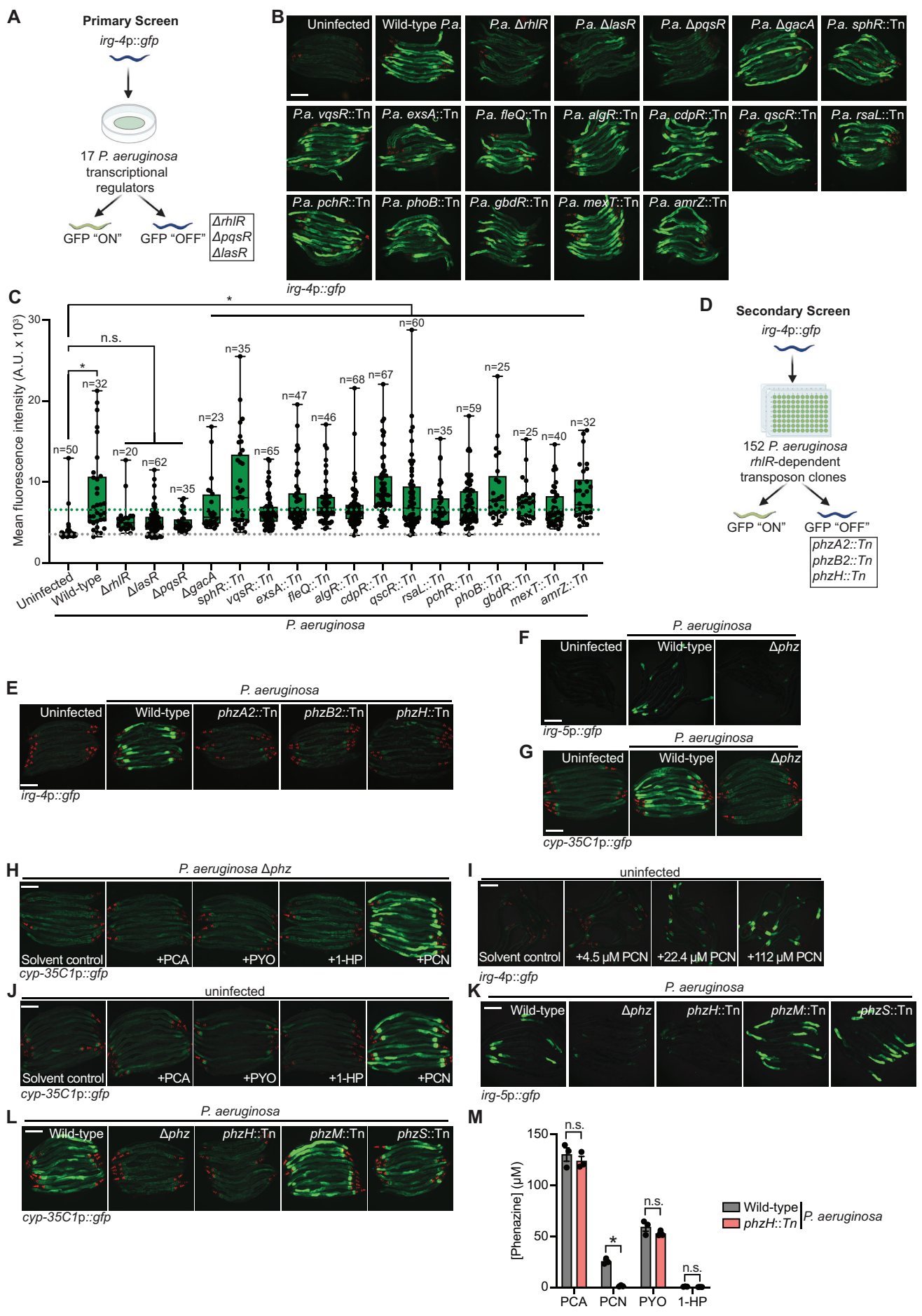
A
Primary Screen
irg-4p::gfp
17 P. aeruginosa transcriptional regulators
GFP "ON"
GFP "OFF"
ΔrhlR
ΔpqsR
ΔlasR
B
Uninfected
Wild-type P.a.
P.a. ΔrhlR
P.a. ΔlasR
P.a. ΔpqsR
P.a. ΔgacA
P.a. sphR::Tn
P.a. vqsR::Tn
P.a. exsA::Tn
P.a. fleQ::Tn
P.a. algR::Tn
P.a. cdpR::Tn
P.a. qscR::Tn
P.a. rsaL::Tn
P.a. pchR::Tn
P.a. phoB::Tn
P.a. gbdR::Tn
P.a. mexT::Tn
P.a. amrZ::Tn
irg-4p::gfp
C
Mean fluorescence intensity (A.U. x 10³)
n.s.
*
n=50
n=32
n=20
n=62
n=35
n=23
n=35
n=65
n=47
n=46
n=68
n=67
n=60
n=35
n=59
n=25
n=25
n=40
n=32
Uninfected
Wild-type
ΔrhlR
ΔlasR
ΔpqsR
ΔgacA
sphR::Tn
vqsR::Tn
exsA::Tn
fleQ::Tn
algR::Tn
cdpR::Tn
qscR::Tn
rsaL::Tn
pchR::Tn
phoB::Tn
gbdR::Tn
mexT::Tn
amrZ::Tn
P. aeruginosa
D
Secondary Screen
irg-4p::gfp
152 P. aeruginosa rhlR-dependent transposon clones
GFP "ON"
GFP "OFF"
phzA2::Tn
phzB2::Tn
phzH::Tn
E
P. aeruginosa
Uninfected
Wild-type
phzA2::Tn
phzB2::Tn
phzH::Tn
irg-4p::gfp
F
P. aeruginosa
Uninfected
Wild-type
Δphz
irg-5p::gfp
G
P. aeruginosa
Uninfected
Wild-type
Δphz
cyp-35C1p::gfp
H
P. aeruginosa Δphz
Solvent control
+PCA
+PYO
+1-HP
+PCN
cyp-35C1p::gfp
I
uninfected
Solvent control
+4.5 μM PCN
+22.4 μM PCN
+112 μM PCN
irg-4p::gfp
J
uninfected
Solvent control
+PCA
+PYO
+1-HP
+PCN
cyp-35C1p::gfp
K
P. aeruginosa
Wild-type
Δphz
phzH::Tn
phzM::Tn
phzS::Tn
irg-5p::gfp
L
P. aeruginosa
Wild-type
Δphz
phzH::Tn
phzM::Tn
phzS::Tn
cyp-35C1p::gfp
M
[Phenazine] (μM)
n.s.
*
n.s.
n.s.
PCA
PCN
PYO
1-HP
Wild-type
phzH::Tn
P. aeruginosa

Figure S1. The pathogen-derived metabolite phenazine-1-carboxamide (PCN) activates anti-pathogen defenses in the *C. elegans* intestine, related to Figure 1. (A) A schematic of the primary screen of 17 *P. aeruginosa* virulence-related transcription factors using *C. elegans irg-4*p::*gfp* transcriptional immune reporter animals. Hits are indicated: *rhlR*, *pqsR*, and *lasR*. **(B)** Representative images of *C. elegans irg-4*p::*gfp* transcriptional reporter animals either uninfected or infected with the indicated *P. aeruginosa* strains. **(C)** Quantification of *irg-4*p::*gfp* transcriptional reporter intensity in animals either uninfected or infected with the indicated *P. aeruginosa* strains. Sample size (*n*) for each strain is indicated. Each data point indicates one animal. Box and whisker plots represent the median with minimum, second quartile, third quartile, and maximum indicated for each condition. *equals $p<0.05$ (one-way ANOVA with Dunnett's multiple comparisons test). **(D)** A schematic of the secondary screen of 152 *P. aeruginosa* mutant strains, each with a mutation in a gene dependent on the transcription factor *rhlR* for full expression. Hits are indicated: *phzA2*, *phzB2*, and *phzH*. **(E-G)** Images of the *C. elegans* transcriptional reporters *irg-4*p::*gfp* **(E)**, *irg-5*p::*gfp* **(F)**, and *cyp-35C1*p::*gfp* **(G)** animals either uninfected or infected with the indicated *P. aeruginosa* strains. **(H-L)** Images of *cyp-35C1*p::*gfp*, *irg-4*p::*gfp*, and *irg-5*p::*gfp* transcriptional reporter expression in animals with indicated conditions and as described in Figure 1. **(M)** Quantification of phenazines in *P. aeruginosa* wild-type and *phzH::Tn* replicates by LC-MS/MS. Data are the mean with errors bars giving SEM of biological replicates (*n*=3). *equals $p<0.05$ (two-way ANOVA with Šídák's multiple comparisons test). See also Table S3 for the LC-MS/MS phenazine retention times. Scale bars in all images equal 200 μm. Source data for this figure is in Table S3.

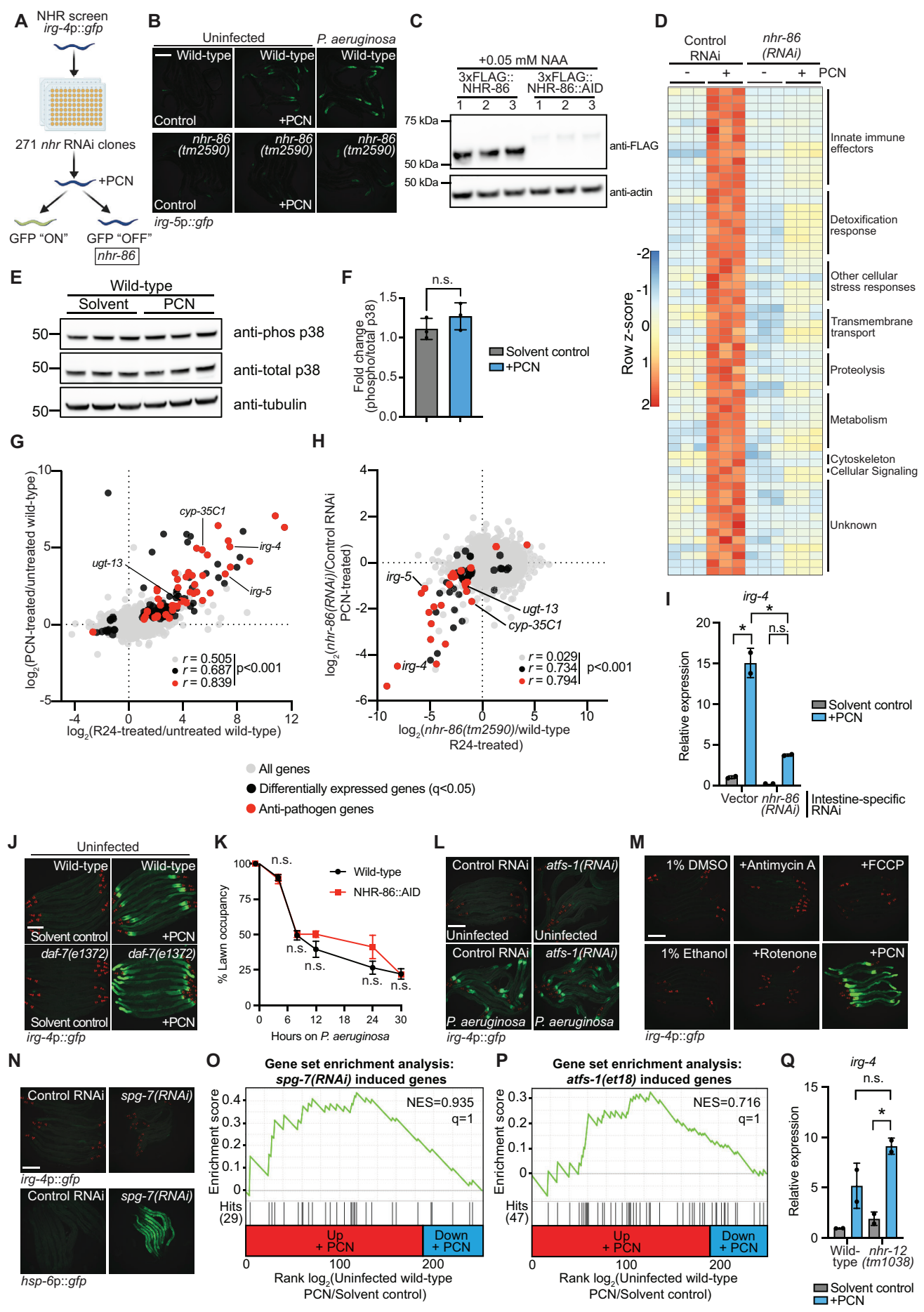
A
NHR screen
irg-4p::gfp
271 nhr RNAi clones
+PCN
GFP "ON"
GFP "OFF"
nhr-86
B
Uninfected
P. aeruginosa
Wild-type
Control
+PCN
nhr-86 (tm2590)
irg-5p::gfp
C
+0.05 mM NAA
3xFLAG:: NHR-86
3xFLAG:: NHR-86::AID
1 2 3
75 kDa
50 kDa
anti-FLAG
anti-actin
D
Control RNAi
nhr-86 (RNAi)
PCN
Row z-score
Innate immune effectors
Detoxification response
Other cellular stress responses
Transmembrane transport
Proteolysis
Metabolism
Cytoskeleton
Cellular Signaling
Unknown
E
Wild-type
Solvent
PCN
anti-phos p38
anti-total p38
anti-tubulin
F
n.s.
Fold change (phospho/total p38)
Solvent control
+PCN
G
cyp-35C1
irg-4
ugt-13
irg-5
r = 0.505
r = 0.687
r = 0.839
p<0.001
log2(PCN-treated/untreated wild-type)
log2(R24-treated/untreated wild-type)
H
irg-5
ugt-13
cyp-35C1
irg-4
r = 0.029
r = 0.734
r = 0.794
p<0.001
log2(nhr-86(RNAi)/Control RNAi PCN-treated)
log2(nhr-86(tm2590)/wild-type R24-treated)
All genes
Differentially expressed genes (q<0.05)
Anti-pathogen genes
I
irg-4
Relative expression
Solvent control
+PCN
Vector
nhr-86 (RNAi)
Intestine-specific RNAi
J
Uninfected
Wild-type
Solvent control
+PCN
daf-7(e1372)
irg-4p::gfp
K
n.s.
Wild-type
NHR-86::AID
% Lawn occupancy
Hours on P. aeruginosa
L
Control RNAi
atfs-1(RNAi)
Uninfected
P. aeruginosa
irg-4p::gfp
M
1% DMSO
+Antimycin A
+FCCP
1% Ethanol
+Rotenone
+PCN
irg-4p::gfp
N
Control RNAi
spg-7(RNAi)
irg-4p::gfp
hsp-6p::gfp
O
Gene set enrichment analysis:
spg-7(RNAi) induced genes
NES=0.935
q=1
Enrichment score
Hits (29)
Up + PCN
Down + PCN
Rank log2(Uninfected wild-type PCN/Solvent control)
P
Gene set enrichment analysis:
atfs-1(et18) induced genes
NES=0.716
q=1
Hits (47)
Q
irg-4
n.s.
Relative expression
Wild-type
nhr-12 (tm1038)
Solvent control
+PCN

Figure S2. The anti-pathogen transcriptional program induced by PCN requires the *C. elegans* nuclear hormone receptor *nhr-86*, related to Figure 2. (A) A schematic of the screen of 271 *nhr* RNAi clones using *C. elegans irg-4*p::*gfp* transcriptional immune reporter animals. The lone hit is indicated: *nhr-86*. **(B)** Images of *irg-5*p::*gfp* transcriptional reporter expression with indicated genotypes and conditions, as described in Fig. 2C. Scale bars in all images equal 200 µm. **(C)** Immunoblot analysis of lysates from biological replicates of 3xFLAG::NHR-86 and 3xFLAG::NHR-86::AID animals expressing the TIR1 transgene in all somatic tissues treated with 50 µM NAA (*n*=3). Lysates were probed with anti-FLAG and anti-actin antibodies. The expected size of 3xFLAG::NHR-86 and 3xFLAG::NHR-86::AID is 49.8 kDa and 54.6 kDa, respectively. **(D)** A heat map of the 63 genes that are induced in *C. elegans* during PCN exposure in an *nhr-86*-dependent manner (q<0.05 RNA-seq analysis, see Materials and Methods). Gene expression in each condition was scaled by calculating a row z-score for each gene. See also Table S1C. **(E)** Immunoblot analysis of lysates from wild-type animals exposed to solvent control (1% DMSO) or PCN (25 µg/mL) (n=3). Lysates were probed using antibodies that recognize the doubly phosphorylated TGY motif of PMK-1 (phos-PMK-1), total PMK-1 protein (total PMK-1) and tubulin (α-tubulin). PMK-1 is a 43.9 kDa protein and tubulin is a 50 kDa protein. **(F)** The band intensities of three biological replicates of the Western blot shown in Fig. S2E were quantified. Error bars represent SEM. n.s.=not significant (unpaired t-test, *n*=3). **(G)** An mRNA-seq experiment as described in Fig. 1H, except the genes differentially expressed in wild-type animals exposed to R24 in the absence of infection is compared to the genes induced by PCN. See also Table S1D. **(H)** Data from an mRNA-seq experiment as described in Fig. 2L, except the genes that require *nhr-86* for their expression during PCN and R24 treatment are compared. See also Table S1E. **(I)** qRT-PCR analysis of *irg-4* using a strain engineered to perform RNAi only in the intestine. Animals of the indicated genotypes were exposed to solvent control or 25 µg/mL PCN (*n*=2). Data are the mean of replicates with error bars giving SEM. *equals p<0.05 (two-way ANOVA with Tukey's multiple comparisons test). **(J)** Images of *C. elegans* wild-type and *daf-7(e1372)* animals expressing *irg-4*p::*gfp* exposed to the indicated conditions. **(K)** Occupancy of wildtype and NHR-86::AID animals on a lawn of *P. aeruginosa* was measured over time. Data points are the average of three replicates and error bars represent SEM. n.s.=not significant for comparison at each time point (two-way ANOVA with Šídák's multiple comparisons test). **(L)** Images of *C. elegans irg-4*p::*gfp* reporter treated with *atfs-1(RNAi)* and exposed to PCN. **(M)** Images of *C. elegans irg-4*p::*gfp* animals exposed to either the solvent controls (1% DMSO or 1% ethanol), the indicated mitochondrial toxins, or PCN. FCCP (trifluoromethoxy carbonylcyanide phenylhydrazone). **(N)** Images of *C. elegans irg-4*p::*gfp* or *hsp-6*p::*gfp* reporters treated with *spg-7(RNAi)*. The induction of *hsp-6*p::*gfp* in *spg-7(RNAi)* animals is the control for this experiment. **(O and P)** Gene set enrichment analysis (GSEA) of genes induced in *spg-7(RNAi)* treated animals **(O)** or in the *atfs-1(et18)* gain-of-function allele **(P)** in the RNA-seq of wild-type *C. elegans* exposed to PCN. In **(O)** and **(P)**, fold change in the expression of the significantly differentially expressed genes (q<0.05) in uninfected animals exposed to PCN in the absence of infection are ranked from higher expression (red) to lower expression (blue). Normalized enrichment score (NES) and q-value are indicated. Genes induced by either condition and found in the PCN transcriptional profile are indicated by hit number in the left margin and black lines. **(Q)** qRT-PCR analysis of *irg-4* in animals of the indicated genotypes, exposed to solvent control or PCN (*n*=2). Data are the mean of biological replicates with error bars giving SEM. *equals p<0.05 (two-way ANOVA with Tukey's multiple comparisons test). Scale bars in all images equal 200 µm. Source data for this figure is in Table S3.

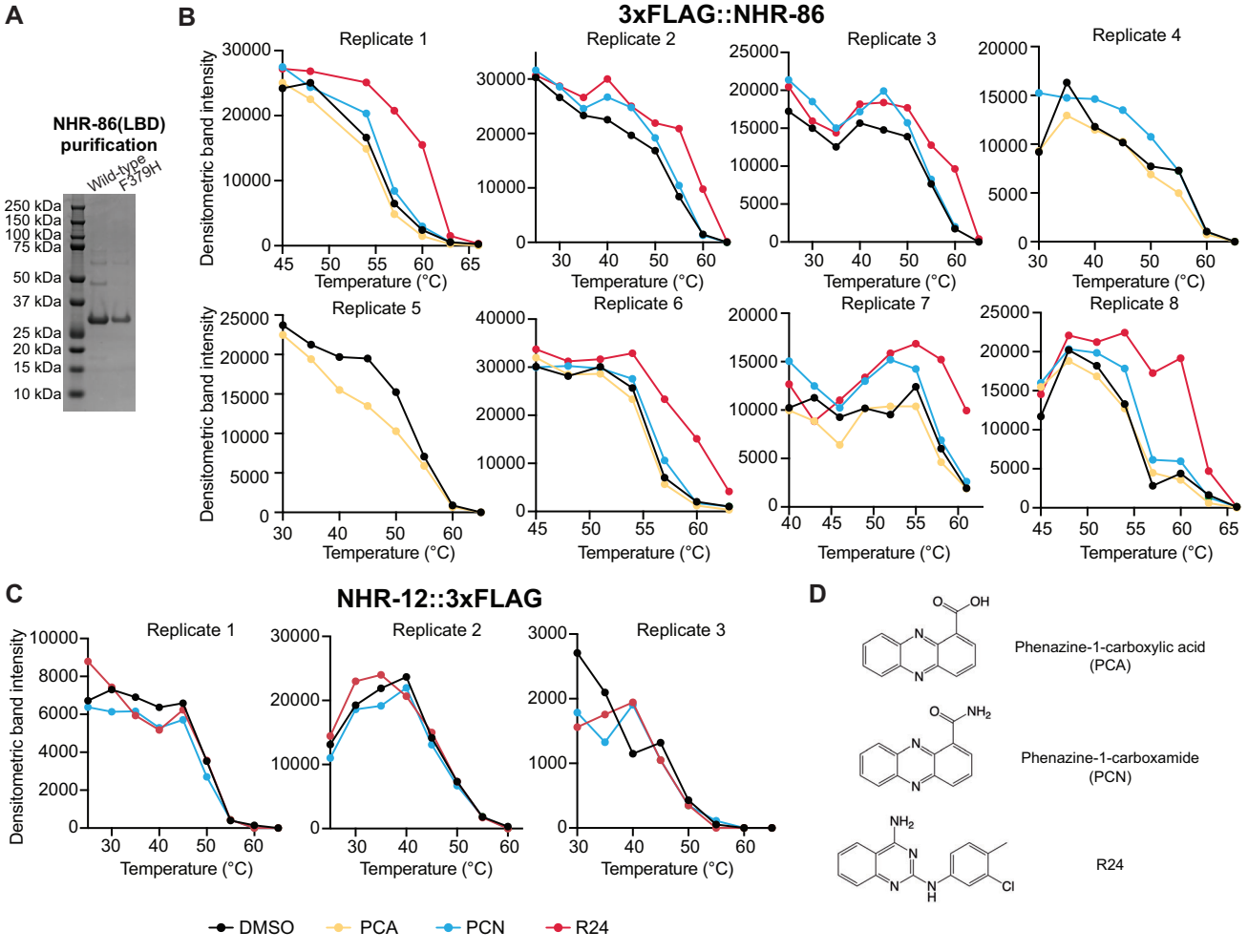

Figure S3. The bacterial metabolite PCN and synthetic immunostimulatory molecule R24 bind to the ligand-binding domain of NHR-86, related to Figure 3. (A) SDS-PAGE analysis of purified NHR(LBD) and NHR(LBD)^{F379H}, expected molecular weight of both proteins is 32.3 kDa. **(B)** Quantification of 3xFLAG::NHR-86 immunoblot band intensities for each treatment condition and temperature for all replicates. **(C)** Quantification of NHR-12::3xFLAG immunoblot band intensities for each treatment condition and temperature for each replicate. **(D)** Chemical structures of PCA, PCN, and R24. Source data for this figure is in Table S3.

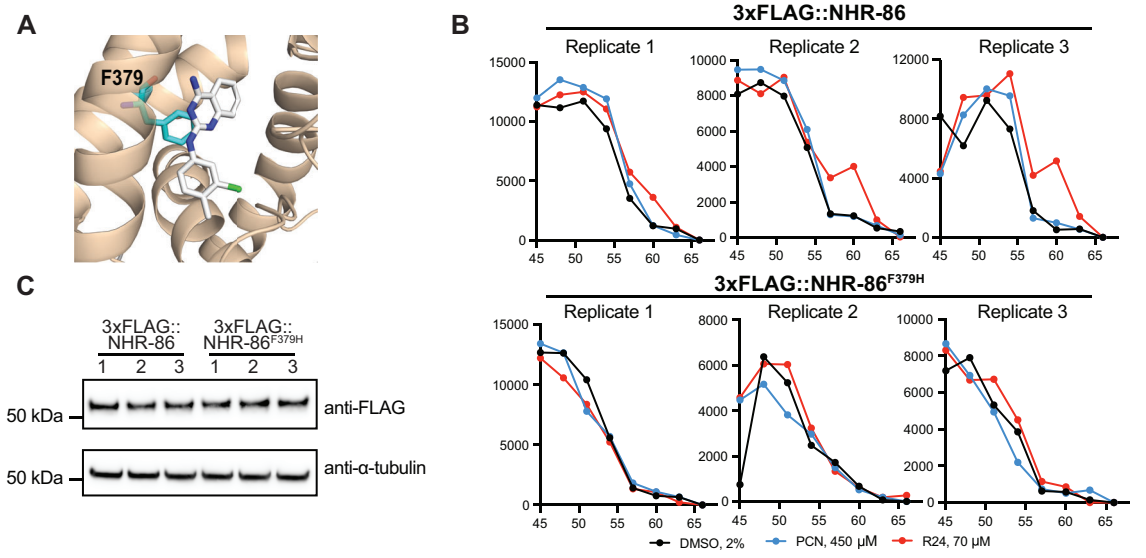

Figure S4. The phenylalanine at position 379 of the NHR-86 is required for the binding of PCN and R24, related to Figure 4. (A) An *in silico* model of R24 bound to the identified binding pocket in the NHR-86(LBD). The interaction of phenylalanine 379 (F379) (cyan) and R24 (white) is shown. **(B)** Quantification of 3xFLAG::NHR-86 and 3xFLAG::NHR-86^{F379H} immunoblot band intensities for each treatment condition and temperature for all replicates. **(C)** Immunoblot analysis of lysates from biological replicates of 3xFLAG::NHR-86 and 3xFLAG::NHR-86^{F379H} animals (*n*=3). Lysates were probed with anti-FLAG and anti-α-tubulin antibodies. The expected size of 3xFLAG::NHR-86 is 49.8 kDa. Source data for this figure is in Table S3.

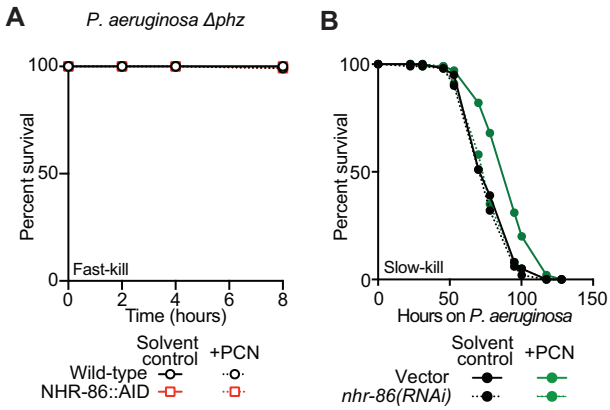

Figure S5. The bacterial metabolite PCN is a pattern of pathogenesis sensed by *C. elegans* NHR-86 to activate innate immunity, related to Figure 5. (A) A phenazine toxicity assay (also called the "fast kill" assay) with *P. aeruginosa* and *C. elegans* of the indicted genotypes either treated with solvent control or PCN. **(B)** *C. elegans- P. aeruginosa* infection assay (also called the "slow kill" assay) with the indicated strains and conditions. Sample sizes, mean lifespan, and p-values for all replicates are shown in Table S2. Source data for this figure is in Table S3.

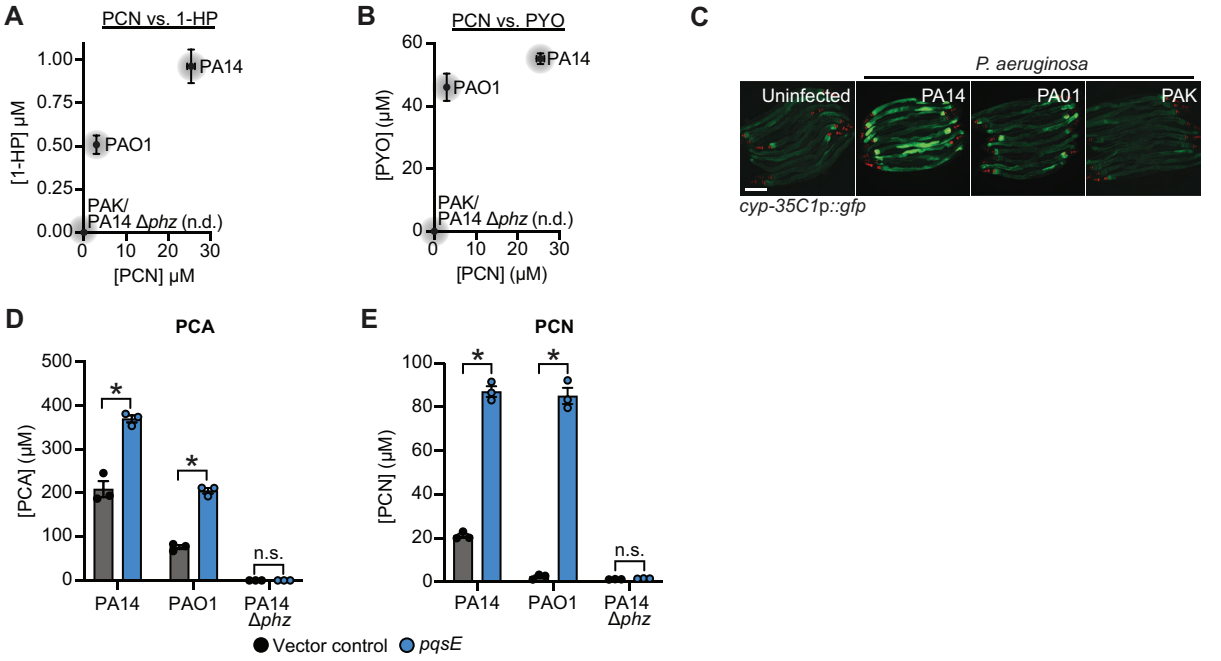

Figure S6. *C. elegans* NHR-86 senses PCN as a marker of pathogen virulence to activate protective anti-pathogen defenses, related to Figure 6. (A-B) HPLC-UV spectroscopy was used to quantify the individual phenazines in the indicated *P. aeruginosa* strains. 1-HP **(A)** and PYO **(B)** were compared to PCN production in biological replicates of the indicated *P. aeruginosa* strains (*n*=3). Phenazines not detected (n.d.). **(C**) Images of *C. elegans cyp-35C1*p::*gfp* animals either uninfected or infected with the indicated *P. aeruginosa* genotypes. Scale bar in all images equals 200 µm. **(D and E).** Quantification of phenazines PCA **(D)** and PCN **(E)** in indicated *P. aeruginosa* strains by HPLC-UV spectroscopy from biological replicates (*n*=3). *equals p<0.05 (two-way ANOVA with Tukey's multiple comparisons test). See also Table S3 for the HPLC-UV and LC-MS/MS phenazine quantification retention times and abundance. Source data for this figure is in Table S3.