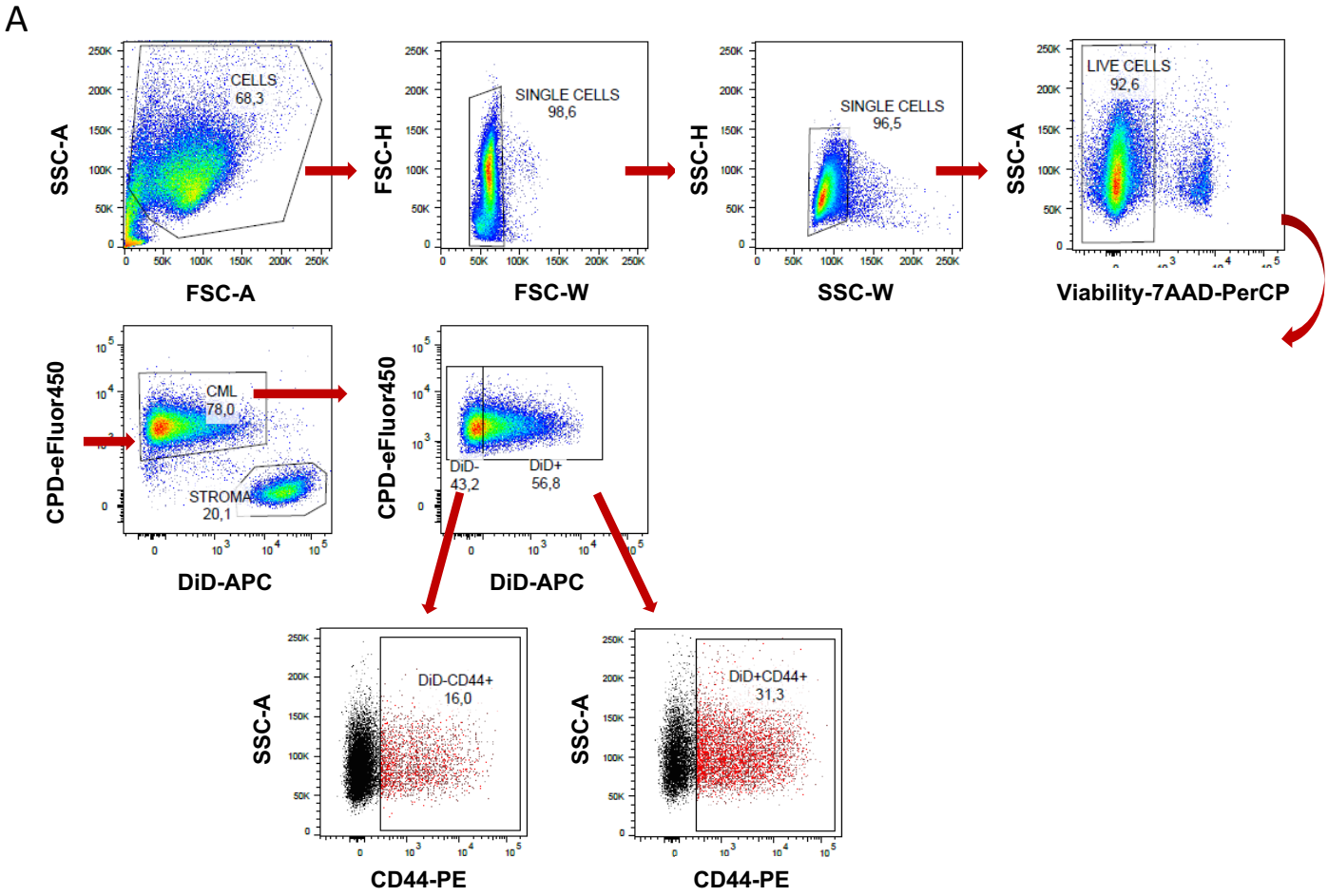

Fig. S1
A Flow cytometry gating strategy used to analyze CD44 levels in K562 DiD^{+} and K562 DiD^{-} populations.

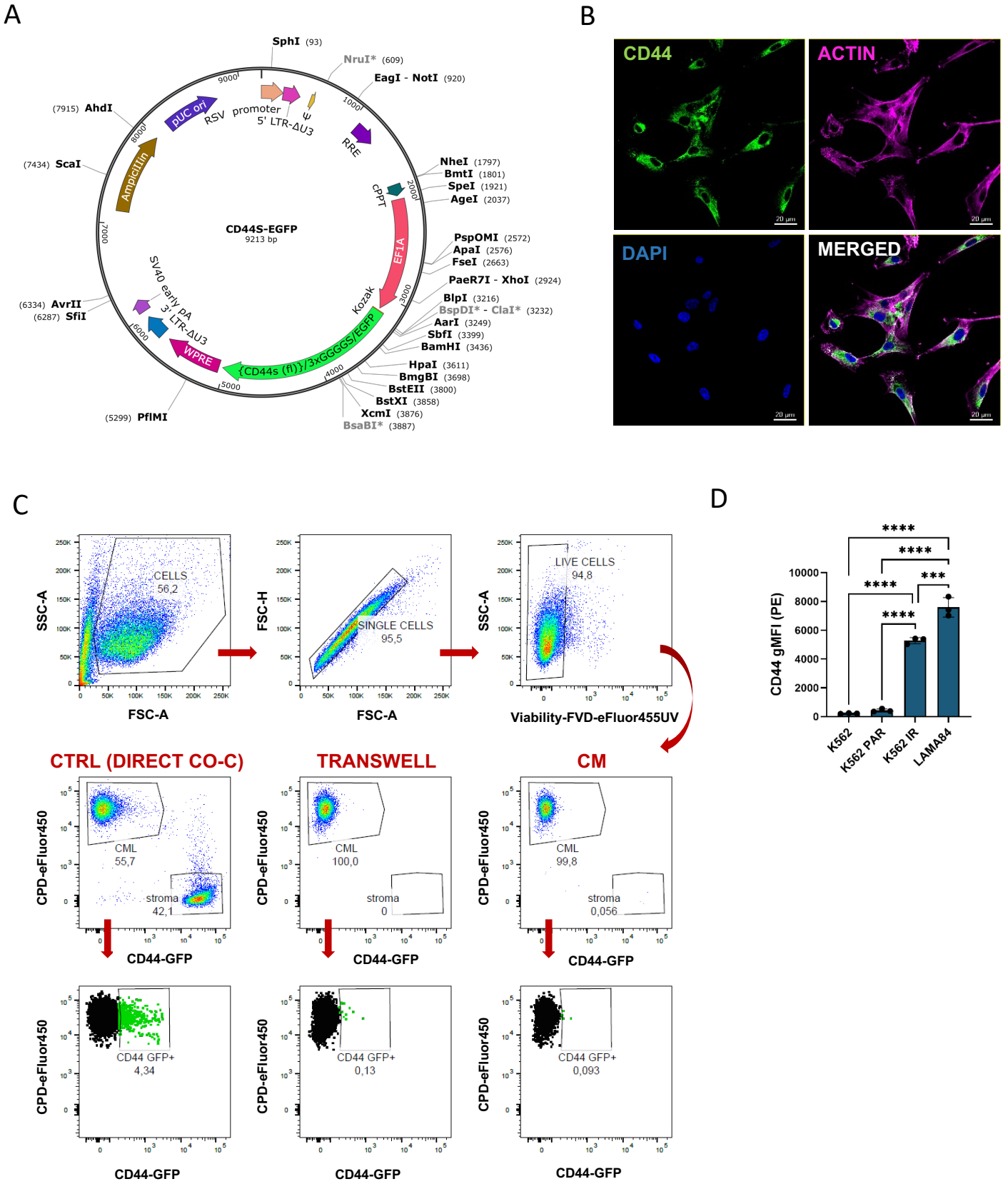

Fig. S2

A Schematic map of the lentiviral construct used for CD44-GFP overexpression in HS-5 cells. CD44 is expressed in-frame with GFP under the EF1A promoter, a 3xGGGGS linker was used between CD44 and GFP to allow proper folding and function. **B** Representative immunofluorescence images of HS-5 cells expressing the CD44-GFP protein (green). Phalloidin-eFluor570 (magenta) was used tu label actin and DAPI to visualize cel nuclei (blue). Scale bar: 20 µm. **C** Flow cytometry gating strategy used for CD44-GFP uptake analysis. **D** CD44 levels (shown as gMFI) in monocultured K562, K562 PAR, K562 IR and LAMA84 cells, measured with flow cytometry. Data expressed as the mean ±SD, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. One-way ANOVA with Tukey's posttest.

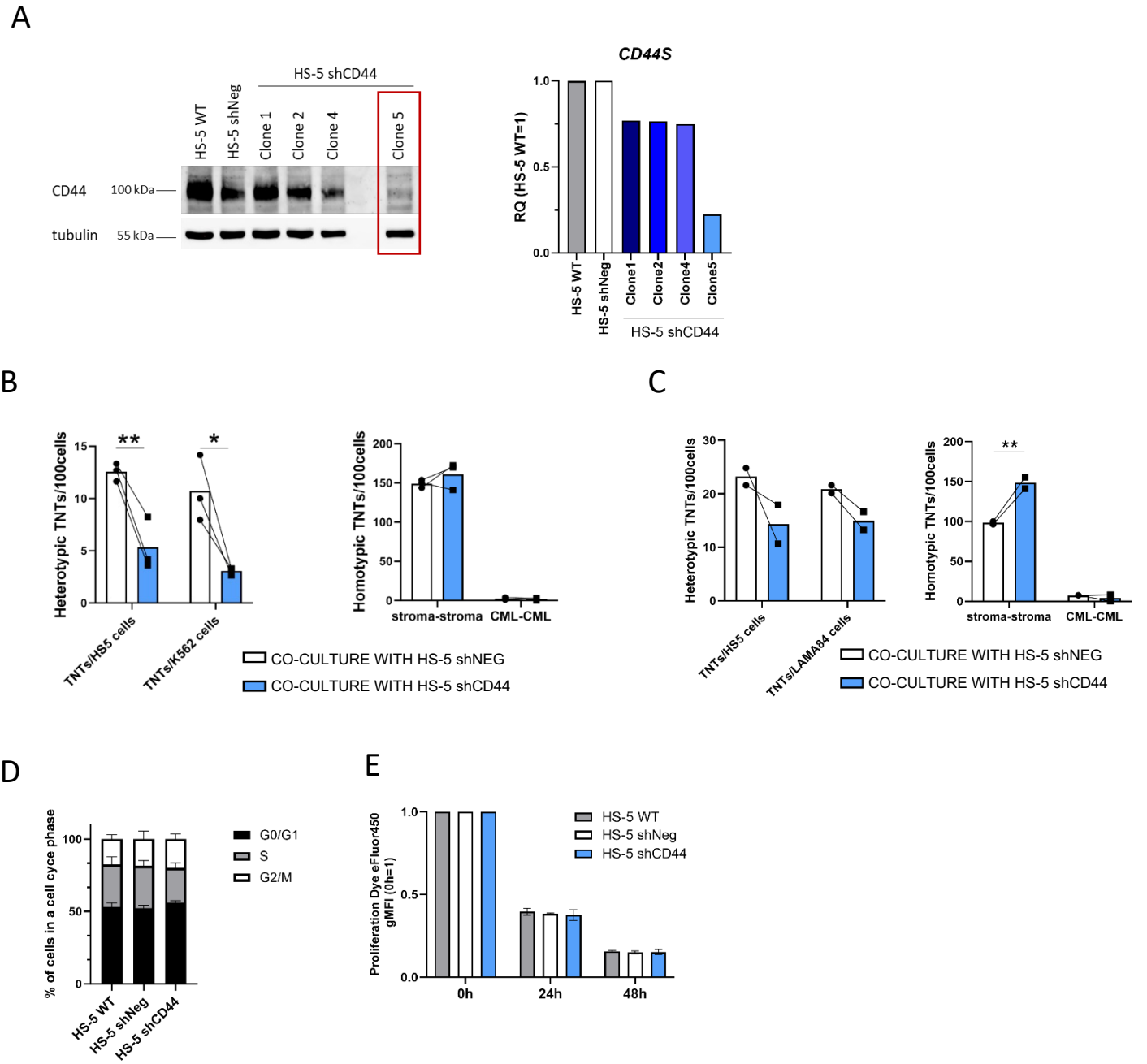

Fig. S3

A Validation of CD44 knockdown in HS-5 cells. **Left:** Western blot showing CD44 protein levels in HS-5 cells untreated, transduced with shNEG or shCD44. Tubulin was used as a loading control. The red frame highlights the clone 5 selected for downstream experiments. **Right:** qPCR analysis of *CD44* mRNA levels in the same clones. KD efficiency correlates with protein reduction observed in western blot analysis. **B** Average numbers of TNTs per 100 cells quantified in co-cultures of K562 cells and HS-5 shNEG or HS-5 shCD44 cells by confocal microscopy after 24 hours of co-culture. Heterotypic TNTs formed between HS-5 and K562 cells (left) and homotypic TNTs interconnecting either HS-5 cells or K562 cells (right). **C** Average numbers of TNTs per 100 cells quantified in co-cultures of LAMA84 cells and HS-5 shNEG or shCD44 cells by confocal microscopy after 24 hours of co-culture. Heterotypic TNTs formed between HS-5 and LAMA84 cells (left) and homotypic TNTs interconnecting either HS-5 or LAMA84 cells (right). **D** Cell cycle distribution of HS-5 WT, shNEG and shCD44 cells measured by propidium iodide staining, shown as stacked bars representing a percentage of cells in G0/G1, S and G2/M phases. **E** Cell proliferation of HS-5 WT, shNEG and shCD44 cells measured with ProliferationDye eFluor450 at 0, 24 and 48 hours. gMFI is normalized to 0h (gMFI=0).

Data expressed as the mean ±SD, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. 2-way ANOVA with Tukey's posttest (**B-E**).

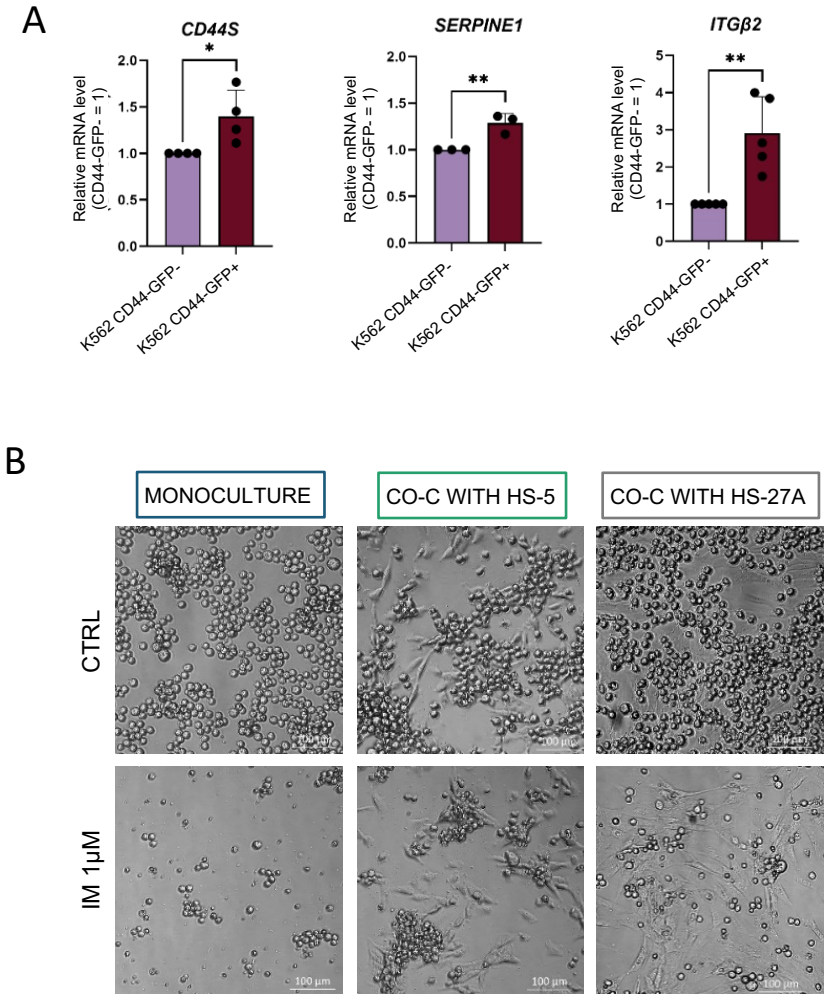

Fig. S4

A RT-qPCR validation of RNAseq results showing relative expression of CD44S, SERPINE1, ITGB2 in CD44 acceptors (K562 CD44-GFP+) versus non-acceptors (K562 CD44-GFP-) (n=3). Expression was normalized to CD44- (set as 1), with actin as the endogenous control. Data expressed as the mean ±SD, unpaired Student's t-test. **B** Representative bright-field images of K562 monocultures or K562 co-cultures with HS-5 or HS-27A stromal cells. Cultures were untreated (CTRL) or treated with 1 µM imatinib for 48 hours. Scale bars: 100 µm.

A

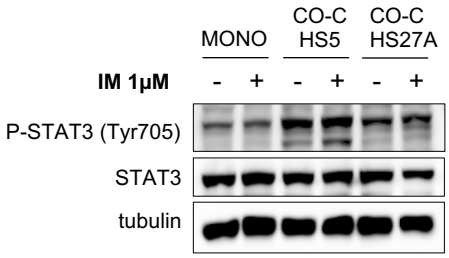

Fig. S5

A Representative immunoblots showing the expression of P-STAT3 (Tyr705) and STAT3 in K562 cells grown in mono- or co-culture with HS-5 WT or HS-27A stromal cells, with or without 1 μM imatinib for 24 hours. Tubulin was used as a loading control.