Supplementary Figure S1: Frequent STAT3 acetylation in tumor and cancer cell lines.
(A) Tumor sections (carcinoid, lymphoma, undifferentiated cancer) from patients were stained for K685-acetylated and Y705-phosphorylated STAT3 and analyzed by confocal microscopy. Scale bar, 50 μm.
(B) STAT3 was immunoprecipitated using whole cell lysates from cancer cell lines as indicated and acetylated STAT3 was analyzed in Western blotting. Pre-immune serum (PIS) was used as a control for STAT3 pull down.

Supplementary Figure S2: Immediate early functional activation of acetylation-deficient STAT3- K685R-YFP. Stat3-YFP constructs as indicated were transiently expressed in mouse MEFStat3Δ/Δ cells and stimulated with 10 ng/ml OSM for 30 min.
(A) Whole cell lysates were prepared and subjected to Western blot analysis of pY705-Stat3. Stat3-SNICQmut-YFP and Stat3D-YFP represent DNA-binding deficient mutants. Stat3-Y705F-YFP is thought to be dysfunctional in immediate early activation.
(B) Nuclear translocation of Stat3-YFP constructs as indicated was analyzed in fixed cells by confocal microscopy. Single channels of each overlay image are split and shown. Scale bar, 10 μm.
Supplementary Figure S3: Loss of STAT3/exportin 7 interaction upon blocking acetylation in vivo.

(A) Growth kinetics of subcutaneously engrafted tumors expressing STAT3-WT or STAT3-K685R was assessed. SD shown; significance: ***) P<0.001.

(B) Loss of STAT3/exportin 7 interaction upon blocking lysine acetylation was assessed by immunoprecipitation using tumor homogenates. STAT3 co-precipitation was analyzed in Western blotting.

Supplementary Figure S4: Unaffected expression of STAT3 and Exportin 7 mRNA upon inhibition of STAT3 acetylation was determined using human HCT colon carcinoma cells expressing STAT3-WT (HCT116wt) or STAT3-K685R (HCT116k685r) by RT-PCR. SD shown.