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Supplementary Data

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Supplementary Figure 1: qRT-PCR for mcl-1, bcl-x, bax and bak at the onset of 3 4 neurogenesis. Endogenous expression of mcl-1, bcl-x, bax and bak mRNA transcripts in the 5 spinal cord of E10 and E12 wild type mice. 6 7 8 9 Supplementary Figure 2: Gene dosage affects the onset of apoptosis in the brainstem. 10 (a) Photomicrograph of cresyl violet stained E11 sagittal embryo. Black box indicates region of 11 brainstem examined. (b) Representative photomicrographs of sagittal brainstem sections from 12 E11 Ctl, Mcl-1 Het:Bcl-x Het, MKO, MKO:Bcl-x Het, BKO, Mcl-1 Het:BKO and DKO 13 animals. Apoptotic cells are labeled by active caspase-3 immunohistochemistry, while cresyl 14 violet staining shows tissue histology. Larger boxed area in **b** indicates region of higher 15 magnification photomicrographs for active caspase-3 stained sections, while smaller inset 16 indicates region shown in cresyl violet photomicrographs. R= rostral, C= caudal, B= basal, A= 17 apical.

18 Supplementary Figure 3: Gene dosage affects the onset of apoptosis in the forebrain. 19 (a) Photomicrograph of cresyl violet stained E11 sagittal embryo. Black box indicates region of 20 forebrain examined. (b) Representative photomicrographs of sagittal forebrain sections from E11 21 Ctl, Mcl-1 Het:Bcl-x Het, MKO, MKO:Bcl-x Het, BKO, Mcl-1 Het:BKO and DKO animals. 22 Apoptotic cells are labeled by active caspase-3 immunohistochemistry, while cresyl violet 23 staining shows tissue histology. Larger boxed area in **b** indicates region of higher magnification 24 photomicrographs for active caspase-3 stained sections, while smaller inset indicates region 25 shown in cresyl violet photomicrographs. R= rostral, C= caudal, B= basal, A= apical. 26 27 Supplementary Figure 4: Expression of Mcl-1 and Bcl-xl protein in spinal cord, brainstem 28 and forebrain CTL, MKO and BKO embryos. Western blots and analysis of Mcl-1 and Bcl-29 xL protein expression in (a, b, g, h) spinal cord (Sp), (c, d, i, j) brainstem (Bs) and (e, f, k, l) 30 forebrain (Fb) tissue. Mcl-1 and Bcl-xL protein was examined in tissue collected from E12 Ctl, 31 Mcl-1 Het and MKO embryos (a - f) and from E12 Ctl, Bcl-x Het and BKO embryos (g - l). 32 Representative blots of three separate experiments. Quantification of Mcl-1 and Bcl-xL protein is

relative to the expression of β -actin, the loading control in each tissue.

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Fogarty_Suppl. Figure 1



Fogarty_Suppl. Figure 2 b CV AC3 AC3 CTL \Box Mcl-1 HET **Bcl-x** HET MKO r E_{rtis} **Bcl-x** HET MKO BKO Ó **Mcl-1 HET** BKO DKO 500 µm 200 µm



a

Fogarty _Suppl. Figure 3 AC3 AC3 CV b CTL Mcl-1 HET Bcl-x HET MKO **Bcl-x HET** MKO BKO **Mcl-1 HET** BKO B DKO 100 µm 50 µm Å

R-

-C

a

Fogarty_Suppl. Figure 4

