Supporting Information

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SI Text: Fine Mapping of 129/SVJ Boundaries in Congenic *Girk4*$^{-/-}$ Mice

Despite extensive backcrossing, a significant differential chromosome segment of ≈9 cM {calculated based on the formula $[200 \times (1 - 2 - N)/N]$, where N is the backcross generation number (1)} containing genetic content from the 129S1/SvImJ strain is predicted to flank the *Girk4* null mutation. Strain-dependent polymorphisms present in the differential chromosome segment could shape or even cause phenotypes seen in *Girk4*$^{-/-}$ mice. To define the size of 129S1/SvImJ-based sequence flanking the *Girk4* null mutation, we probed genomic DNA from *Girk4*$^{-/-}$ mice and wild-type counterparts using microsatellite markers and SNP markers. The *Girk4* (*Kcnj5*) gene is located on mouse chromosome 9 (MMU9: 32.1 Mb). Nineteen different microsatellites on MMU9 between 21.7 and 37.0 Mb were tested for strain-dependent differences by using PCR amplification, as described (2). Of these, only three were polymorphic between C57BL/6J and 129/SvImJ strains: D9Mit297, 33.7 Mb; D9Mit224, 36.4 Mb; and D9Mit247, 36.7 Mb). Restriction fragment length polymorphism assays were also developed for four single-nucleotide polymorphic markers (rs33651098, rs33777057, rs13480121, and rs3720858) located between 29.6 and 34.1 Mb. Primers and restriction enzymes in this analysis are detailed in Table S1. A list of genes found within the differential chromosome segment can be found in Table S2.

1. Silver L (1995) *Mouse Genetics: Concepts and Applications* (Oxford Univ Press).
2. Farber CR, Medrano JF (2007) Fine mapping reveals sex bias in quantitative trait loci affecting growth, skeletal size and obesity-related traits on mouse chromosomes 2 and 11. *Genetics* 175:349–360.

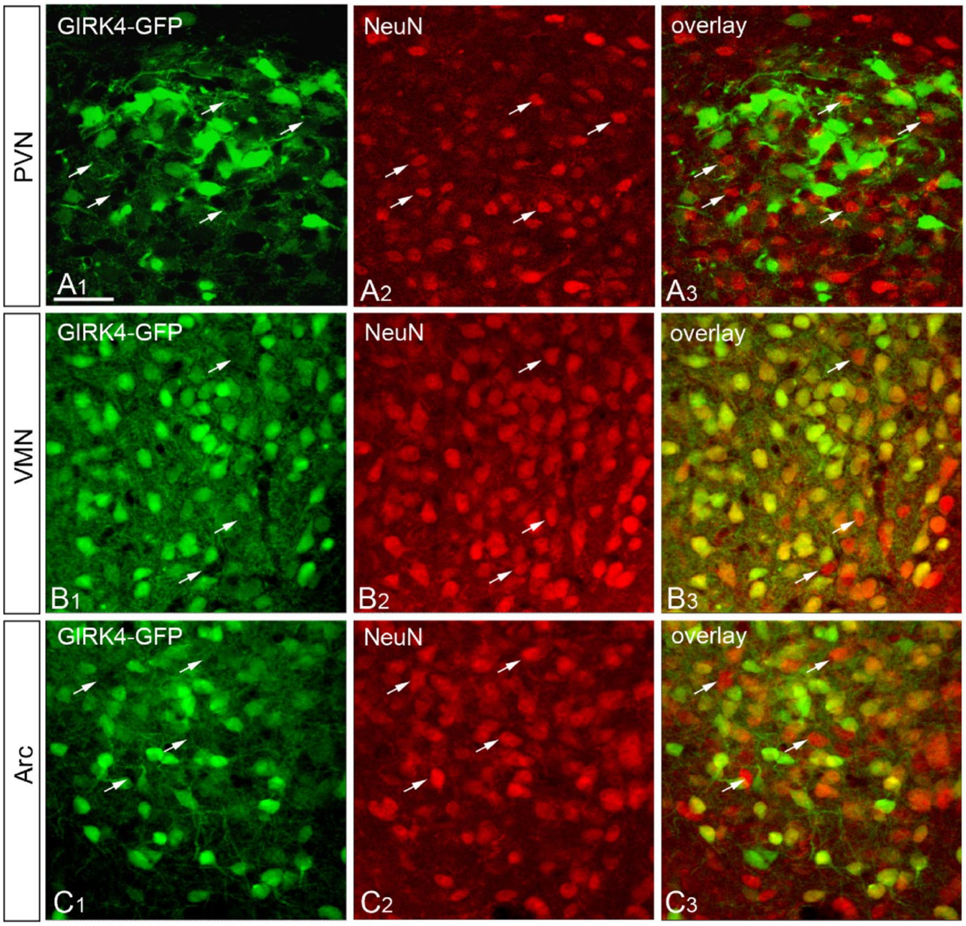

Fig. S1. Overlap between EGFP expression and the neuronal marker NeuN in the VMN, PVN, and posterior ARC from the Tg(*Kcnj5*-EGFP)49sat mouse. Neurons were labeled with a NeuN antibody (1:1,000; Chemicon International) and a Cy3-conjugated goat anti-mouse secondary antibody (1:500; Jackson ImmunoResearch). (Scale bar: 35 μm.)

Table S1. PCR primers and restriction enzyme sites used for fine mapping

SNP ID	Position (MMU9), bp	Primer sequence, 5′ to 3′		Enzyme
		Forward	Reverse	
rs33651098	29690892	CAGCAGCAGAAACAGCAGACT	AGGCCTTTGTAGTGTTGTTTATGG	BsrI
rs33777057	34997582	CTTGATTTTGAGCAGACTAATGCGC	GGTAAGTGCTGCCCCAAAACAA	FspI
rs13480120	30229441	GATGGCTGTAGACAGTGGGGC	ATTTGGTCTCCCTGGGGACCTA	HaeIII
rs3720858	34000554	TCTAAAGGTTCTATGTCCATCCAAAA	AGGAGTAGGCAGAAGGTCTGGAT	Tsp509I

SNP markers used for the fine mapping of the differential genomic segment of MMU9 derived from the 129/SvImJ mouse strain. Primer sequences and restriction enzymes used for restriction fragment length polymorphism (RFLP) analysis are listed. All PCRs were run in the presence of 2 mM $MgCl_2$ and with 55°C annealing temperatures.

Table S2. Genes of 129/SvImJ origin on MMU9 that flank the *Girk4* null mutation

Gene	GenBank accession no.	Name(s)	Function(s)	Position
Hnt	NM_172290	Neurotrimin	Synaptogenesis	28803548(−)
Snx19	NM_028874	Sortin nexin 19	Insulin secretion	30234913(+)
Adamts15	NM_001024139	disintegrin-like and metallopeptidase with thrombospondin type 1 motif, 15	Metalloendopeptidase	30706739(−)
Adamts8	NM_013906	disintegrin-like and metallopeptidase with thrombospondin type 1 motif, 8	Metalloendopeptidase	30750147(+)
Zbtb44	NM_172765	Btbd15	—	30838228(+)
St14	NM_011176	Suppressor of tumorigenicity 14, matriptase	Serine protease	30896174(−)
Aplp2	NM_001102456	Apolipoprotein 2	Synaptogenesis	30957141(−)
Prdm10	NM_001080817	PR domain containing 10	—	31122691 (+
Nfrkb	NM_172766	Nuclear factor related to kappa B binding protein	T cell activation	31193796(+)
Tmem45b	NM_144936	Transmembrane protein 45b	—	31233780(−)
Barx2	NM_013800	Bar-like homeobox 2	Hair follicle remodeling	31653628(−)
Grit	NM_177379	RICS, p250GAP, p200RhoGAP	Neurite morphology, growth	32015784(+)
Kcnj5	NM_010605	GIRK4, Kir3.4	Heart rate regulation	32122367(−)
Kcnj1	NM_019659	Kir1.1, ROMK, ROMK-2	Renal absorption (K^+,NaCl)	32201569(+)
Fli1	NM_008026	Friend leukemia integration 1, EWSR2, Sic1	Induction of erythroleukemia	32229792(−)
Ets1	NM_011808	E26 avian leukemia oncogene 1, Tpl1	Transcription factor	32503626(+)
Kirrel3	NM_026324	Kin of IRRE like 3, NEPH2, SST4	Synapse formation	34296315(+)
St3gal4	NM_009178	ST3 β-galactosidase α-2,3-sialyltransferase 4	Hemostasis, inflammation	34854163(−)
Dcps	NM_027030	Histidine triad protein member 5	mRNA decapping	34931998(−)
Tirap	NM_054096	Toll-interleukin 1 receptor domain-containing adaptor protein	Pulmonary defense	34994360(−)