Supplementary Figure Legends

Figure-S1 Molecular basis of ASS1 deficiency in myxofibrosarcoma: (A)

High-resolution oligonucleotide-based array comparative genomic hybridization
shows normal DNA copy number at loci spanning ASS1 gene at 9q34.1 in the vast
majority (14/15, 93.3%) of myxofibrosarcoma tissue and cell line samples, except for
one featuring hemizygous deletion. In contrast, there are frequent DNA losses,
including homozygous deletions near 9p21, on 9p in myxofibrosarcoma, which
almost involve the whole chromosomal arm. (B) Quantitative pyrosequencing
ascertains ASS1 methylation status detected by methylation-specific PCR. (a) Cell
lines samples display results concordant with those detected by methylation-specific
PCR. High degree of ASS1 promoter methylation is seen in NMFH-1 (left upper) and
OH931 (right upper) myxofibrosarcoma cells, with the percentage of methylation
>50% as shown at most representative CpG islands. Despite barely detectable ASS1
expression at the mRNA and protein levels in Figure-1B, there are comparatively less
methylated CpG islands in NMFH-2 cells by pyrosequencing (data not shown),
corresponding to presence of both methylated and unmethylated bands with similar
intensity by methylation-specific PCR in Figure-1C. In contrast, there is no
remarkable DNA methylation found in CCD966SK fibroblasts (left lower) and
LPS510 dedifferentiated liposarcoma cells (right lower). (b) Formalin-fixed tissue
samples of 10 cases with informative methylation-specific PCR data are selected for
confirmatory pyrosequencing. Representative cases of highly methylated (MFS7, left)
and hypomethylated (MFS25, right) tumors in pyrosequencing are illustrated, which
show only methylated or unmethylated bands by methylation-specific PCR,
respectively. Both methods of methylation assessment reveal concordant results in
general, as summarized in Table-S2. (C) ASS1 mRNA expression in NMFH-1 cells
is not restored with separate or combined use of trichostatin, 3-Dznep, UNC0638,
**and/or EZH2 knockdown.** (a) By quantitative RT-PCR, neither 3-Dznep nor UNC0638 could recover ASS1 mRNA in NMFH-1 cells, regardless of the presence or absence of trichostatin and/or 5-aza-2′-deoxycytidine. 3-Dznep is a novel inhibitor that suppresses H3K27 histone methyltransferation mediated by polycomb recessive complex 2-associated EZH2, while UNC0638 is an inhibitor that targets G9a-mediated H3K9 histone methyltransferation. (b) Similar to 5-aza-2′-deoxycytidine alone, ASS1 mRNA expression level in NMFH-1 still remains barely detectable when the cells are alternatively or additionally treated with trichostatin, irrespective of the presence or absence of stable silencing of EZH2 expression by shEZH2. **Inset:** Western blotting and RT-PCR cross validate successful suppression of EZH2 expression with RNA interference. Histone H2B nuclear protein, shLacZ, and POLR2A transcript are run in parallel as the controls in Western blotting, RNA interference, and quantitative RT-PCR assays, respectively.

**Figure-S2 ADI-PEG20 treatment in myxofibrosarcoma cell lines and xenografts:**

(A) **Pharmacokinetic curve of ADI-PEG20** demonstrates its dose-dependent capability in deprivation of arginine in culture medium of OH931 cells within the range from 0 to 100 ng/ml. (B) **Macroscopic appearances of xenograft specimens treated with PBS versus various doses of ADI-PEG20.** After 28 days of treatment, mice are scarified and tumor sizes are measured, demonstrating apparent regressing fibrotic appearance in the ADI-PEG20-treated groups even at a dose as low as 2.5 IU/kg. (C) **ADI-PEG20 treatment impairs cell proliferation of myxofibrosarcoma and causes S phase arrest of cell cycle.** (a) By using an ELISA-based, colorimetric assay to assess the rate of BrdU uptake, cell proliferation is significantly reduced in OH931 (upper), NMFH-1 (middle), and NMFH-2 (right) cell lines incubated with 150 IU of ADI-PEG20 for 72 h, as compared to the corresponding PBS-treated controls. (b) As analyzed by flow cytometry for OH931 cell line, cell cycle distribution in one
each representative experiment is demonstrated for treatment with vehicle alone (upper), 75 IU of ADI-PEG20 (middle), and 150 IU of ADI-PEG20 (lower), showing dose-dependent S phase arrest induced by ADI-PEG20.

Figure-S3 Validation of anti-angiogenic mediators and anti-proliferative function of ASS1: (A) ELISA assay to measure secreted protein concentration in the conditioned media of ASS1-reexpressing versus control transfectants of myxofibrosarcoma. (a) The histogram shows statistically significantly decreased protein concentration of MMP-9 in ASS1-reexpressing group, which is a consistent phenomenon in all three cell lines. (b) By contrast, this finding of lowered concentration in the ASS1-reexpressing group was not corroborated for other proteins analyzed. For instance, CXCL10 only displays decreased concentration in the conditioned medium of ASS1-reexpressing NMFH-2 cells, unlike the results of corresponding Western blotting assays for cell lysates seen in Figure-5B. Experiments are performed in triplicate and results expressed as mean ± SD. (B) ASS1 reexpression abolishes cell proliferative activity of myxofibrosarcoma cells. By using an ELISA-based, colorimetric assay to assess the rate of BrdU uptake, cell proliferation is significantly reduced in stable ASS1-transfected NMFH-1 (left upper), OH931 (right upper), and NMFH-2 (left lower) cell lines compared to the corresponding empty controls. In addition, the cell number is significantly decreased in NMFH-2 cells stably transfected with the ASS1-expressing plasmid as detected in a real-time manner by using ECIS technique (right lower).

Figure-S4 ASS1 reexpression induces cell cycle arrest at the G1 phase in myxofibrosarcoma cells with consistent downregulation of cyclin E expression.

Left panel: As analyzed by flow cytometry, cell cycle distribution in one each representative experiment is shown for OH931 (upper), NMFH-1 (middle), and NMFH-2 (lower) cells stably transfected with either ASS1-expressing or empty
control vectors. Right panel: In the corresponding histograms, a significant increase of tumors cells distributed at the G1 phase with concomitantly decreased cell percentages at the S and G2 phases are detected in OH931 and NMFH-2 cell lines, while this effect on cell cycle is not apparent in NMFH-1 cell line. Error bars denote the range of values derived from triplicate experiments. Lower panel: Western blotting assays against cyclins and cyclin-dependent kinases associated with regulation of G1 phase and G1/S transition show consistent suppression of cyclin E in all three myxofibrosarcoma cell lines upon stable transfection of ASS1. However, CDK2 is randomly downregulated in ASS1-reexpressing NMFH-2 cells alone.

Figure-S5 ASS1 reexpression attenuates cell migration and invasiveness of myxofibrosarcoma cells. (A) Wound healing assay (left panel) reveals that the capability of wound closure is significantly impaired in both stable ASS1-transfected OH931 (upper row), NMFH-1 (middle row), and NMFH-2 (lower row) cell lines, indicating the anti-migratory function of ASS1. Experiments are performed in triplicate and results expressed as mean ± SD (Right panel). (B) Results of transwell invasion assay reveal that the capability of cell invasion is significantly impaired in both stable ASS1-transfecting OH931 (left panel) and NMFH-1 (right panel) cell lines, indicating the inhibitory role of ASS1 in invasive function of sarcoma cells.

Figure-S6 ASS1 knockdown promotes cell proliferation, viability, migration, and invasion in LPS510 cells that endogenously express ASS1. (A) Among the 4 liposarcoma cell lines tested, endogenous ASS1 mRNA and protein are only readily detected in LPS510 cells by quantitative RT-PCR (upper) and Western blotting assays (lower), respectively. CCD966SK fibroblasts are run in parallel as a positive control. (B) Stable silencing of ASS1 gene with shASS1 in LPS510 cells show significantly downregulated expression at the mRNA and protein levels as detected by real-time RT-PCR (upper) and Western blotting (lower) assays, respectively. The shLacZ
plasmid, *POLR2A* transcript, and GADPH protein are utilized as controls in RNA interference, quantitative RT-PCR, and Western blotting assays, respectively. (C) Results of Brdu (*left upper*), XTT (*right upper*), transwell migration (*left lower*), and matrigel invasion (*right lower*) assays reveal that the functions of cell proliferation, cell viability, cell motility, and cell invasiveness are significantly impaired in *shLacZ* control (all $p < 0.05$) as compared to the LPS510 cells stably silenced against ASS1 expression. All functional experiments are performed in triplicate and results expressed as mean ± SD.