Figure S1. Islet gating strategy and examination. The technique for the isolation of islets of Langerhans and exocrine pancreas (pancreatic stroma) includes a filtration step of the digested pancreas (which separates islets from stroma). Islets are further enriched by dithizone staining followed by manual "hand picking." (A) Gating strategy on dispersed islets performed on live singlets and CD45+ cells. CD45+ cells are then analyzed for their expression of F4/80 and CD11b. (B) Pooled quantitative data from 9–22 independent experiments evaluating dispersed islets for percentages of CD45+ cells (from live/singlets gate, percentages on left y axis) and for the expression of F4/80, CD11b, CD11c, and MHC-II (from CD45+ gated cells, percentages on right y axis). Bars indicate median. (C) Hand-picked enriched islet isolation gated on live single (singlet) cells showing percentage of CD45+ from total dispersed islets (left). Further analysis performed on gated CD45+ islets cells showing the presence of one cell component (F4/80+ CD11b+) and the absence of B cells (B220+), pDCs (Siglec-H+ CD11c+), CD103+ and CD8α+ DCs (gated on CD11c- MHC-II-), and neutrophils (Ly6G+). (D) Crude islet isolation (without filtering or hand-picking) "contaminated islets" showing CD45+ percentage from total dispersed islets (left). CD45+ gated cells showing the presence of leukocyte contaminants [monocytes, B cells, pDCs, CD103+ and CD8α+ DCs (gated on CD11c- MHC-II-), and neutrophils]. The leukocyte contaminants derive from the pancreatic stroma. Experiments shown in hand-picked enriched islets (A) and crude islet isolation (without filtering or hand-picking) contaminated islets are representative of 20 and 5 independent experiments, respectively.
Figure S2. Stroma evaluation of F4/80− CD11b− leukocytes. Gated F4/80− CD11b− stromal leukocytes (from top left) plotted for CD11c and MHC-II expression (top middle) shows three main subsets: (1) CD11c+ MHC-II+, representing CD103+ and CD103− DCs (top right; black line, isotype; red line, CD103); (2) CD11c+ MHC-II+, representing B cells by their CD19 and B220 expression (bottom right); and (3) CD11c− or low MHC-II−, representing two main subsets, T cells expressing either CD4 or CD8 (bottom middle) and ILCs (CD4− CD8− gated) expressing CD127 (IL-7Rα; bottom left; black line, isotype; red line, CD127). Flow cytometry panels are representative of three to five independent experiments. MFI, mean fluorescent intensity.
Figure S3. Mouse models to evaluate ontogeny of pancreatic macrophages. (A) Flt3-cre x Rosa<sup>mTmG</sup> reporter mouse model and the alternative lineage of islet macrophages (M<sub>Φ</sub>) in the adult mouse: (a) Embryonic lineage derived (yolk sac and/or fetal liver monocyte derived); (b) mixed origin; (c) definitive hematopoiesis derived (adult HSC derived). (B) Csf1r-Mer-iCre-Mer x Rosa<sup>mTmG</sup> reporter mouse model labeling yolk sac-derived macrophages at E8.5 by tamoxifen treatment and the alternative outcomes in the adult mouse: (A) yolk sac derived; (B) mixed origin; (C) definitive hematopoiesis derived. PH, primitive hematopoiesis; DH, definitive hematopoiesis; FL, fetal liver; EMP, erythromyeloid precursor; ST-HSC, short-term HSC; MPP, multipotent progenitor; CMP, common myeloid progenitor; CLP, common lymphoid progenitor; GMP, granulocyte-macrophage progenitor; MEP, megakaryocyte-erythroid progenitor; GM, granulocyte/macrophage cells; Pit, platelets; RBC, red blood cells; Pre B, pre-B cell; Pre T, pre-T cell. Figure adopted and modified from Boyer et al. (2011) with permission from Elsevier.
Figure S4. The multiple origins of pancreatic macrophages and their maintenance. Illustration simplifies the origin of islet-resident macrophages (adult HSC derived, shown in green) and their self-maintenance by in situ proliferation. These show basal M1 features. In contrast, the exocrine pancreatic (stroma) macrophages are composed of two subsets: one in continual replacement by circulating monocytes (adult HSC derived) and not self-maintained and the second derived from yolk sac and fetal liver monocytes (shown in red) and self-maintained. Half the interacinar macrophages expressed CD206 and CD301 and were preferentially situated among pancreatic ducts. The second set derives from circulating monocytes and did not express CD206 or CD301. All macrophages in the interacinar stroma have M2 features.

REFERENCE