Supplemental Information

Table S1. Lmx1b-ChIP-seq intervals in E12.5 mouse limb.

Tabulated format of the genomic intervals bound by Lmx1b (LBI) that were identified in mouse limbs (E12.5) in both ChIP-seq replicates. Corresponding chromosome (chr), genomic location in mouse (mm10 reference genome) (LBI-Start and LBI-end), interval length, peak summit, peak value, average value (Avg Val) and bin count are listed (1).

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Table S2. Correlation between Lmx1b-bound Intervals and Chromatin Regulatory Marks.

Comparative analysis of Lmx1b-bound (LBI) intervals to p300, H3K27Ac, H3K4me2, RNA Pol II, Med12, and H3K27me3 ChIP-seq data. Conservation of each Lmx1b-bound interval is also indicated. The data is sorted by the number of chromatin regulatory marks (# of Marks) and by whether both active and repressor marks were present (Both marks). The shaded columns were not used to determine the number of regulatory marks. A potential regulatory modules (PCRM) is an Active-PCRM if has at least two chromatin regulatory marks associated.

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Table S3. Target genes of Lmx1b-bound potential regulatory regions

Comparison of Lmx1b-bound potential regulatory modules (PCRMs) and genes differentially expressed in the presence of Lmx1b. A total of 292 PCRMs are associated to 254 genes (Assoc gene). Note that there are multiple PCRMs associated to different genes and several genes are associated to multiple PCRMs. LBI corresponds to Lmx1b-bound interval number from table S1 for reference. The PCRMs are categorized base on their chromatin regulatory marks (Reg Marks).. If both active and repressor chromatin regulatory marks are present the PCRM is classified as Both-PCRM (See S2 for reference). The background color in this column indicates whether the associated genes are upregulated (red) downregulated (green) or whether the PCRM has both upregulated and downregulated (yellow) gene associations. The distance from the gene to the PCRM (Distance) is included with the fold change (Fold) and p-values from the published gene array data (Feenstra et al., 2012).

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Table S4. Lmx1b-PCRM-associated genes present within functional categories

PCRM associated genes classified according to functional categories and annotated functions as outlined in Figure 2C & D. P-value, number and names of molecules in each assigned category are specified if available**.**

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Table S5. Lmx1b bound Potential Cis Regulatory Modules (PCRMs) that correspond to functionally validated elements from the VISTA enhancer browser.

List of 91 Lmx1b bound PCRMs and corresponding element ID of the VISTA elements - (Visel et al., 2007), followed by the candidate target gene ID and enhancer activity of the tested element. Note that 71 Lmx1b bound PCRMs are functionally active according to the VISTA elements available form the VISTA enhancer browser.

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Table S6. Primers used for ChIP-qPCR Validation

Lmx1b bound Interval (LBI) number and primers sequence for the specified potential target validation by ChIP-qPCR. Primers are in a 5' to 3' orientation.

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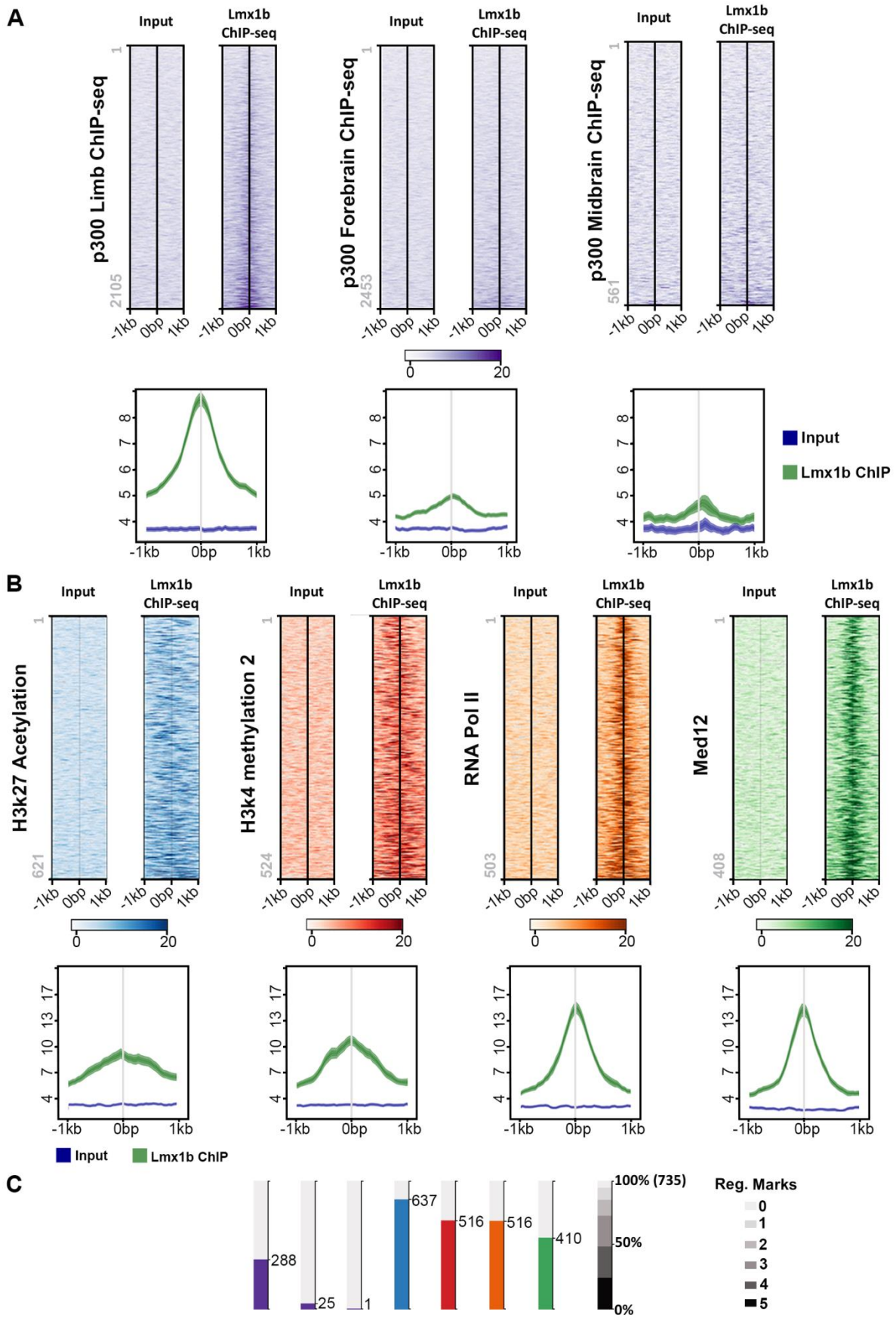

Figure S1. Lmx1b bound intervals display a distribution that corresponded to a limb specific pattern and are enriched in genomic regions associated to active regulation.

A) Heatmap (top) and summarized average plots (bottom) showing the distribution of tagged sequences retrieved from the Lmx1b ChIP-seq and input DNA from limb tissue around p300 ChIP-seq intervals in limb, forebrain and midbrain (Visel et al., 2009). More Lmx1b tagged sequences overlap with p300 intervals in the limb than in the forebrain or midbrain with a 3-fold enrichment for the Lmx1b ChIP-seq retrieved tagged sequences over input DNA. B) Distribution of Lmx1b ChIP-seq and input retrieved tagged sequences around regions associated to active regulation determined by ChIP-seq (H3k27Ac, H3Kme2, RNA Pol II and Med12) (Visel et al., 2009, Berlivet et al., 2013, Cotney et al., 2013, DeMare et al., 2013) that overlapped Lmx1b bound intervals (LBI). Lmx1b ChIP-seq tagged sequences are enriched (~3-fold) within genomic regions associated to chromatin regulatory marks (H3K27Ac, H3K4me2) in comparison to input DNA and it is greater (4-fold) around regulatory regions undergoing active transcription (RNA Pol II, Med12). C) Bar graph depicting the number of LBIs that overlap with the different marks associated to cis-regulatory activity, where the number the percentage of LBIs overlapping chromatin regulatory marks. Note that the colors for each of the marks matched those used above for the heatmaps.

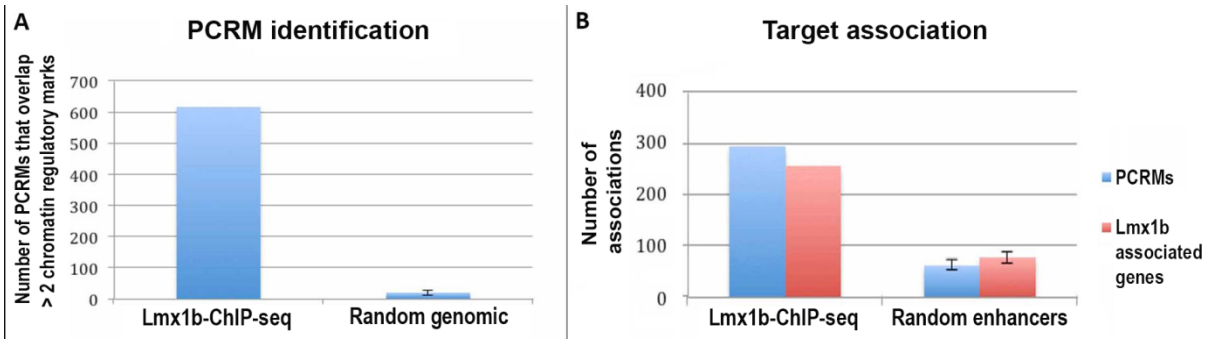

Figure S2. Enrichment of marks associated to potential cis-regulatory modules and Lmx1b regulated genes in Lmx1b bound intervals potential cis regulatory modules.

A) Overlap with at least 2 chromatin regulatory marks yields a 30 times higher number of potential cis-regulatory modules (PCRM) identified in the Lmx1b ChIP-seq dataset (617) in comparison to randomly selected genomic regions (n=5 groups, each with 735 random genomic intervals, One sample t-test p<1e-4, mean19.6 ±6.6). B) Lmx1b bound PCRMs are enriched within Lmx1b regulated genes. The number of PCRM associated to Lmx1b regulated genes is ~5 times higher within Lmx1b identified PCRMs (292) compared to randomly selected enhancer regions based on H3K27Ac (Cotney et al., 2013) (n=5 groups, each with 292 random genomic intervals, One sample t-test p<1e-4, mean 60.6 ±7.1) and ~3 times higher for the number of Lmx1b regulated genes associated to a PCRM (n=5 groups, each with 292 random genomic intervals, One sample t-test p<1e-4, mean 75.8 ±10.8).

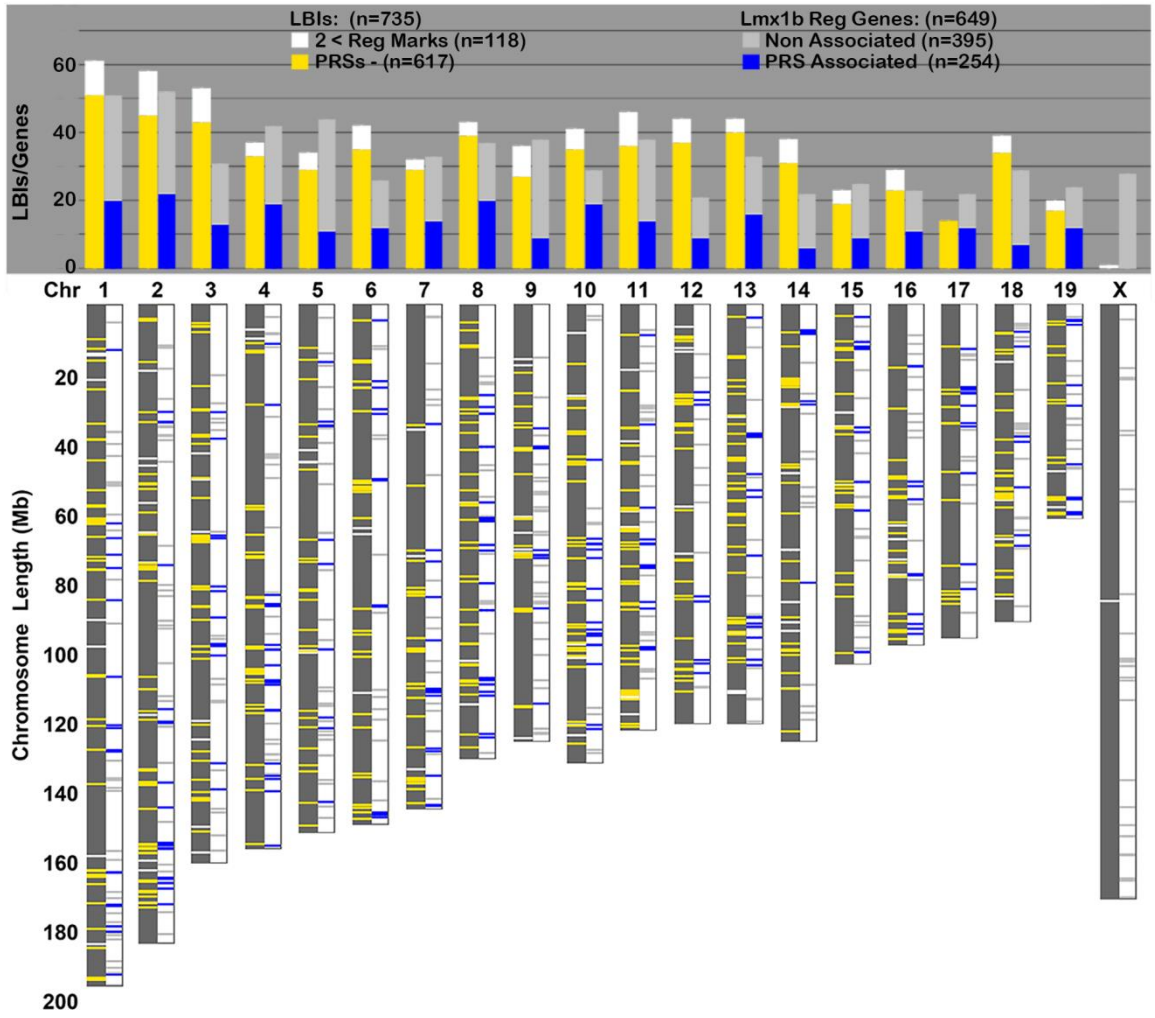

Figure S3. Distribution of Lmx1b-bound PCRMs and associated Lmx1b-regulated genes

The genomic distribution of Lmx1b bound intervals (LBIs) is represented on mouse chromosomes. Yellow marks along the chromosome indicate potential *cis*-regulatory modules (PCRMs), while light grey marks indicate LBIs that do not meet our criterion of a PCRM (≥ 2 chromatin regulatory marks). Location of Lmx1b-regulated genes at E12.5

(Feenstra et al., 2012) are indicated beside each chromosome, blue indicates association with a PCRM and dark grey indicates non-associated genes. A summary of the PCRM and gene distribution is shown above the chromosomes. No Lmx1b-bound PCRMs are identified within the X chromosome.

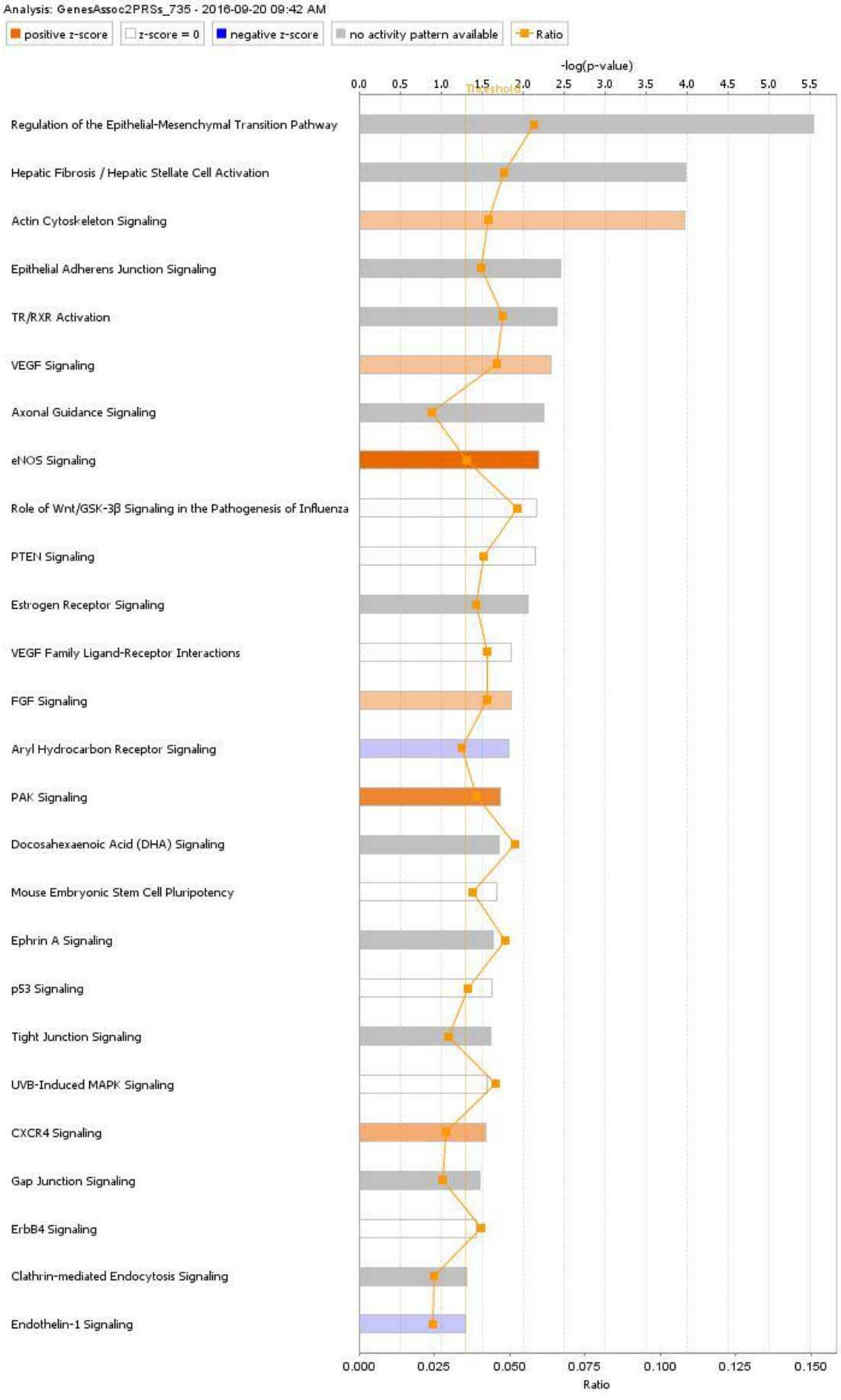

Figure S4. Prediction of canonical pathways affected

Bar-chart representation of pathways affected according to PCRM-associated genes. Orange bars correspond to an overall upregulation of indicated pathways whereas blue is designated for downregulated ones.

References

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