**Supplementary information**

* 1. *Ethics Statement*

These studies were approved and conducted in accredited facilities in accordance with The Home Office UK Animals (Scientific Procedures) Act 1986 (Home Office license number PPL 70/7302 and 70/9066).

* 1. *Flow cytometry*

Cells were stained and analysed as described elsewhere[1](#_ENREF_1) with Abs specific for the following markers: CD4 (GK1.5), FoxP3 (FJK-16s), CD11c (N418), H2Kd (SF1-1.1.1) and CD25 (PC61) (BD Biosciences).

B cells staining antibodies B220 (RA3-6B2), CD43 (S11), Ly51 (BP-1), CD24 (M1/69), IgM (RMM-1), IgD (11-26c.2a), CD20 (SA275A11), CD19 (6D5), CD21/35 (7E9), CD23 (B3B4), CD93 (AA4.1), CD11b (M1/70). For intracellular staining, cells were fixed and permeabilised with Foxp3/Transcription Factor Staining Buffer according to manufacturer’s protocols (Thermo Fisher Scientific). Data was acquired on either a BD Accuri C6™, BD FACSCalibur™ or BD LSRFortessa™ (BD Biosciences) and analysed using CFlow or FlowJo 10 software (Tree Star).

* 1. *B cell isolation*

B cell purification was achieved using untouched CD43 conjugated Dynal beads (Thermofisher) following manufactures instructions. RBC depleted splenocytes were obtained before proceeding with the bead and Dynal Magnet isolation. To confirm purity of B cells, samples were stained using anti-B220/CD45R fluorescently labeled antibody followed by flow cytometer. The purity of isolated B cells was greater than 95%.

* 1. *Generation of CD4+CD25+ Treg line.*

Tregs with indirect specificity were created as outlined by Tsang et al [2](#_ENREF_2). These CD4+CD25+ cells were stimulated and expanded once per week in the presence of immature B6 DCs pulsed with the Kd54–68 peptide and 10 U/ml of IL-2.

* 1. *CFSE labeling of DCs and isolation of EVs*

Bone marrow derived dendritic cells (BM-DCs) were generated as previously described [3](#_ENREF_3). Briefly, BM from B6 mice was passed through a 70 μm cell strainer to obtain a single‐cell suspension and erythrocytes were lysed using ACK buffer (0.15 M NH4Cl/1 mM KHCO3/0.1 mM Na2EDTA). BM cells were then incubated with supernatants from the following hybridoma cultures: YTS 191 (anti‐CD4), M5/114 (anti-class II), RA3–3A1 (anti‐B220), and YTS 169 (anti‐CD8). Mouse depletion Dynabeads (coated with a polyclonal sheep anti-rat IgG antibody) were added and the bead-bound cells were depleted using a magnet. BM cells were grown in RPMI1640 medium supplemented with 10%FCS, PSG, Hepes and 2-mercaptoethanol (2-ME) and 20 ng/mL of murine recombinant GM‐CSF. Media was changed on days 2 and 4 with fresh GM‐CSF containing media. The purity of CD11c+ BM‐DCs was greater than 90% (data not shown). On day 6 of culture, DCs were harvested and labeled with 1 µg/ml of CFSE dye (ThermoFisher) as per manufacturer’s instruction. Two million CFSE labeled DCs were placed in EV free media (RPMI1640 supplemented with 10% ultracentrifuged FCS, PSG, Hepes and 2-ME) and incubated at 37°C overnight. EVs were extracted from cell culture supernatants using ExoQuickTC (SBI). Briefly the supernatants were centrifuged at 300g for 10 minutes, at 2000g for 10 minutes, filtered through a 0.22µm pore-sized filter (Merck Millipore, MA, USA) and incubated with ExoQuickTC (SBI) following manufacturer’s instructions. Following isolation, EVs were dissolved in EV free media and used immediately. EV enriched pellets were stored in -80°C until analysis via NanoSight LM-10 or CD63-Exo ELISA. Unfortunately we could not assess MHC expression directly on EVs since we have previously found that EVs isolated by Exoquick did not attach to microbeads we used to assess molecules on EVs via flow cytometry.

**1.** Smyth LA, Ratnasothy K, Moreau A, et al. Tolerogenic Donor-Derived Dendritic Cells Risk Sensitization In Vivo owing to Processing and Presentation by Recipient APCs. *J Immunol.* May 1 2013;190(9):4848-4860.

**2.** Tsang JY, Ratnasothy K, Li D, et al. The potency of allospecific Tregs cells appears to correlate with T cell receptor functional avidity. *Am J Transplant.* Aug 2011;11(8):1610-1620.

**3.** Smyth LA, Ratnasothy K, Tsang JY, et al. CD73 expression on extracellular vesicles derived from CD4+ CD25+ Foxp3+ T cells contributes to their regulatory function. *European journal of immunology.* Sep 2013;43(9):2430-2440.