Supplementary Materials: Differentiation, Quantification and Identification of Abrin and *Abrus precatorius* Agglutinin

Sylvia Worbs, Bettina Kampa, Martin Skiba, Eva-Maria Hansbauer, Daniel Stern, Hervé Volland, François Becher, Stéphanie Simon, Martin B. Dorner and Brigitte G. Dorner

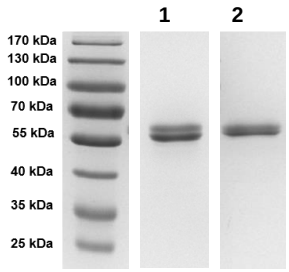

Figure S1. Purified abrin and *A. precatorius* agglutinin (APA) analyzed by SDS-PAGE and Coomassie staining. Two µg of abrin (lane 1) or APA (lane 2) each were separated on 12% gels by SDS-PAGE under non-reducing conditions followed by staining with colloidal Coomasssie Brilliant Blue over night.

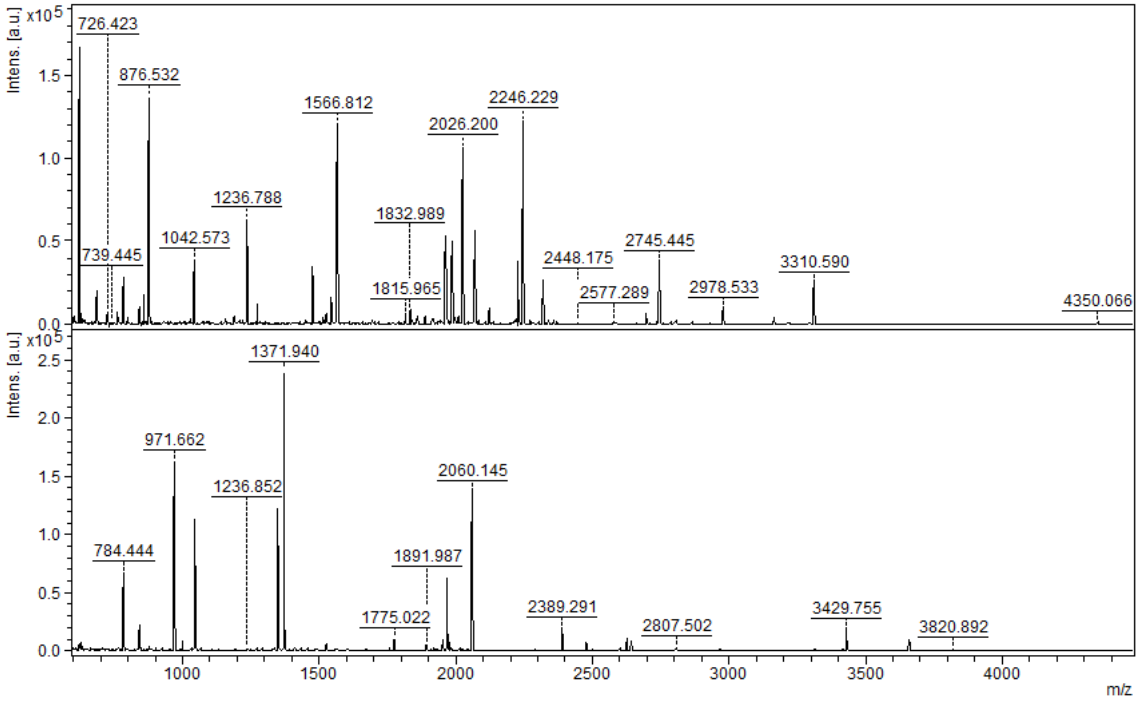

Figure S2. Purified abrin and *A. precatorius* agglutinin (APA) analyzed by MALDI-TOF MS. Overview of peptide mass fingerprinting (PMF) MALDI-TOF MS spectra of approximately 600 ng of purified abrin (top) or APA (bottom) after reduction, alkylation and tryptic *in-solution* digest. Peaks are labelled with the corresponding *m*/*z* value.

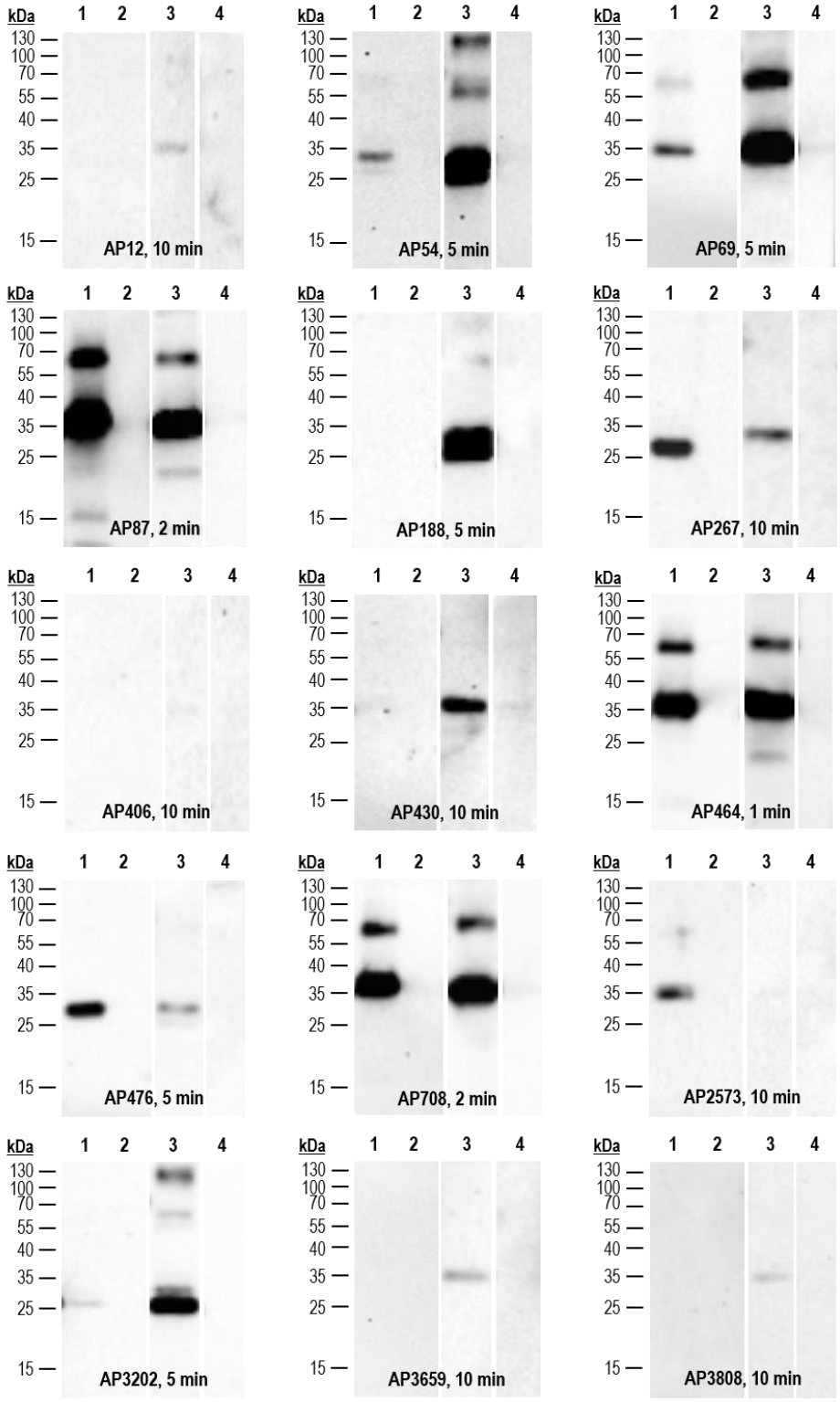

Figure S3. Detection of purified abrin, APA or ricin by Western blot using the monoclonal antibodies generated in this work. Purified APA (lane 1), BSA (as negative control, lane 2), abrin (lane 3) and ricin (lane 4), each 100 ng, were separated by SDS-PAGE under reducing conditions and blotted onto PVDF membranes. For detection the indicated monoclonal antibodies were used. For development a biotin-coupled anti-mouse antibody followed by streptavidin alkaline phosphatase and CDP star as

chemiluminescent substrate were used. Exposure time of the blots was 1–10 min as indicated. As positive control the polyclonal rabbit antibody KAP142 was used in Western blotting which detected both purified *Abrus* lectins (abrin and APA, main reactivity) as well as ricin (lower signals, data not shown).

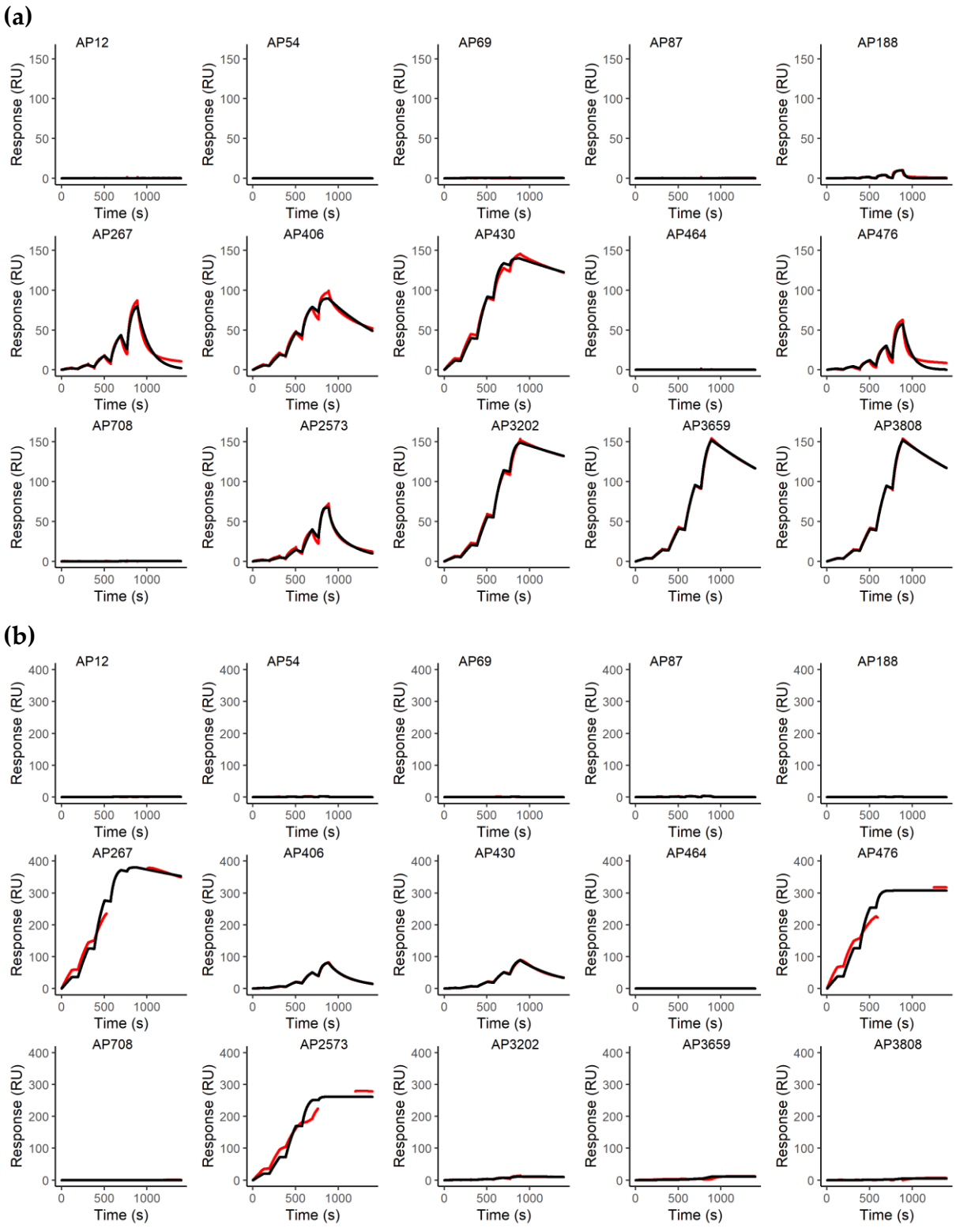

Figure S4. Binding kinetics of the newly generated monoclonal antibodies to abrin and APA. Shown are binding responses (in resonance units RU) of double referenced binding curves (red lines) overlaid with fitting curves (black lines) from a 1:1 binding model for single cycle kinetic measurements of the indicated mAbs binding to (**a**) abrin or (**b**) APA. Five increasing concentrations of abrin or APA were

injected consecutively for 120 s before buffer was injected for 600 s after injection of the highest concentration (333.33 nM corresponding to 20 μg/mL abrin or 40 μg/mL APA).

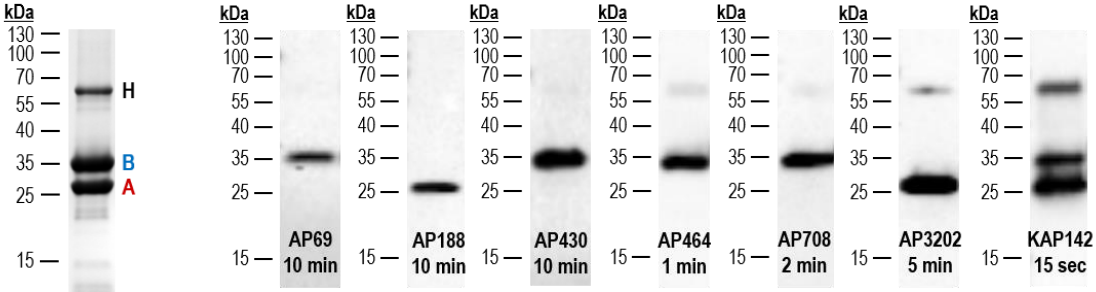

Figure S5. Binding specificity of selected monoclonal antibodies targeting the A- or B-chain of purified abrin-a. (**a**) 15 µg of purified abrin-a were separated by SDS-PAGE under reducing conditions followed by staining with colloidal Coomasssie Brilliant Blue overnight. H: band corresponding to non-reduced abrin-a holotoxin; B: band corresponding to B-chain of abrin-a; A: band corresponding to A-chain of abrin-a [1,2]. The assignments of the A- and B-chains were confirmed by in-gel digest followed by MALDI-TOF MS analysis. (**b**) Purified abrin-a was analyzed by Western blot using several monoclonal antibodies generated in this work. 100 ng of abrin-a were separated by SDS-PAGE under reducing conditions and blotted onto PVDF membranes. For detection the indicated monoclonal antibodies were used. As a positive control the polyclonal rabbit antibody KAP142 was used to detect both sub-units of purified abrin-a (A- and B-chain). For development a biotin-coupled anti-mouse antibody (for the detection of monoclonal antibodies) or a biotin-coupled anti-rabbit antibody (for the detection of KAP142) followed by streptavidin alkaline phosphatase and CDP star as chemiluminescent substrate was used. Exposure time of the blots was 15 s to 10 min as indicated.

P11140, **Abrin-a**; Protein sequence coverage: 60%

```
   1 QDRPIKFSTE GATSQSYKQF IEALRERLRG GLIHDIPVLP DPTTLQERNR
  51 YITVELSNSD TESIEVGIDV TNAYVVAYRA GTQSYFLRDA PSSASDYLFT
 101 GTDQHSLPFY GTYGDLERWA HQSRQQIPLG LQALTHGISF FRSGGNDNEE
 151 KARTLIVIIQ MVAEAARFRY ISNRVRVSIQ TGTAFQPDAA MISLENNWDN
 201 LSRGVQESVQ DTFPNQVTLT NIRNEPVIVD SLSHPTVAVL ALMLFVCNPP
 251 NANQSPLLIR SIVEKSKICS SRYEPTVRIG GRDGMCVDVY DNGYHNGNRI
 301 IMWKCKDRLE ENQLWTLKSD KTIRSNGKCL TTYGYAPGSY VMIYDCTSAV
 351 AEATYWEIWD NGTIINPKSA LVLSAESSSM GGTLTVQTNE YLMRQGWRTG
 401 NNTSPFVTSI SGYSDLCMQA QGSNVWMADC DSNKKEQQWA LYTDGSIRSV
 451 QNTNNCLTSK DHKQGSTILL MGCSNGWASQ RWVFKNDGSI YSLYDDMVMD
 501 VKGSDPSLKQ IILWPYTGKP NQIWLTLF
```

Q06077, **Abrin-b**; Protein sequence coverage: 60%

```
   1 QDQVIKFTTE GATSQSYKQF IEALRQRLTG GLIHGIPVLP DPTTLQERNR
  51 YISVELSNSD TESIEAGIDV SNAYVVAYRA GNRSYFLRDA PTSASRYLFT
 101 GTQQYSLRFN GSYIDLERLA RQTRQQIPLG LQALRHAISF LQSGTDDQEI
 151 ARTLIVIIQM ASEAARYRFI SYRVGVSIRT NTAFQPDAAM ISLENNWDNL
 201 SGGVQQSVQD TFPNAVTLRS VNNQPVIVDS LTHQSVAVLA LMLFVCNPPN
 251 ANQSPLLIRS IVEKSKICSS RYEPTVRIGG RNGMCVDVYD DGYHNGNRII
 301 AWKCKDRLEE NQLWTLKSDK TIRSNGKCLT TEGYAPGNYV MIYDCTSAVA
 351 EATYWEIWDN GTIINPKSAL VLSAESSSMG GTLTVQTNEY LMRQGWRTGN
 401 NTSPFVTSIS GYSDLCMQAQ GSNVWLAYCD NNKKEQQWAL YTDGSIRSVQ
 451 NTNNCLTSKD HKQGSPIVLM ACSNGWASQR WLFRNDGSIY NLHDDMVMDV
 501 KRSDPSLKEI ILHPYHGKPN QIWLTLF
```

P28590, **Abrin-c**; Protein sequence coverage: 55%

```
   1 MDKTLKLLIL CLAWTCSFSA LRCAARTYPP VATNQDQVIK FTTEGATSQS
  51 YKQFIEALRQ RLTGGLIHDI PVLPDPTTVE ERNRYITVEL SNSERESIEV
 101 GIDVTNAYVV AYRAGSQSYF LRDAPASAST YLFPGTQRYS LRFDGSYGDL
 151 ERWAHQTREE ISLGLQALTH AISFLRSGAS NDEEKARTLI VIIQMASEAA
 201 RYRYISNRVG VSIRTGTAFQ PDPAMLSLEN NWDNLSGGVQ QSVQDTFPNN
 251 VILSSINRQP VVVDSLSHPT VAVLALMLFV CNPPNANQSP LLIRSIVEES
 301 KICSSRYEPT VRIGGRDGMC VDVYDDGYHN GNRIIAWKCK DRLEENQLWT
 351 LKSDKTIRSN GKCLTTEGYA PGNYVMIYDC TSAVAEATYW EIWDNGTIIN
 401 PKSALVLSAE SSSMGGTLTV QTNEYLMRQG WRTGNNTSPF VTSISGYSDL
 451 CMQAQGSNVW LADCDNNKKE QQWALYTDGS IRSVQNTNNC LTSKDHKQGS
```

501 PIVLMACSNG WASQRWLFKN DGSIYNLHDD MVMDVKRSDP SLKEIILHPY

551 HGKPNQIWLT LF

Q06076, **Abrin-d**; Protein sequence coverage: 53%

1 QDQVIKFTTE GATSQSYKQF IEALRQRLTG GLIHDIPVLP DPTTVEERNR

51 YITVELSNSE RESIEVGIDV TNAYVVAYRA GSQSYFLRDA PASASTYLFP

101 GTQRYSLRFD GSYGDLERWA HQTREEISLG LQALTHAISF LRSGASNDEE

151 KARTLIVIIQ MASEAARYRC ISNRVGVSIR TGTAFQPDPA MLSLENNWDN

201 LSGGVQQSVQ DAFPNNVILS SINRQPVVVD SLSHPTVAVL ALMLFVCNPP

251 NANQSPLLIR SIVEESKICS SRYEPTVRIG GRDGMCVDVY DDGYHNGNRI

301 IAWKCKDRLE ENQLWTLKSD LTIRSNGKCL TTEGYAPGNY VMIYDCTSAV

351 AEATYWEIWD NGTIINPKSA LVLSAESSSM GGTLTVQTNE YLMRQGWRTG

401 NNTSPFVTSI SGYSDLCMQA QGSNVWLADC DNNKKEQQWA LYTDGSIRSV

451 QNTNNCLTSK DHKQGSPIVL MACSNGWASQ RWLFKNDGSI YSLYDDMVMD

501 VKGSDPSLKQ IILWPYTGKP NQIWLTLF

Q9M6E9, **Agglutinin-1**; Protein sequence coverage: 39%

1 MKFETTKNKL HGNAYYQAQF QDPIKFTTGS ATPASYNQFI DALRERLTGG

51 LIYGIPVLRD PSTVEKPNQY VTVELSYSDT VSIQLGIDLT NAYVVAYRAG

101 SESFFFRNAP ASASTYLFTG TQQYSLPFDG NYDDLEKWAH QSRQRISLGL

151 EALRQGIKFL RSGASDDEEI ARTLIVIIQM VAEAARFRYV SKLVVISLSN

201 RAAFQPDPSM LSLENTWEPL SRAVQHTVQD TFPQNVTLIN VRQERVVVSS

251 LSHPSVSALA LMLFVCNPLN ATQSPLLIRS VVEQSKICSS HYEPTVRIGG

301 RDGLCVDVSD NAYNNGNPII LWKCKDQLEV NQLWTLKSDK TIRSKGKCLT

351 TYGYAPGNYV MIYDCSSAVA EATYWDIWDN GTIINPKSGL VLSAESSSMG

401 GTLTVQKNDY RMRQGWRTGN DTSPFVTSIA GFFKLCMEAH GNSMWLDVCD

451 ITKEEQQWAV YPDGSIRPVQ NTNNCLTCEE HKQGATIVMM GCSNAWASQR

501 WVFKSDGTIY NLYDDMVMDV KSSDPSLKQI ILWPYTGNAN QMWATLF

Figure S6. Protein sequence coverage of proteins identified in the purified abrin preparation. Approximately 75 µg of the purified abrin preparation used in this work were subjected to immuno-affinity enrichment using a mixture of four mAbs, namely AP430, AP3808, AP3659 and AP476, coupled to magnetic Dynabeads, followed by protein reducing, alkylation, tryptic digest and non-targeting LC-ESI-MS/MS analysis. From top to bottom, sequences of identified abrin isoforms (UniProt P11140, UniProt Q06077, UniProt P28590, UniProt Q06076) and *Abrus precatorius* Agglutinin APA; UniProt Q9M6E9) are shown after MASCOT server search against a self-assembled UniProt/NCBI database containing all abrin isoforms and *Abrus precatorius* agglutinin as well as an NCBI database containing all *Abrus precatorius* proteins. Asparagine (N) highlighted in turquoise represents potential N-linked glycosylation sites. The linker peptide sequence between the two chains of each abrin isoform is underlined. Amino acids highlighted in red were experimentally identified. Amino acids in position 1–34 in abrin-c or in position 1–20 in APA represent the signal peptide

(highlighted in grey). CAVE: Unambigous identification of abrin-c in the presence of the other three isoforms (abrin-a, -b and -d) is not possible with our current LC-MS/MS setup-up so that no proteotypic peptides for abrin-c can be detected.

Q9M6E9, **Agglutinin-1**; Protein sequence coverage: 52%

```
  1 MKFETTKNKL HGNAYYQAQF QDPIKFTTGS ATPASYNQFI DALRERLTGG
 51 LIYGIPVLRD PSTVEKPNQY VTVELSYSDT VSIQLGIDLT NAYVVAYRAG
101 SESFFFRNAP ASASTYLFTG TQQYSLPFDG NYDDLEKWAH QSRQRISLGL
151 EALRQGIKFL RSGASDDEEI ARTLIVIIQM VAEAARFRYV SKLVVISLSN
201 RAAFQPDPSM LSLENTWEPL SRAVQHTVQD TFPQNVTLIN VRQERVVVSS
251 LSHPSVSALA LMLFVCNPLN ATQSPLLIRS VVEQSKICSS HYEPTVRIGG
301 RDGLCVDVSD NAYNNGNPII LWKCKDQLEV NQLWTLKSDK TIRSKGKCLT
351 TYGYAPGNYV MIYDCSSAVA EATYWDIWDN GTIINPKSGL VLSAESSSMG
401 GTLTVQKNDY RMRQGWRTGN DTSPFVTSIA GFFKLCMEAH GNSMWLDVCD
451 ITKEEQQWAV YPDGSIRPVQ NTNNCLTCEE HKQGATIVMM GCSNAWASQR
501 WVFKSDGTIY NLYDDMVMDV KSSDPSLKQI ILWPYTGNAN QMWATLF
```

P11140, **Abrin-a**; Protein sequence coverage: 53%

```
  1 QDRPIKFSTE GATSQSYKQF IEALRERLRG GLIHDIPVLP DPTTLQERNR
 51 YITVELSNSD TESIEVGIDV TNAYVVAYRA GTQSYFLRDA PSSASDYLFT
101 GTDQHSLPFY GTYGDLERWA HQSRQQIPLG LQALTHGISF FRSGGNDNEE
151 KARTLIVIIQ MVAEAARFRY ISNRVRVSIQ TGTAFQPDAA MISLENNWDN
201 LSRGVQESVQ DTFPNQVTLT NIRNEPVIVD SLSHPTVAVL ALMLFVCNPP
251 NANQSPLLIR SIVEKSKICS SRYEPTVRIG GRDGMCVDVY DNGYHNGNRI
301 IMWKCKDRLE ENQLWTLKSD KTIRSNGKCL TTYGYAPGSY VMIYDCTSAV
351 AEATYWEIWD NGTIINPKSA LVLSAESSSM GGTLTVQTNE YLMRQGWRTG
401 NNTSPFVTSI SGYSDLCMQA QGSNVWMADC DSNKKEQQWA LYTDGSIRSV
451 QNTNNCLTSK DHKQGSTILL MGCSNGWASQ RWVFKNDGSI YSLYDDMVMD
501 VKGSDPSLKQ IILWPYTGKP NQIWLTLF
```

Q06077, **Abrin-b**; Protein sequence coverage: 54%

```
  1 QDQVIKFTTE GATSQSYKQF IEALRQRLTG GLIHGIPVLP DPTTLQERNR
 51 YISVELSNSD TESIEAGIDV SNAYVVAYRA GNRSYFLRDA PTSASRYLFT
101 GTQQYSLRFN GSYIDLERLA RQTRQQIPLG LQALRHAISF LQSGTDDQEI
151 ARTLIVIIQM ASEAARYRFI SYRVGVSIRT NTAFQPDAAM ISLENNWDNL
201 SGGVQQSVQD TFPNAVTLRS VNNQPVIVDS LTHQSVAVLA LMLFVCNPPN
251 ANQSPLLIRS IVEKSKICSS RYEPTVRIGG RNGMCVDVYD DGYHNGNRII
301 AWKCKDRLEE NQLWTLKSDK TIRSNGKCLT TEGYAPGNYV MIYDCTSAVA
351 EATYWEIWDN GTIINPKSAL VLSAESSSMG GTLTVQTNEY LMRQGWRTGN
401 NTSPFVTSIS GYSDLCMQAQ GSNVWLAYCD NNKKEQQWAL YTDGSIRSVQ
451 NTNNCLTSKD HKQGSPIVLM ACSNGWASQR WLFRNDGSIY NLHDDMVMDV
```

501 KRSDPSLKEI ILHPYHGKPN QIWLTLF

Q06076, **Abrin-d**; Protein sequence coverage: 43%

1 QDQVIKFTTE GATSQSYKQF IEALRQRLTG GLIHDIPVLP DPTTVEERNR

51 YITVELSNSE RESIEVGIDV TNAYVVAYRA GSQSYFLRDA PASASTYLFP

101 GTQRYSLRFD GSYGDLERWA HQTREEISLG LQALTHAISF LRSGASNDEE

151 KARTLIVIIQ MASEAARYRC ISNRVGVSIR TGTAFQPDPA MLSLENNWDN

201 LSGGVQQSVQ DAFPNNVILS SINRQPVVVD SLSHPTVAVL ALMLFVCNPP

251 NANQSPLLIR SIVEESKICS SRYEPTVRIG GRDGMCVDVY DDGYHNGNRI

301 IAWKCKDRLE ENQLWTLKSD LTIRSNGKCL TTEGYAPGNY VMIYDCTSAV

351 AEATYWEIWD NGTIINPKSA LVLSAESSSM GGTLTVQTNE YLMRQGWRTG

401 NNTSPFVTSI SGYSDLCMQA QGSNVWLADC DNNKKEQQWA LYTDGSIRSV

451 QNTNNCLTSK DHKQGSPIVL MACSNGWASQ RWLFKNDGSI YSLYDDMVMD

501 VKGSDPSLKQ IILWPYTGKP NQIWLTLF

Figure S7. Protein sequence coverage of proteins identified in the purified APA preparation. Approximately 85 µg of the purified APA preparation used in this work were subjected to immuno-affinity enrichment using a mixture of four mAbs, namely AP430, AP3808, AP3659 and AP476, coupled to magnetic Dynabeads, followed by protein reducing, alkylation, tryptic digest and non-targeting LC-ESI-MS/MS analysis. From top to bottom, sequences of identified *Abrus precatorius* Agglutinin (APA; UniProt Q9M6E9) and three abrin isoforms (UniProt P11140, UniProt Q06077, UniProt Q06076) are shown after MASCOT server search against a self-assembled UniProt/NCBI database containing all abrin isoforms and *Abrus precatorius* agglutinin as well as an NCBI database containing all *Abrus precatorius* proteins. Asparagine (N) highlighted in turquoise represents potential N-linked glycosylation sites. The linker peptide sequence between the two chains of each abrin isoform is underlined. Amino acids highlighted in red were experimentally identified. Amino acids in position 1–20 in APA represent the signal peptide (highlighted in grey).

References

1. Herrmann, M.S.; Behnke, W.D. A characterization of abrin A from the seeds of the *Abrus precatorius* plant. *Biochim. Biophys. Acta* **1981**, *667*, 397–410, doi:10.1016/0005-2795(81)90206-3.
2. Hegde, R.; Maiti, T.K.; Podder, S.K. Purification and characterization of three toxins and two agglutinins from Abrus precatorius seed by using lactamyl-Sepharose affinity chromatography. *Anal. Biochem.* **1991**, *194*, 101–109, doi:10.1016/0003-2697(91)90156-n.