# FSHR-1 and Innate Immune Response

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Molecular Biology Building

# Background

- Innate Immunity
  - Critical in the defense of pathogens
  - Signals the adaptive immune response
- Genetic analyses have identified many molecular pathways used in signaling when pathogens invade a host

# Background Cont.

- Invading pathogens have Pattern Associated Molecular Patterns (PAMPs) that are recognized by Pattern Recognition Receptors (PRRs)
  - A feature of many PPRs is ligand binding domains that are composed of multiple leucine-rich repeats (LLRs)
- Powell et al hypothesized that *C. elegans* uses LLR domains in pathogen recognition.
  - A Follicle Stimulating Hormone Receptor (FSHR-1) was shown to be critical in innate immune response

# FSHR-1

- Powell's work showed three things
  - Mutants of FSHR-1 show decreased survival time on pathogenic bacteria
  - FSHR-1 acts in the intestine of *C. elegans*
  - Mutants of FSHR-1 show similar survival on non-pathogenic bacteria
- My Project was to learn more about the molecular details of FSHR-1 with respect to Innate Immunity

# Methods

- *S. aureus* and *E. faecalis* grown at $37^0$C and spotted onto sorbitol plate
  - Dried overnight
- 100 worms were placed onto two soribitol plates of each bacteria.
- All strains were tested with controls
  - FSHR-1 deletion
  - N2 wild type
  - WY-254-deletion replaced with multiple copies of FSHR-1

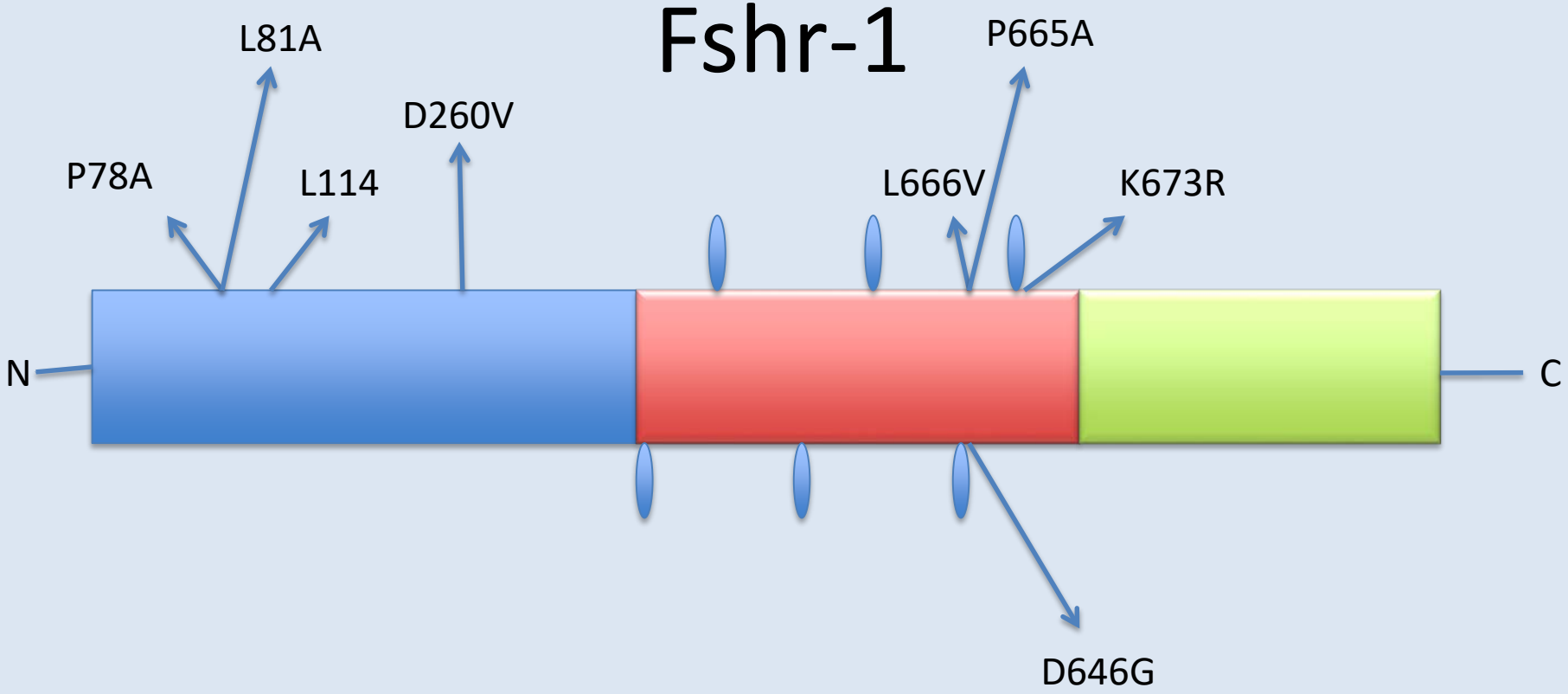
# Methods Continued

- Worms were scored for death each day
  - Scored as dead when they failed to respond to mechanical stimuli.
- An experiment was finished when all the worms being tested were dead.
- The results were put into Microsoft Excel© and graphs were created.

# Previously in the Fay Lab

| Strain Number | Variant Type | FSHR-1 Allele | Tested? |
|---|---|---|---|
| WY-487 | Partial LOF | D260V | No |
| WY-488 | Partial LOF | P665A | No |
| WY-489 | Partial LOF | L666V | No |
| WY-493 | Signaling Def. | K673R | Yes |
| WY-553 | Ligand Binding Def. | P78A | Yes |
| WY-554 | Ligand Binding Def. | L81A | Yes |
| WY-556 | Ligand Binding Def. | L114A | Yes |
| WY-557 | Strong LOF | Bar 1 excised | Yes |
| WY-490 | GOF | D646G | Yes |
| WY-551 | GOF | M469T | Yes |
| WY-552 | GOF | D632K | Yes |

# Fshr-1



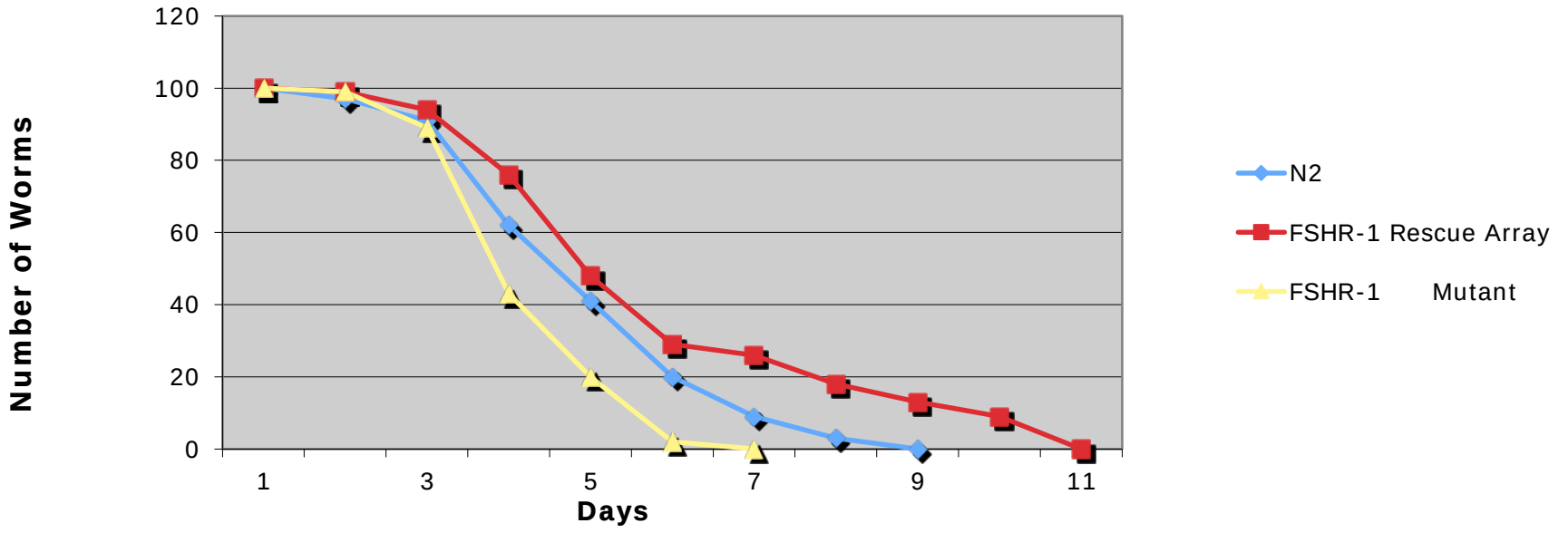

D260V-WY487 p LOF
P665A-WY488 p LOF
L666V-WY489 p LOF

D646G-WY490 GOF
M469T-WY551 GOF
D632K-WY552 GOF

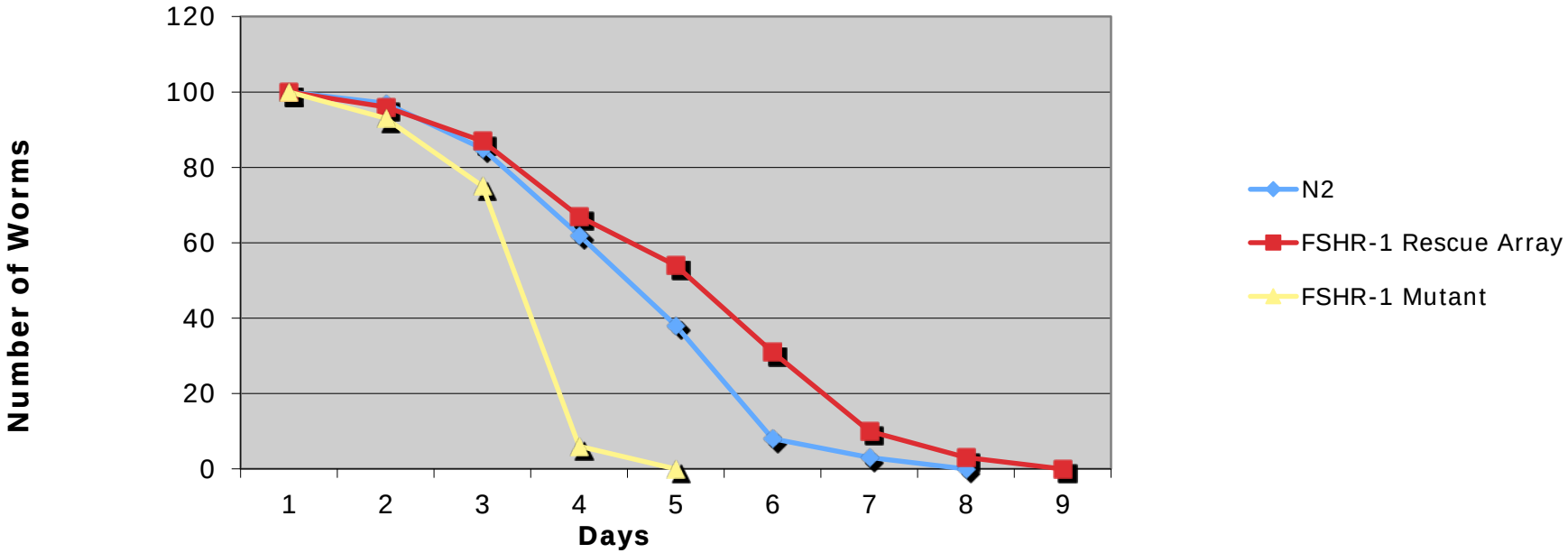
K673R-WY493 Signaling def
P78A-WY553 Ligand binding
L81A-WY554 Ligand binding
L11A-WY556 Ligand binding

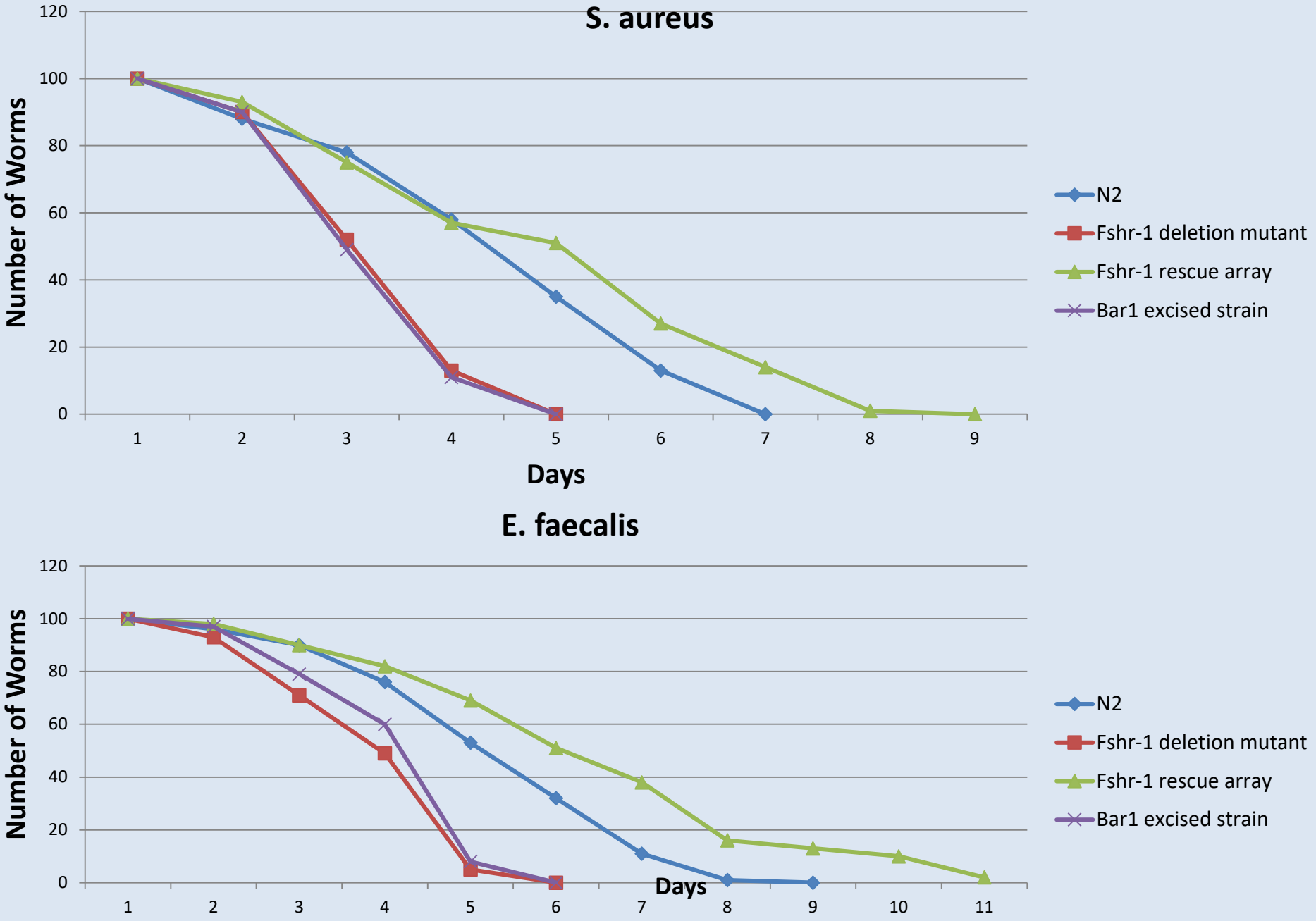
BarI excised-WY557 strong LOF

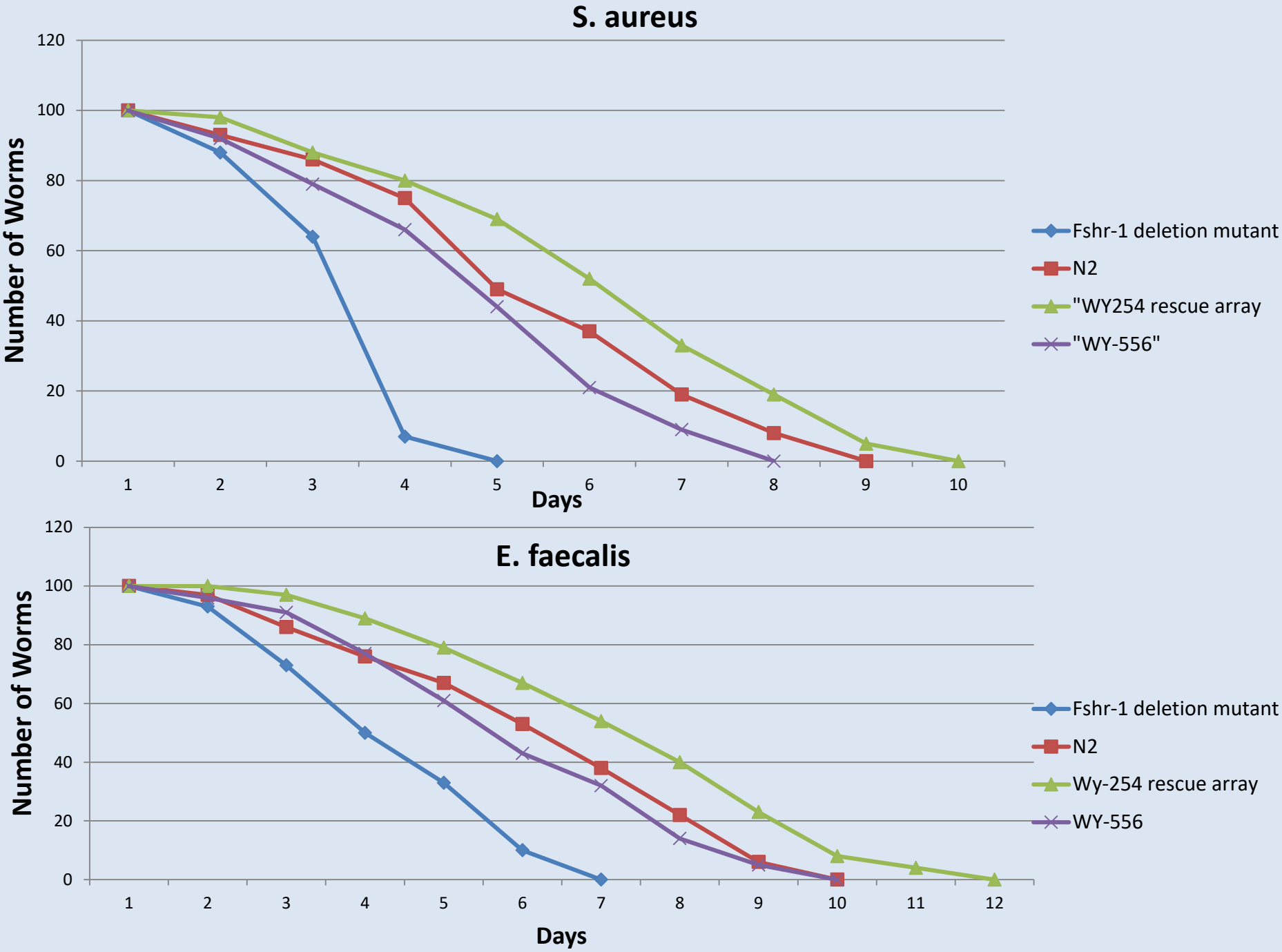
## *E. Faecalis* Kill Curve

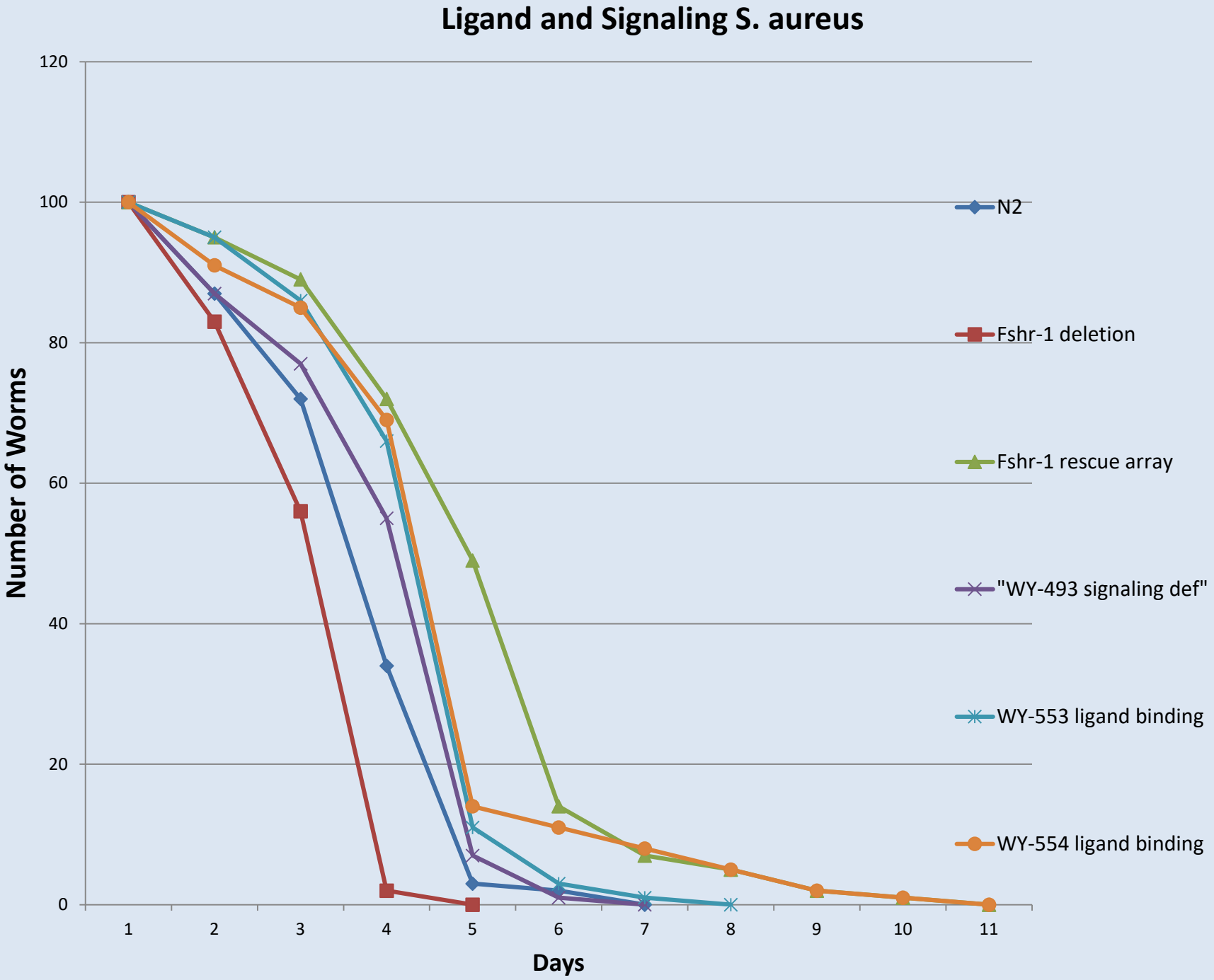


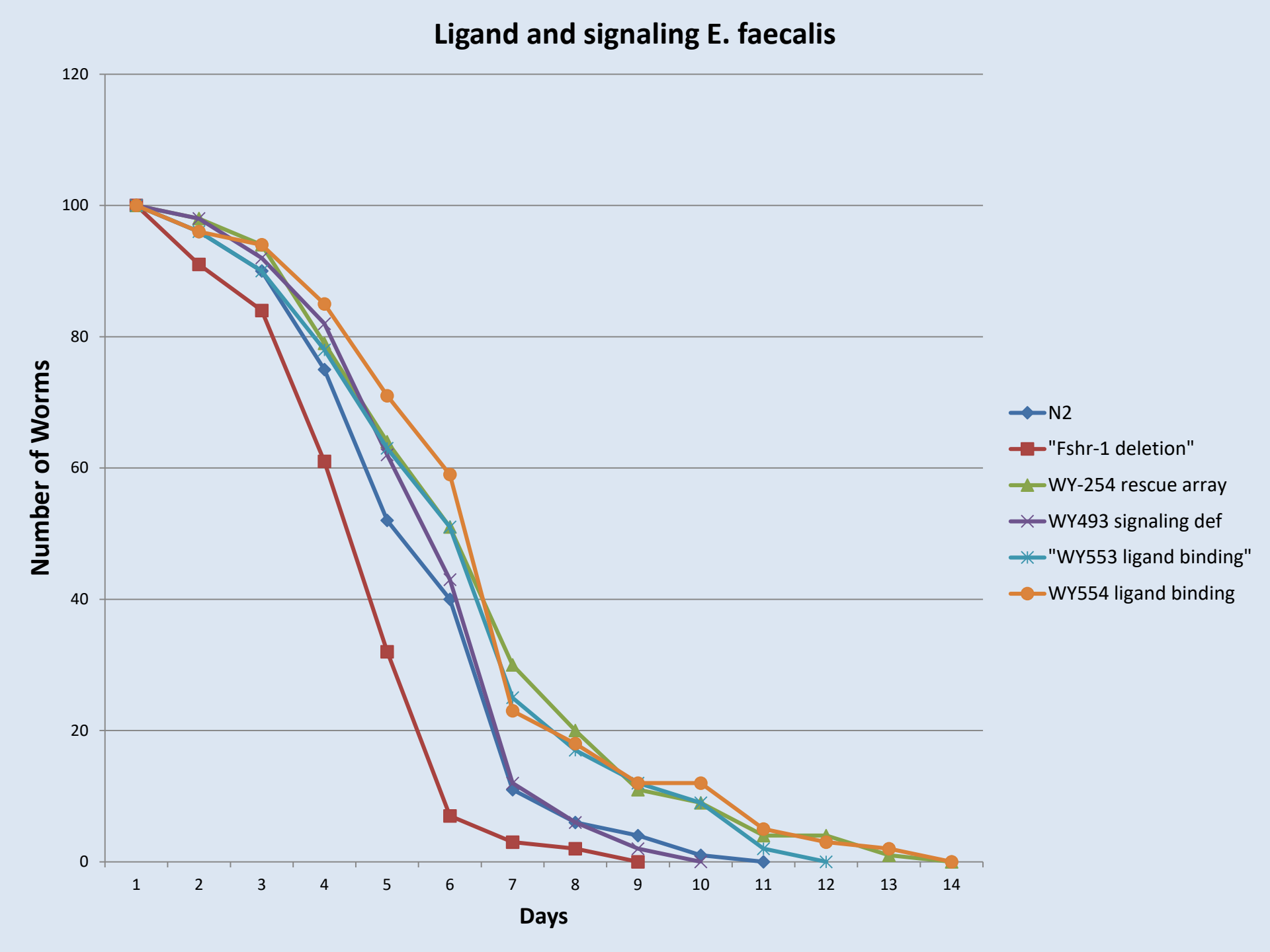

## *S. aureus* Kill Curve

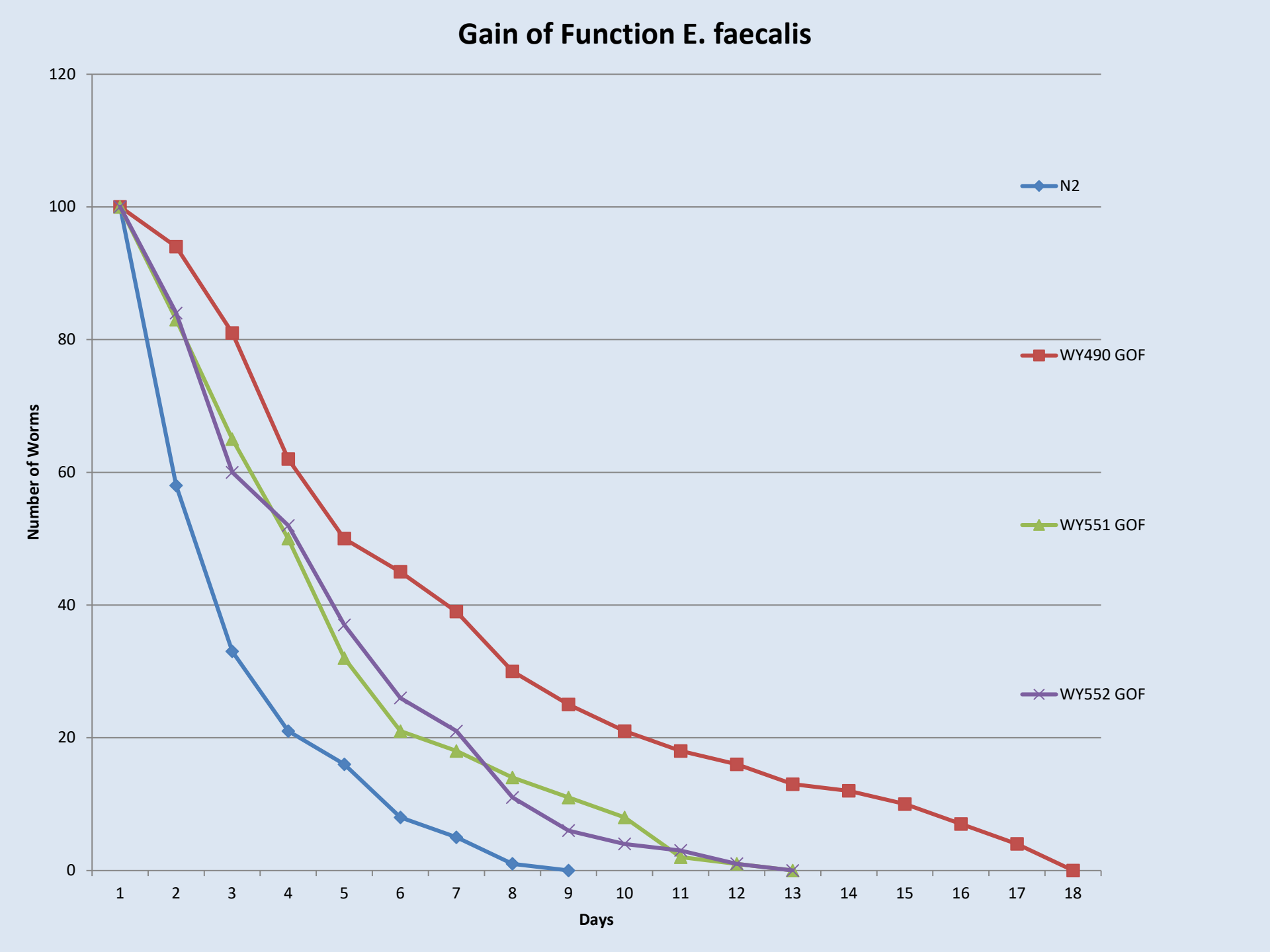



S. aureus
Number of Worms
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
Days
N2
Fshr-1 deletion mutant
Fshr-1 rescue array
Bar1 excised strain
E. faecalis
Number of Worms
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
Days
N2
Fshr-1 deletion mutant
Fshr-1 rescue array
Bar1 excised strain


S. aureus
Number of Worms
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
Days
Fshr-1 deletion mutant
N2
"WY254 rescue array
"WY-556"
E. faecalis
Number of Worms
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
Days
Fshr-1 deletion mutant
N2
Wy-254 rescue array
WY-556


Ligand and Signaling S. aureus
120
100
80
60
40
20
0
Number of Worms
1
2
3
4
5
6
7
8
9
10
11
Days
N2
Fshr-1 deletion
Fshr-1 rescue array
"WY-493 signaling def"
WY-553 ligand binding
WY-554 ligand binding


Ligand and signaling E. faecalis
Number of Worms
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Days
N2
"Fshr-1 deletion"
WY-254 rescue array
WY493 signaling def
"WY553 ligand binding"
WY554 ligand binding


Gain of Function E. faecalis
120
100
80
60
40
20
0
Number of Worms
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Days
N2
WY490 GOF
WY551 GOF
WY552 GOF

# The Caveats

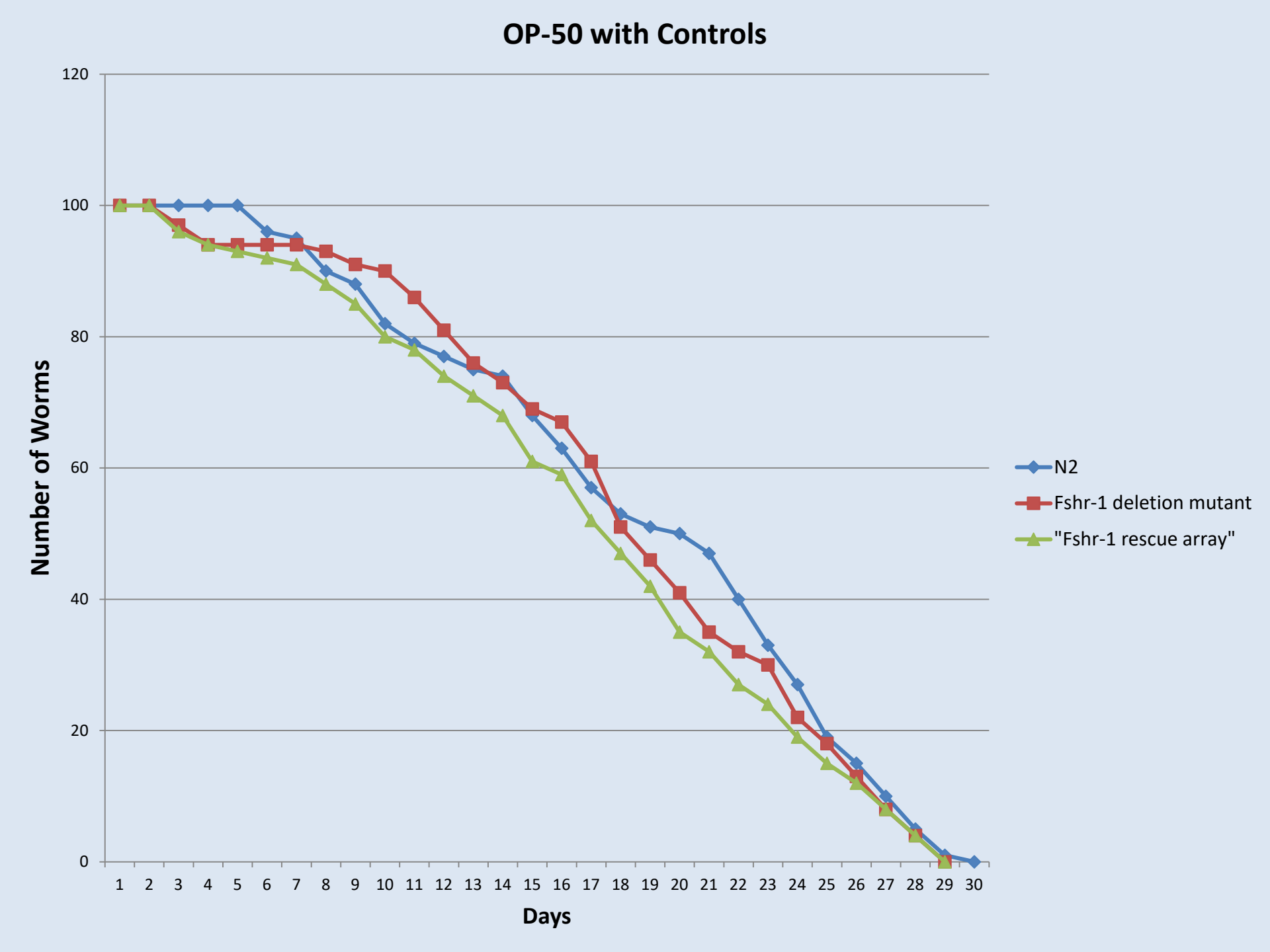
- No data has been complied for any of these strains showing expression levels compared to wild type.
  - A strain could have 5% activity but be over expressed by having multiple copies
- Ligand and Signaling Residues may not be critical in FSHR-1 function

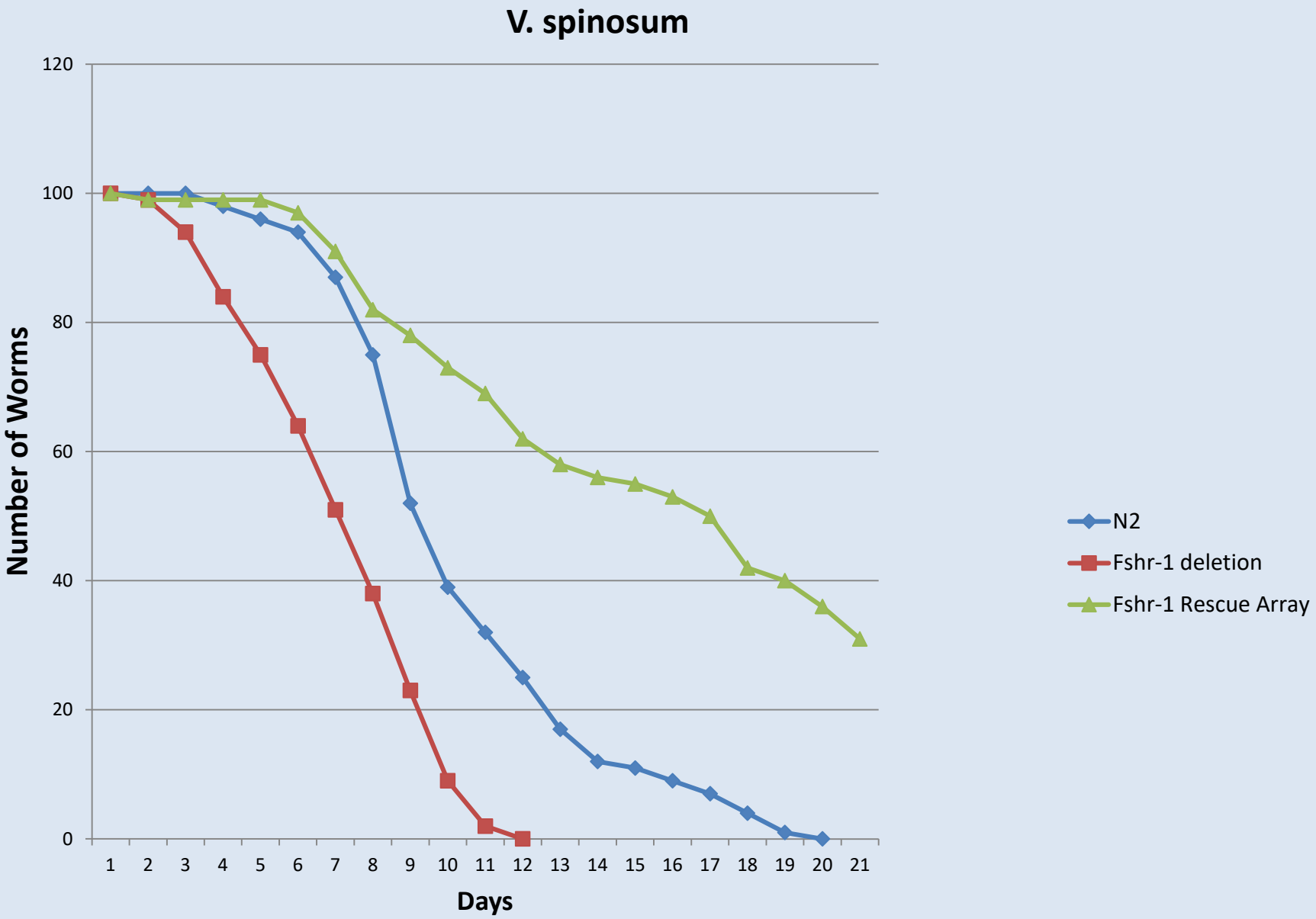
# More Caveats

- An over expressed N2 strain was not tested with the gain of function mutants
- Low graphical resolution and crowding
- Lack of homology correlation in signaling and ligand binding deficient strains

# *Verrucomicrobium spinosum*

- Recently discovered, free living soil microbe.
- Genome sequencing suggests that is has virulence factors including a Type-III secretion system.
- Separate line of experimentation done with this bacteria.


OP-50 with Controls
Number of Worms
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Days
N2
Fshr-1 deletion mutant
"Fshr-1 rescue array"


V. spinosum
Number of Worms
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
Days
N2
Fshr-1 deletion
Fshr-1 Rescue Array

# The End Result

- Work with FSHR-1 had to be stopped
  - More questions than answers
  - Results from *V. spinosum* give strong evidence that it is pathogenic against *C. elegans*

- Quesitons?