Supplementary Materials for

Assessing gene-level translational control from ribosome profiling

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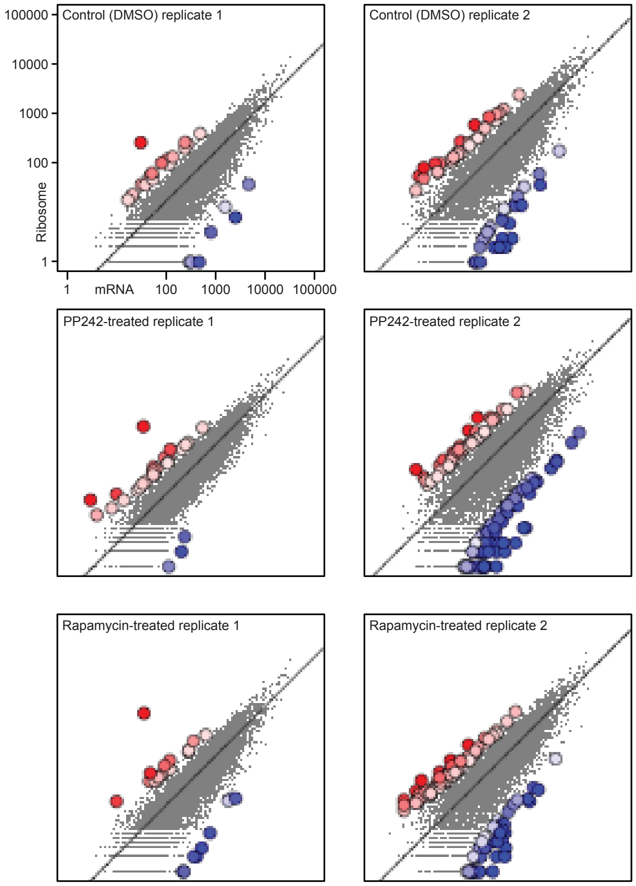
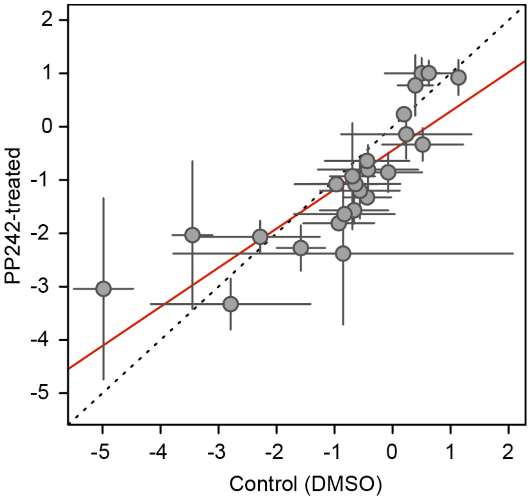
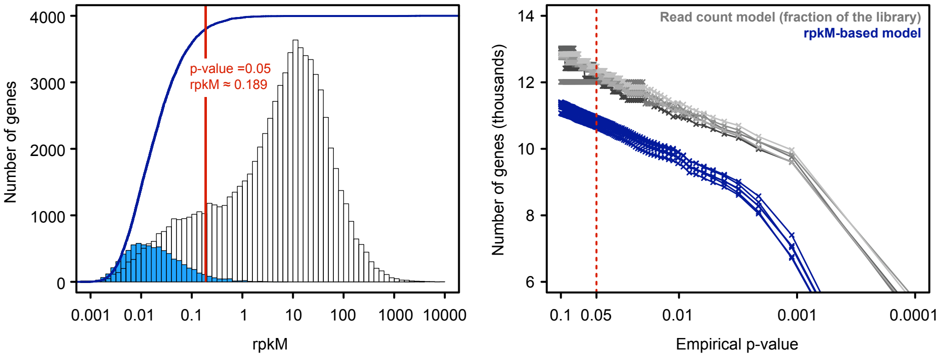
SUPPLEMENTARY FIGURES



Supplementary Figure 1: mRNAs with significant translational regulation. Babel analysis identifies genes within and between conditions with significant mRNA translation (red and blue are greater or less than expected ribosome association given mRNA abundance levels; for graphical purposes, p-value < 0.001 in each sample). Here, the top row is control while the middle and bottom are PP242- and Rapamycin-treated PC3 cells, respectively, from sequencing data obtained in Hsieh et al. The black line represents the fit of the trimmed regression described in the Methods.



Supplementary Figure 2: Presumptive false negatives from Babel analysis. Among 24 genes that were identified in the original analysis, but were not significant in Babel, the average translational efficiency (calculated as in Hsieh et al.) is shown in both treated (PP242) and control (DMSO) cells, as is its variability between replicates in each condition (red line; regression, dashed line is the diagonal).



Supplementary Figure 3: Determining expressed genes. A. The distribution of normalized rpkMs in gene models is juxtaposed to a distribution of random regions of the genome masked of transcribed content (light blue; left panel). The distribution is estimated from the latter (dark blue) and p-values are assigned to each gene in each sample. Expressed genes are those with a nominal p-value < 0.05, which in this example corresponds to an rpkM of greater than 0.189 (see Methods). **B.** The number of expressed genes at multiple cutoffs is stable across replicates and conditions (in blue; based on an rpkM model, as in panel A). While a similar method based on read counts alone (gray) produces more seemingly expressed genes at a given p-value, it does not incorporate gene size.

SUPPLEMENTARY TABLES

			Proportion of p-values			
Replicates	**Mean**	**Median**	**>0.999**	**>0.95**	**<0.05**	**<0.001**
Within-condition						
1	0.4919 (0.005)	0.4837 (0.007)	0.0014 (5e-04)	0.0508 (0.005)	0.0462 (0.004)	4e-04 (2e-04)
2	0.4882 (0.004)	0.4836 (0.007)	0.0011 (4e-04)	0.0471 (0.003)	0.0521 (0.003)	7e-04 (3e-04)
3	0.4858 (0.005)	0.4792 (0.007)	0.0010 (4e-04)	0.0454 (0.003)	0.0543 (0.003)	9e-04 (3e-04)
Between-condition						
2	0.4960 (0.002)	0.4944 (0.003)	0.0010 (3e-04)	0.0496 (0.002)	0.0503 (0.002)	0.0011 (3e-04)

Supplementary Table 1: Simulations for size (type I error). Shown are averages (and standard deviations) across all samples as well as the proportion of p-values above and below the given significance levels.

			Sample size ($\alpha = 0.05$)		
Direction of change	**RNA level[a]**	**Fold change**	**1**	**2**	**3**
Within-condition					
Up	Low	2	0.32 (0.06)	0.46 (0.07)	0.57 (0.07)
		3	0.58 (0.07)	0.73 (0.07)	0.83 (0.06)
		4	0.73 (0.06)	0.86 (0.06)	0.93 (0.03)
	Mid	2	0.38 (0.07)	0.49 (0.07)	0.61 (0.08)
		3	0.64 (0.07)	0.77 (0.06)	0.86 (0.06)
		4	0.78 (0.06)	0.87 (0.05)	0.93 (0.04)
	High	2	0.39 (0.07)	0.50 (0.08)	0.61 (0.07)
		3	0.65 (0.07)	0.78 (0.07)	0.87 (0.05)
		4	0.79 (0.06)	0.89 (0.05)	0.95 (0.04)
Down	Low	2	0.10 (0.05)	0.20 (0.06)	0.29 (0.06)
		3	0.19 (0.06)	0.43 (0.07)	0.62 (0.06)
		4	0.28 (0.06)	0.60 (0.06)	0.77 (0.06)
	Mid	2	0.23 (0.06)	0.39 (0.08)	0.56 (0.08)
		3	0.44 (0.08)	0.75 (0.07)	0.90 (0.05)
		4	0.61 (0.07)	0.92 (0.04)	0.99 (0.02)
	High	2	0.24 (0.06)	0.42 (0.07)	0.59 (0.08)
		3	0.47 (0.07)	0.79 (0.06)	0.93 (0.04)
		4	0.67 (0.07)	0.95 (0.03)	1.00 (0.01)
Between-condition					
Up	Low	2	0.11 (0.04)	0.19 (0.05)	0.27 (0.06)
		3	0.25 (0.06)	0.42 (0.07)	0.59 (0.08)
		4	0.37 (0.06)	0.62 (0.07)	0.80 (0.05)
	Mid	2	0.17 (0.05)	0.26 (0.07)	0.38 (0.07)
		3	0.33 (0.07)	0.55 (0.08)	0.72 (0.07)
		4	0.49 (0.07)	0.73 (0.07)	0.88 (0.05)
	High	2	0.18 (0.05)	0.28 (0.06)	0.41 (0.07)
		3	0.35 (0.07)	0.57 (0.07)	0.75 (0.06)
		4	0.51 (0.07)	0.76 (0.07)	0.90 (0.04)
Down	Low	2	0.05 (0.03)	0.09 (0.04)	0.14 (0.04)
		3	0.08 (0.04)	0.17 (0.06)	0.29 (0.06)
		4	0.11 (0.04)	0.25 (0.06)	0.41 (0.07)
	Mid	2	0.10 (0.04)	0.17 (0.06)	0.27 (0.07)
		3	0.17 (0.06)	0.34 (0.07)	0.52 (0.07)
		4	0.24 (0.06)	0.49 (0.08)	0.70 (0.07)
	High	2	0.10 (0.04)	0.18 (0.06)	0.28 (0.07)
		3	0.18 (0.05)	0.36 (0.07)	0.55 (0.07)
		4	0.26 (0.06)	0.53 (0.07)	0.74 (0.06)

[a] Genes in either the bottom 5% (low), middle 90% (mid), or top 5% (high) of the distribution of mRNA expression

Supplementary Table 2: Simulations for power. Here, values are averages (and standard deviations) across samples for the power to identify altered genes within and between conditions at the significance level indicated.

		One-sided P-value			PP242 versus DMSO		
ID	**Symbol**	**DMSO**	**Rapamycin**	**PP242**	**Z-statistic**	**P-value**	**Q-value**
ENSG00000139675.10	HNRNPA1L2	4.50E-08	5.93E-05	0.5511	7.306	2.74E-13	2.89E-09
ENSG00000231500.2	RPS18	0.1517	0.9306	0.9999	6.127	8.96E-10	4.72E-06
ENSG00000244165.1	P2RY11	0.4745	0.9369	0.9996	5.330	9.82E-08	0.0002
ENSG00000105717.5*	PBX4	0.2222	0.9356	0.9965	5.251	1.52E-07	0.0002
ENSG00000154589.2*	LY96	0.0014	0.6512	0.8115	5.223	1.76E-07	0.0002
ENSG00000143632.8	ACTA1	0.0009	0.4487	0.5294	4.811	1.50E-06	0.0011
ENSG00000189167.6	ZAR1L	0.0055	0.0373	0.8384	4.536	5.73E-06	0.0032
ENSG00000104369.4*	JPH1	0.2482	0.5513	0.9862	4.491	7.09E-06	0.0037
ENSG00000105649.3	RAB3A	0.0566	0.2962	0.9685	4.430	9.43E-06	0.0045
ENSG00000149300.5*	C11orf52	0.0200	0.0588	0.9384	4.429	9.46E-06	0.0045
ENSG00000204271.4	SPIN3	0.0386	0.0070	3.06E-07	-4.395	1.11E-05	0.0050
ENSG00000036448.5	MYOM2	0.0414	0.6197	0.7790	4.336	1.45E-05	0.0058
ENSG00000108272.8	DHRS11	0.4152	0.2090	0.9985	4.165	3.12E-05	0.0094
ENSG00000173267.9*	SNCG	0.0638	0.2376	0.9501	4.158	3.21E-05	0.0094
ENSG00000185519.8*	FAM131C	0.2534	0.5337	0.9668	4.129	3.64E-05	0.0101
ENSG00000186868.9	MAPT	0.1259	0.6869	0.9413	4.079	4.53E-05	0.0111
ENSG00000181826.5*	RELL1	0.7674	0.0148	0.0080	-4.044	5.26E-05	0.0126
ENSG00000122696.8	MCART1	0.1807	0.0078	0.0001	-4.037	5.41E-05	0.0127
ENSG00000167525.8*	PROCA1	0.4498	0.9920	0.9955	3.995	6.48E-05	0.0135
ENSG00000198633.5	ZNF534	0.0627	0.3249	0.0004	-3.901	9.60E-05	0.0181
ENSG00000258920.1*	RP11-33N16.1.1	0.9952	0.7239	0.6124	-3.887	0.0001	0.0187
ENSG00000175787.11*	ZNF169	0.6456	0.1089	0.0153	-3.877	0.0001	0.0189
ENSG00000119608.7*	PROX2	0.9140	0.9610	1.0000	3.877	0.0001	0.0189
ENSG00000119227.3*	PIGZ	0.6201	0.1483	0.9994	3.825	0.0001	0.0227
ENSG00000197444.5	OGDHL	0.1662	0.9447	0.9827	3.755	0.0002	0.0290
ENSG00000145088.4*	EAF2	0.0133	0.7132	0.6631	3.747	0.0002	0.0294
ENSG00000117010.11	ZNF684	0.4299	0.1574	0.0038	-3.635	0.0003	0.0424
ENSG00000204257.8*	HLA-DMA	0.3564	0.9267	0.9754	3.615	0.0003	0.0452
ENSG00000212127.5*	TAS2R14	0.9977	0.9572	0.7442	-3.578	0.0003	0.0500
ENSG00000188763.3*	FZD9	0.1366	0.1566	0.8063	3.572	0.0004	0.0504
ENSG00000151846.6	PABPC3	0.9597	0.9998	1.0000	3.542	0.0004	0.0551
ENSG00000188992.6*	LIPI	0.3335	0.8160	0.9882	3.524	0.0004	0.0574
ENSG00000188037.6*	CLCN1	0.4584	0.3305	0.9905	3.514	0.0004	0.0588
ENSG00000133460.13*	SLC2A11	0.6192	0.9990	0.9986	3.478	0.0005	0.0658
ENSG00000180574.3	EIF2S3L.1	0.0012	0.1199	0.3256	3.470	0.0005	0.0663
ENSG00000189221.5*	MAOA	0.1715	0.9821	0.9746	3.469	0.0005	0.0663
ENSG00000240303.3	ACAD11	0.0957	0.7912	0.9290	3.446	0.0006	0.0706
ENSG00000114948.7*	ADAM23	0.9957	0.8746	0.6600	-3.421	0.0006	0.0747
ENSG00000165689.12	SDCCAG3	0.9998	0.9987	0.8387	-3.413	0.0006	0.0759
ENSG00000117983.12	MUC5B	0.0464	0.0222	0.7712	3.405	0.0007	0.0767
ENSG00000204539.3*	CDSN	0.3032	0.3972	0.9825	3.389	0.0007	0.0773
ENSG00000107581.6	EIF3A	0.0003	0.0071	0.1783	3.385	0.0007	0.0773
ENSG00000180176.10*	TH	0.3571	0.0417	0.9374	3.385	0.0007	0.0773
ENSG00000175390.7	EIF3F	0.2147	0.5498	0.9749	3.385	0.0007	0.0773
ENSG00000213139.3*	CRYGS	0.9990	0.9955	0.8313	-3.380	0.0007	0.0773
ENSG00000167595.8	C19orf55	0.6083	0.0484	0.0119	-3.378	0.0007	0.0773
ENSG00000180346.2	TIGD2	0.5058	0.7542	0.0065	-3.377	0.0007	0.0773

ENSG00000099365.5*	STX1B	0.9968	0.4617	0.5316	-3.364	0.0008	0.0799
ENSG00000183496.5	MEX3B	0.9966	0.9642	0.5346	-3.362	0.0008	0.0799
ENSG00000177570.9	SAMD12	0.0130	0.0124	1.93E-06	-3.347	0.0008	0.0826
ENSG00000166069.9	TMCO5A	0.0475	0.8299	0.7545	3.347	0.0008	0.0826
ENSG00000172053.10	QARS	0.0231	0.1686	0.7808	3.342	0.0008	0.0833
ENSG00000185339.4*	TCN2	0.2023	0.5799	0.7191	3.319	0.0009	0.0898
ENSG00000110090.7	CPT1A	0.0000	0.0001	0.0117	3.296	0.0010	0.0957
ENSG00000203724.6*	C1orf53	0.7011	0.4060	0.9789	3.275	0.0011	0.1011
ENSG00000135722.3*	FBXL8	0.6311	0.9390	0.9634	3.275	0.0011	0.1011
ENSG00000143545.4	RAB13	0.2340	0.4487	0.9764	3.252	0.0011	0.1088
ENSG00000165496.3*	RPL10L	0.2066	0.9144	0.8340	3.204	0.0014	0.1252
ENSG00000136942.9	RPL35	0.2061	0.3337	0.9608	3.204	0.0014	0.1252
ENSG00000120662.10	MTRF1	0.1934	0.5507	0.6411	3.200	0.0014	0.1253
ENSG00000174370.5*	C11orf45	0.3819	0.4550	0.9565	3.199	0.0014	0.1253
ENSG00000104129.4	DNAJC17	0.1175	0.1818	0.8014	3.180	0.0015	0.1327
ENSG00000165730.10*	STOX1	0.2112	0.1670	0.9655	3.166	0.0015	0.1362
ENSG00000188610.7	FAM72B	0.3712	0.0585	0.0050	-3.165	0.0016	0.1362
ENSG00000177932.6	ZNF354C	0.0609	0.7408	0.8664	3.165	0.0016	0.1362
ENSG00000198246.7	SLC29A3	0.2722	0.4577	0.9708	3.161	0.0016	0.1367
ENSG00000125864.7*	BFSP1	0.2099	0.5362	0.9609	3.155	0.0016	0.1385
ENSG00000100290.2*	BIK	0.0278	0.1775	0.7026	3.145	0.0017	0.1421
ENSG00000102409.9	BEX4	0.8834	0.2411	0.0832	-3.121	0.0018	0.1531
ENSG00000130347.8	RTN4IP1	0.0939	0.1037	0.0002	-3.112	0.0019	0.1568
ENSG00000166206.8	GABRB3	0.9818	0.7704	0.3312	-3.103	0.0019	0.1600
ENSG00000156345.10*	CDK20	0.1596	0.0435	0.8826	3.093	0.0020	0.1646
ENSG00000180953.7*	ST20	0.9986	0.8343	0.9492	-3.086	0.0020	0.1668
ENSG00000116396.9*	KCNC4	0.8887	0.9795	0.9993	3.074	0.0021	0.1711
ENSG00000142798.11	HSPG2	0.0560	0.0097	0.8411	3.061	0.0022	0.1774
ENSG00000065150.12	IPO5	0.0494	0.3856	0.8252	3.043	0.0023	0.1867
ENSG00000214021.10*	TTLL3	0.8793	0.3992	0.9980	3.038	0.0024	0.1889
ENSG00000238243.2*	OR2W3	0.9143	0.9634	0.9985	3.035	0.0024	0.1892
ENSG00000113971.13	NPHP3	0.1498	0.6713	0.9027	3.031	0.0024	0.1904
ENSG00000205129.4*	C4orf47	0.5320	0.0246	0.0094	-3.004	0.0027	0.2038
ENSG00000104408.5	EIF3E	0.0243	0.2498	0.7035	3.000	0.0027	0.2049
ENSG00000204390.7	HSPA1L	0.0001	0.0135	0.0684	2.978	0.0029	0.2160
ENSG00000215790.2*	SLC35E2	0.8684	0.9821	0.9991	2.977	0.0029	0.2160
ENSG00000234444.3	ZNF736	0.2645	0.0041	0.0261	-2.973	0.0029	0.2163
ENSG00000103426.7*	CORO7	0.2185	0.0157	0.9572	2.970	0.0030	0.2163
ENSG00000148459.10	PDSS1	0.1670	0.0703	0.9198	2.968	0.0030	0.2163
ENSG00000196409.6*	ZNF658	0.7990	0.4423	0.0540	-2.946	0.0032	0.2253
ENSG00000155034.13	FBXL18	0.0983	0.4271	0.8453	2.944	0.0032	0.2253
ENSG00000141577.7	AZI1	0.6248	0.1246	0.9919	2.941	0.0033	0.2253
ENSG00000127415.8*	IDUA	0.4142	0.9082	0.9716	2.939	0.0033	0.2253
ENSG00000144395.12*	CCDC150	0.6998	0.9899	0.9933	2.938	0.0033	0.2253
ENSG00000146574.10*	CCZ1B	0.5251	0.1204	0.0182	-2.937	0.0033	0.2253
ENSG00000186136.1	TAS2R42	0.0002	0.0000	0.0064	2.935	0.0033	0.2253
ENSG00000205517.6*	RGL3	0.8356	0.9166	0.9998	2.933	0.0034	0.2253
ENSG00000153898.8	MCOLN2	0.0245	0.2354	0.6511	2.925	0.0034	0.2299
ENSG00000198088.5*	NUP62CL	0.9202	0.3414	0.2937	-2.919	0.0035	0.2330

ENSG00000171368.10*	TPPP	0.8975	0.9533	0.9998	2.912	0.0036	0.2367
ENSG00000162639.11*	HENMT1	0.8466	0.8086	0.1208	-2.898	0.0038	0.2443
ENSG00000105409.8	ATP1A3	0.0071	0.1027	0.4239	2.896	0.0038	0.2443
ENSG00000187583.5	PLEKHN1	0.3282	0.0927	0.9569	2.896	0.0038	0.2443
Genes also identified in Hsieh et al. (ref 4 in main text)							
ENSG00000118181.5	RPS25	0.0051	0.1106	0.9036	5.715	1.10E-08	3.86E-05
ENSG00000144713.8	RPL32	0.0901	0.8279	0.9977	5.371	7.83E-08	0.0002
ENSG00000026025.8	VIM	0.0131	0.0665	0.9747	5.213	1.86E-07	0.0002
ENSG00000100316.11	RPL3	0.3350	0.9577	0.9998	5.146	2.67E-07	0.0003
ENSG00000204628.7	GNB2L1	0.0234	0.2860	0.9817	5.140	2.75E-07	0.0003
ENSG00000108107.7	RPL28	0.0791	0.3941	0.9953	5.108	3.26E-07	0.0003
ENSG00000197756.4	RPL37A	0.1047	0.8223	0.9967	5.070	3.97E-07	0.0003
ENSG00000109971.8	HSPA8	0.0149	0.0708	0.9680	4.957	7.14E-07	0.0006
ENSG00000177600.4	RPLP2	0.0790	0.8218	0.9918	4.879	1.07E-06	0.0008
ENSG00000142676.8	RPL11	0.0760	0.6459	0.9832	4.696	2.65E-06	0.0016
ENSG00000161970.7	RPL26	0.3621	0.9494	0.9992	4.688	2.76E-06	0.0016
ENSG00000170889.9	RPS9	0.1487	0.4601	0.9961	4.685	2.80E-06	0.0016
ENSG00000112306.7	RPS12	0.8796	0.9994	1.0000	4.389	1.14E-05	0.0050
ENSG00000008988.5	RPS20	0.2398	0.7082	0.9976	4.348	1.38E-05	0.0058
ENSG00000198755.6	RPL10A	0.0961	0.6051	0.9815	4.331	1.48E-05	0.0058
ENSG00000142541.10	RPL13A	0.9352	0.9977	1.0000	4.278	1.89E-05	0.0071
ENSG00000170275.8	CRTAP	0.6913	0.9692	0.9999	4.269	1.96E-05	0.0071
ENSG00000149273.8	RPS3	0.0941	0.7106	0.9842	4.255	2.09E-05	0.0073
ENSG00000104529.12	EEF1D	0.3218	0.6687	0.9967	4.229	2.35E-05	0.0080
ENSG00000197958.8	RPL12	0.8591	0.9873	0.9999	4.217	2.48E-05	0.0081
ENSG00000140988.9	RPS2	0.9074	0.9764	0.9999	4.201	2.66E-05	0.0085
ENSG00000163584.13	RPL22L1	0.1387	0.5828	0.9904	4.181	2.91E-05	0.0090
ENSG00000198034.6	RPS4X	0.1826	0.9079	0.9910	4.137	3.51E-05	0.0100
ENSG00000101210.6	EEF1A2	0.0562	0.0901	0.9559	4.118	3.82E-05	0.0103
ENSG00000005022.5	SLC25A5	0.0056	0.0217	0.7855	4.097	4.19E-05	0.0110
ENSG00000188846.8	RPL14	0.2300	0.6697	0.9939	4.079	4.52E-05	0.0111
ENSG00000090621.8	PABPC4	0.2992	0.7515	0.9972	4.078	4.54E-05	0.0111
ENSG00000148303.12	RPL7A	0.4941	0.9736	0.9986	4.024	5.72E-05	0.0131
ENSG00000065978.12	YBX1	0.5918	0.9097	0.9992	4.010	6.07E-05	0.0135
ENSG00000162244.5	RPL29	0.3485	0.8358	0.9976	4.001	6.31E-05	0.0135
ENSG00000156508.12	EEF1A1	0.6632	0.9981	0.9997	3.998	6.38E-05	0.0135
ENSG00000101444.7	AHCY	0.1245	0.2899	0.9817	3.992	6.56E-05	0.0135
ENSG00000130741.5	EIF2S3	0.0392	0.3855	0.9395	3.971	7.17E-05	0.0145
ENSG00000111640.8	GAPDH	0.1257	0.1295	0.9776	3.953	7.73E-05	0.0154
ENSG00000137154.8	RPS6	0.2433	0.8593	0.9920	3.909	9.28E-05	0.0179
ENSG00000110700.2	RPS13	0.0530	0.2643	0.9377	3.907	9.36E-05	0.0179
ENSG00000161016.10	RPL8	0.2280	0.5500	0.9895	3.823	0.0001	0.0227
ENSG00000254772.4	EEF1G	0.7727	0.9958	0.9998	3.801	0.0001	0.0245
ENSG00000125691.7	RPL23	0.2930	0.8450	0.9914	3.719	0.0002	0.0324
ENSG00000122406.6	RPL5	0.1556	0.8827	0.9762	3.712	0.0002	0.0328
ENSG00000131269.10	ABCB7	0.0006	0.0037	0.2989	3.702	0.0002	0.0337
ENSG00000205339.5	IPO7	0.0552	0.4454	0.9219	3.646	0.0003	0.0412
ENSG00000171858.11	RPS21	0.2330	0.5493	0.9728	3.595	0.0003	0.0481
ENSG00000163931.10	TKT	0.0251	0.0245	0.8346	3.588	0.0003	0.0488

ENSG00000138326.12	RPS24	0.1031	0.6285	0.9570	3.542	0.0004	0.0551
ENSG00000156482.6	RPL30	0.3497	0.8755	0.9923	3.525	0.0004	0.0574
ENSG00000173599.9	PC	0.0428	0.0175	0.8824	3.492	0.0005	0.0631
ENSG00000171863.8	RPS7	0.0587	0.3194	0.9061	3.463	0.0005	0.0670
ENSG00000174444.9	RPL4	0.1724	0.6487	0.9742	3.442	0.0006	0.0707
ENSG00000142937.7	RPS8	0.6083	0.7882	0.9970	3.422	0.0006	0.0747
ENSG00000070756.8	PABPC1	0.9744	0.9999	1.0000	3.405	0.0007	0.0767
ENSG00000063177.7	RPL18	0.1226	0.3589	0.9501	3.399	0.0007	0.0773
ENSG00000145592.9	RPL37	0.4290	0.8322	0.9941	3.377	0.0007	0.0773
ENSG00000147677.6	EIF3H	0.0674	0.4743	0.8952	3.301	0.0010	0.0950
ENSG00000135390.12	ATP5G2	0.0041	0.0164	0.3490	3.214	0.0013	0.1232
ENSG00000137955.10	RABGGTB	0.1182	0.6948	0.9144	3.080	0.0021	0.1692
ENSG00000134419.10	RPS15A	0.8890	0.9863	0.9993	3.028	0.0025	0.1907
ENSG00000187109.8	NAP1L1	0.3603	0.9182	0.9815	3.003	0.0027	0.2038
ENSG00000178035.6	IMPDH2	0.0235	0.0900	0.6855	2.979	0.0029	0.2160
ENSG00000100129.11	EIF3L	0.1485	0.7605	0.9323	2.969	0.0030	0.2163
ENSG00000167526.9	RPL13	0.8475	0.9673	0.9989	2.961	0.0031	0.2198
ENSG00000164587.7	RPS14	0.2637	0.7238	0.9621	2.941	0.0033	0.2253
ENSG00000186468.8	RPS23	0.1339	0.5645	0.9039	2.935	0.0033	0.2253

Supplementary Table 3: mRNA translation downstream of mTOR. Genes identified by Babel analysis as having significant mRNA translational changes (FDR < 25%) upon inhibition of PC3 cells with the mTOR inhibitor PP242.

	Significance				
	P-value	Q-value	Complex formation[b]	Subunit status	Refs[c]
Expressed					
EIF3A	7.11E-04	0.077	Essential	Conserved	
EIF3F	7.12E-04	0.077	Essential	Non-conserved	
EIF3H	9.65E-04	0.095	Essential	Non-conserved	[4,6]
EIF3E	2.70E-03	0.205	Essential	Non-conserved	
EIF3L	2.99E-03	0.216	Dispensable	Non-conserved	[4,6]
EIF3D	0.080	-	Dispensable	Non-conserved	
EIF3M	0.092	-	N.A.	N.A.	
EIF3I	N.S.	-	Dispensable	Conserved	
EIF3B	N.S.	-	Essential	Conserved	
EIF3J	N.S.	-	Dispensable	N.A.	
Not expressed[a]					
EIF3C	-		Essential	Conserved	
EIF3G	-		Dispensable	Conserved	
EIF3K	-		Dispensable	Non-conserved	

[a] Unexpressed genes not assessed by Babel analysis

[b] Subunit is part of the functional core essential for mammalian eIF3 complex formation

[c] Previously identified mTOR-mediated mRNA translational change

Supplementary Table 4: mRNA translation of eukaryotic initiation factor 3. Shown here is the landscape of mTOR-dependent mRNA translation of genes encoding subunits of the eIF3 complex.

SUPPLEMENTARY NOTE

Low expressing genes

An important issue in analyzing ribosome profiling data, and more generally all count-based RNA data, is how to handle genes with low expression in a given transcriptome. In previous ribosome profiling studies (Bazzini, et al., 2012; Guo, et al., 2010; Hsieh, et al., 2012; Ingolia, et al., 2009; Ingolia, et al., 2011; Thoreen, et al., 2012), genes with low mRNA counts were eliminated from consideration and analysis, typically on the basis of an absolute threshold in read counts irrespective of gene size (such as 128 or 256). While such an approach is rational, we believe it does not fully utilize the information in such data. Here, we sought to preserve the largest fraction of the expressed transcriptome that is supported by data. Therefore, we developed a simulation-based method, described in the Methods, to estimate whether genes were expressed based on mRNA count, size of the gene, and size of the mRNA library that is similar to a previous approach (Ramskold, et al., 2009). The result is that we typically analyze a higher proportion of genes (typically greater than 10,000 transcripts per transcriptome).

Nevertheless, this approach does not avoid completely the issue of low counts, but rather shifts it to the ribosome-protected fragments (RPF) data. Here, using our parametric bootstrap method for estimating p-values, two issues arise: (1) it is not possible to find significantly decreased ribosome counts when the expected count is low, and (2) the p-values for low count data can be inaccurate. The latter complication is subtle, as the number of unique RPF counts in the bootstrap distribution limits the number of potential p-values for a particular mRNA value. If the former is small, then every increase in RPF count leads to a substantial jump in the p-value. In addition, a few false positive reads could have a large impact on the p-value, which amplifies the importance of the approach used for read alignment.

The solution to the low count problem is not straightforward, as it depends on both the mRNA and RPF dispersion and the RPF library size. Additionally, while greater depth of coverage for the library may resolve some cases, it is by no means a solution for all genes, as has been demonstrated in RNA-seq data previously. The Babel framework is robust to including low RPF-count genes, so a sensible strategy is to fit all genes that pass the expression filter and then to give additional scrutiny to those genes that have low expected RPF counts, or more simply, low observed RPF counts.

Histone genes

In every ribosome profiling data set that we have analyzed we have observed the apparent and persistent translational regulation (greater than expected ribosome association given mRNA abundance) of genes encoding members of the histone complex (H1, H2A, H2B, H3, and H4). However, non-variant histone proteins are encoded by replication-dependent genes that are not polyadenylated, rather they are transcriptionally and translationally regulated by a unique 3' stem loop (Marzluff, et al., 2008). As a result, the poly(A)+ selection step in the mRNA library preparation will result in an under-representation of non-polyadenylated transcripts, altering the relationship between observed ribosome association and mRNA levels. Therefore, of the 71 genes encoding subunits of histone complexes present in the Gencode-derived unified gene model used by Babel, the subset determined to be expressed in a given transcriptome (see Methods) are excluded from the statistical assessment of mRNA translation (48 histone-encoding genes among 10,582 expressed genes in the PC3 data analyzed here).

SUPPLEMENTARY REFERENCES

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