Supplemental Table 1. Oligonucleotide sequence of RT-qPCR primers.

Gene	sequence (5' to 3')
S100A10	Forward: GGCTACTTAACAAAGGAGGACC
	Reverse: GAGGCCCGCAATTAGGGAAA
S100a10 (mouse)	Forward: TGGAAACCATGATGCTTACGTT
	Reverse: GAAGCCCACTTTGCCATCTC
NANOG	Forward: TTTGTGGGCCTGAAGAAAACT
	Reverse: AGGGCTGTCCTGAATAAGCAG
SOX2	Forward: GCCGAGTGGAAACTTTTGTCG
	Reverse: GGCAGCGTGTACTTATCCTTCT
KLF4	Forward: CGGACATCAACGACGTGAG
	Reverse: GACGCCTTCAGCACGAACT
POU5F1	Forward: TTCAGCCAAACGACCATCTG
	Reverse: CACGAGGGTTTCTGCTTTGC
ANXA2	Forward: TCTACTGTTCACGAAATCCTGTG
	Reverse: AGTATAGGCTTTGACAGACCCAT
SUPT6H	Forward: GGATGAGCAAGGCAACTTGAA
	Reverse: CACGCCGGTACTTTTGTCCT
KDM6A	Forward: TTCCTCGGAAGGTGCTATTCA
	Reverse: GAGGCTGGTTGCAGGATTCA
18S	Forward: CGGCGACGACCCATTCGAAC
	Reverse: GAATCGAACCCTGATTCCCCGTC

Supplemental Table 2. Primary antibody information for immunoblot assays.

Antibody	Manufacture	Catalog #
HIF-1α	BD Biosciences	610959
S100A10	Novus Biologicals	AF2377
ANXA2	Novus Biologicals	NBP1-31310
SPT6	Novus Biologicals	NB100-2582
KDM6A	Novus Biologicals	NBP1-80628
KDM6B	Novus Biologicals	NBP1-06640
NANOG	Novus Biologicals	AF1997
SOX2	Novus Biologicals	NB110-37235
OCT4	Novus Biologicals	NB100-2379
KLF4	Novus Biologicals	NBP1-83940
α-TUBULIN	Novus Biologicals	NB100-690
H3K27me3	Novus Biologicals	NBP2-59206
HISTONE H3	Novus Biologicals	NB500-171
ACTIN	Santa Cruz	SC-47778

Supplemental Table 3. Antibody information for co-immunoprecipitation assays.

Antibody	Manufacturer	Catalog #
S100A10	Novus Biologicals	NBP1-89370
ANXA2	Novus Biologicals	NBP1-31310
SPT6	Novus Biologicals	NB100-2582
KDM6A	Novus Biologicals	NBP1-80628

Supplemental Table 4. shRNA information.

shRNA	Nucleotide sequence (5' to 3')
S100A10 #1	CCGGCCATGATGTTTACATTTCACACTCGAGTGTGAAATGTAAACATCATGGTTTTTG
S100A10 #2	CCGGTGAGCAGATCAGGACACTTAGCTCGAGCTAAGTGTCCTGATCTGCTCATTTTTTG
ANXA2 #1	CCGGTGAGGGTGACGTTAGCATTACCTCGAGGTAATGCTAACGTCACCCTCATTTTTG
ANXA2 #2	CCGGGCAGGAAATTAACAGAGTCTACTCGAGTAGACTCTGTTAATTTCCTGCTTTTTG
SPT6 #1	CCGGCCCTTGAAGAAATCTTGGAAACTCGAGTTTCCAAGATTTCTTCAAGGGTTTTTG
SPT6 #2	CCGGCCAACCGTGAATGGACTGTTTCTCGAGAAACAGTCCATTCACGGTTGGTTTTTG
KDM6A #1	CCGGTGAATCTACATCGTCAGATAACTCGAGTTATCTGACGATGTAGATTCATTTTTG
KDM6A #2	CCGGTGGAACAGCTCCGCGCAAATACTCGAGTATTTGCGCGGAGCTGTTCCATTTTTG

Supplemental Table 5. Antibody information for chromatin immunoprecipitation assays.

Antibody	Manufacturer	Catalog number
HIF-1α	Novus Biologicals	NB100-479
HIF-2α	Novus Biologicals	NB100-122
HIF-1β	Novus Biologicals	NB100-110
OCT4	Novus Biologicals	AF1759
S100A10	Novus Biologicals	H00006281-M09
SPT6	Novus Biologicals	NB100-2582
H3K27me3	Novus Biologicals	NBP2-59206
Histone H3	Novus Biologicals	NB500-171
KDM6A	Novus Biologicals	NBP1-80628

Supplemental Table 6. Nucleotide sequence of ChIP-qPCR primers.

S100A10 HIF-1 binding site	Forward: CGGACCTCCTAGGGCTAATC
	Reverse: TCCTCTCGGGTTTGGTTTTA
NANOG OCT4 binding site	Forward: GTTGGAAACGTGGTGAACCT
	Reverse: CCTACTGACCCACCCTTGTG
SOX2 OCT4 binding site	Forward: TCATCAATGAGAATTAGATGAGAGAGA
	Reverse: GATAACAATTGCTGTTTCAGTCCA
KLF4 OCT4 binding site	Forward: GGGCTTCCCCCTTTAAGAAG
	Reverse: AGTGCCAATGTGGAGGAAAA
POU5F1 OCT4 binding site	Forward: TCGATCTCAGCTCACTGCAC
	Reverse: CTGGCCAACATGGTGAAAC

Supplemental Figure Legends

Supplemental Figure 1. Changes in S100 family mRNA expression in paclitaxel-treated breast cancer cell lines. mRNA expression of twenty S100 family members was accessed from Gene Expression Omnibus (GEO) dataset GSE50811, which contains transcriptome profiles of 27 breast cancer cell lines treated with vehicle or paclitaxel. Log_2 fold change (FC) of S100 mRNAs (paclitaxel vs. vehicle) in each cell line is presented as a heat map.

Supplemental Figure 2. Chemotherapy induces HIF-1-dependent S100A10 expression. (A) Levels of S100A10 mRNA and 10 mRNAs encoded by HIF-target genes are shown for primary breast cancer samples accessed from TCGA database, which were classified based on the expression of a 50-mRNA signature (PAM50) that defines four molecular subtypes (Basal, HER2-enriched, Luminal B, and Luminal A). Color code: blue, less than the median; red, greater than the median. **(B)** MCF7 subclones were treated with vehicle (V), 10 nM paclitaxel (P), or 250 μM carboplatin (C) for 72 hours and immunoblot assays were performed. **(C)** MCF7 cells were treated with V, P, or C, either alone or in combination with 100 nM digoxin (D) for 72 hours and RT-qPCR was performed to quantify S100A10 mRNA (mean ± SEM; n = 3); $^{*}p < 0.05$ vs. V; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. P or C (one-way ANOVA with Bonferroni post-test). **(D** and **E)** MCF7 cells were treated with V or P for 72 hours (**D**), or exposed to 20% or 1% O_2 for 16 hours (**E**), and chromatin immunoprecipitation (ChIP) assays were performed. Primers flanking the HIF binding site in the *S100A10* gene were used for qPCR (mean ± SEM; n = 3); $^{***}p < 0.001$ vs. corresponding V or 20%; ns, not significant (two-way ANOVA with Bonferroni post-test).

Supplemental Figure 3. S100A10 knockdown blocked chemotherapy-induced BCSC enrichment. (A and **B)** MCF7 cells were cultured on standard polystyrene tissue culture plates (Adherent) or ultra-low adherence plates (Sphere) for 6 days and harvested for analysis of S100A10 mRNA (**A**) and protein (**B**) expression. Results were normalized to Adherent (mean ±

SEM; n = 3); ***p < 0.001 (Student's t test). **(C - E)** MCF7 subclones stably transfected with vector encoding non-targeting control shRNA (NTC) or either of two different shRNAs targeting S100A10 (#1 and #2) were treated with vehicle (V) or 10 nM paclitaxel (P) for 72 hours. The percentage of $ALDH^+$ cells (**C**; mean ± SEM; n = 3) and the number of mammospheres formed per 1,000 cells seeded (**D**; mean ± SEM; n = 4) were determined. **p < 0.01, ***p < 0.001 vs. NTC-V; $^{###}p$ < 0.001 vs. NTC-P (two-way ANOVA with Bonferroni post-test). Representative photomicrograph of mammospheres is shown (**E**; scale bars, 100 μm). **(F)** MDA-MB-231 subclones stably transfected with vector encoding non-targeting control shRNA (NTC) or either of two different shRNAs targeting S100A10 (#1 and #2) were treated with vehicle (V) or 10 nM paclitaxel (P) for 72 hours. Representative photomicrograph of mammospheres formed per 1,000 cells seeded is shown (scale bars, 100 μm). **(G)** MCF7 subclones transfected with NTC or S100A10 shRNA vector were treated with V or P, and RT-qPCR was performed (mean ± SEM; n = 3). **p < 0.01, ***p < 0.001 vs. NTC-V; $^{#}p$ < 0.05, $^{##}p$ < 0.01, $^{###}p$ < 0.001 vs. NTC-P (two-way ANOVA with Bonferroni post-test).

Supplemental Figure S4. S100A10 regulates paclitaxel sensitivity in vitro, and regulates paclitaxel-induced pluripotency factor expression and BCSC enrichment in vivo. (A) MDA-MB-231 subclones transfected with NTC or S100A10 shRNA vector were treated with vehicle (V) or 10 nM paclitaxel (P) for 72 hours and the numbers of live cells were counted (mean ± SEM; n = 6). ***p < 0.001 vs. NTC-V; $^{###}p$ < 0.001 vs. NTC-P (two-way ANOVA with Bonferroni post-test). **(B)** Representative photomicrographs of mammospheres that were quantified in Figure 4C. **(C)** Densitometric analysis of immunoblots presented in Figure 4D.

Supplemental Figure 5. ANXA2 knockdown blocked chemotherapy-induced BCSC enrichment. (A and **B)** MCF7 cells were transfected with vector encoding NTC or either of two different shRNAs targeting ANXA2 (#1 and #2), and RT-qPCR (**A**; mean ± SEM, n = 3; **p < 0.01 vs. NTC; one-way ANOVA with Bonferroni post-test) and immunoblot (**B**) assays were

performed to analyze ANXA2 expression. **(C** and **D)** MCF7 subclones transfected with NTC or ANXA2 shRNA vector were treated with vehicle (V) or 10 nM paclitaxel (P) for 72 hours. The percentage of $ALDH^+$ cells was determined (**C**), and RT-qPCR was performed in (**D**; mean ± SEM; n = 3). ***$p < 0.001$ vs. NTC-V; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. NTC-P (two-way ANOVA with Bonferroni post-test).

Supplemental Figure 6. SPT6 interacts with the S100A10-ANXA2 complex and regulates expression of pluripotency factors. (A) MCF7 cells were treated with vehicle (V) or 10 nM paclitaxel (P) for 72 hours. Cytosolic and nuclear lysates were prepared, and immunoblot assays were performed. **(B** - **D)** MCF7 cells were transfected with vector encoding NTC or either of two different shRNAs targeting SPT6 (#1 and #2), and RT-qPCR (**B**; mean ± SEM, n = 3; ***$p < 0.001$ vs. NTC; one-way ANOVA with Bonferroni post-test), immunoblot (**C**), and cell proliferation (**D**; mean ± SEM, n = 6; ns, not significant; one-way ANOVA) assays were performed. **(E)** MCF7 subclones transfected with NTC or SPT6 shRNA vector were treated with V or P for 72 hours, and RT-qPCR was performed (mean ± SEM; n = 3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. NTC-V; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. NTC-P (two-way ANOVA with Bonferroni post-test).

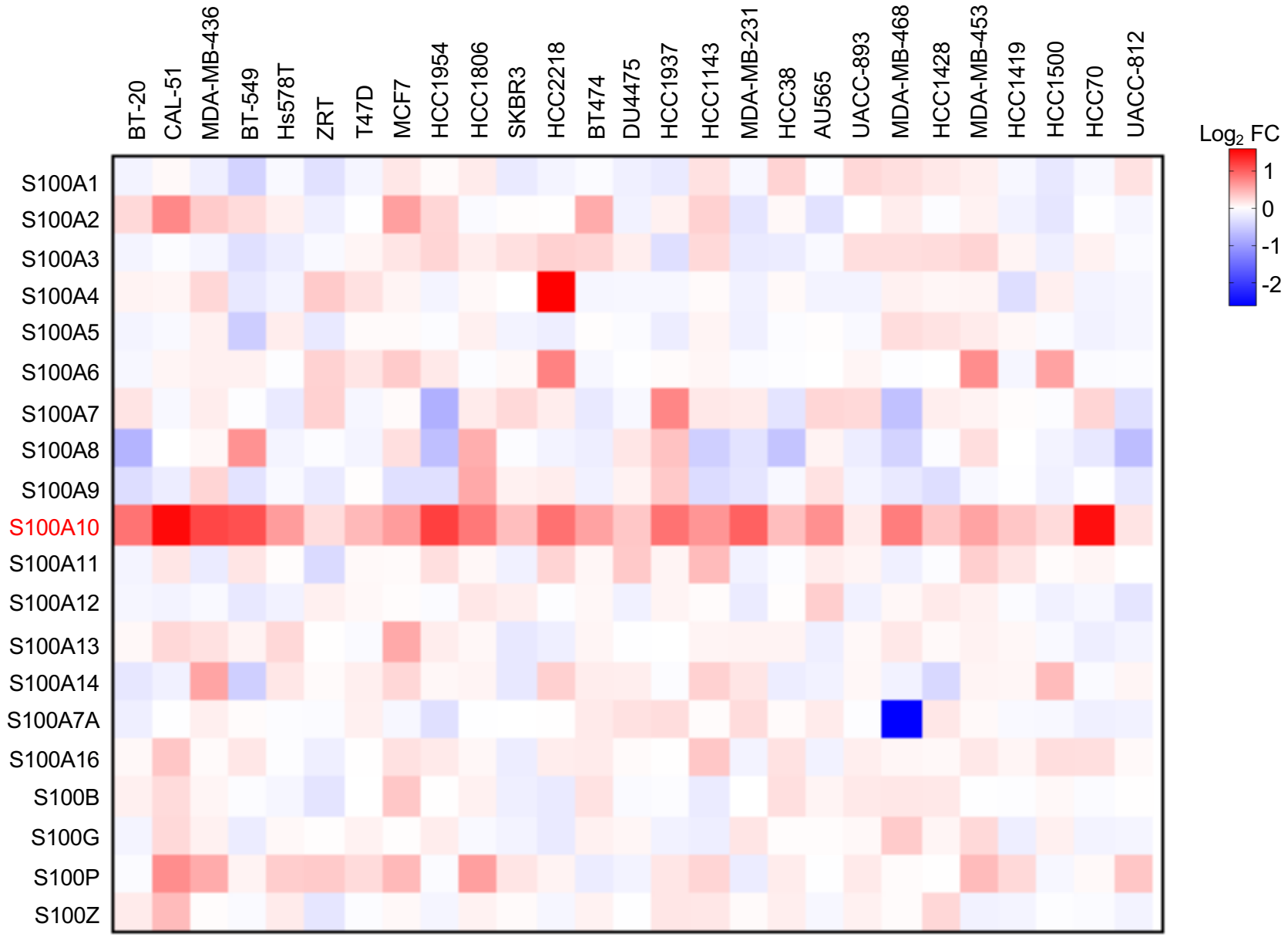
Supplemental Figure 7. KDM6A knockdown blocked chemotherapy-induced BCSC enrichment. (A - **C)** MCF7 cells were transfected with vector encoding NTC or either of two different shRNAs targeting KDM6A (#1 and #2), and RT-qPCR (**A**; mean ± SEM, n = 3, ***$p <$ 0.001 vs. NTC; one-way ANOVA with Bonferroni post-test), immunoblot (**B**), and cell proliferation (**C**; mean ± SEM, n = 6; ns, not significant; one-way ANOVA) assays were performed. **(D** and **E)** MCF7 subclones transfected with NTC or KDM6A shRNA vector were treated with vehicle (V) or 10 nM paclitaxel (P) for 72 hours. The percentage of $ALDH^+$ cells was determined (**D**) and RT-qPCR was performed (**E**) (mean ± SEM; n = 3). *$p < 0.05$, **$p < 0.01$,

***p < 0.001 vs. NTC-V; $^{\#\#}p$ < 0.01, $^{\#\#\#}p$ < 0.001 vs. NTC-P (two-way ANOVA with Bonferroni post-test).

Supplemental Figure 8. Pharmacological inhibition of EZH2 fails to block paclitaxel-induced pluripotency factor expression and BCSC enrichment. (A, **C,** and **D)** MDA-MB-231 cells were treated with vehicle (V) or 10 nM paclitaxel (P), in combination with 0 μM, 0.2 μM, or 1 μM EZH2 inhibitor EPZ-6438, for 72 hours. Immunoblot (**A**) and RT-qPCR (**C**) assays were performed, and the percentage of $ALDH^+$ cells was determined (**D**) (mean ± SEM; n = 3). ***P < 0.001 vs. V and EPZ-6438 0 μM; ns, not significant (two-way ANOVA with Bonferroni post-test). **(B)** MDA-MB-231 cells were treated with V or P, in combination with 0 μM or 1 μM EPZ-6438, for 72 hours, and ChIP assays were performed using antibody against H3K27me3 or total histone H3. Primers flanking OCT4 binding sites in the *NANOG, SOX2, KLF4, and POU5F1* genes were used for qPCR (mean ± SEM; n = 3). **P < 0.01 vs. V and EPZ-6438 0 μM (two-way ANOVA with Bonferroni post-test).

Supplemental Figure 9. GSK-J4 increases global H3K27me3 modification in vivo. (A and **B)** 2 x 10^6 MDA-MB-231 cells were implanted into the MFP of female SCID mice. When tumor volume reached 200 mm^3 (day 0), mice were grouped randomly and treated with vehicle (V), paclitaxel (P; 10 mg/kg, days 0, 5, and 10), GSK-J4 (10 mg/kg, days 0-13), or the combination of P and GSK-J4. Tumors were harvested on day 13 for immunoblot assay to detect global H3K27me3 levels (**A**). Densitometric analysis was performed (**B**; mean ± SEM; n = 4). **p < 0.01 vs. vehicle; $^{\#}p$ < 0.05 vs. paclitaxel (one-way ANOVA with Bonferroni post-test).

Supplemental Figure 1



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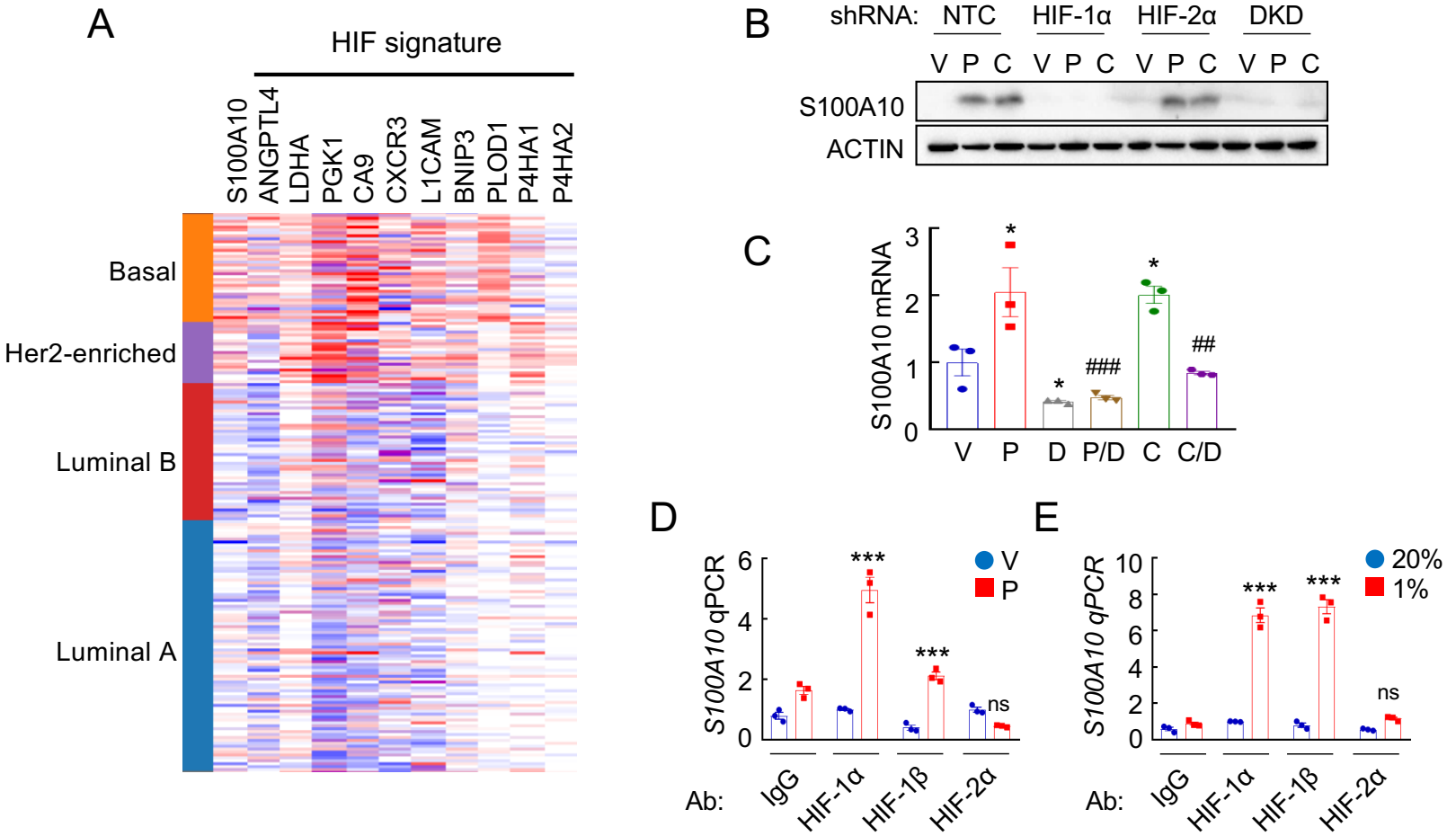

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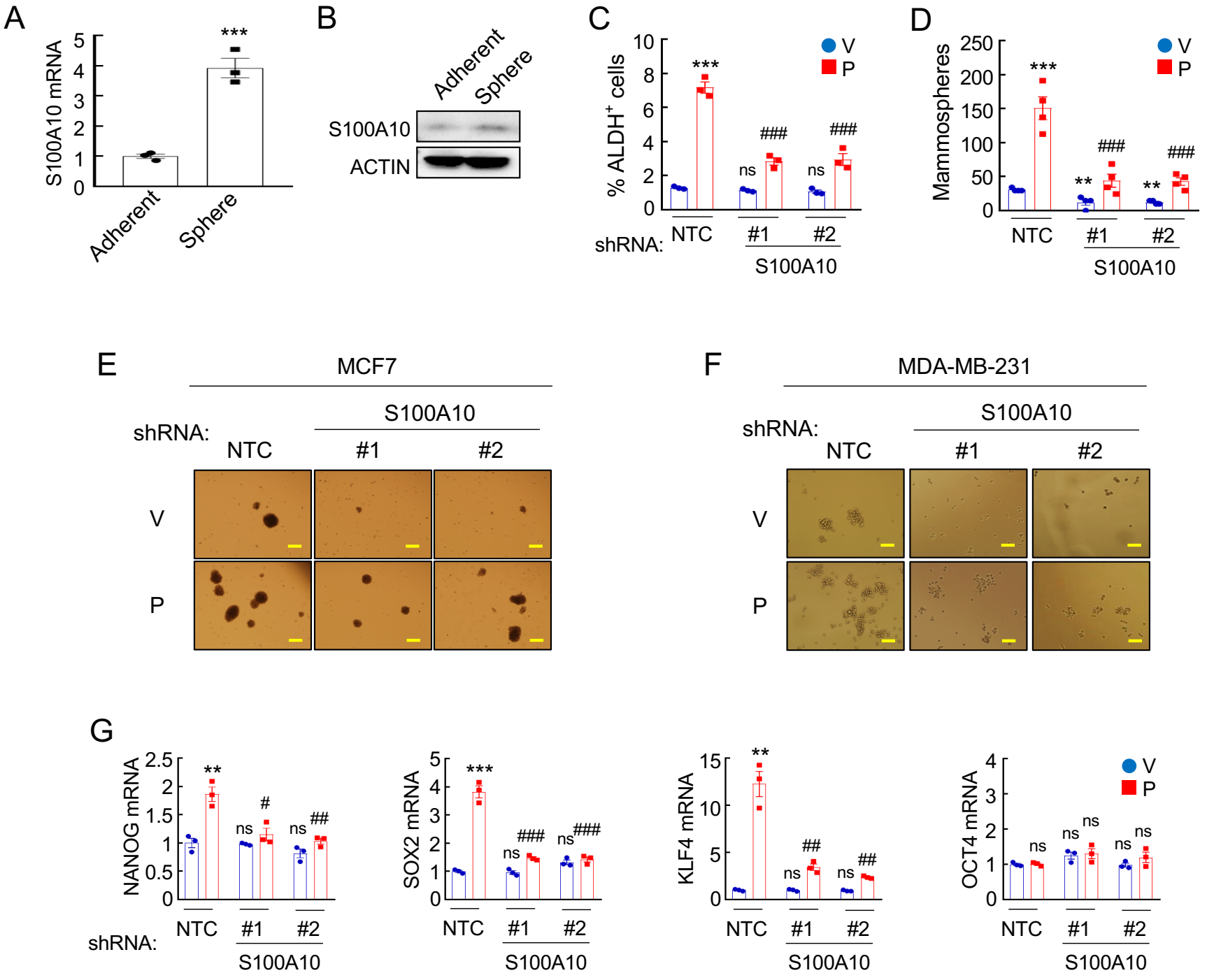

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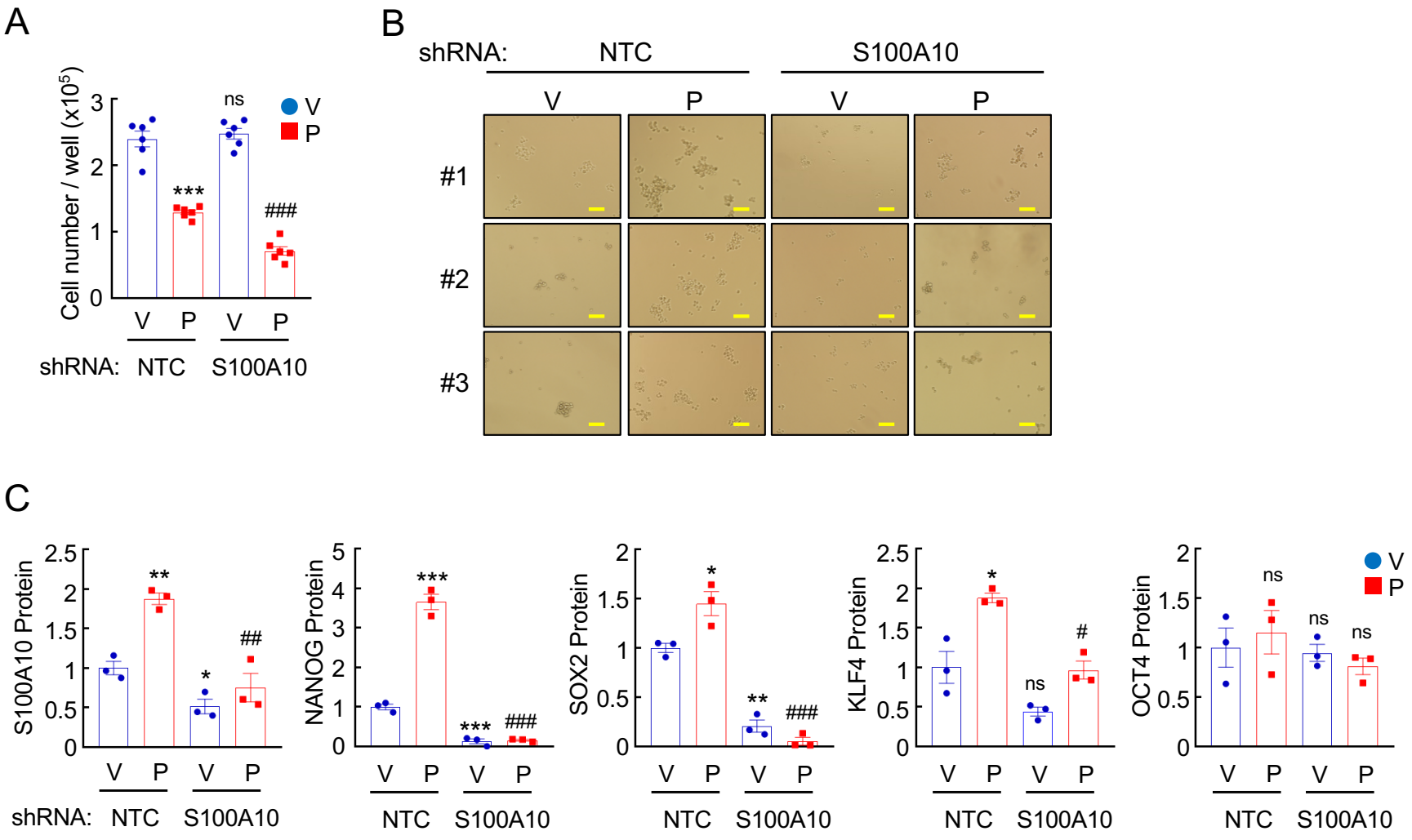

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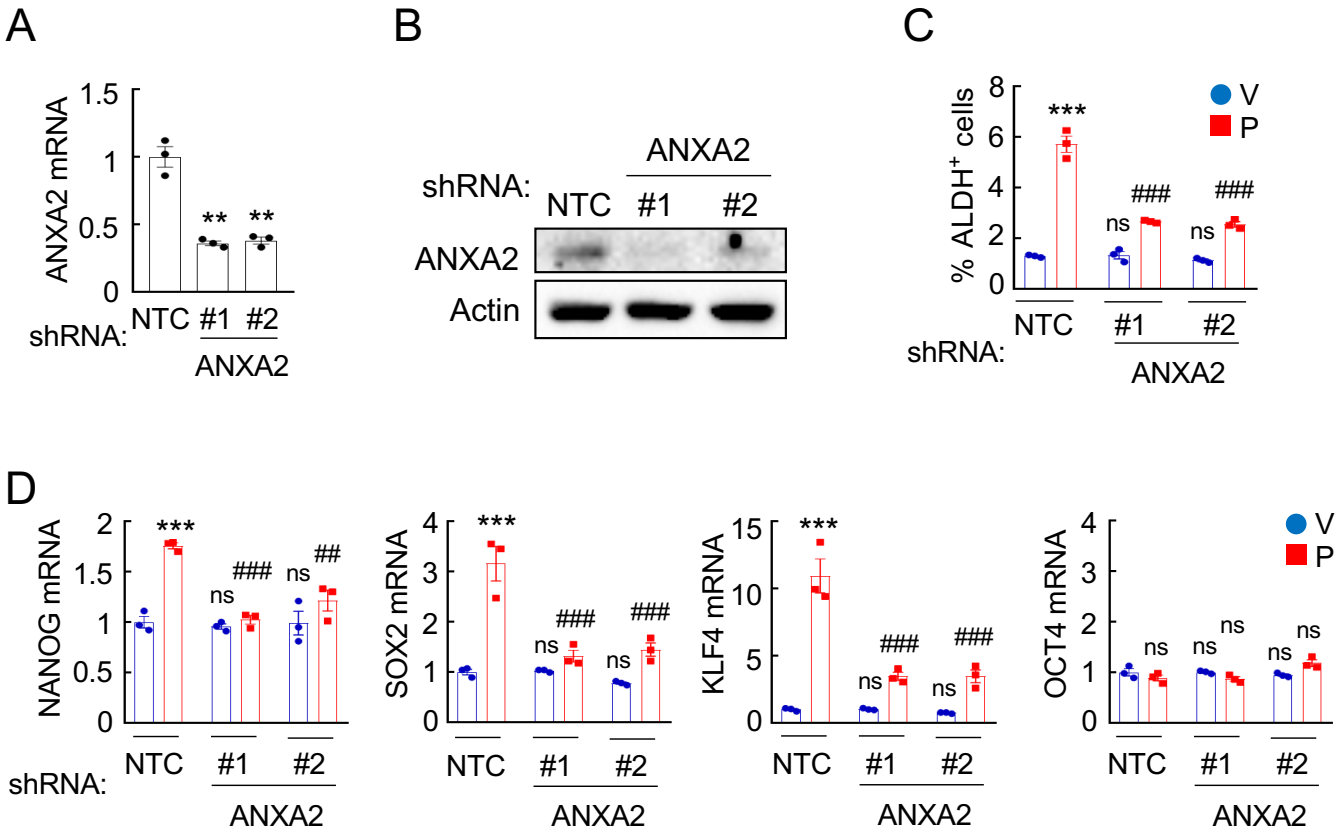

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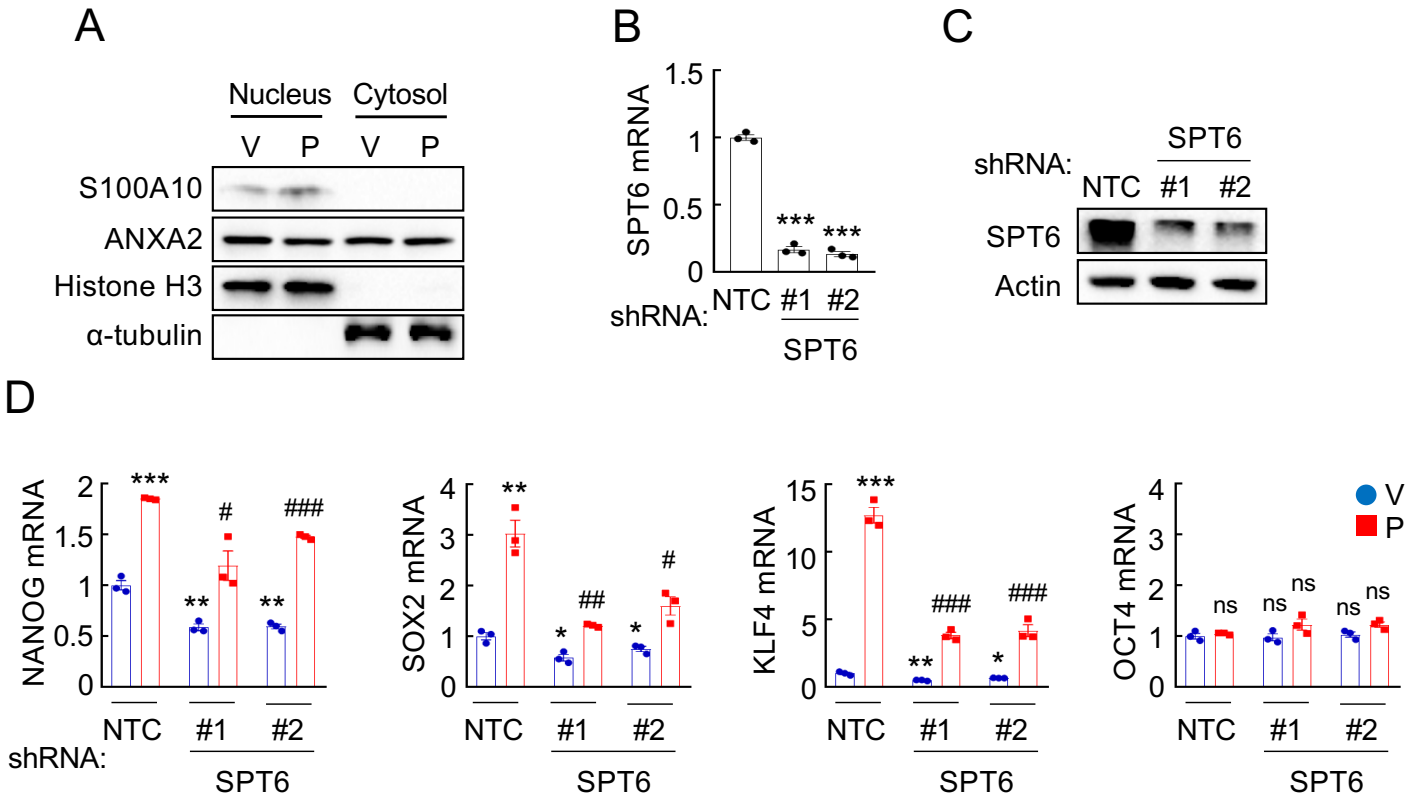

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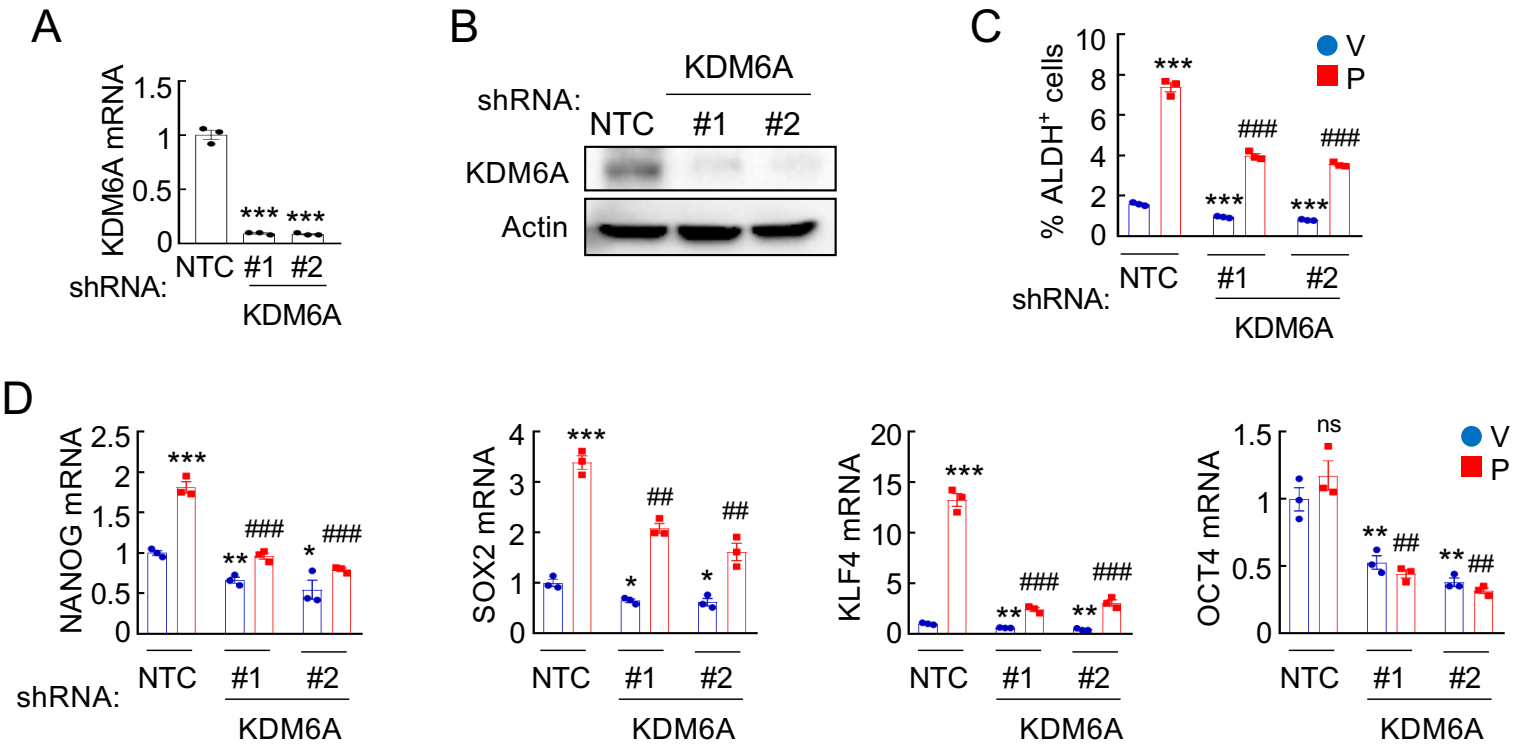

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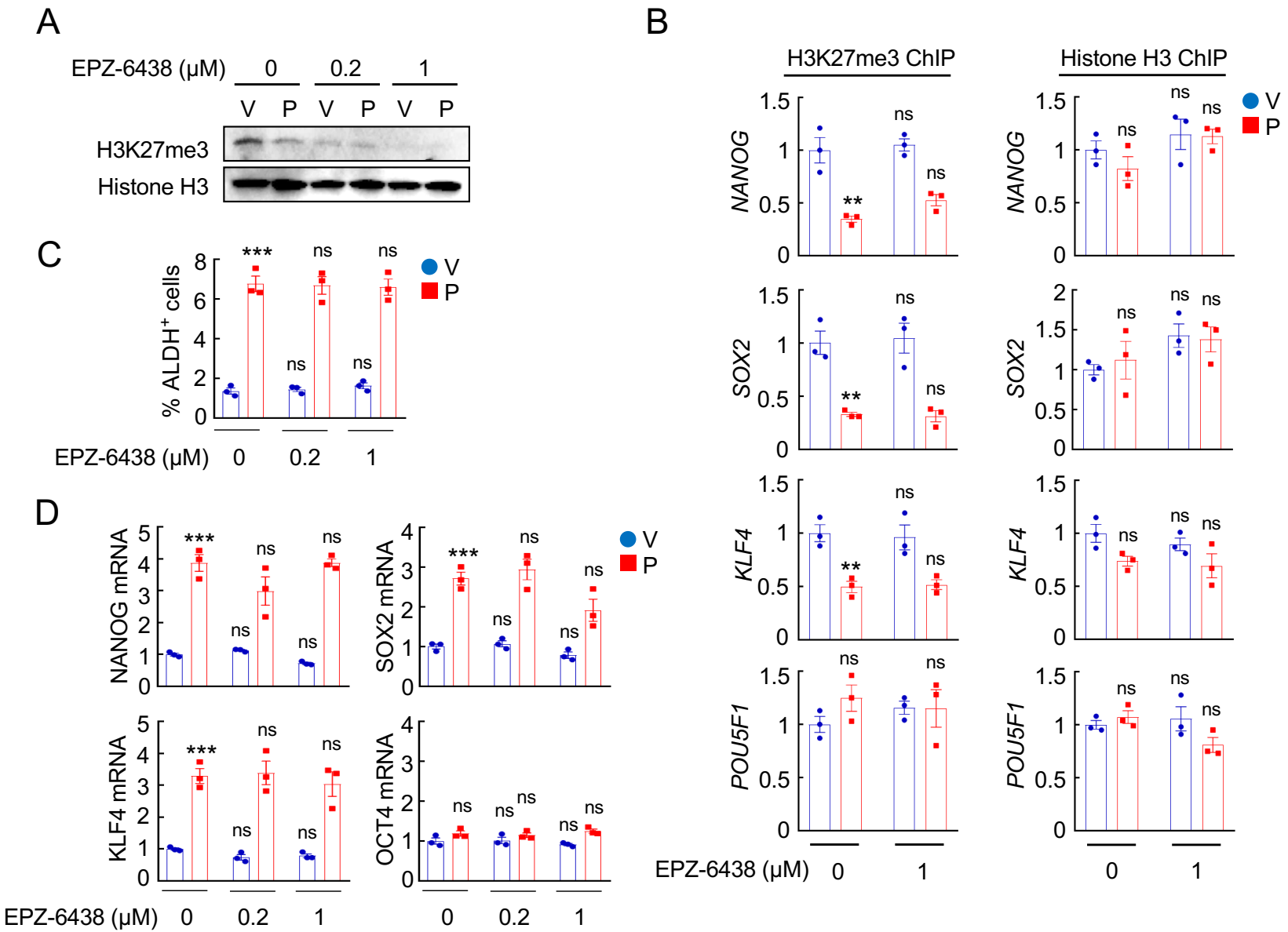

Supplemental
Figure 9