Title: Cxcr1 mediates recruitment of neutrophils and supports proliferation of tumor-initiating astrocytes *in vivo*

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Supplementary Information

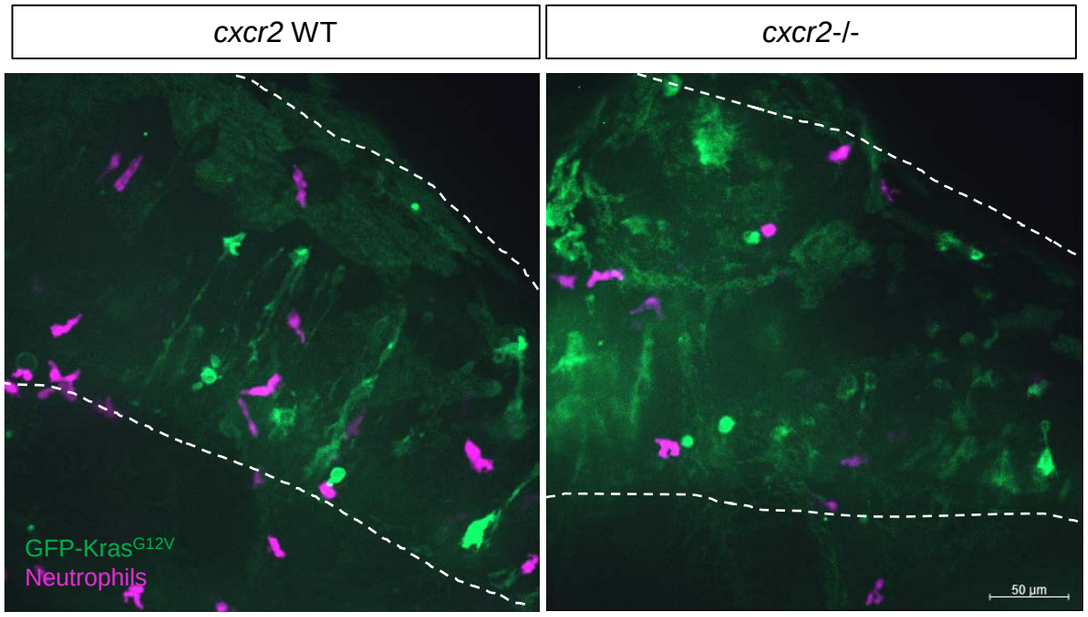
Includes:

Supplemental Figures 1-3

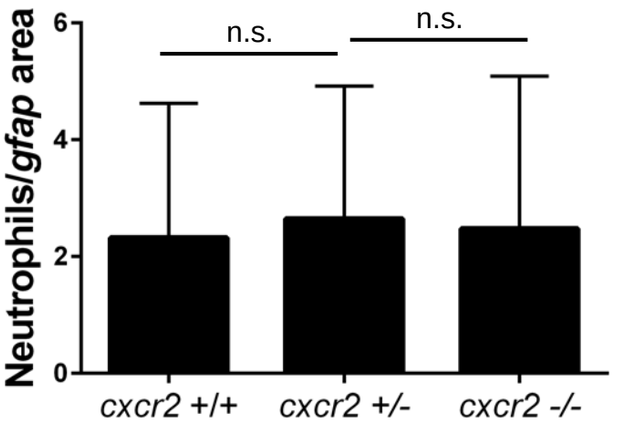
Supplemental Figure Legends

Supplemental Movie Legends

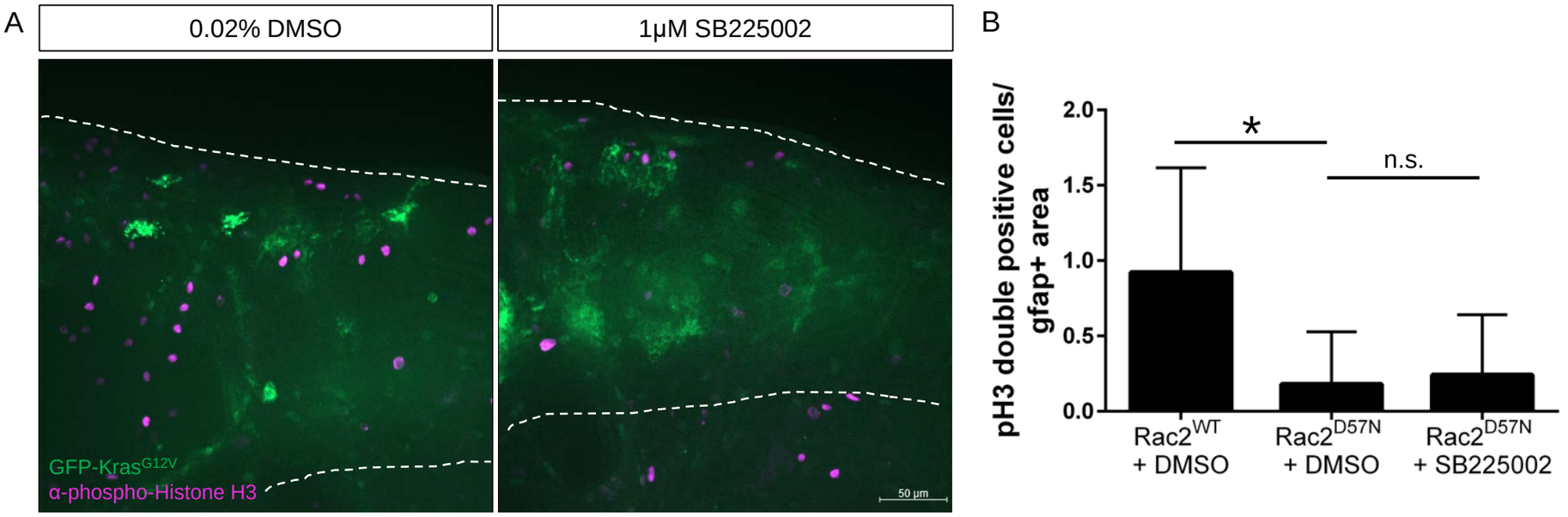
A



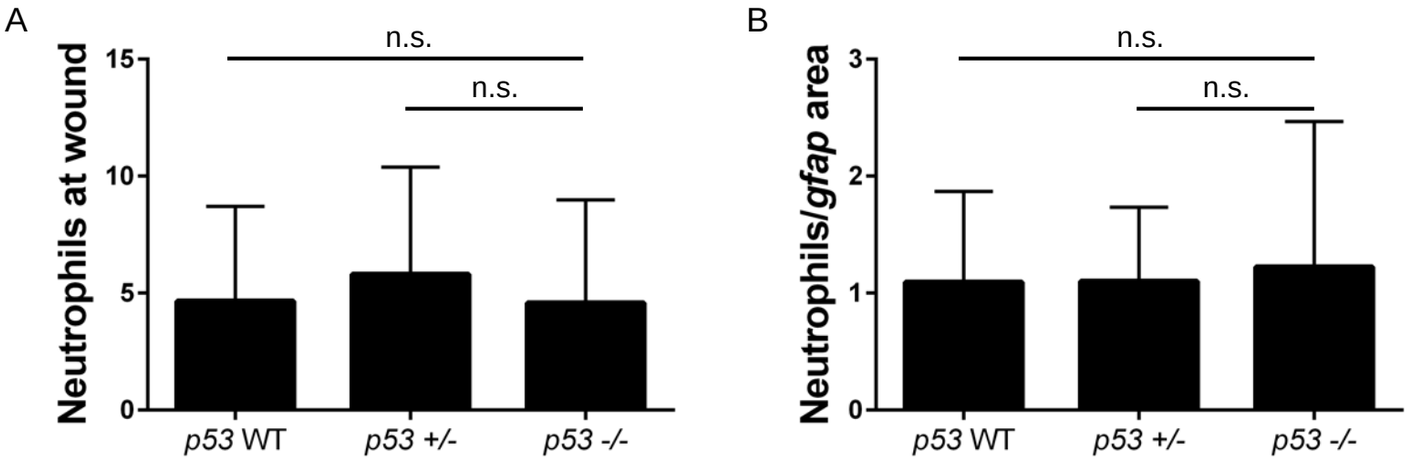
B



Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3

Supplemental Figure Legends:

Supplemental Figure 1. Cxcr2 is dispensable for neutrophil recruitment to tumor-initiating astrocytes. Adult *cxcr2* heterozygote zebrafish expressing Tg(*mpx*:mCherry) were in-crossed, injected with *gfap*:kras^{G12V}, imaged for neutrophil recruitment to the hindbrain at 3 dpf, and then processed for single-embryo genotyping (A). Neutrophils recruited to the field of view were quantified in *cxcr2* mutants and WT or heterozygote siblings (no difference observed between +/+ and +/-) and normalized to total *gfap*-expressing area (B, n= 24 WT, 23 *cxcr2*+/-, 7 *cxcr2*-/-). Data pooled from 2 replicates.

Supplemental Figure 2. Cxcr1/2 inhibition with neutrophil-specific motility loss.

Tg(*mpx*:mCherry-2a-Rac2^{WT}) or Tg(*mpx*:mCherry-2a-Rac2^{D57N}) embryos were injected with *gfap*:GFP-kras^{G12V} and treated with 1µM SB225002 or DMSO control as described in Figure 4. Larvae were fixed at 3 dpf and immunostained for phospho-Histone H3 (A). pH3-GFP double positive cells were quantified by optical section and normalized as above (B, n= 10 Rac2^{WT} + DMSO, 19 Rac2^{D57N} + DMSO, 17 Rac2^{D57N} + SB225002). Data pooled from 2 replicates, *p<0.05.

Supplemental Figure 3. Neutrophil recruitment is normal in *p53* mutant background. Adult *p53* heterozygote zebrafish expressing Tg(*mpx*:mCherry) were in-crossed, exposed to a tail-transection wound at 3 dpf, then imaged 2 hours later for neutrophil recruitment to the wound edge followed by single embryo genotyping. Neutrophils caudal to the notochord were quantified (A). *p53* heterozygote zebrafish expressing Tg(*mpx*:mCherry) were in-crossed, injected with *gfap*:kras^{G12V}, imaged for neutrophil recruitment to the hindbrain at 3 dpf, and then processed for single-embryo

genotyping. Neutrophils recruited to the field of view were quantified and normalized to total *gfap*-expressing area (B). Data pooled from 2 replicates.

Supplemental Movie Legends:

Supplemental Movie 1. Time-lapse imaging of neutrophil recruitment to *gfap*:Kras^{WT} cells. Live-imaging of neutrophils in the hindbrain of Tg(*mpx*:mCherry) larvae expressing *gfap*:GFP-kras^{WT}. Time-lapse imaging by spinning disc confocal microscope was performed for 9 hours beginning at 3 dpf. Total neutrophils (magenta) recruited to the hindbrain during the period of imaging were quantified in Figure 2C.

Supplemental Movie 2. Time-lapse imaging of neutrophil recruitment to *gfap*:Kras^{G12V} cells. Live-imaging of neutrophils in the hindbrain of Tg(*mpx*:mCherry) larvae expressing *gfap*:GFP-kras^{G12V}. Time-lapse imaging by spinning disc confocal microscope was performed for 9 hours beginning at 3 dpf. Total neutrophils (magenta) recruited to the hindbrain during the period of imaging were quantified in Figure 2C.