



Supplemental Figure S2. Analyses of ADAR1 stable knockdown H9 lines upon differentiation.

(A) A schematic view of forebrain neuron (FB) and motor neuron (MN) differentiation from hESCs (Top). Bottom, representative images of hESCs at critical neural induction steps upon lineage-specific differentiation in the WT control and ADAR1 KD2 H9 lines. Green fluorescence (GFP) from d0 to d35 in ADAR1 KD2 H9 lines indicated the persistent expression of shRNAs targeting ADAR1.

(B) Knockdown of ADAR1 led to morphologically aberrant EBs. Left, representative images of EBs induced from control and two ADAR1 knockdown H9 cell lines. Right, the percentage of morphologically normal EBs in controls and two ADAR1 knockdown H9 cell lines (n>200). Note that healthy cell aggregates (EBs) are bright, clear and round (in WT and Scram. groups), while numerous unhealthy EBs (green arrows) with rough edges were observed in two ADAR1 knockdown lines. Morphologically healthy EBs were manually picked up for the subsequent attachment and neural induction.

(C) Knockdown of ADAR1 did not alter the expression of neural genes that specify regional identity, although the maturation of FB neurons was repressed. Left, the expression of *drd2*, a DAergic marker(Korecka et al., 2013), in control and ADAR1 KD H9 lines upon FB neuron induction; Right, the expression of *hoxb6*, a marker for hindbrain and spinal cord(Oosterveen et al., 2003).