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Molecular Complexity Orchestrates Modulation of Phagosome Biogenesis and Escape to the Cytosol of macrophages by *Francisella tularensis*

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Abstract

Upon entry of *Francisella tularensis* to macrophages, the *Francisella*-containing phagosome (FCP) is trafficked into an acidified late endosome-like phagosome with limited fusion to the lysosomes followed by rapid escape into the cytosol where the organism replicates. Although the *Francisella* Pathogenicity Island (FPI), which encodes a type VI-like secretion apparatus, is required for modulation of phagosome biogenesis and escape into the cytosol, the mechanisms involved are not known. To decipher the molecular bases of modulation of biogenesis of the FCP and bacterial escape into the macrophage cytosol, we have screened a comprehensive mutant library of *F. tularensis* subsp *novicida* for their defect in proliferation within human macrophages, followed by characterization of modulation of phagosome biogenesis and bacterial escape into the cytosol. Our data show that at least 202 genes are required for intracellular proliferation within macrophages. Among the 125 most defective mutants in intracellular proliferation, we show that the FCP of at least 91 mutants co-localize persistently with the late endosomal/lysosomal marker LAMP-1 and fail to escape into the cytosol, as determined by fluorescence-based phagosome integrity assays and transmission electron microscopy. At least 34 genes are required for proliferation within the cytosol but do not play a detectable role in modulation of phagosome biogenesis and bacterial escape into the cytosol. Our data indicate a tremendous adaptation and metabolic reprogramming by *F. tularensis* to adjust to the micro-environmental and nutritional cues within the FCP, and these adjustments play essential roles in modulation of phagosome biogenesis and escape into the cytosol of macrophages as well as proliferation in the cytosol. The plethora of the networks of genes that orchestrate *F. tularensis*-mediated modulation of phagosome biogenesis, phagosomal escape, and bacterial proliferation within the cytosol is novel, complex, and involves an unusually large portion of the genome of an intracellular pathogen.

Keywords

endosome; lysosome; phagosome; cytosol; intracellular; tularemia; Listeria

Introduction

Francisella tularensis is an intracellular bacterium that causes tularemia, a fatal zoonotic disease that infects small mammals and humans (Ellis et al., 2002; Pechous et al., 2009; Santic et al., 2010). There are four subspecies of *F. tularensis*, which are subsp *tularensis*, *holarctica*, *mediasiatica* and *novicida* (Keim et al., 2007; Nigrovic and Wingerter, 2008). Subspecies *tularensis* is most virulent, while subsp *holarctica* and *mediasiatica* cause a mild

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form of tularemia (Santic et al., 2006; Pechous et al., 2009). All subspecies share about 97% genome identities (Champion et al., 2009; Larsson et al., 2009). Recent studies have shown that the high virulence of subsp *tularensi*s and *holarctica* may be due to loss of gene functions or an increase in the copy number of genes such as duplication of the *Francisella* Pathogenicity Island (FPI) (Champion et al., 2009; Larsson et al., 2009). Because of low infectivity, ease of dissemination, and high morbidity and mortality, *F. tularensis* is classified by the CDC as a category A select bioterrorism agent (Dennis et al., 2001).

Clinical manifestation of tularemia depends on the route of infection and it includes glandular, ulceroglandular, oculoglandular, oropharyngeal, pneumonic and typhoidal tularemia (Ellis et al., 2002). Tularemia often presents with nonspecific flu-like symptoms such as headache, fever, chills, nausea, diarrhea, and myalgia (Oyston et al., 2004; Nigrovic and Wingerter, 2008).

The ability of *F. tularensis* to cause disease is due to its capacity to replicate within cells (Oyston et al., 2004). Like other intracellular pathogens, *F. tularensis* must overcome the host innate immune response to successfully colonize the intracellular niche. The primary host defense is centered on the antimicrobial properties of the phagosome. Most successful intracellular pathogens either escape the phagosome or divert phagosome maturation to an idiosyncratic niche where they replicate. Bacteria that escape from the phagosome include *Shigella flexneri* and *Listeria monocytogenes* (Goebel and Kuhn, 2000; Ray et al., 2009). Escape of *S. flexneri* and *L. monocytogenes* from the acidified phagosome into the host cell cytosol is mediated by a pore-forming cytolysin and phospholipases (Ray et al., 2009). Similarly, the *Francisella*-containing phagosome (FCP) transiently acquires early and late endosomal markers as well as the vacuolar ATPase, which acidifies the phagosome followed by rapid escape of the bacteria into the cytosol within 30–60 min (Golovliov et al., 2003a; Clemens et al., 2004; Santic et al., 2005a; Santic et al., 2005b; Checroun et al., 2006; Santic et al., 2007; Bonquist et al., 2008; Santic et al., 2008; Qin et al., 2009). Mutants that are unable to escape into the cytosol do not replicate (Santic et al., 2005b; Bonquist et al., 2008; Qin et al., 2009) and are attenuated in animal models (Lauriano et al., 2004; Weiss et al., 2007; Mohapatra et al., 2008). Similar to trafficking within human macrophages, *F. tularensis* transiently occupies a late endosome-like phagosome in *Drosophila melanogaster*-derived S2 cells followed by rapid bacterial escape into the cytosol, where the bacteria proliferate robustly (Santic et al., 2009). This may suggest that common mechanisms are utilized by *F. tularensis* to modulate phagosome biogenesis, escape into the cytosol, and to proliferate within the cytosol of mammalian and arthropod-derived cells. *F. tularensis* subsp *novicida* is very similar to the virulent subspecies in modulation of phagosome biogenesis, phagosomal escape, proliferation within the cytosol of mammalian macrophages, and manifestation of disease in animal models (Santic et al., 2005a; Santic et al., 2005b; Santic et al., 2006; Santic et al., 2007; Santic et al., 2009; Pechous et al., 2009). These characteristics render *F. tularensis* subsp *novicida* a very useful model to dissect the molecular bases of intracellular proliferation of *F. tularensis* under BSL2 containment.

The *Francisella* Pathogenicity Island (FPI) encodes a type VI-like secretion apparatus, which is required for modulation of phagosome biogenesis and escape into the cytosol (Barker et al., 2009). The VgrG and IglI proteins are secreted into the host cell cytosol, and the translocation of IgII is FPI-dependent (Barker et al., 2009). The three FPI-encoded VgrG, IglI, and IglC proteins, the MglA global regulator, four acid phosphatases (AcpA, AcpB, AcpC and Hap) and a lipoprotein (FTT1103) have been shown to play important roles in phagosomal escape of *F. tularensis* into the cytosol of macrophages, but the molecular bases of phagosomal escape are not known (Barker et al., 2009; Golovliov et al., 2003b; Santic et al., 2005a; Santic et al., 2005b; Checroun et al., 2006; Santic et al., 2007; Bonquist et al., 2008; Santic et al., 2008; Qin et al., 2009). Interestingly, the two FPI genes

iglC, *iglD*, and their regulator MglA are also required for intracellular proliferation within arthropod-derived cells (Santic et al., 2009). The phagosome containing the *mglA* and *iglC* mutants matures into a phagolysosome and bacteria fail to escape into the cytosol within macrophages and arthropod-derived cells (Santic et al., 2005b; Santic et al., 2009; Bonquist et al., 2008). In addition, genes involved in oxidative stress, protein turnover, capsule or lipopolysaccharide (LPS) biosynthesis, type IV pilin assembly, iron uptake, outer membrane channels, purine biosynthesis, and regulation through *mglAB*, *sspA* or *pmrA* are required for intracellular growth and virulence of *F. tularensis* (see (Pechous et al., 2009; Santic et al., 2010) for recent reviews).

Genome-wide screens using transposon-based mutagenesis have identified genes involved in various aspects of virulence or dissemination in animal models of tularemia (Qin and Mann, 2006; Maier et al., 2007; Su et al., 2007; Weiss et al., 2007; Kraemer et al., 2009). An *in vivo* negative selection mutant screen has identified genes of *F. tularensis* subsp *novicida* required for virulence in the pulmonary routes of infection in the mice model (Kraemer et al., 2009). On the other hand, genes required for growth of *Francisella in vivo* by the subcutaneous route have been identified (Weiss et al., 2007). *In vivo* negative selection screen in mice using signature-tagged mutagenesis in the LVS strain has identified genes required for growth in the lung during respiratory tularemia (Su et al., 2007). Two other mutant screens have identified *F. tularensis* genes required for replication in macrophages and HepG2 cells, respectively, but the mutant libraries used in these screens are biased and do not cover the entire genome (Qin and Mann, 2006; Maier et al., 2007). Although modulation of phagosome biogenesis and escape into the cytosol are the two crucial steps in the intracellular infection and manifestation of disease, no studies have been reported to identify the genes repertoire involved in these crucial pathogenic processes. Therefore, the molecular bases of phagosome biogenesis and bacterial escape into the cytosol by *F. tularensis* remain unknown, and are the goals of this study.

To decipher the molecular bases of phagosome biogenesis and bacterial escape and proliferation within the cytosol, we utilized a comprehensive transposon insertion mutant library of *F. tularensis* subsp *novicida* (Gallagher et al., 2007). We identified 202 genes that contribute to intracellular growth in human macrophages. Among the mutants defective in replication in human macrophages, 137 of them are required for replication in *D. melanogaster* S2 cells (see accompanying manuscript). In contrast to the wild type strain that co-localize transiently with late endosomal/lysosomal markers prior to rapid escape into the cytosol, 91 of the mutants that are severely defective in intracellular growth in macrophages co-localize persistently with late endosome/lysosome markers and fail to escape into the cytosol. Another thirty four mutants severely defective in intra-macrophages growth but successfully escape into the cytosol fail to proliferate. Our findings are surprising, since phagosomal escape of other bacteria such as *L. monocytogenes* and *S. flexineri* is mediated by few loci (Ray et al., 2009), which indicates a novel molecular complexity governing phagosomal escape of *F. tularensis*.

Results

Replication of *F. tularensis* mutants in U937 macrophages

The ability of *F. tularensis* to cause tularemia is dependent on its proliferation within the macrophage cytosol after rapid phagosomal escape. To identify the bacterial genes involved in phagosomal escape, our experimental design was based on a two-step screen. First, we identified the mutants defective in intracellular proliferation; and second, these mutants were analyzed for phagosome biogenesis and phagosomal escape. Therefore, we performed a primary screen of a comprehensive library of 3,050 sequence-defined insertion mutants of *F. tularensis* subsp *novicida* corresponding to 1448 genes with a minimum of two mutant

alleles for most genes (Gallagher et al., 2007) for their defect in intracellular proliferation in human-derived U937 macrophages. Infections were performed at MOI of 10 for 1 h followed by 1 h of gentamicin treatment. At 24 h post-infection, cells were lysed and serial dilutions were plated on agar plates for colony enumeration. To exclude the mutants with mild defective phenotype from our analyses, a mutant was considered defective in intracellular proliferation if it showed $\geq 10^2$ fold reduction in intracellular growth compared to the wild type strain at 24 h post-infection.

Among the 3,050 mutants tested in the primary screen, we identified 425 mutant alleles with $\geq 10^2$ fold reduction in the number of cfus recovered at 24h post-infection compared to the wild type strain. Since all the FPI genes have been reported to be involved in intracellular proliferation (Barker et al., 2009; Pechous et al., 2009; Santic et al., 2010), we focused our screen on the non-FPI genes. To confirm the phenotype of the primary screen, growth kinetics of the non-FPI mutants was re-examined twice. When the defect was re-examined, the OD of all the 425 mutants was determined after overnight culture in broth, and equivalent OD for all the mutant bacteria was used for infection to ensure equivalent input for all the 425 mutants. Our data confirmed that 271 mutant alleles corresponding to 202 genes showed a consistent $\geq 10^2$ fold reduction in the number of cfus recovered at 24h post-infection compared to the wild type strain (Table 1). Remarkably, defect in at least 125 genes caused $\geq 10^3$ fold reduction in the number of cfus recovered at 24h post-infection compared to the wild type strain, and these were selected for further analyses of phagosome biogenesis and bacterial escape into the cytosol (see below).

There was a lack of consistent phenotype for the two mutant alleles for some of the mutants, which may be due to the site of the insertion that may generate a functional or partially functional protein in some of the mutants. The growth defect for most of the mutants identified was not due to a growth defect *in vitro*, since more than 98% of the mutants exhibited normal growth in vitro, compared to the wild type strain. It is likely that the defect for few of the mutants was due to a defect in attachment and/or entry into macrophages. It is likely that the reduction in intracellular growth for some of the mutants was due to or amplified by a polar effect of the transposon insertion on downstream genes. However, this would implicate the identified disrupted operon in intracellular proliferation. Similar findings were also observed in our screen for mutants defective in S2 cells (see accompanying manuscript).

The 202 mutants defective in intracellular proliferation were not skewed to any particular protein functional group but rather distributed across several different functional groups (Fig. 1). The largest percentage of mutants comprising ~30% of all the mutants identified had mutations in metabolic genes (Fig. 1). Interestingly, about 10% of the mutants had insertions in genes required for the transport of nutrients (Fig. 1), which supports findings about the fastidious nature of *F. tularensis*. The metabolic genes were grouped according to their putative biochemical pathways. Our analysis shows that mutations in genes involved in carbohydrate, amino acid, and nucleotide metabolism are defective in intracellular replication (Table 2).

Approximately, 15% of the mutants had transposon insertion in genes encoding proteins of unknown function and 12% were hypothetical proteins (Fig. 1). Identifying the functions of these genes, which make up about 30% of all the genes identified, will shed more light on the molecular mechanism required for the intracellular infection of macrophages by *F. tularensis*.

Approximately, 25% of the mutants that we identified in our screen have been identified in other screens for various aspects of virulence of *F. tularensis* (Table 3). This indicates the

power of our comprehensive screen that was aimed at identification of genetic loci required for modulation of phagosome biogenesis and escape into the cytosol of human macrophages, and both of these processes are essential for subsequent proliferation within the cytosol. Interestingly, 83% of the identified genes in our screen are conserved in the virulent *F. tularensis* subsp *tularensis*. This indicates that most of the genes that are necessary for intracellular replication in human macrophages are common to the highly virulent subsp of *F. tularensis.* This indicate that adaptation to the intracellular life in mammalian cells occurred before the subspecies diverged (Champion et al., 2009;Larsson et al., 2009).

To confirm that the defect in replication was not limited to the U937 macrophage cell line, six mutants were selected randomly and tested in human monocytes-derived macrophages (hMDMs). Our data showed that all six mutants exhibited similar defective phenotypes in both U937 macrophages and hMDMs (Table 3). This indicates that the growth defect observed for the mutants is not restricted to the U937 human macrophage cell line but is also exhibited in primary human-derived macrophages.

Modulation of phagosome biogenesis by the mutants defective in intracellular replication

Since modulation of phagosome biogenesis and bacterial escape into the cytosol is essential for intra-macrophage proliferation, we decided to focus our additional studies on the most defective mutants in intra-macrophage proliferation to analyze the biogenesis of the FCP and bacterial escape into the cytosol. We determined whether the defect in intra-macrophage growth of the mutants with $\geq 10^3$ fold reduction in intracellular growth was due to a defect in modulation in phagosome biogenesis. Since the FCP transiently co-localizes with the LAMP-1 late endosomal/lysosomal marker for ~ 30 min after which this co-localization is rapidly lost by 1–4h, we determined whether the FCP of the 125 most defective mutants co-localized transiently or persistently with LAMP-1 at 6h post-infection. We selected this time point because most FCPs harboring the wild type strain do not co-localize with LAMP-1 at this time points. This would exclude mutants that are mildly defective in modulation of phagosome biogenesis and would allow us to focus on the most defective mutants. We noticed that growth of the kan^r mutant bacteria in presence of the kanamycin antibiotic before infection caused a significant increase in co-localization with LAMP-1 compared to growth in the absence of the antibiotic. Since the wild type strain is sensitive to kanamycin, we used the kan^r *Sua5* mutant as our negative control, since this mutant had no detectable defect in intracellular proliferation, co-localization with late endosomal/lysosomal markers, or phagosomal escape. As our positive control, we used the *iglC* mutant. To exclude the mildly defective mutants, a mutant was considered persistently co-localized with LAMP-1 if it exhibited more than 50% co-localization with LAMP-1 at 6h post infection, which is significantly different from the positive and negative controls (Student *t*-test, $p<0.001$). In contrast to the wild type strain that co-localized transiently with LAMP-1, 91 of the 125 defective mutants co-localized persistently with LAMP-1, similar to the *iglC* mutant positive control (Table 5). Most of these 91 mutants exhibited 65–75% co-localization with LAMP-1 (Fig. 2 and 3), while the negative control showed only 24% co-localization (Fig. 3 and Table 5) (Santic et al., 2005a;Santic et al., 2005b;Santic et al., 2007;Santic et al., 2008). The mutants that showed aberrant trafficking were distributed across several functional categories (Fig. 3 and Table 5).

To confirm that our observations of alterations in trafficking was not limited to the U937 macrophage cell line, the six mutants that were tested for intracellular proliferation in human monocytes-derived macrophages (hMDMs) were also examined for co-localization of the FCP with LAMP-1. Our data showed that the FCP of all the six mutants co-localized with LAMP-1 at a similar level in both U937 macrophages and hMDMs (Table 3). This indicates that the observed phenotypes for the mutants is not restricted to the U937 human macrophage cell line but is also exhibited in primary human-derived macrophages. These

results indicate that most of the mutants defective in intracellular replication exhibit aberrant trafficking within human macrophages. Our findings indicate the complexity of the regulatory mechanisms that control modulation of biogenesis of the FCP.

Escape of the defective mutants into the host cell cytosol

To decipher the molecular bases of phagosomal escape, we examined the 125 mutants with $\geq 10^3$ fold reduction in CFU at 24h post-infection in human-derived macrophages to examine their ability to escape into the macrophage cytosol. We utilized the fluorescence-based phagosome integrity assay to differentially label bacteria that are cytosolic/or within a compromised phagosome and those enclosed within an intact phagosome. This is achieved by loading the host cell cytosol with anti-*F. tularensis* antibody after preferential permeabilization of the plasma membrane, as previously described (Checroun et al., 2006; Santic et al., 2008). Among the 125 tested mutants, 31 bound the polyclonal anti-*F. tularensis* antibody, indicating their escape into the cytosol or localization in a compromised phagosome, similar to the positive control (Table 5 and Fig. 4). The other 94 mutants were not labeled with the anti-*F. tularensis* antibody loaded into the macrophage cytosol, indicating they were localized within intact phagosomes that were not permeable to the polyclonal antibody (Table 5). However, it is possible that the epitope recognized by the antibody might be altered, masked, or absent in few of the mutants that successfully escaped into the cytosol and were accessible to the antibody (see below). Results of the phagosomal escape assays were further confirmed by TEM for 6 randomly selected mutants, 4 of which escaped similar to the wild type strain while 2 (*pyrB and wzb*) were defective in phagosomal escape, similar to the *iglC* mutant (Fig 5). It is most likely that not all the genes are directly involved in phagosomal escape but are required for general bacterial fitness and adaptation to the acidified micro-environment within the FCP. Our data indicate that tremendous metabolic reprogramming is exhibited by *F. tularensis* during its short residence within the FCP. Overall, the results indicate a remarkable molecular complexity governs phagosomal escape of *F. tularensis*.

Surprisingly, there was discrepancy in the result of the LAMP-1 co-localization and the phagosome integrity assay for 5 mutants. One of the mutants (FTN_0788) that co-localized persistently with LAMP-1 had disrupted phagosome, as determined by fluorescence analyses. In contrast, the FCPs of the other 4 mutants (FTN_0384, FTN_0696, FTN_1619, and FTN_FTN_1441) were intact but were LAMP-1 negative (Table 5). Therefore, TEM was performed on the 5 mutants to decipher the reason for this discrepancy at the ultra-structural level. Our results showed that the four mutants that did not co-localize with LAMP-1 escaped into the cytosol, when examined by TEM (Fig. 5B). The other mutant that co-localized with LAMP-1 was found within intact phagosome. This indicated that our findings by TEM were more consistent with our finding related to LAMP-1 co-localization for these 5 mutants. Thus, at least 91 loci are required for modulation of phagosome biogenesis and escape of *F. tularensis* into the cytosol and at least 34 loci are required for proliferation within the cytosol but play no detectable role in phagosome biogenesis.

Comparison of the phenotype of the intra-macrophage growth-defective mutants to the phenotype within arthropod-derived cells

A concurrent study from our lab identified mutants that exhibited growth defect in *D. melanogaster*-derived S2 cells (see accompanying manuscript). To assess whether similar molecular mechanisms are utilized by *F. tularensis* to infect arthropod-derived cells and human macrophages, we compared the mutants identified to be defective in human macrophages to the mutants identified to be defective in *D. melanogaster* S2 cells. Among the 202 mutants defective in replication in human macrophages 135 of them were also required for replication in *D. melanogaster* S2 cells (see accompanying manuscript). This

large number of loci indicates that common loci are utilized by *F. tularensis* to proliferate within both human macrophages and insect-derived cells. However, there are distinct molecular mechanisms required for replication in the two evolutionarily-distant host cells as more than 30% of the genes required for intracellular replication are specific to human macrophages.

Discussion

Most intracellular pathogens either escape the phagosome or divert phagosome maturation to an idiosyncratic niche where they replicate. Like other cytosolic bacteria, *F. tularensis* escapes from the phagosome into the cytosol where it replicates (Golovliov et al., 2003b; Santic et al., 2005a; Santic et al., 2005b; Checroun et al., 2006; Santic et al., 2007; Bonquist et al., 2008; Santic et al., 2008; Qin et al., 2009). Previous studies have shown that phagosomal escape is indispensable for the pathogenesis of tularemia (Santic et al., 2005b; Bonquist et al., 2008; Qin et al., 2009). Various mutagenesis screens have been performed to identify genes that are essential for the replication of *F. tularensis* in mouse models, in macrophages and hepatic cells (Qin and Mann, 2006; Maier et al., 2007; Su et al., 2007; Weiss et al., 2007; Kraemer et al., 2009), but none identified mutants that fail to modulate phagosome biogenesis or fail to escape into the cytosol, which are the two major steps in the ability of *F. tularensis* to proliferate intracellularly and cause disease. Previous studies have shown that the three FPI gene products IglC, VgrG, and IglI, the MglA regulator, four acid phosphatases, and a lipoprotein are required for escape of *F. tularensis* subsp *novicida* into the host cell cytosol but the mechanism is not known (Barker et al., 2009; Santic et al., 2005b; Bonquist et al., 2008; Mohapatra et al., 2008; Qin et al., 2009). The FPI encodes a type VI-like secretion apparatus, and at least VgrG and IglI are secreted into the host cell cytosol, and the translocation of IglI is FPI-dependent (Barker et al., 2009). Since the FPI-encoded type VI-like secretion apparatus is essential for phagosome biogenesis and bacterial escape into the cytosol, it is most likely that the bacterial effectors directly involved in modulation of phagosome biogenesis and lysis of the phagosomal membranes are translocated by the type VI-like secretion apparatus encoded by the FPI.

Since mutants defective in phagosomal escape do not replicate in macrophages, our primary screen focused on identification of mutants that do not replicate in human macrophages followed by studies on phagosome biogenesis and a fluorescence-based phagosome integrity assay to identify the mutants that fail to escape into the cytosol. Transposon insertion in 202 genes result in ≥100 fold reduction in the number of cfus at 24h post-infection, compared to the wild type strain. Our data do not exclude mutants defective in attachment/entry into macrophages as well as polar effects of the insertion on downstream genes. Remarkably, the genes required for intracellular replication are involved in various physiological functions particularly in metabolic activities and nutrient transport. Interestingly, a large number of the identified genes encode proteins of unknown function or hypothetical proteins. Analysis of the function of these genes would shed light on the mechanisms of intracellular proliferation of *F. tularensis*. Importantly, only 25% of the genes we identified have been identified in other screens to be required for various aspects of virulence of *F. tularensis* (Qin and Mann, 2006; Maier et al., 2007; Su et al., 2007; Weiss et al., 2007; Kraemer et al., 2009). This indicates the comprehensiveness and the power of our screen that is aimed at identification of bacterial loci required for phagosome biogenesis and escape into the cytosol, which are the two major steps essential for subsequent proliferation and manifestation of disease.

The FCP co-localizes transiently with late endosomal/lysosomal markers before bacterial escape into the cytosol (Pechous et al., 2009; Santic et al., 2010). Although the FCP harboring the *mglA*, *iglC* and *FTT1103* mutants co-localize persistently with LAMPs and mature into a phagolysosome (Santic et al., 2005b; Bonquist et al., 2008; Qin et al., 2009), it

is not known whether these mutants are defective in evasion of lysosomal fusion or escape into the cytosol. Our results show that the FCP of 91/125 mutants severely defective in intra-macrophage growth co-localize persistently with the late endosomal marker LAMP-1, and these mutants fail to escape into the cytosol of human macrophages. However, it is unlikely that all these genes are directly involved in bacterial escape into the cytosol but are required for general bacterial fitness and adaptation to the acidic micro-environment within the FCP, which is essential for modulation of phagosome biogenesis and bacterial escape into the cytosol (Santic et al., 2005b; Bonquist et al., 2008; Qin et al., 2009). Indeed, depending on the availability of pyrimidine nucleotide, auxotrophic mutants (carA, carB and pyrB) of *F. tularensis* subsp *holarctica*-derived LVS strain can either escape from the phagosome and grow in the cytosol or remain trapped in the phagosome (Schulert et al., 2009). Similar to LVS, it is most likely that similar mechanisms of nutritional and micro-environmental stresses within the FCP affect phagosomal escape of *F. tularensis* subsp *novicida*. The large numbers of various functional groups of mutants defective in escape into the cytosol suggest that the phagosome is not a hospitable environment for the growth of *F. tularensis* and that tremendous adaptation to the phagosomal micro-environment along with extensive metabolic reprogramming is required for successful phagosomal escape.

Interestingly, 4 of the mutants that have been shown to be defective in phagosomal escape, based on the fluorescence-based phagosome integrity assays, do not co-localize persistently with LAMP-1. In addition, one of the mutants that escape into the cytosol co-localize with LAMP-1. However, ultra-structural studies have clearly shown that this mutant is localized to an intact FCP while the other 4 mutants that are LMAP-1 negative are cytosolic, which is consistent for all the 5 mutants for correlation of LAMP-1 co-localization with the FCP. It is likely that in the phagosome integrity assay, failure of the antibody to bind cytosolic bacteria is due to alteration, masking, or absence of the epitope recognized by the antibody.

Many other bacteria, including *L. monocytogenes, S. flexneri, B. pseudomallei* and *Rickettsia spp*. escape into the host cell cytosol where they replicate (Ray et al., 2009). The mechanisms of phagosomal escape are best studied in *L. monocytogenes* which requires the activity of listeriolysin as well as two other phospholipases to escape into the cytosol (Ray et al., 2009). Similarly, *F. tularensis* resides in phagosomes that transiently acquire endosomal markers and become acidified within 15–30 min prior to rapid escape into the cytosol by 30–60 min (Pechous et al., 2009; Santic et al., 2010). The MglA regulator, four acid phosphatases and a lipoprotein are required for escape of *F. tularensis* subsp *novicida* in addition to the 3 FPI-encoded proteins IglC, VgrG, and IglI, but the mechanism is not known (Barker et al., 2009; Pechous et al., 2009; Santic et al., 2010). However, none of these proteins possess any properties of a cytoylsin. Although *F. tularensis* subsp *novicida* exhibits hemolytic activity (Lai et al., 2003), no hemolysin homologues have been identified in any of the *F. tularensis* subspecies whose genomes have been sequenced (Larsson et al., 2005; Petrosino et al., 2006; Beckstrom-Sternberg et al., 2007; Chaudhuri et al., 2007; Rohmer et al., 2007). Importantly, 3 out of the 4 acid phosphatase identified previously (Mohapatra et al., 2008) have been found to be required for phagosomal escape in the present study. The diversity of genes that affect trafficking and phagosomal escape shows that *F. tularensis* is unlike other cytosolic pathogens that utilize cytolysins, pore-forming toxins, or hydrolytic enzymes to escape into the host cell cytosol. It rather requires a complex array of network of genes involved in various physiological functions to orchestrate its fitness to the acidic micro-environment in response to the environmental and nutritional cues within the FCP to enable efficient phagosomal escape. Importantly the type VI-like secretion apparatus encoded by the FPI seems to be essential for translocation of some secreted proteins (Barker et al., 2009) and is required for phagosomal escape (Barker et al., 2009; Pechous et al., 2009; Santic et al., 2010), suggesting that the effector involved in escape is likely to be translocated through this system.

Importantly, 34 of the 125 mutants with severe defect in intracellular replication escape into the cytosol. The growth defect of some of these mutants in the cytosol may be due to increased sensitivity to host antimicrobial cytosolic factors, inability to acquire nutrients, failure to modulated host cytosolic processes, or failure to adapt to the cytosolic micro-environment.

Interestingly, among 135 common *F. tularensis* loci identified to be required for intracellular proliferation within both human macrophages and *D. melanogaster*-derived S2 cells, 59 are required for phagosomal escape in human-derived macrophages (see accompanying manuscript). These data suggest that some common molecular mechanisms are utilized by *F. tularensis* to escape from the phagosome in mammalian and arthropod-derived cells. This may not be surprising, considering our recent findings that phagosome biogenesis and bacterial escape into the cytosol are very similar in both evolutionarily-distant host cells (Santic et al., 2009). However, our data clearly show that distinct mechanisms are also employed by *F. tularensis* to escape into the cytosol of the two evolutionarily distant host cells.

Our results indicate that *F. tularensis* requires a plethora of networks of genes to orchestrate its fitness during transient residence within the acidified FCP for efficient phagosomal escape and subsequent replication within the host cell cytosol, and that at least 34 loci are indispensable for proliferation within the cytosol. These networks of genes are potential targets for therapy and vaccination against tularemia, since phagosomal escape is the major step in the ability of this pathogen to proliferate intracellularly and cause disease.

Experimental procedures

Bacterial strains, tissue culture and Media

F. tularensis subsp. *novicida* strain U112 and its isogenic mutants *mglA* and *iglC* have been described previously (Lauriano et al., 2004). The construction of the *F. tularensis* subsp. *novicida* mutants library which was obtained from Biodefence and Emerging Infections Resource Repository (http://www.beiresources.org) has been described previously (Gallagher et al., 2007). All *F. tularensis* subsp *novicida* strains were grown on tryptic soy agar (TSA) plates for 2 days or in tryptic soy broth (TSB) supplemented with 0.1% cysteine and 10 mg/ml of kanamycin overnight. U937 macrophages were maintained at 37°C and 5% CO2 in RPMI-1640 tissue culture medium (Gibco BRL) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco BRL). The U937 macrophages were differentiated for 48 h using 50μg/ml of phorbol 12-myristate 13-acetate (PMA). The hMDMs were obtained and maintained as we described previously

Intracellular growth in U937 Macrophages

Infection of U937 macrophages with *F. tularensis* subsp. *novicida* was performed as described previously (Santic et al., 2005a). Briefly, U937 cells were seeded in 96-well plate at a concentration of 1×10^6 cells/ml of RPMI and differentiated for 48 h with PMA. Differentiated cells were infected with *F. tularensis* subsp. *novicida* and its isogenic mutants at MOI of 10 (1×10^7 bacteria/ml of RPMI) for 1 h followed by 1 h of gentamicin ($50\mu g ml^{-1}$) treatment. MOI was determined for each infecting strain by measuring absorbance of bacterial suspension at 550 nm. Infections were done in duplicate for each strain. To synchronize the infection, infected cells were centrifuged at 150 x g for 5 min before incubation at 37°C in 5% CO_2. Cells were then incubated with fresh RPMI for 22 hrs. The supernatant was removed and infected cells were lysed with 200μl of sterile water. The supernatant was recombined with the lysate and serial dilutions were plated on agar plates for colony enumeration. The experiment was done twice to confirm the results.

Co-localization of F. tularensis with LAMP-1 in U937 Cells

Infections were performed as described above in 24-well plate with glass coverslips. At 6 h post-infection, cells were fixed with 3.7% formaldehyde for 30 min. After washing 3 times with 1X PBS, cells were permeabilized with 0.1% triton X 100 for 15 min on ice followed by 3 times wash with 1X PBS. Cells were subsequently treated with goat polyclonal anti *F. tularensis* subsp. *novicida* antibody (Genscript) at 1:4000 dilution and mouse monoclonal anti LAMP-1 antibody (Hybridoma library, University of Iowa) at 1:500 dilution. After 1 hr incubation the cells were washed 3 times and treated with Alexa fluor 488-conjugated anti goat antibody and Alexa fluor 555-conjugated anti mouse antibody (Molecular probes) at 1:4000 dilution. Co-localization of bacteria with LAMP-1 was analyzed with FV1000 Olympus confocal microscope as described previously (Santic et al., 2005a; Santic et al., 2005b; Santic et al., 2008). At least 100 infected cells from more than 10 different fields were analyzed. Experiments were done in duplicate for each strain.

Phagosome integrity assays

We utilized fluorescence-based phagosome integrity assay to determine phagosomal escape into the macrophage cytosol, as described previously (Checroun et al., 2006; Santic et al., 2008). Infections were performed as described for the LAMP-1 experiment above. At 6 h post-infection, infected macrophages washed quickly with 1X PBS and permeabilized with 80μg/ml digitonin in 1X PBS containing. 1:1000 dilution of goat polyclonal anti *F. tularensis* subsp *novicida* antibody (Genscript) for 10 min. Subsequently, 1:1000 dilution of goat polyclonal anti *F. tularensis* subsp. *novicida* antibody was loaded into the macrophage cytosol for another 40 min., as we described previously (Santic et al., 2005a; Santic et al., 2005b; Santic et al., 2008). The infected cells were was 3 times with 1X PBS and fixed for 30 min with 3.7% formaldehyde. The cells were washed 3 times with 1X PBS and permeabilized with 0.1% triton X 100. After washing 3 times with 1X PBS, the cells were treated with mouse monoclonal anti *F. tularensis* subsp. *novicida* antibody (a gift from John Gunn, Ohio State University) at 1:200 dilution followed by 3 times washing with 1X PBX and treatment with Alexa fluor 555-conjugated anti goat antibody and Alexa fluor 488-conjugated anti mouse antibody at 1:4000 dilution. Localization of bacteria was analyzed with FV1000 Olympus confocal microscope at our imaging suite. At least 100 infected cells from more than 10 different fields were analyzed.

Transmission Electron Microscopy

The use of Transmission Electron Microscope (TEM) to analyze escape of *F. tularensis* subsp. *novicida* into the cytosol of U937 macrophages has been described previously (Santic et al., 2005a). Briefly, monolayers of U937 macrophages were infected with *F. tularensis* subsp. *novicida* in 12-well plates at an MOI of 10 for 1 h followed by gentamicin treatment. At 6 h, post-infection, the infected U937 macrophages were processed and sections were stained with Uranyl Acetate and lead citrate and examined with a Hitachi H-7000/STEM electron microscope at 80 kV as described previously (Santic et al., 2005a).

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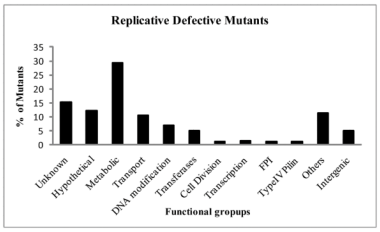

Fig. 1. Functional groups of mutants defective in intra-macrophage growth

U937 macrophages were infected with each of the mutants of *F. tularensis* at MOI of 10 for 1 h followed by 1 h of gentamicin treatment. Growth of the mutants was compared to the wild type strain at 24 h post-infection, and the relative reduction in the number of cfu relative to the wild type strain was determined. After the primary screen, 425 mutants were tested twice in triplicate.

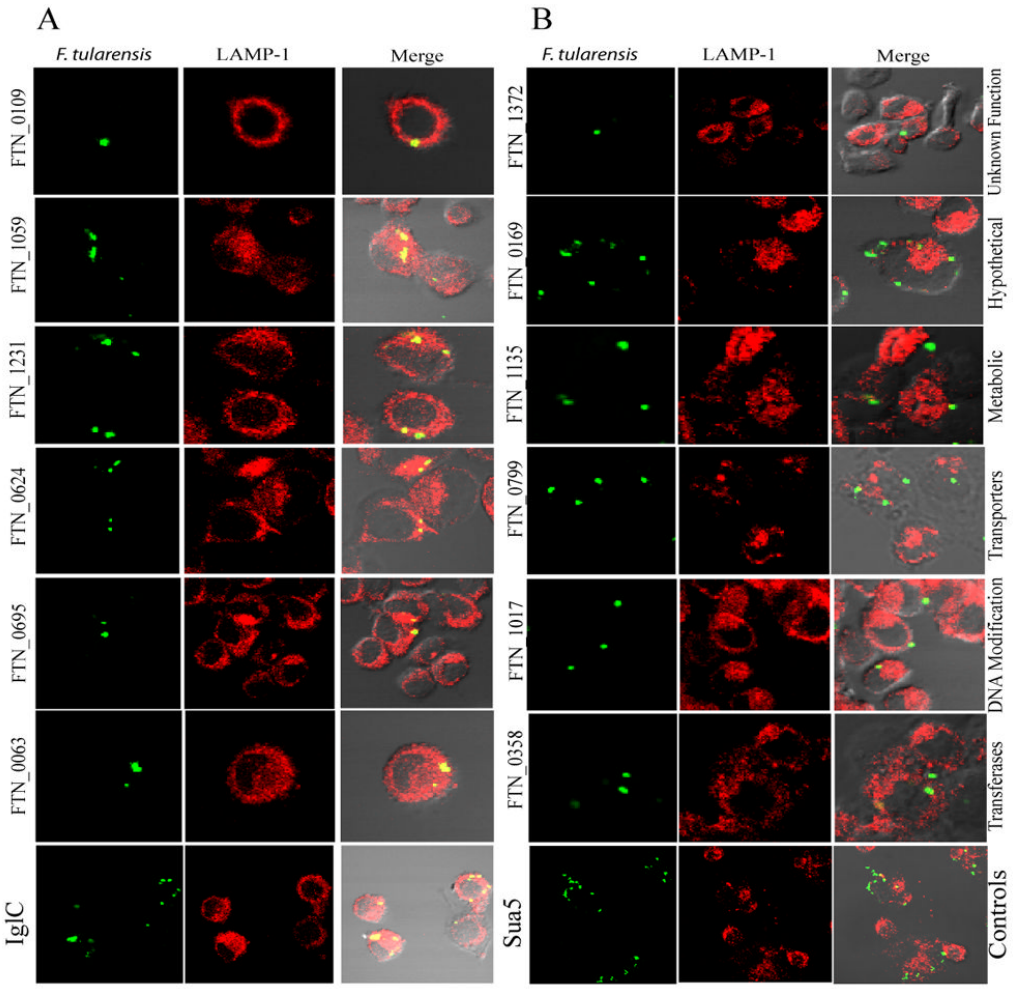

Fig. 2. Co-localization of the FCP of selected mutants with LAMP-1 in U937 macrophages

The cells were infected with each of the 125 mutants defective in intra-macrophage proliferation to determine co-localization of the phagosome with LAMP-1 at 6h post-infection. Bacteria were labeled with goat polyclonal antibody (green) and LAMP-1 was labeled with mouse monoclonal antibody (red). Representative confocal images of phagosomes harboring representative mutants from each functional group showing co-localization with Lamp-1 similar to the IglC mutant (A) or no co-localization, similar to the Sua5 WT-like phenotype (B). Data analyses were based on 100 infected cells analyzed from two different coverslips and the data were reproducible in two independent experiments.

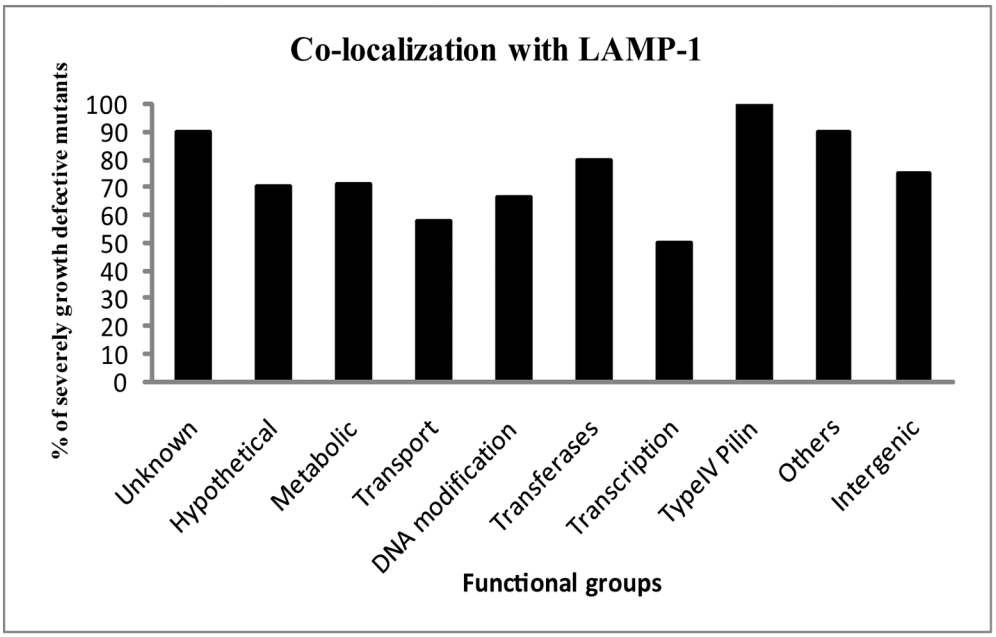

Fig. 3. Functional categories of mutants that co-localize with LAMP-1

U937 macrophages were infected with each of the 125 mutants severely defective in intra-macrophage proliferation to determine co-localization of the phagosome with LAMP-1 at 6h post-infection. Mutants were grouped according to the function of the mutated genes. Percentages of mutants in each functional group that co-localized persistently with LAMP-1 are shown.

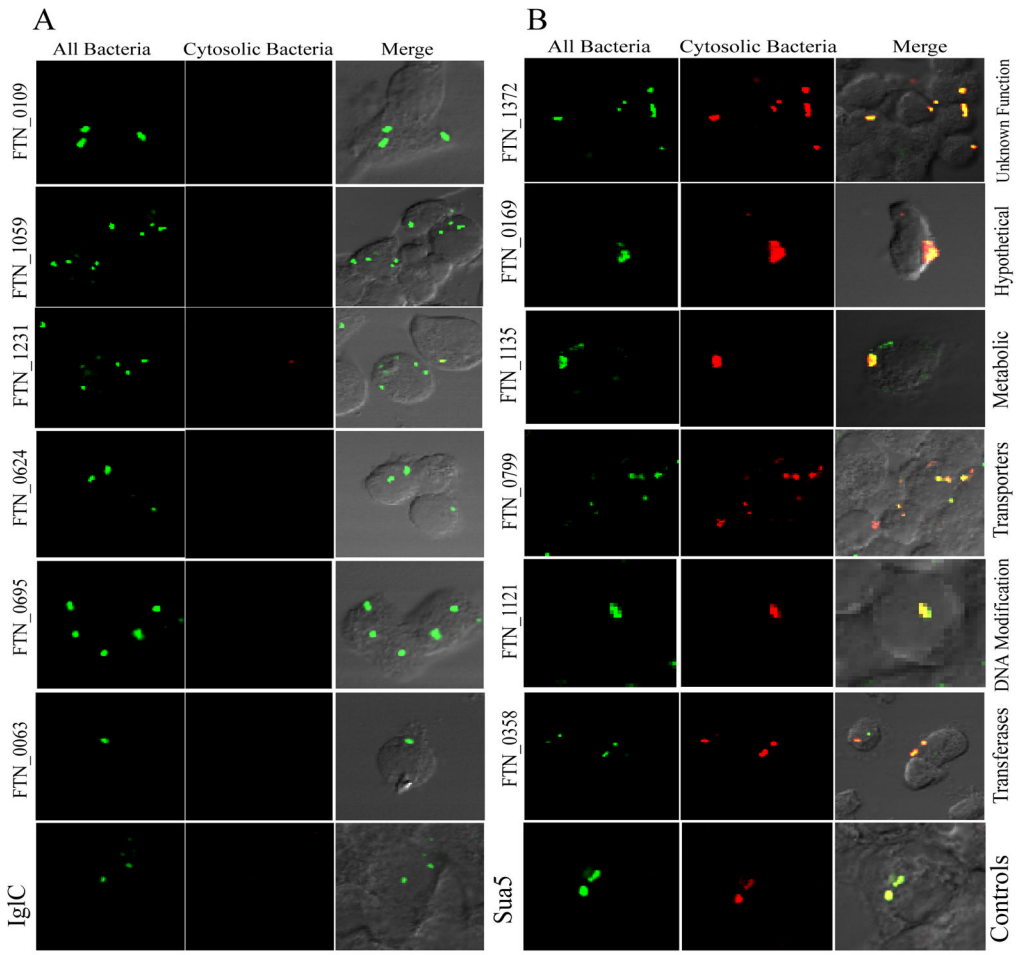

Fig. 4. Representative mutants analyzed for their escape into the cytosol of U937 macrophages

U937 macrophages were infected with each of the 125 mutants of *F. tularensis* defective in intra-macrophage proliferation. Cytosolic bacteria were labeled with goat polyclonal antibody (red) loaded into the macrophage cytosol followed by permeabilization of all cellular membranes and labeling of all intracellular bacteria using mouse monoclonal antibody (green). Representative confocal images of *F. tularensis* mutant defective in phagosomal escape similar to the *iglC* mutant (A) and mutants that exhibit wild type-like Sua5 phenotype (B). Data analyses were based on 100 infected cells analyzed from two different coverslips and the data were reproducible in two independent experiments.

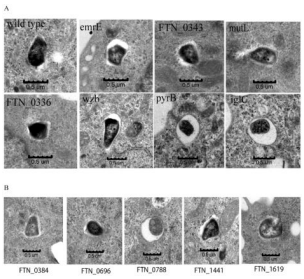

Fig. 5. Ultra-structural characterization of phagosomal escape

U937 macrophages were infected with *F. tularensis* for 1 h followed by 1 h of gentamicin treatment. After a total of 6 h, the infected cells were analyzed by TEM to determine whether bacteria were within intact or disrupted phagosomes. A) Six mutants were selected randomly to determine their phagosomal escape; B) Five mutants with discrepancy in the results of the fluorescence-based phagosome integrity assays and LAMP-1 co-localization were examined by TEM for their phagosomal escape.

Table 1

List of Growth-defective mutants of *F. tularensis* in U937 macrophages and S2 cells grouped according to function.

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
Controls					
Wild type				0	0
Intracellular growth locus C		IglC		5	5
Proteins of unknown Function					
tnfn1_pw060323p08q148	FTN_0027		conserved protein of unknown function	4	6*
tnfn1_pw060510p03q161	FTN_0027		conserved protein of unknown function	2	2*
tnfn1_pw060323p03q103	FTN_0041		protein of unknown function	5	2#
tnfn1_pw060420p01q149	FTN_0041		protein of unknown function	2	3#
tnfn1_pw060420p04q143	FTN_0149		conserved protein of unknown function	5	5
tnfn1_pw060323p02q193	FTN_0275		conserved protein of unknown function	2	2#
tnfn1_pw060419p03q124	FTN_0275		conserved protein of unknown function	2	2#
tnfn1_pw060510p02q121	FTN_0275		conserved protein of unknown function	3	2#
tnfn1_pw060420p04q134	FTN_0297		conserved protein of unknown function	7	7
tnfn1_pw060328p05q119	FTN_0444		membrane protein of unknown function	6	6#
tnfn1_pw060420p03q175	FTN_0444		membrane protein of unknown function	5	5#
tnfn1_pw060323p07q141	FTN_0788		conserved protein of unknown function	5	5
tnfn1_pw060420p04q176	FTN_0855		protein of unknown function	5	2
tnfn1_pw060323p03q147	FTN_0930		protein of unknown function	6	3#
tnfn1_pw060323p05q150	FTN_0930		protein of unknown function	6	3#
tnfn1_pw060510p01q108	FTN_0977		conserved protein of unknown function	7	7
tnfn1_pw060510p01q128	FTN_1170		conserved protein of unknown function	2	3*
tnfn1_pw060418p02q157	FTN_1170		conserved protein of unknown function	2	4*
tnfn1_pw060420p04q196	FTN_1256		membrane protein of unknown function	4	5

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List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060323p01q113	FTN_1343		conserved protein of unknown function	4	4#
tnfn1_pw060418p02q105	FTN_1343		conserved protein of unknown function	4	4#
tnfn1_pw060328p02q110	FTN_1457		protein of unknown function	5	5#
tnfn1_pw060420p02q183	FTN_1457		protein of unknown function	6	6#
tnfn1_pw060328p01q172	FTN_1542		conserved protein of unknown function	2	2#
tnfn1_pw060328p02q177	FTN_1713		protein of unknown function	2	2
tnfn1_pw060328p06q155	FTN_1764		protein of unknown function	6	7#
Hypothetical Proteins					
tnfn1_pw060323p03q142	FTN_0030		hypothetical membrane protein	4	3#
tnfn1_pw060420p02q155	FTN_0030		hypothetical membrane protein	3	3#
tnfn1_pw060328p06q180	FTN_0038		hypothetical protein	4	4#
tnfn1_pw060419p02q127	FTN_0038		hypothetical protein	2	2#
tnfn1_pw060420p02q173	FTN_0169		conserved hypothetical membrane protein	6	6*
tnfn1_pw060510p01q193	FTN_0169		conserved hypothetical membrane protein	5	5*
tnfn1_pw060328p05q136	FTN_0384		conserved hypothetical protein	4	7
tnfn1_pw060328p05q130	FTN_0534		conserved hypothetical membrane protein	5	7
tnfn1_pw060418p01q143	FTN_0556		hypothetical protein	7	7
tnfn1_pw060419p03q188	FTN_0696		hypothetical membrane protein	2	2#
tnfn1_pw060323p01q155	FTN_0696		hypothetical membrane protein	5	3#
tnfn1_pw060328p06q185	FTN_0709		hypothetical protein	2	7
tnfn1_pw060323p07q129	FTN_0759		conserved hypothetical protein	4	2
tnfn1_pw060419p02q102	FTN_0792		hypothetical protein	5	6#
tnfn1_pw060420p01q167	FTN_0792		hypothetical protein	2	2#
tnfn1_pw060323p02q140	FTN_0895		hypothetical protein	2	2*

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List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060323p07q105	FTN_0895		hypothetical protein	4	2*
tnfn1_pw060328p08q188	FTN_1098		conserved hypothetical membrane protein	2	2#
tnfn1_pw060510p03q192	FTN_1098		conserved hypothetical membrane protein	2	2#
tnfn1_pw060510p04q192	FTN_1098		conserved hypothetical membrane protein	7	6#
tnfn1_pw060419p04q117	FTN_1156		hypothetical protein	2	4
tnfn1_pw060328p02q129	FTN_1612		hypothetical protein	2	2
Metabolic Proteins					
tnfn1_pw060323p08q120	FTN_0020	carB	carbamoyl-phosphate synthase large chain	5	7
tnfn1_pw060419p01q106	FTN_0111	ribH	riboflavin synthase beta-chain	4	5
tnfn1_pw060328p06q174	FTN_0125	ackA	propionate kinase 2/acetate kinase A	4	4#
tnfn1_pw060418p03q133	FTN_0199	cyoE	heme O synthase	2	4
tnfn1_pw060323p04q102	FTN_0211	pcp	pyrrolidone carboxylylate peptidase	1	1#
tnfn1_pw060418p03q177	FTN_0211	pcp	pyrrolidone carboxylylate peptidase	3	4#
tnfn1_pw060418p01q187	FTN_0319		amino acid-polyamine-organocation family protein	6	7
tnfn1_pw060323p06q113	FTN_0420		SAICAR synthetase/phosphoribosylamine-glycine ligase	7	5
tnfn1_pw060323p05q182	FTN_0504		lysine decarboxylase	4	4
tnfn1_pw060510p01q124	FTN_0507	gcvP1	glycine cleavage system P protein, subunit 1	5	7
tnfn1_pw060510p02q154	FTN_0511		shikimate 5-dehydrogenase	2	2#
tnfn1_pw060510p02q157	FTN_0511		shikimate 5-dehydrogenase	6	6#
tnfn1_pw060510p04q157	FTN_0511		shikimate 5-dehydrogenase	3	2#
tnfn1_pw060323p06q194	FTN_0527	thrC	threonine synthase	7	7#
tnfn1_pw060510p01q172	FTN_0527	thrC	threonine synthase	5	5#
tnfn1_pw060510p03q172	FTN_0527	thrC	threonine synthase	2	2#
tnfn1_pw060323p03q127	FTN_0567		tRNA synthetase class II (D, K and N)	5	2
tnfn1_pw060510p03q171	FTN_0588		asparaginase	2	2

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060419p03q116	FTN_0593	sucD	succinyl-CoA synthetase, alpha subunit	2	2
tnfn1_pw060418p02q128	FTN_0633	katG	peroxidase/catalase	7	7
tnfn1_pw060328p06q130	FTN_0692	nadA	quinolinate sythetase A	3	2#
tnfn1_pw060419p04q164	FTN_0692	nadA	quinolinate sythetase A	2	2#
tnfn1_pw060510p01q159	FTN_0695	add	deoxyadenosine deaminase/adenosine deaminase	3	7
tnfn1_pw060328p06q156	FTN_0811	birA	biotin--acetyl-CoA-carboxylase ligase	6	7
tnfn1_pw060328p01q128	FTN_0840	mdaB	NADPH-quinone reductase (modulator of drug activity B)	5	5
tnfn1_pw060420p02q175	FTN_0877	cls	cardiolipin synthetase	7	5
tnfn1_pw060328p06q142	FTN_0954		histidine acid phosphatase	4	4
tnfn1_pw060420p01q130	FTN_0965		metal-dependent exopeptidase	3	3
tnfn1_pw060328p01q151	FTN_0983		bifunctional protein: glutaredoxin 3/ribonucleotide reductase beta subunit	5	3#
tnfn1_pw060328p06q189	FTN_0995	hslV	ATP-dependent protease HslVU, peptidase subunit	2	2#
tnfn1_pw060420p04q195	FTN_0995	hslV	ATP-dependent protease HslVU, peptidase subunit	2	2#
tnfn1_pw060510p02q187	FTN_1018		aldolase/adducin class II family protein	3	3
tnfn1_pw060323p02q168	FTN_1046	wzb	low molecular weight (LMW) phosphotyrosine protein phosphatase	2	2
tnfn1_pw060328p06q184	FTN_1061		acid phosphatase, HAD superfamily protein	2	2#
tnfn1_pw060420p02q103	FTN_1061		acid phosphatase, HAD superfamily protein	3	3#
tnfn1_pw060510p04q113	FTN_1121	phrB	deoxyribodipyrimidine photolyase	5	7
tnfn1_pw060328p02q175	FTN_1131	putA	bifunctional proline dehydrogenase, pyrroline-5-carboxylate dehydrogenase	6	6
tnfn1_pw060328p02q174	FTN_1135	aroB	3-dehydroquinate synthetase	3	4#
tnfn1_pw060328p03q107	FTN_1222	kpsF	phosphosugar isomerase	4	3
tnfn1_pw060510p02q164	FTN_1231	gloA	lactoylglutathione lyase	4	4*
tnfn1_pw060420p04q194	FTN_1231	gloA	lactoylglutathione lyase	3	5*
tnfn1_pw060510p04q146	FTN_1231	gloA	lactoylglutathione lyase	2	2*
tnfn1_pw060510p01q142	FTN_1333	tktA	transketolase I	5	5

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060418p02q109	FTN_1376		disulfide bond formation protein, DsbB family	4	4
tnfn1_pw060328p06q150	FTN_1494	aceE	pyruvate dehydrogenase complex, E1 component, pyruvate dehydrogenase	4	7
tnfn1_pw060419p01q104	FTN_1523		amino acid-polyamine-organocation family protein	4	4#
tnfn1_pw060328p02q165	FTN_1523		amino acid-polyamine-organocation family protein	4	5#
tnfn1_pw060419p02q191	FTN_1523		amino acid-polyamine-organocation family protein	2	2#
tnfn1_pw060510p01q118	FTN_1553	nudH	dGTP pyrophosphohydrolase	5	5#
tnfn1_pw060418p01q131	FTN_1557		oxidoreductase iron/ascorbate family protein	7	7
tnfn1_pw060420p04q105	FTN_1584	glpD	glycerol-3-phosphate dehydrogenase	3	5
tnfn1_pw060419p04q130	FTN_1585	glpK	glycerol kinase	3	3
tnfn1_pw060510p01q146	FTN_1597	prfC	peptide chain release factor 3	5	5
tnfn1_pw060419p02q112	FTN_1619	appC	cytochrome bd-II terminal oxidase subunit I	5	7
tnfn1_pw060328p02q105	FTN_1620	appB	cytochrome bd-II terminal oxidase subunit II	6	3
tnfn1_pw060418p04q111	FTN_1621		predicted NAD/FAD-dependent oxidoreductase	3	3#
tnfn1_pw060418p04q112	FTN_1621		predicted NAD/FAD-dependent oxidoreductase	2	2#
tnfn1_pw060420p04q169	FTN_1621		predicted NAD/FAD-dependent oxidoreductase	4	4#
tnfn1_pw060323p04q160	FTN_1655	rluC	ribosomal large subunit pseudouridine synthase C	7	7#
tnfn1_pw060510p02q165	FTN_1655	rluC	ribosomal large subunit pseudouridine synthase C	2	2#
Transporter Proteins					
tnfn1_pw060420p04q149	FTN_0008		10 TMS drug/metabolite exporter protein	4	4#
tnfn1_pw060420p02q151	FTN_0018	sdaC	serine permease	2	4
tnfn1_pw060418p04q168	FTN_0141		ABC transporter, ATP-binding protein	5	6#
tnfn1_pw060418p03q147	FTN_0299	putP	proline:Na+ symporter	2	2#
tnfn1_pw060510p02q139	FTN_0299	putP	proline:Na+ symporter	2	2#
tnfn1_pw060323p03q141	FTN_0619		pseudogene: nicotinamide ribonucleoside (NR) uptake permease (PnuC) family protein	3	3*

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	Locus Tag	Gene	Description	U937	S2
tnfn1_pw060328p06q129	FTN_0619		pseudogene: nicotinamide ribonucleoside (NR) uptake permease (PnuC) family protein	2	5*
tnfn1_pw060510p02q156	FTN_0624		serine permease	2	2*
tnfn1_pw060323p06q164	FTN_0624		serine permease	2	2*
tnfn1_pw060418p01q161	FTN_0636	glpT	glycerol-3-phosphate transporter	7	7
tnfn1_pw060419p04q142	FTN_0687	galP1	galactose-proton symporter, major facilitator superfamily (MFS) transport protein	2	3*
tnfn1_pw060510p04q158	FTN_0687	galP1	galactose-proton symporter, major facilitator superfamily (MFS) transport protein	2	2*
tnfn1_pw060328p06q132	FTN_0728		predicted Co/Zn/Cd cation transporter	2	5
tnfn1_pw060418p03q103	FTN_0739	potG	ATP-binding cassette putrescine uptake system, ATP-binding protein	2	2#
tnfn1_pw060328p08q153	FTN_0739	potG	ATP-binding cassette putrescine uptake system, ATP-binding protein	2	5#
tnfn1_pw060510p04q103	FTN_0799	emrE	putative membrane transporter of cations and cationic drugs, multidrug resistance protein	2	2
tnfn1_pw060323p01q177	FTN_0799	emrE	putative membrane transporter of cations and cationic drugs, multidrug resistance protein	4	3
tnfn1_pw060328p04q109	FTN_0885		proton-dependent oligopeptide transporter (POT) family protein, di-or tripeptide:H+ symporter	5	2
tnfn1_pw060328p04q167	FTN_0997		proton-dependent oligopeptide transporter (POT) family protein, di-or tripeptide:H+ symporter	5	3
tnfn1_pw060323p05q110	FTN_1215	kpsC	capsule polysaccharide export protein KpsC	2	5
tnfn1_pw060323p07q172	FTN_1344		major facilitator superfamily (MFS) transport protein	4	4*
tnfn1_pw060420p04q148	FTN_1344		major facilitator superfamily (MFS) transport protein	5	5*
tnfn1_pw060323p01q175	FTN_1441		sugar porter (SP) family protein	4	4#
tnfn1_pw060420p02q182	FTN_1441		sugar porter (SP) family protein	6	6#
tnfn1_pw060419p02q126	FTN_1581		small conductance mechanosensitive ion channel (MscS) family protein	3	3
tnfn1_pw060323p03q106	FTN_1593	oppA	ABC-type oligopeptide transport system, periplasmic component	2	2*
tnfn1_pw060420p03q104	FTN_1593	oppA	ABC-type oligopeptide transport system, periplasmic component	4	6*
tnfn1_pw060420p01q189	FTN_1611		major facilitator superfamily (MFS) transport protein	7	5

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060328p02q121	FTN_1716	kdpC	potassium-transporting ATPase C chain	2	1*
tnfn1_pw060420p02q159	FTN_1716	kdpC	potassium-transporting ATPase C chain	2	2*
tnfn1_pw060418p03q187	FTN_1733		nicotinamide ribonucleoside (NR) uptake permease (PnuC) family protein	2	4
Transferases					
tnfn1_pw060323p02q177	FTN_0019	pyrB	aspartate carbamoyltransferase	2	2#
tnfn1_pw060323p03q119	FTN_0019	pyrB	aspartate carbamoyltransferase	2	2#
tnfn1_pw060510p01q103	FTN_0063	ilvE	branched-chain amino acid aminotransferase protein (class IV)	3	5
tnfn1_pw060323p03q121	FTN_0343		aminotransferase	7	2
tnfn1_pw060328p03q179	FTN_0358		tRNA-methylthiotransferase MiaB protein	4	4*
tnfn1_pw060419p01q169	FTN_0358		tRNA-methylthiotransferase MiaB protein	2	2*
tnfn1_pw060323p06q168	FTN_0545		glycosyl transferase, group 2	4	4#
tnfn1_pw060419p01q187	FTN_0545		glycosyl transferase, group 2	5	5#
tnfn1_pw060328p01q142	FTN_0928	cysD	sulfate adenylyltransferase subunit 2	3	3#
tnfn1_pw060323p03q182	FTN_1428	wbtO	transferase	3	2#
tnfn1_pw060510p01q119	FTN_1428	wbtO	transferase	2	6#
DNA modifying					
tnfn1_pw060323p03q125	FTN_0133		ribonuclease II family protein	2	2
tnfn1_pw060510p02q141	FTN_0133		ribonuclease II family protein	5	5
tnfn1_pw060323p03q122	FTN_0577	mutL	DNA mismatch repair enzyme with ATPase activity	7	6#
tnfn1_pw060510p01q148	FTN_0577	mutL	DNA mismatch repair enzyme with ATPase activity	5	5#
tnfn1_pw060510p04q193	FTN_0680	uvrC	excinuclease ABC, subunit C	6	3
tnfn1_pw060328p04q156	FTN_1027	ruvC	holliday junction endodeoxyribonuclease	3	4#
tnfn1_pw060510p01q132	FTN_1027		holliday junction endodeoxyribonuclease	3	3#
tnfn1_pw060510p01q114	FTN_1073		DNA/RNA endonuclease G	5	6*

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060510p02q114	FTN_1073		DNA/RNA endonuclease G	2	2*
tnfn1_pw060510p01q153	FTN_1154		type I restriction-modification system, subunit S	5	6
tnfn1_pw060323p03q167	FTN_1197	recR	RecFOR complex, RecR component	2	4#
tnfn1_pw060510p02q106	FTN_1197	recR	RecFOR complex, RecR component	3	3#
tnfn1_pw060328p06q158	FTN_1293	rnhB	ribonuclease HII	2	5
tnfn1_pw060323p07q175	FTN_1487		restriction endonuclease	3	6
Cell Division					
tnfn1_pw060328p03q149	FTN_0162	ftsQ	cell division protein FtsQ	2	2#
tnfn1_pw060328p01q167	FTN_0330	minD	septum formation inhibitor-activating ATPase	2	2
Type IV Pilin					
tnfn1_pw060323p03q109	FTN_1137	pilQ	Type IV pili secretin component	2	2
tnfn1_pw060418p02q167	FTN_1137	pilQ	Type IV pili secretin component	4	4
tnfn1_pw060323p06q157	FTN_1139	pilO	Type IV pili glycosylation protein	2	2
Others					
tnfn1_pw060323p06q138	FTN_0107	lepA	GTP-binding protein LepA	2	4#
tnfn1_pw060418p02q123	FTN_0107	lepA	GTP-binding protein LepA	2	4#
tnfn1_pw060420p04q150	FTN_0155		competence protein	2	7*
tnfn1_pw060510p04q189	FTN_0155		competence protein	6	3*
tnfn1_pw060418p04q181	FTN_0338		MutT/nudix family protein	2	2
tnfn1_pw060328p06q137	FTN_0465		Sua5/YciO/YrdC family protein	2	2#
tnfn1_pw060323p03q111	FTN_0465		Sua5/YciO/YrdC family protein	2	2#
tnfn1_pw060323p06q115	FTN_0768	tspO	tryptophan-rich sensory protein	3	3#
tnfn1_pw060420p03q193	FTN_0768	tspO	tryptophan-rich sensory protein	3	3#
tnfn1_pw060510p01q120	FTN_0768	tspO	tryptophan-rich sensory protein	3	3#
tnfn1_pw060328p06q167	FTN_0985		DJ-1/PfpI family protein	6	6#

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060328p06q167	FTN_0985		DJ-1/PfpI family protein	5	5#
tnfn1_pw060420p04q127	FTN_1031	ftnA	ferric iron binding protein, ferritin-like	2	6
tnfn1_pw060419p02q137	FTN_1034	rnfB	iron-sulfur cluster-binding protein	2	3
tnfn1_pw060420p03q121	FTN_1064		PhoH family protein, putative ATPase	2	4
tnfn1_pw060328p06q178	FTN_1241		DedA family protein	4	5
tnfn1_pw060418p01q185	FTN_1355		regulatory factor, Bvg accessory factor family	6	7
tnfn1_pw060328p03q154	FTN_1453		two-component regulator, sensor histidine kinase	2	2
tnfn1_pw060323p06q110	FTN_1518	relA	GDP pyrophosphokinase/GTP pyrophosphokinase	2	2*
tnfn1_pw060323p07q167	FTN_1518	relA	GDP pyrophosphokinase/GTP pyrophosphokinase	4	4*
Intergenic					
tnfn1_pw060323p03q164	intergenic			3	2
tnfn1_pw060328p06q190	intergenic			3	3
tnfn1_pw060419p03q131	intergenic			2	2
tnfn1_pw060419p04q189	intergenic			5	3
tnfn1_pw060323p08q139	intergenic			4	4
List of growth defective mutants in only U937 Cells					
Proteins of unknown function					
tnfn1_pw060328p06q147	FTN_0109		protein of unknown function	3#	
tnfn1_pw060418p04q193	FTN_0109		protein of unknown function	4#	
tnfn1_pw060510p01q123	FTN_0132		protein of unknown function	2	
tnfn1_pw060323p07q115	FTN_0290		protein of unknown function	5	
tnfn1_pw060328p04q122	FTN_0428		protein of unknown function	2*	
tnfn1_pw060510p04q109	FTN_0428		protein of unknown function	2*	
tnfn1_pw060419p03q140	FTN_0477		conserved protein of unknown function	2	
tnfn1_pw060420p02q178	FTN_0915		conserved protein of unknown function	7	
tnfn1_pw060419p04q188	FTN_0925		protein of unknown function	4	

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060420p02q181	FTN_0933		protein of unknown function	7	
tnfn1_pw060419p04q118	FTN_1172		conserved protein of unknown function	2	
tnfn1_pw060420p01q127	FTN_1175		membrane protein of unknown function	4	
tnfn1_pw060420p01q109	FTN_1367		protein of unknown function	2	
tnfn1_pw060420p01q132	FTN_1624		conserved protein of unknown function	4	
tnfn1_pw060420p02q184	FTN_1696		protein of unknown function	7	
Hypothetical Proteins					
tnfn1_pw060323p01q181	FTN_0336		hypothetical protein	3	
tnfn1_pw060510p01q147	FTN_0403		hypothetical membrane protein	4	
tnfn1_pw060323p01q163	FTN_0727		hypothetical membrane protein	3	
tnfn1_pw060418p03q110	FTN_0847		conserved hypothetical protein	2#	
tnfn1_pw060510p02q108	FTN_0847		conserved hypothetical protein	4#	
tnfn1_pw060419p02q152	FTN_0888		hypothetical membrane protein	2	
tnfn1_pw060418p01q191	FTN_1349		hypothetical protein	4#	
tnfn1_pw060328p06q182	FTN_1395		conserved hypothetical protein	4	
tnfn1_pw060328p04q136	FTN_1406		conserved hypothetical membrane protein	4	
tnfn1_pw060420p02q127	FTN_1656		conserved hypothetical protein	2	
tnfn1_pw060420p02q176	FTN_1686		hypothetical membrane protein	5	
tnfn1_pw060418p03q159	FTN_1736		hypothetical protein	2	
Metabolic Proteins					
tnfn1_pw060419p02q150	FTN_0090	acpA	acid phosphatase	5	
tnfn1_pw060419p03q169	FTN_0218	nfnB	dihydropteridine reductase	2	
tnfn1_pw060420p01q123	FTN_0524	asd	aspartate semialdehyde dehydrogenase	5	
tnfn1_pw060323p06q168	FTN_0545		glycosyl transferase, group 2	5#	
tnfn1_pw060419p01q187	FTN_0545		glycosyl transferase, group 2	5#	
tnfn1_pw060510p03q168	FTN_0598		tRNA-dihydrouridine synthase	3	

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060328p04q196	FTN_0746	alr	alanine racemase	6#	
tnfn1_pw060420p04q108	FTN_0822		para-aminobenzoate synthase component I	5	
tnfn1_pw060420p04q140	FTN_0957		short chain dehydrogenase	4	
tnfn1_pw060420p02q174	FTN_1233		haloacid dehalogenase-like hydrolase	6	
tnfn1_pw060420p04q116	FTN_1421	wbtH	glutamine amidotransferase/asparagine synthase	3	
tnfn1_pw060419p04q135	FTN_1415		thioredoxin	6	
tnfn1_pw060510p04q185	FTN_1701		glutamate decarboxylase	3	
tnfn1_pw060510p04q136	FTN_1767	rbsK	ribokinase, pfkB family	3	
tnfn1_pw060328p05q154	FTN_1777	trpG	anthranilate synthase component II	2#	
Transporter Proteins					
tnfn1_pw060420p04q158	FTN_0800		ArsB arsenite/antimonite exporter	2	
tnfn1_pw060510p01q152	FTN_1711	tyrP	tyrosine permease	6	
DNA Modification					
tnfn1_pw060419p04q116	FTN_0287		type I restriction-modification system, subunit R (restriction)	2	
tnfn1_pw060420p03q134	FTN_0710		type I restriction-modification system, subunit R (restriction)	4	
tnfn1_pw060510p04q179	FTN_0838	xthA	exodeoxyribonuclease III	3	
tnfn1_pw060419p04q152	FTN_1017		pseudogene: DNA-3-methyladenine glycosylase	5	
tnfn1_pw060323p04q111	FTN_1176	uvrB	excinuclease ABC, subunit B	2	
Transferases					
tnfn1_pw060420p02q180	FTN_0483		bifunctional NMN adenylyltransferase/Nudix hydrolase	7	
tnfn1_pw060510p01q158	FTN_0988	prmA	50S ribosomal protein L11, methyltransferase	7	
tnfn1_pw060510p02q144	FTN_1234	queA	S-adenosylmethionine:tRNA ribosyltransferase-isomerase	6	
Transcription/Translation					
tnfn1_pw060323p03q127	FTN_0567		tRNA synthetase class II (D, K and N)	2	
tnfn1_pw060510p03q168	FTN_0598		tRNA-dihydrouridine synthase	3	
tnfn1_pw060419p04q129	FTN_1290	mglA	macrophage growth locus, protein A	3#	
Others					

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060328p08q161	-	isftu1	isftu1	2	
tnfn1_pw060510p04q176	FTN_0182		ATP-binding cassette (ABC) superfamily protein	2	
tnfn1_pw060323p08q110	FTN_0286		transposase	3	
tnfn1_pw060420p01q168	FTN_0646	cscK	ROK family protein	5	
tnfn1_pw060328p04q123	FTN_0672	secA	preprotein translocase, subunit A (ATPase, RNA helicase)	2	
tnfn1_pw060328p04q112	FTN_1002	blaA	beta-lactamase class A	2#	
tnfn1_pw060419p02q192	FTN_1002	blaA	beta-lactamase class A	2#	
tnfn1_pw060420p02q177	FTN_1145	era	GTP-binding protein	6	
tnfn1_pw060418p03q107	FTN_1217		ATP-binding cassette (ABC) superfamily protein	2	
tnfn1_pw060328p06q171	FTN_1263	comL	competence lipoprotein ComL	2#	
tnfn1_pw060420p02q179	FTN_1263	comL	competence lipoprotein ComL	7#	
tnfn1_pw060323p06q110	FTN_1518	relA	GDP pyrophosphokinase/GTP pyrophosphokinase	2*	
tnfn1_pw060323p07q167	FTN_1518	relA	GDP pyrophosphokinase/GTP pyrophosphokinase	4*	
Intergenic					
tnfn1_pw060328p03q108	intergenic			2	
tnfn1_pw060419p04q165	intergenic			5	
tnfn1_pw060510p01q102	intergenic			5	
tnfn1_pw060510p01q112	intergenic			4	
tnfn1_pw060510p01q135	intergenic			4	
List of growth defective mutants in only S2 cells according to their functions					
Proteins of unknown Function					
tnfn1_pw060419p01q176	FTN_0043		conserved protein of unknown function		2
tnfn1_pw060418p01q155	FTN_0044		protein of unknown function		3
tnfn1_pw060418p02q158	FTN_0050		protein of unknown function		4
tnfn1_pw060328p08q104	FTN_0051		conserved protein of unknown function		3
tnfn1_pw060420p01q142	FTN_0052		protein of unknown function		2

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	Locus Tag	Gene	Description	U937	S2
tnfn1_pw060419p04q191	FTN_0077		protein of unknown function		3#
tnfn1_pw060323p06q122	FTN_0077		protein of unknown function		2#
tnfn1_pw060510p04q143	FTN_0099		conserved protein of unknown function		2
tnfn1_pw060418p04q193	FTN_0109		protein of unknown function		4
tnfn1_pw060418p04q117	FTN_0207		protein of unknown function containing a von Willebrand factor type A (vWA) domain		2
tnfn1_pw060328p04q119	FTN_0325		membrane protein of unknown function		2
tnfn1_pw060328p08q156	FTN_0340		protein of unknown function		2
tnfn1_pw060323p03q157	FTN_0364		conserved protein of unknown function		2*
tnfn1_pw060418p04q136	FTN_0364		conserved protein of unknown function		3*
tnfn1_pw060328p08q149	FTN_0439		protein of unknown function		4#
tnfn1_pw060418p01q142	FTN_0482		protein of unknown function		6
tnfn1_pw060328p04q110	FTN_0573		protein of unknown function		2
tnfn1_pw060418p02q126	FTN_0573		protein of unknown function		4
tnfn1_pw060328p08q173	FTN_0584	araJ	conserved inner membrane protein of unknown function		5
tnfn1_pw060510p04q147	FTN_0599		protein of unknown function		2#
tnfn1_pw060328p06q173	FTN_0599		protein of unknown function		2#
tnfn1_pw060510p01q183	FTN_0782		protein of unknown function		5
tnfn1_pw060323p03q129	FTN_0786		protein of unknown function		7#
tnfn1_pw060323p05q127	FTN_0791		protein of unknown function		3#
tnfn1_pw060419p03q107	FTN_0791		protein of unknown function		2#
tnfn1_pw060418p01q141	FTN_0817		conserved protein of unknown function		2
tnfn1_pw060328p05q126	FTN_0828		protein of unknown function		5
tnfn1_pw060510p04q111	FTN_0861		conserved protein of unknown function		4
tnfn1_pw060418p04q148	FTN_0878		protein of unknown function		2
tnfn1_pw060328p02q106	FTN_0884		drug/metabolite transporter superfamily protein		2#

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	Locus Tag	Gene	Description	U937	S2
tnfn1_pw060328p03q163	FTN_0884		drug/metabolite transporter superfamily protein		4#
tnfn1_pw060323p03q150	FTN_0900		protein of unknown function with predicted hydrolase and phosphorylase activity		2#
tnfn1_pw060418p03q108	FTN_0900		protein of unknown function with predicted hydrolase and phosphorylase activity		6#
tnfn1_pw060323p04q104	FTN_0918		conserved protein of unknown function		2#
tnfn1_pw060418p02q131	FTN_0918		conserved protein of unknown function		3#
tnfn1_pw060419p04q188	FTN_0925		protein of unknown function		5
tnfn1_pw060419p04q179	FTN_1001		protein of unknown function		2#
tnfn1_pw060323p07q181	FTN_1001		protein of unknown function		3#
tnfn1_pw060418p02q145	FTN_1020		conserved protein of unknown function		5
tnfn1_pw060419p01q172	FTN_1044		conserved protein of unknown function		3
tnfn1_pw060420p01q111	FTN_1053		outer membrane protein of unknown function		3
tnfn1_pw060420p02q158	FTN_1071		protein of unknown function		5
tnfn1_pw060418p02q133	FTN_1093		protein of unknown function		5
tnfn1_pw060420p01q134	FTN_1103		protein of unknown function		2#
tnfn1_pw060328p01q140	FTN_1103		protein of unknown function		3#
tnfn1_pw060323p08q143	FTN_1235		protein of unknown function		2
tnfn1_pw060510p03q135	FTN_1254		protein of unknown function		4
tnfn1_pw060323p03q102	FTN_1257		membrane protein of unknown function		3#
tnfn1_pw060419p03q150	FTN_1257		membrane protein of unknown function		4#
tnfn1_pw060418p04q121	FTN_1261		protein of unknown function		2
tnfn1_pw060323p08q134	FTN_1270		conserved membrane protein of unknown function		2
tnfn1_pw060418p01q149	FTN_1298		GTPase of unknown function		7
tnfn1_pw060419p01q143	FTN_1334		conserved protein of unknown function		2#
tnfn1_pw060328p08q108	FTN_1334		conserved protein of unknown function		3#

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060328p05q124	FTN_1372		protein of unknown function		5
tnfn1_pw060323p04q183	FTN_1386		protein of unknown function		3
tnfn1_pw060328p01q156	FTN_1442		conserved protein of unknown function		2#
tnfn1_pw060420p01q165	FTN_1442		conserved protein of unknown function		4#
tnfn1_pw060418p02q186	FTN_1448		protein of unknown function		3
tnfn1_pw060328p02q116	FTN_1449		conserved protein of unknown function		3#
tnfn1_pw060419p03q173	FTN_1449		conserved protein of unknown function		2#
tnfn1_pw060323p07q176	FTN_1534		conserved protein of unknown function		3
tnfn1_pw060328p02q177	FTN_1713		protein of unknown function		3
tnfn1_pw060328p05q185	FTN_1734		protein of unknown function		5
tnfn1_pw060328p08q107	FTN_1774		protein of unknown function		3
Hypothetical Protein					
tnfn1_pw060418p04q139	FTN_0011		hypothetical protein		2#
tnfn1_pw060420p02q108	FTN_0012		hypothetical protein		2
tnfn1_pw060420p02q139	FTN_0013		hypothetical protein		3
tnfn1_pw060328p01q141	FTN_0014		conserved hypothetical protein		3
tnfn1_pw060419p04q178	FTN_0028		conserved hypothetical membrane protein		3#
tnfn1_pw060323p04q145	FTN_0028		conserved hypothetical membrane protein		2#
tnfn1_pw060418p04q143	FTN_0053		hypothetical protein		2
tnfn1_pw060328p06q157	FTN_0170		conserved hypothetical membrane protein		5
tnfn1_pw060418p03q151	FTN_0212		hypothetical membrane protein		3
tnfn1_pw060323p08q114	FTN_0326		conserved hypothetical protein		3
tnfn1_pw060328p05q165	FTN_0360		hypothetical protein		5#
tnfn1_pw060419p01q145	FTN_0368		hypothetical protein		2
tnfn1_pw060419p03q186	FTN_0375		hypothetical protein		3
tnfn1_pw060420p02q163	FTN_0398		hypothetical membrane protein		3

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060420p04q104	FTN_0466		conserved hypothetical protein		4
tnfn1_pw060328p08q148	FTN_0548		conserved hypothetical protein		2#
tnfn1_pw060418p04q176	FTN_0548		conserved hypothetical protein		2#
tnfn1_pw060328p06q164	FTN_0630		hypothetical protein		5
tnfn1_pw060328p05q141	FTN_0701		conserved hypothetical protein		5
tnfn1_pw060418p02q152	FTN_0706		hypothetical membrane protein		3
tnfn1_pw060418p02q175	FTN_0717		conserved hypothetical membrane protein		5
tnfn1_pw060328p06q126	FTN_0732		hypothetical protein		5
tnfn1_pw060323p07q129	FTN_0759		conserved hypothetical protein		2
tnfn1_pw060323p04q134	FTN_0938		hypothetical protein		2#
tnfn1_pw060418p02q170	FTN_0938		hypothetical protein		4#
tnfn1_pw060419p03q187	FTN_1123		conserved hypothetical protein		3
tnfn1_pw060418p04q105	FTN_1180		hypothetical membrane protein		3
tnfn1_pw060420p04q159	FTN_1223		conserved hypothetical membrane protein		7
tnfn1_pw060323p08q166	FTN_1232		conserved hypothetical membrane protein		2
tnfn1_pw060328p03q180	FTN_1260		hypothetical membrane protein		2
tnfn1_pw060510p01q184	FTN_1299		hypothetical protein		5
tnfn1_pw060419p04q127	FTN_1342		conserved hypothetical protein		3
tnfn1_pw060328p05q157	FTN_1379		pseudogene: hypothetical membrane protein, fragment		5
tnfn1_pw060323p06q178	FTN_1389		conserved hypothetical membrane protein		3#
tnfn1_pw060420p01q172	FTN_1389		conserved hypothetical membrane protein		2#
tnfn1_pw060420p01q153	FTN_1458		conserved hypothetical protein		2
tnfn1_pw060323p04q147	FTN_1761		pseudogene: hypothetical protein, fragment		3
tnfn1_pw060418p04q149	FTN_1765		conserved hypothetical protein		2
Metabolic					
tnfn1_pw060510p02q160	FTN_0021	carA	carbamoyl-phosphate synthase small chain		2

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060418p04q115	FTN_0095		nitroreductase		7
tnfn1_pw060420p02q191	FTN_0113	ribC	riboflavin synthase alpha chain		6
tnfn1_pw060328p05q159	FTN_0118		serine peptidase, S49 family		3#
tnfn1_pw060420p02q187	FTN_0118		serine peptidase, S49 family		5#
tnfn1_pw060328p06q139	FTN_0127	gabD	succinate semialdehyde dehydrogenase (NAD(P)+ dependent)		5
tnfn1_pw060510p01q130	FTN_0154	rimK	glutathione synthase/ribosomal protein S6 modification enzyme		3
tnfn1_pw060328p01q150	FTN_0168	lysU	lysyl-tRNA synthetase		2#
tnfn1_pw060510p02q178	FTN_0217		L-lactate dehydrogenase		2
tnfn1_pw060323p07q113	FTN_0362		deoxyribodipyrimidine photolyase-related protein		4
tnfn1_pw060323p04q144	FTN_0406		sterol desaturase		3#
tnfn1_pw060418p01q189	FTN_0406		sterol desaturase		6#
tnfn1_pw060328p06q134	FTN_0443	maeA	NAD-dependent malic enzyme		5#
tnfn1_pw060328p06q125	FTN_0496	slt	soluble lytic murein transglycosylase		3
tnfn1_pw060418p04q116	FTN_0512	glgX	pullulanase		4
tnfn1_pw060510p03q154	FTN_0516	glgA	glycogen synthase		7
tnfn1_pw060420p01q135	FTN_0540	pckA	phosphoenolpyruvate carboxykinase		2
tnfn1_pw060419p04q153	FTN_0597		protein-disulfide isomerase		2
tnfn1_pw060510p02q110	FTN_0603	mutM	formamidopyrimidine-DNA glycosylase		2
tnfn1_pw060328p02q139	FTN_0621	eno	enolase (2-phosphoglycerate dehydratase)		2#
tnfn1_pw060510p03q188	FTN_0627	chiA	chitinase, glycosyl hydrolase family 18		6
tnfn1_pw060418p01q120	FTN_0651	cdd	cytidine deaminase		5#
tnfn1_pw060419p01q168	FTN_0651	cdd	cytidine deaminase		2#
tnfn1_pw060328p04q151	FTN_0661	guaB	IMP dehydrogenase/GMP reductase		6#
tnfn1_pw060328p06q131	FTN_0674	glxK	glycerate kinase		3
tnfn1_pw060420p01q148	FTN_0694	nadB	L-aspartate oxidase		4
tnfn1_pw060323p06q103	FTN_0711		predicted metal-dependent hydrolase		2

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	Locus Tag	Gene	Description	U937	S2
tnfn1_pw060328p04q116	FTN_0765		choloylglycine hydrolase family protein		2
tnfn1_pw060510p03q119	FTN_0806		glycosyl hydrolase family 3		3
tnfn1_pw060323p07q185	FTN_0814	bioF	8-amino-7-oxononanoate synthase		3#
tnfn1_pw060419p02q138	FTN_0814	bioF	8-amino-7-oxononanoate synthase		3#
tnfn1_pw060328p04q175	FTN_0818		lipase/esterase		5
tnfn1_pw060418p02q142	FTN_0826		aldo/keto reductase family protein		3
tnfn1_pw060328p08q145	FTN_0907		D-alanyl-D-alanine carboxypeptidase		4#
tnfn1_pw060418p04q131	FTN_0907		D-alanyl-D-alanine carboxypeptidase		4#
tnfn1_pw060418p04q167	FTN_0935	asnB	asparagine synthase		2
tnfn1_pw060510p02q145	FTN_0945	rsuA	16S rRNA pseudouridine synthase		4
tnfn1_pw060328p08q120	FTN_0987		tRNA-dihydrouridine synthase		3#
tnfn1_pw060323p08q141	FTN_1015		isochorismatase family protein		3#
tnfn1_pw060420p01q129	FTN_1015		isochorismatase family protein		2#
tnfn1_pw060323p05q141	FTN_1033	grxB	glutaredoxin 2		3#
tnfn1_pw060420p01q193	FTN_1033	grxB	glutaredoxin 2		4#
tnfn1_pw060418p01q153	FTN_1055	lon	DNA-binding, ATP-dependent protease La		2
tnfn1_pw060328p06q184	FTN_1061		acid phosphatase, HAD superfamily protein		3#
tnfn1_pw060420p02q103	FTN_1061		acid phosphatase, HAD superfamily protein		7#
tnfn1_pw060510p04q113	FTN_1121	phrB	deoxyribodipyrimidine photolyase		6
tnfn1_pw060328p02q175	FTN_1131	putA	bifunctional proline dehydrogenase, pyrroline-5-carboxylate dehydrogenase		4
tnfn1_pw060328p02q174	FTN_1135	aroB	3-dehydroquinate synthetase		5#
tnfn1_pw060328p08q131	FTN_1174	murI	glutamate racemase		2#
tnfn1_pw060419p03q164	FTN_1186	pepO	M13 family metallopeptidase		7
tnfn1_pw060418p01q124	FTN_1245	iscS	cysteine desulfurase		7#
tnfn1_pw060323p04q139	FTN_1264	rluD	ribosomal large subunit pseudouridine synthase D		2#

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	Locus Tag	Gene	Description	U937	S2
tnfn1_pw060510p03q183	FTN_1264	rluD	ribosomal large subunit pseudouridine synthase D		6#
tnfn1_pw060328p06q166	FTN_1273		long chain fatty acid CoA ligase		2
tnfn1_pw060419p03q126	FTN_1278	nadE	NAD synthase		5
tnfn1_pw060328p05q128	FTN_1329	fbaA	fructose bisphosphate aldolase Class II		3
tnfn1_pw060323p06q195	FTN_1390		Zn-dependent hydrolase		5
tnfn1_pw060510p04q137	FTN_1425	wbtF	NAD dependent epimerase		2
tnfn1_pw060419p03q166	FTN_1431	wbtA	dTDP-glucose 4,6-dehydratase		2
tnfn1_pw060323p07q169	FTN_1438		bifunctional protein: 3-hydroxacyl-CoA dehydrogenase/acyl-CoA-binding protein		4#
tnfn1_pw060418p02q122	FTN_1438		bifunctional protein: 3-hydroxacyl-CoA dehydrogenase/acyl-CoA-binding protein		3#
tnfn1_pw060328p08q196	FTN_1459		short chain dehydrogenase		5
tnfn1_pw060328p06q128	FTN_1530	lysA	diaminopimelate decarboxylase		6
tnfn1_pw060328p05q101	FTN_1532	gdhA	glutamate dehydrogenase (NADP+)		2#
tnfn1_pw060419p04q163	FTN_1532	gdhA	glutamate dehydrogenase (NADP+)		6#
tnfn1_pw060418p02q178	FTN_1536		amino acid-polyamine-organocation (APC) superfamily protein		4
tnfn1_pw060323p06q106	FTN_1552		acid phosphatase, PAP2 family		5
tnfn1_pw060510p01q118	FTN_1553	nudH	dGTP pyrophosphohydrolase		2
tnfn1_pw060323p04q110	FTN_1678	nuoC	NADH dehydrogenase I, C subunit		5#
tnfn1_pw060328p05q160	FTN_1729	dapB	dihydrodipicolinate reductase		4#
tnfn1_pw060510p01q178	FTN_1729	dapB	dihydrodipicolinate reductase		3#
tnfn1_pw060328p04q104	FTN_1730	lysC	aspartate kinase III		2
tnfn1_pw060328p03q174	FTN_1768	pepN	aminopeptidase N		3
Transporter proteins					
tnfn1_pw060323p03q117	FTN_0005	corA	divalent inorganic cation transporter		2#
tnfn1_pw060420p01q131	FTN_0005	corA	divalent inorganic cation transporter		3#
tnfn1_pw060420p01q180	FTN_0097		hydroxy/aromatic amino acid permease (HAAAP) family protein		4#

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060419p03q162	FTN_0115		Na+/H+ antiporter		4
tnfn1_pw060323p08q162	FTN_0151		ABC-type nitrate/sulfonate/bicarbonate transport system, ATPase component		2
tnfn1_pw060419p01q165	FTN_0183		manganese/Zinc/Iron chelate uptake transporter family protein		3#
tnfn1_pw060419p04q103	FTN_0183		manganese/Zinc/Iron chelate uptake transporter family protein		2#
tnfn1_pw060510p02q174	FTN_0184		major facilitator superfamily (MFS) transport protein		2
tnfn1_pw060323p03q161	FTN_0276	mviN	multidrug/oligosaccharidyl-lipid/polysaccharide (MOP) transporter		2*
tnfn1_pw060510p02q151	FTN_0276	mviN	multidrug/oligosaccharidyl-lipid/polysaccharide (MOP) transporter		3*
tnfn1_pw060323p08q118	FTN_0345		DNA uptake protein, SMF family		2
tnfn1_pw060419p03q195	FTN_0363		sodium bile acid symporter family protein		4
tnfn1_pw060420p03q115	FTN_0566		mechanosensitive ion channel protein		3
tnfn1_pw060328p08q167	FTN_0579		major facilitator superfamily (MFS) transport protein		2
tnfn1_pw060419p04q167	FTN_0620		major facilitator superfamily (MFS) transport protein		5
tnfn1_pw060328p06q114	FTN_0631		metabolite:H+ symporter (MHS) family protein		2#
tnfn1_pw060510p02q115	FTN_0631		metabolite:H+ symporter (MHS) family protein		5#
tnfn1_pw060510p02q167	FTN_0631		metabolite:H+ symporter (MHS) family protein		5#
tnfn1_pw060418p02q189	FTN_0640	dctA	C4-dicarboxylate transport protein		3
tnfn1_pw060510p02q159	FTN_0688	galP2	galactose-proton symporter, major facilitator superfamily (MFS) transport protein		3
tnfn1_pw060510p03q140	FTN_0741		proton-dependent oligopeptide transporter (POT) family protein, di-or tripeptide:H+ symporter		5
tnfn1_pw060328p05q107	FTN_0767	betT	betaine/carnitine/choline transporter (BCCT) family protein		4
tnfn1_pw060420p03q116	FTN_0824		major facilitator superfamily (MFS) transport protein		2
tnfn1_pw060510p04q173	FTN_0872		small conductance mechanosensitive ion channel (MscS) family protein		5
tnfn1_pw060328p06q175	FTN_0875		metabolite:H+ symporter (MHS) family		2
tnfn1_pw060328p02q106	FTN_0884		drug/metabolite transporter superfamily protein		1#
tnfn1_pw060328p03q163	FTN_0884		drug/metabolite transporter superfamily protein		4#

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List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	Locus Tag	Gene	Description	U937	S2
tnfn1_pw060328p01q188	FTN_0910		sugar:cation symporter family protein		2#
tnfn1_pw060419p04q109	FTN_0910		sugar:cation symporter family protein		2#
tnfn1_pw060419p01q175	FTN_0984		ABC transporter, ATP-binding protein		2
tnfn1_pw060419p01q170	FTN_1006		transporter-associated protein, HlyC/CorC family		4
tnfn1_pw060418p02q160	FTN_1010		major facilitator superfamily (MFS) transport protein		2
tnfn1_pw060419p01q133	FTN_1014		nicotinamide ribonucleoside (NR) uptake permease (PnuC) family protein		2
tnfn1_pw060328p02q109	FTN_1107	metlQ	methionine uptake transporter (MUT) family protein, membrane and periplasmic protein		2
tnfn1_pw060323p05q139	FTN_1166		metabolite:H+ symporter (MHS) family protein		7
tnfn1_pw060419p02q107	FTN_1267		ATP-binding Cassette (ABC) superfamily protein		4
tnfn1_pw060418p02q182	FTN_1275		drug:H+ antiporter-1 (DHA2) family protein		5
tnfn1_pw060420p04q186	FTN_1404		ATP-binding cassette (ABC) superfamily protein		2
tnfn1_pw060510p02q118	FTN_1409		major facilitator superfamily (MFS) transport protein		6
tnfn1_pw060328p06q119	FTN_1549		drug:H+ antiporter-1 (DHA1) family protein		3
tnfn1_pw060419p02q126	FTN_1581		small conductance mechanosensitive ion channel (MscS) family protein		2
tnfn1_pw060418p01q150	FTN_1586		sugar transporter, MFS superfamily		2
tnfn1_pw060420p01q146	FTN_1681	fur	ferric uptake regulation protein		2*
tnfn1_pw060510p04q167	FTN_1681	fur	ferric uptake regulation protein		2*
tnfn1_pw060323p03q163	FTN_1683		drug:H+ antiporter-1 (DHA1) family protein		3*
tnfn1_pw060328p02q192	FTN_1683		drug:H+ antiporter-1 (DHA1) family protein		4*
tnfn1_pw060323p06q117	FTN_1685		drug:H+ antiporter-1 (DHA1) family protein		3
tnfn1_pw060418p02q140	FTN_1685		drug:H+ antiporter-1 (DHA1) family protein		5
tnfn1_pw060328p05q182	FTN_1707	nhaD	Na+:H+ antiporter		5
tnfn1_pw060328p02q121	FTN_1716	kdpC	potassium-transporting ATPase C chain		2*
tnfn1_pw060420p02q159	FTN_1716	kdpC	potassium-transporting ATPase C chain		2*
tnfn1_pw060510p03q118	FTN_1717	kdpB	potassium-transporting ATPase B chain		3

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060420p01q113	FTN_1752	nhaA	Na+:H+ antiporter		3
Transferase					
tnfn1_pw060510p04q127	FTN_0071		LPS fatty acid acyltransferase		2#
tnfn1_pw060419p03q160	FTN_0080		SAM-dependent methyltransferase		4
tnfn1_pw060328p08q125	FTN_0120		rhodanese-related sulfurtransferase		4
tnfn1_pw060328p01q137	FTN_0153		RimI-like acetyltransferase		3
tnfn1_pw060418p01q110	FTN_0200		UDP-3-O-[3-fatty acid] glucosamine N-acyltransferase		2#
tnfn1_pw060510p02q131	FTN_0200		UDP-3-O-[3-fatty acid] glucosamine N-acyltransferase		2#
tnfn1_pw060420p02q146	FTN_0300		glycosyl transferase, group 2		5
tnfn1_pw060328p03q179	FTN_0358		tRNA-methylthiotransferase MiaB protein		4*
tnfn1_pw060419p01q169	FTN_0358		tRNA-methylthiotransferase MiaB protein		2*
tnfn1_pw060420p01q152	FTN_0453		glycosyl transferase		5
tnfn1_pw060419p02q135	FTN_0560	ksgA	dimethyladenosine transferase		3
tnfn1_pw060419p04q168	FTN_1091	aroA	3-phosphoshikimate 1-carboxyvinyltransferase		2
tnfn1_pw060418p03q185	FTN_1400		S-adenosylmethionine-dependent methyltransferase		5
tnfn1_pw060418p04q172	FTN_1418	manC	mannose-1-phosphate guanylyltransferase		4
DNA Modification					
tnfn1_pw060510p04q169	FTN_0122	recA	recombinase A protein		2
tnfn1_pw060328p06q179	FTN_0492	parC	DNA topoisomerase IV subunit A		2#
tnfn1_pw060510p04q168	FTN_0666	uvrA	excinuclease ABC, subunit A		2
	FTN_0704		type I restriction-modification system, subunit M (methyltransferase)		5
tnfn1_pw060510p02q180	FTN_0704		type I restriction-modification system, subunit M (methyltransferase)		2
tnfn1_pw060510p03q158	FTN_1294		rRNA methylase, SpoU family		2
tnfn1_pw060510p02q176	FTN_1413		ATPase, AAA family, related to the helicase subunit of the Holliday junction resolvase		2
tnfn1_pw060328p08q179	FTN_1491		adenine specific DNA methylase		2
tnfn1_pw060328p06q176	FTN_1544	hemK	modification methylase, HemK family		5

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060328p05q164	FTN_1594	uvrD	DNA helicase II		6
Cell Division					
tnfn1_pw060328p01q167	FTN_0330	minD	septum formation inhibitor-activating ATPase		2
tnfn1_pw060323p08q146	FTN_0331	minC	septum formation inhibitor		4#
tnfn1_pw060420p02q170	FTN_0331	minC	septum formation inhibitor		4#
Transcription/Translation					
tnfn1_pw060328p06q196	FTN_0552	yhbY	RNA-binding protein		5#
tnfn1_pw060510p03q150	FTN_0949	rplI	50S ribosomal protein L9		2
tnfn1_pw060328p06q170	FTN_1099		transcriptional regulator, LysR family		7
tnfn1_pw060419p03q165	FTN_1300		transcriptional regulator, LysR family		2
tnfn1_pw060328p02q148	FTN_1393		transcriptional regulator, ArsR family		3#
tnfn1_pw060418p01q138	FTN_1393		transcriptional regulator, ArsR family		2#
tnfn1_pw060419p02q151	FTN_1628		transcriptional regulator, LysR family		2#
tnfn1_pw060510p03q194	FTN_1628		transcriptional regulator, LysR family		2#
FPI					
tnfn1_pw060328p01q144	FTN_1313		hypothetical protein		3
tnfn1_pw060323p03q179	FTN_1314		conserved hypothetical protein		1
tnfn1_pw060328p06q163	FTN_1315		protein of unknown function		5
tnfn1_pw060328p06q115	FTN_1322	iglC	intracellular growth locus protein C		5
tnfn1_pw060419p04q108	FTN_1325	pdpD	protein of unknown function		2
Type IV Pili					
tnfn1_pw060418p04q123	FTN_0070	pilE	Type IV pili, pilus assembly protein		3
tnfn1_pw060510p03q129	FTN_0070	pilE	Type IV pili, pilus assembly protein		3
tnfn1_pw060323p06q179	FTN_0305		pilus assembly protein		4
tnfn1_pw060419p01q196	FTN_0414		Type IV pili, pilus assembly protein		2
tnfn1_pw060419p03q141	FTN_0664	fimT	Type IV pili, pilus assembly protein		2

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060328p05q146	FTN_0946	pilF	Type IV pili, pilus assembly protein		5
tnfn1_pw060418p02q167	FTN_1137	pilQ	Type IV pili secretin component		4
Others					
tnfn1_pw060328p08q161		isftu1	isftu1		2
tnfn1_pw060323p03q115		isftu3	isftu3		1
tnfn1_pw060328p04q157		isftu2	isftu2		1
tnfn1_pw060510p02q150		isftu6	isftu6		2
tnfn1_pw060328p01q179	FTN_0010		phage terminase, small subunit		3
tnfn1_pw060328p08q114	FTN_0266	htpG	chaperone Hsp90, heat shock protein HtpG		2
tnfn1_pw060328p04q152	FTN_0322		VacJ like lipoprotein		3#
tnfn1_pw060418p01q140	FTN_0322		VacJ like lipoprotein		2#
tnfn1_pw060328p08q155	FTN_0357	pal	peptidoglycan-associated lipoprotein, OmpA family		4*
tnfn1_pw060419p01q158	FTN_0357	pal	peptidoglycan-associated lipoprotein, OmpA family		2*
tnfn1_pw060510p02q122	FTN_0367		phage integrase		4
tnfn1_pw060328p08q132	FTN_0372		regulatory protein, AlpA family		4
tnfn1_pw060323p07q171	FTN_0585	cutC	copper homeostasis protein CutC family protein		2
tnfn1_pw060328p06q127	FTN_0713	ostA2	organic solvent tolerance protein OstA		5#
tnfn1_pw060419p01q180	FTN_0713	ostA2	organic solvent tolerance protein OstA		4#
tnfn1_pw060323p06q105	FTN_0810		ROK family protein		4
tnfn1_pw060419p01q139	FTN_0836		kinase-like protein		2
tnfn1_pw060418p04q134	FTN_1051	hfq	host factor I for bacteriophage Q beta replication		2
tnfn1_pw060420p03q121	FTN_1064		PhoH family protein, putative ATPase		4
tnfn1_pw060328p05q177	FTN_1192		chitin-binding protein		6
tnfn1_pw060419p04q183	FTN_1240		BolA family protein		4
tnfn1_pw060418p02q190	FTN_1242		DedA family protein		5
tnfn1_pw060419p01q120	FTN_1488		prophage maintenance system killer protein (DOC)		6

List of growth defective mutants in both U937 and S2 Cells					
				Log reduction in Growth relative to WT	
Strain Name	**Locus Tag**	**Gene**	**Description**	**U937**	**S2**
tnfn1_pw060419p01q135	FTN_1665		magnesium chelatase		2
tnfn1_pw060419p04q180	FTN_1682	frgA	siderophore biosynthesis protein		5
tnfn1_pw060510p04q122	FTN_1698		Dam-replacing family protein		2
Intergenic					
tnfn1_pw060418p01q125	intergenic				7
tnfn1_pw060323p06q165	intergenic				5
tnfn1_pw060323p08q117	intergenic				3
tnfn1_pw060328p05q195	intergenic				6
tnfn1_pw060419p01q148	intergenic				3
tnfn1_pw060420p03q148	intergenic				3
tnfn1_pw060420p01q164	intergenic				2
tnfn1_pw060510p02q127	intergenic				2
tnfn1_pw060328p08q109	intergenic				2
tnfn1_pw060510p04q116	intergenic				2

[#] Mutants for which all the mutant alleles showed similar growth defect

[*] Mutants for which two out of three or three out of four alleles showed growth defect

Table 2

List of growth defective or dissemination defective mutants identified in previous screens and are defective in both U937 macrophages and S2 cells

Strain Name	Locus Tag	Gene	Description
tnfn1_pw060323p03q172$^{\alpha}$	FTN_0008		10 TMS drug/metabolite exporter protein
tnfn1_pw060420p02q151$^{\beta\gamma\delta}$	FTN_0018	sdaC	serine permease
tnfn1_pw060323p02q177$^{\beta\gamma\delta}$	FTN_0019	pyrB	aspartate carbamoyltransferase
tnfn1_pw060323p08q120$^{\beta\gamma\delta}$	FTN_0020	carB	carbamoyl-phosphate synthase large chain
tnfn1_pw060510p02q160$^{\beta\gamma\delta}$	FTN_0021	carA	carbamoyl-phosphate synthase small chain
tnfn1_pw060419p04q178$^{\alpha}$	FTN_0028		conserved hypothetical membrane protein
tnfn1_pw060418p04q123$^{\beta}$	FTN_0070	pilE	Type IV pili, pilus assembly protein
tnfn1_pw060420p01q180$^{\beta\gamma\delta\pi}$	FTN_0097		hydroxy/aromatic amino acid permease (HAAAP) family protein
tnfn1_pw060419p01q106$^{\alpha}$	FTN_0111	ribH	riboflavin synthase beta-chain
tnfn1_pw060510p04q169$^{\delta}$	FTN_0122	recA	recombinase A protein
tnfn1_pw060510p01q123$^{\alpha}$	FTN_0132	lpsA	protein of unknown function
tnfn1_pw060323p03q125$^{\alpha}$	FTN_0133		ribonuclease II family protein
tnfn1_pw060420p02q173$^{\alpha}$	FTN_0169		conserved hypothetical membrane protein
tnfn1_pw060418p03q133$^{\alpha}$	FTN_0199	cyoE	heme O synthase
tnfn1_pw060323p04q102$^{\beta\gamma\delta}$	FTN_0211	pcp	pyrrolidone carboxylylate peptidase
tnfn1_pw060510p02q178$^{\alpha}$	FTN_0217		L-lactate dehydrogenase
tnfn1_pw060420p04q134$^{\alpha}$	FTN_0297		conserved protein of unknown function
tnfn1_pw060418p03q147$^{\alpha}$	FTN_0299	putP	proline:Na+ symporter
tnfn1_pw060420p02q146$^{\beta\gamma\delta}$	FTN_0300		glycosyl transferase, group 2
tnfn1_pw060328p01q167$^{\beta\gamma\delta}$	FTN_0330	minD	septum formation inhibitor-activating ATPase
tnfn1_pw060323p08q146$^{\delta}$	FTN_0331	minC	septum formation inhibitor
tnfn1_pw060328p08q156$^{\alpha}$	FTN_0340		protein of unknown function
tnfn1_pw060323p06q113$^{\beta\gamma\delta}$	FTN_0420	purCD	SAICAR synthetase/phosphoribosylamine-glycine ligase
tnfn1_pw060328p06q134$^{\beta\gamma\delta}$	FTN_0443	maeA	NAD-dependent malic enzyme
tnfn1_pw060328p05q119$^{\beta\gamma\delta\pi}$	FTN_0444		membrane protein of unknown function
tnfn1_pw060323p05q182$^{\beta\gamma\delta}$	FTN_0504	cadC	lysine decarboxylase
tnfn1_pw060510p01q124$^{\beta\gamma\delta}$	FTN_0507	gcvP1	glycine cleavage system P protein, subunit 1
tnfn1_pw060323p06q168$^{\beta\gamma\delta}$	FTN_0545		glycosyl transferase, group 2
tnfn1_pw060419p02q135$^{\beta}$	FTN_0560	ksgA	dimethyladenosine transferase
tnfn1_pw060419p03q116$^{\beta\gamma\delta}$	FTN_0593	sucD	succinyl-CoA synthetase, alpha subunit
tnfn1_pw060510p04q147$^{\beta\gamma\delta}$	FTN_0599		protein of unknown function

Strain Name	Locus Tag	Gene	Description
tnfn1_pw060323p06q164$^{\beta\gamma\delta}$	FTN_0624		serine permease
tnfn1_pw060418p02q128$^{\delta}$	FTN_0633	katG	peroxidase/catalase
tnfn1_pw060420p01q168$^{\alpha}$	FTN_0646	cscK	ROK family protein
tnfn1_pw060419p01q168$^{\beta\gamma\delta}$	FTN_0651	cdd	cytidine deaminase
tnfn1_pw060510p04q168$^{\delta}$	FTN_0666	uvrA	excinuclease ABC, subunit A
tnfn1_pw060328p04q123$^{\beta\gamma\delta}$	FTN_0672	secA	preprotein translocase, subunit A (ATPase, RNA helicase)
tnfn1_pw060420p03q134$^{\alpha}$	FTN_0710		type I restriction-modification system, subunit R (restriction)
tnfn1_pw060328p06q127$^{\alpha}$	FTN_0713	ostA2	organic solvent tolerance protein OstA
tnfn1_pw060328p06q132$^{\beta\gamma\delta}$	FTN_0728		predicted Co/Zn/Cd cation transporter
tnfn1_pw060323p06q115$^{\alpha}$	FTN_0768	tspO	tryptophan-rich sensory protein
tnfn1_pw060323p06q105$^{\alpha}$	FTN_0810		ROK family protein
tnfn1_pw060323p07q185$^{\beta\gamma\delta}$	FTN_0814	bioF	8-amino-7-oxononanoate synthase
tnfn1_pw060418p01q141$^{\beta}$	FTN_0817		conserved protein of unknown function
tnfn1_pw060420p04q108$^{\beta\gamma\delta\pi}$	FTN_0822		para-aminobenzoate synthase component I
tnfn1_pw060420p03q116$^{\alpha}$	FTN_0824		major facilitator superfamily (MFS) transport protein
tnfn1_pw060328p01q128$^{\alpha}$	FTN_0840	mdaB	NADPH-quinone reductase (modulator of drug activity B)
tnfn1_pw060420p04q176$^{\beta\gamma\delta}$	FTN_0855		protein of unknown function
tnfn1_pw060420p02q175$^{\alpha}$	FTN_0877	cls	cardiolipin synthetase
tnfn1_pw060323p04q104$^{\alpha}$	FTN_0918		conserved protein of unknown function
tnfn1_pw060419p04q188$^{\beta\gamma\delta}$	FTN_0925		protein of unknown function
tnfn1_pw060420p02q181$^{\alpha}$	FTN_0933		protein of unknown function
tnfn1_pw060323p04q134$^{\alpha}$	FTN_0938		hypothetical protein
tnfn1_pw060419p01q170$^{\alpha}$	FTN_1006		transporter-associated protein, HlyC/CorC family
tnfn1_pw060323p08q141$^{\alpha}$	FTN_1015		isochorismatase family protein
tnfn1_pw060418p01q153$^{\alpha}$	FTN_1055	lon	DNA-binding, ATP-dependent protease La
tnfn1_pw060510p01q114$^{\pi}$	FTN_1073		DNA/RNA endonuclease G
tnfn1_pw060419p04q168$^{\beta\gamma\delta}$	FTN_1091	aroA	3-phosphoshikimate 1-carboxyvinyltransferase
tnfn1_pw060328p08q188$^{\alpha}$	FTN_1098		conserved hypothetical membrane protein
tnfn1_pw060328p02q109$^{\alpha}$	FTN_1107	metlQ	methionine uptake transporter (MUT) family protein, membrane and periplasmic protein
tnfn1_pw060328p02q175$^{\beta\gamma\delta}$	FTN_1131	putA	bifunctional proline dehydrogenase, pyrroline-5-carboxylate dehydrogenase
tnfn1_pw060418p03q107$^{\beta\gamma\delta}$	FTN_1217		ATP-binding cassette (ABC) superfamily protein
tnfn1_pw060323p08q166$^{\alpha}$	FTN_1232		conserved hypothetical membrane protein
tnfn1_pw060328p06q178$^{\beta\gamma\delta}$	FTN_1241		DedA family protein

Strain Name	Locus Tag	Gene	Description
tnfn1_pw060510p03q135[β]	FTN_1254		protein of unknown function
tnfn1_pw060420p04q196[βγδπ]	FTN_1256		membrane protein of unknown function
tnfn1_pw060323p03q102[βγδ]	FTN_1257		membrane protein of unknown function
tnfn1_pw060420p02q179[βγδ]	FTN_1263	comL	competence lipoprotein ComL
tnfn1_pw060418p01q149[βγδ]	FTN_1298		GTPase of unknown function
tnfn1_pw060328p01q144[βγδ]	FTN_1313		hypothetical protein
tnfn1_pw060328p06q163[β]	FTN_1315		protein of unknown function
tnfn1_pw060510p01q110[αβγδ]	FTN_1321	iglD	intracellular growth locus protein D
tnfn1_pw060328p06q115[βγδ]	FTN_1322	iglC	intracellular growth locus protein C
tnfn1_pw060419p04q108[βγδ]	FTN_1325	pdpD	protein of unknown function
tnfn1_pw060510p01q142[βγδ]	FTN_1333	tktA	transketolase I
tnfn1_pw060418p01q191[α]	FTN_1349		hypothetical protein
tnfn1_pw060418p01q185[α]	FTN_1355		regulatory factor, Bvg accessory factor family
tnfn1_pw060418p02q109[π]	FTN_1376		disulfide bond formation protein, DsbB family
tnfn1_pw060418p03q185[α]	FTN_1400		S-adenosylmethionine-dependent methyltransferase
tnfn1_pw060419p04q135[α]	FTN_1415		thioredoxin
tnfn1_pw060420p04q116[βγδ]	FTN_1421	wbtH	glutamine amidotransferase/asparagine synthase
tnfn1_pw060510p04q137[βγδ]	FTN_1425	wbtF	NAD dependent epimerase
tnfn1_pw060419p03q166[βγδπ]	FTN_1431	wbtA	dTDP-glucose 4,6-dehydratase
tnfn1_pw060418p02q122[βγδ]	FTN_1438		bifunctional protein: 3-hydroxacyl-CoA dehydrogenase/acyl-CoA-binding protein
tnfn1_pw060328p08q196[α]	FTN_1459		short chain dehydrogenase
tnfn1_pw060323p06q110[βγδ]	FTN_1518	relA	GDP pyrophosphokinase/GTP pyrophosphokinase
tnfn1_pw060328p06q128[β]	FTN_1530	lysA	diaminopimelate decarboxylase
tnfn1_pw060323p07q176[α]	FTN_1534		conserved protein of unknown function
tnfn1_pw060418p02q178[α]	FTN_1536		amino acid-polyamine-organocation (APC) superfamily protein
tnfn1_pw060418p01q150[γ]	FTN_1586		sugar transporter, MFS superfamily
tnfn1_pw060510p01q146[βγδ]	FTN_1597	prfC	peptide chain release factor 3
tnfn1_pw060420p01q189[α]	FTN_1611		major facilitator superfamily (MFS) transport protein
tnfn1_pw060323p04q160[βγδ]	FTN_1655	rluC	ribosomal large subunit pseudouridine synthase C
tnfn1_pw060419p04q180[δ]	FTN_1682	frgA	siderophore biosynthesis protein
tnfn1_pw060323p03q163[βγδ]	FTN_1683		drug:H+ antiporter-1 (DHA1) family protein
tnfn1_pw060328p05q154[βγδ]	FTN_1777	trpG	anthranilate synthase component II

[α](Kraemer et al., 2009)

[β](Weiss et al., 2007)

$^{\gamma}$(Qin and Mann, 2006)

$^{\delta}$(Su et al., 2007)

$^{\pi}$(Maier et al., 2007)

Table 3

List of 6 growth-defective mutants of *F. tularensis* and the co-localization of the FCP with LAMP-1 in hMDMs

Strain Name	Locus Tag	Gene	%Co-localization with Lamp1	Log reduction in Growth relative to WT
Control				
Sua5/YciO/YrdC family protein			17	0
Intracellular growth locus C		IglC	88	6
Selected Mutants				
tnfn1_pw060328p05q136	FTN_0384		20	2
tnfn1_pw060323p07q141	FTN_0788		75	4
tnfn1_pw060323p01q177	FTN_0799	emrE	26	3
tnfn1_pw060510p01q158	FTN_0988	prmA	84	4
tnfn1_pw060420p02q179	FTN_1263	comL	78	5
tnfn1_pw060323p01q113	FTN_1343		72	2

Table 4

Classification of metabolic genes according to metabolic pathways

Amino acid metabolism			
tnfn1_pw060323p08q120	FTN_0020	carB	carbamoyl-phosphate synthase large chain
tnfn1_pw060328p06q174	FTN_0125	ackA	propionate kinase 2/acetate kinase A
tnfn1_pw060323p05q182	FTN_0504		lysine decarboxylase
tnfn1_pw060510p01q124	FTN_0507	gcvP1	glycine cleavage system P protein, subunit 1
tnfn1_pw060510p02q154	FTN_0511		shikimate 5-dehydrogenase
tnfn1_pw060510p02q157	FTN_0511		shikimate 5-dehydrogenase
tnfn1_pw060510p04q157	FTN_0511		shikimate 5-dehydrogenase
tnfn1_pw060510p04q157	FTN_0511		shikimate 5-dehydrogenase
tnfn1_pw060323p06q194	FTN_0527	thrC	threonine synthase
tnfn1_pw060510p01q172	FTN_0527	thrC	threonine synthase
tnfn1_pw060510p03q172	FTN_0527	thrC	threonine synthase
tnfn1_pw060510p03q171	FTN_0588		asparaginase
tnfn1_pw060328p04q196	FTN_0746	alr	alanine racemase
tnfn1_pw060328p06q142	FTN_0954		histidine acid phosphatase
tnfn1_pw060328p02q175	FTN_1131	putA	bifunctional proline dehydrogenase, pyrroline-5-carboxylate dehydrogenase
tnfn1_pw060328p04q116	FTN_9765		choloylglycine hydrolase family protein
tnfn1_pw060510p04q185	FTN_1701		glutamate decarboxylase
Carbohydrate metabolism			
tnfn1_pw060420p01q123	FTN_0524	asd	aspartate semialdehyde dehydrogenase
tnfn1_pw060419p03q116	FTN_0593	sucD	succinyl-CoA synthetase, alpha subunit
tnfn1_pw060328p02q139	FTN_0621	eno	enolase (2-phosphoglycerate dehydratase)
tnfn1_pw060510p02q187	FTN_1018		aldolase/adducin class II family protein
tnfn1_pw060328p03q107	FTN_1222	kpsF	phosphosugar isomerase
tnfn1_pw060328p06q150	FTN_1494	aceE	pyruvate dehydrogenase complex, E1 component, pyruvate dehydrogenase
tnfn1_pw060420p04q105	FTN_1584	glpD	glycerol-3-phosphate dehydrogenase
tnfn1_pw060419p04q130	FTN_1585	glpK	glycerol kinase
tnfn1_pw060419p02q112	FTN_1619	appC	cytochrome bd-II terminal oxidase subunit I
tnfn1_pw060328p02q105	FTN_1620	appB	cytochrome bd-II terminal oxidase subunit II
tnfn1_pw060510p04q136	FTN_1767	rbsK	ribokinase, pfkB family
Nucleotide metabolism			
tnfn1_pw060323p08q120	FTN_0020	carB	carbamoyl-phosphate synthase large chain
tnfn1_pw060510p02q160	FTN_0021	carA	carbamoyl-phosphate synthase small chain
tnfn1_pw060418p03q133	FTN_0199	cyoE	heme O synthase
tnfn1_pw060323p06q113	FTN_0420		SAICAR synthetase/phosphoribosylamine-glycine ligase
tnfn1_pw060510p01q159	FTN_0695	add	deoxyadenosine deaminase/adenosine deaminase
tnfn1_pw060328p01q151	FTN_0983		bifunctional protein: glutaredoxin 3/ribonucleotide reductase beta subunit
tnfn1_pw060419p04q135	FTN_1415		thioredoxin

tnfn1_pw060419p04q181	FTN_1415		thioredoxin
tnfn1_pw060420p04q116	FTN_1421	wbtH	glutamine amidotransferase/asparagine synthase
tnfn1_pw060510p01q118	FTN_1553	nudH	dGTP pyrophosphohydrolase
tnfn1_pw060323p04q160	FTN_1655	rluC	ribosomal large subunit pseudouridine synthase C
tnfn1_pw060510p02q165	FTN_1655	rluC	ribosomal large subunit pseudouridine synthase C
Reductive Metabolism			
tnfn1_pw060419p03q169	FTN_0218	nfnB	dihydropteridine reductase
tnfn1_pw060418p02q128	FTN_0633	katG	peroxidase/catalase
tnfn1_pw060328p01q128	FTN_0840	mdaB	NADPH-quinone reductase (modulator of drug activity B)
tnfn1_pw060420p03q153	FTN_0840	mdaB	NADPH-quinone reductase (modulator of drug activity B)
tnfn1_pw060420p04q194	FTN_1231	gloA	lactoylglutathione lyase
tnfn1_pw060510p02q164	FTN_1231	gloA	lactoylglutathione lyase
tnfn1_pw060510p04q146	FTN_1231	gloA	lactoylglutathione lyase
tnfn1_pw060418p01q131	FTN_1557		oxidoreductase iron/ascorbate family protein
tnfn1_pw060418p04q111	FTN_1621		predicted NAD/FAD-dependent oxidoreductase
tnfn1_pw060418p04q112	FTN_1621		predicted NAD/FAD-dependent oxidoreductase
tnfn1_pw060420p04q169	FTN_1621		predicted NAD/FAD-dependent oxidoreductase
Lipid Metabolism			
tnfn1_pw060420p02q175	FTN_0877	cls	cardiolipin synthetase
tnfn1_pw060420p04q140	FTN_0957		short chain dehydrogenase
conenzyme synthesis			
tnfn1_pw060328p06q130	FTN_0692	nadA	quinolinate sythetase A
tnfn1_pw060419p04q164	FTN_0692	nadA	quinolinate sythetase A
tnfn1_pw060328p06q156	FTN_0811	birA	biotin--acetyl-CoA-carboxylase ligase
tnfn1_pw060418p01q124	FTN_1245	iscS	cysteine desulfurase
tnfn1_pw060419p03q126	FTN_1278	nadE	NAD synthase
tnfn1_pw060323p04q110	FTN_1678	nuoC	NADH dehydrogenase I, C subunit
tnfn1_pw060419p01q106	FTN_0111	ribH	riboflavin synthase beta-chain
tnfn1_pw060420p02q191	FTN_0113	ribC	riboflavin synthase alpha chain
Peptidoglycan biosynthesis			
tnfn1_pw060323p04q102	FTN_0211	pcp	pyrrolidone carboxylylate peptidase
tnfn1_pw060418p03q177	FTN_0211	pcp	pyrrolidone carboxylylate peptidase
Aromatic compound biosynthesis			
tnfn1_pw060420p04q108	FTN_0822		para-aminobenzoate synthase component I
tnfn1_pw060323p08q141	FTN_1015		isochorismatase family protein
tnfn1_pw060420p01q129	FTN_1015		isochorismatase family protein
tnfn1_pw060328p02q174	FTN_1135	aroB	3-dehydroquinate synthetase
ppGpp biosynthesis			
tnfn1_pw060323p06q110	FTN_1518	relA	GDP pyrophosphokinase/GTP pyrophosphokinase
tnfn1_pw060323p07q167	FTN_1518	relA	GDP pyrophosphokinase/GTP pyrophosphokinase

Table 5

Vacuolar vs. cytosolic localization of the mutants within U937 cells

Strain Name	Locus Tag	Gene	%Co-localization with Lamp1	Localization
Control				
Sua5/YciO/YrdC family protein			24	Cytoplasmic
Intracellular growth locus C		IglC	81	Phagosomal
Proteins of unknown function				
tnfn1_pw060323p08q148	FTN_0027		76	Phagosomal
tnfn1_pw060323p03q103	FTN_0041		26	Cytoplasmic
tnfn1_pw060418p04q193	FTN_0109		70	Phagosomal
tnfn1_pw060420p04q143	FTN_0149		67	Phagosomal
tnfn1_pw060420p04q134	FTN_0297		64	Phagosomal
tnfn1_pw060328p05q119	FTN_0444		81	Phagosomal
tnfn1_pw060323p07q141	FTN_0788		68	Phagosomal*
tnfn1_pw060420p04q176	FTN_0855		89	Phagosomal
tnfn1_pw060420p02q178	FTN_0915		70	Phagosomal
tnfn1_pw060419p04q188	FTN_0925		72	Phagosomal
tnfn1_pw060323p03q147	FTN_0930		55	Phagosomal
tnfn1_pw060420p02q181	FTN_0933		66	Phagosomal
tnfn1_pw060510p01q108	FTN_0977		69	Phagosomal
tnfn1_pw060420p01q127	FTN_1175		80	Phagosomal
tnfn1_pw060420p04q196	FTN_1256		83	Phagosomal
tnfn1_pw060323p01q113	FTN_1343		60	Phagosomal
tnfn1_pw060328p02q110	FTN_1457		32	Cytoplasmic
tnfn1_pw060420p01q132	FTN_1624		70	Phagosomal
tnfn1_pw060420p02q184	FTN_1696		45	Cytoplasmic
tnfn1_pw060328p06q155	FTN_1764		76	Phagosomal
Hypothetical Proteins				
tnfn1_pw060323p03q142	FTN_0030		63	Phagosomal
tnfn1_pw060328p06q180	FTN_0038		79	Phagosomal
tnfn1_pw060420p02q173	FTN_0169		32	Cytoplasmic
tnfn1_pw060323p01q181	FTN_0336		40	Cytoplasmic
tnfn1_pw060328p05q136	FTN_0384		28	Cytoplasmic*
tnfn1_pw060510p01q147	FTN_0403		77	Phagosomal
tnfn1_pw060419p03q188	FTN_0696		45	Cytoplasmic*
tnfn1_pw060323p01q163	FTN_0727		68	Phagosomal
tnfn1_pw060419p02q102	FTN_0792		80	Phagosomal
tnfn1_pw060510p02q108	FTN_0847		64	Phagosomal
tnfn1_pw060323p07q105	FTN_0895		30	Cytoplasmic

Strain Name	Locus Tag	Gene	%Co-localization with Lamp1	Localization
tnfn1_pw060510p03q192	FTN_1098		75	Phagosomal
tnfn1_pw060418p01q191	FTN_1349		56	Phagosomal
tnfn1_pw060328p06q182	FTN_1395		80	Phagosomal
tnfn1_pw060328p04q136	FTN_1406		84	Phagosomal
tnfn1_pw060328p02q129	FTN_1612		69	Phagosomal
tnfn1_pw060420p02q176	FTN_1686		89	Phagosomal
Metabolic Proteins				
tnfn1_pw060419p02q150	FTN_0090		52	Phagosomal
tnfn1_pw060419p01q106	FTN_0111	ribH	45	Cytoplasmic
tnfn1_pw060328p06q174	FTN_0125	ackA	80	Phagosomal
tnfn1_pw060323p06q113	FTN_0420		37	Cytoplasmic
tnfn1_pw060323p05q182	FTN_0504		67	Phagosomal
tnfn1_pw060510p01q124	FTN_0507	gcvP1	28	Cytoplasmic
tnfn1_pw060510p02q157	FTN_0511		72	Phagosomal
tnfn1_pw060420p01q123	FTN_0524	asd	64	Phagosomal
tnfn1_pw060323p06q194	FTN_0527	thrC	58	Phagosomal
tnfn1_pw060510p03q168	FTN_0598		42	Cytoplasmic
tnfn1_pw060328p06q130	FTN_0692	nadA	68	Phagosomal
tnfn1_pw060328p04q196	FTN_0746	alr	72	Phagosomal
tnfn1_pw060328p06q156	FTN_0811	birA	78	Phagosomal
tnfn1_pw060420p04q108	FTN_0822		76	Phagosomal
tnfn1_pw060420p03q153	FTN_0840	mdaB	81	Phagosomal
tnfn1_pw060420p02q175	FTN_0877	cls	70	Phagosomal
tnfn1_pw060328p06q142	FTN_0954		85	Phagosomal
tnfn1_pw060420p04q140	FTN_0957		24	Cytoplasmic
tnfn1_pw060420p01q130	FTN_0965		83	Phagosomal
tnfn1_pw060328p01q151	FTN_0983		66	Phagosomal
tnfn1_pw060328p06q184	FTN_1061		70	Phagosomal
tnfn1_pw060328p02q174	FTN_1135	aroB	18	Cytoplasmic
tnfn1_pw060328p03q107	FTN_1222	kpsF	68	Phagosomal
tnfn1_pw060420p04q194	FTN_1231	gloA	87	Phagosomal
tnfn1_pw060420p02q174	FTN_1233		34	Cytoplasmic
tnfn1_pw060510p01q142	FTN_1333	tktA	85	Phagosomal
tnfn1_pw060418p02q109	FTN_1376		78	Phagosomal
tnfn1_pw060328p06q150	FTN_1494	aceE	87	Phagosomal
tnfn1_pw060328p02q165	FTN_1523		46	Cytoplasmic
tnfn1_pw060510p01q118	FTN_1553	nudH	90	Phagosomal
tnfn1_pw060420p04q105	FTN_1584	glpD	23	Cytoplasmic
tnfn1_pw060510p01q146	FTN_1597	prfC	80	Phagosomal

Strain Name	Locus Tag	Gene	%Co-localization with Lamp1	Localization
tnfn1_pw060419p02q112	FTN_1619	appC	42	Cytoplasmic*
tnfn1_pw060420p04q169	FTN_1621		70	Phagosomal
tnfn1_pw060323p04q160	FTN_1655	rluC	72	Phagosomal
Transporter Proteins				
tnfn1_pw060420p04q149	FTN_0008		35	Cytoplasmic
tnfn1_pw060418p04q168	FTN_0141		25	Cytoplasmic
tnfn1_pw060323p03q141	FTN_0619		26	Cytoplasmic
tnfn1_pw060323p06q164	FTN_0624		83	Phagosomal
tnfn1_pw060328p06q132	FTN_0728		65	Phagosomal
tnfn1_pw060323p01q177	FTN_0799	emrE	34	Cytoplasmic
tnfn1_pw060328p04q109	FTN_0885		76	Cytoplasmic
tnfn1_pw060328p04q167	FTN_0997		80	Phagosomal
tnfn1_pw060323p07q172	FTN_1344		70	Phagosomal
tnfn1_pw060420p02q182	FTN_1441		24	Cytoplasmic*
tnfn1_pw060420p01q189	FTN_1611		87	Phagosomal
tnfn1_pw060510p01q152	FTN_1711	tyrP	72	Phagosomal
DNA Modification				
tnfn1_pw060510p02q141	FTN_0133		81	Phagosomal
tnfn1_pw060323p03q122	FTN_0577	mutL	31	Cytoplasmic
tnfn1_pw060510p04q193	FTN_0680	uvrC	60	Phagosomal
tnfn1_pw060420p03q134	FTN_0710		75	Phagosomal
tnfn1_pw060419p04q152	FTN_1017		23	Cytoplasmic
tnfn1_pw060328p04q156	FTN_1027	ruvC	60	Phagosomal
tnfn1_pw060510p01q114	FTN_1073		86	Phagosomal
tnfn1_pw060510p01q153	FTN_1154		69	Phagosomal
tnfn1_pw060323p03q167	FTN_1197	recR	27	Cytoplasmic
Transferases				
tnfn1_pw060323p03q119	FTN_0019	pyrB	63	Phagosomal
tnfn1_pw060510p01q103	FTN_0063	ilvE	68	Phagosomal
tnfn1_pw060323p03q121	FTN_0343		26	Cytoplasmic
tnfn1_pw060328p03q179	FTN_0358		28	Cytoplasmic
tnfn1_pw060420p02q180	FTN_0483		77	Phagosomal
tnfn1_pw060323p06q168	FTN_0545		68	Phagosomal
tnfn1_pw060510p01q158	FTN_0988	prmA	78	Phagosomal
tnfn1_pw060510p02q144	FTN_1234	queA	67	Phagosomal
tnfn1_pw060418p04q172	FTN_1418	manC	68	Phagosomal
tnfn1_pw060510p01q119	FTN_1428	wbtO	82	Phagosomal
Transcription/Translation				
tnfn1_pw060510p03q168	FTN_0598		42	Cytoplasmic

Strain Name	Locus Tag	Gene	%Co-localization with Lamp1	Localization
tnfn1_pw060419p04q129	FTN_1290	mglA	71	Phagosomal
Type IV Pilin				
tnfn1_pw060418p02q167	FTN_1137	pilQ	66	Phagosomal
tnfn1_pw060323p06q157	FTN_1139	pilO	67	Phagosomal
Others				
tnfn1_pw060323p08q110	FTN_0286		73	Phagosomal
tnfn1_pw060420p01q168	FTN_0646	cscK	77	Phagosomal
tnfn1_pw060323p06q115	FTN_0768	tspO	74	Phagosomal
tnfn1_pw060328p06q167	FTN_0985		37	Cytoplasmic
tnfn1_pw060419p02q137	FTN_1034	rnfB	78	Phagosomal
tnfn1_pw060420p02q177	FTN_1145	era	84	Phagosomal
tnfn1_pw060328p06q178	FTN_1241		76	Phagosomal
tnfn1_pw060420p02q179	FTN_1263	comL	79	Phagosomal
tnfn1_pw060328p03q154	FTN_1453		73	Phagosomal
tnfn1_pw060323p07q167	FTN_1518	relA	81	Phagosomal
Intergenic				
tnfn1_pw060323p03q164	intergenic		28	Cytoplasmic
tnfn1_pw060323p08q139	intergenic		80	Phagosomal
tnfn1_pw060328p06q190	intergenic		35	Cytoplasmic
tnfn1_pw060419p04q165	intergenic		65	Phagosomal
tnfn1_pw060419p04q189	intergenic		89	Phagosomal
tnfn1_pw060510p01q102	intergenic		90	Phagosomal
tnfn1_pw060510p01q112	intergenic		78	Phagosomal
tnfn1_pw060510p01q135	intergenic		87	Phagosomal

* Mutants with discrepancy between LAMP-1 co-localization and Phagosomal integrity assay.