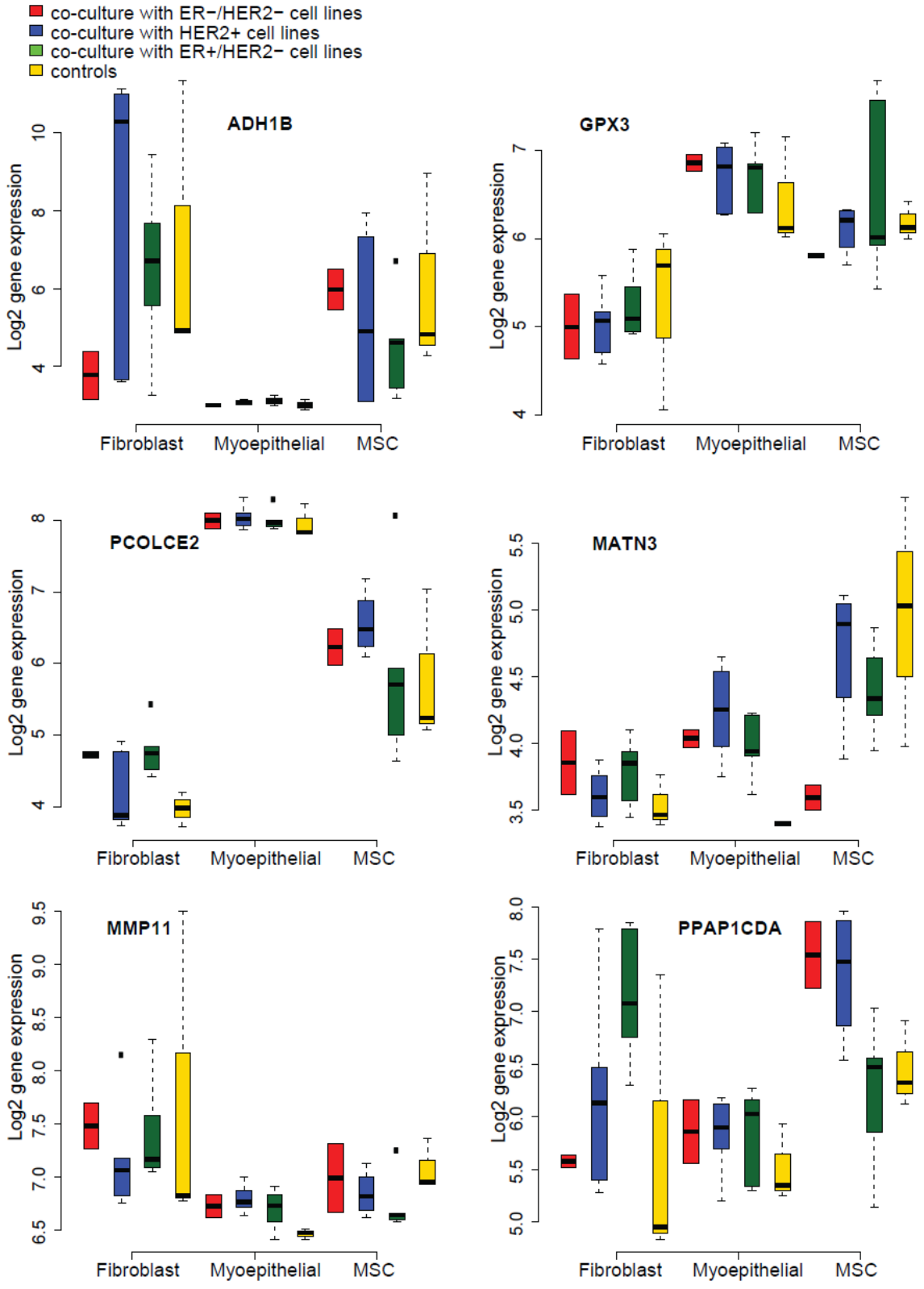
Supplementary Figure 3:



Supplementary Figure 4: