Supporting Information

Characterization of Aryloxyalkanoate Dioxygenase-12 (AAD-12), a Non-Heme Fe(II)/α-Ketoglutarate-Dependent Dioxygenase, Expressed in Transgenic Soy and *Pseudomonas fluorescens*

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Figure S1. SDS-PAGE of AAD-12 proteins isolated from transgenic soybean and Pf. Lane 1, Invitrogen molecular weight markers; lanes 2 and 3, event DAS-444Ø6-6-derived AAD-12; lane 4, PF-derived AAD-12.

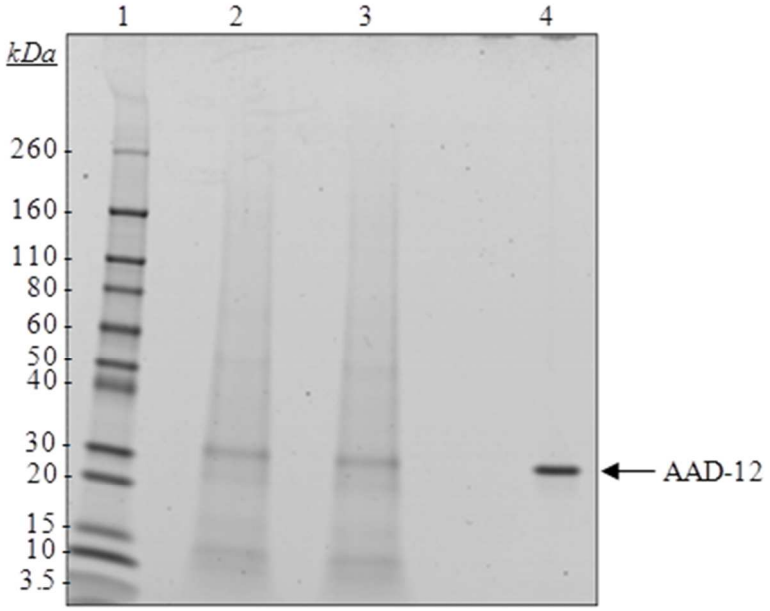


Figure S2. SDS-PAGE gels stained with (**A**) Coomassie blue (**B**) glycoprotein stain. Lane M, Invitrogen molecular weight markers; lane 1, horseradish peroxidase (positive control); lane 2, soybean trypsin inhibitor (negative control); Lane 3, bovine serum albumin (negative control); Lane 4, event DAS-444Ø6-6-derived AAD-12; lane 5, Pf-derived AAD-12; lane P, Novex prestained molecular weight markers**.**

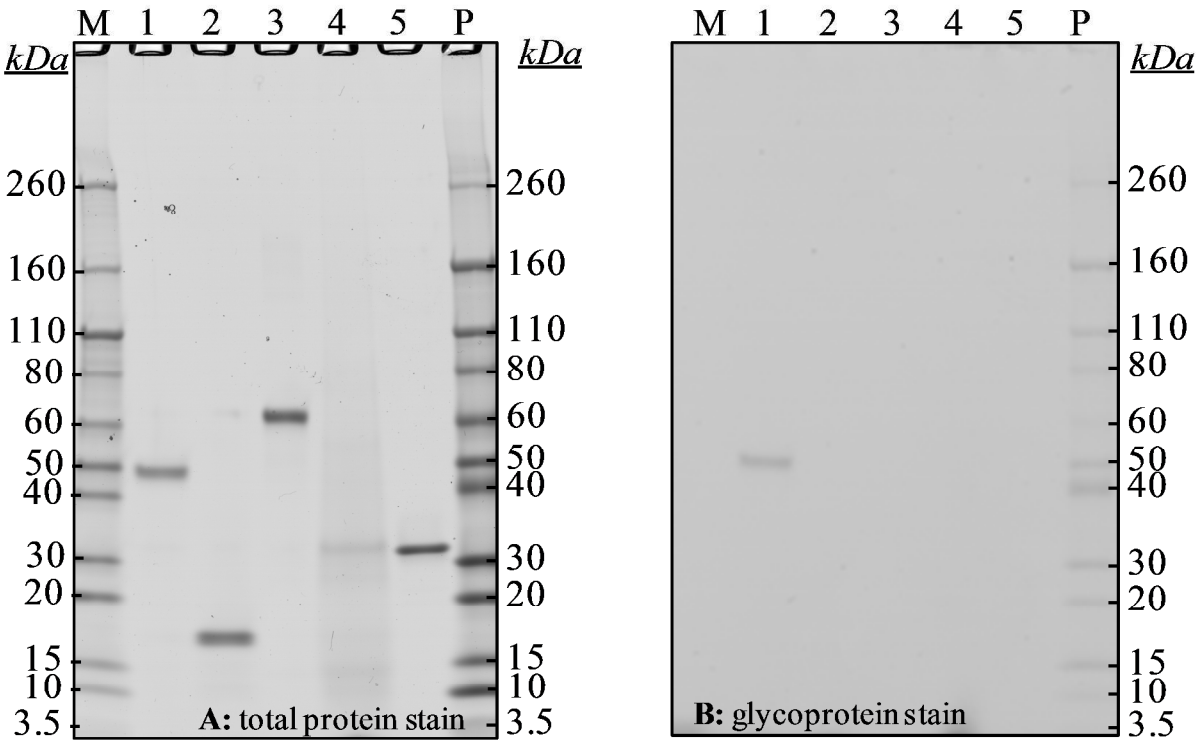


Figure S3. Sequence coverage map for event DAS-444Ø6-6 soybean derived AAD-12.

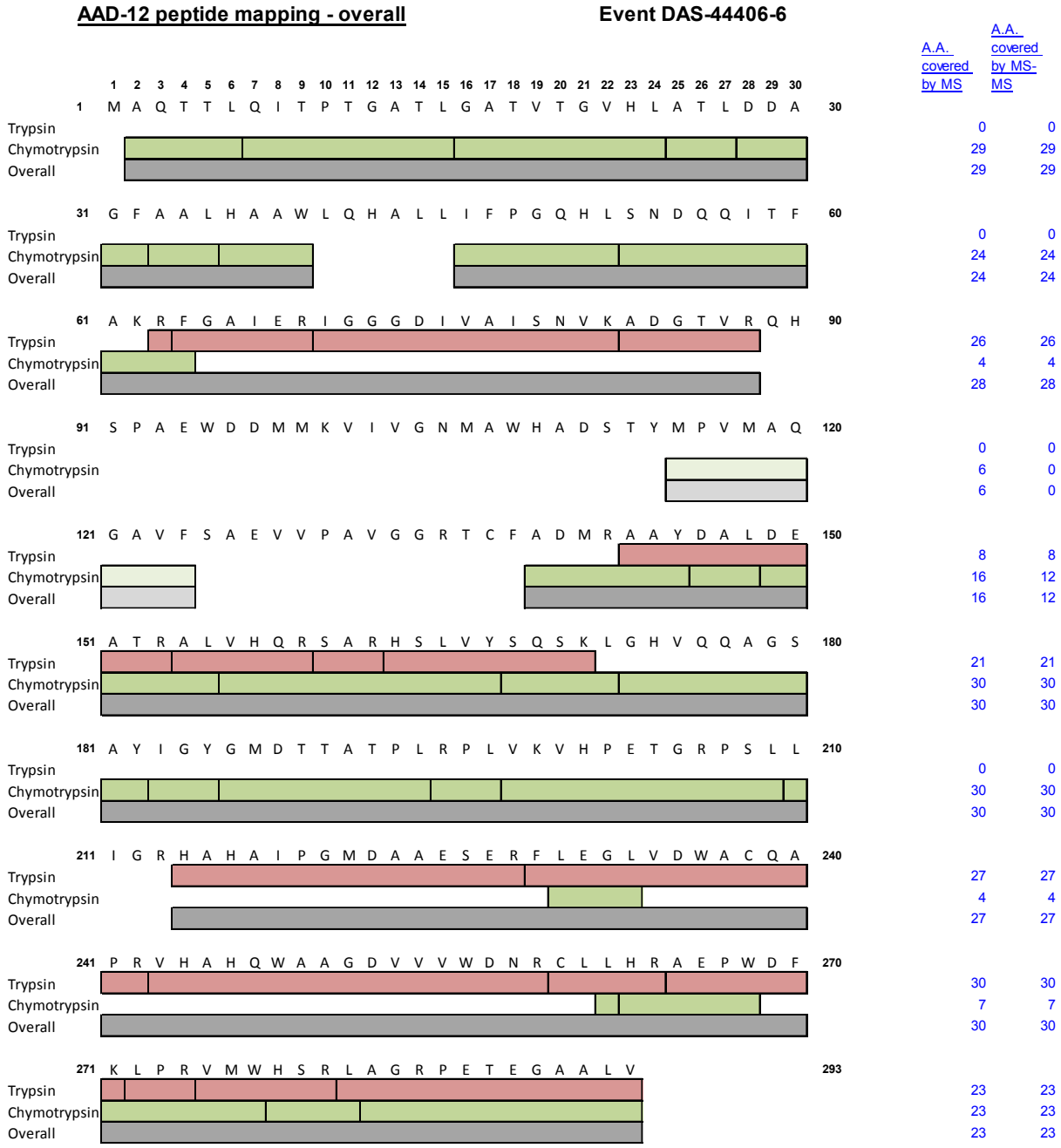


Figure S4. Sequence coverage map for event DAS-68416-4 soybean derived AAD-12.

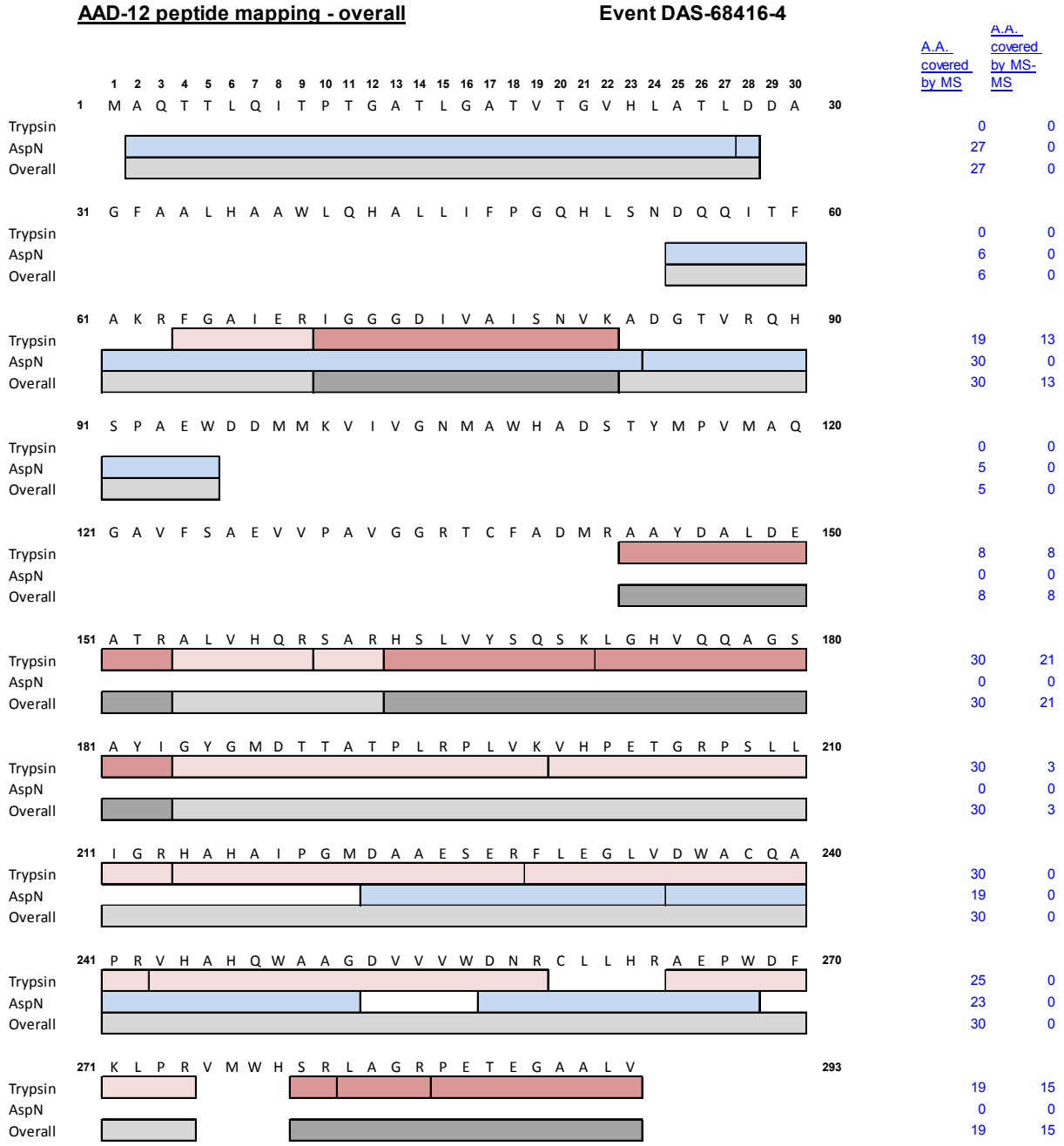


Figure S5. Sequence coverage map for purified Pf-derived AAD-12.

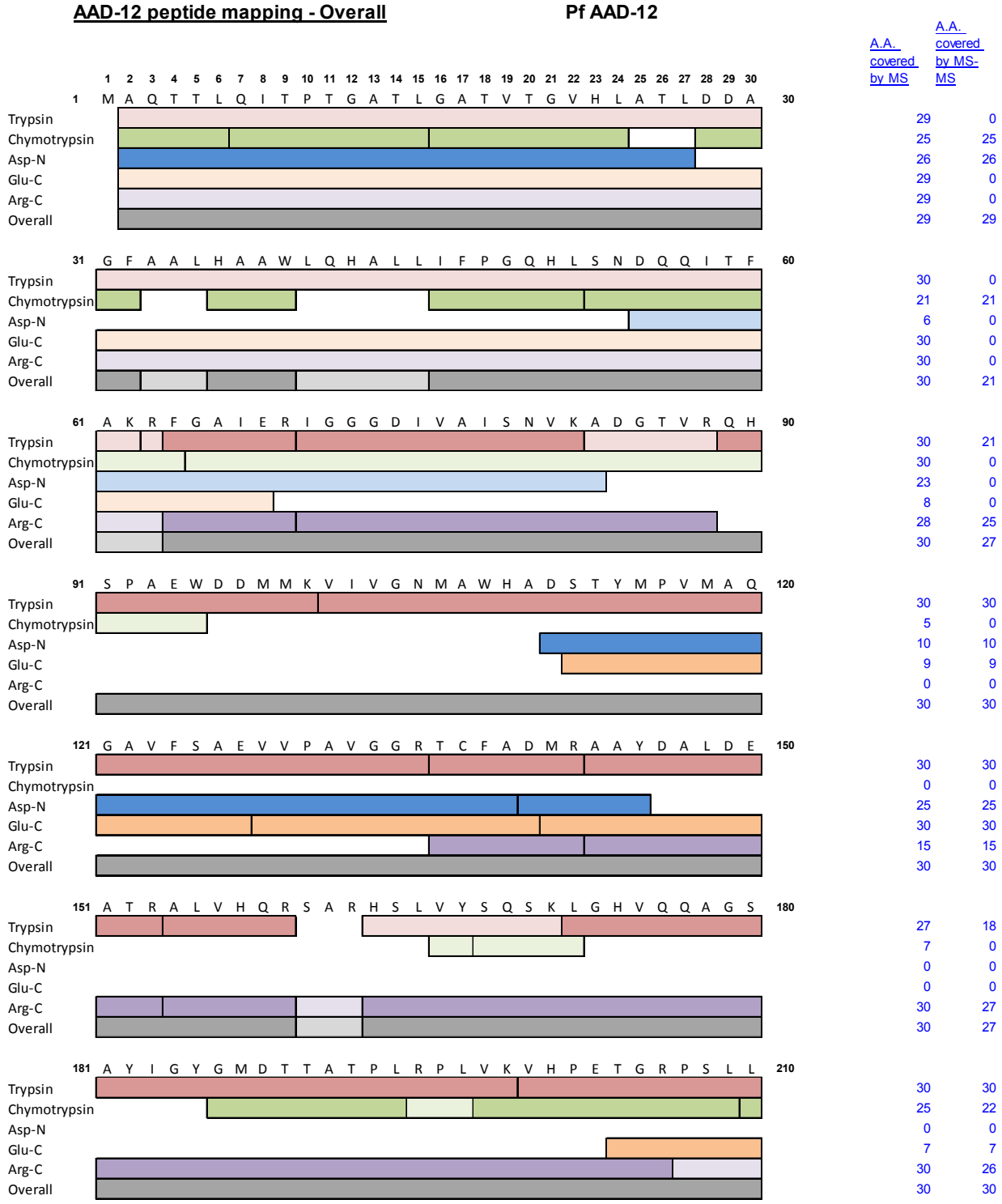
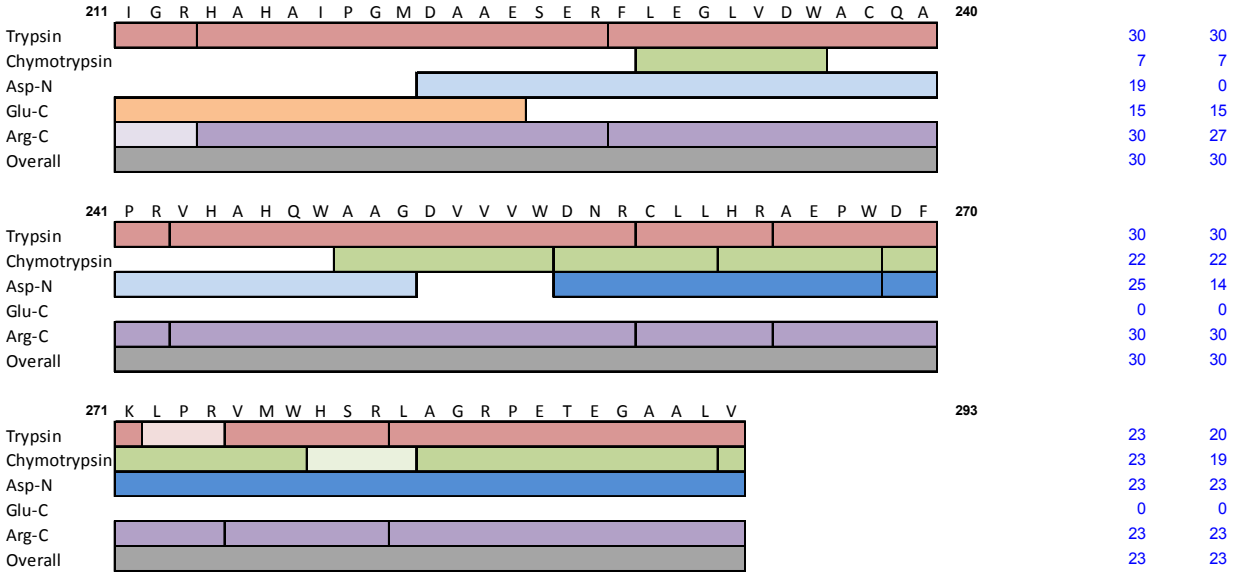
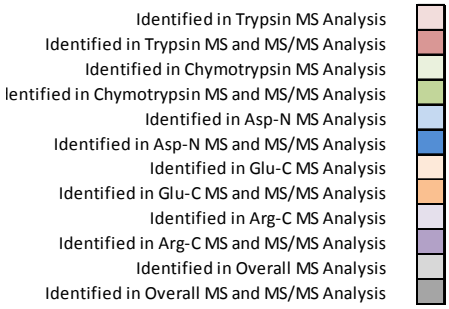


Figure S5. (continued)





Trypsin # of AA covered	**289**	**209**
Chymotrypsin # of AA covered	**165**	**116**
Asp-N # of AA covered	**157**	**98**
Glu-C # of AA covered	**128**	**61**
Arg-C # of AA covered	**245**	**173**
Overall # of AA covered	**292**	**277**
Trypsin Percent Coverage	**98.6**	**71.3**
Chymotrypsin Percent Coverage	**56.3**	**39.6**
Asp-N Percent Coverage	**53.6**	**33.4**
Glu-C Percent Coverage	**43.7**	**20.8**
Arg-C Percent Coverage	**83.6**	**59.0**
Overall Percent Coverage	**99.7**	**94.5**

Figure S6. Reaction rates as a function of *S*-dichlorprop concentration for Soybean AAD-12 (A) and Pf AAD12 (B).

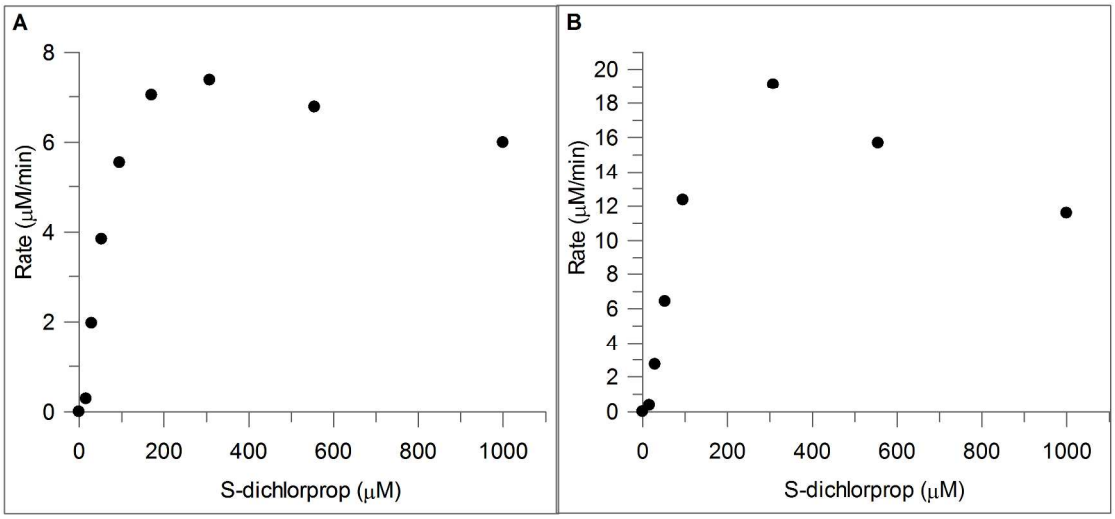


Figure S7. Relative rate of succinate production using L-amino acids versus 2,4-D.

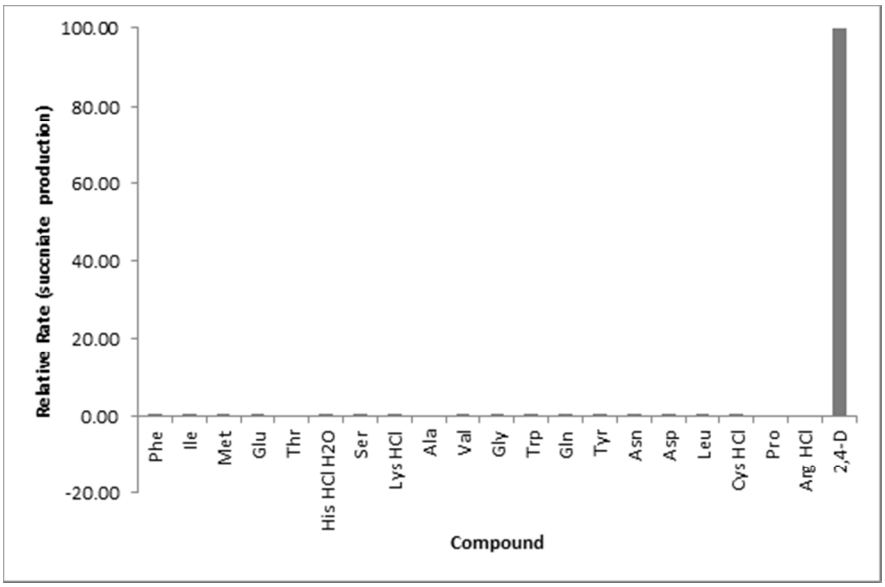
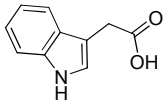
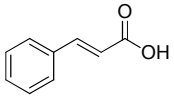


Figure S8. Structures of Endogenous and Xenobiotic Compounds tested in *in vitro* AAD-12 Assays.

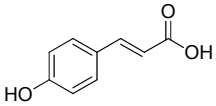
Plant endogenous compounds



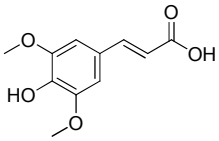
indole-3-acetic acid



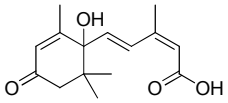
trans-cinnamate



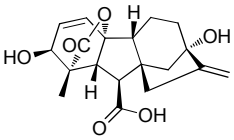
p-courmarate



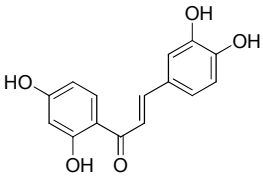
Sinapate



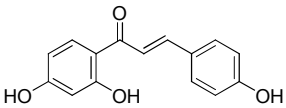
Abscisic acid



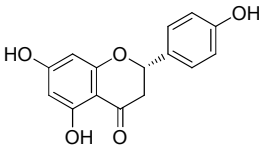
Gibberellic acid



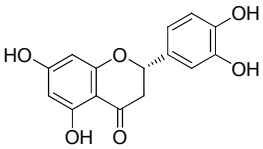
Butein



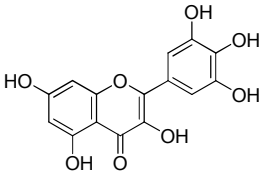
Isoliquiritigenin



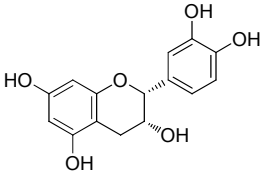
Naringenin



Eriodictyol

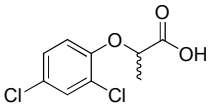


Myricetin

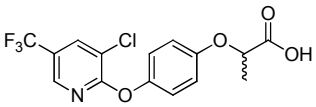


(-)Epicatechin

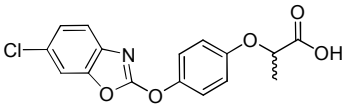
Xenobiotic compounds



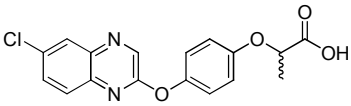
R,S-dichlorprop



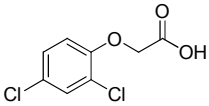
R,S-haloxyfop



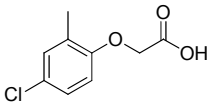
R,S-fenoxaprop



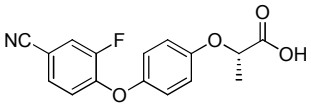
R,S-quizalofop



2,4-D



MCPA



S-cyhalofop

Table S1. LC-MS/MS Instrument Parameters

	2,4-dichlorophenylglycidate		3-phenylglycidic acid epoxide	
	Transition 1	**Transition 2**	**Transition 1**	**Transition 2**
Q1	230.8	230.8	162.8	162.8
Q3	186.8	114.9	119	100.9
DP	-40	-40	-55	-55
EP	-10	-10	-10	-10
CE	-14	-14	-16	-24
CXP	-25	-11	-15	-13

Instrument Settings	
Polarity	negative
Ion Source	turbo spray
Resolution Q1	unit
Resolution Q3	unit
CUR	30.00
CAD	medium
TEM	500.00
GS1	40.00
GS2	60.00
IS	-3500
EP	-10.00
CEM	2200

Table S2. Comparison of initial rates for succinate and oxidized products from *trans*-2,4-dichlorocinnamate and *trans*-cinnamate reactions.

Substrate	Substrate Concentration (μM)	Succinate (μM min^{-1})	Epoxide (μM min^{-1})	Coupling Efficiency
trans-cinnamate	500	9.03	4.15	0.46
trans-2,4-dichlorocinnamate	62.5	12.1	3.52	0.29
	500	26.8	4.91	0.18