Double negative feedback loop between reprogramming factor LIN28 and microRNA *let-7* regulates aldehyde dehydrogenase 1-positive cancer stem cells

Supporting Figure Legends

Figure S1. *Let-7* was stably or inducibly expressed in tumor cells by viral infection

A. Illustration of the expression vector, pmiRvect-let7, which was used to stably express *let-7* in tumor cells. **B.** Illustration of the expression vector, pRev-DsRed-*let-7*, which was used to inducibly express *let-7* in tumor cells. **C**. Polyclonal tet-on *let-7* inducible HeLa cells were treated with 1,000 ng/ml doxycycline. The reporter gene, DsRed, was observed in more than 30% of the cells by fluorescence microscopy. **D**. FACS analysis showing that the dose-dependent response of DsRed expression in the tet-on *let-7* HeLa cells. **E**. Real-time RT-PCR showing that the dose-dependent response of mature *let-7* expression in the tet-on *let-7* HeLa cells.

Figure S2. Full-length Western blots

A. Full-length Western blots of Figure 2A. **B**. Full-length Western blots of Figure 2C. **C**. Full-length Western blots of Figure 6B.

Figure S3. LIN28 functionally regulates ALDH1^{br} cancer stem cell population *in vivo*

A. Six to eight week old female immune deficient mice were used in these studies. Using lentiviral shRNA vectors, LIN28 was stably knocked down in A2780 cells (Figure 2A), and then 1×10^5 LIN28 shRNA cells or control cells were injected subcutaneously into the flanks of these mice. Once tumors were detectable, tumor size was measured daily. **B.** The mice were sacrificed 6 weeks after injection, and fresh specimens of the xenograft tumors were collected. The percentage of ALDH1^{br} tumor cells in the xenograft specimens was measured using the ALDEFLUOR assay. Knockdown of LIN28 expression in these cells significantly decreased the brightly fluorescent ALDH1 (ALDH1^{br}) cell population *in vivo* ($p<0.05$). **C.** The tumors with LIN28 knockdown were significantly smaller than the control tumors ($p<0.05$).

Figure S4. LIN28 regulates cancer stem cell population *in vitro*

A. Knocking down LIN28 expression significantly reduced the CD133$^+$ cell population in A2780 cells. The percentage of CD133$^+$ tumor cells in the A2780 cell line was measured by FACS analysis. Knock down of endogenous LIN28 expression via shRNAs significantly reduced the percentage of CD133$^+$ tumor cells ($p<0.05$). **B.** Overexpression of LIN28 significantly increased the CD24^{low}/CD44^{high} cell population in MCF7 cells. The percentage of CD24^{low}/CD44^{high} tumor cells in the MCF7 cell line was measured by FACS analysis. Overexpression of LIN28 significantly increased the percentage of CD24^{low}/CD44^{high} tumor cells ($p=0.001$).

Figure S5. Knocking down LIN28 expression in T47D cells remarkably increased *let-7* activity

A. A luciferase *let-7*b sensor assay, containing a constitutively expressed firefly luciferase reporter bearing sequences complementary to *let-7*b in the downstream 3'UTR, was used to monitor *let-7*b activity in T47D cells. **B**. Knocking down endogenous LIN28 expression significantly reduced luciferase activity of the *let-7*b sensor, indicating that *let-7*b function was remarkably increased in the LIN28 knockdown cells (T47D).

Figure S6. Overexpression of LIN28 significantly increased progenitor cell population in primary cultured murine mammary gland epithelial cells

A. The percentage of $CD24^{low}/CD49f^{high}$ cells in primary cultured murine mammary gland epithelial cells was measured by FACS analysis. Overexpression of LIN28 significantly increased the percentage of $CD24^{low}/CD44^{high}$ mammary gland epithelial progenitor cells ($p<0.05$). **B.** LIN28 expression regulates the number of mammospheres in mammary gland epithelial cells ($p<0.05$).