MT1-MMP cleaves Dll1 to negatively regulate Notch signaling to maintain normal B cell development

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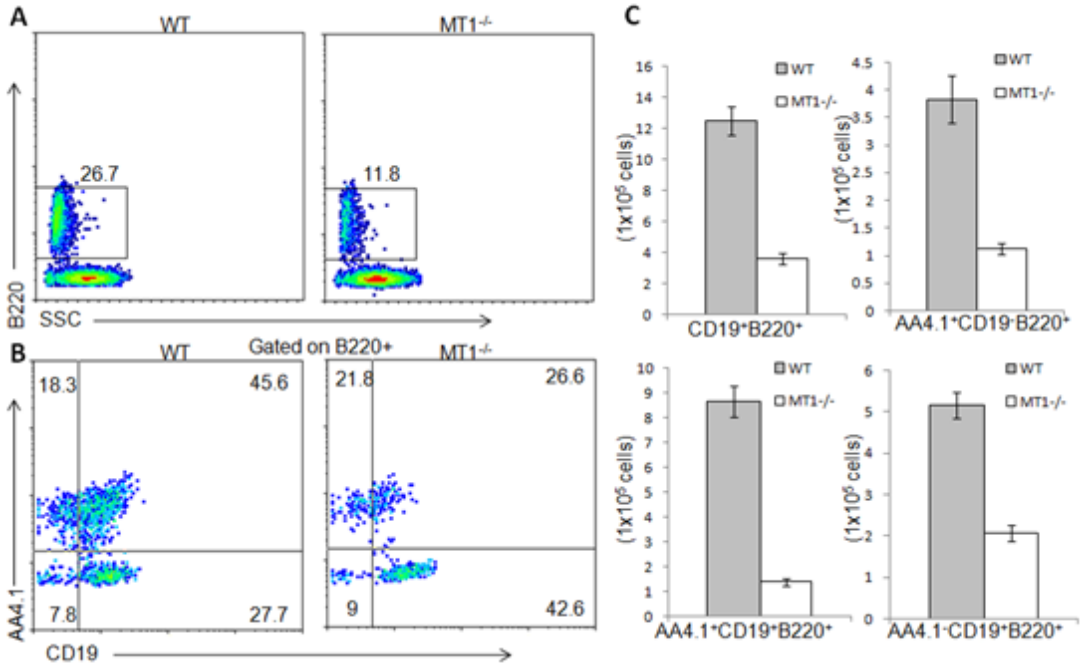
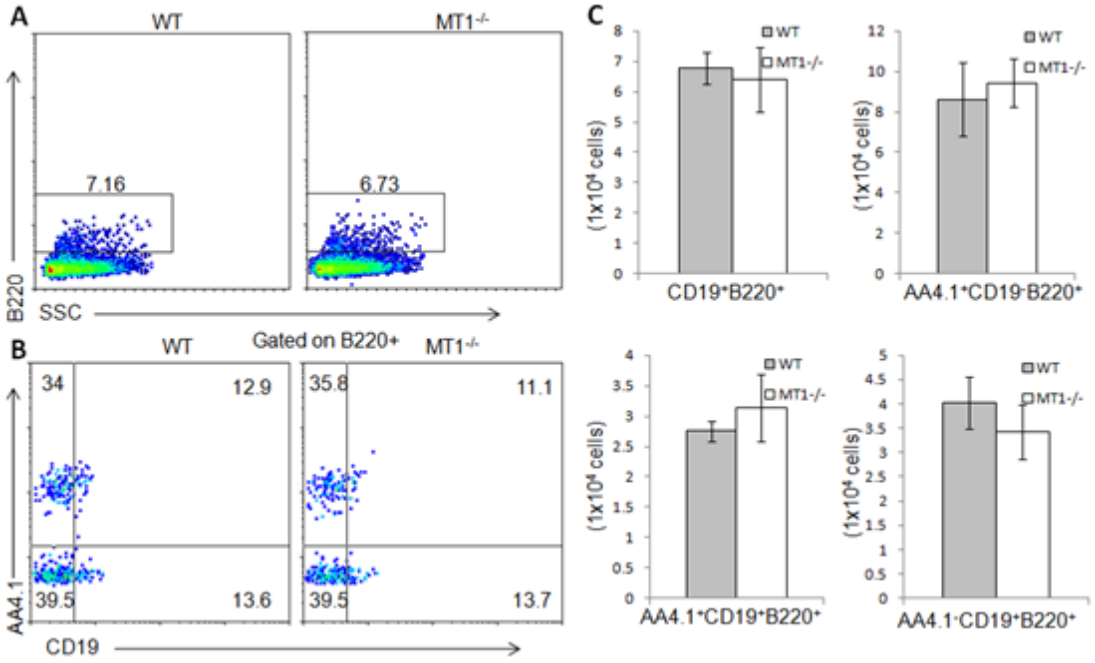
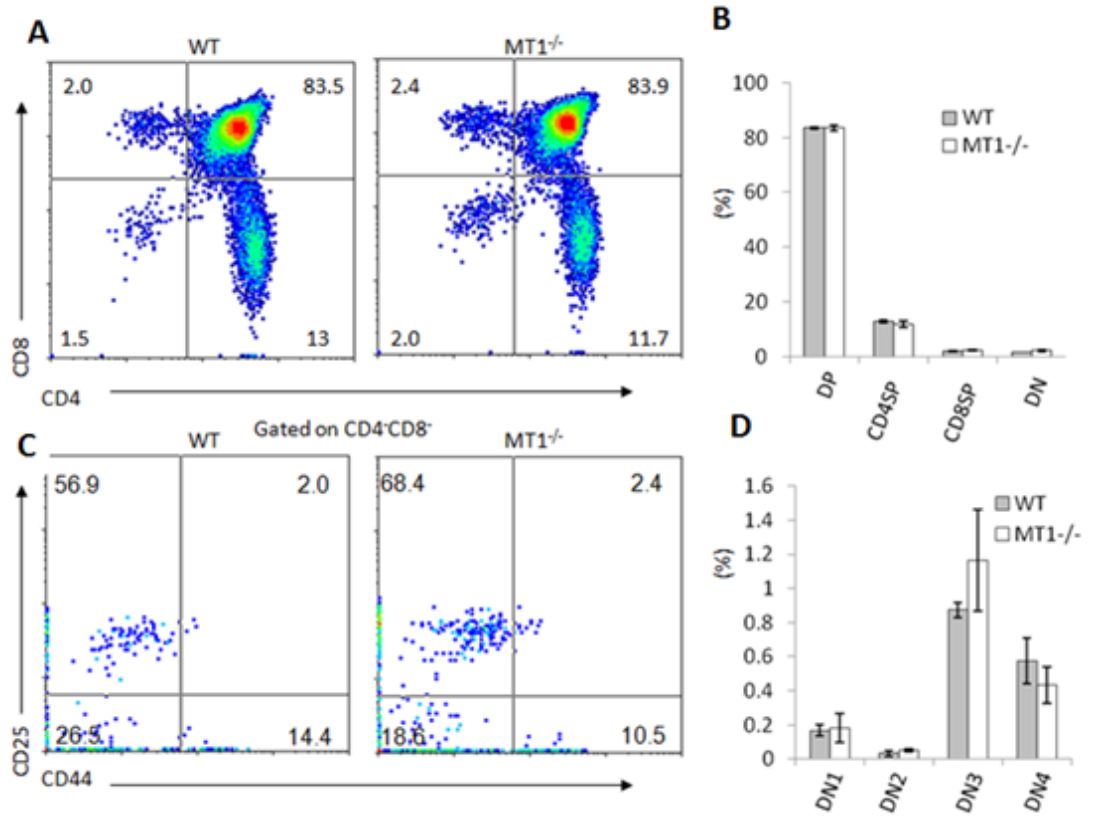


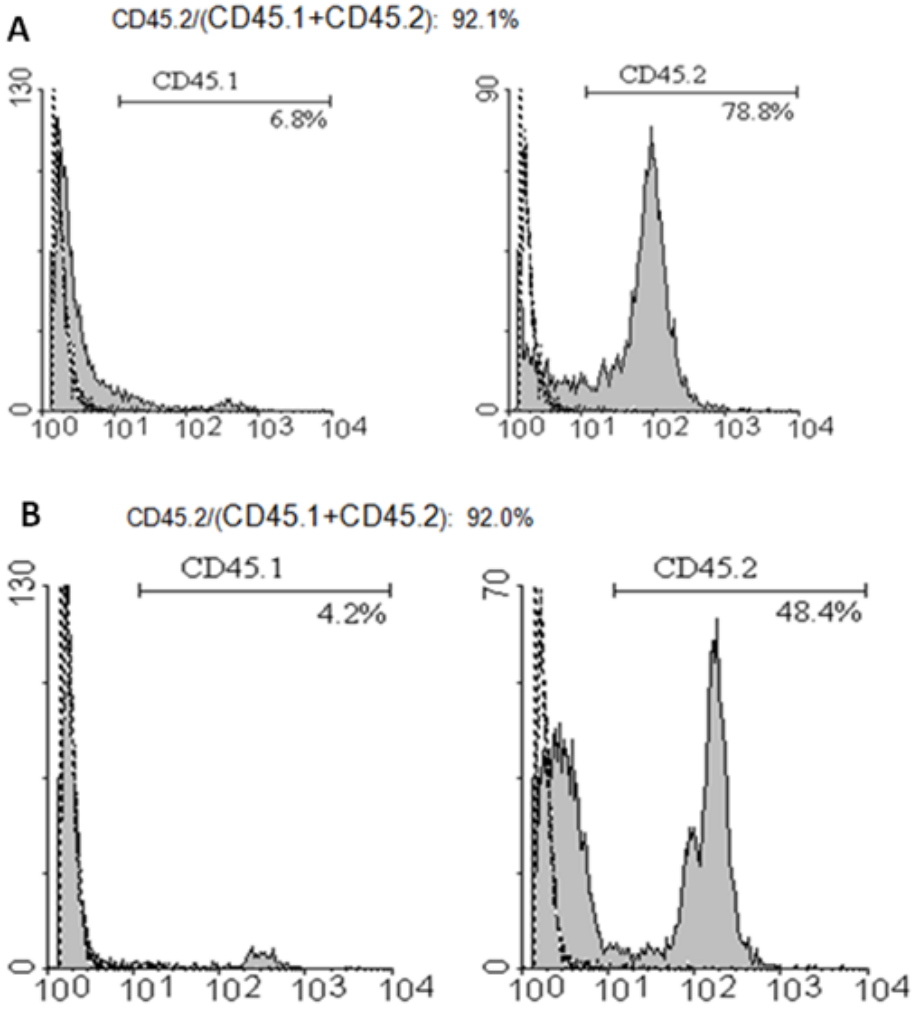
Supplementary Figure 1 MT1-MMP deficiency impairs B cell development in bone marrow. (A) Flow cytometry analyses of total $B220^+$ B cells in either wild-type (WT) or MT1-MMP deficient ($MT1^{-/-}$) bone marrow. Numbers adjacent to the outlined areas indicate the percentage of $B220^+$ cells. **(B)** Flow cytometry analyses of B cells gated on the $B220^+$ population by the expression of AA4.1 and CD19. Numbers in the plots indicate the percentage of $AA4.1^+CD19^-$ cells (up-left), $AA4.1^+CD19^+$ cells (up-right), $AA4.1^-CD19^+$ cells (bottom-right) and $AA4.1^-CD19^-$ cells (bottom-left) in the $B220^+$ population. **(C)** Statistical analyses of different stages of B cell populations in bone marrow; P=0.0051 ($B220^+CD19^+$ cells), P=0.0155 ($B220^+CD19^-AA4.1^+$ cells), P=0.0042 ($B220^+CD19^+AA4.1^+$ cells), P=0.0081 ($B220^+CD19^+AA4.1^-$ cells), Student's *t*-test.



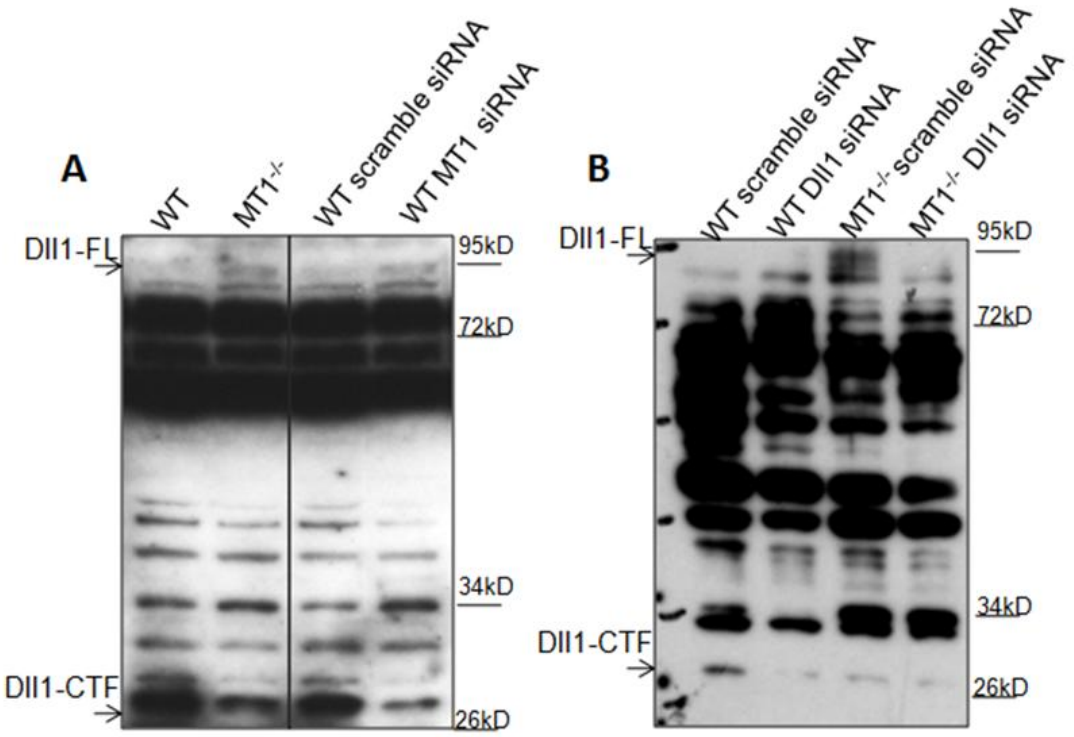
Supplementary Figure 2 Normal fetal liver B cell development in MT1-MMP deficient mice. (A) Flow cytometry analyses of total $B220^+$ B cells in either wild-type or MT1-MMP deficient E17.5 fetal liver. Numbers adjacent to the outlined areas indicate the percentage of $B220^+$ cells. **(B)** Flow cytometry analyses of B cells gated on the $B220^+$ population by the expression of AA4.1 and CD19. Numbers in the plots indicate the percentage of $AA4.1^+CD19^-$ cells (up-left), $AA4.1^+CD19^+$ cells (up-right), $AA4.1^-CD19^+$ cells (bottom-right) and $AA4.1^-CD19^-$ cells (bottom-left) in the $B220^+$ population. **(C)** Statistical analyses of different stages of B cell populations in E17.5 fetal liver; $P>0.05$, Student's *t*-test. Data are representative of two independent experiments (mean±s.d.).



Supplementary Figure 3 Normal T cell development in thymus of MT1-MMP deficient mice. (A) Flow cytometry analyses of T cells in thymus. Numbers in the plots indicate the percentages of CD4 SP (bottom-right), CD8 SP (up-left), DP (up-right) and DN (bottom left) cells. **(B)** Statistical analyses of CD4 SP, CD8 SP, DP and DN cells in thymus; *P*>0.05, Student's *t*-test. Data are representative of two independent experiments (mean±s.d.). **(C)** Flow cytometry analyses of thymus DN T cell progenitors. Numbers in the plots indicate the relative percentage of DN1 (bottom-right), DN2 (up-right), DN3 (up-left) and DN4 (bottom-left) T cell progenitors in the DN subset. **(D)** Statistical analyses of DN T cell progenitors in the thymus; *P*>0.05, Student's *t*-test. Data are representative of two independent experiments (mean±s.d.). DP: CD4CD8 double positive; DN: CD4CD8 double negative; CD4 SP: CD4 single positive; CD8 SP: CD8 single positive.



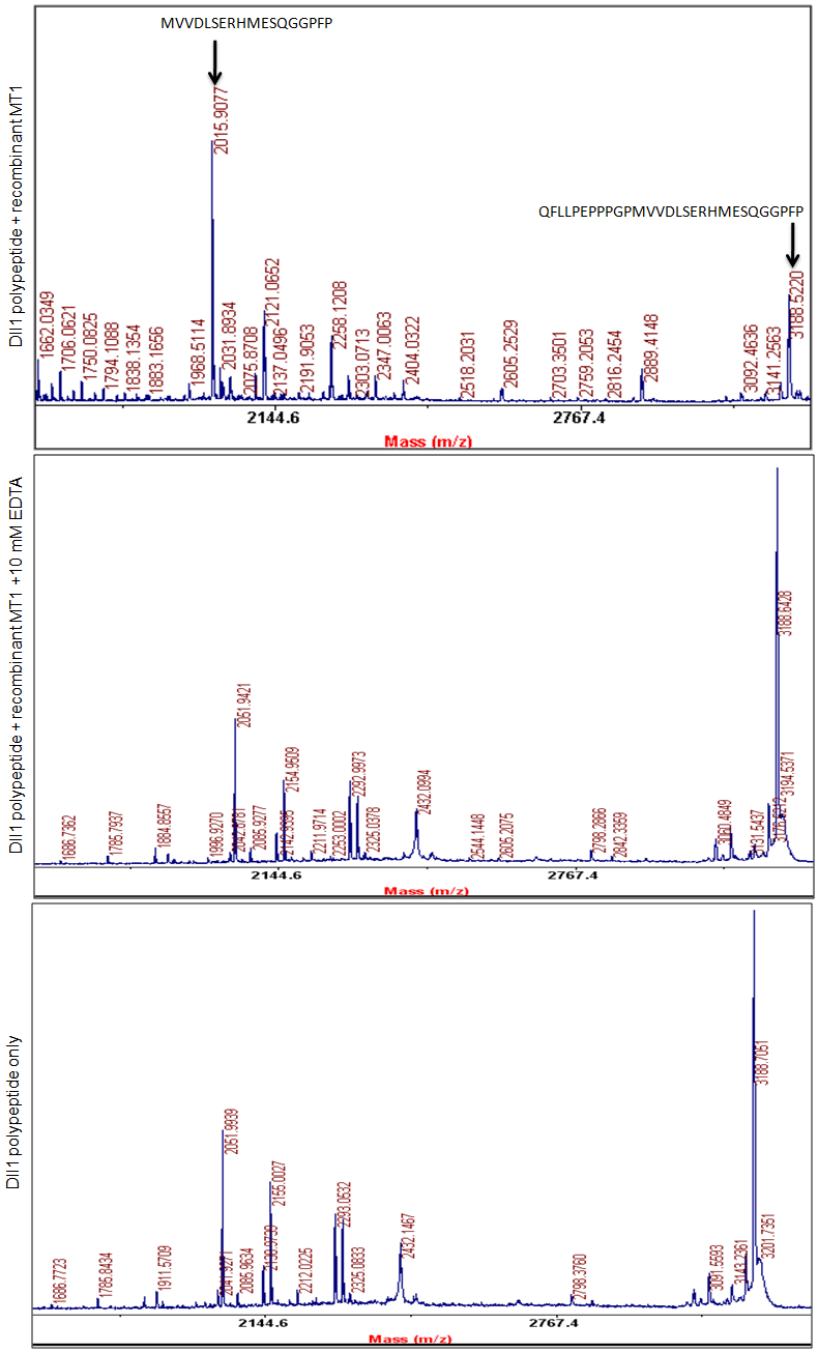
Supplementary Figure 4 Bone marrow reconstitution efficiency. (A) Flow cytometry analyses of bone marrow CD45 positive cells at 2 months after bone marrow reconstitution. Numbers in the plots indicate the percentage of recipient CD45.1 (left) or donor CD45.2 (right) positive cells. **(B)** Flow cytometry analyses of blood CD45 positive cells 2 months after bone marrow reconstitution. Numbers in the plots indicate the percentage of recipient CD45.1 (left) or donor CD45.2 (right) positive cells. The percentage of CD45.2 positive cells in total CD45 positive cells represents the reconstitution efficiency. The dotted lines represent the isotype controls. Data are representative of two independent experiments.



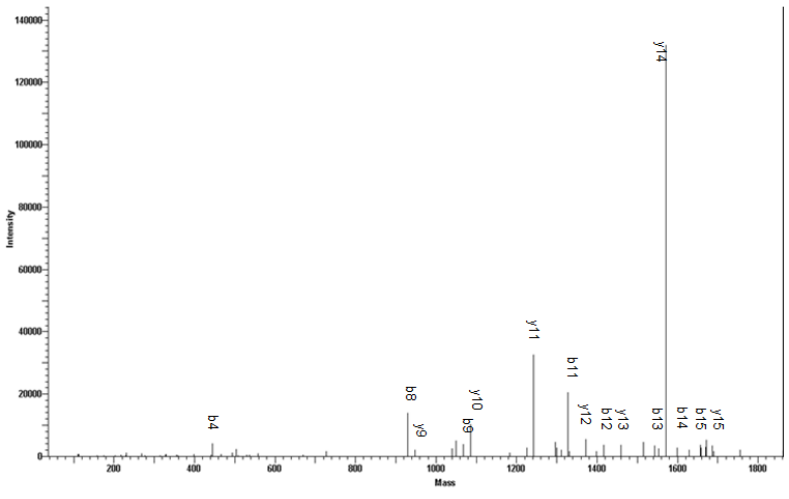
Supplementary Figure 5 Full Western blot of Dll1 in BMSCs presented in Fig. 8B.

(A) Immunoblot analyses of Dll1 in wild-type and MT1-MMP deficient BMSCs. The cleavage of Dll1 is also analyzed in wild-type BMSCs in which MT1-MMP is knocked-down by siRNA (Polyclonal anti-Dll1, sc-8155). **(B)** In both wild-type BMSCs and MT1-MMP deficient BMSCs, Dll1 is knocked-down to confirm the specific bands for Dll1 (Polyclonal anti-Dll1, sc-9102). Dll1-FL represents unprocessed full-length Dll1 and Dll1-CTF represents C-terminal cleaved fragment of Dll1.

A



B



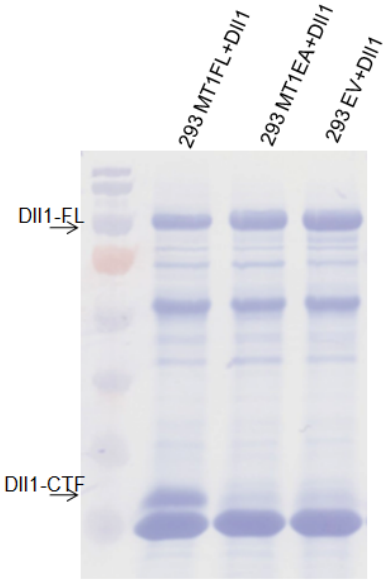
b ion 4 8 9 11 12 13 14 15

M/V/V/D/L/S/E/R/H/M/E/S/Q/G/G/P/F/P

y ion 15 14 13 12 11 10 9

Supplementary Figure 6 Recombinant MT1-MMP cleaves Dll1 peptide. (A) Mass spectrometry analysis after incubating Dll1 polypeptide (^{516}QFLLPEPPP GPMVVDLSERHMESQGGPFP) with recombinant catalytic domain of MT1-MMP. The control experiments are performed in the presence of EDTA inhibition or without MT1-MMP. The fragment at 3188.5 Da is the intact peptide. The fragment at 2015.9 Da is further identified as ^{527}MVVDLSERHMESQGGPFP by tandem MS/MS **(B)**.

A



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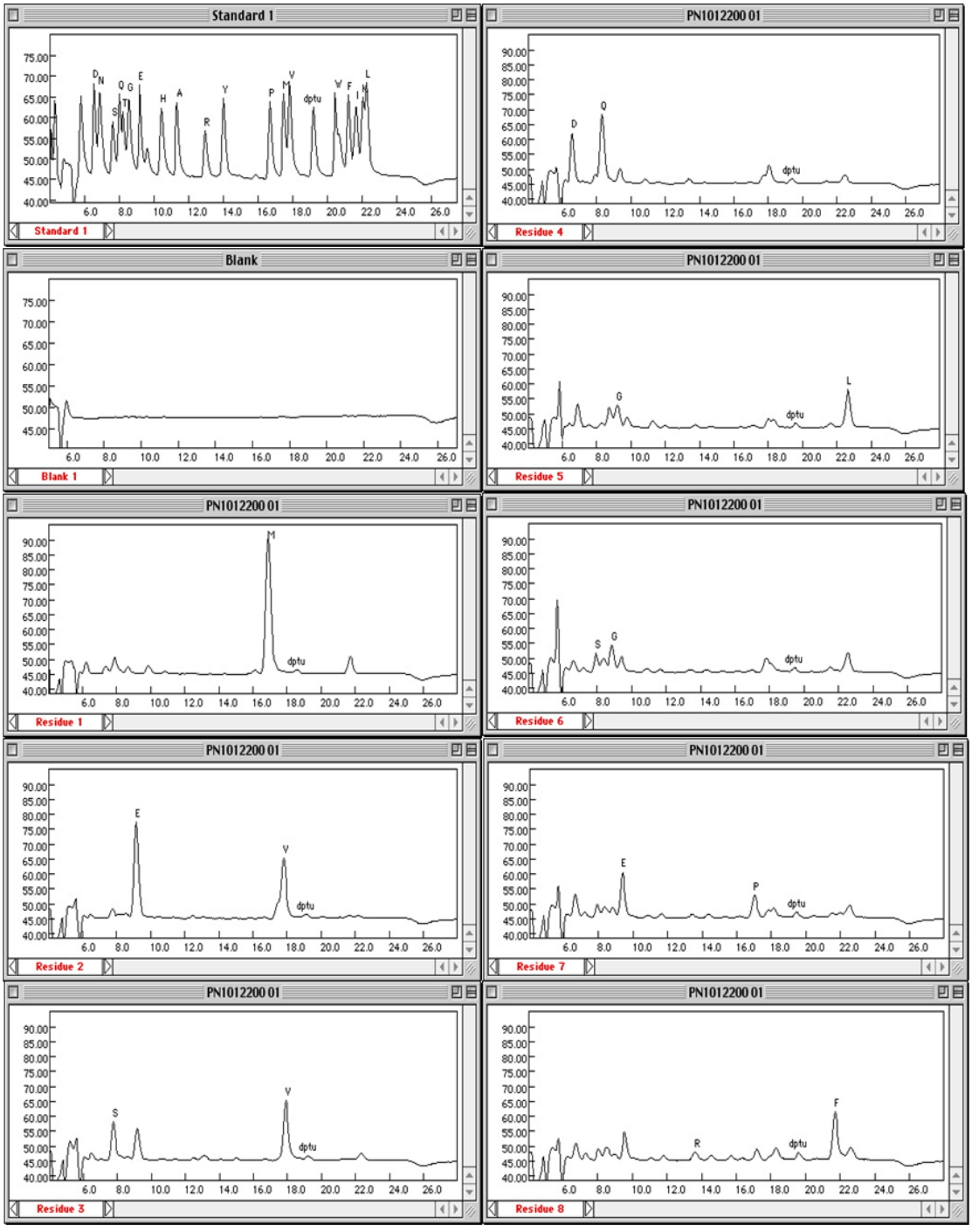
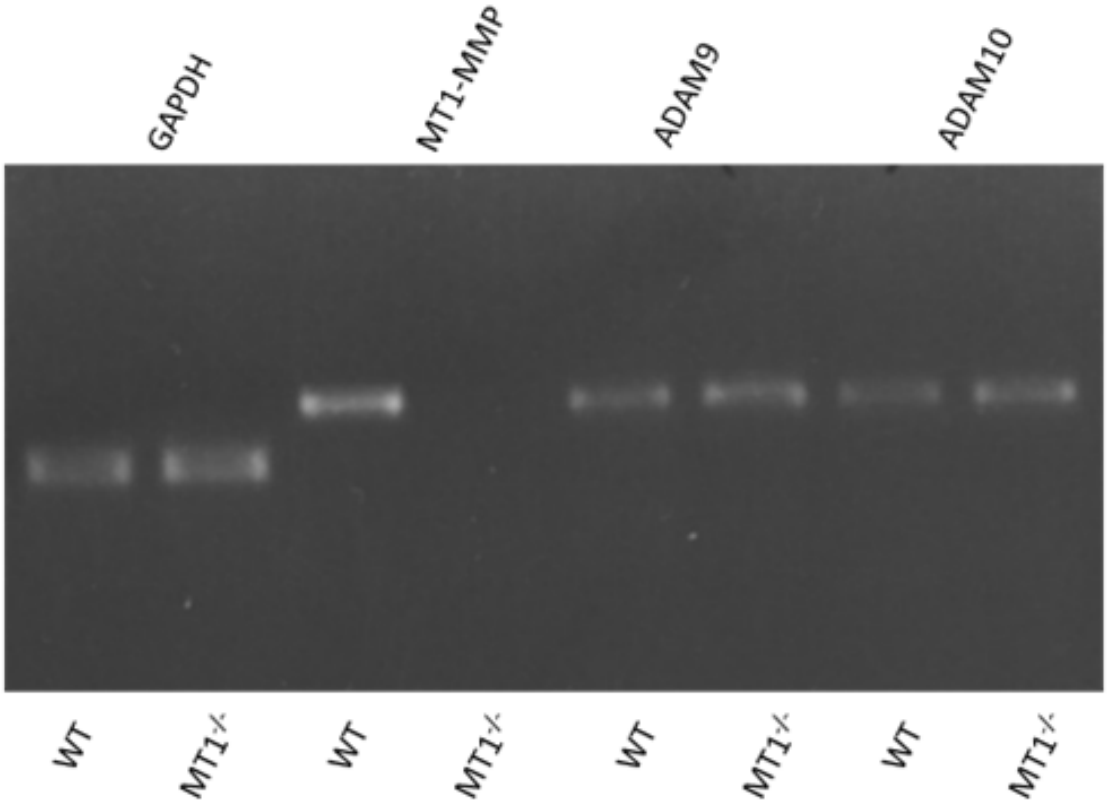


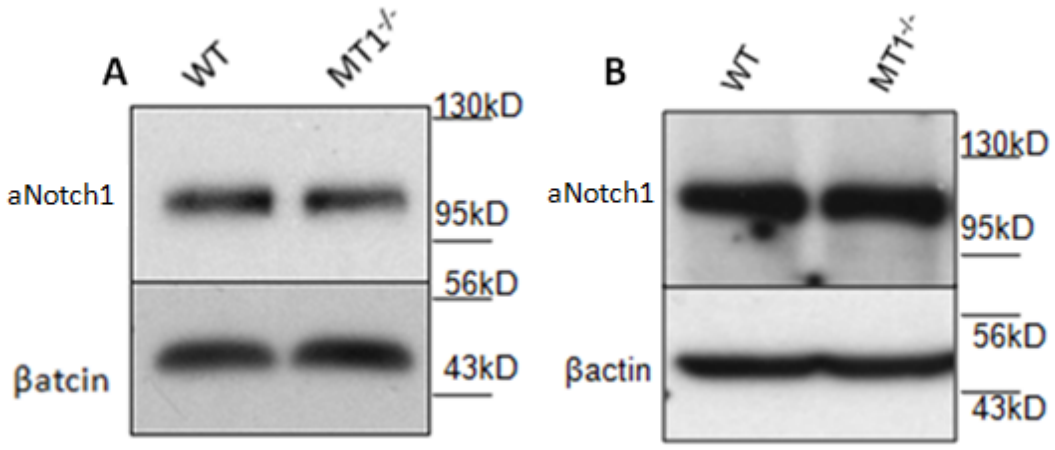
Sequence 1: [527]MVVDLSER Sequence 2: [536]MESQGGPF

Supplementary Figure 7 Edman N-terminal sequencing. (A) Coommassie blue staining of purified Dll1 full length protein (Dll1-FL) and C-terminal fragment (Dll1-CTF) from co-transfected 293 cells with Dll1 and active MT1-MMP (293MT1FL+Dll1), or inactive MT1-MMP (293MT1EA+Dll1), or empty vector (293EV+Dll1). **(B)** Edman N-terminal sequencing of Dll1-CTF purified from Dll1 and active MT1-MMP co-transfected 293 cells.



Supplementary Figure 8 Expression of ADAM9 and ADAM10 in BMSCs.

Semi-quantitative RT-PCR analyses of *ADAM9* and *ADAM10* mRNA levels in BMSCs.



Supplementary Figure 9 Protein level of aNotch1 in thymus and E17.5 fetal liver. (A) Immunoblot analyses of aNotch1 in wild-type and MT1-MMP deficient thymus. **(B)** Immunoblot analyses of aNotch1 in wild-type and M1-MMP deficient fetal liver (E17.5). β -actin serves as internal loading controls.