

VGLL4 Selectively Represses YAP-Dependent Gene Induction and Tumorigenic Phenotypes in Breast Cancer

Yinglong Zhang^{1,2#}, He Shen^{2#}, Henry G. Withers², Nuo Yang², Kayla E. Denson², Ashley L. Mussell², Alexander Truskinovsky³, Qingyu Fan⁴, Irwin H. Gelman², Costa Frangou^{2,*} and Jianmin Zhang^{2,*}

¹ Department of Orthopedics, the First Affiliated Hospital of Chinese People's liberate Army General Hospital, Beijing, 100048, China

² Department of Cancer Genetics & Genomics,

³ Department of Pathology, Roswell Park Cancer Institute, Buffalo, NY 14263, USA

⁴ Orthopedic Oncology Institute, Tangdu Hospital, Fourth Military Medical University, Xi'an, Shaanxi 710038, China

*To whom correspondence should be addressed. Email: gus.frangou@RoswellPark.org;

jianmin.zhang@roswellpark.org

these authors contributed equally to this work

Supplementary information

Supplementary figure legends

Supplemental Table 1. Univariate Cox Regression analysis of VGLL4 gene expression and breast cancer patient survival.

Supplemental Table 2. VGLL4 regulated pathways.

Supplemental Table 3. VGLL4 regulated GO process.

Supplemental Figure Legends

Figure S1. VGLL1-4 gene expression and correlations with mutation status and clinical breast cancer features.

- (a) Comparison of VGLL1-4 gene expression, mutation frequency and copy number alterations.
- (b) The relationship between VGLL1-4 gene expression and tumor stage.

Figure S2. Heat map analysis of VGLL1-4 and TEAD1-4 gene expression in a panel of breast cancer cell lines.

- (a) VGLL1-4 relative quantities by qPCR.
- (b) TEAD1-4 relative quantities by qPCR from a panel of breast cancer cell lines. Each row shows expression of a single gene and colored according to the fold change relative to the median. Cell line names are labeled on the right.

Figure S3. VGLL4 overexpression inhibits the proliferation, colony-formation and transformation abilities of breast cancer cells *in vitro*.

- (a) Cell proliferation by the MTT assay for vector- or VGLL4-transduced CAL-51 and CAL-120 cells.

Representative images of colony formation (b) and anchorage-independent growth in soft agar

- (c) for vector- or VGLL4-transduced CAL-51 and CAL-120 cells.

Figure S4. Knockdown of YAP inhibits breast cancer cell proliferation.

Representative images of colony formation (a), anchorage-independent growth in soft agar (b) for shGFP- or shYAP-transduced CAL-51 and CAL-120 cells.

Figure S5. Transcriptome profiling of the VGLL4 domain mutants in CAL-51 cells.

(a) Heat map generated from the *VGLL4* transcriptome data reflecting gene expression values.

Fold-change relative to control cells rendered in a blue–white–yellow pseudo color scheme for all genes with $P < 0.005$ and $FC_{\log 2} > 1.5$ or $FC_{\log 2} < -1.5$.

(b) Venn diagram drawn by the VennPlex program summarizing gene overlap between VGLL4 and TDU domain mutants. The numbers in the 4-set Venn diagram indicate the output set orders in the resultant extracted data panel. To indicate the polarity of numerical regulation of each factor within each set or intersection, a two or three-way key system was employed. The number of factors unique to a set, with a positive regulation polarity, is identified with an italic numeral. The number of factors unique to a set, with a negative regulation polarity, is identified with an underlined numeral. The number of factors common between multiple sets is indicated with a red colored numeral.

(c) Heat map for GSEA result analysis to assess enrichment of YAP gene expression signature (CORDENONSI_YAP_CONSERVED_SIGNATURE). Bar color reflects gene expression fold change from blue (low), white (medium) to yellow (high).

Figure S6. Cellular viability of breast cancer cells expressing TET-inducible VGLL4.

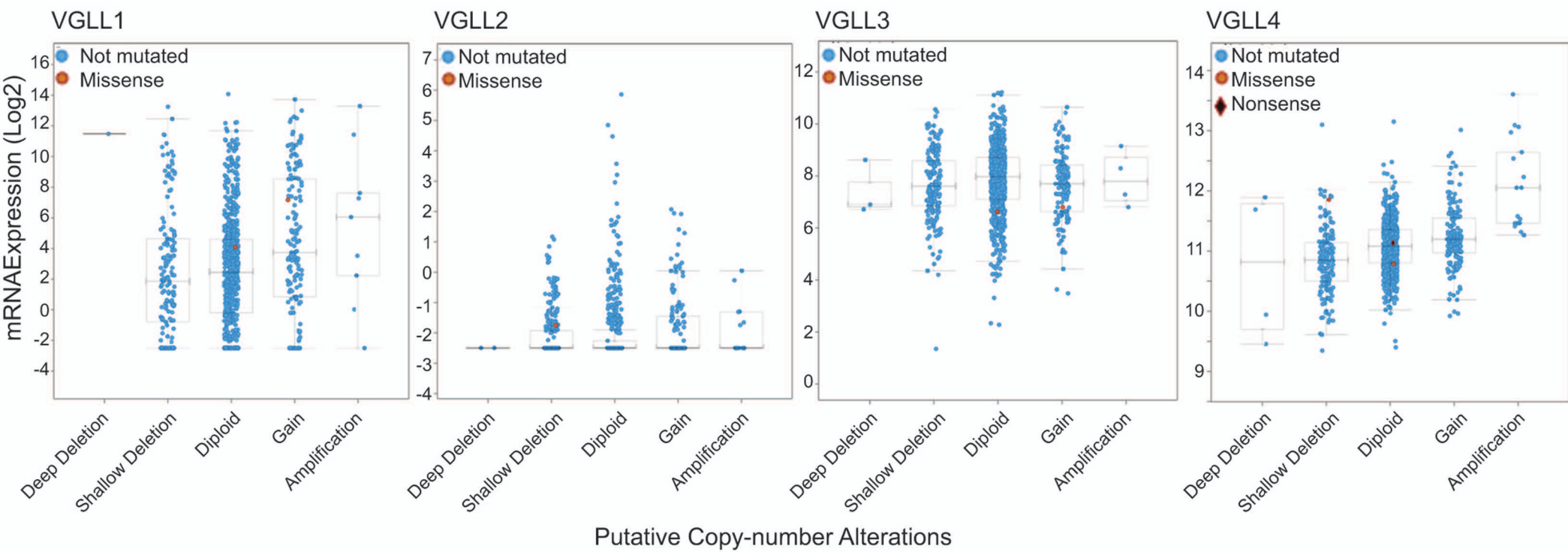
(a) List of a panel of breast cancer cell lines with different genetic lesions.

(b) Cellular viability measured 1, 5 and 9 days post VGLL4 induction by doxycycline in a panel of breast cancer cell lines using CellTiter-Glo (Promega). All error bars represent SD, $n=3$. (***) $p < 0.001$)

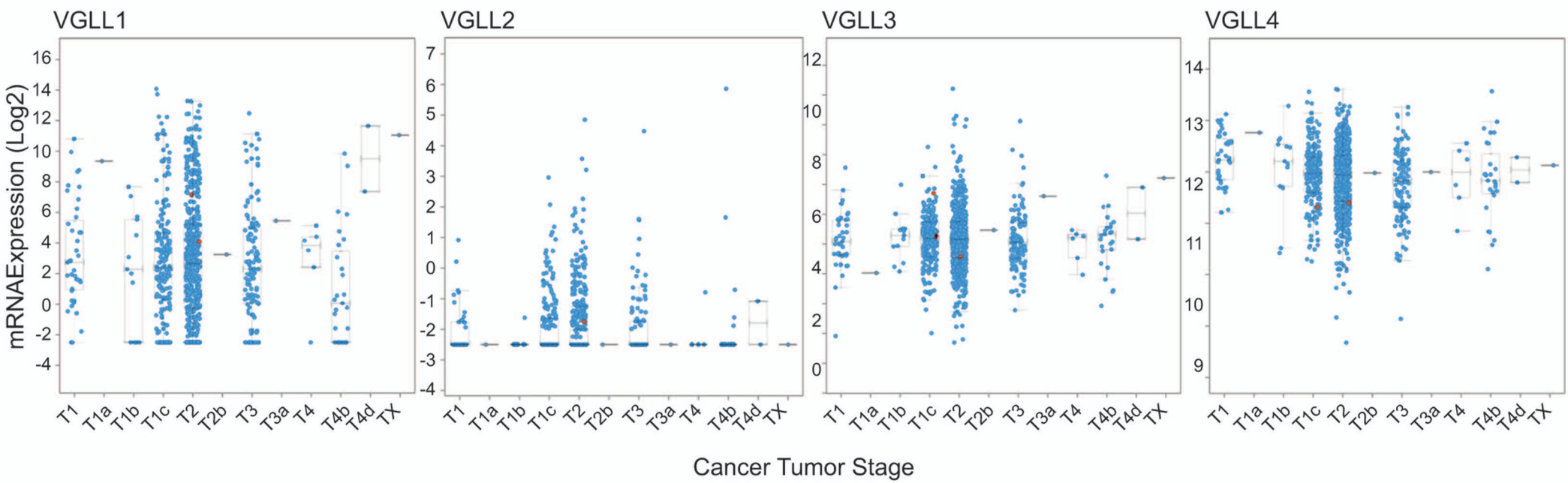
Figure S7. TDU2 domain deletion rescues the effect of VGLL4 suppression on cell proliferation and colony-formation *in vitro*.

Quantification of colony-formation assay (a), anchorage-independent growth in soft agar (b) and cell migration (c) for vector, WT or mutant VGLL4 transduced CAL-51 cells. (*P < 0.05, **P<0.01, ***P<0.001)

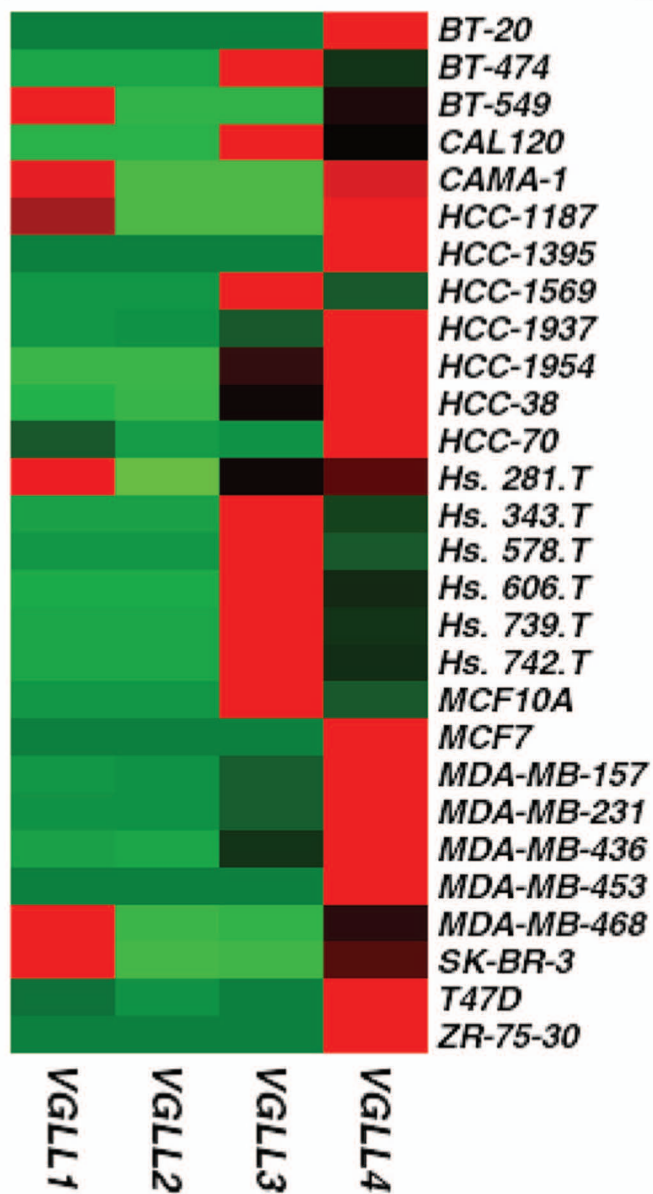
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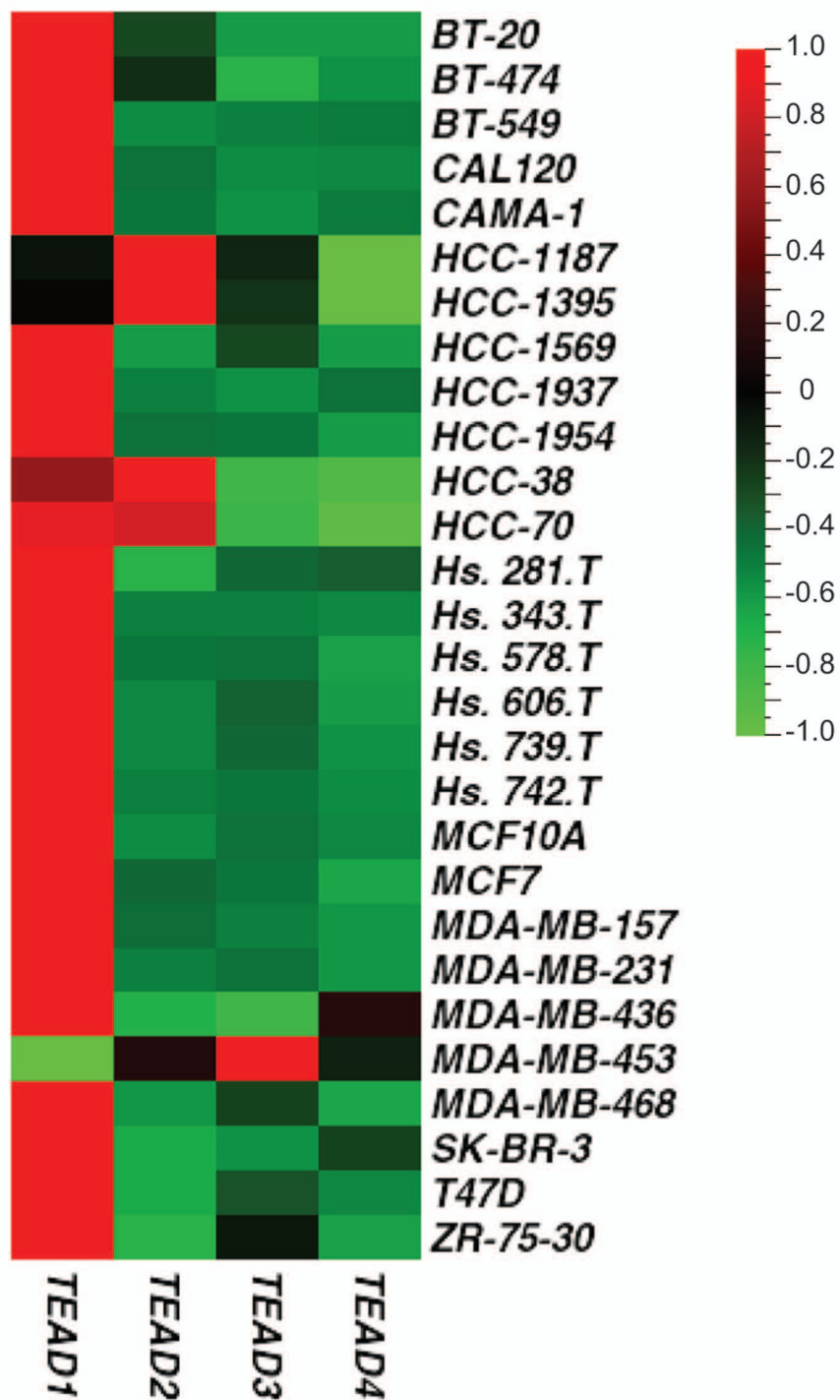
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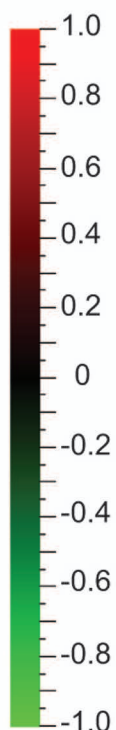
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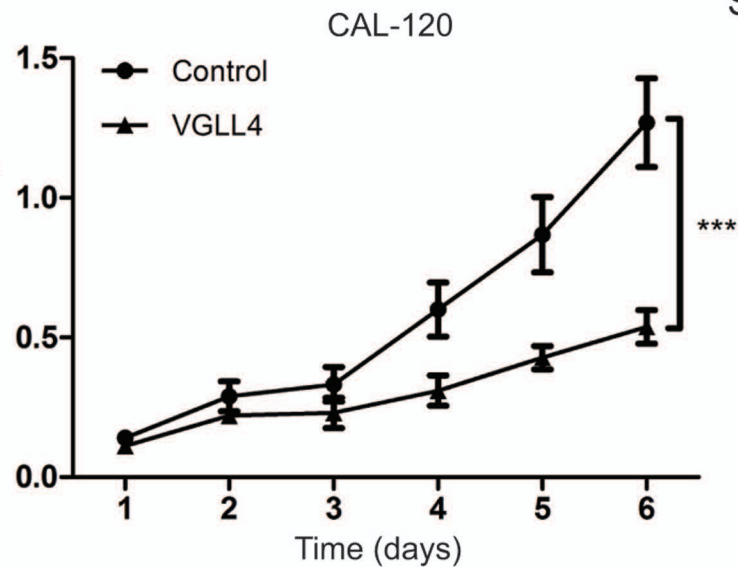
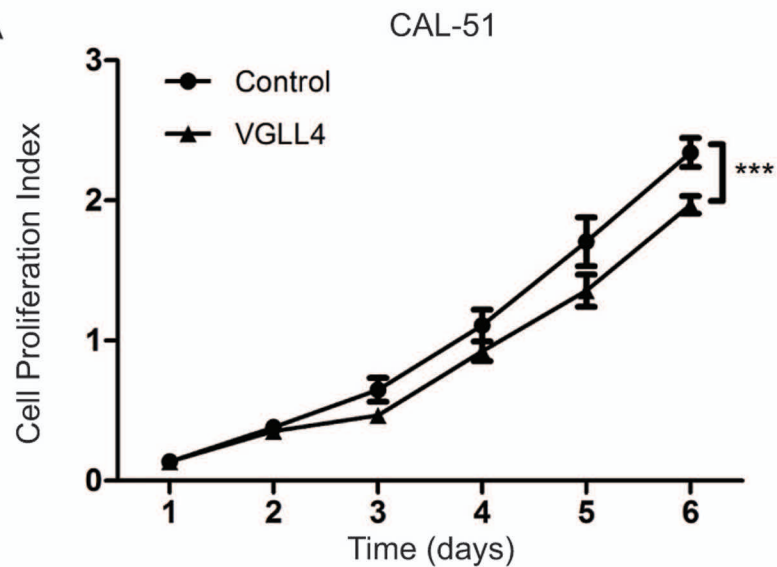
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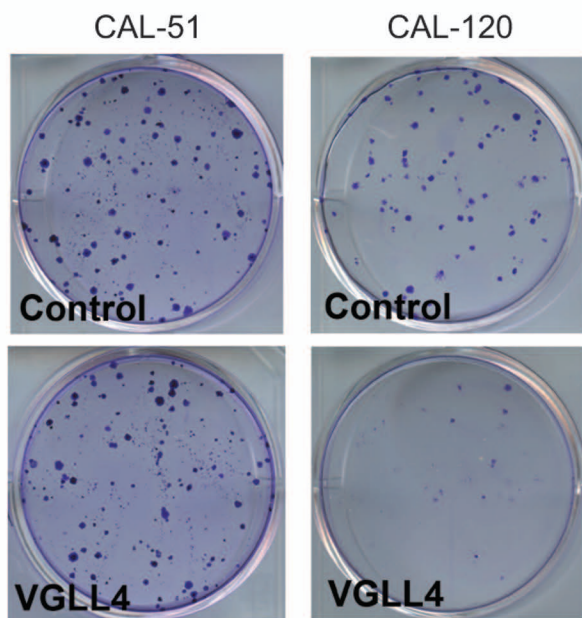
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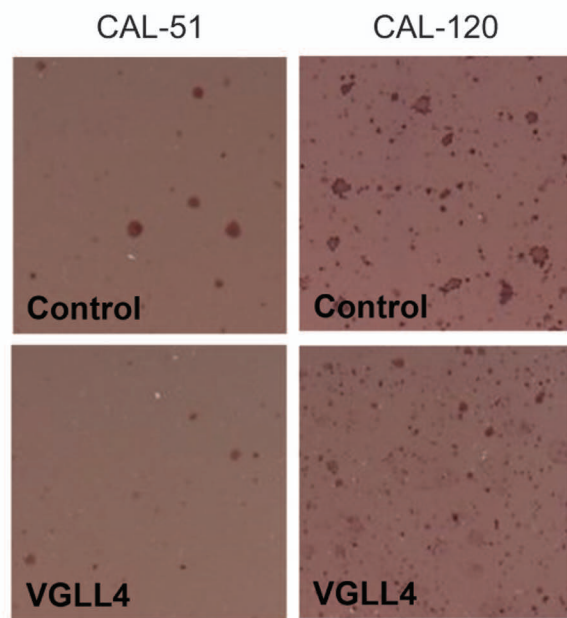
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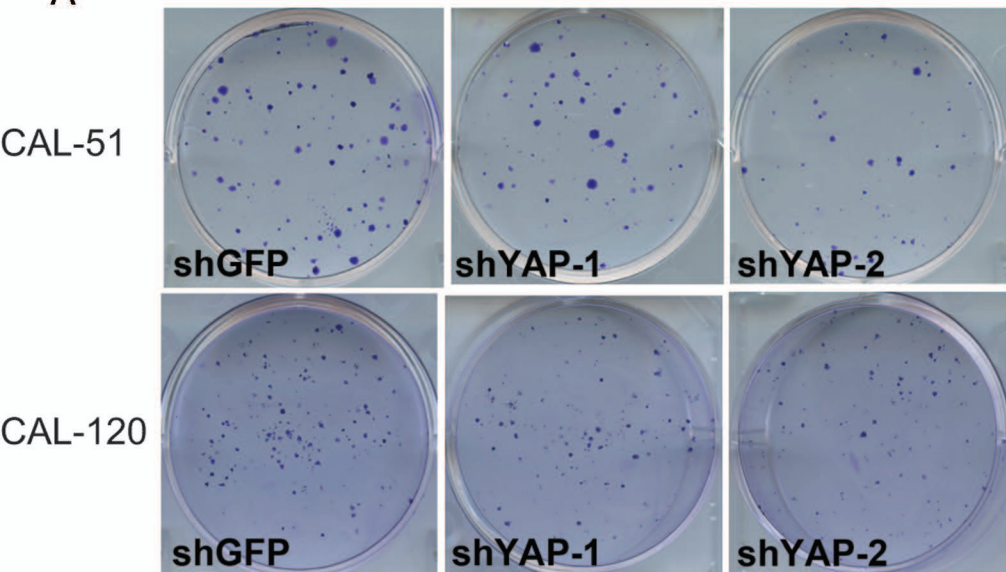
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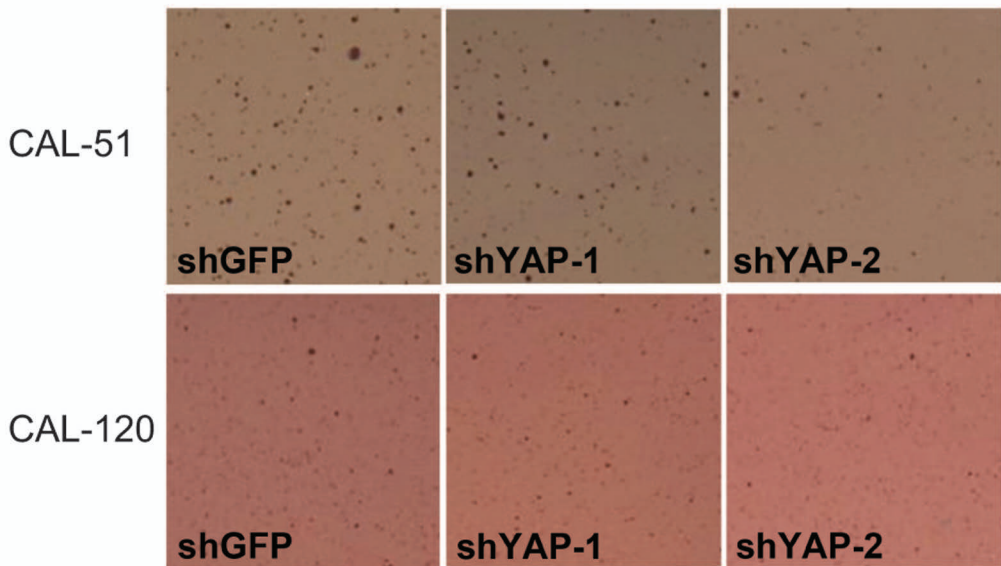
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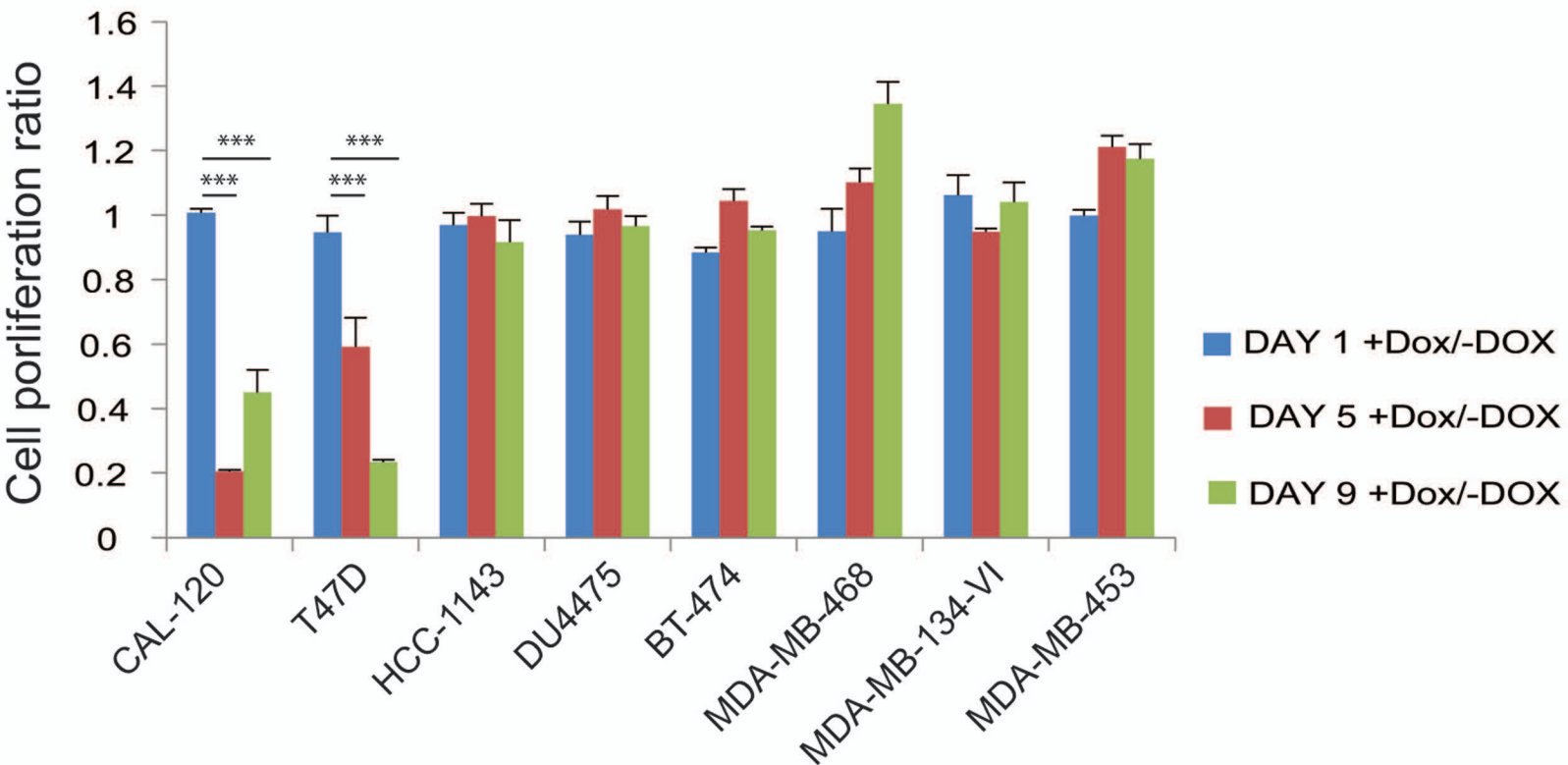
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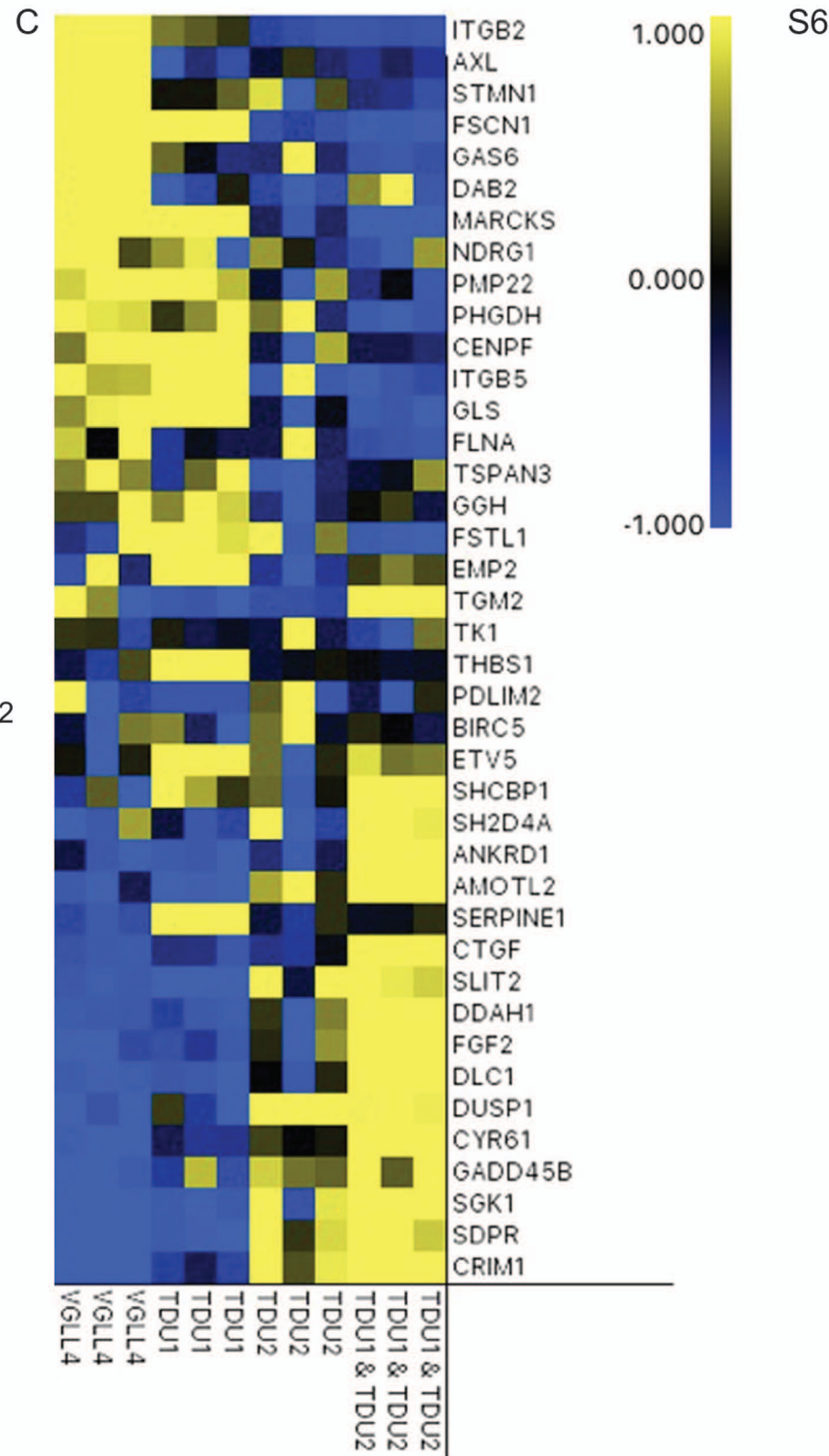
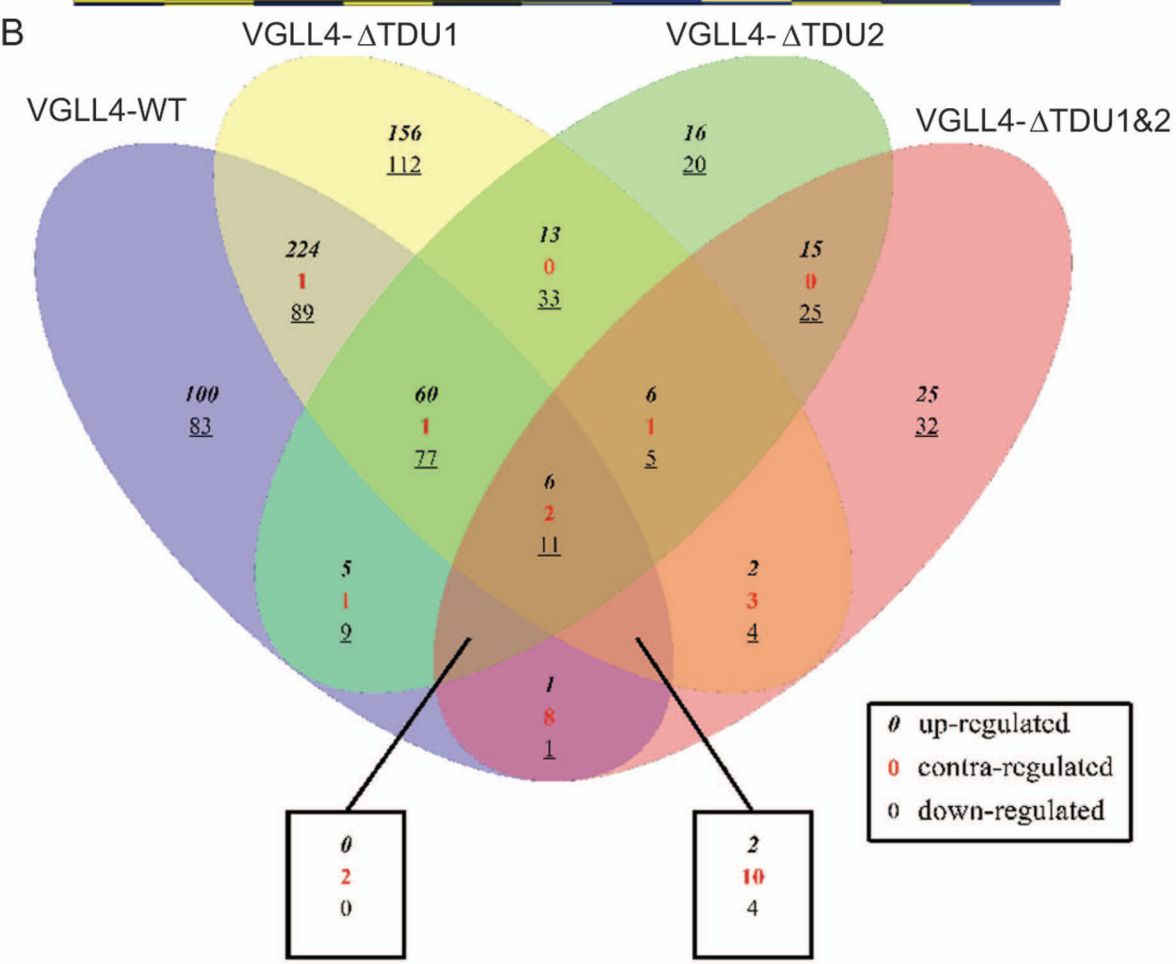
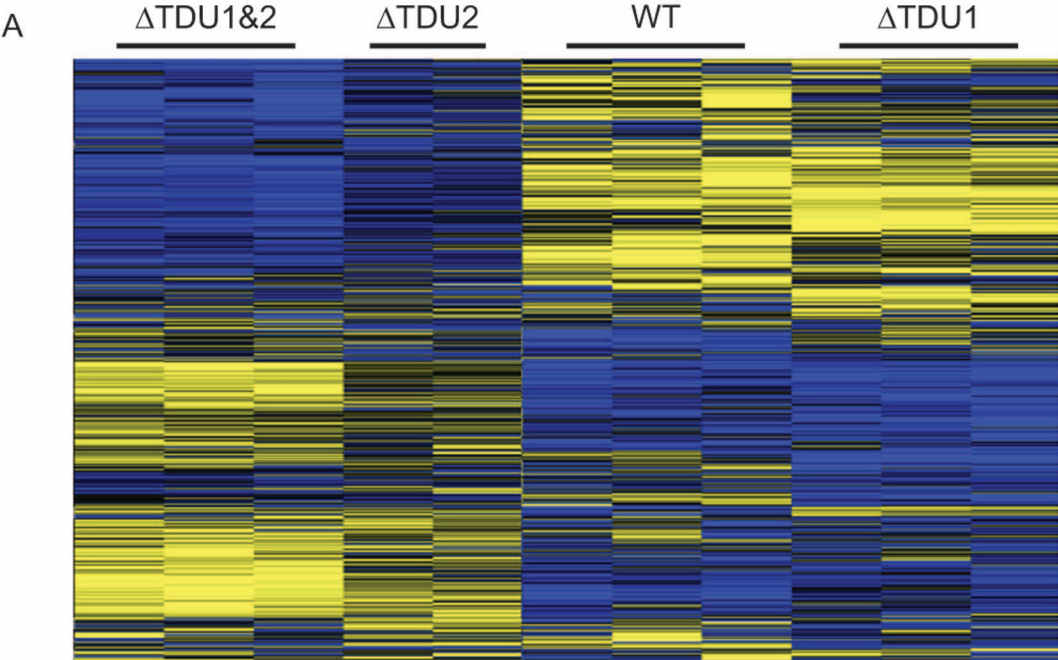


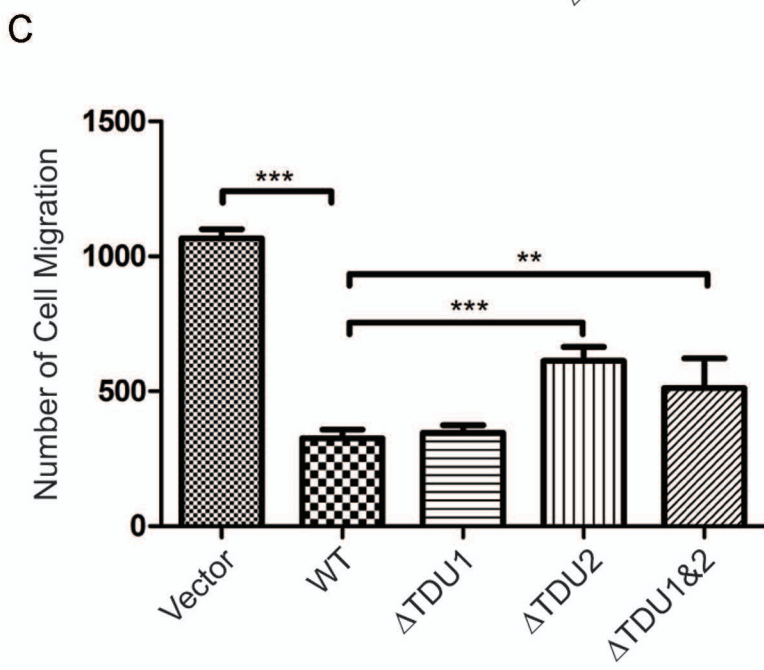
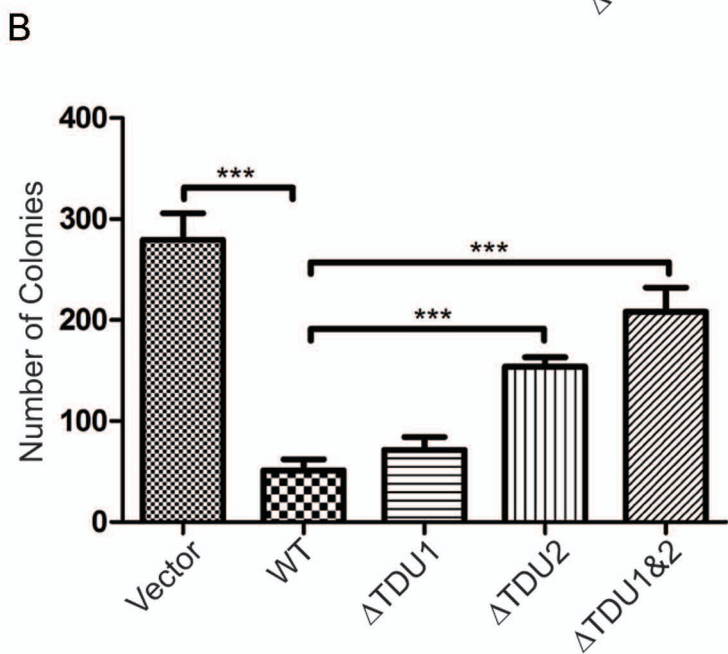
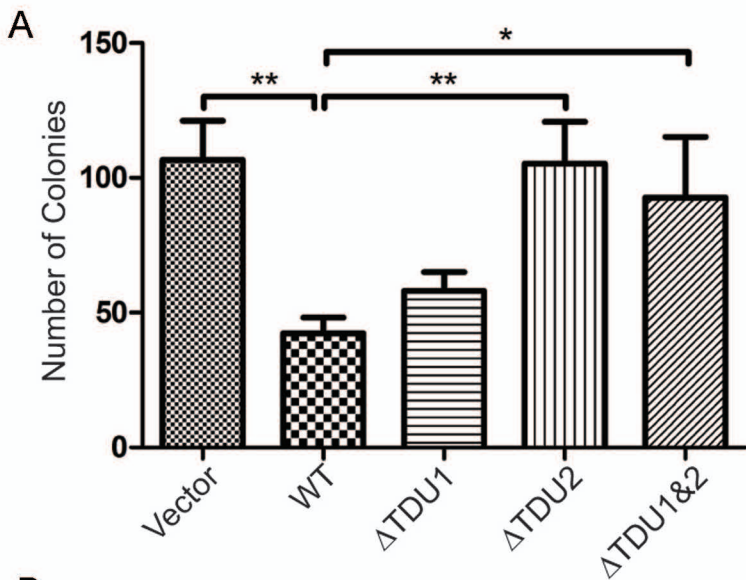
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Cell line	Genetic alterations	Tumor source
CAL-120	YAP hyperactivation	Breast
T47D	YAP hyperactivation	Breast
HCC1143	AKT1 amplification	Breast
DU4475	BRAF mutation (V600E)	Breast
MDA-MB-468	EGFR amplification	Breast
BT-474	ERBB2 amplification	Breast
MDA-MB-134-VI	FGFR1 amplification	Breast
MDA-MB-453	PIK3CA mutation (H1047R)	Breast

B







Supplemental table 1. Univariate Cox Regression analysis of VGLL4 gene expression and breast cancer patient survival.

GO	SAMPLES		HAZARD RATIO	LCI (95%)	UCI (95%)	p VALUE
	VGLL4- High	VGLL4- Low				
GSE3143	79	78	0.44	0.23	0.81	0.009
GSE6130	17	16	0.05	0	0.78	0.032
GSE1456	80	79	0.57	0.36	0.91	0.018
GSE22226	64	64	0.45	0.25	0.81	0.008