Maternal hematopoietic TNF, via milk chemokines, programs hippocampal development and memory

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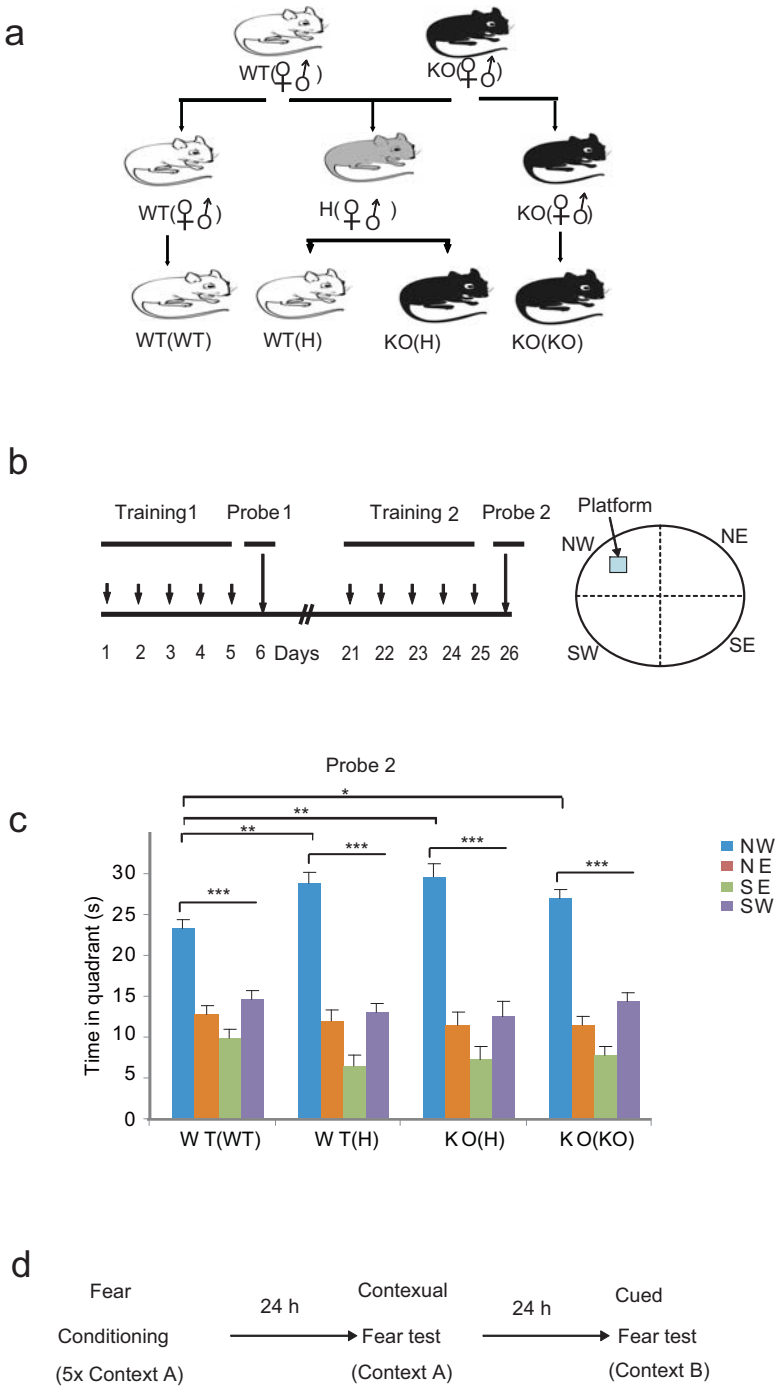
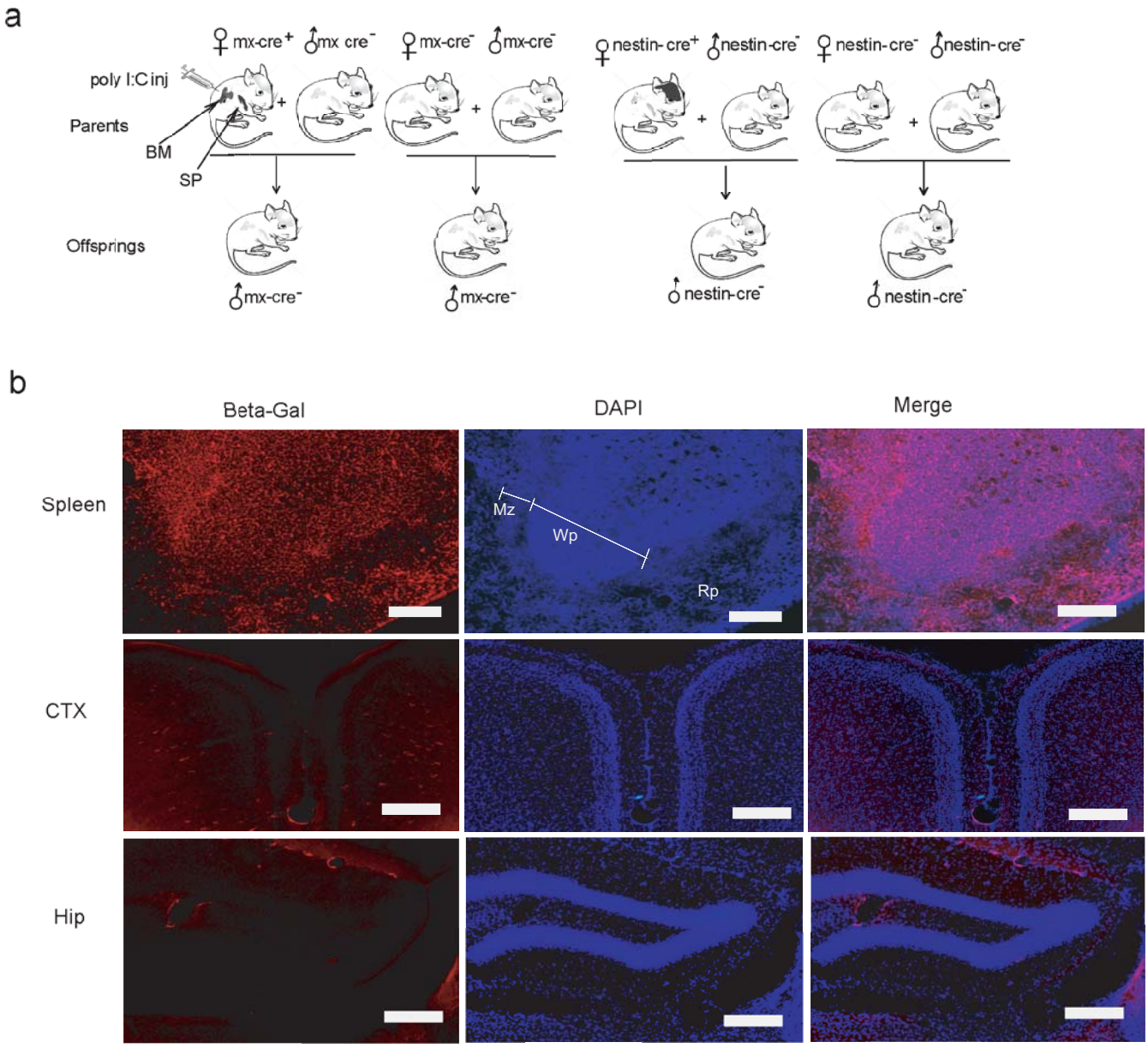
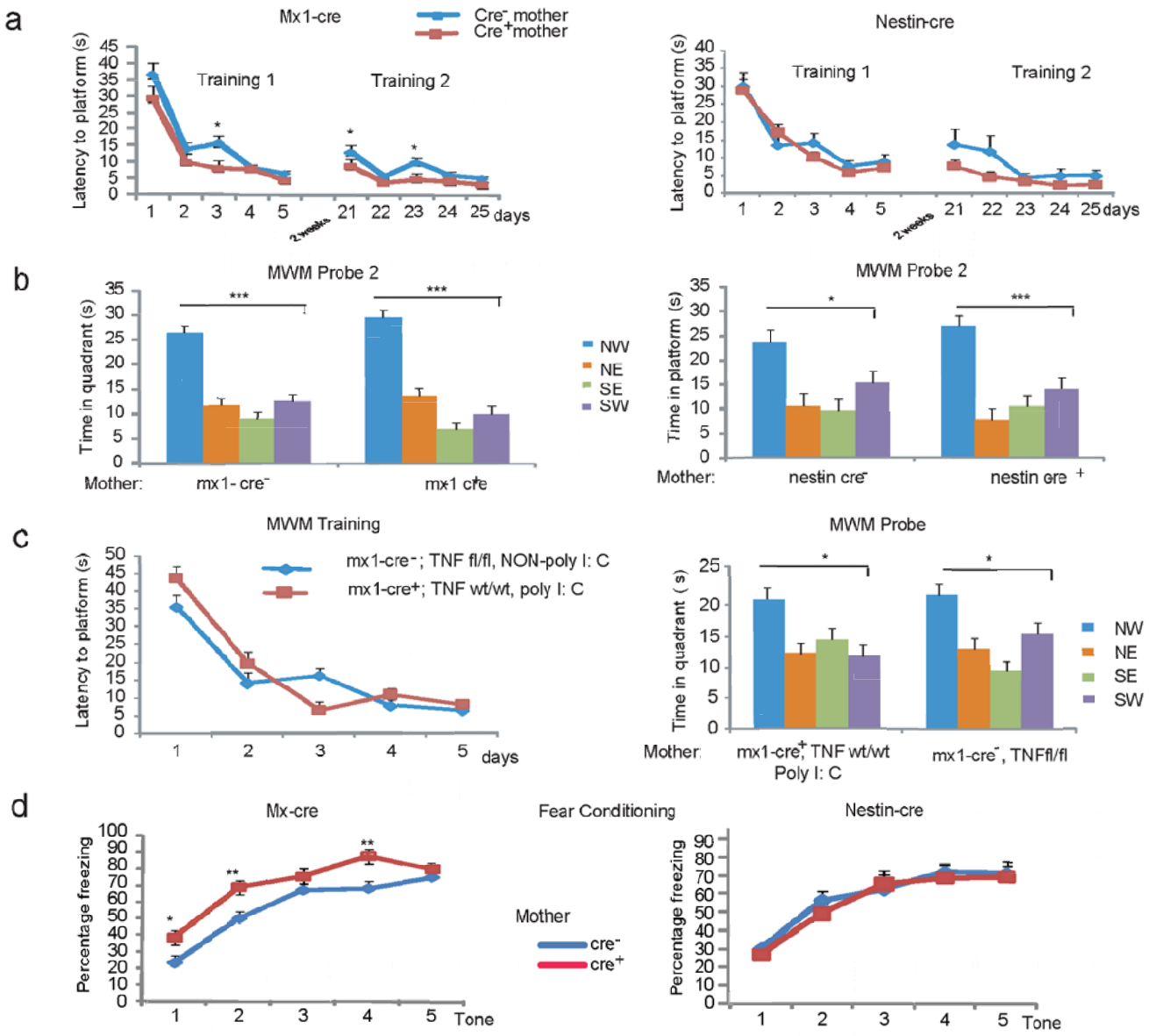


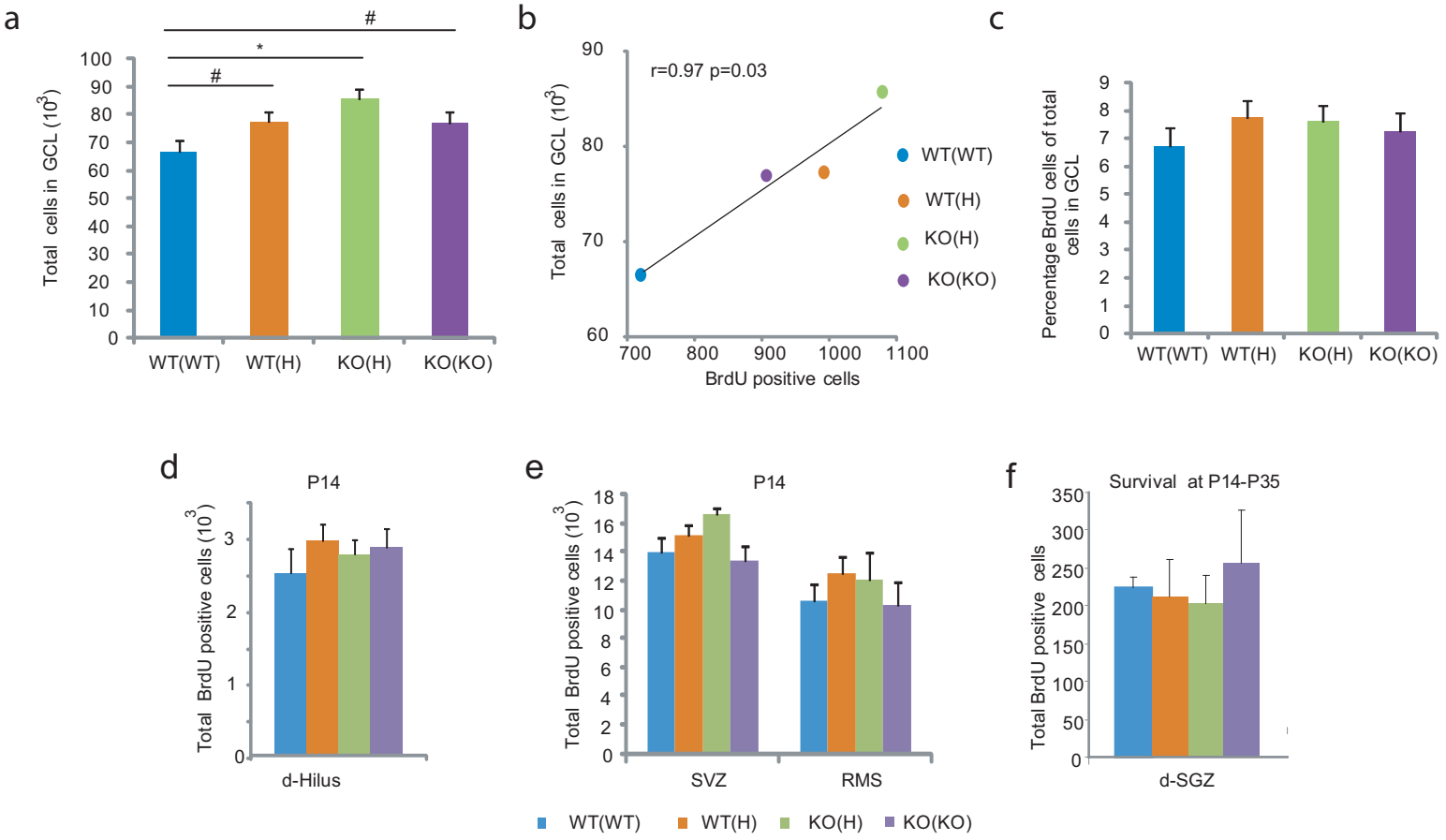
Supplementary Figure 1. Effect of maternal TNF deficit on offspring cognitive functions. **(a)** Breeding strategy to generate WT and $TNF^{-/-}$ mice born to and raised by $TNF^{+/+}$, $TNF^{+/-}$, $TNF^{-/-}$ parents. **(b)** Design of the MWM experiment to test the spatial reference memory of offspring of TNF mutant parents. **(c)** Second probe trial following extensive training in MWM. While a second round of training improved the recall of the platform location of WT(WT) mice in probe trial 2, offspring of mutant parents still spent more time in the NW target quadrant (quadrant $F_{3,148}=172.69$, $P<10^{-5}$; quadrant x group, $F_{9,148}=2.84$, $P=0.004$; LSD posthoc; N= 8,11,12,13/group). **(d)** Design of the contextual and cued fear conditioning experiments with the offspring of TNF mutant parents.



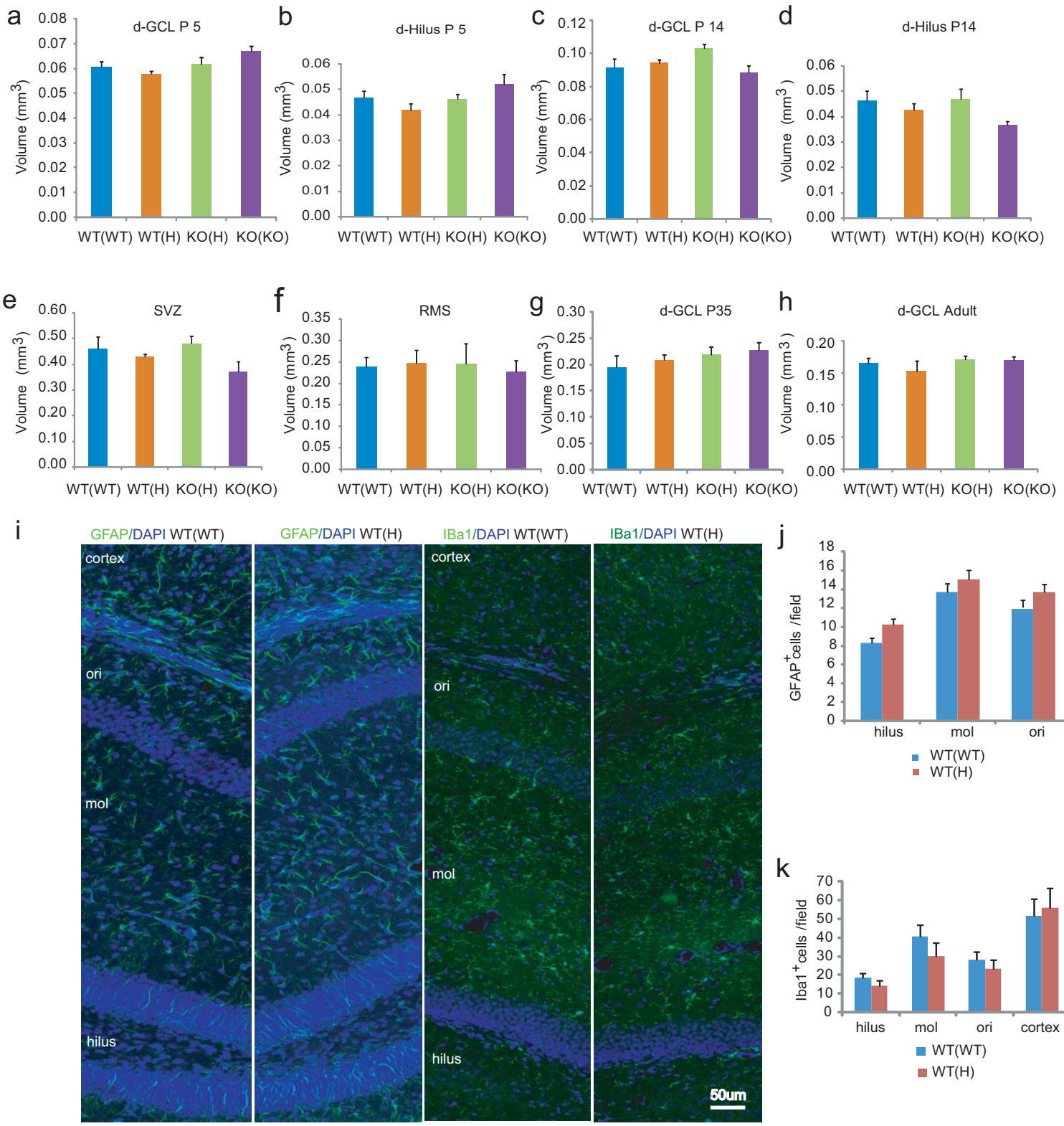
Supplementary Figure 2. Hematopoietic system specific inactivation of the *TNF* gene in the mother. **(a)** Generation of male WT offspring of mothers with hematopoietic (mx-cre) or brain (nestin-cre) specific *TNF* deletion. SP=spleen, BM=bone marrow. **(b)** Hematopoietic system specificity of mx-cre mediated recombination demonstrated by the cre-reporter *Gt(ROSA)26Sor*^{tm1Sor}/J strain. LacZ immunostaining in the macrophage rich white pulp (WP), including the marginal zone (MZ), and in the red blood cells and macrophages rich red pulp (RP) in mx-Cre/ *Gt(ROSA)26Sor*^{tm1Sor}/J mice indicates recombination. No apparent immunoreactivity in cortex (Ctx) and hippocampus (Hip). Bar represents 50 μm.



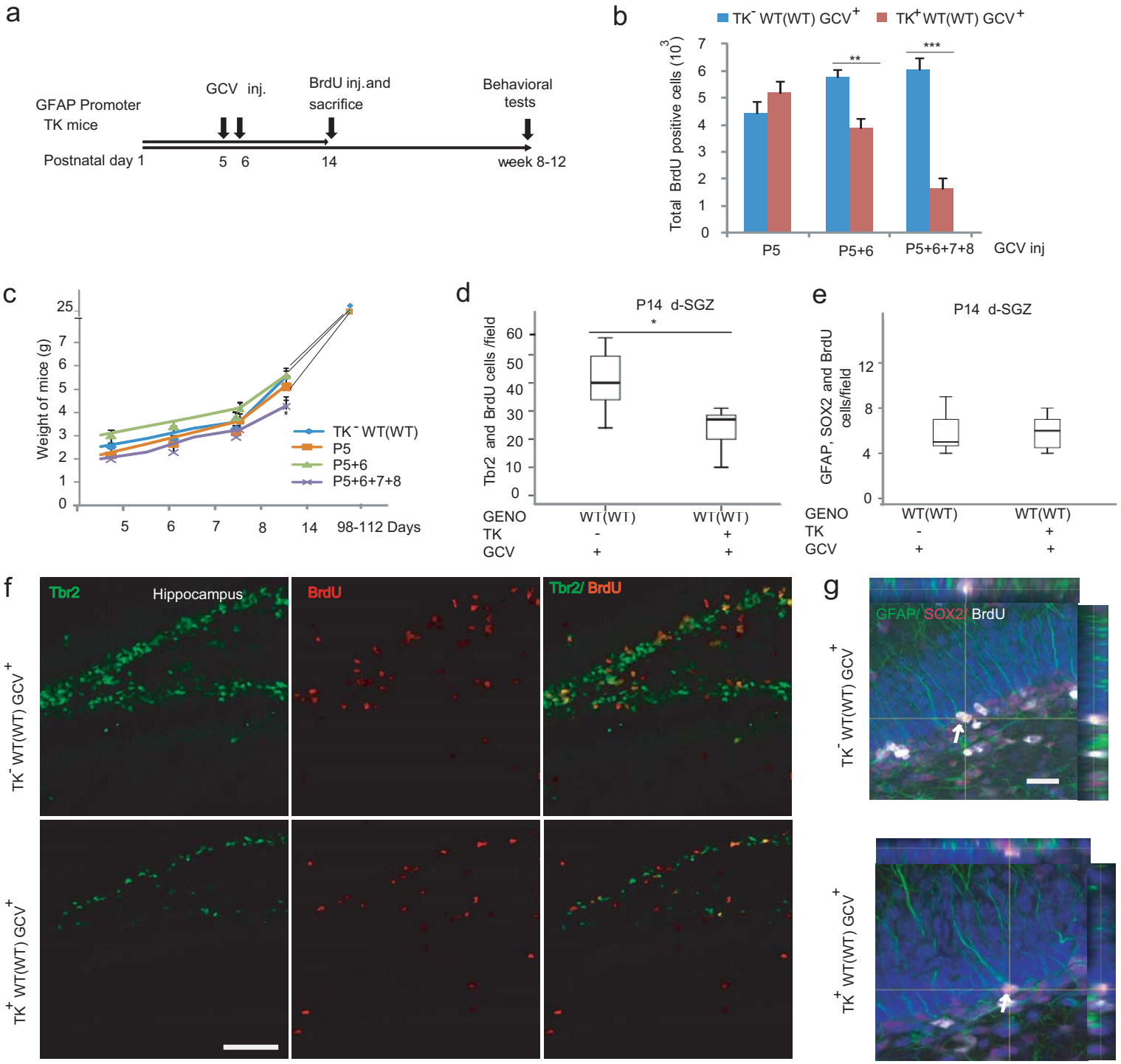
Supplementary Figure 3. Performance in the MWM and conditional fear test of the offspring born to conditional $TNF^{-/-}$ mothers. **(a)** All offspring improved in finding the platform during the first (Repeated measures of ANOVA: $F_{4,244}$=45.10, $P<10^{-5}$, N=7,9/group and $F_{4,324}$=28.63, $P<10^{-5}$, N=9,13 for mx-cre and nestin-cre, respectively) and second training periods ($F_{4,240}$=6.71, $P<10^{-5}$and $F_{4,128}$=6.02, $P<10^{-5}$). There was also a genotype effect in the mx-cre ($F_{1,62}$=6.23, P=0.015 and $F_{1,60}$=9.15, P=0.004 for the first and second training trial, respectively; LSD posthoc *p<0.05) but not in the nestin-cre group comparison. **(b)** Performance of the offspring born to mx-cre and nestin-cre mothers in probe trial 2 of MWM. A second period of 5 training sessions increased spatial memory in all groups in probe trial 2, eliminating the difference caused by the maternal hematopoietic deletion of TNF-±, seen in probe trial 1 (ANOVA: effect of quadrant for mx-cre and nestin-cre; $F_{3,56}$=68.35, $P<10^{-5}$, N=7,9 and $F_{3,28}$=19.77, $P<10^{-5}$, N=9,13; no group x location interaction). **(c)** PolyIC and the mx transgene do not confound the behavior in MWM. In WT mice, e.g. in the absence of floxed TNF alleles, poly IC and the mx-cre transgene in mothers have no effect on offspring learning and memory. The behavior of the offspring of mx-cre^{+} polyIC; $TNF^{wt/wt}$ mothers is indistinguishable from the behavior of the control offspring of mx-cre^{-}; $TNF^{flox/flox}$ mothers (control data are same as shown in Fig. 2c). **(d)** A temporal increase in freezing in the offspring of mx-cre^{+} but not nestin-cre^{+} mothers during tone-shock pairing (Repeated measures ANOVA; session effect $F_{4,96}$=53.34, $P<10^{-5}$, N=13,13/group and $F_{4,60}$=23.80, $P<10^{-5}$, N=7,10/group for mx-cre and nestin-cre, respectively; group effect $F_{1,24}$=11.49, P=0.002 and $F_{1,15}$=0.26, P=0.6; LSD posthoc. *p<0.05, **<0.005). The difference between the groups disappeared at the end of the training.



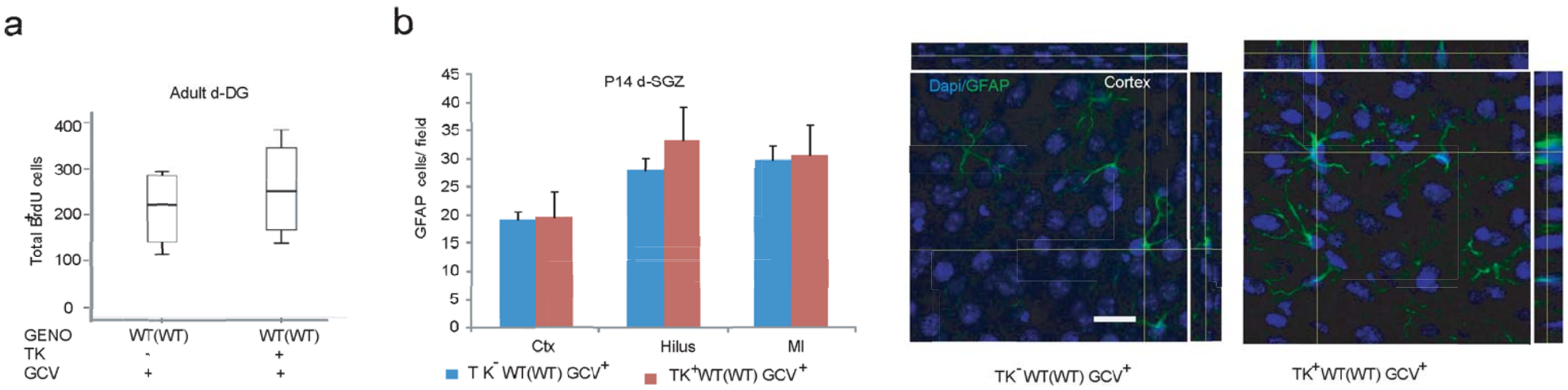
Supplementary Figure 4. Proliferation in the developing DG in the offspring of TNF mutant mothers. **(a)** Total number of cells ($F_{3,14}$=3.95, *P*=0.03, N=4,5,5,5; *p<0.05, #p=0.05-0.1.), **(b)** correlation between total and BrdU positive cells, and **(c)** the fraction of BrdU positive cells in percent of all cells ($F_{3,14}$=0.52, *P*=0.67, N=4,5,5,5.) in the GCL at P14. **(d,e)** No change in proliferation in the d-hilus, SVZ, and RMS at P14 (N=4,5,5,5). **(f)** No significant effect of the maternal genotype on the number of surviving BrdU positive cells in the DG, 3 weeks after pulse labeling with BrdU at P14 (p>0.05, N=5,5,5,6).



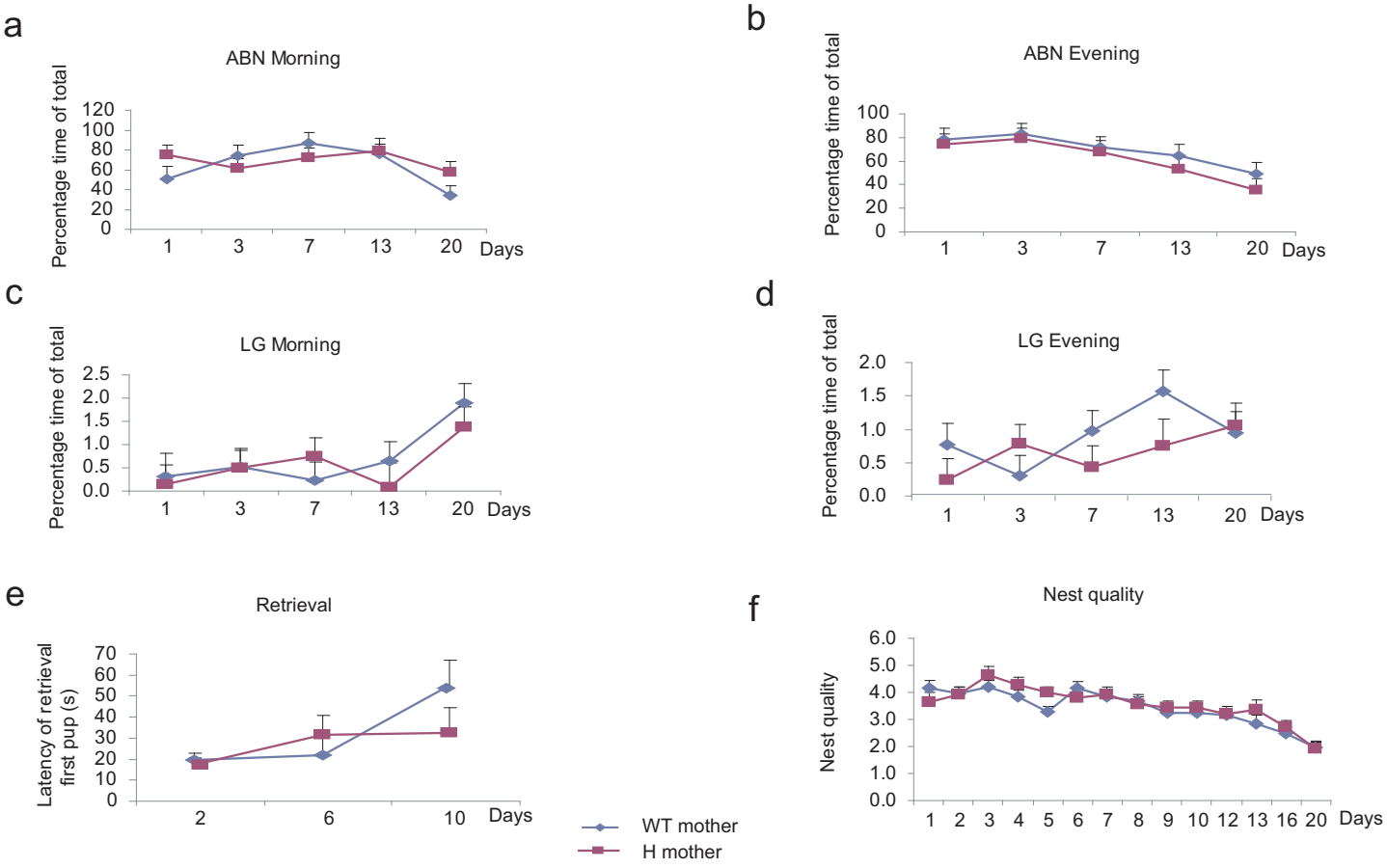
Supplementary Figure 5. Characteristics of the developing DG in the offspring of TNF mutant mothers. **(a-h)** No volumetric changes in neurogenic areas in the offspring of TNF deficient mothers. Volume of GCL (a, c, g, h), hilus (b, d), SVZ (e), RMS (f) at P5 (a and b), P14 (c-f) and adolescent/adult age (g,h). The maternal effect has no significant impact on the volume at any region and age (N=5 per group).



Supplementary Figure 6. Constraining postnatal hippocampal proliferation by GCV. **(a,b)** Repeated GCV administration results in a cumulative dose dependent reduction in P14 proliferation (ANOVA and LSD postoc test: $F_{6,112}=6.85$, $P<10^{-3}$; N=4,4,6,8,4,5/per group; **p<0.005, ***p<0.0005). GCV administered at P5+6 is sufficient to reduce proliferation by approximately 30%. **(c)** Postnatal administration of GCV did not alter weigh gain (N=7,7,4,7/grop). **(d,f)** GCV (P5+6) reduces ANP proliferation in P14 DG (*t* test, p< 0.05, N=5/group). Box-whisker plots represent the first three quartiles (25%, median and 75%) and values 1.5× the interquartile range below the first quartile (lower horizontal line) and above the third quartile (upper horizontal line).. Representative micrographs showing $BrdU^+/Tbr2^+$ ANPs in the DG of GCV treated TK^+ and TK^- mice. Bar=50µm. **(e,g)** GCV (P5+6) does not alter QNP proliferation in P14 DG. Representative confocal images with orthogonal views of SGZ showing BrdU-labeled Sox-2 and GFAP positive QNPs in the DG of GCV treated TK^+ and TK^- mice. Arrows indicate triple stained QNPs. Bar=20µm.



Supplementary Figure 7. No long term consequence of GCV administration on proliferation and reactive gliosis. **(a)** GCV (P5+6) resulted in no change in adult DG proliferation. **(b)** GCV (P5+6) resulted in no change in GFAP positive cell number in the neocortex (Ctx), hilus and molecular layer (ML) of the DG in P14 animals. Representative confocal images with orthogonal views of cortex showing no obvious loss of GFAP positive cells in 2x GCV injected mice at P14. Bar=20μm.



Supplementary Figure 8. TNF deficiency does not alter maternal behavior. **(a,b)** Arched-back nursing (ABN) of H mothers during the light and dark periods, respectively. Data show an effect of time ($F_{4,73}$=3.13, P=0.02 and $F_{4,78}$=4.58, P=0.02) but no group effect (n=7). **(c,d)** Licking/grooming (LG) of H mothers during the light and dark periods, respectively. Effect of time only in the dark period ($F_{4,77}$=34, p=0.013) but no group effect. **(e)** No difference between WT and H mothers in the latency to pup retrieval. Effect of time ($F_{2,24}$= 4.87 P=0.017). **(f)** No difference in nest quality between WT and H mothers.

Supplementary Table 1. Gene ontology analysis of genes differentially expressed in adult WT(H) vs. WT(WT) DG granule cells identified the related functional categories of "Neurotransmission at the synapse" (p=6.48E-6; Ingenuity Pathway Analysis) and "Neurotransmission" (p=1.91E-4).

Neurotransmission at the synapse

ID	Genes		Control FPKM	Sample FPKM	FC log2 S/C	TestStat	Pval	Qval
ENSMUSG00000021919	Chat	choline O-acetyltransferase	0.0488929	0.703504	3.84686	-5.42292	5.86E-08	0.000006
ENSMUSG00000032303	Chrna3	cholinergic receptor, nicotinic, alpha 3 (neuronal)	0.524488	3.7488	2.83745	-7.63431	2.26E-14	0
ENSMUSG00000031492	Chrnb3	cholinergic receptor, nicotinic, beta 3 (neuronal)	0.0858336	1.40069	4.02845	-8.47339	0	0
ENSMUSG00000035200	Chnrb4	cholinergic receptor, nicotinic, beta 4 (neuronal)	0.150738	2.22328	3.88258	-8.55866	0	0
ENSMUSG00000023945	Slc5a7	solute carrier family 5 (choline transporter), member 7	0.220928	1.70128	2.94497	-7.24775	4.24E-13	0
ENSMUSG00000023064	Sncg	synuclein, gamma (breast cancer-specific protein 1)	3.92477	11.5267	1.55429	-4.70875	2.49E-06	0.000172

Neurotransmission

ID	Genes in dataset (in addition to synaptic genes above)		Control FPKM	Sample FPKM	FC log2 S/C	TestStat	Pval	Qval
ENSMUSG00000031654	Cbln1	cerebellin 1 precursor	1.39842	5.13847	1.87754	-6.07971	1.20E-09	0
ENSMUSG00000024907	Gal	galanin	3.43954	0.544129	-2.66019	5.30069	1.15E-07	0.000012
ENSMUSG00000028778	Hcrtr1	hypocretin	1.71439	0.644644	-1.41112	4.73697	2.17E-06	0.000151
ENSMUSG00000033774	Npbwr1	neuropeptides B/W receptor 1	0.363859	0.0770603	-2.23932	3.83279	0.0001267	0.004745
ENSMUSG00000074006	Omp	olfactory marker protein	0.458313	0.0547013	-3.06669	4.21555	2.49E-05	0.001196
ENSMUSG00000004366	Sst	somatostatin	183.118	71.3544	-1.3597	6.35952	2.02E-10	0
ENSMUSG00000035431	Sstr1	somatostatin receptor 1	7.75869	2.04115	-1.92643	7.70041	1.35E-14	0

Supplementary Table 2. Cytokine levels in the P10 offspring of WT and TNF mutant mothers

	WT(WT)			WT(H)			KO(KO)		
	Mean		SE	Mean		SE	Mean		SE
KC/GRO (ng/ml)	0.062667	±	0.009984	0.084	±	0.009455	0.0515	±	0.01158
FGF-9 (ng/ml)	3.9	±	0.374759	3.766667	±	0.34312	4.05	±	0.420235
IP-10 (pg/ml)	79.33333	±	17.17104	161	±	25.0408	133	±	30.66859
Lymphotactin (pg/ml)	138	±	13.8532	146.3333	±	13.11364	117	±	16.06087
MIP-1 beta (pg/ml)	166.3333	±	16.65066	208.6667	±	18.93471	137	±	23.19019
MIP-2 (pg/ml)	20.33333	±	3.955306	21	±	4.075673	16	±	4.99166
MCP-1 (pg/ml)	52.33333	±	9.148163	70.33333	±	14.59959	65	±	17.88078
MCP-3 (pg/ml)	150.6667	±	14.59833	182	±	24.94995	161	±	30.55732
MCP-5 (pg/ml)	16	±	1.725624	21.33333	±	2.420017	23	±	2.963903
SCF (pg/ml)	1333.333	±	118	1260	±	116.5416	1340	±	142.7337
Eotaxin (pg/ml)	366	±	87.0129	759.6667	±	210.3114	705	±	257.5778
LIF (pg/ml)	1590	±	129	1846.667	±	141.998	1620	±	173.9113
M-CSF-1 (ng/ml)	13.33333	±	1.054093	13	±	0.964653	12	±	1.181454
MDC (pg/ml)	1170.667	±	164	1593.333	±	200.1811	1365	±	245.1708
MIP-1 alpha (ng/ml)	7.6	±	0.563718	8.533333	±	0.528975	8.1	±	0.64786
MIP-3 beta (ng/ml)	3.9	±	0.229734	5.266667	±	0.40546	4.45	±	0.496586

Two way ANOVA; group: F2-26=0.207, P=0.81; group x cytokine: F10-26=0.085, P=1