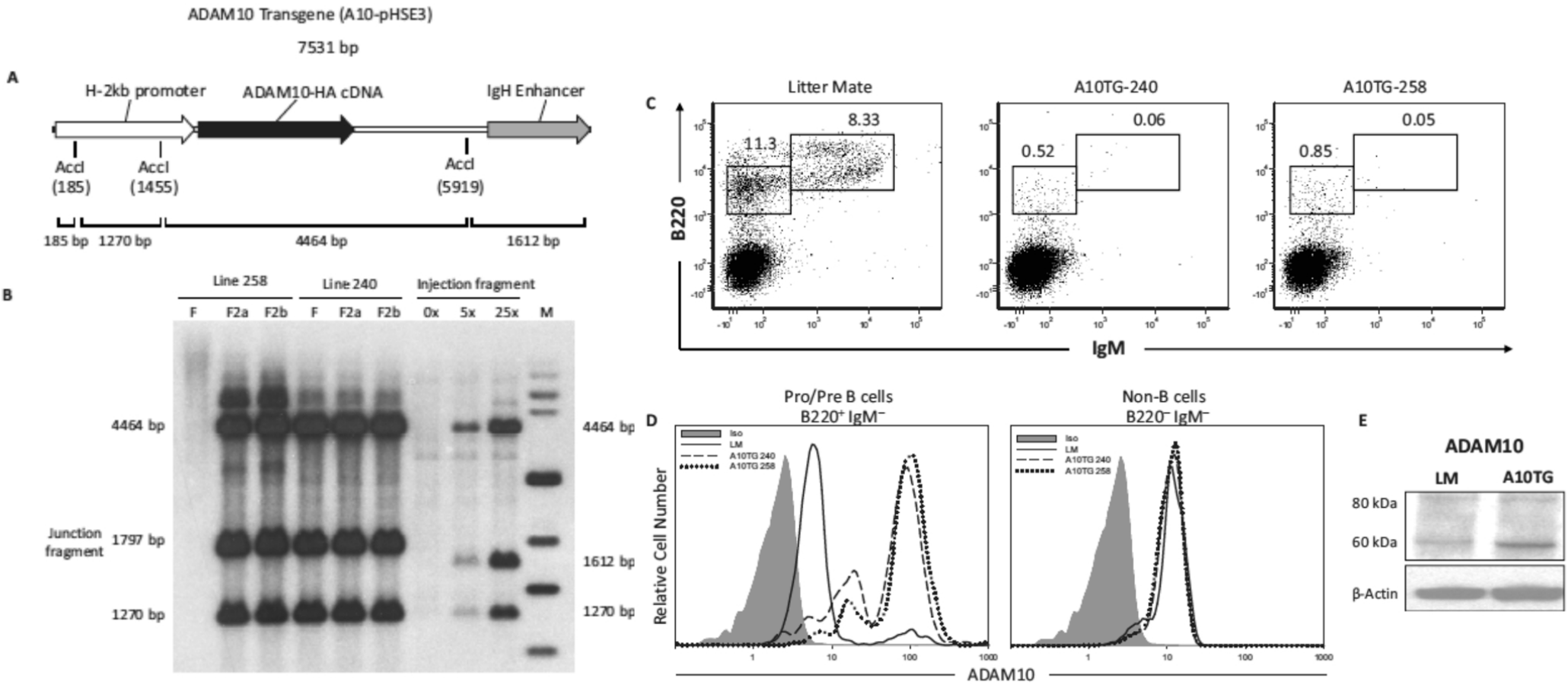
Figure S1 - Generation of A10Tg mice: (A) Schematic of the 7.5-kb XhoI injection fragment containing the murine ADAM10-HA cDNA and the murine H-2Kb promoter and IgH enhancer regulatory elements. (B) Southern blot analysis of genomic tail DNA from both founders (F) and their F2 progeny (F2a and F2b) digested with AccI and electrophoresed on a 0.9% agarose gel along with 1 kb DNA Ladder markers (M). The injection fragment shown in (A) was used as both probe and copy number control (5x and 25x). A10Tg lines 240 and 258 possess greater than 25 copies of the transgene, generating 1797 bp junction fragments (1612 bp + 185 bp) indicative of head-to-tail arrays, as well as 1270 bp and 4464 bp internal fragments. The founder of line 258 appears to be mosaic, identifiable as a transgenic by PCR only. (C) Flow cytometric analysis of pro/pre B cells (B220+Ig IgM_) and immature B cells (B220hiIgM+) in BM of littermate (LM) and A10Tg progeny. (D) ADAM10 surface expression by B220+IgM_cells and B220_IgM_ cells shown in (C). Dot plots and histograms in (C,D) are representative of 6 independent experiments. (E) Western blot analysis of ADAM10 and_-actin protein levels in whole cell lysates of BM cells from indicated mice; ADAM10 pro-form (80 kDa) and ADAM10 mature form (60 kDa), representative of 3 independent experiments.

Generation of ADAM10 Transgenic mice: ADAM10 transgenic (A10Tg) mice were generated with the ADAM10-pHSE3' transgene construct. ADAM10-pHSE3' was produced by subcloning the murine ADAM10-HA cDNA from mADAM10-pcDNA3.1/Zeo (27) into the previously described pHSE3'vector (28), containing the murine H-2Kb promoter and IgH enhancer regulatory elements. Briefly, the ADAM10-HA cDNA was excised using BamHI/SalI and ligated into BamHI/XhoI cut pHSE3'.

ADAM10-pHSE3' was amplified and analyzed by restriction endonuclease digestion and sequence analysis. A 7.5-kb XhoI fragment containing both cDNA and regulatory elements (Fig. S1A) was excised from ADAM10-pHSE3' and injected into C57BL/6 (A10Tg line 240) or C57BL/6 x Balb/c (A10Tg line 258) embryos by the Virginia Commonwealth University Transgenic/Knockout Mouse Core. The resulting offspring were screened for the presence of the ADAM10-HA cDNA by PCR analysis of genomic tail DNA using ADAM10 cDNA sense (5'-CCGACAGTGTTAATTCTGCTCC-3') and anti-sense (5'-TTCTTTCAGCCAGAGTTGTGCG-3') primers. Amplification of DNA from A10Tg founders generated a 652-bp PCR product. Transgene integrity was verified and transgene copy number determined for both A10Tg lines by Southern blot analysis (Fig. S1B). Briefly, genomic tail DNA from both founders and their F2 progeny was digested with AccI and electrophoresed on a 0.9% agarose gel, and the injection fragment from ADAM10-pHSE3' was used as both probe and copy number control. A10Tg line 258 was backcrossed with C57BL/6 mice for at least five generations.

Western blots: Whole cell lysates (30ug) of BM cells generated with lysis buffer containing 0.5% NP40 and protease inhibitors (Roche) were applied to SDS-PAGE and blotted on nitrocellulose membranes. Blots were probed with HRP-conjugated anti-mouse β-actin (Sigma-Aldrich) or unlabeled rabbit anti-ADAM10 (AnaSpec Inc.) followed by HRP-conjugated anti-rabbit IgG (Southern Biotech). Chemiluminescence was visualized with the Pico chemiluminescent kit (Pierce).


A
ADAM10 Transgene (A10-pHSE3)
7531 bp
H-2kb promoter
ADAM10-HA cDNA
IgH Enhancer
AccI (185)
AccI (1455)
AccI (5919)
185 bp
1270 bp
4464 bp
1612 bp
B
Line 258
Line 240
Injection fragment
F
F2a
F2b
F
F2a
F2b
0x
5x
25x
M
4464 bp
Junction fragment
1797 bp
1270 bp
4464 bp
1612 bp
1270 bp
C
Litter Mate
A10TG-240
A10TG-258
8.33
11.3
0.06
0.52
0.05
0.85
B220
IgM
D
Pro/Pre B cells
B220+ IgM−
Non-B cells
B220− IgM−
Iso
LM
A10TG 240
A10TG 258
Relative Cell Number
ADAM10
E
ADAM10
LM
A10TG
80 kDa
60 kDa
β-Actin