

Supplemental Table 1. List of reagents and suppliers			
Chemical Supplies	Chemical Name	Supplier	Part Number
ExM Gel or Preparation	Sodium Acrylate (purity note:*)	Sigma	408220
	Acrylamide	Sigma	A9099
	N,N'-Methylenebisacrylamide	Sigma	M7279
	Ammonium Persulfate	Sigma	A3678
	N,N,N',N'-Tetramethylethylenediamine	Sigma	T7024
	VA-044	Wako	27776-21-2
	4-Hydroxy-TEMPO	Sigma	176141
Hybridization Buffer	Dextran Sulfate	Sigma	D8906-50g
	SSC	Thermo Fisher	AM9765
	Formamide	Thermo Fisher	AM9342
Fixation and Permeabilization	Paraformaldehyde	Electron Microscopy Sciences	15710
	Tissue-prep Buffered 10% Formalin	Electron Microscopy Sciences	15742-10
	Triton X-100	Sigma	93426
	Ethyl Alcohol	Sigma	E7023
	Glycine	Sigma	50046
	10x PBS	Thermo Fisher	AM9624
Protein Digestion	Proteinase K	New England Biolabs	P8107S
	Ethylenediaminetetraacetic acid	Sigma	EDS
	Sodium Chloride	Sigma	S9888
	Tris-HCl	Life Technologies	AM9855
HCR Amplification	Amplification Buffer	Molecular Instruments	N/A
	Tween 20	Sigma	P1379
LabelX Preparation	Label-IT ® Amine	Mirus Bio	MIR 3900
	Acryloyl-X, SE	Thermo Fisher	A20770
LabelX Treatment	MOPS	Sigma	M9381-25G
Reembedded Gels Staining	DNase I	Sigma	4716728001
Bind-silane Treatment	Bind-Silane	Sigma	GE17-1330-01
	* check for yellow color upon resuspension: that indicates poor quality; solution should be clear (see http://expansionmicroscopy.org)		

Decades (Transcript Abundance)	Mean (Ratio of # spots detected in individual cells after ExM, to # spots detected before ExM)	Standard Deviation	Sample size (<i>n</i>)	<i>p</i> -Value
10s	1.082	0.177	14	0.107
100s	1.105	0.138	29	3.24×10 ⁻⁴
1000s	1.596	0.562	16	7.09×10 ⁻⁴

Supplementary Table 2. Statistical Analysis of RNA FISH spots detected in individual cells before and after ExFISH. For RNA molecules detected before vs after expansion, spots were counted by an automated algorithm. The ratio of the number of spots after ExM to spots counted before ExM was determined for each cell. Spot counts were grouped into decades based on the pre-expansion spot count. The table shows the results of a one-sample T-test performed on the ratio of spots counts for each decade to determine significant deviation from the expected mean ratio value of one.

Target	Total Spot Count (Averaged Across Both Red and Blue Channels)	Co-localized Spots	Co-localization %	Hybridization Efficiency	Volume analyzed (μm^3 in unexpanded coordinates)	Density (Co- localized Puncta per μm^3)
<i>ActB</i>	27504	15866	0.577	0.76	236749	0.067
<i>Dlg4</i>	9795	5174	0.528	0.727	236749	0.022
<i>Camk2a</i>	14440	8799	0.609	0.781	147968	0.059
<i>Dlg4</i> Missense	1540	4	0.003	0.051	147968	0
mCherry	1209	0	0	0	147968	0

Supplementary Table 3. Analysis of two-color colocalization of FISH probes with HCR amplification in expanded slices.

Supplementary Video 1. Volume rendering of Thy1-YFP (green) brain tissue acquired by lightsheet microscopy with HCR-ExFISH targeting *YFP* (red) and *Gad1* (blue) mRNA. Movie of volume in **Supp. Fig. 9**.