

1 Supplemental Fig. 1 and 2 of “ZINC- $\alpha$ 2-GLYCOPROTEIN IS AN INHIBITOR OF AMINE  
2 OXIDASE COPPER-CONTAINING 3”, Romauch M, Open Biology, 2019

### 3 **Plasma enhances AOC3 activity**

4 Endogenous plasma amine oxidase activity has previously been described in both human and  
5 mouse plasma [1–3]. This activity derives from membrane-bound AOC3 which has been  
6 released by metalloprotease activity [4]. A pronounced increase in levels of cleaved AOC3 is  
7 observed during diabetes, congestive heart failure and liver cirrhosis [5–7]. Incubating  
8 recombinant AOC3 with IEX fractions lacking ZAG (Fig. 4B) increased amine oxidase  
9 activity, which might be due to plasma-derived AOC3 activity or other plasma components.  
10 To test this hypothesis, the amine oxidase activity in plasma of wt, AOC3 k.o. and ZAG k.o.  
11 mice was measured directly using radioactive benzylamine as substrate. The activity linearly  
12 increased with measured plasma volume of wt and ZAG k.o. mice, and the activity could be  
13 blocked by the highly selective AOC3 inhibitor LJP1586 (Supplemental Fig. 1, A and B).  
14 However, AOC3 activity in AOC3 k.o. plasma does not increase with increasing plasma  
15 volume and residual activity cannot be further significantly reduced by adding LJP1586  
16 (Supplemental Fig. 1, C). This suggests that AOC3 is the main plasma enzyme responsible for  
17 benzylamine deamination, but it does not exclude other amine oxidases that are not sensitive  
18 to LJP1586 or that have a higher affinity for other substrates. One such category of enzymes  
19 could be the lysyl oxidases, which are also members of the copper amine oxidase family. The  
20 lysyl oxidase family is made up of lysyl oxidase (LOX) and the four lysyl oxidase-like enzymes  
21 (LOXL1 – 4); these enzymes catalyze the final reaction required for cross-linking of collagens  
22 and elastin [8]. Comparison of wt and ZAG k.o. plasma-derived AOC3 activity reveals that  
23 lack of ZAG has no influence on activity, which is supported by the fact that the same level of  
24 AOC3 protein expression is found in the gonadal white adipose tissue of both wt and ZAG k.o.

25 mice (Supplemental Fig. 2, D). However, this contradicts the assumption that lack of ZAG  
26 automatically leads to significantly higher plasma-derived AOC3 activity.

27 Since plasma IEX fractions lacking ZAG enhanced recombinant AOC3 activity in a dose-  
28 dependent manner (Fig. 4, B), it tested whether plasma in general is able to enhance amine  
29 oxidase activity. Indeed, plasma from wt, AOC3 k.o. and ZAG k.o. mice did enhance  
30 recombinant AOC3 activity in a dose-dependent manner, reaching maximal activity at 50  
31  $\mu\text{g/ml}$  (Supplemental Fig. 1, D). Since no significant difference among genotypes was  
32 observed, it was concluded that a plasma component present in all three genotypes must be  
33 responsible. Therefore, bovine serum albumin (BSA; fatty acid-free) was tested and was also  
34 found to enhance AOC3 activity in a dose-dependent manner (Supplemental Fig. 2, A).  
35 However, combining both plasma (50  $\mu\text{l}$  = 5 mg/ml) and BSA (2 mg/ml) does not further  
36 enhance plasma-derived or recombinant AOC3 activity, which indicates that AOC3 activity is  
37 already maximally enhanced by the albumin within plasma (Supplemental Fig. 2, B).  
38 Interestingly, when amounts of ZAG-IEX fractions and IEX fractions lacking ZAG (Fig. 4, B)  
39 were increased, there was no corresponding increase in basal activity (i.e. without addition of  
40 recombinant of AOC3) (Supplemental Fig. 2, C), as observed for wt and ZAG k.o. plasma  
41 (Supplemental Fig. 1, A and B). This might be due to dilution of plasma-derived AOC3  
42 activity, but also suggests the existence of a non-enzymatic component in IEX fractions lacking  
43 ZAG that boosts recombinant AOC3 activity. The latter notion is supported by the work of  
44 Dalfo et al., who described a low molecular weight plasma component that, in association with  
45 lysophosphatidylcholine (LPC), boosts AOC3 activity by up to 5-fold [9]. This is similar to the  
46 effect of incubating recombinant AOC3 with 200  $\mu\text{l}$  IEX fractions lacking ZAG (Fig. 4, B).

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## 48 References

- 49 [1] Aalto, K., Maksimow, M., Juonala, M., Viikari, J., Jula, A., Kähönen, M., Jalkanen, S., Raitakari, O.  
50 T. & Salmi, M. 2012 Soluble vascular adhesion protein-1 correlates with cardiovascular risk  
51 factors and early atherosclerotic manifestations. *Arteriosclerosis, thrombosis, and vascular*  
52 *biology* **32**, 523–532. (doi:10.1161/ATVBAHA.111.238030).
- 53 [2] Stolen, C. M., Yegutkin, G. G., Kurkijärvi, R., Bono, P., Alitalo, K. & Jalkanen, S. 2004 Origins of  
54 serum semicarbazide-sensitive amine oxidase. *Circulation research* **95**, 50–57.  
55 (doi:10.1161/01.RES.0000134630.68877.2F).
- 56 [3] Boomsma, F., Hut, H., Bagghoe, U., van der Houwen, A. & van den Meiracker, A. 2005  
57 Semicarbazide-sensitive amine oxidase (SSAO): from cell to circulation. *Medical science monitor*  
58 *: international medical journal of experimental and clinical research* **11**, RA122-6.
- 59 [4] Abella, A., García-Vicente, S., Viguerie, N., Ros-Baró, A., Camps, M., Palacín, M., Zorzano, A. &  
60 Marti, L. 2004 Adipocytes release a soluble form of VAP-1/SSAO by a metalloprotease-  
61 dependent process and in a regulated manner. *Diabetologia* **47**, 429–438. (doi:10.1007/s00125-  
62 004-1346-2).
- 63 [5] Boomsma, F., Derkx, F. H., van den Meiracker, A. H., Man in 't Veld, A. J. & Schalekamp, M. A.  
64 1995 Plasma semicarbazide-sensitive amine oxidase activity is elevated in diabetes mellitus and  
65 correlates with glycosylated haemoglobin. *Clinical science (London, England : 1979)* **88**, 675–  
66 679.
- 67 [6] Boomsma, F., van Veldhuisen, D. J., Kam, P. J. de, Man in't Veld, A. J., Mosterd, A., Lie, K. I. &  
68 Schalekamp, M. A. 1997 Plasma semicarbazide-sensitive amine oxidase is elevated in patients  
69 with congestive heart failure. *Cardiovascular research* **33**, 387–391. (doi:10.1016/s0008-  
70 6363(96)00209-x).

- 71 [7] Boomsma, F., Bhaggoe, U. M., van der Houwen, A. M. B. & van den Meiracker, A. H. 2003  
72 Plasma semicarbazide-sensitive amine oxidase in human (patho)physiology. *Biochimica et*  
73 *biophysica acta* **1647**, 48–54.
- 74 [8] Trackman, P. C. 2016 Enzymatic and non-enzymatic functions of the lysyl oxidase family in  
75 bone. *Matrix biology : journal of the International Society for Matrix Biology* **52-54**, 7–18.  
76 (doi:10.1016/j.matbio.2016.01.001).
- 77 [9] Dalfó, E., Hernandez, M., Lizcano, J. M., Tipton, K. F. & Unzeta, M. 2003 Activation of human  
78 lung semicarbazide sensitive amine oxidase by a low molecular weight component present in  
79 human plasma. *Biochimica et biophysica acta* **1638**, 278–286.

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81 **Figure captions:**

82 **Supplemental Fig. 1 A, B and C, [<sup>14</sup>C]-benzylamine assay:** Different volumes of murine wt,  
83 AOC3 k.o. and ZAG k.o. plasma were tested for amine oxidase activity. In parallel, the same  
84 volumes were tested in the presence of the highly selective AOC3 inhibitor LJP1586. **D, [<sup>14</sup>C]-**  
85 **benzylamine assay:** recombinant AOC3 (50 ng) activity in the presence of plasma of wt,  
86 AOC3 k.o and ZAG k.o mice (male, C57Bl/6 genetic background).

87 **Supplemental Fig. 2 A, [<sup>14</sup>C]-benzylamine assay:** Recombinant AOC3 (50 ng) activity in the  
88 presence of BSA (bovine serum albumin; fatty acid-free). **B, [<sup>14</sup>C]-benzylamine assay:**  
89 Comparison of AOC3 activity in wt and ZAG k.o. plasma. For basal plasma AOC3 activity  
90 (i.e. without recombinant AOC3, but with 50 ng GST), 50 µl (final concentration 5 mg/ml) of  
91 plasma with and without BSA (final concentration 2 mg/ml) were tested. In parallel, the same  
92 conditions were tested in the presence of AOC3 (50 ng). **C, [<sup>14</sup>C]-benzylamine assay:** AOC3  
93 activity of ZAG-IEX fractions and IEX-fractions lacking ZAG (Fig. 4, B) without addition of  
94 recombinant AOC3. AOC3 was replaced with GST (50 ng). **D, WB:** Plasma membrane

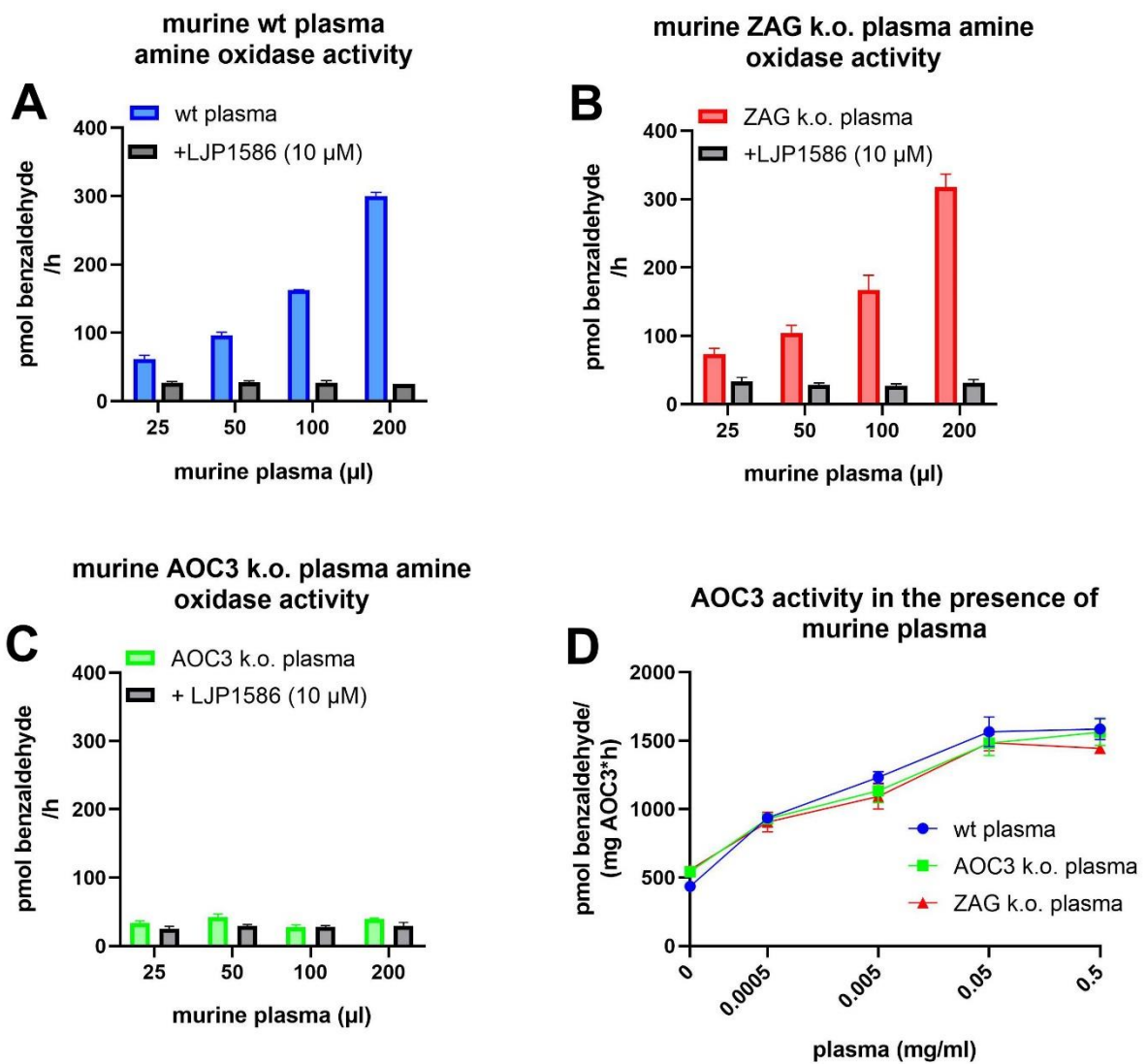
95 proteins from gonadal white adipose tissue (1  $\mu$ g) of wt and ZAG k.o. mice (four per genotype)  
96 were separated by SDS-PAGE and probed using  $\alpha$ -AOC3 antibody.

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98 **Figures:**

99 **Supplemental Fig. 1**

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