

Figure S1. Lin28 and Oct4 expression in hES cells and EC cells. Immunofluorescence was performed on H1 and PA-1 cells using anti-Oct4 (red) and anti-Lin28 (green) antibodies. Dapi staining (blue) indicates cell nuclei. Panels a-d, staining of H1 cells; e-h, staining of PA-1 cells. Panels a and e, cells stained with Dapi; b and f, cells stained with anti-Oct4; c and g, cells stained with anti-Lin28. Panel d is a merge of a-c, and h, a merge of e-g. Photomicrographs taken at x400 magnification.

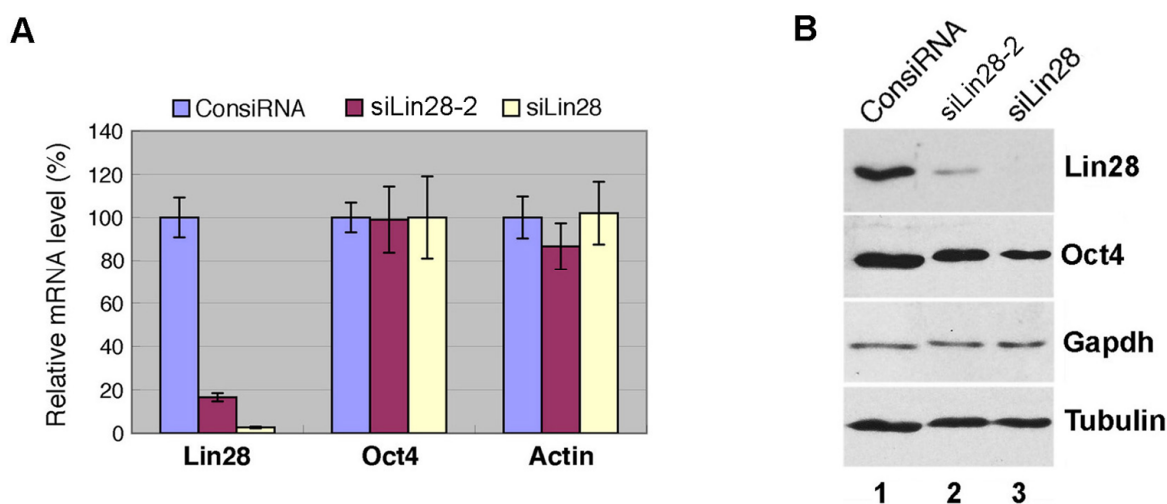


Figure S2. PA-1 cells were transfected with control siRNA (consiRNA) or Lin28-specific siRNA (siLin28 or siLin28-2). Seventy-two hours later, RNAs were extracted from transfected cells, and the levels of mRNAs (Lin28, Oct4, and beta-actin) measured by RT-qPCR. In the left panels, relative mRNA levels are shown after normalization against beta-tubulin control. mRNA levels in cells transfected with consiRNA were arbitrarily set as 1. Numbers are mean \pm SD (n=3). In the right panels, in lanes 1, 2 and 3, cells were transfected with consiRNA, siLin28-2, and siLin28, respectively. The antibodies used in the Western blot analyses are labeled on the right. Protein levels on Western gels were determined using Bio-Rad Quantity One software, and calculated after normalization against beta-tubulin loading control.

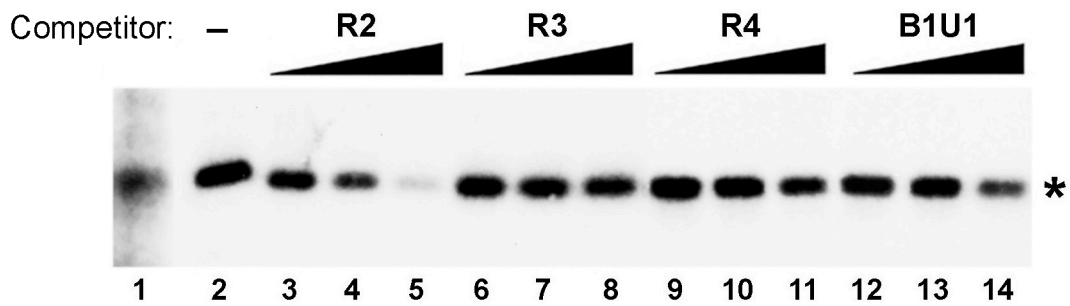


Figure S3. XL and competition assays. Flag-Lin28 was transfected into HEK293 cells and cell extract prepared. XL were carried out using radioactively labeled R3 RNA in the absence (lane 2) or presence of increasing amounts of unlabeled competitor R2 (lanes 3-5), R3 (lanes 6-8), R4 (lanes 9-11), and B1U1 (lanes 12-14), followed by IP using anti-Flag antibody. The unlabeled RNAs were at 7 (lanes 3, 6, 9, 12), 21 (lanes 4, 7, 10, 13), and 63 (lanes 5, 8, 11, 14) molar excess relative to the labeled R2 RNA. 5% of total crosslinked product (lane 1) and anti-Flag IP product were resolved by SDS-PAGE, followed by autoradiography. The bands marked with * indicate Flag-Lin28.

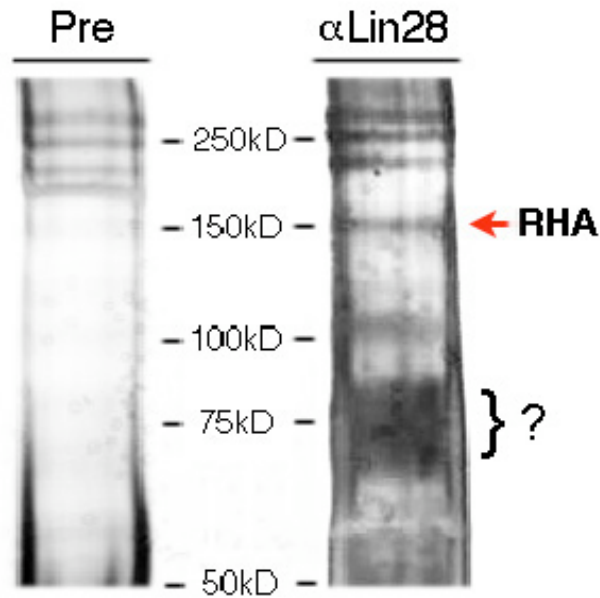


Figure S4. A silver-stained SDS-PAGE image of protein samples derived from co-IP using pre-immune (Pre) or anti-Lin28 (αLin28) antibody. The band marked by the red arrow was excised from the gel and subjected to mass spectrometric analysis. Numbers are molecular size markers. The identities of the bands in the 75 kDa range marked by a “?” are currently under investigation.

