Supplementary Figures

S1.

(A-B) *P2ry14* expression was assessed by (A) genotyping (upper arrow: WT; lower arrow: KO) and (B) q-PCR analysis with Lin- cells, The white vertical line in panel A indicates that the lanes were run on the same gel but were noncontiguous, * denotes "undetectable"; (C) The absence of the P2Y14 protein was confirmed by flow analysis. Left: WT Lin- BM cells stained with isotype control (normal rabbit IgG), Middle: WT Lin- BM cells stained with anti-P2Y14 antibody, Right: P2Y14 KO Lin- BM cells stained with anti-P2Y14 antibody

S2.

Blood from WT (n= 3) and *P2ry14*$^{-/-}$ (n= 3) mice was analyzed for white blood cell (WBC), red blood cell (RBC), lymphocyte, monocyte, granulocyte, platelet (PLT), hemoglobin (HGB), and hematocrit (HCT) with VetABC^{TM}(Scil).

S3.

Freshly isolated BM cells from *P2ry14*$^{-/-}$ and littermate control (WT, *P2ry14*$^{+/+}$) mice were analyzed for CD150+ CD48- LSK, CD34- LSK and LSK cells. The graph shows the absolute numbers of HSPCs. Data are represented as absolute numbers of cells ± s.d. of at least four independent experiments. The two-tailed Student's t-test was used.

S4.

Thymus cells were exposed to various stresses as indicated. cDNA was synthesized using 2 µg of total RNA. qPCR assays were done in duplicate, at least three times. The expression was normalized to GAPDH. Control untreated samples (white bars) were set

to 100% and treated samples (black bars) are shown as a percentage of controls. 5FU: 5-Fluorouracil; LPS: Lipopolysaccharides

S5.

Mice of the indicated genotypes were exposed to 3 Gy and 6 Gy of TBI. Cell death was measured by quantification of DAPI+ cells within LSK cells. The data are representative of three independent experiments. At doses of greater than 8 Gy, only a very small number of LSK cells were alive (data not shown), making it difficult to obtain enough cells for the further analysis. The two-tailed Student's t-test was used.

S6.

Early passage MEFs were analyzed using qPCR for the expression of *P2ry14* mRNA expression. Human immature myeloid cell line KG-1 from which *P2RY14* gene was originally cloned (62) was used as a positive control. *P2ry14*$^{-/-}$ MEFs were used as negative control. *P2ry14* transcript was assessed using human- (for KG-1) and mouse (for MEFs) specific primers, respectively. The expression was normalized to human and mouse GAPDH, respectively. *P2RY14* mRNA level of KG-1 was arbitrarily set as 1. Q-RT-PCR was done in duplicate at least three times.

S7.

Early passage MEFs (P2–P3) from *P2ry14*$^{-/-}$ and *P2ry14*$^{+/+}$ mice were exposed to a single dose of 20 Gy radiation. Left: SA-β-Gal activity was analyzed eight days after exposure to IR; Right: Western analysis of irradiated WT and *P2ry14*$^{-/-}$ MEF cells.

S8.

Mice (n=2) of the indicated genotypes were exposed to 6 Gy of TBI. The levels of H2AX phosphorylation on gated LSK cells were quantified using anti–phospho-histone H2AX (Ser139) antibody. Numbers denote mean fluorescent intensity (MFI).

S9.

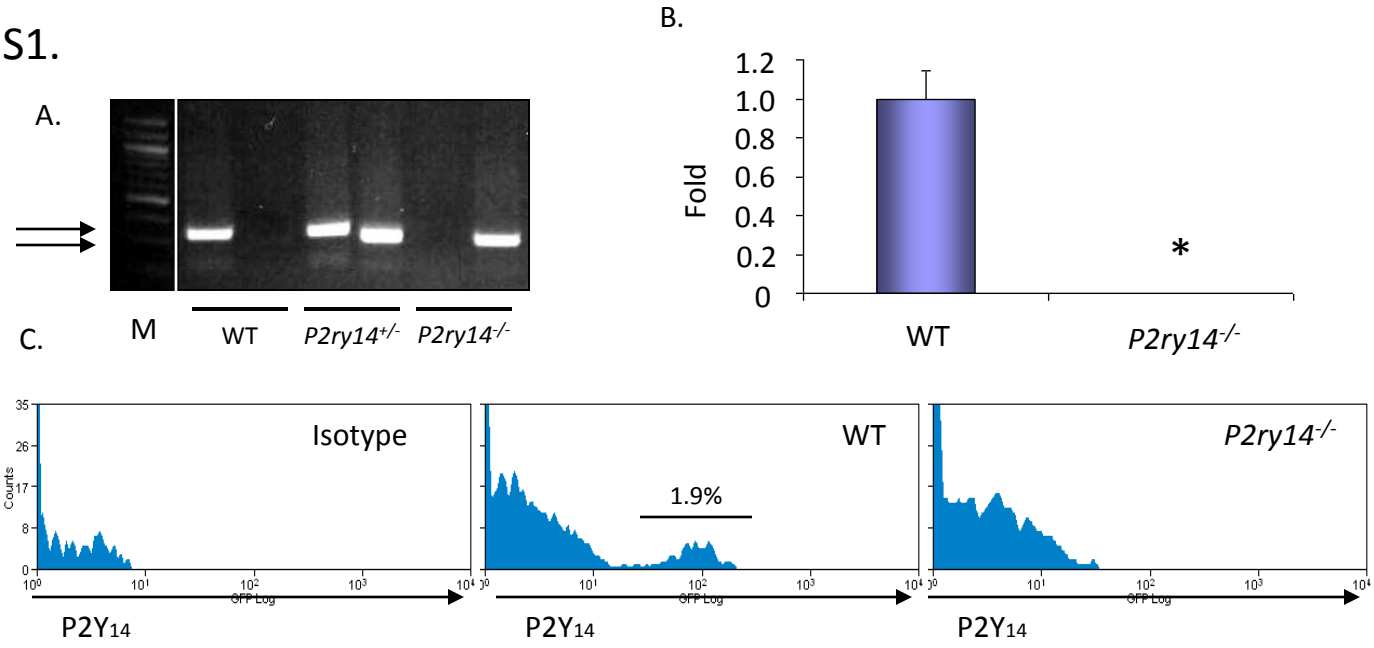
Mice (n=3) of the indicated genotypes were exposed to 6 Gy TBI. Bone marrow cells were harvested at 5-6 hours after TBI and cell cycle status of HSPCs (LSK) was analyzed by Ki-67 and DAPI staining. For the cell cycle analysis in LSK cells, at least 10^6 bone marrow cells were acquired. Ki67 positive gate was set based on the intensity of Ki67 in cycling cells (e.g., S/G_2/M). Lower panels show the histogram of the gated G_0 (Ki67−DAPI−), G_1 (Ki67+DAPI−) and S/G_2/M (Ki67+DAPI+) cells within LSK cells. The number denotes the percentage of cells in G_0, G_1, and S/G_2/M.

S10.

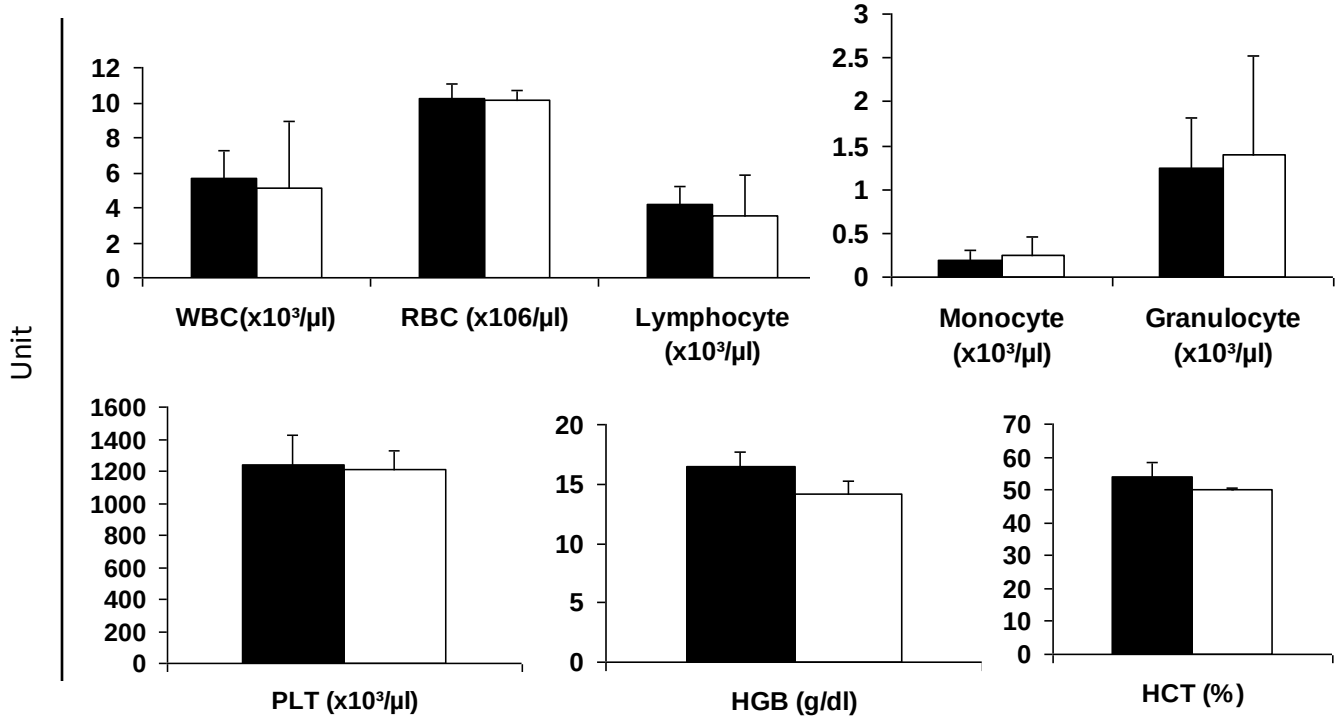
Representative dot plots and histogram that correspond with data in Fig. 8B are shown. Numbers are percentages of gated cells.

Supplementary Figures

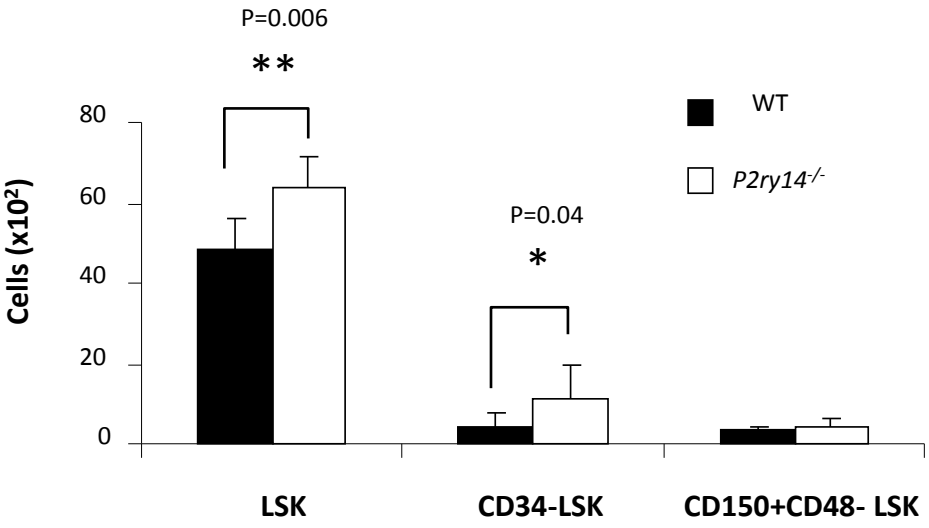
S1.



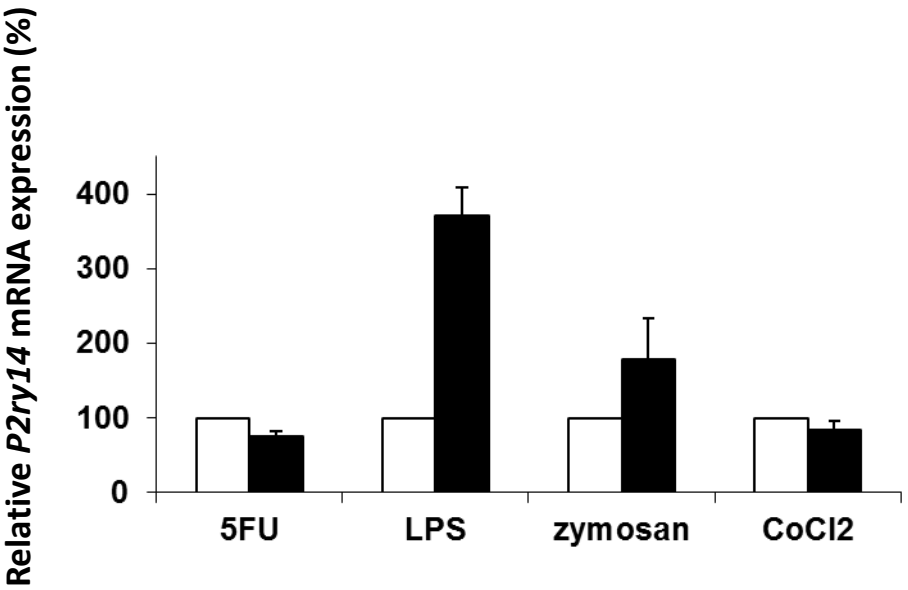
S2.



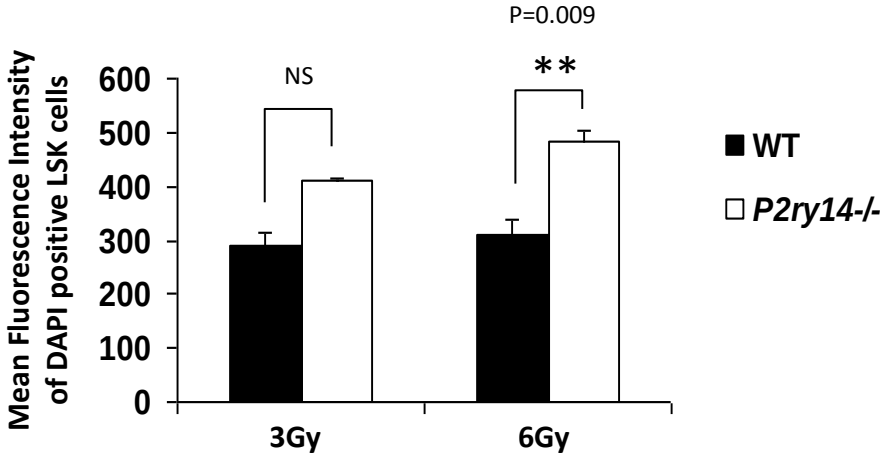
S3.


P=0.006
**
WT
P2ry14-/-
80
60
40
20
0
Cells (x10²)
P=0.04
*
LSK
CD34-LSK
CD150+CD48- LSK

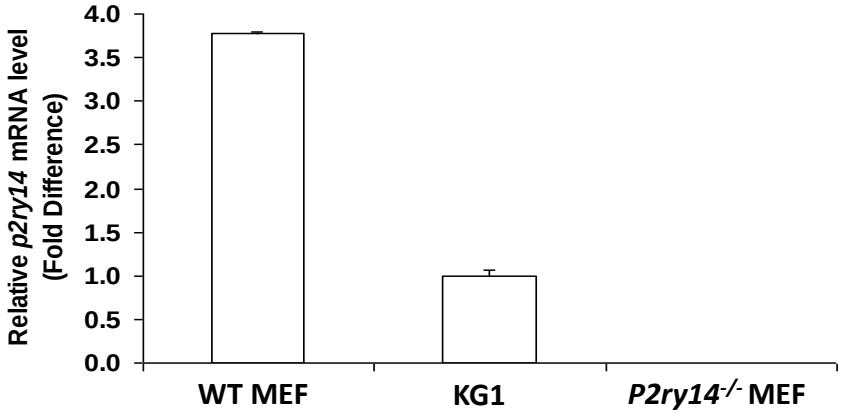
S4.


Relative P2ry14 mRNA expression (%)
400
300
200
100
0
5FU
LPS
zymosan
CoCl2

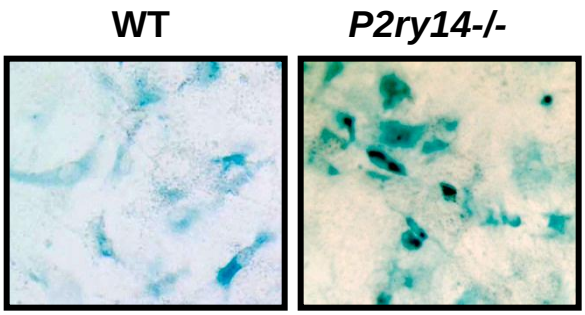
S5.

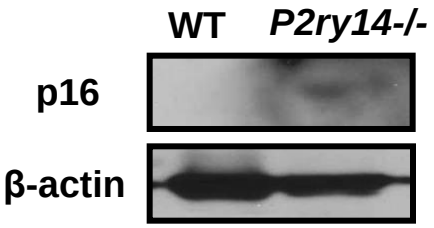

P=0.009
NS
**
Mean Fluorescence Intensity of DAPI positive LSK cells
600
500
400
300
200
100
0
3Gy
6Gy
WT
P2ry14-/-

S6.

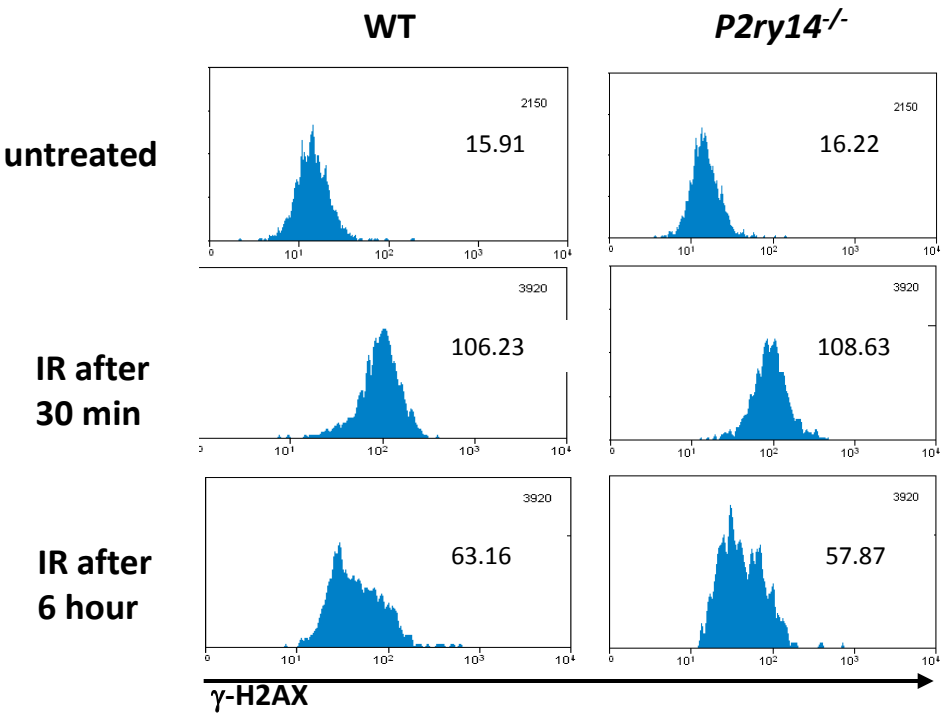

Relative p2ry14 mRNA level (Fold Difference)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
WT MEF
KG1
P2ry14-/- MEF

S7.

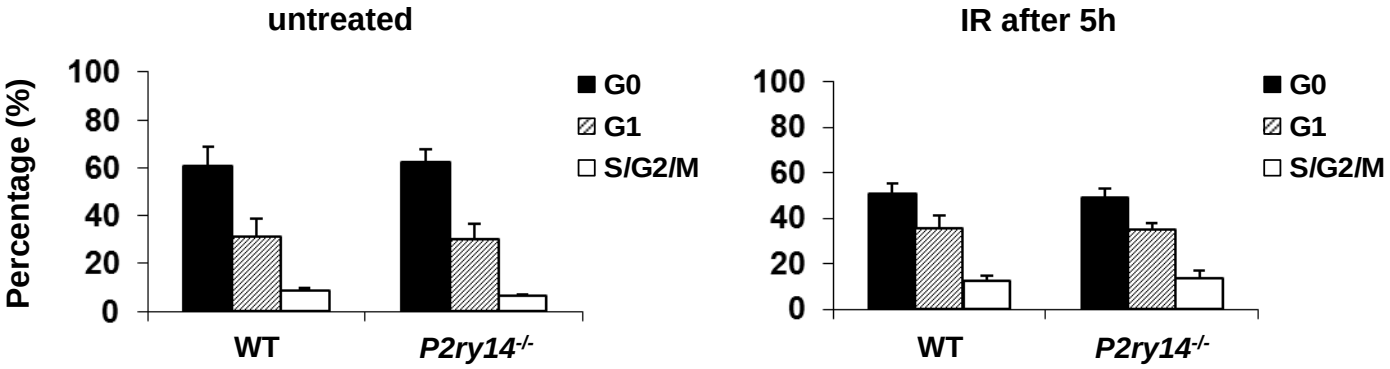

WT
P2ry14-/-

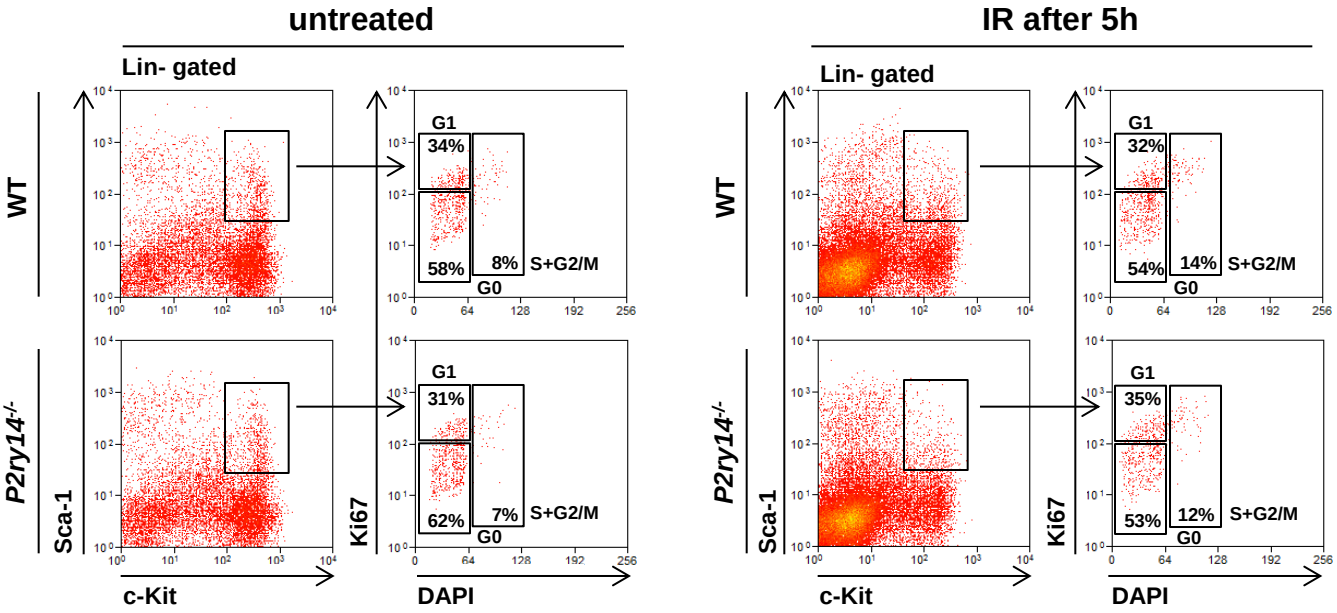

WT
P2ry14-/-
p16
β-actin

S8.


WT
P2ry14-/-
untreated
IR after 30 min
IR after 6 hour
2150
15.91
2150
16.22
3920
106.23
3920
108.63
3920
63.16
3920
57.87
γ-H2AX

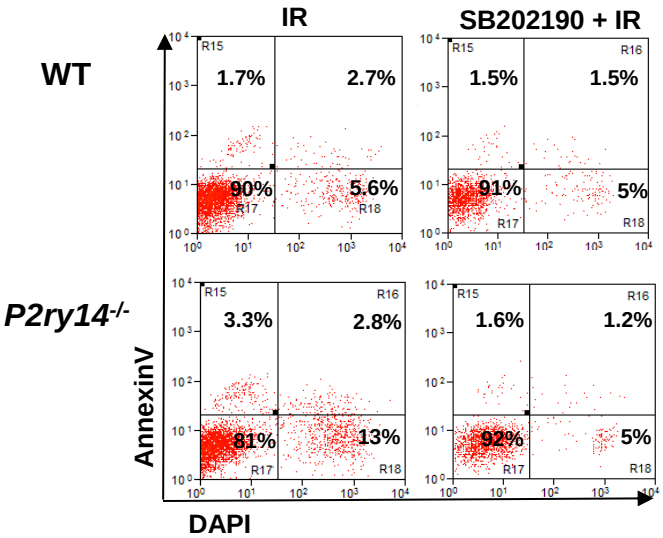
S9


untreated
IR after 5h
Percentage (%)
100
80
60
40
20
0
G0
G1
S/G2/M
WT
P2ry14-/-


untreated
IR after 5h
Lin- gated
WT
P2ry14-/-
Sca-1
c-Kit
Ki67
DAPI
G1
34%
58%
8%
S+G2/M
G0
31%
62%
7%
32%
54%
14%
35%
53%
12%

S10

LSK



CD150+CD48- LSK