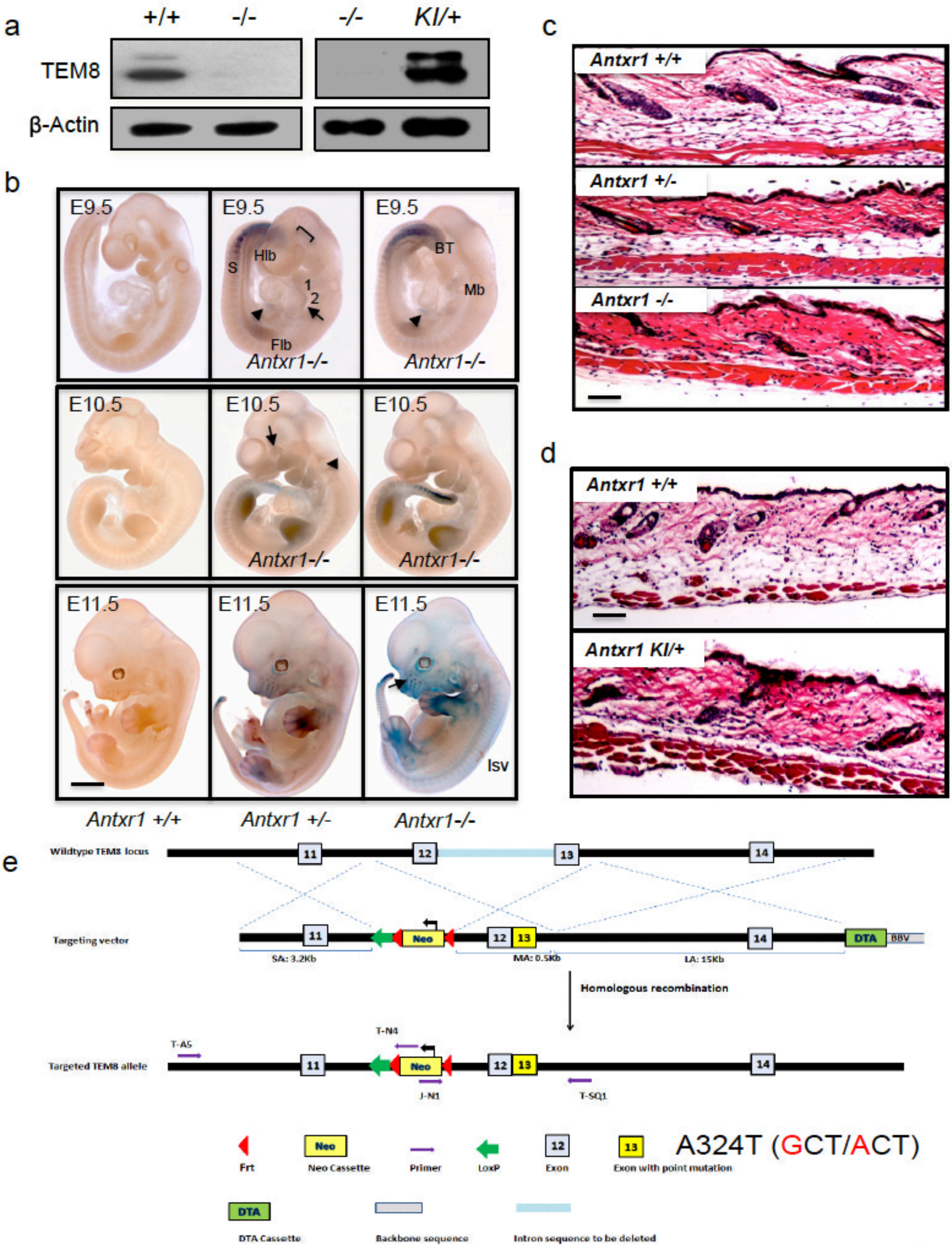
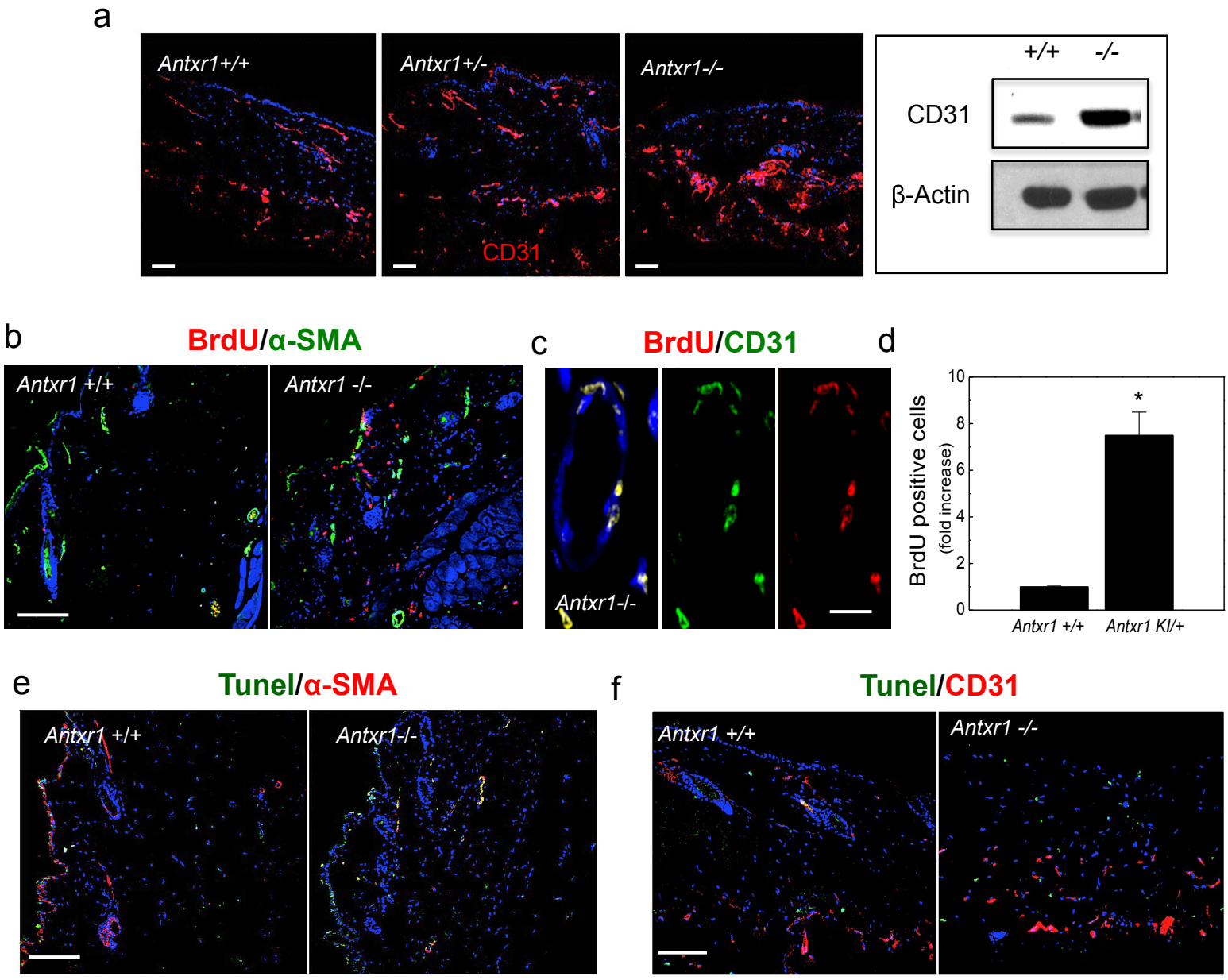
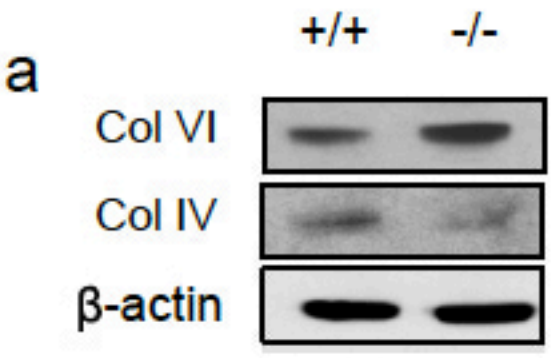
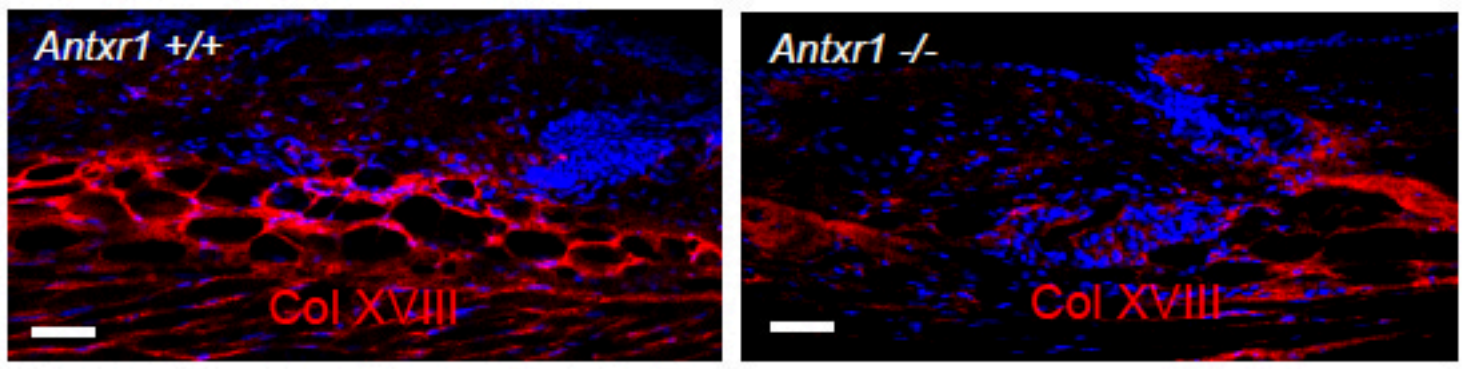


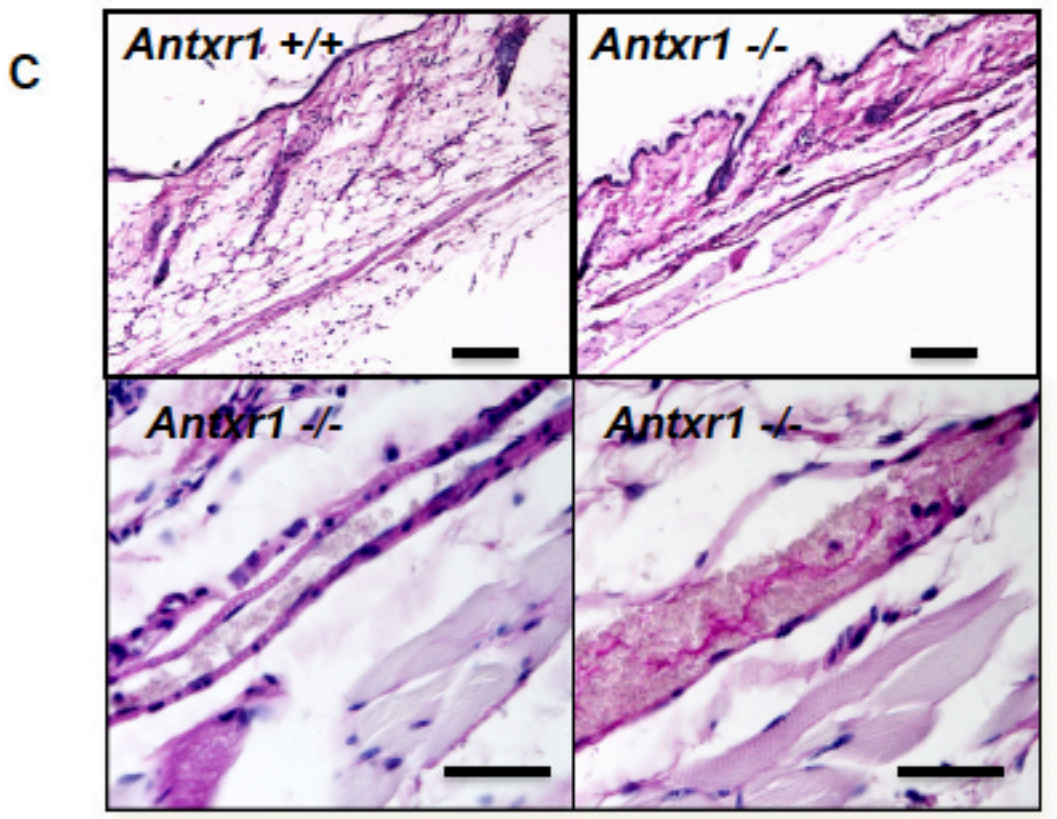
Supplemental Figure 1: TEM8 protein levels, LacZ staining of embryos, and skin histology of adult animals
(**a**) Western-blots of skin lysates of 7-weeks old wild-type (+/+), *Antxr1* -/- and 5-weeks old *KI*/+ mice. (**b**) Whole-mount staining of embryos. Indicated at E9.5 are: First and second pharyngeal arches (1 and 2); bud tail (BT); mandible (Mb); somites (S); forelimb (Flb); hindlimb (Hlb); primary head vein (bracket); common atrial chamber (arrow); sinus venosus (arrowhead); at E10.5: Optic vesicle and perioptic vascular plexus (arrow); otic vesicle (arrowhead); at E11.5: Vibrissae (arrow); intersomitic vessels (Isv). Scale bar 1mm. (**c**, **d**) Representative images of H&E stained 5 μm skin sections of mutant and littermate control mice. Scale bars 100 μm. (**e**) Diagrams showing the strategy used to generate mice with A324T mutation knocked into *Antxr1* locus.

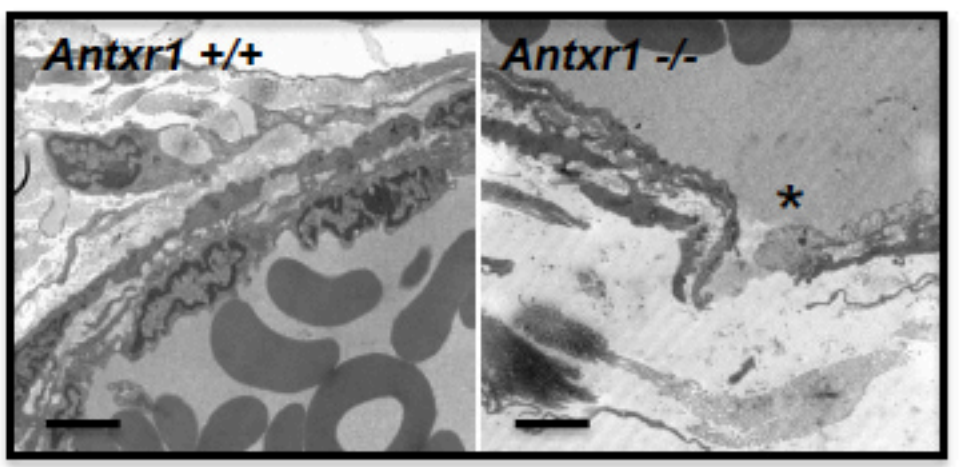


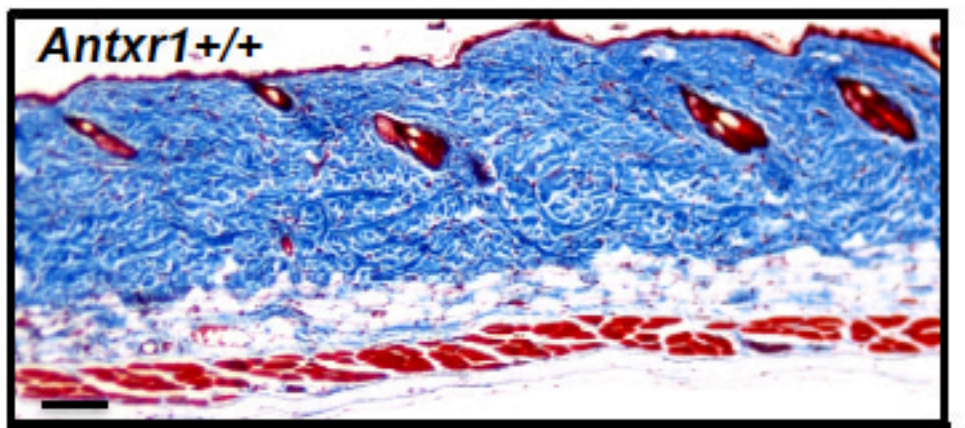
Supplemental Figure S2: Antxr1-/- vasculature is affected by loss of TEM8.
Antxr1 −/− vasculature is affected by loss of TEM8. (a) Left: Immunostaining for CD31 in skin sections from control and mutant mice. Scale bars 50 µm. Right: Western blot of total skin lysates shows increased level of CD31 in skin of mutant animals. (b) Double BrdU (red)/α-SMA (green) staining of skin sections. The α-SMA positive cells are BrdU negative in control and mutant sections. Scale bar 50 µm. (c) Double BrdU (red)/CD31 (green) staining shows correlation between CD31 and BrdU positive cells in mutant vessels. Scale bar 20 µm. (d) Increased numbers of BrdU positive cells in skin sections of *KI/+* mice (n = 6; (*P < 0.05)). (e) Double Tunel (green)/α-SMA (red) staining showing colocalization of Tunel- and α-SMA-positive cells (yellow/merged signal) in mutant skin. Scale bar 50 µm. (f) No correlation between CD31 positive cells (red) and Tunel positive cells (green) in skin sections of control and mutant mice. Scale bar 50 µm.

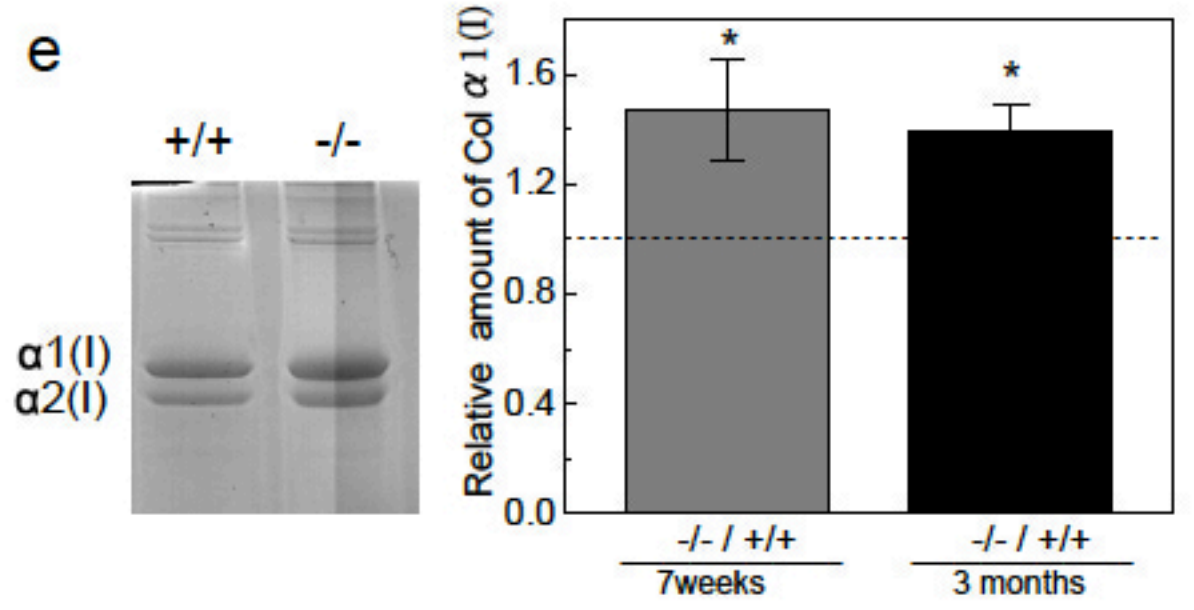






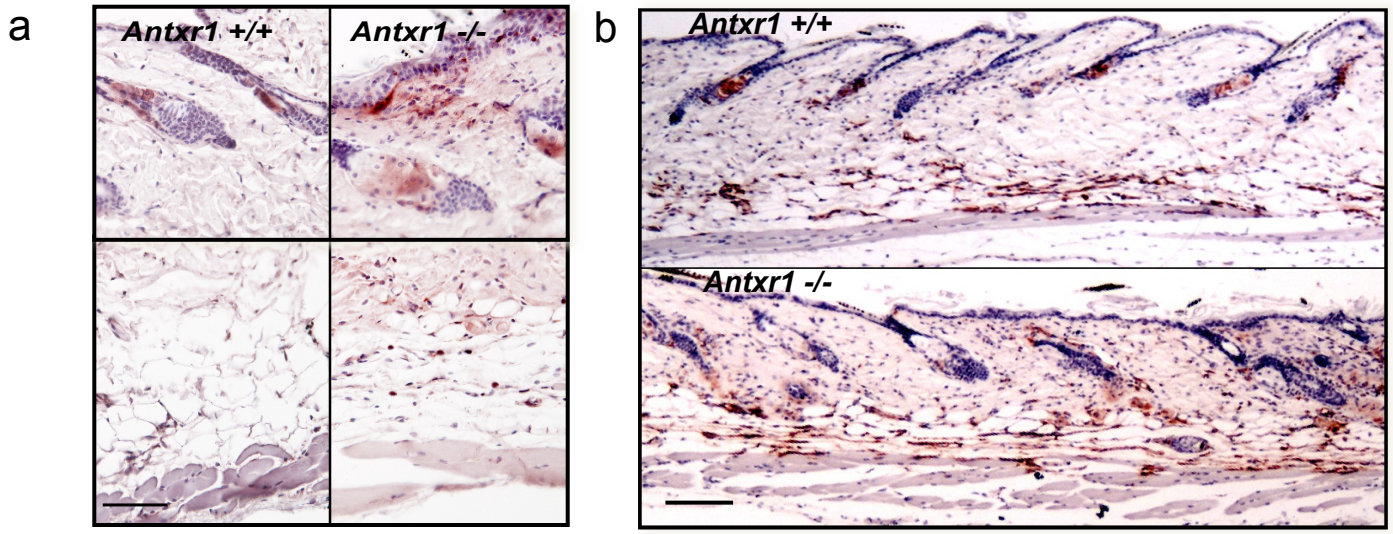




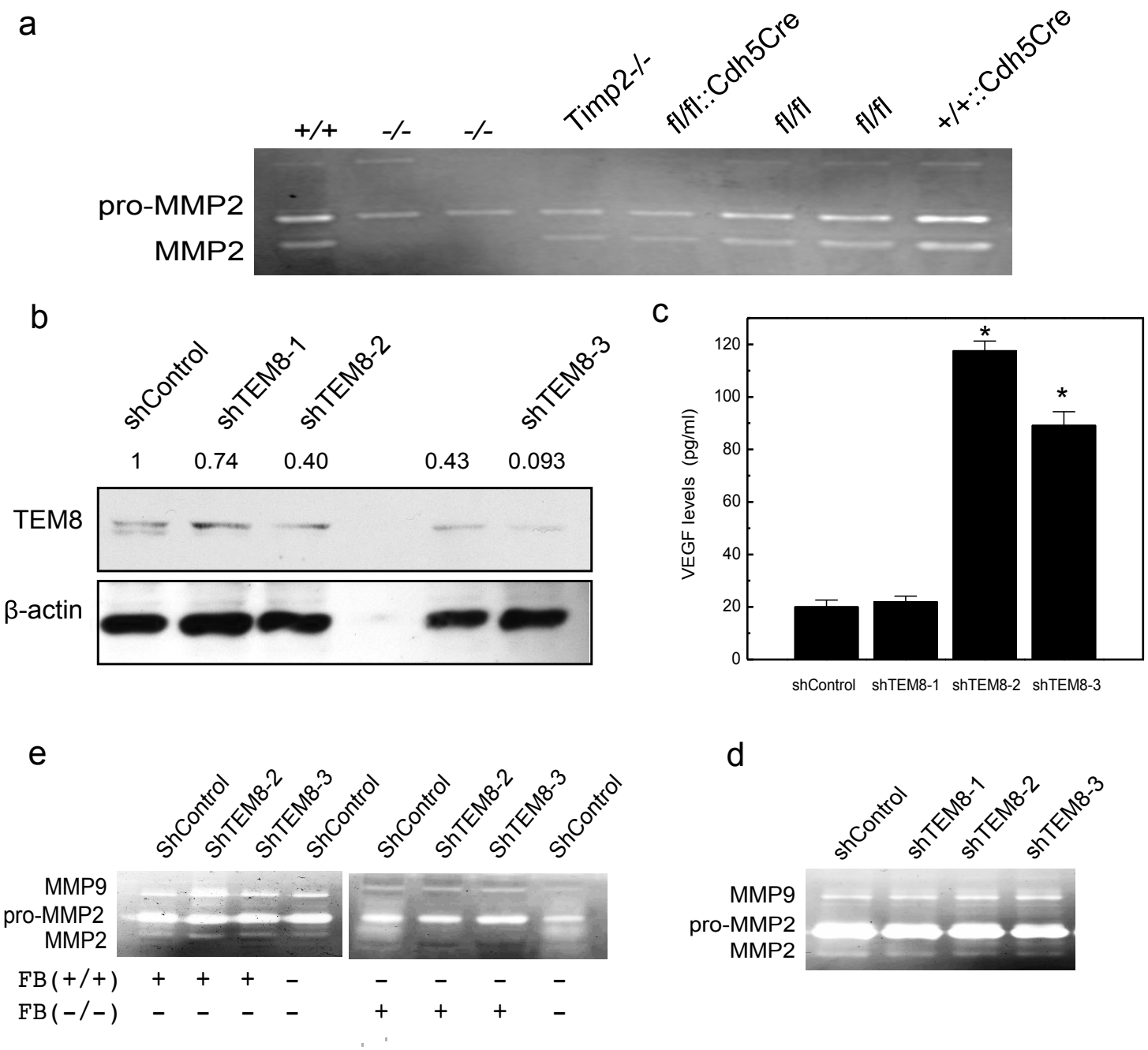


Supplemental Figure 3: Effect of TEM8 loss on extracellular matrix

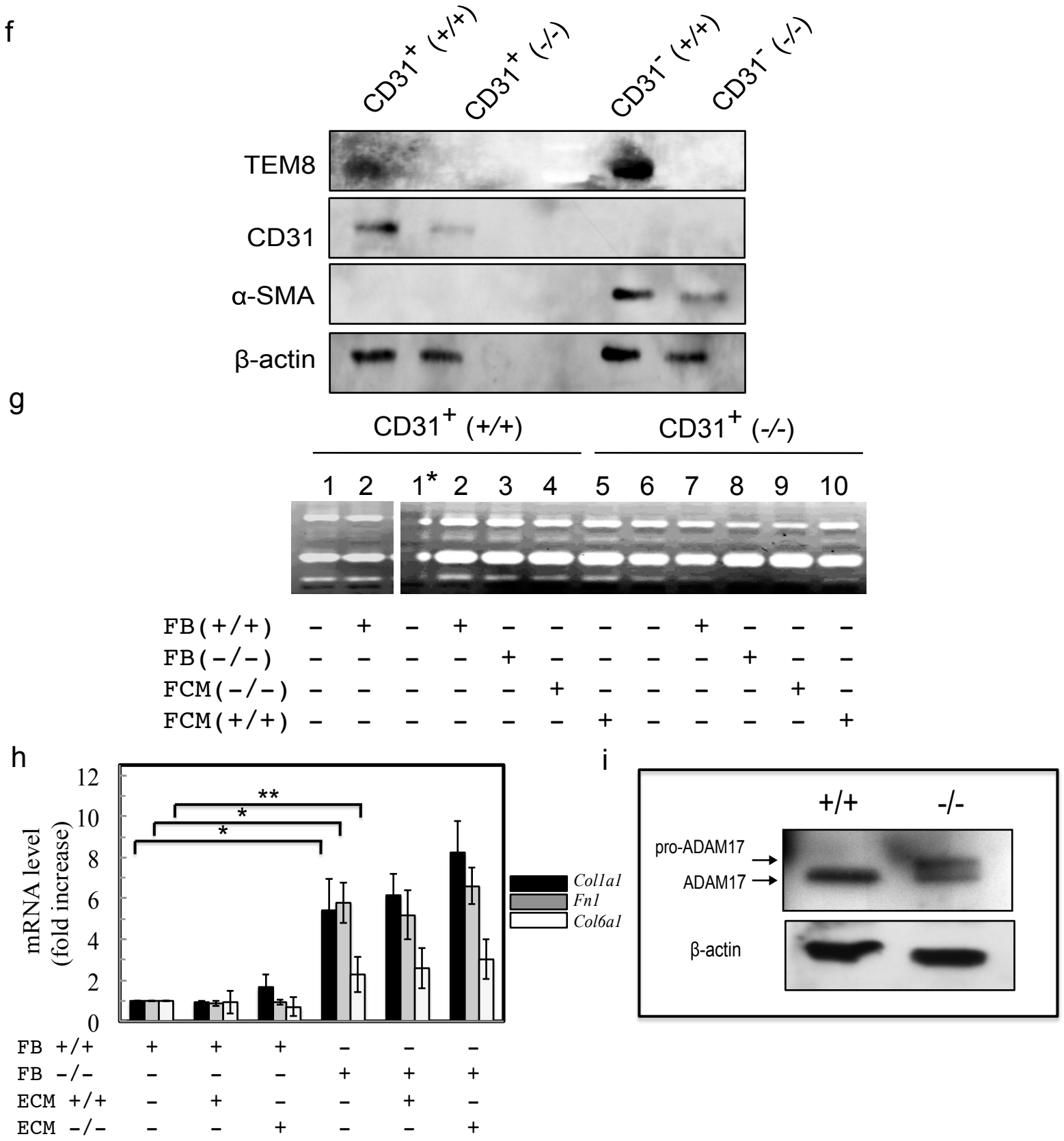
(**a**) Western blotting of total skin lysates shows increased level of collagen VI and reduced level of collagen IV in mutant mice. (**b**) Reduced mmunostaining for collagen XVIII (red) in cutaneous vasculature of mutant mice. Scale bars 50 μm. (**c**) PAS staining shows abnormal thickening of blood vessel walls (top; scale bars 50 μm) and accumulation of PAS-stained material in vessels of mutant mice (bottom; scale bars 25 μm). (**d**) Electron microscopy of cutaneous vasculature; star indicates ruptured vessel. Scale bars 500 nm. (**e**) Left: PAGE of pepsin-resistant extract from skin of control and mutant littermates. Right: Mass spectrometry data for collagenα1(I) band in PAGE of pepsin-extracted triple-helical collagen from skin of 7-week and 3-month old control and mutant mice (n=4; * $P < 0.05$). (f) Masson's trichrome staining of skin sections of 3-month old control and mutant mice. Scale bar 100 μm.



Supplemental Figure 4: Effect of TEM8 loss on ECM proteolysis
(**a**) Immunohistochemical staining of skin sections for MMP9 shows staining in epidermis, hair follicles, sebaceous glands, and in interstitial/dermal cells (Scale bar, 50 μm). (**b**) Strong immunohistochemical staining for MMP2 in skin vascular region (Scale bar, 100 μm).



Supplemental Figure S5: Effect of TEM8 loss on MMP2 activity.
(a) Gelatin zymography data on which Fig. 6d is based. (b) Western blots of shTEM8-1, shTEM8-2 shTEM8-3 and control endothelial cell lysates with antibodies against TEM8. Relative TEM8 band intensities are shown above. (c) ELISA of secreted levels (pg/ml) of VEGF in shTEM8-1, shTEM8-2 shTEM8-3 and control shTEM8 endothelial cell lines (n = 3; * $P < 0.05$). (d) Representative gelatin zymography data of lysates of shTEM8-1, shTEM8-2, shTEM8-3 and shControl endothelial cell lines on which Fig. 7b is based. (e) Representative zymogram on which Fig. 7c is based.



Supplemental Figure S5: Effect of TEM8 loss on MMP2 activity

(f) Immunoblotting with antibody against TEM8 of lysates of primary CD31-positive (CD31+) and CD31-negative (CD31−) cells isolated from skin of 7-week old *Antxr1*+/+ and *Antxr1* −/− mice. The purity of CD31− positive and CD31− negative cells confirmed by western blotting with antibodies against CD31 and α-SMA. (g) Gelatin zymography data, on which Fig. 7d is based, of lysates of primary endothelial (CD31+) cells from *Antxr1* +/+ and *Antxr1* −/− mice either co-cultured with fibroblasts (FB) from *Antxr1* +/+ or *Antxr1* −/− mice or exposed to conditioned medium (FCM) from such fibroblasts. Because of technical problem with lane 1 (*), lanes 1 and 2 from a different gel are shown at left. (h) Transcript levels of *Col1a1, Col6a1* and *Fn1* in *Antxr1* +/+ and *Antxr1* −/− fibroblasts (FB +/+ and FB −/−) cultured in conditioned media of wild type (ECM +/+) or mutant (ECM −/−) CD31-positive cells (n = 4; *P < 0.05, **P < 0.005). (i) Western blotting of ADAM17 in total skin lysates of 7-week old mutant and control mice.

Supplemental Table S1

Gene	Forward primer 5'-	Reverse primer 5'-
Vegfr1	CTCAGGGTCGAAGTTAAAAGTGC	TTGCCTGTTATCCCTCCCACA
Vegfr2	TCCAGAATCCTCTTCCATGC	CCAGAGACCCTCGTTTTCAG
Tie2	CAGCTTGCTCCTTTATGGAGTAG	ATCAGACACAAGAGGTAGGGAAT
Itgb1	ATGCCAAATCTTGCGGAGAAT	TTTGCTGCGATTGGTGACATT
Vegfa	GGAGATCCTTCGAGGAGCACTT	GGCGATTTAGCAGCAGATATAAGAA
Vegfb	GGCAACACCAAGTCCGAATG	GCTGTGTTCTTCCAGGGACATC
Vegfc	GGCTGCTCCAAACTCCTTC	CTCAATACCAGGACAGGGGA
Angpt1	CATTCTTCGCTGCCATTCTG	GCACATTGCCCATGTTGAATC
Angpt2	TGCACCACATTCTGTTGGAT	GACGACTCAGTGCAAAGGCT
Col6a1	CATGGTTCCTTGTAGCCCTC	CAAGTACTTCGGGAAAGGCA
Col6a2	CATCGGTGACATGTTCCACG	TGAGGAAGACGAAGGAGAGC
Col6a3	TCTTCAGCAACAAGCCCACC	ACTGGAACAGTGTGGTAGCC
Col6a4	TCGCATTATTCGTGCTCGCC	GCCTACACCATGTCTCACACTC
Col6a5	GCTCAGTGATTGACAGCTTCC	GTGTGTCTCCCCTCTCTAACTC
Col6a6	TTCCTCAGCCTACAACCTG	CCAACAGGAACACCACATCAG
Col1α1	GGTTTCCACGTCTCACCATT	CGGCTCCTGCTCCTCTTAG
Col18a1	CTCATCAGGACTTTCAGCCAGT	CTGACCTGTAGCCCCAGTAGTT
Col4a1	CAAGCATAGTGGTCCGAGTC	AGGCAGGTCAAGTTCTAGCG
Lama5	TGCCTCCTCCAAGAGGGATTGTTT	TTCGCGAGTATTCTGTGGTGCAGA
Fn1	ACTGGATGGGGTGGGAAT	GGAGTGGCACTGTCAACCTC
Col3a1	TGCCCACAGCCTTCTACACCT	CCAGCTGGGCCTTTGATACCT
Col2a1	GTGTCACACACACAGATGCG	CTACGGTGTCAGGGCCAG
Mmp2	GCGCTTTTCTCGAATCCAT	GGGTATCCATCTCCATGCTC
Mmp9	ACGACATAGACGGCATCCA	GCTGTGGTTCAGTTGTGGTG
Timp1	TGGGGAACCCATGAATTTAG	ATCTGGCATCCTCTTGTTGC
Timp2	GAATCCTCTTGATGGGGTTG	CGTTTTGCAATGCAGACGTA
Timp3	GCTTCTTTCCCACCACTTTG	GTGCTCCTGAGCTGTTGGA
Cxcl12	CAGAGCCAACGTCAAGCA	AGGTACTCTTGGATCCAC
Cxcr4	AGCATGACGGACAAGTACC	GATGATATGGACAGCCTTACAC
Cxcl12 (h)[*]	TGCCAGAGCCAACGTCAAG	CAGCCGGGCTACAATCTGAA
Cxcr4 (h)[*]	TGACGGACAAGTACAGGCTGC	CCAGAAGGGAAGCGTGATGA
Gapdh	TGGCAAAGTGGAGATTGTTGCC	AAGATGGTGATGGGCTTCCCG

(h)[*] human