Finding the minimal set of gene functions needed to sustain life is of both fundamental and practical importance. Minimal gene lists have been proposed by using comparative genomics-based core proteome definitions. A definition of a core proteome that is supported by empirical data, is understood at the systems-level, and provides a basis for computing essential cell functions is lacking. Here, we use a systems biology-based genome-scale model of metabolism and expression to define a functional core proteome consisting of 356 gene products, accounting for 44% of the *Escherichia coli* proteome by mass based on proteomics data. This systems biology core proteome includes 212 genes not found in previous comparative genomics-based core proteome definitions, accounts for 65% of known essential genes in *E. coli*, and has 78% gene function overlap with minimal genomes (*Buchnera aphidicola* and *Mycoplasma genitalium*). Based on transcriptomics data across environmental and genetic backgrounds, the systems biology core proteome is significantly enriched in nondifferentially expressed genes and depleted in differentially expressed genes. Compared with the noncore, core gene expression levels are also similar across genetic backgrounds (two times higher Spearman rank correlation) and exhibit significantly more complex transcriptional and posttranscriptional regulatory features (40% more transcription start sites per gene, 22% longer 5'UTR). Thus, genome-scale systems biology approaches rigorously identify a functional core proteome needed to support growth. This framework, validated by using high-throughput datasets, facilitates a mechanistic understanding of systems-level core proteome function through in silico models; it de facto defines a paleome.