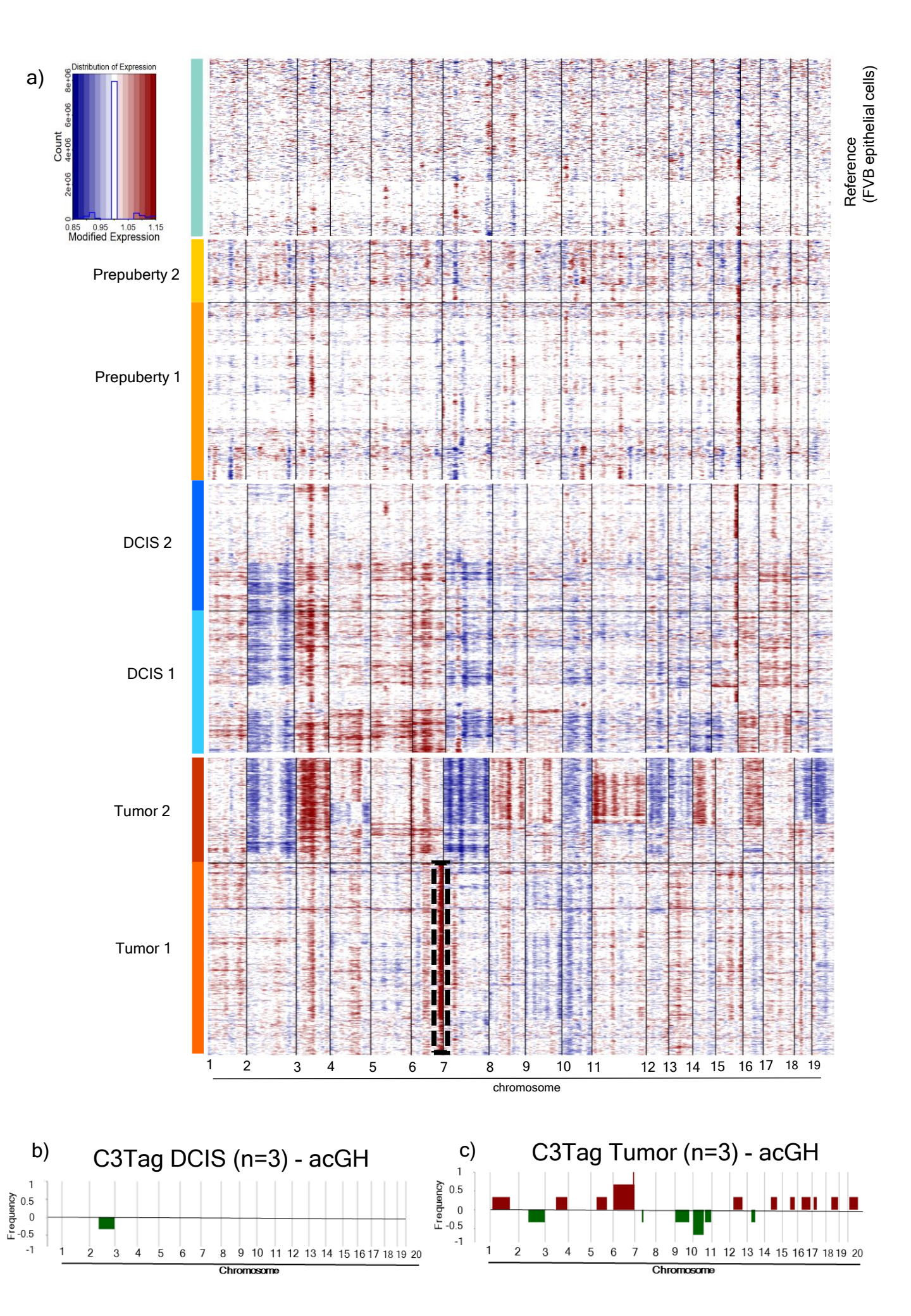
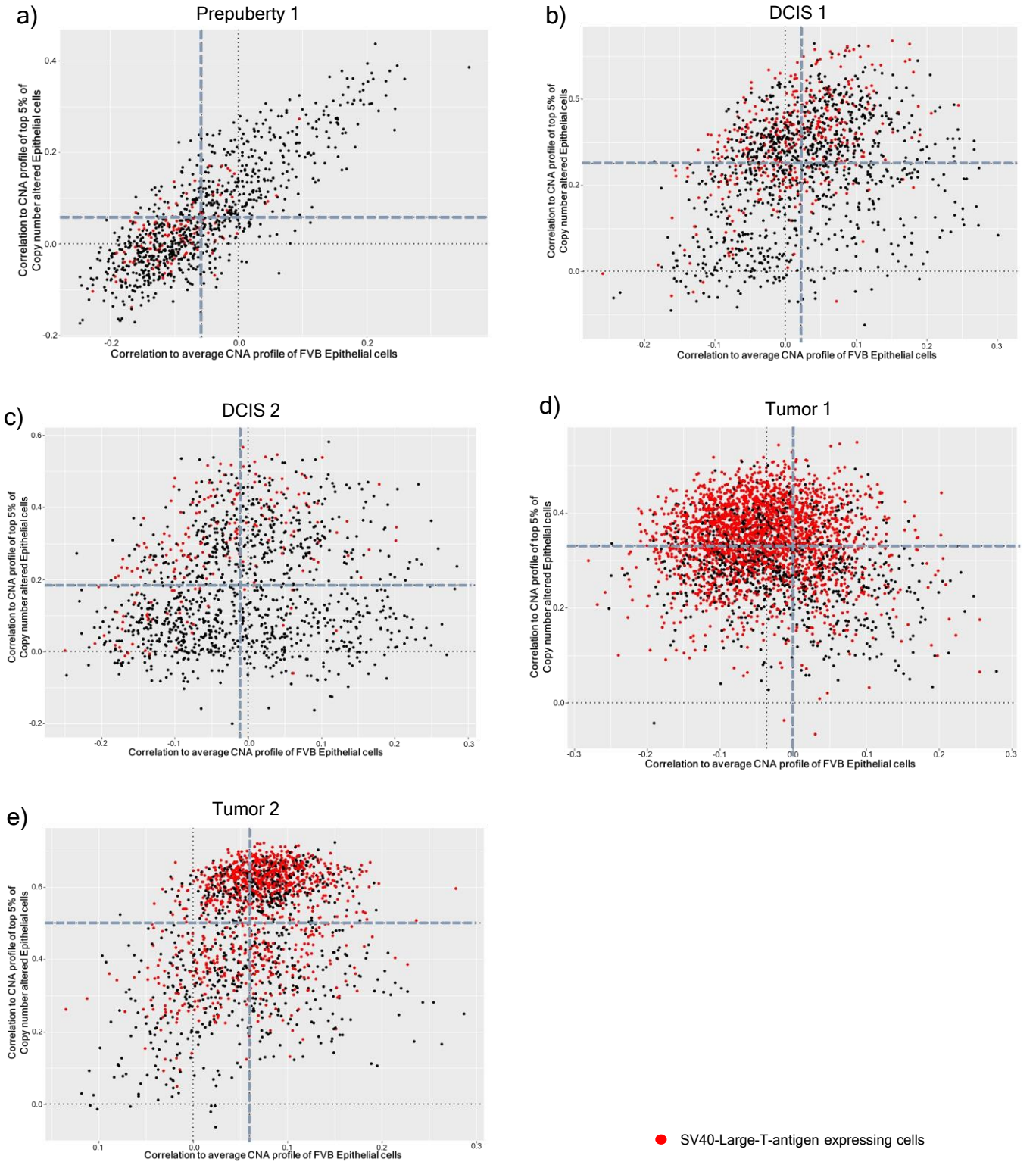
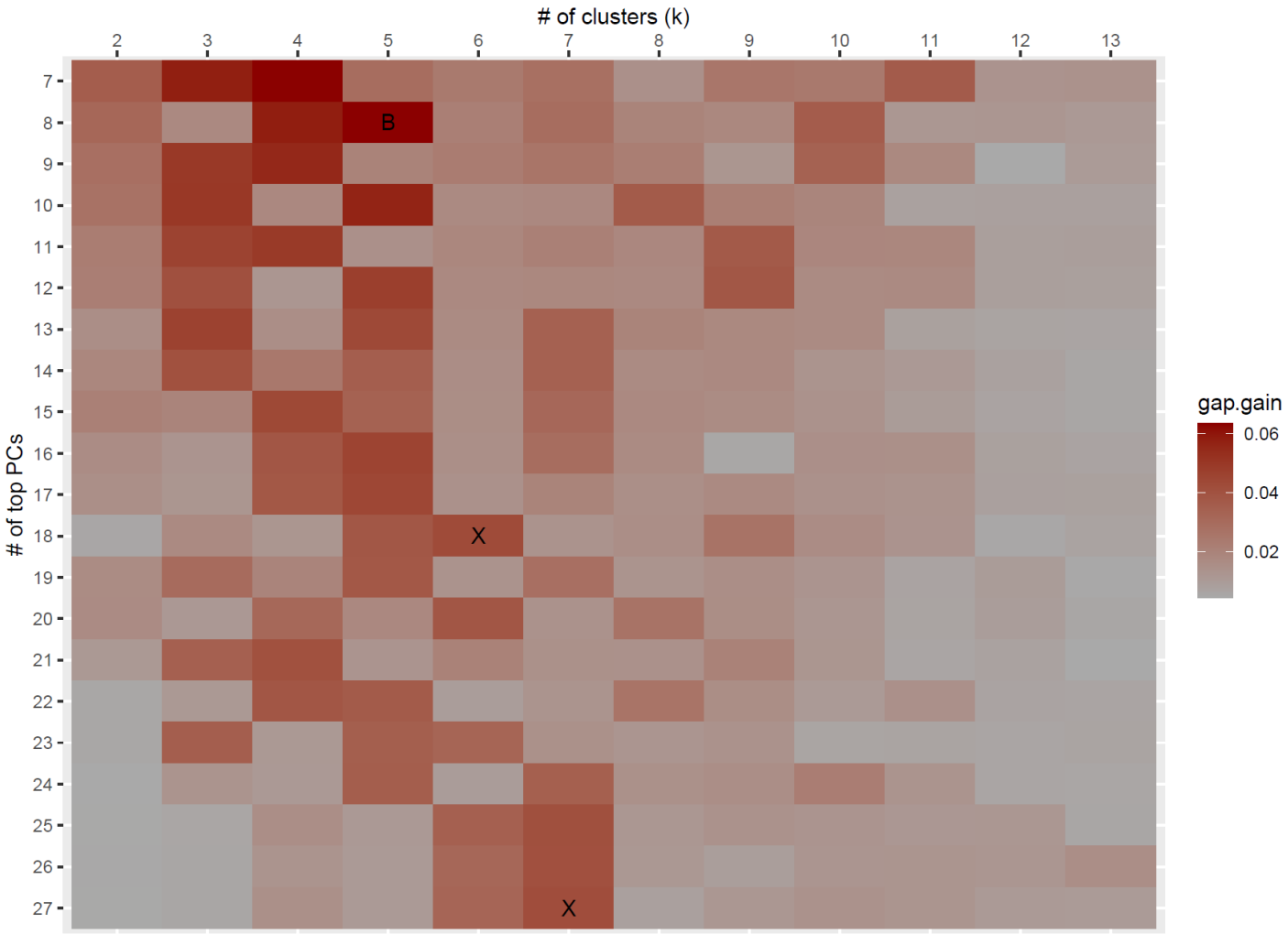

a)
Distribution of Expression
Count
Modified Expression
Reference
(FVB epithelial cells)
Prepuberty 2
Prepuberty 1
DCIS 2
DCIS 1
Tumor 2
Tumor 1
chromosome
b)
C3Tag DCIS (n=3) - acGH
Frequency
Chromosome
c)
C3Tag Tumor (n=3) - acGH
Frequency
Chromosome

**Supplementary Figure 1. The scRNAseq inferred and bulk copy number events in C3Tag mice (a)** InferCNV produced copy number heatmaps of epithelial cells from Prepuberty1 and Prepuberty2**;** DCIS1 and DCIS2 and Tumor1 and Tumor2**.** KRAS amplification is highlighted in the InferCNV plot of Tumor1. **(b)** SWITCH plots identifying copy number segments from aCGH performed on bulk DNA harvested from C3Tag DCIS disease states (n=3). **(c)** SWITCH plots identifying copy number segments from aCGH performed on bulk DNA harvested from C3Tag Tumor disease states (n=3). X-axis represents chromosomes in SWITCH plots and y-axis represents copy number frequency.

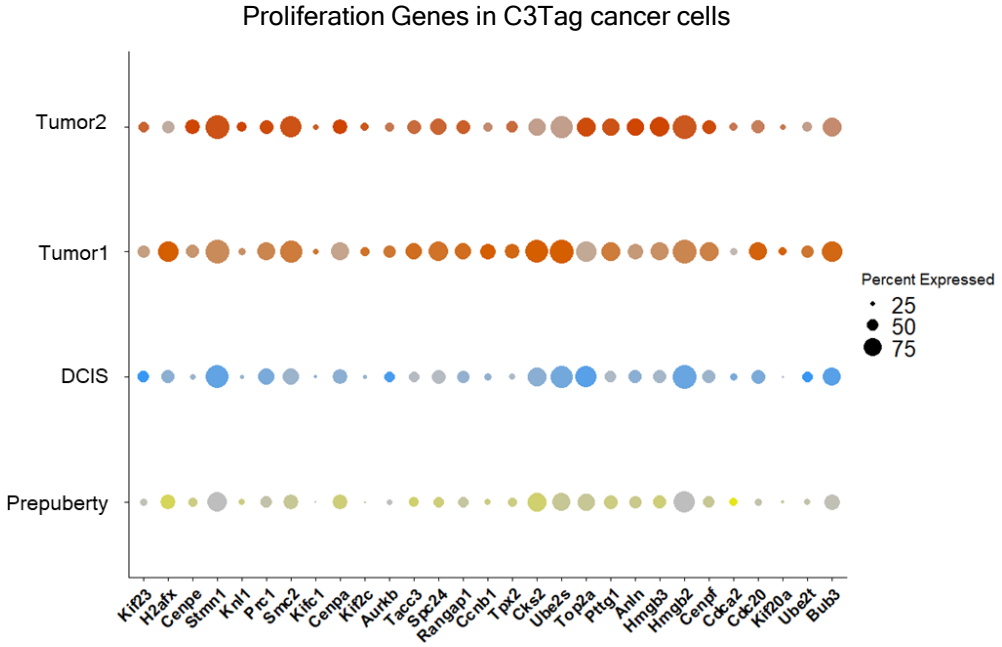



**Supplementary Figure 2. The high CNA+ cells identified from scRNAseq inferred CNA profiles (a-e)** Correlation plots of InferCNV derived values for Prepuberty1, Prepuberty2, DCIS1, DCIS2, Tumor1 and Tumor2. X-axis for individual plot indicates correlation values to the normal (FVB epithelial cells) for all cells of that sample. Y-axis for individual plot indicates correlation values to the top CNA profile of the cells within that sample. Red dots indicate positive expression of the SV40-large-T-antigen. Grey dotted lines indicate the median cutoffs for both correlation scores on x and y-axis indicating the top left quadrant of cells in each individual plot with highest correlation to CNA profile and lowest correlation to the normal cells.

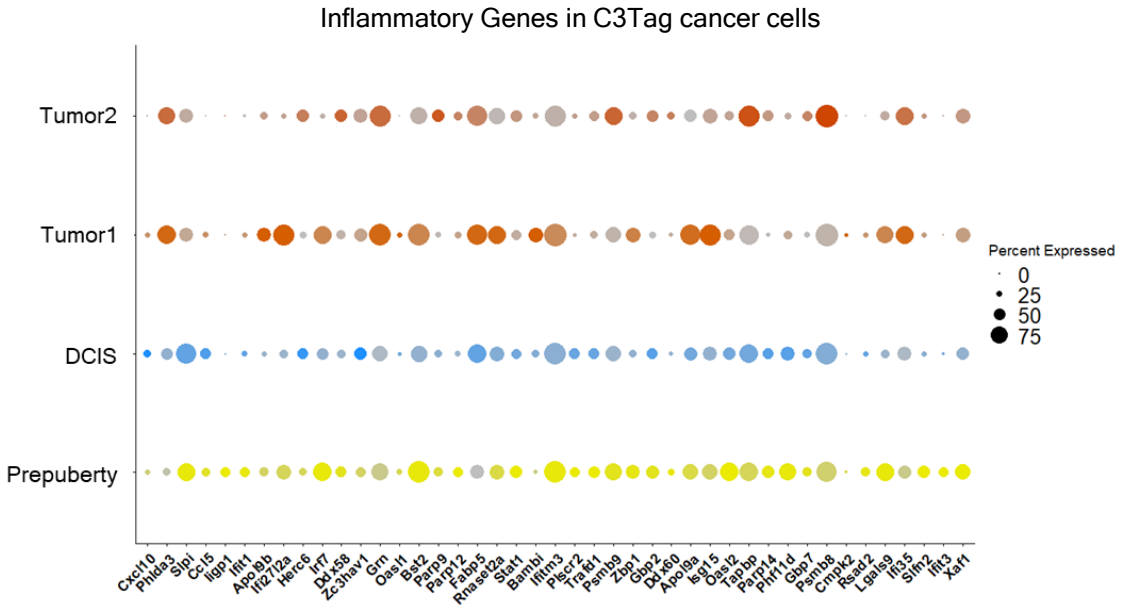



**Supplementary Figure 3. Number of possible clusters in high CNA+ cells.** IKAP generated plot indicating optimal number of clusters in the InferCNV correlation analysis derived 2025 cancer cell single cells**.** B = Best cluster number; X = Next best cluster numbers.

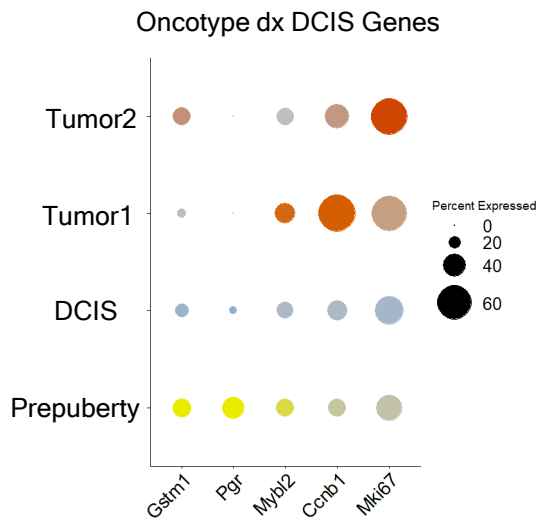
a)


Proliferation Genes in C3Tag cancer cells
Tumor2
Tumor1
DCIS
Prepuberty
Percent Expressed
25
50
75
Kif23
H2afx
Cenpe
Stmn1
Knl1
Prc1
Smc2
Kifc1
Cenpa
Kif2c
Aurkb
Tacc3
Spc24
Rangap1
Ccnb1
Tpx2
Cks2
Ube2s
Top2a
Pttg1
Anln
Hmgb3
Hmgb2
Cenpf
Cdca2
Cdc20
Kif20a
Ube2t
Bub3

b)


Inflammatory Genes in C3Tag cancer cells
Tumor2
Tumor1
DCIS
Prepuberty
Percent Expressed
0
25
50
75
Cxcl10
Phlda3
Slpi
Ccl5
Iigp1
Ifit1
Apol9b
Ifi27l2a
Herc6
Irf7
Ddx58
Zc3hav1
Grn
Oasl1
Bst2
Parp9
Parp12
Fabp5
Rnasel2a
Stat1
Bambi
Ifitm3
Plscr2
Tra1d1
Psmb9
Zbp1
Gbp2
Ddx60
Apol9a
Isg15
Oasl2
Tapbp
Parp14
Phf11d
Gbp7
Psmb8
Cmpk2
Rsad2
Lgals9
Ifi35
Slfn2
Ifit3
Xaf1

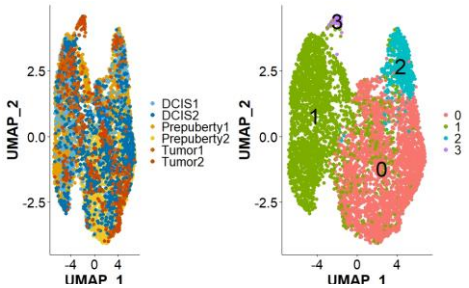
c)


Oncotype dx DCIS Genes
Tumor2
Tumor1
DCIS
Prepuberty
Percent Expressed
0
20
40
60
Gstm1
Pgr
Mybl2
Ccnb1
Mki67

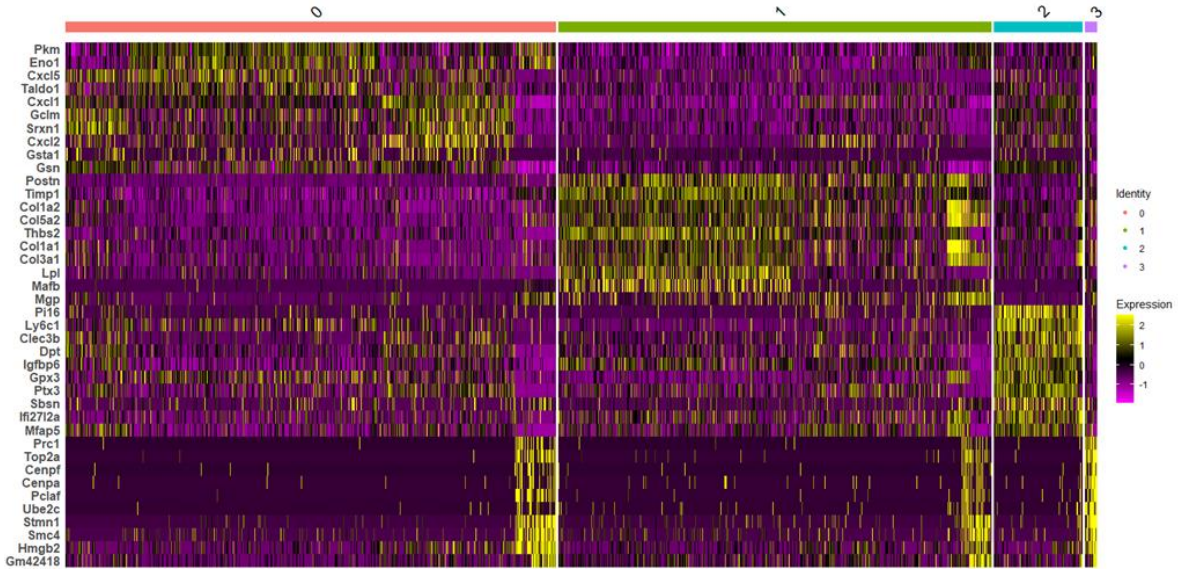
**Supplementary Figure 4. Sustained genes present across the high CNA+ cells across the C3Tag pre-DCIS, DCIS, and Tumor disease states. (a)** Dot plot representation of 30 conserved genes associated with proliferation across the disease states**. (b)** Dot plot representation of all conserved genes associated with pro-inflammatory/interferon signaling across the disease states**. (c)** Dot plot representation of 5/7 of the human Oncotype Dx DCIS genes in the 2025 C3Tag cancer cells**.**

a)

Fibroblasts

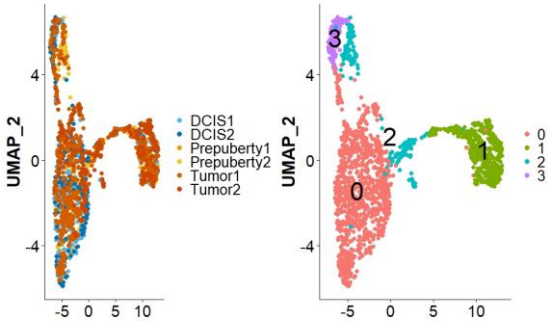



b)

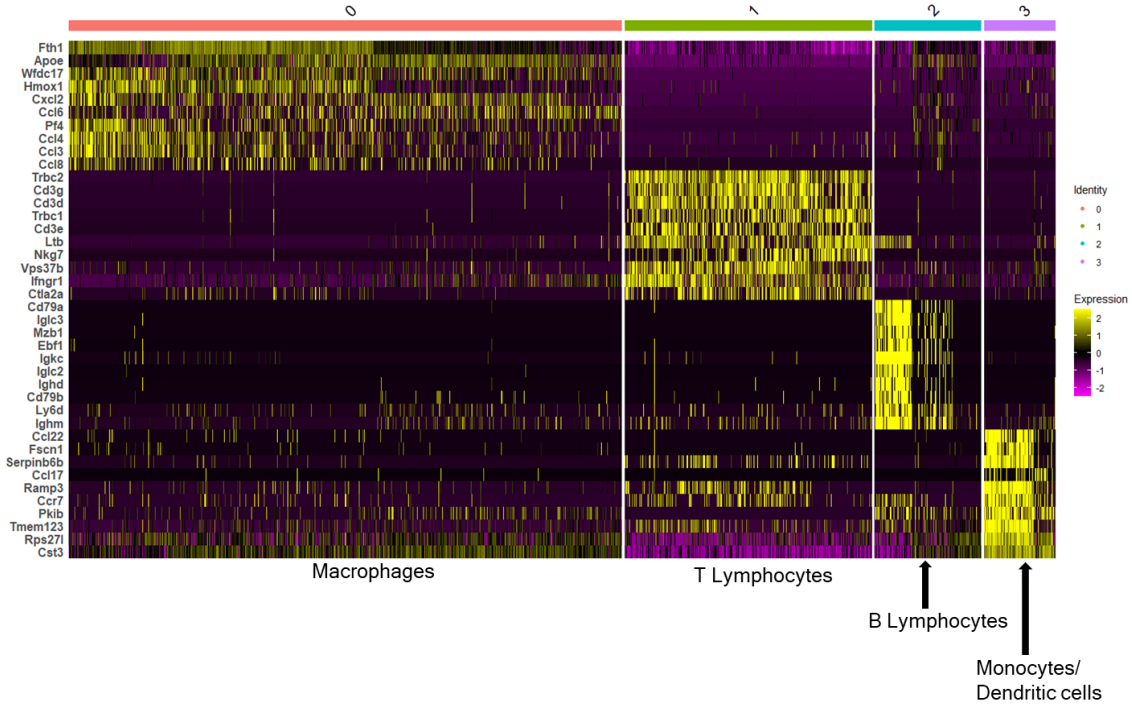



c)

Immune cells



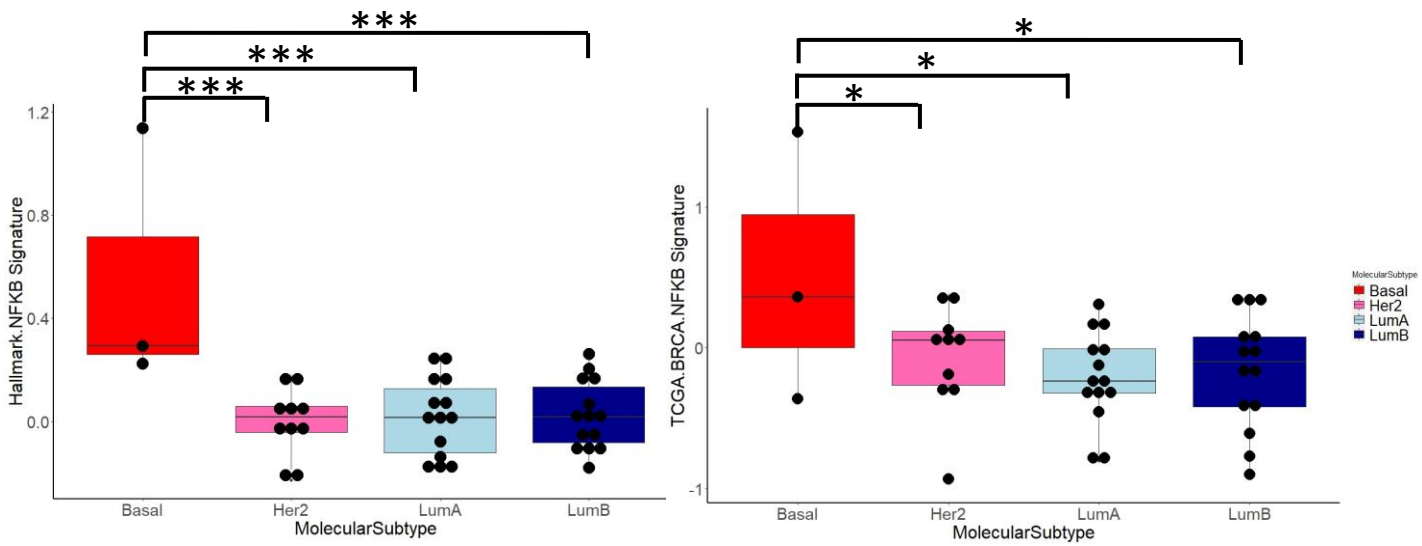

d)



**Supplementary Figure 5. scRNAseq profile of individual analysis on C3Tag fibroblasts and immune cells.** **(a)** UMAP plot of all fibroblasts with RNAseq data colored according to the disease state **(a; Left panel)** and UMAP plot of all fibroblasts according to cell populations identified by IKAP **(a; Right panel). (b)** Heatmap of top 10 significant upregulated genes identified per fibroblast subpopulation by Wilcoxon rank sum test**. (c)** UMAP plot of all Immune cells with RNAseq data colored according to the disease state **(c; Left panel)** and UMAP plot of all Immune cells according to cell populations identified by IKAP **(c; Right panel). (d)** Heatmap of top 10 significant upregulated genes identified per Immune cells subpopulation by Wilcoxon rank sum test**.** Major Immune populations are annotated based on specific gene markers present in the heatmap.

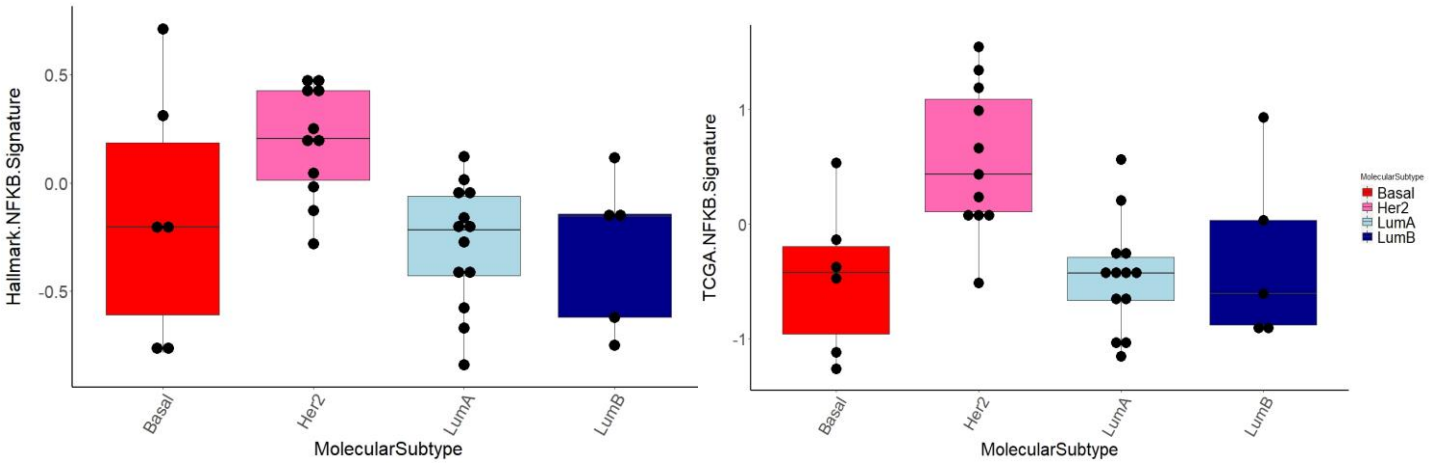
a)

Balleine et al.



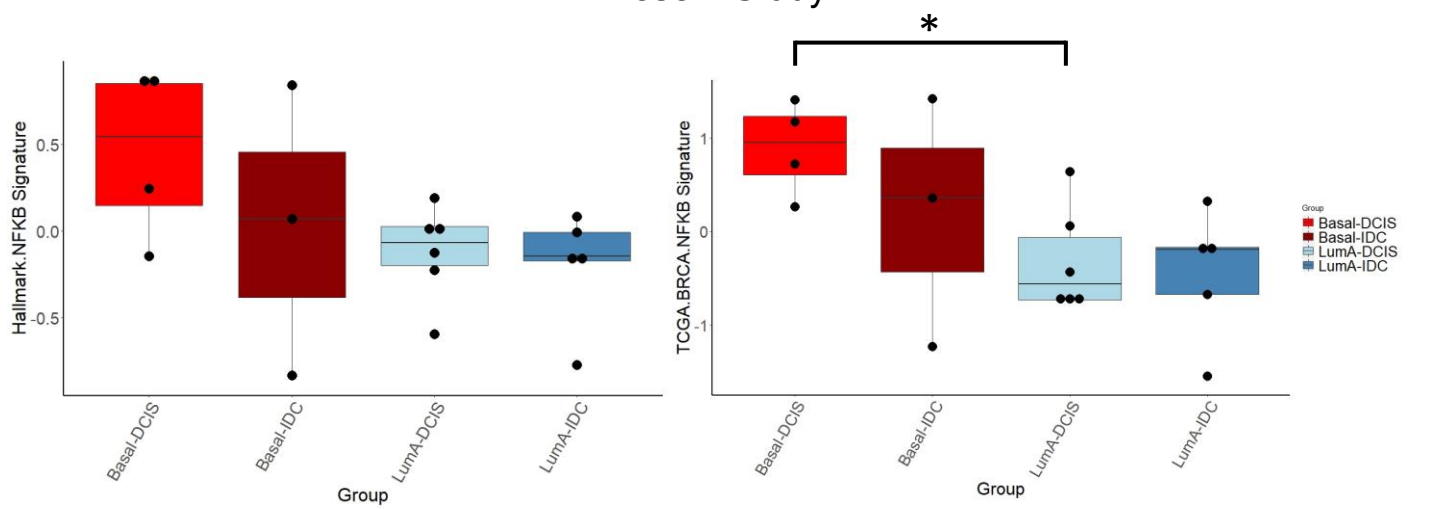

b)

LeSurf et al.




c)

Present Study



**Supplementary Figure 6. NFKB gene signature analysis on human DCIS datasets. (a)** Box and whisker plots of DCIS microarray data from Balleine et al[37] where x axis denotes the PAM50 subtype and y axis shows the hallmark NFKB gene signature (left) and TCGA breast cancer NFKB gene signature (right)**. (b)** Box and whisker plots of DCIS microarray data from LeSurf et al[12] where x axis denotes the PAM50 subtype and y axis shows the hallmark NFKB gene signature (left) and TCGA breast cancer NFKB gene signature (right)**. (c)** Box and whisker plots of Ribo-Zero RNAseq data from human FFPE DCIS-IDC tumor pairs of the present study where x axis denotes the PAM50 subtype and y axis shows the hallmark NFKB gene signature (left) and TCGA breast cancer NFKB gene signature (right). T-test with BH correction was used for all pair-wise comparisons. The upper and lower edges of the boxes represent the upper and lower quartile respectively. The middle line represents the median value. [* = $p<0.05$, ** = $p<0.001$, ***=$p<0.0001$]