

*Supplementary Movie 1: Atomistic molecular dynamics simulations of the FGF2/ $\alpha$ 1-subCD3 interface based on the WT1 system.*

Comparison of the interaction of FGF2-wt and FGF2-K54/60E with  $\alpha$ 1-subCD3 using MD simulations. While the FGF2-wt/ $\alpha$ 1-subCD3 interface (left panel) remains stable during the whole simulation time (only the last 100 ns are shown in the video), FGF2-K54/60E dissociates from the  $\alpha$ 1-subCD3 domain already during the first 100 ns of simulation. See Materials and Methods for simulation details. The color coding and the representation styles for FGF2 and  $\alpha$ 1-subCD3 correspond to Figure 6. In addition, the licorice style is used to highlight the residues that are in contact with K54 and K60 from FGF2.

*Supplementary Movie 2: Single particle analysis of FGF2 membrane recruitment at the inner plasma membrane leaflet in living cells*

Time-lapse TIRF videos (100 ms/frame) of CHO-K1 cell lines expressing the FGF2-GFP fusion proteins introduced in Supplementary Figure 5A and in Fig. 8A (FGF2-K54E, FGF-K60E and FGF2-K54/60E). Background fluorescence was subtracted. Scale bar = 6  $\mu$ m.

*Supplementary Movie 3: Single particle analysis of FGF2 membrane recruitment at the inner plasma membrane leaflet in living cells*

Time-lapse TIRF videos (100 ms/frame) of CHO-K1 cell lines expressing the FGF2-GFP fusion proteins introduced in Supplementary Figure 5B and in Fig. 8B (FGF2-K54E, FGF-K60E and FGF2-K54/60E combined with K127Q/R128Q/K133Q). Background fluorescence was subtracted. Scale bar = 6  $\mu$ m.