

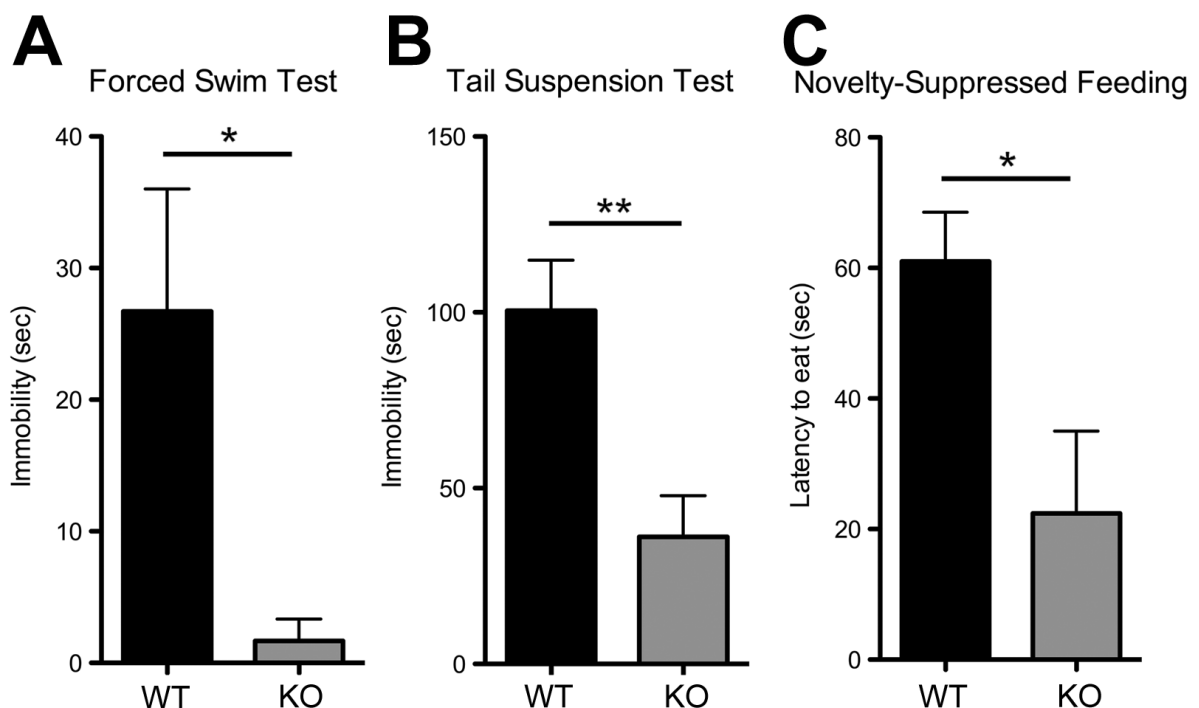
# Serotonin depletion causes valproate-responsive manic-like condition and increased hippocampal neuroplasticity that are reversed by stress

Giacomo Maddaloni, Sara Migliarini, Francesco Napolitano, Andrea Giorgi, Serena Nazzi, Daniele Biasci, Alessia De Felice, Marta Gritti, Anna Cavaccini, Alberto Galbusera, Sara Franceschi, Francesca Lessi, Marco La Ferla, Paolo Aretini, Chiara Maria Mazzanti, Raffaella Tonini, Alessandro Gozzi, Alessandro Usiello & Massimo Pasqualetti

## SUPPLEMENTARY INFORMATIONS

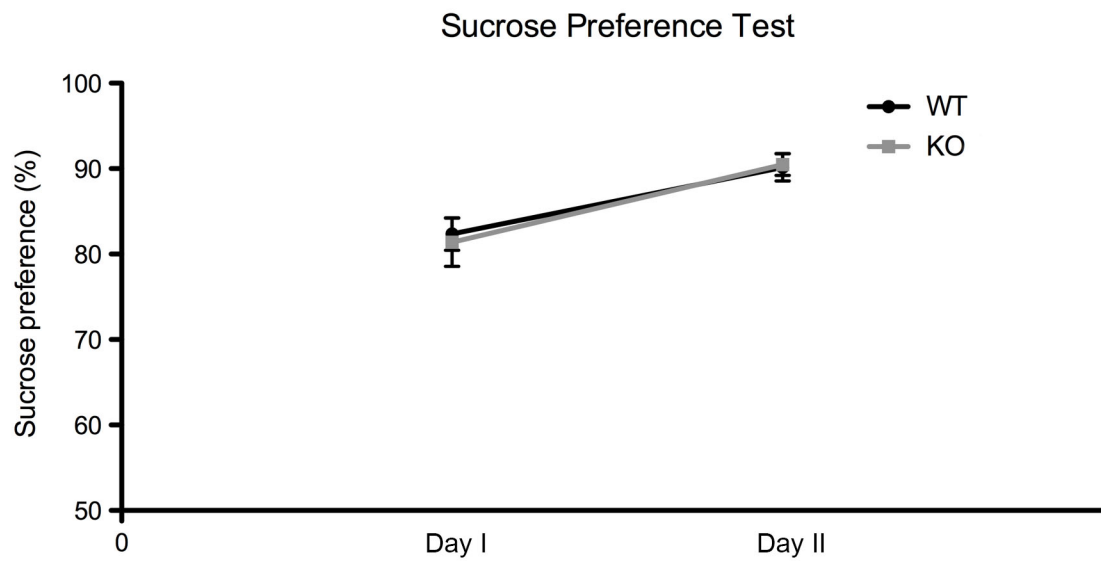
### Supplementary figures and legends

#### Supplementary Figure S1



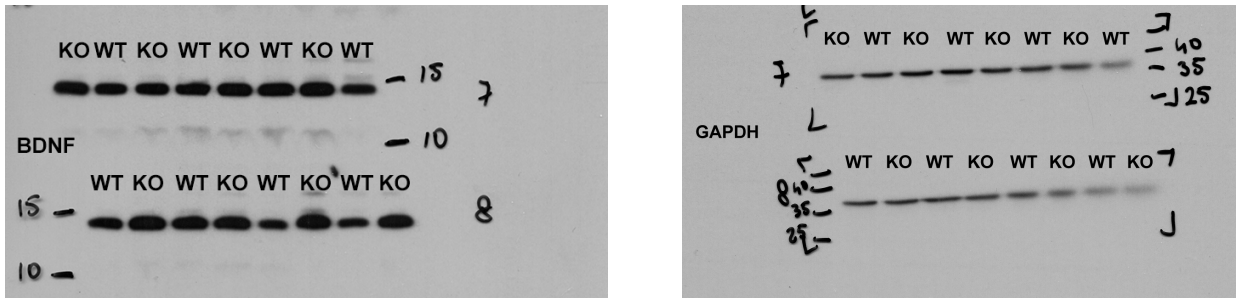
**Supplementary Figure S1:** Behavioural analysis in aged animals. (A) Immobility time in the FST and (B) TST. (C) Latency to eat in the NSF. Data are expressed as mean  $\pm$  s.e.m., \* $p < 0.05$ , \*\* $p < 0.01$ .

## Supplementary Figure S2



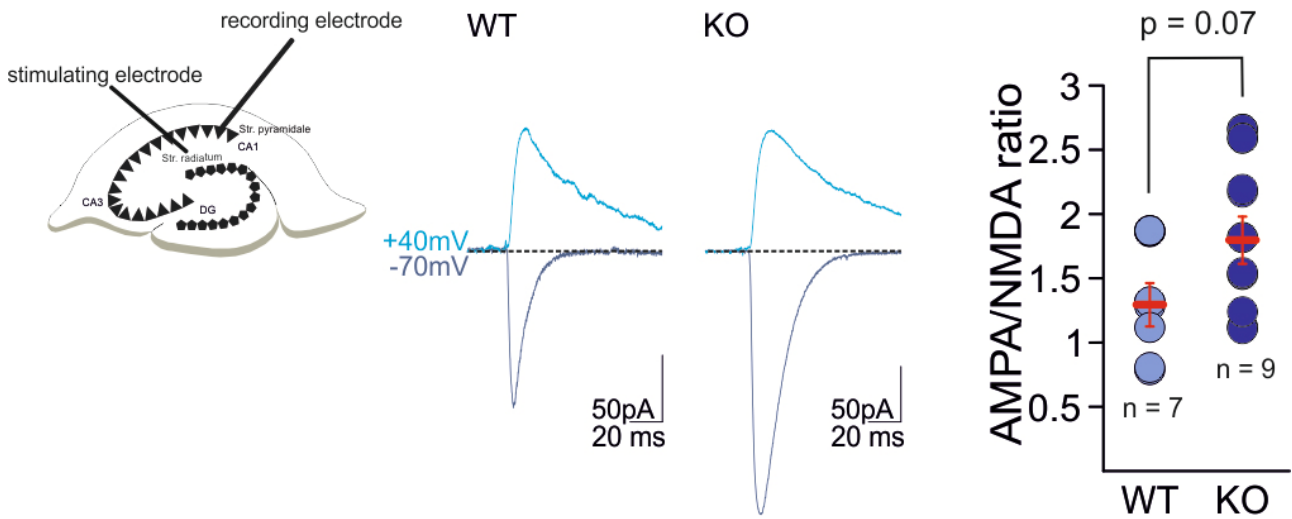
**Supplementary Figure S2:** Hedonic behaviour in *Tph2* KO mice. Results showed no difference in sucrose preference (calculated as percentage of sucrose intake over the total volume of fluid intake) between WT and KO mice both on day I and day II. Moreover, both WT and KO mice showed increased preference on day II. Data are expressed as mean  $\pm$  s.e.m.

**Supplementary Figure S3**



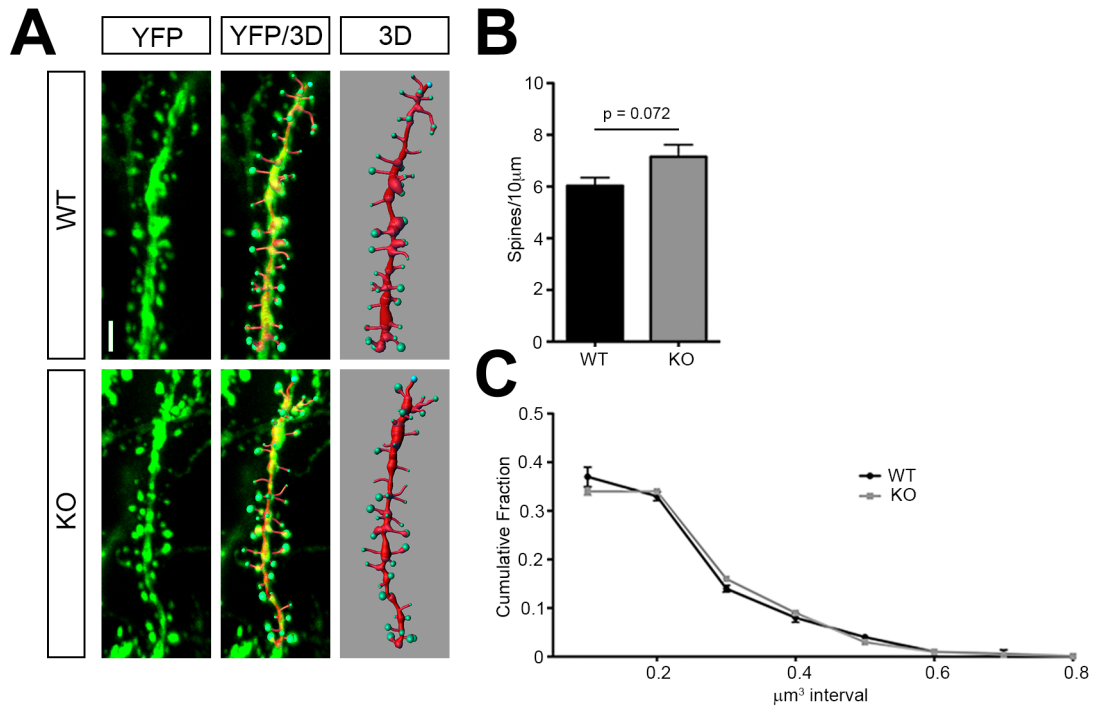
**Supplementary Figure S3:** Full length blots showing BDNF protein levels supporting figure 2C.

### Supplementary Figure S4



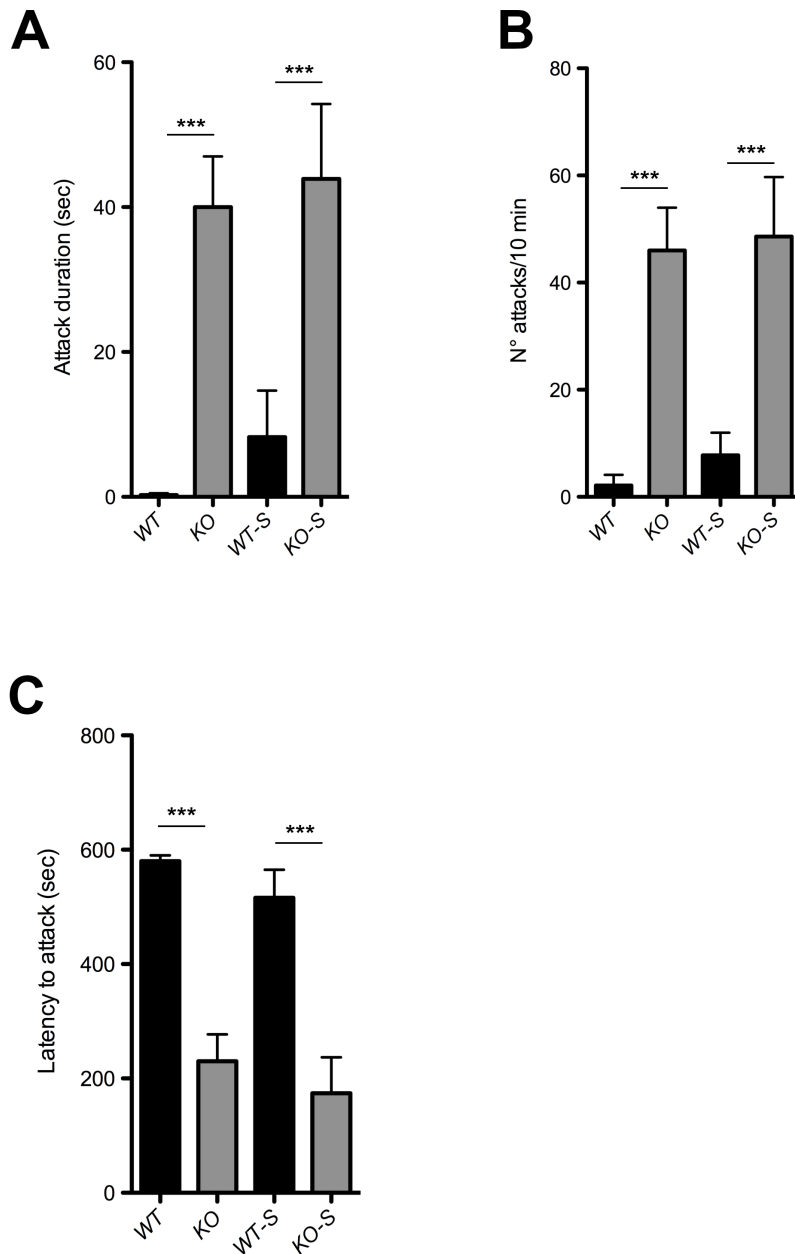
**Supplementary Figure S4:** AMPA to NMDA ratio in CA1 hippocampal neurons. (left) Schematic of recording configuration; (middle) Representative traces from whole-cell voltage-clamp experiments showing AMPA receptor- and NMDA-receptor mediated currents in CA1 pyramidal neurons of WT and KO mutant mice; (right) AMPA to NMDA ratio in the two mouse cohorts.

## Supplementary Figure S5



**Supplementary Figure S5:** Dendritic spine analysis in CA1 apical dendrites. **(A)** Representative images of YFP-labelled CA1 apical dendrites and their 3D reconstruction. **(B, C)** Quantification of spine density (WT  $n = 7$ ; KO  $n = 8$ , for spine density one-way ANOVA,  $F_{1,13} = 3.624$ ,  $p = 0.072$ ) and volume distribution. Data are expressed as mean  $\pm$  s.e.m.  $n$  indicates biological replicates. Scale bar =  $2\mu\text{m}$ .

## Supplementary Figure S6



### Supplementary Figure S6: Aggressive behaviour characterization in uCMS exposed mice. (A)

Attack duration (n = 12; two-way ANOVA followed by Fisher's post-hoc test, effect of genotype

$F_{1,44} = 27.073$ ,  $p < 0.0001$ ; KO vs WT  $p < 0.0001$ , KO-S vs WT-S  $p < 0.0001$ ) (B) latency to the first

attack (n = 12; two-way ANOVA followed by Fisher's post-hoc test, effect of genotype  $F_{1,44} =$

$27.127$ ,  $p < 0.0001$ ; KO vs WT  $p < 0.0001$ , KO-S vs WT-S  $p < 0.0001$ ) and (C) attack frequency (n

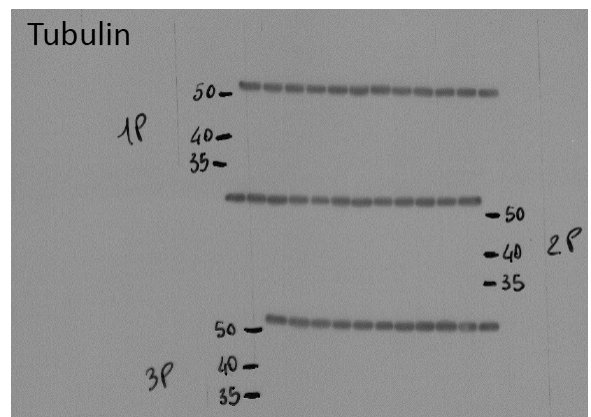
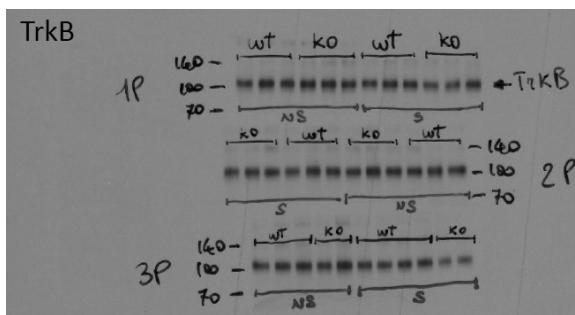
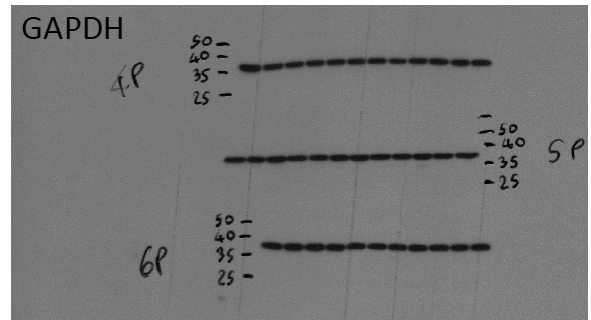
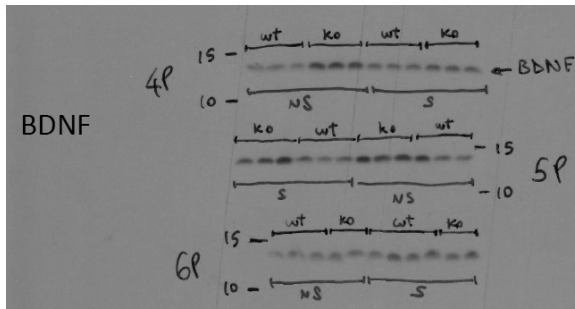
= 12; two-way ANOVA followed by Fisher's post-hoc test, effect of genotype  $F_{1,44} = 58.592$ ,  $p <$

$0.0001$ ; KO vs WT  $p < 0.0001$ , KO-S vs WT-S  $p < 0.0001$ ) in the NAAT. Data are expressed as

mean  $\pm$  s.e.m. n indicates biological replicates. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . NAAT, Neutral

Arena Aggression Test.

**Supplementary Figure S7**



**Supplementary Figure S7.** Full length blots showing BDNF and TrkB protein levels supporting figure 4C and 4D, respectively. NS = non-stress group; S = stress group.

## Supplementary tables

**Supplementary Table S1: Biological process Gene Ontology categories for KO/WT comparison**

Biological process	p-value	Biological process	p-value
single-multicellular organism process (GO:0044707)	0,000000	anion transmembrane transport (GO:0098656)	0,001550
ion transport (GO:0006811)	0,000000	localization (GO:0051179)	0,001930
anion transport (GO:0006820)	0,000000	neurogenesis (GO:0022008)	0,002450
		positive regulation of metabolic process	
multicellular organism development (GO:0007275)	0,000000	(GO:0009893)	0,002870
single-organism developmental process			
(GO:0044767)	0,000000	single-organism cellular process (GO:0044763)	0,003620
developmental process (GO:0032502)	0,000000	pattern specification process (GO:0007389)	0,004920
multicellular organismal process (GO:0032501)	0,000000	regulation of biological process (GO:0050789)	0,005490
anatomical structure development (GO:0048856)	0,000000	nervous system development (GO:0007399)	0,005800
single-organism process (GO:0044699)	0,000001	regulation of cell proliferation (GO:0042127)	0,005940
system development (GO:0048731)	0,000008	regulation of localization (GO:0032879)	0,010200
single-organism transport (GO:0044765)	0,000009	regulation of response to stimulus (GO:0048583)	0,010600
		regulation of multicellular organismal development	
ion transmembrane transport (GO:0034220)	0,000011	(GO:2000026)	0,010800
single-organism localization (GO:1902578)	0,000022	regulation of cellular process (GO:0050794)	0,011100
biological regulation (GO:0065007)	0,000082	nitrogen compound transport (GO:0071705)	0,011800
sensory organ development (GO:0007423)	0,000129	eye development (GO:0001654)	0,013500
positive regulation of biological process			
(GO:0048518)	0,000181	regulation of biological quality (GO:0065008)	0,013600
transport (GO:0006810)	0,000192	muscle contraction (GO:0006936)	0,013800
animal organ development (GO:0048513)	0,000204	amino acid transport (GO:0006865)	0,015300
anatomical structure morphogenesis (GO:0009653)	0,000298	cell development (GO:0048468)	0,015700
transmembrane transport (GO:0055085)	0,000340	regulation of metabolic process (GO:0019222)	0,016900
cell differentiation (GO:0030154)	0,000569	response to organic substance (GO:0010033)	0,019600
		positive regulation of cellular process	
organic anion transport (GO:0015711)	0,000590	(GO:0048522)	0,021000
establishment of localization (GO:0051234)	0,000610	dicarboxylic acid transport (GO:0006835)	0,022600
regulation of multicellular organismal process			
(GO:0051239)	0,000714	neuron development (GO:0048666)	0,025700
generation of neurons (GO:0048699)	0,000763	regulation of transport (GO:0051049)	0,025900
cellular process (GO:0009987)	0,000841	renal system process (GO:0003014)	0,027500
cellular developmental process (GO:0048869)	0,000909	neuron projection development (GO:0031175)	0,038000



		antigen processing and presentation of peptide	
carboxylic acid transport (GO:0046942)	0,000973	antigen via MHC class I (GO:0002474)	0,038500
organic acid transport (GO:0015849)	0,001110	positive regulation of transport (GO:0051050)	0,038600
		cellular response to external stimulus	
response to external stimulus (GO:0009605)	0,001230	(GO:0071496)	0,039300
		positive regulation of nitrogen compound metabolic	
		process (GO:0051173)	0,044000

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**Supplementary Table S2: Cellular component Gene Ontology categories for KO/WT comparison**

<b>Cellular Component</b>	<b>p-value</b>	<b>Cellular Component</b>	<b>p-value</b>
plasma membrane part (GO:0044459)	0,000000	cell surface (GO:0009986)	0,004330
cell periphery (GO:0071944)	0,000002	membrane part (GO:0044425)	0,005220
vesicle (GO:0031982)	0,000002	membrane protein complex (GO:0098796)	0,006040
plasma membrane (GO:0005886)	0,000003	extracellular exosome (GO:0070062)	0,006070
membrane region (GO:0098589)	0,000005	cell part (GO:0044464)	0,006070
whole membrane (GO:0098805)	0,000007	cell (GO:0005623)	0,006390
plasma membrane region (GO:0098590)	0,000016	extracellular vesicle (GO:1903561)	0,006720
extracellular region part (GO:0044421)	0,000450	ion channel complex (GO:0034702)	0,007970
neuron projection (GO:0043005)	0,000704	endoplasmic reticulum lumen (GO:0005788)	0,009700
intrinsic component of plasma membrane (GO:0031226)	0,000884	MHC protein complex (GO:0042611)	0,012300
endoplasmic reticulum part (GO:0044432)	0,001230	endoplasmic reticulum (GO:0005783)	0,013600
cell projection (GO:0042995)	0,001400	transmembrane transporter complex (GO:1902495)	0,017500
MHC class I protein complex (GO:0042612)	0,001430	transporter complex (GO:1990351)	0,020600
endoplasmic reticulum exit site (GO:0070971)	0,001430	organelle (GO:0043226)	0,022600
integral component of plasma membrane (GO:0005887)	0,001480	smooth endoplasmic reticulum (GO:0005790)	0,024500
neuron part (GO:0097458)	0,002100	plasma membrane protein complex (GO:0098797)	0,025600
extracellular organelle (GO:0043230)	0,002340	endomembrane system (GO:0012505)	0,033300
extracellular region (GO:0005576)	0,002580	endoplasmic reticulum chaperone complex (GO:0034663)	0,040800
Golgi medial cisterna (GO:0005797)	0,002920	cell projection membrane (GO:0031253)	0,044600

### Supplementary Table S3: Molecular functions Gene Ontology categories for KO/WT comparison

Molecular function	p-value	Molecular function	p-value
protein binding (GO:0005515)	0,000002	beta-2-microglobulin binding (GO:0030881)	0,002020
binding (GO:0005488)	0,000004	T cell receptor binding (GO:0042608)	0,002710
ion transmembrane transporter activity (GO:0015075)	0,000011	unfolded protein binding (GO:0051082)	0,004240
substrate-specific transmembrane transporter activity (GO:0022891)	0,000037	sodium ion transmembrane transporter activity (GO:0015081)	0,010900
anion transmembrane transporter activity (GO:0008509)	0,000054	metal ion transmembrane transporter activity (GO:0046873)	0,015700
transporter activity (GO:0005215)	0,000063	ion channel activity (GO:0005216)	0,016000
substrate-specific transporter activity (GO:0022892)	0,000116	substrate-specific channel activity (GO:0022838)	0,020700
transmembrane transporter activity (GO:0022857)	0,000156	cation transmembrane transporter activity (GO:0008324)	0,025800
gated channel activity (GO:0022836)	0,001130	monovalent inorganic cation transmembrane transporter activity (GO:0015077)	0,028400
TAP binding (GO:0046977)	0,001470	passive transmembrane transporter activity (GO:0022803)	0,039100
protein dimerization activity (GO:0046983)	0,001560	channel activity (GO:0015267)	0,039100

**Supplementary Table S4: Biological process Gene Ontology categories for KO-S/KO comparison**

<b>Biological Process</b>	<b>p-value</b>	<b>Biological Process</b>	<b>p-value</b>
ion transport (GO:0006811)	0,000007	multicellular organism development (GO:0007275)	0,006060
single-multicellular organism process (GO:0044707)	0,000027	transport (GO:0006810)	0,006500
single-organism transport (GO:0044765)	0,000259	cellular component organization (GO:0016043)	0,008000
		cellular component organization or biogenesis	
animal organ development (GO:0048513)	0,000477	(GO:0071840)	0,008710
		positive regulation of biological process	
single-organism developmental process (GO:0044767)	0,000584	(GO:0048518)	0,009230
single-organism process (GO:0044699)	0,000618	anion transport (GO:0006820)	0,010300
single-organism localization (GO:1902578)	0,000656	blood circulation (GO:0008015)	0,011500
developmental process (GO:0032502)	0,000729	regulation of biological quality (GO:0065008)	0,011800
ion transmembrane transport (GO:0034220)	0,000749	circulatory system process (GO:0003013)	0,012900
extracellular matrix organization (GO:0030198)	0,001780	system development (GO:0048731)	0,013200
extracellular structure organization (GO:0043062)	0,001870	establishment of localization (GO:0051234)	0,019100
multicellular organismal process (GO:0032501)	0,002190	single-organism cellular process (GO:0044763)	0,021800
localization (GO:0051179)	0,002260	positive regulation of cellular process (GO:0048522)	0,025100
anatomical structure morphogenesis (GO:0009653)	0,003590	anatomical structure development (GO:0048856)	0,032400
response to chemical (GO:0042221)	0,003880	response to inorganic substance (GO:0010035)	0,041400
transmembrane transport (GO:0055085)	0,003910	cellular process (GO:0009987)	0,049500

**Supplementary Table S5:** Cellular component Gene Ontology categories for KO-S/KO comparison

<b>Cellular Component</b>	<b>p-value</b>	<b>Cellular Component</b>	<b>p-value</b>
membrane-bounded organelle (GO:0043227)	0,000008	extracellular organelle (GO:0043230)	0,003230
organelle (GO:0043226)	0,000015	cell periphery (GO:0071944)	0,005020
extracellular matrix (GO:0031012)	0,000131	plasma membrane (GO:0005886)	0,005750
extracellular region part (GO:0044421)	0,000259	extracellular region (GO:0005576)	0,005770
cell surface (GO:0009986)	0,000391	extracellular exosome (GO:0070062)	0,007680
vesicle (GO:0031982)	0,001060	cell part (GO:0044464)	0,010300
endoplasmic reticulum chaperone complex (GO:0034663)	0,001080	cell (GO:0005623)	0,010900
membrane region (GO:0098589)	0,001530	plasma membrane region (GO:0098590)	0,016900
plasma membrane part (GO:0044459)	0,001810	basolateral plasma membrane (GO:0016323)	0,035600
whole membrane (GO:0098805)	0,002610	basement membrane (GO:0005604)	0,041800
extracellular vesicle (GO:1903561)	0,003070		

**Supplementary Table S6: Molecular function Gene Ontology categories for KO-S/KO comparison**

<b>Molecular Function</b>	<b>p-value</b>
binding (GO:0005488)	0,000000
transporter activity (GO:0005215)	0,000276
ion transmembrane transporter activity (GO:0015075)	0,000739
substrate-specific transporter activity (GO:0022892)	0,001640
transmembrane transporter activity (GO:0022857)	0,001970
substrate-specific transmembrane activity (GO:0022891)	0,002230
protein binding (GO:0005515)	0,003300
metal ion transmembrane transporter activity (GO:0046873)	0,011500
cation transmembrane transporter activity (GO:0008324)	0,028800

**Supplementary Table S7: Biological process Gene Ontology categories for WT-S/WT comparison**

<b>Biological process</b>	<b>p-value</b>	<b>Biological process</b>	<b>p-value</b>
anatomical structure development (GO:0048856)	0,00000000	regulation of hormone levels (GO:0010817)	0,00048100
developmental process (GO:0032502)	0,00000000	establishment of localization (GO:0051234)	0,00058000
positive regulation of multicellular organismal process (GO:0051240)	0,00000000	cation transport (GO:0006812)	0,00061800
cellular process (GO:0009987)	0,00000006	response to endogenous stimulus (GO:0009719)	0,00064900
collagen fibril organization (GO:0030199)	0,00000009	muscle system process (GO:0003012)	0,00066800
extracellular matrix organization (GO:0030198)	0,00000012	organic acid transport (GO:0015849)	0,00104000
anion transmembrane transport (GO:0098656)	0,00000020	ion transport (GO:0006811)	0,00116000
		cellular component organization or biogenesis (GO:0071840)	0,00169000
response to organonitrogen compound (GO:0010243)	0,00000024	organic anion transport (GO:0015711)	0,00205000
connective tissue development (GO:0061448)	0,00000043	anatomical structure morphogenesis (GO:0009653)	0,00213000
single-organism developmental process (GO:0044767)	0,00000067	receptor protein serine/threonine kinase signaling pathway (GO:0090092)	0,00000079
		single-organism process (GO:0044699)	0,00215000
system process (GO:0003008)	0,00000181	regulation of cell differentiation (GO:0045595)	0,00224000
cellular component organization (GO:0016043)	0,00000236	circulatory system process (GO:0003013)	0,00229000
positive regulation of developmental process (GO:0051094)	0,00000256	hormone transport (GO:0009914)	0,00335000
cell-cell signaling (GO:0007267)	0,00000419	single-multicellular organism process (GO:0044707)	0,00362000
nitrogen compound transport (GO:0071705)	0,00002190	tissue development (GO:0009888)	0,00405000
localization (GO:0051179)	0,00003320	regulation of developmental process (GO:0050793)	0,00518000
blood circulation (GO:0008015)	0,00003380	response to chemical (GO:0042221)	0,00519000
organ morphogenesis (GO:0009887)	0,00004250	response to nitrogen compound (GO:1901698)	0,00763000
single-organism transport (GO:0044765)	0,00004510	anion transport (GO:0006820)	0,00986000
transmembrane transport (GO:0055085)	0,00005260	regulation of biological quality (GO:0065008)	0,01160000
animal organ development (GO:0048513)	0,00005590	metal ion transport (GO:0030001)	0,01220000
response to organic substance (GO:0010033)	0,00006270	amino acid transport (GO:0006865)	0,01350000
		regulation of multicellular organismal development (GO:2000026)	0,01360000
cell differentiation (GO:0030154)	0,00008380	cellular developmental process (GO:0048869)	0,02140000
carboxylic acid transport (GO:0046942)	0,00010700	single-organism localization (GO:1902578)	0,02400000
sensory organ development (GO:0007423)	0,00013000	single-organism cellular process (GO:0044763)	0,02570000
extracellular structure organization (GO:0043062)	0,00024000	transport (GO:0006810)	0,02700000
system development (GO:0048731)	0,00024300	ion transmembrane transport (GO:0034220)	0,03050000
multicellular organism development (GO:0007275)	0,00027500		

muscle contraction (GO:0006936)	0,00031500	neurotransmitter transport (GO:0006836)	0,03630000
		regulation of multicellular organismal process (GO:0051239)	0,03740000

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**Supplementary Table S8:** Cellular component Gene Ontology categories for WT-S/WT comparison

Cellular Component	p-value	Cellular Component	p-value
vesicle (GO:0031982)	0,00000077	extracellular vesicle (GO:1903561)	0,00444000
		extracellular organelle (GO:0043230)	0,00468000
collagen trimer (GO:0005581)		integral component of plasma membrane (GO:0005887)	0,00571000
	0,00000293	proteinaceous extracellular matrix (GO:0005578)	0,00816000
extracellular matrix component (GO:0044420)	0,00000974	extracellular exosome (GO:0070062)	0,01090000
extracellular space (GO:0005615)	0,00004060	plasma membrane part (GO:0044459)	0,02080000
extracellular region part (GO:0044421)	0,00005290	cytoplasmic vesicle membrane (GO:0030659)	0,02160000
complex of collagen trimers (GO:0098644)	0,00015000	basal part of cell (GO:0045178)	0,03060000
extracellular matrix (GO:0031012)	0,00015900	whole membrane (GO:0098805)	0,03380000
extracellular region (GO:0005576)	0,00057400	cytoplasmic vesicle part (GO:0044433)	0,03590000
fibrillar collagen trimer (GO:0005583)	0,00115000	membrane-bounded organelle (GO:0043227)	0,04090000
banded collagen fibril (GO:0098643)	0,00110000	cell periphery (GO:0071944)	0,04370000
organelle (GO:0043226)	0,00290000	plasma membrane (GO:0005886)	0,04950000
intrinsic component of plasma membrane (GO:0031226)	0,00392000		

**Supplementary Table S9:** Molecular Function Gene Ontology categories for WT-S/WT comparison

<b>Molecular Function</b>	<b>p-value</b>	<b>Molecular function</b>	<b>p-value</b>
transporter activity (GO:0005215)	0,0000000	growth factor binding (GO:0019838)	0,0037400
substrate-specific transporter activity (GO:0022892)	0,0000051	ion binding (GO:0043167)	0,0050100
		cation transmembrane transporter activity	
transmembrane transporter activity (GO:0022857)	0,0000054	(GO:0008324)	0,0071600
		metal ion transmembrane transporter activity	
ion transmembrane transporter activity (GO:0015075)	0,0000072	(GO:0046873)	0,0135000
substrate-specific transmembrane transporter activity		monovalent inorganic cation transmembrane	
(GO:0022891)	0,0000270	transporter activity (GO:0015077)	0,0203000
anion transmembrane transporter activity		active ion transmembrane transporter activity	
(GO:0008509)	0,0000396	(GO:0022853)	0,0222000
		inorganic cation transmembrane transporter activity	
binding (GO:0005488)	0,0002460	(GO:0022890)	0,0251000
		sodium ion transmembrane transporter activity	
symporter activity (GO:0015293)	0,0005840	(GO:0015081)	0,0324000
secondary active transmembrane transporter activity		amino acid transmembrane transporter activity	
(GO:0015291)	0,0006650	(GO:0015171)	0,0342000
active transmembrane transporter activity		organic anion transmembrane transporter activity	
(GO:0022804)	0,0008350	(GO:0008514)	0,0351000
		transcription factor activity, RNA polymerase II	
anion:cation symporter activity (GO:0015296)	0,0019100	proximal region binding (GO:0000982)	0,0371000
platelet-derived growth factor binding (GO:0048407)	0,0028600		

