

Supplemental Figure 1 –Experimental design of mouse studies. Two separate mouse experiments were performed to examine the effect of G-CSF on spermatogenic recovery after busulfan treatment. **(A)** In both experiments, Five-week old C57BL/6 males were treated with G-CSF or vehicle seven days (green triangles) and given one injection of DMSO or Busulfan on day 3. **(B)** In both experiments, animals were randomly assigned to three treatment groups: Control, Busulfan Only, and Busulfan + G-CSF. Shown is the specific treatment regimen for animals in each group and the number of animals per group. **(C)** Animals in Experiment 1 were euthanized 10 weeks after treatment and testis weight, testis histology and epididymal sperm counts were measured (Figure 1). **(D)** Animals in Experiment 2 were euthanized 24 hours following the last treatment (on day 8) and used for immunofluorescent analysis of the frequency of PLZF+ spermatogonia and their labeling indices with apoptotic measures (Figure 2).

Supplemental Figure 2 – Gross tubule cross-section morphometry (Experiment 1).

Automated image analysis using a custom Matlab program was used to measure gross morphometric characteristics of round seminiferous tubule cross-sections in testes from mice in Experiment 1 treated with busulfan ± G-CSF. Round seminiferous tubules were defined as having a shape factor of ≥ 0.8 (shape factor = $4\pi \text{area} / \text{circumference}^2$), where a value closer to 1 is a more perfect circle. Morphometrics were reported for only seminiferous tubule cross-sections

containing complete spermatogenesis (**A, C, E, G, I**) or for all round tubule cross-sections (**B, D, F, H, J**). Shown are (**A-B**) equivalent diameter (equivalent diameter = $\sqrt{(4\text{area}/\pi)}$ which provides the diameter of a circle with the equivalent area as the noted tubule cross-section, (**C-D**) major and minor axes (largest and smallest diameters), (**E-F**) area, (**G-H**) perimeter (circumference), and (**I-J**) shape factor. All values are average \pm SEM. Labels above bars signify statistically-significant differences between groups as determined by student's t-test. Pairwise t-test p-values for these data are shown in Supplemental Table 4.

Supplemental Table 1 – Antibodies used in this study.

Experiment(s)	Antigen	Primary Antibody (dilution, cat#, vendor)	Secondary Antibody (dilution, cat#, vendor)
Testis Immunofluorescence (Experiment 2)	PLZF	goat anti-PLZF IgG (1ug/ml, AF2944, R&D Systems, Minneapolis, MN)	donkey anti-goat AlexaFluor568 IgG (1:800, A-11057, Life Technologies)
Testis Immunofluorescence Experiment 2)	Cleaved Caspase 3	rabbit anti-cleaved Caspase 3 IgG (10ug/ml, ab2302, Abcam, Cambridge, MA)	goat anti-rabbit AlexaFluor488 IgG (1:800, A-11034, Life Technologies)
Western blot	CSF3R	Sheep anti-CSF3R IgG (1:1000, AF6039, R&D Systems)	donkey anti-sheep IgG (1:10,000; sc-2916, Santa Cruz Biotechnology)
Flow cytometry	CSF3R	mouse anti-CSF3R IgG2b, κ (3 μ g/10 ⁶ cells; ab1947, Abcam)	APC-conjugated goat anti- mouse IgG (0.25ug/10 ⁶ cells; 405308, Biolegend)
Flow cytometry	Isotype control	Ms IgG2b, κ (3 μ g/10 ⁶ cells; 400301, Biolegend, San Diego, CA)	APC-conjugated goat anti- mouse IgG (0.25ug/10 ⁶ cells; 405308, Biolegend)
Immunofluorescence in Thy1+ sg cultures	CSF3R	Sheep anti-CSF3R IgG (2ug/ml, AF6039, R&D Systems)	donkey anti-sheep AlexaFluor488 IgG (1:800; A-11015, Life Technologies)
Immunofluorescence in Thy1+ sg cultures	SALL4	rabbit anti-SALL4 IgG (1.6ug/ml, ab29112, Abcam)	donkey anti-rabbit AlexaFluor568 IgG (1:800; A-10042, Life Technologies)

Supplemental Table 2 – Oligodeoxynucleotide primers.

Target gene	Method	Forward primer (5'- 3')	Reverse Primer (5'- 3')	Amplicon (bp)	Location
<i>Csf3r</i>	RT-PCR	GTACTCTTGTCCACTACCTGT	CAAGATACAAGGACCCCCAA	630bp	Exons 15-17
<i>Gapdh</i>	RT-PCR	ATCACCATCTTCCAGGAGCGAGA	GATGGCATGGACTGTGGTCATGA	324bp	Exons 3-5
<i>Csf3r</i>	qRT-PCR*	GCGTCCAACCTCCTGGATCA	GAGGTGCATGAGGCAGGATA	83bp	Exons 4-5
<i>Dazl</i>	qRT-PCR*	CTTACATGCAGCCTCCAACC	GTGATGACCTGAACTGGTGAAC	91bp	Exons 6-7
<i>Ddx4</i>	qRT-PCR*	TGATGGCTTGTGCTCAAACA	CCCGCATCATATGAGCCAAA	75bp	Exons 14-15
<i>Foxo1</i>	qRT-PCR*	GAAGAGCGTGCCCTACTTCA	GGACAGATTGTGGCGAATTGAA	82bp	Exons 1-2
<i>Gfra1</i>	qRT-PCR*	CCAATGTATCGGGCAGTACACA	CCAGCGAGACCATCCTTTCC	67bp	Exons 9-10
<i>Sall4</i>	qRT-PCR*	TTCACCACGAAAGGCAACC	TCTATGGCCAGCTTCCTTCC	89bp	Exons 2-3
<i>Sohlh1</i>	qRT-PCR*	GCAAGCCAGACTCCGGTATA	TGTATCCAGCATCCCAAAGCA	80bp	Exons 4-5
<i>Sohlh2</i>	qRT-PCR*	GATCAGCACTGGGGAGCTA	TCTTGTGGCATCTCCAACCA	76bp	Exons 1-2
<i>Zbtb16</i>	qRT-PCR*	TGGCAAGAAGTTCAGCCTCA	GTGGCAGAGTTTGCCTCAA	88bp	Exons 6-7
<i>Gdnf</i>	qRT-PCR*	CCCGCTGAAGACCACTCC	CTGGTCAGGATAATCTTCAGGCATA	87bp	Exons 2-3
<i>Hsd3b1</i>	qRT-PCR*	TCCCAGGCAGACCATCCTA	CTGGCACACTTGCTTGAACA	80bp	Exons 4-5
<i>Actb</i>	qRT-PCR*	CCCTAAGGCCAACCGTGAAA	AGCCTGGATGGCTACGTACA	82bp	Exons 3-4
<i>Gapdh</i>	qRT-PCR*	AGACGGCCGCATCTTCTT	TTCACACCGACCTTCACCAT	68bp	Exons 1-2
<i>Rpl7</i>	qRT-PCR*	CAAAGCTGGCCTTTGTCATCA	AAGACGAAGGAGCTGCAGAA	83bp	Exons 3-4

*Single-cell qRT-PCR

Supplemental Table 3 – Tubule cross-section counting statistics from mice 10 weeks after busulfan ± G-CSF treatment (Experiment1).

Treatment Group	Animal	Category (# round tubule cross-sections)				
		Complete	Round Std	Pachytene Sct	Empty/Sg	All
Control	1	1508	0	0	1	1509
	2	287	0	0	0	287
	3	533	22	9	0	564
Busulfan Only	1	8	0	0	597	605
	2	26	9	8	468	511
	3	91	21	16	569	697
Busulfan + G-CSF	1	102	17	38	681	838
	2	238	43	21	660	962
	3	117	23	12	549	701
	4	98	15	23	545	681
Control	Sum	2328	22	9	1	2360
Busulfan Only	Sum	125	30	24	1634	1813
Busulfan + GCSF	Sum	555	98	94	2435	3182
Control	Avg±SEM	776±457	7±9	3±4	0.3±0.4	787±453
Busulfan Only	Avg±SEM	42±31	10±7	8±6	545±48	604±66
Busulfan + GCSF	Avg±SEM	139±39	25±7	24±6	609±41	796±76

Supplemental Table 4 – P-values of pairwise t-test comparisons of tubule morphometrics (Experiment 1).

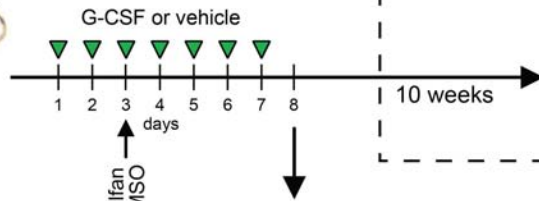
Tubule type	Comparison groups	Measurement					
		Equivalent diameter	Perimeter	Major Axis	Minor Axis	Area	Shape Factor
All round	Control to Busulfan Only	8.8818E-173	1.8503E-176	1.6955E-146	7.1083E-186	3.8381E-225	0.769411277
	Control to Busulfan + GCSF	0*	0*	5.0311E-260	0*	0*	0.025741627
	Busulfan Only to Busulfan + GCSF	7.08151E-08	1.4229E-07	4.68496E-05	6.74707E-10	3.01461E-07	0.185102919
Round with complete spermatogenesis	Control to Busulfan Only	8.51959E-59	6.45497E-57	2.76657E-36	4.3319E-79	1.38328E-70	4.01479E-07
	Control to Busulfan + GCSF	4.2078E-147	4.3913E-143	1.51448E-94	1.6467E-170	8.4803E-169	8.02502E-15
	Busulfan Only to Busulfan + GCSF	0.405229249	0.407494917	0.602036078	0.003678421	0.34779885	0.741391701

*Note: P-values of 0 are derived from rounding and reflect values very close to 0.

Supplemental Table 5 – Quantification of PLZF+ spermatogonia and overlap with apoptotic measures from mice after busulfan ± G-CSF (Experiment 2).

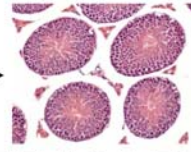
Treatment Group	Animal	Combined PLZF+ (round only)			PLZF+ (round and non-round)			PLZF+ (round and non-round)		
		Round tubule X-sections	PLZF+ in Round tubules	PLZF+ per cross-section	Caspase3+	Caspase3-	Caspase3+ % of PLZF+	TUNEL+	TUNEL-	TUNEL+ % of PLZF+
Control	1	549	1747	3.18	3	1581	0.19	3	1942	0.15
	2	224	524	2.34	0	930	0	3	1354	0.22
	3	232	469	2.02	0	1011	0	1	1064	0.09
	4	197	388	1.97	0	608	0	0	1030	0
	5	173	388	2.24	0	628	0	5	857	0.58
	6	231	559	2.42	0	646	0	0	923	0
	7	143	385	2.69	0	542	0	0	1260	0
	8	227	545	2.4	0	543	0	0	931	0
Busulfan Only	1	107	62	0.58	6	321	1.83	4	228	1.72
	2	175	77	0.44	2	258	0.77	7	230	2.95
	3	254	114	0.45	8	222	3.48	2	249	0.80
	4	269	68	0.25	4	75	5.06	9	112	7.43
	5	203	69	0.34	3	151	1.95	0	203	0
	6	180	63	0.35	6	107	5.31	4	157	2.48
	7	160	80	0.50	2	112	1.75	2	171	1.16
	8	550	137	0.25	2	104	1.89	0	118	0
	9	545	133	0.24	7	93	7.00	2	126	1.56
Busulfan + G-CSF	1	262	159	0.61	6	272	2.16	6	281	2.09
	2	284	143	0.50	4	187	2.09	12	170	6.59
	3	257	106	0.41	5	180	2.70	17	201	7.80
	4	175	82	0.47	3	169	1.74	4	205	1.91
	5	247	139	0.56	7	184	3.66	3	207	1.43
	6	99	76	0.77	7	247	2.76	4	253	1.56
	7	301	101	0.34	11	111	9.01	0	157	0
	8	241	110	0.46	4	157	2.48	4	163	2.40
	9	266	93	0.35	9	157	5.42	1	187	0.53
Control	Sum	1976	5005	-	3	6489	-	12	9361	-
Busulfan Only	Sum	2443	803	-	40	1443	-	30	1594	-
Busulfan + GCSF	Sum	2132	1009	-	56	1664	-	51	1824	-
Control	Avg±SEM	247±48	626±173	2.40±0.15	0.38±0.40	811±135	0.024±0.025	1.5±0.73	1170±134	0.13±0.08
Busulfan Only	Avg±SEM	271±58	89±11	0.38±0.04	4.44±0.78	160±29	3.23±0.70	3.33±1.01	177±17	2.01±0.76
Busulfan + GCSF	Avg±SEM	237±22	112±10	0.50±0.05	247±48	247±48	3.56±0.77	5.67±1.82	203±14	2.70±0.89

A 5 week ♂
C57BL/6 mice



C - Experiment 1

Endpoints: Testis weight,
Histology, Sperm count



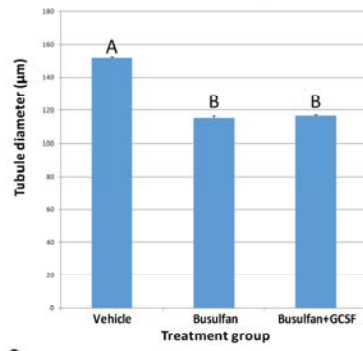
D - Experiment 2

Endpoints: Immunohistochemical analysis
(PLZF, activated Caspase3, TUNEL)

B

Treatment Group	G-CSF (days 1 through 7)	Busulfan (day 3)	Experiment 1 Animal #	Experiment 2 Animal #
Control	0.1% BSA/DPBS	DMSO	3	8
Busulfan only	0.1% BSA/DPBS	44mg/kg in DMSO	3	9
Busulfan + G-CSF	50ug/kg in 0.1% BSA/DPBS	44mg/kg in DMSO	4	9

A *Round cross-sections with complete spermatogenesis*



B *All round cross-sections*

