Transcriptome In Vivo Analysis (TIVA) of spatially defined single cells in intact live mouse and human brain tissue

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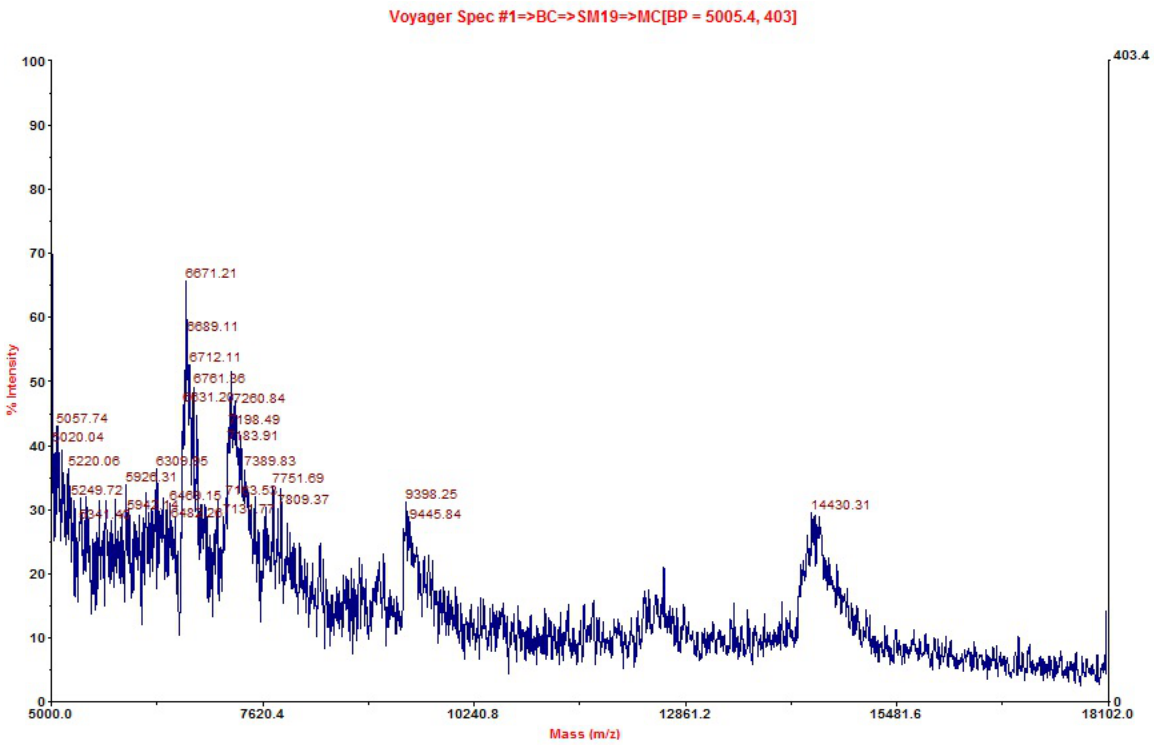
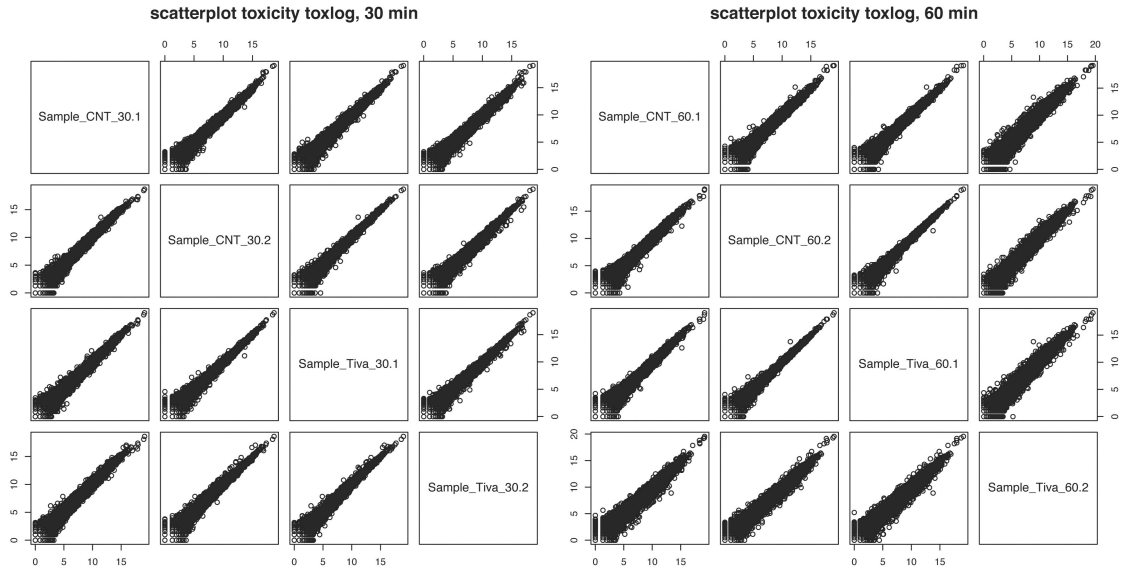
Supplementary Figure 1 | MALDI-TOF mass spectrum of $(D\text{-}Arg)_9$ TIVA-tag



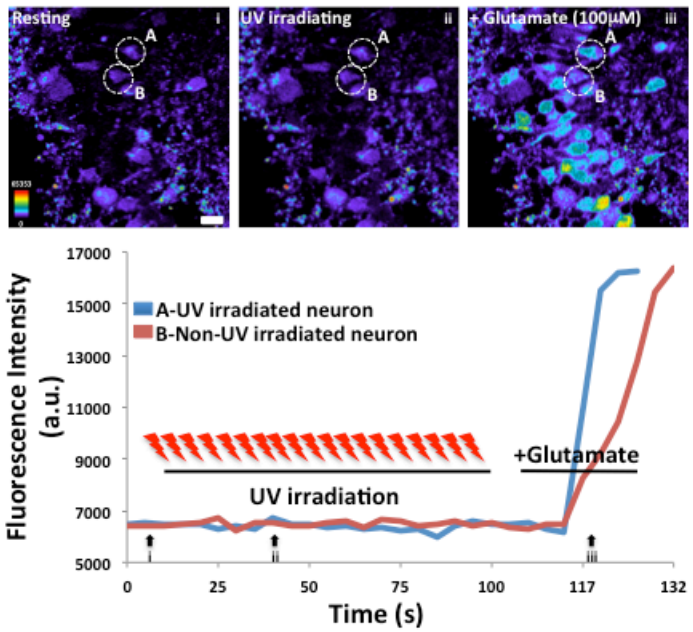
Supplementary Figure 1. MALDI-TOF mass spectrum of $(D\text{-}Arg)_9$ TIVA-tag. Spectrum was recorded on an Applied Biosystems Voyager System 6030 operated in negative ion mode. The peak at 14,430.31 corresponds to the intact $(D\text{-}Arg)_9$ TIVA-tag (expected mass is 14,413). Lower mass peaks are indicative of photolysis products resulting from oligonucleotide cleavage due to MALDI laser exposure.

Supplementary Figure 2 | Global gene expression changes are not induced during TIVA-tag loading



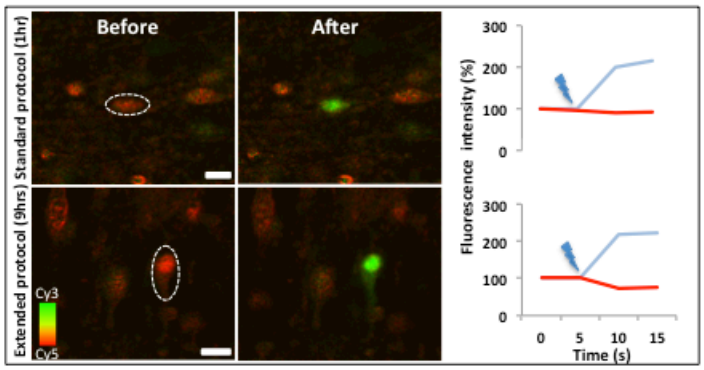
Supplementary Figure 2: Global gene expression changes are not induced during TIVA-tag loading. Scatter plots of transcriptomes among slices with TIVA-tag (30 min: Sample_CNT30.1, Sample_CNT30.2; 60 min: Sample_CNT60.1, Sample_CNT60.2) and without TIVA-tag (30 min: Sample_TIVA30.1, Sample_TIVA30.2; 60 min: Sample_TIVA60.1, Sample_TIVA60.2). RNA was bulk extracted from tissue and amplified one round followed by library generation and RNA-seq.

Supplementary Figure 3 | Multiple UV irradiations did not alter calcium excitability



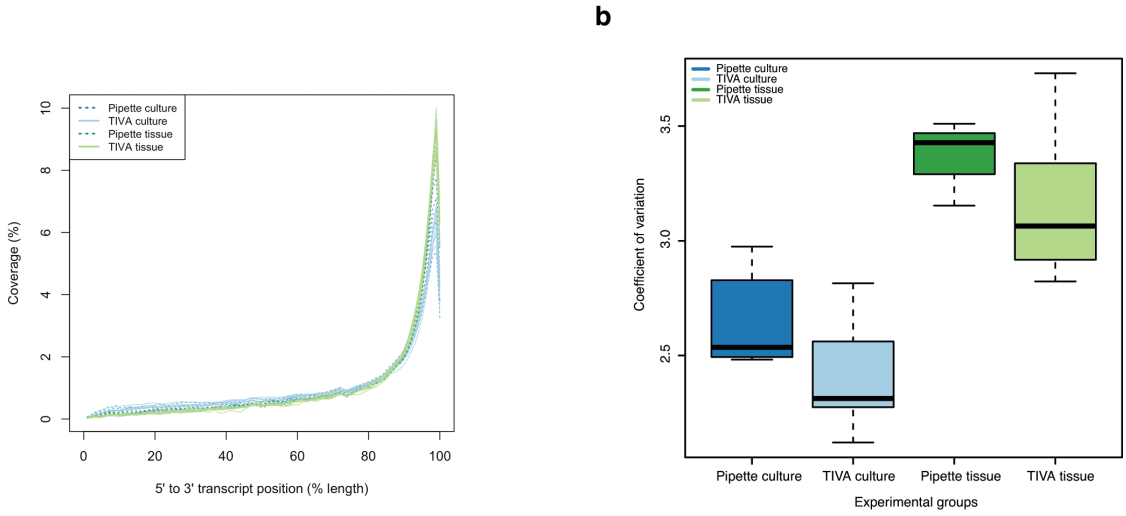
Supplementary Figure 3: Multiple UV irradiations did not alter calcium excitability. In order to evaluate whether any photodamage occurred during TIVA-tag uncaging with the 405 nm laser, excessive repetition (20 times more than uncaging protocol) of irradiation was performed on targeted neuron. Top: Acute brain slice loaded with the Ca^{2+} indicator, fluo-4 AM (left: before (i), middle: UV irradiating (ii), right: after glutamate stimulation (iii)). Bottom: 20 UV irradiations of the target cell (A-blue trace) caused no Ca^{2+} response. Both irradiated and non-irradiated neurons responded to glutamate treatment showing that the irradiated cell responded normally to stimulation. Time interval for image capturing was modified after 100 second time point from 3 to 5 seconds interval. Imaging depth 50 μm; Scale bar: 10 μm.

Supplementary Figure 4 | TIVA-tags in neurons were stable for up to 9 hours following loading



Supplementary Figure 4: TIVA-tags in neurons were stable for up to 9 hours following loading. Left: (upper panel) Neuron loaded with TIVA-tag 1 h after loading, and (lower panel) after 9 hours of loading. Neurons in circles were uncaged. Right: The corresponding fluorescence plots on the right show fluorescence signal changes. Scale bar: 10 μm.

Supplementary Figure 5 | Evenness of read coverage for TIVA isolated RNAs

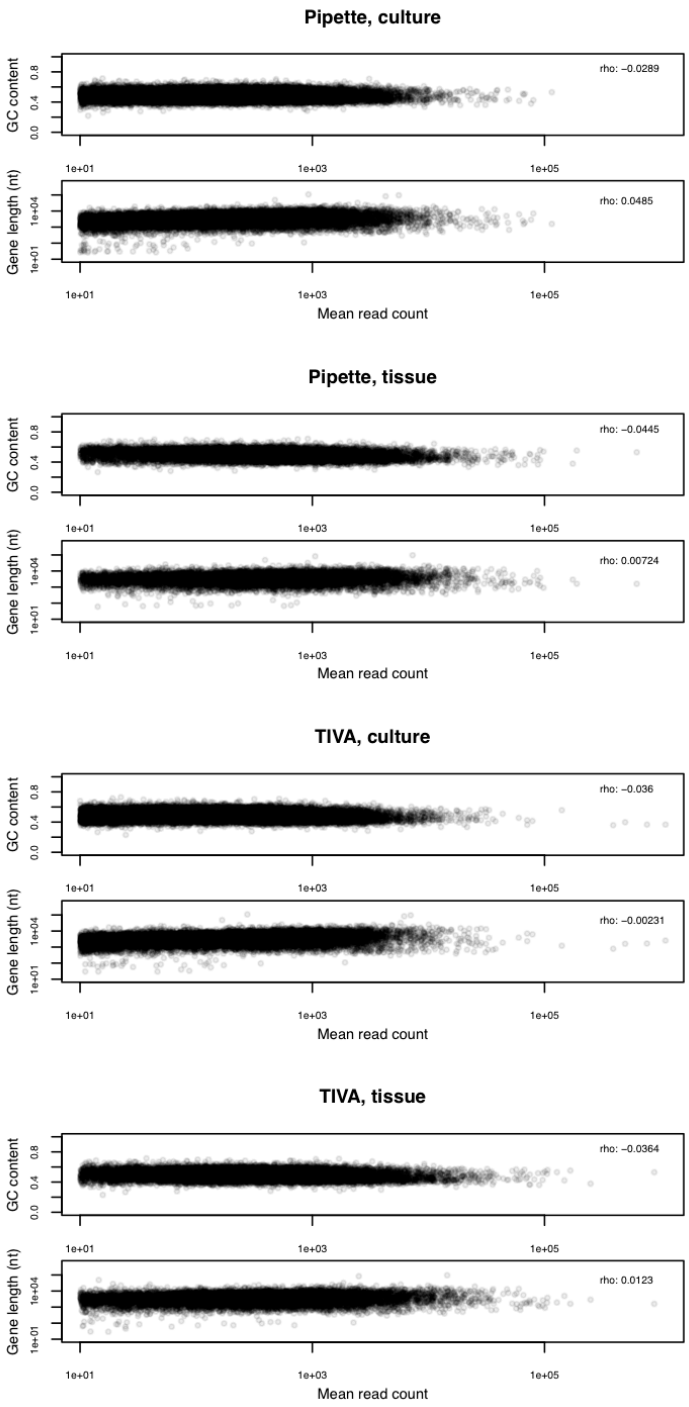


Supplementary Figure 5: Evenness of Read Coverage for TIVA Isolated RNAs: To assess the effect of TIVA collection on evenness of coverage, we employed two methods used by Adiconis et al. (Adiconis X, Borges-Rivera D, Satija R, DeLuca DS, Busby MA, Berlin AM, Sivachenko A, Thompson DA, Wysoker A, Fennell T, Gnirke A, Pochet N, Regev A, Levin JZ. Nat Methods. 2013 Jul;10(7):623-9). (A) We assessed evenness of coverage across the length of a transcript, from 5' to 3'. To do this we selected highly expressed genes, retaining those with greater than 500 uniquely aligned reads. We divided each gene into 100 equally sized bins by length and then calculated the relative read-depth observed for each bin. Finally, for each sample, we examined the average relative coverage across all transcripts. (B) As a global measure of evenness of coverage we calculated the average coefficient of variation in per-nucleotide coverage across the 1000 most highly expressed transcripts for each sample. For all coverage analysis, we considered only reads where both mates aligned uniquely to genes on autosomal chromosomes. Transcripts shorter than 100 nucleotides in length were excluded from analysis. For some uses of RNA sequencing data, such as isoform detection or discovery, evenness of read coverage across a transcript is beneficial. To determine whether TIVA collection influences evenness of coverage as a function of transcript length, we examined relative coverage over 100 evenly spaced bins along highly expressed transcripts for both TIVA and pipette collected samples. We observe no difference in coverage due to collection technique. We additionally computed the average coefficient of variation in coverage depth across highly expressed transcripts for each sample. Again, we observe no difference in evenness of coverage due to collection method (Welch's t-test p-value > 0.05).

Reference: Adiconis X, Borges-Rivera D, Satija R, DeLuca DS, Busby MA, Berlin AM, Sivachenko A, Thompson DA, Wysoker A, Fennell T, Gnirke A, Pochet N, Regev A, Levin JZ. Nat Methods. 2013 Jul;10(7):623-9

Supplementary Figure 6 | The Effect of GC content and gene length on TIVA-tag captured and pipette collected samples

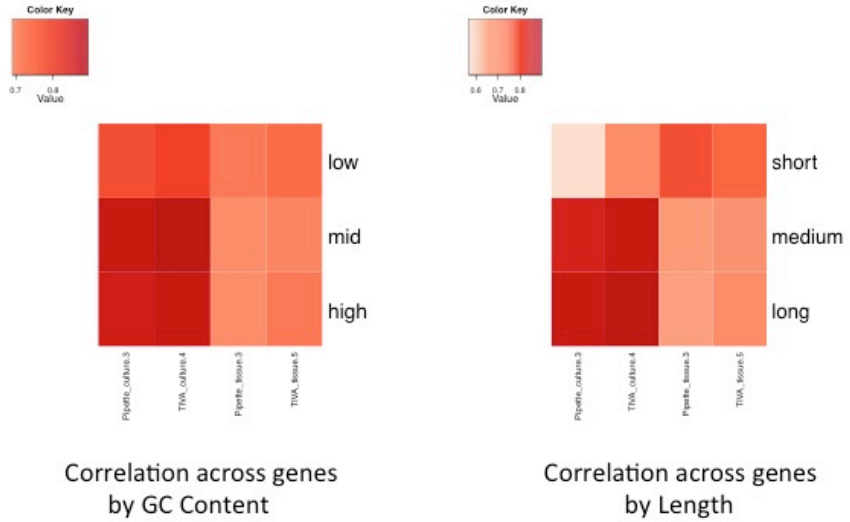
a



Supplementary Figure 6: The Effect of GC content and gene length on TIVA-tag captured and pipette collected samples. (A) Correlation of GC content and gene length with normalized read abundances. Gene traits for UCSC known genes is shown on the y-axis, plotted against average read depth on the x-axis for four different experimental groups. Top panel: pipette-collected samples from culture (n=7); Second panel: TIVA-captured samples from culture (n=8); Third panel: pipette-collected samples from tissue (n=3); Fourth panel: TIVA-captured samples from tissue (n=7). Genes with an average read-depth of 10 or more. (B) To detect bias in read counts due to sequence traits (such as GC content or length) that may be unique to TIVA-collection, we examined correlations in abundance measurements for TIVA- and pipette-collected samples over sets of genes binned by these traits. As in Adiconis et al. Nature Methods 2013, we categorized genes as having low (<37%), medium (>37% up to 62%) or high (>62%) GC content, and as being short (<1000 nucleotides), medium length (1000 to 5000 nucleotides) or long (>5000 nucleotides). To minimize the confounding effect of biological variation on this comparison, we restricted our attention to hippocampal neurons. To further test for differences in abundance

Supplementary Figure 6 | The Effect of GC content and gene length on TIVA-tag captured and pipette collected samples

b

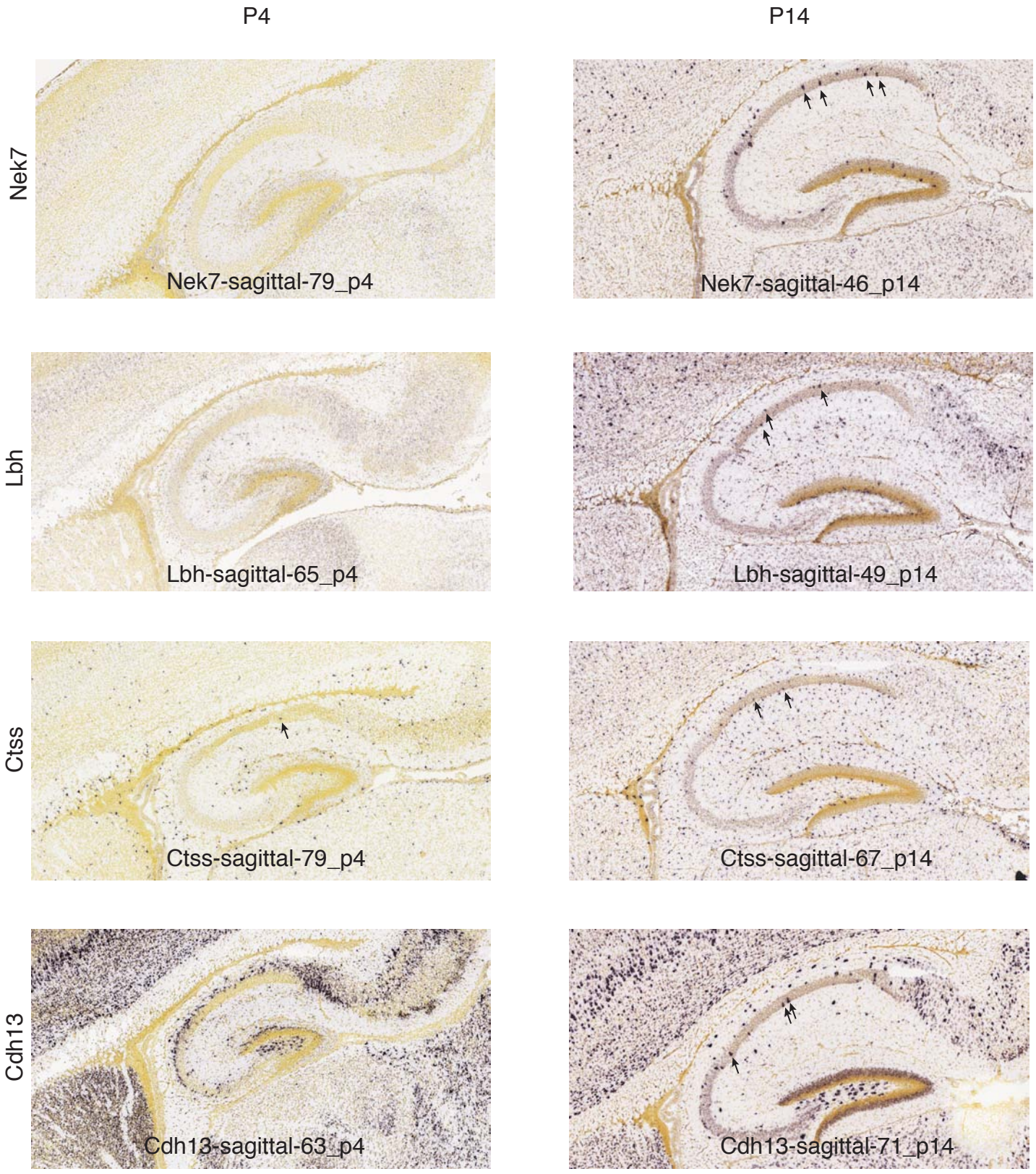


estimates due to an interaction between sequence traits and collection method, we performed ANOVA on genes categorized by trait bin (as described above) and collection method. Because RNA-sequencing counts are over-dispersed, we perform this analysis on $\log_{10}$ counts (after incrementing all counts by one). Additionally, because we are concerned with accuracy of abundance estimation, we examined only genes with reads observed in all samples. As a more sensitive measure, we compared correlations in abundance measurements across TIVA- and pipette-collected samples for genes binned by GC-content and by length. We estimated how much effect the interaction of sequence traits and collection technique has on abundance estimates using ANOVA, both for GC content and for length. We treated culture samples and tissue samples separately in this analysis. Due to the large number of genes included in these tests, most examined interactions are significant; however, the effect sizes are small in all cases, accounting for less than 0.2% of observed variation in mean abundance (Supplementary table 5).

Supplementary Figure 7 | Selected Allen Brain Atlas images displaying bimodally expressed genes

Images derive from the Allen Brain Atlas (ABA) (http://www.brain-map.org/) of the developing mouse brain at P4 and P14 days of age. All genes were picked based on the bimodal gene expression extracted from the transcriptomes among single hippocampal CA1 neurons collected using TIVA-tag mediated RNA isolation. Mining the ABA allowed us to retrieve instant validation of 87 bimodally identified genes, of which, 14 are shown here. In general, the expression pattern was either i) strong bimodal expression at either or both time points, or ii) clear upregulation of the gene over the developmental window of P4 to P14 days. Note, the single neurons collected with TIVA were performed on 9 days old tissue. Arrows indicate the presence of clear and strong bimodal expression in individual hippocampal cells. Inserted text in image indicates the “gene symbol” - “section cut direction” - “section number” - “developing age” provied by the ABA

Supplementary Figure 7 | Selected Allen Brain Atlas images displaying bimodally expressed genes



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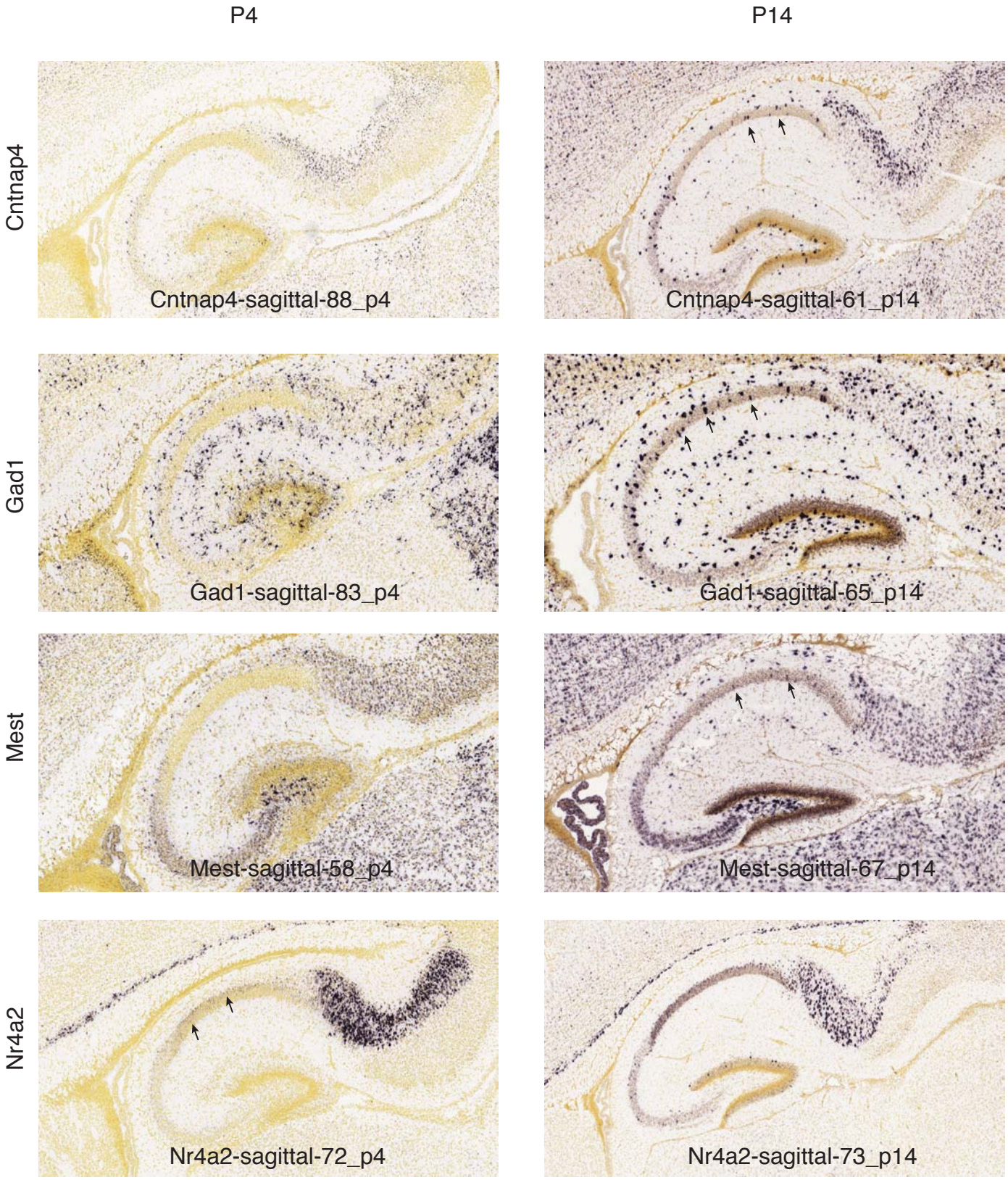

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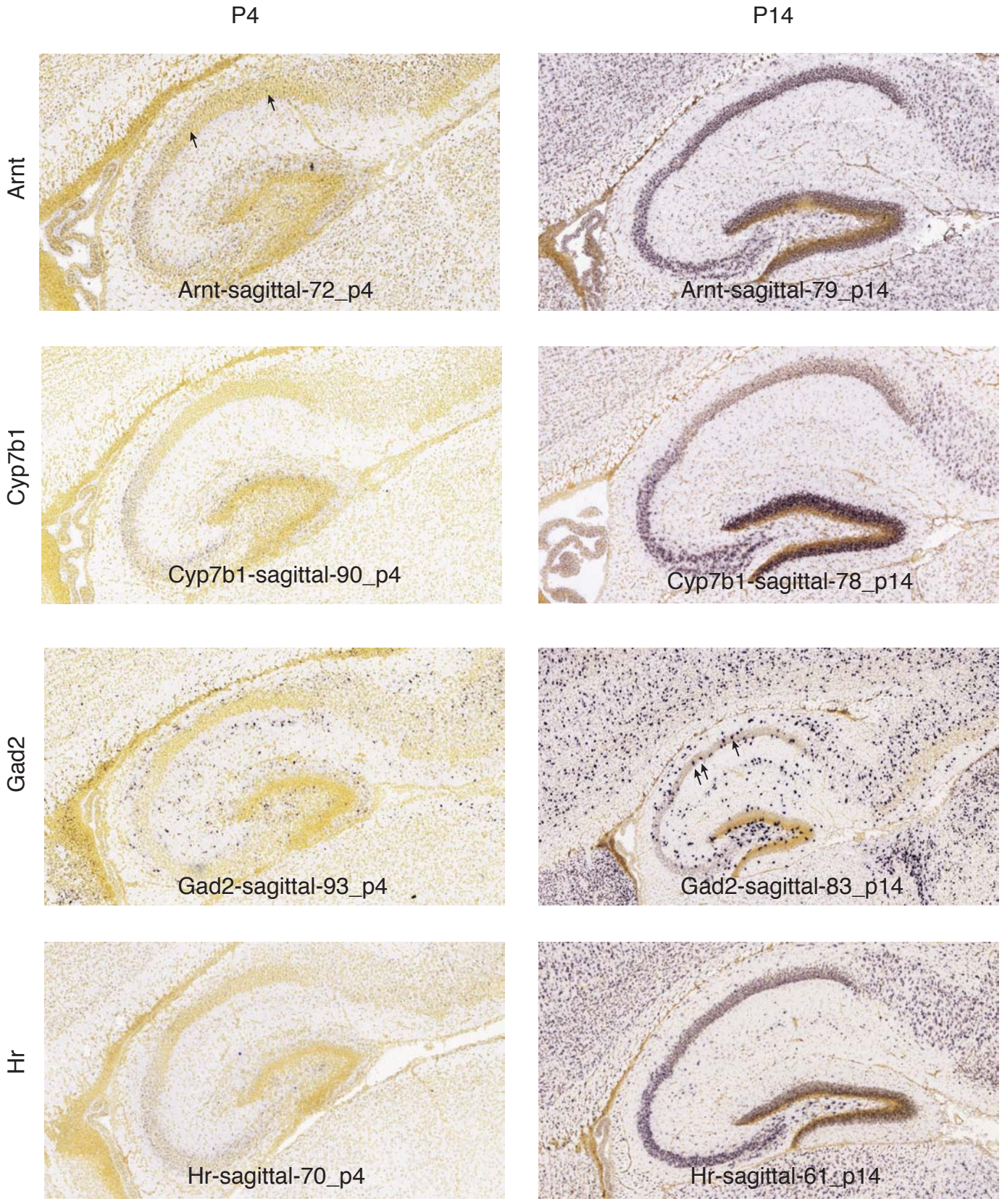

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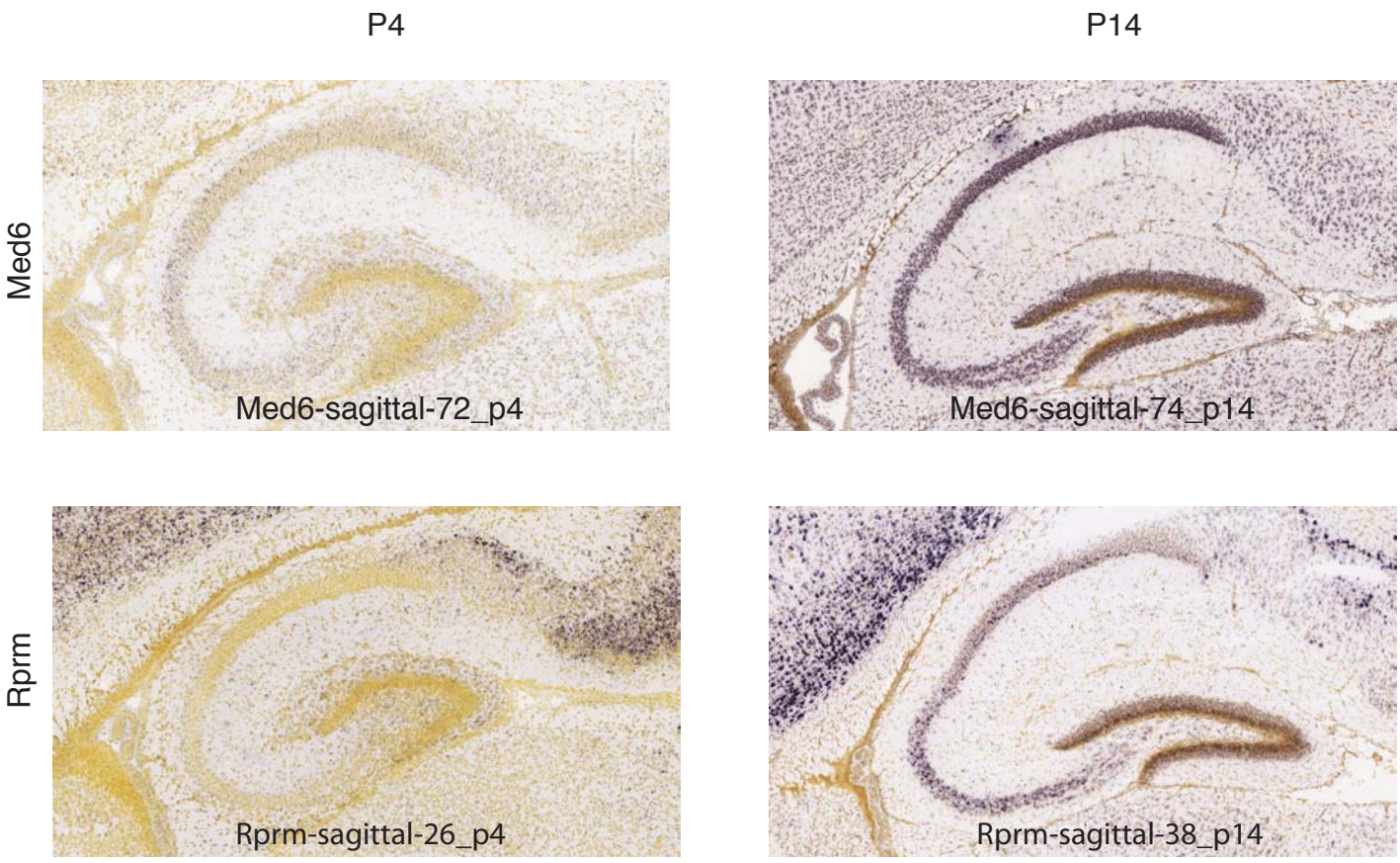

Supplementary Table 1 | Global gene expression changes are not induced during TIVA-tag loading.

Correlation coefficient between slices with and without TIVA-tag (n = 2 slices per group and time point) after 30 min and 60 min loading. RNA was bulk extracted from tissue and amplified one round followed by library generation and RNA-seq.

Incubation time (min)	Correlation coefficient control vs. test
30	0.97±0.02
60	0.97±0.02

Supplementary Table 2 | Expression Tlr3 gene targets and the Tlr3 receptor

The transcriptomes for bulk tissue and single tissue cells were mined for the expression of the Tlr3 receptor as well as the downstream genes that are expressed resulting from dsRNA acting on the Tlr3 receptor (Ganes C Sen & Saumendra N Sarkar, Nature Immunology 6, 1074 - 1076 (2005)). Numbers indicate DE-normalized read counts.

	Bulk tissue			**Single tissue neurons**				
Gene symbol	**Bulk.4b**	**Bulk.5a**	**Bulk.3b**	**Vivo.2E**	**Vivo.T7**	**Vivo.T9**	**Vivo.T10**	**Vivo.2F**
Ccl5	6	1	1	0	0	0	0	0
Cxcl10	0	32	10	0	0	0	0	0
Ifih1	60	68	58	0	0	1	0	0
Ifnb1	0	0	0	0	0	0	0	0
Il6	0	1	0	0	0	0	0	0
Sele	0	0	0	0	0	0	0	0
Tnf	0	8	0	0	0	2	2	0
Tlr3	10	110	168	0	0	1	1	0

Note that Supplementary Table 3 & Table 6 can be found in separate files.

Supplementary Table 4 | 5'-coverage of TIVA-Isolated mRNAs.

To measure the extent of coverage at the five prime (5') end of expressed genes, we calculated the fraction of genes demonstrating read coverage within the 250 most 5' bases. We limited our attention to expressed genes, defined here as those with at least ten read counts.

		Fraction 5' covered	Fraction 3' covered	Fraction 5' covered / Fraction 3' covered
Pipette culture	CTX_11	23%	72%	32%
	CTX_7	24%	75%	32%
	CTX_8	24%	72%	34%
	CTX_10	21%	69%	30%
	Sample_36H	39%	84%	46%
	Sample_40H	30%	79%	38%
	Sample_43H	39%	86%	46%
TIVA culture	Sample_DL.cs2	49%	89%	55%
	Sample_DL.cs3	30%	81%	37%
	Sample_DL.cs4	30%	84%	35%
	Sample_DL.cs5	25%	81%	31%
	Sample_DL.cs10	26%	83%	32%
	Sample_DL.cs6	38%	85%	44%
	Sample_DL.cs7	38%	88%	44%
	Sample_DL.cs8	24%	85%	28%
Pipette tissue	Sample_Tiva_44	13%	79%	16%
	Sample_Tiva_45	12%	79%	15%
	Sample_Tiva_46	16%	80%	20%
TIVA tissue	Sample_3T5	11%	77%	15%
	Sample_3T19	9%	77%	11%
	Sample_Tiva_2E	15%	82%	18%
	Sample_T7	19%	84%	22%
	Sample_T9	13%	81%	17%
	Sample_T10	17%	80%	21%
	Sample_Tiva_2F	14%	81%	17%
Bulk	HPs4b	50%	92%	54%
	Sample_DL.HP3b	49%	91%	54%
Experimental summaries	**TIVA**	**24%**	**83%**	**28%**
	TIVA culture	**33%**	**85%**	**38%**
	TIVA tissue	**14%**	**80%**	**17%**
	Pipette	**24%**	**77%**	**31%**
	Pipette culture	**29%**	**77%**	**37%**
	Pipette tissue	**13%**	**79%**	**17%**
	Bulk	**50%**	**92%**	**54%**
	Overall average	**26%**	**81%**	**31%**

Supplementary Table 5 | ANOVA Testing of the Effect of GC content and gene length on TIVA-tag captured and pipette collected samples.

Summary of ANOVA results testing the effect of collection technique and GC-content or length on abundance estimates.

GC content ANOVA

Bins used

Low: 0% to 37%
Medium: >37% to 62%
High: >62% to 100%

Culture samples

Mean expression level by category

		Collection method		Difference in median, Pipette - TIVA
		Pipette (n=3)	TIVA (n=4)	
GC bin	low (n=157)	2.06	2.51	-0.44
	mid (n=12020)	2.29	2.38	-0.11
	high (n=185)	2.27	2.12	0.16

ANOVA results

	Sum Sq	Df	F value
(Intercept)	8.59E+10	1.00	3572.24
GC content	1.10E+09	2.00	22.92
experimental group	5.78E+08	1.00	24.02
GC content:experimental group	1.96E+09	2.00	40.67
Residuals	2.08E+12	86528.00	
GC content by technique effect	**0.090%**		

Tissue samples

Mean expression level by category

		Collection method		Difference in median, Pipette - TIVA
		Pipette (n=3)	TIVA (n=5)	
GC bin	low (n=84)	2.64	2.57	0.07
	mid (n=6143)	2.63	2.63	-0.03
	high (n=102)	2.24	2.44	-0.22

ANOVA results

	Sum Sq	Df	F value
(Intercept)	1.97E+11	1.00	3315.37
GC_content	7.39E+09	2.00	62.21
experimental_group	5.82E+04	1.00	0.00
GC_content:experimental_group	4.79E+08	2.00	4.04
Residuals	3.01E+12	50626.00	
GC content by technique effect	0.015%		

Supplementary Table 5 | ANOVA Testing of the Effect of GC content and gene length on TIVA-tag captured and pipette collected samples.

Continued…

Length ANOVA

Bins used

Short: 1 to 999 nt
Medium: 1000 to 5000 nt
Long: > 5000 nt

Culture samples

Mean expression level by category

		Collection method		Difference in median, Pipette - TIVA
		Pipette (n=3)	TIVA (n=4)	
Length bin	short (n=787)	2.28	2.38	-0.07
	mid (n=7996)	2.23	2.30	-0.08
	long (n=3579)	2.39	2.57	-0.18

ANOVA results

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	8.05E+11	1.00	33968.23	0.00
length	2.47E+10	2.00	521.43	0.00
experimental_group	3.04E+09	1.00	128.12	0.00
length:experimental_group	5.76E+09	2.00	121.46	0.00
Residuals	2.05E+12	86528.00		
Length by technique effect	**0.199%**			

Tissue samples

Mean expression level by category

		Collection method		Difference in median, pipette - TIVA
		Pipette (n=3)	TIVA (n=5)	
Length bin	short (n=450)	2.61	2.61	-0.04
	mid (n=3831)	2.60	2.59	-0.03
	long (n=2048)	2.66	2.69	-0.03

ANOVA results

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	1.81E+12	1.00	30571.66	0.00
length	7.61E+09	2.00	64.06	0.00
experimental_group	3.60E+08	1.00	6.06	0.01
length:experimental_group	2.82E+07	2.00	0.24	0.79
Residuals	3.01E+12	50626.00		
Length by technique effect	0.001%			

Note that Supplementary Table 3 & Table 6 can be found in separate files.