

Cell Reports, Volume 42

Supplemental information

**DUX4 expression in cancer induces
a metastable early embryonic totipotent program**

Andrew A. Smith, Yee Nip, Sean R. Bennett, Danielle C. Hamm, Richard J.L.F. Lemmers, Patrick J. van der Vliet, Manu Setty, Silvère M. van der Maarel, and Stephen J. Tapscott

Figure S1

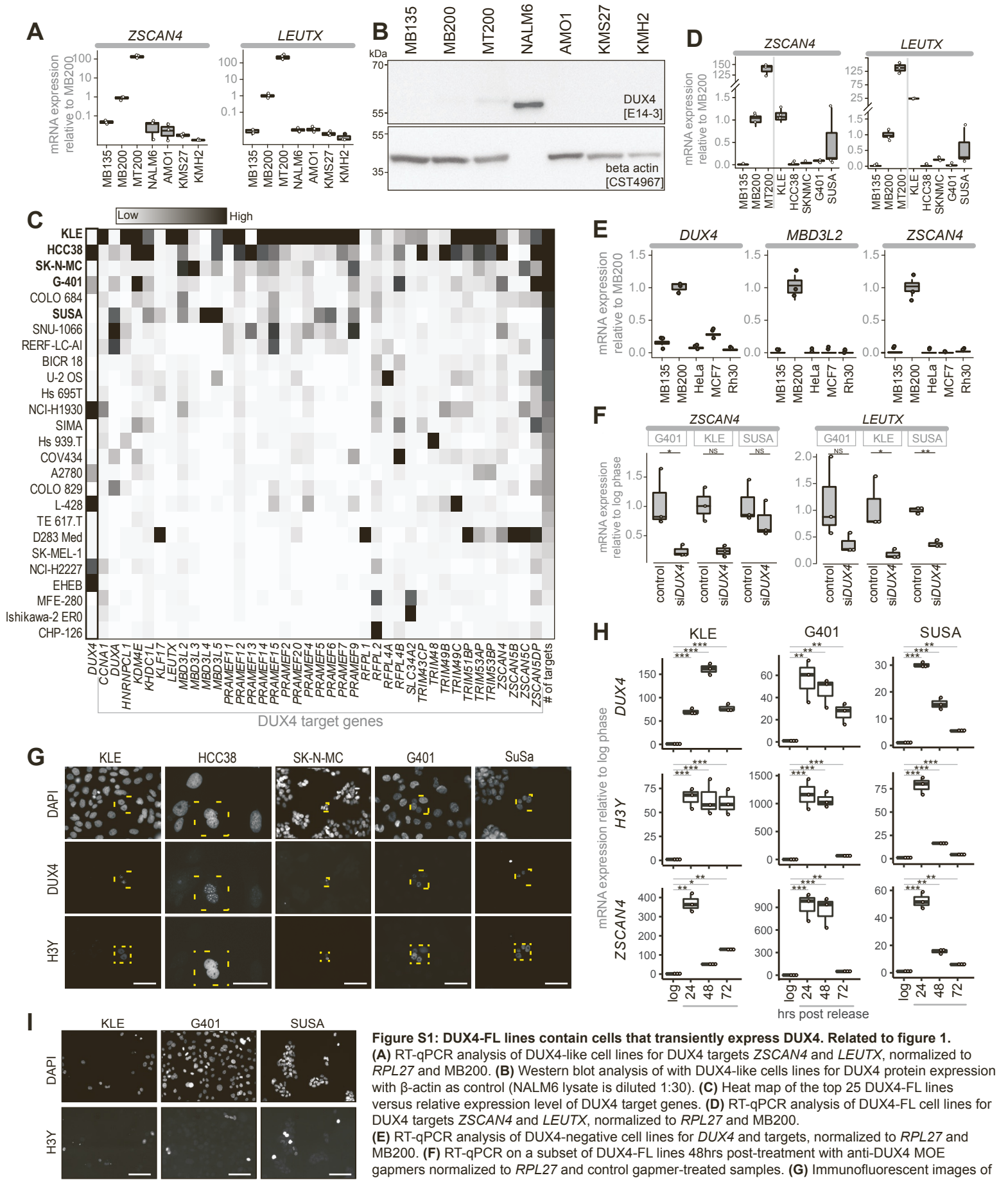


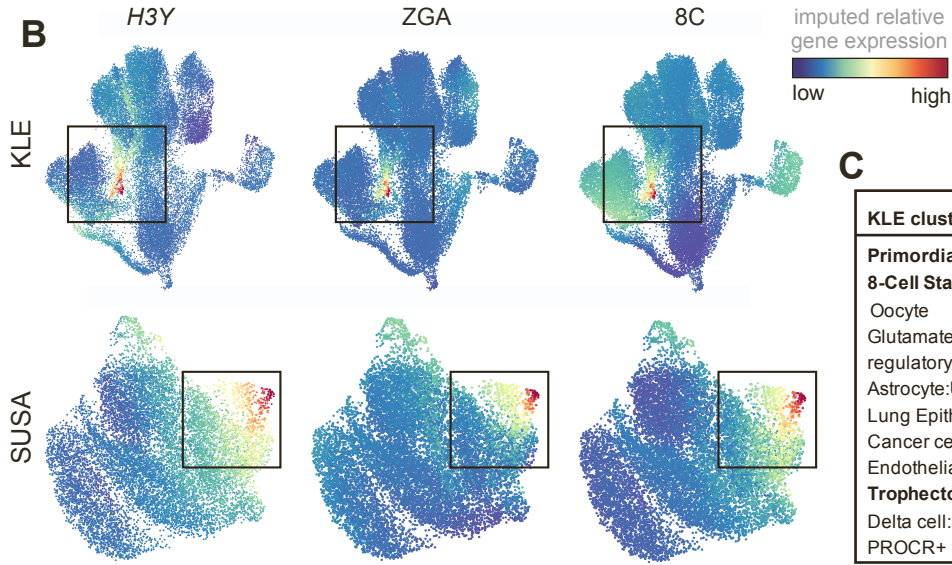
Figure S1: DUX4-FL lines contain cells that transiently express DUX4. Related to figure 1.
(A) RT-qPCR analysis of DUX4-like cell lines for DUX4 targets *ZSCAN4* and *LEUTX*, normalized to *RPL27* and MB200. **(B)** Western blot analysis of with DUX4-like cells lines for DUX4 protein expression with β -actin as control (NALM6 lysate is diluted 1:30). **(C)** Heat map of the top 25 DUX4-FL lines versus relative expression level of DUX4 target genes. **(D)** RT-qPCR analysis of DUX4-FL cell lines for DUX4 targets *ZSCAN4* and *LEUTX*, normalized to *RPL27* and MB200. **(E)** RT-qPCR analysis of DUX4-negative cell lines for *DUX4* and targets, normalized to *RPL27* and MB200. **(F)** RT-qPCR on a subset of DUX4-FL lines 48hrs post-treatment with anti-DUX4 MOE gupmers normalized to *RPL27* and control gupmer-treated samples. **(G)** Immunofluorescent images of DUX4-FL lines stained for DUX4 [E14-3] and H3Y [6H8-2111] showing a larger field of the images in main Figure 1D; yellow dashed line = cropped region. **(H)** RTqPCR on a subset of DUX4-FL lines after release from confluence. **(I)** Immunofluorescence for H3Y in DUX4-FL lines post-release from a confluent state. Scale bars = 200uM. For all RTqPCR experiments, n=3 biological replicates. P-values: * < 0.05; **<0.005, ***<0.0005, NS = no significance, calculated with one-tailed Welch's t-tests..

Figure S2

A

	n_cells	n_genes	n_counts	total_counts_mt	pct_counts_mt	total_counts_ribo
KLE_Log_Rep1	5356.00	5166.93	34940.29	1551.58	4.28	7625.58
KLE_24hr_Rep1	5931.00	4248.62	20620.63	1013.40	5.36	3451.28
KLE_48hr_Rep1	9837.00	3674.60	14841.59	624.79	4.50	2441.77
KLE_72hr_Rep1	7839.00	3493.04	15343.70	762.26	4.97	2596.92
SuSa_Log_Rep1	3573.00	4516.80	28812.00	1737.70	5.71	7513.20
SuSa_24hr_Rep1	3531.00	3189.04	15931.64	589.33	5.91	4021.80
SuSa_48hr_Rep1	2814.00	3791.63	26066.28	1358.23	4.73	6310.09
SuSa_72hr_Rep1	3304.00	3994.35	20853.99	1497.83	6.57	4808.30

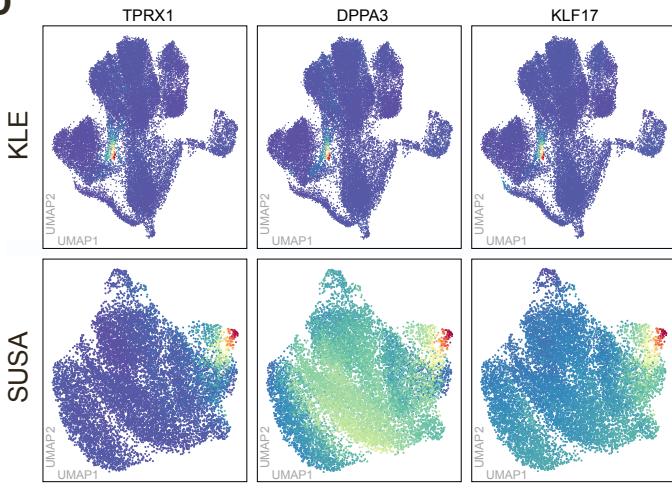
B



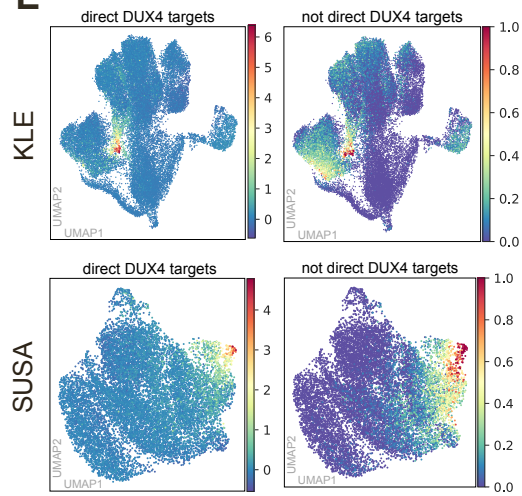
C

CellMarker_Augmented_2021	
KLE cluster 14 upregulated	SUSA cluster 16 upregulated
Primordial Germ cell	Epithelial cell:Corneal Epithelium
8-Cell Stage Cell (Blastomere): Embryo	Astrocyte:Brain
Oocyte	Microglial cell:Embryonic Prefontal cortex
Glutamatergic neuron:Undefined	Monocyte:Fetal Kidney
regulatory T (Treg) cell	Natural Killer (T) cell:Fetal Kidney
Astrocyte:Undefined	Dicidual cell
Lung Epithelial cell:Lung	Pancreatic Polypeptide cell
Cancer cell:undefined	8-Cell Stage Cell (Blastomere)
Endothelial cell:Fetal gonad	Brush Cell (Tuft cell)
Trophectoderm cell: Embryo	Neuroendocrine cell
Delta cell:Pancreas	Mitotic Arrest Phase Fetal Germ cell
PROCR+ Progenitor cell	Primordial Germ Cell

D



E



F

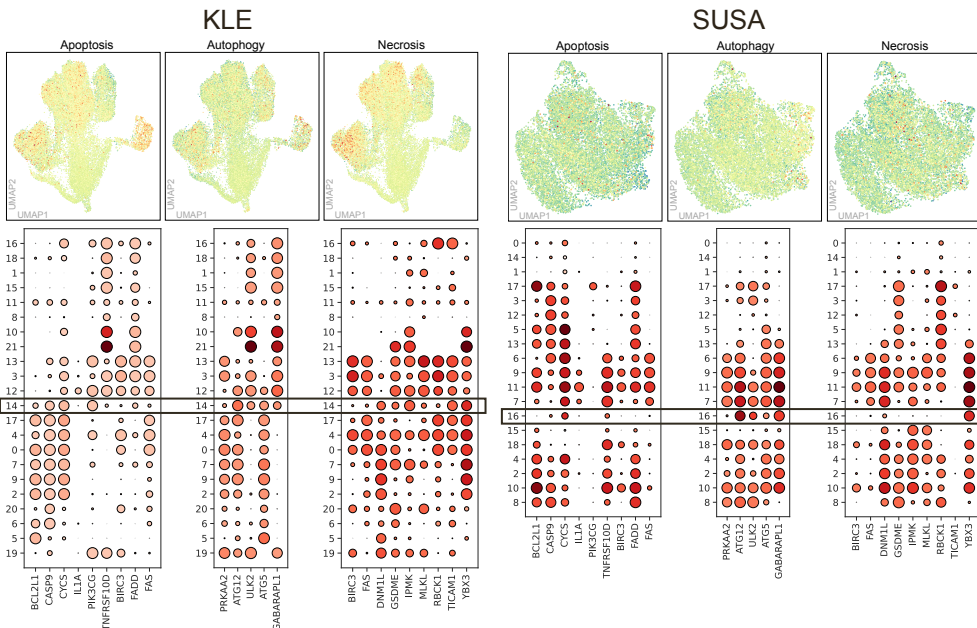


Figure S2: DUX4 activates ZGA and 8CLC programs, but does not affect cell death. Related to Figure 2.

(A) Mean single-cell RNA-sequencing cell counts processed with ScanPy.

(B) Full UMAPs indicated that are shown in Fig 2B.

(C) Most significant EnrichR Cell Marker expression patterns for KLE and SUSa.

(D) Single-cell RNA-seq as UMAPs demonstrating relative expression of indicated 8C genes.

(E) Single cell RNA-sequencing UMAPs demonstrating relative expression of 8CLC gene identified as direct targets of DUX4 or as not direct targets of DUX4.

(F) Single-cell RNA-seq as UMAPs and dot plots of relative expression of composite scores for the indicated cell death pathways.

Figure S3

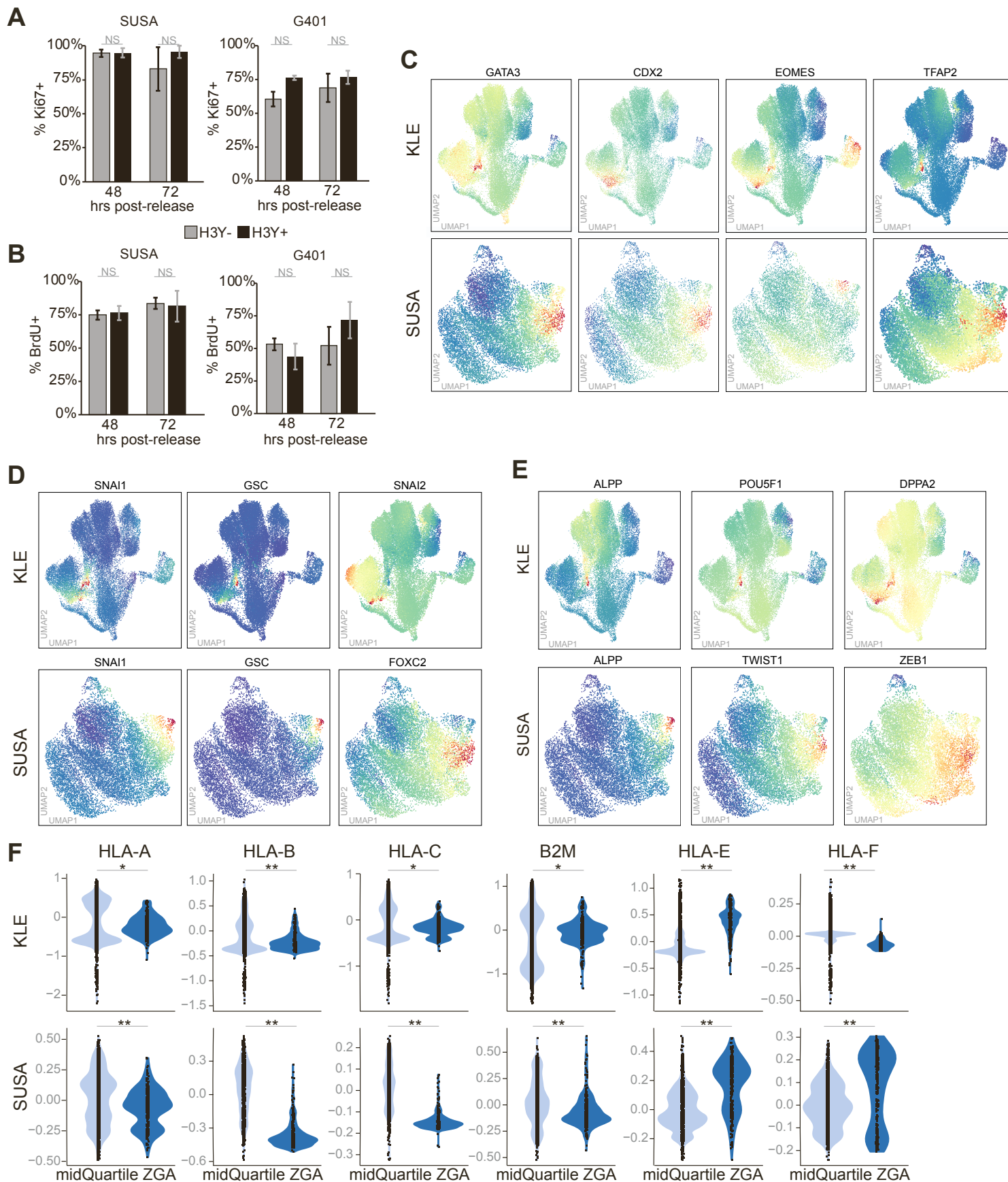


Figure S3: DUX4 does not affect cell division, but activates early embryonic genes and suppresses MHC-I. Related to Figure 2.
(A-B) Immunofluorescent quantification of (A) Ki67+ or (B) BrdU+ cells after release from confluence, n= 3 separate experiments; >240 cells per timepoint; expressed as mean +SD.
(C-E) Single-cell RNA-seq as UMAPs of relative expression of composite scores for the indicated genes.
(F) Violin plots comparing HLA gene expression in the mid quartile or top 350 cells based on imputed ZGA gene composite scores.
P-values: * < 0.05; **<0.005, ***<0.0005, NS = no significance, calculated with a one-tailed welch's t-test.

Figure S4

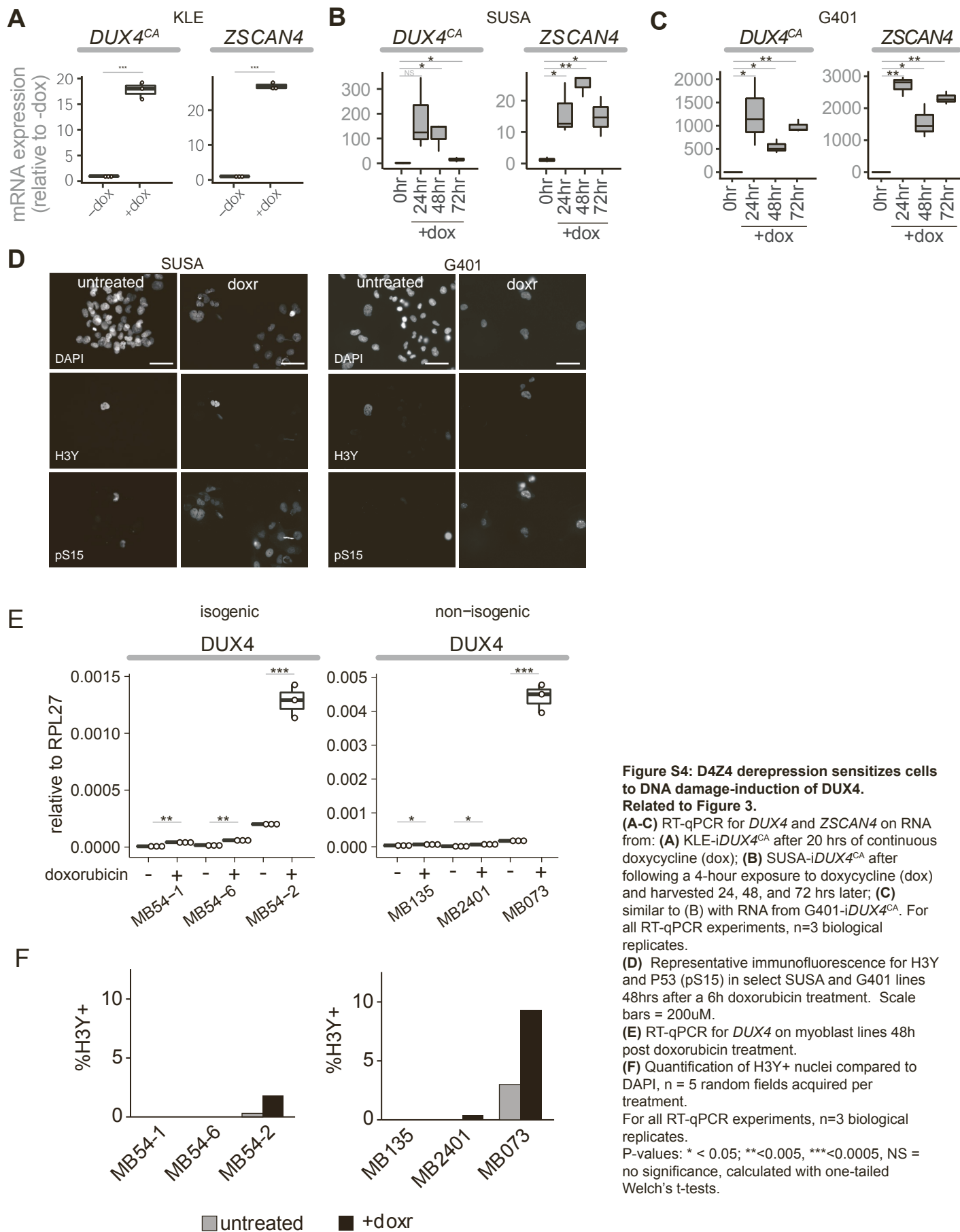


Figure S4: D4Z4 derepression sensitizes cells to DNA damage-induction of DUX4. Related to Figure 3.

(A-C) RT-qPCR for *DUX4* and *ZSCAN4* on RNA from: (A) KLE-*iDUX4^{CA}* after 20 hrs of continuous doxycycline (dox); (B) SUSAs-*iDUX4^{CA}* after following a 4-hour exposure to doxycycline (dox) and harvested 24, 48, and 72 hrs later; (C) similar to (B) with RNA from G401-*iDUX4^{CA}*. For all RT-qPCR experiments, n=3 biological replicates.

(D) Representative immunofluorescence for H3Y and P53 (pS15) in select SUSAs and G401 lines 48hrs after a 6h doxorubicin treatment. Scale bars = 200uM.

(E) RT-qPCR for *DUX4* on myoblast lines 48h post doxorubicin treatment.

(F) Quantification of H3Y+ nuclei compared to DAPI, n = 5 random fields acquired per treatment.

For all RT-qPCR experiments, n=3 biological replicates.

P-values: * < 0.05; ** < 0.005, *** < 0.0005, NS = no significance, calculated with one-tailed Welch's t-tests.