Supplementary Materials for

An IL-10/DEL-1 axis promotes granulopoiesis and survival from sepsis in early life

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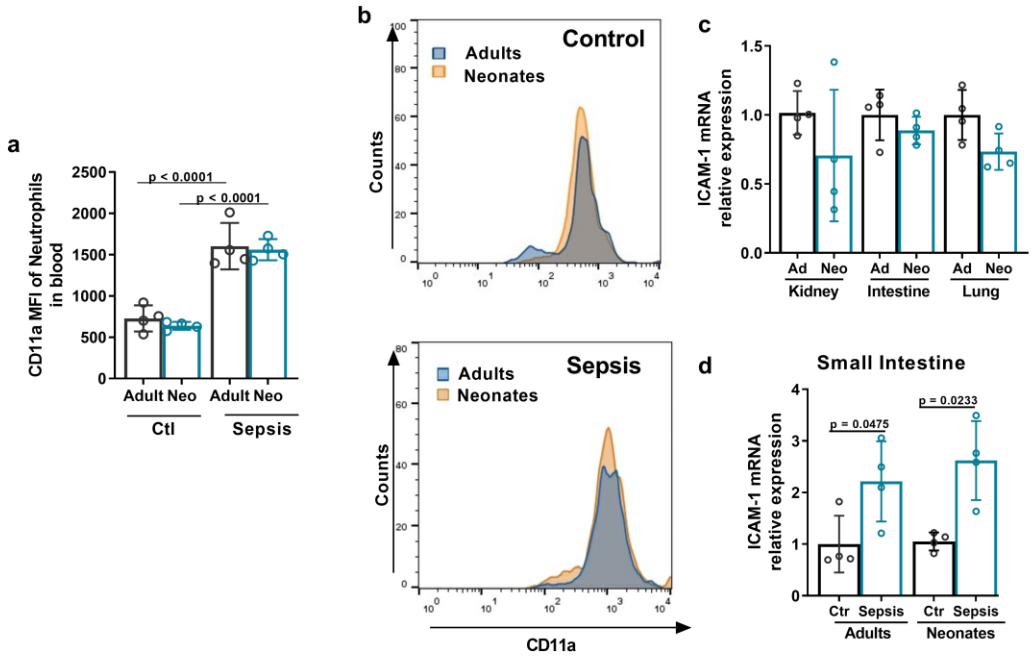
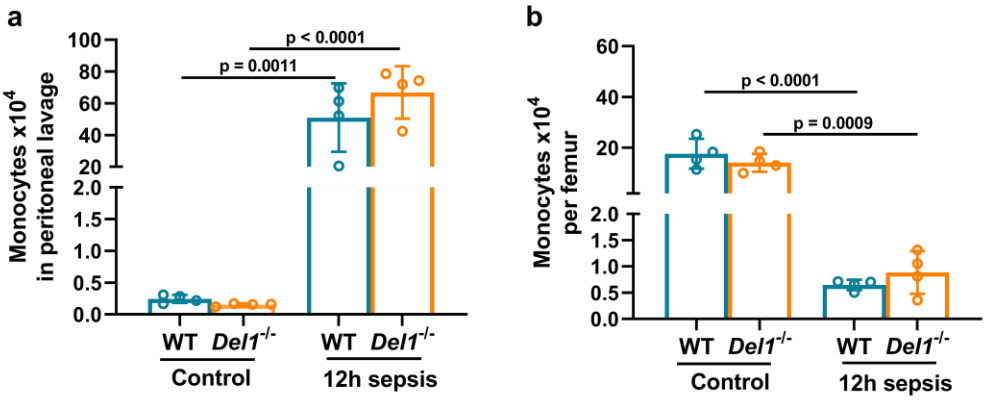
The PDF file includes:

Supplementary Figure 1



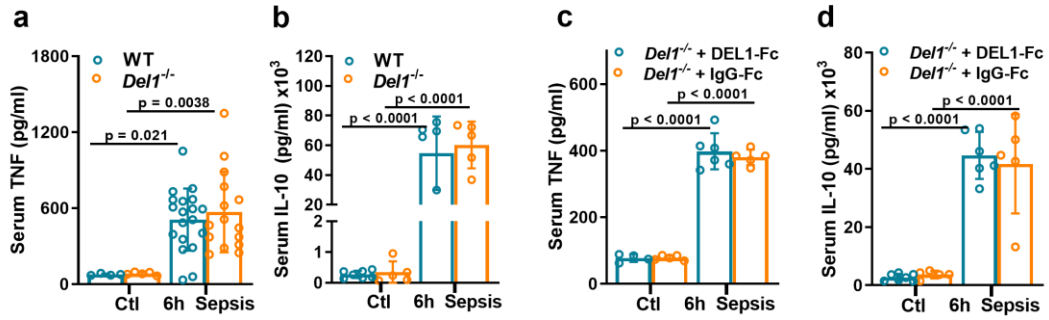
Supplementary Figure 1. **The expression of ICAM-1 and CD11a in neonates**. **a** CD11a mean fluorescence intensity (MFI) and **b** representative flow cytometry plots of (a) in neutrophils (identified as $CD11b^{+}Ly6G^{+}$) from control and septic adult and neonate C57BL/6 mice (n=4 animals per group). **c** ICAM-1 relative mRNA levels from kidney, intestine and lung tissue from adult and neonate C57BL/6 mice. ICAM-1 mRNA expression in each adult tissue was set as 1 (n=4 animals per group). **d** ICAM-1 relative mRNA levels from intestine of adult and neonate C57BL/6 mice after 6 hours of cecal slurry induced sepsis. ICAM-1 mRNA expression in control adult mice was set as 1 (n=4 animals per group). Statistical analysis by one-way ANOVA with Bonferroni's multiple comparison post-test (a, c) or two-sided unpaired *t* test (d). Mean ± SD is depicted (a, c, d). Source data are provided as a Source Data file. Ctl: control, Ad: adult, Neo: neonate.

Supplementary Figure 2



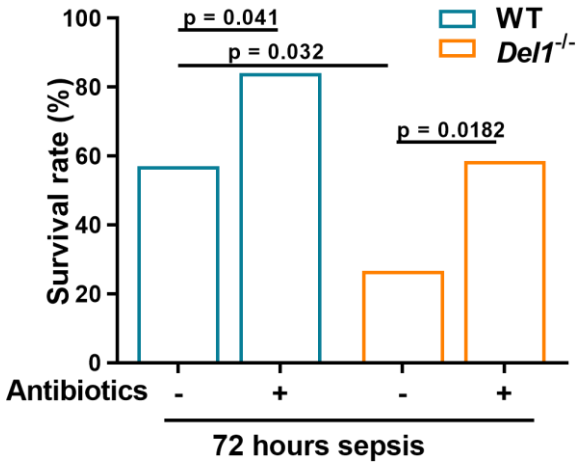
Supplementary Figure 2. **Effect of DEL-1 on monocyte population. a** Total number of monocytes (gated as $CD11b^{+}Ly6C^{+}Ly6G^{-}$) in peritoneal lavage (n=4 animals per group) and **b** total number of monocytes ($CD11b^{+}Ly6C^{+}Ly6G^{-}$) in bone marrow (n=4 animals per group) in WT and *Del1*$^{-/-}$ C57BL/6 healthy neonate mice and following 12 hours of cecal slurry - induced sepsis. Mean ± SD is depicted. Statistical analysis by one-way ANOVA with Bonferroni's multiple comparison post-test. Source data are provided as a Source Data file. h: hours.

Supplementary Figure 3



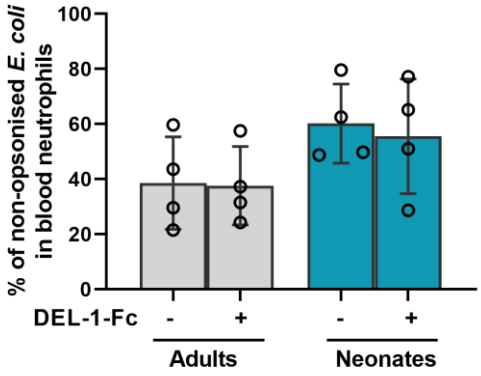
Supplementary Figure 3. **Regulation of cytokine responses by DEL-1.** Protein expression of **a** TNF (pg/ml) in serum from WT and *Del1*$^{-/-}$ C57BL/6 neonate mice in steady state and upon 6 hours of cecal slurry (CS) sepsis (n =4 animals in WT control group, n=5 animals in *Del1*$^{-/-}$ control group, n=19 animals in WT sepsis group and n=15 animals in *Del1*$^{-/-}$ sepsis group) **b** IL-10 (pg/ml) in serum from WT and *Del1*$^{-/-}$ C57BL/6 neonate mice in steady state and upon 6 hours of CS sepsis (n =6 animals in WT control group, n=5 animals in *Del1*$^{-/-}$ control group, n=6 animals in WT sepsis group and n=5 animals in *Del1*$^{-/-}$ sepsis group) **c** TNF (pg/ml) in serum from *Del1*$^{-/-}$ C57BL/6 neonate pups treated with either i.v DEL-1-Fc or IgG-Fc, in steady state and upon 6 hours after CS-induced sepsis (n=5 animals in *Del1*$^{-/-}$+IgG-Fc control group, n=4 animals in *Del1*$^{-/-}$ +DEL-1-Fc control group, n=5 animals in *Del1*$^{-/-}$ + IgG-Fc sepsis group and n=6 animals in *Del1*$^{-/-}$ +DEL-1-Fc sepsis group). **d** IL-10 (pg/ml) in serum from *Del1*$^{-/-}$ C57BL/6 neonate pups treated with either i.v DEL-1-Fc or IgG, in steady state and upon 6 hours after CS-induced sepsis (n= 5 animals in *Del1*$^{-/-}$+IgG-Fc control group, n=6 animals in *Del1*$^{-/-}$ +DEL1-Fc control group, n=5 animals in *Del1*$^{-/-}$ +IgG-Fc sepsis group and n=6 animals in *Del1*$^{-/-}$ +DEL1-Fc sepsis group). Statistical analysis by one way ANOVA with Bonferroni's multiple comparison post-test (a-d). Mean ± SD is depicted (a-d). Source data are provided as a Source Data file. Ctl: control. h: hours.

Supplementary Figure 4



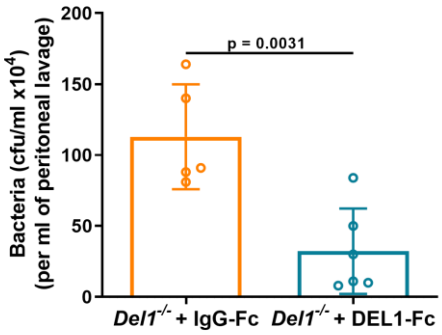
Supplementary Figure 4. Survival of WT and *Del1*$^{-/-}$ neonate mice, treated with antibiotics. Survival rate (%) of WT and *Del1*$^{-/-}$ C57BL/6 neonate mice, treated with or without antibiotics (meropenem). (n=28 animals in WT without antibiotics septic group, n=25 animals in WT with antibiotics septic group, n=30 animals in *Del1*$^{-/-}$ without antibiotics septic group and n=29 animals in *Del1*$^{-/-}$ with antibiotics septic group). Frequency % is depicted. Statistical analysis with Fisher's exact test. Source data are provided as a Source Data file.

Supplementary Figure 5



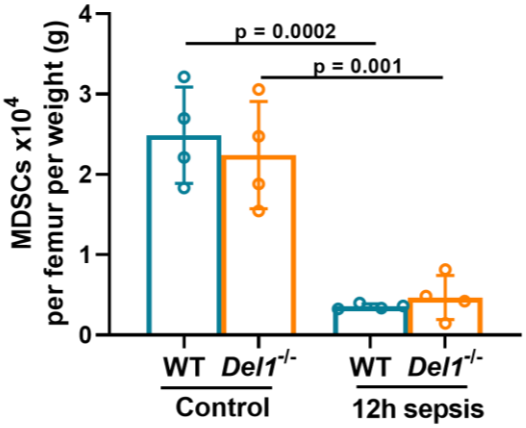
Supplementary Figure 5. The impact of DEL-1 on neutrophil phagocytosis of non-opsonised *E. coli*. Blood neutrophils ($CD11b^{+}Ly6G^{+}$) from healthy, non-septic WT adult and WT neonate mice were treated *ex vivo* with either DEL-1-Fc (10μg/ml) or same amount of IgG-Fc control and their capacity to phagocytose *ex vivo* non-opsonized FITC-labeled *E. coli* particles was assessed by flow cytometry (n=4 animals per group). Neutrophils that phagocytosed FITC-labeled *E. coli* were identified as single cells, CD11b positive, Ly6G positive and FITC - *E. coli* positive. Statistical analysis by one-way ANOVA with Bonferroni's multiple comparison post-test. Mean ± SD is depicted. Source data are provided as a Source Data file.

Supplementary Figure 6



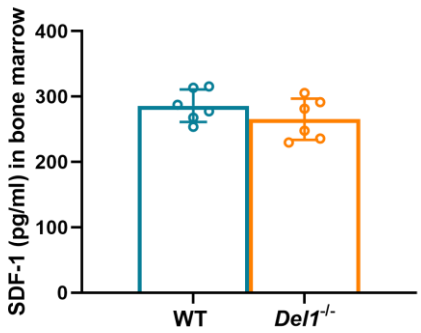
Supplemental Figure 6. The impact of DEL-1 in bacterial clearance at the site of infection (source control). Bacteria load (colony forming units, cfu) in the peritoneal lavage of septic *Del1* $^{-/-}$ C57BL/6 neonate mice (12 hours following cecal slurry induced sepsis) treated with either DEL-1-Fc (5μg/ml) or IgG-Fc control (n=5 animals in *Del1* $^{-/-}$ +IgG-Fc group and n=6 animals in *Del1* $^{-/-}$ +DEL1-Fc group). Statistical analysis by two-sided unpaired *t* test. Mean ± SD is depicted. Source data are provided as a Source Data file.

Supplementary Figure 7



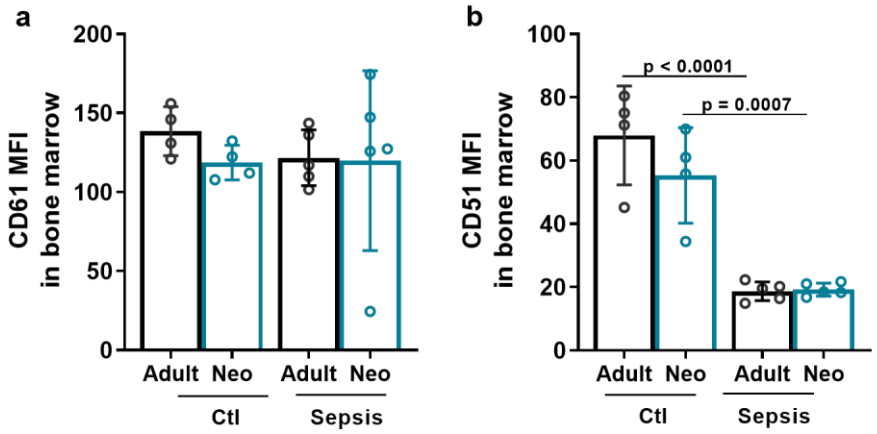
Supplementary Figure 7. Myeloid-Derived Suppressor Cell (MDSC) population in the bone marrow of WT and DEL-1 deficient mice. Total number of MDSCs (gated as $CD11c^{-}CD11b^{+}Ly6G^{+}Ly6C^{+}$) per femur per mouse weight (grams) in the bone marrow of WT and *Del1*$^{-/-}$ C57BL/6 neonate mice upon steady state and 12 hours of cecal slurry - induced polymicrobial sepsis. n= 4 mice per group. Mean ± SD is depicted. Statistical analysis by one-way ANOVA with Bonferroni's multiple comparison post-test. Source data are provided as a Source Data file. h: hours, g: grams.

Supplementary Figure 8



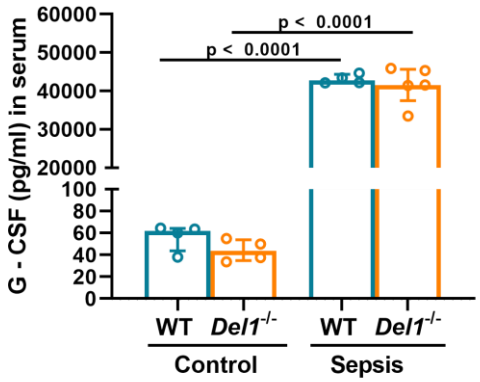
Supplementary Figure 8. SDF-1 protein expression in the bone marrow of septic WT and *Del1*$^{-/-}$ neonate mice. Protein expression of stromal cell-derived factor 1 (SDF-1) (pg/ml) in the bone marrow of WT and *Del1*$^{-/-}$ C57BL/6 neonate mice upon 12 hours of cecal slurry induced sepsis (n = 6 animals per group). Statistical analysis by two-sided unpaired *t* test. Mean ± SD is depicted. Source data are provided as a Source Data file.

Supplementary Figure 9



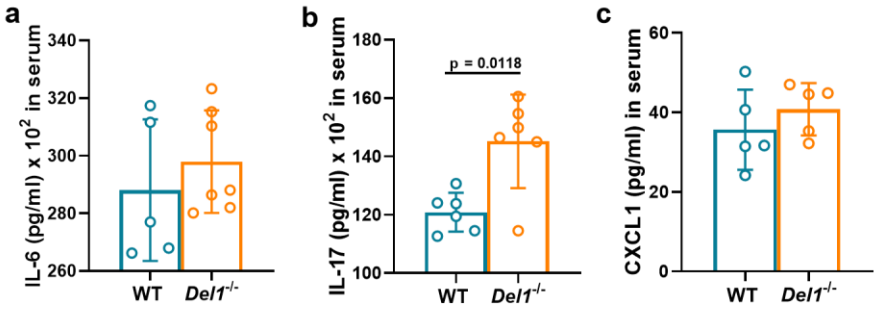
Supplementary Figure 9. **Expression of DEL-1 receptor CD61/CD51 in neonates**. **a** CD61 mean fluorescence intensity (MFI) in total bone marrow cells from adult and neonate C57BL/6 mice in steady state and upon 6 hours of cecal slurry induced sepsis (single live cells, CD61 positive, n=4 animals in adult control group, n=4 animals in neonate control group, n=5 animals in adult sepsis group and n=5 animals in adult sepsis group). **b** CD51 MFI in total bone marrow cells from adult and neonate C57BL/6 mice in steady state and upon 6 hours of cecal slurry induced sepsis (single live cells, CD51 positive, n=4 animals in adult control group, n=4 animals in neonate control group, n=5 animals in adult sepsis group and n=5 animals in adult sepsis group). Statistical analysis by one-way ANOVA with Bonferroni's multiple comparison post-test (a, b). Mean ± SD is depicted (a, b). Source data are provided as a Source Data file. Ctl: control, Neo: Neonate.

Supplementary Figure 10



Supplementary Figure 10. G-CSF protein expression in the serum of WT and *Del1*$^{-/-}$ neonate mice. Protein expression of granulocyte colony stimulating factor (G-CSF) (pg/ml) in the serum of WT and *Del1*$^{-/-}$ C57BL/6 neonate mice upon steady state and 12 hours of cecal slurry induced sepsis (n = 4 animals in WT control group, n = 4 animals in *Del1*$^{-/-}$ control group, n = 4 animals in WT sepsis group and n = 5 animals in *Del1*$^{-/-}$ sepsis group). Statistical analysis by Kruskal-Wallis test with Dunn's multiple comparison post-test. Median ± interquartile range is depicted. Source data are provided as a Source Data file.

Supplementary Figure 11



Supplementary Figure 11. Expression of inflammatory cytokines in the serum of WT and *Del1*$^{-/-}$ neonate mice upon 12 hours of sepsis. Protein expression of **a** interleukin (IL) – 6 (pg/ml) in serum (n = 5 animals in WT group and n=7 animals in *Del1*$^{-/-}$ group) **b** IL-17 (pg/ml) in serum (n = 6 animals per group) and **c** CXCL-1(pg/ml) in serum (n = 5 animals in each group), of septic WT and *Del1*$^{-/-}$ C57BL/6 neonate mice (12 hours of cecal slurry sepsis). Statistical analysis by two-sided unpaired *t* test (a, b, c). Mean ± SD is depicted (a, b, c). Source data are provided as a Source Data file.

Supplementary Table 1. Clinical characteristics and outcomes of the low DEL-1 and high DEL-1 groups in neonates with sepsis.

	All cases n = 24	Low DEL-1 n = 12	High DEL-1 n = 12	p value*
Demographics				
Sex (males)	15/24 (62.5%)	8/12 (66.7)	7/12 (58.3%)	>0.9999
Postnatal days†	2 (1 -4)	1.5 (1 – 3)	2.5 (1 – 4)	0.8877
Gestational age ǂ (weeks)ǂ	34.36 (±3.6)	34.37 (±2.9)	34.35 (±3.4)	0.9848
Type of sepsis				
Early onset	20/24 (83.3%)	10/12 (83.3%)	10/12 (83.3%)	>0.9999
Late onset	4/24 (16.6%)	2/12 (16.6%)	2/12 (16.6%)	>0.9999
Bacteremia	7/24 (29.2%)	4/12 (33.3%)	3/12 (25%)	>0.9999
Laboratory data				
IL-6 (pg/ml) †	188.7 (87-391)	186.2 (62-338)	188.7 (101-2578)	0.10774
IL-10 to IL-17 ratio ǂ	7.26 (20.03)	0.32 (±0.32)	14.20 (±20.07)	**0.0355**
ANC ($x10^3$/µl) ǂ Day 0	6,040 (±3.390)	6,880 (± 4,320)	5,190 (±3,890)	0.3269
ANC ($x10^3$/µl)† Day 1	6,003(3,525-8,520)	5,280 (2,076-8,266)	6,444 (3,685-13,250)	0.5563
ANC (fold increase) †	1.62 (±1.3)	1 (±0.4)	2.3 (±1.28)	**0.00717**
Outcome				
ICU length of stay†	14 (10-30.7)	12 (10-30.50)	14 (10-31)	0.8051
28 – day mortality	4/24 (16.7%)	3/12 (25%)	1/12 (8.33%)	0.5901

Abbreviations: Low DEL-1 group: serum DEL-1 levels less than 700pg/ml; ANC: absolute neutrophil count, ICU: intensive care unit.

Definitions: early onset: sepsis in neonates less than 7 days old; late-onset: sepsis in neonates≥ 7 days old; *Comparison among low and high DEL-1 group; statistical analysis was performed with Fisher's exact test or Chi-squared test (for categorical data) and two-sided unpaired *t* test (ǂ) or by two-sided Mann-Whitney test (†) for numerical data. ǂMean ±SD and †median (interquartile range) are depicted. In ANC (fold increase) the Low DEL-1 group value was set to 1. Source data are provided as a Source Data file. The DEL-1 protein level data, the ANC on day 0 and day and the mortality rate data presented in this table are also depicted in Figure 5 and figure 9h of the main manuscript.

Supplementary Table 2. Clinical characteristics and outcomes in the low DEL-1 and high DEL-1 groups in adults with sepsis

	All cases n = 40	**Low DEL-1 n = 25**	**High DEL-1 n = 15**	**p value***
Demographics				
Sex (males)	27/40 (67.5%)	17/25 (68%)	10/15 (66.7%)	>0.9999
Age in years†	66 (46 - 72)	68 (47 - 75)	54 (41 - 68)	0.1071
SOFA score (median, range)	8 (6-10)	8 (6-10)	9 (6.5-12.5)	0.5025
Source of sepsis				
Pulmonary	24/40 (60%)	13/25 (52%)	11/15 (73.3%)	0.3176
Abdominal	10/40 (25%)	8/25 (20%)	2/15 (13.3%)	0.2686
Other	6/40 (15%)	4/25 (26.5%)	2/15 (13.3%)	>0.9999
Positive Blood cultures	13/40 (32.5%)	8/25 (20%)	5/15 (33.3%)	>0.9999
Laboratory data				
IL-6 (pg/ml) †	410 (179 – 1400)	403 (161 – 1834)	378 (193–1093)	0.6422
Outcome				
ICU length of stay †	21 (13-29)	21 (13-31)	17 (12-30)	0.6221
28 – day mortality	13/40 (32.5%)	11/25 (44%)	2/15 (13.3%)	**0.0450#**

Abbreviations: Low DEL-1 Group: serum DEL-1 levels less than 125pg/ml, ANC: absolute neutrophil count. ICU: intensive care unit.

*Comparison among low and high DEL-1 group; statistical analysis was performed with Fisher's exact test or Chi-squared test (#) (for categorical data) and by two-sided Mann-Whitney test (†) for numerical data. † Median (interquartile range) is depicted. Source data are provided as a Source Data file. The DEL-1 protein level data and the mortality rate data presented in this table are also depicted in Figure 5 of the main manuscript.