Extracellular vesicles are independent metabolic units with asparaginase activity

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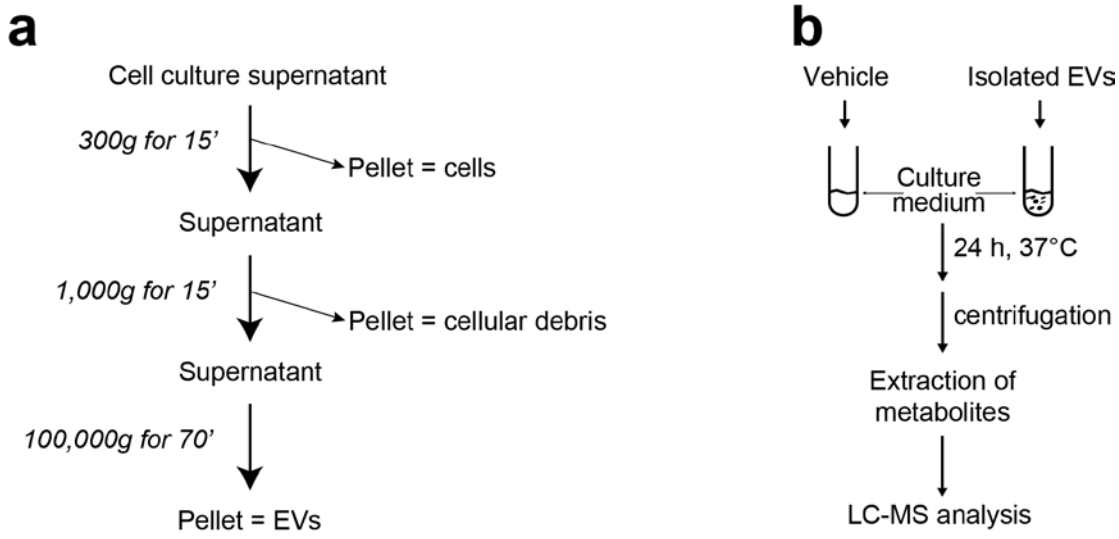
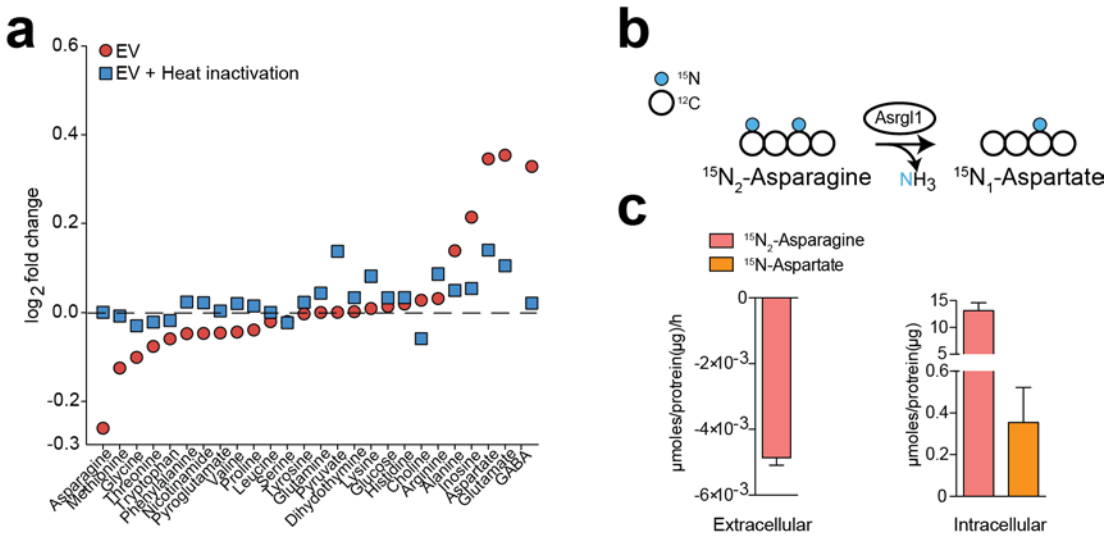
Metabolite	log2FC Exp#1	log2FC Exp#2
Alanine	0.246700732	0.138503108
Inosine	0.316723446	0.213925772
Arginine	-0.136704011	0.032036196
Asparagine	-0.543564683	-0.26045801
Aspartate	0.471548081	0.345474331
Choline	0.289704115	0.027983298
Dihydothymine	-0.126562997	0.002232793
GABA	1.339122336	0.395749701
Glucose	-0.032562674	0.014290393
Glutamate	1.336853427	0.353822205
Glutamine	-0.129516457	0.000706108
Glycine	-0.074366796	-0.10061939
Histidine	-0.086343988	0.019392119
Leucine	0.13234056	-0.01995848
Lysine	-0.135236264	0.009537467
Methionine	-0.083941825	-0.12485433
Methyladenine	0.016463544	0.020225401
Nicotinamide	-0.069080268	-0.04669718
Phenylalanine	-0.054332221	-0.04714899
Proline	-0.087400611	-0.0387874
Pyroglutamate	-0.125757661	-0.0454492
Pyruvate	-0.048202959	0.000966917
Serine	-0.069080769	-0.019728
Succinate	0.138747094	0.039571835
Threonine	-0.093929959	-0.07616743
Tryptophan	0.011793229	-0.0588765
Tyrosine	-0.043199375	-0.00251545
Valine	-0.047982667	-0.04362321

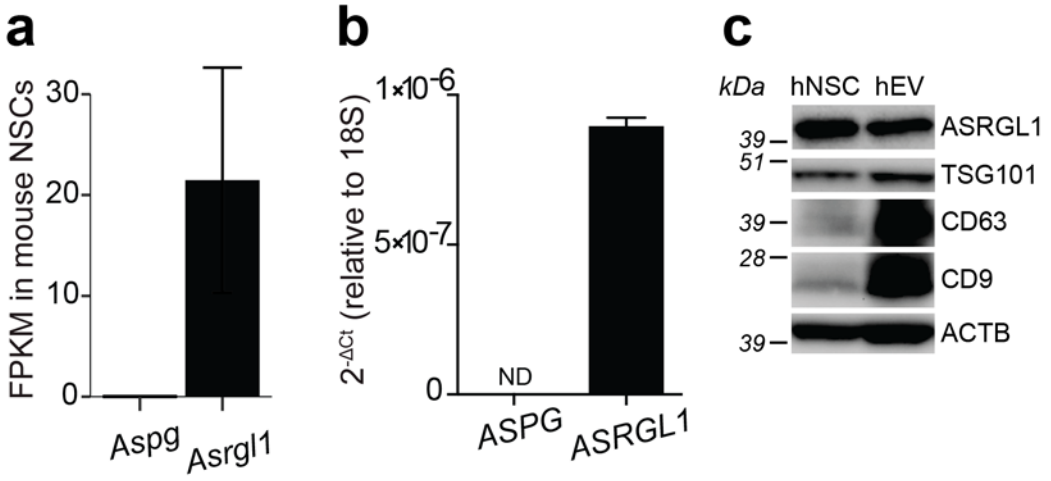
Supplementary Table 1. Identified metabolites in medium + EVs *vs*. fresh medium as in **Fig. 1a** and **b**.



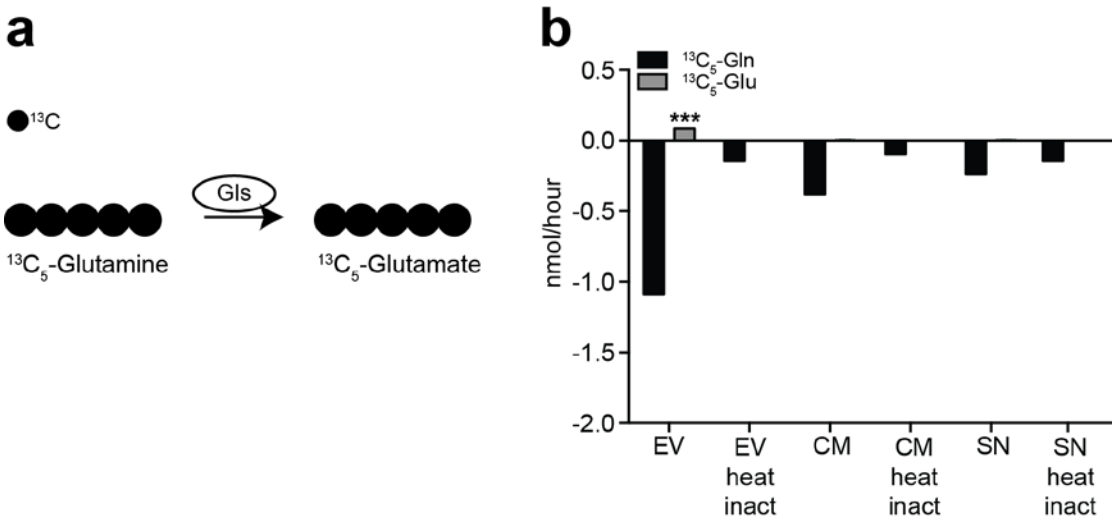
Supplementary Figure 1. (**a**) Schematic representation of the protocol used for the purification of EVs starting from NSC culture supernatants. (**b**) Schematic representation of the metabolomic experiment in medium + EVs *vs*. fresh medium (Vehicle).



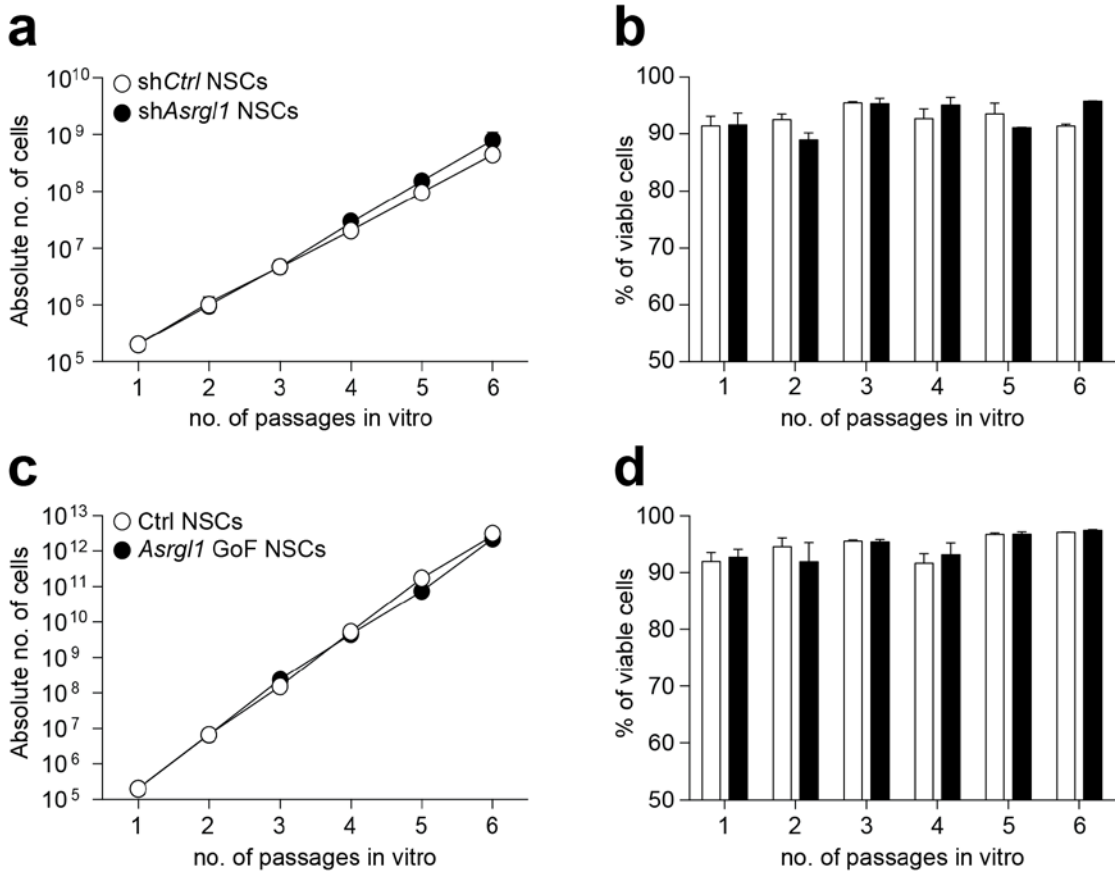
Supplementary Figure 2. (a) Scatter plot indicating fold changes (log_2) of metabolite levels between EV or EV subjected to heat inactivation and vehicle control medium. **(b)** Schematic representation of isotope tracing experiment. Reactions carried out by L-asparaginase like 1 (Asrgl1); carbon and nitrogen atoms are depicted as circles. **(c)** Consumption of $^{15}N_2$-Asn from extracellular medium (left) and accumulation of intracellular $^{15}N_2$-Asn and ^{15}N-Asp as measured in cultures of NPCs. Data are mean ± SD from three independent cultures.



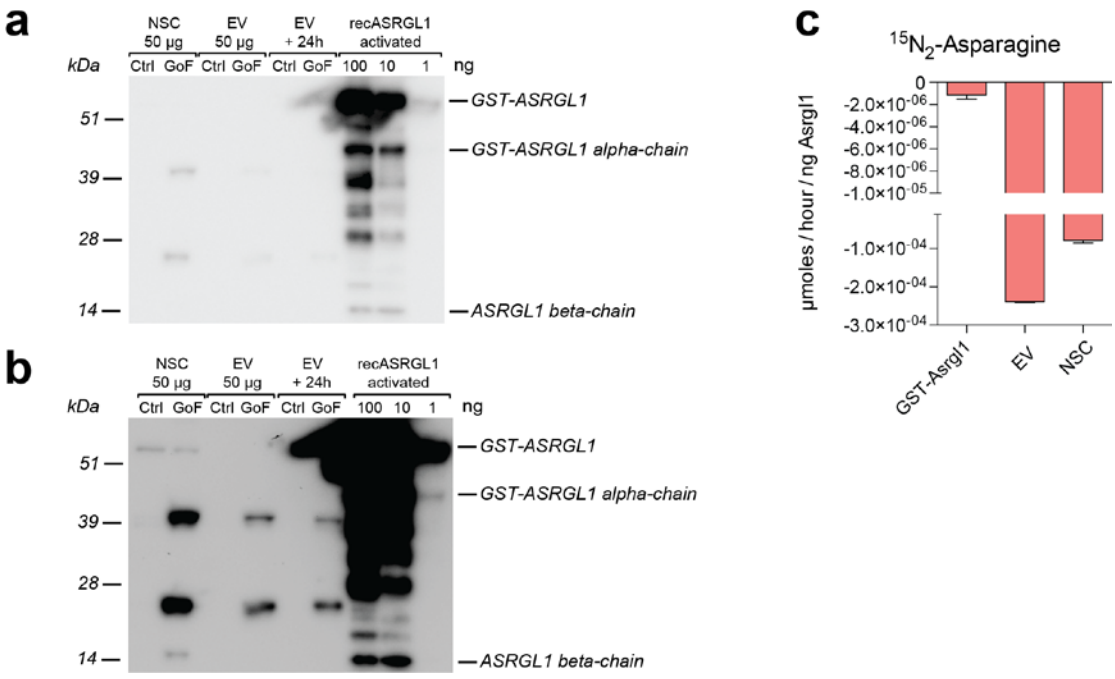
Supplementary Figure 3. Expression of Aspg and Asrgl1 in mouse and human NSCs. (a) RNA-Seq data for *Aspg* and *Asrgl1* in mouse NSCs. The y-axis indicates the expression levels normalized as fragments per kilobase of transcript per million reads mapped (FPKM). Data were retrieved from [6]. **(b)** qPCR analyses of *ASPG* and *ASRGL1* expression in human NSCs. qPCR data are represented as fold change ± SEM of the *ASPG* and *ASRGL1* mRNA levels in human NSCs, and analysed with the $2^{-\Delta Ct}$ method over 18S ribosomal RNA, used as housekeeping gene. Data have been obtained from n= 3 independent experiments. **(c)** WB analyses of exosomal markers in hNSC and hEV. Data are representative of n= 3 independent protein preparations showing the same trends. For uncropped blots see **Supplementary Figure 10**.



Supplementary figure 4. The L-asparaginase activity of EVs is devoid of Glutaminase activity. (a) Schematic representation of isotope tracing experiment. Reaction carried out by glutaminase (Gls). Labelled carbon atoms are indicated in black. Nitrogen atoms are not indicated in this schematic. **(b)** Barplot of the consumption of $^{13}C_5$-Gln and production of $^{13}C_5$-Glu mediated by EV, conditioned medium (i.e. CM, medium with EV) and supernatant (i.e. SN, medium deprived of EV), with or without heat inactivation (100ºC for 10'). Data are mean ± SEM and have been obtained from n= 2 independent experiments. Statistical analysis was performed using one-way ANOVA, followed by Bonferroni's test correction. *** $p < 0.001$.

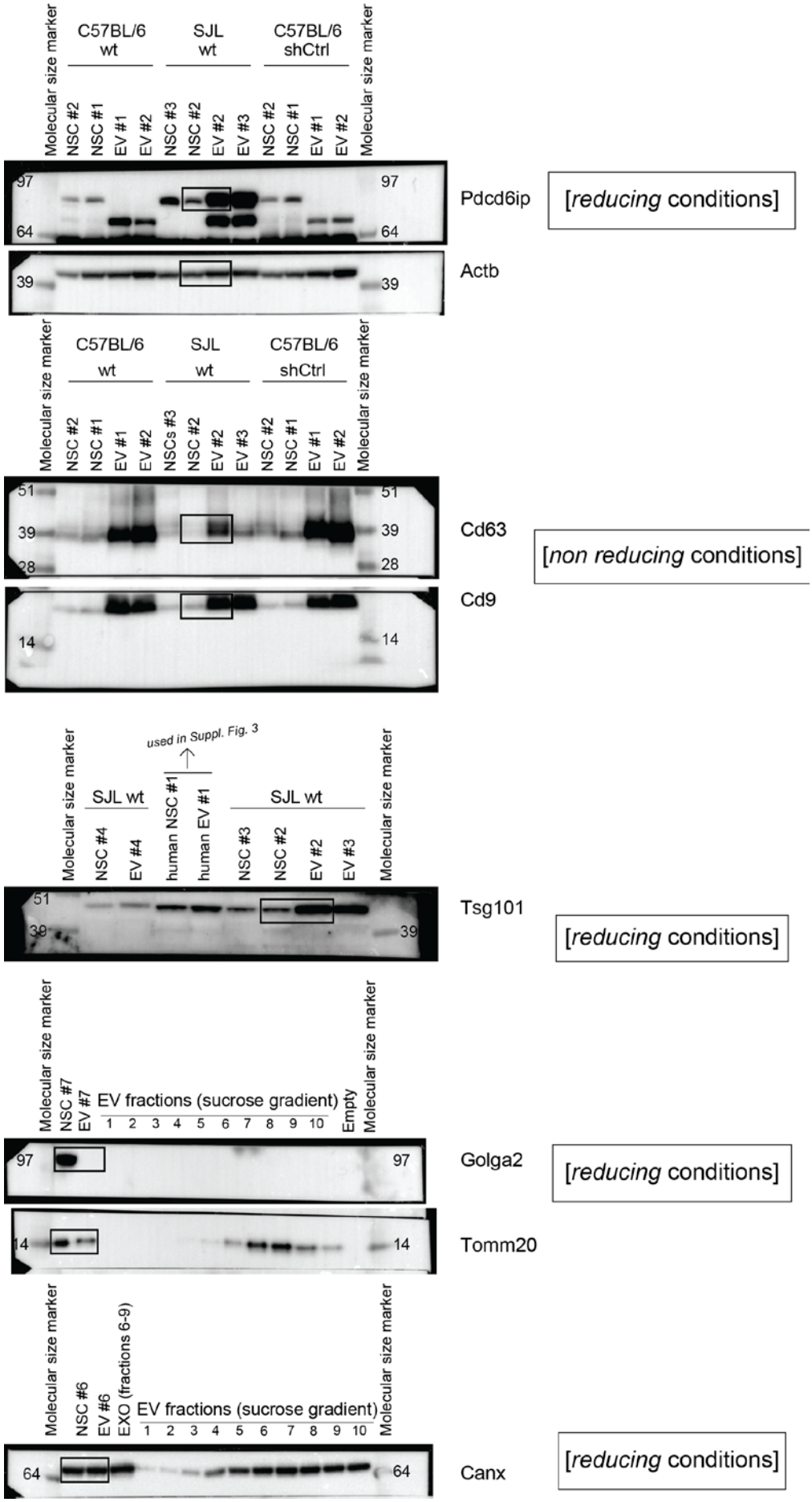


Supplementary Figure 5. Viability of NSCs subjected to viral transduction of shAsrgl1 and Asrgl1 GoF. *shAsrgl1* **(a, b)** and *Asrgl1* GoF **(c, d)** NSCs were passaged every 4 days and stained with Trypan blue (TB) for viability. The numbers of (TB-negative) live cells were collected and absolute numbers of cells plotted to generate the growth curves in **a** and **c**. **(b-d)** Same as in **a**-**b** for the % of viable cells over passaging *in vitro*. The data are showed as mean numbers ± SEM from n= 3 independent experiments.

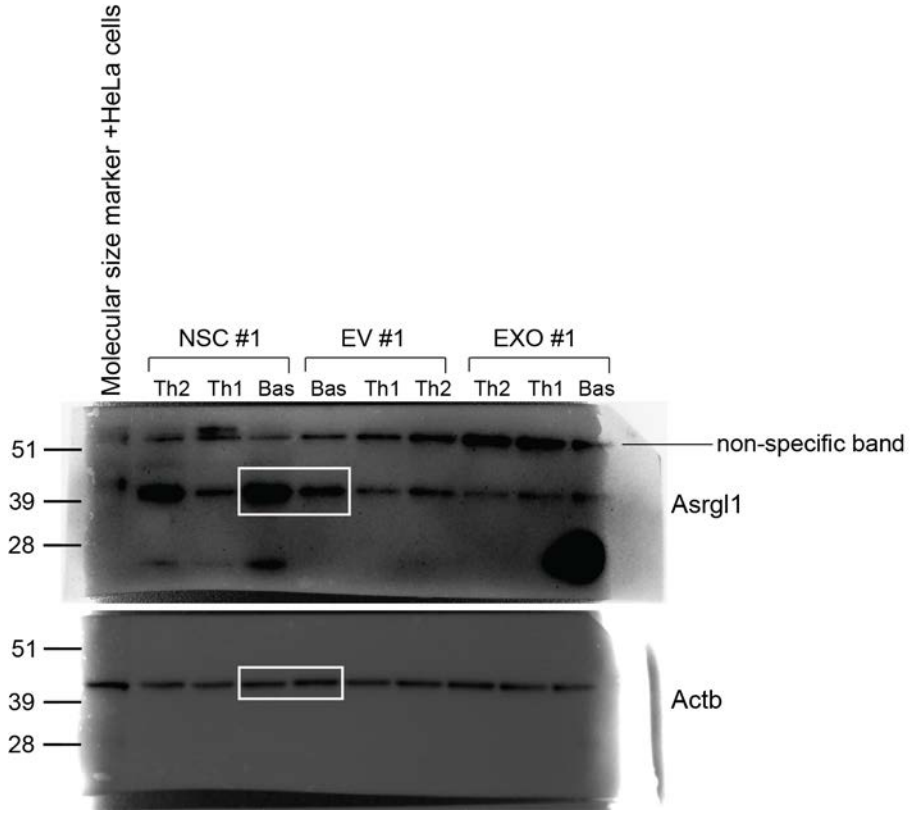


Supplementary Figure 6. Comparison of asparaginase activity among recombinant protein, EVs, and NSCs. (a, b) The indicated amounts of protein extract of NSC and EV, and of recombinant GST-tagged ASRGL1 were loaded on a 4-12% gel and blotted onto a PVDF membrane. Short (10'') (**a**) and long (5') **(b)** exposures are presented. **(c)** Consumption of ^{15}N-L-asparagine by recombinant Asrgl1, EV and NSC. Data are normalised on Asrgl1 protein abundance.

Supplementary Results

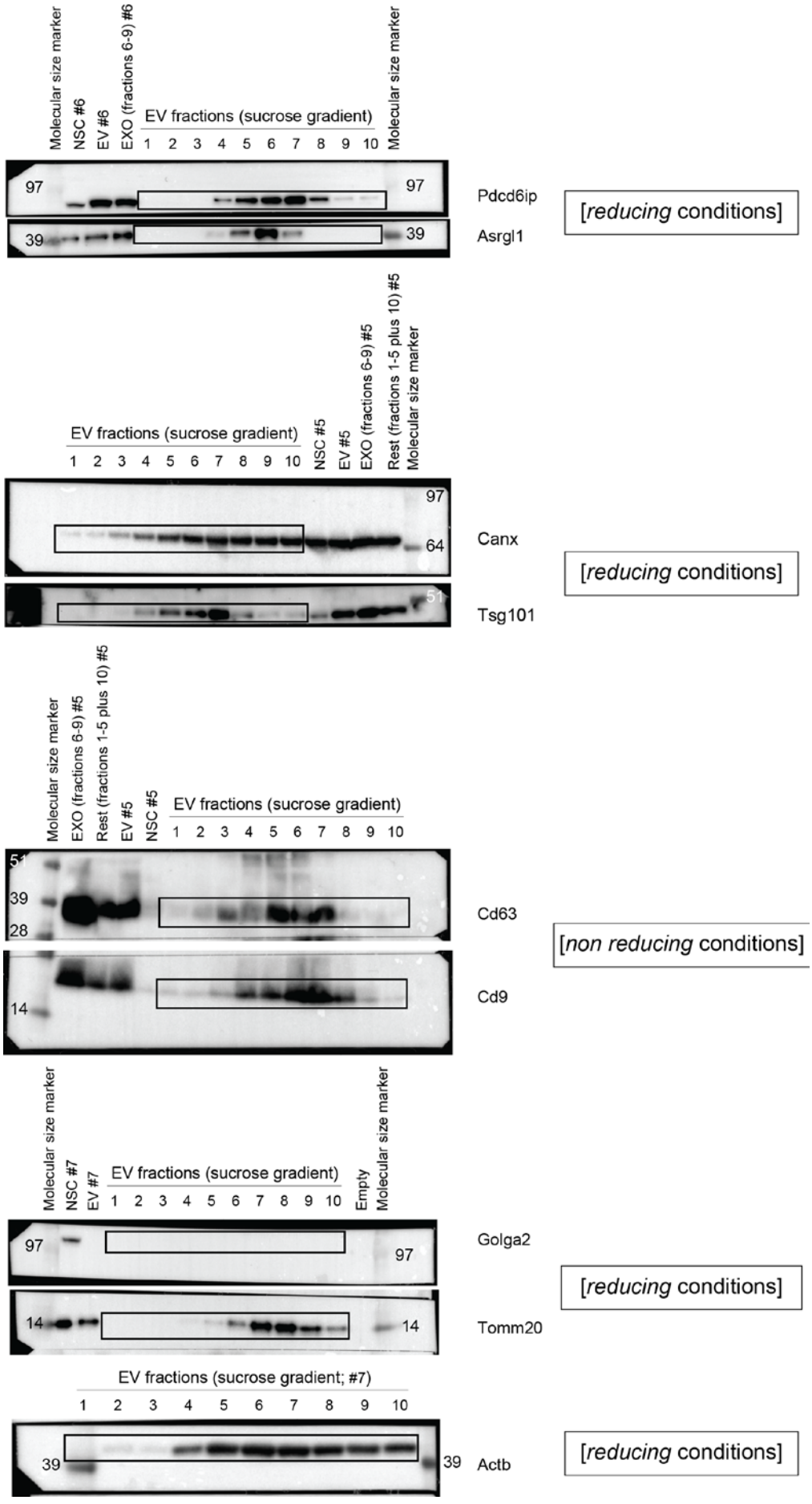


Supplementary Figure 7. Uncropped blots for Figure 1c. Total of (#) seven independent wild type (wt) SJL NSC and EV preparations were used.
Protein extracts from C57BL/6 NSC and EV were not used for this paper. Cd63 and Cd9 were obtained in *non reducing* conditions (described in methods). The lanes with human NSC and human EV were used in Supplementary Figure 3 (see also Supplementary Figure 10). EXO proteins were derived pooling together sucrose fractions from 6 to 9 as described[6], and were not used in this work. Equal amount of proteins were loaded for NSC, EV and EXO. Actin was used as a sample processing control. Lanes used for main figures are indicated by a rectangle.



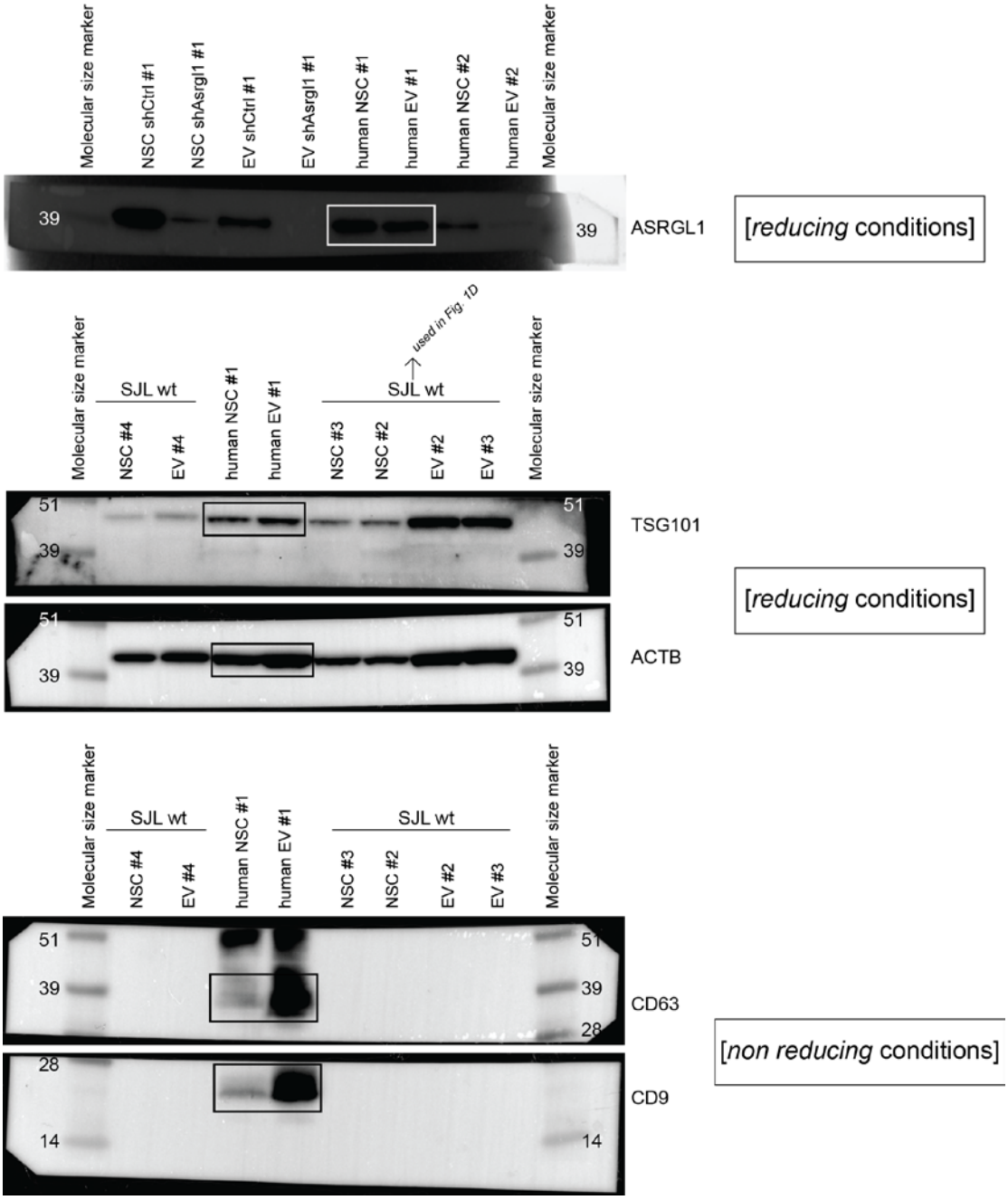
Supplementary Figure 8. Uncropped blots for Figure 3b. Bas= basal conditions (corresponding to wild type SJL NSC and EV). Th1 and Th2 indicate NSC treatments prior to EV collection as described[6]. Th1= 500 IU/ml recombinant mouse IFN-γ + 200 UI/ml recombinant mouse TNF-α + 100 UI/ml recombinant mouse IL-1β; Th2= 10 ng/ml recombinant murine IL-4 + 10 ng/ml recombinant mouse IL-5 + 10 ng/ml recombinant mouse IL-13. Th1 and Th2 NSC and EV preparations were not used in this work. Equal amounts of protein were loaded for NSC and EV. Actin was used as a sample processing control. Lanes used for main figures are indicated by a rectangle.

Supplementary Results

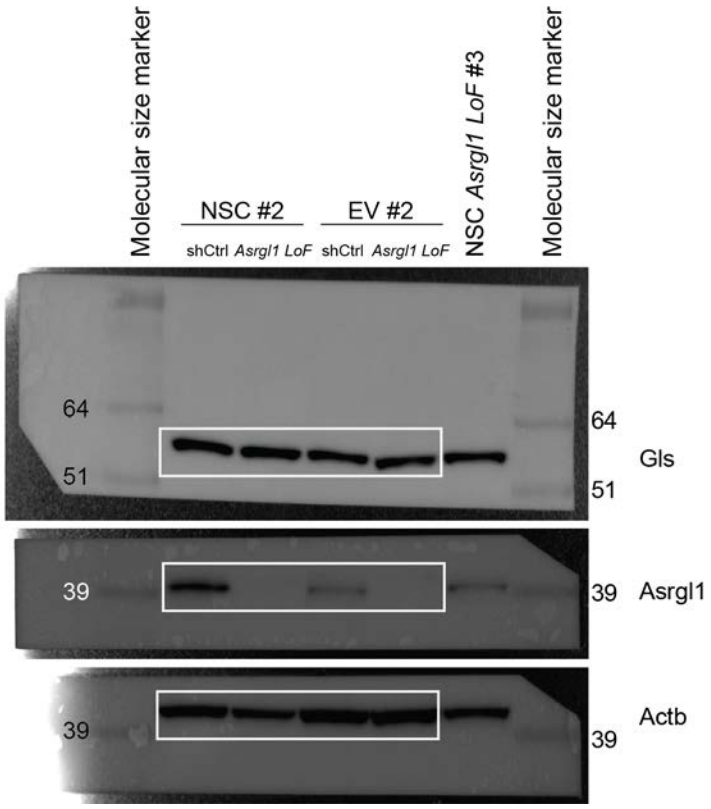


Supplementary Figure 9. Uncropped blots for Figure 3c.

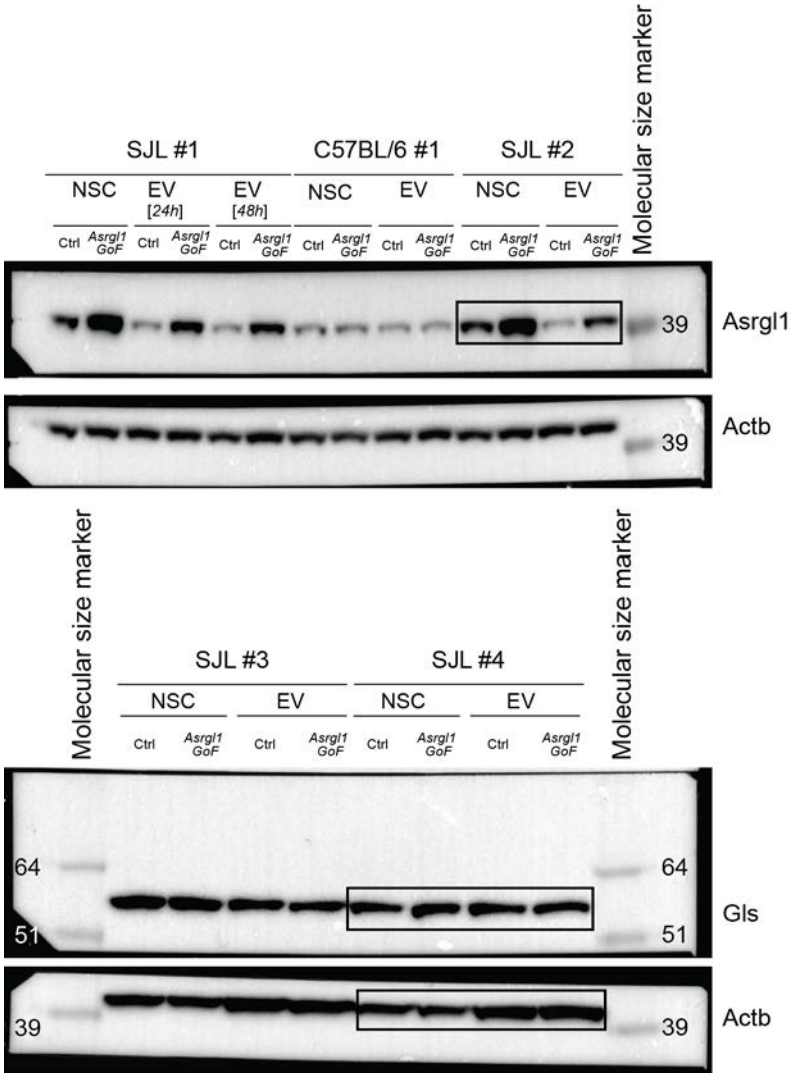
Total of three independent sucrose gradients were prepared. EXO proteins were derived pooling together sucrose fractions from 6 to 9, whereas Rest was obtained pooling fractions 1 to 5 and 10, as described[6], and were not used in this work. Cd63 and Cd9 were obtained in *non reducing* conditions. Equal amounts of protein were loaded for NSC, EV, EXO and Rest. Equal volumes were loaded for each sucrose gradient fraction. Each fraction was obtained from matched NSC preparation. Actin was used as a sample processing control. Lanes used for main figures are indicated by a rectangle.



Supplementary Figure 10. Uncropped blots for Supplementary Figure 3c. Total of (#) three independent human NSC and human EV preparations were used. Cd63 and Cd9 were obtained in *non reducing* conditions. Equal amounts of protein were loaded for human NSC and human EV. Actin was used as a sample processing control. Lanes used for main figures are indicated by a rectangle.



Supplementary Figure 11. Uncropped blots for Figure 4a. Total of (#) three independent preparations of shCtrl and *Asrgl1 LoF* NSC and EV were prepared. Equal amounts of protein were loaded for NSC and EV. Actin was used as a loading control when comparing shCtrl NSC with *Asrgl1 LoF* NSC and shCtrl EV with *Asrgl1 LoF* EV. Lanes used for main figures are indicated by a rectangle.



Supplementary Figure 12. Uncropped blots for Figure 4b. Total of (#) four independent preparations of Ctrl and *Asrgl1 GoF* NSC and EV were prepared. In preparation #1 proteins from EV were extracted after 24 or 48 hours of incubation as in Figure 4d. Protein extracts from C57BL/6 NSC and EV were not used for this paper. Equal amounts of protein were loaded for NSC and EV. Actin was used as a loading control when comparing Ctrl NSC with *Asrgl1 GoF* NSC and Ctrl EV with *Asrgl1 GoF* EV. Actins from all the blots are shown. Lanes used for main figures are indicated by a rectangle.