

Supplementary Information

Title

Arsenic exposure impels CD4 commitment in thymus and suppresses T cell cytokine secretion by increasing regulatory T cells

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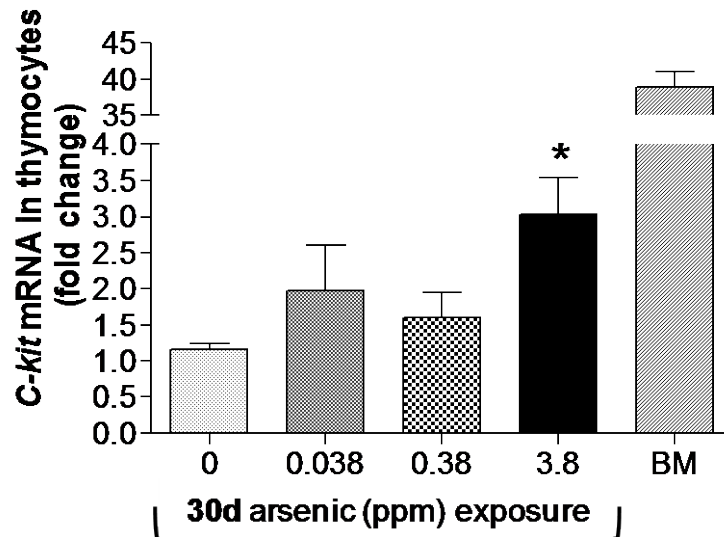
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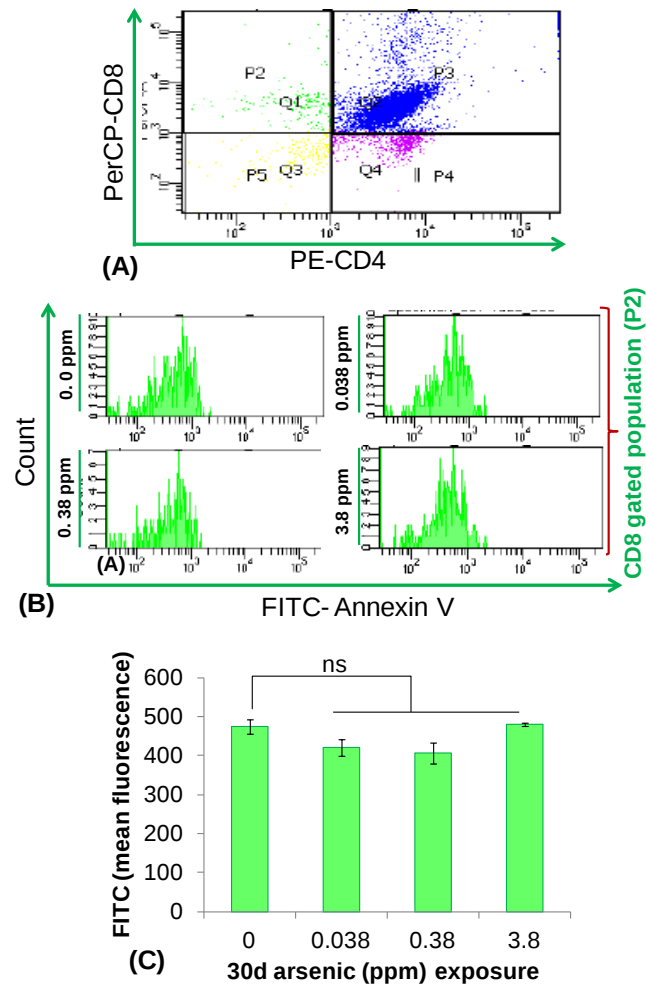
Arsenic exposure promoted influx of DN cells into thymus from bone marrow:



Supplementary Figure S1. Arsenic-induced alteration in *c-kit* expression in thymocyte.

Total RNA was extracted from thymus tissue of all 30 days arsenic-exposed groups. *c-kit* mRNA level was measured under QuantStudio 6 Flex instrument with SYBR Green by normalizing with *Hprt*. Bone marrow (BM) cells were isolated from femur bone of mice and used as a positive control for *c-kit* expression. Values are mean $\pm$ S.E. (n=3 per group). *p<0.05 compared to control using one way ANOVA followed by Newman-Keuls test with 95% confidence intervals.

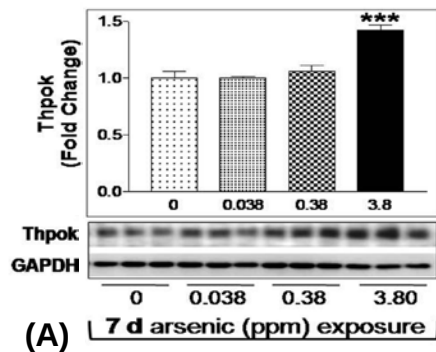
Arsenic promoted percentage of CD4⁺ cells without inducing apoptosis in CD8⁺ cells:



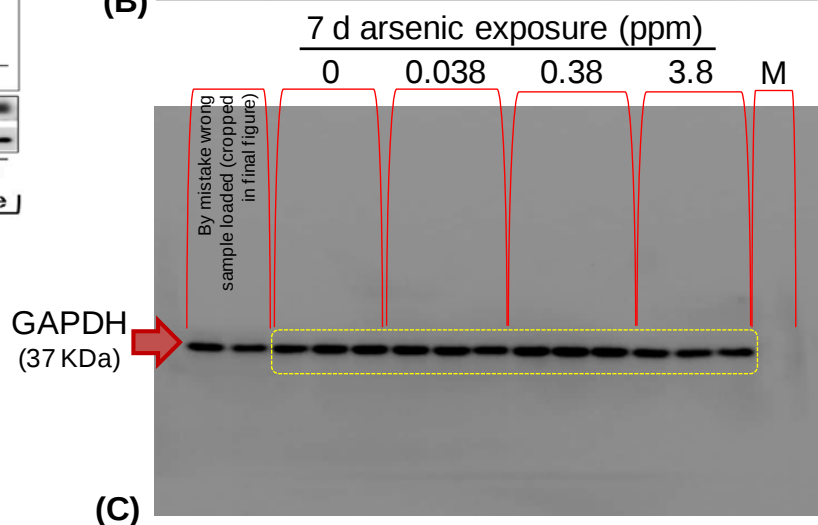
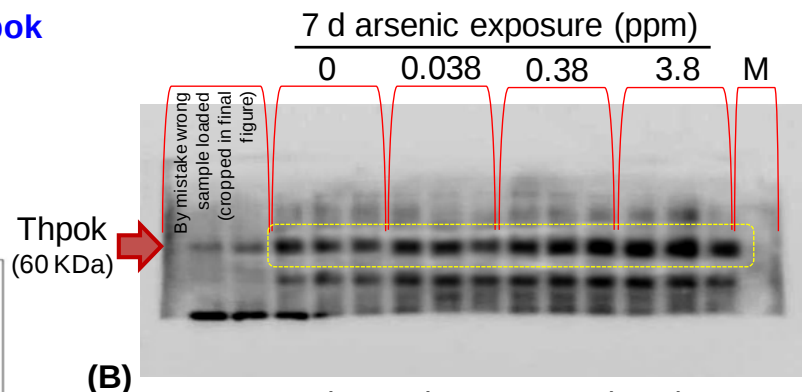
Supplementary Figure S2. Arsenic-induced alteration in Annexin-FITC labeling in thymic CD8⁺ cells. Thymocyte suspension was prepared from thymus obtained from 30 days arsenic-exposed mice. Thymocytes (0.2×10^6 cells) were stained with PE and PerCP conjugated anti-CD4 and anti-CD8 antibodies respectively along with FITC conjugated annexin-V for 1hr, run in BD-FACSCANTO II and analyzed using FACS-Diva software. **(A)** Representative dot plot showing the strategy used to gate CD8⁺ cells as P2 population (green color) in thymocyte suspension. **(B)** Representative histograms showing annexin-V staining of CD8⁺ gated cells. **(C)** Bar diagram reflecting the mean fluorescence of FITC conjugated with annexin-V in CD8⁺ cells. Values are mean $\pm$ S.E. (n=3 per group). ns = not significant when compared to control using One way ANOVA followed by Newman-Keuls test with 95% confidence intervals.

Original full-length western blots of Thpok, RunX3 and FoxP3 with corresponding GAPDH (loading control):

Original western blot of **Thpok**
(7 d arsenic exposure)

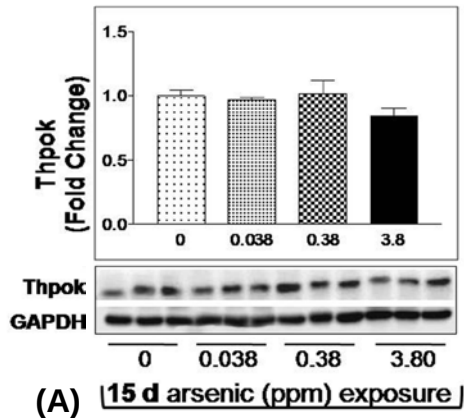


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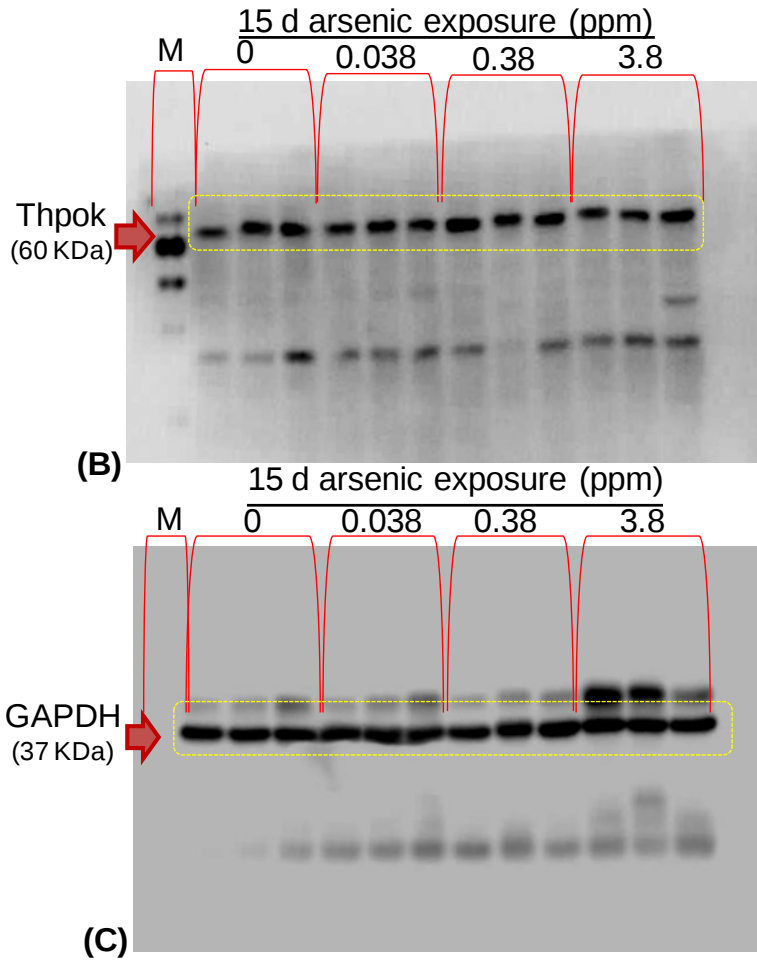


Supplementary Figure S3: Arsenic-induced alteration in Thpok expression following 7 days arsenic exposure. Thymus dissected from mice treated with arsenic (0.00, 0.038, 0.38 and 3.8 ppm) orally for 7days were homogenized for protein preparation and examined for the expression of (A) Thpok and GAPDH. Full-length blot for (B) Thpok and, (C) corresponding GAPDH.

Original western blot of **Thpok**
(15 d arsenic exposure)

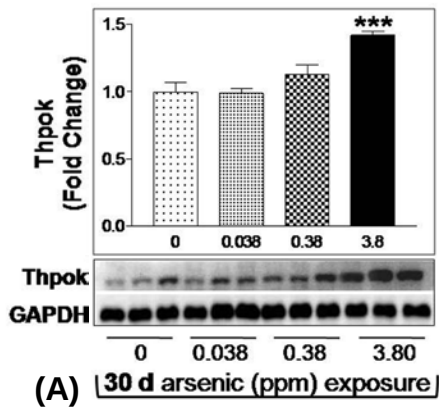


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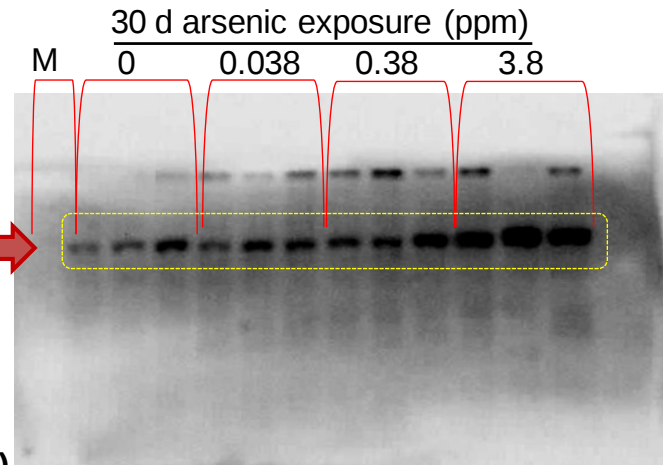
Supplementary Figure S4: Arsenic-induced alteration in Thpok expression following 15 days arsenic exposure. Thymus dissected from mice treated with arsenic (0.00, 0.038, 0.38 and 3.8 ppm) orally for 15 days were homogenized for protein preparation and examined for the expression of (A) Thpok and GAPDH. Full-length blot for (B) Thpok and, (C) corresponding GAPDH.

Original western blot of **Thpok**
(30 d arsenic exposure)



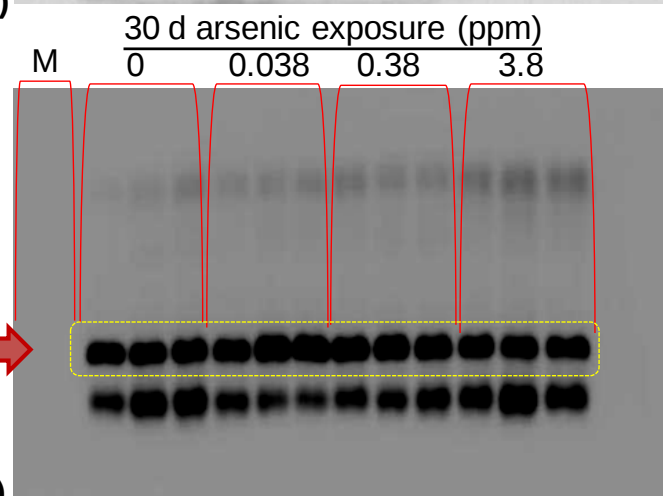
Thpok
(60 KDa)

(B)



GAPDH
(37 KDa)

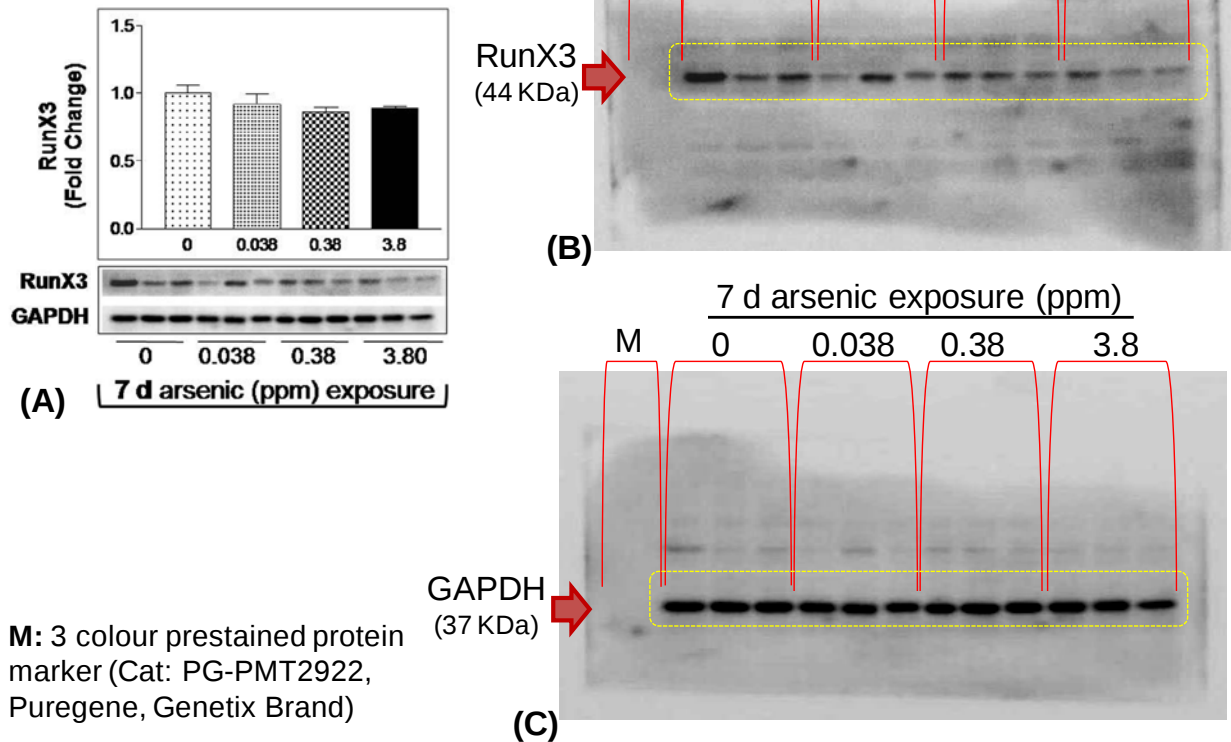
(C)



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marker (Cat: PG-PMT2922,
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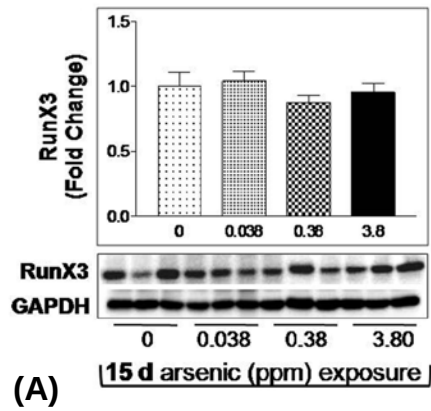
Supplementary Figure S5: Arsenic-induced alteration in Thpok expression following 30 days arsenic exposure. Thymus dissected from mice treated with arsenic (0.00, 0.038, 0.38 and 3.8 ppm) orally for 30 days were homogenized for protein preparation and examined for the expression of (A) Thpok and GAPDH. Full-length blot for (B) Thpok and, (C) corresponding GAPDH.

Original western blot of **RunX3**
(7 d arsenic exposure)

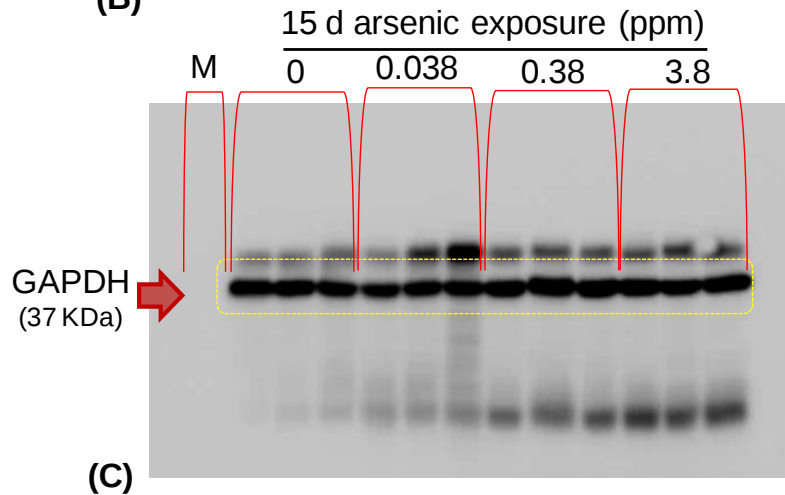
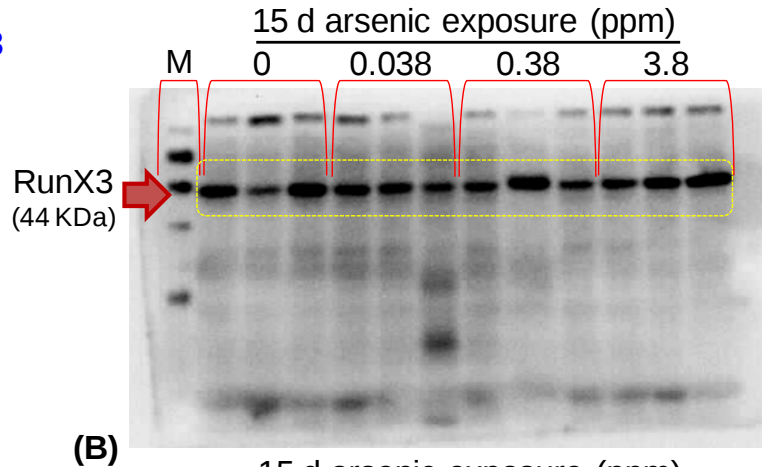


Supplementary Figure S6: Arsenic-induced alteration in RunX3 expression following 7 days arsenic exposure. Thymus dissected from mice treated with arsenic (0.00, 0.038, 0.38 and 3.8 ppm) orally for 7 days were homogenized for protein preparation and examined for the expression of **(A)** RunX3 and GAPDH. Full-length blot for **(B)** RunX3 and, **(C)** corresponding GAPDH.

Original western blot of **RunX3**
(15 d arsenic exposure)

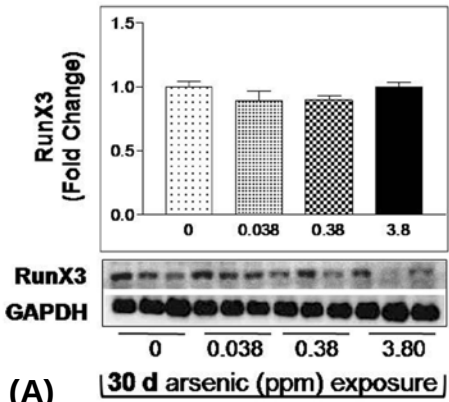


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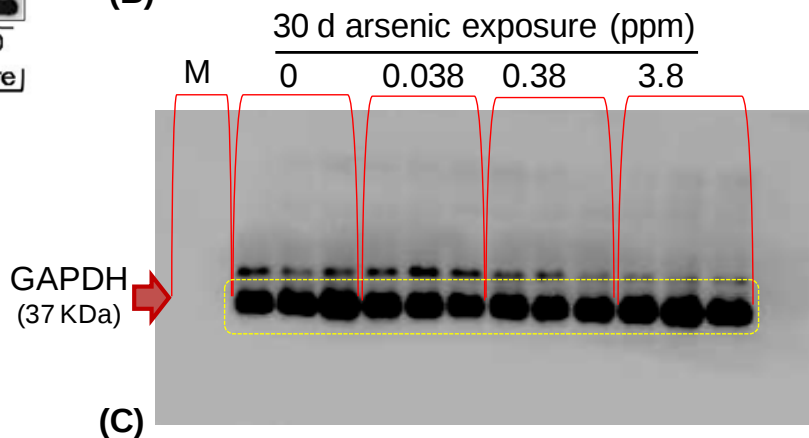
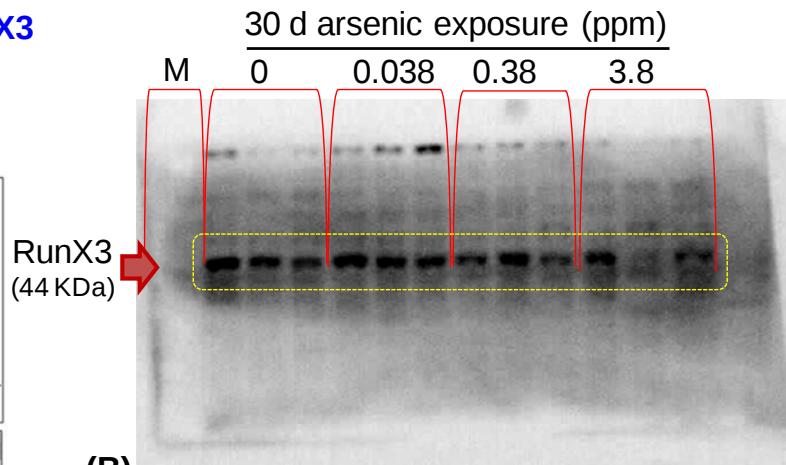


Supplementary Figure S7: Arsenic-induced alteration in RunX3 expression following 15 days arsenic exposure. Thymus dissected from mice treated with arsenic (0.00, 0.038, 0.38 and 3.8 ppm) orally for 15 days were homogenized for protein preparation and examined for the expression of (A) RunX3 and GAPDH. Full-length blot for (B) RunX3 and, (C) corresponding GAPDH.

Original western blot of **RunX3**
(30 d arsenic exposure)

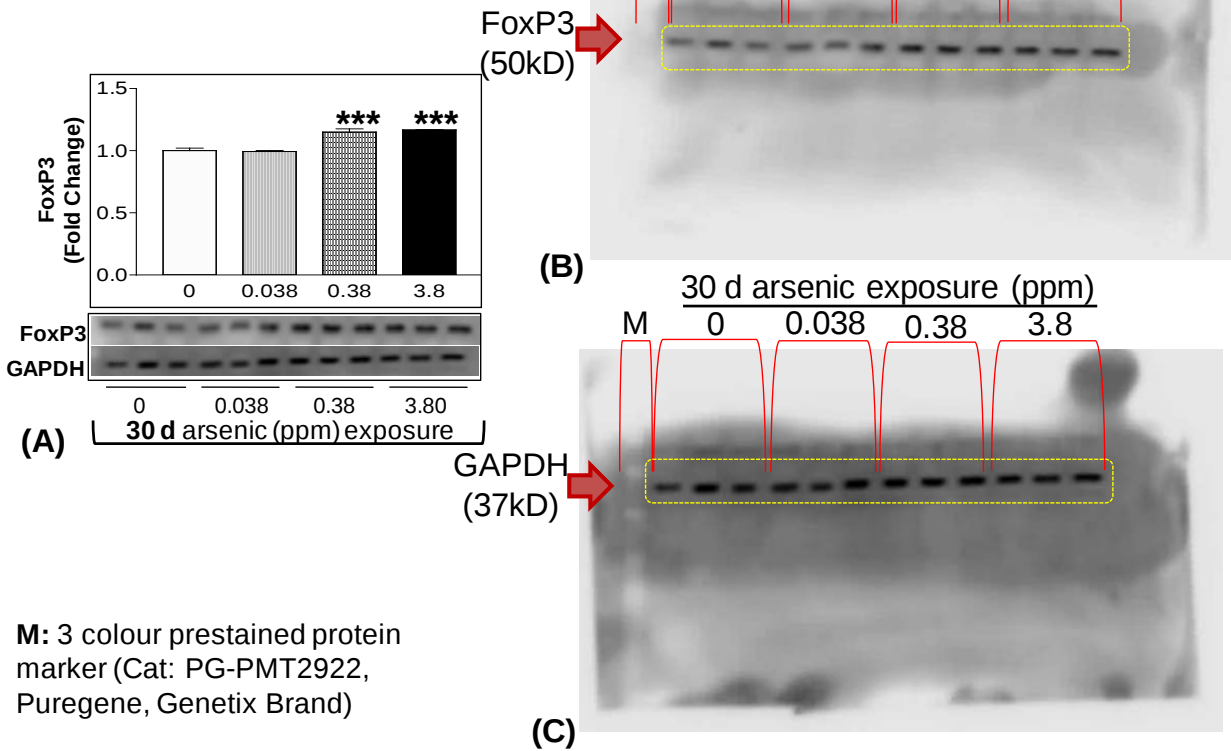


M: 3 colour prestained protein marker (Cat: PG-PMT2922, Puregene, Genetix Brand)



Supplementary Figure S8: Arsenic-induced alteration in RunX3 expression following 30 days arsenic exposure. Thymus dissected from mice treated with arsenic (0.00, 0.038, 0.38 and 3.8 ppm) orally for 30 days were homogenized for protein preparation and examined for the expression of (A) RunX3 and GAPDH. Full-length blot for (B) RunX3 and, (C) corresponding GAPDH.

Original western blot of **FoxP3**
(30 d arsenic exposure)



Supplementary Figure S7: Arsenic-induced alteration in FoxP3 expression following 30 days arsenic exposure. Spleen dissected from mice treated with arsenic (0.00, 0.038, 0.38 and 3.8 ppm) orally for 30 days were homogenized for protein preparation and examined for the expression of **(A)** FoxP3 and GAPDH. Full-length blot for **(B)** FoxP3 and, **(C)** corresponding GAPDH.