

Supplementary Materials for  
**The E3 ubiquitin ligase Idol controls brain LDL receptor expression,  
ApoE clearance, and A $\beta$  amyloidosis**

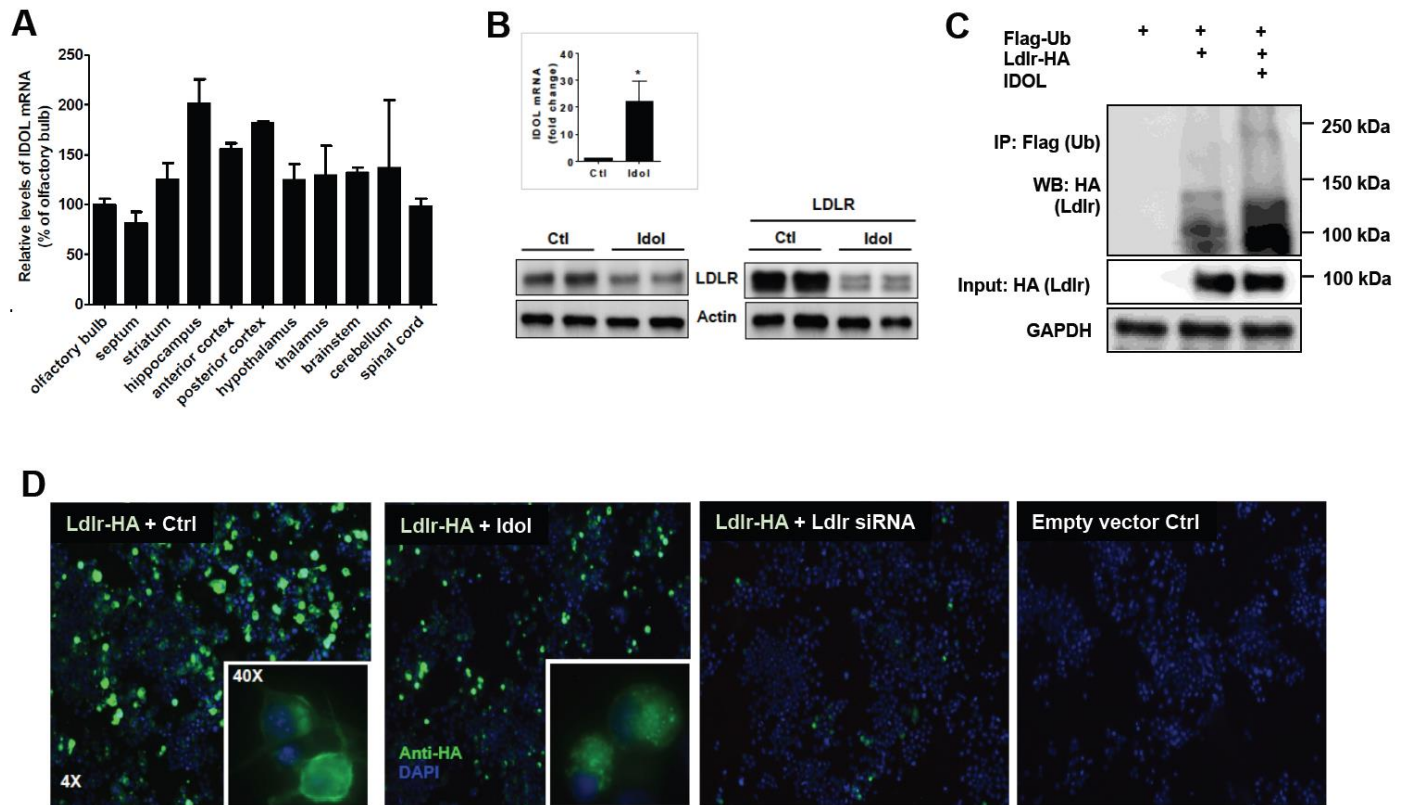
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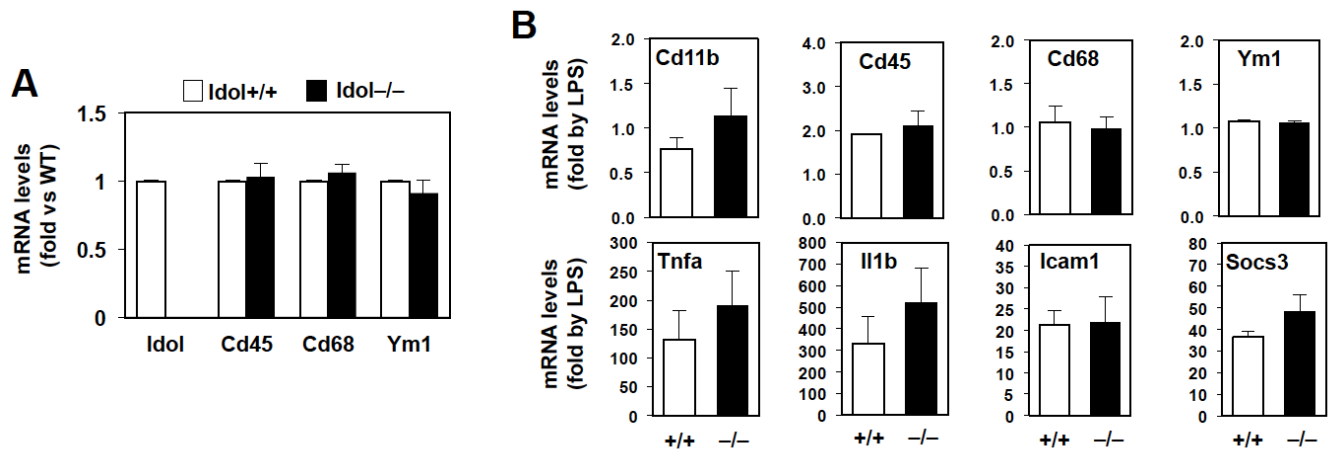
**The PDF file includes:**

- Fig. S1. Idol decreases LDLR protein and promotes LDLR ubiquitination in mouse brain cells.
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- Fig. S3. Idol inhibition promotes the clearance of A $\beta$  by microglia.
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- Table S6. Quantification of Iba<sup>+</sup> cells in Fig. 4D.

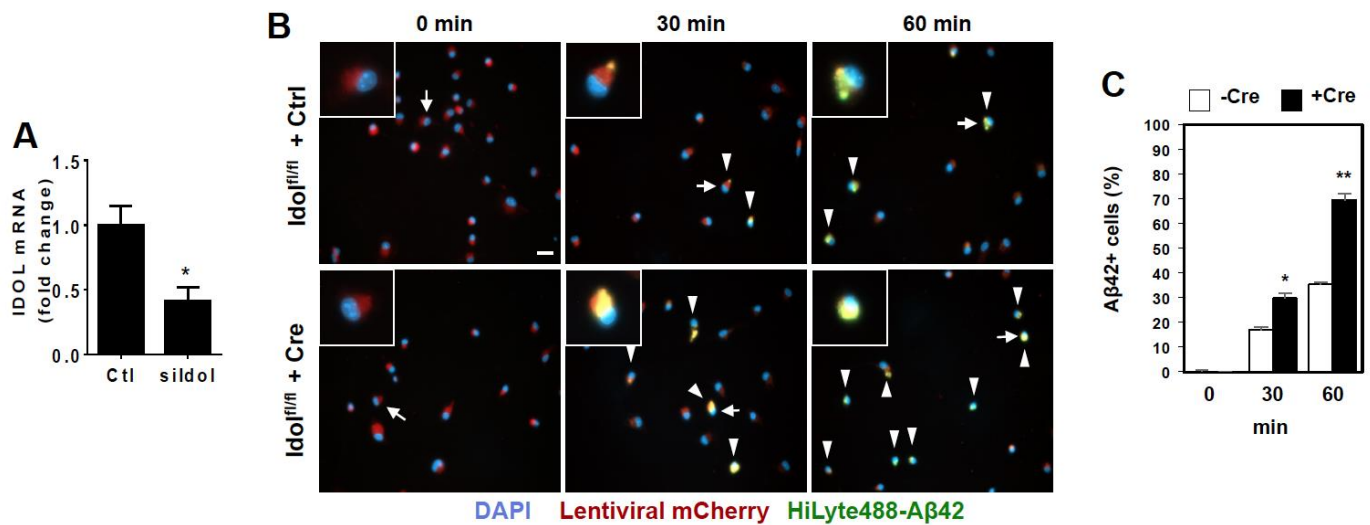


**Fig. S1. Idol decreases LDLR protein and promotes LDLR ubiquitination in mouse brain cells.**

(A) Real-time PCR analysis of Idol expression in different compartments of the central nervous system, isolated from 3.5 month-old C57BL6J mice. Data are normalized to olfactory bulb. Error bars represent SEM. N = 3. (B) Real-time PCR analysis of Idol expression levels and immunoblot analysis of LDLR protein levels after Idol knockdown. Idol expression plasmid (Idol) or control plasmid (Ctl) was transfected to Neuro2a cells with or without LDLR expression plasmid (LDLR). Error bars represent SEM. \*,  $p < 0.05$  by Student's t-test. (C) Immunoblot analysis of LDLR protein level and ubiquitination in the presence of Idol. Flag-tagged ubiquitin (Ub) and HA-tagged LDLR were expressed in Neuro2a cells with or without Idol co-expression. 24 h post-transfection, cell were incubated with 400 nM bafilomycin A1 for 24 h. Cell lysates were immunoprecipitated with Flag antibody and immunoblotted with HA antibody. (D) Representative micrographs showing immunofluorescence staining of Neuro2a cells co-transfected with LDLR-HA expression plasmid and either control or Idol expression plasmid. Green color indicates LDLR-HA stained by anti-HA antibody.



**Fig. S2. Loss of Idol does not directly affect inflammatory responses.** (A) Real-time PCR analysis of *Idol* and microglial marker gene expression in primary microglia isolated from *Idol*<sup>+/+</sup> or *Idol*<sup>-/-</sup> mice. Cells were cultured in DMEM containing 10% FBS prior to harvest. Data are normalized to wild-type control. Error bars represent SEM. (B) Real-time PCR analysis of neuroinflammatory gene expression in response to LPS in primary microglia isolated from *Idol*<sup>+/+</sup> or *Idol*<sup>-/-</sup> mice. Cells were treated with serum-free DMEM containing 100 ng/ml LPS for 4 h prior to harvest. mRNA levels are presented as fold induction by LPS vs. vehicle control. Error bars represent SEM.



**Fig. S3. Idol inhibition promotes the clearance of A $\beta$  by microglia.** (A) Real-time PCR analysis of Idol in Neuro2a cells transfected with Idol siRNA (siIdol) or scrambled negative control (Ctl), related to Figure 6. Data are from RNA harvested at 48 h post-transfection and are normalized to negative control. Error bars represent SEM. \*,  $p < 0.05$  by Student's t-test. (B) Representative micrographs showing uptake of HiLyte488 fluorescently labeled aggregated A $\beta_{42}$  in primary microglia from *Idol<sup>fl/fl</sup>* mice treated *in vitro* with control mCherry-expressing or Cre/mCherry-expressing lentiviral vector. DAPI (blue); Lentiviral mCherry (red); A $\beta_{42}$  (green). Arrowheads denote A $\beta_{42}$ -positive cells. Arrows denote representative cells shown in the magnified field. Scale bar, 20  $\mu$ m. (C) Quantification of results shown in (B). Error bars represent SEM. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$  by Student's t-test.

**Table S1. Data for A $\beta$ 40 shown in Fig. 2A.**

<b>A<math>\beta</math>40 (pg/mg tissue) ELISA data for Fig. 2A</b>			
<b>Male</b>			
<b>soluble</b>		<b>insoluble</b>	
<b>WT</b>	<b>KO</b>	<b>WT</b>	<b>KO</b>
2.344073	1.796427	1989.072	846.0812
2.638793	1.539898	1638.396	433.6560
3.500615	1.691221	2020.978	346.9319
4.366740	1.528893	2801.148	871.2848
2.045750	1.593942	2526.885	797.9648
3.236280		2366.212	
<b>Female</b>			
<b>soluble</b>		<b>insoluble</b>	
<b>WT</b>	<b>KO</b>	<b>WT</b>	<b>KO</b>
4.502602	6.021950	2746.788	2550.714
5.722557	2.156720	3202.000	2272.099
6.390170	3.135278	2991.323	2082.000
6.345493	3.352174	3232.591	2847.890
8.836700	3.239042	3237.861	2707.436
6.542100	3.542100	3805.518	2114.403

**Table S2. Data for A $\beta$ 42 shown in Fig. 2A.**

<b>A<math>\beta</math>42 (pg/mg tissue) ELISA data for Fig. 2A</b>					
<b>Male</b>					
<b>soluble</b>			<b>insoluble</b>		
<b>WT</b>	<b>het</b>	<b>KO</b>	<b>WT</b>	<b>het</b>	<b>KO</b>
12.63166	12.123290	6.201966	10294.18	10436.830	5749.680
13.08260	8.336532	3.293293	13319.18	8983.113	3804.303
14.87140	15.271710	3.511020	11783.94	6122.472	3383.310
19.93512	9.101973	5.563617	12339.33	8270.967	6338.289
28.30721	10.238130	5.123914	14421.38	12513.560	5902.533
17.43271	11.348670		14061.08	8910.378	
			12768.13		
<b>Female</b>					
<b>soluble</b>			<b>insoluble</b>		
<b>WT</b>	<b>het</b>	<b>KO</b>	<b>WT</b>	<b>het</b>	<b>KO</b>
25.32183	20.97352	8.803017	14818.67	14564.04	12854.29
17.99380	21.83572	11.369410	15370.41	17460.52	11056.42
23.98040	21.02117	13.860700	15877.29	13108.66	15528.55
30.86358	17.60413	16.977340	17702.61	10668.22	14206.94
49.99380	23.74065	16.977340	15867.00	11161.34	14217.97
27.12650	22.56790	12.163700	18703.69	16881.73	11757.61

**Table S3. Quantification of A $\beta$  plaque load in Fig. 2E.**

<b>Data values for quantification of A<math>\beta</math> plaque load found in Fig. 2E</b>				
Sample	Cortex		Hippocampus	
	Particle area	Particle % area	Particle area	Particle % area
Male WT 1	6.748	2.759	3.001	0.968
Male WT 2	5.550	2.426	1.395	0.510
Male WT 3	2.135	0.905	3.041	1.016
Male WT 4	2.625	1.172	2.285	0.715
Male WT 5	2.308	0.967	2.504	0.877
Male WT 6	5.057	2.183	2.595	0.839
Male het 1	2.818	1.239	2.978	1.040
Male het 2	2.622	1.095	2.554	0.933
Male het 3	2.118	0.907	2.769	0.968
Male het 4	2.096	0.846	2.091	0.704
Male het 5	2.007	0.925	1.409	0.526
Male het 6	2.648	1.189	2.678	0.906
Male KO 1	2.223	0.923	1.946	0.687
Male KO 2	1.562	0.655	0.638	0.217
Male KO 3	2.260	1.039	1.423	0.517
Male KO 4	2.314	0.849	0.414	0.145
Male KO 5	1.989	0.761	0.724	0.231
Male KO 6	2.185	0.924	1.830	0.563
Female WT 1	4.816	2.004	3.188	1.171
Female WT 2	7.827	3.026	4.940	1.615
Female WT 3	7.547	2.859	3.387	1.172
Female WT 4	4.688	1.978	3.047	1.065
Female WT 5	8.561	3.572	2.428	0.928
Female WT 6	6.674	2.829	4.506	1.648
Female het 1	9.708	4.024	3.737	1.371
Female het 2	3.919	1.764	4.562	1.436
Female het 3	6.020	2.371	2.197	1.026
Female het 4	5.148	2.242	3.661	1.258
Female het 5	2.961	1.091	2.875	1.029
Female het 6	5.187	2.143	3.388	1.140
Female KO 1	1.651	0.741	0.606	0.203
Female KO 2	2.282	1.076	1.086	0.381
Female KO 3	1.654	0.789	1.650	0.566
Female KO 4	2.687	1.173	0.939	0.323
Female KO 5	2.257	0.966	2.704	0.974
Female KO 6	3.157	1.457	3.674	1.283

**Table S4. Quantification of thioflavin S plaque load in Fig. 3B.**

<b>Data values for quantification of ThioS plaque load found in Fig. 3B</b>				
Sample	Cortex		Hippocampus	
	Particle area	Particle %area	Particle area	Particle %area
Male WT 1-1	0.594	0.300	0.895	0.261
Male WT 1-2	1.322	0.544	0.803	0.215
Male WT 2-1	1.169	0.577	0.939	0.332
Male WT 2-2	0.986	0.473	1.036	0.320
Male WT 3-1	1.746	0.831	1.330	0.455
Male WT 3-2	1.469	0.654	0.789	0.272
Male WT 4-1	1.072	0.463	1.247	0.432
Male WT 4-2	1.033	0.521	0.843	0.318
Male WT 5-1	0.867	0.426	0.449	0.177
Male WT 5-2	1.359	0.647	0.926	0.311
Male WT 6-1	0.999	0.438	0.900	0.264
Male WT 6-2	2.197	1.045	0.941	0.276
Male het 1-1	0.940	0.477	0.410	0.136
Male het 2-1	1.470	0.708	0.624	0.220
Male het 2-1	1.788	1.136	0.789	0.246
Male het 2-2	0.295	0.123	0.956	0.315
Male het 3-1	1.783	0.687	0.880	0.303
Male het 3-2	0.646	0.314	0.210	0.070
Male het 4-1	0.883	0.490	0.497	0.187
Male het 4-2	0.663	0.302	0.547	0.212
Male het 5-1	0.937	0.419	1.332	0.461
Male het 5-2	0.753	0.419	0.749	0.279
Male het 6-1	0.764	0.384	1.036	0.347
Male het 6-2	0.849	0.557	0.284	0.090
Male KO 1	0.846	0.442	0.550	0.190
Male KO 2	0.504	0.224	0.662	0.219
Male KO 3-1	0.383	0.274	0.641	0.212
Male KO 3-2	0.739	0.336	1.342	0.387
Male KO 4-1	0.446	0.219	0.671	0.218
Male KO 4-2	0.635	0.277	0.539	0.168
Male KO 5-1	0.556	0.249	0.435	0.131
Male KO 5-2	0.211	0.078	0.229	0.076
Male KO 6-1	0.590	0.284	1.130	0.377
Male KO 6-2	0.376	0.206	0.525	0.182
Female WT 1	1.788	0.711	0.937	0.282
Female WT 2	1.473	0.712	1.746	0.546



Female WT 3-1	0.995	0.608	1.025	0.386
Female WT 3-2	2.560	1.052	0.709	0.258
Female WT 4-1	0.699	0.432	1.008	0.386
Female WT 4-2	1.388	0.704	0.867	0.323
Female WT 5-1	1.011	0.419	0.860	0.296
Female WT 5-2	1.298	0.561	0.921	0.301
Female WT 6-1	2.151	1.050	0.479	0.177
Female WT 6-2	2.511	1.257	1.497	0.480
Female het 1	1.904	0.973	1.138	0.364
Female het 2	1.394	0.757	2.116	0.655
Female het 3	1.748	0.798	1.085	0.383
Female het 4	1.472	0.613	1.252	0.421
Female het 5-1	1.005	0.439	0.563	0.197
Female het 5-2	0.774	0.308	0.589	0.211
Female het 6-1	1.361	0.699	0.943	0.314
Female het 6-2	1.300	0.609	0.877	0.290
Female KO 1	1.566	0.704	0.979	0.337
Female KO 2	1.037	0.451	0.612	0.209
Female KO 3-1	0.978	0.521	0.714	0.239
Female KO 3-2	1.213	0.665	0.659	0.239
Female KO 4-1	0.123	0.069	0.635	0.224
Female KO 4-2	0.635	0.319	0.692	0.236
Female KO 5-1	0.541	0.294	0.566	0.196
Female KO 5-2	0.921	0.514	0.741	0.249
Female KO 6-1	0.833	0.358	0.535	0.181
Female KO 6-2	0.505	0.244	0.398	0.131

Replicates were included when samples from different hemispheres were available.

**Table S5. Quantification of CD45 load in Fig. 4B.**

<b>Data values for quantification of CD45 load found in Fig. 4B</b>						
% area	Male WT	Male het	Male KO	Female WT	Female het	Female KO
cortex 1	0.728	0.995	0.332	1.199	1.244	0.367
cortex 2	1.430	0.544	0.236	2.286	0.817	0.418
cortex 3	1.233	0.510	0.694	1.630	0.969	0.410
cortex 4	0.889	0.542	0.535	0.785	0.761	0.254
cortex 5	0.706	0.909	0.229	0.753	0.936	0.221
cortex 6	0.883	0.807	0.285	0.567	0.967	0.399
% area	Male WT	Male het	Male KO	Female WT	Female het	Female KO
hippocampus 1	1.213	0.455	0.436	0.859	1.300	0.294
hippocampus 2	1.170	1.124	0.175	1.018	1.120	0.246
hippocampus 3	0.551	0.547	0.400	1.016	0.941	0.523
hippocampus 4	0.785	0.762	0.596	1.264	0.705	0.427
hippocampus 5	0.719	0.690	0.601	1.104	1.092	0.765
hippocampus 6	0.581	0.734	0.283	0.915	0.751	0.641

**Table S6. Quantification of Iba<sup>+</sup> cells in Fig. 4D.**

<b>Data values for quantification of Iba1<sup>+</sup> cells/mm<sup>2</sup> found in Fig. 4D</b>												
# per .3125 mm <sup>2</sup>												
	hippocampal CA1						Cortex					
	Male			Female			Male			Female		
Pic #	WT	het	KO	WT	het	KO	WT	het	KO	WT	het	KO
1	65	49	46	70	60	54	31	30	24	43	34	33
2	64	70	49	74	71	44	34	33	38	41	34	21
3	60	52	40	62	52	45	38	31	33	37	28	38
4	56	52	55	52	51	39	29	28	31	42	35	30
5	69	30	40	76	58	39	37	17	36	44	43	26
6	78	43	40	65	67	53	30	26	24	37	35	25
7	54	50	46	90	64	83	32	28	27	47	43	40
8	65	62	41	81	72	50	44	37	23	50	31	28
9	52	51	37	70	70	39	45	46	17	52	58	25
10	63	56	33	73	72	66	49	38	25	36	33	36
11	58		41	92	70	31	33		22	70	34	30
12				84		56				56		36

Each picture was taken from a unique hemisphere.  
Two pictures were taken from one mouse when samples were available.  
Number of mice analyzed for each group was 6.