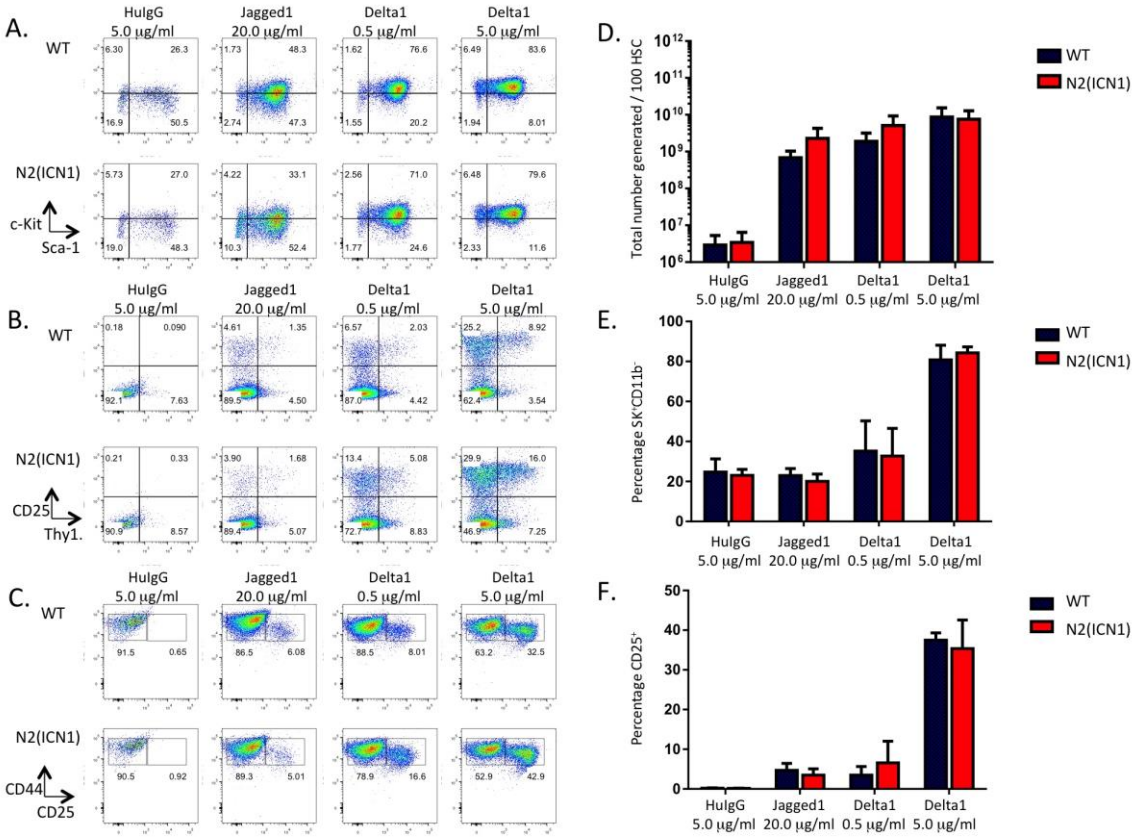

Fig. S1. **Notch1 ICD swapped into Notch2 locus functionally replaces Notch2 ICD during Notch2-dependent ex vivo culture with Notch ligands Delta and Jagged. (**A-C) Representative dot plots after 14 day bone marrow (BM) HSC culture on plastic coated with either different densities of immobilized Delta1, Jagged1 (extracellular domain Delta1 or Jagged1 fused to Fc domain of Human IgG) or control Human IgG. Boxes in C indicate DN1 ($CD44^+CD25^-$) and DN2 ($CD44^+CD25^+$) subpopulations. (D) Total number of cells, (E) percent Sca^+ Kit^+ $CD11b^-$ cells, and (F) percent $CD25^+$ cells generated in cultures initiated with 100 FACS isolated HSC after 14 days of culture. Data represent mean +/- SEM from 4 independent experiments. p values were determined with 2-tailed paired Student's t test. Numbers within dot plots denote percentage of events within the respective gates.

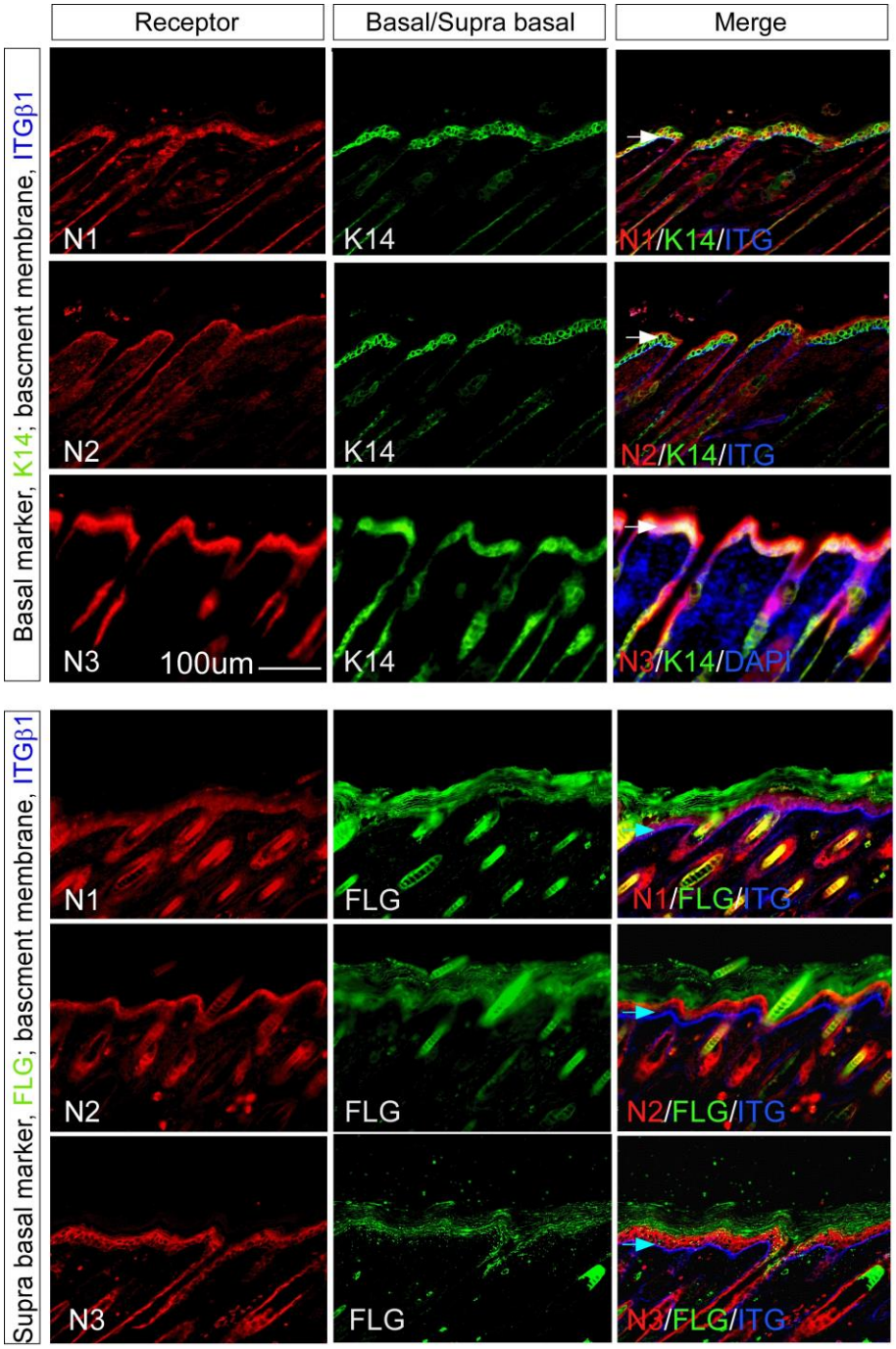

Fig. S2. Detailed analysis of the expression pattern of Notch1, Notch2 and Notch3 in the epidermis of wild type postnatal day 9 (P9) pups. Whereas Notch1 and Notch3 are expressed in both basal (marked by K14 antibody staining) and supra basal (marked by filagrin (FLG) antibody staining) cell layers, Notch2 is only expressed in supra basal cells. Arrows in the merged images indicate the basal cell layer, whose basal membrane is marked by the expression of β1 integrin (ITGβ1).

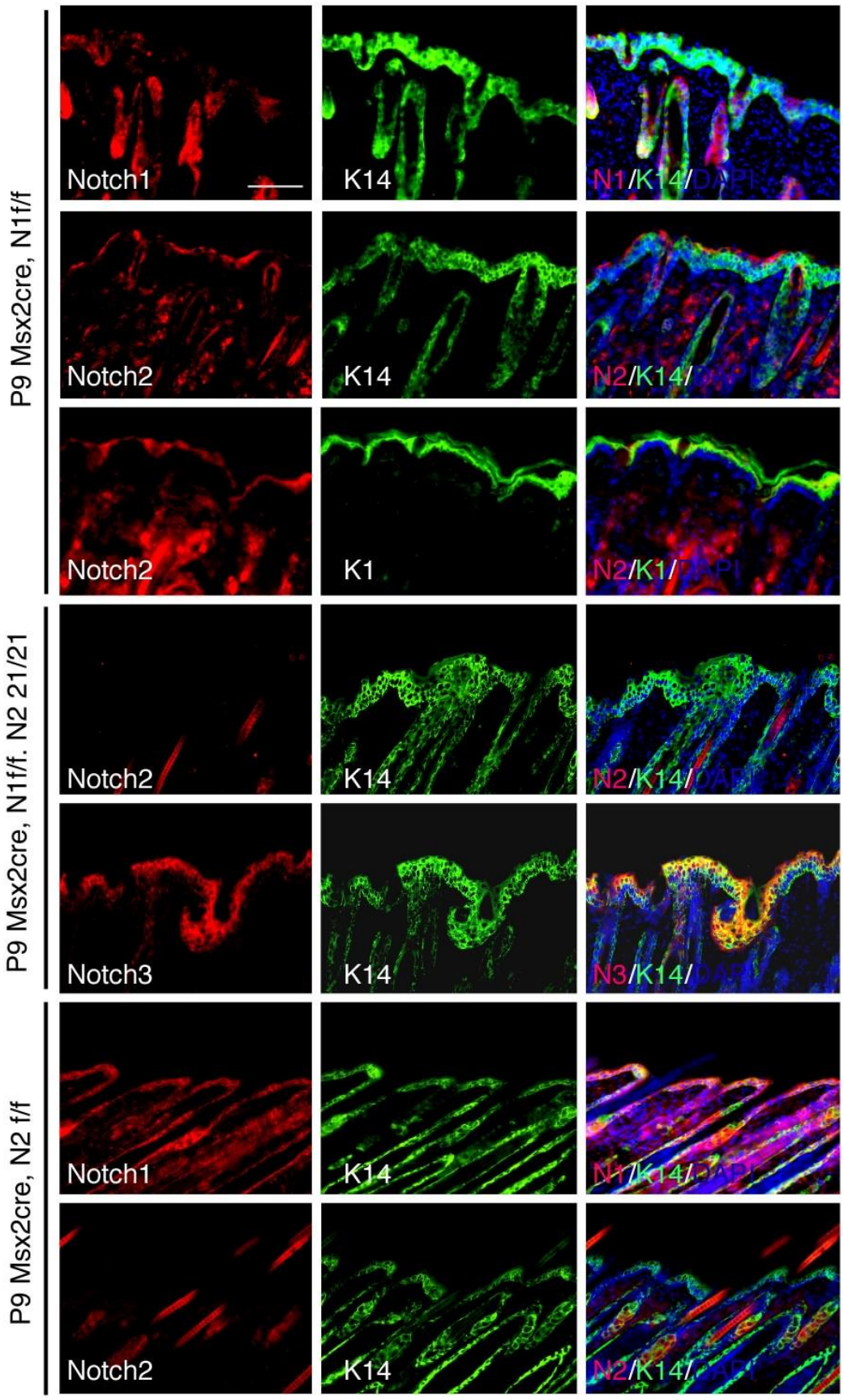
P9 Msx2cre, N1f/f
Notch1
K14
N1/K14/DAPI
Notch2
K14
N2/K14/
Notch2
K1
N2/K1/
P9 Msx2cre, N1f/f. N2 21/21
Notch2
K14
N2/K14/
Notch3
K14
N3/K14/
P9 Msx2cre, N2 f/f
Notch1
K14
N1/K14/
Notch2
K14
N2/K14/

Fig. S3. The expression of Notch1, Notch2 and Notch3 in various mutant skin at P9. Basal cells are marked with K14 and supra basal cells with K1. In the skin of Msx2cre, $N1^{f/f}$ pups, complete loss of Notch1 is observed in mutant area marked by hyper-proliferation of basal cells. N2ICD is replaced by N1ICD in the skin of Msx2Cre, $N1^{f/f}$; $N2^{21/21}$ pups, but basal cell hyper proliferation is still evident. In the skin of Msx2Cre, $N2^{f/f}$, the complete loss of Notch2 does not result in basal cell hyper-proliferation.

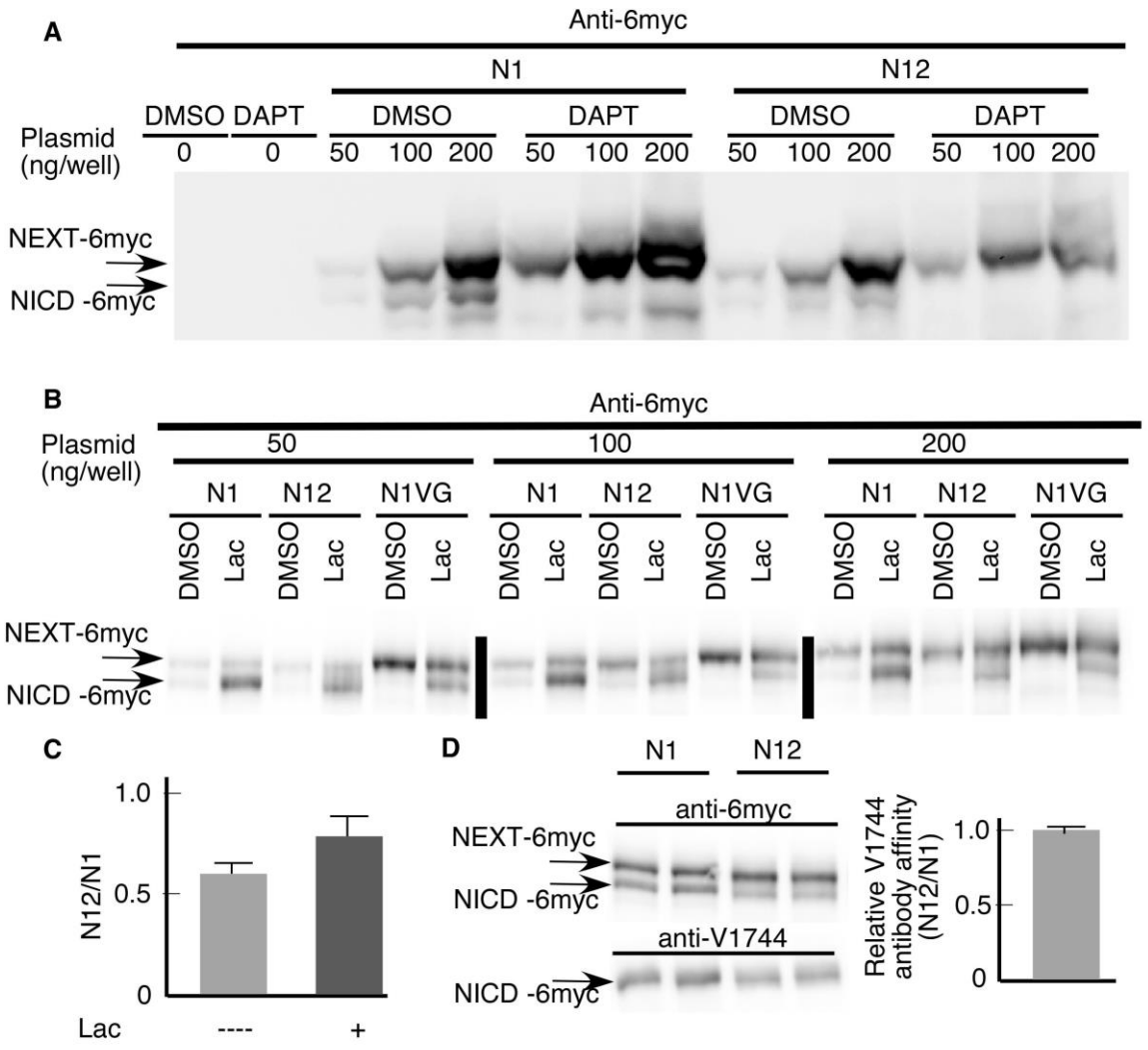

Fig. S4. Analysis of cleavage efficiency of the N12 protein and the stability of N12ICD in cultured HEK293 cells. (A-B) HEK293 cells were transfected with various amount of plasmid expressing a c-terminal tagged proteins; pCS2ΔE-Notch1-6xmyc (N1), pCS2ΔE-Notch12-6xmyc (N12) (A, B), or pCS2ΔE-Notch1 V1744G-6xmyc (N1VG) plasmid (B). (A) Uncleaved fragment (NEXT-6myc) and γ-secretase cleaved fragment (NICD-6myc) were detected with anti-Myc antibody. In the presence of γ-secretase inhibitor DAPT, the production of the cleaved fragment is dramatically reduced whereas the uncleaved fragment increases, validating the identity of the NICD. (B) The presence of proteasome inhibitor Lactacystin (Lac) dramatically increased the amount of the NICD. (C) Quantification of relative NICD level shows that in the absence of Lac, the cleaved N1ICD level in cells transfected with pCS2ΔE-

Notch12-6xmyc (N12) is about 50% of that of pCS2ΔE-Notch1-6xmyc (N1). The presence of Lac increases this ratio to ~75%, suggesting that both the stability of the cleaved ICD from N12 and the cleavage efficiency of the N12 chimeric protein are reduced relative to the wild type N1 locus in cultured 293 cells. (D) To examine whether the binding affinity of anti-V1744 antibody is affected by intracellular amino acids, the same lysates from cells transfected with pCS2ΔE-Notch1-6xmyc (N1) and pCS2ΔE-Notch12-6xmyc (N12) were probed with anti-Myc tag and anti-V1744 antibodies, respectively, and the relative signal intensity was calculated. The results show that the affinity of anti-V1744 antibody was unaffected by the intracellular composition.

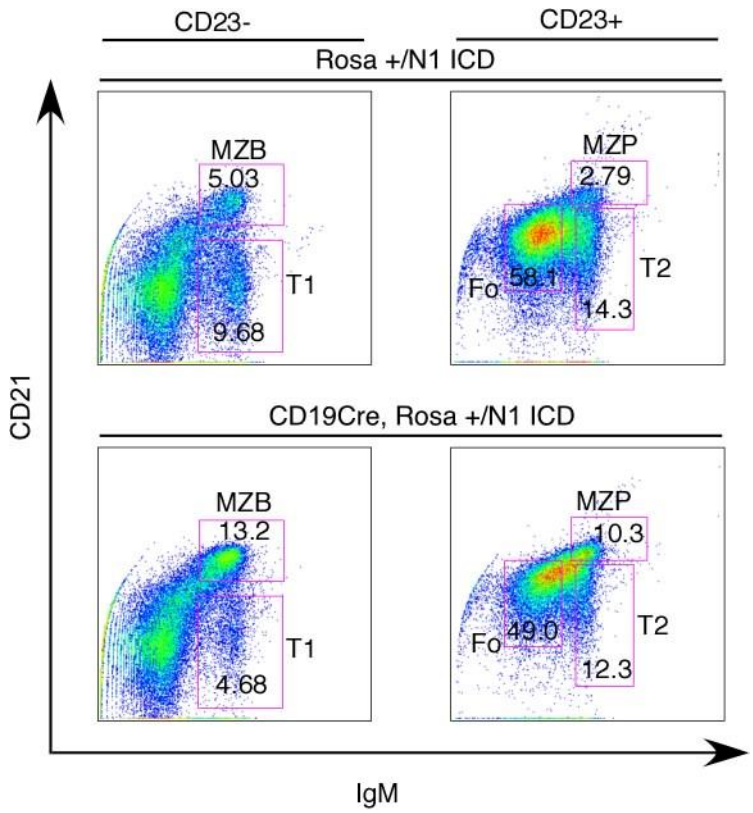

Fig. S5. Rosa^{Notch}; CD19-Cre mice released N1ICD in the B cell lineage and could efficiently drive the differentiation of MZP and MZB cells when overexpressed.