Effect of anti-inflammatory treatment with AMD3100 and CX_3CR1 deficiency on $GABA_A$ receptor subunit and GAD isoforms expression after stroke

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MOLECULAR NEUROBIOLOGY

SUPPLEMENTARY MATERIAL

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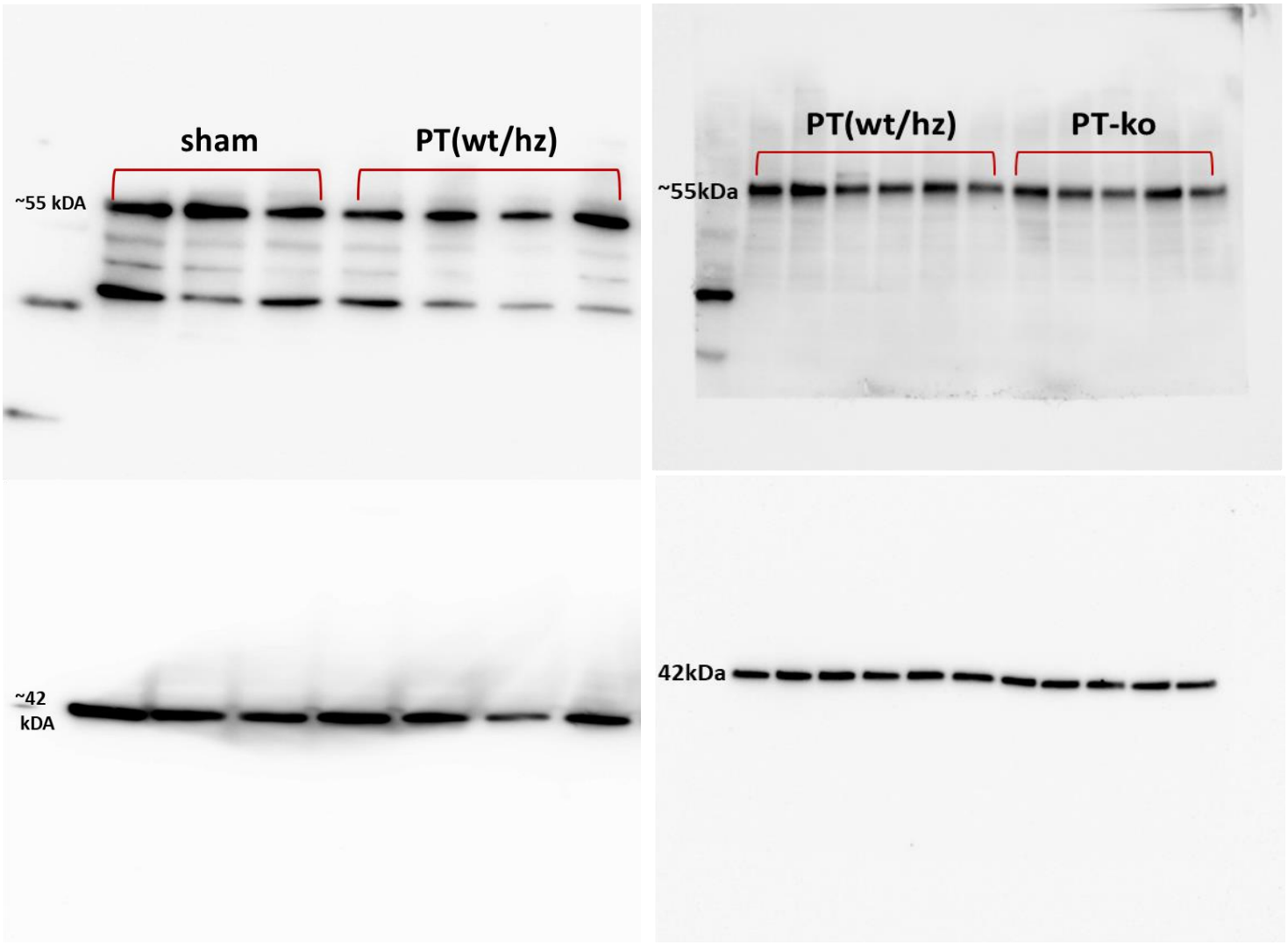
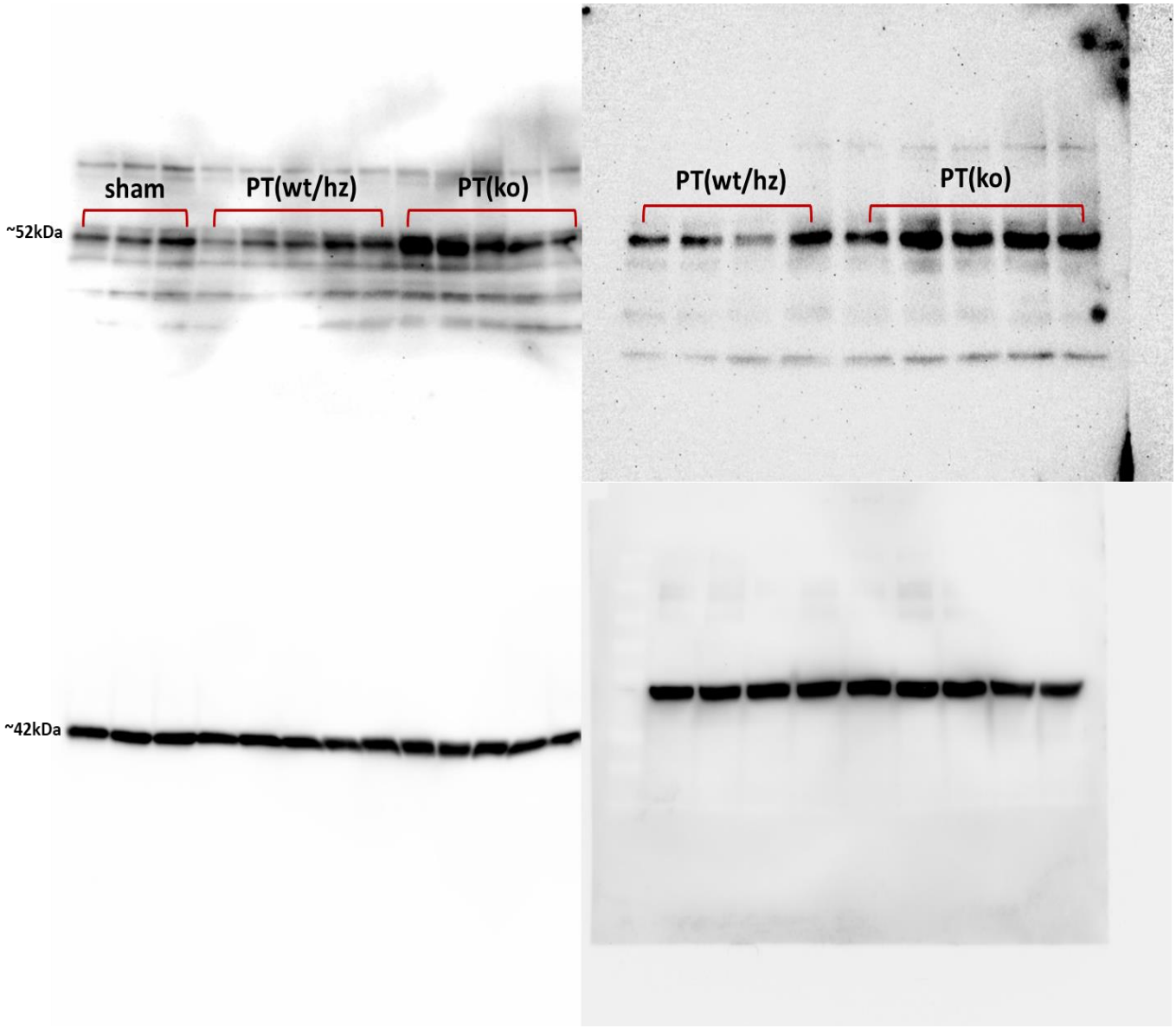
20 **Table 1** Sequences of gene specific primers and associated amplicon lengths

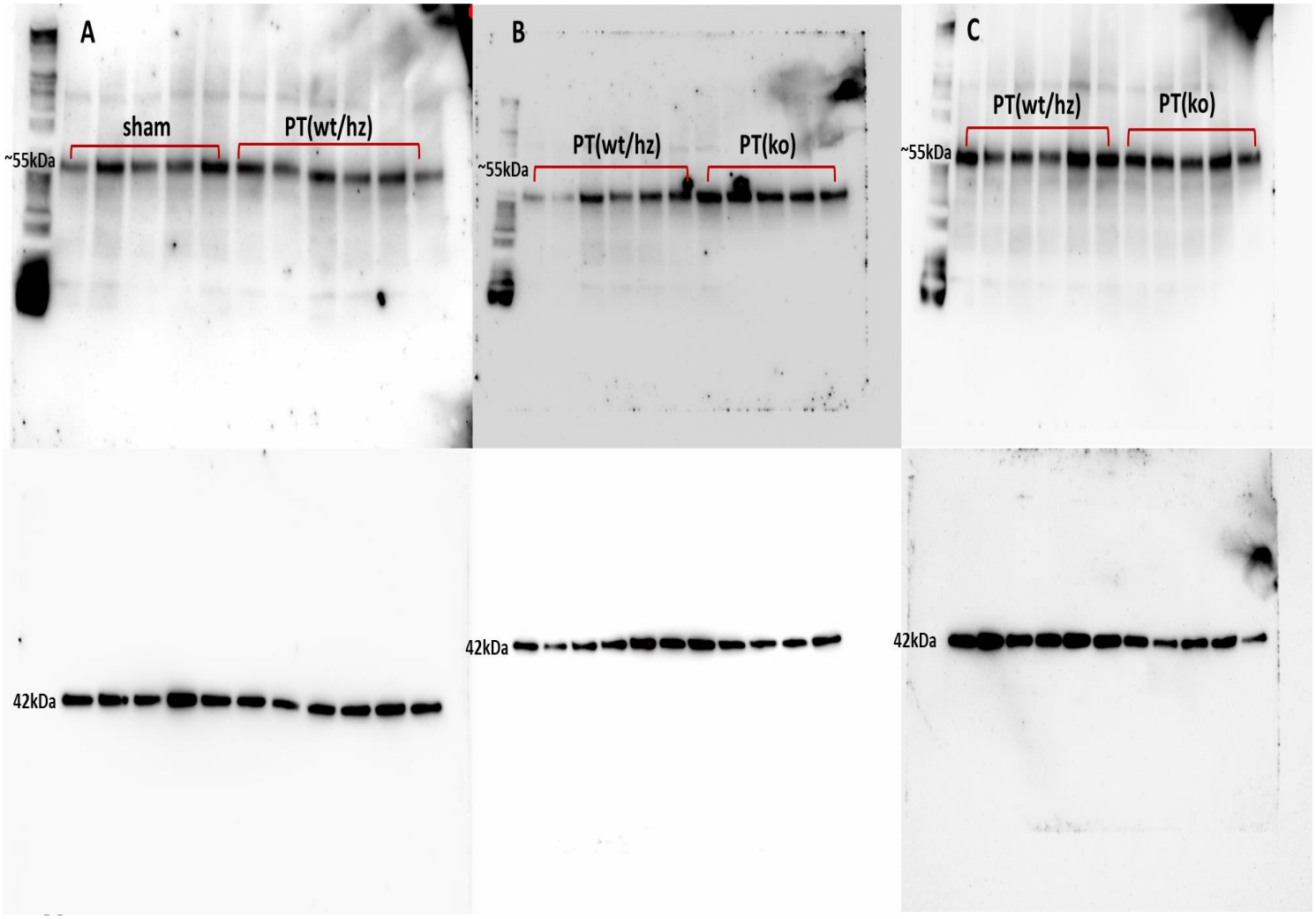
Gene	Forward (5′→3′)	Reverse (5′→3′)	Product size (bp)
GAPDH	TTCTCAAGCTCATTTCCTGGTATG	GGATAGGGCCTCTCTTGCTCA	143
GABA(A) α3	GTTCTCACCATGACCACCTT	GTTGGAGCTGCTGGTGTTT	153
GABA(A) δ	CTCCCTGACACCTTCATCGT	GTACTTGGCGAGGTCCATGT	153
GABA(A) β3	GCCTTGATGACAGCCCTTTA	CATTGGCAACACAACCACTC	174
GAD67	GAACAACCATGGTGGGCTAC	GCCGATGATTCTGGTTCTGT	166
CXCR4	TCAGTGGCTGACCTCCTCTT	CTTTTCAGCCAGCAGTTTCC	222



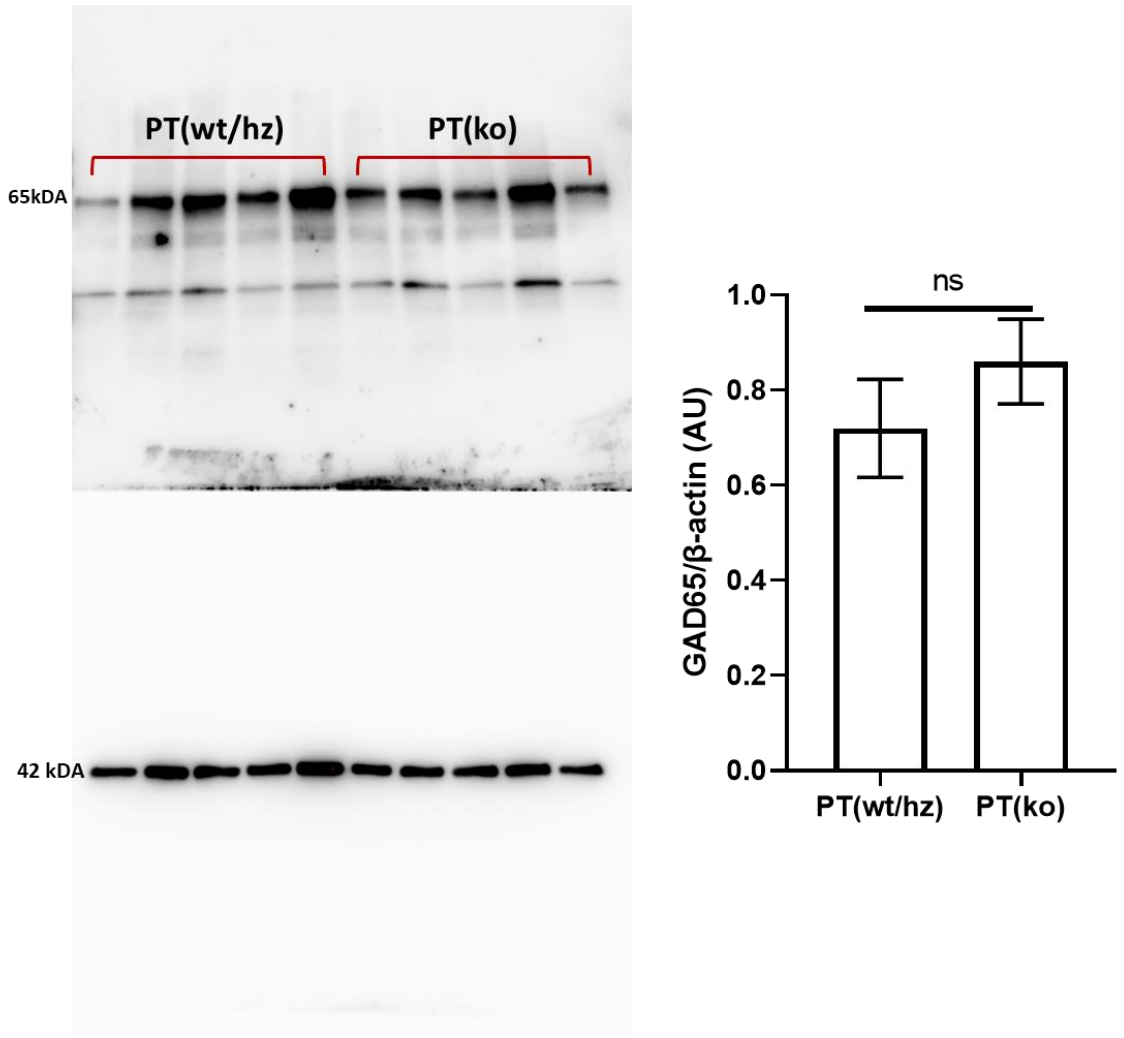
Supp. Figure 1 Western Blot of the $GABA_A$ α3 subunit in the contralateral cortex of mice which underwent sham surgery and stroke-mice of day 14 after PT regarding CX3CR1 deficiency. Corresponding band size were 55kDa for α3 subunit and 42kDA for β-actin.



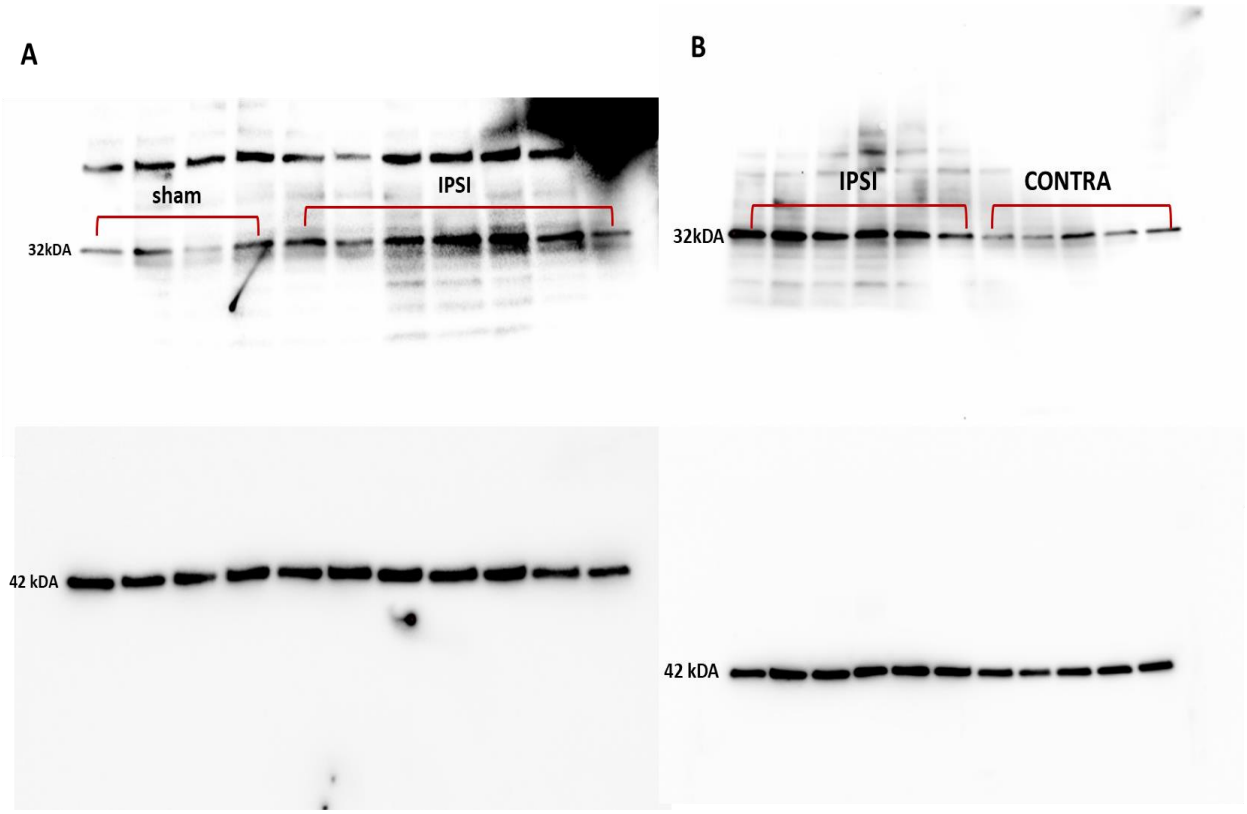
Supp. Figure 2 Western Blot of the $GABA_A$ δ subunit in the contralateral cortex of mice which underwent sham surgery and stroke-mice of day 14 after PT regarding CX3CR1 deficiency. Corresponding band sizes were approximately 52kDa for δ subunit and 42kDA for β-actin. The comparison PT (wt/hz) VS PT (ko) was repeated in a second Western Blot in order to confirm results.



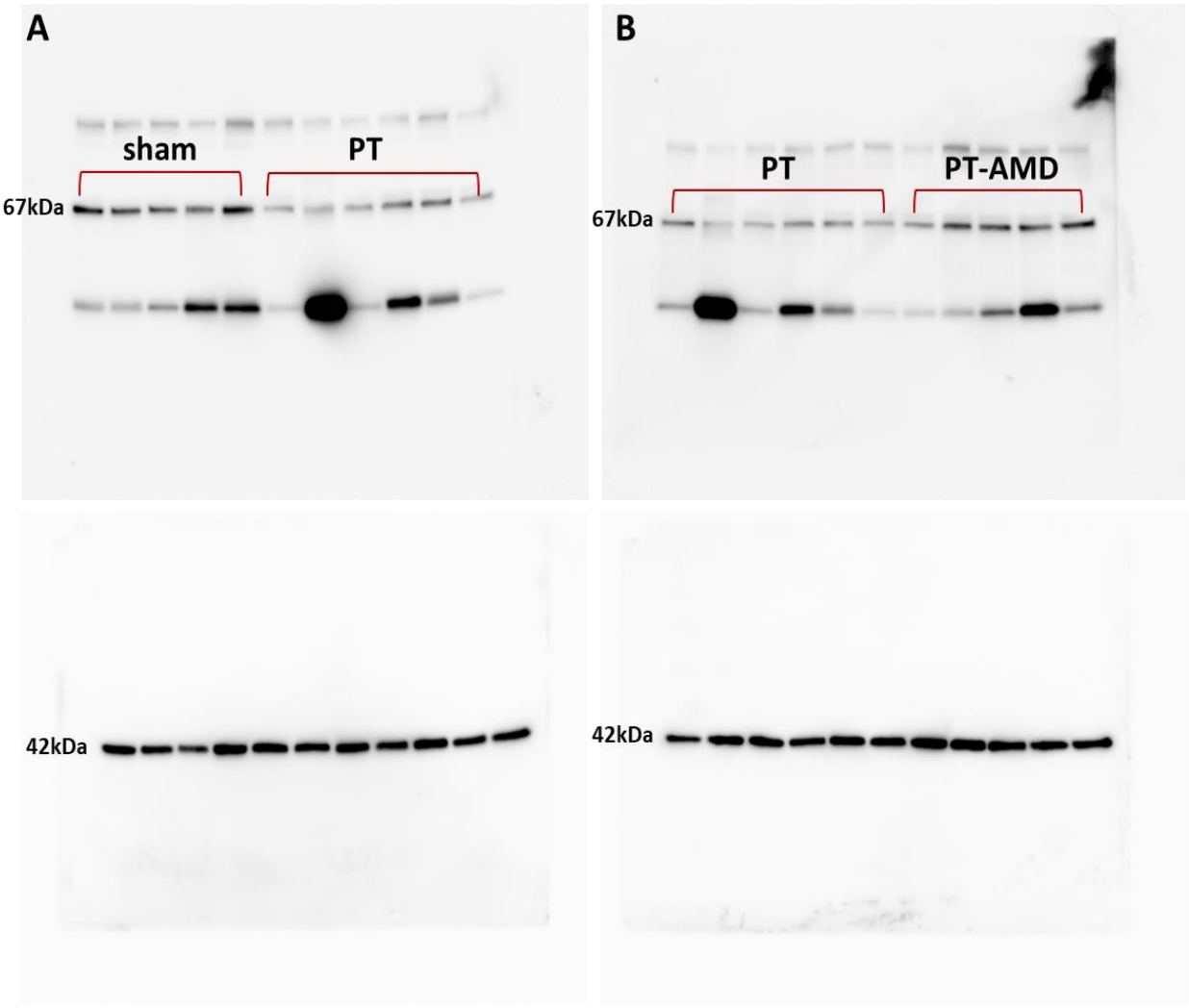
Supp. Figure 3 Western Blot of the $GABA_A$ β3 subunit in the contralateral cortex of mice which underwent sham surgery and stroke-mice of day 14 after PT regarding CX3CR1 deficiency. **(A)** Comparison between contralateral cortex of sham and stroke mice and **(B, C)** comparison between wt/hz littermates and ko mice which underwent stroke. Corresponding band sizes were approximately 55kDa for β3 subunit and 42kDA for β-actin. The comparison PT (wt/hz) VS PT (ko) was repeated twice **(B, C)** in order to confirm results.



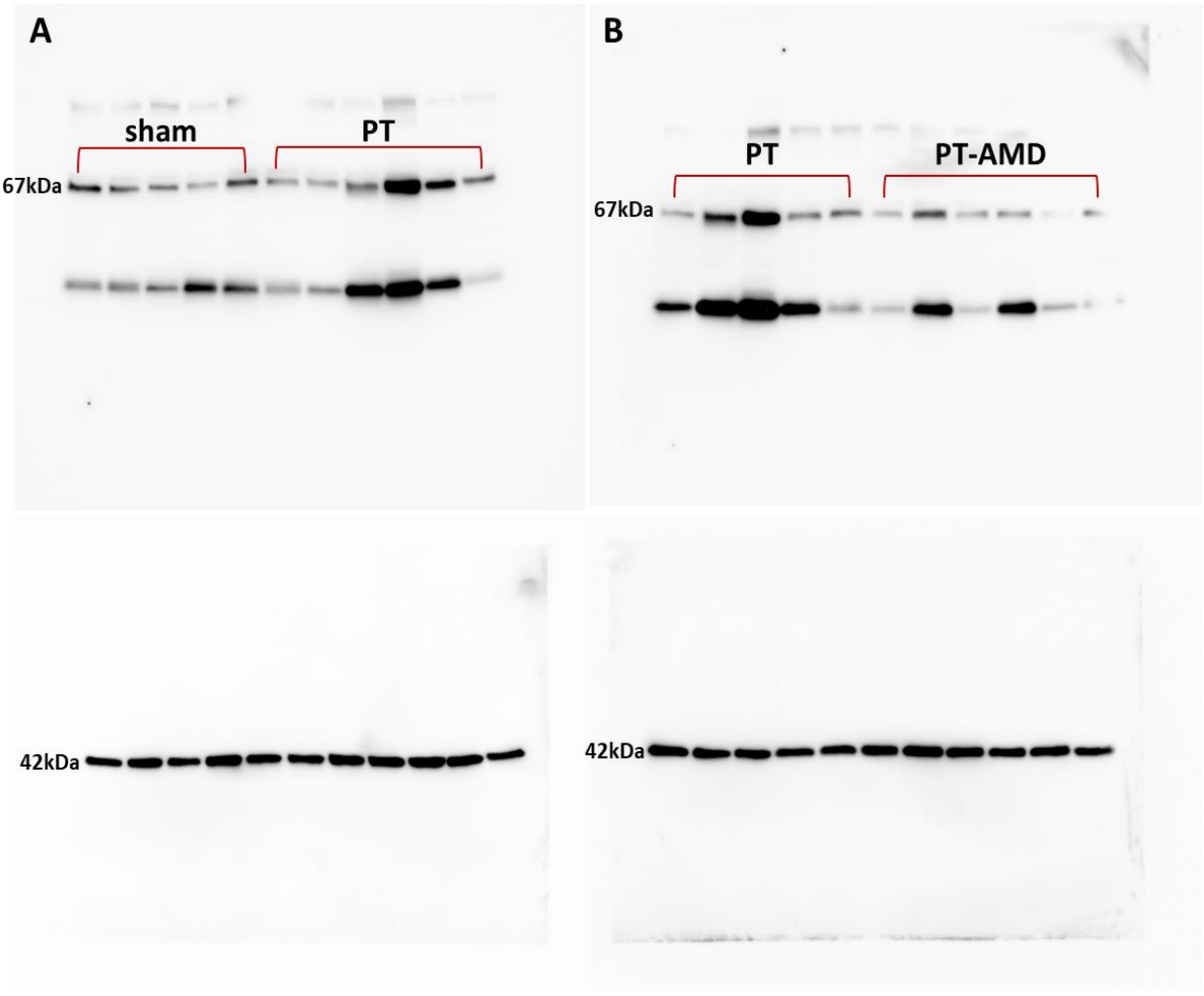
Supp. Figure 4 Western Blot of GAD65 in stroke-mice of day 14 after PT in the contralateral cortex regarding the effect of CX3CR1 deficiency. Comparison between wt/hz and ko stroke mice. Corresponding band size were 65kDa for GAD65 and 42kDA for β-actin. No significant differences were observed with Student's t test (*p = 0.33*).



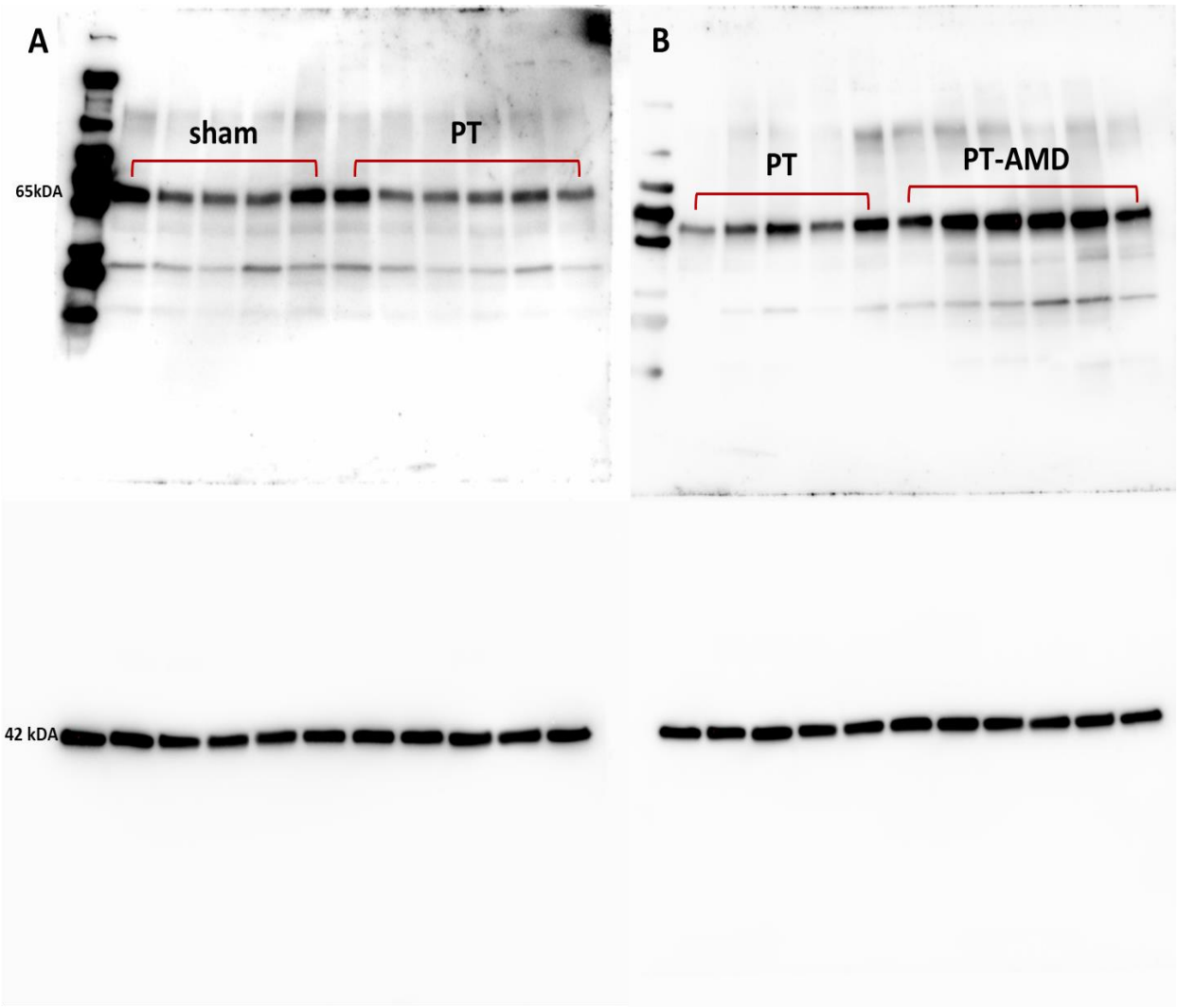
Supp. Figure 5 Western Blot of CXCR4 in stroke-mice of day 14 after PT regarding the effect of AMD3100 treatment. **(A)** Comparison between ipsilateral cortex of sham and stroke mice and **(B)** comparison of ipsilateral vs contralateral cortex of stroke mice. Corresponding band size were 32kDa for CXCR4 and 42kDA for β-actin.



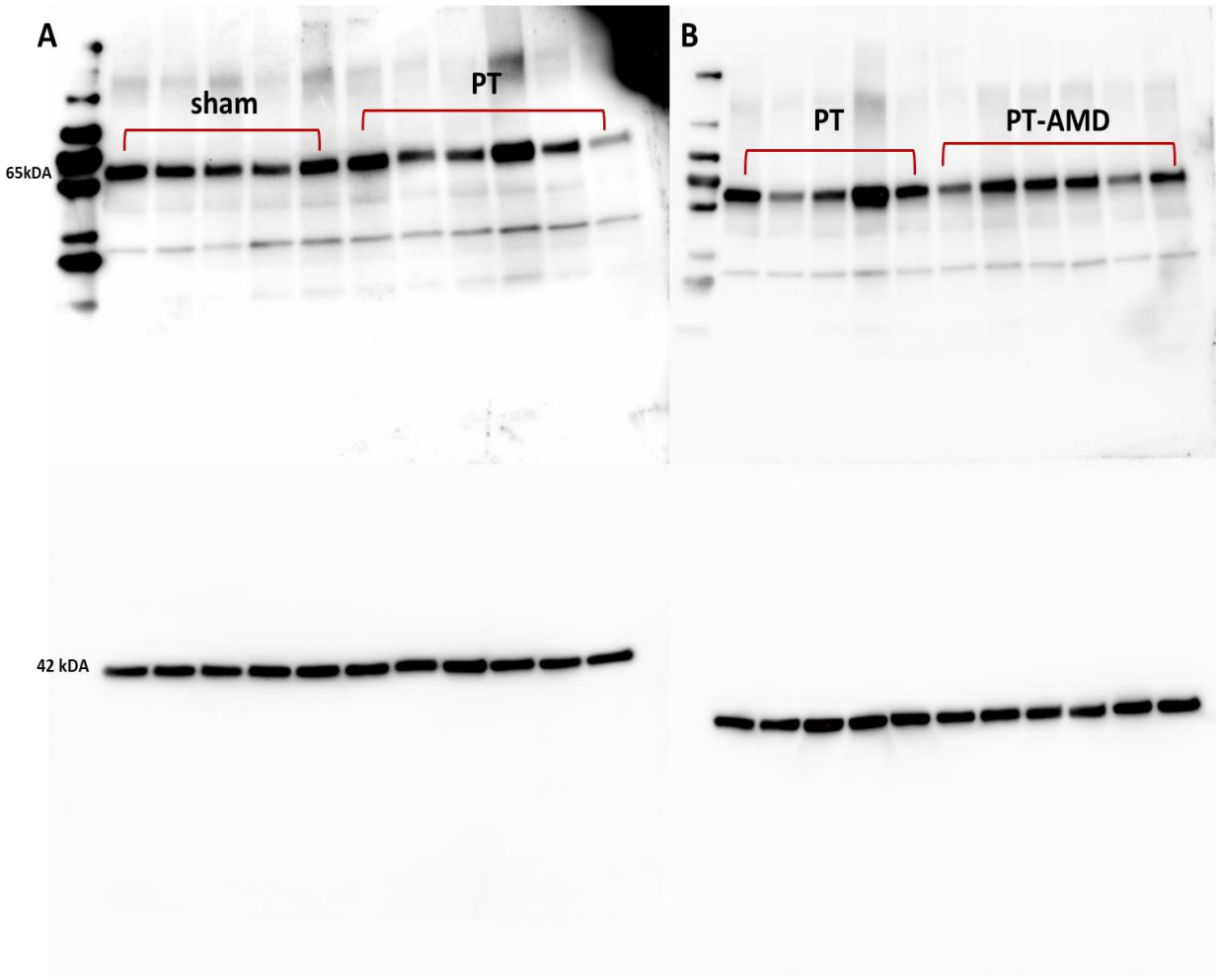
Supp. Figure 6 Western Blot of GAD67 in the **contralateral** cortex of stroke-mice of day 14 after PT regarding the effect of AMD3100 treatment. **(A)** Comparison of sham vs PT and **(B)** comparison of stroke-mice treated with saline or AMD3100. Corresponding band size were 67kDa for GAD67 and 42kDA for β-actin.



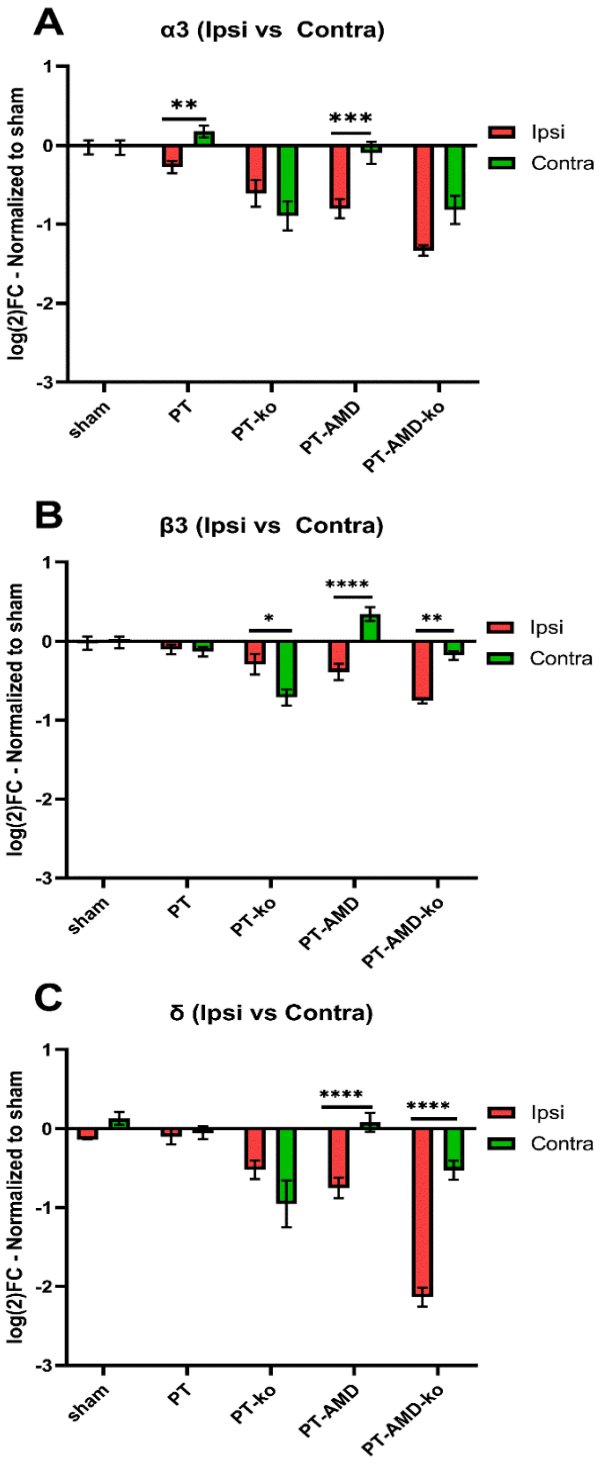
Supp. Figure 7 Western Blot of GAD67 in the **ipsilateral** cortex of stroke-mice of day 14 after PT regarding the effect of AMD3100 treatment. **(A)** Comparison of sham vs PT and **(B)** comparison of stroke-mice treated with saline or AMD3100. Corresponding band size were 67kDa for GAD67 and 42kDA for β-actin.



Supp. Figure 8 Western Blot of GAD65 in the **contralateral** cortex of stroke-mice of day 14 after PT regarding the effect of AMD3100 treatment. **(A)** Comparison of sham vs PT and **(B)** comparison of stroke-mice treated with saline or AMD3100. Corresponding band size were 65kDa for GAD65 and 42kDA for β-actin.



Supp. Figure 9 Western Blot of GAD65 in the **ipsilateral** cortex of stroke-mice of day 14 after PT regarding the effect of AMD3100 treatment. **(A)** Comparison of sham vs PT and **(B)** comparison of stroke-mice treated with saline or AMD3100. Corresponding band size were 65kDa for GAD65 and 42kDA for β-actin.



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105 **Supp. Figure 10. Comparison of mRNA levels between the two hemispheres under different**
106 **experimental conditions** mRNA levels of the α3 **(A)**, the δ **(B)** and the β3 **(C)** GABAA subunits in the
107 contralateral and ipsilateral cortex of mice. All data depicted are normalized to expression levels of

sham/naive mice and shown as a log2 fold-change. Bars represent the mean ± S.E.M. Statistically significant differences were observed through Two-Way ANOVA and *post-hoc* Sidak's Multiple Comparisons (* $p < 0.05$, ** $p < 0.005$, **** $p < 0.0001$).