Ceramide synthase inhibition by fumonisins: a perfect storm of perturbed sphingolipid metabolism, signaling and disease

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Supplement A

Expansion of Figure 2 to indicate the current understanding of SL metabolism, trafficking through the cell, and efflux before, during and shortly after cessation of CerS inhibition by fumonisins

General legend for Figures 2a and 2b. An overview of sphingolipid biosynthesis and turnover in mammalian cells with a focus on ceramide synthases (CerS) and their products. The box between the nucleus and Golgi represents the location of the enzymes of de novo sphingoid base biosynthesis in the ER and mitochondria associated membranes (MAM) (CerS is also present in mitochondria), which initially produce 3-ketosphinganine (not shown) then sphinganine, Sa, (likewise, 1-deoxysphinganine, 1-dSa, from Ala) which are N-acylated to dihydroceramides (DHCer, and 1-dDHCer) by CerS. DHCer is mostly desaturated to Cer and both are incorporated into more complex sphingolipids, SL (mainly sphingomyelin, SM, and glycosphingolipids, GSL) beginning in the ER for Gal(DH)Cer and during trafficking via vesicles and transport proteins (Glc(DH)Cer beginning in the cis-Golgi and more complex GSL throughout the Golgi; SM in the trans-Golgi and plasma membrane), eventually arriving at their destinations (cell membranes or release from the cell). Sphingoid bases (SphB) and 1-phosphates are present in multiple locations in the cell as intermediates of de novo biosynthesis, SL (in these diagrams, SL refers to SM and GSL) turnover (from which they can be reutilized), and signaling, and also efflux from the cell (S1P to serve as a signal to activate S1P receptors, S1PR).

For additional information, consult the following reviews on sphingolipid metabolism, trafficking & function:

1. Merrill, A. H., Jr. 2011. Sphingolipid and glycosphingolipid metabolic pathways in the era of sphingolipidomics. *Chemical reviews* 111: 6387-6422.
2. Yamaji, T., and K. Hanada. 2015. Sphingolipid metabolism and interorganellar transport: localization of sphingolipid enzymes and lipid transfer proteins. *Traffic* 16: 101-122.
3. Harrison, P. J., T. M. Dunn, and D. J. Campopiano. 2018. Sphingolipid biosynthesis in man and microbes. *Nat Prod Rep* 35: 921-954.
4. Sandhoff, R., and K. Sandhoff. 2018. Emerging concepts of ganglioside metabolism. *FEBS Lett* 592: 3835-3864.
5. Hannun, Y. A., and L. M. Obeid. 2018. Sphingolipids and their metabolism in physiology and disease. *Nat Rev Mol Cell Biol* 19: 175-191.

Figure 2a highlighting the biosynthetic processes. These steps have been described on the preceding page of this Supplement and the main text of the manuscript. The main processes that are associated with SL biosynthesis are highlighted in dark blue.

Figure 2b highlighting turnover at the plasma membrane for signaling and in lysosomes and autophagosomes (including mitophagy). Turnover of SM and GSL in lysosomes mainly produces So that is released to the cytosol where it can be reutilized via CerS, or phosphorylated to S1P that can be dephosphorylated or cleaved to hexadecenal (or hexadecanal from Sa1P) and ethanolamine phosphate.

Highlighted in purple are the main processes that are associated with SL hydrolysis for cell signaling or turnover with recycling or degradation of the sphingoid base backbones.

The asterisk by Cer* at the plasma membrane highlights that it can be recycled directly to SM by plasma membrane SM synthase.

For all of the figures, the abbreviations that have been used are:
1-dSa, 1-deoxysphinganine; 1-DHCer, N-acyl-1-dSa; Cer, ceramide; CerS, ceramide synthase; DHCer, dihydroceramide; ER, endoplasmic reticulum; FB1, Fumonisin B1; Golgi/TGN, Golgi/trans-Golgi network; GSL, glycosphingolipids; S1P, sphingosine 1-phosphate; Sa, sphinganine; Sa1P, sphinganine 1-phosphate; SL, sphingolipid; SM, sphingomyelin; So, sphingosine; SphB, sphingoid base(s)*; SPT, serine palmitoyltransferase.
*For the supplement, SphB is used where multiple sphingoid bases have these features, not just Sa or S.

Schematic of **sphingolipid biosynthesis** *de novo* (No inhibition)

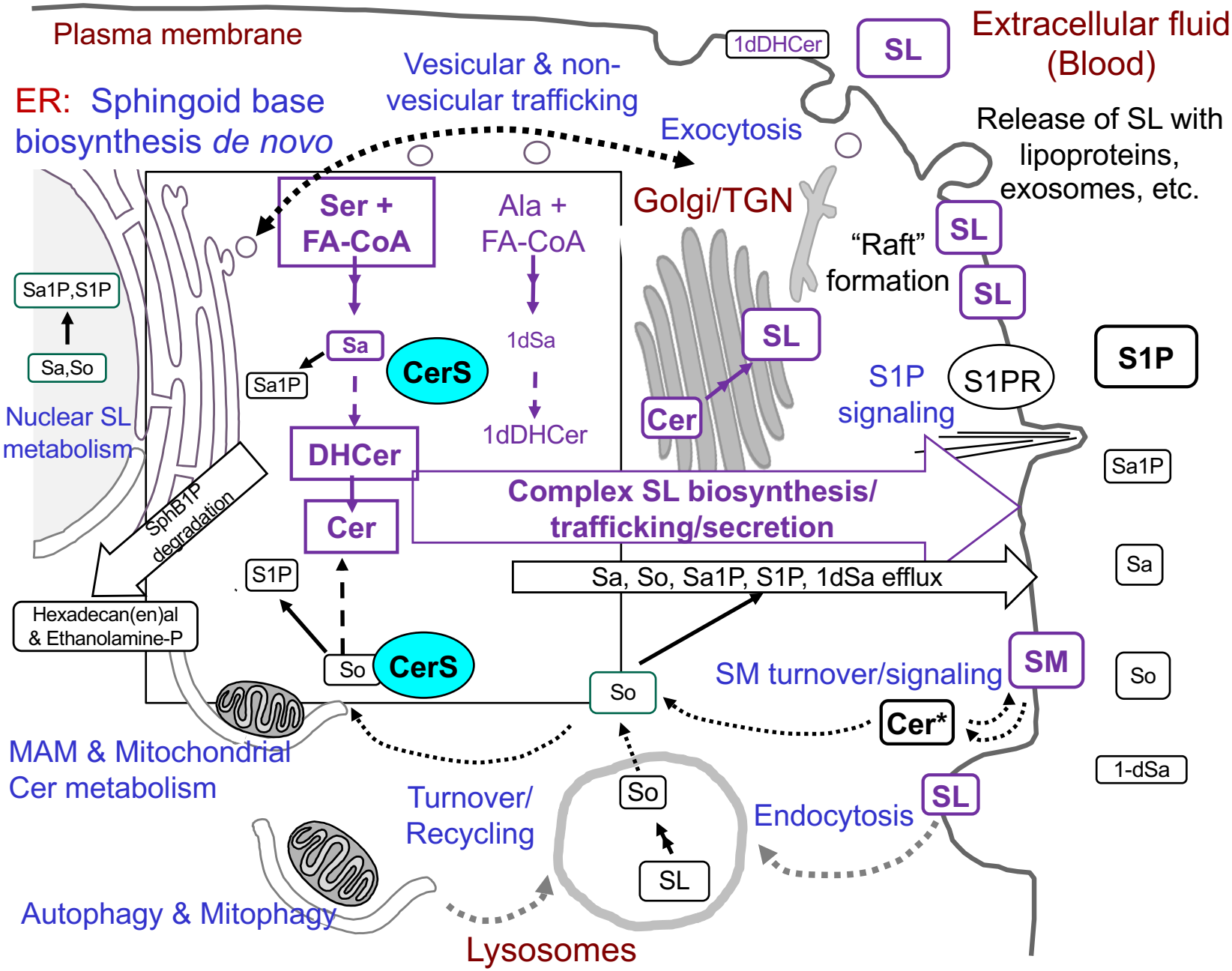

Fig. S2a

Schematic of **sphingolipid turnover, signaling and recycling** (No inhibition)

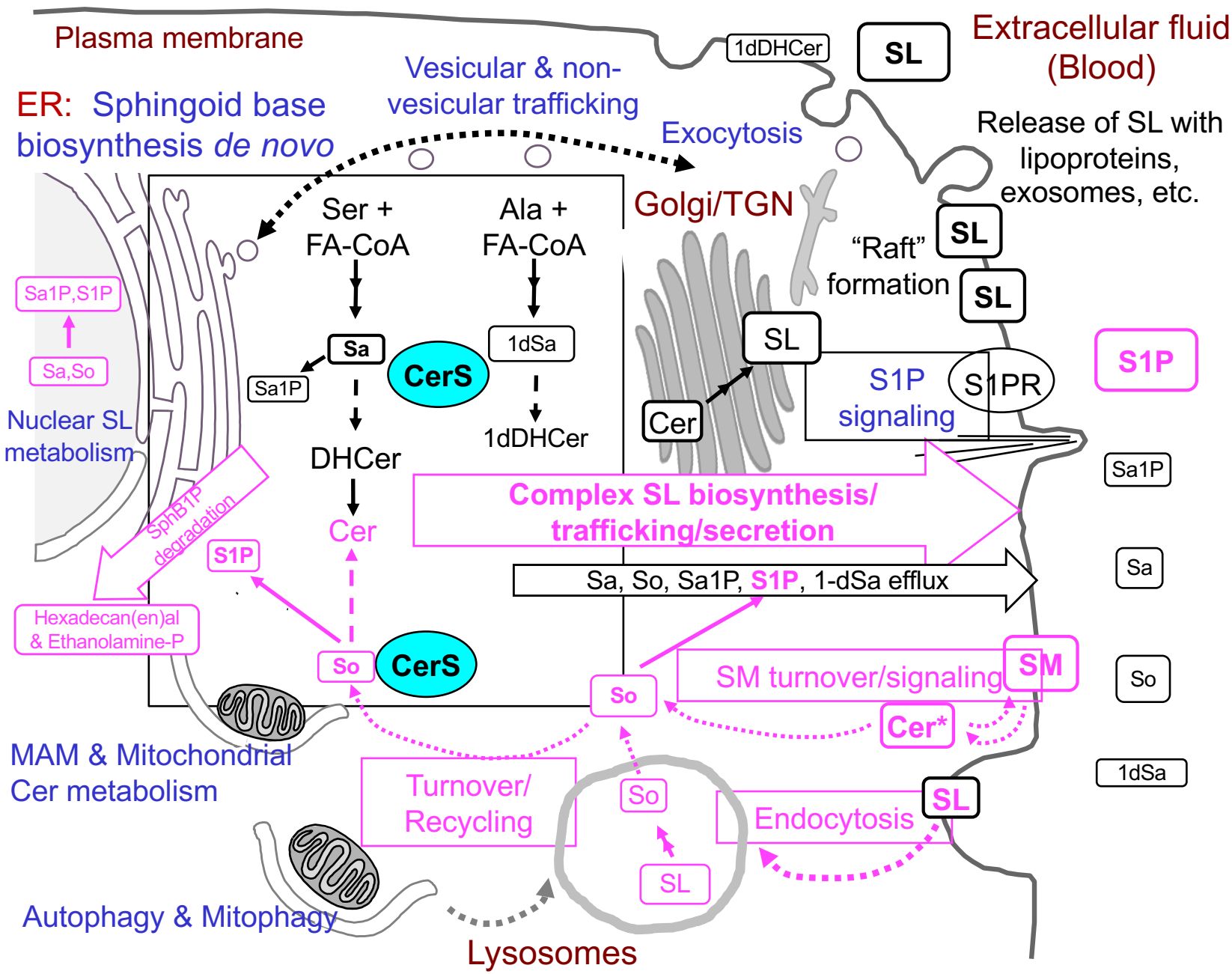

Fig. S2b

General legend for Figures 2c-2e. An overview of sphingolipid biosynthesis and turnover in mammalian cells with highlighted metabolites that are affected by inhibition of ceramide synthases (CerS) by fumonisins (FB1). See the legend for Fig. 2a-b for a general description of the components of the figure.
The metabolites in green are generally elevated when CerS is inhibited by FB1; the metabolites in red eventually decrease depending on the rate of turnover.

Figure 2c. Likely temporal changes in SL metabolism with fumonisin exposure during early inhibition. Very soon after fumonisin administration, Sa is elevated and formation of DHCer and Cer decrease. Sa1P elevations often accompany increases in Sa. Under some circumstances, there are detectable increases in So and S1P, but these are typically smaller, and often later, than increases in Sa and Sa1P. Release of Sa and Sa1P into circulation is often seen early after fumonisin administration; the changes in nuclear sphingoid bases are based on N. M. Gardner *et al*., *Toxicol Appl Pharmacol* 298:56-65 (2016).

Figure 2d. Likely temporal changes in SL metabolism with fumonisin exposure for longer term, typically with tissue damage/ toxicity. This diagram illustrates that after prolonged fumonisin exposure, there is further accumulation of sphingoid bases and 1-phosphates as well as reductions in complex sphingolipids due to their not being replaced after turnover from cell signaling and/or endocytosis/autophagic processes.

Figure 2e. Likely changes in SL metabolism after fumonisin exposure. The main point of this diagram is to illustrate that after sphingoid bases have become elevated by fumonisins, when the fumonisins are removed and cleared from cells, the sphingoid bases are reacylated by CerS (as seen in K. A. Voss *et al., Toxicol Sci* 112:459–467, 2009); therefore, there is a short-term increase in the amounts until homeostasis is restored.

Likely temporal changes in SL metabolism with fumonisin exposure: **Early inhibition**

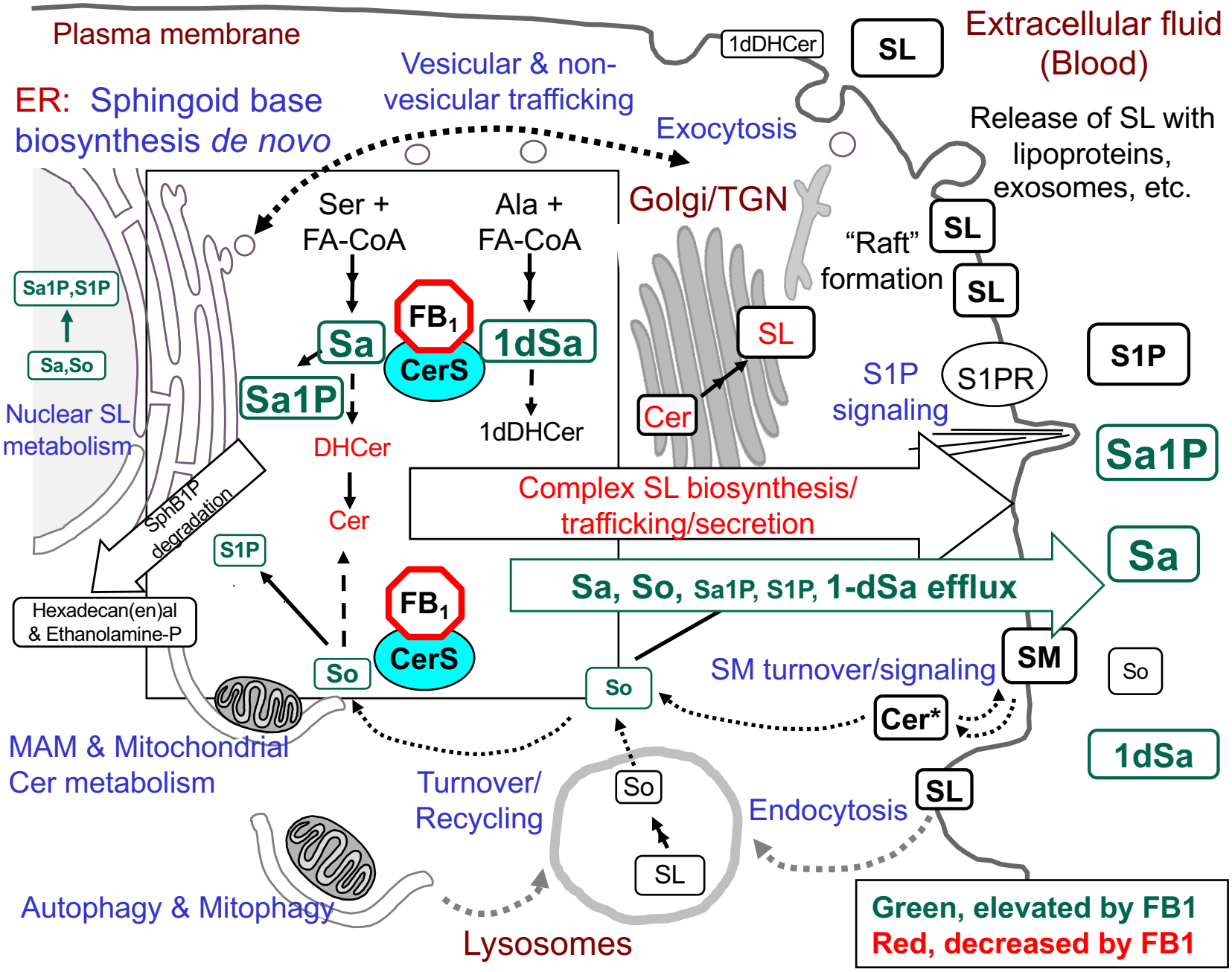

Fig. S2c

Likely temporal changes in SL metabolism with fumonisin exposure: **Late inhibition** with tissue damage/ toxicity

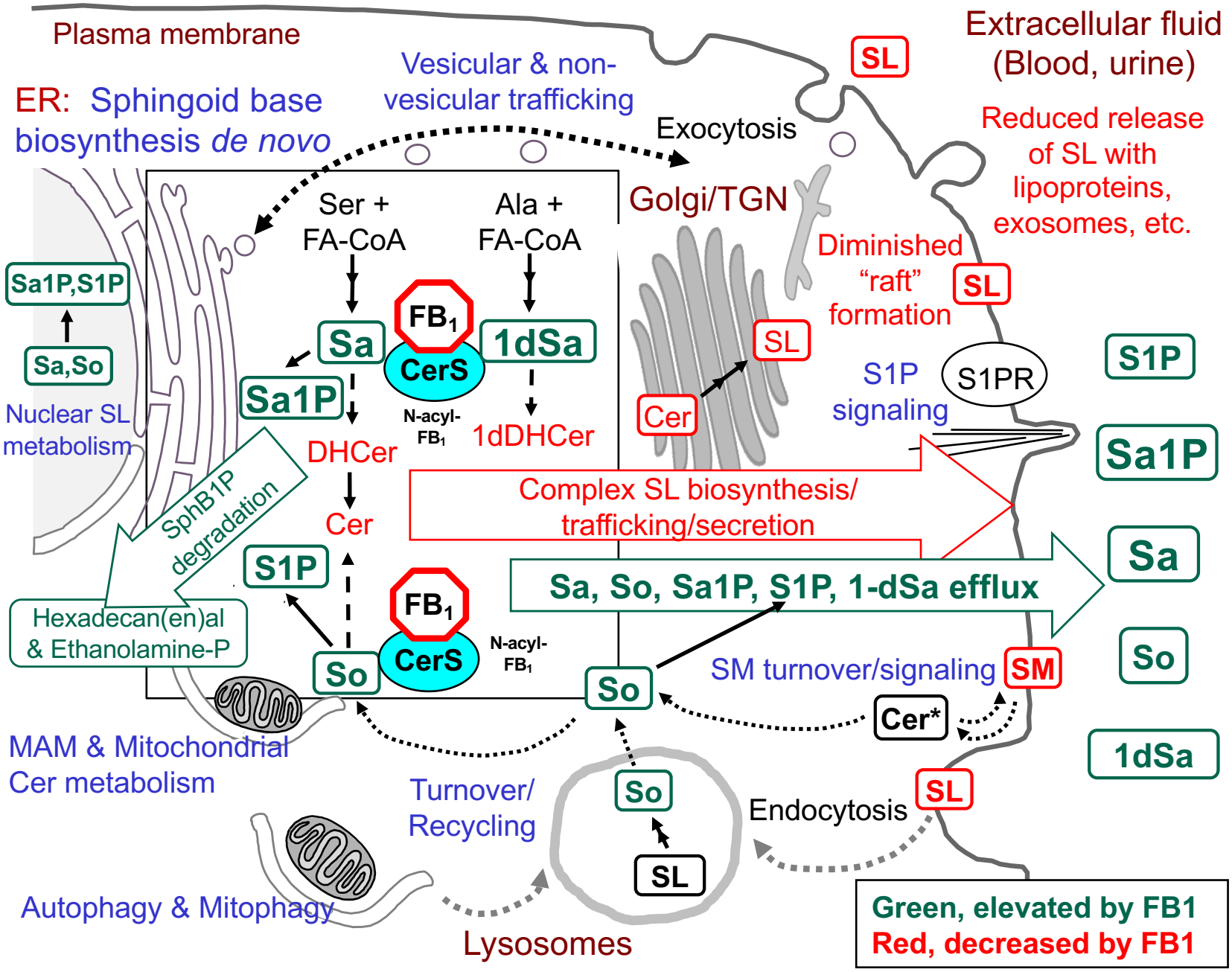

Fig. S2d

Likely changes in SL metabolism **after fumonisin exposure** (Post inhibition)

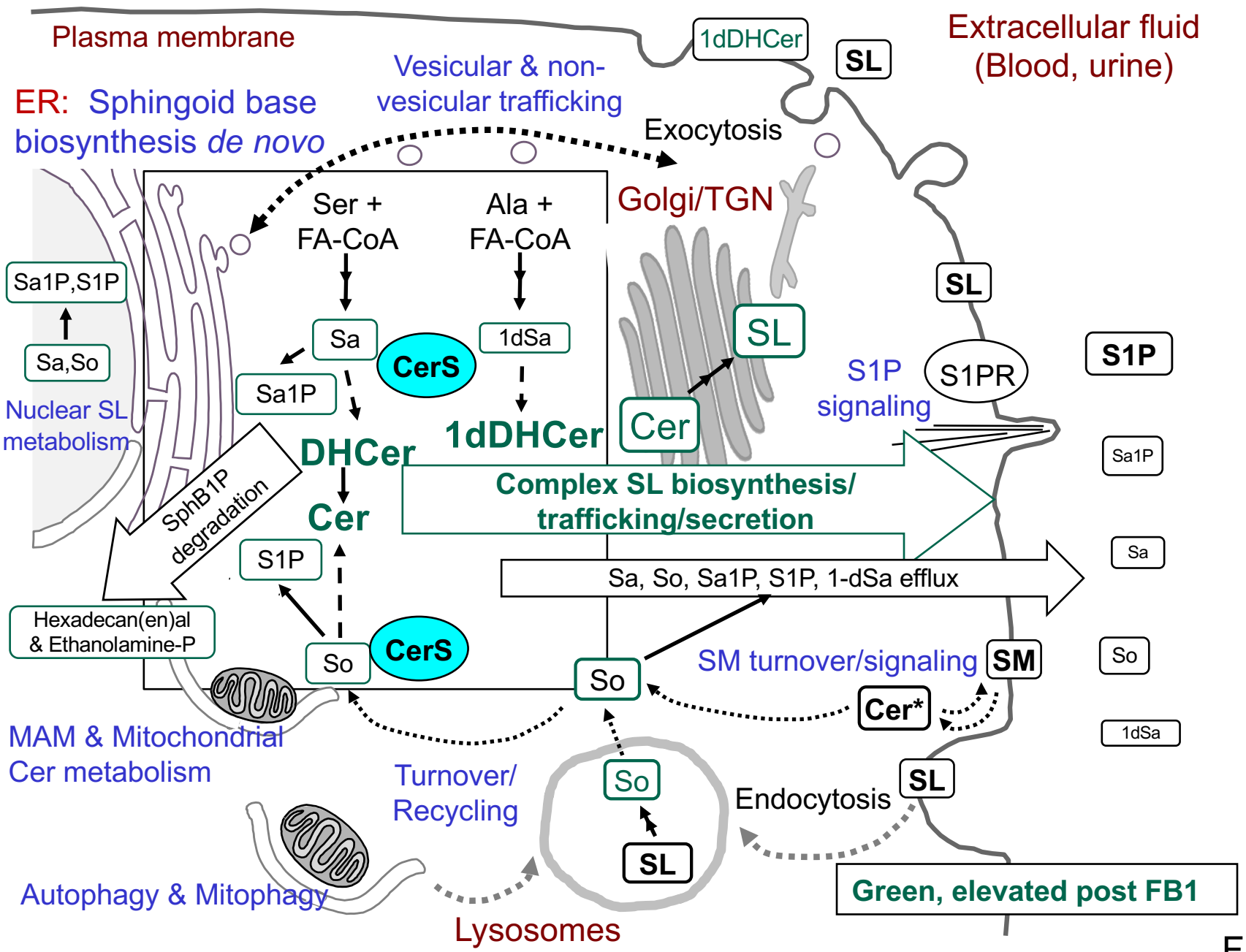

Fig. S2e