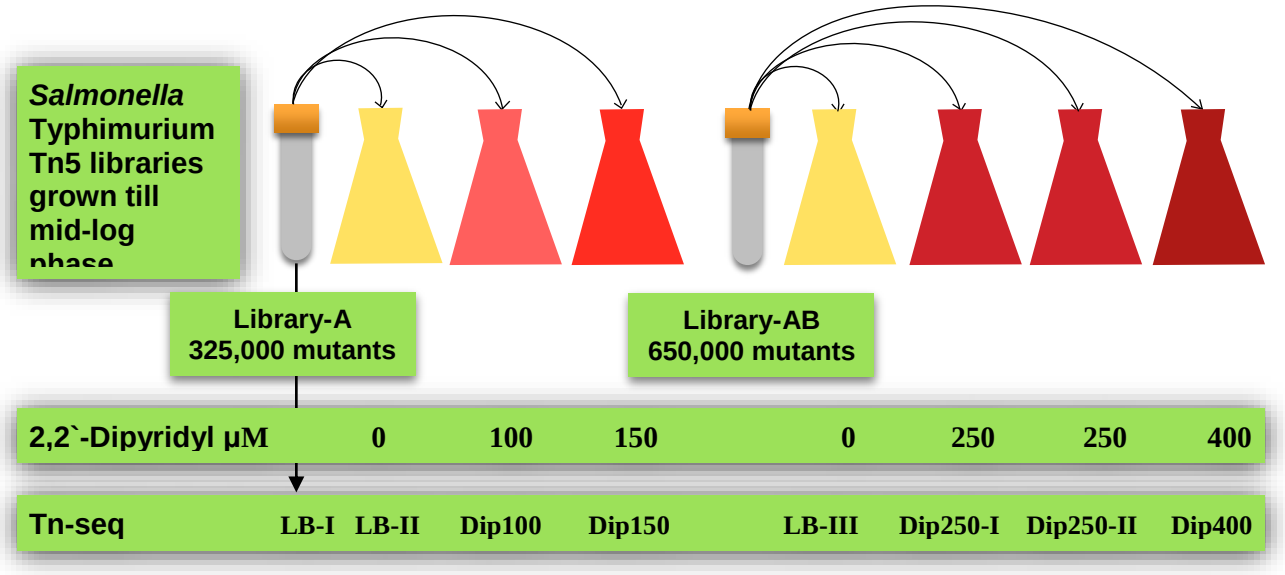


Figure S1. Schematic representation of the study design. Library-A was inoculated to LB medium (LB-II) or LB medium containing either 100 µM iron chelator Dip (Dip100) or 150 µM Dip (Dip150). Library-A was also directly subjected to Tn-seq analysis without growth (LB-I). Library-AB was inoculated to LB medium (LB-III) or LB medium containing either 250 µM Dip (Dip250) or 400 µM Dip (Dip400). The cultures were grown till mid-log phase and then subjected to Tn-seq analysis.

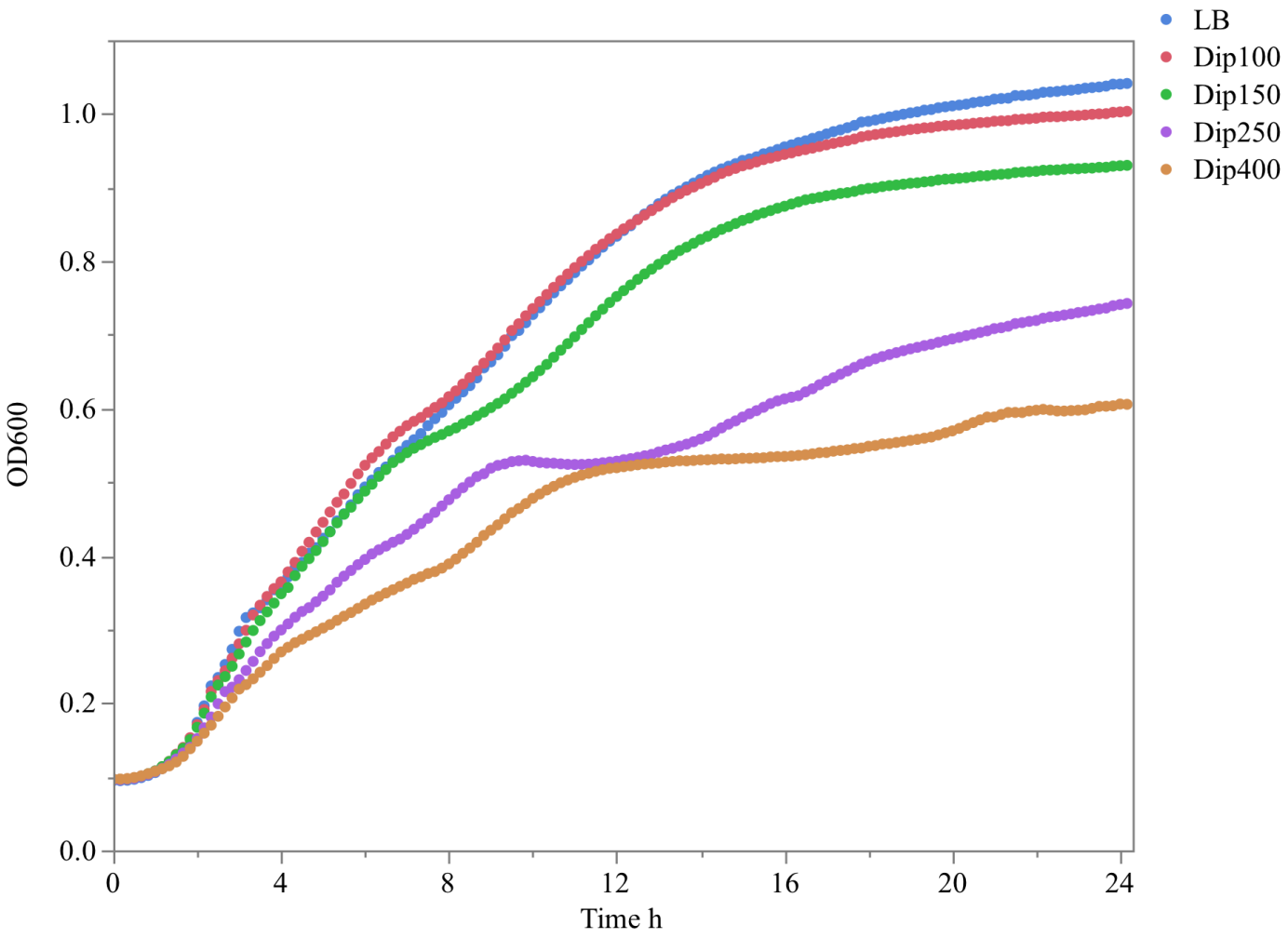


Figure S2. Effect of 2,2`-Dipyridyl (Dip) on *S.* Typhimurium growth. An overnight culture of the wild type *S.* Typhimurium 14028 was diluted 1:200 in LB medium supplemented with 0 µM Dip (LB), 100 µM Dip (Dip150), 150 µM Dip (Dip150), 250 µM Dip (Dip250) or 400 µM Dip (Dip400). The cultures were transferred to the wells of a 96-well plate and incubated at 37˚C in a plate reader with reading OD_{600} every 10 minutes.

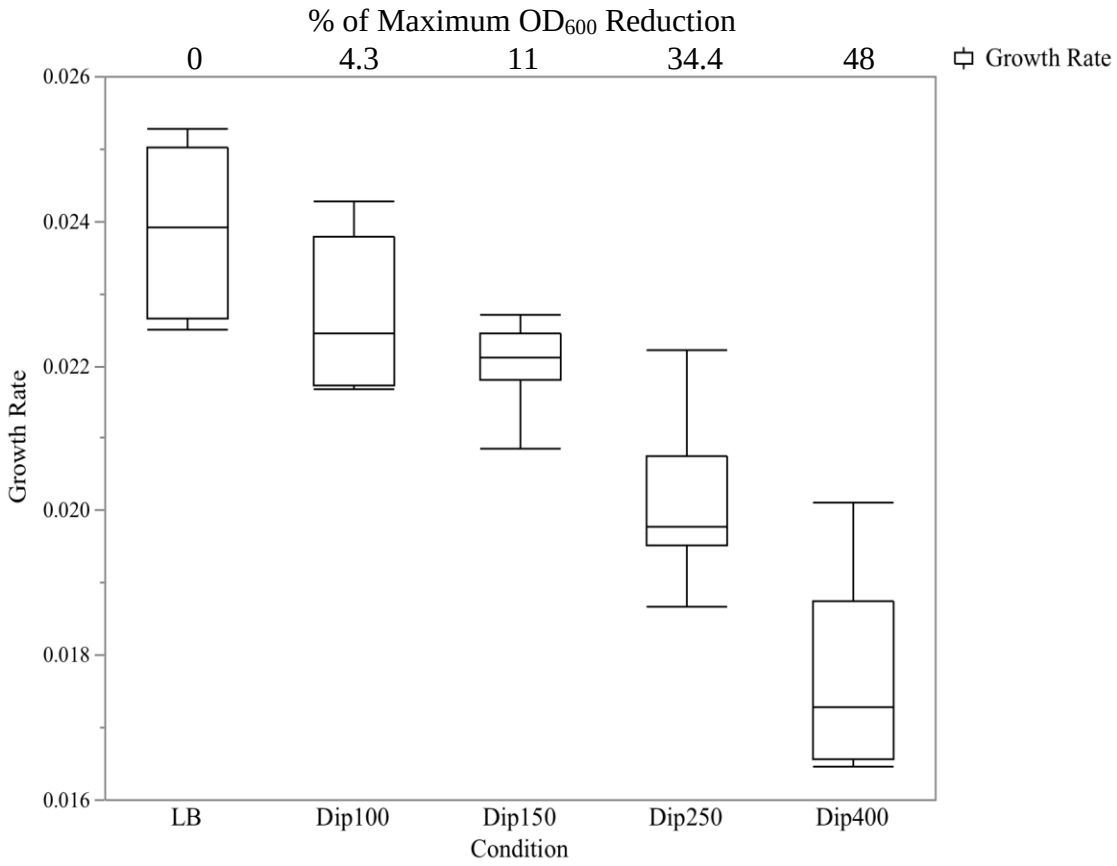
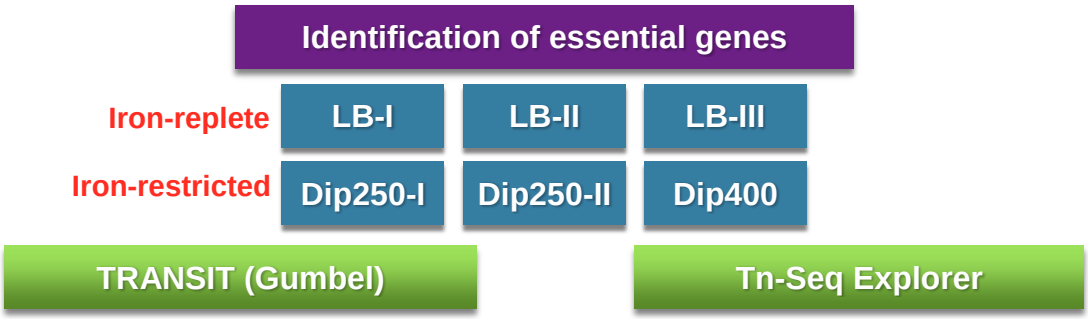


Figure S3. Effect of 2,2`-Dipyridyl (Dip) on *S.* Typhimurium growth rate and cell density. An overnight culture of the wild type *S.* Typhimurium 14028 was diluted 1:200 in LB medium supplemented with 0 μM Dip (LB), 100 μM Dip (Dip150), 150 μM Dip (Dip150), 250 μM Dip (Dip250) or 400 μM Dip (Dip400). The cultures were transferred to the wells of a 96-well plate and incubated at 37°C in a plate reader with reading OD_{600} every 10 minutes. The maximum OD_{600} reduction is shown as a percentage in reference to LB.



A		LB-III Essentiality Index < 3
B	LB-III Essential p < 0.05	
C	The gene has to be essential in at least 5 of the 6 essentiality analyses	

Essential genes in iron-replete condition

Gene	LB-I	LB-II	LB-III		LB-I	LB-II	LB-III	Call
murA	**E**	**E**	**E**		**0**	**0**	**0**	**E**
dapD	**NE**	**E**	**E**		**1**	**1**	**0**	**E**
acpP	**NE**	**NE**	**E**		**2**	**1**	**1**	**NE**
gmhA	**NE**	**NE**	**E**		**5**	**1**	**0**	**NE**
ssb	**E**	**NE**	**E**		**3**	**0**	**1**	**NE**
cydC	**E**	**E**	**E**		**3**	**0**	**1**	**E**

Essential genes in iron-restricted condition

Gene	Dip250-I	Dip250-II	Dip400		Dip250-I	Dip250-II	Dip400	Call
murA	**NE**	**E**	**E**		**4**	**2**	**4**	**NE**
dapD	**E**	**NE**	**E**		**0**	**2**	**2**	**E**
acpP	**NE**	**E**	**NE**		**2**	**1**	**2**	**NE**
gmhA	**NE**	**E**	**NE**		**1**	**0**	**1**	**NE**
ssb	**NE**	**NE**	**E**		**4**	**2**	**3**	**NE**
cydC	**E**	**E**	**E**		**2**	**4**	**5**	**NE**

Figure S4. Algorithm used for essential gene calling. Two methods were used for essential gene analysis, TRANSIT (Gumbel) and Tn-Seq Explorer. Tn-seq data from LB-I, LB-II, and LB-III were analyzed separately by both methods for identification of essential genes in iron-replete condition. A gene was considered essential if 5 out of 6 analyses (3 groups x 2 methods) were essential (E; TRANSIT) or essentiality index (EI; Tn-Seq Explore) < 3. The essential genes in iron-restricted condition were identified using the same algorithm but with Tn-seq data from Dip250-I, Dip250-II, and Dip400.

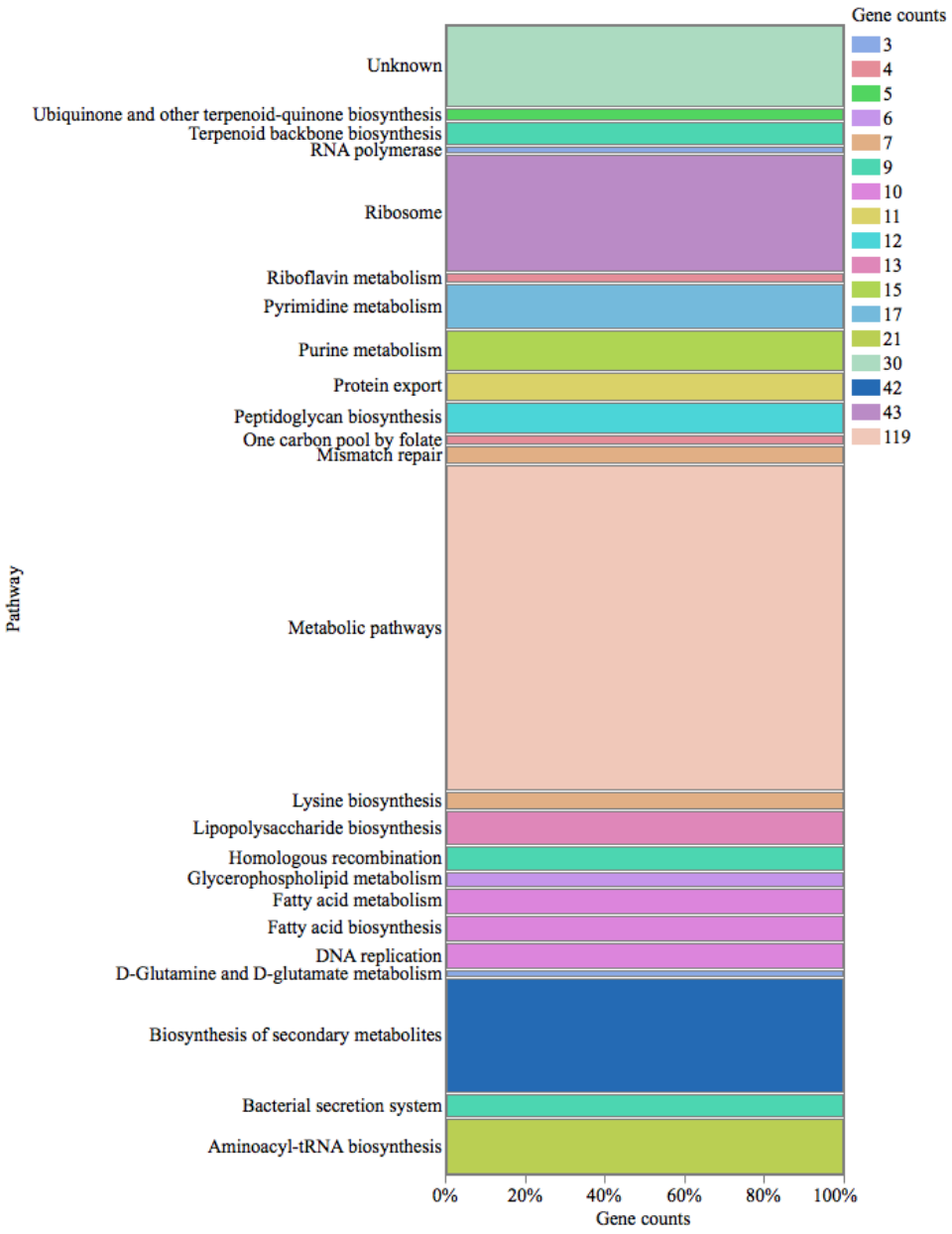


Figure S5. KEGG pathway analysis of the 336 essential genes of *S.* Typhimurium 14028 in LB medium identified in this study. All 336 essential genes were categorized into 23 essential KEGG pathways.