## **Supporting Information**

## Larsson et al. 10.1073/pnas.1006821107



**Fig. S1.** Scatter plots of effects and *p*-values for all genes when using the log ratio approach and anota for three datasets. (*A*) Ingolia et al. (1). (*B*) Kitamura et al. (2). (*C*) Otulakowski et al. (3). The numbers indicate how many genes fall within each area generated by the dotted lines. The lines were drawn at a >2-fold effect or a *p*-value <0.01. Loess fits are shown as green lines.

- 1 Ingolia NT, Ghaemmaghami S, Newman JR, Weissman JS (2009) Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. Science 324:218–223.
- 2 Kitamura H, et al. (2008) Genome-wide identification and characterization of transcripts translationally regulated by bacterial lipopolysaccharide in macrophage-like J774. 1 cells. *Physiol Genomics* 33:121–132.
- 3 Otulakowski G, Duan W, O'Brodovich H (2009) Global and gene-specific translational regulation in rat lung development. Am J Respir Cell Mol Biol 40:555–567.



**Fig. 52.** A comparison of the top 3 mRNAs identified as differentially translated by the log ratio approach and anota. The top 5% genes ranked by *p*-value from the Kitamura et al. study (1) using either the log ratio approach or anota were collected and separated into three sets: those identified by log ratio only (top row); those identified by anota only (middle row); and those identified by both log ratio and anota (bottom row). Translational activity data (indicated as translation on each y-axis) and cytosolic mRNA data (indicated as transcription on each x-axis) for the top 3 ranked genes (by *p*-value) for each set is plotted using a common scale for all graphs (the two sample classes are shown as circles and triangles). The effects from anota and the log ratio approach are indicated together with their corresponding *p*-values. The differences in means between the sample classes for the translational activity data only and the cytosolic mRNA data only are indicated as delta transcription, respectively. Lines represent the regression lines estimated by anota for the two sample categories.

1 Kitamura H, et al. (2008) Genome-wide identification and characterization of transcripts translationally regulated by bacterial lipopolysaccharide in macrophage-like J774. 1 cells. *Physiol Genomics* 33:121–132.



**Fig. S3.** A comparison of the top 3 mRNAs identified as differentially translated by the log ratio approach and anota. The top 5% genes ranked by *p*-value from the Otulakowski et al. study (1) using either the log ratio approach or anota were collected and separated into three sets: those identified by log ratio only (top row); those identified by anota only (middle row); and those identified by both log ratio and anota (bottom row). Translational activity data (indicated as translation on each x-axis) and cytosolic mRNA data (indicated as transcription on each x-axis) for the top 3 ranked genes (by *p*-value) for each set is plotted using a common scale for all graphs (the two sample classes are shown as circles and triangles). The effects from anota and the log ratio approach are indicated together with their corresponding *p*-values. The differences in means between the sample classes for the translational activity data only and the cytosolic mRNA data only are indicated as delta transcription, respectively. Lines represent the regression lines estimated by anota for the two sample categories.

1 Otulakowski G, Duan W, O'Brodovich H (2009) Global and gene-specific translational regulation in rat lung development. Am J Respir Cell Mol Biol 40:555–567.



**Fig. S4.** RVM improves the performance of APV within anota [the Kitamura et al. dataset (1)]. (A). Volcano plots for anota and anota RVM. The  $-\log 10$  *p*-value is plotted as a function of the effect. (B). A comparison of statistical power between anota and anota RVM. Shown is the cumulative distribution function (CDF) of all *p*-values from anota or anota RVM. CDFs for both the raw *p*-values and *p*-values adjusted as described in the text are shown.

1 Kitamura H, et al. (2008) Genome-wide identification and characterization of transcripts translationally regulated by bacterial lipopolysaccharide in macrophage-like J774. 1 cells. *Physiol Genomics* 33:121–132.



**Fig. S5.** RVM improves the performance of APV within anota [the Otulakowski et al. dataset (1)]. (A). Volcano plots for anota and anota RVM. The – log 10 *p*-value is plotted as a function of the effect. (*B*). A comparison of statistical power between anota and anota RVM. Shown is the CDF of all *p*-values from anota or anota RVM. CDFs for both the raw *p*-values and *p*-values adjusted as described in the text are shown.

1 Otulakowski G, Duan W, O'Brodovich H (2009) Global and gene-specific translational regulation in rat lung development. Am J Respir Cell Mol Biol 40:555–567.



**Fig. S6.** Functional analysis of genes identified as differentially translated using the log ratio approach and anota from the study by Ingolia et al (1). All genes that were identified by anota as differentially expressed were further divided into up or down-regulated; equivalent lists were generated for those genes that were identified by the log ratio approach. Each of these 6 lists was used as input when searching for enrichment of biological themes as defined by the Gene Ontology Consortium using GO::Termfinder. Hierarchical cluster analysis was performed on the significant biological themes from each list (adjusted *p*-value <0.1) paired with their corresponding GO terms. The values represented by the color codes are multiple test corrected – log 10 *p*-values (i.e., a value of 2 equals a *p*-value of 0.01).

1 Ingolia NT, Ghaemmaghami S, Newman JR, Weissman JS (2009) Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. Science 324:218-223.



**Fig. 57.** Functional analysis of genes identified as differentially translated using the log ratio approach and anota from the study by Kitamura et al. (1). All genes that were identified by anota as differentially expressed were further divided into up or down-regulated; equivalent lists were generated for those genes that were identified by the log ratio approach. Each of these 6 lists was used as input when searching for enrichment of biological themes as defined by the Gene Ontology Consortium using GO::Termfinder. Hierarchical cluster analysis was performed on the significant biological themes from each list (adjusted *p*-value <0.1) paired with their corresponding GO terms. The values represented by the color codes are multiple test corrected – log 10 *p*-values (i.e., a value of 2 equals a *p*-value of 0.01).

1 Kitamura H, et al. (2008) Genome-wide identification and characterization of transcripts translationally regulated by bacterial lipopolysaccharide in macrophage-like J774. 1 cells. *Physiol Genomics* 33:121–132.



**Fig. S8.** Functional analysis of genes identified as differentially translated using the log ratio approach and anota from the study by Otulakowski et al. (1). All genes that were identified by anota as differentially expressed were further divided into up or down-regulated; equivalent lists were generated for those genes that were identified by the log ratio approach. Each of these 6 lists was used as input when searching for enrichment of biological themes as defined by the Gene Ontology Consortium using GO::Termfinder. Hierarchical cluster analysis was performed on the significant biological themes from each list (adjusted *p*-value <0.1) paired with their corresponding GO terms. The values represented by the color codes are multiple test corrected – log 10 *p*-values (i.e., a value of 2 equals a *p*-value of 0.01).

1 Otulakowski G, Duan W, O'Brodovich H (2009) Global and gene-specific translational regulation in rat lung development. Am J Respir Cell Mol Biol 40:555–567.