Supplementary Material

Supplemental Table 1 | qPCR primer list.

Housekeeping gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
Gapdh	TGGATCTGACGTGCCGC	TGCCTGCTTCACCACCTTC

Negative control gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
Gfp	GAAGCGCGATCACATGGT	CCATGCCGAGAGTGATCC

GPCR-encoding genes	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
Adcyap1r1	TATGGACTTCAAGCACCGGC	TCTTGCTCAGGATGGACAGC
Adora2a	GTTAGGTAGGCAGAGGGACAGG	CTGCGATTGCTTCCCTTCTCTG
Adora2b	GGAACCGAGACTTCCGCTAC	GACTGAGAGTAGACTGCGCC
Adrb1	CTACAACGACCCCAAGTGCT	ACGTAGAAGGAGACGACGGA
Adrb2	TACACAGGGGAGCCAAACAC	TCAACGCTAAGGCTAGGCAC
Adrb3	CAGGCTCTGTGTCTCTGGTTA	GAGGAGACAGGGATGAAACCTC
Agtr1a	CTTAGGGTTGGAACCTGCGG	TCATCCAGTCCCTCCCAACT
Bdkrb1	CCGCTACAGGTTGCTGGTAT	TTGACGGAACGCAGAAGGAA
Cckar	ACTGCCAAGTCCACGTTCAA	TCATCTGGGGCGTTCCAAAA
Chrm2	ACTGCCATTGCGGCTTTCTA	TATTCTGCTCTTGCTCGCCC
Chrm4	GCCTCTGGCTAGTTCCGCC	TCGCCATGCTGAACCCAAC
Drd2	GACACCACTCAAGGGCAACT	ATCCATTCTCCGCCTGTTCA

Drd5	CGAACCTACGCCATCTCCTC	GCGCGTGTAGGTCACTATCA
Gabbr2	ACAGGCGATTCCAGTTCACA	CGTAGGCGGTGGTTTTCTGA
Gpr3	ATCTACGCCTTTCGCAACCA	CGGGACCGGAATGGAATCTT
Gpr65	CATGGGCTACGCAATACCCT	TGTTTTCCGTGGCTTGGTTG
Gpr68	ACGATACCAGCCCAAGTGTG	CACCTTAACCAGTCCTCTGGC
Grm4	TACCAGTACCAACGTCGCAA	GCATCCGCTCTATTCTGAGGT
Grm8	TGTGCTCCTAACGGGGATTT	GATGATTGTGTCAGGTGCCG
Hcar1	AGTGTGAAGGAAACCGTGGG	CGCTTTTCTCAGCCATGCAA
Hcar2	GCGGCCATCATTTCTTGCTT	GCCTCGCCATTTTTGGTCAT
Hrh3	TTAGAGCATCAACCCGGCAG	CACTCCAGTTCCACCAACGA
Htr7	GTGGTCAAAATGGGAAACGGA	CCATTCTGCCTCACGGGGTA
Lpar2	GGCAGATGACTTGACTTCGC	GCCTCCCTGAATGTTTGCTC
Oprd1	TGGATGCTTTTGGGGTTCCT	AAACAAAGGGTCTCGGTGCT
Oprl1	TCCTCAGGCACACCAAGATG	GAAGGGCAGTGTCAGCAAGA
P2ry12	AACGCCAGTGTCATTTGCTG	TCTCCTTTTATTCTTGCACTGTGAC
Pthr1	AGCGAGTGCCTCAAGTTCAT	TCCCACGGTGTAGATCATGC
S1pr5	AACTCGCTGCTGAATCCCAT	GGAGGAGTCTTGGTTGCAGG
Tacr1	AGGTGTCTGGGGGTTTCTTTA	CCTAGAAGTGACAGGTGACCA

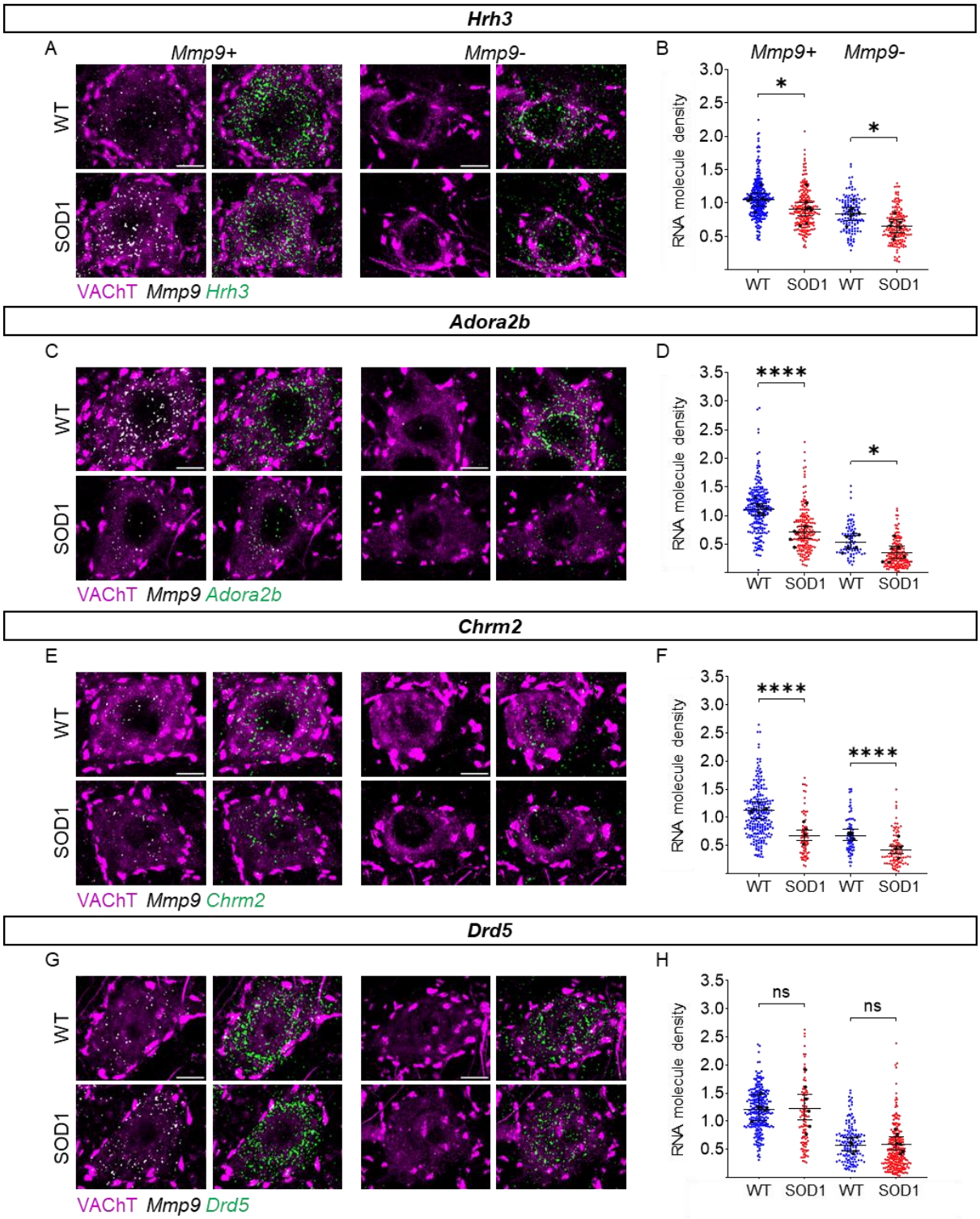
Immediate-early genes	**Forward primer sequence (5’-3’)**	**Reverse primer sequence (5’-3’)**

ΔFosB	AGGCAGAGCTGGAGTCGGAGAT	GCCGAGGACTTGAACTTCACTCG
Arc	GCACAAAAGCCATGACCCAT	TCTCCCTAGTCCCCAGGGC
c-Fos	CCTGCCCCTTCTCAACGAC	GCTCCACGTTGCTGATGCT
Egr1	GCCGAGCGAACAACCCTAT	TCCACCATCGCCTTCTCATT
Egr2	GTTGACTGTCACTCCAAGAAATGG	AGCGCAGCCCTGTAGGC
NPas4	GCTATACTCAGAAGGTCCAGAAGGC	TCAGAGAATGAGGGTAGCACAGC

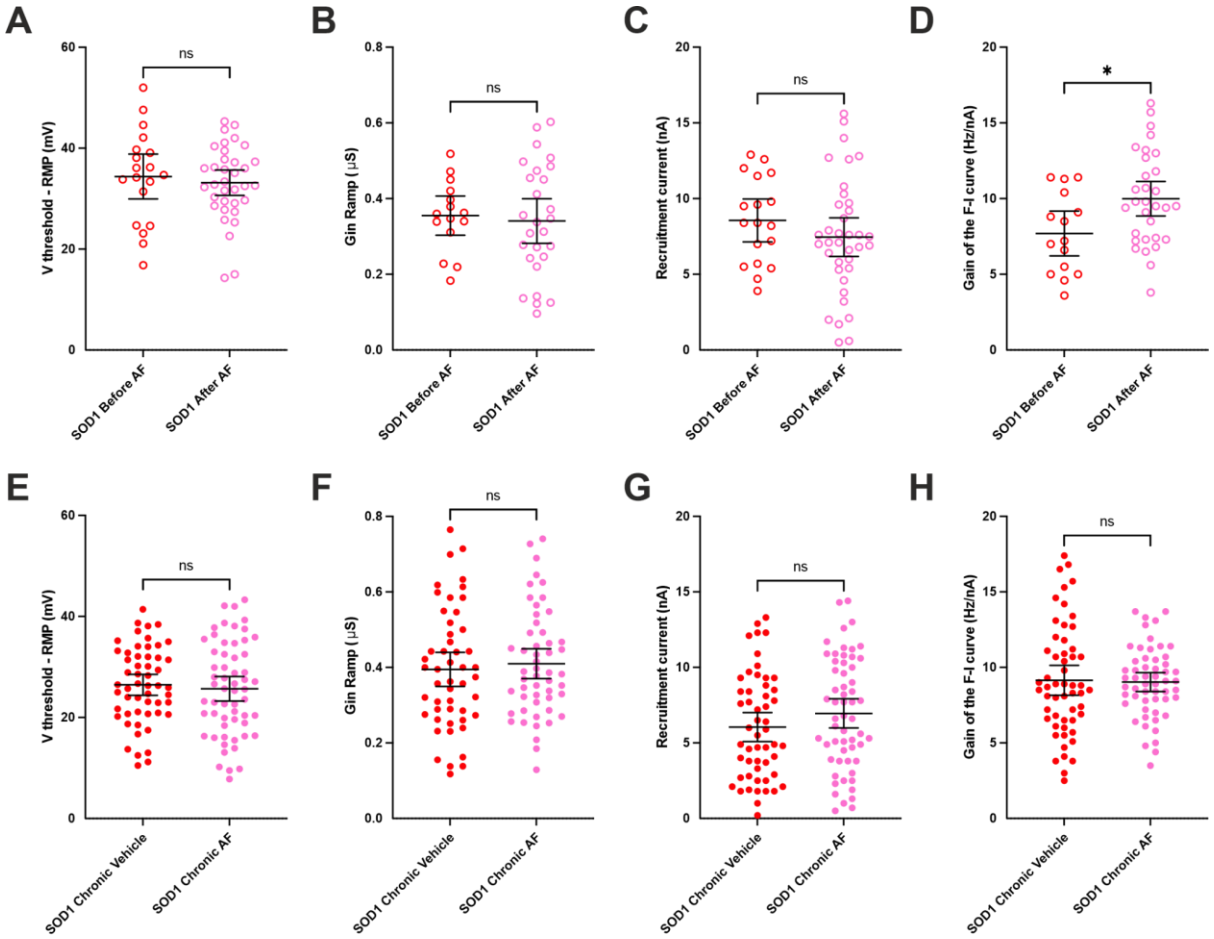
Ion channel-encoding genes	**Forward primer sequence (5'-3')**	**Reverse primer sequence (5'-3')**
Cacna1d (isof.1)	GCTCGGTGGCTGTATTTTCAA	CCGTGCTTTCTACCGCACTT
Cacna2d3	GCAGATCGCAGGAAGCTTTG	ACGGGAGATTTCCGCTCATC
Hcn1	CGTGAAGCATGACCGAGAGA	GTAGACTGGCGGAGATTGGG
Hcn2	CATCCACACCAAAGCCATGC	CCCGCCTCCTAAGCTACCTA
Kcna1	TGCTGTGTGTCGCTCAATCT	TCTCCGAACTGGACACTTGC
Kcna2	TACCCATCTGCAAGGGCAACG	CGACTTGAGGAGGAGAGTGGA
Kcnab1	TCTTGGACTGGTCCCCTACC	AGATTCCCCTACCCCAGCAT
Kcnb1	GAGAGGGCGTGGCTAAGAAG	GCCCTCTTGGTCCATTTCCA
Kcnj14	GCCGAGGACAGACCTGAACAC	ACTGGGGGTTCCTCTGCTCA
Kcnn3	ATCCACCGTCATCCTGCTTG	GTAGGTCATGGCTATCCGCC
Kcnn2	ACAAGGCGTCGCTGTATTCT	CTGTATTTCCCTGGCGTGGT

Kcnq2	GCCATTTTGTACGTGCCCTT	TAGAAGACAGCGTCGTGTGC
Kcnq3	AGTCTTGCTTCCCTGGTGATTC	TCGTCCTGCATTTGGCTGATA
Kcnq5	GCAGCCACCAGACTAAAGGA	CTGCCGCTTCCAATTCCAAA
Kcnt2	CTGTGCACTTAAAAGCAATACAGT	AGCATTTTCCACATCCATGACT
Scn1a	TACAGAAGCAGACCGTAGGC	TGTGATTAGCATCATTTTGGGCT
Scn8a	CCTTCTTACGAGACCCGTGG	ACCCTGAAAGTGCGTAGAGC
Tmem16f	TGGAACCCTGATCTTCGCTG	TTGCTGTAGCTCAACGGTGT
Trpm5	GAATGGGGACTACAGAGGCTG	CGAATGTTTCCTGTGGAGGC

Supplementary Figure



Supplemental Figure 1 | Additional disease-driven transcriptional changes in pre-symptomatic SOD1 MNs at single-molecule single-MN resolution. A, C, E, G) Representative confocal images of individual p45 WT and SOD1 *Mmp9*+ and *Mmp9*- MNs for *Hrh3*, *Adora2b*, *Chrm2* and *Drd5* ISH, respectively. MNs are identified by VAChT+ C-boutons and diffuse cytoplasmic signal (magenta); cytoplasmic spots define single mRNA molecules of *Mmp9* (white) or the GPCRs of interest (green). Scale bar=10µm. **B, D, F, H)** Quantification of the density of single mRNA molecules of *Hrh3* **(B)**, *Adora2b* **(D),** *Chrm2* (**G**) and *Drd5* **(H)** per MN cross-section area. In each dot plot, small dots correspond to individual MN, whereas large dots indicate individual mouse means; the error bar delineates the overall mean and corresponding 95% confidence interval (for *Chrm2* and *Drd5* it is back-propagated from the log scale). N = 4-6 mice per genotype group. **p*<0.05, ***p*<0.01, ****p*<0.001, *****p*<0.0001, ns - non significant.



Supplemental Figure 2 | Replication of acute and prolonged delivery of adrenergic β2/β3 agonists on a different cohort. A-H) Electrophysiological properties were obtained from slow ramps of current, as in Figure 3 and 7 but were performed in Poznan. **A-D)** Effect of the acute treatment on Voltage threshold - resting membrane potential **(A)**, ramp input conductance **(B)**, recruitment current **(C)**, gain of the F-I relationship **(D),** in MNs from SOD1 mice. **E-H)** Effect of the chronic treatment on Voltage threshold - resting membrane potential **(E)**, ramp input conductance **(F)**, recruitment current **(G)**, gain of the F-I relationship **(H),** in MNs from SOD1 mice. In all graphs, each point represents one MN and the mean ± 95% CI are shown. Significances on top bars are for treatment effects. N =7 Acute SOD1 mice and N = 11 Chronic SOD1 mice. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns - non significant.