**Legends for Supplemental Figures**

**Supplemental Figure S1**: *The effect of -secretase inhibition on ectodomain cleavage*

**A**: RPM-MC cells were transfected and treated as in Figure 1, either without DAPT or with DAPT. Absence of gamma secretase inhibition caused loss of the intracellular cleavage product.

**B**: RPM-MC cells were transfected with an N-terminally truncated CD44E mimicking the cleavage product of ADAM10. CD44E is substrate for gamma secretase. Without DAPT the intracellular domain is released.

**Supplemental Figure S2.** *Regulated cleavage targets substrates after surface expression*

(**A** and **B**) Biotinylation of cell surface proteins shortly prior to cleavage stimulation proves that the release of the CD44 and NRG1 ectodomains occurs by cleavage of pre-formed and already plasma membrane-associated substrates. Transfected RPM-MC cells were subjected to cell surface biotinylation and shortly thereafter stimulated with TPA (100 ng/ml, 2 hours). Biotinylated proteins were pulled down from supernatant and cell lysate using streptavidin beads; immunoblots as indicated. Un-bound proteins were also analyzed. Cell lysates revealed biotinylated CD44fl and mature ADAM10, but not proform of ADAM10. The cell supernatant contained no biotin-carrying solCD44E in the control, but increased release after TPA, which is blocked by batimastat. Biotinylated neuregulin (B) is visible in the control – compatible with the relatively higher spontaneous cleavage – and elevated upon TPA treatment.

**Supplemental Figure S3**:*Interaction between CD44 and Merlin.*

**A**: Merlin dephosphorylation at high cell density

RPM-MC cells were stably transfected with expression constructs encoding either CD44 wt or CD44-KR-Mt. The cells were plated at low or high cell density in 12-well plates. Lysates were subjected to 10 % SDS-PAGE and immunoblotted with merlin specific antibody (C-18).

**B**: Co-immunoprecipitation of CD44 and Merlin

Cells as in A were seeded at high cell density and treated with the cross-linker DSP. Lysates were immune-precipitated with CD44-specific antibody (5G8). The immune precipitates (and aliquots of the lysates before immune precipitation) were subjected to 10 % SDS-PAGE and immunoblotted with merlin specific antibody (C-18).

**Supplemental Figure S4**: *The tumor suppressor merlin (Nf2) inhibits CD44 cleavage.*

NIH3T3 cells co-transfected with a plasmid encoding CD44Wt and plasmids encoding wt merlin, constitutively active merlin, NFS518A, and the phospho-mimicking mutant NFS518D, were grown at low cell density. The dephosphorylation-mimicking merlin mutant NFS518A inhibited CD44 cleavage (shown for the released solCD44E and the residual membrane-bound fragment CD44ΔE). The phospho-merlin mimicking inactive mutant NFS518D did not affect the cleavage induction as in Fig. 2.

**Supplemental Figure S5.** *Exemplified analysis of the cells used in the migration assay of Figure 7A and Supplemental Figure 6*

The MEFs carrying CD44fl/fl and GT(Rosa)26-CRE were immortalized by p19 downregulation through introduction of sh-ARF (courtesy of Zhao Qi Wang). shNF2 was stably introduced using a lentiviral construct (courtesy of Cui Yan). The floxed MEFs were treated with 5 M 4-hydroxytamoxifen for two weeks to excise the *cd44* genes. The immunoblots show also the levels of reintroduced CD44 wt and mutants. In the non-cleavable CD44Δstalk the sequence between the transmembrane domain and the first globular domain of CD44 was deleted. It was generated by site-directed mutagenesis usingstandard CD44 in the pFLAG-myc-CMV-21 vector as a template and the following primers: Fw: 5’- gtgatggcacctggcttatcatc **– 3’** andRev: 5’- GATGATAAGCCAGGTGCCATCAC –3’.

**Supplemental Figure S6:** *Exemplified photographs of one of the scratch assays with MEFs as shown in Figure 7A.*

Scratch assays using immortalized mouse embryonic fibroblasts from mice with floxed *cd44*alleles (CD44fl/fl; GT(Rosa)26-CRE (B6/129) and derivatives of these cells. **(1)** CD44 expressing cells. **(2)** CD44fl/fl cells with stably downregulated *nf2* expression. **(3)** CD44 deletion of the cells in (2) upon CRE induction by tamoxifen. **(4)** Cells of (3) with stable overexpression of CD44wt. **(5)** Cells of (3) with stable overexpression of CD44-KR-Mt. **(6)** Cells of (3) with stable overexpression of the CD44 stalk deletion mutant. For details of the assay see Supplemental Material and Methods, and in the legend to Supplemental Figure S5.

**Supplemental Figure S7:** *Loading diagrams of Figures 3B amd 4C*