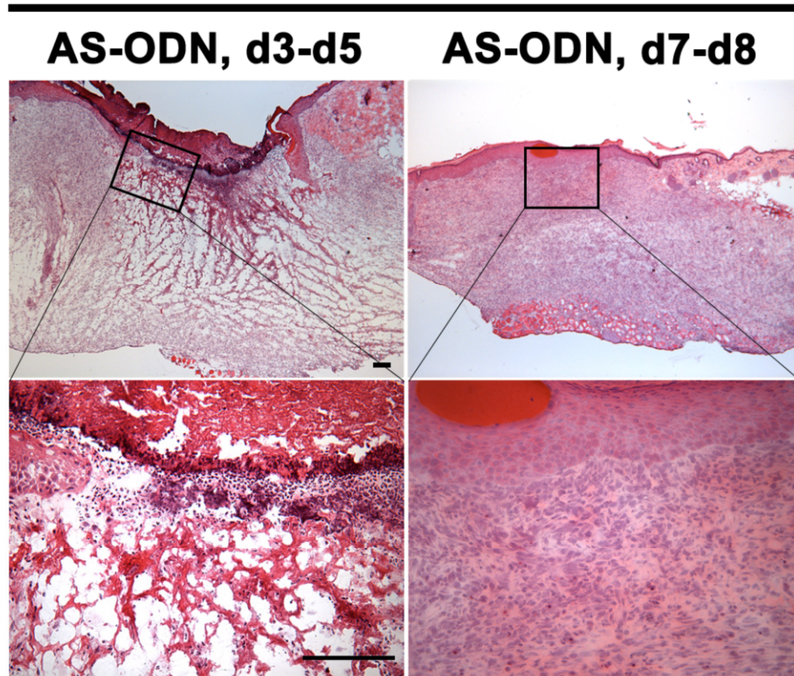
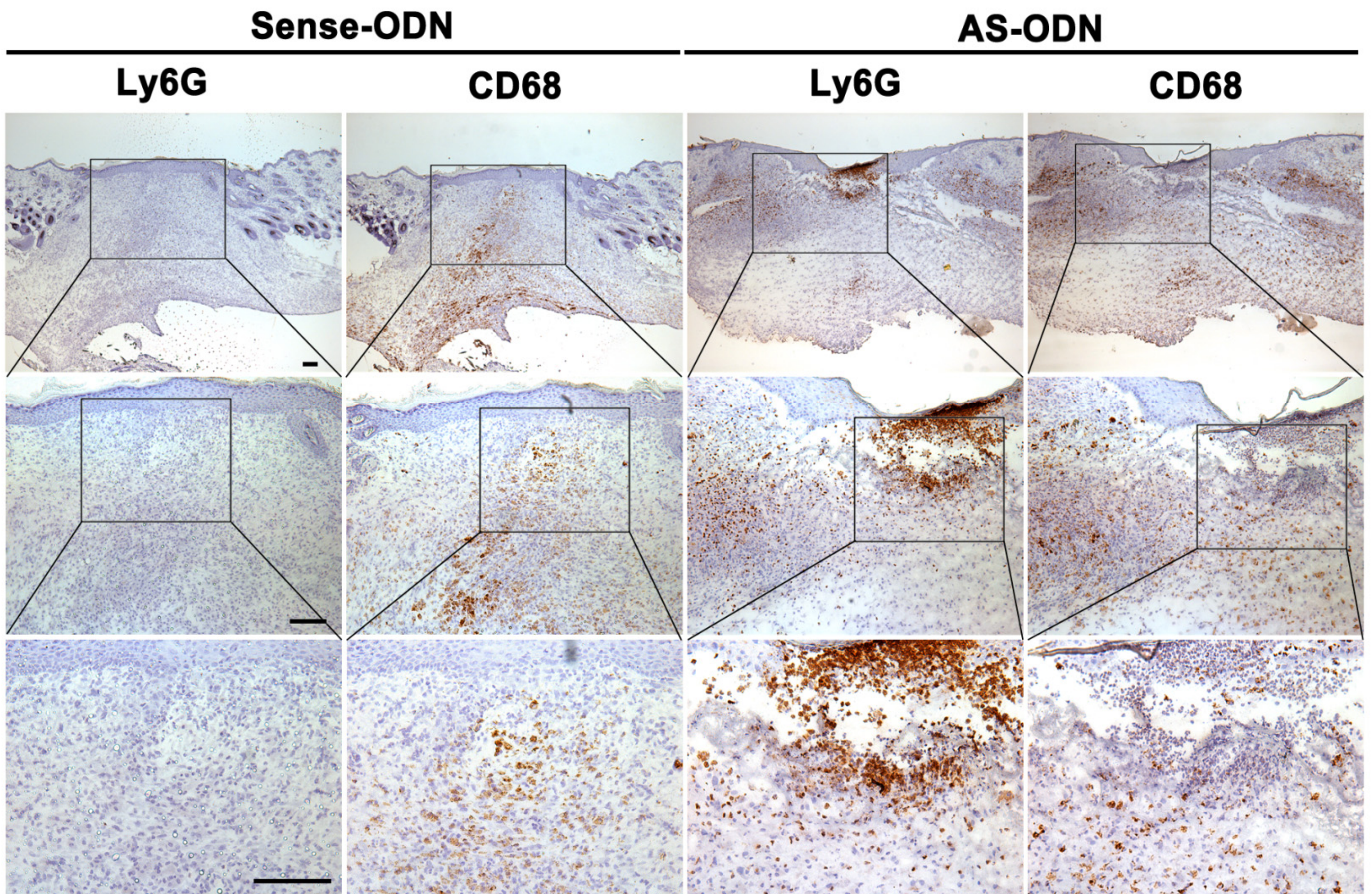
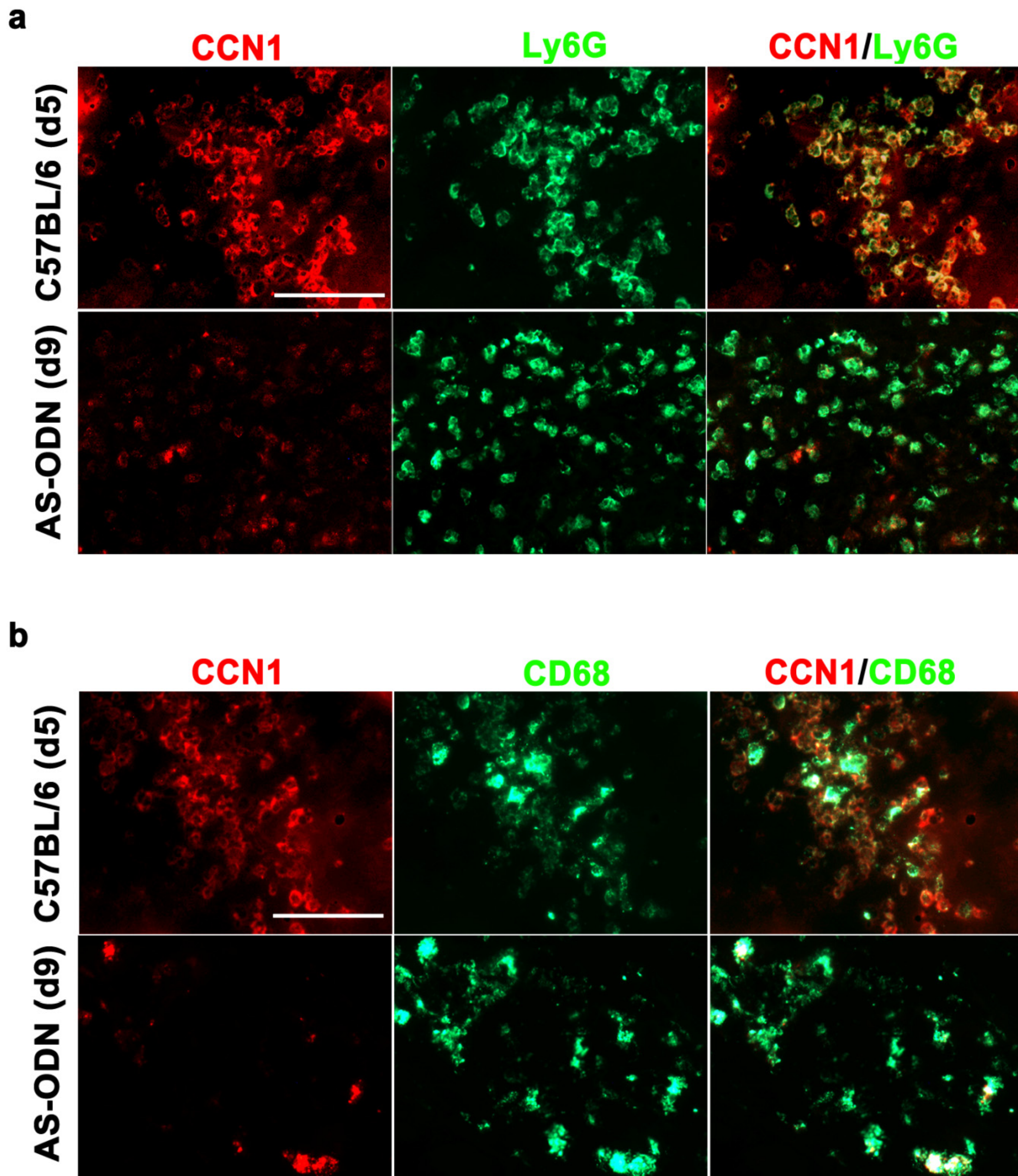


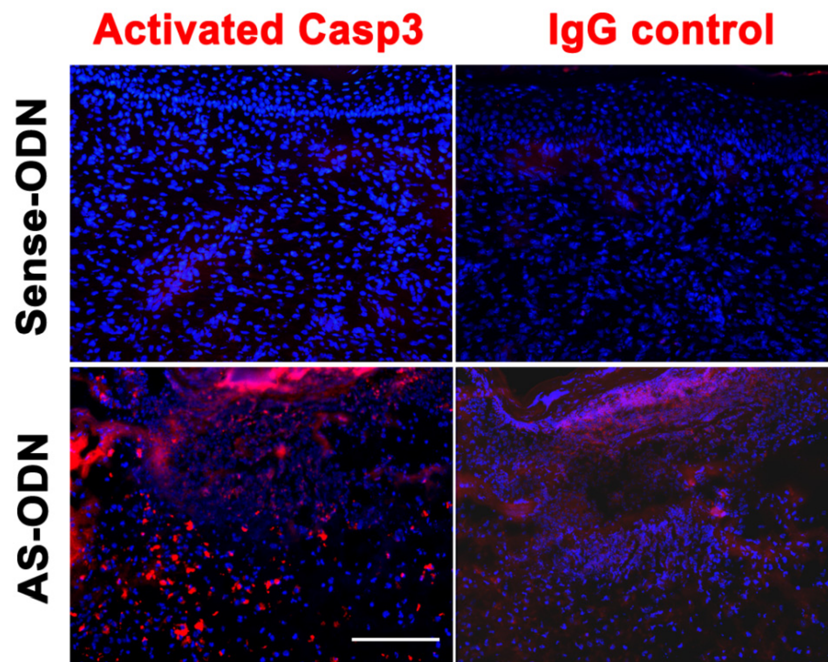
Supplementary Figure S1. Targeting early *Ccn1* expression in wound healing results in persistent inflammatory cells infiltration. Mouse excisional wounds were treated with *Ccn1* antisense oligonucleotides (AS-ODN) either from days 3-5 to target early expression or days 7-8 to target late expression, and day 9 wounds were sectioned and stained with H&E (Bars=100μm). Boxed areas were enlarged with higher magnification. Persistent inflammatory cells infiltration and impaired granulation tissue formation were observed only in wounds with knockdown of early *Ccn1* expression



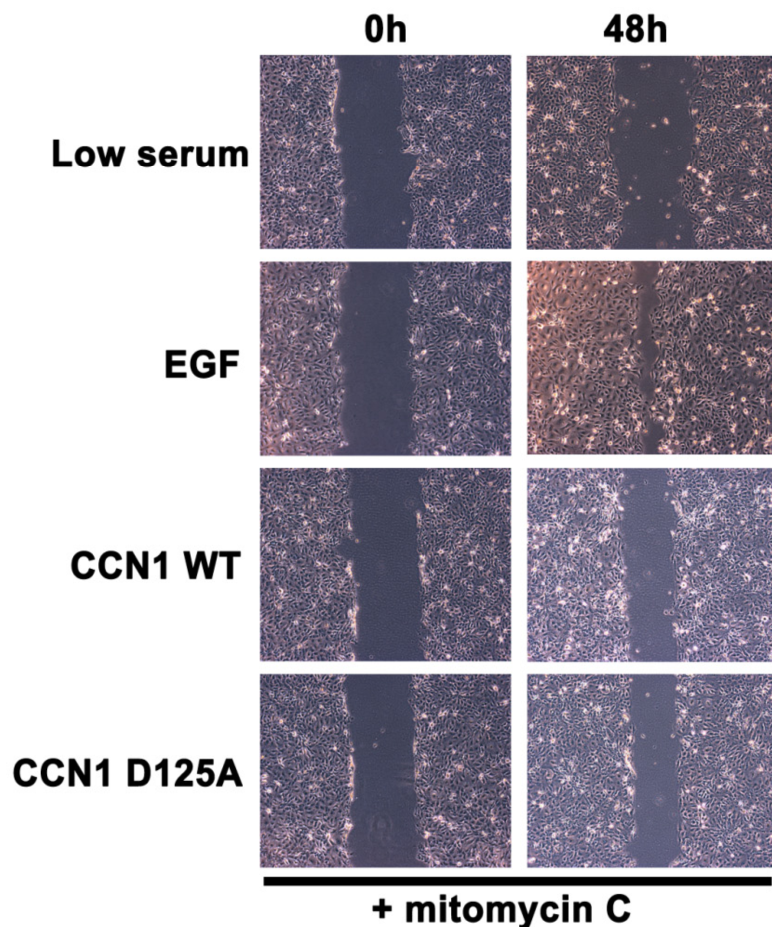
Supplementary Figure S2. AS-ODN-treated wounds exhibit accumulation of neutrophils. Both sense-ODN and AS-ODN-treated wounds of C57BL/6 mice (day 9) were stained with antibodies against Ly6G (neutrophils) or CD68 (macrophages). Boxed areas were enlarged with higher magnification. Bars=100μm

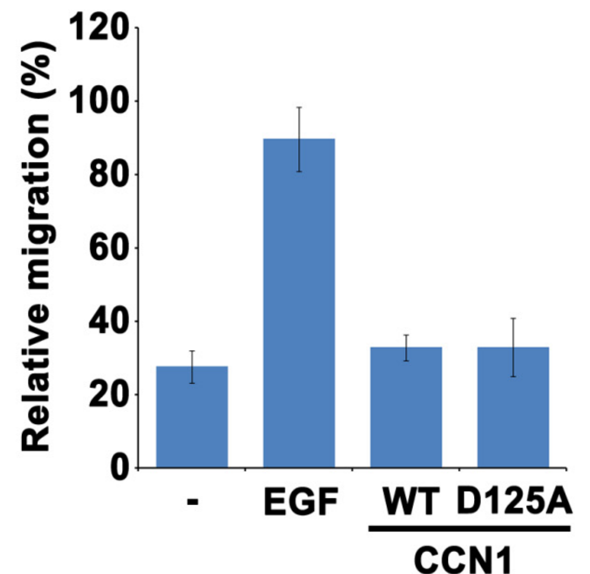


Supplementary Figure S3. CCN1 expression and knockdown in both neutrophils and macrophages. Cutaneous wounds from untreated C57BL/6 mice (day 5) or mice with AS-ODN treatment (day 9) were visualized by double-immunofluorescence staining using antibodies against CCN1 (red) and Ly6G (green, panel **a**) or CD68 (green, panel **b**). (Bar=50 μm).

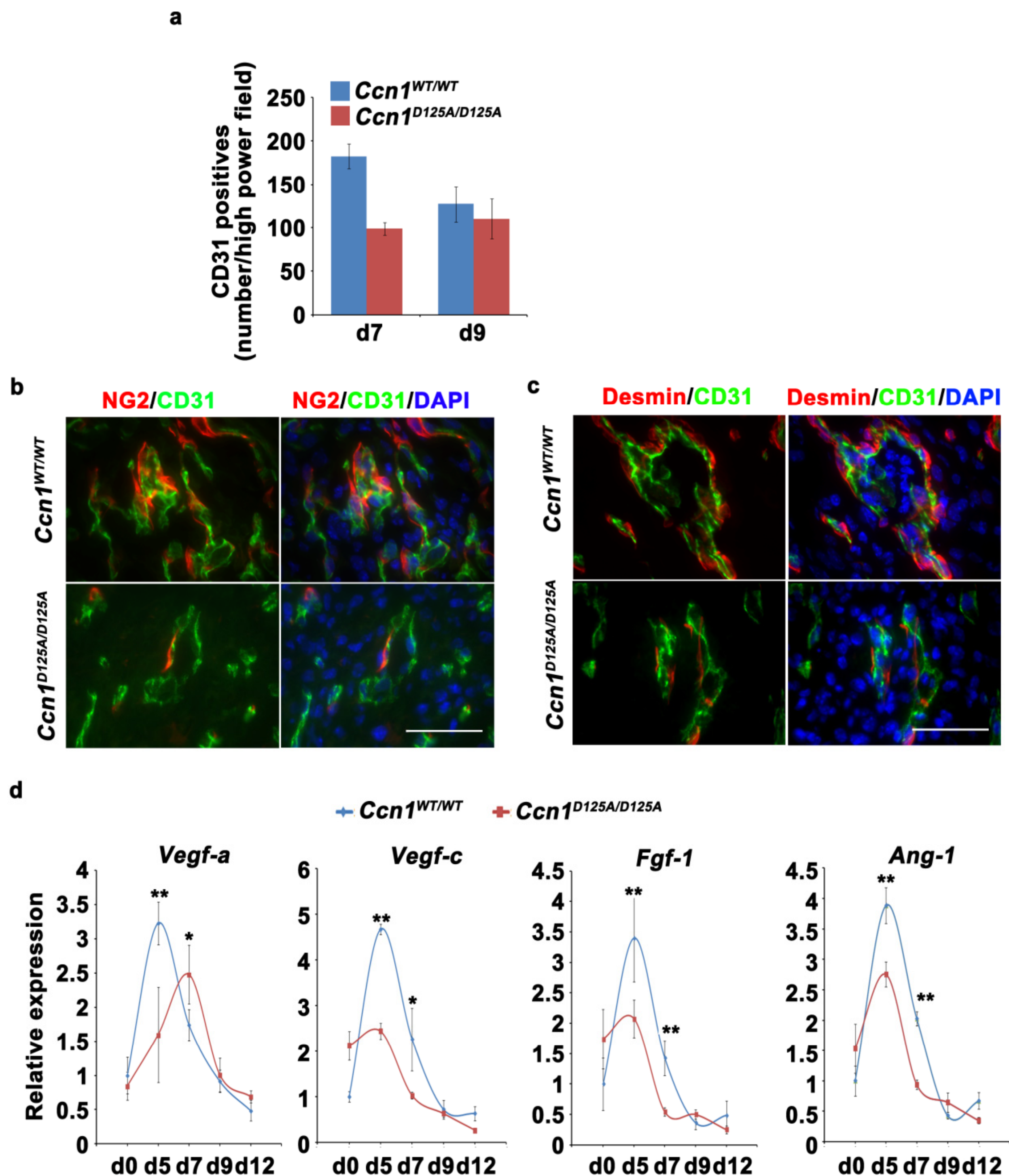


Supplementary Figure S4. Increased detection of apoptotic cells in AS-ODN-treated wounds. Wound tissue sections (day 9) from mice treated with AS-ODN or sense-ODN from day3 to day7 were stained with anti-active caspase3 (red) and counterstained with DAPI (blue). Diffused staining seen on the top of AS-ODN-treated wound is the scab area, as these wounds have not re-epithelialized (see Fig.1i). Isotype IgG was used as a control. Bar=100μm.

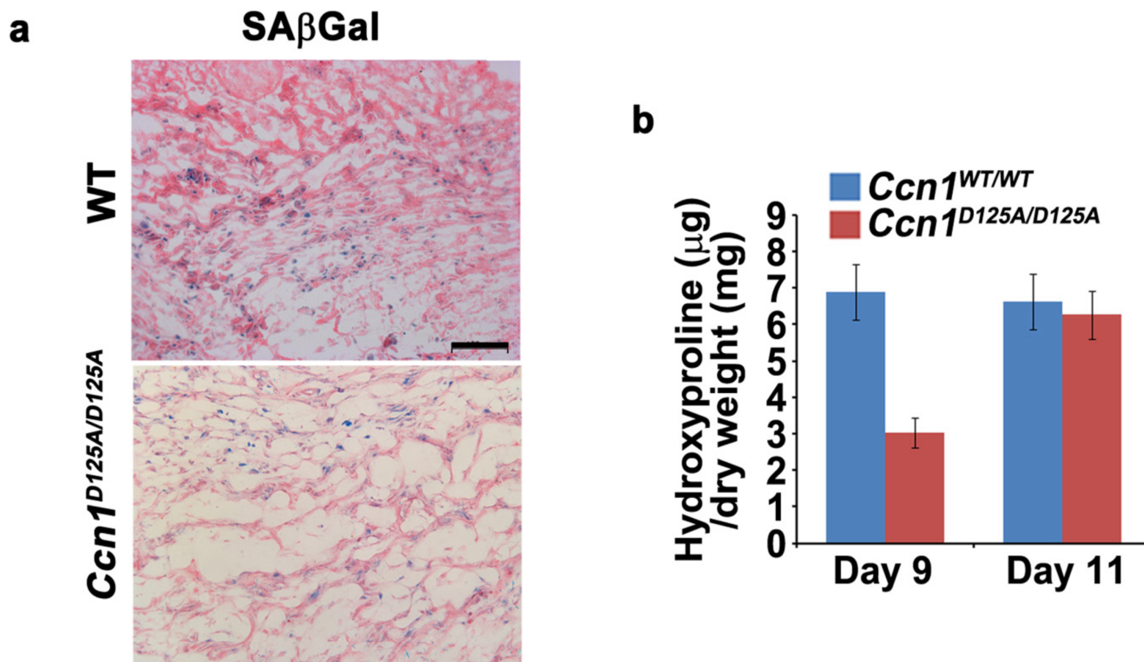




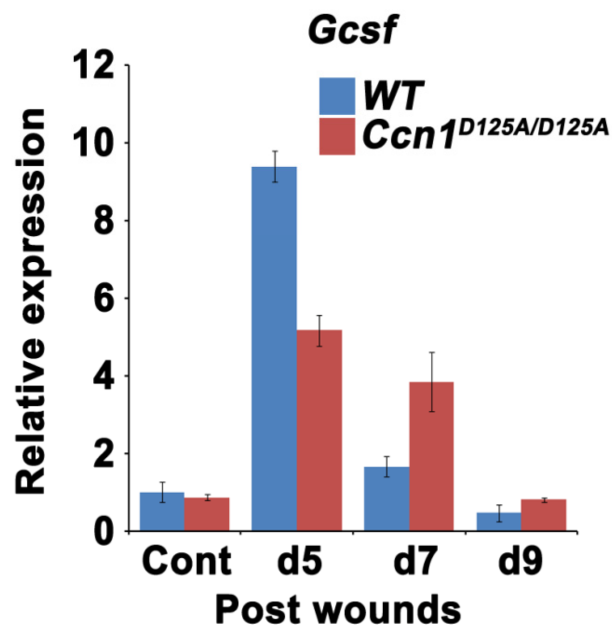
Supplementary Figure S5. CCN1 shows no effect on keratinocytes migration. (**a and b**) Human immortalized keratinocytes (HaCaT) were plated on collagen-coated plate and serum-starved for 48h. Cells were pre-treated with mitomycin C (10 µg per ml), followed by the treatment of recombinant CCN1 proteins (WT and D125A; 2 µg per ml each) or rhEGF (10 ng per ml). Cell migration was analyzed after 48 h. Representative images were shown (**a**) and cell migration was quantified using ImageJ software (n=3 per treatment). (**b**) Data represent means ± standard deviations. Experiments were conducted in triplicate.



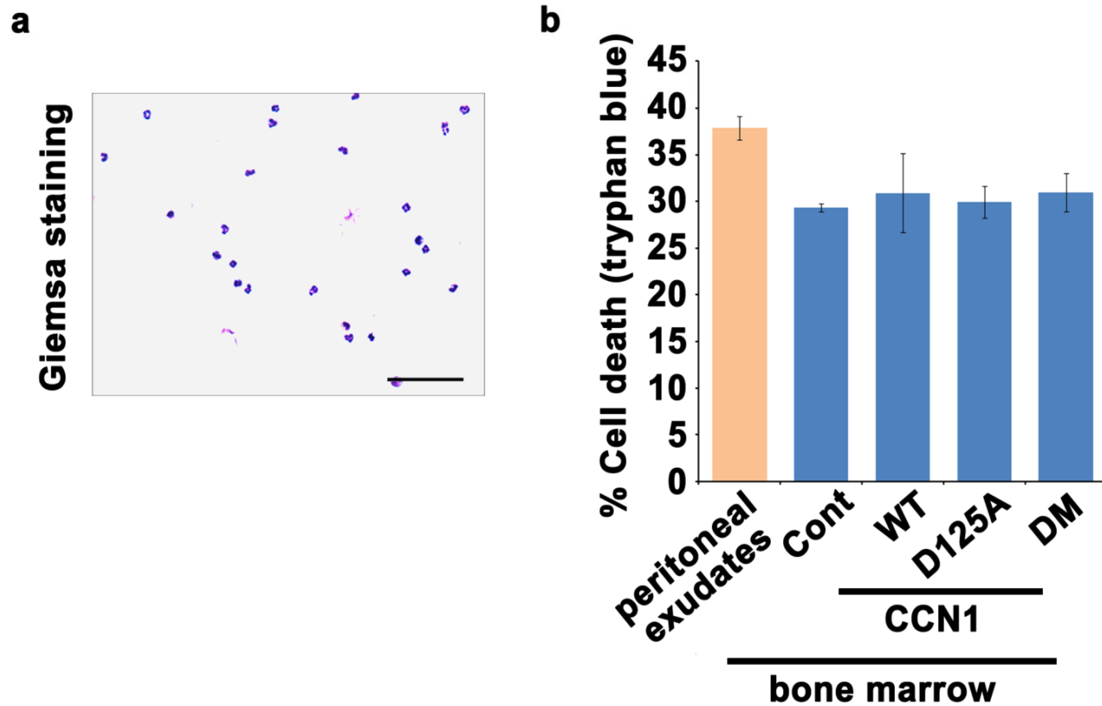
Supplementary Figure S6. ***Ccn1$^{D125A/D125A}$* mice exhibit impaired angiogenesis in wound healing.** (**a**) Wound sections were stained for CD31, and the number of CD31 positive endothelial cells were counted in randomly selected high-powered fields (n=6 per genotype). (**b**) Double immunofluorescence staining for CD31 (endothelial cells; green) and NG2 (pericytes; red) or (**c**) CD31 and desmin (pericytes; red) was performed to visualize pericyte recruitment by blood vessels. Extensive association and juxtaposition of pericytes with endothelial cells was observed in *WT* wounds but not in wounds of *Ccn1$^{D125A/D125A}$* mice. Bar=40μm. (**d**) Expression of angiogenesis-related genes (*Vefg-a*, *Vegf-c*, *Fgf-1*, and *Ang-1*) in *WT* and *Ccn1$^{D125A/D125A}$* wounds was analyzed using qRT-PCR (n=4 per genotype). Statistical analyses were conducted using one-sided two-sample *t*-tests (qRT-PCR). *$p<0.03$, **$p<0.006$. Data represent means ± standard deviation. All experiments were conducted in triplicates.



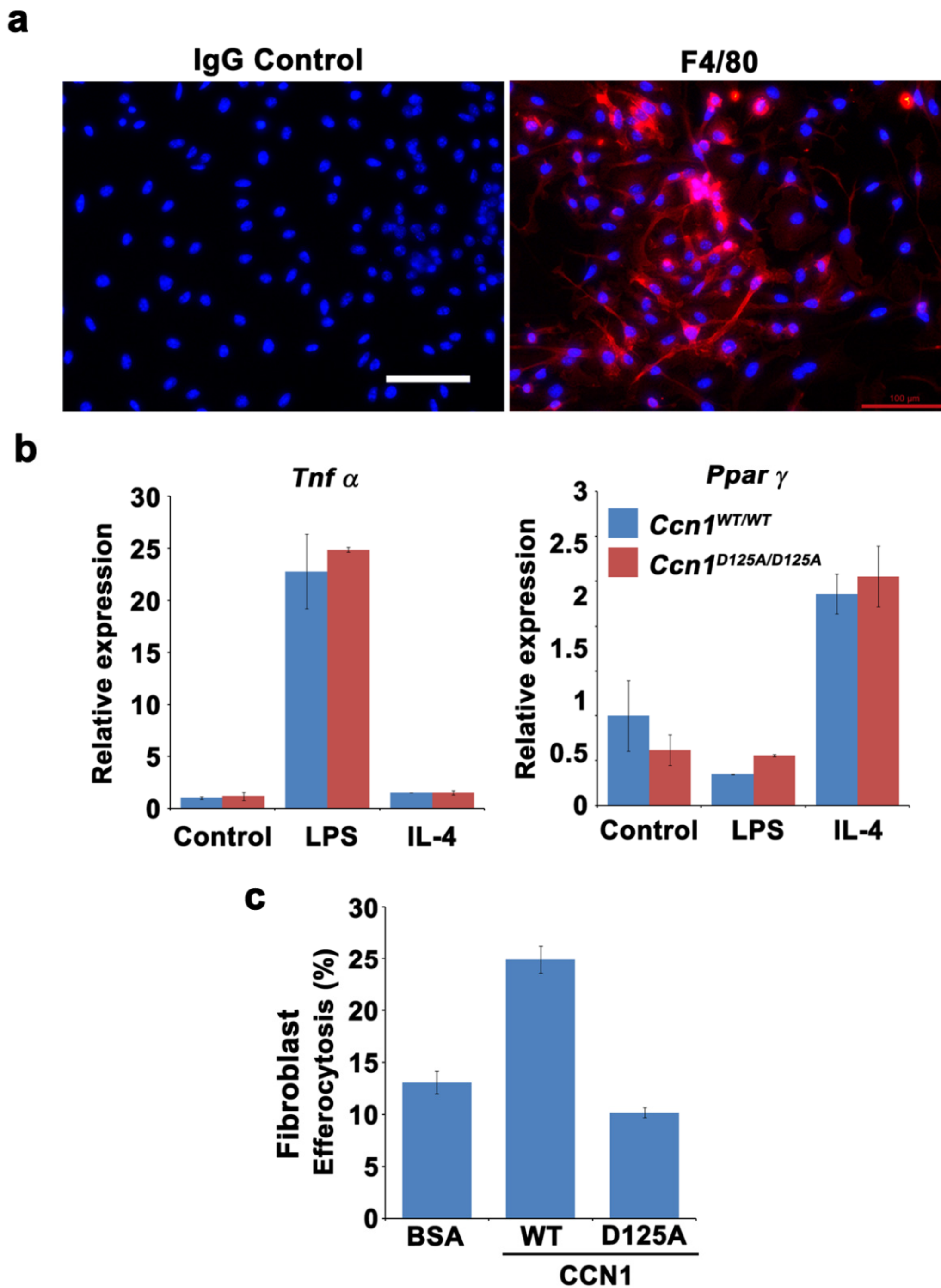
Supplementary Figure S7. **Cellular senescence occurs during wound healing in *Ccn1$^{D125A/D125A}$* mice.** (**a**). Wound tissues from either *WT* or *Ccn1$^{D125A/D125A}$* mice (day 9) were evaluated for senescence by SA-β- gal staining. Both genotypes showed comparable staining for the senescent cell marker. Bar=100 µm. (**b**) Wound tissues were harvested at indicated days and hydroxyproline contents were measured to evaluate collagen deposition (n=4 per genotype). Wounds of *Ccn1$^{D125A/D125A}$* mice initially showed lower levels of collagens but later held similar levels to *WT* wounds, consistent with delayed healing. Data represent means ± standard deviation. Experiments were performed in triplicate.



Supplementary Figure S8. **Analysis of *Gcsf* expression during wound healing.** Wound tissues from either *WT* or *Ccn1*$^{D125A/D125A}$ mice were analyzed for *Gcsf* expression using qRT-PCR (n=4 for post wounds day per each genotype). Data represent means ± standard deviation. Experiments were performed in triplicate.



Supplementary Figure S9. **Characterization of neutrophils isolated from bone marrow.** (**a**) Neutrophils were isolated from bone marrow of either *WT* or *Ccn1*$^{D125A/D125A}$ mice using Percoll density gradient centrifugation and cellular film was created on the slide, and the purity of the neutrophil preparation was evaluated by Giemsa staining. Representative image from *WT* neutrophils was shown (Bar=40μm). (**b**) Isolated neutrophils were incubated with CCN1 proteins (WT, D125A, and DM mutants) for 1 day and cell death was measured using tryphan blue exclusion assay (n=3 per treatment). Neither WT nor mutant CCN1 proteins showed any effect on neutrophil cell death. Neutrophils from the peritoneal cavity were used as a control. Data represent means ± standard deviation. Experiments were conducted in triplicates.



Supplementary Figure S10. Characterization of bone marrow-derived macrophages. (**a**) Bone marrow-derived monocytes were differentiated into macrophages and stained with anti-F4/80 (red), and counterstained with DAPI (blue). Bar=100mm. (**b**) Isolated macrophages from either *WT* or *Ccn1*$^{D125A/D125A}$ mice were treated with mLPS (50 ng per ml) or mIL-4 (20 ng per ml) to evaluate their responses to M1 or M2 activation, as monitored by expression of *Tnfα* or *Pparγ*, respectively (n=3). Macrophages from *WT* or *Ccn1*$^{D125A/D125A}$ mice responded similarly to stimulation by mLPS or mIL-4. (**c**) Bone marrow-derived macrophages were examined for efferocytosis of apoptotic fibroblasts. Cultured human skin fibroblasts (1077SK) were induced to apoptosis using staurosporine (1 µM; 16h). Apoptotic cells were pre- incubated with WT and D125A CCN1 proteins (2 µg per ml) for 1 h and co-incubated with bone marrow-derived macrophages (*WT*) for additional 1 h. Efferocytosis was measured using fluorescence microscopy (n=3). Data represent means ± standard deviation. All experiments were performed in triplicate.

Fig1b

WT wound CCN1

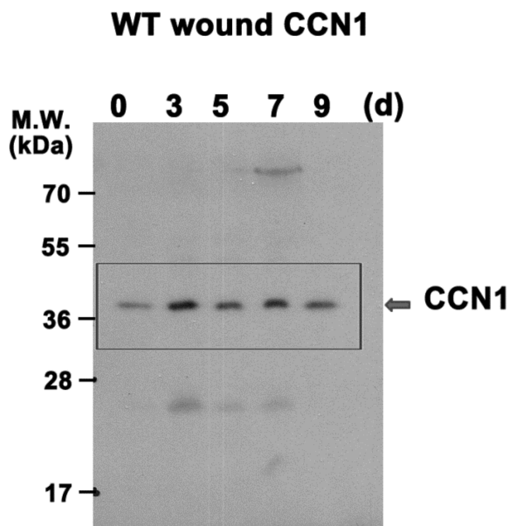


Fig8g

db wound CCN1

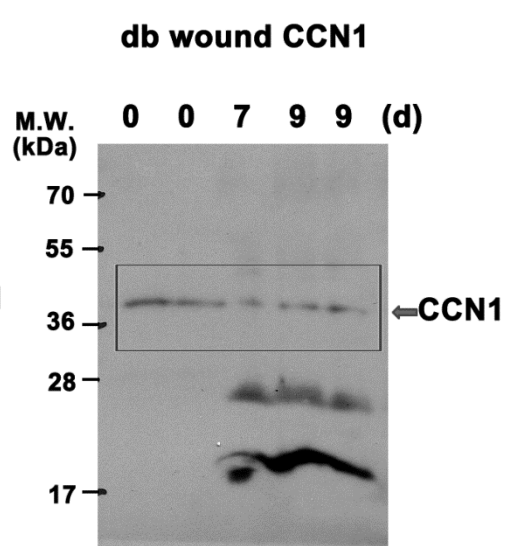


Fig1b

WT wound beta actin

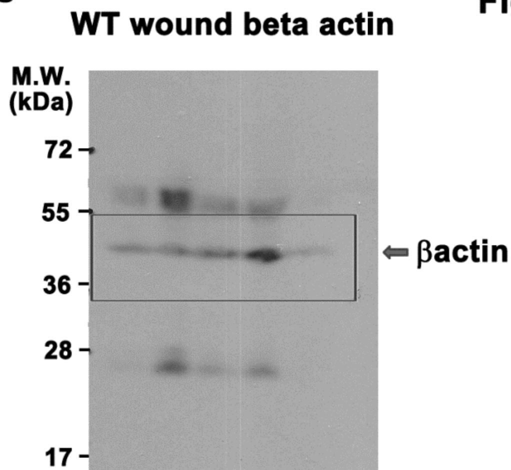


Fig8g

db wound beta actin

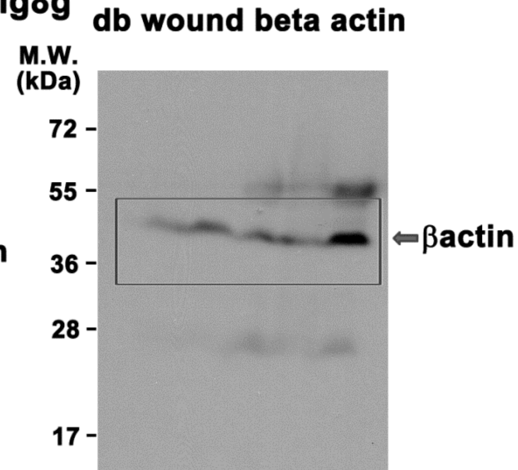


Fig6c

IP:FAK WB: p-FAK

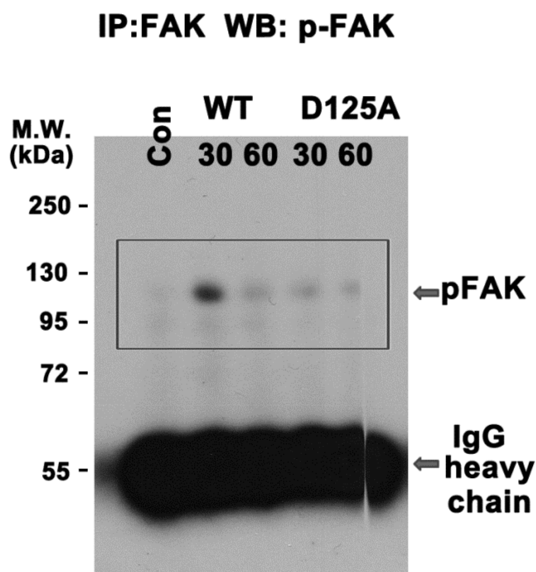
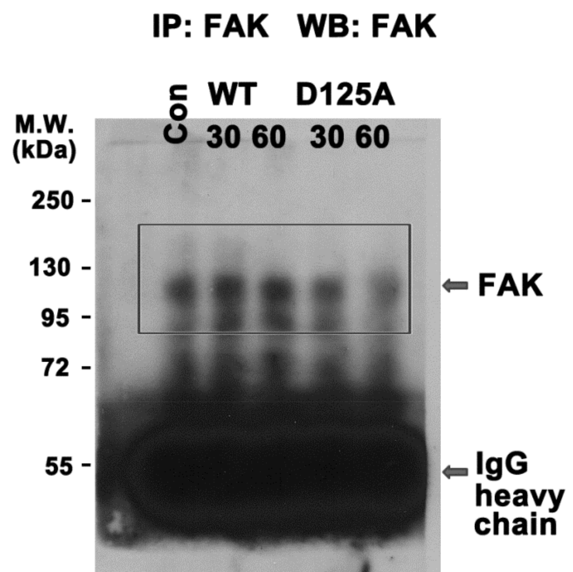


Fig6c

IP: FAK WB: FAK



Supplementary Figure S11. Uncropped Western blots.

Fig6a

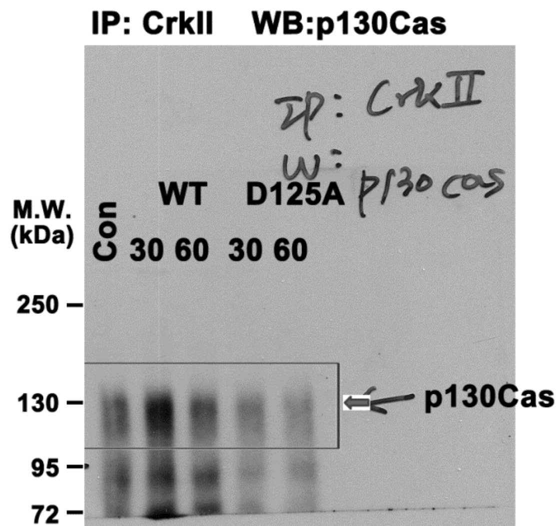


Fig6b

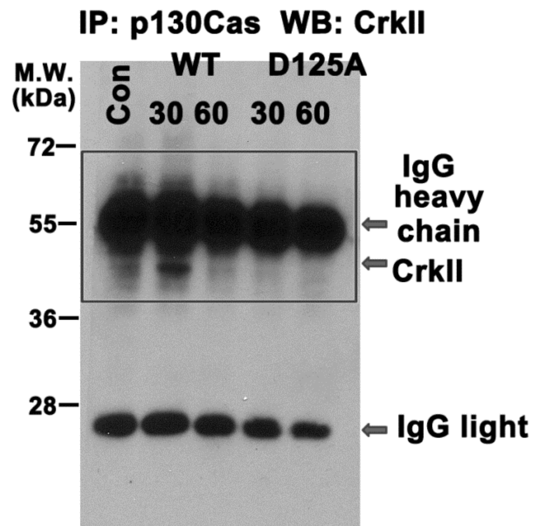


Fig6a

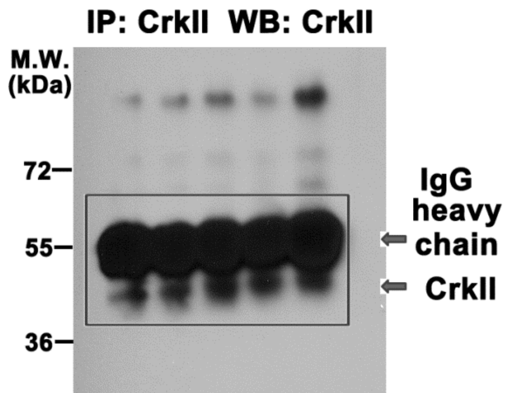


Fig6b

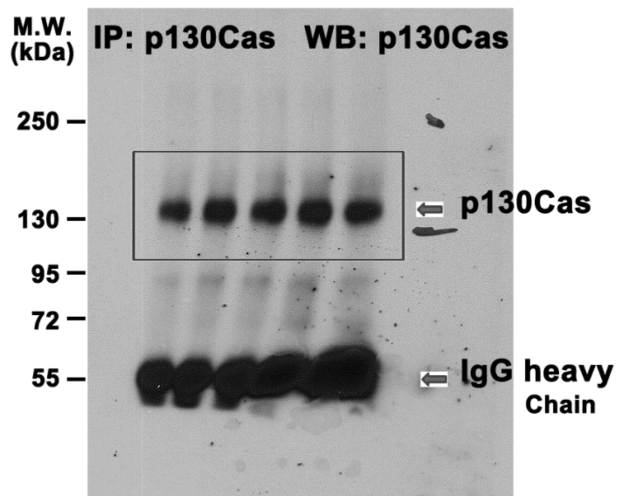


Fig6d

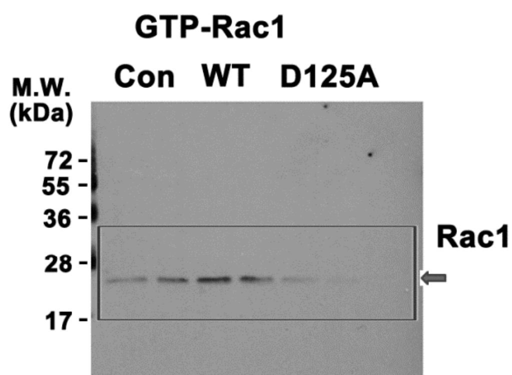
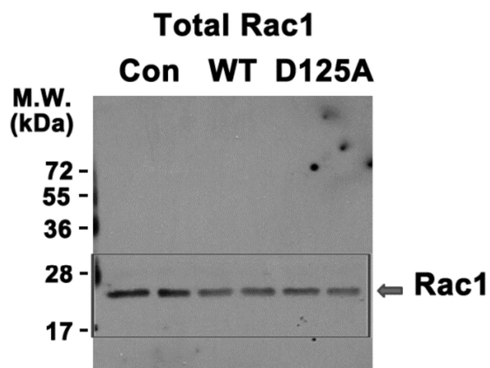


Fig6d



Supplementary Figure S11 (Continued). Uncropped Western blots.

Supplementary Table.1 Primer sequences for qRT-PCR

Gene		Sequence (5'→3')
Ccn1	forward	CCAGTGTACAGCAGCCTAAA
	reverse	CTGGAGCATCCTGCATAAGTAA
Tnfa	forward	CATCTTCTCAAAATTCGAGTGACAA
	reverse	TGGGAGTAGACAAGGTACAACCC
Il1b	forward	CAACCAACAAGTGATATTCTCCATG
	reverse	GATCCACACTCTCCAGCTGCA
Cxcl1	forward	CGAAGTCATAGCCACACTCAA
	reverse	GAGCAGTCTGTCTTCTTTCTCC
Cxcl5	forward	TGAACTCCCTGCTTTGATGAG
	reverse	CCGATAGTGTGACAGATAGGAAAG
Cxcr2	forward	CCTCACACAGGAACATAGCATAG
	reverse	GAACTTCGGACCTTTGGAAGA
Ccl2	forward	CTCGGACTGTGATGCCTTAAT
	reverse	TGGATCCACACCTTGCATTTA
Gcsf	forward	CATTCTCTCCACTTCCGAGTTT
	reverse	GGTATTTACCCATCTCCTTCCC
Vegfa	forward	TCACCAAAGCCAGCACATAGGAGA
	reverse	TTTCTCCGCTCTGAACAAGGCTCA
Vegfc	forward	TTTGGAGCAGCCACAAACACCTTC
	reverse	TGAGGTAACCTGTGCTGGTGTTCA
Fgf1	forward	AGTGAAGCATGGTTTCAAGGCACC
	reverse	TTCTAATTTGCTGGGCACTTGGGC
angiopoetin1	forward	GCGCTGGCAGTACAATGACAGTTTCA
	reverse	CATTGCCCATGTTGAATCCGGT
cyclophilin E	forward	TTCACAAACCACAATGGCACAGGG
	reverse	TGCCGTCCAGCCAATCTGTCTTAT