MluI_PRA1_1+FWD	GCTGCG**ACGCGT**GCGGCCCAGAAGGACCAGCAG
BglII_Stop_PRA1-185_RVRS	GCTGCG**AGATCT**TTACACAGGTTCCATCTGCAGCTCCTC
XhoI_PRA1_1+_FWD	GCTGCG**CTCGAG**ATGGCGGCCCAGAAGGACCAGCAG
MscI_PRA1_185+_RVRS	GATACAC**TGGCCA**CCACAGGTTCCATCTGCAGCTC
XbaI_XhoI_ATG_EGFP_FWD	GCTGCG**TCTAGA**CTCGAG**ATG**GTGAGCAAGGGCGAGGAG
BglII_STOP_MluI_EGFP_RVRS	GCTGCG**AGATCT**TCATCA**ACGCGT**CTTGTACAGCTCGTCCATGCC
mCHERRY_FWD_5'Cloning	GCTGCG**GAATTC**GACGCT**ACGCGT**GTGAGCAAGGGCGAGGAGGATAAC
mCHERRY_RVRS_5'Cloning	GCTGCG**AGATCT**TTA**CTTGTCATCGTCGTCCTTGTAGTC**CTTGTACAGCTCGTCCATGCCGCC
mCHERRY_FWD_3'Cloning	GCTGCG**GAATTC**CGCCACC**ATGGACTACAAGGACGACGATGACAAG**GTGAGCAAGGGCGAGGAGGATAAC
mCHERRY_RVRS_3'Cloning	GCTGCG**AGATCT**TTACGT**ACGCGT**CTTA**GGTACC**CTTGTACAGCTCGTCCATGCCGCC
KpnI_mSec61b_FWD	GCTGCG**GGTACC**CCGGGTCCAACGCCCAGTGGCACC
KpnI_mSec61b_RVRS	GCTGCG**ACGCGT**TTATGATCGCGTGTACTTGCCCCAAAT
XbaI_mMannII_FWD	GCTGCG**TCTAGA**ATGAAGTTAAGTCGCCAGTTCACCGTG
Mlu_mMannII-aa116_RVRS	GCTGCG**ACGCGT**CAAACAGTCTCTGGGGTCAGCCTG
KpnI_ PRA1_131+RVRS	GCTGCG**GGTACC**CTGATGTGCTGGGCTCACCTC
PRA1_R37/38/39/43A_FWD	**GAGGCCGCCGCCGCGACCATCGCCCCCTGG**

PRA1_R37/38/39/43A_RVRS	GGCGATGGTCGCGGCGGCGGCCTCCAG
PRA1_R37/38/39K_FWD	CTGGAGAAGAAGAAGGCGACCATCCGG
PRA1_R37/38/39K_RVRS	CCGGATGGTCGCCTTCTTCTTCTCCAG
PRA1_80-86_OE_FWD	TTCGTGTTTCTCGGCCTCATC
PRA1_72-78A_RVRS	**GCCGAGAAACACGAA**CACGGCGGCGGCGGCGGCGGCGGCCACGTT
mPRA1_66-86_72-78 Δ _RVRS	GATGAGGCCGAGAAACACGAACACCACGTTGCGTACCAGGCG
mPRA1_66-86_72-79 Δ _RVRS	GATGAGGCCGAGAAACACGAACACGTTGCGTACCAGGCG
mPRA1_YYQSNY-FFDAQE	GAGAAACACGAACAC**CTCCTGGGCGTCGAAGAA**CTCCAC

Table S1. Primer sequences used in this study

Supplementary Methods

ShRNA design

ShRNA constructs were made according to previously described protocols (Fellmann et al., 2013). Mir30 based 97nt hairpins were amplified using the following primers: 5'-cagaaggctcgagaaggtatattgctgttgacagtgagcg-3' and 5'-tctcgaattctagccccttgaagtccgaggcagtaggc-3' with the reverse primer containing the "CNNC" motif retrofitted for more efficient processing (Fellmann et al., 2013). The resulting PCR fragment was cut with XhoI/EcoRI and ligated into the pCAG-mir30 vector (Addgene plasmid #14758) (Matsuda and Cepko, 2007).

Contructs generated (5'-3'): hPRA1-a-mir30, [tgctgttgacagtgagcgCGCGCAGAAGGACCAGCAGAAAtagtgaagccacagatgtaTTTCTGCTGGTCCTTCTGCGCTtgcctactgcctcgga]

hPRA1-b-mir30,
[tgctgttgacagtgagcgAACCCTGCTGCCGAAGCTGATTtagtgaagccacagatgtaAATCAGCTTCGGCAGCAGGGTCtgcctactgcctcgga]
hPRA1-c-mir30,
[tgctgttgacagtgagcgCGGTGGCTCTGGCTGTCTTTTTtagtgaagccacagatgtaAAAAAGACAGCCAGAGCCACCAtgcctactgcctcgga]
GFP-mir30,
[tgctgttgacagtgagcg**aagccacaacgtctatatcatg**tagtgaagccacagatgta**catgatatagacgttgtggctg**tgcctactgcctcgga]. Prior to use, constructs were verified via Sanger sequencing at Eurofins Sequencing (Louisville, KY, USA).

Untagged PRA1 construct

PRA1 was amplified from mouse cDNA purchased from Origene (see Materials and Methods). The amplified product was cloned into the PCAGIG vector (Addgene-see Materials and Methods) using the XhoI and BglII restriction enzyme sites.

Generation of Stable ShRNA cell lines (Abgent anti-PRA1 antibody confirmation)

To generate selectable ShRNA constructs, the generated mir30 constructs were amplified from the pCAG-mir30 backbone and subcloned within the pcDNA 3.1 (-) vector XbaI/BamHI restriction enzyme sites. Resulting constructs were sequence verified and linearized using the BglII restriction enzyme site. 1.5 μg of each construct was then transfected into Hek293T cells using Lipofectamine 2000 (12 well plates). 24 hrs later, cells were trypsinized and moved to 100 mm cell culture plates where selection was carried out over a 10 day period. An untransfected control plate showed complete cell death after the selection period was finished. Media was changed every 3 days with fresh Hygromycin B added each time.

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Matsuda, T. and Cepko, C. L. (2007). Controlled expression of transgenes introduced by in vivo electroporation. *Proc Natl Acad Sci U S A* **104**, 1027-32.