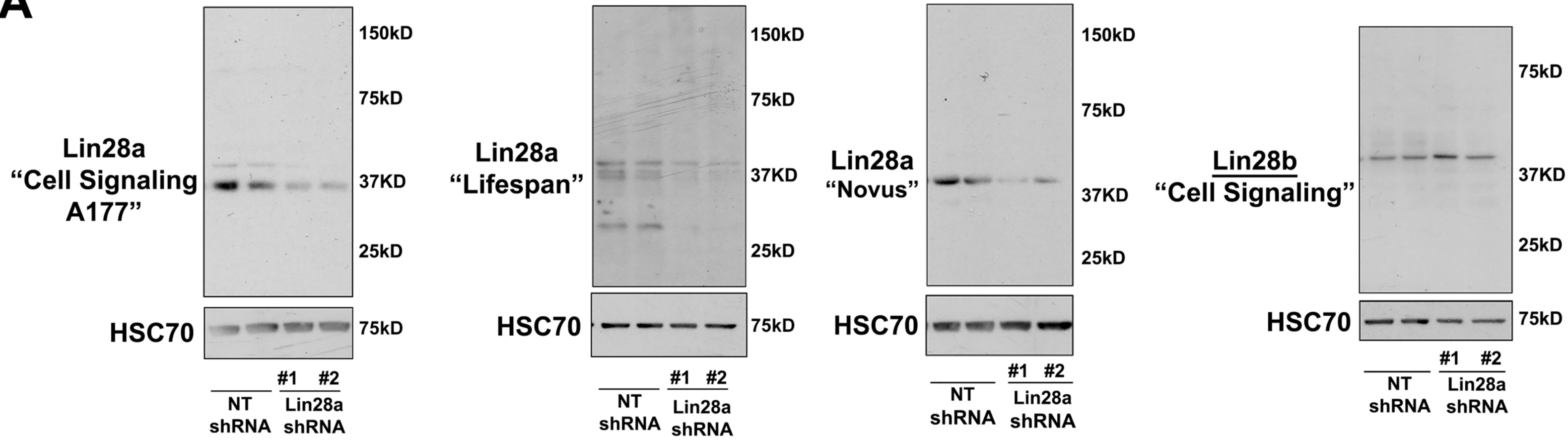
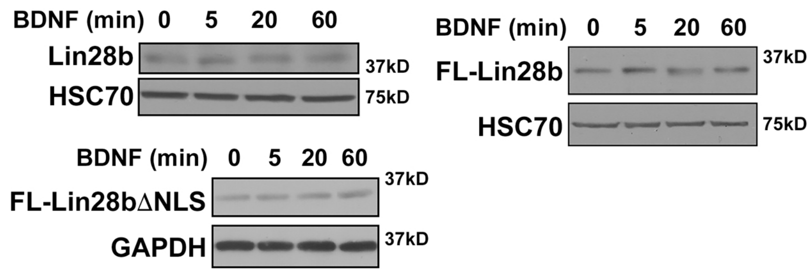
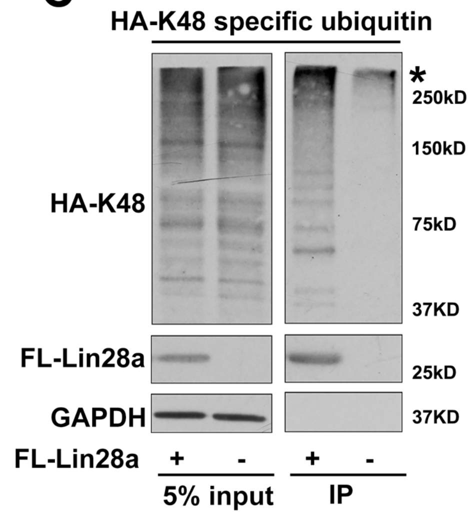
S1



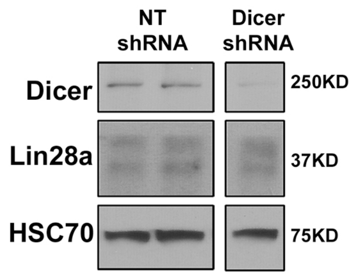
B

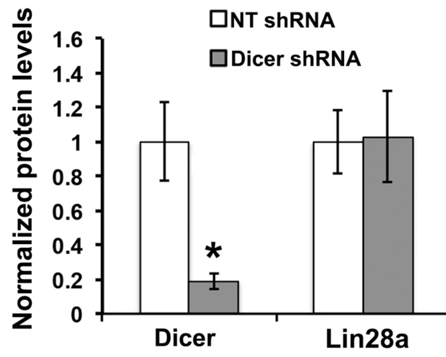


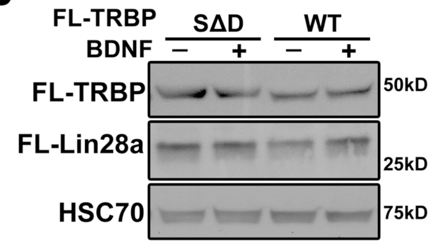
C

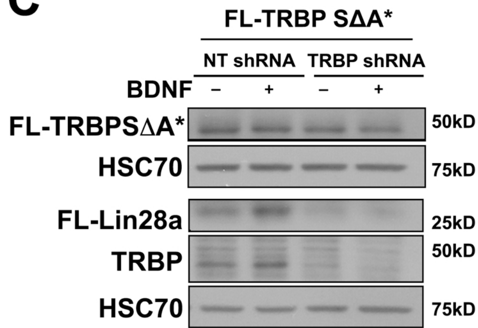


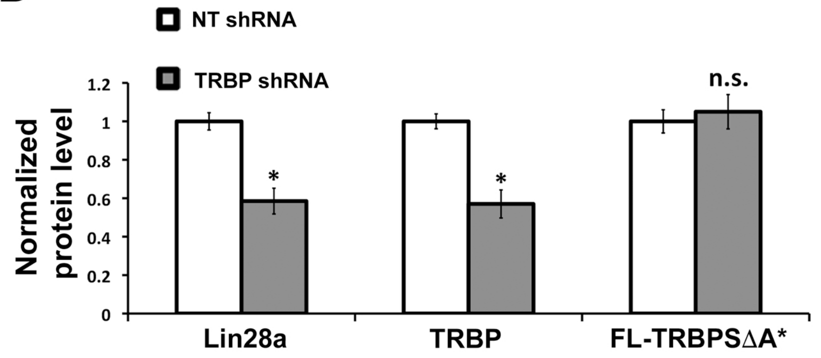
S2



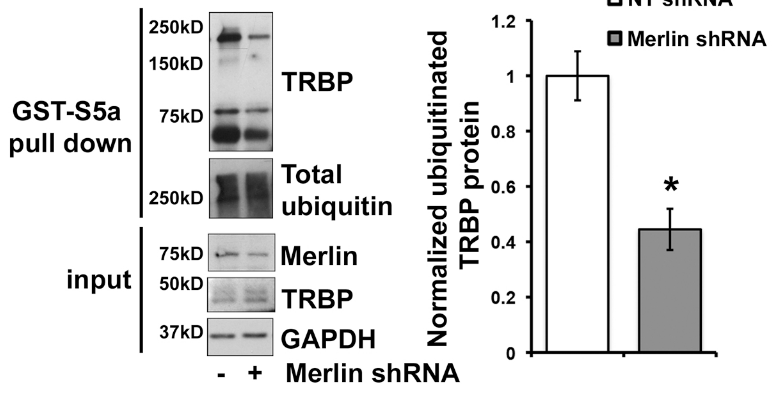


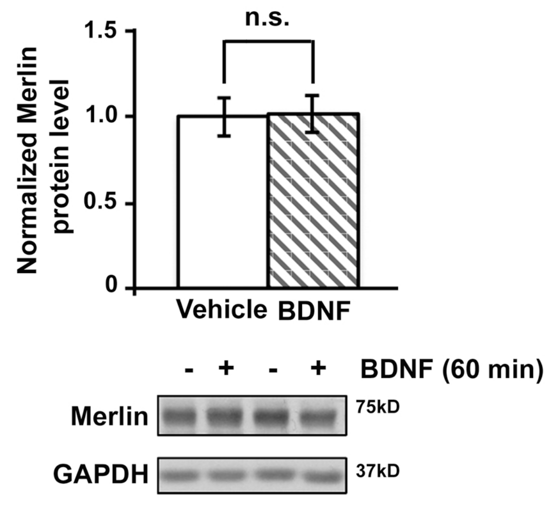




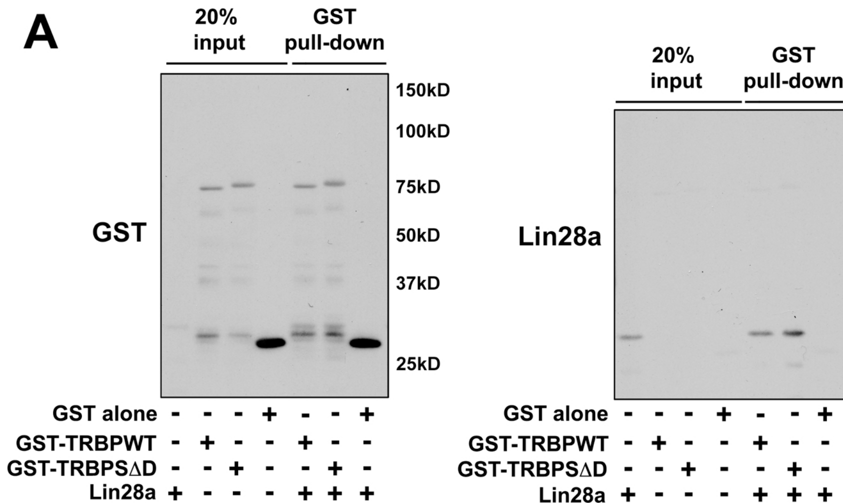


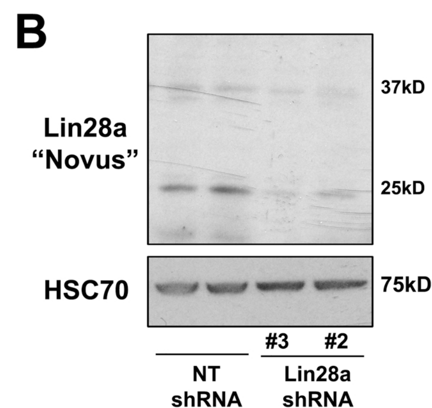
S3

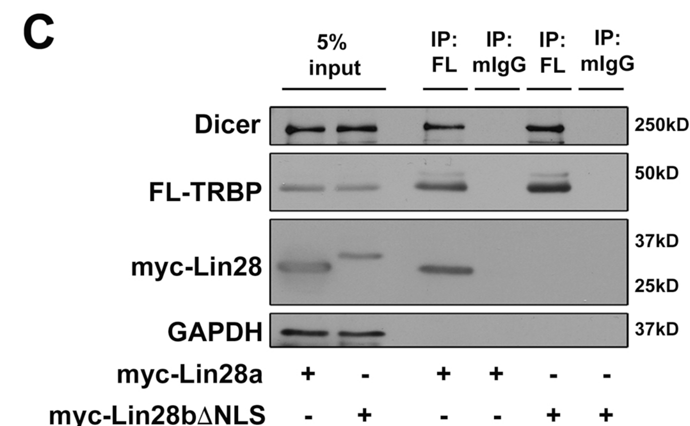


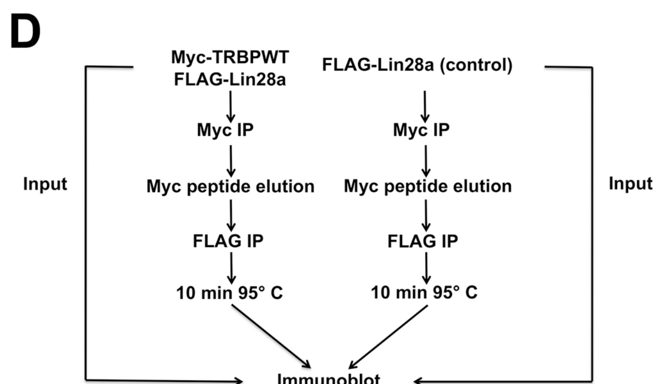


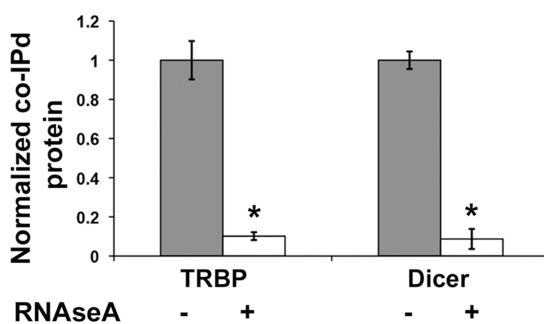
S4

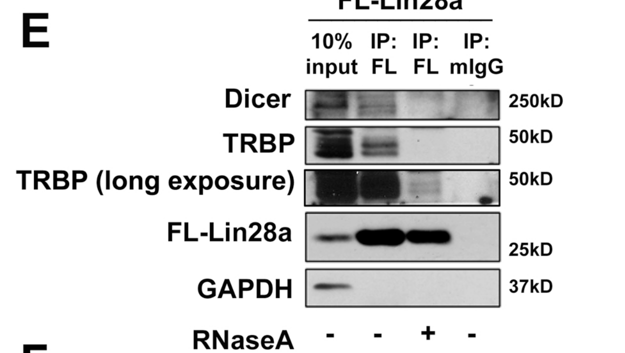


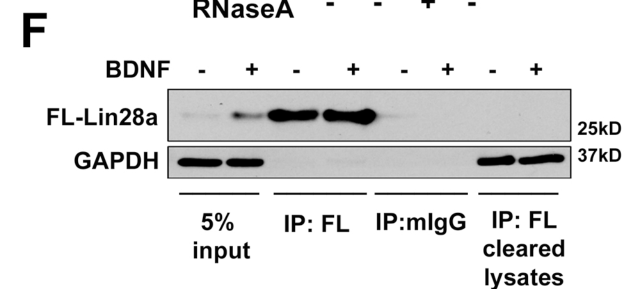


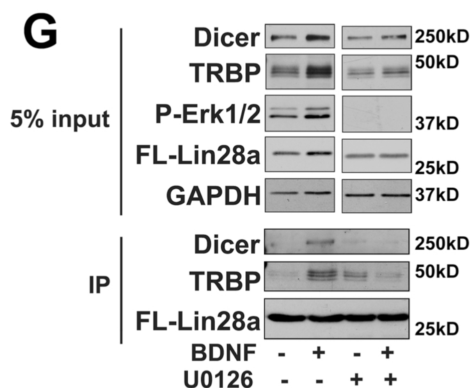


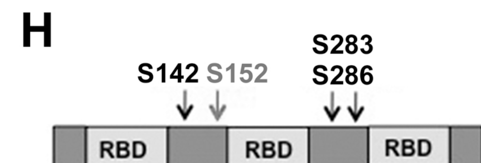


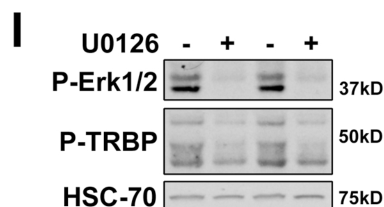


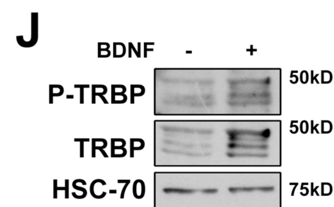




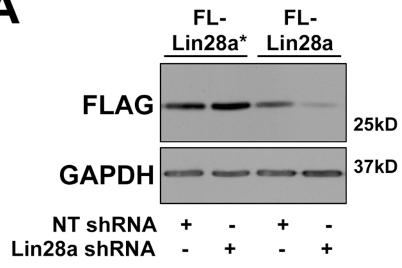




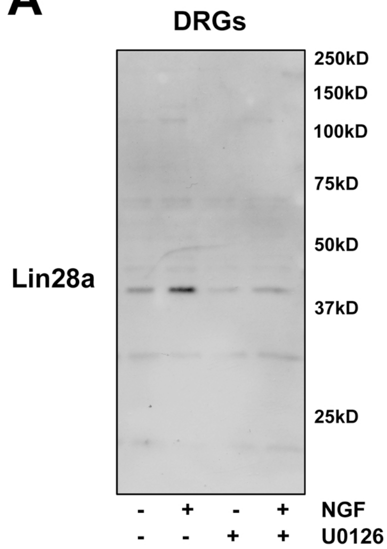


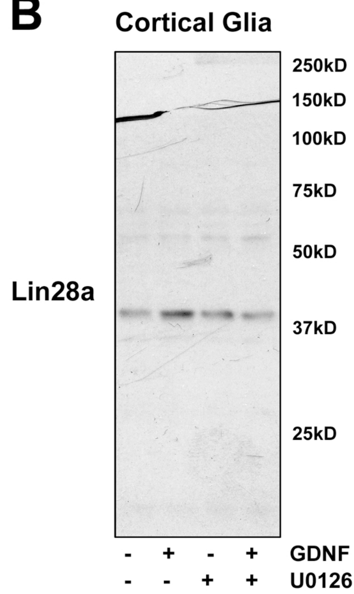


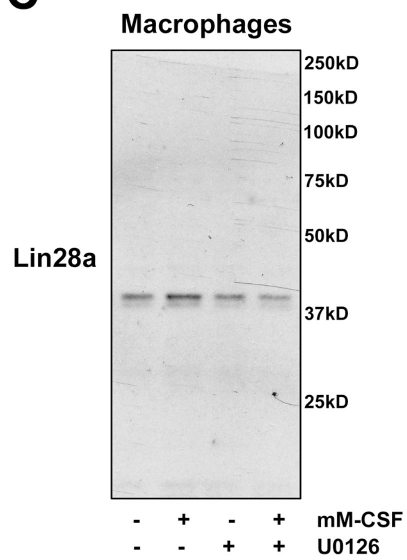
S5

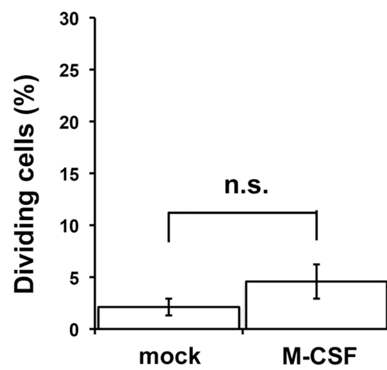


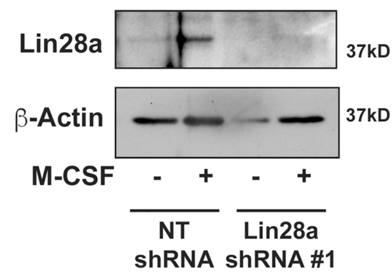
S6











Supplemental Information:

Figure S1: Immunoblots for Lin28a and Lin28b proteins.

Related to Figure 1. (**A**) Immunoblots using three antibodies directed towards three different Lin28a eptiopes (Cell Signaling A177, Lifespan LS-C165782 now LS-B11566, and Novus NBP-149537), and one towards Lin28b, in lysates from hippocampal neurons infected with two different shRNAs targeting Lin28a compared to a non-target shRNA (NT shRNA, control). At the titers tested, Lin28a shRNA #1 reduces Lin28a protein detection by 84% (Cell Signaling), 62% (Lifespan), and 86% (Novus), but does not alter Lin28b detection. Lin28a shRNA #2 reduces Lin28a protein detection by 88% (Cell Signaling), 85% (Lifespan), and 75% (Novus), but does not alter Lin28b detection. Endogenous Lin28a protein is detected by immunobot in differentiated neurons as a predominant singlet or doublet band near 37 kD (Cell Signaling A177, Lifespan LC-C165782, and Novus NBP-149537); a band near the predicted molecular weight of Lin28a (25 kD) is also detected by LS-C165782 (now LS-B11566). These Lin28a-immunoreactive bands are each sensitive to Lin28a knockdown, and have been previously observed in differentiated cells (Huang et al., 2012, Moss and Tang, 2003, Nowak et al., 2014, Seggerson et al., 2002). Lin28a protein bands near 37 kD have been previously reported to be reflective of post-translational modification and alternative splicing (Seggerson et al., 2002). (**B**) Representative immunoblot from Figure 1B of (top left) endogenous Lin28b protein, (top right) FL-Lin28b protein, or (bottom) FL-Lin28bΔNLS protein in hippocampal neurons undergoing a BDNF timecourse. (**C)** Lysates from HEK293T cells co-expressing either FLAG-Lin28a (FL-Lin28a) or PCDNA3.1 alone (control) with HA-K48 ubiquitin were IPd under stringent conditions with anti-FLAG antibody, followed by immunoblot with anti-FL and anti-HA antibodies. Representative immunoblot demonstrates HA-K48 ubiquitin laddering in the IPd Lin28a condition. Asterix (*) denotes an HA signal detected in the absence of FL-Lin28a (and non-reactive with anti-FLAG Ab). N=3-4 independent replicates per panel.

Figure S2: Knockdown of TRBP, but not Dicer, reduces Lin28a.

Related to Figure 2. (**A**) (Left) Representative immunoblot and (right) quantification of Dicer and Lin28a protein levels in hippocampal neurons expressing non-target shRNA (NT shRNA, control) or shRNA targeting Dicer (Dicer shRNA). (**B**) Representative immunoblot from Figure 2E showing FL-Lin28a protein levels in hippocampal neurons co-expressing WT or ΔD TRBP, in the presence or absence of BDNF. (**C**) Representative immunoblot from Figure 2E showing FL-Lin28a protein levels in hippocampal neurons co-expressing shRNA-resistant SΔA TRBP (FL-TRBPSΔA*), in the presence or absence of BDNF. Neurons were additionally treated with either non-target shRNA (NT shRNA, control) or shRNA targeting endogenous TRBP (TRBP shRNA). Technical replicate samples were run on two separate gels to allow blotting for both FL-tagged and endogenous TRBP. (**D**) Quantification of Lin28a, TRBP, and FL-TRBPSΔA* protein levels from lysates from hippocampal neurons under non-target shRNA (NT shRNA, control) or TRBP knockdown (TRBP shRNA) conditions, normalized to loading control (HSC70) and

plotted relative to NT shRNA condition, set as 1.0. Quantified data represent mean -/+ SEM.. *p < 0.05 by unpaired Student's *t* test. N=3-16 independent replicates per panel.

Figure S3: Merlin protein regulates TRBP ubiquitination and is not induced by BDNF.

Related to Figure 3. (**A**) (Left) representative immunoblot and (right) quantification of TRBP protein bound to GST-S5a using lysates from HEK293T cells treated with non-target shRNA (NT shRNA, control) or shRNA targeting Merlin protein (Merlin shRNA). Ubiquitinated TRBP protein was normalized to total ubiquitin pull-down and plotted relative to NT shRNA (set as 1.0). We note that total TRBP protein levels are increased by Merlin shRNA (inputs), revealing a striking reduction in the proportion of ubiquitinated TRBP following Merlin knockdown. (b) Merlin protein levels in lysates from hippocampal neurons treated with vehicle (growth media) or BDNF (60 min). (Left) Representative immunoblot and (right) quantification of Merlin protein, normalized to GAPDH and plotted relative to vehicle (set as 1.0). *$p < 0.05$ by *t* test for all experiments. Quantified data represent mean -/+ SEM. N=4-8 independent replicates per panel.

Figure S4: BDNF-induced TRBP phosphorylation regulates binding to Lin28a, but not Lin28b.

Related to Figure 4. (**A**) Uncropped blots of (left) purified GST-TRBP and GST alone (control) pull-downs and (right) co-associated Lin28a from Figure 3A. (**B**) Lin28a immunoblot using antibody from Novus (NBP-149537) to detect Lin28a protein in lysates from HEK293T cells infected with two different shRNAs targeting Lin28a compared to a non-target shRNA (NTshRNA, control). At the titers tested, Lin28a shRNA #3 reduces Lin28a protein detection by 86%. Lin28a shRNA #2 reduces Lin28a protein detection by 76%. In the HEK293T cell line, the predominant Lin28a immunoreactive band is near its predicted molecular weight (25 kD). (**C**) Representative immunoblot of lysates from HEK293T cells co-expressing FL-TRBPWT and either myc-Lin28a or myc-Lin28bΔNLS. Lysates were IPd with anti-FLAG antibody, followed by immunoblot with anti-FLAG, anti-myc, and anti-Dicer antibodies. (**D**) Sequential IP flowchart. HEK293T cells were transfected with either FL-Lin28a and myc-TRBP constructs together (left side), or FL-Lin28a alone as a control (right side). Input samples from both transfection conditions were saved for immunoblot (indicated by side arrows). Each transfection condition underwent sequential IP (indicated by straight arrows). Initial myc IP was followed by elution with a myc peptide competitive inhibitor and subsequent FLAG IP. Final eluents were boiled at 95° C and subjected to immunoblot along with input samples. (**E**) Lysates from HEK293T cells expressing FL-Lin28a were treated with or without RNAseA and IPd with anti-FLAG or control mouse IgG (mIgG) antibody. (Left) Representative immunoblot. (Right) Densitometric quantification of co-IPd proteins normalized to input and plotted relative to no RNAse condition (set as 1.0). (**F**) Depletion of lentiviral FL-Lin28a following neuronal IP was verified by running cleared lysate samples taken from the remaining neuronal cell lysate after tumbling with FLAG antibody-bound G-

sepharose beads. FL-Lin28a signal is detectable in input samples prior to FLAG IP, but not in cleared lysate samples. (**G**) Representative immunoblot of lysates from hippocampal neurons expressing FL-Lin28a that were pre-treated (20 min) with either vehicle (DMSO) or MEK/Erk inhibitor U0126, following by incubation with either vehicle (growth media) or BDNF (90 min). Lysates were IPd with anti-FLAG antibody. Inputs were run on separate gels from IPd samples. (**H**) Mass spectrometry has demonstrated TRBP phosphoryation at serine residues 142, 152, 283, and 296. An antibody was developed in our lab towards phosphorylation at S152 (red), a perfect Erk phosphorylation consensus site. (**I**) Assay of phospho-TRBP antibody reactivity with U0126-treated lysates. HEK293T cells were incubated with either vehicle (DMSO) or MEK/Erk inhibitor U0126 for 48 hours, followed by immunoblot with anti-phospho-TRBP antibody. (**J**) Immunoblot of TRBP and phospho-TRBP protein levels in lysates from hippocampal neurons treated with vehicle (growth media) or BDNF (60 min). *$p < 0.05$ by unpaired Student's *t* test. Quantified data represent mean -/+ SEM. N=3-6 independent replicates per panel.

Figure S5: Validation of shRNA-resistant Lin28a construct.
Related to Figure 5. (**A**) Representative immunoblot of HEK293T cells co-transfected with either FL-Lin28a or shRNA-resistant Lin28a (FL-Lin28a*), alongside either non-target shRNA (NTshRNA, control) or Lin28a shRNA#1. N=4 independent replicates.

Figure S6: Lin28a immunoblotting in multiple primary cell types.
Related to Figure 6. (**A-C**) Uncropped immunoblots from Figure 6 of Lin28a protein using Lin28a Novus antibody (validated in Figure S1A) in lysates from (**A**) DRG neurons (Figure 6B), (**B**) cortical glia (Figure 6C), and (**C**) macrophages (Figure 6D). Cells were treated with vehicle (growth media) or (**A**) NGF, (**B**) GDNF, or (**C**) M-CSF following a 30 min pretreatment with either DMSO or pharmacological MEK/Erk inhibitor, U0126. (**D**) BrdU incorporation in peritoneal macrophages incubated with either BrdU in empty media (control) or BrdU in media containing M-CSF, for 12 hours. Graph shows percent (out of 100) of peritoneal macrophages that underwent division, in the presence and absence of M-CSF. N=7-11 microscopic fields per condition. (**E**) Immunoblot using Lin28a Novus antibody of lysates from peritoneal macrophages (imaged in Figure 6E) treated with either Lin28a shRNA#1 or non-target shRNA (NT shRNA, control) for 56 hours, in the presence or absence of M-CSF for 48 hours.