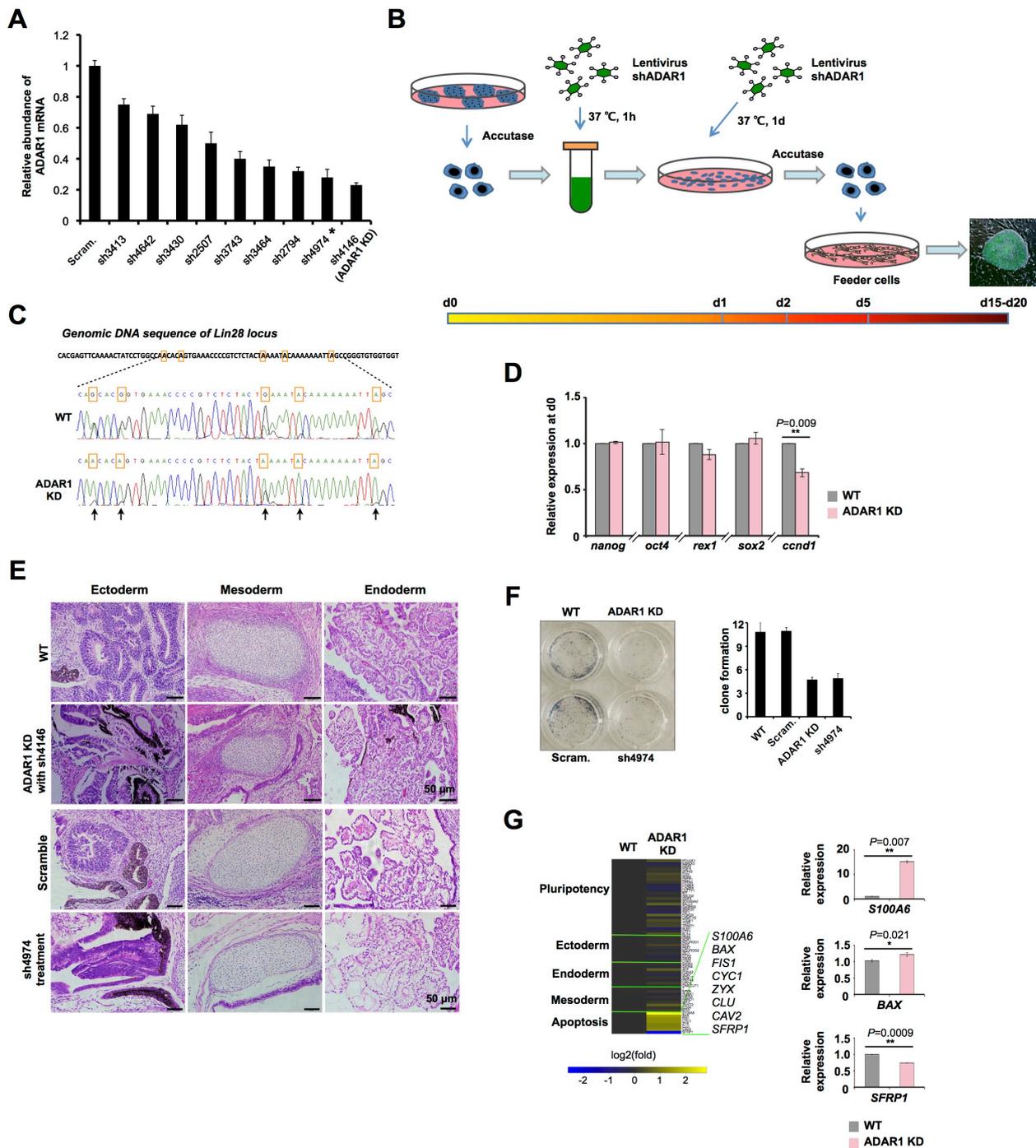


Supplemental Figures and Figure Legends



Supplemental Figure S1. Generation and characterization of ADAR1 stable knockdown H9 lines.

(A) Selection of efficient shRNAs for human ADAR1. Nine shRNAs were designed to target different sequences of ADAR1 and tested for their activity in SH-SY5Y cells. A new

shRNA, sh4146 (KD) and a reported shRNA, sh4974(Toth et al., 2009) were selected to knock down ADAR1 expression in H9 cells.

(B) A schematic view of the procedure for stable ADAR1 knockdown H9 cell lines selection by lentiviral particles infection.

(C) RNA editing analysis confirmed the knockdown of ADAR1 in H9 cells. The known ADAR1 substrate(Chen and Carmichael, 2009), *AluSc* in the 3'UTR of *Lin28* mRNA was analyzed in the WT control and ADAR1 KD H9 lines. Note that after knockdown of ADAR1, all examined A-to-I editing ratios in *AluSc* were significantly reduced (in red boxes and black arrowheads), demonstrating the successful generation of the ADAR1 KD H9 cells.

(D) RT-qPCR revealed that knockdown of ADAR1 in H9 cells did not affect the expression of pluripotency-related marker genes.

(E) ADAR1 KDs did not affect the pluripotency of hESCs, as revealed by teratoma assays and H/E staining.

(F) ADAR1 knockdown reduced colony formation of H9 cell lines. Left panels, representative images of colony formation from control and two ADAR1 KD H9 cell lines. Right panel, the efficiency of colony formation in different H9 cell lines (n>200).

(G) RNA-seq (left) and RT-qPCR analyses revealed that knockdown of ADAR1 has little effect on pluripotency, but induces apoptosis in H9 cells. Left, Heatmap of affected apoptosis-related genes and known pluripotency-/lineage-specific marker genes in ADAR1 KD H9 cells is shown (log₂ ratio). Note that pro-apoptotic genes, such as BAX(Pawlowski and Kraft, 2000), were up-regulated, while anti-apoptotic genes, such as SFRP1(Han and Amar, 2004; Joesting et al., 2005), were down-regulated in ADAR1 KD H9 line. Right panels, RT-qPCR analyses confirmed the RNA-seq data shown in left. Error bars represent \pm SD in triplicate experiments. *P* values from one-tail t tests are shown.