## Supplemental Figures

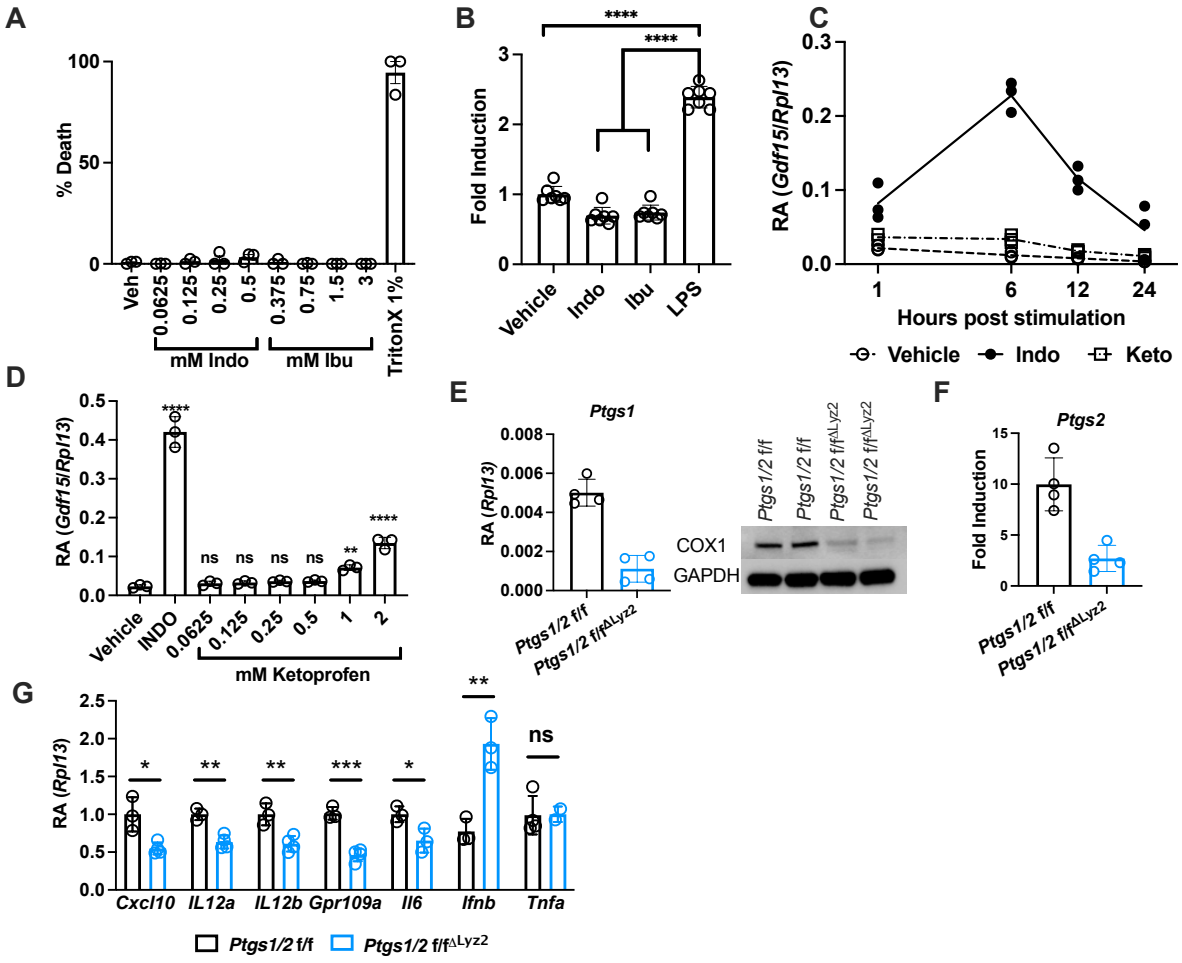


**Figure S1. Indomethacin induces GDF15 independent of cyclooxygenases, Related to Figure 1.** (A) LDH assay performed from the supernatants of BMDMs treated for 24 hours with increasing concentrations of indomethacin and ibuprofen (n=3 per group). (B) Fold change in reactive oxygen species following BMDM treatment with indomethacin, ibuprofen and LPS 5 hrs (n=7 per group). (C) *Gdf15* mRNA expression from BMDMs following treatment with vehicle, 0.5mM indomethacin or 0.5 mM ketoprofen diluted in 1% DMSO for 1, 6, 12 and 24 hours (n=3 per group). (D) *Gdf15* mRNA expression from BMDMs following 6h treatment with 0.5 mM indomethacin and increasing concentrations of ketoprofen diluted in 1% DMSO (n=3 per group). (E) *Ptgs1* mRNA expression and COX1 protein levels by Western Blot from BMDMs derived from *Ptgs1/2* f/f or *Ptgs1/2*$^{f/fLyz2}$ (n=4 per group). (F) *Ptgs2* mRNA expression from BMDMs derived from *Ptgs1/2* f/f or *Ptgs1/2* f/f$^{Lyz2}$ mice that were treated with

indomethacin to induce *Cox2* expression (n=4 per group). (G) mRNA expression of the indicated prostaglandin-sensitive transcripts from BMDMs derived from *Ptgs1/2* f/f or *Ptgs1/2* f/f$^{Lyz2}$ mice that were treated with LPS for 3 hours (n=3 per group). All experiments are representative and repeated at least once. Statistics by ANOVA or t-test. Data are represented as mean ± standard deviation. **P*<.05, ***P*<0.01, ****P*<.001, *****P*<.0001, ns *P*>.05

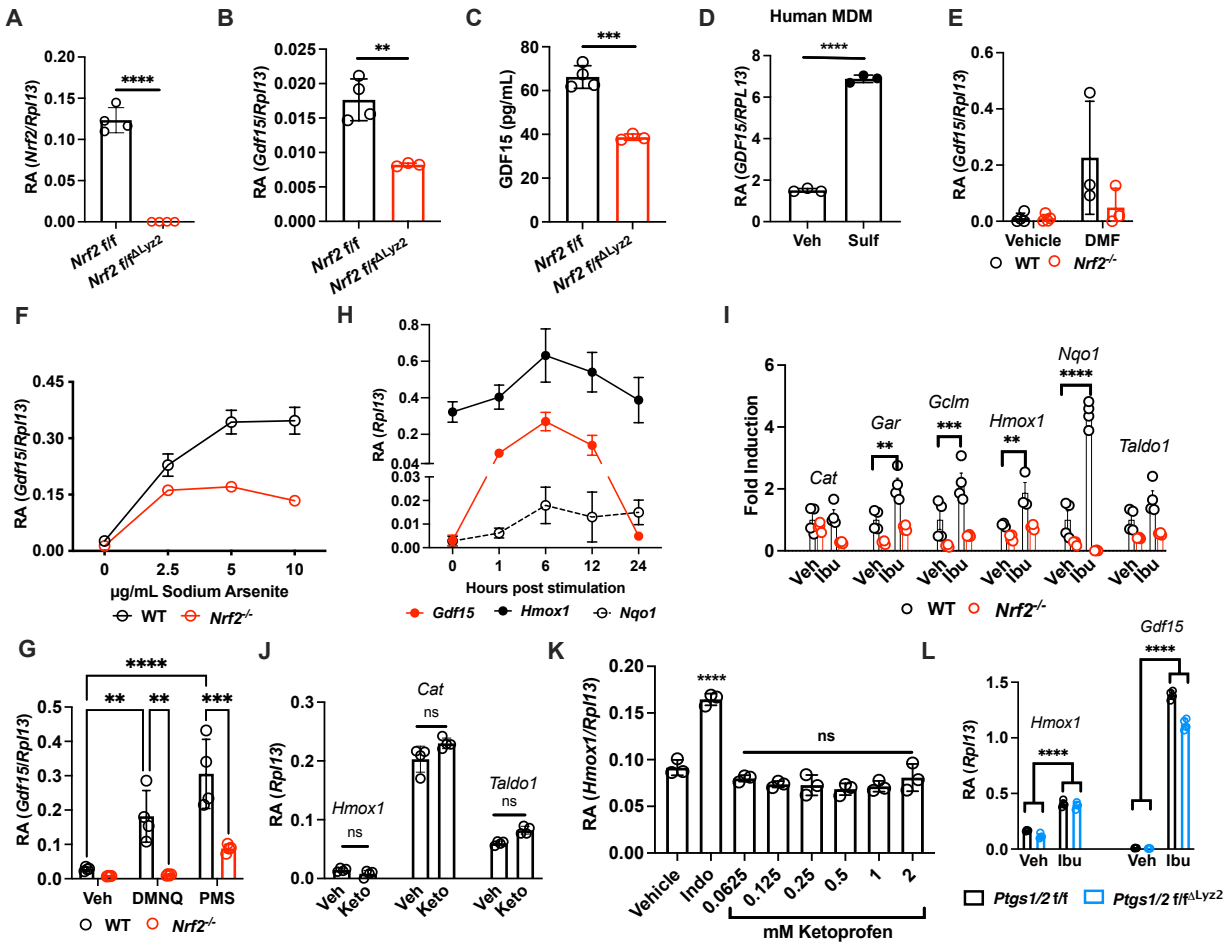


**Figure S2. NRF2 is necessary and sufficient for GDF15 secretion and some NSAIDs activate NRF2, Related to Figure 2.** (A) *Nrf2* and (B) *Gdf15* mRNA expression and (C) GDF15 protein levels from BMDMs derived from *Nrf2* f/f or *Nrf2* f/f$^{Lyz2}$ (n=3-4 per group). (D) *GDF15* mRNA expression in human MDM at 6 hours post stimulation with 8.3 mg/mL sulforaphane (n=3 per group). (E) *Gdf15* mRNA expression from BMDMs at 6 hours stimulation with dimethylfumarate (n=3-4 per group). (F) *Gdf15* mRNA expression from BMDMs at the indicated doses of sodium arsenite at 6 hours post stimulation (n=3 per group). (G) *Gdf15* mRNA expression from BMDM 6 hours after stimulation with 2,3-dimethoxy-1,4-naphthalenedione (DMNQ), and phenazine methosulfate (PMS) (n=4 per group). (H) *Gdf15, Hmox1,* and *Nqo1* expression at the indicated timepoints after indomethacin stimulation in BMDM (n=4 per group). (I) Fold induction of *Cat, Gar, Gclm, Hmox1, Nqo1, Taldo1* mRNA expression in BMDMs derived from B6 and *Nrf2*$^{-/-}$ mice treated for 16 hours with vehicle or 1.5

mM ibuprofen diluted in 1% DMSO (n=4 per group). (J) mRNA expression of *Hmox1, Cat, Taldo1* from BMDMs derived from B6 mice treated with vehicle and 0.5 mM ketoprofen diluted in 1% DMSO (n=4 per group). (K) *Hmox1* mRNA expression from BMDMs derived from B6 mice treated for 16 hours with increasing doses of ketoprofen or 0.5 mM indomethacin diluted in 1% DMSO (n=3 per group). (L) *Hmox1* and *Gdf15* mRNA expression from BMDMs derived from *Ptgs1/2* f/f or *Ptgs1/2* f/f$^{Lyz2}$ mice treated with vehicle or 0.5 mM ibuprofen diluted in 1% DMSO (n=4 per group). All experiments are representative and repeated at least once. Statistics by ANOVA or t-test. Data are represented as mean ± standard deviation. ***P*<0.01, ****P*<.001, *****P*<.0001, ns *P*>.05

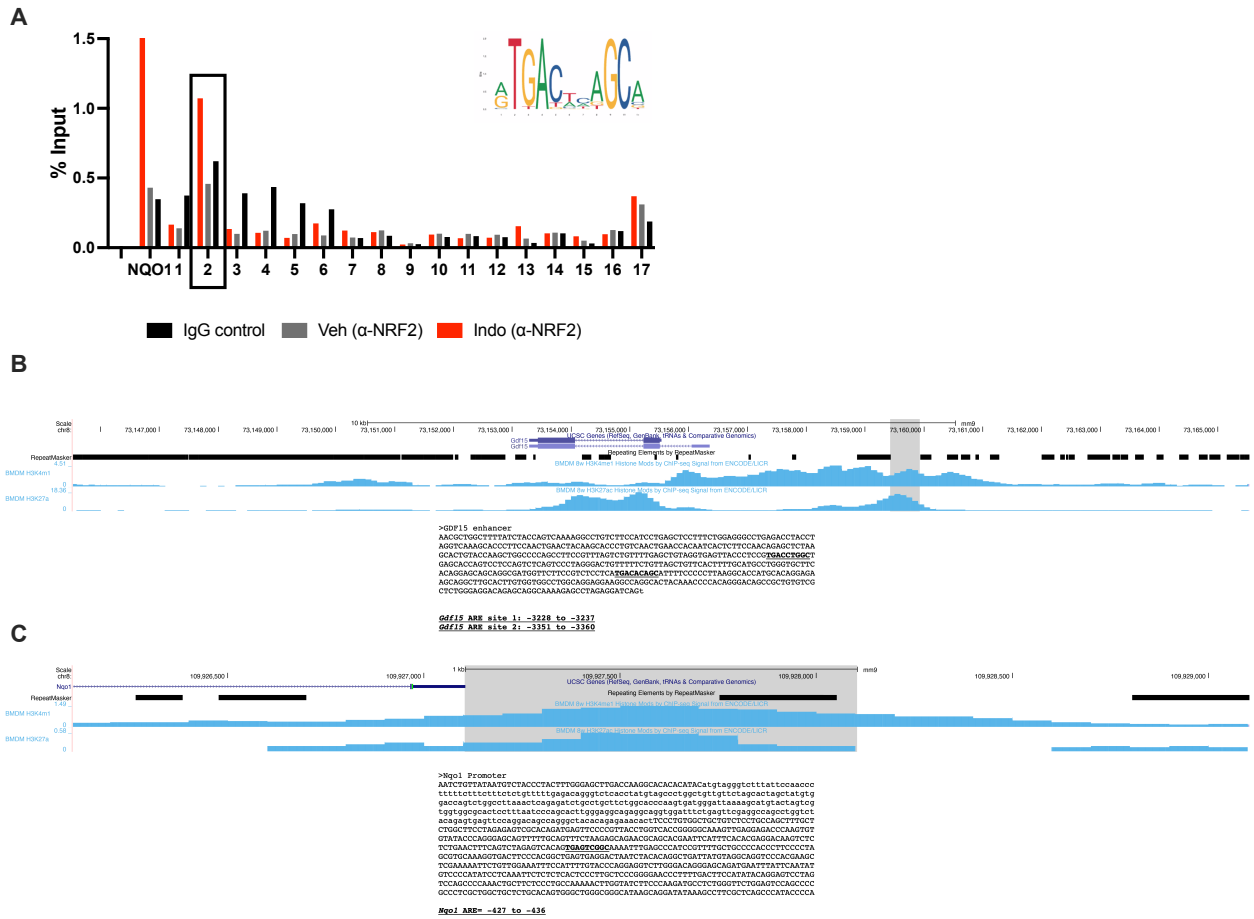


**Figure S3. *In silico* identification of NRF2 regulatory elements in Gdf15 and Nqo1, Related to Figure 2.** To identify putative NRF2 binding sites, ENCODE data for enhancer histone marks (H3Kme1 and/or H3k27Ac) were used in conjunction with ARE sequences (sequence from JASPAR, inset) identified 10 kb upstream and downstream of the *Gdf15* transcriptional start site. (A) BMDM were stimulated with indomethacin or vehicle for four hours and ChIP-qPCR was performed. Primers were designed for the top 20 putative NRF2-binding sites. *Nqo1* was used as a positive control. Primer names, genomic positions, and sequences can be found in Table S1. Region 2 (containing ARE sequences -3328 to -3237 and -3351 to -3360, boxed) is the only putative site that bound NRF2 inducibly after indomethacin above antibody control. This region was then tiled 1 kb upstream and downstream from this ARE position (Figure 2H). (B) The genomic position and activating histone marks from ENCODE data for the identified NRF2-

binding site for *Gdf15* and (C) the canonical NRF2 target gene *Nqo1*. The ARE consensus sequences are underlined and bolded.

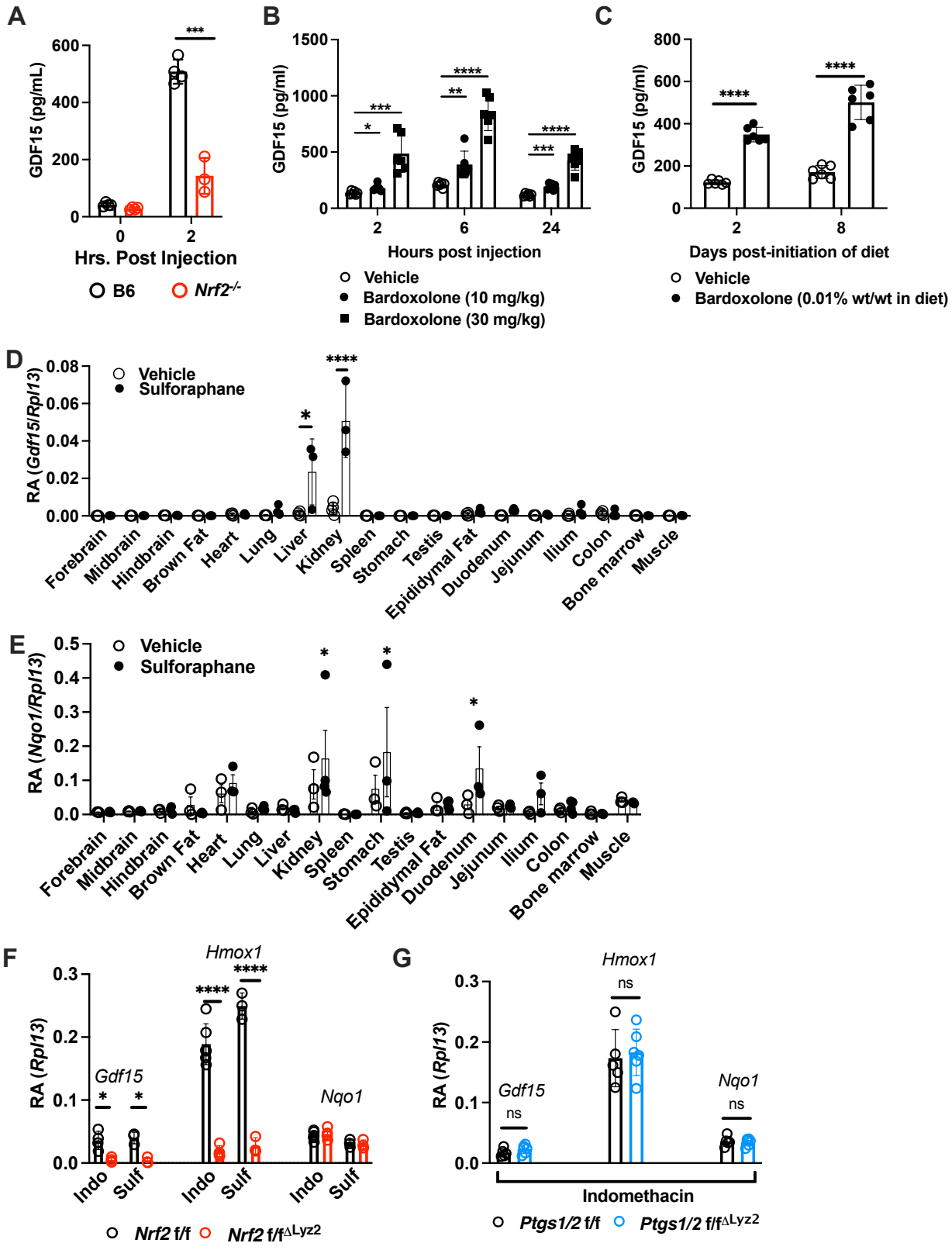
A
GDF15 (pg/mL)
600
400
200
0
***
0
2
Hrs. Post Injection
B6
Nrf2-/-
B
GDF15 (pg/ml)
1500
1000
500
0
*
***
**
****
***
****
2
6
24
Hours post injection
Vehicle
Bardoxolone (10 mg/kg)
Bardoxolone (30 mg/kg)
C
GDF15 (pg/ml)
800
600
400
200
0
****
****
2
8
Days post-initiation of diet
Vehicle
Bardoxolone (0.01% wt/wt in diet)
D
RA (Gdf15/Rpl13)
0.08
0.06
0.04
0.02
0.00
Vehicle
Sulforaphane
*
****
Forebrain
Midbrain
Hindbrain
Brown Fat
Heart
Lung
Liver
Kidney
Spleen
Stomach
Testis
Epididymal Fat
Duodenum
Jejunum
Ilium
Colon
Bone marrow
Muscle
E
RA (Nqo1/Rpl13)
0.5
0.4
0.3
0.2
0.1
0.0
Vehicle
Sulforaphane
*
*
*
Forebrain
Midbrain
Hindbrain
Brown Fat
Heart
Lung
Liver
Kidney
Spleen
Stomach
Testis
Epididymal Fat
Duodenum
Jejunum
Ilium
Colon
Bone marrow
Muscle
F
RA (Rpl13)
0.3
0.2
0.1
0.0
Gdf15
*
*
Hmox1
****
****
Nqo1
Indo
Sulf
Indo
Sulf
Indo
Sulf
Nrf2 f/f
Nrf2 f/fΔLyz2
G
RA (Rpl13)
0.3
0.2
0.1
0.0
Gdf15
ns
Hmox1
ns
Nqo1
ns
Indomethacin
Ptgs1/2 f/f
Ptgs1/2 f/fΔLyz2

**Figure S4. Indomethacin activates Nrf2 target genes in a manner dependent on Nrf2, and independent of COX1/2, in *Lyz2* expressing cells *in vivo,* Related to Figure 3.** (A) Serum GDF15 protein levels after injection with vehicle or 25 mg/kg sulforaphane in B6 and *Nrf2*$^{-/-}$ animals (n=3-4 per group). (B) Serum GDF15 protein levels 2, 6, 24 hours post injection with vehicle or Bardoxolone (10 and 30 mg/kg) (n=6 per group). (C) Serum GDF15 levels in mice fed diet with 0.01% wt/wt Bardoxolone for 2 and 8 days (n=6 per group). *Gdf15* (D) and *Nqo1* (E) mRNA expression extracted from the organs of B6 mice 6h after injection with vehicle (EtOH) or 50 mg/kg sulforaphane (n=3 per group). (F) *Gdf15, Hmox1,* and *Nqo1* mRNA expression from the liver of *Nrf2* f/f or *Nrf2* f/f$^{Lyz2}$ mice 6h following intraperitoneal injection of 15 mg/kg indomethacin or 50 mg/kg sulforaphane (n=3-6 per group). (G) *Gdf15, Hmox1,* and *Nqo1* mRNA expression from the liver of *Ptgs1/2* f/f or *Ptgs1/2* f/f$^{Lyz2}$ mice 6h following intraperitoneal injection of 15 mg/kg indomethacin (n=5-6 per group). All experiments are representative and repeated at least once. Statistics by ANOVA. Data are represented as mean ± standard deviation. **P*<.05, ***P*<0.01, ****P*<.001, *****P*<.0001, ns *P*>.05

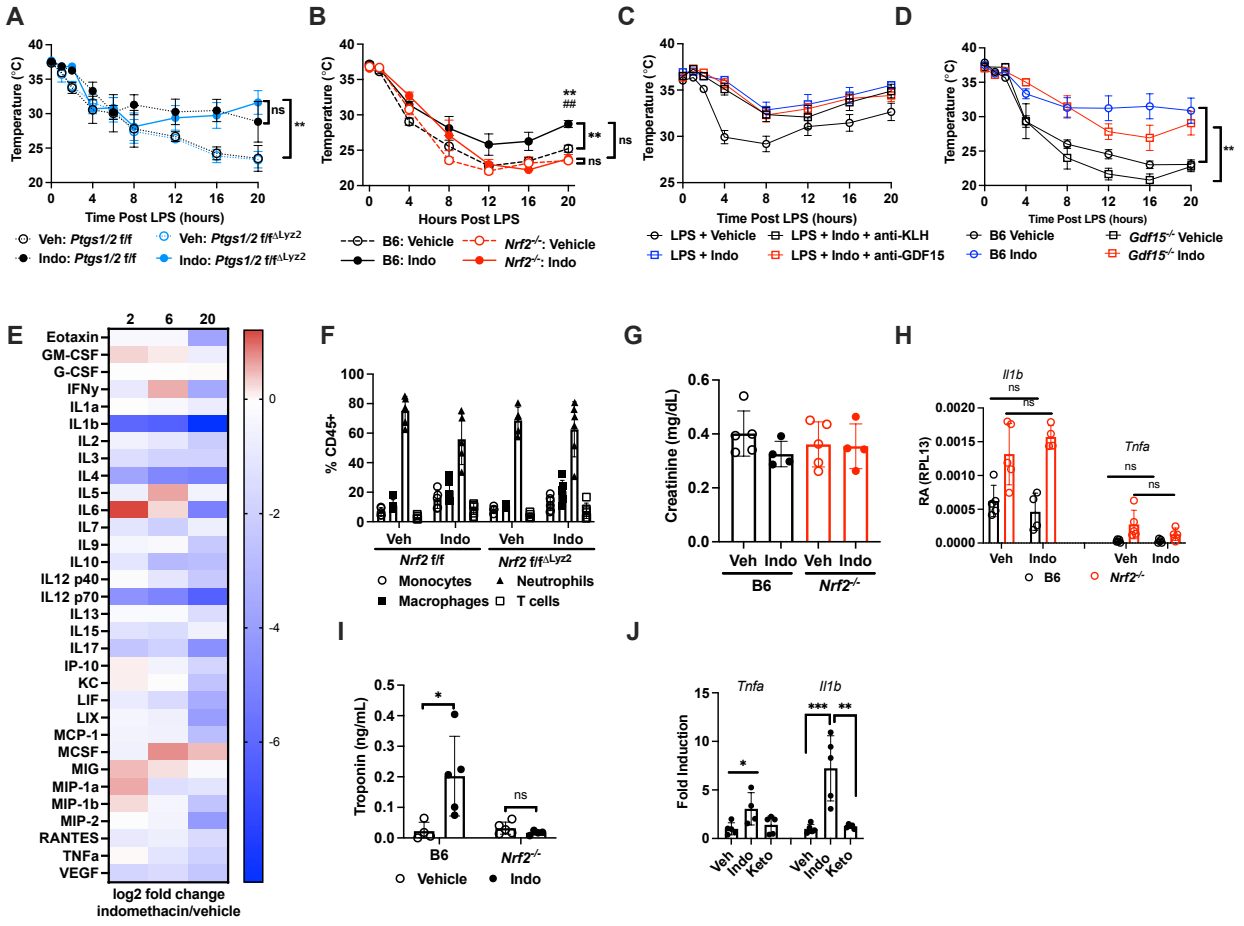


**Figure S5. Indomethacin reduces inflammation in endotoxemia and gout via an Nrf2 dependent, GDF15-independent and COX1/2 independent mechanism, Related to Figure 5.** Temperature curve of *Ptgs1/2* f/f (A) and *Nrf2*$^{-/-}$ (B) mice following intraperitoneal injection LPS and vehicle or indomethacin (AUC p <0.0085 and 0.0210, respectively, n=4-5 per group). (C) Temperature curve for B6 animals challenged with LPS with or without indomethacin with or without pre-treatment with GDF15 blocking antibody or isotype control (KLH) (n=4-5 per group). (D) Temperature curve for *Gdf15*$^{-/-}$ and littermate control animals challenged with LPS with or without indomethacin (AUC p<0.01) (n=6-9 per group). (E) Heat map of serum cytokine levels in B6 mice 2, 6, or 20 hours following intraperitoneal injection with LPS and vehicle or indomethacin, expressed as a log2 fold change (n=5 per group). (F) Flow cytometry analysis of inflammatory cells composing the liver 20 hours following intraperitoneal injection with vehicle of indomethacin in *Nrf2* f/f or *Nrf2* f/f$^{Lyz2}$ mice (n=5-6 per group). (G) Serum creatinine levels 20 hours after intraperitoneal injection of LPS and vehicle or indomethacin in B6 and *Nrf2*$^{-/-}$ mice

(n=4-5 per group). (H) *Il1b* and *Tnfa* mRNA expression in the kidney of B6 and *Nrf2*$^{-/-}$ mice 20 hours following intraperitoneal injection LPS and vehicle or indomethacin (n=4-5 per group). (I) Serum troponin levels 20 hours following intraperitoneal injection of vehicle or indomethacin in B6 and *Nrf2*$^{-/-}$ mice (n=4-5 per group). (J) *Il1b* and *Tnfa* mRNA expression in the heart of B6 mice 24 hours following intraperitoneal injection LPS and vehicle, indomethacin or ketoprofen (n=5 per group). All experiments are representative and repeated at least once. Statistics by ANOVA. Data are represented as mean ± standard deviation. **P*<.05, ***P*<0.01, ****P*<.001, ns *P*>.05

Table S1. Primer sequences used for identifying NRF2 binding sites in *Gdf1,* Related to Figure 2.

| Primer Name | Primer Sequence | mm9 coordinates |
|---|---|---|
| 1 GTGACTCAGCA Con L | GCTTGCCTATCCGATTCCTT | chr8:73167029+73167133 |
| 1 GTGACTCAGCA Con R | CTCTGAGACAGGGCTCAACC | chr8:73167029+73167133 |
| 2 TGCTGTGTCAT revcomp L | GCTTCTCCTGTGCATGGTG | chr8:73159549+73159640 |
| 2 TGCTGTGTCAT revcomp R | CCTGGGTGCTTCACAGGA | chr8:73159549+73159640 |
| 3 TACTGAGTCTT comp L | TTATGGAAGGGGTCACATGG | chr8:73162894+73163000 |
| 3 TACTGAGTCTT comp R | CAGTTCTGCGTGCCCAATA | chr8:73162894+73163000 |
| 4 ACGGCTCAGTC Conrev L | GCAGGTCTGGAGCCTCATTA | chr8:73137215+73137320 |
| 4 ACGGCTCAGTC Conrev R | ACAGGGGCAGGTACAGGAAT | chr8:73137215+73137320 |
| 5 ATGAATCTGCA Con L | TGCTGGTAATGCATGCTAGTCT | chr8:73135846+73135949 |
| 5 ATGAATCTGCA Con R | TGAGGGTCAGGAGAGAATGG | chr8:73135846+73135949 |
| 6 CTGACTCAGCG Con L | GGTAGGCTTCGGGGAGAC | chr8:73153996+73154097 |
| 6 CTGACTCAGCG Con R | CCGGTGTTCTGGTTTCTTCT | chr8:73153996+73154097 |
| 7 ATGACCCGGCA Con L | TCCCCATTCCTATAAATGCAA | chr8:73165844+73165950 |
| 7 ATGACCCGGCA Con R | CTTTTGAATTCCTGGGGTGA | chr8:73165844+73165950 |
| 8 ATGAGTCAGGA Con L | CTCTCAATTGCCCCCTTGT | chr8:73174403+73174501 |
| 8 ATGAGTCAGGA Con R | CCAGCTCTGACAGCCATGTA | chr8:73174403+73174501 |
| 9 GGCTCAGTCAT revcomp L | GCAGGTCTGGAGCCTCATTA | chr8:73137215+73137320 |
| 9 GGCTCAGTCAT revcomp R | ACAGGGGCAGGTACAGGAAT | chr8:73137215+73137320 |
| 10 TGCAGGGTCAT revcomp L | GTAGCCACGTGACAGGAACC | chr8:73151874+73151983 |
| 10 TGCAGGGTCAT revcomp R | TGAGAGGAAGCCTGGTAAGG | chr8:73151874+73151983 |
| 11 CTGCCTCAGCA Con L | GGTCTCTGGAACTCACCATCTC | chr8:73136141+73136240 |
| 11 CTGCCTCAGCA Con R | TACCAGCTGAGCCTCCCTAA | chr8:73136141+73136240 |
| 12 TGCTGATTCCT revcomp L | CCAAGCACAGGGCTTAATTC | chr8:73135704+73135801 |
| 12 TGCTGATTCCT revcomp R | AGGCAGGTTGCTTTGTCACT | chr8:73135704+73135801 |
| 13 TGTTGGGTCAT revcomp L | CCCACAAGTGTAGGGAAGGA | chr8:73148362+73148454 |
| 13 TGTTGGGTCAT revcomp R | TTCTGTCAATGCAGCCCTAA | chr8:73148362+73148454 |
| 14 GTGACAAAGCA Con L | CAGCGTTGGTACAACTGCATA | chr8:73135744+73135839 |
| 14 GTGACAAAGCA Con R | AAATGAGGAACCGGTGTTTG | chr8:73135744+73135839 |
| 15 GTCTGAGTCAT revcomp L | TGCTCCAAAATGCTGCATAG | chr8:73151141+73151242 |
| 15 GTCTGAGTCAT revcomp R | CTGCTCAGGGAAGGAAACTG | chr8:73151141+73151242 |
| 16 GTGAGTCACCA Con L | GTCACCCCAGGAATTCAAAA | chr8:73165930+73166023 |
| 16 GTGAGTCACCA Con R | TGTGATCAAGTGGCATTCTCTT | chr8:73165930+73166023 |
| 17 TATTGAGTCCT comp L | TGGATTACCTCTGGGGACAG | chr8:73148464+73148565 |
| 17 TATTGAGTCCT comp R | CAGCCACTGCATTGAAACC | chr8:73148464+73148565 |

Consensus (Con), Consensus reverse (Conrev), Reverse complementary (Revcomp), Compliment (Comp).