

Appendix: Validation of our intron retention pipeline

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Summary of assays for the IR pipeline validation

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PCR assays performed for the IR pipeline validation

In all conditions, DRB (50 μ M) was applied to mouse primary neocortical cells (14 days in culture) for 2hrs; 1hr before cell collection, bicuculline (20 μ M) was added to the cultures.

Left: RT-PCR validations of IR-transcripts identified as regulated (green, "Valid. PCR" column) or not (red, "Valid. PCR" column) upon bicuculline stimulation with our pipeline. The expression of the IR- and spliced isoforms were assessed with semi-quantitative or with real-time qPCR using three different primer sets, as represented in the top scheme. Mean PIR values are indicated beneath the PCR assay panels.

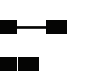
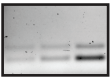
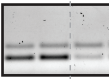
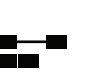
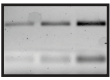
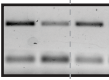
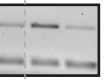
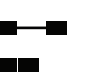
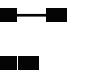
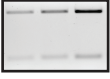
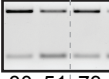
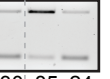
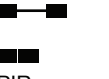
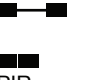
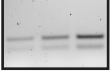
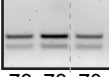
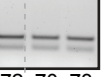
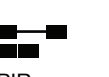
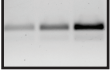
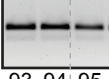
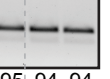
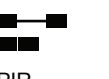
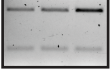
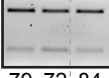
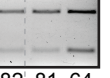
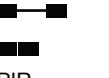
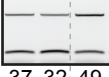
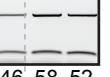
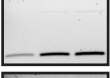
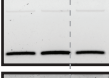
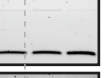
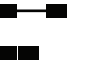
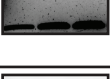
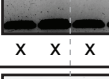
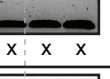
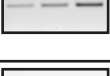
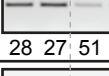
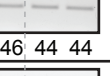
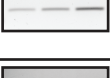
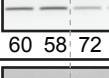
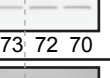
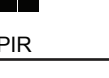
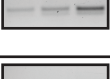
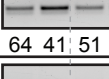
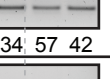
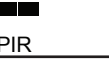
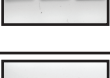
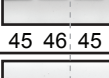
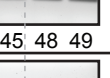
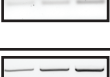
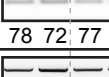
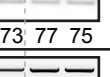
Right: For each IR candidate, the PIR values in control condition (DRB), the PIR values in stimulated condition (DRB + Bicuculline) and the PIR fold-change values are indicated in the column "Our pipeline". Those values were calculated with our IR pipeline.

PIR: Percentage of intron retention

Appendix TableS1. Summary of assays for the IR pipeline validation

	Numbers	Percentages
True positive	16/20	80.0%
False positive	4/20	20.0%
True negative	13/15	86.7%
False negative	2/15	13.3%

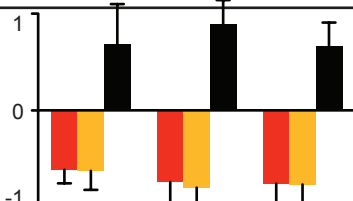
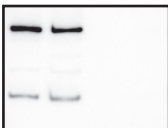
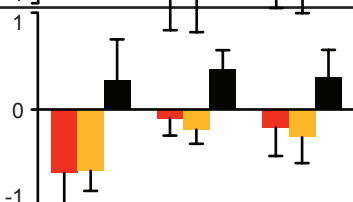
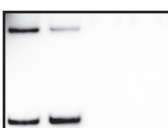
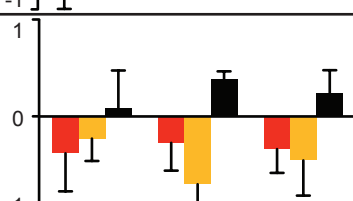
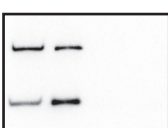
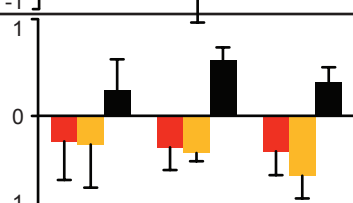
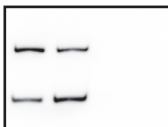
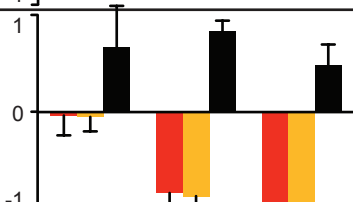
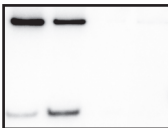
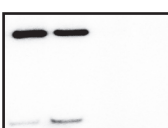
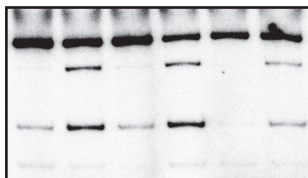
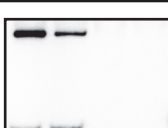
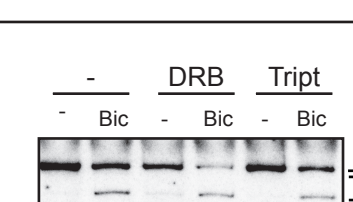
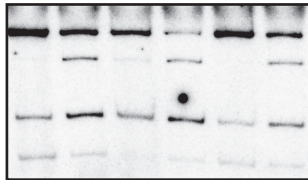
Appendix Figure S1. PCR assays performed for the IR pipeline validation

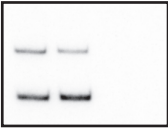
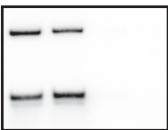
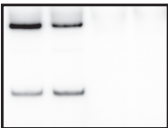
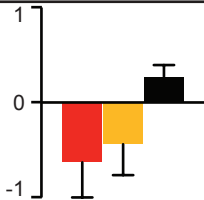
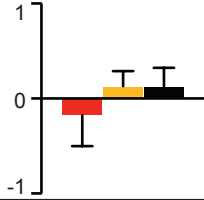
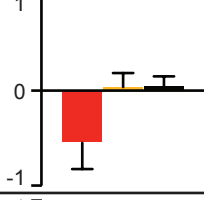
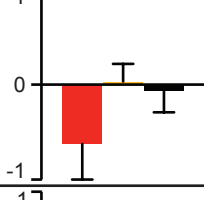
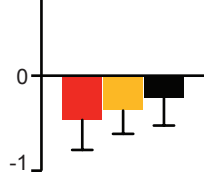
RT (volume)				-		DRB		Tript		1hr Bicuculline	Valid. PCR		Our pipeline
	1	2	4	-	+	-	+	-	+				
Aurkaip1 i1											No	PIR DRB	39
				24 34 35 31 28 26				PIR		PIR DRB+Bic		32	
												Fold Change	0.83
Kdm2a i17											No	PIR DRB	57
				31 30 76 75 89 75				PIR		PIR DRB+Bic		45	
												Fold Change	0.79
Oxsr1 i15											Yes	PIR DRB	28
				60 39 64 37 57 27				PIR		PIR DRB+Bic		20	
												Fold Change	NR
Zscan26 i3											Yes	PIR DRB	46
				80 57 74 34 87 37				PIR		PIR DRB+Bic		28	
												Fold Change	0.61
Zscan26 i4											Yes	PIR DRB	59
				80 51 73 30 85 24				PIR		PIR DRB+Bic		41	
												Fold Change	0.69
Ccdc9 i5											No	PIR DRB	26
				x x x x x x				PIR		PIR DRB+Bic		24	
												Fold Change	NR
Ppfibp1 i9											No	PIR DRB	NI
				73 73 70 72 70 73				PIR		PIR DRB+Bic		NI	
												Fold Change	NI
Rubcn i7											No	PIR DRB	NI
				93 94 95 95 94 94				PIR		PIR DRB+Bic		NI	
												Fold Change	NI
Selenoo i5											No	PIR DRB	34
				79 72 84 82 81 64				PIR		PIR DRB+Bic		32	
												Fold Change	NR
Unc80 i60											No	PIR DRB	30
				37 32 49 46 58 52				PIR		PIR DRB+Bic		28	
												Fold Change	NR
Srcap i27											No	PIR DRB	NI
				x x x x x x				PIR		PIR DRB+Bic		NI	
												Fold Change	NI
Hbp1 i5											No	PIR DRB	2
				28 27 51 46 44 44				PIR		PIR DRB+Bic		1	
												Fold Change	NR
Kif20a i18											No	PIR DRB	0
				60 58 72 73 72 70				PIR		PIR DRB+Bic		1	
												Fold Change	NR
Pcgf6 i7											Yes	PIR DRB	NI
				64 41 51 34 57 42				PIR		PIR DRB+Bic		NI	
												Fold Change	NI
Yipf2 i2											No	PIR DRB	0
				45 46 45 45 48 49				PIR		PIR DRB+Bic		0	
												Fold Change	NR
Zfp335 i24											No	PIR DRB	1
				78 72 77 73 77 75				PIR		PIR DRB+Bic		2	
												Fold Change	NR
Daam2 i16											No	PIR DRB	NI
				x x x x x x				PIR		PIR DRB+Bic		NI	
												Fold Change	NI

NI Not identified as intron
NR Not Retained

Regulated

Not Regulated

semi-quantitative PCR		quantitative PCR		Valid. PCR		Our pipeline
						
RT+ RT- Bicuculline - + - +		log2(Expression Fold Change) - DRB Tript				
Tia1 i7  PIR 88 78 ±5 ±8				Yes	PIR DRB	48
					PIR DRB+Bic	38
					Fold Change	0.78
Ddh2 i13  PIR 73 63 ±8 ±7				Yes	PIR DRB	55
					PIR DRB+Bic	45
					Fold Change	0.82
Ahctf1 i19  PIR 60 46 ±2 ±3				Yes	PIR DRB	37
					PIR DRB+Bic	21
					Fold Change	0.57
Sptbn4 i33  PIR 47 31 ±5 ±4				Yes	PIR DRB	51
					PIR DRB+Bic	36
					Fold Change	0.71
Fnbp1l i9  PIR 51 24 ±3 ±1				Yes	PIR DRB	39
					PIR DRB+Bic	27
					Fold Change	0.70
Clk1 i3  PIR 86 58 ±2 ±8				Yes	PIR DRB	63
					PIR DRB+Bic	43
					Fold Change	0.68
Clk1 i4  PIR 95 85				Yes	PIR DRB	60
					PIR DRB+Bic	40
					Fold Change	0.67
Clk4 i4  PIR 67 37 ±2 ±4				Yes	PIR DRB	32
					PIR DRB+Bic	20
					Fold Change	0.64
Clk4 i5  PIR 87 77				Yes	PIR DRB	39
					PIR DRB+Bic	27
					Fold Change	0.69

semi-quantitative PCR  OR quantitative PCR 		Valid. PCR		Our pipeline
Bicuculline	RT+ RT- - + - +			
Fam98a i7	 	Yes	PIR DRB	29
	PIR DRB+Bic		24	
	Fold Change		0.82	
Fnbp1l i10	 	Yes	PIR DRB	32
	PIR DRB+Bic		26	
	Fold Change		0.80	
Kcnc2 i4	 	Yes	PIR DRB	56
	PIR DRB+Bic		43	
	Fold Change		0.76	
Rimbp2 i21	 	Yes	PIR DRB	66
	PIR DRB+Bic		51	
	Fold Change		0.78	
Smg7 i16		Yes	PIR DRB	40
	PIR DRB+Bic		30	
	Fold Change		0.74	
Arhgef1 i2		No	PIR DRB	61
	PIR DRB+Bic		36	
	Fold Change		0.59	
Dcun1d5 i8		No	PIR DRB	74
	PIR DRB+Bic		59	
	Fold Change		0.79	
Ovgp1 i18		No	PIR DRB	33
	PIR DRB+Bic		16	
	Fold Change		0.49	
Prpf39 i5		No	PIR DRB	65
	PIR DRB+Bic		54	
	Fold Change		0.83	