Figure S1:

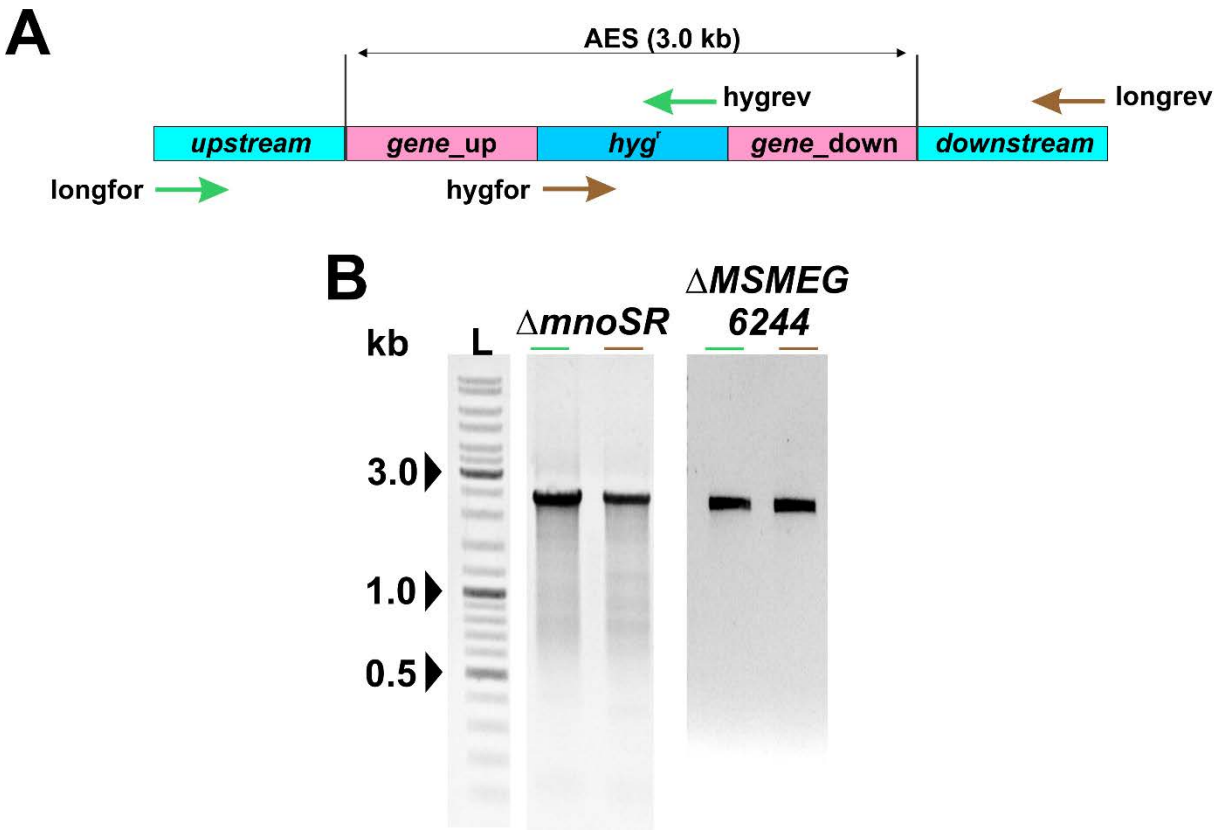

Figure S1. Confirmation of *mnoSR* and *MSMEG_6244* knockout by PCR. Genetic knockout of *mnoSR* and *MSMEG_6244* was confirmed by PCR. Panel A shows the PCR based stratergy for the knockout confirmation. The primers used in each set are mentioned near the arrows (green or brown). Two sets of PCR reactions, brown bars andgreen bars, were perfromed. PCR reaction with brown bar indicates amplification of the fragment consisting of upstream of the allelic exchange substrate (AES) and *hyg*^{r} cassette. Similarly, PCR reaction green bar shows the amplification of the fragment consisting of downstream of the AES and *hyg*^{r} cassette. Amplification of ~2.5 kb is a confirmation of the knockout. PCR amplicons were further gel purified and confirmed by DNA sequencing. 'L' represents the DNA ladder with three bands marked.

Figure S2:

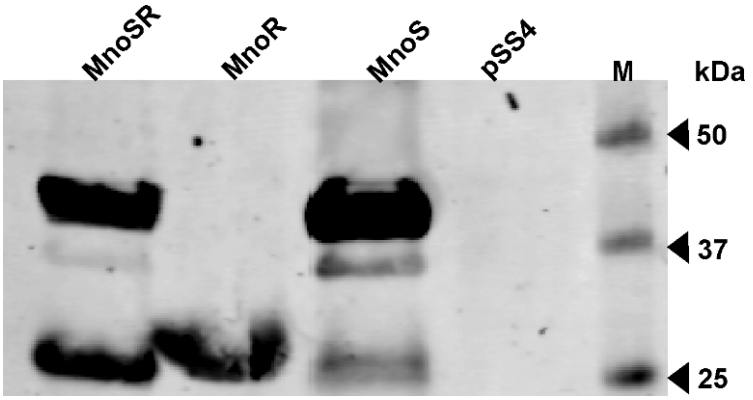

Figure S2. Expression of MnoS and MnoR proteins from recombinant plasmid in *M. smegmatis*. The panel represents the Western blot showing the production of MnoS and MnoR proteins upon induction with acetamide. Western blotting was performed using anti-His antibodies to confirm the expression of MnoS and MnoR from their respective plasmids; lane MnoSR represents the co-expression of both MnoS and MnoR. pSS4 empty vector was used as control. 'M' represents protein molecular weight marker with few bands marked.

Figure S3:

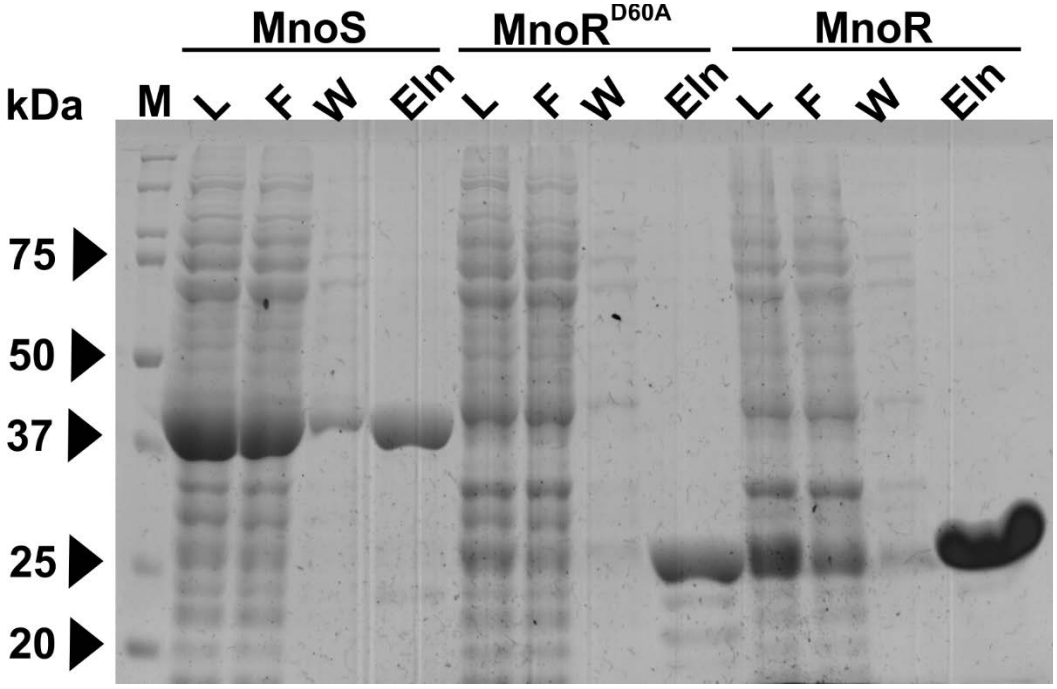

Figure S3. Purification of proteins and assessment of their purity on SDS-PAGE. Coomassie-stained SDS PAGE shows the purification of MnoS (41.7 kDa), MnoR (23.2 kDa), and $MnoR^{D60A}$ (23.1 kDa), which were purified on a Ni-NTA affinity chromatography. The loaded fractions for each protein (marked on the top of the lanes) correspond to lysate (L), flow through (F), wash (W), and elution (Eln). 'M' represents protein molecular weight marker with few bands marked.

Figure. S4

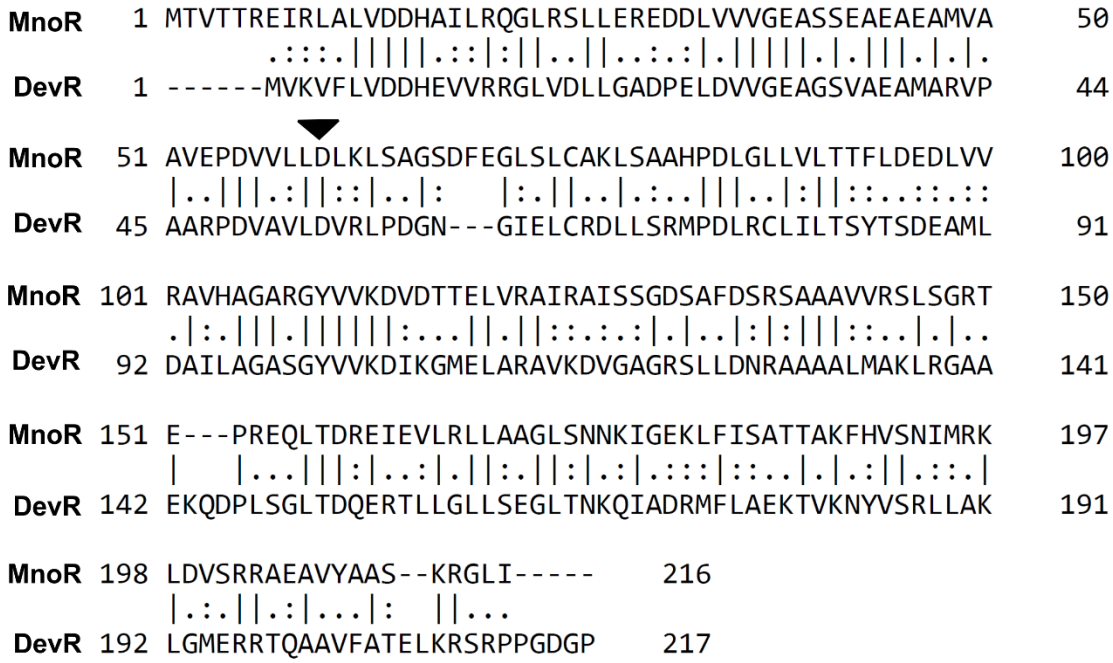

Figure S4. Pairwise sequence alignment of MnoR and DevR. Pairwise sequence alignment of the MnoR and DevR proteins sequences (obtained from Mycobrowser) was carried our using EMBOSS-Needle pairwise alignment tool. The two proteins show 41% identity. The inverted triangle shows the conserved Asp60 in MnoR, which is spatially similar to the Asp54 in DevR.