Supplementary Materials:

Supplementary text S1

Materials and Methods

Fig S1 – S4

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Movie S1, and S2

Supplementary text:

Supplementary text S1: JA signalling stimulates expression of vesicle acidification–associated transcripts.

To accumulate high vacuolar H^+ concentrations, lemon fruits engage unique P-type ATPase (48) at the tonoplast. The orthologous ATPase in *Dionaea* DmAHA10 is expressed in resting *Dionaea* glands and is upregulated upon insect, mechanical and COR stimulation (Fig. S1, http://tbro.carnivorom.com and (7)). While in the lemon system protons are accompanied by the citrate anion, in *Dionaea* chloride is the counter anion (Fig. 2F and (15)). In bacteria and plants, ClC-type anion channels operate as H^+ / anion antiporters (49-51). *Dionaea* glands express a ClC-type anion channel already in the resting state, which is transcriptionally further induced upon trap stimulation (DmCLCc, Fig. S1, (http://tbro.carnivorom.com and (7)). Interestingly, the JA-signalling inhibitor Coronatine-Methyloxime (COR-MO) suppressed mechanostimulation-induced transcription (Fig. S1 C & D). The fact that gland DmCLC expression and chloride release (Fig. 2E and S1) are triggered by touch and the touch hormone mimic COR complements early findings that stimulated head cells of the gland accumulate chloride and disperse it after vacuolar fragmentation (2, 15). In these studies, the appearance of Cl^- in the outer walls of stimulated *Dionaea* glands was correlated to vacuolar fragmentation into vesicles and their fusion with the plasma membrane (15). This assumption is supported by

our ion-selective MIFE studies monitoring the release of Cl^- and H^+ about one hour after gland stimulation (Fig. 2).

Materials and Methods:

Plant growth and harvesting

Dionaea plants were grown as described previously (12). Briefly, *D. muscipula* plants were purchased from CRESCO Carnivora and grown in plastic pots at 22°C in a 16-h : 8-h light–dark photoperiod. For coronatine treatments, traps were directly sprayed with a 100 **µ**M COR solution (Sigma - Aldrich). Isolation of secretory gland complexes was achieved by gently abrading the inner trap surface using a sharp razor blade. For mechanical induction of gene expression, trigger hairs were stimulated 60 times (frequency: 1/min), and samples were collected 24 h after the first stimulus. In inhibitor tests, 100 µM COR-MO (3) was sprayed 4 h before mechanical stimulation.

Electron microscopy

Traps from intact *Dionaea* plants were either stimulated with coronatine solution (100 µM) or remained unstimulated. After 48 h intact leaves were fixed with 2% glutaraldehyde in cacodylate buffer (75 mM, pH 7,0) for 1 h, cut into small trap sections with a razor blade and further incubated for 24 h in total. Samples were postfixed with 1% osmium tetroxide at 4°C overnight, subsequently. Dehydrated through a series of graded acetone concentrations (30% - 100%) samples were finally embedded in plastic according to Spurr (52). Ultrathin sections were obtained with an ultramicrotome (Ultracut E, Leica-Reichert-Jung, Nußloch, Germany), transferred onto copper grids coated with Mowital and stained with uranyl acetate followed by lead citrate (53). Sections were viewed with a LEO 906 E TEM (LEO, Oberkochen, Germany) at 100 kV equipped with the MultiScan CCD Camera (Model 794, Gatan, Munich, Germany)

using the Digital Micrograph 3.3 software (Gatan) to acquire, visualize, analyze, and process image data.

MIFE studies

Plant material handling

A single lobe containing a mid-rib was cut and immobilised around a hollow plastic cylinder of 8 mm diameter using medical adhesive (VH355, Ulrich AG, St Gallen, Switzerland) and Parafilm. The immobilized lobe was mounted in a petri dish containing basic salt medium (BSM) (0.5 mM KCl, 0.1 mM $CaCl_2$, and 0.1 mM NaCl, pH 5.8). Following recovery for 1 h net Ca^{2+} and H^+ fluxes were measured in response to mechanical or chemical stimulation. Mechanical stimulation was achieved by touching a trigger hair 5 to 10 times with a pipette tip. Chemical stimulation was performed by applying either 1 mM methyl jasmonate or 100 µM coronatine to the glands. Net ion fluxes were monitored for 5 min in BSM followed by stimulation and continuous recordings for about 10 h.

Ion flux measurements

Net Ca^{2+}, Cl^- and H^+ fluxes were measured using non-invasive microelectrode ion flux measuring (the MIFE) technique (Univ. Tasmania, Australia) (54). Briefly, microelectrodes with an external tip diameter of ~2 µm were pulled, silanized and filled with selective cocktail (H^+ (catalogue No 95297), Cl^- (99408) or Ca^{2+} (99310; all from Sigma-Aldrich). Electrodes were mounted on a 3D-micromanipulator (MMT-5, Narishige, Toyko, Japan) and calibrated with an appropriate set of standards. A measuring chamber with the immobilized trap lobe was placed into a Faraday cage. Ion-selective microelectrodes were positioned, with their tips aligned, ~50 µm above the lobe's surface using a 3D-hydraulic micromanipulator. During measurements, a computer-controlled stepper motor moved microelectrodes in a slow 6s/6s square-wave cycle between two positions 100 µm apart in distance. The potential difference

between two positions was recorded by the MIFE CHART software (55) and converted into an electrochemical potential difference using the calibrated Nernst slopes of the electrodes. Net ion fluxes were calculated using the MIFEFLUX software for cylindrical diffusion geometry.

RNA extraction, sequencing, and qPCR

RNA was isolated separately from each flytrap sample using a modified cetyltrimethylammonium bromide (CTAB)–based protocol. In brief, 0.1 g *Dionaea* plant material powdered in liquid nitrogen was thoroughly mixed with 0.7 mL of hot (65°C) RNA-extraction buffer (2% CTAB, 2% polyvinylpyrrolidone K 25 [PVP], 100 mM TRIS/HCl at pH 8.0, 25 mM Na-EDTA at pH 8.0, 2 M NaCl, with 2.5% [v/v] 2-mercaptoethanol added immediately before use). Following 10 min incubation at 65°C and extraction with 1 vol of chloroform/isoamyl alcohol (24:1, v/v), RNA was precipitated from the supernatant by adding 175 µL of 8 M LiCl overnight (4°C). RNA was collected by centrifugation, resuspended in DEPC H_2O, and precipitated in the presence of 0.1 vol 3 M Na acetate (pH 5.2) and 2.5 vol of 96% EtOH. After a washing step with 70% EtOH, RNA was dissolved in 40 µl of DEPC H_2O. DNA contamination was removed by DNase I treatment on a micro column (Roche). RNA quantity and quality were determined by capillary electrophoresis (Experion automated electrophoresis system and Experion RNA high sense analysis kit, Bio-Rad Laboratories). Individual transcript levels were analyzed by quantitative real-time PCR (qPCR). qPCR was performed using a Realplex Mastercycler (Eppendorf), 1:20 diluted cDNA, and the Absolute QPCR SYBR green capillary mix (Thermo Scientific). Expression levels were quantified using a standard for each primer pair and normalized to 10,000 molecules of actin (DmACT) cDNA transcripts. Gene specific primers were designed using the software LightCycler Probe Design 2.0 (Roche Life Science) based on the transcriptomic information available under http://tbro.carnivorom.com (release 1.03; (7)). Individual transcripts deposited there are given in parentheses. The following primers were used: DmACT (comp226979_c1_seq1),

DmACTLCfw: 5´-TCTTTGATTGGGATGGAAGC-3´; DmACTLCrev: 5´-GCAATGCCAGGGAACATAGT-3; DmAHA10 (comp234095_c1.1_seq9), DmAHA10LCfw: 5´-GACTTTACATGGGCTG-3´; DmAHA10LCrev: 5´-GCCCGAAAACTATTTATC-3; DmCLCc (comp214625_c0.0_seq1), DmCLCcLCfw: 5´-ATATACGGTTGTTGAGAC-3´; DmCLCcLCrev: 5´-AATCTTCAGATCCCAC-3´; DmOASTL (comp199845_c0.0_seq1) DmOASTLLCfw: 5´- AAGTTATCACCGTGTC-3´; DmOASTLLCrev: 5´- AGAGTGCAAGGTAAATC-3´; DmAPR3 (comp215379_c1.0_seq1), DmAPR3LCfw: 5´-GGAACTGGCTGACAAG-3´; DmAPR3LCrev: 5´-TGGATTACACTTAAAAG-3´; DmOPT6 (comp225114_c0_seq7), DmOPT6LCfw: 5´-GCCCGCTACAACTATA-3´; DmOPT6LCrev: 5´- CTTGCTTTGGAACTCTTAC-3´.

UPLC measurements

Glutathione and its metabolic precursors γ-glutamylcysteine and cysteine were quantified as monobromobimane (mBBr) derivatives (34, 35) by a modification of the UPLC method previously described (56). For this purpose, digestive fluids of stimulated flytraps were collected, immediately frozen in liquid N_2 and stored at -80 °C until analyses. For derivatization, digestive fluids were diluted with 0.1 HCl as required (1:5 to 1:20) and aliquots of 50 µl were treated with mBBr (56). Stimulated flytraps were cut off, washed in ddH_2O, immediately frozen in liquid N_2, ground with mortar and pistil to a homogenous powder, and stored at -80 °C until analyses. Thiols were determined in aliquots of 50 mg frozen powder after derivatization with mBBr (56). Thiol derivatives in samples from digestive fluids and trap tissues were separated by UPLC, detected by fluorescence at 380 nm / 480 nm, and quantified by comparison with external standards (56). Ascorbic acid was determined in aliquots of 50 µl digestive fluids using the colorimetric method previously described (36).

Gas Exchange (IRGA) measurements

Fluid phase secretion of COR stimulated Venus flytraps was recorded in a setup of two whole plant cuvettes. Two customized Infra-Red-Gas-Analyzers (IRGA) (HCM-1000, Walz, www.walz.com) were used to measure the increase of water vapor concentration in the air stream passing the flytraps. To avoid transpiration from the soil the surface and the plant except one trap sprayed with a 100 **µ**M COR solution (Sigma- Aldrich) was covered with water-tight foil. The composition of the gas stream of 1 l min^{-1} through each cuvette was adjusted by mass flow meters (red-y smart series; www.voegtlin.com) and set to 24 °C, 47% relative humidity and 350 ppm CO_2. Illumination was provided by three LEDs, providing light at 655 nm at a photon fluence rate of 100 µmol m^2 s^{-1} (Winger WEPDR3-S1 Power LED Star tiefrot 3W), at 455 nm at 8 µmol m^2 s^{-1} (Philips, Luxeon, Royal Blue) and at 395 nm (Winger WEPUV3-S1 UV Power LED Star 395 nm). The three light beams were collected by dichroic mirrors (Q525 LPXR and DCLP 425, Chroma, www.chroma.com) and guided through fiber optics to the cuvettes (Fiber Illuminator FL-460; www.walz.com).

MRI

Sample preparation: Plants including roots were prepared to carry a single flytrap only and were placed in a 50 mL Eppendorf tube filled with tap water or water with a concentration of 50 mM of $GdCl_3$ and mounted in the MRI tube. COR was applied to trigger flytrap's slow closure and secretion (6).

MRI experiments were performed on a vertical 11.7 T superconducting magnet (Bruker BioSpin GmbH, Rheinstetten). We used an actively shielded Micro 2.5 gradient system (inner diameter 40mm, maximum strength 660mT/m). A custom-built birdcage-coil (i.d. 15mm) was used for the measurements. MRI measurements were conducted using an adjusted custom UTE (ultra-short echo time) sequence. The repetition time TR was set to 50 ms, for frequency encoding 128 points were acquired at a bandwidth of 200 kHz. The echo-delay between excitation pulse and start of the acquisition was 10 µs. A total of 59157 (digestion) or 61995

156 ($GdCl_3$) spokes were acquired. The measurements were averaged (NA = 2) for SNR-
157 optimization. The experiment was performed over a course of 12 days (digestion) or 3 days
158 ($GdCl_3$).
159 Reconstruction was based on the method proposed by Duyn et al. (57) by using in-house written
160 programs in MATLAB (The Mathworks, Inc., Natick, MA, USA).
161 In the $GdCl_3$-experiment, the *Dionaea* was placed in the tube with $GdCl_3$ applied to the root
162 medium and COR to the trap surface. Trigger hairs were touched to cause fast trap closure
163 before MRI measurement was started. *Dionaea* plants absorbed the Gd-Ions and closed traps
164 started to seal slowly (phase 3). Due to the presence of Gd the longitudinal relaxation-parameter
165 in the stem decreased significantly, showing the distribution of the contrast agent within the
166 plant due to the increase of signal within the plant tissue. When *Dionaea* reached phase 4, no
167 droplets of the digestive fluid could be detected on the trap's surface.
168

Figures S1-S4:

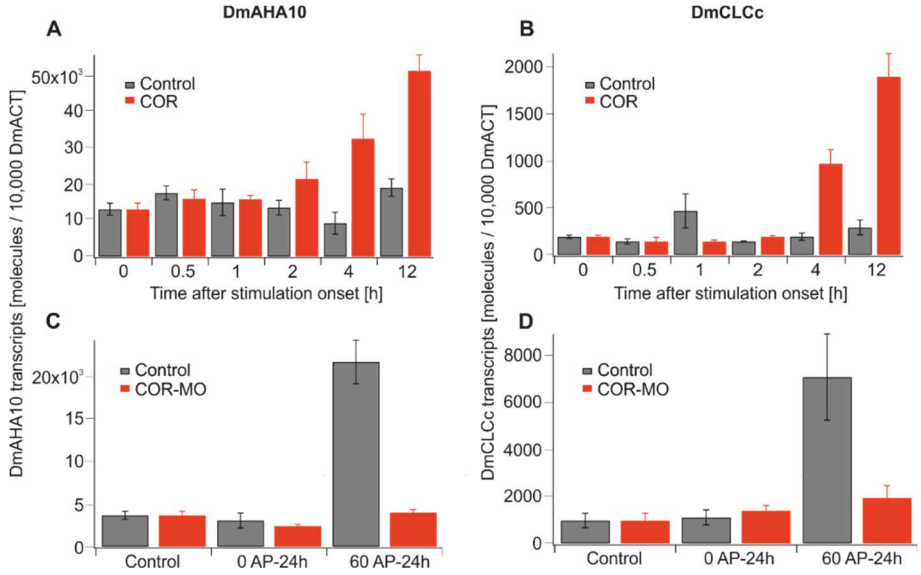

Fig. S1: Expression of DmAHA10 and DmCLCc is induced in activated *Dionaea* gland complexes in a JA-dependent manner.

A) and **B)**. Traps were sprayed with water (control, grey) or 100µM coronatine (red) and gland complexes were harvested after the time points indicated. **C)** and **D)** Effect of the JA-antagonist coronatine-O-methyloxime (COR-MO) on electro-mechanical induction of DmAHA10 and DmCLCc expression. Traps were pretreated 4 h before application of zero or 60 action potentials (APs) with H_2O (grey) or 100 µM COR-MO (red). RNA was sampled 24 h after onset of mechanostimulation. Transcript numbers are given relative to 10,000 molecules of DmACT1; Data are given as mean ± SE, n = 6.

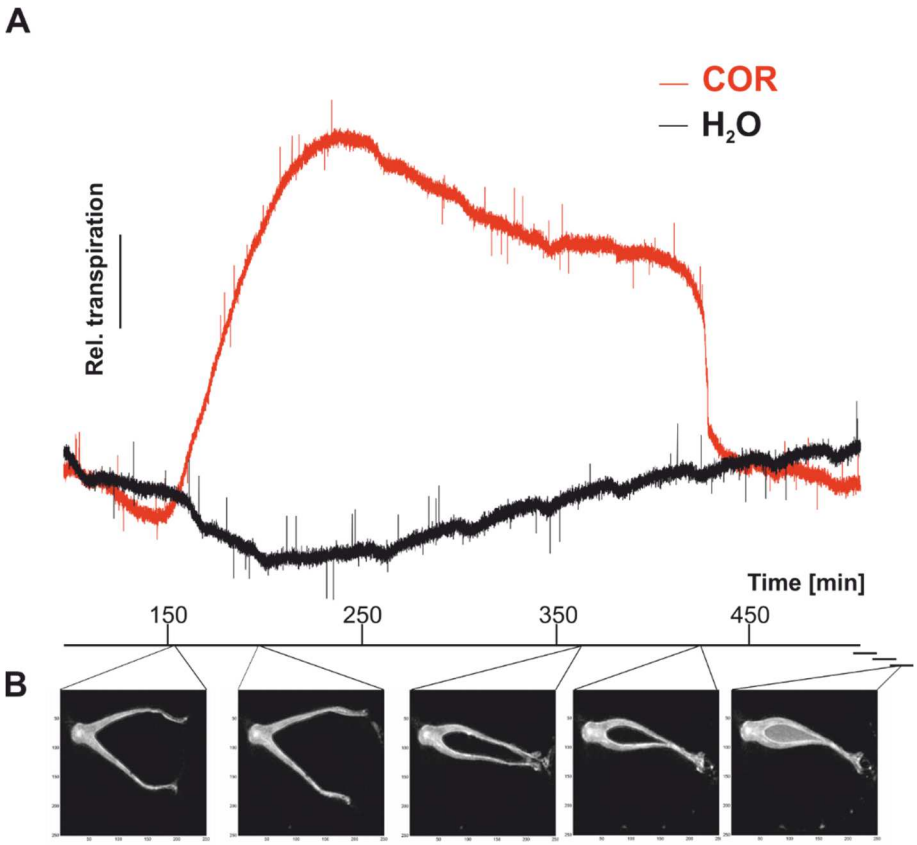

Fig. S2: COR induces exocytosis-based fluid phase secretion.

A) COR stimulated kinetics of relative transpiration. The trap was sprayed with 100 µM COR and placed in the IRGA-gas exchange chamber. The increase in rel. humidity reflects start of secretion. The drop in rel. humidity was paralleled by hermetical sealing during trap closure. Representative experiment is shown. **B)** MRI imaging of a COR stimulated flytrap. Representative pictures according to the different phases of *Dionaea* stimulation are shown. (Left to right) Unstimulated trap, phase 1-2 - trap transiently opens-up wider and starts to produce fluid film, phase 3 - trap closes slowly to reach a position similar to mechanically induced fast trap closure, phase 4 – trap lobes seal hermetically, trap completely filled by a fluid, acidic hydrolase moiety.

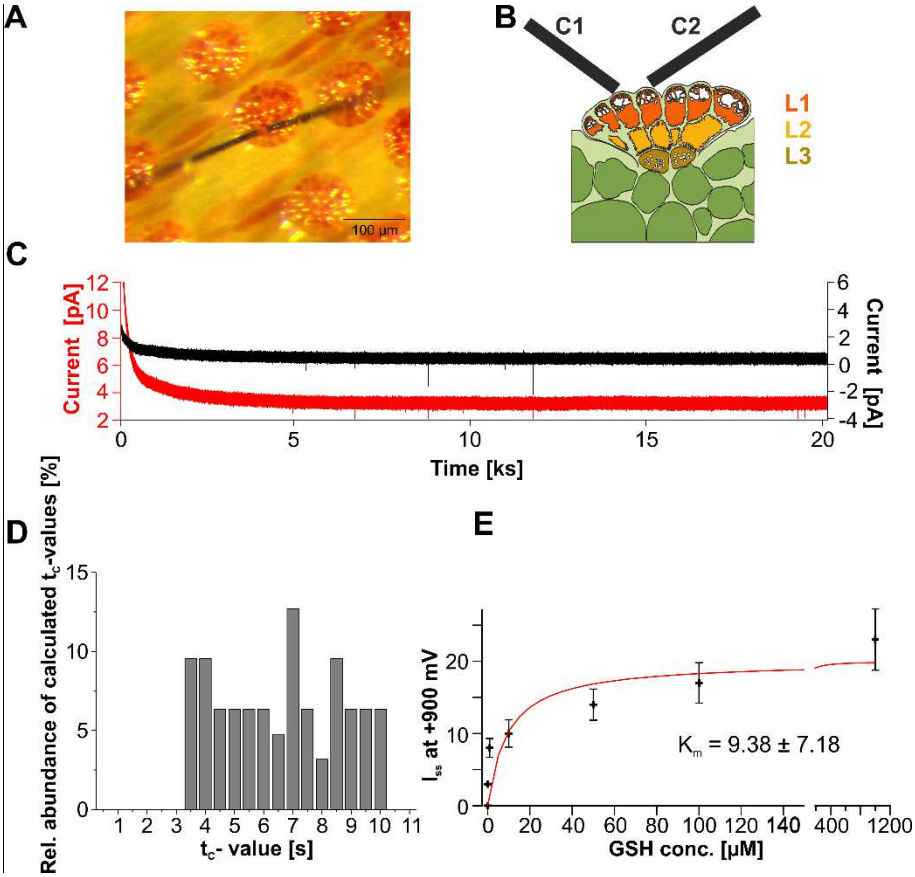

Fig. S3: Amperometric detection of secretion

A) Overview of the inner *Dionaea* trap surface. Two amperometrical carbon fibers were clamped to +900 mV and placed on top of a gland complex to follow exocytotic secretion. **B)** Modell of two carbon fibers (C1 and C2) attached to the upper layer (L1) of a gland complex as shown in **A**. The three functional layers (L1–L3): upper secretory layer L1 (red), inner layer L2 (yellow), and endodermoid layer L3 (brown) are indicated. **C)** Long-time amperometrical response measured with two separate carbon fiber electrodes clamped to +900 mV. Electrodes were placed on top of an unstimulated Venus flytrap´s gland. After initial settling, currents resulted in a steady state value at about 30 min. Under these conditions artificial spiking of the electrodes was characterised as very fast spiking mostly pointing towards negative values. **D)** Calculated t_c-values derived from equation (1) plotted against the relative signal abundance. In 63 analyzed spikes the t_c-values distributed broadly homogenous between 3.25 – 10.0 s. **E)** Michaelis-Menten fit of a dose-response curve of amperometric current detected at carbon

fibers held at +900 mV in solutions containing different concentrations of reduced glutathione (n = 5, mean ± SD).

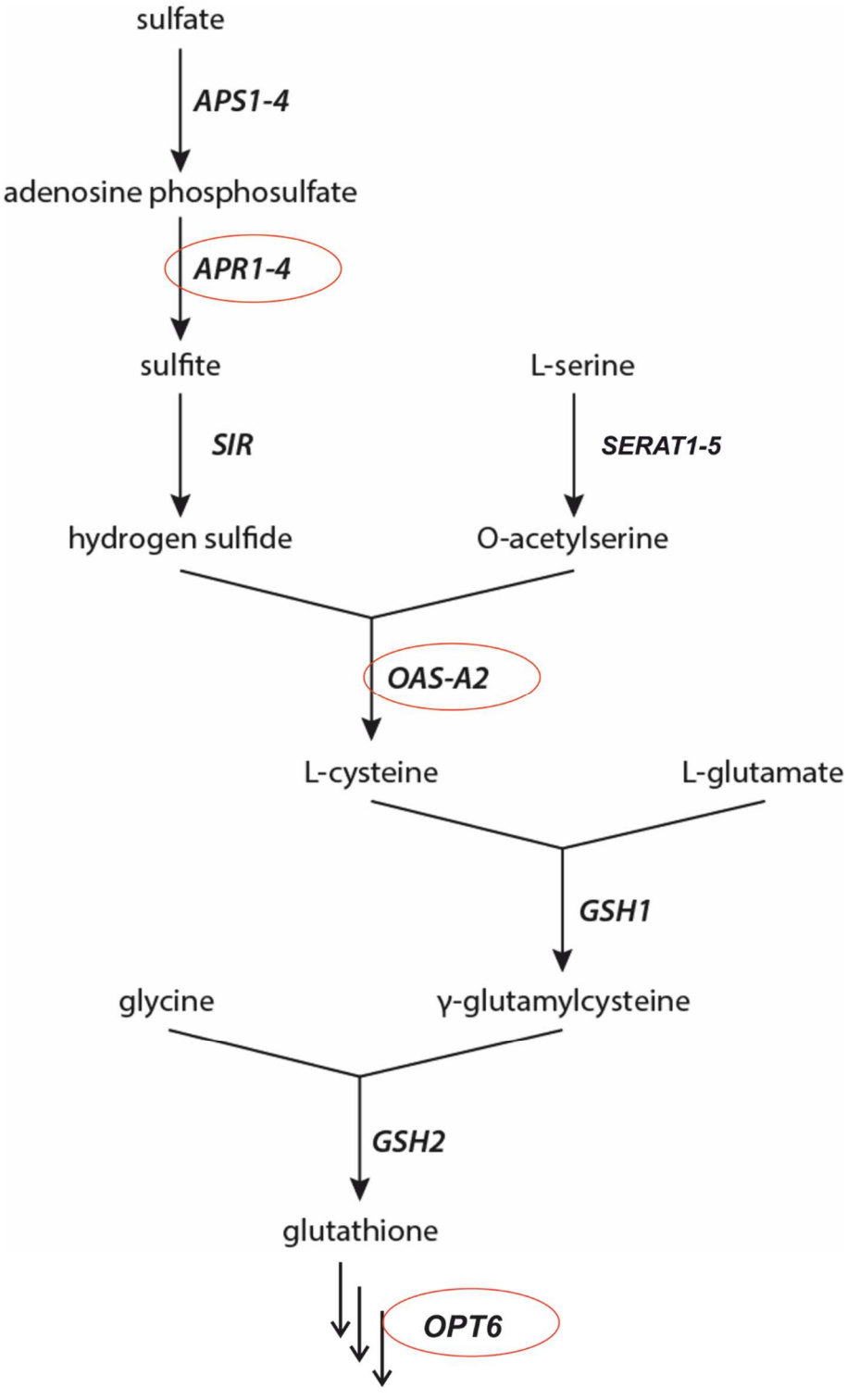

Fig. S4: Biosynthetic pathway of Glutathione

APS1-4: ATP Sulfurylases 1-4; APR1-4: Adenosine Phosphosulfate Reductase 1-4; SIR: Sulfite Reductase; SERAT: Serine Acetyl Transferase; OASTL: O-Acetylserine (thiol) Lyase; GSH1 & 2: Glutathione Synthetase 1 & 2; OPT6: Oligopeptide Transporter 6.

Movies S1-S2

Supplementary Movie 1: MRI imaging of a coronatine stimulated flytrap.

A *Dionaea* flytrap was stimulated for secretion by application of coronatine. In the following phases representing *Dionaea´s* hunting cycle, the trap transiently opens-up wider and starts to produce fluid film. After that phase the trap starts to close slowly reaching a position similar to mechanically induced fast trap closure. In the final phase the trap lobes seal hermetically and the emerging external stomach is filled by the digestive fluid.

Supplementary Movie 2: MRI imaging of the Ca^{2+} channel blocker gadolinium reducing the extruded fluid volume a stimulated flytrap.

In the $GdCl_3$-experiment, a *Dionaea* plant was placed and stimulated for secretion. The trap started to close and absorbed the Gd-Ions dissolved in the water. Due to the influence of the Gd on the longitudinal relaxation-parameter, the signal in the stem increased significantly, showing the distribution of the contrast agent within the plant. When *Dionaea* closed its trap lobes, no droplets of the digestive fluid could be detected on the trap's surface. After 3 days the signal derived from the *Dionaea* was deteriorated in such a way that no further MRI experiments were rendered possible and no secreted fluid was detected in the trap.