Supplemental Material and Methods

Cell lines, culture conditions and reagents

To ensure integrity, cell lines were frozen in small aliquots and thawed at frequent intervals. No cell lines were used beyond 30-40 passages. Cell morphology was monitored for each cell line. Proper media and growth conditions were selected.

Guadecitabine was provided by Astex Pharmaceuticals Inc. (Pleasontin, CA, USA). Cisplatin (CDDP) was purchased from Calbiochem (Billerica, MA, USA), and carboplatin was purchased from Sigma. Human recombinant IL-6 was purchased from (BioVision Inc., Milpitas, CA, USA, Cat# 4243-100) and IL-6 neutralizing antibody and IgG control was purchased from InvivoGen (San Diego, California, USA, Cat# mabg-hIL-6-3; Cat#mabg1-ctrlm). Control scrambled oligo (Cat #sc-108060), shIL-6 (Cat # sc-39627-SH-IL-6) and shALDH1A1 (Cat # sc-41442-SH-ALDH1A1) were purchased from Santa Cruz Biotechnology (Dallas, Texas, USA). pGL3_prom ALDH1A1 firefly luciferase plasmid was designed and purchased from Keyclone Technologies (San Diego, CA USA). Stattic was purchased from Abcam (Cat #ab120952, Cambridge, MA, USA)

Co-culture OC cells with NOFs. 10^5 NOFs were seeded into 10cm plate on day 1, and OC cells (10^5 or $0.5X10^5$) were plated into the same plate on day 2. Co-cultured cells were washed with (1X) PBS and incubated with serum starving media for 24h (DMEM with 0.5% FBS). Cells were treated with CDDP (half of IC_{50} of OC cells), guadecitabine (100nM, 4 days) alone, or in combination. Conditioned media were collected at 24h, 48, 72h, and 96h exposure to CDDP, and the total number of cells were counted.

Transwell co-culture OC cells with NOFs. 10^5 NOFs/well were seeded on the bottom chamber of 6 well plates on day1 in DMEM with 10% FBS, and $0.5X10^5$ Kuramochi derived ALDH(-), or A2780 derived ALDH(-) cells were seeded on the top insertion with 0.45 micron pores (Cat# 5140001, control inserts, 6-well plate 0.45 micron, Corning, Bedford, MA, USA) on day 2. Co-cultured cells were treated daily with low dose guadecitabine (100nM) for three days (day 3-5), and then followed by washing 1X with PBS and starved for 24h in DMEM media with 0.5% FBS (day 6). Then, cells were treated with CDDP (Kuramochi derived ALDH(-), 3μM; A2780 derived ALDH(-) cells, 2μM) for 3 hours, IL-6 Nab (1μg/ml) alone or in combination. Conditioned media of co-cultured cells were collected at 24 hour after CDDP withdrawal, and cells cultured on both top and bottom chambers were lysed in the RLT buffer for DNA and RNA extraction preparation.

Clonogenic survival assay. OC cells (n=500) or OC cells pretreated with guadecitabine, IL-6 Nab or in combination (n=500 cells per condition) were plated in triplicate into 6-well plates (A2780_CR5, A2780_CR5-derived ALDH(+)/(-) cells, Kuramochi, Kuramochi-ALDH(+)/(-)) and cells were treated with vehicle (0.9% NaCl) or CDDP for 3 h, allowed 6–14 days of cell growth to form colonies. Once visible colonies formed, they were washed with (1x) PBS, fixed with 4% paraformaldehyde and stained with 0.05% crystal violet. The number of the colonies/well was determined.

Cell survival assay (MTT assay). Briefly, 10,000 OC cells or pretreated OC cells with CDDP, IL-6, guadecitabine, IL-6 Nab, or guadecitabine+IL-6 Nab were plated in 96-well plates. The quantity of viable cells was determined on Day 1, 2, 3, and 4 by MTT assay as

described previously (1). For the CDDP treatment cells, after a 48-hour recovery period from cell seeding, cells were treated with serial dilutions of CDDP (0 to 500 μM) for 3 hours, followed by an additional 3-day recovery. Cell viability was determined by MTT (1mg/mL, Invitrogen) assays as described previously (2, 3) using a Bio-Tek (Winooski, VT, USA) microplate spectrophotometer at 600nm wavelength. IC_{50} dose values for cisplatin were used (previously determined by Prism 6, GraphPad Software, San Diego, CA using logarithm normalized sigmoidal dose curve fitting).

Luciferase assay. Cells (Kuramochi derived ALDH(-) cells) were seeded in 96-well plates (10^4 cells/well) and co-transfected with pGL3-promALDH1A1 or pGL (500 ng construct DNA/transfection) with PGL4 Renilla plasmid (100 ng construct DNA /transfection) using Turbofect (Thermo Scientific). Twenty-four hours after transfection, cells were washed 1x with PBS and starved in RPMI supplemented with 0.5% FBS for another twenty-four hours. Then cells were treated with IL-6 (100ng/ml) for 3 hours. Luciferase activity was analyzed using the Dual Luciferase Reporter Assay System (Promega, Madison, WI, USA) and a Thermo Scientific Multilabel Plate Reader (Thermo Scientific, Florence, KY, USA). Relative luciferase units were calculated over renilla luciferase signals.

Caspase 3/7 cleavage apoptosis assay. Cells were treated with CDDP (half of IC_{50}, 3h), IL-6 Nab, guadecitabine, guadecitabine +IL-6 Nab, CDDP+guadecitabine, CDDP+IL-6 Nab, and CDDP+IL-6 Nab +guadecitabine. 48h after treatment, cells were washed with PBS, trypsinized and collected. 10^4 cells/well were reseeded into 96 well plates and cultured in RPMI supplemented with 10% FBS and antibiotics for 24 hours. Caspase 3/7 cleavage luciferase signals were detected by using Caspase 3/7 GloAssay, according to the

manufacturer's instruction (Promega, Cat#G8090, Madison, WI, USA).

Spheroid formation assay. 250 Kuramochi/A2780 cells, which were treated with IL-6 (100ng/ml), IL-6 Nab (1μg/ml), CDDP (half of IC_{50}), guadecitabine (100nM, 3 days) and in combination were sorted into duplicate rows of 96- well ultra low attachment plates (Corning, Tewkesbury, MA, USA) with 200ul stem cell media (DMEM/F12 (Corning, REF#10-017-cv) with P/S (100U), 0.4% BSA, 10ng/ml bFGF (Introgen, REF#13256029), 20ng/ml EGF (Cat 585506, EGF BioLegend) and 5ug/ml Insulin (Sigma, 10mg/ml, 19278)). The number of spheroids was counted under the microscope and images were acquired on day 14.

OC xenograft tumors were mechanically and enzymatically digested into single cell suspension. Then red blood cells were lysed and removed from xenografts by using (1x) red blood lysis buffer (Biolegend, Cat#420301) according to the manufacture's instruction. Cell dilution adjusted to 10^5 cells /ml and 10^4 cells were plated into 24 ultra-low attachment plates with stem cell media. After two weeks, tumorspheres were counted under a microscope and images were captured at that time.

Western blotting. Primary antibodies for ALDH1A1 (Rabbit, Cat. #12035s, 1:500), DNMT1 (Rabbit, Cat. #5032s, 1:1000) and IL-6 (Rabbit, Cat. #12153S, 1:500) were from cell signaling technology. Antibodies for IL-6R (Rabbit, Cat. #sc661, 1:1000), beta-tubulin (mouse, Cat. #sc101527, 1:4000), GAPDH (Rabbit, Cat. #sc25778, 1:2000), pSTAT3 Tyr705 (Rabbit, Cat. #sc7993, 1:1000), and STAT3 (Rabbit, Cat. #sc482, 1:1000) were purchased from Santa Cruz Biotechnology. HRP-conjugated secondary

antibodies including goat-anti-rabbit (1:4000) and goat-anti-mouse antibody (1:4000) were purchased from Kirkegaard and Perry Laboratories (Gaithersburg, MD, USA). Protein bands were visualized using SuperSignal West Pico chemiluminescence substrate system (Lot# RC227166; ThermoFisher Scientific), following the manufacturer instructions.

Infection. 100,000 OVCAR3 cells were transfected with shctrl (MISSION shRNA lentiviralSHC001V, Sigma-Aldrich), shALDH1A1(MISSION shRNA lentiviralTRCN0000026415, TRCN0000026498, Sigma-Aldrich) or shIL-6 (MISSION shRNA lentiviralTRCN0000372667, TRCN0000059203, Sigma-Aldrich) with MOI=2.5. Cells with stable infection were selected and maintained by 1.0 ug/ml puromycin containing medium.

***In vivo* xenograft experiments**. Tumor were mechanically and enzymatically digested in DMEM/F12 media supplemented with 300U/ml collagenase and 300U/ml hyaluronidase into single cell suspensions for FACS analysis of OCSCs population in xenograft and spheroid formation assays. Xenograft tumors were also directly transferred into RLT (Qiagen) buffer or RIPA buffer and then stored at −80°C until DNA, RNA and protein were extracted. Xenograft tumors were minced in RLT buffer and then homogenized by using 1.5 mL Rino tissue homogenization bead lysis kits (Next Advance Inc., Averill Park, NY, Lot# CAP3260), according to manufacturer's cancer tissue homogenization protocol. Blood (200μl) was drawn from mice via the vein prior to tumor cell injection and carboplatin treatment and after completion of carboplatin and maintenance treatments.

Blood samples were stored on ice for 1 hour prior to centrifugation at 1000g for 15 min at 4°C. Plasma was then frozen and stored at -80°C. IL-6 ELISA assays were performed according to manufacturer's instructions (eBioscience).

References

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2. Fan MY, Yan PS, Hartman-Frey C, Chen L, Paik H, Oyer SL, et al. Diverse gene expression and DNA methylation profiles correlate with differential adaptation of breast cancer cells to the antiestrogens tamoxifen and fulvestrant. *Cancer Res.* 2006;66(24):11954-66.
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Supplemental Table S1. IC_{50} dose values of OC cells for 3 hours cisplatin exposure

Cell lines	**IC_{50} of CDDP for 3h**	**The range of IC_{50}**
A2780	14.73 μM	13.09-16.58 μM
A2780_CR5	33.99 μM	28.70-40.25 μM
OVCAR4	21.16μM	17.72-25.28 μM
Kuramochi	40.43μM	39.09-51.89 μM
Kuramochi_CDDP	79.65μM	51.94-89.2 μM

Supplemental Table S2. Guadecitabine -IL-6 Nab prevents OC tumor relapse. Xenograft model derived from A2780 treated with carboplatin (50mg/ml, weekly for 3 weeks) were randomized to 2 weeks of twice-weekly treatment with vehicle+IgG, guadecitabine, IL-6 Nab and in combination (n=6 per group). The number of mice that formed intraperitoneal tumors is shown.

Group	Control	Carboplatin treated tumor residuals	Carboplatin treated recurrent tumors	Carboplatin+ Guadecitabin-treated recurrent tumors	Carboplatin+ IL-6 Nab treated recurrent tumors	Carboplatin+ Guadecitabine +IL-6 Nab treated recurrent tumors
# of Mice Formed Tumors	6/6	4/6	6/6	5/6	4/6	3/6

Supplemental Table S3. Ovarian cancer cells, cell culture media, and phenotypes (classified based on their genomic profiles (4))

Cell lines	Media	Phenotype	Cell Source
A2780	RPMI1640 (Invitrogen, Carlsbad, CA) medium with 10%FBS, 2mM L-glutamine, 50U/ml penicillin, 50mg/ml streptomycin	Unlikely high-grade serous	Sigma
A2780_CR5	RPMI1640 medium with 10%FBS, 2mM L-glutamine, 50U/ml penicillin, 50mg/ml streptomycin	Unlikely high-grade serous	Sigma
COV318	DMEM (Invitrogen, Carlsbad, CA) with 10% FBS, 2mM L-glutamine, 50U/ml penicillin, 50mg/ml streptomycin	Likely high-grade serous	ATCC
EFO27	RPMI1640 medium with 20%FBS, 2mM L-glutamine, 50U/ml penicillin, 50mg/ml streptomycin	Hypermutaed	ATCC
HeyA8	DMEM medium with 10%FBS, 2mM L-glutamine, 50U/ml penicillin, 50mg/ml streptomycin	Unlikely high-grade serous	ATCC
Kuramochi	RPMI1640 medium with 10%FBS, 2mM L-glutamine, 50U/ml penicillin, 50mg/ml streptomycin	Likely high-grade serous	ATCC
OVCAR4	DMEM medium with 10%FBS, 2mM L-glutamine, 50U/ml penicillin, 50mg/ml streptomycin	Likely high-grade serous	ATCC
OVCAR3	ATCC-formulated RPMI-1640 Medium (Catalog No. 30-2001) with 20% FBS, 0.01 mg/ml bovine insulin, 50U/ml penicillin, 50mg/ml streptomycin	Likely high-grade serous	ATCC
SKOV3	McCoy'S 5A medium with10% FBS, 50U/ml penicillin, 50mg/ml streptomycin	Unlikely high-grade serous	ATCC

Supplemental Table S4. Primer sequences of selected genes

Gene Name	Primer Sequence	
	Forward (5' to 3')	Reward (5' to 3')
ALDH1A1	TCCCGTTGGTTATGCTCATTTG	GGAGTTTGCTCTGCTGGTTTGAC
Bmi-1	CTCATCCACAGTTTCCTCACATTTC	GTCTATTGGCAAAAGAAGATTGGTG
EEF1A1	GCCCCAGGACACAGAGACTTTATC	CAACACCAGCAGCAACAATCAG
Interleukin 6	CCCCCAGGAGAAGATTCCAAAG	TGAAGAGGTGAGTGGCTGTCTGTG
Interleukin 6 Receptor	AAGAGCCCCCTCAGCAATGTT G	ACCAAGAGCACAGCCTTTGTC G
Sox2	GCGCGGGCGTGAACCAG	CGGCGCGGGGAGATACA

Supplemental Figure Legends

Supplemental Figure S1. ALDH(+) population is positively correlated with ALDH1A1 expression in majority of OC cells. (A) Average percentage of ALDH(+) cells across human OC cell lines were measured by FACS (N=3). **(B)** ALDH1A1 mRNA expression in human OC cells (ng/ml) were measured by q-RTPCR. Bars represent average measurements of three independent experiments ±SD. **(C)** Correlation of average percentage of ALDH(+) cells population with average ALDH1A1 expression (pg/ml) across human OC cells. Pearson r value was calculated and shown (p=0.0126).

Supplemental Figure S2. Role of IL-6 in OCSCs maintenance. (A) Average fold change of mRNA expression of ALDH1A1 in ovarian cancer cell Kuramochi, A2780 and OVCAR4 derived ALDH(+) cells over their respective ALDH(-) cells (N=3). **(B)** OVCAR3 cells were transfected with scrambled shRNA, shALDH1A1 or shIL-6 RNA. Side scatter of FACS analysis of the percentage of ALDH(+) cell population in Kuramochi cells with stable ALDH1A1 and IL-6 knockdown expression. Average percentage of ALDH(+) cells (± SD) is shown in bar graph below (N=3). **(C)** Kuramochi cells transfected with stable shcontrol scrambled oligo, shALDH1A1 or shIL-6 RNA plasmid DNA. Protein expression of ALDH1A1, IL-6, IL-6R, STAT3 and GAPDH expression in OC Kuramochi cells (N=2). **(D)** OVCAR3 cells transfected with stable shcontrol, shALDH1A1 or shIL-6 RNA plasmid DNA. Protein expression of ALDH1A1, IL-6, STAT3 and beta tubulin expression in OC OVCAR3 cells (N=2). **(E)** Protein expression of ALDH1A1, IL-6R, pSTAT3, STAT3, cyclinD1 and beta tubulin expression in Kuramochi-derived ALDH(+) and ALDH(-) cells are shown (N=2).

Supplemental Figure S3. Platinum/ IL-6 upregulates ALDH1A1 expression in ALDH(-) OCs. (A) Side scatter of FACS analysis of the percentage of ALDH(+) in OC cells (A2780) treated with CDDP (1/2 of IC_{50}, 3h), CDDP+IL-6 Nab and IL-6 (50ng/ml) for 72h. Average percentage of ALDH(+) cells ± SD is shown on the graph and quantification shown in bar graph below. **(B)** Morphology of spheroids formed by A2780, A2780_ALDH(-) and A2780_ALDH(-) cells treaded with IL6 (100ng/ml, 3days). (Bottom) Quantification of the total number of spheroids. Scale bar indicates 100µm. **(C)** Side scatter of FACS analysis of the percentage of ALDH(+) cells treated with different doses of IL-6 (0, 100ng/ml, 250ng/ml, 500ng/ml and 1000ng/ml) for 72h. Average percentage of ALDH(+) cells (± SD) is shown on the graph. **(D)** ALDH1A1, Sox2 and Bmi-1 mRNA expression were determined in Kuramochi derived ALDH(-) cells treated with different doses of IL-6 (1000ng/ml) for 72h. Average fold change (± SD) of three independent experiments over untreated cells is shown (*P< 0.05, **P<0.01, and ***P<0.001). **(E)** Side scatter of FACS analysis of the percentage of ALDH(+) cells in A2780_CR5 derived ALDH(-) cells treated with a serial dose of IL6 (0, 100, 250, 500, 1000 ng/ml). **(F)** mRNA expression of ALDH1A1, Sox2 and Bmi-1 in A2780_ALDH(-) cells treated with IL-6 (1000 ng/ml). 2 tailed student's *t*-test was used to analyze statistical significance (*P< 0.05, **P<0.01, and ***P<0.001, N=3).

Supplemental Figure S4. Platinum-induced IL-6 induces ALDH1A expression and OCSC enrichment. (A) The morphology of A2780 platinum sensitive (A2780) and resistant variant (A2780_CR5) OC cells. Scale bar indicates 100µm. **(B)** Side scatter of

FACS analysis of the percentage of ALDH(+) cells in A2780 and A2780_CR5. **(C)** The average fold change of mRNA expression of ALDH1A1, IL-6, IL-6R, Sox2 and Bmi-1 in A2780_CR5 over A2780 parental cells. 2 tailed student's *t*-test was used to analyze statistical significance (*P< 0.05, **P<0.01, and ***P<0.001, N=3). **(D)** Morphology of Kuramochi_ALDH(-) cells, and cisplatin (CDDP)-treated Kuramochi cells (IC_{50}, weekly, 3 weeks). Scale bar indicates 100μm. **(E)** mRNA expression of ALDH1A1 expression in CDDP-treated Kuramochi versus control Kuramochi cells (N=3, 2 tailed student's *t*-test). **(F)** Protein expression of pSTAT3, STAT3, IL-6 and beta-tubulin in Kuramochi and CDDP-treated Kuramochi cells (N=2). **(G)** A2780 OC cells were starved for 24 hours and then treated with IL-6 (100ng/ml) for 30 mins. Cells were collected at the indicated time points (0, 0.5, 1, 1,5, 2, 2.5, 3, 4, 12, 24, 48 hours) after IL-6 removal. The expression of pSTAT3, STAT3, Cyclin D1 and beta-tubulin were examined by western blot (images shown above) (N=1). **(H)** mRNA expression of ALDH1A1, IL-6 and IL-6R in A2780 OC cells treated with CDDP (IC_{50}, 3h), IL-6 (50 ng/ml), CDDP+IL-6 Nab (200 ng/ml) and IL-6+IL6Nab. 2 tailed student's *t*-test was used to analyze statistical significance (*P< 0.05, **P<0.01, and ***P<0.001, N=3). **(I)** Protein expression level of ALDH1A1 and GAPDH in OC cells (A2780) treated with IL-6 (50 ng/ml), CDDP (IC_{50}, 3h), IL-6 Nab (200 ng/ml), CDDP+IL-6 Nab or IL-6+IL-6 Nab are shown. Cells were collected 72 hours after exposure to CDDP (N=2).

Supplemental Figure S5. **IL-6 Nab-guadecitabine-combination depletes ALDH(+) OCSCs by inhibition of ALDH1A1 expression**. **(A)** Protein DNMT1 and GAPDH expression in Kuramochi derived ALDH(-) cells treated with IL6 (0, 100, 250 ng/ml)

(N=2). **(B)** Kuramochi cells transfected with stable shcontrol scrambled oligo, or shIL-6 RNA plasmid DNA. Protein expression of DNMT1 and GAPDH expression in OC Kuramochi cells. Samples were run separately on the same gel (N=2). **(C)** Side scatter of FACS analysis of the percentage of ALDH(+) cells in A2780_CR5 derived ALDH(+) cells which were treated with IL-6 Nab (1 μg/ml, 4Days), guadecitabine (100 nM, 4 Days), or IL-6 Nab + guadecitabine. Average percentage of ALDH(+) cells ± SD and quantification shown in bar graph (N=3, 2 tailed student's *t*-test); **(D)** the average fold change ± SD of mRNA expression of ALDH1A1 in A2780_CR5 derived ALDH(+) cells treated as in (A) (*P< 0.05, **P<0.01, and ***P<0.001, N=3). **(E)** ALDH1A1, DNMT1, pSTAT3, STAT3 and GAPDH levels determined by western blot (N=1). **(F)** Kuramochi cells were treated with cisplatin (half of IC_{50}), IL-6 Nab (1 μg/ml), guadecitabine (100 nM, 3 Days), cisplatin+guadecitabine, cisplatin+IL-6 Nab, cisplatin+IL-6 Nab+guadecitabine or guadecitabine+IL-6 Nab. ALDH1A1, DNMT1, IL-6, pSTAT3, STAT3 and beta-tubulin levels determined by western blot (N=2).

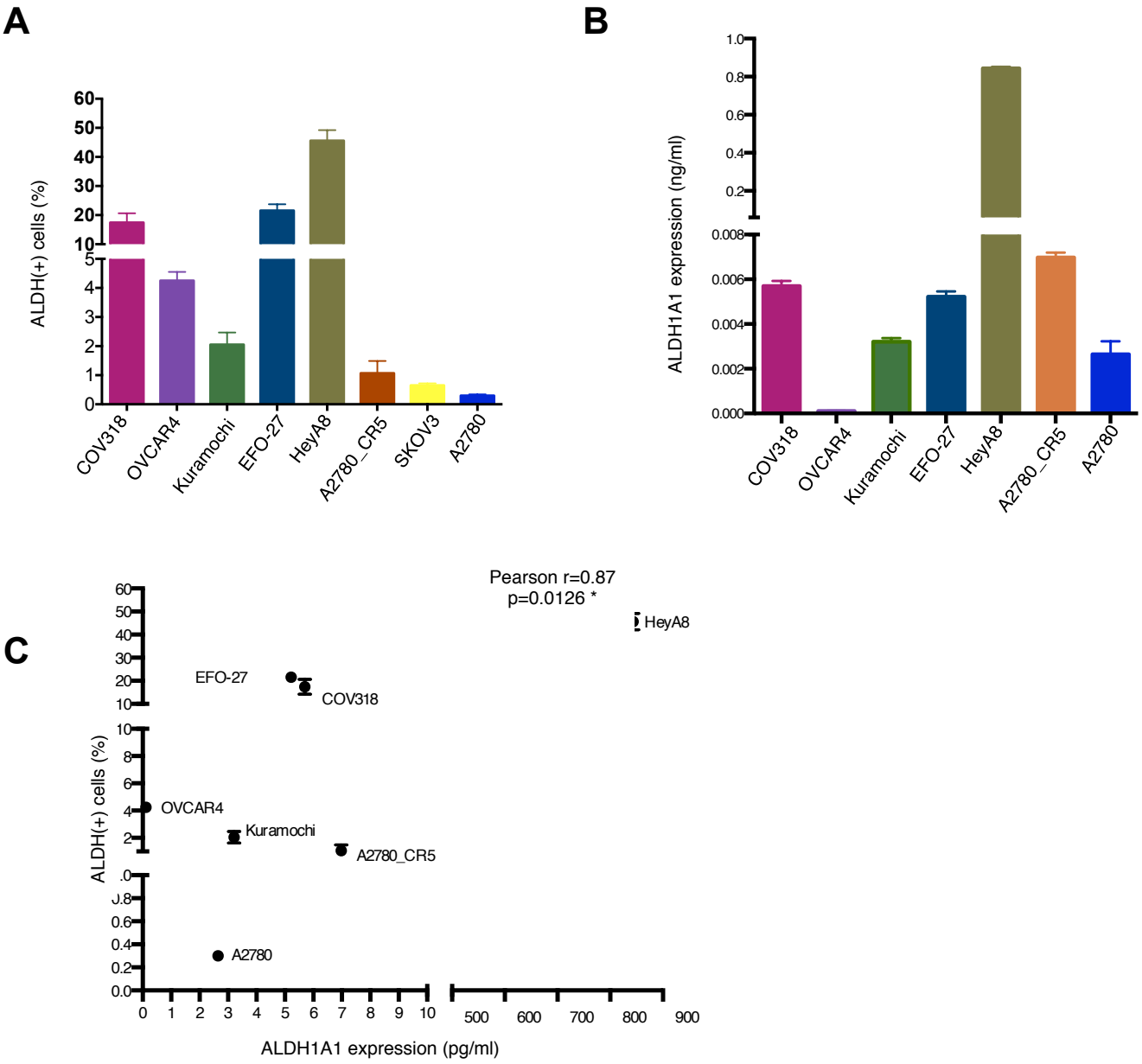
Supplemental Figure S6. IL-6 Nab-guadecitabine combination inhibits ALDH(+) OCSCs clonogenicity and re-sensitizes OC cells to cisplatin therapy. **(A)** Colony formation assay of 500 OC cells (Kuramochi) treated with cisplatin (3μM), IL-6 Nab (400ng/ml), guadecitabine (100nM, 3 days), IL-6 Nab + guadecitabine, guadecitabine + cisplatin, IL-6 Nab+cisplatin, or guadecitabine + IL-6 Nab + cisplatin. Cells were seeded in triplicate. Average total number of colonies ± SD is shown in the graph (N=3, 2 Way ANOVA, Dunnett's and Sidak's multiple comparisons tests were used for multiple comparison, *P< 0.05, **P<0.01, and ***P<0.001, ****P<0.0001). **(B)**

A2780_CR5_ALDH(+) cells were treated daily with IL-6 Nab (1μg/ml), guadecitabine (100 nM) or IL-6 Nab + guadecitabine for 4 days. 500 pretreated cells were reseeded into 6 well plates for clonogenic survival assays. The average number and of colonies formed by pretreated A2780_CR5_ALDH(+) cells with the conditions described previously and pretreated A2780_ALDH(+) cells after exposure to cisplatin (1μM) for 3 hours ± SD are shown in the graph (N=3). **(C)** Kuramochi_ALDH(+), **(D)** A2780_ALDH(+) cells were treated daily with IL-6 Nab (1μg/ml), guadecitabine (100nM) or IL-6 Nab + guadecitabine for 4 days. 1000 cells were seeded into 96 well plates for detection of drug-induced apoptosis measured in the cell lysates by caspase 3/7 activity. Average caspase 3/7 relative luciferase units (RLU) ± SD was normalized by the total number of cells (N=3, 2 tailed student's *t*-test, *P< 0.05, **P<0.01, and ***P<0.001).

Supplemental Figure S7. *In vivo* the combination approach of IL-6 Nab plus guadecitabine as maintenance therapy inhibits platinum-recurrent tumor. (A) Average body weight of mice bearing A2780 derived i.p. xenograft tumors treated with vehicle, carboplatin, carboplatin + vehicle, carboplatin + guadecitabine, carboplatin + IL-6 Nab and carboplatin + IL-6 Nab + guadecitabine (N=5/group). **(B)** Representative images of A2780 derived i.p. xenograft tumors treated with vehicle, carboplatin, carboplatin + vehicle, carboplatin + guadecitabine, carboplatin + IL-6 Nab and carboplatin + IL-6Nab + guadecitabine. **(C)** Representative images of total A2780 derived i.p. xenograft tumors/ mouse, which were treated with vehicle, carboplatin, carboplatin+vehicle, carboplatin+ guadecitabine, carboplatin+IL-6 Nab and carboplatin+ guadecitabine +IL-6 Nab.

Supplemental Figure S8. ***In vivo*** **anti-OCSCs effect of the combination approach of IL-6 Nab plus guadecitabine as maintenance therapy in platinum-treated tumor residuals.** **(A)** Side scatter of FACS analysis of the percentage of ALDH(+) cells in xenografts treated with vehicle, carboplatin, carboplatin+vehicle, carboplatin+ guadecitabine, carboplatin+IL-6 Nab or carboplatin+ guadecitabine +IL-6 Nab. Average percentage of ALDH(+) population ± SD is indicated in the graph (N=5/group). **(B)** Representative images spheroids formed by 10,000 cells dissociated from xenografts, which were treated with vehicle, carboplatin, carboplatin+vehicle, carboplatin+ guadecitabine, carboplatin + IL-6 Nab or carboplatin + IL-6 Nab + guadecitabine (N=5/group). Scale bar indicates 200μm. **(C)** Protein expression of ALDH1A1, DNMT1, pSTAT3, STAT3 and Beta-tubulin in xenograft tumors collected from the mice which were treated with vehicle, carboplatin, carboplatin +vehicle, carboplatin + guadecitabine, carboplatin + IL-6 Nab and carboplatin + guadecitabine+IL-6 Nab (N=2). **(D)** (Top) Schematic diagram describing the time points of blood sample collection. Blood samples were collected at baseline (Day 0), the day prior to platinum treatment (Day4), two days after 3-week- carboplatin treatment (Day21) and two days after two-week- maintenance therapy (Day 37). Relative IL-6 levels in plasma at collection time points (red arrows) determined by ELISA and shown in graph below. Bars represent average measurements ±SD; * $P < 0.05$ (N=3, 2 tailed student's *t*-test).

Supplemental Figure S1



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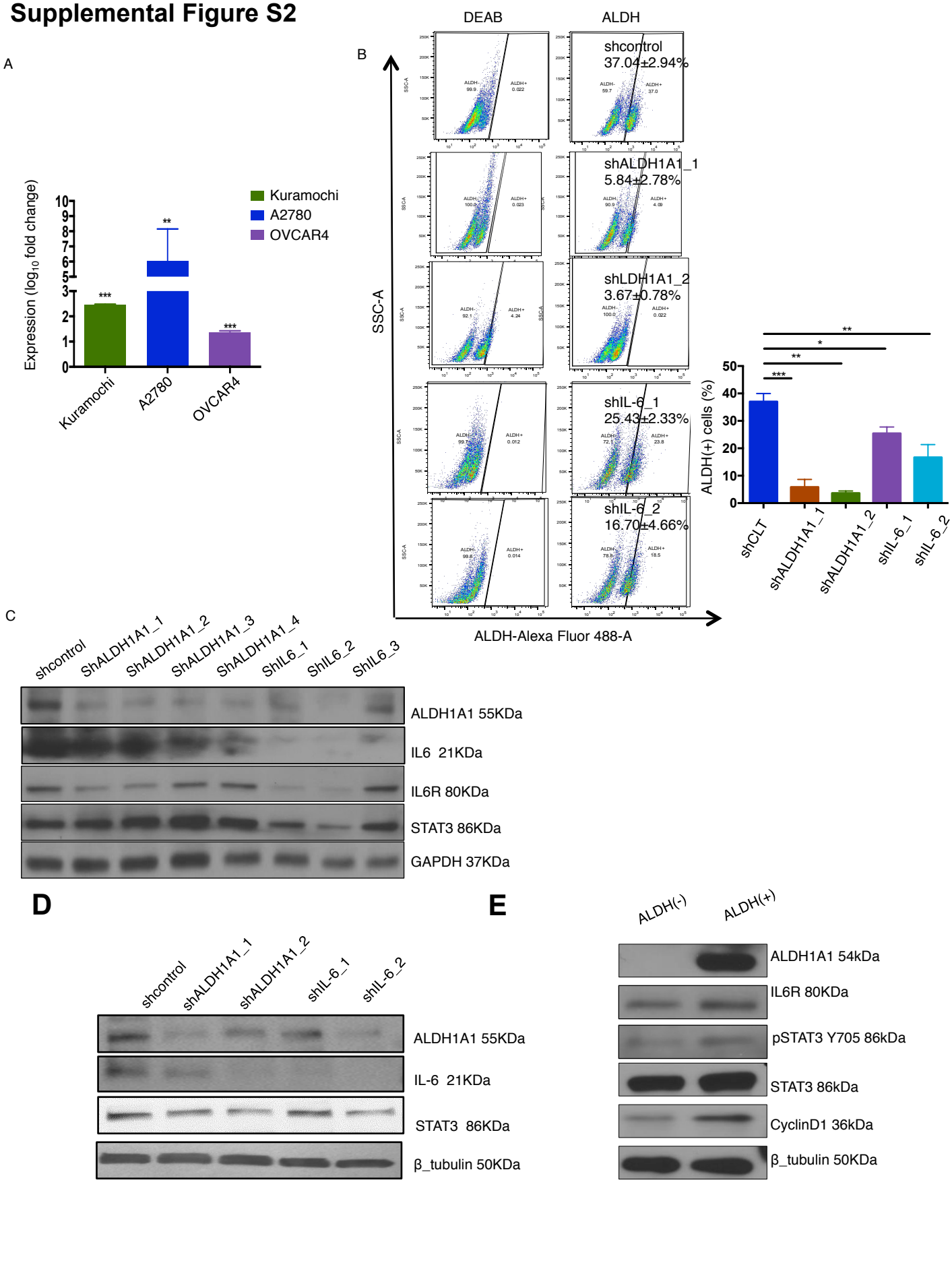

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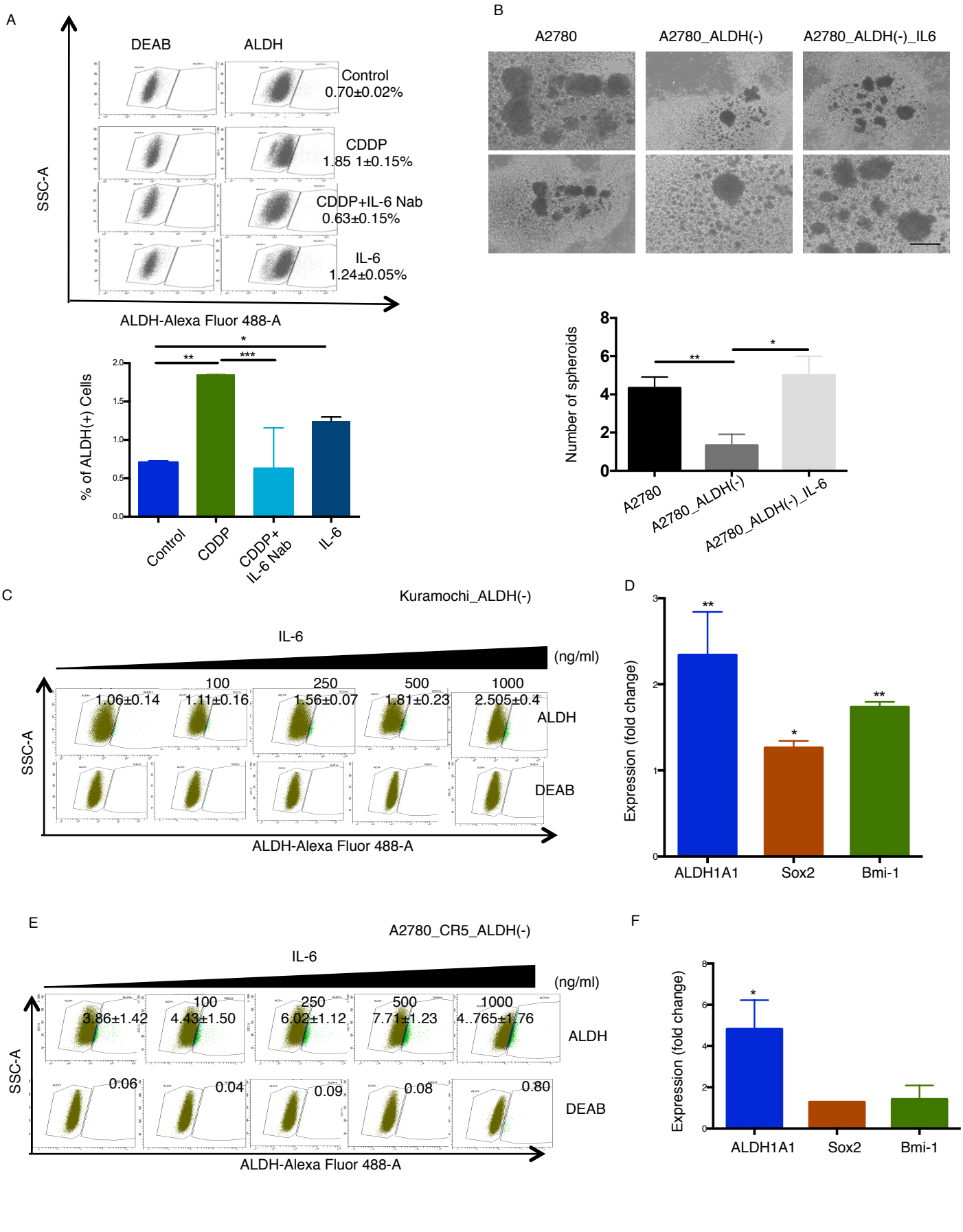

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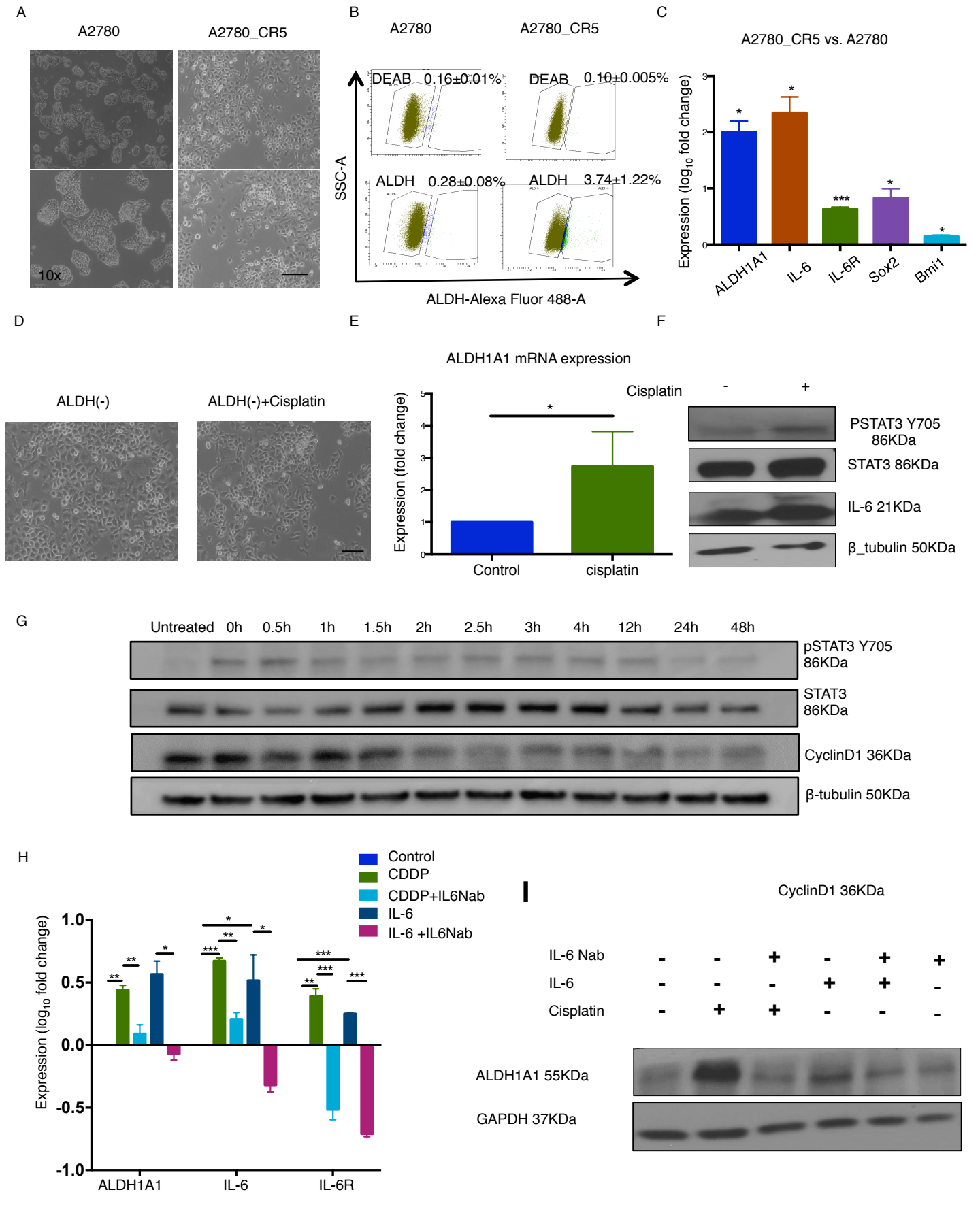

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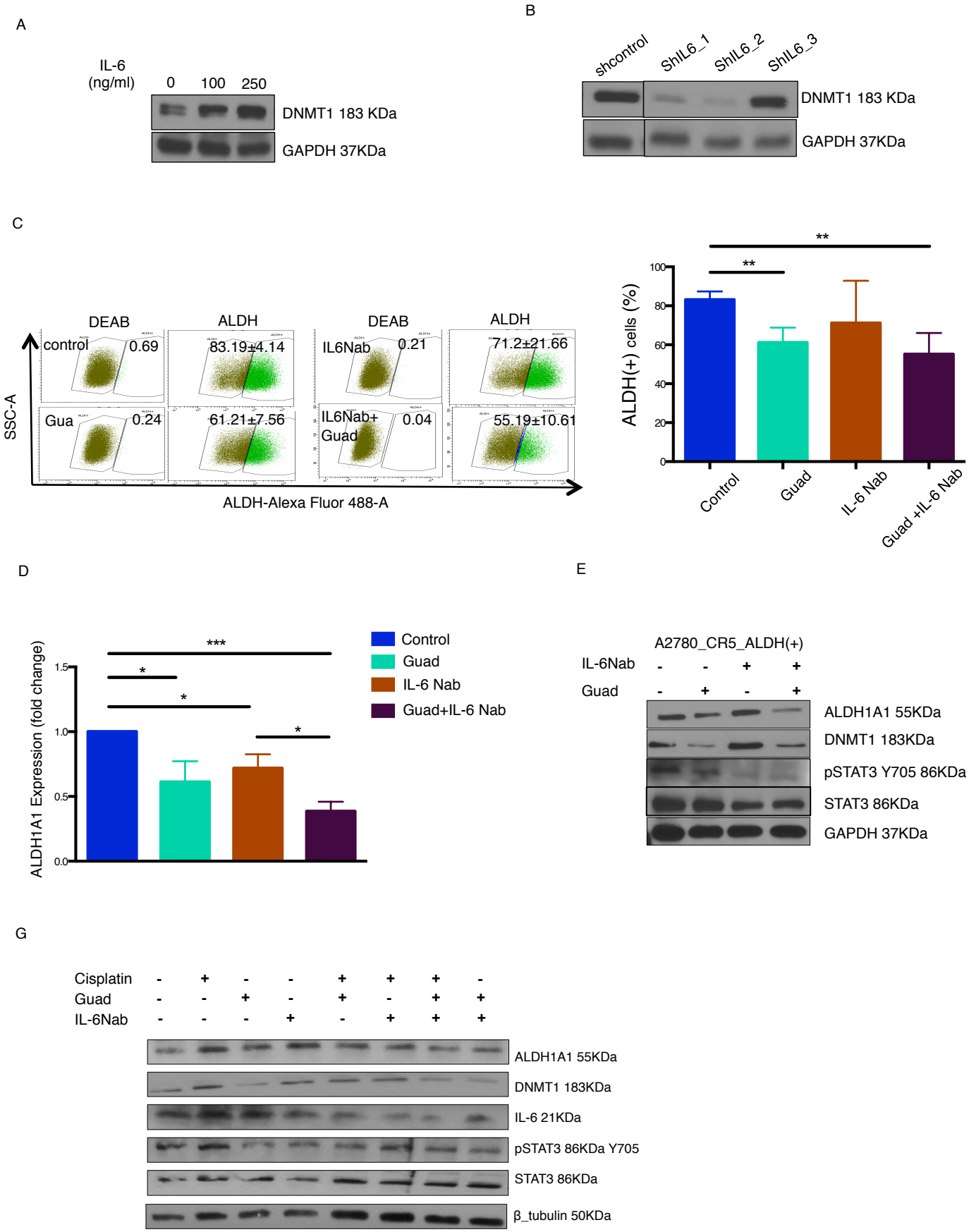

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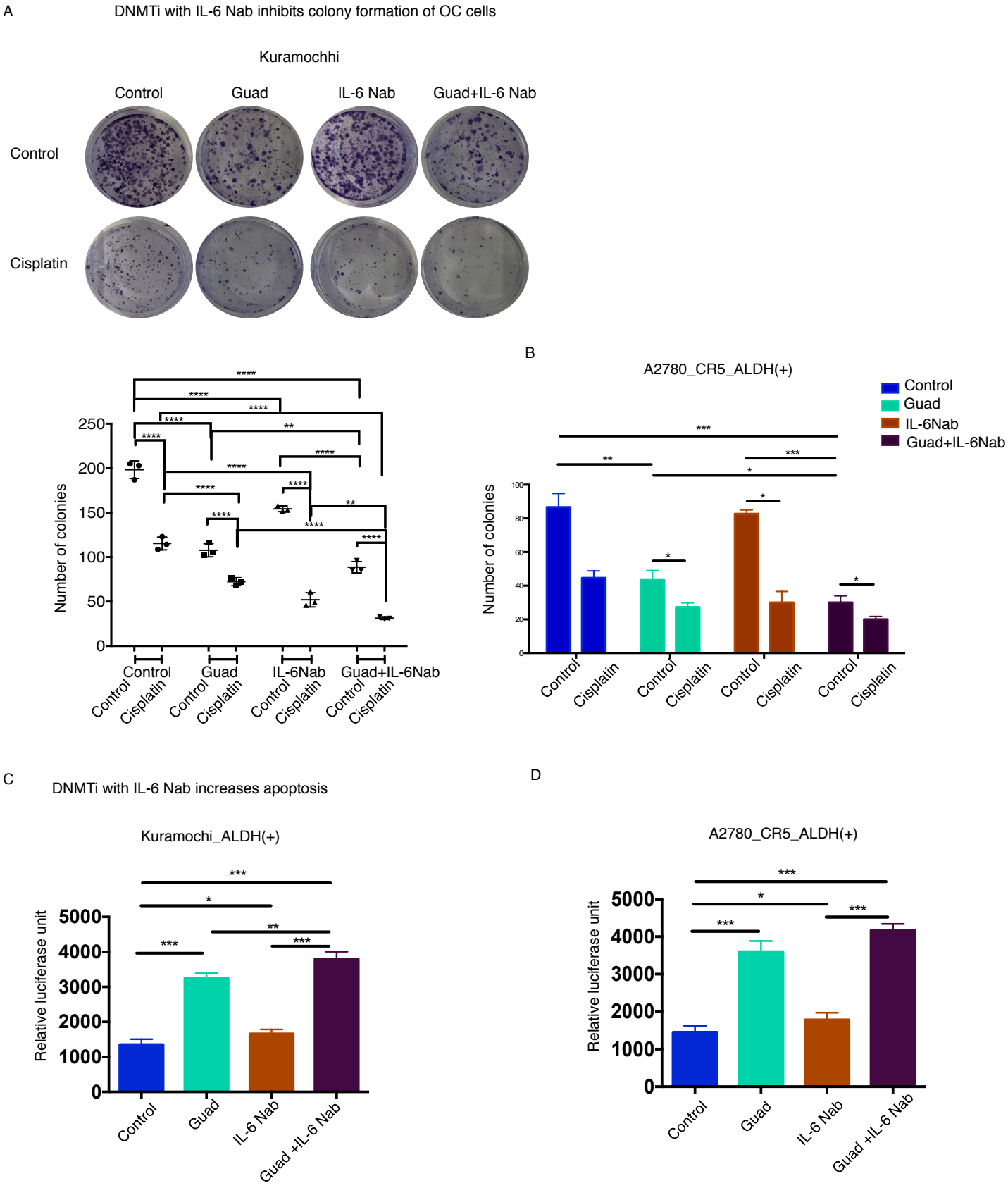

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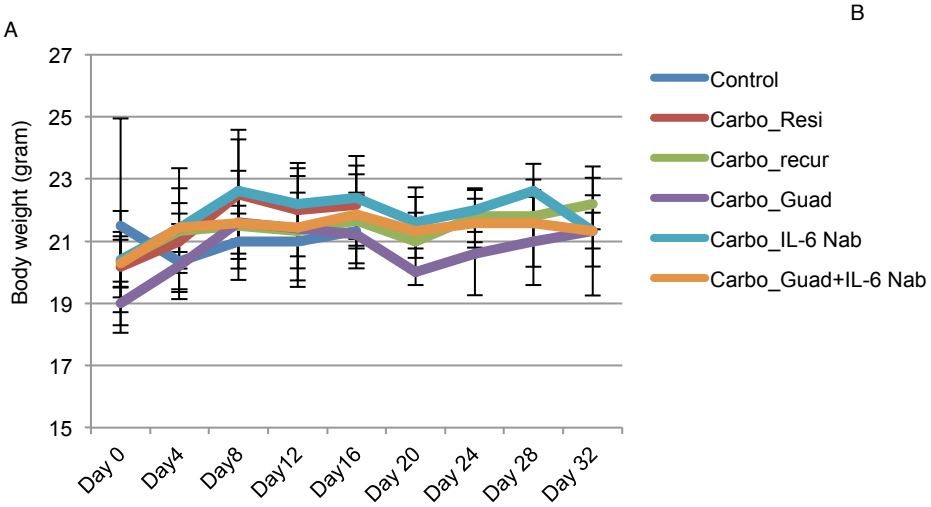

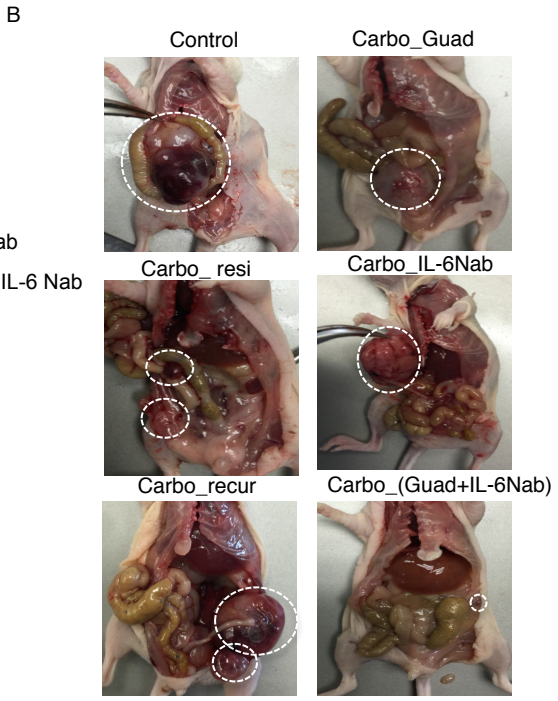

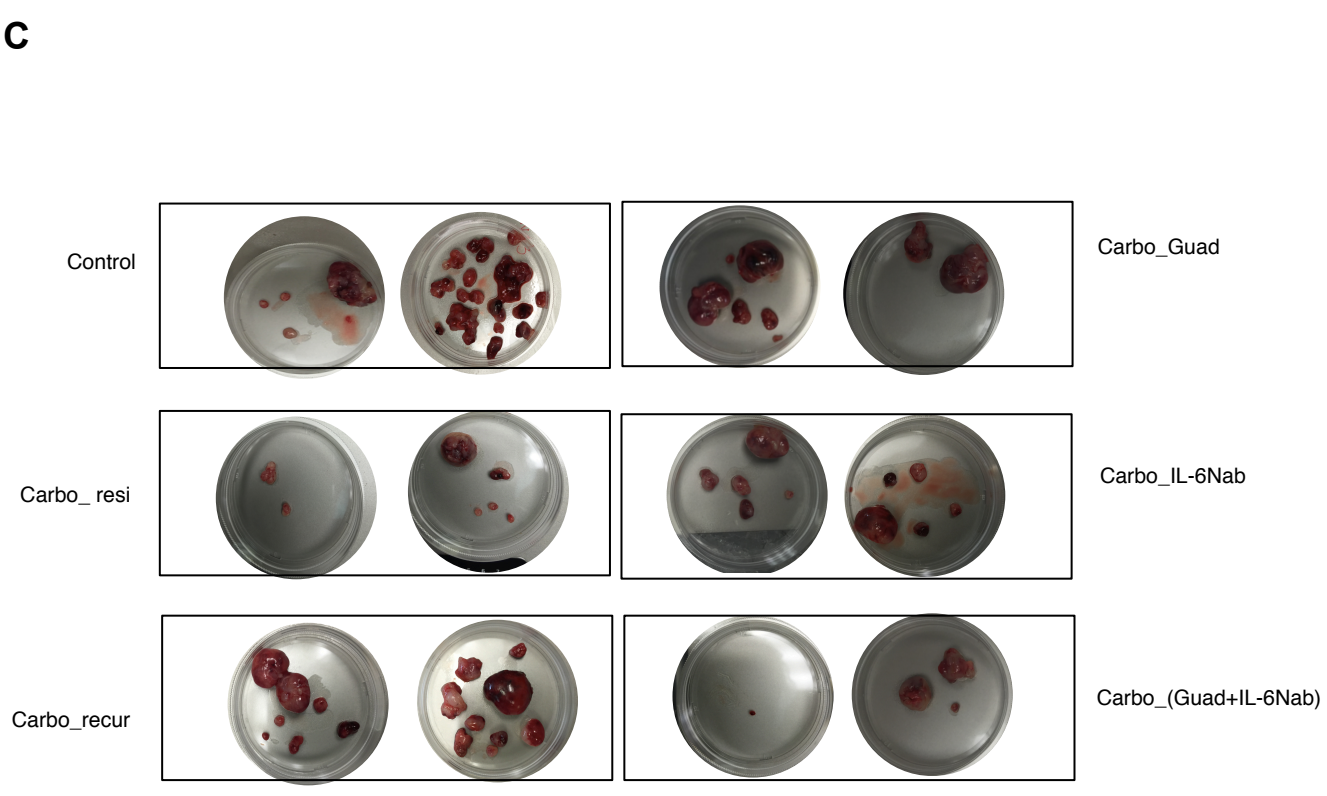

Supplemental Figure S8