SUPPLEMENTARY DISCUSSION

Mortality Effects of LPS and $A_{2A}R$ KO

Data support a survival effect of A_{2A}Rs specific to aged male mice (**Fig S3**). This is somewhat consistent with Lukashev *et al.* [S1] who reported exaggerated mortality after 48 hrs in A_{2A}R KO mice (age un-reported) challenged with LPS, whereas Sullivan *et al.* [S2] report no major survival effect of A_{2A}R KO in this setting (though A_{2A}R agonism potently improved survival). In direct contrast to these mortality effects in acute endotoxemia, it has been reported that A_{2A}R KO (or receptor antagonism) improves survival in polymicrobial sepsis [S3,S4]. Immunosuppressive functions of the A_{2A}R and associated reductions in bacterial clearance are thus detrimental to survival, whereas anti-inflammatory actions may be protective in the setting of endotoxemia (absent infection). In terms of age- and sex-dependent mortality with LPS, it is known that aging worsens outcomes from endotoxemia or sepsis in animal models [S5,S6]. There is also evidence female rats exhibit greater myocardial tolerance to endotoxemic dysfunction [S7,S8], potentially reflecting enhanced AKT/eNOS signalling and reduced NFκB activation. The A_{2A}R may thus be more important (or less redundant) in limiting inflammation and associated mortality in intrinsically less tolerant aged and male mice.

Transcriptional Profile of Endotoxemic Myocardium

1. The Most LPS-Responsive Transcripts

The transcripts most highly induced and repressed by LPS are shown in **Fig 3** in the main body of the paper. The most induced was lipocalin-2/NGAL (almost +600), in accordance with recent observations of Ding *et al.* [S9] in rodent and human myocarditis. Lipocalin-2 is expressed in cardiomyocytes [S10], and is specifically induced by IL-1ß. The novel NFκB regulator IkBζ, induced by IL-1R and TLR ligands and substantially up-regulated here (+13), mediates induction of *Lcn2*. Recent studies document increased cardiac lipocalin-2 with infarction and inflammation [S11,S12], correlations between lipocalin-2 and adverse outcomes in heart failure [S10], and

lipocalin-2 dependence of myocardial apoptosis and mitochondrial dysfunction [S12, S13]. However, in other cell types lipocalin-2 may inhibit LPS triggered cytokine release and detrimental effects of inflammatory mediators [S14] and is attributed with protective actions [S15], contrasting pro-inflammatory [S16] and pro-death [S12,S13] responses in the heart. Absence of $A_{2A}R$ activity almost doubled lipocalin-2 induction (+950), reflecting suppression of this injurious mediator by intrinsic $A_{2A}R$ activity.

The second most induced transcript was serum amyloid A3 [S*aa3*), an acute-phase protein with monocyte chemotactic activity. SAA proteins may induce cytokines and remodeling enzymes including collagenase, stromelysin and MMPs. The third most induced, *Serpina3n* (+122), encodes the serine protease inhibitor SPI-3 (α1-antichymotrypsin), with *Serpina3g* also highly induced. These are considered stress-response genes, and SPI-3 is reportedly up-regulated in other tissues in response to inflammatory challenge [S17]. Induction may counter damaging proteolysis during inflammation, and limit apoptosis, a response apparently insensitive to $A_{2A}R$ activity based on our findings.

Transcript for metallothionein-2 was highly induced (+54), and metallothionein-1 also up-regulated (+7). Metallothionein-2 protects against oxidant stress and cardiac dysfunction [S18], is induced by STAT-3 signaling to protect the heart [S19], and was recently found to limit contractile dysfunction in sepsis [S20]. Substantial induction 24 hrs post-LPS could reflect an adaptive response to limit cardiac injury and dysfunction, and was insensitive to $A_{2A}R$ KO.

Many of the most repressed genes were involved in cell growth and tissue remodeling, with others relevant to inflammatory and cardiac responses, whereas several are of obscure function. The most repressed was muscle specific carbonic anhydrase 3 (-38), a cytosolic protein whose low enzymatic activity suggests roles other than CO_2 hydration. The protein reportedly protects against oxidative stress and apoptosis via inhibition of ROS generation, and has been linked to muscle fatigue. However, recent analysis of its deletion found no changes in either muscle fatigue or resistance to oxidative stress [S21]. Similarly, *H19* transcript was one of the most repressed (-11),

yet its function is poorly understood. The gene generates a 2.3-kb non-coding RNA expressed primarily during embryogenesis, but also in adult tissue including myocardium. Recent studies identify a role for the RNA (or miRNA partner) in trans-regulation of components of the imprinted gene network. Based on potential roles in embryogenesis and tumor suppression, one might speculate that repression with LPS reflects a shift in transcriptional control of tissue growth/remodeling.

The 3rd most repressed transcript was for adiponectin (-17), which is anti-inflammatory, protects against LPS-dependent injury, promotes cell survival via enhanced autophagy, and is cardioprotective [S22,S23]. Adiponectin deficiency worsens LPS-dependent inflammation and mortality, though interestingly a murine KO model exhibits unaltered responsiveness to LPS [S24]. Adiponectin is protective and exerts anti-oxidant actions in ischemic-reperfused myocardium [S22, S25]. Marked repression may sensitize cardiac tissue to oxidative damage, limit autophagy, and promote the cardiac injury process during LPS challenge. The apelin-APJ receptor (or angiotensin receptor-like 1) was also markedly repressed (-14), and is known to improve contractility, induce NO-dependent vasodilatation, and also protect against cardiac injury and heart failure [S26]. Repression by LPS may thus be relevant to evolution of contractile and vascular dysfunctions with myocardial inflammation.

2. Pathway Modifications in LPS-Challenged Intact Myocardium

Cell death signaling: Cell death pathways were up-regulated by LPS (**Table S6**, **Figs S13** and **S14**). Within death receptor signaling, transcripts for both major receptors (*Fas*, *Tnfr1*) and ligands (*Trail*, *Apo2l*) were increased with LPS challenge. Signaling components coupled to FAS and TNFα receptors were up-regulated (*Tbk1*, *Rip*), and transcripts for the distal apoptotic effectors caspase-7, caspase-8/10, Bid and Apaf1 were also induced. On the other hand, anti-apoptotic *Daxx*, *Cflar* (FLIP) and c-IAP components (*Birc2, Birc3*) were up-regulated, with other distal effectors repressed (*Map3k5*, *Mapk8*). In non death-receptor triggered apoptosis, pro-apoptotic *Bak*, ER-

dependent *Casp12*, *p53* and *Bax* were all induced, while anti-apoptotic *Bcl2a1* (Bfl-1) and *Bcl2l1* (Bcl-XL) were also increased. Substantial induction of upstream apoptotic elements is thus likely to favor cell death, though such effects may be tempered by parallel induction of Daxx, FLIP, Bfl-1, c-IAP and Bcl-XL.

Endoplasmic reticulum (ER) stress: The ER stress response is important in myocardial stress-resistance [S27] and inflammatory responses to LPS [S28,S29]. Activation of ER chaperones by XBP-1, regulating the ER stress and ATF6-dependent unfolded protein (UPR) responses [S30, S31], may induce cardioprotection, and transcripts for both XBP-1 and ATF6 were up-regulated. In contrast, PERK/ATF4/CHOP-dependent UPR signaling may be pro-apoptotic, and data reveal LPS represses CHOP/Gadd153 (which may limit later apoptosis). In addition, *Dnajc3* (which inhibits PERK and is involved in CHOP induction) and XBP-1 dependent *Edem1* (involved in degrading unfolded proteins) were both induced by LPS. Other ER-stress related genes modified included *Ramp4*, *Casp12*, and *Ask1*. The cell survival outcome from these varied transcriptional changes in ER stress signaling pathways is unclear.

Antigen-antibody removal and complement: Inflammatory challenge with LPS substantially modifies antigen-antibody removal and the complement system (**Fig S15**; **Tables 5**, **S5** and **S7**). Although an augmented complement response may facilitate antigen-antibody complex removal, this can also exacerbate cardiac injury [S32]. Modifications to the complement path (**Fig S15**) include 15-fold induction of complement factor B (key to alternate path activation), and up-regulation of *C1r* and *C1s* (+6), the integrin *C3aR/Itgam* (+6), *C3* (+4), *C2* (+3), *Cr3ß/Itgb2* (+3), the C1 inhibitor *Serping1* (+2), *C1qb* (+2), and Fcγ receptor transcripts (IIIA, IIB, IA, 1, and IIA; in descending order of change). The lectin path component *Masp1* was also induced (+3), while we detect 3- to 4-fold repression of rate-limiting complement factor D (adipsin). This complex regulation could either limit classical (increased C1 inhibitor) and alternate paths (reduced adipsin), or enhance classical (enhanced Fcγ receptors, C1R, C1S, C1Q, and C2) or alternate (increased factor B and C3) pathways, the end-result dependent upon the rate-limiting properties of each

component, and their roles in other responses. For example, C3aR/ITGAM may protect against inflammation and sepsis-related mortality [S33,S34], induction potentially reflecting compensatory protection. Atkinson *et al.* recently showed that the alternate pathway increases ischemic injury and inflammation following cardiac transplantation [S35]. Complement activation could thus contribute to cardiac injury/dysfunction in sepsis.

Cardiac growth/remodeling: As noted above, hepatic fibrosis paths are modulated by LPS, and tissue remodeling and cell turnover is evidenced by induction of transcripts previously identified by Wong *et al*. [S36] (*eg*. 10-fold induction of lysyl oxidase and p21 protein Cip1), together with other remodeling mediators (*eg. Mmp3*, +11; *Mmp14*, +2; *Mmp8,* +2; *Timp4,* +2). In addition, induced *Serpine1* (PAI-1) is pro-fibrotic, and vascular remodeling may be favored by this induction together with *Plaur*, *Icam1*, *Vcam1* and integrin changes. There is high representation within the 50 most repressed transcripts of factors regulating cardiac structure and mass (including *Igf1*, *Pcsk6/Pace4, Fgf12, Tgfb2, Tgfb3*, *Ogn*), together with structural/cytoskeletal/ECM components (*Aspn, Itga8, Tuba4, Tuba8, Col14a1, Fmod, Stmn2, Col6a3, Tagln*). LPS also significantly modified canonical hypertrophy pathways (**Tables 6** and **S8**; **Figs S16** and **S17**). Thus LPS initiates major shifts in cardiovascular growth and remodeling processes.

Hepatic fibrosis/stellate cell activation and DC maturation: Identification of hepatic fibrosis/stellate cell activation (regulating cell growth, differentiation and fibrogenesis with liver injury) and DC maturation evidences myocardial roles for the encoded proteins. Canonical cardiac growth/remodeling processes were indeed modified by LPS, and molecules implicated in hepatic injury (*eg.* induced TIMP1 and CSF1; repressed IGF-1, TIMP2, VEGFc, and IGFBP5) likely also influence cardiac remodeling/fibrosis.

Molecules involved in dendritic cell (DC) maturation were modified by LPS, however these are also key myocardial regulators. Identification of this path could reflect some signal contamination from invading inflammatory cells. However, DC gene markers themselves are variably modified, with induction of more widely expressed cytokines (IL-6) and adhesion

molecules (ICAM-1), together with IgG Fc receptor II-b, and IRF8/ICSBP, whereas *Cd83* was repressed and more select DC/myeloid markers and cytokines remained unchanged (*eg. Cd58, Cd86, Ccr7, Trem2, Stat4, Il12* and *Il23*). Torri *et al*. recently identified a 54-transcript suite predictive of LPS activation in DCs [S37]. Of these, we detect changes in only 15 with the majority unaltered. These data are inconsistent with a major contribution or contamination from DC RNAs to the cardiac gene expression profile.

3. Transcriptional Determinants of Endotoxemic Dysfunction

Gene expression data support potential contributions from multiple pathways to the contractile dysfunction arising with endotoxin, including repression of ß-adrenergic signaling, mitochondrial dysfunction, altered Ca^{2+} signaling, and impaired myofibrillar electromechanical coupling. As already discussed, TLR and NFκB signaling paths known to depress contractile function [S38,S39,S40] are also substantially up-regulated (**Figs 5**, **6** and **S8**).

ß-adrenergic and PKA signaling: Data support impairment of ß-adrenergic signaling (**Fig S18**), with reduced transcripts for $ß_1$-adrenergic receptor, G-protein, adenylate cyclase, PKA and related signal components. Repressed *Pkia, Pde7a*, and protein phosphatase-1 components could counter such effects to some degree, reducing inhibition of cAMP and PKA signaling. However, the PKA pathway itself was repressed (**Fig S19**). Thus, positive ß-AR/PKA control of contractile function may be transcriptionally suppressed in endotoxemic myocardium.

Ca^{2+} signaling: Determinants of Ca^{2+} signaling were also substantially modified (**Fig S20**), with largely repression of major regulatory elements. Repression of calsequestrin (*Casq2*) will impact on ryanodine receptor Ca^{2+} channel function, predisposing to premature Ca^{2+} release and arrhythmogenesis, while repression of *Asph*/junctin may impact SERCA2a function and SR Ca^{2+} uptake and arrhythmogenesis (though roles of the protein are poorly defined). Another SERCA2a regulator - histidine-rich Ca^{2+}-binding protein (*Hrc*) - was also repressed. The HRC protein interacts with triadin, which was induced and could sensitize ryanodine receptor channels and

enhance SR Ca^{2+} release. This may compensate for reduced HRC in LPS hearts. Phospholamban was also repressed, and is a well established determinant of SR Ca^{2+} uptake.

Electromechanical coupling: Other myofibrillar and EC-coupling changes are evident in LPS-treated myocardium. For example, LPS down-regulated determinants of cardiac structure and function that are linked to heart failure, hypertrophy and cardiomyopathy: α-tropomyosin (-2), troponin T (-1.5), cardiac alpha actin (-1.6), titin (-2), and PKA γ-subunit PRKAG2 (-2). As already noted, canonical hypertrophy paths were significantly modified (**Figs S16** and **S17**). We also observe profound induction of transcript for Ca^{2+} binding proteins S100a8 and S100a9 (**Table S4**). Wong *et al.* [S36] reported major induction of S100a8 and S100a9 in endotoxemic myocardium, and Boyd et al. [S41] confirmed induction in cardiac tissue, with evidence of involvement in RAGE-dependent contractile depression. The proteins associate with SERCA2a, which may be important in contractile changes.

Mitochondrial dysfunction: Mitochondrial abnormalities are evidenced by: repression of transcripts for multiple respiratory genes within complexes I, II and III (**Fig S21**); and up-regulation of genes for uncoupling proteins, proteins promoting mitochondrial injury and apoptosis, and for the key mediator of mitochondrial biogenesis *Ppargc1a*. Mitochondrial dysfunction is further supported by induction of *Bax, Bak,* and *Bid*, together with ROS generating molecules such as NOX2 (*Cybb/Gp91*), which promote mitochondrial injury and associated apoptosis.

Other potential determinants of endotoxemic dysfunction: Fibroblast factors influence myocyte structure and function and contribute to acute shifts in contractility [S42]. Of 10 factors implicated by LaFramboise *et al.* we detect induction of *Rantes/Ccl5* (+10), *Gro/Kc* (+35), *Mcp1/Ccl2* (+12), and *Il6* (+34), together with up-regulation of *Tgfb2* (+2) and repression of *Tgfb3* (-2). These data support modification of TGF-ß signaling, also evidenced by up-regulation of TNF-α sensitive transcripts (*Myc*, +8; *Ctgf*, +2; *Klf10/Tieg1*, +3; *Tsc22d1/Tgfb1i4*, +2) and growth factor receptor *Tgfbr2* (+2), together with repression of *Ltbp1* (-2) and *TgfbI* (-2).

Another possible determinant of evolving contractile dysfunction is substrate metabolism.

Feingold et al. [S43] documented repression of key components of fatty acid uptake and metabolism during the acute phase response, including nuclear hormone receptors, co-activators required for transcriptional activities of RXR-PPAR and RXR-thyroid receptors, and target enzymes of fatty acid handling. Since we observe dysfunction in hearts supplied with glucose as sole substrate, shifts in fatty acid metabolism are unlikely to be the primary driver of contractile dysfunction. Nonetheless, they may impact *in vivo*, and we do detect modulation of orphan nuclear receptor (RXR, PXR, FXR) and PPARα signaling paths by LPS (**Table S5**). This again largely involves gene repression, with reduced transcripts for RXRγ, THRα, THRβ, PPARα, NCOA1, CREB-binding protein and co-activators PPARGC1α and PPGARC1β.

Additional to evidence of pathway dysfunctions outlined above, several individual gene changes are relevant to contractile dysfunction following LPS challenge. IGF-1 was substantially reduced (-5), and exerts positive inotropic effects in post-ischemic [S44] and potentially healthy myocardium [S45], and can alleviate LPS-induced dysfunction [S46]. Repression and alterations in IGF-1 signaling (**Fig S22**) may thus promote evolving cardiac dysfunction. Myocardial *Icam1* (+9) and *Vcam1* (+3) have been previously shown to be up-regulated by LPS, and may contribute to contractile dysfunction [S47]. Increases in ICAM-1 and VCAM-1 can also enhance injury via increased neutrophil adhesion and ROS generation [S48]. NOX2 (*Cybb/Gp91*) was also induced several-fold by LPS, generates superoxide and TNFα in LPS-challenged myocytes [S49], and may exert a cardiodepressant action. COX-2 also enhances TNFα and cardiac depression in sepsis, and was induced 2- to 3-fold here. Although IL1ß and TNFα are implicated in LPS-induced cardiac depression, we detect rather modest IL1ß induction (+1.5) at 24 hrs and no change in TNFα. On the other hand, *Il1ß* sensitive *Ptx3* was elevated 31-fold and TNFα sensitive *Tnfaip3* (MAD6) 9-fold. It is possible very early shifts in IL1ß and TNFα are missed at 24 hrs, whereas downstream targets of these signals remain modified. Transcripts for the TNFα receptors *Tnfr1* and *Fas* were also up-regulated, as was LPS-induced TNF transcription factor (*Litaf*), a mediator of inflammation that promotes cytokine expression (including TNFα) and reduces survival in sepsis.

4. Comparison With Prior Transcriptional Analyses

In more restricted array analysis of LPS-induced myocarditis (from <9000 genes and ESTs), Wong *et al.* [S36] identified 592 transcript changes at 24 hrs, consistent with roles for: enhanced antigen–antibody complex removal; cardiac remodeling/cell turnover; leukocyte chemotaxis; enhanced immunoactivation; and thrombophilia. They observed expansion of cardiac transcriptional responses over 24 hr following LPS challenge, consistent with subsequent data supporting a mix of transient and more sustained shifts in gene transcription after 6 or 24 hrs [S50]. We assessed transcriptional responses at 24 hrs, as this broader and less transient profile may be more informative regarding evolving cardiac dysfunction and myocarditis. Many responses highlighted by Wong *et al.* are also evidenced here. As already noted, antigen-antibody removal/complement and cardiac remodeling are highly influenced by LPS. Chemotaxis signaling is also apparent (induction of MIP1α receptor, GRO, PLAUR, ICAM-1, VCAM-1, P-selectin, L-selectin, and E-selectin among others), with modulation of the acute phase response and DC maturation (**Table S5, Fig S4**). Immunoactivation is reflected in up-regulation of innate immunity pathways, including the acute phase response (**Fig S4**), TLR (**Fig 5**), interferon and IRF (**Figs 5** and **S5**), and PRR/RIG-1 like signaling pathways (**Figs S6** and **S7**). Thrombophilia was not identified here, though could arise through observed *Serpine1* induction, although *Serpine2* (an inhibitor of thrombin, urokinase, and plasmin) and thrombolytic PLAUR were also up-regulated.

More recently Rudiger *et al*. reported on transcriptomic changes in hearts from a rat model of early sepsis (following faecal peritonitis) [S51]. Their observations generally mesh with the current study, though with some differences. They report several major changes akin to those observed in LPS-treated mice, including up-regulation of TLR2/MyD88 and JAK-STAT inflammatory pathways, and repression of β-adrenergic signaling and cellular Ca^{2+} handling. We also report amplification of TLR/MyD88 and JAK-STAT signal paths, though in terms of TLR signaling we observe up-regulation of both MyD88-dependent and independent pathways, with greater amplification of TLR4/CD14 signal elements in the mouse *vs.* the rat (**Fig 5**). Similarly,

while we also report repression of adrenergic and Ca^{2+} signaling (**Figs S18** and **S20**), changes appear more extensive in the murine endotoxemia model, which additionally exhibits impaired downstream PKA signaling (**Fig S19**).

While the above studies are the only broad-scale interrogations of myocardial transcription during inflammatory challenge, Goren *et al*. [S52] examined inflammatory gene expression in cultured myocytes. They highlighted NFκB-sensitive genes, many responsive in the current model, including induction of transcripts for ICAM1, IκBß, NFκB2/p100, TNFAIP3/A20, COX-2/PTGS2, lipocalin-2, MMP9, CSF1, CSF3, CXCL9, LBP, LDLR, and JunB. As already outlined, cardiac NFκB signaling is highly responsive to LPS (**Fig S8**). However, distinct from outcomes in the intact organ, Goren and colleagues suggest the isolated cardiomyocyte NFκB response is attenuated, thereby limiting cellular sensitivity to LPS and associated injury.

In another cell-based study Maresh *et al.* [S53] assessed effects of LPS on cardiac endothelial gene transcription, identifying >100 fold induction of *Lcn2* (the most highly induced in the current study; +590) and *Cxcl2* (+36 in hearts here), together with major up-regulation of *Isg20, Igtp, Cts1, Pde4b* and *B2m*, which were also induced by LPS in hearts (**Tables S4** and **S5**). Unique induction of *Jak1* was not detected in the heart, though *Jak2* was increased ~3-fold. They also reported repression of *Gfra2*, which is similarly reduced by LPS in heart. Thus, many responses reported for cardiac endothelium are generally replicated in the intact myocardium.

Kristof *et al.* [S54] examined STAT1-dependent gene induction in epithelial adenocarcinoma cells subjected to LPS/IFN-γ treatment, detecting a sub-set of pro-apoptotic and pro-inflammatory genes sensitive to mTOR/PI3K and STAT1. We detect profound induction of *Stat1*, *Stat2* and *Stat3* (+3 to +12) and modulation of JAK/Stat signaling (**Fig S9**), while mTOR signaling appears repressed by LPS (~3-fold increase in inhibitory *Eif4ebp1*, 3-fold repression of the target *Eif4e*). Beneficial effects of mTOR on protein translation may thus be impaired in endotoxemia. We also detect modulation of many apoptosis transcripts previously identified by Kristof and colleagues (*Cflar, Bid, Fas, Hsp40, Nmi*). Diverse paths of apoptosis/cell death thus

appear to be up-regulated in endotoxemic tissues (**Tables S7** and **S8**; **Figs S13** and **S14**).

5. Effects of $A_{2A}R$ KO on the Myocardial Response to LPS

Table S9 details cardiac gene responses to LPS that are either enhanced or repressed by $A_{2A}R$ KO, with **Table S10** showing toxicological processes in LPS hearts sensitive to $A_{2A}R$ KO. Many of the latter responses reflect a damping of changes in adiponectin (*Adipoq*) and heme-oxygenase 1 (*Hmox1*), together with amplification of responses for *Serpine1* (encoding PAI-1), *Kckn3* (encoding TWIK-related acid-sensitive K^+ channel-1, or TASK1) and *Ppargc1a* (PPARγ co-activator 1a), all of which are potentially relevant to myocardial outcomes. Anti-inflammatory adiponectin reduces LPS and ischemic damage [S22,S25,S55], and repression may thus promote cardiac injury. Curiously, this response appears partially reliant upon intrinsic $A_{2A}R$ activity. Conversely, induction of anti-oxidant and anti-inflammatory heme-oxygenase 1 following LPS may reflect a protective response [S56], which appears to be augmented by ~40% by $A_{2A}R$ activity. Near doubling of *Serpine1* induction by KO indicates $A_{2A}Rs$ reduce the LPS-responsiveness of this transcript, which may also be beneficial since encoded PAI-1 predicts unfavorable outcomes in sepsis. However, KO studies also support protective and anti-inflammatory roles for PAI-1 [S57, S58]. *Ppargc1a* encodes PPARγ co-activator 1a (PGC-1α), key to fatty acid metabolism and mitochondrial biogenesis [S59], and prior studies report its repression by LPS (which is implicated in detrimental outcomes) [S60]. Myocardial repression with LPS, a response that may exaggerate mitochondrial dysfunction, is exaggerated in the absence of $A_{2A}Rs$. Sweeney *et al.* [S61] recently reported a novel MyD88-independent path linking TLR2/TLR4 signaling to *Ppargc1a* regulation. While we observe induction of elements of this path here (including *Tlr2*, *Tlr4*, *Tlr3* and *Irf7*), *Ppargc1a* was repressed in LPS-treated myocardium.

Repressed *Kcnk3*/*Task1* encodes an outwardly rectifying K^+ channel protein sensitive to extracellular pH and anesthetics, that is implicated in electrophysiological dysfunction/remodeling in heart failure. Down-regulation with LPS may be detrimental, a response also countered by $A_{2A}R$

activity.

LPS responses counteracted by $A_{2A}R$ activity: The responses of 294 LPS-sensitive genes were increased by (in some cases required) $A_{2A}R$ KO (**Table S9**). Many of these $A_{2A}R$-sensitive transcripts were associated with pro-inflammatory signaling, with data supporting inhibitory effects of the $A_{2A}R$ on acute phase response, glucocorticoid receptor, Erk/MAPK, HIF1α and JAK/Stat signaling responses to LPS (**Tables 6** and **7**). The $A_{2A}R$-dependence of these paths can be linked to a small number of transcripts - by inhibiting responses of this gene sub-set (*Rras2, Stat1, Vegfc, Pgf, Pak6*), key to multiple cellular processes, intrinsic $A_{2A}R$ activity may influence a range of LPS-responsive pathways.

The Ras-like small GTPase r-Ras2 activates Raf/MAPK signaling and PI3K and NFκb responses [S62], and is important to cell proliferation and survival. Induction may promote inflammatory signaling in response to LPS, with $A_{2A}R$ activity limiting this change. STAT-1 underpins many cardiac responses, including myocyte death, and is activated by IFNα/γ EGF, PDGF and IL-6 (among other factors). As outlined by Kristof *et al.* [S54], STAT-1 is key to LPS-induced apoptosis in other cell types. Myocardial induction, potentially involved in cardiac inflammation and damage with LPS, is dampened by $A_{2A}R$ activity. Interestingly, recent work supports a novel anti-inflammatory action of $A_{2A}Rs$, promoting the polyubiquitylation and proteasomal degradation of JAK-phosphorylated STATs [S63]. The current data suggest an additional mechanism, whereby $A_{2A}Rs$ may transcriptionally repress STAT-dependent signaling.

Absence of $A_{2A}Rs$ promoted transcriptional responses of VEGF-c and placental growth factor (PGF) to LPS. VEGF-c and its receptors are highly expressed in heart, and regulate lymphangiogenesis and coronary vascularization [S64]. While VEGF's are induced by hypoxia/ischemia via PGC-1α and HIF-1α, VEGF-c transcript was repressed by LPS (possibly reflecting repression of regulatory PGC-1α). This response was almost halved by $A_{2A}R$ activity. On the other hand, PGF (known to be induced with hypoxia/ischemia, and promoting angiogenesis) was induced >5-fold with LPS. Yano *et al*. [S65] found that systemic PGF reduces mortality in sepsis, though

myocardial actions are unclear. Induction, adaptive or otherwise, appears to be limited by intrinsic $A_{2A}R$ activity.

The ATF4 transcription factor (or CREB-2) was induced with LPS, and is involved in PERK control of apoptosis and the UPR, autophagy activation by ER stress or hypoxia [S66], and mediation of muscle atrophy [S67]. Repression of the response by $A_{2A}Rs$ may be protective given evidence ATF4 inhibition reduces cell damage in other tissues [S68].

Repression of *Kcnip2* with LPS was also doubled in the absence of $A_{2A}Rs$. Since the encoded protein positively regulates cardiac ion currents, with repression potentially contributing to electrophysiological abnormalities in endotoxemia [S69,S70], $A_{2A}R$ inhibition of the response may be protective. The ankyrin repeat and suppressor of cytokine signaling box-containing protein (ASB) 15 activates protein synthesis, muscle differentiation and growth via PI3K/Akt/mTOR and MAPK signaling [S71], which may reflect adaptation to myocyte stress. Repression by LPS could impair such adaptation, a response countered by $A_{2A}R$ activity. LPS-dependent induction of transcripts for cyclin-dependent kinase inhibitor 1A (*Cdkn1a*) and its target kinases (*Pak6*, *Pak3*) was enhanced up to 2-fold by $A_{2A}R$ KO. *Cdkn1a* is a stress-responsive transcript induced with infarction [S72], the encoded protein inhibiting cyclin A-associated kinase activity and cardiac apoptosis [S73]. Cyclin-dependent kinase inhibitor 1A also regulates phenotypic switching of cardiac fibroblasts to myofibroblasts [S74], maintains myocyte withdrawal from the cell cycle [S75], and promotes cardiac inflammation [S76]. Inhibition of the *Cdkn1a* response by $A_{2A}Rs$ may this limit injurious inflammation and modify remodeling effects of LPS.

LPS responses promoted by $A_{2A}R$ activity: The LPS-responsiveness of 134 genes was partially or entirely countered by $A_{2A}R$ KO (**Table S9**). These changes support modulation of canonical pathways including orphan nuclear receptor and PPARα activation, PRR responses to infection, AMPK and NFκB signaling, together with several cardiac toxicological functions (**Tables 6**, **7**, **S9** and **S10**). Many responses can again be linked to a small sub-set of genes (**Table 6**): through facilitating changes in 6 transcripts (*Rac2*, *Nfkbie*, *Prkar2b*, *Hmox1* and *Eif2ak2*) $A_{2A}R$

activity can impact most of the top 50 modified pathways. These data support $A_{2A}R$ modulation of key signaling responses, including G-protein, PKA, Stat1 and NF-κB dependent processes.

Interestingly, $A_{2A}R$ KO represses the LPS responsiveness of *Rac2*, involved in control of kinase signaling, cell growth, cytoskeletal organization and inflammatory processes. Encoded RAC2 is crucial for PI3K-dependent neutrophil chemotaxis and inflammation [S77,S78], and enhances nuclear signaling and IFN-ß induction via NFκB. Reduced induction following $A_{2A}R$ KO (**Tables 6** and **S9**) suggests $A_{2A}Rs$ promote this response. Deletion of $A_{2A}Rs$ also nearly halved induction of *Nfkbie* (encoding the NFκB signal inhibitor IκBε). The $A_{2A}R$ may thus be important in inducing inhibitory IκBε during sepsis, limiting detrimental NFκB signaling. As already discussed, *Hmox1* induction may be protective, and this response is promoted by $A_{2A}R$ activity. Eukaryotic translation initiation factor 2-alpha kinase 2 (*Eif2ak2*) or PKR (double-stranded RNA-activated protein kinase) is a critical cell regulator induced by LPS, interferon, TNF-α and dsRNA. PKR induces inflammatory cytokine expression via NF-κB- and p38-dependent signaling [S79,S80]. Curiously, approximately 4-fold induction with LPS was reduced by $A_{2A}R$ KO, supporting some $A_{2A}R$ dependence of this response.

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Fig. S1. *Adora* gene expression in hearts from WT and $A_{2A}R$ KO mice.

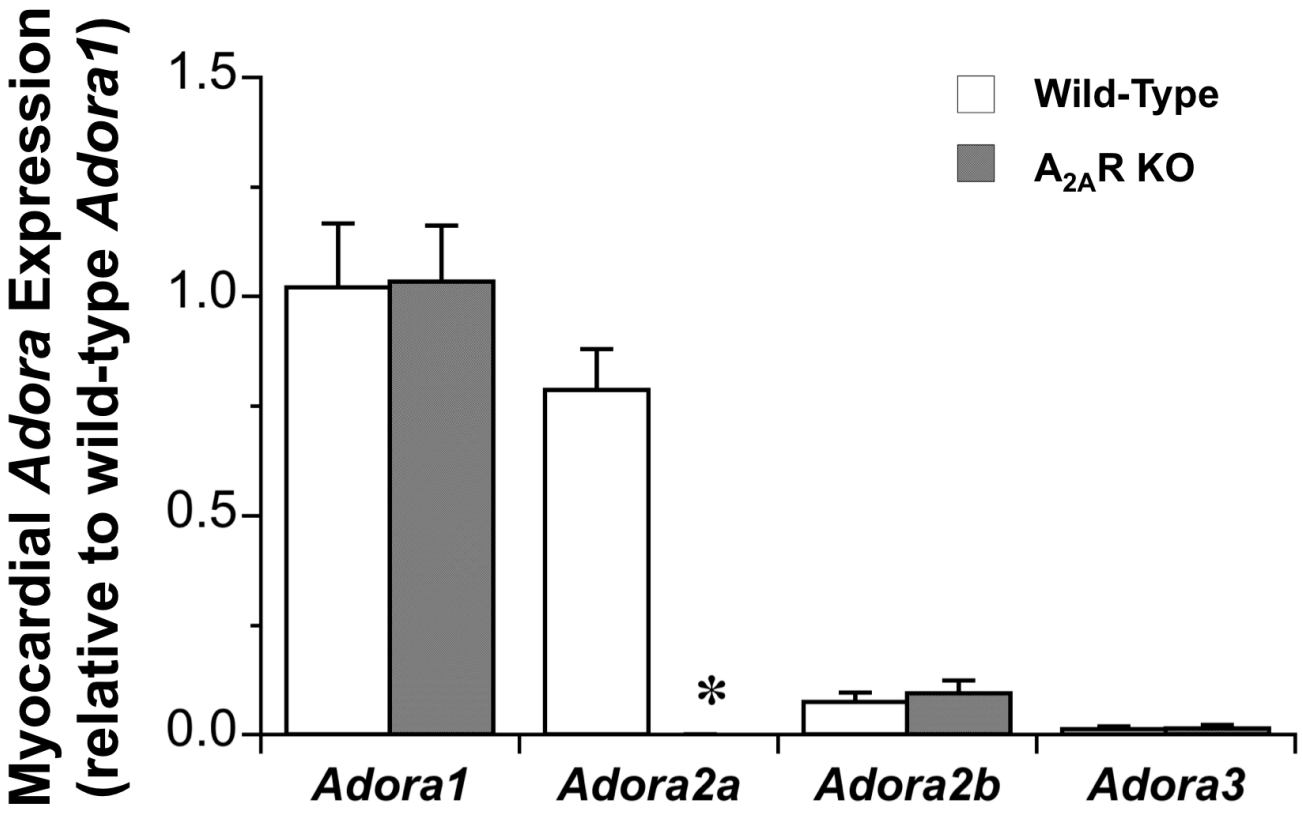


Fig. S2. Summary of analysis employed in interrogating array data. The array platform consisted of ~39,000 probe sets. After RMA normalization and data reduction of probe sets, 25,646 reliable genes remained for statistical analysis. Two-factor ANOVA (P<0.005) and multi-class SAM (FDR<1.0%) were independently performed to generate 2 sets of significantly expressed genes. These sets were compared, resulting in 8,110 consistently expressed genes for ratio analysis and annotation.

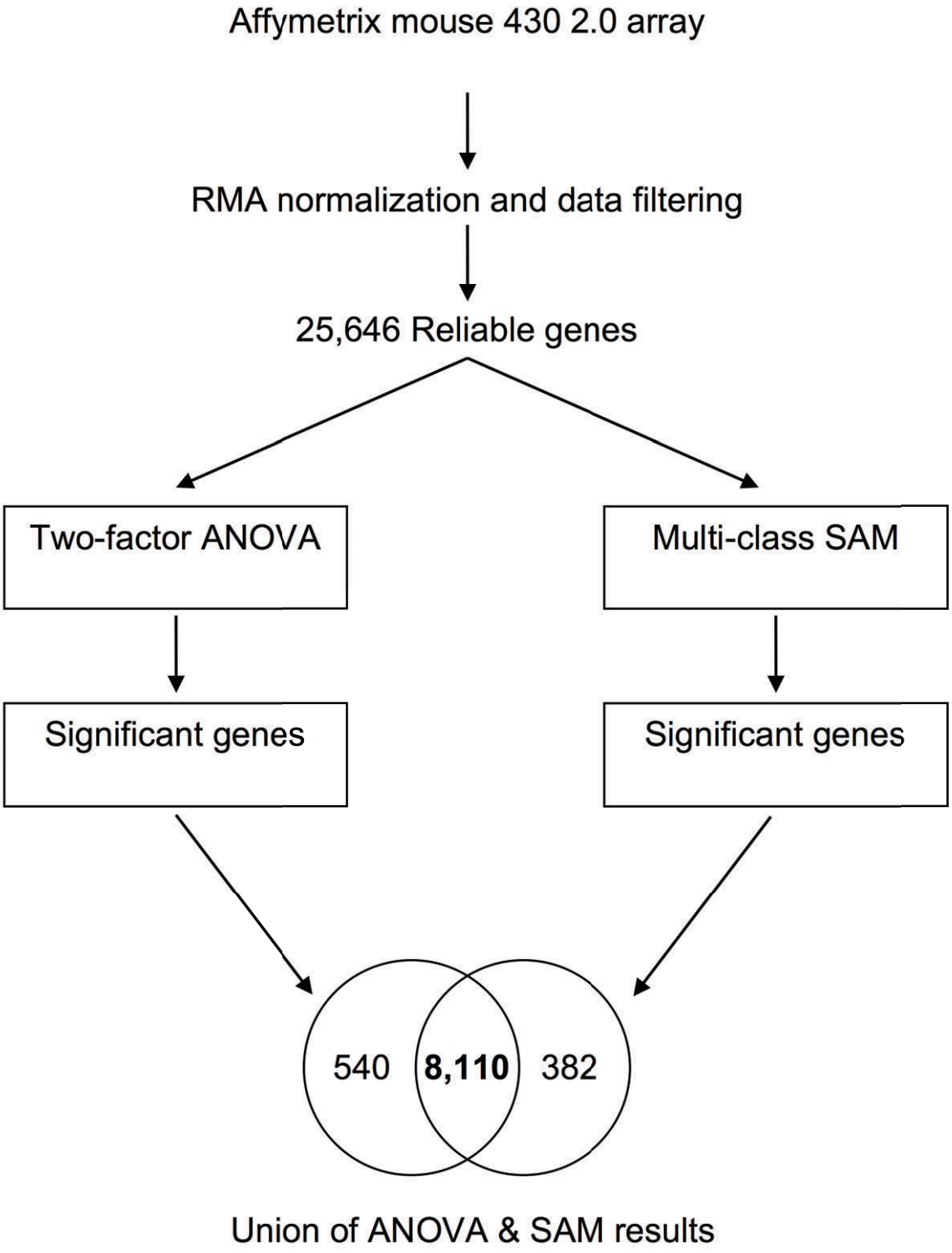
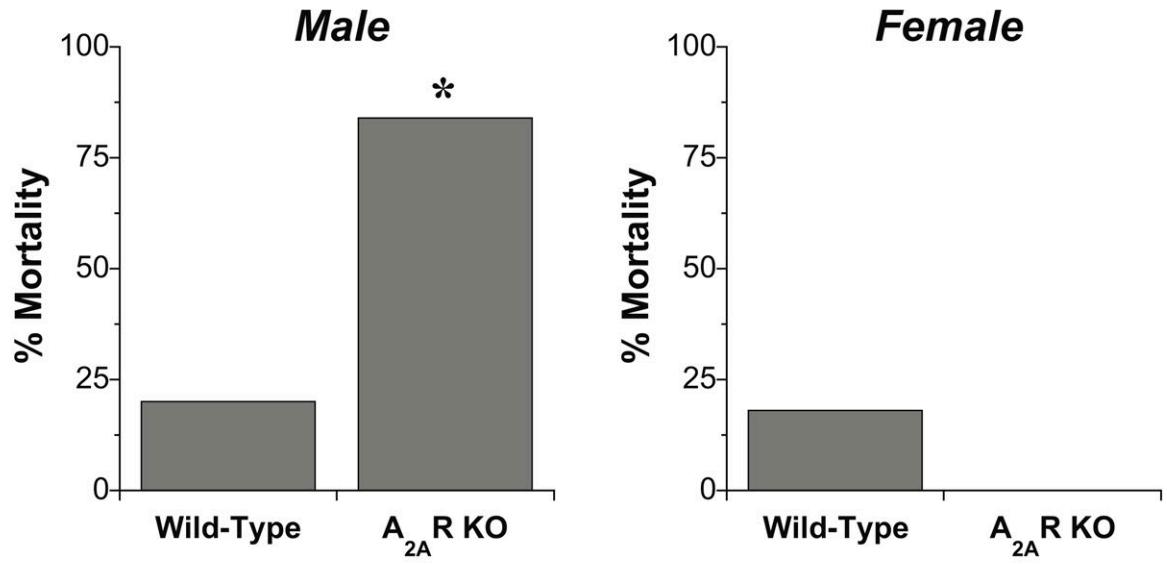


Fig. S3. Mortality in old mice (10-12 months) over 24 hrs LPS challenge. Wild-type and $A_{2A}R$ KO mice (male and female) were injected with 20 mg/kg LPS or saline vehicle and monitored for 24 hrs prior to tissue sampling. Note that no mortality was evident in young (2-3 month) mice ultimately assessed for transcriptomic responses in this study. Mortality was assessed in WT (*n*=6 male, *n*=6 female) and $A_{2A}R$ KO (*n*=7 male, *n*=5 female) mice. Data are means ± S.E.M. * $P < 0.05$ $A_{2A}R$ KO *vs.* corresponding WT mice.



Key for subsequent signaling diagrams:

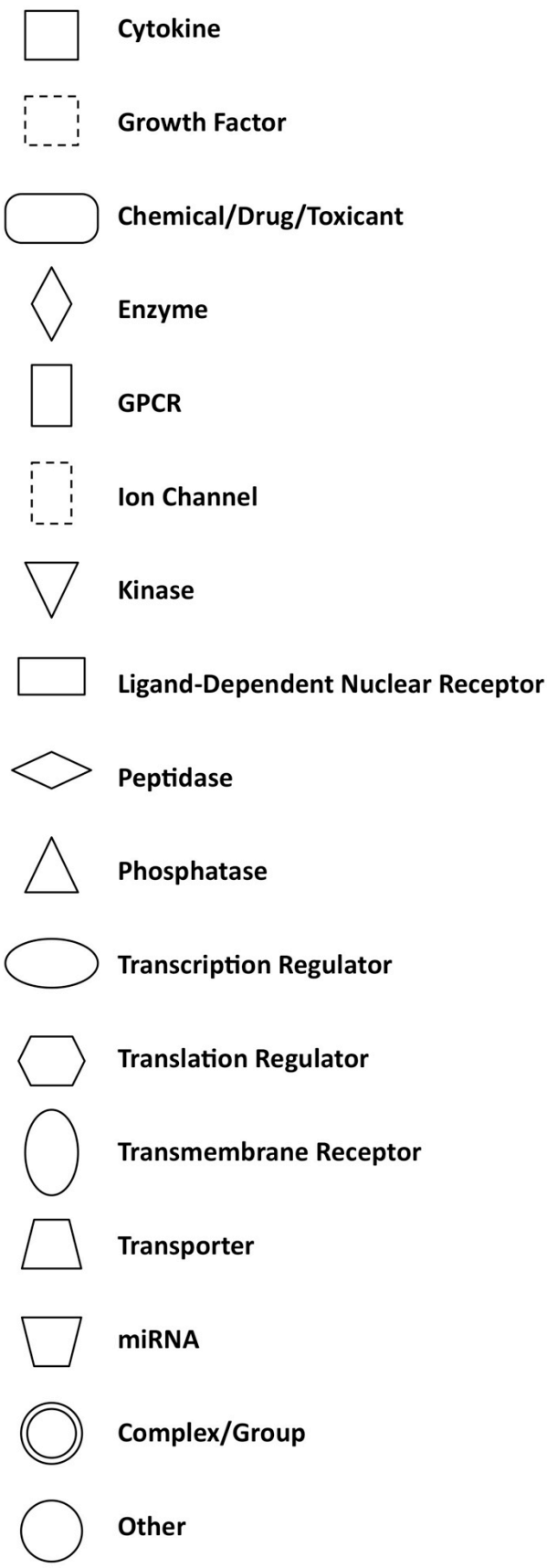


Fig. S4. Diagrammatic representation of transcriptional effects of LPS on the acute phase response after 24 hrs of treatment (red, induction; green, repression).

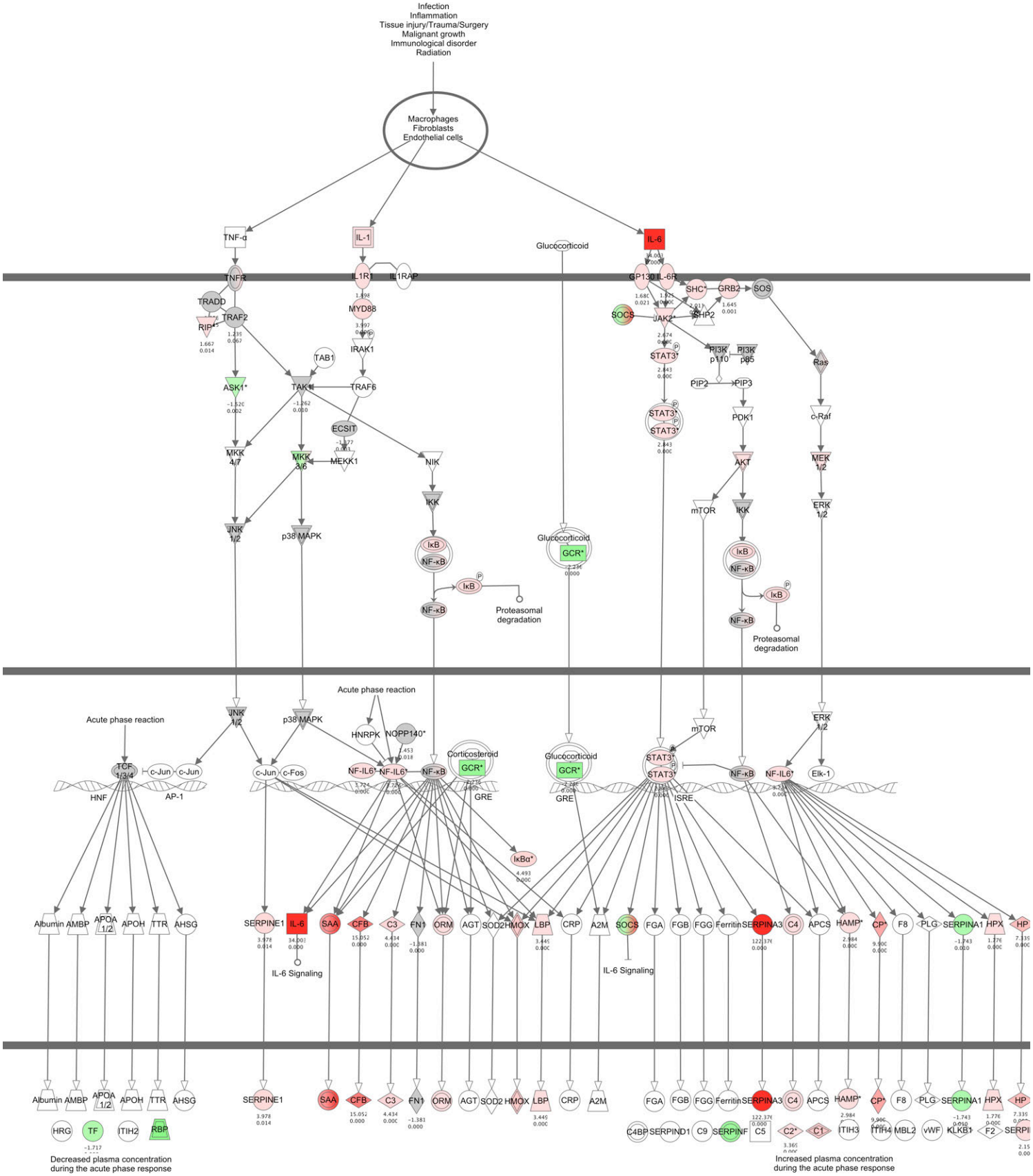


Fig. S5. Diagrammatic representation of transcriptional effects of LPS on IRF activation by cytosolic PRRs after 24 hrs of treatment (red, induction; green, repression).

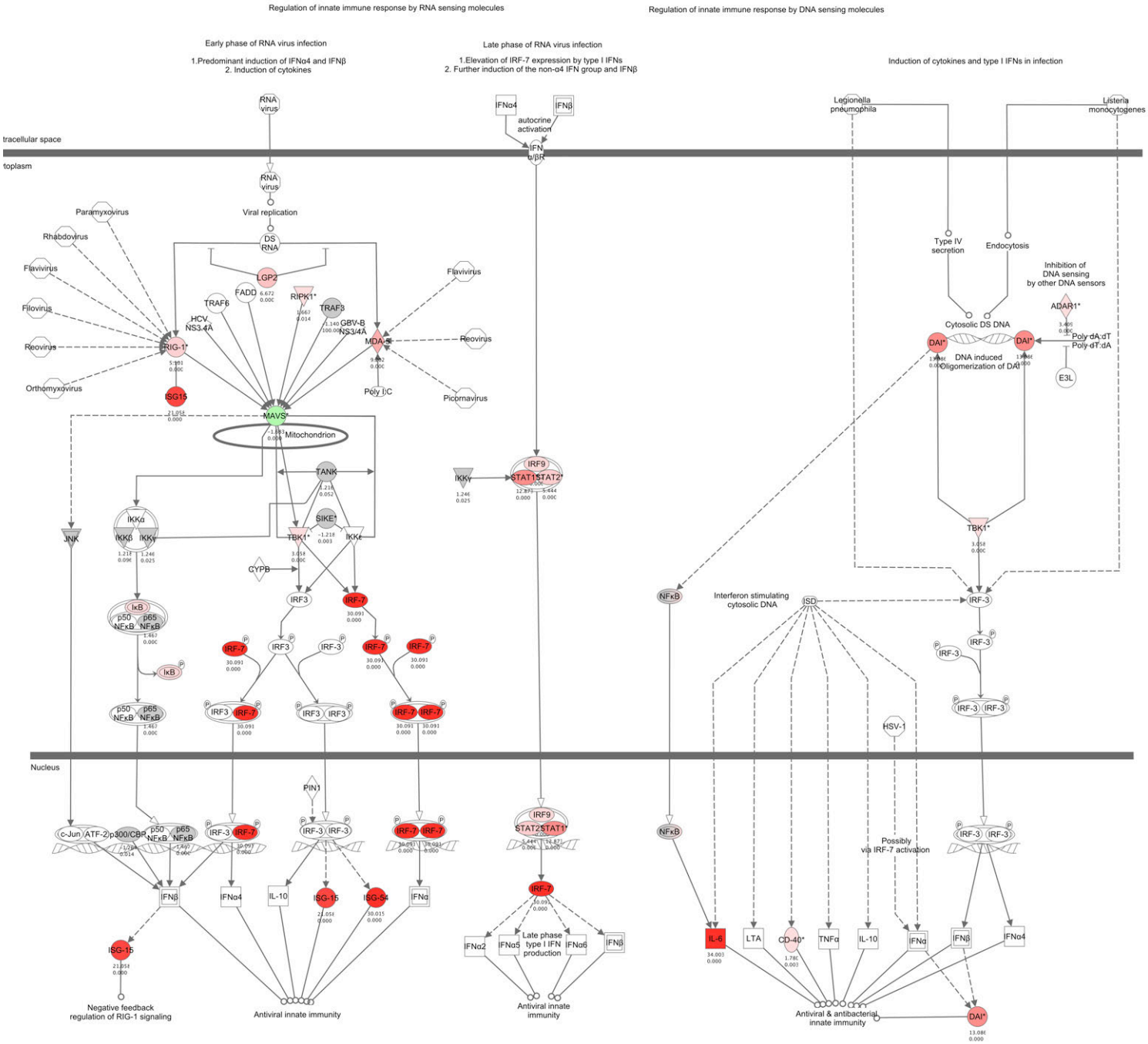


Fig. S6. Diagrammatic representation of transcriptional effects of LPS on PRR involvement in infection, after 24 hrs of treatment (red, induction; green, repression).

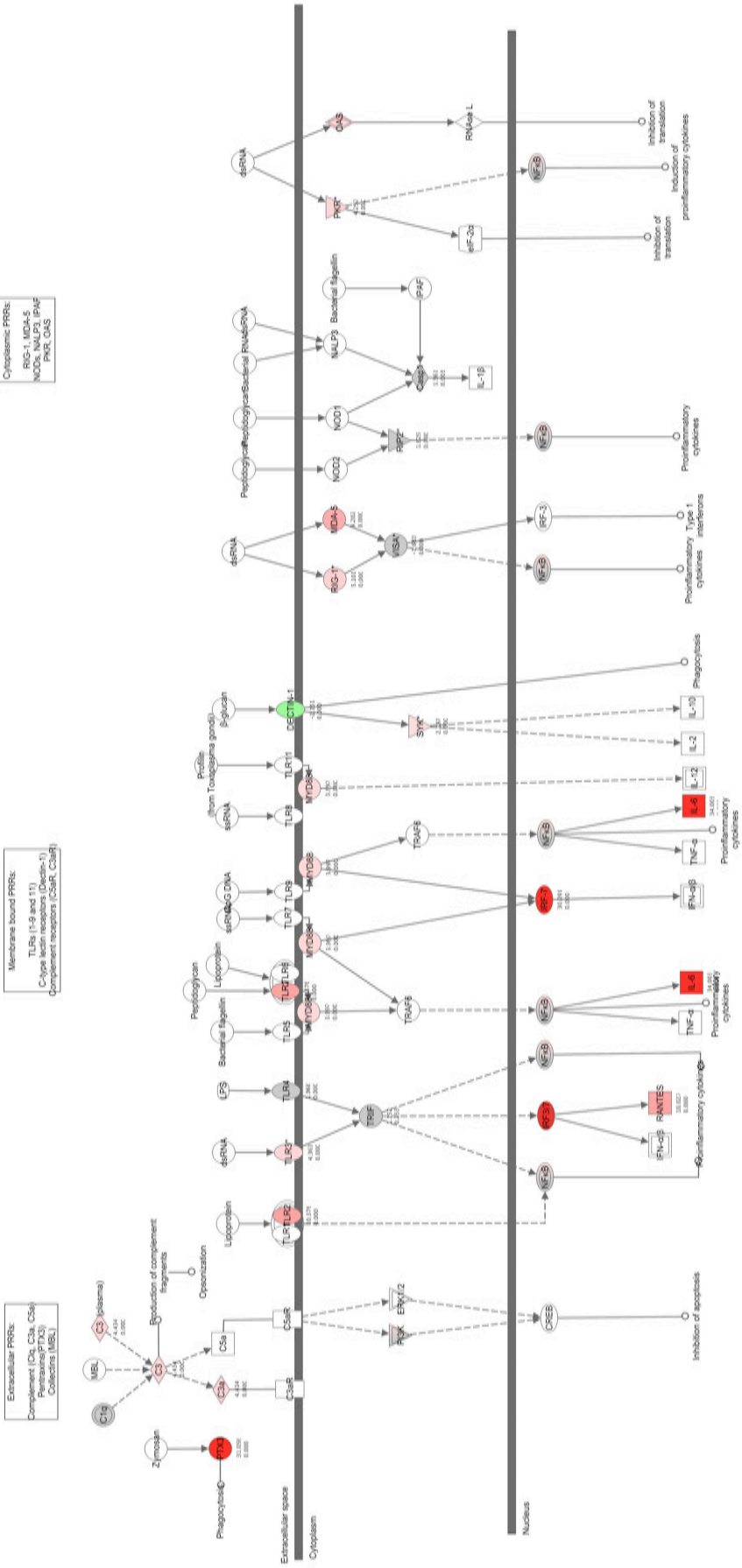


Fig. S7. Diagrammatic representation of transcriptional effects of LPS on RIG-1 like signaling in immunity, after 24 hrs of treatment (red, induction; green, repression).

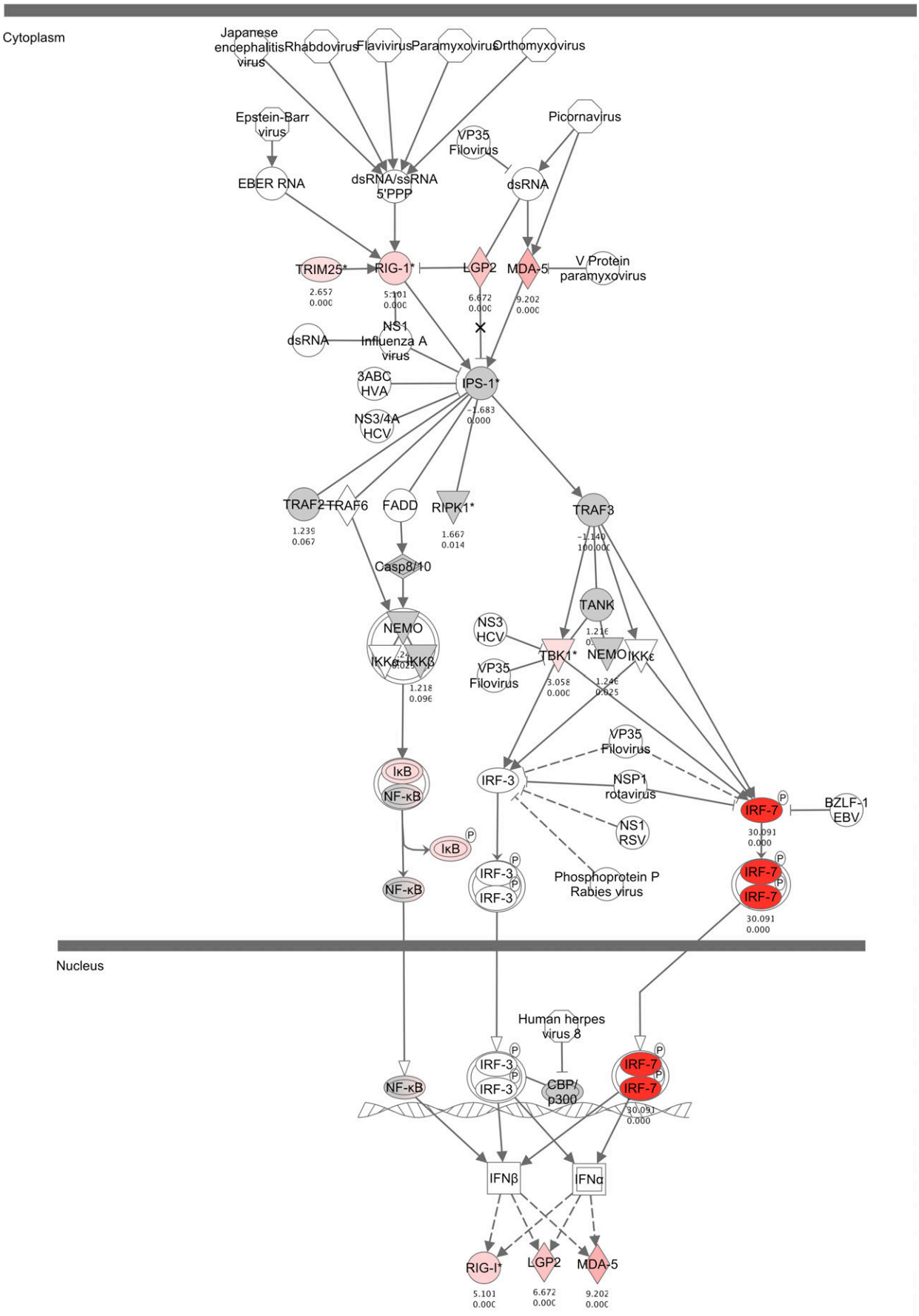


Fig. S8. Diagrammatic representation of transcriptional effects of LPS on NfκB signaling, after 24 hrs of treatment (red, induction; green, repression).

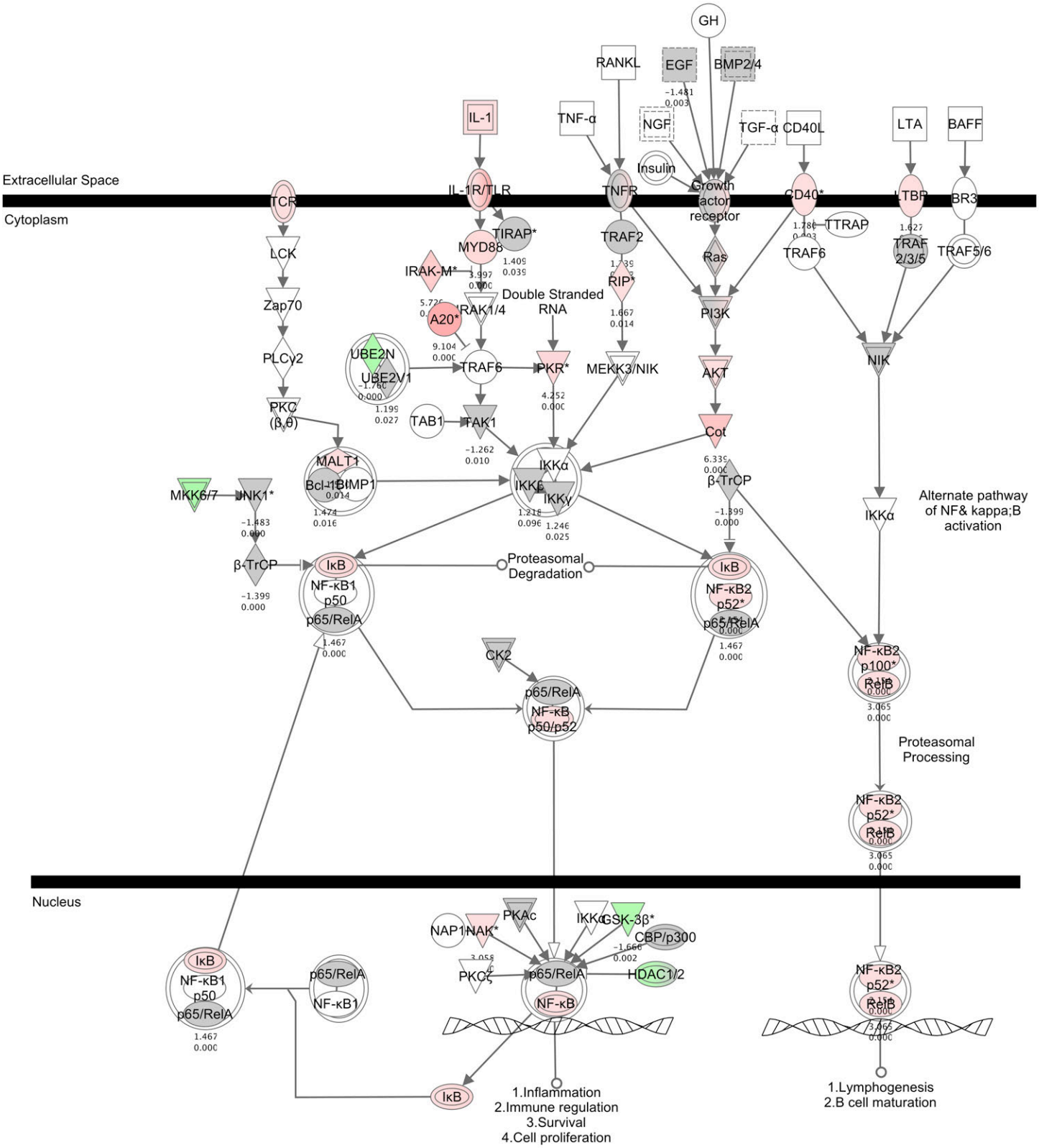


Fig. S9. Diagrammatic representation of transcriptional effects of LPS on JAK/Stat signaling, after 24 hrs of treatment (red, induction; green, repression).

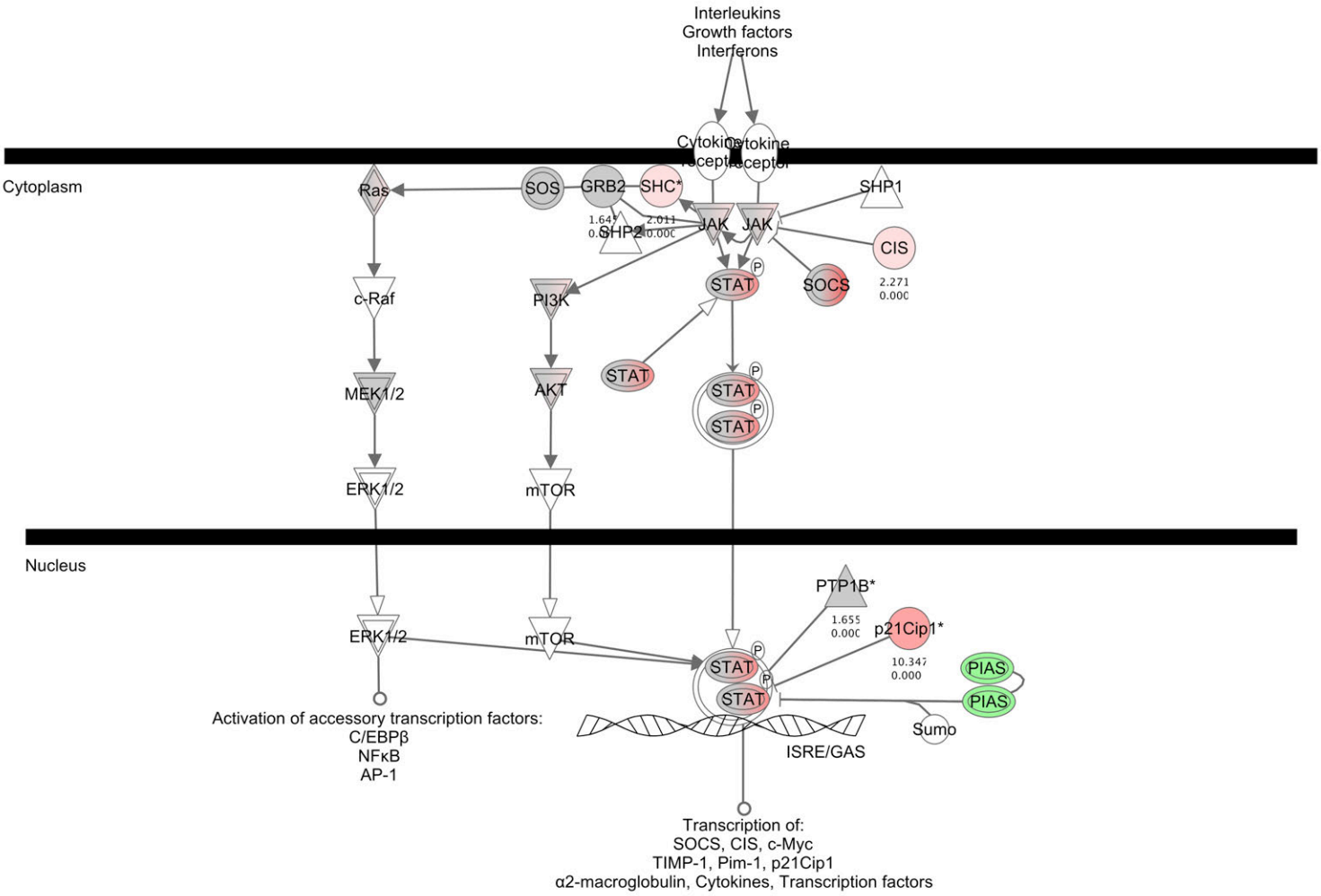


Fig. S10. Diagrammatic representation of transcriptional effects of LPS on MAPK activation by LPS signaling, after 24 hrs of treatment (red, induction; green, repression).

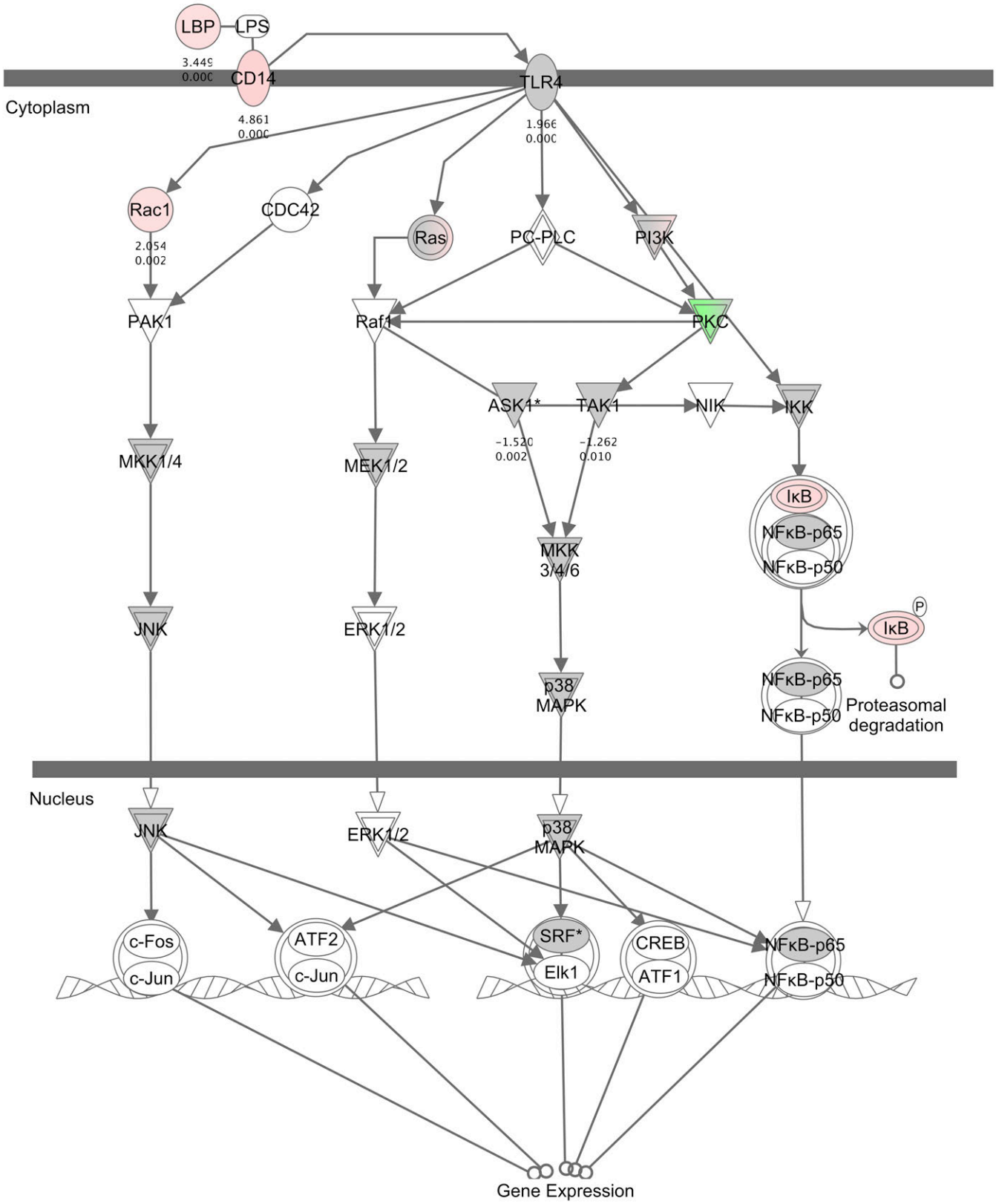


Fig. S11. Diagrammatic representation of transcriptional effects of LPS on p38-MAPK signaling, after 24 hrs of treatment (red, induction; green, repression).

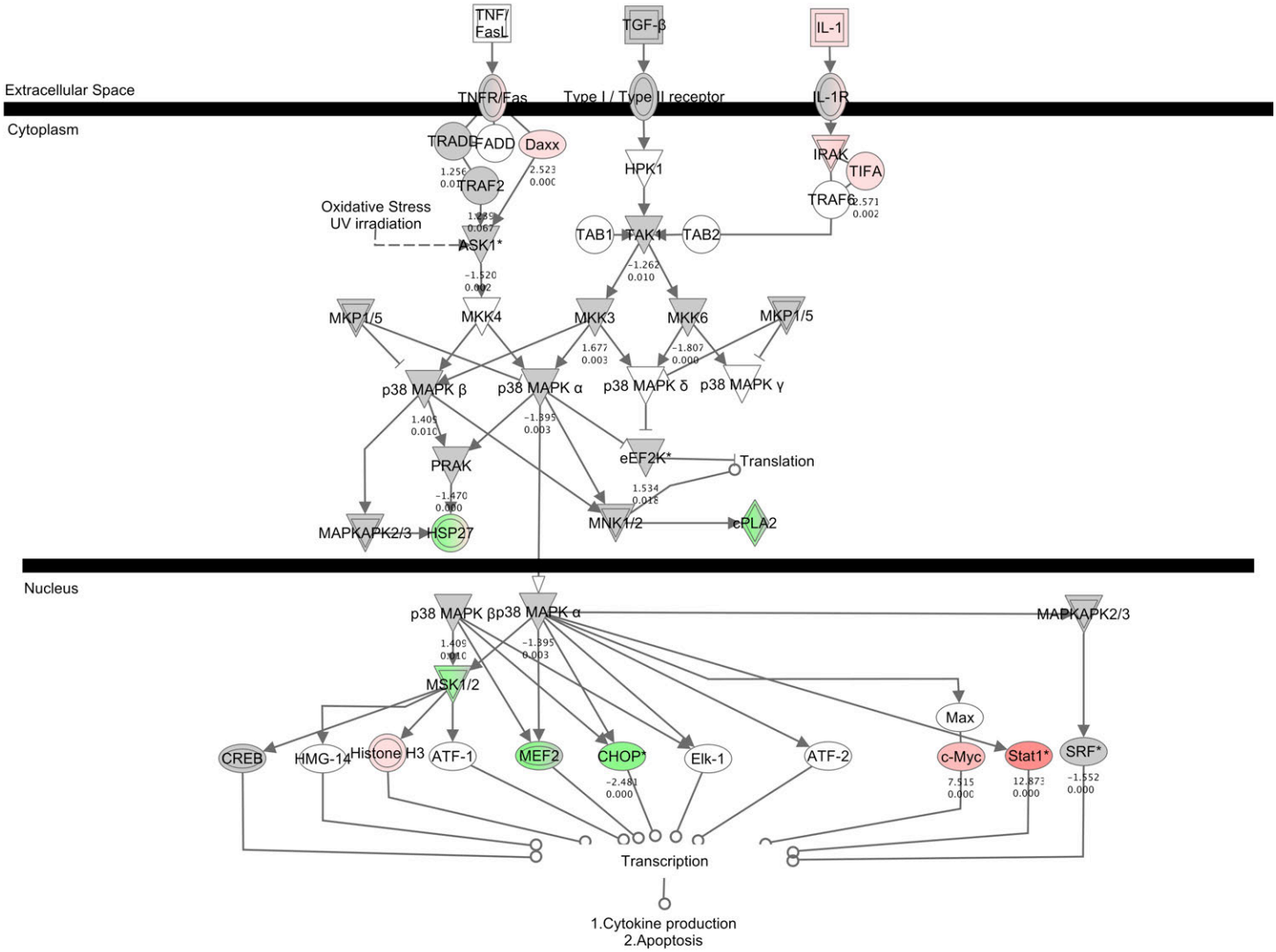


Fig. S12. Diagrammatic representation of transcriptional effects of LPS on PI3K signaling, after 24 hrs of treatment (red, induction; green, repression).

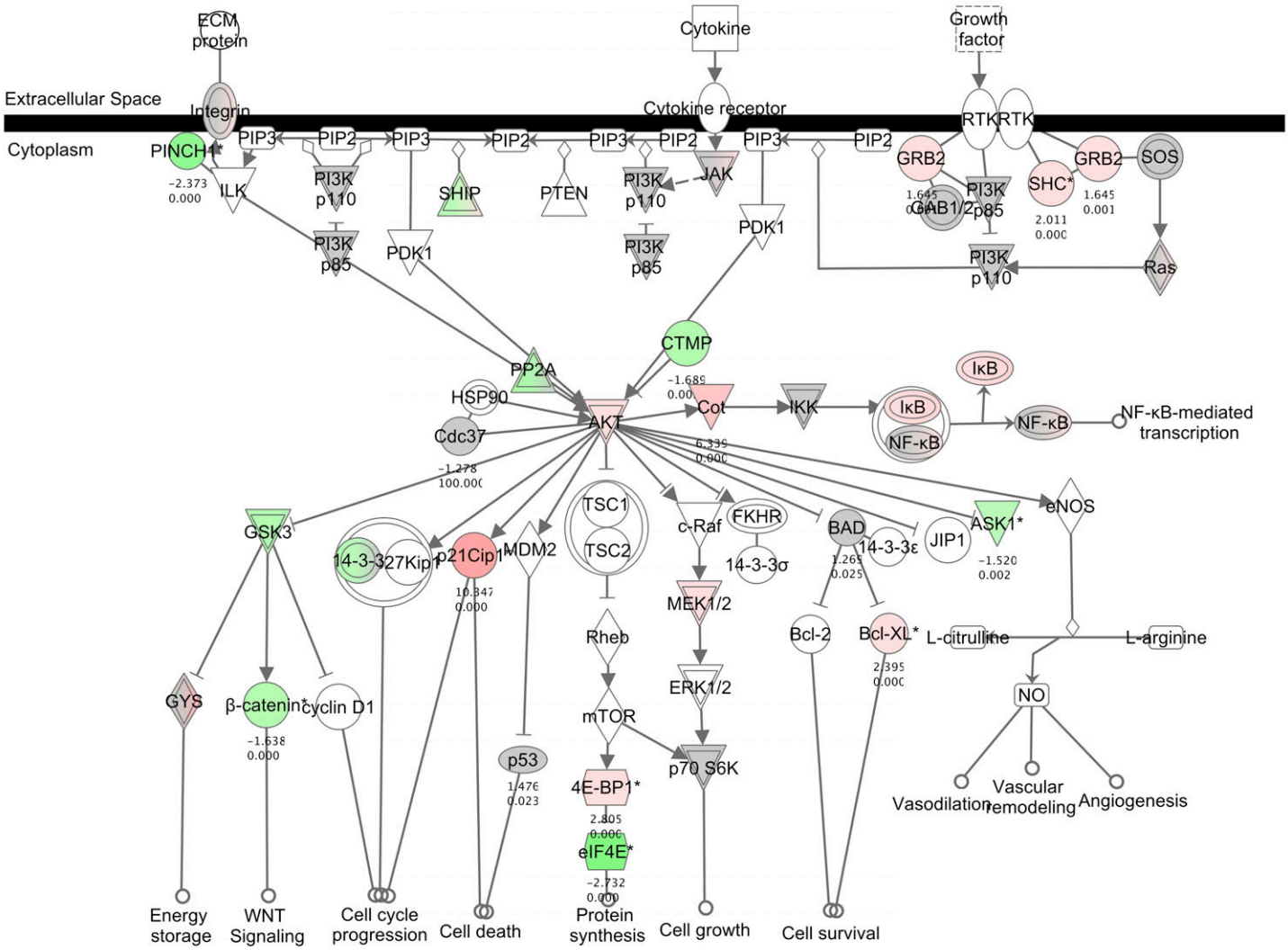


Fig. S13. Diagrammatic representation of transcriptional effects of LPS on apoptosis signaling, after 24 hrs of treatment (red, induction; green, repression).

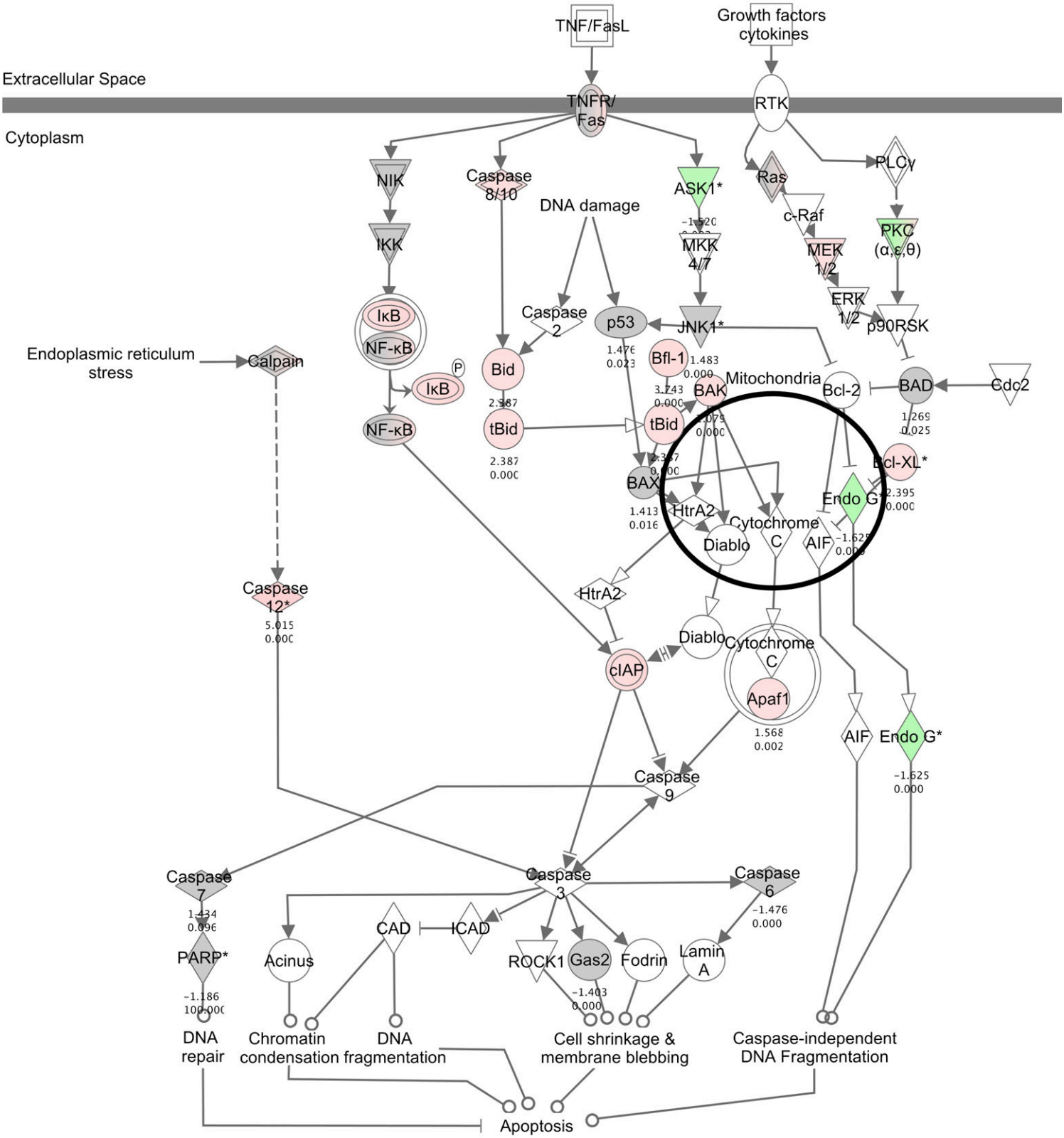


Fig. S14. Diagrammatic representation of transcriptional effects of LPS on death receptor signaling, after 24 hrs of treatment (red, induction; green, repression).

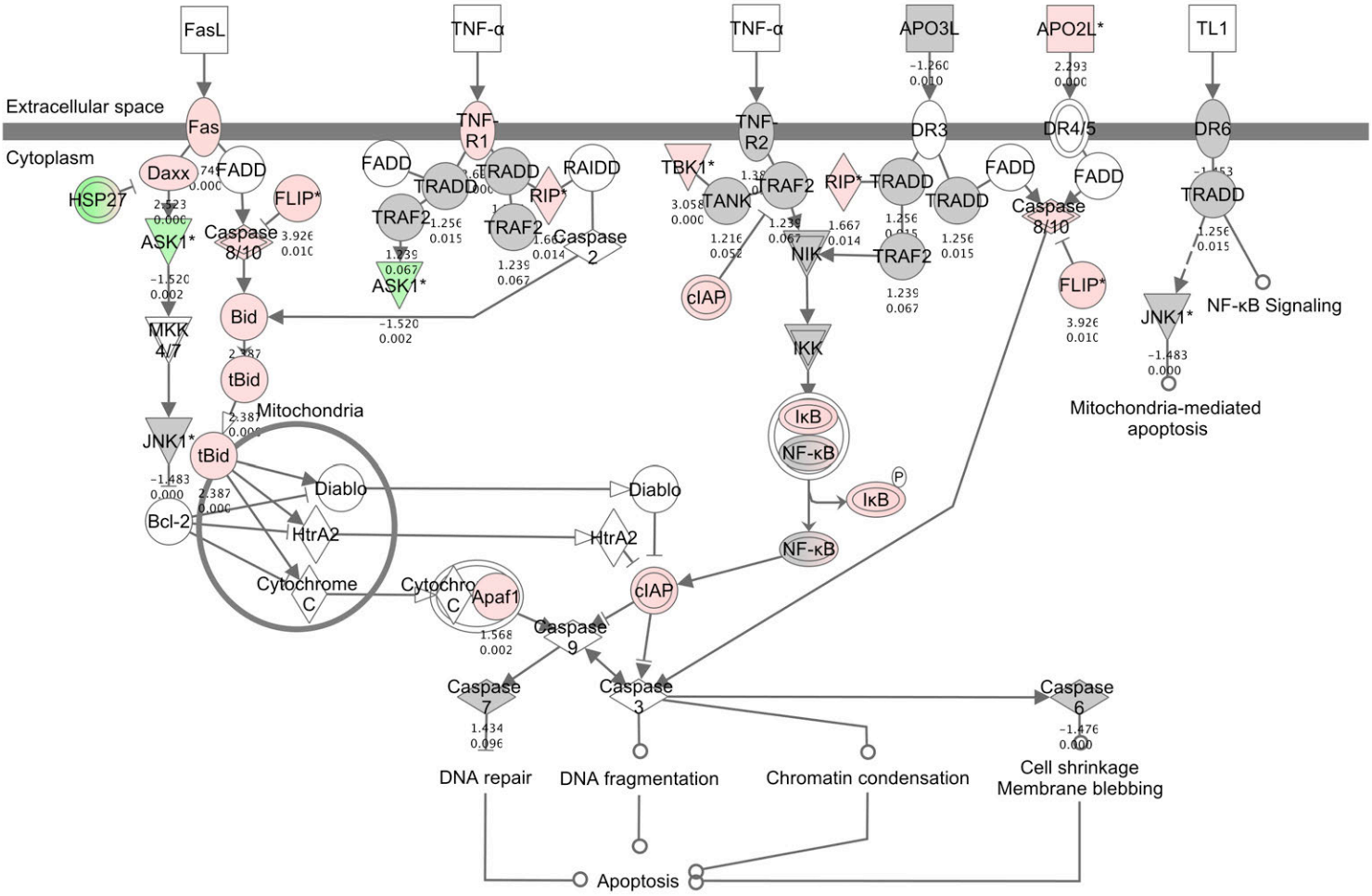


Fig. S15. Diagrammatic representation of transcriptional effects of LPS on complement signaling, after 24 hrs of treatment (red, induction; green, repression).

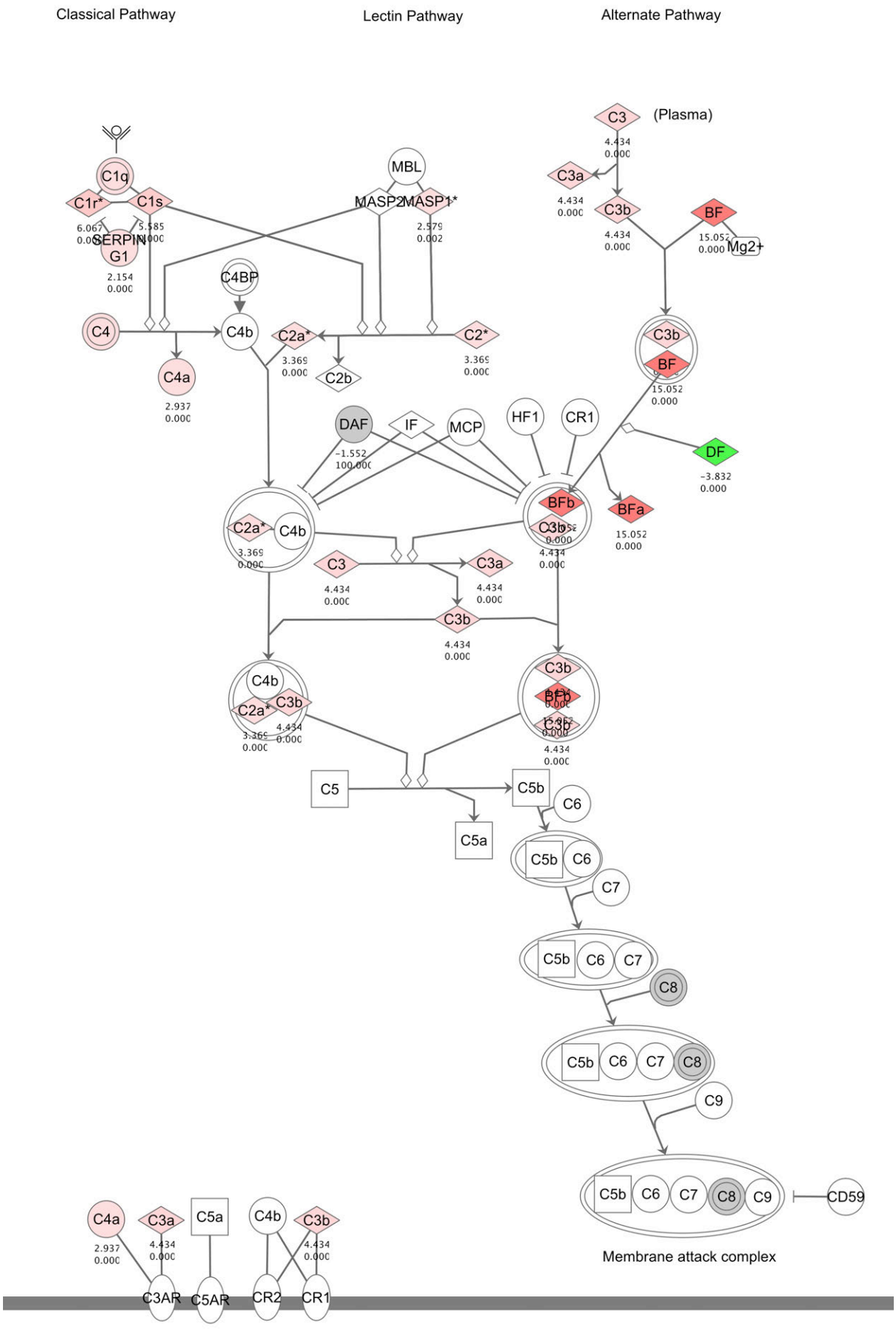


Fig. S16. Diagrammatic representation of transcriptional effects of LPS on cardiac hypertrophy signaling, after 24 hrs of treatment (red, induction; green, repression).

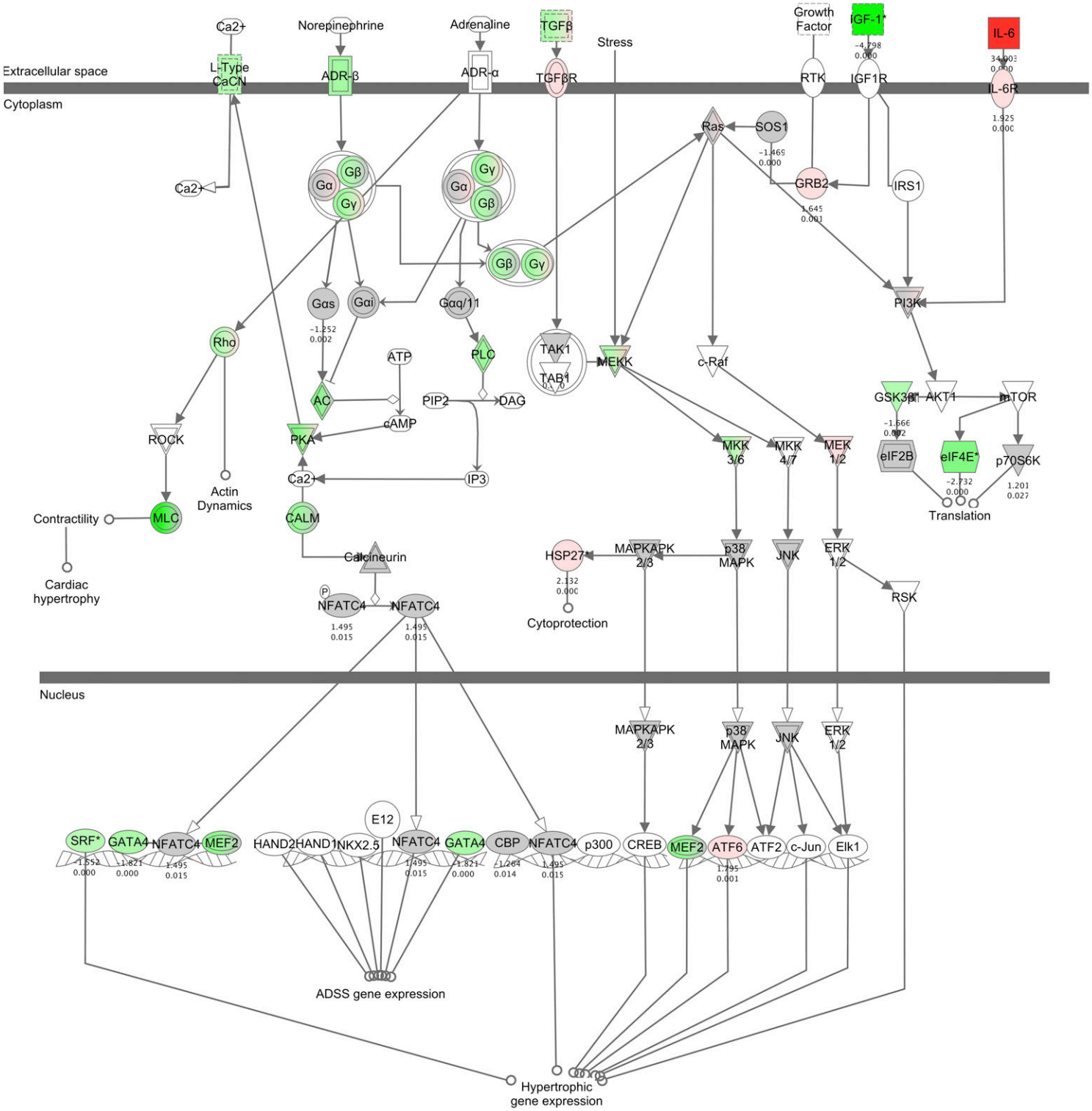


Fig. S17. Diagrammatic representation of transcriptional effects of LPS on NFAT signaling in hypertrophy, after 24 hrs of treatment (red, induction; green, repression).

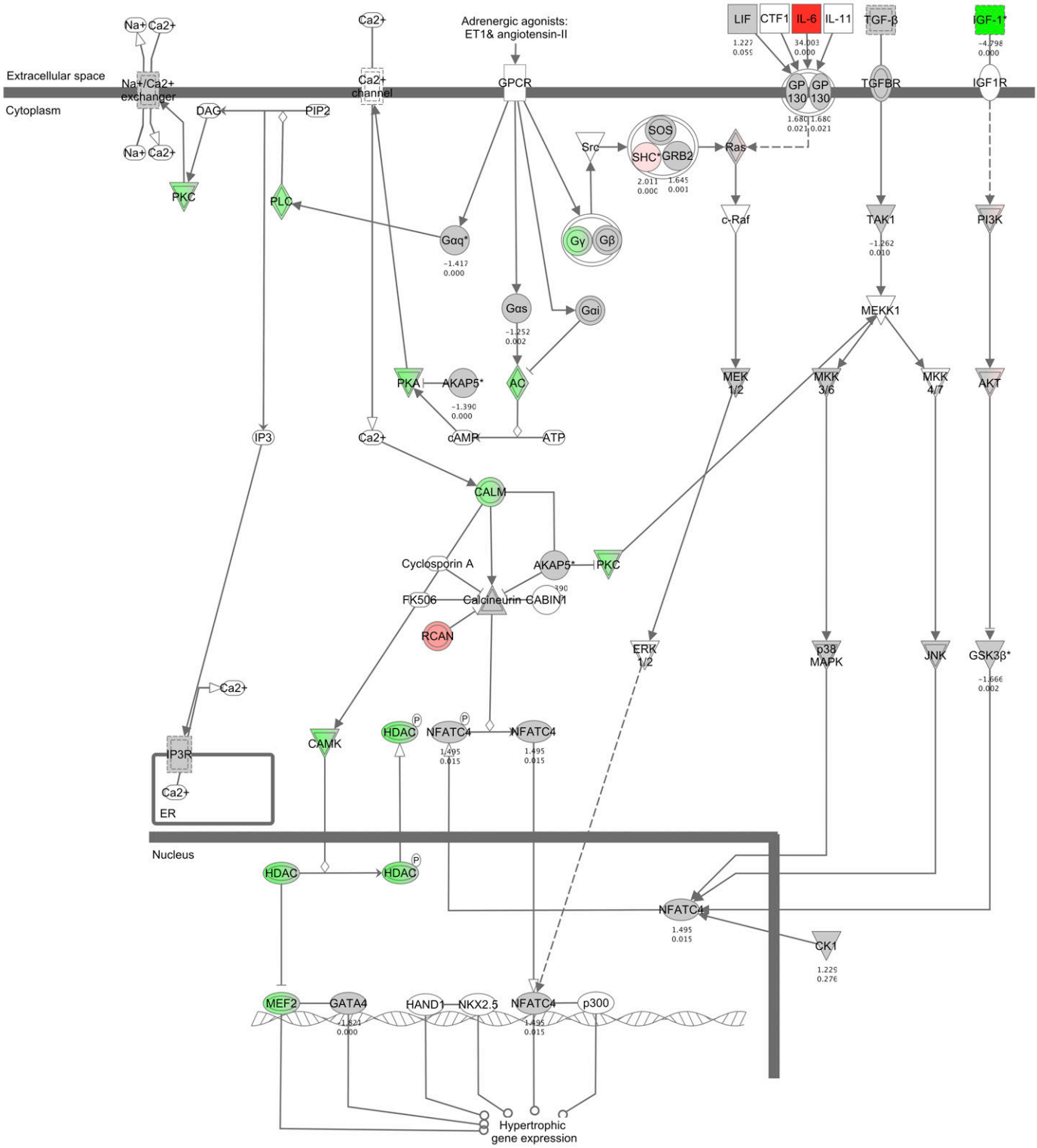


Fig. S18. Diagrammatic representation of transcriptional effects of LPS on ß-adrenergic signaling after 24 hrs of treatment (red, induction; green, repression).

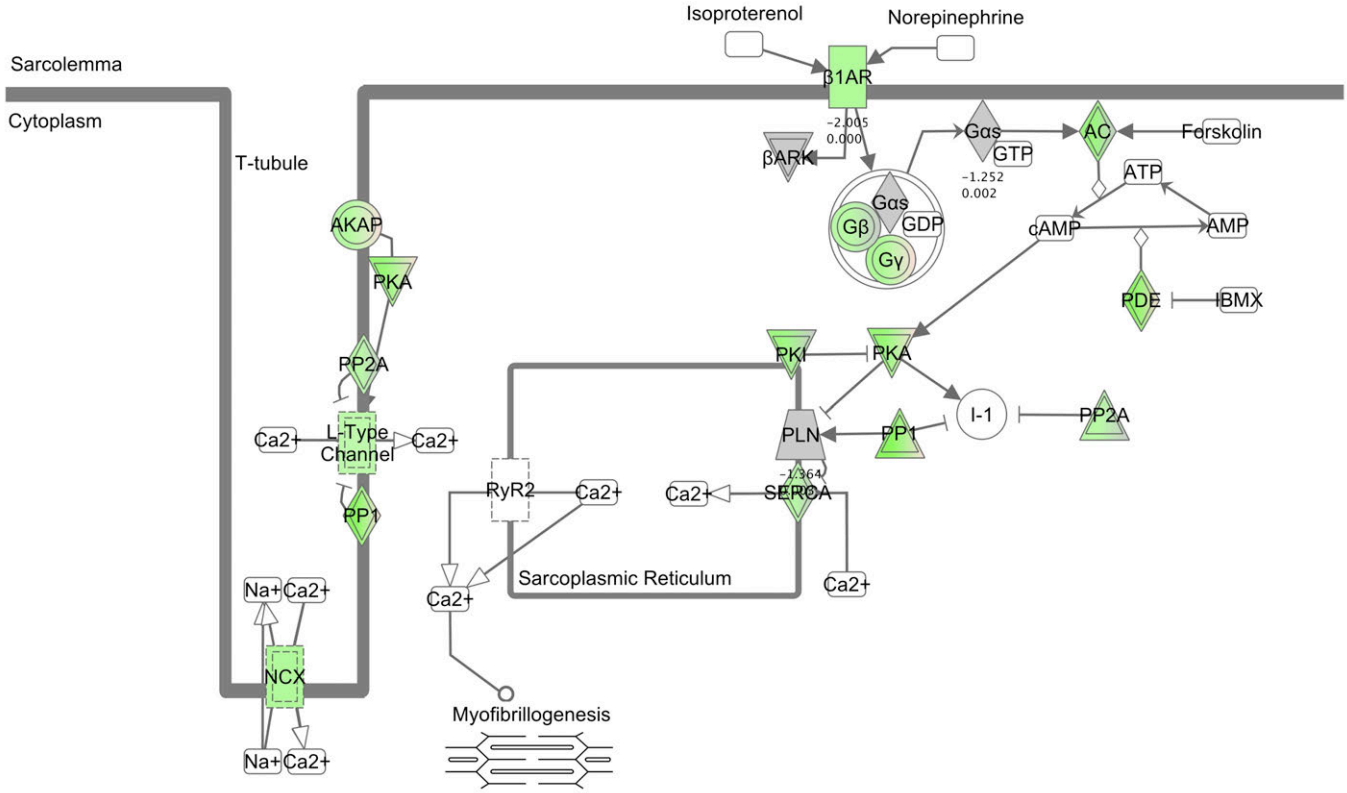


Fig. S19. Diagrammatic representation of transcriptional effects of LPS on PKA signaling, after 24 hrs of treatment (red, induction; green, repression).

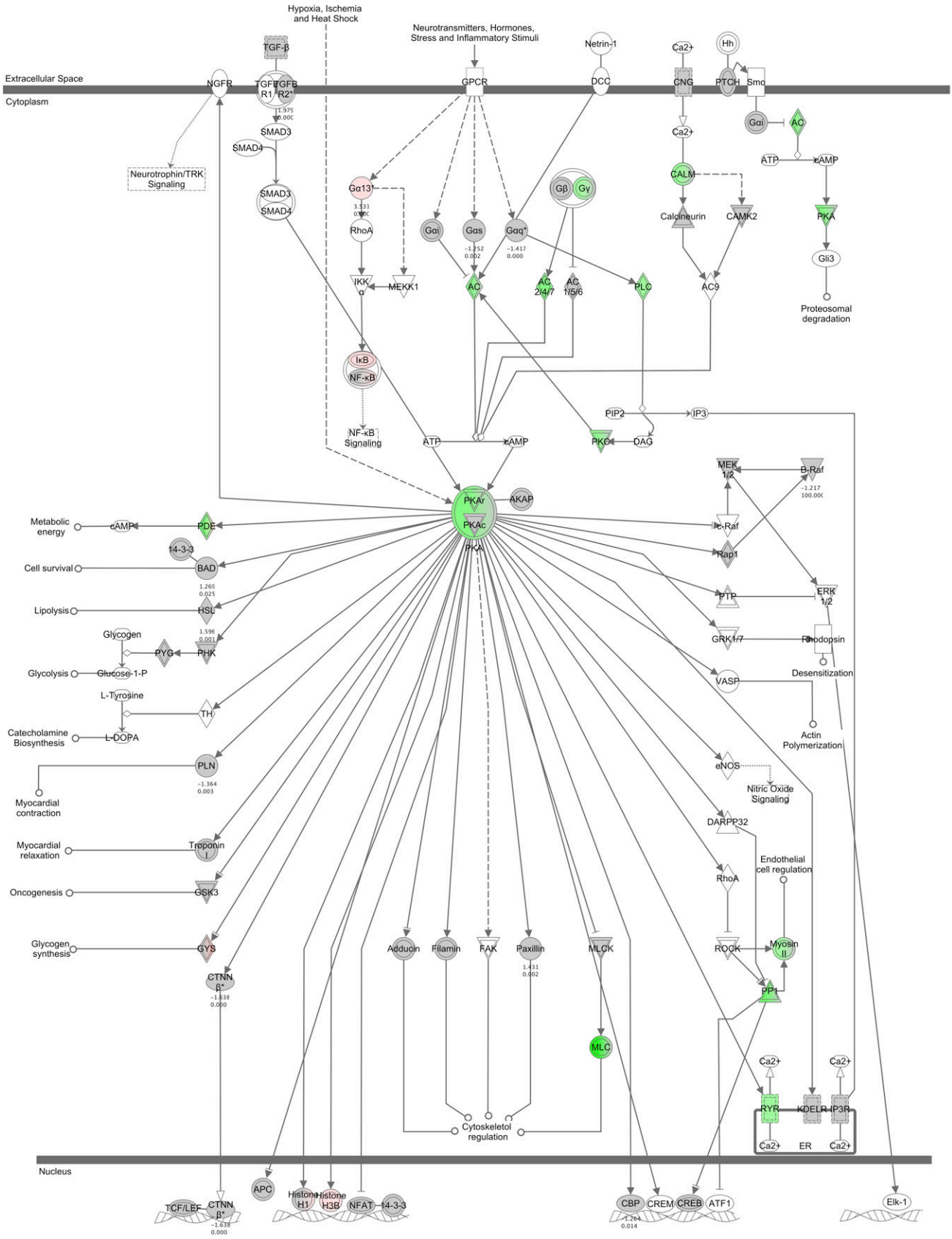


Fig. S20. Diagrammatic representation of transcriptional effects of LPS on Ca^{2+} signaling, after 24 hrs of treatment (red, induction; green, repression).

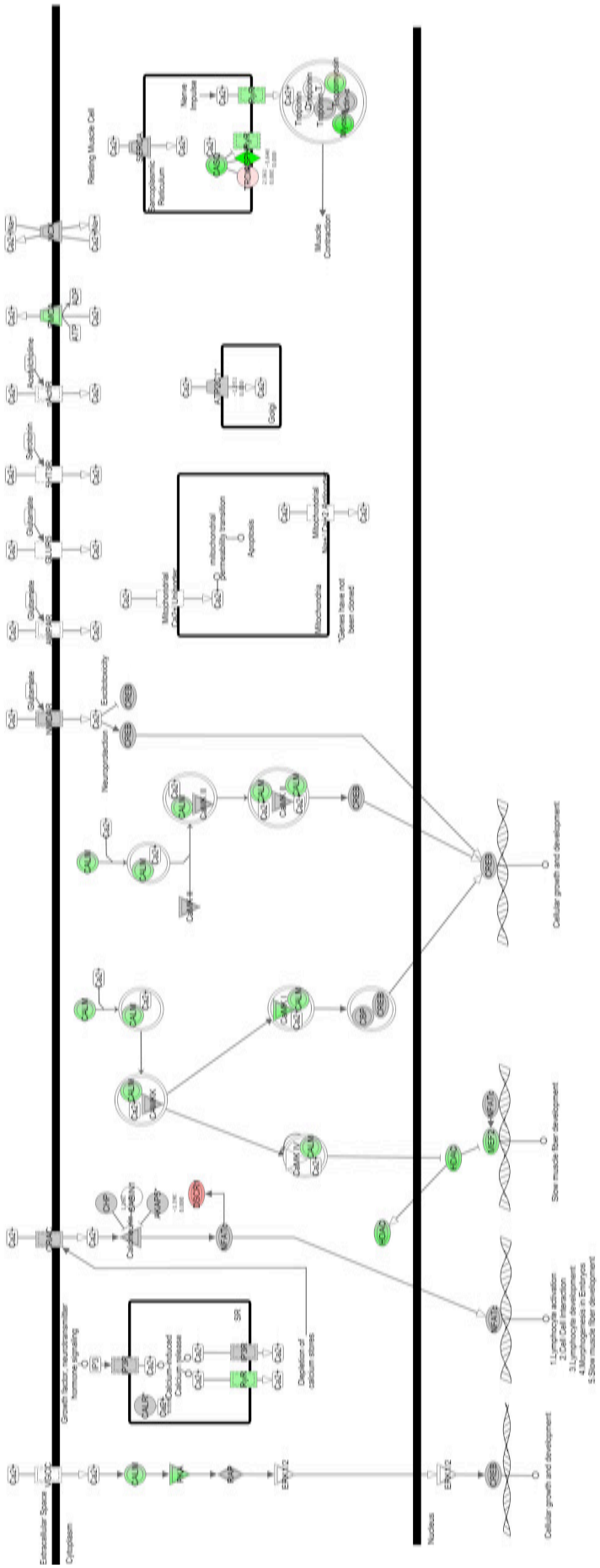


Fig. S21. Diagrammatic representation of transcriptional effects of LPS on mitochondrial function, after 24 hrs of treatment (red, induction; green, repression).

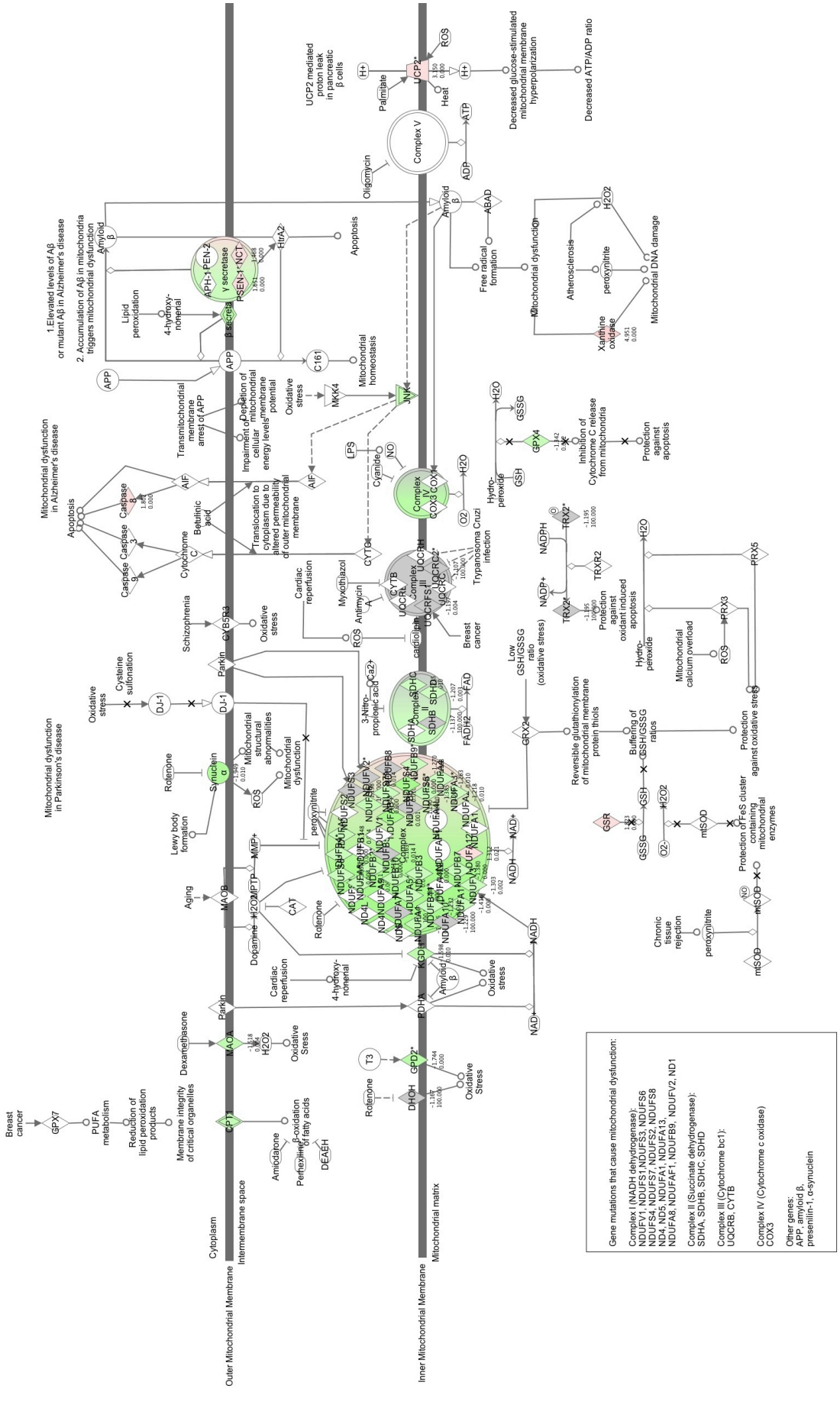


Fig. S22. Diagrammatic representation of transcriptional effects of LPS on IGF-1 signaling, after 24 hrs of treatment (red, induction; green, repression).