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Supplemental Material

Temporal Modulation of Differential Alternative Splicing in HaCaT Human Keratinocyte Cell Line Chronically Exposed to Arsenic for up to 28 Wk

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Figure S1. RT-PCR products from amplification of (A) ELK4 and (B) SHC1 resolved on 6% Polyacrylamide gel. (C) Nested RT-PCR products from amplification of the internal SHC1 fragment resolved on 6% Polyacrylamide gel. All the bands that had sizes corresponding to annotated isoforms on Ensembl database have been labeled in addition to the predicted included and skipped isoforms. "Novel isoform" refers to band sizes that would be amplified from possible permutations and combinations of annotated exons in between the "flanking exons" but are not yet recognized as annotated isoform. Those isoforms labeled in bold and italics font have been confirmed by sequencing.

Figure S2. Immunoblot analyses of selected alternative splicing events in HaCaT cells exposed to arsenic for 7 weeks. (A) Immunoblot analyses of well-characterized isoforms of SHC1. The blot shows three specific bands corresponding to p66, p52 and p46 forms of SHC1, but not any other isoforms. (B) Densitometric analysis of SHC1 isoforms (p66, p52 and p46). (C) Immunoblot analyses of ELK4 main isoform at 45 kDa, no other isoforms were seen. (D) Densitometric analysis of ELK4 main isoform. (E) Table depicting the expression levels of SHC1 isoforms (p62, p52, p46) detected by immunoblot in panel B and that of ELK4 canonical isoform detected by immunoblot in panel C. For each protein isoform, the mean expression of control samples was considered to be 100% and the expression of As-Treated samples were expressed as % Mean Control. (F) Immunoblot analyses of XRRA1 in HaCaT and HepG1 cell lysates.

Figure S3. Alternative splicing-proteomic relationship at 7-week time point. (A) Schematic representation of filtering strategy to shortlist predicted differential splicing events for proteomic validation. (B) Representation of which of the splicing related enriched GO terms at 7-weeks involved each of the shortlisted genes. Grey squares represent that the gene was involved in that specific pathway, while white squares represent it was not.

Additional File- Excel Document