Supplemental data
Figure legends

Figure 1. EGFR and ErbB2 transactivation by the PAR1 specific agonist peptide. Serum-deprived MDA-MB-231 cells were incubated with 100 μM TFLLRNPNDK for various times at 37°C. Cells were lysed, immunoprecipitated with anti-PY99 antibody and immunblotted with anti-EGFR or anti-ErbB2 antibody. Total cellular lysates were immunoblotted for EGFR or ErbB2 as a control. The time-course of TFLLRNPNDK-induced EGFR and ErbB2 transactivation shown is from a representative experiment. The data (mean ± S.E.) are expressed as fold increase over basal from three independent experiments.

Figure 2. PAR1 and PAR2 are expressed in MDA-MB-231 cells. Total RNA was isolated from MDA-MB-231 cells using TRIzol reagents (Invitrogen), treated with DNase and quantified by OD_{260}. Reverse transcriptase (RT) reactions were performed with 2 μg of total cellular RNA, 100 ng random primers, and 1 μl of SuperScript II RT (Invitrogen) in a 20 μl- reaction volume according to the manufacturer's instructions and is shown in lanes marked as plus "+". Mock RT reactions of total cellular RNA were performed with no reverse transcriptase added is shown in lanes marked as minus "-". RT product (2 μl) was amplified by PCR in a 50 μl-volume containing a final 0.2 μM PAR specific primers (see below) and 5 U of *Taq* polymerase (Invitrogen). Reaction conditions were one cycle of 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 30 s, and then one cycle of 72°C for 10 min. (**a**) The following PAR primer sets were used to generate specific PCR where the nucleotide numbering is such that 1 equals the A of the start ATG as previously described M. L. Kahn *et al*. *J. Clin. Invest.* 1999 103: 879-887. The primer set for PAR1 PCR reaction included the forward primer 5' CAG TTT GGG TCT GAA TTG TGT CG 3' (1148-1129) and reverse primer 5' TGC ACG AGC TTA TGC TGC TGA 3' yielding a 592 kb PCR product (lanes 1 and 2). PAR2 cDNA was PCR amplified using the forward primer 5' TGG ATG AGT TTT CTG CAT CTG TCC 3' and reverse primer 5' CGT GAT GTT CAG GGC AGG AAT G 3' resulting in a PCR product of 491 bp (lanes 3 and 4). PAR3 was PCR amplified using forward primer 5' TCC CCT TTT CTG CCT TGG AGG 3' and reverse primer 5' AAA CTG TTG CCC ACA CCA GTC CAC 3' resulting in a PCR 513 kb product (lanes 5 and 6). (**b**) PAR1 was amplified using the same primer set described in above (lanes 1 and 2), whereas PAR4 was PCR amplified using the forward primer 5' GGC AAC CTC TAT GGT GCC TA 3' and reverse 5'AC TGG AGC AAA GAG GAG TGG 3' yielding an expected product size of 211 bp (lanes 3 and 4). (**c**) PCR amplification of PAR1 and PAR4 cDNAs for assessing primer pair amplification ability. PAR1 pBJ, PAR4 pBJ or pBJ vector at 0.10 ng were PCR amplified with the gene specific primers for varying numbers of cycles to demonstrate the comparable ability of the primers to amplify equal amount of template.