Title: AMD1 IS ESSENTIAL FOR ES CELL SELF-RENEWAL AND IS TRANSLATIONALLY DOWN REGULATED ON DIFFERENTIATION TO NEURAL PRECURSOR CELLS

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Supplemental Information

Figure S1. Induction of neural progenitor cells.

S1A. Immunofluorescence staining for ESC marker Oct4 and NPC markers Nestin and Sox2 following differentiation to NPCs. Sox1 is GFP-tagged. Scale bars: 20uM. S1B. qRT-PCR of pluripotency markers and neural differentiation markers in ESC and NPCs. S1C. Flow cytometry profile of Sox1-GFP cells following differentiation to NPCs. S1D. Quantification of Sox1-GFP cells during NPC differentiation. S1E. Tuj1 and GFAP staining of neurons and astrocytes respectively differentiated from NPCs.

Figure S2. Validation of Microarray data.

Expression levels of 40 mRNAs were measured using qRT-PCR in the different polysomal groups. The correlation coefficient was calculated between microarray and qRT-PCR data.

Figure S3. Luciferase mRNA levels of translationally controlled candidates.

qRT-PCR showing the relative levels of luciferase RNA following transfection into ESCs and NPCs. Firefly luciferase mRNA levels are normalized to control Renilla levels.

Figure S4. Amd1 over-expression in NPCs.

Immunofluorescence staining for the NPC markers Nestin and Sox1 following differentiation to NPCs after transfection with the Amd1 over-expressing vector or empty vector. Scale bars: 20uM.
Figure S5. miR-762 targeting the Amd1 3’UTR.

**S5A.** qRT-PCR showing the relative expression level of miR-762 in ES cells, NPCs, EBs and BMP4 treated NPCs. **S5B.** Relative luciferase assays for the constructs shown in Figure 5B after transfection into HeLa cells. Values are normalized to control luciferase and empty vector. **S5C.** qRT-PCR analysis of the relative luciferase mRNA levels after transfection into HeLa cells. Renilla luciferase is normalized to firefly luciferase mRNA levels. **S5D.** A bar chart showing the effect on luciferase activity of the Amd1 3’UTR in ES cells, NPCs, and BMP4 treated NPCs.

Figure S6. Expression of miR-762 mimic and siRNA knockdown of Amd1 in ES cells.

**S6A.** qRT-PCR analysis of mRNAs 3 days after transfection of ES cells with negative control mimics or with miR-762 mimic with or without the addition of spermine (SPM) or spermindine (SPD). **S6B.** Immunofluorescence staining for ESC marker Sox2 in ES cells transfected with pools of siRNA targeting Amd1 or scrambled siRNA. Scale bars: 20μM.