Evidence of efficient stop codon readthrough in four mammalian genes

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Supplementary Figure 1. Comparative evidence for 3 new readthrough candidates: (A-C) Genomic alignments of the readthrough extensions of \textit{ACP2}, \textit{AQP4}, and \textit{MAPK10} with other eutherian mammal genomes, colour coded using CodAlignView ("CodAlignView: a tool for visualizing protein-coding constraint", I Jungreis, M Lin, M Kellis, in preparation). The high concentrations of synonymous substitutions (light green) and conservative amino acid changes (dark green), and relatively low concentrations of radical amino acid changes (red) and frame shifted regions (orange) is characteristic of protein-coding regions. PhyloCSF scores for these extensions are higher than 99\% of similar-sized non-coding regions, indicating that they have been functional at the amino acid level in much of the eutherian mammal tree. Horizontal lines in the \textit{ACP2} alignment indicate paired bases in the predicted RNA stem loop. (D) Conserved RNA stem loop 3' of the UGA stop codon in \textit{ACP2}, as predicted by RNAz.

Supplementary Figure 2. Per-codon PhyloCSF scores of the predicted readthrough extensions of the seven readthrough candidates (blue stars) relative to the distributions for coding and non-coding regions. Non-coding distribution was computed from "third ORFs", i.e., the region from the first in-frame stop codon after an annotated stop codon to the next in-frame stop codon, excluding regions that overlap the coding region of another annotated transcript. Coding distribution was computed from same-sized regions directly 5' of the annotated stop codon. Regions less than 10 codons or for which the aligned species encompass less than 10\% of the branch length of the 29-mammals tree were excluded because PhyloCSF has insufficient power on such regions. All seven readthrough regions have scores near the middle of the coding distribution and higher than 99.96\% of non-coding regions.

Supplementary Figure 3. Nucleotide and codon alignments of the regions corresponding to the end of the main ORF, the readthrough ORF and the flanking sequences of (A) \textit{AQP4}, (B) \textit{MAPK10}, (C) \textit{OPRK1}, (D) \textit{OPRL1} and (E) \textit{SACM1L}. The alignments in A, B, C and D were generated by custom python scripts and clustalW2. First the given region in the main ORF was conceptually translated into a peptide sequence. The conceptual peptide sequences were aligned using ClustalW2. This amino acid alignment was back-translated to a corresponding nucleotide alignment based on codons corresponding to amino acids in the alignment using the initial nucleotide sequences. Finally, the common ancestral (root) sequence was inferred using PyCogenet python module v. 1.5.3 (1) using a ClustalW guide tree. The alignment shown in E used a variant of the procedure for A-D where an alignment was performed directly on the nucleotide sequence with ClustalX2. This nucleotide alignment was then manually adjusted and converted into a corresponding protein sequence alignment which was used to generate a guide tree and evaluate the root sequence as described earlier. Synonymous and non-synonymous substitutions were determined relative to the inferred root sequence shown on top of each alignment. In these alignments, consensus (root) codons (and gaps) are highlighted in white, synonymous codons are highlighted in green, non-synonymous codons are highlighted in brown and stop codons are highlighted in magenta. Abbreviated species identifiers of the sequences used are indicated on the left. Full names of the species whose sequences are used in A-E: \textit{Ailuropoda melanoleuca}, \textit{Alligator mississippiensis}, \textit{Anolis carolinensis}, \textit{Bos Taurus}, \textit{Callithrix
The sequences used in the alignments here were obtained from GenBank using the BLAST (tblastn) algorithm where the translated human sequence was used as bait. The target databases during this search were Expressed Sequence Tags (EST), the Whole-Genome Shotgun contigs, and Transcriptome Shotgun Assembly. All non-mammalian orthologous sequences that could be identified, as well as representative number of mammalian sequences were collected and used in the alignments.

Supplementary Figure 4. Amino acid conservation of OPRK1 and OPRL1 readthrough extensions. The conservation is shown as amino acid logogram (A) of the OPRK1, and (B) the ORPL1 readthrough extensions, respectively. The five highly conserved amino acids near their C-terminal ends, that also show sequence similarity between the two paralogs, are boxed in read. To build the conservation plots the nucleotide sequence alignment for the OPRK1 and OPRL1 readthrough extensions (Supplementary Figures 3C and D) were converted into amino acid sequence alignments and used to generate logogram utilizing WebLogo (2).

Supplementary Figure 5. (a) Dual luciferase assay indicating readthrough efficiencies of OPRL1 constructs with only one 5’ nt and with 3’ nested deletions showing the importance of the nt immediately 3’. (B) The effect of nt identity immediately 5’ of the OPRL1 UGA on readthrough efficiency. (C) The effect of replacing the UGA stop codon with either UAA or UAG on the readthrough efficiencies of wild type OPRL1 and OPRL1-CARYYA.

REFERENCES
