Supplementary information

Supplementary Figure 1

Representative flow cytometry dot plots illustrating gating strategy and post-sort purity of CD45.2+CD11b- and CD45.2+CD11b+ cell populations (a). Equal number of each cell population was sorted from C57BL/6 or K14.E7 mice after one day of DNCB treatment. Gating strategy and post-sort purity of different myeloid subsets based on the expression of F4/80 and Gr1 or Ly6C and Ly6G antigens (b, c).

Supplementary figure 2

Concentration effect of nor-NOHA on arginase activity in DNCB treated K14.E7 skin. K14.E7 mice (n=4) were injected i.p. with 500 µg, 100 µg of nor-NOHA or saline buffer (PBS) alone. The treatment was repeated two hours prior DNCB/vehicle application. Arginase activity in ear tissues after 24 hours of exposure to DNCB was determined by arginase assay. Means ± SEM, $*p<0.05$, ns= not significant.

Supplementary figure 3

Sensitized K14.E7 mice exhibit enhanced ear swelling response, arginase-1 mRNA and arginase activity after DNCB challenge. K14.E7 and C57BL/6 mice were sensitized topically with 5% of DNCB on the abdomen. Five days later, all mice were challenged with 1% of DNCB (left ear) or vehicle (right ear). The ear swelling was measured within five day after DNCB challenge (a). Arginase activity in ear tissues harvested 24 hours after DNCB challenge was assessed by arginase assay (b). Relative gene expression of arginase (c) and arginase-2 (d) were determined by

real-time PCR in ear tissues of C57BL/6 and K14.E7 mice 1 day after DNCB challenge. Means ± SEM, n= 5 mice per group, *p<0.05, **p<0.01, *** p<0.001, ns= not significant.

Supplementary Table 1

Primers used for real time PCR determinations

Gene	Forward 5' – 3'	Reverse 3' – 5'
Arginase-1	AAGAATGGAAGAGTCAGTGTGG	GGGAGTGTTGATGTCAGTGTG
Arginase-2	GATCTCTGTGTCATCTGGGTTG	AATCCTGGCAGTTGTGGTAC
IL-4	CGAATGTACCAGGAGCCATATC	TCTCTGTGGTGTTCTTCGTTG
IL-10	GGAGTCGGTTAGCAGTATGTTG	AGCCGGGAAGACAATAACTG
IFN-γ	GAACTGGCAAAAGGATGGTGA	TGTGGGTTGTTGACCTCAAAC
Ptge2s	CAGTATTACAGGAGTGACCCAG	AAATGTATCCAGGCGATCAGAG
IL-6	CAAAGCCAGAGTCCTTCAGAG	GTCCTTAGCCACTCCTTCTG
IL-1β	GTTGATTCAAGGGGACATTA	GTTGATTCAAGGGGACATTA

Supplementary methods

Inhibition of arginase activity

Mice were injected i.p. with either 500 µg of Nor-NOHA/500 µl PBS or 500 µl of PBS alone one day prior to DNCB treatment. The treatment was repeated daily for 5 consecutive days. Nor-NOHA is a synthetic analog of NOHA, an intermediate of L-arginase pathway. The inhibitor specifically interacts with the manganese-cluster of the enzyme active site of arginase and does not function as a substrate or as an inhibitor for other factors (Custot *et al.*, 1997). There is extensive literature confirming specificity of nor-NOHA as a specific inhibitor of arginase *in-vitro* (Tenu *et al.*, 1999) and *in-vivo* (Bratt *et al.*, 2009).

Arginase activity

Briefly, ear tissues were homogenized in PBS buffer containing protease inhibitor cocktail (Roche Diagnostics, New South Wales, Australia), centrifuged and supernatants collected. Protein concentration was determined by using bicinchoninic acid (BCA) assay (Thermo Fisher Scientific, Victoria, Australia). 50 µl of tissue lysate was preactivated by adding 50 µl of 10 mM MnCl2/50 mM Tris/HCl at pH 7.5 and heating for 10 min at 56oC. 50 µl of 0.5 M L-arginine substrate (pH 9.5) was added to the samples and further incubated at 37oC for 120 min. The reaction was stopped by adding 400 µl of H3SO4: H3PO4: H2O (1:3:7, v/v/v). After adding 25 µl of 9% 1-phenyl-1,2-propanedione-2-oxime, the samples were then boiled for 45 min at 100oC. Urea concentration was determined using a

spectrophotometer at 540 nm. Using a standard curve, arginase activity was calculated as units/ml/100 mg protein lysate.

Real-time PCR reaction

For cDNA synthesis, 500 ng of total RNA was reverse transcribed in 20 µl reaction containing 5 mM MgCl2; 1.6 mM dNTP mix, 2.5 mM oligo-dT, 1 µl of MuLv reverse transcriptase 5000U (Applied Biosystems, Victoria, Australia) and 10 units of RNaseOUT (Invitrogen, Victoria, Australia) at 42oC for 60 min. The reactions were heated at 70oC for 15 min to inactivate reverse transcriptase enzyme. cDNA product was diluted at 1:4 and 2.5 µl was subjected to real-time PCR reaction using SYBR TAKA kit (Scientifix, Victoria, Australia) and primers (Integrated DNA Technologies, Iowa, United States) listed in supplementary table 1. The relative expression of arginase-1 and arginase-2 was determined by normalizing against the house keeping gene encoded ribosomal protein RPL32.

Flow cytometry and cell sorting

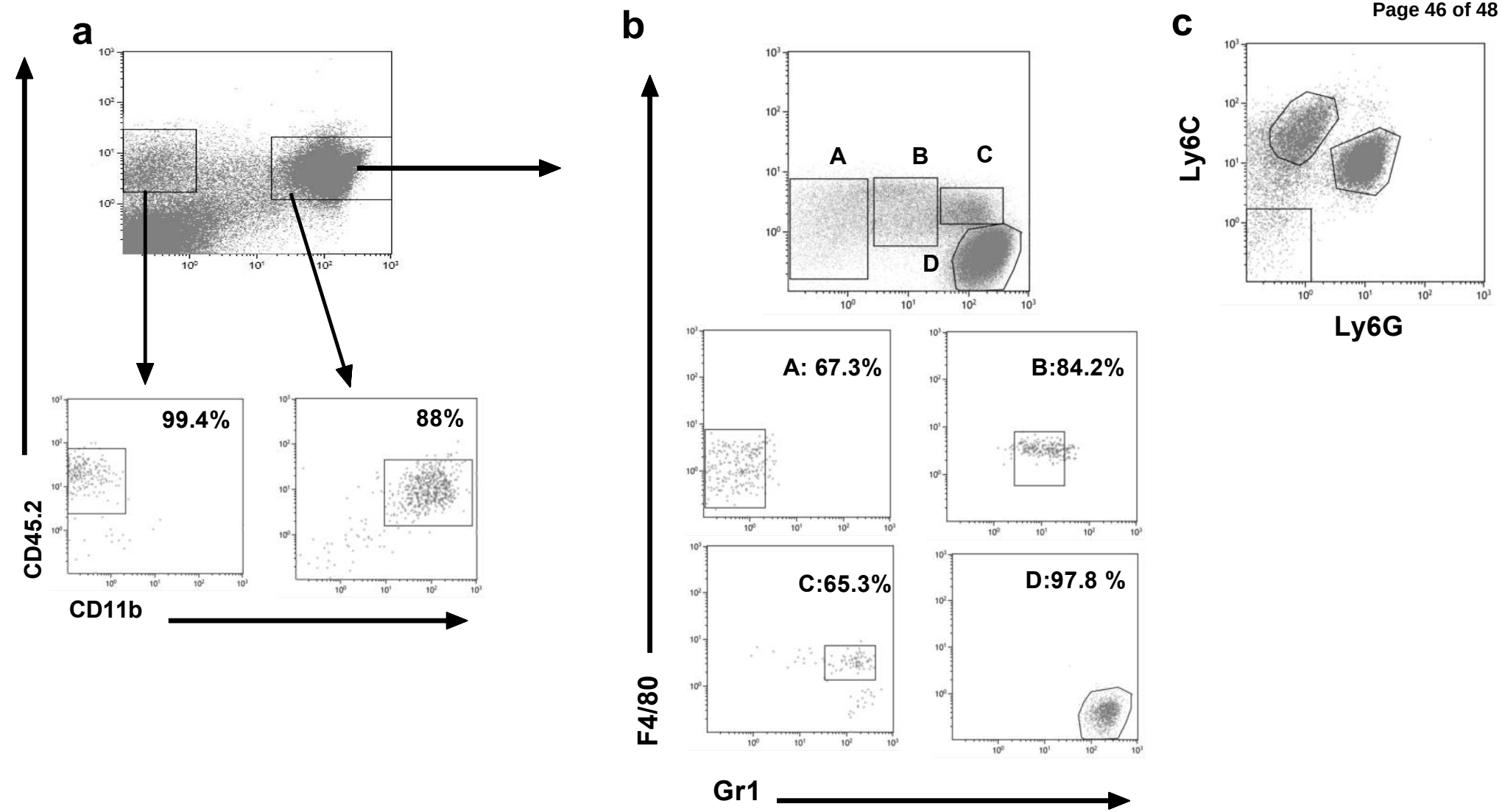
Single cell suspensions were prepared from ear tissues by using collagenaseD/dispase (Roche Diagnostics) and grinding through a 70µm cell strainer. After adding Fcγ receptor block (Fcγ III/II receptor; BD Biosciences, Auckland, New Zealand, 1/50) and incubating on ice for 10 min, cells were stained with live/dead aqua dyes (Live/Dead fixable aqua dead cell stain kit, Invitrogen, 1/1000) and antibodies specific for the following markers for 30 min at 4˚C: CD45.2-PE-Cy7 (BD Biosciences, 1/200); CD11b-PE (BD Biosciences, 1/100), F4/80-FITC

(eBioscience, San Diego, United States 1/100), and Gr1(Ly6G/Ly6C)-APC (eBioscience, 1/200), Ly6G-Alexa 700 (Biolegend, San Diego, United State, 1/200), Ly6C-APC.Cy7 (Biolegend, 1/100). Stained cells were acquired on a LSIR II or FACSCanto flow cytometer (BD Biosciences) and analyzed using Kaluza software (version 1.2, Beckman Coulter, New South Wales, Australia).

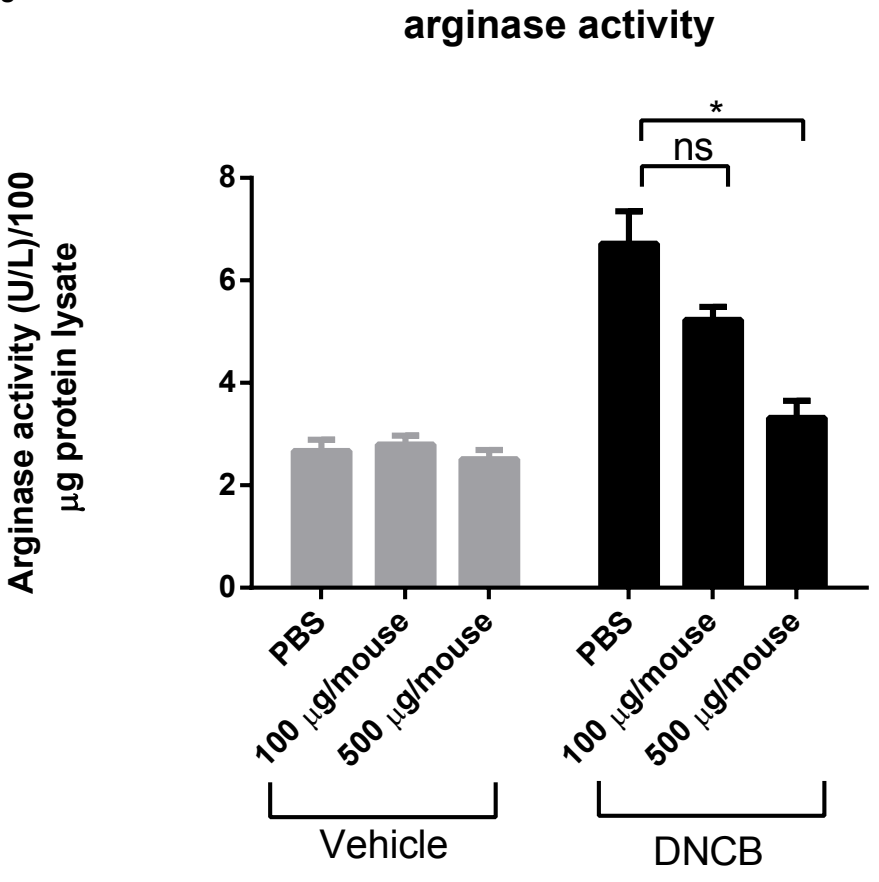
For cell sorting, ear tissues were processed as described above. Cells were stained in FACS staining buffer with anti–CD11b-PE, anti–CD45.2-PeCy7, anti-F4/80-FITC and anti-Gr1 (Ly6G/Ly6C)-APC. Viable $CD45.2^{+}CD11b^{-}$; $CD45.2^{+}CD11b^{+}$; and 5 different subsets of $CD45.2^{+}CD11b^{+}$ cells (Gr1-; F4/80hiGr1int; F4/80lowGr1int; $F4/80^{hi}Gr1^{hi}$; $F4/80^{-}Gr1^{hi}$) were sorted under BSL2 conditions on MoFlo Astrios cell sorter (BD Biosciences).

RNA from sorted cells was isolated by the RNAzol method (Sigma).

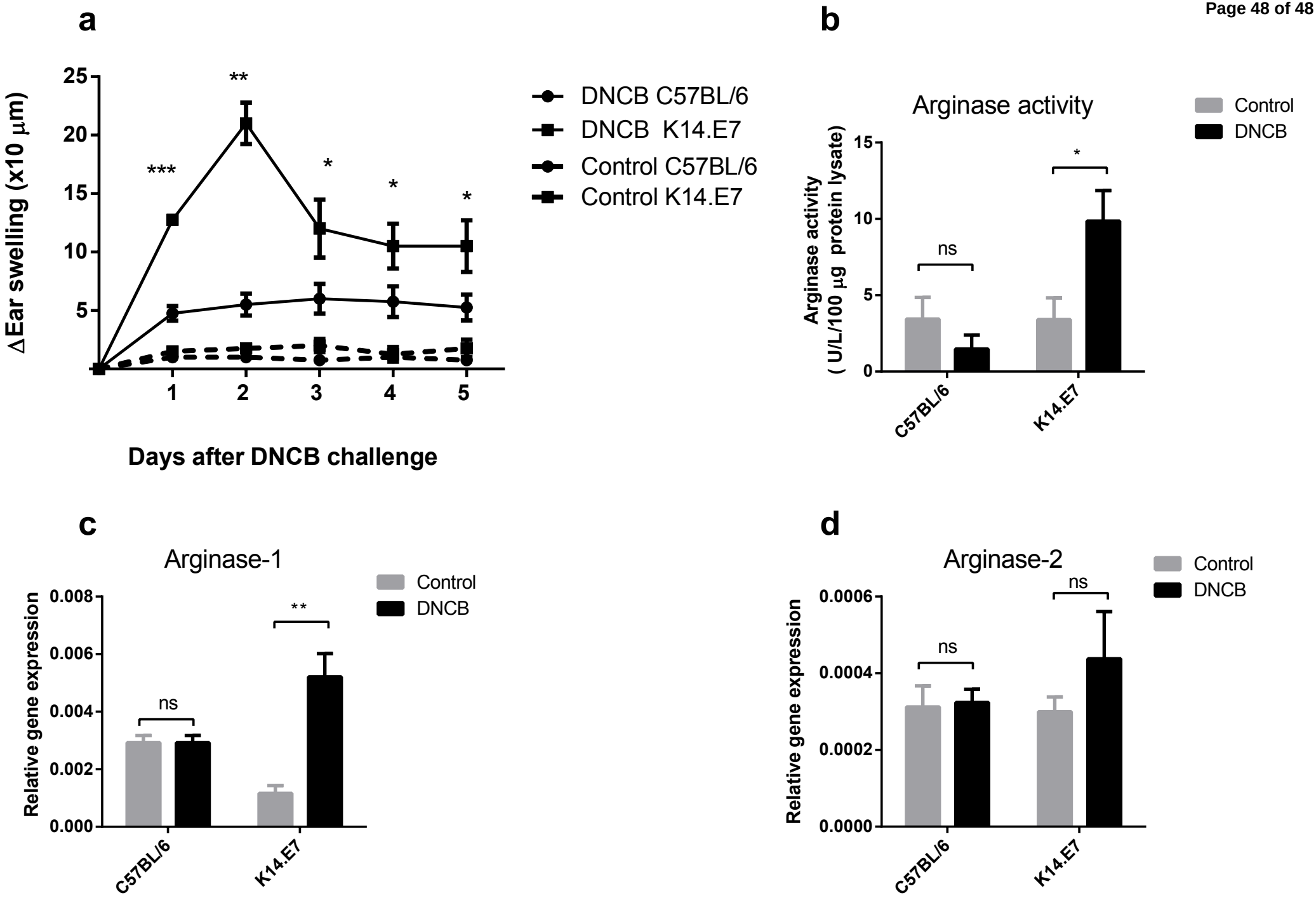
Total protein was isolated from sorted cells by resuspending cells in 50 µl of lysis buffer (20 mM Tris, 100 mM NaCl, 1 mM EDTA, 0.5% Triton X100) and incubating for 20 min. After centrifugation at 13,000 g at $4^{o}C$ for 20 min, the supernatant was collected for protein quantification (BCA) and arginase assay.



Supplementary figure 1 Tran *et al.*



Supplementary figure 2 Tran *et al.*



Supplementary figure 3 Tran *et al.*