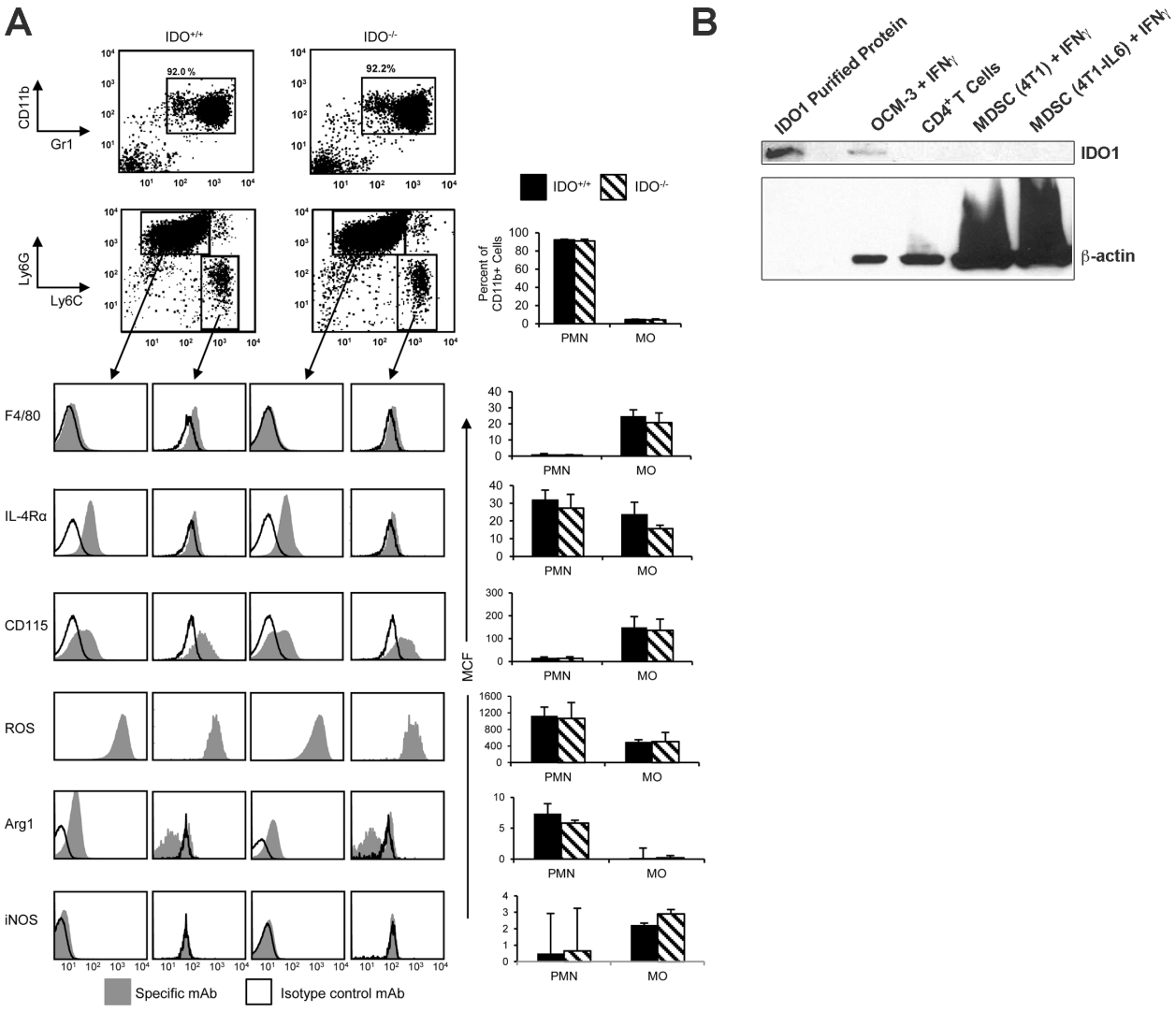

Supplementary Figure S3. (**A**) Phenotypic characterization reveals no demonstrable differences between MDSCs from WT and *Ido1*$^{-/-}$ mice. BALB/c strain WT and *Ido1*$^{-/-}$ mice were inoculated in the abdominal mammary gland with 10^5 4T1 mammary carcinoma cells. MDSC were harvested from the blood when WT and *Ido1*$^{-/-}$ mice had primary tumors that were not significantly different in size (12.2 ± 1.36 and 11.5 ± 0.4 mm in diameter, respectively). Red blood cells were removed by lysis and the remaining leukocytes were stained with mAbs to Gr1 and CD11b, or with mAbs to CD11b, Ly6C, Ly6G, and either arginase I, iNOS, CD115, F4/80, IL-4Rα and their respective isotype control mAbs, or with DCFDA to detect ROS. Gated CD11b$^+$

cells were analyzed for Ly6C and/or Ly6G expression. Granulocytic (PMN) and monocytic (MO) MDSC were identified as per Youn et al. (J. Immunol. 2008, 18:5791-802) as $CD11b^{+}Ly6G^{+}Ly6C^{-}$ or $CD11b^{+}Ly6G^{-}$ $Ly6C^{+}$ cells, respectively. Flow cytometry dotplots and histograms show MDSC from representative individual mice; graphs depict the average percent $Gr1^{+}CD11b^{+}$ cells or average mean channel fluorescence (MCF) for three mice per group. The values for total MDSC ($Gr1^{+}CD11b^{+}$) and for MO and PMN MDSC for iNOS, arginase, CD115, F4/80, IL-4Rα, and ROS are not statistically significantly different between MDSC from WT and *Ido1*$^{-/-}$ mice (Student's two-tailed *t* test with equal variance). (**B**) MDSCs from 4T1 tumor bearing mice lack detectable IDO1 expression. Western blot analysis using antibodies to IDO1 (top panel) and β-actin (bottom panel; loading control) with each lane individually labeled at the top. (Lanes 1,2) purified IDO1 protein for size confirmation with the adjacent lane left blank to avoid spillover contamination, (Lane 3; positive control) IFNγ-induced expression of IDO1 in OCM-3, a human melanoma cell line, (Lane 4; negative control) CD4 T cells lacking IDO1 expression, (Lane 5,6; experimental) MDSC isolated from 4T1 or 4T1-IL6 tumor-bearing mice and stimulated with IFNγ for 24 hours prior to analysis.