

Supplementary Table S1

Summary of directional RNA sequencing

	Sample	Total # reads	# Valid reads ^a	(%) ^b	# Mapped reads	(%) ^c	# Uniquely mapped reads	(%) ^d	# Uniquely mapped reads after removal of duplication	Top strand-mapped reads / Bottom strand-mapped reads ^e
1st run	Chimpanzee Cerebral Cortex (n=1)	75,012,641	70,894,403	94.5%	61,404,334	86.6%	55,436,357	78.2%	18,894,958	1.03
	Chimpanzee Cerebral Cortex (n=2)	77,141,984	72,913,619	94.5%	63,089,059	86.5%	56,935,814	78.1%	19,057,212	1.03
	Chimpanzee Cerebellum (n=1)	76,342,191	72,237,582	94.6%	64,283,120	89.0%	59,273,070	82.1%	22,562,341	1.04
	Chimpanzee Cerebellum (n=2)	76,081,033	71,963,443	94.6%	63,973,178	88.9%	58,955,648	81.9%	22,396,483	1.04
	Mouse Cerebral Cortex (n=1)	76,557,221	72,322,648	94.5%	68,635,440	94.9%	62,286,305	86.1%	18,552,890	1.02
	Mouse Cerebral Cortex (n=2)	76,687,574	72,437,179	94.5%	68,665,190	94.8%	62,418,231	86.2%	19,814,914	1.02
2nd run	Mouse Cerebellum (n=1)	220,787,053	192,894,809	87.4%	181,763,145	94.2%	164,728,033	85.4%	30,097,533	1.03
	Mouse Cerebellum (n=2)	238,989,445	207,347,311	86.8%	195,402,638	94.2%	177,060,051	85.4%	30,571,226	1.03
	Mouse Heart (n=1)	233,051,369	201,311,951	86.4%	190,937,364	94.8%	146,153,387	72.6%	18,243,843	1.01
	Mouse Heart (n=2)	220,364,813	191,584,172	86.9%	181,795,500	94.9%	139,227,099	72.7%	17,875,501	1.01

^aThe number of reads passing the read trimming

^bThe percentage of valid reads in the total reads

^cThe percentage of mapped reads in the valid reads

^dThe percentage of uniquely mapped reads in the valid reads

^eThe ratio of top strand- to bottom strand-mapped reads after removal of duplication

Supplementary Table S2

Comparison of the number of the uniquely mapped reads in directional RNA-seq data of chimpanzee samples mapped onto the human versus the chimpanzee genome

Sample	Reference genome	# Mapped reads	(%) ^a	# Uniquely mapped reads	(%) ^b
Chimpanzee Cerebral Cortex	human	62,246,697	86.6%	56,186,086	78.1%
	chimpanzee	60,036,046	83.5%	52,078,154	72.4%
Chimpanzee Cerebellum	human	64,128,149	88.9%	59,114,359	82.0%
	chimpanzee	62,768,664	87.1%	57,441,695	79.7%

^aThe percentage of mapped reads in the valid reads

^bThe percentage of uniquely mapped reads in the valid reads

Supplementary Table S3

The percentage of transcribed regions in the whole genome in various chimpanzee tissues

	Transcribed regions	Unidirectionally transcribed regions ^a	Bidirectionally transcribed regions ^b
Cerebral cortex	24.5%	23.6%	0.9%
Cerebellum	23.4%	22.2%	1.2%

^aRegions where either sense or antisense transcripts (but not both) originated.

^bRegions where both sense and antisense transcripts originated.

Supplementary Table S4

RPKM of the upstream and downstream regions of TSSs of genes belonging to each subgroup in each indicated tissue

Sample	Subgroup	Upstream region		Downstream region	
		Antisense RPKM	Sense RPKM	Antisense RPKM	Sense RPKM
Mouse Cerebellum	Total genes	19.4	11.3	8.1	174.8
	Top 100 ranked antisense RPKM located upstream ^a	790.7	15.2	213.2	370.5
	& Low antisense RPKM located downstream ^b	463.0	7.4	32.3	206.1
	& Middle antisense RPKM located downstream ^b	190.4	7.3	65.6	124.6
	& High antisense RPKM located downstream ^b	137.3	0.6	115.3	39.8
	Bottom 100 ranked antisense RPKM located upstream ^c	0.0	8.4	0.5	88.1
	Top 100 ranked sense RPKM located downstream ^d	50.1	236.3	22.7	1736.3
	Bottom 100 ranked sense RPKM located downstream ^e	2.1	0.3	1.9	0.0
Mouse Heart	Total genes	15.7	10.5	6.5	211.2
	Top 100 ranked antisense RPKM located upstream ^a	848.6	19.4	207.2	489.1
	& Low antisense RPKM located downstream ^b	579.0	15.2	27.9	246.8
	& Middle antisense RPKM located downstream ^b	120.7	1.1	41.5	129.3
	& High antisense RPKM located downstream ^b	148.9	3.1	137.9	113.0
	Bottom 100 ranked antisense RPKM located upstream ^c	0.0	8.9	0.5	121.6
	Top 100 ranked sense RPKM located downstream ^d	70.4	181.6	40.9	2542.2
	Bottom 100 ranked sense RPKM located downstream ^e	2.0	0.3	1.6	0.0
Chimpanzee Cerebral Cortex	Total genes	16.2	10.0	5.9	114.3
	Top 100 ranked antisense RPKM located upstream ^a	653.6	7.3	150.0	231.0
	& Low antisense RPKM located downstream ^b	401.4	5.1	18.7	146.6
	& Middle antisense RPKM located downstream ^b	147.7	1.7	48.5	44.0
	& High antisense RPKM located downstream ^b	104.4	0.5	82.8	40.4
	Bottom 100 ranked antisense RPKM located upstream ^c	0.0	9.5	0.9	78.4
	Top 100 ranked sense RPKM located downstream ^d	25.3	94.9	14.5	1636.6
	Bottom 100 ranked sense RPKM located downstream ^e	5.4	1.0	5.1	0.0
Chimpanzee Cerebellum	Total genes	16.1	10.7	6.3	102.4
	Top 100 ranked antisense RPKM located upstream ^a	649.9	26.6	211.2	214.5
	& Low antisense RPKM located downstream ^b	402.1	24.2	27.9	131.2
	& Middle antisense RPKM located downstream ^b	85.0	0.7	24.7	22.8
	& High antisense RPKM located downstream ^b	162.8	1.6	158.6	60.4
	Bottom 100 ranked antisense RPKM located upstream ^c	0.0	9.3	0.5	65.1
	Top 100 ranked sense RPKM located downstream ^d	59.9	119.2	44.8	1484.9
	Bottom 100 ranked sense RPKM located downstream ^e	4.4	0.6	4.8	0.0

The values in this table are normalized by the number of genes.

^aThe genes with the top 100 ranked antisense RPKM in the upstream region of their TSSs

^bThe values are divided into three groups. The genes whose antisense transcript expression level from the upstream region of the TSS was five times higher than that from the downstream region (Condition 1; Low antisense RPKM in downstream region). The genes whose antisense transcript expression level from the upstream region of the TSS was two times lower than that from the downstream region (Condition 2; High antisense RPKM in downstream region). The remaining genes (Middle antisense RPKM in downstream region).

^cThe genes with the bottom 100 ranked antisense RPKMs in the upstream region of their TSSs

^dThe genes with the top 100 ranked sense RPKMs in the downstream region of their TSSs

^eThe genes with the bottom 100 ranked antisense RPKMs in the downstream region of their TSSs

Supplementary Table S5

RPKM of the upstream and downstream regions of TSSs of genes with tissue-specific pancRNAs in various tissues.

			Upstream region		Downstream region	
			Antisense RPKM	Sense RPKM	Antisense RPKM	Sense RPKM
Cerebellum vs Heart (mouse)	Cerebellum-specific pancRNA-bearing genes	cerebellum	96.5	29.2	14.8	410.6
		heart	2.5	11.1	2.1	192.2
	Heart-specific pancRNA-bearing genes	cerebellum	5.2	9.1	3.7	203.3
		heart	144.8	16.7	7.7	486.1
	Cerebral cortex-specific pancRNA-bearing genes	cerebral cortex	74.6	16.1	7.8	304.3
		cerebellum	4.9	15.1	5.3	201.5
Cerebral cortex vs Cerebellum (mouse)	Cerebellum-specific pancRNA-bearing genes	cerebral cortex	4.5	16.0	1.3	220.8
		cerebellum	70.5	21.8	7.8	302.6

The values in this table were normalized by the number of genes. In this analysis, we defined a pancRNA whose RPKM was higher than 0.3 in one tissue and lower than 0.1 in the other as a tissue-specific pancRNA.

Supplementary Table S6

Pearson correlation coefficient between the expression level of pancRNA and the corresponding mRNA

Loci	Pearson correlation coefficient	P-Value
<i>Sh3rf3</i>	0.85	4.95 x 10 ⁻⁶
<i>Vwa5b2</i>	0.93	7.81 x 10 ⁻⁹
<i>Pacsin1</i>	0.81	3.13 x 10 ⁻⁵

Supplementary Table S7

The bias of the pancRNA-bearing protein-coding genes for CpG islands in various chimpanzee tissues

Candidate pancRNA-bearing genes	
With CpG islands in their promoter regions ^a	
Cerebral cortex	94.7%
Cerebellum	93.4%

^aThe percentage of pancRNA-bearing protein-coding genes harboring CpG islands in their promoter regions

Supplementary Table S8

The percentage of genes expressed in various chimpanzee tissues with both “CCGCCG” and “CGGCGG” sequences

All genes	Candidate pancRNA-bearing genes	
	in cerebral cortex	in cerebellum
36.8%	61.2%	60.9%

Supplementary Table S9

Primers for strand-specific RT-PCR analysis

Locus	Name	Primer sequence
<i>Pacsin1</i>	a	CGATCTGATAAGCCCCCTTCGTAA
	b	AGCTGCTGCCTTGTATTCTAACT
	c	ATCCCTCGGTTCCCTGACTAATA
	d	CTTGAGAGACCTGGATGTTTACA
	e	CTTCTCTCACCTCCGGATCTCTC
	f	GGAGACTCTTTGGTGGACTCTAT
	g	GGAGAAGTATGAGAAGGTGCTGG
	h	AGATGTGACATTGGGTAAAGTCC
	q	CGCTCCTAATACCTGCTTCTCAT
	r	ATGAGAAGCAGGTATTAGGAGCG
	s	CTTCCTCCTTCCCGCAGC
<i>Kcnmb4</i>	i	GTTTTTCCAAGTCCTGCAGCC
	j	TGCGAAGCGGGAATCTTCAA
	k	GCCAGCTAAGGGTGGCAATA
	l	CGTGCAGGCTAACACGATTG
	m	ACGTGAACAACCTCCGAGTCC
	n	CCAACTGTGCCTGTTTCTGC
	o	GATCGGTTCCCAGCCATTCA
	p	ACCACGATGAGAACACCCAC
	v	GAGCTGCTCTCGGAATCCTC
	w	AGGAGCAGCCTCGCTCAA
	x	TTCGAGTGACCTTCACCTG
	y	GGAGTTGGACTCGGAGTTGTT

Supplementary Table S10

shRNA sequences

Target	Sequence
<i>pancSh3rf3</i>	TGCAGATTCTCCTAAGCCATGTTTCAAGAGAACATGGCTTAGGAGAATCTGCTTTTTTGGAAC
<i>pancVwa5b2</i>	TGATATGAACAAATACTAAAGATTCAAGAGATCTTTAGTATTTGTTTCATATCTTTTTTGGAAC
<i>pancPacsin1</i>	TGCTGCTGCCTTGTTATTCTAACTTCAAGAGAGTTAGAATACAAGGCAGCAGCTTTTTTGGAAC

Target sequence in BOLD

Supplementary Table S11

Primers for quantitative RT-PCR analysis

Target		Primer sequence
<i>Sh3rf3</i>	Forward	CATCTGTCCTGCACTGTCCC
	Reverse	ACTTTGGGAGACATGCCCTG
<i>pancSh3rf3</i>	Forward	CAGCCCTTAGCCTGTAGTCC
	Reverse	GAGAGGGTCCAGAATGCCTG
<i>Vwa5b2</i>	Forward	CCAGAGGAGGTGTTATCCGC
	Reverse	GTCAGAGCTTCCATACTCTGCT
<i>pancVwa5b2</i>	Forward	GGGAAGTGAGCGAAGGTAAGT
	Reverse	AGTTCTGACTGCTCCACCT
<i>Pacsin1</i>	Forward	TGATGGTGTCCGGTGCTC
	Reverse	TTGTAGTTCCCCACCTCCCA
<i>pancPacsin1</i>	Forward	TGCATGCAGACGCTTGTATTG
	Reverse	TTAGTCAGGGAACCGAGGGA
<i>Gapdh</i>	Forward	TCCACCACCCTGTTGCTGTA
	Reverse	ACCACAGTCCATGCCATCAC