

Non-homologous end joining factors XLF, PAXX and DNA-PKcs are required to maintain the neural stem and progenitor cell population

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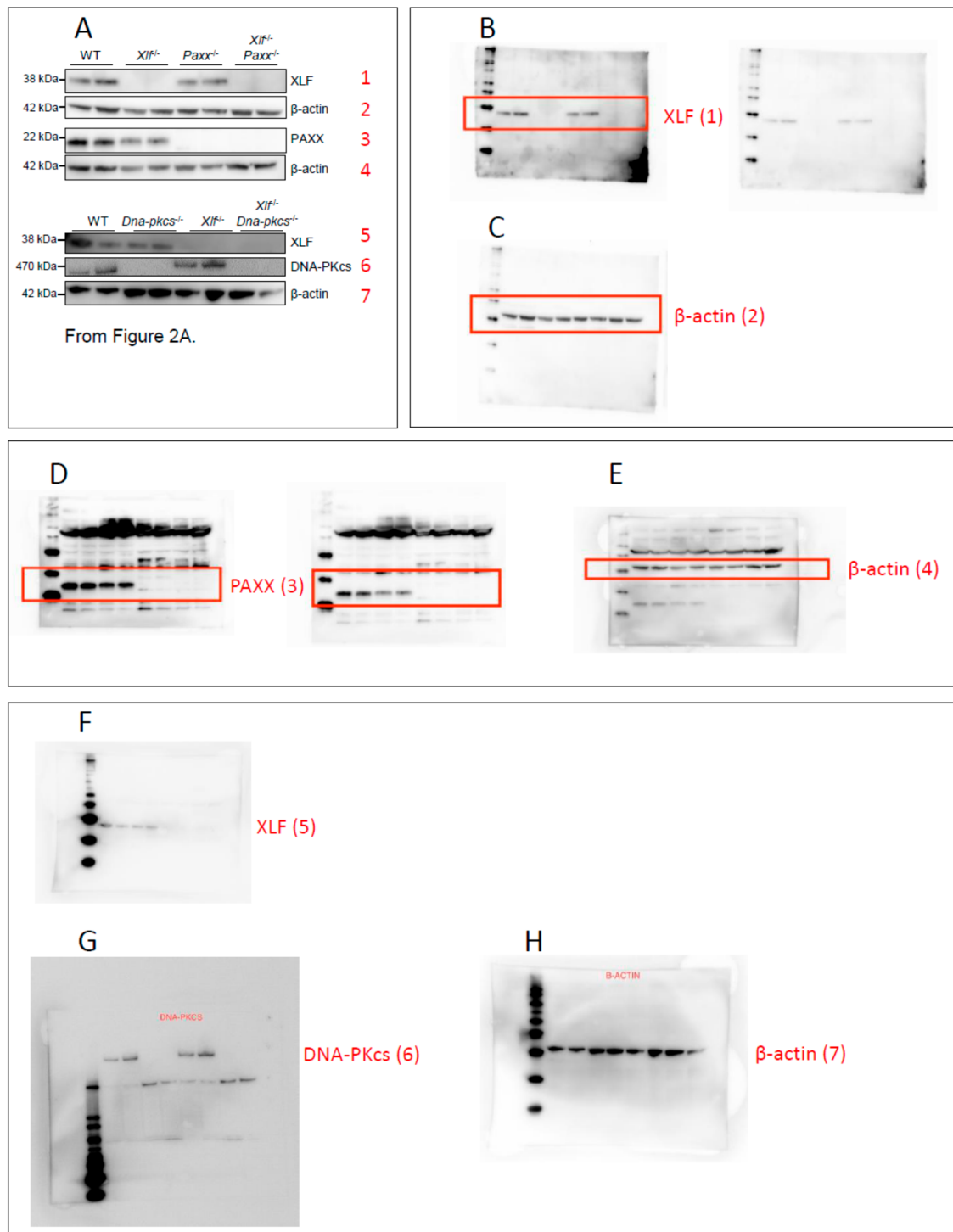
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Supplementary Figure S1. Uncropped western blots



Supplementary Figure S1. Uncropped western blots for Figure 2A.

- (A) Original Figure 2A. 1-7 indicate western blot numbers, uncropped images are presented in B-H.
- (B) Western blot detecting XLF (1). Longer (left) and shorter (right) exposures are provided.
- (C) Western blot detecting a loading control for XLF gel (1), β-actin (2).
- (D) Western blot detecting PAXX (3). Longer (left) and shorter (right) exposures are presented.
- (E) Western blot detecting a loading control for PAXX gel (3), β-actin (4).
- (F) Western blot detecting XLF (5).
- (G) Western blot detecting DNA-PKcs (6).
- (H) Western blot detecting a loading control for XLF (5) and DNA-PKcs (6) gel, β-actin (7).

Supplementary Table S1. Commercial reagents

Reagent	Catalogue number, Company, Country
<i>Proteinase K</i>	#1703001, Invitrogen, Carlsbad, CA, USA
<i>Trizma</i>	#T3253, Sigma, St. Louis, MO, USA
<i>KCl</i>	#P9541, Sigma, St. Louis, MO, USA
<i>NP-40</i>	#127087-87-0, Sigma, St. Louis, MO, USA
<i>Tween-20</i>	#9005-64-5, Sigma, St. Louis, MO, USA
<i>GoTaq®G2 Green Master Mix</i>	# M7823, Promega, Madison, WI, USA
<i>DMEM/F12 Medium</i>	#11330-057, Thermo Fisher Scientific, Waltham, MA, USA
<i>Penicillin/Streptomycin</i>	#15140-122, Thermo Fisher Scientific, Waltham, MA, USA
<i>B27 supplement</i>	#17504044, Thermo Fisher Scientific, Waltham, MA, USA
<i>Epidermal Growth Factor (EGF)</i>	# AF-100-15, PeproTech, Sweden
<i>basic-Fibroblast Growth Factor (b-FGF)</i>	#100-18B, PeproTech, Sweden
<i>Trypsin-EDTA 0.25 %</i>	#T3924, Sigma, St. Louis, MO, USA
<i>PrestoBlue™ Cell Viability Reagent</i>	#A13262, Invitrogen, Carlsbad, CA, USA
<i>Neurobasal A Medium</i>	#10888-022, Thermo Fisher Scientific, Waltham, MA, USA
<i>Poly-D-lysine</i>	# p0899, Sigma, St. Louis, MO, USA
<i>Laminin</i>	# L2020, Sigma, St. Louis, MO, USA
<i>B27 supplement without vitamin A</i>	#12587010, Thermo Fisher Scientific, Waltham, MA, USA
<i>GlutaMAX</i>	#35050-038, Thermo Fisher Scientific, Waltham, MA, USA
<i>Triton X-100</i>	#T8787, Sigma, St. Louis, MO, USA
<i>Bovine serum albumin (BSA)</i>	#A2153, Sigma, St. Louis, MO, USA
<i>Goat antiserum</i>	#10000C, Invitrogen, Carlsbad, CA, USA
<i>4'-diamidino-2-phenylindole (DAPI)</i>	#62248, Molecular Probes, Eugene, OR, USA
<i>RIPA</i>	#R0278, Sigma, St. Louis, MI, USA
<i>cOmplete™ EDTA-free Protease Inhibitor</i>	#11873580001, Roche, USA
<i>Phenylmethane sulfonyl fluoride (PMSF)</i>	#70137720, Sigma, St. Louis, MI, USA
<i>Bradford reagent</i>	#5000006, BioRad, Hercules, CA, USA
<i>Phosphate-Buffered Saline (PBS)</i>	#BR0014G, Oxoid Limited, Hampshire, UK
<i>20x NuPAGE Transfer Buffer</i>	#NP0006-1, Life Technologies, Carlsbad, CA, USA
<i>SuperSignal™ West Femto</i>	#34095, Thermo Fisher Scientific, Waltham, MA, USA

Supplementary Table 2. Antibodies

Antibody	Catalogue number, Dilution, Company, Country
<i>Mouse anti-neuron specific β-tubulin (Tuj1)</i>	#MAB1195, 1:600, R&D Systems, USA
<i>Mouse anti-glial fibrillary acid protein (GFAP)</i>	#G3893, 1:600, Sigma, USA
<i>Rabbit anti-glial fibrillary acid protein (GFAP)</i>	#Z0334, 1:1000, Dako, Denmark
<i>Goat anti-mouse Alexa 488</i>	#A11001, 1:500, Molecular Probes, USA
<i>Goat anti-rabbit Alexa 594</i>	#A11037, 1:500, Molecular Probes, USA
<i>Rabbit anti-XLF</i>	#A300-730A , 1:1000, Bethyl, USA
<i>Rabbit anti-C9orf142 (PAXX)</i>	#126353, 1:200, Novus Biologicals, USA
<i>Mouse anti-DNA-PKCS</i>	#MA5-13404, 1:1000, Invitrogen, Carlsbad, USA
<i>Mouse anti-β-actin</i>	#Ab8226, 1:2000, Abcam, UK
<i>Swine anti-rabbit</i>	#P0399, 1:2000, Dako, Denmark
<i>Goat anti-mouse</i>	#P0447, 1:2000, Dako. Denmark

Supplementary Table 3. Equipment and software

Equipment, software	Company, Country
<i>FLUOstar Omega</i>	BMG Labtech, Ortenberg, Germany
<i>EVOS microscope</i>	Invitrogen, Carlsbad, USA
<i>ChemiDoc™ Touch Imaging System</i>	BioRad, Hercules, USA
<i>ImageJ</i>	National Institute of Health, Bethesda, USA
<i>GradhPad Prism software</i>	GradhPad Prism, La Jolla, CA, USA

Supplementary Table 4. Solutions and cell culture media

Solution, medium	Composition
DNA lysis solution	10 mM pH 9 Trizma, 1 M KCl, 0.4% NP-40 and 0.1% Tween20
Proliferation medium	DMEM/F12 medium supplemented with 1% penicillin/streptomycin, 2% B27 without vitamin A, 10 ng/ml EGF and 20 ng/ml bFGF
Differentiation medium	NeuroBasal A medium supplemented with 1% penicillin/streptomycin, 2% B27, 1 % GlutaMAX and 10 ng/ml bFGF
Blocking solution (10x)	10% BSA (<i>Sigma, USA</i>), 10% goat serum and 0.1% Triton X-100
PBST	10% Tween20 in PBS

Supplementary Table 5. Genotyping primers

Gene	Sequence
<i>Xlf</i> wild type (650 bp)	<i>Forward</i> : CATGTTGGCTCTGCGAATAGA <i>Reverse</i> : GAGCTCGGATATGAGCGCTCAG
<i>Xlf</i> knockout (950 bp)	<i>Forward</i> : CTGTCTTGTGGGCATAGTAGGC <i>Reverse</i> : GAGCTCGGATATGAGCGCTCAG
<i>Paxx</i> (965 bp wild type; 298, 312, 329 bp knockout)	<i>Forward</i> : ACAGAGGGTGGTGACTCAGACAATGG <i>Reverse</i> : GGAAATGCTATTAGAACCACTGCCACG
<i>Dna-pkcs</i> wild type (250 bp)	<i>Dnapkcs-1</i> : CCCTCCAGACAGCCAGCTAAGACAGG <i>Dnapkcs-2</i> : GAAAAAGTCTATGAGCTCCTGGGAG
<i>Dna-pkcs</i> knockout (427 bp)	<i>Dnapkcs-1</i> : CCCTCCAGACAGCCAGCTAAGACAGG <i>Dnapkcs-3</i> : ACGTAACTCCTCTTCAGACCT
<i>Trp53</i> wild type (321 pb)	<i>Trp53-1</i> : TGGATGGTGGTATACTCAGAGC <i>Trp53-2</i> : AGGCTTAGAGGTGCAAGCTG
<i>Trp53</i> knockout (110 bp)	<i>Trp53-1</i> : TGGATGGTGGTATACTCAGAGC <i>Trp53-3</i> : CAGCCTCTGTTCCACATACACT