Protocol 1:

Bone marrow cultures (adapted from {Sokol:2007ht}**)**

For the production of DCs, bone marrow was cultured for 5 d in media supplemented with granulocyte-macrophage colony-stimulating factor (Peprotech). Cultures were then stimulated for 18 h with heat-inactivated or active protease (100 g/ml) or with CpG dinucleotide (5 M).

For the production of mast cells and basophils, erythrocyte-depleted mouse bone marrow was initially seeded at density of **5 10^6 cells/ml**, followed by replating every **3–4 d at a density of 1 10^6 cells/ml** for a total of 12 d culture in RPMI medium supplemented with IL-3 (30 ng/ml; Peprotech) and 10% (vol/vol) FCS.

Samples were enriched for basophils by positive selection of $DX5^+$ cells with magnetic-activated cell sorting.

Reactivation:

Cultures were stimulated with ionomycin (500 ng/ml; Calbiochem), lipopolysaccharide (100 ng/ml; Sigma) or heat-inactivated or active protease (100 g/ml). Activation by IgE crosslinking was accomplished by incubation with mouse IgE (10 g/ml) followed by incubation with anti–mouse IgE (10 g/ml; R35-118).

Protocol 2 (shorter):

Isolation of bone marrow (BM) cells from femurs and tibias of 8-12 week old mice.

Culture whole BM cells at 2 to 2.5 × 10^6 cells/ml in 10 ml of penicillin and streptomycin, 2mM L-glutamine, 50 μM β-mercaptoethanol, 10% fetal bovine serum-containing RPMI 1640 medium supplemented with recombinant mouse IL-3 (5 ng/ml) for 9 days with medium changed every 3 days.

Enrich BM-derived basophils by depleting mast ($c\text{-}kit^+$) cells using IMag system or autoMACS as instructed by the supplier.