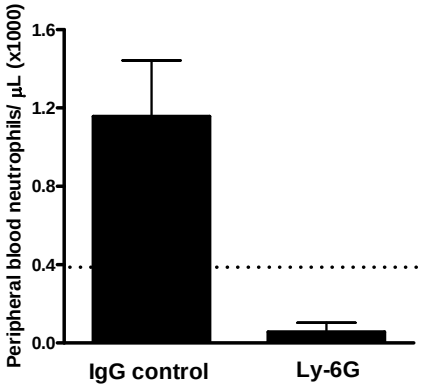
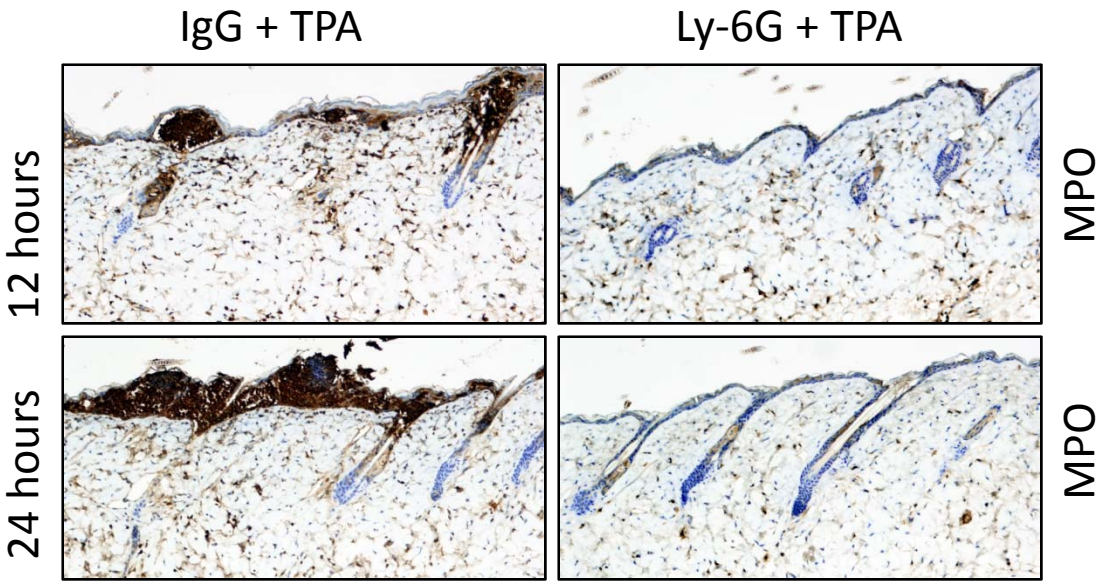
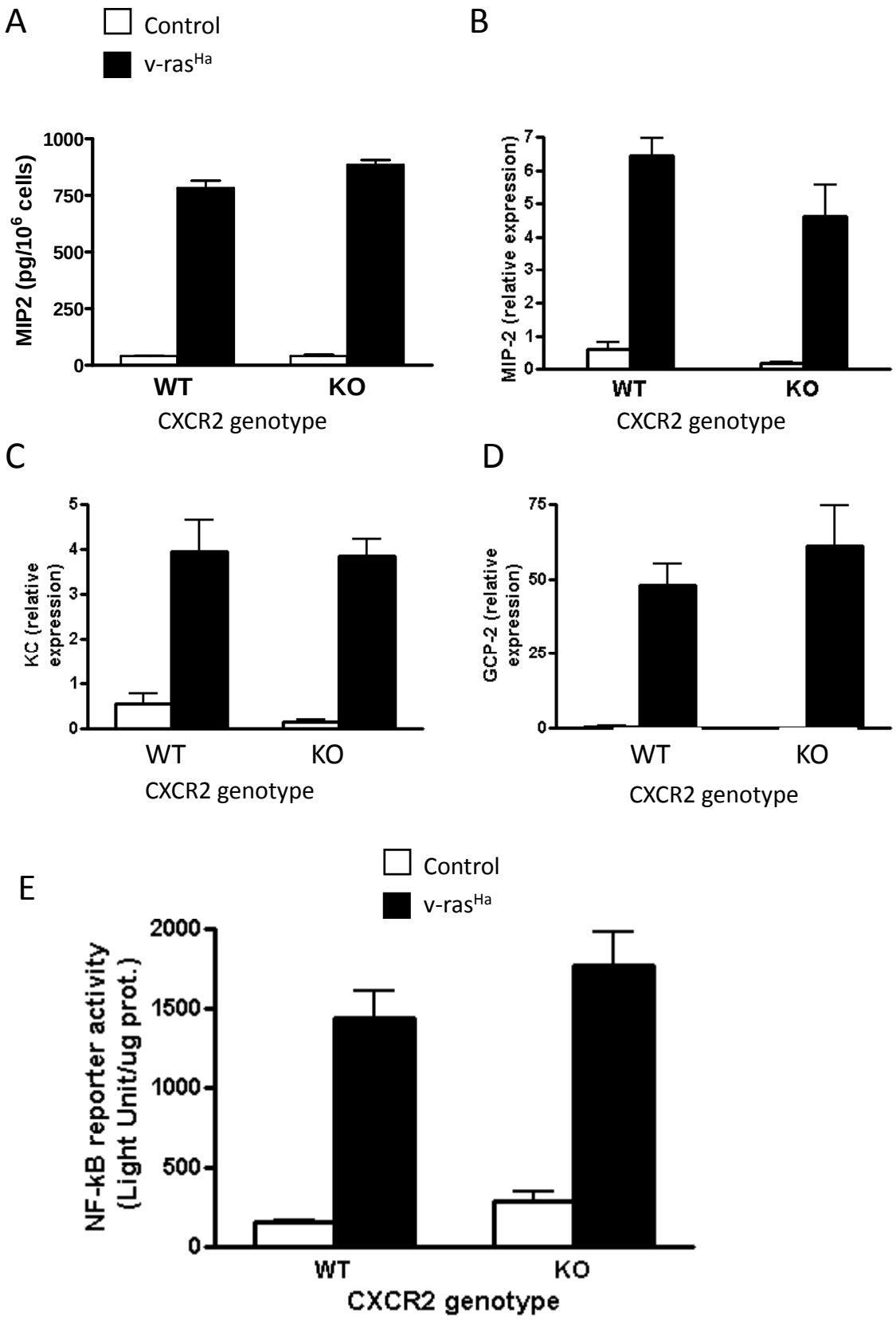
A

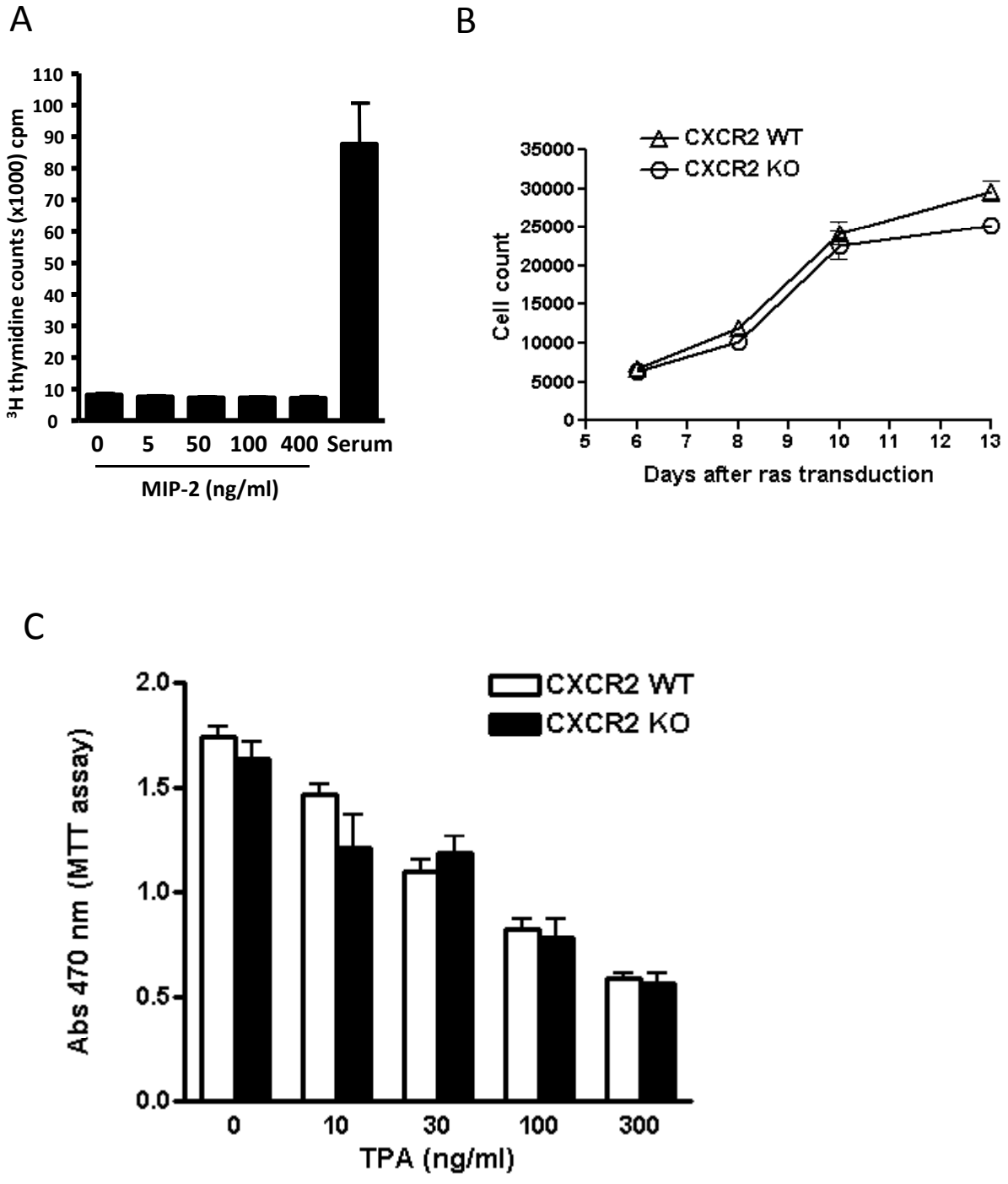

Peripheral blood neutrophils/ µL (x1000)
1.6
1.2
0.8
0.4
0.0
IgG control
Ly-6G

B


IgG + TPA
Ly-6G + TPA
12 hours
24 hours
MPO
MPO


A
Control
v-ras^Ha
MIP2 (pg/10^6 cells)
1000
750
500
250
0
WT
KO
CXCR2 genotype
B
MIP-2 (relative expression)
7
6
5
4
3
2
1
0
WT
KO
CXCR2 genotype
C
KC (relative expression)
5
4
3
2
1
0
WT
KO
CXCR2 genotype
D
GCP-2 (relative expression)
75
50
25
0
WT
KO
CXCR2 genotype
E
Control
v-ras^Ha
NF-kB reporter activity (Light Unit/ug prot.)
2000
1500
1000
500
0
WT
KO
CXCR2 genotype

Supplemental Figure 3 Cataisson et al.



Supplemental figure 1: Ly-6G treatment of K5-PKCa mice prevented systemic neutrophilia and neutrophil infiltration and microabscess formation in K5-PKCa mice after topical TPA treatment. A single dose of TPA (1 mg) in acetone was applied to the shaved backs of K5-PKCa transgenic mice previously inoculated with control IgG or Ly-6G antibodies by IP injection. Blood was drawn at 12h, and differential white blood cell counts were performed. Bars represent the mean number of neutrophils per microliter of blood ± SEM from 5-7 mice per group. The baseline values for neutrophil count is represented by the dotted line (415±85 neutrophil/ml of blood) in non TPA treated K5-PKCa mice. B, Skin sections from similarly treated mice were immunostained using myeloperoxidase (MPO) antibody that recognizes granulocytes. Inflammatory cells infiltrating the hair follicles and epidermis and forming intraepidermal microabscesses are seen in K5-PKCa mice treated with control IgG but not in Ly-6G treated animals.

Supplemental figure 2: CXCR2 ligands are induced by oncogenic ras (v-ras^{Ha}) in primary keratinocytes independent of CXCR2 genotype. A, culture supernatants from mock or v-ras^{Ha}-transduced CXCR2 WT or KO primary keratinocytes were collected over 24 hours for cells in culture for 5 days. MIP-2 concentrations were determined by ELISA. Bars represent the mean ± SEM of triplicate determinations. B, C and D, real-time PCR analysis of MIP-2 (panel B), KC (panel C) or GCP-2 (panel D) mRNA expression in CXCR2 WT or CXCR2 KO keratinocytes 3 days after mock or v-ras^{Ha}-transduction. Bars represent mean value of triplicate determinations ± SEM. E) Three days after control or v-ras^{Ha} transduction, CXCR2 WT and KO primary keratinocytes were transiently transfected with the NF-kB reporter activity plasmid construct, and 48h later cells were harvested for reporter activity. Values are expressed as relative light units (RLU) per 100ug total protein as determined by Bradford. Bars represent the mean ± SEM of six culture wells. Results are representative of two independent experiments.

Supplemental figure 3: CXCR2 status does not affect the growth of v-ras^{Ha}-transduced keratinocytes. A, three days after ras transduction primary keratinocytes were starved overnight in 0.2% serum containing media, then treated with indicated concentration of MIP-2 or complete media (serum) for 24 hours. Cells were pulsed with ^{3}H-thymidine for the last 4 hours. Bars represent the mean ± SEM of quadruplicate determinations. Results are representative of two independent experiments. B, growth of CXCR2 WT and KO keratinocytes following transduction by the v-ras^{Ha} oncogene as described in methods. Primary keratinocytes were grown in 24-well tissue culture plates and cell counts were performed on the indicated days. Points represent value based on counts from four wells, ± SEM. C, primary keratinocytes from CXCR2 WT and KO mice were cultured in 0.05 mM Ca^{2+} medium and treated overnight with TPA on the fourth day after ras transduction. Cell viability was assayed by MTT assay. Bars represent the mean ± SEM of quadruplicate determinations. Results are representative of two independent experiments.