Leptin Enhances TH2 and ILC2 Responses in Allergic Airway Disease

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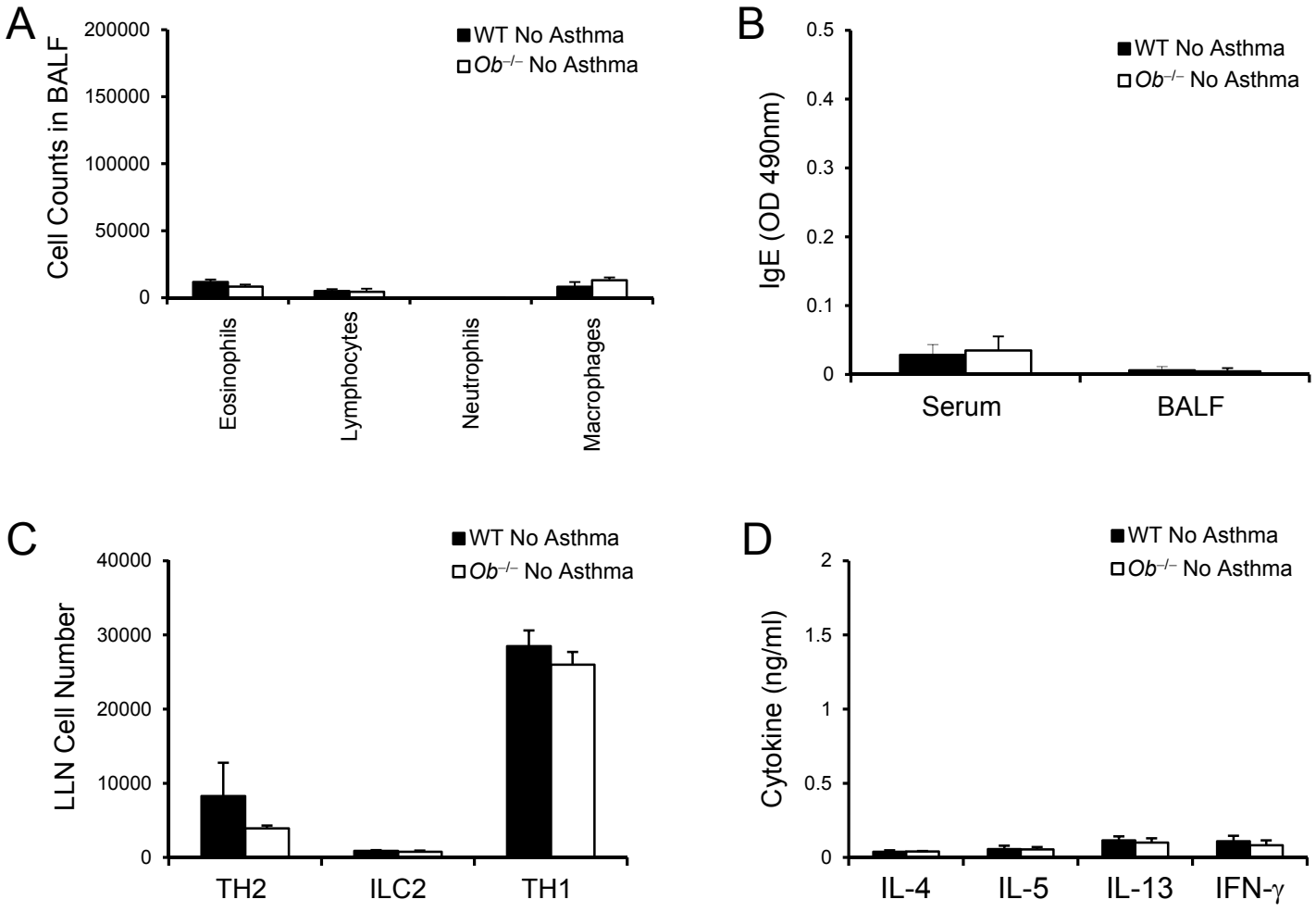

Fig. S1. Characterization of mice without allergic asthma. (A) Cellular profile of BALFs from unchallenged WT and *Ob*$^{-/-}$ mice. (B) ELISA of Ova-specific IgE in sera and BALFs. (C) Quantification of TH2 cells, ILC2s and TH1 cells in LLNs. (D) ELISA of cytokines in LLN cells after *ex vivo* recall with Ova (100 μg/ml). Values are means and SD. Student's *t*-test. Data represent two experiments (n = 3-4 per group).

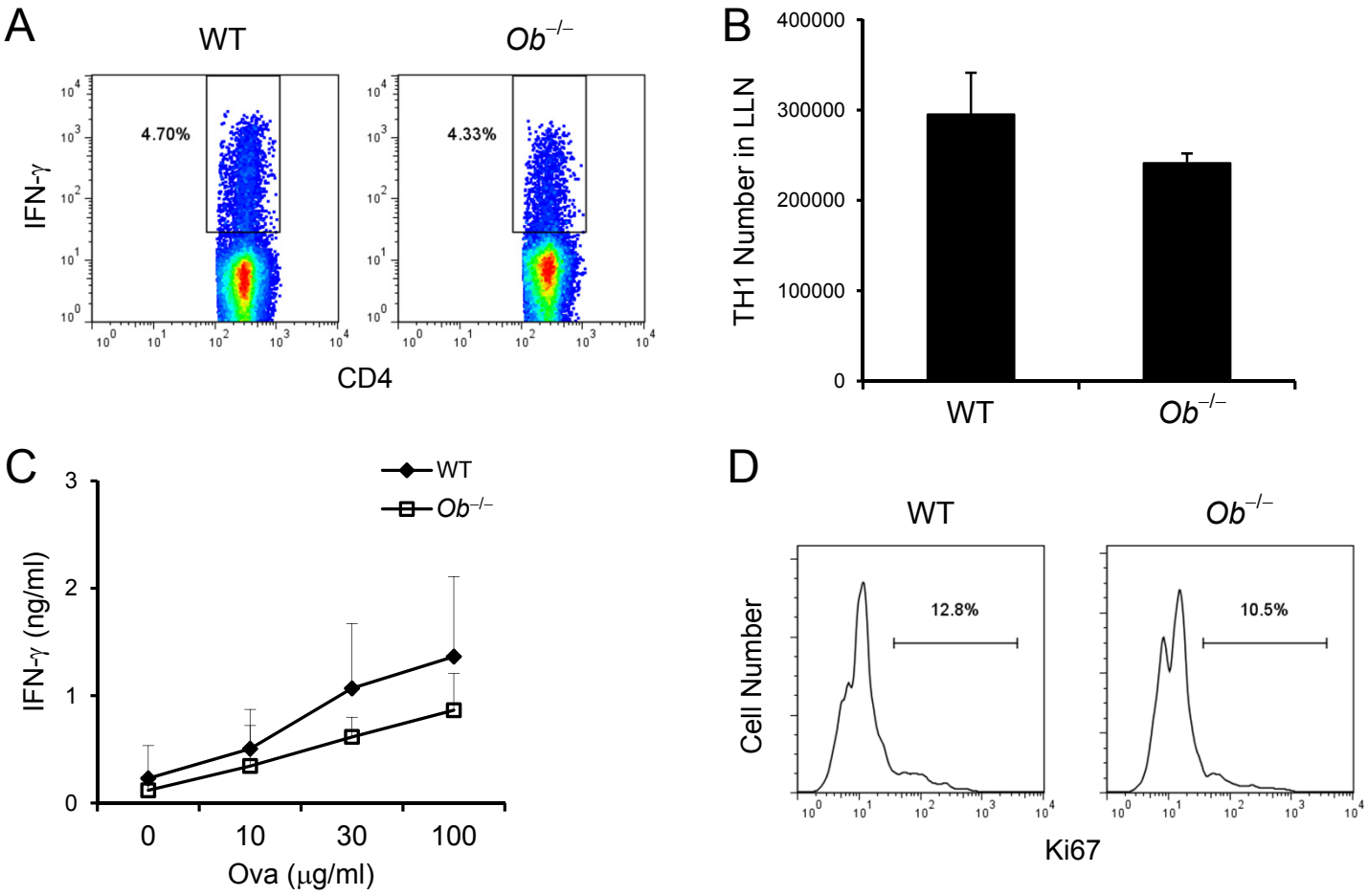

Fig. S2. Leptin-deficiency has minimal effects on TH1 responses in allergic asthma. (A) Intracellular stain of TH1 cells ($LIN^+CD4^+IFN\gamma^+$) in LLN cells from WT and *Ob*$^{-/-}$ mice after induction of experimental asthma. (B) Quantification of TH1 cells in LLNs. (C) ELISA of IFNγ expression by LLN cells after ex vivo recall with Ova at indicated concentrations. (D) Intracellular stain of Ki67 in LLN TH1 cells. (B, C) Values are means and SD. Student's *t*-test, no significance. Data represent two experiments (n = 5-6 per group).

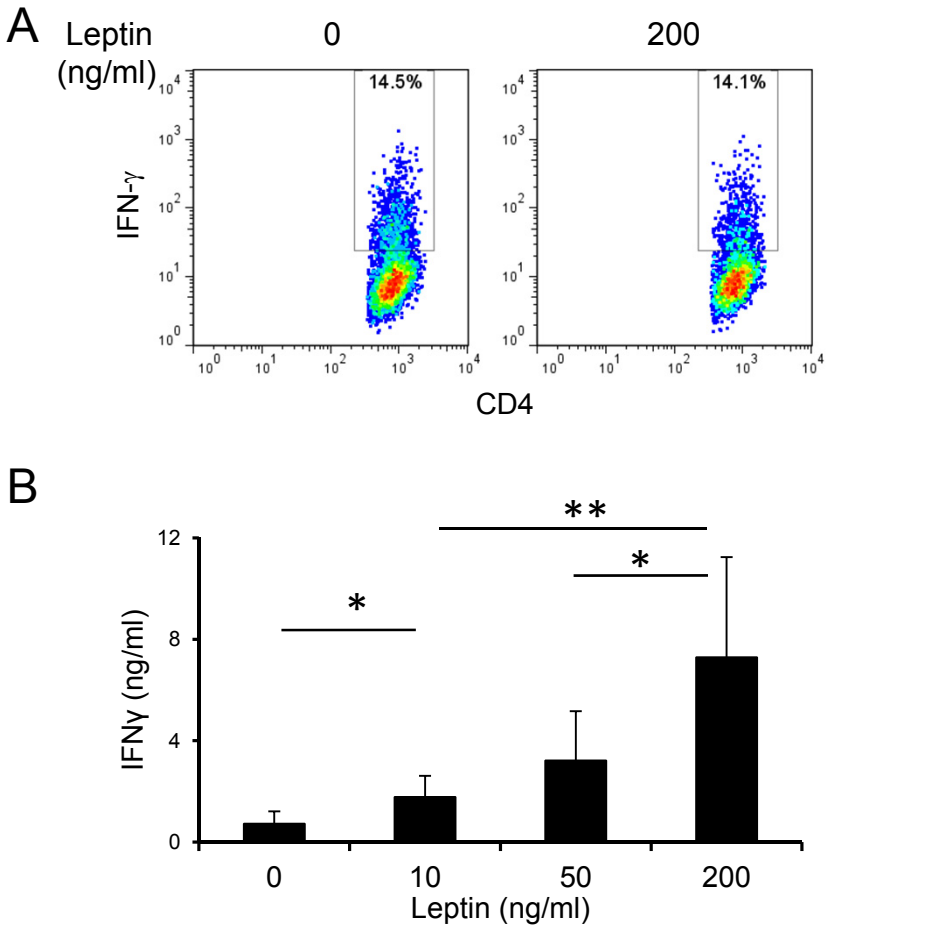

Fig. S3. Leptin promotes TH1 cell responses *in vitro*. (A) Intracellular stain of TH1 cells differentiated *in vitro* with or without leptin treatment. (B) ELISA of IFNγ expression by TH1 cells re-stimulated overnight with plate-bound anti-CD3 in the presence of leptin at indicated concentrations. Values are means and SD (n = 4 in each group). Student's *t*-test, * $p \leq 0.05$ and ** $p \leq 0.005$. Data represent three experiments.

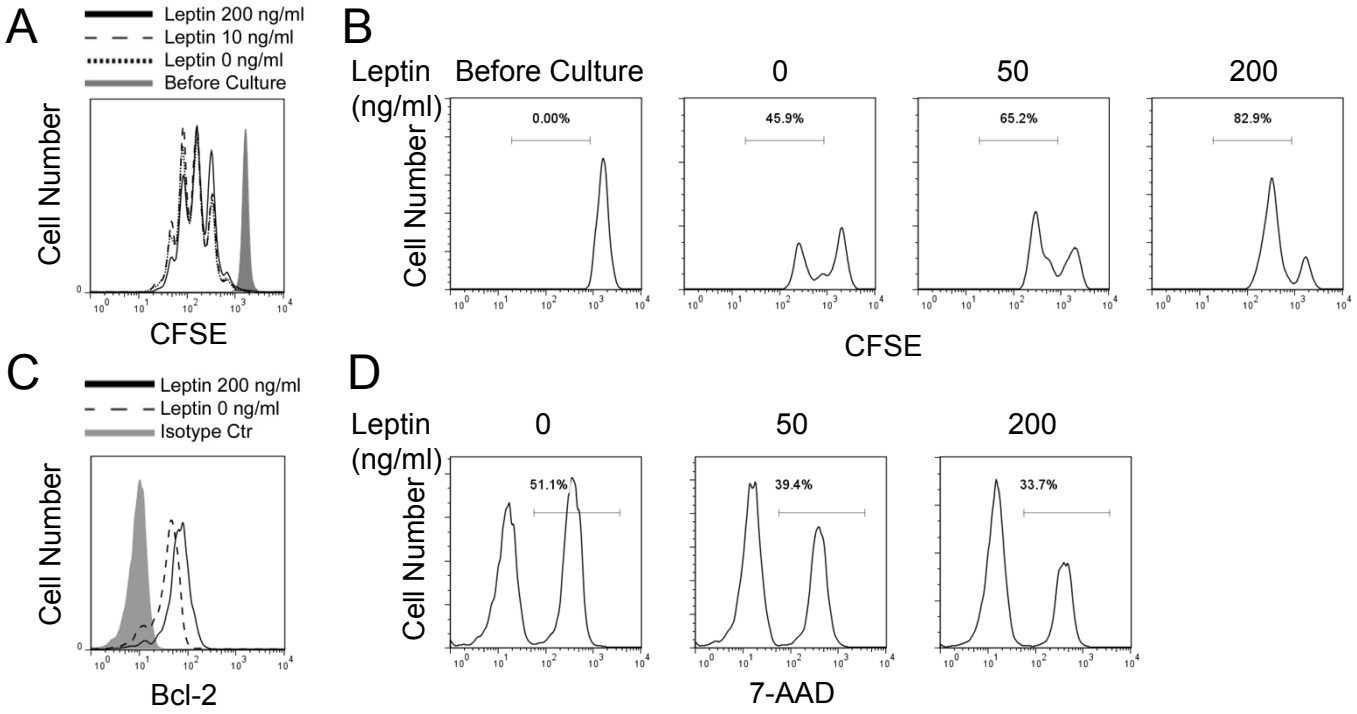

Fig. S4. Leptin promotes proliferation of TH1 cells and protects TH1 cells from activation induced cell death. (A-D) Cells were cultured in the absence or presence of leptin at indicated concentrations. (A) Flow cytometry of CFSE dilution in TH1 cells during *in vitro* differentiation. (B) Flow cytometry of CFSE dilution in TH1 cells during 1-day restimulation with plate-bound anti-CD3. (C) Intracellular stain of Bcl-2 in *in vitro* polarized TH1 cells. (D) Flow cytometry of activation induced cell death in TH1 cells after restimulation with plate-bound anti-CD3 for 24 h. Data represent three experiments.

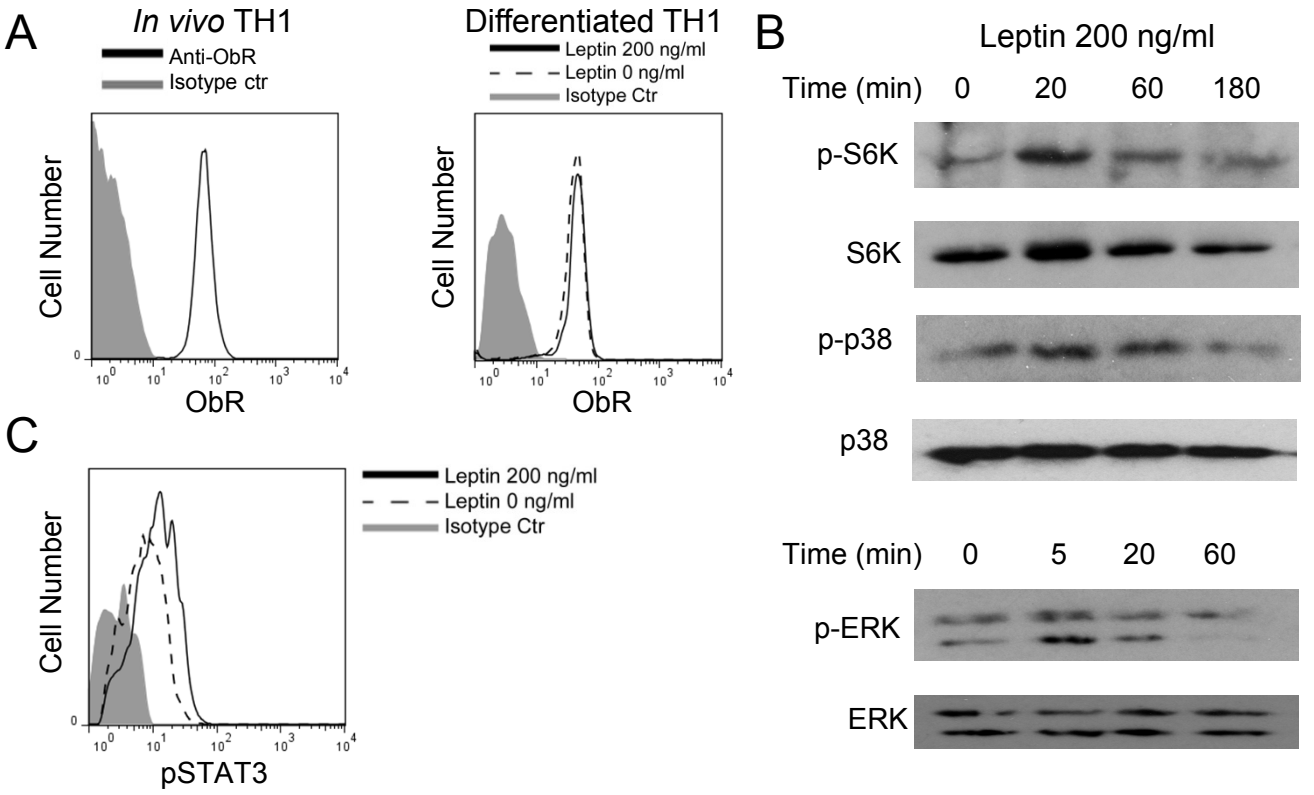

Fig. S5. Leptin activates STAT3, MAPK and mTOR signal pathways through targeting leptin receptor (ObR) in TH1 cells. (A) Flow cytometry of ObR expression on LLN TH1 cells generated in the asthmatic responses and on *in vitro* differentiated TH1 cells. (B) Immunoblot of phospho- and total S6K, p38 MAPK and ERK1/2 in TH1 cells generated *in vitro* treated with leptin for indicated times. (C) Intracellular stain of p-STAT3 in TH1 cells treated with leptin for 20 m. Data shown represent two experiments.